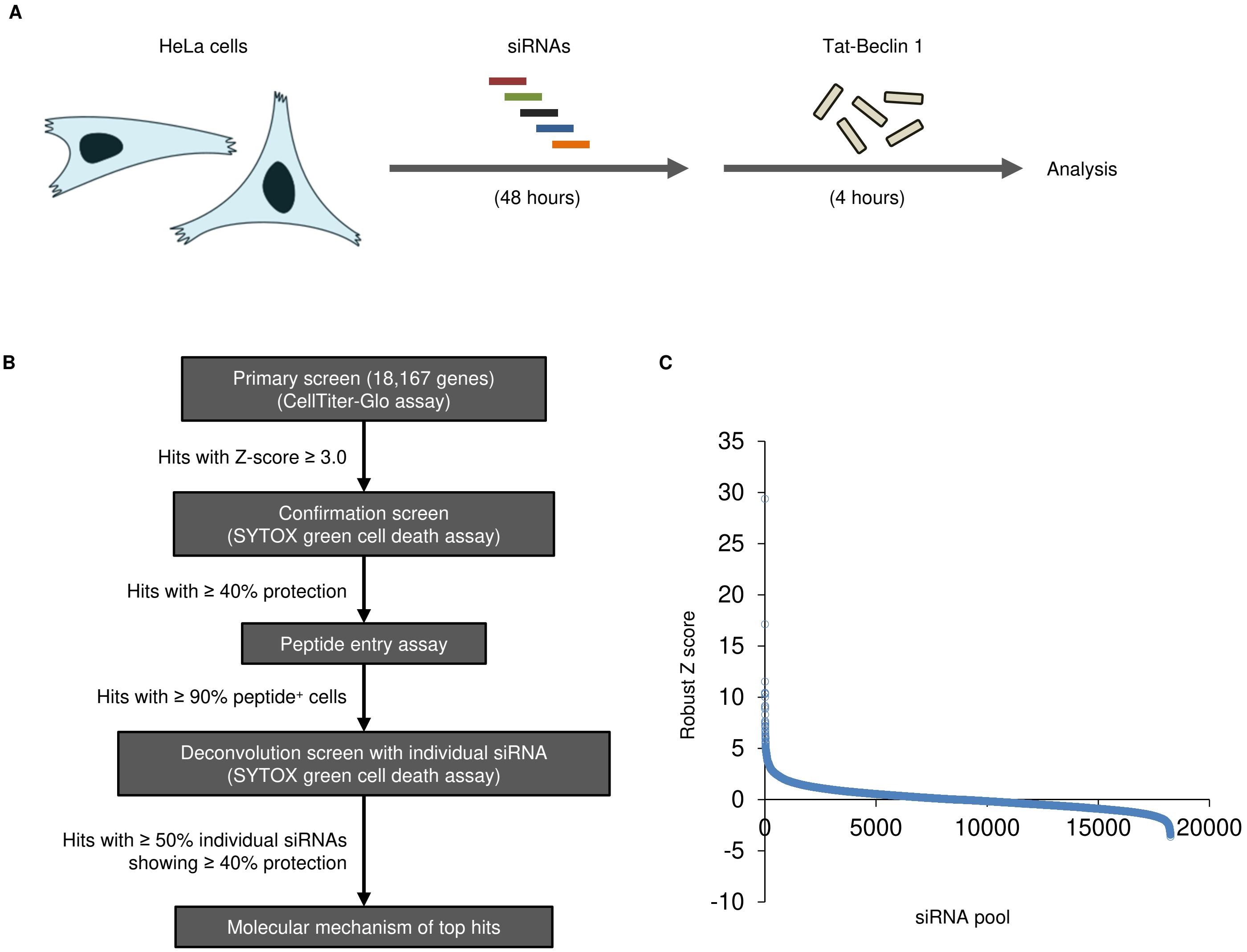
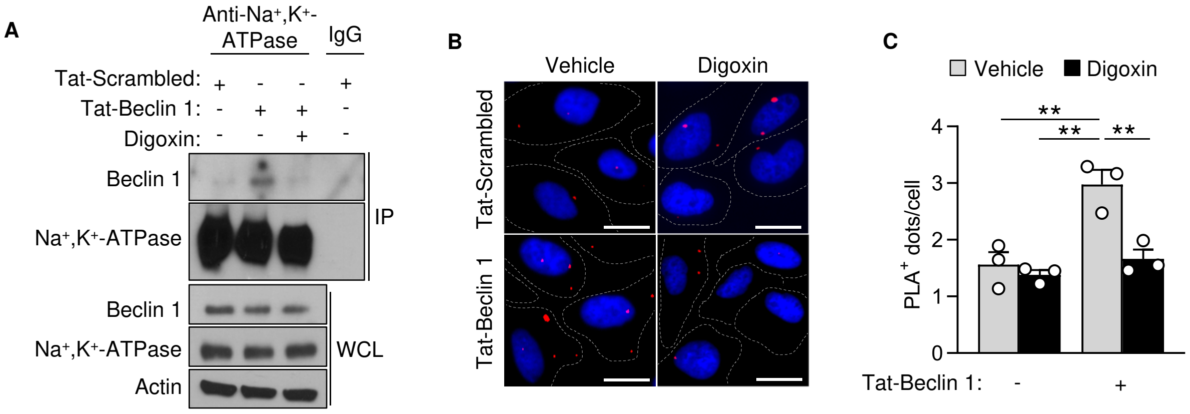
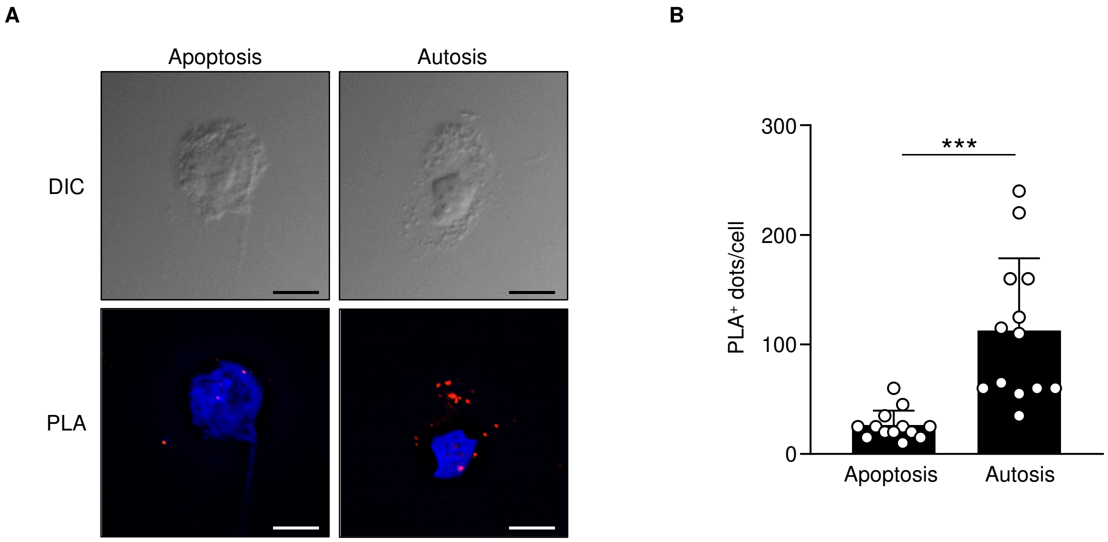


Supplemental Figure 1. Identification of autosis regulators in a genome-wide siRNA screen. (A, B), Schematic diagrams of genome-wide siRNA screening for regulators of Tat-Beclin 1-induced autosis. See Methods for details. (C), Ranked distribution of z-score averages for each siRNA pool in primary genome-wide siRNA screen. See Supplemental Table 1 for top hits from primary screen, Supplemental Table 2 for top hits from conformational screen and Supplemental Table 3 for top hits from the final deconvolution screen. All assays were performed in triplicate.



Supplemental Figure 2. Beclin 1 and Na$^+$,K$^+$-ATPase interact during autophagy-inducing peptide treatment. (A) Co-immunoprecipitation of Beclin 1 with the α-subunit of Na$^+$,K$^+$-ATPase in HeLa cells after 3 hours of treatment with Tat-Scrambled peptide or Tat-Beclin 1 active peptide supplemented with either vehicle or 10 μM digoxin. The same cell lysate from cells treated with Tat-Scrambled peptide was used as a control for IgG immunoprecipitation. Similar results were observed in three independent experiments. (B, C) Representative images (B) and quantitation (C) of proximity ligase assay (PLA) of Beclin 1 and the α-subunit of Na$^+$,K$^+$-ATPase in the indicated conditions. In (C) mean values ± SEM from three independent experiments (value for each experiment represent mean value of at least 100 cells per condition). Scale bars, 10 μm. $^{**}P < 0.01$, Two-way ANOVA test.



Supplemental Figure 3. Increased interaction between Beclin 1 and Na$^+$,K$^+$-ATPase during autosis but not apoptosis. (A) Representative images of differential interference contrast (DIC) microscopy and proximity ligation assays (PLA) of Beclin 1 and Na$^+$,K$^+$-ATPase in apoptotic and autotic-like HeLa cells after 24 hours of starvation. Apoptotic cells were defined as those cells with rounded morphology and cytoplasmic and cell surface blebbing. Autotic-like cells were defined as cells with perinuclear ballooning. (B), Quantitation of (A). Bars represent mean ± SD (n = 13 cells per condition). ***$P < 0.001$; Two-tailed unpaired Student's t-test.

Supplemental Table 1: Top hits from primary screen

Symbol	Name	Function	Average Z-scores
ATP1A1	ATPase Na+/K+ transporting subunit alpha 1	Component of ion pump	25.151
TRIM25	tripartite motif containing 25	E3 ubiquitin ligase in immunity	17.780
ZNF185	zinc finger protein 185 with LIM domain	Cytoskeleton dynamics	11.798
RFWD3	ring finger and WD repeat domain 3	E3 ubiquitin ligase in DNA repair	10.259
WFDC1	WAP four-disulfide core domain 1	Control of stromal-epithelial interaction	10.250
KCNN4	potassium calcium-activated channel subfamily N member 4	Component of ion channel	9.090
SOAT2	sterol O-acyltransferase 2	Acyl-CoA:cholesterol acyltransferase	9.044
RNASET2	ribonuclease T2	Lysosomal degradation of RNA	8.566
ACP5	acid phosphatase type 5. tartrate resistant	Dephosphorylation of osteopontin	8.380
KCNQ2	potassium voltage-gated channel subfamily Q member 2	Component of ion channel	8.120
SAMD4	sterile alpha motif domain containing 4	RNA-binding post-transcriptional regulator	8.054
TMEM132E	transmembrane protein 132E	Cellular adhesion	7.837
PPFIA3	PTPRF interacting protein alpha 3	Regulation of focal adhesions	7.370
AIRE	autoimmune regulator	Transcription factor in T cell negative selection	7.324
TRIM46	tripartite motif containing 46	Microtubule organization in neuronal polarization	7.095
MEX3B	mex-3 RNA binding family member B	RNA-binding post-transcriptional regulator	7.053
ECEL1	endothelin converting enzyme like 1	Endopeptidase in regulation of neuropeptides	6.865
MRPL22	mitochondrial ribosomal protein L22	Mitochondrial protein synthesis	6.790
IFNAR1	Interferon alpha and beta receptor subunit 1	Receptor for IFN-alpha and IFN-beta	6.787
PTPNS1 (SIRPA)	signal regulatory protein alpha	Inhibitory receptor of CD47	6.769
PVRL4 (NECTIN4)	nectin cell adhesion molecule 4	Regulation of cadherin-based adherens junctions	6.750
PPP1R14A	protein phosphatase 1 regulatory inhibitor subunit 14A	Smooth muscle contraction	6.578
PABPC1	poly(A) binding protein cytoplasmic 1	mRNA regulation	6.412
SYS1	SYS1 golgi trafficking protein	Targeting of ARFRP1 to the Golgi apparatus	6.264
AQP2	aquaporin 2	Renal water homeostasis	5.919
SSX6	SSX family member 6. pseudogene	Probable transcriptional repressor	5.867
HECTD1	HECT domain E3 ubiquitin protein ligase 1	E3 ubiquitin ligase in different processes	5.712
LDAH	lipid droplet associated hydrolase	Lipid hydrolase involved in triacylglycerol dynamics	5.599
PTPRN2	protein tyrosine phosphatase receptor N2	Phosphatidylinositol phosphatase in insulin secretion	5.531
RAB40AL	RAB40A like	Protein targeting for proteasomal degradation	5.498
TRAF3	TNF receptor associated factor 3	Signal transducer of different immune receptors	5.465
MIB1	mindbomb E3 ubiquitin protein ligase 1	E3 ubiquitin ligase in different processes	5.259
G3BP1	GRBP stress granule assembly factor 1	Helicase in DNA sensing; stress granule assembly	5.239
SSX2	SSX family member 2	Probable transcriptional repressor	5.218
TRIM56	tripartite motif containing protein 56	E3 ubiquitin ligase in immunity	5.164
COQ10B	coenzyme Q10B	Coenzyme Q chaperone	5.161
ZFH4	zinc finger homeobox 4	Transcription factor in neural cell differentiation	5.117
ZNF278 (PATZ1)	POZ/BTB and AT hook containing zinc finger 1	Transcriptional repressor	5.111
CCNE2	cyclin-E2	Cell cycle regulation	5.022

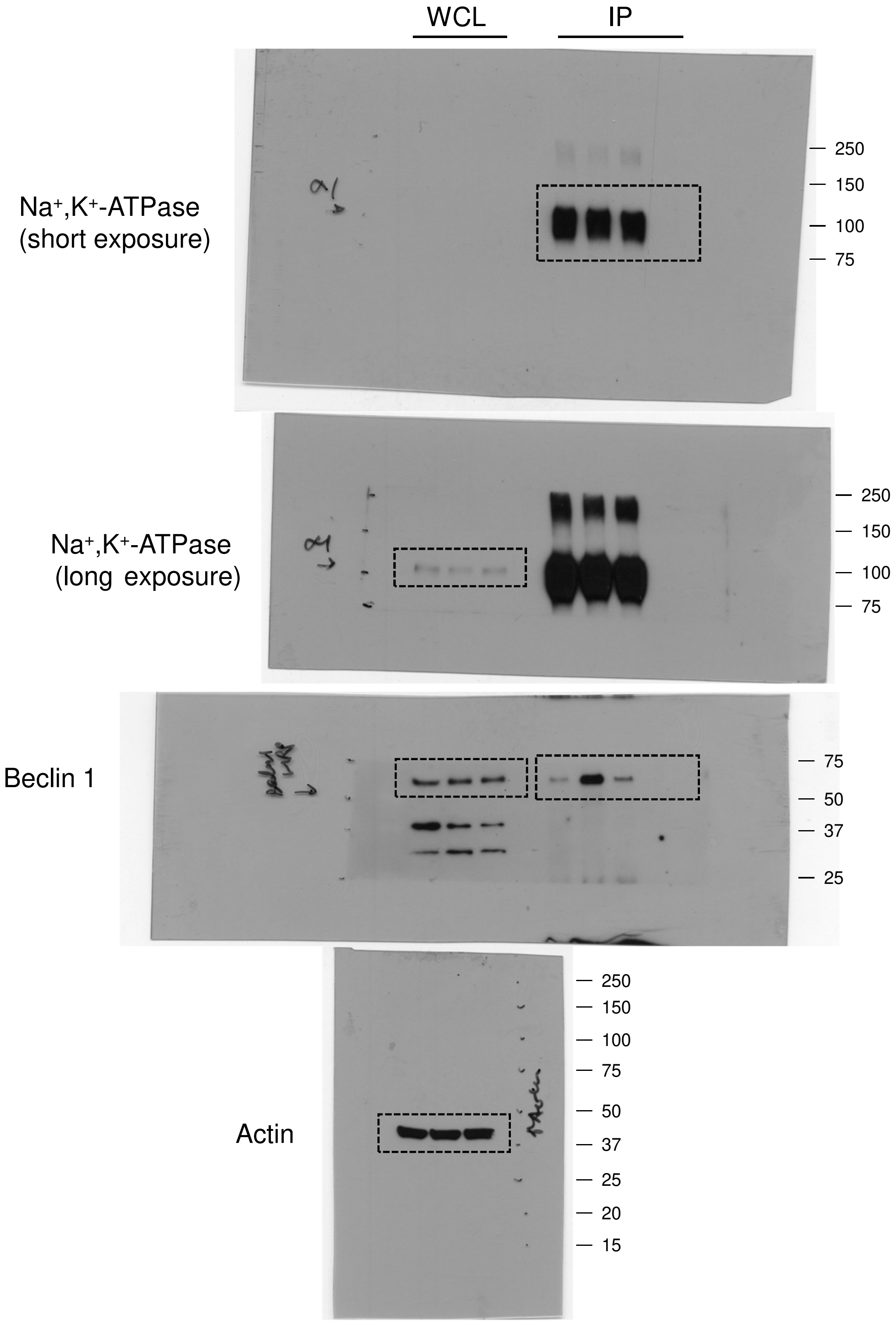
Supplemental Table 2: Top hits from confirmation screen

Symbol	**Name**	**Function**	**Cell death protection (%)**
ATP1A1	ATPase Na+/K+ transporting subunit alpha 1	Component of ion pump	118.27%
HIST1H2AI (H2AC13)	H2A clustered histone 13	Core component of nucleosome	106.19%
CBX1	chromobox 1	Epigenetic repression in heterochromatin	103.03%
IGSF9	Immunoglobulin superfamily 9	Cell adhesion molecule in synapses	92.01%
STAU2	staufen double-stranded RNA binding protein 2	mRNA transport in heart and brain	88.52%
ARSI	arylsulfatase family member I	Hydrolysis of sulfate esters	87.42%
FLJ40142	Unknown	Unknown	87.10%
RNASET2	ribonuclease T2	Lysosomal degradation of RNA	87.00%
SYT9	synaptotagmin 9	Ca2+-dependent exocytosis	85.77%
WFDC1	WAP four disulfide core domain 1	Control of stromal-epithelial interaction	82.88%
SAMD4	sterile alpha motif domain containing 4	RNA-binding post-transcriptional regulator	80.78%
SYS1	SYS1 golgi trafficking protein	Targeting of ARFRP1 to the Golgi apparatus	80.41%
TRIM25	tripartite motif containing 25	E3 ubiquitin ligase in immunity	80.12%
ATP2B2	ATPase plasma membrane Ca2+ transporting 2	Transport of calcium	71.78%
EVPL	evnoplakin	Cytoskeletal linker protein	69.27%
PPFIA3	PTPRF interacting protein alpha 3	Regulation of focal adhesions	68.84%
SLC6A8	solute carrier family 6 member 8	Creatine transporter	66.42%
THBS3	thrombospondin 3	Cell-to-cell. cell-to-matrix interactions	65.17%
TRPT1	tRNA phosphotransferase 1	tRNA splicing	64.27%
TMEM132E	transmembrane protein 132E	Cellular adhesion	63.60%
HIST1H2BL (H2BC13)	H2B clustered histone 13	Core component of nucleosome	63.34%
SLC22A5	solute carrier family 22 member 5	Carnitine transporter	61.85%
MRPL22	mitochondrial ribosomal protein L22	Mitochondrial protein synthesis	60.55%
RAB40L	RAB40A	Protein targeting for proteasomal degradation	60.48%
AQP2	aquaporin-2	Renal water homeostasis	57.45%
PGLYRP3	peptidoglycan recognition protein 3	Antimicrobial peptide	57.28%
VPS28	VPS28 subunit of ESCRT-I	Vesicular trafficking	55.45%
HES2	hes family bHLH transcription factor 2	Transcriptional repressor	54.81%
NFKBIZ	NFKB inhibitor zeta	Nuclear inhibitor of NF-kB	54.57%
PPP1R3A	protein phosphatase 1 regulatory subunit 3A	Regulation of PP1 in muscle	53.51%
PSCD2 (CYTH2)	cytohesin 2	Activator of Arf-family small GTPases	53.18%
SLCO4A1	solute carrier organic anion transporter family 4A1	Transport of organic anions	52.98%
TM4SF5	transmembrane 4 L six family member 5	Lysosomal arginine sensor. mTORC1 activator	52.50%
AIRE	autoimmune regulator	Transcription factor in T cell negative selection	52.07%
MCMDC1 (MCM9)	minichromosome maintenance 9 homologous recombination repair factor	Repair of double-stranded DNA breaks and DNA interstrand cross-links	51.30%
TDRD3	tudor domain containing 3	Methylarginine recognition	50.62%
DRD4	dopamine receptor D4	Dopamine receptor in many processes	50.11%

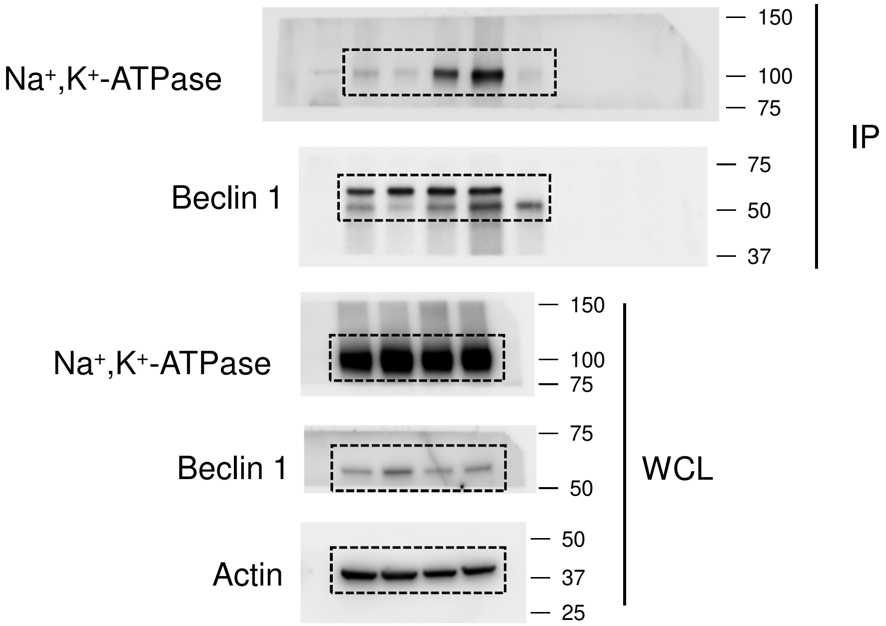
Supplemental Table 3: Top hits from deconvolution screen

Symbol	Name	Function	% Dharmacon siRNA (Total = 4)	% Invitrogen siRNA (Total = 3)
ATP1A1	ATPase Na+/K+ transporting subunit alpha 1	Component of ion pump	100%	100%
KCNN4	potassium calcium-activated channel subfamily N member 4	Component of ion channel	100%	67%
NICN1	nicolin 1	Nuclear protein of unknown function	50%	100%
SAMD4A	sterile alpha motif domain containing 4	RNA-binding post-transcriptional regulator	75%	67%
SPG7	SPG7 matrix AAA peptidase subunit, paraplegin	Mitochondrial metalloprotease (AAA protein family)	50%	100%
ARSI	arylsulfatase I	Hydrolysis of sulfate esters	75%	33%
CSPP1	centrosome and spindle pole associated protein 1	Cell-cycle progression; cilia formation	75%	33%
ADGRF1	adhesion G protein-coupled receptor F1	Adhesion-GPCR involved in brain development	50%	67%
KCNQ2	potassium voltage-gated channel subfamily Q member 2	Component of ion channel	75%	33%
SYS1	SYS1 golgi trafficking protein	Targeting of ARFRP1 to the Golgi apparatus	75%	33%
RAB40AL	Ras-related protein Rab-40A-like	Protein targeting for proteasomal degradation	75%	0%
THBS3	thrombospondin 3	Cell-to-cell, cell-to-matrix interactions	75%	0%
TRPT1	tRNA 2’-phosphotransferase 1	tRNA splicing	75%	0%

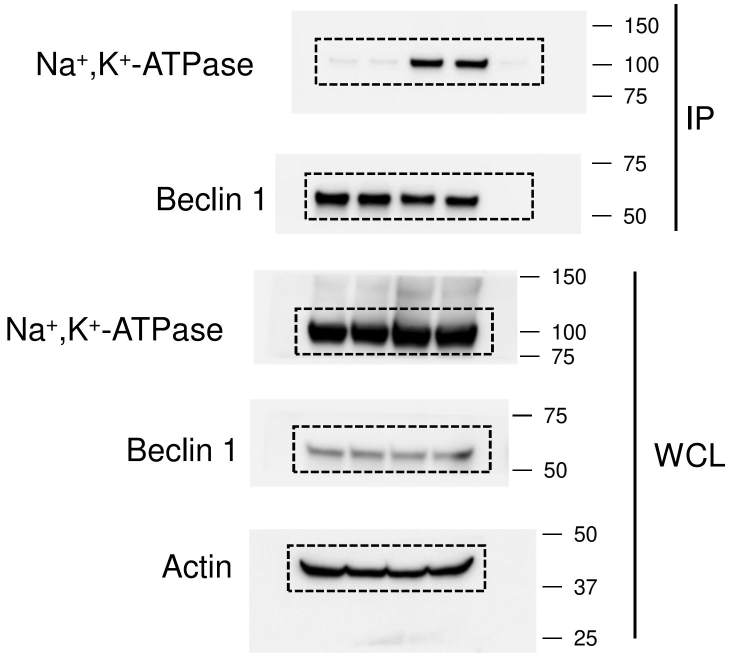
Full unedited gels for Figure 1A



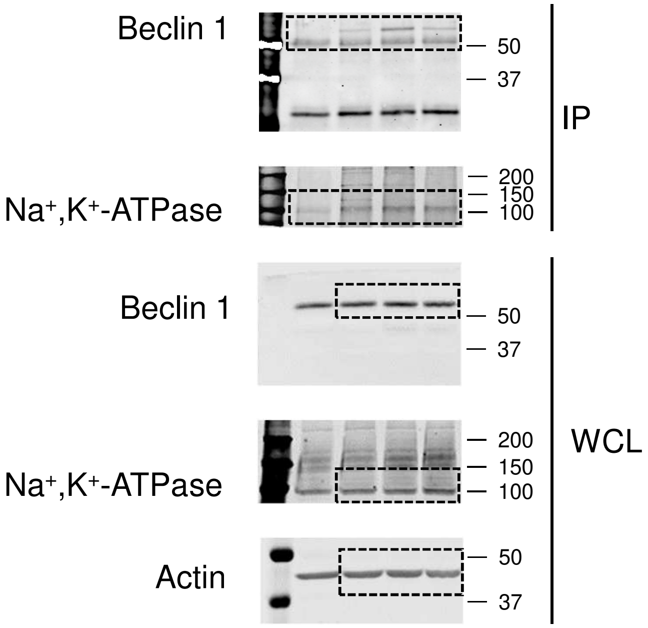
Full unedited gels for Figure 2A



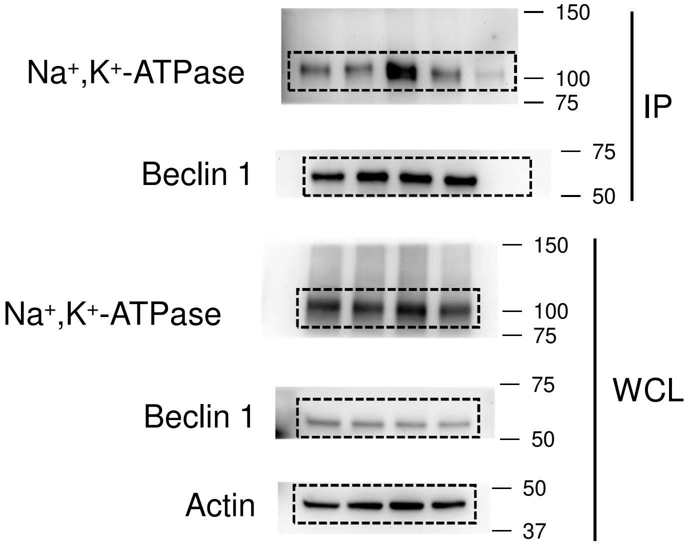
Full unedited gels for Figure 2C



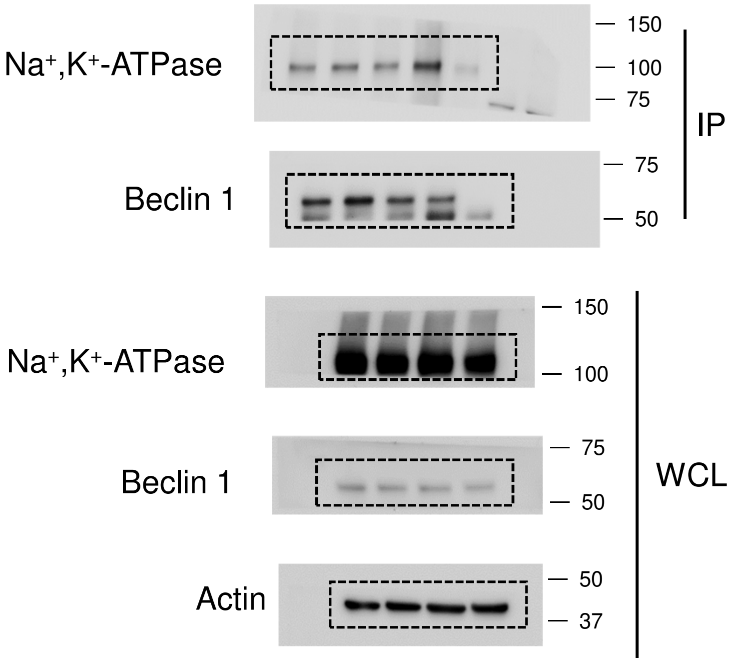
Full unedited gels for Figure 3A



Full unedited gels for Figure 3E



Full unedited gels for Figure 5A



Full unedited gels for Supplemental Figure 2A