SUPPLEMENTAL FIGURES

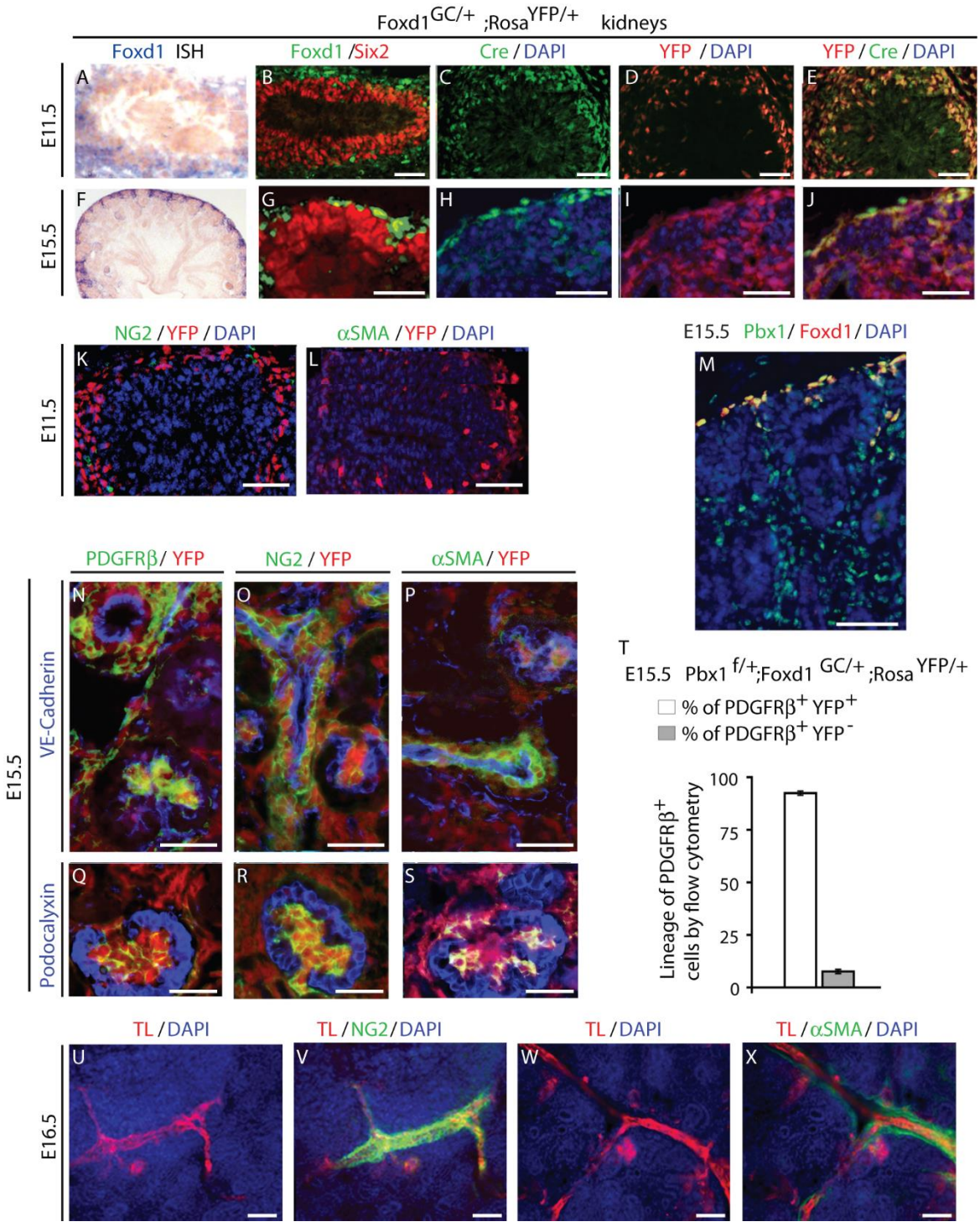

Fig. S1, related to Fig. 1. Differentiation of the Foxd1 lineage into VMCs in the developing kidney.

(A-J) Cre expression in the *Foxd1*$^{GC/+}$*;Rosa*$^{YFP/+}$ line faithfully recapitulates *Foxd1* promoter activity and imparts a YFP tag to cells derived from Foxd1^{+} progenitors. E11.5 (A-E) and

E15.5 (F-J) *Foxd1*$^{GC/+}$;*Rosa*$^{YFP/+}$ kidneys assayed for in situ detection of *Foxd1* mRNA (A,F); IF detection of Foxd1 and Six2 (B,G) and Cre (C,E,H,J). Foxd1-derivatives were detected by YFP florescence (D,E,I,J). Scale bar B-J=50 µm. (K-L) Foxd1^{+} progenitors lack VMC markers at the onset of kidney development. E11.5 *Foxd1*$^{GC/+}$;*Rosa*$^{YFP/+}$ kidneys assayed by IF for the detection of VMC markers NG2 (K) and αSMA (L). Scale bar =50 µm. (M) E15.5 kidneys were analyzed by IF for the detection of Pbx1 and Foxd1. Scale bar =100 µm. (N-S) E15.5 *Foxd1*$^{GC/+}$;*Rosa*$^{YFP/+}$ kidneys assayed by IF for the detection of VECAD (N-P) to reveal endothelial structures, Podocalyxin (Q-S) to reveal podocytes and vascular mural cell markers including *PDGFRβ* (N,Q) and NG2 (O,R) and αSMA (P,S). Foxd1 derivatives were detected by YFP florescence. Scale bar (N-P)=50 µm; (Q-S)=30 µm. (T) Flow cytometry of dissociated E15.5 *Pbx1*^{Ctrl};*Rosa*^{YFP} kidneys (n=18) after immunostaining for PDGFRβ reveal that the vast majority of PDGFRβ^{+} cells in the developing kidney derive from Foxd1^{+} progenitors. (U-X) Cells expressing VMC markers localize around blood-conducting vessels. E16.5 kidneys were processed by intravital dye labeling Tomato Lectin (TL) to identify blood-conducting vessels (U-X) and IF to detect the VMC markers NG2 (V) and αSMA (X). Scale bar =50 µm.

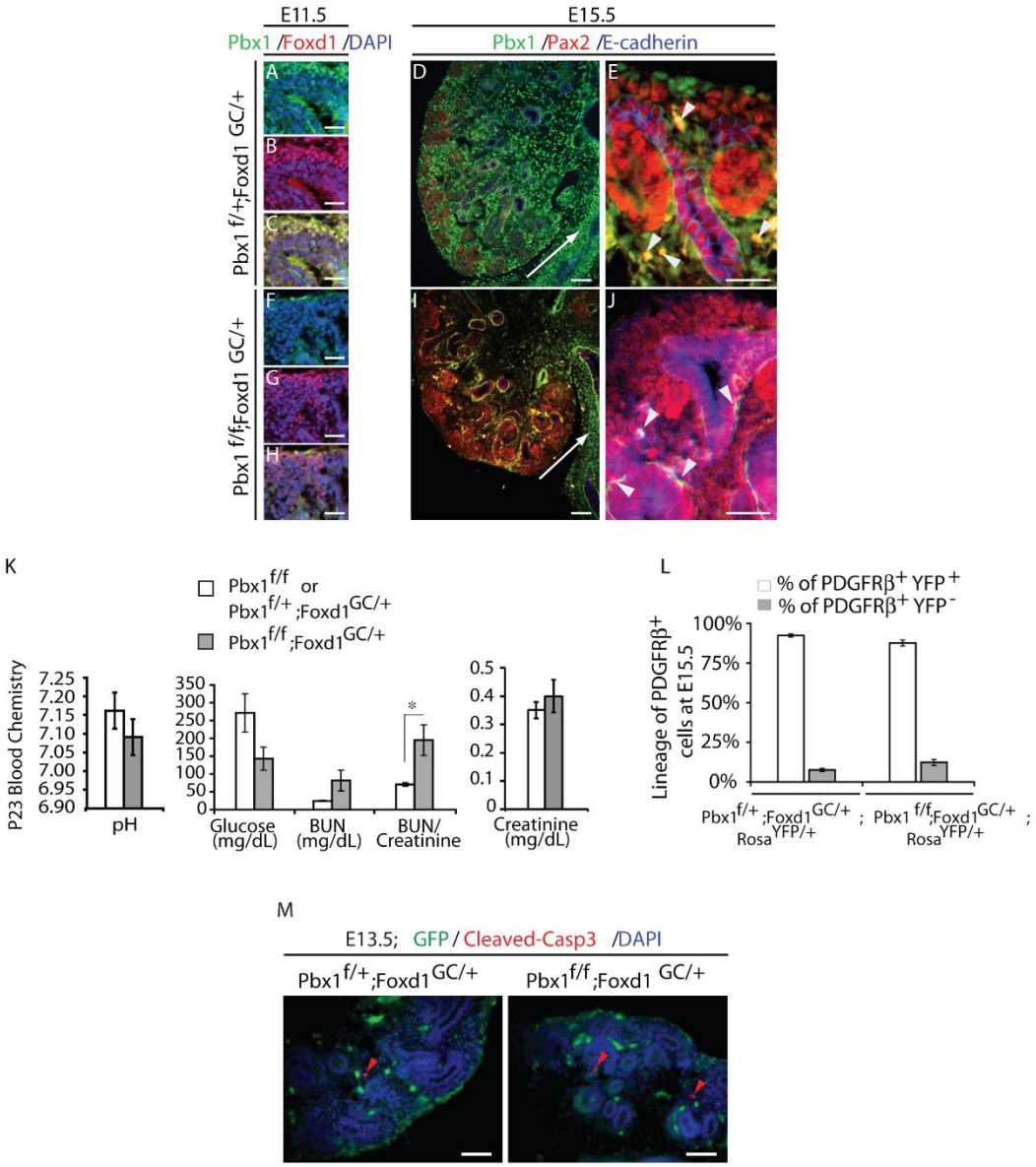

Fig. S2, related to Fig. 2. Foxd1-Cre mediated *Pbx1* inactivation results in impaired kidney function and lethality within the first weeks of life. (A-J) Efficient Foxd1-Cre mediated *Pbx1* inactivation. Control *Pbx1*$^{f/+}$;*Foxd1*$^{GC/+}$ and mutant *Pbx1*$^{f/f}$;*Foxd1*$^{GC/+}$ kidneys at E11.5 (A-C, F-H); scale bar =30 µm and E15.5 (D,E,I,J) kidneys assayed for the detection of Pbx1 and Foxd1 (A-H); Pax2 and E-cadherin (D,E,I,J). Scale bar D,I =50 µm; E,J =25 µm. (D,I) White arrows indicate ureteric mesenchyme. (E,J) White arrowheads indicate nephron progenitors co-expressing Pbx1 and Pax2. (K) Postnatal renal function was assessed by measuring plasma glucose concentration, blood pH, blood urea nitrogen (BUN) and creatinine levels in mutant *Pbx1*$^{f/f}$;*Foxd1*$^{GC/+}$ (n=3) and control *Pbx1*$^{f/+}$;*Foxd1*$^{GC/+}$ or *Pbx1*$^{f/f}$ littermates (n=4) (*i-STAT*® system). Error bars;± s.e.m. **P=0.019*, ***P=0.007*.

(L) Quantitation of the percent of PDGFRβ^{+} cells that express YFP in E15.5 control *Pbx1$^{f/+}$;Foxd1$^{GC/+}$;Rosa$^{YFP/+}$* (n=18 kidney pairs) and mutant *Pbx1$^{f/f}$;Foxd1$^{GC/+}$; Rosa$^{YFP/+}$* kidneys (n=14 kidney pairs) as determined by FACS analyses. (M) IF analysis of apoptosis by Cleaved Caspase-3 staining in E13.5 control *Pbx1$^{f/+}$;Foxd1$^{GC/+}$* and mutant *Pbx1$^{f/f}$;Foxd1$^{GC/+}$* kidneys (n=2 per genotype). Red arrowheads indicate apoptotic cells. Scale bar=50 µm.

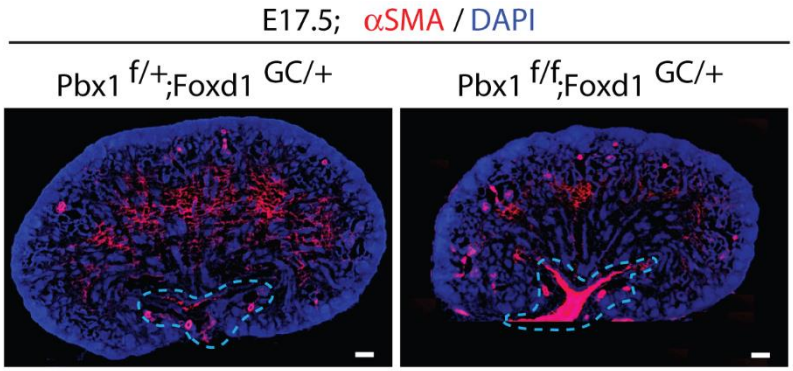

Fig. S3, related to Fig. 3. Medullary interstitium development is impaired in *Pbx1$^{f/f}$;Foxd1$^{GC/+}$* kidneys.

Immunodetection of αSMA^{+} medullary interstitium in E17.5 kidney sections of indicated genotypes. Dashed blue outline indicates the αSMA^{+} ureteric mesenchyme that is excluded from quantification. Scale bar= 50 µm.

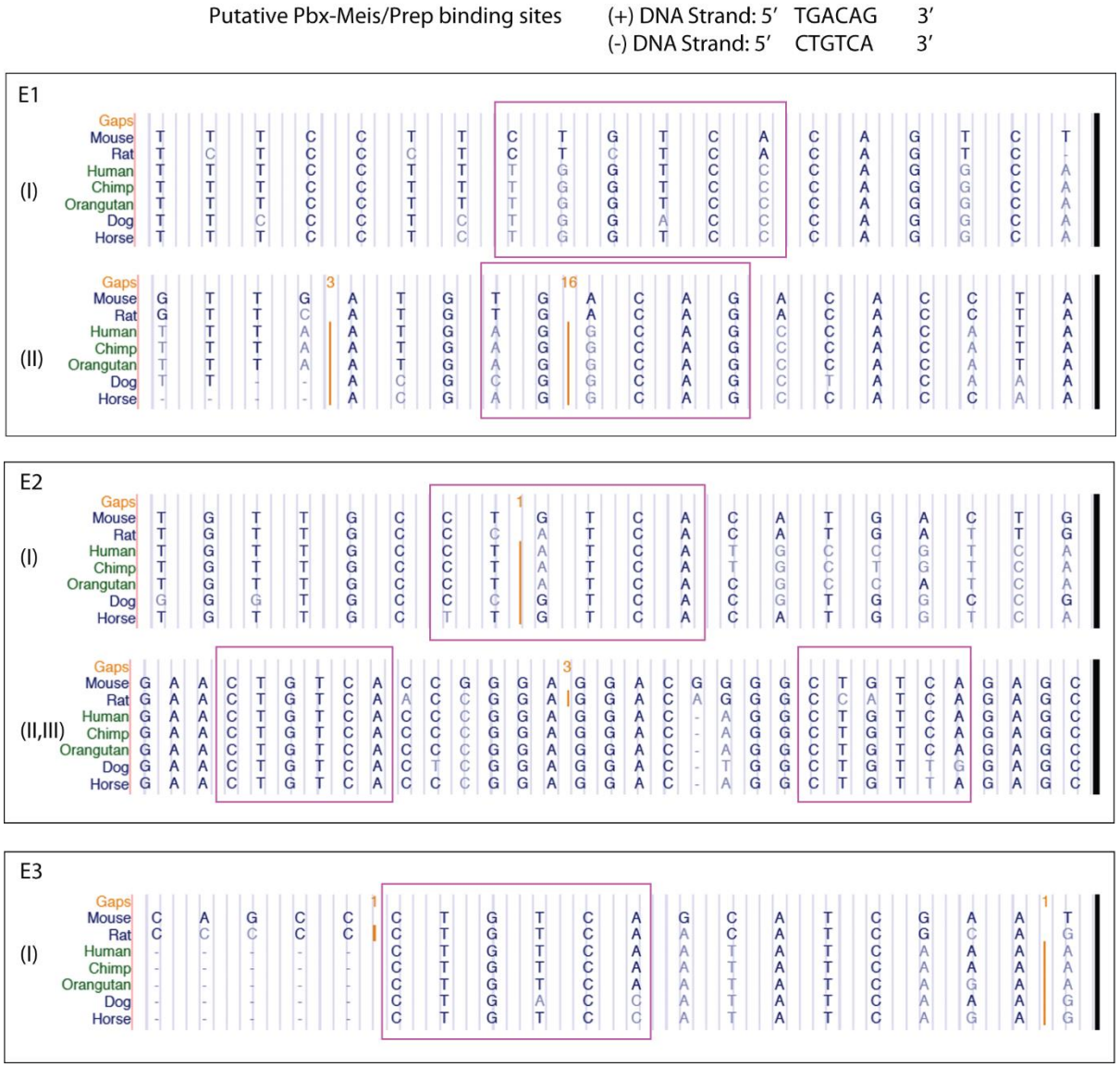

Figure S4, related to Figure 5. Sequence conservation of Pbx-Prep/Meis binding sites within E1, E2, E3 across select mammalian species UCSC genome browser (mm9) generated sequence alignment of Pbx-Prep/Meis binding sites within E1, E2, E3 across select mammalian species

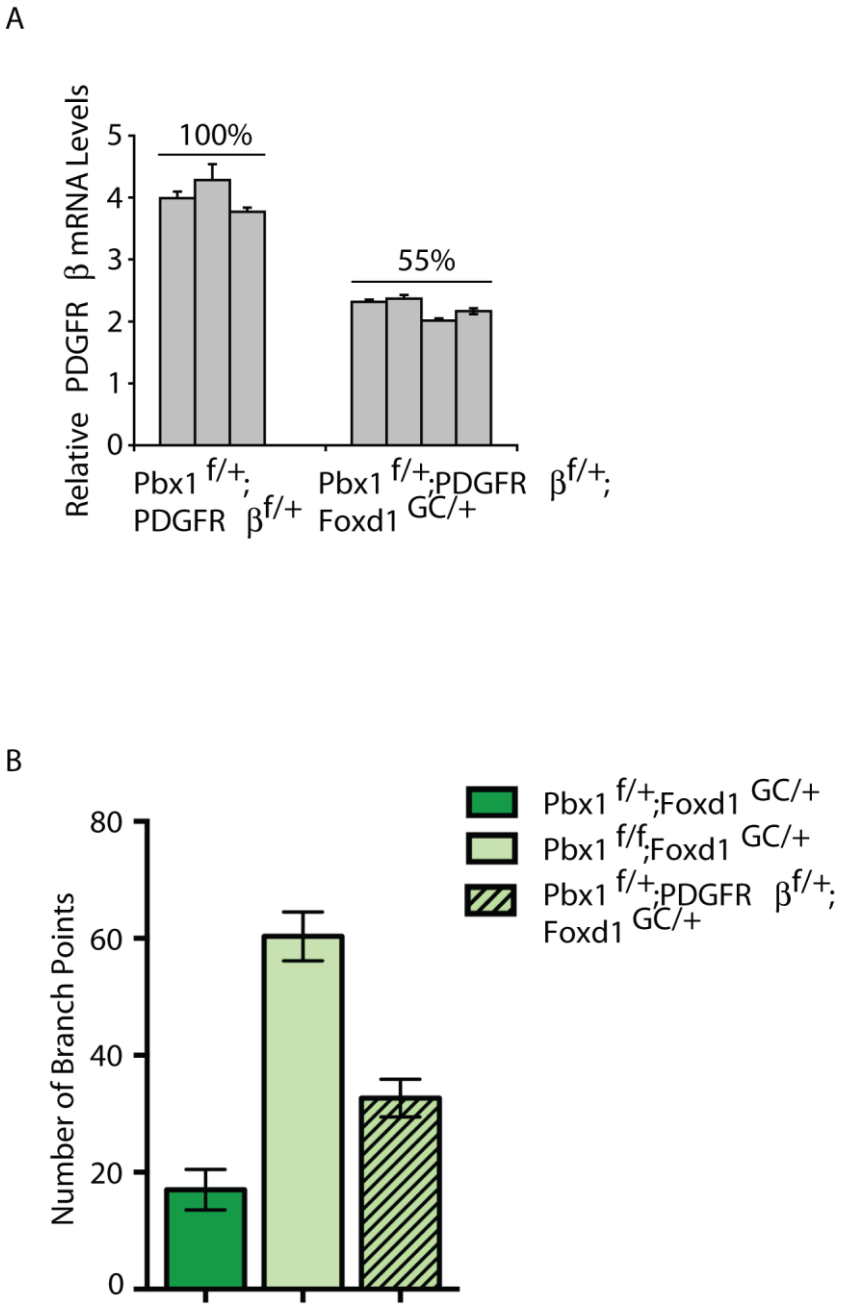

Fig. S5, related to Fig. 6. Inactivation of one allele of *PDGFRβ* by Foxd1-Cre efficiently reduces *PDGFRβ* mRNA levels

(A) Relative expression levels of *PDGFRβ* transcripts in E15.5 control *Pbx1*$^{f/+}$;*Foxd1*$^{GC/+}$ (n=3 kidney pairs) and *Pbx1*$^{f/+}$;*PDGFRβ*$^{f/+}$;*Foxd1*$^{GC/+}$ (n=4 kidney pairs) by RT-qPCR. Normalization to *Tbp* mRNA levels. Error bars ± s.e.m. (B) Quantification of arterial branch points (mean±s.d.) in E13.5 control *Pbx1*$^{f/+}$;*Foxd1*$^{GC/+}$, mutant *Pbx1*$^{f/f}$;*Foxd1*$^{GC/+}$ and *PDGFRβ*^{het} rescue *Pbx1*$^{f/f}$;*PDGFRβ*$^{f/+}$;*Foxd1*$^{GC/+}$ kidneys. n>3 per genotype.

SUPPLEMENTARY MATERIALS AND METHODS

Antibodies

Primary antibodies used include anti-αSMA-Cy3 (Sigma C6198), anti-PDGFRβ (Cell Signaling Technology 3169, Novus Biologicals NBP1-43349), anti-Pbx1 (Cell Signaling Technology 4342), anti-GFP (Aves Labs GFP-1020), anti-CD31/Pecam1 (BD Pharmingen 550274), anti-Foxd1 (Novus Biologicals NBP1-30959), anti-VECAD (R&D Systems AF1002), Anti-Cre (Millipore MAB5320), anti-Six2 (Santa Cruz sc-67837), anti-Pax2 (Cell Signaling 9666), anti-NG2 (Millipore MAB5320), anti-E-cadherin (R&D Systems AF748), anti-Villin-1 (Cell Signaling 2369), anti-Podocalyxin (R&D Systems AF1556), anti-Calbindin-28 (Sigma C9848), anti-cleaved-Caspase3 (Promega G7481).

TaqMan Gene Expression Assays

Pbx1 Mm04207617_m1 *Pbx2* Mm00479560_m1

Pbx3 Mm00479413_m1 *Tbp* Mm00446971_m1

PDGFRβ Mm00435546_m1 *PDGFRβ* Mm00435564_m1

Oligonucleotides

Oligonucleotides were synthesized by Operon Biotechnology. All the oligonucleotides used for PCR assays were designed using the Primer3 website (http://frodo.wi.mit.edu/primer3/).

Chromatin Immunoprecipitation Primers

E1 For TTGCCCAAATCTCTCTGCTT

E1 Rev CTCAGAGTGCTGGGTTTGGT

E2 For AGACACGGTAGGTGGAGGTG

E2 Rev GGAGAGAGGAGCCAGGTTCT

E3 For CTCCGTTGGTTTCAACCTGT

E3 Rev GCTGACAGGGCTGGAGTTAG

OUT For ATGGCTGGGTATCTCCAGTG

OUT Rev TCTAATCCCAAATGGCAAGC

Transcriptional Reporter Assay Primers

E1wtFOR GGGGTACCTCCTCTGTGAGGAGGGATTG

E1wtREV CGAGCTCCCGTGGGGTAGAGGAAACTT

E2FOR CCGCTCGAGCTGGGCAGCCTTACAGTCTC

E2REV GAAGATCTCCCAAATGCTCTCCTCTCTG

E3FOR GGGGTACCCACCTTCTCCAGTGTGCTGA

E3REV CCGCTCGAGTCATCGCTGGCCATATAACA

E1Mut FOR.1 TCCTTCATTGCCAGTCTGTTGTTTTCC

E1Mut REV.1 GGAAAACAACAGACTGGCAATGAAGGA

E1Mut FOR.2 CCTTTCTCTATGTTGATGGCAATGACACC

E1Mut REV.2 GGTGTCATTGCCATCAACATAGAGAAAGG