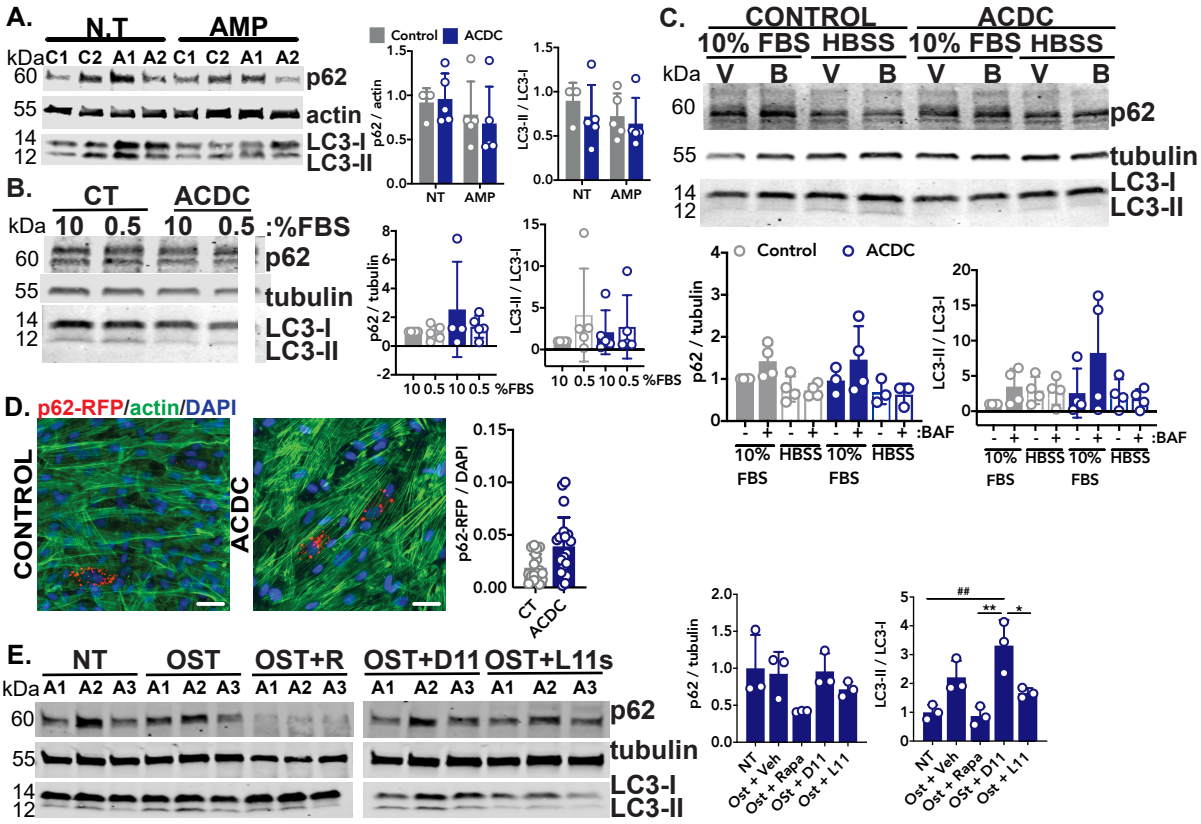
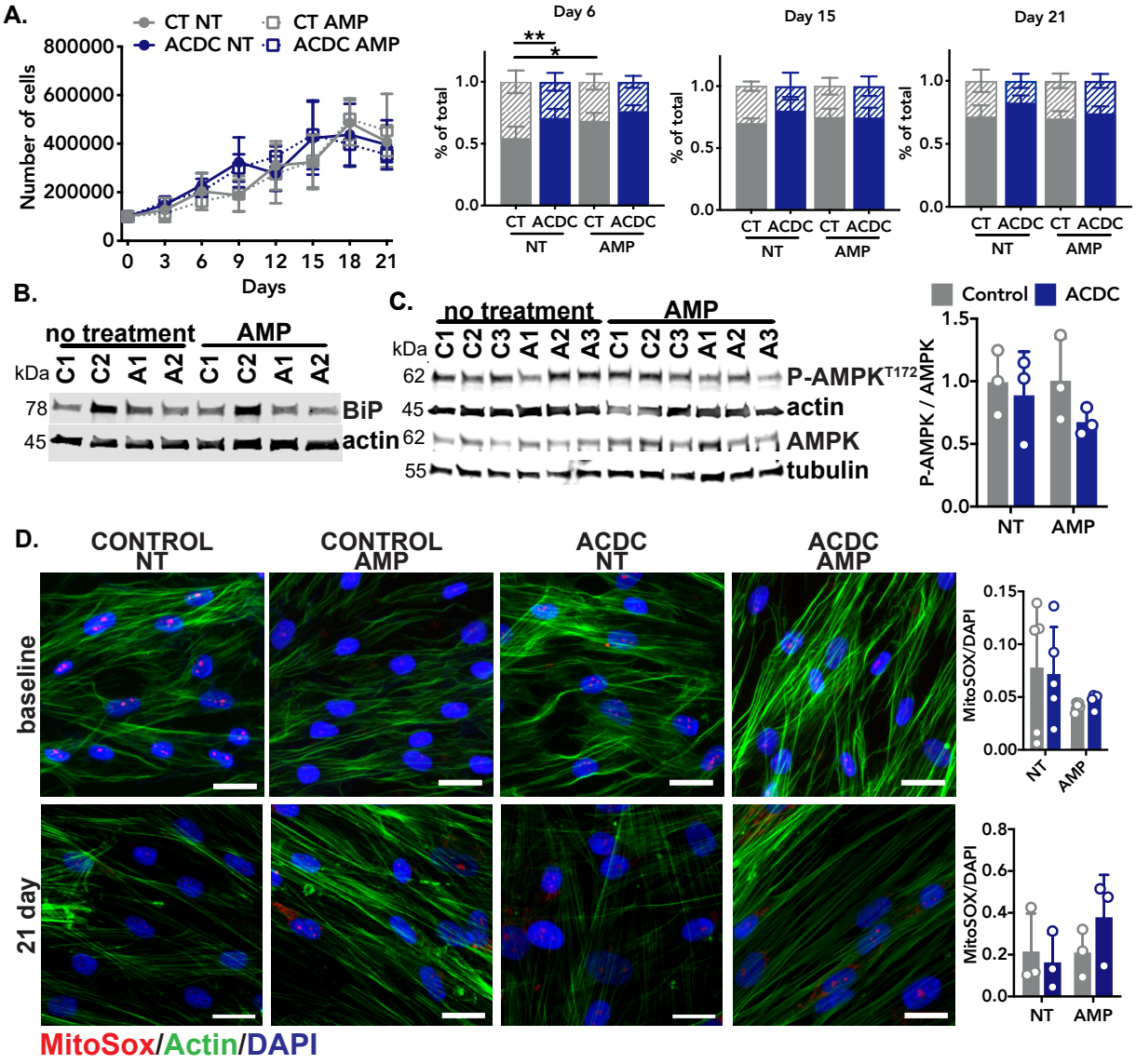
SUPPLEMENTAL FIGURE I



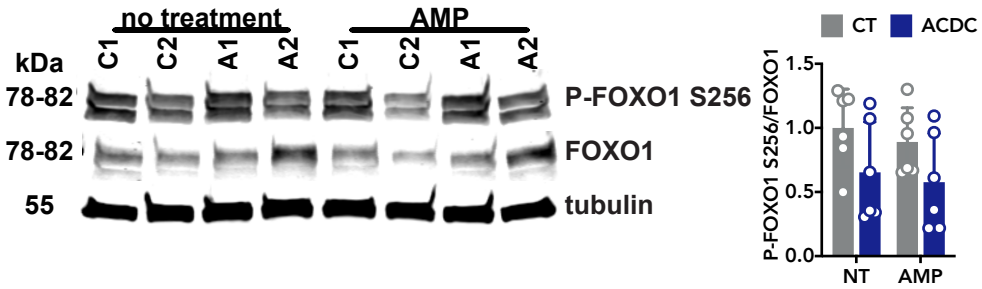
SUPPLEMENTAL FIGURE I: Autophagy flux is not altered due to CD73 deficiency. **A.** Western blot of cells serum-starved overnight then treated with vehicle or 10μM exogenous AMP for 24hr. Results representative of n=5 replicates using 3 control and 3 ACDC patient cell lines. **B.** Western blot of cells grown under normal growth conditions or under serum-starved conditions (10% FBS and 0.5% FBS, respectively). Results representative of n=3 replicates of 3 control and 3 ACDC patient cell lines. **C.** Western blot of cells in normal growth conditions or amino acid starvation (HBSS) with vehicle or 10nM bafilomycin for 4 hours. Results representative of n=3-4 per group using 3 control and 3 ACDC patient cell lines. In A-C no statistical differences detected using two-way ANOVA with Tukey's multiple comparisons test. **D.** Immunofluorescent staining of cells cultured in osteogenic media for 21 days and then treated with the Premo™ Autophagy Sensor RFP-p62 kit. Intensity of RFP-p62 over DAPI staining determined using ImageJ software. Results representative of 5-11 images from 3 control and 3 ACDC patient cell lines using unpaired Student's t-test with Welch's correction. Scale bar represents 50μm. **E.** Western blot of control and ACDC fibroblasts cultured in osteogenic conditions with vehicle (DMSO), 200nM rapamycin, 10μM Tat-Beclin D11 peptide, or 10μM Tat-Beclin L11s peptide for 21 days. ##p=0.0019, **p=0012, *p=0.0193 using one-way ANOVA with multiple comparisons for statistical analysis. Results representative of n=3 replicates of 3 control patient cells and 3 ACDC patient cells.

SUPPLEMENTAL FIGURE II



SUPPLEMENTAL FIGURE II: Exogenous AMP does not alter proliferation, cell death, or cellular stress in CD73 deficient cells. A. Control and ACDC fibroblasts were treated with or without 100μM AMP for 21 days. Cells were counted every 3 days. The proportion of live/total and dead/total was compared between control and ACDC cells. **p=0.0063, *p=0.0289. Statistical significance was determined by two-way ANOVA with Tukey's multiple comparisons test. Data representative of n=4 replicates of 3 control and 3 ACDC patient fibroblast lines. B. Western blot analysis of cells serum-starved overnight then treated with vehicle or 10μM exogenous AMP for 24hr. Results representative of n=3 per group using 3 control patient and 3 ACDC patient cell lines. C. Western blot analysis of cells treated with vehicle or 100μM exogenous AMP for 10min. No statistical significance was detected using two-way ANOVA with Tukey's multiple comparisons test. Results representative n=3 replicates of 3 control and 3 ACDC patient cell lines. D. Control and ACDC fibroblasts were treated with or without 100μM AMP for 2 hours at baseline and after 21 days of osteogenic treatment and stained with MitoSOX red reagent. Data representative of n=4-5 images each of 3 control and 3 ACDC patient fibroblast lines. No statistical significance detected by two-way ANOVA with Tukey's multiple comparison test. Scalebar represents 25μm.

SUPPLEMENTAL FIGURE III



SUPPLEMENTAL FIGURE III: FOXO1 is not phosphorylated in cells treated with exogenous AMP. Control and ACDC fibroblasts were given 100μM exogenous AMP for 30 minutes and total cell lysates were analyzed by western blotting for FOXO1 phosphorylation. Results representative of n=6 per group using 3 control patient and 3 ACDC patient cell lines. No statistical difference detected by two-way ANOVA with Tukey's multiple comparisons test.

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Not applicable

Genetically Modified Animals

Not applicable

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot #	Persistent ID / URL
α-tubulin	LI-COR	926-42213	1:2000 (WB)	C40624-01	https://www.licor.com/bio/reagents/alpha-tubulin-mouse-monoclonal-antibody-for-normalization
β-tubulin	LI-COR	926-42211	1:2000 (WB)	C40702-01	https://www.licor.com/bio/reagents/beta-tubulin-rabbit-polyclonal-antibody-for-normalization
β-actin	LI-COR	926-42212	1:2000 (WB)	C70302-01	https://www.licor.com/bio/reagents/beta-actin-mouse-monoclonal-antibody-for-normalization
p62	Cell Signaling	5114S	36.2ng/mL (WB)	6	https://www.cellsignal.com/products/primary-antibodies/sqstm1-p62-antibody/5114
LC3	Cell Signaling	12741S	64.5ng/mL (WB)	4	https://www.cellsignal.com/products/primary-antibodies/lc3a-b-d3u4c-xp-rabbit-mab/12741
CD73	Abcam	ab124725	1.37µg/mL (WB)	GR260532-7	https://www.abcam.com/cd73-antibody-epr6115-ab124725.html
CD73	Atlas	HPA017357	0.3µg/mL (IFC)	B96770	https://www.atlasantibodies.com/products/antibodies/primary-antibodies/triple-a-polyclonals/nt5e-antibody-hpa017357/
P-VASP^{S157}	Cell Signaling	3111	0.146µg/mL (WB)	5	https://www.cellsignal.com/products/primary-antibodies/phospho-vasp-ser157-antibody/3111
VASP	BD Transduction Laboratories	610447	0.25µg/mL (WB)	7228895	https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/cell-biology-reagents/cell-biology-antibodies/purified-mouse-anti-vasp-43vasp/p/610447
P-Akt^{T308}	Cell Signaling	2965	0.704µg/mL (WB)	5	https://www.cellsignal.com/products/primary-antibodies/phospho-akt-thr308-c31e5e-rabbit-mab/2965
P-Akt^{S473}	Cell Signaling	4060P	0.182µg/mL (WB)	19	https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060
Akt1	Cell Signaling	2967	1:1000 (WB)	17	https://www.cellsignal.com/products/primary-antibodies/akt1-2h10-mouse-mab/2967

DOI [to be added]

Akt1/2/3	Santa Cruz Biotechnology	sc-8312	0.8µg/mL (WB)	A0406	https://www.scbt.com/p/akt1-2-3-antibody-h-136
P-AMPK^{T172}	Cell Signaling	2535	27.0ng/mL (WB)	16	https://www.cellsignal.com/products/primary-antibodies/phospho-ampka-thr172-40h9-rabbit-mab/2535
AMPK	Cell Signaling	2793S	1.602µg/mL (WB)	6	https://www.cellsignal.com/products/primary-antibodies/ampka-f6-mouse-mab/2793
P-FoxO1^{S256}	Cell Signaling	9461	0.214µg/mL (WB)	8	https://www.cellsignal.com/products/primary-antibodies/phospho-foxo1-ser256-antibody/9461
P-FoxO1^{T24}	Cell Signaling	9464T	0.1µg/mL (WB)	7	https://www.cellsignal.com/products/primary-antibodies/phospho-foxo1-thr24-foxo3a-thr32-antibody/9464
FoxO1	Cell Signaling	2880	88ng/mL (WB) 1.76µg/mL (IFC) 1.76µg/mL (IHC)	11	https://www.cellsignal.com/products/primary-antibodies/foxo1-c29h4-rabbit-mab/2880
BiP	Cell Signaling	3177	98.0ng/mL (WB)	9	https://www.cellsignal.com/products/primary-antibodies/bip-c50b12-rabbit-mab/3177
SMA-α	Abcam	Ab7817	10µg/mL (IFC)	GR241210-2	https://www.abcam.com/alpha-smooth-muscle-actin-antibody-1a4-ab7817.html
TNAP	Novus Biologicals	NBP2-67295	1:50 (IFC)	HM0128	https://www.novusbio.com/products/alkaline-phosphatase-tissue-non-specific-antibody-sa40-00_nbp2-67295
IRDye goat anti-mouse 680	LI-COR	926-68070	0.1µg/mL		https://www.licor.com/bio/reagents/irdye-680rd-goat-anti-mouse-igg-secondary-antibody
IRDye goat anti-rabbit 680	LI-COR	926-68071	0.1µg/mL		https://www.licor.com/bio/reagents/irdye-680rd-goat-anti-rabbit-igg-secondary-antibody
IRDye goat anti-mouse 800	LI-COR	926-32210	0.1µg/mL		https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody
IRDye goat anti-rabbit 800	LI-COR	926-32211	0.1µg/mL		https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-rabbit-igg-secondary-antibody
AlexaFluor Goat anti-rabbit 594	Invitrogen	A11012	0.2mg/mL		https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11012

DNA/cDNA Clones

DOI [to be added]

Clone Name	Sequence	Source/ Repository	Persistent ID / URL
pEZX-LvPG04 plasmid	GGCACCAGGCCCCAGTTCTGGCTCCAACCTTACT GAGAAGTCACATGATCTCTCTGGGCCTCAGTTTTC TCCTCTGGAAAATGGAGCTTTTGGAAGTTAATGC ATGCACAGTGCCTGGCCCTGAGACTGGCATGGAG TGAGTGGAAGAGAGGTTGCCTGACCTGGCTAAAA TTGGTTCTCTGGGCAGACATTTTCCCAAGGGCCAC TGAGAAGACCCTCCTGTTAGGAGTCAGTGAGCTC TGTCGCTGGAGGCATTCAAACAGAGCCTGCGGAC TTCTCACTGCGAAGTTGCCCAGAGAATTCAGTGCT CAGAGGAAAGGGAGTGGTTATTCCATCAGAGCTG GTTCCCCAGGAGCGGGAGCAGGGCCTGTAGCACC CAGCCTCTGTCCCTGGCTCCCGTCTATCCGGGATT TTAGCGTTTCCTCTGTAGTTTTCAAGCACTGTCTC ATATGACTCTCGCACCAGCGAGAGGCCAGGGGAG ATGGTGTCTGCCTGTTAAAGAGGGGCAGGCTGGT CCACATAGGTCAAGTGACTTGGCCAAGGTCACCA GAGCAGAGTTTTGAACTTGAGCTGTCTGACTCAA CTGCCTGGGAAGTGCCTGCCCCTCCTCTGGCATCC AGGGAGCATGTCCTGGGGCTCTGGCTGGGACATA GCCGGACACCTGCGGGCCCTTTACGTCTCTAAAG AGAGAAAGAGGGAAGGGCCCCTGTCTAGGGGGT GGTTTCCCTCCAGATGCCACCCCTCCGAGGTCCCC TTCTGCTTCTTCTTGCGGTAGCCAGGGAGGCAGCC CACGGGCAGGGAAGCGGGGGTGGGGGTGCAGAG TCAGAGGTGCACGTGGACAGAGACAGAGAGACA GGGACACGTGGGCAGAGACGGATAAAGACAGAG ACCCAGAGAAAGCCAGATATGTTGACAGACACAG AGACAGACGCCAGAGAGGAAGGCAGACAAAGAG ACGGGTGGAGACAAAGACTCCCACCAAGAGACG CAGAAGGAAGATGCCGACGGTAAAGACAAAACA GGAGACGCGCGCAAGGAGCAGGTCAGAGCCCAG GCTCGCTGAGAGAGGAAGGGCTGGGCTGGGGCA GCCCGGAGGCAGAGAGACCGAGAGTGCGGGGCG GGCGAGGGACGCCAGGGCCGCGTCACCCCAGCCC GTTCCTAGCTCCGCTCCCGGCAGGGGGCGCCCTG GCCTCGTGGCACGACCGGCCCGCGGGGCGCGGGG CTCGGGCCGGGGGCGGGGCCGGGGCCGGGCTGG GGAGGGGTTGGGGCCGGGGGCGGGGGAGGGGGC GGGCTGCCCGGGCCTCACTCGGGCCCCGCGGCCG CCTTTATAAGGCGGCGGGGGTGGTGGCCCGGGCC GCGTTGCGCTCCCGCCACTCCGCGCCCGCTATCCT GGCTCCGTGCTCCCACGCGCTTGTGCCTGGACGG ACCCTCGCCAGTGCTCTGCGCAGGTAAGGATTCG ACGCTGCCCCGCGCCCTGGTTCCCCAGGGCCCCA GCGGACGTGGTCCATCCCCTTCTGCATCCTCCGCT GGCCCCGTGG	GeneCopoeia	https://www.genecopoeia.com/product/search/detail.php?prt=22&cid=58705&key=HPRM30446

DOI [to be added]

Primers

Gene	Forward Sequence (5' -> 3')	Reverse Sequence (5' -> 3')	Source
h18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG	IDT
hCD73	GGGCGGAAGGTTCCTGTAG	GAGGAGCCATCCAGATAGACA	IDT
hA1AR	TGGGCCACAGACCTACTTC	TACCGGAGAGGGATCTTGACC	IDT
hA2aAR	TGACTCCCATGCTAGGTTGGA	CATCCTCAAAGAGACAGGCCA	IDT
hA2bAR	TGTCCCGCTCAGGTATAAAAGT	CCCAGGAATGGAGTCAATCCG	IDT
hA3AR	GGCCAATGTTACCTACATCACC	CCAGGGCTAGAGAGACAATGAA	IDT
hALPL	ATGGGATGGGTGTCTCCACA	CCACGAAGGGGAACTTGTC	IDT
Mutagenesis	TCTGTCTCTGTGTCTGTTAATATATC TGG CTTTCTCTGGGTC	GACCCAGAGAAAGCCAGATATATT AACAG ACACAGAGACAGA	IDT

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Control patient fibroblasts	Skin biopsy	F & M	N/A
ACDC patient fibroblasts	Skin biopsy	F & M	N/A

Data & Code Availability

Not applicable

Other

Description	Source / Repository	Persistent ID / URL
β-glycerol phosphate disodium salt pentahydrate	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/50020
L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/a8960
dexamethasone	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/d4902
EHNA hydrochloride	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/e114
adenosine 5′-monophosphate monohydrate (AMP)	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/a2252
Rapamycin	Gemini Bio-Products	https://www.gembio.com/product/rapamycin-powder
AS1842856	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/mm/506081?lang=en&region=US
bafilomycin A1	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/b1793
3-isobutyl-1-methylxanthine	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/i5879

DOI [to be added]

Forskolin	Tocris	https://www.tocris.com/products/forskolin_1099
8-Bromoadenosine 3′,5′-cyclic monophosphate sodium salt	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/b7880
Alizarin Red S	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/a5533
Tat-Beclin 1 D11 Autophagy Inducing Peptide	Novus Biologicals	https://www.novusbio.com/products/tat-beclin-1-autophagy-inducing-peptide_nbp2-49888
Tat-Beclin 1 L11S Autophagy Inducing Peptide	Novus Biologicals	https://www.novusbio.com/products/tat-beclin-1-peptide_nbp2-49887#protocols-faqs
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS)	Promega	https://www.promega.com/products/cell-health-assays/cell-viability-and-cytotoxicity-assays/celltiter-96-aqueous-one-solution-cell-proliferation-assay-_mts_/
RNeasy Mini Kit	Qiagen	https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/rneasy-mini-kit/
High-Capacity cDNA Reverse Transcription Kit	Thermo Fisher	https://www.thermofisher.com/order/catalog/product/4368814#/4368814
Direct cAMP ELISA kit	Enzo	https://www.enzolifesciences.com/ADI-900-066/direct-camp-elisa-kit/
SIGMA*FAST*™ 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) tablets	Sigma-Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/b5655
Premo™ Autophagy Sensor RFP-p62 Kit	Invitrogen	https://www.thermofisher.com/order/catalog/product/P36241
MitoSOX™ Red Mitochondrial Superoxide Indicator	Invitrogen	https://www.thermofisher.com/order/catalog/product/M36008
QuikCHange II XL Site-Directed Mutagenesis Kit	Agilent	https://www.agilent.com/store/en_US/Prod-200521/200521
Monarch® Plasmid Miniprep Kit	New England Biolabs	https://www.neb.com/products/t1010-monarch-plasmid-miniprep-kit
Lipfectamine LTX Reagent with PLUS Reagent	Invitrogen	https://www.thermofisher.com/order/catalog/product/15338030
Secrete-Pair™ Dual Luminescence Assay Kit	GeneCopoeia	https://www.genecopoeia.com/product/secrete-pair/secrete-pair-gaussia-luciferase-assay-kit/
Von Kossa Method of Calcium Kit	Polysciences	https://www.polysciences.com/default/catalog-products/life-sciences/histology-microscopy/staining-histology-cytology/silver-stains/von-kossa-method-for-calcium-kit/

DOI [to be added]

SUPPLEMENTAL FIGURE IV: Final unedited western blot images and Major Resources table.