

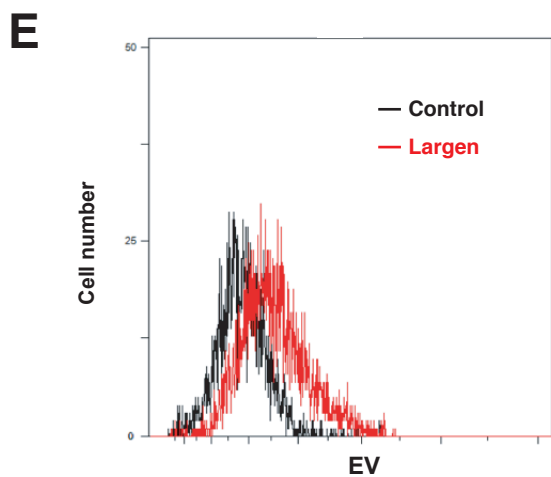
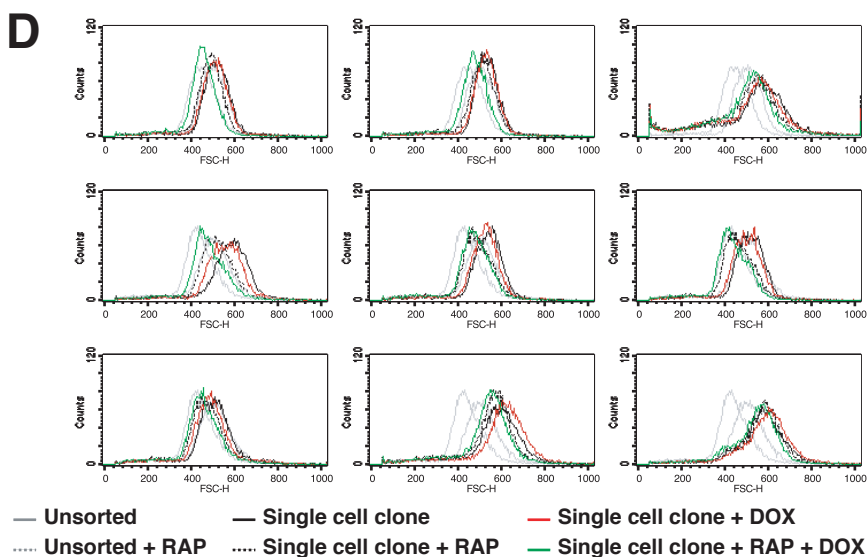
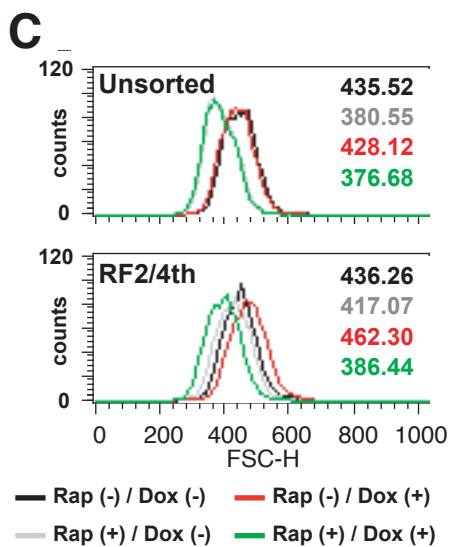
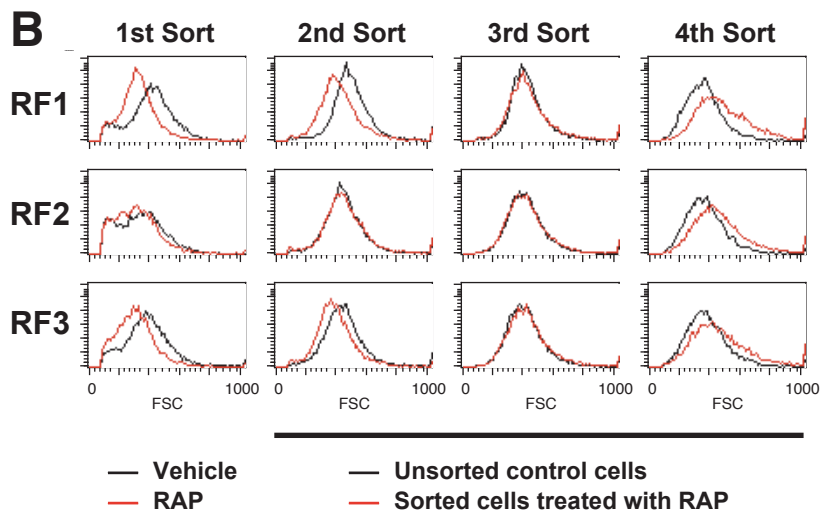
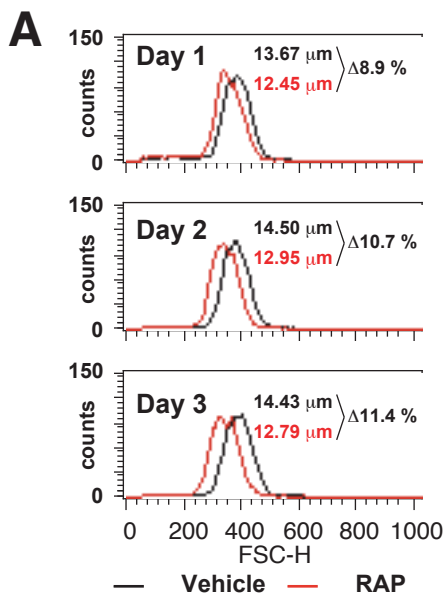
**Molecular Cell, Volume 53**

**Supplemental Information**

**Largen: A Molecular Regulator**

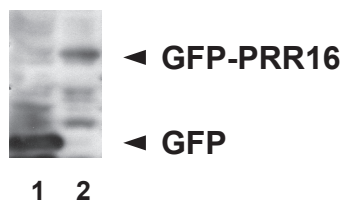
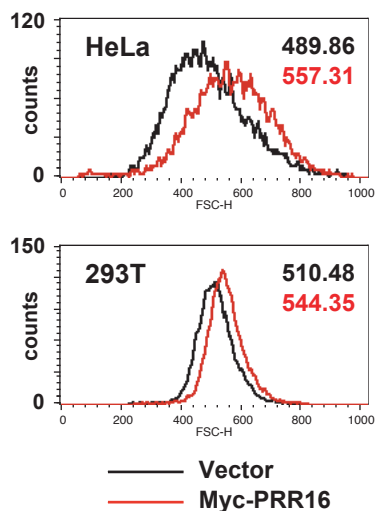
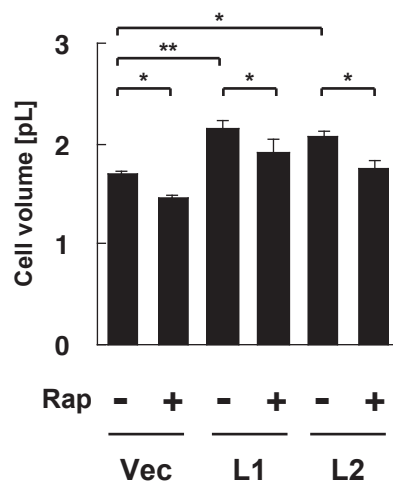
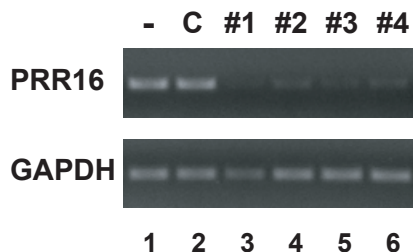
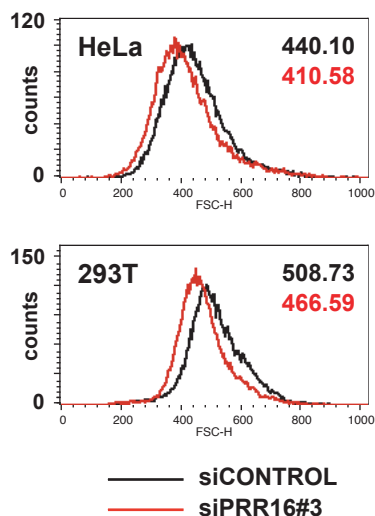
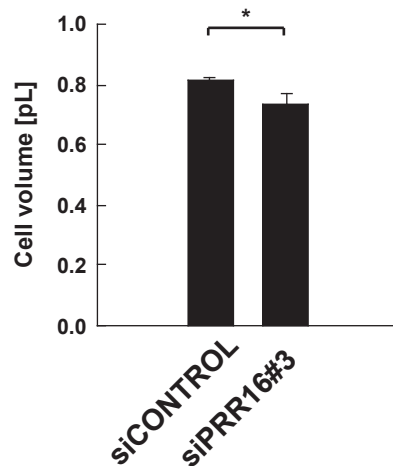
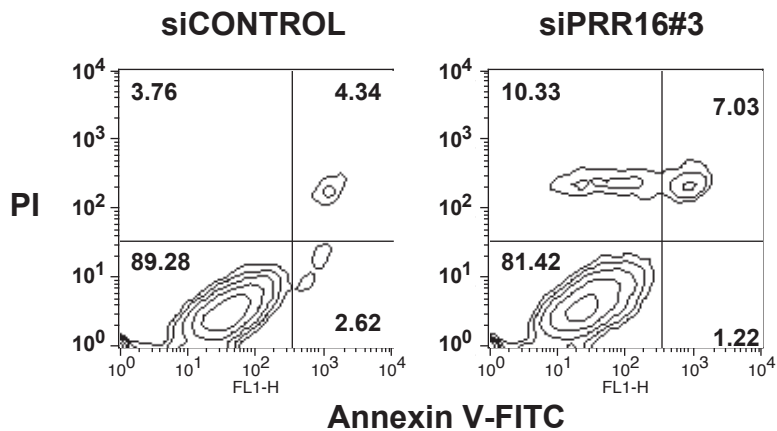
**of Mammalian Cell Size Control**

**Kazuo Yamamoto, Valentina Gandin, Masato Sasaki, Susan McCracken, Wanda Li, Jennifer Liepa Silvester, Andrew J. Elia, Feng Wang, Yosuke Wakutani, Roumiana Alexandrova, Yathor D Oo, Peter Mullen, Satoshi Inoue, Momoe Itsumi, Valentina Lapin, Jillian Haight, Andrew Wakeham, Arda Shahinian, Mitsuhiko Ikura, Ivan Topisirovic, Nahum Sonenberg, and Tak W. Mak**



### Figure S1, related to Figure 1

(A) Rapamycin (RAP) induces cell size reduction. Jurkat cells were cultured in the presence of 20 nM RAP for 1, 2 or 3 days and cell size distribution was determined by flow cytometry. Inset numbers are the mean cell diameter for each group.  $\Delta$ , calculated % difference between RAP-treated and untreated cells. (B) Repeated sorting increases the “stay-large” population in bulk cell clones. Solid black lines represent the size distribution of unsorted control cells. Solid red lines in the 1<sup>st</sup> Sort columns represent the size distribution of RAP-treated cells. The top <2% largest cells were isolated (1<sup>st</sup> Sort) from among these RAP-treated cells and expanded in culture until they reached  $>1 \times 10^7$ . RAP treatment was repeated and the top <2% largest cells were again recovered by flow cytometric sorting (2<sup>nd</sup> Sort). These steps were repeated two more times to generate the 3<sup>rd</sup> and 4<sup>th</sup> Sorts. (C) Flow cytometric measurement of the cell size distribution of bulk clones after the 4<sup>th</sup> sorting cycle (RF2/4<sup>th</sup>) and culture in the absence (-) or presence (+) of RAP and/or DOX, as indicated. (D) Different single cell clones show different cell size responses to RAP. Solid and dashed grey lines represent size distributions of parental Jurkat cells in the absence or presence of RAP, respectively. Solid and dashed black lines represent size distributions of representative single cell clones in the absence or presence of RAP, respectively. Red and green lines indicate size distributions of single cell clones treated with DOX in the absence or presence of RAP, respectively. (E) Comparison of cell volumes of G1 phase-gated cells. Control and Largen-O/E cells were gated to detect cells in G1 phase, and cell volumes were measured electronically.

**A****B****C****D****E****F****G**

## Figure S2, related to Figure 2

(A) Immunoblot of cell lysates prepared from Jurkat cells stably transformed by infection with retrovirus expressing GFP (lane 1) or GFP-Largen (PRR16) fusion protein (lane 2). The indicated proteins were detected using anti-GFP antibody. (B) Flow cytometric determination of cell size distribution of (top) HeLa cells transiently transfected with plasmid expressing Myc-tagged Largen or vector control, and (bottom) 293T cells stably transformed with Myc-tagged Largen or vector control. Inset numbers are the mean forward scatter (FSC) values for each group. (C) Cell volumes of two independent 293T cell clones overexpressing *Prr16* (L1 and L2), as well as a control clone transformed with the empty vector (Vec), cultured with/without RAP. Numbers are the mean cell volume in picolitres  $\pm$  SD of 3 independent measurements using a Moxi Z cell counter. (\* $p$ <0.05, \*\* $p$ <0.001) (D) Semi-quantitative RT-PCR of total RNA prepared from untransfected U2-OS cells (-) or U2-OS cells transfected with non-targeting control siRNA (C), or one of 4 PRR16/Largen siRNAs, each of which targeted a different site in the PRR16/Largen mRNA (#1 - #4). Unless otherwise noted, siRNA#3 was used for all experiments described in the main text. (E) Flow cytometric measurement of cell size distribution of (top) HeLa and (bottom) 293T cells transiently transfected with siRNA against PRR16/Largen (#3) or with non-targeting siControl. Inset numbers are the mean FSC values for each group. (F) Cell volumes of Jurkat cells transiently transfected with siRNA against PRR16/Largen (#3) or with non-targeting siControl. Numbers are the mean cell volume in picolitres  $\pm$  SD of 4 independent measurements using a Moxi Z cell counter. (\* $p$ <0.05) (G) Flow cytometric

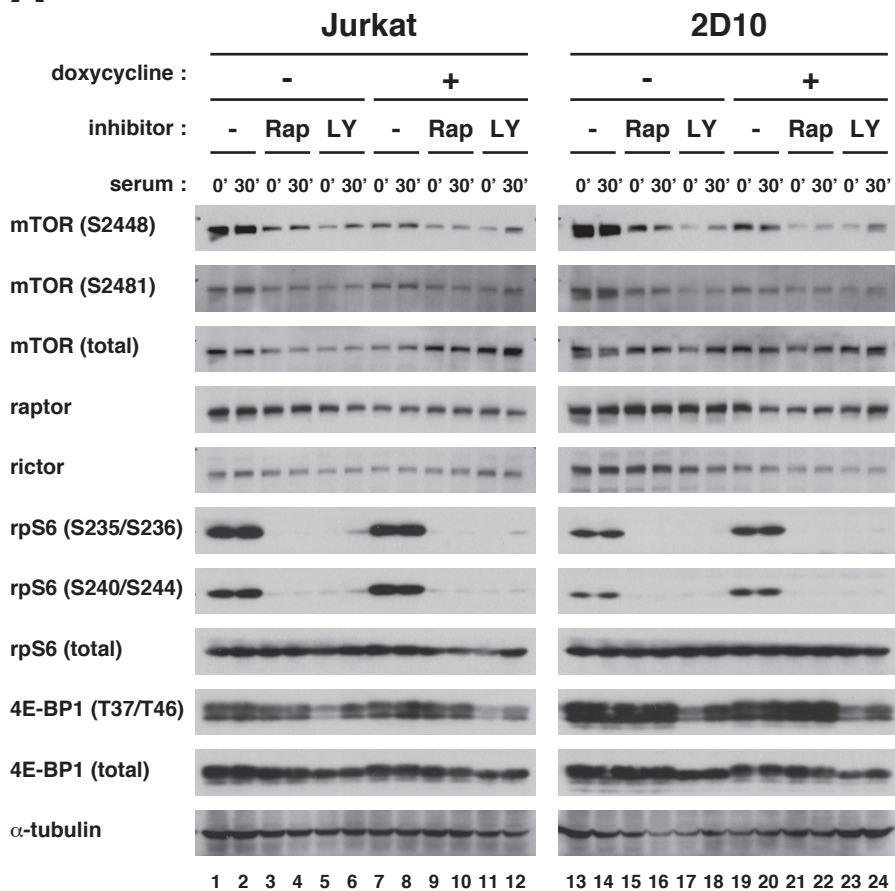
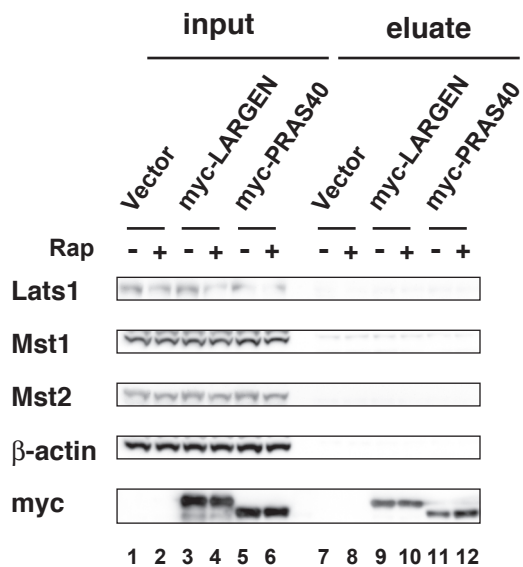
determination of apoptosis of U2-OS cells transfected with siRNA#3 against PRR16/Largen or with non-targeting siControl. Transfected cells were incubated with FITC-conjugated Annexin V followed by staining with propidium iodide (PI).

Human	1	MSAKSKGN	PSSSC	PAEG	PPAASKTKVKEQIKIIVEDLELVLGDLKDVAKELKE	53														
Chimp	1	-----	-----	-----	-----	53														
Rat	1	-----	SAA	-----	-----	53														
Mouse	1	-----	SAA	-----	-----	53														
Zebrafish	1		M--SA--	P	DAGV--V--R--RS-----NI-----	44														
Bovine	1	METGKLFKSGFTHI	P	KSQ	LFN	NYQ	NTETLQ	AP	GH	VK	LICITEEMSSMLMFSYAFYR	59								
Chick	1	MILDTSYKERLWELGLFSL	PKRV	P	RGDRIVTFQDLE	GAYKN	NGERLLS	FDV	STEV	STDELMELL	PP	66								
Human	54	VVDQIDTLTSDLQLEDEMTDSSKTD	TLN	SSSSG	TASSL	EKIKVQANAP	PLIK	PPAH	P	SAILTVLR	118									
Chimp	54	-----	-----	-----	-----	-----	-----	-----	-----	-----	118									
Rat	54	-----	-----	-----	I	-----	E	-----	-----	-----	118									
Mouse	54	-----	-----	-----	I	-----	E	-----	-----	-----	118									
Zebrafish	45	--QE--C----	H--E-Q-----	S--TTTTASS--	I	Y	PEETLFR	PTSL-	PSV----	K	110									
Bovine	60	-----	-----	-----	I	-----	-----	-----	-----	-----	124									
Chick	67	-----	S-----	--V--	RGST----	N-V-----	-----	-----	-----	-----	131									
Human	119	KPN	PPPP	RLT	PVKCED	PKRVV	TAN	PVK	TNGTLLR	NGGL	PGG	NKI	P	NGDICCI	P	NSNL	DKAPV	184		
Chimp	119	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	184		
Rat	119	-----	R--E-Q	KAA--	L-----	R-----	V-----	L	184											
Mouse	119	-----	R--E-Q-----	A-R-----	L	184														
Zebrafish	111	R-----	SA-SRS-E	V--A-V	LSR	137														
Bovine	125	----A-----	SQ-----	V-----	F--A-T-----	Y-K-S-----	G	190												
Chick	132	-----	DKQL-	PP-S-----	--SR-----	L-----	SVAI	192												
Human	185	QLLMHR	PEKDRCP	QAG	PRERVRFNEK	VQYHGYC	P	DCDTRYNIKNREVHLHSE	PVHP	PKI	PHQG	PP	250							
Chimp	185	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	250							
Rat	185	-S-----	S-----	E-----	L--Y--V	250														
Mouse	185	-S-----	S-----	ES-----	L	250														
Zebrafish	138	EKGHTG----	T T-----	S-----	LQ-DMNSTDL---	T-LLDNK	P	SPV--	CS	197										
Bovine	191	-P	-----	HV-----	L-P----	256														
Chick	193	PP-----	E-----	A-----	S-----	V-----	A-LL-AL---	C--	258											
Human	251	L	PP	PHL	PP	F	LENGGMGISHS	NSF	PP	IR	P	ATV	PP	P	TAP	K	P	QK	TILRKSTTTT	304
Chimp	251	-	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	304
Rat	251	-	P	-----	T-----	L-----	H-----	P	305											
Mouse	251	-	H-P	-----	N-----	L-----	P-----	304												
Zebrafish	198		----	LAF-SQ	-----	S----	222													
Bovine	257	H	-L	P	-----	L-----	L-----	I-----	310											
Chick	259	-P-	P-P	-----	L--S-----	P--V--	-Q-----	-----	313											

### **Figure S3, related to Figure 2**

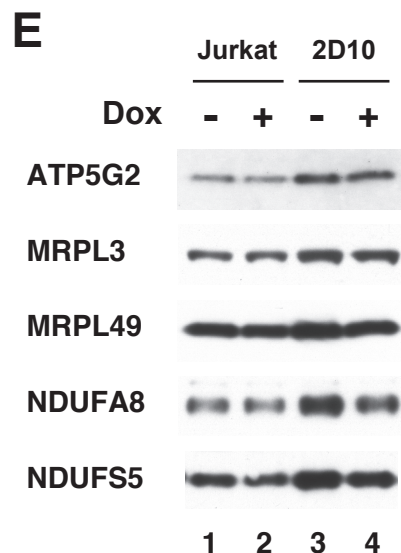
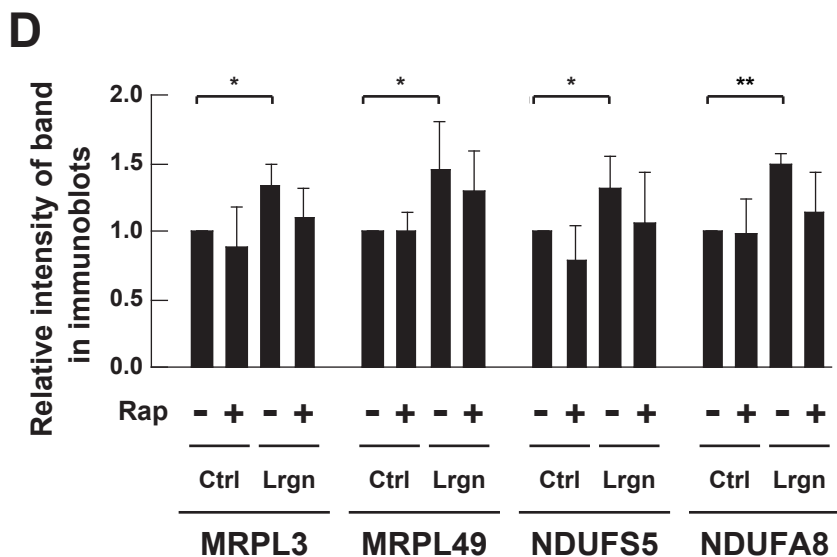
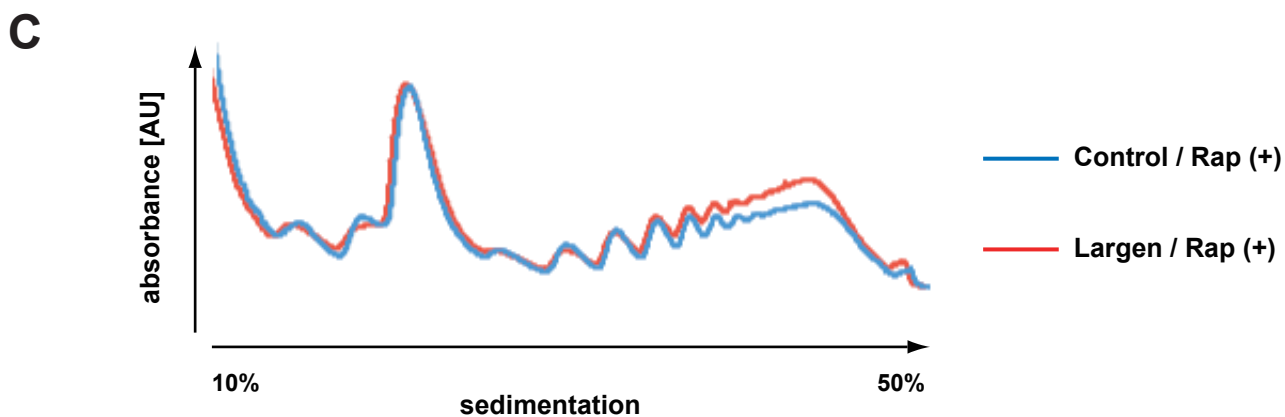
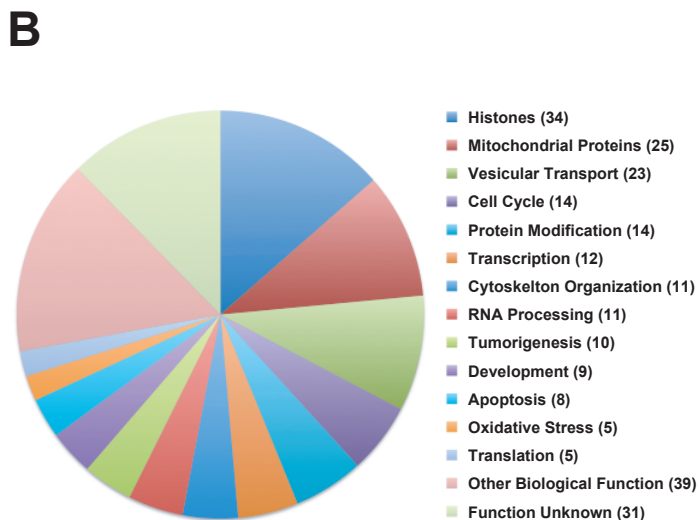
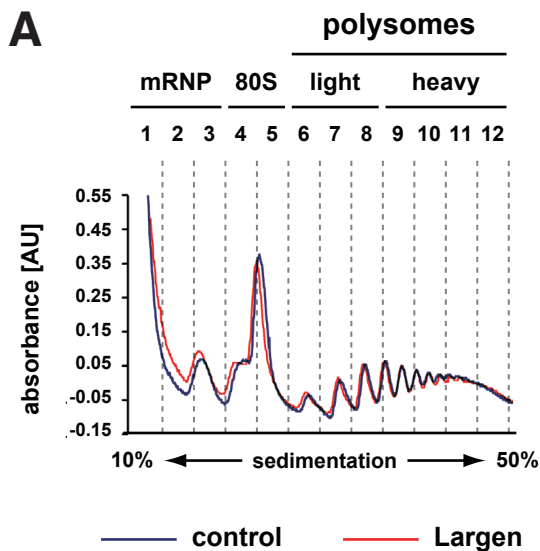
Comparison of the primary structures of Largen proteins among vertebrates. Amino acid sequences of human Largen and its orthologues from chimpanzee, rat, mouse, zebrafish, bovine, and chick are aligned. A dash '-' indicates that the orthologue has the same amino acid present in the same position as the human sequence. Differences in amino acids at a given position are shown for each orthologue. A gap indicates that there is no corresponding amino acid in the human sequence at the position where an orthologue has an extra amino acid or *vice versa*. Prolines are marked in red in the human sequence and in yellow in the orthologue sequences.



**A****B**

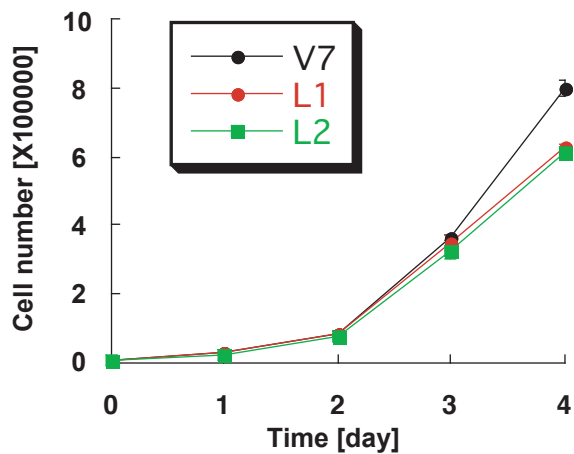
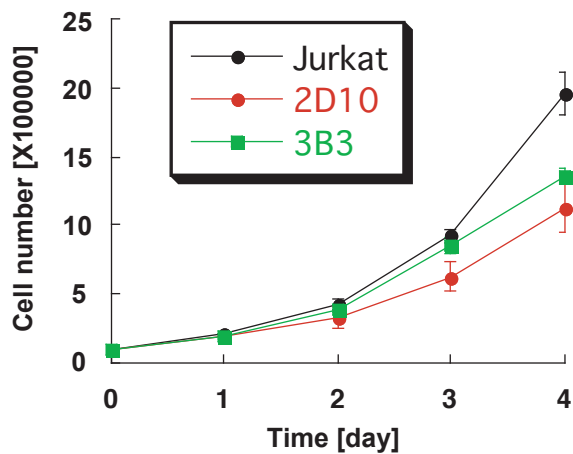
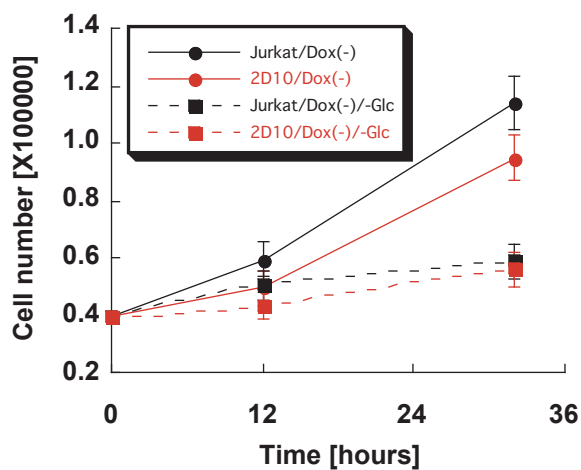
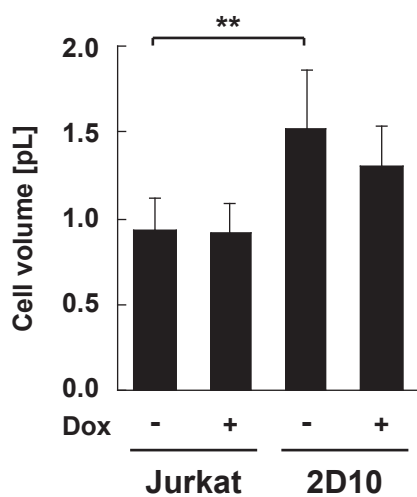
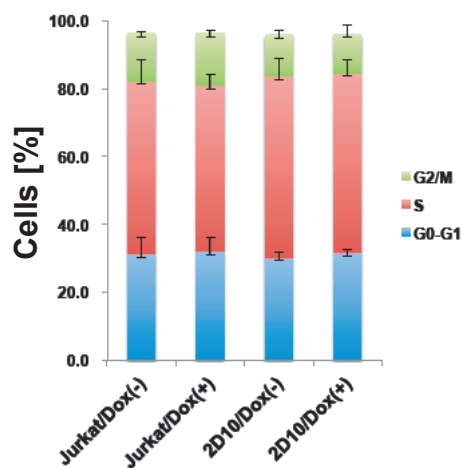
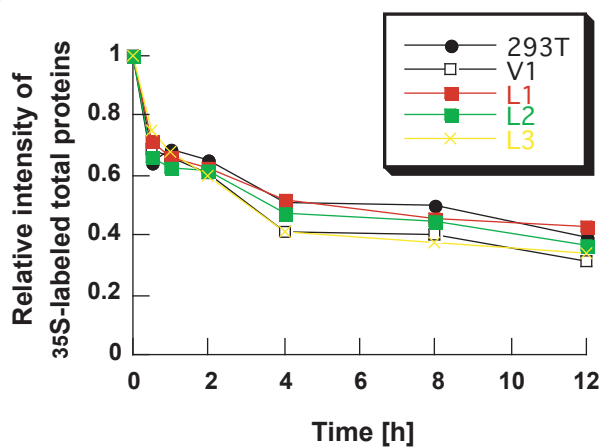
**Figure S4, related to Figure 3**

**(A)** Phosphorylation status of molecules in the mTOR signaling pathway. Jurkat and 2D10 cells were serum-starved overnight in the absence (-) or presence (+) of DOX. Cells were further treated for 1 hr with vehicle (-), RAP (Rap, 20 nM), or LY294002 (LY, 50  $\mu$ M) prior to stimulation by serum. Lysates were prepared either before (0') or after 30 min incubation and immunoblotted as described above.  $\alpha$ -tubulin, loading control. **(B)** Immunoblot of proteins eluted from anti-Myc Ab-conjugated beads that were incubated with lysates of 293T cells transfected with empty vector (Vector), or plasmid expressing Myc-tagged Largin (myc-LARGEN) or Myc-tagged PRAS40 (myc-PRAS40; control). Proteins in the input, and proteins eluted from the beads using Myc-peptides, are shown for each lysate.  $\beta$ -actin, loading control. Results are representative of 3 experiments.



**Figure S5, related to Figure 4**

(A) UV absorption profiles of ribosomes from control and Largen-O/E cells. Cytoplasmic extracts were fractionated by sucrose gradient sedimentation with continuous monitoring of absorbance at 254 nm. Fractions containing light polysomes (1-3 ribosomes per mRNA molecule) or heavy polysomes (>4 ribosomes per mRNA) were collected and applied to microarray analysis. Results are expressed as arbitrary units (AU) and are representative of multiple sedimentations. (B) Pie graph representation of 251 transcripts enriched in heavy polysome fractions of 2D10 cells as determined by differential microarray analysis of the fractions in (A). Transcripts are categorized by primary biological function. Numbers are the number of genes in each category. (C) Polysome profiles of control and Largen-O/E cells cultured in the presence of RAP. Measurement and analysis were performed as in (A). (D) The intensity of each band in the immunoblots in Figure 4C was quantitated by Image-J and the values were normalized against that of  $\alpha$ -tubulin. Results are the mean  $\pm$  SD of values relative to untreated controls (n>3). (\* $p$ <0.05, \*\* $p$ <0.001). (E) Immunoblot of the indicated mitochondrial proteins in extracts of purified mitochondria from control or 2D10 cells with/without DOX. Results are representative of >3 trials.

**A****B****C****D**

### Figure S6, related to Figure 7

(A) Growth rate in cultures of (left) Jurkat, 2D10 and 3B3 cells, and (right) 293T clones stably transformed with empty vector (V7) or plasmid expressing Myc-tagged Largin (L1 and L2). Cell density was measured daily. Data are the mean  $\pm$  SD of >3 independent measurements. (B) Cell volumes (left) and proliferation rates (right) of Jurkat and 2D10 cells cultured in glucose-depleted medium with/without DOX. Data are the mean  $\pm$  SD of 3 independent experiments. (\*\* $p < 0.001$ ). (C) Cell cycle analysis of Jurkat and 2D10 cells treated with/without DOX. Cells were seeded at  $2.5 \times 10^5$ /ml and cultured overnight in the presence (+) or absence (-) of 1  $\mu$ g/ml DOX. The percentages of the total cell population that were in the G0-G1, S or G2/M phases were determined by flow cytometry. Results are the mean percentage of two independent assays. (D) Protein degradation profiles of parental 293T cells, 293T cells stably transformed with control plasmid (V1), and three independent 293T clones stably transformed with Myc-tagged Largin (L1, L2, L3). Cells were cultured in labeling medium plus [ $^{35}$ S]-methionine for 30 min as described in EXPERIMENTAL PROCEDURES. Labeled cells were washed and cultured in standard medium for the indicated times. Cell lysates were prepared and equal amounts of total protein were fractionated by SDS-PAGE followed by autoradiography. Relative amounts of [ $^{35}$ S]-methionine-labeled proteins were quantitated by densitometric scanning with Image-J. The relative density of radiolabeled proteins in each lane was normalized to that of the initial (time 0) sample for each cell line.

**Table S1 Identification and mapping of the candidate genes in single cell clones, related to Figure 1**

Screen	ERM-tag	Clone ID	Intergenic integration <sup>1</sup>	Intragenic integration	CC <sup>2</sup>
Pilot	RF2	1A2	<i>Myc proto-oncogene protein / Gasdermin-C</i>	C1orf186	8q24.2
		1C2			1q32.1
		2A1	<i>Myc proto-oncogene protein / Gasdermin-C</i>		8q24.2
		2A2	<i>POU class 5 homeobox 1B / myc proto-oncogene protein</i>		8q24.21
		2B1			3q21
		2B4	<i>Myc proto-oncogene protein / Gasdermin-C</i>		8q24.2
		2C1		Triple functional domain protein	5q15.2
		2D10	<i>Proline-rich protein 16 / Ferritin mitochondrial</i>		5q23.1
		3B2	<i>Prostaglandin D2 receptor 2 / Zona pellucida glycoprotein 1</i>		11q12.2
		3B3	<i>Proline-rich protein 16 / Ferritin mitochondrial</i>		5q23.1
		3B4	<i>POU class 5 homeobox 1B / myc proto-oncogene protein</i>		8q24.21
Core	Normal1	1C6		Down syndrome critical region protein 3 GNPTAB	21q22.2
		1D2			12q23.2
	Normal2	1A1	NACC family member 2 / C9orf69		9q34.3
		1B6	<i>Myc proto-oncogene protein / Gasdermin-C</i>		8q24.2
		1C1	CD83 / Jumonji, AT rich interactive domain 2 protein		6p23
		2B5			Adenylate cyclase activating polypeptide 1 18p11
	Normal3	1A1	Methionine adenosyltransferase II, beta / Teneurin-2	Mucin 4 <i>Myocyte-specific enhancer factor 2C isoform 1</i> PR domain containing 8	3q23
		1A3			5q14
		1B5			4q21
		1C1			5q34
		1C2			Metastasis-associated protein MTA1 14q32.3
	1D1	<i>Myocyte-specific enhancer factor 2C isoform 1</i> 5q14			
	NLS1	1A6	<i>Rho GDP-dissociation inhibitor 2 / Phosphodiesterase 6H</i> <i>Rho GDP-dissociation inhibitor 2 / Phosphodiesterase 6H</i>	Cytohesin 1	12p13
		1D5			12p13
		1B2			17q25
		1D1			Gamma-glutamyltransferase light chain 1 / SYNDIG1 20p11
		1D3			C12orf70 / Liprin-beta-1 isoform 4 12p12
		2A6			C11orf96 / ACCSL 11p11.2
	NLS2	1B6	MYCN/FAM49A	Topoisomerase (DNA) II alpha 170kDa	2q24.2
		1C4	<i>Proline-rich protein 16 / Ferritin mitochondrial</i>		5q23.1
1D1			17q21		
NLS3	1D1		Nuclear receptor interacting protein 1 CDC28 protein kinase regulatory subunit 1B	21q11.2	
	2A5			1q21.2	
	2C1			<i>Myc proto-oncogene protein / Gasdermin-C</i> 8q24.2	
MEM1	1A3	C1orf229 / Zinc finger protein 124	Slingshot homolog 2 Cryptochrome 1 ELOVL fatty acid elongase 5 Keratin 1	1q44	
	1A5			17q11.2	
	1A6			12q23	
	1B2			6p21.1	
	2D6			12q13.13	
MEM2	1C6		MOB kinase activator 3A Solute carrier family 43, member 2 Hexamethylene bis-acetamide inducible 1	19p13.3	
	1D1			17p13.3	
	1D5			17q21.31	
MEM3	1A2	<i>FYN binding protein / Complement component 9</i> <i>FYN binding protein / Complement component 9</i>	<i>FYN binding protein</i> <i>FYN binding protein</i> <i>FYN binding protein</i>	8p23.1	
	1B3			3p14.1	
	1B4			5p13.1	
	1B5			5p13.1	
	1C1			5p13.1	
	1C2			5p13.1	
	1C6			5p13.1	

<sup>1</sup> Sequenced tag was assigned in the noncoding region between the genes indicated.

<sup>2</sup> Cytogenetic Coordinates

**Table S2 mRNAs enriched in heavy polysome fractions in Largen-overexpressing cells, related to Figure 4**

Gene Symbol	EntrezGene ID	ProbeName	GenbankAccession	Description	FCAbsolute	p-value	logFC RNA
<b>Histones</b>							
H1FX	8971	A_23_P96087	NM_006026	H1 histone family, member X (H1FX), mRNA [NM_006026]	3.876878	0.004709158	0.003404416
H2AFJ	55766	A_24_P236003	NM_177925	H2A histone family, member J (H2AFJ), transcript variant 2, mRNA [NM_177925]	4.7487535	0.002603759	-0.098778236
H3F3B	3021	A_23_P152516	NM_005324	H3 histone, family 3B (H3.3B) (H3F3B), mRNA [NM_005324]	3.7573144	5.21E-05	0.092142511
HIST1H1C	3006	A_23_P122443	NM_005319	histone cluster 1, H1c (HIST1H1C), mRNA [NM_005319]	6.4905505	0.003481256	0.314208212
HIST1H1D	3007	A_24_P260639	NM_005320	histone cluster 1, H1d (HIST1H1D), mRNA [NM_005320]	4.631392	0.003491658	0.279042989
HIST1H2AB	8335	A_24_P223384	NM_003513	histone cluster 1, H2ab (HIST1H2AB), mRNA [NM_003513]	5.941576	0.010168375	0.307751065
HIST1H2AC	8334	A_23_P372860	NM_003512	histone cluster 1, H2ac (HIST1H2AC), mRNA [NM_003512]	9.26241	0.005724356	0.084224222
HIST1H2AD	3013	A_23_P428184	NM_021065	histone cluster 1, H2ad (HIST1H2AD), mRNA [NM_021065]	6.2743306	0.001889621	-0.103736596
HIST1H2AE	3012	A_23_P59045	NM_021052	histone cluster 1, H2ae (HIST1H2AE), mRNA [NM_021052]	4.3202634	0.005601537	-0.017556739
HIST1H2AH	85235	A_23_P81859	NM_080596	histone cluster 1, H2ah (HIST1H2AH), mRNA [NM_080596]	5.948902	0.007667313	0.475197631
HIST1H2AK	8330	A_24_P217848	NM_003510	histone cluster 1, H2ak (HIST1H2AK), mRNA [NM_003510]	7.610374	0.003799502	-0.012348964
HIST1H2AM	8336	A_24_P86389	NM_003514	histone cluster 1, H2am (HIST1H2AM), mRNA [NM_003514]	5.9595299	0.008196656	0.338437496
HIST1H2BD	3017	A_24_P146211	NM_021063	histone cluster 1, H2bd (HIST1H2BD), transcript variant 1, mRNA [NM_021063]	4.247539	0.011999182	0.019796474
HIST1H2BE	8344	A_23_P40470	NM_003523	histone cluster 1, H2be (HIST1H2BE), mRNA [NM_003523]	4.2729325	0.002672735	0.302068077
HIST1H2BG	8339	A_23_P167997	NM_003518	histone cluster 1, H2bg (HIST1H2BG), mRNA [NM_003518]	4.501325	0.007847901	-0.257993148
HIST1H2BI	8346	A_23_P111041	NM_003525	histone cluster 1, H2bi (HIST1H2BI), mRNA [NM_003525]	3.9637601	0.003272965	0.019796474
HIST1H2BL	8340	A_23_P8013	NM_003519	histone cluster 1, H2bl (HIST1H2BL), mRNA [NM_003519]	4.291472	0.009281887	0.044985383
HIST1H2BM	8342	A_24_P3783	NM_003521	histone cluster 1, H2bm (HIST1H2BM), mRNA [NM_003521]	3.988967	0.002465271	-0.275218894
HIST1H2BN	8341	A_23_P402081	NM_003520	histone cluster 1, H2bn (HIST1H2BN), mRNA [NM_003520]	4.3840466	0.010517041	-0.065151793
HIST1H3B	8358	A_24_P174924	NM_003537	histone cluster 1, H3b (HIST1H3B), mRNA [NM_003537]	9.451302	0.003595352	-0.148710017
HIST1H3C	8352	A_23_P133814	NM_003531	histone cluster 1, H3c (HIST1H3C), mRNA [NM_003531]	4.7221163	0.004147666	0.06954691
HIST1H3D	8351	A_24_P217834	NM_003530	histone cluster 1, H3d (HIST1H3D), mRNA [NM_003530]	9.012119	0.008122187	0.127656124
HIST1H3G	8355	A_23_P42198	NM_003534	histone cluster 1, H3g (HIST1H3G), mRNA [NM_003534]	6.084677	0.011490446	0.172193562
HIST1H3I	8354	A_24_P9321	NM_003533	histone cluster 1, H3i (HIST1H3I), mRNA [NM_003533]	4.908716	0.001970914	0.115697593
HIST2H2AA3	8337	A_23_P309381	NM_003516	histone cluster 2, H2aa3 (HIST2H2AA3), mRNA [NM_003516]	7.234894	0.001999636	0.156047113
HIST2H2AB	317772	A_24_P68631	NM_175065	histone cluster 2, H2ab (HIST2H2AB), mRNA [NM_175065]	5.14886	0.009893297	-0.230590403
HIST2H2AC	8338	A_24_P8721	NM_003517	histone cluster 2, H2ac (HIST2H2AC), mRNA [NM_003517]	8.251695	0.004336114	-0.332275715
HIST2H2BE	8349	A_24_P156911	NM_003528	histone cluster 2, H2be (HIST2H2BE), mRNA [NM_003528]	4.568563	0.010834846	-0.163684688
HIST2H3A	333932	A_23_P115375	NM_001005464	histone cluster 2, H3a (HIST2H3A), mRNA [NM_001005464]	13.457071	0.003003158	N.I.
HIST3H2A	92815	A_23_P149301	NM_033445	histone cluster 3, H2a (HIST3H2A), mRNA [NM_033445]	6.1659255	0.009170881	-0.139142341
HIST3H2BB	128312	A_23_P332992	NM_175055	histone cluster 3, H2bb (HIST3H2BB), mRNA [NM_175055]	4.0308	0.003272943	0.943382169
HIST3H3	8290	A_23_P137909	NM_003493	histone cluster 3, H3 (HIST3H3), mRNA [NM_003493]	7.293737	2.98E-04	-0.882899372
HIST4H4	121504	A_23_P388871	NM_175054	histone cluster 4, H4 (HIST4H4), mRNA [NM_175054]	12.452248	8.03E-04	N.I.
LOC440093	440093	A_32_P12214	NM_001013699	similar to H3 histone, family 3B (LOC440093), mRNA [NM_001013699]	4.569646	1.84E-06	0.05925017
<b>Mitochondrial Proteins</b>							
AFG3L2	10939	A_32_P117338	NM_006796	AFG3 ATPase family gene 3-like 2 (yeast) (AFG3L2), mRNA [NM_006796]	3.7661774	0.00134532	0.925972262
C12orf65	91574	A_23_P339003	NM_152269	chromosome 12 open reading frame 65 (C12orf65), mRNA [NM_152269]	3.9480476	3.13E-04	-0.037543333
CHCHD3	54927	A_32_P159150	NM_017812	coiled-coil-helix-coiled-coil-helix domain containing 3 (CHCHD3), mRNA [NM_017812]	3.5508165	0.012479872	-0.044155526
COQ4	51117	A_24_P389491	NM_016035	coenzyme Q4 homolog (S. cerevisiae) (COQ4), mRNA [NM_016035]	3.7592945	2.66E-04	0.150944543
CYB5B	80777	A_23_P206697	NM_030579	cytochrome b5 type B (outer mitochondrial membrane) (CYB5B), mRNA [NM_030579]	4.4442697	0.002271432	-0.009484903
DHRS4	10901	A_23_P162982	NM_021004	dehydrogenase/reductase (SDR family) member 4 (DHRS4), mRNA [NM_021004]	3.7148697	0.002045734	0.13558824
ECH1	1891	A_23_P153853	NM_001398	enoyl Coenzyme A hydratase 1, peroxisomal (ECH1), mRNA [NM_001398]	4.5474977	0.00141372	0.179311318
ENDOG1	9941	A_24_P24444	NM_005107	endonuclease G-like 1 (ENDOG1), mRNA [NM_005107]	5.0326395	0.004892046	0.018150387
ERAL1	26284	A_23_P71981	NM_005702	Era G-protein-like 1 (E. coli) (ERAL1), mRNA [NM_005702]	3.5932307	7.37E-04	0.002852379
GRPEL1	80273	A_24_P174563	NM_025196	GrpE-like 1, mitochondrial (E. coli) (GRPEL1), mRNA [NM_025196]	4.5121446	2.95E-04	0.306687816
MLYCD	23417	A_23_P88817	NM_012213	malonyl-CoA decarboxylase (MLYCD), mRNA [NM_012213]	7.3015447	0.002779396	-0.288203997
MRPL3	11222	A_23_P7030	NM_007208	mitochondrial ribosomal protein L3 (MRPL3), mRNA [NM_007208]	5.576191	0.003042849	0.210408669
MRPL38	64978	A_23_P54963	NM_032478	mitochondrial ribosomal protein L38 (MRPL38), mRNA [NM_032478]	3.774992	0.003000696	-0.024578551
MRPL49	740	A_23_P138819	NM_004927	mitochondrial ribosomal protein L49 (MRPL49), mRNA [NM_004927]	5.9330945	3.67E-04	0.241870942
MRPS11	64963	A_24_P935318	NM_022839	mitochondrial ribosomal protein S11 (MRPS11), mRNA [NM_022839]	3.7820513	0.007972163	-0.052741851
NDUFS5	4725	A_23_P10463	NM_004552	NADH dehydrogenase (ubiquinone) Fe-S protein 5, 15kDa, mRNA [NM_004552]	5.196022	1.76E-04	-0.094677741
PKD3	5165	A_23_P250478	NM_005391	pyruvate dehydrogenase kinase, isozyme 3 (PKD3), mRNA [NM_005391]	4.045194	8.71E-04	0.244788039
PECR	55825	A_23_P91140	NM_018441	peroxisomal trans-2-enoyl-CoA reductase (PECR), mRNA [NM_018441]	3.5095732	1.20E-04	-0.127270831
SDHC	6391	A_24_P233850	NM_003001	succinate dehydrogenase complex, subunit C, 15kDa (SDHC), mRNA [NM_003001]	3.9940686	0.001516132	-0.237860906
SH3BP5	9467	A_24_P148750	NM_004844	SH3-domain binding protein 5 (BTK-associated) (SH3BP5), mRNA [NM_004844]	4.2188463	0.003000793	0.42225273



SSBP1	6742	A_23_P31536	NM_003143	single-stranded DNA binding protein 1 (SSBP1), mRNA [NM_003143]	3.5105083	3.05E-04	-0.331059187
TFAM	7019	A_24_P134727	NM_003201	transcription factor A, mitochondrial (TFAM), mRNA [NM_003201]	3.647071	1.51E-05	-0.157639354
TOMM34	10953	A_23_P57033	NM_006809	translocase of outer mitochondrial membrane 34 (TOMM34), mRNA [NM_006809]	6.9714594	3.74E-04	-0.33241783
TOMM40	10452	A_24_P178093	NM_006114	translocase of outer mitochondrial membrane 40 homolog, mRNA [NM_006114]	4.6797104	0.004695755	0.069700186
TTC19	54902	A_24_P126393	NM_017775	tetratricopeptide repeat domain 19 (TTC19), mRNA [NM_017775]	4.35522	0.002828887	-0.050970013
<b>Vesicular Transport</b>							
AP2M1	1173	A_23_P155624	NM_004068	adaptor-related protein complex 2, mu 1 subunit (AP2M1), mRNA [NM_004068]	3.5457442	0.010316623	-0.100125098
ARF3	377	A_23_P36521	NM_001659	ADP-ribosylation factor 3 (ARF3), mRNA [NM_001659]	4.039595	2.22E-04	0.092200104
ATP6V1D	51382	A_23_P140115	NM_015994	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D (ATP6V1D), mRNA [NM_015994]	3.6205635	5.20E-05	-0.141244361
CHMP7	91782	A_32_P70220	NM_152272	CHMP family, member 7 (CHMP7), mRNA [NM_152272]	4.464436	5.32E-04	0.295837767
COPE	11316	A_24_P399622	NM_199444	coatamer protein complex, subunit epsilon (COPE), transcript variant 3, mRNA [NM_199444]	5.664686	3.12E-04	0.056479237
DENND1B	163486	A_23_P201605	NM_144977	DENN/MADD domain containing 1B (DENND1B), mRNA [NM_144977]	4.927893	5.23E-04	0.585976807
GOLGA3	2802	A_23_P98864	NM_005895	golgi autoantigen, golgin subfamily a, 3 (GOLGA3), mRNA [NM_005895]	4.899892	0.001383066	-0.07094354
GOLPH2	51280	A_24_P394865	NM_016548	golgi phosphoprotein 2 (GOLPH2), transcript variant 1, mRNA [NM_016548]	4.9918213	8.62E-04	0.051037511
GORASP2	26003	A_24_P328320	NM_015530	golgi reassembly stacking protein 2, 55kDa (GORASP2), mRNA [NM_015530]	4.6763873	2.44E-04	0.371436299
LAPTM4B	55353	A_24_P414999	NM_018407	lysosomal associated protein transmembrane 4 beta (LAPTM4B), mRNA [NM_018407]	4.750426	0.001584326	-0.228927305
NAPA	8775	A_23_P55990	NM_003827	N-ethylmaleimide-sensitive factor attachment protein, alpha (NAPA), mRNA [NM_003827]	5.545775	4.41E-04	0.126750582
RAB1B	81876	A_23_P64090	NM_030981	RAB1B, member RAS oncogene family (RAB1B), mRNA [NM_030981]	7.8021164	0.007477358	-0.750527084
RAB21	23011	A_24_P247749	NM_014999	RAB21, member RAS oncogene family (RAB21), mRNA [NM_014999]	7.293949	0.002303502	0.295837876
RAB5C	5878	A_23_P107211	NM_201434	RAB5C, member RAS oncogene family (RAB5C), transcript variant 1, mRNA [NM_201434]	4.1075897	0.001544687	0.146006932
RAB7A	7879	A_24_P234572	NM_004637	RAB7A, member RAS oncogene family (RAB7A), mRNA [NM_004637]	5.5856795	5.86E-04	-0.069336447
SCAMP2	10066	A_23_P385081	NM_005697	secretory carrier membrane protein 2 (SCAMP2), mRNA [NM_005697]	3.9955194	5.63E-04	-0.18358624
SCAMP3	10067	A_23_P97274	NM_052837	secretory carrier membrane protein 3 (SCAMP3), transcript variant 2, mRNA [NM_052837]	3.5104465	2.30E-04	0.122917478
SFT2D2	375035	A_23_P148785	NM_199344	SFT2 domain containing 2 (SFT2D2), mRNA [NM_199344]	3.840829	0.005326902	-0.126058272
SNX17	9784	A_23_P28238	NM_014748	sorting nexin 17 (SNX17), mRNA [NM_014748]	5.549111	0.012332906	-0.191836146
STX18	53407	A_24_P388622	NM_016930	syntaxin 18 (STX18), mRNA [NM_016930]	3.641451	7.37E-04	-0.274393366
TRAPPC6A	79090	A_24_P390928	NM_024108	trafficking protein particle complex 6A (TRAPPC6A), mRNA [NM_024108]	3.7415912	3.54E-05	0.587678106
VAPA	9218	A_23_P382199	NM_003574	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa, mRNA [NM_003574]	3.5910416	0.002880531	-0.148040567
VPS39	23339	A_23_P100103	NM_015289	vacuolar protein sorting 39 homolog (S. cerevisiae) (VPS39), mRNA [NM_015289]	4.4489408	0.006613969	0.770155558
<b>Cell Cycle</b>							
CBX5	23468	A_23_P23355	NM_012117	chromobox homolog 5 (HP1 alpha homolog, Drosophila) (CBX5), mRNA [NM_012117]	4.150486	2.95E-05	-0.033699817
CDK6	1021	A_23_P168651	NM_001259	cyclin-dependent kinase 6 (CDK6), mRNA [NM_001259]	7.8250427	0.001233212	-0.002011879
MAPK1	5594	A_23_P257895	NM_138957	mitogen-activated protein kinase 1 (MAPK1), transcript variant 2, mRNA [NM_138957]	3.5353088	2.61E-04	-0.247222475
MAPK14	1432	A_24_P397566	NM_139013	mitogen-activated protein kinase 14 (MAPK14), transcript variant 3, mRNA [NM_139013]	3.903526	0.002112991	-0.203185847
PTMA	5757	A_24_P264207	NM_002823	prothymosin, alpha (gene sequence 28) (PTMA), mRNA [NM_002823]	10.714185	3.79E-04	-0.251064844
RAN	5901	A_32_P506600	NM_006325	RAN, member RAS oncogene family (RAN), mRNA [NM_006325]	4.397727	1.53E-04	-0.092000636
RHOA	387	A_24_P174550	NM_001664	ras homolog gene family, member A (RHOA), mRNA [NM_001664]	5.449942	0.002297695	0.103015448
RUVBL1	8607	A_32_P30693	NM_003707	RuvB-like 1 (E. coli) (RUVBL1), mRNA [NM_003707]	3.947765	0.009505489	0.106850576
SCAND1	51282	A_23_P6196	NM_016558	SCAN domain containing 1 (SCAND1), transcript variant 1, mRNA [NM_016558]	3.852981	4.39E-04	0.003989829
SEPT2	4735	A_24_P43092	NM_001008491	septin 2 (SEPT2), transcript variant 1, mRNA [NM_001008491]	4.463853	0.00208986	0.271663537
SMEK1	55671	A_24_P369691	NM_032560	SMEK homolog 1, suppressor of mek1 (Dictyostelium) (SMEK1), mRNA [NM_032560]	3.9156673	0.002503073	-0.213158833
STK4	6789	A_23_P143199	NM_006282	serine/threonine kinase 4 (STK4), mRNA [NM_006282]	3.5498006	7.61E-04	-0.240837343
TIMELESS	8914	A_24_P231004	NM_003920	timeless homolog (Drosophila) (TIMELESS), mRNA [NM_003920]	4.95406	0.002980027	0.325204334
YWHA8	7529	A_23_P500251	NM_003404	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide, mRNA [NM_003404]	3.8492427	4.76E-05	0.124945406
<b>Transcription</b>							
AFF4	27125	A_24_P394408	NM_014423	AFF4/FMR2 family, member 4 (AFF4), mRNA [NM_014423]	5.251953	0.002908462	-0.275774495
LCOR	84458	A_23_P328836	NM_032440	ligand dependent nuclear receptor corepressor (LCOR), mRNA [NM_032440]	3.5043979	0.0040297	-0.08703071
MGC29891	126626	A_24_P191207	NM_144618	hypothetical protein MGC29891 (MGC29891), mRNA [NM_144618]	4.77659	0.001411601	0.15723534
NR1H2	7376	A_23_P55926	NM_007121	nuclear receptor subfamily 1, group H, member 2 (NR1H2), mRNA [NM_007121]	3.866714	0.001633707	0.024037451
NXN	64359	A_23_P61778	NM_022463	nucleoredoxin (NXN), mRNA [NM_022463]	4.837317	7.17E-04	0.438540439
REXO4	57109	A_23_P157861	NM_020385	REX4, RNA exonuclease 4 homolog (S. cerevisiae) (REXO4), mRNA [NM_020385]	4.2764845	7.08E-04	0.136332191
SCYL1	57410	A_23_P75470	NM_020680	SCY1-like 1 (S. cerevisiae) (SCYL1), transcript variant A, mRNA [NM_020680]	3.5090451	0.004178692	0.070811768
SETD7	80854	A_23_P80966	NM_030648	SET domain containing (lysine methyltransferase) 7 (SETD7), mRNA [NM_030648]	8.036785	0.011688318	-0.231608287
SMARCD1	6602	A_23_P204745	NM_139071	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, mRNA [NM_139071]	4.0041122	0.007162517	-0.159161725
SSBP3	23648	A_24_P375453	NM_001009955	single stranded DNA binding protein 3 (SSBP3), transcript variant 3, mRNA [NM_001009955]	3.734599	0.011738982	-0.280069375
TMEM113	80335	A_24_P388536	NM_025222	transmembrane protein 113 (TMEM113), mRNA [NM_025222]	3.783626	0.005087834	-0.187407351
ZXDC	80292	A_24_P922397	NM_01040653	ZXDC family zinc finger C (ZXDC), transcript variant 2, mRNA [NM_01040653] Agilent	4.018627	1.49E-04	-0.198037002
<b>Cytoskeleton Organization</b>							
AAMP	14	A_23_P56529	NM_001087	angio-associated, migratory cell protein (AAMP), mRNA [NM_001087]	3.6952455	0.001701752	-0.497122693

ARHGDB	397	A_23_P151075	NM_001175	Rho GDP dissociation inhibitor (GDI) beta (ARHGDB), mRNA [NM_001175]	6.1358	5.05E-05	-0.156837872	
ARPC4	10093	A_23_P29566	NM_005718	actin related protein 2/3 complex, subunit 4, 20kDa (ARPC4), transcript variant 1, mRNA [NM_005718]	5.1617007	0.001829339	0.00080338	
C6orf206	221421	A_24_P52189	AK055407	cDNA FLJ30845 fis, clone FEBRA2002727. [AK055407]	6.330771	0.003550302	-0.130088224	
MAPRE2	10982	A_24_P193911	NM_014268	microtubule-associated protein, RP/EB family, member 2 (MAPRE2), mRNA [NM_014268]	8.343991	5.92E-04	-0.129261351	
MYL6	4637	A_23_P344973	NM_079423	myosin, light chain 6, alkali, smooth muscle and non-muscle (MYL6), transcript variant 2, mRNA [NM_079423]	3.6509323	1.55E-04	-0.038605236	
MYO9B	4650	A_24_P922921	NM_004145	myosin IXB (MYO9B), mRNA [NM_004145]	5.3462934	4.27E-04	-0.165941073	
PDPK1	5170	A_24_P830690	NM_002613	3-phosphoinositide dependent protein kinase-1 (PDPK1), transcript variant 1, mRNA [NM_002613]	5.129891	1.32E-04	-0.367326597	
PIP5K1A	8394	A_23_P23572	NM_003557	phosphatidylinositol-4-phosphate 5-kinase, type I, alpha (PIP5K1A), mRNA [NM_003557]	4.061195	0.007036914	-0.094774677	
TRIO	7204	A_24_P42603	NM_007118	triple functional domain (PTPRF interacting) (TRIO), mRNA [NM_007118]	3.5016432	0.001180741	0.108991931	
TUBGCP2	10844	A_23_P127150	NM_006659	tubulin, gamma complex associated protein 2 (TUBGCP2), mRNA [NM_006659]	3.7539735	2.49E-04	0.127277631	
<b>RNA Processing</b>								
ADAT1	23536	A_24_P48139	NM_012091	adenosine deaminase, tRNA-specific 1 (ADAT1), mRNA [NM_012091]	3.633133	0.008537588	-0.022681704	
DCP2	167227	A_23_P256868	NM_152624	DCP2 decapping enzyme homolog (S. cerevisiae) (DCP2), mRNA [NM_152624]	4.8735456	6.77E-04	-0.121276001	
EXOSC5	56915	A_24_P211151	NM_020158	exosome component 5 (EXOSC5), mRNA [NM_020158]	4.063116	4.32E-04	-0.18149941	
HNRPAB	3182	A_23_P19084	NM_004499	heterogeneous nuclear ribonucleoprotein A/B (HNRPAB), transcript variant 2, mRNA [NM_004499]	5.0968943	0.002330112	N.I.	
RNPS1	10921	A_24_P725630	NM_006711	RNA binding protein S1, serine-rich domain (RNPS1), transcript variant 1, mRNA [NM_006711]	3.7806795	0.008888845	0.360513499	
RPUSD3	285367	A_23_P255916	NM_173659	RNA pseudouridylylase domain containing 3 (RPUSD3), mRNA [NM_173659]	3.5483909	5.25E-04	0.299408828	
SNRPB	6628	A_23_P154675	NM_198216	small nuclear ribonucleoprotein polypeptides B and B1 (SNRPB), transcript variant 1, mRNA [NM_198216]	3.792452	0.005617595	0.35746232	
TNRC6B	23112	A_24_P786172	NM_015088	trinucleotide repeat containing 6B (TNRC6B), transcript variant 1, mRNA [NM_015088]	3.6123264	0.006184844	0.011847781	
TSR2	90121	A_23_P414269	NM_058163	TSR2, 20S rRNA accumulation, homolog (S. cerevisiae) (TSR2), mRNA [NM_058163]	3.9734452	0.001964119	-0.143831849	
WDR55	54853	A_24_P200549	NM_017706	WD repeat domain 55 (WDR55), mRNA [NM_017706]	4.4930825	0.001128758	-0.261034191	
ZMAT5	55954	A_23_P132248	NM_019103	zinc finger, matrin type 5 (ZMAT5), transcript variant 1, mRNA [NM_019103]	6.9478564	7.52E-04	-0.392138986	
<b>Tumorigenesis</b>								
ABL1	25	A_24_P393711	NM_005157	v-abl Abelson murine leukemia viral oncogene homolog 1 (ABL1), transcript variant a, mRNA [NM_005157]	4.5879054	9.47E-04	9.47E-04	
ANP32A	8125	A_32_P133670	NM_006305	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A (ANP32A), mRNA [NM_006305]	4.961744	0.010472801	0.010472801	
ANP32C	23520	A_23_P92520	NM_012403	acidic (leucine-rich) nuclear phosphoprotein 32 family, member C (ANP32C), mRNA [NM_012403]	6.3007956	0.005655026	0.005655026	
C6orf173	387103	A_32_P143245	NM_001012507	chromosome 6 open reading frame 173 (C6orf173), mRNA [NM_001012507]	5.9531865	1.71E-05	1.71E-05	
ENOX2	10495	A_24_P391468	NM_182314	cytosolic ovarian carcinoma antigen 1 (COVA1), transcript variant 2, mRNA [NM_182314]	3.8306012	0.001708381	0.001708381	
PPP1CA	5499	A_23_P434710	NM_001008709	protein phosphatase 1, catalytic subunit, alpha isoform (PPP1CA), transcript variant 3, mRNA [NM_001008709]	4.696808	0.003334807	0.098455499	
PTOV1	53635	A_23_P325080	NM_017432	prostate tumor overexpressed gene 1 (PTOV1), mRNA [NM_017432]	4.7075696	4.43E-04	-0.219509738	
RAB8A	4218	A_23_P164752	NM_005370	RAB8A, member RAS oncogene family (RAB8A), mRNA [NM_005370]	4.5002646	0.002299181	-0.167841813	
SELENBP1	8991	A_23_P74619	NM_003944	selenium binding protein 1 (SELENBP1), mRNA [NM_003944]	24.007956	5.61E-04	0.319437728	
SKI	6497	A_24_P338603	NM_003036	v-ski sarcoma viral oncogene homolog (avian) (SKI), mRNA [NM_003036]	5.084857	0.008819896	0.407098105	
<b>Development</b>								
ACVR2B	93	A_23_P109950	NM_001106	activin A receptor, type IIB (ACVR2B), mRNA [NM_001106]	5.5561876	4.69E-04	4.69E-04	
ADAMT5	11173	A_23_P37624	AF140675	zinc metalloprotease ADAMT5 (ADAMT5) mRNA, complete cds. [AF140675]	3.8011234	0.001162121	0.001162121	
BB55	129880	A_23_P5785	NM_152384	Bardet-Biedl syndrome 5 (BB55), mRNA [NM_152384]	4.0974236	0.007936392	0.007936392	
CREBBP	1387	A_24_P342279	NM_004380	CREB binding protein (Rubinstein-Taybi syndrome) (CREBBP), transcript variant 1, mRNA [NM_004380]	4.289346	0.008835916	0.008835916	
FGFR1	2260	A_24_P4171	NM_023111	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome), mRNA [NM_023111]	5.3067718	0.011039325	0.011039325	
HOXA10	3206	A_24_P914411	S69027	HOX C6=class I homeodomain [fragment M13, homeodomain] [S69027]	3.803851	0.007220427	N.I.	
TAOK1	57551	A_23_P38457	NM_020791	TAO kinase 1 (TAOK1), mRNA [NM_020791]	4.648342	3.21E-04	0.096721255	
THRAP2	23389	A_24_P911508	NM_015335	thyroid hormone receptor associated protein 2 (THRAP2), mRNA [NM_015335]	5.411525	0.003208764	-0.001601489	
TRIM44	54765	A_24_P192821	NM_017583	tripartite motif-containing 44 (TRIM44), mRNA [NM_017583]	3.55853	3.02E-04	-0.072582369	
<b>Apoptosis</b>								
BOK	666	A_23_P61112	AF089746	Bcl-2 related ovarian killer (BOK) mRNA, complete cds. [AF089746]	3.7052493	0.00432121	0.00432121	
C1D	10438	A_23_P56590	NM_006333	nuclear DNA-binding protein (C1D), transcript variant 1, mRNA [NM_006333]	3.5132194	1.31E-04	1.31E-04	
DAD1	1603	A_23_P106056	NM_001344	defender against cell death 1 (DAD1), mRNA [NM_001344]	3.635886	3.32E-04	3.32E-04	
DFB	1677	A_24_P370626	NM_001004285	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase), mRNA [NM_001004285]	5.925662	0.003967213	0.003967213	
FAM82C	55177	A_24_P296280	NM_018145	family with sequence similarity 82, member C (FAM82C), mRNA [NM_018145]	4.3146267	0.005278487	0.005278487	
MAP2K6	5608	A_24_P416489	NM_00000359094	Dual specificity mitogen-activated protein kinase kinase 6 (EC 2.7.12.2) (MAP kinase kinase 6) [ENST00000359094]	5.6349616	0.004105873	-0.293578389	
MAP4K4	9448	A_23_P102192	NM_145686	mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4), transcript variant 2, mRNA [NM_145686]	3.610419	0.008489762	-0.173558829	
PDCL3	79031	A_32_P157531	NM_024065	phosducin-like 3 (PDCL3), mRNA [NM_024065]	3.736076	0.002133665	-0.067209927	
<b>Protein Degradation</b>								
FBXO36	130888	A_24_P254702	NM_174899	F-box protein 36 (FBXO36), mRNA [NM_174899]	3.8612113	7.82E-04	7.82E-04	
HERC2	8924	A_23_P48973	NM_004667	hect domain and RLD 2 (HERC2), mRNA [NM_004667]	6.202575	0.003272481	0.003272481	
HUWE1	10075	A_24_P932016	NM_031407	HECT, UBA and WWE domain containing 1 (HUWE1), mRNA [NM_031407]	3.765481	0.001565655	0.647979861	
PSMD13	5719	A_23_P75889	NM_175932	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13 (PSMD13), transcript variant 2, mRNA [NM_175932]	4.1500163	0.003752919	-0.125131845	
PSME3	10197	A_24_P352864	NM_005789	proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki), transcript variant 1, mRNA [NM_005789]	3.8185866	7.01E-04	0.210002213	
SERPINB8	5271	A_24_P147461	NM_198833	serpin peptidase inhibitor, clade B (ovalbumin), member 8 (SERPINB8), transcript variant 2, mRNA [NM_198833]	4.107185	8.53E-04	-0.190785216	

UBE2NL	389898	A_23_P350234	NM_001012989	ubiquitin-conjugating enzyme E2N-like (UBE2NL), mRNA [NM_001012989]	4.8517766	1.55E-05	-0.425252929
USP22	23326	A_23_P207068	BC110499	ubiquitin specific peptidase 22, mRNA (cDNA clone IMAGE:40027756), partial cds. [BC110499]	9.620007	0.001757989	0.239943214
<b>Protein Folding</b>							
AHSA1	10598	A_23_P117599	NM_012111	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast) (AHSA1), mRNA [NM_012111]	4.744326	0.001122977	0.001122977
ASF1B	55723	A_23_P119254	NM_018154	ASF1 anti-silencing function 1 homolog B ( <i>S. cerevisiae</i> ) (ASF1B), mRNA [NM_018154]	3.5671954	9.48E-05	9.48E-05
DNAJC8	22826	A_23_P157192	NM_014280	DnaJ (Hsp40) homolog, subfamily C, member 8 (DNAJC8), mRNA [NM_014280]	3.5431235	0.00625256	0.00625256
PFDN1	5201	A_32_P41127	NM_002622	prefoldin subunit 1 (PFDN1), mRNA [NM_002622]	4.1542726	0.005784741	0.096757483
PPIA	5478	A_32_P68459	NM_021130	peptidylprolyl isomerase A (cyclophilin A) (PPIA), mRNA [NM_021130]	4.4811387	1.95E-05	-0.515456986
PPIAL4	164022	A_32_P61061	NM_178230	peptidylprolyl isomerase A (cyclophilin A)-like 4 (PPIAL4), mRNA [NM_178230]	4.524395	1.31E-04	-0.359406777
<b>Oxidative Stress</b>							
GSTM4	2948	A_24_P396660	NM_147148	glutathione S-transferase M4 (GSTM4), transcript variant 2, mRNA [NM_147148]	4.5142026	0.001594553	0.001594553
PRDX1	5052	A_23_P11995	NM_002574	peroxiredoxin 1 (PRDX1), transcript variant 1, mRNA [NM_002574]	4.0355334	0.00231451	-0.053466491
PRDX2	7001	A_23_P142045	NM_005809	peroxiredoxin 2 (PRDX2), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA [NM_005809]	4.4623494	8.69E-05	0.251570318
SOD1	6647	A_23_P154840	NM_000454	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult)) (SOD1), mRNA [NM_000454]	5.698108	4.87E-05	-0.020962833
TXNRD3	114112	A_32_P170925		Thioredoxin reductase 3 (Thioredoxin reductase TR2) (Fragment). [ENST00000360201]	4.0982194	0.007455865	N.I.
<b>Translation</b>							
DHPS	1725	A_23_P501887	NM_013407	deoxyhypusine synthase (DHPS), transcript variant 3, mRNA [NM_013407]	3.9081845	0.003835373	0.003835373
DOHH	83475	A_23_P125408	NM_031304	deoxyhypusine hydroxylase/monooxygenase (DOHH), mRNA [NM_031304]	3.8361614	0.001960568	0.001960568
EIF1	10209	A_32_P53049	NM_005801	eukaryotic translation initiation factor 1 (EIF1), mRNA [NM_005801]	4.1642256	3.83E-04	3.83E-04
EIF4H	7458	A_24_P149390	NM_031992	eukaryotic translation initiation factor 4H (EIF4H), transcript variant 2, mRNA [NM_031992]	4.375157	0.007858049	0.007858049
PAIP2B	400961	A_24_P860797		cDNA FLJ37016 fis, clone BRACE2010632. [AK094335]	4.1392784	2.67E-04	N.I.
<b>DNA Repair</b>							
CINP	51550	A_23_P88134	NM_032630	cyclin-dependent kinase 2-interacting protein (CINP), mRNA [NM_032630]	5.064411	5.98E-05	5.98E-05
MGMT	4255	A_23_P104323	NM_002412	O-6-methylguanine-DNA methyltransferase (MGMT), mRNA [NM_002412]	3.5433805	2.48E-05	-0.042636253
NSMCE1	197370	A_23_P95823	NM_145080	non-SMC element 1 homolog ( <i>S. cerevisiae</i> ) (NSMCE1), mRNA [NM_145080]	3.512327	0.006473885	-0.28311469
TYMS	7298	A_23_P50096	NM_001071	thymidylate synthetase (TYMS), mRNA [NM_001071]	5.172912	6.59E-05	-0.029334326
<b>Immune Function</b>							
BST1	683	A_23_P7325	NM_004334	bone marrow stromal cell antigen 1 (BST1), mRNA [NM_004334]	4.3530526	0.009666925	0.009666925
DBNL	28988	A_24_P43681	NM_014063	drebrin-like (DBNL), transcript variant 1, mRNA [NM_014063]	4.0787334	0.001101922	0.001101922
TNIP1	10318	A_23_P19036	NM_006058	TNFAIP3 interacting protein 1 (TNIP1), mRNA [NM_006058]	4.310057	3.49E-04	-0.110491034
TYK2	7297	A_23_P141917	NM_003331	tyrosine kinase 2 (TYK2), mRNA [NM_003331]	4.161575	0.001457419	0.029139847
<b>Cell Adhesion</b>							
JAM3	83700	A_23_P217998	NM_032801	junctional adhesion molecule 3 (JAM3), mRNA [NM_032801]	3.6390152	0.01173648	-0.145035888
SH3PXD2A	9644	A_23_P345220	NM_014631	SH3 and PX domains 2A (SH3PXD2A), mRNA [NM_014631]	7.402252	0.005191974	-0.569549557
SPA17	53340	A_23_P104876	NM_017425	sperm autoantigenic protein 17 (SPA17), mRNA [NM_017425]	3.538727	0.001317423	0.49505745
<b>Other Biological Processes</b>							
ABHD11	83451	A_23_P362712	NM_148912	abhydrolase domain containing 11 (ABHD11), mRNA [NM_148912]	4.131526	3.22E-04	3.22E-04
ACOT8	10005	A_23_P143218	NM_005469	acyl-CoA thioesterase 8 (ACOT8), transcript variant 1, mRNA [NM_005469]	3.720359	2.13E-06	2.13E-06
ADA	100	A_23_P210482	NM_000022	adenosine deaminase (ADA), mRNA [NM_000022]	4.4052896	3.22E-04	3.22E-04
ADPRHL2	54936	A_23_P34568	NM_017825	ADP-ribosylhydrolase like 2 (ADPRHL2), mRNA [NM_017825]	3.6460505	0.009126779	0.009126779
AKR7A3	22977	A_23_P103968	NM_012067	aldo-keto reductase family 7, member A3 (aflatoxin aldehyde reductase) (AKR7A3), mRNA [NM_012067]	3.9732635	2.38E-04	2.38E-04
ALDH3A2	224	A_23_P129896	NM_000382	aldehyde dehydrogenase 3 family, member A2 (ALDH3A2), transcript variant 2, mRNA [NM_000382]	4.423245	0.003354911	0.003354911
ATP6V1C2	245973	A_24_P276932	NM_144583	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2 (ATP6V1C2), transcript variant 2, mRNA [NM_144583]	3.7277849	0.009451731	0.009451731
C11orf60	56912	A_23_P116207	NM_020153	chromosome 11 open reading frame 60 (C11orf60), mRNA [NM_020153]	3.678062	0.003257254	0.003257254
CA8	767	A_23_P83838	NM_004056	carbonic anhydrase VIII (CA8), mRNA [NM_004056]	3.502614	0.00188713	0.00188713
CYB5R3	1727	A_24_P100277	NM_007326	cytochrome b5 reductase 3 (CYB5R3), transcript variant 5, mRNA [NM_007326]	3.940876	0.001437201	0.001437201
DHDH	27294	A_23_P67367	NM_014475	dihydrodiol dehydrogenase (dimeric) (DHDH), mRNA [NM_014475]	4.744879	2.37E-04	2.37E-04
GK5	256356	A_23_P347562	NM_001039547	glycerol kinase 5 (putative) (GK5), mRNA [NM_001039547]	3.8568692	0.008392108	0.008392108
GMPPB	29925	A_23_P92202	NM_021971	GDP-mannose pyrophosphorylase B (GMPPB), transcript variant 2, mRNA [NM_021971]	3.882554	0.002558592	0.002558592
GRAP	10750	A_23_P49638	NM_006613	GRB2-related adaptor protein (GRAP), mRNA [NM_006613]	4.054854	2.94E-05	2.94E-05
GRB2	2885	A_24_P39654	NM_002086	growth factor receptor-bound protein 2 (GRB2), transcript variant 1, mRNA [NM_002086]	4.5788116	0.003430953	0.003430953
IMPAD1	54928	A_24_P240732	NM_017813	inositol monophosphatase domain containing 1 (IMPAD1), mRNA [NM_017813]	6.9755464	6.40E-04	0.135935915
LYPLA2	11313	A_24_P276490	NM_007260	lysophospholipase II (LYPLA2), mRNA [NM_007260]	5.225022	0.008499536	-0.078183226
NECAP2	55707	A_24_P302332	NM_018090	NECAP endocytosis associated 2 (NECAP2), mRNA [NM_018090]	3.9905655	0.002929441	0.499318836
NUDT11	55190	A_24_P345002	NM_018159	nudix (nucleoside diphosphate linked moiety X)-type motif 11 (NUDT11), mRNA [NM_018159]	3.8240397	0.009355441	-0.06435149
ORST2	219464	A_24_P307785	AK098491	cDNA FLJ25625 fis, clone STM02974. [AK098491]	4.545266	5.03E-05	-0.140089893
PEBP1	5037	A_23_P13604	NM_002567	phosphatidylethanolamine binding protein 1 (PEBP1), mRNA [NM_002567]	5.396274	1.09E-04	0.241636996
PKD2	5311	A_23_P167324	NM_000297	polycystic kidney disease 2 (autosomal dominant) (PKD2), mRNA [NM_000297]	4.252228	0.001213257	-0.304167678
PNPO	55163	A_23_P89708	NM_018129	pyridoxamine 5'-phosphate oxidase (PNPO), mRNA [NM_018129]	6.484336	5.48E-05	-0.360310981

RBKS	64080	A_23_P9523	NM_022128	ribokinase (RBKS), mRNA [NM_022128]	3.617256	0.001252149	0.286431955
SLC39A3	29985	A_24_P160460	NM_144564	solute carrier family 39 (zinc transporter), member 3 (SLC39A3), transcript variant 1, mRNA [NM_144564]	3.5092661	0.011392389	0.272708367
SNX8	29886	A_23_P414252	NM_013321	sorting nexin 8 (SNX8), mRNA [NM_013321]	3.5666678	1.25E-04	-0.30038026
TP1	7167	A_32_P95739	NM_000365	triosephosphate isomerase 1 (TP1), mRNA [NM_000365]	4.4984965	1.42E-04	0.445172593
WBSCR22	114049	A_23_P71014	NM_017528	Williams Beuren syndrome chromosome region 22 (WBSCR22), mRNA [NM_017528]	3.6100235	0.002973945	0.019936947
<b>Function Unknown</b>							
C17orf85	55421	A_24_P176572	NM_018553	chromosome 17 open reading frame 85 (C17orf85), mRNA [NM_018553]	4.0953345	8.10E-04	8.10E-04
C20orf196	149840	A_23_P401709	NM_152504	chromosome 20 open reading frame 196 (C20orf196), mRNA [NM_152504]	4.465778	8.07E-04	8.07E-04
C20orf199	441951	A_32_P157385	AK124175	cDNA FLJ42181 fis, clone THYMU2031368. [AK124175]	5.4482255	0.003258362	0.003258362
C20orf43	51507	A_24_P914590	BC002769	chromosome 20 open reading frame 43, mRNA (cDNA clone IMAGE:3615348), complete cds. [BC002769]	3.571246	6.65E-04	6.65E-04
C20orf77	58490	A_23_P91207	NM_021215	chromosome 20 open reading frame 77 (C20orf77), mRNA [NM_021215]	6.0635233	0.009970715	0.009970715
C21orf56	84221	A_23_P371765	NM_032261	chromosome 21 open reading frame 56 (C21orf56), mRNA [NM_032261]	3.5951922	2.55E-04	2.55E-04
C3orf37	56941	A_23_P251377	NM_001006109	chromosome 3 open reading frame 37 (C3orf37), transcript variant 1, mRNA [NM_001006109]	3.54688	0.011563594	0.011563594
C9orf40	55071	A_24_P43876	NM_017998	chromosome 9 open reading frame 40 (C9orf40), mRNA [NM_017998]	4.304017	3.97E-04	3.97E-04
C9orf64	84267	A_23_P60339	NM_032307	chromosome 9 open reading frame 64 (C9orf64), mRNA [NM_032307]	4.6827483	0.001417922	0.001417922
CCDC32	90416	A_32_P98975	NM_052849	coiled-coil domain containing 32 (CCDC32), transcript variant 2, mRNA [NM_052849]	3.7351253	0.002678538	0.002678538
COMMD4	54939	A_23_P117767	NM_017828	COMM domain containing 4 (COMMD4), mRNA [NM_017828]	4.6958866	0.001497977	0.001497977
CTA-126B4.3	27341	A_23_P29303	NM_015703	CGI-96 protein (CGI-96), mRNA [NM_015703]	4.122841	5.37E-04	5.37E-04
DHRS41	728635	A_24_P903378		isolate 1261292 dehydrogenase/reductase SDR family member 4-like 1 mRNA, [GQ871922] Agilent	3.5196078	0.003623696	0.003623696
FAM102B	284611	A_24_P237927	NM_001010883	family with sequence similarity 102, member B (FAM102B), mRNA [NM_001010883]	3.6320534	0.003446399	0.003446399
FAM103A1	83640	A_23_P65712	NM_031452	family with sequence similarity 103, member A1 (FAM103A1), mRNA [NM_031452]	4.145468	6.72E-06	6.72E-06
FAM63B	54629	A_24_P392333	NM_001040453	family with sequence similarity 63, member B (FAM63B), transcript variant 2, mRNA [NM_001040453]	4.2302322	6.88E-04	6.88E-04
FLJ32658	147872	A_23_P431284	NM_144688	hypothetical protein FLJ32658 (FLJ32658), mRNA [NM_144688]	3.6755083	0.001644313	0.001644313
HN1	51155	A_23_P100632	NM_001002033	hematological and neurological expressed 1 (HN1), transcript variant 3, mRNA [NM_001002033]	3.6078517	6.04E-04	N.I.
KIAA0241	23080	A_24_P272967	NM_015060	KIAA0241 (KIAA0241), mRNA [NM_015060]	4.4414825	0.001038222	-0.074528231
LIX1L	128077	A_23_P342744	NM_153713	Lix1 homolog (mouse)-like (LIX1L), mRNA [NM_153713]	3.5574625	0.00178892	0.347932557
LRRC37A2	474170	A_24_P666482	NM_001006607	leucine rich repeat containing 37, member A2 (LRRC37A2), mRNA [NM_001006607]	3.6066716	0.005008937	0.095475878
MIAT	440823	A_24_P595223	NR_003491	myocardial infarction associated transcript (non-protein coding) (MIAT) on chromosome 22 [NR_003491]	3.890917	1.20E-04	N.I.
NBPF15	284565	A_32_P171181	NM_173638	neuroblastoma breakpoint family, member 15 (NBPF15), mRNA [NM_173638]	3.9074104	0.00402691	-0.066741306
NOL7	51406	A_23_P82068	NM_016167	nucleolar protein 7, 27kDa (NOL7), mRNA [NM_016167]	4.034611	2.54E-04	0.05051315
OTUD3	23252	A_24_P8575	AB007928	mRNA for KIAA0459 protein, partial cds. [AB007928]	5.1292386	0.00425537	-0.140089893
PNMA6A	84968	A_23_P84892	NM_032882	paraneoplastic antigen like 6A (PNMA6A), mRNA [NM_032882]	3.7837152	0.002480922	0.198139089
STOX2	56977	A_23_P251364	NM_020225	storkhead box 2 (STOX2), mRNA [NM_020225]	3.699861	0.002620557	0.356154185
TAGLN2	8407	A_32_P194848	NM_003564	transgelin 2 (TAGLN2), mRNA [NM_003564]	3.7862806	0.001943818	0.030681935
TPD52L2	7165	A_23_P131771	NM_199360	tumor protein D52-like 2 (TPD52L2), transcript variant 1, mRNA [NM_199360]	5.294338	0.001024691	-0.020674294
YIPF4	84272	A_23_P424080	NM_032312	Yip1 domain family, member 4 (YIPF4), mRNA [NM_032312]	4.895983	0.001400962	0.043654987
ZMAT2	153527	A_24_P226248	NM_144723	zinc finger, matrin type 2 (ZMAT2), mRNA [NM_144723]	4.33067	0.006994322	0.009221831

**Table S3 Oligonucleotides used in this study, related to Experimental Procedures**

<b>Primers for RT-PCR screening</b>		
<b>Primer name</b>		<b>Sequence 5'-3'</b>
RT-1		GCTAAATACGACTCACTATAGGGATCCNNNSACG (N = A, C, G, or T, S = C or G)
HA-specific		TATCCGTACGACGTCCCAGACTAC
RT-0		GCTAAATACGACTCACTATAGGG
<b>Primers for qRT-PCR of polysomal RNAs</b>		
<b>Primer name</b>		<b>Sequence 5'-3'</b>
GUSB	Forward	TGCCATCGTGTGGGTGAATG
	Reverse	CCCTCATGCTCTAGCGTGTC
HIST2H3A	Forward	CAAGGCCCCGAGGAAGCAGCTG
	Reverse	AGGGCAGCTTGCGGATCAGCAG
MRPL49	Forward	CCACCCAACCTGCCTTACTTT
	Reverse	TGTCCTTGTAGACGGGGATGT
NDUFS5	Forward	TGCACATGGAATCGGTTATACTC
	Reverse	CCTTCCTTTATCAGCTTATCCCG
<b>Primers for qRT-PCR of mtDNA quantitation</b>		
<b>Primer name</b>		<b>Sequence 5'-3'</b>
Human ND1	Forward	CCCTAAAACCCGCCACATCT
	Reverse	GAGCGATGGTGAGAGCTAAGGT
Human hemoglobin	Forward	GTGCACCTGACTCCTGAGGAGA
	Reverse	CCTTGATACCAACCTGCCAG
Mouse COX2	Forward	GCCGACTAAATCAAGCAACA
	Reverse	CAATGGGCATAAAGCTATGG
Mouse $\beta$ -globin	Forward	GAAGCGATTCTAGGGAGCAG
	Reverse	GGAGCAGCGATTCTGAGTAGA
<b>Primers for qRT-PCR of luciferase mRNA quantitation</b>		
<b>Primer name</b>		<b>Sequence 5'-3'</b>
FFluc	Forward	AGGTGGCTCCCGCTGAAT
	Reverse	CATCGTCTTTCCGTGCTCCA
18S rRNA	Forward	CGGCGACGACCCATTCTGAAC
	Reverse	GAATCGAACCCCTGATTCCCCGTC

**Table S4. Antibodies used in this study, related to Experimental Procedures**

<b>Supplier</b>	<b>Antigen</b>	<b>Species</b>	<b>Cat. No</b>
Aviva Systems Biology	ATP5G2	rabbit polyclonal	ARP40228_P050
Bioworld Technology	NDUFA8	rabbit polyclonal	BS3336
Bethyl Laboratories	4E-BP1	rabbit polyclonal	BL895
	eIF4B	rabbit polyclonal	A301-767A
	rictor	goat polyclonal	A300-506A
Cell Signaling Technology	eIF4A	rabbit monoclonal	2013
	eIF4E	rabbit polyclonal	9742
	Lats1	rabbit monoclonal	3477
	Mst1	rabbit polyclonal	3682
	Mst2	rabbit polyclonal	3952
	Myc-tag	mouse monoclonal	2273
	phospho-4E-BP1 (S65)	rabbit polyclonal	9451
	phospho-4E-BP1 (T37/T46)	rabbit monoclonal	2855
	phospho-mTOR (S2448)	rabbit polyclonal	2971
	phospho-mTOR (S2481)	rabbit polyclonal	2974
	phospho-S6 ribosomal protein (S235/S236)	rabbit monoclonal	4857
	phospho-S6 ribosomal protein (S240/S244)	rabbit monoclonal	2215
	raptor	rabbit monoclonal	2280
	S6 ribosomal protein	rabbit monoclonal	2217
GeneTex	NDUFS5	rabbit polyclonal	101829
Proteintech	MRPL49	rabbit polyclonal	15542-1-AP
Santa Cruz Biotechnology	eIF3b	goat polyclonal	sc-16377
	eIF4G	goat polyclonal	sc-9602
	FRAP/mTOR	goat polyclonal	sc-1549

## **SUPPLEMENTAL EXPERIMENTAL PROCEDURES**

### **Cell Culture and Plasmid Transfection**

Plasmids expressing Myc-tagged or EGFP-tagged Largin or other proteins were constructed in the pcDNA3 and/or pIRES2-EGFP vectors according to standard subcloning procedures. Jurkat cells were cultured in RPMI1640 medium supplemented with 10% FBS and antibiotics. HeLa, U2-OS, and 293T cells were cultured in DMEM supplemented with 10% FBS and antibiotics. All cells were incubated in a humidified chamber at 37°C in 5% CO<sub>2</sub>. Transfection of plasmids or siRNA into the above cells was performed using Lipofectamine 2000 (Invitrogen) in Opti-MEM supplemented with 10% FBS without antibiotics and according to the manufacturer's instructions. Unless otherwise noted, cells were cultured for 2 days after transfection before use in reporter assays or for protein extractions. Stable 293T transformants were selected by culture in the presence of Geneticin (Gibco) at 0.6 mg/ml. To ensure a higher frequency of cells expressing the transgene, GFP-positive cells among stable transformants were isolated by FACS.

### **Preparation of Cell Lysates and Immunoblotting**

Cells ( $\sim 10^7$ ) were washed twice with PBS(-), transferred into a microtube, and resuspended in CHAPS Lysis Buffer containing 40 mM HEPES (pH 7.5), 120 mM NaCl, 1 mM EDTA, 0.3% CHAPS, 50 mM NaF, 1.5 mM Na<sub>3</sub>VO<sub>4</sub>, 10 mM glycerophosphate, 10 mM pyrophosphate, and 1 mM PMSF. Tubes were rocked at 4°C for 10 min and centrifuged at

10,000 X g for 10 min at 4°C. Supernatants were recovered as the cell lysates, which were snap-frozen on dry ice and stored at -80°C until used in experiments.

For immunoblotting, equal amounts of protein (~20-40 µg) were fractionated on SDS-PAGE under reducing conditions and transferred onto a PVDF membrane. Proteins of interest were identified using the primary antibodies listed in Table S4. Primary antibodies were then visualized by incubation for 1 hr with the appropriate secondary antibodies, which were: horseradish peroxidase-conjugated secondary antibody, HRP-labeled goat anti-rabbit IgG (cat.#JM-6401-05), and rabbit anti-mouse IgG (cat.#JM-6402-05) (both from MBL International); and donkey anti-goat IgG-HRP (cat.#sc-2056) (Santa Cruz Biotechnology). Bands were detected using ECL-Plus (GE Healthcare).

### **Polysome Fractionation and Microarray Analysis**

Cytoplasmic extracts prepared from exponentially-growing 293T cell clones were centrifuged on pre-chilled 10-50% sucrose gradients at 35,000 rpm for 2 hr at 4°C in a Beckman SW40Ti rotor, as described previously (Mamane et al., 2007; Dowling et al., 2010). Gradients were fractionated and the UV-absorbance at 254 nm was continuously recorded using an ISCO fractionator (Teledyne ISCO). Light and heavy polysome fractions were prepared from three independent sucrose gradient fractionations, and RNA from each fraction was isolated using Trizol (Invitrogen). RNA was subjected to microarray analysis at the UHN Microarray Centre (<http://www.microarrays.ca/>) using Whole Human



Genome 4x44k arrays (Agilent Technologies). The unpaired Student t-test was used to evaluate differences in mRNA levels between the heavy and light polysome groups, and the Benjamini & Hochberg FDR method (Benjamini and Hochberg, 1995) was used for multiple testing corrections. A list of genes exhibiting a fold-change >3.5 (using corrected  $p < 0.05$ ) was compared between parental control cells and 2D10 cells to create Table S2.

### **Quantitative Real-Time PCR (qRT-PCR)**

RNA from individual sucrose gradient fractions was isolated using Trizol (Invitrogen). Purified RNA (1  $\mu\text{g}$ ) was subjected to reverse transcription (RT) using the iScript cDNA Synthesis Kit (BIO-RAD) according to the manufacturer's instructions. Equal amounts of each RT reaction were amplified on a 7900HT Fast Real-Time PCR System (Applied Biosystems) using Power SYBR Green PCR Master Mix (Applied Biosystems) and the primer sets listed in Table S3. Relative quantitation of the abundance of an mRNA in a given polysome fraction was performed using the  $\Delta\Delta\text{Ct}$  method. Values were normalized to the value obtained for each mRNA in the corresponding input RNA.

For mitochondrial DNA/nuclear DNA quantitation, genomic DNA was purified from liver or cultured cells and serially diluted to estimate the range of templates necessary for linear amplification. Typically, 8-32 ng of genomic DNA was used for qRT-PCR, which was performed as described above using the primer sets listed in Table S3.

For luciferase mRNA quantitation, total RNAs were purified from transfected cells using the NucleoSpin RNAII kit (Macherey-Nagel) and converted to 1<sup>st</sup> strand cDNAs as

described above. qRT-PCR was performed as described above using the primer sets listed in Table S3.

### **Purification of Mitochondria**

Mitochondria were isolated from cultured cells or mouse liver using the Mitochondria Isolation Kit for Cultured Cells or Tissue (Thermo Scientific), respectively, following the manufacturer's instructions. Mitochondrial pellets were resuspended in 1xTBS containing 2% CHAPS followed by centrifugation at 20,000 x g for 2 min at 4°C. Supernatants were recovered as mitochondrial protein extracts and subjected to immunoblotting as described above.

### **Mitochondrial Mass Measurement**

Cells were stained with 50 nM Mitotracker Red FM or Mitotracker Green FM (Invitrogen) in PBS buffer at 37°C for 15 min, followed by washing and resuspension in PBS. Samples were evaluated on a FACSCalibur (BD Biosciences) and data were analyzed with CellQuest Pro version 5.2 software (BD Biosciences). The mean fluorescence intensity in the FL1 or FL3 channel was used as an estimate of mitochondrial mass.

### **Immunocytochemistry**

Cells ( $2 \times 10^5$ ) of individual 293T clones were seeded in a glass bottom 35 mm dish, cultured overnight, washed with PBS(-), and fixed in 4% formaldehyde. To visualize

mitochondria and nuclei, cells were counterstained with Mitotracker Red FM (Invitrogen) and 4',6-diamino-2-phenylindole (DAPI) (Invitrogen). Microscopic observations were made and photographic images captured using an Olympus confocal microscope (FV1000).

### **Oxygen Consumption Rate**

Measurement of oxygen consumption was performed using a Seahorse XF96 analyzer (Seahorse Bioscience). Exponentially-growing Jurkat and 2D10 cells were collected by centrifugation, resuspended in unbuffered RPMI1640 medium, and seeded at  $1.5 \times 10^5$  cells/well in XF96 plates precoated with CELL-TAK (BD Biosciences). Cells were equilibrated in unbuffered medium for 45 min at 37°C in a CO<sub>2</sub>-free incubator prior to transfer to the XF96 analyzer. The basal oxygen consumption rate (OCR) was measured before sequential injections of 1 μM (final concentration) oligomycin (Sigma-Aldrich) were applied to halt ATP synthesis (negative control).

### **ATP Measurement**

Total ATP levels in cells ( $4 \times 10^5$ ) of individual 293T clones were measured by chemiluminescence using the ATPlite ATP detection kit (Perkin Elmer) according to the manufacturer's protocol. Absolute ATP levels were determined using an ATP standard curve established in parallel in each 96-well plate assayed.

## **Cell Cycle Analysis**

For cell cycle and proliferation analyses, BrdU incorporation was assessed using the APC BrdU Flow Kit (BD Pharmingen). Briefly, Jurkat or 2D10 cells were seeded in 6-well plates ( $1 \times 10^5$ /well) and cultured with or without 1  $\mu\text{g/ml}$  DOX. After 24 hrs, 10  $\mu\text{M}$  BrdU was added to each well for an additional 30 min incubation. Cells were then fixed and immunostained according to the manufacturer's protocol. Flow cytometric analysis of incorporated BrdU was performed using a FACSCalibur (BD Biosciences) and FlowJo software (Tree Star).

## **SUPPLEMENTAL REFERENCES**

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