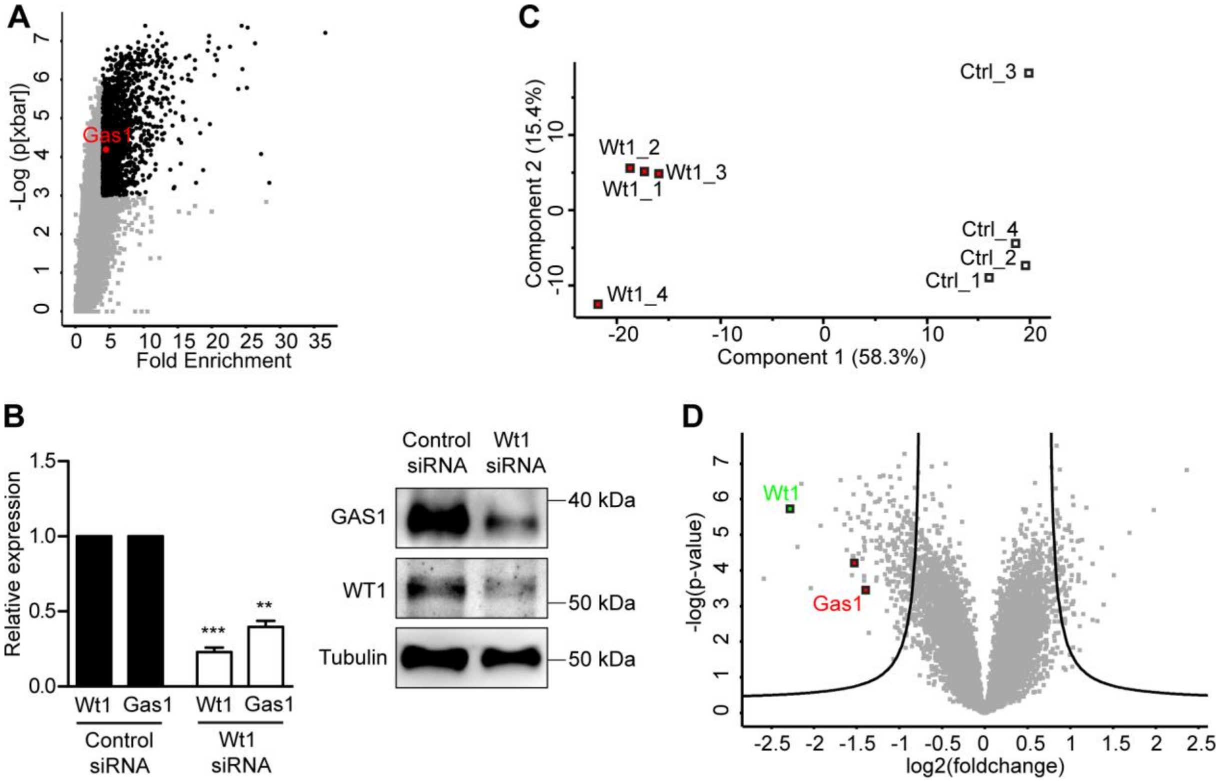

Figure S1. Identification of *Gas1* as a candidate WT1 target gene from high throughput experiments. A Volcano plot of previously published WT1 ChIP-on-chip data. Significantly enriched probes shown in black. *Gas1* probe is labeled in red. **B** Confirmation of siRNA-mediated Wt1 knockdown and decreased Gas1 expression in *Wt1*-knockdown LB-22 cells by qPCR and western blot. (** $p < 0.01$, *** $p < 0.001$, error bars = s. e. m., n =3). **C** Principal component analysis of gene expression array data shown in **A**. **D** Volcano plot identifying significant differentially regulated genes between siRNA-mediated *Wt1* knockdown and control LB-22 cells. *Gas1* and *Wt1* probes are highlighted. Black lines indicate an FDR threshold of 0.0001.

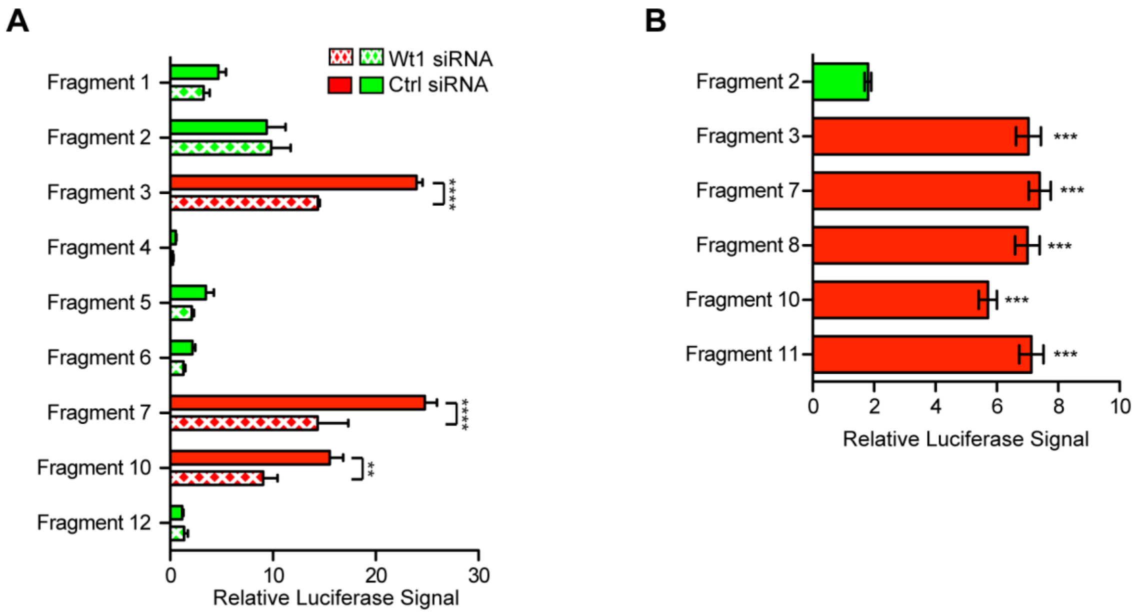

Figure S2. An endogenous 200 bp *Gas1* promoter fragment is WT1 responsive and sufficient to drive reporter transcription in the absence of a minimal promoter. A Bar graph representing dual luciferase reporter assay results in LB-22 cells normalized to empty vector. Location of fragments is provided in Fig. 1A. Fragments containing the WT1 binding site show reduced reporter signals upon transfection of WT1 siRNA. (** $p < 0.01$, **** $p < 0.0001$, n = 3, error bars = s. e. m.). **B** Dual luciferase reporter assays using a reporter vector missing a minimal promoter. The WT1-responsive element is sufficient to endogenously drive reporter activation. (*** $p < 0.001$, n = 3, error bars = s. e. m.)

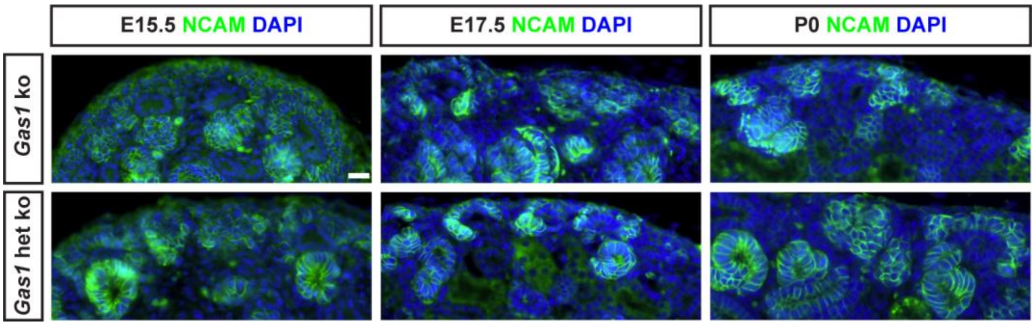

Figure S3. *Gas1* knockout kidneys show reduced nephrogenesis in later stages of kidney development. Immunofluorescence stainings for NCAM showing nephron progenitor cells and primordial nephrons from the RV to S-shaped body stage at various stages in kidney development. Normal induction of nephrons is present in *Gas1* knockout kidneys at E15.5. At later stages, reduced numbers of progenitors and induced nephrons become evident. Scale bar: 10 µm.

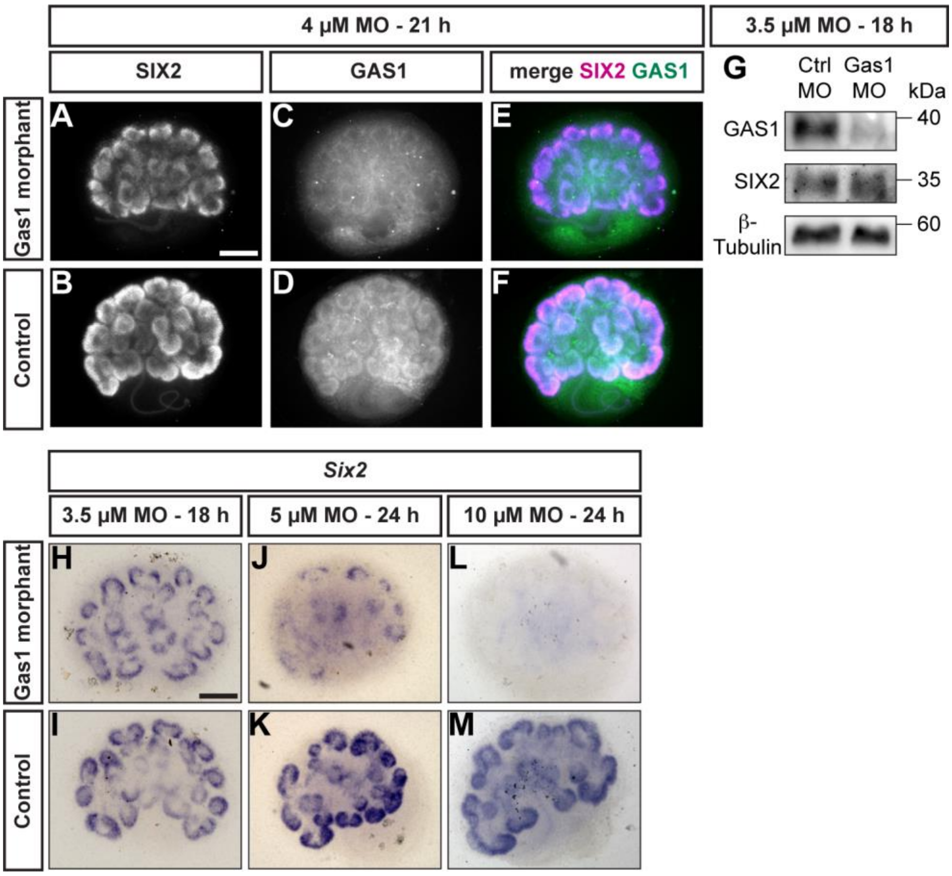

Figure S4. Knock-down of GAS1 in kidney organ cultures confirms a role of Gas1 in maintaining nephron progenitor cells. A – F Whole-mount immunofluorescence staining of kidney organ cultures exposed to Gas1 MO at indicated concentrations or control MO. GAS1 expression is reduced beyond the depletion of SIX2+ NPC in Gas1 morphant cultures indicating an effective knockdown. Scale bar: 100 µm **G** Western blot of pooled whole cell lysates of explants cultured in a mild knockdown condition (2.1 µM Gas1 MO1, 1.4 µM Gas1 MO2, 18 hours) or in a control condition (3.5 µM Control MO, 18 hours). Efficient knockdown of GAS1 is evident, while SIX2 signals are preserved. **H – M** Whole-mount in situ hybridization of Gas1 morphant and control organ cultures for *Six2* when cultured in the presence of increasing concentrations of Gas1 MO. A reciprocal relationship between the degree of Gas1 knockdown and preservation of the nephron progenitor pool is evident, indicating a role for Gas1 in maintaining nephron progenitor cells in this *ex vivo* model. Scale bar: 100 µm.

Table S1. Primer, siRNA and morpholino sequences.

Primer name		Sequence (5’ to 3')	
Genotyping primers			
Six2-Cre	f	ATG TCC AAT TTA CTG ACC GT	
	r	CGC CGC ATA ACC AGT GAA AC	
Wt1 shRNA	f	CGA AGT TAT CTA GAG TCG AC	
	r	AAA CAA GGC TTT TCT CCA AGG	
Gas1 mutant	f	ACT ACG CGT ACT GTG AGC CAG AG	
	r	AGT GAC CAG CGA ATA CCT GTT CC	
Gas1 wild type	f	TAC TGC GGC AAG CTT TTC AAC GG	
	r	AGC GCG CTG CTC GTC GTC ATA TTC	
ChIP qPCR			
Gapdhs	f	CAG GAG CCC AGG GAA GAT ACA AAT A	
	r	ACG CAT ACA CAT ATA CAA CCA GTC A	
Jmjd1a	f	GCA GCT CCA TTC TTC CAT TT	
	r	GCT CAT GAT CCT GGG TCT C	
Gas1 Amplicon 1	f	GAG CTG GAT AAG CGC CGA GAT G	
	r	GCC AGT ACG CCG AGG CTT GT	
Gas1 Amplicon 2	f	GTG CGG AGC GGA GAC GAG G	
	r	GAT GAG GAC GCC CAT GCC AGA A	
Gas1 Amplicon 3	f	CCG GGG GTG CAT GGT GCT	
	r	GGG TAG AGG GGG AGG GGA C	
Gas1 Amplicon 4	f	CCG CGA GGC TTT AAA TAC AA	
	r	CGG AGA GTG GAG AAA GGA GA	
Gas1 Amplicon 5	f	CAG CGG GCA GGG CTT AGG G	
	r	ACA AAA AGG GGG CGG AGG AAG G	
Gas1 Amplicon 6	f	CCA GCT TTC AGT CCC CCT TTC C	

	r	TGC TAA GAT TTG TCA TTG CCG TTC GT	
Gas1 Amplicon 7	f	AAA GCA AAA GCC ACC AGC AGA G	
	r	AGG GGA GAG GAA GGA GCC AGT T	
RT-qPCR			
Wt1	f	GAG AGC CAG CCT ACC ATC C	
	r	GGG TCC TCG TGT TTG AAG GAA	
Gas1	f	CGA ATC GGT CAA AGA GAA CA	
	r	GCT CGT CGT CAT ATT CTT CG	
Gapdh	f	CAA TGA AGG GGT CGT TGA T	
	r	GGT GAA GGT CGG TGT GAA	
Wnt11	f	GCT GGC ACT GTC CAA GAC TC	
	r	CTC CCG TGT ACC TCT CTC CA	
Etv5	f	TCA GTC TGA TAA CTT GGT GCT TC	
	r	GGC TTC CTA TCG TAG GCA CAA	
Cxcr4	f	TCC AAC AAG GAA CCC TGC TTC	
	r	TTG CCG ACT ATG CCA GTC AAG	
Crlf1	f	CTC CCT GCA AGC TAC CTG C	
	r	AGG GTG GAG GTG TTA AGG AGG	
Cloning Gas1 promoter constructs			
Fragment 1	f	CGG CCA GGA CGC TTG GT	
	r	CAC CGC TCC CGG CCC ACT TTT GTA TTT A	
Fragment 2	f	CGG AGC CCG GCT GCG GAC TAG C	
	r	CAC CAG GGC GCC GGA GAG TGG AGA AAG G	
Fragment 3	f	TTG CTC GGC CCC CTT CCC CGG CTT	
	r	CAC CGC CCT GCC CGC TGG CTC TGT GCG	
Fragment 4	f	TTA GGG CCA GCT GCC TGC	
	r	CAC CGA GGA ACT GAA ACG TGC AGT TTA AAG C	
Fragment 5	f	GCT GTG TTT AAA GCA AAA GCC ACC AG	

	r	CAC CCA CAG CTC AGT CTG CCT CTC TCA TCC	
Fragment 3 mutagenesis motif 1	f	TGG AGT GGG GCT GCA GAG T**T**G G**AT** AGG GCG GTG G	
	r	CCA CCG CCC T**AT** CC**A** ACT CTG CAG CCC CAC TCC A	
Fragment 3 mutagenesis motif 2	f	GAC ACA GGC CCA GCT CCT **TA**C A**A**T CTT CAG GAG GCT GG	
	r	CCA GCC TCC TGA AGA **T**TG **TA**A GGA GCT GGG CCT GTG TC	
siRNAs & shRNAs			
Gas1 siRNA 1	f	CCG CUG AUC UAU ACU GCC AGC UCU A	
	r	UAG AGC UGG CAG UAU AGA UCA GCC G	
Gas1 siRNA 2	f	UAG AUC AGC CGG UUA UCU CUG CAG C	
	r	GCU GCA GAG AUA ACC GGC UGA UCU A	
Gas1 Ctrl siRNA	f	CGG CUA GAU AUC GUC CGA CUU CCU A	
	r	UAG GAA GUC GGA CGA UAU CUA GCC G	
WT1 siRNA	f	UUC CAG GUC AUG CAU UCA AGC UGG G	
	r	CCC AGC UUG AAU GCA UGA CCU GGA A	
WT1 Ctrl siRNA	f	UUC UCC GAU GAC GUA CUU ACG AGG G	
	r	CCC UCG UAA GUA CGU CAU CGG AGA A	
WT1 shRNA	f	GCC CAG CTT GAA TGC ATG ACC TGG AAC AAG AGA TTC CAG GTC ATG CAT TCA AGC TGG GTT TTT TCC ATG GGC	
	r	GGC CGC CCA TGG AAA AAA CCC AGC TTG AAT GCA TGA CCT GGA ATC TCT TGT TCC AGG TCA TGC ATT CAA GCT GGG C	
Vivo-Morpholino oligonucleotides			
WT1 MO		CAG GTC CCG CAC GTC GGA ACC CAT G	
WT1 mismatch MO		CAG cTC CgG CAC cTC GcA ACC gAT G	
GAS1 MO 1		TCC CTG AGA AGC GCG GCG ACG CA	
GAS1 MO 2		GCG TCC TCA TCC ATC CAT GCC GG	
Control MO		CCT CTT ACC TCA GTT ACA ATT TAT A	