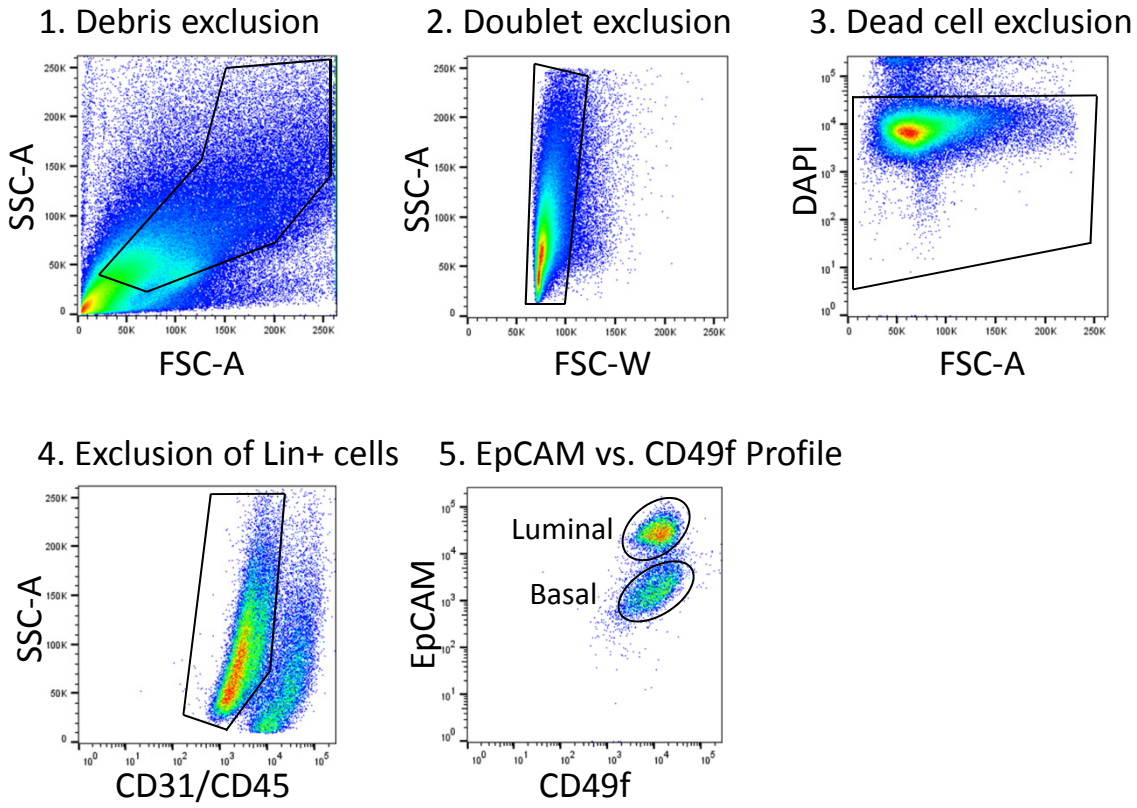
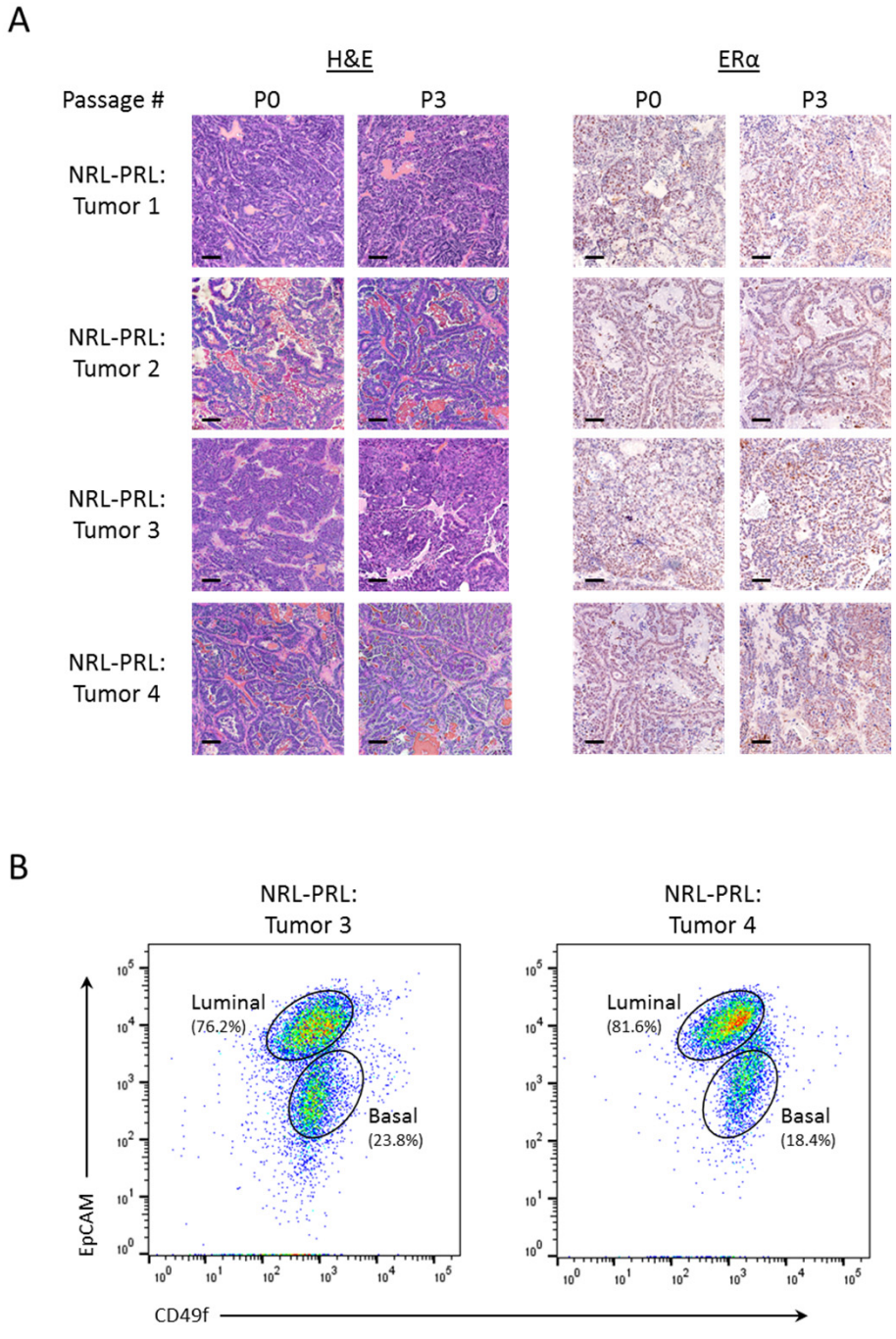
Supplementary Fig. 1



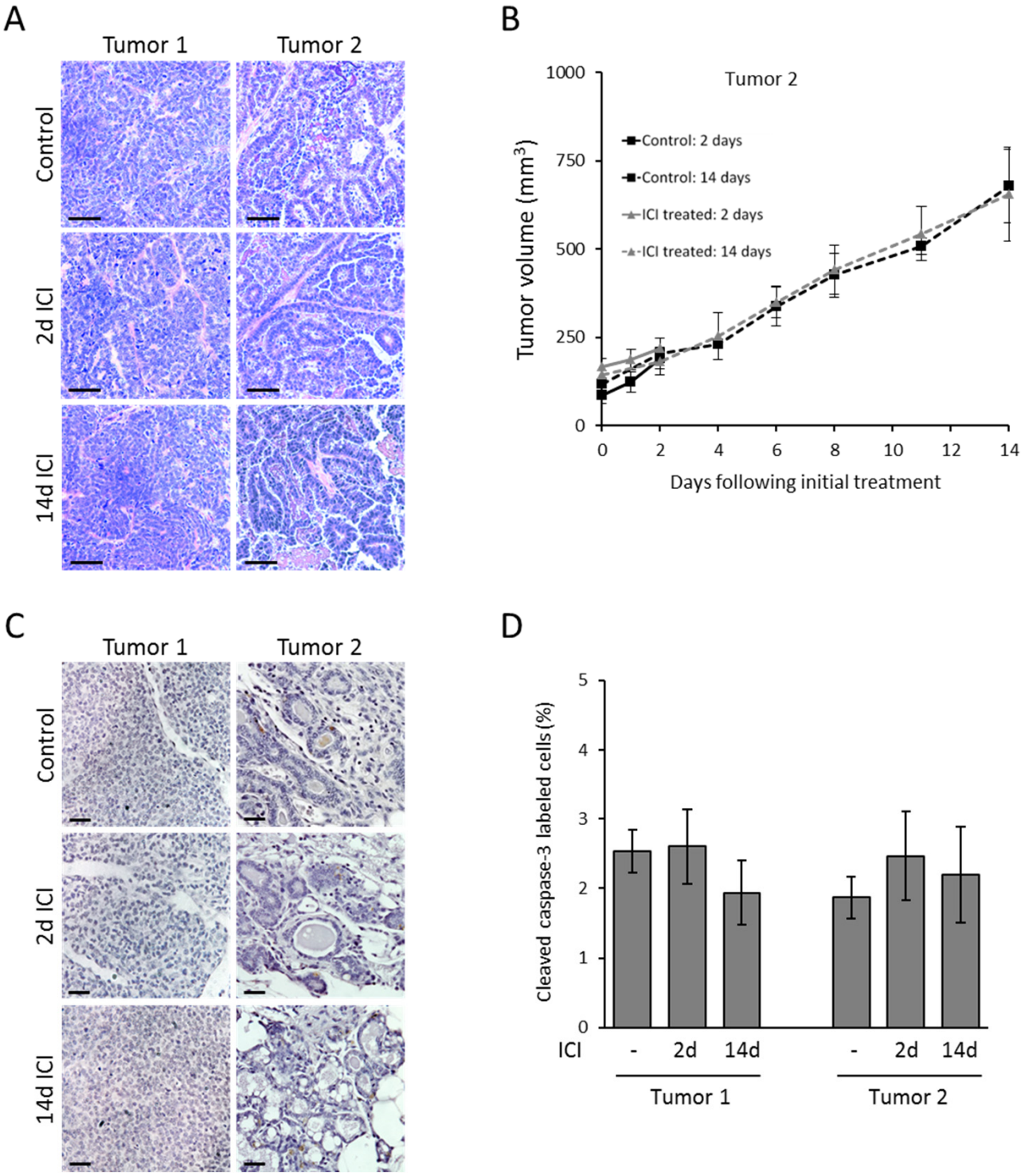
Supplementary Figure 1: Flow cytometric gating strategy for isolation and analysis of tumor epithelial subpopulations.

Supplementary Fig. 2



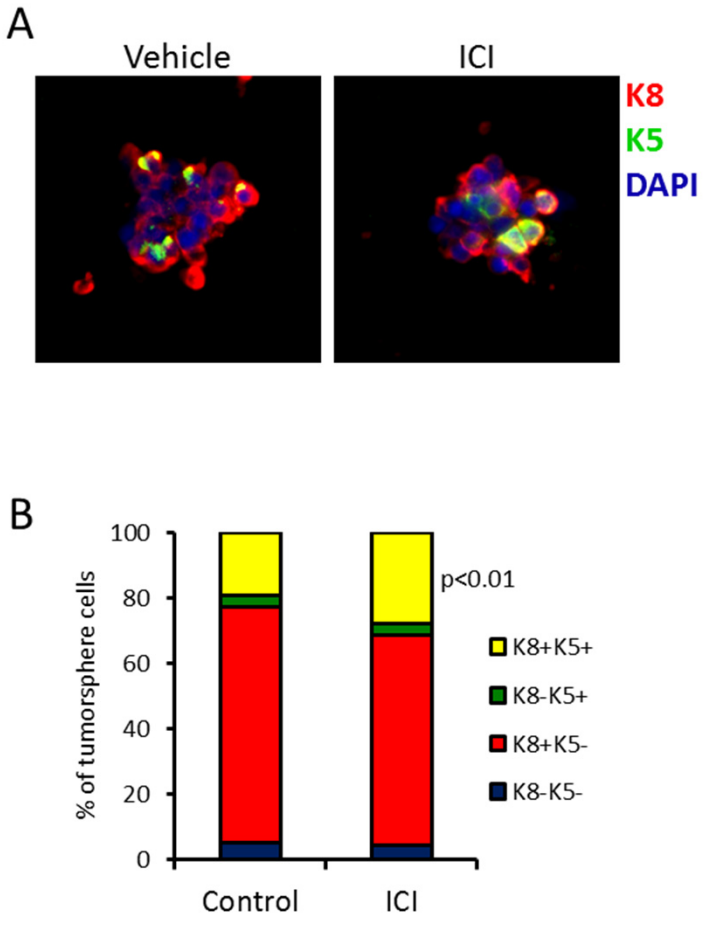
Supplementary Figure 2: (A) Representative photomicrographs showing unchanged histotype and ERα expression of four independent spontaneous NRL-PRL tumors (P0) and daughter tumors serially passaged three times (P3) into mammary fat pads of syngeneic mice. Scale bars, 50 µm. (B) Tumors 3 and 4 display tumor subpopulation profiles by flow cytometry using EpCAM and CD49f which are similar to Tumors 1 and 2 (shown in Fig. 1B).

Supplementary Fig. 3



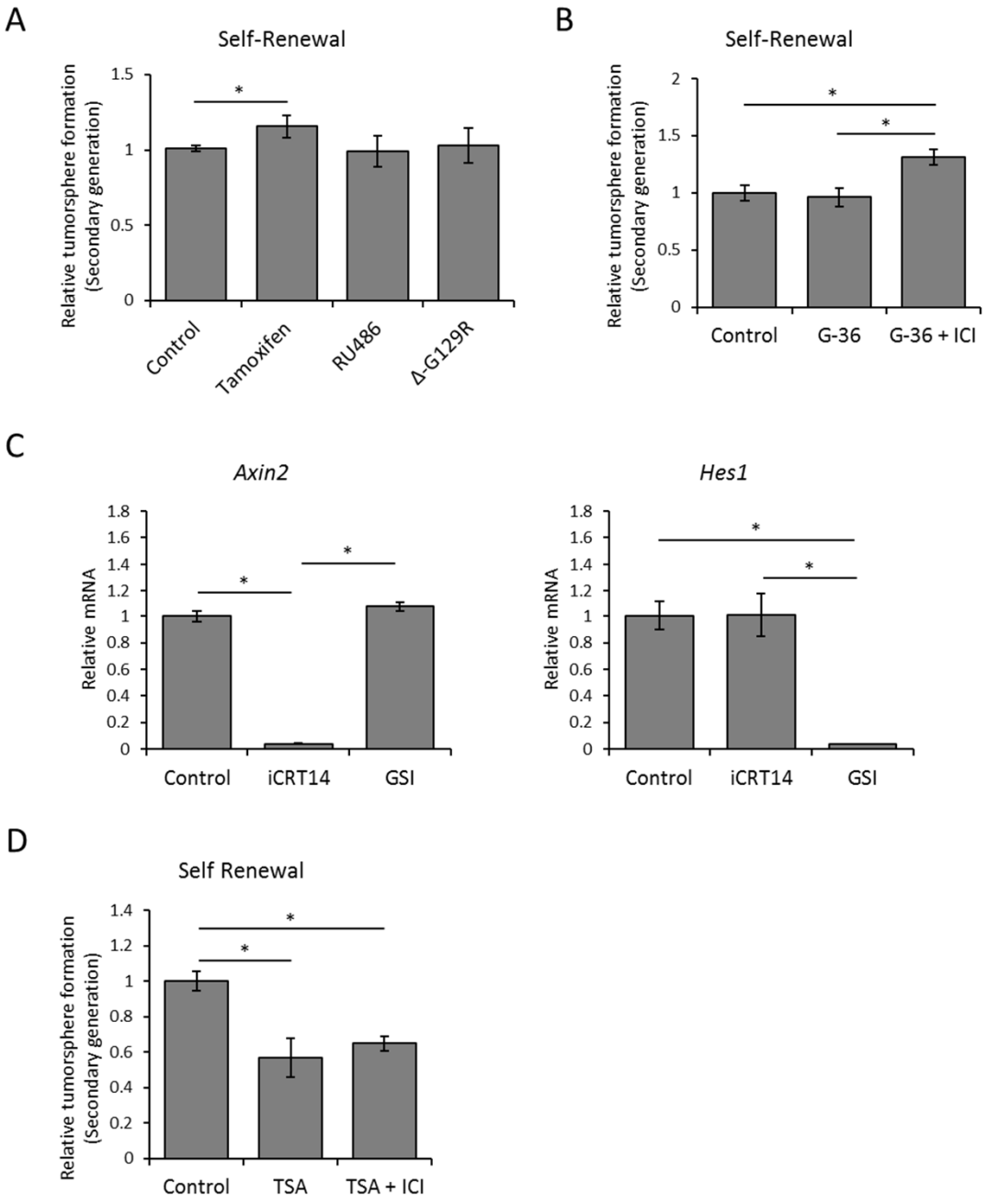
Supplementary Figure 3: (A) Morphology of Tumors 1 and 2 is not altered by ICI treatment (representative photomicrographs, hematoxylin and eosin stain). (B) Growth of Tumor 2 is not altered by ICI treatment (Tumor 1 is shown in Fig. 3C). (C,D) ICI does not alter cleaved caspase-3 expression of either tumor. (C) Immunochemistry of cleaved caspase-3 in control and ICI-treated tumors. (D) Quantification of cleaved caspase-3 labeled cells in untreated and ICI-treated tumors. N=3 tumors; mean±S.D. Scale bars, 50 µm.

Supplementary Fig. 4



Supplementary Figure 4: (A) Representative photomicrographs showing K8 (red) and K5 (green) expression by cells within primary tumorspheres generated under *in vitro* treatment of vehicle (0.1% ethanol) or ICI. Quantification of K8/K5 staining was performed as described in the Methods. (B) Quantification of K8/K5 staining was performed as described in the Methods. N= 10 independent tumorspheres. ICI increases the proportion of K8+/K5+ cells, $p<0.01$.

Supplementary Fig. 5



Supplementary Figure 5: (A,B) Relative secondary tumorsphere formation, a measure of self-renewal capacity, of cells harvested from primary tumorspheres grown in the presence 1 nM Tamoxifen (selective estrogen receptor modulator, EMD Millipore, 579002), 100 nM RU486 (PR antagonist, Sigma-Aldrich, M8046), 1 µg/mL Δ1-9-G129R-hPRL (PRLR antagonist (33)), 10 µM G-36 (GPER antagonist, AZ0001303, Azano Pharmaceuticals Inc.), or cotreatment of 10 µM G-36 with 1 µM ICI. (C) Relative mRNA levels of *Axin2* and *Hes1* from primary tumorspheres treated for 6 hours with 100 µM iCRT14 or 50 µM GSI. (D) Relative secondary tumorsphere formation of primary tumorspheres treated with 100 nM of the HDAC inhibitor, trichostatin A (TSA) in the absence and presence of ICI. Asterisks indicate significant differences (one-way ANOVA followed by Tukey's multiple comparison tests, $p<0.05$).