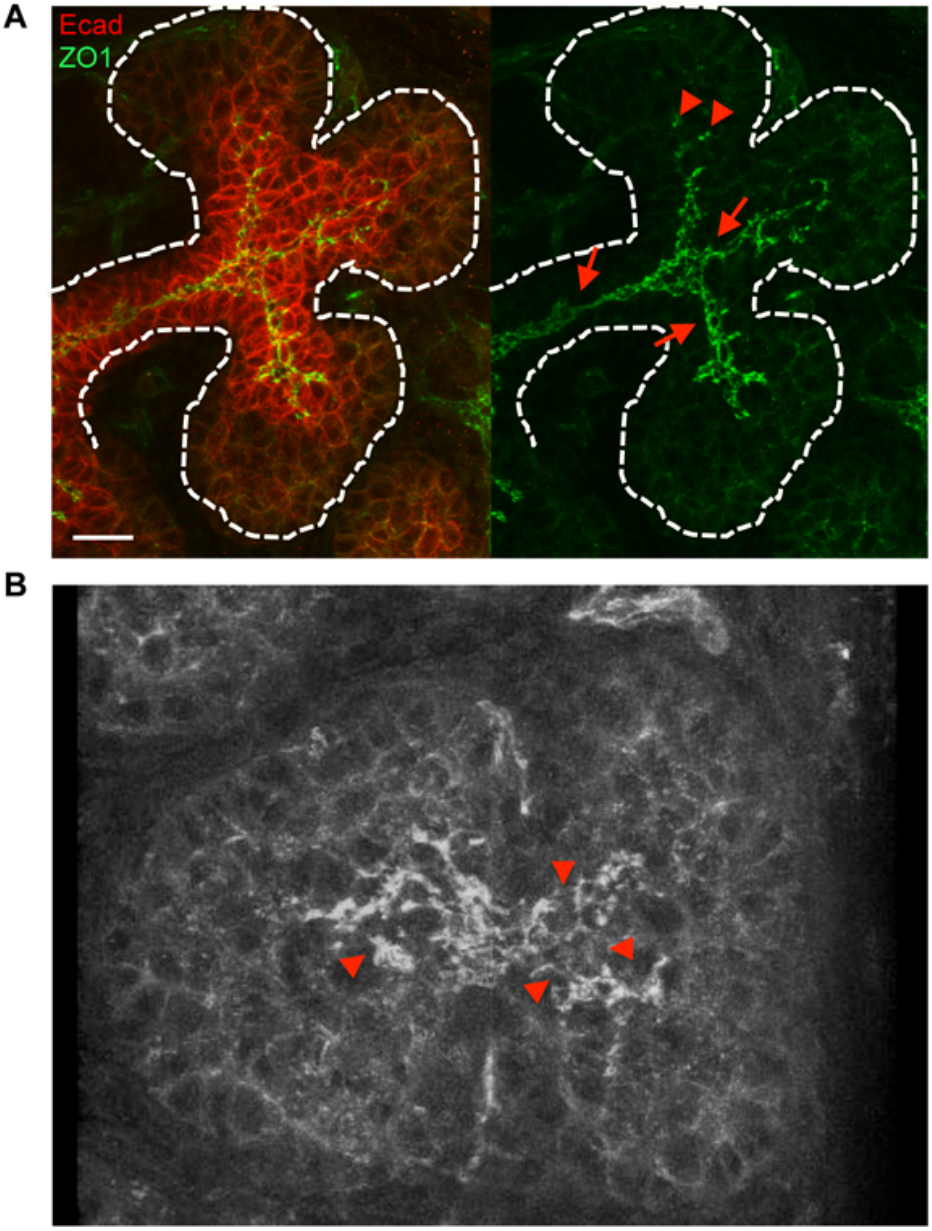

Figure S1. Microlumens are present in developing SMG, related to Figure 1. A, ZO1 staining of a freshly isolated E14.5 SMG demonstrate presence of contiguous lumen (arrows) in proximal parts and microlumens (arrowheads) in distal ducts. SMG were stained for ZO1 (green) and E-Cadherin (red). Scale bar 25μm. **B,** 3D projection of presumptive ducts showing ZO1(+) microlumens that will fuse to form a single lumen (see Movie S2 for 3D rotation). Arrowheads indicate the lack of connection between individual microlumens. Samples were imaged using a Leica Sp5 LSM confocal microscope. Image is a 30μm stack of 0.8μm section.

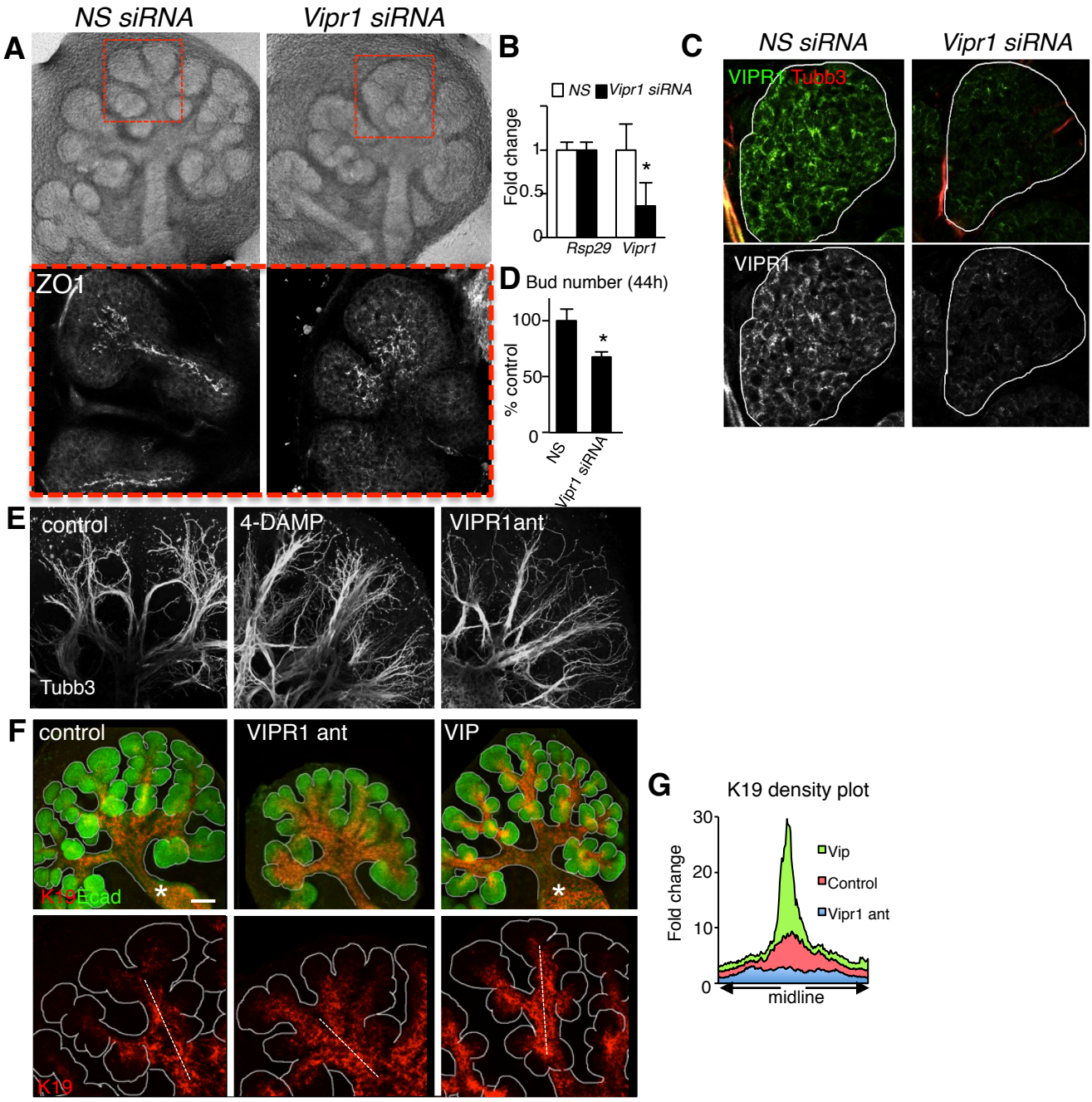

Figure S2. VIP/VIPR1 signaling regulates accumulation of ZO1+ tight junctions and K19(+) cells at the ductal midline and VIP induced duct elongation is PKA-dependent, related to Figure 2. (A-D) E12.5 SMG were cultured with non-silencing (NS) or Vipr1 siRNA (400 nM) for 44 h. Presumptive lumens were immunolabeled for ZO1 (**A**, **lower panel**). Knockdown in *Vipr1* gene and protein expression was confirmed by qPCR (**B**) and immunostaining (**C**), respectively, and the number of end buds was counted (**D**). Students t-test was performed on log transformed data; *P<0.05. **(E)** 4-DAMP but not VIPR1ant treatment increases innervation of SMG epithelium. E13 SMG were treated for 24h with DMSO, 10μM 4-DAMP or 30μM VIPR1ant before being fixed and stained for nerves (Tubb3). (**F** and **G)**. E13 SMG were cultured with or without VIPR1ant (30 μM) or VIP (200 nM) for 24 h and immunostained for K19 (red) and Ecadherin (green). **G** is a representative density plot of the distribution of K19(+) fluorescent intensity across the duct midline (dashed line in **F,** lower panel). 5 ducts per 3 SMG were analyzed for each condition. Images are a 20 μm projection of 2 μm confocal sections. Asterisk (**F**, upper panel) indicates primary duct expansion in the presence of exogenous VIP. Scale bar = 100 μm.

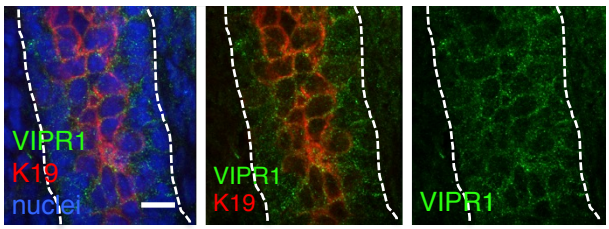

Figure S3. VIPR1 is expressed by K19+ cells, related to Figure 3. E14 SMG was fixed and immunostained for Vipr1 (green), K19 (red) and nuclei (blue). Image is 1μm confocal section. Scale bar = 10μm.

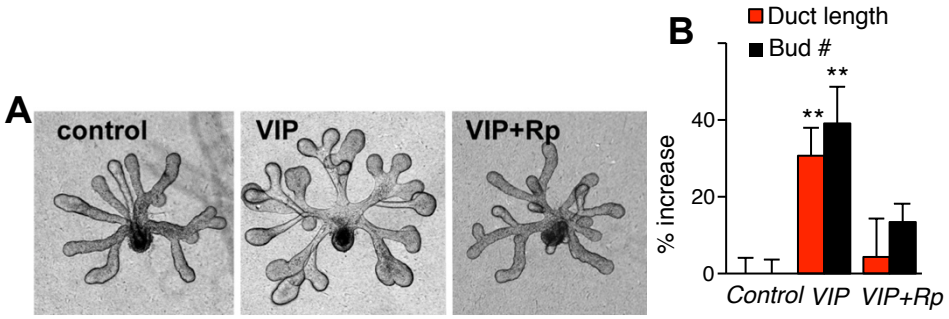

Figure S4. Inhibition of PKA reduces VIP-mediated epithelial duct elongation and bud number, related to Figure 4. (**A** and **B**) E13 epithelial rudiments were cultured untreated (control) or treated with VIP (200 nM) with or without the PKA inhibitor, Rp-CPT-cAMPS (Rp, 200 μM) for 48 h. Data in **B** are means ± s.d. of 3 experiments and was analyzed using a Students t-test. **P<0.01.

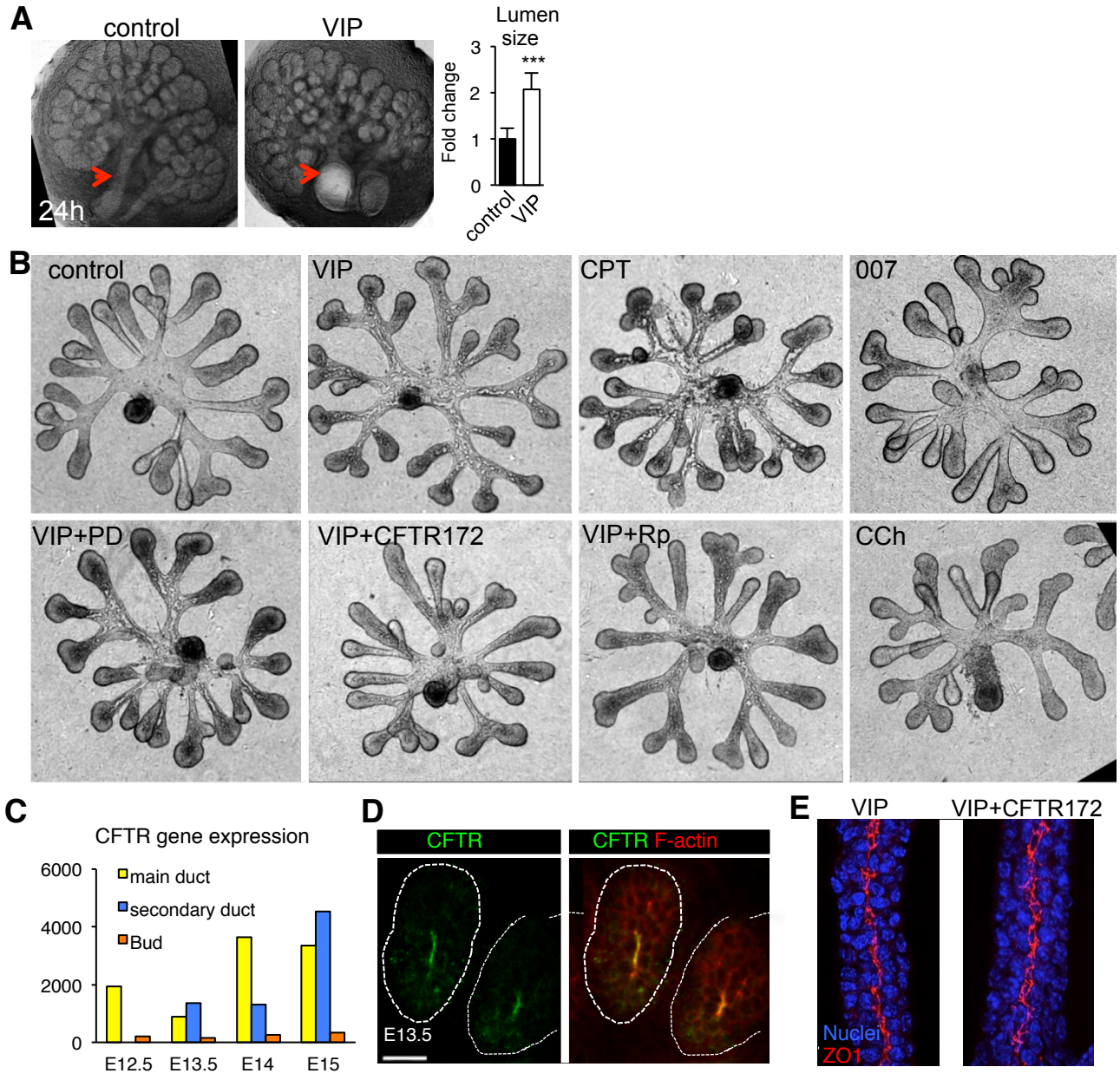

Figure S5. Lumen expansion depends on VIP/VIPR1/PKA/CFTR but not on muscarinic or EGFR signaling, related to Figure 6. A, Primary duct lumens of cultured SMG is dilated in the presence of VIP. E13 SMG were cultured with or without 500 nM VIP for 24 h. Lumen size was measured as the distance at widest point. Red arrows point to primary lumens. Graph shows the mean ±SD of 5 SMG from 2-3 experiments., Student t-test was performed. ***P<0.001. **B**, E13 epithelia cultured in FGF10 and HBEGF for 28 h before the addition of 200 nM VIP, 200 nM CCh, 200 μM 8-CPT-cAMP (CPT; PKA/Epac activator), or 200 μM 8-CPT-2'-O-Me-cAMP (007; Epac activator). When indicated, samples were treated by combination of VIP and the EGFR inhibitor PD168393 (PD; 5 μM), the CFTR inhibitor CFTR-172 (40 μM) or the PKA inhibitor Rp-CPT-cAMPS (Rp; 250 μM) was added. **C and D**, CFTR is upregulated in the ducts during SMG development (**C**) and colocalizes with apical F-actin at the presumptive lumen (**D**). **E**, E13 epithelia were treated with 200nM VIP with or without 20μM CFTR172 for 48h before staining for ZO1 (red) and nuclei (blue). Images are 3μm confocal sections. Data in **C** was derived from an existing microarray data set (http://sgmap.nidcr.nih.gov/sgmap/sgexp.html). Scale bar in **D** = 25μm.

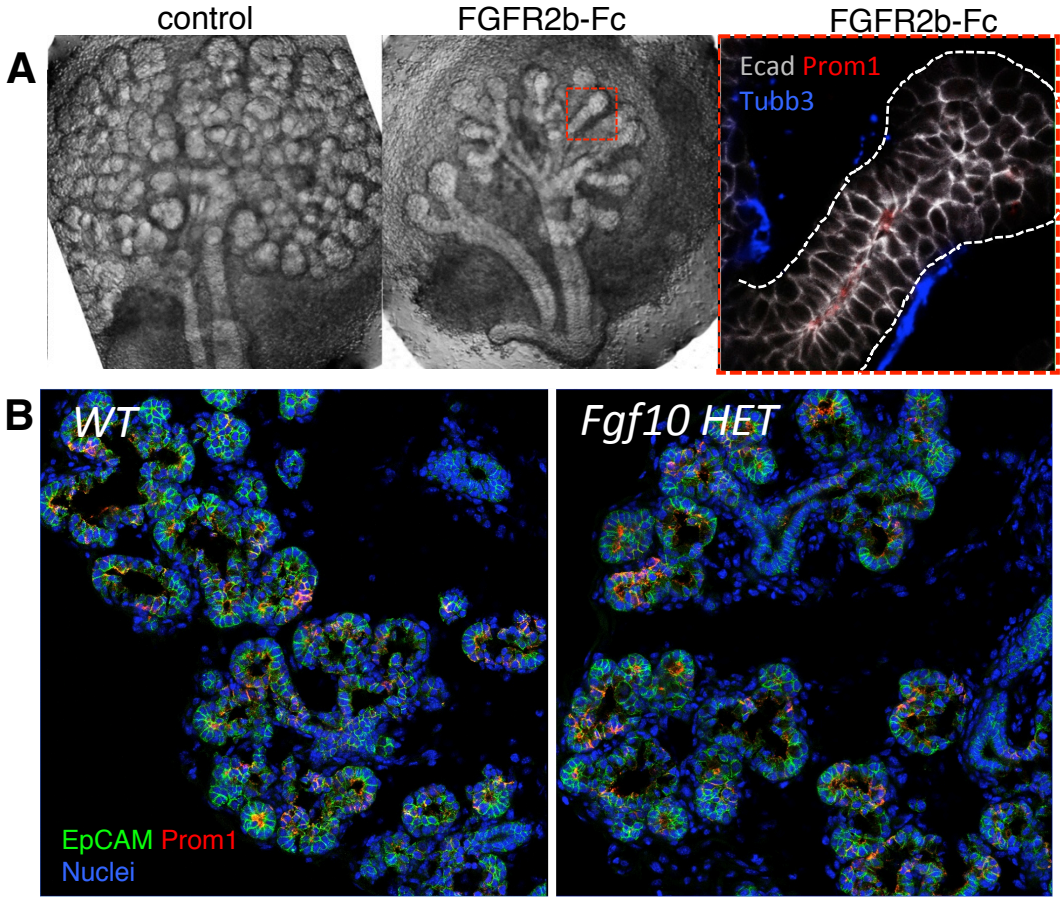

Figure S6: Reducing FGF10/FGFR2b signaling does not inhibit tubulogenesis but salivary gland devoid of CFTR exhibits reduced ductal development, related to Figure 7. (**A**) Inhibition of FGFR2b signaling reduced branching with fewer endbuds and ductal epithelium. E13 SMG were treated with 2.5µg/ml IgG (control) or FGFR2b-Fc for 48h before being fixed and immunostained for the epithelium (Ecad, grey), lumens (Prom1, red) and axons (Tubb3, blue). (**B**) Genetic reduction in Fgf10 reduces epithelial branching but has no effect on lumen formation and expansion. E17.5 salivary glands from wild type and *Fgf10* heterozygous mice were fixed and immunostained for the epithelium (EpCAM, green), luminal membrane (Prom1, red) and nerves (Tubb3, blue).