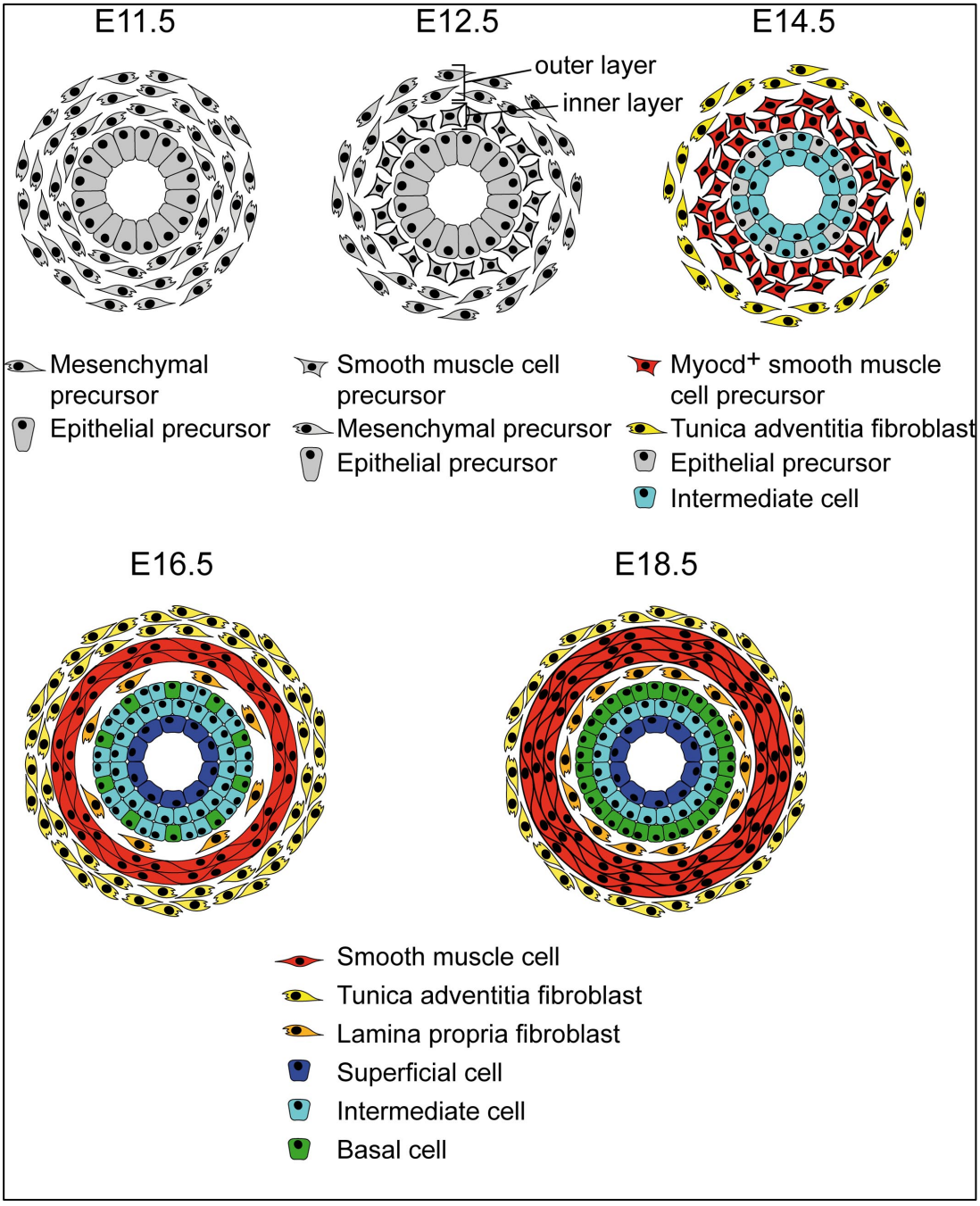


Fig. S1. Scheme of patterning and differentiation of the ureter during embryogenesis. At E11.5, the mono-layered epithelium of the (distal) stalk region of the ureteric bud is surrounded by a homogenous mesenchyme of loosely associated cells of a fibroblast-like appearance. At E12.5, the ureteric mesenchyme is radially subdivided into an inner layer of condensed cells with a rhomboid-shape, and an outer layer with fibroblast-like cells that remain loosely associated. At E14.5, the inner layer becomes MYOCD-positive, the outer layer expresses adventitial markers. The ureteric epithelium has started to stratify and expresses intermediate cell markers. At E16.5, a functional SMC layer is established. Fibroblast-like cells appear between the SMC layer and the epithelium. The epithelium becomes three-layered with a contiguous superficial layer, and some cells that start to express basal markers. At E18.5, all cell layers of the ureter are established. In the mesenchymal wall: the inner *lamina propria*, the middle *tunica muscularis*, and the outer *tunica adventitia*. In the epithelium: superficial cells, intermediate cells and basal cells.

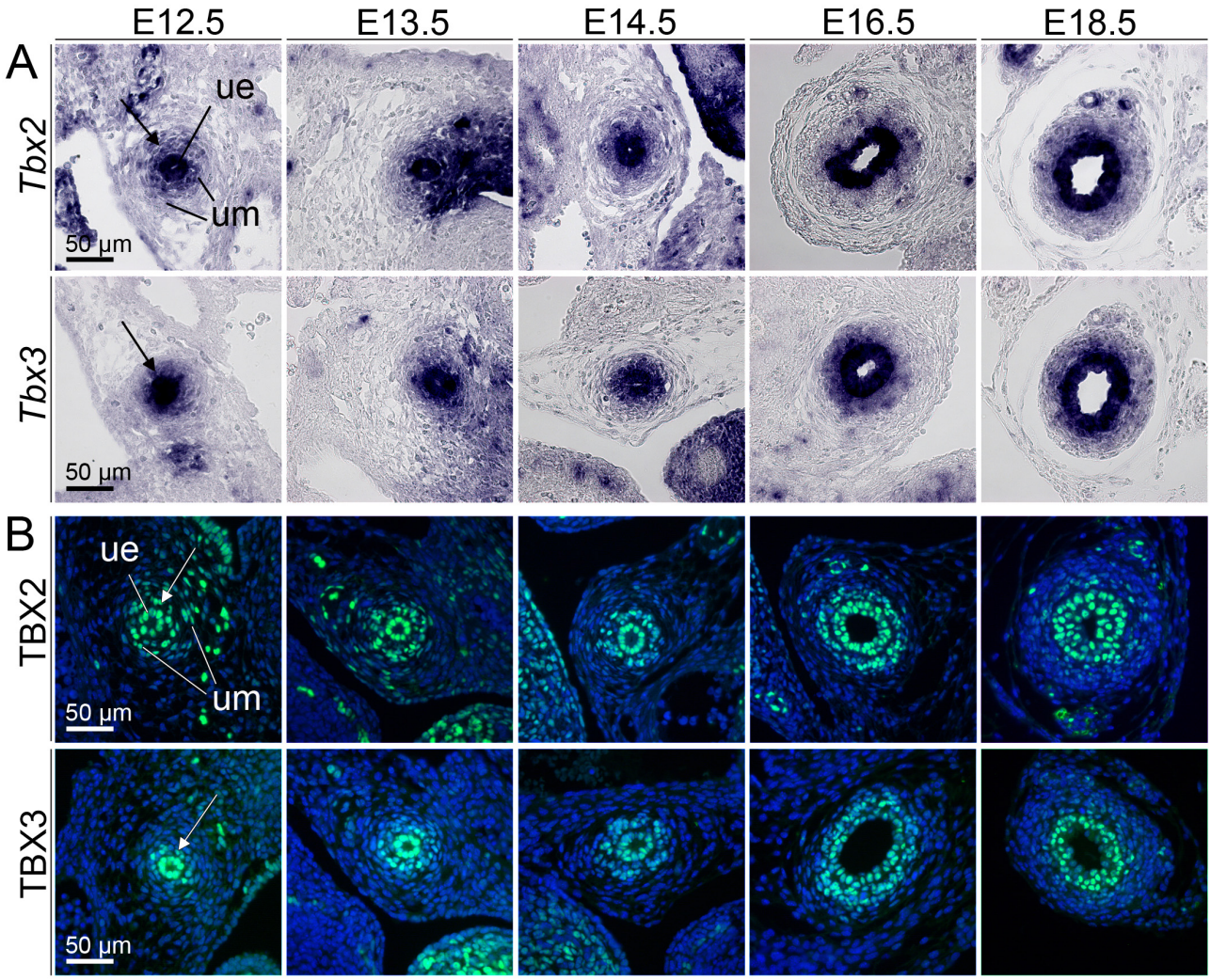


Fig. S2. *Tbx2* and *Tbx3* are expressed at distal levels of the developing ureter. (**A**) RNA *in situ* hybridization analysis on transverse sections through the posterior trunk region of wildtype embryos detects strong *Tbx2* and *Tbx3* expression in the epithelial compartment and weak expression in the inner mesenchymal region at distal levels (close to the bladder) of the developing ureter. Note that the color reaction was prolonged to visualize the weak mesenchymal expression domain. (**B**) Immunofluorescence analysis on transverse sections through the posterior trunk region of wildtype embryos shows that expression of TBX2 and TBX3 protein at distal levels (close to the bladder) of the developing ureter follows that of the mRNA. Nuclei (blue) are counterstained with DAPI. Arrows (in **A** and **B**) point to the mesenchymal expression domain of *Tbx2*/TBX2 and *Tbx3*/TBX3 at E12.5. Stages and probes are as indicated. ue, ureteric epithelium; um, ureteric mesenchyme.

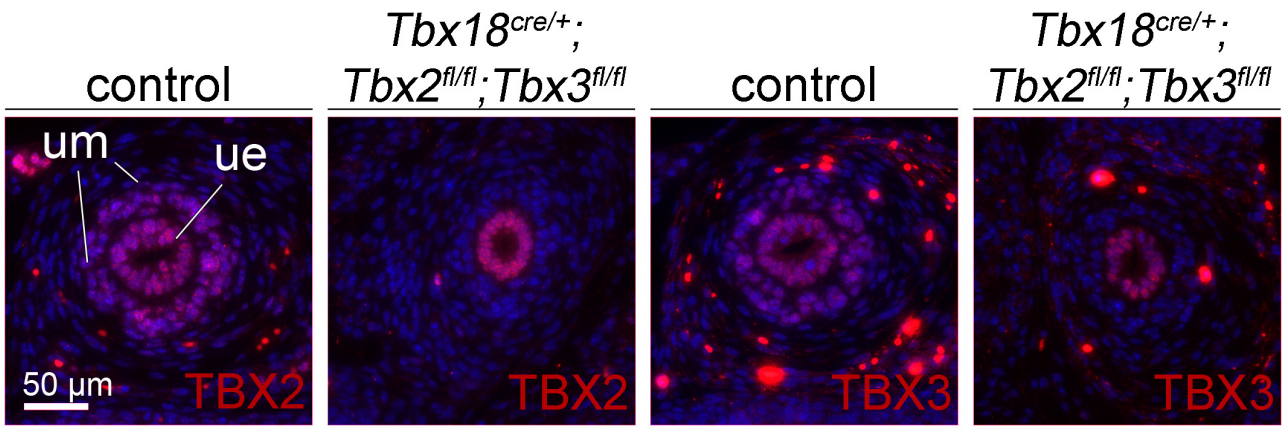


Fig. S3. *Tbx18*^{cre} mediates recombination of floxed alleles of *Tbx2* and *Tbx3* to null alleles in the ureteric mesenchyme. Immunofluorescence analysis on transverse sections at the proximal ureter level (close to the kidney) shows that expression of TBX2 and TBX3 is lost in the mesenchymal compartment in E12.5 *Tbx18*$^{cre/+}$*;Tbx2*$^{fl/fl}$*;Tbx3*$^{fl/fl}$ ureters. Epithelial expression remains unaffected. Nuclei (blue) are counterstained with DAPI. ue, ureteric epithelium; um, ureteric mesenchyme.

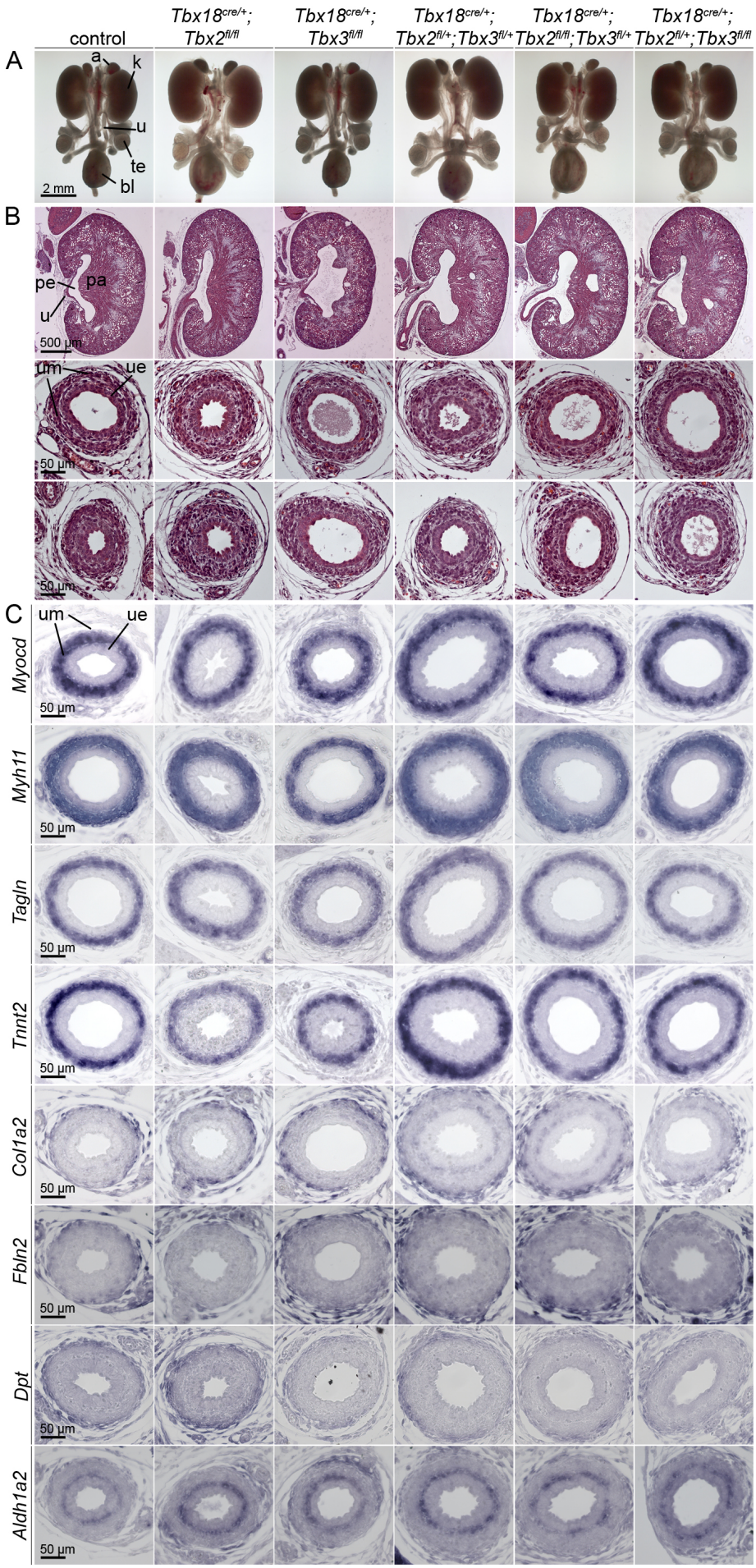


Fig. S4. Cytodifferentiation is not affected in E18.5 ureters with conditional (*Tbx18*^{cre}-mediated) inactivation of two or three alleles of *Tbx2* and *Tbx3* in the ureteric mesenchyme. (**A**) Morphology of whole urogenital systems of E18.5 male embryos; wildtype n=11, *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$ n=9, *Tbx18*$^{cre/+}$;*Tbx3*$^{fl/fl}$ n=7, *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/+}$;*Tbx3*$^{fl/+}$ n=18, *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/+}$ n=12, *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/+}$;*Tbx3*$^{fl/fl}$ n=17. **(B)** Haematoxylin and eosin staining of sagittal sections of kidneys (**row 1**) and of transverse sections at the proximal level (close to the kidney) (**row 2**) and the distal ureter level (close to the bladder) (**row 3**) does not reveal changes in the morphological and histological appearance of mutant kidneys and ureters. (**C)** Cytodifferentiation of the ureteric mesenchyme as revealed by *in situ* hybridization analysis on transverse sections of the proximal ureter at E18.5. SMC markers *(Myocd, Myh11, Tagln, Tnnt2),* markers of the outer *tunica adventitia* (*Col1a2, Fbln2, Dpt*), and of the *lamina propria* (*Aldh1a2*) are not changed in the mutant, or appear slightly reduced (*Tnnt2* in *Tbx2*- and *Tbx3*-single mutants). Genotypes and probes are as indicated. a, adrenal; bl, bladder; k, kidney; pa, papilla; pe, pelvis; te, testis; u, ureter; ue, ureteric epithelium; um, ureteric mesenchyme.

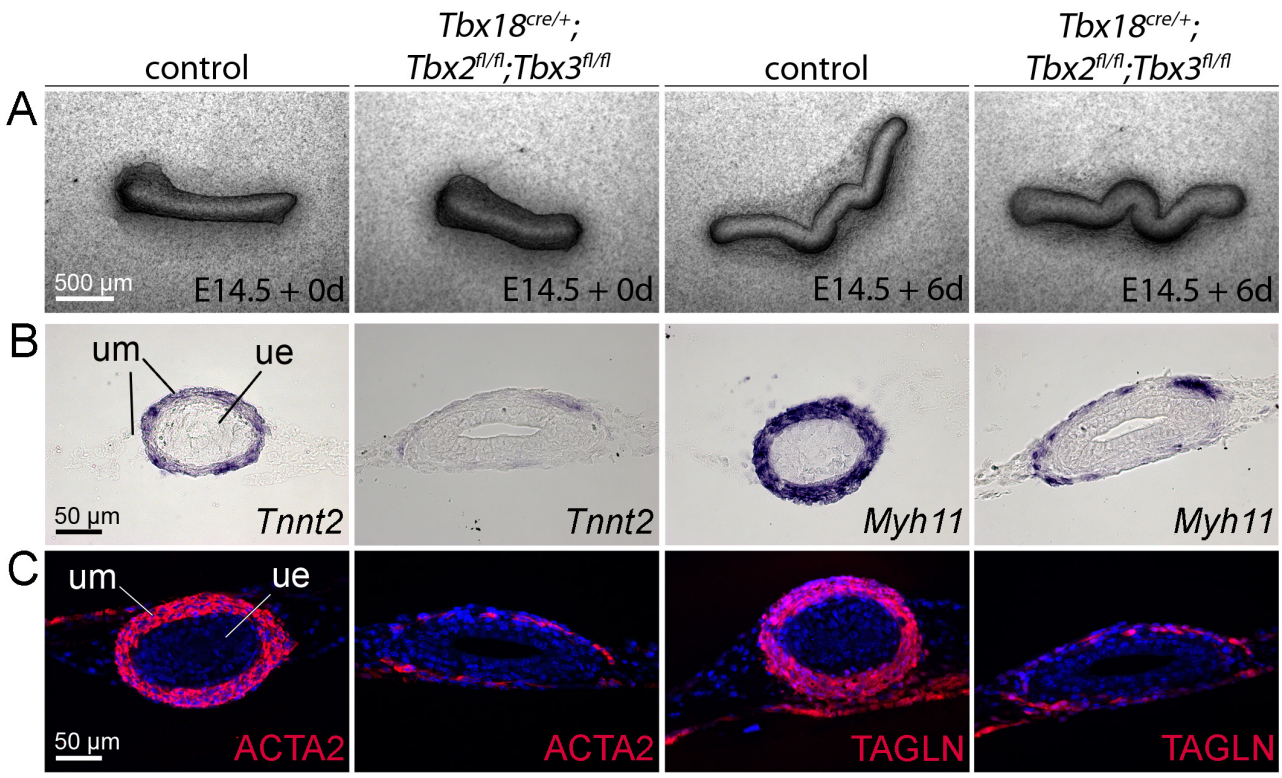


Fig. S5. SMC investment is severely reduced in explants of E14.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ ureters after 6 days of culture. (A) Brightfield images of E14.5 ureters after 0 and 6 d of culture (n=9 for the control, n=5 for the mutant). (**B**) *In situ* hybridization analysis of *Tnnt2* and *Myh11* expression, and (**C**) immunofluorescence analysis of ACTA2 and TAGLN expression on transverse sections of the proximal region of ureters cultured for 6 d. Nuclei are counterstained with DAPI (blue). The number of mesenchymal cells expressing these markers is severely reduced in the mutants. Genotypes and probes are as indicated. ue, ureteric epithelium; um, ureteric mesenchyme.

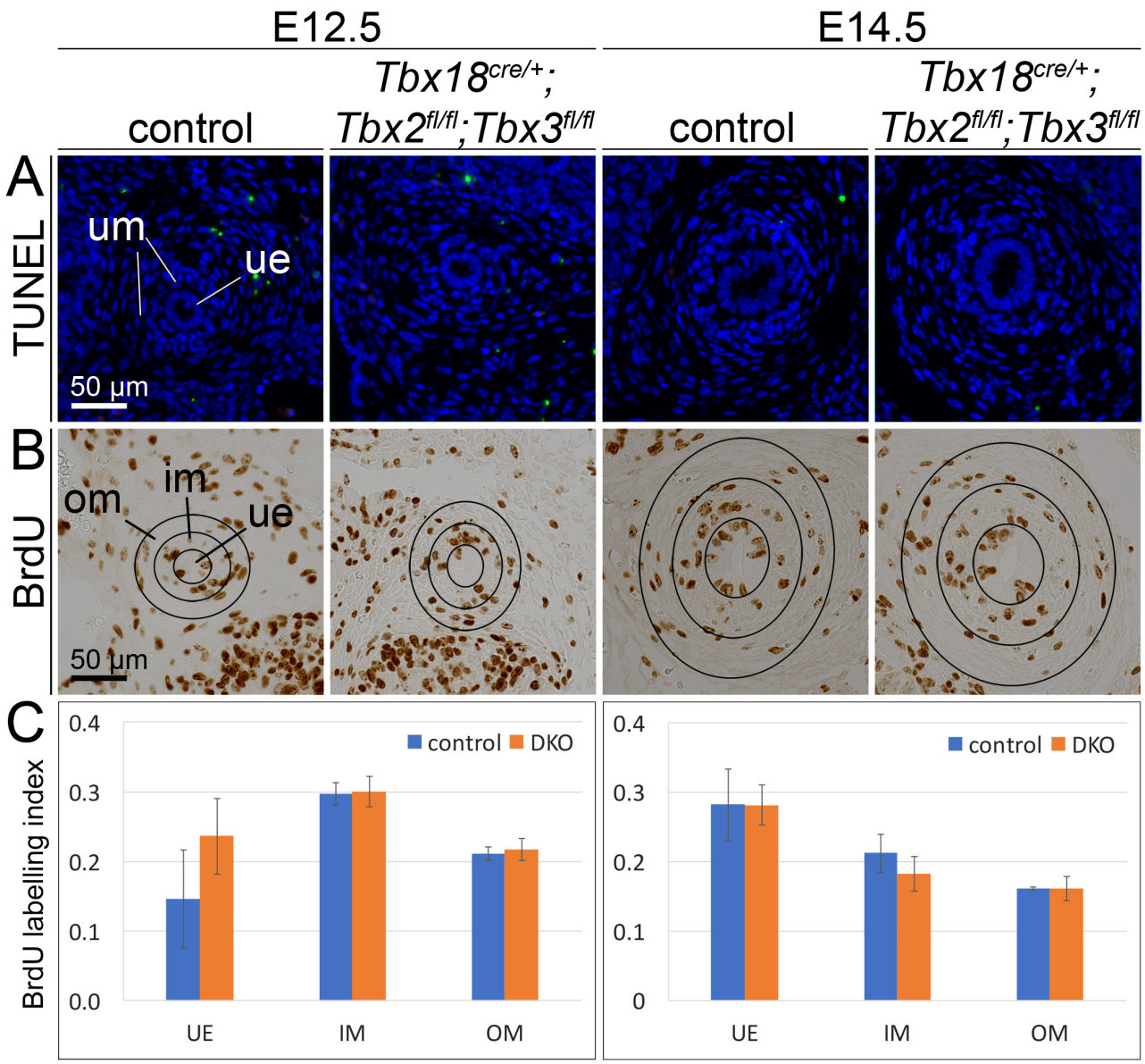


Fig. S6. Apoptosis and proliferation are not affected in *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ ureters at E12.5 and E14.5. (**A**) Cell death as detected by the TUNEL assay (green) does not show changes between control and *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ ureters at E12.5 or E14.5 (n=3 each). Nuclei are counterstained with DAPI. (**B**) Determination of cellular proliferation by the BrdU incorporation assay on transverse sections of the proximal ureter at E12.5 and E14.5. Black circles mark the epithelium (ue), and the inner (im) and outer (om) mesenchymal compartments of the ureter that were analyzed to quantify proliferation. Nuclei (blue) are counterstained with DAPI. (**C**) Quantification of the BrdU assay does not reveal altered proliferation patterns in the mutant ureter (n=5 independent specimens each). E12.5 control versus mutant: UE, 0.146±0.070 vs 0.236±0.054, P=0.154; IM, 0.297±0.017 vs 0.300±0.021, P=0.828; OM, 0.212±0.009 vs 0.217±0.015, P=0.641. E14.5 control versus mutant: UE, 0.282±0.051 vs 0.281±0.029, P=0.977; IM, 0.212±0.028 vs 0.182±0.025, P=0.232; OM, 0.161±0.002 vs 0.161±0.018, P=0.641. Values are shown as mean±sd. P<0.05 is considered significant; two-tailed Student's t-test.

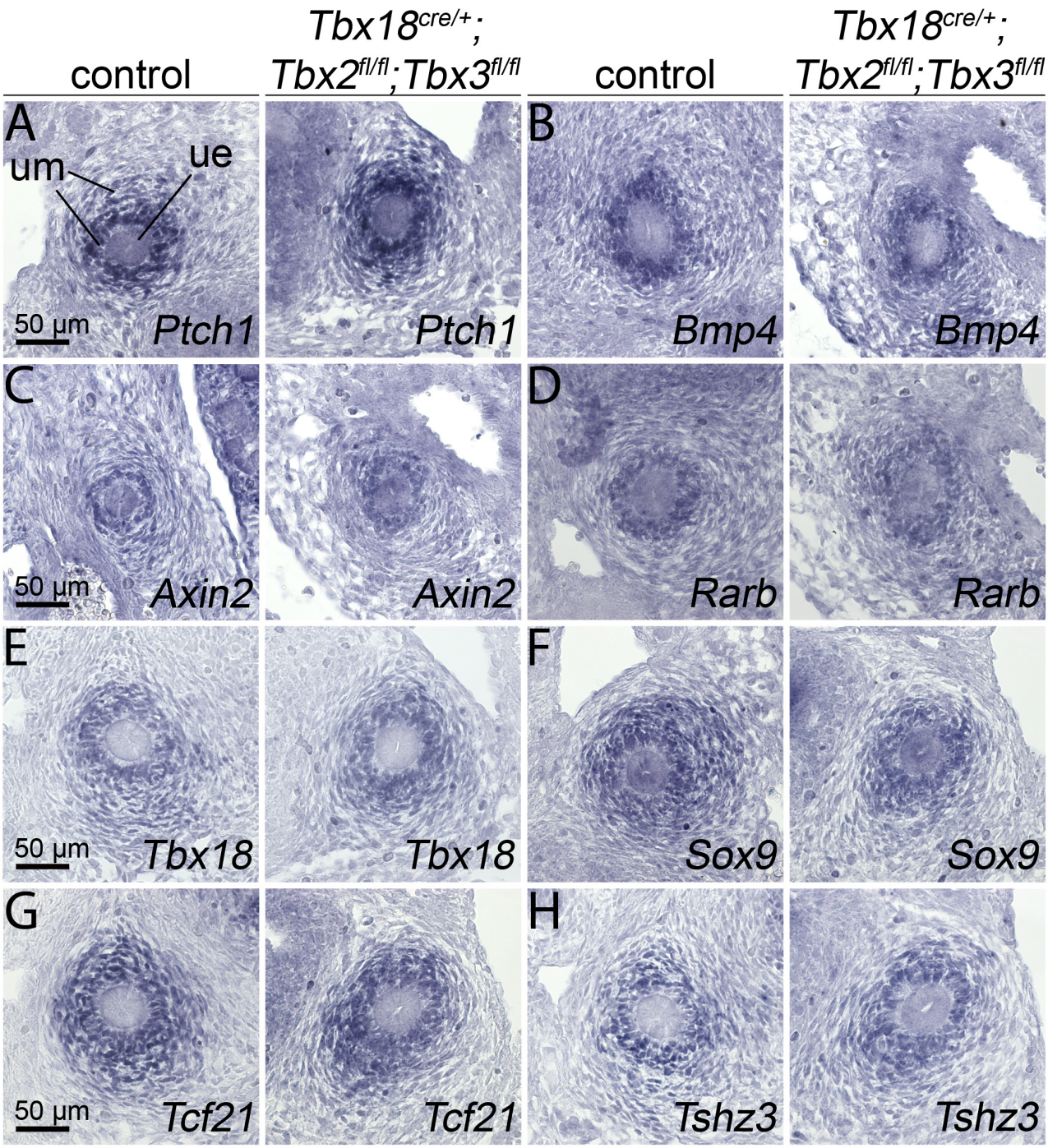


Fig. S7. Expression of signaling activities and transcription factors is not changed in the mesenchyme of *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ ureters at E12.5. *In situ* hybridization analysis on proximal ureter sections of control and *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Genotypes and probes are as indicated. ue, ureteric epithelium; um, ureteric mesenchyme.

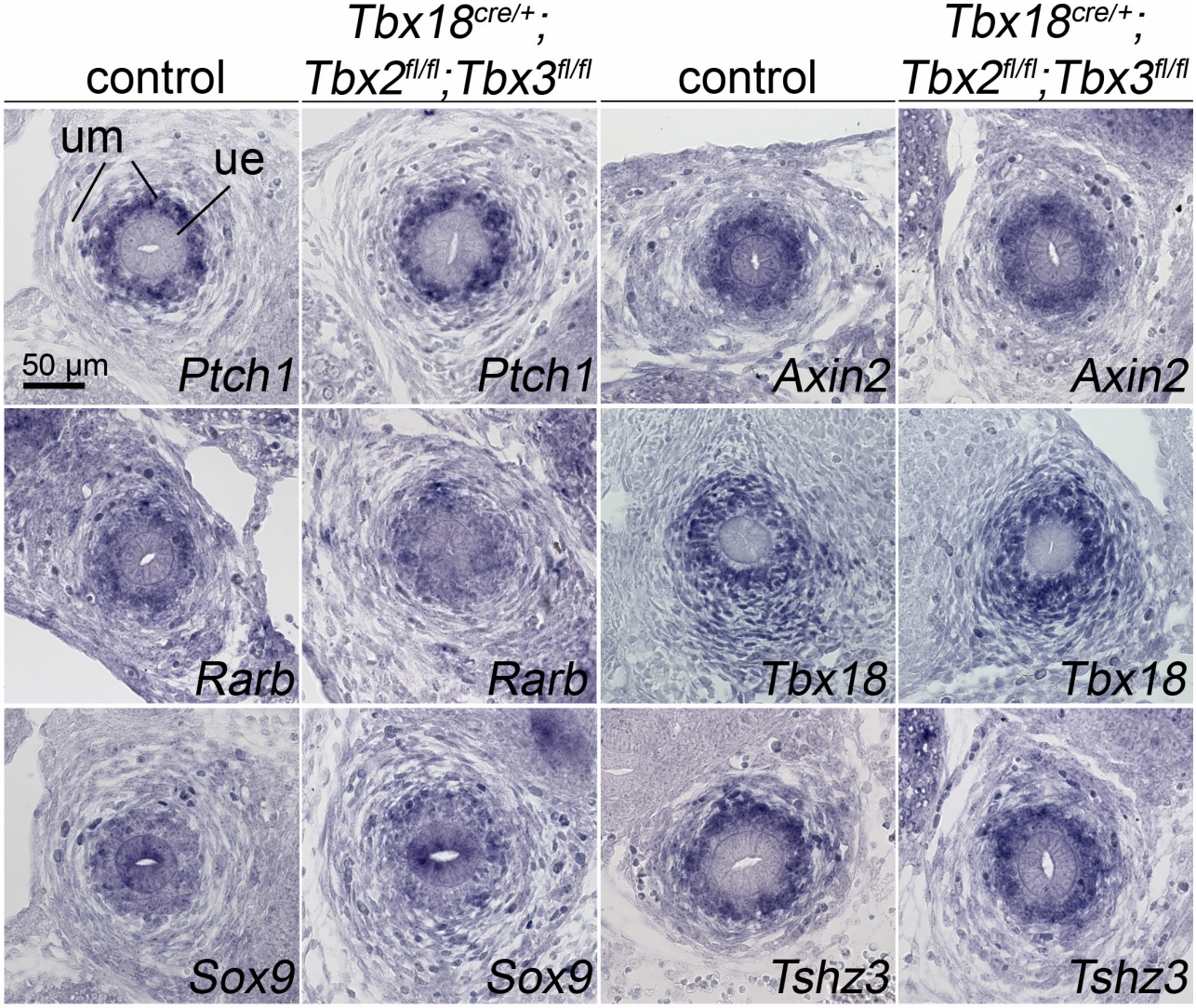


Fig. S8. Expression of signaling activities and transcription factors is not changed in the mesenchyme of *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ ureters at E14.5. *In situ* hybridization analysis on proximal ureter sections of control and *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Genotypes and probes are as indicated. ue, ureteric epithelium; um, ureteric mesenchyme.

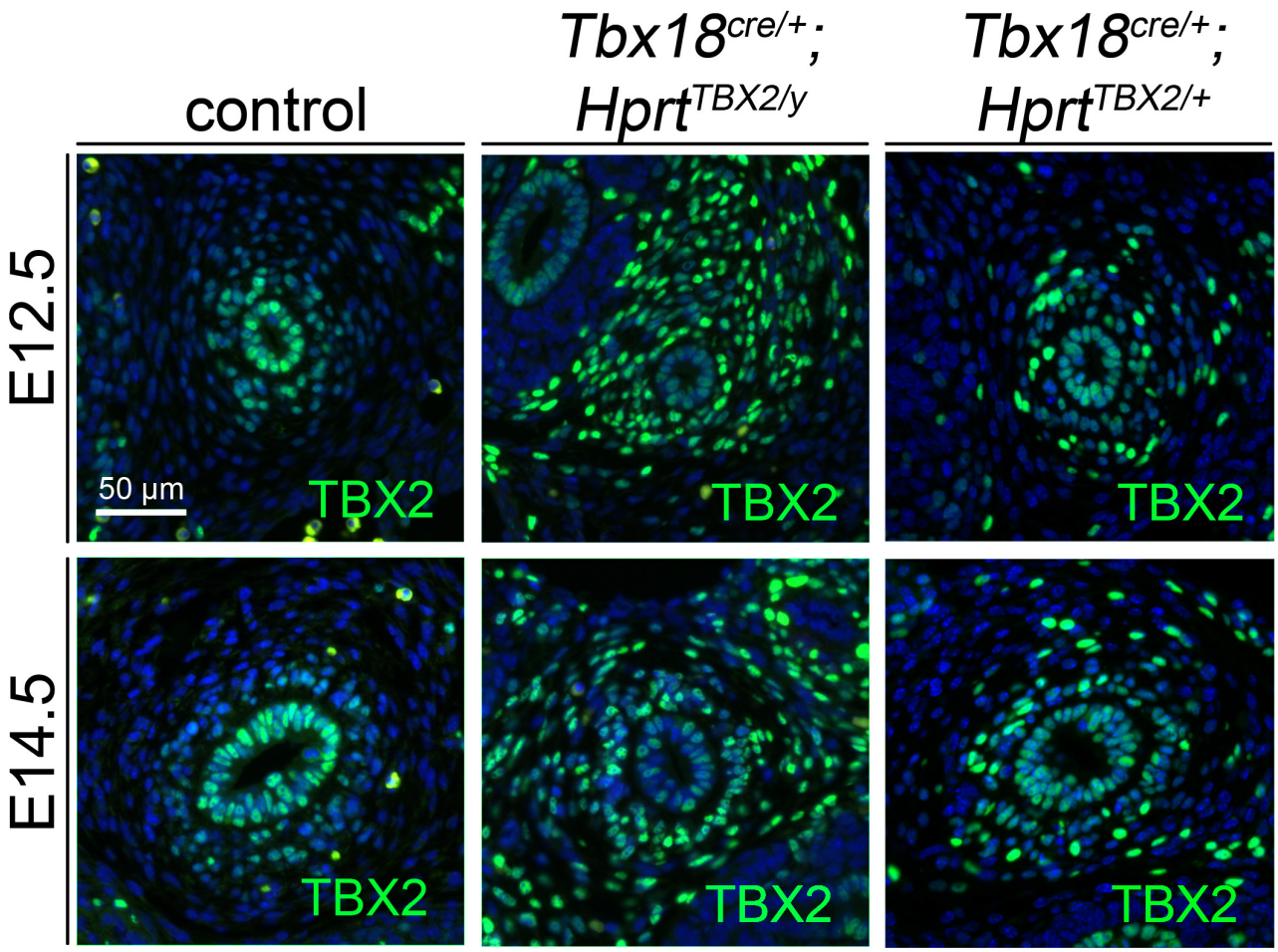


Fig. S9. *Tbx18*^{cre} mediated recombination of a *Hprt*^{TBX2} allele leads to ectopic expression of TBX2 in the ureteric mesenchyme. Immunofluorescence analysis on transverse sections at the proximal ureter level shows that TBX2 is expressed throughout the mesenchymal compartment in E12.5 and E14.5 *Tbx18*$^{cre/+}$;*Hprt*$^{TBX2/y}$ ureters. In female mutants, TBX2 is detected in a mosaic pattern in the inner and outer domain of the ureteric mesenchyme. Epithelial expression remains unaffected. Nuclei (blue) are counterstained with DAPI. ue, ureteric epithelium; um, ureteric mesenchyme.

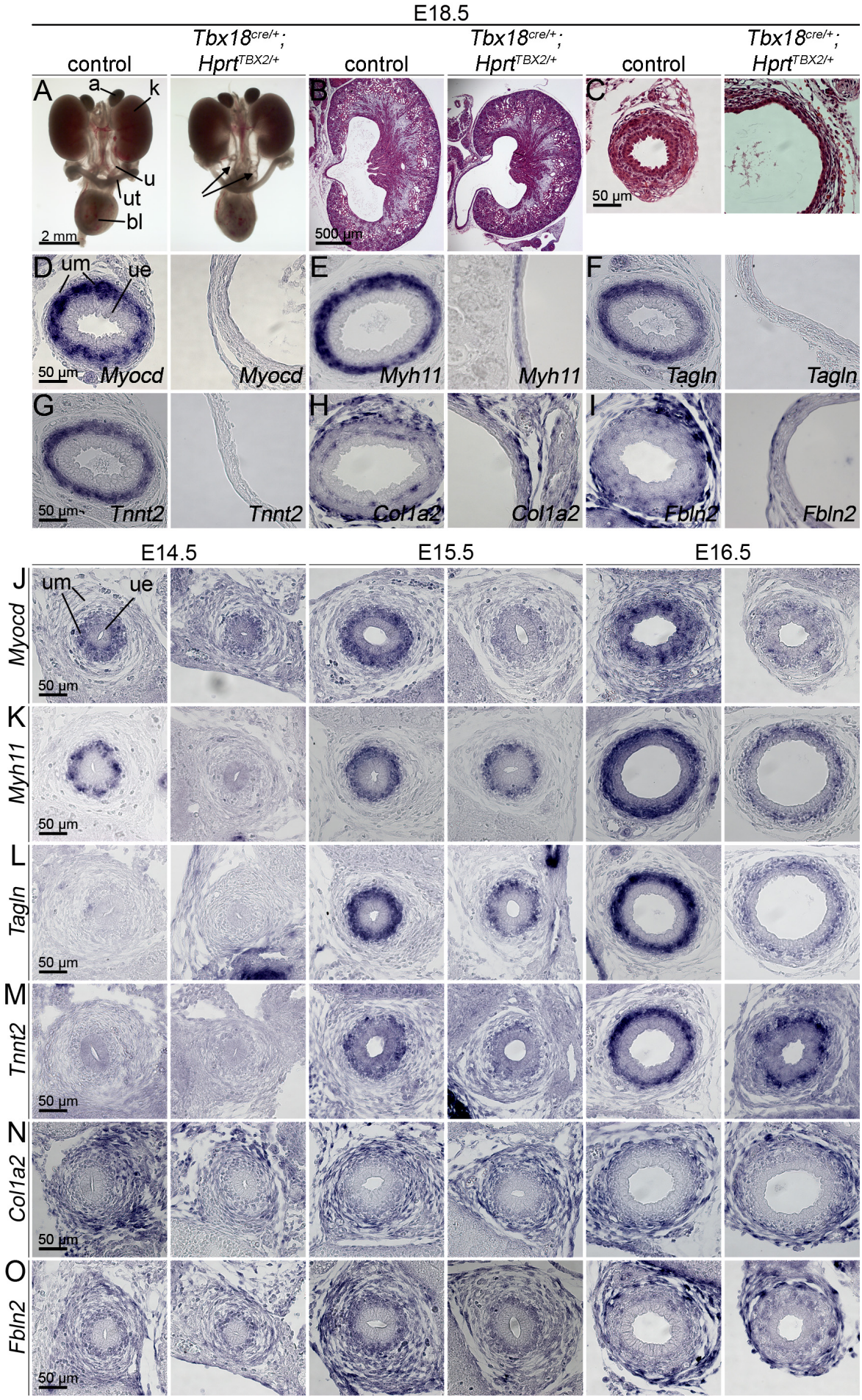


Fig. S10. Female *Tbx18*$^{cre/+}$;*Hprt*$^{TBX2/+}$ embryos develop bilateral hydroureter and exhibit delayed and reduced SMC differentiation. (**A**) Morphology of whole urogenital systems of female E18.5 embryos reveals dilated ureters (arrows) in the mutant (n=5) but not in control embryos (n=6). **(B,C)** Haematoxylin and eosin staining of sagittal sections of kidneys (**B**) and of transverse sections of the dilated proximal ureter (**C**). (**D-O**) *In situ* hybridization analysis on proximal ureter sections at E18.5 (**D-I**) and at E14.5 to E16.5 (**J-O**) for expression of SMC markers (**D-G, J-M**) and *tunica adventitia* markers (**H,I,N,O**). Expression of SMC markers is delayed and reduced. *Fbln2* is reduced, *Col1a2* is unaffected. Histological and molecular stainings were performed on at least 3 independent specimens. Genotypes and probes are as indicated. a, adrenal; bl, bladder; k, kidney; te, testis; u, ureter; ue, ureteric epithelium; um, ureteric mesenchyme.

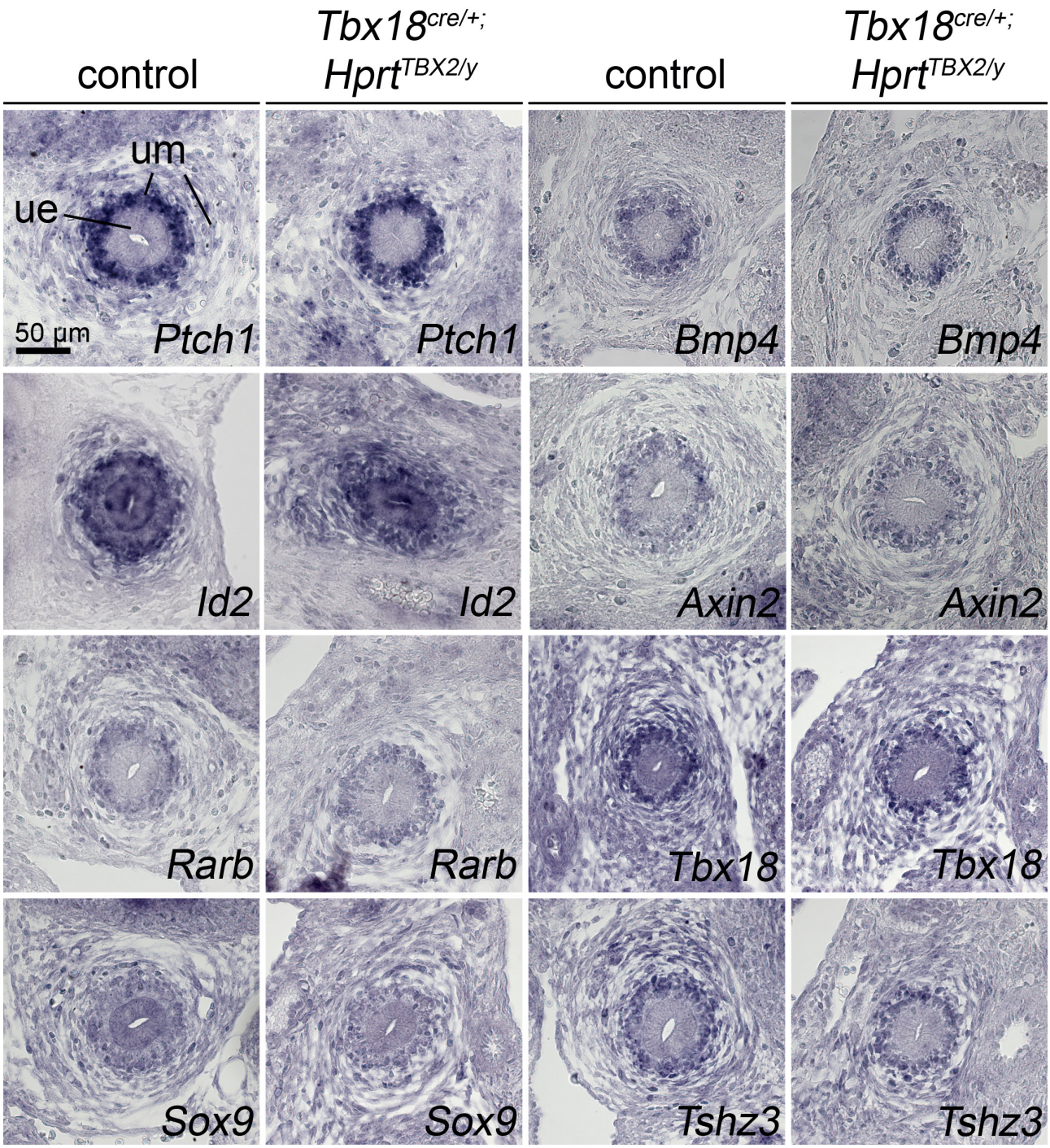


Fig. S11. Expression of signaling activities and transcription factors is not changed in the mesenchyme of *Tbx18*$^{cre/+}$;*Hprt*$^{TBX2/y}$ ureters at E14.5. *In situ* hybridization analysis on proximal ureter sections of control and *Tbx18*$^{cre/+}$;*Hprt*$^{TBX2/y}$ embryos. Genotypes and probes are as indicated. ue, ureteric epithelium; um, ureteric mesenchyme.

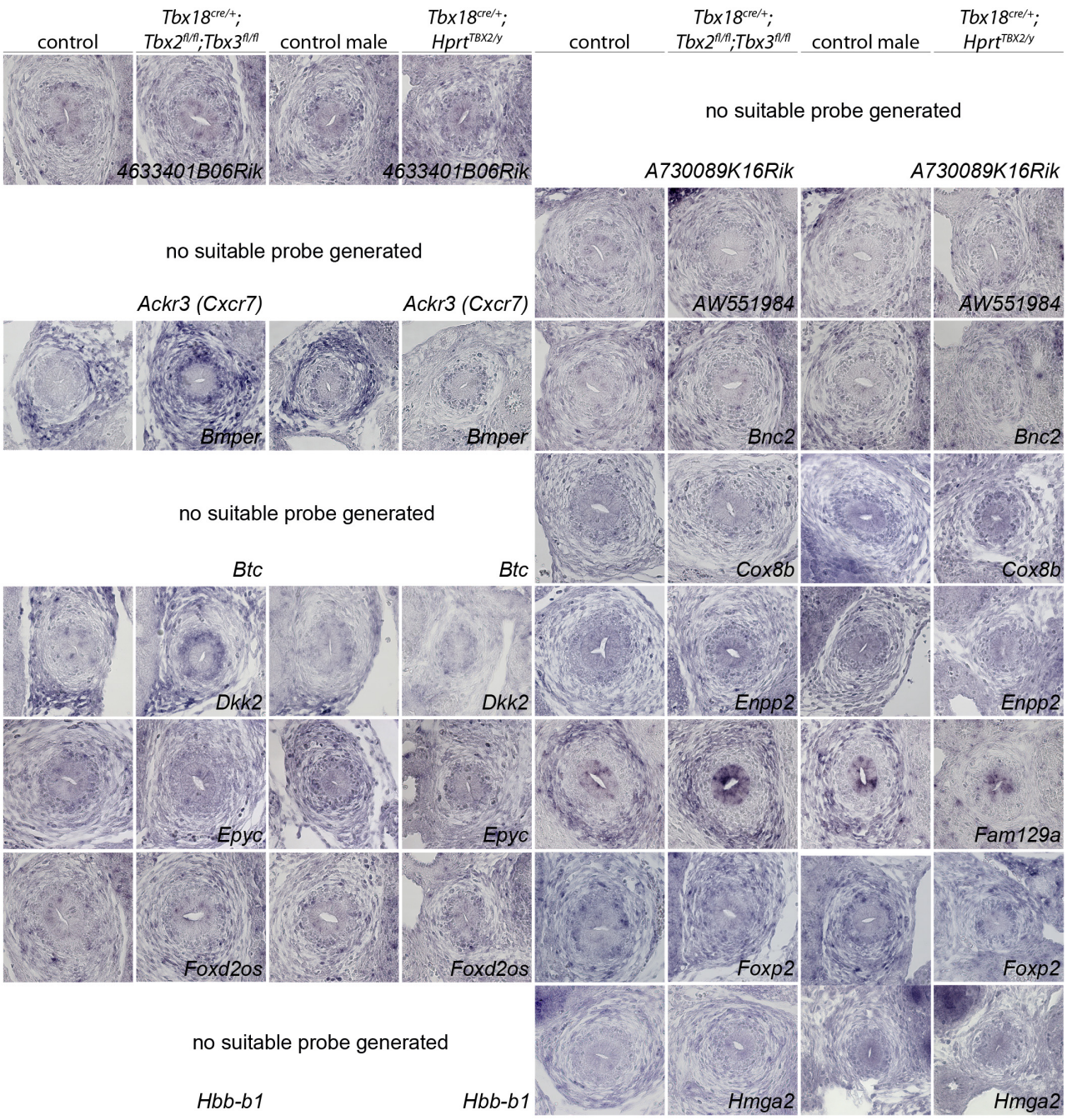


Fig. S12. Expression of few microarray candidates can be validated by *in situ* hybridization analysis. *In situ* hybridization analysis on transverse sections of the proximal ureter region in E14.5 wildtype, *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ and *Tbx18*$^{cre/+}$;*Hprt*$^{TBX2/y}$ embryos. Probes and genotypes are as indicated.

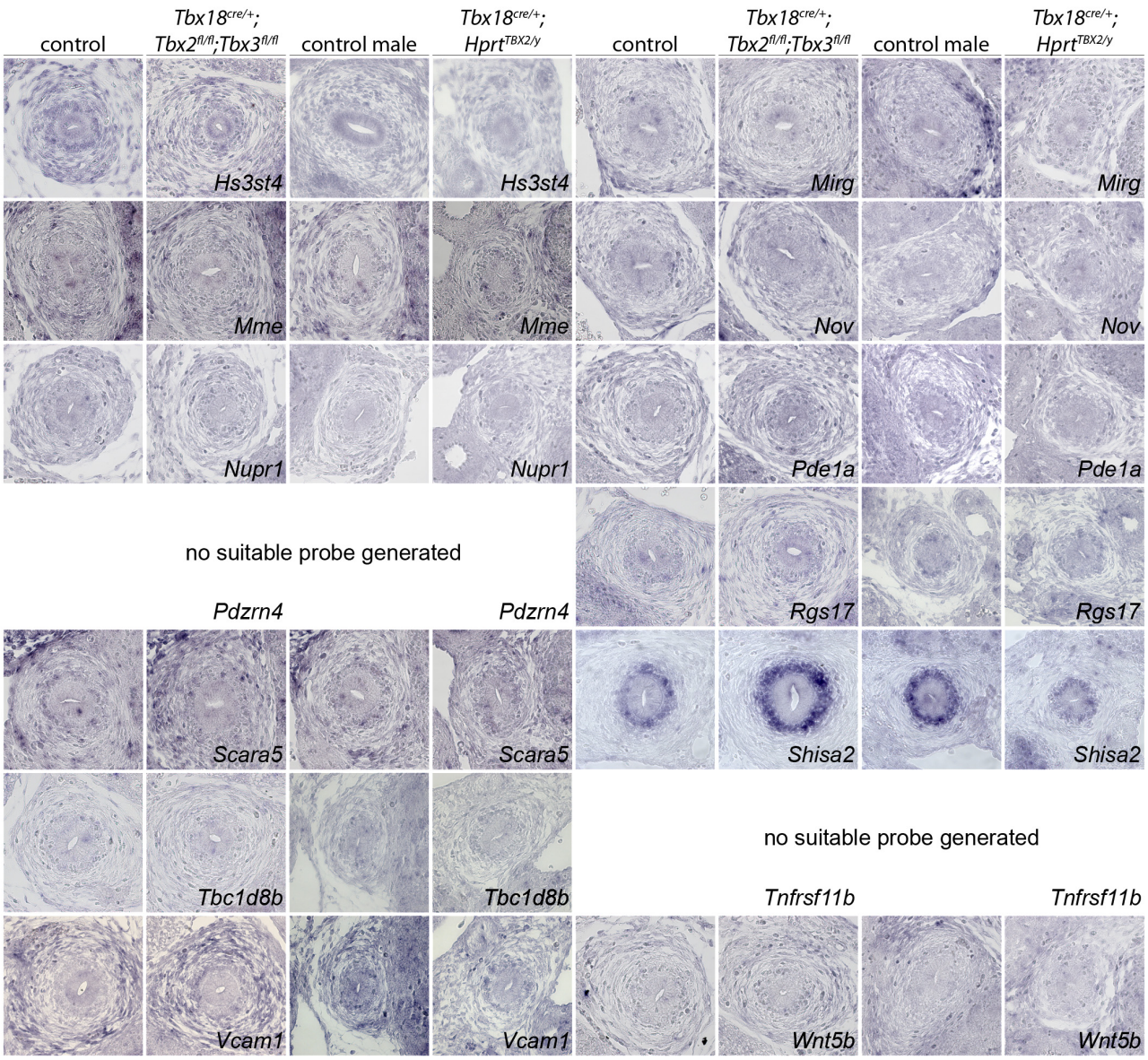


Fig. S13. Expression of few microarray candidates can be validated by *in situ* hybridization analysis. *In situ* hybridization analysis on transverse sections of the proximal ureter region in E14.5 wildtype, *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ and *Tbx18*$^{cre/+}$;*Hprt*$^{TBX2/y}$ embryos. Probes and genotypes are as indicated.

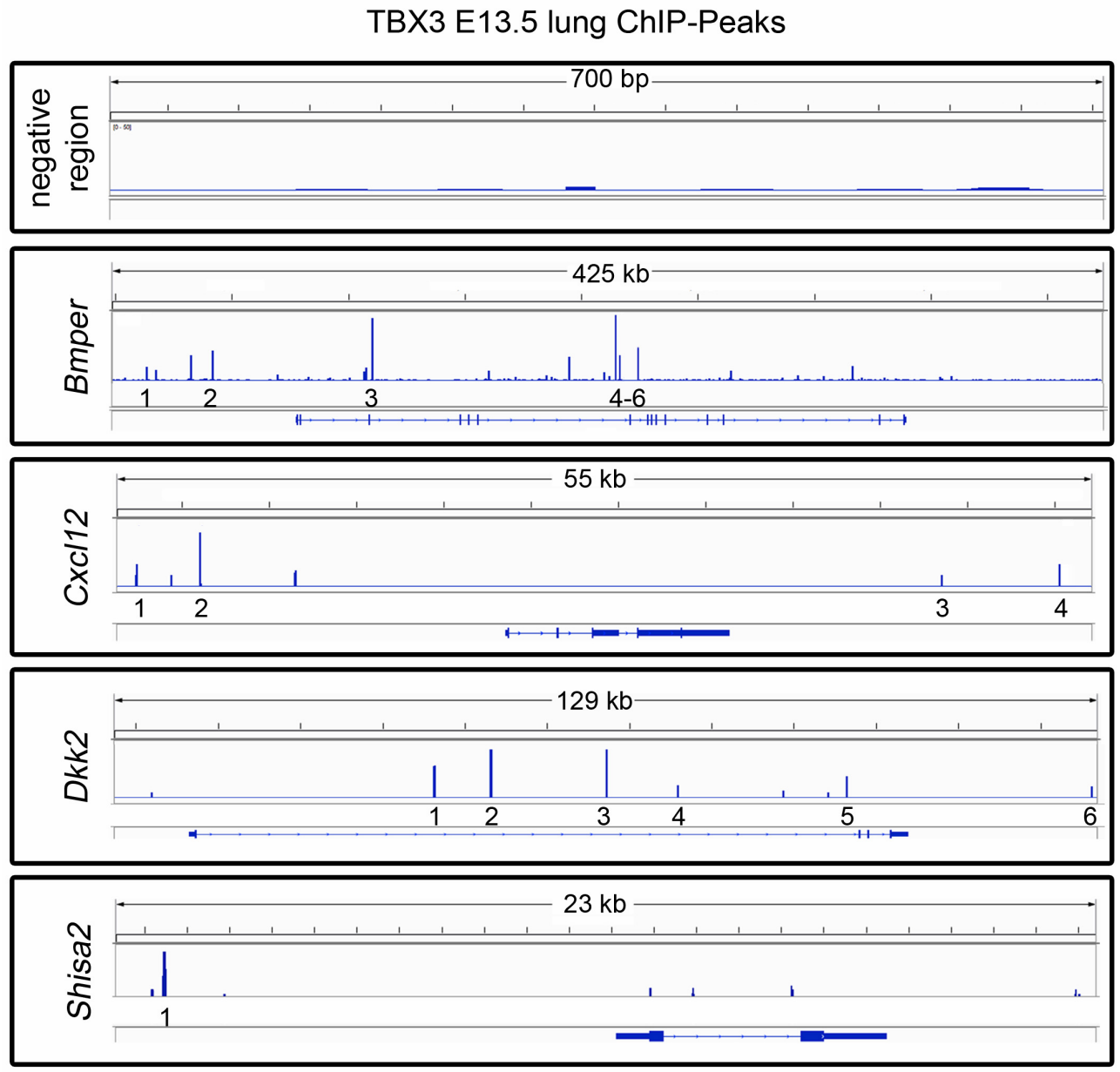


Fig. S14. *Bmper*, *Cxcl12, Dkk2* and *Shisa2* show TBX3 binding peaks in ChIP-Seq experiments from embryonic lung tissue. Schemes depicting the genomic organization of a negative control region, and of the *Bmper, Cxcl12, Dkk2* and *Shisa2* locus including up- and downstream DNA sequences. Exon coding sequences are shown as big blue boxes, and 3'- and 5'-UTRs are indicated as smaller blue boxes. Binding peaks as detected by ChIP-seq analysis are plotted as blue vertical lines and are numbered.

Table S1. Transcripts identified by microarray analysis that were upregulated in E18.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ ureters. Two pools of mutant ureters were compared to wildtype controls and the resulting fold changes (FC) in expression are displayed. Intensity thresholds were ≥100; Fold changes were > 1.3.

Table S2. Transcripts identified by microarray analysis that were downregulated in E18.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ ureters. Two pools of mutant ureters were compared to wildtype controls and the resulting fold changes (FC) in expression are displayed. Intensity thresholds were ≥100; Fold changes were > 1.3.

Table S3. Functional enrichment analysis for transcripts that were upregulated in ureters of E18.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Functional enrichment analysis for 327 genes was performed with DAVID 6.8 websoftware (https://david.ncifcrf.gov) using default settings. Shown are enriched terms for the annotation categories/databases Gene Ontology (GO), KEGG Pathway and UniProt (UP) with a p-value p<0.05.

Table S4. Functional enrichment analysis for transcripts that were downregulated in ureters of E18.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Functional enrichment analysis for 405 genes was performed with DAVID 6.8 websoftware (https://david.ncifcrf.gov) using default settings. Shown are enriched terms for the annotation categories/databases Gene Ontology (GO), KEGG Pathway and UniProt (UP) with a p-value p<0.05.

Table S5. Transcripts identified by microarray analysis that were upregulated in E14.5 ureters of *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Two pools of mutant ureters were compared to controls and the resulting fold changes (FC) in expression are displayed. Intensity thresholds were ≥100; Fold changes were > 1.3.

Table S6. Transcripts identified by microarray analysis that were downregulated in E14.5 ureters of *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Two pools of mutant ureters were compared to controls and the resulting fold changes (FC) in expression are displayed. Intensity thresholds were ≥100; Fold changes were > 1.3.

Table S7. Functional enrichment analysis for transcripts that were upregulated in ureters of E14.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Functional enrichment analysis for 238 genes was performed with DAVID 6.8 websoftware (https://david.ncifcrf.gov) using default settings. Shown are enriched terms for the annotation categories/databases Gene Ontology (GO), KEGG Pathway and UniProt (UP) with a p-value p<0.05.

Table S8. Functional enrichment analysis for transcripts that were downregulated in ureters of E14.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ embryos. Functional enrichment analysis for 260 genes was performed with DAVID 6.8 websoftware (https://david.ncifcrf.gov) using default settings. Shown are enriched terms for the annotation categories/databases Gene Ontology (GO), KEGG Pathway and UniProt (UP) with a p-value p<0.05.

Table S9. Transcripts identified by microarray analysis that were downregulated in E13.5 ureters of *Tbx18*$^{cre/+}$;*Hprt*$^{Tbx2/Y}$ embryos. Two pools of mutant ureters were compared to controls and the resulting fold changes (FC) in expression are displayed. Intensity thresholds were ≥100; Fold changes were > 1.3.

Table S10. Transcripts identified by microarray analysis that were upregulated in E13.5 ureters of *Tbx18*$^{cre/+}$;*Hprt*$^{Tbx2/Y}$ embryos. Two pools of mutant ureters were compared to controls and the resulting fold changes (FC) in expression are displayed. Intensity thresholds were >100; Fold changes were > 1.3.

Table S11. Functional enrichment analysis for transcripts that were downregulated in ureters of E13.5 *Tbx18*$^{cre/+}$;*Hprt*$^{Tbx2/Y}$ embryos. Functional enrichment analysis for 298 genes was performed with DAVID 6.8 websoftware (https://david.ncifcrf.gov) using default settings. Shown are enriched terms for the annotation categories/databases Gene Ontology (GO), KEGG Pathway and UniProt (UP) with a p-value p<0.05.

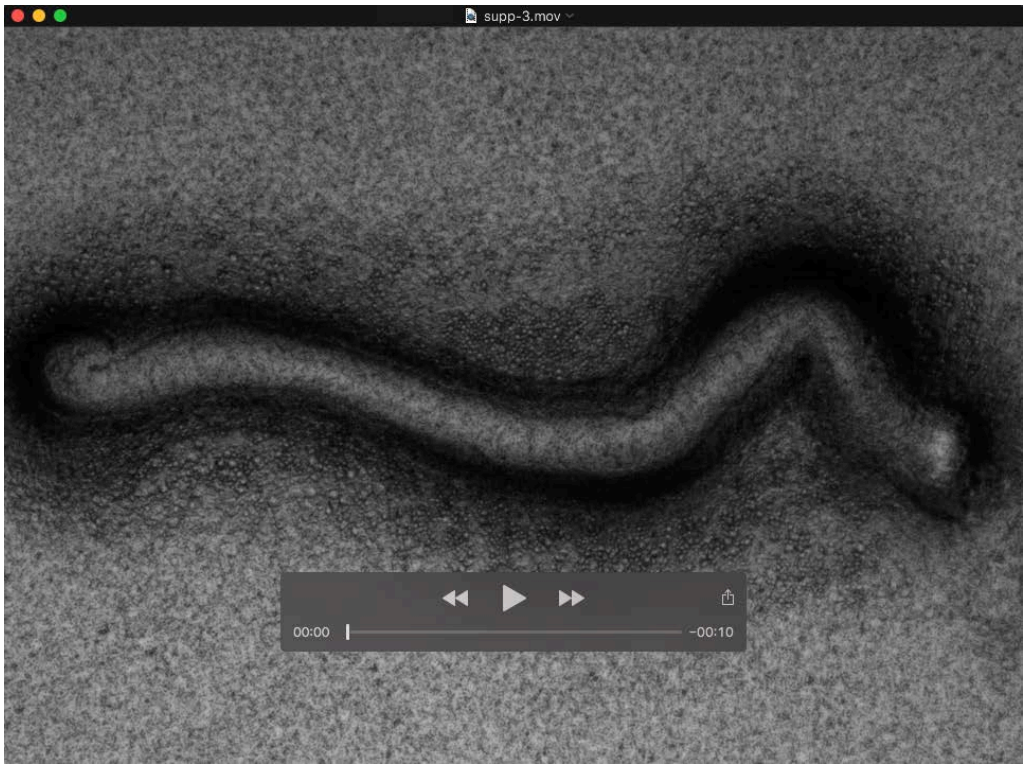
Table S12. Functional enrichment analysis for transcripts that were upregulated in ureters of E13.5 *Tbx18*$^{cre/+}$;*Hprt*$^{Tbx2/Y}$ embryos. Functional enrichment analysis for 442 genes was performed with DAVID 6.8 websoftware (https://david.ncifcrf.gov) using default settings. Shown are enriched terms for the annotation categories/databases Gene Ontology (GO), KEGG Pathway and UniProt (UP) with a p-value p<0.05.

Table S13. Transcripts identified by microarray analysis that were upregulated in E14.5 and E18.5 ureters of *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ (LOF) embryos and downregulated in E13.5 ureters of *Tbx18*$^{cre/+}$;*Hprt*$^{Tbx2/Y}$ (GOF) embryos. FC>1.3, INT≥100, *n.d., not detected (INT<100)*

Click here to download Table S1 - S13

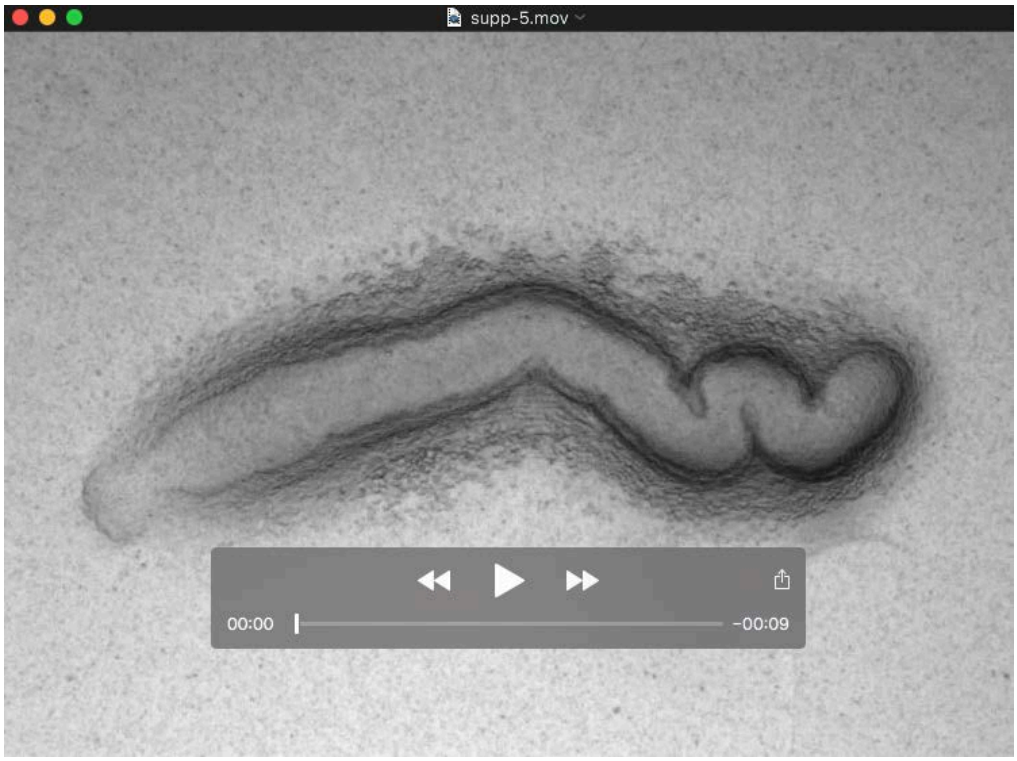
Table S14. List of oligos used for chromatin immunoprecipitation (ChIP) experiments with anti-TBX2 antibodies on E14.5 ureters. Shown are the oligo name, the sequence, the target region and the chromosomal localization.

oligo name	sequence	target region	locus
AKO7063	TAGTGCCTGGCCTAAGTGAG	*Dkk2* region 1	chr3:131,780,330-131,780,886
AKO7064	CCCTTCAAACCCTTGTGGTG		
AKO7065	AGTGAACAGCAATGTGTGGG	*Dkk2* region 2	chr3:131,787,956-131,788,431
AKO7066	GTAAACTGCCAACAGACAGTG		
AKO7067	CCTGAAACTGACACGAAGAC	*Dkk2* region 3	chr3:131,803,155-131,803,634
AKO7068	TCACACCCAGATTTCAGCAG		
AKO7069	GGGACTGCAGTGTGAAGGA	*Dkk2* region 4	chr3:131,812,620-131,813,028
AKO7070	TGGCTCAGTGAAGCTTTAAGT		
AKO7071	TTTGCCCCCAGGATCACAC	*Dkk2* region 5	chr3:131,835,025-131,835,417
AKO7072	CGATGTCCTGACACCACAC		
AKO7073	GCCCCCAAGCAAATAACCC	*Dkk2* region 6	chr3:131,867,305-131,867,510
AKO7074	GGGACAGGAAGTGGTAAGC		
AKO7075	GGAATTCTGTCCTGTGGAAC	*Shisa2* region 1	chr14:60,233,097-60,233,572
AKO7076	GCCCAGCTAAGCATTTCCAG		
AKO7077	AGCATGTGTGCTTTGTGTGG	negative control region	chr14:59,614,060-59,614,758
AKO7078	AAGGGGCTCCAAGCAGCTA		
AKO7079	CCAGGTTTGTGTGTTTTCTGG	*Cxcl12* region 1	chr6:117,100,961-117,101,405
AKO7080	TGATGCTCTTGGGGTGGTC		
AKO7081	AAATCTCAAAACTCAGGCTGC	*Cxcl12* region 2	chr6:117,097,334-117,097,708
AKO7082	ACCCCAAACCCATCACCAG		
AKO7083	TGTGGGTTCAAGGTCTAAGG	*Cxcl12* region 3	chr6:117,143,321-117,143,755
AKO7084	GTTTGGTTTCAAGGCAACCC		
AKO7085	GAGGGGTCCCTTACCATAC	*Cxcl12* region 4	chr6:117,150,812-117,151,140
AKO7086	CTTTCCCCCAAACATTCCGC		
AKO7250	ATTTGGCAGACTGAGGCAAGG	*Bmper* region 1	chr9:22,962,500-22,963,128
AKO7251	TGAGGTGAGGGAAGAACACA		
AKO7252	CCCCATATGCCGTAGCTCTG	*Bmper* region 2	chr9:22,981,599-22,981,958
AKO7253	TACAGCAAGCCCAGGAATCG		
AKO7254	TGCTAAAGGCTCTTTCTCCCC	*Bmper* region 3	chr9:22,986,649-22,987,003
AKO7255	GCAGCTTTTCCCTTTCCTCC		
AKO7256	CTAAGCTCCTTCCCACTGGC	*Bmper* region 4	chr9:23,166,470-23,167,060
AKO7257	CCCTTCAGGAGCTACCCAAC		
AKO7258	ACAAACAAACAACATACTCACGAA	*Bmper* region 5	chr9:23,167,208-23,167,508
AKO7259	GGAGATCCTAGTGGAATTACAACCT		
AKO7260	TGTGCTATTAATTCCTTCCCACA	*Bmper* region 6	chr9:23,174,349-23,174,705
AKO7261	AGCATGGGACTTTCAAGTGGA		

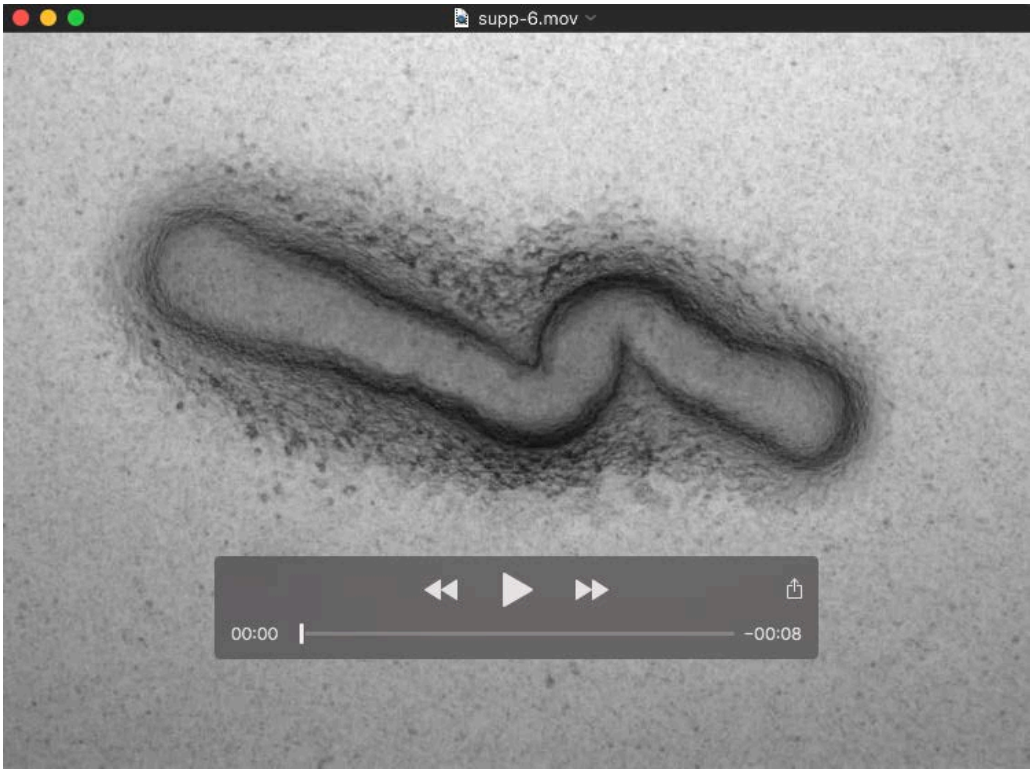


Movie 1. E18.5_Control_D6. Videomicroscopy to monitor the peristaltic activity of an E18.5 wildtype ureter after 6 days of culture. Magnification is 5x. The video was accelerated from 20 sec to 6 sec.

Movie 2. E18.5_DKO_D6. Videomicroscopy to monitor the peristaltic activity of an E18.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ (DKO) ureter after 6 days of culture. Magnification is 5x. The video was accelerated from 20 sec to 6 sec.



Movie 3. E14.5_Control_D6. Videomicroscopy to monitor the peristaltic activity of an E14.5 wildtype ureter after 6 days of culture. Magnification is 5x. The video was accelerated from 20 sec to 6 sec.



Movie 4. E14.5_DKO_D6. Videomicroscopy to monitor the peristaltic activity of an E14.5 *Tbx18*$^{cre/+}$;*Tbx2*$^{fl/fl}$;*Tbx3*$^{fl/fl}$ (DKO) ureter after 6 days of culture. Magnification is 5x. The video was accelerated from 20 sec to 6 sec.