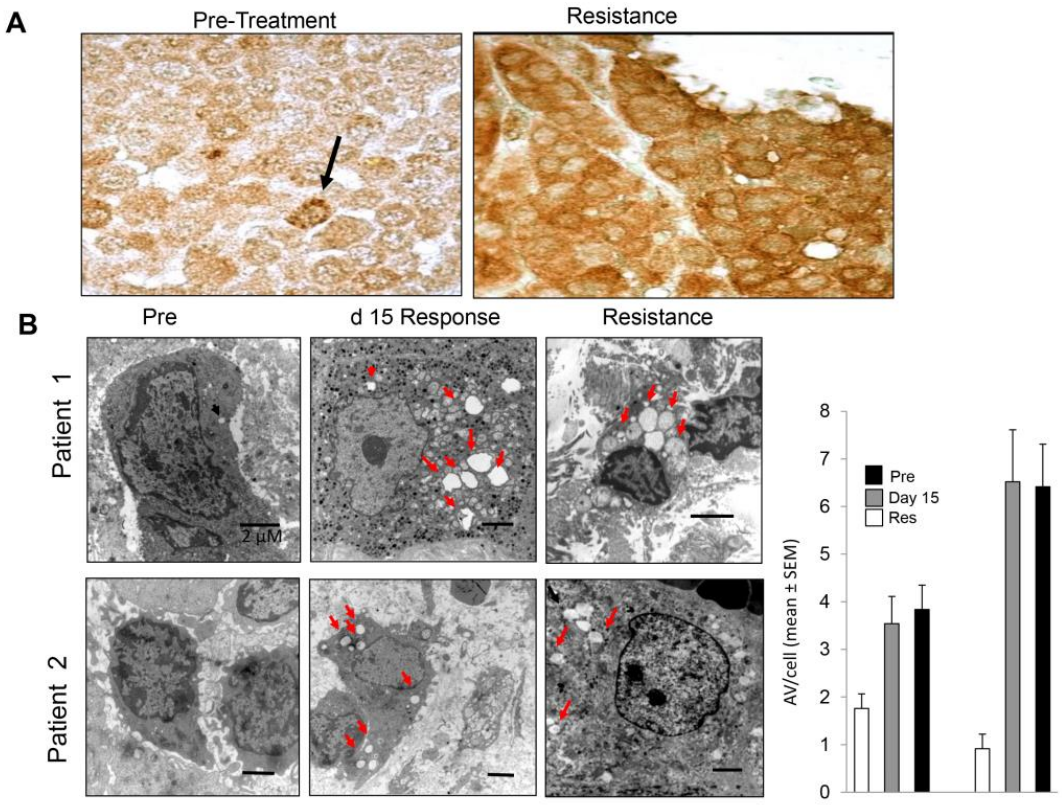
Supplemental Table, Figures and Legends

Supplemental Table 1. Patient Characteristics

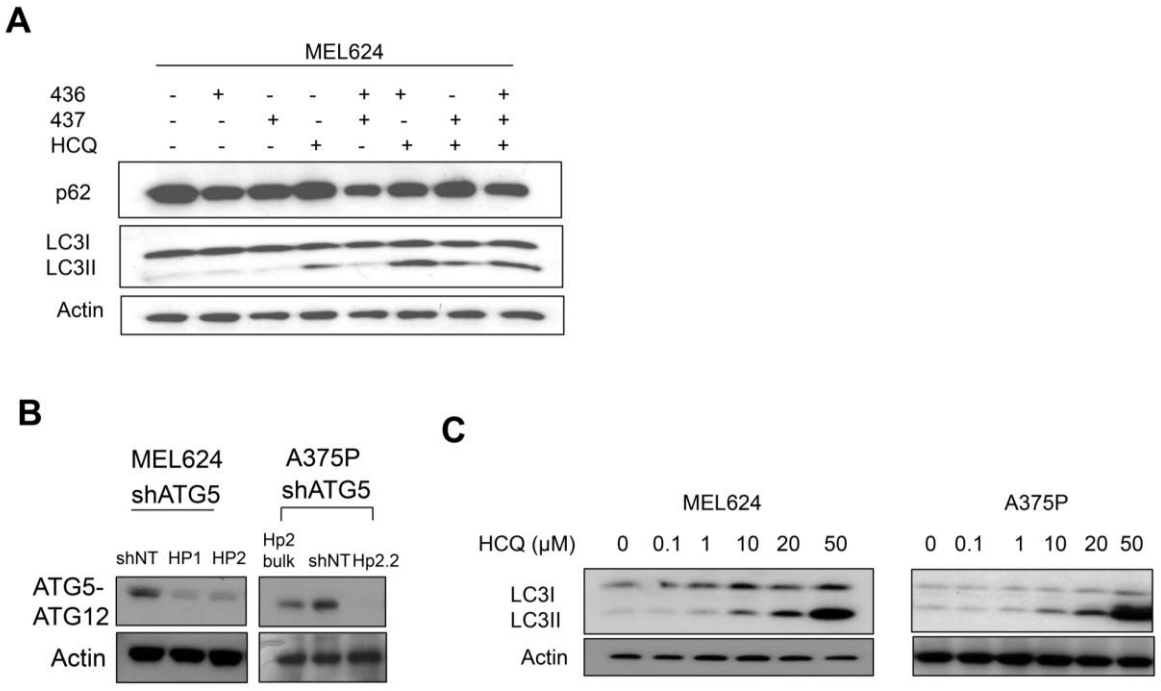
Pt#	Stage	LDH	ECOG PS	Trial	Agent(s)	Best response	Time to progression (days)	Confirmed PR	Any LC3 increase	2+ LC3 increase	4+ LC3 at progression
578	IVM1c	<ULN	0	B	Vemurafenib	SD	374	No	No	No	No
503	IVM1c	<ULN	0	A	Vemurafenib	PR	489	Yes	No	No	No
12_161	IIIc	<ULN	0	F	Vemurafenib	PR	neoadjuvant	Yes	No	No	No
636	IVM1c	>2XULN	0	B	Vemurafenib	PR	270	Yes	No	No	No
11_29	IVM1c	NA	1	D	Dabrafenib	PR	247	Yes	Yes	No	No
11_20	IVM1c	>ULN	1	G	Dabrafenib + trametinib	PR	179	Yes	Yes	No	No
11_6	IVM1c	>ULN	0	G	Dabrafenib + trametinib	PR	275	Yes	Yes	No	No
667	IVM1a	<ULN	0	B	Vemurafenib	PR	306	Yes	Yes	No	No
610	IVM1b	<ULN	1	B	Vemurafenib	SD	291	No	Yes	Yes	Yes
674	IVM1c	>ULN	1	C	Vemurafenib	SD	203	No	Yes	Yes	Yes
11_3	IVM1c	<ULN	1	C	Vemurafenib	SD	252	No	Yes	Yes	Yes
11_23	IVM1c	<ULN	1	D	Dabrafenib	PR	270	Yes	Yes	Yes	Yes
11_47	IVM1c	>ULN	1	E	Vemurafenib	PR	229	No	Yes	Yes	Yes
11_59	IVM1c	<ULN	0	E	Vemurafenib	PR	110	No	Yes	Yes	Yes
12_158	IVM1c	>ULN	0	G	Dabrafenib + trametinib	PR	103	No	Yes	Yes	Yes

LDH: Lactate dehydrogenase, ULN: upper limit of normal ; ECOG PS: Eastern Cooperative Group Performance Status, SD: stable disease; PR: Partial response

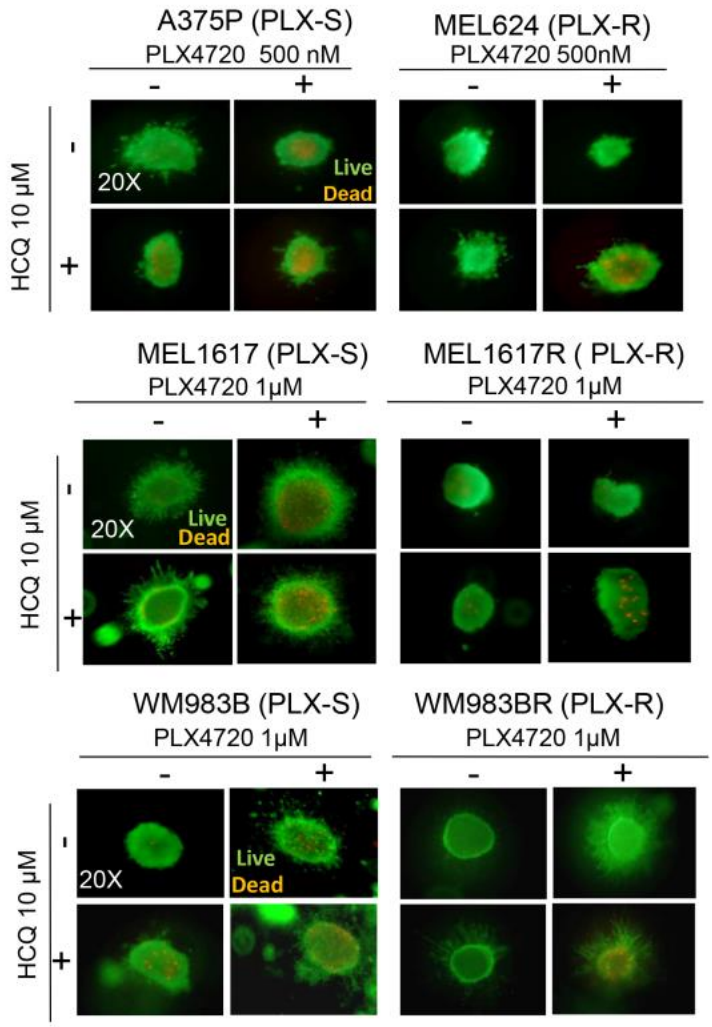
Trial Code	Trial description	NCT	Reference
A	Phase I trial of vemurafenib in patients with advanced BRAF mutant solid tumors	NCT00405587	Flaherty et al. NEJM 2010
B	BRIM2: Phase II trial of vemurafenib in advanced BRAF mutant melanoma	NCT00949702	Sosman et al. NEJM 2012
C	BRIM3: Randomized phase III trial of vemurafenib v. DTIC in advanced BRAF mutant melanoma	NCT01006980	Chapman et al. NEJM 2011
D	BREAK2: Phase II trial of dabrafenib in patients with advanced BRAF mutant melanoma	NCT01153763	Ascierto et al. JCO 2013
E	Expanded access vemurafenib	NCT01248936	
F	Commercial vemurafenib		
G	Phase I trial of dabrafenib and trametinib in advanced BRAF mutant melanoma	NCT01072175	Flaherty et al NEJM 2012



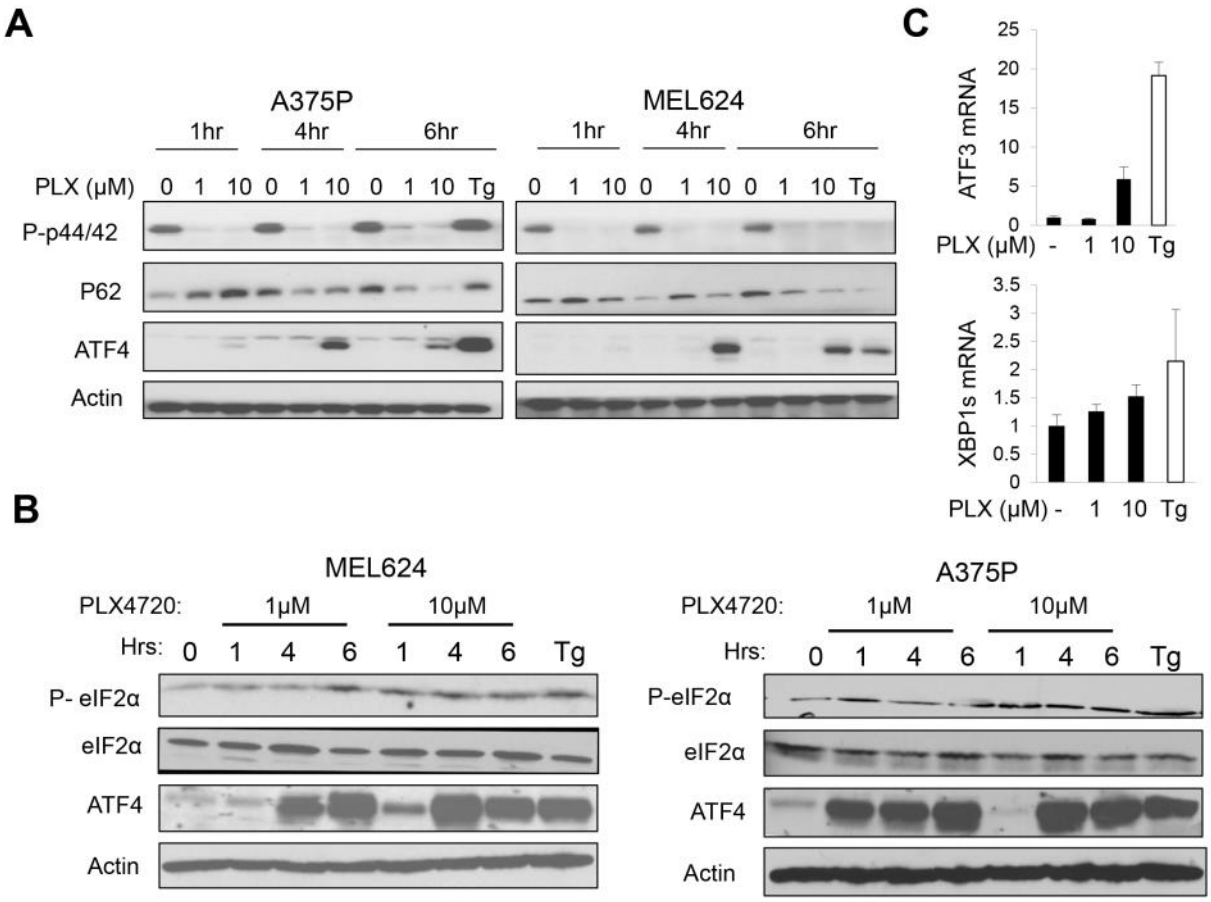
Supplemental Figure S1. Vemurafenib-induced autophagy in tumor samples from Stage IV BRAF mutant melanoma patients. **(A)** High powered image of tissues from one patient that demonstrates the punctate staining pattern for LC3 (arrow) reflecting accumulation of autophagic vesicles. **(B)** Representative electron micrographs of cutaneous tumor biopsies of melanoma metastases from patients treated with vemurafenib. Arrows: Autophagic vesicles; scale bar 2 µm. Graph shows quantification of mean +/- SEM of number of autophagic vesicles per cell. BRAFi: BRAF inhibitor.



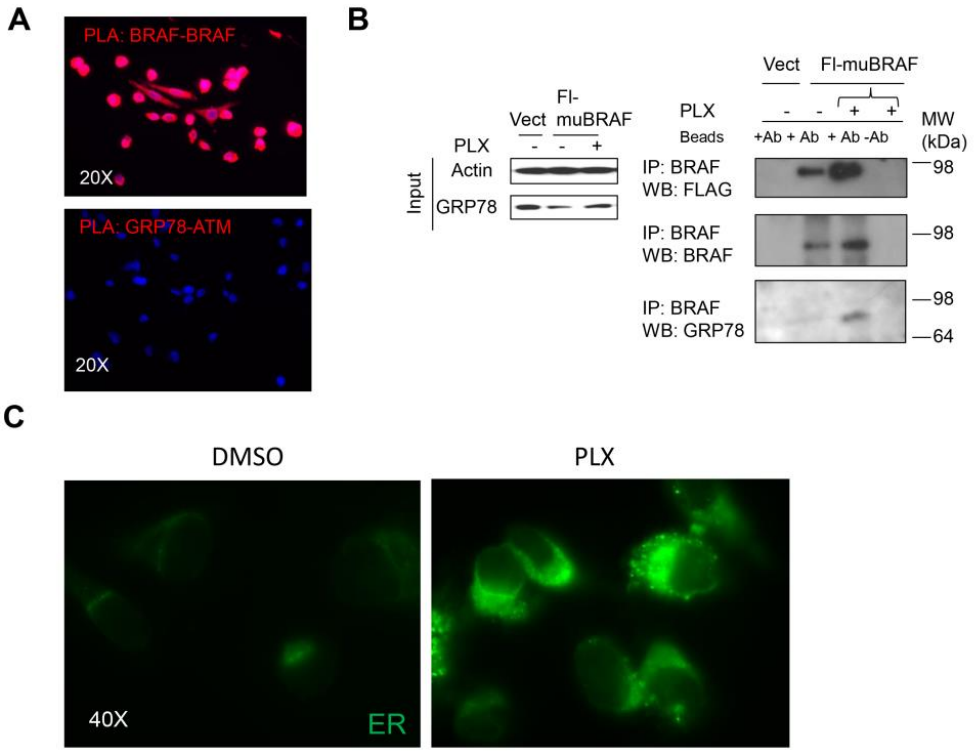
Supplemental Figure S2. BRAF inhibitors induce cytoprotective autophagy in BRAF mutant melanoma. **(A)** MEL624 cells were treated with vehicle, BRAF inhibitor GSK2118436 (436), MEK inhibtior GSK1120212 (212), HCQ 10 μM or the indicated combinations. 48 hour immunoblots directed against the proteins indicated. **(B)** Knockdown of ATG5 in BRAF inhibitor sensitive (A375P) and BRAF inhibitor resistant (MEL624) cells. Short hairpin (sh); Non Target Control (NT). **(C)** Dose response for HCQ and LC3 immunoblotting at 48 hours.



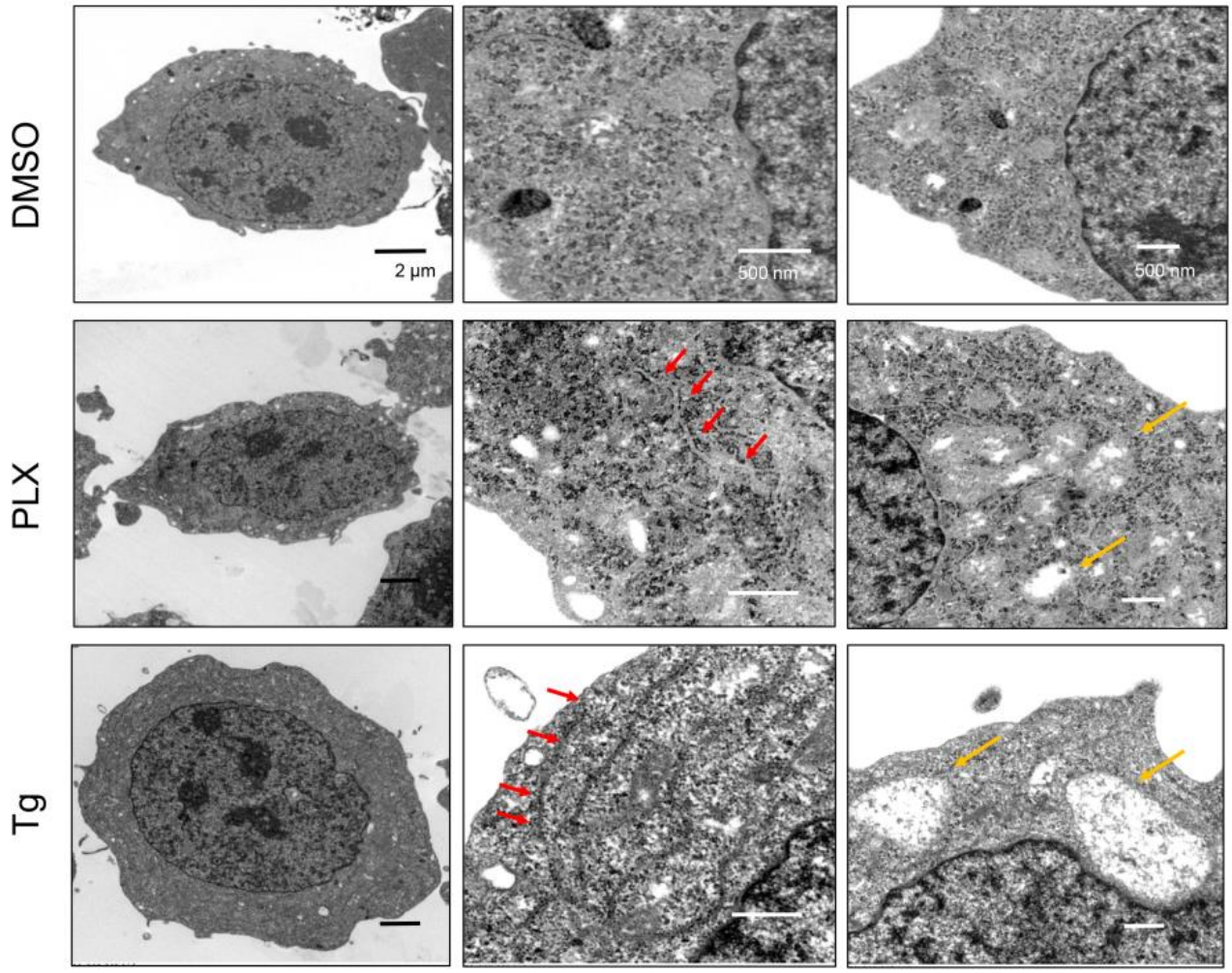
Supplemental Figure S3. Effects of combined BRAF and autophagy inhibition on growth impairment and cell death in 3D culture. 50-100 3D spheroids were generated and implanted into a collagen matrix in triplicate, and cells were treated as indicated. The Live (green)/Dead (orange) assay was performed after 72 hours. Representative images are shown.



Supplemental Figure S4. BRAF inhibitors induce a dose dependent ER stress response. **(A)** Mel624 and A375P cells were treated with PLX at the indicated doses and timepoints or ER stress inducer thapsigargin (Tg) 0.5 μM. Whole cell lysates were used for immunoblotting against indicated proteins. **(B)** Nuclear lysates of Mel624 and A375P cells treated with PLX at indicated doses and times. (**C)** Quantitative real time PCR of ATF3 and spliced XBP1 8 hours after the indicated treatments in MEL624 cells.

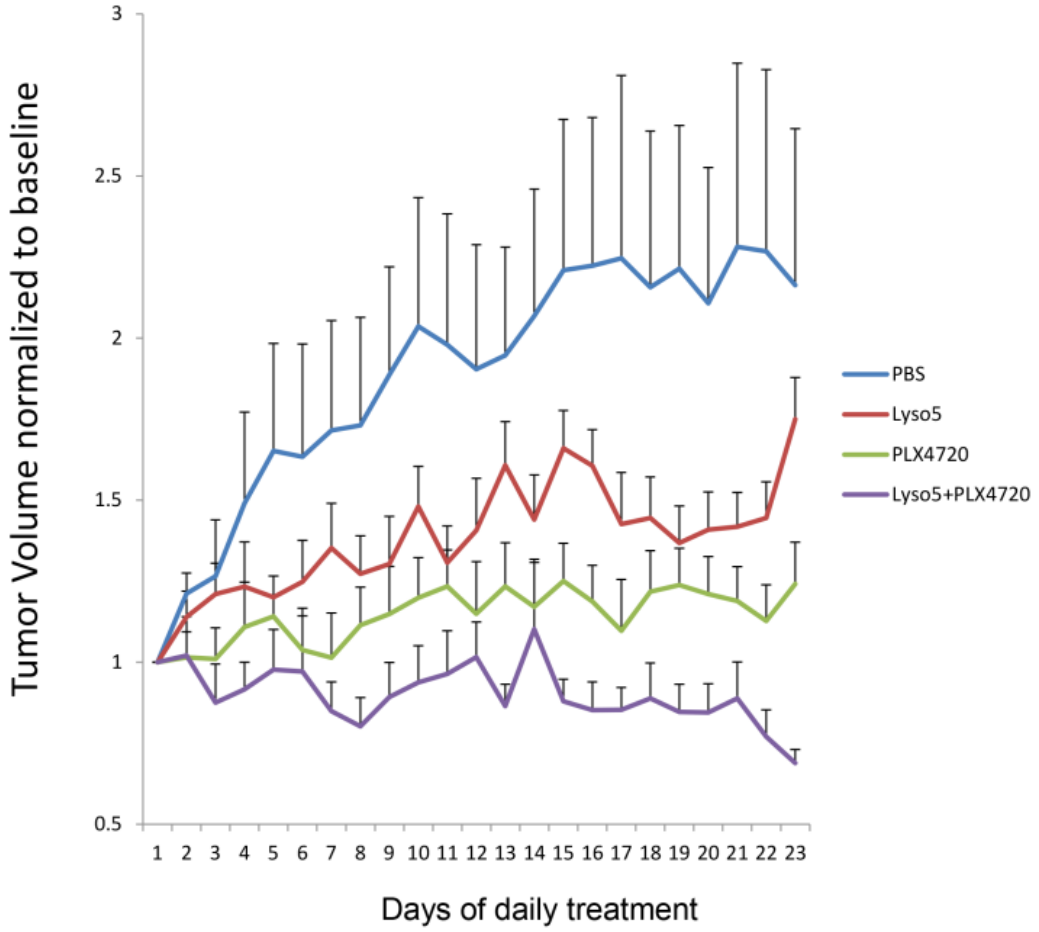


Supplemental Figure S5. Specific drug-induced binding of BRAF and GRP78. (A) Positive proximity ligation assay (PLA) control consisted of Rabbit anti BRAF antibody followed by Rabbit PLUS and Rabbit MINUS secondary PLA detection. Negative PLX control consisted of GRP78 and ATM antibodies. Cells were counterstained with DAPI. **(B)** Mel624 cells were transduced with vector (Vect) or Flag-BRAF^{V600E} (Fl-muBRAF), after 72hours of antibiotic selection and 24 hours of recovery, cells were treated with DMSO or PLX4720 10 μM for 30 minutes. Lysate was reacted with beads conjugated with and without pulldown antibody. Co-immunoprecipitation (IP), and immunoblotting (WB) were performed as indicated. Bracket indicates one input sample split into two immunoprecipitation samples. **(C)** ER expansion observed with BRAF inhibition. Immunofluorescence against protein disulfide isomerase, the most abundant protein within the endoplasmic reticulum. Representative images of MEL624 cells treated with vehicle or PLX4720 10 μM for 30 minutes.

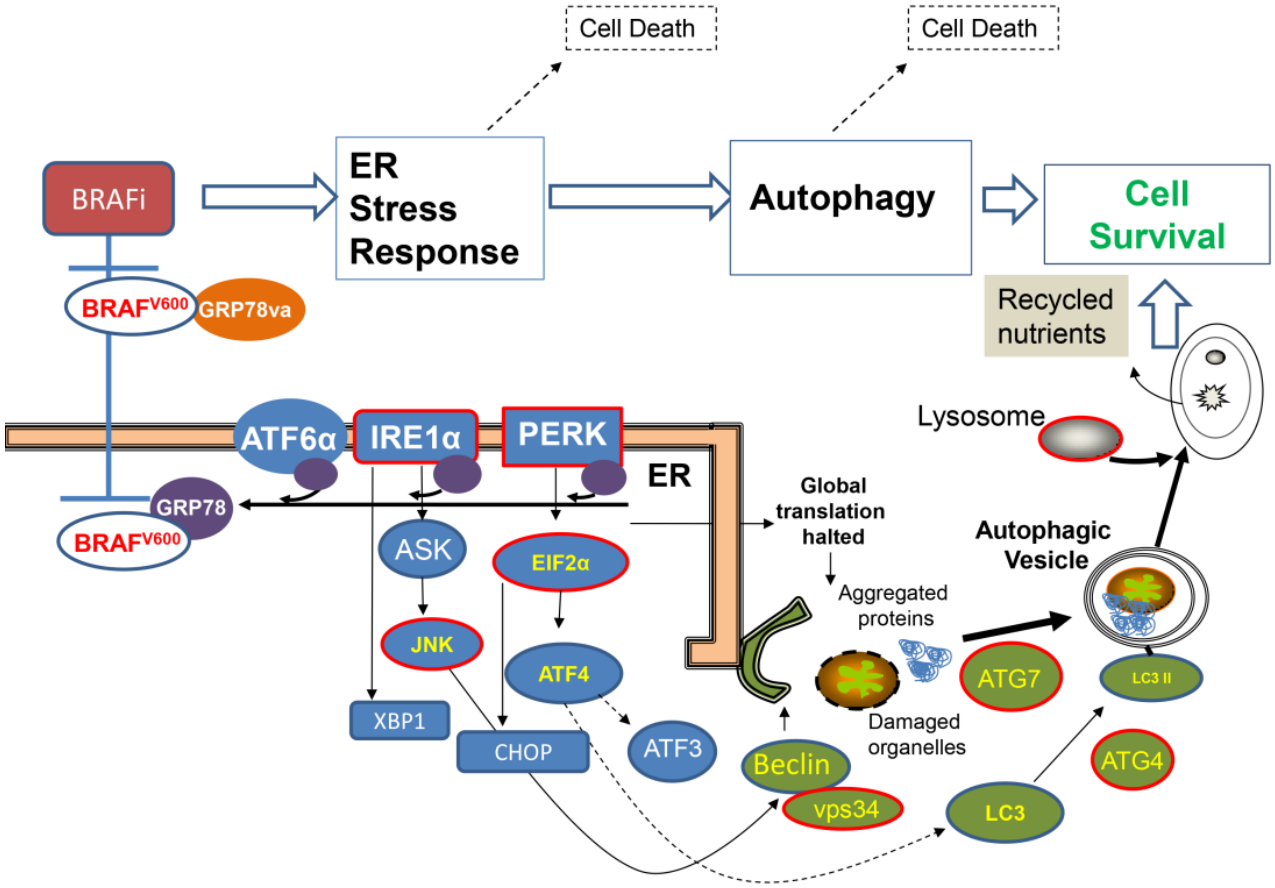


Supplemental Figure S6. Electron microscopy of MEL624 cells treated with vehicle, PLX 1µM or Tg 1 µM for 6 hours. Red arrows: Dilated/ disorganized ER; Orange arrows: dilated ER with unfolded proteins.

Supplemental Figure S7.
Knockdown of PERK in A375P and MEL624 cells at 72 hours



Supplemental Figure S8. Normalized tumor volume (Mean +/- SEM) over time for Nude mice bearing MEL624 xenografts treated with PBS intraperitoneally (i.p.) daily + Control Chow, PBS i.p. daily + PLX4720 chow (PLX), Lys05 40 mg/kg i.p daily + Control Chow (Lys05), and Lys05 40 mg/kg i.p. daily + PLX4720 chow.



Supplemental Figure S9. Molecular pathway linking BRAF inhibition with ER stress response (blue circles) and autophagy (green circles). There are three arms of unfolded proteins and ER stress: PERK, activating transcription factor 6 (ATF6α), and inositol requiring transmembrane kinase and endonuclease 1α (IRE1α). Under non-stressed conditions, these ER-resident proximal transmembrane receptors are bound by the master modulator GRP78 within the ER and kept in an inactive, latent state.The cytoplasmic variant of GRP78, GRP78va, which lacks the ER localization signal is bound constituively to mutant BRAF. , r, Treatment with specific BRAF inhibitors promote ER resident mutant BRAF to associate with GRP78, resulting in the dissociation of GRP78 from and he subsequent activation of PERK, and possibly other transmemberane ER stress components ATF6α, and IRE1α Active PERK phosphorylates the eukaryotic initiation factor 2-alpha (eIF2α) blocking protein synthesis, but paradoxically activating translation of specific mRNAs, including activating transcription factor 4 (ATF4), ATF3, and C/EBP homologous protein (CHOP). Autophagy consists of the sequestration of damaged organelles and proteins in autophagic vesicles and degradation and recycling of building blocks that fuel further growth following fusion with the lysosome. The lipid kinase Beclin-vps34 complex is responsible for nucleation of nascent membrane from the ER or other subcellular compartments. Once the AV begins to form, a pair of ubiquitin-like conjugation systems involving the ATG5-ATG12 complex and ATG7-ATG10 complex, results in nucleation of the AV and conjugation of the ubiquitin-like cytoplasmic protein LC3, which is cleaved, activated, and removed by the redox sensing protease ATG4, to the surface of forming AV permitting maturation of the AV. Conjugated LC3II migrates separately from unconjugated LC3I on gel electrophoresis and the LC3II/LC3I ratio reflects the number of AV that have accumulated within a cell. While an uncontrolled ER stress response or autophagy can produce cell death, a well-orchestrated activation of the ER-stress response- autophagy program allows degradation of damaged organelles and proteins and recycling of macromolecules contributes to cell survival. Druggable targets in the ER stress response-autophagy pathway are encircled in red.