Table S1. Antibodies

Antigen	Vendors	Catalog #	Host	dilution
Six2	Proteintech	11562-1-AP	rabbit	1:500
GFP	Aves	GFP-1020	chick	1:500
FLAG	Sigma-Aldrich	F3165	mouse	1:500
Jag1	DSHB	TS1.15H	rat	1:20
Calbindin	Abcam	ab82812	mouse	1:500
Lhx1	DSHB	4F2	mouse	1:20
Pax2	Covance	PRB-276P	rabbit	1:200
Wt1	Santa Cruz	sc-192	rabbit	1:200
biotin-LTL	Vector Laboratories	B-1325		1:900
Slc12a1	Proteintech	18970-1-AP	rabbit	1:500
Slc12a3	Sigma-Aldrich	HPA028748	rabbit	1:300
Cdh1	Santa Cruz	sc-59778	rat	1:500
Cytokeratin	Sigma-Aldrich	C2562	mouse	1:200
pHH3	Cell Signaling Tech.	9701S	rabbit	1:100
Rbpj	Cosmo bio USA	SIM-2ZRBP1	rat	1:1000
β-gal	MPbio	559761	rabbit	1:15000

Supplemental Experimental Procedures

Reporter assays

Six2 promoter-driven reporter assays were done as previously described (Brodbeck et al., 2004). Human embryonic kidney HEK293 cells were plated on a 24-well dish to near confluency and transiently transfected with the firefly luciferase reporter plasmid pGL3B-Six2p-luc (p414) or empty vector (pGL3B) along with Pol III-renilla luciferase plasmid (p130) using Lipofectamine 2000 (Invitrogen), according to manufacturer's instructions. SuperTopFlash cells (Xu et al., 2004) were plated as described above. In addition to reporters, we transfected plasmids expressing a stable form of β-catenin (ΔN-β-catenin, p427), untagged Six2 (p420), 3xFLAG-tagged Six2 (p416), or empty vector (p383). Duplicate or triplicate wells of transfected cells were processed for firefly and renilla luciferase assays using the Dual-Glo Luciferase Assay System (Promega) 2 days after transfection. Firefly luciferase activities were divided by renilla luciferase activity for calculation of normalized fold activity.

Brodbeck, S., Besenbeck, B. and Englert, C. (2004). The transcription factor Six2 activates expression of the Gdnf gene as well as its own promoter. *Mech Dev* **121**, 1211-1222.

Xu, Q., Wang, Y., Dabdoub, A., Smallwood, P. M., Williams, J., Woods, C., Kelley, M. W., Jiang, L., Tasman, W., Zhang, K., et al. (2004). Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. *Cell* **116**, 883-895.

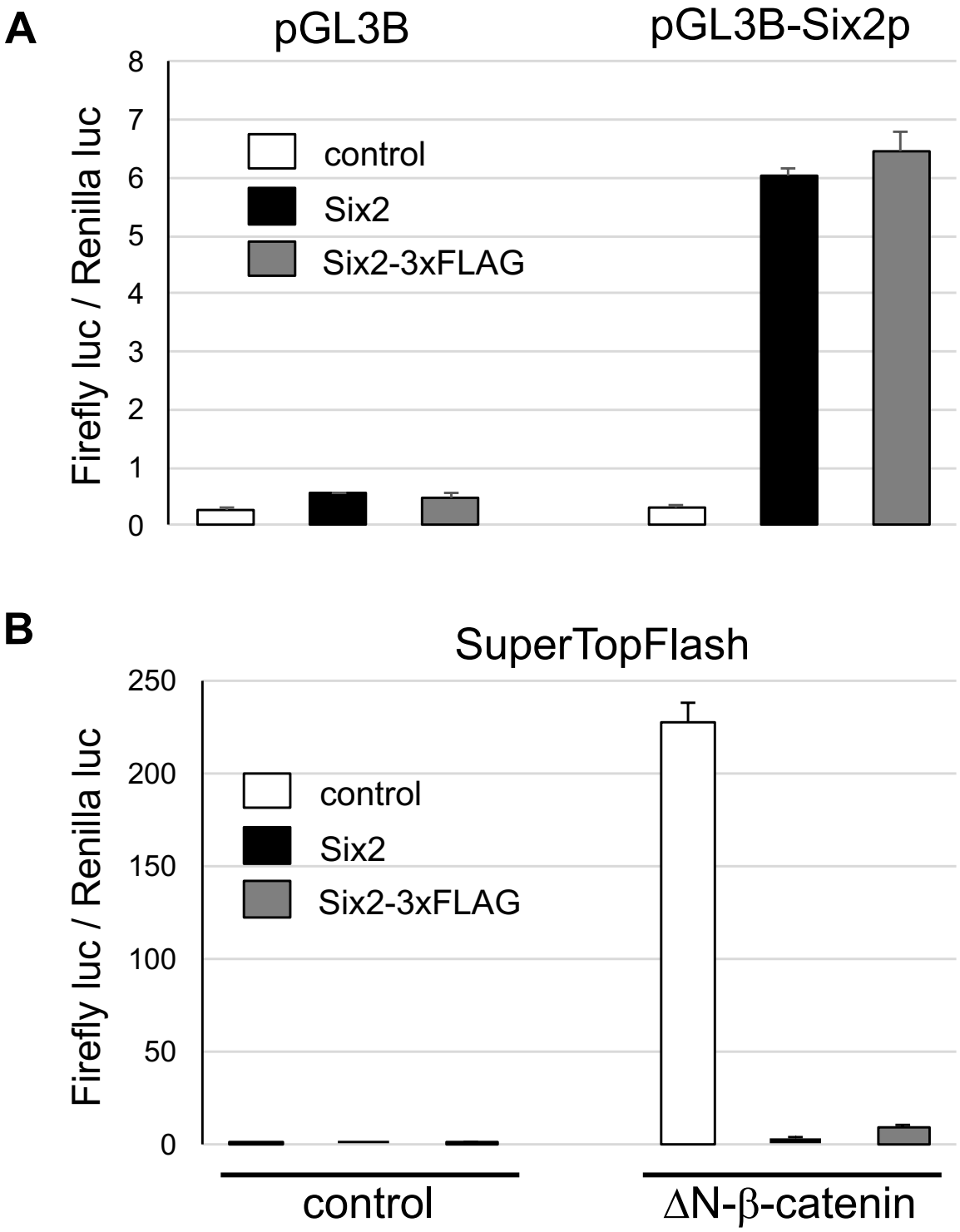

Fig. S1 3xFLAG-tagged Six2 is functional (A) Both untagged and 3xFLAG-tagged Six2 can activate the Six2 promoter-driven luciferase reporter. (B) Both untagged and 3xFLAG-tagged Six2 can repress β-catenin-mediated activation of SuperTopFlash reporter.

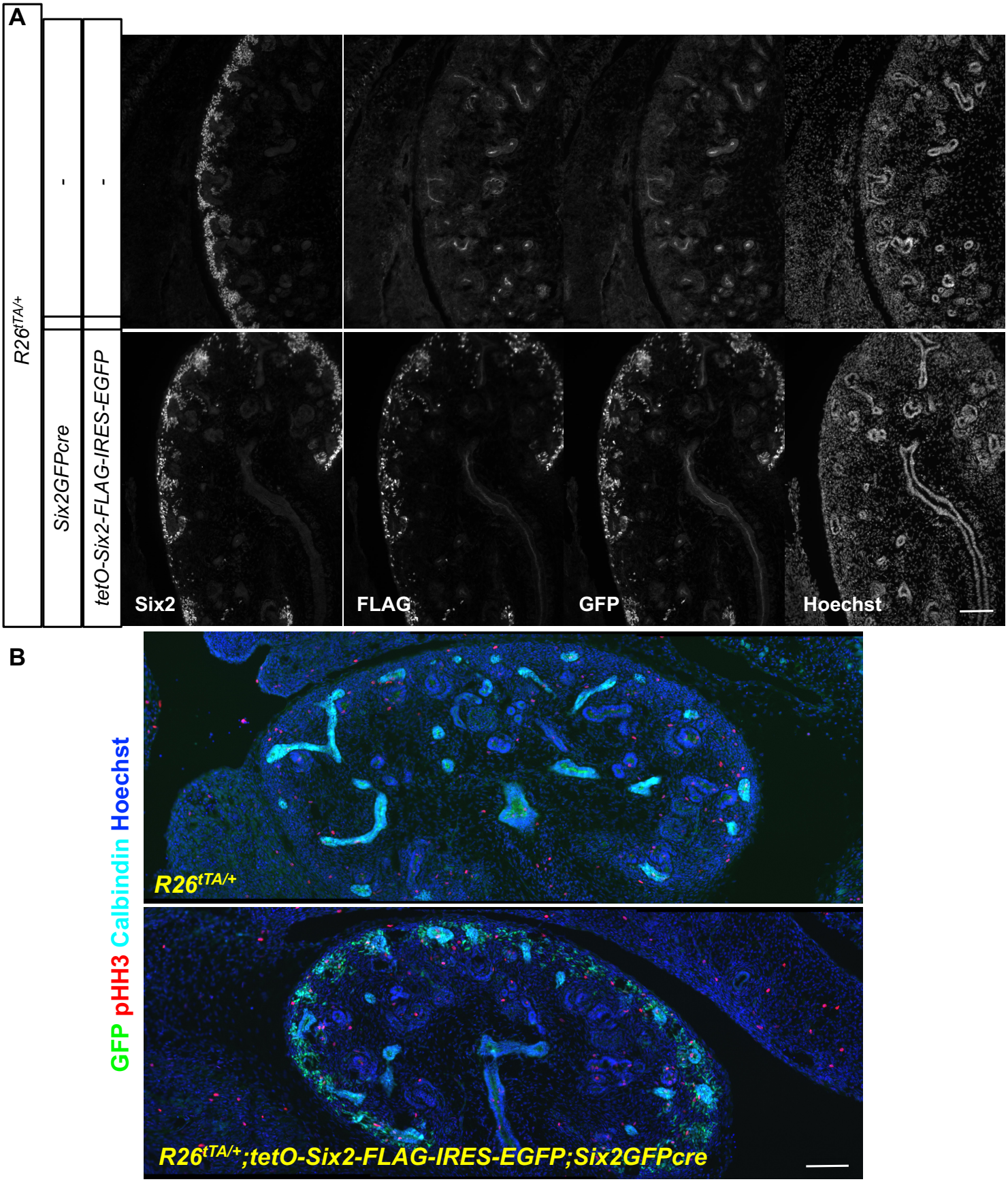

Fig. S2 Characterization of the *Six2* gain-of-function (GOF) mutant kidney (A) Six2 expression levels in the *Six2* GOF mutant and control kidneys are similar. (B) Proliferation of nephron progenitors in the *Six2* GOF mutant and control kidneys is similar. Phospho-histone H3 (pHH3) was used as a proliferation marker.

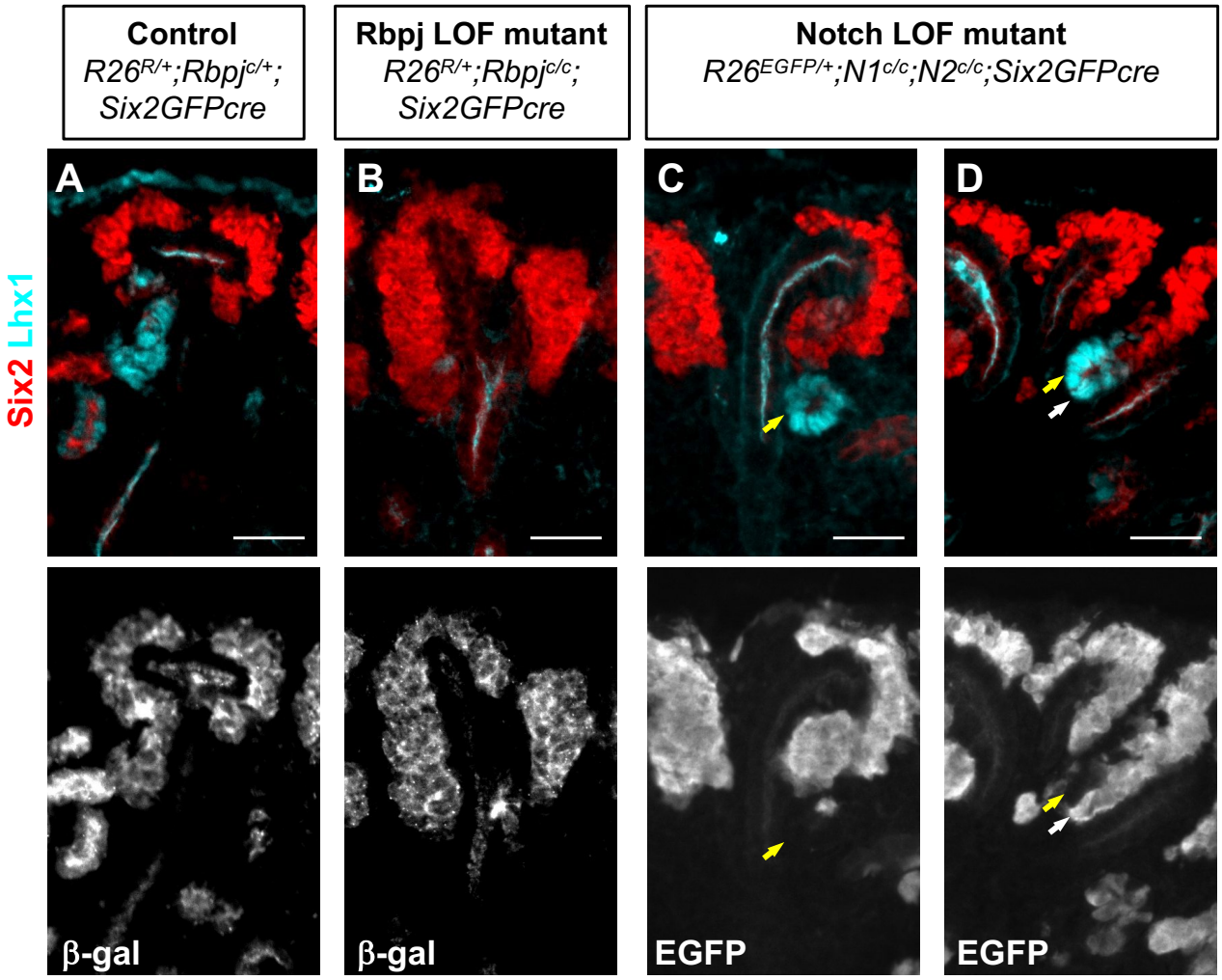

Fig. S3 Expression of a differentiation marker Lhx1 in the loss-of-function (LOF) mutant kidneys of *Rbpj* and *Notch*. Lineage tracer β-gal (A and B) or EGFP (C and D) was used to follow LOF cells. (A) In the control kidney, β-gal+ nephron progenitors differentiate into Lhx1+ cells. (B) In the *Rbpj* mutant kidney, Lhx1 expression is reduced. (C) In the *Notch* double mutant kidney, some Lhx1+ cells are seen that are not labeled with EGFP (yellow arrow), meaning that these cells have escaped Cre-mediated recombination. (D) In the *Notch* double mutant kidney, some EGFP+ cells (white arrow) differentiate into Lhx1+ cells. These cells are often adjacent to EGFP- Lhx1+ wild type cells (yellow arrow).

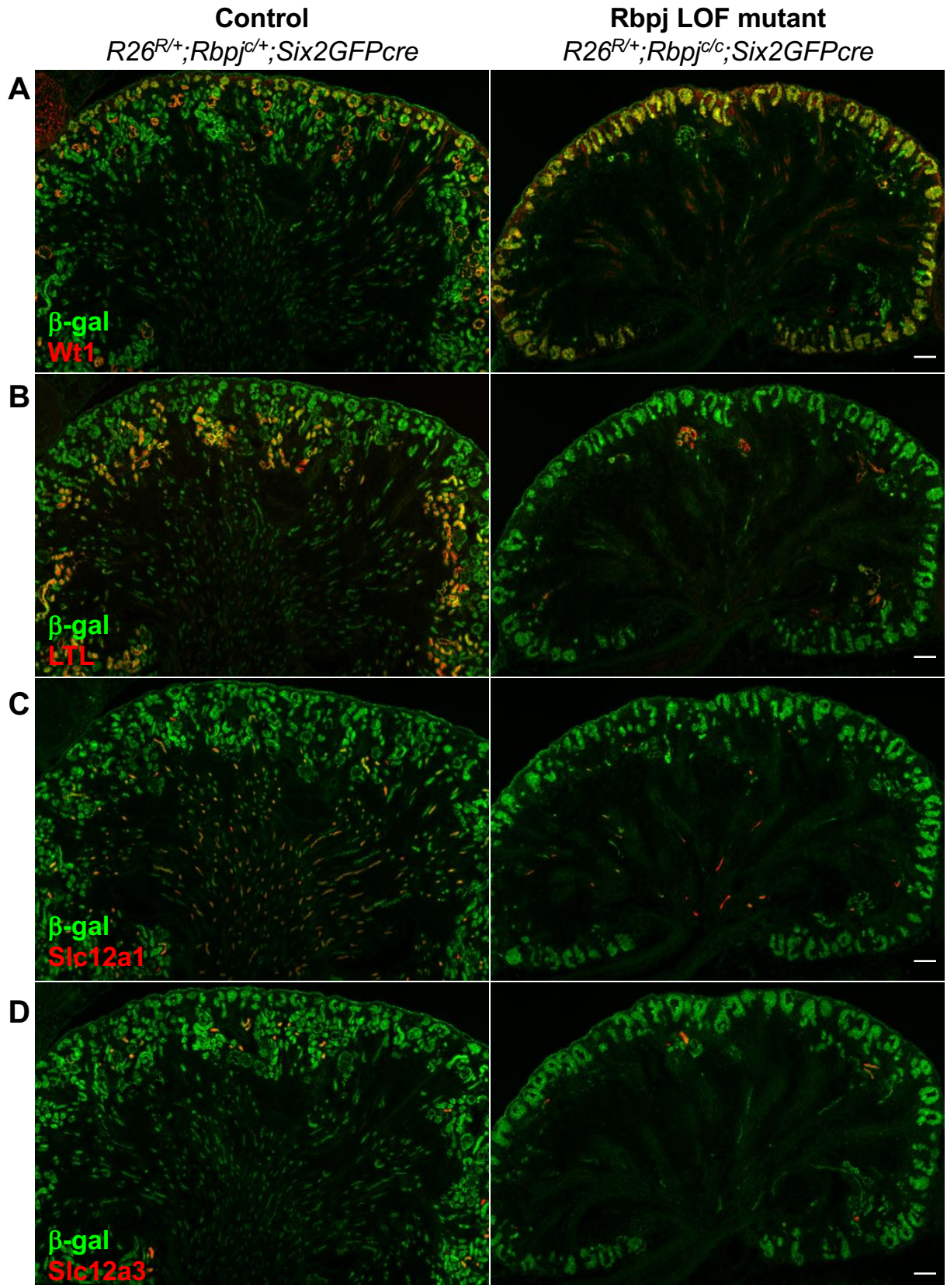

Fig. S4 The nephron progenitors in the *Rbpj* LOF mutant kidney differentiate into nephron segments inefficiently Lineage analysis of Six2+ cells shows that nephrogenesis is almost blocked in the *Rbpj* mutant kidney. In the control kidney, the Rosa GFP reporter positive cells form Wt1+ podocytes (A), LTL+ proximal tubules (B), Slc12a1+ loop of Henle (C), and Slc12a3+ distal tubules (D). In the *Rbpj* mutant kidney, GFP positive cells poorly differentiate into any segment of the nephron. P0 kidneys. Images are representative of two independent experiments. Scale bars, 100 mm.

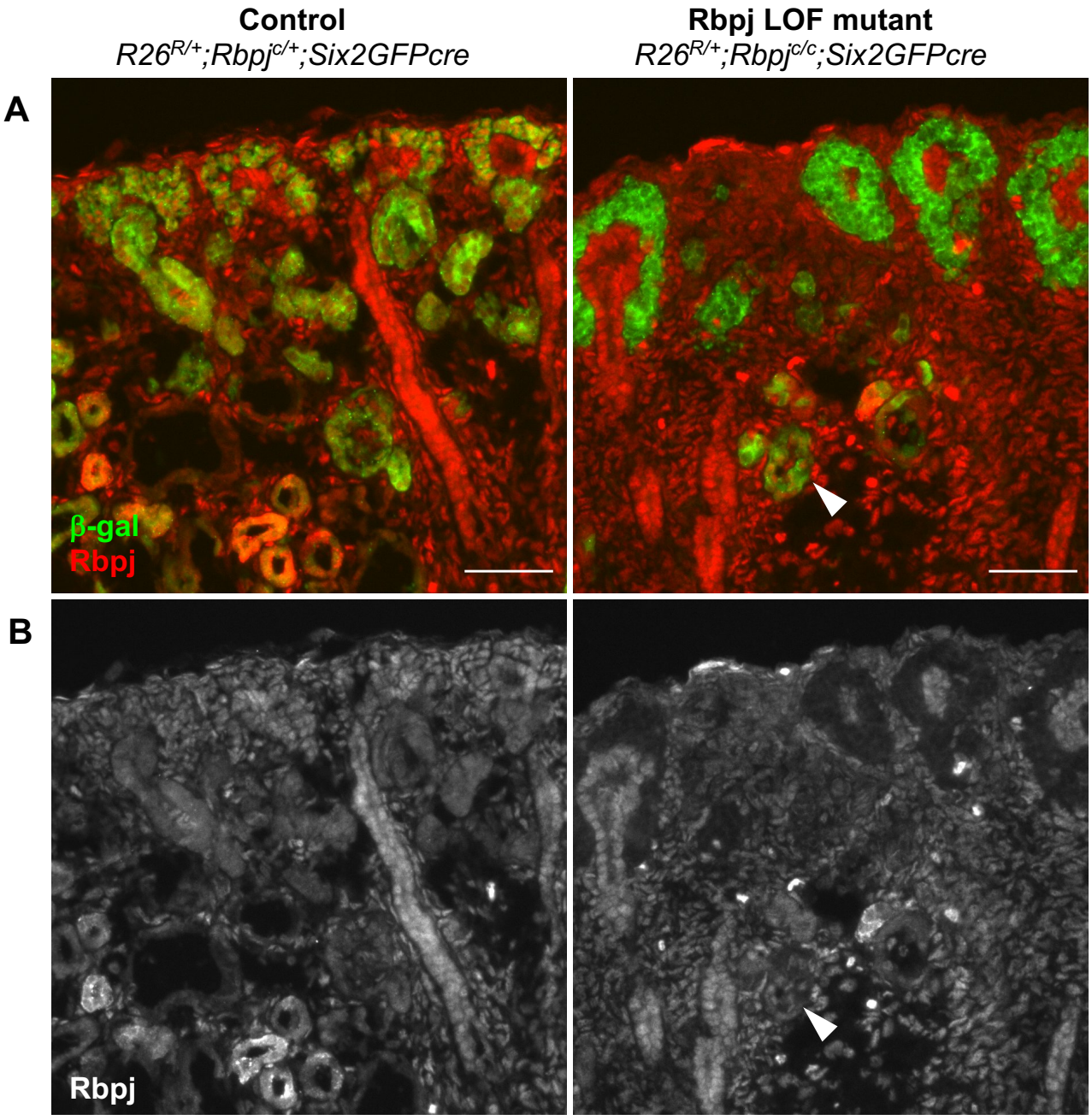

Fig. S5 Expression of Rbpj in the *Rbpj* LOF mutant kidney (A) In the control kidney, expression of Rbpj is high in the cap mesenchyme and collecting duct. Rbpj expression is reduced after nephron progenitors undergo differentiation. (B) In the *Rbpj* mutant kidney, while Rbpj is largely absent in β-gal+ cells, still a small minority of β-gal+ cells are Rbpj+ (white arrowhead).