



UNIVERSITY OF PITTSBURGH

EIGHTEENTH

*POSTDOCTORAL  
RESEARCH  
SYMPOSIUM*

April 14, 2025 | 12:30-4pm  
William Pitt Union

April 14, 2025

Dear Colleagues,

Welcome to the 18th Annual Postdoctoral Research Symposium! This year's event is dedicated to the postdoctoral fellows at the University of Pittsburgh—a highly talented and innovative pool of early-stage investigators whose passion and commitment to research fuels new discoveries every day. We are thrilled to have you join us in recognizing and sharing the innovative work being conducted across disciplines.

Formerly known as Data & Dine, this symposium celebrates the outstanding contributions of our postdocs while providing a valuable opportunity to connect, exchange ideas, and build collaborations with each other. We encourage you to engage with your peers, explore the diverse research on display, and take advantage of this networking space. Additionally, UPPDA board elections are coming up—if you're interested in getting involved, we'd love to chat!

Continuing our tradition, we are proud to present professional development awards for the best poster presentations. Thanks to the generous support of our academic community, UPPDA will recognize 10 postdoctoral fellows for their exceptional work.

This event would not be possible without the support of the Office of Academic Career Development, the Office of the Senior Vice Chancellor for the Health Sciences, the Office of the Provost, and our departmental sponsors. We also extend our gratitude to the poster judges and volunteers who have contributed to making this event a success.

Finally, thank you to all fellows, faculty, and administrators for being part of this special day. We hope you find inspiration, connections, and opportunities to further your research journey.

Enjoy the symposium!

---- 2025 UPPDA Executive Board

President: Ashley Shafer, PhD

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**Executive Board Officers 2024-2025**

|                                      |                                                                                                                                                          |
|--------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
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| <b>Networking Chair</b>              | <b>Tamara Kadi, MD</b><br>School of Medicine<br>Department of Medicine<br><a href="mailto:tak244@pitt.edu">tak244@pitt.edu</a>                           |

**AGENDA**  
**April 14, 2025 | 12:30-4:00 PM**  
**WILLIAM PITT UNION**

|                          |                                                                                                   |
|--------------------------|---------------------------------------------------------------------------------------------------|
| <b>10:00 AM-12:00 PM</b> | <b>Poster Set-Up &amp; Check-in</b><br>Lobby                                                      |
| <b>12:00 – 12:30 PM</b>  | <b>Judge and Guest Check-In</b>                                                                   |
| <b>12:30-1:30 PM</b>     | <b>Poster Session 1</b><br>Odd Numbered Posters<br>Ballroom, Kurtzman Room, and<br>Assembly Room  |
| <b>1:30-2:30 PM</b>      | <b>Poster Session 2</b><br>Even Numbered Posters<br>Ballroom, Kurtzman Room, and<br>Assembly Room |
| <b>2:30-4:00 PM</b>      | <b>Networking &amp; Awards Reception</b><br>William Pitt Union Lower Lounge                       |

## POSTDOCTORAL LEADERSHIP AWARD WINNER



### **Dr. Rachael Rush**

Dr. Rachael Rush is a third-year Postdoctoral Scholar in the Center for Vaccine Research (CVR) and the Department of Infectious Diseases and Microbiology at the University of Pittsburgh's School of Public Health. She received her PhD in Immunology and Microbial Pathogenesis and a University Teaching Certificate from WVU School of Medicine in 2022, following a B.S. in Microbiology and a Life Sciences Research Certificate from the University of Pittsburgh in 2017. Dr. Rush's research focuses on the pathogenesis of high containment arboviruses. As a team lead in her lab under PI Dr. Amy Hartman, Dr. Rush oversees graduate students, undergraduate students, and technicians.

Beyond her research, Dr. Rush has made significant contributions to the University of Pittsburgh postdoctoral community. She served as the 2023-2024 president of the University of Pittsburgh Postdoctoral Association (UPPDA) and currently serves as the postdoctoral representative on the Senate Research Committee. While president of the UPPDA, she represented the Pitt postdoctoral community at the 2024 NPA meeting in Seattle. She has earned the "Leading People in Organizations" micro-credential from the Katz School of Business and has also participated in numerous career development panels and programs organized by the OACD and others. Beyond the lab, Dr. Rush was awarded an NIH Loan Repayment Program (LRP) award and actively serves as a program ambassador, helping fellow postdocs navigate the program and application process. She has been actively involved in various departmental committees, including the CVR Trainee Day and Invited Speaker committees, and regularly volunteers for science outreach events. Additionally, Dr. Rush serves as the lead advisor for the Delta Phi Epsilon undergraduate chapter at the University of Pittsburgh, demonstrating her commitment to mentoring and engaging with the broader Pitt community. Outside of work, Dr. Rush enjoys cooking, exploring new restaurants with her husband Dylan, and spending time with her family and cats, Sam and Sadie. She is deeply honored to receive the Postdoctoral Leadership Award from the UPPDA and looks forward to continuing to serve the Pitt postdoctoral community.

**ABOUT THE POSTDOCTORAL LEADERSHIP AWARD** The University of Pittsburgh Postdoctoral Association offers an annual Postdoctoral Leadership Award, formally known as the Alumni Award, to an individual who has demonstrated a profound, sustained, or leadership contribution to the Postdoctoral Association. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

#### **Past Alumni Award Recipients**

|                                                                    |                                              |
|--------------------------------------------------------------------|----------------------------------------------|
| <a href="#">2024</a> – Mohammadreza Tabary, MD                     | <a href="#">2012</a> – Aaron Bell, PhD       |
| <a href="#">2023</a> – David Gau, PhD                              | <a href="#">2011</a> – Natacha De Genna, PhD |
| <a href="#">2019</a> – Kimberly Payne, PhD                         | <a href="#">2010</a> – Lei Hong, PhD         |
| <a href="#">2018</a> – Chelsea Stillman, P<br>Collin Diedrich, PhD | <a href="#">2009</a> – David Robinson, PhD   |
| <a href="#">2017</a> – Karen Carney, PhD                           | <a href="#">2008</a> – Richard Bodnar, PhD   |
| <a href="#">2016</a> – John Merriam, PhD                           | <a href="#">2007</a> – Steven Wendell, PhD   |
| <a href="#">2014</a> – Catherine Haggerty, PhD                     | <a href="#">2006</a> – Elsa Strotmeyer, PhD  |
| <a href="#">2013</a> – Timothy Maul, PhD                           | <a href="#">2005</a> – Stuart Olmsted, PhD   |

## POSTDOCTORAL ADVOCATE AWARD WINNER



### **Dr. Laura J. Miller**

Laura Miller, PhD, PC, is the Associate Director of the Office of Academic Career Development (OACD) for the University of Pittsburgh Schools of the Health Sciences. She is responsible for the strategic planning, development, marketing, execution, and assessment of career development programs, workshops, and special events for biomedical scientists, clinical researchers, and trainees across all six schools of the health sciences to develop leaders and drive career excellence. Dr. Miller works closely with the UPPDA Executive Board to support their success and develops important programming for postdoctoral researchers that enhances postdoctoral career development opportunities and meets the needs of the postdoc community throughout the year. This includes the development of unique postdoctoral resources like the PerLL program

and the establishment of the F32 writing program. In this role, she also collaborates with researchers, academic personnel, and thought leaders across campus to create innovative programs that keep the university at the cutting-edge. Dr. Miller graduated with a PhD in Medical Neurobiology from Indiana University School of Medicine, completed postdoctoral training in the field of behavioral neuroscience, earned the "Leading People in Organizations" professional credential from the Katz Graduate School of Business, and obtained specialized training in project management and teaching. She also completed Duquesne University's Palumbo-Donahue School of Business Professional Coaching Program to become a professional coach (PC). Dr. Miller combines her passion, educational background, and experience to create transformative programming in support of the University of Pittsburgh's role as a national leader in biomedical research.

**ABOUT THE POSTDOCTORAL ADVOCATE AWARD** The University of Pittsburgh Postdoctoral Association offers an annual Postdoctoral Advocate Award to an individual who has demonstrated a profound, sustained, or leadership contribution to the Postdoctoral Association. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

#### **Past Advocate Award Recipients**

|                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>2024 – Ian Sigal, PhD</p> <p>2023 – Amanda Godley, PhD</p> <p>2019 – Nathan Urban, PhD</p> <p>2018 – Thomas Smithgall, PhD</p> <p>2017 – Arvind Suresh, MS</p> <p>2016 – Jennifer Woodward, PhD</p> <p>2015 – Ora Weisz, PhD</p> <p>2014 – Alan Sved, PhD</p> <p>2013 – Charles Nieman, PhD, DSO, ARO</p> | <p>2012 – Darlene Zellers, PhD</p> <p>2011 – Christopher Martin, PhD</p> <p>2010 – Patricia Beeson, PhD</p> <p>2009 – Bruce Freeman, PhD</p> <p>2008 – Andrew Blair, PhD</p> <p>2007 – Joan Lakoski, PhD</p> <p>2006 – Elizabeth Baranger, PhD</p> <p>2005 – Arthur Levine, MD</p> |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## POSTDOCTORAL MENTOR AWARD WINNER



### Dr. Michael S. Gold

Michael S. Gold, Ph.D., is a Professor of Neurobiology at the University of Pittsburgh. Dr. Gold received his B.A. from UC Berkeley, Ph.D. from UCLA, and completed postdoctoral fellowships at USCF and UCLA. The focus of Dr. Gold's research is the neurobiology of pain. He has made important contributions to injury-induced plasticity in nociceptive afferents, and their contribution to the manifestation of persistent pain. Toward this end, he has employed an array of approaches ranging from the study of isolated cells to the development of novel preclinical behavioral assays with which to assess the presence of persistent hypersensitivity, and more recently the study of clinical populations suffering from persistent pain. Dr. Gold is actively involved in the greater pain community working to form the [US Association for the Study of Pain](#) and most recently

serving as Treasurer for the [International Association for the Study of Pain](#).

Dr. Gold has trained 18 postdoctoral fellows and 8 predoctoral students, with several postdocs already transitioning to independent research roles and even faculty positions at Pitt. His mentorship not only drives significant scientific advancements but also lays the foundation for the long-term success of his trainees, ensuring that they are well-equipped to lead and innovate in the broader scientific community. He routinely provides instruction in specialized techniques and ensures that every team member has the opportunity to share progress and receive constructive feedback during lab meetings. His efforts extend beyond his own lab, as he actively encourages participation in programs like journal clubs, seminars, pain classes, and international learning opportunities. He even serves as a co-mentor for postdocs from other labs. This organic fostering of diversity and inclusion has led to a vibrant and multicultural research group. Dr. Gold's long-term influence is apparent in the way he prepares his trainees for independent success. He equips them with essential project management and networking skills, ensuring they are well-prepared for careers beyond the lab. His ability to create a low-pressure yet intellectually rigorous environment, combined with his constant availability and genuine enthusiasm for his work, distinguishes him as an exceptional mentor. Anecdotes such as his involvement in extracurricular events—organizing group activities at PCPR, dressing up as Santa Claus for the holiday party, or sharing fresh eggs from his chicken coop, bringing Oakmont Bakery Doughnuts every (well almost) Friday—highlight his unique approach to building a strong, cohesive community that values both professional excellence and personal well-being.

**ABOUT THE POSTDOCTORAL MENTOR AWARD** The University of Pittsburgh Postdoctoral Association Mentor Award criterion is modeled from the National Postdoctoral Association (NPA) Mentor Award, which recognizes a faculty member who has been engaged in exceptional mentoring of postdoctoral fellows and postdoctoral scholars. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

#### Past Mentor Award Recipients

2024 – Michele D. Levine, PhD

2023 – Ian Scott, PhD

2019 – Ronald Buckanovich, PhD

2018 – Tina Goldstein, PhD

2017 – Brooke Molina, PhD

2016 – Melissa Bilec, PhD

2015 – Jennifer Silk, PhD

## POSTER PRESENTATION & AWARD GUIDELINES

Posters will be judged from 12:30 PM to 2:30 PM.

Presenters with **odd** numbered posters will be judged from **12:30 PM to 1:30 PM**.

Presenters with **even** numbered posters will be judged from **1:30 PM to 2:30 PM**.

*Presenters are to be present at their posters at their assigned times.*

The top poster presenters will each receive a \$500 Professional Development Award. These awards will provide funds for professional development, including participation in a scientific conference, and are to be used by recipients between July 2025 and May 2026. The award categories include:

- *Basic Biomedical and Pharmacological Sciences*
- *Behavioral Sciences and Humanities, Education and Curriculum, and DEI*
- *Cellular and Molecular Biology*
- *Clinical and Surgical Sciences*
- *Engineering, Physical, and Computational Sciences*

All posters have been assigned to one of these categories for judging purposes. The judges will not be identified during the poster sessions. Scoring will be based upon creativity, style, content, impact, presentation, and overall impressions.

Award winners will be announced during the reception following the poster sessions.

## POSTER PRESENTATION AWARD SPONSORS

### GOLD SPONSORS

**\$501 - \$1,000**

Department of Immunology  
School of Medicine

Department of Neurobiology  
School of Medicine

### SILVER SPONSORS

**\$251 - \$500**

Department of Computational and Systems Biology  
School of Medicine

Department of Pediatrics  
School of Medicine

Department of Psychiatry  
School of Medicine

Department of Radiology  
School of Medicine

Pittsburgh Institute for Neurodegenerative Diseases  
School of Medicine

Vascular Medicine Institute  
School of Medicine

## INDEX OF POSTER PRESENTERS

**Abstracts are in alphabetical order starting on page 16.**

| Poster | Name                         | Category                                                              | Email                    |
|--------|------------------------------|-----------------------------------------------------------------------|--------------------------|
| 98     | Abdelhady, Ali               | Clinical and Surgical Sciences                                        | AOA90@pitt.edu           |
| 1      | Acharya, Vibha               | Basic Biomedical and Pharmacological Sciences                         | via16@pitt.edu           |
| 99     | Ajam, Ali                    | Clinical and Surgical Sciences                                        | ala449@pitt.edu          |
| 100    | Alimohamadi, Sahar           | Clinical and Surgical Sciences                                        | alimohamadis@upmc.edu    |
| 50     | Amalric, Amandine            | Cellular and Molecular Biology                                        | ama527@pitt.edu          |
| 37     | Antezana, Ligia              | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | antezanald@upmc.edu      |
| 38     | Anujoo, Kenneth              | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | koa51@pitt.edu           |
| 51     | Barrenada, Odei              | Cellular and Molecular Biology                                        | barrenadatalebo@upmc.edu |
| 101    | Bauer-Negrini, Guilherme     | Clinical and Surgical Sciences                                        | gbnegrini@pitt.edu       |
| 52     | Beaulieu, Delphine           | Cellular and Molecular Biology                                        | dpb61@pitt.edu           |
| 2      | Becker, Casey                | Basic Biomedical and Pharmacological Sciences                         | cab648@pitt.edu          |
| 3      | Behzadi, Parya               | Basic Biomedical and Pharmacological Sciences                         | pab222@pitt.edu          |
| 53     | Bhat, Aditi                  | Cellular and Molecular Biology                                        | adb344@pitt.edu          |
| 4      | Bhise, Abhinav               | Basic Biomedical and Pharmacological Sciences                         | abb195@pitt.edu          |
| 54     | Boosi Narayana Rao, Kannan   | Cellular and Molecular Biology                                        | KAB660@PITT.EDU          |
| 55     | Budai, Shanmukha Vivek Vinod | Cellular and Molecular Biology                                        | bus15@pitt.edu           |
| 102    | Campion, Jacques-Yves        | Clinical and Surgical Sciences                                        | jpc211@pitt.edu          |
| 122    | Campos, Vinicius             | Engineering, Physical, and Computational Sciences                     | vpc24@pitt.edu           |
| 49     | Campos Leite, Taiana         | Cellular and Molecular Biology                                        | tal117@pitt.edu          |
| 5      | Chagas, Brisa                | Basic Biomedical and Pharmacological Sciences                         | bca23@pitt.edu           |
| 6      | Chen, Jialiang               | Basic Biomedical and Pharmacological Sciences                         | JIC374@pitt.edu          |
| 7      | Chen, Jialiang               | Basic Biomedical and Pharmacological Sciences                         | JIC374@pitt.edu          |
| 8      | Chen, Shangyu                | Basic Biomedical and Pharmacological Sciences                         | shc315@pitt.edu          |

| Poster | Name                | Category                                                              | Email                |
|--------|---------------------|-----------------------------------------------------------------------|----------------------|
| 123    | Chen, Baixi         | Engineering, Physical, and Computational Sciences                     | baixi.chen@pitt.edu  |
| 39     | Chou, Aileen        | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | aileen.chou@pitt.edu |
| 31     | Christian, Caroline | Behavioral Sciences                                                   | carolinec2@upmc.edu  |
| 56     | Chronis, Ian        | Cellular and Molecular Biology                                        | ibc10@pitt.edu       |
| 9      | Crummy, Elizabeth   | Basic Biomedical and Pharmacological Sciences                         | eac124@pitt.edu      |
| 57     | Culbertson, Ed      | Cellular and Molecular Biology                                        | emc217@pitt.edu      |
| 58     | Cvammen, William    | Cellular and Molecular Biology                                        | whc26@pitt.edu       |
| 59     | Das, Arun           | Cellular and Molecular Biology                                        | ard212@pitt.edu      |
| 60     | Das Ghatak, Piya    | Cellular and Molecular Biology                                        | pid12@pitt.edu       |
| 61     | DeMarco, Andrew     | Cellular and Molecular Biology                                        | demarcoag@upmc.edu   |
| 62     | Duan, Bingbing      | Cellular and Molecular Biology                                        | bid14@pitt.edu       |
| 10     | Ekhtor, Ese         | Basic Biomedical and Pharmacological Sciences                         | ese33@pitt.edu       |
| 40     | Eldeeb, Safaa       | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | eldeeb@upmc.edu      |
| 32     | Feldman, Julia      | Behavioral Sciences                                                   | feldmanjs2@upmc.edu  |
| 103    | Felix, Cynthia      | Clinical and Surgical Sciences                                        | cyf9@pitt.edu        |
| 63     | Filingeri, Domenic  | Cellular and Molecular Biology                                        | domfil@pitt.edu      |
| 64     | FNU, Navdezda       | Cellular and Molecular Biology                                        | NAF94@pitt.edu       |
| 41     | Fulay, Aarohee      | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | apf31@pitt.edu       |
| 104    | Furman, Leah        | Clinical and Surgical Sciences                                        | furmanl@upmc.edu     |
| 105    | Furman, Leah        | Clinical and Surgical Sciences                                        | furmanl@upmc.edu     |
| 11     | Galgano, Luca       | Basic Biomedical and Pharmacological Sciences                         | lucag@pitt.edu       |
| 65     | Gandhi, Jaishree    | Cellular and Molecular Biology                                        | vvg13@pitt.edu       |
| 66     | Gandhi, Vishwa      | Cellular and Molecular Biology                                        | jag581@pitt.edu      |
| 124    | Ganjee, Razieh      | Engineering, Physical, and Computational Sciences                     | r_ganjee@pitt.edu    |
| 12     | Ghelich, Pejman     | Basic Biomedical and Pharmacological Sciences                         | peg72@pitt.edu       |
| 67     | Glathar, Alexandra  | Cellular and Molecular Biology                                        | alg507@pitt.edu      |

| Poster | Name                | Category                                                              | Email               |
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| 42     | Griffith, Rebecca   | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | griffithr3@upmc.edu |
| 106    | Helou, Vanessa      | Clinical and Surgical Sciences                                        | vah73@pitt.edu      |
| 107    | Helou, Vanessa      | Clinical and Surgical Sciences                                        | vah73@pitt.edu      |
| 68     | Islam, Rahnuma      | Cellular and Molecular Biology                                        | rai31@pitt.edu      |
| 69     | Issa, Dalia         | Cellular and Molecular Biology                                        | dar258@pitt.edu     |
| 70     | Jauro, Solomon      | Cellular and Molecular Biology                                        | soj22@pitt.edu      |
| 13     | Kargl, Chris        | Basic Biomedical and Pharmacological Sciences                         | ckk23@pitt.edu      |
| 14     | Katana, Dzana       | Basic Biomedical and Pharmacological Sciences                         | dzk7@pitt.edu       |
| 15     | Kim, Elena          | Basic Biomedical and Pharmacological Sciences                         | ysk13@pitt.edu      |
| 71     | Kumar, Amit         | Cellular and Molecular Biology                                        | amk550@pitt.edu     |
| 72     | Kumar, Amit         | Cellular and Molecular Biology                                        | amk550@pitt.edu     |
| 108    | Li, Yu              | Clinical and Surgical Sciences                                        | liy23@upmc.edu      |
| 109    | Liang, Hai-Wei      | Clinical and Surgical Sciences                                        | hal137@pitt.edu     |
| 110    | Lovence, Keisha     | Clinical and Surgical Sciences                                        | KEL358@pitt.edu     |
| 111    | Lukan, Caiden       | Clinical and Surgical Sciences                                        | cjl224@pitt.edu     |
| 16     | Ma, Denglei         | Basic Biomedical and Pharmacological Sciences                         | dam471@pitt.edu     |
| 73     | Mabry, Samuel       | Cellular and Molecular Biology                                        | sjm255@pitt.edu     |
| 74     | Maestas, David      | Cellular and Molecular Biology                                        | drm154@pitt.edu     |
| 75     | Maneekul, Jindanuch | Cellular and Molecular Biology                                        | jim119@pitt.edu     |
| 33     | McChesney, Eric     | Behavioral Sciences                                                   | erm216@pitt.edu     |
| 125    | Mcloughlin, Kiran   | Engineering, Physical, and Computational Sciences                     | kjm257@pitt.edu     |
| 17     | Mills, Amber        | Basic Biomedical and Pharmacological Sciences                         | AVM108@pitt.edu     |
| 112    | Mroue, Rayan        | Clinical and Surgical Sciences                                        | ram518@pitt.edu     |
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| Poster | Name                     | Category                                                              | Email                |
|--------|--------------------------|-----------------------------------------------------------------------|----------------------|
| 126    | Mustafaev, Tamerlan      | Engineering, Physical, and Computational Sciences                     | t.mustafaev@pitt.edu |
| 113    | Naame, Elissa            | Clinical and Surgical Sciences                                        | eln58@pitt.edu       |
| 76     | Oliveira Junior, Markley | Cellular and Molecular Biology                                        | MAS1505@pitt.edu     |
| 77     | Packard, Jessica         | Cellular and Molecular Biology                                        | jep329@pitt.edu      |
| 43     | Park, Sangmi             | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | sap545@pitt.edu      |
| 34     | Park, Yul Min            | Behavioral Sciences                                                   | yulminpark@pitt.edu  |
| 20     | Pastukh, Vasyi           | Basic Biomedical and Pharmacological Sciences                         | vap81@pitt.edu       |
| 78     | Pearce, Oluwaseyi        | Cellular and Molecular Biology                                        | sap465@pitt.edu      |
| 79     | Pearson, Andrew          | Cellular and Molecular Biology                                        | pearsonac4@upmc.edu  |
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| 80     | Pinero, Carlos           | Cellular and Molecular Biology                                        | CAP215@pitt.edu      |
| 21     | Pinto, Alipio            | Basic Biomedical and Pharmacological Sciences                         | ave21@pitt.edu       |
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| 81     | Prabhu, Sathyen          | Cellular and Molecular Biology                                        | sap447@pitt.edu      |
| 45     | Prakash, Krithika        | Behavioral Sciences and Humanities, Education and Curriculum, and DEI | krp228@pitt.edu      |
| 23     | Qi, Meiyuzhen            | Basic Biomedical and Pharmacological Sciences                         | meq8@pitt.edu        |
| 82     | Rafael Guimaraes, Thais  | Cellular and Molecular Biology                                        | thr38@pitt.edu       |
| 24     | Raminfard, Samira        | Basic Biomedical and Pharmacological Sciences                         | raminfards@upmc.edu  |
| 83     | Rani, Annu               | Cellular and Molecular Biology                                        | anr525@pitt.edu      |
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| 114    | Rocha, Andreia           | Clinical and Surgical Sciences                                        | anr436@pitt.edu      |
| 85     | Romero, Daniela          | Cellular and Molecular Biology                                        | djr154@pitt.edu      |
| 115    | Rosenblum, Lauren        | Clinical and Surgical Sciences                                        | ltr20@pitt.edu       |
| 86     | Roy, Abhishek            | Cellular and Molecular Biology                                        | ABR171@PITT.EDU      |

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| 127    | Saha, Anumeha                | Engineering, Physical, and Computational Sciences                     | ans1201@pitt.edu      |
| 87     | Saleh Ziabari, Omid          | Cellular and Molecular Biology                                        | oms81@pitt.edu        |
| 117    | Scarpatto Rodrigues, Matheus | Clinical and Surgical Sciences                                        | msr104@pitt.edu       |
| 35     | Schroeder, Rachel            | Behavioral Sciences                                                   | schroederra@upmc.edu  |
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| 94     | Yang, Yue                    | Cellular and Molecular Biology                                        | yueyang@pitt.edu      |
| 95     | Yee, Eric                    | Cellular and Molecular Biology                                        | egy11@pitt.edu        |
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# UPPDA

University of Pittsburgh Postdoctoral Association

| Poster | Name                        | Category                                          | Email             |
|--------|-----------------------------|---------------------------------------------------|-------------------|
| 30     | Yu, Guoning                 | Basic Biomedical and Pharmacological Sciences     | i.ygn97@gmail.com |
| 129    | Zamani Ashtiani, Shaghayegh | Engineering, Physical, and Computational Sciences | shz110@pitt.edu   |
| 97     | Zheng, Wenxiao              | Cellular and Molecular Biology                    | wenxiao@pitt.edu  |
| 121    | Zynda, Aaron                | Clinical and Surgical Sciences                    | ajz61@pitt.edu    |

## **Role of MUC1 Transmembrane Glycoprotein in the Urinary Tract Host Defense against Infections**

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Urinary tract infections (UTIs), mainly caused by uropathogenic *Escherichia coli* (UPEC), are among the most common bacterial infections. The rise in antimicrobial resistance and recurrent infections underscore the need for alternative approaches. Understanding host defense mechanisms in the bladder and kidneys may offer new therapeutic targets for selective, antimicrobial-sparing therapeutic approaches. MUC1, a transmembrane glycoprotein expressed on the apical surface of epithelial cells in the bladder and kidneys, has a known role in various infections, yet its involvement in UTIs is unclear. We *hypothesize* that MUC1 acts as a decoy receptor for bacterial pathogens and modulates immune responses during UTIs, potentially serving as a diagnostic biomarker.

To test this hypothesis, we analyzed MUC1 levels in urine samples from 75 UTI-positive and 75 age- and sex-matched UTI-negative patients admitted to an emergency department. We found that age, UTI status, and NGAL levels significantly influenced MUC1 levels. MUC1 levels were negatively correlated with UTI status ( $p = 0.0008$ ), suggesting that higher MUC1 levels reduce UTI risk. Interestingly, MUC1 and NGAL were positively correlated ( $p = 0.0001$ ). In addition, we investigated MUC1 haploinsufficiency in MUC1 Kidney Disease (MKD) patients, who express about 50% normal MUC1. Female MKD patients had significantly higher UTI rates compared to unaffected family members (odds ratio = 8.63;  $p = 0.02$ ). Further analysis revealed that MUC1 levels were positively correlated with pro-inflammatory Th2 cytokines which are important for immune cell development and bacterial clearance.

In mouse models of experimental UTI, *Muc1* KO females displayed increased susceptibility to UTIs, with more severe urothelial damage, inflammation, and higher bacterial counts compared to wild-type (WT) littermates.

Our findings suggest that MUC1 plays a key role in regulating the host's dynamic response to bacterial infections, involving both innate and adaptive immune mechanisms. This knowledge opens new avenues for targeted, antimicrobial-sparing therapies for UTIs.

**Cis-Expression Quantitative Trait Loci (eQTL) Analysis on Blood-Derived Gene Expression in Older Adults**

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Genome-wide association studies (GWAS) have successfully identified thousands of genetic variants involved in complex traits, including aging and neurodegenerative diseases. However, elucidating the functional mechanisms of these genetic variants are difficult as the vast majority of them lie in the non-coding regions. Gene expression can help to map variants to genes and elucidate the functional role of the gene in disease/trait. We performed cis-expression quantitative loci (eQTL) analysis using blood-derived gene expression from 327 older adults aged 65 and above. Salmon was used to align the bulk RNA-seq reads to GRCh38.p14 homo sapiens reference transcriptome and to quantify transcripts. Genes with Transcripts per million (TPM) values less than 0.1% and read counts less than 6 in 20% of the samples or belonging to sex-chromosomes were excluded from the analysis. Cis-eQTL analysis was conducted on a total of 14181 genes (protein coding=14019, long noncoding RNA=161 and transcriptional element-associated transcript= 1) in TensorQTL using linear regression of the gene expression with genome-wide genotype data, including the first four principal components, race and sex as covariates. We adapted the multiple testing correction methods implemented by GTEx to define significant genes and variants. A total of 97806 variants were found to regulate the gene expression of 1921 genes. Our eQTL analysis provides knowledge on regulatory variants involved in gene expression in blood among older adults and can aid in functional annotation of GWAS associations.

**Cholesterol-mTOR Nexus: Dissecting the Impact of Hypercholesterolemia on Lysosomal Degradation Pathways**

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Atherosclerosis is the leading global cause of death. Cholesterol buildup in arterial walls triggers inflammation, recruiting monocytes and macrophages. These cells exhibit impaired autophagy, creating a hyperinflammatory, proatherogenic state unaddressed by cholesterol-lowering therapies. We hypothesize that blood low-density lipoprotein cholesterol (LDL-c) correlates with circulating monocyte mammalian target of rapamycin complex 1 (mTORC1) activity, impairing autophagy and promoting atherogenesis.

Peritoneal macrophages (pMACs) and human monocyte-derived macrophages (HMDMs) were treated with LDL-c to assess mTORC1 activation, autophagy, and apoptosis. ApoE-null mice were fed a Western diet for 10 days followed by a Chow diet for 10 days. Plasma cholesterol and monocyte mTORC1 activity were analyzed using FACS and western blot for phosphorylated S6.

In the clinical study, hypercholesterolemic patients (LDL>160 mg/dL, n=10) and healthy controls (LDL<130 mg/dL, n=10) were enrolled. Blood samples collected at baseline to isolate monocytes and measure cholesterol levels. After 12 weeks of lipid-lowering therapy (e.g., statins), hypercholesterolemic patients will provide a second sample.

LDL activated mTORC1 in pMACs and HMDMs in a time- and dose-dependent manner, suppressing autophagy and promoting apoptosis. ApoE-null mice on a Western diet had ~4-fold higher plasma cholesterol and ~10-fold greater monocyte mTORC1 activity, which declined sharply after switching to a Chow diet. Preliminary data show increased monocyte mTORC1 activation in hypercholesterolemic patients. Ongoing post-treatment analysis will assess lipid-lowering therapy's impact. These findings highlight the cholesterol-mTORC1 axis as a potential therapeutic target for cardiovascular disease.

**Hypercoagulable patients have increased long-term risk of limb loss following acute limb ischemia**

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Acute limb ischemia (ALI) is associated with high rates of amputation. Hypercoagulable states are associated with an increased risk of failure after elective revascularization; however, the impact on outcomes of ALI is not well documented.

Patients presenting with ALI to a multi-institution healthcare system from 2016 to 2023 were identified. Patients were separated into two cohorts: those with existing peripheral arterial disease (PAD; acute on chronic) and those without (de novo thrombosis). Within each cohort, patients with hypercoagulable disorders were compared to those without hypercoagulable disorders. The primary outcome was amputation and reintervention at one year.

Of the 638 patients (age  $64 \pm 13$ ; 44% female) included in the analysis, 347 (27 [9.3%] hypercoagulable) patients were included in the acute on chronic cohort and 291 (27 [7.8%] hypercoagulable) were in the de novo thrombosis cohort. The most common hypercoagulable state was antiphospholipid syndrome. Patients with hypercoagulable disorders were younger (55 vs 65,  $p < 0.05$ ), more female (69% vs 41%,  $p < 0.05$ ), had higher BMIs (31 vs. 27,  $p < 0.05$ ), and fewer comorbidities including coronary artery disease, heart failure, arrhythmias, and COPD ( $p < 0.05$ ).

Patients in both cohorts presented with similar Rutherford class (39% vs 32% class 2b and above,  $p = 0.073$ ). Hypercoagulable patients in the de novo thrombosis group were more likely to have tibial interventions (77% vs 54%,  $p < 0.05$ ) and had an increased risk of amputation at one year (HR= 2.50 [1.01, 6.17]) after adjusting for Rutherford class, discharge on anticoagulation, and diabetes. However, there was no difference in risk of reintervention at one year. In the acute on chronic cohort, hypercoagulable patients had no difference in risk of amputation or reintervention compared to non-hypercoagulable patients.

Although patients with hypercoagulable disorders were younger with fewer comorbidities, those presenting with ALI without history of PAD had an increased risk of amputation.

**Nuclear fatty acid metabolism in ovarian cancer.**

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Cancers adopt metabolic adaptations for survival and excessive cell division, including differential metabolic compartmentalization allowing cells to maximize certain pathways. However, how nuclear metabolic compartmentalization is reprogrammed in cancer cells and what the biological consequences of this differential compartmentalization is unclear. Focusing on ovarian cancer, our data reveal the presence of 30 metabolic enzymes compartmentalized within the nucleus of ovarian cancer cells, many of which are typically annotated to the cytoplasm. Interestingly, many of these enzymes are related to fatty acid metabolism, including long-chain Acyl-CoA synthetases (ACSL) 3 and 4. Consistently, lipidomics on isolated nuclei detect specific fatty acyl species that can be activated by ACSLs. *In silico* analysis identified a putative nuclear localization signal (NLS) within ACSL4, and inhibition of nuclear import/export confirms compartmentalization of these enzymes between cytoplasm and nucleus. While ACSLs are canonically localized to the mitochondria and the endoplasmic reticulum, these data provide the first evidence of their presence in the nucleus. Future studies will determine the biological significance of nuclear ACSL3/4-mediated fatty acid metabolism and explore the feasibility of targeting nuclear metabolic enzymes or their translocation in cancer.

## Characterizing Patterns and Correlates of Nonsuicidal Self-Injury in Autistic Adults

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While ~50% of autistic adults report nonsuicidal self-injury (NSSI), it remains understudied despite an association with suicide. In the limited work in autistic people, alexithymia and sensory sensitivities are correlates of NSSI. Interestingly, repetitive behaviors, cognitive rigidity, emotion dysregulation, impulsivity, and social anhedonia have not yet been examined in the context of NSSI in autism. Though these constructs are linked to self-injurious behaviors in autistic samples and/or with NSSI in nonautistic samples. This study aimed to examine group differences and correlates of NSSI in autistic vs. nonautistic adults.

Data were from the Pitt Autism Center of Excellence (Projected total N=300 [Autistic n=200]). To date, 100 participants (Autistic n=75; M(SD) age= 32.96(10.77) years) were administered NSSI questions. Participants completed self-reports, including measures of repetitive behaviors, cognitive rigidity, sensory sensitivity, alexithymia, emotion dysregulation, impulsivity, and social anhedonia. Chi-square tests were used for group differences in history of NSSI. Within the autistic group, hierarchical logistic regressions were used to examine correlates of history of NSSI. Covariates for all regressions included age, sex, and IQ.

Autistic adults were more likely to report history of lifetime NSSI compared to nonautistic adults (64% vs. 24%),  $p<.001$ . In autistic participants, more repetitive behaviors and worse cognitive reappraisal associated with history of lifetime NSSI,  $ps<.001$ . Significant models emerged for 6-month NSSI history, 6-month NSSI frequency, and number of NSSI methods with similar patterns of older age, and more repetitive behaviors associations,  $ps<0.05$ . No other models were significant.

Older age and more repetitive behaviors are strongly linked with NSSI in autistic adults. Future work should parse the ways in which repetitive behaviors and NSSI may be intertwined. Uncovering mechanisms related to repetitive behaviors will allow better understanding of the emergence and maintenance of NSSI and may aid in development of targeted NSSI prevention and intervention for autistic people.

**Exploring correlates of Mental Health and Employment in individuals with Long COVID:  
A secondary data analysis**

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Background: Employment is a basic human right as stated in Article 23 of the Universal Declaration of Human Rights (UDHR) and everyone deserves equal opportunity for employment. Long COVID, which emerged as a long-term consequence of COVID-19 pandemic has been a major public health challenge, affecting no fewer than 200 million individuals globally. Long Covid has mental health and socioeconomic consequences for affected individuals.

Objective: The objective of this study is to examine the mental health and socioeconomic correlates of long COVID using a secondary dataset from a population-based survey of Detroit Metro Area Communities Study (DMACS).

Design and Method: This is a secondary data analysis of the DMACS study. A total of 1,901 participants were investigated. The outcome variable was employment, and the major predictor variable was Long COVID. The association between long COVID, mental disorders and employment was assessed using logistic regression reporting odd ratios (OR).

Results: Long-COVID was associated with lower rates of employment. 60.9% participants with long COVID reported being employed compared to 61.7% participants without long COVID. This indicates that participants who had long COVID were more likely to report lower employment compared to those who did not have long COVID. Mental disorder was reversely associated with employment. 53.2% participants with mental health reported being employed compared to 44.7% participants without mental health.

Conclusion: Although, this study does not infer causality, it highlights the existing gap in employment among individuals with long COVID and mental disorders, suggesting that negative health consequences of long COVID and mental disorders have the potential to limit employment opportunities. There is a need to develop interventions on how to support the mental health and socioeconomic needs of individuals with long COVID.

**Raising Queens: Transcriptional Landscape of the Naked Mole-Rat Ovary**

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The naked mole-rat (NMR) is well known for being the longest-lived rodent (maximum lifespan >40 years). Female NMRs are exceptional in showing no decline in fertility or fecundity into their third decade of life, whereas comparably sized mice have reduced fertility by 9 months of age. However, most individuals in a naked mole-rat colony will never be reproductively active, it is only those that become dominant breeding females (queen) that will breed until they die. However, the eusocial structure is not fixed, in the absence of a queen, other females can raise as new breeders from the subordinate females. We recently established that NMRs do not exhaust their oocyte stores and continue to produce primordial germ cells that could be a source for newly developed oocytes both in vivo and in vitro. To understand how this process of shifting from an inactive ovary to a fully functional one is regulated we explored the transcriptional dynamics using 10x Genomics Multiome Chromium Single Cell Multiome ATAC + Gene Expression on three different NMR reproductive status: Queens, subordinates, and ex-subordinates. Our results will allow us to understand the regulatory mechanisms that underlays in this animals' incredible reproductive longevity.

## Longitudinal Progression and Harmonization of tau-PET Tracers

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Tau-PET tracers have been used to monitor the progression of Alzheimer's disease (AD). However, different tracers present distinct patterns of binding throughout the brain, challenging the harmonization of their findings. Leveraging the HEAD Study, we recently developed the Unit scale, which cross-sectionally harmonizes Flortaucipir and MK6240 onto a universal tau-PET measurement. Here, we provide a preliminary evaluation of the Unit scale's longitudinal performance in HEAD and two independent cohorts.

We assessed 422 individuals across the AD spectrum with longitudinal tau-PET from three cohorts: HEAD, ADNI, and TRIAD. Standardized uptake ratios (SUVRs) were harmonized to Unit using the Unit Ecosystem (unitau.app). Braak I-II and the Meta-Temporal regions were used as regions of interest. Annual tau-PET uptake changes and the effect sizes were calculated.

In HEAD, annual change in tau-PET uptake in Braak I-II across CU and CI was only detectable in MK6240. However, both tracers detected significant annual changes in the Meta-Temporal ROI for CI. In both ADNI (Flortaucipir) and TRIAD (MK6240), changes in tau-PET uptake in Braak I-II regions were not significant. However, across all cohorts, the Meta-Temporal ROI consistently showed detectable annual tau-PET increases—most pronounced among CI—leading to larger effect sizes than in Braak I-II. The Unit harmonization did not fundamentally alter the pattern of the findings. However, in ADNI (Flortaucipir), Unit yielded a slightly higher effect size than SUVR in CU for both Braak I-II and Meta-Temporal regions.

In this large longitudinal sample, our findings confirm that Flortaucipir and MK6240 can detect tau PET changes over time likely associated with the progression of tau tangle pathology. While MK6240 appears to show greater progression in CU, both tracers progress similarly in CI. Our data also suggested that Unit harmonized tau PET measurements maintain the longitudinal characteristics of each tau PET tracer for use in clinical trials.

**A Novel ECM Human Lung Proteome Atlas of Healthy Aging and COPD**

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Aging is a complex biological process associated with impaired pulmonary function and an increased risk to develop chronic lung diseases. We applied comprehensive proteomic analysis on lung tissues of a human cohort of 20 healthy donors, aged 4 months to 95 years. We observed a dramatic remodeling of the lung proteome with aging and progressive changes of proteins involved in extracellular matrix (ECM) homeostasis, immune and inflammatory responses. Parallel senescence induction of human tissue derived precision-cut lung slices (PCLS) highlighted cell-specific alteration of senescence-driven ECM proteins, associated with lung aging, such as matrix metalloproteinases, and serpins as revealed by multi-omic technologies (proteomics/single nuclear RNA Sequencing). Dysregulation of collagens with aging and senescence suggested compositional and structural changes as key lung aging paradigm. ECM proteome trajectories were developed to compare healthy aging with accelerated aging, as observed in chronic obstructive pulmonary disease (COPD). We provide insights into the molecular changes and trajectories associated with aging, senescence, and COPD.

**Neurodynamics of Active Face Perception During Free Viewing with Eye Tracking and Intracranial EEG**

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When you look at a face, you first glimpse it in your peripheral vision before moving your eyes to examine it more closely. Your eyes then dart across its features, updating your interpretation of the person's character and intentions. Yet most research on face perception uses static faces that appear suddenly at the centre of a screen, with no eye movements allowed. We took a more naturalistic approach. Using eye tracking and intracranial EEG, we presented happy, fearful, and neutral faces in the periphery and allowed participants to look at them freely. In some trials, the stimulus changed (emotion or orientation) during the eye movement so that the face seen in peripheral vision differed from the one seen on fixation. This allowed us to examine how previews shape face perception. We also analysed saccades and fixations across the face. Natural fixations produced markedly different neural responses compared to those triggered by faces that appeared suddenly. Using Multi-Temporal Pattern Analysis, we found neural patterns that predicted the direction of the next eye movement, even before the eyes moved. This preparatory activity involved both surface and deep cortical regions. Our findings indicate that face perception is an active process driven by eye movements and continuous shifts in attention. Our analysis of valid and invalid previews is ongoing. This work offers new insights into how the brain processes faces in real-world situations rather than in tightly controlled experimental settings.

**SMC Ercc1 Deficiency Induces a DNA Damage Response and Leads to Structural Abnormalities in the urinary bladder**

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**Background:**

Arterial calcification due to deficiency of CD73 (ACDC) is a rare genetic disease causing medial arterial calcification (MAC) in lower-extremity arteries. ACDC is caused by inactivating mutations in the gene encoding the CD73 enzyme, which converts extracellular AMP to adenosine. We previously reported that reduced CD73 levels are found in non-genetic common forms of MAC, suggesting that CD73 is critical for peripheral vascular health. Excision Repair Cross-Complementation Group 1 (Ercc1) is a protein involved in DNA repair. We found that DNA damage response is activated in calcified tissue with the lack of CD73. To study whether the DNA damage response induces calcification in SMCs, we generated a mouse model with Ercc1 knockout in smooth muscle cells (SMCs).

**Hypothesis:** The DNA damage response in Ercc1<sup>SMC-ko</sup> mice induces medial arterial calcification.

**Methods:** Ercc1<sup>fl/fl</sup> mice were crossed with Myh11-CreERT2-RAD R26R-tdTomato to generate Ercc1<sup>SMC-ko</sup>. Myh11 encodes a SMC contractile protein and drives Cre recombinase expression, with tamoxifen (10 mg/day for 10 days) inducing recombination in SMCs. Calcification, tissue morphology, and collagen were assessed histologically, while DNA damage (H2ax, p53, Ercc1) and SMC contractile (α-SMA) markers were analyzed via immunofluorescence.

**Results:** Ercc1<sup>SMC-ko</sup> mice exhibited lower body weight at six months, and developed a pronounced phenotype, including an enlarged bladder compared to Ercc1<sup>SMC-wt</sup>. Histological analysis found that calcification content in the aortas was unchanged between Ercc1<sup>SMC-ko</sup> and Ercc1<sup>SMC-wt</sup>, however the bladder exhibited structural changes and developed injury in Ercc1<sup>SMC-ko</sup> mice compared to Ercc1<sup>SMC-wt</sup>. Ercc1 and SMA were elevated in Ercc1<sup>SMC-wt</sup> mice aorta and bladder compared to Ercc1<sup>SMC-ko</sup>. Ercc1<sup>SMC-ko</sup> mice aorta and bladder exhibited higher DNA damage response, increased of H2ax and p53, than Ercc1<sup>SMC-wt</sup>.

**Conclusion:** The DNA damage response is activated in the aorta and bladder of Ercc1<sup>SMC-ko</sup> mice and leads to injury and structural abnormalities in the bladder due to the absence of Ercc1.

**Discovery of Novel Receptor-Like Kinases in Regulation of Root Microbiome in *Arabidopsis Thaliana***

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Microbiota offer various benefits to their plant hosts, including enhanced nutrient uptake and protection against pathogens and pests. In return, plants selectively assemble a microbiome that differs from the surrounding environment. While the genetic regulation of microbiome assembly is crucial for maintaining its function, the mechanisms underlying its composition remain largely unknown, partly due to complexity of microbial communities. To study the complex host-microbiome interactions, we use the model plant *Arabidopsis thaliana* and its interactions with single bacterial strains as genetically tractable rhizosphere microbiome models. Previous work identified two receptor-like kinases (RLKs) that are necessary for recruiting and maintaining appropriate immune responses to beneficial microbiota, namely FERONIA and PSKR1 respectively. Functionally, RLKs are a large family of transmembrane proteins in plants that play crucial roles in sensing external signals and initiating intracellular responses. In *Arabidopsis* genome, there are over 600 RLKs, however majority of them have not been functionally characterized yet. Given the previous screen in the lab identified two RLKs being implicated in regulating the microbiome, we hypothesized that additional uncharacterized RLKs may provide a functional link between endogenous or environmental signals and microbiome structure and function. To this end, we used a collection of 135 homozygous T-DNA lines with insertions in 69 root-expressed LRR-RLKs (Leucine-rich repeats RLKs) as a toolkit to probe plant interactions with microbiota. We identified several candidate RLKs where the mutants showed dramatically shorter primary roots in presence of a beneficial *Pseudomonas fluorescens* strain as well as an altered abundance of *P. fluorescens* when grown in natural soil (changes in microbiome structure). More experiments are currently underway to study the changes in microbial community in the rhizosphere of these mutants. Taken together, our study will facilitate the characterization of signaling network of uncharacterized LRR-RLKs and identify novel genes that are important for shaping the microbiome.

## Optimizing Fc-Engineered VH-Fc Fusion Proteins for MSLN-Targeted PET Imaging

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Mesothelin (MSLN) is an attractive biomarker for targeted cancer imaging and therapy due to its overexpression in mesothelioma, pancreatic, colorectal, and ovarian cancers. However, conventional monoclonal antibodies face challenges like limited tumor penetration and suboptimal pharmacokinetics. To address these, we developed anti-MSLN VH-Fc fusion proteins with improved PK profiles and greater tumor uptake and penetration. Initial studies revealed non-specific binding due to FcγR interactions, limiting tumor uptakes in immunodeficient mouse, which are ideal for PDX models to mimic human tumor biology and are crucial for evaluating therapeutic efficacy and personalized treatment strategies. To overcome this, we engineered VH-Fc proteins with FcγR silencing mutations to improve Fc-mediated stability and favorable pharmacokinetics.

Radiolabeled with zirconium-89, these VH-Fc mutants were assessed as a pan imaging agent via PET imaging in multiple tumor models, including HCT116, AsPC-1, and engineered A431-G9 and A431-H9 cells with high and low MSLN expression, respectively. Compared to wild-type (WT) VH-Fc protein, the Fc-engineered mutants exhibited enhanced tumor uptake with reduced liver and spleen retention. *In vitro* and *in vivo* assays confirmed FcγR silencing, with LALAPG variant performing best.

In conclusion, our engineered VH-Fc proteins with FcγR silencing mutations demonstrated enhanced tumor uptake, reduced off-target retention, and improved tumor-to-background contrast. By minimizing non-specific immune uptake, these refinements support the translational potential of our approach for pan-MSLN targeted imaging and precision oncology applications in future studies.

**Screening of endogenous cannabinoid receptor 1 expression and elucidation of its role in various brain cancer lines**

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The endocannabinoid system along with the various G-protein coupled receptors play a vital role in the modulation of the human brain signaling pathways that maintain homeostasis. Cannabinoid receptor (CB1) is a key GPCR protein involved in maintenance of neuronal metabolism. CB1 activates various downstream signaling pathways by which it regulates energy metabolism and respiration through cAMP pathway. Using animal models, recent studies characterized CB1 receptor and its localization by knockout approach; however, the molecular mechanism of endogenous expression and activation of various downstream signaling pathways is not completely understood. Since CB1 is expressed in neuronal cells, we performed in-silico screening using already available data set. Interestingly, CB1 RNA expression was high in both adult (Grade IV medulloblastoma) and pediatric tumor lines (Grade IV glioblastoma), However, we did not find similar patterns of expression in respective protein lysates. To further study functional consequences of CB1 expression and its downstream signaling in these lines, we performed lentiviral mediated CB1 knock-down and further evaluated the changes in growth, morphology and localization in cellular compartments. The preliminary data suggests that CB1 has compartment specific localization and signaling events in some brain tumors. Studying this basic mechanism would pave the way to develop novel drugs for CB1 receptor and therapeutics in future.

**Proteomic Screening Identifies PHF19 as a Caspase Substrate and EBV Restriction Factor**

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Epstein-Barr virus (EBV) infects 95% of the human population and contributes to approximately 2% of all human cancers. Our previous studies demonstrated site-specific cleavage of host restriction factors is a new way to promote EBV reactivation from latency. To identify novel EBV restriction factors that are cleaved by caspase, we utilized immunoprecipitation coupled with quantitative mass spectrometry to monitor protein cleavage in Akata (EBV+) cells upon lytic induction. Among over 1000 identified proteins that are cleaved during EBV replication, we focused on polycomb repressive complexes 2 (PRC2) proteins, specifically PHF19, for further functional analysis. PHF19 is responsible to recruit PRC2 enzymes SUZ12/EZH2 to chromatin to facilitate H3K27 methylation, a marker for transcription repression. PHF19 contains N-terminal Tudor, two plant Homeodomains (PHDs) and Extended Homology (EH) domains and a C-terminal domain (CD). CD is a region essential for PHF19-PRC2 interactions and Tudor and EH are reported to bind histone and DNA, respectively. Here, using CRISPR-Cas9 genomic editing method to disrupt PHF19, we found that PHF19 depletion significantly promotes EBV lytic replication. We further demonstrated that PHF19 is efficiently cleaved upon lytic induction and caspase-mediated cleavage separates Tudor and EH domains from CD, providing a way to disable PRC2 and facilitate viral gene expression. Together our study identifies PHF19 as a novel caspase substrate and host restriction factor that represses EBV lytic replication. Ongoing study will determine the molecular mechanism by which PHF19 represses EBV replication through chromatin remodeling and how PHF19 is disrupted by caspase-mediated cleavage to promote the EBV replication.

**Predicting Worry-Related Mental States using Regional Brain Activity with Long Short-Term Memory (LSTM) Recurrent Deep Neural Networks**

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Worry is common, spontaneous and evolutionary adaptive phenomenon. By contrast severe worry is a pathological condition prevalent in late-life mood and anxiety disorders. While previous research mapped neural correlates using traditional statistical approaches, this study employed an explainable long short-term memory (LSTM) neural network to predict worry-related mental states through brain activity timeseries. The research involved two functional magnetic resonance imaging (fMRI) studies: FINA (n=116) and RAW (n=88), recruiting older adults across a worry spectrum. Participants underwent a clinical interview to identify personalized worry induction, neutral and reappraisal statements, which were presented in a block design fMRI task. Data were preprocessed and normalized in MNI space, extracting fMRI timeseries from 481 cortical, subcortical, and cerebellar regions. The LSTM model was bidirectional with two layers, using a 20-second sequence length, MAE loss, and 32-sample batch size, trained over 250 epochs. The FINA dataset was split into training and validation sets, with the RAW dataset serving as a test set. Model's accuracy in predicting mental states was, with validation and full length, AUC of 0.89 for worry, 0.77 for reappraisal, and 0.91 for neutral states. The independent test set showed lower performance with 0.78, 0.63, and 0.81. Key predictive networks for worry included dorsal attention, anterior hippocampus, cerebellum, left executive control, and basal ganglia networks. The study's conclusion highlights the LSTM model's success in predicting worry-related mental states in older adults, potentially offering a neural marker for treatment response in severe worry. This approach provides a novel method for understanding complex worry-related brain activity patterns.

**Denoising Diffusion MRI Data: Principal Components Meet Non-Local Block-Matching**

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Diffusion MRI (dMRI) provides valuable insights into the brain tissue microstructure. Recent hardware advancements have enabled stronger diffusion gradient strengths (higher b-values) and improved spatial and angular resolution. However, increasing the b-value and/or resolution results in a loss of SNR. Additionally, modern acquisition and reconstruction protocols may introduce spatially correlated (non-white) noise in the diffusion-weighted images (DWI). Here, we propose a novel denoising method for dMRI that utilizes global singular value decomposition on DWI data and subsequently applies a powerful denoiser (BM4D) to each Principal Component (PC). We compared our proposed BM4D-PC method with two widely used approaches: MPPCA and NORDIC. Three datasets were utilized: (1) The publicly available EDDEN dataset featuring an in-vivo human acquisition with parameters TE/TR=91/6569 ms; multiband = 3; in-plane GRAPPA = 2; b-values = 0, 1000, and 2000 s/mm<sup>2</sup>; resolution = 0.9 mm isotropic; (2) An in-vivo human  $\mu$ FA acquisition, characterized by a single-shot variable-density spiral trajectory with parameters TE/TR = 80/9000 ms; multiband = 3; in-plane acceleration = 3; b-values = 0 (6 LTE), 150 (6 LTE), 1000 (26 LTE), 2000 (30 STE + 26 LTE) s/mm<sup>2</sup>; resolution = 1.2 mm isotropic; (3) An ex-vivo marmoset acquisition with parameters: TE/TR = 53/500 ms; b-values = 0 (6), 2400 (30), 3600 (45), and 4800 (60) s/mm<sup>2</sup>; resolution = 60  $\mu$ m isotropic. Our method outperformed several state-of-the-art algorithms by enhancing DWI image quality, achieving noise suppression without signal loss, and preserving tissue features. Moreover, our results demonstrated higher accuracy in estimating diffusion metrics such as FA, NDI, and excess kurtosis. The innovative BM4D-PC reduces noise in DWI, enhancing diagnostic capabilities and sensitivity to tissue microstructure and degeneration. It operates fully automatically, requires no training (unlike deep learning methods), and can be applied across various acquisition types, from Cartesian encoding to advanced spiral techniques.

## **Mechanisms of Macrophage Polarization in Periodontal Disease**

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Macrophages play a critical role in the promotion and resolution of inflammation. However, mechanisms that control macrophage polarization are not yet fully understood. Triggering receptor expressed on myeloid cells 2 (TREM2) dysregulation is implicated in certain inflammatory conditions, but its role in the oral mucosa remains unknown. Our objective was to investigate the immunoregulatory role of TREM2 on macrophages in periodontal disease (PD).

PD was induced in wild-type (WT) and TREM2<sup>-/-</sup> mice using a standard ligature model. In the induction arm, ligatures remained for seven days. In the resolution arm, ligatures were removed, and the animals were allowed to recover for five additional days. Macrophages were isolated from the oral mucosa and analyzed via flow cytometry and single cell RNA-sequencing. Oral mucosa was analyzed for linear bone loss and gene expression of proinflammatory cytokines and proteases, and soluble TREM2 (sTREM2) was measured in plasma.

We showed that TREM2 was expressed on macrophages in the oral mucosa, and we demonstrated its differential regulation, with significantly decreased surface expression during PD and increased expression during resolution ( $p < 0.05$ ). To further explore TREM2 regulatory mechanisms, we investigated ADAM10/17 activity, known proteases that cleave TREM2 and produce sTREM2. ADAM10/17 was upregulated during PD and the decreased TREM2 surface expression during disease was associated with increased sTREM2. The periodontal phenotype in TREM2<sup>-/-</sup> mice was characterized by increased PD severity with significantly increased alveolar bone loss and local inflammatory cytokine expression ( $p < 0.05$ ). Finally, transcriptional characterization of macrophages of the oral mucosa in mice demonstrated subpopulations of TREM2<sup>+</sup> macrophages characterized by distinct gene signatures and pathways consistent with immunoregulatory functions.

This study improves our understanding of mechanisms of macrophage polarization and introduces TREM2 as an important immunoregulator on macrophages in the oral mucosa. Targeting TREM2 may be an important immunoregulatory strategy for improved management of PD.

**Functional Studies on the Cytochrome P450 Splice Variants CYP4F3A and CYP4F3B Unveil the Basis for their Distinct Physiological Functions**

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The cytochrome P450 (CYP, P450) 4F family are human fatty acid  $\omega$ -hydroxylases. The isoforms CYP4F3A and CYP4F3B are expressed in different tissues and have distinct functions in the body. Despite this, they share 93% amino acid sequence identity due to their shared gene locus. The CYP4F3 gene undergoes alternative splicing, resulting in the isoforms CYP4F3A and CYP4F3B, which differ only in a specific amino acid region from residues 67 to 114. CYP4F3A is exclusively expressed in monocytes and deactivates leukotriene B<sub>4</sub>, contributing to the anti-inflammatory response. CYP4F3B is expressed in liver and kidney and produces the lipid mediator 20-hydroxyeicosatetraenoic acid (20-HETE) from arachidonic acid. Overproduction of 20-HETE is linked to diseases such as hypertension, diabetes, and cancer. We hypothesize that amino acid differences caused by alternative splicing lead to an altered substrate preference and, ultimately, different functions. Furthermore, both enzymes are attractive potential drug targets for human disease. However, there is a significant lack of in-depth biochemical and biophysical studies to further shed light on enzymes function.

This work presents the first reported expression and purification protocols for CYP4F3A and CYP4F3B. Total protein yield was increased to 15-fold with high purity and quality. Kinetic analysis reveals crucial differences in substrate preference and catalytic activity. UV-vis spectroscopic binding assays indicate that isoforms have different affinities to the respective substrates. Strikingly, CYP4F3B exhibits a 100-fold higher affinity for the common CYP4 inhibitor HET0016 compared to CYP4F3A which provides valuable clues for the design of selective drugs. Those differences in drug binding likely arise from the exon swap region.

The first reported generation of recombinant CYP4F3A and CYP4F3B and their characterization will shed light on their function and regulation in health and disease. The specific differences in both enzymes can be exploited for the design of drugs specifically targeting one enzyme over another.

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## **Normalizaion of Magnesium Deficiency with Magnesium-L-Threonate Alleviates Bladder Overactivity in Cyclophosphamide-Induced Cystitis: Mechanisms of Inflammatory Modulation and Barrier Restoration**

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**Background:** Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) emerges as a chronic urological syndrome with escalating incidence (2.7~6.5%) and intricate pathogenetic complexity. Bladder-related pain and lower urinary tract symptoms are primary clinical manifestations of BPS/IC. Inflammation and disruption of the urothelial barrier are the two primary mechanisms. Our previous research demonstrated that normalization of magnesium deficiency with Magnesium-L-Threonate (L-TAMS) alleviates mechanical allodynia, depressive-like behaviors, and memory deficits in cyclophosphamide (CYP)-induced cystitis by suppressing neuroinflammatory responses. In the current study, we aim to elucidate how L-TAMS modulates bladder overactivity in CYP-induced cystitis rats.

**Methods:** We established a rat model of BPS/IC through intraperitoneal CYP injections at 50 mg/kg every 3 days for three doses. L-TAMS was administered via drinking water at 604 mg·kg<sup>-1</sup>·day<sup>-1</sup>. We evaluated micturition frequency using cystometry and assessed bladder tissue through various techniques including hematoxylin and eosin staining, TUNEL assay for apoptotic cells, toluidine blue staining for mast cell infiltration, and Western blotting to examine protein expressions.

**Results:** Administration of Magnesium-L-Threonate ameliorates CYP-induced cystitis rats through restoration of magnesium homeostasis. It mitigated bladder overactivity by prolonging intercontractile intervals. Histopathological alterations like epithelial defects, edema, and vascular congestion were alleviated. The increased apoptosis and mast cell infiltration were reversed. We found that L-TAMS normalized the upregulation of inflammatory markers, particularly TNF- $\alpha$ /NF- $\kappa$ B signaling and IL-1 $\beta$ . Additionally, it restored the integrity of bladder tight junction proteins.

**Conclusion:** Oral administration of L-TAMS effectively reduced bladder overactivity in CYP-induced cystitis rats by inhibiting inflammatory signaling pathways and restoring the bladder's tight junction barrier integrity. Our findings suggest L-TAMS may provide therapeutic benefits for managing lower urinary tract symptoms in BPS/IC.

## **A Novel Implantable Neuroprosthesis to Restore Bladder Function After Spinal Cord Injury**

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**Aims:** Spinal cord injury (SCI) leads to complex urological complications, including detrusor overactivity (DO) and detrusor sphincter dyssynergia (DSD). While intermittent self-catheterization remains the standard treatment, it carries risks of recurrent infections. This study proposes a novel implantable pudendal nerve stimulation system (PSTIM) to restore urinary continence and micturition in SCI-induced neurogenic bladder, offering a daily therapeutic alternative to catheterization.

**Methods:** A complete T9-T10 spinal cord transection was performed in three cats to establish SCI models. PSTIM devices were surgically implanted: two cats received bilateral pudendal nerve cuff electrodes (3 in total), while one cat received an additional unilateral lead electrode. Bladder emptying was achieved via manual expression or PSTIM activation. Weekly urodynamic assessments in awake cats evaluated PSTIM efficacy. Continuous 12-week recordings of daily stimulated voiding were obtained in one awake chronic SCI cat. Low-pressure voiding was further validated under anesthesia.

**Results:** Channel 1 (CH1) stimulation at 30 Hz under isovolumetric conditions induced low bladder pressures (~60 cmH<sub>2</sub>O) via spinal reflex-mediated detrusor contraction. When combined with bilateral 10 kHz stimulation (CH2/3) to relax the external urethral sphincter, CH1 triggered highly efficient weekly and daily voiding. PSTIM-induced low-pressure voiding was replicated in anesthetized cats. Additionally, 5 Hz CH1 stimulation restored continence by suppressing non-voiding contractions and increasing bladder capacity. In the cat with four electrodes, PSTIM achieved sustained daily voiding efficiency over 12 weeks (77.95±1.48% AM, 56.50±2.97% PM).

**Conclusions:** PSTIM enables selective pudendal nerve stimulation and blockade to restore both continence and micturition post-SCI. This implantable neuroprosthesis demonstrates potential as a long-term, catheterization-free therapy for SCI patients, addressing critical limitations of current clinical practices.

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**Targeting DPP4 to Enhance T Cell Infiltration and Improve Cancer Immunotherapy**

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Cancer immunotherapy has transformed cancer treatment, yet its efficacy remains limited by a “cold” tumor immune microenvironment (TIME) characterized by poor T cell infiltration. Induction of immunogenic cell death (ICD) has been shown to improve T cell infiltration by promoting the release of T-cell-recruiting chemokines, such as CXCL10. However, the overall outcome is limited by dipeptidyl peptidase IV (DPP4), a serine protease that degrades CXCL10 and related chemokines. We herein report that ICD-inducing chemotherapeutic agents transcriptionally upregulate DPP4 in cancer cells, potentially acting as a negative feedback mechanism that dampens the magnitude of immune response. To improve the delivery of both DPP4 inhibitor and ICD-inducing agent while minimizing the systemic off-target side effect, we developed a hyaluronic acid (HA)-dendrimer nanocarrier for tumor-targeted co-delivery of the two therapeutic agents. HA is a natural ligand for CD44 that is overexpressed on both tumor cells and tumor endothelial cells, enabling precise targeting. In preclinical animal tumor models, this strategy significantly enhanced T cell infiltration, anti-tumor immunity, and the overall therapeutic efficacy while minimizing systemic toxicity. Our work establishes a novel immunochemotherapy platform that synergizes chemotherapy and immunotherapy via targeted DPP4 inhibition, offering a promising strategy to improve clinical outcomes in immunotherapy-resistant cancer.

## **Reliability-Oriented Evaluation of 3D Printing Concrete Process Via Data-Driven Uncertainty Propagation Analysis**

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Material uncertainties are critical factors in the 3D concrete printing process, causing significant variability in buildability and posing challenges for quality control of 3D printed concrete structure. To address this issue, this study presents a reliability-oriented evaluation of 3D printing process through a data-driven approach. Material uncertainties of concrete are firstly quantified through maximum likelihood-based stochastic parameter estimation. Combined with Monte Carlo simulations, the developed data-driven model can effectively propagate the quantified concrete uncertainties through the printing process and reveal the stochastic behavior of 3D printed concrete for reliability analysis. The impact of other uncertainty sources, including model error, on reliability-oriented buildability is analyzed, showing minimal effect when these sources are less than 30% as intense as material uncertainty. Then, the effects of various printing configurations, such as print speed, layer height, and curvature, on the printing process are also examined. Additionally, an empirical model is developed for quick buildability assessment at given reliability levels, offering practical insights for 3D concrete printing designers.

**Exercise Treatment for Chronic Low Back Pain: A Longitudinal Analysis of Trajectory Patterns and Psychosocial Factors**

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Background Context: Exercise is a common and effective treatment for chronic low back pain (cLBP), but adherence is often low. It is unclear whether exercise adherence trends exist across time and if these trends impact future outcomes.

Purpose: This study aims to 1) identify patterns of unsupervised exercise over one year, 2) explore group differences in baseline participant characteristics, and treatment utilization. 3) and predict physical function one year later.

Study Design and Sample: This retrospective longitudinal study included participants with cLBP from a larger study (n=1000).

Outcome Measures: Self-reported exercise frequencies were measured at 30-90 day intervals for 1 year. PROMIS physical function T-scores (PROMIS-PF) measured at the end of the study (1 year) were used.

Methods: Group-based trajectory modeling (GBTM) classified participants based on exercise frequency. Baseline characteristics including socioeconomic status (SES) and treatment utilization were compared using Kruskal-Wallis or chi-squared tests. A multivariate linear regression was conducted using baseline characteristics and exercise trajectories to predict the PROMIS-PF T-scores at 1 year.

Results: On average, participants were 59 years old with 12 years of pain (n=618). GBTM identified three stable exercise frequency trajectories (High (5.0 days/week(SD=1.8)), Moderate (2.2(SD=1.9), Low (0.6(SD=1.3)). Most high-exercisers were older, white, married, males, retired, with higher education and income. This group reported the lowest proportion of substance use and highest for non-pharmacologic treatment for cLBP. Most low-exercisers were the opposite and reported lowest rates of non-pharmacological treatment and highest anxiety and depression. The adjusted mean 1-year PROMIS-PF T-scores were significantly higher in moderate and high exercisers (39.79(0.35); 40.95(0.43)) compared to low exercisers (39.08(0.35)).

Conclusion: All groups had similar baseline pain and physical function scores. However, high-frequency exercisers had a higher SES with better physical function 1 year later compared to moderate and low-frequency exercisers. Exercise for cLBP may be unattainable for individuals with lower SES due to resource constraints.

**Implicit Approach-Avoidance Bias for Body and Food-related Stimuli among Individuals with Anorexia Nervosa in Partial Remission**

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**Objective.** Partial remission from anorexia nervosa (AN) is an important and understudied stage of illness, during which individuals experience improvements in behavioral/nutritional symptoms, but continue to experience cognitive and affective symptoms, which can contribute to relapse. Studying factors that may impact the transition to full recovery vs. relapse during partial remission may help improve the relatively low rates of full remission in AN. Fear and avoidance of eating disorder-related stimuli (e.g., feared foods, body size) have been shown to be important maintaining factors in AN. However, no empirical research to date has investigated fear and avoidance of eating disorder stimuli during this stage of remission. **Methods.** The current pilot study (N = 35 individuals with AN in partial remission) investigates implicit approach-avoidance bias towards food and body stimuli using a computer-based, behavioral task. Reaction times were measured to capture how quickly individuals approached (i.e., moved closer) or avoided (i.e., pushed away) images of high- and low-calorie foods and larger and smaller bodies. **Results.** Participants on average had an avoidance bias towards larger bodies and an approach bias towards smaller bodies. Contrary to our hypothesis, participants on average had an avoidance bias towards lower calorie foods. Body-related implicit biases were associated with momentary avoidance, fear, and behavioral urges. **Discussion.** These findings suggest that implicit avoidance of body-related stimuli (i.e., larger bodies) may be present during the partial remission stage of AN; however, avoidance bias towards higher calorie foods may diminish by this stage of recovery. These results are consistent with current treatment models of AN, which often prioritize behavioral and nutritional rehabilitation first, and persistent body image preoccupations later in treatment. Pending future research, step-down care and relapse prevention for AN during partial remission may benefit from targeting these body-related fear and avoidance biases.

**Arrestin-dependent co-trafficking of GPR3 and amyloid precursor protein in neurons: relevance for Alzheimer's disease**

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Alzheimer's disease (AD) is simultaneously among the most widespread and least treatable public health crises in the United States. Despite decades of research, there remain no FDA-approved medications that effectively treat disease progression. The prevalence, severity, and intractability of AD demonstrate the need for novel therapeutic strategies.

G protein-coupled receptors (GPCRs) are a diverse class of transmembrane signaling proteins that are among the most successful classes of drug targets. The GPCR GPR3 regulates multiple AD-associated phenotypes in mouse models of the disease, including amyloid  $\beta$  (A $\beta$ ) generation, A $\beta$  plaque burden, glial cell reactivity, and cognitive and behavioral function. The multifaceted impact of GPR3, combined with the exceptional druggability of GPCRs, makes understanding the mechanistic function of GPR3 in AD a high priority in characterizing novel therapeutic targets for this disorder.

GPR3 promotes A $\beta$  generation through phosphorylation of the receptor's C-terminal domain and association with arrestin, which regulates GPCR trafficking and can scaffold effectors to activate signaling cascades. Here, we characterize the impact of C-terminal phosphorylation on GPR3 localization and co-trafficking with the amyloid precursor protein (APP), which is cleaved to form the A $\beta$  peptides that aggregate to form the hallmark A $\beta$  plaques in AD.

Blocking arrestin recruitment to GPR3 decreases association of APP with GPR3 and impairs residence of both proteins at the Golgi apparatus in human-derived neurons *in vitro*. We also observe decreased presence of phosphodeficient GPR3 at recycling endosomes but, conversely, increased presence of APP at these structures in the same cells. Both the Golgi and endosomes are major sites of sequential APP cleavage to form A $\beta$ , indicating that GPR3 may regulate A $\beta$  production through arrestin-dependent changes in APP localization to these compartments. Further investigation into the impact of GPR3 on APP trafficking and processing will allow us to identify targetable points for regulating A $\beta$  production in neurons.

**Investigating the Neural Substrates of Active Avoidance in the Bed Nucleus of the Stria Terminalis**

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Obsessive-Compulsive Disorder (OCD), is a psychiatric disorder characterized by obsessions and compulsive behaviors performed to alleviate anxiety and avoid perceived threats. The bed nucleus of the stria terminalis (BNST) is known to regulate anxiety, and BNST deep brain stimulation reduces OCD symptom severity, making it a promising treatment target. However, how the BNST regulates active avoidance behaviors is unknown. We hypothesized that BNST neurons are recruited and encode avoidance responses following acquisition of conditioned active avoidance. Mice were trained to lever press to avoid footshocks across 7 sessions. To monitor BNST activity during avoidance acquisition, mice were injected with GCaMP6M and implanted with a GRIN lens in the BNST for *in vivo* calcium imaging. Modulation of BNST neurons was examined during avoid presses and escape presses. To determine if there increased BNST modulation following task acquisition, the proportion of cells excited, inhibited, or unmodulated was compared between day 1 and day 7. Lever press-modulated neurons emerged within the first session and were largely selective to avoid-only or escape-only responses. To examine if BNST activity around lever presses could predict press type, we input average calcium activity around lever presses into a support vector classifier. Decoder performance was significantly higher than shuffled distributions. In addition, proportion of cells inhibited during avoid presses increased from day 1 to day 7. To determine if this increased inhibition is necessary for avoidance expression, a separate cohort of mice were injected with channelrhodopsin to stimulate BNST neurons during avoid periods. Stimulating BNST reduced the trials avoided and increased avoid latencies. Our data suggest that BNST neurons are modulated following learned defensive actions specific and that BNST activation during the avoid period impairs avoidance expression. These findings help elucidate mechanisms for development of avoidant behaviors and may inform how these behaviors become maladaptive in OCD.

## Co-option and evolution of TIR domains across the tree of life

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In recent years, researchers have discovered many bacterial antiphage immune defenses which are related to animal immune proteins. Phylogenetic analyses have revealed that a subset of these immune proteins have been horizontally transferred between bacteria and eukaryotes. Yet it remains unclear how eukaryotes can co-opt bacterial immune modules into their existing immune systems. Here, we investigate Toll-interleukin-1 receptor (TIR) domains as a case study of immune protein co-option, focusing on the TIR domains of amoeba, which lie at an important location on the Tree of Life. Prior to this work, the amoeba *Dictyostelium discoideum*, was known to have three TIR domain-containing proteins, TirA, TirB, and TirC. Only TirA has been characterized, having a role in amoeba antibacterial immunity.

We asked; are there other TIR domain-containing proteins in amoeba, if so, what might they be doing? We used deep homology to search for TIR domains in amoeba genomes and across the eukaryotic tree of life. We found additional TIR domain-containing proteins in *Dictyostelium* amoeba, which we named TirD and TirE. TirD is like TirB and C, while TirE is highly diverged and shares some of its domain architecture with TirA. We find that the TirB, C, and D family arose from a horizontal gene transfer (HGT) event from bacteria into eukaryotes. TirA and E are more ancient, like other TIR-containing proteins across eukaryotes. To test if these TIR genes are used for immunity, we generated mutants of TirB, TirC, and TirE in *D. discoideum*. Here, we present data on the role of these proteins in amoeba growth, their ability to consume bacteria, and their susceptibility to infection by bacterial pathogens. Overall, this work addresses a gap in our understanding of the origin and evolution of eukaryotic immunity and investigates how bacterial immune modules are co-opted and deployed by eukaryotes.

**Trabectedin and Lurbinectedin Disrupt Transcription-Coupled Nucleotide Excision Repair, Leading to DNA Breaks in Actively Transcribed Genes**

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Our previous studies demonstrated that trabectedin exerts its cytotoxicity through transcription-coupled nucleotide excision repair (TC-NER), where it induces persistent single-strand breaks (SSBs) due to blockage of the second incision reaction of XPG (Son et al., Nat. Commun., 2024). By leveraging this mechanism, we developed TRABI-Seq (trabectedin-induced break sequencing) to map TC-NER and transcriptional activity at a genome-wide scale. Given the mechanistic similarity between trabectedin and lurbinectedin, we extend this approach to investigate lurbinectedin's activity in small cell lung cancer (SCLC), a transcription-driven malignancy for which lurbinectedin is an approved treatment.

Utilizing the methodologies established in our previous work, we observe that lurbinectedin induces DNA break patterns highly similar to trabectedin, both in frequency and genomic distribution, reinforcing their shared mechanism of action. This study aims to further elucidate lurbinectedin's mode of action and to enhance its potential for precision oncology by refining its application in transcriptionally driven malignancies. These findings will contribute to a deeper understanding of TC-NER dynamics and provide a foundation for improving the clinical utility of lurbinectedin in SCLC.

**A Spatial Single-Cell Atlas of Kaposi's Sarcoma Reveals KSHV Tropism, Cellular Reprogramming, and Immune Evasion during Tumor Progression**

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Kaposi's sarcoma (KS) is a type of soft tissue sarcoma that is characterized by angiogenesis and chronic inflammation in immunocompromised individuals. However, the spatial dynamics and molecular underpinnings within the tumor microenvironment remain incompletely defined. To elucidate these complexities, we performed spatial single-cell in situ transcriptomic analysis (SSTA) by employing a targeted 310-gene panel covering both host and Kaposi's sarcoma-associated herpesvirus (KSHV) transcripts. In total, we collected data from 16 tissue microarrays comprising 20 patch, 23 plaque, and 200 nodular KS cores from 46 patients, alongside 13 normal skin cores from three healthy individuals.

Our analysis encompassed approximately 684 million transcripts from over 5.8 million cells, classifying them into 12 major cell populations, including lymphatic and vascular endothelial cells, fibroblasts, pericytes, keratinocytes, and diverse immune cell subsets including macrophages, T-cells, B-cells, and dendritic cells. Approximately 35% of the cells harbored KSHV transcripts, predominantly localized to endothelial populations, with increasing viral burdens correlating with disease progression from patch (6%) to nodular stages (40%). Notably, viral interleukin-6 expression doubled in nodular KS lesions compared to earlier stages, suggesting a critical role in tumor advancement. Further, cellular trajectory analyses revealed significant phenotypic plasticity and transdifferentiation among fibroblasts, macrophages, and endothelial cells, indicative of profound cellular reprogramming. Spatial mapping highlighted pronounced neoangiogenesis and inflammatory responses, with immune cells preferentially infiltrating stromal compartments but excluded from tumor niches, underscoring immune evasion as a pivotal tumor survival mechanism. This comprehensive spatial single-cell atlas created at the Hillman Cancer Center advances our understanding of KS pathogenesis by uncovering stage-specific cellular reprogramming, viral tropism patterns, and immune evasion strategies, thus offering novel therapeutic insights into targeting the intricate tumor microenvironment interactions in Kaposi's sarcoma.

***Staphylococcus aureus* Biofilm Infection Impairs Macrophage Dead Cell Clearance Necessary for Wound Healing**

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Background: Biofilm infection (BFI), implicated in 60% chronic wounds, involves *Staphylococcus aureus* (SA) as a predominant bacterial species. SA-BFI derails resolution of inflammation, necessary for wound healing. Macrophages perform cellular debridement by phagocytosing dead or dying cells via efferocytosis. Optimal efferocytosis helps resolve inflammation enabling wound healing. This work tested the hypothesis that SA-BFI impairs efferocytosis thus prolonging the inflammatory phase.

Methods: To specifically address the biofilm component of SA pathogenicity we employed a hypobiofilm ( $\Delta$ sarA) and hyperbiofilm ( $\Delta$ rexB) isogenic mutant strains of wildtype SA USA300. Mechanistic studies were performed on monocyte-derived macrophages (MDM) isolated from human peripheral blood and subjected to conditioned media (CM) derived from  $\Delta$ sarA and  $\Delta$ rexB biofilm cultures.

Results: Efferocytosis index or the ratio of the number of macrophages that have phagocytosed apoptotic cells to the total number of MDMs was analyzed. Efferocytosis index was significantly compromised ( $p < 0.05$ ,  $n = 5$ ) in high biofilm forming  $\Delta$ rexB CM treated macrophages compared to treatment with low biofilm forming  $\Delta$ sarA CM. RNAseq studies identified Macrophage receptor with collagenous structure (MARCO) as a biofilm-inducible surface protein that was significantly upregulated ( $p < 0.05$ ;  $n = 5$ ) both at the mRNA and protein levels in MDM treated with  $\Delta$ rexB CM compared to treatment of MDM with low biofilm forming  $\Delta$ sarA CM. Blocking MARCO with a neutralizing antibody in  $\Delta$ rexB CM treated MDMs restored biofilm-induced suppression of macrophage efferocytosis index pointing towards a key role of MARCO in such dysfunction. MDM treated with  $\Delta$ rexB CM, compared to  $\Delta$ sarA CM, demonstrated high levels ( $p < 0.001$ ;  $n = 6$ ) of pro-inflammatory CCL3. To dissect the independent significance of myeloid cell MARCO, MARCO was overexpressed in wound macrophages using an in vivo myeloid cell targeted gene expression vector in a mouse excisional wound model. Over expression of MARCO in myeloid cells in excisional wound resulted in compromised ( $p < 0.001$ ;  $n = 6$ ) phosphatidylserine<sup>+</sup> dead cell clearance and a pro-inflammatory environment in the granulation tissue.

Conclusion: This study identifies a specific cellular and molecular mechanism by which SA-BFI may complicate wound inflammation and healing. Specifically, MARCO causes SA-BFI dependent impairment of wound-site efferocytosis and sharp rise in wound-site phosphatidylserine<sup>+</sup> apoptotic cells. In SA-BFI affected wounds, MARCO is a candidate therapeutic target.

## Mapping the Schizophrenia Interactome

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Signaling pathways are composed of interacting molecules working in concert to control neurophysiology. These macromolecular interactions can be broadly placed into three categories: input, signaling, and output, with interactions spanning molecular classes. Genetic risk for schizophrenia (Sz) is distributed across each level, including receptors and channels (e.g., GRIN2A and CACNA1C), signaling proteins such as TRIO and MAPK3, and SETD1A and DMN3 at the output level, implicating interactions between different signaling modalities including protein phosphorylation, small molecules, and lipids. This level of complexity demands an integrated assessment of multiple “-omes” composing the “interactome.” To accomplish this, we characterized the proteome, phosphoproteome, lipidome, and metabolome in dorsal postmortem anterior cingulate cortex (dACC) grey matter from 56 schizophrenia patients and matched controls to provide novel insights into the disease. Proteomics, phosphoproteomics, metabolomics, and lipidomics analysis were individually performed for all cohort members. Data from each omic tranche was individually imputed, followed by mixed linear modeling accounting for clinical co-variants and performing case-control statistics. Dysregulated features in each tranche were characterized with KEGG, Lipid Ontology, and Metabolanalyst. Biologically directed multiomics analysis utilizing defined interactions extracted from KSEA, STRING, and STITCH were segregated into multiomics communities utilizing k-core clustering, resulting in 23 discrete communities, which were characterized by KEGG. 3 of the 23 communities were significantly enriched for dysregulated features; the top 3 most connected nodes from these communities included the Sz risk gene MAPK3.

**Determining residue interactions in RNA polymerase active site by integrated genetic approaches**

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Multi-subunit RNA Polymerases (msRNAPs) are responsible for transcription in all kingdoms of life. At the heart of these enzymes is an ultra-conserved active site domain, the trigger loop (TL), which coordinate transcription speed and fidelity by critical conformational changes impacting multiple steps in substrate selection, catalysis, and translocation.

Previous studies have observed several types of genetic interactions between TL residues in eukaryotic RNA polymerase II (Pol II), suggesting that TL function is shaped by residue functional interactions within and surround the TL. Moreover, identical mutations in a residue conserved between yeast Pol I and Pol II TLs yielded different biochemical phenotypes, implying even functions of conserved residues are shaped by context specific residue interactions (epistasis). The extent of these interaction networks and how they control RNA polymerase function and evolution remain largely unknown.

To address this, we are using integrated genetic and computational approaches, including spontaneous suppressor screening, deep mutational scanning, and statistical coupling analysis, to map and compare residue interaction landscapes in the active sites of three yeast RNA polymerases.

Our preliminary data suggests residue interactions could be different even within these highly conserved active sites of RNA polymerases. Additionally, substituting residues creates allele-specific networks and propagates epistatic effects across the Pol II active site. Furthermore, statistical coupling analyses reveal groups of co-evolving residues across RNA polymerase that converge on the active site, where evolutionary constraints interface with pervasive epistasis. Our studies provide the first example of comparing interaction landscapes in a highly conserved and constrained domain within three homologous essential enzymes, providing a powerful system to understand the influence of residue interactions on function and evolution of any protein.

**Neuroprotection and LRRK2 Inhibition by a Small Molecule Nitroalkene in Parkinson's Disease**

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Mutations in the leucine-rich- repeat kinase 2 (LRRK2) gene are associated with an increased risk of developing Parkinson's disease (PD) and elevated LRRK2 kinase activity. Even in idiopathic PD patients, LRRK2 is hyperactivated, though its regulation remains unclear. LRRK2's catalytic domain contains cysteines (Cys2024, Cys2025) that are highly susceptible to electrophilic modifications that alter kinase function and structural integrity. Under oxidative stress conditions, the lipid peroxidation by-product 4-hydroxynonenal (4-HNE) forms adducts with Cys2024 and Cys2025, significantly increasing kinase activity. In this context, Aldehyde dehydrogenase 1A1 (ALDH1A1), a member of the detoxifying ALDH superfamily, is expressed by approximately 70% of nigrostriatal dopaminergic neurons and actively catabolizes reactive aldehydes such as 4-HNE. Post-mortem analyses of PD brains revealed a marked downregulation of ALDH1A1 mRNA levels and an accumulation of toxic aldehydes in SNpc, supporting the hypothesis that compromised aldehyde detoxification may contribute to the pathogenesis of the disease.

In this study, we investigated the neuroprotective properties of the electrophilic small molecule nitroalkene 10-nitro-oleic acid (10-NO<sub>2</sub>-OA) in a chronic rotenone rat model of PD. We propose novel direct and indirect mechanisms underlying the modulation of LRRK2 kinase activity by 10-NO<sub>2</sub>-OA. Preliminary results show that oral administration of 10-NO<sub>2</sub>-OA (45 mg/Kg/day) readily crosses the blood-brain barrier, impeding striatal lesions, the progression of nigrostriatal degeneration, and mitigating motor impairment symptoms. Furthermore, 10-NO<sub>2</sub>-OA inhibits rotenone-induced LRRK2 hyperactivity, 4-HNE accumulation, and LRRK2-4-HNE adduct formation while simultaneously upregulating the expression of ALDH1A1 in SNpc. Click chemistry experiments with "clickable" alkenyl-10-NO<sub>2</sub>-OA shows direct LRRK2 inhibition through post-translational modification of Cys2024-2025. These results suggest that 10-NO<sub>2</sub>-OA's neuroprotective effects may arise from direct LRRK2 inhibition and indirect inhibition via ALDH1A1 upregulation and reduced 4-HNE levels. This dual mechanism highlights 10-NO<sub>2</sub>-OA's potential as a novel therapeutic strategy for slowing or possibly preventing the progression of PD.

**Variability in Suicidal Thoughts and Behavior Among Autistic Adolescents and Adults: Subgroup Identification, Predictive Features, and Group Differences**

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Autistic people experience high rates of suicidal thoughts and behaviors (STB) and are three times more likely to die by suicide than non-autistic people. However, understanding who is most at risk remains limited. Identifying subgroups with varying STB levels could improve prevention efforts. This study aimed to use machine learning clustering to identify and characterize STB subgroups.

The sample included 831 autistic people who participated in an online study (age 37±12; range 14-86). We applied a random forests clustering to item-level data from the Autism Suicidality Inventory, Columbia-Suicide Severity Rating Scale, and Suicidal Thoughts and Behaviors Questionnaire. Feature importance analyses identified key items for subgroup membership, while demographics, health, and mental health variables were examined for subgroup differences.

Our results identified five distinct groups of STB risk based on suicidal thought severity and past attempts. Groups range from lowest risk (G1, n=97: no current suicidal thoughts, 6.2% lifetime attempts) to highest risk (G5, n=128: 97% current thoughts, 62.5% past attempts). G2 (n=101) and G3 (n=166) differ in STB, while G3 and G4 (n=339) share past similarities but differ in current STB. G5 had the highest percentage of females (41% vs. 19% in G1), and exhibited the most psychiatric diagnoses, food and housing insecurity, physical pain, masking and low self-compassion. The STUQ item on suicidal thought frequency was the strongest factor influencing STB clustering, followed by ASI items on disclosure concerns, social challenges, and emotional masking.

This study highlights new areas to consider, such as food and housing insecurity, physical pain, and self-compassion, in addition to known correlates of STB in autism (e.g. masking and ED). Key factors influencing STB subgroups highlight areas for prevention and intervention, such as overcoming disclosure barriers, enhancing social support, and alternative ED strategies. Future work should examine short-term STB increases related to these risk factors.

**The Relation Between Paternal Emotion Regulation and Inconsistent Parenting is Dependent on Maternal Emotion Regulation**

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Emotion regulation (ER) is important for navigating daily life, including parenting. Most research on ER and parenting has focused on mothers. Although it is likely that similar relations exist for fathers, this remains untested. The link between paternal ER and parenting is likely moderated by contextual factors, such as co-parent ER. This study tested longitudinal relations between paternal ER and parenting. Paternal ER was expected to be associated with lower levels of inconsistent parenting and higher levels of supportive parenting at 1-year follow-up. Maternal ER was hypothesized to moderate these relations, such that the negative impact of low paternal ER would be buffered in families with mothers who have high ER.

Participants come from the Pittsburgh Attention-Deficit/Hyperactivity Disorder (ADHD) Risk in Infancy Study (N = 73 families). Parents rated their ER skills (child age 4) and fathers rated their supportive and inconsistent parenting practices (child age 5). Paternal ADHD symptoms were assessed via clinical interview (baseline in infancy) and controlled for in analyses.

Path analysis revealed that paternal ER was not directly related to parenting one year later. Maternal ER moderated relations between paternal ER and inconsistent parenting (interaction B = 0.03, SE = 0.01,  $p < .05$ ), but not supportive parenting (interaction B = -0.02, SE = 0.01,  $p > .24$ ). Simple slopes were not significant across levels of maternal ER. However, the presence of a significant interaction term implies that the relation between paternal ER and inconsistent parenting varied by level of maternal ER.

Implications include the importance of considering the role of paternal ER in challenges in parenting and including mothers *and* fathers in studies of paternal caregiving.

## Usefulness of MoCA in detecting preclinical AD

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Preclinical AD is difficult to detect using traditional clinical tools. Yet, it can exhibit a heterogeneous pattern of subtle clinical abnormalities. Identifying common clinical tools to detect preclinical AD can help tackle the disease early. We aim to evaluate the link between Montreal Cognitive Assessment (MoCA) scores and AD pathology in cognitively unimpaired (CU) individuals. We studied 204 CU older adults who underwent both <sup>18</sup>F-FTP and <sup>18</sup>F-MK6240 scans, who were stratified based on their amyloid PET visual reading, into CU A $\beta$ + (n = 35) and CU A $\beta$ - (n = 137), from the HEAD study. CU adults had Clinical Dementia Rating (CDR) of 0 and were clinically identified as non-MCI and non-demented. Voxel-wise linear regression models tested the association between MoCA and tau pathology. CU A+T+ individuals had significantly lower total MoCA scores than A-T- individuals. Lesser MoCA total score was significantly associated with greater mediobasal temporal tau deposition, using <sup>18</sup>F-MK6240 and <sup>18</sup>F-FTP. These results were driven by the CU A+ group and absent in the CU A-. Thus, the MoCA, a simple routine in-office clinical test, can detect subtle cognitive dysfunction associated with preclinical AD. This is an option for prescreening community-dwelling older adults in primary care settings, the initial interface for most older adults without cognitive complaints.

## **RIMOC1, A Novel Regulator of Mitochondrial Function and Mitophagy In $\beta$ -cells**

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**Introduction/ Purpose:** Literature supports preserving  $\beta$ -cell mass and function to slow diabetes progression. To do so,  $\beta$ -cells withstand stressors including inflammation and increased insulin demand. Mitochondria emerge as an area of focus, as respiration drives ATP generation required for glucose-stimulated insulin secretion (GSIS), and damaged mitochondria lead to ROS generation. Mitochondria recycle via mitophagy, and defects have been linked to  $\beta$ -cell death. We isolated RIMOC1 (RAB7A-Interacting MON1-CCZ1 Complex Subunit 1) as a protein highly expressed in  $\beta$ -cells. While its function is unknown, human GEO datasets highlight a negative correlation with glucose load and HgbA1c. Considering this, our hypothesis is that RIMOC1 preserves mitophagy and mitochondrial function in  $\beta$ -cells, and a loss of RIMOC1 function in  $\beta$ -cells would lead to  $\beta$ -cell failure.

**Methods:** We generated a stable INS2 RIMOC1 KO cell line and an INS1-Cre RIMOC1 KO mouse model using CRISPR-Cas9. Mitochondrial membrane potential was quantified via TMRE, mitophagy via mtKeima probe, and mitochondrial respiration via Seahorse. Ongoing in vivo experiments include glucose and insulin tolerance testing (GTT/ITT), and ex vitro GSIS experiments.

**Results:** Human GEO datasets show that RIMOC1 expression is positively correlated with  $\beta$ -cell maturity and negatively correlated with glucose load and HgbA1c. Our in vitro data supports this, demonstrating RIMOC1 KO leads to decreased insulin mRNA expression, decreased insulin content, diminished TMRE staining, altered mitophagy, impaired mitochondrial respiration, and increased  $\beta$ -cell cycle inhibition. Early in vivo data shows significant impairment of glucose tolerance in oral GTT vs IP GTT.

**Conclusion:** RIMOC1 is enriched in  $\beta$ -cells and has a significant role in maintaining euglycemia and mitochondrial function, and ongoing studies will further explore  $\beta$ -cell specific RIMOC1 KO. This research highlights RIMOC1 as a promising drug target for preserving  $\beta$ -cell function for the prevention or treatment of diabetes.

## **Unravelling the mysteries of VLT and its protein in Varicella Zoster Virus Latency**

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Varicella zoster virus (VZV) is a human alpha-herpesvirus that causes chickenpox on a primary infection, and shingles when it reactivates from latency in neuron ganglia. During latency, alpha-herpesviruses silence lytic genes, but encode long noncoding RNAs known as latency-associated transcripts (LAT). In human trigeminal ganglia, the VZV LAT (VLT) is transcribed antisense to the ORF61 of VZV, an important regulatory protein that is key to lytic/latent decisions. There are multiple spliced forms of VLT, some which encode proteins of unknown function in both the lytic and latent stages. The molecular mechanisms underlying VZV latency and how VLT affects reactivation are still ambiguous. Our goal is to understand how they might act, including the contribution of the VLT and/or VLT-ORF63 encoded proteins to latency and VZV lytic/latent decisions in human neurons. We are conducting a genetic dissection of the VLT regions by generating different VLT mutants and determining their impact on viral replication and latency. We present preliminary work in which the VLT proteins are shown not to influence viral growth in epithelial cell models. We are now examining these in latency using and *in-vitro* human neuron culture models. The exploration of these mutants in the influence on the onset, maintenance or reactivation from latency in *in-vitro* neurons will provide insight into the mechanisms, paving the way to potential target VZV latency itself for therapeutic intervention.

**Novel Nutrition Equity Method: Older Adult Nutrition Equity Index (NEI) Developed in Health ABC & Expanding to PHRESH**

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Nutrition equity – no barriers to obtaining healthy foods – is critical for older adults. Current nutrition equity research lacks sufficient attention to multiple and concurrent processes that may contribute to acquiring healthy food. For example, improving food security, i.e. fiscal ability to obtain healthy foods, alone may not improve diet without accounting for geospatial food access or individual physical limitations. We designed a novel nutrition equity index (NEI) for older adults which combines food security, food access, and physical limitations data from older Black and White adults in Pittsburgh, PA and Memphis, TN from the Health, Aging, and Body

Composition Study (Health ABC; 51.7% women; 37.9% Black; 74.7±2.9 years). In multivariable linear regression, low (12.1%) and moderate nutrition equity (32.5%) vs. high were associated with lower dietary quality (measured via Healthy Eating Index), though after adjusting for race (which was included as a marker for social/structural factors), this NEI association attenuated to non-significance, suggesting effect modification. We will extend our analysis to examine nutrition equity with a historically marginalized population living in two predominantly Black neighborhoods by using recent data from Pittsburgh Hill/Homewood Research on Neighborhood Change and Health (PHRESH; 81.1% women; 93.4% Black; 67.3±9.8 years). We will expand the NEI to include additional food access (e.g. distance to major food shopping venue) and functional ability measures in an expanded population of middle-aged to older adults. Expansion of the NEI and its application in this new population/setting will highlight the structural nutrition equity components which are critical to address.

**Unique Pattern of Coagulopathy Among Patients with Severe Traumatic Brain Injury**

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Introduction: Trauma-induced coagulopathy may be distinct among patients with severe traumatic brain injury (TBI). We sought to identify unique patterns of coagulopathy associated with TBI.

Methods: Data were harmonized from 6 major hemorrhagic shock trials: COMBAT, CriSP-HS, PAMPer, PPOWER, PROPPR, and STAAMP. Subjects in the placebo groups of each trial who had admission TEG were included. Strata were defined by 1) isolated severe TBI (iTBI), with head AIS $\geq$ 3; 2) severe polytrauma (POLY), TBI plus other body region AIS $\geq$ 3; and 3) severe trunk/extremity trauma only (noTBI). Stratified principal component (PC) analysis was performed including INR, alpha angle (AA), K time (K), maximum amplitude (MA), and LY30. After reduction to PCs, associations between PC scores and a) 24-hr transfusion and b) 24-hr mortality were examined with logistic regression.

Results: The analysis included 506 subjects: 51 iTBI, 115 POLY, 340 noTBI. For each stratum, two PCs were identified that accounted for  $\geq$  65% of the variance. For both iTBI and POLY, the dominant phenotype was associated with INR, AA, K, and MA, and had greater odds of 24-hr mortality (OR 1.57, p=0.049 for iTBI; OR 1.55, p=0.006 for POLY) and transfusion (OR 3.57, p=0.004 for iTBI; OR 2.29, p=0.026 for POLY). For noTBI, a different phenotype was identified that was associated with INR, AA, and MA, with lower odds of 24-hr mortality (OR 0.71, p=0.001) and transfusion (OR 0.51, p<0.001).

Conclusion: PC analysis demonstrated a unique predominant pattern of coagulopathy among patients with severe TBI associated with increased mortality and transfusion requirement.

**Early Plasma Associated with Lower Odds of Midline Shift in Pediatric Brain Injury**

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**Introduction:** In adults with traumatic brain injury (TBI), early plasma may improve outcomes. We examined the impact of early plasma on midline shift (MLS), a radiologic surrogate for significant intracranial hemorrhage or edema, in children.

**Methods:** The National Trauma Data Bank from 2020-2022 was queried for children age 1-17 years with severe TBI (head Abbreviated Injury Scale  $\geq 3$ ) and non-missing MLS variable. MLS was defined by the NTDB as  $>5\text{mm}$  shift past midline within 24-hrs post-injury. The association between plasma administration within 4 hours and MLS was examined with logistic regression after adjusting for age, sex, trauma mechanism, interfacility transfer, shock, injury severity, Glasgow Coma Scale, binary receipt of platelets, red blood cells, and whole blood, and weight-adjusted total transfusion volume, clustered by facility. Subgroup analysis was performed for age  $< \text{or} \geq 14$  years.

**Results:** Overall, 36,557 children met inclusion criteria, 3,082 (8.4%) of whom had MLS. The cohort was mostly male (66.1%) with blunt injury (85.0%), median (IQR) ISS = 16 (10-25), and a 30-day mortality rate of 7.1%. Children who received plasma had lower odds of MLS than those who did not (adjusted odds ratio (aOR) 0.79,  $p=0.019$ ). Treatment effect was dichotomized by age: children  $<14$  years had significantly lower odds (aOR 0.60,  $p<0.001$ ) of MLS with plasma, while children  $\geq 14$  years did not (aOR 1.21,  $p=0.206$ ).

**Conclusions:** Early plasma was associated with lower odds of MLS within 24 hours in children with severe TBI. Age was an important factor in this relationship. Further prospective data are needed.

## **The Focal Adhesion Kinases Regulate Central Energy Homeostasis Through Leptin Signaling**

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Leptin is secreted from fat tissue and is required for the maintenance of energy homeostasis. In obesity, hyperleptinemia and leptin resistance block the anorexigenic potential of leptin and contribute to the maintenance of adiposity. Resensitizing obese individuals to their endogenous leptin is a promising approach to combat obesity.

We previously showed that histone deacetylase 6 (HDAC6) inhibition increases central leptin sensitivity in a non-cell-autonomous manner, with concomitant weight loss in diet-induced obese (DIO) mice. However, the mechanism is unclear.

Using an integrative analysis of leptin-responsive hypothalamic gene expression signatures, we identified that the anorectic effect of leptin and the anti-obesity effect of the HDAC6 inhibitors require focal adhesion kinases, FAK and PYK2. The weight reducing effect of the HDAC6 inhibitor tubastatin A (TubA) in DIO mice is compromised in the PYK2-knockout (KO) background. In wild-type (WT) DIO mice, central inhibition of FAK/PYK2 blocks the TubA-induced weight loss. Using genetic and pharmacological approaches, we show that FAK/PYK2 interact with the transcription factor signal transducer and activator of transcription 3 (STAT3), the main downstream regulator of leptin action, leading to its phosphorylation and activation. STAT3 activation is attenuated if either FAK or PYK2 are knocked down or if their kinase activity is inhibited. Accordingly, PYK2-KO mice have compromised leptin-induced STAT3 phosphorylation and weight loss. Furthermore, central inhibition of FAK/PYK2 in WT mice reduces hypothalamic STAT3 activity and the anorectic action of leptin. Using RNAscope in situ hybridization, we show that FAK is ubiquitously expressed throughout the hypothalamus, whereas PYK2 expression is higher in the dorsomedial hypothalamus (DMH). Finally, dual hypothalamic knockdown of FAK/PYK2 blunts leptin action, leads to hyperphagic obesity, and attenuates the anti-obesity effect of HDAC6 inhibitors.

Altogether, these findings reveal FAK and PYK2 as a previously uncharacterized component of the leptin receptor signaling and critical mediators of central leptin sensitization.

## **Human Immune Recognition of Ocular Surface Bacteria**

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**Purpose:** The role of the ocular microbiome on human physiology remains a contentious topic. Specifically, it is unclear how ocular bacteria may interact positively or negatively with the host immune system. Here, we aim to describe how host immunity recognizes ocular surface bacteria.

**Methods:** Culturomics was performed on conjunctival swabs of 70 healthy human subjects. To maximize the possibility of expanding eye-associated fastidious microbes. In parallel, conjunctival immune cells, peripheral blood mononuclear cells (PBMCs), and serum were isolated from the same subjects. Flow cytometry was used to characterize ocular surface immune cells, while PBMCs were incubated for 3 days with bacteria isolated from culturomics assays to assess cellular immune responses. Similarly, serum was incubated with bacteria to identify potential bacteria-specific antibody responses.

**Results:** 26% of culturomics swabs yielded culturable bacteria and over 50% of bacteria were *Staphylococci sp.* Upon incubating PBMCs with *S. epidermidis*, *P. lautus*, *B. tropicus*, or *C. mastididis*, we observed that humans had blood circulating CD8<sup>+</sup> T cells and gd T cells recognize ocular surface bacteria through proliferation by Ki67 expression and the upregulation of cell activation marker, CD69. Notably, only *P. lautus* and *B. tropicus* were recognized by antibodies within the serum of healthy human subjects.

**Conclusion:** Our data lay the framework for beginning to use the immune system to interrogate how ocular bacteria may affect host physiology. Specifically, local and circulating immune cells may be able to provide information on whether specific bacteria remain associated with the host long-term.

**Quercetin: A Natural Flavonoid Compound Radio Sensitizes Pancreatic Ductal Adenocarcinoma Cells**

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most treatment resistant cancers, accounting for 7% of total cancer deaths worldwide. One of the major challenges in treating PDAC with radiotherapy (RT) effectively is its proximity to sensitive GI organs. One strategy to increase the efficacy of RT is combination with radiosensitizing drugs, to selectively increase the sensitivity of tumor cells killing by RT. With this aim, we investigated Quercetin, a nutraceutical flavonoid compound. In the present study, we investigated whether Quercetin could radiosensitize mouse (PK5L1940) and human (AsPC-1, PANC-1 and BXP-3) PDAC cells. To understand the effect of quercetin on radiation response of various PDAC lines, we performed clonogenic assays. We pre-treated PDAC cells for 24 hrs. with Quercetin(25  $\mu$ M), then exposed to increasing doses RT (0-8 Gy), and incubated for 7 days to determine clonogenic survival. Quercetin (25  $\mu$ M) treatment sensitized the PDAC cells lines with dose modification factor (DMF) at  $D_{50}$  of 0.53, 0.07, 0.45 and 0.58 in PK5L1940, AsPC-1, PANC-1 and BXP-3, respectively. We further validated these *in vitro* results *in vivo*. We implanted flank tumors with PK5L1940 cells in C57BL/6 mice and treated with vehicle, Quercetin (100 mg/kg of body weight), 16 Gy RT + Vehicle, or 16Gy RT + Quercetin. Tumors were measured twice a week and mice were euthanized when the average tumor volume was reached 2000 mm<sup>3</sup>. Quercetin and RT individually did not significantly reduce the tumor volume or delay time to tumor endpoint. The combination of Quercetin and RT significantly reduced tumor size at all time points, and on the day of experiment termination, the average tumor volume of the combination group was 1127 mm<sup>3</sup> compared to all 3 other groups whose average volume surpassed 2000 mm<sup>3</sup>.

## Testing Validity of FFDM-Based Density Evaluating AI Algorithm for Synthetic Mammograms

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**Introduction:** Digital Breast Tomosynthesis (DBT) is the standard screening method in the US. Synthetic mammograms (SM) were developed to reduce patient dose and eliminate the need for combination imaging of Full-field Digital Mammogram (FFDM) and DBT. However, SM image quality can differ from FFDM, potentially emphasizing false lesion-like features (1). We previously developed an AI-based algorithm for breast density evaluation using 2D FFDM (2). Its performance on SM is uncertain, as it is new to this image type and may introduce errors.

**Objectives:** This study aims to evaluate the validity of our FFDM-based AI algorithm for assessing breast density on SM images reconstructed from DBT.

**Methods:** Under an IRB-approved protocol, we curated 226 asymptomatic women (screening BIRADS 1 or 2, clinical density BIRADS A-D) who had simultaneous FFDM and DBT scans. Both image types were used in their “for presentation” versions. We applied our FFDM-based AI algorithm to both FFDM and SM images. Breast percent density (PD) was computed as the ratio of dense to total breast area, and average PD (APD) was calculated from four standard views (L/R-CC and L/R-MLO) for both FFDM and SM. We assessed agreement between the density estimates from FFDM and SM using the proportion of agreement within 0.05 and the Bland-Altman plot.

**Results:** The APD estimates showed a high level of agreement between FFDM and SM, with 89% (CI: 84-93%) of the estimates within 0.05, and Bland-Altman limits of agreement from -0.06 to 0.06.

**Conclusion:** Without any fine-tuning on SM images, our FFDM-based AI algorithm can reliably segment breast tissue and estimate breast density from SM images. Few algorithms currently exist that can estimate breast density from both FFDM and SM images. As our algorithm works on SM images as well, it could facilitate DBT screening and risk assessment processes.

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## **Whipped-Cream Inspired Injectable Hydrogel for Chronic Skin Wound Treatment**

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Chronic skin wounds impact over 6.5 million Americans annually, costing the healthcare system approximately \$25 billion. These wounds, including burns and battle field wounds, require rapid intervention to prevent worsening. Current treatments like antibacterial agents, growth factor therapies, and negative pressure wound therapy mainly address symptoms rather than the root causes. The healing process is complex, with macrophages playing a key role. Recent shifts toward immunomodulatory therapies, especially using macrophages to naturally produce healing factors, show promise. Recombinant human PRG4 (rhPRG4), a lubricating proteoglycan, has demonstrated potential as an immunomodulatory agent, influencing macrophage polarization. Higher PRG4 levels in newborns motivate further research on its use in wound healing. rhPRG4 has shown encouraging results in rodent models, but topical application has limitations in control and efficiency. This study introduces a whipped cream-inspired foam dressing that can be applied directly to wounds with different geometries, crosslinking in situ while acting as an immunomodulatory scaffold to enhance healing. In both in vitro and in vivo mouse model, the foam demonstrated promising effects on wound healing and tissue regeneration by increase in IGF-1, TGF- $\beta$ , IL10, angiogenesis (CD31), and collagen deposition levels and decrease in inflammatory biomarkers such as TNF- $\alpha$ , and IL6. This innovative technology holds potential for clinical use, offering new possibilities for treating acute and chronic skin wounds and providing hope for patients.

**Leveraging single-cell transcriptomic and spatial modalities to dissect the tumor microenvironment in patients with head and neck cancer**

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Understanding cell states and spatial localization is essential to dissect immune responses within tissues. The tumor immune microenvironment (TME) involves complex interactions among immune, stromal, and tumor cells. While immune-checkpoint blockade (ICB) has improved outcomes in Head and Neck Squamous Cell Carcinomas (HNSCC), most patients do not achieve a clinical benefit. We hypothesize that the composition, spatial distribution, and transcriptomic profiles of immune, stromal and tumor components together dictate response to ICB. Single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) enable detailed tumor analysis, yet integrating their strengths remains challenging. To address this, we combined an HNSCC single-cell atlas with a cohort of 20 HNSCC patients analyzed via the Bruker CosMx platform to infer transcriptional states and spatial organization. Our joint-clustering approach maps cell populations into spatial contexts, identifying distinct cellular neighborhoods through unsupervised clustering. Utilizing these datasets, we have been able to identify recurrent cellular neighborhoods across a cohort of HNSCC patients. Of particular interest, one cellular neighborhood was present at the tumor margin, and featured interactions between immune cells, fibroblasts, and tumor cells. This neighborhood may restrict immune infiltration into the tumor core, potentially limiting antitumor immunity. Evaluating its presence and activity could reveal its role in shaping the TME and influencing therapeutic response. This research will advance our understanding of HNSCC's complex cellular landscape, potentially uncovering novel therapeutic targets and improving patient stratification for personalized treatment strategies.

**Longitudinal Associations Between Shared and Unique Components of Executive Function and Externalizing Subdimensions: Findings from the ABCD Study**

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Deficits in executive function—including inhibition, working memory, and cognitive flexibility—are robustly linked to externalizing behaviors in youth. However, limited research has examined how shared versus unique components of executive function relate to distinct externalizing dimensions over time. This study investigated differential associations between executive function components and developmental trajectories of externalizing behaviors in a large, diverse sample of youth (N = 11,875; Mage = 9.51; 48% girls). Executive function was assessed at baseline using the NIH Toolbox Cognition Battery, and externalizing behaviors were measured through parent-reported Child Behavior Checklist (CBCL) scores collected at four timepoints spaced six months apart. A confirmatory factor analysis identified a shared latent executive function factor, and latent growth curve models were estimated for a broad externalizing factor and three subdimensions: conduct problems, irritability, and neurodevelopmental difficulties. Models included time-varying covariates (i.e., other externalizing subdimensions) and time-invariant covariates (age, sex, site, income, and IQ proxy).

Results showed that both shared and unique components of executive function were associated with higher initial levels of broad externalizing behavior, with the shared executive function factor showing the strongest effect ( $\beta = -0.15$ ) and a small association with increases over time ( $\beta = 0.057$ ). When examining subdimensions, these effects were primarily driven by neurodevelopmental problems. The latent executive function factor and individual executive function indicators were consistently associated with higher levels of neurodevelopmental difficulties ( $\beta$ s = -0.053 to -0.184), while associations with conduct problems and irritability were negligible or non-significant.

These findings suggest that the influence of executive function on externalizing behavior may be more strongly related to the development of neurodevelopmental problems rather than conduct problems or irritability. This underscores the need to account for heterogeneity within externalizing behaviors when studying executive function as a risk factor, with potential implications for understanding etiology and tailoring interventions.

## **Circulating Tumor DNA in Non-Viral Head and Neck Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis.**

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Non-viral head and neck squamous cell carcinoma (HNSCC) has poor survival and high recurrence rates. Circulating tumor DNA (ctDNA) is a promising biomarker for understanding tumor biology, assessing treatment response, and monitoring disease progression. While extensively studied in virally mediated HNSCC, its role in non-viral HNSCC remains underexplored.

This systematic review and meta-analysis consolidates evidence on the diagnostic, prognostic, and therapeutic value of ctDNA in non-viral HNSCC. A systematic search across Medline, PubMed, Embase, and the Cochrane Library identified 1,915 records, of which 47 were included. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to report our study. We abstracted the study characteristics, patient demographics, ctDNA assay techniques, and clinical outcomes. Survival outcomes, including overall survival (OS), progression-free survival (PFS), and recurrence-free survival (RFS), were pooled as hazard ratios (HRs) with 95% confidence intervals (CIs) using a fixed-effect model. Study quality was assessed using the Newcastle-Ottawa Scale.

Among 3,574 patients, the most common tumor sites were the oral cavity (35%) and oropharynx (22%), with the majority presenting with stage IVA/IVB disease (29%). Pre-treatment ctDNA detection rates ranged from 50% to 100% (median: 83%), while post-treatment detection rates varied between 28% and 100% (median: 48%). ctDNA detected recurrence in 80% of patients, with a median lead time of 4.6 months. ctDNA detection was significantly associated with worse OS (HR 10.26, 95% CI 3.58–29.40;  $P < .0001$ ). Residual ctDNA was strongly correlated with worse PFS (HR 7.32, 95% CI 4.17–12.86;  $P < .00001$ ) and RFS (HR 7.33, 95% CI 2.75–19.58;  $P < .0001$ ).

ctDNA holds potential for improving diagnostic accuracy, monitoring progression, and predicting survival outcomes in non-viral HNSCC. However, further large-scale studies and standardized guidelines are needed for validation and clinical implementation.

## **Visualizing Natural Sleep State During Nasolaryngoscopy in Infants: A Feasibility Study**

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### **Introduction**

Otolaryngology consultation for infant airway obstruction is common, with nasolaryngoscopy serving as the primary diagnostic tool. However, traditional awake nasolaryngoscopy is often limited by infant crying and brief examinations. This study explores Time-Induced Sleep Endoscopy (TISE) as a relatively brief, non-invasive alternative for evaluating sleep-related airway dynamics in infants.

### **Methods**

This prospective study enrolled infants under three months of age at the University of Pittsburgh Medical Center Children's Hospital. Hospitalized infants with indications for flexible nasolaryngoscopy for airway evaluation at bedside were eligible. A flexible nasolaryngoscope was inserted through the nose and left in place until the patient fell asleep, naturally, or after five minutes. Endoscopic videos were scored by two attending pediatric otolaryngologists using the Sleep Endoscopy Rating Scale. Demographics and time to sleep were recorded. Logistic regression and statistical analyses examined factors associated with successful TISE. Inter-rater reliability was assessed using Cohen's Kappa.

### **Results**

A total of 19 infants were included, with a mean gestational age of 38.1 weeks (SD 1.7 weeks) and a median post-menstrual age of 40.3 weeks (range: 37.1–51.5). TISE was successful in 11 (57.9%), with an average time to sleep of 3.1 minutes (SD 1.3). Logistic regression analysis revealed a negative correlation between successful TISE and both chronological and post-menstrual age. Infants younger than three weeks were eight times more likely to fall asleep compared to older infants (OR=8.00, 95% CI: 1.00–63.96,  $p=.05$ ). Compared to awake endoscopy, TISE revealed higher airway obstruction scores, particularly for hypopharyngeal obstruction (100% compared to 18%,  $p=.004$ ). Inter-rater reliability was moderate for awake endoscopy ( $\kappa=0.55$ ) and fair for TISE ( $\kappa=0.38$ ).

### **Conclusion**

This feasibility study supports TISE as a minimally invasive and effective method for evaluating suspected upper airway obstruction in infants. Further validation and standardization are necessary for broader use.

## **Stochastic Modeling of Extinction During Viral Reproductive Cycle**

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We present a stochastic model in immunology describing the evolution of diseases in the human organism. Our analysis includes two types of viral production: bursting and budding. Euler-Maruyama simulation algorithm and jump-diffusion process are used to analyze the stochastic model. Numerical simulations explore the dynamic behavior of the reproduction system in vitro in the absence of any immune component. There are two absorbing states for the dynamics of our system: extinction of virus cells and extinction of host cells. The simulation also investigates the mean times of absorption and probabilities of absorption depending on the parameters of the system.

## **Macrophage Polarization Directs the Migration and Proliferation of Post-natal Prx1-Expressing Cells**

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**Objectives:** Post-natal skeletal stem cells expressing Paired related homeobox 1 (pnPrx1+ cells) play an important role in bone regeneration. Meanwhile, macrophages help in tissue regeneration by recruitment of numerous cell types including stem cells. However, whether macrophages contribute to tissue regeneration by direct effect on skeletal stem cells remains unclear. In the present in vitro study, we aimed to identify the effect of macrophages' polarization on proliferation and migration of pnPrx1+ cells.

**Methods:** Unpolarized (RAW 264.7 cells) macrophages (M0) were polarized using lipopolysaccharides to obtain pro-inflammatory macrophages (M1) and interleukin-13 to obtain anti-inflammatory macrophages (M2). Then, to explore their effects on the migration of pnPrx1+ cells, the conditioned media of M0, M1, and M2 were utilized on the lower chamber of a transwell co-culture system, while DMEM medium was used as negative control. Immortalized pnPrx1+ cells were incubated in culture inserts with 5 um pores. pnPrx1+ cells migrated to the lower chamber were detected by CellTiter-Glo® 2.0 Assay at 1, 3, and 6 hours. For pnPrx1 cells proliferation, pnPrx1+ cells were directly cultured using the same media using 96 well plates. After 24 hours, pnPrx1+ cells' proliferation was examined using MTT assay.

**Results:** M2 conditioned medium showed the highest levels of pnPrx1+ cells' migration at all time points, followed by the M0 conditioned medium. In terms of pnPrx1+ cells' proliferation, M2 conditioned medium showed the highest pnPrx1 cells' proliferation. There was no statistically significant difference between all other conditioned media.

**Conclusion:** In vitro, the macrophages' transition to M2 creates a favorable environment that directly contributes to the migration and proliferation of pnPrx1+ cells. This may stimulate tissue regeneration by enhancing recruitment of pnPrx1+ cells. Additional studies are being performed to evaluate the effect of immunomodulatory molecules on macrophages' polarization to induce migration and proliferation of pnPrx1+ cells and tissue regeneration.

**Protecting Simian Immunodeficiency Virus-Infected Macaques From *Mycobacterium Tuberculosis* by Intravenous Bacillus Calmette-Guérin Vaccination Requires Viral Control**

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a major global health challenge, especially for people living with HIV (PLWH) not on antiretroviral therapy (ART). We previously showed that intravenous Bacillus Calmette-Guérin (IV BCG) provides robust protection in SIV+ macaques. This study evaluated the immunogenicity and protective efficacy of IV BCG in SIV+ macaques treated for 8 weeks with an anti-BCG regimen of isoniazid, rifampicin, and ethambutol (HRE), starting either 1 week (early) or 3 weeks (late) post-BCG. Control groups included IV BCG-vaccinated macaques without HRE and those vaccinated with intradermal (ID) BCG. Five months post-vaccination, macaques were challenged with Mtb, and TB progression was monitored by PET-CT imaging and bacterial load at necropsy 12 weeks post-challenge. IV BCG induced stronger immune responses than ID BCG. Protection conferred by IV BCG was significant regardless of HRE timing. However, SIV non-controllers, marked by high viremia and reduced CD4+ T cells in the lungs, showed less protection than SIV controllers. This study confirms the robust efficacy of IV BCG in SIV+ macaques, even with short BCG exposure times. It further demonstrates the critical importance of viral control in vaccine-elicited protection from TB in PLWH.

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**Effects of Contraception and Menstrual Cycle Phase on Circulating Metabolites in Response to Resistance Exercise**

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Background: Women are often underrepresented in exercise studies, partly due to the complexity of controlling for menstrual cycle phase and hormonal contraception (HC) use. Recent evidence indicates a slight impact of these variables on exercise performance. Exercise is a powerful metabolic modulator and circulating metabolites can have important signaling properties. Extracellular vesicles (EVs) are lipid bilayer particles that transport signaling factors like metabolites. No studies have examined the effects of menstrual cycle or HC on plasma and EV metabolites following exercise.

Methods: 40 women participated in this study (n=10/group): Follicular (FOL), Luteal (LUT), oral contraceptive pill (OCP) users, or women with hormonal intrauterine devices (IUD). Menstrual cycle information was determined from self-reports. Blood was drawn before and after a 10x10 bout of weighted plyometric jumps (PlyoPress). Progesterone and 17- $\beta$  Estradiol were measured and plasma EVs were isolated via SEC. Metabolomics was run via LC-HRMS. Differential comparisons generated significant compounds ( $p < 0.05$ ; Log FC>2) and pathway analysis was run on MetaboAnalyst.

Results: LUT had greater progesterone than all other groups, and greater 17- $\beta$  Estradiol than FOL. Targeted plasma metabolomics showed no major group differences in exercise-responsive metabolites. Differentially expressed plasma metabolites differed slightly between groups with FOL having the largest exercise response (60 metabolites changed). EVs and plasma differed greatly in metabolite overlap, with only 49 shared metabolites. For EV metabolites, LUT women had the strongest exercise response with 51 altered metabolites and 60 pathways that were predicted to be altered by exercise.

Conclusion: Major exercise-responsive metabolites were not impacted by menstrual phase or HC usage. EV and plasma metabolite responses to exercise varied slightly depending on phase with LUT having greater EV metabolite changes and FOL having greater plasma metabolite changes. These patterns should be further investigated to see if specific metabolites are linked with exercise performance and adaptation.

**Synthetic Nerve Conduits: Comparative Assessment of Dip-Coated and Electrospun Methods in Peripheral Nerve Repair**

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Peripheral nerve injuries significantly impact patient quality of life and functionality. Autografts, the current gold standard, present limitations including donor-site morbidity, neuroma formation, and graft-size mismatch. Synthetic nerve conduits offer an alternative, providing structural guidance and localized therapeutic delivery.

This study comparatively evaluated two polymeric nerve guide fabrication methods: dip-coated polycaprolactone (PCL) and electrospun polycaprolactone/polyurethane (PCL/PU) conduits. Both conduit types incorporated double-walled microspheres (DWMS) loaded with lysozyme, a surrogate molecule for glial cell-derived neurotrophic factor, assessing release kinetics and microsphere-conduit integration.

Dip-coated conduits were fabricated using an automated process involving sequential layering of PCL with embedded lysozyme-DWMS, whereas electrospun conduits employed optimized parameters yielding a porous nanofiber architecture. Scanning electron microscopy confirmed uniform integration of DWMS within both conduit types, with electrospun conduits exhibiting higher porosity and fiber alignment suitable for cellular infiltration.

Mechanical evaluations via tensile tests revealed significant differences; electrospun PCL/PU conduits demonstrated greater flexibility (elongation:  $367 \pm 125\%$ ) compared to dip-coated conduits ( $20 \pm 5\%$ ,  $p < 0.001$ ), whereas dip-coated conduits exhibited superior stiffness ( $472 \pm 233 \text{ N}\cdot\text{m}^{-1}$ ) compared to electrospun conduits ( $70 \pm 53 \text{ N}\cdot\text{m}^{-1}$ ,  $p < 0.01$ ), beneficial for structural integrity. Suture pull-out tests indicated no statistically significant differences (average pull-out  $\sim 3 \text{ N}$ ,  $p > 0.05$ ), demonstrating comparable suture retention.

In vitro lysozyme release profiles highlighted distinct dynamics: electrospun conduits showed rapid initial release ( $\sim 29.3\%$  total protein in 72 h), followed by sustained linear kinetics plateauing at 16 weeks. Dip-coated conduits presented a biphasic release (initial  $\sim 16\%$  total protein,  $p < 0.05$  compared to electrospun), achieving plateau by 8 weeks with significantly different long-term kinetics ( $p < 0.0001$  at week 4 peak).

These findings highlight fabrication methods and polymer composition as critical determinants for nerve conduit performance, warranting further in vivo validation and clinical translation.

**Role of profilin-1 (Pfn-1) in tumor microenvironment of renal carcinoma**

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Renal carcinoma murine model was used to characterize kidney cancer tumor microenvironment. As we demonstrated earlier, Pfn1 is predominantly overexpressed in tumor-associated vascular endothelial cells. In this study we further analyzed the tumor cellular composition and differential gene expression under Pfn1 upregulation, which were dissected using cutting-edge technology of single cell RNAseq (scRNAseq). We annotated cell type populations and identified cell type specific differentially expressed genes (DEGs), along with those which shared upregulation across several types of cells, demonstrating trends during tumor progression under elevated Pfn1. The latter are linked to promotion of autophagy (decidual protein induced by progesterone 1 (Depp1)), inflammation, activation of macrophages (serum amiloid A3 (Saa3)). This study confirmed upregulation of Serine Protease Inhibitor, Clade E, Member 1 (Serpine1), which we identified earlier as a Pfn1-dependent pro-angiogenic factor and proposes adrenomedullin (Adm) as an additional factor increasing tumor progression via promoting its vascularization. Adm was found to be upregulated in several cell types including podocytes, endothelial cells, tumor cells and Pfn1/Ftl1-positive tumor cells. Other factors affect metabolic pathways participating in lipids metabolism (Hilpda) and preventing iron-associated apoptosis (Selenium-binding protein 1 (Selenbp1)). Interestingly, a part of tumor cells formed a distinct cluster with upregulated Ferritin light chain 1 (Ftl1) gene, participating in iron intracellular storage. This could serve as sign of elevated concentration of iron, leading to ferroptosis, which is counteracted by Selenbp1.

## **Circadian Misalignment Activates Inflammasome to Drive MASH**

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Shift work is linked to obesity and metabolic disorders, including MASLD and MASH. Emerging studies highlight circadian regulation of immune function, lipid metabolism, mitochondrial and ER stress. We propose that circadian disruption and dietary stress worsen MASLD/MASH by driving mitochondrial dysfunction, macrophage activation and pyroptosis via NLRP3 inflammasome activation. Hepatocyte injury in MASH may further augment AIM2 inflammasome activation under these stressors.

C57BL/6 mice were fed chow or high-fat diet (HFD) *ad-libitum* and subjected to regular or shift cycles (CD) for 6 months. Shift cycles mimicked shift work with alternating 12-hour LD phase shifts. Body weight, GTT and liver clock gene expression were measured. Liver histology was analyzed for steatosis, macrophage infiltration and fibrosis. Inflammatory and fibrotic markers were quantified through qRT-PCR, while western blots were assessed for NLRP3 inflammasome components. Ex vivo, BMDMs from Bmal1 KO and KI mice were treated with CRE, LPS, and total DNA from these livers to study AIM2-mediated NLRP3 inflammasome activation.

Mice fed HFD combined with CD (HFD+CD) showed higher body weight and blood glucose levels than other groups. Liver clock gene analysis indicated disrupted circadian regulation in HFD+CD. H&E staining and triglyceride assays revealed increased steatosis and Mallory-Denk bodies. Immune activation, linked to hepatocyte damage, was evident with elevated F4/80 staining and increased *Ly6c*, *Tnfa*, *Cxcl2* and *Inos* expression in the livers of HFD+CD mice. CD alone upregulated key proinflammatory markers, emphasizing its role in inflammation. Fibrosis, assessed by Picro-Sirius Red staining and fibrotic gene expression, was significantly elevated in HFD+CD mice. Mechanistically, NLRP3 inflammasome activation was heightened, with increased NLRP3, cleaved caspase-1, IL-1 $\beta$  and cleaved GSDMD, identifying pyroptosis as a key event in MASH progression.

Our findings suggest that circadian disruption worsens MASH by enhancing NLRP3 inflammasome activation under dietary stress. Thus, augmenting the circadian clock may reduce inflammation and alleviate MASH.

**TEAD1 Control of Osteopontin in the Liver is Sufficient to Protect Against Primary Sclerosing Cholangitis**

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Primary sclerosing cholangitis (PSC) is a bile duct disease where inflammation-driven fibrosis leads to duct narrowing and severe liver damage, often requiring a transplant. We hypothesized that TEAD1, the transcription factor downstream of hippo signaling, is required in the pathogenesis of PSC by triggering hepatocyte-derived osteopontin (OPN) levels, promoting inflammation and fibrosis. We tested this hypothesis in the hepatocyte-specific TEAD1 knockout and DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine)-induced biliary obstruction/liver injury model of PSC.

Hepatocyte-specific TEAD1KO (TEAD1<sup>fl/fl</sup>;Alb-Cre<sup>+</sup>) and floxed controls were fed DDC-supplemented diet for 8 days (with Chow-fed controls). Body weight and liver weight were assessed. Liver histopathology was analyzed for hepatocyte proliferation, macrophage infiltration, and fibrosis through IHC and picosirius red staining. We also assessed the expression of TEAD1, bile transporters, inflammation and fibrosis via qRT-PCR and western blotting.

Our results demonstrate a significant reduction in the body weights of mice supplemented with DDC. However, the DDC-supplemented Hep-TEAD1KO group showed a significant increase in liver weight compared to other groups. DDC significantly increased macrophage infiltration (F4/80), hepatic stellate cell activation (αSMA) and porto-portal fibrosis in floxed controls as compared to Hep-TEAD1KO. TEAD1 and OPN expression were elevated in DDC-induced liver injury. However, this increase in OPN was mitigated in Hep-TEAD1KO mice. This suggests that TEAD1 was required for the upregulation of OPN induced by DDC. Further studies to test the direct transcriptional regulation of OPN by TEAD1 and its direct role in promoting liver injury in PSC model are underway.

Taken together this study demonstrates the requirement of TEAD1 in PSC pathogenesis and identifies TEAD1 modulation as a potential target for regulating inflammation and fibrosis in Primary sclerosing cholangitis.

## **Inpatient Health Outcomes and Resource Utilization in Patients with Simple Congenital Heart Disease**

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**Background:** Children with congenital heart disease (CHD) have higher healthcare utilization and mortality rates compared to their peers. However, most CHD cases involve isolated defects that require minimal intervention with no need for follow-up. Limited data exist on whether children with simple CHD receive different management during hospitalization for common pediatric conditions or experience distinct health outcomes. This study aims to compare the length of stay and health outcomes of hospitalized young children with simple CHD to those without CHD.

**Methods:** The Pediatric Health Information System (PHIS) was used to identify patients 5 years or younger with ten pre-defined pediatric hospital medicine diagnoses who were discharged between 2020 and 2024. Patients with complex CHD or complex chronic conditions were excluded. The association between having a simple CHD diagnosis and length of stay was analyzed using ordinal logistic regression.

**Results:** Among a total of 242,163 admissions, 4,235 (1.7%) involved patients with simple CHD. Gender and race distributions were similar between groups, but patients with simple SCD were younger and had lower median household incomes. Simple CHD patients had higher ICU admission rates (36.3% vs 25.7%), were more likely to receive mechanical ventilation (14.8 vs 5.6%), and had higher rates of medical or surgical complications. Mortality was rare in both groups (0.1% vs <0.1%). Hospital stays were longer for simple CHD patients, with 43.4% having a length of stay (LOS) of 3-7 days and 23.0% had LOS of  $\geq 8$  days, compared to 38.7% and 5.3%, respectively, in those without simple CHD. These differences remained significant in multivariable models (OR = 1.52 [95%CI, 1.42, 1.63] for LOS 3-7 days and OR = 4.46 [4.09, 4.95] for LOS  $\geq 8$  days).

**Conclusion:** Young children with simple CHD have longer LOS, higher rates of ICU admission and complications compared to their healthy peers.

**Leveraging Health Data and Machine Learning to Predict No-Show Propensity Factors in Glaucoma Patients: A Health Informatics Approach to Improve Clinic Efficiency and Patient Care**

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Glaucoma requires lifelong care, but missed appointments ("no-shows") disrupt treatment and threaten eye health. Traditional no-show predictions treat it as a binary outcome, useful for hospital resource planning but less informative for individualized patient support. This study develops algorithms to predict no-show propensity for new patients and identify key clinical and demographic risk factors. The goal is to target patients needing support and determine effective interventions—such as social services or tailored engagement strategies—to improve care continuity.

Data from the University of Pittsburgh Medical Center's electronic health records for ophthalmology encounters (Jan 2013–Dec 2023) identified glaucoma patients by billing codes. A no-show propensity factor (NSPF) was calculated to adjust for variability in no-show rates and to weight by appointment frequency. NSPF was classified as low or high based on a 31.7% no-show rate reported for resident ophthalmology clinics. Machine learning models (logistic regression, random forest, SVM, XGBoost) with 10-fold cross-validation assessed performance based on sensitivity, helping identify patients who may need additional support. Meanwhile, log-logistic regression models were used to calculate relative risks (RR) for baseline characteristics and NSPF, highlighting key factors associated with poor appointment compliance.

8504 unique glaucoma patients had 163,385 appointments, 7400 (4.5%) were no-shows. The XGBoost model performed best, achieving 89% sensitivity. Higher NSPF was associated with younger age (<60 vs. 80+, RR = 1.58[1.44,1.75]), black race (vs. White, RR = 2.89[2.71,3.09]), unmarried status (RR = 1.87[1.69,2.09]), Medicaid insurance (RR = 2.40[2.19,2.63]), higher Distressed Communities Index (RR = 2.32[2.06,2.61]), and worse clinical conditions (severe-stage glaucoma, RR = 1.40[1.24,1.58]).

This study identifies high-risk patients and informs targeted interventions to reduce no-shows, enhance appointment adherence, and improve care continuity while optimizing resource allocation. Findings may guide future efforts to improve patient outcomes.

**Every Step Counts: Reported Walking Ability as a Predictor of All-Cause Mortality**

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Evaluation of mortality risk with respect to physical function has focused on limitation. Here, we evaluated all-cause mortality risk in relation to increments of capacity in mobility intact older adults using the Walking Ability Index (WAI). In the Health, Aging and Body Composition study (Health ABC, N=3075, aged 70-79), self-reported ease/difficulty walking ¼ mile and 1 mile, scored zero (worst) to nine (best) was assessed. Per study eligibility requirements, the WAI ranged from 4-9 at baseline. This analysis includes 2812 participants (57% White; 49% men; aged 73.6±2.9 years). Mortality over 15 years was ascertained from death certificates and proxy reports. Analyses used Kaplan-Meier plots, and Cox regression adjusting for demographic, lifestyle, and health factors. For baseline WAI, 673 (24%) scored 4-6, 855 (30%) scored 7-8, and 1284 (46%) scored 9. During a follow-up of 15 years, 1578 (56%) participants died. Kaplan-Meier analysis demonstrated significant association between higher WAI scores and better survival (log-rank test  $p<0.001$ ). A 1-point higher WAI score corresponded to a lower adjusted mortality risk (HR=0.88, 95% CI: 0.85–0.92). A dose-response relationship was observed, with scoring 9 on the WAI associated with lowest mortality risk (0.61[0.46–0.81]) referent to scoring 4. Findings strongly indicate that attention to functional capacity provides important information about the likelihood of longevity and argues for including capacity assessments within research and health care settings involving older adults. Future work should interrogate relationships between self-reported walking ability and other aging outcomes.

**Automating the Update of the Acute Kidney Intervention and Pharmacotherapy (AKIP) List: A Dynamic Approach to Identifying Renally Eliminated and Nephrotoxic Medications**

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The Acute Kidney Intervention and Pharmacotherapy (AKIP) list is an evidence-based standardized list designed to aggregate all renally-dosed and/or potentially nephrotoxic medications. The AKIP list sourced data from primary literature, FDA package inserts, tertiary references, and clinical decision support system rules. The list as developed is static, but there is great potential to leverage technology to facilitate automated updates as new drugs or data become available. The goal of this project was to automate the discovery of new renally dosed drugs or nephrotoxins suitable for addition to the AKIP list.

A systematic PubMed search was created by a research librarian for 2022 to 2024, corresponding to a one-year overlap with the terminal date of the existing AKIP search and two additional years. Each returned article was reviewed by at least two pharmacists and labeled according to whether it identified a potentially renally dosed or nephrotoxic drug. A spacy text classification model was fine-tuned to select included and excluded articles. The best performing model was identified from the highest recall (sensitivity). Based on the model outputs, articles that containing medications already included on the list will be excluded. This model will be implemented in a webapp that will be used on a bi-yearly basis for a pharmacist to review new literature and identify new renally dosed or nephrotoxic medications.

The recall of the best performing model in identifying an article that would produce a new renally dosed drug or nephrotoxin to be added to the AKIP list has a recall of 0.9643. The precision, F1 score, accuracy, and ROC AUC were 0.6522, 0.7781, 0.75, and 0.9002 respectively.

**Brd4 Regulates Microglial Activation and Synapse Elimination in PS19 Tauopathy Mice**

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Progressive supranuclear palsy (PSP) is a fatal 4-repeat (4R) tauopathy. We recently reported that (+)JQ1, an inhibitor of bromodomain-containing protein 4 (Brd4), rescued neurological deficits in a zebrafish model of PSP by preventing microglial synapse elimination. The current study was designed to determine whether (+)JQ1 shows similar activity in PS19 mice, which express human 4R-Tau causing early microglial activation and synapse loss. We tested the hypothesis that: *(+)JQ1-mediated prevention of Tau-dependent microglial synapse elimination is conserved in a mammalian model.*

There were three experimental groups: (i) non-transgenic siblings treated with vehicle (n=12); (ii) PS19 mice treated with vehicle (n=12); and (iii) PS19 mice treated with (+)JQ1 (n=13). (+)JQ1 at 50 mg/kg was administered by intraperitoneal injection daily from 3 to 5 months of age. Mice in all groups showed an increase in body weight throughout treatment, indicating that (+)JQ1 was well-tolerated. Animals were perfused, and brains harvested for histopathological analysis, at 5 months of age.

Compared with siblings, the abundance of Iba1-labeled microglia in the hippocampus of PS19 mice, and both the number and volume of CD68-immunoreactive puncta within microglia (a marker of microglial activation), were increased robustly. Both of these abnormalities were prevented by (+)JQ1 treatment. PS19 mice showed a decreased density of PSD95-immunoreactive synaptic puncta, and an increased frequency of intra-microglial PSD95-immunoreactive puncta, indicating microglial synaptic phagocytosis. Both of these changes were prevented by (+)JQ1 treatment. The proportion of PSD95-immunoreactive synaptic puncta labeled with complement C1q and C3 (early markers of pathological synaptic pruning in neurodegeneration) was increased in PS19 mice. These changes were prevented by (+)JQ1 treatment.

Together, these new data suggest that Brd4 is a key regulator of microglial synaptic phagocytosis in tauopathy. These findings are potentially important, since the pharmacological inhibition of Brd4 presents a possible therapeutic strategy to prevent neurological deficits in PSP.

**The Combined Roles of Vesicular Release and Dopamine Reverse Transport on the Psychostimulant Properties of Amphetamine**

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Amphetamine (AMPH), a psychostimulant commonly prescribed for treatment of neuropsychiatric disorders, also has high abuse liability. Both abuse and psychostimulant properties of AMPH are primarily associated with its ability to increase dopamine (DA) neurotransmission. It is understood that DA efflux, sometimes referred to as DA reverse transport, is critical for AMPH-induced behavioral effects. This phenomenon occurs when the DA transporter (DAT), through a channel-like intermediate, transports DA out of the cytosol into the synapse. Recent evidence showed that phosphorylation of DAT and subsequent interactions with SNARE proteins like syntaxin 1 are critical for DAT-mediated reverse transport of DA. Importantly, when AMPH is uptaken by DAT, it causes appreciable DA neuron depolarization. This depolarization can initiate vesicular DA release, offering AMPH a potential secondary mechanism for increasing DA neurotransmission beyond DA efflux. We propose a mechanism where AMPH-induced depolarization is critical for the psychostimulant properties of the drug. Using the tractable *Drosophila melanogaster* model, we probed the behavioral effects of AMPH along with genetic and pharmacological approaches to block vesicular release and DA efflux. Blocking either vesicular release via tetanus toxin (TeNT) expression in DA neurons or blocking DA efflux via a casein kinase II (CK2) inhibitor significantly reduced AMPH's psychomotor effects. Furthermore, both TeNT expression and CK2 inhibition blocked AMPH-induced DA release in an *ex vivo* *Drosophila* brain preparation. We therefore propose a two-part mechanism for AMPH-induced DA release. The initial rapid phase of DA release in response to AMPH is caused by DAT-mediated depolarization due to uptake of AMPH, which enhances vesicular DA release. Subsequent DA reuptake raises cytoplasmic DA levels, priming cells for a prolonged phase of DAT-mediated efflux which raises synaptic DA to drive the prolonged psychostimulant effects of AMPH. Elucidating molecular mechanisms of AMPH actions offers the promise of more effective treatments with less addictive potential.

## **A 3D Artery-Vascular Graft Culture System for Dynamic Matrix Remodeling Analysis**

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Tissue engineered vascular grafts (TEVGs) are promising candidates for small diameter blood vessel replacements, such as coronary bypass grafts. However, their clinical translation remains limited due to high failure rates and the extensive bottlenecks that occur during long-term animal implantation studies. These studies can require up to a year for *in vivo* evaluations, skilled microsurgical team members, and substantial investments in animal procurement, housing, and post-operative care. Collectively, these represent significant logistical and financial barriers to TEVG development. While some *in vitro* models exist for screening TEVGs in early development, they tend to be either overly simplistic (e.g., 2D cultures) or too costly and complex for widespread adoption. There is a need for scalable and biologically relevant *in vitro* systems capable of evaluating TEVGs prior to animal implantation.

We present a novel three-dimensional (3D) *ex vivo* TEVG-arterial culture model that enables rapid, biologically informative screening of graft designs. This platform integrates rat or mouse aortic explants in direct contact with TEVGs to assess integration. This system supports long-term culture in a controlled environment, and is compatible with non-destructive, label-free two-photon imaging to dynamically assess cell infiltration and extracellular matrix (ECM) remodeling over time.

Using this system, we evaluated five different TEVG biomaterial designs and tracked de novo collagen deposition and stromal cell activity along the TEVG-adventitia interface. This platform offers a new approach to streamline TEVG development by enabling systematic exploration of graft-material interactions, stromal integration, and ECM remodeling; each without immediate reliance on surgical implantation and animal monitoring. By bridging the gap between early-stage TEVG design and *in vivo* validation, this system has the potential to accelerate translational progress and reduce the bottlenecks that currently hinder clinical implementation.

## Characterizing Anti-Mycobacterial Activity of Phage-Encoded Lysterase Enzymes

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*Mycobacterium abscessus* (*Mab*) is a commonly acquired non-tuberculous mycobacterium (NTM) respiratory pathogen in cystic fibrosis patients. Due to its antibiotic resistance and clinical challenges, alternative approaches to manage *Mab* infections are urgently needed. The therapeutic use of bacteriophages is currently being explored but there are key challenges including narrow phage specificities among *Mab* clinical isolates, challenges in phage purification, and the potential for phage resistance. Phage-encoded endolysins (such as lysin A), that hydrolyze peptidoglycan have been developed as exogenously administered antimicrobials (enzybiotics) for some Gram-positive bacteria, without development of resistance. However, such endolysins are of limited use for mycobacteria as the mycolic acid-rich outer membrane blocks the access to the peptidoglycan. Mycobacteriophages code for a second lysin protein (Lysterase, or lysin B), which is a serine-esterase that cleaves mycolylarabinogalactan–peptidoglycan (mAGP) to complete cell lysis during lytic growth. In this study, six diverse recombinant lysterase enzymes from different phages were purified and their enzymatic activity was tested on *M. smegmatis* mc<sup>2</sup>155. All six enzymes were shown to have activity against *M. smegmatis* and able to kill cells within one hour of treatment. We also tested these enzymes for activities against a diverse panel of 24 *Mab* clinical strains, revealing modest variations in susceptibilities to the different enzymes. Further studies will determine if a cocktail of several enzymes will act broadly on *Mab* clinical isolates, have synergistic activities with antibiotics, and have potential therapeutic utility.

**Engagement Re-Examined: A More Sophisticated Empirical Model of Student Learning and Success**

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This study breaks new ground by presenting a new, more sophisticated model of learning engagement that goes beyond the current state of the art embodied in the widely used Affective-Behavioral-Cognitive model. The work synthesizes and builds upon neglected lines of research in the structure of affective engagement. It also integrates entirely novel theoretical considerations in the form of activity spaces—the different learning spaces associated with different course-defined activities, which in turn afford different forms of cognitive and behavioral engagement. To empirically test these theoretical notions, data from a sample of 655 students across multiple sections of a genetics course and from 772 students across sections of a biology course were analyzed using structural equation modeling that linked different elements of learning engagement to academic performance outcomes like exam grades, quizzes, attendance, and assignment performance, and to the development of attitudes like self-efficacy, fascination, identity, and growth mindset. As predicted, affective learning engagement separated into positive and negative components, and cognitive and behavioral engagement further separated by activity spaces. Crucially, each of these engagement factors contribute to different outcomes. They are not interchangeable, and cannot be conflated with one another, but have distinct functions. The more sophisticated engagement model presented here reveals deeper patterns of student engagement for the research community and can be used by faculty members to improve student engagement, performance, and psychosocial development in their courses. They also have implications for promoting equitable engagement, learning, and performance in the classroom.

**Extracellular Vesicles (EVs) and Silk Fibroin (SF) Can Deliver and Enhance the Therapeutic Efficacy of Multiple Cell Populations**

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Cell therapy involves the substitution of damaged or dead cells with healthy replacements. Cell delivery has proved challenging, as cells may be lost upon injection or unable to integrate into the disease milieu. Silk fibroin (SF) is an FDA-approved, biodegradable material with low immunogenicity. Extracellular vesicles (EVs) are novel therapeutics that promote repair and regulate inflammation. They can be soak-loaded into SF before use to further enhance its regenerative potential. Enrichment of SF with EVs represents a novel bioengineering platform for the delivery of cell therapeutics. This study investigated the use of SF+EVs as a delivery platform for multiple cell types for regenerative medicine.

EVs were purchased/characterized from RoosterBio, Inc. Mature human endothelial cells (ECs), fibroblasts (HDFs) and smooth muscle cells (SMCs) were treated with EVs to assess effects on viability/proliferation using AlamarBlue. Cells were grown on either tissue culture plastic (TCP) or silk films. Proliferation was assessed at either 24/72h (films) or 48/96h (TCP) post-treatment. Growth media was used as a positive control. Adhesion to films was assessed by seeding cells on EV pre-loaded films. Cells were fixed/stained for DAPI and phalloidin to assess the number of cells adhered, and cytoplasmic area respectively. Proliferation was analyzed using two-way ANOVA and adhesion was analyzed using one-way ANOVA. All data is presented as fold change to corresponding vehicle control.

EVs were internalized by all cell types. EVs enhanced the proliferative potential of all cells on TCP after 96h ( $P < 0.0005$ ). On films, EVs enhanced the proliferative potential of all cell types after 24 and 72h post-seeding ( $P < 0.0001$ ). For adhesion, all cells showed increased cell number adherence and cytoplasmic spreading ( $P < 0.0005$ ).

SF can be enhanced with EVs to promote the adhesion and proliferation of multiple cell types. Future work will investigate the migratory potential and gene expression of these populations on this platform.

## **Loss of PPAR $\alpha$ in Murine Hearts Abolishes Sex-Specific Cardioprotection in Females Cardiac Physiology**

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The heart has high metabolic demand, relying primarily on fatty acids (FA) for energy production. Doxorubicin (DOX), a chemotherapeutic agent is well known to cause cardiotoxicity, oxidative stress and mitochondrial dysfunction specifically FA modulation. PPAR $\alpha$  (Peroxisome Proliferator Activated Receptor alpha) is essential for cardiac FA utilization including FA uptake and mitochondrial  $\beta$ -oxidation. Additionally, expression of PPAR $\alpha$  is sex-specific with females having higher expression compared to males, leading to better cardiovascular outcomes in females even with exposure to chemotherapy like DOX. Thus, we tested the hypothesis that loss of PPAR $\alpha$  would eliminate sex-specific cardioprotection in females during DOX treatment. To test this, we used quadruple-parametric optical mapping to simultaneously image transmembrane potential, calcium handling, NADH, and FAD intensity in male and female murine hearts. Groups were 1. Wild type (WT) (n=5m/f) 2. WT+DOX (n=6m/f) 3. PPAR $\alpha$  KO (n=3m/f), and 4. PPAR $\alpha$  KO+DOX (n=2-3m/f). DOX was intraperitoneally injected 5-days before optical mapping (20mg/kg). In WT+DOX males, transverse conduction-velocity was decreased ( $0.26 \pm 0.04$ ,  $0.30 \pm 0.06$ ), and calcium rise-time was increased ( $9.45 \pm 1.91$ ,  $6.93 \pm 0.83$ ) compared to WT males, respectively, ( $p < 0.05$ ). WT+DOX females did not have any significant differences compared to control females. Male PPAR $\alpha$  KO+DOX hearts had a significant increase in voltage rise-time ( $5.77 \pm 0.22$ ,  $4.81 \pm 0.12$ ) and a decrease in longitudinal conduction-velocity ( $0.40 \pm 0.07$ ,  $0.45 \pm 0.05$ ) compared to male PPAR $\alpha$  KO, respectively, ( $p < 0.05$ ). Female PPAR $\alpha$  KO+DOX hearts had a significant increase in calcium rise-time ( $8.97 \pm 0.40$ ,  $5.76 \pm 0.026$ ) and calcium transient-duration ( $53.79 \pm 8.49$ ,  $42.63 \pm 7.42$ ) compared to female PPAR $\alpha$  KO, respectively, ( $p < 0.05$ ). In WT mice DOX treatment altered cardiac electrophysiology in males but not females. However, when PPAR $\alpha$  was deleted, sex-specific differences were abolished with electrical excitation parameters being altered in males and calcium handling parameters being altered in females. This preliminary data adds to the long-term goal of developing sex-specific cardioprotective strategies for patients undergoing cancer chemotherapy.

## **Different Patterns of Propagation of Tau Tangle Pathology in Typical Alzheimer's Disease Determine Clinical Sub-Phenotypes**

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One of the hallmarks of Alzheimer's disease (AD) is the progressive spread of tau pathology. However, heterogeneity in the spatial patterns of tau deposition at the individual level may contribute to distinct clinical presentations.

We investigated 106 tau PET positive individuals (16% cognitively unimpaired, 84% amnesic cognitively impaired) from the HEAD study using head-to-head Flortaucipir and MK6240 tau PET. For each tracer, we extracted the SUVR values from seven major brain networks: Limbic, Visual (Vis), Default Mode (Default), Dorsal Attention (DorsAttn), Frontoparietal, Somatomotor (SomMot) and Salience Ventral Attention (SalVentAttn) and the Entorhinal cortex. Pathways were identified by ranking regions from highest to lowest SUVR, with the region displaying the highest SUVR value being defined as the peak region. Individuals in late stages of tau deposition displaying distinct pathways were compared. Image averages and voxel-wise group comparisons using tau negative individuals as the reference were generated for each pathway and tau PET tracer. Cognitive differences were assessed using ANOVA for MoCA score and domain-specific composite scores for Memory, Attention and Visuospatial skills.

In all individuals and tracers, tau in the Entorhinal cortex progressed to the Limbic network, supporting a common starting point for tau propagation. Beyond these initial stages, tau propagation followed three main distinct pathways following either the Vis, Default or DorsAttn networks. Voxel-wise comparison between individuals across these three pathways captured clear and similar differences in tau PET uptake as measured with Flortaucipir and MK6240. Although MoCA and Memory scores were similar across groups, individuals in the Vis network showed significantly lower Attention scores than those in the Default and DorsAttn networks, and lower Visuospatial scores than the Default group.

Our results indicate a model in which, although tau progresses hierarchically, its deposition peaks mainly follow specific networks, which drive tau deposition and, consequently, the clinical manifestations of AD.

**Phosphorylated Amelogenin Controls Dental Enamel Growth and Organization in Secretory Stage of Amelogenesis**

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Enamel mineralization is a natural example of biomineralization process. Amelogenin (AMELX) is the predominant enamel matrix protein involved in enamel formation. Amelogenesis imperfecta is a genetically disordered disease associated with dental enamel leads to abnormal growth of enamel. This research focused on how amelogenin phosphorylation regulates the growth, phase transformation and organization of developing tooth enamel mineral in wild-type (WT) and mutant novel knock-in (KI) mouse model. The appositional growth and organization of enamel crystals during secretory stage of amelogenesis were revealed from this study. The thick (0.35  $\mu$ m thickness) enamel sections were prepared using ultramicrotome and stained with toluidine blue stain followed by light microscope visualization. For transmission electron microscopic (TEM) studies, ultra-thin sections (70-100 nm thickness) were mounted immediately on Cu grids followed by visualization. The TEM studies revealed that WT enamel showed an interwoven decussating enamel rod pattern during mid-secretory stage of amelogenesis. However, KI mice had a rod less enamel structure. The selected area electron diffraction (SAED) pattern also elucidated about the different amorphous and crystalline phase transformations of these enamel mineral formation. In summary, phosphorylated AMELX stabilizes and regulates mineral phase transformation during crystal growth followed by enamel biomineralization.

## **Microbiota-Derived Metabolites Control Type 2 Immune-Related Ovarian Fibrosis**

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Pathological fibrosis in the ovary can lead to premature ovarian aging, which increases infertility and ovarian cancer risk. Recent evidence has demonstrated the involvement of the gut microbiota and its metabolites in the fibrosis of various tissues; however, its role in ovarian fibrosis remains unknown. We hypothesize that the microbiota regulates ovarian fibrosis by modulating ovarian immune pathways. We found that germ-free (GF) mice lacking microbiota exhibited extensive ovarian fibrosis at 10 weeks old, which is substantially earlier than in conventionally housed mice. Using bulk RNA sequencing, we identified *Il33*, a cytokine that regulates type 2 immune responses such as wound healing and fibrosis, was upregulated in GF ovaries. Colonizing GF mice with microbiota or directly supplementing with microbial-derived metabolites rescued the fibrosis and normalized IL-33 expression in GF ovaries. To understand how *Il33* upregulation impacts the ovarian immune compartment, we performed single-cell RNA sequencing of ovarian CD45+ cells. This revealed a significant type 2 innate lymphoid cells (ILC2s) expansion in GF ovaries. These ovarian ILC2s expressed the *Il33* receptor ST2 and type 2 immune response cytokines *Il5*, *Il4*, and *Il13*. Furthermore, alternatively activated macrophages, which are activated by these type 2 cytokines and play a crucial role in tissue repair and fibrosis, were more abundant in GF ovaries. Our results demonstrate that microbially derived metabolites are critical regulators of gene networks involved in immunity, collagen deposition, and fibrosis, revealing a novel microbiota-immune crosstalk essential for ovarian homeostasis. This may lead to new immunological targets to treat female reproductive pathologies.

## **Exploring Anatomical Race Differences in Mammographic Images Using AI: Preliminary Results**

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### **Purpose:**

Exploring race-wise differences is crucial for overcoming model bias and recognizing anatomical differences linked to disease risks. Identifying features used by AI for race prediction reveals important traits and distinctions among races.

The purpose of this study was to conduct multiple ablation experiments to identify anatomical breast features that differentiate Black and Asian races.

### **Materials and Methods:**

We used mammograms from Asian and Black women taken from the Emory EMBED dataset. We matched the Asian and Black cohorts by age and BI-RADS breast density for a total of 1039 Asian and 1016 Black women. Also, we had an independent test set consisting of Asian/Black women from our institution. Input images were given to a ViT (transformer-based) model. We used a segmentation model to segment breast area and fibro-glandular tissue. We produced three different ViT models trained and tested on the original images, binary breast area images (breast shape and size), and binary fibro-glandular tissue images (amount and distribution of fibroglandular tissue).

### **Results:**

Our ViT model trained on original images achieved an AUC of 0.90/0.82 (EMBED/Independent test set). With binary fibro-glandular tissue images, the AUC was 0.76/0.73, highlighting density distribution differences between Asian and Black races. For the breast area, the AUC was 0.81/0.80. Using the breast size (total number of pixels in the breast area) as a feature for ROC analysis, the resulting AUC was 0.72/0.73, indicating its importance for distinguishing races, but breast shape also had added discriminatory power. For Black women, the AI model focused on the pectoral muscle/axillary lymph node area. There was also some discriminatory power in the amount and distribution of fibroglandular tissue.

### **Clinical Relevance/Application:**

Identifying race-specific anatomical differences and variations in breast density distribution may help develop less racially biased AI algorithms and establish a link between different races and disease risk.

**Remote Monitoring and Parent Engagement for Type 1 Diabetes (T1D) Care in Schools**

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**Objectives:** Though school nurses (SN) are the primary school health care providers for children with T1D in the United States, there are gaps in their diabetes device training. We aimed to understand how parents engage with SN in daily decisions for diabetes management in school in the setting of devices, particularly continuous glucose monitors (CGM).

**Methods:** We conducted semi-structured interviews with parents of school-age children with T1D about their experiences and attitudes toward CGM use in school. Interviews were analyzed by two reviewers using consensus coding.

**Results:** Parents (n=24) were mainly female (83%) and non-Hispanic white (75%); their children (n=25) were between 6-15 years with a T1D duration of 1-8 years. Most used T1D devices (92% used CGM, 44% insulin pumps, 24% automated insulin delivery systems). Three themes emerged. 1) Most parents engaged in remote monitoring of their child's CGM. For some, this enhanced frequent communication with the SN to facilitate needed interventions. Others elected to bypass the SN and communicate directly with their child for hypo- and hyperglycemia management. Though this practice excluded the SN, parents saw this as less disruptive to class time. 2) Opinions were mixed on whether SN should remotely monitor the CGM. Some parents relied on SN remote monitoring for active co-management, which increased parent reassurance that their child was safe. Others considered it to be potentially intrusive, unnecessary, or burdensome for the SN. 3) Parents were polarized on whether the SN is an extension of their child's medical team. Parents with less favorable school health care experiences or concerns about SN knowledge of devices may want more guidance from the diabetes medical team to SN.

**Conclusions:** Parent and SN interactions are influenced by use of remote monitoring. Best practices for shared remote monitoring with SN may facilitate efficient bidirectional communication and minimize classroom disruptions.

## White Matter Changes and Oligodendrocyte Dysfunction in Alzheimer's Disease

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**Background:** Demyelination is a key feature of Alzheimer's disease (AD) and varies across Braak stages, but the role and distribution of oligodendrocytes remain unclear. While preclinical studies suggest that demyelination follows oligodendrocyte-tau pathology, this is not well-characterized in AD. Here, we examined myelin, oligodendrocyte distribution, and tau pathology across Braak stages in an AD brain.

**Methods:** Brain tissue from a male AD patient, deceased 8 years post-diagnosis, was analyzed. Neuropathological assessment showed moderate cerebral amyloid angiopathy with no signs of Lewy body disease, cortical microinfarcts, or other neurodegenerative conditions. Formalin-fixed, paraffin-embedded sections were examined in regions corresponding to Braak stages: entorhinal cortex and hippocampus (I–II), anterior cingulate cortex (III–IV), and inferior parietal cortex (V–VI). Luxol Fast Blue (LFB) staining assessed myelin content (signal intensity/ $\mu\text{m}^2$  and % demyelination) using ImageJ-FIJI (v2.9). Immunofluorescence for MBP, CC1-APC, and pTau217 was used to identify and quantify mature and pathological oligodendrocytes.

**Results:** White matter demyelination was more severe in early Braak stages (I–II), with increased myelin preservation, yet with high percentage of demyelinated areas in Braak III–IV and V–VI, as shown by LFB and MBP staining (Figure 1K–M). Quantification confirmed reduced myelin content and greater demyelinated area in early stages. Oligodendrocyte analysis revealed a progressive increase in CC1<sup>+</sup>/MBP<sup>−</sup> and CC1<sup>+</sup>/pTau217<sup>+</sup>/MBP<sup>−</sup> cells across Braak stages (Figure 2D, 2E), indicating an accumulation of tau-positive, non-functional oligodendrocytes in later disease.

**Discussion:** Our study indicates that early Braak stages (I–II) in AD exhibit more severe demyelination than later stages, with lower LFB levels and absence of mature myelinating oligodendrocytes. In contrast, later stages show more surviving oligodendrocytes, though many display tau pathology, which may impair recovery. This preliminary analysis reveals a distinct tau-related pattern in AD, warranting further investigation with additional cases.

## **VP1 and VP3 Mutations in EV-D68: Implications for Neurotropism in Human Spinal Cord Organoids**

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EV-D68 is a non-polio RNA virus that causes respiratory illness in children. However, in 2014, EV-D68 infections were linked to a polio-like disease that results in paralysis in once healthy children known as acute flaccid myelitis (AFM). The mechanism by which paralysis occurs is currently unknown. It has recently been demonstrated that EV-D68 containing point mutations in viral structural proteins VP1 and VP3 resulted in decreased neurovirulence and paralysis in different neonatal mouse models. However, the phenotypes of these mutations have not been tested in a human central nervous system (CNS) model. Our lab developed a human spinal cord organoid (hSCO) model from induced pluripotent stem cells (iPSC) to model EV-D68 infection. hSCOs are multicellular and have the capacity to be infected by EV-D68 strains that have circulated since at least 2014. We hypothesize that mutations in VP1 and VP3 will both result in decreased infection of hSCO, corresponding to decreased neurotropism in animal models. To investigate the effect of previously published mutants, we recreated a single point mutation in VP3 (I88V), and two different four-amino acid point mutations in VP1 (L11I/N2D/T98A/E283K and L1P/V148A/K282R/G283E). We then infected hSCOs with the infectious clones to determine the effect on neurotropism via immunofluorescence and titer. Initial results suggest that the mutation in VP3, but not the mutations in VP1, impact neurotropism in this human CNS model, in contrast to the results in prior mouse systems. Results from this work will identify genetic drivers of EV-D68 neurotropism in humans.

**Educational Impact of a Board Game on Changes in Understanding, Empathy, and Awareness of Community Mobility Among Mobility Device Users: A Study with Occupational Therapy Trainees**

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The lack of standardized educational materials for wheelchair skill training in rehabilitation education curricula has been reported. HERL-Town is an educational board game consisting of 58 validated scenarios that address transportation barrier situations, with four strategies provided for each situation to assist mobility device users (MDUs). While the game's educational benefits have been confirmed in MDUs and their travel companions, its impact on rehabilitation trainees has not been explored. This study aimed to evaluate the educational impact of HERL-Town on rehabilitation students.

In this pre-post design study, 11 groups of occupational therapy (OT) students played the game once. To earn game money and progress, participants reviewed game scenarios and discussed the best strategy with their classmates. Before advancing to the next scenario, game facilitator provided players feedback on how their choice aligned with strategies used by MDUs. During the 60-minute session, students reviewed 20-25 scenarios.

Understanding was assessed with 20 questions (10/test) based on scenarios not used in the game. Empathy and awareness were measured through self-ratings on a 10-point Likert scale. Paired t-tests were conducted to examine differences between pre- and post-test scores using SPSS 29.

A convenience sample of 90 students (ages 18-44, 88.9% female) from 1st to 3rd year of two accredited OT programs located in Pittsburgh participated in the study. Statistically significant improvements were observed in understanding (Pre:  $6.15 \pm 1.68$ , Post:  $6.82 \pm 1.41$ ,  $t(89) = -0.66$ ,  $p < .01$ ), empathy (Pre:  $8.48 \pm 1.57$ , Post:  $9.48 \pm 0.83$ ,  $t(89) = -7.25$ ,  $p < .001$ ), and awareness (Pre:  $5.36 \pm 1.49$ , Post:  $7.97 \pm 1.25$ ,  $t(89) = -15.99$ ,  $p < .001$ ).

Engaging in this one-hour board game on transportation barriers faced by MDUs could help fill gaps in the OT curriculum, providing an enjoyable and educational supplement to wheelchair skill training, particularly in community mobility, for OT trainees.

**Issue Ownership, Electoral Accountability, and Politicians' Social Media Posts: Evidence from the George Floyd Protests in the United States**

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Under what conditions do politicians express views on media channels? How do they phrase their positions on social media? This study explores issue ownership and electoral accountability in shaping politicians' behavior, focusing on discourse surrounding the 2020 George Floyd protests in the United States. Leveraging an original, computationally-gathered dataset of social media posts from members of Congress, the study examines patterns of political communication and partisan messaging.

Findings reveal strong partisan divides: a multi-level analysis indicates Democrats were significantly more likely to discuss Floyd-related events than Republicans, consistent with the Democratic Party's ownership of social justice issues. Furthermore, non-white politicians and representatives from Democratic-leaning and racially diverse constituencies exhibited higher engagement on the topic, underscoring the influence of electoral incentives.

Topic modeling demonstrates stark contrasts in narrative framing between the two parties. Democrats predominantly emphasized systemic racism and policy accountability, framing the protests as part of a broader racial justice movement. Republicans, on the other hand, largely portrayed the events as violent riots, reinforcing law and order narratives. This divergence highlights how officials strategically craft their messaging to align with partisan priorities and electoral considerations.

The study suggests that digital platforms, while facilitating direct political communication, also contribute to ideological echo chambers, exacerbating divisions. By analyzing politicians' digital messaging, this research offers critical insights into the intersection of social media, political behavior, and party polarization in contemporary democracies.

**Use of a Biomimetic Polymer Adhesive Improves Tibia Bone Fracture Healing in Rats**

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**Introduction:** Bone healing occurs through primary or secondary osteogenesis. Fractured bones are capable of repair and regeneration, however, approximately 10% of fractures result in delayed union, malunion, or nonunion. In this study, we developed an amino acid - glycerol-based polymer adhesive to improve bone fracture healing.

**Methods:** The polymer adhesive exhibits non-toxic, biodegradable, biomimetic, and bio-adhesive properties. A foam filling the gap between fracture fragments serves as a scaffold for cell migration – osteoconductive factor and amino acids serve as osteoinductive factors attracting cell migration. The partial tibia fracture model was made in rats followed by wound suturing in the control group or polymer injection in the fracture site and wound suturing in the experimental group. The fracture healing was assessed 8 weeks post-surgery with Micro-CT and histological methods.

**Results:** Micro-CT shows the formation of sponge bone with a deformed cortical layer in control group – early remodeling phase. Whereas in the polymer group, we observed intensive thick and smooth cortical layer formation – late remodeling phase. Increased bone tissue volume in the polymer group – 36.27 mm<sup>3</sup> compared to 26.10 mm<sup>3</sup> in the control group. SOFG-stained histological slides indicated the formation of disorganized sponge-like bone tissue with excessive inclusions of fibrovascular tissue in the control group. While in the polymer group, we observed well-organized sponge and cortical bone layer formation with proteoglycan expression in the periosteum area, indicating a late remodeling phase.

**Discussion:** We demonstrated that our polymer stimulates bone tissue formation at the fracture healing site compared to the control group and accelerates the callus remodeling phase.

## **Transcriptomic Analysis of Microglial Gene Expression Changes in Tau Zebrafish**

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Progressive supranuclear palsy (PSP) is an incurable neurodegenerative disease characterized by neurodegeneration, neuroinflammation, and 4-repeat (0N/4R) Tau accumulation in the central nervous system (CNS). Median age of onset is 67 and median survival is 8 years from initial symptoms. There is an urgent need for effective treatments.

To analyze the molecular mechanisms underlying 4-repeat tauopathy, we generated transgenic zebrafish that express wildtype human 0N/4R-Tau in CNS neurons. Tau zebrafish replicate multiple features of PSP including impaired survival, motor and oculomotor deficits, neurodegeneration, neuroinflammation, and tauopathy. A small molecule screen using this model identified a Brd4 inhibitor, (+)JQ1, that rescued hypokinesia, survival, microgliosis, and synapse loss. To elucidate the molecular mechanisms underlying these observations, we analyzed Tau zebrafish by single-cell RNA sequencing.

The study design included six independent biological replicates, each with three experimental groups (siblings, untreated- and (+)JQ1-treated Tau larvae). In each replicate, brains from 100 larvae per group were dissected, pooled, and dissociated, so that 1800 brains were sequenced in total. Transcriptional profiles identified multiple CNS cell types including neurons, microglia, astrocytes and oligodendrocytes. Compared with siblings, microglial abundance in Tau zebrafish was increased by  $\approx 75\%$ , and rescued by (+)JQ1, consistent with our previous histological findings. Analysis of differentially expressed microglial genes is ongoing. Preliminary results suggest that genes associated with microglial dynamics and activation are robustly up-regulated in response to Tau.

Overall, our data are compatible with a model in which neuronal accumulation of human 0N/4R-Tau induces dramatic changes in microglial transcription. We previously showed that Brd4-dependent microglial synapse elimination mediates neurological phenotypes in this model. Our current working hypothesis is that Brd4 regulates expression of the microglial genes responsible for microgliosis and synaptic phagocytosis in response to tauopathy. Our ongoing work aims to identify targets for therapeutic intervention, with strong translational potential for PSP and other tauopathies.

**Hyaluronan Promotes Ovarian Longevity: Lessons from the Un-Aging Naked Mole Rat**

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The ovary is the first organ to experience the effects of aging, with reproductive decline occurring midway through a woman's life. In contrast, the naked mole-rat (NMR) is a unique, long-lived rodent which maintains reproductive capacity throughout its >40-year lifespan. The NMR uniquely produces very high molecular weight hyaluronan (vHMW-HA), known for its anti-inflammatory and anti-fibrotic properties, which contribute to its resistance to cancer and other age-related pathology. HA is a ubiquitous and polydisperse extracellular glycosaminoglycan which regulates tissue homeostasis. High levels of HA are associated with stem cell proliferation and protection from differentiation during early embryogenesis. In our previous work in the NMR ovary, we have identified cells positive for pluripotency transcription factors and primordial germ cell markers (VASA+OCT4+, VASA+SOX2+, and VASA+BLIMP1+) to be enriched at early postnatal ages (P1-P8), followed by reductions that led to the lowest level at P90. This indicates that in the NMR ovary, primordial germ cells are still present at postnatal day 90. We hypothesize that vHMW-HA regulates the establishment of the ovarian reserve and maintains reproductive longevity in NMRs. To determine if vHMW-HA preserves germ cell pluripotency, we created knock-in mouse models which express NMR vHMW-HA in germ cells or somatic cells of the ovary. Preliminary analysis shows SOX2+ cells at P1, P5, and P10. These results are expected to provide a strong, evidence-based proof of principle for the further development of drugs that protect HMW-HA levels in the ovary, ultimately providing new opportunities for the development of novel therapies for ovarian rejuvenation.

**Understanding the Relation Between SES and Math Skills: Exploring the Role of Stress**

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Socioeconomic disparities in STEM learning have been extensively studied, yet the mechanisms linking these disparities to essential skills for STEM learning are not well understood. The Family Stress Model suggests that economic hardship results in financial pressure and increased family stress ultimately impacting a child's development. Most research on the family stress model has concentrated on socioemotional outcomes and psychopathology, with less focus on key cognitive skills related to academic outcomes. Spatial skills are early predictors of STEM success, yet the impact of family income and stress on these skills is underexplored. The present study draws data from the Parents Promoting Early Learning. Parents' experiences with financial hardship (e.g., borrowing money, difficulty paying bills, cut-off utilities) were averaged to create a composite score. Children's mental transformation skills were assessed using the mental transformation task and spatial relation skills were assessed using the geometric sensitivity task. Data on income and financial hardship were collected at age 4, and spatial skills were assessed at ages 4 and 5. Age, gender, and the spatial outcomes at age 4 were included as covariates in analyses. No significant associations of income on changes in spatial skills were detected. However, when financial hardship was added to the model significant indirect effects of family income and spatial relation skills through financial hardship were evident (bootstrapped unstandardized indirect effect = .003, 95% CI [.0008, .01],  $p = .002$ ). An indirect effect was not detected for family income and mental transformation skills. These findings highlight the importance of financial hardship by demonstrating its association on spatial skills. Results also demonstrate differences by spatial thinking type showing an impact on only mental transformation skills. Understanding how family income influences these skills is vital for developing interventions aimed at reducing opportunity gaps in STEM.

## **HPV+ Carrying OSCC-EVs Transfer Viral DNA Into Recipient Cells**

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**Objectives:** To identify HPV DNA as a component of small extra cellular vesicles (EVs) isolated from supernatants of HPV(+) oral squamous cell carcinoma (OSCC) cells. To demonstrate that EVs produced by HPV(+)OSCC cells transfer viral DNA to HPV<sup>-</sup> cells in vitro.

**Methods:** OSCC47 cell line supernatants were centrifuged at 10000 rpm for 30 min, followed by filtration on 0.22µm filters. Supernatants were concentrated (vivaspin concentrators, MWCO 100000), and 1 ml was then loaded on size exclusion chromatography (SEC) columns to separate EVs. Numbers of isolated HPV(+)OSCC47-EVs measured by nanoparticles tracking (NTA) and protein concentration assay BCA, were cocultured with HPV<sup>-</sup> PCI-13 (tumor cells) and HaCat (non tumor cells) for 6 and 12h. RT-PCR was used to confirm HPV DNA in isolated EVs and to detect viral DNA in HPV<sup>-</sup> recipient cells after coincubation. HPV+ EVs were labeled with PKH26 stain and coincubated with PCI-13 and HaCat for 30 min, 3h and 6h. Uptake of stained HPV (+) EVs by HPV<sup>-</sup> recipient cells was quantified by flow cytometry.

**Results:** Preliminary PCR results showed an average of 30306 copies of HPV DNA in PCI-13 cells after 6h of coincubation. Flow cytometry analysis showed PKH26-HPV(+)EVs uptake by PCI-13 and HaCat cells after 30min, 3 and 6h.

**Conclusion:** Our preliminary results demonstrated that HPV DNA is a component of sEV produced by HPV(+) cells. HPV+ OSCC-EVs can transfer viral DNA in HPV<sup>-</sup> recipient cells after coincubation.

**Rap1–AC9 Signaling as a Novel Mechanism for Trypanosoma cruzi Invasion**

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The protozoan Trypanosoma cruzi is responsible for Chagas disease, a condition affecting eight million people worldwide. Untreated infections can lead to chronic heart disease with significant mortality, posing a growing threat in non-endemic regions, including the United States. Current treatments—nifurtimox and benznidazole—are decades old, poorly tolerated, and minimally effective in chronic cases. A deeper understanding of the host-pathogen interactions driving T. cruzi invasion is urgently needed to develop novel therapies. As an obligate intracellular parasite, T. cruzi relies on host signaling pathways to invade cells, manipulating cAMP and Ca<sup>2+</sup> signaling to trigger lysosomal exocytosis and parasitophorous vacuole formation. We previously proposed a three-wave cAMP model and recently demonstrated that the second, internalization-dependent cAMP wave is regulated by a GαS-independent Rap1–AC9 module. Here, we investigate the role of Rap1–AC9 signaling in T. cruzi invasion. Using FRET-based assays in HC-1 cells, we demonstrated that AC9, but not AC2, is a Rap1-GTP-sensitive cyclase. In HEK Gαs-KO cells, Rap1 activated AC9 but not AC2. Using confocal microscopy-based assays, we found that Rat-1 cells transfected with shRNA targeting AC9 (sh-AC9) showed a 75% reduction in T. cruzi infection, whereas AC9 expression rescued the low infectivity in HC-1 cells, an effect not observed with AC2. Rap1b and AC9 were recruited during early parasite-host contact in Rat-1 cells, contributing to infection establishment. Moreover, cells transfected with a Rap1b mutant (D69A), which disrupts Rap1–AC9 interaction, exhibited reduced infection, acting as a dominant negative, while the constitutively active Rap1b mutant (G12V) significantly increased basal infection. These findings reveal that T. cruzi exploits a Rap1–AC9 signaling axis to establish infection, bypassing the canonical GαS-cAMP pathway. This previously unrecognized mechanism of host cAMP regulation provides a potential target for therapeutic intervention.

## Improved Synthesis and Radiolabeling of a Selective PET Radiotracer for Imaging Cerebral Amyloid Angiopathy (CAA)

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Cerebral amyloid angiopathy (CAA) results from the deposition of amyloid-beta (A $\beta$ ) peptides in the cerebral vasculature and is distinct from parenchymal A $\beta$  plaques in Alzheimer's disease (AD). Positron emission tomography (PET) imaging of A $\beta$  aggregates with current PET tracers cannot distinguish between plaques and CAA. Thus, a PET radiotracer that can selectively image CAA would provide a tool for studying CAA in the living human brain. We have previously reported a candidate CAA ligand (*Nucl. Med. Biol.* **2021**,92,85) and evaluated it with fluorescence and ex-vivo autoradiography studies. Here we report an improved synthesis of the compound, as well as synthesis of the radiolabeling precursor and preliminary radiolabeling with carbon-11 (C-11).

Our original multi-step synthesis required two separate Horner-Wadsworth-Emmons olefination reactions to create two separate *trans*-alkene bonds. Additionally, phenol and carboxylic acid functionalities required protecting groups throughout the entire synthetic sequence. Our improved procedure utilizes separate Heck coupling reactions to create the two *trans*-alkene bonds and allows the benzoic acid fragment to remain unprotected. This method was then modified to enable synthesis of the radiolabeling precursor with the central phenol group unprotected, the second phenol group MOM-protected, and the carboxylic acid MEM-protected. C-11 radiolabeling was performed by *O*-methylation (O<sup>11</sup>CH<sub>3</sub>) of the unprotected phenol, followed by dual deprotection with H<sub>2</sub>SO<sub>4</sub>.

In summary, we have developed an improved synthesis of the target compound and the radiolabeling precursor. Key improvements were Heck coupling reactions that allow the benzoic acid group of the target compound to remain unprotected, and that allow the central phenol of the radiolabeling precursor to remain unprotected.

## Investigation of LARP1 as a Novel Substrate of MNK1

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The MAP kinase pathway (MAPK) activates the MAPK-interacting serine/threonine-protein kinases 1 and 2 (MNK1/2), which play crucial roles in disease progression across numerous malignancies. While the role of MNK1/2 in oncogenic mRNA translation through the phosphorylation of eIF4E at serine 209 is well-established, our understanding of the breadth of their activity remains limited. Identifying additional substrates has been challenging, prompting us to employ a proteomics approach to uncover new interactors and substrates. Notably, we identified the RNA-binding protein LARP1 as a candidate interactor of MNK1. Similar to eIF4E, LARP1 binds to mRNA at the 5'cap; moreover, comparable to eIF4E, the activity of LARP1 is dependent on mTOR. Investigation of this novel interaction revealed that LARP1 specifically interacts with MNK1 but not MNK2. Moreover we discovered that MNK1 interacts with and phosphorylates the La-Module of LARP1. Mechanistically, we demonstrate that a phosphomimetic LARP1 mutant exhibited reduced RNA binding compared to wild-type or phospho-deficient forms. Overall, our findings expand our understanding of MNK1/2 signaling beyond eIF4E, and highlight LARP1 as a previously unrecognized effector of MNK1 activity to potentially regulate 5' TOP mRNA translation.

**Cumulative Effects of Chronic and Acute Stress and Discrimination on Momentary Alcohol Craving Across Black and White Young Adults**

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Naturalistic studies have shown that general stressors and interpersonal discrimination are related to acute alcohol craving. Additionally, chronic stress is related to retrospective reports of alcohol craving, suggesting there may be a synergistic effect of chronic and acute experiences. This study tested how chronic and acute forms of stress and discrimination relate to alcohol craving for Black and White young adults. Participants included 76 Black and 105 White individuals (N=181; ages 21-30; 66.9% assigned female at birth) who consumed alcohol at least weekly. As part of a larger study, participants completed a baseline survey and Ecological Momentary Assessment (EMA) surveys 4x/day for 17 days. At baseline, chronic stress was measured using the Perceived Stress Scale, State-Trait Anxiety Inventory (STAI), and Difficult Life Circumstance Scale, while discrimination was assessed using the Everyday Discrimination Scale (EDS), Major Experiences of Discrimination, and Racial and Ethnic Microaggressions Scale. During EMA, craving was measured with two items on craving intensity, while acute stress and discrimination were measured using the STAI and EDS. Initial Confirmatory Factor Analysis (CFA) indicated that some chronic stress and discrimination indicators functioned differently for Black and White participants. This partial invariance model had acceptable fit (CFI = 0.85, TLI = 0.82, RMSEA = 0.06) and was used for subsequent multilevel analyses. In the multilevel model, chronic stress but not discrimination predicted between-person craving for Black participants ( $\beta=0.340$ ,  $p=0.043$ ), but not for White participants. Within-person interactions between chronic discrimination and acute stress did not significantly predict craving. Measurement differences suggest stress experiences differ between Black and White participants, with chronic stress having a stronger influence on craving for Black participants. These findings demonstrate the importance of examining general stressors and discrimination experiences across timescales to understand influences of alcohol-related inequities.

**Women's Midlife Systolic and Diastolic Blood Pressure Multi-trajectories and Future Cognitive Decline: The Study of Women's Health Across the Nation (SWAN)**

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**Objective:** Hypertension is more predictive of dementia risk at midlife than later in life. When evaluated separately, midlife women experience rises and declines in their systolic (SBP) and diastolic blood pressure (DBP) that could be menopause-specific in some women. Yet, the joint patterns of SBP and DBP during midlife may not be clear. We aimed to identify latent clusters of women sharing joint changes in SBP and DBP over the menopause transition (MT) and evaluate their associations with cognitive decline.

**Methods:** Group-based multi-trajectory model was used to identify distinct joint patterns of SBP and DBP over the MT; a linear trajectory suggests a dominant aging contribution while a non-linear would more likely be related to the MT. Multivariable piecewise mixed-effect models were applied to assess associations of the identified SBP/DBP trajectory groups with longitudinal cognitive measures of working and episodic memory, immediate and delayed recall.

**Results:** Three distinct trajectories were identified in 2700 women (aged 46

± ±

2.65 years at the 1<sup>st</sup> BP measure): Group 1: low menopause-related rise (low levels with menopause-related SBP rise but linear DBP rise, 30.2%), Group 2: medium linear rise (medium levels with linear SBP and DBP rises, 45.2%), and Group 3: high stable menopause-related decline (high levels with persistently high SBP and DBP decline around menopause, 24.6%). Nonlinear declines in working memory, immediate recall, and delayed recall started at ages 63, 62, and 58, respectively. Compared to group 1, group 3 showed greater working memory decline **before** 63 years old, while group 2 showed less immediate and delayed recall declines **before** 62 and 58 years old, respectively.

**Conclusion:** Blood pressure trajectories characterized by SBP rises or DBP declines close to menopause were linked to greater declines in episodic and working memory later in life, respectively.

**From Marmosets To Man: Generating A Protocol For Directly Converted Neurons To Advance Alzheimer's Disease Research**

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The progress of pre-clinical research on Alzheimer's Disease (AD) has been hindered by research models that do not faithfully recapitulate human aging and frank AD neuropathology. The common marmoset (*Callithrix jacchus*) is a New World non-human primate (NHP) with aging, genetic variability, and social behaviors that closely resemble those of humans, making it an invaluable model for studying age-related disorders such as AD. Furthermore, research on marmosets overcomes a major limitation of human studies: the correlation of longitudinal *in vitro* models with *in vivo* assessments. Fibroblasts can be directly converted into induced neurons (iNs), bypassing pluripotent stages and thereby maintaining age-associated phenotypes that are crucial for modeling the pathobiology of AD. However, while reproducible protocols for obtaining marmoset-derived iNs are still in their early stages, they offer a tremendous opportunity to explore disease pathogenesis and identify therapeutic avenues for AD. We implemented a well-validated human direct reprogramming protocol on marmoset fibroblasts. Surprisingly, marmoset fibroblasts did not survive the standard 21-day human protocol for iN conversion. Consequently, we conducted an unbiased RNAseq study comparing the conversion trajectory of marmoset and human iNs, which revealed significant species-specific differences. These differences guided modifications to our conversion strategies for marmoset iNs (e.g., length of conversion, starting number of cells, media composition, frequency of media changes, substrate coating, pro-survival supplementation, and gradual induction). Upon extensive optimization, we developed a robust, high-efficiency protocol for marmoset iN conversion that preserved cell survival, enhanced neuronal maturity, and produced synaptically functional neurons in culture. Our novel protocol facilitates the study of cellular mechanisms with minimally invasive techniques, maximizing insights gained from marmoset models. This invaluable *in vitro* platform will also permit high-throughput drug screening, safer toxicology assessment, and in-depth mechanistic investigation of normal brain aging and AD pathogenesis, thus advancing translatable therapeutic opportunities for AD intervention.

**Diffusion-Derived Subcortical Microstructural changes Associated with Clozapine Response in Treatment-Resistant Schizophrenia**

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**Introduction:** Schizophrenia is a chronic psychiatric disorder. Up to 35% of individuals with schizophrenia do not respond to first-line antipsychotic drugs and meet broad criteria for treatment-resistant schizophrenia (TRS). Clozapine (CLZ), has demonstrated superior efficacy for psychosis in people with TRS. However, CLZ's mechanism of action remains unclear. As of yet, microstructural alterations in cortical and subcortical regions remain underexplored. Thus, in this study, we examined microstructural changes in these regions using multi-shell diffusion-weighted imaging, exploring their relationship with changes in the clinical symptoms of schizophrenia following CLZ administration.

**Methods:** Twenty-nine TRS underwent 3T multi-shell diffusion imaging at baseline and after 12 weeks of clozapine (CLZ) treatment. Longitudinal data were available for 19 patients. Diffusion data were preprocessed using MRtrix. Neurite density index (NDI) and orientation dispersion index (ODI) mean kurtosis (MK) and radial kurtosis (RK) were extracted. Microstructural changes in frontal and basal ganglia regions were assessed. Along tract measures were also calculated using TractSeg.

**Clinical symptoms** were measured with the Brief Psychiatric Rating Scale (BPRS) at multiple time points. Correlations between symptoms and microstructural changes were examined using a general linear model ( $p < 0.05$ ).

**Result:** Association between changes in MK and total BPRS score was noted in the left ventral caudate ( $R^2=0.42$   $p = 0.46$  FDR corrected), the left globus pallidus ( $R^2=0.40$   $p = 0.46$  FDR corrected) and the left dorsolateral putamen ( $R^2=0.41$   $p = 0.46$  FDR corrected). Additionally, we examined white matter alterations along tracts and identified the predominant striatal endpoints that correlated with BPRS scores within the left striato-premotor tract. However, those endpoints did not remain significant after FDR correlation.

**Conclusion:** These results highlight the dimension of CLZ's impact on brain structure, suggesting that improvements in schizophrenia symptoms may be supported by microstructural changes within the basal ganglia.

**Investigating the Role of Srebf1/Srebf2 Pathway in Retinal Lipid Composition and Vascular Network**

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The retina is a light-sensitive neuronal tissue at the back of the eye. It has a unique phospholipid composition, highly enriched in polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (DHA), arachidonic acid (AA), and very long-chain polyunsaturated fatty acids (VLC-PUFAs), which are critical for human vision. Depletion of polyunsaturated fatty acids has been observed in various forms of retinal diseases. However, the mechanisms controlling this unique fatty acid composition in the retina, as well as the biological processes critically dependent on the high content of polyunsaturated fatty acids, remain poorly defined.

Our previous studies demonstrated that the retina responds to DHA deficiency through compensatory fatty acid remodeling and a robust upregulation of the Srebf1/Srebf2 signaling pathway, a master regulator of lipogenesis. In this study, we used mouse genetics to identify biological processes critically dependent on the basal activity of the Srebf1/Srebf2 pathway. Mice with attenuated Srebf1/Srebf2 pathway activity showed a shift to a simpler fatty acid profile, characterized by the loss of major polyunsaturated fatty acids. Transcriptional analysis revealed reduced levels of multiple genes involved in fatty acid transport and degradation, which could explain the observed lipidomic changes. Notably, preliminary observations identified defects in retinal vascularization as a major phenotypic feature of Srebf1/Srebf2-deficient mice. These findings underscore the previously underappreciated role of Srebf1/Srebf2 signaling and the PUFA composition of retinal membranes in developing and maintaining a healthy retinal vasculature network.

## **Unsupervised Machine Learning Analysis of Movement Similarity Predicts Body Composition and Performance Outcomes**

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Markerless motion capture (MMC) is an emerging technology that evaluates movement kinematics without the need for traditional markers. This capability has led to more kinematic testing to evaluate movement kinematics due to being field expedient, featuring a lower time and cost demand per participant, and mobile. Although this modality has been utilized by clinicians, sports scientists, biomechanists, coaches, and athletes themselves, the value of MMC in stratifying movement similarity has not been clearly established.

**Purpose:** To determine the applicability of MMC in stratifying performance outcomes and body composition based on similarity of movement in several common body weight exercises.

**Methods:** Body composition, aerobic capacity, maximal strength, upper and lower body power, and military performance were assessed in 79 healthy, active participants (age 18-35; 36 F, 43 M). Kinematics were assessed using MMC for 8 movements: overhead squat, front lunge, lateral lunge, unilateral jump, vertical jump, multiple hops, lateral bounds, and drop jump. Movement similarity clusters were generated by K-means clustering with a K of 2, which was indicated with Elbow Method. Welch’s t-tests were performed to assess for differences in body composition and performance between the K-means clustering derived groups.

**Results:** Based off kinematic results-based clustering, both the male and female populations were different within group ( $\alpha < 0.05$ ) in body composition metrics. These included: Lean Mass (LM), Leg LM, Trunk LM, Arm LM, Fat Mass (FM), Trunk FM, Leg FM, Arm FM, and Total Weight. We observed performance differences in the male groups for: Medicine Ball Chest Toss, 3-RM Back Squat, Casualty Drag Time, 3-RM Deadlift,  $VO_{2\max}^2$ , and Bench Press.

**Conclusion:** These data show that MMC-based kinematic analysis paired with unsupervised machine learning are associated with differences in body composition in both men and women, and musculoskeletal performance outcomes in men.

**Effects of Conditional CK2 $\alpha$ 1 Knockout on Dental and Skeletal Tissues Formation in Mice**

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Dental and skeletal mineralization is a complex process involving the deposition of hydroxyapatite crystals (calcium-phosphate complex) into an extracellular matrix rich in collagenous and non-collagenous proteins. Phosphorylation of non-collagenous proteins within the matrix is crucial for proper mineralization. Casein kinase 2 (CK2), a serine/threonine protein kinase, phosphorylates various substrates and regulates diverse cellular functions, including cell survival, proliferation, and apoptosis. CK2 role in mineralization is poorly understood. We hypothesized that CK2 is required for skeletal and dental tissues formation. We generated a conditional knockout (cKO) mouse by depleting *CK2 $\alpha$ 1* in cells expressing the Sp7 promoter. We characterized *CK2 $\alpha$ 1* cKO mice phenotype by micro-computed tomography ( $\mu$ -CT) and histological staining of 4-week-old male and female mice. *CK2 $\alpha$ 1* cKO mice presented reduced body weight and length compared to *CK2 $\alpha$ 1<sup>fl/fl</sup>* control mice.  $\mu$ -CT analysis of cKO demonstrated a significant reduction in alveolar bone volume and thickness, reduced enamel thickness, shorter and thinner roots, and an enlarged pulp chamber compared to control mice.  $\mu$ -CT evaluation of the femur's trabecular bone revealed a significant decrease in bone volume, connectivity density, and trabecular thickness in cKO compared to control mice. The cortical bone of the femur was significantly smaller, more porous, and less dense in cKO than in control mice. Histological assessment with Safranin-O stain revealed less proteoglycans in cartilaginous tissues in cKO than control mice. Picrosirius red staining demonstrated a reduced collagen network under bright light, and fewer type I and III collagen bundles, under polarized lights in cKO than in control mice. Alizarin red and Von Kossa stains detected fewer calcium and phosphate deposits in cKO than in control mice. Our data suggests CK2 is required for dental and skeletal tissues formation and homeostasis. We plan to study the mechanisms of CK2 regulation of mineralization by studying its interactions with other proteins involved in mineralization.

**Head-To-Head in Vivo Braak Staging with Two tau PET Tracers: MK6240 and Flortaucipir**

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Background: Braak staging is a widely used framework for classifying Alzheimer's disease (AD) tau pathology progression. Originally derived from post-mortem histopathological examinations, over the past decade, the Braak scheme has been increasingly applied to in vivo imaging studies using various tau PET tracers. However, compatibility and consistency of Braak staging across different tracers have not yet been confirmed, particularly given tracer-specific differences in binding sensitivity and specificity. This study aims to compare and test harmonization procedures for Braak staging with the two most extensively used tau PET tracers: Flortaucipir and MK6240.

Methods: We assessed 437 participants (245 cognitively unimpaired (CU), 192 cognitively impaired; mean age  $68.5 \pm 8.6$  years) with head-to-head MK6240 and Flortaucipir scans, alongside MRI, A $\beta$ -PET, and clinical evaluations. Several cutoff methods and region-of-interest (ROI) optimizations were tested. Braak stage concordance was examined both within tracers (intra-tracer; sequential Braak pattern adherence) and between tracers (inter-tracer; staging match) using seven (0–VI) and simplified four-stage groupings (0, I–II, III–IV, V–VI).

Results: Intra-tracer seven-stage Braak concordance ranged from 63% to 94%, with Gaussian mixture modeling yielding highest concordance (MK6240: 94%; Flortaucipir: 89%). Inter-tracer concordance ranged from 56% to 76%, with the highest agreement (76%) achieved using CU A $\beta$ -mean + 2SD cutoffs and optimizing the Braak II ROI to reduce off-target spill-off. The simplified four-stage approach enhanced intra-tracer concordance (MK6240: 94%, Flortaucipir: 89%) and inter-tracer agreement (86.5%). Most discrepancies between tracers occurred in Braak stages II–IV.

Conclusion: Our preliminary results demonstrate substantial discrepancies in Braak staging between Flortaucipir and MK6240 tracers, with up to 44% of cases classified differently depending on the chosen ROIs and cutoffs. Adjusting these parameters can partially reduce differences within and between tracers, highlighting the importance of harmonizing methods for consistent tau PET staging.

## **Matrix-Bound Nanovesicles: Modulating the Esophageal Cancer Phenotype**

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Esophageal adenocarcinoma (EAC) is rising at an alarming rate and remains a leading cause of cancer-related mortality. The current standard treatment, esophagectomy, is associated with high morbidity and reduced quality of life, underscoring the need for alternative therapeutic strategies. Matrix-Bound Nanovesicles (MBV), a distinct subset of extracellular vesicles (EV), have emerged as key regulators of tissue homeostasis within the extracellular matrix (ECM). Unlike exosomes, which are secreted into body fluids for cell-cell communication, MBV remain embedded within ECM collagen fibers and are released during matrix remodeling—an active process in wound healing, inflammation, and cancer progression. MBV carry bioactive proteins and miRNAs that influence cellular behavior, particularly in immune modulation, yet their role in shaping the tumor microenvironment in EAC remains poorly understood.

In this study, we explored the potential of MBV to influence the neoplastic phenotype of esophageal epithelial cells beyond their known immunomodulatory effects on macrophages. Results show that MBV derived from porcine urinary bladder (UBM-MBV) can be internalized by epithelial cells, leading to the downregulation of the neoplastic phenotype. Interestingly, while normal esophageal cells maintain their physiological characteristics in culture, neoplastic esophageal cells show a pronounced cytotoxic response to MBV, partially dependent on caspase-3 activity. These observations suggest that MBV possess cytotoxic potential against esophageal cancer cells, offering pivotal insights for advancing next-generation therapeutic strategies in the treatment of esophageal diseases.

**Fluorescence Guided Surgery with Dinutuximab-DTPA-IR800 In A Rat Model of Neuroblastoma**

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Complete resection is a critical component of treatment for high-risk neuroblastoma, though 5-year-survival remains near 50%. Identifying and resecting the cancer remains challenging due to hidden tumor deposits, encasement of neurovascular structures, and indistinct tumor borders. Tracers can be targeted to cancer to improve visualization and detection, in techniques known as fluorescence or radio-guided surgery (FGS or RGS), or more generally as intraoperative molecular imaging (IMI). Dinutuximab-DTPA-IR800 is an IMI tracer that targets GD2, which is highly overexpressed in neuroblastoma, with a near-infrared fluorophore, IR800. Here, we present an animal experiment which simulates an established clinical trial design for FGS.

SK-N-BE(2) cells were injected into the left adrenal of RNU rats (5 male, 6 female) to generate orthotopic xenograft neuroblastomas 5 weeks later. Four days after dinutuximab-DTPA-IR800 was injected IV, complete resection of tumor and nodules was attempted with standard-of-care white light (non-IMI) surgery. The SPY-PHI near-infrared camera was then used to assess the fluorescence of previously resected tumor and to identify and resect residual fluorescent tissue. Presence of neuroblastoma was then assessed pathologically for all resected tissue as well as tumor bed.

Eleven primary tumors and 5 additional nodules were resected with white-light surgery, all of which were confirmed neuroblastoma by pathology, and 13 of which were fluorescent. An additional 20 nodules that were only identified by FGS were resected from 7 of the rats using FGS, of which 14 were confirmed neuroblastoma by pathology. Two tumor beds had microscopic residual disease deposits following FGS, while 9 had no evidence of neuroblastoma on pathology. Collectively, FGS had a sensitivity of 84%, specificity of 60%, PPV of 81%, and NPV of 64%.

We conclude that FGS with dinutuximab-DTPA-IR800 in a rat model of neuroblastoma enables a more complete oncologic resection, supporting further development and clinical testing of the tracer.

**Essential Role of Nfe2l1 in UPS Function and Retinal Integrity**

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Nuclear factor erythroid-2-like 1 (Nfe2l1) is a ubiquitously expressed stress response factor implicated in the regulation of multiple cellular processes, including the targeted degradation of proteins via the ubiquitin-proteasome system (UPS). Our previous studies demonstrated that Nfe2l1 overexpression supports retinal viability in a mouse model of blindness driven by protein misfolding. However, a comprehensive understanding of Nfe2l1-mediated cellular functions in the retina is necessary to advance Nfe2l1-targeted therapeutic strategies.

The retina is a light-sensitive tissue composed of six major cell types originating from a common progenitor: photoreceptors, bipolar cells, ganglion cells, horizontal cells, amacrine cells, and Müller glia. To dissect Nfe2l1's contributions to retinal biology, we generated retina-specific Nfe2l1 knockout (KO) mice and performed functional, molecular, and transcriptomic analyses. Loss of Nfe2l1 disrupted key processes including protein degradation, synaptic integrity, retinal function. Unexpectedly, despite its ubiquitous expression, Nfe2l1 loss led to the selective degeneration of retinal ganglion and horizontal cells, as confirmed by transcriptional profiling and immunolocalization studies. Further analysis demonstrated that this degeneration occurs postnatally and is not attributable to defects in cell fate specification. Retinal ganglion cells (RGCs) are critical for transmitting visual signals to the brain, and their loss leads to irreversible blindness. Similarly, horizontal cells, which are essential for integrating and modulating photoreceptor responses, are irreversibly lost in advanced retinal diseases. These findings underscore a previously unrecognized connection between Nfe2l1-controlled UPS function and the long-term viability of these neurons. Deciphering its role in retina may pave the way for Nfe2l1-targeted therapies to preserve retinal neurons and prevent human blindness.

## Harmonizing Visual Readings of tau PET Tracers Using the HEAD Study

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**Background:** Tau-PET provides critical topographic information on tau deposition in the living brain. Integrating research-based practices into clinical settings through qualitative methods, such as visual reads, is becoming increasingly important. This study aims to compare the performance of existing visual reading methods for [<sup>18</sup>F]Flortaucipir (FTP) and [<sup>18</sup>F]MK-6240 (MK) and provide preliminary data toward a unified visual reading approach for all tau-PET tracers using a head-to-head dataset.

**Methods:** To evaluate previously published visual reading methods, two blinded raters conducted FTP and MK visual reads on 340 participants from the HEAD study. A unified visual reading method was developed and tested on 101 participants, using a composite of adjusted Braak regions to determine 3 stages of severity while harmonizing between tracers.

**Results:** Inter-rater agreement using previous methods showed high Cohen's kappa values (0.71-0.77) for the low and high tau burden categories in both FTP and MK visual reads. However, agreement was substantially lower in the non-AD-like category, leading to overall agreement rates of 0.55 for FTP and 0.69 for MK. In contrast, the method developed here improved inter-rater agreement across all categories (0.87-0.96) and introduced a moderate tau (0.79-0.83) burden group, leading to higher overall agreement rates of 0.81 for FTP and 0.87 for MK. For inter-tracer agreement, previous methods resulted in discordant visual reads for FTP and MK in 24.7-34.4% of cases. The developed method reduced this disagreement to 11.9-15.8%, demonstrating improved consistency across different tracers.

**Conclusion:** Previously published visual reading methods produced varying classifications depending on the tracer used. The method proposed here improved inter-rater and inter-tracer visual read agreements by developing a unified, tracer-agnostic approach easy to be used. Importantly, this clinician-friendly method has the potential for widespread adoption, offering a single harmonized visual reading technique for all tau-PET tracers.

## **Behind the Curtain: Insights into Showerhead Spray Pattern Impacts on the Genomic Landscape of Non-Tuberculous Mycobacteria**

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Non-tuberculous mycobacteria (NTM) are emerging pathogens with a growing prevalence and responsible for rising incidence of respiratory infections in the U.S., particularly among immunocompromised individuals. While NTM exposure can occur through various pathways, inhalation of waterborne aerosols is thought to be the primary route of infection. Showering, in particular, is a significant exposure source, however, little is known about whether different showerhead design choices impact the type and abundance of aerosolized NTM.

To bridge this gap, NTM isolates were obtained from the water collected from nine identical showerheads operating with either mist, jet, or rain spray patterns and were subjected to whole genome sequencing. Through genomic approach, this study aimed to identify them and provide insights into the genomic repertoire of NTMs, emphasizing antibiotic resistance genes (ARGs), virulence factors, biosynthetic gene clusters, and nutritional preferences relevant to virulence.

The results showed that the isolates were taxonomically affiliated to *Mycobacterium phocaicum*, *M. gordonae*, *M. abscessus*, and *M. paragordonae*, harboring diverse combinations of virulence factors. Genomic analysis revealed all isolates contained multiple ARGs such as *mtrA*, *mfpA*, *katG*, and *AAC(2')-I*, and genes associated with survival in oligotrophic environments (e.g., *sqr*, *frmA*, and *arsC*). Additionally, multiple isolates show prophage sequences indicating scope for phage-mediated evolution with implications for virulence. Overall, the research findings are expected to leverage the existing knowledge of diversity and metabolic versatility of canonical NTMs reported from the built environment and deemed avirulent, thus accentuating the pathobiological outcomes of NTM research and comparative studies. Furthermore, the study is slated to provide genomic evidence for virulence and aid in prioritizing strains for future host-pathogen interaction studies.

**Gene duplication captures morph-specific promoter usage in the evolution of aphid wing dimorphisms**

**Saleh Ziabari, Omid<sup>1,2</sup>; Liu, Fangzhou<sup>1</sup>; Deem, Kevin D.<sup>1</sup>; Liu, Xiaomi<sup>1</sup>; Kholwadwala, Akhil<sup>1</sup>; Brisson, Jennifer A.<sup>1</sup>**

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Understanding how morphology evolves requires identifying the types of mutations that contribute to changes in development. We integrated comparative genomics and transcriptomics to reconstruct the evolution and regulation of follistatin paralogs in relation to the evolution of aphid winged and wingless morphs. We find that different pea aphid follistatin duplicates play an essential molecular role in both the male and female wing dimorphisms, linking the genetic and environmental control of morph determination in each sex, respectively. We also find that an ancestral follistatin gene likely had multiple promoters and that the follistatin duplicates that evolved wingless-specific expression retained only the ancestral wingless-specific promoter. Our work provides a roadmap for how alternative promoter usage and subsequent gene duplication can enable the evolution of animal form.

**Gene Duplication Captures Morph-Specific Promoter Usage in the Evolution of Aphid Wing Dimorphisms**

**Ziabari, Omid Saleh<sup>1,2</sup>; Liu, Fangzhou<sup>1</sup>; Deem, Kevin, D.<sup>1</sup>; Liu, Xiaomi<sup>1</sup>; Kholwadwala, Akhil<sup>1</sup>; Brisson, Jennifer, A.<sup>1</sup>**

**<sup>1</sup>Department of Biology, University of Rochester;**

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**Effects of AD Modifiable Risk Factors to Tau-PET Tracer Uptake and its Association with Cognition in Early Braak Stages**

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**Introduction:** Many risk factors can contribute to the occurrence of Alzheimer's Disease (AD). However, little is known about the impact of dementia risk factors to the uptake of tau-PET tracers. Therefore, in this work we aim to investigate the influence of dementia risk factors on <sup>18</sup>F-Flortaucipir (FTP) and <sup>18</sup>F-MK6240 (MK) tau-PET tracers' uptake.

**Methods:** We accessed 436 individuals across the aging and AD spectrum (251 amyloid negative and 185 amyloid positive) from the HEAD study, with available A $\beta$ -PET, FTP, MK, and clinical assessments. Linear regression models corrected for age, sex, clinical diagnosis, and study site tested the association of factors with tau-PET tracers in the medial temporal lobe (MTL). A tau-PET  $\times$  risk factor term was added to test the influence of risk factors to the association of tau with cognition.

**Results:** Dementia risk factor exert different impact on tau-PET tracers' uptake in the MTL, according to amyloid- $\beta$  pathology, with high BMI being positively associated with the uptake of both FTP and MK in amyloid- $\beta$  negative individuals, whereas in amyloid- $\beta$  positive individuals high BMI were negatively associated with the uptake of both tau-PET tracers in the MTL. Using MMSE scores as the outcome, we found that amyloid- $\beta$  negative individuals with high BMI showed poorer cognition related to both MK and FTP in the MTL, while those with vision or hearing loss were affected by MK only. Amyloid- $\beta$  positive individuals with hypercholesterolemia and hypertension showed worse cognition associated with both MK and FTP in the MTL.

**Conclusion:** In this preliminary analysis, we show that the effects of dementia risk factors vary according to amyloid- $\beta$  pathology. These prevalent factors in the elderly also changed the association between tau-PET and cognition, underscoring the need for further studies to better understand their role in modulating this relationship.

**Associations of Estradiol and APOE4 Genotype with Hippocampal Connectivity during Verbal Encoding in Postmenopausal Women**

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High levels of endogenous estradiol in postmenopausal women are associated with beneficial effects on memory circuitry after menopause. Here we examine how estradiol and hippocampal functional connectivity (HippfConn) vary as a function of APOE4 carrier status, and whether estradiol-associated patterns of HippfConn relate to adverse AD biomarker profiles among APOE4+ women.

Participants were enrolled in MsBrain, a cohort study of postmenopausal women, a subsample of whom completed 3T MRI neuroimaging, estradiol assessment, and plasma AD biomarker (pTau181, pTau231, amyloid-beta 42/40) measures (n=172, mean age 59.3±3.9 years, 83.1% white, 23.33% APOE4 carriers). Interactive associations of estradiol levels and APOE4 genotype (APOE4+ versus APOE4-, excluding APOE2) with whole-brain left and right HippfConn during a word encoding fMRI task were analyzed via linear regression. Next, the magnitude of association between each estradiol-related HippfConn and AD biomarkers was examined, stratified by APOE4 genotype. Models controlled for age, education, and BMI, and were cluster-corrected at p<.05.

APOE4+ status modified the association of estradiol with both left and right HippfConn during encoding. Among APOE4+ women, higher estradiol was associated with lower connectivity between the left HippfConn to several bilateral brain regions; in contrast, in APOE4- women, those associations were beneficial or neutral. Among APOE4+ women, higher estradiol was associated with increased right HippfConn to several bilateral brain regions; in contrast, associations in APOE4- women were neutral or negative. HippfConn was more strongly related to AD pathology in APOE4+ than APOE4- individuals.

These findings challenge previous assumptions that estradiol is universally beneficial to the brain.

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**Age Dependent Changes in 24 hour Gene Expression Rhythms Across Cells of the Human Dorsolateral Prefrontal Cortex**

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The importance of circadian rhythms in health and disease has become increasingly apparent, particularly in aging during which circadian behavior and biology is known to change drastically. Additionally, disruptions in circadian rhythms are associated with impaired cognition and neurodegeneration. As such, we are interested in exploring how circadian rhythms change at the molecular level in brain regions like the dorsolateral prefrontal cortex (DLPFC), which is both central to higher cognitive functions and particularly vulnerable to age-related decline. To do so, we leveraged a single-cell transcriptomic atlas of human postmortem DLPFC and used a nonlinear cosinor-based regression model to identify differences in gene expression rhythms between Adult (21 – 60 years; n= 116) and Late Adult (61-97 years; n = 76) subjects within 26 cell type subclasses. In Adults, core circadian clock genes were the top rhythmic genes in most neuronal subclasses, suggesting that an intact, rhythmic, molecular clock, which is crucial for regulating sleep-wake patterns, cognitive function, and cellular metabolism, is a fundamental component of the DLPFC transcriptomic neuronal landscape during adulthood. However, in Late Adults, these core circadian gene rhythms are lost, while different genes gain rhythms. Intriguingly, oligodendrocytes and microglia in particular gain rhythmicity in genes associated with the unfolded protein response, indicating a potential adaptive response to increased protein synthesis demands and cellular stress during aging. We believe these age-related differences may represent a compensatory clock that becomes active with the loss of canonical clock function and/or in response to the stressor of aging. The impact of our findings is important for consideration in both healthy aging biology, and in future research on age-related disorders.

**Parallel HIV-1 Fitness Landscapes Shape Viral Dynamics in Humans and Macaques that Develop Broadly Neutralizing Antibodies**

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Human immunodeficiency virus (HIV)-1 exhibits remarkable genetic diversity. An effective HIV-1 vaccine must therefore elicit antibodies that can neutralize many variants of the virus. While broadly neutralizing antibodies (bnAbs) have been isolated from HIV-1 infected individuals, a general understanding of the virus-antibody coevolutionary processes that lead to their development remains incomplete. We performed a quantitative study of HIV-1 evolution in humans and rhesus macaques, including individuals who developed bnAbs. We observed strong selection early in infection for mutations affecting HIV-1 envelope glycosylation and escape from autologous strain-specific antibodies, followed by weaker selection for bnAb resistance. The inferred fitness effects of HIV-1 mutations in humans and macaques were remarkably similar. Moreover, we observed a striking pattern of rapid HIV-1 fitness gains that precedes the development of bnAbs. Our work highlights strong parallels between infection in rhesus macaques and humans, and it reveals a quantitative evolutionary signature of bnAb development.

**Profiling Amyloid-Negative, Tau-Positive Individuals with Two Tau PET Tracers: Implications for tauopathies**

**Soares, Carolina<sup>1</sup>; Ruppert, Emma<sup>1</sup>; Ferreira, Pamela L.<sup>1</sup>; Medeiros, Marina S.<sup>1</sup>; Rocha, Andreia<sup>1</sup>; Rodrigues, Matheus Scarpatto<sup>1</sup>; Oliveira Jr, Markley<sup>1</sup>; Bellaver, Bruna<sup>1</sup>; Povala, Guilherme<sup>1</sup>; Amaral, Livia<sup>1</sup>; Lussier, Firoza<sup>1</sup>; Karikari, Thomas<sup>1</sup>; Masdeu, Joseph<sup>2</sup>; Tudorascu, Dana L.<sup>1</sup>; Soleimani-Meigooni, David<sup>3</sup>; Fortea, Juan<sup>4</sup>; Lowe, Val<sup>5</sup>; Oh, Hwamee<sup>6</sup>; Pascual, Belen<sup>2</sup>; Gordon, Brian A.<sup>7</sup>; Rosa-Neto, Pedro<sup>8</sup>; Baker, Suzanne<sup>9</sup>; Pascoal, Tharick A.<sup>1</sup>**

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Little is known about individuals outside the Alzheimer's disease (AD) spectrum, such as those with amyloid-negative (A-), tau-positive (T+) positron emission tomography (PET) scans. While tau-PET imaging has been used to study this group, differences between tau tracers can impact identification. This study aimed to investigate A-/T+ individuals using two tau-PET tracers in the HEAD cohort. Here, we studied 433 individuals from the HEAD cohort (244 cognitively unimpaired, CU), 138 mild cognitive impairment (MCI), and 51 with dementia). Participants underwent clinical assessments, MRI, A-PET, both MK-6240 and Flortaucipir scans, and a subset had plasma biomarkers (n=332). A-PET positivity was defined as Centiloid > 24. Tau-positivity was determined by MK-6240 and Flortaucipir SUVR values exceeding the mean + 1.5SD in at least one Braak region anchored in CU A- individuals followed by visual confirmation in A-/T+ cases. Biomarker concordance was assessed across A/T groups. Plasma GFAP, p-tau217, NFL levels, and Centiloids were compared across A/T groups using adjusted linear regressions. We found a total of 109 (25.1%) cases classified as A-/T+ defined by at least one tau tracer (67 ± 8.5 years, 63% females, 76 CU, 27 MCI, 6 with dementia). Detection of A-/T+ cases varied by tracer: MK-6240 (n=41, 10%), Flortaucipir (n=53, 13%). Among A-/T+ individuals, MK-6240 identified 28 CU, 10 MCI, and 3 dementia cases, while Flortaucipir identified 32 CU, 18 MCI, and 3 dementia cases. Concordance between MK-6240 and Flortaucipir in A-/T+ cases was low (n=29, 41%). A-/T+ individuals showed lower levels of plasma p-tau217 than A-/T-: MK-6240: P<0.001; Flortaucipir: P=0.002. Levels of other plasma biomarkers evaluated were not different between groups. In conclusion, A-/T+ individuals exhibited varying tau tracer detection rates and lower plasma p-tau217 levels compared to controls, suggesting that plasma p-tau217 could be a useful tool for identifying this group. These findings emphasize the need for studies to account for tracer variability.

**Elucidating Myeloid and B Cell Signatures Associated with Treatment Response in Head and Neck Squamous Cell Carcinoma**

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The complex interplay between cancer cells and the immune microenvironment critically influences treatment response in head and neck squamous cell carcinoma (HNSCC). This study aimed to delineate single-cell RNA signatures associated with myeloid and B cells in HNSCC and to elucidate their roles in modulating response to immune checkpoint inhibition (ICI).

We analyzed single-cell RNA sequencing data (GSE232240, n=17) from pre- and post-treatment HNSCC tumors of patients treated with anti-PD-1 and anti-CTLA-4 therapy. A comparative analysis of non-responders (n = 7) versus responders (n = 10) revealed signatures that were predominantly expressed in non-responders. Cell-cell communication analysis revealed responder-specific interactions between B cells and myeloid cells, absent in non-responders. Dysregulated immunomodulatory pathways, including those involving CD40 and FLT3, were prominent in non-responders, alongside reduced crosstalk between cytotoxic T cells and NK cells and myeloid cells.

A transcriptional signature strongly correlated with ICI response at pre-treatment (AUC = 0.861) and post-treatment (AUC = 0.88). Increased expression of B cell-associated genes (e.g., MS4A1, CD79A) correlated with improved overall survival in an independent TCGA cohort (n=521). Conversely, elevated expression of non-responder genes (e.g., GSN, SERPINH1, SERPINF1) was linked to poor survival outcomes, suggesting a role for fibroblast activation and extracellular matrix remodeling in resistance.

This study highlights novel immune-mediated determinants of treatment response and disease progression in HNSCC. Identification of myeloid and B cell signatures associated with ICI resistance may inform rational combination therapies to improve efficacy. Understanding these immune interactions provides a foundation for tailoring therapeutic strategies to overcome resistance and enhance patient outcomes.

**"Not Only Do I Have to Break the Family Generational Curse, I Have to Set an Example":  
The Journey of Black College Students from Foster Care to College**

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The decision for college students to pursue further education is influenced by a variety of factors, and each student's path is unique. Black foster youth face distinctive challenges navigating educational pathways. Systematic oppressive systems often contribute to their overrepresentation in foster care and lack of educational opportunities. Currently, there is little research on Black college-going foster youth. This research adds to the literature, challenging the deficit views of this group. This study explores: What structural challenges do Black foster youth face to enroll in college? What motivates them to pursue higher education?

BlackCrit and Community Cultural Wealth were used as theoretical frameworks to examine the structural challenges Black foster youth traverse and the motivational factors that led Black college-going foster youth to pursue higher education.

This study took on a phenomenological qualitative methodological approach to unpack the experiences of 32 Black college-going students with foster care experiences across the country. Four preliminary findings emerged: (a) Navigating desperate family dynamics, (b) A passion for learning, (c) The sense of serenity offered by school, and (d) The drive to reimagine themselves and prove others wrong.

The findings highlight factors motivating participants to pursue postsecondary enrollment and challenge public discourse that overlooks their successes despite added hardships. This study shares stories of achievement within this often-overlooked population, offering valuable insights for social workers, college personnel, and foster youth advocates. It also presents implications for those aiming to improve educational outcomes for current and former Black foster youth, who are intelligent, resourceful, and capable of thriving with the right opportunities.

## **Phage Resistance in *Klebsiella* via Disruption of O-antigen Biosynthesis**

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*Klebsiella* are among the most prevalent multidrug-resistant Gram-negative pathogens responsible for severe hospital-associated infections. While bacteriophage (phage) therapy has emerged as a promising alternative, its efficacy is often limited by the diversity of *Klebsiella* capsular polysaccharide (K-antigen) compositions, which serve as the primary receptor of most *Klebsiella*-targeting phages.

To explore if *Klebsiella* phages may target other receptors, we examined the host ranges of 10 *Klebsiella* phages against a panel of >40 *Klebsiella* clinical isolates encompassing diverse species, sequence types (STs), and polysaccharide profiles (K and O-antigen). Two phages isolated from a *K. oxytoca* strain, KLO08 and KLO10, were able to infect both *K. oxytoca* and an acapsular *K. pneumoniae* isolate. This result suggested that these phages utilize a receptor other than the capsule.

To test this hypothesis, we generated phage-resistant mutants of both the host *K. oxytoca* and the *K. pneumoniae* acapsular strain. Whole-genome sequencing revealed mutations in conserved O-antigen loci (WbbM, WbbO, WbdD, or wzt) that disrupted the biosynthesis of O-antigen. Phenotypic assays confirmed that the phage-resistant mutants had diminished O-antigen. These findings indicate that KLO08 and KLO10 phages target the O-antigen, which may be a conserved component of the *Klebsiella* lipopolysaccharide.

Unlike most *Klebsiella* phages that target the K-antigen for infection, O-antigen-targeting phages likely has broader host range potential. Incorporating such phages into therapeutic cocktails alongside K-antigen targeting phages could enhance the efficacy of phage therapy against *Klebsiella* infections.

## **Maternal Parenting-Related Confidence Predicts Neural Co-Regulation Among Mother-Infant Dyads**

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Background: During co-regulation, infants and their caregivers coordinate behavioral, affective and neural signals which over time support the child's ability to self-regulate (Feldman, 2012). Maternal risk factors, such as high stress, may disrupt neural co-regulation with the infant (Azhari et al., 2019; Nguyen et al., 2020). Conversely, maternal *strengths*—such as higher parenting-related confidence—may relate to higher levels of neural co-regulation in the prefrontal cortex (PFC), an important region for emotional expression and regulation.

Methods: Twenty-nine mothers and their 3-month-old infants [mean age (SD) = 3.6(.59) months; 45% female] participated. *Neural co-regulation* was measured during 3 minutes of face-to-face play using Near-Infrared Spectroscopy (NIRSport2 system). To measure *Parenting Confidence*, mothers completed an 11-item Competency Subscale (BIMF; Barkin et al., 2010). Data analysis was performed in MatLab using the AnalyzIR toolbox (Santosa et al., 2018).

Results: Overall, mothers experienced high parenting-related confidence [mean(SD) = 58.48(5.86); range = 43-66]. Greater maternal confidence was associated with stronger coupling of maternal medial and lateral PFC with infant right lateral PFC (5 associations;  $t = 4.09$ ,  $p = .0004$ ,  $q\text{-corrected} = .005$ ;  $t = 3.21$ ,  $p = .003$ ,  $q = .029$ ;  $t = 4.90$ ,  $p < .0001$ ,  $q = .001$ ;  $t = 4.04$ ,  $p = .0004$ ,  $q = .005$ ;  $t = 3.47$ ,  $p = .002$ ,  $q = .012$ ). Higher maternal confidence was also associated with weaker coupling of mother medial PFC and infant left lateral PFC ( $t = -3.06$ ;  $p = .005$ ,  $q = .04$ ).

Conclusions: Greater maternal confidence in parenting was associated with stronger activation between maternal brain regions implicated in emotional expression (medial PFC) and regulation (lateral PFC) and infant areas implicated in emotional regulation (right lateral PFC). Clinically, increasing mothers' parenting-related confidence may have a positive cascading effect on mother-infant co-regulation and support the development of babies' early emotional self-regulation.

## **Mental Health Service Use in a National Sample of Children Ages 5-12**

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Mental health disorders among school-aged children are rising, yet many do not receive adequate services. Current estimates of service use, particularly for those with severe symptoms, are crucial for informing research and policy. This study examined mental health service utilization in a nationally representative survey of 1,000 parents with children ages 5-12 conducted in 2024. Using the Service Assessment for Children and Adolescents (SACA), we assessed service use across outpatient, school-based, residential, child welfare, and juvenile justice settings. Results indicated that 22.4% of all children received mental health services, with higher rates among those above the 70<sup>th</sup> and 90<sup>th</sup> percentiles for symptoms of depression (46.2%; 73.3%), anxiety (46.6%; 62.7%), conduct disorder (43.7%; 60.4%), oppositional defiant disorder (37.0%; 59.1%), and ADHD (39.6%; 57.0%). Outpatient services were used most, followed by school-based services. Children above the 70<sup>th</sup> and 90<sup>th</sup> percentiles for symptoms of depression were the most likely to access nearly all types of services. Children with multiple comorbid mental health categories above the 70<sup>th</sup> or 90<sup>th</sup> percentiles were more likely to access acute services (e.g., inpatient stays, contact with the juvenile justice system) and multiple service sectors. A sizable minority of children did not receive intervention, highlighting significant gaps in care. Providing updated estimates of service use is essential to addressing gaps in care and mitigating adverse outcomes associated with untreated childhood mental health conditions.

## **Examining Coadministration of Nephrotoxins and Acute Kidney Injury in Hospitalized Patients at Two Hospital Systems**

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### **Introduction**

Medications are a common cause of acute kidney injury (AKI) contributing to kidney damage and deterioration in renal function. Coadministration of nephrotoxins causes burden on the kidney increasing the likelihood of development and worsening of AKI. Our objective was to examine the coadministration of nephrotoxins and progression from no AKI or stage 1 AKI to AKI stage  $\geq 2$  for those in non-intensive care units (ICUs).

### **Method**

We performed a retrospective cross-sectional study in patients admitted to University of Pittsburgh Medical Center (UPMC) between 2018-2022 and University of Florida Health (UFH) between 2012-2019. AKI was defined and staged by the Kidney Disease Improving Global Outcome guidelines. Nephrotoxins evaluated were selected from a published list that used a Delphi method for inclusion. Exclusion criteria included patients with end-stage kidney disease, a hospital length of stay less than 48 hours, AKI stage  $\geq 2$  upon admission, those with no serum creatinine measured within 48 hours of admission, and patients in an ICU. A descriptive assessment of nephrotoxin exposure and progression from no AKI or stage 1 AKI to AKI stage  $\geq 2$  was conducted.

### **Results**

At UPMC, 4.7% (1,871/39,431) of patients progressed from no AKI or stage 1 AKI to AKI stage  $\geq 2$  and 1.5% (1,800/121,415) at UFH. Patients administered  $\leq 1$  nephrotoxin accounted for 47.1% (881/1871) of patients who progressed in AKI at UPMC and 15.8% (284/1800) at UFH. Patients who had coadministration of nephrotoxins accounted for 52.9% (990/1871) of patients who progressed in AKI at UPMC and 84.2% (1516/1800) at UFH.

### **Conclusions**

AKI progression was more prevalent in patients who received coadministration of nephrotoxins at both hospital systems. Avoiding coadministration of nephrotoxins should be an approach to prevention of AKI progression as part of nephrotoxin stewardship efforts. Future studies should include assessment of drug-AKI causality and nephrotoxin exposure duration.

**$\alpha$ KG-mediated Carnitine Synthesis Promotes Homologous Recombination Via Histone Acetylation**

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Homologous recombination (HR) deficiency enhances sensitivity to DNA damaging agents commonly used to treat cancer. In HR-proficient cancers, metabolic mechanisms driving response or resistance to DNA damaging agents remain unclear. We identified that depletion of  $\alpha$ KG sensitizes HR-proficient cells to DNA damaging agents by metabolic regulation of histone acetylation.  $\alpha$ KG is required for the activity of  $\alpha$ KG-dependent dioxygenases ( $\alpha$ KGDDs), and prior work has exclusively shown that changes in  $\alpha$ KGDD affect demethylases. Using a targeted CRISPR knockout library consisting of 64  $\alpha$ KGDDs, we discovered that Trimethyllysine Hydroxylase Epsilon (TMLHE), the first and rate-limiting enzyme in *de novo* carnitine synthesis, is necessary for survival of HR-proficient cells in the presence of DNA damaging agents. Unexpectedly,  $\alpha$ KG-mediated TMLHE-dependent carnitine synthesis was required for histone acetylation. The increase in histone acetylation via the  $\alpha$ KG-carnitine axis promoted HR-mediated DNA repair through site-specific histone acetylation. Finally, we observed a positive correlation between TMLHE and histone acetylation in patient samples and found that high TMLHE or acetylcarnitine correlates with worse progression free survival in patients treated with DNA damaging agents. This study demonstrates for the first time that  $\alpha$ KG affects site-specific histone acetylation and provides a mechanism of HR-proficiency via carnitine synthesis. Moreover, these data provide a metabolic avenue to induce HR-deficiency and sensitivity to DNA damaging agents.

## **Dendritic inhibition terminates plateau potentials in CA1 pyramidal neurons**

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Neurons actively integrate information in their dendrites, where multiple forms of dendritic spikes boost voltage signals to amplify synaptic potentials. In hippocampal CA1 pyramidal neurons (CA1-PYRs), plateau potentials are a form of dendritic spikes that control synaptic plasticity and memory formation. Somatostatin interneurons (SST-INs) form inhibitory synapses on CA1-PYRs dendrites, a strategic position to regulate dendritic activity. How inhibition controls plateaus is incompletely understood.

Here, we used whole-cell recordings and optogenetic stimulation to show that dendritic inhibition terminates plateaus in an all-or-none manner. Plateaus were initially resistant to inhibition but became increasingly susceptible to termination as they progressed. Postsynaptic current injection mimicking inhibitory potentials sufficed to terminate plateaus. Between two genetically distinct subpopulations of SST-INs, NDNF-INs generated slower postsynaptic currents that terminated plateaus more effectively than ChRNA2-INs. Voltage-gated Ca<sup>2+</sup> channels (VGCCs), but not Na<sup>+</sup> channels, were necessary for plateaus and plateau duration was extended by blockade of small-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels (SK). A single-compartment biophysical model with these two conductances reproduced core experimental findings and provided a mechanistic explanation for plateau termination. Finally, two-photon Ca<sup>2+</sup> imaging showed that plateaus evoked large dendritic Ca<sup>2+</sup> transients, and plateau termination transformed these binary events into graded Ca<sup>2+</sup> signals. Overall, our results show how synaptic inhibition controls plateau duration to grade dendritic Ca<sup>2+</sup> signals, possibly modulating synaptic plasticity and memory.

**Longitudinal characterization of Retinal Ganglion Cells during the chick embryonic development: peaking at the HAA**

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**Purpose:** The molecular and cellular mechanisms governing the formation of a high acuity area (HAA), also known as fovea, during retinal development remain mostly unknown. It has previously been described that retinoic acid (RA) is modulated during development of the chick HAA by exclusion of RA in the central retina, becoming necessary for a complete absence of rods and peak cellular density in the ganglion cell layer in this area. While the highest density of retinal ganglion cells (RGCs) is a well established attribute of mature HAAs, little is known about progression and acquisition of this RGCs peak density during development.

**Methods:** To identify HAA-specific attributes regarding RGCs during retinal development, chick retinas across development were used. A pharmacological-genetic approach based on all-trans Retinoic Acid in ovo injections during the predicted "foveogenesis" period, at E4.5, followed by RNAseq analysis, was performed to detect potential RGCs specific gene expression responses after RA treatment. Following this, combination of in situ Hybridizations Chain Reaction, cell death detection and longitudinal immunofluorescence analyses with unreported RGCs antibodies were conducted over flat-mount preparations and evaluated by advanced imaging of flat-mounts reconstructions, culminating in the acquisition of RGCs isodensity maps.

**Results:** After Retinoic Acid treatment, analysis of central retinas at E4 revealed differentially gene expression mostly related to RGCs differentiation when compared with control retinas, without obvious changes in cell death. Analysis of multiple markers of RGCs during development by immunofluorescence studies on control retinas reveal an enrichment in the centro-ventronasal region, starting at the first embryonic week and further consolidated during the second embryonic week.

**Conclusion:** Our longitudinal evaluation of RGCs populations on flat-mounts of developing chick retinas reveal the progression and acquisition of RGCs peak density at the HAA, thus validating this particular cell type as a valid proxy to analyze foveal development.

**Older Cancer Survivor Perception of Utilizing Digital Technology to Support Healthy Survivorship: A Qualitative Study**

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While technology is a promising way to address barriers to delivering behavior change intervention and supportive care to older cancer survivors, exploration of the usability and interest in employing digital tools to promote lifestyle change among this population is needed. This study aimed to explore the knowledge and perception of utilizing digital technology for health promotion among older cancer survivors. Semi-structured interviews were conducted remotely with 22 cancer survivors over the age of 55 years. Participants were recruited from the community and an online survey panel site. The interview guide was grounded in the Diffusion of Innovation (DOI) Theory and the Technology Acceptance Model (TAM) to explore survivor perception of relative advantage, complexity, compatibility, and ease of use of digital technology. Participants were queried about features they desire in technology to support lifestyle behavior change. Interviews analyzed using a directed content analysis approach in Dedoose software with thematic codes derived from the DOI theory and TAM. Participants were predominately non-Hispanic White and female (17/22, 77.3%), an average age of 66.8 ( $\pm$  6.2) years, and 40.9% (9/22) had less than a college education. Key themes included: (1) high perceived technology aptitude among older cancer survivors, (2) common use of technology for health tracking, (3) the perception of technology as a convenient and efficient way to track health, (4) desire to learn and adapt to new technology to support health, and (5) avoidance of technology that is too challenging to learn individually. Survivors identified five key features they want to see incorporated in future digital technology-delivered lifestyle behavior change interventions: accountability, feedback, gamification, socialization, and tracking. The interviews provide insight into how older cancer survivors perceive technology and use it for health promotion. Results support the acceptability and feasibility of implementing digital technology-based cancer survivorship programming with older adult survivors of cancer.

## Reducing Human Annotation Effort in Image-Based Machine Learning

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**Background:** Annotating images is a bottleneck for training machine learning models, often requiring detailed analysis of hundreds or thousands of images by domain experts. This is costly from a time, resource, and monetary perspective. Poor annotations negatively impact model performance, making it crucial to balance annotation efficiency and quality. Machine-generated “pseudo-annotations” can be efficiently created by leveraging pretrained models from similar tasks to imperfectly predict annotations for a new task. Filtering these predictions based on customized criteria can enhance the quality of the pseudo-annotations, allowing large-scale annotations with reduced need for expert involvement.

**Objective:** We aimed to identify the best filtering methods for identifying high-quality pseudo-annotations of amyloid-beta (A-beta) plaques with cored and diffuse subtypes in whole-slide images (WSIs) of brain autopsy tissue, leveraging a published model trained to detect cored plaques and cerebral amyloid angiopathy.

**Methods:** We collected 4,378 tiles from brain autopsy WSIs and developed color-based filtering criteria using hue, saturation, and value characteristics. A previously published model was used to produce pseudo-annotations for plaques after being tuned to increase its sensitivity. We evaluated three filtering methods of increasing complexity to identify tiles from which the algorithm captured plaque pseudo-annotations well, with the goal of filtering out tiles with suboptimal pseudo-annotations.

**Results:** The highest complexity filter performed best on this task, detecting all definitive plaques for 76 consecutive tiles. The intermediate and lower complexity methods detected all definitive plaques for 63 and 52 consecutive tiles.

**Conclusion:** Pseudo-annotation of WSIs is a viable approach for efficiently obtaining high-quality annotations of pathologic features with minimal domain expert intervention. Performance in our task depended on filtering criteria complexity, and work remains to evaluate larger sample sizes and more sophisticated filtering criteria. Different pseudo-annotation and filtering methods will be necessary for different tasks.

**Alternative Splicing of the Mitochondrial Fission Inducer Drp1 in Cell Health and Disease**

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Mitochondrial regulation, through fission and fusion, is essential for mitochondrial function, cell proliferation and death regulation, and stress responses. As such, these phenomena play integral roles in organ system function and disease progression. A protein key to regulation of fission is Dynamin Related Protein 1 (Drp1). It is well understood that Drp1 activity is regulated by post-translational modifications. Literature on the role of alternative splicing on regulation of Drp1 function, however, is not as well studied. Drp1 is alternatively spliced at 3 exons, exon 3 in the GTPase domain, and exons 16 and 17 in the variable domain. Several studies, including work of ours, has demonstrated that alternative splicing of exons 16 and 17 impacts protein localization and fission induction capacity. Additionally, Drp1 Further, our preliminary data, in agreement with existing literature, suggests that Drp1 variable domain variants are differentially expressed across tissue types in mice. This suggests that Drp1 variable domain variants likely serve a functional purpose that remains to be understood. With the help of the Innovative Technologies Development Core at the University of Pittsburgh, we have generated mice that express single variable domain variants of Drp1 (Drp1 X00 which lacks exons 16 and 17 and Drp1 X11 which contains exons 16 and 17) using CRISPR-Cas9. Using these mice and mouse embryonic fibroblasts (MEFs), we have begun to characterize the impact of Drp1 alternative splicing of the variable domain on mitochondrial function, cell behaviors, and tissue development and function.

**The Impact of For-Profit Hospice Providers on End-of-Life Care for Prostate Cancer Decedents**

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Since 1983, the Medicare hospice benefit has provided palliative and supportive care for patients and families at the end of life. The growing presence of for-profit hospices raises concerns about financial incentives shaping care delivery and quality, particularly for cancer patients with intensive end-of-life needs. However, empirical evidence on these effects remains limited.

This study examines the impact of for-profit hospice care on hospice use among 63,926 prostate cancer decedents in their final year of life, using Surveillance, Epidemiology, and End Results (SEER)-Medicare linked cancer registry and claims data (2008–2017). Prostate cancer follows a prolonged course, often necessitating hospice care. To address potential selection bias, we employ a two-stage least squares (2SLS) instrumental variables approach. The instrument, derived from the Centers for Medicare & Medicaid Services (CMS) Provider of Services file, measures the local share of for-profit hospices (Mean = 0.537; F-stat = 8079.21). We estimate effects on hospice length of stay, live discharge, and care transitions in the final week of life, controlling for patient demographics and clustering standard errors at the ZCTA level.

Our IV estimates show that for-profit hospice patients have significantly longer stays ( $\beta=13.33$  days,  $p < 0.01$ , Mean = 44.73) but also face higher risks of live discharge ( $\beta=0.07$ ,  $p < 0.01$ , Mean=0.098) and provider changes ( $\beta = 0.05$ ,  $p < 0.01$ , Mean = 0.032). Additionally, burdensome transitions in the last week of life are more frequent ( $\beta = 0.009$ ,  $p < 0.01$ , Mean = 0.0076). These findings suggest that while for-profit hospices extend hospice use, they also contribute to more disruptions, complicating care coordination.

By linking for-profit hospice density to patient outcomes, this study informs policy efforts to enhance quality, accountability, and equitable hospice access. Future research will expand to other cancers and sociodemographic disparities to guide policy improvements in end-of-life care.

**Differential Response of Tissue-Specific vs. Whole-Body Modulation of NAD Metabolism on Aging**

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Decline in NAD<sup>+</sup> levels with age is one the major contributing factors for aging as well as for the development of age associated diseases. Hence several studies have focused on ways to boost NAD<sup>+</sup> levels to promote healthy aging. Multiple pre-clinical studies in rodents provide evidence for the beneficial effects of NAD supplementation however the preclinical studies in humans have mixed results where the benefits were not observed consistently fostering the need for a better understanding of the cellular mechanisms underlying the beneficial effects of NAD supplementation *in vivo*. Additionally, the supplementation studies cannot determine tissue-specific effects of an altered NADH/NAD<sup>+</sup> ratio. To get a better understanding of how boosting NAD levels impacts aging and associated functional deficits we generated transgenic *Drosophila* expressing a genetically encoded xenotopic tool LbNOX to directly manipulate the cellular NADH/NAD<sup>+</sup> ratio. We find that LbNOX expression impacts both NAD(H) and NADP(H) metabolites in a sex-specific manner. It rescues the reductive stress induced rough eye phenotype in *Drosophila*. Utilizing LbNOX, we demonstrate that targeting NAD metabolism in different tissues may have drastically different outcomes, as the expression of LbNOX solely in the muscles extends lifespan in both male and female flies, provides resistance towards oxidative stress, supports significantly improved muscle function at old age, and rescues aging associated sleep deficits. Expression of LbNOX in neurons results in mixed response where beneficial effects were observed on sleep with no effect on lifespan, and detrimental impact on stress tolerance in male flies. We also report identification of non-neuronal inputs modulating sleep in *Drosophila*. In summary, we identify tissues and metabolic processes that benefit the most from the modulation of the NAD metabolism thereby highlighting important aspects of rebalancing the NAD and NADP pools, all of which can be translated into novel designs of NAD-related human clinical trials.

**Distinct transmission sites within a synapse for strengthening and homeostasis**

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At synapses, miniature synaptic transmission forms the basic unit of evoked transmission, thought to utilize one canonical transmission site. Two general types of synaptic plasticity, associative plasticity to change synaptic weights and homeostatic plasticity to maintain an excitatory balance, are so far thought to be expressed at individual canonical sites in principal neurons of the cortex. Here, we report two separate types of transmission sites, termed silenceable and idle-able, each participating distinctly in evoked or miniature transmission in the mouse visual cortex. Both sites operated with a postsynaptic binary mode with different unitary sizes and mechanisms. During postnatal development, silenceable sites were unsilenced by associative plasticity with AMPA-receptor incorporation, increasing evoked transmission. Concurrently, miniature transmission remained constant, where AMPA-receptor state changes balanced unsilencing with increased idling at idle-able sites. Thus, individual cortical spine synapses mediated two parallel, interacting types of transmission, which predominantly contributed to either associative or homeostatic plasticity.

**Riding the Waves: Heatwave-induced shifts in metabolomic profiles of Duckweed (*Spirodela polyrhiza*) reveal differences in tolerance strategies amongst polyploid and diploid plants**

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We are in the midst of a global environmental crisis where extreme temperature anomalies like heatwaves have increased in duration, intensity, and frequency. Aquatic plant species are especially vulnerable to these extreme temperature events, as they have adapted to typically thermally stable freshwater ecosystems like lakes and ponds. However, some aquatic plants can be polyploid (containing >2 sets of chromosomes), often resulting in highly stress-tolerant phenotypes that can resist these extreme temperatures. Additionally, metabolites are some of the first responders of any stress event in plants, and polyploid plants are known to contain higher basal metabolite concentrations and rates of biochemical activity which may give them an advantage in surviving increasingly severe, frequent, and long heatwave events. To investigate the heatwave response of polyploid plants, I will conduct a series of massive, controlled experiments using next-generation techniques to quantify the impact of heatwave dimensions, including duration and frequency, on the metabolomic profiles of diploid and neoautotetraploid populations of duckweed (*Spirodela polyrhiza*, L. Schleid). I predict that 1) longer heatwaves will be more detrimental for diploids than polyploid duckweed, as polyploid plants have larger metabolite pools that lie closer to the activation thresholds for heat resistance, therefore minimizing damage, 2) more frequent heatwaves will decrease diploid survivorship as heat-related damage will occur before resistance metabolites can be upregulated, resulting in a trade-off between repair/survival vs. bet-hedging against future shocks, and 3) metabolomic diversity in polyploid plants will be greater because faster heatwave response enables them to divert resources to synergistic, alternative pathways and morphological responses that could confer long-term resistance. Here, I present preliminary data that demonstrates that not only does metabolomic diversity differ between diploid and polyploid duckweed, but differences in the relative intensities (i.e., concentrations) of metabolites suggests different mechanistic strategies of heatwave response.

## **Lipophagy is a Critical Mediator of Lipid Metabolism in Adipocytes**

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Adipose tissue is the primary reservoir for neutral lipids, mobilized via lipolysis to meet energy demands during fasting or cold-induced non-shivering thermogenesis. Canonical lipolysis occurs in the cytoplasm, driven by adipose triglyceride lipase (ATGL), which hydrolyzes triglycerides into free fatty acids (FFAs) and glycerol. However, the persistence of lipolysis in ATGL-deficient models suggests the existence of alternative pathways. Lysosomal lipolysis, mediated by lysosomal acid lipase (LAL), has been highlighted as a non-canonical pathway, though the mechanisms governing lipid trafficking to lysosomes for degradation remain poorly understood.

We hypothesize that lipophagy, a form of autophagy specialized in lipid degradation, plays a pivotal role in adipocyte lipid metabolism by facilitating lysosomal lipolysis. Our study demonstrated that autophagic activity is stimulated in adipose tissue during fasting, cold exposure, and treatment with a  $\beta$ -adrenergic agonist mimicking cold-induced lipolysis. Inhibition of autophagy in cultured adipocytes resulted in intracellular triglyceride accumulation and reduced lipolysis. To further elucidate the role of lipophagy, we generated adipose-specific Atg5 knockout mice. Atg5 deficiency resulted in blunted cold-enhanced plasma FFAs and impaired thermogenesis, along with reduced ex vivo lipolysis in response to  $\beta$ -adrenergic agonist stimulation or nutrient-deprived conditions. Notably, Atg5 deficiency did not alter ATGL or LAL expression levels. The inhibition of ATGL with Atglistatin additively suppressed Atg5-deficiency-impaired lipolysis, underscoring the synergistic effect of cytosolic lipolysis and lipophagy pathways.

Overall, our findings establish lipophagy as a critical mediator of lipid metabolism in adipocytes.

**Contributions and synaptic basis of diverse cortical neuron responses to flexible task performance**

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Flexible behaviors are critical for survival in diverse and dynamic environments. However, the synaptic mechanisms underlying flexible behaviors are largely unknown. Neuronal responses during behavior are diverse, ranging from reliable “classical” responses (e.g. pure tone tuning in auditory cortex) to irregular “non-classically responsive” firing. Previous work has shown both neuron types contain significant information about sensory stimuli and behavioral decisions and are necessary for task performance, suggesting that non-classically responsive cells play an underappreciated role in perception and behavior. To explore the synaptic origin and functional contribution of diverse response profiles to flexible task performance and dynamics, we adapted a recently developed, spiking recurrent neural network (RNN) model incorporating excitatory and inhibitory spike-timing-dependent plasticity (STDP). We demonstrate that this model can be trained to perform a reversal learning task similar to that of behaving animals where the rewarded target tone is spontaneously changed (i.e. reversed) partway through the task. This model recapitulates the distribution of classically and non-classically responsive neurons measured from the cortex of behaving rodents. While the network can be trained to perform the initial, pre-reversal task over a wide range of model parameters, we find that it can only successfully learn the full reversal task when network parameters result in a balance of excitatory and inhibitory input to individual units. Interestingly, balanced networks also demonstrate the greatest diversity of response types and the emergence of distinct functional roles for classically and non-classically responsive units as demonstrated by detailed network perturbations. We identify a specific parameter regime for these flexible, balanced networks where tonic inhibition lies between baseline and evoked excitation in a “see-saw” mechanism that facilitates stimulus-choice remapping. Network weights subspace analysis further indicates that balanced networks facilitate behavioral flexibility through readout plasticity. These results elucidate the relationship between synaptic dynamics, response type diversity, and behavioral flexibility.

**Data-Driven Approximation of Bladder Remodeling and Function in Bladder Outlet Obstruction**

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Benign prostatic hyperplasia is a common condition in older men that often leads to bladder outlet obstruction (BOO) due to prostate enlargement. This obstruction causes various urinary symptoms, such as difficulty urinating and incomplete bladder emptying, which may require surgical intervention. Although the exact biological mechanisms underlying BOO remain unclear, our recent studies using rat models have been conducted to further investigate the condition.

Using extensive in vivo and in vitro data from both healthy and obstructed bladders, our group previously developed a computational model to simulate bladder growth and structural remodeling in rats. The model captures bladder mechanics, voiding function (micturition), and tissue adaptation in response to altered biomechanical conditions. While a single simulation runs relatively quickly, incorporating uncertainty across multiple parameters requires exploring a broad range of values, significantly increasing computational cost.

To address this challenge, we propose a data-driven, physics-informed machine learning algorithm that accelerates computation and enables real-time prediction of outcomes across a defined range of parameter uncertainties. The algorithm was validated using repeated random sub-sampling validation across all outputs and demonstrated strong performance.

**Protein Kinase D2 Promotes Neuroendocrine Phenotypes of Prostate Cancer by regulating Aurora A Kinase and N-Myc Complex**

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Neuroendocrine prostate cancer (NEPC) is a lethal subset of metastatic castration-resistant prostate cancer (mCRPC) with aggressive clinical features and poor overall survival. Although it rarely arises de novo, it is more commonly diagnosed in advance mCRPC as a mechanism of resistance to therapy. The protein kinase D family is an emerging therapeutic target for prostate cancer. In this study, we aim to elucidate the roles of PKD2 in NEPC and assess the therapeutic potential of PKD inhibitors for treating this cancer. Knockdown of PKD2 and 3, the predominant PKD isoforms in NEPC cells, reduced NE markers and inhibited cell proliferation/survival. Conversely, PKD2 overexpression promoted NE programming by inducing NE markers and suppressing AR-targeted genes, mirroring the effects of N-Myc, a key driver of NEPC. PKD2 overexpression also induced androgen independence and conferred resistance to multiple therapeutic agents in androgen-sensitive prostate cancer cells. Mechanistically, PKD regulated Aurora-A expression at both protein and transcriptional levels. Knockdown of PKD2 downregulated Aurora-A and N-Myc and reduced the expression of N-Myc target genes, suggesting that PKD may promote NEPC progression by stabilizing the Aurora-A/N-Myc complex. Moreover, inhibition of PKD by the PKD inhibitor CRT0066101 blocked NEPC-like tumor cell proliferation/survival and suppressed NEPC xenograft growth in mice. CRT0066101 also significantly suppressed tumor development in a transgenic mouse model of NEPC. Collectively, our study indicates that PKD2 is a promising therapeutic target for NEPC, and PKD inhibitors may offer a potential effective treatment for this lethal disease.

**Randomized Controlled Trial of Brock String Vision Therapy for Receded Near Point of Convergence Following Concussion: Preliminary Findings**

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**Purpose:** To compare the effectiveness of Brock string vision therapy to usual care for receded near point of convergence (NPC) following concussion.

**Methods:** This study employed a single-blind, randomized controlled trial design involving participants aged 11-30 years within 10 days of a concussion. Participants with receded NPC (i.e., mean NPC>5cm) were randomized to either Brock string vision therapy (BSVT) or usual care (UC). The BSVT group was prescribed BSVT exercises (5 reps, 2x daily), whereas the UC group was prescribed symptom-specific management strategies (e.g., breaks during reading, computer work) and behavioral management (e.g., routine sleep, hydration, diet) until follow-up (7-14 days post-enrollment). Two-way mixed effects analyses of variance examined the interaction between group (BSVT, UC) and time (enrollment, follow-up) on mean NPC (primary outcome) and secondary outcomes (e.g., Concussion Clinical Profile Screening [CP Screen]).

**Results:** 50 participants were enrolled, with 25 in the BSVT group (age=15.8±4.1, 44% F, 84% SRC) and 25 in the UC group (age=17.9±4.8, 36% F, 60% SRC). Results supported a significant interaction in the BSVT group for NPC ( $F[1,48]=5.3$ ,  $p=0.026$ ,  $\eta_p^2=0.10$ ) and CP Screen Neck ( $F[1,43]=4.9$ ,  $p=0.033$ ,  $\eta_p^2=0.10$ ). The BSVT group exhibited greater improvement in mean NPC distance (-8.9 cm, 95% CI, -12.1, -5.7,  $p<0.001$ ) than the UC group (-3.8 cm, 95% CI, -6.9, -0.6,  $p=0.02$ ), and greater improvement in CP Screen neck symptoms (-0.6, 95% CI, -1.1, -0.03,  $p=0.03$ ) than the UC group (0.27, 95% CI, -0.3, 0.8,  $p=0.32$ ).

**Conclusion:** Brock string vision therapy prescribed following concussion significantly improved receded NPC and neck symptoms within 1-2 weeks compared to usual care consisting of behavioral management and symptom-specific management strategies. This study provides evidence for a simple and cost-effective intervention for treating receded NPC following concussion and highlights the benefits of an active, precision medicine approach for patients with concussion.

**Trial Registration:** [www.clinicaltrials.gov](http://www.clinicaltrials.gov); identifier: NCT04076657.

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