Supplementary figures

Supplementary Figure S1. Six2 inactivation strategy. (A) Six2 was inactivated by targeted disruption of exon 1, including the transcription start site, the homeobox domain (yellow), and the Six domain (red). (B) The targeted locus resulted in the removal of a *Hin*dIII site, which was visualized in *Six2* heterozygotes and *Six2*-null as a 15.6-kb band on a Southern blot. The wild-type band was 3-kb long.

Supplementary Figure S2. Six2 is expressed in E14.5 MM progenitors. (A) At this stage, Pax2 is normally expressed in condensing MM, developing nephrons (arrow), and the UB tips (asterisk) in the cortex of the kidney. (B) *Six2* is expressed only in the condensing MM reserved for future rounds of nephrogenesis and is absent from the MM derivatives (i.e. developing nephron; arrow) which express *Wnt4* (C). All sections are adjacent. Scale bar, 100 μm.

Supplementary Figure S3. *Six2*-null kidneys exhibited premature and arrested nephrogenesis. (A) The mean number of nephrons detected in each wild-type and *Six2*$^{-/-}$ kidney at E12.5, E14.5, and E15.5 was determined by using different nephron-specific markers in embryonic sections and explant cultures. Wild-type kidney: E12.5 (n=33), E14.5 (n=13), and E15.5 (n=10); *Six2*-null: E12.5 (n=26), E14.5 (n=6), and E15.5 (n=4). At E12.5, *Six2*-null kidneys develop more nephrons than controls due to premature nephrogenesis; however, later on the number of nephrons present in the wild-type kidney surpasses that of *Six2*$^{-/-}$. In *Six2*-null kidneys at E15.5, *Slc34a1* expression (C) confirmed the presence of proximal tubules, that of *Slc12a1* (E) identified the presence of tubules

corresponding to the thick ascending limb of Henle's loop, and that of *Slc12a3* (G) detected the presence of the distal tubules. (I) No obvious changes in the expression of *Calbindin-3* were seen in the connecting segments of the *Six2*-null kidney. Scale bar, 100 μm.

Supplementary Figure S4. Molecular characterization of the *Six2*-null kidney. (B) In the E11.5 *Six2*-null kidney, the expression of *Foxd1* in the stromal population, and that of *Wnt9b* (C) and *Lim1* (E) in the UB were normal. (G) At E12.5, the stromal population in the *Six2*-mutant kidney was reduced in size but maintained normal levels of *Foxd1*. (I) *Wnt9b* continued to be expressed at normal levels at this stage in the UB of *Six2*-null kidney and *Lim1* (K) was expressed in the ectopic renal vesicles (arrows) although its expression was reduced in the UB (asterisk). Scale bar, 100 μm.

Supplementary Figure S5. Analysis of the *Six2*$^{-/-}$ kidney at later developmental stages. (B) At E14.5, *cRet* expression (blue) was absent from the *Six2*$^{-/-}$ UB. Pax2 expression (brown) remained in the renal tubules (arrow) and UB. (D) *Eya1* expression was absent from the *Six2*$^{-/-}$ kidney at this stage. The *Six2*$^{-/-}$ kidney anlagen is outlined by the dashed lines. *Wnt4* (F) and *Foxd1* (H) expression was normal in *Six2*$^{-/-}$ renal tubules and stroma respectively. Scale bar, 100 μm.