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

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CONTRACTING ORGANIZATION: Harvard Medical School, Boston, MA

REPORT DATE: January 2022

TYPE OF REPORT: Final

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
January 2022	Final	30Sep2018-29Sep2021

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

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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Boston, MA 02115-6027

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
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	25	19b. TELEPHONE NUMBER *(include area code)*

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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 vulnerabilities. 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?

Specific Aim 1: Elucidate the mechanism underlying the synthetic lethal interaction between CTNS and TSC1/2. *90% 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). *Complete.* We have shown that CTNS is 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. *Complete.* We performed targeted metabolomics for metabolites in CTNS KO and found significant defects in nucleotides metabolism.

Task 3 Test the effect of cystine-loading or cysteine depletion on Drosophila S2 cells and mammalian cell (mouse 105K cells) models of TSC. *Complete.* Our combined work in *Drosophila* and mouse TSC2-deficient cells revealed the essential nature of two independent routes of cytosolic Cys acquisition in these cell-based models of TSC, lysosomal efflux Cys through CTNS and exogenous uptake of cystine (Cys_2) through SLC7a11.

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. *Complete.* We found that a major driver of the demand for cytosolic Cys in TSC2-deficient cells was to produce glutathione, the most abundant and important anti-oxidant in cells, and this relates to the oxidative stress put on these cells due to their aberrantly high level of anabolic metabolism. To facilitate this adaptation, mTORC1 induces Cys uptake and glutathione synthesis, protecting cells from an oxidative form of cell death called ferroptosis. This culminated in our finding of a synergistic effect of rapamycin and the ferroptosis-inducing compound Erastin (an SLC7a11 inhibitor), revealing a potential new combination therapy to selectively kill TSC tumor cells.

Task 5 In vivo preclinical testing of CTNS inhibition in a mouse TSC tumor model. *25% complete.* While we have successfully established the syngeneic 105K xenograft tumor model in the lab and have demonstrated the critical nature of de novo purine nucleotide synthesis in this model, a process influenced by CTNS, we are still working on establishing cell lines for tumor studies with inducible CTNS knockdown.

Specific Aim 2: Genetic screen for rapamycin-synergistic vulnerabilities in TSC deficient cells. 90% Complete.

Task 1 Perform rapamycin-sensitized CRISPR screens using wild-type, TSC1, and TSC2 deficient *Drosophila* cell lines. *Complete.*

Gain of function screen

We established a CRISPR based transcriptional activator SAM system in *Drosophila* cells and performed genome-wide CRISPR activation screens to globally identify genes associated with rapamycin resistance. We characterized in details the candidates from the genome-wide CRISPR activation screen for rapamycin resistance. *CG5399*, a *Drosophila* lipocalin gene, is one of the top hits with FDR < 0.05 in two replicates (Fig 1A), suggesting that overexpression of *CG5399* confers resistance to rapamycin. Four of six sgRNA vectors targeting *CG5399* were enriched in rapamycin treated samples compared with DMSO treated samples (Fig 1B). To further verify the screen results, we established stable cell lines expressing the same sgRNA vectors as in the genome-wide sgRNA library targeting *CG5399*. Interestingly, only the enriched sgRNA vectors in the genome wide pooled screen can activate *CG5399* (Fig 2A). To validate that *CG5399* overexpression could confer resistance to rapamycin, we mixed wildtype cells (GFP negative cells) and *CG5399* sgRNA vectors expressing cells (GFP positive cells) together and treated with rapamycin. Cell competition results showed that cells expressing sgRNA activating *CG5399* had a growth advantage compared with wildtype cells in the presence of rapamycin. However, cells expressing sgRNAs which failed to activate *CG5399* did not have the growth advantage (Fig 2B). Importantly, *CG5399* overexpression activates InR-Akt-mTOR signaling, and cholesterol depletion by methyl-beta-cyclodextrin treatment abolished the activation of InR-Akt-mTOR by *CG5399* overexpression in a dose-dependent manner (Fig 3A). As cholesterol is required for different microdomains formation on plasma membrane, we further showed that clathrin coated pits are required for the activation of InR-Akt-mTOR by *CG5399* overexpression (Fig 3B). In summary, we identified a novel regulator of InR-Akt-mTOR signaling by genome-wide CRISPR activation screening.

Characterization of REPTOR/CREBRF

We have characterized in details REPTOR which we identified from a genome-wide screen in *Drosophila* for rapamycin-resistance and enhanced sensitivity in wild-type cells. We found that REPTOR is sufficient to affect mitochondrial biogenesis and morphology, alter glucose metabolism, reduce total protein content, and trigger muscle wasting (Fig 4,5). Importantly, CREBRF, the mammalian ortholog of REPTOR, is likewise a potent metabolic regulator. In mouse myotubes CREBRF is induced by nutrient deprivation. Furthermore, forced expression of CREBRF in myotubes causes a shift from glycolytic to oxidative metabolism and alters the expression of genes controlling mitochondrial biogenesis (Fig 6). Altogether, our studies indicate that REPTOR/CREBRF are novel major regulators of cellular energy metabolism and that perturbed

REPTOR/CREBRF function affects the balance between anabolism and catabolism at the tissue level, in part by shifting cellular energy substrate choice. REPTOR/CREBRF may therefore be adaptive in the context of starvation, but maladaptive if perturbed, contributing in one extreme to excessive energy expenditure and wasting, and in the other to excessive energy storage and obesity.

Task 2 Data analysis using MAGeCK. *Complete.* From the CRISPR LOF and CRISPRa GOF pooled *Drosophila* screens we have established a list of candidates for testing in mammalian cells (Fig. 7,8).

Task 3 For candidates for which small molecule inhibitors already exist, synergistic effects of those compounds and target-specific RNAi with rapamycin in TSC deficient *Drosophila* S2 cells and mammalian cell models will be tested. 75% Complete. Given the intriguing findings from Aim 2, Task 1 described in Figures 1-3 on the role of GG5399, the *Drosophila* ortholog of lipocalin involved in lipid and cholesterol uptake into cells, we have decided to focus efforts on defining the primary sources of cholesterol in cells with chronically activated mTORC1 signaling and its importance in cell proliferation. Importantly, profiling of over 2000 lipids via LC/MS-based lipidomics revealed that the most common cholesterol-ester species in cells are the most sensitive lipid species to rapamycin treatment of *Tsc2*-deficient cells (Fig 9A). Our previous work demonstrated that mTORC1 signaling promotes activation of the SREBP1 and SREBP2 transcription factors to drive global lipid synthesis in cells, with SREBP2 being the major driver of *de novo* cholesterol synthesis (PMID: 20670887; PMID: 21723501; PMID: 26028026). We found that cells with RNAi-mediated knockdown of SREBP2 proliferate well in lipid-rich fetal bovine serum (FBS), demonstrating that the de novo synthesis of cholesterol is dispensable under such growth conditions (Fig 9B). However, removal of lipid from this serum prevents SREBP2-knockdown cells from proliferating, and this effect can be fully rescued with exogenous addition of cholesterol, but not the cholesterol precursor mevalonate or the fatty acid oleate. Thus, these data demonstrate a likely mechanism for the buffering effects of CG5399 overexpression on rapamycin sensitivity, as it would enhance the uptake of exogenous cholesterol and bypass the effects of rapamycin on the mTORC1-SREBP2-cholesterol synthesis circuit. As there are not readily available or reasonably selective small molecule inhibitors for the other candidate targets identified, genetic approaches will be explored in subsequent studies

Task 4 *In vivo* preclinical testing of targets that synergize with rapamycin in a mouse TSC tumor model. *25% complete.* While we have successfully established the syngeneic 105K xenograft tumor model in the lab, the lack of reasonably selective and pharmacologically relevant small molecule inhibitors for the candidate targets identified have stalled further progress on this task. Ongoing and subsequent studies will focus on genetic approaches and preclinical testing in cell lines under Task 3 to select those to test in this tumor model.

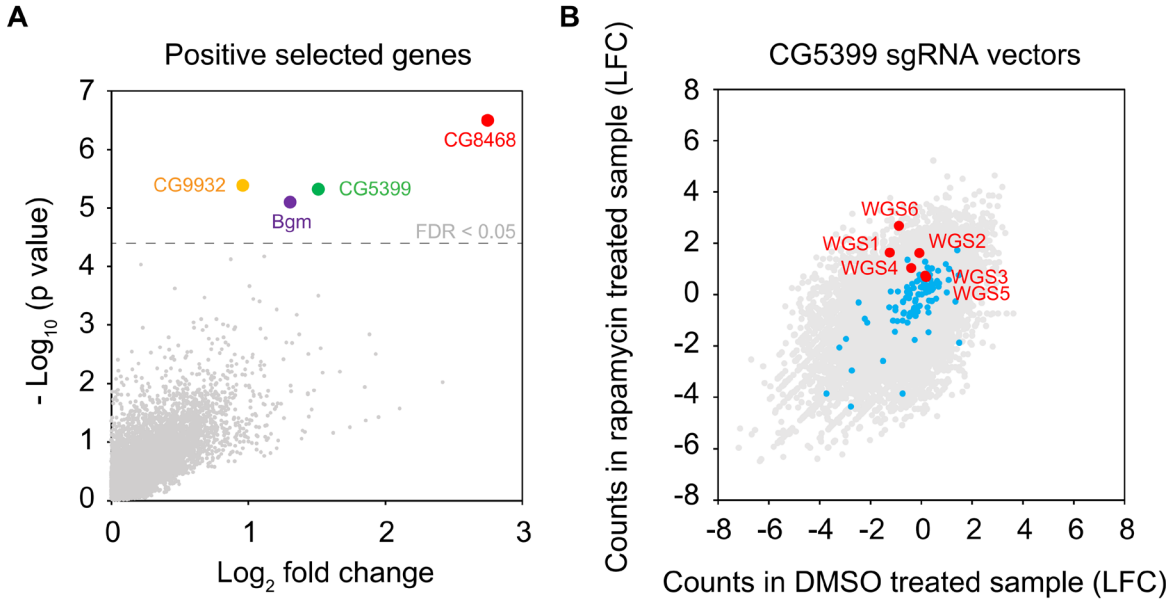

Figure 1. Genome-wide CRISPR activation screening for rapamycin resistance. (A) Positive selected genes for rapamycin resistance. Genes with FDR < 0.05 are highlighted. (B) sgRNA vector counts in DMSO treated sample and rapamycin treated sample. sgRNA vectors targeting *CG5399* are shown as red dots, sgRNA vectors targeting intergenic region are shown as blue dots.

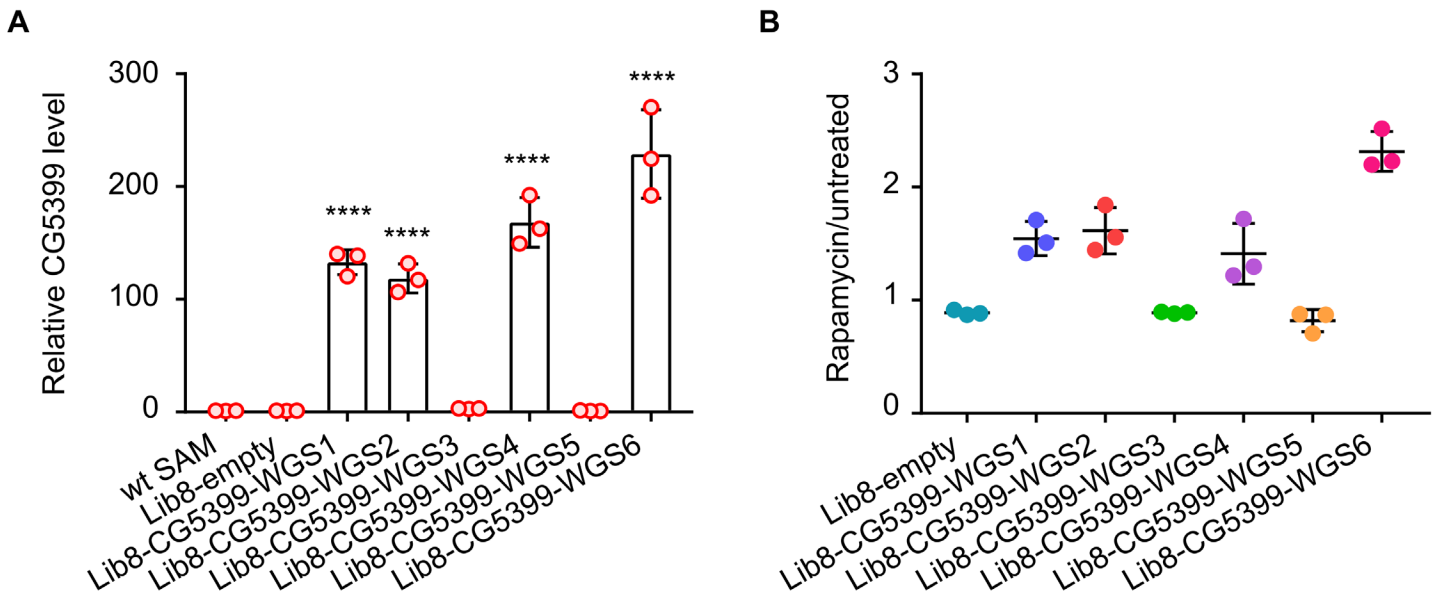

Figure 2. Stable cell lines expressing sgRNA vectors targeting CG5399. (A) qPCR analysis of *CG5399* expression level in stable cell lines expressing the same sgRNA vectors as in the genome-wide sgRNA library targeting CG5399. Expression relative to empty vector-expressing cells is graphed as mean ± SD from three biological replicates (n=3). ****p < .0001. (B) Cell proliferation of stable cell lines expressing *CG5399* sgRNA vectors (GFP positive cells) compared with wildtype cells (GFP negative cells) under rapamycin treatment. GFP proportion is graphed as mean ± SD from three biological replicates (n=3).

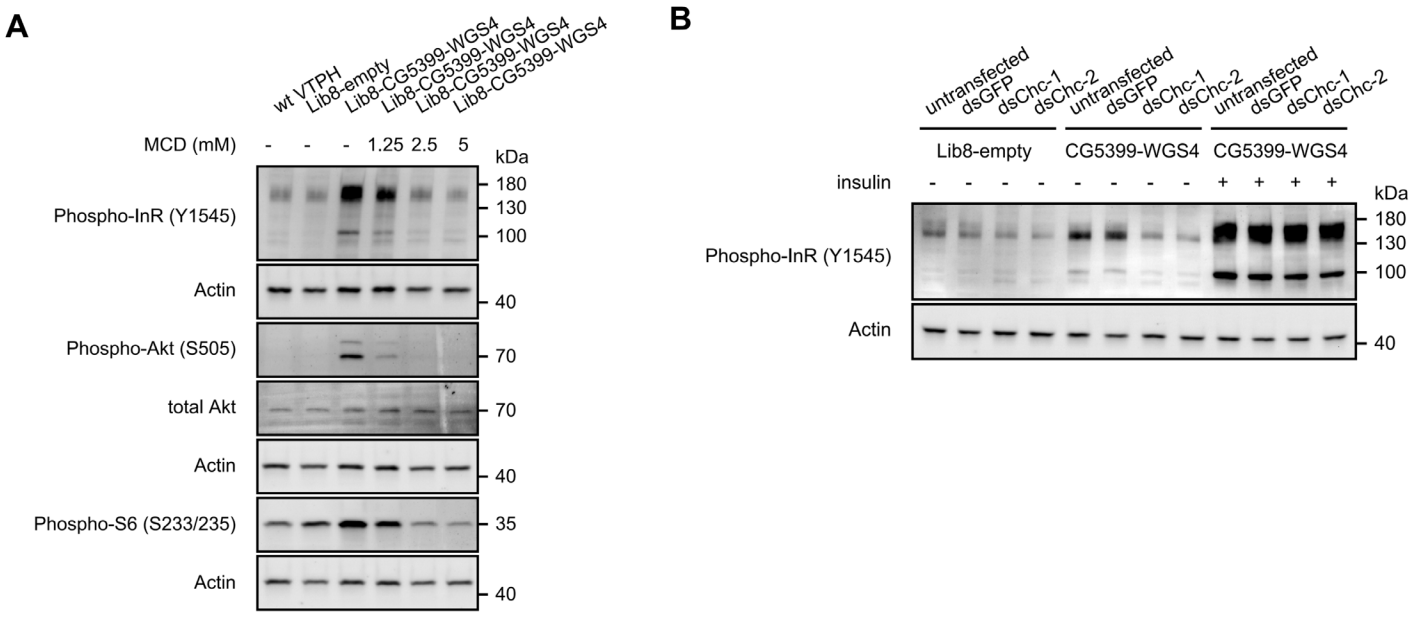

Figure 3. *CG5399* overexpression activates InR-Akt-mTOR signaling in cholesterol and clathrin-coated pits dependent manner. (A) Immunoblotting analysis of phosphorylation of InR, Akt and S6 in *CG5399* overexpressing cells and methyl-beta-cyclodextrin treated cells. MCD, methyl-beta-cyclodextrin. (B) Immunoblotting analysis of phosphorylation of InR in *CG5399* overexpressing cells and clathrin heavy chain knockdown cells.

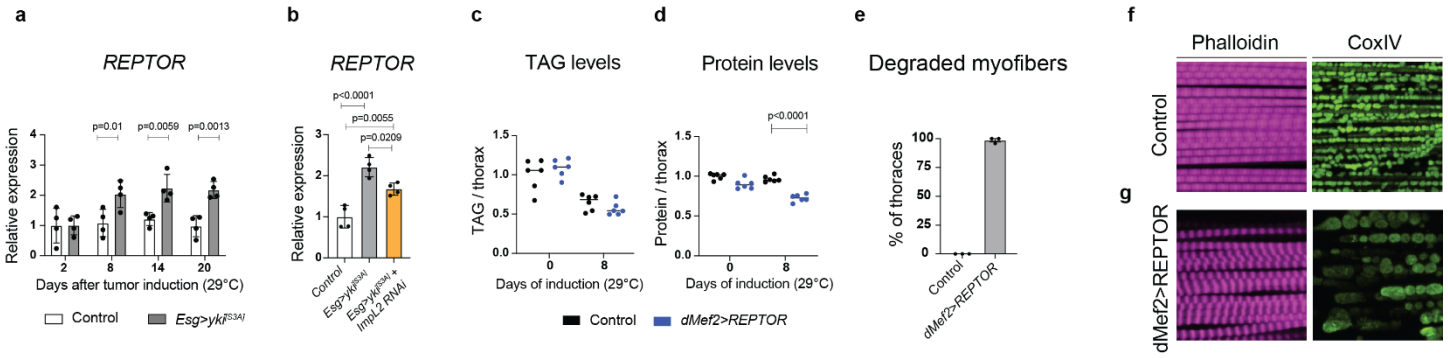

Figure 4: *REPTOR* gain-of-function in muscles induces muscle wasting. a, b) *REPTOR* mRNA in a) *esg>yki* thoraces or b) *esg>yki* thoraces and *esg>yki* + *ImpL2* RNAi after 20 days of tumor induction. *ImpL2* knockdown in *yki*-tumors partially rescues *REPTOR* upregulation observed in *esg>yki* thoraces. In a) values are normalized to the mean of control samples at 2 days after tumor induction. c, d) Quantification of (c) TAG and (d) protein in thoraces. Values are normalized to the mean of respective control samples of the same day. *REPTOR* increase in muscles reduces protein content in thoraces after 8 days of overexpression using *dMef2-GAL4* at 29°C. e) Percentage of thoraces showing myofiber degradation after 8 days of REPTOR induction in the muscle. f,g) Immunostaining of muscles after 8 days of expression *REPTOR* overexpression with *dMef2-GAL4*. Myofibrils are labeled with phalloidin (magenta) and mitochondria with CoxIV (green). *REPTOR* overexpression induces striking changes in mitochondrial morphology.

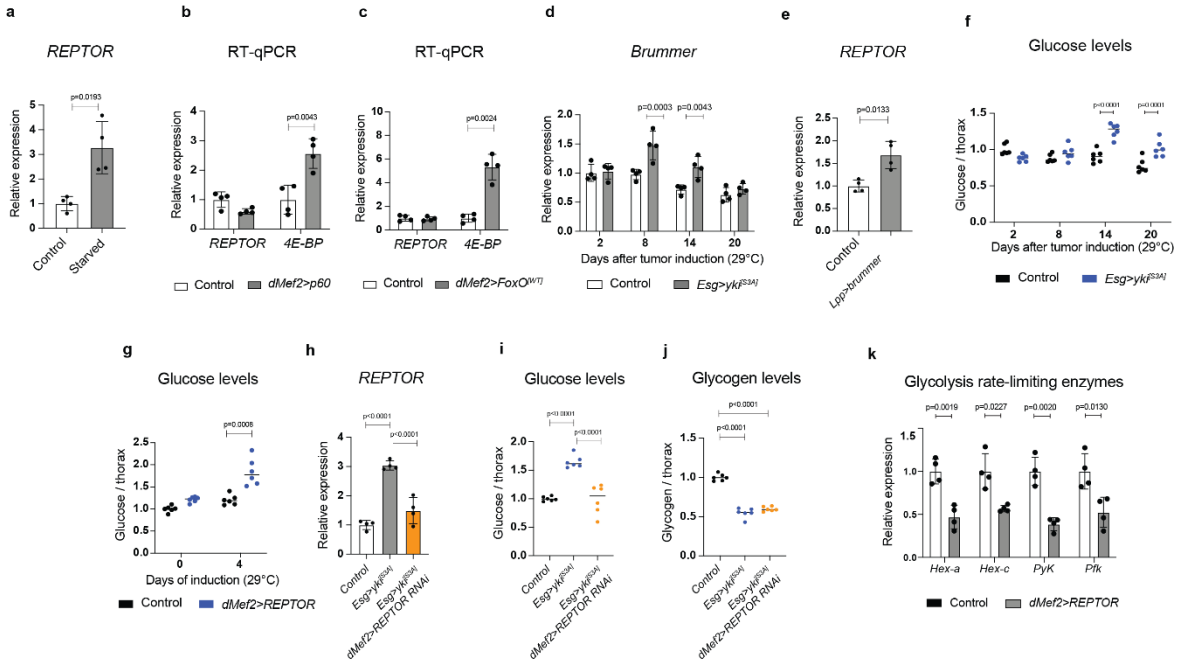

Figure 5: REPTOR regulates metabolism and is upregulated by starvation or increased lipolysis in the fat body. a) *REPTOR* mRNA in thoraces after 3 days of starvation in 1% sucrose. b, c) mRNA levels in thoraces of *REPTOR* and *4E-BP* after 4 days of overexpression of (b) *dMef2>p60* and (c) *dMef2>FoxO[WT].* d) *brummer* mRNA in *esg>yki* thoraces. Values are normalized to the mean of control samples of 2 days of tumor induction. e) *REPTOR* mRNA in thoraces after 4 days of *brummer* overexpression in the fat body using *lpp-GAL4*. Induction of lipolysis increases *REPTOR* expression in thoraces. f, g) Quantification of glucose normalized to number of thoraces in (f) *esg>yki* and (g) overexpression of *REPTOR* in the muscle for 8 days. Values are normalized to the mean of control samples of the earliest time point. h-j) Quantification of (h) *REPTOR* mRNA levels, (i) glucose and (j) glycogen in thoraces in which *yki*-tumors were induced using

esg.LexA/LexAop-yki whereas knockdown of *REPTOR* in muscles was induced using dMef2-GAL4. *REPTOR* knockdown in muscles suppresses glucose accumulation but does not stop glycogen breakdown. Values are normalized to the mean of control samples. k) mRNA expression of glycolytic enzymes in thoraces when *REPTOR* is overexpressed in muscles for 4 days.

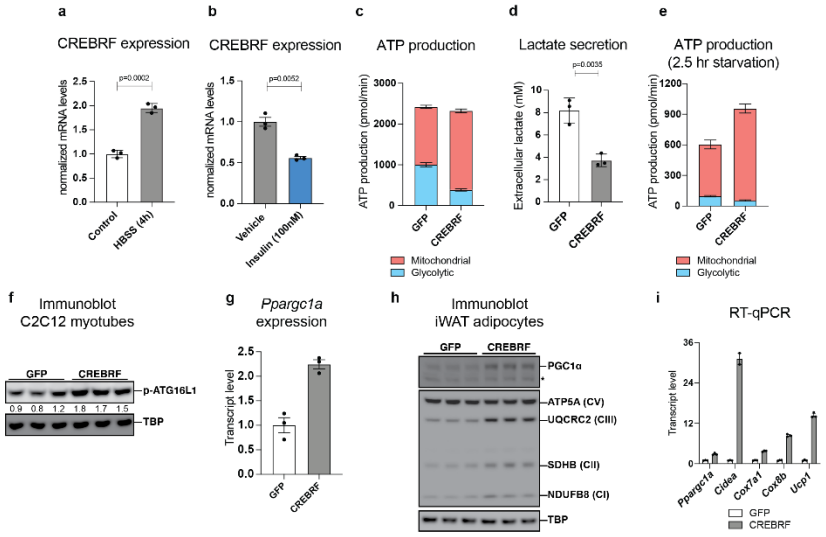

Figure 6: *Crebrf*, the mammalian ortholog of *REPTOR*, is regulated by signals of nutrient availability and alters energy metabolism. a-b) *Crebrf* mRNA in C2C12 myotubes after 4 hr starvation in HBSS medium (a) or after 4 hr treatment with 100 nM insulin (b). c) Contribution of glycolysis and mitochondrial respiration to cellular ATP production in C2C12 myotubes transduced with adenovirus encoding GFP or CREBRF. Cells were assessed in a Seahorse XFe24 instrument by sequential treatment with oligomycin (1.5 μM) and rotenone/antimycin A (1 μM each) in the presence of 10 mM glucose and 1 mM pyruvate. d) Extracellular lactate in DMEM medium conditioned 48 hr by C2C12 myotubes after forced expression of GFP or CREBRF. e) Contribution of glycolysis and mitochondrial respiration to cellular ATP production in C2C12 myotubes as in (c) in the presence of 0 mM glucose and 0 mM pyruvate. f) Immunoblot assessing autophagy marker phospho-ATG16L1 in C2C12 myotubes expressing GFP or CREBRF. phospho-ATG16L1 densitometry values are shown. g) RT-qPCR assessing *Ppargc1a* mRNA level in C2C12 myotubes overexpressing GFP or CREBRF. h) Immunoblot of PGC1α or respiratory complex components in adipocytes overexpressing GFP or CREBRF. i) RT-qPCR assessing *Ppargc1a* and selected mRNAs encoding proteins involved in adipocyte thermogenesis upon CREBRF expression.

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

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

Known Rapamycin/mTOR/PI3K involvement

Figure 7. 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. Top candidate genes involved in synergizing or buffering interactions are listed with known rapamycin/mTOR/PI3K pathway-involved genes highlighted.

Synergizing

Rank	Symbol	Ortholog
1	ATP7	ATP7A
2	lncRNA:CR44597	none
3	CG15641	none
4	AIMP3	EEF1E1
5	mtj	DNAJB2
6	Toll-6	TLR3
7	CG8195	SLC35F5
8	Gr23a	none
9	r2d2	TARBP2
10	CG9220	CHSY1
11	CG33645	none
12	lncRNA:CR44459	none
13	asRNA:CR46144	none
14	Acp98AB	none
15	CG9222	TSSK4
16	CG9452	ACP2
17	lncRNA:CR44369	none
18	DIP-kappa	LSAMP
19	Chrac-14	POLE3
20	CG31848	none
21	Or22b	none
22	CG14212	PHOSPHO1
23	CG4613	TMPRSS3
24	LysP	LYZ
25	lncRNA:CR44274	none

Buffering

Rank	Symbol	Ortholog
1	CG8468	SLC16A8
2	CG5399	none
3	lncRNA:CR43417	none
4	CG34459	none
5	CG9932	ZNF462
6	CG7142	PRSS45P
7	Psc	BMI1
8	cib	TMSB4X
9	CG43980	ABTB2
10	Zip89B	SLC39A1
11	CG34303	none
12	eIF3d2	EIF3D
13	CG15210	none
14	ppk22	ASIC5
15	Lmx1a	LMX1B
16	Gyf	GIGYF2
17	tor	TEK
18	yellow-f	RGN
19	CG8675	C9orf85
20	lncRNA:CR45461	none
21	lncRNA:CR46148	none
22	Pka-C3	PRKX
23	cn	KMO
24	CG5681	none
25	CG30345	SLC46A3

Figure 8. Whole-genome CRIPSRa screen for genetic interactions with rapamycin treatment. Gene list of synergizing or buffering interactions from CRISPRa screening. Known Insulin/mTOR/autophagy pathway-involved genes are highlighted.

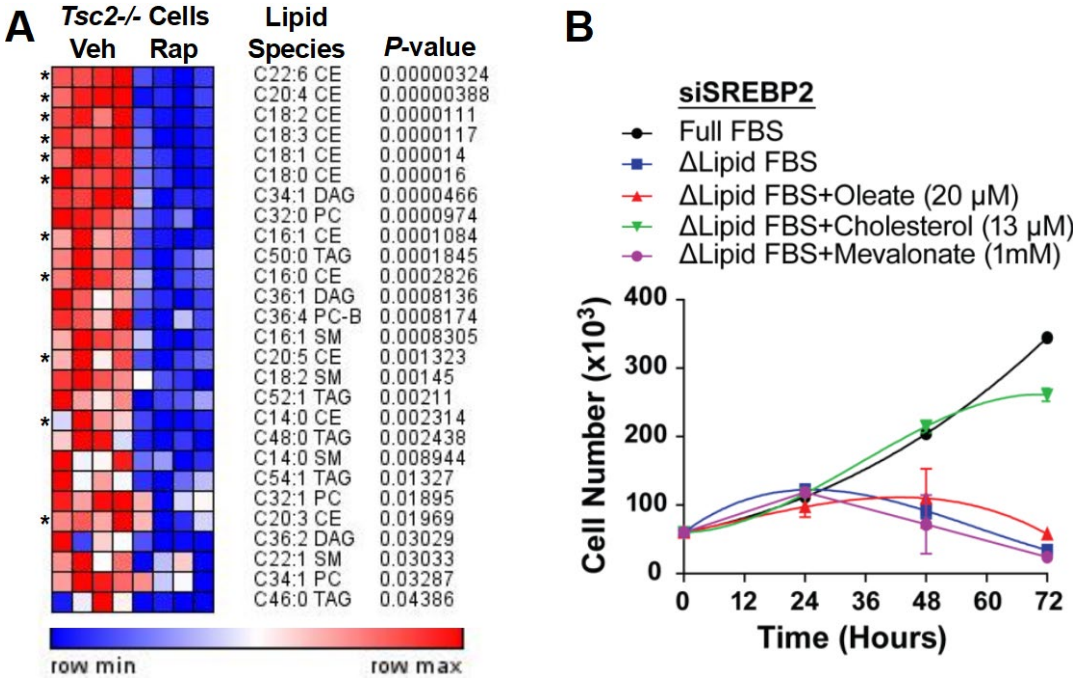

Figure 9. Rapamycin decreases endogenous cholesterol ester levels and exogenous cholesterol is essential for cell proliferation upon SREBP2 knockdown. (A) Lipidomic profiling of *Tsc2-/-* cells treated with vehicle or rapamycin (20 nM) for 24 hours (N=4 biological replicates per treatment), with the most significantly decreased ($p<0.05$) lipid species upon rapamycin treatment shown, ranked by p-value. Lipids species are named as total number of carbons: unsaturated carbons and lipid class. Cholestoryl-ester (CE) species are denoted * and are the most significantly decreased with rapamycin. (C) Cells with siRNA-mediated knockdown of SREBP2 were grown for 72 hours in full serum (FBS) or lipid-stripped (ΔLipid) FBS supplemented as indicated, with cells counted every 24 hours.

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

Harvard Medical School – Perrimon Laboratory
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?

Some of the results of this project were presented by Dr. Manning at the International Tuberous Sclerosis Complex Research Conference 2021: A vision for the future in June of 2021, which is attended by TSC researchers, clinicians, advocates, families, and patients. Dr. Manning gave both a scientific and lay audience talk as part of this virtual conference, which was also attended by three members of his lab.

Publications: See sections #6

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

Nothing to report.

4. IMPACT

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

These collective studies have revealed the critical nature of two distinct nutrients for the proliferation, survival, and rapamycin sensitivity of TSC cells, namely cysteine (Task 1) and cholesterol (Task 2). These are areas of intense interest for further study in TSC tumor models and patient samples, with a need to understand their limitations within the tumor microenvironment associated with TSC – an area that is currently understudied. It is possible that these discoveries will lead to alternative treatments to supplement the use of rapamycin anologs in TSC.

What was the impact on other disciplines?

As aberrantly mTORC1 signaling is a feature of many different diseases and rapamycin is used in other clinical settings, the findings from this study, as with all TSC research, have broader applications to these other pathological settings.

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.

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., Li., Z., Rodiger, J., Merckaert, P., Chung, V., Mohr, S. and Perrimon, N. (2019) Pooled CRISPR screens in Drosophila cells. Current Protocols in Molecular Biology. 129(1):e111. doi: 10.1002/cpmb.111. PMID:31763777. Acknowledges federal support.

Tang, H-W., Weng, J-H., Lee, W. X. Hu, Y., Gu, L., Cho, S., Lee, G., Binari, R., Li, C., Cheng, M. E., Kim, A-R., Xu, J., Shen, Z., Xu, C., Asara, J. M., Blenis, J. and Perrimon, N. (2021) mTORC1-chaperonin CCT signaling regulates m6A RNA methylation to suppress autophagy. PNAS. 118:e2021945118. doi: 10.1073/pnas.2021945118. PMID: 33649236.

Cho, S., Lee, G., Pickering, B. F., Jang, C., Park, J., He, L., Mathur, L., Kim, S-S., Jung, S., Tang, H-W., Monette, S., Rabinowitz, J. D., Perrimon, N., Jaffrey, S. R. and Blenis, J. (2021) mTORC1 promotes cell growth via m6A-dependent mRNA degradation. Molecular Cell. 19:S1097-2765(21)00178-7. doi: 10.1016/j.molcel.2021.03.010. PMID: 33756105.

Jouandin, P., Marelja, Z., Parkhitko, A. A., Dambowsky, M., Asara, J. M. Nemazanyy, I., Dibble, C., Simons, M. and Perrimon, N. (2022) Lysosomal cystine mobilization shapes the response of mTORC1 and tissue growth to fasting. Science. In Press.

Gu, X., Jouandin, P., Binari, R., Reid, M. A., Allen, A. E., Locasale, J. W., Sabatini, D. M. and Perrimon, N. (2022) Sestrin-mediated leucine sensing by mTORC1 is essential for detecting and adapting to a low leucine diet in Drosophila. Nature. In Press.

Liu, G. Y., Jouandin, P., Perrimon, N. and Sabatini, D. M. (2022) The S-adenosylmethionine sensor Unmet expectations reveals evolutionary mechanisms to assimilate new nutrient sensors into the mTORC1 pathway. Submitted.

Saavedra, P., Dumesic, P. A., Hu, Y., Binari, R., Jouandin, P., Wilensky, S. E., Rodiger, J., Wang, H., Spiegelman, B., and Perrimon, N. (2022) REPTOR/CREBRF encode key regulators of muscle energy metabolism. Submitted.

Hoxhaj G, Manning BD. (2019) The PI3K–AKT network at the interface of oncogenic signaling and cancer metabolism. Nat Rev Cancer. 20:74-8. doi: 10.1038/s41568-019-0216-7. PMID: 31686003. Acknowledges federal support.

Torrence ME, MacArthur MR, Hosios AM, Valvezan AJ, Asara JM, Mitchell JR, Manning BD. The mTORC1-mediated activation of ATF4 promotes protein and glutathione synthesis downstream of growth signals. eLife 2021; 10:e63326. doi:10.7554/eLife.63326. PMID: 33646118. Acknowledges federal support.

Byles V, Cormerais Y, Kalafut K, Barrera V, Hughes Hallett JE, Sui SH, Asara JM, Adams CM, Hoxhaj G, Ben-Sahra I, Manning BD. Hepatic mTORC1 signaling activates ATF4 as part of its metabolic response to feeding and insulin. Mol Metab 2021; 101309. doi: 10.1016/j.molmet.2021.101309. PMID: 34303878. 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
Research Identifier (e.g. ORCHID ID): 0000-0001-7542-472X
Nearest person month worked: 3
Contribution to Project: Experimental design and data interpretation for outlined experiments in Drosophila systems.
Funding Support: Howard Hughes Medical Institute

Name: Brendan Manning
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0003-3895-5956
Nearest person month worked: 3
Contribution to Project: Experimental design and data interpretation for outlined experiments in mammalian systems.

Name: Alexander Valvezan
Project Role: Postdoctoral Fellow, Manning Laboratory
Researcher Identifier (e.g. ORCID ID): 0000-0002-4369-6074
Nearest person month worked: 12
Contribution to Project: Design and execution of outlined experiments in mammalian systems.

Name: Madi Cisse
Project Role: Postdoctoral Fellow, Manning Laboratory
Researcher Identifier (e.g. ORCID ID): 0000-0001-6760-3381
Nearest person month worked: 6
Contribution to Project: Design and execution of outlined experiments in mammalian systems.

Name: Raghuvir Viswanatha
Project Role: Postdoctoral Fellow, Perrimon Laboratory
Research Identifier (e.g. ORCHID ID): 0000-0002-9457-6953
Nearest person month worked: 29
Contribution to Project: Design and execution of outlined experiments in Drosophila systems

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

Please see Other Support documents for Dr. Perrimon and Dr. Manning, included as appendices.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Collaborative Awards

Not applicable.

Quad Charts

Not applicable.

9. APPENDICES

Other Support documents for Dr. Perrimon and Dr. Manning are appended.

OTHER SUPPORT – Norbert Perrimon

ACTIVE

Research Awards

Project Number: 5R01AR057352
Sponsor/Funding Source: NIH / NIAMS
Contact PI: Perrimon, Norbert
Effort (person months): .46
Award Period (dates): 08/01/2020 – 5/31/2025
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Characterization of the Insulin to Autophagy Pathway in Muscles
Major Goals: *Understanding the molecular mechanisms underlying muscle growth and wasting is highly relevant to conditions such as anorexia and sarcopenia, and diseases such as cachexia. We will study these mechanisms in Drosophila, a well-established model for the study of muscle biology.*

Project Number: 5R24OD030002
Sponsor/Funding Source: Office of the Director, NIH
Contact PI: Perrimon, Norbert
Effort (person months): 1.49
Award Period (dates): 07/01/2020 – 03/31/2024
Total Amount (DC+IDC) for Award Period: Annual Direct
Costs:
Project Title: TRiP resources for modeling human disease
Major Goals: *This project is to expand the TRiP resource of Drosophila transgenic lines relevant to human diseases and develop tools to make them even more useful and accessible to the research community. The resources we build contribute to the knowledge of Drosophila and help researchers develop Drosophila models of human diseases, and as such are relevant to almost all NIH institutes and relevant in particular to diseases.*

Project Number: 5R01NS101745
Sponsor/Funding Source: NIH / NINDS
Contact PI: Shen, Jie
Effort (person months): .23
Award Period (dates): 03/15/2017 – 02/28/2022
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Identification of Presenilin downstream targets in neuronal survival
Major Goals: *This grant supports the characterization of Drosophila neurodegenerative models.*

Project Number: 5R01DK121409
Sponsor/Funding Source: NIH / NIDDK

OTHER SUPPORT – Norbert Perrimon

Contact PI: Perrimon, Norbert
Effort (person months): .46
Award Period (dates): 09/25/2018 – 06/30/2023
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Mapping protein communication between organs in homeostasis and disease
Major Goals: *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.*

Project Number: 5P01CA120964
Sponsor/Funding Source: NIH / NCI
Contact PI: Kwiatkowski, David J.
Effort (person months): .91
Award Period (dates): 09/17/2018 – 07/31/2023
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Molecular Pathogenesis of the Hamartoma Syndromes: Project 1 - Identifying new therapeutic avenues to selectively target tumors with uncontrolled mTORC1 activation
Major Goals: *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.*

Project Number: N/A
Sponsor/Funding Source: American Federation for Aging Research
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 07/01/2019 – 06/30/2022
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Regulation of the aging process by molecules and pathways involved in organ communication
Major Goals: *This project takes a systematic approach to identify molecules involved in organ communication that influence aging. Specifically, we: 1) Characterize proteins and metabolites present in the Drosophila blood at different age points and in different genetic context; 2) Identify genes in one tissue that sense the aging of other tissues using tissue-specific translatome profiling, and characterize changes in the mitochondrial proteome (mitochondriome) during aging and in response to relevant genetic perturbations; and 3) Identify molecules involved in organ communication and test their roles during aging.*

(NEW)
Project Number: Fund 365274
Sponsor/Funding Source: Fairbairn Family Lyme Research Initiative at Harvard Medical School
Contact PI: Perrimon, Norbert

Effort (person months): .12
Award Period (dates): 09/01/2021 – 08/31/2022
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Establishing genome-wide pooled CRISPR-Cas9 screening in Ixodes scapularis (deer tick) cultured cells for the study of tick cell responses to the Lyme pathogen Borrelia burgdorferi
Major Goals: *To establish CRISPR knockout screening in Ixodes cells by using CRISPR knock-in to generate RMCE-competent I. scapularis cells; to develop expression vectors for Cas9 and for sgRNAs; to introduce Cas9 and confirm activity; and to test the efficiency of the method on selected genes.*

Active Projects Supporting the Drosophila Community and not the Perrimon Laboratory

Project Number: 5P41GM132087
Sponsor/Funding Source: NIH / NIGMS
Contact PI: Perrimon, Norbert
Effort (person months): 3.00
Award Period (dates): 08/01/2019 – 04/30/2024
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Functional genomics resources for the Drosophila and broader research communities
Major Goals: *This project builds on our existing infrastructure, expertise, and track-record to form the Drosophila Research and Screening Center-Biomedical Technology Research Resource (DRSC-BTRR) at Harvard Medical School. We will focus on technology development in the areas of functional genomics and proteomics in Drosophila and mosquito vectors of disease, working together with collaborators and the community to ensure that relevant technologies are developed, improved, applied to a broad set of biomedical projects, and disseminated to ensure long-term access.*

Project Number: 5R24OD019847
Sponsor/Funding Source: Office of the Director, NIH
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 09/18/2017 – 08/31/2022
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Next-generation Drosophila cell lines to elucidate the cellular basis of human diseases
Major Goals: *This project involves the generation of mutant cell lines and cell lines tagged with fluorescent markers for performing CRISPR screens.*

Project Number: 5R01GM084947
Sponsor/Funding Source: NIH / NIGMS

OTHER SUPPORT – Norbert Perrimon

Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 08/04/2016 – 07/31/2022
Total Amount (DC+IDC) for Award Period: Annual Direct Costs:
Project Title: Drosophila Transgenic RNAi Resource Project
Major Goals: *Dr. Perrimon is the PI on this grant that supports funding for the Drosophila Transgenic RNAi Project at Harvard Medical School.*

Project Number: 5R24OD021997
Sponsor/Funding Source: Office of the Director, NIH
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 06/01/2016 – 04/30/2022
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Drosophila resources for modeling human diseases
Major Goals: *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.*

Project Number: 5R24OD026435
Sponsor/Funding Source: Office of the Director, NIH
Contact PI: Perrimon, Norbert
Effort (person months): .61
Award Period (dates): 07/01/2018 – 06/30/2022
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Using CRISPR technology to study the function of paralogous genes
Major Goals: *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.*

Project Number: 5U41HG000739
Sponsor/Funding Source: NIH / NHGRI
Contact PI: Perrimon, Norbert
Effort (person months): .76
Award Period (dates): 04/03/2018 – 03/31/2023
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: FlyBase: A Drosophila Genomic and Genetic Database
Major Goals: *This grant supports the development and maintenance of the FlyBase database project in its aim to provide a crucial openly accessible centralized resource for Drosophila genetic and genomic data to enable researchers and educators worldwide both in the Drosophila*

community and broader biomedical sciences community to further their research.

Project Number: Fund 028942
Sponsor/Funding Source: Harvard Medical School, Dean's Initiatives Program
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 11/01/2019 – 10/31/2021
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Harvard Medical School Foundry: 2019 Award
Major Goals: *This internal award funds infrastructure updates to support Functional Genomics Screening and Bioinformatics Resources at the DRSC/TRiP-Functional Genomics Resources.*

Project Number: DBI-2035515
Sponsor/Funding Source: National Science Foundation
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 08/01/2020 – 7/31/2024
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title BBSRC-NSF/BIO: Integrative analysis and Visualisation of Fly Cell Atlas datasets to enable cross-species comparisons
Major Goals: *This project will work towards the cell type annotation of the Fly Cell Atlas data sets, ensuring a coordinated data flow and community online access across the different public resources (FlyBase; Single Cell Expression Atlas; HCA Data Coordination Platform; and the proposed Fly Cell Atlas Portal).*

(NEW)
Project Number: DBI-2039324
Sponsor/Funding Source: National Science Foundation
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 04/01/2021 – 03/31/2025
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Sustaining FlyBase: The Drosophila genomic and genetic database
Major Goals: *This funding allows FlyBase to maintain its main activities by supporting personnel costs for a computational programmer and biocurator to assist in the development and maintenance of the computational database pipeline infrastructure and curation of FlyBase data.*

(NEW)
Project Number: U24HG010859

Sponsor/Funding Source: NIH / NHGRI
Contact PI: Sternberg, Paul
Effort (person months): .12
Award Period (dates): 09/01/2019 – 07/31/2022
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Alliance Central: A platform for sustainable development of next generation genome knowledgebases
Major Goals: *This funding supports the FlyBase project's involvement in the Alliance of Genome Resources by: developing a platform for ingesting, curating, storing, integrating, and harmonizing data for model organism databases; implementing standards for accessing data and annotations; and implementing a framework to support the development and deployment of novel software applications and analysis workflows.*

Other

Project Number: N/A
Sponsor/Funding Source: Howard Hughes Medical Institute
Contact PI: Perrimon, Norbert
Award Period (dates): 09/01/1997 – 08/31/2024
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Howard Hughes Medical Institute Investigator Program
Major Goals: *HHMI is committed to providing core support to Dr Perrimon's research program, which focuses on studies of Drosophila signal transduction pathways and cell polarity in patterning the Drosophila embryo and imaginal discs. In addition to this funding, Dr. Perrimon receives his salary and benefits from HHMI. His appointment is currently until 8/31/2024; however, budgets are set annually. The total award value represents the budget for the current HHMI fiscal year which ends 8/31/2022.*

INACTIVE

(THIS AWARD)
Project Number: W81XWH1810659
Sponsor/Funding Source: U.S Department of Defense
Contact PI: Perrimon, Norbert
Effort (person months): .91
Award Period (dates): 09/30/2018 – 09/29/2021
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: An Evolutionary Approach to Vulnerability Mapping in Order to Identify Alternative and Synergistic Therapeutic Strategies for TSC and Related Diseases

Major Goals: *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. We will also characterize a promising drug target that has already emerged from our screens for the treatment of TSC.*

Project Number: 2R01GM067858
Sponsor/Funding Source: NIH / NIGMS
Contact PI: Bellen, Hugo J.
Effort (person months): .58
Award Period (dates): 08/15/2019 – 05/31/2021
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: A Comprehensive Resource for Manipulating the Drosophila Genome
Major Goals: *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.*

Project Number: 5R01HG009352
Sponsor/Funding Source: NIH / NHGRI
Contact PI: Celniker, Susan E.
Effort (person months): .12
Award Period (dates): 09/01/2017 – 06/30/2021
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Systematic, Genome-Scale Functional Characterization of Conserved smORFs
Major Goals: *This project involves the identification of smORFs and characterization of their mutant phenotypes.*

Project Number: Fund 028942
Sponsor/Funding Source: Harvard Medical School, Dean's Initiatives Program
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 11/01/2019 – 10/31/2021
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Harvard Medical School Foundry: 2019 Award
Major Goals: *This internal award funds infrastructure updates to support Functional Genomics Screening and Bioinformatics Resources at the DRSC/TRiP-Functional Genomics Resources.*

KEY PERSONNEL PREVIOUS/CURRENT/PENDING SUPPORT

MANNING, BRENDAN

PREVIOUS SUPPORT (last five years)

Tuberous Sclerosis Alliance 194641 (PIs: Manning & Valvezan) 12/01/2015 – 11/30/2018
Repurposing clinically approved inhibitors of purine synthesis for the treatment of TSC
DC/YR, 0.6 calendar months
Tuberous Sclerosis Alliance
801 Roeder Road, Suite 750, Silver Spring, MD 20910-4487
Grants Officer: Kari Luther Rosbeck
This project screened nucleotide synthesis inhibitors for selective effects in TSC1/2-deficient cells, determined the underlying mechanism, and demonstrated anti-tumor efficacy in preclinical TSC tumor models.
Aim 1: Characterize the response of TSC1/2-deficient cells to available inhibitors of purine synthesis
Aim 2: Preclinical trials of IMPDH inhibitors in mouse models of TSC
Aim 3: Define the mechanism underlying the selective response of TSC cells to purine synthesis inhibitors
No overlap.

Zafgen, No Award Number, (PIs: Manning & Mitchell) 09/01/2016 – 09/30/2019
Determining the mechanism of action of derivatives of the anti-obesity drug, fumagillin
DC/YR, 0.6 calendar months
Zafgen, Inc.
3 Center Plaza, Suite 610, Boston, MA 02108
CFO: Patricia Allen
Under this grant, we are characterizing the effects of anti-obesity drugs on cellular and systemic metabolism, and nutrient signaling pathways.
No overlap.

Department of Defense (PI: Manning) 09/01/2018 – 07/31/2021
Mapping the Routes to Tumor Cell Death in TSC
W81XWH-18-1-0370-TS170026
DC/YR, 1.2 calendar months
U.S. Army Medical Research Acquisition Activity
820 Chandler Street, Fort Detrick, MD 21702-5014
Grants Specialist: Christopher Meinberg
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.
Aim 1: Define the status of pro- and anti-apoptotic proteins of the BCL-2 family and apoptotic priming in TSC.
Role: PI
***This award has moved from "Current" to "Completed" since the last report.**

Department of Defense (PI: Perrimon) 09/30/2018 – 09/29/2021
An evolutionary approach to vulnerability mapping in order identify alternative and synergistic therapeutic strategies for TSC and related diseases
DC/YR, 0.6 calendar months
U.S. Army Medical Research Acquisition Activity
Fort Detrick, Maryland 21702-5012
Grant Specialist: Mark Wilkison

This project aims to identify synergistic interactions with Rapamycin.
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 cysteine 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 mice, 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 human or mouse 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 rapamycinsensitized genome-wide screens in wild-type and TSC deficient Drosophila cells. The results will be validated in a collection of 4 different mammalian cell models of TSC (2 mouse and 2 human), prioritizing hits against which small molecule inhibitors exist. The most promising hits from the screening and validation experiments will be tested in a preclinical mouse TSC tumor model for synergistic elimination of TSC-associated tumors in combination with rapamycin. The results of this work are likely to contribute new combinatorial therapeutic options for TSC and related diseases.
Role: Co-PI
No overlap.
***This award has moved from "Current" to "Completed" since the last report, and is the award that this final technical report is for.**

CURRENT SUPPORT

NIH/NCI Outstanding Investigator Award: R35-CA197459 (PI: Manning) 07/01/2015 – 06/30/2022
Decoding and targeting the PI3K-mTOR signaling network in cancer
DC/YR, 6 calendar months
National Cancer Institute
BG 9609 MSC 9760, 9609 Medical Center Drive, Bethesda, MD 20892-9760
Grants Management Specialist: Marianne Galczynski
There are no specific aims in this award, but research is focused on defining the upstream regulation and downstream functions of the PI3K-mTOR network.
No overlap.
***A competing renewal application was submitted for this award on 11/01/2021**

NIH/NCI P01 CA120964 (PI: Kwiatkowski; Project leader: Manning) 08/01/2018 – 07/31/2023
Molecular Pathogenesis of the Hamartoma Syndromes. **Project 1 (Manning and Perrimon): Identifying new therapeutic avenues to selectively target tumors with uncontrolled mTORC1 activation.**
DC/YR, 1.2 calendar months
National Cancer Institute
BG 9609 MSC 9760, 9609 Medical Center Drive, Bethesda, MD 20892-9760
Grants Management Specialist: Rogers Gross
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.
No overlap.

PENDING SUPPORT

NIH R21 (PI: Manning) 04/01/2022 – 03/31/2024
Neurodevelopmental Function of TBC1D7: A Core Component of the TSC Complex
DC/YR, 0.6 calendar months
National Institutes of Health
Defects in the control of neuronal growth underlie a myriad of human neurological disorders, including epilepsy, autism, brain overgrowth, neurocognitive deficits, and neuropsychiatric disorders. The protein TBC1D7 is a key component of growth control pathways that has been found to be defective in neurological disorders, but its function is unknown. This study will use a newly established genetic model to define the function of TBC1D7 as it relates to brain development and neuronal growth.
No Overlap.