

BOSTON  
TAIWANESE  
BIOTECHNOLOGY  
ASSOCIATION



# 2019 Annual Symposium Program Book

08/03 - 08/04

HARVARD, Northwest Building  
Cambridge, MA

**Official Website** <http://btbatw.org/2019>  
**BTBA** <https://www.facebook.com/btbatw/>



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## ***WELCOME MESSAGE***

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Dear Attendees,

Welcome to the 2019 BTBA Symposium!

This is the 7<sup>th</sup> BTBA Symposium since our establishment in 2012. Thanks to the endeavors of all previous co-chairs and committees, BTBA Symposium has become an event that attracts attendees from all over the U.S. We are excited to meet all of you!

This year, we are grateful to have Dr. Seng H. Cheng, Senior Vice President and Chief Scientific Officer at Pfizer and Dr. Jenny P-Y Ting, William Rand Kenan Professor at University of North Carolina at Chapel Hill as our keynote speakers. Our two keynotes will share their career trajectories and insights in the development of the biomedical field with us. Like previous years, we have a spectrum of different sessions to discuss potential career options in academia and industry, as well as tactics to advance our careers in the U.S and Taiwan.

In addition to these popular signature sessions, our committee members pulled their hearts out to design brand new sessions every year to spark more discussions and interactions. This year, we have a Drug Modality session to provide an overview of the various modalities of drug research and development. We also have a session focused on management. In the Managing Up, Down, and Across session, the panelists will share their experiences to guide senior researchers on how to manage your team, communicate with your manager, or accomplish tasks with your peers. Moreover, we invite the Massachusetts Life Science Center (MLSC), an agency dedicated to supporting the growth and development of life sciences in Massachusetts, to introduce the productive biotech ecosystem in the Great Boston Area! Following this session, we also invite leaders from our sister TBAs (TTBA, BATBA, MTBA) to present the uniqueness of biotech development in their respective regions. Finally, this year's program features trending topics in Synthetic Biology in biomedicine, where invited experts will tell us about how the newest innovations are transforming the field.





As an active member of the Taiwanese community in the Greater Boston Area, one of our goals is always to facilitate scientific interactions and professional networking. BTBA hosts various networking events and workshops throughout the year. These include a brand-new Career Development Program for all researchers to strengthen the soft skills during their career path, and Peer-Mentoring Programs from industry or academia to share different tips for helping junior researchers toward a leadership path. BTBA also engages with other communities in Boston or from Taiwan to launch programs that aim to build more connections. We also continue our mentoring program in collaboration with Mount Jade New England and a series of seminars for members to share their research findings. If you are new to the area, stay tuned for more activities to come!

We would not be able to put together a symposium that covers so many different topics without the help of many people. We'd like to thank our speakers and panelists for generously sharing their expertise and experiences. We'd like to thank our sponsors who echo with our mission to foster collaboration and networking among the community. We'd also like to thank every member of our symposium organizing committee for their effort to make this symposium come true!

We hope you enjoy the 2019 BTBA Symposium in the next two days, and continue to support BTBA in the future!

Sincerely,

Yung-Chih Cheng and Chih-Chi (Jimmy) Chu  
2019 BTBA co-chairs





# ORGANIZERS

## Boston Taiwanese Biotechnology Association

### 2019 Symposium Committees

#### Co-Chairs

Yung-Chih Cheng	鄭永志	Senior Scientist	Q-State Biosciences
Chih-Chi (Jimmy) Chu	朱治齊	Scientist I	Bluebird Bio

#### Field Director

Jerry Lin	林致中	Postdoc	Massachusetts General Hospital
Kun-Po Li	李坤珀	Laboratory Supervisor	Sanofi

#### Biotech Forum: Drug Modality

Hung-Yi Wu	吳泓儀	Graduate student	Harvard University
Margaret Wey	魏嘉英	Attending Research Pharmacist	Massachusetts General Hospital
Yi-Shan Chen	陳怡珊	Scientist II	CRISPR Therapeutics
Yi-Dong Lin	林意棟	Scientist I	Sanofi
Yvonne Meng	孟憲薇	Senior Scientist	Merck Co.
Ariel Wei Hsi Yeh		Graduate student	Harvard University
Ho-Chou Tu	杜荷洲	Senior Scientist	Alnylam Pharmaceuticals

#### Poster and Elevator Pitch

Bin-Kuan Chou	周秉寬	Postdoctoral Researcher	Massachusetts General Hospital
Yu-Huan Shih	施毓桓	Postdoctoral Researcher	University of Massachusetts Medical School
Hsiao-Ying (Monica) Wey	魏曉英	Assistant Professor	Massachusetts General Hospital/Harvard Medical School
Fu-Kai Hsieh	謝富凱	Postdoctoral Researcher	Massachusetts General Hospital/Harvard Medical School

#### Careers in Biotech

Agnes Lin	林穎柔	Senior Associate Scientist	Bluebird bio
Kun-Po Li	李坤珀	Laboratory Supervisor	Sanofi
Freya Liao	廖心瑋	Scientist	Sanofi
Jie-Kai Wu	吳介凱	Scientist	Biolegend
Joyce Chen		BI Analyst	BIB
Sidney Hsieh	謝欣康	Senior Scientist	Merck Co.





## Managing Up, Down and Across

I-Ju Lee	李以如	Postdoctoral Researcher	Dana-Farber Cancer Institute/Harvard Medical School
Ting-Ya Chang	張婷雅	PhD Candidate	Boston University
Priscilla Lee	李靜蓉	Scientist	Sanofi

## Academic Panel Discussion

Jason Lu	盧彥君	Research Fellow	MIT/Boston Children's Hospital
Wei-Ting Kuo	郭瑋庭	Research Fellow	Brigham and Women's Hospital
Chih-Hao Wang	王志豪	Postdoctoral Researcher	Harvard University
Hung-Yi Wu	吳泓儀	Graduate Student	Harvard University
Jerry Lin	林致中	Postdoctoral Researcher	Massachusetts General Hospital

## Breakfast and Meet

Georgia Lin	Project Manager	Siemens Healthcare
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## Biotech Clusters Across the U.S

Yung-Chi Huang	黃詠琪	Postdoctoral Associate	MIT/Picower Institute
Chih-Hao Wang	王志豪	Postdoctoral Researcher	Harvard Medical School/Joslin Diabetes Center
Kun-Po Li	李坤珀	Laboratory Supervisor	Sanofi
Shu-Fan Chou	周書帆	Postdoctoral Researcher	Harvard Medical School

## Trending Topic: Synthetic Biology

Huey-Ming Mak	麥惠明	Strain Engineer	Ginkgo Bioworks
Ming-Yueh Wu	吳明玥	Organism Engineer	Ginkgo Bioworks

## Fundraising

Shu-Fan Chou	周書帆	Postdoc Fellow	Harvard Medical School
Ting-Ya Chang	張婷雅	PhD Candidate	Boston University
Shiao-Chi Chang	張筱琦	Senior Associate Scientist	Pfizer

## Website

Yu-An Chen	陳語安	Research Scientist	Harvard Medical School
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## Promotion and Program book

Kuei-Ting Chen (Tim)	陳奎廷	Data Analyst	Foundation medicine
Amy Kao	高士婷	Graduate Student	Lesley University
Shu-Wei Wu	吳淑璋	Associate Scientist II	Codiak Bioscience





Chun Liu	劉駿		
Chih-Hung Chou	周致宏	Postdoc Associate	Broad Institute of MIT and Harvard
Pei-Ting Chang	張佩婷	Statistical Programmer	Vertex Pharmaceuticals

## Administrative

Hung-Yi Wu	吳泓儀	Graduate Student	Harvard University
Angela Hsia	夏智筠	Postdoctoral Researcher	Harvard Medical School
I-Ting Huang	黃意庭	Graduate Student	Harvard University
Yu-Tien Hsu	許祐活	Research Fellow	Massachusetts General Hospital
Pin-Kuang Lai	賴品光	Postdoc Associate	Massachusetts Institute of Technology
Jacky (Chieh-Chi Chen)	陳杰祺	Software Engineer	TriNetX, Inc.
Jie-Kai Wu (JK)	吳介凱	Scientist	BioLegend
Geng-Min Lin	林庚民	Postdoc Associate	Massachusetts Institute of Technology
Ya-Wen Chen	陳雅文	Research Fellow	Massachusetts General Hospital
Wan-Ling Tsai (Brenda)	蔡宛玲	PhD Student	Boston College
Yupu Wu	吳育蒲	Graduate Student	Harvard
Ming-Shan Kao	高銘杉	PhD Student	Harvard Medical School
Yi-Wei Lee	李翊璋	PhD Student	UMass Amherst
Andrew Hung	洪梓恩	Graduate Student	Boston University
Chun Liu	劉駿		
Chih-Hung Chou	周致宏	Postdoc Associate	Broad Institute of MIT and Harvard
Chih-Hao Wang	王志豪	Postdoc	Harvard University
Wei-Ting Kuo	郭璋庭	Research Fellow	Brigham and Women's Hospital
Tien-yu Hsin	辛天妤	Recent Graduate	Harvard Medical School





## CO-ORGANIZER



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## SPONSORS



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Boston

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ITRI

Industrial Technology  
Research Institute





# DIRECTIONS and PARKING

## Northwest Building, Harvard University 52 Oxford St., Cambridge, MA, 02138

### By Subway:

- Take the MBTA (subway) **RED Line** to **Harvard Square Station**.

### By Train or Bus:

- Amtrak and the bus terminal are located at South Station. Transfer to **RED Line at South Station to Harvard Square Station**.
- Local bus #1 #66 #68 #69 #71 #72 #73 #74 #75 #77 #78 #86 #96 can also bring you to Harvard Square.

### By Car:

- From the Massachusetts Turnpike:** Take Exit 18 (Allston or Brighton/Cambridge). At 2nd traffic light, turn left onto Storrow Drive (Soldiers Field Road). Exit at Harvard Square. Turn right to cross the bridge and you will be on JFK Street headed into Harvard Square.
- From the South (I-93 North):** Head north on Route 93, take the Mass Pike.
- From the North (I-93 South):** Head south on Route 93 exit onto Storrow Drive west. Take Harvard Square/Cambridge exit. Turn right to cross the bridge and you will be on JFK Street headed into Harvard Square.
- From Logan Airport:** As you leave the airport, follow signs for Rt. 90, Massachusetts Turnpike West

### Parking

On-street parking is scarce in Cambridge, but there are several public parking lots and garages around the square.

#### *If it is a rental car,*

We recommend you to check public parking in the Harvard Square:

<http://www.transportation.harvard.edu/parking/visitors/public-parking-square>

#### *If you have your own car,*

We recommend you to use Harvard University Daily Visitor Parking Permits Online Purchase System to purchase your parking permit:

<https://onedaypermit.vpcs.harvard.edu/cgi-bin/permit/purchase.pl>

Please see the next page for the instruction to purchase your parking permit.

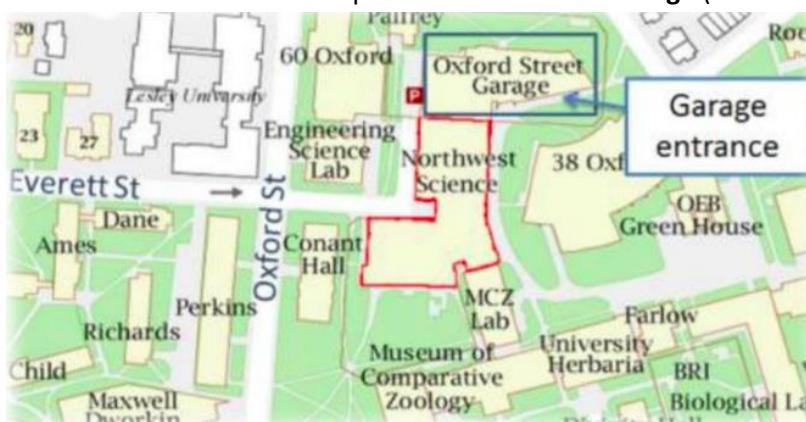




Please note that parking permit can only be purchased from **two weeks to one day in advance**, but **NOT on the day of the event**.

To use the online purchase system, please have your vehicle license plate number ready at hand and then follow the instructions below:

1. First-time users need to register. To complete the registration (as "visitor"), use "**Visitor to Campus**" as your department and department code **7700**.
2. You will receive a confirmation e-mail from Harvard University Daily Visitor Parking Permits Online Purchase System. Click the provided link to confirm registration.
3. You can now log in as a visitor with username and password you just created.
4. You will be asked to provide department information and department code again, which will be the same as in step 1. This information has to be provided every time after you log in.
5. After entering into the system, **select a parking lot** to begin at the bottom of the webpage. We recommend our attendee to park at **52 Oxford St. Garage** (see the map below for its location.)



6. Weekend parking hours are 7am – 11:30pm. Please state yourself as "Event Participant" as your affiliation and specify yourself as "HTSA event participant" in "Adding Parking Permits" form.
7. Choose the intended date(s) to park on campus.
8. Provide your vehicle's plate number and issued state.
9. Hit "Add Parking Permit" button when completed.
10. Confirm/Modify your purchase and then hit "Checkout"
11. Agree with the disclaimer before proceed.
12. After you hit "complete order," you will be re-directed to PayPal.com to pay for the permit.  
**Please note that if errors occur during re-directing, try to use a different browser (different browsers may work on different computers.)** After logging into the system with a different browser, you should be able to find your unfinished order in "My basket" tab.
13. Use either PayPal account or a debit/credit card to finish purchase.
14. Remember to print the permit and bring it with you on the day coming to the symposium. Put the permit on your dashboard before you leave.
15. Enjoy the symposium!





# ***SYMPOSIUM AGENDA - DAY 1***

## **Saturday, August 3, 2019**

<b>8:00 – 9:00</b>	<b>Registration and Poster Setup (B100 Prefunction area)</b>	
<b>9:00 – 9:10</b>	<b>Opening Remarks (Room B103)</b>	
<b>9:10 – 10:10</b>	<b>Keynote Speech: Dr. Seng Cheng</b> <i>Senior Vice President &amp; Chief Scientific Officer, Rare Diseases Research Unit at Pfizer</i> <b>(Room B103)</b>	
<b>10:10 – 10:30</b>	<b>Group Photo &amp; Coffee Break (B100 Prefunction area)</b>	
<b>10:30 – 12:15</b>	<b>Biotech Forum: Drug Modality (Room B103)</b>	
<b>12:15 – 13:45</b>	<b>Lunch &amp; Elevator Pitch (B100 Prefunction area)</b>	
<b>13:45 – 14:45</b>	<b>Careers in Biotech Industry (Room B103)</b>	<b>Academic Panel Discussion (Room B101)</b>
<b>14:45 – 15:45</b>	<b>Managing Up, Down and Across (Room B103)</b>	<b>Academic Roundtable (Room B109 &amp; B110)</b>
<b>15:45 – 17:00</b>	<b>Poster Session (w/ Coffee &amp; Snacks) (B100 Prefunction area)</b>	

\*Please see the venue map after agenda for the location of meeting rooms





## ***SYMPOSIUM AGENDA – DAY 2***

**Sunday, August 4, 2019**

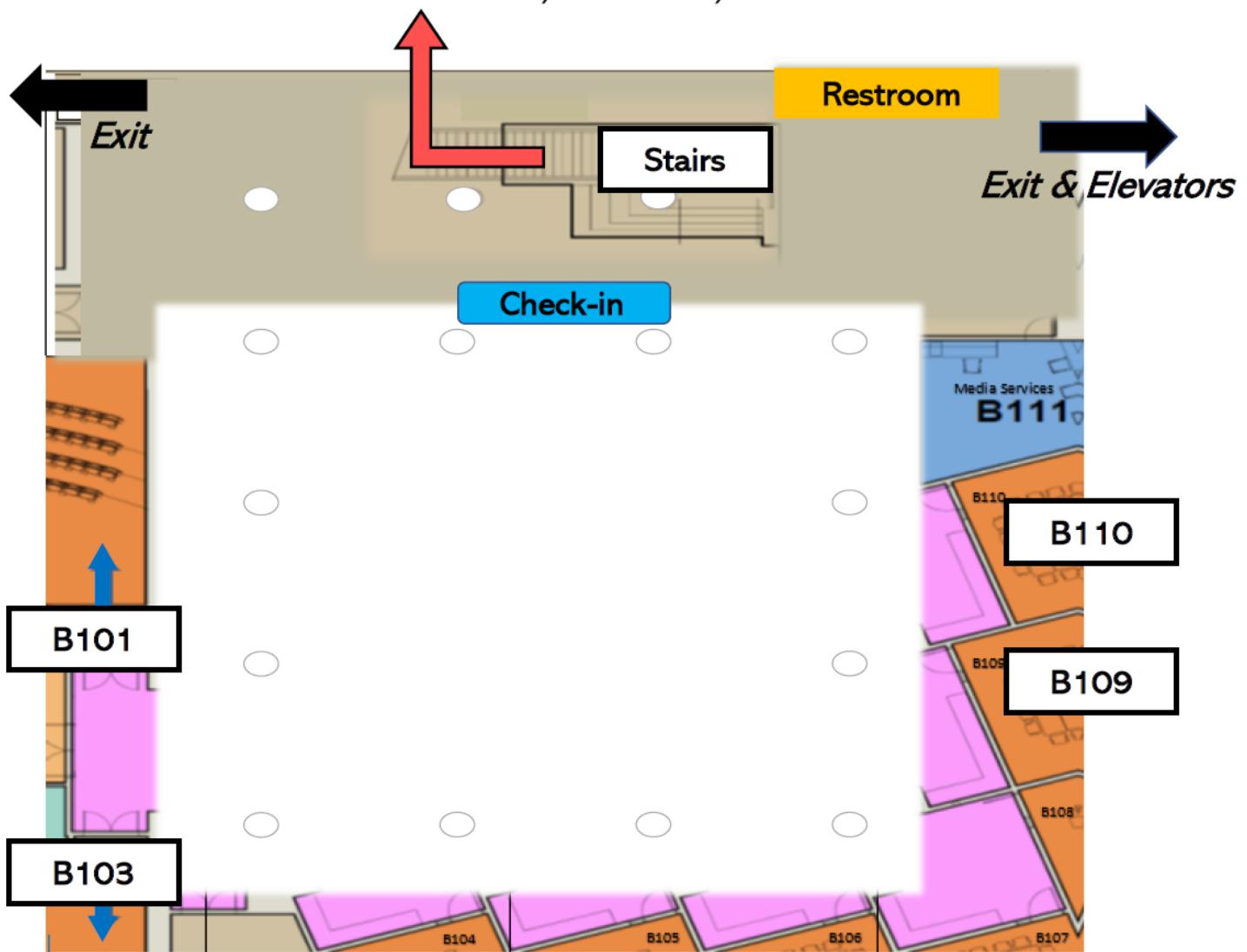
<b>8:00 – 9:00</b>	<b>Breakfast and Meet (B100 Prefunction area)</b>
<b>9:00 – 10:00</b>	<b>Keynote Speech: Dr. Jenny P-Y Ting</b> <i>William Rand Kenan Professor of Genetics, Microbiology and Immunology at UNC-Chapel Hill</i> <b>(Room B103)</b>
<b>10:00 – 10:10</b>	<b>Intermission (B100 Prefunction area)</b>
<b>10:10 – 10:40</b>	<b>Massachusetts Life Science Center (MLSC): Overview of Boston Biotech Ecosystem</b> <b>(Room B103)</b>
<b>10:40 – 11:40</b>	<b>Biotech Clusters Across the U.S</b> <b>(Room B103)</b>
<b>11:40 – 12:40</b>	<b>Trending Topic: Synthetic Biology</b> <b>(Room B103)</b>
<b>12:40 – 13:00</b>	<b>Closing Remarks &amp; Poster Award Ceremony</b> <b>(Room B103)</b>

\*Please see the venue map after agenda for the location of meeting rooms





*1<sup>st</sup> Floor café area, Restroom, Exit*





## KEYNOTE SPEAKER

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**Seng H. Cheng,**

**Ph.D.**

*Senior Vice President & Chief Scientific Officer, Rare Diseases Research Unit at Pfizer*

Dr. Seng H. Cheng, Ph.D., is Senior Vice President and Chief Scientific Officer of the Rare Disease Research Unit at Pfizer. Dr. Cheng obtained his PhD in Biochemistry at the University of London and trained as a postdoctoral fellow at the National Institute of Medical Research, U.K., in the field of tumor biology. He was Group Vice President of Genetic Diseases Science at Genzyme for 25 years researching solutions for patients with rare diseases, notably lysosomal storage disorders and cystic fibrosis. Most recently, he was the Global Head of Research of Rare Diseases at Sanofi where he led researchers in the pursuit of transformative treatments for patients living with rare metabolic, neurological, neuromuscular, renal, pulmonary and hematological diseases.

Dr. Cheng is experienced in bringing projects from discovery through to clinical development and approval. He has co-authored 276 research articles and reviews, and is a named co-inventor on 50 issued US patents in the area of biotechnology. As the Chief Scientific Officer at Pfizer, he is charged with building upon the foundation of rare diseases-associated initiatives at Pfizer, with emphasis on gene therapy as an emerging therapeutic paradigm.





# KEYNOTE SPEAKER

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**Jenny P-Y Ting,**

**Ph.D.**

*William Rand Kenan Professor of Genetics,  
Microbiology and Immunology at UNC-  
Chapel Hill*

Dr. Jenny Ting, Ph.D., is a William Rand Professor of Genetics, Microbiology, and Immunology at the University of North Carolina-Chapel Hill Lineberger Comprehensive Cancer Center. She is also the Director for the Center for Translational Immunology and Co-Director for the Inflammatory Disease Institute at UNC. She received her Ph.D. from Northwestern University.

Dr. Ting's research focuses on the application of molecular biology to unveil new pathways that impact immunity and inflammation. Her group first described the human NLR gene family to include a few known and sixteen new genes, include NLRC3 and NLRP12. In addition to the inflammasome NLRs, she has published numerous papers documenting that two regulate gene expression, including class I and II MHC genes. With translational and clinical relevance, she has published several papers indicating that while expression of the inflammasome NLRs is enhanced inflammatory conditions and cancers. She has also found roles of NLRs which are highly expressed in T cells and exert a regulatory function in T cells. Her work showed that NLR proteins are increasingly found to affect diseases of high impact in human health, including autoimmune disorders, cancer, and metabolic diseases.





## FEATURED SESSION

### Biotech Forum: Drug Modality

Time	Location
Aug. 3 (Sat.) 10:30 - 12:15	Room B103
Description	
<p>This session aims to provide our audience with a general overview of the various modalities of drug research and development in the pharmaceutical industry. From traditional small molecules to biologics and all the way to the new gene therapy and cell therapy. We will discuss basic mechanisms of each modality, delivery route, unique properties and challenges in the discovery/development process. We are excited to have a diverse group of panelists; each has first-hand experiences with these respective drug types. This would be a great discussion! We welcome our audience to bring your specific questions for an interactive session!</p>	



**Chi-Wang Lin**  
Senior research investigator II, Bristol-Myers Squibb



**Yvonne Meng**  
Senior Scientist, Merck & Co.



**April Kuo**  
Scientist II, Acceleron Pharma



**Wei-Chiang Chen**  
Senior Scientist, Solid Biosciences



**Leslie Wu**  
Scientist, Sarepta Therapeutics



**Ho-Chou Tu**  
Senior Scientist, Alnylam Pharmaceuticals



**Hon-Ren Huang**  
Associate Director, Intellia Therapeutics



**DeKuan Chang**  
Senior Scientist, Takeda Pharmaceuticals



**Pam Shou-Ping Wang**  
Scientist, Moderna Therapeutics





## FEATURED SESSION

### Careers in Biotech Industry

Time	Location
Aug. 3 (Sat.) 13:45 - 14:45	Room B103
Description	
<p>As many people dream about entering the biotech/pharmaceutical industry after receiving their science diplomas, do you know what real life is like working in a biotech/pharmaceutical organization? What are the career pathways you can choose and the challenges you will eventually face as you climb the corporate ladder? How to maximize your strengths, expand skills in the industry as well as build your value over time?</p> <p>Come join us for an hour discussion with some industrial pioneers -- from R&amp;D to Patient Safety, Regulatory, Legal, Business Development and Equity Investment. Let's find out how to prepare yourself step by step towards your dream lifestyle!</p>	



**Leyi (Colin) Wang**  
Vice President of Business Development  
at Phoenix Tissue Repair



**Seng-Lai (Thomas) Tan**  
Vice President of R&D at  
Elstar Therapeutics



**Joy Chen**  
Senior Manager of Regulatory  
at Pfizer



**I-Hung Shih**  
Biotech Analyst (equity investment) at  
Wellington Management



**Allyson Hatton**  
Principal Counsel of Biologics  
Patents US at Sanofi



**Curtis Chang**  
Senior Medical Director of Global  
Patient Safety at Takeda

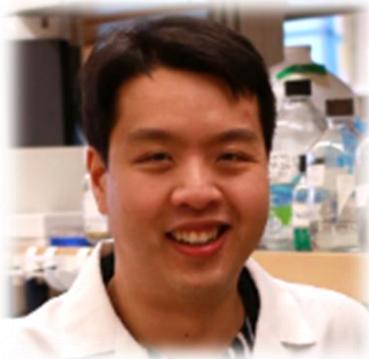




## FEATURED SESSION

### Academic Panel Discussion

Time	Location
Aug. 3 (Sat.) 13:45 - 14:45	Room B101
Description	
<p>Do you wonder what a career in academia would be like and how to be successful in this path? Join us in an academic panel featuring professors with rich experiences, who will share their tips on how to thrive in this rapidly expanding field of academia!</p>	



**Isaac M. Chiu**  
Assistant professor at Harvard Medical School,  
Department of Immunology



**Gene-Wei Li**  
Assistant professor at MIT,  
Department of Biology



**Chih-Hao Lee**  
Professor, Department of Genetics and Complex  
Diseases, Division of Biological Sciences,  
Harvard T.H. Chan School of Public Health



**Lee-Yuan Liu-Chen**  
Professor of Pharmacology at Center for  
Substance Abuse Research, Lewis Katz School  
of Medicine, Temple University





## FEATURED SESSION

### Managing Up, Down and Across

Time	Location
Aug. 3 (Sat.) 14:45 - 15:45	Room B103
Description	
<p>Trained as scientists, we pursue a better understanding of the world and aim to improve human well-being. In the real world, achieving these goals often takes the effort from the whole team, if not multiple teams. Graduate school never taught us how to work with people! Worry not. In this session, we invited 4 respected professionals from both the academia and the industry to share with us their experiences in managing or collaborating with groups of different sizes and expertise. Our panelists will also be giving practical advice for young professionals to advance their managing skills strategically. Come join us and learn how to build a team, resolve conflicts, and manage up.</p>	



**Albert Hwa**  
Operations Director, Joslin's Center  
for Cell-Based Therapy for Diabetes



**Lih-Ling Lin**  
Cluster Head, Immunology and  
Inflammation, Sanofi



**Mary Ellen Lane**  
Dean of the Graduate School of  
Biomedical Sciences, University of  
Massachusetts Medical School

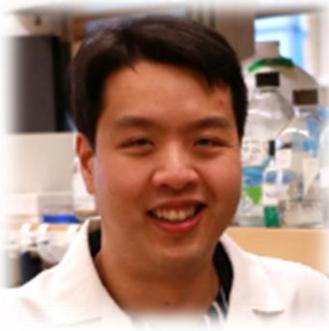




## FEATURED SESSION

### Academic Roundtable

Time	Location
Aug. 3 (Sat.) 14:45 - 15:45	Room B109 & B110
Description	
Would you like to ask more questions about the academic field? The roundtable discussion offers a chance to network with outstanding professors in a small group setting, allowing you to get insights among local academic circumstance as well as to discover and develop your strengths!	



**Isaac M. Chiu**  
Assistant professor at Harvard Medical School,  
Department of Immunology



**Gene-Wei Li**  
Assistant professor at MIT,  
Department of Biology



**Chih-Hao Lee**  
Professor, Department of Genetics and Complex  
Diseases, Division of Biological Sciences,  
Harvard T.H. Chan School of Public Health



**Lee-Yuan Liu-Chen**  
Professor of Pharmacology at Center for  
Substance Abuse Research, Lewis Katz School  
of Medicine, Temple University





## FEATURED SESSION

### Breakfast and Meet

Time	Location
Aug. 4 (Sun.) 8:00 - 9:00	B100 Prefunction area

Are you graduating and thinking about landing a job in the biotech industry? Are you considering a career change but don't know what options you have? We understand that the job hunting process can often feel frustrating and overwhelming! Come to our networking session to chat with young professionals with advanced degrees. Learn about useful skills to get your first job and explore the different fields in the biotech industry.



**Jimmy Chu**  
Scientist I, mRNA Process Development at Bluebird Bio



**Chien-Chung (James) Wang**  
Senior Supervisor at Siemens Healthineer



**Kuei Ting (Tim) Chen**  
Clinical Bioinformatics Analyst at Foundation Medicine



**Jun-Yuan Huang**  
Research Scientist at CuraCloud Corporation



**Eric Chen**  
Principal Design Quality Engineer at Cardinal Health



**Yian Ruan**  
Quality Analyst at Siemens Healthineer



**Jessica Huang**  
Senior Associate Scientist II, Analytical Development at Solid Biosciences



**Yi-Dong Lin**  
Scientist / Flow Cytometry Core Manager at Sanofi



**Pi-i Debby Lin**  
Research Scientist/Harvard Pilgrim Health Care Institute



**Ling-Fang Tseng**  
Preclinical Scientist II/Lyra Therapeutics



**Po-Jen (Will) Yen**  
Business Development professional/Voyager Therapeutics





## FEATURED SESSION

### Massachusetts Life Science Center (MLSC): Overview of Boston Biotech Ecosystem

Time	Location
Aug. 4 (Sun.) 10:10 - 10:40	Room B103
Description	
<p>Have you ever heard of Biotech clusters? These clusters are geographic regions active in bioscience and can range from small emerging groups to large, full-blown clusters like Boston. Despite the commonly shared key factors to make a top-tier biocluster: Talents and Capital. Each cluster has its advantages and focuses. In this session, we are honored to have Mass Life Science Center (MLSC) to give us an overview of Boston Biotech Ecosystem explaining why the Great Boston is the most successful Biotech Cluster.</p>	



**Sandhya Iyer**  
*Associate Director of Business Development,  
Massachusetts Life Sciences Center*





## FEATURED SESSION

### Biotech Clusters Across the U.S

Time	Location
Aug. 4 (Sun.) 10:40 - 11:40	Room B103
Description	
<p>Have you ever heard of Biotech clusters? These clusters are geographic regions active in bioscience and can range from small emerging groups to large, full-blown clusters like Boston. Despite the commonly shared key factors to make a top-tier biocluster: Talents and Capital. Each cluster has its advantages and focuses. In this session, three young professionals across the U.S will share their valuable experiences and observations of their local biotech environments. Join us to learn more about the culture and career opportunities in these biotech clusters!</p>	



**Chih-Hsu (Jack) Lin**  
TTBA president | Bioinformatics Intern at Illumina | PhD Candidate at Baylor College of Medicine



**Hao-Wei Chang**  
MTBA president, Graduate Student at Washington University in St. Louis



**Eric Du**  
BATBA core member, Senior Project Management Associate at Gilead Sciences





## FEATURED SESSION

### Trending Topic: Synthetic Biology

Time	Location
Aug. 4 (Sun.) 11:40 - 12:40	Room B103
Description	
<p><b>Imagine</b> a future where microbe acts as fertilizers for cereal crops, where wastewater is purified by bacteria, where cultured ingredients are produced by microbes in a sustainable way, and genetic diseases are cured with engineered microorganisms. Welcome to the world of synthetic biology, an innovative field that brings chemistry, biology, engineering, and computer science together. Join us as we explore this multi-billion-dollar industry that is making a huge impact across agriculture, energy, pharmaceutical, and manufacturing industries, and learn about its present and promising future.</p>	



**Dawn Thompson**  
Chief Scientific Officer at Directed Genomics



**Sergio Florez**  
Senior Fermentation Engineer at Ginkgo Bioworks



**Felipe Sarmiento**  
Associate Director, Commercial Operations at Ginkgo Bioworks



**Chin Giaw (Ryan) Lim**  
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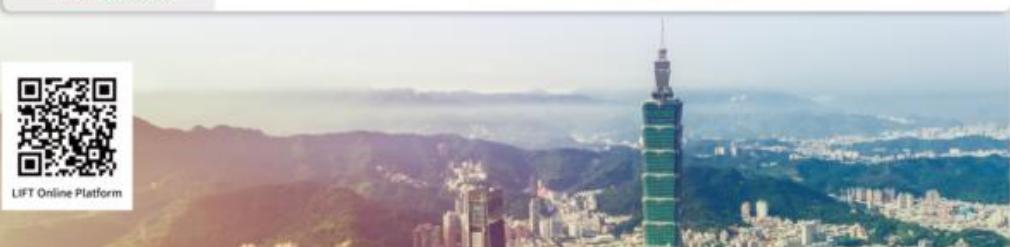


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## Elevator Pitch & Abstracts

Number	Title and Presenter
1	<b>Genome scale <i>in vivo</i> CRISPR screen identifies PHX2 as a modifier of beta cell vulnerability in type 1 diabetes</b> Erica P. Cai, Section for Islet Cell and Regenerative Biology, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA
2	<b>Synthetic sequence entanglement augments stability and containment of genetic information in cells</b> Hsing-I Ho, Department of Systems Biology, Columbia University, New York, NY, USA.
3	<b>Screening for targets promoting regeneration after injury using human iPSC-derived neurons</b> Tammy Szu-Yu Ho, Kirby Neurobiology Center, Boston Children's Hospital and Department of Neurobiology, Harvard Medical School, Boston, 02115, MA, USA,
4	<b>Confined Migration Induces Heterochromatin Formation in Cancer Cells</b> Chieh-Ren Hsia, Department of Molecular Biology and Genetics and Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY 14853, USA
5	<b>Revealing Inhibitory Effects of Small-molecule Drugs on Amyloid-forming Peptides Using Computational Modeling Approaches</b> Chungwen Liang, Computational Modeling Core Facility, Institute for Applied Life Sciences (IALS), University of Massachusetts Amherst, Amherst, MA 01003, USA
6	<b>Identification of <i>Escherichia coli</i> ClpAP in regulating susceptibility to type VI secretion system-mediated attack by <i>Agrobacterium tumefaciens</i></b> Hsiao-Han Lin, Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan; Institute of Biotechnology, National Taiwan University, No. 81, Chang-Xing St., Taipei 10617, Taiwan
7	<b>Muscle as an antigen reservoir in IDLV induced long term immunity</b> Yi-Yu Lin, Department of Medicine and Duke Human Vaccine Institute, Duke University Medical Center, Durham, 27710 NC, USA
8	<b>CTL-derived exosomes enhance the activation of CTLs stimulated by low affinity peptides</b> Shu-Wei Wu, Department of Animal and Avian Sciences, University of Maryland, College Park, MD, United States



## Genome scale *in vivo* CRISPR screen identifies *PHX2* as a modifier of beta cell vulnerability in type 1 diabetes

Erica P. Cai<sup>1</sup>, Yuki Ishikawa<sup>2</sup>, Wei Zhang<sup>2</sup>, Nayara C. Leite<sup>3</sup>, Jian Li<sup>1</sup>, Badr Kiaf<sup>2</sup>, Jennifer Hollister-Lock<sup>1</sup>, Doug A. Melton<sup>3</sup>, Stephan Kissler<sup>2</sup> & Peng Yi<sup>1</sup>

<sup>1</sup>Section for Islet Cell and Regenerative Biology and <sup>2</sup>Section for Immunobiology, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA

<sup>3</sup>Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Harvard University, Cambridge, MA 02138, USA

Insulin deficiency in type 1 diabetes (T1D) is caused by the immune-mediated destruction of pancreatic beta cells<sup>1</sup>. Advances in pluripotent stem cell differentiation protocols have raised the prospect of restoring insulin sufficiency in T1D using stem cell-derived beta cells<sup>2</sup>. However, recurring autoimmunity remains a critical hurdle to a successful beta cell replacement therapy. In an effort to overcome this hurdle, we performed a genome-scale CRISPR screen *in vivo* to identify gene edits capable of protecting beta cells against autoimmunity. Here we report that the disruption of *PHX2*, a gene previously implicated in T1D by GWAS<sup>3,4</sup>, confers resistance to ER stress-mediated cell death and protects beta cells against immune-mediated killing in a mouse model for T1D. Deleting *Phx2* diminished the unfolded protein response upon cellular stress and promoted beta cell survival. These effects were replicated in *PHX2* deficient human stem cell-derived beta cells. Our results identify *PHX2* as a novel modifier of beta cell vulnerability and suggest that a gene edit that confers ER stress resistance could avert immune-mediated beta cell loss in T1D without compromising immunity and immune surveillance.

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# Synthetic sequence entanglement augments stability and containment of genetic information in cells

Hsing-I Ho<sup>1,‡</sup>, Tomasz Blazejewski<sup>1,‡</sup>, Harris H. Wang<sup>1</sup>

<sup>1</sup>Department of Systems Biology, Columbia University, New York, NY, USA.

**Abstract:** Stabilizing genetically engineered functions and confining recombinant DNA to intended hosts are key challenges for synthetic biology when facing natural mutation accumulation and pervasive lateral gene flow. Here we present a generalizable strategy to preserve and constrain genetic information through computational design of overlapping genes. Overlapping a sequence with an essential gene altered its fitness landscape and produced a constrained evolutionary path even for synonymous mutations. Embedding a toxin gene in a gene of interest restricted its horizontal propagation. We further demonstrated a multiplex and scalable approach to build and test >7,500 overlapping sequence designs, yielding functional yet highly divergent variants from natural homologs. This work enables deeper exploration of natural and engineered overlapping genes and facilitates enhanced genetic stability and biocontainment in emerging applications.

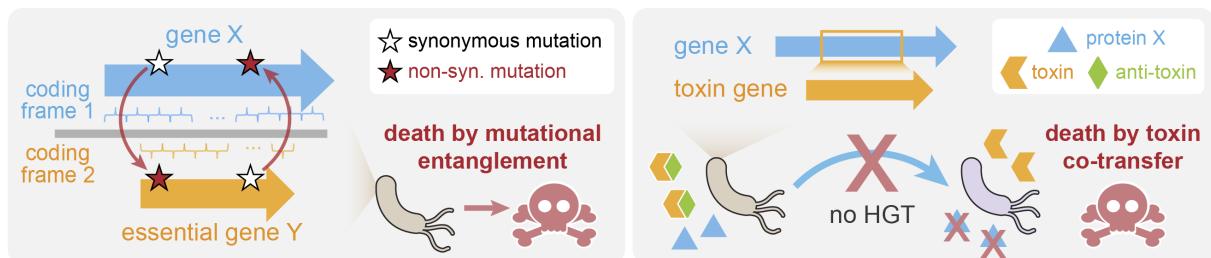


Figure 1. Schematic of mutational restriction or horizontal transfer confinement due to engineered sequence entanglement of two genes.

# **Screening for targets promoting regeneration after injury using human iPSC-derived neurons**

Tammy Szu-Yu Ho<sup>1</sup>, Bhagat Singh<sup>1</sup>, Zhuqiu Xu<sup>2</sup>, Tabitha Hees<sup>3</sup>, Yung-Chih Cheng<sup>1</sup>, Clifford J. Woolf<sup>1</sup>

<sup>1</sup>Kirby Neurobiology Center, Boston Children's Hospital and Department of Neurobiology, Harvard Medical School, Boston, 02115, MA, USA, <sup>2</sup>Plastic Surgery Hospital, Peking Union Medical College, Beijing, China,

<sup>3</sup>Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands

Once neurons in the brain and spinal cord are damaged after injury or in degenerative diseases, they cannot regrow and thus lead to permanent functional deficits. Currently there is no clinically approved drug treatment targeting regeneration of damaged axons. Progress in developing therapies to promote axon regeneration has been slow, which may be contributed to by the lack of robust human-relevant assays suitable for large scale drug screens. We have developed a high throughput phenotypic screen using human induced pluripotent stem cell (iPSC)-derived neurons to identify targets for promoting regeneration. We are able to generate large numbers of human derived motor and cortical neurons consistently and efficiently for drug screening. The differentiated neurons undergo an axonal injury surrogate by re-plating and are cultured on a major component of the extracellular matrix in the injured central nervous system. The bioactive small molecule libraries we use are composed of target-annotated compounds for identifying novel pathways regulating regeneration and for compounds that have potential for drug development. After we identify positive hits, we run secondary screens including studying the drug effects over time in a novel live human neuron spot culture assay. Furthermore, we perform laser cutting the axons of human neuron spots and study live axon regeneration after drug treatment. The new “injury in a dish” model also have high-throughput ability for drug screening. From a screen of more than 10,000 compounds we have identified some promising hits that dramatically promotes axon regrowth in human induced neurons. In addition, we have shown that some top hits promotes axon regeneration after a sciatic nerve injury model *in vivo*. Our goal is to find new therapeutic potentials for axon regeneration after injury and diseases.

# Confined Migration Induces Heterochromatin Formation in Cancer Cells

Chieh-Ren Hsia<sup>1,2</sup>, Ovais Hasan<sup>2</sup>, Chao-Yuan Chang<sup>2,3</sup>, and Jan Lammerding<sup>1,2,3</sup>

<sup>1</sup> Department of Molecular Biology and Genetics, <sup>2</sup>Weill Institute for Cell and Molecular Biology,

<sup>3</sup>Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY 14853, USA

During cancer metastasis, cancer cells migrate through confined interstitial spaces, requiring extensive deformation of the cell body and nucleus. The severe physical stress on the nucleus during this frequently results in rupture of the nuclear envelope (NE) and nuclear fragmentation<sup>1</sup>. Previous studies found that mechanical force application on cells can induce mechanosensitive gene expression<sup>2</sup> and chromatin rearrangement<sup>3</sup>. However, it remains unclear whether physical stress on the nucleus during confined migration can induce histone modification and gene expression changes. Here, we performed live-cell and immunofluorescence imaging of HT1080 fibrosarcoma and MDA-MB-231 breast cancer cells migrating through collagen gel matrices and custom-made microfluidic migration devices that mimic interstitial spaces *in vivo*. Cell migration in confined 3D environments resulted in increased H3K9me3 and H3K27me3 heterochromatin marks and DNA methylation (5-methylcytosine) compared to unconfined conditions. The heterochromatin enrichment was particularly prominent in cells with visible nuclear deformation and in chromatin protrusions that formed inside nuclear blebs. In addition, we observed GFP-labeled HP1 $\alpha$  enrichment within nuclear blebs in HT1080 and MDA-MB-231 cells during confined migration. By using a fluorescence recovery after photobleaching (FRAP) assay, we verified those GFP-HP1 $\alpha$  enrichments to be heterochromatin. Treating cells with a pan histone methyltransferase inhibitor 3-Deazaneplanocin A (DZnep) significantly reduced the extent of heterochromatin formation after confined migration, suggesting an active enzymatic process underlying the increased heterochromatin formation. In particular, enriched phosphorylated (pSer424) HDAC3 nuclear staining suggested activation of HDAC3, potentially due to the mechanical force cells encountered during confined migration. Consistent with this idea, chemical inhibition of HDAC3 significantly reduced heterochromatin formation during confined migration. Interestingly, cells treated with DZnep showed significantly slower migration when compared to control treatment, suggesting the importance of chromatin condensation for efficient migration in 3D environment. Taken together, our research indicates that migration of cancer cells through confined spaces can induce heterochromatin formation, which is expected to both alter the physical properties of the nucleus and to modulate gene expression. These modifications could promote further metastatic cancer progression. Targeting the ability of chromatin condensation upon confined migration may potentially serve as a novel approach in treating metastatic cancers.

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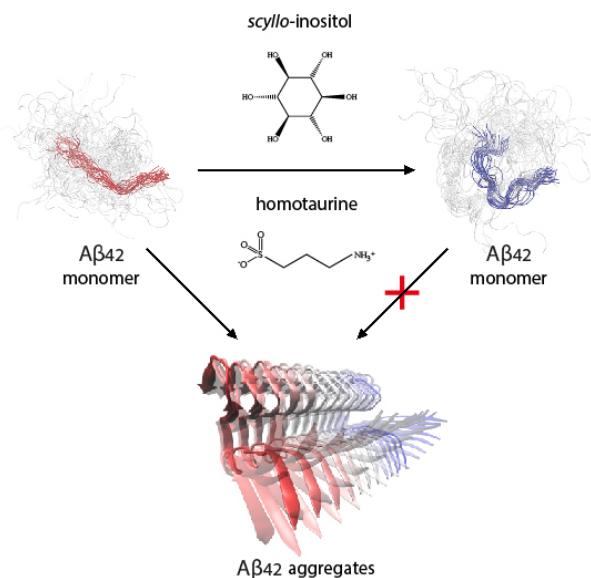
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# Revealing Inhibitory Effects of Small-molecule Drugs on Amyloid-forming Peptides Using Computational Modeling Approaches

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Molecular modeling and simulation provide crucial insights into the structure, dynamics, and interactions of various systems at the molecular level, to both complement and enrich experimental measurements. Our recent work focuses on identifying the key interaction between small-molecule drugs and amyloid forming proteins. Aggregation of amyloid- $\beta$  (A $\beta$ ) peptides from soluble monomers to insoluble amyloid fibrils has been hypothesized to be one of the crucial steps in the progression of Alzheimer's disease (AD). Due to the disordered nature of A $\beta$  peptides, identifying aggregation inhibitors as potential drug candidates against AD has been a great challenge. We identified the inhibition mechanism of two small molecules, homotaurine and *scyllo*-inositol, which are AD drug candidates currently under investigation. Using a replica exchange molecular dynamics method to extensively explore the conformational space of A $\beta$ 42 monomer with and without the presence of the small molecule agents, we found that both drug candidates reduce the  $\beta$ -strand propensity of the C-terminus region (I31-A42) and promote a conformational change of A $\beta$ 42 monomer toward a more collapsed phase through a non-specific binding mechanism. These findings provide atomistic-level insights into understanding of the inhibitory mechanisms of two potential small-molecule drug candidates for AD treatment in the future.



## Identification of *Escherichia coli* ClpAP in regulating susceptibility to type VI secretion system-mediated attack by *Agrobacterium tumefaciens*

Hsiao-Han Lin<sup>1,2</sup>, Manda Yu<sup>1</sup>, Manoj Kumar Sriramoju<sup>3</sup>, Shang-Te Danny Hsu<sup>3</sup>, Chi-Te Liu<sup>2,4\*</sup>, Erh-Min Lai<sup>1\*</sup>

<sup>1</sup>Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan; <sup>2</sup>Institute of Biotechnology, National Taiwan University, No. 81, Chang-Xing St., Taipei 10617, Taiwan; <sup>3</sup>Institute of Biological Chemistry, and the <sup>4</sup>Agricultural Biotechnology Research Center, Academia Sinica, Taipei 11529, Taiwan

Type VI secretion system (T6SS) is an effector delivery system used by gram-negative bacteria to kill other bacteria or eukaryotic host to gain fitness. In *Agrobacterium tumefaciens*, T6SS has been shown to kill other bacteria such as *Escherichia coli* [1]. Interestingly, the *A. tumefaciens* T6SS killing efficiency differs when using different *E. coli* strains as recipient cells. Thus, we hypothesize that a successful T6SS killing not only relies on attacker T6SS activity but also depends on recipient factors. To test the hypothesis, a high-throughput interbacterial competition assay was employed to screen for mutants with reduced killing outcomes caused by *A. tumefaciens* strain C58. From the 3909 *E. coli* Keio mutants screened, 16 candidate mutants were filtered out. One strain,  $\Delta clpP::\text{Kan}$ , showed ten times more resistant to T6SS-mediating killing but restored its susceptibility when complemented with *clpP* in *trans*. ClpP is a universal and highly conserved protease that is found in both prokaryotes and eukaryotic organelles. In *E. coli*, ClpP uses either ClpA or ClpX as its adaptor for substrate specificity. Therefore, the susceptibility of  $\Delta clpA::\text{Kan}$  and  $\Delta clpX::\text{Kan}$  was also tested. The T6SS attack susceptibility of  $\Delta clpA::\text{Kan}$  is at the same level as  $\Delta clpP::\text{Kan}$ , while  $\Delta clpX::\text{Kan}$  showed no difference as compared to that of wild-type BW25113 (Figure 1). The data also suggest that ClpA-ClpP interaction, rather than its protease activity, is responsible for enhancing susceptibility to T6SS killing. This study highlights the importance of recipient factors in determining the outcome of T6SS killing.

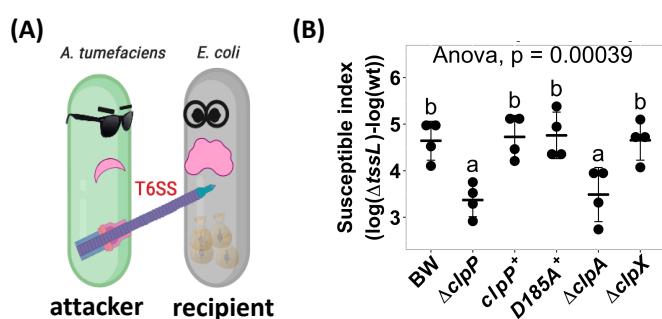


Figure 1. Recipient ClpP but not its protease activity, together with ClpA, plays a critical role in enhancing *A. tumefaciens* T6SS killing outcome. (A) Cartoon illustration of the *A. tumefaciens* T6SS killing against *E. coli*. (B) *A. tumefaciens* T6SS killing against *E. coli*  $\Delta clpP$  and its complement strain,  $\Delta clpA$  and  $\Delta clpX$ . The *A. tumefaciens* and the *E. coli* were co-cultured on AK agar medium for 16 h. Afterwards, the recovery of *E. coli* strains was quantified and the susceptible index (SI) was calculated by subtraction difference of the recovered log(cfu) of that attacked by  $\Delta tssL$  to that by wild type *A. tumefaciens* C58.

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## Muscle as an antigen reservoir in IDLV induced long term immunity

Yi-Yu Lin<sup>1,2</sup>, Ian Belle<sup>1</sup>, Maria Blasi<sup>1,2</sup>, Mary Klotman<sup>1,2</sup>, Andrea Cara<sup>1,2,3</sup>, Donatella Negri<sup>1,2,4</sup>

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The development of vaccines eliciting protective and durable immune response remains a global health priority. We developed Integrase-Defective Lentiviral Vectors (IDLVs) as efficient delivery of strategic immunogens, able to induce a strong and persistent immune response in mice and non-human primates<sup>1,2</sup>. Here, we evaluated the association between the persistence of antigen expression *in vivo* and the long-term immunity induced by IDLV. Following a single intramuscular (IM) or subcutaneous (SC) injection of IDLV delivering GFP as a model antigen in mice, we evaluated antigen expression, cellular infiltration at the site of injection and persistence of antigen-specific T cell response by IFN $\gamma$  ELISpot at early and late time points. IM immunization with IDLV-GFP resulted in durable antigen expression in the muscle up to 90 days. Conversely, antigen expression was detectable in the skin only at early time points after SC immunization. Moderate cellular infiltration was evident soon after the injection in all injected mice and detectable up to 30 days at lower levels only in IM immunized mice. The GFP-specific T cell response was more persistent in IM compared to SC injected mice. Interestingly, we identified GFP positive muscle cells expressing MHC I *in vivo* and we demonstrated *in vitro* that primary myoblasts and myocytes are able to present the antigen to GFP-specific T effector cells through MHC I. We also demonstrate that myocytes but not myoblasts are sensitive to Fas-dependent CTL killing activity. Overall these data indicate that muscle cells may serve as “antigen reservoir” for maintaining the long term immunity induced by IDLV.

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## **CTL-derived exosomes enhance the activation of CTLs stimulated by low affinity peptides**

Shu-Wei Wu\*, Lei Li\* and Zhengguo Xiao\*

\*Department of Animal and Avian Sciences, University of Maryland, College Park, MD, United States

CTLs (cytotoxic T cells) with low affinity to peptides presented by MHC I (pMHC) induce and maintain a detectable and most time substantial population during an adaptive immune response, although CTLs with the highest affinity receive competitive activation signals. Low-affinity CTLs are important to induce effector response and retain a diverse memory repertoire against viral infection and cancer. However, the mechanism of generating and maintaining the expansion of lower affinity CTLs is still unknown. We hypothesized that exosome (Exo), the secreted extracellular vesicles, can deliver a message of high affinity to low-affinity T cells to enhance the activation of CTLs. Here, naïve CTLs were first purified and stimulated with high-affinity peptide, co-stimulators, and cytokine, interleukin-12. The supernatant was collected and isolated the Exo through differential centrifugation. The purified Exo was characterized and further used to stimulate naïve CTLs. The results showed that the CTLs with the highest affinity underwent dramatic expansion, expressed higher activation marker, increased the production of Granzyme B (GZB) and IFN- $\gamma$ , and more importantly, they could produce the functional Exo. The Exo was 30-150nm and expressed typical exosomal protein markers. Next, naïve CTLs were treated with peptides with diverse affinities and with/without the purified Exo. The treatment with the Exo together with peptide produced stronger proliferation and increased the expression of T cell functional molecules (CD25, GZB, IFN- $\gamma$ ) than treatment with peptide only. Moreover, the Exo had more profound effects on the group of CTLs treated with lower affinity peptides than the group treated with higher affinity peptides. Also, a critical transcriptional regulator of T cell activation, T-bet, was upregulated in the treatment with low-affinity peptides and Exo. Together, these results identified the important role of the Exo derived from the high affinity CTLs in activating the CTLs stimulated with lower affinity. This research provided the distinct mechanism of activating low-affinity CTLs and sustaining immune response during infection.

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## Posters & Abstracts

Poster Board Number	Title and Presenter
1	<b>Can Artificial Intelligence See the Pain? Assessment of Knee Pain from MRI Imaging using a deep Siamese network</b> Gary H. Chang, Section of Computational Biomedicine, Department of Medicine, Boston University School of Medicine, Boston, MA, USA – 02118
2	<b>Impaired cell cycle progression and self-renewal of fetal neural stem cells in mouse fetus with intrauterine growth restriction (IUGR)</b> Chu-Yen Chen, Department of Pediatrics, University of Kansas Medical Center, Kansas City, KS
3	<b>Kinetochoore tension induces microtubule unbinding but inhibits depolymerization upon activating Aurora B kinase</b> Geng-Yuan Chen, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA
4	<b>In vivo three-photon excited fluorescence imaging in the spinal cord of awake, locomoting mice</b> Yu-Ting Cheng, Department of Neurobiology and Behavior, Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY
5	<b>AlleleHMM: a data-driven method to identify allele specific differences in distributed functional genomic marks</b> Shao-Pei Chou, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA; Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA,
6	<b>Ventromedial prefrontal parvalbumin neurons signal active avoidance</b> Yi-Yun Ho, Neurobiology & Behavior, Cornell University, Ithaca, NY
7	<b>Confined Migration Induces Heterochromatin Formation in Cancer Cells</b> Chieh-Ren Hsia, Department of Molecular Biology and Genetics and Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY 14853, USA
8	<b>Label-free chemical image cytometry reveals metabolic signatures of cancer cells under stress</b> Kai-Chih Huang, Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA.
9	<b>Identification of C2CD4A as a human diabetes susceptibility gene with a role in <math>\beta</math>-cell insulin secretion</b> Taiyi Kuo, Department of Medicine and Berrie Diabetes Center, Columbia University College of Physicians and Surgeons, New York, NY; Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA





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**10 Highly Selective *in vivo* Editing of Macrophages through Systemic Delivery of CRISPR-Cas9-Ribonucleoprotein-Nanoparticle Nanoassemblies**

Yi-Wei Lee, Department of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, Massachusetts, 01003, U.S.A.

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**11 Functional Studies of Bacterial Stress Response Metabolite AHRAs**

Jhe-Hao Li, Department of Chemistry, Yale University, New Haven, Connecticut, 06516, USA

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**12 Revealing Inhibitory Effects of Small-molecule Drugs on Amyloid-forming Peptides Using Computational Modeling Approaches**

Chungwen Liang, Computational Modeling Core Facility, Institute for Applied Life Sciences (IALS), University of Massachusetts Amherst, Amherst, MA 01003, USA

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**13 Inhibition of FGFR1 in Breast Cancer Metastasis**

Hang Lin, Purdue University Department of Medicinal Chemistry and Molecular Pharmacology, Purdue Center of Cancer Research, Purdue University, West Lafayette, IN 47907, USA

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**14 Identification of Escherichia coli ClpAP in regulating susceptibility to type VI secretion system-mediated attack by *Agrobacterium tumefaciens***

Hsiao-Han Lin, Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan; Institute of Biotechnology, National Taiwan University, No. 81, Chang-Xing St., Taipei 10617, Taiwan

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**15 Muscle as an antigen reservoir in IDLV induced long term immunity**

Yi-Yu Lin, Department of Medicine and Duke Human Vaccine Institute, Duke University Medical Center, Durham, 27710 NC, USA

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**16 The dynamics and structural interactions between p53 and RNA Polymerase II by using Single Molecule Tracking and Cryo-EM**

Shu-hao Liou, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY

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**17 Identification of cancer drivers at CTCF insulator in 1962 whole genomes**

Eric Minwei Liu, Meyer Cancer Center, Weill Cornell Medicine, New York, New York 10065, USA; Department of Physiology and Biophysics, Weill Cornell Medicine, New York, New York 10065, USA; Institute for Computational Biomedicine, Weill Cornell Medicine, New York, New York 10021, USA;

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**18 SRNS-associated *MYO1E* mutations have differential effects on Myo1e activity and stability**

Pei-Ju Liu, Department of Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY, United States

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**19 Immunity to Invasive Infection by Intestinal Commensal Microbes**

Tzu-Yu Shao, Division of Infectious Diseases, Immunology Graduate Program, Cincinnati Children's Hospital, University of Cincinnati

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**Identification of cellular mechanisms for memory by cell-type-specific RNA-seq of *Drosophila* neurons**

**20**

Meng-Fu Maxwell Shih, Anesthesiology, Stony Brook School of Medicine, Stony Brook University

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**Regulation of HIV-1 gene expression by post-transcriptional acetylation of cytidine in viral RNA transcripts**

Kevin Tsai, Department of Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC, USA

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**22**

**CTL-derived exosomes enhance the activation of CTLs stimulated by low affinity peptides**

Shu-Wei Wu, Department of Animal and Avian Sciences, University of Maryland, College Park, MD, United States

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**23**

**The Hippo Pathway Effector TAZ Regulates Ferroptosis in Renal Cell Carcinoma**

Wen-Hsuan Yang, Department of Molecular Genetics and Microbiology, Center for Genomic and Computational Biology, Department of Biochemistry, Department of Medicine, Duke University School of Medicine, Durham, NC 27710, USA

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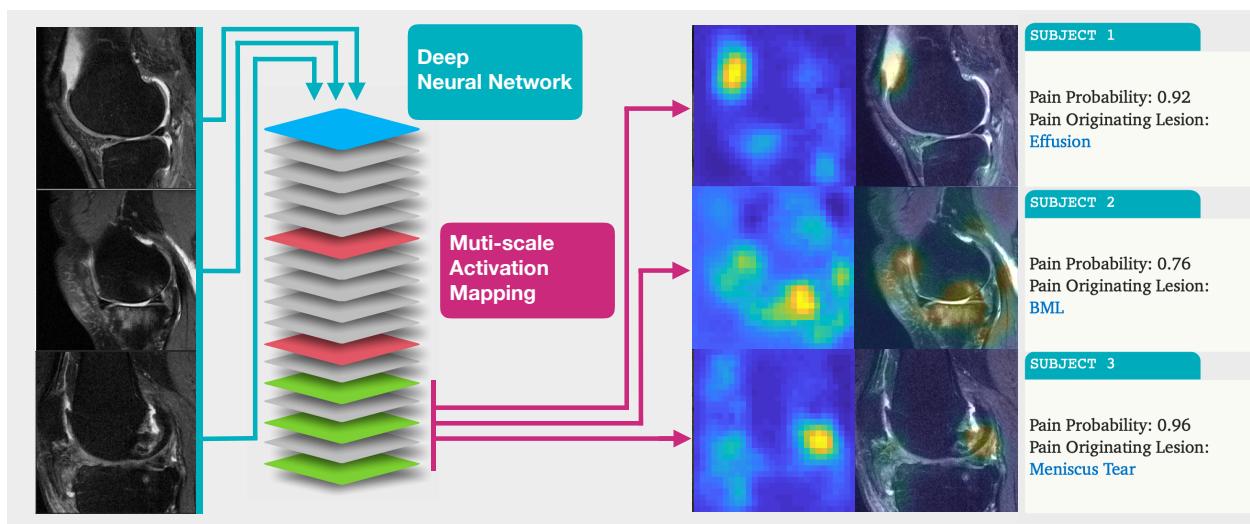
# Can Artificial Intelligence See the Pain? Assessment of Knee Pain from MRI Imaging Using a Deep Siamese Network

Gary H. Chang and Vijaya B. Kolachalama

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Despite significant efforts by several studies, the existence of knee pain has not been found to be strongly correlated with radiographic osteoarthritis (OA). On the other hand, recent studies have attempted to correlate knee pain with MRI findings but these findings have been inconsistent and success has been only moderate. This limited the development of targeted treatment as lesions associated with pain are hard to be localized and specified. We therefore developed a machine learning (ML) approach to objectively and accurately associate MR imaging with knee pain, independent of other risk factors.

The Siamese network architecture enabled pairwise learning of information from two-dimensional (2D) sagittal intermediate-weighted turbo spin echo slices obtained from similar locations on both knees. Class activation mapping (CAM) was utilized to create saliency maps, which highlighted the regions most associated with knee pain. The MRI scans and the CAMs of each subject were reviewed by a radiologist to identify the presence of abnormalities within the model-predicted regions of high association. Using 10-fold cross validation, our model achieved an area under curve (AUC) value of 0.808. When individuals whose knee WOMAC pain scores were not discordant were excluded, model performance increased to 0.853. The radiologist review revealed that about 86% of the cases that were predicted correctly had effusion-synovitis within the regions that were most relevant to pain. To the best of our knowledge, our work is the first to leverage a deep learning framework to associate MR images of the knee with knee pain.



# **Impaired cell cycle progression and self-renewal of fetal neural stem cells in mouse fetus with intrauterine growth restriction (IUGR)**

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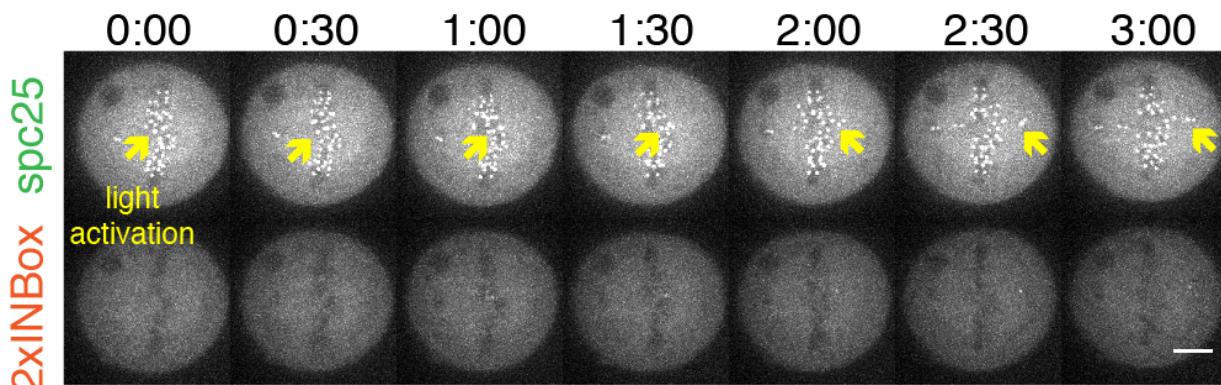
Individuals with IUGR are at significantly increased risk for neurodevelopmental abnormalities. Clinical imaging showed decreased total brain and cortical grey matter volume in IUGR infants, suggesting decreased neurogenesis. Fetal cortical neurogenesis is a time-sensitive process in which fetal NSCs follow a distinct pattern of layer-specific neuron generation to populate the cerebral cortex as the gestation progresses. It has been shown in an *in vitro* system that profound hypoxia induces cell cycle arrest in NSCs. However, the *in vivo* effect of antenatal maternal hypoxia in fetal NSCs remains unclear. We used a murine maternal hypoxia-induced IUGR model to study the impact of IUGR on fetal NSC development. In this model, timed-pregnant mice were exposed to hypoxia during the active stage of neurogenesis, followed by fetal brain collection. In the IUGR fetal brains, we found a significant reduction in cerebral cortical thickness accompanied by decreases in layer-specific neurons. Using EdU labeling, we demonstrated that cell cycle progression of fetal NSCs was delayed. Interestingly, we also observed a defect in self-renewal in a subset of NSCs, leading to premature neuronal differentiation. Following relief from maternal hypoxia exposure, the remaining fetal NSCs re-established their neurogenic ability and resumed production of layer-specific neurons. Surprisingly, the newly generated neurons matched their control counterparts in layer-specific marker expression, suggesting preservation of the fetal NSC temporal identity despite IUGR effects. As expected, the number of neurons generated in the IUGR group remained lower compared to that in the control group due to premature depletion of fetal NSCs. Transcriptome analysis identified hundreds of genes affected by maternal hypoxia-induced IUGR. Finally, the IUGR offspring mice exhibited poorer cognitive functions than the control offspring mice. Taken together, maternal hypoxia-induced IUGR is associated with a defect in cell cycle progression and self-renewal of fetal NSCs, and has a long-term impact on offspring cognitive development. Our findings also pointed to a possible scenario where the temporal identity of the fetal NSCs may not be affected by IUGR.

# Kinetochore tension induces microtubule unbinding but inhibits depolymerization upon activating Aurora B kinase

Geng-Yuan Chen<sup>1</sup>, Mike Lampson<sup>1</sup>

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Error-free chromosome segregation requires Aurora B kinase regulating the interaction between kinetochores and associated microtubules. Two mechanisms by which Aurora B resets chromosome configuration have been proposed: inducing kinetochore-microtubule unbinding or inducing microtubule depolymerization while maintaining kinetochore attachment. To distinguish between these models, we acutely recruited Aurora B to kinetochores using a photo-activatable chemical dimerizer. Because kinetochore-microtubule unbinding relaxes inter-kinetochore tension whereas microtubule depolymerization generates tension, the contrasting mechanisms would cause opposite directions of chromosome movement. For a monopolar spindle, we find that Aurora B recruitment drives kinetochore poleward movement, consistent with Aurora B promoting microtubule depolymerization under low resisting loads. In contrast, Aurora B recruitment to a single kinetochore of a bioriented sister kinetochore pair can trigger movement of the targeted kinetochore away from its attached pole (Figure 1), indicating kinetochore-microtubule unbinding under high tension. Furthermore, the frequency of unbinding under these conditions increases with Aurora B recruitment efficacy and the instant inter-kinetochore tension. Thus, the response of Aurora B activity depends on the cellular context: depolymerization at low tension and unbinding at higher tension. These findings suggest distinct mechanisms to correct different microtubule attachment errors, under the control of a single kinase.



**Figure 1. An example montage of Aurora B recruitment to a single kinetochore of a sister kinetochore pair promoting kinetochore-microtubule detachment.** A single kinetochore was activated at a given position (yellow arrow) at the initial frame. The recruitment reaches maximal intensity at 1 min, followed by moving the kinetochore against its attached pole. Scale bar: 5  $\mu$ m

My poster title “*In vivo* three-photon excited fluorescence imaging in the spinal cord of awake, locomoting mice” has been accepted to present at 2018 society for Neuroscience at San Diego, CA. This project covers most of my PhD work and the presentation was intended to attract potential collaborators and reviews once the project is submitted.

*In vivo* three-photon excited fluorescence imaging in the spinal cord of awake, locomoting mice

Yu-Ting Cheng<sup>1,2</sup>, SallyAnne L. Ness<sup>2,4</sup>, Jason Jones<sup>2</sup>, Daniel Rivera<sup>2</sup>, Sofia Hu<sup>2</sup>, Jared Raikin<sup>2</sup>, Dimitre G. Ouzounov<sup>3</sup>, Tianyu Wang<sup>3</sup>, Xin Li<sup>2</sup>, Jean C. Cruz Hernandez<sup>2</sup>, Isle M. Bastille<sup>2</sup>, Nozomi Nishimura<sup>2</sup>, Joseph R. Fetscho<sup>1</sup>, Chris Xu<sup>3</sup>, and Chris B. Schaffer<sup>2</sup>

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Nonlinear optical microscopy is a powerful technology for non-invasively recording the firing activity of a large population of neurons using calcium sensitive fluorescent reporters. Spinal cord neurons in central pattern generator (CPG) circuits control rhythmic locomotor behaviors but sit below the highly optically scattering white matter, making imaging of these cells challenging, even with two-photon microscopy. Recent work has shown that utilizing higher order nonlinear optical processes, such as three-photon excited fluorescence (3PEF), enables deeper penetration into scattering tissue. Here, we explore the use of 3PEF imaging using a 1.3-μm excitation source to image cellular structure and function in the mouse spinal cord. We first imaged the topology of the microvascular network and measured blood flow speed throughout the vascular hierarchy, from the lateral arterioles, through the capillary bed, and to the dorsal spinal vein. Next, we examined the response of microglia (expressing GFP) and dorsal ascending axons (expressing YFP) to occlusion in the venules that drain the spinal cord, all using 3PEF microscopy. The surgical preparation we use to gain optical access to the spinal cord enables us to spine fix the mice, while awake, under the microscope. Mice can then “run” on a spinning disk while we image their spinal cord. Once trained, mice exhibited a normal running gait and grooming behaviors while spine fixed atop the disk. We then used 3EPF imaging of the genetically-encoded calcium sensor GCaMP6s to measure neural activity in spinal cord neurons. In mice expressing GCaMP in sensory neurons (CaMKIIαTA-GCaMP6s), we observed stimulus-locked neural responses ( $dF/F > 50\%$ ) in response to electric shocks to the hind paw. When these animals were awake under the microscope, the neural firing frequency, as well as the amplitude of the calcium response, increased as the mouse went from a resting state to continuously running on the disk. In efforts to image activity in some of the CPG neurons that control limb motion, we delivered AAV9-LoxP-GCaMP6s virus into the spinal cord of Chx10-Cre animals. This led to GCaMP6s expression in V2a cells, which reside ~500 μm underneath the

cord surface. When combined with quantitative tracking of limb kinematics, this capability for 3PEF imaging of cell-resolved neural activity could enable detailed studies of how activity patterns in CPG circuits coordinate rhythmic locomotion.

# AlleleHMM: a data-driven method to identify allele specific differences in distributed functional genomic marks

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How DNA sequence variation influences gene expression remains poorly understood. Diploid organisms have two homologous copies of their DNA sequence in the same nucleus, providing a rich source of information about how genetic variation affects a wealth of biochemical processes. However, few computational methods have been developed to discover allele specific differences in functional genomic data. Existing methods either treat each SNP independently [1, 2], limiting statistical power, or combine SNPs across gene annotations [3], preventing the discovery of allele specific differences in unexpected genomic regions. Here we introduce AlleleHMM, a new computational method to identify blocks of neighboring SNPs that share similar allele specific differences in mark abundance. AlleleHMM uses a hidden Markov model to divide the genome into three hidden states based on allele frequencies in genomic data: a symmetric state (state S) which shows no difference between alleles, and regions with a higher signal on the maternal (state M) or paternal (state P) allele. AlleleHMM substantially outperformed naive methods using both simulated and real genomic data, particularly when input data had realistic levels of overdispersion. Using global run-on sequencing (GRO-seq) data, AlleleHMM identified thousands of allele specific blocks of transcription in both coding and non-coding genomic regions. AlleleHMM is a powerful tool for discovering allele specific regions in functional genomic datasets.

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## Ventromedial prefrontal parvalbumin neurons signal active avoidance

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The medial prefrontal cortex (mPFC) plays an essential role in the suppression of passive behaviors and the facilitation of active behaviors in high-threat environments. mPFC parvalbumin (PV) neurons, which innervate the axon initial segment, cell body, and proximal dendrites of pyramidal neurons, gate mPFC signaling and play an important role in reward and fear extinction, working memory, and the allocation of attention. Here, we investigate the role of ventral mPFC (vmPFC) PV neurons in actions to avoid punishment and to obtain reward in freely moving mice.

Animals were placed in an operant chamber and trained to cross to the opposite side of the chamber following an auditory cue. In the avoidance task, crossing the chamber within 5 seconds allowed the mouse to avoid receiving a shock. If the mouse failed to cross, the shock was turned on until escape occurred. In the approach task the mouse was required to cross the chamber within 5 seconds to obtain a water reward, and failure led to an aborted trial. We used fiber photometry to monitor the real-time population calcium dynamics of genetically defined vmPFC PV neurons in freely behaving mice. We injected AAV1-CAG-Flex-GCaMP6f into the vmPFC of PV-cre mice to express a genetically-encoded calcium indicator, and implanted an optical fiber over the vmPFC to monitor calcium-dependent fluorescence.

vmPFC PV neurons were strongly activated immediately prior to and during chamber crossing in successful avoidance trials, but were slightly suppressed during chamber crossing in successful approach trials. In addition, vmPFC PV neurons were active during chamber-crossing movements during the inter-trial interval during avoidance sessions, a response that only emerged after animals had experienced shock. No response to locomotion in the open field or during approach behavior was detected, and neural activity was not correlated with speed of movement. Since the auditory tone was turned off the moment the mouse successfully crossed the chamber, we also recorded neural activity during a modified version of the task, in which short auditory cues were used in some trials and auditory cues persisting beyond chamber crossing were used in others. We found that PV neural responses during shock avoidance were better explained by chamber crossing than by tone termination. Our results reveal that vmPFC PV neurons respond during actions to avoid threats.

# Confined Migration Induces Heterochromatin Formation in Cancer Cells

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During cancer metastasis, cancer cells migrate through confined interstitial spaces, requiring extensive deformation of the cell body and nucleus. The severe physical stress on the nucleus during this frequently results in rupture of the nuclear envelope (NE) and nuclear fragmentation<sup>1</sup>. Previous studies found that mechanical force application on cells can induce mechanosensitive gene expression<sup>2</sup> and chromatin rearrangement<sup>3</sup>. However, it remains unclear whether physical stress on the nucleus during confined migration can induce histone modification and gene expression changes. Here, we performed live-cell and immunofluorescence imaging of HT1080 fibrosarcoma and MDA-MB-231 breast cancer cells migrating through collagen gel matrices and custom-made microfluidic migration devices that mimic interstitial spaces *in vivo*. Cell migration in confined 3D environments resulted in increased H3K9me3 and H3K27me3 heterochromatin marks and DNA methylation (5-methylcytosine) compared to unconfined conditions. The heterochromatin enrichment was particularly prominent in cells with visible nuclear deformation and in chromatin protrusions that formed inside nuclear blebs. In addition, we observed GFP-labeled HP1 $\alpha$  enrichment within nuclear blebs in HT1080 and MDA-MB-231 cells during confined migration. By using a fluorescence recovery after photobleaching (FRAP) assay, we verified those GFP-HP1 $\alpha$  enrichments to be heterochromatin. Treating cells with a pan histone methyltransferase inhibitor 3-Deazaneplanocin A (DZnep) significantly reduced the extent of heterochromatin formation after confined migration, suggesting an active enzymatic process underlying the increased heterochromatin formation. In particular, enriched phosphorylated (pSer424) HDAC3 nuclear staining suggested activation of HDAC3, potentially due to the mechanical force cells encountered during confined migration. Consistent with this idea, chemical inhibition of HDAC3 significantly reduced heterochromatin formation during confined migration. Interestingly, cells treated with DZnep showed significantly slower migration when compared to control treatment, suggesting the importance of chromatin condensation for efficient migration in 3D environment. Taken together, our research indicates that migration of cancer cells through confined spaces can induce heterochromatin formation, which is expected to both alter the physical properties of the nucleus and to modulate gene expression. These modifications could promote further metastatic cancer progression. Targeting the ability of chromatin condensation upon confined migration may potentially serve as a novel approach in treating metastatic cancers.

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# Label-free chemical image cytometry reveals metabolic signatures of cancer cells under stress

Kai-Chih Huang<sup>1</sup>, Junjie Li<sup>1</sup>, Chi Zhang<sup>1</sup>, Yuying Tan<sup>1</sup>, Chien-Sheng Liao<sup>1</sup>, and Ji-Xin Cheng<sup>1,2,3,4</sup>

<sup>1</sup>Department of Biomedical Engineering, <sup>2</sup>Department of Chemistry, <sup>3</sup>Department of Electrical and Computer Engineering, <sup>4</sup>Photonics Center, Boston University, Boston, MA 02215, USA.,

Understanding the cell-to-cell heterogeneity is a fundamental challenge of biology. Altered metabolism is being recognized as one of the hallmarks of cancer. Conventional single cell analysis method to study metabolism i.e. mass spectrometry and fluorescence labeled flow cytometry, could not provide spatial-composition of metabolites or with the drawback of sample destruction. We present a chemical image cytometry as a high-content high-throughput single cell analysis platform with molecular chemical information in the label-free manner. We achieved high-throughput Raman spectral acquisition by multiplex stimulated Raman scattering (SRS) technique at every 5  $\mu$ s covering 200 wavenumbers<sup>1,2</sup>, which is six orders of magnitude higher than spontaneous Raman scattering spectral acquisition. Multiple chemical signatures, featuring different cellular organelles such as lipids, endoplasmic reticulum (ER), nucleus, and cytoplasm can be segmented. Using this system, we found lipid-accumulated protrusion as a metabolic biomarker for cancer cells to deal with stress. Our finding highlights the potential of targeting lipid metabolism for elimination of starvation-resistant and chemotherapy-resistant cancer cells.

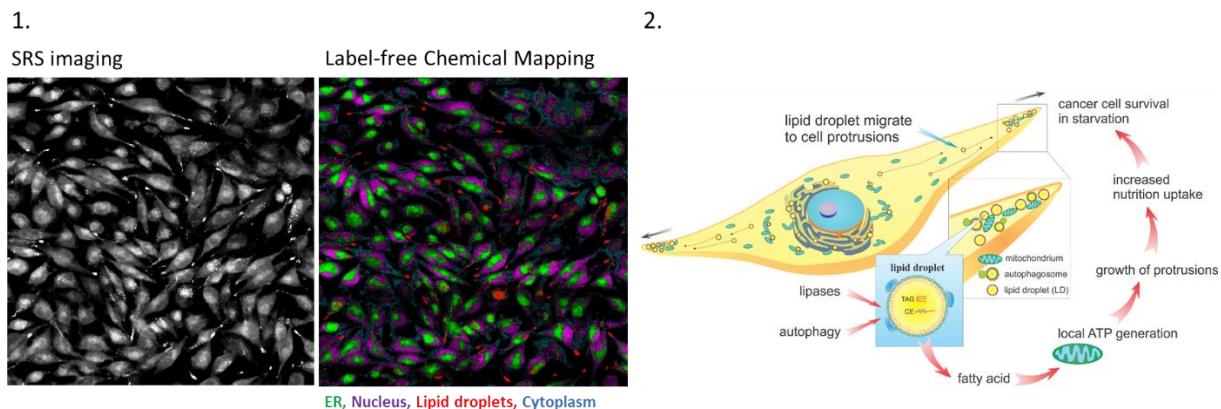


Figure 1. Left panel: SRS imaging of G3K cell (gemcitabine resistant pancreatic cell); Right panel: label-free chemical mapping of sub-cellular organelles based on their Raman signature.

Figure 2. A schematic showing how lipid droplets in cell protrusions promote cancer cell survival under stress.

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# Identification of *C2CD4A* as a human diabetes susceptibility gene with a role in $\beta$ -cell insulin secretion

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Fine mapping and validation of genes causing  $\beta$ -cell failure from susceptibility loci identified in type 2 diabetes genome-wide association studies (GWAS) poses a significant challenge. The *VPS13C-C2CD4A-C2CD4B* locus on chromosome 15 confers diabetes susceptibility in every ethnic group studied to date. However, the causative gene is unknown. FoxO1 is involved in the pathogenesis of  $\beta$ -cell dysfunction, but its link to human diabetes GWAS has not been explored. Here we generated a genome-wide map of FoxO1 super-enhancers in chemically identified  $\beta$ -cells using 2-photon live-cell imaging to monitor FoxO1 localization. When parsed against human super-enhancers and GWAS-derived diabetes susceptibility alleles, this map revealed a conserved super-enhancer in *C2CD4A*, a gene encoding a  $\beta$ -cell/stomach-enriched nuclear protein of unknown function. Genetic ablation of *C2CD4A* in  $\beta$ -cells of mice phenocopied the metabolic abnormalities of human carriers of *C2CD4A*-linked polymorphisms, resulting in impaired insulin secretion during glucose tolerance tests as well as hyperglycemic clamps. *C2CD4A* regulates glycolytic genes, and notably represses *lactate dehydrogenase*, the key  $\beta$ -cell “disallowed” gene. We propose that *C2CD4A* is a transcriptional coregulator of the glycolytic pathway whose dysfunction accounts for the diabetes susceptibility associated with the chromosome 15 GWAS locus.

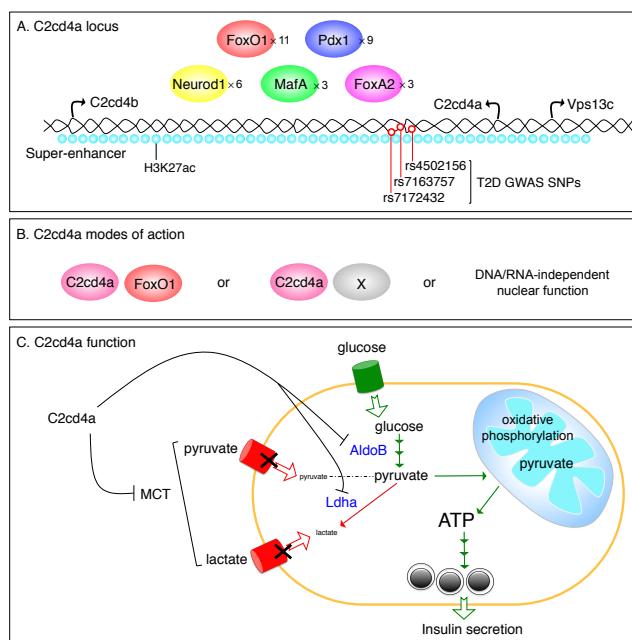


Figure 1. Proposed model of *C2cd4a* function in the pancreatic  $\beta$ -cell. (A) *C2cd4a* resides in a transcription factors-, histone acetylation-, and type 2 diabetes GWAS SNPs-enriched super-enhancer. (B) *C2cd4a* may act as a coregulator for transcription factors, including FoxO1 and others (indicated as X), or DNA/RNA-independent nuclear function. (C) *C2cd4a* represses key  $\beta$  cell disallowed genes, such as lactate dehydrogenase A (*LdhA*), aldolase B (*AldoB*), and monocarboxylate transporter (MCT). These disallowed genes are expressed at extremely low amount to ensure the coupling of glucose metabolism through efficient oxidative phosphorylation to insulin secretion.

# Highly Selective *in vivo* Editing of Macrophages through Systemic Delivery of CRISPR-Cas9-Ribonucleoprotein-Nanoparticle Nanoassemblies

Yi-Wei Lee, Rubul Mout, David C. Luther, Yuanchang Liu, Laura Castellanos-García, Amy S. Burnside, Moumita Ray, Gulen Yeşilbag Tonga, Joseph Hardie, Harini Nagaraj, Riddha Das, Erin L. Phillips, Tristan Tay, Richard W. Vachet and Vincent M. Rotello\*

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Macrophages are key effectors of host defense and metabolism, making them promising targets for transient genetic therapy. Gene editing through delivery of the Cas9-ribonucleoprotein (RNP) provides multiple advantages over gene delivery-based strategies for introducing CRISPR machinery to the cell. There are, however, significant physiological, cellular, and intracellular barriers to the effective delivery of the Cas9 protein and guide RNA (sgRNA) that have to date, restricted *in vivo* Cas9 protein-based approaches to local/topical delivery applications. Herein we describe a new nanoassembled platform featuring co-engineered nanoparticles and Cas9 protein that has been developed to provide efficient Cas9-sgRNA delivery and concomitant CRISPR editing through systemic tail-vein injection into mice, achieving >8% gene editing efficiency in macrophages of the liver and spleen.<sup>1</sup>

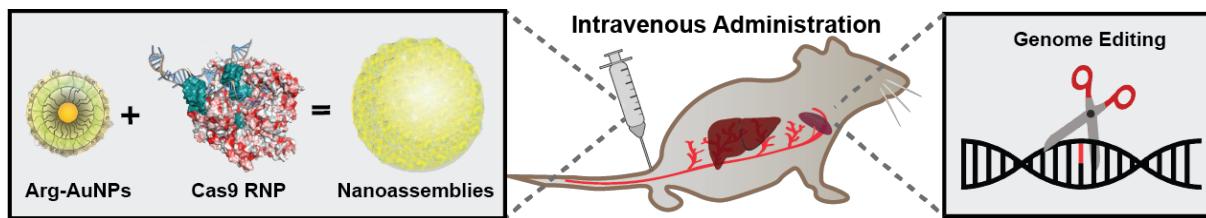


Figure 1. The nanoassemblies provide efficient CRISPR editing through systemic tail-vein injection into mice, achieving >8% gene editing efficiency in macrophages of the liver and spleen.<sup>1</sup>

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## Functional Studies of Bacterial Stress Response Metabolite AHRA

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Bacteria respond to a variety of environmental stresses through different intracellular signaling pathways. Though much is known about the biochemistry, little is known about changes in the metabolome. In this study, we analyzed the metabolic effects of erythromycin antibiotic stress and paraquat-induced oxidative stress applied to *Xenorhabdus bovienii*, *Pseudomonas aeruginosa*, and different *E. coli* strains. Several metabolites (AHRA analogs) resembling the known aryl hydrocarbon receptor agonist (AHRA) ITE were identified with increased production (up to 20-fold) upon stress. In *E. coli*, our AHRA analogs showed a mild protecting effect under heat stress and antibiotic stress. These AHRA analogs also showed aryl hydrocarbon receptor (AhR) activation in human cell lines at micromolar level. In order to investigate the mechanism of action in bacteria, an AHRA probe with an alkyne functional group was synthesized and used to pull down interacting proteins in *E. coli*. Photo-crosslinking followed by streptavidin enrichment and protein ID suggested possible interaction partners including elongation factor Tu (EF-Tu). Preliminary fluorescence assays suggested binding between AHRA-272 and EF-Tu. We are currently analyzing the binding affinity.

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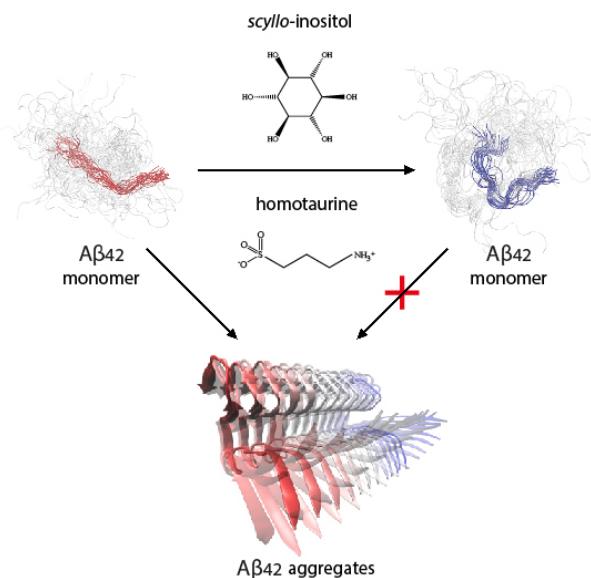
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# Revealing Inhibitory Effects of Small-molecule Drugs on Amyloid-forming Peptides Using Computational Modeling Approaches

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Molecular modeling and simulation provide crucial insights into the structure, dynamics, and interactions of various systems at the molecular level, to both complement and enrich experimental measurements. Our recent work focuses on identifying the key interaction between small-molecule drugs and amyloid forming proteins. Aggregation of amyloid- $\beta$  (A $\beta$ ) peptides from soluble monomers to insoluble amyloid fibrils has been hypothesized to be one of the crucial steps in the progression of Alzheimer's disease (AD). Due to the disordered nature of A $\beta$  peptides, identifying aggregation inhibitors as potential drug candidates against AD has been a great challenge. We identified the inhibition mechanism of two small molecules, homotaurine and *scyllo*-inositol, which are AD drug candidates currently under investigation. Using a replica exchange molecular dynamics method to extensively explore the conformational space of A $\beta$ 42 monomer with and without the presence of the small molecule agents, we found that both drug candidates reduce the  $\beta$ -strand propensity of the C-terminus region (I31-A42) and promote a conformational change of A $\beta$ 42 monomer toward a more collapsed phase through a non-specific binding mechanism. These findings provide atomistic-level insights into understanding of the inhibitory mechanisms of two potential small-molecule drug candidates for AD treatment in the future.



# Inhibition of FGFR1 in Breast Cancer Metastasis

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Metastatic breast cancer is the most advanced stage of breast cancer. However, our understanding of the molecular mechanisms which drive metastatic breast cancer remain incomplete. Epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) promote tumor invasion and metastasis. Previously, it has been reported that fibroblast growth receptor 1 (FGFR1) plays a key role during EMT: MET cycle [1]. Therefore, optimizing FGFR inhibitors is crucial for therapeutic targeting of late-stage breast cancer. Here, we examined the efficacies of four FGFR kinase inhibitors, AZD4547, JNJ-42756493, FIIN4, and TAS-120, in the metastatic D2.A1 cell line using a 3D spheroid assay. This 3D culture approach combines tumor spheroid formation in a non-adherent round bottom dish followed by placement on a bed of matrix. Our results demonstrate that these FGFR kinase inhibitors have potent anti-proliferative activities in 3D culture. Metastasis can arise from disseminated tumor cells (DTCs) which can enter dormancy and escape therapies after primary tumor removal. Therefore, we seek to investigate whether inhibition of FGFR1 can target dormant breast cancer cells. Here, we tested different FGFR1 inhibitors in D2.OR cells, in which dormancy depends on soft matrices. The data show that FGF2 could break the D2.OR dormancy in the 3D environment. In vivo, inhibition of FGFR is highly effective against the 4TO7 pulmonary microenvironment (Figure 1). Importantly, FIIN4 showed the least toxicity. Therefore, we compared the efficacies of FIIN4 and TAS-120 two covalent inhibitors, in the 4T-1 primary tumor model. TAS-120 induced tumor regression efficiently in 4T-1 primary tumor model.

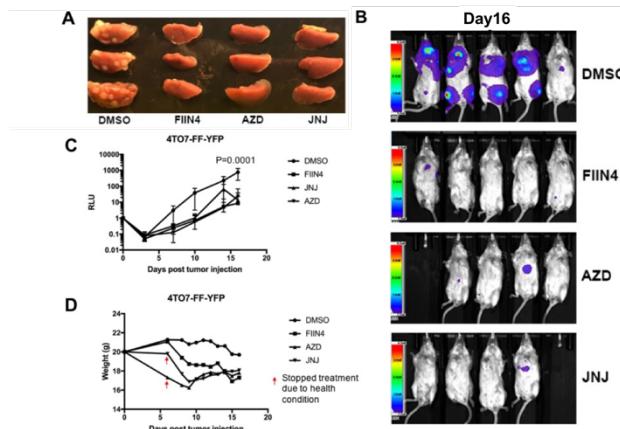


Figure 1. Inhibition of FGFR is highly effective against 4TO7 tumor in the pulmonary microenvironment. (A) Gross anatomical images of lungs 16 days after tail vein inoculation. (B) BLI images of mice 16 days after tail vein inoculation. (C) BLI quantification of pulmonary tumor growth normalized to injected values. (D) Body weight of the indicated treatment groups.

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## Identification of *Escherichia coli* ClpAP in regulating susceptibility to type VI secretion system-mediated attack by *Agrobacterium tumefaciens*

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Type VI secretion system (T6SS) is an effector delivery system used by gram-negative bacteria to kill other bacteria or eukaryotic host to gain fitness. In *Agrobacterium tumefaciens*, T6SS has been shown to kill other bacteria such as *Escherichia coli* [1]. Interestingly, the *A. tumefaciens* T6SS killing efficiency differs when using different *E. coli* strains as recipient cells. Thus, we hypothesize that a successful T6SS killing not only relies on attacker T6SS activity but also depends on recipient factors. To test the hypothesis, a high-throughput interbacterial competition assay was employed to screen for mutants with reduced killing outcomes caused by *A. tumefaciens* strain C58. From the 3909 *E. coli* Keio mutants screened, 16 candidate mutants were filtered out. One strain,  $\Delta clpP::\text{Kan}$ , showed ten times more resistant to T6SS-mediating killing but restored its susceptibility when complemented with *clpP* in *trans*. ClpP is a universal and highly conserved protease that is found in both prokaryotes and eukaryotic organelles. In *E. coli*, ClpP uses either ClpA or ClpX as its adaptor for substrate specificity. Therefore, the susceptibility of  $\Delta clpA::\text{Kan}$  and  $\Delta clpX::\text{Kan}$  was also tested. The T6SS attack susceptibility of  $\Delta clpA::\text{Kan}$  is at the same level as  $\Delta clpP::\text{Kan}$ , while  $\Delta clpX::\text{Kan}$  showed no difference as compared to that of wild-type BW25113 (Figure 1). The data also suggest that ClpA-ClpP interaction, rather than its protease activity, is responsible for enhancing susceptibility to T6SS killing. This study highlights the importance of recipient factors in determining the outcome of T6SS killing.

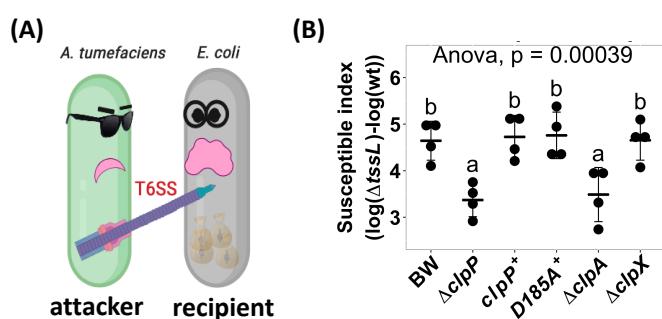


Figure 1. Recipient ClpP but not its protease activity, together with ClpA, plays a critical role in enhancing *A. tumefaciens* T6SS killing outcome. (A) Cartoon illustration of the *A. tumefaciens* T6SS killing against *E. coli*. (B) *A. tumefaciens* T6SS killing against *E. coli*  $\Delta clpP$  and its complement strain,  $\Delta clpA$  and  $\Delta clpX$ . The *A. tumefaciens* and the *E. coli* were co-cultured on AK agar medium for 16 h. Afterwards, the recovery of *E. coli* strains was quantified and the susceptible index (SI) was calculated by subtraction difference of the recovered log(cfu) of that attacked by  $\Delta tssL$  to that by wild type *A. tumefaciens* C58.

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## Muscle as an antigen reservoir in IDLV induced long term immunity

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The development of vaccines eliciting protective and durable immune response remains a global health priority. We developed Integrase-Defective Lentiviral Vectors (IDLVs) as efficient delivery of strategic immunogens, able to induce a strong and persistent immune response in mice and non-human primates<sup>1,2</sup>. Here, we evaluated the association between the persistence of antigen expression *in vivo* and the long-term immunity induced by IDLV. Following a single intramuscular (IM) or subcutaneous (SC) injection of IDLV delivering GFP as a model antigen in mice, we evaluated antigen expression, cellular infiltration at the site of injection and persistence of antigen-specific T cell response by IFN $\gamma$  ELISpot at early and late time points. IM immunization with IDLV-GFP resulted in durable antigen expression in the muscle up to 90 days. Conversely, antigen expression was detectable in the skin only at early time points after SC immunization. Moderate cellular infiltration was evident soon after the injection in all injected mice and detectable up to 30 days at lower levels only in IM immunized mice. The GFP-specific T cell response was more persistent in IM compared to SC injected mice. Interestingly, we identified GFP positive muscle cells expressing MHC I *in vivo* and we demonstrated *in vitro* that primary myoblasts and myocytes are able to present the antigen to GFP-specific T effector cells through MHC I. We also demonstrate that myocytes but not myoblasts are sensitive to Fas-dependent CTL killing activity. Overall these data indicate that muscle cells may serve as “antigen reservoir” for maintaining the long term immunity induced by IDLV.

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## **The dynamics and structural interactions between p53 and RNA Polymerase II by using Single Molecule Tracking and Cryo-EM**

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The master tumor suppressor p53 responds to cellular stresses by activating the transcription of tumor-suppress genes. Herein I present the latest studies of p53-Pol II dynamics *in vivo* and structure mechanism by Single-Molecule Tracking and cryo-Electron Microscopic (cryo-EM). Single-molecule Tracking WT p53/Pol II hubs form dynamically co-localization binding hubs. The consistent dynamics between p53 and Pol II hubs indicates that p53 recruits Pol II to form a transcriptional convoy. In addition, three-dimensional reconstruction of Single-Particle cryo-EM determines two p53-Pol II density maps with global resolution around 6-8Å. This map suggests that p53 regulates the transcriptional elongation. These studies shed light on the interactions between p53 and transcriptional machinery in response to the cellular stress.

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## Identification of cancer drivers at CTCF insulator in 1962 whole genomes

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10. These authors contributed equally to this work.

Recent studies have shown that mutations at non-coding elements, such as the *TERT* promoter and *ESR1* enhancer, can act as cancer drivers. However, an important class of non-coding elements, namely CTCF insulators, has been overlooked in the previous driver analyses. We used insulator annotations from CTCF and cohesin ChIA-PET and analyzed somatic mutations in 1,962 whole-genomes from 21 cancer types. Using the heterogeneous patterns of transcription-factor-motif-disruption, functional impact and recurrence of mutations, we developed a computational method that revealed 21 insulators showing signals of positive selection. Most mutations in these insulators tend to be clonal. CTCF motif orientations at constitutive insulators allow prediction of potential loop rewiring events and associated gene expression changes. We find 16% of melanoma patients show mutations in an insulator that are associated with *TGFB1* up-regulation. Using CRISPR-Cas9 in human melanoma A375 cells, we find that two of the most frequent mutations in this region increase cell growth by 40-50%, validating their role as cancer drivers. Thus, our study reveals CTCF insulators as a novel class of non-coding cancer drivers.

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## SRNS-associated *MYO1E* mutations have differential effects on Myo1e activity and stability

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Mutations in the *MYO1E* gene, encoding myosin 1e (Myo1e), are associated with steroid resistant nephrotic syndrome (SRNS) [1]. We set out to characterize novel *MYO1E* mutations identified in SRNS patients [2]. Using adenoviral transduction of EGFP-Myo1e constructs into cultured podocytes, we compared steady-state expression levels, protein turnover rates, and localization of the wild type (wt) Myo1e and several Myo1e mutants, including mutants with point mutations in the motor domain and a frameshift mutant d3094-7 lacking the C-terminal SH3 domain.

We found the T119I motor domain mutant and the d3094-7 frameshift mutant had low expression levels and high turnover rates and did not localize to cell-cell junctions, where Myo1e is thought to aid in the assembly of the slit diaphragm complexes [3]. In an attempt to restore Myo1e protein stability, we treated cells expressing these mutants with a proteasome inhibitor and observed accumulation of the mutant proteins. The restoration of protein expression in cells expressing the d3094-7 mutant resulted in a partial recovery of the junctional localization and dynamics of this protein. In contrast, an increase in the protein level of the T119I mutant achieved using proteasome inhibition was accompanied by formation of random, seemingly insoluble, aggregates (as determined using fluorescence recovery after photobleaching (FRAP)). Further investigating the properties of those Myo1e mutants that did not exhibit decreased protein stability, we found that the dynamics of one of the motor domain mutants at the junctions was decreased compared to the wt Myo1e. Using FRAP, we found that the mobile fraction of this mutant was lower than that of the wt Myo1e while the half time of fluorescence recovery was higher.

Overall, our findings indicate that while some SRNS-associated mutations affect Myo1e stability and folding, others may have more specific effects on motor domain activity. Furthermore, proteasome inhibition needs to be further examined as a potential therapeutic approach for alleviating the effects of those mutations that affect Myo1e stability without disrupting its motor function.

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## **Immunity to Invasive Infection by Intestinal Commensal Microbes**

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Commensal microbes that colonize the intestine are an important cause of invasive infection. However, how colonization of individuals impacts their susceptibility to invasive infection remains undefined. This knowledge gap reflects technical roadblocks in models of long-term commensal colonization by microbes that can also cause invasive infection, and conceptual appreciation for systemic antigen-specific immune modulation primed by commensals. To address this knowledge gap, a model of intestinal colonization with the human commensal and pathogenic fungi, *Candida albicans* was developed. We show *C. albicans* intestinal colonization protects against subsequent invasive *C. albicans* infection. These benefits require tonic stimulation since protection is eliminated in mice eradicated of commensal fungi. Protection against invasive infection parallels the systemic expansion of *C. albicans*-specific ROR $\gamma$ <sup>+</sup> Th17 cells and enhanced IL-17 responsiveness by circulating neutrophils. Reciprocally, the protective benefits of intestinal colonization are overturned by depletion of CD4<sup>+</sup> T cells or Ly6G<sup>+</sup> neutrophils, or IL-17 neutralization. These results highlight a new facet in the symbiotic relationship between host and microbe in mucosal tissues.

# **Identification of cellular mechanisms for memory by cell-type-specific RNA-seq of *Drosophila* neurons**

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The ability to remember past experiences is a fundamental aspect of brain function, and memory formation displays three universal behavioral properties from mollusks to humans [1]: (1) the consolidation from a short-lived, labile form to a long-lasting, stable form, (2) disruptive treatments cause retrograde amnesia by blocking memory consolidation, (3) stronger, longer-lasting memory forms after spaced repetitive training. Ample evidence in the fly has demonstrated these universal behavioral properties, as well as universal molecular mechanisms such as the cAMP signaling pathway. However, functional manipulations of neural circuits in flies have revealed that memory formation involves ongoing neural activity and parallel processing among a circuit that includes ~ 50 cell types [2]. Thus, cell-type-specific investigation is required for a detailed mechanistic understanding of memory formation. Here we use cell-type-specific RNA-seq to identify cellular mechanisms for memory.

For the mushroom body (MB) Kenyon cells that sparsely encode the odor information, we used Tandem Affinity Purification of Intact Nuclei and RNA-seq (TAPIN-seq) to profile the nuclear transcriptomes of the whole population of each of 7 cell types [3]. For individual identified cell types, such as the dorsal paired medial (DPM) neuron, we have developed a robust single-cell RNA-seq (scRNA-seq) pipeline. Using these TAPIN-seq and scRNA-seq pipelines we have obtained high-quality transcriptomes of each of the seven MB cell subtypes and of 31 individual DPM neurons. We have compared transcriptomes in each cell type between flies subjected to different training protocols. The naive transcriptomes provide a full accounting of the input-output properties within each of these cell types, including 23 neuropeptide genes other than *amnesiac* in the DPM neuron. We also identified more than 50 candidate genes that are transcriptionally regulated in specific cell types during memory formation. Taken together, our results establish pipelines to profile the naive transcriptome and the transcriptional response to behavioral training in both populations of neurons and single neurons that participate in memory. *Drosophila* provides an arsenal of genetic tools to perform subsequent manipulation of gene function with cell type specificity, enabling us to identify novel cellular mechanisms for a better mechanistic understanding of memory.

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# **Regulation of HIV-1 gene expression by post-transcriptional acetylation of cytidine in viral RNA transcripts**

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As obligate parasites, viruses need to navigate a variety of cellular regulatory systems while infecting and replicating in the host cell. Post-transcriptional modifications have recently emerged as an important layer of regulation of viral RNA function. For example, our lab and others have shown that the RNA modification N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) can enhance the replication of multiple viruses in *cis*, including Human Immunodeficiency virus 1 (HIV-1), Influenza A virus, SV40 and Kaposi's sarcoma-associated herpesvirus (KSHV). Recent reports have revealed the presence of another RNA modification, N<sup>4</sup>-acetylcytidine (ac4C) on cellular mRNAs and have shown that ac4C can enhance mRNA translation. Here, we demonstrate that ac4C is present at multiple sites on HIV-1 mRNAs and on the viral genomic RNA. Through ac4C RNA immunoprecipitation (RNA-IP) and RNA-seq, we found ac4C on HIV-1 mRNAs as well as the virion genomic RNA, with ac4C sites in the coding regions of the pol, env, nef genes, and the trans-activation response (TAR) hairpin. Phenotypically, we observe that increasing the expression level of the ac4C acetyltransferase NAT10 leads to an increase in viral replication that is dependent on the RNA binding and enzymatic domains of NAT10. Moreover, both CRISPR-depletion of NAT10 ( $\Delta$ NAT10) and treatment with the small molecule NAT10 inhibitor Remodelin, diminishes HIV-1 replication in T cells. Our data suggest that HIV-1 has evolved to incorporate ac4C in essential viral gene coding regions and regulatory RNA structures, and that NAT10-dependent ac4C addition enhances HIV-1 replication. We will present the latest developments from this project addressing how ac4C regulates HIV-1 infection, providing us with new potential points of intervention.

## **CTL-derived exosomes enhance the activation of CTLs stimulated by low affinity peptides**

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CTLs (cytotoxic T cells) with low affinity to peptides presented by MHC I (pMHC) induce and maintain a detectable and most time substantial population during an adaptive immune response, although CTLs with the highest affinity receive competitive activation signals. Low-affinity CTLs are important to induce effector response and retain a diverse memory repertoire against viral infection and cancer. However, the mechanism of generating and maintaining the expansion of lower affinity CTLs is still unknown. We hypothesized that exosome (Exo), the secreted extracellular vesicles, can deliver a message of high affinity to low-affinity T cells to enhance the activation of CTLs. Here, naïve CTLs were first purified and stimulated with high-affinity peptide, co-stimulators, and cytokine, interleukin-12. The supernatant was collected and isolated the Exo through differential centrifugation. The purified Exo was characterized and further used to stimulate naïve CTLs. The results showed that the CTLs with the highest affinity underwent dramatic expansion, expressed higher activation marker, increased the production of Granzyme B (GZB) and IFN- $\gamma$ , and more importantly, they could produce the functional Exo. The Exo was 30-150nm and expressed typical exosomal protein markers. Next, naïve CTLs were treated with peptides with diverse affinities and with/without the purified Exo. The treatment with the Exo together with peptide produced stronger proliferation and increased the expression of T cell functional molecules (CD25, GZB, IFN- $\gamma$ ) than treatment with peptide only. Moreover, the Exo had more profound effects on the group of CTLs treated with lower affinity peptides than the group treated with higher affinity peptides. Also, a critical transcriptional regulator of T cell activation, T-bet, was upregulated in the treatment with low-affinity peptides and Exo. Together, these results identified the important role of the Exo derived from the high affinity CTLs in activating the CTLs stimulated with lower affinity. This research provided the distinct mechanism of activating low-affinity CTLs and sustaining immune response during infection.

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# The Hippo Pathway Effector TAZ Regulates Ferroptosis in Renal Cell Carcinoma

Wen-Hsuan Yang<sup>1,2,3</sup>, Chien-Kuang Cornelia Ding<sup>1,2</sup>, Tianai Sun<sup>1,2</sup>, Gabrielle Rupprecht<sup>2,4</sup>, Chao-Chieh Lin<sup>1,2</sup>, David Hsu<sup>2,4</sup>, Jen-Tsan Chi<sup>1,2,\*</sup>

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Despite recent advances, the poor outcomes of renal cell carcinoma (RCC) still necessitate novel therapeutics. Ferroptosis is a form of regulated cell death which may have therapeutic potential toward RCC; however, much remains unknown about the determinants of ferroptosis susceptibility. We found that ferroptosis susceptibility is highly influenced by cell density and confluence. Because cell density regulates the Hippo-YAP/TAZ pathway, we investigated the roles of Hippo pathway effector in the ferroptosis. TAZ is abundantly expressed in RCC and undergoes density-dependent nuclear/cytosolic translocation. TAZ removal confers ferroptosis resistance, while overexpression of TAZS89A sensitizes cells to ferroptosis. Furthermore, TAZ regulates the expression of EMP1 that, in turn, induces the expression of NOX4, a renal-enriched ROS-generating enzyme essential for ferroptosis. These findings reveal that the cell density-regulated ferroptosis is mediated by TAZ through the regulation of EMP1-NOX4, suggesting its therapeutic potential for RCC and other TAZ-activated tumors.

