

## Supplemental data

### Supplementary Figure 1. Analysis of the *Sall1* mutant mice

(A) (A': higher magnification) Nephrons formed in the *Six2<sup>GFP</sup>Cre;Sall1<sup>fllox/fllox</sup>* mice at E14.5 retain *Sall1* expression. *Sall1* is stained red and *Six2* is green. Scale bar=40  $\mu\text{m}$ .

(B)(C) *In situ* hybridization of *Etv4* (FGF activity) in *Sall1<sup>+fllox</sup>* and *Sall1<sup>CreER/fllox</sup>* kidneys at E14.5 (48hr after tamoxifen treatment), showing the intact expression in the progenitors (arrowheads). Expression in ureteric buds reflects *Gdnf* activity. Scale bar=100  $\mu\text{m}$ .

(D)(E) Immunostaining of *Six2* (green) and cytokeratin 8 (red) in *Sall1<sup>fllox/fllox</sup>* (D) and *Six2<sup>Cre</sup>;Sall1<sup>fllox/fllox</sup>* (E) kidneys in culture. E12.5 kidneys were treated with FGF2 (50ng/ml) and heparin (1ug/ml) and cultured for 2 days, but the phenotypes of the *Sall1* mutants were not rescued. Scale bar=100  $\mu\text{m}$ .

(F)(G) Immunostaining of *Lef1* (Wnt activity) *Sall1<sup>+fllox</sup>* and *Sall1<sup>CreER/fllox</sup>* kidneys at E14.5 (48hr after tamoxifen treatment). *Lef1* is expressed in the differentiating nephrons (arrows) and excluded from the progenitors (arrowheads) in both samples. Scale bar=100  $\mu\text{m}$ .

(H)(I) Whole mount *in situ* hybridization of *Wnt9b* in *Sall1<sup>+fllox</sup>* (H) and *Sall1<sup>CreER/fllox</sup>* (I) kidneys 48hr after tamoxifen treatment. Scale bar=100  $\mu\text{m}$ .

(J)(K) Whole mount *in situ* hybridization of *Wnt11* in *Sall1<sup>+fllox</sup>* (J) and *Sall1<sup>CreER/fllox</sup>* (K) kidneys 48hr after tamoxifen treatment. Scale bar=100  $\mu\text{m}$ .

(L) Quantitative RT-PCR of E14.5 kidneys. *Sall1<sup>+fllox</sup>* mice and *Sall1<sup>CreER/fllox</sup>* mice were treated with tamoxifen at E12.5. The columns represent means  $\pm$  SD (n=5). Primers are listed in Supplementary Table 4.

### Supplementary Figure 2. *Sall1* binding to gene loci that are essential for kidney development

(A) *Sall1* and *Six2* co-occupy *Pax2*, *Wt1*, *Gdnf*, and *Sall1* loci. Asterisk: peaks co-occupied by *Sall1* and *Six2*. Diamond: peaks reported in a study by Park et al. <sup>3</sup>

(B) *Sall1* but not *Six2* binds to the loci of *Hox* gene clusters.

(C) *Six2* but not *Sall1* binds to the *Fgf8* and *Bmp7* loci. The peak marked by # is an artifact, which is also detected in the IgG control sample.

### Supplementary Figure 3. Generation and analysis of *Sall1*Flag mouse

(A) Targeting strategy for *Sall1*Flag knock-in mouse. The stop codon of *Sall1* was replaced by the Flag tag. H: *HindIII*; E: *EcoRV*. The genomic DNA was digested with

*EcoRV* or *HindIII* and hybridized with 5' or 3' probes, respectively.

(B) Flag and Sall1 immunostaining of Sall1Flag mouse kidneys at E15.5. Scale bar=100  $\mu\text{m}$ .

(C) ChIP-qPCR analysis of the *Six2* locus using Sall1Flag mouse kidneys and the anti-Flag antibody. A binding peak is observed at region 2, which corresponds to the marked peak in Figure 6A. Primers are listed in Supplementary Table 4.

#### **Supplementary Figure 4. Overexpression of Nkx6.1 *in vivo* affects kidney development**

(A) Successful overexpression of Nkx6.1 in *Six2Cre: Nkx6.1 OE* kidneys at E14.5, shown by immunostaining using the anti-Nkx6.1 antibody.

(B) A mild size reduction of the *Six2Cre: Nkx6.1 OE* kidneys at P0. Hematoxylin-eosin staining.

(C) Scattered Six2-positive nephron progenitors in the *Six2Cre: Nkx6.1 OE* kidneys at P0. Scale bar=100  $\mu\text{m}$ .

#### **Supplementary Figure 5. *Wnt4Cre*-mediated deletion is not specific to differentiating nascent nephrons**

(A) *Wnt4Cre*-mediated *Sall1* deletion at E14.5.

(B) Lineage trace analysis of *Wnt4Cre* analyzed at E14.5. tdTomato is stained by the anti-RFP antibody (blue). Arrowheads: nephron progenitors; arrows: differentiating nephrons; ub: ureteric bud. Scale bar=50  $\mu\text{m}$ .

#### **Supplementary Video 1. Time-lapse analysis of nephron progenitor development in the control kidney**

*Six2CreGFP* kidneys were cultured at E12.5 on Millicell Cell Culture Inserts placed in dishes containing DMEM with 10% serum. The confocal time-lapse images were taken using a CellVoyager CV1000. GFP-positive nephron progenitors expand as the ureteric bud branches.

#### **Supplementary Video 2. Time-lapse analysis of nephron progenitor depletion in *Sall1* deficiency**

*Six2CreGFP; Sall1<sup>flox/flox</sup>* kidneys were cultured at E12.5 on Millicell Cell Culture Inserts placed in glass-bottomed dishes containing DMEM with 10% serum. Confocal time-lapse images were taken using a CellVoyager CV1000. GFP signal decreased rapidly in the absence of *Sall1*.

**Supplementary Table 1. Down-regulated genes in the absence of *Sall1***

Decreased expression of a series of genes in *Sall1* mutant kidneys compared with that in their respective controls. Most of the down-regulated genes in the absence of *Sall1* are expressed more abundantly in *Six2*(+) cells than in *Six2*(-) cells. *Sall1*- and *Six2*-binding peaks detected in our ChIP-Seq analysis were compared with *Six2*-binding peaks in the study by Park et al.<sup>3</sup> (mm9 coordinates). Asterisks and diamonds correspond to those in Figure 6 and Supplementary Figure 2.

**Supplementary Table 2. Up-regulated genes in the absence of *Sall1***

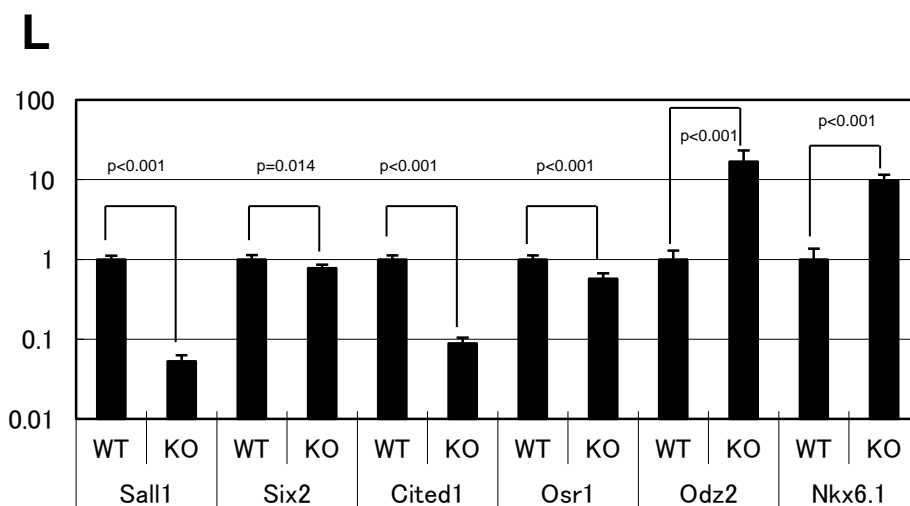
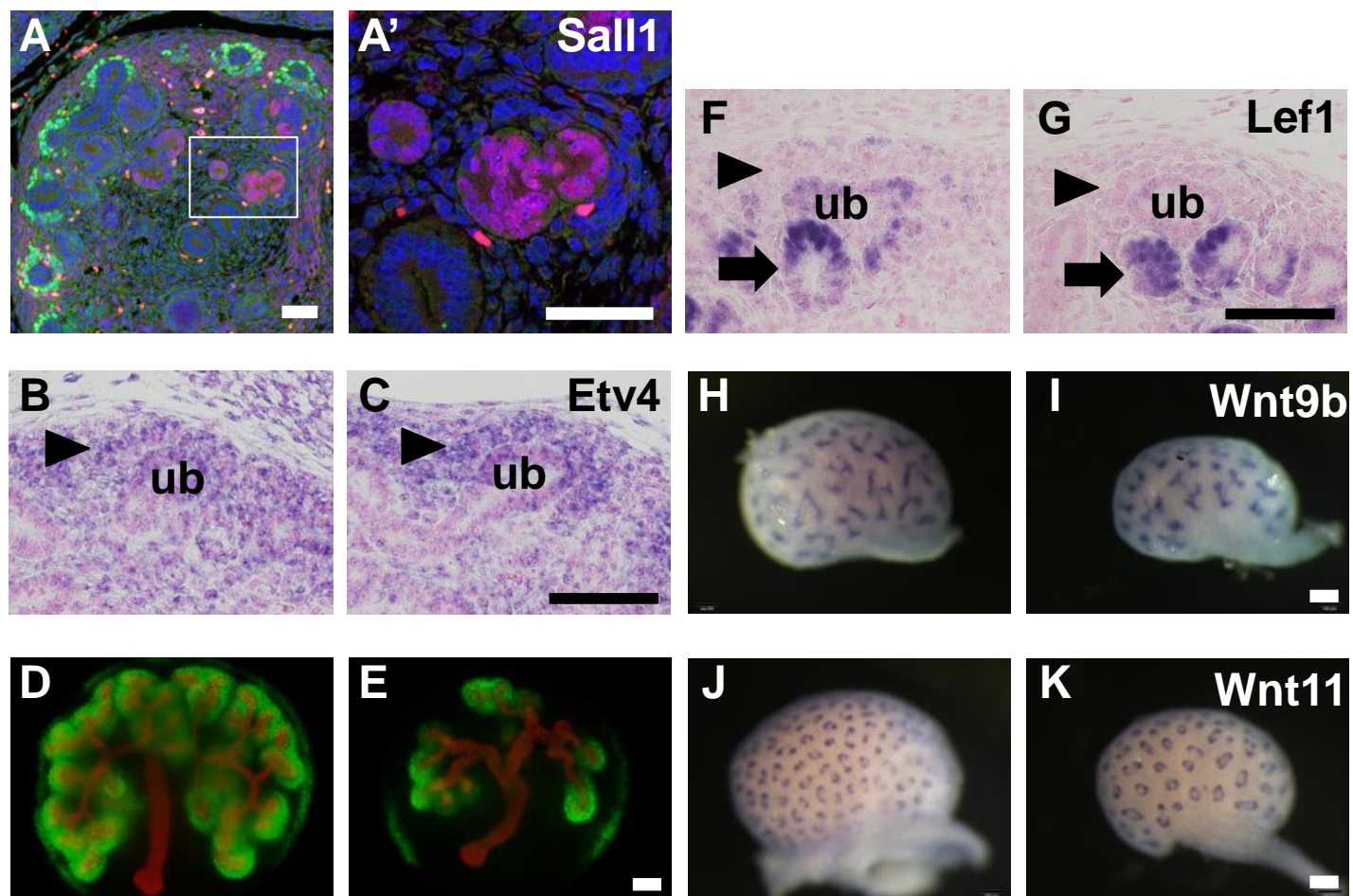
Increased gene expression in a series of *Sall1* mutant kidneys compared with their respective controls. Most of the up-regulated genes are expressed more abundantly in *Six2*(-) cells than in *Six2*(+) cells. *Sall1*- and *Six2*-binding peaks detected in our ChIP-Seq analysis were compared with *Six2*-binding peaks in the study by Park et al.<sup>3</sup> (mm9 coordinates).

**Supplementary Table 3. Expression and ChIP-seq analysis of kidney developmental genes**

*Sall1*- and *Six2*-binding peaks detected in our ChIP-Seq analysis were compared with *Six2*-binding peaks in the study by Park et al.<sup>3</sup> (mm9 coordinates). Asterisks and diamonds correspond to those in Figure 6 and Supplementary Figure 2.

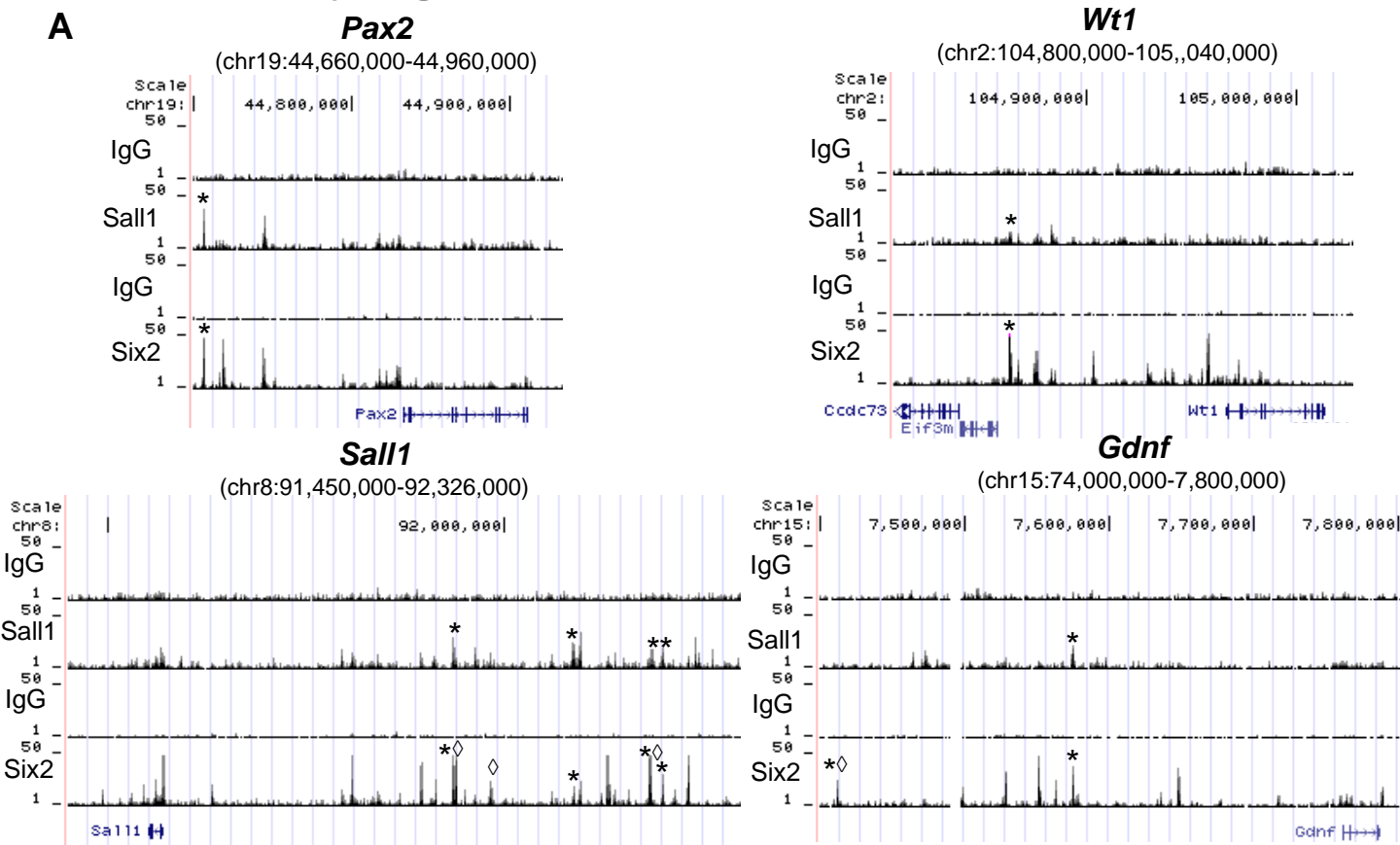
**Supplementary Table 4. Primers for ChIP-qPCR and quantitative RT-PCR, and oligonucleotides for EMSA.**

# Supplementary Figure 1

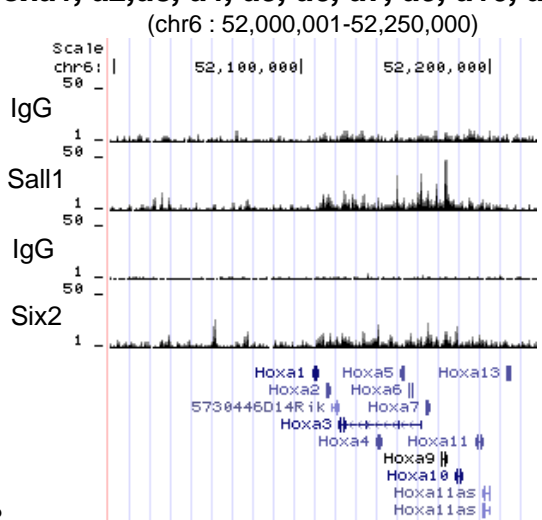


# Supplementary Figure 2

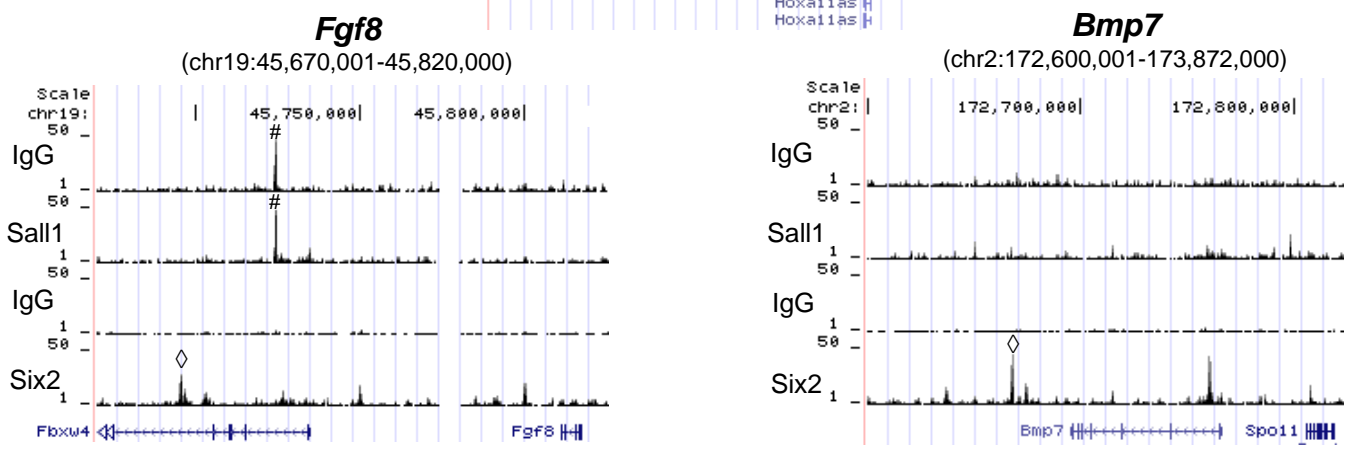
**A**



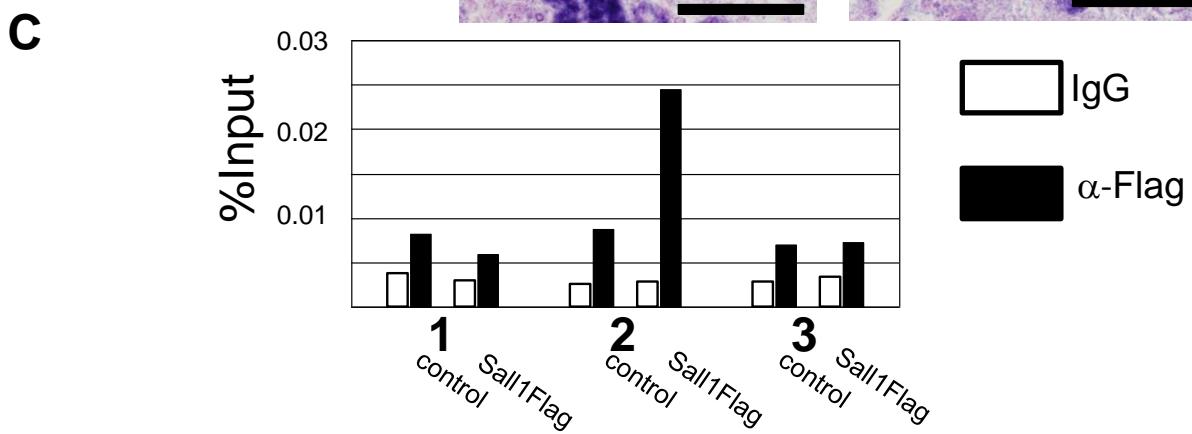
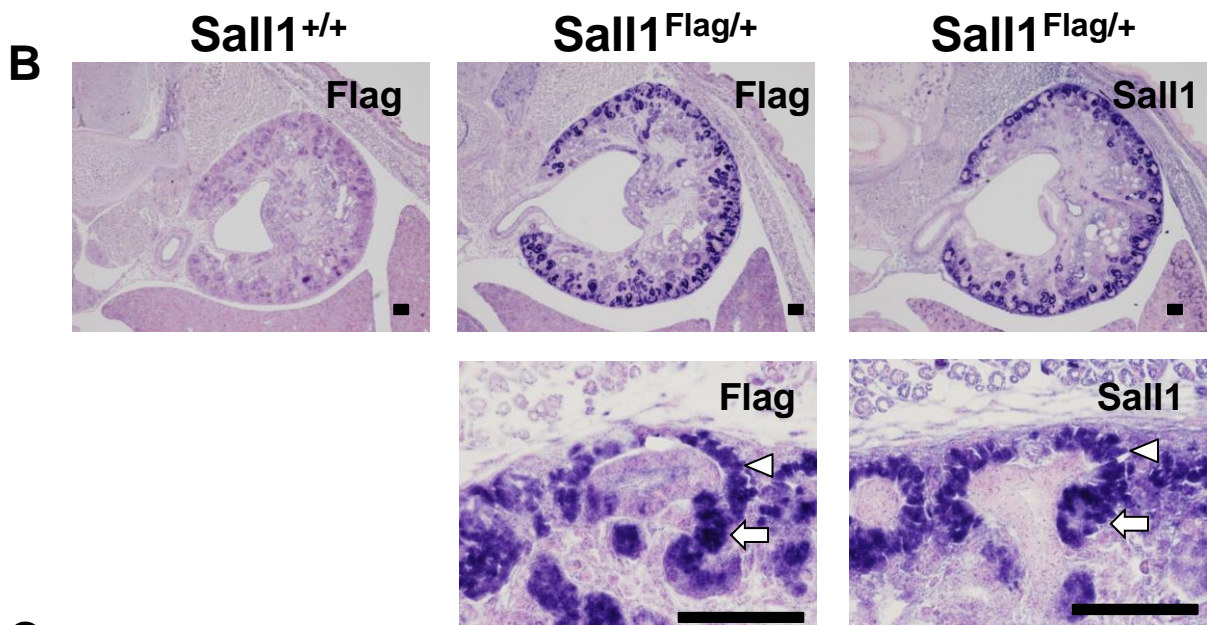
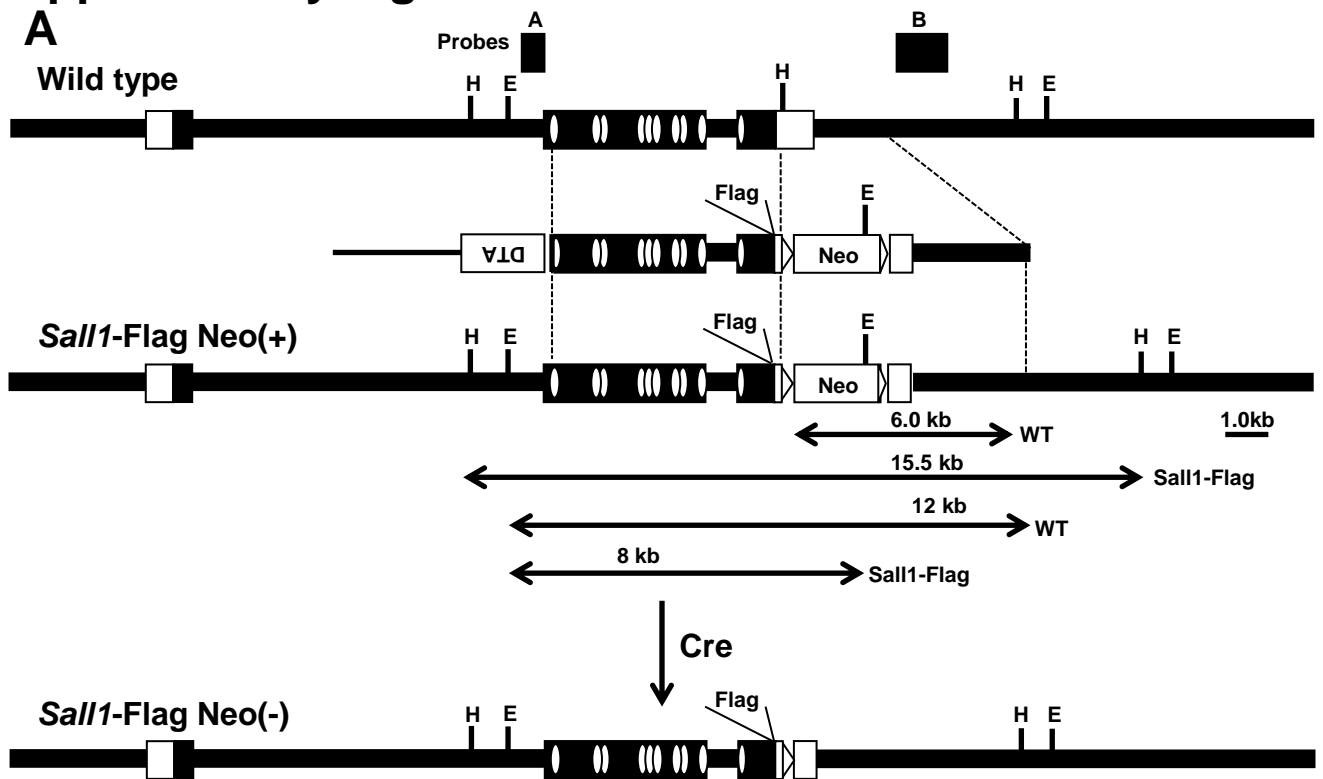
**B** *Hoxa1, a2, a3, a4, a5, a6, a7, a9, a10, a11, a13*



**C**



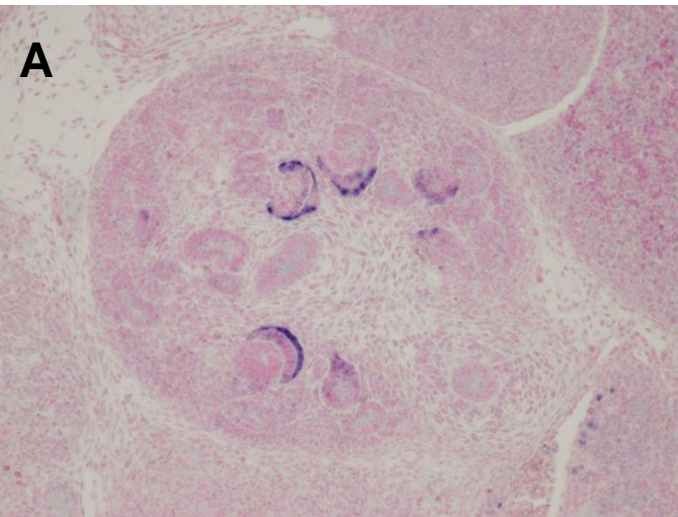
# Supplementary Figure 3



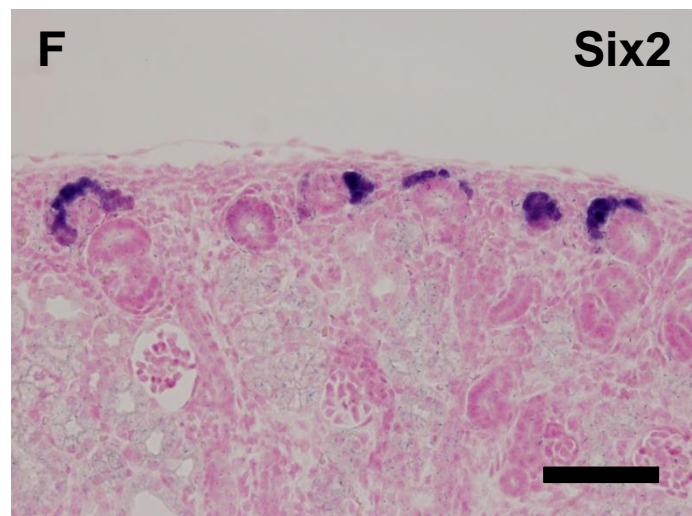
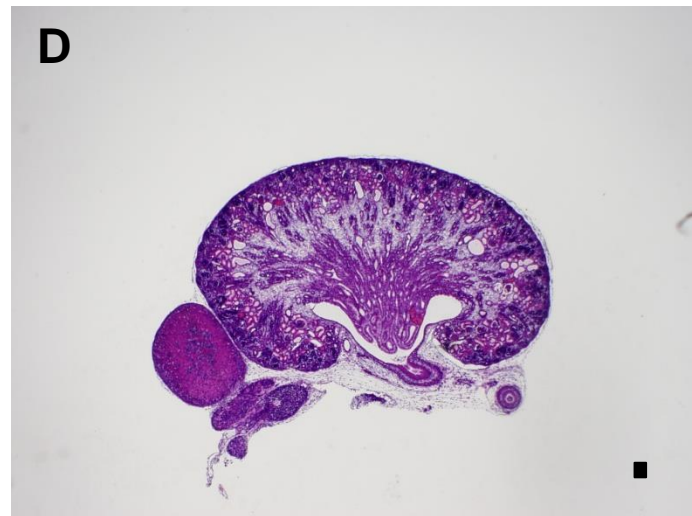
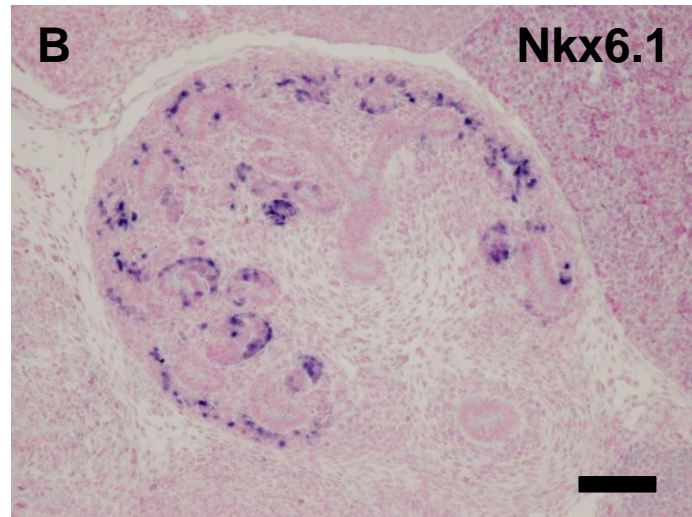


# Supplementary Figure 4

**Nkx6.1OE**



**Six2GFP-Cre;Nkx6.1OE**



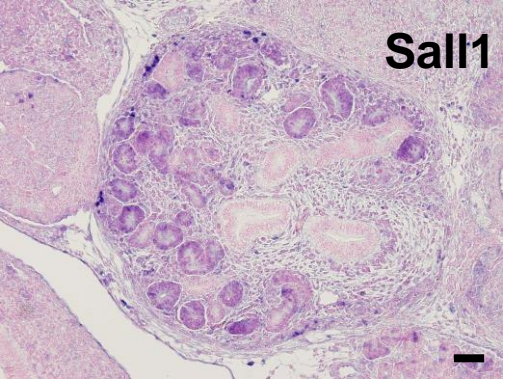
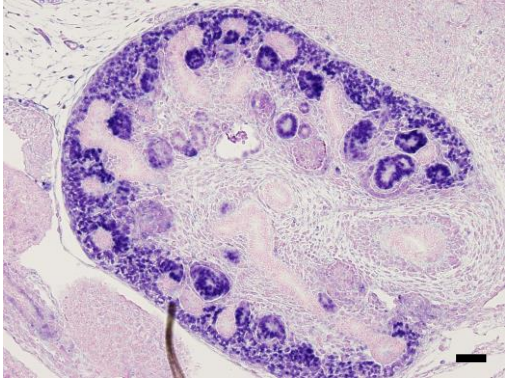
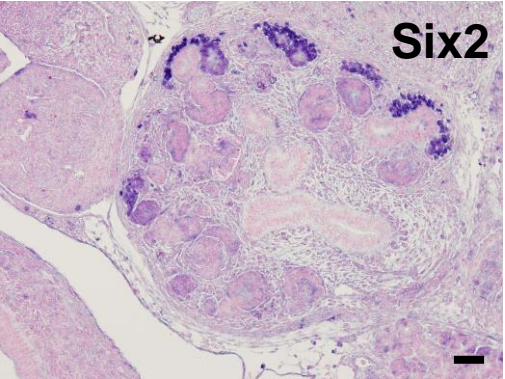
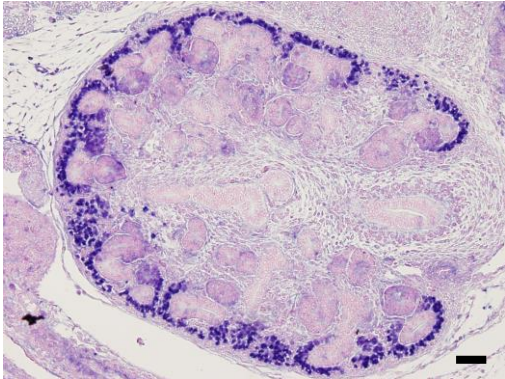
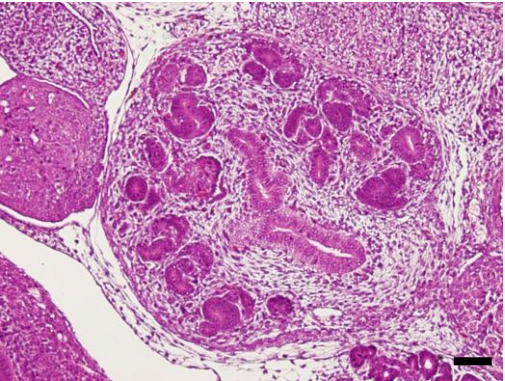
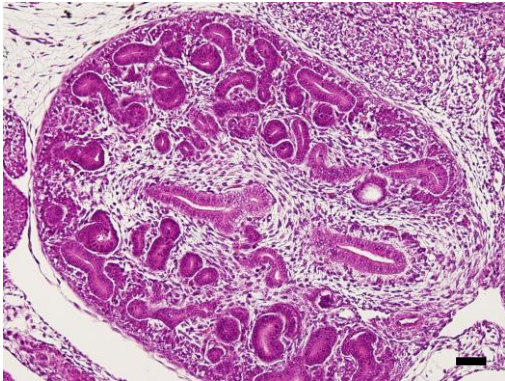


# Supplementary Figure 5

**A**

**Sall1<sup>flx/flx</sup>**

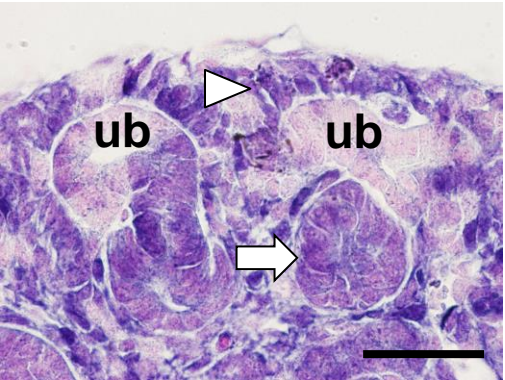
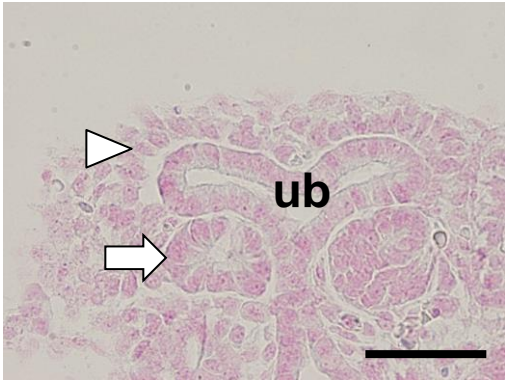
**Wnt4GFP-Cre:  
Sall1<sup>flx/flx</sup>**



**B**

**tdTomato**

**Wnt4GFP-Cre;  
tdTomato**





Supplementary Table 1

GeneSymbol	GeneName	Log2 (Sal1 <sup>CreER/lox</sup> 48hr)	Log2 (Sal1 <sup>CreER/lox</sup> 24hr)	Log2 (Six2Cre; Sal1 <sup>lox/lox</sup> )	Log2 (Six2 GFP+ vs GFP-)	Sal1 binding	Six2 binding	Six2 binding (Park et al.; ref3)
Ocm	oncomodulin	-3.78	-2.59	-1.78	7.05	absent	chr5:144,793,500-144,793,600	chr5:144,793,471-144,793,622
Cled1	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	-2.85	-2.20	-4.16	4.80	absent	chrX:99,515,160-99,515,460	absent
Sal1	sal-like 1 (Drosophila)	-2.78	-0.74	-1.25	1.64	chr8:91,559,382-91,564,748 chr8:91,504,911-914,563	chr8:91,569,591-91,571,276 chr8:91,912,350-91,913,805	
						chr8:91,935,147-91,937,179* chr8:91,962,070-91,964,145	chr8:91,935,304-91,937,293* chr8:91,938,409-91,940,376	chr8:91,936,103-91,936,284 chr8:91,939,275-91,939,431
						chr8:92,085,399-92,088,663 chr8:92,092,399-92,094,667* chr8:92,094,825-92,096,530	chr8:92,085,787-92,088,204 chr8:92,129,157-92,130,804*	
						chr8:92,182,190-92,186,278*	chr8:92,182,364-92,184,152*	chr8:92,130,054-92,130,238
						chr8:92,198,196-92,199,608*	chr8:92,184,846-92,186,022	
						chr8:92,230,090-92,242,534	chr8:92,198,196-92,199,608*	
Dync11	dynein cytoplasmic 1 intermediate chain 1	-2.30	-1.12	-1.85	3.30	absent	chr6:5,683,998-5,685,382	chr6:5,684,379-5,684,728
Dkk1	dickkopf homolog 1 (Xenopus laevis)	-2.15	-1.00	-1.89	-5.32	absent	absent	absent
Dkk1	dickkopf homolog 1 (Xenopus laevis)	-2.03	-1.00	-1.73	-6.57	shown in the other column		
Galnt2	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-like 2	-1.98	-2.46	-2.66	4.14	absent	absent	absent
Pdm	podoplanin	-1.93	-1.30	-3.93	5.35	absent	chr2:129,523,851-129,526,042	absent
Fgf1	fibroblast growth factor 1	-1.83	-2.48	-1.68	5.40	absent	chr18:39,034,094-39,035,438 chr18:39,040,772-39,042,700	chr18:39,034,516-39,034,806 chr18:39,041,961-39,042,307
Mxd1	monooxygenase, DBH-like 1	-1.42	-1.11	-1.20	1.57	absent	absent	absent
Fgf1	fibroblast growth factor 1	-1.17	-0.61	-1.62	4.35	shown in the other column		
Gm5213	predicted gene 5213	-1.16	-0.63	-1.65	5.01	absent	absent	absent
Capn6	calpain 6	-1.10	-0.67	-0.85	2.99	absent	absent	absent
Lbx2	ladybird homeobox homolog 2 (Drosophila)	-1.07	-0.78	-1.29	2.79	absent	absent	absent
Tcfap2c	transcription factor AP-2, gamma	-1.07	-0.69	-0.80	-4.06	absent	absent	absent
Wnt6	wingless-related MMTV integration site 6	-1.04	-1.06	-1.45	3.01	absent	absent	absent
H2-Eb1	histocompatibility 2, class II antigen E beta	-1.02	-0.65	-1.13	0.49	absent	absent	absent
Osr1	odc-skipped related 1 (Drosophila)	-1.01	-0.85	-0.88	5.15	chr12:9,265,136-9,266,406		
						chr12:9,583,063-98,855,408		
							chr12:9,663,412-9,665,066	
							chr12:9,990,482-9,906,011*	
							chr12:10,134,583-10,136,241	
							chr12:10,141,955-10,143,714	
Robo2	roundabout homolog 2 (Drosophila)	-1.00	-0.70	-0.66	3.56	chr16:73,529,347-73,531,168	chr12:10,237,565-10,239,324 chr16:73,530,336-73,530,636 chr16:73,533,609-73,533,909	chr12:10,238,413-10,238,627 absent
						chr16:74,457,123-74,459,457*	chr16:74,457,244-74,459,474* chr16:74,464,261-74,466,231 chr16:74,467,369-74,469,755	chr16:74,458,499-74,458,753
						chr16:74,513,516-74,516,233*	chr16:74,514,010-74,516,020*	chr16:74,514,689-74,515,212
C2cd4b	C2 calcium-dependent domain containing 4B	-0.99	-1.27	-1.41	5.18	absent	absent	absent
Nefl	neurofilament, light polypeptide	-0.95	-0.79	-0.92	3.70	absent	absent	absent
Sik3a	serine/threonine kinase 32A	-0.95	-1.14	-2.31	3.50	absent	chr18:43,442,666-43,444,253	chr18:43,443,559-43,443,715
Osr1	odc-skipped related 1 (Drosophila)	-0.94	-0.85	-0.82	4.72	shown in the other column		
Snap91	synaposomal-associated protein 91	-0.93	-1.10	-3.60	3.30	absent	chr9:86,878,689-86,882,629 chr9:86,891,164-86,893,355	absent
Pcnx2	pecanex-like 2 (Drosophila)	-0.92	-1.09	-1.29	2.75	absent	chr8:128,395,717-128,397,635	chr8:128,396,405-128,396,560
Has2	hyaluronan synthase 2	-0.92	-0.76	-1.16	4.18	absent	chr15:56,349,183-56,350,888	absent
Nefl	neurofilament, light polypeptide	-0.87	-0.79	-0.99	4.50	shown in the other column		
Spock2	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2	-0.86	-1.08	-1.89	3.74	absent	chr10:59,559,940-59,561,286	chr10:59,568,493-59,569,043
Has2	hyaluronan synthase 2	-0.84	-0.76	-1.30	4.81	shown in the other column	chr10:59,567,851-59,570,032	
Gadd45gip1	growth arrest and DNA-damage-inducible, gamma interacting protein 1	-0.83	-0.88	-1.01	0.37	absent	absent	absent
1700024P16Rik	RIKEN cDNA 1700024P16 gene	-0.83	-0.57	-0.70	-1.92	absent	absent	absent
Sik3a	serine/threonine kinase 32A	-0.82	-2.95	-1.87	2.20	shown in the other column		
Robo2	roundabout homolog 2 (Drosophila)	-0.80	-0.49	-0.75	3.93	shown in the other column		
Wnt6	wingless-related MMTV integration site 6	-0.79	-1.06	-1.16	-1.59	shown in the other column		
Pnmt	phenylethanolamine-N-methyltransferase	-0.79	-0.70	-1.78	5.00	absent	absent	absent
Ppm1e	protein phosphatase 1E (PPP2C domain contain)	-0.75	-0.83	-1.47	3.13	absent	absent	absent
Robo2	roundabout homolog 2 (Drosophila)	-0.72	-0.70	-1.25	3.43	shown in the other column		
2610028E06Rik	RIKEN cDNA 2610028E06 gene	-0.72	-0.63	-3.77	3.74	absent	absent	absent
Spock2	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2	-0.71	-1.08	-1.53	4.30	shown in the other column		
Krt23	keratin 23	-0.66	-1.02	-0.62	-6.09	absent	absent	absent
Meg9	multiple EGF-like-domains 9	-0.63	-0.69	-0.94	1.51	chr4:70,149,257-70,152,337* chr4:70,157,125-70,159,298* chr4:70,160,472-70,162,492 chr4:70,163,076-70,164,382 chr4:70,195,701-70,198,887	chr4:70,149,909-70,151,851* chr4:70,157,444-70,159,250* chr4:70,195,594-70,199,117	absent
B3gal5	UDP-Gal betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5	-0.62	-1.01	-1.70	1.04	absent	chr5:150,477,007-150,478,593	chr5:150,477,654-150,477,982

Supplementary Table 2

GeneSymbol	GeneName	Log2 (Sall1 <sup>CreERTox</sup> 48hr)	Log2 (Sall1 <sup>CreERTox</sup> 24hr)	Log2 (Six2Cre; Sall1 <sup>lox/lox</sup> )	Log2 (Six2 GFP+ vs GFP-)	Sall1 binding	Six2 binding	Six2 binding (Park et al., ref3)
Odz2	odd Oz/ten-m homolog 2 (Drosophila)	3.70	2.48	2.13	-1.63	absent	chr11:36,685,840-36,687,519	chr11:36,686,562-36,686,793
Odz2	odd Oz/ten-m homolog 2 (Drosophila)	3.62	2.48	2.10	-1.42	absent	chr11:36,810,638-36,812,020	
Odz2	odd Oz/ten-m homolog 2 (Drosophila)	3.47	2.48	1.47	0.18	absent		
Nkx6-1	NK6 homeobox 1	3.10	2.14	1.53	-1.47	absent		
3110082D06Rik	RIKEN cDNA 3110082D06 gene	2.68	1.54	2.40	-2.74	absent		
Trhr	thyrotropin releasing hormone receptor	2.64	1.07	1.86	0.12	chr15:44,020,317-44,022,935	absent	absent
Krt36	keratin 36	2.32	2.08	2.65	2.07	absent	chr11:99,964,150-99,964,350	absent
3110082D06Rik	RIKEN cDNA 3110082D06 gene	2.31	1.54	2.32	-1.56	absent		absent
Krt36	keratin 36	2.13	1.99	2.67	2.00	shown in the other column	shown in the other column	shown in the other column
Gm6792	predicted gene 6792	2.04	0.61	0.63	-3.21	absent		absent
Rnf152	ring finger protein 152	1.81	1.33	1.68	-1.26	absent		absent
Kera	keratocan	1.78	1.97	2.05	-1.90	absent	chr10:97,053,826-97,055,372	chr10:97,054,393-97,054,612
Pcdh9	protocadherin 8	1.66	0.78	1.02	4.37	absent		absent
Rnf152	ring finger protein 152	1.47	1.33	1.76	-0.86	shown in the other column	shown in the other column	absent
Cc221a	chemokine (C-C motif) ligand 21A	1.45	1.30	1.48	-1.39	absent		shown in the other column
Lrrc4c	leucine rich repeat containing 4C	1.42	0.60	1.68	-0.86	absent		absent
Sltk6	SLIT and NTRK-like family, member 6	1.40	1.40	0.69	-5.30	absent		absent
Saa2	serum amyloid A 2	1.36	0.98	0.75	5.13	absent		absent
Ebf2	early B-cell factor 2	1.36	0.66	0.69	-1.03	absent		absent
Fap	fibroblast activation protein	1.34	0.76	0.89	-0.37	absent		absent
Fap	fibroblast activation protein	1.34	0.76	1.56	-5.15	shown in the other column	shown in the other column	shown in the other column
Krt15	keratin 15	1.30	0.87	2.08	2.63	absent		absent
Rnf152	ring finger protein 152	1.28	1.33	1.93	-0.65	shown in the other column	shown in the other column	shown in the other column
Snph	synthaphilin	1.24	1.01	1.02	1.46	absent		absent
Vegfc	vascular endothelial growth factor C	1.24	0.62	1.17	-1.20	absent		absent
Gdp2	glycerophosphodiester phosphodiesterase domain	1.24	0.89	0.74	-0.03	absent		absent
Angpt1	angiotensin-like 1	1.21	1.45	0.88	-2.97	absent		absent
Gabbr3	gamma-aminobutyric acid (GABA) A receptor, subunit 3	1.20	0.63	0.72	-1.77	absent		absent
Oik3	oncoprotein induced transcript 3	1.18	0.84	1.09	-3.46	absent		absent
Foxp2	forkhead box P2	1.18	1.09	1.36	-3.50	absent		absent
Lypd1	Ly6/Plaur domain containing 1	1.16	0.87	0.82	-0.05	absent		absent
Egfl6	EGF-like domain, multiple 6	1.12	0.78	0.97	-1.57	chrX:162,947,271-162,948,745	absent	absent
Aspm	asperm	1.09	2.14	1.52	-5.27	absent		absent
5430435G22Rik	RIKEN cDNA 5430435G22 gene	1.05	0.67	0.68	-1.45	absent		absent
Dtna	dystrobrein alpha	1.05	0.83	0.80	2.06	absent		absent
Napsa	napsin A aspartic peptidase	1.05	0.47	0.98	-2.72	absent		absent
1700028P14Rik	RIKEN cDNA 1700028P14 gene	1.04	0.61	0.73	-0.46	absent		absent
Epha5	Eph receptor A5	0.99	1.37	1.32	-0.59	chr5:84,813,557-84,815,455	absent	absent
Rspo2	R-spondin 2 homolog (Xenopus laevis)	0.97	1.80	1.51	-0.42	absent	chr5:85,097,634-85,099,671	absent
Ebf3	early B-cell factor 3	0.96	0.62	1.77	-3.46	absent		absent
Foxp2	forkhead box P2	0.95	0.78	1.36	-2.18	shown in the other column	shown in the other column	shown in the other column
Dtna	dystrobrein alpha	0.95	0.83	0.70	1.90	shown in the other column	shown in the other column	shown in the other column
Shisa9	shisa homolog 9 (Xenopus laevis)	0.95	0.85	0.74	-2.70	absent		absent
Ebf3	early B-cell factor 3	0.94	0.62	1.38	0.00	shown in the other column	shown in the other column	shown in the other column
Sfrp4	secreted frizzled-related protein 4	0.92	1.26	1.95	-4.32	absent		absent
Htr2b	5-hydroxytryptamine (serotonin) receptor 2B	0.90	0.80	0.62	1.60	absent	chr1:88,015,600-88,017,322	absent
Fibr1	fin bud initiation factor homolog (zebrafish)	0.87	0.75	1.18	-4.77	absent		absent
Ryr3	ryanodine receptor 3	0.86	0.60	0.75	-2.07	absent		absent
Nov	nephroblastoma overexpressed gene	0.84	1.41	1.30	-2.10	chr15:54,477,140-54,478,129	absent	absent
Dtna	dystrobrein alpha	0.82	0.83	0.73	0.71	shown in the other column	shown in the other column	shown in the other column
Col5a3	collagen, type V, alpha 3	0.76	0.64	1.17	-3.92	chr9:20,557,700-20,558,700	absent	absent
Ddx4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	0.76	1.01	0.64	-0.20	absent		absent
		0.75	0.93	1.16	-4.17	chr5:13,325,149-13,327,960	absent	absent
Sema3a	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A					chr5:13,397,505-13,402,459		
						chr5:13,402,643-13,406,956		
						chr5:13,426,453-13,427,898		
Bmp5	bone morphogenetic protein 5	0.75	0.72	0.86	-2.33	absent		absent
Oik3	oncoprotein induced transcript 3	0.75	0.84	0.77	-0.83	shown in the other column	shown in the other column	shown in the other column
Cldn1	claudin 1	0.75	1.30	1.55	-4.40	absent		absent
Tbx1	T-box 1	0.75	0.72	1.03	-3.54	absent		absent
Trem2	triggering receptor expressed on myeloid cells 2	0.72	0.61	0.68	0.06	absent		absent
Ms4a7	membrane-spanning 4-domains, subfamily A, member 7	0.72	0.66	1.38	0.71	absent		absent
Fxyd7	FXD domain-containing ion transport regulator 7	0.70	0.68	1.22	-0.78	absent		absent
Cldn1	claudin 1	0.69	1.30	1.28	-5.01	shown in the other column	shown in the other column	shown in the other column
Ms4a7	membrane-spanning 4-domains, subfamily A, member 7	0.69	0.66	1.43	0.75	shown in the other column	shown in the other column	shown in the other column
Col12a1	collagen, type XII, alpha 1	0.68	0.74	0.81	-4.30	absent		absent
Trem2	triggering receptor expressed on myeloid cells 2	0.65	0.61	0.77	-0.00	shown in the other column	shown in the other column	shown in the other column
Sema3a	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	0.64	0.83	1.26	-5.00	shown in the other column	shown in the other column	shown in the other column
Pcdh20	protocadherin 20	0.64	0.88	1.37	-3.22	absent		absent
March11	membrane-associated ring finger (C3HC4) 11	0.62	0.72	0.66	-4.09	absent		absent
Pam1	prostate androgen-regulated mucin-like protein 1	0.61	0.64	1.13	-1.91	absent		absent
Bmp5	bone morphogenetic protein 5	0.61	0.72	0.75	0.44	shown in the other column	shown in the other column	shown in the other column
Fgf1	c-fos induced growth factor	0.60	0.82	1.18	-4.93	absent		absent
Ccdc113	coiled-coil domain containing 113	0.60	0.91	1.13	-4.03	absent		absent
Slurp1	secreted Ly6/Plaur domain containing 1	0.59	0.49	1.25	-0.70	absent		absent

Supplementary Table 3

	Gene Symbol	Log2 (Sall1 <sup>CreER/lox</sup> 48hr)	Log2 (Sall1 <sup>CreER/lox</sup> 24hr)	Log2 (Six2Cre; Sall1 <sup>lox/lox</sup> )	Log2 (Six2 GFP+ vs GFP-)	Sall1 binding	Six2 binding	Six2 binding (Park et al.; ref3)	
Progenitor-related		-2.8	-0.72	-1.27	1.65	chr8:91,559,382-91,564,748	chr8:91,569,591-91,571,276		
	Sall1					chr8:91,910,504-91,914,563	chr8:91,913,350-91,913,805		
						chr8:91,935,147-91,937,179*	chr8:91,935,304-91,937,293*	chr8:91,936,103-91,936,284	
						chr8:91,962,070-91,964,145	chr8:91,938,409-91,940,376	chr8:91,939,275-91,939,431	
							chr8:91,982,978-91,984,393		
						chr8:92,085,399-92,088,663	chr8:92,085,787-92,088,204		
						chr8:92,092,399-92,094,667*	chr8:92,129,157-92,130,804*		
						chr8:92,094,825-92,096,530			
						chr8:92,182,190-92,186,278*	chr8:92,182,364-92,184,152*	chr8:92,130,054-92,130,238	
							chr8:92,184,846-92,186,022		
						chr8:92,197,793-92,199,765*	chr8:92,198,196-92,199,608*		
	Six2	-0.52	-0.5	-1.62	5.87	chr8:92,239,090-92,242,534	chr8:92,230,587-92,232,637	chr17:86,087,682-86,087,809	
						chr17:86,087,020-86,088,664	chr17:86,146,706-86,149,340*	chr17:86,147,979-86,148,273	
						chr17:86,145,975-86,149,207*	chr17:86,196,249-86,200,295	chr17:86,198,467-86,198,745	
	Pax2	0.29	0.17	-0.57	1.22	chr19:44,706,202-44,707,935*	chr19:44,705,424-44,708,047*		
						chr19:44,743,902-44,746,867	chr19:44,718,402-44,720,395		
							chr19:44,743,623-44,745,539		
	Eya1	-0.35	0.09	-1.09	0.04		chr1:14,234,375-14,235,693	chr1:14,235,103-14,235,289	
						chr1:14,451,499-14,453,661			
						chr1:14,558,658-14,561,609	chr1:14,560,514-14,560,929		
							chr1:14,577,583-14,579,048		
						chr1:14,624,565-14,627,013*	chr1:14,624,969-14,626,797*	chr1:14,625,742-14,625,927	
	Wt1	0.15	0.09	-0.49	1.15	chr2:104,863,154-104,864,824*	chr2:104,862,758-104,865,023*	chr1:14,706,626-14,706,934	
						chr2:104,882,731-104,886,149			
							chr2:104,867,037-104,868,414		
							chr2:104,902,608-104,904,049		
							chr2:104,928,528-104,931,383		
	Cited1	-2.87	-2.18	-4.18	4.8	absent	chrX:99514,619-99,515,712	absent	
	Osr1	-0.99	-0.82	-0.87	5.11		chr12:9,265,136-9,266,406		
						chr12:9,583,063-98,855,408			
							chr12:9,663,412-9,665,066		
							chr12:9,990,485-9,990,011*		
							chr12:10,134,583-10,136,241		
	FGF1	-1.52	-1.52	-1.67	5.52	absent	chr18:39,034,094-39,035,438	chr18:39,034,516-39,034,806	
							chr18:39,040,772-39,042,700	chr18:39,041,961-39,042,307	
	FGF2	0.07	0.14	-0.15	-0.47	absent	absent	absent	
	FGF9	0.04	-0.1	-0.68	1.7	absent	chr14:59,107,122-59,108,879	chr14:59,107,730-59,108,069	
	FGF20	-0.34	-0.4	-0.32	6.85	absent	chr14:59,134,560-59,136,448	chr14:59,135,528-59,135,796	
	BMP7	-1.08	-0.27	-0.74	1.15	absent	chr8:41,324,122-41,326,059	chr8:41,325,166-41,325,419	
							chr2:172,667,102-172,668,820	chr2:172,667,933-172,668,149	
	Hoxa2					chr6:52,112,980-52,116,365	absent	absent	
	Hoxa5					chr6:52,159,318-52,165,129			
	Hoxa9					chr6:52,175,369-52,182,159			
Differentiation	Wnt9b	-0.01	-0.06	0.5	-3.52	absent	absent	absent	
	Wnt4	0.37	-0.02	-0.71	-1.59	absent	chr4:136,777,571-136,774,226	chr4:136,773,187-136,773,353	
	LEF1	0.19	-0.1	0.29	-4.26	absent	absent	chr3:130440576-130440757	
	Dkk1	-2.1	-0.98	-1.73	-5.71	absent	absent	absent	
	FGF8	0.14	-0.13	-1.05	1.3	absent	chr19:45,695,222-45,697,760	chr19:45695446-45695814	
	Jag1	-0.07	0.06	-0.83	-3.87	absent	chr2:136,990,923-136,992,487	absent	
	Notch1	0.16	0.29	-0.75	-0.41	absent	chr2:26,396,629-26,398,109	absent	
	Notch2	0.07	0.22	-0.14	0.24	absent	chr3:97,740,586-97,742,523	absent	
	Hnf1b	-0.14	0.37	-0.82	-3.62	chr11:83,629,065-83,631,720	absent	absent	
	Adhesion/repulsion	Integrin α3	0.18	0.13	0.05	0.84	absent	absent	absent
		Integrin α8	-0.12	-0.31	-0.82	2.28	absent	chr2:11,940,289-11,942,124	chr2:11,941,243-11,941,615
		Integrin β1	-0.26	-0.03	0.05	-0.67	absent	chr8:130,948,221-130,949,370	chr8:130,948,920-130,949,219
	Robo2	-0.75	-0.57	-1.01	3.42	chr16:73,529,347-73,531,168	chr16:73,530,336-73,530,636		
						chr16:73,533,609-73,533,909			
						chr16:74,457,123-74,459,457*	chr16:74,457,244-74,459,474*	chr16:74,458,499-74,458,753	
						chr16:74,464,261-74,466,231	chr16:74,467,369-74,469,755		
	Slit2	0.01	-0.05	-0.01	-0.4	chr5:47,947,543-47,949,658	chr5:47,947,543-47,949,658	chr5:47,948,014-47,948,303	
Extracellular matrix	Nephronectin	0.16	-0.1	-0.84	-2.71	absent	absent	absent	
	Laminin alpha1	-0.28	0	-1.17	-2.95	absent	absent	chr17:67982218-67982477	
	Laminin alpha5	0.05	0.07	-0.31	0.03	absent	absent	absent	
	Laminin beta1	0.12	0.14	-0.06	-1.56	absent	absent	absent	
	Laminin beta2	0.22	0.17	0.2	0.04	absent	absent	absent	
	Laminin gamma1	0.08	0.18	-0.22	-1.1	absent	absent	absent	
	COL4A3	0.94	0.25	0.38	absent	absent	absent	absent	
	COL4A4	0.45	0.07	0.58	-1.56	absent	absent	absent	
	COL4A5	0.05	-0.01	0.58	-0.2	absent	absent	absent	
	Ureteric bud attraction		-0.76	-0.66	-0.06	1.11	chr15:7,411,984-7,413,234	chr15:7,411,984-7,413,234	chr15:7,412,321-7,412,817
	Gdnf					chr15:7,463,342-7,465,268			
						chr15:7,472,514-7,474,370			
							chr15:7,551,156-7,552,891		
							chr15:7,562,479-7,563,621		
							chr15:7,574,199-7,575,862*		
	Ret	-0.5	-0.25	-0.24	-6.67	absent	chr15:7,647,427-7,648,858	absent	
	Etv4	-0.06	-0.27	-1.22	1.37	absent	chr11:101,632,947-101,634,979	chr11:101,633,863-101,634,122	
	Etv5	0.04	-0.1	-1.3	0.49	absent	absent	absent	
	Sprouty1	-0.12	-0.15	-0.6	-0.46	absent	absent	chr3:37,538,702-37,538,895	
	FGF10	0.04	-0.07	-0.3	2.19	absent	absent	absent	
	BMP4	-0.15	0.23	-0.21	-1.06	absent	chr14:47,195,029-47,195,717	chr14:47,195,735-47,195,901	
						absent	chr14:47,213,357-47,215,049	chr14:47,213,638-47,214,076	
	Gremlin	-0.43	0.05	-1.38	-0.83	absent	chr14:47,337,540-47,338,880	chr14:47,338,153-47,338,279	
	Klf26b	-0.29	-0.32	-1.67	2.85	chr1:180,457,002-180,459,602	absent	absent	
						chr1:180,613,106-180,614,972	chr1:180,613,939-180,614,184		



**Supplementary Table 4**

Primers for ChIP-qPCR

Six2-1F 5'-CAG CAG CTG TAT CCA GGT CA-3'  
 Six2-1R 5'-CAA TGG GCA CAT GAA CAA AG-3'  
 Six2-2F 5'-GGG GTT TAT GGT TCA CAA CG-3'  
 Six2-2R 5'-GGG AAG AAA AAC CAT GTC CA-3'  
 Six2-3F 5'-CCA TGA GAG ATT CCC TTC CA-3'  
 Six2-3R 5'-CCG GAG TAC TGA AGC CAG AG-3'

Primers for qRT-PCR

b-actin F 5'-CATCCGTAAGACCTCTATGCCAAC-3'  
 b-actin R 5'-ATGGAGCCACCGATCCACA-3'  
 Sall1 F 5'-TGTCAAGTTCCAGAAATGTTCCA-3'  
 Sall1 R 5'-ATGCCGCGTCTGAAATGA-3'  
 Six2 F 5'-GCAACTTCCGCGAGCTCTAC-3'  
 Six2 R 5'-GCCTTGAGCCACAACCTGCTG-3'  
 Osr1 F 5'-ACTGATGAGCGACCTTACACCTG-3'  
 Osr1 R 5'-ACTTGTGAGTGAGCGCTTTGTGGA-3'  
 Cited1 F 5'-GTCGAGGCGCTGCACTTGATG-3'  
 Cited1 R 5'-CCAAGGTTGGAGTAGGCCAGAG-3'  
 Odz2 F 5'-GAGGTCCCAGATTACATCAGTCA-3'  
 Odz2 R 5'-TTTCCATCCAGGCGTTCCA-3'  
 Nkx6.1 F 5'-AGCACGCTTGGCCTATTCTC-3'  
 Nkx6.1 R 5'-CACTGGTCTCGGTTCTG-3'

Oligonucleotides for EMSA

	Name	Sequence	Position of Sall1 probe	Position of Six2 binding motif	Six2 consensus GNAACNNNANNC	
1	Osr1-Wt-Fw	5'-GTATATATTATCCTGCAAAAAGAAAGCATAAC-3'	chr12:9,905,826-9,905,856	chr12:9,905,421-9,905,432	GGAACCTGACAC	394bp up stream of Sall1 binding motif
2	Osr1-Mt-Fw	5'-GTATATATTgTCTGCAAAAAGAAAGCAcAAC-3'				
3	Six2-Wt-Fw	5'-CTGGAGAAATAAACTGAGTTATTGTTCC-3'	chr17:86,147,858-86,147,887	chr17:86,148,253-86,148,264	GAAACCCGACCC	366bp down stream of Sall1 binding motif
4	Six2-Mt-Fw	5'-CTGGAGAAAcAAAACAGTTgTTGTTCC-3'				
5	Eya1-Wt-Fw	5'-CATAAATAGAGTAAATACATTTAATTTTCATTTCTATATTATC-3'	chr1:14,625,520-14,625,562	chr1:14,625,819-14,625,830	GTAACACTCCC	257bp down stream of Sall1 binding motif
6	Eya1-Mt-Fw	5'-CAcAcAGAGTAggTACATTTggccTTCATccTATATggTC-3'				
7	Robo2-Wt-Fw	5'-CAGATAGTTTCATAATGAAATTTCTTTTATGTTGAATCC-3'	chr16:74,514,526-74,514,565	chr16:74,515,053-74,515,063	GTTTGAGTTAC (complementary)	435bp down stream of Sall1 binding motif
8	Robo2-Mt-Fw	5'-CAGATAGccTCAcAATGGGATtCTTctgTGTTGggTCC-3'				
9	Megf9-Wt-Fw	5'-GGCACTAAATAAAACGGCAAAGTCATAAAAATTTAATTTG-3'	chr4:70,150,276-70,150,315	chr4:70,150,664-70,150,675	GTTTTAGTTCC (complementary)	349bp down stream of Sall1 binding motif
10	Megf9-Mt-Fw	5'-GGCACTAgAcAggACGGCAAAGTCAcAccAATcTccTTTG-3'				
11	Cited1-Wt-Fw	5'-TTACAAGCAATTAATAACACTAAAATAGATTTATACAGG-3'	chrX:99,515,395-99,515,434	chrX:99,515,343-99,515,353	GGAAATGACTC	42bp up stream of Sall1 binding motif
12	Cited1-Mt-Fw	5'-TTACAAGCAAccAggATACACTAggATAGAggTcTACAGG-3'				