SUPPLEMENTAL INFORMATION

Loss of *Zeb2* in mesenchyme-derived nephrons causes congenital atubular glomeruli and primary glomerulocystic kidney disease

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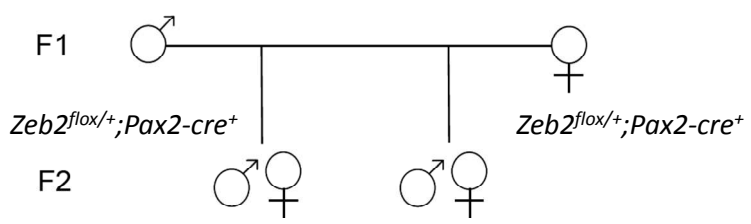
SUPPLEMENTAL FIGURES

Supplemental Figure 1

a



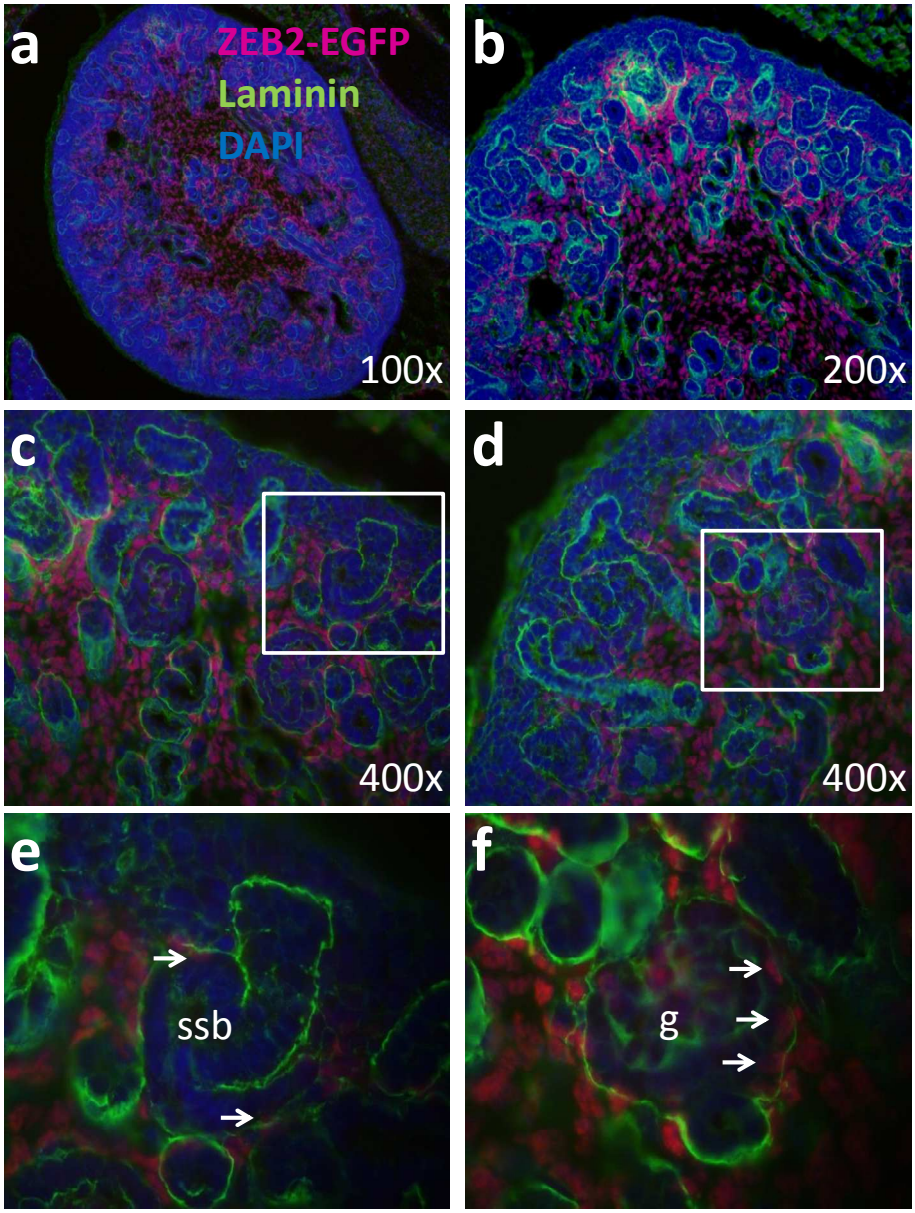
b

Genotype	(i) 3 weeks		(ii) E18.5	
	Observed	Expected	Observed	Expected
Zeb2 flox/+;Pax2-cre+	23 (24%)	36 (38%)	10 (18%)	20.625 (38%)
Zeb2 +/+;Pax2-cre+	28 (29%)	18 (19%)	10 (18%)	10.3125 (19%)
Pax2-cre-	45 (47%)	24 (25%)	14 (26%)	13.75 (24%)
Zeb2 flox/flox;Pax2-cre+	0	18 (19%)	21 (38%)	10.3125 (19%)
Total	96		55	

Supplemental Figure 1. *Zeb2$^{flox/flox}$;Pax2-cre* homozygous mice die at birth.

(a) Breeding scheme for generating *Zeb2* conditional knockout mice using the *Pax2-cre^{+}* allele. (b) Distributions of genotypes in weaned mice at 3 weeks from 20 litters (i) and at E18.5 from 10 litters (ii). Observed: the number of mice with each genotype obtained after breeding experiments; Expected: the number of mice with each genotype expected based on the parents genotypes and normal Mendelian distribution.

Supplemental Figure 2

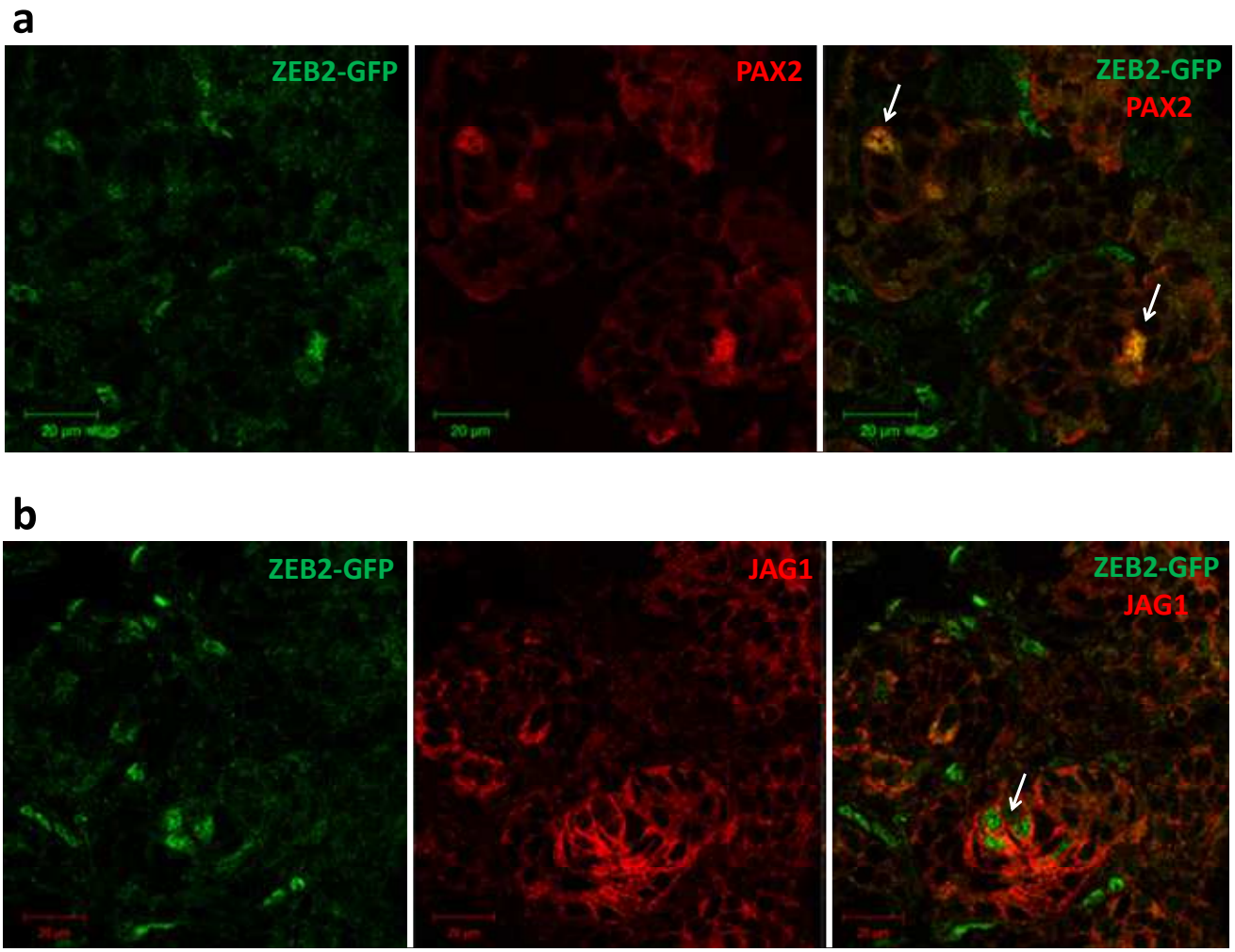


Supplemental Figure 2: ZEB2 expression in E16.5 developing mouse kidney.

(**a-f**) ZEB2 expression (red) in the developing kidney was detected using the ZEB2-EGFP reporter mouse at E16.5 with an anti-GFP antibody (red). The structure of the nephrons and collecting ducts were delineated with an anti-Laminin antibody (green). Magnification of 100x (**a**), 200x (**b**), and 400x (**c, d**) are indicated. (**e**) Enlarged boxed area from **c** shows

ZEB2 positive cells (arrows) in an S-shaped body (ssb). (**f**) Enlarged boxed area from **d** shows ZEB2 positive cells (arrows) in the Bowman's capsule of a glomerulus (g).

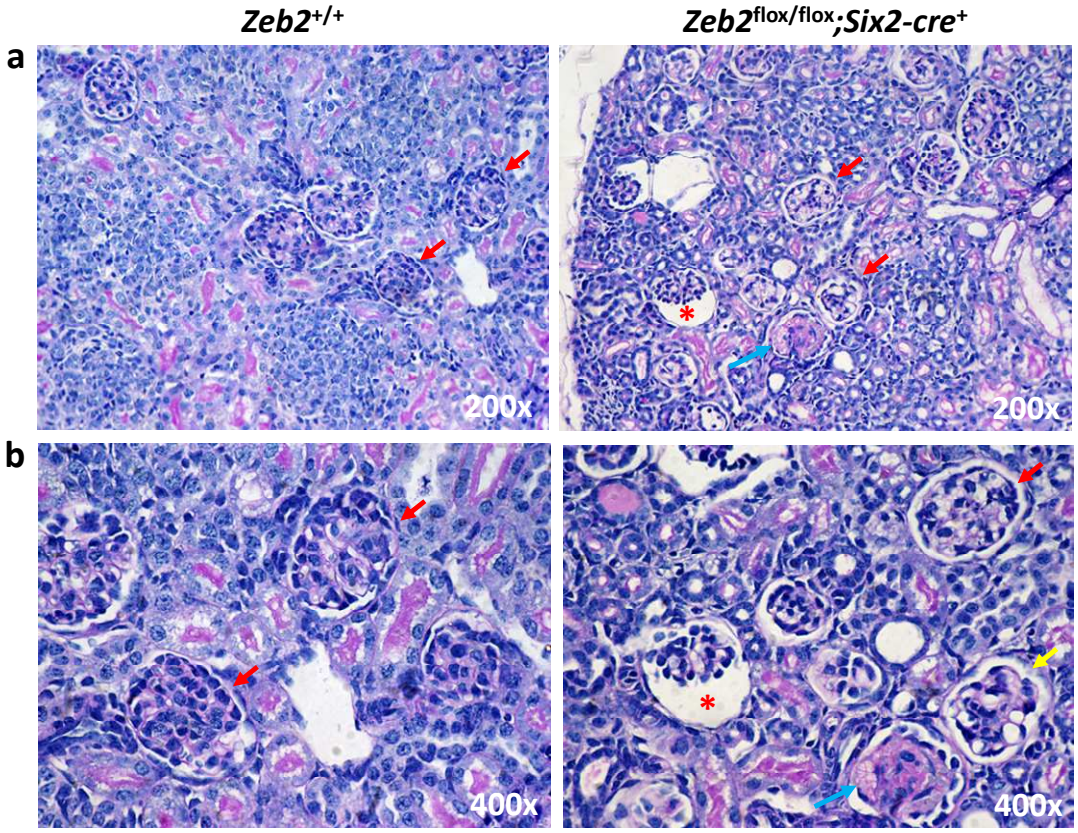
Supplemental Figure 3



Supplemental Figure 3: ZEB2 and PAX2/JAG1 co-expression in E15.5 and E16.5 developing nephron from ZEB2-GFP transgenic mice.

(**a**) Co-localization of ZEB2 (green) and PAX2 (red) on an E15.5 Zeb2-GFP reporter mouse kidney shows co-expression of ZEB2-GFP and PAX2 in the same cell nuclei (arrows) of developing nephron. (**b**) ZEB2 (green) is expressed in the same cells (arrow) labeled by membrane protein JAG1 (red) in the S-shaped body of an E16.5 Zeb2-GFP reporter mouse kidney.

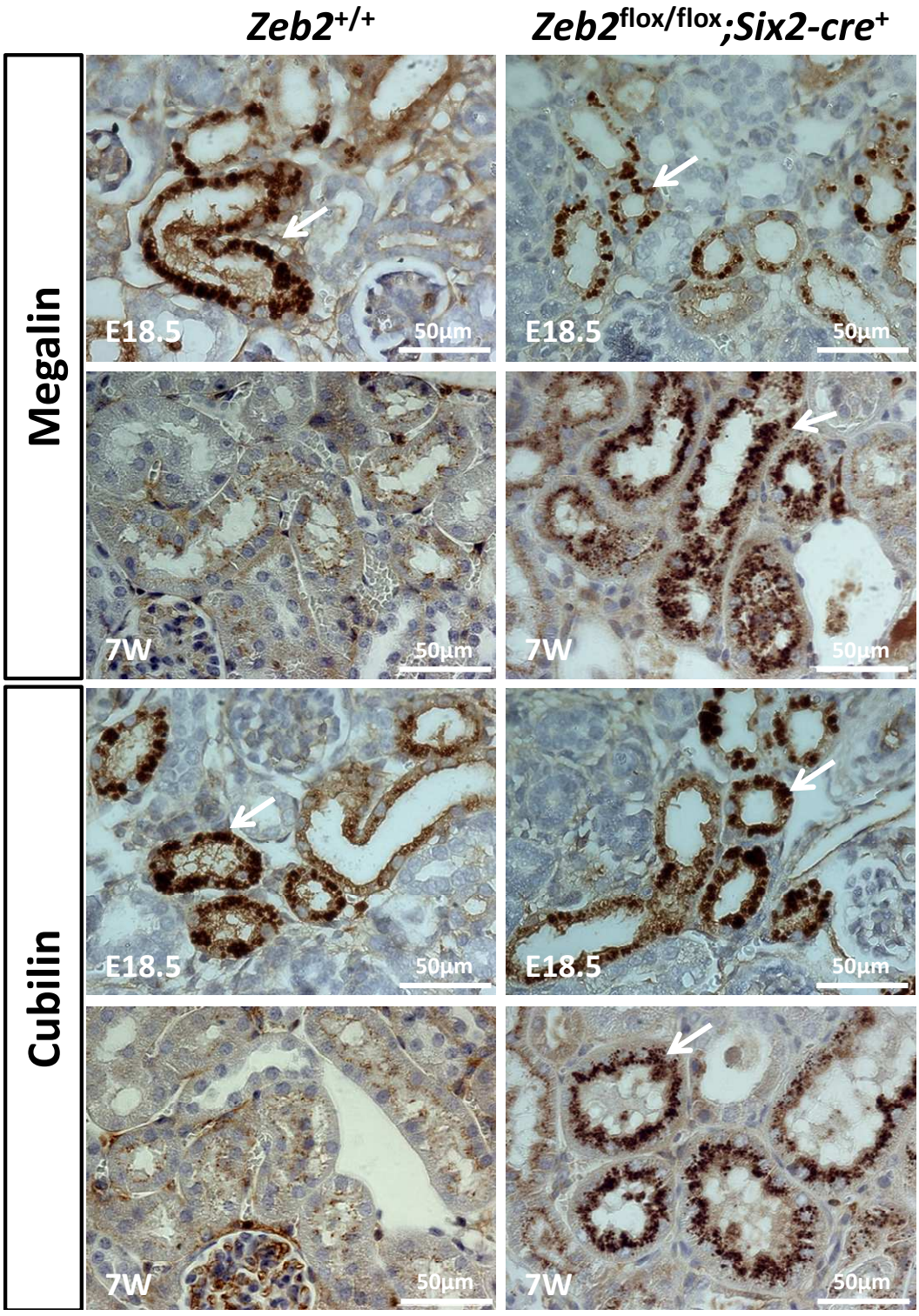
Supplemental Figure 4



Supplemental Figure 4: Morphology of non-cystic glomeruli in *Zeb2* cKO kidneys compared to wild-type littermate controls.

PAS staining of P8 kidneys from *Zeb2*$^{flox/flox}$;*Six2-cre*$^{+}$ cKO and *Zeb2*$^{+/+}$ wild-type littermate controls show difference of the histomorphometry of cKO mutant non-cystic glomeruli (red arrows) compared with those of wild-type mice, including increased number of sclerotic glomeruli (blue arrow) and glomerular capillary dilation (yellow arrow) in the cKO mutant kidneys. Magnification: 200x in (a) and 400x in (b). Red asterisk marks glomerular cyst in cKO mutant kidney.

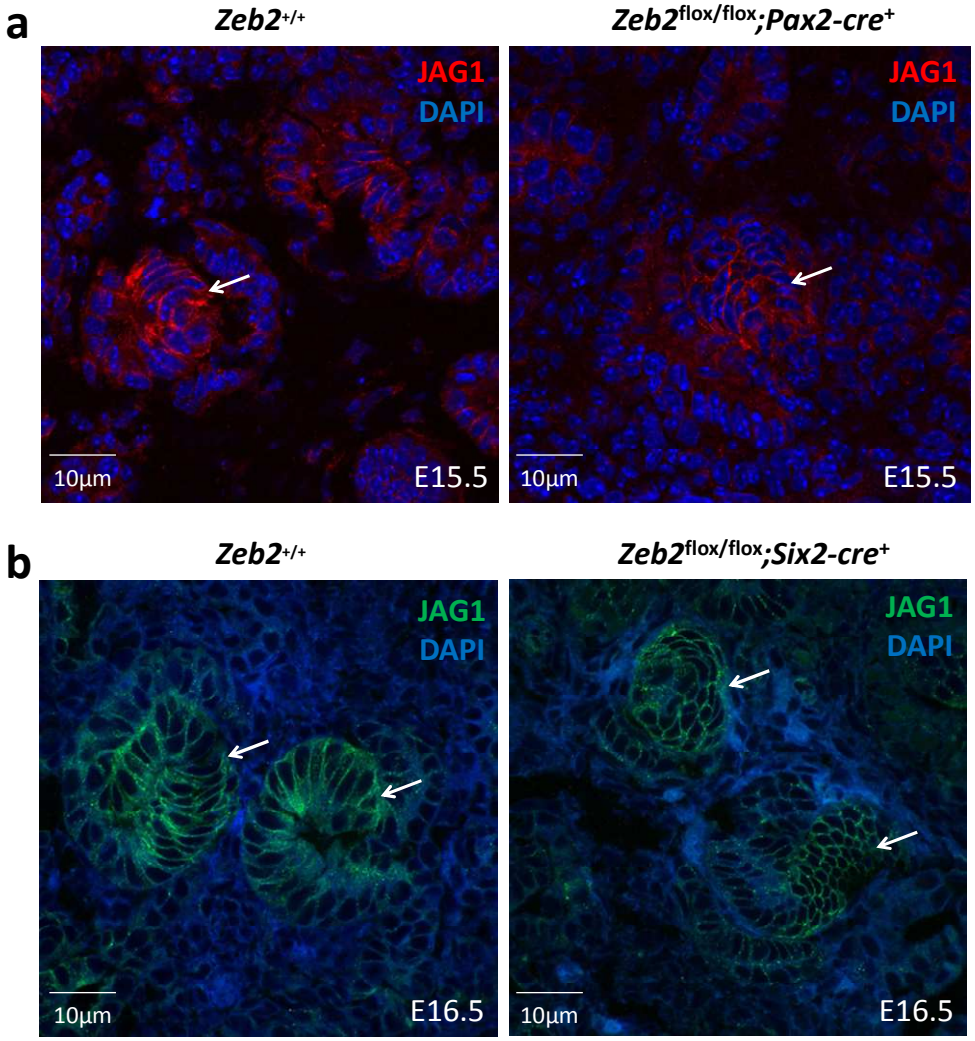
Supplemental Figure 5



Supplemental Figure 5: Megalin and cubilin are upregulated in adult *Zeb2* cKO renal proximal tubules.

Immunohistochemistry (IHC) staining with anti-megalin and anti-cubilin antibodies show similar levels of megalin and cubilin expression (arrows) in the proximal tubules in embryonic E18.5 kidneys of *Zeb2* cKO and wild-type littermate controls. The expressions of megalin and cubilin are significantly upregulated in the proximal tubules of 7-week old adult *Zeb2* cKO in comparison to the age-matched wild-type littermate controls, suggesting increased proximal tubule endocytosis of albumin in *Zeb2* cKO. The albuminuria in *Zeb2* cKO is probably coming through those nephrons that are patent and connected to the hyperfiltrative glomeruli.

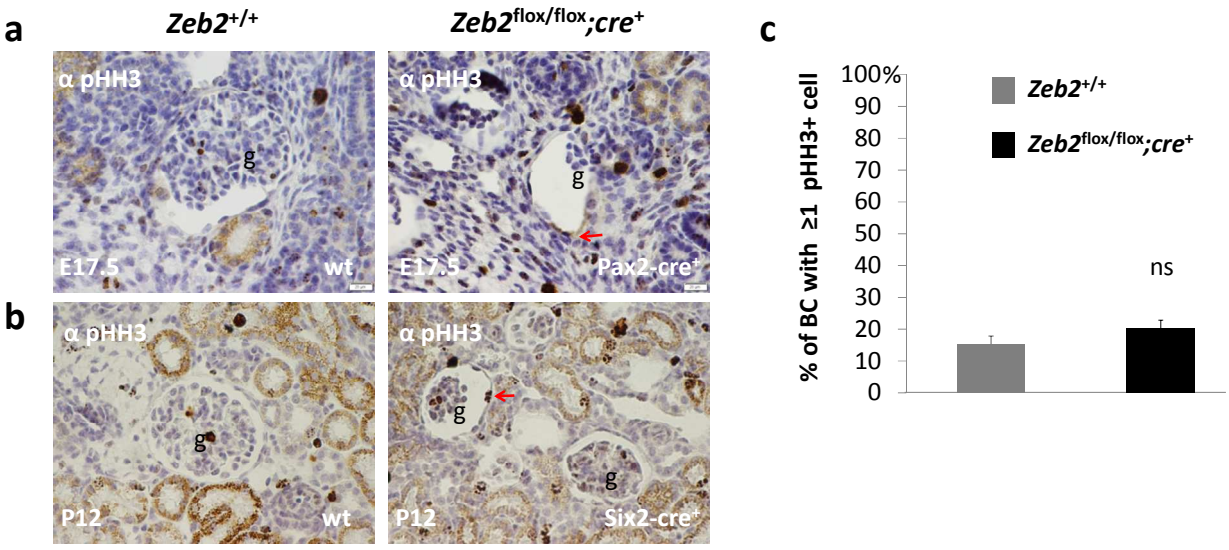
Supplemental Figure 6



Supplemental Figure 6: No major difference of JAG1 expression in E15.5 and E16.5 *Zeb2* cKO kidney compared to littermate controls

(**a**) No major difference of JAG1 expression patterns in developing nephron (arrow) of *Zeb2*$^{flox/flox}$*;Pax2-cre*$^{+}$ cKO and wild-type littermates at E15.5. (**b**) No major difference of JAG1 expression patterns in developing nephron (arrows) of *Zeb2*$^{flox/flox}$*;Six2-cre*$^{+}$ cKO and wild-type littermates at E16.5.

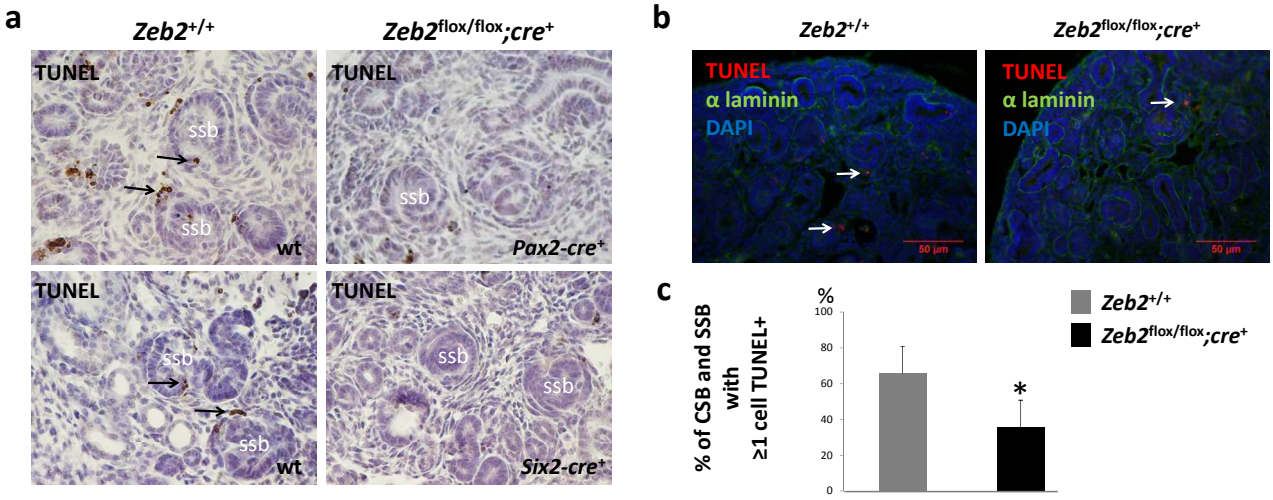
Supplemental Figure 7



Supplemental Figure 7: No significant changes in cell proliferation in the Bowman's capsule of *Zeb2* knockout mice.

(**a**) Staining with an antibody against Phospho-histone H3 (pHH3) as a marker of cell proliferation in E17.5 *Zeb2*$^{flox/flox}$*;Pax2-cre*$^{+}$ embryonic kidneys (two kidneys in each group, 400x magnification). (**b**) Staining with an antibody against pHH3 as a marker of proliferation in P12 *Zeb2*$^{flox/flox}$*;Six2-cre*$^{+}$ kidneys (two kidneys in each group, 400x magnification). (**c**) No significant difference in cell proliferation between the wild-type and *Zeb2* conditional knockout mice in the Bowman's capsule (cKO mutant n=235 glomeruli analyzed, wild-type n=220 glomeruli analyzed from four kidneys in each group, ns - non significant).

Supplemental Figure 8



Supplemental Figure 8: Decreased apoptosis in the C-shaped and S-shaped bodies in *Zeb2* knockout mice.

(**a**) TUNEL (rhodamine fluorochrome) staining shows reduced positive apoptotic cells (arrows) in *Zeb2* conditional knockout kidneys using both *Pax2-cre*$^{+}$ and *Six2-cre*$^{+}$ (n=5 mutant kidneys and n=4 wild-type kidneys, 400x magnification). (**b**) Immunofluorescence staining with TUNEL (red) and an antibody against Laminin (green) to delineate the C-shaped bodies and the S-Shaped bodies. (**c**) Reduced apoptosis in the C-shaped bodies and S-shaped bodies of the *Zeb2* conditional knockouts (n=34 mutant C-shaped and S-shaped bodies and n=39 wild-type C-shaped and S-shaped bodies analyzed, *p-value < 10^{-2}).