SUPPLEMENTARY INFORMATION

Seifert et al. *Sonic hedgehog controls growth of external genitalia by regulating cell cycle kinetics.*

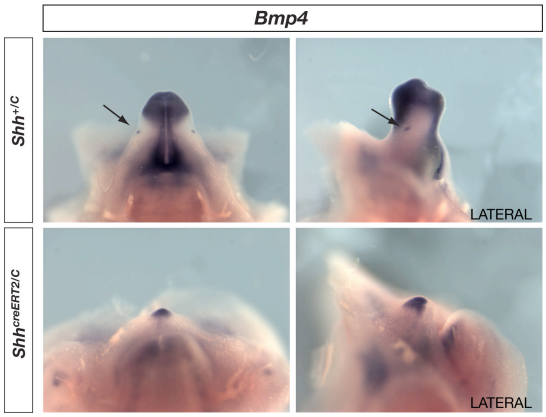

Figure S1 ShhcreERT2 embryos show regionalized reduction of Bmp4 expression. *Bmp4* expression in E13.5 *Shh*$^{creERT2/C}$ embryos and wildtype littermate 48 hours after Shh pathway inactivation. In mutant embryos, *Bmp4* expression remains at the distal tip of the genital tubercle, but is down-regulated in the mesenchyme adjacent to the urethral plate and is undetectable in the region where the preputial glands normally develop (black arrows).

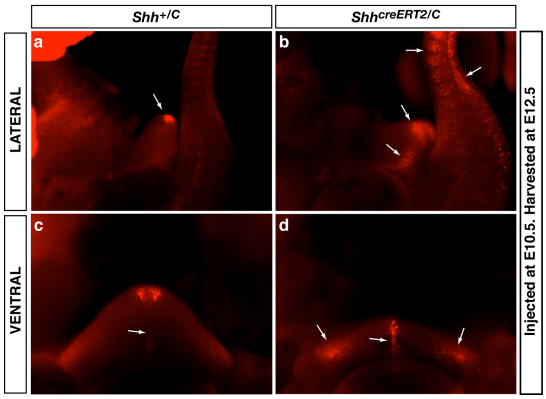

Figure S2 Loss of Shh signaling does not result in widespread cell death in the genital tubercle. Comparison of cell death in control *Shh*$^{C/+}$ (a, c) and *Shh*$^{creERT2/C}$ (b, d) genitalia 48 hours after injection of tamoxifen at E10.5. Red staining shows cells that are positive for Lysotracker Red, which labels dying cells. (**a, b)** Lateral views of the genital tubercles with the tail to the right. Arrows show regions of abundant cell death. **(c, d)** Ventral views of genital tubercles. Lysotracker red showed similar low levels of mesodermal and endodermal cell death in *Shh*$^{creERT2/C}$ and wild type genital tubercles, except for two small domains of dying cells that were seen at the proximal-lateral edges of mutant tubercle (vertical arrows in d). Horizontal arrows in (c, d) show cell death in urethral plate.

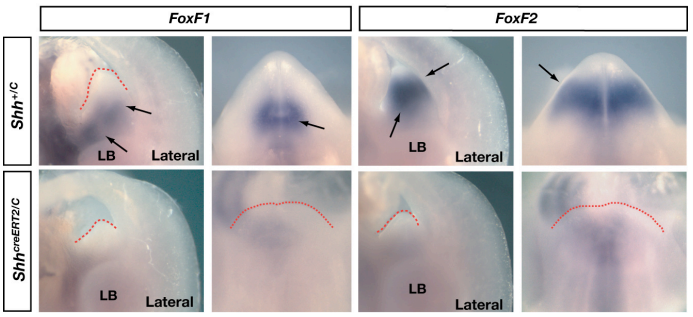

Figure S3. Loss of Shh signaling results in down-regulation of *FoxF1* and *FoxF2*. *In situ* hybridization of *Foxf1* and *Foxf2* in control *Shh*$^{C/+}$ and *Shh*$^{creERT2/C}$ embryos. Twenty-four hours after loss of Shh signaling at E11.5 (tamoxifen injection at E10.5), both *Foxf1* and *Foxf2* are down-regulated in genital mesenchyme (black arrows). Arrows mark normal regions of expression in control embryos. Red outlines show tubercle in *Shh*$^{creERT2/C}$ embryos. LB, Limb bud.