Supplemental Experimental Procedures

Plasmids, antibodies, and reagents

Sources of the plasmids utilized are as follows: pcDNA3.1-CFTR, pcDNA3.1-ΔF508 CFTR, pEGFP-GFP-CFTR, pEGFP-GFP-ΔF508 CFTR, pcDNA3.1-GnRHR-GFP, pcDNA3.1-E90K GnRHR-GFP, pcDNA3.1-S168R GnRHR-GFP, pcDNA3.1-E90K GnRHR, pcDNA3.1-HA-ubiquitin, pcDNA3.1-DNAJB12-myc, pcDNA3.1- DNAJB12-FLAG, pcDNA3.1-HA-derlin-1, pcDNA3.1 FLAG-RMA1, pcDNA3.1-calnexin, pcDNA3.1-ERp57(Ayala Yanez and Conn, 2010; Brothers et al., 2004; Brothers et al., 2003; Grove et al., 2011). The plasmids pRc/RSV-ATM and pRc/RSV-ATZ were a generous gift from David Perlmutter (University of Pittsburgh)(Hidvegi et al., 2010). The plasmid pCMV6-SAR1A-DDK-myc was purchased from OriGene (Rockville, MD). The plasmids pEGFP-Orai1-CFP (plasmid 19757) and pcDNA4-beclin1-HA (plasmid 24399) were obtained from Addgene (Cambridge, MA) (Prakriya et al., 2006; Sun et al., 2008). FLAG-JB12 and Beclin-1-HA has been shown to function similar to untagged forms (Grove et al., 2011; Sun et al., 2008). Mutant forms of indicated proteins were created via the QuikChange protocol (Stratagene, Santa Clara, CA) with the following primers:

Plasmid	**template**	**Primers**
E90K,ΔK191 GnRHR-GFP pcDNA3.1	E90K GnRHR-GFP pcDNA3.1	5'-CAGCTCTGGACAGACAGTTTTCTCTCAATGTG-3', 5- CACATTGAGAGAAAACTGTCTGTCCAGAGCTG-3'
E90K,C114S GnRHR-GFP pcDNA3.1	E90K GnRHR-GFP pcDNA3.1	5'-GTATGCTGGAGAGTTACTCAGCAAAGTTCTCAGTTATC-3', 5- GATAACTGAGAACTTTGCTGAGTAACTCTCCAGCATAC-3'
E90K,C14S,C114S GnRHR-GFP pcDNA3.1	E90K,C114S GnRHR-GFP pcDNA3.1	5'-CTGAACAGAATCAAAATCACAGCTCAGCCATCAACAACAGCATC-3', 5'-GATGCTGTTGTTGATGGCTGAGCTGTGATTTTGATTCTGTTCAG-3'
E90K,S168R GnRHR-GFP pcDNA3.1	E90K GnRHR-GFP pcDNA3.1	5'-CTGGATCCTCAGTAGGGTCTTTGCAGGACC -3', 5'-GGTCCTGCAAAGACCCTACTGAGGATCCAG-3'
H79G Sar1a-DDK-myc pCMV6	WT Sar1a-DDK-myc pCMV6	5'-CTTTTGATCTTGGTGGGGGCGAGCAAGCACGTCGC-3', 5'-GCGACGTGCTTGCTCGCCCCCACCAAGATCAAAAG-3'

For knockdown of JB12, a pLKO.1 plasmid encoding an shRNA against human JB12 (TRCN0000022297) was purchased from Open Biosytems (Hunstville, AL) and packaged into a lentiviral vector. For all other knockdown experiments, Silencer Select control and pre-designed siRNAs (with LNA modifications) for ATG5, p97, p62, NBR1, and SEL1L were purchased from Life Technologies (Carlsbad, CA):

target	cat#	sequence (sense)	sequence (antisense)
SEL1L	s12675	GCACCGAUGUAGAUUAUGAtt	UCAUAAUCUACAUCGGUGCca
	s12674	GGCUUAUGACUGCCUAUAAtt	UUAUAGGCAGUCAUAAGCCtt
p62	s16962	CUUCCGAAUCUACAUUAAAtt	UUAAUGUAGAUUCGGAAGat
	s16960	GGAGCACGGAGGGAAAAGAtt	UCUUUUCCCUCCGUGCUCCac
NBR1	s8381	GGGCUGAUAUCGAAGCUAUtt	AUAGCUUCGAUAUCAGCCCaa
	s57876	GGUCUGGUGCAGUAUCAUAtt	UAUGAUACUGCACCAGACCcg
ATG5	s18159	GAACCAUACUAUUUGCUUUtt	AAAGCAAAUAGUAUGGUUCtg
p97	s14765	GAAUAGAGUUGUUCGGAAUtt	AUUCCGAACAACUCUAUUCat
Control	4390846		

No reliable antibodies against GnRHR exist, so GFP tagged forms of GnRHR were used for analysis. Antibodies were custom made as previously described or purchased from commercial sources, as indicated below:

Antibody	source	catalog #	purpose
goat anti-mouse IgG (HRP conjugated)	Thermo Fisher Scientific	31430	WB
goat anti-rabbit IgG (HRP conjugated)	Thermo Fisher Scientific	31461	WB
mouse anti-CFTR	Millipore	05-581	WB
mouse anti-GFP	Roche	11 814 460 001	WB
mouse anti-HA	Sigma-Aldrich	H9658	WB
mouse anti-NBR1	abcam	ab55474	WB
mouse anti-p97	Research Diagnostics Inc.	RDI-PRO65278	WB
mouse anti-tubulin	Sigma-Aldrich	T-9026	WB

rabbit anti-Hdj1	Stressgen	SPA-400	WB
rabbit anti-ATG5	Medical and Biological Laboratories	PM050	WB
rabbit anti-ERp57	Pierce	PA5-17117	WB
rabbit anti-myc	Sigma-Aldrich	C3956	WB
rabbit anti-BiP	Cell Signaling Technology	3183	WB
rabbit anti-beclin-1	Novus Biologicals	NB500-249	WB
rabbit anti-phospho-beclin-1 (Ser93/96)	Cell Signaling Technology	12476	WB
rabbit anti-Hsp70	Stressgen	SPA-811	WB
rabbit anti-Vps34	Cell Signaling Technology	4263	WB
rabbit anti-LC3B	Sigma-Aldrich	L7543	WB
rabbit anti-OS9	Sigma-Aldrich	HPA013694	WB
mouse anti-p62	Abnova	H00008878-M01	WB, IF
mouse anti-α1-antitrypsin	Millipore	MAB1261	WB, IF
mouse anti-DNAJB12	Cyr Lab (Grove et al., 2011)		WB, IF
mouse anti-RMA1	Cyr Lab (Grove et al., 2011)		WB,IF
rabbit anti-SEL1L	Sigma-Aldrich	S3699	WB,IF
rabbit anti-Derlin1	Sigma-Aldrich	D4443	WB,IF
rabbit anti-calnexin	Sigma-Aldrich	C4731	WB,IF
goat anti-mouse IgG (oregon green 488 conjugated)	Life Technologies	O-6380	IF
goat anti-mouse IgG (texas red conjugated)	Life Technologies	T-862	IF
goat anti-rabbbit IgG (oregon green 488 conjugated)	Life Technologies	A-11034	IF
goat anti-rabbbit IgG (texas red conjugated)	Life Technologies	T-2767	IF
mouse anti-CD107b (LAMP2)	BD Biosciences	555803	IF
mouse anti-ERGIC53	Enzo Life Sciences	ALX-804-602	IF
rabbit anti-Derlin2	Sigma-Aldrich	D1194	IF
rabbit anti-GM130	abcam	ab52649	IF
rabbit anti-GP78	Cell Signaling Technology	9590	IF
rabbit anti-proteasome subunit S5a	Affinity Bioreagents	PA1-966	IF
rabbit anti-Sec16	Bethyl Laboratories	A300-648A	IF
rabbit anti-YFP (also reacts with GFP)	Dr. C.J. Beckers		IP
goat anti-rabbit IgG (0.8nm Gold)	Electron Microscopy Sciences	25101	EM
rabbit anti-GFP	Cell Signaling Technology	2956	EM

Cell culture , transfections, and chemical treatments

Cos-7 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in accordance with ATCC recommendations. Cells were grown in DMEM (GIBCO, Carlsbad, CA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and 10U/ml penicillin, 10µg/ml streptomycin (GIBCO, Carlsbad, CA) on 75cm^2 cell culture flasks (Corning, Corning, NY). Cells were incubated in a sterile, humidified tissue culture incubator at 37˚C with 5% CO_2. For maintenance of cell lines, confluent cells were detached from flasks using 0.05% trypsin-EDTA (GIBCO, Carlsbad, CA) and re-seeded at 20% confluency. All experiments were performed using cells between passages 3 and 10 (ATCC stock = passage 1). Cell stocks in 50% DMEM (GIBCO, Carlsbad, CA), 10% DMSO, 40% fetal bovine serum were frozen in a cryo freezing container (Nalgene, Penfield, NY) at a rate of -1˚C/min and stored in liquid nitrogen vapor phase for long term storage.

For experimentation, cells were seeded into a 6-well, Costar cell culture treated, polystyrene plate (Corning, Corning, NY) at a density of 1.5x10^5 cells/well (for microscopy) or 2x10^5 cells/well (for all other experiments). For microscopy, sterile cover glass (Thermo Fisher, Waltham, MA) was placed into each well prior to seeding of cells. Cells were transfected with plasmid DNA using the Effectene transfection reagent (Qiagen, Alameda, CA) 18hours post-seeding. Transfection reagent was removed after 5 hours and replaced with fresh media.

For siRNA knockdown experiments, cell were seeded into a 6-well, Costar cell culture treated, polystyrene plate (Corning, Corning, NY) at a density of 2x10^5 cells/well. Eighteen hours post-seeding, siRNA transfections were performed with Lipofectamin 2000 (Life Technologies, Carlsbad, CA) using 20nM siRNA in DMEM (GIBCO, Carlsbad, CA) supplemented with 10% FBS(Hyclone, Logan, UT), with no antibiotic. Where indicated, two siRNAs to the same target were used simultaneously to increase knockdown efficiency. Four-hours post-transfection, media was replaced with DMEM (GIBCO, Carlsbad, CA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and 10U/ml penicillin, 10µg/ml

streptomycin (GIBCO, Carlsbad, CA). Eighteen-hours later, cells were harvested with 0.05% trypsin-EDTA (GIBCO, Carlsbad, CA) and reseeded into 6-well plates for plasmid transfections (as described above).

To investigate the effects of various chemicals, cells were transfected with 0.75µg pcDNA3.1-GnRHR-GFP, 0.75µg pcDNA3.1-CFTR, 0.3µg pRc/RSV-ATZ, or 100ng pcDNA3.1-beclin1-HA. As indicated, transfected cells in a 6-well plate were treated with, 5µg/ml brefeldin A (Life Technologies, Carlsbad, CA), 500 nM rapamycin (Cayman Chemical, Ann Arbor, MI), 100nM Bafilomycin A1(Sigma-Aldrich, St. Louis, MO), 15 µM chloroquine (Sigma-Aldrich, St. Louis, MO), 10 µM bortezomib (LC Laboratories, Woburn, MA), 200 nM wortmannin (LC Laboratories, Woburn, MA), 10µM LY-294002 (Cayman Chemical, Ann Arbor, MI), 5mM castanospermine (Sigma-Aldrich, St. Louis, MO), 10µM DTT (Thermo Fisher, Waltham, MA), 15µM DBeQ (Sigma-Aldrich, St. Louis, MO), or 15µM Eeyarstatin-I (Sigma-Aldrich, St. Louis, MO) for indicated amounts of time prior to harvesting. For western-blot analysis, cells were harvested with cold citric saline and frozen prior to lysis. For microscopy, slides were placed into cold methanol prior to fixation.

To investigate the effects of overexpression of different factors, cells were co-transfected with 0.75µg pcDNA3.1-GnRHR-GFP, 0.75µg pcDNA3.1-CFTR, or 0.3µg pRc/RSV-ATZ and 5ng pCMV6-SAR1A-DDK-myc , 100ng pcDNA3.1-DNAJB12-myc, 300ng pcDNA3.1-FLAG-RMA1, 100ng HA-Derlin-1, 100ng calnexin, or 100ng of ERp57. For western-blots, cells were harvested with cold citric saline (24 hours post-transfection) and frozen prior to lysis. For microscopy, cells were grown 36 hours post-transfection and then fixed and stored in cold methanol prior to additional fixation steps (see below).

Cycloheximide chases

Cos-7 cells were transfected with 0.75µg GnRHR-GFP, 0.5µg Orai1-CFP, or 0.3µg ATZ. Twenty-four hours post-transfection, cells in a 6-well plate were incubated with 5µg/ml cycloheximide (CHX) in the presence and absence of 15µM chloroquine or 10µM bortezomib. Where indicated, 10µM DTT or

5mM castanospermine were added 4 hours prior to CHX addition. Cells were harvested with cold citric saline at t = 0, 0.5, 1, 1.5, and 2 hours post-CHX and immediately frozen prior to lysis.

The half-lives were calculated using the values obtained from densitometry. Values were fit to the half-life decay equation, an exponential regression:

$$N(t) = N(0 \text{ hours}) * e^{-\lambda t}$$

Where N(t) is the normalized densitometry value at time t, such that N(0 hours) is normalized to 1. λ is the decay constant. Half-lives ($t_{1/2}$) were calculated using the formula:

$$t_{1/2} = \ln(2) / \lambda$$

Immunoprecipitation (IP)

For ubiquitination IPs, Cos-7 cells were transfected with 1.0µg pcDNA3.1-GnRHR-GFP + 200g pcDNA3.1-HA-ubiquitin. Twenty-four hours post-transfection, cells were treated with 15µM Eeyarstatin-I or DBeQ for 5 hours, then harvested with cold citric saline, and isolated with centrifugation at 5,000g. Cell pellet was lysed in 200µl of RIPA buffer + N-ethyl-malemide for 1 hour at 4˚C. Lysate was sonicated for 10seconds pulses at 25% intensity using a digital sonifier (Branson, Danbury, CT). Protein concentrations of lysate were determined using the Protein-Dc assay kit (Bio-Rad, Hercules, CA)and lysates were diluted to 0.5mg/ml total protein concentration. 50µl of sample was removed for "input". 1µl of rabbit α-YFP was added to 200µl of lysate and incubated for 1 hour at 4˚C. Samples were then added to 25µl of protein-G agarose (pre-blocked in 5% BSA; Roche, Basel, Switzerland) and incubated for 1 hour at 4˚C. Samples were centrifuged for 5 minutes at 8,000g and supernatant was aspirated. Protein-G agarose was washed 3x with RIPA buffer. Proteins were eluted from the agarose with 65µl of 1x SDS-sample buffer with 1.25% β-mercaptoethanol by incubating at 50˚C for 10 minutes. Input and eluate samples were sonicated with two 30-second pulses at 25% intensity using a digital sonifier

(Branson, Danbury, CT) prior to SDS-PAGE.

For GnRHR-GFP coIPs, Cos-7 cells were transfected with 1.0µg pcDNA3.1-GnRHR-GFP. Twenty-four hours post-transfection, cells were harvested with cold citric saline, and isolated with centrifugation at 5,000g. Cell pellets were lysed in 250µl of 50mM Tris-Cl, 150mM NaCl, 1% Triton X-100, pH 7.6 (TBSt) for 1 hour at 4˚C. Protein concentrations of lysate were determined using the Protein-Dc assay kit (Bio-Rad, Hercules, CA) and lysates were diluted to 0.7mg/ml total protein concentration. 50µl of sample was removed for "input". 1µl of rabbit α-YFP was added to 200µl of lysate and incubated for 1 hour at 4˚C. Samples were then added to 25µl of protein-G agarose (pre-blocked in 5% BSA; Roche, Basel, Switzerland) and incubated for 1 hour at 4˚C. Samples were centrifuged for 5 minutes at 8,000g and supernatant was aspirated. Protein-G agarose was washed 3x with TBSt. Protein were eluted from the agarose with 65µl of 1x SDS-sample buffer with 1.25% β-mercaptoethanol by incubating at 50˚C for 10 minutes. Input and eluate samples were sonicated with two 30-second pulses at 25% intensity using a digital sonifier prior to SDS-PAGE.

For JB12-FLAG coIPs, wells were transfected with 50ng pcDNA3.1-DNAJB12-FLAG in the presence or absence of various concentrations of pcDNA3.1-GnRHR-GFP, pcDNA3.1-ΔF508 CFTR, pcDNA3.1-A103E OraI1-CFP, 100ng of pcDNA3.1-Vps34-myc, and 100ng of pcDNA3.1-beclin1-HA. Twenty-four hours post-transfection, cells were harvested with cold citric saline. Cells were lysed in 200µl of PBS with 1% triton X-100 (PBSt(1%)) for 1 hour at 4˚C. Lysates were added to 50µl of packed anti-FLAG M2 affinity agarose (Sigma-Aldrich, St. Louis, MO) and incubated for 1hour at 4˚C. Samples were centrifuged for 10 minutes at 5,000xg at 4˚C and supernatants were discarded. Agarose was washed 3 times in PBSt(1%) and 3 times in 50mM ammonium bicarbonate (pH 7.0). Samples were eluted from agarose in two 50µl elutions of 100µg/mL FLAG peptide (Sigma-Aldrich, St. Louis, MO) in PBSt(1%) at 37˚C for 30 minutes.

Triton X-100 solubility assay

Harvested cell pellets were lysed for 1 hour at 4˚C in 240µl of 50mM Tris-Cl, 150mM NaCl, 1% Triton X-100, pH 7.6 (TBSt). Protein concentrations of lysate were determined using the Protein-Dc assay kit (Bio-Rad, Hercules, CA) and normalized across all samples. 40µl of lysate was removed for "total" sample and remaining lysate was subjected to centrifugation at 20,000xg for 15minutes at 4˚C. Supernatant was removed and added to 67µl of 4x SDS-sample buffer with 1.25% β-mercaptoethanol. Pellet was dissolved in 267µl of 1x SDS-sample buffer with 1.25% β-mercaptoethanol. Samples were sonicated with two 30-second pulses at 25% intensity using a digital sonifier prior to SDS-PAGE.

Limited trypsin proteolysis

Harvested Cos-7 cell pellets were lysed in 250µl TBSt for 1hour at 4˚C. and diluted to 0.5mg/ml total protein concentration in TBSt. Lysates were divided into 4 x 75µl aliquots and 25µl of various concentrations of trypsin (Worthington Biochemical, Lakewood, NJ) in TBSt were added to aliquots. Samples were digested on ice for 15 minutes and trypsin was inhibited by the addition of 100µl of 50mM Tris-Cl, pH8.0, 150mM NaCl, 1.6% SDS, 20mM EDTA, 1x complete protease inhibitor cocktail (Roche, Basel, Switzerland), 100µM phenylmethylsulfonyl fluoride, 2µg/ml turkey egg-white trypsin inhibitor (Sigma-Aldrich, St. Louis, MO). Samples were then sonicated for 30seconds at 25% intensity using a digital sonifier (Branson, Danbury, CT). 65µl of 4x SDS-sample buffer + 5% β-mercaptoethanol was added to sample prior to SDS-PAGE and western blotting.

Cell lysis and western blotting

For all non-immunoprecipitation or TX-100 solubility GnRHR experiments, harvested cell pellets were lysed for 1 hour at 4˚C in 200µl of 50mM Tris-Cl pH 7.7, 150mM NaCl, 0.8% SDS, 10mM EDTA with 1x Complete protease inhibitor cocktail (Roche, Basel, Switzerland). For experiments to look at phosphorylation of beclin-1, cells were lysed in 2x SDS-sample buffer with 1x PHOSstop phosphatase

inhibitor cocktail (Roche, Basel, Switzerland). Lysate was then subjected to two 30-second sonication pulses at 25% intensity using a digital sonifier, and lysates were diluted to 0.5mg/ml total protein concentration. Lysate (30µl) was added to 1x SDS sample buffer (10µl) that contained 1.25% β-mercaptoethanol, incubated at 4˚C for 30 minutes, and then incubated at 37˚C for 2 minutes. Proteins in a 30µl aliquot of sample were then resolved on a 9% SDS-polyacrylamide gel. Protein was transferred to a 0.4µM nitrocellulose membrane (GE Life Sciences, Pittsburgh, PA). Membranes were blocked for 1 hour with 5% milk in PBS with 0.1% triton X-100 (PBSt(0.1%)) and washed 3x in PBSt(0.1%) for 5minutes. Membranes were then incubated for 1 hour with primary antibody in 2.5% BSA, 1mM NaN_3, PBSt(0.1%) and washed 3x in PBSt(0.1%) for 5minutes. Membranes were then incubated for 1 hour in secondary antibody and washed 3x in PBSt(0.1%) for 15minutes. Membranes were then treated with ECL reagent and visualized using an LAS4000 luminescence imager (GE Life Sciences, Pittsburgh, PA). Data was analyzed with ImageQuant TL software (GE Life Sciences, Pittsburgh, PA).

Fluorescence Microscopy

Fixation of cells for microscopy was performed using a method similar to that previously described (Bhattacharyya et al., 2010). Cos-7 cells were grown and transfected on sterile coverslips. Thirty-six hours post transfection, coverslips were removed from media, placed into -20˚C methanol , and incubated for 24 hours at -20˚C. Coverslips were then removed from methanol and allowed to air dry at room temperature for 1 hour. A humidified chamber was prepared using a wet paper-towel, coverslips were placed in the chamber, and covered with 100µl of the cross-linker bis[sulfosuccinimidyl] suberate (BS3,100µM) for 30 minutes. BS3 solution was aspirated off the coverslips and replaced with 100µl of 1M glycine for 15 minutes. Glycine was then aspirated, coverslips were washed 3-times in PBS, and 100µl of blocking buffer (PBS with 1% FBS, 1% milk) was put on coverslips for 1 hour. Blocking buffer was aspirated and replaced with primary antibody diluted in blocking buffer for 1 hour. Primary antibody was then aspirated, coverslips were washed three times, and secondary antibody diluted in

blocking buffer with DAPI stain was added. After 1 hour, solution was aspirated, coverslips were washed three times with PBS, and mounted onto slides using mounting media. Slides were allowed to dry in the dark at room temperature for at least 12 hours before imaging. Cells were imaged using an IX81 motorized inverted microscope (Olympus, Center Valley, PA) equipped with standard DAPI (blue), FITC (green), and TRITC (red) filter cubes. Images were acquired using MetaMorph (Molecular Devices, Sunnyville, CA). In each experiment 100 cells were scored for a punctate staining pattern. A punctate cell is defined as a one that contained more than 10 discrete puncta, of any size, in the indicated channel. Where indicated, Pearson's correlation coefficients were calculated using a plug-in for ImageJ (Collins, 2007).

Immunogold Electron Microscopy

Cells were harvested 24 hours post transfection with 0.05% Trypsin-EDTA (Gibco) from a 6-well plate and reseeded on Nunc Permanox chamberslides. Cells were allowed to grow for another 24 hours and then were fixed with 2% paraformaldehyde/0.5% glutaraldehyde in 0.15M sodium phosphate buffer, pH 7.4, for 1 hour. Following free aldehyde inactivation with 0.2M glycine in 0.15M sodium phosphate buffer (PB), cells were permeabilized with 0.1% saponin in PB for one hour, and incubated in a 1:100 dilution of Rabbit anti-GFP (Cell Signaling, #2956) overnight at 4˚C. After buffer washes, samples were incubated in secondary antibody, 1:100 goat anti-rabbit IgG 0.8nm immunogold (Aurion, Electron Microscopy Sciences), for 16 hours at 4˚C. The cells were post-fixed in 2% glutaraldehyde in PB, and silver-enhanced for 90 minutes using an Aurion R-Gent SE-EM Silver Enhancement Kit. The monolayers were post-fixed in 0.1% osmium tetroxide, dehydrated in ethanol and embedded in Polybed 812 epoxy resin (Polysciences, Inc., Warrington, PA). 80nm ultrathin sections were cut, mounted on copper grids, and post-stained with 4% uranyl acetate and lead citrate. Sections were observed using a LEO EM-910 transmission electron microscope operating at 80kV (LEO Electron Microscopy, Thornwood, NY) and

images were taken using a Gatan Orius SC1000 CCD camera with Digital Micrograph 3.11.0 (Gatan, Inc., Pleasanton, CA).

Cell Viability (MTT) Assay

To assess viability JB12 knockdown cells the MTT assay was utilized (Mosmann, 1983). Cells in which JB12 was knocked down were seeded into a 6-well, Costar cell culture treated, polystyrene plate (Corning, Corning, NY) at a density of $1x10^5$ cells/well and incubated at 37˚C for 24 hours. Next, media containing 0.1% MTT was added and incubated for 2 hours at 37˚C prior to lysis in 800µl of 1% SDS at 37˚C. A 150µl aliquot of cell lysate was transferred to a Costar 96-well, flat bottom, medium binding microplate (Corning, Corning, NY), and absorbance at 570nm was measured with a FlexStation 3 plate reader (Molecular Devices, Sunnyville, CA). Cells to which no MTT was added were used for background substraction. The signal for MTT in control knockdown cell lysated was used for normalization of values to 100% of control.

Supplemental References

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