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Supplemental Material to:

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Regulation of autophagy and chloroquine sensitivity by oncogenic RAS in vitro is context-dependent

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Figure S1

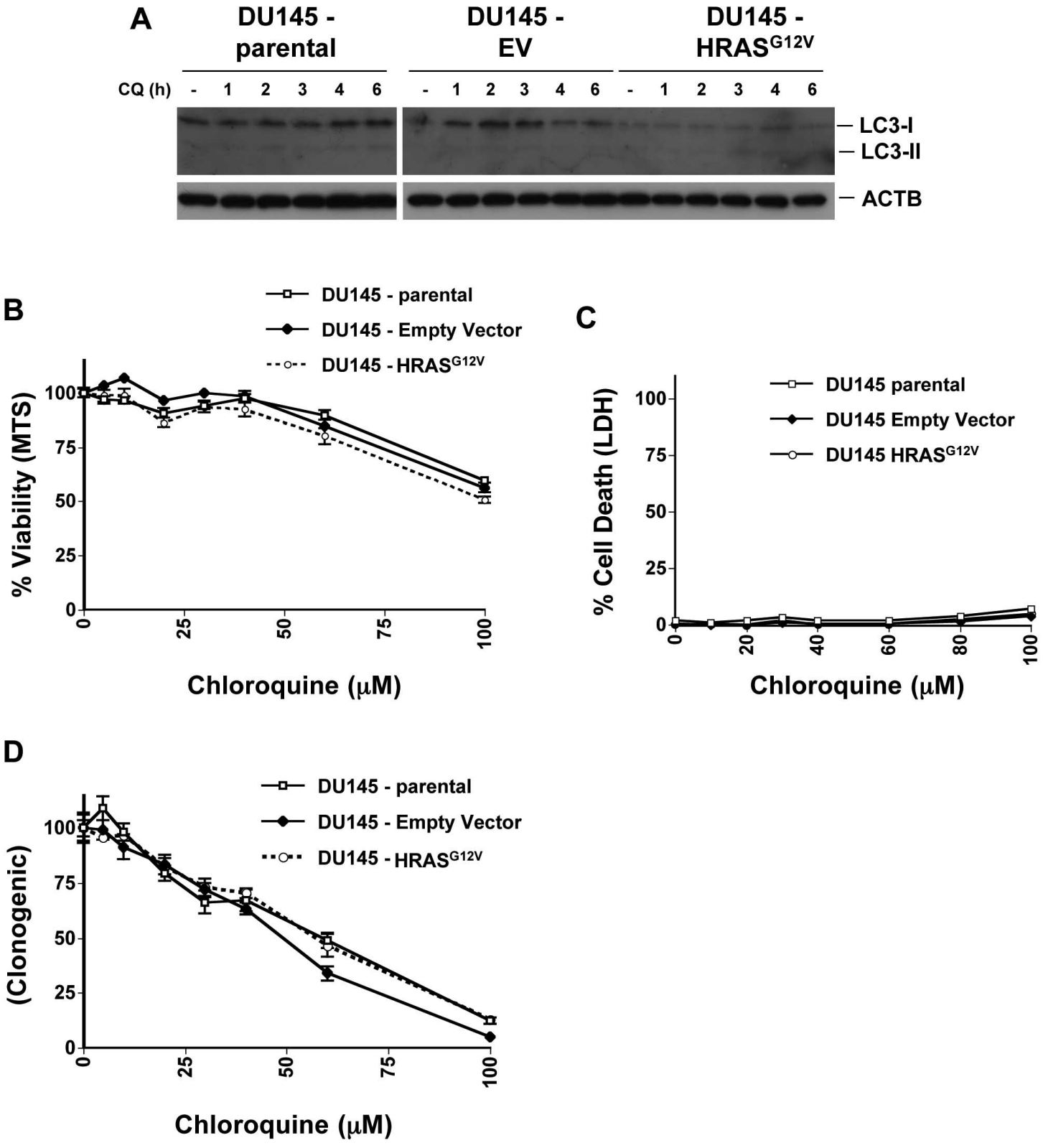
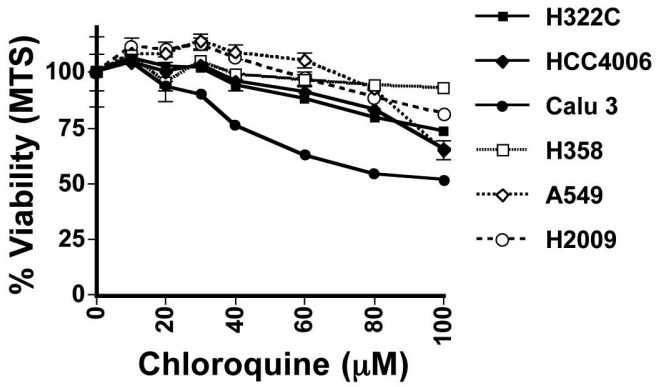


Figure S1. RAS does not confer sensitivity or resistance to CQ in DU145 cells. **(A)** DU145 cells or DU145 cells stably integrated with empty vector control or $HRAS^{G12V}$ were treated with chloroquine (30 μM) for increasing lengths of time and then cells were lysed and immunoblotted for LC3 and ACTB. **(B, C, D)** Parental DU145 cells or DU145 cells stably integrated with empty vector control or $HRAS^{G12V}$ were treated with CQ for 48 h and cell viability, cytotoxicity, and growth were measured by **(B)** MTS viability assay, **(C)** LDH release, and **(D)** clonogenic growth assay as measured by crystal violet staining 5 days after removing the CQ and adding growth media.

Figure S2

A



B

Cell line	KRAS status	TP53 status	CQ sensitivity
H322C	WT	mutant (G12C)	intermediate
HCC4006	WT	WT[#]	intermediate
Calu3	WT	mutant (M237I)	high
H358	mutant (G12C)	deleted	moderately low
A549	mutant (G12S)	WT	low
H2009	mutant (G12A)	mutant (G12C)	intermediate

C

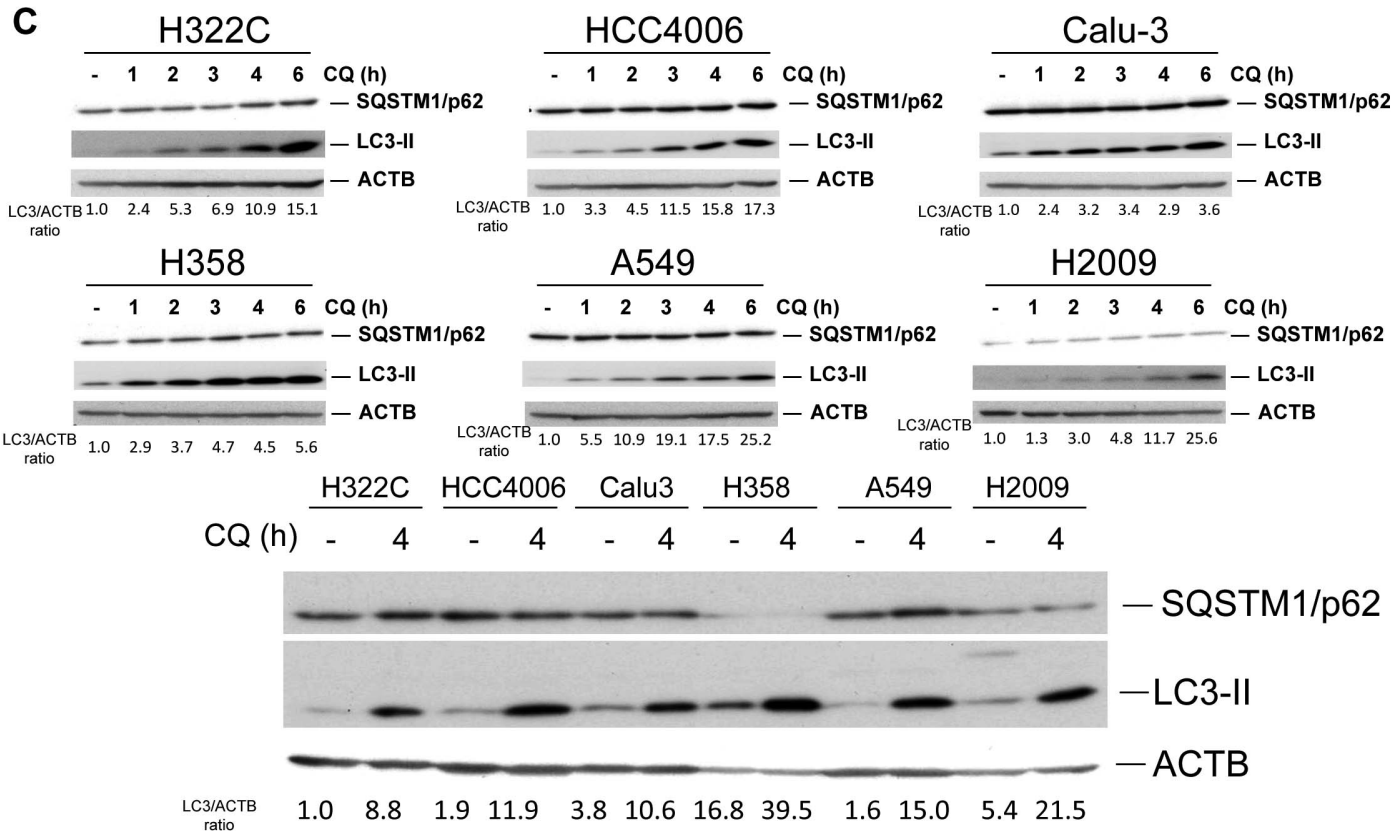


Figure S2. RAS status or basal autophagy does not correlate with autophagy dependence in NSCLC lung cell lines. **(A)** H322C, HCC4006, and Calu3 (wt RAS, indicated by filled symbols) and H358, A549, and H2009 (oncogenic KRAS mutant, indicated by unfilled symbols) NSCLC cancer cell lines were treated with chloroquine and assayed by MTS viability assay at 48 h. (**B)** The RAS and TP53 mutation status of the 6 cell lines and their approximate CQ sensitivity are shown. [#] No mutations in TP53 have been described for HCC4006, but to our knowledge, wild-type status has not yet been verified. (**C)** H322C, HCC4006, Calu-3 and H358, A549, and H2009 NSCLC cancer cell lines were treated with CQ (30 μM) for the indicated timepoints and then cells were lysed and immunoblotted for SQSTM1, LC3, and ACTB (top panels). Similarly, for comparison, these cells were treated with or without CQ (30 μM) for 4 h and then cells were lysed and lysates on the same blot were immunoblotted for SQSTM1, LC3, and ACTB (bottom panels).

Figure S3

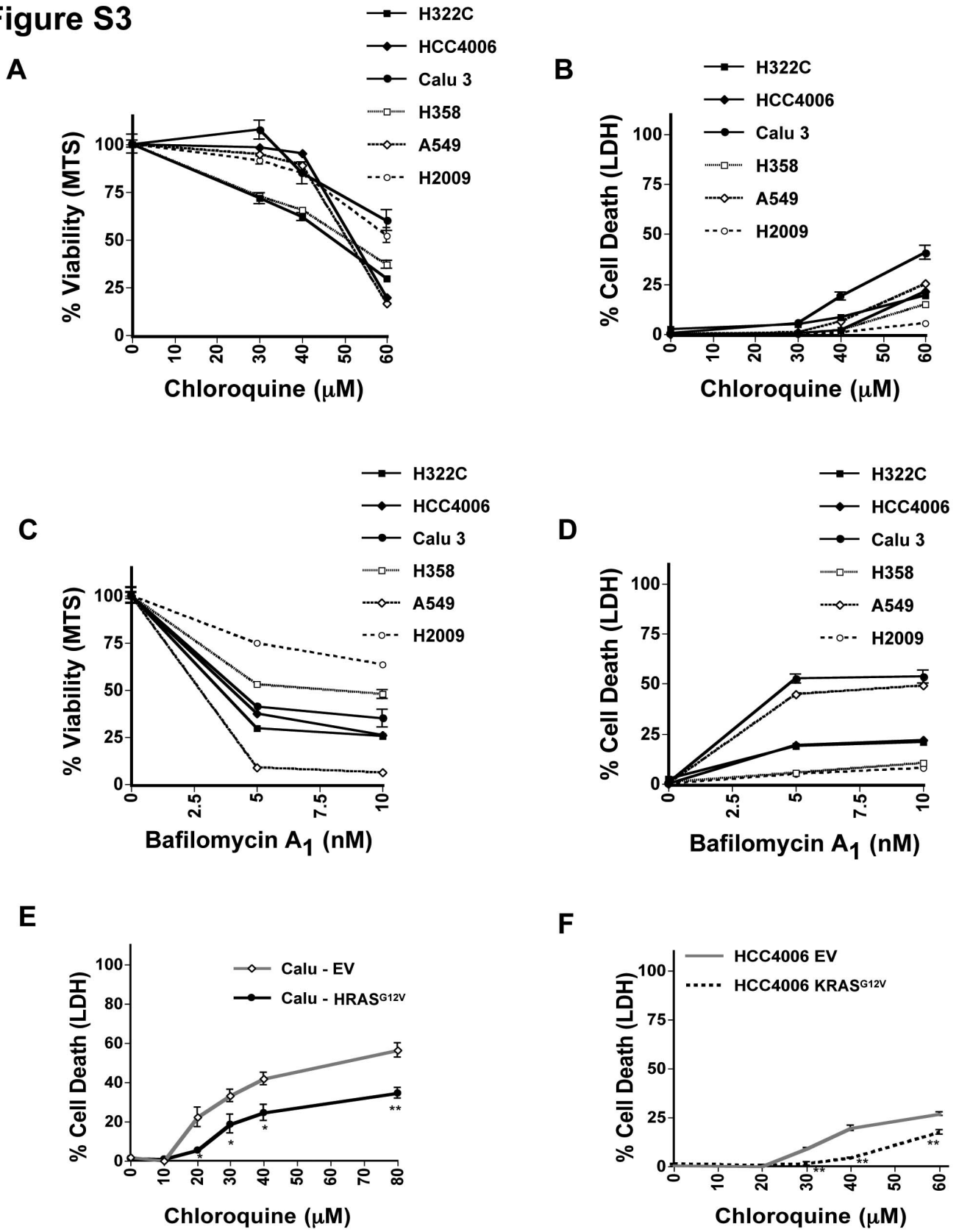


Figure S3. When grown in basal media, RAS status does not correlate with sensitivity to autophagy inhibition in NSCLC cell lines. **(A-C)** H322C, HCC4006, and Calu3 (WT RAS, indicated by filled symbols) and H358, A549, and H2009 (oncogenic KRAS mutant, indicated by unfilled symbols) NSCLC cell lines grown in basal media (RPMI, 10% FBS) were treated with CQ or bafilomycin A_1 and assayed by (**A, C**) MTS viability assay (72 h) or (**B, D**) LDH release cytotoxicity assay (72 h). (**E)** Calu3 cells stably infected with retrovirus expressing $HRAS^{G12V}$ or empty vector control were treated with CQ and assayed at 72 h for LDH release. (**F)** HCC4006 cells stably infected with retrovirus expressing $KRAS^{G12V}$ or the empty vector control were treated with CQ and assayed at 72 h for LDH release. ** P<0.01, * P<0.05

Figure S4

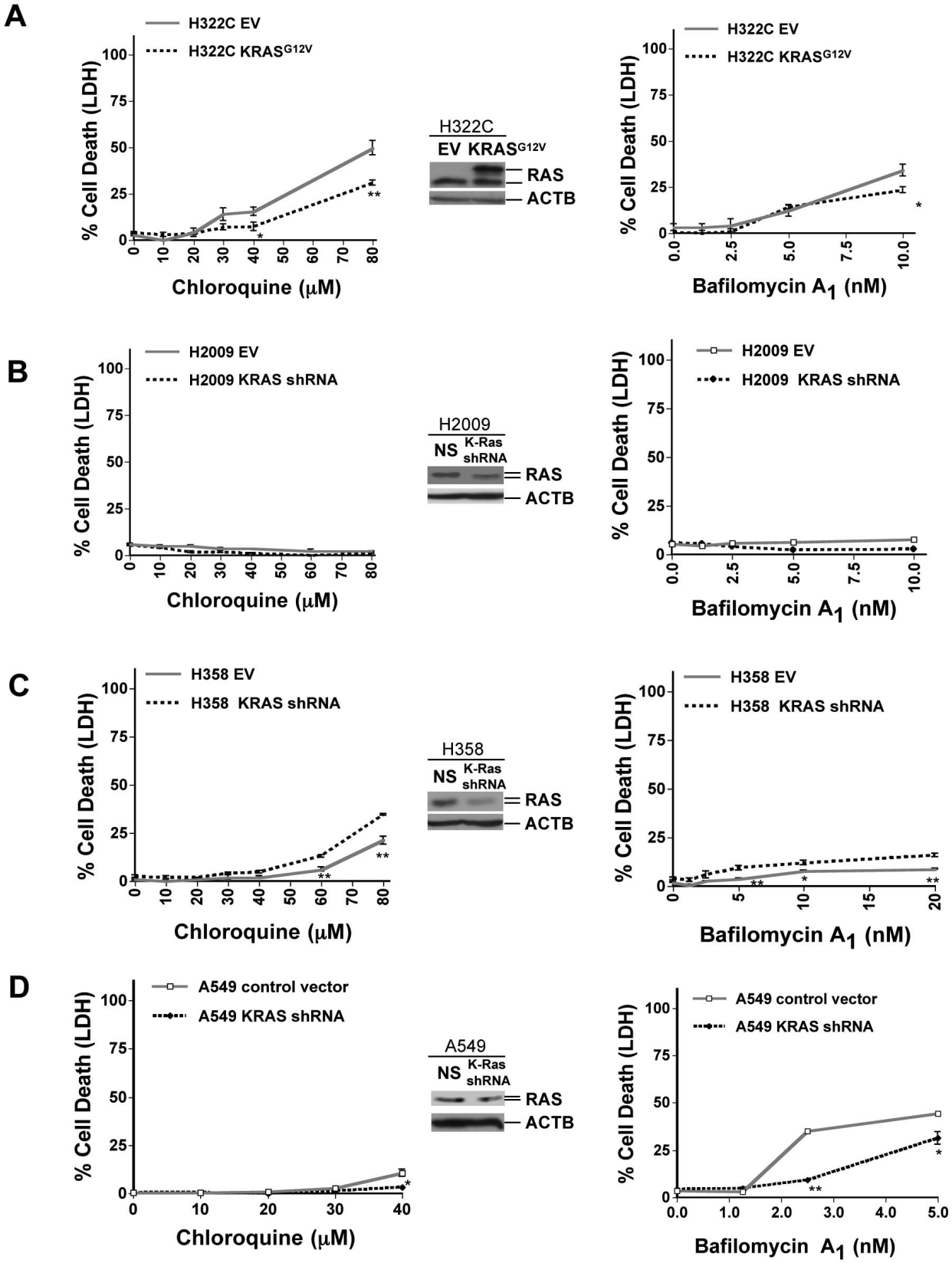


Figure S4. RAS can have opposing effects sensitivity to autophagy inhibition in isogenic NSCLC cell lines **(A)** H322C cells stably infected with retrovirus expressing $KRAS^{G12V}$ or the empty vector control were treated with CQ (left) or bafilomycin A_1 (right) and assayed at 72 h for LDH release. (**B-D)** Oncogenic KRAS mutant cells were stably infected with lentivirus expressing KRAS shRNA or a nonsilencing control and were treated with CQ (left panels) or bafilomycin A_1 (right panels) and assayed at 72 h for LDH release. Shown are (**B)** H2009, (**C)** H358, and (**D)** A549 cells lines. ** $P<0.01$, * $P<0.05$.

Figure S5

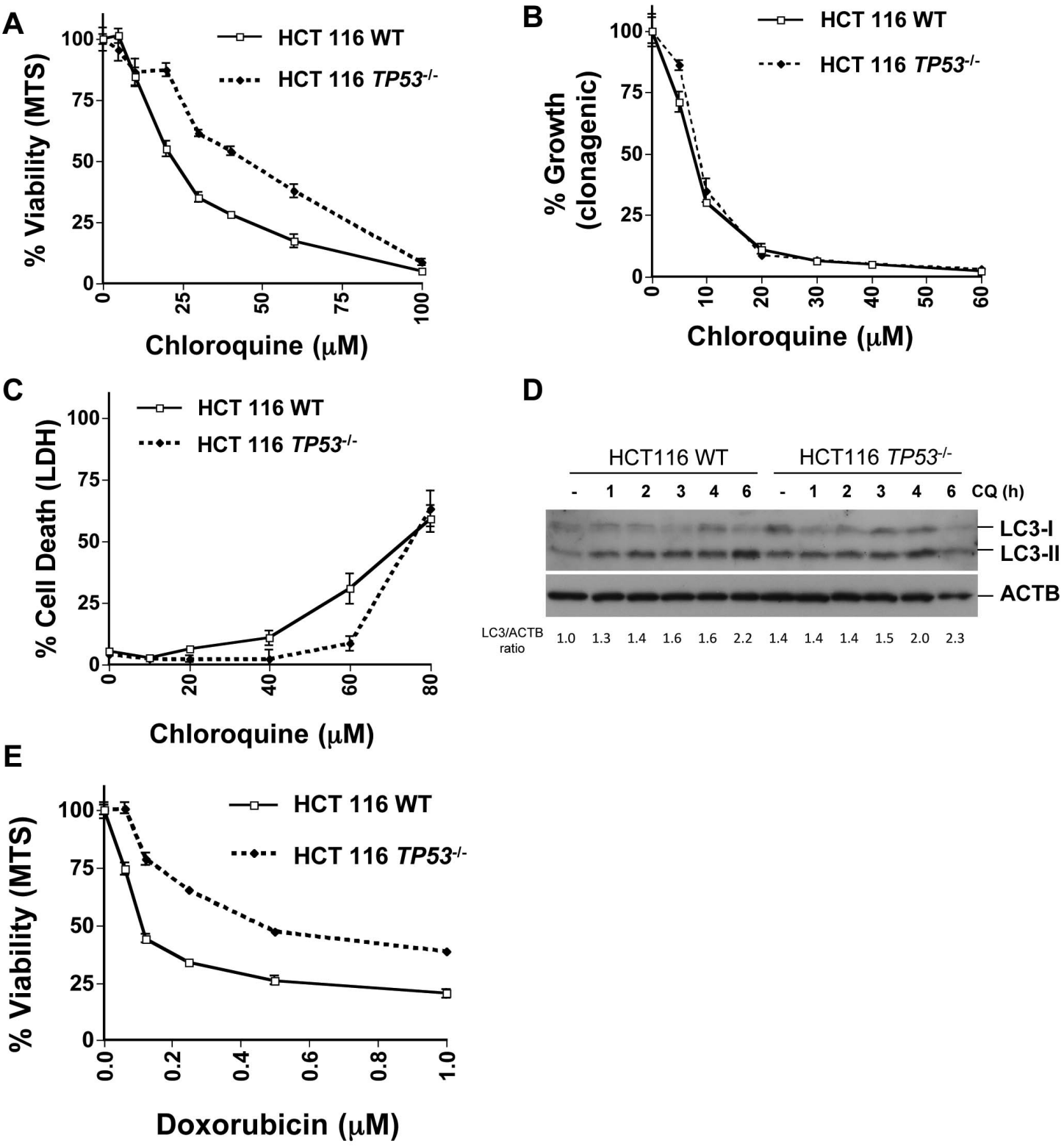
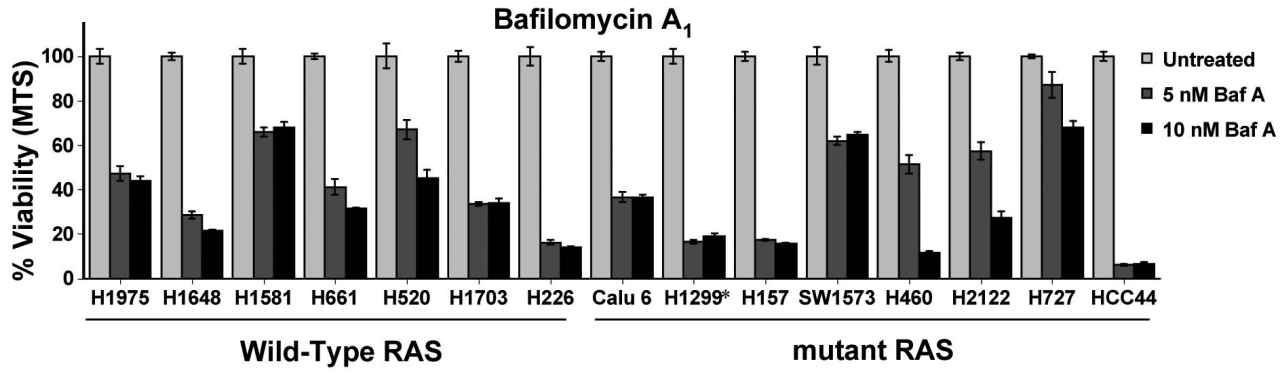


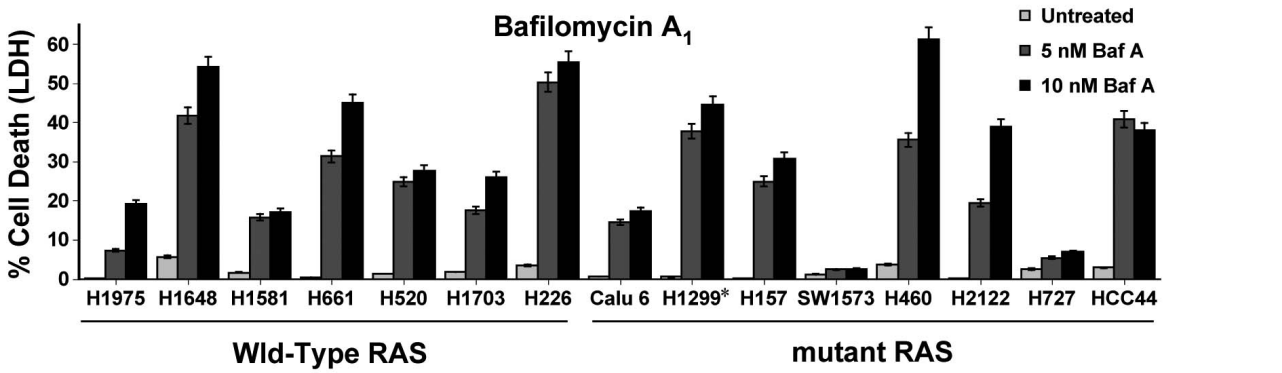
Figure S5. Loss of TP53 makes HCT116 cells (KRAS mutant) resistant to toxicity for autophagy inhibition. **(A**) Matched HCT116 cells with or without somatic TP53 deletion were exposed CQ for 48 h, and cell viability was examined by MTS viability assay. (**B)** HCT116 cells were exposed to CQ for 48 h, and cell growth was examined by clonogenic growth assay as measured by crystal violet staining 5 days after removing the CQ and adding growth media. (**C)** HCT116 cells were treated with CQ for 48 h, and cell death was measured by LDH assay. **(D**) HCT116 cells were treated with CQ (30 μM) for the indicated timepoints and then cells were lysed and immunoblotted for LC3 and ACTB. (**E**) HCT116 cells were treated with doxorubicin for 48 h, and cell viability was examined by MTS viability assay

Figure S6

A



B



C

Cell line	RAS Status	TP53 status
H322C	WT	R248L
HCC4006	WT	WT#
Calu3	WT	M237I
H358	mutant KRAS (G12C)	null
A549	mutant KRAS (G12S)	WT
H2009	mutant KRAS (G12A)	R273L
H1975	WT	R273H
H1648	WT	WT, L35 Framesshift
H1581	WT	WT, Q144X
H661	WT	R158L, S215I
H520	WT	W146X
H1703	WT	E285K
H226	WT	R158L, P309A
Calu6	mutant KRAS (Q61K)	R196X
H1299	mutant NRAS (Q61K)	WT, deletion
H157	mutant KRAS (G12R)	E298X
SW1573	mutant KRAS (G12C)	WT
H460	mutant KRAS (Q61H)	WT
H2122	mutant KRAS (G12C)	Q16L, C176F
H727	mutant KRAS (G12C)	3AA in frame insertion (Y166/K167/Q168)
HCC44	mutant KRAS (G12C)	R175L, S94X

Figure S6. Neither RAS status nor TP53 status correlates with sensitivity to autophagy inhibition in NSCLC lung cell lines. **(A and B)** Multiple NSCLC cancer cell lines with wild-type or oncogenic RAS status as designated were treated with the indicated doses of bafilomycin A_1 and then assayed by (**A)** MTS viability assay (72 h) or **(B**) LDH release cytotoxicity assay (72 h). *H1299 has an oncogenic mutation in NRAS instead of KRAS. **(C)** The RAS and TP53 mutation status of the cell lines as given in multiple databases (COSMIC, IARC TP53 Database, etc.) is shown. # No mutations in TP53 have been described for HCC4006, but to our knowledge, wild-type status has not yet been verified.

Figure S7

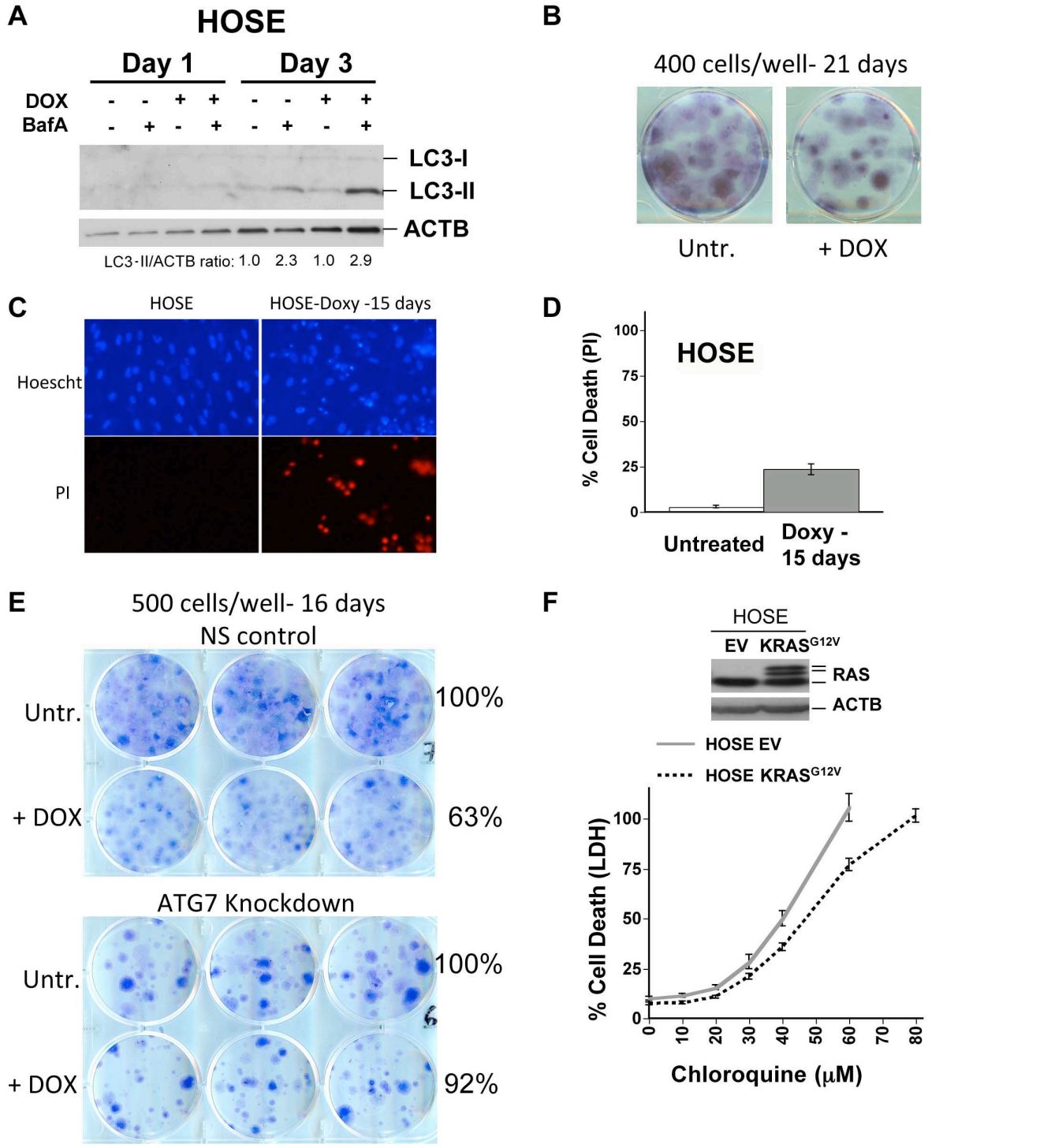


Figure S7. Oncogenic RAS expression activates autophagy and promotes cell death in a human ovarian surface epithelial (HOSE) cell line. **(A)** HOSE cells with a tetracycline-inducible HRAS^{G12V} transgene (HOSE-HRAS^{G12V}) were left untreated or were treated with or without doxycycline (100 ng/mL) for 1 or 3 days. In some cells bafilomycin A_1 (10 nM) was added for 4 h to block flux and then cells were lysed and immunoblotted for LC3 and ACTB to measure basal autophagy. **(B)** HOSE-HRAS^{G12V} cells (400) were plated in a 6-well plate and left untreated or were treated with doxycycline (100 ng/mL) for 21 days and analyzed by crystal violet staining. (**C and D**) HOSE-HRAS^{G12V} cells were treated with doxycycline (100 ng/mL) for 15 d and analyzed by PI exclusion using fluorescence microscopy. Sample picture is shown in (**C**), and quantification is shown in (**D**). (**E**) Similar to (**B**), 500 HOSE-HRAS^{G12V} cells infected with lentivirus expressing *ATG7* shRNA or a non-silencing control shRNA were plated on in a 6-well plate and left untreated or were treated with doxycycline (100ng/mL) for 16 days. Cells were fixed and analyzed by crystal violet staining. Quantification of the dye at 540 nm was done after dissolving in 33% acetic acid. (**F**) HOSE-HRAS^{G12V} cells (uninduced) were infected with a KRAS^{G12V} retrovirus or empty vector virus and, after selection, were exposed to various doses of CQ for 48 h, and cell death was measured by LDH assay.

Figure S8

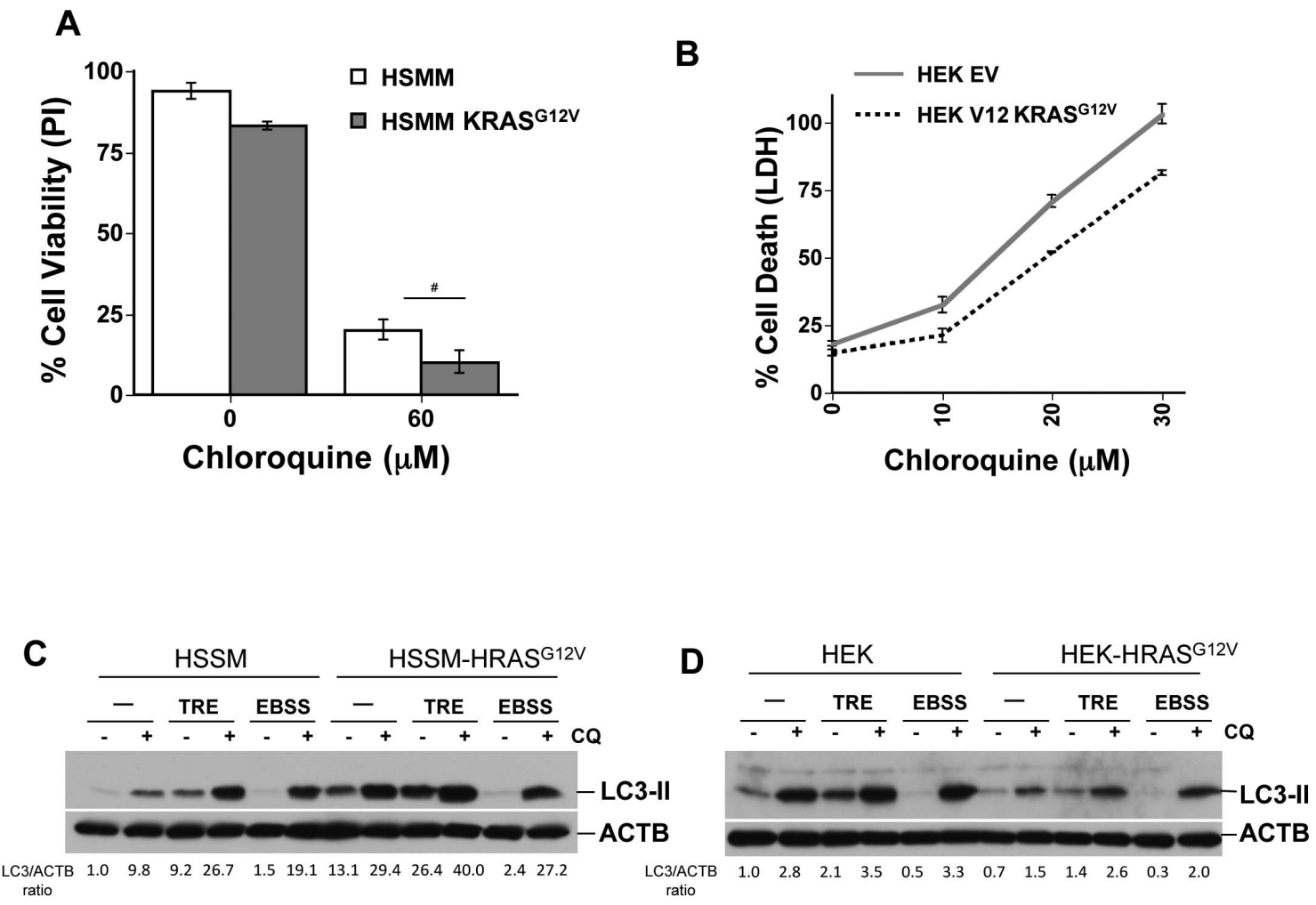


Figure S8. RAS has opposing effects on autophagy and CQ sensitivity in genetically defined immortalized human skeletal muscle myoblasts (HSMM) and human embryonic kidney cells (HEK) in stimulated or unstimulated conditions. **(A and B)** Immortalized HSMM (**A)** or HEK cells (**B)** with or without stable expression of KRAS^{G12V} were treated with varying doses of CQ for 48 h and assayed by (**A**) propidium iodide exclusion as counted under a fluorescence microscope or (**B)** LDH release cytotoxicity assay. (**C and D)** Immortalized HSMM (**C**) or HEK cells (**D**) with or without stable expression of HRAS^{G12V} were treated for 4 h with or without chloroquine CQ (30 μM), in combination with or without trehalose (100 mM) or EBSS as indicated and then cells were lysed and immunoblotted for LC3, and ACTB. ** P<0.01, * P<0.05, $^{\#}$ P< 0.08.

Figure S9

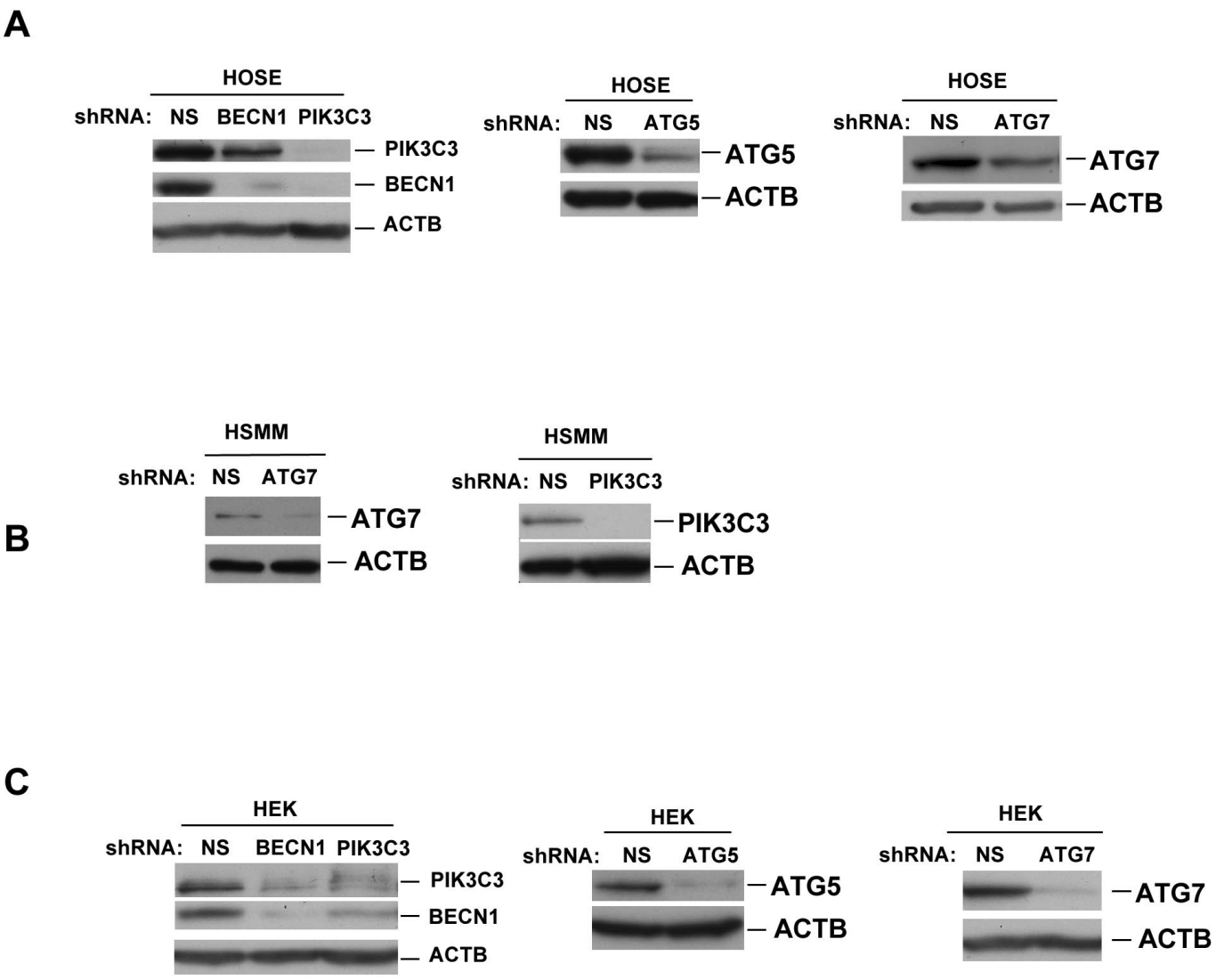


Figure S9. Knockdown of autophagy proteins in HOSE, HSMM and HEK cell lines.
(A to C) Representative western blots show knockdown of autophagy proteins in (**A)** HOSE cells, (**B)** HSMM and (**C)** HEK cell lines.