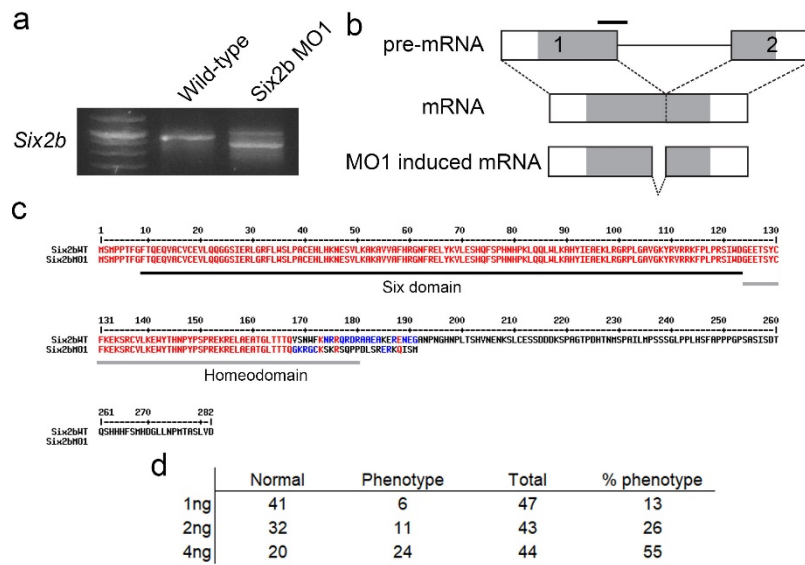
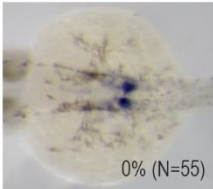
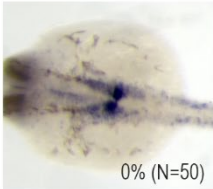
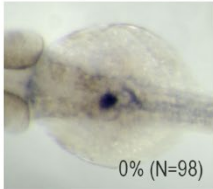
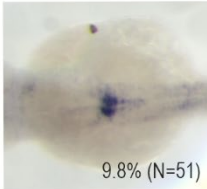
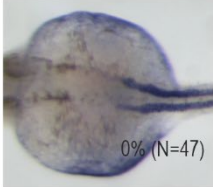
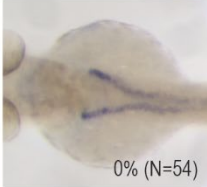
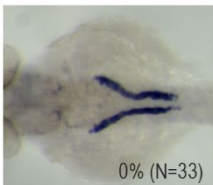
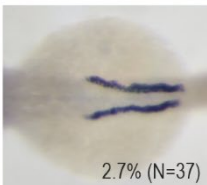




Supplemental Figure 2: Characterization of *six2b* MO1. (a) PCR amplification of full-length *six2b* in wild-type and MO1 injected embryos. A smaller PCR amplicon is detected along with the wild-type band following MO1 injection corresponding to a deletion from improper splicing. The presence of both bands most likely represents mosaic effect of the MO. Both PCR fragments from MO1 injected embryos were isolated and sequenced confirming the MO1 induced deletion. (b) Schematic of *six2b* splicing in embryos injected with splice donor inhibitor MO1. In the presence of the morpholino, a cryptic splice in exon 1 is utilized resulting in a 59-nucleotide deletion. (c) Amino acid alignment of wild-type and MO1 induced Six2b protein. Injection of MO1 is predicted to result in a protein that has a disrupted homeodomain and missing C-terminus. (d) MO1 injection series at increasing concentrations. Embryos were assessed at 48 hpf using the *wt1a* glomerular marker. Increasing percentage of *wt1a* phenotype was observed with increased MO1 amounts.

b

	24hpf TUAB	24hpf <i>Six2b</i> ^{T35-3/+}	48hpf TUAB	48hpf <i>Six2b</i> ^{T35-3/+}
<i>wt1a</i>	 0% (N=55)	 0% (N=50)	 0% (N=98)	 9.8% (N=51)
<i>cdh17</i>	 0% (N=54)	 0% (N=47)	 0% (N=125)	 0% (N=54)
<i>slc20a1a</i>	N/A	N/A	 0% (N=33)	 2.7% (N=37)

Chi square = 1.833 2 degrees of freedom; two-tailed P = 0.3998

Supplemental Figure 3: Phenotypic analysis of *Six2b*^{T35-3/+} incrosses. (a) Nucleotide alignment of wild-type *six2b* NM_001128734 with the CRISPR/Cas9 induced mutation *six2b*^{T35-3} to show the genomic DNA lesion. (b) *Six2b* mutant heterozygous parents were crossed and resulting embryos were processed for *in situ* hybridization at 24 and 48 hpf. No morphological defects were found at 24 hpf. Some pronephric marker phenotypes were detected at 48 hpf but not at expected frequencies. (c) Chi square analysis of embryos resulting from heterozygous crosses. Genotypes of embryos were at expected frequencies.

Supplemental Table 1: Reagents for *six2b* CRISPR/Cas9 mutagenesis

Target without PAM sequence	GCAGTAGTAGCTTTCCACCG
sgRNA with Sp6 promoter and overlap region	ATTAGGTGACACTATAGCAGTAGTAGCTTTCCACCGTTTTAGAGCTAGAAATAGCAAG
Forward primer	5' GCACTTAGCAATGTCTATGCCAC 3'
Reverse primer	5' GGAGAAAACCTGGTGGCTCTCTA 3'
Forward primer with T7 sequencing site	cacTAATACGACTCACTATAGGGcacGCACTTAGCAATGTCTATGCCAC 3'