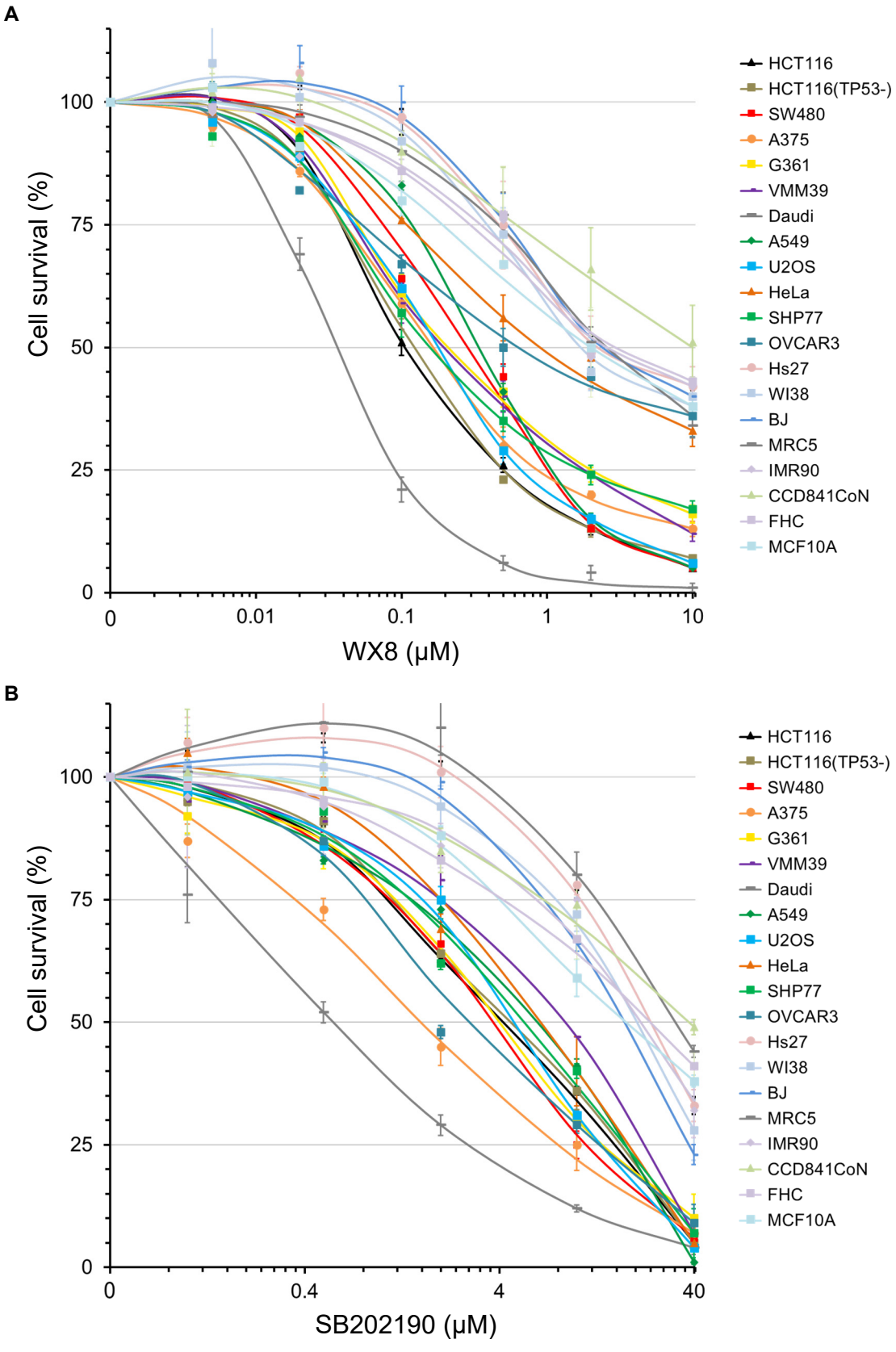
O'Connell and Vassilev. 2021. Supplementary Figure S1



Supplementary Figure S1. Sensitivity of 20 human cell cancer and normal cell lines to PIKfyve and p38MAPK inhibition.
(A) The indicated cells were cultured for 3 days in the presence of the indicated concentration of the PIKfyve inhibitor WX8.
(B) The indicated cells were cultured for 3 days in the presence of the indicated concentration of the p38MAPK inhibitor SB202190.
All cells were collected, counted and analyzed by FACS. The number of viable cells for each treatment was presented as a percentage from the vehicle treated viable cells "Cell Survival (%)". To plot logarithmic scale, the vehicle was set to 0.001 or 0.04 μM instead of 0 μM. Values are mean ±SEM (n = 3).

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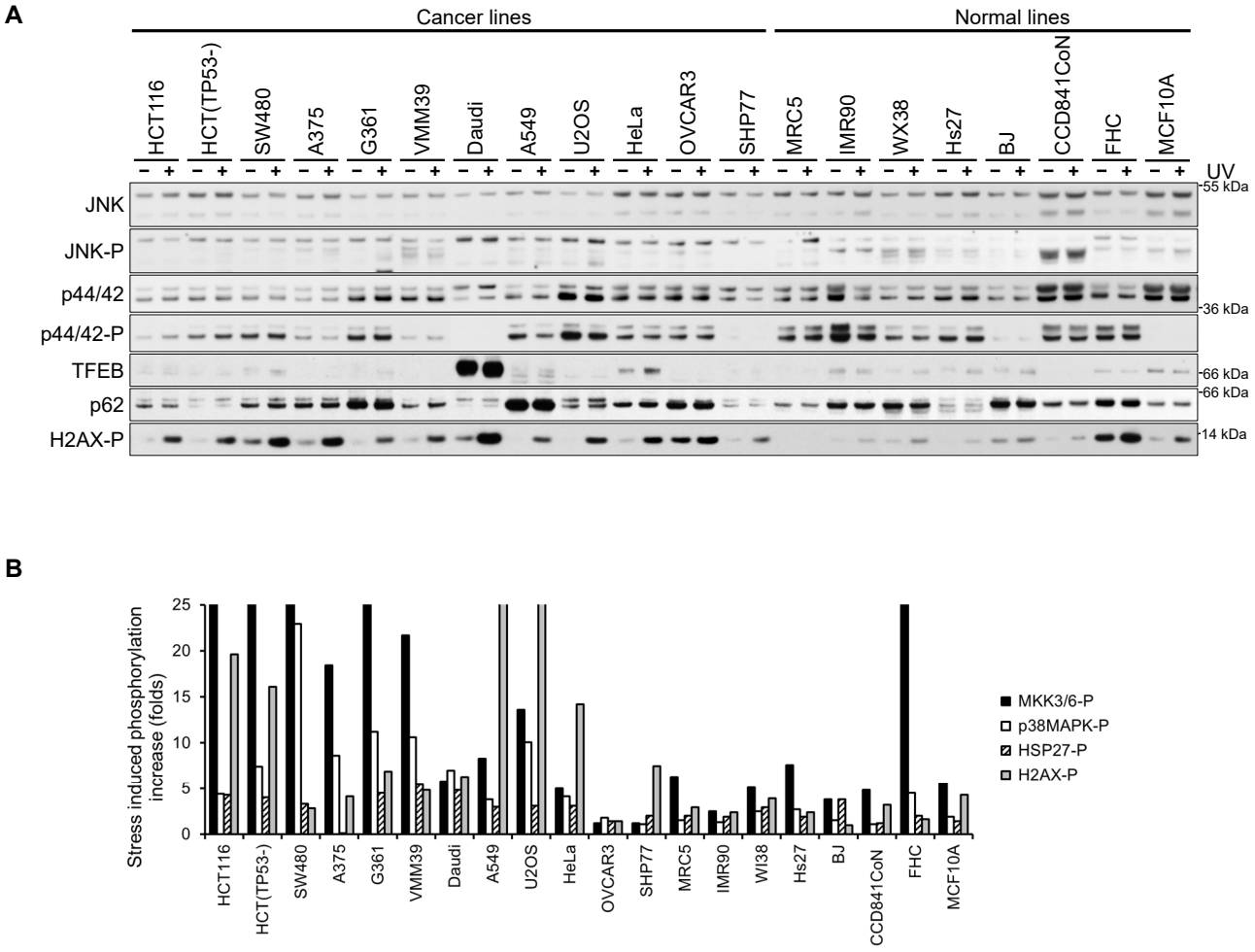

Supplementary Figure S2. The protein and phosphorylation levels of JNK and p44/42 (Erk1/2) mitogen-activated protein kinases and TFEB and p62 protein levels do not correlate with sensitivity to PIKfyve inhibition.
(A) Immunoblot analysis of JNK, p44/42 (Erk1/2), TFEB, p62 and H2AX proteins amount and/or phosphorylation in exponentially growing and UV-irradiated cells from 20 human cell lines.
(B) The p38MAPK stress response is active in all tested cell lines. Quantification of the relative increase of UV-induced phosphorylation of p38MAPK, HSP27, MKK3/6 and H2AX in 20 human cell lines.

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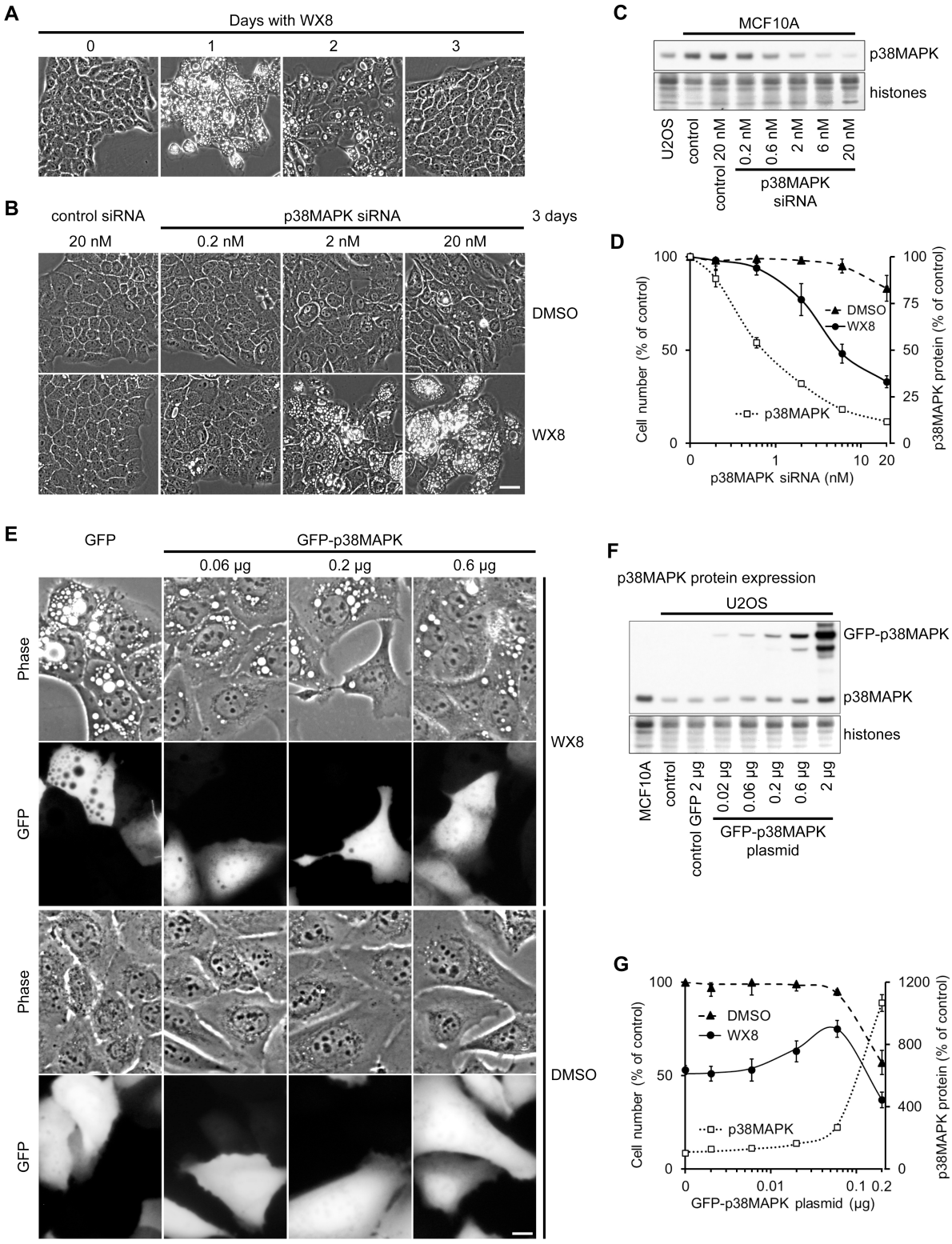

Supplementary Figure S3. The cellular p38MAPK protein levels directly modulate the effects of PIKfyve inhibition
(A) MCF10A cells were incubated with 0.5 µM WX8 and imaged for 3 days. (B) p38MAPK was depleted in MCF10A cells with the indicated concentration of p38MAPK siRNA or control siRNA and incubated with 0.5 µM WX8 or DMSO. Cells were imaged and collected after 3 days, and the counts plotted as a percentage of the control siRNA-treated cells (D). The amount of p38MAPK protein determined by immunoblot (C), quantified and the results presented as a percentage of the vehicle-treated cells. Values are mean ±SEM (n = 3). Scale bar: 20 µm.
(E) GFP-p38MAPK fusion protein and/or GFP was expressed transiently in U2OS cells by transfection with 2 µg plasmid. One day later, the media was replaced with fresh containing 0.5 µM WX8 or DMSO. Cells were imaged after 3 days using fluorescent microscopy, collected and the counts plotted as a percentage of vehicle-treated GFP transfected cells (G). The amount of p38MAPK protein was determined by immunoblot (F), quantified and the results presented as a percentage of the vehicle-treated GFP transfected cells. Values are mean ±SEM (n = 3). Scale bar: 10 µm.
Samples of U2OS and MCF10A cells were included in the immunoblots to compare protein levels between cell lines.

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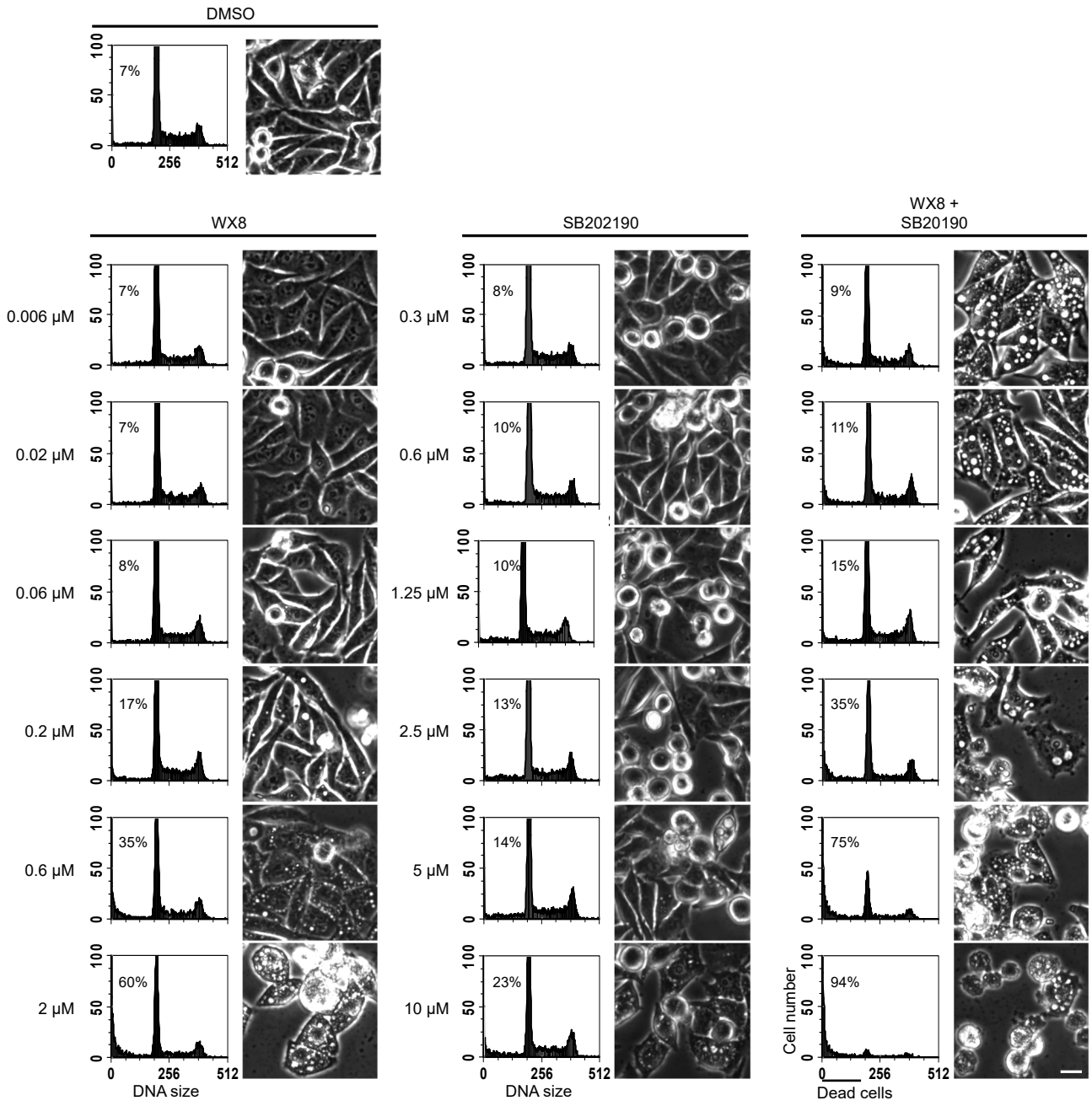

Supplementary Figure S4. p38MAPK and PIKfyve inhibitors synergistically and selectively reduce cancer cell viability
(A) WX8 and SB202190 dosage matrix was performed with SW480 cells cultured for three days with the indicated drug concentrations. All cells were phase contrast imaged, collected, counted and analyzed by FACS. Selected FACS generated DNA histograms include the percentage of dead cells.

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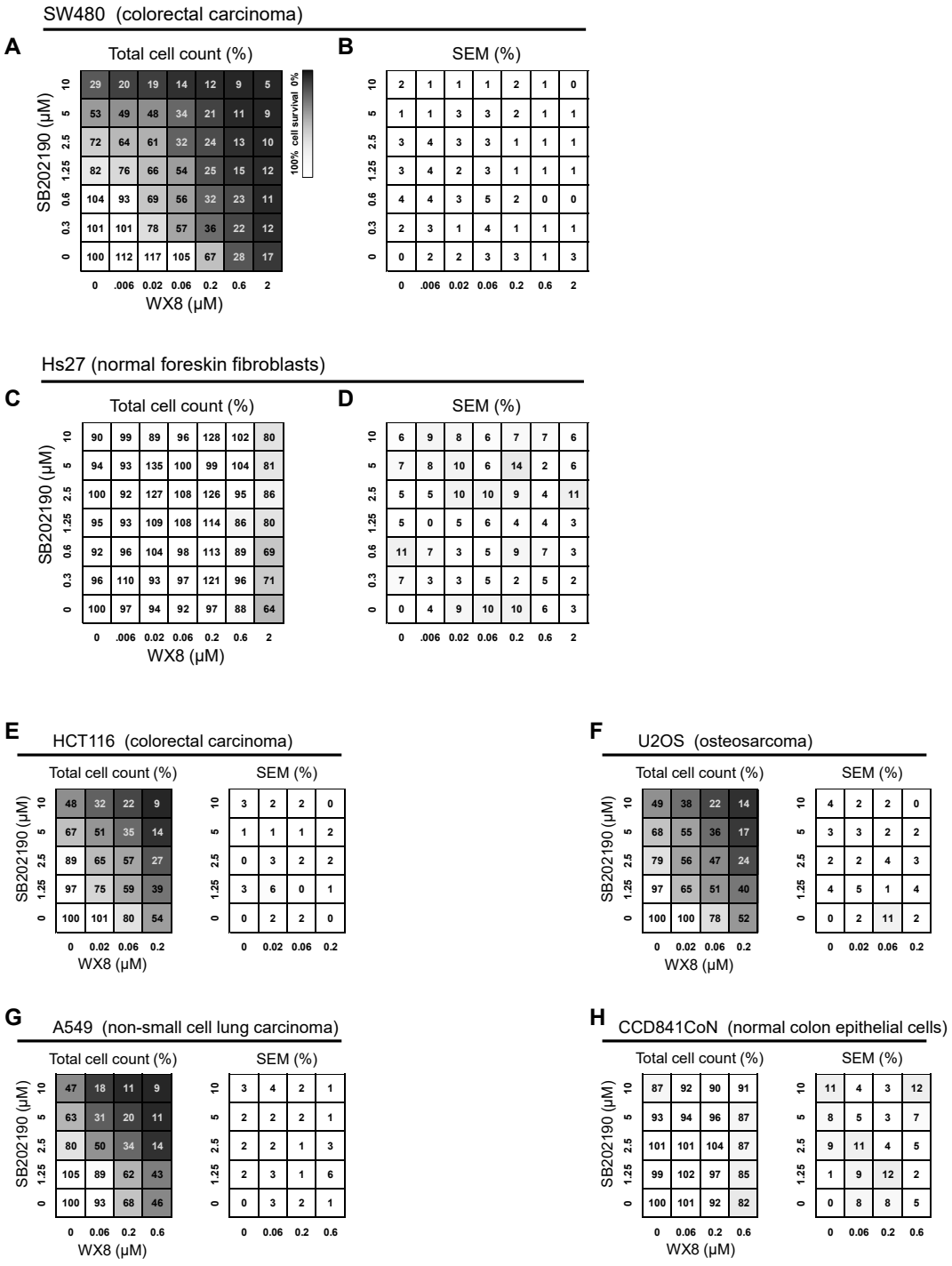

Supplementary Figure S5. p38MAPK and PIKfyve inhibitors synergistically and selectively reduce cancer cell viability
WX8 and SB202190 dosage matrix was performed with the indicated cell lines cultured for 3 days with the indicated drug concentrations. All cells were collected, counted and analyzed by FACS. Total cell count and SEM for each treatment is indicated.

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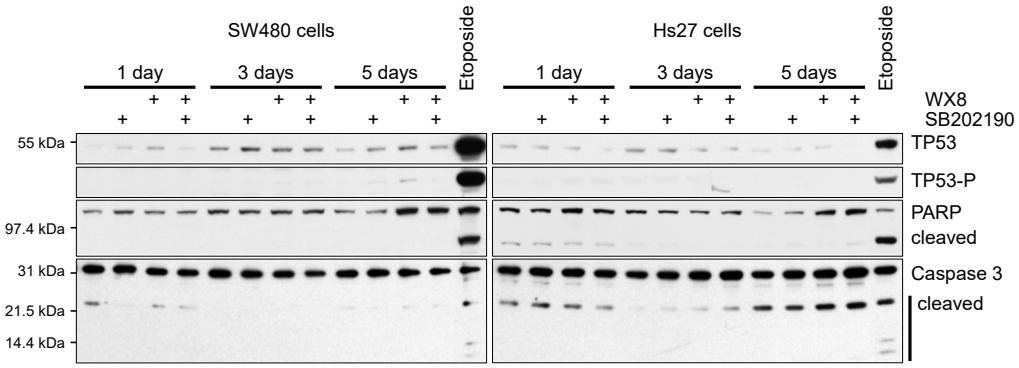

Supplementary Figure S6. Inhibition of autophagy induces cell death without activation of the p53 stress response or activation of the canonical apoptotic pathway.
(A) SW480 and Hs27 cells were cultured with either 0.125 μM WX8 or 5 μM SB202190 or both together, for the times indicated. Caspase 3 and PARP full length and cleaved proteins and p53 protein and phosphorylation at Ser15 levels were determined by immunoblot. A sample of cells treated for 1 day with 2 μM Etoposide was included as a positive control of p53 stress response and apoptosis activation.

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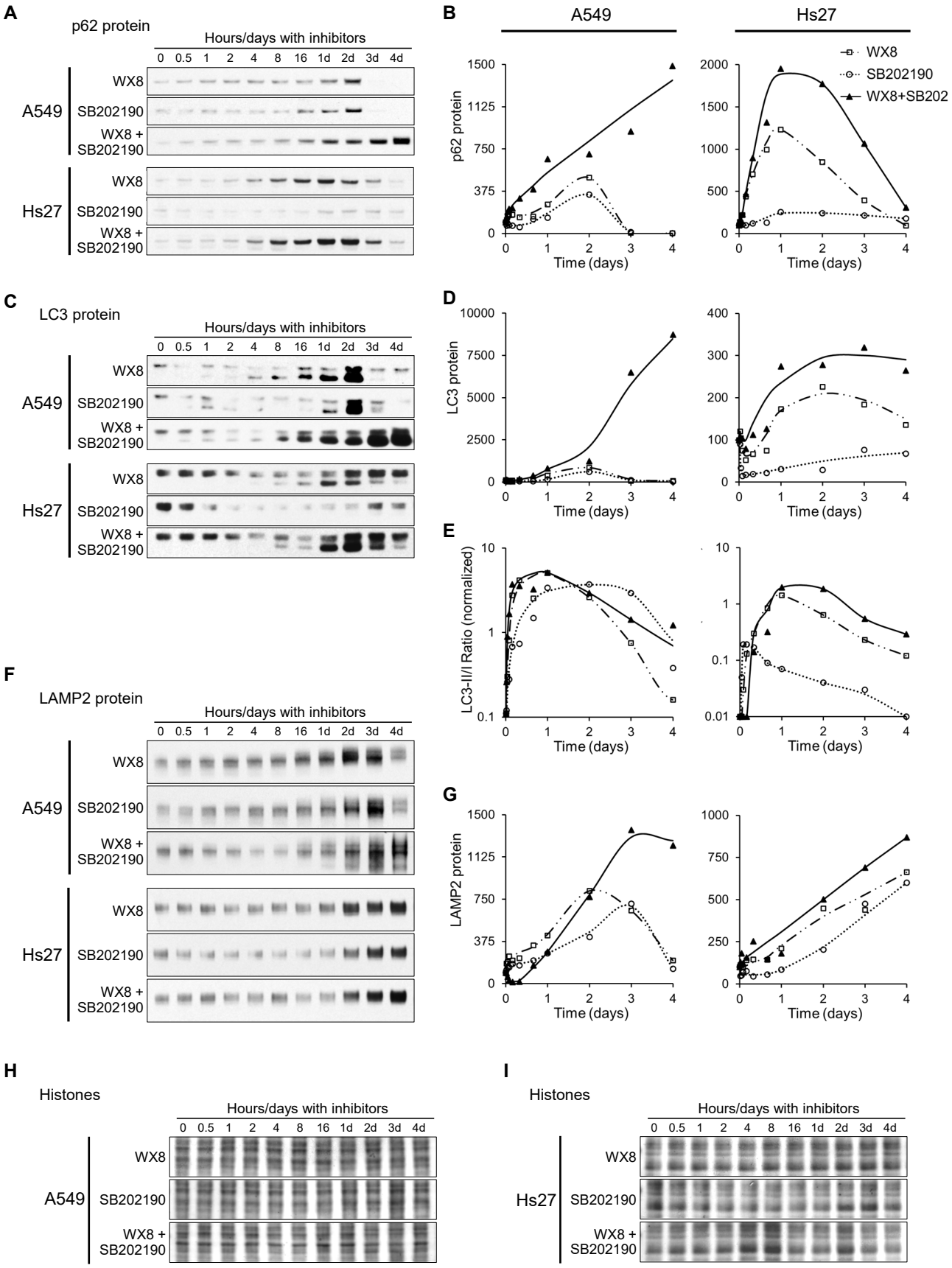

Supplementary Figure S7. p38MAPK and PIKfyve inhibitors synergistically block autophagic protein degradation in all cells. A549 and Hs27 cells were incubated with WX8 (0.375 μM) or SB202190 (5 μM) alone and in combination, and samples were collected at indicated time. The amounts of p62 (A,B), LC3 (C,D,E), LAMP2 (F,G) proteins were determined by immunoblot and plotted as a fraction of the protein present at time point 0 after normalization by histone content (H,I).

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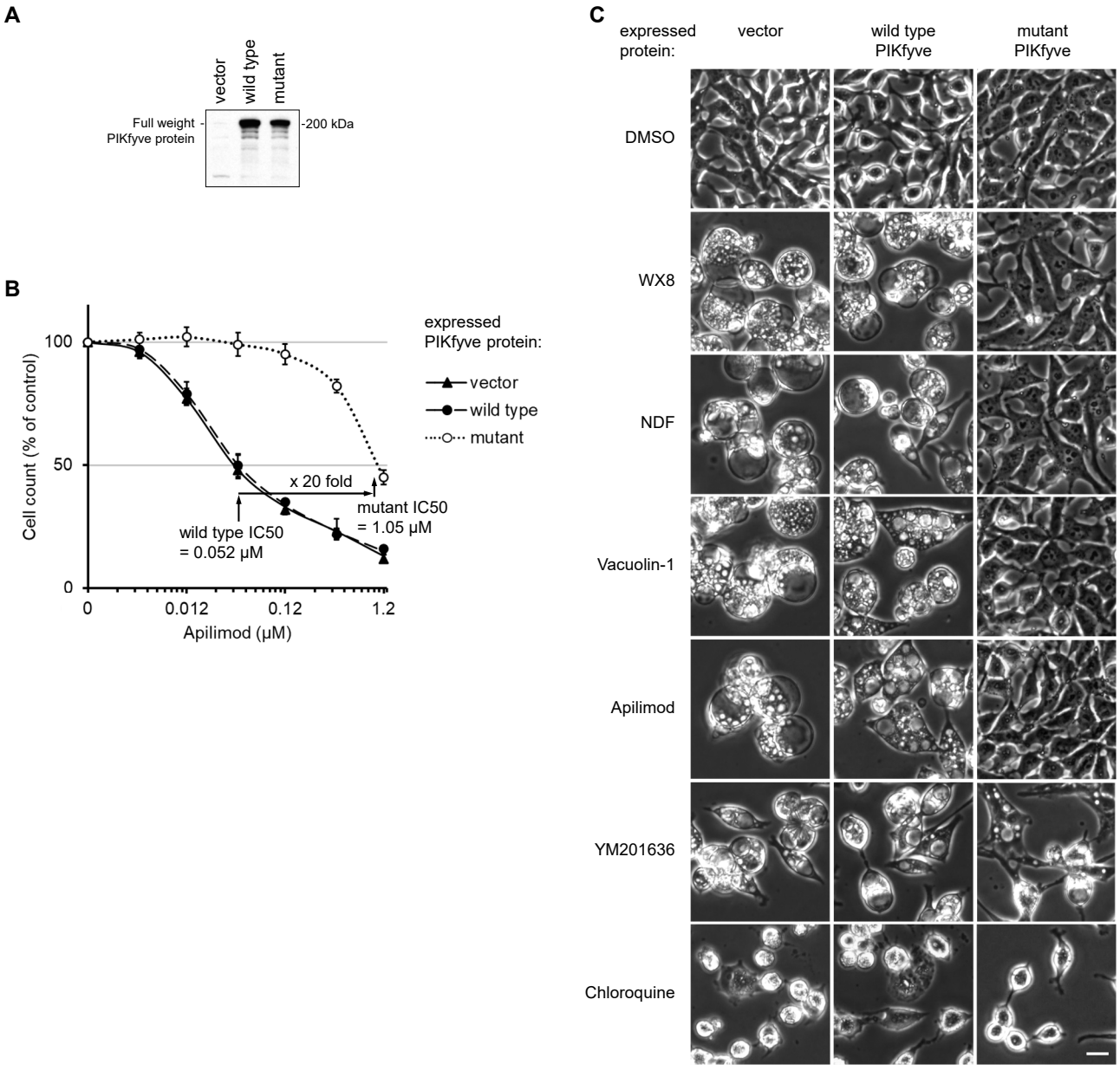

Supplementary Figure S8. WX8 is a specific inhibitor of PIKfyve.
(A) Wild-type and N1939K mutant PIKfyve proteins were stably overexpressed is A375 cells and total cells lysates analyzed by immunoblot to determine the amount of PIKfyve protein.
(B) 6-point Apilimod concentration titration was performed with vector, PIKfyve wild-type and N1939K mutant stably expressing A375 cells. Values are mean ±SD (n = 3).
(C) Cells were incubated for 3 days with IC75 concentrations of inhibitors (3.75 μM WX8, 6 μM NDF, 5 μM Vacuolin-1, 0.3 μM Apilimod, 12.5 μM YM201636, 30 μM chloroquine), counted and imaged (C). Scale bar: 20 μm.