Supplemental information

Sall1 in renal stromal progenitors non-cell autonomously restricts the excessive expansion of nephron progenitors

Tomoko Ohmori, Shunsuke Tanigawa, Yusuke Kaku, Sayoko Fujimura, and Ryuichi Nishinakamura*

Department of Kidney Development and Liaison Laboratory Promotion Facility, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan

* E-mail: ryuichi@kumamoto-u.ac.jp

Supplementary Figure S1 | Sall1 expression in the stroma is absent in the *Foxd1GFPCre; Sall1*^{flox/flox} mice.

Immunostaining for Sall1, Six2, and GFP of *Sall1^{flox/flox}*, *Foxd1GFPCre; Sall1^{flox/+}*, and *Foxd1GFPCre; Sall1^{flox/flox}* kidneys at E12.5 (A) and E14.5 (B). Serial sections of each genotype were stained. Sall1 in the stroma (asterisks) is absent, while GFP indicating *Foxd1* expression is retained in the homozygous mutant. Sall1 is not expressed in the medullary regions at E14.5. ub: ureteric bud. Scale bars = $100 \,\mu$ m

Supplementary Figure S2 | Survival, proliferation, and lineage commitment of the stromal progenitors are not affected in the *Sall1* mutants.

(A) TUNEL and Aldh1a2 staining of *Sall1^{flox/flox}* and *Foxd1Cre; Sall1^{flox/flox}* kidneys at E14.5.

(B) The percentages of TUNEL-positive cells in the Aldh1a2-positive cortical stromal cells at E13.5 and E14.5.

(C) PHH3 and Aldh1a2 staining of *Sall1^{flox/flox}* and *Foxd1Cre; Sall1^{flox/flox}* kidneys at E14.5.

(D) The percentages of PHH3-positive cells in the Aldh1a2-positive cortical stromal cells at E13.5 and E14.5.

(E) tdTomato expression in *Foxd1Cre; R26R-tdTomato* and *Foxd1Cre; Sall1^{flox/flox}; R26R-tdTomato* kidneys at E15.5, immunostained using an anti-RFP antibody. Arrows: glomeruli.

(F, G) Survival and proliferation of the neonatal nephron epithelia are not affected in the

Sall1 mutants. The percentages of TUNEL-positive (F) or PHH3-positive (G) cells are shown for the E-cadherin-positive nephron epithelia in the cortex and medulla.

Scale bars: (A, C) = 20 μ m; (E) = 100 μ m. Results are presented as the mean ± standard error. NS: not significant statistically (p>0.05).

Supplementary Figure S3 | Expression of stromal genes in the control and *Sall1* mutant kidneys.

(A-C) Whole mount *in situ* hybridisation of the embryonic kidney at E14.5. The expression levels of *Meis1* (A), *Hoxd10* (B), and *Tcf21* (C) are unaffected. *Meis1* and *Hoxd10* are also expressed in the neighbouring nephron progenitors, while *Tcf21* is expressed in the medullary stroma located deep inside the tissue. Therefore, the border of the expression domain is somewhat vague. Scale bars = 100 μ m.

Primers for quantitative RT-PCR		
Gene	Sequence (5' - 3')	
Aldh1a2	F	AGCCCATTGGAGTGTGTGGAC
	R	TGCTCAGCGGGTTTGATGAC
eta -Actin	F	CATCCGTAAAGACCTCTATGCCAAC
	R	ATGGAGCCACCGATCCACA
Decorin	F	CTGGGCTGGCACAGCATAAGTA
	R	GAACGCACATAGACACATCTGAAGG
Fat4	F	GTGTTTAACGTCACCGATGCAGA
	R	TCCACTGCAAACTGCCCAAG
<i>Pdgfr</i> β	F	GAACGACCATGGCGATGAGA
	R	GCATCGGATAAGCCTCGAACA
Six2	F	GCAACTTCCGCGAGCTCTAC
	R	GCCTTGAGCCACAACTGCTG

Supplementary Table | Primer sequences used for qPCR.

Supplementary Figure S1

Sall1 flox/flox

Foxd1GFPCre; Sall1 ^{flox/+} Foxd1GFPCre; Sall1 ^{flox/flox}



GFP

Six2

Α





ub







В



















Supplementary Figure S2



Foxd1Cre; R26R-tdTomato

Ε

F

Foxd1Cre; Sall1 ^{flox/flox}; R26R-tdTomato









Supplementary Figure S3 Sall1 ^{flox/flox}

Foxd1Cre; Sall1 flox/flox

