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Supplemental Information

Selective elimination of pluripotent stem cells by PIKfyve specific inhibitors

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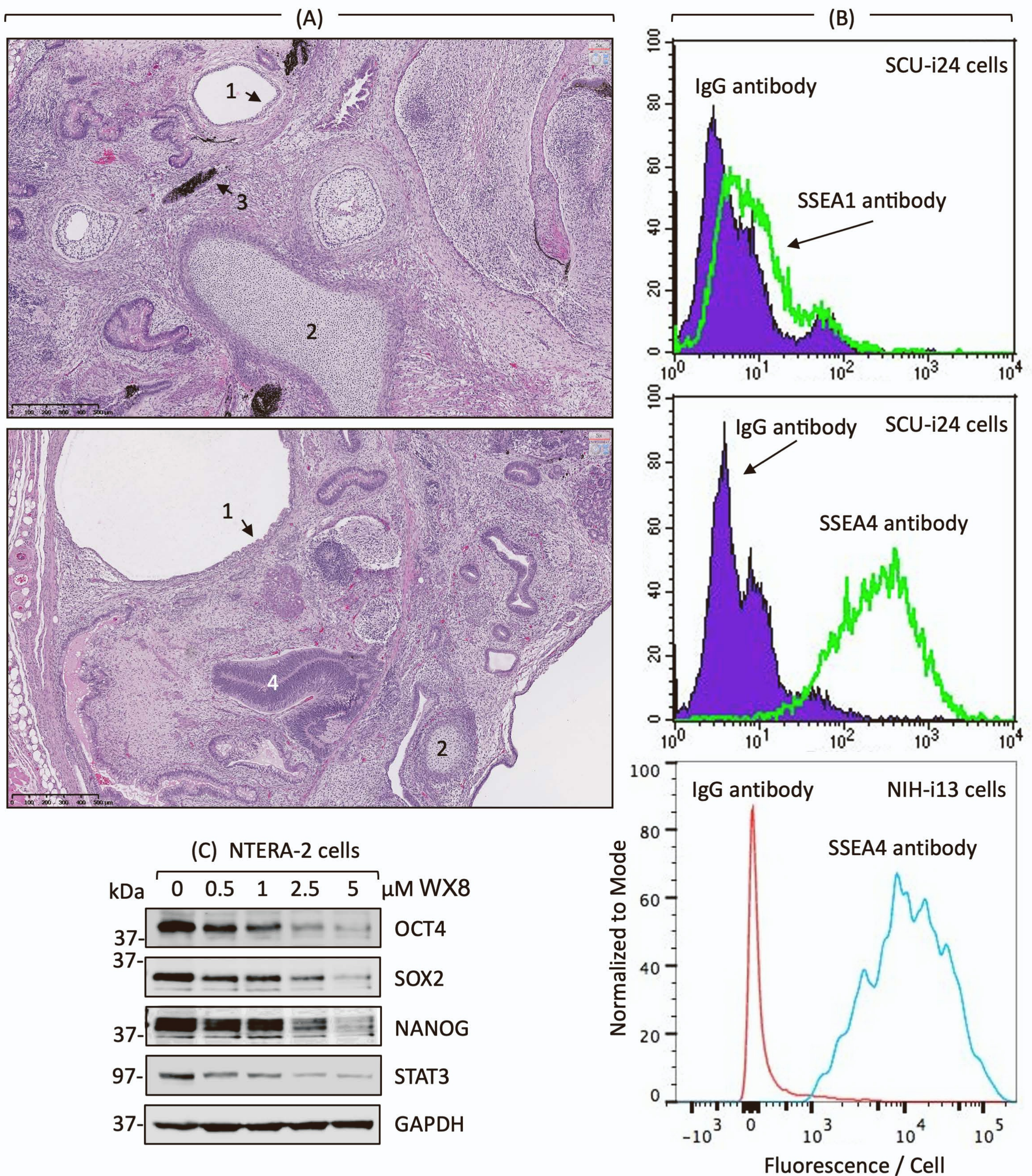


Figure S1. ESCs, iPSCs and ECCs were pluripotent. (A) Tumors were harvested 12 weeks after subcutaneous inoculation of 5 million SCU-i24 cells into nude mice. Histology identified them as well-differentiated teratomas containing (1) Epithelial Cells (endoderm), (2) cartilage (mesoderm), (3) pigment cells and (4) neural rosette-like structures (ectoderm). Magnification is 5X. **(B)** The cell surface human pluripotency marker SSEA-4 was detected by FACS analysis of SCU-i24 and NIH-i13 cells stained with either PE-conjugated anti-SSEA-4 antibody, FITC-conjugated anti-SSEA-1 antibody, or isotype-matched normal IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Mouse specific pluripotency marker SSEA1 was used as negative control. **(C)** NTERA-2 cells were cultured in the presence of the indicated concentrations of WX8 for 4 days. Total cell extracts were then immunoblotted to detect the indicated proteins.

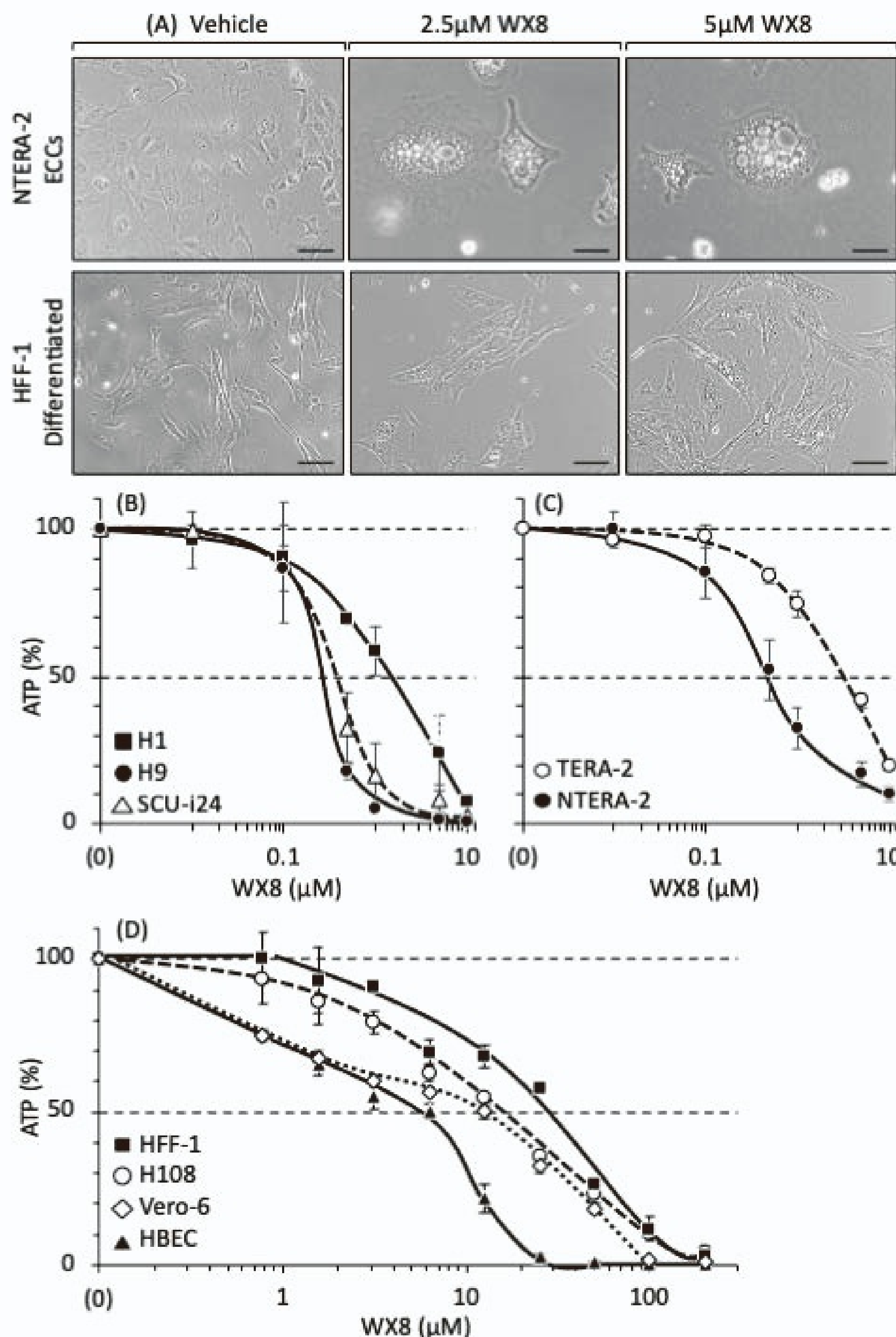


Figure S2. WX8 inhibited viability selectively in human pluripotent stem cells. (**A**) Cytoplasmic vacuolation in NTERA-2 ECCs and HFF-1 fibroblasts cultured for 24 hours with 2.5µM WX8. Scale bars are 10µm. (**B-D**) Human ESCs, iPSCs, ECCs and differentiated cells were seeded in 96-well plates (6,200 cells/cm^2) and cultured for 4 days in the presence of the indicated concentration of WX8 and then their viability was quantified as the fraction of ATP present relative to cells cultured in the presence of vehicle. ATP levels were quantified using the CellTiter-Glo Luminescent Cell Viability Assay kit. (**B**) ESCs H1 (■), H9 (●) and iPSCs NIH-i13 (◊), SCU-i24 (△). (**C**) ECCs TERA-2 (○), NTERA-2 (●). (**D**) Fibroblasts HFF-1 (■), H108 (○), Vero-6(◊), HBEC(▲). Error bars indicate SEM for 3 independent assays. To generate a logarithmic scale, time '0 hours' was plotted as '0.001 hours' in panels B and C, and 0.1 hours in panel D. IC_{50} values are in Table 1.

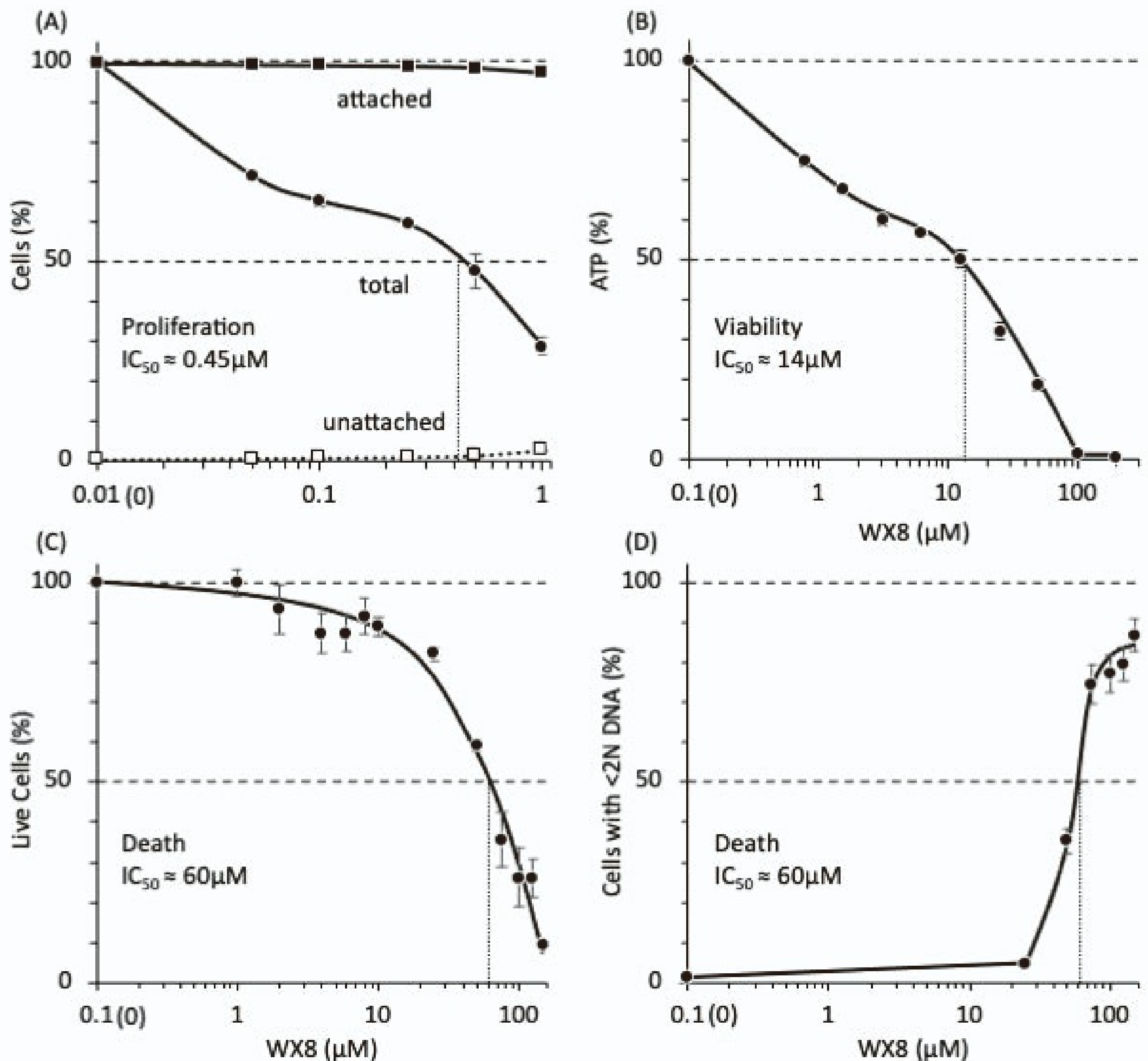


Figure S3. WX8 inhibited proliferation of differentiated cells under conditions that did not induce cell death. Vero-6 kidney cells were cultured with the indicated concentration of WX8 for 96 hours. Control cells were cultured in the absence of WX8, but zero was plotted as a real number on logarithmic scales. Results are given as a percent of the control cells cultured in the absence of WX8. (A) Cell proliferation was quantified as the fraction of attached cells (■), unattached cells (□), and total cells (●, attached + unattached). (B) Cell viability was quantified as the fraction of cellular ATP. (C) Live cells were quantified as the fraction of cells not stained with either annexin-V or propidium iodide, as determined by FACS. (D) The fraction of cells containing less than 2N DNA (less than G1 or G0 cells) were quantified by staining permeabilized cells with propidium iodide and then measuring cellular DNA content by FACS. The IC_{50} value is the concentration of WX8 that inhibited 50% of the cells. SEM is for 3 independent assays.

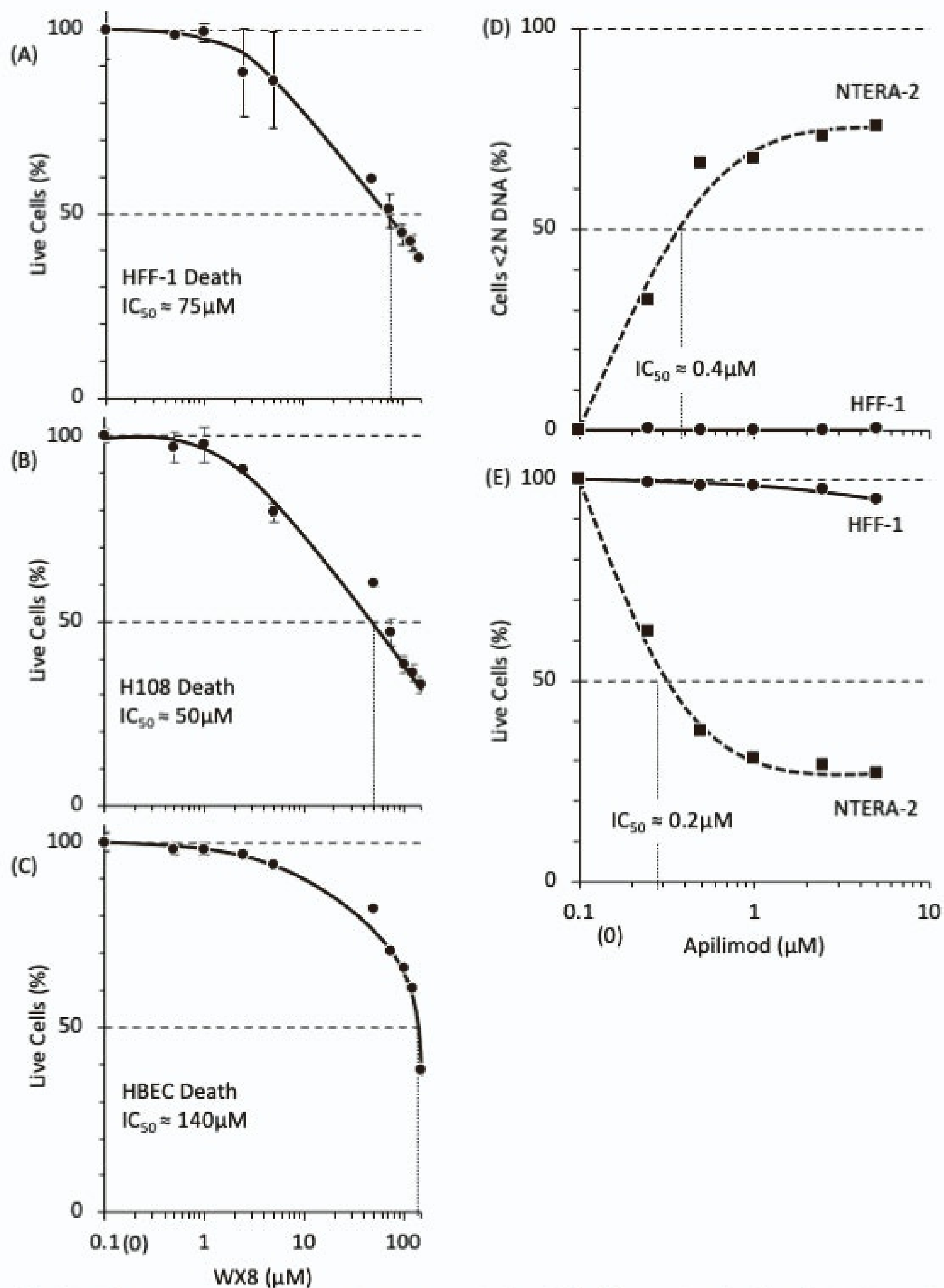


Figure S4. (A-C) High concentrations of WX8 were required to kill differentiated cells. The indicated cells were cultured for 96 hours with either WX8 or vehicle (0µM WX8). '0µM WX8' was plotted as 0.1µM for logarithmic scales. The fraction of live cells is the fraction of cells not stained with either annexin-V or propidium iodide relative to the control cells. **(D, E) Apilimod induced programmed cell death in NTERA-2 ECCs under conditions where the viability of HFF-1 fibroblasts was not affected.** Cells were cultured with Apilimod for 96 hours. (**D**) The fraction of cells containing <2N DNA was quantified by staining permeabilized cells with propidium iodide and then measuring cellular DNA content by FACS. (**E**) Live cells were quantified as panels A-C. SEM is indicated for 3 independent assays.

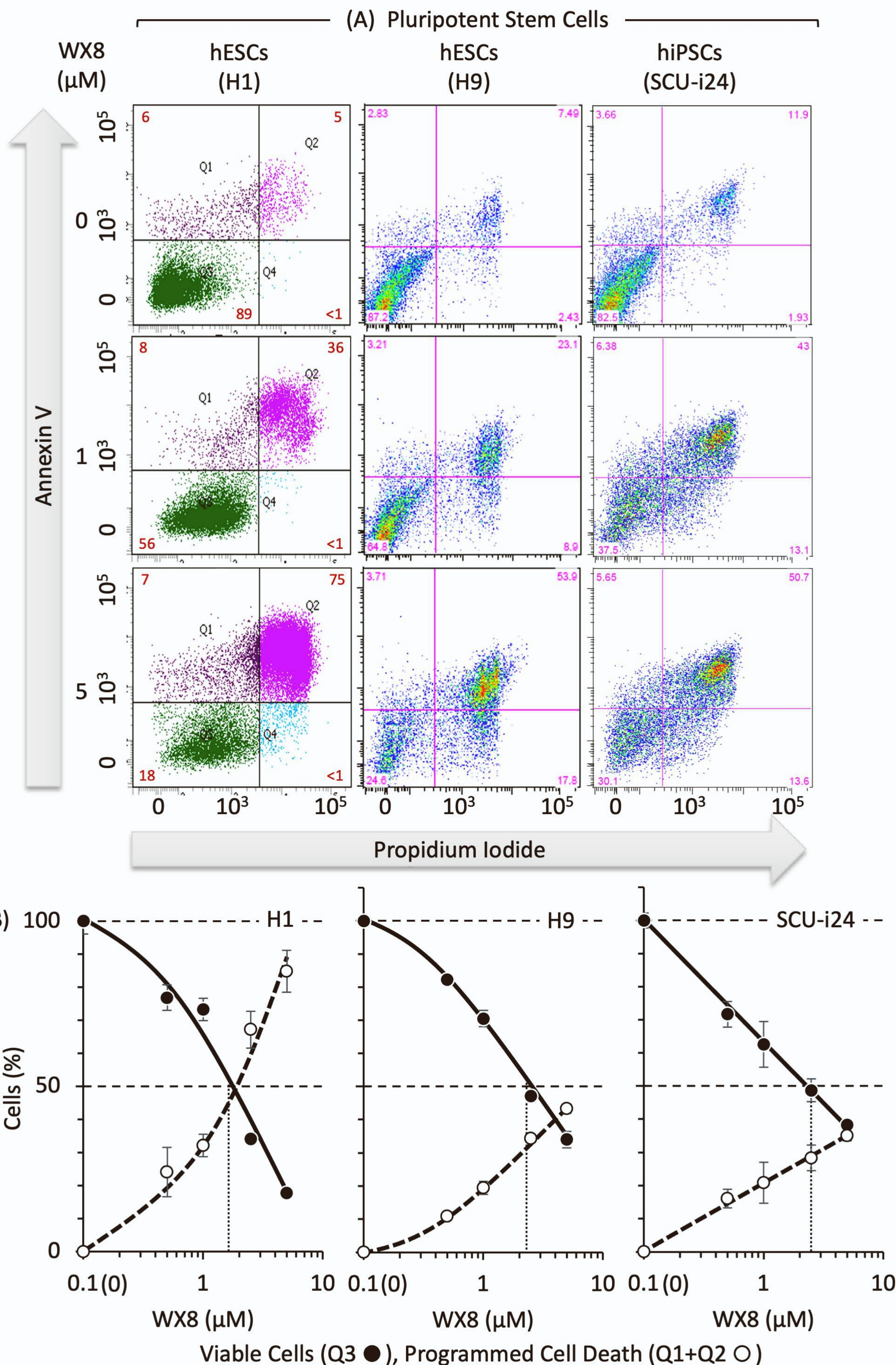
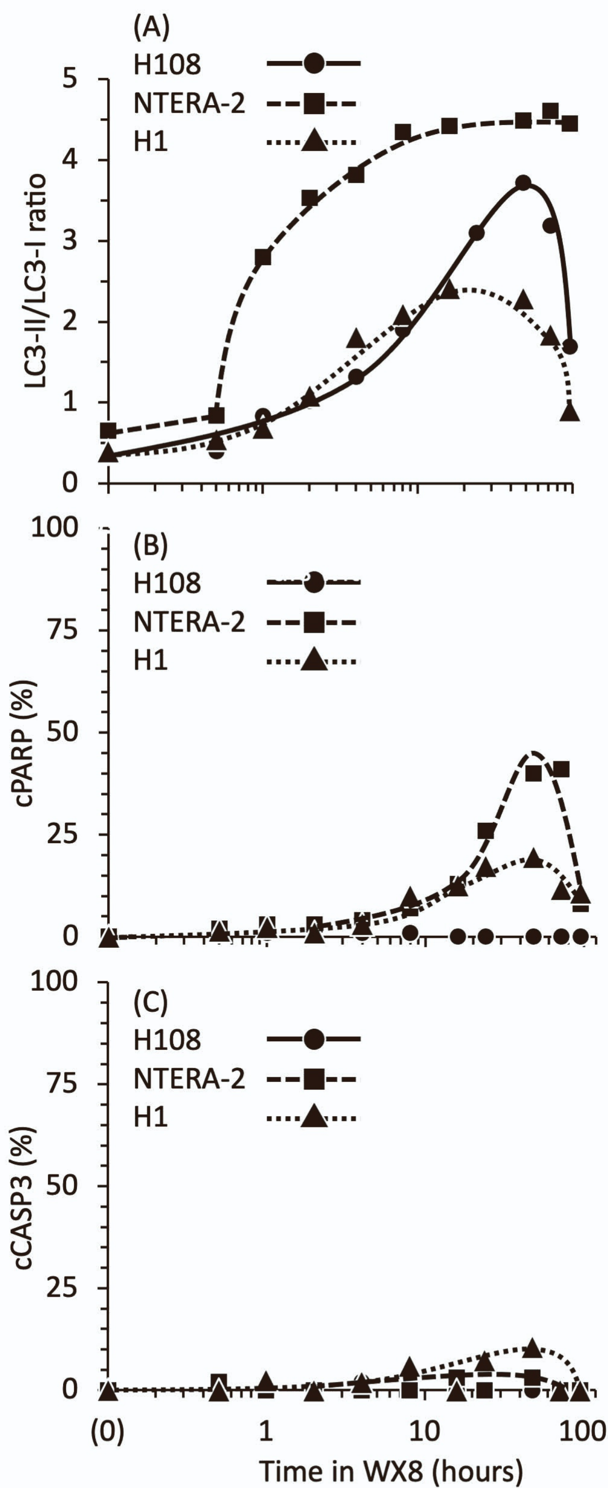
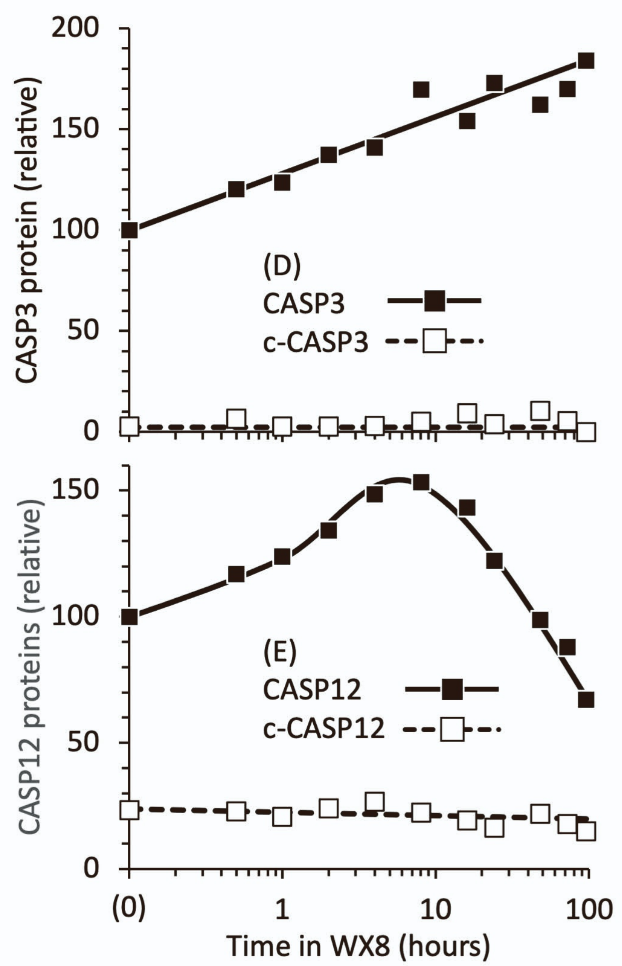
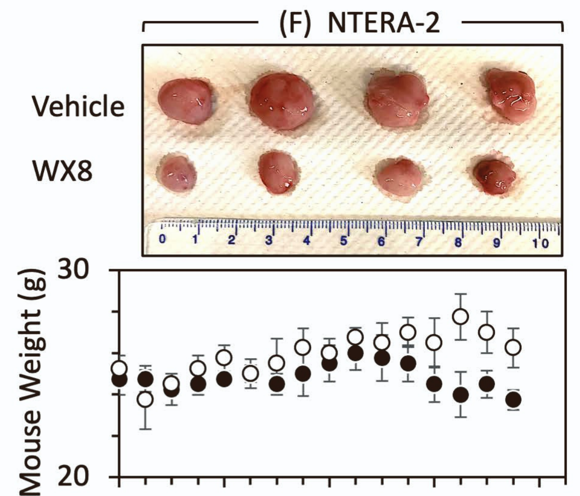


Figure S5. WX8 induced programmed cell death selectively in pluripotent stem cells. (**A**) H1 and H9 ESCs, and SCU-i24 iPSCs were cultured for 4 days in the presence of WX8, stained with annexin-V and propidium iodide (PI), and then analyzed by FACS as in figure 4. Q1 contained cells stained only with annexin-V. Q2 contained cells stained with both annexin-V and PI. Q3 contained unstained viable cells. Q4 contained cells stained only with PI. (**B**) Cells cultured in the presence of vehicle (0μM WX8) were defined as 100% viable (Q3) and 0% dead (Q1+Q2) so that only changes due to WX8 were represented. SEM for 3 independent assays is indicated. ‘0 WX8’ was plotted as ‘0.1 WX8’ for log scale.







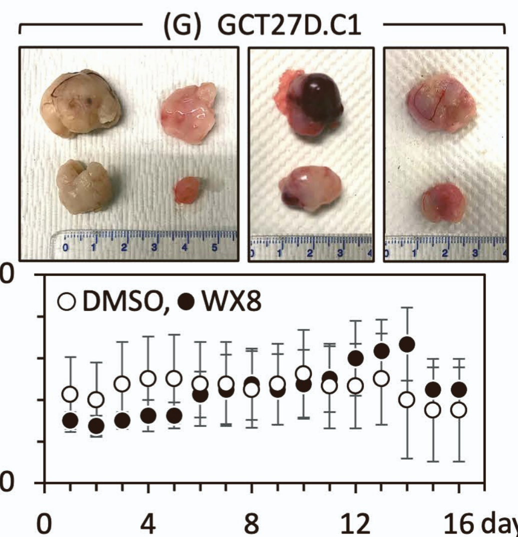


Figure S6. Time dependent changes induced by WX8 in LC3-II, PARP, CASP3 & CASP12 in WX8 sensitive (H1, NTERA-2) and insensitive (H108) cells. (**A**) total LC3-II / LC3-I ratios. (**B**) % PARP cleavage. (**C**) % CASP3 cleavage, (**D, E**) total CASP3 and CASP12 protein in NTERA-2 cells at 0 hours defined as 100 units. '0 Hours' was plotted as '0.1 hours' to generate a logarithmic scale.

(F, G) WX8 reduced tumor volumes without visible harm to the mice. Tumors were harvested after 15 days of IP treatment. Photos in panel G are the same magnification. Rulers are in cm. Mouse body weights after injection of either DMSO vehicle or 40mg WX8/kg mouse. Error bars indicate ±SEM for 6 mice.

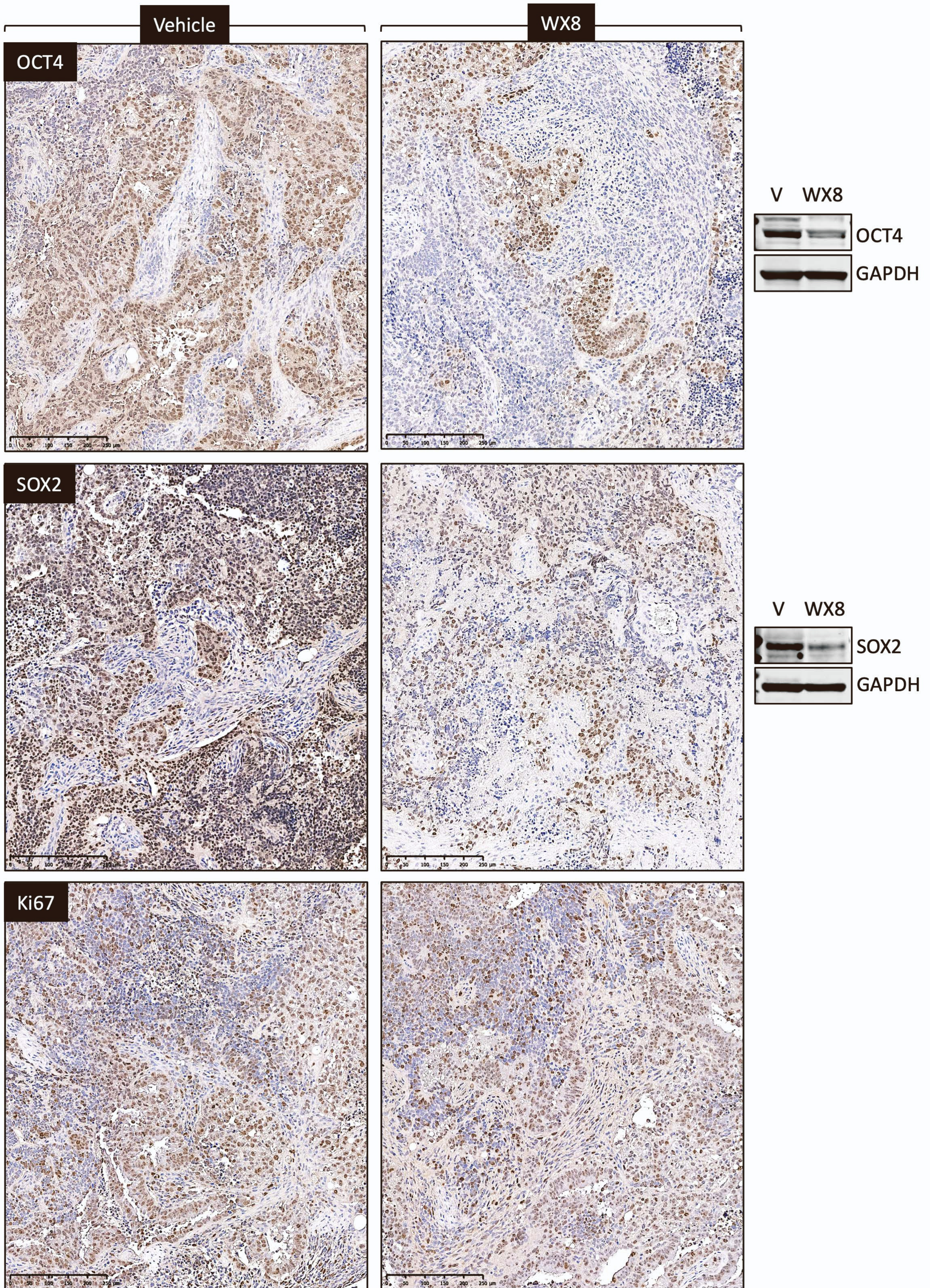


Figure S7. WX8 selectively eliminated pluripotent stem cells from ECC GCT27D.C1 derived tumors. Large field images of tumor sections stained for OCT4, SOX2 or Ki67 in figure 6.