AWARD NUMBER: W81XWH-21-1-0260

TITLE: Toward Pharmacological Rescue of TSC Loss of Function

PRINCIPAL INVESTIGATOR: Jonathan Weissman

CONTRACTING ORGANIZATION: Whitehead Institute for Biomedical Research
Cambridge, MA

REPORT DATE: MaMay 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE

May 2022

2. REPORT TYPE

Annual

3. DATES COVERED

01Apr2021-31Mar2022

4. TITLE AND SUBTITLE

Toward Pharmacological Rescue of TSC Loss of Function

5a. CONTRACT NUMBER

W81XWH-21-1-02600260

5b. GRANT NUMBER

GRANT13165114

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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E-Mail: weissman@wi.mit.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Whitehead Institute
for Biomedical Research
455 Main Street
Cambridge
MA 02141

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command

Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT

	NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

BACKGROUND. This project aims to address the FY20 TSCRP Area of Emphasis: Eradicating tumors associated with TSC and TSC-associated lymphangioleiomyomatosis (LAM), including gaining a deeper mechanistic understanding of TSC signaling pathways. Currently, the only approved pharmacological intervention for TSC is via the direct mTOR inhibition by rapamycin or its synthetic analogs (rapalogs). Acute rapalog treatments initiate apoptosis within tumors of TSC mouse models, and chronic treatment blocks tumor formation and causes tumor regression. However, once the treatment is discontinued, the tumors regrow and the function of affected organs continues to decline. A would-be solution might involve chronic administration of the drug. However, prolonged exposure to rapalogs is associated with many adverse effects which might make it undesirable for widespread use in TSC patients. These include immunosuppression, insulin resistance andimpaired glucose homeostasis. Consequently, there is an urgent unmet need to develop truly potent therapies with no or limited side effects for the treatment of TSC.

HYPOTHESIS. We hypothesize that small-molecule chemical probes specifically targeting mTORC1 docking on the lysosomal surface will lead to effective therapies against tuberous sclerosis complex. We propose a shift in target focus — from the mTOR kinase itself to the molecular event of mTORC1 docking on lysosomal membranes.

SPECIFIC AIMS. The general aim of this proposal is to develop chemical probes that bind to and/or disrupt protein machinery responsible for anchoring mTORC1 on the lysosomal surface. Such disruption will limit the number of mTORC1 molecules that are activatable by upregulated Rheb in mutant TSC tissues. With the aid of our recent cryo-EM structure of Raptor (the defining subunit of mTORC1) bound to its lysosomal anchoring complex, Rag-Ragulator, we identified a number of potentially druggable pockets, and designed a three-pronged strategy of complementary drug discovery approaches that share a common goal.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
				19	USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified		19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			

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1. **Introduction**

Rapamycin and its analogs have showed some promise in the clinic for the treatment of TSC. However, prolonged use of these drugs causes many adverse effects which make it undesirable for widespread use in TSC patients. These include immunosuppression, insulin resistance andimpaired glucose homeostasis. The general aim of this proposal is to begin developing a novel therapeutic that has a different, more specific mechanism that rapamycin, and therefore is more likely to be safe for use in patients. The key protein, the hyperactivation of which causes TSC is mTORC1, and which for its function docks on the surface of lysosomes. This project focuses on disrupting the ability of mTORC1 to dock on lysosomes by interfering with the protein machinery responsible for facilitating this process. Such disruption will limit the number of mTORC1 molecules that are activated in mutant TSC tissues. With the aid of our recent cryo-EM structure of Raptor (the defining subunit of mTORC1) bound to its lysosomal anchoring complex, Rag-Ragulator, we identified a number of potentially druggable pockets, and designed a strategy of complementary drug discovery approaches that share a common goal.

2. **Keywords**

mTORC1, rapamycin, TSC, drug discovery, lysosome

3. **Accomplishments**

What were the major goals of the project?

- **Major Task 1:** Create a library of circular peptides that compete with the Raptor claw, thus disrupting the ability of Raptor to secure mTORC1 on lysosomes.
    - In progress. 25% completed.
- **Major Task 2:** Discover novel Raptor binder molecules and develop a proof-of-concept degrader.
    - In progress. 25% completed.

What was accomplished under these goals?

- **TASK #1**
    - Computationally designed plasmids, carrying hundreds of Raptor-claw macrocyclic peptide mimics.
    - Began cloning of the designed mimic sequences into the intein-circularization plasmids, in order to establish a larger library.
- **TASK #2**
    - Expressed and purified Avi- and Flag-tagged Raptor from mammalian cells.

- We recloned Raptor, to carry an Avi tag, and scaled up our previously established purification protocol to express and purify Raptor.
- This involved initial cell lysis using detergent (0.4% CHAPS), followed by centrifugal clarification of cellular debris. Once cleared, cell lysate was incubated with anti-Flag agarose beads to capture Flag-tagged Raptor molecules. Captured Raptor was eluted from beads via competition with an excess of Flag peptide, and the resulting protein was concentrated and further purified via size-exclusion chromatography (SEC).

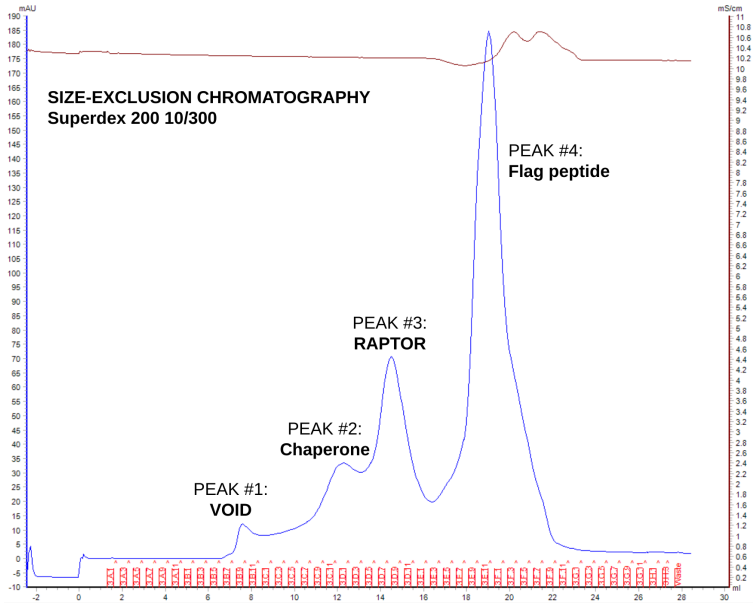


Figure 1. Raptor comes off from SEC in the 3rd peak. Blue — UV (280 nm) absorbance of protein molecules. Brown — conductivity readings.

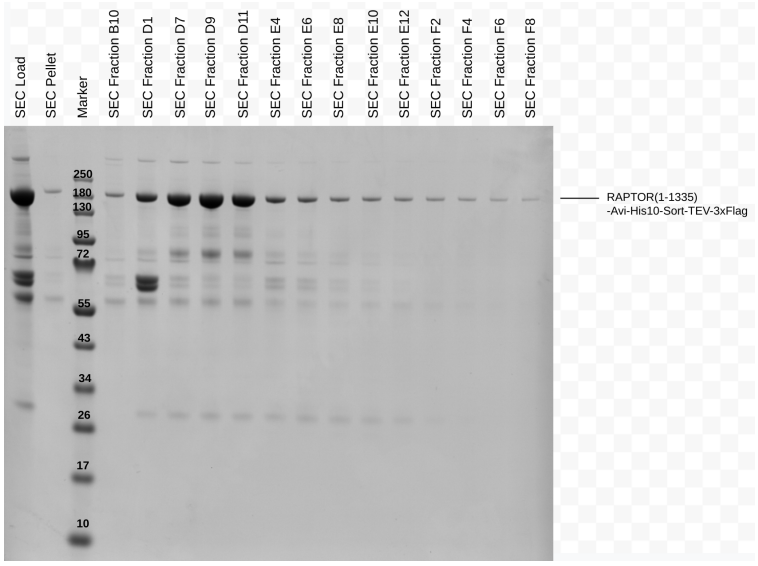


Figure 2. Raptor appears to be interacting with the size-exclusion matrix to a small extent, which can be visualized by SDS-PAGE. Please note the presence of Raptor throughout the entire elution profile. Peak #3 contains the highest amount of Raptor, and this is the protein we used for further experiments. Chaperones come off early, and the contaminating Flag peptide elutes late, providing a solid final purification step. Please note that some Raptor degradation products are carried along in Peak #3.

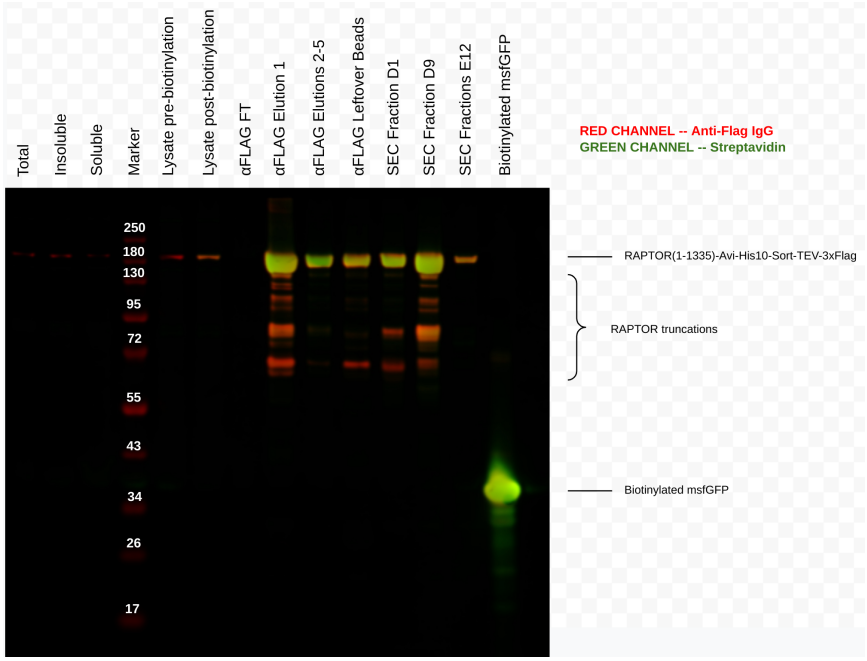
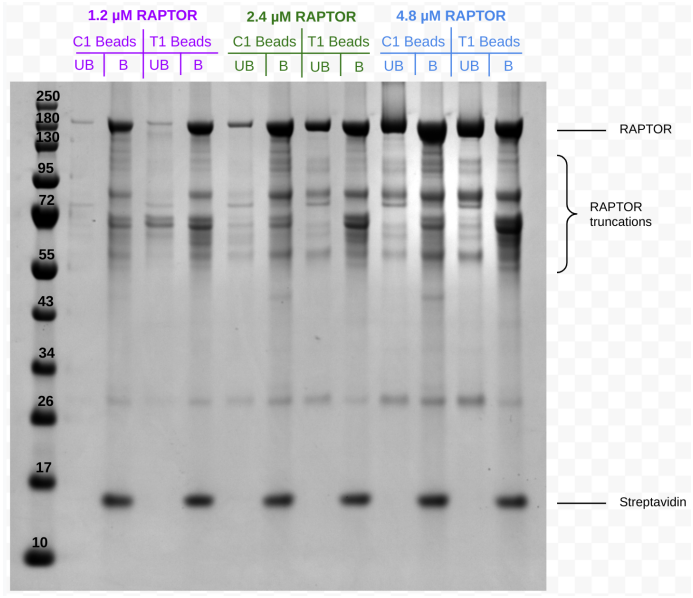
- Performed in vitro enzymatic biotinylation of Raptor, and confirmed its high efficiency.
  - In order to accomplish this goal, we needed to first apply our previously established method to in vitro biotinylate Rag GTPases and Ragulator — to Raptor.
  - This worked flawlessly, and we obtained a well-biotinylated Raptor at a single site — the Avi tag.
  - 

Figure 3. Western blot of Raptor purification/biotinylation. We used BirA enzyme to in-vitro biotinylate partially-purified Raptor in solution. We considered a number of potential steps that could accommodate a biotinylation reaction during the purification process of Raptor, and found that the reaction proceeds rather well during the anti-Flag bead-binding step. This allows for the reaction to be performed relatively early in the purification process, so that the contaminating BirA gets washed off in the subsequent steps. RED CHANNEL — anti-Flag. GREEN CHANNEL — Streptavidin (targets biotinylated proteins). Please note a highly efficient biotinylation of Raptor, in comparison to a positive control — Avi-tagged GFP protein.

- Immobilized Raptor on Streptavidin magnetic beads, and performed a series of quality control checks to ensure that the protein is well behaved and ready for a large DEL compound screen.
  - This step was rather difficult to accomplish, because of the inherent “stickiness” of Raptor that we discovered in the SEC purification step.
  - To address this issue, we tested various buffer conditions, and different types of magnetic beads to ensure that we only campture “healthy” Raptor molecules. And also that these beads (and Raptor itself!) will be intert enough so that no non-specific DNA-encoded molecules will bind to them. Such non-specific interactions will produce a number of false positive hits, and largely pollute our downstream efforts to identify specific binders.



- **Figure 4.** Immobilization trials of biotinylated Raptor on Streptavidin-coated magnetic Dynabeads. We tested different buffer conditions (here only showing the best trial), and different amounts of Raptor to be loaded on beads. We wanted to establish what amount of Raptor is necessary to saturate these beads, and whether too much of too little Raptor would lead to non-specific binding on beads. We also tested two different types of Dynabeads, C1 and T1, with different chemical properties — more hydrophilic versus more hydrophobic, respectively. UB — unbound. B — beads (bound to beads). Please note that Raptor truncations are associating much more strongly with T1 beads than with C1. This might be indicative of these truncations having exposed hydrophobic patches and therefore non-specifically interacting with the more hydrophobic T1 beads.
- With this experiment, we optimized our bead-immobilization conditions for Raptor, and we are currently in the process of screening Raptor for binders with a 3M-compoud library of molecules.

- OTHER ACHIEVEMENTS:
  - We expanded the same strategy of immobilizing Raptor on magnetic beads — to it's anchor protein Ragulator. We already had produced a biotinylated Ragulator, so this was a rather straightforward extension of our work. We hope that screening for molecules that bind to Ragulator will give us extra potential new molecules that might serve to disrupt mTORC1 landing on the lysosome.
- GOALS NOT MET:
  - The pace of our research has been affected by a number of recent events — including staffing and the most recent pandemic waves. We are a little behind on the schedule, however, we feel that we have now overcome the most challenging hurdles, and we are now on a good path to bring this project to completion.
  - TASK #1: Clone the Raptor Claw macrocycle mimic library — in progress.
  - TASK #1: Run a pilot in-cell experiment of mTORC1 docking disruption with a macrocyclic peptide coding for the exact Raptor claw sequence — in progress.

- TASK #1: Perform the macrocyclic peptide screen — we will enter this stage once the full library has been cloned and once the pilot in-cell experiment has been completed.
- TASK #2: Perform a DEL screen — in progress.
- TASK #2: Synthesise most promising binders — we will enter this stage once the DEL screen is completed and analyzed.

What opportunities for training and professional development has the project provided?

- Karen Linde-Garelli joined this project as research assistant, and was mentored by Dr. Kacper Rogala. Through this arrangement, Ms. Linde-Garelli received instruction and mentorship on how to effectively work with proteins, and how to perform quality control experiments for downstream assays. Likewise, Dr. Rogala had an opportunity to learn how to mentor a junior lab member, which will be invaluable for his future development as an independent group leader.
- Dr. Rogala has been participating in the following meeting series:
  - Binders project (every two months) - discussing the development of DNA-encoded libraries and strategies for binder screening — between the laboratories of Dr. Stuart Schreiber (Broad / Harvard) and Dr. Benjamin Cravatt (UCSD).
  - Chemical Biology Supergroup of the Broad Institute, discussing the latest research in the chemical biology space happening at the Broad / Harvard / MIT.
- Dr. Rogala participated in the following external conferences, workshops, seminars:
  - Hit-Finding Success with DNA Encoded Libraries in Academia (June 29, 2021) — Chemical and Engineering News (webinar)
  - S2C2 CryoEM Map Modeling and Validation Workshop (September 8-10, 2021) — Stanford University
  - Cancer Chemical Biology and Metabolism (CCBM) Annual Retreat (October 12, 2021) — Dana-Farber Cancer Institute
  - 7th Semi-Annual New England Cryo-EM Symposium (November 16-17, 2021) — University of Massachusetts Medical School

How were the results disseminated to communities of interest?

- Nothing to report yet.

What do you plan to do during the next reporting period to accomplish the goals?

- With the majority of hurdles behind us, we are planning to finalize the current “in progress” tasks, which include:
  - The DEL screen of Raptor and Ragulator.
  - Pilot experiment to test whether a macrocyclic peptide mimicking Raptor can interfere with mTORC1 lysosomal docking.
  - Cloning of the macrocycle library of Raptor-claw mimicking peptides.
- Once these are completed, I will move directly to:

- Analyzing DEL hits, and synthesizing the resulting compounds for in vitro validation with purified proteins and biophysical methods (SPR and BLI).
- Fuse validated hit molecules with E3 moieties (e.g. VHL and CRBL), and perform in-cell screen to determine degradation properties of these chimeric molecules (towards Raptor and Ragulator).
- Perform the large in-cell screen to test whether other permutations of the Raptor claw macrocylic peptide can yield more beneficial results. And then validate these results with cell signaling experiments using a range of KO cell lines.

4. **Impact**

What was the impact on the development of the principal discipline(s) of the project?

- Nothing to report yet.

What was the impact on other disciplines?

- Nothing to report yet.

What was the impact on technology transfer?

- Nothing to report yet.

What was the impact on society beyond science and technology?

- Nothing to report yet.

5. **Changes/Problems**

Changes in approach and reasons for change.

- We received a formal permission to simplify the original goals of this project due to staffing challenges (see W81XWH2110260 P00001). The new goals are in line with the original idea, and we are working towards their completion without any changes.

Actual or anticipated problems or delays and actions or plans to resolve them.

- We encountered some delays due to staffing issues, pandemic and supply-chain disruptions. The situation appears to have stabilized now, and we are back on track to complete these project.

Changes that had a significant impact on expenditures.

- Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

- Nothing to report.

Significant changes in use or care of human subjects.

- Nothing to report.

Significant changes in use or care of vertebrate animals.

- Nothing to report.

Significant changes in use of biohazards and/or select agents.

- Nothing to report.

6. **Products**

Nothing to report yet.

7. **Participants & Other Collaborating Organizations**

What individuals have worked on the project?

- **Name: Kacper Rogala, D. Phil.**
  - Project Role: Senior Research Associate
  - Researcher Identifier (ORCID ID): 0000-0001-5997-7770
  - Nearest person month worked: 5 months
  - Contribution to Project: This project was Dr. Rogala's idea and implementation. He wrote the research proposal, and performed the initial experiments — together with Max Valenstein and Karen Linde-Garelli.
  - Funding Support: This grant, and K99 Pathway to Independence Award.

- **Name: Karen Linde-Garelli**
  - Project Role: Research Assistant
  - Researcher Identifier (ORCID ID): N/A
  - Nearest person month worked: 6 calendar months
  - Contribution to Project: Karen Linde-Garelli assisted with the generation of plasmids and purified proteins for this project. She also performed magnetic bead binding experiments for Raptor and Ragulator.
  - Funding Support: This grant and Whitehead funding

- **Name: Max Valenstein**
  - Project Role: Graduate Student
  - Researcher Identifier (ORCID ID): 0000-0001-7616-0148
  - Nearest person month worked: 6 calendar months
  - Contribution to Project: Max Valenstein assisted with the generation of plasmids and purified proteins for this project.
  - Funding Support: This grant and Whitehead funding

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- Dr. David Sabatini is no longer the PI on this grant. That role was taken by Dr. Jonathan Weissman.
- Please see Dr. Weissman's other support attached.

What other organizations were involved as partners?

- Organization Name: Broad Institute

- Location of Organization: 415 Main St, Cambridge, MA 02142
- Partner's contribution to the project:
  - Facilities — Dr. Rogala regularly uses biophysical instrumentation at the Broad Institute.
  - Collaboration — Dr. Stuart Schreiber's lab is actively collaborating with us on this project. They developed a large DNA-encoded library of small molecules using diversity-oriented synthesis, and we are currently in the process of using it to screen for novel binders against Raptor and Ragulator.

8. **Special Reporting Requirements**

Not applicable.

9. **Appendices**

Not applicable.

Name of Individual: WEISSMAN, JONATHAN SETH
Commons ID: WEISSMAN

PHS 398 OTHER SUPPORT

PROJECT/PROPOSAL

ACTIVE SUPPORT

Title: An IND-Enabling Platform for CBRN Threat Protection via Transient, RNA-guided, Targeted Epigenome Editing In Vivo
Major Goals: The major goals of this project are to build and deploy a vertically integrated, target-agnostic, and IND-enabling platform for clinic-ready, transient, RNA-guided, targeted epigenome editing *in vivo*, for prophylactic (radioprotectant) and post-exposure (radiomitigation) protection of hematopoiesis and the gastrointestinal (GI) tract from high-dose radiation exposure and resulting acute radiation syndrome (ARS).
Status of Support: Active
Project Number: HR0011-19-2-0007
PI/PD: Jonathan Weissman
Source of Support: DOD DARPA
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 04/2017 - 7/2022 (NCE)
Total Award Amount (including IDC): (Weissman allocation)
Calendar Months per Budget Period:

Year	Person Months
1. 2017	2.4 calendar months
2. 2018	2.4 calendar months
3. 2019	2.4 calendar months
4. 2020	2.4 calendar months
5. 2021	2.4 calendar months

Title: Center for Genome Editing and Recording
Major Goals: The major goals of this project are to create technologies to enable robust, comprehensive exploration of genes and genetic pathways responsible for human disease.
Status of Support: Active
Project Number: 1RM1 HG009490-01
PI/PD: Jennifer Doudna, UC Berkeley
Source of Support: NIH/NHGRI
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 08/2017 - 05/2022
Total Award Amount (including IDC): (Weissman allocation)
Calendar Months per Budget Period:

Year	Person Months
1. 2017	1.8 calendar months
2. 2018	1.8 calendar months
3. 2019	1.8 calendar months
4. 2020	1.8 calendar months
5. 2021	1.8 calendar months

Title: The Cancer Target Discovery and Development Network at UCSF
Major Goals: To directly bridge the gap between the enormous volumes of data generated by the comprehensive molecular characterization of a number of cancer types– and the ability to use these data for the development of human cancer therapeutics.
Status of Support: Active

Name of Individual: WEISSMAN, JONATHAN SETH
Commons ID: WEISSMAN

Project Number: 1U01 CA217882-01
PI/PD: Michael McManus, UCSF
Source of Support: NIH/NCI
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 08/2017 - 07/2022
Total Award Amount (including IDC): (Weissman allocation) Calendar Months per Budget Period:

Year	Person Months
1. 2017	1.2 calendar months
2. 2018	1.2 calendar months
3. 2019	1.2 calendar months
4. 2020	1.2 calendar months
5. 2021	1.2 calendar months

Title: Towards pharmacological rescue of TSC loss-of-function
Major Goals: The general aim of this proposal is to develop chemical probes that bind to and/or disrupt protein machinery responsible for anchoring mTORC1 on the lysosomal surface. Such disruption will limit the number of mTORC1 molecules that are activatable by upregulated Rheb in mutant TSC tissues.
Status of Support: Active
Project Number: W81XWH-21-1-0260
PI/PD: Jonathan Weissman
Source of Support: TSCRP — EHDA, Department of Defense
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 04/2021 - 08/2022
Total Award Amount (including IDC):
Calendar Months per Budget Period:

Year	Person Months
1. 2021	0 calendar months
2. 2022	0 calendar months

Title: Novel Components of the mTORC1 and mTORC2 Pathways
Major Goals: The major goal of this grant is to identify and characterize the cellular functions of novel components of the mTORC1 or mTORC2 Pathways.
Status of Support: Active
Project Number: 5 R01 AI047389-22
PI/PD: Jonathan Weissman
Source of Support: NIAID
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 05/2021 - 04/2022
Total Award Amount (including IDC):
Calendar Months per Budget Period:

Year	Person Months
1. 2022	0.12 calendar months

Title: Defining Molecular Signatures Underlying Lysosomal Dysfunction in Alzheimer's Disease
Major Goals: We will combine novel tools developed in our laboratory with quantitative proteomic and metabolomic approaches to study lysosomes isolated from murine neurons and microglia and define the cell type-specific lysosomal alterations in mouse models of Alzheimer’s disease.
Status of Support: Active

Name of Individual: WEISSMAN, JONATHAN SETH
Commons ID: WEISSMAN

Project Number: 1 R21 AG072511-01,
PI/PD: Jonathan Weissman
Source of Support: NIH
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 05/2021 - 04/2023
Total Award Amount (including IDC):
Calendar Months per Budget Period:

Year	Person Months
1. 2022	0.6 calendar months
2. 2023	0.6 calendar months

Title: Mapping Spatiotemporal Dynamics of Single Cells During Carcinogenesis
Major Goals: Developing and testing the computational framework related to investigating the spatiotemporal dynamics of carcinogenesis at single-cell resolution.
Status of Support: Active
Project Number: 6941120/6945659
PI/PD: Weissman
Source of Support: Jameel Clinic at MIT
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 06/2021 - 05/2022
Total Award Amount (including IDC):
Calendar Months per Budget Period:

Year	Person Months
1. 2022	0.24 calendar months

Title: Ludwig Fund for Cancer Research
Major Goals: The major goal of this research is to better understand cancer cells by studying routes of metastases and mechanisms of drug resistance.
Status of Support: Active
Project Number: 659216
PI/PD: Jonathan Weissman
Source of Support: Virginia and D.K. Ludwig Fund for Cancer Research at MIT
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 07/2021 - 06/2022
Total Award Amount (including IDC):
Calendar Months per Budget Period:

Year	Person Months
1. 2022	0.12 calendar months

Title: Exploiting mitochondrial heteroplasmy for cancer chemotherapy
Major Goals: The major goal of this project is a systemic study of how heteroplasmy affects susceptibility to a variety of relevant inhibitors and how these inhibitors may drive changes in heteroplasmy leading to drug resistance.
Status of Support: Active
Project Number: 5 R01 CA219859-05
PI/PD: Jonathan Weissman
Source of Support: NIH/NCI
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 08/2021 - 07/2022
Total Award Amount (including IDC):

Name of Individual: WEISSMAN, JONATHAN SETH
Commons ID: WEISSMAN

Calendar Months per Budget Period:

Year	Person Months
1. 2022	0.24 calendar months

Title: Defining Molecular Signatures Underlying Lysosomal and Mitochondrial Dysfunction in Aging
Major Goals: The major goal of this project is to leverage novel transgenic mouse lines to perform unprecedented analyses of organelles in vivo to identify and study organellar deficiencies underlying aging.
Status of Support: Active
Project Number: N/A
PI/PD: Jonathan Weissman
Source of Support: Impetus Grants
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 12/2021 - 11/2024
Total Award Amount (including IDC):
Person Months per Budget Period:

Year	Person Months
1. 2022	0.24 calendar months
2. 2023	0.24 calendar months
3. 2024	0.24 calendar months

Title: An Atlas of Cellular Rejuvenation Cocktails: from discovery to mechanism
Major Goals: The major goals of this project are to identify novel combinations of gene perturbations that induce cellular rejuvenation by screening high-complexity libraries using a directed search strategy informed by machine learning, to define the proteomic and metabolomic changes that accompany aging, focusing on lysosomal/mitochondrial function and protein synthesis and to test novel rejuvenation cocktails for the ability to restore cellular health *in vivo*, and assess the most promising combinations for their ability to reverse age-related decline in multiple tissues.
Status of Support: Active
Project Number: N/A
PI/PD: Jonathan Weissman
Source of Support: Milky Way Research Foundation
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 12/2021 - 12/2024
Total Award Amount (including IDC): (Weissman allocation)
Person Months per Budget Period:

Year	Person Months
1. 2022	1.8 calendar months
2. 2023	1.8 calendar months
3. 2024	1.8 calendar months

PENDING SUPPORT

Title: Center for Genome Editing and Recording: Development and Application of Next-Generation Genome and Epigenome Editing Methods to Advance the Study and Treatment of Human Disease
Major Goals: To create technologies to enable robust, comprehensive exploration of genes and genetic pathways responsible for human disease in addition to the development of higher-level multichannel molecular recorders that will allow us to track and reconstruct the life history of cells in an *in vivo* setting. Collectively, these technologies have profound implications for genome science, therapeutic strategies for somatic disorders, and genetic diseases as well as understanding normal development and disease processes such as tumor evolution and the mechanism of metastases and response to therapeutic

challenges. In addition, we aim to create a vibrant environment in which scholars across educational and socioeconomic levels can engage without barriers, where diverse students at every stage of development will be exposed to novel opportunities for training, research and skill development, and new ideas in a dynamic interdisciplinary research environment.
Status of Support: Pending
Project Number: 1RM1 HG009490 (Renewal)
PI/PD: Jonathan Weissman
Source of Support: NIH/CEGS
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 06/2022 - 05/2027
Total Award Amount (including IDC): (Weissman allocation)
Person Months per Budget Period:

Year	Person Months
1. 2023	1.8 calendar months
2. 2024	1.8 calendar months
3. 2025	1.8 calendar months
4. 2026	1.8 calendar months
5. 2027	1.8 calendar months

Title: Bay Area Cancer Target Discovery and Development
Major Goals: To bridge the gap between the enormous volumes of data generated by the comprehensive molecular characterization of several cancer types – and the ability to use these data for the development of human cancer therapeutics. Our end goal is to generate game-changing reagents, and data valuable for the development of cancer therapeutics.
Status of Support: Pending
Project Number: N/A
PI/PD: Michael McManus, UCSF
Source of Support: NIH/NCI
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 07/2022 - 06/2027
Total Award Amount (including IDC): (Weissman allocation)
Person Months per Budget Period:

Year	Person Months
1. 2023	1.2 calendar months
2. 2024	1.2 calendar months
3. 2025	1.2 calendar months
4. 2026	1.2 calendar months
5. 2027	1.2 calendar months

Title: Cellular Barcoding to Define Melanoma Drug Resistance and Cell of Origin
Major Goals: The major goal of this project is to identify the most lethal cancer cells across melanoma subtypes (including drug resistant BRAF-mutant melanomas, BRAF wildtype, mucosal and acral melanomas) using “barcoding” technology that will improve melanoma tracking in both animal models and in patients to lead to new therapies for melanoma patients.
Status of Support: Pending
Project Number: N/A
PI/PD: E. Elizabeth Patton, University of Edinburgh
Source of Support: Melanoma Research Alliance
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 09/2022 - 08/2025

Name of Individual: WEISSMAN, JONATHAN SETH
Commons ID: WEISSMAN

Total Award Amount (including IDC): (Weissman allocation) Person Months per Budget Period:

Year	Person Months
1. 2023	0.12 calendar months
2. 2024	0.12 calendar months
3. 2025	0.12 calendar months

Title: Defining Signatures of Lysosomal and Mitochondrial Dysfunction in Aging to Guide the Restoration of Cellular Health
Major Goals: We propose to understand how mitochondria and lysosomes change during aging by profiling organelles isolated from several tissues and four main cell types of the mouse brain; to dissect mechanistically how the observed age-related changes in mitochondrial and lysosomal contents arise and further impact cellular physiology using *in vivo* and *in vitro* models; and to explore whether novel rejuvenation factors that are currently being defined in my lab can restore organellar and cellular health *in vivo*.
Status of Support: Pending
Project Number: N/A
PI/PD: Jonathan Weissman
Source of Support: National Academy of Medicine Healthy Longevity Catalyst Award
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 11/2022 - 10/2023
Total Award Amount (including IDC):
Person Months per Budget Period:

Year	Person Months
1. 2023	0.12 calendar months

IN-KIND

Summary of In-Kind Contribution: Howard Hughes Medical Institute Investigator
This renewable funding supports Weissman Lab studies of how cells ensure that proteins fold into their correct shape, as well as the role of protein misfolding in disease and normal physiology. It also supports developing experimental and analytical approaches for exploring the organizational principles of biological system.
Status of Support: Active
Primary Place of Performance: Whitehead Institute for Biomedical Research
Project Start and End Dates: 09/2021 - 08/2022
Calendar Months per Budget Period: N/A
Estimated Dollar Value of In-Kind:

OVERLAP: There is no scientific or budgetary overlap for any of the projects listed.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: Jonathan Weissman

Name of Individual: WEISSMAN, JONATHAN SETH
Commons ID: WEISSMAN

Date: 04/01/2022