Supplemental Figure 1: Levels of Sprouty gene expression vary across tissues. Quantitative real-time PCR was used to measure expression of *Spry1, Spry2,* and *Spry4* in lung, kidney, and GTs of E14.5 male wild-type embryos.

Supplemental Figure 2: Female GT development is normal in *Spry1*$^{-/-}$;*Spry2*$^{-/-}$ embryos. Scanning electron micrographs of control and mutant female GTs at E14.5 and E16.5 (A-D). Coronal sections of E18.5 control and mutant GTs stained with hematoxylin and eosin (E, F). GT, genital tubercle; PS, preputial swelling; Pre, prepuce; UO, urethral opening; Ur, urethra; LS, labioscrotal fold. Scale bar, 500 μm.

Supplemental Figure 3: Urorectal septation is complete in *Spry1*$^{-/-}$;*Spry2*$^{-/-}$ GTs. Sagittal sections of E16.5 control and *Spry1*$^{-/-}$;*Spry2*$^{-/-}$ embryos stained with hematoxylin and eosin (A, B). In control embryos, the lumen of the urethra (green arrow) begins at the bladder neck and extends to the tip of the GT, whereas the rectal lumen (blue arrow) is separated from the urethra by the urorectal septum and forms a separate channel caudally (A). Septation of the urethra from the rectum is also complete in *Spry1*$^{-/-}$;*Spry2*$^{-/-}$ embryos, but the urethral lumen terminates at the base of the GT rather than at the distal tip. (B) GT, genital tubercle; re, rectum; bl, bladder. Scale bar, 500 μm.

Supplemental Figure 4: Deletion of *Spry1* and *Spry2* in the urethral epithelium, but not the GT mesenchyme, is sufficient to induce abnormal development of the GT. *Shh*^{CreEGFP} mice were used to delete *Spry1* and *Spry2* from the endoderm-derived

urethral epithelium, while *Tbx4*^{Cre} mice were used to delete them in the GT mesenchyme. Domains of Cre activity in the E14.5 and E16.5 GT were characterized by crossing either *Shh*^{CreEGFP} or *Tbx4*^{Cre} mice with mice carrying the *R26R-lacZ* reporter allele, followed by whole-mount lacZ staining (A-D). Gross morphology (E-G) and coronal histological sections (H-J) of E18.5 GTs show that genital development is relatively unchanged in *Tbx4*^{Cre}*;Spry1*$^{-/-}$*;Spry2*$^{-/-}$ male mice, while *Shh*^{CreEGFP}*;Spry1*$^{-/-}$*;Spry2*$^{-/-}$ male mice displayed the same urethral closure defects observed in global *Spry1/Spry2* mutant mice (J, asterisk). Scale bars, 500 μm (A-G), 400 μm (H-J).