PCXGI Penn Center for Genome Integrity

Penn Center for Genome Integrity

A Review of Achievements

A Message from the Director

The Penn Center for Genome Integrity (PCGI) was founded in 2019 to investigate provocative questions at the interface of genome integrity and different aspects of organismal biology. As we embark on the fifth year of operations, I wanted to take a moment to highlight how much we've been able to accomplish in the past few years despite the obstacles presented by a global pandemic, while also emphasizing exciting plans for the coming year.

The mission of the PCGI is to understand how genome integrity impacts human biology. This requires a wholistic approach that connects laboratories engaged in the study the basic tenets of genome integrity with others that focus on the biological sequelae that are affected by genome instability. This philosophy distinguishes the PCGI from genome integrity centers at other institutions. We bring together research in basic mechanisms of DNA replication, DNA repair, and mitosis with laboratories that focus on immunology, stem cell function, virology, and development. Another distinguishing feature of the PCGI is the breadth of methodologies taken by participating laboratories, which range from molecular and cellular biology, structural biology, synthetic biology, evolutionary biology, chemical biology, and bioengineering. Such wide-ranging approaches were instrumental to the successful P01 application that examines the impact of genome instability on immune responses in cancer. Complementary approaches between PCGI laboratories have also led to additional grant applications, trainee awards and fellowships, high impact collaborative publications, and translation of basic research.

An important mission of the PCGI is to provide an environment that allows trainees to develop a broad range of skills and a network of colleagues from different fields. We have had 36 trainee speakers in the past year and made trainees a focal point of our mini-symposia series that include prominent faculty from other institutions. Trainees present at these symposia and attend dinner with the keynote speakers, giving them unique access to a side of science not often experienced by students and postdocs. We hope this broadens the horizons of our trainees and gives them the opportunity to acquire important scientific and social interactions that are among the most enjoyable sides of academic science. We have also featured trainees at our annual PCGI retreats, which included keynote talk from scientific luminaries Nicholas Plachta, Jennifer Phillips-Cremins, TJ Ha, and WHYY's "The Pulse" host Maiken Scott. Retreats have had additional enriching activities such as trainee roundtable discussions on entrepreneurial opportunities in academic science, strategies to land an academic faculty position, and bench to bedside translation of laboratory science.

The 2023/2024 academic year will bring more exciting developments, with planned joint symposia with the Institute on Aging entitled "Threats to the Neuronal Genome", and PCGI mini- symposia on repetitive DNA maintenance and on DNA repair and cancer. We will cap the 2024 academic year with a symposium centered on the P01 grant on Genomic instability and immune responses in cancer with keynote talks by P01 external advisory board members Drs. Douglas Green, Karlene Cimprich, and Tarun Kapoor. We look forward to continued growth this year by welcoming new PCGI laboratories, purchasing shared equipment and resources, and expanding our presence on campus to shine light on how genome integrity shapes human biology.

-Roger A. Greenberg, MD, PhD

Leadership

Roger Greenberg, MD, PhD

PCGI Director

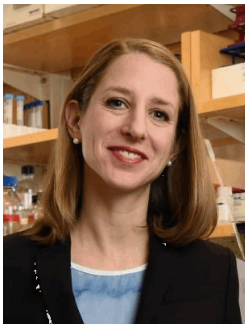
Ben E. Black, PhD

PCGI Co-Director

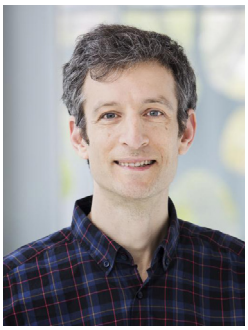
Matthew Weitzman, PhD

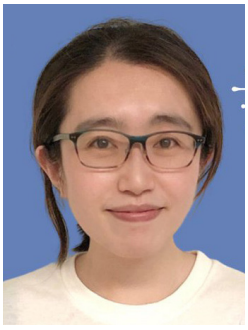
PCGI Co-Director

Core Investigators







From top left: Kara Bernstein, PhD; Luca Busino, PhD; Dennis Discher, PhD; Matthew C. Good, PhD; Malay Haldar, MD, PhD; Michael Lampson, PhD; Mia Levine, PhD; Kathy Liu, PhD; Sunny Shin, PhD; and Wei Tong, PhD

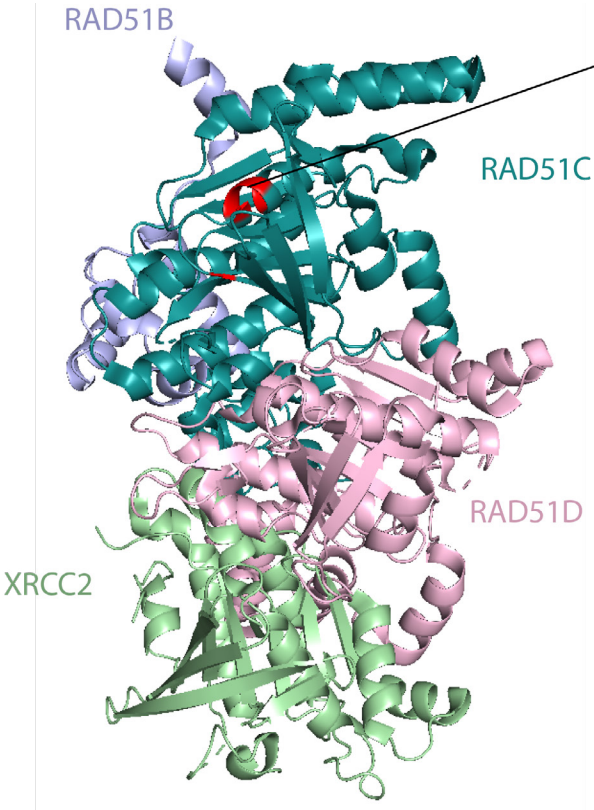
High-Profile Papers

Bernstein Lab

- Luong, TT, Li Z, Priedigkeit N, Parker PS, Böhm S, Rapchak K, Lee AV, **Bernstein KA**. Hrq1/RECQL4 regulation is critical for preventing aberrant recombination during DNA intrastrand crosslink repair and is upregulated in breast cancer. *PLoS Genetics*, 2022 Sep 20;18(9): e1010122.

- Using yeast to perform a large functional screen to identify pathogenic variants in RAD51C, identified a key region in RAD51C that is important for its function in cancer prevention. Prakash R*, Rawal Y, Sullivan MR, Grundy MK, Bret H, Mihalevic MJ, Rein, HL, Baird JM, Darrah K, Zhang F, Wang R, Traina TA, Radke MR, Kaufmann SH, Swisher EM, Guérois R, Modesti M, Sung P, Jasin M*, **Bernstein KA***. Homologous recombination-deficient mutation cluster in tumor suppressor RAD51C identified by comprehensive analysis of cancer variants. *Proc Natl Acad Sci USA* 2022 Sep 20;119(38):e2202727119. *Co-corresponding.

- Performed a genome-wide unbiased approach to examine mutation signatures in RAD51 paralog deficient yeast cells and identified a function for the Shu complex in bypassing abasic sites and 3-methyl-cytosine (3MeC). This work answered a long-standing question of how specific base damage, 3MeC, is tolerated in yeast despite not having the ALKB enzymes like other organisms. Bonilla B, Brown AJ, Hengel SR, Rapchak KS, Mitchell D, Pressimone CA, Fagunloye AA, Luong TT, Russell RA, Vyas RK, Mertz TM, Zaher HS, Mosammaparast N, Malc EP, Mieczkowski PA, Roberts SA*, **Bernstein KA**.* The Shu complex prevents mutagenesis and cytotoxicity of single-strand specific alkylation lesions. *eLife*. 2021 Nov 1;10:e68080. doi: 10.7554/eLife.68080. *Co-corresponding.



RAD51C ATPase mutants in the Walker A region found in cancer are highlighted in the cryoEM BCDX2 structure (PDB-8GBJ).

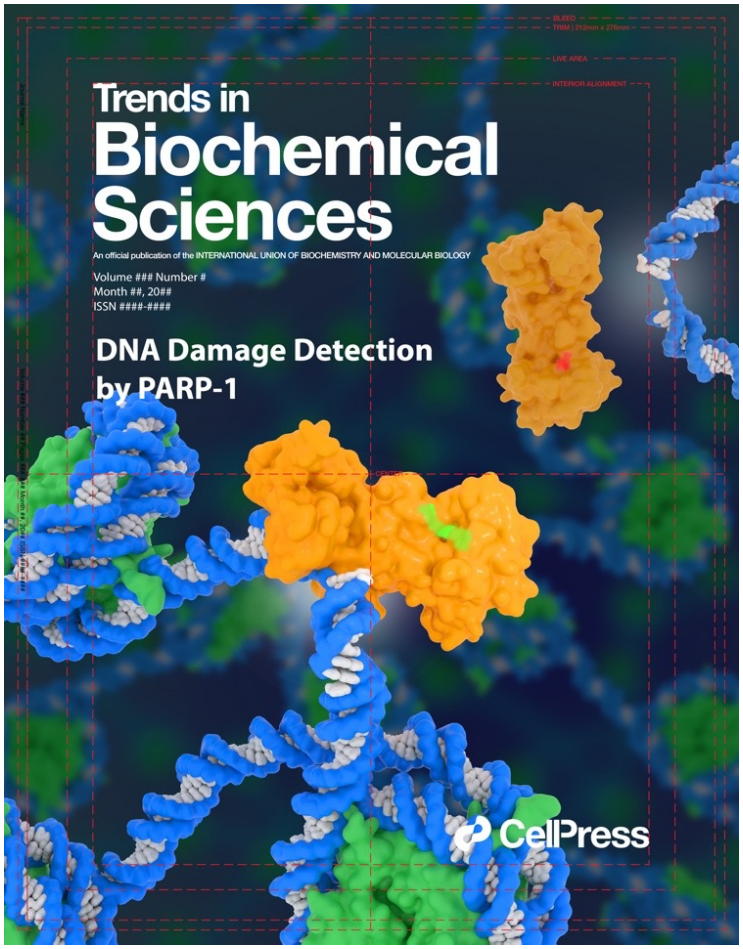
This figure was featured in functional insight into the structure of the BCDX2 complex cryo-EM structure by analyzing cancer variants in RAD51C.

Rawal Y, Jia L, Meir A, Zhou S, Kaur H, Ruben EA, Kwon, Y, **Bernstein KA,** Jasin M, Taylor AB, Burma S, Hromas R, Mazin AV, Zhao W, Zhou D, Wasmuth EV, Greene EC, Sung P, Olsen SK. Structural insights into BCDX2 complex function in homologous recombination. *Nature* 2023 Jul;619(7970):640-649. doi: 10.1038/s41586-023-06219-w.

High-Profile Papers

Black Lab

- 2020 study that showed that small molecule binding to the active site of PARP1 can have diverse allosteric effects. This opens the door to small molecule development for those that would trap PARP1 on DNA breaks (i.e. for targeting it in cancer) or cause its release from DNA breaks (i.e. for inhibiting PARP1 activity but not trapping it on DNA, as is ideal for treating neuroinflammation as seen in diverse disorders including Parkinson's disease). Zandarashvili L, Langelier M-F, Velagapudi UK, Hancock MA, Steffen JD, Billur R, Hannan ZM, Wicks AJ, Krastev DB, Pettitt SJ, Lord CJ, Talele TT, Pascal JM, **Black BE**. Structural basis for allosteric PARP-1 retention on DNA breaks. *Science* 2020 Apr 3;368(6486):eaax6367. doi: 10.1126/science.aax6367.



- The Lab's review on PARP1 in detecting DNA damage, and targeting it in human disease, was selected for the cover of Trends in Biochemical Sciences (see cover image here). They also highlight their emerging work on developing useful artificial chromosomes (featured in a preprint: Gambogi et al., 2023, Biorxiv) that is garnering positive attention as it makes its way through the peer review process. Pandey N, **Black B**E. Rapid Detection and Signaling of DNA Damage by PARP-1. *Trends Biochem Sci*. 2021 Sep;46(9):744-757. doi: 10.1016/j.tibs.2021.01.014. Epub 2021 Mar 3.

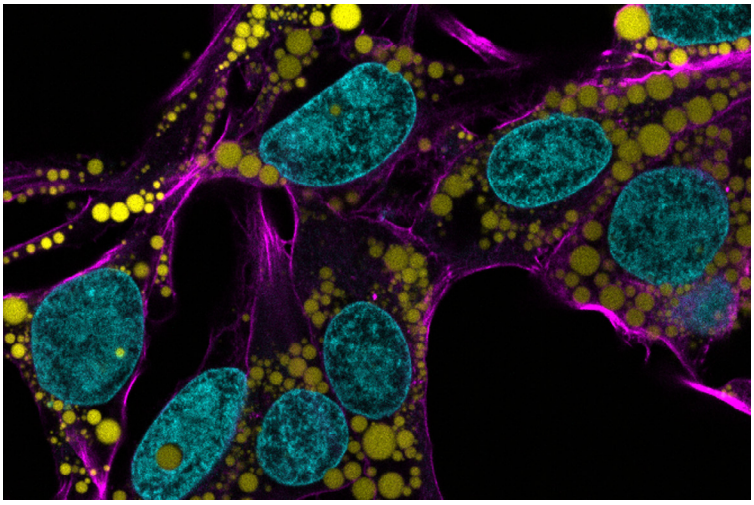
- A product of their close PCGI collaboration with the Lampson lab, co-mentored postdoc Runi Das discovered that CENP-A is a maternal effect gene and established the mechanisms governing centromere inheritance in the mouse model system. Das A, Iwata-Otsubo A, Destouni A, Dawicki-McKenna JM, Boese KG, **Black BE**, **Lampson MA**. Epigenetic, genetic and maternal effects enable stable centromere inheritance. *Nat Cell Biol*. 2022 May;24(5):748-756. doi: 10.1038/s41556-022-00897-w.

Busino Lab

- This paper from the Busino lab opens the doorway for therapeutic strategies aimed at targeting the oncogenic pathway activated in KLHL6- or NOTCH2-mutated DLBCL. Zhou N, Choi J, Grothusen G, Kim BJ, Ren D, Cao Z, Liu Y, Li Q, Inamdar A, Beer T, Tang HY, Perkey E, Maillard I, Bonasio R, Shi J, Ruella M, Wan L, **Busino L**. DLBCL-associated NOTCH2 mutations escape ubiquitin-dependent degradation and promote chemoresistance. *Blood* 2023 Sep 14;142(11):973-988. doi: 10.1182/blood.2022018752.

High-Profile Papers

Discher Lab



Pictured here: Aforementioned fat-filled lipid droplets (in green)

- The Discher Lab was featured in Penn Today for their article published in the *Journal of Cell Biology* for their discovery of lipid droplets' capability to indent and puncture the nucleus, causing loss of diffusible DNA repair factors. Ivanovska IL, Tobin MP, Bai T, Dooling LJ, **Discher DE**. Small lipid droplets are rigid enough to indent a nucleus, dilute the lamina, and cause rupture. *J Cell Biol*. 2023 Aug 7;222(8): e202208123. doi: 10.1083/jcb.202208123. Epub 2023 May 22. Also featured in Penn Engineering Today's Blog.
- The Discher and Lampson labs collaborated on a new method to literally see genetic changes as they occur -- even under physical stress. Hayes BH, Zhu PK, Wang M, Pfeifer CR, Xia Y, Phan S, Andrechak JC, Du J, Tobin MP, Anlas A, Dooling LJ, Vashisth M, Irianto J, **Lampson MA**, **Discher DE**. Confinement plus myosin-II suppression maximizes heritable loss of chromosomes, as revealed by live-cell ChReporters. *J Cell Sci*. 2023 Jun 1;136(11):jcs260753. doi: 10.1242/jcs.260753. Epub 2023 Jun 8.
- The Discher lab is beginning to optimize macrophage-based anti-cancer cures with addition of genome instability: Dooling LJ, Andrechak JC, Hayes BH, Kadu S, Zhang W, Pan R, Vashisth M, Irianto J, Alvey CM, Ma L, **Discher DE**. Cooperative phagocytosis of solid tumours by macrophages triggers durable anti-tumour responses. *Nat Biomed Eng*. 2023 Apr 24. doi: 10.1038/s41551-023-01031-3.
- Finally, an article preprint: Hayes BH, Wang M, Zhu H, Phan SH, Andrechak JC, Chang AH, Dooling LJ, Tobin MP, Marchena T, **Discher DE**. Chromosomal instability can favor macrophage-mediated immune response and induce a broad, vaccination-like anti-tumor IgG response. eLife. 2023 Apr 4:2023.04.02.535275. doi.org/10.7554/eLife.88054.1.

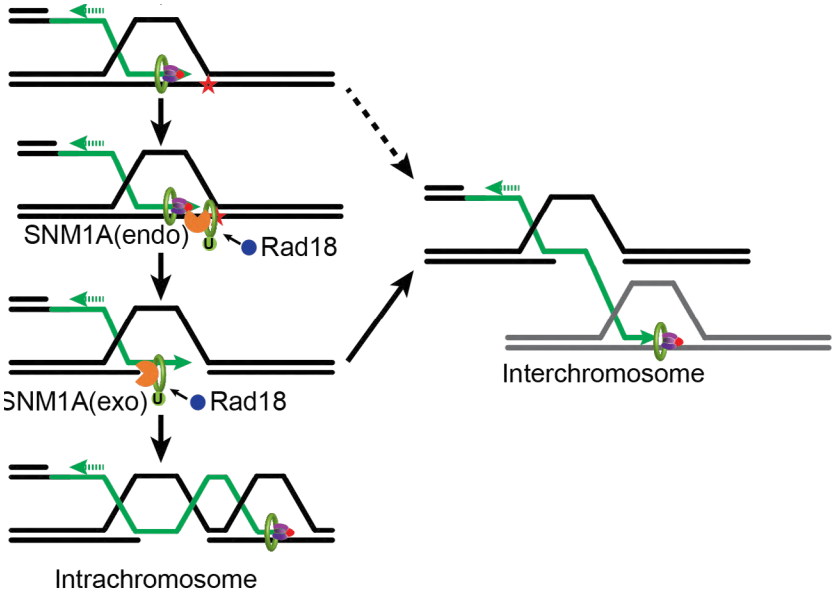
Good Lab

- Research Associate, Hui Chen, discovered a link between blastomere cell size and cell cycle elongation that sets the time of onset for widespread embryonic genome activation. Using nascent transcriptomics, he also revealed the zygotic genes that undergo a spatially graded pattern of transcriptional initiation in a model vertebrate blastula embryo. Chen H, **Good MC*** Nascent transcriptome reveals orchestration of zygotic genome activation in early embryogenesis. *Curr Biol* 32:4314-4324 (2022).
- Determined the sequence and chain rules governing the selectivity of disordered protein self-assembly into discrete membraneless compartments: Welles RM, Sojitra KA, Garabedian MV, Xia B, Wang W, Guan M, Regy RM, Gallagher ER, Hammer, DA, Mittal J*, **Good MC*** Determinants of Disordered Protein Co-Assembly Into Discrete Condensed Phases. Preprint on bioRxiv. Under revision at *Nature Chemistry*. doi: 10.1101/2023.03.10.532134 (2023).

Greenberg Lab

- Postdoctoral Researcher Tianpeng Zhang was first author on a paper in *Nature* describing PCNA-Ub-directed resection for template switch during break-induced replication at telomeres: Zhang T, Rawal Y, Jiang H, Kwon Y, Sung P, **Greenberg RA**. Break-induced replication orchestrates resection-dependent template switching. *Nature* 2023 Jul;619(7968):201-208. doi: 10.1038/s41586-023-06177-3. Epub 2023 Jun 14.

- Discovery of a new drug target for BRCA mutant cancers. ALC1 loss increased PARP inhibitor sensitivity by several hundred-fold and overcame known mechanisms of clinical resistance specifically in homologous recombination deficient cells. ALC1 inhibitors are being developed by several companies for the treatment of homologous recombination deficient cancers. Verma P, Zhou Y, Cao Z, Deraska PV, Deb M, Arai E, Li W, Shao Y, Puentes L, Li Y, Patankar S, Mach RH, Faryabi RB, Shi J, Greenberg RA. ALC1 links chromatin accessibility to PARP inhibitor response in homologous recombination-deficient cells. *Nat Cell Biol*. 2021 Feb;23(2):160-171. doi: 10.1038/s41556-020-00624-3.



Zhang et al. Nature, 2023

- Using a combination of crosslinking mass spectrometry, structural and cellular biology, the Greenberg lab discovered a new mechanism of regulation for the BRCA1-A complex. The complex becomes decorated with lysine63-linked ubiquitin chains that interact with the RAP80 ubiquitin binding sites and prevent recognition of DNA damage. BRCC36 contains K63-Ub deubiquitylase activity to remove these chains internally in the BRCA1-A complex and license its DNA damage response activities. Jiang Q, Foglizzo M, Morozov YI, Yang X, Datta A, Tian L, Thada V, Li W, Zeqiraj E, **Greenberg RA**. Autologous K63 deubiquitylation within the BRCA1-A complex licenses DNA damage recognition. J Cell Biol. 2022 Sep 5;221(9):e202111050. doi: 10.1083/jcb.202111050 and Walden et al. Nature 2019.

- Chen et al. *Cell Reports* 2020. Building on the discovery that DNA damage activates anti-tumor immune responses through cGAS recognition of missegregated genomic DNA in micronuclei (Harding, *Nature* 2017), the Greenberg lab discovered that progression through mitosis after damage also activated double-strand RNA pattern recognition through RIG-I. Chen J, Harding SM, Natesan R, Tian L, Benci JL, Li W, Minn AJ, Asangani IA, **Greenberg RA**. Cell Cycle Checkpoints Cooperate to Suppress DNA- and RNA-Associated Molecular Pattern Recognition and Anti-Tumor Immune Responses. *Cell Rep*. 2020 Sep 1;32(9):108080. doi: 10.1016/j.celrep.2020.108080.

High-Profile Papers

Haldar Lab

- Identified an immune evasive pathway active in some solid tumors whereby tumor cells produce retinoic acid that acts on local monocytes to promote their differentiation into an immunosuppressive and tumor-promoting macrophage. Devalaraja S, Jerrick To TK, Folkert IW, Natesan R, Alam MZ, Li M, Tada Y, Budagyan K, Dang MT, Zhai L, Lobel GP, Ciotti GE, Eisinger-Mathason TSK, Asangani IA, Weber K, Simon MC, **Haldar M**. Tumor-Derived Retinoic Acid Regulates Intratumoral Monocyte Differentiation to Promote Immune Suppression. *Cell* 2020 Mar 19;180(6):1098-1114.e16. doi: 10.1016/j.cell.2020.02.042.

- Described how intratumoral macrophages in medulloblastoma undergo treatment-specific changes in their composition, which can be leveraged from therapeutic purposes. Dang MT, Gonzalez MV, Gaonkar KS, Rathi KS, Young P, Arif S, Zhai L, Alam Z, Devalaraja S, Jerrick To TK, Folkert IW, Raman P, Rokita JL, Martinez D, Taroni JN, Shapiro JA, Greene CS, Savonen C, Mafra F, Hakonarson H, Curran T, **Haldar M**. Macrophages in SHH subgroup medulloblastoma display dynamic heterogeneity that varies with treatment modality. *Cell Rep*. 2021 Mar 30;34(13):108917. doi: 10.1016/j.celrep.2021.108917.

Lampson Lab

- As listed with the Black lab, Co-mentored postdoc Runi Das discovered that CENP-A is a maternal effect gene and established the mechanisms governing centromere inheritance in the mouse model system. Das A, Iwata-Otsubo A, Destouni A, Dawicki-McKenna JM, Boese KG, Black BE, **Lampson MA**. Epigenetic, genetic and maternal effects enable stable centromere inheritance. 2022 May;24(5):748-756. doi: 10.1038/s41556-022-00897-w.

- Dudka D, **Lampson MA**. Centromere drive: model systems and experimental progress. *Chromosome Res*. 2022 Sep;30(2-3):187-203. doi: 10.1007/s10577-022-09696-3. Epub 2022 Jun 22.

- Kumon T, Lampson MA. Evolution of eukaryotic centromeres by drive and suppression of selfish genetic elements. *Semin Cell Dev Biol.* 2022 Aug;128:51-60. doi: 10.1016/j.semcdb.2022.03.026. Epub 2022 Mar 26.

- The Lampson lab has a preprint on an automated molecular evolution tool that they developed for cell biologists, which was just accepted for publication in the Journal of Cell Biology. Here's the preprint: https://pubmed.ncbi.nlm.nih.gov/36909479/.

- **Michael Lampson** wrote a feature in *The Scientist* titled "Probing "Selfish" Centromeres Unveils an Evolutionary Arms Race": https://www.the-scientist.com/features/probing-selfish-centromeres-unveils-an-evolutionary-arms-race-71017.

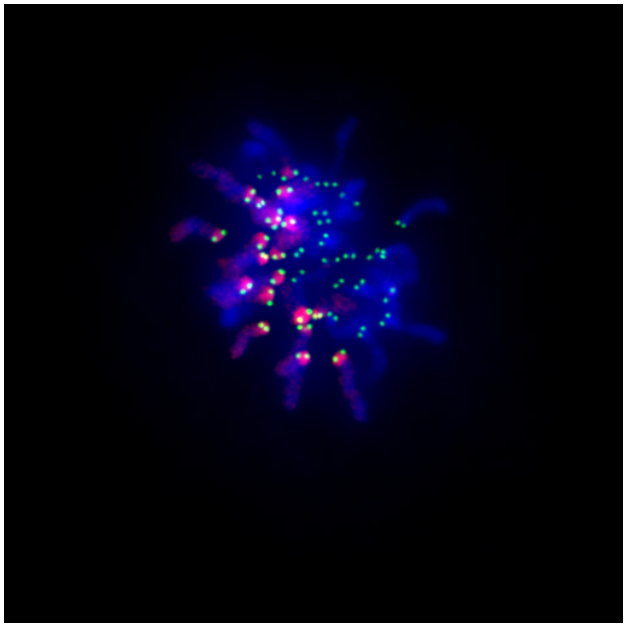
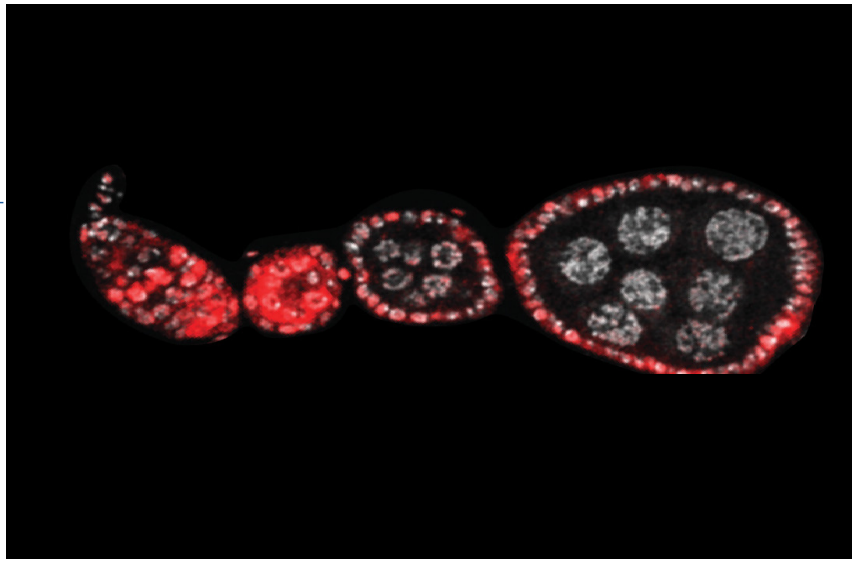


Image shows chromosomes (blue) in a mouse zygote with maternal chromosomes labeled in red and centromeres in green. The centromeres of the maternal chromosomes are larger, which is one of the findings of our study.

High-Profile Papers

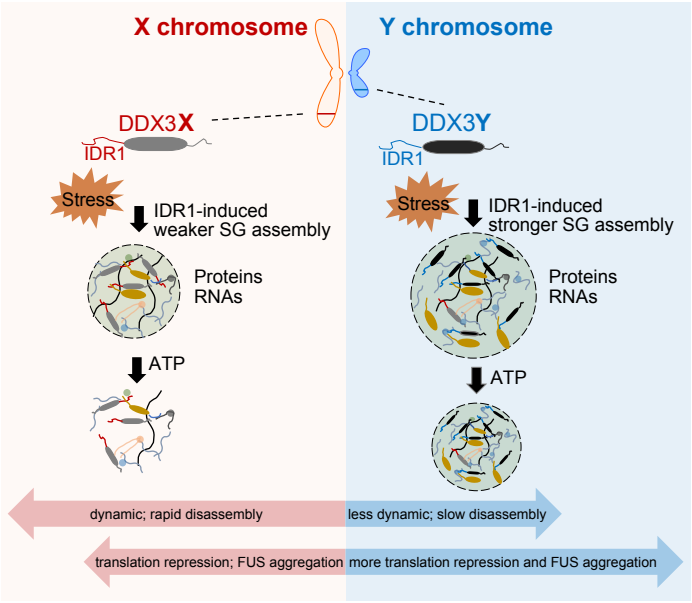
Levine Lab

- This article reported on the causes and consequences of rapid evolution of a DNA repair gene, maternal haploid, the Drosophila homolog of human Spartan: Brand CL, **Levine MT**. Cross-species incompatibility between a DNA satellite and the Drosophila Spartan homolog poisons germline genome integrity. *Curr. Biol.* 2022 Jul 11;32(13):2962-2971.e4. doi: 10.1016/j.cub.2022.05.009. Epub 2022 May 27. The discoveries show, for the first time, that DNA repeat-enriched regions of the genome require finely tuned DNA-protein crosslink repair to preserve germline genome integrity.
- This study identified two histone modifications that determine reproductive lifespan extension in Drosophila: Divito-Evans A, Fairbanks R, Schmidt P, **Levine MT**. Histone methylation regulates reproductive diapause in Drosophila melanogaster. PLoS Genetics 2023 Sep 13;19(9):e1010906. doi: 10.1371/journal.pgen.1010906.



The fruit fly ovary (white = DNA) shows elevated levels of DNA damage (red) in the presence of a toxic DNA repair protein derived from a closely related species.

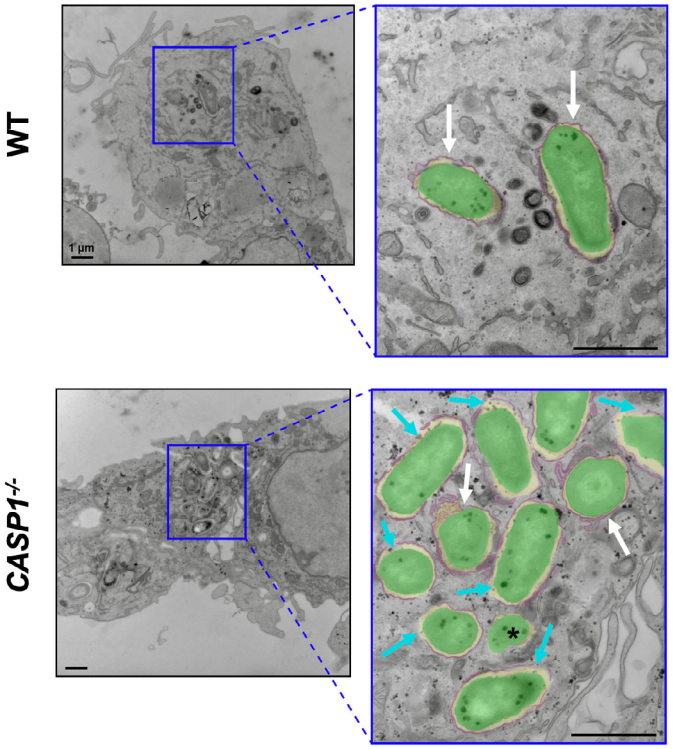
Liu Lab



- Gonskikh Y†, Stoute J†, Shen H†, Budinich K, Pingul B, Schultz K, Elashal H, Marmorstein R, Shi J, **Liu KF**. Non-catalytic regulation of 18S rRNA methyltransferase DIMT1 in acute myeloid leukemia. *Genes Dev* 2023 Apr 1;37(7-8):321-335. doi: 10.1101/gad.350298.122. Epub 2023 Apr 6. † shared first authorship.
- Shen H†, Yanas A†, Owens M, Zhang C, Fritsch C, Fare CM, Copley KE, Shorter J, Goldman, YE, **Liu KF.** Sexually dimorphic RNA helicases DDX3X and DDX3Y differentially regulate RNA metabolism through phase separation. *Mol Cell* 2022 Jul 21;82(14):2588-2603.e9. doi: 10.1016/j.molcel.2022.04.022. Epub 2022 May 18.

Shin Lab

- This study revealed that inflammatory caspases and pyroptotic factors mediate inflammasome responses that restrict the subcellular localization of intracellular Salmonella replication within human macrophages. Egan MS, O'Rourke EA, Mageswaran SK, Zuo B, Martynyuk I, Demissie T, Hunter EN, Bass AR, Chang Y-W, Brodsky IE, **Shin S**. Inflammasomes primarily restrict cytosolic Salmonella replication within human macrophages. eLife 2023 Aug 23:https://doi.org/10.7554/eLife.90107.1



- The Shin lab revealed species-specific differences in how a family of IFN-inducible GTPases, termed guanylate-binding proteins, function to target and promote damage to phagosomes containing bacterial pathogens in order to promote inflammasome activation: Bass AR, Egan MS, Alexander-Floyd J, Fischer NL, Doerner J, **Shin S**. Human GBP1 facilitates the rupture of the Legionella-containing vacuole and inflammasome activation. *mBio* 2023 Sep 22:e0170723. doi: 10.1128/mbio.01707-23.

- This study revealed species- and cell type-specific differences in the mechanisms of activation and evasion of inflammasomes by the bacterial pathogen Yersinia pseudotuberculosis. Zhang J, Brodsky IE, **Shin S**. *Yersinia* deploys type III-secreted effectors to evade caspase-4 inflammasome activation in human cells. *mBio* 2023 Aug 24:e0131023.. doi: 10.1128/mbio.01310-23

- This study revealed how the cytokine TNF activates rapid inflammasome-dependent cell death mediates by the caspases-1, -11, and -8 to restrict intracellular bacterial infection: Pollock TY, Vázquez Marrero VR, Brodsky IE, Shin S. TNF licenses macrophages to undergo rapid caspase-1, -11, and -8-mediated cell death that restricts Legionella pneumophila infection. *PLOS Pathog*. 2023 Jun 6;19(6):e1010767. doi: 10.1371/journal.ppat.1010767.

- This review describes species- and cell type-specific differences in inflammasome responses to infection: Egan M, Zhang E, **Shin S**. Human and mouse NAIP/NLRC4 inflammasome responses to bacterial infection. Curr Opin Microbiol. 2023 Jun:73:102298. doi: 10.1016/j.mib.2023.102298.

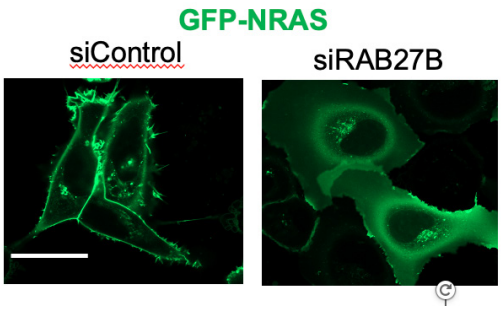
- The Shin lab identified a key role for the human NAIP and NLRP3 inflammasomes in detection and control of Salmonella Typhimurium infection in macrophages. Naseer N, Egan MS, Reyes Ruiz VM, Scott WP, Hunter EN, Demissie T, Rauch I, Brodsky IE, **Shin S**. Human NAIP/NLRC4 and NLRP3 inflammasomes detect Salmonella type III secretion system activities to restrict intracellular bacterial replication. *PLoS Pathog*. 2022 Jan 24;18(1):e1009718. doi: 10.1371/journal.ppat.1009718.

High-Profile Papers

Tong Lab

- Ren JG, Xing B, Lv K, O'Keefe RA, Wu M, Wang R, Bauer K, Ghazaryan A, Burslem GM, Zhang J, O'Connell RM, Pillar V, Hexner EO, Philips MR, **Tong W**. RAB27B controls palmitoylation-dependent NRAS trafficking and signaling in myeloid leukemia. *J Clin Invest*. 2023 Jun 15;133(12):e165510. doi: 10.1172/JCI165510.

- Lv K, Gong C, Aruljothi C, Han X, Ren J, Donaghy R, Cheng Y, Pellegrino S, Warren AJ, Paralkar VR, **Tong W**. HectD1 controls hematopoietic stem cell regeneration by coordinating ribosome assembly and protein synthesis. *Cell Stem Cell* 2021 Jul 1;28(7):1275-1290.e9. doi: 10.1016/j.stem.2021.02.008.



Schematic illustration of of NRAS localization at the plasma membrane is dependent upon RAB27B.

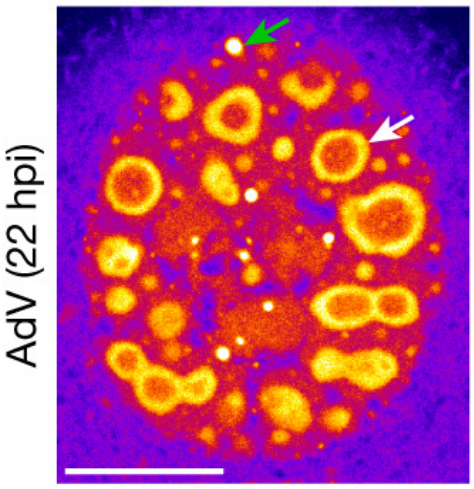
- Holdreith N, Lee GY, Chandra V, Salas Salinas C, Nicholas P, Olson PS, **Tong W**. LNK (SH2B3) Inhibition Expands Healthy and Fanconi Anemia Human Hematopoietic Stem and Progenitor Cells. *Blood Adv*. 2022 Feb 8;6(3):731-745. doi: 10.1182/bloodadvances.2021004205.

- Lv K, Ren, J, Han X, Ren J, Gui J, Gong C, **Tong W**. Depalmitoylation Rewires FLT3-ITD Signaling and Exacerbates Leukemia Progression. *Blood* 2021Dec 2;138(22):2244-2255. doi: 10.1182/blood.2021011582.

Weitzman Lab

- Charman M, Grams N, Kumar N, Halko E, Dybas JM, Abbott A, Lum KK, Blumenthal D, Tsopurashvili E, **Weitzman MD**. A viral biomolecular condensate coordinates assembly of progeny particles. *Nature* 2023 Apr;616(7956):332-338. doi: 10.1038/s41586-023-05887-y.

- Petljak M, Green AM, Maciejowski J, **Weitzman MD**. Addressing the benefits of inhibiting APOBEC3-dependent mutagenesis in cancer. Nat Genet. 2022 Nov;54(11):1599-1608. doi: 10.1038/s41588-022-01196-8.



(Pictured right): The nucleus of an adenovirus infected human bronchial epithelial cell. Newly synthesized protein is enriched at virus-induced subcompartments including viral nuclear bodies (green arrow) and viral replication compartments (white arrow). Scale bar = 10 µm.

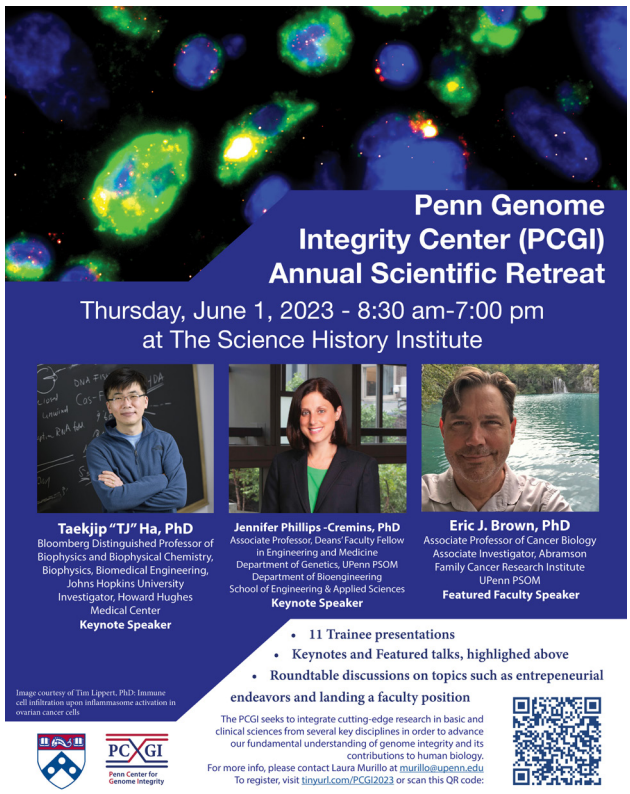
Events Hosted

9 Trainee Seminars

8 Working Group Meetings

3 Mini Symposia

In the 2022-2023 Academic Year, along with the Annual PCGI retreat at the Science History Institute, and a joint symposium with the Mark Foundation, we featured 36 trainee talks, three mini symposia on Repetitive DNA & Development, Recombination, and Molecular Machines:



[Click here to see full-sized images of all our event fliers]

Events Hosted

Penn Center for Genome Integrity (PCGI) Mini Symposium

Monday, April 17, 2023

2:00 - 5:00 pm in the BRB II/III Auditorium

Session Topic:
"Molecular machines in genome stability"

Tarun Kapoor, PhD
The Pels Family Professor
Head of the Selma and Lawrence Ruben Laboratory of Chemistry and Cell Biology
The Rockefeller University

"Regulating when and where a microtubule assembles: put a gamma-tubulin ring on it"

Vidhya Krishnamoorthy, PhD
Postdoctoral Researcher
Laboratory of Roger Greenberg

"55LCC: A new macromolecular machine in DNA replication protein quality control"

Edward Twomey, PhD
Assistant Professor of Biophysics and Biophysical Chemistry, The Johns Hopkins University School of Medicine, Co-Director, Beckman Center for Cryo-EM at Johns Hopkins

"Molecular Machines in Action: Developing Time-Resolved Cryo-EM"

Hee Jong Kim
Graduate Student, BMB
Laboratory of Kenji Murakami

"Structural insights into histone deposition by a transcriptional histone chaperone complex"

Coffee break in-between talks & happy hour at conclusion in the BRB Lobby

PCGI Penn Center for Genome Integrity

The PCGI seeks to integrate cutting-edge research in basic and clinic
key disciplines in order to advance our fundamental understanding of
contributions to human biology. For more information, please
(www.med.upenn.edu/pcgi). For more information, contact Laura Mur

Image courtesy of Timothy Lippert, PhD, Po

Penn Center for Genome Integrity (PCGI) Special Seminar

Weixing Zhao, PhD
Assistant Professor, Department of Biochemistry and Structural Biology, University of Texas Health San Antonio

"Deciphering Mechanisms of BRCA1 Tumor Suppression: A Journey from Biochemical Dissection of BRCA1 Network"

Wednesday, March 15, 2023
10:00 - 11:00 am
Room 1412 BRB II/III

PCGI Penn Center for Genome Integrity

The PCGI seeks to integrate cutting-edge research in basic and clinical sciences from several key disciplines in order to advance our fundamental understanding of genome integrity and its contributions to human biology. For more information, please visit our website (www.med.upenn.edu/pcgi). For more information, contact Laura Murillo at murillo@upenn.edu.

Image courtesy of Timothy Lippert, PhD, Postdoctoral Researcher, Greenberg Lab

Penn Center for Genome Integrity (PCGI) Trainee Seminar

Monday, March 27, 2023
4:00 - 5:00 pm in BRB II/III Auditorium

Featured Speakers:

Yulia Gonskikh, PhD
Postdoctoral Researcher, Laboratory of Kathy Liu
PSOM Department of Biochemistry & Biophysics

"The molecular mechanism of 5-methylcytosine installation on tRNAs by NSUN2 to support neuronal development"

and

Neha Nataraj
PhD Student, Immunology, Laboratory of Sunny Shin
PSOM Department of Microbiology

"Yersinia blockade of immune signaling induces RIPK1-independent apoptosis in human macrophages"

Join us at the conclusion of the talks for a Happy Hour in the BRB Lobby.

PCGI Penn Center for Genome Integrity

The PCGI seeks to integrate cutting-edge research in basic and clinical sciences from several key disciplines in order to advance our fundamental understanding of genome integrity and its contributions to human biology. For more information, please visit our website (www.med.upenn.edu/pcgi). For more information, contact Laura Murillo at murillo@upenn.edu.

Image courtesy of Timothy Lippert, PhD, Postdoctoral Researcher, Greenberg Lab

[Click here to see full-sized images of all our event fliers]

PCGI Penn Center for Genome Integrity

Honors

Bernstein Lab
- Dr. Kara Bernstein has been nominated for an Endowed Chair of Biochemistry and Biophysics.

Black Lab
- In 2021, the Black/Lampson collaborative team was granted a Transformative Research Award (T-R01) from the NIH Director's Office.
- In 2022, the Black Lab was awarded an R01 from NCI to study PARP1 and efforts to target it more effectively in the treatment of cancer.
- Black Lab trainees have been advancing onto independent positions at leading educational and research universities. Some recent examples are former postdoc, Viridiana Herrera (now Asst. Prof. at Lincoln University); current postdoc, Runi Das (co-mentored by Mike Lampson; starting her lab at Cornell University in 2024); and former PhD student, Glennis Logsdon (starting her lab at UPenn in 2024).

Greenberg Lab
- Dr. Roger Greenberg was selected as the recipient of the 2023 William L. Gerald Award, which is given annually by the Department of Pathology and Laboratory Medicine at Memorial Sloan Kettering Cancer Center.
- Dr. Greenberg was named Vice Chair, Gordon Research Conference on Mammalian DNA Repair, in Ventura, CA, on February 5-10, 2023, which the Center also sponsored with a gift (highlighted in funding initiatives).

Good Lab
- Matt was added to the Board of Directors for the International Xenopus Committee and co-chaired the 19th International Xenopus Conference in Aug 2023.
- Matt was promoted to associate professor at Penn in with tenure in July 2022
- Matt's former postdoc, Dr. Hui Chen, was hired as an associate professor in the department of Biological Sciences at USC.

Lampson Lab
- Arunika Das was awarded the 2022 S. Walter Englander Prize for Postdoctoral Research from the Department of Biochemistry & Biophysics (UPenn).
- Arunika also received Honorable Mention for the 2022 ASCB Porter Prize for Research Excellence

Levine Lab
- Dr. Mia Levine received the 2023 Biology Department Teaching Award, an acknowledgement of her teaching excellence in two courses: the Molecular Biology and Genetics lecture course and the Genetics of Adaptation seminar course.
- Dr. Levine was elected to be a 2023-2024 Penn Faculty Fellow. This program offers a select group of mid-career Penn faculty a one-year course of leadership training. The program includes opportunities to build alliances, engage with senior leaders at Penn, and cultivate strategic thinking around university governance.

Liu Lab
- In 2021, Dr. Kathy Liu was named a Scialog Fellow.
- Dr. Liu was also awarded a Chemical Machinery of the Cell Fellow in 2021.
- Dr. Liu received the Damon Runyon-Rachleff Innovation Award in 2022.
- Also in 2022, Dr. Liu was named American Cancer Research Scholar, with the American Cancer Society.
- Dr. Liu was presented with the Linda Pechenik Montague Investigator Award in 2023.

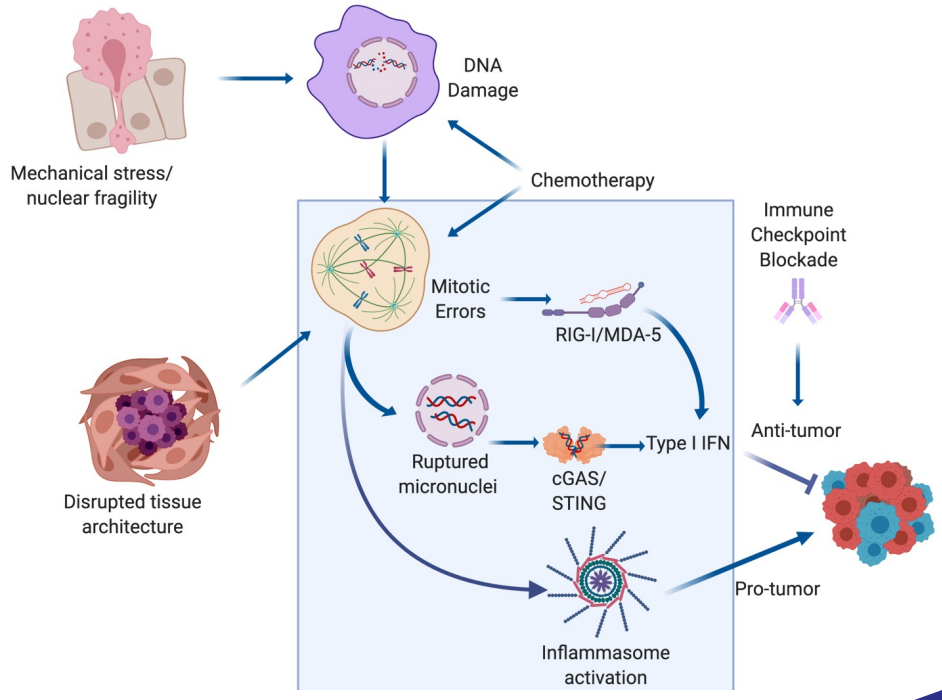
Shin Lab
- Dr. Shin was elected as a Fellow of the American Academy of Microbiology in 2023.
- Postdoctoral fellow Xin Liu started a tenure-track faculty position in the Department of Biochemistry at Case Western Reserve University in 2023.
- Dr. Shin was the Philip J. Bassford Memorial Lecturer at the University of North Carolina at Chapel Hill in 2022.

P01 CA265-79401 - Funded May 2023

From left: **Roger A. Greenberg**, Principal Investigator, Project 1: *Tumor Cell intrinsic DNA Damage Signaling to the Immune Response*; **Michael Lampson**, Co-PI, Project 2: *Mechanics of Cells & Tissues Impact Chromosome Instability & Phagocytic Interactions*; **Dennis Discher**, Co-PI, Project 2; **Sunny Shin**, Co-PI, Project 3: *Delineating How nucleic acid sensing in tumor cells regulate anti-tumor immune responses*; **Malay Haldar**, Co-PI, Project 3; **Ben E. Black**, Core 1: *Mammalian Artificial Chromosome (MAC)*; : *Delineating How nucleic acid sensing in tumor cells regulate anti-tumor immune responses*; **David Chenoweth**, Core 2, *Chemical Biology & Materials Tools (CBMT); and* **Laura Murillo**, Administrative Core Coordinator

We seek to investigate the following with this P01:

- How does genome instability promote recognition of endogenous nucleic acids?
- How do endogenous nucleic acid sensing pathways impact tumor immune microenvironment and tumor progression?
- Strategies to implement immune therapies in chromosomally unstable cancers (BRCA mutant and others).
- The P01 addresses goals of cancer interception in BRCA mutant cancers, which is a primary objective of the Basser Center for BRCA.



Grants

In addition to the multi-investigator P01 (above), our investigators also received the following funding:

Bernstein Lab

- In April 2021, the Bernstein Lab was awarded an R01 titled "RAD51 paralog function in cancer predisposition and genome integrity." Direct costs total $1,425,380.
- The Bernstein Lab received a Department of Defense grant for a project titled "Molecular targeting of homologous recombination-deficient breast cancers" that began July 2021. Direct costs: $1,000,000.

Black Lab

- In 2021, the Black/Lampson collaborative team was granted a Transformative Research Award (T-R01) from the NIH Director's Office. In 2022, the Black Lab was awarded an R01 from NCI to study PARP1 and efforts to target it more effectively in the treatment of cancer.
- Black Lab trainees have been advancing onto independent positions at leading educational and research universities. Some recent examples are former postdoc, Viridiana Herrera (now Asst. Prof. at Lincoln University); current postdoc, Runi Das (co-mentored by Mike Lampson; starting her lab at Cornell University in 2024); and former PhD student, Glennis Logsdon (starting her lab at UPenn in 2024).

Busino Lab

- In 2020, the Busino Lab renewed an R01 grant from NCI for their study on ubiquitin mediated proteolysis in Diffuse Large B-cell Lymphoma.
- The Busino Lab was awarded an R01 grant from NHLBI on emergency hematopoiesis in 2021.
- The Busino Lab received a TR01 as co-PI grant from NCI titled „Chemical Space of Cancer-Associated Perturbations in 2022.

Greenberg Lab

- Dr. Roger Greenberg is the PI of recently-funded P01 *Genome Instability Anti-Tumor Immune Responses*, with Co-PIs Ben Black, David Chenoweth, Dennis Discher, Malay Haldar, Michael Lampson, and Sunny Shin.
- Successfuly renewed his GM R01 award last year, which will run through March 2026.
- Postdoctoral researcher Timothy Lippert is entering his third year of a Cancer Research Institute fellowship. Timothy also had funding from the Basser Center for BRCA.
- Tianpeng Zhang received a Discovery fellowship from the Abramson Family Cancer Research Institute.
- Postdoc Stefano Misino received the Italian-American Postdoctoral Fellowship.

Good Lab

- Dr. Matt Good was named co-leader of the NSF Material Research Science and Engineering Center interdisciplinary research group, IRG2. This project focuses on building materials through controlled phase separation from macromolecular to cellular length scales.
- Dr. Good (PSOM) and Dr. Amish Patel (SEAS) were awarded a CPE4H pilot grant on "Versatile Coacervating Peptides as Carriers and Synthetic Organelles for Cell Engineering" Sep 2022.
- Dr. Mikael Garabedian, a postdoc in the Good Lab co-mentored by Roger Greenberg, was awarded a K99 from NIGMS entitled "Characterizing the role of tumor suppressor phase separation and chromatin...

Good Lab continued on the next page...

Grants

Good Lab continued...

maintaining genomic integrity," beginning in the summer of 2023.
- Dr. Hui Chen, a Research Associate in the Good lab, was awarded an R03 from NICHD on "Hierarchical Onset of Germ Layer Specification" last Spring 2022.

Haldar Lab
- This September 2023, the Haldar lab received an R01 titled "Role of glutamine metabolism in dendritic cell development" to examine how the levels of amino acid glutamine regulates development of dendritic cell subsets.

Lampson Lab
- The Lampson lab's R35 grant titled Cell biological mechanisms of centromere drive" was successfully renewed, 6/1/2022 – 5/31/2027.

Levine Lab
- During the 2022-2023 academic year, Mia Levine's lab renewed their 5-year NIH NIGMS R35 grant, "Causes and functional consequences of chromatin evolution."
- Levine Lab postdoc, Dr. Cara Brand, received an NIH NIGMS K99/R00 award, which supports Cara's salary for two years along with providing a small research budget and continued funding during the first three years of leading her own independent lab.

Liu Lab
- During the 2022-2023 academic year, the Liu Lab obtained a collaborative R01 with the Barry Cooperman Lab at Penn.

National Heart Lung and Blood Institute

American Heart Association.

Shin Lab
- The Shin Lab renewed an R01 grant from NIAID on studying how inflammasome-mediated crosstalk between myeloid cells and alveolar epithelium orchestrates inflammatory responses in the lung in 2021.
- The Shin Lab received an R21 grant from NIAID on studying mechanisms of apoptosis in dendritic cells during bacterial infection in 2023.
- The Shin Lab received an R01 Diversity Supplement to support graduate student Jaydeen Sewell in 2023.
- Postdoctoral researcher Xin Liu received an NHLBI K99/R00 Pathway to Independence Award in 2021.
- Postdoctoral researcher Mikel Haggadone received an American Heart Association Postdoctoral Fellowship in 2022.
- Graduate student Stephanie Schreiner received a National Science Foundation Graduate Research Fellowship in 2022.

NSF

Tong Lab
- This September 2023, the Tong lab received an R01 for their project "Regulation of FLT3 Signaling in Leukemia"
- The Tong lab received a new R01 entitles, "Novel Regulation of Oncogenic NRAS Signaling in Myeloid Malignancies", from NCI in 2022.

$750,000 funded

in support of PCGI Trainees

Established sponsored research funding we call "Provocative Questions" to support our trainees. Any PCGI Lab can apply. We're currently working on establishing trainee fellowships with a formal application process. See more details on page ###.

$50,000 committed

to support PCGI Undergrads

Undergraduate research is the groundwork of every scientific career. This committment recognizes the importance of supporting early research endeavors.

$10,000 contributed

in support of conferences

- Gordon Research Conference (GRC) on "DNA Damage, Mutation, and Cancer" from March 10-15, 2024 in Ventura, CA.
- Cell and Molecular Biology Graduate Group (CAMB) Symposium on October 19-20, 2023. We also supported the 2021 CAMB Symposium.
- Gordon Research Conference (GRC), on "Mammalian DNA Repair" held from February 5-10, 2023.
- Gordon Research Conference (GRC), on Genomic Instability held from July 10-15, 2022.

Continued next page...

$4,000 salary support

for Teacher's Assistant

Funding provided to pay a Teacher's Assistant, for CAMB 530 course "The Cell Cycle, Genome Integrity and Cancer" in 2022 and 2023, Directed by Dr. David Feldser.

$250,000 distributed

Equipment and Shared Resources

The PCGI has invested in equipment that will be accessible to all PCGI Core members:

- ImageXpress Micro Confocal High-Content Imaging System for 3D image analysis. Overseen by Eric Joyce.
- MACsQuant Vyb multicolor high-throughput flow cytometer. Overseen by Roger Greenberg.
- FiberComb DNA Molecular Combing System for DNA replication fiber analyses. Overseen by Roger Greenberg.
- Nikon Ti2E Motorized Microscope. Overseen by Roger Greenberg.
- S3e Cell Sorter, 405, 488, and 640 nm 100, mW lasers, includes 4 fluorescence detectors with filters. Overseen by Malay Haldar.
- Qubit instrument for nucleic acid quality control. Overseen by Ben Black.

Webpage on the PCGI Website coming soon with additional details.

Provocative Questions

We have provided approximately $750,000 in support of projects led by trainees in PCGI Laboratories, leading to the following accomplishments:

Black Lab: Development of mammalian artificial chromosome technology

- A paper has been submitted for publication and posted as a pre-print: Gambogi CW, Mer E., Brown DM, Yankson G, Gavade JN, Logsdon GA, Heun P, Glass JI, **Black BE***. Efficient formation of single-copy human artificial chromosomes. submitted for publication. (*corresponding author) https://www.biorxiv.org/content/10.1101/2023.06.30.547284v1.

Busino Lab: Determining the role of NT5DC2-proteolysis in regulating nucleotide levels and genome instability

- A paper is nearing publication, first-author Grant Grothusen, titled "CRL4-DCAF15 control of cohesin dynamics sustains cell proliferation" for *Nature Communication* (in Revision).

Discher Lab: Engineering anti-tumor immune responses

- The Discher Lab was featured in Penn Today for their article published in the *Journal of Cell Biology* for their discovery of lipid droplets' capability to indent and puncture the nucleus, the organelle which contains and regulates a cell's DNA. Ivanovska IL, Tobin MP, Bai T, Dooling LJ, **Discher DE**. Small lipid droplets are rigid enough to indent a nucleus, dilute the lamina, and cause rupture. *J Cell Biol*. 2023 Aug 7;222(8): e202208123. doi: 10.1083/jcb.202208123. Epub 2023 May 22. Also featured in Penn Engineering Today's Blog.
- Dooling LJ, Andrechak JC, Hayes BH, Kadu S, Zhang W, Pan R, Vashisth M, Irianto J, Alvey CM, Ma L, **Discher DE**. Cooperative phagocytosis of solid tumours by macrophages triggers durable anti-tumour responses. *Nat Biomed Eng*. 2023 Apr 24. doi: 10.1038/s41551-023-01031-3. Online ahead of print.
- Hayes BH, Zhu H, Andrechak JC, Dooling LJ, **Discher DE**. Titrating CD47 by mismatch CRISPR-interference reveals incomplete repression can eliminate IgG-opsonized tumors but limits induction of antitumor IgG. *PNAS Nexus*. 2023 Jul 27;2(8):pgad243. doi: 10.1093/pnasnexus/pgad243. eCollection 2023 Aug.
- Hayes BH, Zhu PK, Wang M, Pfeifer CR, Xia Y, Phan S, Andrechak JC, Du J, Tobin MP, Anlas A, Dooling LJ, Vashisth M, Irianto J, Lampson MA, **Discher DE**. Confinement plus myosin-II suppression maximizes heritable loss of chromosomes, as revealed by live-cell ChReporters. *J Cell Sci*. 2023 Jun 1;136(11):jcs260753. doi: 10.1242/jcs.260753. Epub 2023 Jun 8.
- Wang M, Phan S, Hayes BH, **Discher DE**. Genetic heterogeneity in p53-null leukemia increases transiently with spindle assembly checkpoint inhibition and is not rescued by p53. *Chromosoma* 2023 May 31. doi: 10.1007/s00412-023-00800-y. Online ahead of print.

Haldar Lab: Dendritic cell responses to genomic instability in cancer

- The Haldar lab has a paper submitted under revision to the Journal *Cancer Immunology Research* titled "RALDH1 inhibitors for the immunotherapy of hepatocellular carcinoma" by authors: Yu P, Cao S, Yang S-M, Rai G, Martinez NJ, Yasgar A, Zakharov AV, Simeonov A, Molina Arocho WA, Lobel GP, Mohei H, Scott AL, Zhai L, Furth EE, Simon MC, and **Haldar M.**

Jin Lab: Determine the role of AIM2 in cancer progression and treatment

- A paper has been submitted for publication: Liu Z, Hu B, Chen E, Zhao C, Dong Q, Meeth K, Bosenberg M, Flavell RA, Ho PC, **Jin C**. The innate receptor AIM2 acts as a dual metabolic and immune

Jin Lab continued on the next page...

Provocative Questions (Cont.)

Jin Lab continued...

sensor to dictate the functional dichotomy in tumor-associated myeloid cells.
- The funded project resulted in a Wistar/Penn skin SPORE Developmental Research Program (DRP) award.

Lampson Lab: Extracellular force control of mitotic fidelity

This funding supported Scott (Geng-Yuan) Chen, a Research Associate in the Lampson Lab, his research since funding resulted in the following:

- **Chen G-Y**, Renda F, Zhang H, Gokden A, Wu DZ, Chenoweth DM, Khodjakov A, **Lampson MA**. Tension promotes kinetochore-microtubule release by Aurora B kinase. J Cell Biol. 2021 Jun 7;220(6):e202007030. doi: 10.1083/jcb.202007030.
- A review: **Chen G-Y**, **Lampson MA**. Chemical tools for dissecting cell division. Nat Chem Biol. 2021 Jun; 17(6):632-640. doi: 10.1038/s41589-021-00798-3.
- Scott also received an Early Career Research Award from the Basser Center during this period.
- Finally, this PCGI funding also contributed to preliminary data from the Lampson lab for the P01 (above).

Levine Lab: Evolution of telomere protection protein complexes

- This funding has supported postdoc Hyuk-Joon Jeon, who has discovered telomere length asymmetry across maternal and paternal chromosomes determines the rate of telomere elongation in the early mouse embryo. These data serve as the foundation of a Levine-Lampson multi-PI R01 slated for submission in early 2024.

Liu Lab: Sex chromosome homologous proteins in stem cell differentiation

- The Liu lab just recently obtained this funding, hope to have progress to report soon!

Shi & Kohli Labs: Expanding the toolbox for controllable base editing

- The labs of Dr. Junwei Shi and Rahul Kohli published two papers with the help of this funding, the first of which was: Berríos KN, Evitt NH, DeWeerd RA, Ren D, Luo M, Barka A, Wang T, Bartman CR, Lan Y, Green AM, **Shi J**, Kohli RM. Controllable genome editing with split-engineered base editors. *Nat Chem Biol.*, 2021 Dec;17(12):1262-1270. doi: 10.1038/s41589-021-00880-w.
- Secondly: Berríos KN, Barka A, Gill J, Evitt NH, Gajula KS, **Shi J**, Kohli RM. Cooperativity between Cas9 and hyperactive AID establishes broad and diversifying mutational footprints in base editors. *BioRxiv*, doi: https://doi.org/10.1101/2022.12.03.518995.

Shin Lab: DNA damage-induced cell death and type I interferon signaling in anti-tumor immunity

- The Shin lab just recently obtained this funding, hope to have progress to report soon!

Weitzman Lab: Mechanisms of HSV-1 replication

- The Weitzman lab just recently obtained this funding, hope to have progress to report soon!

Entrepreneurial Efforts

Genome Integrity from Bench to Bedside

Targeting cancer, autoimmunity, and other indications

Basic studies leading to clinical trials

- Small molecule therapeutics
- Immune therapy
- Radiopharmaceuticals
- Prognostic biomarkers

Companies founded by PCGI labs

RADD PHARMACEUTICALS

JAMM THERAPEUTICS

ATRIN Pharmaceuticals

TREVARX BIOMEDICAL

HYSPLEX Therapeutics