Table S1. Antibodies

Antigen	Vendors	Catalog #	Host	dilution
Six2	Proteintech	11562-1-AP	rabbit	1:500
GFP	Aves	GFP-1020	chick	1:500
Jag1	DSHB	TS1.15H	rat	1:20
Lhx1	DSHB	4F2	mouse	1:20
Pax2	Proteintech	21385-1-AP	rabbit	1:200
Wt1	Santa Cruz	sc-192	rabbit	1:200
Wt1	Santa Cruz	sc-7385	mouse	1:100
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
β-gal	MPbio	559761	rabbit	1:15000
β-gal	Abcam	ab9361	chick	1:300
Hnf1b	Proteintech	12533-1-AP	rabbit	1:200
Mafb	Sigma	HPA005653	rabbit	1:300
Lama1	Sigma	L9393	rabbit	1:10000

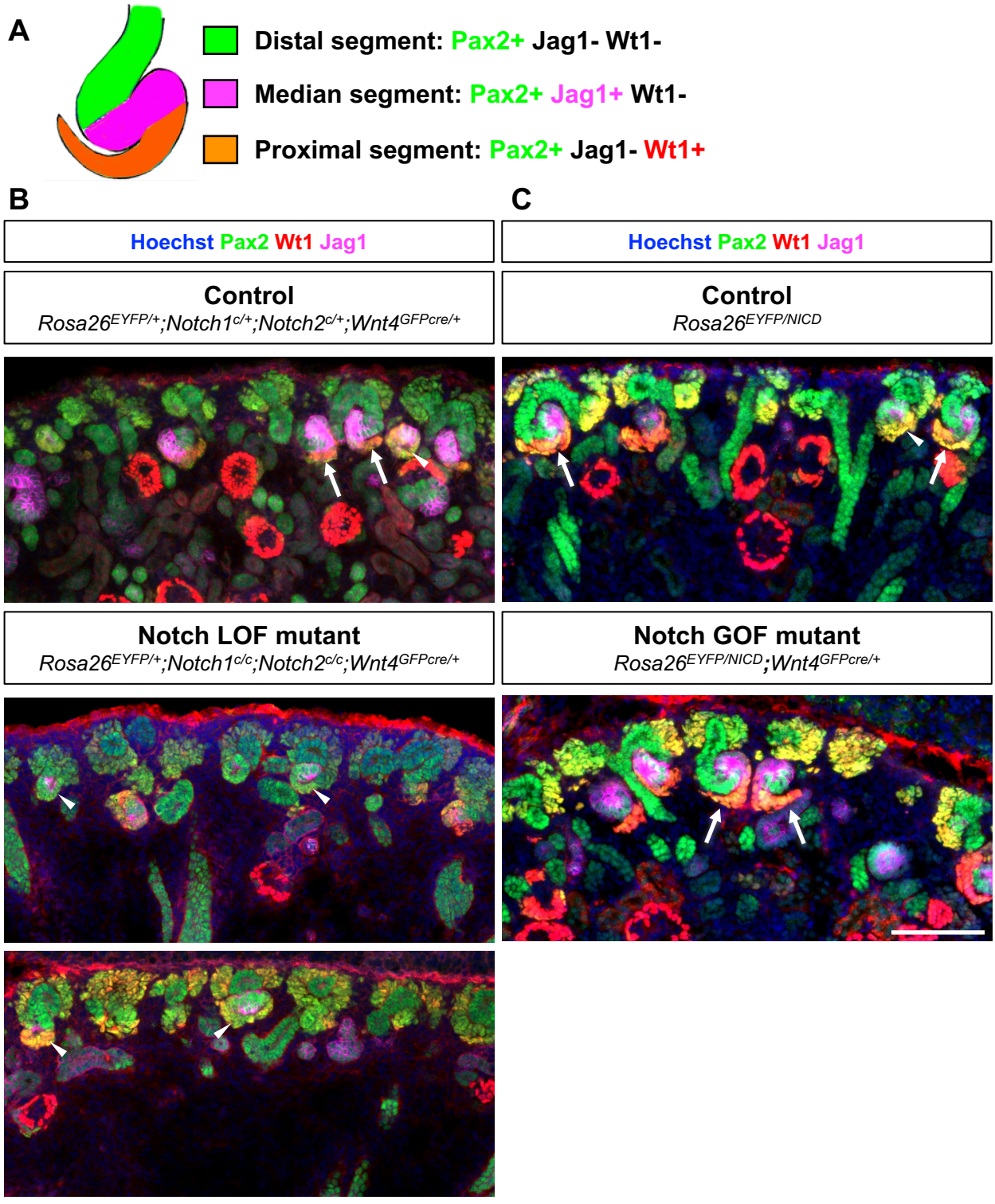

Fig. S1. The S-shaped body (SSB) is absent in the Notch loss-of-function (LOF) mutant kidney but present in the Notch gain-of-function (GOF) mutant kidney (A) Schematic diagram of SSB segments and markers (B) The Notch LOF mutant kidney forms renal vesicles (marked with white arrowheads) but fails to form SSB (marked with white arrows). (C) The Notch GOF mutant kidney forms SSB (marked with white arrows). (B-C) Embryonic kidneys at E18.5 are shown. Scale bar, 100μm

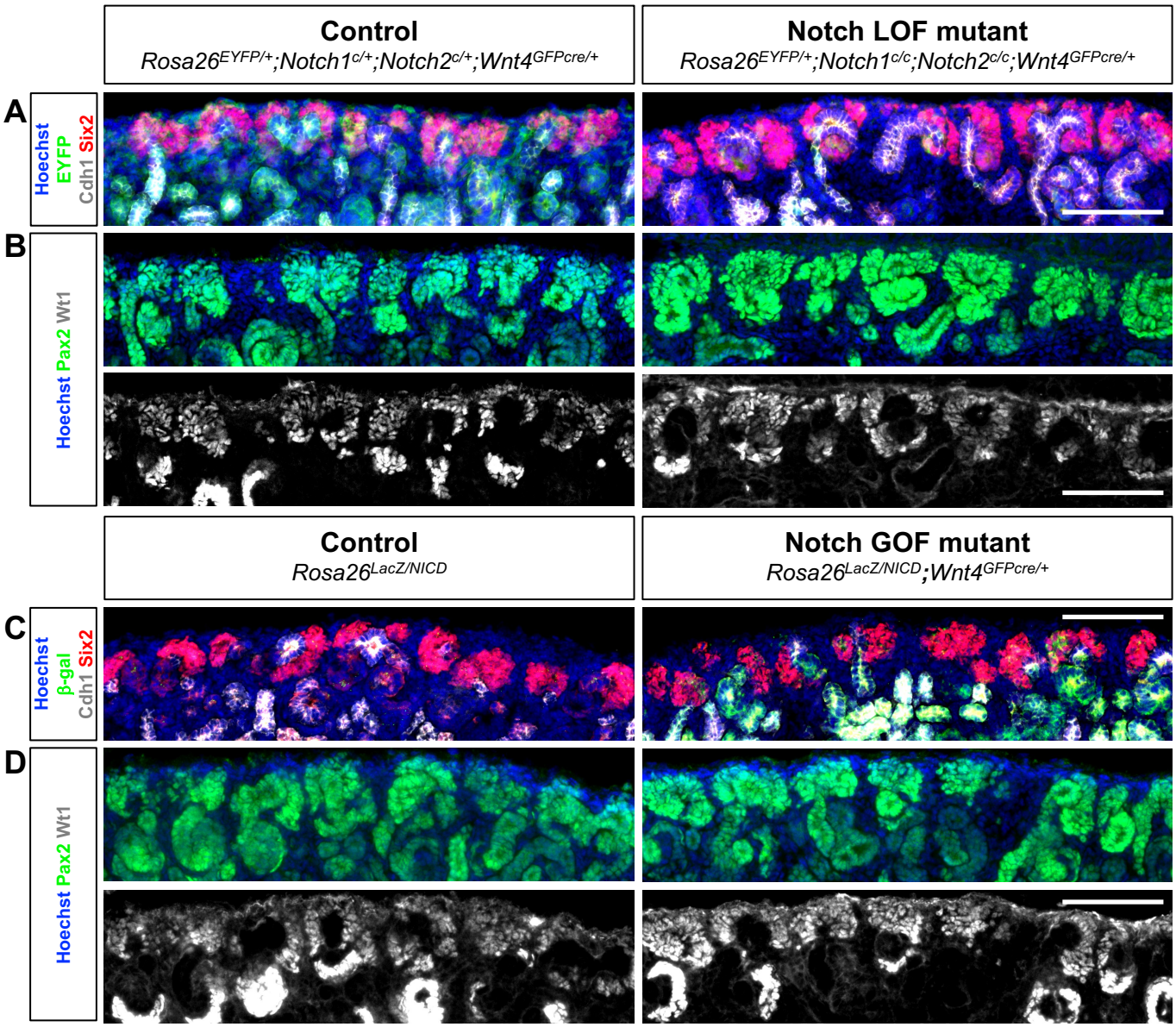

Fig. S2. Genetic manipulation of Notch signaling with *Wnt4GFPcre* does not affect mesenchymal nephron progenitor cells (A) Six2+ cells are not prematurely depleted in the Notch LOF mutant kidney even with *Wnt4GFPcre* targeting a subset of Six2+ cells. (B) Deletion of Notch by *Wnt4GFPcre* does not affect *Pax2* and *Wt1* expression in the mesenchymal nephron progenitors. *Pax2* is also expressed in the collecting duct and developing nephrons. (C) Six2+ cells are not prematurely depleted in the Notch GOF mutant kidney. The *R26R* reporter is inactive in the control kidney because it lacks *Wnt4GFPcre.* (D) Constitutive activation of Notch signaling by *Wnt4GFPcre* does not affect *Pax2* and *Wt1* expression in the mesenchymal nephron progenitors. (A-D) Embryonic kidneys at E18.5 are shown. Scale bars, 100μm

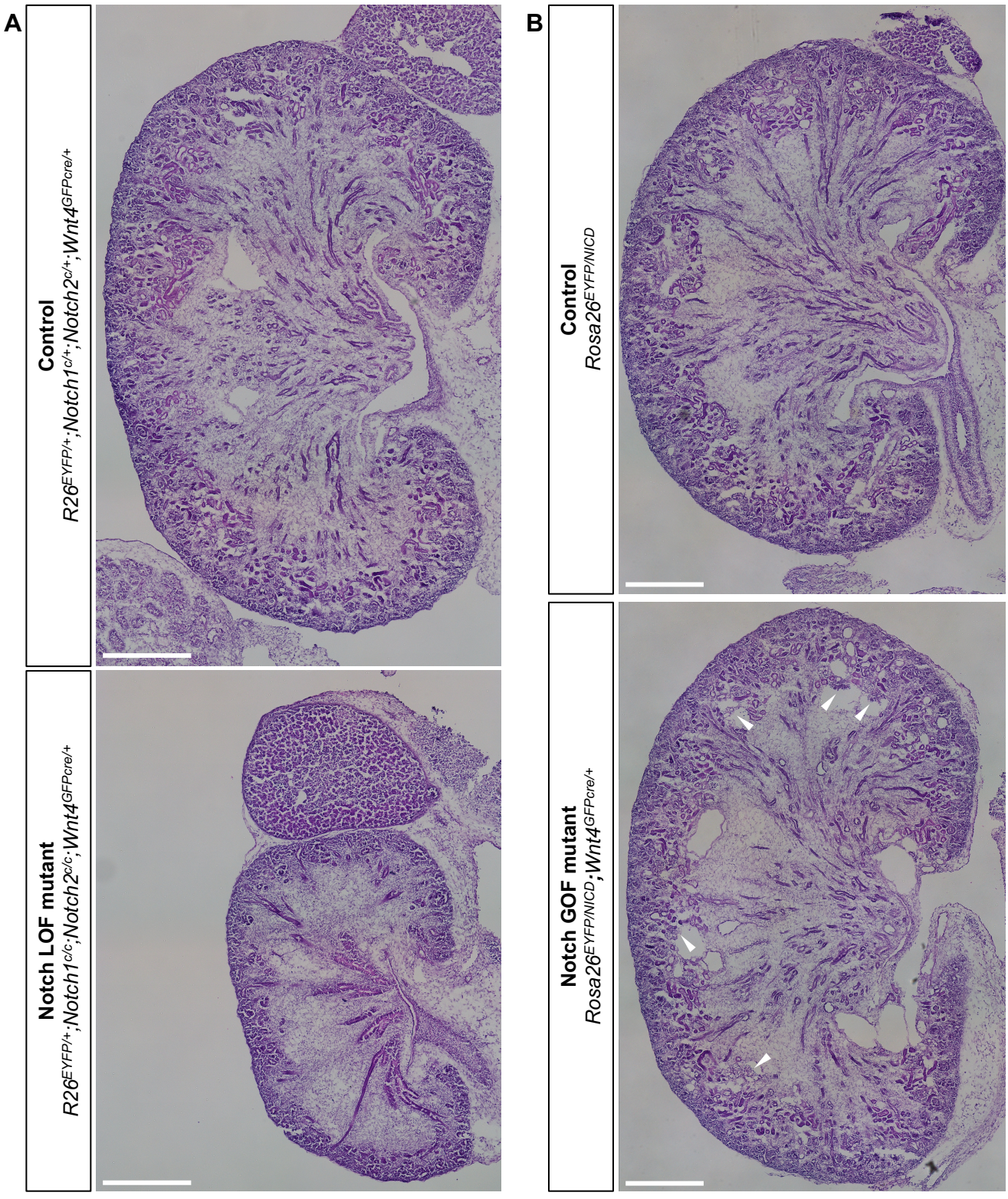

Fig. S3. Histology of Notch LOF and GOF mutant kidneys (A) H&E staining shows paucity of nephron tubules in the Notch LOF mutant kidney (B) The Notch GOF mutant kidney shows relatively normal nephrogenesis except for the formation of glomerulocysts (marked with white arrowheads). (A-B) Embryonic kidneys at E18.5 are shown. Scale bars, 500μm

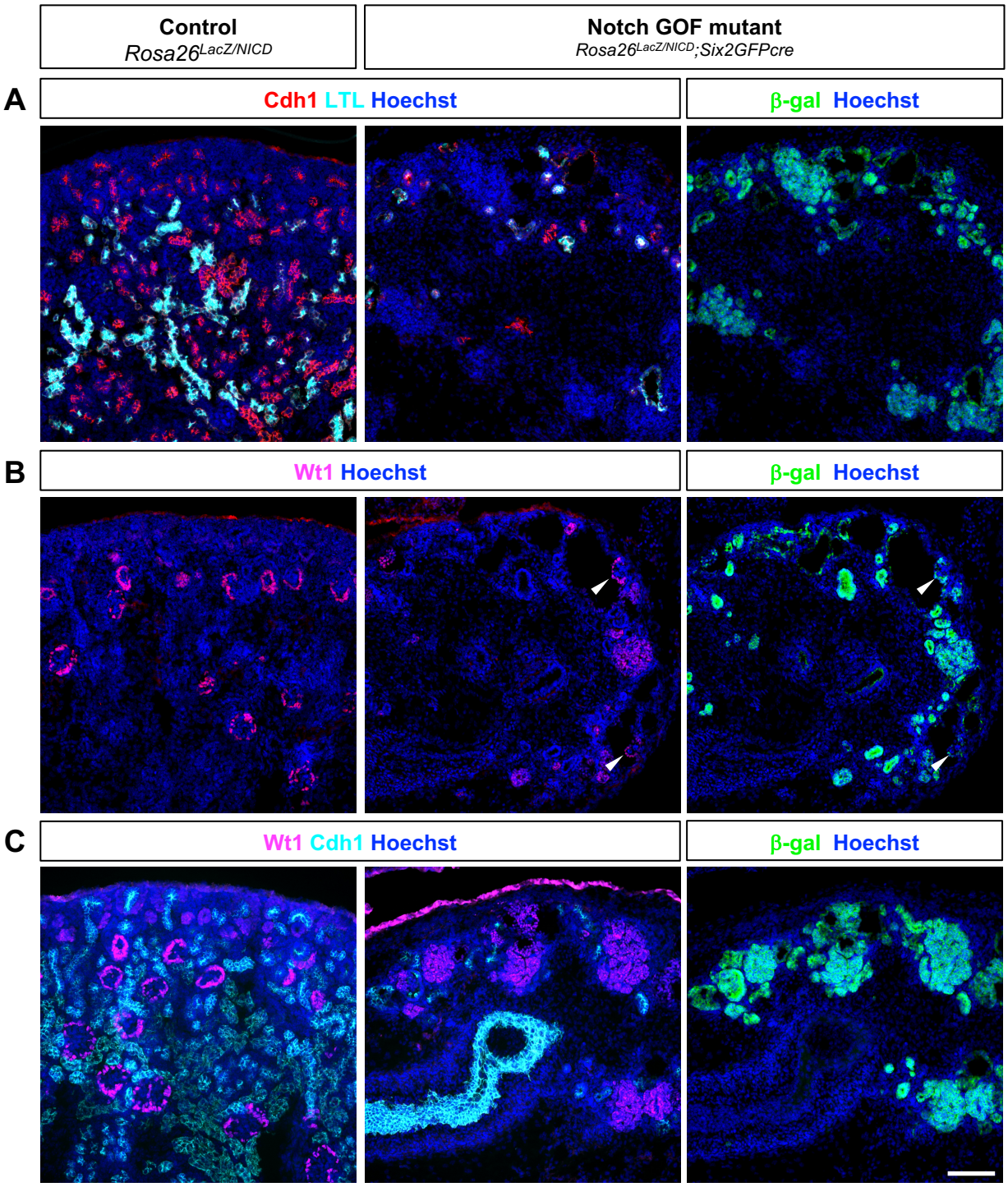

Fig. S4. Constitutive activation of Notch signaling by *Six2GFPcre* leads to the formation of a heterogeneous population of cells, rather than proximal tubules exclusively (A-C) Notch GOF mutant cells are labeled with the *R26R* reporter (β-gal). The *R26R* reporter is inactive in the control kidney because it lacks *Six2GFPcre.* P0 kidneys are shown. Scale bar, 100μm (A) Only a subset of the Notch GOF mutant cells differentiate into LTL+ proximal tubule cells. Cdh1 was used as an epithelial marker. LTL+ proximal tubules are positive for Cdh1 in both control and mutant kidney. (B) The Notch GOF kidney forms glomerulocysts (marked with white arrowheads). (C) A significant portion of Notch GOF mutant cells are positive for Wt1 and negative for Cdh1.

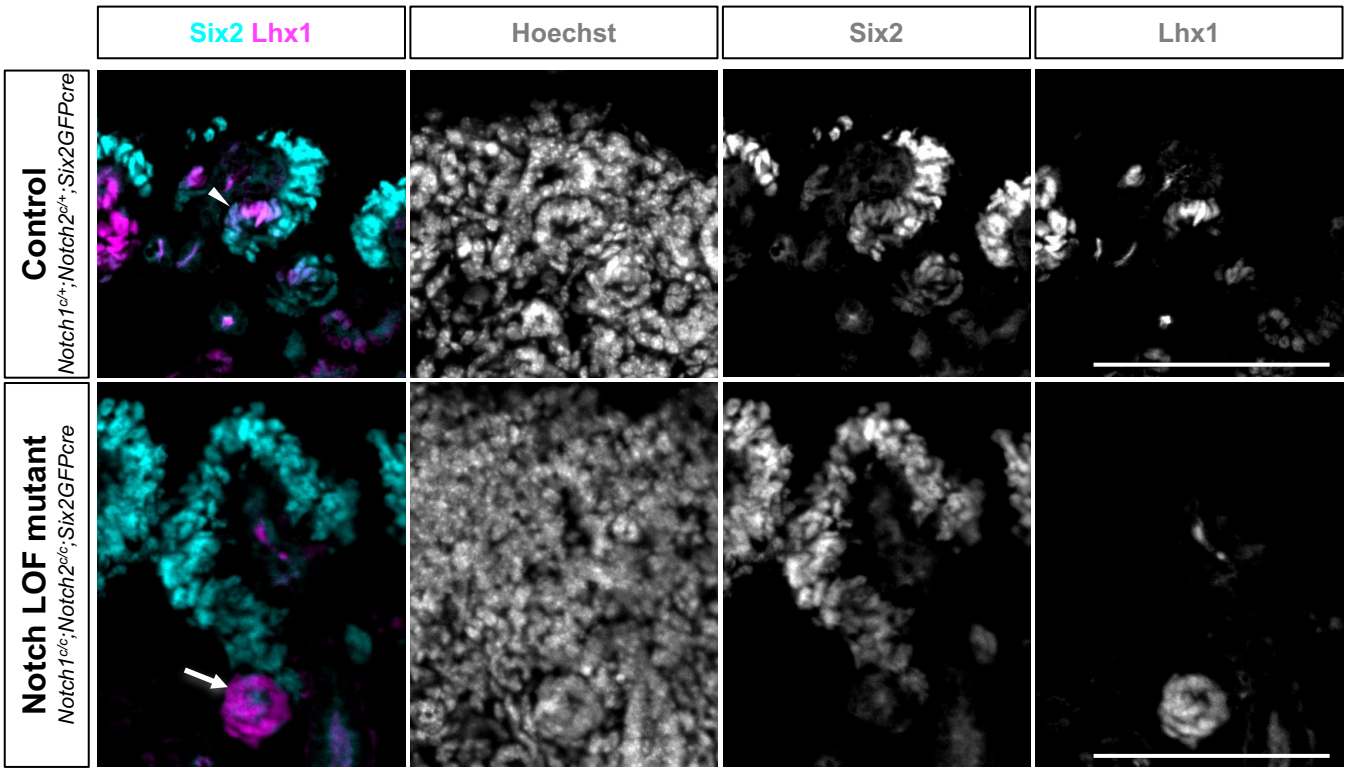

Fig. S5. Notch signaling is required for the establishment of proper proximodistal axis in the renal vesicle In the control kidney, *Lhx1* is expressed in the distal part of the renal vesicle (marked with a white arrowhead), indicating the existence of the proximodistal axis in the renal vesicle. When *Notch1* and *Notch2* are deleted with *Six2GFPcre*, *Lhx1* is expressed in the entire renal vesicle (marked with a white arrow), suggesting that the mutant renal vesicle fails to establish a proper proximodistal axis. Note that the renal vesicle in the Notch loss-of-function mutant kidney is located deeper toward the medullary side due to inefficient downregulation of *Six2*. P0 kidneys are shown. Scale bars, 100μm