McKnight et al Supplementary Materials

Supplementary Figure Legends

Supplementary Table S1. Top 1000 hits from the primary screen. The 500 best increasers, and 500 best decreasers from the primary screen are presented separately. The analysis was done as described in Methods. SCPO (spot count per object), STAPO (spot total area per object), STIPO (spot total intensity per object), OC (object count) for three replicates (r1-r3) are shown. Dharmacon catalogue numbers for the siGenome SMARTpools are also indicated.

Supplementary Table S2. Autophagy genes identified in screen. 17 known autophagy genes and isoforms were selected from the primary screen and shown here are the median Z-scores and corresponding ranks as decreasers for SCPO, STIPO, STAPO, and rank products (RP).

Supplementary Table S3. Deconvolution screen of 190 genes. 760 siRNA duplexes were tested in triplicates and their median scores are expressed as percent of control as described in Methods. For each parameter (STIPO, SCPO, STAPO) scores above 1.2 are classified as positive, denoted as 1 in the positive column, scores below 0.8 are classified as negative, denoted as 1 in the negative column. Successful deconvolution is noted as 'yes' in 2 out of 4 duplexes column and is achieved when at least 2 out of 4 duplexes for any parameter repeat the primary screen and when the other 2 duplexes do not have the opposite effect in more than one parameter. 3 or 4 out of 4 duplexes is achieved when at least 3 out of 4 duplexes repeat the primary screen in any of the parameters. GAB1, previously suggested to be required for basal autophagy (Lipinski et al, 2010), is one such case where two duplexes had the opposite effect.

Supplementary Table S4. 51 validated-by-deconvolution hits.
51 candidates are listed with official gene and protein annotations, and GO slim terms, cellular components and panther analysis. 51 hits were analysed for GO term annotations using the Generic GO term Mapper and generic GO slim terms (http://go.princeton.edu/), (http://go.princeton.edu/GOTermMapper/goSlimFiles/goslim_generic.obo) and analysed using Panther annotation for protein class (http://www.pantherdb.org). The

p-values for GO terms were obtained using g:profiler (http://biit.cs.ut.ee/gprofiler/). Genes were also manually annotated using publically available data.

Supplementary Figure S1. qRTPCR of 11 increasers. qRTPCR was performed as described in Methods. Data shown is from a representative experiment; error bars represent standard deviation of triplicates. The number of times the experiment was performed is indicated.

(A) ADHFE1 (n=2)

(B) CNOT1 (n=2 duplex02 n=1 duplex04)

(C) FBXL14 (n=1 duplex01 n=2 duplex04)

(D) KIF25 (n=4 duplex01 n=3 duplex03)

(E) RASGRF2 (n=3)

(F) RASIP1 (n=3 duplex03 n=2 duplex04)

(G) RBM12 (n=1 duplex03 n=2 duplex04)

(H) SNX20 (n=1)

(I) STAT2 (n=1 duplex01 n=2 duplex04)

(J) TLK2 (n=4 duplex01 n=2 duplex03)

(K) WDR6 (n=2)

Supplementary Figure S2. qRTPCR of 9 decreasers. qRTPCR was performed as described in Methods. Data shown is from a representative experiment; error bars represent standard deviation of triplicates. The number of times the experiment was performed is indicated.

(A) C1orf198 (n=1)

(B) CDH19 (n=3)

(C) GHSR (n=2)

(D) LARP1 (n=1)

(E) NAA25 (n=1)

(F) PAFAH1B2 (n=1)

(G) SCOC (n=4)

(H) SUPT5H (n=1)

(I) WAC (n=3)

Supplementary Figure S3. Validation using LC3 lipidation in HeLa cells.

(A)-(I), HeLa cells were treated with RISCfree or indicated siRNA duplexes, incubated in full medium (FM), starvation medium (ES), and ES with leupeptin (EL) for two hours. Note: candidate siRNA knock-downs were performed in duplicate. LC3 lipidation was analysed by anti-LC3 and anti-Actin western blotting, duplicates were quantified (see Methods), averaged and shown as LC3II/LC3I normalised to FM. When multiple experiments were performed (n=x) averaged values were averaged again from the multiple experiments. **(A)** KIF25 duplex-03 (n=3), bars represent the average of 3 experiments; **(B)** RASIP1 duplex-03 (n=2), bars represent the average of 2 experiments; **(C)** TLK2 duplex-03 (n=2), bars represent the average of 2 experiments; **(D)** WDR6 duplex-02 (n=2), bars represent the average of 2 experiments; **(E)** PAFAH1B2 duplex-04 (n=1), siPAFAH1B2 bars represent the average of duplicates; **(F)** LARP1 duplex-01 (n=1), siLARP1 bars represent the average of duplicates; **(G)** SCOC duplex-01 (n=4), bars represent the average of 4 experiments; error bars represent s.e.m, significance was determined using a two-tailed paired t-test: RF FM vs siSCOC-03 FM p=0.0238; RF EL vs siSCOC-03 EL p=0.0166; **(H)** SUPT5H duplex-01 (n=2), bars represent the average of 2 experiments; **(I)** WAC duplex-03 (n=3), bars represent the average of 3 experiments; error bars represent s.e.m, significance was determined using a two-tailed paired t-test: RF ES vs siWAC-03 ES p=0.0203.

Supplementary Figure S4. Validated-by-deconvolution hits in autophagy interaction network (AIN). NRBF2 co-immunoprecipitates with SCOC. WAC co-immunoprecipitates with Beclin1.

(A) List of 9 hits from our siGenome screen with the corresponding autophagy gene "Bait" to which the hit protein was found to bind during a proteomic analysis of the autophagy interaction network (Behrends et al, 2010). P-value, WD_Score and Z-Score from the proteomics screen is indicated. **denotes our siRNA screen hits that passed further validation (summarised in Fig. 2B). **(B)** Anti-Myc and -FLAG blots after indicated co-expression of FLAG-SCOC and Myc-NRBF2 in HEK293 cells and co-immunoprecipitation with anti-FLAG (left) or anti-Myc (right). Co-immunoprecipitation (IP), input lysate (Inp), unbound supernatant (Unb). **(C)** Anti-WAC and -GFP blots after indicated co-expression in HEK293 cells and indicated medium conditions. Co-immunoprecipitation of Myc-WAC plus GFP or plus GFP-Beclin with anti-GFP antibody.

Supplementary Figure S5. Knock-down of SCOC inhibits p62 degradation.
Western blots of p62 and actin from three separate experiments done as described in Figure 4B (siRNA depletion of SCOC, middle five lanes 6 – 10) and Figure 5A (siRNA depletion of FEZ1, last five lanes 11-15). Quantification is shown in Figure 4B and 5A for siSCOC and siFEZ1, respectively.

Supplementary Figure S6. FEZ1 is a negative regulator of autophagy.
(A) Knock-down of FEZ1 enhances autophagy. Anti-Actin and -LC3 blot after indicated siRNA in HEK293 cells. A representative blot is shown. Quantification of LC3II/actin from the average of two experiments with duplicate samples; values are normalised to RISCfree EL. qRTPCR showing FEZ1 mRNA levels after indicated knock-down in GFP-LC3-HEK cells (error bars represent SD of triplicates). **(B)** Overexpression of FEZ1 inhibits autophagy. Anti-Actin, -LC3, and -GFP blot after transfection with pcDNA or FEZ1-GFP in HEK293 cells. A representative blot is shown. Quantification of LC3II/actin from the average of three experiments with duplicate samples; values are normalised to pcDNA EL; error bars represent s.e.m. (n=3); pcDNA EL vs FEZ1-GFP EL p=0.0444.

Supplementary Figure S7. FEZ1 mutant that does not bind SCOC still binds to ULK1, and SCOC does not bind directly to ULK1.
(A) MBP, MBP-FEZ1, either the wild-type (WT) or point mutations inhibiting binding to SCOC (L254P/L260P) were expressed in bacteria and purified. In vitro translated ^{35}S-Myc-ULK1 was added and the MBP-tagged proteins were immobilized and the bound ULK1 was detected by autoradiography (top). The input MBP, and MBP-FEZ1 proteins were detected by coomassie blue staining (CB) bottom panel. 10% input is derived from ^{35}S-Myc-ULK reaction mix. **(B)** SCOC does not bind to ULK1. In vitro translated ^{35}S-myc-ULK1 failed to bind to GST-SCOC in pull-down assays.

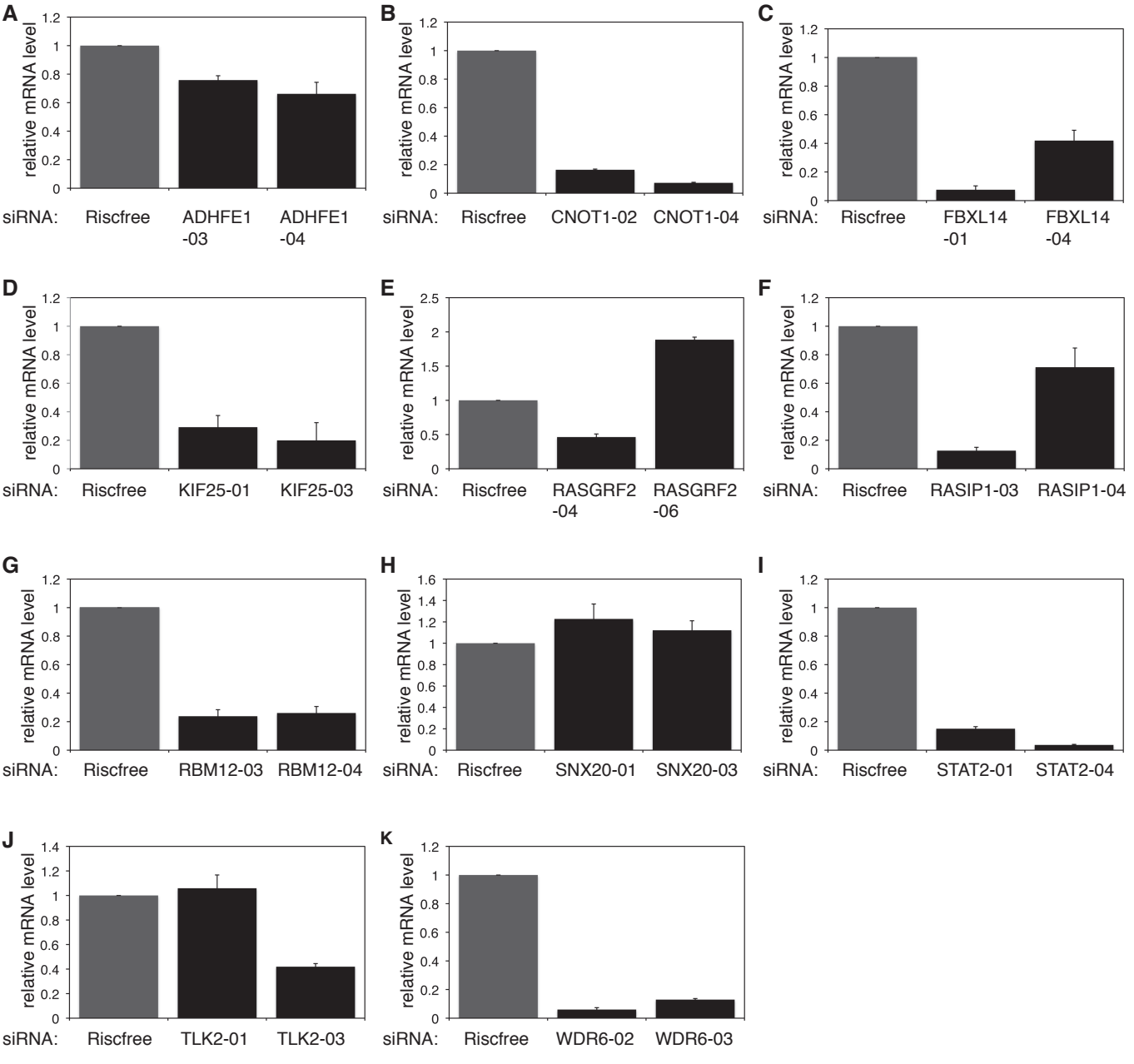
References

Behrends C, Sowa ME, Gygi SP, Harper JW (2010) Network organization of the human autophagy system. *Nature* **466:** 68-76

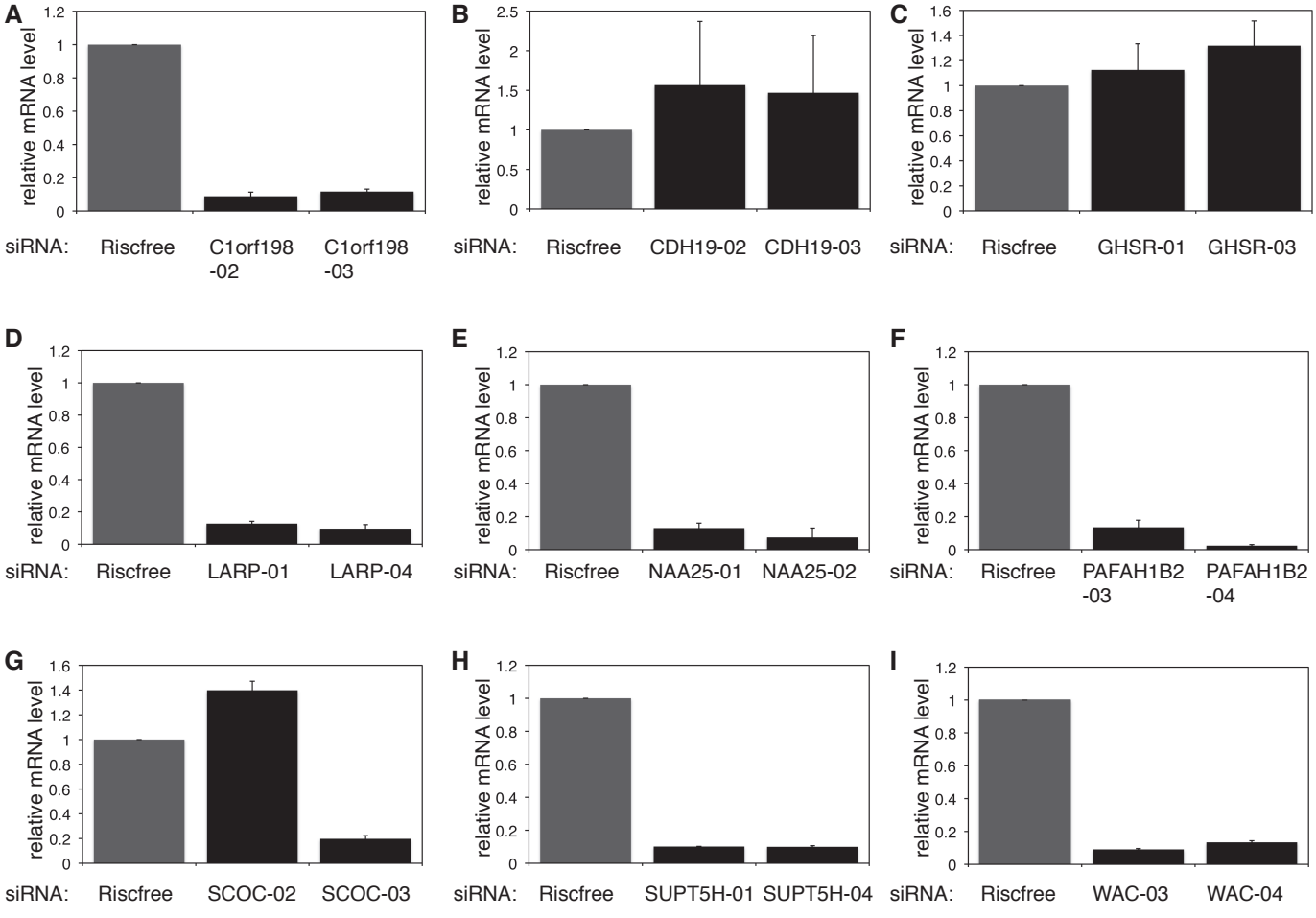
Lipinski MM, Hoffman G, Ng A, Zhou W, Py BF, Hsu E, Liu X, Eisenberg J, Liu J, Blenis J, Xavier RJ, Yuan J (2010) A Genome-Wide siRNA Screen Reveals Multiple mTORC1 Independent Signaling Pathways Regulating Autophagy under Normal Nutritional Conditions. *Dev Cell* **18:** 1041-1052

Accession no	Gene ID	Gene Symbol	Dharmacon name	Dharmacon cat no	Median SCPO	Rank by SCPO	Median STIPO	Rank by STIPO	Median STAPO	Rank by STAPO	Rank product (RP) as decreasers
NM_032852	84938	**ATG4C**	APG4C	M-005788-00	-2.199	115	-2.073	73	-2.451	32	64.5
NM_003565	8408	**ULK1**	ULK1	M-005049-00	-2.012	192	-2.207	40	-1.969	164	108.0
XM_375080	22863	**ATG14/Barkor**	KIAA0831	M-020438-00	-1.558	666	-1.686	295	-1.051	1906	720.8
NM_024085	79065	**ATG9A**	FLJ22169	M-014294-00	-1.536	699	-1.401	731	-1.38	883	767.0
NM_014781	9821	**FIP200**	RB1CC1	M-021117-00	-1.538	695	-1.225	1209	-1.573	562	778.7
NM_006395	10533	**ATG7**	APG7L	M-020112-00	-1.006	2218	-1.026	1850	-0.992	2129	2059.5
NM_022818	81631	**MAP1LC3B**	MAP1LC3B	M-012846-00	-0.884	2881	-1.328	886	-0.674	4041	2176.8
NM_022488	64422	**ATG3**	APG3	M-015375-00	-0.73	3707	-0.954	2246	-1.204	1365	2248.3
NM_004707	9140	**ATG12**	APG12L	M-010212-01	-0.765	3494	-0.697	3739	-0.845	2876	3349.3
NM_003766	8678	**BECN1**	BECN1	M-010552-00	-0.506	5370	-0.593	4694	-0.634	4403	4805.7
NM_014683	9706	**ULK2**	ULK2	M-005396-01	-0.355	6601	-0.375	6297	-0.287	7065	6646.9
NM_014741	9776	**ATG13**	KIAA0652	M-020765-00	0.253	12362	-0.219	7715	-0.361	6466	8511.8
NM_013325	23192	**ATG4B**	APG4B	M-005786-00	-0.131	8581	-0.063	9215	-0.09	8998	8927.4
NM_052936	115201	**ATG4A**	APG4A	M-005789-00	0.004	9965	-0.011	9703	0.038	10548	10065.9
XM_290517	23130	**ATG2A**	KIAA0404	M-026591-00	-0.037	9416	0.024	10158	0.0865	10912	10143.6
NM_173681	285973	**ATG9B**	NOS3AS	M-018082-00	0.152	11510	-0.009	9668	0.303	12988	11306.2
NM_015610	26100	**WIPI2**	DKFZP434J154	M-020521-00	0.27	12546	0.03	10322	0.282	12713	11807.9
NM_004849	9474	**ATG5**	APG5L	M-004374-02	0.415	13888	0.293	12610	0.266	12656	13038.2
NM_017974	55054	**ATG16L1**	APG16L	M-021033-00	0.486	14485	0.633	15301	0.122	11281	13572.6
NM_031482	83734	**ATG10**	APG10L	M-019426-00	1.347	19048	2.111	19814	1.364	19034	19295.3

McKnight Supplementary Table S2



McKnight Supplementary Figure S1



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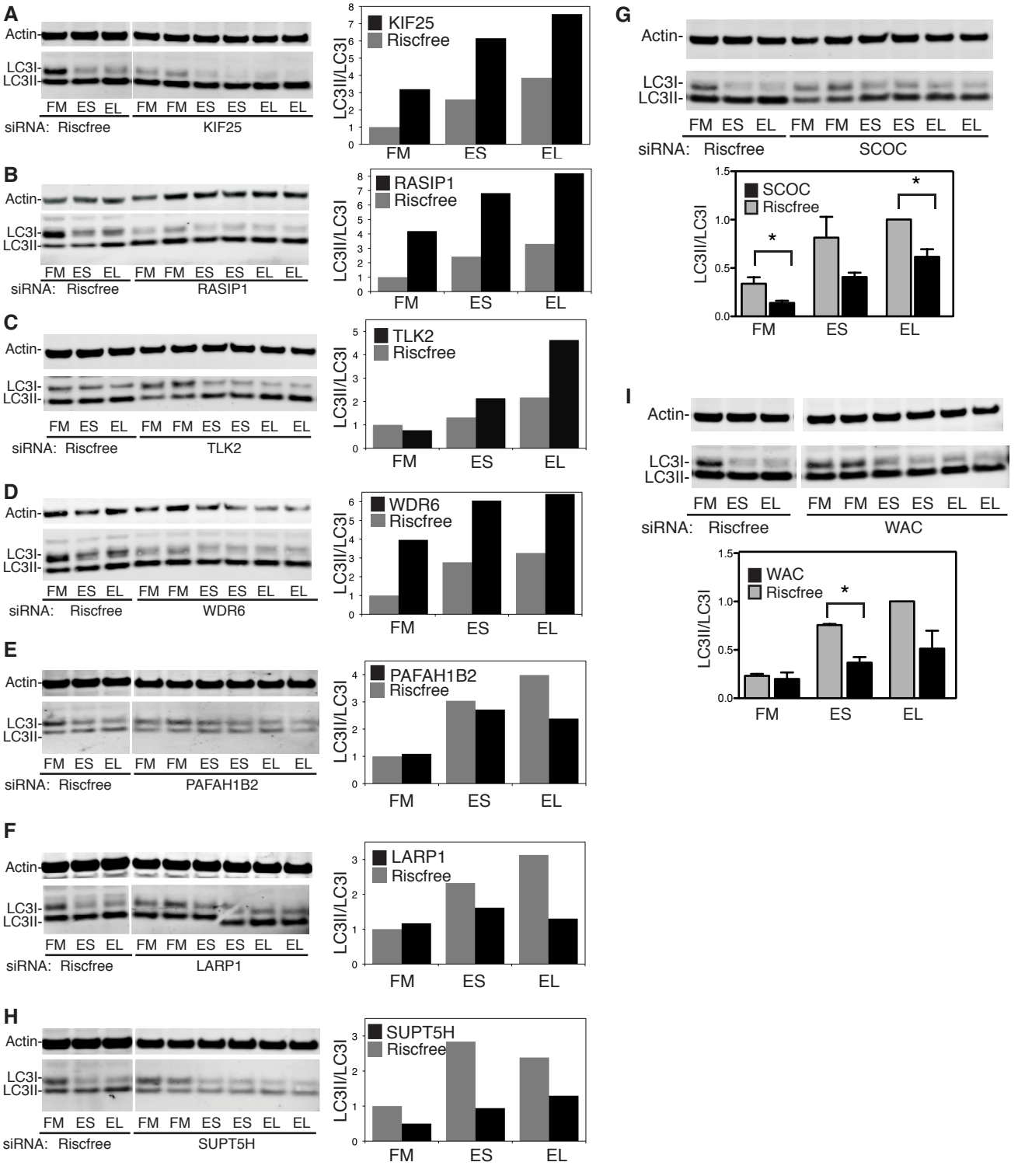


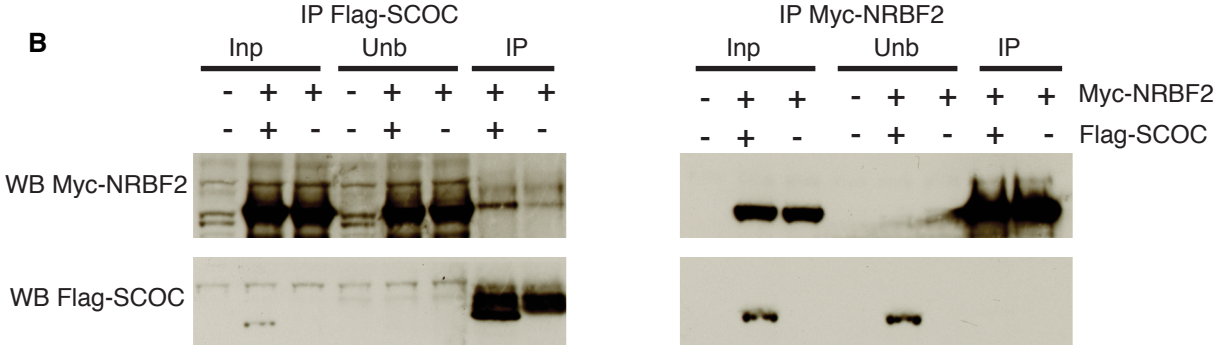
McKnight Supplementary Figure S3

A

siRNA screen hit	Bait*	p_value*	WD_Score*	Z_Score	siRNA screen hit	Bait*	p_value*	WD_Score*	Z_Score
WAC **	BECN1	0.28773972	236.17	12.88	CSTB	PRKAB1		3.73	2.99
SCOC **	UVRAG	0.28773972	118.09	9.11	CSTB	PRKAA2		2.15	0.67
SCOC **	NRBF2	0.28773972	118.09	9.11	CSTB	PRKAA1		2.15	0.67
DNAJA3	WIPI2		18.89	5.86	CSTB	PIK3C3		9.28	4.15
DNAJA3	UVRAG		4.48	1.97	CSTB	PDPK1		2.15	0.67
DNAJA3	SQSTM1		9.45	1.19	CSTB	NEK9		3.73	2.99
DNAJA3	RABGAP1		2.58	0.41	CSTB	MAP1LC3A		3.05	1.83
DNAJA3	PRKAB2		11.57	1.97	CSTB	KIAA0265		3.05	1.83
DNAJA3	ATG2A		18.89	5.86	CSTB	HIF1A		3.73	2.99
DNAJA3	GBAS		3.66	1.19	CSTB	GOSR1		2.15	0.67
DNAJA3	CLN3		5.78	3.52	CSTB	GABARAPL1		6.56	1.83
DNAJA3	CAMKK2		5.17	2.75	CSTB	FYCO1		6.56	1.83
DNAJA3	BECN1		3.66	1.19	CSTB	TECPR1		6.56	1.83
DNAJA3	ATG5		2.58	0.41	CSTB	ATG10		9.28	4.15
DNAJA3	ATG4C		3.66	1.19	CSTB	ATG4C		2.15	0.67
NUP160	WIPI2		7.46	4.15	CSTB	ATG4B		6.56	1.83
NUP160	GBAS		26.24	2.69	LARP1 **	WDR45		3.44	-0.04
NUP160	C12orf44		6.09	2.69	LARP1 **	ULK2		4.22	0.42
C1orf25	ULK1		2.53	0.9	LARP1 **	ULK1		1.86	-0.49
C1orf25	PRKAA1		14.36	6.1	LARP1 **	RASSF5		3.44	-0.04
C1orf25	CLN3		3.58	2.2	LARP1 **	RABGAP1		4.87	0.87
C8orf33	ULK1		3.05	1.41	LARP1 **	PRKAB2		1.86	-0.49
C8orf33	PRKAA1		4.31	3.14	LARP1 **	PRKAA1		5.96	1.77
C8orf33	PDPK1		3.05	1.41	LARP1 **	NSMAF		3.44	-0.04
C8orf33	KBTBD7		3.05	1.41	LARP1 **	NSF		2.27	-0.26
CSTB	WIPI2		2.15	0.67	LARP1 **	KIAA0831		2.62	-0.04
CSTB	ULK2		2.15	0.67	LARP1 **	KIAA0265		4.87	0.87
CSTB	TRAF2		3.05	1.83	LARP1 **	TECPR1		1.31	-0.71
CSTB	STK4		2.15	0.67	SUPT5H **	FYCO1		10.01	5.23
CSTB	SQSTM1		9.28	4.15					

** Passed further validation experiments

B



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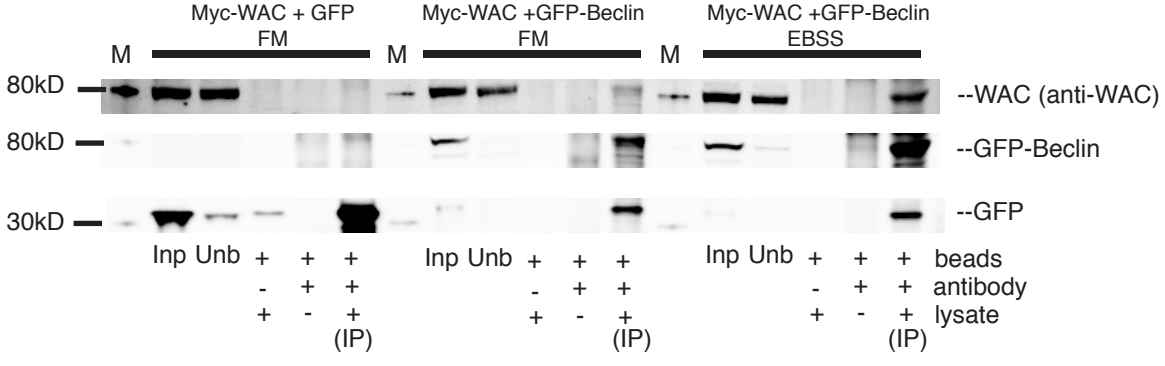
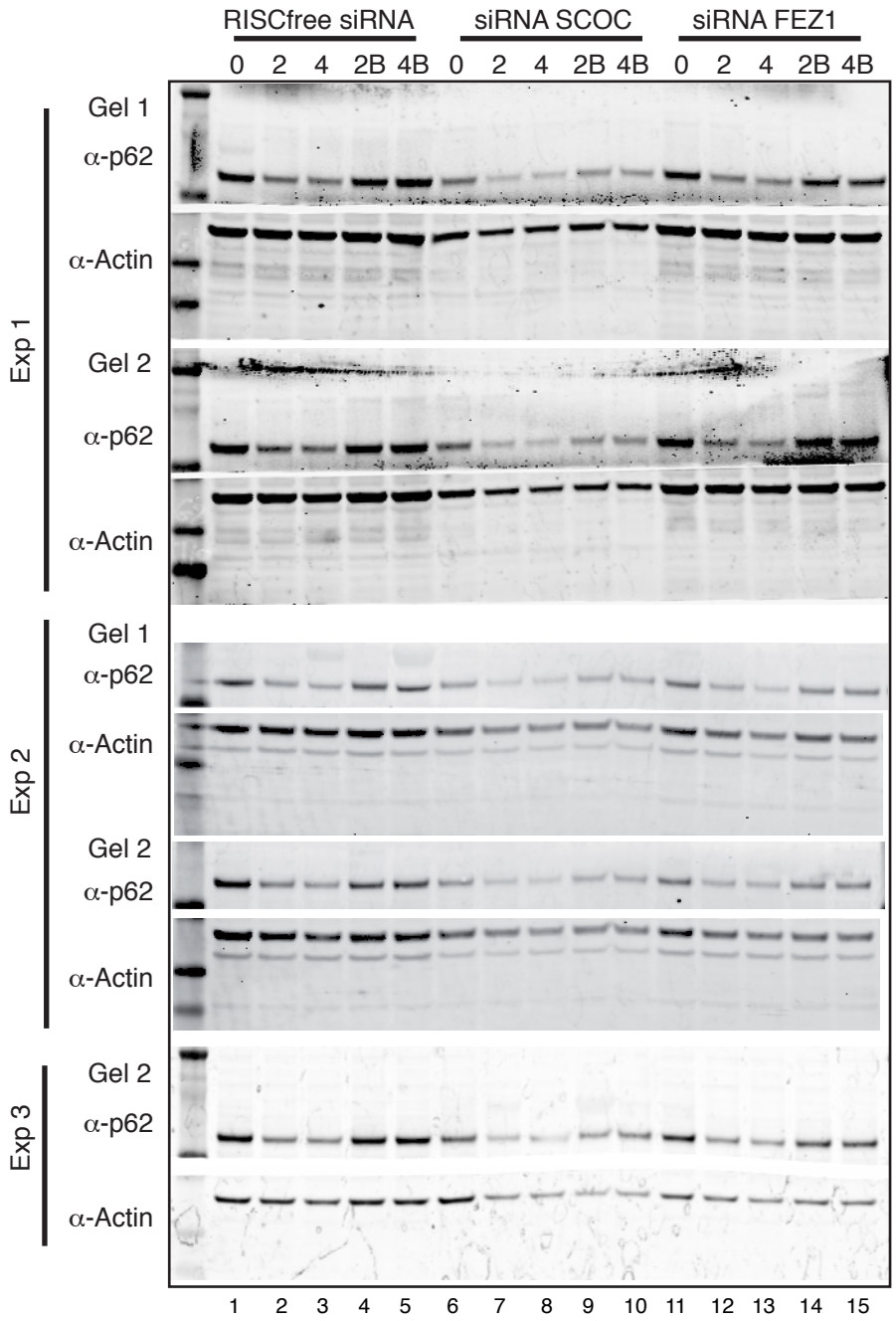
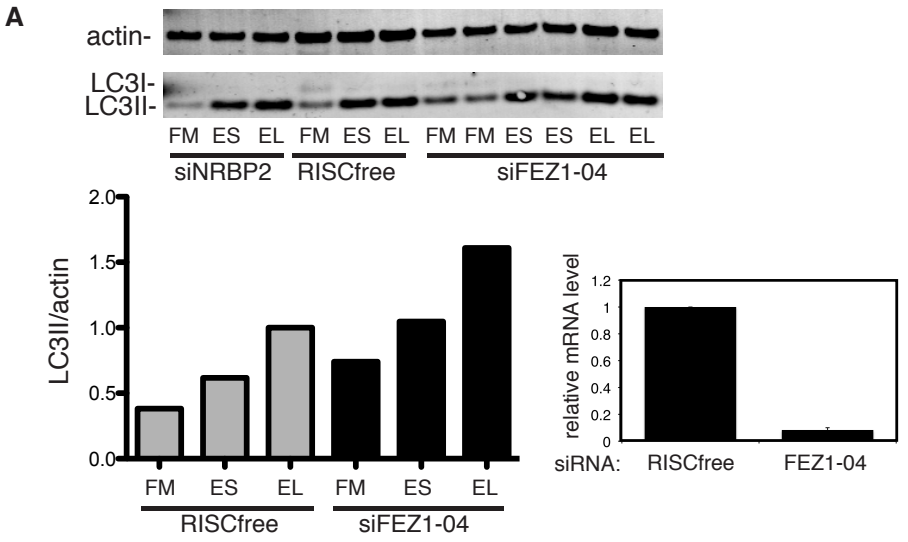
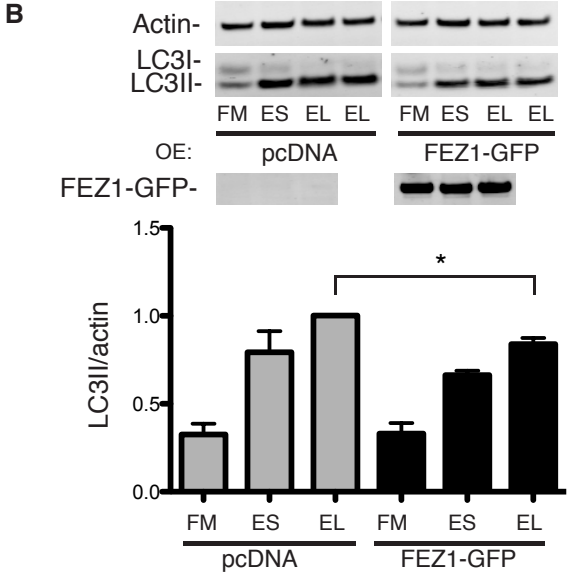


McKnight Supplementary Figure S4

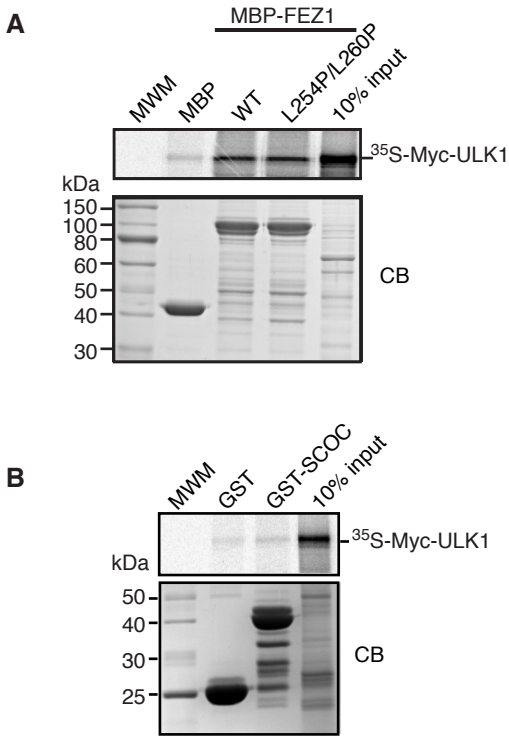


McKnight Supplementary Figure S5





McKnight Supplementary Figure S6



McKnight Supplementary Figure S7