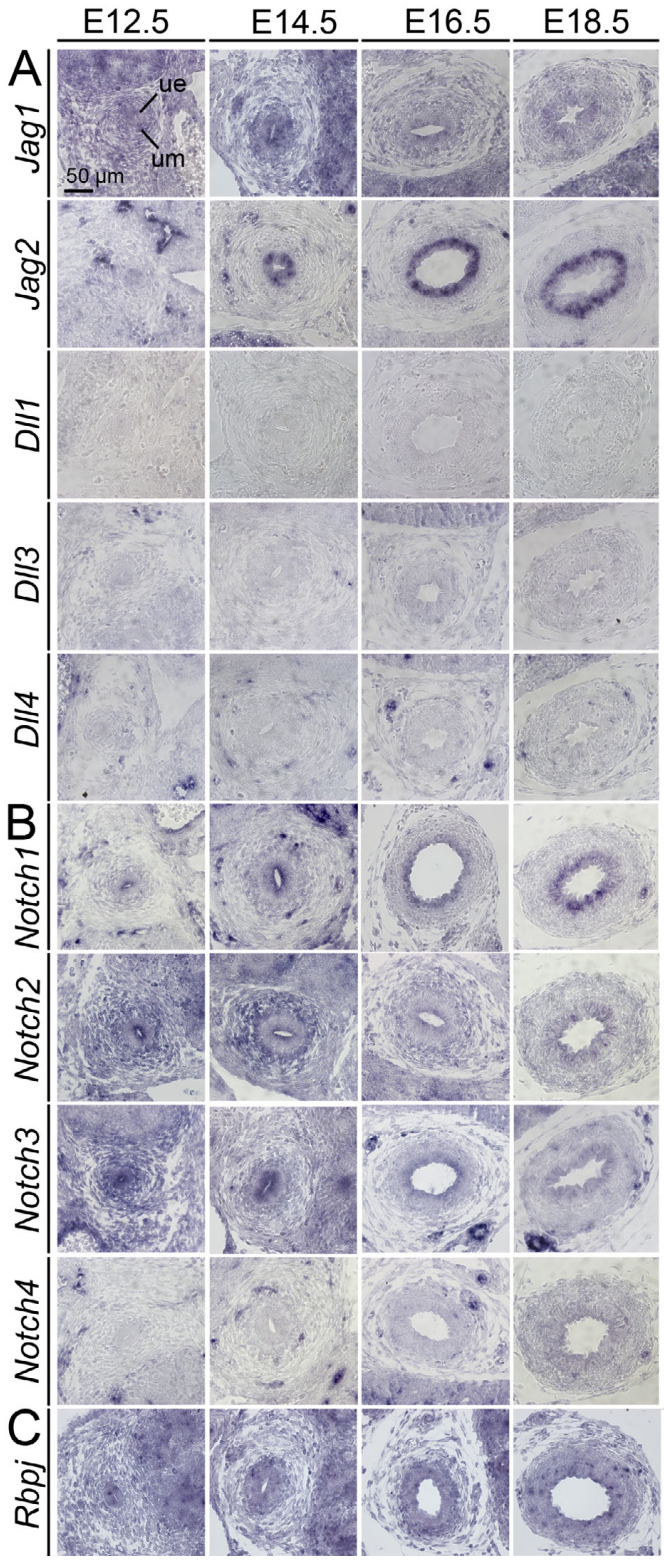

Fig. S1. Notch signaling components are expressed during murine ureter development. (**A-C**) RNA *in situ* hybridization analysis on transverse sections of the proximal ureter for expression of genes encoding Notch ligands (**A**), Notch receptors (**B**) and the signaling mediator *Rbpj* (**C**). n>=3 for all probes and stages. ue, ureteric epithelium; um, ureteric mesenchyme.

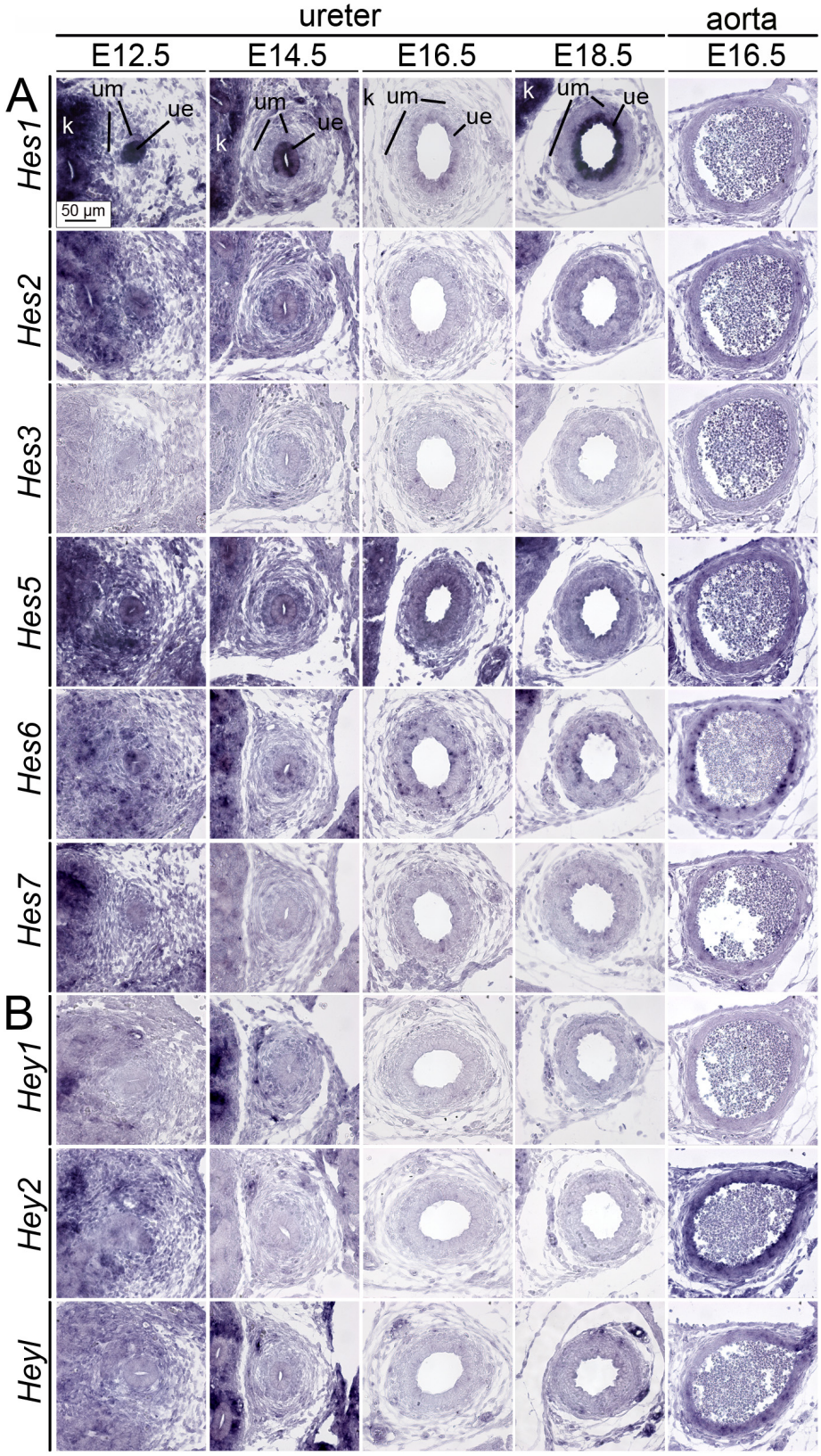

Fig. S2. Notch target genes are expressed during murine ureter development. (**A**,**B**) RNA *in situ* hybridization analysis on transverse sections of the proximal ureter and the dorsal aorta for expression of *Hes* (**A**) and *Hey* (**B**) genes. n>=3 for all probes and stages. k, kidney; ue, ureteric epithelium; um, ureteric mesenchyme.

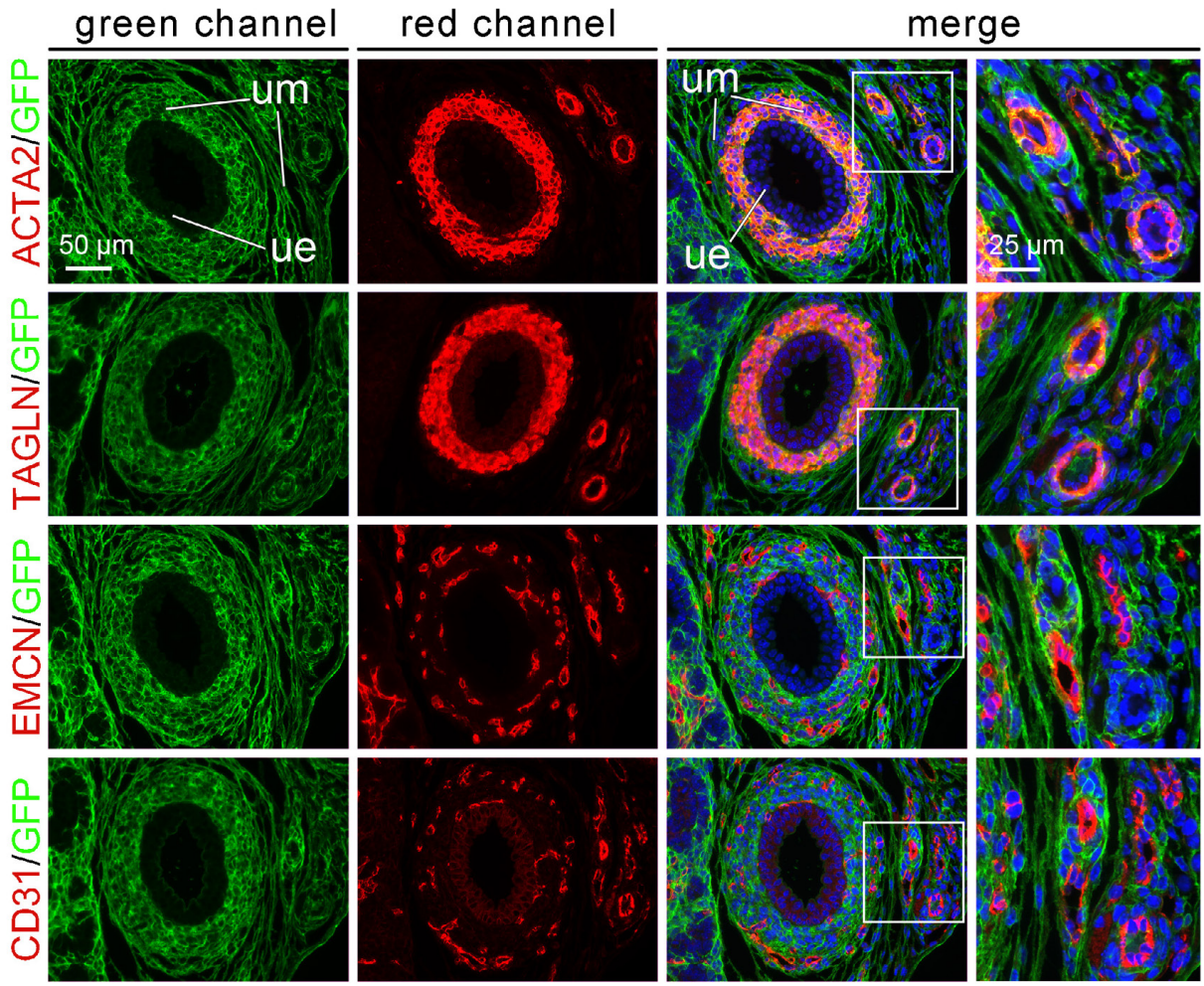

Fig. S3. Lineage analysis of *Tbx18*$^{+}$ descendants in the ureter. Co-immunofluorescence analysis on transverse sections of the proximal ureter of E18.5 *Tbx18*$^{cre/+}$;*R26*$^{mTmG/+}$ embryos for expression of the lineage marker GFP and of differentiation markers for SMCs (ACTA2, TAGLN) and endothelial cells (EMCN, CD31). Shown are the green channel for GFP expression (first column), the red channel for the differentiation marker (second column), and a merge of the two channels (third and fourth column). The fourth column shows higher magnification images of the regions marked by a white square in the third column, which contain vessels with SMC investment. n=5 for all markers. Note that visceral and vascular SMCs arise from *Tbx18*$^{+}$ mesenchymal progenitors whereas endothelial cells are of a different origin. ue, ureteric epithelium; um, ureteric mesenchyme.

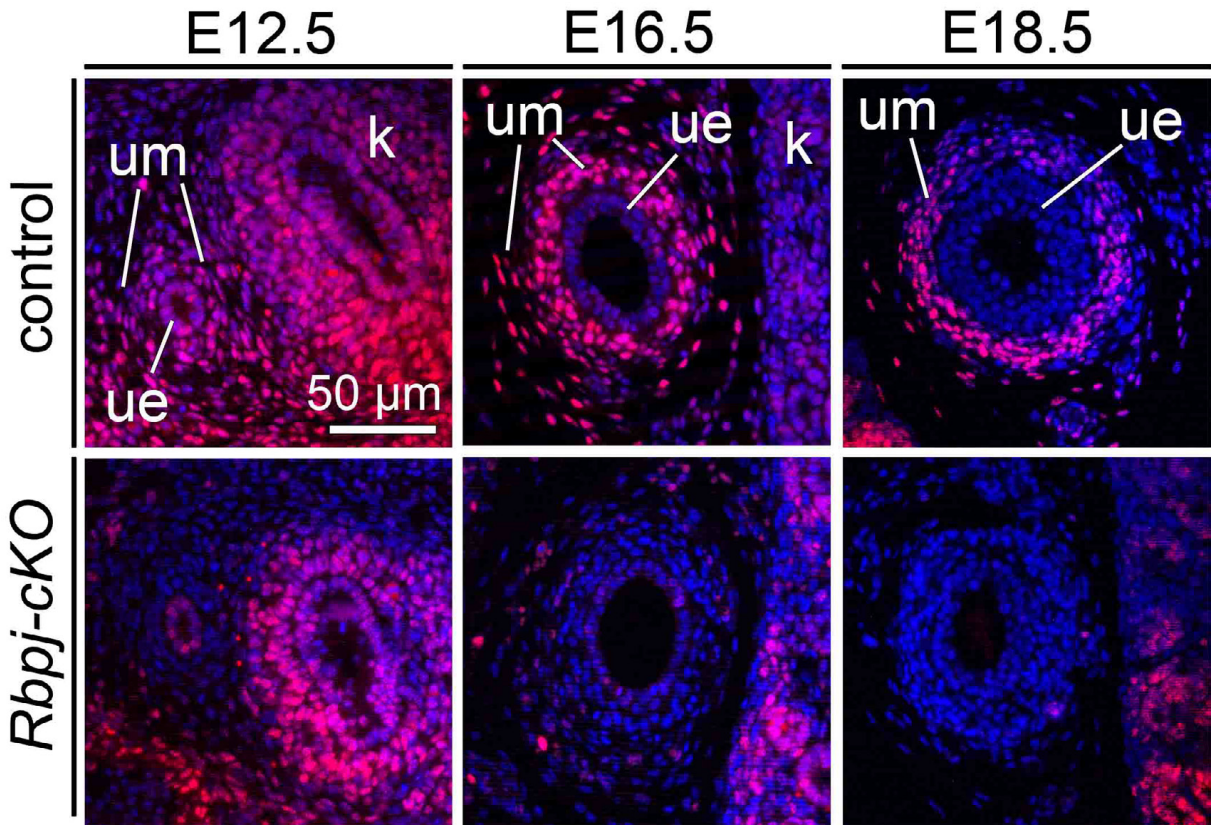

Fig. S4. Loss of RBPJ expression in the UM of *Rbpj-cKO* embryos. Immunofluorescence analysis of RBPJ expression on transverse sections of the proximal region of control and *Rbpj-cKO* ureters at E12.5, E16.5 and E18.5; n=4 for both genotypes and stages. k, kidney; ue, ureteric epithelium; um, ureteric mesenchyme.

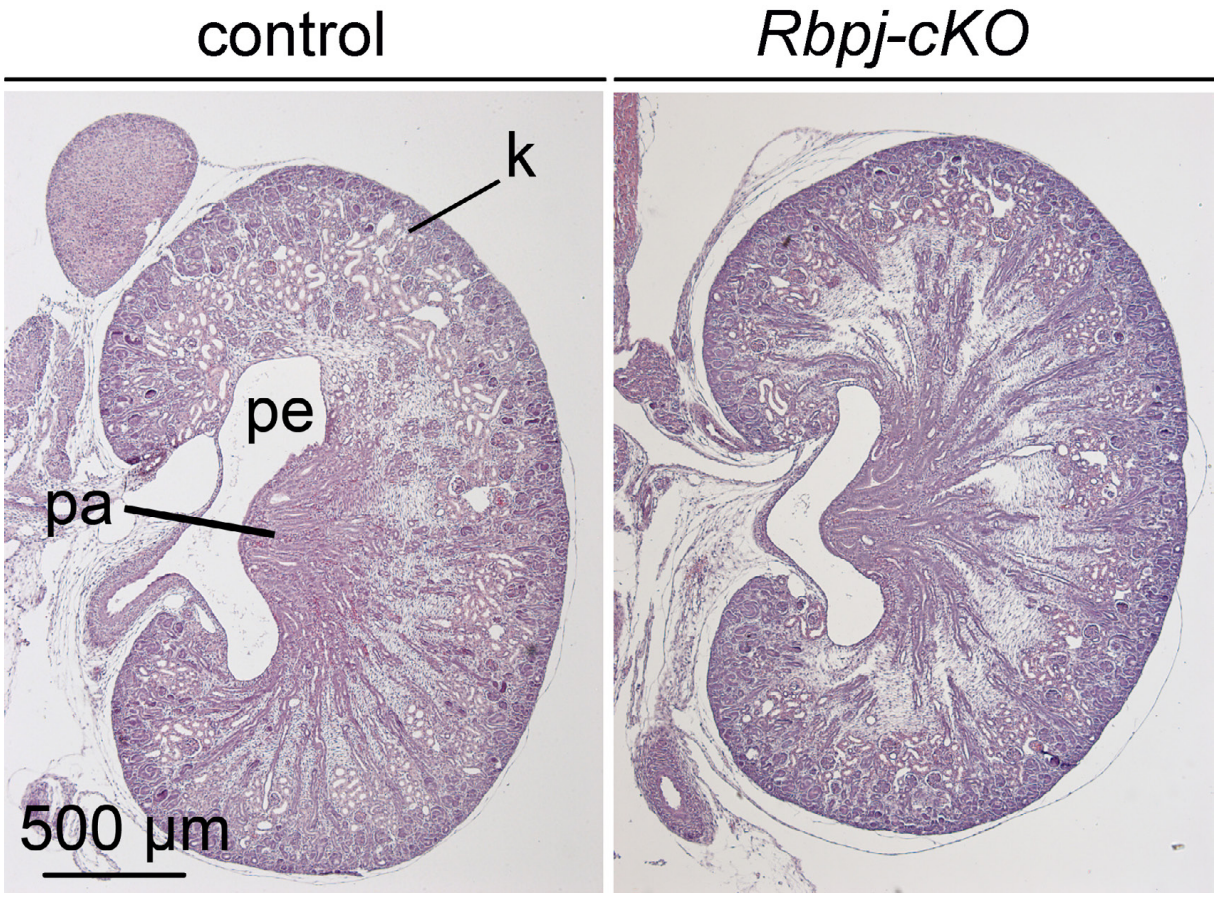

Fig. S5. Renal histology is unaltered in *Rbpj-cKO* embryos at E18.5. Hematoxylin and eosin staining of sagittal sections of control and *Rbpj-cKO* kidneys at E18.5. n=3 for both genotypes. k, kidney; pa, papilla; pe, pelvis;

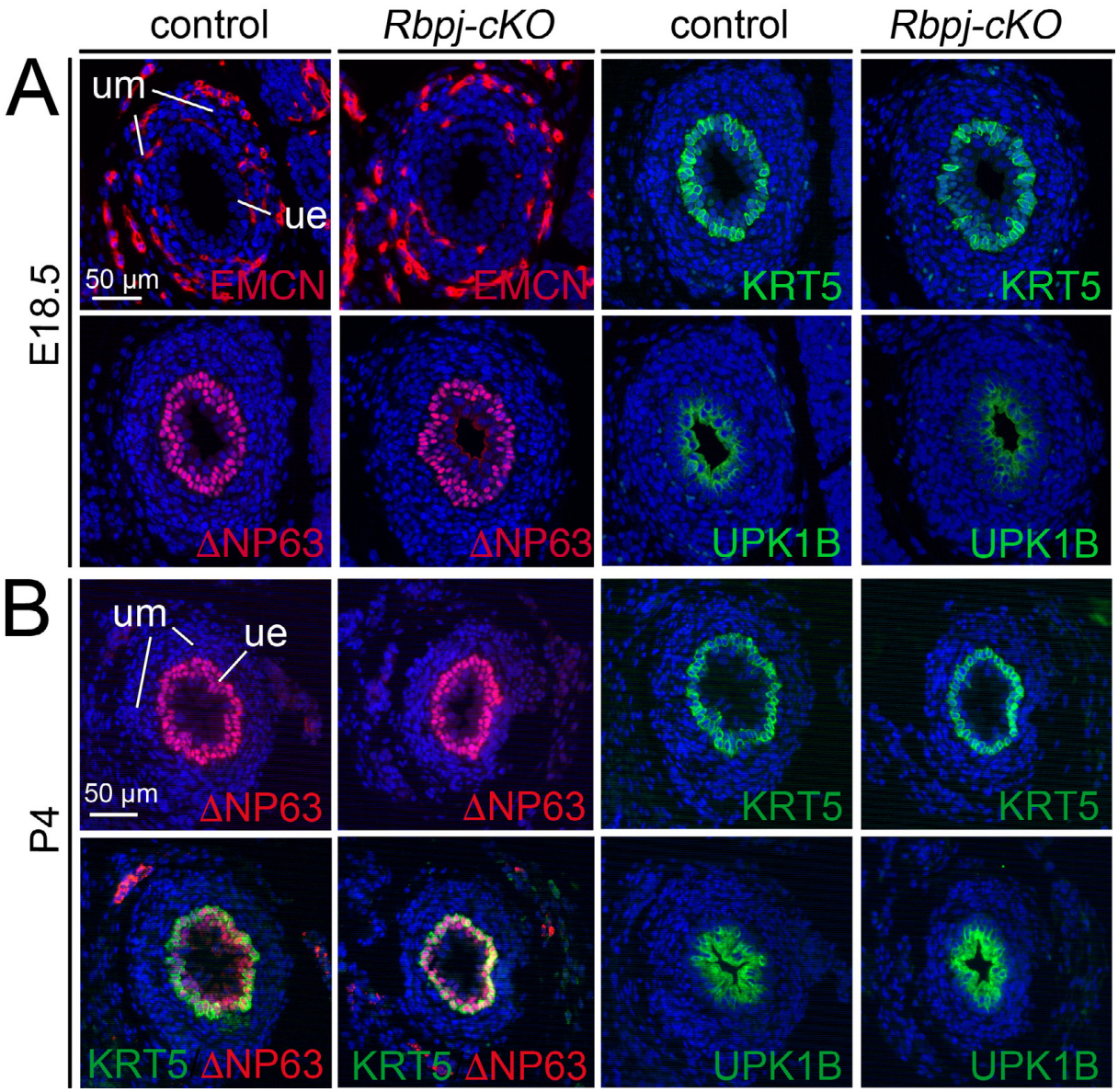

Fig. S6. *Rbpj-cKO* ureters do not show urothelial defects at E18.5 and P4. (A,B) Immunofluorescence analysis for endothelial (EMCN) and urothelial (KRT5, ΔNP63, UPK1B) differentiation markers on proximal sections of E18.5 (**A**) and P4 ureters (**B**). Nuclei (blue) are counterstained with DAPI. KRT5, ΔNP63 and UPK1B combinatorially mark basal cells ($KRT5^{+}\Delta NP63^{+}UPK1B^{-}$), intermediate cells ($KRT5^{-}\Delta NP63^{+}UPK1B^{+}$) and superficial cells ($KRT5^{-}\Delta NP63^{-}UPK1B^{+}$). n=4 for each marker, genotype and stage. ue, ureteric epithelium; um, ureteric mesenchyme.

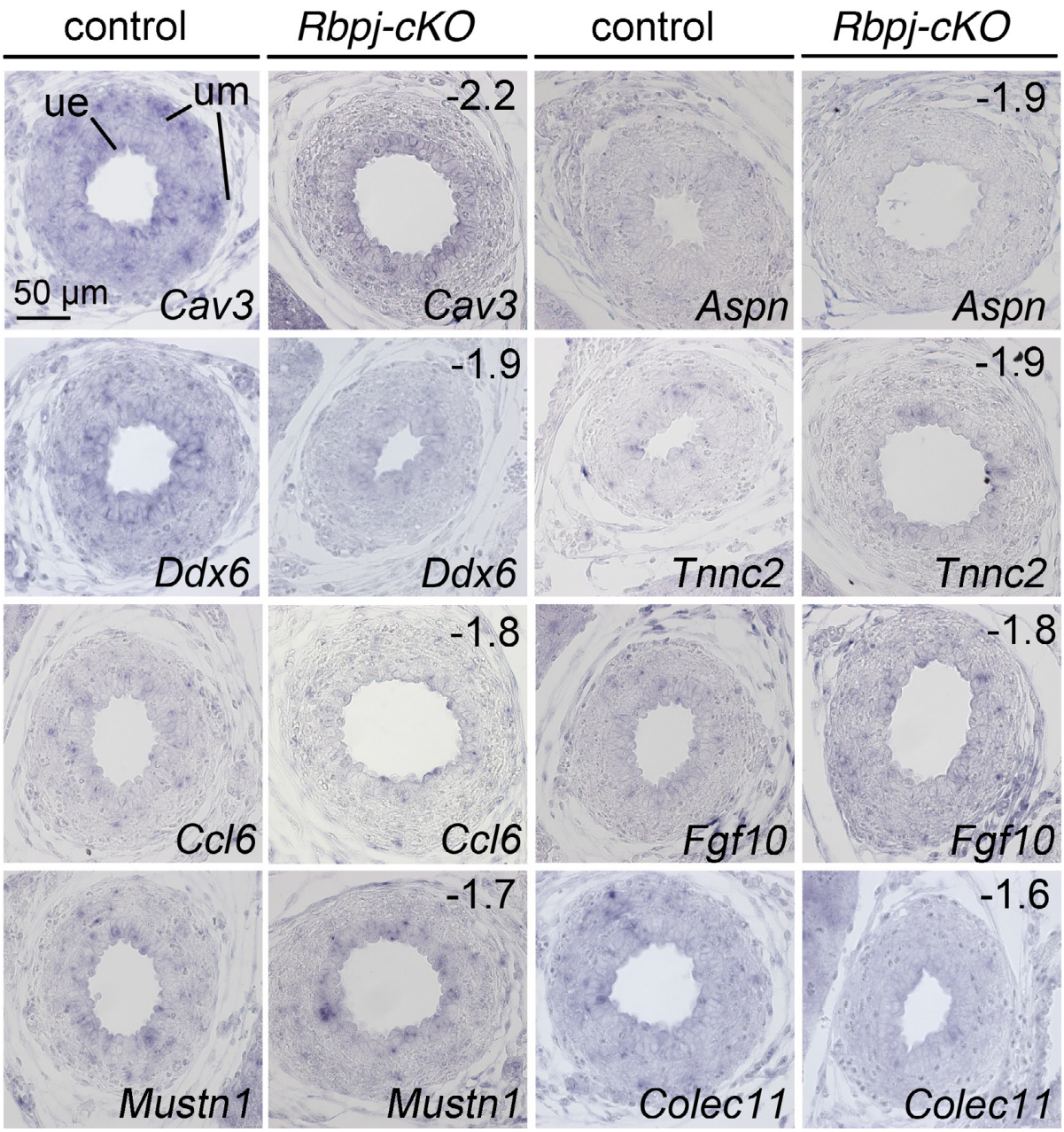

Fig. S7. RNA *in situ* hybridization analysis of candidate genes with decreased expression in microarrays of E18.5 *Rbpj-cKO* ureters. RNA *in situ* hybridization analysis of selected candidate genes with decreased expression in microarrays of E18.5 *Rbpj-cKO* ureters was performed on transverse sections of the proximal ureter region of control and *Rbpj-cKO* embryos at E18.5. Probes, genotypes and fold changes in the microarray are as indicated. n>=3 for all probes and genotypes. ue, ureteric epithelium; um, ureteric mesenchyme.

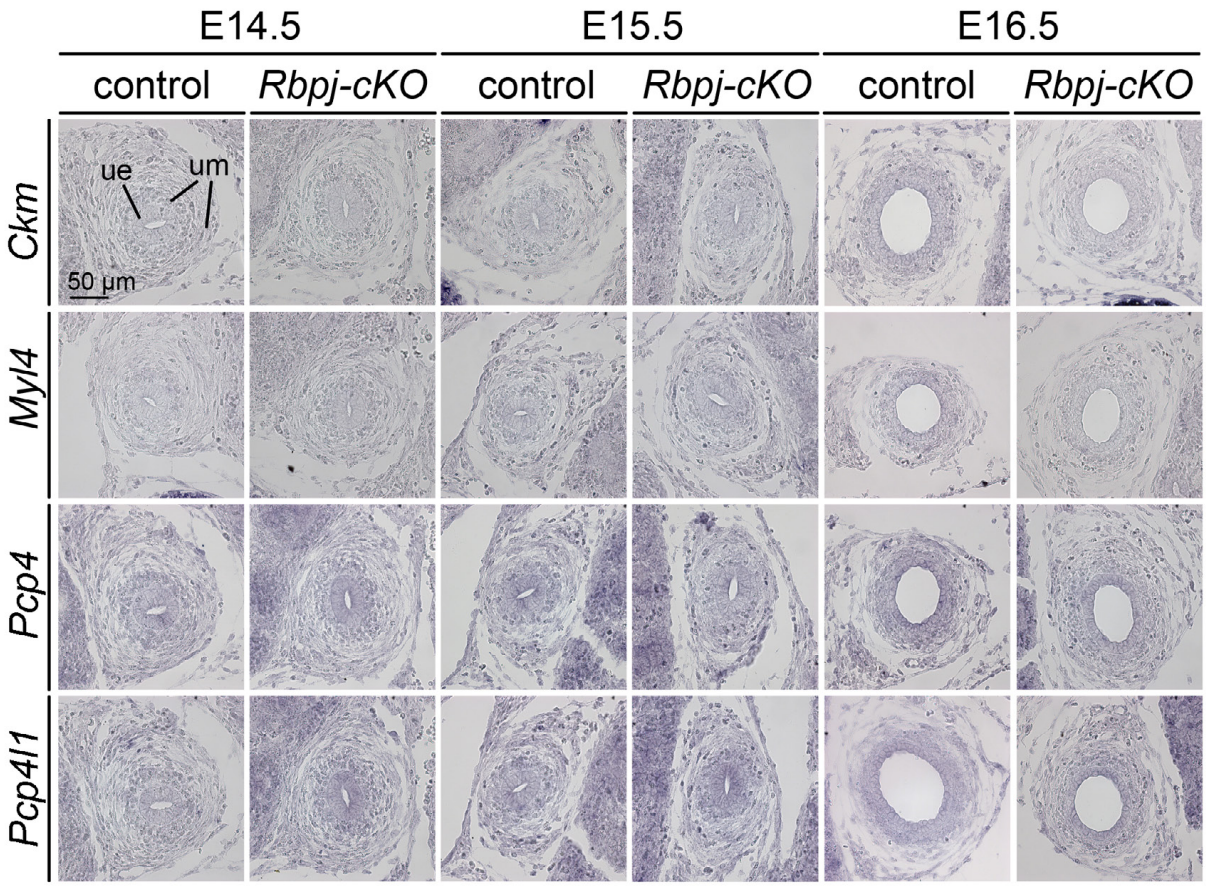

Fig. S8. RNA *in situ* hybridization analysis of selected SMC genes in ureter development. RNA *in situ* hybridization analysis of selected SMC genes was performed on transverse sections of the proximal ureter region of control and *Rbpj-cKO* embryos at E14.5, E15.5 and E16.5. n>=3 for all probes and genotypes. ue, ureteric epithelium; um, ureteric mesenchyme.

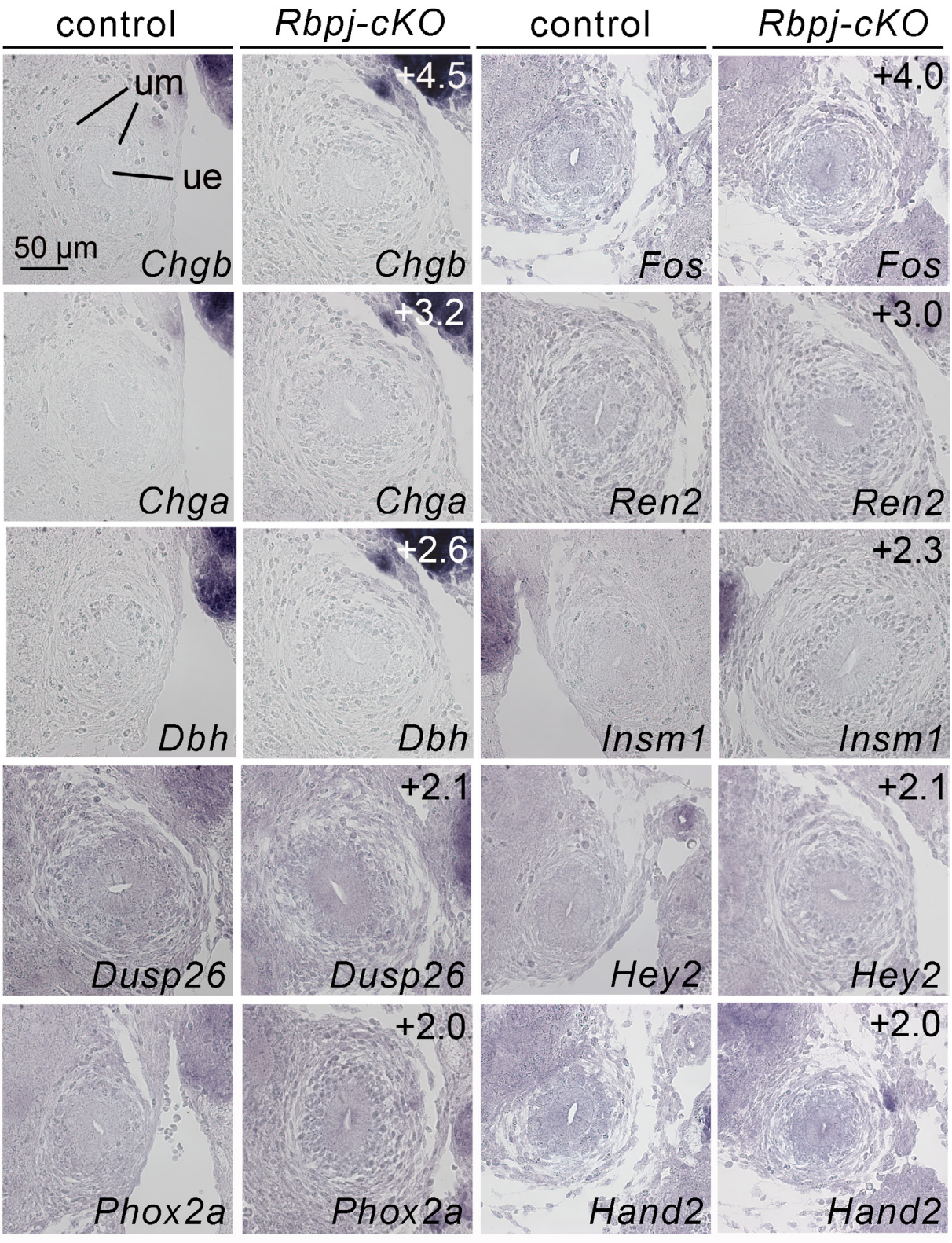

Fig. S9. RNA *in situ* hybridization analysis of candidate genes with increased expression in microarrays of E14.5 *Rbpj-cKO* ureters. RNA *in situ* hybridization analysis of selected candidate genes with increased expression in microarrays of E14.5 *Rbpj-cKO* ureters was performed on transverse sections of the proximal ureter region of E14.5 control and *Rbpj-cKO* embryos. Numbers refer to fold increase in the microarray. n>=3 for all probes and genotypes. ue, ureteric epithelium; um, ureteric mesenchyme.

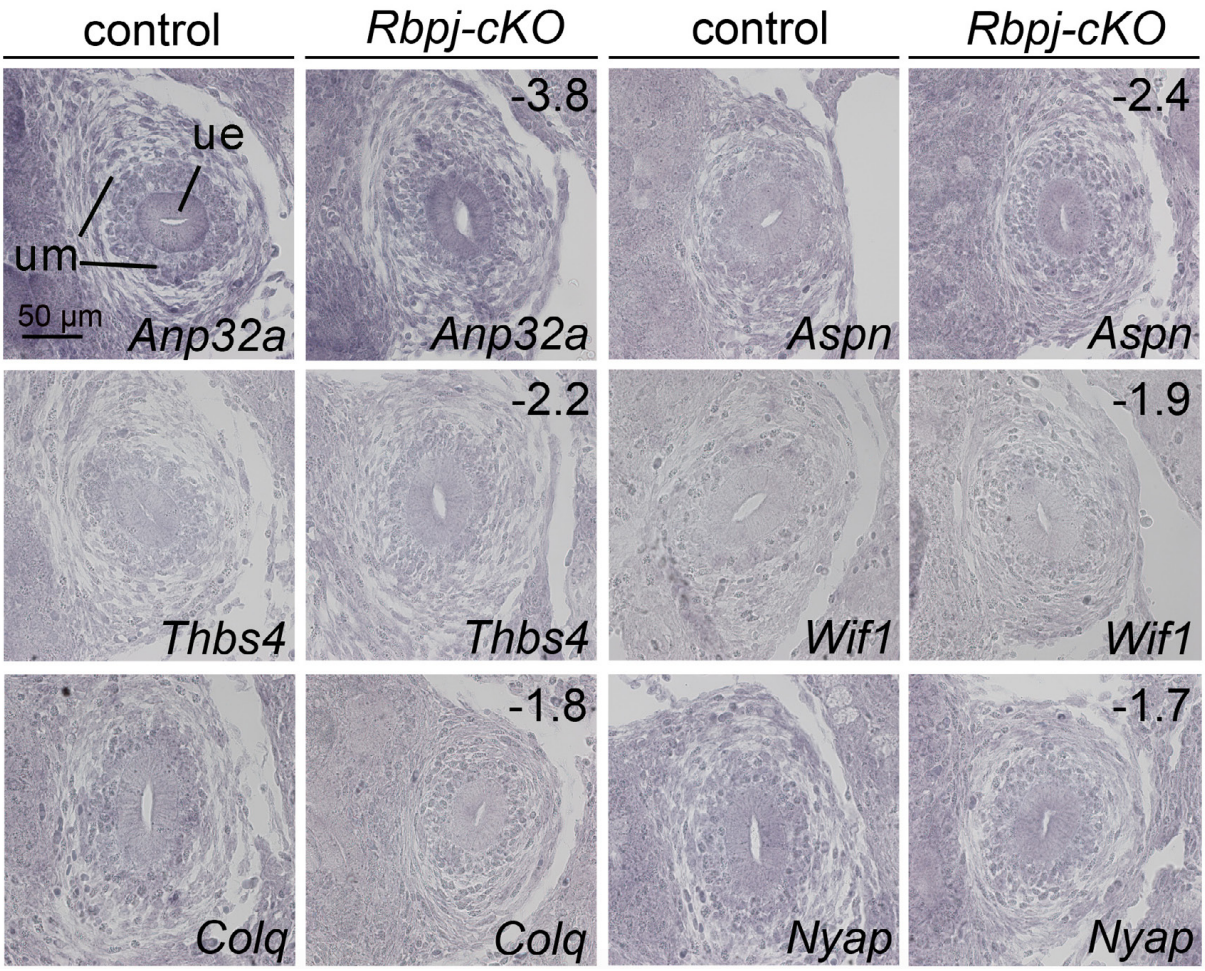

Fig. S10. RNA *in situ* hybridization analysis of candidate genes with decreased expression in microarrays of E14.5 *Rbpj-cKO* ureters. RNA *in situ* hybridization analysis of selected candidate genes with decreased expression in microarrays of E14.5 *Rbpj-cKO* ureters was performed on transverse sections of the proximal ureter region of E14.5 control and *Rbpj-cKO* embryos. Numbers refer to fold change in the microarray. n>=3 for all probes and genotypes. ue, ureteric epithelium; um, ureteric mesenchyme.

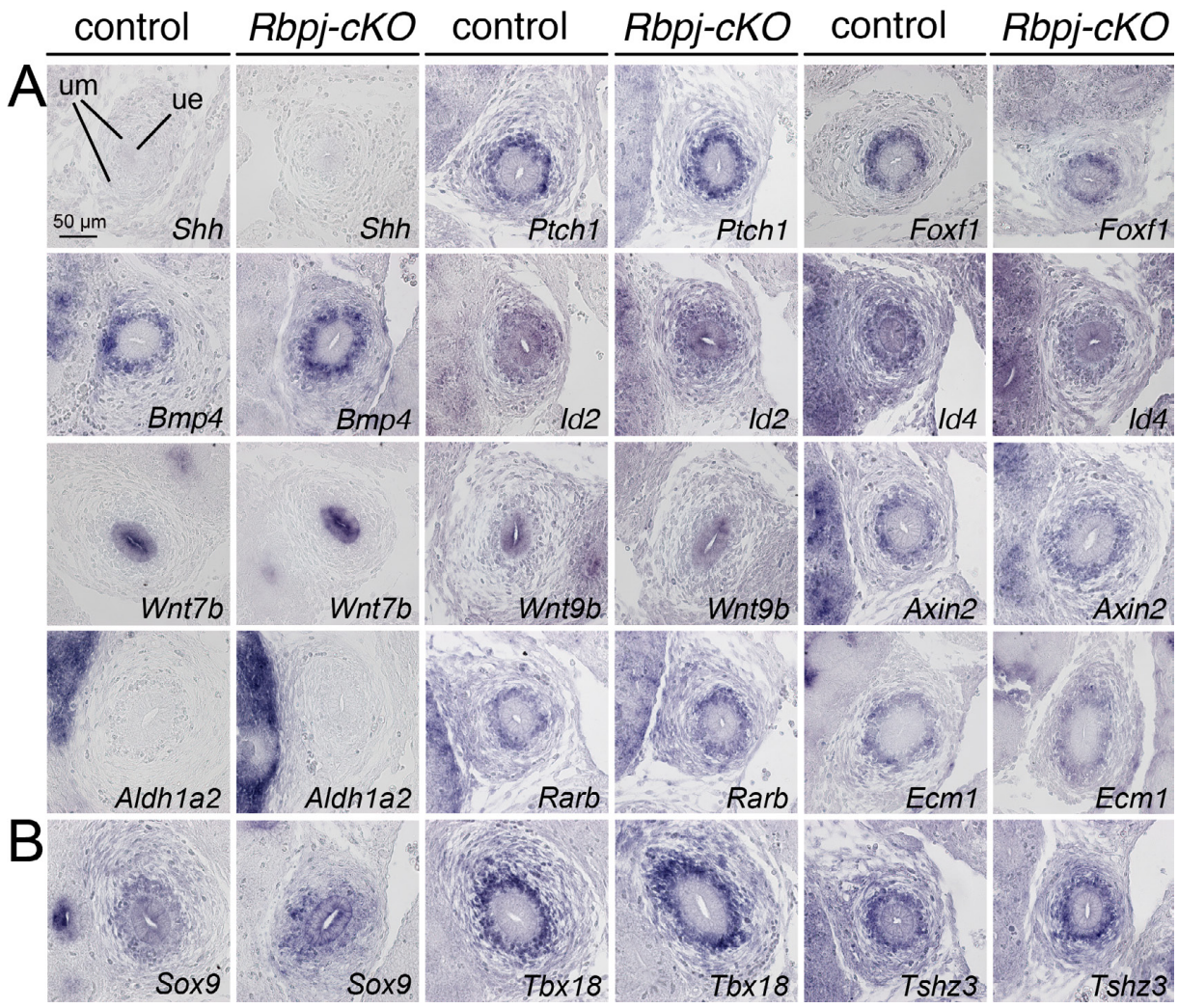

Fig. S11. Signaling pathways and transcription factor genes relevant for SMC differentiation are unchanged in their activity/expression in *Rbpj-cKO* ureters at E14.5. (**A**,**B**) RNA *in situ* hybridization analysis of of expression of *Shh*, its target gene *Ptch1* and its effector gene *Foxf1*; of *Bmp4*, its target genes *Id2* and *Id4*; of *Wnt7b* and *Wnt9b*, and the WNT target gene *Axin2*; of the gene encoding the RA synthesizing enzyme *Aldh1a2*, and the targets of RA signaling activity in the UM, *Rarb* and *Ecm1* (**A**) and of the transcription factor genes *Sox9*, *Tbx18* and *Tshz3* (**B**) on transverse sections of the proximal ureter of control and *Rbpj-cKO* embryos at E14.5. Genotypes, probes and fold change in the microarray are shown. n>=3 for all probes and genotypes. ue, ureteric epithelium; um, ureteric mesenchyme.

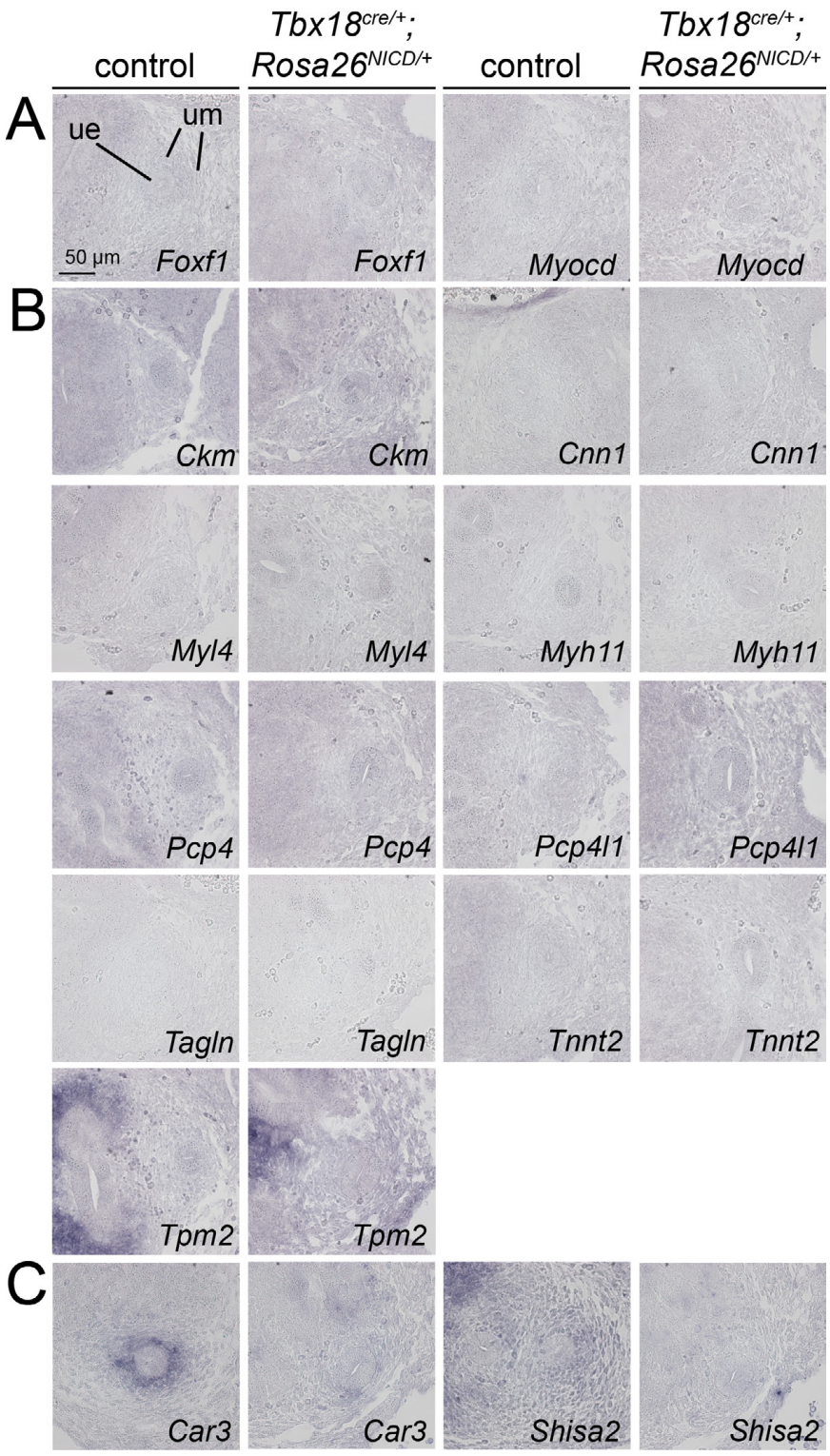

Fig. S12. Ectopic expression of the Notch1 intracellular domain (N1ICD) does not induce premature expression of SMC regulatory and structural genes in the UM. (**A**) RNA *in situ* hybridization analysis on transverse sections of E12.5 control and *Tbx18*$^{cre/+}$;*Rosa26*$^{NICD/+}$ ureters for expression of SMC regulatory genes (**A**), SMC structural genes (**B**) and genes with reduced expression in E14.5 *Rbpj-cKO* microarray, *Car3* and *Shisa2* (**C**); n=3 for all probes and genotypes. ue, ureteric epithelium; um, ureteric mesenchyme.

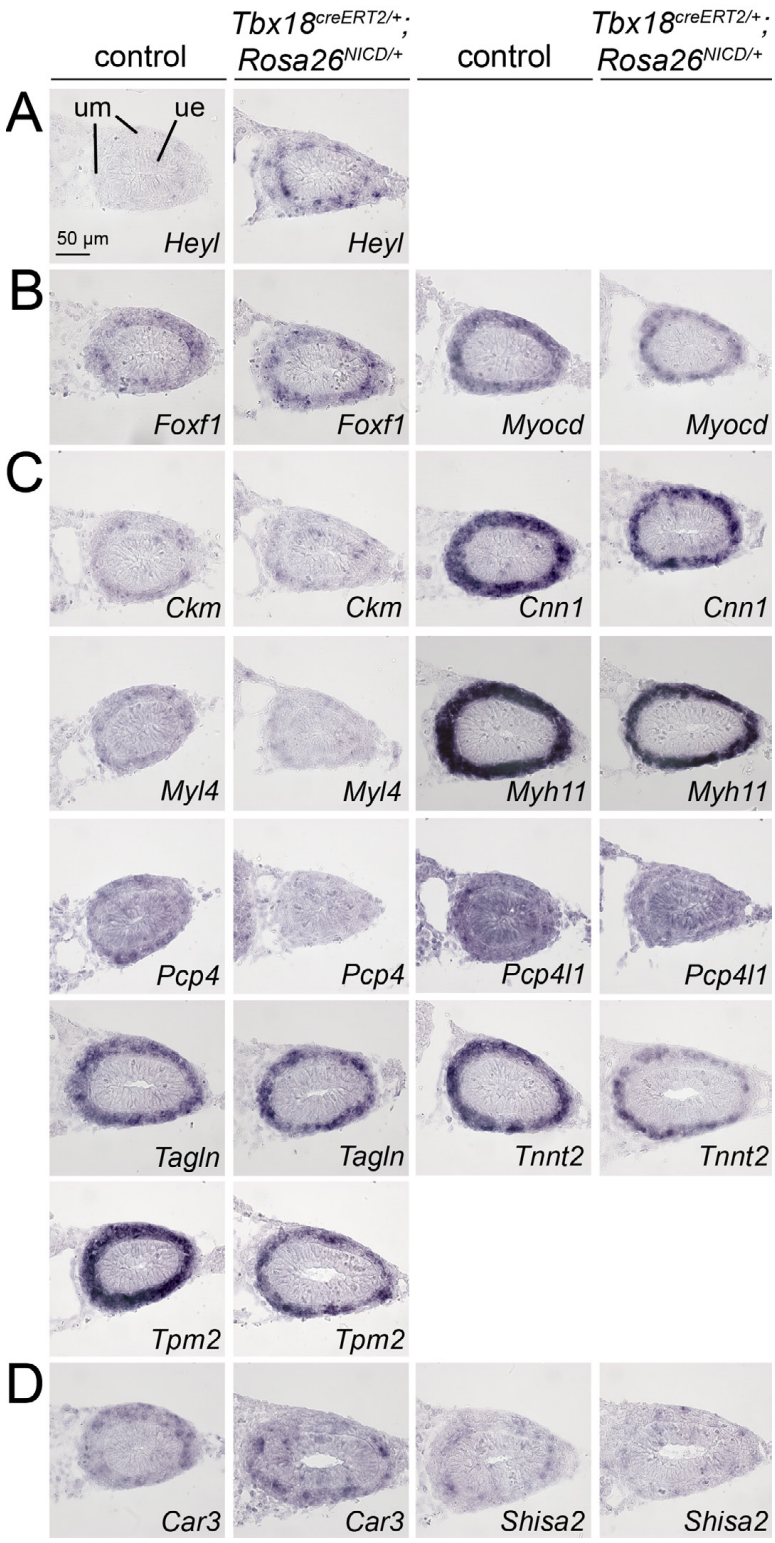

Fig. S13. Ectopic expression of the Notch1 intracellular domain (N1ICD) affects the homogeneity of expression of SMC regulatory and structural genes in the UM. (**A**) RNA *in situ* hybridization analysis on transverse sections of organ explants of 13.5 control and *Tbx18*$^{creERT2/+}$;*Rosa26*$^{NICD/+}$ ureters cultured for 4 days in the presence of 4-hydroxytamoxifen for expression of the Notch target gene *Heyl* (**A**), of SMC regulatory genes (**B**), of SMC structural genes (**C**), and of genes with reduced expression in the E14.5 *Rbpj-cKO* microarray, *Car3* and *Shisa2* (**D**); n=4 for all probes and genotypes. ue, ureteric epithelium; um, ureteric mesenchyme.

Table S1. Genes with altered expression in microarrays of E18.5 *Rbpj-cKO* ureters.

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Table S2. Functional annotation and clustering of genes with decreased expression in E18.5 *Rbpk-cKO* ureters.

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Table S3. Functional annotation and clustering of genes with increased expression in E18.5 *Rbpk-cKO* ureters.

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Table S4. RT-qPCR analysis of expression of SMC genes in different conditions.

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Table S5. Genes with altered expression in microarrays of P4 *Rbpj-cKO* ureters.

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Table S6. Functional annotation of genes with decreased expression in the microarray of P4 *Rbpj-cKO* ureters.

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Table S7. Functional annotation of genes with increased expression in the microarray of P4 *Rbpj-cKO* ureters.

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Table S8. Functional annotation of genes with decreased expression in the microarrays of both E18.5 and P4 *Rbpj-cKO* ureters.

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Table S9. Statistical analysis of contraction frequencies and intensities of E14.5 control and *Rbpj-cKO* ureters over 8 days of culture.

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Table S10. Statistical analysis of contraction frequencies and intensities of E18.5 control and *Rbpj-cKO* ureters over 6 days of culture.

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Table S11. Genes with altered expression in microarrays of E14.5 *Rbpj-cKO* ureters.

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Table S12. Functional annotation of genes with altered expression in microarrays of *E14.5 Rbpk-cKO* ureters.

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Table S13. Statistical analysis of ureter contraction frequency in contralateral explanted E12.5 ureters treated with either DMSO or 1 μM DAPT or 2.5 μM DAPT over 10 days of culture (relates to Figure 7A,B).

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Table S14. Statistical analysis of ureter contraction frequency in contralateral explanted E18.5 ureters treated with either DMSO or 1 μM DAPT over 6 days of culture (relates to Figure 7C).

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Table S15. Statistical analysis of ureter contraction frequency in contralateral explanted P4 ureters treated with either DMSO or 1 μM DAPT over 6 days of culture (relates to Figure 7E).

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Table S16. Primer for RT-qPCR analysis of gene expression.

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