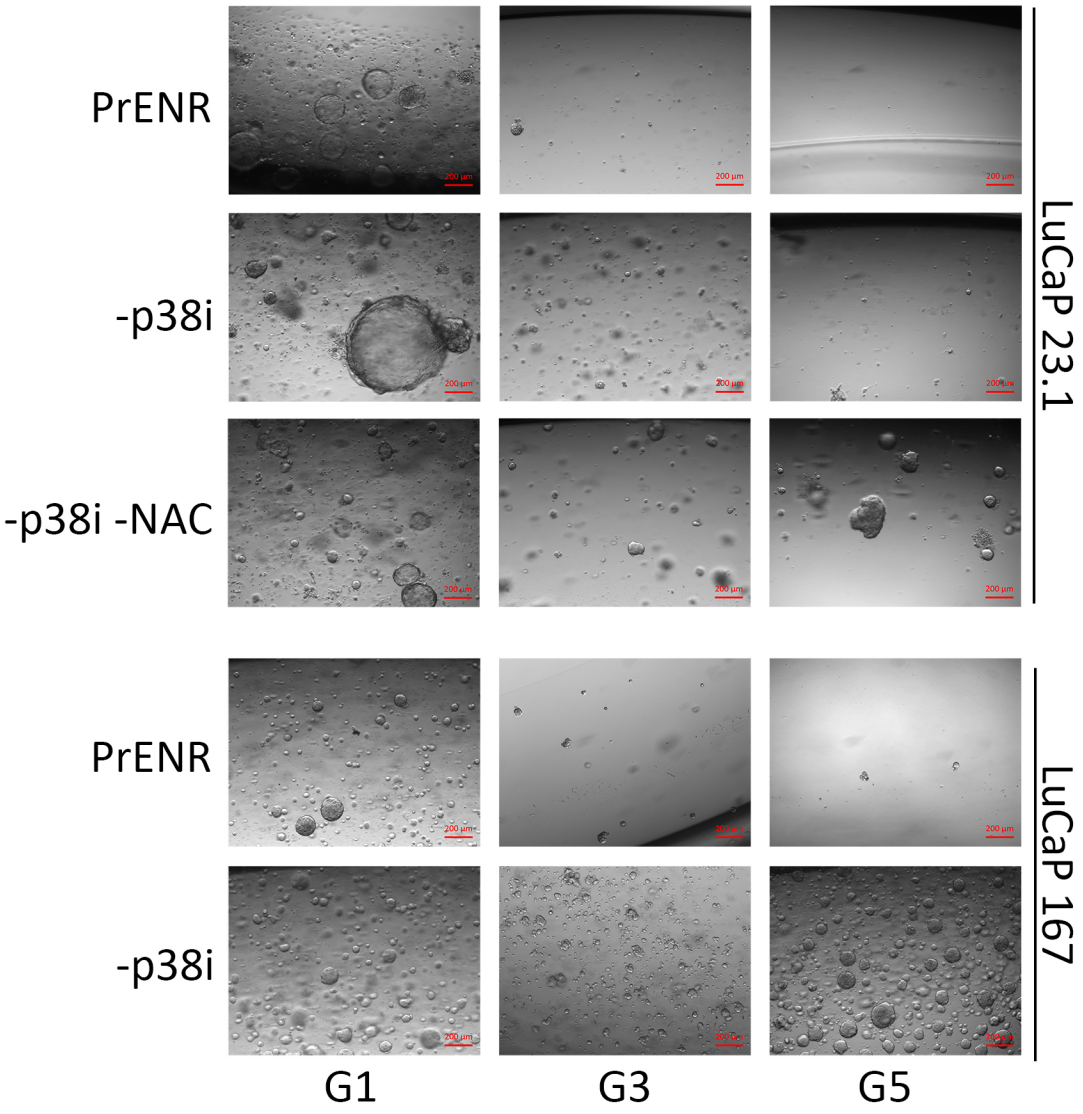
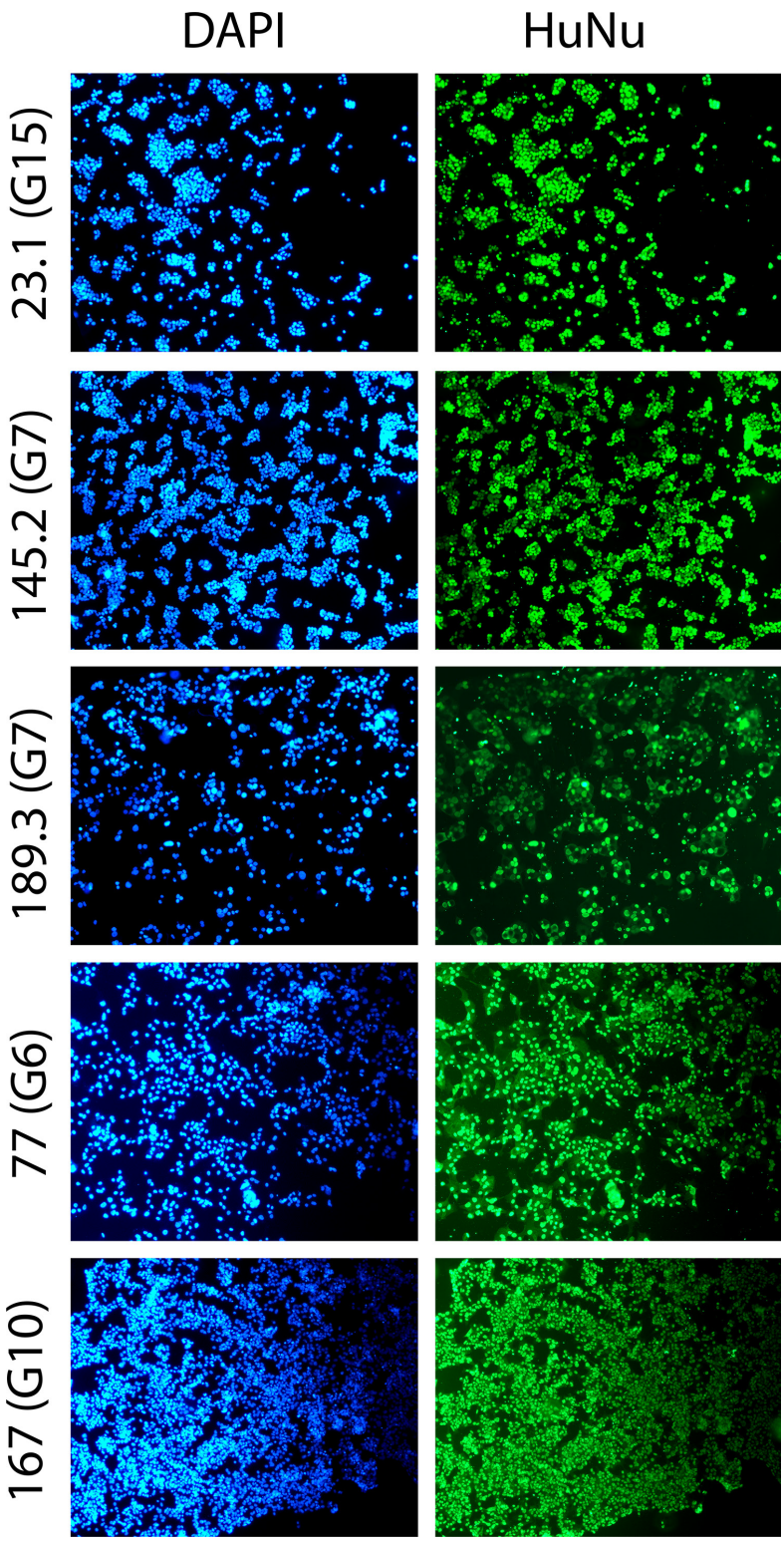


Supplementary Fig. S1.

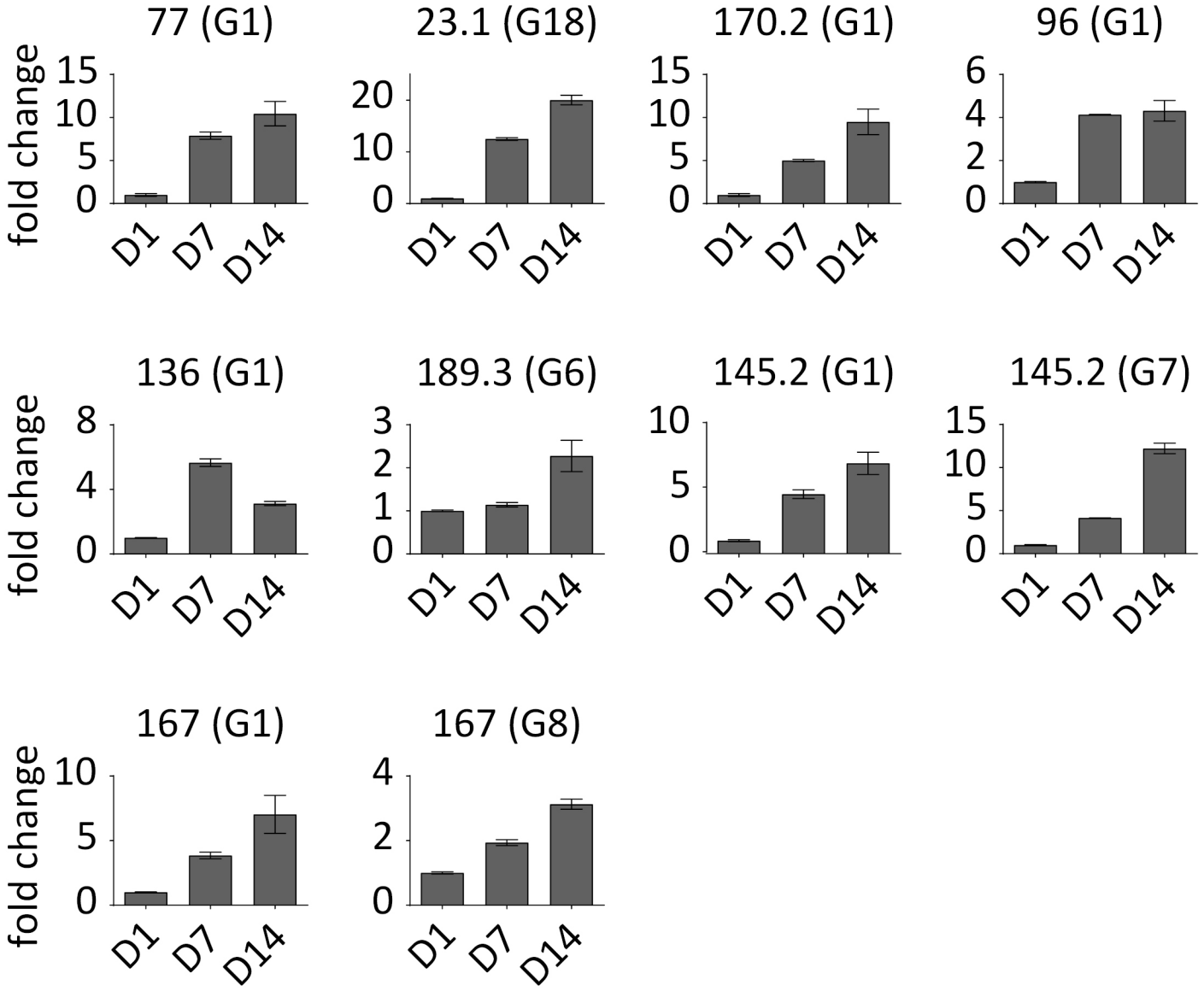
Growth/survival advantages measured in short-term assays with G1 organoids leads to a pronounced effect with additional passages. (upper) LuCaP 23.1 grown in PrENR, PrENR –p38i, and PrENR –p38i –NAC for five generations. (lower) LuCaP 167 grown in PrENR and PrENR –p38i for five generations. Bright field images taken with 5x objective at generations G1, G3 and G5. Scale bars represent 200 μM. "G" indicates generation number, with G1 indicating the first organoid culture established from PDX tumors.



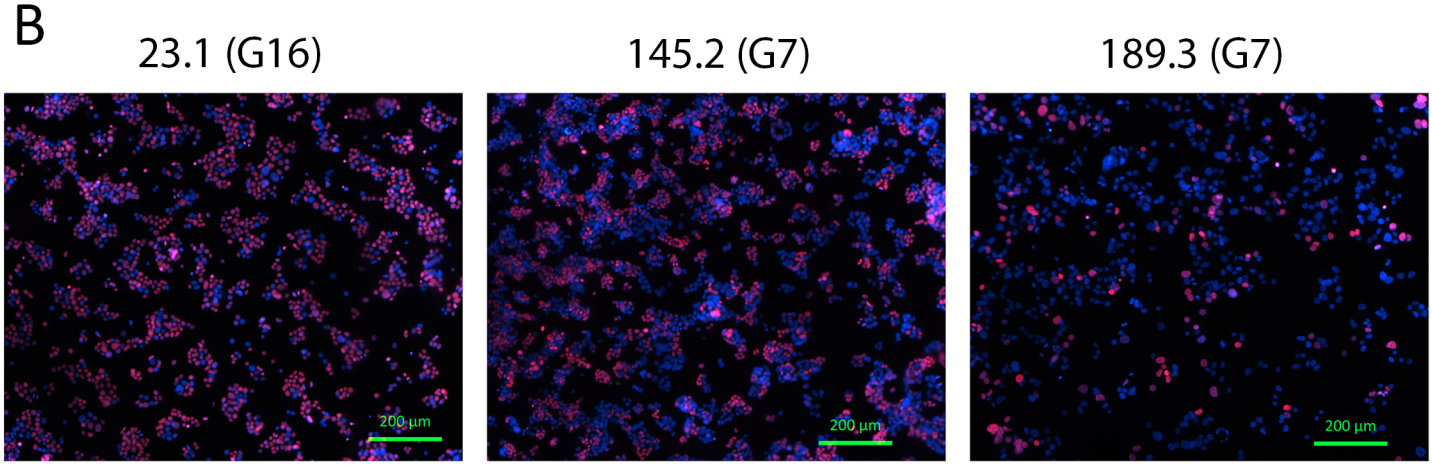
Supplementary Fig. S2.

Late generation LuCaP-derived organoids are of human origin. The indicated LuCaPs were stained for the human-specific nuclear antigen HuNu (green) and DAPI (blue). The LuCaP generation number is indicated in parentheses.

A



B



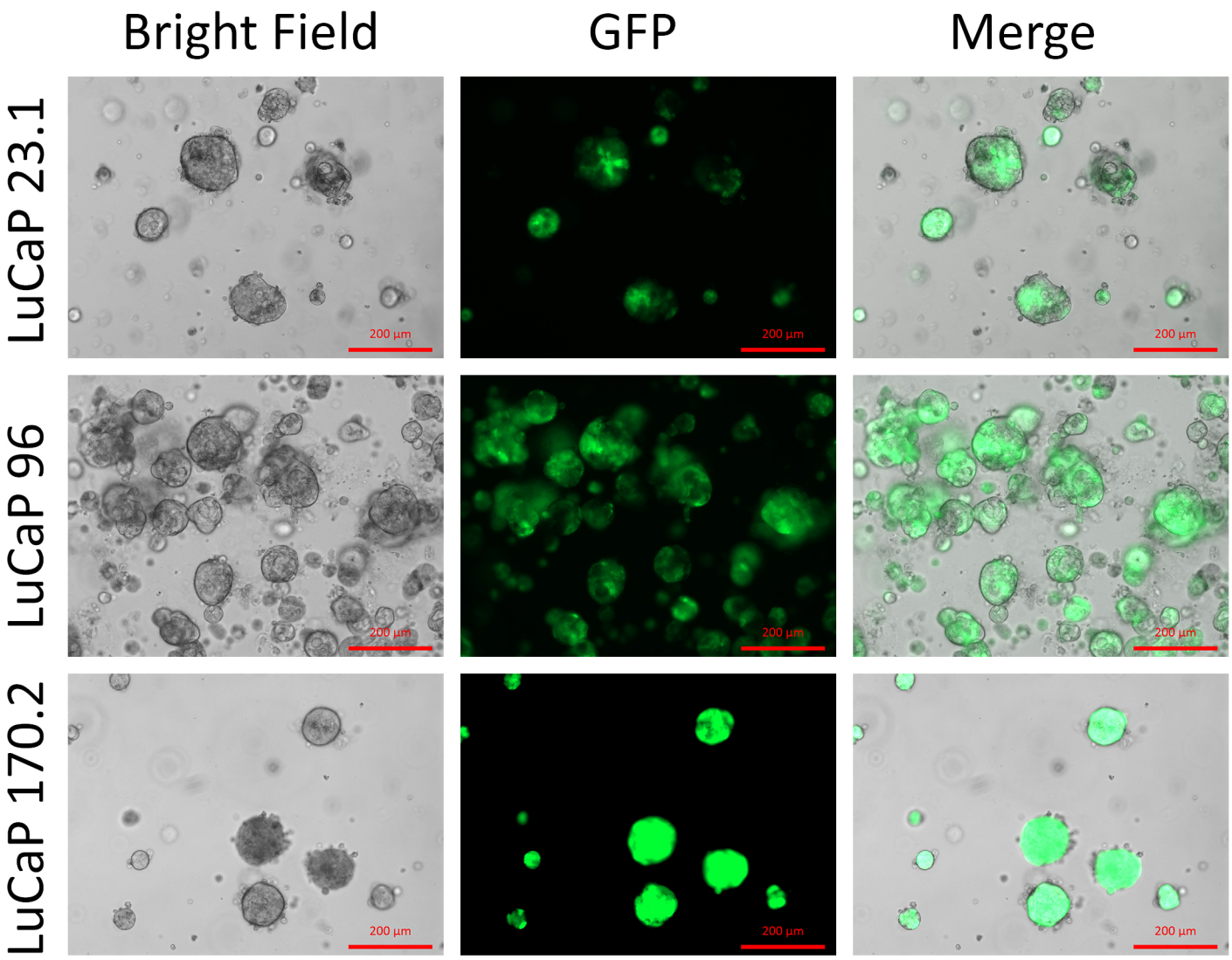
EdU positive
87% ± 0.02

EdU positive
56% ± 0.03

EdU positive
24% ± 0.02

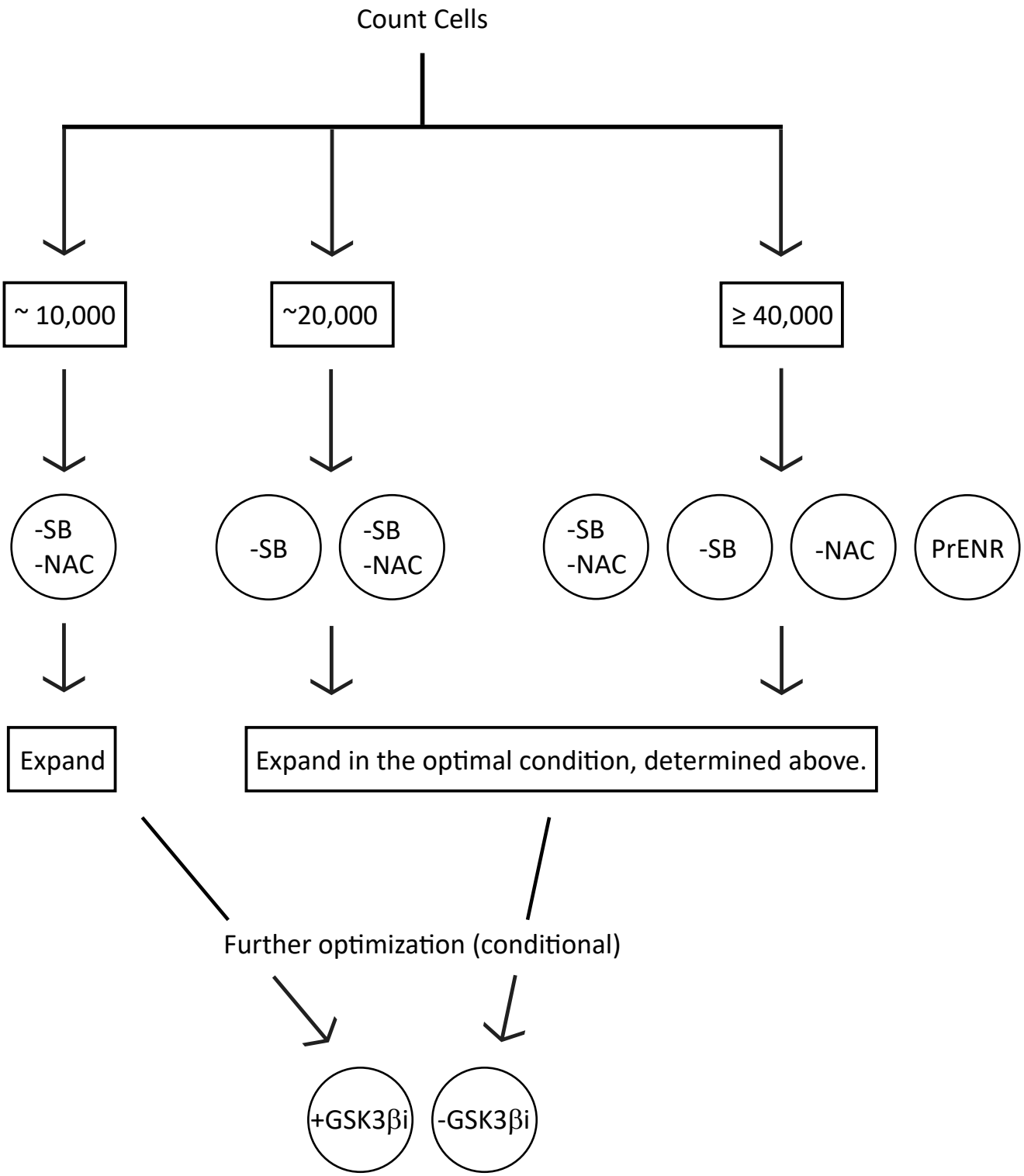
Supplementary Fig. S3.

LuCaP organoids proliferate in culture. A, Cells were plated at the indicated generation number in the previously determined optimal growth condition, and assayed by CellTiter Glo 3D at days 1, 7, and 14. Standard error of the mean of three replicates is shown. **B,** Cells were plated at the indicated generation number, incubated for 24 hours with 10 μM EdU, then stained for EdU and DAPI. EdU incorporation was quantified and is presented as percentage of total cells that are positive for EdU. Standard error of the mean of three replicates is shown.



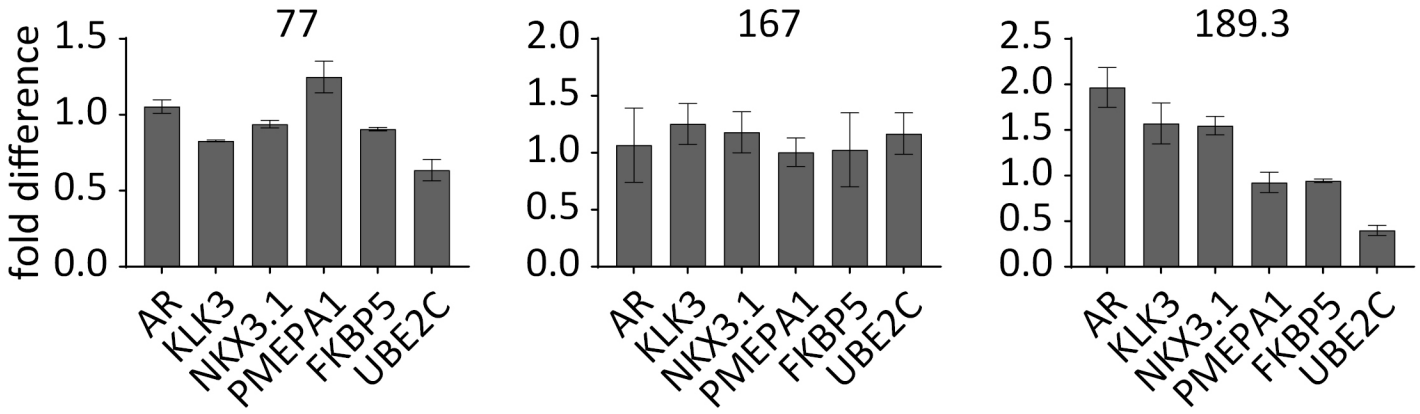
Supplementary Fig. S4.

LuCaP organoids transduced with Lenti-pLVX-GFP. Images taken three days after transduction.



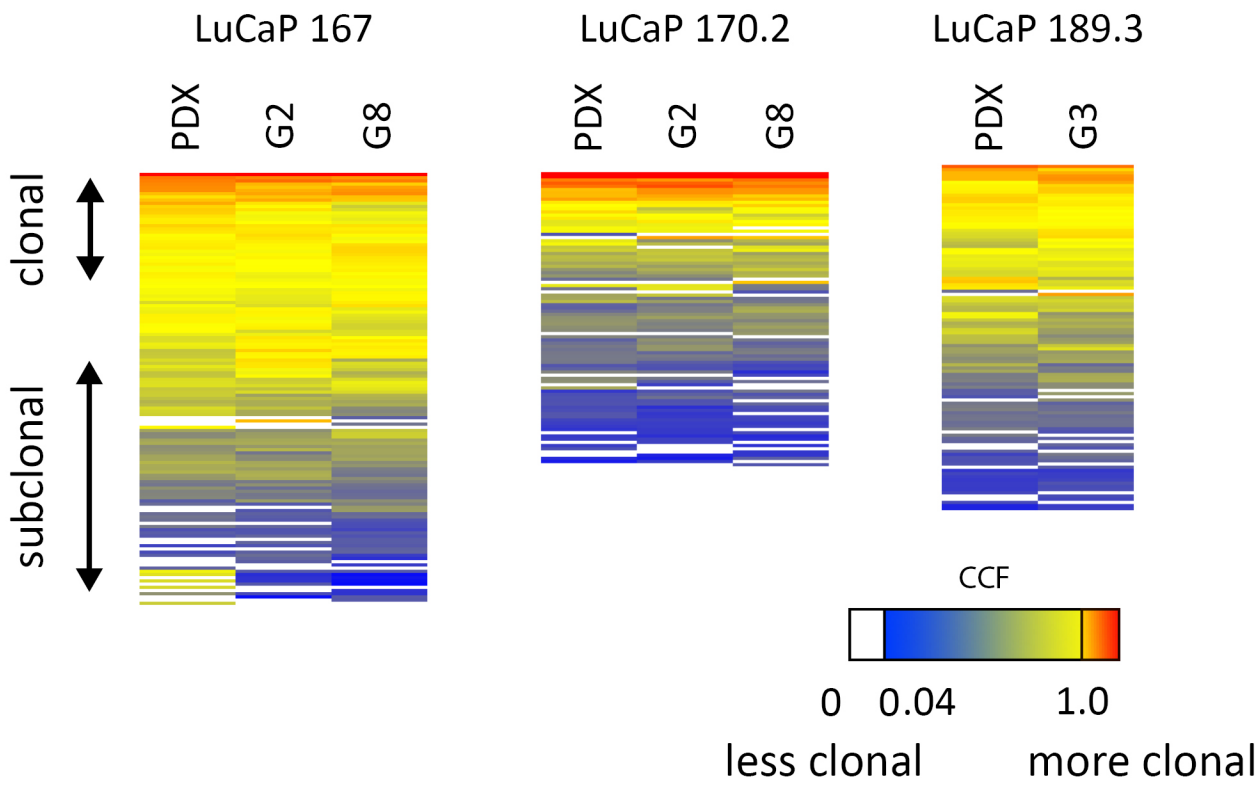
Supplementary Fig. S5.

Decision tree for selection of media conditions when initiating cultures from patient-derived needle biopsies. Cells are counted and plated on a 24-well plate. For yields ≤ 10,000 cells, a single well is plated. Yields of 15,000 -20,000 cells are plated in two wells, and yields of ≥ 40,000 cells are plated in at least four wells. When cell numbers are limiting, different media conditions are used in the following order of priority: (1) -SB -NAC; (2) -SB; (3) -NAC; (4) PrENR. When multiple conditions can be used, the optimal condition is determined by observation of growth, as well as by quantification of secreted PSA, which is usually applicable for AR+ samples. If organoids are successfully established, then they are expanded in the condition determined to be optimal. Additional optimization steps can be tested on growing cultures.



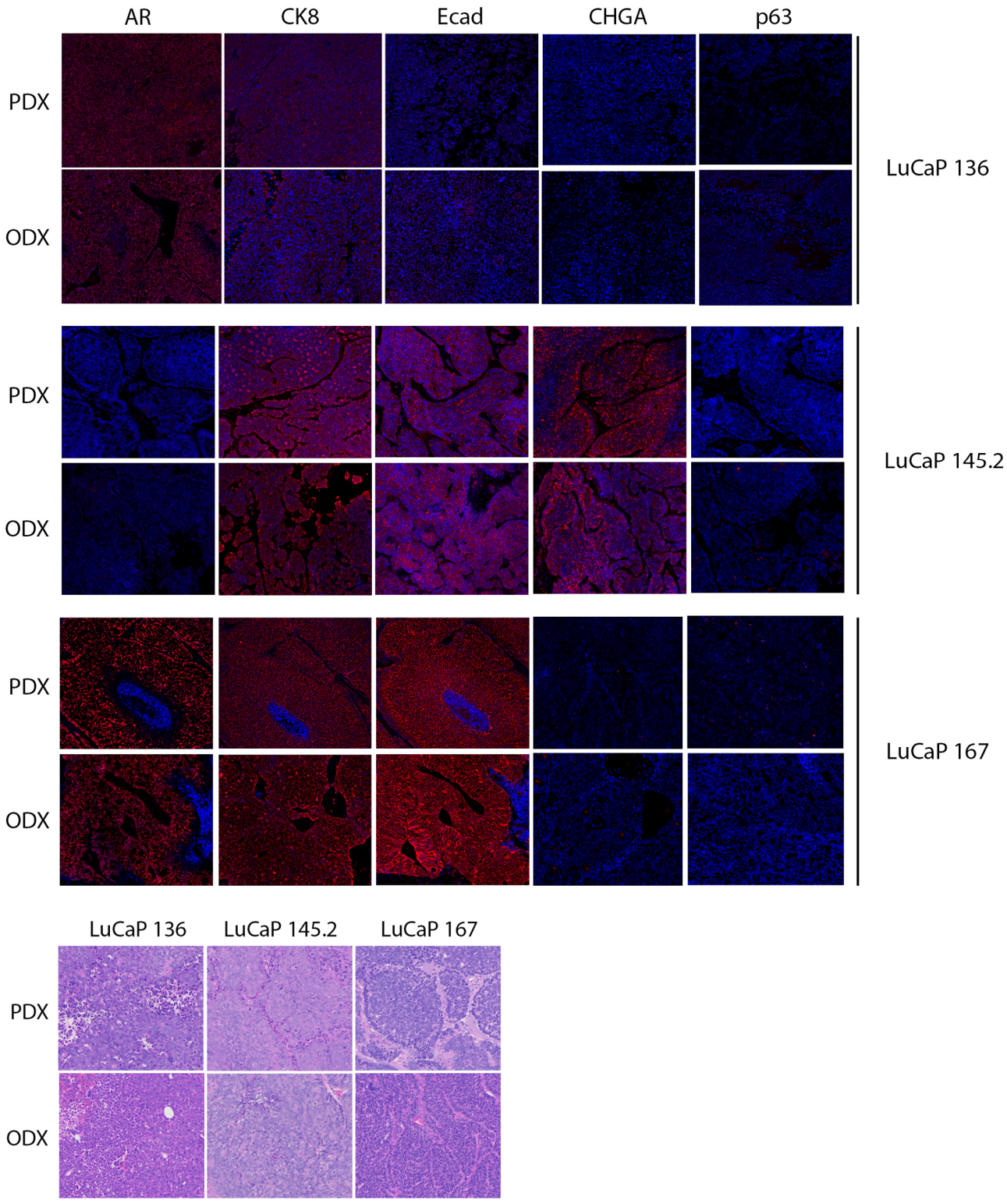
Supplementary Fig. S6.

The effect of p38i treatment on the expression of AR responsive genes is minimal. LuCaP 77, LuCaP 167, and LuCaP 189.3 organoids were grown with or without p38i for two weeks. The difference in expression of the indicated genes was determined by real-time PCR and presented as the fold difference of the p38i treated condition relative to untreated. Error bars represent the standard error of the mean. Two independent experiments were performed.



Supplementary Fig. S7.

Somatic mutations are stable between PDX and early and later generation organoids. Heatmaps indicate the frequency and stability of somatic mutations across PDX and organoid models. Columns represent the sample type (PDX or organoid). Rows represent somatic mutations for each of the three LuCaPs arranged by hierarchical clustering. Each cell of the heatmap is colored to indicate the fraction of the total cell population that contains a given mutation. Mutations were filtered so that at least one column for a given LuCaP contained a minimum fraction of 0.2. The color scale bar shows the range of cell fractions (CCF) containing a given mutation, from 0.0 (white), 0.04 (blue) to 1.0 (yellow). Values above 1.0 (orange) indicate that two alleles are affected through copy neutral loss of heterozygosity or biallelic conversion.



Supplementary Fig. S8

Organoid-derived xenografts maintain the histological phenotype of continually-passaged PDXs. Organoids cultured from the indicated LuCaP PDXs were reinjected into mice as organoid-derived xenografts. Fixed tumor sections were immune-stained for the indicated markers (upper panel) or by H&E (lower panel).