AWARD NUMBER: W81XWH-18-1-0659

TITLE: An Evolutionary Approach to Vulnerability Mapping in Order to Identify Alternative and Synergistic Therapeutic Strategies for TSC and Related Diseases

PRINCIPAL INVESTIGATOR: Norbert Perrimon & Brendan Manning

CONTRACTING ORGANIZATION:
President and Fellows of Harvard College

Boston Ma 02115-6027

REPORT DATE: Oct 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

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1. REPORT DATE Oct 2019

2. REPORT TYPE Annual

3. DATES COVERED 09/30/2018 - 09/29/2019

4. TITLE AND SUBTITLE

An Evolutionary Approach to Vulnerability Mapping in Order to Identify Alternative and Synergistic Therapeutic Strategies for TSC and Related Diseases

5a. CONTRACT NUMBER W81XWH-18-1-0659

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)

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

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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

The aim of this research project is to develop new approaches to the treatment of diseases resulting from mutations in the Tuberous sclerosis complex (TSC) genes. TSC mutations lead to the formation of tumors in tissues including the brain, skin, kidneys, heart and lungs and affect an estimated 1 in 6,000 to 10,000 births. Furthermore, disruption of TSC can produce varied neurological and cognitive deficits, representing the most severe features of TSC. The currently available approaches to treating TSC-related diseases are limited and generally block or slow down tumor growth, rather than killing the diseased cells. Therefore there is an urgent need to develop new therapeutic strategies to treat TSC related diseases. The purpose of our work is to identify new drug targets that selectively kill TSC cells either alone or in combination with Rapamycin/Rapalogs that are used today for the treatment of TSC. Rapalogs have shown some success in treating TSC tumors but their effects are cytostatic and tumors rapidly regrow upon cessation of treatment, highlighting the urgent need to identify new drugs for the treatment of TSC. To achieve this goal, we will use state-of-the art functional genomics methods in the fruit fly, *Drosophila*, a proven model to study TSC, to identify drug targets that synergize with Rapalogs in the treatment of TSC. In addition, we will characterize in details a promising drug target that has already emerged from our screens for the treatment of TSC.

15. SUBJECT TERMS

Synthetic lethality, tumor suppressors, tuberous sclerosis complex. Rapamycin, TOR signaling, *Drosophila*

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION:

The aim of this research project is to develop new approaches to the treatment of diseases resulting from mutations in the Tuberous sclerosis complex (TSC) genes. TSC mutations lead to the formation of tumors in tissues including the brain, skin, kidneys, heart and lungs and affect an estimated 1 in 6,000 to 10,000 births. Furthermore, disruption of TSC can produce varied neurological and cognitive deficits, representing the most severe features of TSC. The currently available approaches to treating TSC-related diseases are limited and generally block or slow down tumor growth, rather than killing the diseased cells. Therefore there is an urgent need to develop new therapeutic strategies to treat TSC related diseases. The purpose of our work is to identify new drug targets that selectively kill TSC cells either alone or in combination with Rapamycin/Rapalogs that are used today for the treatment of TSC. Rapalogs have shown some success in treating TSC tumors but their effects are cytostatic and tumors rapidly regrow upon cessation of treatment, highlighting the urgent need to identify new drugs for the treatment of TSC. To achieve this goal, we will use state-of-the art functional genomics methods in the fruit fly, *Drosophila*, a proven model to study TSC, to identify drug targets that synergize with Rapalogs in the treatment of TSC. In addition, we will characterize in details a promising drug target that has already emerged from our screens for the treatment of TSC.

2. KEYWORDS:

Synthetic lethality, tumor suppressors, tuberous sclerosis complex. Rapamycin, TOR signaling, *Drosophila*

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. Elucidate the mechanism underlying the synthetic lethal interaction between CTNS and TSC1/2. To expand our list of high-confidence candidate genes that show synthetic lethality with *TSC*, we recently performed genome-wide CRISPR knockout screening and RNAi screens to search for TSC vulnerabilites. A strong hit in all fly screens, which also had similar effects in mouse TSC cells, was the lysosomal cystine transporter, *CTNS*. Preliminary evidence suggests altered cystine levels in *TSC*-mutated fly cells, hinting at a mechanistic link at the level of cystine metabolism. Therefore, we propose to determine how the levels of cystine and related metabolites affects growth rates in TSC-deficient mouse cell-lines and in mouse tumor models, and how and if these interface with mTOR signaling.

Aim 2. Use of a rapamycin-sensitized screen in Drosophila cells to identify synergistic vulnerabilities to be characterized in mammalian TSC deficient cell-lines. A promising approach for the treatment of TSC is to identify synergistic interactions with rapamycin, as these could lead to combinatorial therapeutic approaches. Thus, we propose to capitalize on our development of CRIPSR knockout screening to perform rapamycin-sensitized genome-wide screens in wild type and *TSC* deficient *Drosophila* cells. The results will be validated in a collection of 5 different isogenic mammalian cell models of TSC, prioritizing hits against which small molecule inhibitors exist. The results of this work are likely to contribute new combinatorial therapeutic options for TSC and related diseases associated with uncontrolled mTOR signaling.

What was accomplished under these goals?

Major goals for year 1:

Specific Aim 1: Elucidate the mechanism underlying the synthetic lethal interaction between CTNS and TSC1/2. ***Mos 1-20 – 50% complete.***

Task 1 Characterize effects of CTNS RNAi on ROS levels, cell death, mTOR activation status, etc. in TSC1/2 deficient *Drosophila* S2 cells and mammalian cell models (mouse 105K cells). ***Mos 1-4 – 40% complete.*** During this period, we have obtained and characterized reagents to test the mechanism of CTNS function in Drosophila (**Figure 1**). dCTNS mutations causes increased mTORC1 reactivation following starvation leading to starvation-sensitivity, and are rescuable by mTORC1 suppression. These striking observations strongly support our cell-based identification of dCTNS as a vulnerability in TSC mutants, because mTORC1 is similarly upregulated in TSC and dCTNS mutants. Further, we observed accumulation of GSSG in metabolomics dataset of CTNS KO. Interestingly, in addition to expected defects in cysteine metabolism, these mutants also exhibit defects in nucleotide metabolism. Since recent work from our labs has demonstrated that nucleotide metabolism is sensed directly by mTOR, these results provide a critical inroad to understanding the reason for CTNS's cross-species synthetic lethality with TSC1/2. Importantly, we find similar selective effects on the viability of TSC2-deficient mammalian cells upon shRNA-mediated knockdown of CTNS and are preparing to test the effects of CTNS depletion in a mouse TSC tumor model in the coming year. We have obtained ACURO approval for these experiments.

CTNS exports cystine out of the lysosome to fuel several important metabolic pathways. Thus, the synthetic lethal interaction between CTNS and TSC1/2 likely rely on a metabolic liability in TSC1/2 cells that cannot be compensated in absence of CTNS. As loss of CTNS and TSC1/2 individually trigger extensive metabolic alterations, we characterized the metabolic defects in CTNS single mutants and matched them to the metabolic fuels that support TSC1/2 mutant cells growth that we recently identified. In addition to the known role of CTNS in antioxidant defense (through glutathione synthesis), we found that CTNS is also implicated in the regulation of nucleotides metabolism. Because both antioxidant defense and nucleotide synthesis are critical for survival of TSC1/2 cells, we believe that we have successfully identified the nature of the synthetic lethal interaction.

Task 2 Perform high throughput metabolomics profiling on TSC deficient *Drosophila* S2 cells and mammalian cells (mouse 105K cells) treated or untreated with CTNS RNAi and analyze data. ***Mos 5-8 – 60% complete.*** In collaboration with Mattias Simons we performed targeted metabolomics for metabolites in CTNS KO and found significant defects in nucleotides metabolism (**Figure 2**). We will now analyze these defects specifically in double KO cells both in S2 and 105K cells. We have all the protocols worked out and reagents and thus will be able to quickly complete this task.

Task 3 Test the effect of cystine-loading or cysteine depletion on *Drosophila* S2 cells and mammalian cell (mouse 105K cells) models of TSC. ***Mos 9-12 – 80% complete.*** There are multiple sources of Cys in cells which is important for producing glutathione and cell survival. Previously we showed that mTORC1 activates ATF4, which contributes to the stimulation of nucleotide synthesis (Issam Ben-Sahra et al. mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle, Science 351, 728 (2016)). We found that ATF4 also controls uptake of exogenous Cystine and de novo synthesis of cysteine through transcriptional targets (Figure 3). Thus, ATF4 acts downstream of the TSC tumor suppressors, but parallel to CTNS, for controlling intracellular Cys levels.

Task 4 Test the synergistic effect of rapamycin treatment combined with CTNS RNAi, cystine-loading or cysteine depletion on Drosophila S2 cells and mammalian cell models (mouse 105K cells) of TSC. ***Mos 13-15 – 0% complete.*** Work will be initiated in Year 2

Task 5 In vivo preclinical testing of CTNS inhibition in a mouse TSC tumor model. Xenograft tumors will be generated with 105K tumor cell derivatives expressing doxycycline-inducible CTNS shRNAs or their non-targeting controls, and tumor growth, regression, and regrowth will be measured with and without doxycycline treatment to control shRNA expression in the tumors. (n=10 mice per conditions; 40 total). ***Mos 16-20 – 0% complete.*** Work will be initiated in Year 2.

Specific Aim 2: Genetic screen for rapamycin-synergistic vulnerabilities in TSC deficient cells. ***Mos 21-36 – 25% complete.*** We have already made significant progress towards this aim that we initially planed to initiate in Year 2.

Task 1 Perform rapamycin-sensitized CRISPR screens using wild-type, TSC1, and TSC2 deficient *Drosophila* cell lines. ***Mos 21-36 – 30% complete.*** We conducted and finalized a genome-wide screen in *Drosophila* for rapamycin-resistance and enhanced sensitivity in wild-type cells (**Figure 4**). Key findings from this list are that REPTOR and REPTOR-BP, transcription factors and sensors of decreased mTOR signaling, are growth-suppressive in the context of rapamycin-treatment, validating previous studies in Drosophila showing that REPTOR and REPTOR-BP are upstream of a context-specific stress-response specific to low mTOR signaling. Our proposed mammalian screens include these candidates as well as ~100 other potential rapamycin-resistance factors and ~100 potential rapamycin synergizing genes for further mammalian CRISPR screens.

Task 2 Data analysis using MAGeCK. ***Mos 27-28 – 50% complete.*** **We analyzed using MAGeCK the results from the rapamycin-resistance and enhanced sensitivity in wild-type cells.** The analysis provided a list of candidates that will be tested in mammalian cells (**Figure 3**).

Task 3 For candidates for which small molecule inhibitors already exist, which will be prioritized, synergistic effects of those compounds and target-specific RNAi with rapamycin in TSC deficient *Drosophila* S2 cells and mammalian cell models will be tested; for other promising targets, synergy between target RNAi and rapamycin will be tested. Four isogenic pairs of TSC2-deficient and wild- type mammalian cell lines (2 mouse: MEFs and 105K cells / 2 human: MCF10A and IMR90 –commercially available from ATCC) will be used for these validation and characterization experiments. ***Mos 29-32 – 0% complete.*** Work will be initiated in Year 2.

Task 4 *In vivo* preclinical testing of targets that synergize with rapamycin in a mouse TSC tumor model. Xenograft tumors will be generated with 105K tumor cell derivatives expressing doxycycline-inducible shRNAs of the top hits from in vitro screening and validation experiments, or their non-targeting controls. Tumor growth, regression, and regrowth will be measured with and without doxycycline treatment to control shRNA expression in the tumors. (n=10 mice per condition; 80 total) ***Mos 29-32 – 0% complete.*** Work will be initiated in Year 2.

Figure 1. Characterization of loss of CTNS in Drosophila reveals profound effect on mTORC1. (A) Drosophila CTNS is predicted to be a lysosomal cysteine efflux transporter. (B) dCTNS mutation results in cysteine-rescuable starvation-sensitivity. (C) dCTNS RNAi causes upregulation of mTORC1 following starvation. (D) Starvation sensitivity of dCTNS mutants is rapamycin-rescuable. (E) dCTNS mutation and TSC1/2 mutation converge on mTORC1.

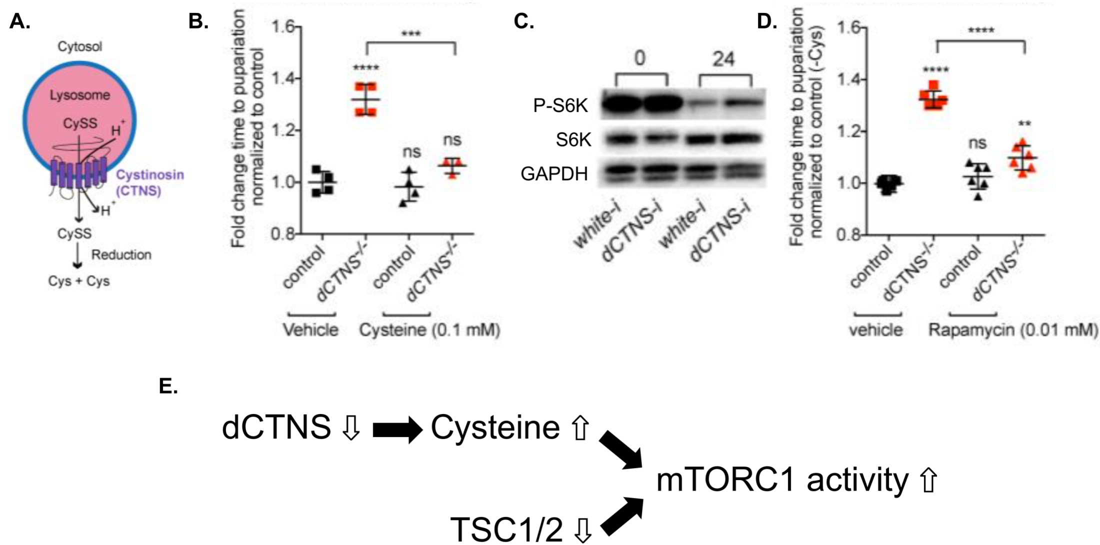


Figure 2. Metabolite heat map of CTNS-/- (R5) vs control (WT) in starvation condition. Note the defects in nucleotides metabolism in the absence of CTNS.

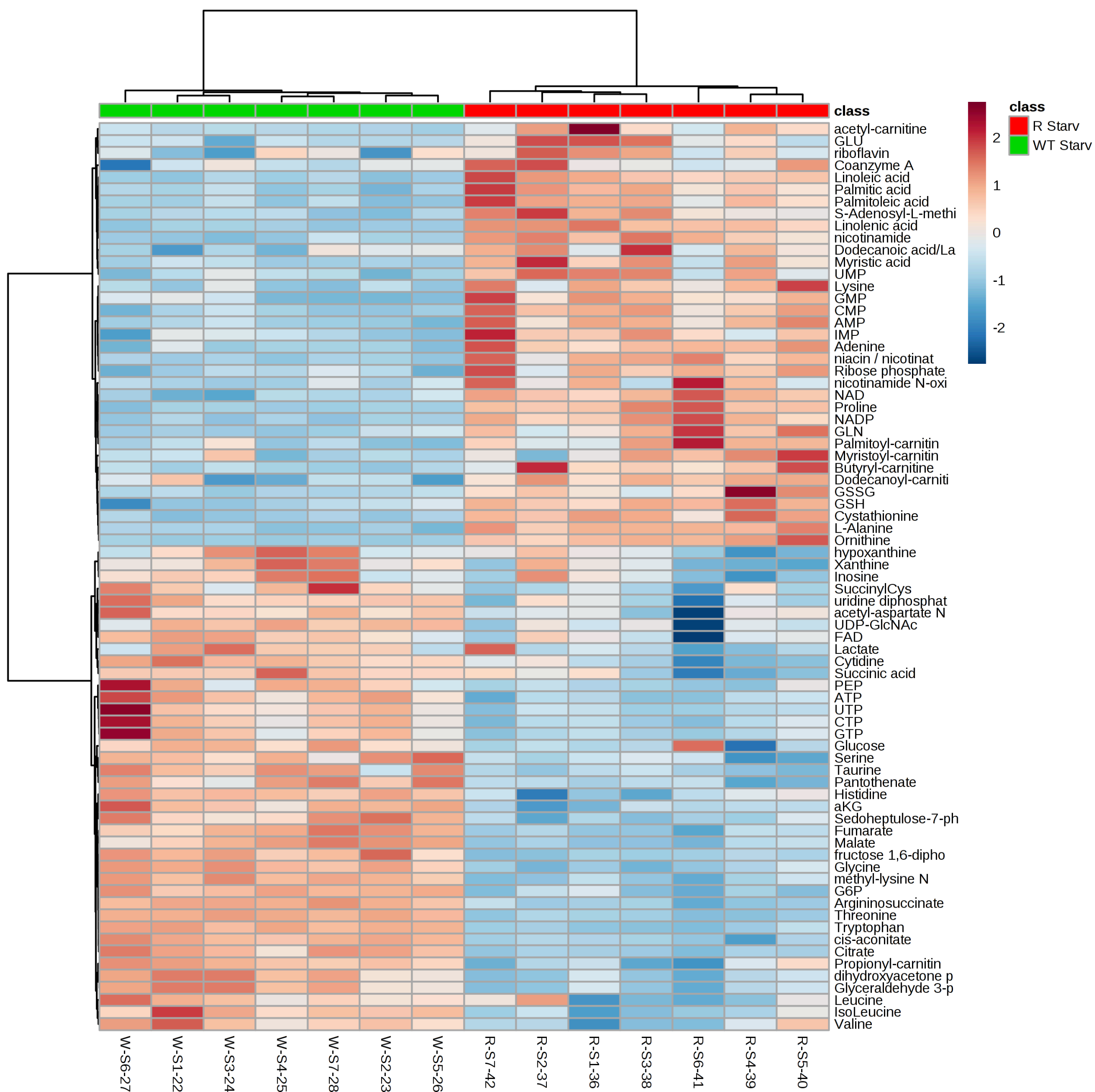


Figure 3. Synthetic *Tsc2, Atf4* lethality and Cys regulation. (A) ATF4 controls uptake of exogenous Cystine and de novo synthesis of Cysteine through transcriptional targets; (B) Gluthatione levels in TSC2, ATF4 double mutant cells; (C) Effects of single and double TSC2, ATF4 mutants on cell viability.

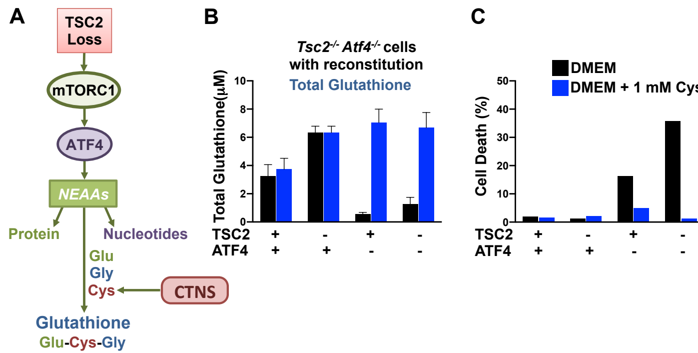
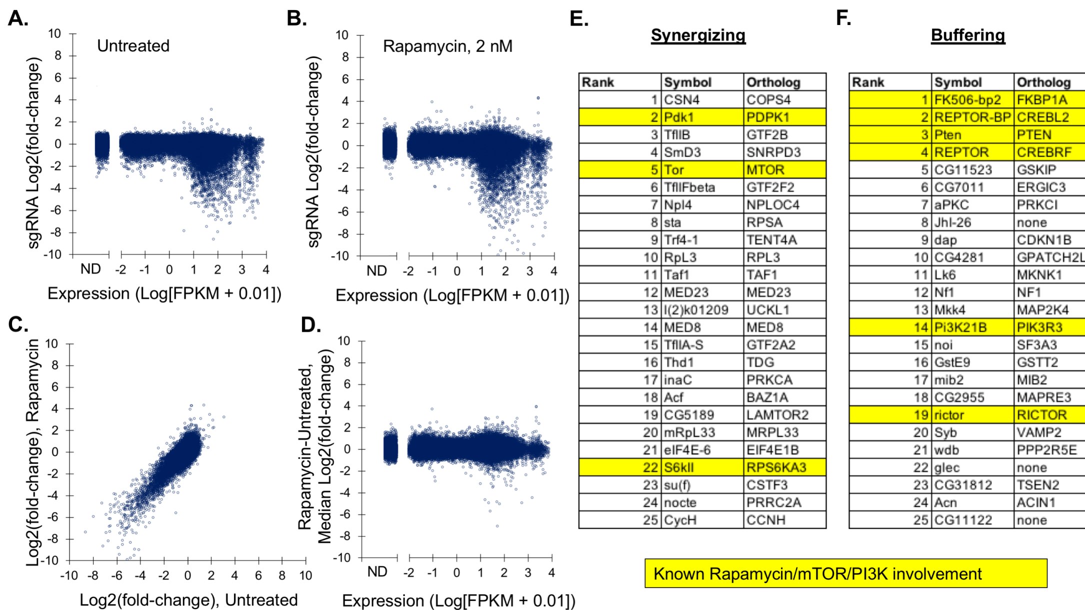


Figure 4. Whole-genome CRIPSR screen for genetic interactions with rapamycin treatment. (A,B) Whole-genome sgRNA fitness screens (79,000 sgRNAs targeting 13,650 genes, N=2 biological replicates) in the presence or absence of rapamycin identify a subset of expressed genes. (C,D,E,F) Quantitative comparison of the two screens at the sgRNA-level (C) and gene-level (D) results in a list of synergizing (E) or buffering (F) interactions that are highly enriched for known rapamycin/mTOR/PI3K pathway-involved genes.



E. **Synergizing**

Rank	Symbol	Ortholog
1	CSN4	COPS4
2	Pdk1	PDPK1
3	TfIIB	GTF2B
4	SmD3	SNRPD3
5	Tor	MTOR
6	TfIIFbeta	GTF2F2
7	Npl4	NPLOC4
8	sta	RPSA
9	Trf4-1	TENT4A
10	RpL3	RPL3
11	Taf1	TAF1
12	MED23	MED23
13	l(2)k01209	UCKL1
14	MED8	MED8
15	TfIIA-S	GTF2A2
16	Thd1	TDG
17	inaC	PRKCA
18	Acf	BAZ1A
19	CG5189	LAMTOR2
20	mRpL33	MRPL33
21	eIF4E-6	EIF4E1B
22	S6kII	RPS6KA3
23	su(f)	CSTF3
24	nocte	PRRC2A
25	CycH	CCNH

F. **Buffering**

Rank	Symbol	Ortholog
1	FK506-bp2	FKBP1A
2	REPTOR-BP	CREBL2
3	Pten	PTEN
4	REPTOR	CREBRF
5	CG11523	GSKIP
6	CG7011	ERGIC3
7	aPKC	PRKCI
8	Jhl-26	none
9	dap	CDKN1B
10	CG4281	GPATCH2L
11	Lk6	MKNK1
12	Nf1	NF1
13	Mkk4	MAP2K4
14	Pi3K21B	PIK3R3
15	noi	SF3A3
16	GstE9	GSTT2
17	mib2	MIB2
18	CG2955	MAPRE3
19	rictor	RICTOR
20	Syb	VAMP2
21	wdb	PPP2R5E
22	glec	none
23	CG31812	TSEN2
24	Acn	ACIN1
25	CG11122	none

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

HARVARD MEDICAL SCHOOL – PERRIMON LAB
Career development for postdoctoral trainees at Harvard Medical School (HMS) is supported at the level of the school through the office of Postdoctoral Fellows, through local activities at the level of the department and in the individual lab through mentorship and annual individual development planning.

The HMS/HSDM Office for Postdoctoral Fellows (OPF) has created programming that aims to enhance postdoc research skills, professional and career development, and social and personal skills while addressing specific issues of early, mid, and late career trainees. Throughout the year, fellows participate in workshops, panel discussions, seminars, and networking opportunities designed to advance lab management skills, grantsmanship, writing and communication,
academic and industry career exploration, as well as work/life and cultural considerations.

The OPF hosts an annual "myIDP" workshop for postdocs to encourage independent planning and goal setting, additionally the OPF provides trainees and faculty mentors with tools for Individual Development Planning that fosters ongoing and recurring discussions involving evaluation, goal setting and feedback. The IDP will be used to address research and professional progress by benchmarking advancement and identifying barriers to success along the training path. This process allows for evaluation of trainee performance and progress while assessing issues related to research, training, or mentoring.

The Department of Genetics offers ample development opportunities for postdoctoral fellows. The department has a weekly internal seminar series where postdocs and graduate students can present their work. We also host a monthly seminar series that invites international leaders in different areas of genetics to speak about their research. The Department is located in the Longwood Medical Area, which is home not only to Harvard Medical School, but also to Beth Israel Deaconess Medical Center, Boston Children's Hospital, Brigham & Women's Hospital, Dana-Farber Cancer Institute, Joslin Diabetes Center, and the Wyss Institute for Biologically Inspired Engineering. Our location fosters intellectual interactions and collaborative research projects with scientists at these neighboring institutions.

How were the results disseminated to communities of interest?

Publications: See sections #6

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

We plan to:

1. Use the CTNS reagents we have generated to examine genetic and molecular interactions with TSC1/2.
2. We will test the synergistic effects of rapamycin treatment combined with manipulations of CTNS and cystine levels on the viability of mammalian TSC cell culture models.
3. We will establish xenograft tumor models for preclinical testing of the effects of CTNS depletion on TSC tumor growth.
4. Select among the 100 candidates, 5 to 10 genes to be tested in different isogenic mammalian cell models of TSC.
5. We have established recently an in vivo model for Reptor activity, whereby overexpression of Reptor in muscles leads to muscle wasting. We will use this assay to validate candidates from the Rapamycin screen.

4. IMPACT:

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

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

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

Nothing to Report

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:

- **Publications, conference papers, and presentations**

  Journal publications.

Jouandin P, Marelja Z, Parkhitko A, Dambowsky M, Asara M, Nemazanyy I, Simons M, **Perrimon N.** Lysosomal cystine efflux opposes mTORC1 reactivation through the TCA cycle. Biorxiv. doi: 10.1101/606541. Submitted (pre-print published). Acknowledges federal support.

Viswanatha R, Li Z, Hu Y, **Perrimon N**. Pooled genome-wide CRISPR screening for basal and context-specific fitness gene essentiality in *Drosophila* cells. eLife. 2018 Jul 27;7. pii: e36333. PMCID: PMC6063728. Acknowledges federal support.

Viswanatha, R., Brathwaite, R., Hu, Y., Rodiger, J., Merckaert, P., Chung, V., Li, Z., Mohr, S. and **Perrimon, N**. (2019) Pooled CRISPR screens in Drosophila cells. Current Protocols in Molecular Biology. In Press. Acknowledges federal support.

Hoxhaj G, **Manning BD**. The PI3K–AKT network at the interface of oncogenic signaling and cancer metabolism. Nat Rev Cancer. 2019, In Press. Acknowledges federal support.

- **Books or other non-periodical, one-time publications.**

Nothing to Report

- **Other publications, conference papers and presentations**.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Norbert Perrimon
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-7542-472X
Nearest person month worked:	1.00 Cal. Mos.
Contribution to Project:	Experimental design and data interpretation for outlined experiments in *Drosophila* systems.
Funding Support:	See "Other Support" below
Name:	Brendan Manning
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-3895-5956
Nearest person month worked:	0.50 Cal. Mos.
Contribution to Project:	Experimental design and data interpretation for outlined experiments in mammalian systems.
Funding Support:	See "Other Support" below
Name:	Raghuvir Viswanatha
Project Role:	Postdoctoral Fellow (N. Perrimon Lab)
Researcher Identifier (e.g. ORCID ID):	0000-0002-9457-6953
Nearest person month worked:	8.10 Cal. Mos.
Contribution to Project:	Design and execution of outlined experiments in *Drosophila* systems
Funding Support:	NIH/NIGMS: T32GM7748-40 (PI: C. Morton)
Name:	Alexander Valvezan
Project Role:	Postdoctoral Fellow (B. Manning Lab)
Researcher Identifier (e.g. ORCID ID):	0000-0002-4369-6074
Nearest person month worked:	6.00 Cal. Mos.
Contribution to Project:	Design and execution of outlined experiments in mammalian systems.
Funding Support:	DoD: W81XWH-18-1-0370 (PI: B. Manning)

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

See "Other Support" for Drs. Perrimon & Manning.

OTHER SUPPORT

PERRIMON, Norbert

ACTIVE

Grant # N.A. (Perrimon) 09/01/2019 – 08/31/2020 0.50 CM
Howard Hughes Medical Institute $800,000 (neg. yearly)
"Pattern formation in *Drosophila*"
The major goals of this project are the studies of *Drosophila* signal transduction pathways and cell polarity in patterning the *Drosophila* embryo and imaginal discs. Dr. Perrimon's salary is not Included in the total amount.

Grants Officer: Mary Ellen Morency
Howard Hughes Medical Institute
300 Longwood Avenue
Enders Building, Room 661
Boston, MA 02115
617-355-7736 | morencym@hhmi.org

5R01NS101745-03 (Shen, Perrimon sub) 03/15/2017 – 02/28/2022 0.30 CM
NIH/NINDS $99,000 (direct/year)
"Identification of Presenilin downstream targets in neuronal survival"
This grant supports the characterization of *Drosophila* neurodegenerative models.

Grants Officer: Roderick A. Corriveau
National Institute of Neurological Disorders and Stroke
P.O. Box 5801
Bethesda, MD 20824
301-496-5680 | roderick.corriveau@nih.gov

5R01AR057352-10 (Perrimon) 07/01/2015 – 06/30/2020 0.46 CM
NIH/NIAMS $220,000 (direct/year)
"Characterization of the Insulin to Autophagy Pathway in Muscles"
These studies will address the role of Insulin and FOXO in regulating anabolic and catabolic pathways during muscle growth and aging in *Drosophila*. Because of the evolutionary conservation of Insulin signaling and the basic cellular machinery involved in protein degradation, our findings in the *Drosophila* model will be directly relevant to the understanding of muscle wasting associated with muscular dystrophies, cachexia, and sarcopenia.

Grants Officer: Aleisha James
National Institute of Arthritis and Musculoskeletal and Skin Diseases
6701 Democracy Blvd, Bldg. Democracy 1, Suite 800
Bethesda MD 20892-4872
301-594-3968 | aleisha.james@nih.gov

I11-0015 (Perrimon/Blenis) 01/01/2018 – 12/31/2019 0.50 CM
Starr Cancer Consortium $208,333 (direct/year)
"Biochemical and genetic investigation of oncogenic TOR-dependent regulation of RNA metabolism and tumorigenesis using cross-species approaches."
These studies will address the role of TOR signaling in regulating mRNA 3'UTR elongation and mRNA splicing.

Grants Officer: Sylvie Le Blancq
Starr Cancer Consortium
646-888-3773 | Leblancs@mskcc.org

5R01HG009352-03 (Celniker/Perrimon sub) 09/01/2017 – 06/30/2020 0.30 CM
NIH/NHGRI $144,000 (direct/year)
"Systematic, Genome-Scale Functional Characterization of Conserved smORFs"
This project involves the identification of smORFs and characterization of their mutant phenotypes.

Grants Officer: Elise Feingold
National Human Genome Research Institute
5635 Fishers Lane, Room 4087, MSC 9305
Rockville, MD 20892-9305
301-480-2770 | elise_Feingold@nih.gov

W81XWH1810659 (Perrimon/Manning) 09/01/2018 – 08/30/2021 1.00 CM
U.S. Department of Defense $75,000 (direct/year)
"An Evolutionary Approach to Vulnerability Mapping in Order to Identify Alternative and Synergistic Therapeutic Strategies for TSC and Related Diseases"
We use state-of-the art functional genomics methods in the fruit fly, *Drosophila*, a proven model to study TSC, to identify drug targets that synergize with Rapalogs in the treatment of TSC. We also seek to characterize a promising drug target that has already emerged from our screens for the treatment of TSC.

Grants Officer: Elizabeth Yu
United States Department of Defense
1077 Patchel Street
Ft. Detrick, MD 21702
301-619-8922 | elizabeth.l.yu2.ctr@mail.mil

[NEW AWARD]
5R24OD026435-02 (Perrimon/Bellen) 07/01/2018 – 06/30/2022 0.65 CM
NIH/OD $298,055 (direct/year)
"Using CRISPR technology to study the function of paralogous genes"
Genetic analysis is a powerful tool for uncovering conserved gene functions but paralogs can have full or partial overlap in function, preventing discovery in single-gene studies. This grant uses state-of-the-art CRISPR technology to generate a resource that will allow gene function to be uncovered through simultaneous disruption of paralogs.

Grants Officer: Donna James
National Institutes of Health, Office of the Director
One Democracy Plaza, Suite 902
6701 Democracy Boulevard, MSC 4877
Bethesda, Maryland 20892-4874
301-496-7484 | jamesd@mail.nih.gov

[NEW AWARD]
5R01DK121409-01 (Perrimon, Sub: Carr, McMahon, Ting) 09/25/2018 – 06/30/2023 1.37 CM
NIH/NIDDK $286,785 (direct/year, Perrimon)
"Mapping protein communication between organs in homeostasis and disease"
This project is to develop the BirA labeling system to identify secreted factors in the mouse. The Perrimon lab will provide its expertise with the use of these reagents.

Grants Officer: Christy Ezell
National Institutes of Health, Office of the Director
6701 Democracy Boulevard
Bethesda, Maryland 20892-4874
301-443-9231 | ezellc@od.nih.gov

[NEW AWARD]
5-P01CA120964-12 (Kwiatkowski, Perrimon/Manning sub) 08/01/2018 – 07/31/2023 0.91 CM
Brigham & Women's Hospital (NIH/NCI) $136,097 (direct/yr., sub only)
"Molecular Pathogenesis of the Hamartoma Syndromes: Project 1 – Molecular wiring and therapeutic targeting of the TSC-Rheb signaling network"
The major goal of this project is to use a dsRNA mini-library containing all kinases and phosphatases encoded in the *Drosophila* genome to search for components regulating AMPK activity.

Grants Officer: Stephanie Draper
National Cancer Institute
9609 Medical Center Drive, Bg 9609 MSC 9760
Bethesda, MD 20892-9760

Grants supporting the Drosophila community and not the Perrimon laboratory.

[NEW AWARD]
1P41GM132087-01 (Perrimon) 08/01/2019 – 04/30/2024 3.00 CM
NIH/NIGMS $800,000 (direct/year)
"Functional genomics resources for the Drosophila and broader research communities"
This project is to renew ongoing support for the DRSC which provides RNAi and gRNA cell-based reagents to the community.

Grants Officer: Jennifer Billington
National Institute of General Medical Sciences
45 Center Drive MSC 6200
Bethesda, MD 20892-6200
301-594-5243 | billingj@nigms.nih.gov

5R01GM084947-12 (Perrimon) 08/01/2017 – 07/31/2020 0.75 CM
NIH/NIGMS $484,868 (direct/year)
"*Drosophila* Transgenic RNAi Resource Project"
Dr. Perrimon is the P.I on this grant that supports funding for the *Drosophila* Transgenic RNAi Project at Harvard Medical School.

Grants Officer: Connie Murphy
National Institute of General Medical Sciences
45 Center Drive MSC 6200, Rm. 2AN24A
Bethesda, MD 20892-6200
301-594-0233 | murphco@mail.nih.gov

5R24OD019847-03 (Perrimon/Simcox sub) 09/18/2017 – 08/31/2021 0.50 CM
NIH/OD $220,000 (direct/year)
"Next-generation *Drosophila* cell lines to elucidate the cellular basis of human diseases"
This project involves the generation of mutant cell lines and cell lines tagged with fluorescent markers for performing CRISPR screens.

Grants Officer: Stephanie Blackford
National Institutes of Health, Office of the Director
6701 Democracy Boulevard
Bethesda, Maryland 20892-4874
301-402-6737 | stephanie.page@nih.gov

2R01GM067858-17 (Bellen/Perrimon sub) 07/01/2018 – 06/30/2020 0.30 CM
NIH/NIGMS $186,783 (direct/year)
"A Comprehensive Resource for Manipulating the *Drosophila* Genome"

The major goal of this project is to expand the Gene Disruption Project (GDP) collection to increase its coverage and provide new methods for analyzing gene function. Generating additional mutant strains and tools will provide valuable resources that will greatly advance the pace of basic and translational research in many laboratories around the world.

Grants Officer: Karen F. Whitaker
National Institute of General Medical Sciences
45 Center Drive, MSC 6200 Bethesda, MD 20892-6200
301-594-6905 | whitakek@nigms.nih.gov

5R24OD021997-04 (Perrimon) 06/01/2016 – 04/30/2020 0.50 CM
NIH/NIGMS $212,250 (direct/year)
"*Drosophila* resources for modeling human diseases"
The major goal of this project is to generate a resource of U6-sgRNA transgenic lines for overexpression targeting rate limiting enzymes implicated in human diseases.

Grants Officer: Karen Brummet
National Institutes of Health, Office of the Director
6701 Democracy Boulevard
Bethesda, Maryland 20892-4874
301-594-6268 | brummettk@mail.nih.gov

5U41HG000739-27 (Perrimon) 04/01/2018 – 03/31/2020 0.58 CM
NIH/NHGRI $1,147,086 (total project funding)
"FlyBase: A *Drosophila* Genomic and Genetic Database"
This grant supports the development and maintenance of the FlyBase database project.

Grants Officer: Diane Patterson
National Human Genome Research Institute
5635 Fishers Lane
Rockville, MD 20892-9305
pattersd@mail.nih.gov

INACTIVE

R01GM067761 (Perrimon) 12/01/2016 – 07/31/2019
NIH/NIGMS
"Functional Genomic Analysis by RNAi Screening in *Drosophila* Cells"
Dr. Perrimon is the P.I on this grant that supports funding for the *Drosophila* RNAi Screening Center at Harvard Medical School.

Grants Officer: Darren D. Sledjeski
National Institute of General Medical Sciences
45 Center Drive, MSC 6200 Bethesda, MD 20892-6200
301-594-2387 | Darren.Sledjeski@NIH.gov

R01HG007118 (Perrimon, PI; Celniker, Vidal, Co-PIs) 09/04/2013 – 06/30/2018
NIH/NHGRI
"Large-Scale High-Confidence Binary Protein Interaction Network for *Drosophila*"
The major goal of this project is to perform a state-of-the-art, high-throughput, quality-controlled analysis of binary protein interactions in *Drosophila*. The resulting next-generation "interactome" will provide a much more complete picture of possible protein interactions in this model system.

Grants Officer: Elise Feingold
National Human Genome Research Institute

5635 Fishers Lane, Room 4087, MSC 9305
Rockville, MD 20892-9305
301-480-2770 | elise_Feingold@nih.gov

PENDING

None.

OVERLAP

There is no budgetary or scientific overlap between these various projects.

OTHER SUPPORT

MANNING, Brendan

ACTIVE

5R35CA197459-05 (Manning) 08/14/2015 – 07/31/2022 6.00 CM
NIH/NCI $580,312 (Direct/year)
"Decoding and targeting the PI3K-mTOR signaling network in cancer"
The major goals of this project include defining how the primary lines of cellular communication function in normal cells and become dysfunctional in cancer cells to promote uncontrolled cell growth. We focus on one of the most commonly activated pathways in human cancers (the PI3K-mTOR pathway) and identifying novel therapeutic strategies to destroy cancer cells displaying activation of this pathway.

Grants Officer: T. Erik Edgerton
National Cancer Institute
9609 Medical Center Drive, Bg 9609 Rm 2W456
Rockville, MD 20850

5-P01CA120964-12 (Kwiatkowski) 07/01/2018 – 06/30/2023 1.20 CM
NIH/NCI $169,000 (Direct/year)
"Molecular Pathogenesis of the Hamartoma Syndromes"
(Project 1 – Manning and Perrimon, Co- Leaders)
This project uses unbiased genomic, proteomic, and genetic approaches to reveal new components, connections, and targets within the TSC-Rheb signaling network. The co-project leaders are focused on identifying novel therapeutic strategies and biomarkers by merging high-throughput *Drosophila* studies with mechanistic biochemical and cell biological studies in mammalian systems.

Grants Officer: Stephanie Draper
National Cancer Institute
9609 Medical Center Drive, Bg 9609 MSC 9760
Bethesda, MD 20892-9760

[NEW AWARD]
W81XWH-18-1-0370-TS170026 (Manning) 09/01/2018 – 08/31/2021 1.20 CM
Dept. of Defense $150,000 (Direct/year)
"Mapping the Routes to Tumor Cell Death in TSC"
Under this grant, we will examine how TSC gene loss and mTORC1 activation influences the cell intrinsic apoptosis machinery in TSC cell and tumor models, and the therapeutic implications.

Grants Specialist: Christopher Meinberg
U.S. Army Medical Research Acquisition Activity
820 Chandler Street
Fort Detrick, MD 21702-5014

[THIS AWARD]
W81XWH-18-1-0659-TS170030 (Manning and Perrimon) 09/01/2018 – 08/31/2021 0.50 CM
Dept. of Defense $150,000 (Direct/year)
"An Evolutionary Approach to Vulnerability Mapping in Order to Identify Alternative and Synergistic Therapeutic Strategies for TSC and Related Diseases"
Under this grant, we will perform synthetic lethality screens in *Drosophila* to identify therapeutically actionable targets conserved in mammalian systems, which will be studied in TSC cell and tumor models.

Grants Specialist: Elizabeth Yu

United States Department of Defense
1077 Patchel Street
Ft. Detrick, MD 21702

INACTIVE

19464 (Manning) 12/01/2015 – 11/30/2018
Tuberous Sclerosis Alliance
"Repurposing clinically approved inhibitors of purine synthesis for the treatment of TSC"
Aim 1. To characterize clinically approved purine synthesis inhibitors that selectively inhibit TSC1/2- deficient cell survival. Aim 2: Determine the effects of structurally distinct IMPDH inhibitors on TSC1/2 deficient cell growth. Aim 3: Establish new cell culture models of TSC for further testing of compounds

Grants Officer: Kari Luther Rosbeck
Tuberous Sclerosis Alliance
801 Roeder Road, Suite 750
Silver Spring, Maryland 20910-4487

Grant # N/A (PI: Mitchell; PD/PI: Manning) 07/05/2016 – 07/04/2018
Zafgen
"Fumagillin, MetAP2, and eIF2alpha"
The goal of this project is to understand the molecular mechanism(s) by which MetAP2 inhibition leads to the primary endpoint of interest, weight loss in vivo. TNP-470 induces weight loss in multiple genetic and diet induced obesity models (Zafgen; Rupnick 2002 PNAS; Brakenheilm 2004 Cir Res), as well as in wild-type lean nheilm 2004 Cir Res).

Grants Officer: Zafgen Inc. Attn: Patricia Allen, CFO
175 Portland Street, 4th Floor
Boston, MA 02114

PENDING

None.

OVERLAP

None. Should pending projects be funded, commitment overlap will be resolved in conjunction with applicant institution officials, the PI, and awarding agency staff.

What other organizations were involved as partners?

Not Applicable

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not Applicable

QUAD CHARTS:

Not Applicable

9. APPENDICES:

Not Applicable