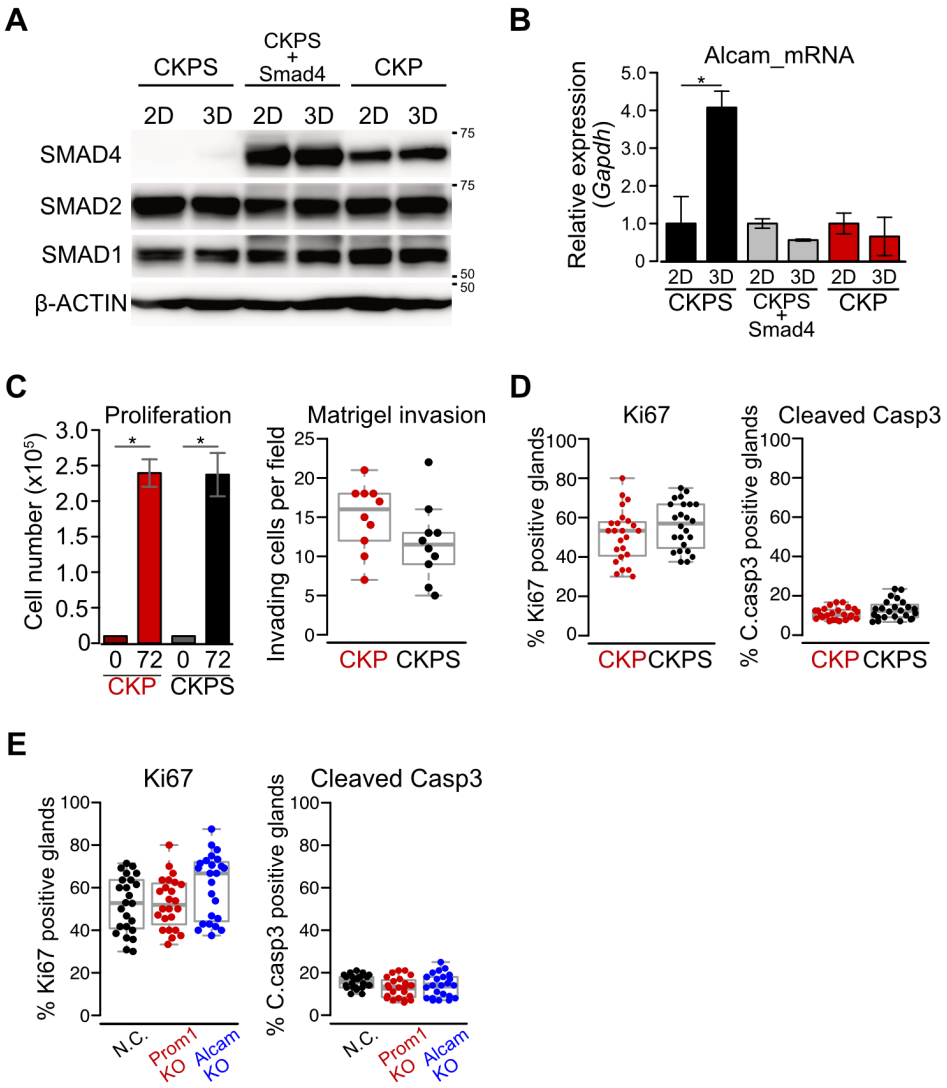
Supplementary Figure S7



Supplementary Figure S7. Characterization of organoid-derived CKP and CKPS cells. (A) Western blot analysis of Smad1, Smad2, and Smad4 in CKPS cells, CKPS cells overexpressing Smad4, and CKP cells cultured in 2D or shifted to 3D conditions for 24 h. (B) RT-qPCR analysis *Alcam* expression in CKPS cells, CKPS cells overexpressing Smad4, and CKP cells cultured in 2D or shifted to 3D condition for 24 h. Data are means ± SD (n=3). (C) Cell proliferation (left) and matrigel invasion ability (right) of CKP and CKPS cells. Numbers of cells in 2D culture were counted 72 h after seeding $1x10^4$ cells.

Data are means ± SD (n=4). Invasion ability of CKP and CKPS cells was evaluated using a Matrigel invasion assay. Numbers of invading cells through matrigel were counted 24 h after seeding $1x10^4$ cells. Data are presented as beeswarm and box plots (n=10). (D) Quantification of percentages of Ki67-positive cells (left) and cleaved caspase 3-positive cells (right) in liver metastases induced by isograft transplantation of 2D-cultured CKP and CKPS cells in the spleen. Data are presented as beeswarm and box plots (n=24 field from 4 mice). (E) Quantification of percentages of Ki67-positive cells (left) and cleaved caspase 3-positive cells (right) in liver metastases induced by isograft transplantation of 2D-cultured Prom1 KO or Alcam KO CKPS cells in the spleen. N.C., negative control for CRISPR. Data are shown as beeswarm and box plots (n=24 field from 4 mice). Data are assessed with one-way ANOVA and Tukey HSD test or with two-tailed Student's t-test. *$p<0.05$.