Extended Experimental Procedures

Plasmid Construction

To generate EGFR expression vectors, wild-type (WT) EGFR, EGFR L858R, and EGFR Δ□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□p□□□□□□□□ □□□□□□□ (Greulich et al., 2005), and inserted in frame into p-IRES-bleo3 (Clontech). Fragments encoding full-length (aa 1-450) and truncated mutants of (aa 1-115, aa1-135, aa 141-277 aa 244-337) Beclin 1 were amplified from pCDNA-flag-*beclin* 1 and cloned in frame into p-Bicep-CMV2 (Sigma-Aldrich). Mutations of Beclin 1 (Y229F, Y233F, Y352F, Y229F/Y233F, Y229F/Y352F, Y233F/Y352F, Y229F/Y233F/Y352F and Y229E/Y233E/Y352E) were generated using QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies). To generate tetracycline-inducible constructs, 3x-Flag-tagged WT and mutant Beclin 1 fragments were amplified from pBicep-CMV2-Beclin 1 constructs and cloned into pcDNA5/TO© (Invitrogen). The tetracycline regulatory vector, pcDNA™6/TR, was purchased from Invitrogen. pcDNA3-AU1-mTOR-S2215Y (Sato et al., 2010) was purchased from Addgene. The viral γHV-68 vBcl-2 coding sequence was amplified from pcDNA3.1-HA-γHV68 M11 (Sinha et al., 2008) and inserted into pIRESpuro3 (Clontech) to generate a final construct with a triple tandem Flag-Myc tag. Viral Bcl-2 mutants (E et al., 2009), Δα1 (lacking the N-terminal 21 residues), ΔBH2 (lacking residues 129-144 of the BH2 domain), and AAA (with alanine substitutions at serine 85, glycine 86, arginine 87 residues) were created using a QuikChange™ Site-Directed Mutagenesis Kit (Agilent Technologies).

Cell Culture and Transfection

Hela cells and the NSCLC tumor cell lines, A549, H1703, H1819, H2073, H1819, HCC827, H1975, H3255, H1993, HCC4017 and H2122, were grown in RPMI-1640 medium (Invitrogen) with 10% fetal bovine serum (FBS) (Invitrogen). A549/Flag-Beclin

1, HCC827/GFP-LC3, H1975/GFP-LC3 and HCC827/Flag-Beclin 1 (WT and mutant) cell lines were generated by selection with 0.5 μg/ml G418. HCC827/GFP-LC3/Flag-Beclin 1 tetracycline-inducible cell lines were generated by sequential transfection of pcDNA/GFP-LC3, pcDNA™6/TR and pcDNA5/TO©/Flag-Beclin 1 followed by sequential selection with 0.5 μg/ml G418, 5 ng/ml blasticidin and 50 μg/ml hygromycin (Invitrogen). HCC827/GFP-LC3/vBcl-2 cells were generated by selection with 50 ng/ml puromycin. Plasmid transfection was performed using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). For autophagy assays, Hela cells were transfected with WT or mutant EGFR constructs and selected with 1 μg/ml Bleomycin (Invitrogen). The positive clones were collected as a pool for subsequent transfection with pcDNA/GFP-LC3.

Western Blot Analysis

Cells were lysed in lysis buffer containing 20 mM Hepes (pH 7.0), 150 mM NaCl, 1 mM EDTA, 0.8% CHAPS, 10% glycerol, Proteinase Inhibitor Cocktail (Roche Applied Sciences) and Halt Phosphatase Inhibitor Cocktail (Thermo Scientific), and subjected to western blot analysis with the following antibodies: anti-LC3 (Novus Biologicals, 1:500 dilution), anti-p62 (BD Biosciences, 1:500 dilution), anti-Beclin 1 (Santa Cruz Biotechnology, 1:200 dilution), anti-EGFR (Santa Cruz Biotechnology, 1:200 dilution), anti-p-EGFR (Santa Cruz Biotechnology, 1:100 dilution), anti-Bcl-2 (Santa Cruz Biotechnology, 1: 100 dilution), anti-4E-BP1 (Cell Signaling, 1:1000 dilution), anti-p-4E-BP1 (Cell Signaling, 1:1000 dilution), anti-Rubicon (MBL international 1:1000 dilution), anti-hVPS34 (Echelon Bioscience, 1:500 dilution), anti-ATG14 (MBL international, 1:1000 dilution), anti-UVRAG (Sigma, 1:200 dilution), anti-Flag (Sigma 1:5000 dilution), anti-ATG7 (Sigma, 1:1000 dilution), anti-c-Met (Santa Cruz, 1:1000 dilution), anti-c-Src (Santa Crus, 1:200 dilution), anti-clathrin heavy chain (Santa Cruz, 1:200 dilution), and anti-actin (Santa Cruz Biotechnology, 1:5000 dilution).

To detected phosphorylated Beclin 1, cells were treated with DMSO, 1 μM erlotinib or 1 μM crizotinib for 2 h, lysed in RIPA Buffer (20 mM Tris-HCl pH7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% Triton X100, 1% sodium deoxycholate, Proteinase Inhibitor Cocktail and Halt Phosphatase Inhibitor Cocktail) on ice for 30 min, followed by immunoprecipitation of Flag-Beclin 1 with anti-Flag-M2 agarose (Sigma, 1:100 dilution) for 30 min or of endogenous Beclin 1 with anti-Beclin 1 (Santa Cruz, 1:100 dilution). Immunoprecipitates were subjected to SDS-PAGE gel electrophoresis and phospho-Beclin 1 was detected by western blot analysis with anti-pTyr-99-HRP (Santa Cruz, 1:2000 dilution) or anti-pTyr233-Beclin 1 (custom-produced by Phosphosolutions Inc., 1:400 dilution).

To extract protein from tumors, frozen tumor samples were homogenized in ice-cold buffer containing 20 mM Hepes (pH 7.0), 150 mM NaCl, 1 mM EDTA, 1% Triton X100, Proteinase Inhibitor Cocktail (Roche Applied Sciences) and Halt Phosphatase Inhibitor Cocktail (Thermo Scientific) for 15 sec using a motor-driven IKA Ultra-Turrax Disperser (Sigma Aldrich). Samples were sonicated for 15 sec and centrifuged at 15,000 rpm for 10 min and total proteins were recovered in the supernatants.

VPS34 Kinase Assay.

Hela or NSCLC cells were lysed in lysis buffer containing 20 mM Hepes (pH 7.0), 150 mM NaCl, 1 mM EDTA, 1.0% triton X-100, Proteinase Inhibitor Cocktail and Halt Phosphatase Inhibitor Cocktail (Thermo Scientific). Immunoprecipitation was performed with a monoclonal anti-Beclin 1 antibody (Santa Cruz, 1:200 dilution). Immune complexes were washed 3x in lysis buffer, followed by an additional wash in TNE buffer (10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 100 uM Na_3VO_4), then resuspended in 60 μl TNE buffer containing 20 μg sonicated phosphatidylinositol (Avanti Polar Lipids). The reaction was started by adding 6 μl cold ATP (0.5 mM in TNE buffer), 4 μl ATP alpha ^{32}P (10 μCi, PerkinElmer, Inc.) and 10 μl 100 mM $MgCl_2$. After

incubation at RT for 20 min, the reaction was terminated by adding 20 µl 8M HCl, and the organic phase was extracted with 160 µl chloroform:methanol (1:1). Extracted phospholipid products were resolved by TLC using a silica-coated gel (EMD Millipore) and a solvent composed of chloroform:methanol:H_2O:ammonium hydroxide (v/v/v/v, 9/7/1.7/0.3), followed by visualization with x-ray autoradiography.

Co-immunoprecipitation Assays

Co-immunoprecipitation of endogenous Beclin 1 complexes in HeLa or NSCLC cells was performed by lysing cells with lysis buffer (25 M Hepes pH 7.0, 150 mM NaCl, 1 mM EDTA, 0.8% CHAPS, 10% glycerol, Halt Phosphatase Inhibitor Cocktail (Thermo Scientific) and Protease Inhibitor Cocktail (Roche Applied Sciences)) on ice for 1 h. To co-immunoprecipitate endogenous Beclin 1 and endogenous Bcl-2, immunoprecipitation was performed using a monoclonal anti-Bcl-2 antibody pre-conjugated to agarose (Santa Cruz, 1:20 dilution). Immunoprecipitates were subjected to SDS-PAGE gel electrophoresis and Beclin 1 was detected by western blot analysis as described above. To immunoprecipitate EGFR, Rubicon, VPS34, ATG14, and UVRAG with Beclin 1, immunoprecipitation was performed overnight at 4^0C with a monoclonal anti-Beclin 1 antibody (Santa Cruz, 1:50 dilution). To immunoprecipitate Beclin 1, Rubicon, VPS34, ATG14, and UVRAG with EGFR, immunoprecipitation was performed overnight at 4^0C with a monoclonal anti-EGFR antibody (Santa Cruz, 1:100 dilution). To immunoprecipitate Beclin 1 and c-Src with c-Met, immunoprecipitation was performed overnight at 4^0C with a monoclonal anti-c-Met antibody (Santa Cruz, 1:100 dilution). Immunoprecipitates were subjected to SDS-PAGE separation, and Beclin 1, EGFR, VPS34, ATG14, UVRAG, Rubicon, and c-Src were detected by western blot analysis as described above.

Immunofluorescence Microscopy

Cells were plated on glass chambers (Nalge Nunc International) at 60% confluency and incubated in normal medium or serum-free medium for 24 h. A549 cells were either untreated or stimulated with EGF (50 ng/ml) for 30 min; NSCLC cells were either untreated or treated with 1 μM erlotinib (LC Labotatories) for 3 h. Cells were washed in phosphate buffered saline (PBS), fixed in 3.7% paraformaldehyde (PFA) for 20 min at RT, blocked in blocking buffer (2% FBS in PBS) at 4°C for 1 h, and permeabilized in 0.05% Saponin/0.2% BSA/PBS (Sigma) for 15 min. Cells were stained with primary and fluorescent-conjugated (Alexa 488-, Alexa 594- or Alexa 647-conjugated goat anti-mouse or goat anti-rabbit) secondary antibodies (Invitrogen) diluted in immunostaining buffer (2% FBS plus 1% BSA in PBS) for 1 h. Coverslips were mounted on microscope chamber slides with VECTASHIELD® Hard Set™ Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA). Z-stacks were acquired with a Zeiss AxioImager Z2 microscope equipped with a Photometrics CoolSnap HQ2 camera and a Zeiss PLAN APO 63X/1.3 NA objective using the same acquisition times for each sample. Z-stacks were deconvolved with AutoDeBlur (Bitplane) using a blind deconvolution algorithm (30 iterations, medium noise) and Imaris version 7.4.0 (Bitplane) was then used to examine the deconvolved images. Background signals from samples stained with secondary antibody alone and acquired with identical exposure times for each antibody pair were used to determine thresholds for positive signal. Representative images were chosen and exported from the Slide module in Imaris after examining >50 cells per sample for triplicate samples for each experimental group using identical settings for each set of control versus experimental conditions. For detection of EGFR and Beclin 1 in the setting of clathrin knockdown, A549/Flag-Beclin 1 cells were transfected with non-silencing control siRNA #3 (Thermo-Scientific), or clathrin siRNA (GAAGAACTCTTTG CCCGGAAATTTA), and incubated in serum-free medium for 24 h followed by 30 min stimulation with EGF (2 ng/ml). This lower dose of EGF is known to induce clathrin-

dependent EGFR endocytosis but not clathrin-independent EGFR endocytosis (Sigismund et al., 2008)

Autophagy Assays

Autophagy was measured during growth in normal medium, after 3 h erlotinib (1 μM) treatment or after 2 h Torin1 (50 ng/ml) (Thoreen et al., 2009) treatment by light microscopic quantitation of numbers of GFP-LC3 puncta per cell in cells transfected with GFP-LC3 or by western blot analysis of the amounts of LC3-I/LC3-II and p62/SQSTM1 as described previously (Wei et al., 2008). For assessment of autophagy *in vivo*, vehicle- or erlotinib-treated mice bearing NSCLC/GFP-LC3 xenografts were anaesthetized by isoflurane inhalation and perfused with 4% PFA. Tumors were dissected and fixed in 4% PFA overnight followed by 15% sucrose for 4 h and 30% sucrose overnight before frozen sections were prepared. The number of GFP-LC3 puncta per unit area of tissue was quantified by fluorescence microscopy. All GFP-LC3 puncta quantitation was performed by an observer blinded to experimental condition.

Clonogenic Cell Survival Assay

HCC827/GFP-LC3 cells expressing tetracycline-inducible Flag-Beclin 1 were untreated or treated with 1 μg/ml doxycycline for 4 days, and then plated in 6-well plates at a density of 500 cells/well. After a 4 h attachment period, the cells were either treated with 0.2 μM erlotinib or mock-treated with DMSO for 48 h, and then were incubated in doxycycline-free media or doxycycline-containing media for observation of colony formation. After 14 days, the cells were fixed and stained in a solution containing 6% glutaraldehyde and 0.5% crystal violet. Colonies with $\geq$ 50 cells were counted using a stereomicroscope. Clonogenic survival assays were performed on HCC827/GFP-LC3/vBcl-2 cells using a similar protocol except for the absence of doxycycline treatment. For assessment of clonogenic survival in HCC827 cells with *ATG7* siRNA knockdown,

cells were mock-transfected or transfected with non-silencing control siRNA #3 (Thermo-Scientific), or *ATG7-1* (GGGUUAUUACUACAAUGGUG) or *ATG7-2* (GCCUGCUGAGGAGCUCUCCAU) siRNA, and 48 h later, treated with 0.2 μM erlotinib or vehicle control for 24 h. Cells were then plated in 6-well plates and colony number was measured at 14 days as described above.

Tumor Xenograft Studies

To measure tumor formation of NSCLC cell lines and their response to erlotinib treatment, 6 week-old NOD SCID® mice (JAX Stock Number 001303, Jackson Laboratories) were shaved on the right flank one day prior to injection and injected subcutaneously with 10^7 tumor cells in 200 μl of PBS. Ten mice were injected with each NSCLC cell line. Tumor growth was monitored by daily measurement of tumor length (L) and width (W) and tumor volume was estimated using the formula (tumor volume = $½(L \times W^2)$). At the end of the experiment (35 days after inoculation), mice were sacrificed and tumors were dissected; half of each tumor was frozen in liquid nitrogen and half was immersion fixed in 4% PFA for subsequent histological examination.

To measure the response of NSCLCs expressing tetracycline-inducible WT or mutants of Flag-Beclin 1 to erlotinib therapy, mice were randomized to two groups after tumors had grown to a palpable size (~400 mm^3). One group received oral administration of doxycycline dissolved in drinking water containing 1% sucrose at a final concentration of 2 mg/ml and one group received sucrose-enriched drinking water lacking doxycycline. Due to its light sensitivity, doxycycline-containing drinking water was provided in shielded flasks that were replaced once a week. Three days after initiation of doxycycline administration, mice were treated daily with 12.5 mg kg^{-1} (body weight) erlotinib via intraperitoneal injection. Tumor size was measured daily until tumors were undetectable or until 28 days after treatment initiation. All animal procedures were performed in

accordance with institutional guidelines and with approval from the Institutional Animal Care and Use Committee.

Histology

Tumors were fixed in 4% PFA, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E). TUNEL staining was performed according to the manufacturer's instructions, using Sigma FAST™ 3, 3′-diaminobenzidine (DAB) tablets as the peroxidase substrate. Immunohistochemical staining of paraffin-embedded tumor tissues was performed using TTF-1 (Santa Cruz, 1:200 dilution) and Ki67 (Abcam, 1:100 dilution) primary antibodies and the ABC Elite immunoperoxidase kit according to the manufacturer's instructions. TTF-1 staining in xenografts was classified as strongly positive, moderately positive or weakly positive using previously described criteria (Saad et al. 2004).

Phosphorylation Site Identification by an *in vitro* Peptide Kinase Assay

To identify the tyrosine phosphorylation sites of Beclin 1, a radiometric protein kinase assay based on streptavidin-coated FlashPlate™ Plus (PerkinElmer) was used for measuring the kinase activity of recombinant active human EGFR L858/T790M. SRMS, human PDGFRβ, and mutationally active PDGFRα T674I were used as control kinases. Five biotinylated sample peptides containing the candidate tyrosine phosphorylation sites (or mutations in the tyrosine residues) were used in this assay. They were WT Beclin 1 aa 223-239 (D_{223}QEEAQY$_{229}$QREY$_{233}$-SEFKRQ$_{239}$), mutant Beclin 1 aa 223-239 (Y229S) (D_{223}QEEAQSS$_{229}$QREY$_{233}$SEFKRQ$_{239}$), mutant Beclin 1 aa 223-239 (Y233S) (D_{223}QEEAQY$_{229}$QRES$_{233}$SEFKRQ$_{239}$), mutant Beclin 1 aa 223-239 (Y229S/Y233S) (D_{223}QEEAQS$_{229}$QRES$_{233}$SEFKRQ$_{239}$), and WT Beclin 1 aa 345-358 (K_{345}SKELPLY$_{352}$CSGGLR$_{358}$). The sample peptides were tested in triplicate at three final assay concentrations: 1 μM, 0.5 μM and 0.25 μM. The reaction cocktails contained

60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μM Na_3VO_4, 1.2 mM DTT, 1 μM cold ATP/[γ-^{33}P]-ATP (9.0 x 10^5 cpm per well), 25 ng EGFR T790M and sample peptides (1/0.5/0.25 μM) which were pipetted into 96-well, V-shaped polypropylene microtiter plates. The assay plates were incubated at 30°C for 60 min. The reaction cocktails were then stopped with 20 μl 4.7 M NaCl/35 mM EDTA, and transferred into 96-well streptavidin-coated FlashPlate™ Plus plates followed by 30 min incubation at RT on a shaker. Subsequently the plates were aspirated and washed 3X with 250 μl of 0.9% NaCl. Incorporation of ^{33}Pi was determined by detecting counts per minute (cpm) with a microplate scintillation counter (Microbeta, Perkin Elmer).

Feulgen Staining and Morphometric Analyses

Nuclear morphometry was performed using similar approaches as previously described (Veltri et al. 2012). Using the AutoCyte Pathology Workstation (APW) [TriPath Inc, Burlington, NC], the tissue of interest and a calibrator slide made of normal rat hepatocyte tetraploid nuclei were Feulgen-stained simultaneously and the 500 rat nuclei from the calibrator were measured and had to have a peak with a CV <2.0% and an IOD between 6500-8500. The APW imaging system is a microspectrophotometer microscope and is used to conduct nuclear morphometry analysis of size, shape and DNA content features. Using the AWP QUIC-DNA v1.201software, we captured 300 nuclear images of malignant lung Feulgen-stained nuclei from each xenograft tumor section (10 tumors per experimental group). We selected only single cancer nuclei for measurement and collected data for nuclear DNA intensity, perimeter, area, and DNA Ploidy. From the nuclear images captured, the DNA intensity in pixels (DNA intensity = n.GVmean), perimeter (length of the cell contour) and area (number of pixels belonging to the cell nucleus) were calculated for each nucleus by using QUIC-DNA v1.20 software. The variance of these measurements was evaluated and a Mann-Whitney U test was applied to assess distributional differences across the four xenograft genotype groups.

Statistical Analyses

For comparisons of the means of two groups, one-way ANOVA was used. For comparison of the magnitude of changes between different conditions in different groups, two-way ANOVA was used. Linear mixed-effect models (which compare differences between groups while accounting for correlations within the same subject) were used to compare different changes for slopes of linear curves among groups. To assess distributional differences of variance across different test groups, the Mann-Whitney U test was used.

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1, associated with Figure 1.

(A) Western blot analysis of clathrin heavy chain in A549 cells 48 h after treatment with non-silencing control (NC) siRNA or siRNA targeting clathrin heavy chain (HC). Clathrin siRNA does not block EGFR receptor homodimerization/activation as measured by detection of phosphorylated EGFR. Actin is shown as a loading control.

(B) Colocalization of EGFR and Beclin 1 in A549/Flag-Beclin 1 cells after EGF stimulation. Cells were transfected with NC or clathrin siRNA for 48 h, cultured overnight in serum-free medium, and then treated EGF (2 ng/ml, 30 min) (a concentration that induces clathrin-dependent endocytosis), and stained with anti-EGFR to detect EGFR (green) and anti-Flag to detect Flag-Beclin 1 (red). Yellow indicates colocalization. Scale bar, 5μm.

Figure S2, associated with Figure 2.

Western blot detection of EGFR in Hela cells, Hela cells transfected with wild-type (WT) EGFR, and HCC827 cells. In this experiment, a higher concentration of anti-EGFR antibody (1:200 dilution vs. 1:1000) dilution and a longer gel exposure (15 sec vs. 1-2 sec) was used compared to anti-EGFR blots in the main figures of the manuscript.

Figure S3, associated with Figure 3.

(A) Schematic diagram of domains of Beclin 1 involved in binding to Bcl-2/Bcl-X_L, Rubicon, and VPS34 (top), and schematic representation of interactions between either WT Beclin 1 or indicated Beclin 1 mutant constructs and EGFR L858R in Hela cells. +, denotes co-immunoprecipitation in (B).

(B) Immunoprecipitation of EGFR with Beclin 1 in Hela cells co-transfected with EGFR (L858R) and indicated Flag-Beclin 1 constructs.

Figure S4, associated with Figure 4.

(A) Representative images of NSLC xenografts treated with vehicle alone (vehicle) or erlotinib (12.5 mg kg^{-1} daily for 2 d). Scale bar, 20 μm.

(B) Quantification of GFP-LC3 puncta in xenografts shown in (A). Bars are mean ± SEM for xenografts from 10 mice per treatment group. For each xenograft, the number of GFP-LC3 puncta per unit area was quantitated for the entire tumor section. NS, not significant, $*P< 0.05$; one-way ANOVA for indicated comparison. $^{\#\#}P<0.01$, two-way ANOVA for comparison of magnitude of changes between different groups.

(C) Effects of erlotinib treatment (50 mg kg^{-1} daily) on tumor volume of NSCLC xenografts. Daily treatment was begun when xenografts reached a volume of 500 mm^3.

(D) Immunoprecipitation of indicated proteins with EGFR in HCC827 cells. Cells were incubated in medium with DMSO or erlotinib (1 μM, 2 h) prior to immunoprecipitation.

Figure S5, associated with Figure 5

(A) Western blot detection of Beclin 1 tyrosine phosphorylation in NSCLC cells +/- EGFR tyrosine kinase treatment (erlotinib, 1 μM, 2 h) or c-Met tyrosine kinase inhibitor treatment (crizotinib, 1 μM, 2 h). Cells were immunoprecipitated with anti-Beclin 1 followed by western blot analysis with anti-pY99. c-Met inhibition with crizotinib treatment was confirmed by western blot analysis of whole cell lysates using an anti-phospho c-Met antibody.

(B) Immunoprecipitation of Beclin 1 and c-Src with c-Met in HCC827 cells. c-Src, a known binding partner of c-Met was used as a positive control.

(C) Identification of Beclin 1 tyrosine phosphorylation sites with an EGFR *in vitro* kinase assay. Synthetic peptides of Beclin 1 were incubated with recombinant active EGFR L858/T790M. Bars are mean EGFR L858R/T790M kinase activity ± SD of triplicate samples.

(D) Beclin 1 phosphotyrosine site identification. HCC827 cells transfected with WT or indicated mutant Flag-Beclin 1 constructs were immunoprecipitated with anti-Flag followed by western blot analysis with an anti-phosphotyrosine antibody (pY99). In lane 2, cells were treated with erlotinib (1 μM, 2 h) prior to immunoprecipitation.

(E) Model of the effects of Beclin 1 phosphorylation on Beclin 1 homodimerization and interaction with Beclin 1 binding partners. Beclin 1 (blue cartoon) contains three functional domains: the N terminal Bcl-2 homology 3 domain (BH3, short cylinder), the central coiled coil region (CC, blue circle), and the C terminal evolutionarily conserved domain (ECD, blue circle). The CC mediates homodimer formation through an anti-parallel interaction (PDB:3q8t, corresponding CC denoted by dotted box), which should place the BH3 and the ECD in close spatial proximity for EGFR binding. Rubicon (red hexagon) interacts with the Beclin 1 ECD in the EGFR complex, while Bcl-2 (red dotted circle, not in the EGFR complex) interacts with the Beclin 1 BH3 domain. EGFR-mediated phosphorylation of Beclin 1 Y229, Y233, and Y352 (residue positions marked by a closed orange circle) stabilizes the homodimer and promotes Rubicon complex formation. Unphosphorylated Beclin 1 monomers (lower panel, open circles) can interact with VPS34 (green square) via the ECD (PDB: 4ddp, corresponding ECD and partial CC denoted by dotted box). The C terminal segment of the Beclin 1 CC region is visible in both homodimer (PDB: 3q8t) and ECD (4ddp) experimental structures, and can either interact with the N terminus of the CC region in a homodimer (upper panel) or the ECD in a monomer (lower panel, interaction depicted by a light blue square), but not both.

Figure S6, associated with Figure 6

(A-C) Quantitation of the variance of three nuclear image features including DNA intensity (A), size of perimeter (B) and area (C) of Feulgen-stained nuclei in indicated HCC827 xenograft tumor genotype. Bars are mean $\pm$ SEM of the variance of all tumors in each genotype (10 per group), with 300 captured nuclear images examined per tumor.

NS, not significant; *P<0.05, **P<0.01, ***P<0.001; Mann-Whitney U test for comparison between groups.

(D) Representative H & E-stained section of a xenograft derived from HCC827/Flag-Beclin 1 EEE NSCLC cells. Arrow denotes cell-in-cell structure (entosis).

(E-F) Quantitation of entotic cells (E) and multinucleated cells (F) in indicated HCC827 xenograft tumor genotype. Bars are mean ± SEM of all tumors in each genotype (10 per group), with 5 randomly selected high power fields (400X) examined for each tumor. Quantitation was performed by examination of H & E-stained slides by a pathologist blinded to experimental condition. NS, not significant, *P<0.05, **P < 0.01, ***P<0.001; one-way ANOVA.

(G) Quantitation of DNA ploidy variance of Feulgen-stained nuclei in indicated HCC827 xenograft tumor genotype. Bars are mean ± SEM of the variance of all tumors in each genotype (10 per group), with 300 captured nuclear images examined per tumor. NS, not significant; ***P<0.001; Mann-Whitney U test for comparison between groups.

WT, wild-type Flag-Beclin 1; FFF, Flag-Beclin 1 Y229F/Y233F/Y352F; Flag-Beclin 1 Y229E/Y233E/Y352E.

Figure S7, associated with Figure 7.

(A) Western blot detection of ATG7 in HCC827 cells 48 h after transfection with indicated siRNA. NC, non-silencing control.

(B) Clonogenic survival of HCC827 cells transfected with indicated siRNA for 48 h, and then treated with vehicle control (DMSO) or erlotinib (0.2 μM, 24 h). Bars are mean ± SEM percentage of colonies in erlotinib vs. control-treated cells after 14 days. Similar results were observed in 3 independent experiments. ***P< 0.001; one-way ANOVA for comparison of *ATG7* siRNA groups vs. NC group.

(C) Immunoprecipitation of endogenous Beclin 1 with Flag-Myc-M11 or of Flag-Myc-M11 with endogenous Bax in HCC827/GFP-LC3 cells that stably express indicated M11

mutants. Shown are data for two clones transfected with WT M11 (WT5, WT8) and two clones transfected with mutant M11 AAA (AAA9, AAA12).

(D) Western blots of LC3-I/II and p62 in indicated HCC827/GFP-LC3/M11 cell lines incubated in medium +/- erlotinib (0.2 μM, 2 h).

(E) Quantification of GFP-LC3 puncta in indicated HCC827/GFP-LC3/M11 cell lines incubated in medium +/- erlotinib (0.2 μM, 2 h). Bars are mean ± SEM of triplicate samples (≥50 cells analyzed per samples). Similar results were observed in 3 independent experiments. $*P < 0.05$ $**P < 0.01$ $***P < 0.001$; one-way ANOVA for comparison between erlotinib treatment and no treatment (DMS0). $^{###}P<0.001$, two-way ANOVA for comparison of magnitude of erlotinib-induced autophagy in cells expressing either WT or AAA vBcl-2 vs. empty vector.

(F) Percentage of cell death in indicated HCC827/GFP-LC3/M11 cell lines following treatment with DMSO or indicated etoposide concentration for 24 h. Bars are mean ± SEM of triplicate wells per condition. Similar results were observed in 3 independent experiments. $**P < 0.01$ $***P < 0.001$; one-way ANOVA for comparison between etoposide treatment and no treatment in each cell line. NS, not significant, $^{###}P<0.001$; two-way ANOVA for comparison of magnitude of etoposide-induced cell death in cells either expressing WT or AAA vBcl-2 vs. in cell expressing empty vector.

(G) Clonogenic survival of indicated HCC827/GFP-LC3/M11 cell lines grown in medium +/- erlotinib (0.2 μM) for 14 d. Bars are mean ± SEM of triplicate wells per condition. Similar results were observed in 3 independent experiments. $**P < 0.01$ $***P < 0.001$; one-way ANOVA for comparison between erlotinib treatment and no treatment (DMSO) in each cell line. $^{###}P<0.001$; two-way ANOVA for comparison of magnitude of erlotinib-induced decrease in colony number in cells expressing AAA vBcl-2 vs. in cell expressing empty vector.

(H) Representative images of GFP-LC3 staining in NSCLC xenografts treated with vehicle alone or 12.5 mg kg^{-1} erlotinib for 2 d. Scale bar, 20 μm.

(I) Quantitation of number of GFP-LC3 puncta per unit area of tumor. Bars are mean ± SEM for 3 mice per genotype, with 50 randomly selected fields for each tumor. ***$P<$ 0.001; one-way ANOVA for comparison between erlotinib treatment and no treatment (vehicle). $^{###}P<0.001$; two-way ANOVA for comparison of magnitude of erlotinib-induced increase in AAA groups vs. in vector group.

(J) Effect of erlotinib on tumor response of xenografts formed by indicated HCC827/GFP-LC3/M11 cell lines. Erlotinib (12.5 mg kg^{-1} daily) was initiated when tumor volume reached ~300 mm^3. Results are mean tumor volume ± SEM per time point for 12 mice per group. $P<0.0001$ for AAA groups vs. vector control group; linear-mixed effect model.

Figure S1, associated with Figure 1

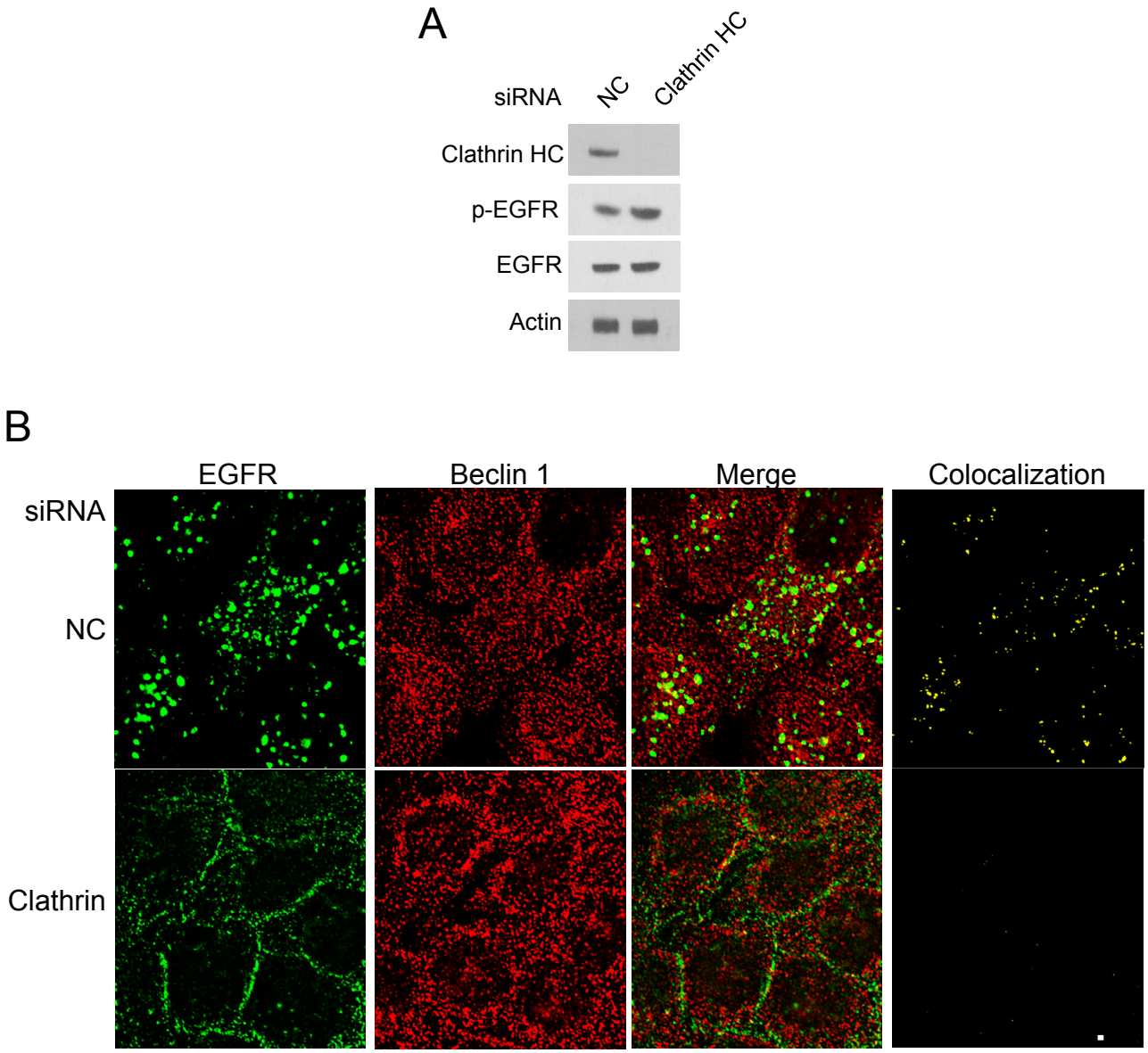
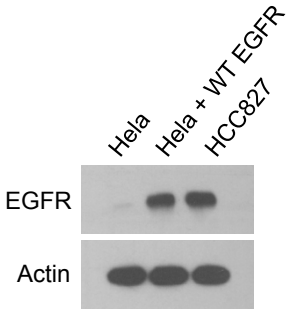
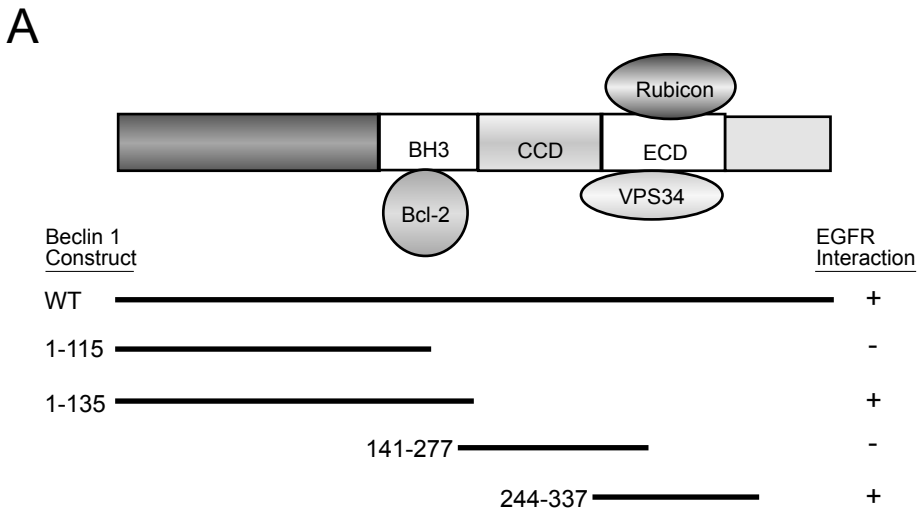


Figure S2, associated with Figure 2

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CCD
ECD
Bcl-2
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Construct
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Interaction
WT
+
1-115
-
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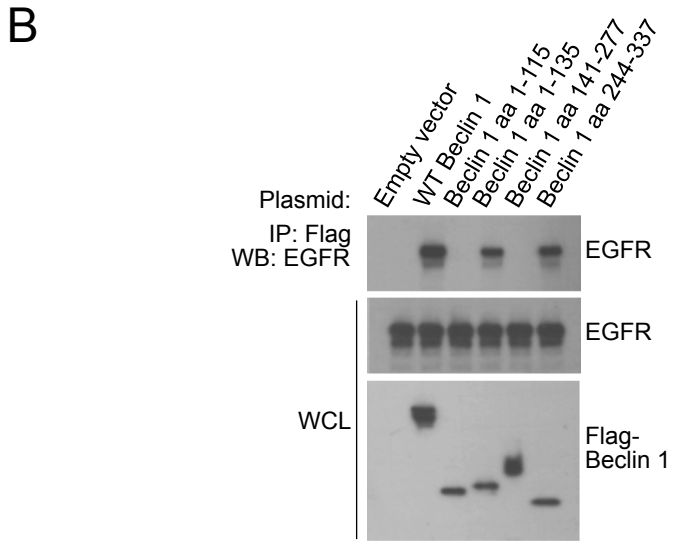
+
141-277
-
244-337
+


B
Empty vector
WT Beclin 1
Beclin 1 aa 1-115
Beclin 1 aa 1-135
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Plasmid:
IP: Flag
WB: EGFR
EGFR
EGFR
WCL
Flag-
Beclin 1

Figure S4, associated with Figure 4

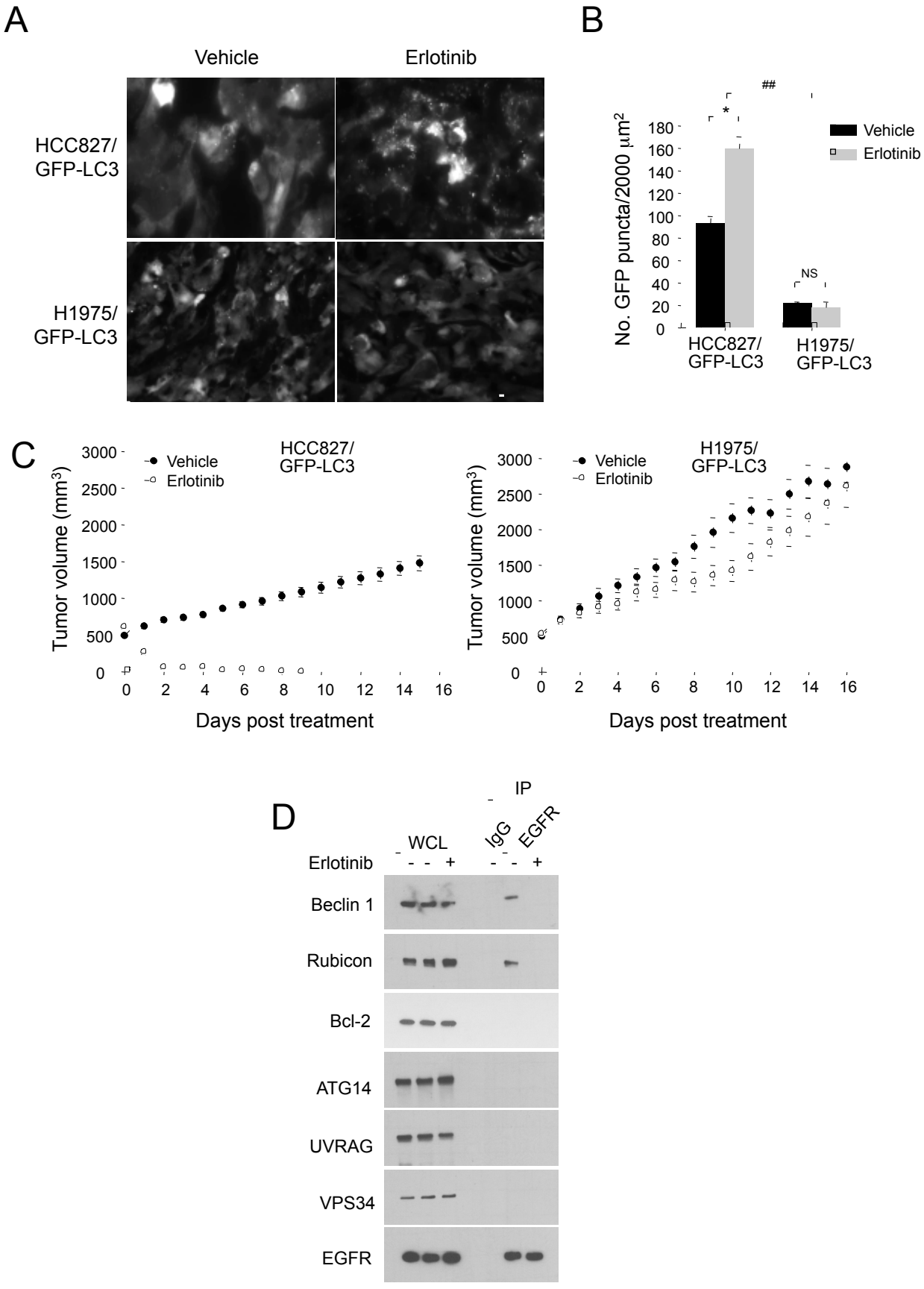

Figure S5, associated with Figure 5

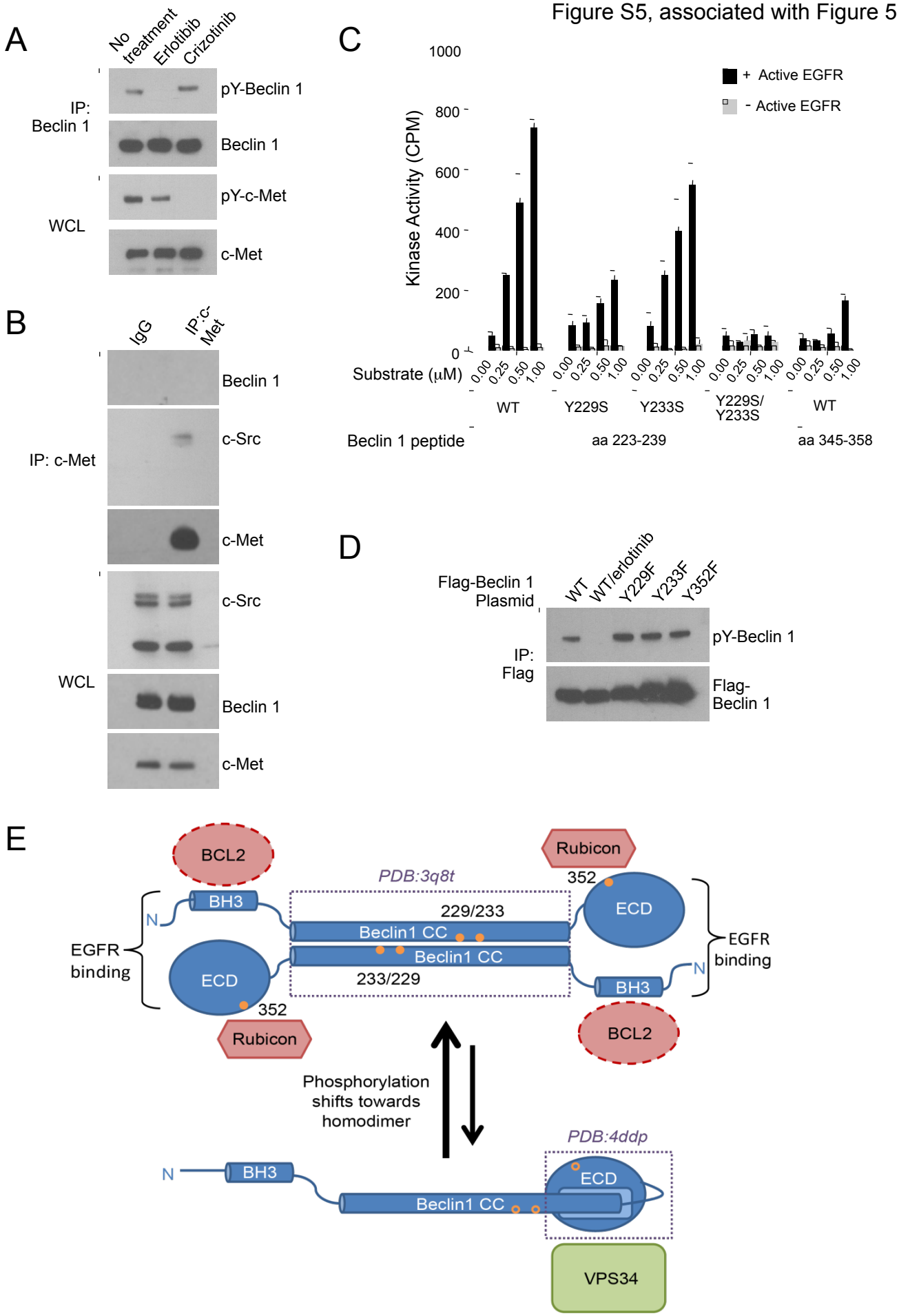

Figure S6, associated with Figure 6

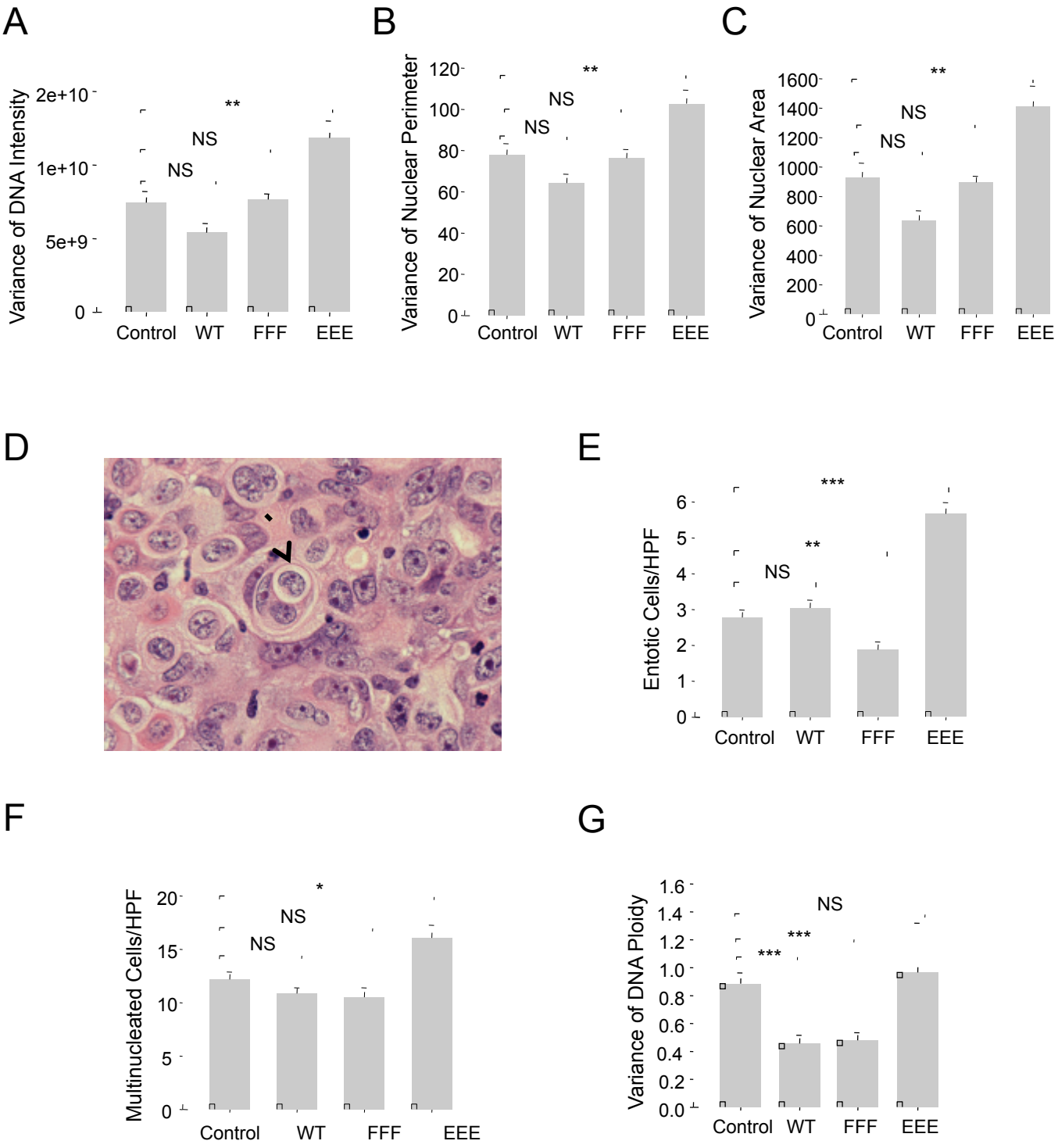

Figure S7, associated with Figure 7