# Supporting Information

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### SI Materials and Methods

**Antibodies and Reagents.** Lapatinib and torin1 were purchased from LC Laboratories. Baf A1 was purchased from Acros (Thermo Scientific). Chloroquine was purchased from Sigma. Antibodies were purchased from the following sources and used at the indicated dilutions: HER2 (no. 2165, 1:1,000 dilution; Cell Signaling), p-HER2 (Y1221/1222) (no. 2243, 1:1,000 dilution; Cell Signaling), p-S6 (S240/244) (no. 2215, 1:1,000 dilution; Cell Signaling), Flag [F1804, 1 μg/mg of protein for immunoprecipitation (IP); Sigma], Flag-HRP (A8592, 1:2,000 dilution; Sigma), total p-tyrosine (sc-7099, 1:1,000 dilution; Santa Cruz Biotechnology), actin-HRP (sc-47778, 1:2,000 dilution; Santa Cruz Biotechnology), Beclin 1 [sc-48341, used for IP at 1 μg of antibody per mg of total protein; sc-11427, used for Western blot (WB), 1:1,000 dilution; Santa Cruz Biotechnology], normal IgG (sc-2025, used for IP at 1 μg of antibody per mg of total protein; Santa Cruz Biotechnology), Bcl-2 (sc-7382, used for WB at 1:100 dilution and IP-WB at 1:500 dilution; Santa Cruz Biotechnology), p62 (GP62-C, 1:2,000 dilution; Progen), and LC3B (NB100-2220, 1:2,000 dilution; Novus Biologicals). Tat-Beclin 1 (YGRKKRRQRRR-GG-VWNATFHIWHD) and Tat-Scrambled (YGRKKRRQRRR-GG-WNHADHTFVWI) were synthesized by the University of Texas Southwestern Protein Technology Center as reported elsewhere (1).

**Mouse Strains and Breeding.** FVB/N-Tg (MMTVneu) 202 Mul/J mice were obtained from The Jackson Laboratory (002376). C57/B6 *Becn1* knock-in *Becn1*$^{F121A}$ mice were generated as described (2). MMTVneu mice were crossed with *Becn1*$^{F121A/F121A}$ mice to generate MMTV/neu transgenic; *Becn1*$^{F121A/WT}$ mice. These mice were intercrossed, and offspring were monitored for tumor development weekly by mammary gland palpation. *Becn1*$^{WT}$ and *Becn1*$^{F121A}$ mice were crossed with GFP-LC3 transgenic mice (3), and mammary glands of the *Becn1*$^{WT}$:GFP-LC3 and *Becn1*$^{F121A}$:GFP-LC3 mice were analyzed for autophagy. Five- to 6-wk-old female *nu/nu* mice were obtained from Taconic Farms.

**Plasmids.** The pBabe-puromycin-HER2 plasmids [WT, KA (A775_G776insYVMA), and KD (D845A)] were obtained from Addgene. The pBabe-GFP-LC3 and pCR3.1-Flag-Beclin 1 plasmids were previously reported (4).

**siRNA and Plasmid Transfection.** Plasmid transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Human Dicer-siRNA for HER2 and nontargeting control were obtained from Integrated DNA Technologies, and transfected with Lipofectamine RNAiMax (Invitrogen) according to the manufacturer's instructions.

**Cell Lysates, IP, and WB Analyses.** Cells were washed in ice-cold PBS and lysed in lysis buffer [50 mM Tris·HCl (pH 7.4), 250 mM NaCl, 0.5% Igepal] with proteases (Roche) and phosphatases (Thermo Scientific) inhibitor mixtures for 10 min at 4 °C. Lysates were centrifuged at 16,000 × *g* for 10 min. Cleared lysates were diluted in 2× SDS/PAGE loading buffer (Bio-Rad) and boiled for 10 min. The resulting samples were separated on a 5–20% gradient gel (Bio-Rad), transferred to nitrocellulose membranes (Bio-Rad), and incubated with the indicated antibodies.

For Flag and endogenous Beclin 1 IPs, cells were lysed and processed as above using IP buffer [50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 0.5% Nonidet P-40]. Lysates were precleared using 30 μL of protein-G Sepharose beads (1:1 in IP buffer; Invitrogen) for 2 h. Samples were incubated with anti-Flag or anti-Beclin 1 antibody (or corresponding IgG controls) overnight at 4 °C. Thirty microliters of Protein-G Sepharose beads were added and incubated for 1 h. Immunoprecipitates were washed three times in IP buffer, resuspended in 2× SDS/PAGE loading buffer, boiled for 5 min, and analyzed by WB.

For Bcl-2 IPs of mammary glands, flash-frozen mammary glands were weighed and homogenized in ice-cold lysis buffer (25 mM Hepes, 150 mM NaCl, 1 mM EDTA, 1% TX100; 1 mL per 100 mg of tissue) containing protease and phosphatase inhibitors for 30 min at 4 °C. Lysates were cleared using QIAshredder columns (Qiagen) and further centrifugation (16,000 × *g* at 4 °C for 30 min). Lysates were precleared with 60 μL of protein-G agarose beads for 2 h and incubated overnight with Bcl-2-agarose (or IgG) antibody. Immunoprecipitates were washed five times, resuspended in 2× SDS/PAGE loading buffer, boiled for 5 min, and analyzed by WB.

For IP of xenograft tumor samples, flash-frozen tumor samples were weighed, diluted in three volumes of IP buffer, and homogenized. Samples were lysed for 30 min at 4 °C and cleared with QIAshredder columns and centrifugation (16,000 × *g* for 10 min at 4 °C).

**Electron Microscopy.** Tumor tissue was cut into 1-mm$^3$ pieces and fixed with 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium cacodylate buffer. Tissue samples were then rinsed in 0.1 M sodium cacodylate buffer and postfixed in 1% osmium tetroxide and 0.8% potassium ferricyanide in 0.1 M sodium cacodylate buffer for 1.5 h at room temperature. After three rinses in water, they were stained en bloc with 4% uranyl acetate in 50% ethanol for 2 h. Next, they were dehydrated with increasing concentrations of ethanol, transitioned into resin with propylene oxide, infiltrated with Embed-812 resin, and polymerized in a 60 °C oven overnight. Blocks were sectioned with a diamond knife (Diatome) on a Leica Ultracut 6-μL tramicrotome (Leica Microsystems) and collected onto copper grids, they were then poststained with 2% aqueous uranyl acetate and lead citrate. Images were acquired on a JEOL JEM 1200 EX II transmission electron microscope using a voltage of 120 kV.

Autophagy induction in xenografts was measured by the presence of autophagosomes and autolysosomes at the ultrastructural level. Double-membrane vesicles filled with cytoplasmic portions were categorized as autophagosomes, while double-membrane vacuoles with inner membrane rupture or partial degradation of their contents were classified as autolysosomes. More than 50 cell profiles were analyzed per sample, and the average value for each xenograft was calculated.

**RNAseq Analyses.** RNAseq analyses were performed by Admera Health. Total RNA was extracted from 30 mg of flash-frozen xenograft tumors using a QIAGEN RNeasy PLUS Universal Kit according to the manufacturer's instructions. The RNA quality check was performed using RNA Bioanalyzer chips. A cDNA library was constructed using a NEBNext Ultra RNA Library Prep Kit, and ribosomal RNA depletion was performed with a Ribo-Zero Gold rRNA Removal Kit (Illumina). Sequencing was performed with NextSeq High-Output 2x75. An NGS QC Toolkit (v2.3.1) (5) was used to assess the sequencing quality of the raw reads, and reads that did not pass the default filter were removed. The quality-filtered reads were mapped to the human reference genome (GRCh38) using TopHat (v2.0.8) (6). An HTSeq Python package (0.6.1) (7) was used to generate read counts. Data normalization and sample comparison were

then performed using the DESeq R Bioconductor package (8), and the differentially expressed genes were selected using the false discovery rate-adjusted $P$ value cutoff of 0.05. Pathway analysis was performed using ConsensusPathDB (9) for gene set overrepresentation analysis.

**Xenograft and Mouse Experiments.** Six- to 7-wk-old female *Nu/Nu* mice (Taconic Farms) were injected with 60-d, slow-release, 1.7-mg estrogen pellets (Innovative Research of America). Two days later, $10^7$ BT-474-VH2 cells were injected into the upper right mammary pad. Tumor volume was measured twice a week, and treatments were started when tumor volume reached 200 $mm^3$. Mice were randomly distributed and treated with daily i.p. injections of the following agents: Tat-Scrambled and Tat-Beclin 1 (16 $mg \cdot kg^{-1}$, diluted in PBS), lapatinib (100 $mg \cdot kg^{-1}$, diluted from a DMSO stock in vehicle containing 5% PEG, 5% Tween 80, 5% dextrose in water), or vehicle alone. Following treatment initiation, tumor volume was monitored daily. At the end of the experiment, tumors were harvested and fixed in 4% paraformaldehyde (PFA) for histopathological and ultrastructural analyses or flash-frozen in liquid nitrogen for biochemical and RNAseq analyses.

All mouse experiments were performed in accordance with approved institutional animal care and use protocols of the University of Texas Southwestern Medical Center.

**Autophagy Analyses.** Autophagic activity was measured in HeLa cells and cultured breast cancer cells by WB analysis of p62 and LC3 or by microscopic quantification of GFP-LC3 puncta (autophagosome numbers) in the absence or presence of 100 nM Baf A1 as described elsewhere (10). For measurement of GFP-LC3 puncta, cells were seeded in four-well chamber slides (Lab-Tek) after GFP-LC3 transfection, incubated with the desired treatments, and fixed in 3.6% PFA in PBS for 20 min at room temperature. Cells were permeabilized in 0.05% saponin and 0.2% BSA in PBS for 15 min at room temperature and blocked in 3% BSA for 1 h at room temperature. Images were acquired at a 20× magnification on a Zeiss AxioImager Z2 microscope. AutoDeBlur and Imaris (Bitplane) were used for Z-stack deconvolution.

Autophagy was measured in vivo in the mammary glands of 2-mo-old homozygous *Becn1*$^{F121A}$;GFP-LC3 or *Becn 1*$^{WT}$:GFP-LC3 mice (three mice per group) treated with either PBS or chloroquine (50 $mg \cdot kg^{-1}$) for 4 h and perfused in 4% PFA in PBS. The total number of GFP-LC3 dots was counted per duct area. All ducts in the slide were quantified (>50 per mammary gland), and the average value for each mammary gland was determined.

**Histopathological Analyses.** Xenografts tumors were fixed in 4% PFA in PBS, embedded in paraffin, and sectioned. Sections were stained with an anti-Ki67 antibody (1:200; Abcam) or processed for TUNEL labeling (ApopTag; Millipore) according to the manufacturer's instructions.

**METABRIC Breast Cancer Dataset Analyses.** The METABRIC dataset, as described elsewhere (11), was used for all analyses. mRNA expression was measured using the Illumina HT-12 platform, and a Gaussian mixture method-based model was used as described (12) to determine the cutoff between HER2 overexpression and non-overexpressed HER2. The copy number profile was measured using the Affymetrix SNP6 array and processed using the GISTIC 2.0 method algorithm (13) to classify patients as having net loss, diploid, or net gain for the genes *BECN1* and *ERBB2/HER2*. The entire dataset consisted of 2,433 patients, and patients for whom HER2 expression or copy number data were unavailable were excluded from analysis. Survival curves were estimated by the Kaplan–Meier method, and statistical significance between groups determined by the log-rank test.


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A

| No. | Gene Symbol | Chromosomal Location |
|---|---|---|
| 1 | *ERBB2* (*HER2*) | chr17:39,700,080-39,728,662 |
| 2 | *BECN1* | chr17:42,810,134-42,824,263 |
| 3 | *BRCA1* | chr17:43,045,629-43,125,483 |

B

| *BECN1* Copy Number | *HER2* Copy Number | | |
|---|---|---|---|
| | Loss | Diploid | Gain |
| Loss | 347 | 23 | 217 |
| Diploid | 30 | 1228 | 146 |
| Gain | 1 | 19 | 162 |

C

D

| *HER2* Overexpression | *BECN1* Copy Number | | |
|---|---|---|---|
| | Loss | Diploid | Gain |
| Yes | 146 | 62 | 7 |
| No | 376 | 1152 | 161 |

E

**Fig. S1.** Association between *BECN1* loss, *HER2* amplification/overexpression, and survival in the METABRIC breast cancer dataset ($n$ = 2,433 patients) (1). (*A*) Schematic depiction of the location of *BECN1*, *BRCA1*, and *ERBB2*/*HER2* on chromosome 17. The map refers to the UCSC Genome Browser (GRCH38/hq38). (*B*) Copy number variation of *BECN1* and *HER2* in the METABRIC breast cancer dataset. The GISTIC 2.0 algorithm was used to determine net loss, diploid normal copy, or net amplification for each gene as described (2, 3). The vast majority of patients with *BECN1* net loss have heterozygous, not homozygous, deletions (3). (*C*) *HER2* expression profiling. Histogram showing two peaks, one around 10.4 and the other around 13.8, indicating that there are two subpopulations, *HER2* overexpression and *HER2* non-overexpression. A Gaussian mixture model-based method was used to determine the boundary of the two subpopulations. Based on this method, the split point was 12.67. Patients with *HER2* non-overexpression are shown in red, and patients with *HER2* overexpression are shown in green. (*D*) Copy number variation of *BECN1* in *HER2* overexpression and *HER2* non-overexpression breast cancers. (*E*) Kaplan–Meier curve showing overall survival of patients with *HER2* overexpression and diploid copy number of BECN1 (green line) or *HER2* overexpression and net loss of BECN1 (red line). Patients with *BECN1* gain ($n$ = 7) were excluded from analysis, as there is not sufficient statistical power to evaluate the association between survival and *BECN1* gain in such a small sample size.

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Fig. S2. Role of Beclin 1 phosphorylation and mTOR in regulation of HER2-mediated inhibition of starvation-induced autophagy. (*A*) Effects of HER2 on Beclin 1 tyrosine phosphorylation in HeLa cells cotransfected with Flag-Beclin 1 and the indicated HER2 construct. V, empty vector; WCL, whole-cell lysates. (*B*) Effects of starvation (HBSS, 3 h) and torin1 (250 nM, 3 h) on HER2 and S6 phosphorylation in HeLa cells transfected with the indicated HER2 expression plasmid. (*C*) Effect of starvation (HBSS, 3 h) or mTOR inhibition (torin1, 250 nM, 3 h) on GFP-LC3 puncta (autophagosome numbers) in HeLa cells expressing the indicated HER2 constructs. (*D*) WB analysis of HeLa cells transfected with the indicated HER2 constructs and either Flag epitope-tagged WT Beclin 1 or a tyrosine phosphorylation site mutant Beclin 1 (Y229F/Y233F/Y352F) and used for autophagy analyses in *E*. (*E*) Effect of starvation (HBSS, 3 h) on GFP-LC3 puncta (autophagosome numbers) in HeLa cells expressing the indicated HER2 and Beclin 1 constructs. Bars are mean ± SEM of triplicate samples (100–150 cells per condition). Similar results were observed in three independent experiments. n.s., not significant. ***$P < 0.001$ vs. normal media control, ANOVA.

**Fig. S3.** *Becn1*$^{WT}$ MEFs (WT) *Becn1*$^{F121A}$ homozygous KI MEFs have similar levels of HER2 expression after transfection of indicated HER2 expression plasmids. Cells were either grown in normal media (starvation, −) or cultured in HBSS for 3 h (starvation, +). V, empty vector.

**Fig. S4.** Effects of lapatinib on autophagy in HER-positive breast cancer cell lines. Quantitation of GFP-LC3 puncta (autophagosomes) in indicated HER-positive breast cancer cells treated with vehicle (DMSO control) or 1 μM lapatinib for 3 h. Bars are mean ± SEM of triplicate samples (100–150 cells per condition). Similar results were observed in three independent experiments. ***$P < 0.001$ for the indicated comparison, ANOVA.

A

# TUNEL-positive cells/ mm² area of tissue

B

% of tumor xenograft cells that are KI67-positive

**Fig. S5.** Quantification of TUNEL (*A*) and Ki-67 staining (*B*) of BT-474-VH2 xenografts 3 d after the indicated treatment. Bars are mean ± SEM for xenografts for four mice per treatment group. For *A*, the value of each mouse xenograft represents the total number of TUNEL-positive cells per unit area in the entire tissue section. For *B*, the value of each mouse xenograft is the average percentage of 20 low-power fields per tissue section. n.s., not significant; T-B, Tat-Beclin 1; T-S, Tat-Scrambled. $*P < 0.05$ and $***P < 0.001$ for indicated comparison; ANOVA.

**Dataset S1. Genes and pathways regulated in lapatinib- vs. vehicle-treated BT-474-VH2 xenografts**

Dataset S1

**Dataset S2. Genes and pathways regulated in Tat-Beclin 1– vs. Tat-Scrambled–treated BT-474-VH2 xenografts**

Dataset S2

**Dataset S3. Genes and pathways regulated in lapatinib- vs. Tat-Beclin 1–treated BT-474-VH2 xenografts**

Dataset S3