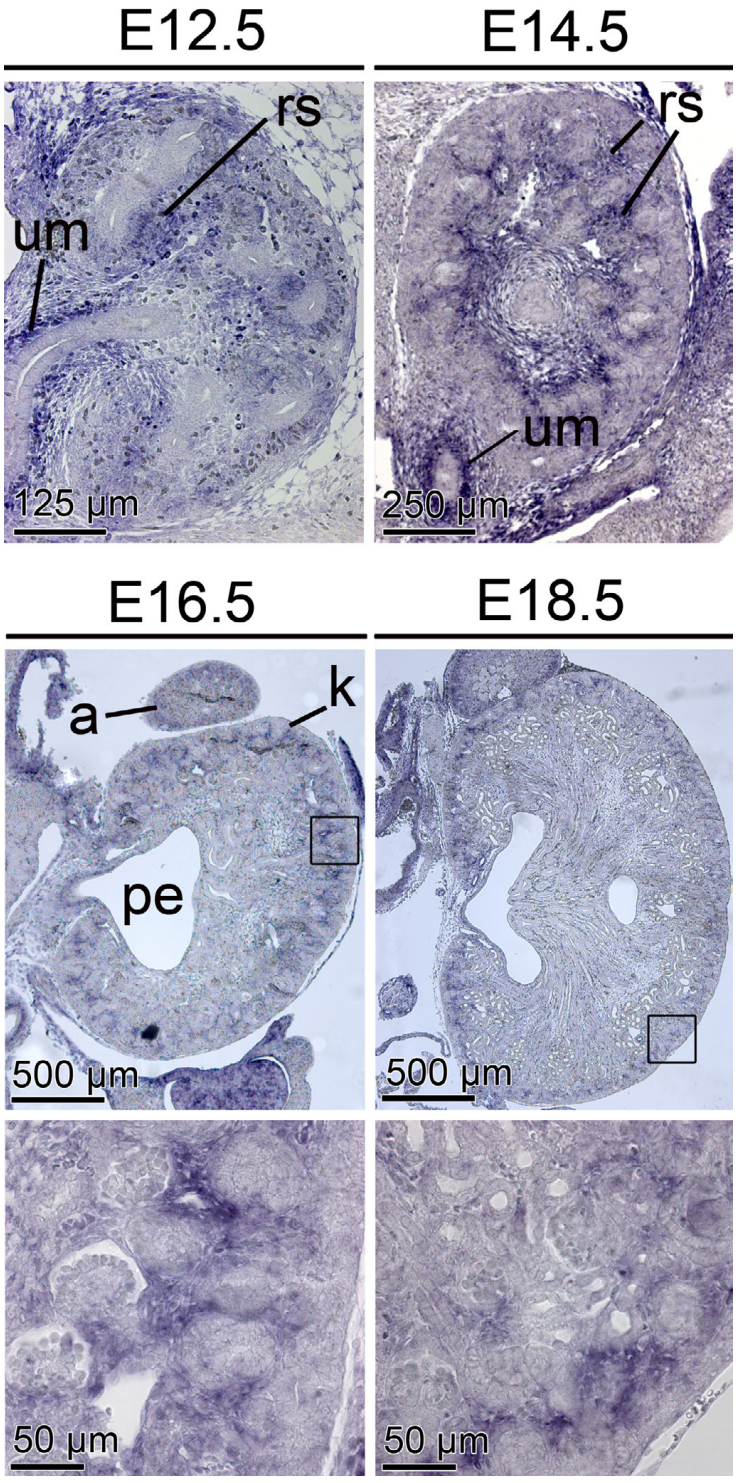

Fig. S1. *Gata6* expression occurs in the renal stroma. *In situ* hybridization analysis of *Gata6* expression on sagittal kidney sections of E12.5, E14.5, E16.5 and E18.5 wildtype embryos. Note that *Gata6* expression occurs in the entire renal stroma at E12.5 and E14.5, but is confined to the stroma at the medullary-cortical border region at E16.5 and E18.5. n≥3 for each stage. a, adrenal gland; k, kidney; pe, renal pelvis; rs, renal stroma; um, ureteric mesenchyme.

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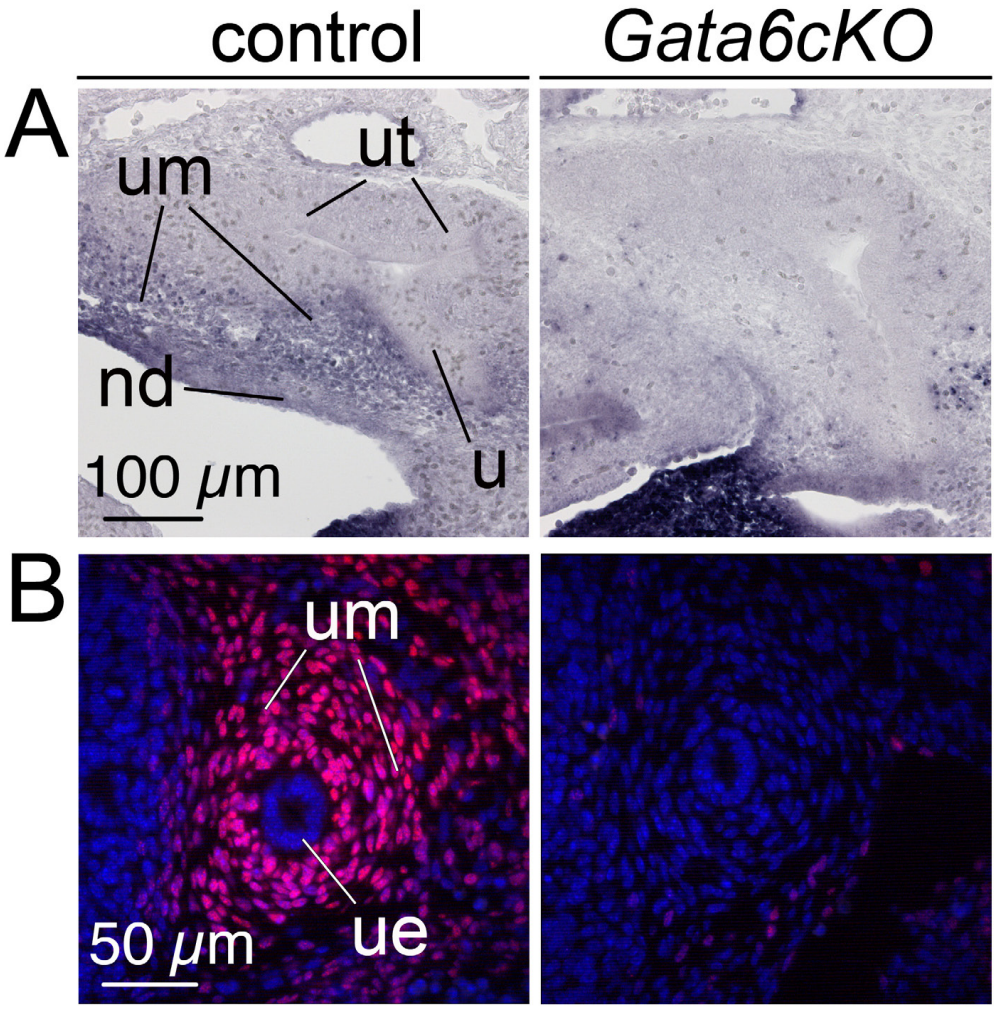

Fig. S2. The *Tbx18*^{cre} driver line mediates conditional deletion of *Gata6* in the UM. (A) *In situ* hybridization analysis of *Gata6* expression on sagittal sections of the metanephros of E11.5 wildtype and *Gata6cKO* (*Tbx18*$^{cre/+}$;*Gata6*$^{fl/fl}$) embryos using a probe against exon2, which is floxed in the *Gata6*^{fl} allele. (B) Immunofluorescence analysis of GATA6 protein on proximal sections of the ureter at E12.5. n≥3 for each assay and genotype. nd, nephric duct; ue, ureteric epithelium; um, ureteric mesenchyme; ut, ureteric tip; u, ureter.

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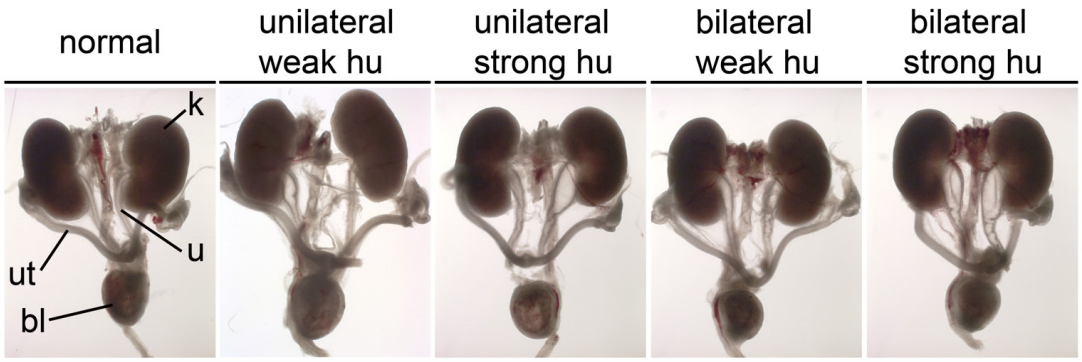
A

Genotype	*Tbx18*$^{+/+}$; *Gata6*$^{fl/+}$	*Tbx18*$^{+/+}$; *Gata6*$^{fl/fl}$	*Tbx18*$^{cre/+}$; *Gata6*$^{fl/+}$	*Tbx18*$^{cre/+}$; *Gata6*$^{fl/fl}$
(expected %)	(25%)	(25%)	(25%)	(25%)
E11.5, n=94	26 (28%)	25 (27%)	21 (22%)	22 (23%)
E12.5, n=896	220 (25%)	206 (23%)	251 (28%)	219 (24%)
E14.5, n=575	132 (23%)	139 (24%)	151 (26%)	153 (27%)
E15.5, n=95	31 (33%)	19 (20%)	19 (20%)	26 (27%)
E16.5, n=102	33 (32%)	22 (22%)	24 (23%)	23 (23%)
E18.5, n=205	57 (28%)	45 (22%)	48 (23%)	55 (27%)

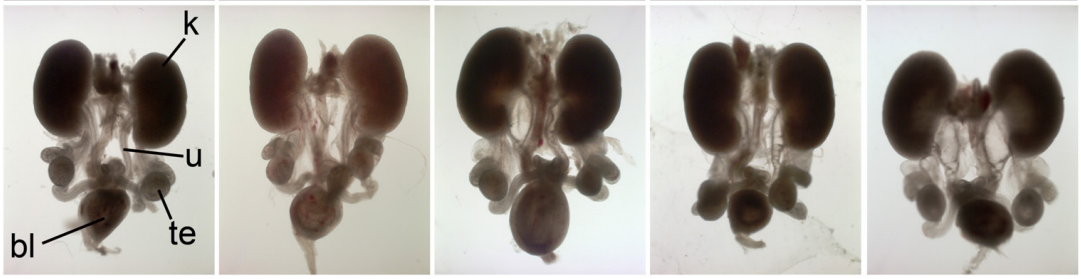
B



C

Genotype female	Control	*Tbx18*$^{cre/+}$; *Gata6*$^{fl/+}$	*Tbx18*$^{cre/+}$; *Gata6*$^{fl/fl}$
normal	26 (93%)	3 (25%)	5 (19%)
unilateral weak hu	1 (3.5%)	2 (17%)	4 (15%)
unilateral strong hu	0	2 (17%)	2 (8%)
bilateral weak hu	1 (3.5%)	5 (42%)	7 (27%)
bilateral strong hu	0	0	8 (31%)
total number	28	12	26

D



E

Genotype male	Control	*Tbx18*$^{cre/+}$; *Gata6*$^{fl/+}$	*Tbx18*$^{cre/+}$; *Gata6*$^{fl/fl}$
normal	33 (89%)	6 (30%)	5 (22%)
unilateral weak hu	3 (8%)	7 (35%)	3 (13%)
unilateral strong hu	0	1 (5%)	2 (9%)
bilateral weak hu	1 (3%)	6 (30%)	8 (35%)
bilateral strong hu	0	0	5 (22%)
total number	37	20	23

Fig. S3.

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Fig. S3. *Gata6cKO* embryos are viable and display ureter dilatation of variable severity at E18.5. (A) Distribution of genotypes in litters of matings of *Tbx18$^{cre/+}$;Gata6$^{fl/+}$* males with *Gata6$^{fl/fl}$* females at the indicated stages. Shown are the stages, the number of embryos, the expected and obtained frequency of the indicated genotypes. (B) Morphology of whole urogenital systems of E18.5 female *Gata6cKO* embryos displaying different grades of hydroureter (hu) used for classification in the Table shown in (C). (C) Distribution of ureter dilatations of different severity in female *Tbx18$^{cre/+}$;Gata6$^{fl/+}$* and *Tbx18$^{cre/+}$;Gata6$^{fl/fl}$* embryos at E18.5. (D) Morphology of whole urogenital systems of E18.5 male *Gata6cKO* embryos displaying different grades of hydroureter (hu) used for classification in the Table shown in (E). (E) Distribution of ureter dilatations of different severity in male *Tbx18$^{cre/+}$;Gata6$^{fl/+}$* and *Tbx18$^{cre/+}$;Gata6$^{fl/fl}$* embryos at E18.5. bl, bladder; k, kidney; te, testis; u, ureter; ut, uterus.

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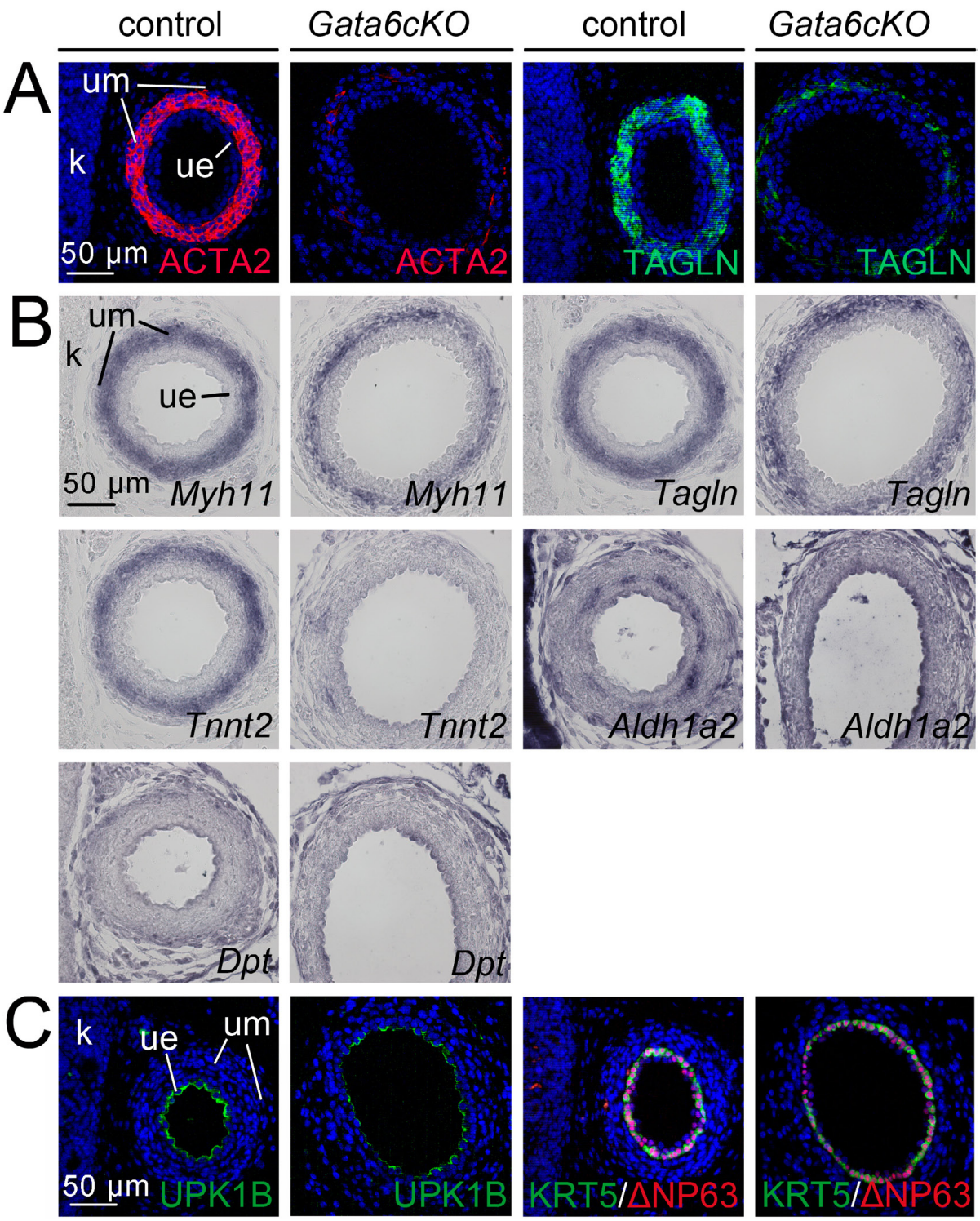

Fig. S4. Weak proximal hydroureter is associated with reduced expression of SMC markers in *Gata6cKO* embryos at E18.5. (A) Immunofluorescence of the SMC markers ACTA2 and TAGLN and (B) RNA *in situ* hybridization analysis of SMC genes (*Tagln, Tnnt2, Myh11*), of the *lamina propria* marker *Aldh1a2*, and of the *tunica adventitia* marker *Dpt* on transverse sections of the proximal ureter. (C) Analysis of urothelial differentiation by immunofluorescent detection of the B-cell marker KRT5, the I/B-cell marker ΔNP63 and the S-cell marker UPK1B on proximal ureter sections. Nuclei are counterstained with DAPI (blue, in A and C). n≥3 for each assay, genotype and probe. k, kidney; ue, ureteric epithelium; um, ureteric mesenchyme.

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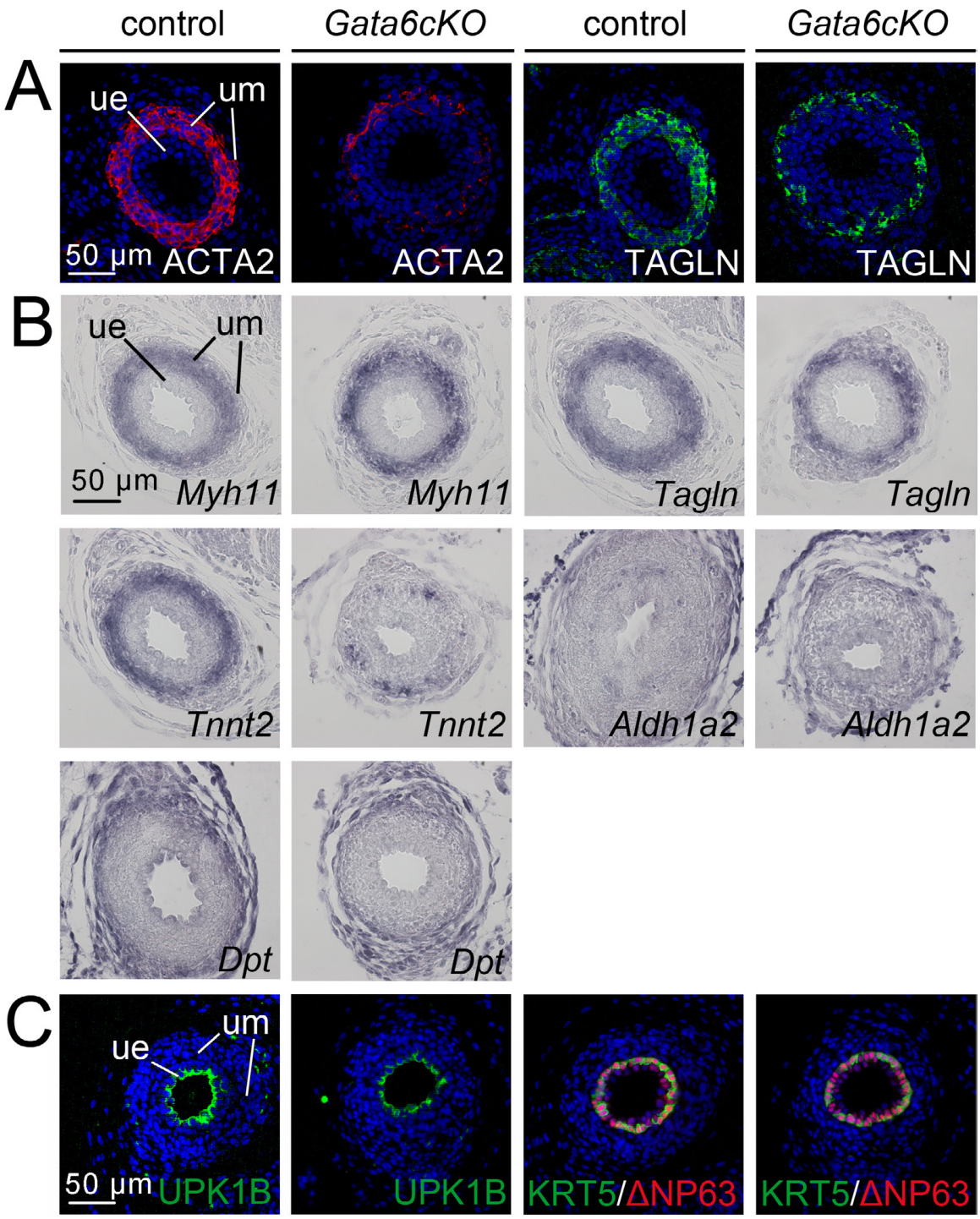

Fig. S5. Expression of SMC markers is partly reduced in distal ureters in *Gata6cKO* embryos at E18.5. (A) Immunofluorescence of the SMC markers ACTA2 and TAGLN and (B) RNA *in situ* hybridization analysis of SMC genes (*Tagln, Tnnt2, Myh11*), of the *lamina propria* marker *Aldh1a2*, and of the *tunica adventitia* marker *Dpt* on transverse sections of the proximal ureter. (C) Analysis of urothelial differentiation by immunofluorescent detection of the B-cell marker KRT5, the I/B-cell marker ΔNP63 and the S-cell marker UPK1B on proximal ureter sections. Nuclei are counterstained with DAPI (blue, in A and C). n≥3 for each assay, genotype and probe. ue, ureteric epithelium; um, ureteric mesenchyme.

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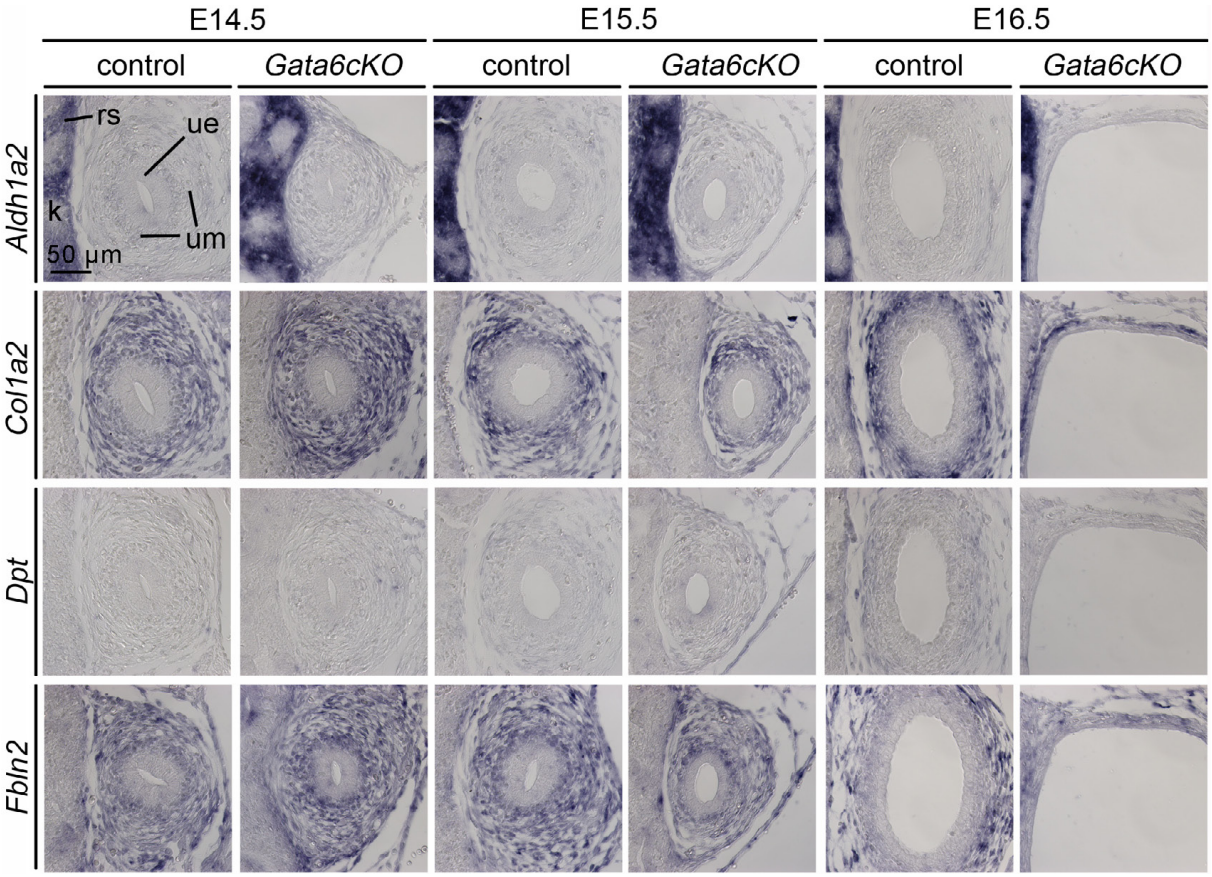

Fig. S6. Differentiation of fibrocytes is unchanged in *Gata6cKO* ureters at E14.5 to E16.5. Shown are RNA *in situ* hybridization analyses on sections of the proximal ureter at E14.5, E15.5 and E16.5 of control and *Gata6cKO* embryos for the *lamina propria* marker *Aldh1a2* and the *tunica adventitia* markers *Col1a2, Dpt, Fbln2*. n≥3 for each probe, stage and genotype. k, kidney; rs, renal stroma; ue, ureteric epithelium; um, ureteric mesenchyme.

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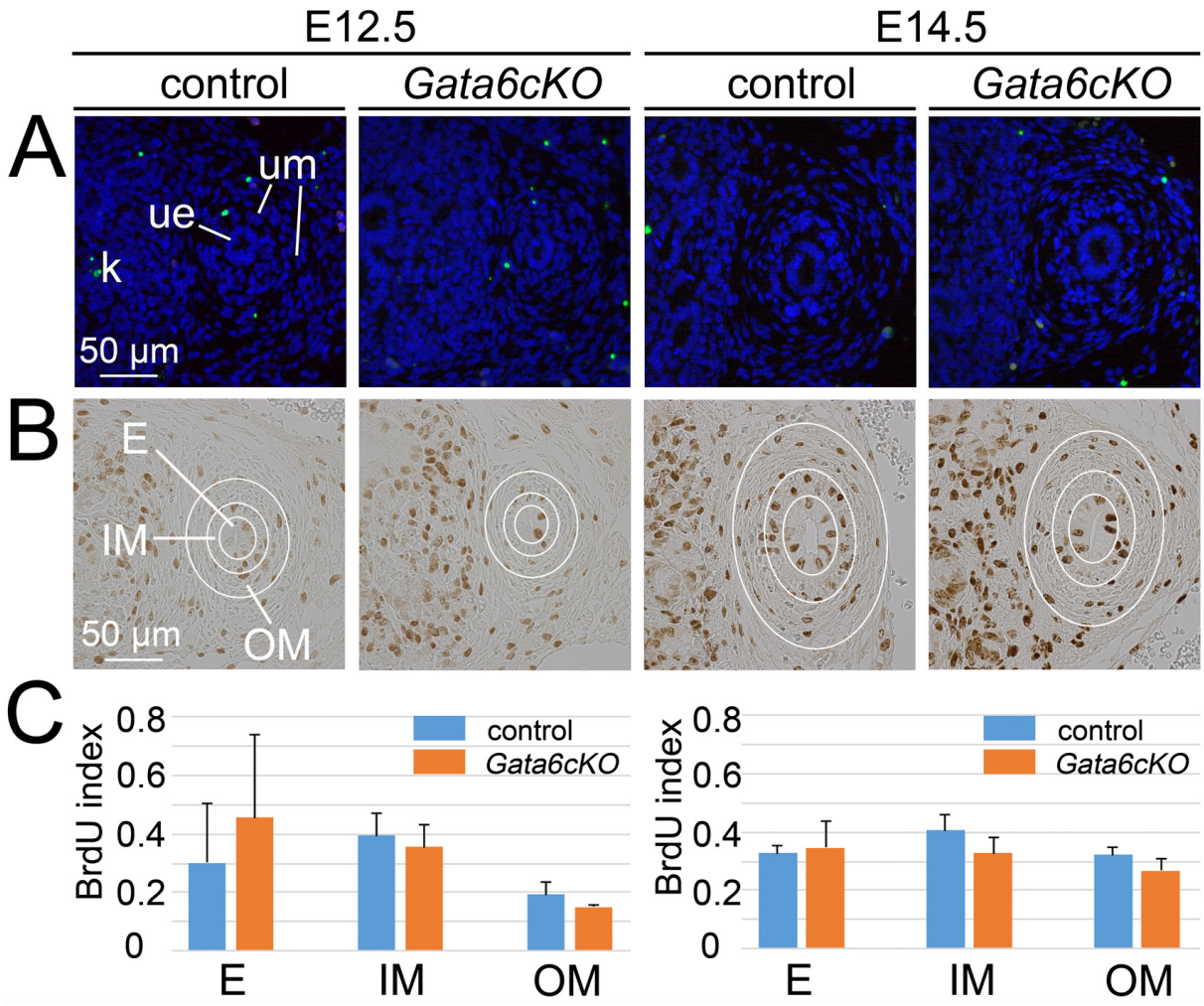

Fig. S7. Proliferation and apoptosis are not affected in *Gata6cKO* ureters at E12.5 and E14.5. (A) Immunofluorescent analysis of apoptosis (green) by the TUNEL assay on proximal ureter sections. Nuclei are counterstained with DAPI (blue). k, kidney; ue, ureteric epithelium; um, ureteric mesenchyme. (B) Immunohistochemical detection of BrdU on proximal ureter sections. White circles demarcate the ureteric epithelium (E), the inner and outer mesenchymal cell populations (IM and OM). n=3, each assay and stage. (C) Quantification of BrdU-positive cells in E12.5 control (n=3) versus mutant (n=3) ureters. Values are displayed as mean ± sd. All values are ns, *i.e.* P>0.05; two-tailed Student's t-test. For source data and statistics see Table S1.

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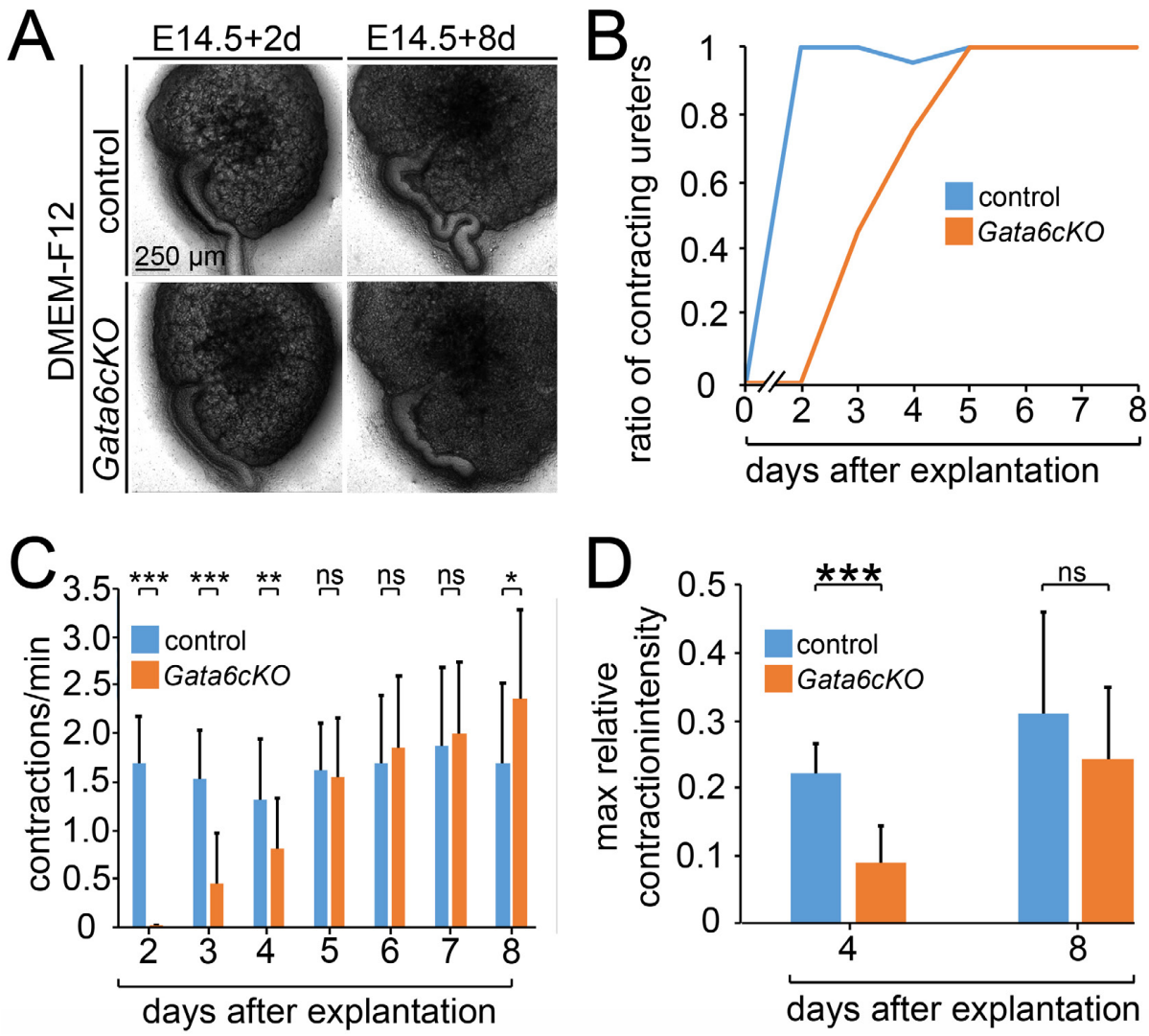

Fig. S8. Peristaltic activity is regained *in Gata6cKO* ureters in serum-free cultures. (A) E14.5 combined ureters and kidneys were explanted and grown for 8 days in culture with minimal medium, i.e. without FCS. Morphology and peristaltic activity were monitored every day from day 2 onwards using video-microscopy. (B) The onset of contraction was delayed by 2 to 3 days in *Gata6cKO* ureters (n=20) compared to control ureters (n=23). (C) Statistical analysis of peristaltic activity (expressed as contractions per min) of control (n=23) and mutant ureters (n=20). Bar graphs display mean±sd. Differences were considered significant with a P-value below 0.05 ($p<0.05$, *), highly significant ($p\leq0.005$, **) and extremely significant ($p\leq 0.0005$, ***), two-tailed Student's *t*-test. For source data and statistics see Table S4. (D) Contraction intensity was reduced after 4 days in culture in *Gata6cKO* mutants (n=15) compared to the control (n=22) but caught up after 8 days in culture (control n=23, *Gata6cKO* n=20). Bar graphs display mean±sd. Significance levels are as in (C). For source data and statistics see Table S4.

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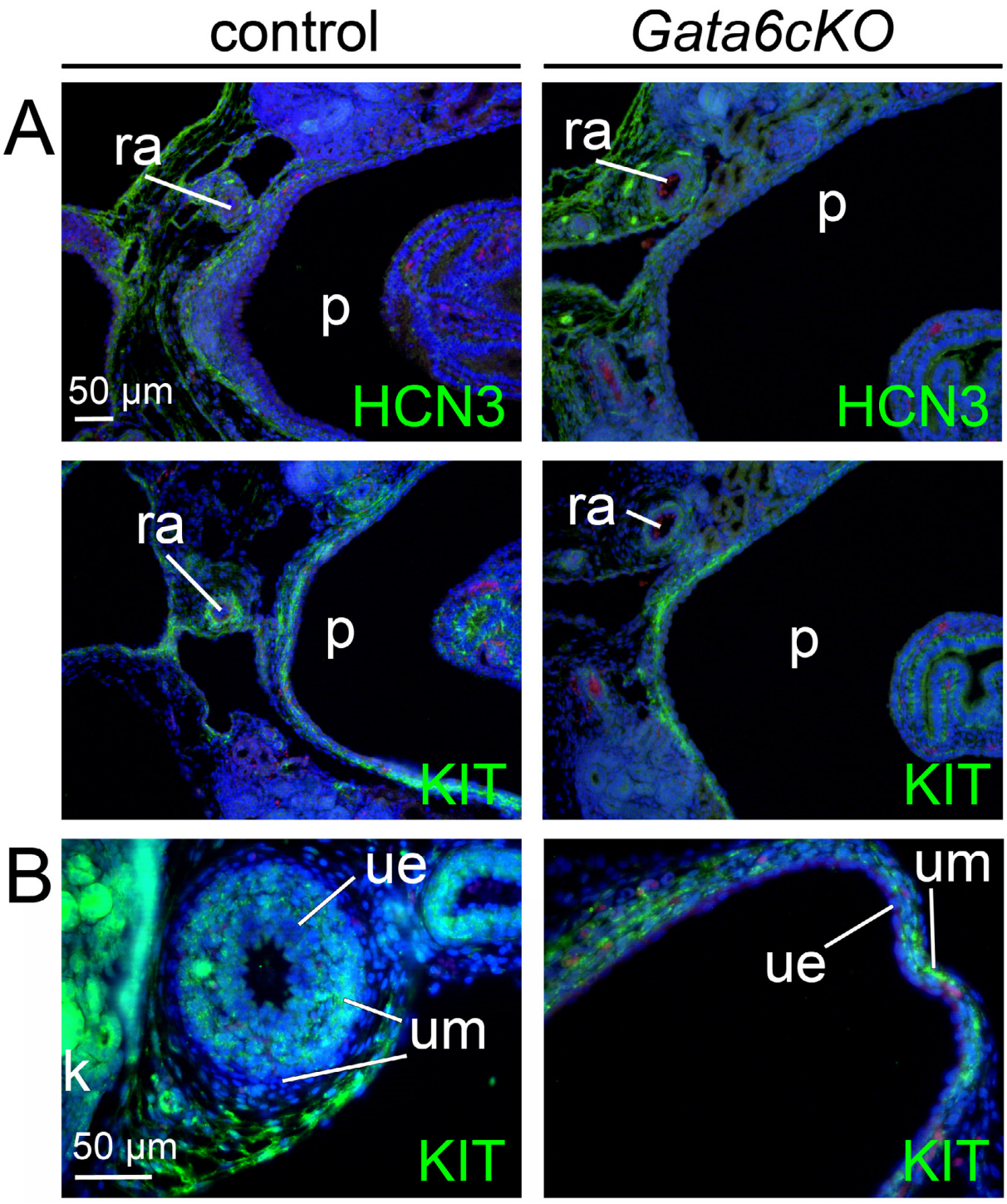

Fig. S9. Loss of *Gata6* in the UM does not affect the excitation/conduction system of the upper urinary tract. Immunofluorescence analysis of sagittal kidney (A) and proximal ureter sections (B) of E18.5 control and *Gata6cKO* embryos. Expression of HCN3, a marker for the pacemaker cells in the renal pelvis, and of KIT, a marker for interstitial Cajal-like cells in the renal pelvis and the mesenchymal region of the ureter is unchanged. k, kidney; p, pelvis; ra, renal artery; ue, ureteric epithelium; um, ureteric mesenchyme.

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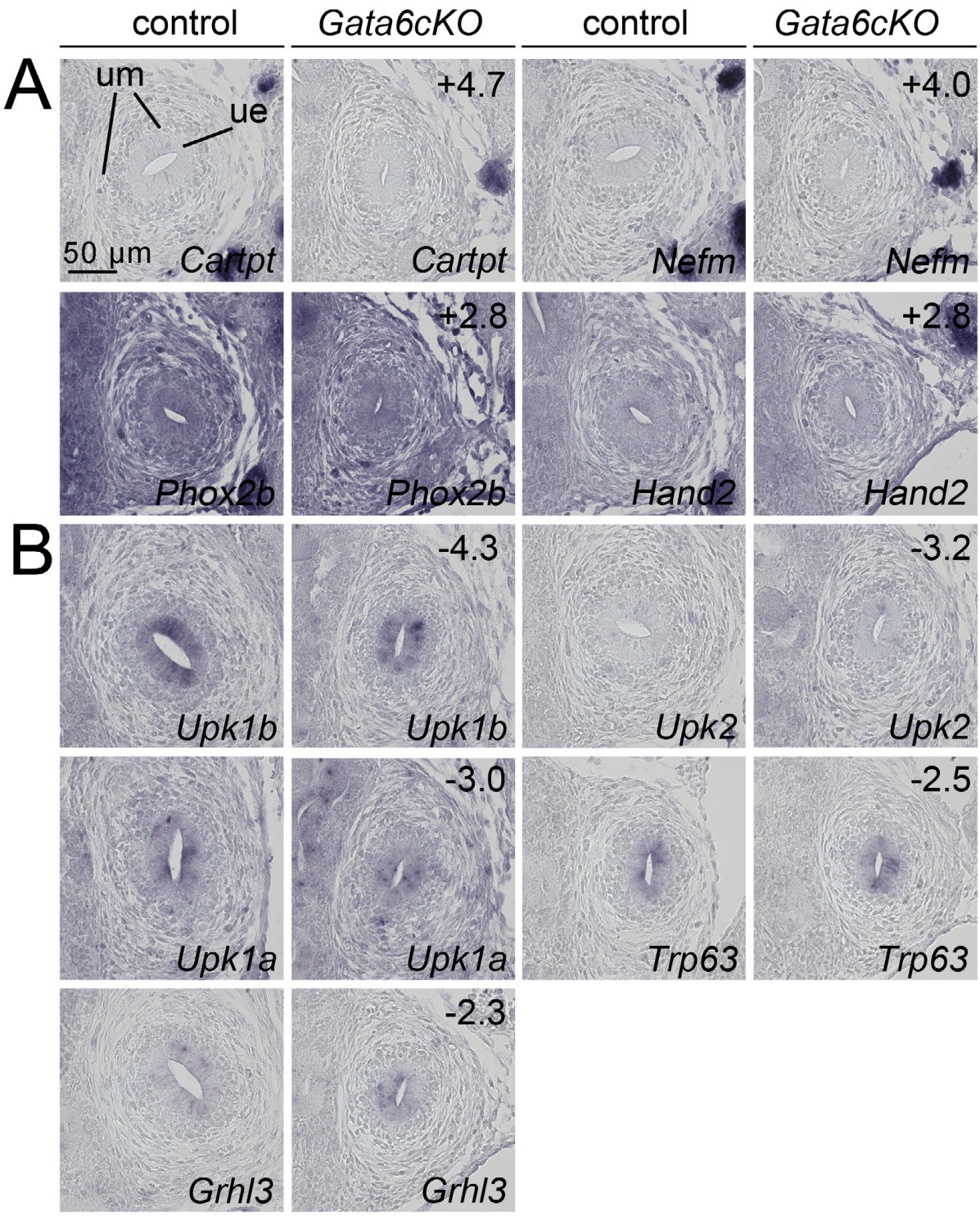

Fig. S10. RNA *in situ* hybridization analysis of genes with altered expression in microarrays of E14.5 *Gata6cKO* ureters. (A,B) Shown are RNA *in situ* hybridization analyses of proximal ureter sections of control and *Gata6cKO* embryos for genes with increased expression (A) and genes with decreased expression (B) in *Gata6cKO* microarrays. Probes, genotypes and fold changes in the microarray are as indicated. n≥3 for each probe and genotype. ue, ureteric epithelium; um, ureteric mesenchyme.

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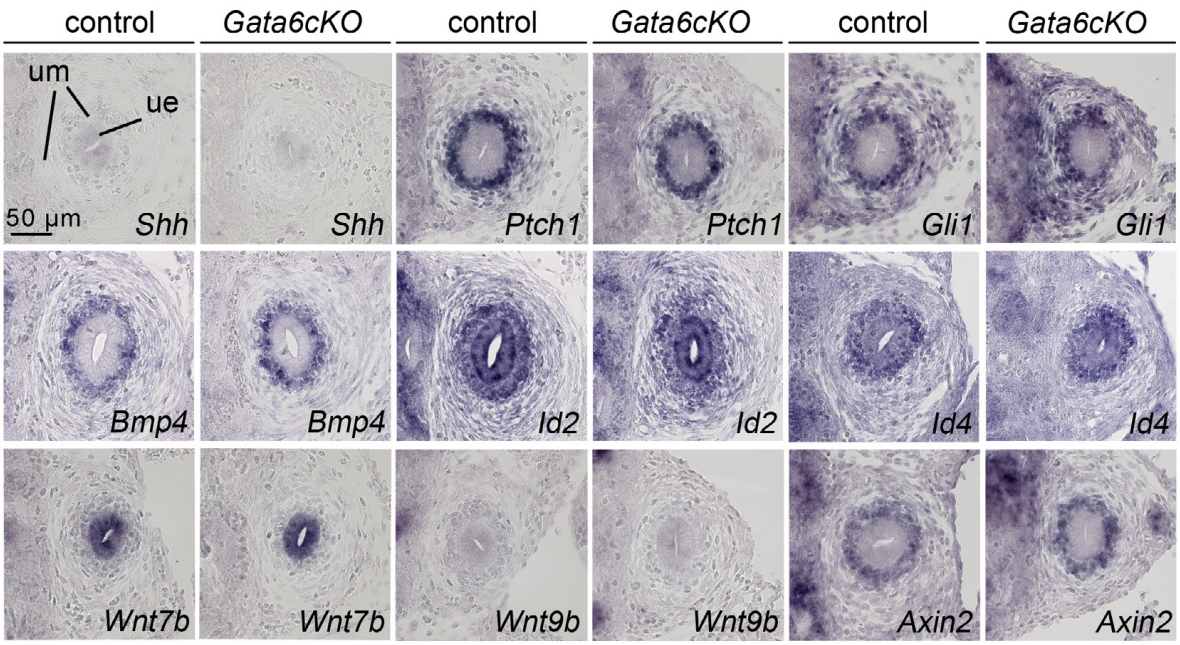

Fig. S11. Signaling pathways relevant for SMC differentiation are unchanged in their activity/expression in *Gata6cKO* ureters at E14.5. Shown are RNA *in situ* hybridization analyses on sections of the proximal ureter of E14.5 control and *Gata6cKO* embryos of *Shh*, and the targets of SHH signaling, *Ptch1* and *Gli1*; of *Wnt7b* and *Wnt9b, a*nd the WNT target gene *Axin2;* of *Bmp4,* and its target genes *Id2* and *Id4*. n≥3 for each probe and genotype. ue, ureteric epithelium; um, ureteric mesenchyme.

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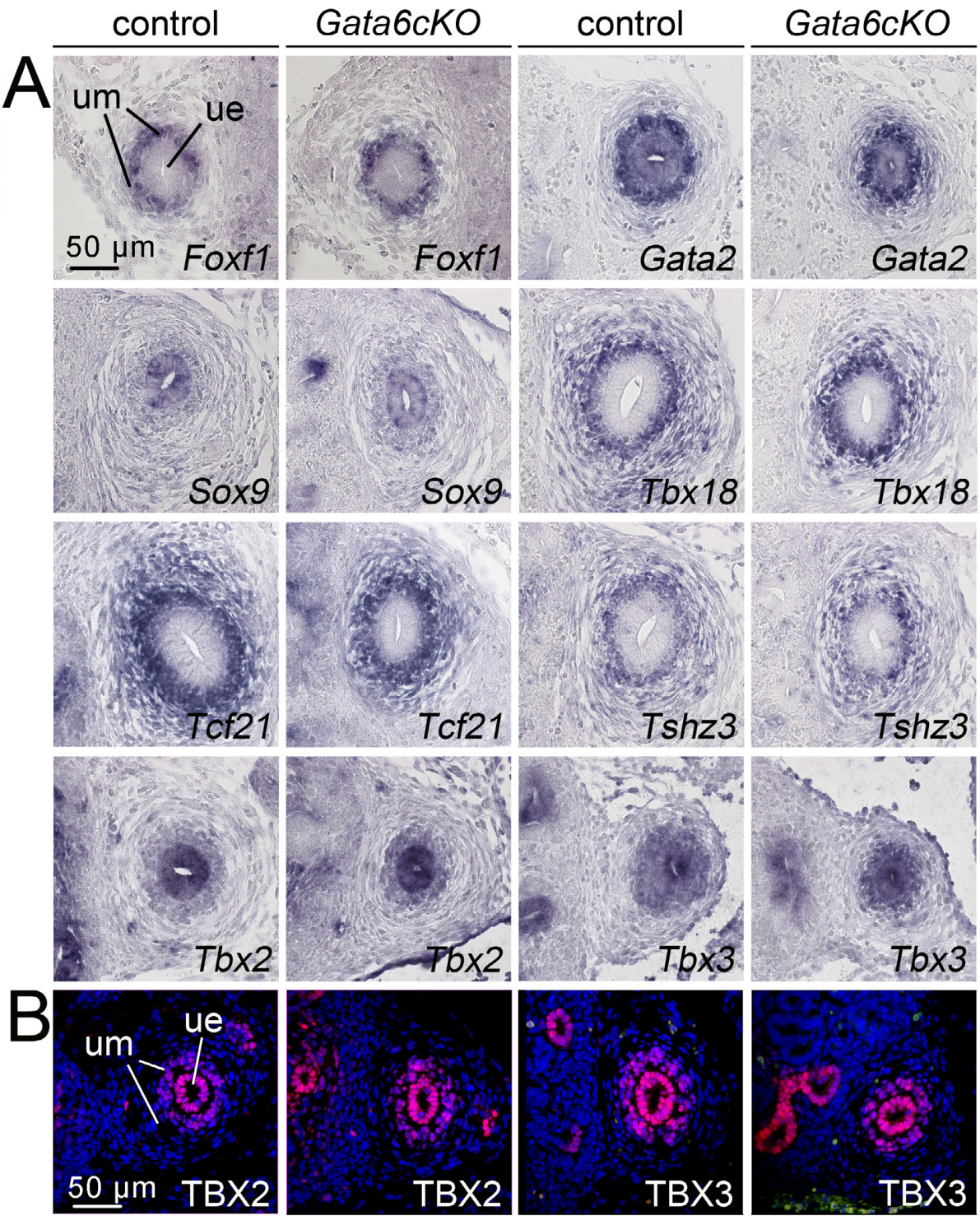

Fig. S12. Transcription factors relevant for SMC differentiation are unchanged in their expression in *Gata6cKO* ureters at E14.5. (A) RNA *in situ* hybridization analysis for expression of *Foxf1*, *Gata2, Sox9, Tbx18*, *Tcf21, Tshz3*, *Tbx2*, *Tbx3* and (B) immunofluorescence analysis of TBX2 and TBX3 on sections of the proximal ureter at E14.5 of control and *Gata6cKO* embryos. Nuclei are counterstained with DAPI (B). n>=3, each probe, assay and genotype. ue, ureteric epithelium; um, ureteric mesenchyme.

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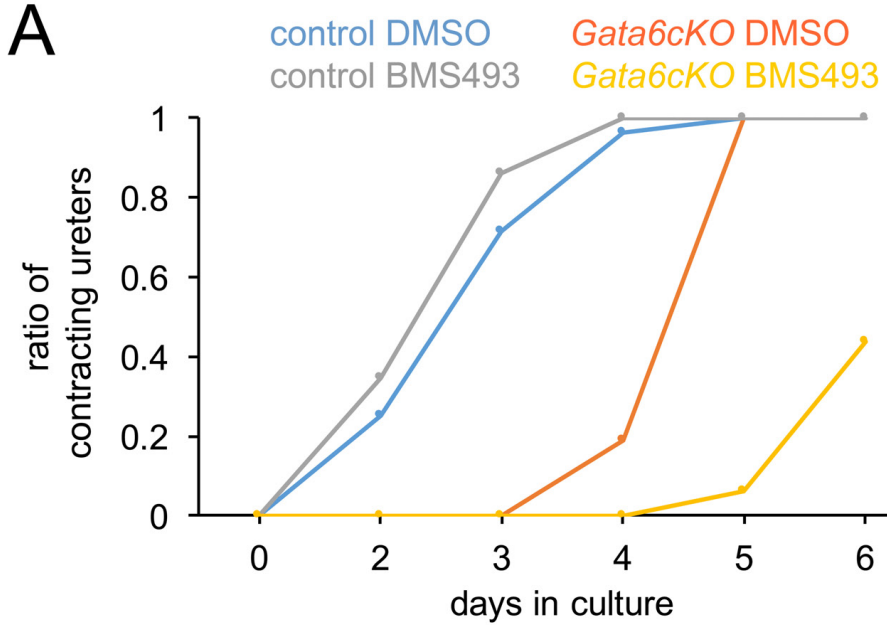
A



B

days in culture	2	3	4	5	6
control DMSO vs. Gata6cKO DMSO	ns	***	***	***	*
control BMS493 vs. Gata6cKO BMS493	*	***	***	***	***
control DMSO vs.control BMS493	ns	ns	***	***	***
Gata6cKO DMSO vs. Gata6cKO BMS493			ns	***	***

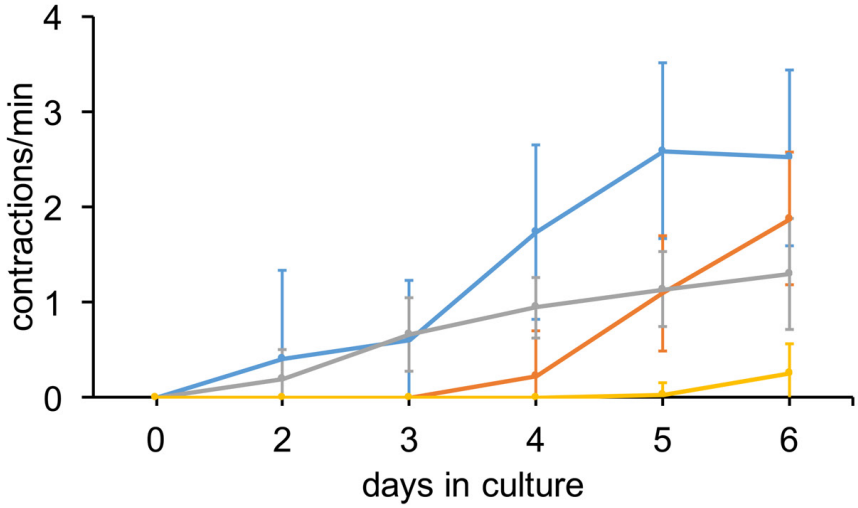


Fig. S13. Reduction of RA signaling does not rescue peristalsis defects in *Gata6cKO* ureters. Kidney rudiments from E13.5 control and *Gata6cKO* embryos were explanted and cultured for 6 days in the presence of DMSO (control) or 1 µM of the RA signaling inhibitor BMS493. Peristalsis was monitored daily in 1-min intervals. (A) Percentage of ureters that show peristaltic activity at the individual days during the culture period. (B) Statistical analysis of peristaltic activity (expressed as contractions per min) of control DMSO-treated (n=28), control BMS493-treated (n=29), mutant DMSO-treated (n=16) and mutant BMS493-treated (n=16) ureters. Bar graphs display mean±sd. Differences were considered significant with a P-value below 0.05 ($p<0.05$, *), highly significant ($p\leq0.005$, **) and extremely significant ($p\leq 0.0005$, ***); two-tailed Student's *t*-test. For source data and statistics see Table S12.

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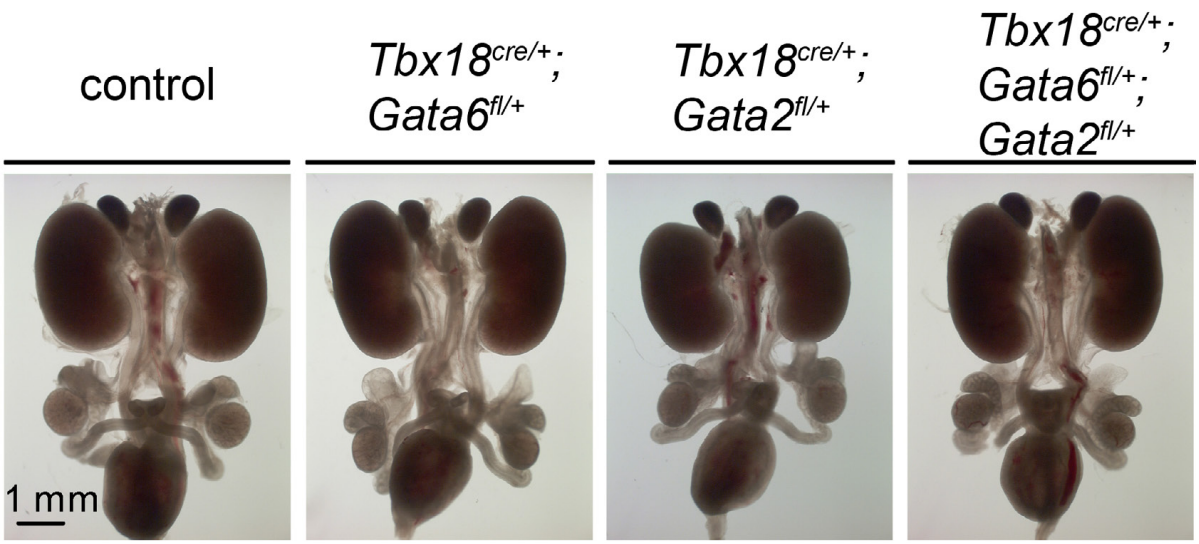

Fig. S14. *Gata2* and *Gata6* do not genetically interact in SMC differentiation in the developing ureter. Morphological analysis of whole urogenital systems of control (n=20), *Tbx18*$^{cre/+}$;*Gata6*$^{fl/+}$ (n=14), *Tbx18*$^{cre/+}$;*Gata2*$^{fl/+}$ (n=3) and *Tbx18*$^{cre/+}$;*Gata6*$^{fl/+}$;*Gata2*$^{fl/+}$(n=20) embryos at E18.5.

Table S1. Statistical evaluation of the BrdU incorporation assay in E12.5 and E14.5 control and Gata6cKO ureters.

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Table S2. Statistics of the peristaltic frequency of explant cultures of E14.5 control and Gata6cKO ureters.

Click here to download Table S2

Table S3. Statistics of the peristaltic intensity of explant cultures of E14.5 control and Gata6cKO ureters.

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Table S4. Statistics on the peristaltic activity of explants of E14.5 Gata6cKO ureters cultured together with kidneys for 8 days in DMEM-F12-only medium (relates to Fig. S8).

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Table S5. Statistics of the peristaltic frequency of explant cultures of E18.5 control and Gata6cKO ureters.

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Table S6. Statistics of the peristaltic intensity of explant cultures of E18.5 control and Gata6cKO ureters.

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Table S7. Genes with increased expression in microarrays of E14.5 Gata6cKO ureters.

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Table S8. Genes with decreased expression in microarrays of E14.5 Gata6cKO ureters.

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Table S9. Functional annotation by DAVID for genes with increased expression in the microarray of E14.5 Gata6cKO ureters.

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Table S10. Functional annotation by DAVID for genes with decreased expression in the microarray of E14.5 Gata6cKO ureters.

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Table S11. RT-qPCR analysis of SMC gene expression in control and Gata6cKO ureters at E14.5.

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Table S12. Statistics of the peristaltic frequency of explant cultures of E18.5 control and Gata6cKO ureters treated with the pan-RAR antagonist BMS493.

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Table S13. GATA6 and FOXF1 cooperate in Myocd activation in NIH3T3 cells (relates to Figure 7A).

Click here to download Table S13

Table S14. Statistical analysis of peristaltic activity of ureters with misexpression of Foxf1 (relates to Figure 7B).

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Table S15. Primers for RT-qPCR analysis of gene expression.

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