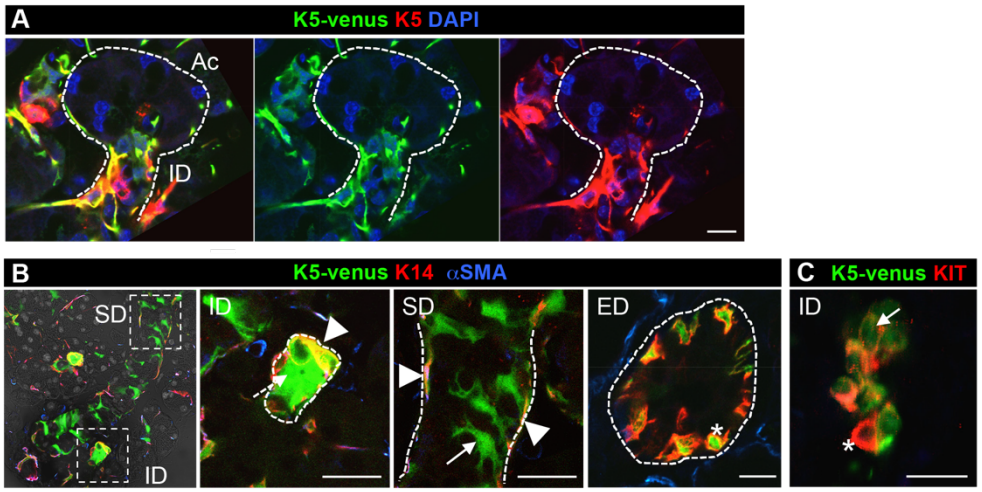
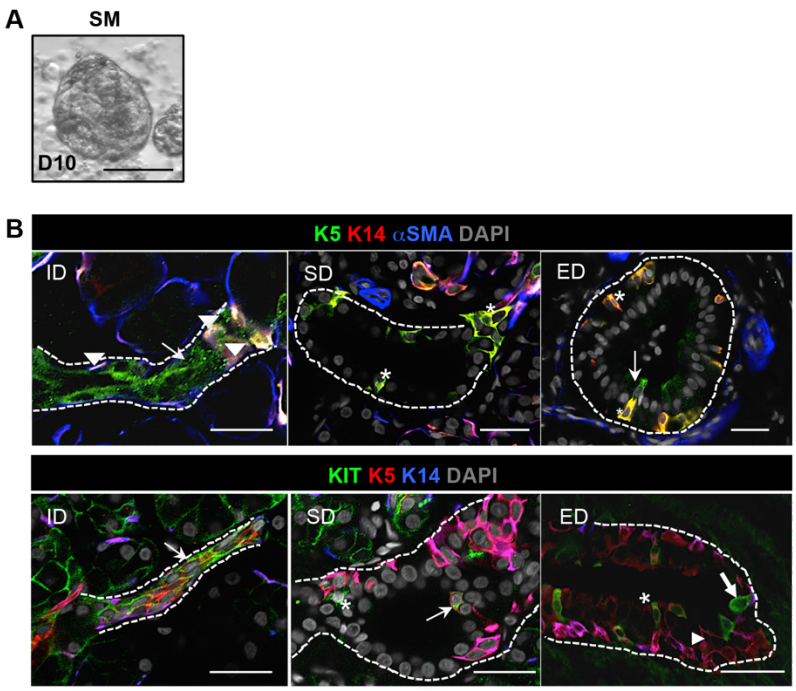
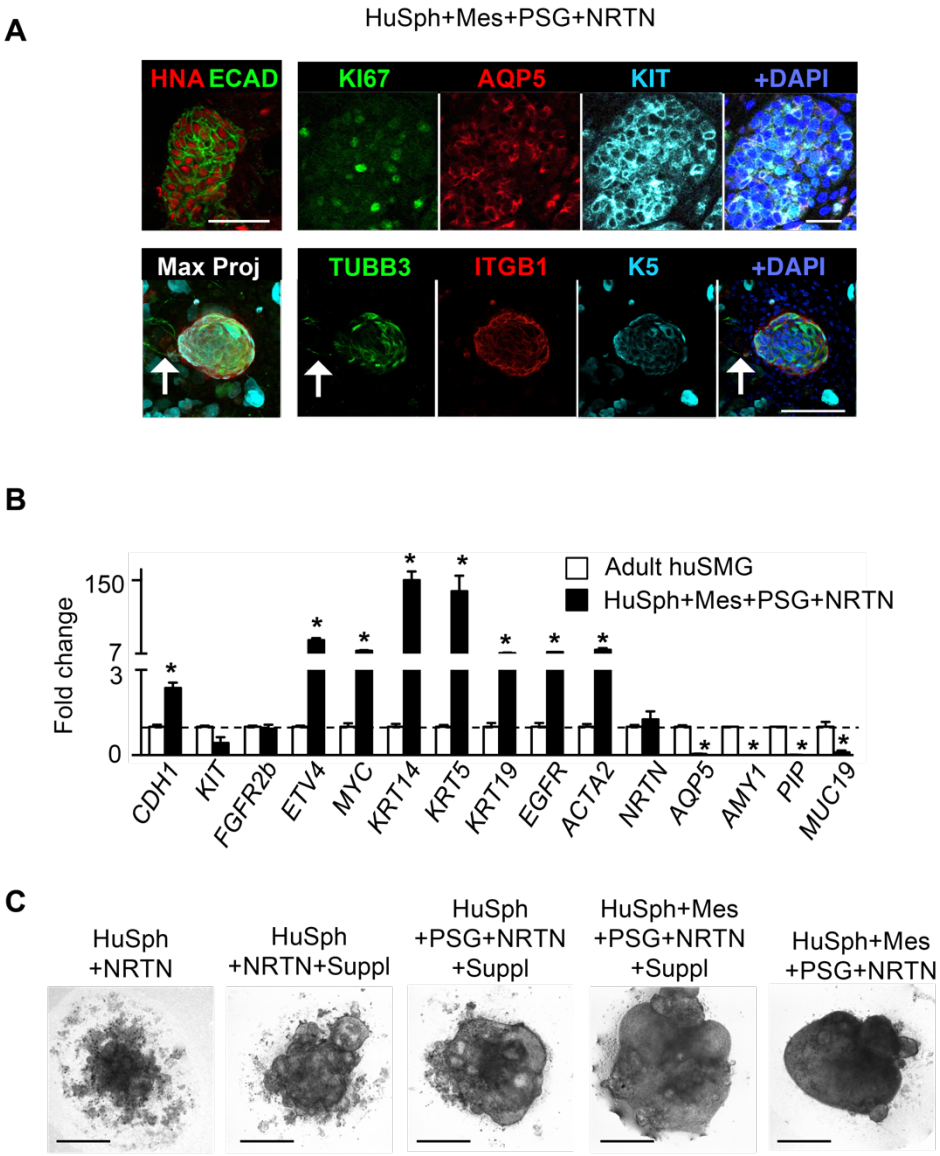


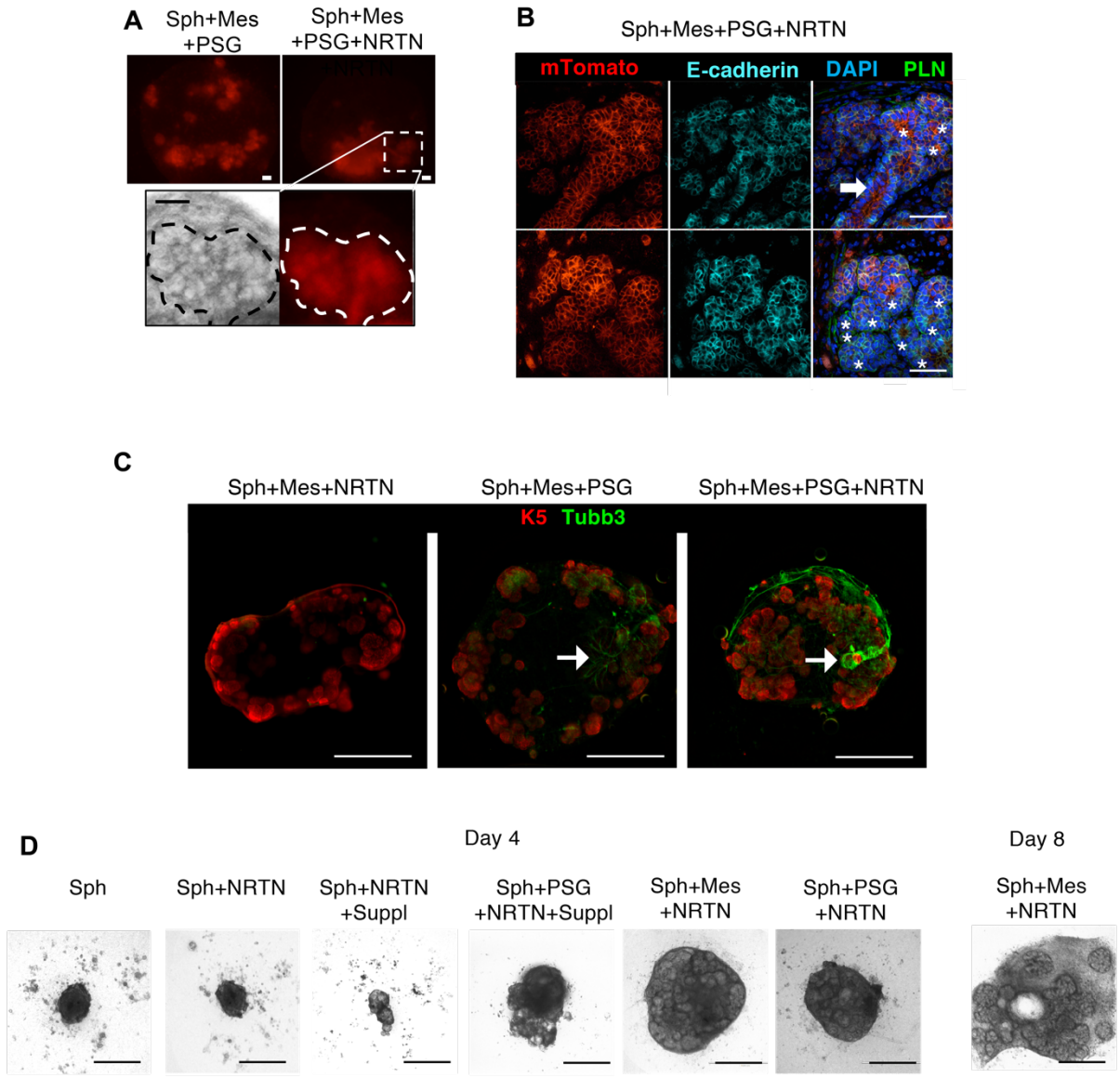
Suppl. Fig. 1. Evaluation of K5-venus adult murine SMG. (A) Confocal section (2μm) of intercalated duct (ID) and acinus (Ac), showing epithelial cells stained for K5-venus (green), endogenous K5 (red), and nuclei (blue, DAPI). Yellow indicates overlap of K5-venus with endogenous K5. Scale bar, 20 μm. **(B-C)** Confocal imaging striated duct (SD), intercalated duct (ID), and excretory duct (ED). K5-venus (green) expressing cells also express K14 (red), αSMA (blue) and/or KIT (red). Arrowhead, myoepithelial cell co-expressing K5-venus, K14 and αSMA. **(B)** Arrow, striated duct (SD) cells expressing K5-venus. Dotted arrow, intercalated duct (ID) cells expressing K5-venus. Asterisk, excretory duct (ED) cells co-expressing K5-venus and K14. **(C)** KIT+ ID cells are K5-venus negative (asterisk) or K5-venus positive (arrow). Scale bar, 20 μm.



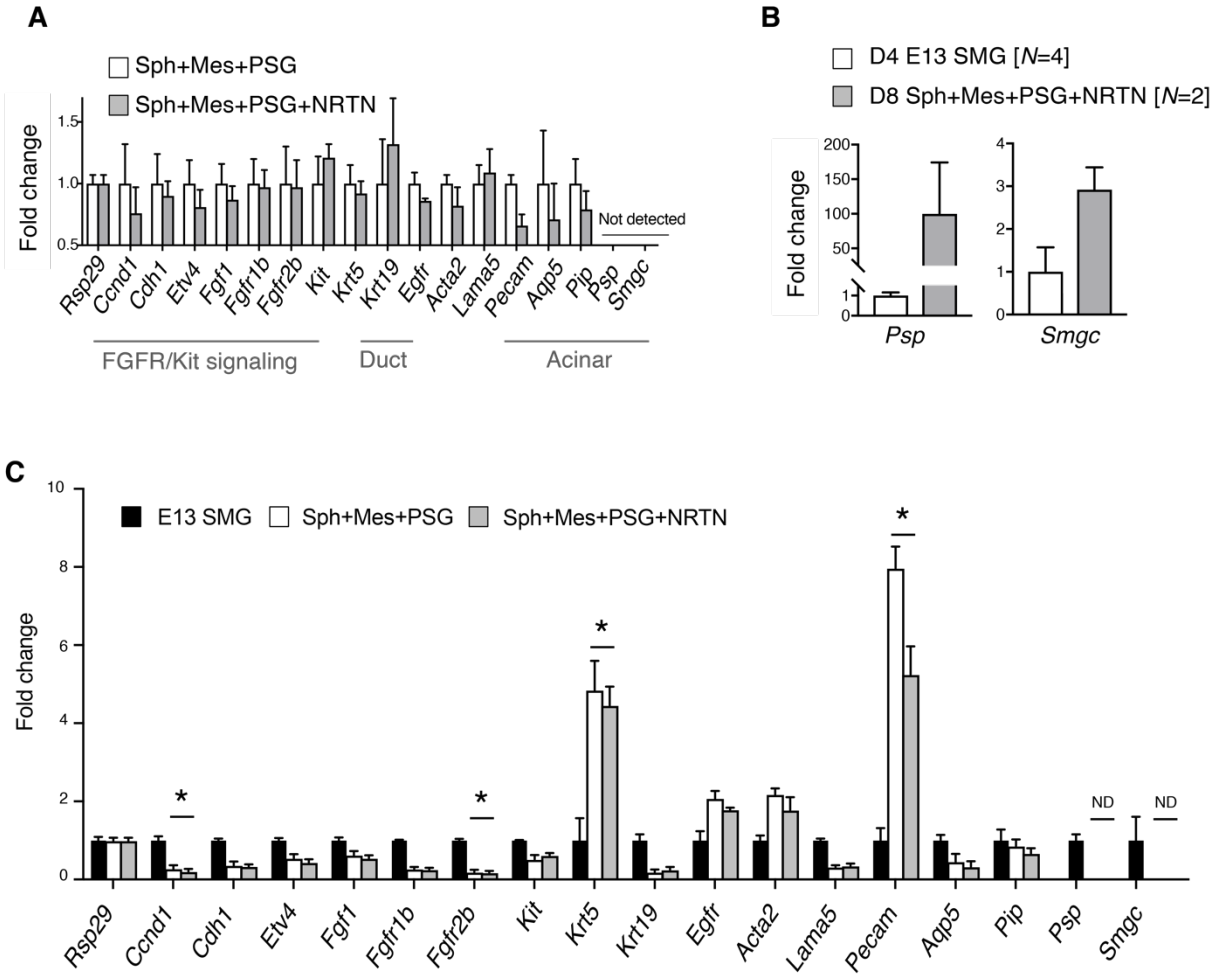
Suppl. Fig. 2. Evaluation of human salivary gland cells in SM culture or in vivo. (A) Representative bright field picture of human salisphere at D10 in SM [FGF7] culture. Scale bar, 0.5 mm. **(B)** Confocal images of human SMG stained for K5, K14, αSMA, KIT and /or nuclei (DAPI). Top panel: intercalated duct (ID), arrow shows K5+ cell, arrowheads indicate K5+K14+αSMA+ cells. Striated duct (SD), asterisks indicate K5+ cells. Excretory duct (ED), arrow shows K5+ cell, asterisks are K5+K14+ cells. Lower panel: ID, arrow indicates KIT+K5+ cell. SD, arrow shows KIT+K5+ cell, asterisk indicates KIT+ cell. ED, arrow shows KIT+ cell, asterisks indicates KIT+K5+ cell, arrowhead shows K5+ cell. Scale bar, 20 μm.



Suppl Fig. 3. Human salispheres recombined in fetal niche express markers of KIT+ and K5+ progenitors. (A) Top row - Immunostaining of human nuclear antigen (HNA, red) and E-cadherin (ECAD, green) indicated the presence of human epithelial cells in the mouse recombined environment. Ki67 staining (green) shows proliferation. AQP5 staining (red) is not organized in apical membranes but is cytoplasmic. KIT (cyan) protein expression is present. Scale bar, 50 µm. Bottom row – Immunostaining of beta-3-tubulin (TUBB3, green), integrin-beta-1 (ITGB1, red), and keratin 5 (K5, cyan) of single confocal sections and a composite maximum intensity projection, which show small TUBB3+ nerves (white arrow) in association with the recombined spheres. Scale bar, 100 µm. **(B)** Fold changes of gene expression of multiple genes present in recombined human salispheres+Mes+PSG+NRTN. Data was normalized to adult human SMGs (huSMG) and housekeeping gene (*RSP29*) (dotted line). Mean±SEM. *N*=6 biological samples. *, $P < 0.05$; Multiple *t*-test with 5% False Discovery Rate (FDR). **(C)** Phase images of human salispheres (HuSph) encapsulated in laminin with controls (+NRTN, +NRTN+Suppl, +PSG+NRTN+Suppl, +Mes+PSG+NRTN+Suppl), as well as standard recombination (+Mes+PSG+NRTN). SM media (without ROCK or PD inhibitors) were added as supplements when indicated and changed after 3 days (+Suppl). Scale bar, 0.5 mm.



Suppl Fig. 4. Branching morphogenesis of mouse salispheres in fetal mesenchyme + PSG +/- NRTN. (A) Salispheres (Sph) expressing mTomato (red) were recombined with wild type Mes+PSG+NRTN and compared to recombination without NRTN. Scale bar, 100 µm. Higher power image: Fluorescence and phase contrast image shows mTomato+ (red) branching structures (dotted curved lines) that developed from recombined salispheres in the presence of NRTN. Scale bar, 100 µm. **(B)** Confocal sections of spheres+Mes+PSG+NRTN recombinations show mTomato+ (red) and E-cadherin+ (cyan) ductal structures (arrow) with basally located nuclei (blue) and a lumen (arrow). Sections through end-bud structures (*) show expression of basement membrane protein perlecan (PLN, green). Scale bar, 50 µm. **(C)** Fluorescence microscopy of keratin 5 (K5, red) and beta-3 tubulin (Tubb3, green) of mouse salispheres in laminin hydrogels recombined with fetal Mes +/- PSG (white arrow) +/- NRTN. Scale bar, 0.5 mm. **(D)** Phase images of salispheres encapsulated in laminin with controls (+NRTN, +NRTN+Suppl, +PSG+NRTN+Suppl, +Mes+NRTN, +PSG+NRTN) after 4 days, as well as after 8 days for +Mes+NRTN, which show enlargement and aggregation of spheres, but visible branching. SM media (without ROCK or PD inhibitors) were added as supplements when indicated and changed after 3 days (+Suppl). Scale bar, 0.5 mm.



Suppl Fig. 5. Mouse salispheres in fetal niche express FGFR/Kit signaling, ductal, and acinar markers. (A) Fold change in gene expression of Spheres+Mes+PSG+/-NRTN at 4 days post-recombination. Data was normalized to Spheres+Mes+PSG and *Rps29*. *N*=3 biological samples. Differences between +/- NRTN were not statistically significant, multiple *t*-test with 5% FDR. **(B)** Qualitative gene expression analysis of *Psp* and *Smgc* in D8 Spheres+Mes+PSG+NRTN compared to D4 intact E13 gland. Data was normalized to D4 intact E13 gland and *Rps29*. *N*=4 biological samples for E13, *N*=2 for Spheres+Mes+PSG+NRTN. **(C)** Fold change in gene expression of Spheres+Mes+PSG+/-NRTN at 4 days post-recombination, compared with an intact E13 SMG at day 4. Data was normalized to E13 SMG D4 and *Rps29*. *, statistically significant differences compared to E13 SMG at D4, two-way ANOVA and Dunnet's multiple comparisons test, *N*=4 biological samples, *P*<0.05. ND, not detected.