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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*

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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?

Major goals for year 2:

Specific Aim 1: Elucidate the mechanism underlying the synthetic lethal interaction between CTNS and TSC1/2. *Mos 1-20 – 65% 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. – 100% 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. *Mos 5-8. – 70% complete.* In Year 1, we performed targeted metabolomics for metabolites in CTNS KO and found significant defects in nucleotides metabolism. In Year 2, we planned to analyze these defects specifically in double KO cells both in S2 and 105K cells with our collaborator Matias Simons. However, this has been delayed as Matias moved his lab from Paris to Germany in fall 2019 and then Covid19 happened. We plan to complete this task when labs are fully functional. We are currently in the process of testing a collection of inducible CTNS shRNAs for use in mammalian cells.

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 – 100% complete.* There are multiple sources of cytosolic Cys in cells, including exogenous uptake, de novo synthesis, and recycling through the lysosome, that latter of which involves cystine efflux through CTNS. To understand the SL relationship of CTNS with TSC2 in both Drosophila and mouse cells, we sought to understand the effects of TSC2 loss and mTORC1 activation on these alternative sources of cytosolic Cys. The primary source of exogenous Cys in plasma is delivered to cells in its oxidized form of Cystine. Importantly, we find that mouse Tsc2-/- cells exhibit rapamycin sensitive cystine uptake, which is also sensitive to Erastin, an inhibitor of xCT (encoded by SLC7a11) (Fig 1A,B). Consistent with this finding, we found that rapamycin suppressed Slc7a11 expression in these cells (Fig 1C). As Slc7a11 is a canonical gene target of the transcription factor ATF4, which we had found in a previous study to be regulated by the TSC-mTOR pathway, we tested a role for ATF4 in this regulation via CRISPR/Cas9 knockout. Indeed, we found that ATF4 knockout strongly decreased Slc7a11 expression and cytsine uptake in Tsc2-/- cells and this was rescued by expression of a rapamycin resistant mutant of ATF4, but not a DNA-binding domain mutant of ATF4 (Fig 1C,D). Importantly, knockout of ATF4 lead to the death of Tsc2-/- cells in a manner that was fully rescued by re-expression of ATF4 or the cystine transporter SLC7A11 (Fig 1E). The rescue of Tsc2-/- Atf4-/- cells by ATF4 required its ability to bind DNA, as the DNA-binding domain mutant failed to rescue (Fig 1F). Interestingly, excess exogenous Cys, which gets into cells through alternative transporters (Fig 1A), could alone partially rescue the proliferation of Tsc2-/- Atf4-/- cells and could fully rescue proliferation, similar to ATF4 addback, when combined with non-essential amino acid (NEAA) supplementation (Fig 1G). However, neither Cystine nor NEAA alone could rescue the proliferation of these cells. Therefore, TSC2 loss leads to enhanced uptake of exogenous cystine through ATF4 and its gene target SLC7A11, which is essential for the survival and proliferation of these cells.

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 – 80% complete.* To determine the consequences of the mTORC1 and ATF4-dependent increase in exogenous Cystine uptake in *Tsc2-/-* cells we next measured the most abundant anti-oxidant in cells, glutathione, the production of which is limited by Cysteine availability. Indeed, treatment of *Tsc2-/-* cells with the mTOR inhibitors rapamycin or torin1, or the glutathione synthesis inhibitor BSO, decreased total intracellular glutathione levels (both oxidized and reduced), and re-expression of TSC2 in these cells also decreased glutathione (Fig 2A,B). Interestingly, treatment of mice bearing *Tsc2-/-* xenograft tumors with rapamycin for just 5 days also significantly reduced tumor glutathione levels (Figure 2C). We found that the effects on glutathione levels were completely dependent on ATF4, SLC7A11, and the acquisition of intracellular cysteine. In Tsc2-/- cells, ATF4 knockout decreased glutathione levels in a manner that was rescued by exogenous expression of ATF4, but not the DNA-binding domain mutant, or SLC7A11, or supplementation of the media with exogenous cysteine, but not cystine (Figure 2D). Finally, to test the synergy between rapamycin and the cystine-dependent synthesis of glutathione, we treated *Tsc2-/-* cells with rapamycin and the SLC7A11 inhibitor erastin, alone or in combination. Erastin alone, but not rapamycin, induced cell death, which was enhanced by co-treatment with rapamycin (Figure 2E). Importantly, the cell death induced by rapamycin plus erastin was ferroptosis, a form of cell death caused by oxidative stress resulting from glutathione depletion, as the ferroptosis inhibitor ferrostatin, but not the apoptosis inhibitor Q-VD-OPh. In the upcoming year we will test whether the same observations are also true in S2 cells.

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 – 10% complete.* We have established the tumor model and collected data on glutathione levels

(Fig 2C), which we find are driven by cytosolic cysteine availability. We are now in the process of testing inducible shRNAs to CTNS to establish the lines for these studies.

Specific Aim 2: Genetic screen for rapamycin-synergistic vulnerabilities in TSC deficient cells. ***Mos 21-36 – 90% complete.***

Task 1 Perform rapamycin-sensitized CRISPR screens using wild-type, TSC1, and TSC2 deficient *Drosophila* cell lines. ***Mos 21-36 – 90% complete.***

Gain of function screen

In Year 1, we completed loss of function pooled CRISPR screens (see previous report). In Year 2, we performed gain of function screens in *Drosophila* cells to identify regulators of sensitivity to Rapamycin using CRISPR based transcriptional activator SAM. To establish the SAM system in *Drosophila* cells, we inserted a vector expressing SAM components under the control of the copper-inducible metallothionein promoter into the genome of *Drosophila* S2R+ cells containing *attP* sites. In the presence of phiC31 helper plasmid, sgRNA vectors were inserted into genome by *attB*/*attP* recombination (Fig 3A). To verify this system can indeed activate target genes, we transfected sgRNA vectors targeting two different genes into SAM cells. qPCR results showed sgRNA vectors can robustly activate target genes after copper induction (Fig 3B).

To characterize genes whose upregulation could confer resistance to rapamycin, we conducted a small-scale screen with a focused sgRNA library covering ~600 genes in SAM cells under different concentrations of rapamycin (1 nM and 0.1 nM). From the focused screen, we identified known positive regulator *HELZ* and negative regulator *DDIT4L* of mTOR signaling. Interestingly, *CG8468* was identified as the top hit from both screen with high concentration of rapamycin (Fig 4A) and screen with low concentration of rapamycin (Fig 4C). sgRNAs targeting *CG8468* were enriched in Rapamycin treated samples compared with control samples (Fig 4B is for screen with high concentration of rapamycin and Fig 4D is for screen with low concentration of rapamycin).

Next, we carried out a genome-wide CRISPR activation screening in SAM cells to globally identify genes associated with rapamycin resistance. We generated genome wide dual-sgRNA library in which two different sgRNAs expressed from one vector target the same gene. Interestingly, *CG8468* was the top hit from two replicates of genome wide rapamycin screening (Fig 5A and Fig 5C). sgRNA vectors targeting *CG8468* were enriched in Rapamycin treated samples compared with control samples (Fig 5B and Fig 5D).

As *CG8468* was identified as the top hit from different screens with different sgRNA libraries, it strongly suggested that upregulation of *CG8468* can confer resistance to Rapamycin. To validate this result, we established stable SAM cell lines expressing the same dual-sgRNA vectors targeting *CG8468* as genome wide library. qPCR results showed these sgRNA vectors can activate *CG8468* expression (Fig 6A). Rapamycin dose-response assay showed cells expressing *CG8468* sgRNA vectors are more resistance to Rapamycin (Fig 6B). To further support this, we mixed wildtype SAM cells (GFP negative cells) and *CG8468* sgRNA vectors expressing SAM cells (GFP positive cells) together and treated with rapamycin. Cell competition results showed cells expressing *CG8468* sgRNA vectors have growth advantage compared with wildtype SAM cells in the presence of rapamycin (Fig 6C).

Characterization of REPTOR/CREBRF

In Year 2, we characterized in details REPTOR which we identified in Year 1 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 7, 8). 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 9). 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. *Mos 27-28. – 100% complete*. From the CRISPR LOF and CRISPRa GOF pooled *Drosophila* screens we have established a list of candidates that will be tested in mammalian cells (Fig. 10,11).

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 3.

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 3.

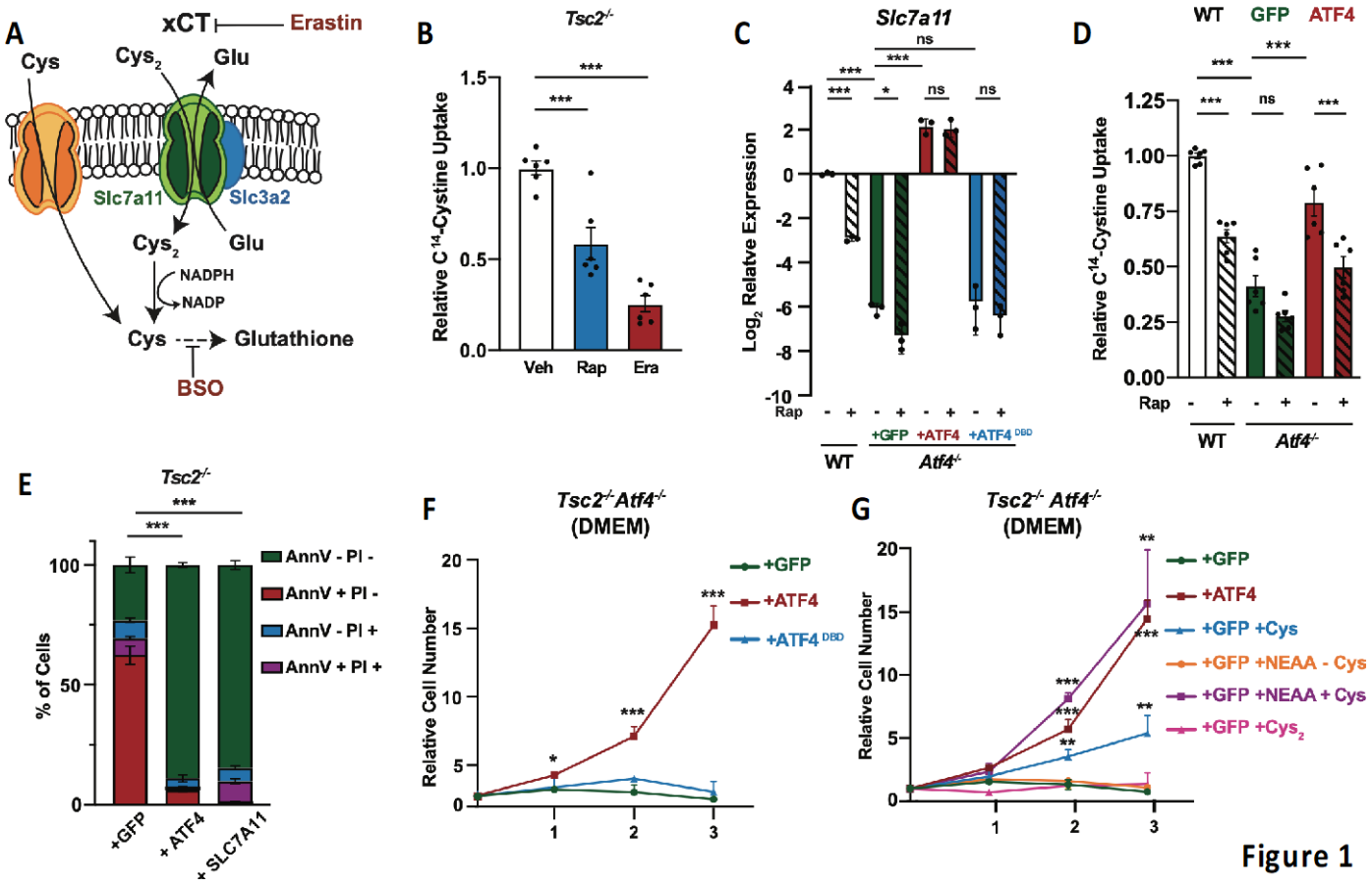

Figure 1

Figure 1: The TSC-mTOR pathway induces Cystine uptake via ATF4 and SLC7A11, a process essential for their survival and proliferation.

(A) Schematic of transporter xCT, encoded by *Slc7a11*, which heterodimerizes with SLC3A2 to serve as a cystine (Cys_2)/glutamate anti-porter. Cystine is reduced to cysteine, which is essential for glutathione synthesis. Cysteine transport is mediated by neutral amino acid trasporters distict from xCT. The targets of Erastin and BSO, two compounds used in this study, are also depicted.

(B) Cystine uptake in serum-deprived *Tsc2*$^{-/-}$ cells treated with vehicle, rapamycin (20 nM), or erastin (10 uM) for 16h is graphed as the mean ± SEM radiolabel incorporation from C^{14}-cystine over the final 10 min relative to vehicle-treated cells from two independent experiments, with three biological replicates each (n=6).

(C) qPCR analysis of *Slc7a11* in *Tsc2*$^{-/-}$ and *Tsc2*$^{-/-}$ *Atf4*$^{-/-}$ cells with stable expression of cDNAs encoding GFP, ATF4 lacking its 5'UTR, or a DNABD mutant (DBD) of this ATF4 treated with vehicle or rapamycin (20 nM, 16 h). Expression relative to WT vehicle-treated cells is graphed as the log_2 mean ± SD from a representative experiment with three biological replicates (n=3).

(D) Cystine uptake in the cells from (C) and graphed as in (B) relative to vehicle-treated WT cells with data from two independent experiments, with three biological replicates each (n=6).

(E) Cell death of *Tsc2*$^{-/-}$ *Atf4*$^{-/-}$ cells with stable expression of cDNAs encoding GFP, ATF4 or SLC7A11 was quantified by Annexin V and PI staining after 72 h in full growth media and graphed as the mean percentage of total cells ± SD from three biological replicates (n=3).

(F,G) Representative growth curves of *Tsc2*$^{-/-}$ *Atf4*$^{-/-}$ cells with stable expression of GFP, ATF4, or $ATF4^{DBD}$ grown in 10% dialyzed FBS with DMEM (F) or DMEM supplemented with cysteine alone (Cys, 1 mM), non-essential amino acids (100 μM each) lacking cysteine (NEAA-Cys), or nonessential amino acids plus either cysteine (1 mM, NEAA+Cys), or cystine (0.5 mM, NEAA+Cys_2) (G). Mean cell numbers ± SD relative to Day 0 are graphed from three biological replicates (n=3).

*p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant.

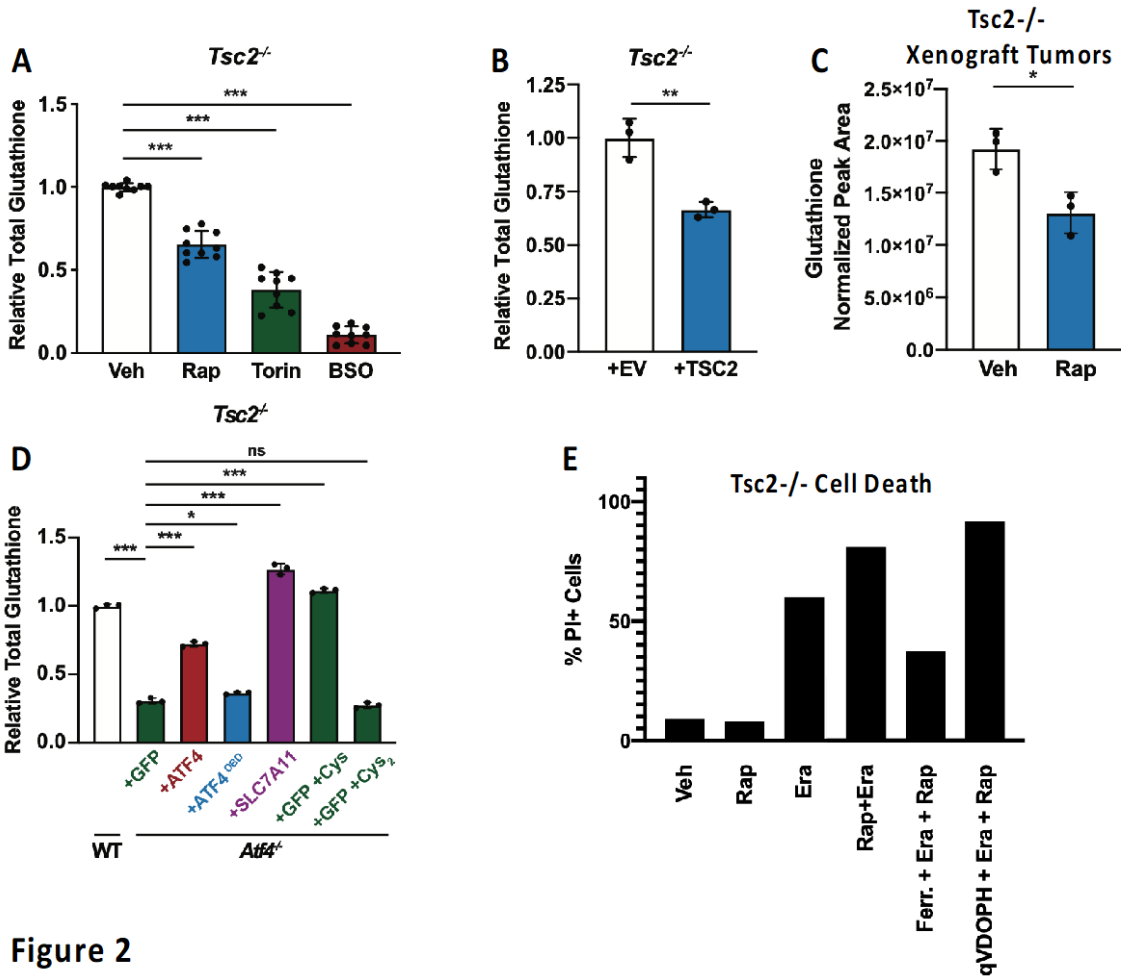

Figure 2

Figure 2: The TSC-mTOR pathway induces the Cystine-dependent synthesis of glutathione, and rapamycin synergizes with SLC7A11 inhibition to induced ferroptosis in *Tsc2-/-* cells.
(A) Total glutathione in serum-deprived *Tsc2*$^{-/-}$ cells treated with rapamycin (20 nM), Torin1 (250 nM), or BSO (10 μM) for 16 h is graphed as mean ± SEM relative to vehicle-treated cells from three independent experiments, each with three biological replicates (n=9).
(B) Relative total glutathione in serum-deprived *Tsc2*$^{-/-}$ cells with stable expression of a cDNA encoding TSC2 or empty vector (EV) control is graphed as mean ± SD from a representative experiment with three biological replicates (n=3).
(C) Relative glutathione levels measured by LC-MS/MS from *Tsc2*$^{-/-}$ xenograft tumors resected from mice treated for 5 days with vehicle or rapamycin (1 mg/kg on days 1, 3, and 5) (n = 3 mice/group).
(D) Total glutathione in *Tsc2*$^{-/-}$ and *Tsc2*$^{-/-}$ *Atf4*$^{-/-}$ cells with stable expression of cDNAs encoding GFP, ATF4, ATF4^{DBD}, or SLC7A11 grown in DMEM and supplemented, where indicated, with cysteine (1 mM) or cystine (0.5 mM) is graphed as mean ± SD relative to WT cells from a representative experiment with three biological replicates (n=3).
(E) Cell death (graphed as % PI+ staining) in *Tsc2-/-* cells treated with vehicle, rapamycin, erastin, ferrostatin, and/or Q-VD-OPh, as indicated for 24 hours.
*p < .05, **p < .01, ***p < .001, ns = not significant.

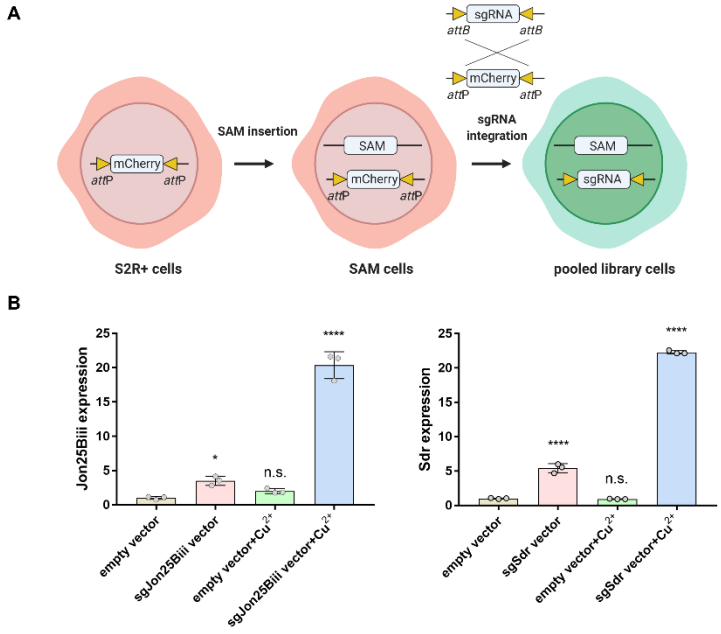

Figure 3: SAM-mediated transcriptional activation in Drosophila cells. (A) Schematic of SAM-mediated transcriptional activation in Drosophila S2R+ cells. Plasmid expressing components of SAM under the control of copper-inducible metallothionein promoter is inserted into the genome of Drosophila S2R+ cells containing attP sites. In the presence of phiC31 helper plasmid, sgRNA vectors were inserted into genome by attB/attP recombination. (B) qPCR analysis of target gene expression in SAM cells transfected with sgRNA vectors targeting Jon25Biii and Sdr. Expression relative to empty vector-transfected cells is graphed as mean ± SD from three biological replicates (n=3). *p < .05, ****p < .0001, n.s. = not significant.

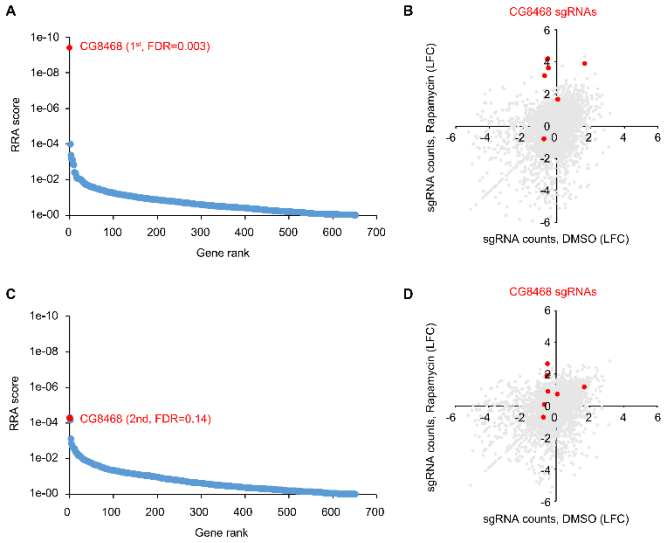

Figure 4: Small scale gene activation screens. (A) CRISPRa screen of rapamycin resistant genes with focused sgRNA library (6336 sgRNAs targeting 652 genes) under high concentration of rapamycin (1 nM) treatment. Genes are ranked by robust rank aggregation (RRA) scores calculated by MAGeCK. (B) sgRNA counts in DMSO treated samples and rapamycin (1 nM) treated samples. sgRNAs targeting CG8468 are showed as red dots. (C) CRISPRa screen of rapamycin resistant genes with focused sgRNA library (6336 sgRNAs targeting 652 genes) under high concentration of rapamycin (0.1 nM) treatment. Genes are ranked by robust rank aggregation (RRA) scores calculated by MAGeCK. (D) sgRNA counts in DMSO treated samples and rapamycin (0.1 nM) treated samples. sgRNAs targeting CG8468 are showed as red dots.

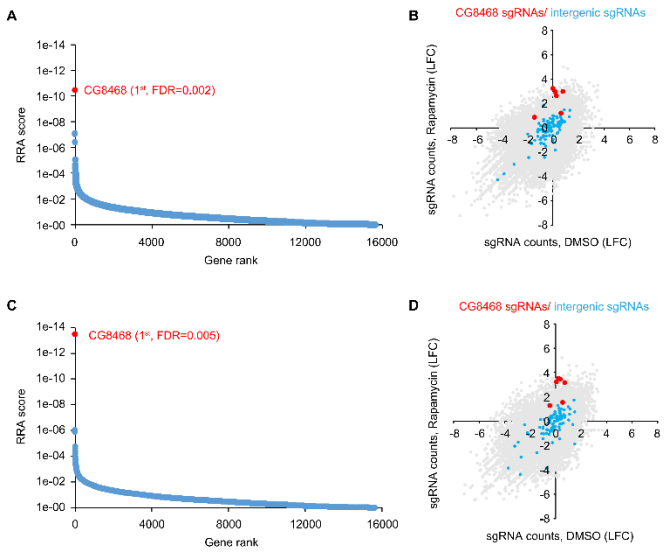

Figure 5: Genome scale gene activation screens. (A) (C) CRISPRa screen of rapamycin resistant genes with genome-wide sgRNA library (83,854 sgRNA vectors targeting 15,625 genes) in two biological replicates. Genes are ranked by robust rank aggregation (RRA) scores calculated by MAGeCK. (B) (D) sgRNA counts in DMSO treated samples and rapamycin treated samples in two biological replicates. sgRNAs targeting CG8468 are showed as red dots, sgRNA targeting intergenic regions are showed as blue dots.

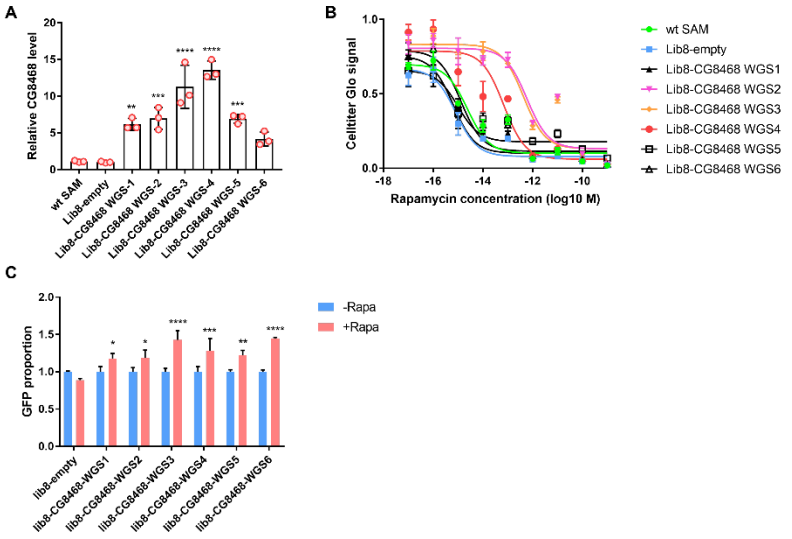

Figure 6: CG8468 confers resistance to rapamycin. (A) qPCR analysis of CG8468 expression in SAM cells expressing CG8468 sgRNA vectors. Expression relative to empty vector-expressing cells is graphed as mean ± SD from three biological replicates (n=3). (B) Cell proliferation of SAM cells expressing CG8468 sgRNA vectors under serial diluted rapamycin. Celltiter glo values relative to cells without Rapamycin treatment are graphed as mean ± SD from three biological replicates (n=3). (C) Cell proliferation of SAM cells expressing CG8468 sgRNA vectors (GFP positive cells) compared with wt SAM cells (GFP negative cells) under rapamycin treatment. GFP proportion is graphed as mean ± SD from three biological replicates (n=3). *p < .05, **p < .01, ***p < .001, ****p < .0001.

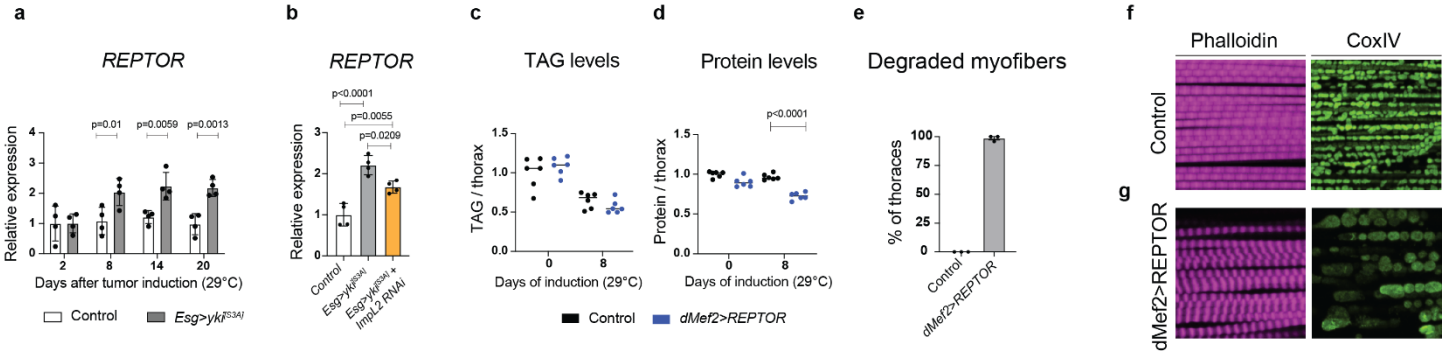

Fig 7: *REPTOR* gain-of-function in muscles induces muscle wasting. a, b) *REPTOR* mRNA in a) *esg>yki* thoraces or b) *esg>yki* thoraces and *esg>yki* + *ImpL2* RNAi after 20 days of tumor induction. *ImpL2* knockdown in *yki*-tumors partially rescues *REPTOR* upregulation observed in *esg>yki* thoraces. 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.

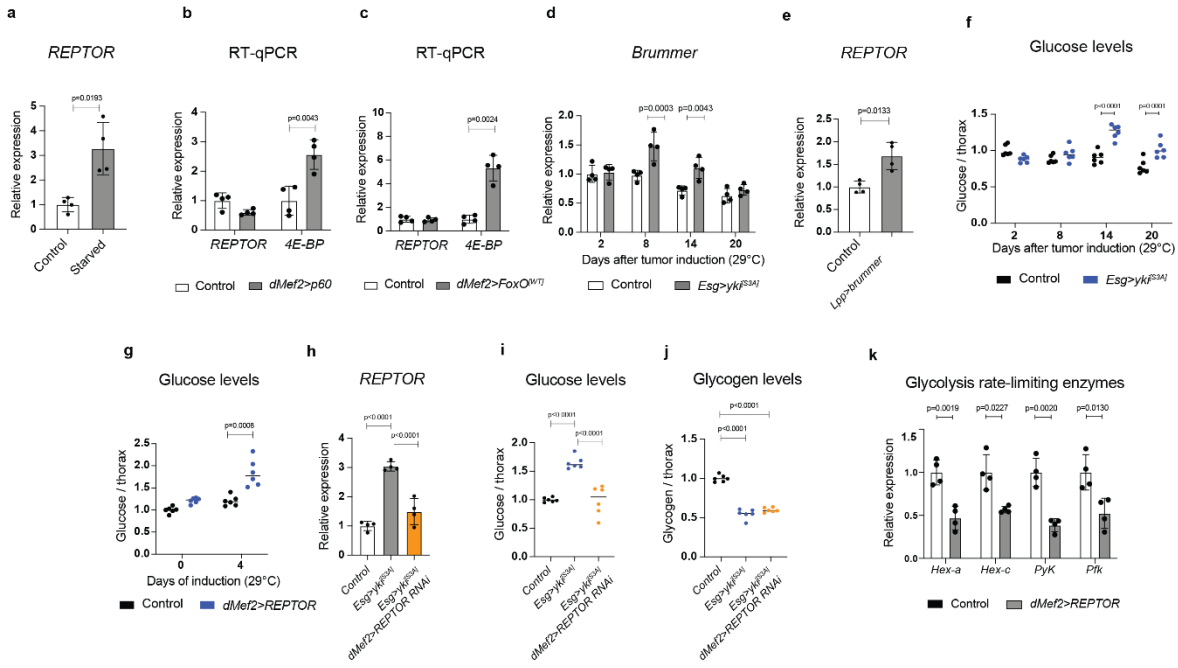

Fig 8: REPTOR regulates metabolism and is upregulated by starvation or increased lipolysis in the fat body. a) *REPTOR* mRNA in thoraces after 3 days of starvation in 1% sucrose. b, c) mRNA levels in thoraces of *REPTOR* and *4E-BP* after 4 days of overexpression of (b) *dMef2>p60* and (c) *dMef2>FoxO[WT].* 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.

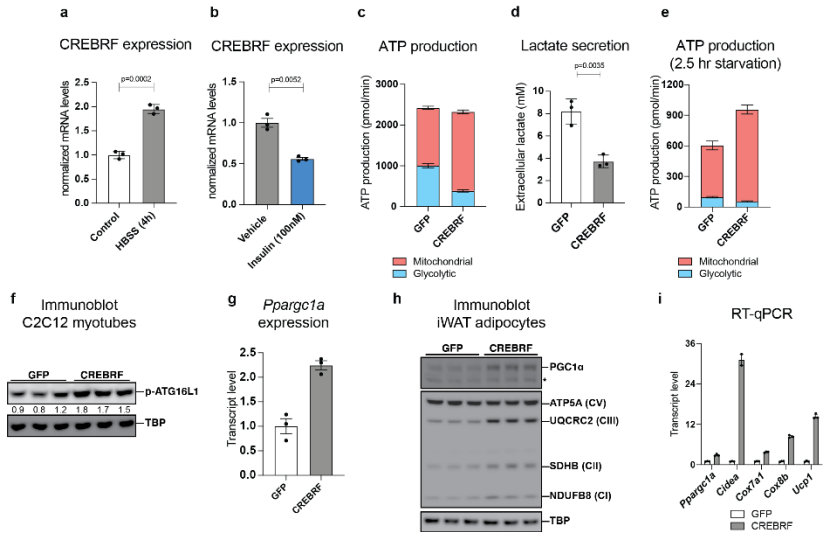

Fig 9: *Crebrf*, the mammalian ortholog of *REPTOR*, is regulated by signals of nutrient availability and alters energy metabolism. a-b) *Crebrf* mRNA in C2C12 myotubes after 4 hr starvation in HBSS medium (a) or after 4 hr treatment with 100 nM insulin (b). c) Contribution of glycolysis and mitochondrial 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 10. 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	CG15041	none
4	AIMP3	EEF1E1
5	mtj	DNAJB2
6	Toll-6	TLR3
7	CG8195	SLC35F5
8	Gz23a	none
9	l2d2	TARBP2
10	CG9220	GHSY1
11	CG33945	none
12	lncRNA:CR44459	none
13	lncRNA:CR41144	none
14	Acp98AB	none
15	CG9222	ITSN1
16	CG9452	ACP2
17	lncRNA:CR40318	none
18	DIP-kappa	LSAMP
19	Chmp-14	POLE3
20	CG31848	none
21	Gz22b	none
22	CG14212	PHOSPHO1
23	CG4813	TMPRSS3
24	Lysp	LYZ
25	lncRNA:CR44274	none

Buffering

Rank	Symbol	Ortholog
1	CG34435	SLC9A5
2	CG5399	none
3	lncRNA:CR43417	none
4	CG34459	none
5	CG9932	ZNF462
6	CG7942	PRSS56P
7	Psc	BMI1
8	cib	TMEM4X
9	CG43580	ABTB2
10	Zip69B	SLC39A1
11	CG34303	none
12	eIF5C	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:CR45401	none
21	lncRNA:CR46148	none
22	Pka-C3	PRKX
23	cn	KMO
24	CG5551	none
25	CG30345	SLC40A3

Figure 11. Whole-genome CRIPSRa screen for genetic interactions with rapamycin treatment. Gene list of synergizing or buffering interactions from CRISPRa screening. Known Insulin/mTOR/autophage pathway-involved genes are highlighted.

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?

See 6. Publications.

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

We plan to:

- test a collection of inducible CTNS shRNAs for use in mammalian cells.
- analyze the regulation of *REPTOR* mRNA and protein in the muscles of flies with *yki*-tumors or when lipolysis is induced.
- identify the transcription factor(s) modulating *REPTOR* expression and if REPTOR is regulated by phosphorylation identify the relevant protein kinases.
- assess the extent of transcriptional and post-translational CREBRF regulation and to define the upstream nutrient cues responsible.
- perform metabolite profiling of *CG8468* overexpressing cells to identified the affected metabolites and their roles in rapamycin sensitivity and mTor signaling.
- perform the in vivo preclinical testing of CTNS inhibition in a mouse TSC tumor model.
- initiate tasks 3 and 4 of Specific Aim 2.

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., 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., Hu, Y., Weng, J-W., Gu, L., Binari, R., Li, C., Kim, A-R., Shen, Z., Xu, C., Asara, J. M., and **Perrimon, N.** (2020) mTORC1- chaperonin CCT signaling regulates m^6A RNA methylation to suppress autophagy. Submitted.

Hoxhaj G, **Manning BD.** (2020) The PI3K–AKT network at the interface of oncogenic signaling and cancer metabolism. Nat Rev Cancer. 20:74-8 PMID: **31686003**. 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):
Nearest person month worked: 1
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):
Nearest person month worked: 1
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):
Nearest person month worked: 6
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):
Nearest person month worked: 1
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):
Nearest person month worked: 12
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 AND INACTIVE PROJECTS/AWARDS

Active

Project Number: N/A
Sponsor/Funding Source: Howard Hughes Medical Institute
Contact PI: Perrimon, Norbert
Effort (person months): .50
Award Period (dates): 09/01/2020-08/31/2021
Annual Direct Costs: (negotiated yearly) Project Title:
Pattern formation in Drosophila
Major Goals: *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 annual direct costs.*

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: 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: 5R01DK121409
Sponsor/Funding Source: NIH / NIDDK
Contact PI: Perrimon, Norbert
Effort (person months): 1.36
Award Period (dates): 09/25/2018 – 06/30/2023
Total Amount (DC+IDC) for Award Period: Annual
Direct Costs:

OTHER SUPPORT – Norbert Perrimon

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.*

(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: 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 – Molecular wiring and therapeutic targeting of the TSC-Rheb signaling network
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: I11-0015
Sponsor/Funding Source: Starr Cancer Consortium
Contact PI: Blenis, John
Effort (person months): .12
Award Period (dates): 01/01/2018 – 12/31/2020
Total Amount (DC+IDC) for Award Period:
Annual Direct Costs:
Project Title: Biochemical and genetic investigation of oncogenic TOR-dependent regulation of RNA metabolism and tumorigenesis using cross-species approaches
Major Goals: *This project aims to validate and characterize phosphorylation sites on RNA processing machineries using knockout/knockin, RNAi, overexpression experiments both in cell line models and in Drosophila mutants. In addition, to identify how the RNA processing of target genes is regulated, we will examine the expression patterns*

of the validated candidate RNA isoforms in various Drosophila cell lines in the presence or absence of small molecule inhibitors.

(NEW)
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.*

<u>Active Projects Supporting the Drosophila Community and not the Perrimon Laboratory</u>

Project Number: 5R24OD019847
Sponsor/Funding Source: NIH / OD
Contact PI: Perrimon, Norbert
Effort (person months): .46
Award Period (dates): 09/18/2017 – 08/31/2021
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.*

(NEW)
Project Number: 1R24OD03002
Sponsor/Funding Source: NIH / OD
Contact PI: Perrimon, Norbert
Effort (person months): .76
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

OTHER SUPPORT – Norbert Perrimon

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: 1P41GM132087
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: 5R01GM084947
Sponsor/Funding Source: NIH / NIGMS
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 08/04/2016 – 07/31/2021
Total Amount (DC+IDC) for Award Period: Annual Direct
Costs:
Project Title: Drosophila Transgenic RNAi Resource Project
Major Goals: *Dr. Perrimon is the P.I on this grant that supports funding for the Drosophila Transgenic RNAi Project at Harvard Medical School.*

Project Number: 2R01GM067858
Sponsor/Funding Source: NIH / NIGMS
Contact PI: Bellen, Hugo J.
Effort (person months): .38
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: 5R24OD021997
Sponsor/Funding Source: NIH / OD
Contact PI: Perrimon, Norbert
Effort (person months): .12
Award Period (dates): 06/01/2016 – 04/30/2021
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: 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: R24OD026435
Sponsor/Funding Source: NIH / OD
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:

OTHER SUPPORT – Norbert Perrimon

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.*

(NEW)
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*

(NEW)
Project Number: BB/T014008/1
Sponsor/Funding Source: BBSRC / NSF
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: Integrative analysis and Visualization 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).*

Inactive

None.

OVERLAP – *None.*

IN-KIND RESOURCES

- Trainee: Post Doctoral Trainee, Ying Liu, sponsored by Sigrid Juselius Foundation
- Use of Mass Spectrometry Facility (courtesy of John Asara, Beth Israel Deaconness Medical Center): 5 hours per month
- Use of Mass Spectrometry Facility (courtesy of Steve Carr, Broad Institute): 4 hours per month
- Discounted scientific supplies: Harvard Medical School Biopolymer Facility

OTHER SUPPORT – Norbert Perrimon

- Discounted rate data analysis, Harvard T.H. Chan School Bioinformatics Core: 15-20 hours per month
- Discounted rate DNA Sequencing, Massachusetts General Hospital Center for Computational and Integrative Biology DNA Core (MGH CCIB): Average of 5 orders per month

KEY PERSONNEL PREVIOUS/CURRENT/PENDING SUPPORT

MANNING, BRENDAN

PREVIOUS SUPPORT (last five years)

NIH/NCI R01 CA122617 (PI: Manning) 07/12/2010 – 04/30/2015
Regulatory mechanisms and role of the PI3K-TSC-mTOR signaling network in tumors
DC/YR, 3 calendar months
National Cancer Institute
BG9606 MSC 9760, 9609 Medical Center Drive, Bethesda, MD 20892-9760
Grants Management Specialist: Sy Shackleford
Research was focused on the mechanisms of mTORC2 regulation in normal cells and misregulation in tumors.
Aim 1: Determine the mechanisms and consequences of our finding that the TSC tumor suppressors, while inhibiting mTORC1, activate mTORC2
Aim 2: Determine the molecular mechanisms leading to mTORC2 activation downstream of receptor tyrosine kinases
Aim 3: Determine the role of mTORC2 activation and downstream signaling in glioblastoma
No overlap

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.

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

Department of Defense: TS170026 (PI: Manning) 09/01/2018 – 08/31/2021
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.
Aim 2: Preclinical studies to enhance apoptotic priming in TSC cell and tumor models with BH3 mimetics developed for clinical use.
Aim 3: Determine the control mechanisms downstream of the TSC complex and mTORC1 altering apoptotic priming in TSC.
No overlap

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

None.