Supplemental Data

Functional Genomics Identifies TOR-Regulated Genes that Control Growth and Division

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Supplemental Experimental Procedures

Drosophila RNAi Methods

In this system RNAi can be initiated by introducing long dsRNAs (generally 500–800 bp) corresponding to target mRNAs into cells through a brief starvation (thought to stimulate endocytosis of the dsRNAs) or through use of a conventional transfection reagent [S1, S2]. Long dsRNAs are then processed into multiple small interfering RNAs that attack the corresponding endogenous mRNA transcript, rendering silencing exceptionally efficient. dsRNAs corresponding to each gene analyzed were synthesized by in vitro transcription (IVT) with a T7 MEGAscript kit (Ambion). Templates for IVT were generated by RT-PCR from total *Drosophila* cellular RNA with primers that incorporate a 5′ and 3′ T7 promoter. In general, we synthesized dsRNAs 500–800 bp in length corresponding to the target cDNA sequence. dsRNA products were purified on a vacuum manifold with Millipore filter plates.

To transfect dsRNA into *Drosophila* cells, one of two methods was employed. For functional analysis of rapamycin-sensitive genes and knockdown expression profiling, we used a starvation-induced uptake method. Briefly, cells are washed and resuspended in *Drosophila* SFM (Invitrogen) at 1×10^6 cells/mL. 1 ml of cells are seeded into each well of a 6 cm dish, and 15 μg of dsRNA corresponding to the target gene is directly added to the media. For 12-well dishes, the cell number and media volume is decreased accordingly. The cells and dsRNA are incubated in SFM at 25ºC for 1 hr. 2 ml of Schneiders medium (Invitrogen) supplemented with 10% serum is then added back to the cells and they are allowed to incubate for 4 days to allow turnover of the target mRNAs. For combination knockdown experiments, we use FuGENE (Roche), a conventional transfection reagent, to reduce the amount of dsRNA used. For this method, 1×10^6 cells were seeded in 2 ml of Schneider's medium in 6-well dishes (or into 12-well dishes by reducing cell number and volume by 50%). FuGENE:dsRNA complexes are prepared by adding 3 μl of FuGENE to 97 μl of SFM, followed by 2 μg total of dsRNA. The mix is incubated at RT for 15 min, then added to cells drop-wise. For combination dsRNA experiments, 1 μg of each dsRNA was added together in the mix. For solo knockdowns, 1 μg dsRNA corresponding to the target gene was mixed with 1 μg of GFP dsRNA. For the GFP alone control, 2 μg of GFP dsRNA was transfected. Cells were incubated for 3 days in the S6K v. eIF4E knockdown experiment to allow a more accurate comparison to rapamycin-treated cells. Otherwise, cells were incubated for 4 days.

shRNA Sequences

SAW_shRNA_1 sense: 15a, 5′CCGGGCACATGAAGATGAGACAATACTTCCTgTCATATTGTCTCATCTTCATGTGCTTTTTG; SAW_shRNA_1 antisense: 15a, 5′AATTCAAAAAGCACATGAAGATGAGACAATATGAcAGGAAGTATTGTCTCATCTTCATGTGC; SAW_shRNA_2 sense: 5′CCGGCCGTGACATGTTTATGTCTAACTTCCTgTCATTAGACATAAACATGTCACGGTTTTTG; SAW_shRNA_2 antisense: 5′AATTCAAAAACCGTGACATGTTTATGTCTAATGAcAGGAAGTTAGACATAAACATGTCACGG; ASH2L_shRNA_1 sense: 5′CCGGGGATGAACATCCGAAGACAATCTTCCTgTCAATTGTCTTCGGATGTTCATCCTTTTTG; ASH2L_shRNA_1 antisense: 5′AATTCAAAAAGGATGAACATCCGAAGACAATTGAcAGGAAGATTGTCTTCGGATGTTCATCC; ASH2L_shRNA_2 sense: 5′CCGGCCTATTTCTGGAGACCTTTACCTTCCTgTCAGTAAAGGTCTCCAGAAATAGGTTTTTG; ASH2L_shRNA_2 antisense: 5′AATTCAAAAACCTATTTCTGGAGACCTTTACTGAcAGGAAGGTAAAGGTCTCCAGAAATAGG.

Cell Lysis

Drosophila S2 cells were resuspended by pipetting up and down gently 4–5 times, then transferred to sterile eppendorf tubes. Cells were pelleted at RT, then rinsed once with cold PBS. Mammalian cells, which adhere more strongly to the cell culture dish, were rinsed directly in the dish with cold PBS. Cells were lysed on ice for 10 min with cold lysis buffer (40 mM HEPES [pH 7.5], 120 mM NaCl, 1 mM EDTA, 10 mM pyrophosphate, 10 mM glycerophosphate, 50 mM NaF, 0.5 mM orthovanadate, and EDTA-free protease inhibitors [Roche]) containing 1% Triton X-100. Lysates were cleared by spinning at 13,000 × g for 10 min, and protein samples were resolved by SDS-PAGE in 8% gels. Proteins were transferred to PVDF membranes and processed for immunoblotting with the indicated antibodies.

Supplemental References

S1. Clemens, J.C., Worby, C.A., Simonson-Leff, N., Muda, M., Maehama, T., Hemmings, B.A., and Dixon, J.E. (2000). Use of double-stranded RNA interference in *Drosophila* cell lines to dissect signal transduction pathways. Proc. Natl. Acad. Sci. USA *97*, 6499–6503.

S2. Sarbassov, D.D., Guertin, D.A., Ali, S.M., and Sabatini, D.M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science *307*, 1098–1101.

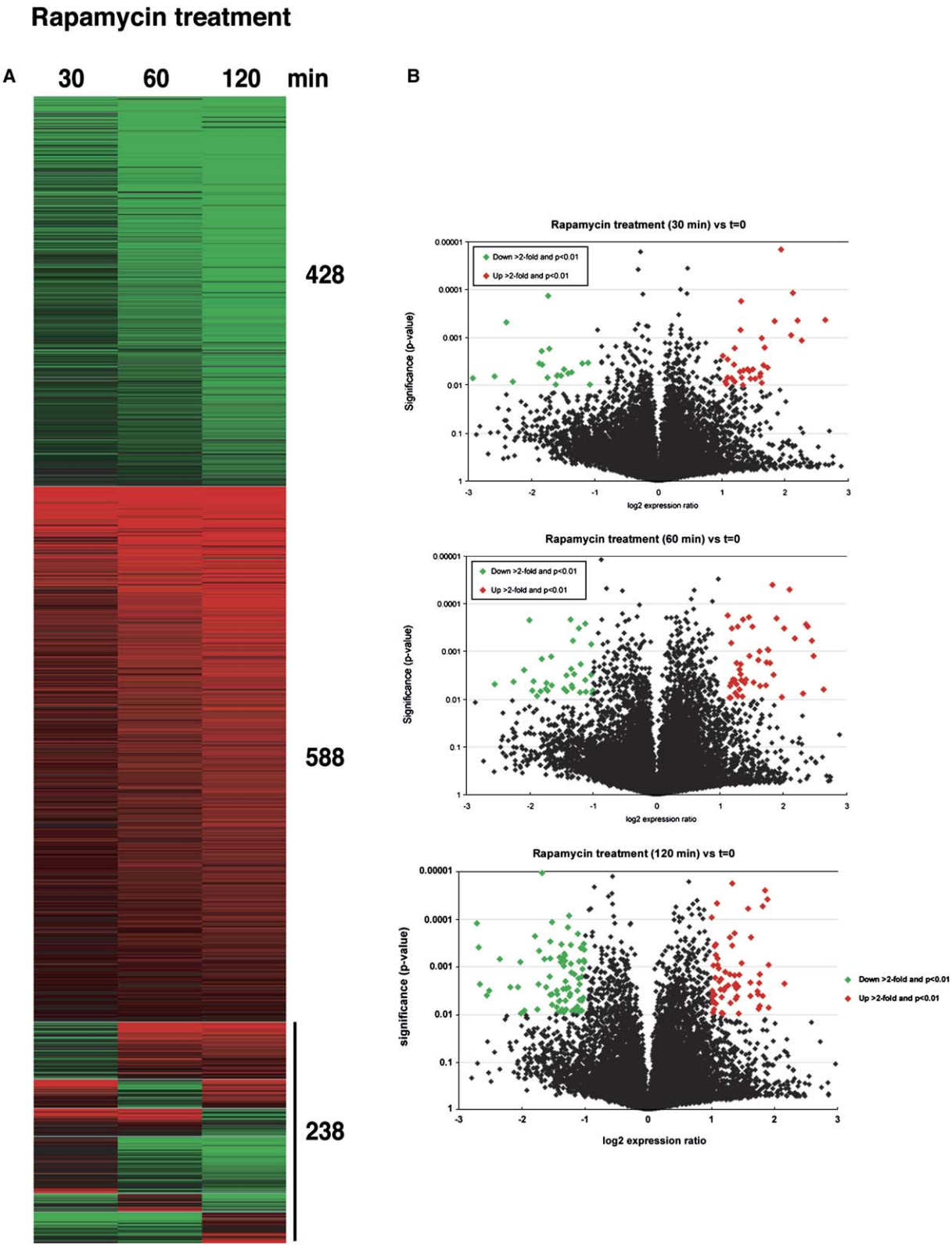

Figure S1. Genes Differentially Expressed after Acute Rapamycin Treatment

(A) A conventional heat map indicating genes decreasing (green) and increasing (red) in expression after 30, 60, or 120 min of 20 nM rapamycin treatment. Expression ratios (log2) were determined for genes exhibiting rapamycin- or ethanol-induced differential expression ($p < 0.01$) compared to T_0 control chips, and genes significantly changing expression for at least one time point were ordered into groups of similar profiles. A very small number of genes significantly responded to ethanol treatment, and these were excluded from further analysis.

(B) Volcano plots indicating genes significantly ($p < 0.01$) decreasing and increasing expression greater than 2.0-fold after 30 (top), 60 (middle), or 120 (bottom) min of rapamycin treatment. Expression values were log transformed and plotted against significance (p value). Genes decreasing

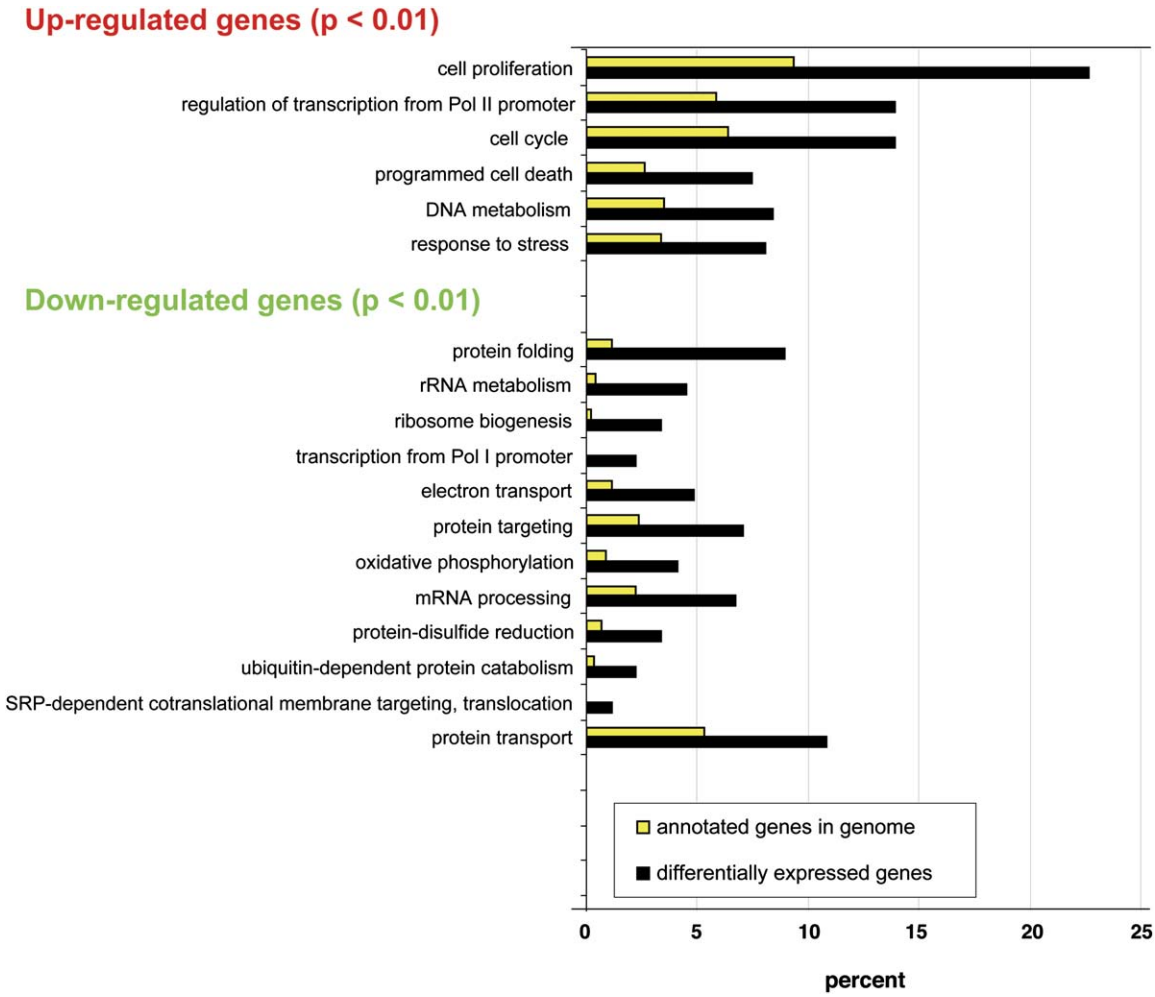

Figure S2. GO Assignments for Genes Differentially Expressed after 120 min of Rapamycin Treatment

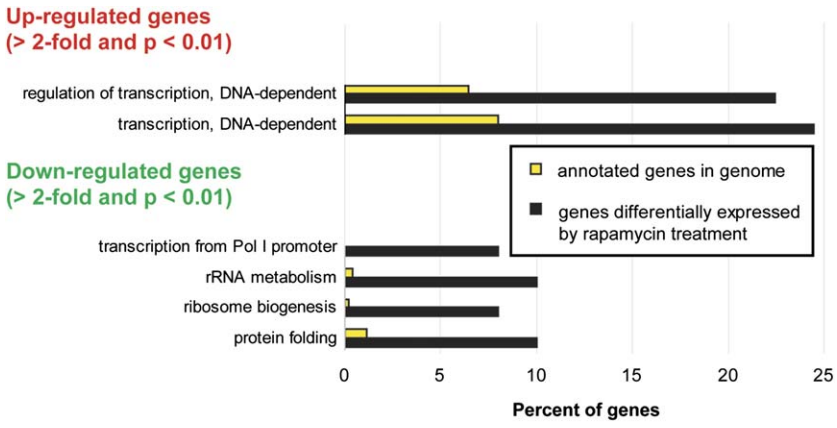

Figure S3. GO Analysis of dTOR-Regulated Genes Chosen for Cell Size Analysis

84 genes met the criteria of significantly ($p < 0.01$) increasing or decreasing expression 2-fold or more in response to rapamycin treatment. GO tags were assigned by means of Flybase annotations.

significantly in expression ($p < 0.01$) 2-fold or more are colored green. Genes significantly increasing expression ($p < 0.01$) 2-fold or more are colored red. These genes define a subset of rapamycin-sensitive genes that were functionally analyzed in the follow-up studies.

Table S1. Cell Size, Number, and Expression Values of Genes Listed in Figure 3

Gene Name	Mean Cell Diameter (μm)	Cell Size versus Control: p Value	Cell Number as % of Control	RAPA 30 min (fold Change in Gene Expression)	p Value	RAPA 60 min (Fold Change in Gene Expression)	p Value	RAPA 120 min (Fold Change in Gene Expression)	p Value
Raptor	8.90	<1e−10	110	na	na	na	na	na	na
Surf6	8.92	<1e−10	71	1.0	0.982	0.9	0.429	0.4	0.004
CG8939	8.98	<1e−10	69	0.9	0.020	0.7	0.024	0.4	0.001
Rheb	9.09	<1e−10	106	na	na	na	na	na	na
TOR	9.12	<1e−10	87	na	na	na	na	na	na
CG6724	9.15	<1e−10	79	0.9	0.378	0.6	0.030	0.5	0.005
CG7845	9.17	<1e−10	66	0.9	0.555	0.5	0.068	0.4	0.005
Nopp140	9.30	<1e−10	90	1.0	0.676	0.8	0.018	0.5	0.002
CG12499	9.35	<1e−10	64	0.8	0.000	0.8	0.192	0.4	0.009
CG5114	9.45	<1e−10	114	0.8	0.097	0.6	0.004	0.3	0.000
Sas10	9.45	<1e−10	84	0.9	0.536	0.6	0.147	0.4	0.010
Nop60B	9.46	<1e−10	77	0.5	0.120	0.3	0.001	0.4	0.003
CG10805	9.50	<1e−10	72	1.0	0.735	0.7	0.005	0.3	0.001
NHP2	9.55	<1e−10	119	0.9	0.527	0.6	0.010	0.4	0.001
bys	9.56	<1e−10	71	0.8	0.042	0.6	0.000	0.4	0.000
CG13096	9.57	<1e−10	69	0.9	0.080	0.7	0.008	0.4	0.003
CG1381	9.62	<1e−10	93	0.9	0.018	0.6	0.001	0.4	0.004
CG12301	9.65	<1e−10	74	1.0	0.218	0.6	0.087	0.5	0.017
CG18600	9.70	<1e−10	101	1.0	0.820	0.7	0.003	0.5	0.004
CG9710	9.72	<1e−10	163	0.9	0.205	0.8	0.144	0.5	0.002
CG5033	9.72	<1e−10	96	1.0	0.166	0.7	0.011	0.4	0.000
G32343	9.79	<1e−10	138	0.8	0.098	0.7	0.023	0.4	0.001
S6K	9.89	<1e−10	128	na	na	na	na	na	na
stai	9.94	<1e−10	97	1.1	0.395	1.5	0.025	2.6	0.000
CG4554	9.95	<1e−10	75	1.0	0.757	0.8	0.129	0.4	0.008
Akt	9.97	<1e−10	126	na	na	na	na	na	na
CG1994	9.97	<1e−10	104	1.0	0.555	0.7	0.063	0.2	0.000
CG11076	10.01	<1e−10	148	0.8	0.021	0.6	0.003	0.4	0.001
mu2	10.06	<1e−10	110	1.5	0.030	1.9	0.003	2.2	0.002
Rrp45	10.12	5.58e−05	68	0.9	0.056	0.7	0.005	0.5	0.000
CG4043	10.13	5.28e−04	76	0.8	0.345	0.7	0.015	0.5	0.003
unk	10.19	0.458	100	1.8	0.007	2.4	0.002	3.2	0.088
CONTROL	10.20	na	100	na	na	na	na	na	na
Ski6	10.22	0.385	115	0.7	0.129	0.5	0.002	0.3	0.025
CG6459	10.24	0.091	89	0.7	0.009	0.6	0.003	0.4	0.001
EP2237	10.47	<1e−10	125	2.6	0.000	2.3	0.223	2.3	0.127
PTEN	10.62	<1e−10	92	na	na	na	na	na	na
TSC2	10.67	<1e−10	108	na	na	na	na	na	na
ash2	10.70	<1e−10	77	1.3	0.013	1.8	0.157	2.2	0.001
CG7993	8.14	<1e−10	55	0.9	0.659	0.7	0.056	0.4	0.001
CG6712	8.75	<1e−10	51	0.8	0.223	0.5	0.005	0.4	0.009
Nnp-1	8.97	<1e−10	59	0.9	0.183	0.8	0.117	0.5	0.001
PPAN	9.06	<1e−10	55	0.8	0.376	0.5	0.010	0.3	0.004
CG1542	9.25	<1e−10	37	0.9	0.693	0.8	0.208	0.5	0.007
CG3756	9.29	<1e−10	42	0.8	0.038	0.6	0.123	0.4	0.001
CG10648	9.30	<1e−10	34	1.0	0.958	0.6	0.023	0.3	0.002
CG3983	9.30	<1e−10	39	0.9	0.322	0.7	0.009	0.5	0.001
CG7006	9.33	<1e−10	62	0.9	0.051	0.7	0.003	0.5	0.000
CG12785	9.34	<1e−10	62	0.7	0.082	0.4	0.005	0.1	0.001
CG3071	9.34	<1e−10	30	0.9	0.195	0.7	0.039	0.4	0.003
CG7516	9.41	<1e−10	54	0.9	0.567	0.7	0.035	0.5	0.010
pit	9.45	<1e−10	28	1.0	0.588	0.7	0.017	0.4	0.014
CG11920	9.54	<1e−10	54	0.9	0.463	0.6	0.019	0.4	0.008
hoip	9.62	<1e−10	10	0.7	0.007	0.4	0.002	0.3	0.010
Rpl135	9.76	<1e−10	55	1.0	0.658	0.8	0.121	0.4	0.005
CG5824	9.80	<1e−10	58	1.0	0.872	0.5	0.026	0.5	0.008
Hsp83	10.20	1	36	0.9	0.034	0.7	0.008	0.3	0.000
CG3838	10.24	0.071	63	1.4	0.200	2.1	0.012	2.4	0.002
Hsp60	10.37	<1e−10	58	0.9	0.036	0.8	0.011	0.4	0.009
bor	10.42	<1e−10	47	0.8	0.018	0.5	0.003	0.3	0.000
CG11267	10.65	<1e−10	16	0.8	0.000	0.6	0.000	0.4	0.012
Hsc70Cb	10.91	<1e−10	52	0.9	0.470	0.8	0.004	0.4	0.000

Table S2. dTOR-Regulated Growth and Division Genes Identified by Combining Gene Expression Profiling and RNAi-Mediated Loss-of-Function Analysis in *Drosophila* Cultured S2 Cells

CG #	Name	GO Biological Process	GO Molecular Function	GO Cellular Component	Human Ortholog
CG4510	*surf6*	ribosome biogenesis	heme transporter activity	nucleolus	*SURF6*
CG8939	na	rRNA metabolism; rRNA processing	rRNA methyltransferase activity	nucleolus	*FTSJ3*
CG6724	na	na	na	na	*WDR12*
CG7845	na	na	na	na	*NSA1*
CG7421	*nopp140*	nucleologenesis	na	nucleolus	*NOLC1*
CG12499	na	na	na	na	na
CG5114	na	na	na	na	*AAMP*
CG4202	na	na	na	na	*SAS10*
CG3333	*nop60B*	pseudouridine synthesis; rRNA metabolism; rRNA processing; ribosome biogenesis; chromosome segregation; germ cell development; mitosis	RNA binding; pseudouridylate synthase activity; centromeric DNA binding; centromeric DNA binding	nucleolus; nucleus	*DKC1*
CG10805	na	na	na	na	*BAP28*
CG5258	*NHP2*	protein biosynthesis	structural constituent of ribosome	nucleolus; ribosome	*NOLA2*
CG1430	*bys*	cell adhesion	na	na	*BYSL*
CG13096	na	na	na	na	*RSL1D1*
CG1381	na	protein biosynthesis; protein metabolism	nucleic acid binding; structural constituent of ribosome	ribosome	*C1orf33*
CG12301	na	na	na		*UTP14A*
CG18600	na	na	na	na	na
CG9710	*nudC*	nuclear migration; oogenesis (sensu Insecta)	na	cytoplasm	na
CG5033		ribosome biogenesis	ribonucleoprotein binding	nucleolus	na
CG32343	na	regulation of transcription, DNA-dependent	transcription factor activity	nucleus	*NP_653219.1*
CG5981	*stai*	germ cell migration; hormone secretion; intracellular signaling cascade; microtubule-based process; neurogenesis; signal transduction	microtubule binding	microtubule associated complex	*STMN1*
CG4554	na	na	na	na	*DRIM*
CG1994	na	na	N-acetyltransferase activity	nucleus	*FLJ10774*
CG11076	na	na	na	na	na
CG1960	*mu2*	na	na	na	na
CG9606	*Rrp45*	mRNA processing	RNA binding; nucleic acid binding; 3′-5′-exoribonuclease activity	cytoplasmic exosome (RNase complex); exosome (RNase complex); nuclear exosome (RNase complex)	*EXOSC9*
CG4043	*Rrp46*	mRNA processing	RNA binding; nucleic acid binding; nucleotidyltransferase activity; 3′-5′-exoribonuclease activity	cytoplasmic exosome (RNase complex); nuclear exosome (RNase complex)	*EXOSC5*
CG4620	*unk*	bristle morphogenesis; eye morphogenesis (sensu Endopterygota); larval development; protein ubiquitination; wing morphogenesis	DNA binding; zinc ion binding; ubiquitin-protein ligase activity	cytoplasm; ubiquitin ligase complex	*ZCC5*
CG15481	*ski6*	RNA processing; nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RNA binding; nucleic acid binding; nucleotidyltransferase activity; 3′-5′-exoribonuclease activity	cytoplasmic exosome (RNase complex); nuclear exosome (RNase complex); polytene chromosome	*EXOSC4*
CG6459	na	defense response to bacteria	na	mitochondrial matrix; mitochondrion	*C1QBP*

Table S2. *Continued*

CG #	Name	GO Biological Process	GO Molecular Function	GO Cellular Component	Human Ortholog
CG4427	*cbt*	JNK cascade; autophagic cell death; dorsal closure; establishment of planar polarity; eye morphogenesis; germ-band shortening; positive regulation of transcription; regulation of transcription from Pol II promoter; salivary gland cell death; sensory organ development	transcriptional activator activity	nucleus	*KLF11*
CG6677	*ash2*	chromatin-mediated maintenance of transcription; transcription from Pol II promoter; wing morphogenesis	transcription regulator activity	nucleus	*ASH2L*
CG7993	na	na	na	na	*BXDC1*
CG6712	na	nucleobase, nucleoside, nucleotide and nucleic acid metabolism; rRNA metabolism	RNA binding; nucleic acid binding		*BXDC5*
CG12396	*Nnp-1*	rRNA metabolism; rRNA processing	na	nucleus	*NNP1*
CG5786	*ppan*	imaginal disc development; larval development; mitosis; oogenesis (sensu Insecta)	na	na	*PPAN*
CG1542	na	processing of 27S pre-rRNA	na	nucleolus	*EBNA1BP2*
CG3756	na	mRNA transcription; transcription from Pol I promoter; transcription from Pol II promoter; transcription from Pol III promoter	DNA binding; nucleic acid binding; DNA-directed RNA polymerase activity; protein dimerization activity	DNA-directed RNA polymerase I complex; DNA-directed RNA polymerase III complex	*POLR1C*
CG10648	na	cell cycle; protein complex assembly; protein metabolism	na	na	*RBM13*
CG3983	na	cell surface receptor linked signal transduction; intracellular protein transport; intracellular signaling cascade; nucleobase, nucleoside, nucleotide and nucleic acid metabolism; nucleobase, nucleoside, nucleotide and nucleic acid transport; protein metabolism; regulation of translation; signal transduction; transport	GTP binding; receptor binding; hormone activity	na	na
CG7006	na	na	na	na	*NP_057185*
CG12785	na	na	na	na	*NOL6*
CG3071	na	retrograde transport, Golgi to ER	na	COPI vesicle coat	*UTP15/SAW*
CG7516	na	na	na	na	*NOL10*
CG6375	*pit*	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	nucleic acid binding; ATP binding; RNA helicase activity; ATP-dependent RNA helicase activity	nucleolus; nucleus	*DDX18*
CG11920	na	rRNA metabolism	RNA binding	small nuclear ribonucleoprotein complex	*IMP4*
CG3949	*hoip*	neurogenesis; nuclear mRNA splicing, via spliceosome; peripheral nervous system development; protein biosynthesis	RNA binding; structural constituent of ribosome	nucleus; ribosome; small nuclear ribonucleoprotein complex; spliceosome complex	*NHP2L1*
CG4033	*Rpl135*	transcription from Pol I promoter	DNA binding; nucleic acid binding; DNA-directed RNA polymerase activity	DNA-directed RNA polymerase I complex; nucleus	*POLR1B*
CG5824	na	na	na	na	*C4orf9*

Table S2. *Continued*

CG #	Name	GO Biological Process	GO Molecular Function	GO Cellular Component	Human Ortholog
CG1242	*Hsp83*	R7 cell fate commitment; actin filament organization; centrosome cycle; defense response; determination of anterior/posterior axis, embryo; protein complex assembly; protein folding; regulation of cell shape; regulation of circadian sleep/wake cycle, sleep; response to heat; spermatogenesis; torso signaling pathway	ATP binding; ATPase activity, coupled; unfolded protein binding	centrosome; cytoplasm	*HSPCA*
CG3838	na	na	DNA binding	na	na
CG12101	*Hsp60*	de novo protein folding; protein folding; protein refolding; protein-mitochondrial targeting; response to heat; response to stress	ATP binding; ATPase activity, coupled; unfolded protein binding		*HSPD1*
CG6815	*bor*	na	ATP binding; nucleotide binding; nucleoside-triphosphatase activity;	cytoplasm	*ATAD3A*
CG11267	na	de novo protein folding	ATP binding; ATPase activity, coupled; unfolded protein binding	mitochondrial matrix; mitochondrion	*CH10*
CG6603	*Hsc70Cb*	defense response; protein folding; response to stress	ATP binding; chaperone binding	na	*HSPA4L*

Genes in table are arranged as ordered in Figure 2.
GO assignments obtained from Flybase annotations (http://flybase.bio.indiana.edu/).
na = none available

Table S3. *Drosophila* Genes Identified that Have a Predicted Yeast Ortholog

CG #	Fly Name	Yeast Name[a]	Predicted Function in Yeast[a]
CG4510	*Surf6*	*RRP14*	Essential protein, constituent of 66S preribosomal particles; interacts with proteins involved in ribosomal biogenesis and cell polarity; member of the SURF-6 family
CG8939	-	*SPB1*	AdoMet-dependent methyltransferase involved in rRNA processing and 60S ribosomal subunit maturation; methylates G2922 in the putative tRNA docking site of the large subunit rRNA and in the absence of snR52, U2921; suppressor of PAB1 mutants
CG6724	-	*YTM1*	Constituent of 66S preribosomal particles, required for maturation of the large ribosomal subunit
CG7845	-	*NSA1*	Constituent of 66S preribosomal particles, involved in 60S ribosomal subunit biogenesis
CG7421	*Nopp140*	*SRP40*	Nucleolar protein, suppressor of rpc40 and rpb10 mutations; SPBC660.06 (*pombe*) not *cerevisiae* otholog—human ortholog, but functions unknown
CG5114	-	*SQT1*	Essential protein involved in a late step of 60S ribosomal subunit assembly or modification; contains multiple WD repeats; interacts with Qsr1p in a two-hybrid assay
CG4202	-	*SAS10*	Part of small (ribosomal) subunit (SSU) processosome (contains U3 snoRNA); Something About Silencing 10; nuclear protein involved in silencing
CG3333	*Nop60B*	*CBF5*	Component of box H/ACA small nucleolar ribonucleoprotein particles (snoRNPs), probable rRNA pseudouridine synthase, binds to snoRNP Nop10p and also interacts with ribosomal biogenesis protein Nop53p
CG10805	-	*UTP10*	Component of the 80S U3 snoRNA complex (SSU processome), which is required for 18S rRNA biogenesis
CG5258	*NHP2*	*NHP2*	Nuclear protein related to mammalian high mobility group (HMG) proteins, essential for function of H/ACA-type snoRNPs, which are involved in 18S rRNA processing
CG1430	*bys*	*ENP1*	Essential nuclear protein required for 35S pre-rRNA processing into 18S rRNA
CG1381	-	*MRT4*	Protein involved in mRNA turnover
CG12301	-	*UTP14*	Nucleolar protein, component of the small subunit (SSU) processome containing the U3 snoRNA that is involved in processing of pre-18S rRNA
CG4554	-	*UTP20*	Possible snoRNA-binding protein, based on computational analysis of large-scale protein-protein interaction data
CG1994	-	*KRE33*	Protein with high similarity to n-acetyltransferase-like protein (human FLJ10774), which is a histone acetyltransferase and transcriptional activator that regulates transcription of human TERT, contains a DUF699 putative ATPase domain
CG9606	*Rrp45*	*RRP45*	Protein involved in rRNA processing; component of the exosome 3->5 exonuclease complex
CG4043	*Rrp46*	*RRP46*	Protein component of the exosome 3′-5′ exoribonuclease complex involved in 3′end processing of multiple small RNA species
CG15481	*Ski6*	*SKI6*	3′-to-5′ phosphorolytic exoribonuclease that is a subunit of the exosome; required for 3′ processing of the 5.8S rRNA; involved in 3′ to 5′ mRNA degradation and translation inhibition of non-poly(A) mRNAs
CG4427	*cbt*	*FZF1*	Transcription factor involved in sulfite metabolism, sole identified regulatory target is SSU1, overexpression suppresses sulfite-sensitivity of many unrelated mutants due to hyperactivation of SSU1, contains five zinc fingers
CG6677	*ash2*	*BRE2*	Subunit of the COMPASS (Set1C) complex, which methylates Rad6p ubiquitinated histone H3 on lysine 4 and is required in transcriptional silencing near telomeres; has similarity to the trithorax-group protein ASH2L
CG7993	-	*RPF2*	Essential protein involved in the processing of pre-rRNA and the assembly of the 60S ribosomal subunit; interacts with ribosomal protein L11; localizes predominantly to the nucleolus; constituent of 66S preribosomal particles
CG6712	-	*RPF1*	Nucleolar protein involved in the assembly of the large ribosomal subunit; constituent of 66S preribosomal particles; contains a sigma(70)-like motif, which is thought to bind RNA
CG12396	*Nnp-1*	*RRP1*	Essential evolutionarily conserved nucleolar protein necessary for biogenesis of 60S ribosomal subunits and processing of pre-rRNAs to mature rRNAs, associated with several distinct 66S preribosomal particles
CG5786	*ppan*	*SSF1*	Protein involved in 27S rRNA processing required for the maturation of 25S and 5.8S rRNA products, contains a BRIX domain and is a member of the Imp4p superfamily containing a sigma70-like motif
CG1542	-	*EBP2*	Essential protein required for the maturation of 25S rRNA and 60S ribosomal subunit assembly, localizes to the nucleolus; constituent of 66S preribosomal particles
CG3756	-	*RPC40*	RNA polymerase subunit, common to RNA polymerase I and III; also known as AC40
CG10648	-	*MAK16*	Essential nuclear protein, constituent of 66S preribosomal particles; required for normal concentration of free 60S ribosomal subunits; required for maintenance of M1 satellite double-stranded RNA of the L-A virus
CG3983	-	*NUG1*	Nuclear protein required for 60S ribosomal subunit export from the nucleus, has similarity to mouse Gna-rs1 protein, which is a putative GTP binding protein
CG7006	-	*NIP7*	Nucleolar protein required for 60S ribosome subunit biogenesis, constituent of 66S preribosomal particles; physically interacts with Nop8p and the exosome subunit Rrp43p
CG12785	-	*UTP22*	Possible U3 snoRNP protein involved in maturation of pre-18S rRNA, based on computational analysis of large-scale protein-protein interaction data

Table S3. *Continued*

CG #	Fly Name	Yeast Name[a]	Predicted Function in Yeast[a]
CG3071	-	*UTP15*	Component of the 80S U3 snoRNA complex (SSU processome), which is required for 18S biogenesis, has WD (WD-40) repeats
CG7516	-	*ENP2*	Essential nucleolar protein of unknown function; contains WD repeats, interacts with Mpp10p and Bfr2p, and has homology to Spb1p
CG6375	*pit*	*HAS1*	ATP-dependent RNA helicase; localizes to both the nuclear periphery and nucleolus; highly enriched in nuclear pore complex fractions; constituent of 66S preribosomal particles
CG11920	-	*IMP4*	Component of the 80S U3 snoRNA complex (SSU processome), which is required for pre-18S rRNA processing, member of superfamily of proteins with a sigma70-like motif
CG3949	*hoip*	*SNU13*	RNA binding protein, part of U3 snoRNP involved in rRNA processing, part of U4/U6-U5 tri-snRNP involved in mRNA splicing, similar to human 15.5K protein
CG4033	*Rpl135*	*RPA135*	RNA polymerase I subunit A135; also known as A135
CG5824	-	*NOP14*	Nucleolar protein, forms a complex with Noc4p that mediates maturation and nuclear export of 40S ribosomal subunits; also present in the small subunit processome complex, which is required for processing of pre-18S rRNA
CG1242	*Hsp83*	*HSC82*	Cytoplasmic chaperone of the Hsp90 family, redundant in function and nearly identical with Hsp82p, and together they are essential; expressed constitutively at 10-fold higher basal levels that HSP82 and induced 2- to 3-fold by heat shock
CG11267	-	*HSP10*	Mitochondrial matrix co-chaperonin that inhibits the ATPase activity of Hsp60p, a mitochondrial chaperonin; involved in protein folding and sorting in the mitochondria; 10 kD heat shock protein with similarity to *E. coli* groES
CG6603	*Hsc70Cb*	*SSE1*	ATPase that is a component of the heat shock protein Hsp90 chaperone complex; binds unfolded proteins; member of the heat shock protein 70 (HSP70) family; localized to the cytoplasm

[a] Yeast orthologs and their known or predicted molecular function were identified with InParanoid v.4.0 (http://inparanoid.cgb.ki.se/index.html) and Proteome (http://www.proteome.com/).

Table S4. Sensitivity of ASH2L Knockdown Cells to Rapamycin Treatment

		−RAPA	+RAPA
No hairpin	cell size	16.21	15.60
	cell number	36,232	37,604
shGFP	cell size	16.36	15.56
	cell number	54,715	45,222
shASH2L_1	cell size	18.03	16.74
	cell number	23,523	21,179
shASH2L_2	cell size	18.64	17.45
	cell number	11,667	14,957

HEK293T cells knocked down for ASH2L were treated with 100 nm rapamycin for 24 hr. Cell size and number of cells counted were measured with a Coulter counter. Numbers are averages of two independent readings. Note that ASH2L knockdown cells show a reduced mean cell size when treated with rapamycin but are still larger than control cells without rapamcyin treatment.