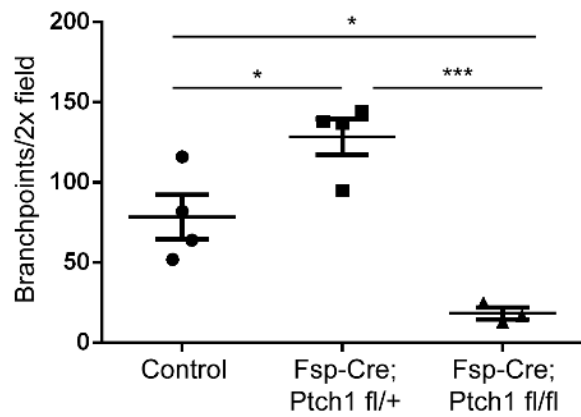
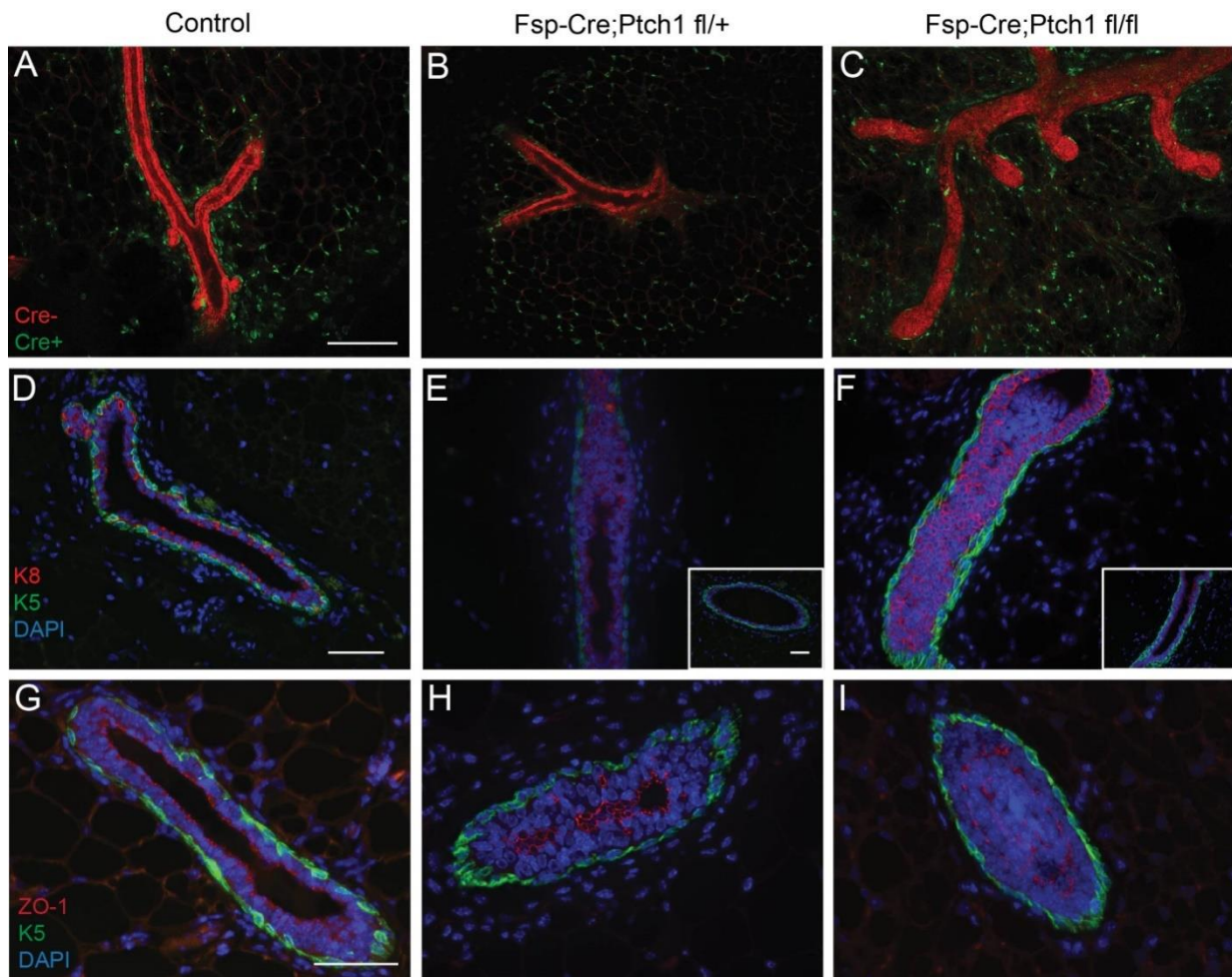


## Supplemental Data



**Fig. S1. Quantification demonstrating increased branching in *Fsp-Cre;Ptch1<sup>fl/+</sup>* mutants, but reduced branching in *Fsp-Cre;Ptch1<sup>fl/fl</sup>* mutants.**

Graph shows mean  $\pm$  SEM. \* indicates  $p < 0.05$ , and \*\*\* indicates  $p < 0.001$  by ANOVA/Tukey's test. Branch points were quantified with a representative 2x field for each gland. Cont

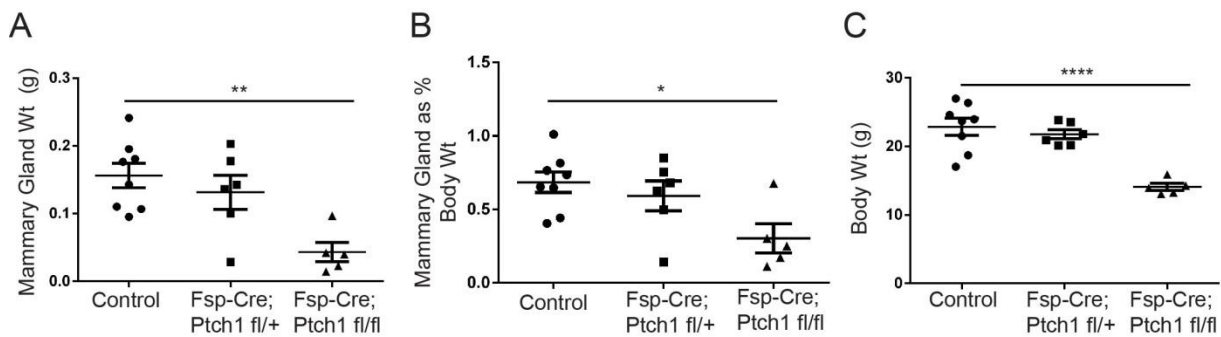


**Figure S2. *Fsp-Cre*-mediated ablation of *Ptch1* produces ducts filled with K8+ luminal cells, and aberrant microlumens.**

A-C: 3D confocal reconstruction of A) *Fsp-Cre, mTmG* B) *Fsp-Cre;Ptch1<sup>fl/+</sup>* and C) *Fsp-Cre;Ptch1<sup>fl/fl</sup>* ducts. *Cre* negative cells express TdTomato Red, and *Cre* positive cells express GFP. There are increased luminal cells inside the ducts of heterozygotes, while the homozygote displays completely filled-in ducts. Scale bar is 100  $\mu$ m.

D-E: K5 (basal), and K8 (luminal) coimmunofluorescence of D) control E) *Fsp-Cre;Ptch1<sup>fl/+</sup>* and F) *Fsp-Cre;Ptch1<sup>fl/fl</sup>* ducts, indicating that the cells filling the ducts of mutants are K8 positive. Insets display ducts with normal histology. Scale bar is 50  $\mu$ m.

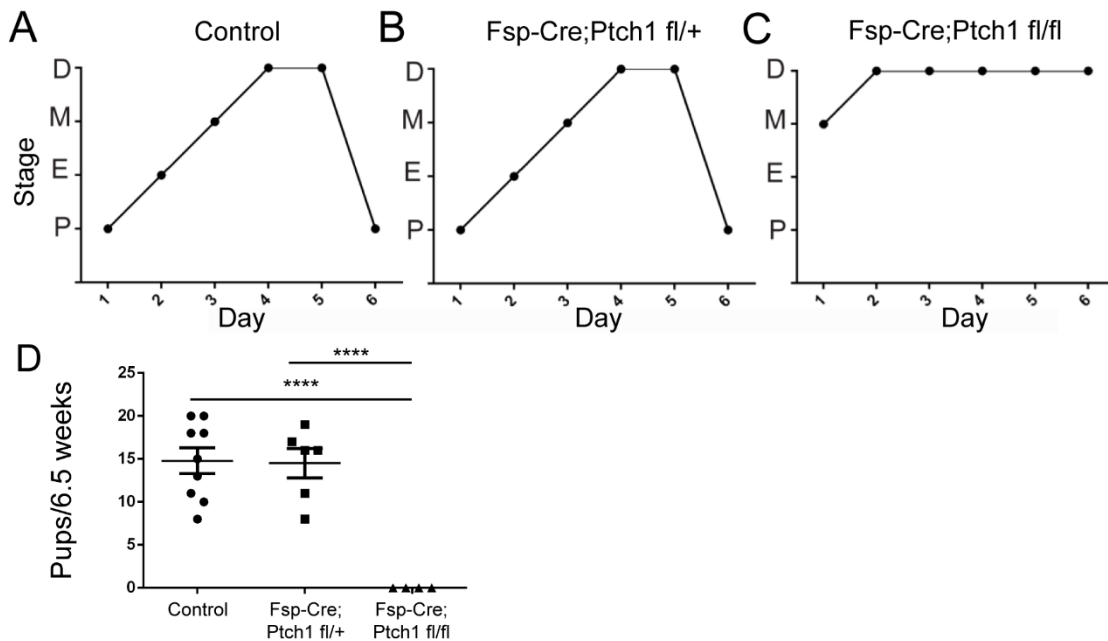
G-I: ZO-1 (apical and tight junction marker) and K5 costained G) control H) *Fsp-Cre;Ptch1<sup>fl/+</sup>* and I) *Fsp-Cre;Ptch1<sup>fl/fl</sup>* ducts. The heterozygote displays extra staining in microlumens, while the homozygous mutant displays aberrant concentric staining.



**Figure S3. *Fsp-Cre* mediated loss of *Ptch1* reduces mammary gland and body weight.**

- A) Mass of inguinal mammary gland of control and mutant animals at 8 weeks, showing a decrease in *Fsp-Cre;Ptch1<sup>fl/fl</sup>* mutants.
- B) Mass of control and mutant animals at 8 weeks as percent body weight, showing a decrease in *Fsp-Cre;Ptch1<sup>fl/fl</sup>* mutants.
- C) Body weight of control and mutant animals at 8 weeks, showing a decrease in *Fsp-Cre;Ptch1<sup>fl/fl</sup>* mutants.

Graphs show data as mean  $\pm$  SEM, and weights are given in grams. \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.01$ , and \*\*\*\* indicates  $p < 0.0001$  by ANOVA/Tukey's test.



**Figure S4. *Fsp-Cre;Ptch1<sup>fl/fl</sup>* animals do not proceed through the estrous cycle normally, and have severely compromised fertility.**

A-C: representative estrous cycle for A) control B) *Fsp-Cre;Ptch1<sup>fl/+</sup>* and C) *Fsp-Cre;Ptch1<sup>fl/fl</sup>* animals. Homozygous mutants do not cycle normally as assayed by vaginal smear in  $\geq 4$  animals per group. D indicates diestrous, M metestrous, E estrous, and P proestrous.

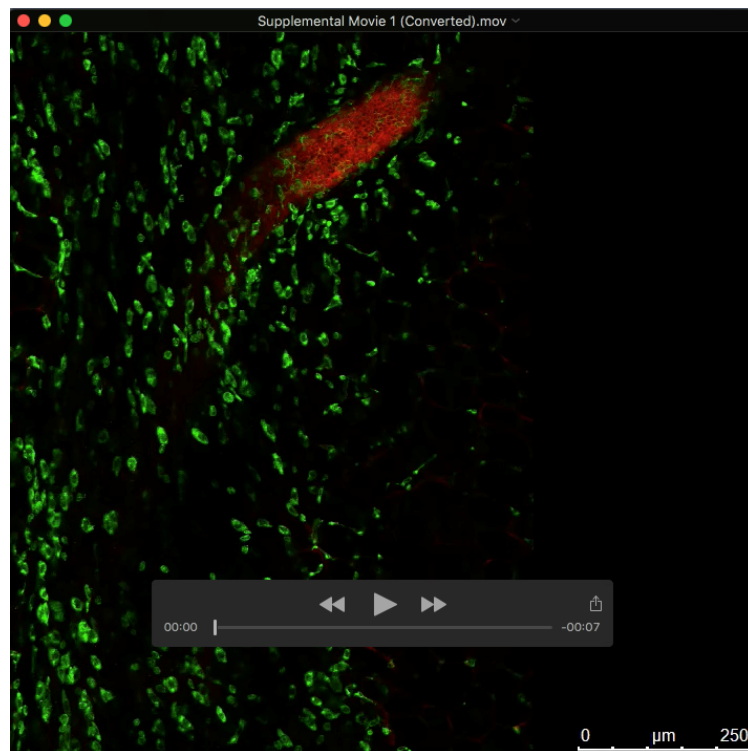
D: Number of pups produced by females of different genotypes over 6.5 weeks showing severe loss of fertility in *Fsp-Cre;Ptch1<sup>fl/fl</sup>* animals. Control animals produced  $14.8 \pm 1.5$ , heterozygotes produced  $14.5 \pm 1.7$ , and homozygotes produced no pups. \*\*\*\* denotes  $p < 0.0001$  by ANOVA/Tukey's test.

Table S1. Antibodies used for immunostaining.

Antibody	Concentration	Antigen Retrieval
Ki67 (Vector Labs, Rabbit Polyclonal VP K451)	1:200	1 mM Sodium citrate buffer, pH 6
GFP (Living Colors, Clontech JL-8)	1:250	Citrate
BrdU (Abcam #6326)	1:250	10 mM Tris, 1 mM EDTA, pH 9.0
ZO-1 (Millipore MABT11)	1:100	Citrate
ER $\alpha$ (Santa Cruz)	1:100	Citrate
PR (DAKO)	BCM Breast Pathology Core	
K5 (Abcam 52635)	1:100	Citrate
K8 (U. of Iowa, Hybridoma bank)	1:100	Citrate
CC3 (Cell Signaling 9661)	1:200	Citrate
Cyclin B1 (Cell Signaling 4138)	1:100	TE pH 9.0
Anti-Rabbit 594 (Secondary) (AlexaFluor)	1:500	either
Anti-Mouse 488 (Secondary) (AlexaFluor)	1:500	either

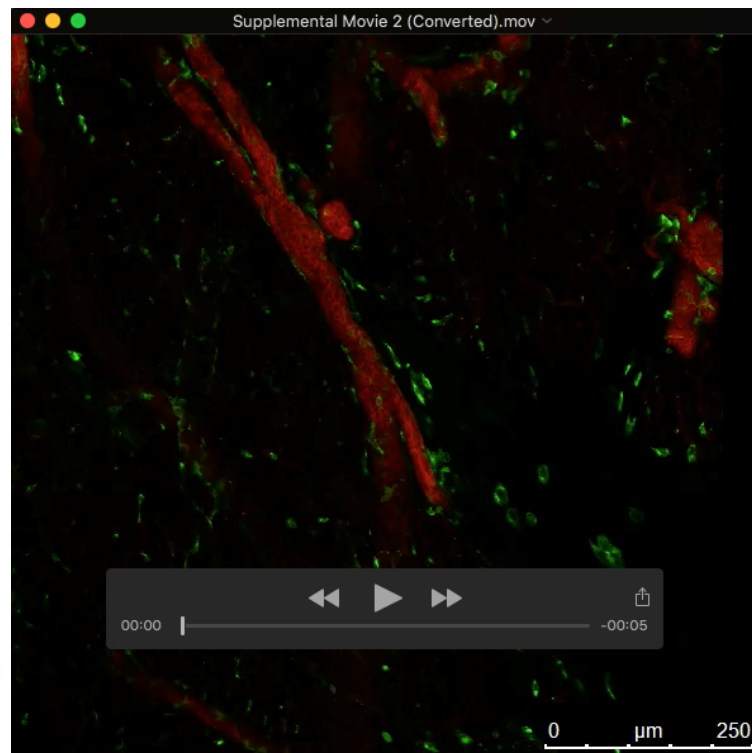
Table S2: Primers used for QPCR

Gene	Primer Ref No. (Taqman)	Probe
18S	Mm03928990_g1	
Ptch1	Mm00436026_m1	Exon 17-18 (start base 2846)
Ptch2	Mm00436047_m1	Exon 19-20 (start 3115)
Smo	Mm01162704_m1	Exon 1-2 (start 856)
Gli1	Mm00494654_m1	Exon 11-12 (start 1476)
Gli2	Mm01293111_m1	Exon 13-14 (start 2598)
Gli3	Mm00492345_m1	Exon 14-15 (start 2867)
Hhip	Mm00469580_m1	Exon 12-13 (start 2406)



#### Supplemental Movie 1:

This movie is a confocal Z-stack from an eight-week-old *Fsp-Cre;Ptch1<sup>+/+</sup>;mTmG+* mammary duct showing a clear ductal lumen. GFP and RFP are endogenous fluorescence from the *mTmG* reporter; GFP indicates *Fsp-Cre+* stromal cells, while RFP is expressed by *Fsp-* cells.



#### Supplemental Movie 2:

This movie is a confocal Z-stack of eight-week-old *Fsp-Cre*; *Ptch1<sup>fl/fl</sup>*; *mTmG*+ mammary ducts showing some ducts with clear lumens, and a duct filled with RFP+ cells (upper right). GFP and RFP are endogenous fluorescence from the *mTmG* reporter; GFP indicates *Fsp-Cre*+ cells, while RFP is expressed by non-*Fsp*+ cells.