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Supplemental Information

Cancer Cells Upregulate NRF2 Signaling to Adapt to Autophagy Inhibition

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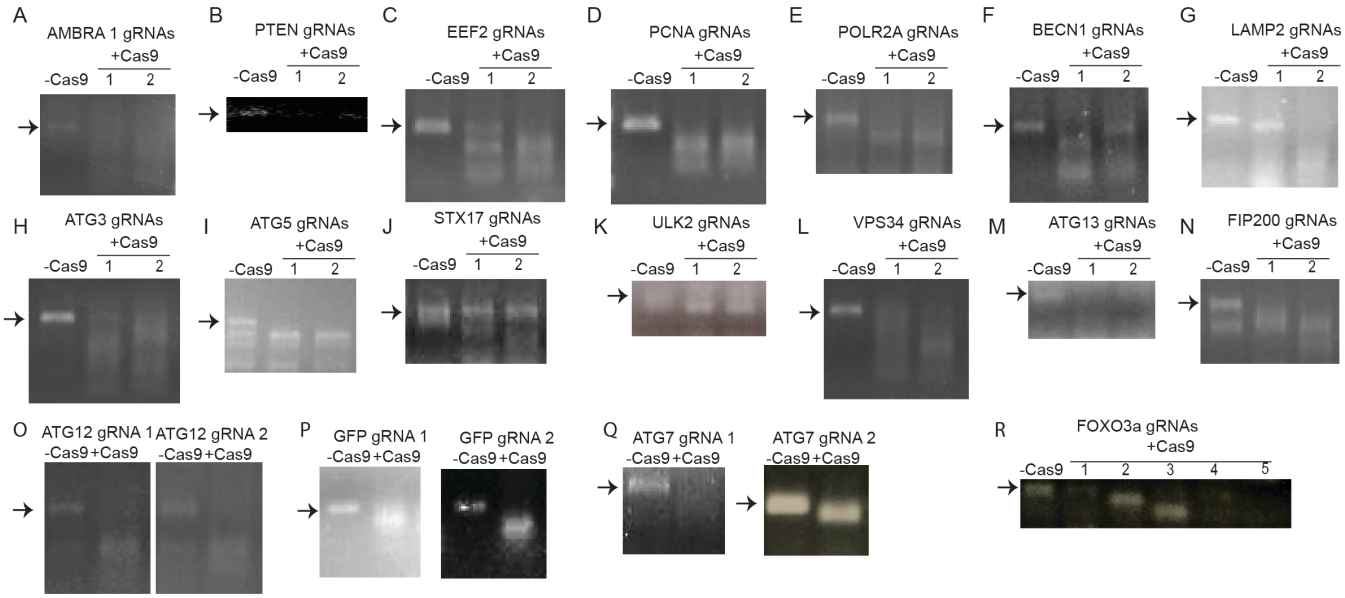


Figure S1, Related to Figure 1: Verification of gRNAs. Agarose gels showing cut DNA before and after In-vitro Cas9 assay for all the indicated gRNAs used in Figure 1 and 2. Black arrows indicate the correct PCR product running at the expected size.

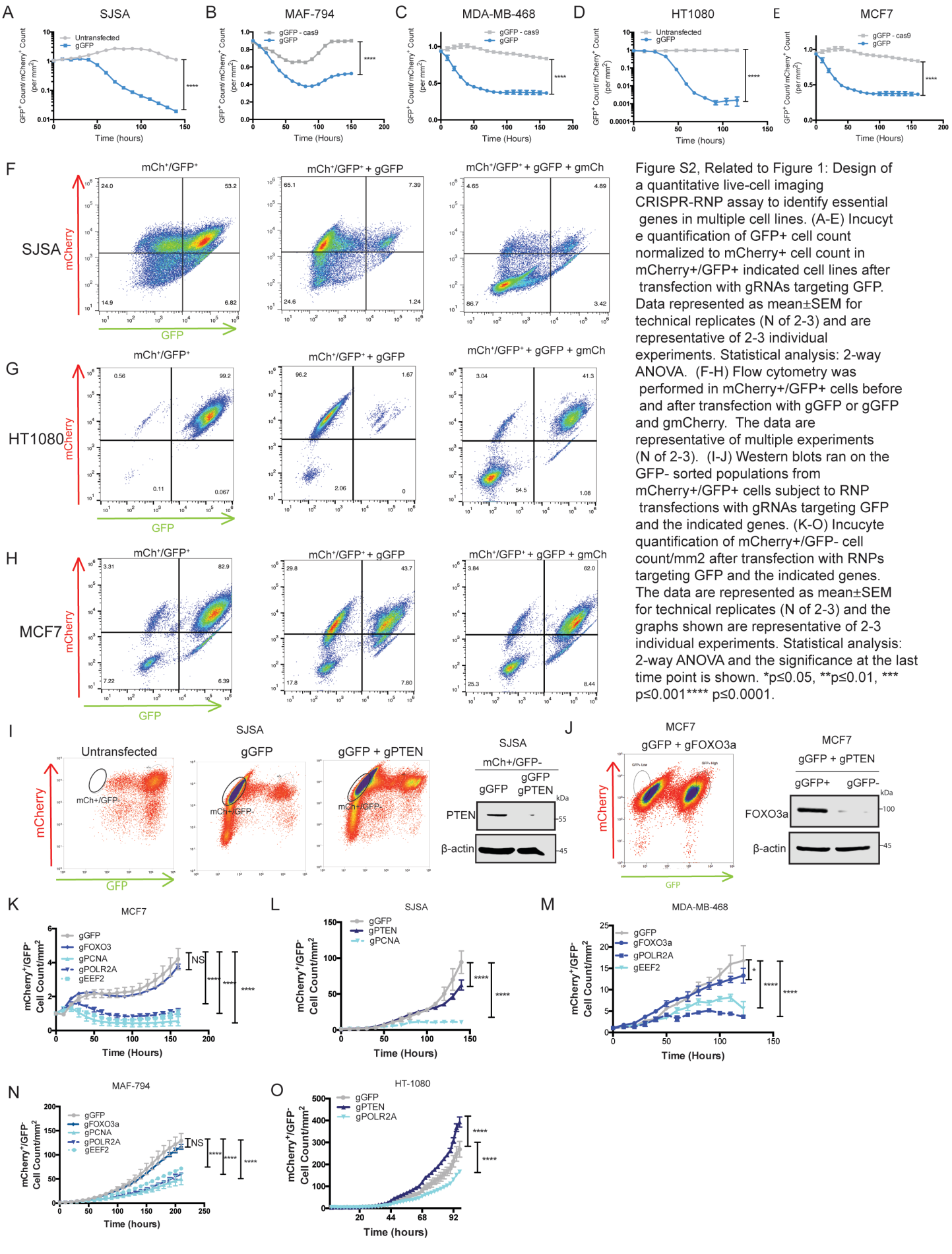
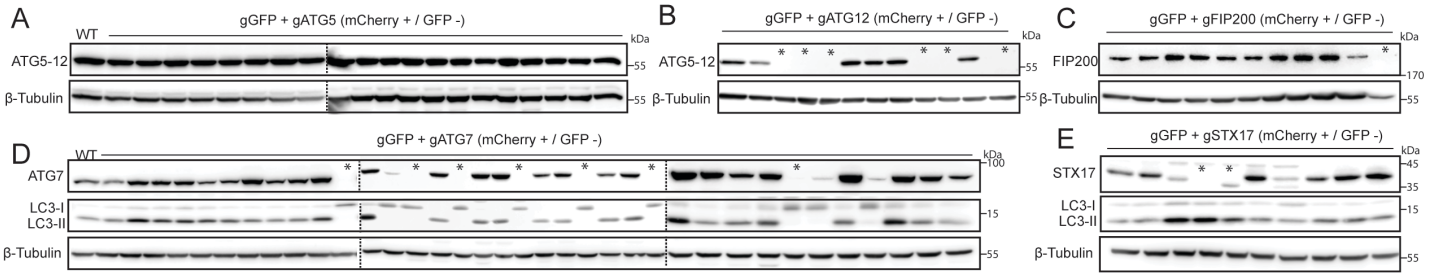


Figure S2, Related to Figure 1: Design of a quantitative live-cell imaging CRISPR-RNP assay to identify essential genes in multiple cell lines. (A-E) Incucyte quantification of GFP+ cell count normalized to mCherry+ cell count in mCherry+/GFP+ indicated cell lines after transfection with gRNAs targeting GFP. Data represented as mean±SEM for technical replicates (N of 2-3) and are representative of 2-3 individual experiments. Statistical analysis: 2-way ANOVA. (F-H) Flow cytometry was performed in mCherry+/GFP+ cells before and after transfection with gGFP or gGFP and gmCherry. The data are representative of multiple experiments (N of 2-3). (I-J) Western blots ran on the GFP- sorted populations from mCherry+/GFP+ cells subject to RNP transfections with gRNAs targeting GFP and the indicated genes. (K-O) Incucyte quantification of mCherry+/GFP- cell count/mm2 after transfection with RNPs targeting GFP and the indicated genes. The data are represented as mean±SEM for technical replicates (N of 2-3) and the graphs shown are representative of 2-3 individual experiments. Statistical analysis: 2-way ANOVA and the significance at the last time point is shown. *p≤0.05, **p≤0.01, *** p≤0.001**** p≤0.0001.



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gRNA	Ratio -/- : Total clones	Percentage
ATG5	0:20	0%
ATG12	6:12	50%
ATG7	7:36	19%
FIP200	1:11	9%
STX17	2:10	20%

Figure S3, Related to Figure 2: Verification of gene KO in H292 cells. (A-E) Western blots ran on clones generated from the GFP- sorted populations from mCherry+/GFP+ cells subject to RNP transfections with gRNAs targeting GFP and the indicated genes. * represent clones with complete KO. (F) Calculated percent of clones with complete loss of protein expression based on western blots from A-E. Lorem ipsum

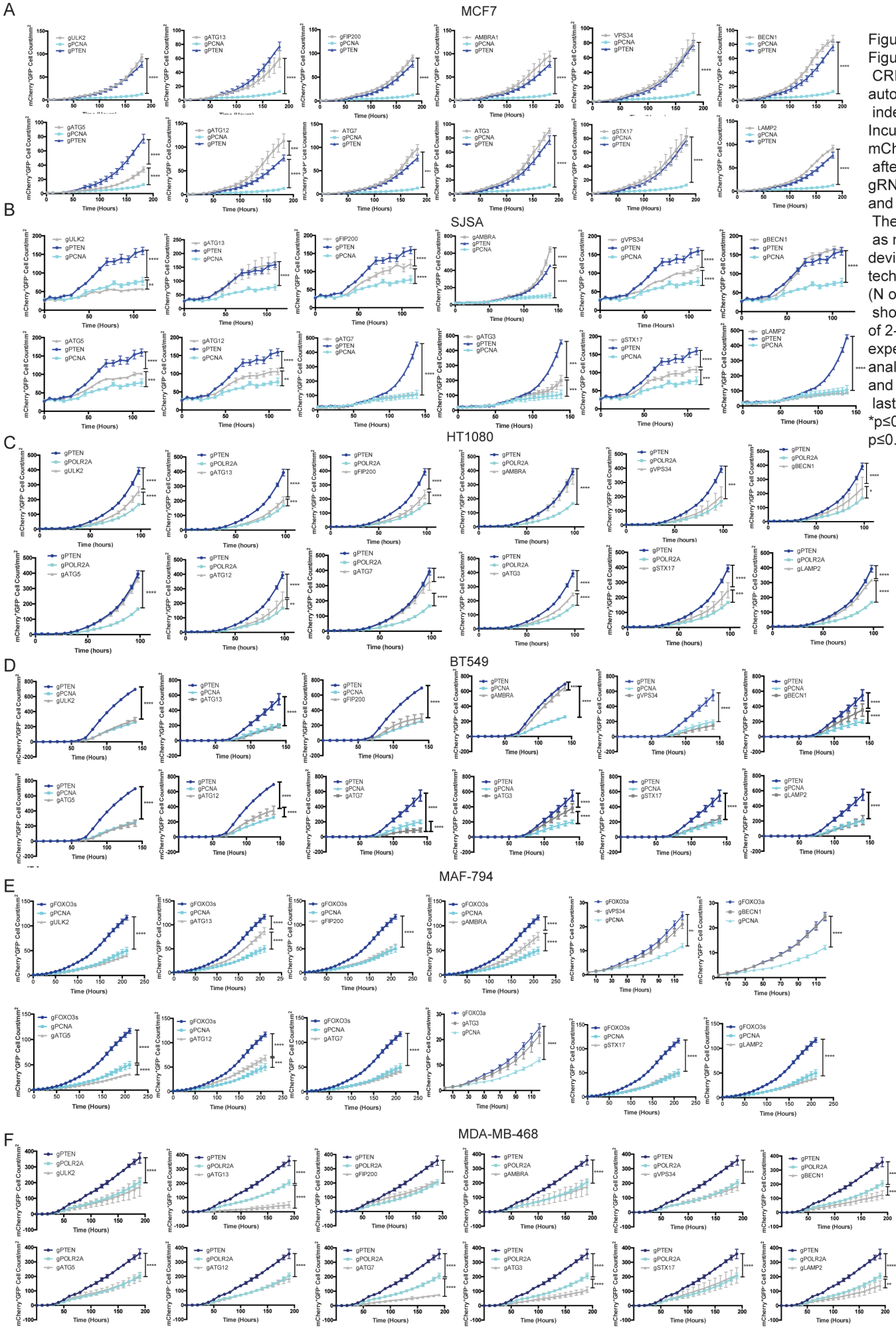

Figure S4, Related to Figure 2: Live-cell imaging CRISPR-RNP identifies autophagy dependent and independent cells. (A-F) Incucyte quantification of mCherry+/GFP- cell count after transfection with gRNAs targeting GFP and the indicated genes. The data are represented as mean±standard deviation (SD) for technical replicates (N of 2-3) and the graphs shown are representative of 2-3 individual experiments. Statistical analysis: 2-way ANOVA and the significance at the last time point is shown. *p≤0.05, **p≤0.01, *** p≤0.001**** p≤0.0001.

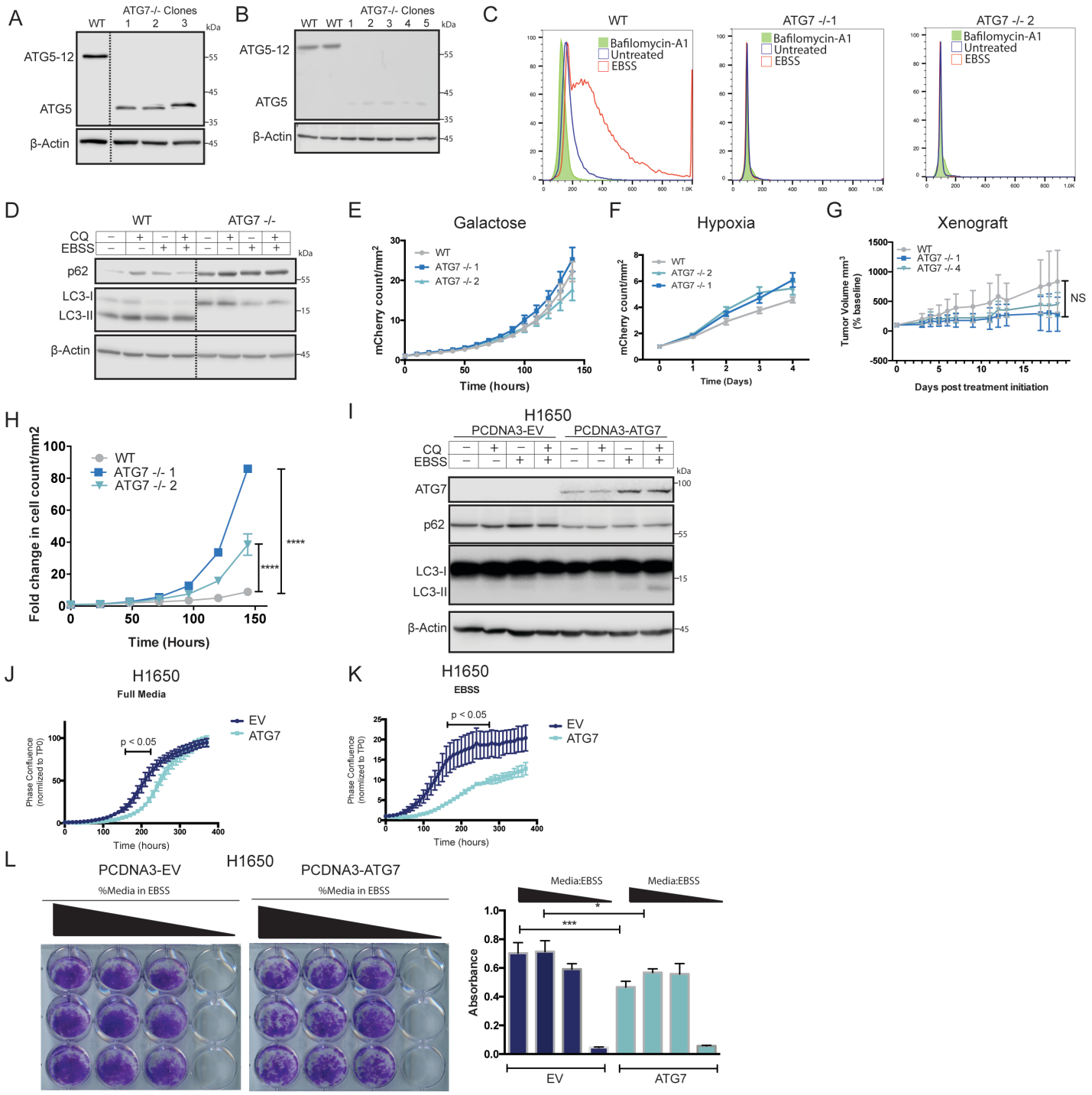

Figure S5, Related to Figures 3, 4: Autophagy-dependent cancer cells can undergo selection to circumvent complete inactivation of an autophagy regulator. (A-B) Western blot analysis in (A) BT549 and (B) H292 WT and ATG7-/- clones. The blots shown are representative of 2 experiments. (C) The graphs show the shift in GFP expression in BT549 WT and ATG7-/- clones with stable expression of LC3-mCherry-GFP 24hrs after starvation or Bafilomycin A1 (10nM). The data are representative of 2 experiments. (D) Western blot in BT549 WT and ATG7-/- clones after 16hr starvation with or without Chloroquine (40μM) for 2hrs. The blots are representative of 3 experiments. (E-F) Incucyte live cell imaging mCherry+ cell counts over time in BT549 WT and ATG7-/- clones grown in (E) media containing 10mM galactose or (F) hypoxia (1% 02). The data are represented as mean±SEM for technical replicates (N of 3) and the graphs shown are representative of 3 individual experiments. (G) The mean tumor volume as a percentage of pre-treatment baseline, in vehicle treated mice bearing H292 WT or ATG7-/- xenograft tumors. N= 5-7 mice/group. The data are represented as mean±SEM. Statistical analysis: 2-way ANOVA. (H) Incucyte quantification of mCherry+ cell count of H292 WT or ATG7-/- clones after treatment with CQ (20μM). The data are represented as mean±SEM for technical replicates (N of 2-3) and the graphs shown are representative of 3 individual experiments. (I-L) NCIH1650 cells. (I) Western blot analysis in parental cells with stable expression of PCDNA3-EV or PCDNA3-ATG7. Representative of 3 experiments. (J-K) Incucyte growth assay monitoring phase confluency in (J) full growth media or (K) nutrient starvation with EBSS. The data are represented as mean±SEM for technical replicates (N of 3) and the graphs are representative of 2 experiments. Statistical analysis: 2-way ANOVA. (L) Left: Long term clonogenic growth assay in EV and ATG7 overexpressing cells treated with 100%, 75%, and 50% media in EBSS for 72 hours and then allowed to grow back in 100% media for 7 days. Right: Quantification of solubilized crystal violet from long term clonogenic growth assay. The data are represented as mean±SEM for technical replicates (N of 3). Statistical analysis: 2-way ANOVA. *p≤0.05, **p≤0.01, **** p≤0.0001

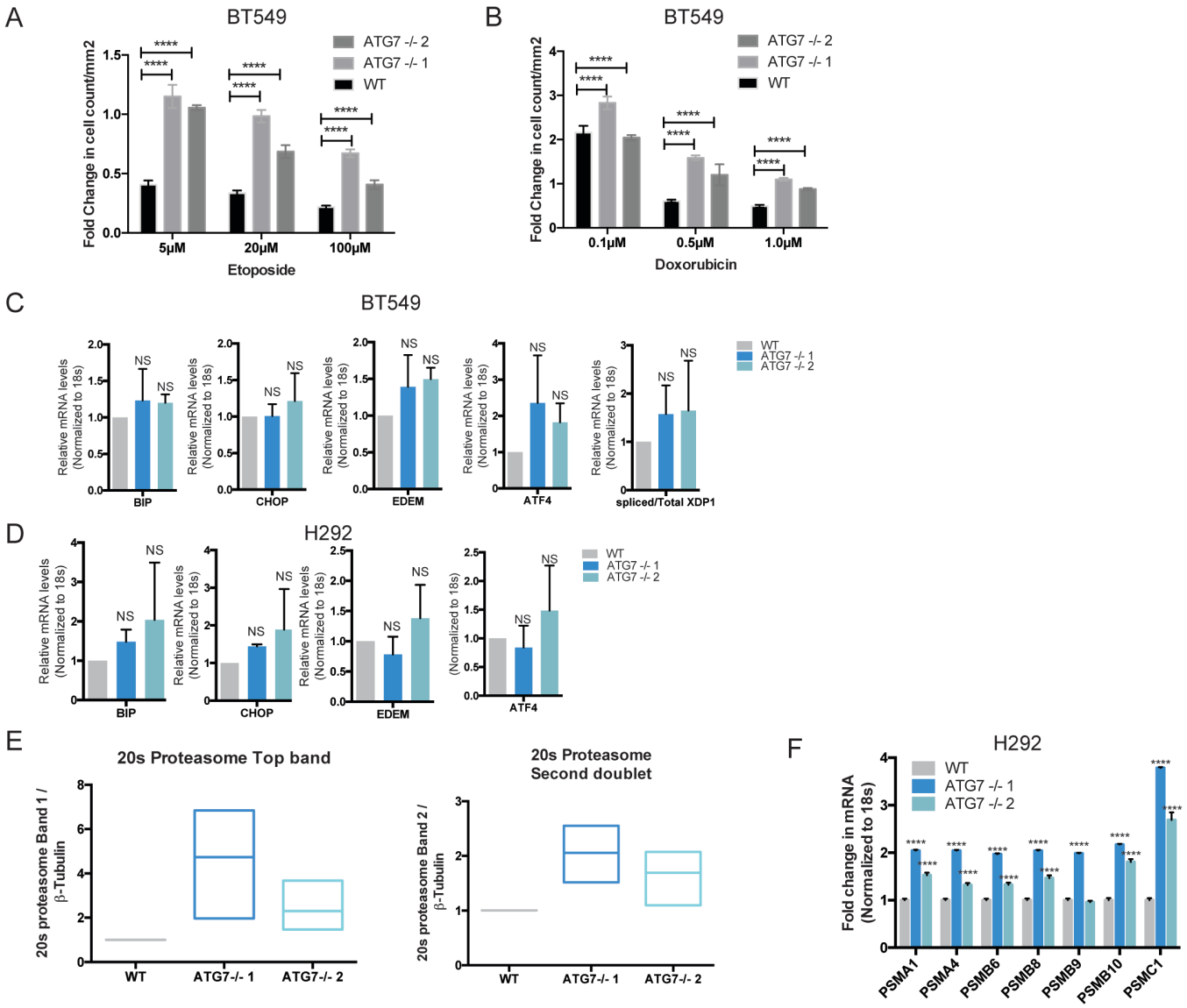

Figure S6, Related to Figure 5: Autophagy-dependent cancer cells are not more sensitive to DNA damaging agents and do not have increased ER stress but do have increased proteasomal subunits. (A-B) The Incucyte mCherry+ cell count/mm2 calculated 4 days after treatment with (A) etoposide or (B) doxorubucin in BT549 WT or ATG7-/- clones normalized to TP0. The data are represented as mean±SD for technical replicates (N of 3) and representative of 2 individual experiments. Statistical analysis: 2-way ANOVA. (C-D) qRT-PCR to measure mRNA levels of ER stress response genes relative to 18s mRNA levels in (C) BT549 and (D) H292 WT and ATG7-/- clones. The data are represented as mean±SEM for experimental replicates (C: N of 3, D: N of 2). Statistical analysis: 1-way ANOVA. (E) Densitometric quantification of western blots probed for the 20s proteasome in Figure 5F for bands indicated with an asterisk. The data shown is from 3 independent experiments. (F) qRT-PCR to measure mRNA levels of 26s proteasomal subunits relative to 18s mRNA levels in H292 WT and ATG7-/- clones. The data are represented as mean±SD for technical replicates (N of 2) and are representative of 2 individual experiments. Statistical analysis: 2-way ANOVA. **** p≤0.0001

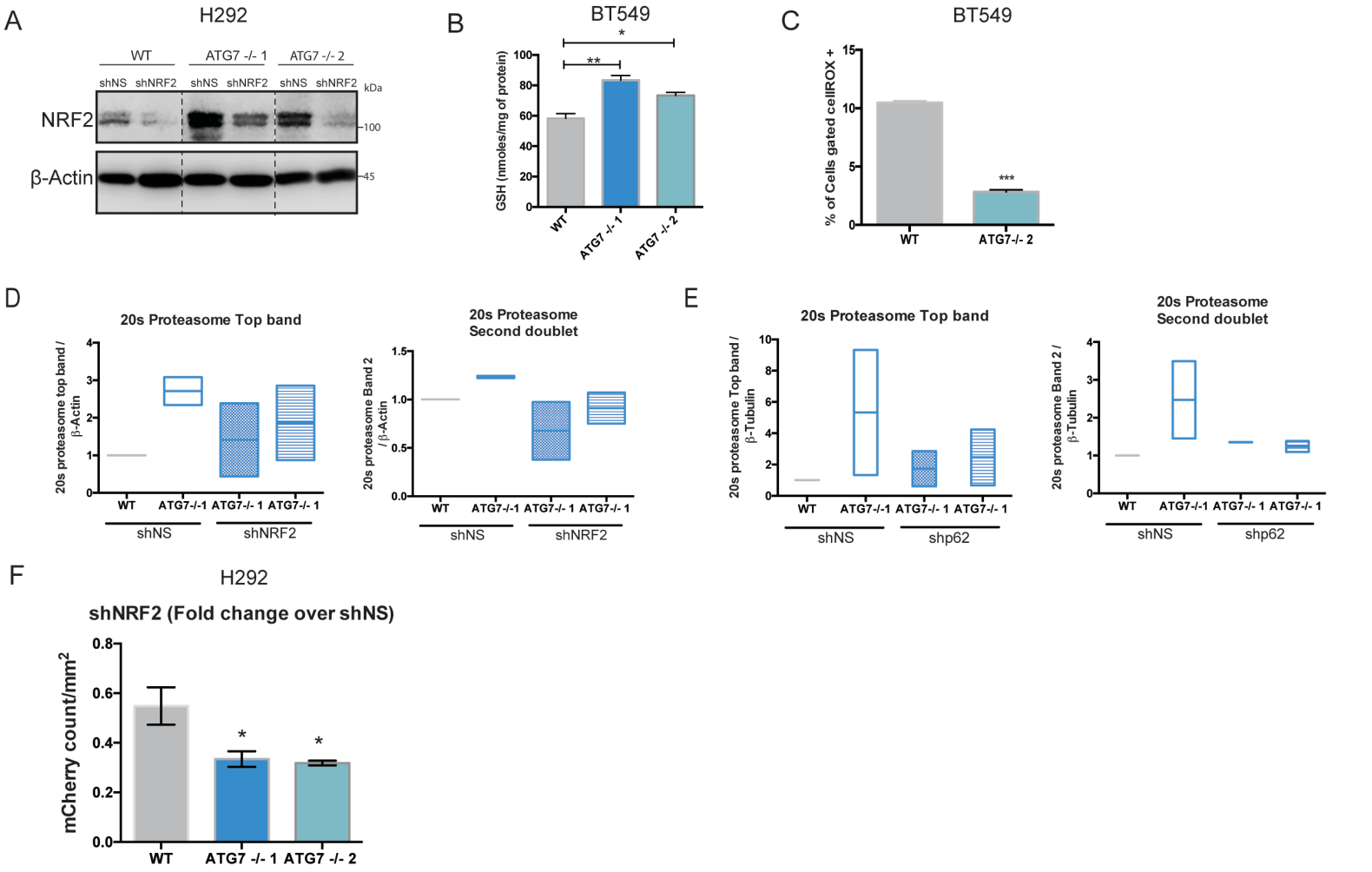

Figure S7, Related to Figures 6 and 7: ATG7-/- clones have an increased dependency on NRF2. (A)Western blot analysis in H292 WT and ATG7-/- clones with KD of NRF2. Dotted line indicates where unnecessary lanes were removed. The blots shown are representative of 2 experiments. (B-C) BT549 WT and ATG7-/-. (B) Glutathione (GSH) levels were measured with a microtiter plate assay and normalized to total protein content. The data are represented as mean±SEM of three biological replicates. Statistical analysis: 1-way ANOVA. (C) Flow cytometry analysis of cellROX green was measured to identify levels of ROS. After gating live singlets based on forward and side scatter, a cellROX+ gate was drawn such that 10% of the untreated WT cells were counted as positive and used to quantify the percent of ATG7-/- cells within that gate. The data is represented as the mean±SEM from two independent experiments. (D-E) Densitometric quantification of western blots probed for the 20s proteasome in (D) Figure 6B and (E) Figure 6D for bands indicated with an asterisk. The data shown is from 2 independent experiments. (F) KD of NRF2 induced effects on growth in H292 cells. The data are represented as mean±SEM of Incucyte mCherry+ cell count/mm2 9 days after cells were transduced with shNRF2 and normalized to shNS for technical replicates (N of 3). The data are representative of 2 individual experiments. Statistical analysis: 1-way ANOVA. *p≤0.05, *** p≤0.001