Genome Editing Shared Resource

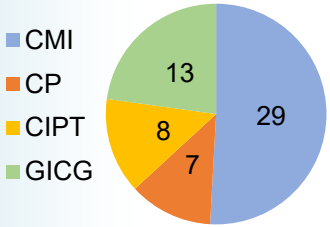
RUTGERS Cancer Institute of New Jersey
RUTGERS HEALTH

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

Aims

- Co-managed by CINJ and Rutgers Office for Research, the GESR has seen significant growth since the last site visit
- The GESR impact on members' science is best highlighted by the central role of the Managing Director in a new NCI P01 and involvement in two NCI R01s awarded to the GICG and CMI programs
- The GESR produces genetically engineered mouse models using CRISPR-CAS9 gene-editing technology, including the design and validation of CRISPR reagents, and performs custom genome editing of human and mouse cell lines

Research Program Support (2018–2022)



57 Members

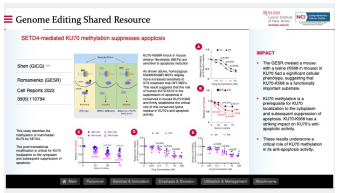
Publications

Total	35
IF>10	9

Peer-Reviewed Grants

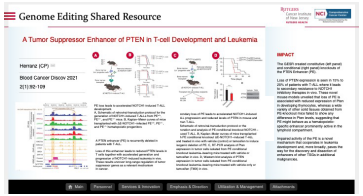
All	41 (2T)
NCI	17 (2T)

GICG



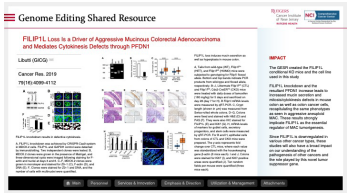
Cell Reports, 2022

CP



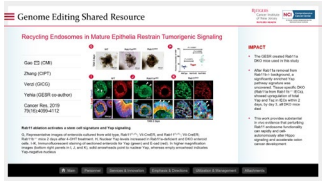
Blood Cancer Discov, 2021

GICG, CIPT



Cancer Res, 2021

CMI, CIPT, GICG



Cancer Res, 2019

Leading Personnel & Roles

Derek Sant'Angelo, PhD
Director

Peter Romanienko, PhD
Managing Director

Yibin Kang, PhD
Co-Director

Ghassan Yehia, PhD
Resource Manager

Services & Innovation

New

- Electroporation to introduce CRISPR reagents into mouse embryos, resulting in a more efficient generation of mouse models and the ability to modify embryos generated by IVF
- Developed novel method to allow for modification of a single target allele

Continuing

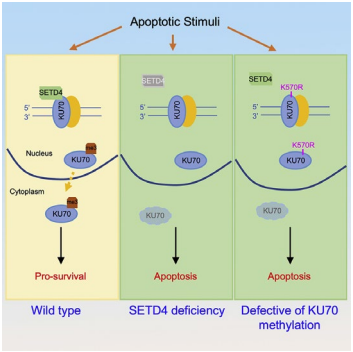
- Cell line editing
- Mouse line cryopreservation
- Mouse line rederivation
- Expression vectors and viral vectors for in vivo gene editing

SETD4-Mediated KU70 Methylation Suppresses Apoptosis

Shen (GICG)

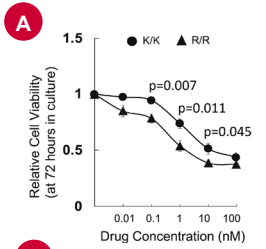
Romanienko (GESR co-author)

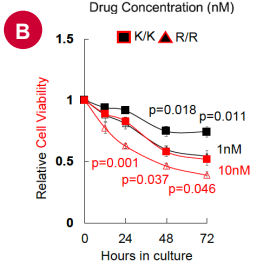
Cell Reports, 2022
39(6):110794



KU70-K568R knock in mouse embryo fibroblasts (MEFs) are sensitive to apoptosis induction

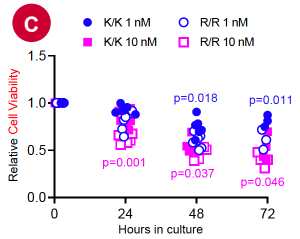
As shown above, homozygous K568R/K568R MEFs display more increased sensitivity to STS treatment than WT MEFs. This result suggests that the role of human KU70-K570 in the suppression of apoptosis is conserved in mouse KU70-K568 and firmly establishes the critical role of this conserved lysine residue in KU70's anti-apoptotic activity

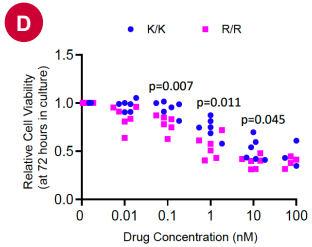


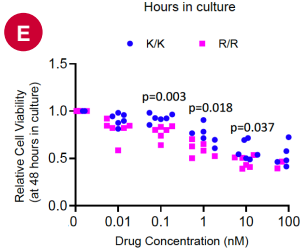


This study identifies the methylation of mammalian KU70 by SETD4.

This post-translational modification is critical for KU70 localization to the cytoplasm and subsequent suppression of apoptosis







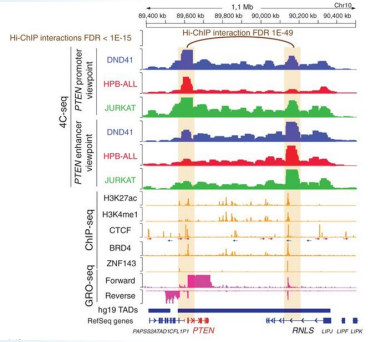
IMPACT

- The GESR created a mouse with a lysine (K568 in mouse) in KU70, which had a significant cellular phenotype, suggesting that KU70-K568 is a functionally important substrate
- KU70 methylation is a prerequisite for KU70 localization to the cytoplasm and subsequent suppression of apoptosis. KU70-K568 has a striking impact on KU70's anti-apoptotic activity
- These results underscore a critical role of KU70 methylation in its anti-apoptosis activity

A Tumor Suppressor Enhancer of PTEN in T-cell Development and Leukemia

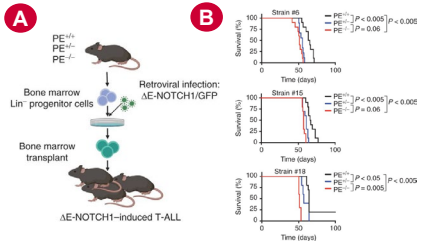
Herranz (CP) ✉

Blood Cancer Discov, 2021
2(1):92-109



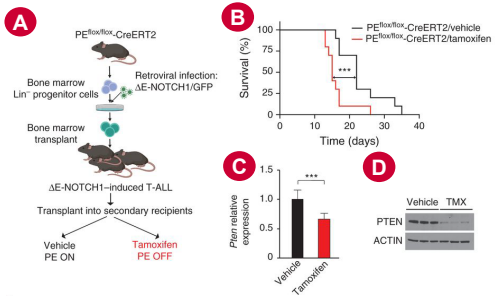
A *PTEN* enhancer (PE) is recurrently deleted in patients with T-ALL

Loss of this enhancer leads to reduced PTEN levels in T cells together with accelerated generation and progression of NOTCH1-induced leukemia in vivo. These results uncover long-range regulation of tumor suppressor genes as a relevant mechanism in cancer



PE loss leads to accelerated NOTCH1-induced T-ALL development

A, Schematic of retroviral-transduction protocol for the generation of NOTCH1-induced T-ALLs from PE$^{+/+}$, PE$^{+/-}$, and PE$^{-/-}$ mice. **B,** Kaplan–Meier curves of mice transplanted with ΔE-NOTCH1–infected PE$^{+/+}$, PE$^{+/-}$, and PE$^{-/-}$ hematopoietic progenitors



Secondary loss of PE leads to accelerated NOTCH1-induced T-ALL progression and reduced levels of PTEN in mouse and human T-ALL

A, Schematic of retroviral-transduction protocol or the generation and analysis of PE conditional knockout NOTCH1–induced T-ALL. **B,** Kaplan–Meier curves of mice transplanted with PE conditional knockout ΔE-NOTCH1–induced T-ALL and treated in vivo with vehicle (control) or tamoxifen to induce isogenic deletion of PE. C, RT-PCR analysis of *PTEN* expression in tumor cells isolated from PE conditional knockout leukemia–bearing mice treated with vehicle or tamoxifen in vivo. D, Western blot analysis of PTEN expression in tumor cells isolated from PE conditional knockout leukemia–bearing mice treated with vehicle only or tamoxifen (TMX) in vivo

IMPACT

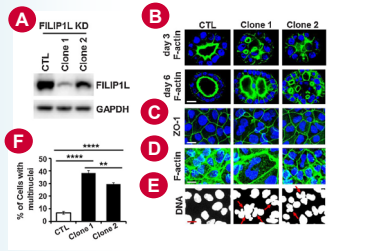
- The GESR created constitutive (left panel) and conditional (right panel) knockouts of the *PTEN* Enhancer (PE)
- Loss of PTEN expression is seen in 15% to 20% of patients with T-ALL where it leads to secondary resistance to NOTCH1 inhibitory therapies in vivo. These novel mouse models unveiled that loss of PE is associated with reduced expression of PTEN in developing thymocytes, whereas a wide variety of other solid tissues obtained from PE-knockout mice failed to show any difference in PTEN levels, suggesting that PE might behave as a hematopoietic-specific enhancer prominently active in the lymphoid compartment
- Impaired activity of the PE is a novel mechanism that cooperates in leukemia development and, more broadly, paves the way for the discovery and dissection of enhancers of other tumor suppressor genes

FILIP1L Loss is a Driver of Aggressive Mucinous Colorectal Adenocarcinoma and Mediates Cytokinesis Defects through PFDN1

Verzi; Libutti (GICG) ✉

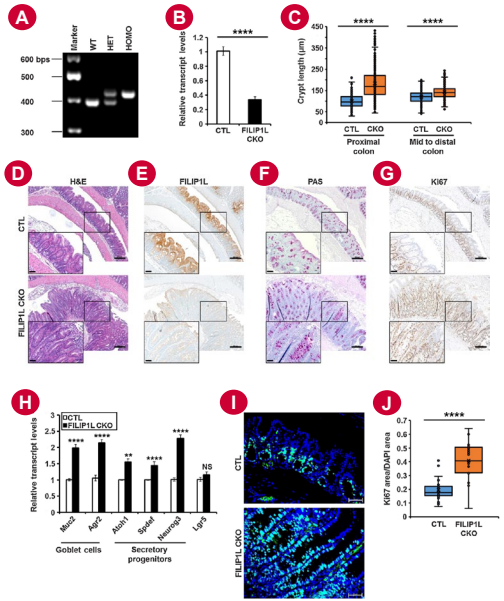
Zhou (CIPT)

Cancer Res, 2021
81(21):5523-5539



FILIP1L knockdown results in defective cytokinesis.

(A) FILIP1L knockdown was achieved by CRISPR-Cas9 system in MDCK.2 cells. FILIP1L and GAPDH control were detected by immunoblotting. **(B)**, MDCK.2 clones were grown on Matrigel, and three-dimensional cysts were imaged following staining for F-actin and nuclei. **(C–F)**, MDCK.2 clones were grown in monolayer and stained and the number of cells with multinuclei quantified.



FILIP1L loss induces mucin secretion as well as hyperplasia in mouse colon.

(A) Tails from wild-type (WT), Filip1l$^{fl/+}$ (HET), and Filip1l$^{fl/fl}$ (HOMO) mice were subjected to genotyping for Filip1l floxed allele. Bottom and top bands indicate PCR products from wild-type and floxed allele, respectively. **(B–J)**, Littermate Filip1l$^{fl/fl}$ (CTL) and Filip1l$^{fl/fl}$; Cdx2-CreER^{T2} (CKO) mice were treated with daily doses of tamoxifen (160 mg/kg) for 5 days and sacrificed on day 28 (day 7 for H). **(B)** Filip1l mRNA levels and **(C)**, Crypt length (shown in μm) was measured from Swiss-rolled whole colons. **(D–G)**, Colons were fixed and stained with H&E **(D)** and PAS **(F)** FILIP1L **(E)** and Ki67 **(G)**. **(H)**, mRNA levels of markers for goblet cells, secretory progenitors, and stem cells were measured by qRT-PCR. For **(B)** and **(H)**, epithelial cells from colons of CTL and CKO mice were prepared and fold change shown standardized with β-actin, Colons were stained for Ki67 **(I)**, and Ki67-positive areas were quantified **(J)**.

IMPACT

- The GESR created the FILIP1L conditional KO mice and the cell line used in this study
- FILIP1L knockdown and the resultant PFDN1 increase leads to increased mucin secretion and mitosis/cytokinesis defects in mouse colon as well as colon cancer cells, recapitulating the same phenotypes as seen in aggressive aneuploid MAC. These results strongly implicate FILIP1L as the essential regulator of MAC tumorigenesis
- Since FILIP1L is downregulated in various other cancer types, these studies will also have a broad impact on our understanding of the pathogenesis of other cancers and the role played by this novel tumor suppressor gene

Recycling Endosomes in Mature Epithelia Restrain Tumorigenic Signaling

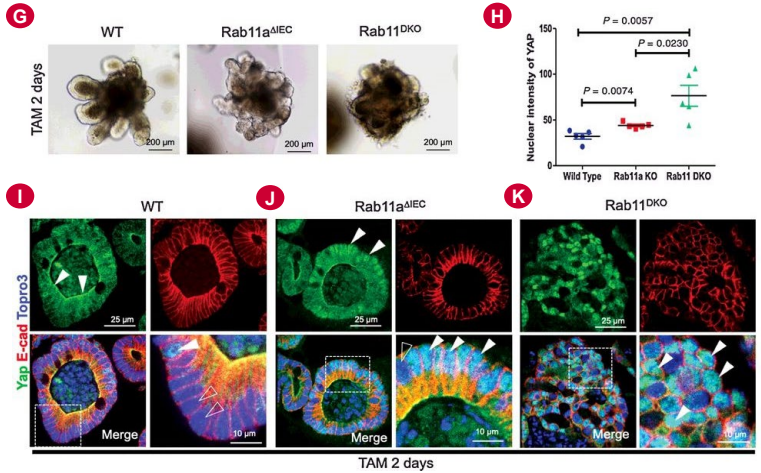
Gao ✉ (CMI)

Zhang (CIPT)

Verzi (GICG)

Yehia (GESR co-author)

Cancer Res, 2019
79(16):4099-4112



Rab11 ablation activates a stem cell signature and Yap signaling

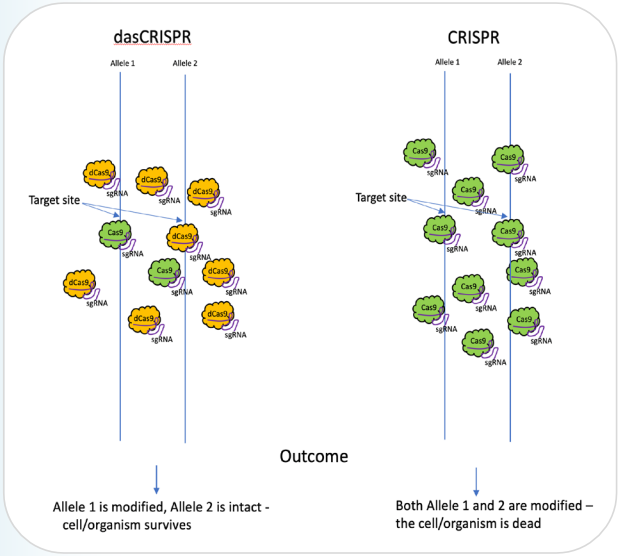
G, Representative images of enteroids cultured from wild type, Rab11$^{FL/FL}$; Vil-CreER, and Rab11$^{FL/FL}$; Vil-CreER; Rab11b$^{-/-}$ mice 2 days after 4-OHT treatment. H, Nuclear Yap levels increased in Rab11a-deficient and DKO enteroid cells. I–K, Immunofluorescent staining of sectioned enteroids for Yap (green) and E-cad (red). In higher magnification images (bottom right panels in I, J, and K), solid arrowheads point to nuclear Yap, whereas empty arrowhead indicates Yap-negative nucleus

IMPACT

- The GESR created Rab11a DKO mice used in this study
- After Rab11a removal from Rab11b-/- background, a significantly enriched Yap pathway signature was uncovered. Tissue specific DKO (Rab11a from Rab11b$^{-/-}$ IECs), showed upregulation of total Yap and Taz in IECs within 2 days, by day 3, all DKO mice died
- This work provides substantial in vivo evidence that perturbing Rab11 endosome functionality can rapidly and cell-autonomously alter Hippo signaling and accelerate colon cancer development

Emphasis & Future Directions

Method development



The GESR has developed an innovative deadCas9 allelic sequestration CRISPR method that incorporates the use of nuclease deficient Cas9 (dCas9) to allow for modification of a single target allele

Improves upon the use of CRISPR technology to modify a single allele in cases where modification of both alleles produces deleterious mutations which could adversely affect the survival of the cell/embryo or alter stem cell properties through sustained DNA damage response

Future Directions

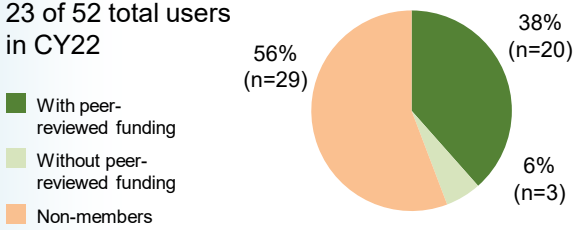
- Refine the generation of conditional mouse models from repository mouse stocks through in vitro fertilization and electroporation of recombinase mRNA
- Focus on generating mouse models de novo
- Adopt the GONAD method that can reduce the size of the mouse colony needed to generate mouse models
- Add a lentivirus production service to improve the variety of transduction methods available for cell line editing

Genome Editing Shared Resource

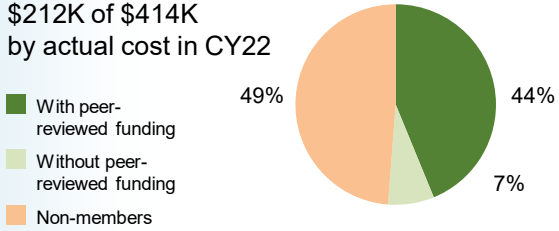
Rutgers Cancer Institute of New Jersey
RUTGERS HEALTH
NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

Utilization & Management

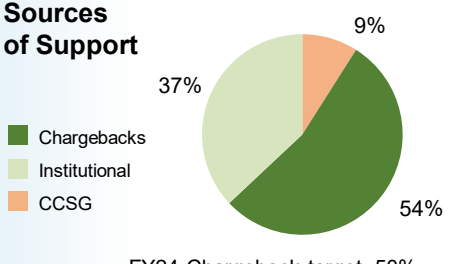
Member Users: 44%
23 of 52 total users in CY22



Member Usage: 51%
$212K of $414K by actual cost in CY22

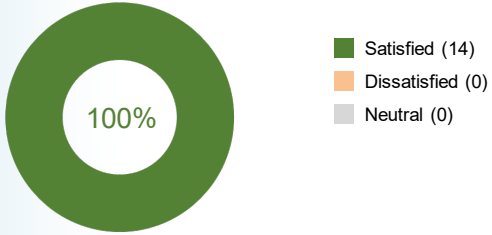


Sources of Support



FY24 Chargeback target: 50%

Satisfaction Survey for CY22 services



Participated: 14 of 23 members (61%)

Organization & Governance

GESR
6.1 FTE

SRACs
- Advisory Committee meets annually
- Discusses operational and scientific progress
- SRM supports organization

SRM
- SR Faculty Directors report to the ADSR
- SRM tracks and supports SRAC recommendations, productivity, service development, outreach

CINJ Director
- RLC
- Finance & Admin
- EAB

Supporting Information

Program Support

- Publications
- Grants
- 5-Year User List

Advisory Committee

- FY23 Presentation
- Action Items
- Notes

Quality Satisfaction

- Annual Survey Action Items

Usage

- CY22 Usage

Submitted Information

- Research Strategy
- Aims
- SRM Research Strategy