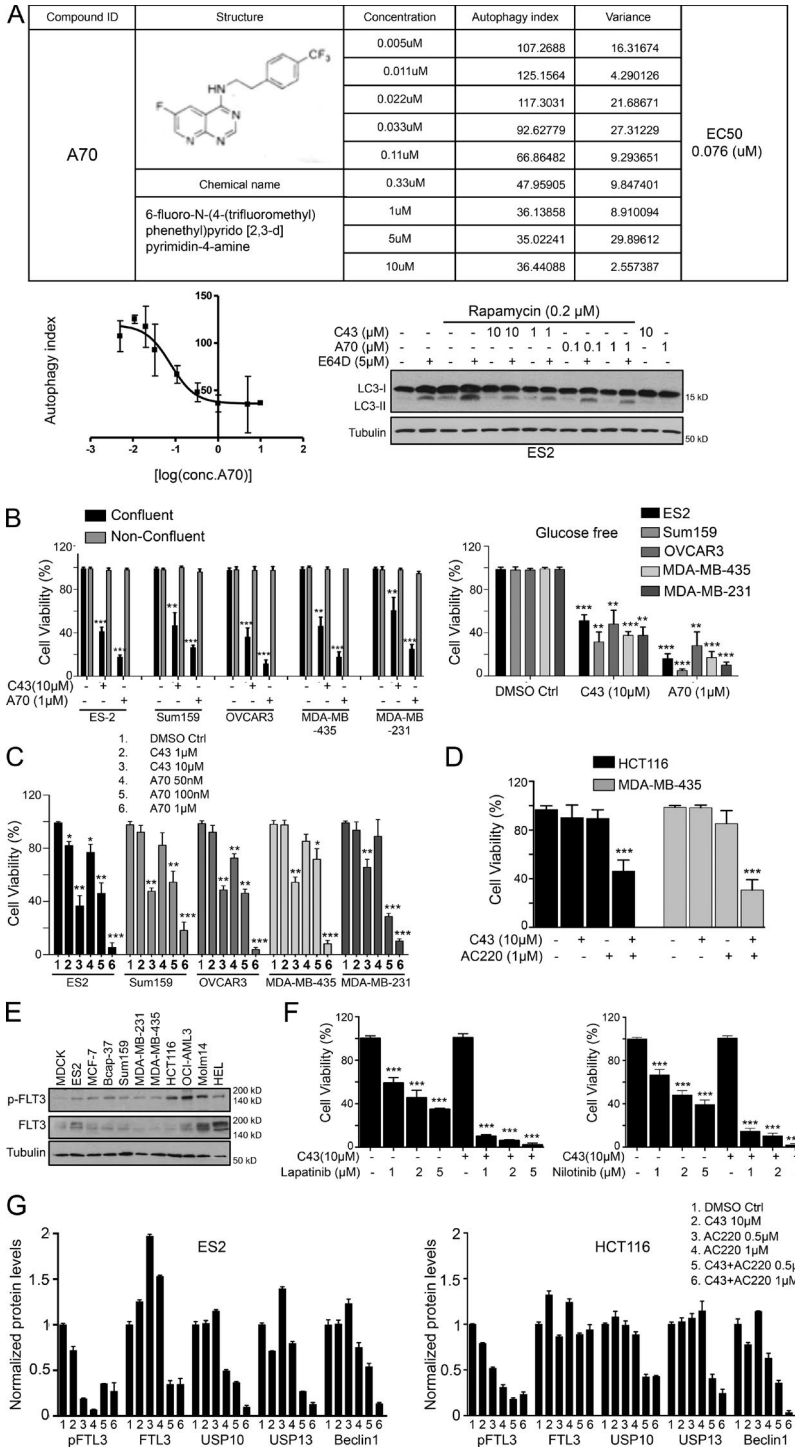

Figure S1. **Development of A70 as a novel and more potent autophagy inhibitor.** (A) The structure of A70 and autophagy index in response to A70 (μM). WB of LC3 protein levels in ES2 cells treated with increasing concentrations of A70 or C43 in the presence of rapamycin for 12 h is shown. (B) Cell viability (%) of indicated cells in confluent, nonconfluent (Non-C; 24 h), or glucose-free (6 h) conditions treated with C43 or A70. (C) Cell viability (%) of indicated cells in confluence treated with increasing concentration of A70 or C43 for 24 h. (D) Cell viability (%) of HCT116 and MDA-MB-435 cells treated with AC220 and C43 for 24 h. (E) Phospho- and total FLT3 protein levels of all indicated cell lines. (F) Cell viability (%) of ES2 cells treated with increasing concentrations of Lapatinib or Nilotinib and C43 for 16 h. (G) The quantification of protein degradation upon AC220 and/or C43 treatment presented in Fig. 1 H as bar graphs. Anti–α-tubulin was used as a loading control. Cells were treated with 0.1% DMSO (control: vehicle) or 1 μM AC220 and 10 μM C43, unless otherwise indicated. In all the experiments, treatment groups were compared with the control group, unless otherwise indicated. Error bars indicate ±SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

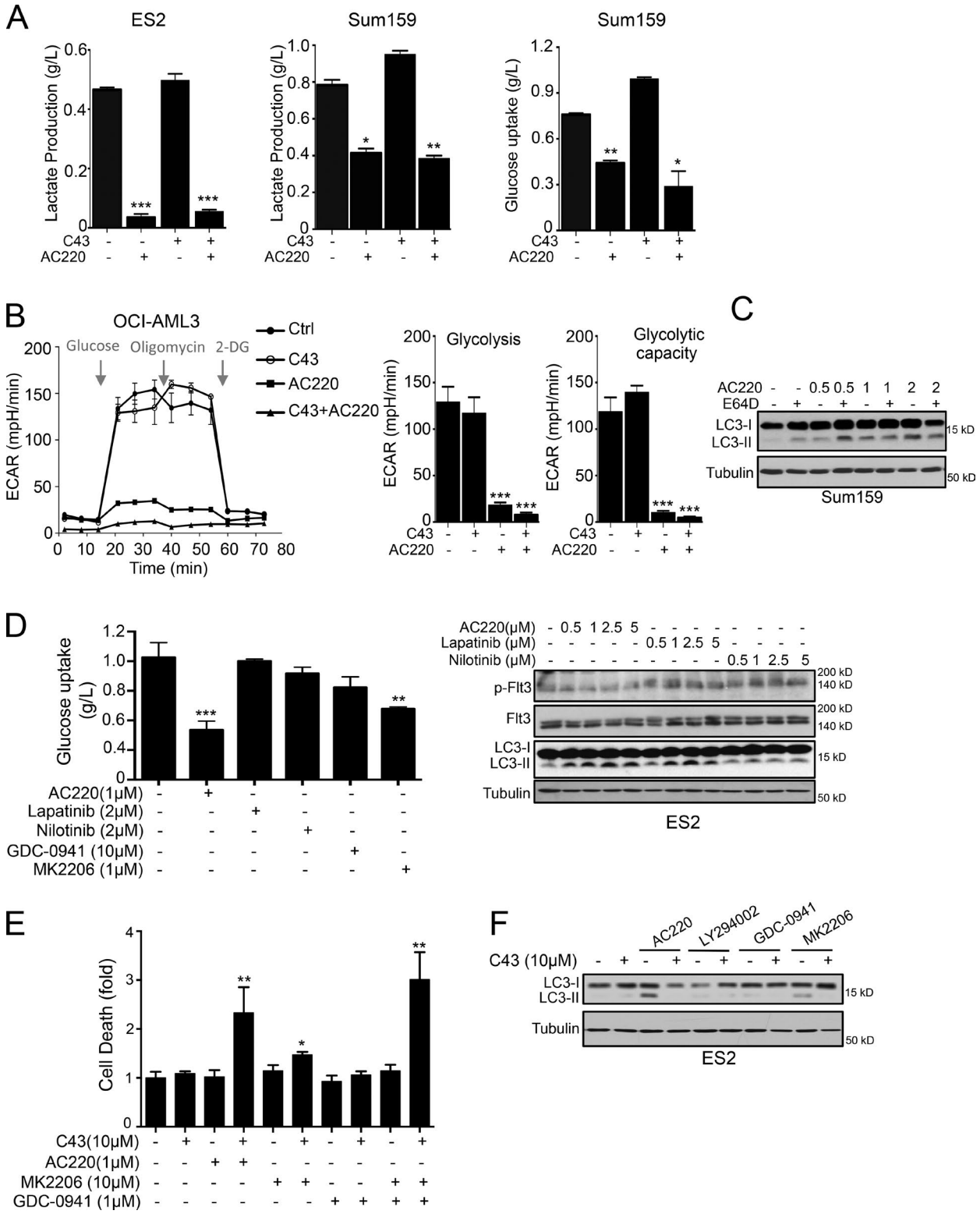

Figure S2. **The effect of combination treatment of AC220 and spautins on metabolism and cellular growth pathways.** (A) Relative changes of glucose or lactate levels in the cell medium of ES2 and Sum159 cells treated with AC220 and/or C43 (normalized to cell numbers) for 16 h. (B) The glycolytic activity and maximum glycolytic capacity of OCI-AML3 cells, determined by ECAR, after AC220 and C43 treatment for 8 h. (C) WB of LC3 protein levels in Sum159 cells, treated with increasing concentrations of AC220 in the presence or absence of 5 μM E64D for 16 h. (D) Relative changes of glucose levels in the culture medium of ES2 cells treated with the indicated inhibitors, and phospho- and total FLT3, LC3 protein levels in ES2 cells treated with increasing concentrations of AC220, Lapatinib, or Nilotinib for 16 h. (E) Cell death (fold) of ES2 cells treated with AC220, MK2206, or GDC-0941 for 24 h. (F) LC3 protein levels in ES2 cells treated with indicated inhibitors for 16 h. Anti–α-tubulin was used as a loading control. Cells were treated with 0.1% DMSO (control: vehicle) or 1 μM AC220 and 10 μM C43, unless otherwise stated. In all the experiments, treatment groups were compared with the control group, unless otherwise indicated. Error bars indicate ±SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

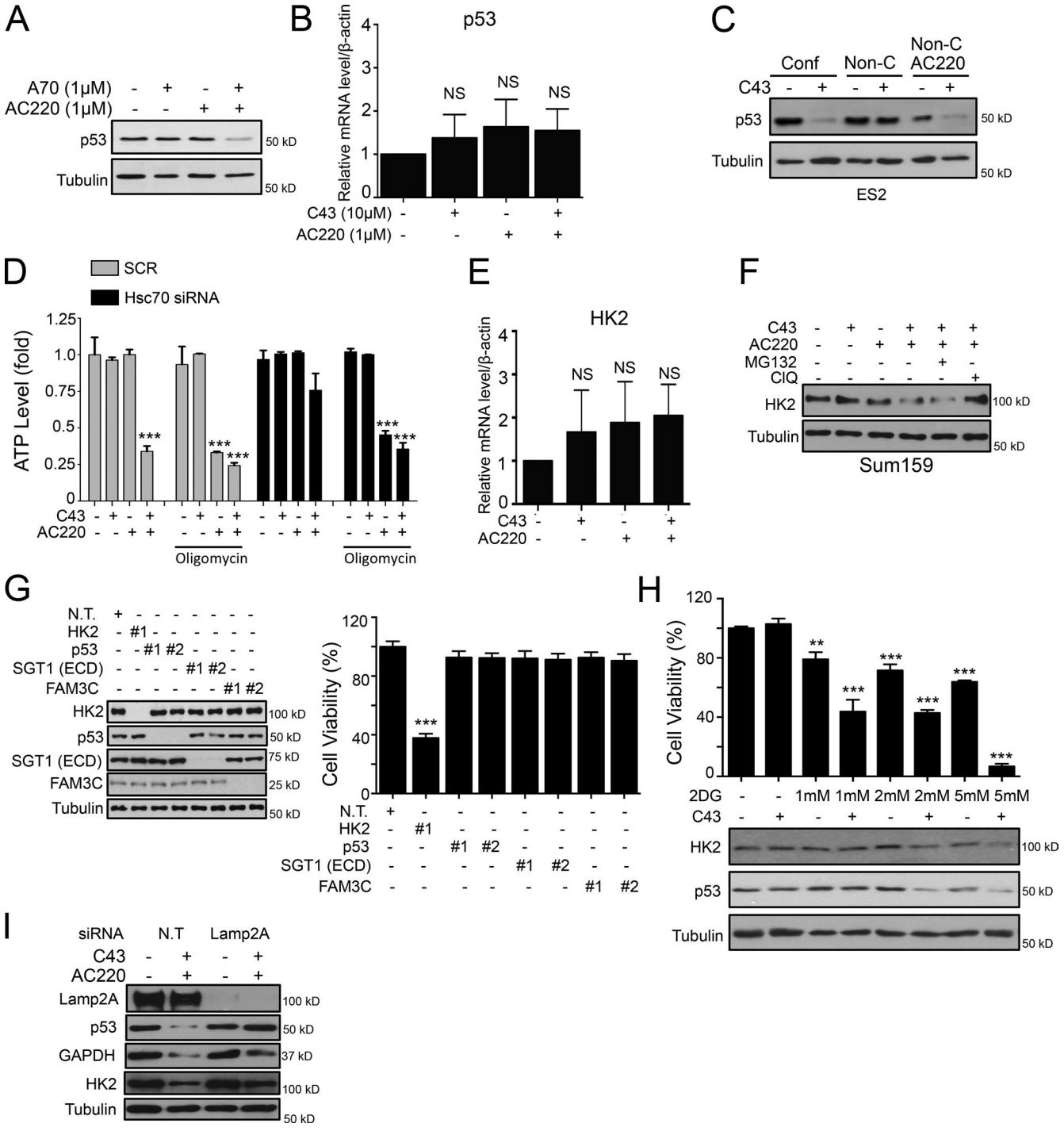

Figure S3. **The effect of combination treatment of AC220 and spautins on expression levels of proteins involved in metabolism and cell death.** (A) Mutant p53 levels in ES2 cells treated with AC220 and/or C43 for 24 h. (B) The levels of HK2 mRNAs in ES2 cells treated with AC220 and/or C43 for 16 h. (C) WB of the mutant p53 levels in ES2 cells treated with AC220 and/or C43 in different growth conditions for 24 h. (D) The cellular ATP levels in scramble (SCR) or Hsc70 siRNA–transfected ES2 treated with AC220 and/or C43 in the absence or presence of oligomycin for 16 h. (E) The expression levels of HK2 mRNAs in ES2 cells treated with AC220 and/or C43 for 16 h. (F) HK2 protein levels in Sum159 cells treated with AC220 and/or C43 for 24 h in the absence or presence of proteasome (MG132) or lysosomal inhibitor (ClQ). (G) Cell viability (%) of nontargeting (N.T.), HK2, p53, SGT1, or FAM3C siRNA–transfected ES2 cells treated with AC220 and/or C43 for 16 h. WB confirmed the siRNA knockdown efficiencies. (H) Cell viability (%) and WB of ES2 cells treated with indicated concentrations of 2DG and/or C43 for 16 h. (I) WB of HK2, p53, and GAPDH levels in ES2 cells transfected with nontargeting (N.T) or Lamp2A siRNA, treated with AC220 and C43 for 12 h. Anti–α-tubulin was used as a loading control. Cells were treated with 0.1% DMSO (control: vehicle) or 1 μM AC220 and 10 μM C43, unless otherwise indicated. In all the experiments, treatment groups were compared with control group, unless otherwise shown. Error bars indicate ±SD. **, $P < 0.01$; ***, $P < 0.001$.

Table S1. **List of statistically significant (P < 0.05) proteins identified with a quantitative proteomics approach using TMT MS spectral data**

Fold decrease	P-value	Gene symbol	Gene description	CMA targeting motif
1.29	0.001	RPL10A	60S ribosomal protein L10a	QKDKR
1.66	0.002	EHD2	EH domain-containing protein 2	FRDIQ/NRRLF/QEELE
1.25	0.002	ABCF2	ATP-binding cassette sub-family F member 2	KLELN
1.38	0.003	SPC24	Kinetochore protein Spc24 (Fragment)	QVVER
2.21	0.004	CKS2	Cyclin-dependent kinases regulatory subunit 2	Not found
1.53	0.004	CENPK	Centromere protein K	QKLRQ/NRLFD
1.45	0.004	ADI1	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase	RRERN
1.72	0.005	CDC123	Cell division cycle protein 123 homolog	QKEEI/KLKRN
1.34	0.005	EHD1	EH domain-containing protein 1	QKIER/QELLQ
1.21	0.005	EIF4A2	Eukaryotic initiation factor 4A-II	RIDVQ/QKERD/VKKEE
1.21	0.006	HK2	Hexokinase-2	QRFEK
1.53	0.007	CPA4	Carboxypeptidase A4	NRPVD
1.45	0.01	RRM2	Ribonucleoside-diphosphate reductase subunit M2	Not found
1.21	0.01	TXLNA	α-Taxilin	QRLEK/FEEFQ/NKRVQ
1.29	0.012	RB1	Retinoblastoma-associated protein	QKNIE/QKKKE/VDLDE/
1.23	0.012	TRIO	Isoform 4 of Triple functional domain protein	QKLLQ/NRLVE/LEELQ/NELFQ/VKLRD
1.2	0.012	HSPA8	Heat shock cognate 71 kD protein OS = Homo sapiens !HSPA8 PE = 1 SV = 1	FEELN/QKLLQ/QKILD/QKELE
1.29	0.013	ATXN10	Ataxin-10	QRVLD
1.50	0.014	TMA7	Translation machinery-associated protein 7	QKKLE
1.60	0.015	NOSIP	Nitric oxide synthase-interacting protein (Fragment)	Not found
1.49	0.015	NUF2	Kinetochore protein Nuf2 (Fragment)	Not found
1.97	0.016	ECD	Isoform 3 of Protein SGT1	RLEVQ/QKYIE/QRFVP
1.32	0.016	DNAJA1	DnaJ homolog subfamily A member 1	VELVD
1.21	0.017	FKBP10	Peptidyl-prolyl cis-trans isomerase FKBP10	Not found
1.63	0.019	COL6A1	Collagen alpha-1(VI) chain	QELKE
1.49	0.021	RPL30	60S ribosomal protein L30 (Fragment)	Not found
1.48	0.022	EHD4	EH domain-containing protein 4	FRDIQ/NKKRE
1.27	0.022	H2BFS	Histone H2B type F-S	Not found
1.24	0.022	TP53	Isoform 7 of Cellular tumor antigen p53	NLRVE/FRELN
1.47	0.023	COMMD9	COMM domain-containing protein 9	Not found
1.28	0.023	GPS1	Isoform 3 of COP9 signalosome complex subunit 1	NVIKV
2.18	0.027	COLEC12	Collectin-12	KEKVQ
1.71	0.027	ITGB5	Integrin beta (Fragment)	Not found
1.34	0.03	CAPRIN1	Caprin-1	VLELQ/QRVQD
1.23	0.03	G3BP1	Ras GTPase-activating protein-binding protein 1	KDFFQ/NVVEL/NVEEK/QRVRE
1.43	0.032	PLAT	Isoform 4 of Tissue-type plasminogen activator	NRVEY/QKFEV
1.26	0.032	TOMM34	Mitochondrial import receptor subunit TOM34	QKLRQ
1.34	0.033	CSDE1	Isoform 3 of Cold shock domain-containing protein E1	VKEVQ
1.22	0.038	NUP188	Nucleoporin NUP188 homolog	QDERQ/VDKLE
1.27	0.039	CKS1B	Cyclin-dependent kinases regulatory subunit	Not found
3.62	0.04	SDF4	Isoform 6 of 45 kD calcium-binding protein	KVDVN/QRWIM
1.24	0.04	CACNA1G	Isoform 11 of Voltage-dependent T-type calcium channel subunit alpha-1G	QEVLE/NRLDF
1.48	0.043	MKI67	Antigen KI-67	QKLDL/QKVEV/QELDQ/QKLDQ/VEKLD/VDVEE
1.21	0.043	PAPOLA	Poly(A) polymerase α	VFEEE
1.27	0.046	FAM3C	Protein FAM3C (fragment)	QVFEI
1.25	0.047	DNAJB6	Isoform C of DnaJ homolog subfamily B member 6	VEVEE
1.74	0.049	HAUS4	HAUS augmin-like complex subunit 4	QEVEE/QRLLQ/VEKVE

Table S2 lists all WB quantifications (fold) for Figs. 1–5. Table S3 lists all WB quantifications (fold) for Figs. S1–S3. Both are available as Excel files.