Figures and figure supplements

Transcription factor *TFCP2L1* patterns cells in the mouse kidney collecting ducts

Max Werth *et al*

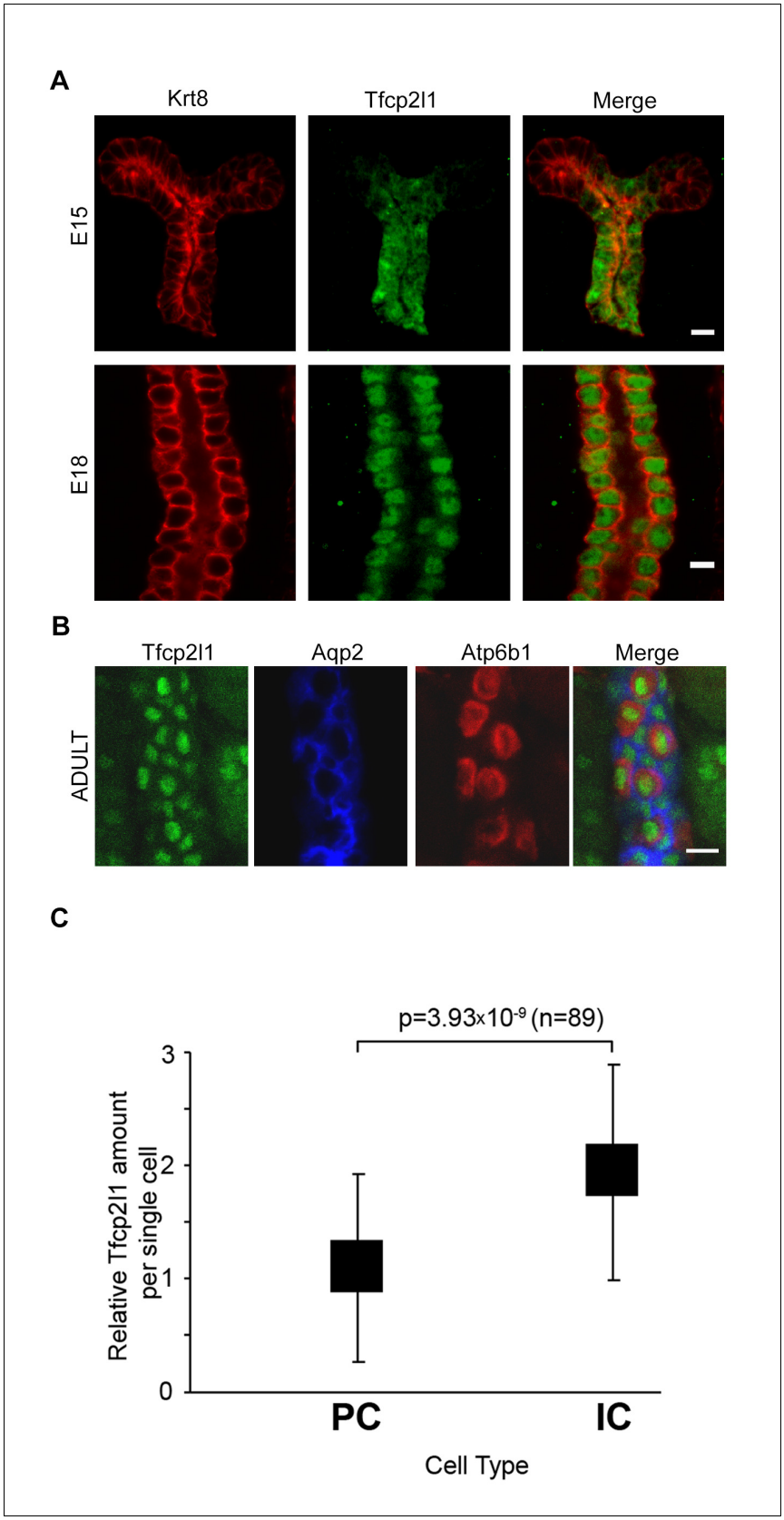

Figure 1. *Tfcp2l1* is a nuclear protein expressed in the collecting ducts. (**A**) Immunofluorescence detection of *Tfcp2l1* (green) in stalks of ureteric-collecting ducts at E15 and at E18. Nuclear localization was prominent at E18.
Figure 1 continued on next page

Figure 1 continued

The ducts were identified by the uniform expression of *Krt8* (red). Bars = 5 µm. (**B**) In adult collecting ducts, *Tfcp2l1* (green) was expressed by both Intercalated Cells (IC), identified by immunodetection of *Atp6v1b1*, abbreviated *Atp6b1* (red), and Principal Cells (PC) identified by immunodetection of *Aqp2* (blue). Z-stack projection. Bar = 10 µm. (**C**) Quantification of *Tfcp2l1* immunoflourescence in adult collecting ducts normalized per measurement area. *Atp6b1*$^{+}$ IC cells expressed higher levels of *Tfcp2l1* than did *Aqp2*$^{+}$ PC cells.

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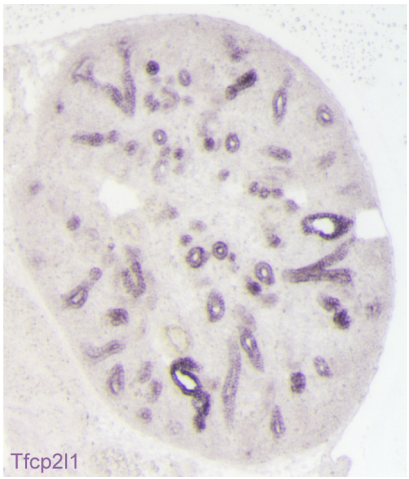

Figure 1—figure supplement 1. Expression of *Tfcp2l1* message in the stalks of the ureteric bud (E15 mouse kidney; in-situ hybridization). All of the *Tfcp2l1*$^{+}$ tubules are cross-sections of the collecting ducts.
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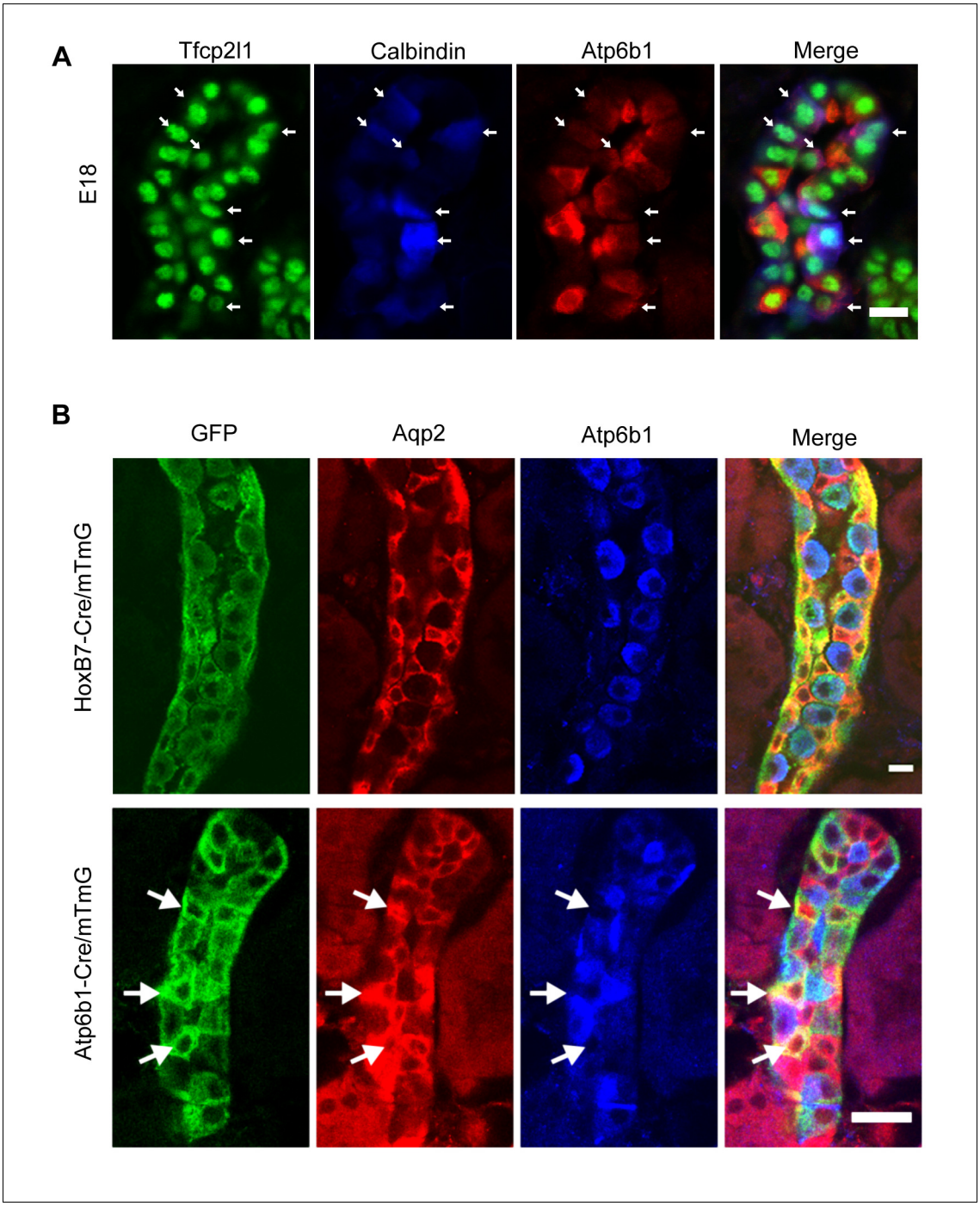

Figure 2. 'Double positive' progenitors populate the E18 collecting duct. (**A**) IC proteins were expressed at E18 in presumptive PC cells. Co-expression of *Atp6b1* (typical of ICs, red) and Calbindin (typical of PCs, blue) is shown in *Tfcp2l1*$^{+}$ cells in the cortical region of the collecting duct. (white arrows) Bar = 10 μm. (**B**) Lineage of ICs and PCs was detected with genetic reporters. *HoxB7-Cre;mTmG* (green) marked every cell in the collecting duct including *AQP2*$^{+}$ PC cells (red-yellow) and *Atp6b1*$^{+}$ IC cells (blue-green). *Atp6b1-Cre;mTmG* labeled every IC cell (endogenous *Atp6b1*$^{+}$; blue-green), as well as some *Aqp2*$^{+}$ PCs (white arrows, yellow). *Atp6b1-Cre;mTmG*-negative PC cells are also found (*Aqp2*$^{+}$, *Atp6b1*$^{-}$, *Atp6b1-Cre;mTmG*$^{-}$, red) (Bars= top 5 μm, bottom 20 μm).

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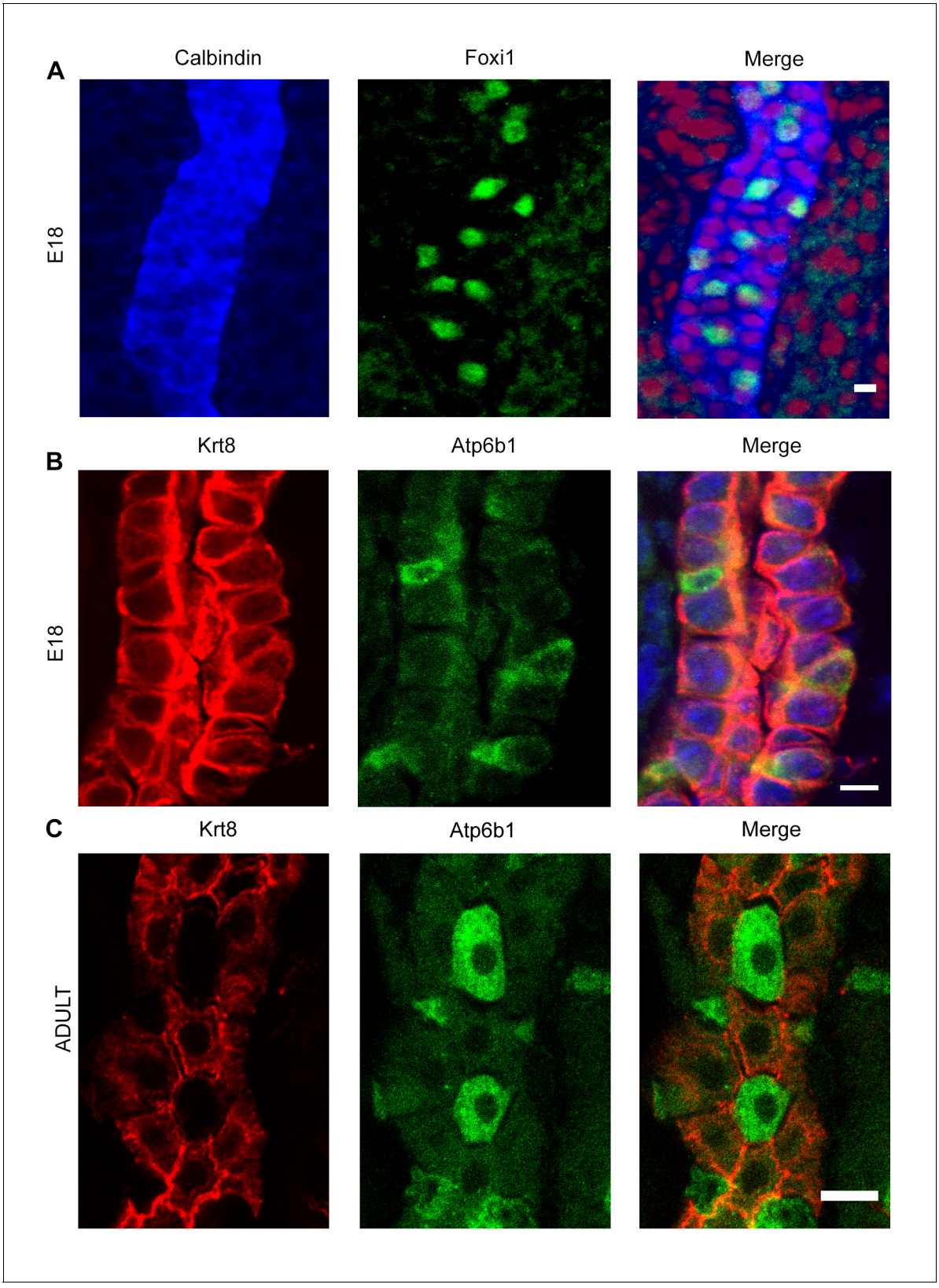

Figure 2—figure supplement 1. Detection of 'double positive' precursors in the embryonic collecting ducts. (**A**,**B**) Detection of 'double positive' precursors in the embryonic collecting ducts. Immunofluorescence analysis of developing collecting ducts using markers of PCs (*Calb1* blue, *Krt8* red) and ICs (*Foxi1* green, *Atp6b1* green) cells. By E18, IC proteins are expressed in PC cells (i.e.*Foxi1*$^{+}$ with *Calb1*$^{+}$ or *Atp6b1*$^{+}$ with *Krt8*$^{+}$; n = 4

Figure 2—figure supplement 1 continued on next page

Figure 2—figure supplement 1 continued

independent mice for each immunofluorescence analysis; Bars = 5 μm). (**C**) In contrast, PC (*Krt8*, red) and IC (*Atp6b1*, green) proteins were expressed in separate cells after birth (n = 4 independent mice for each immunofluorescence analysis; Bar = 10 μm).

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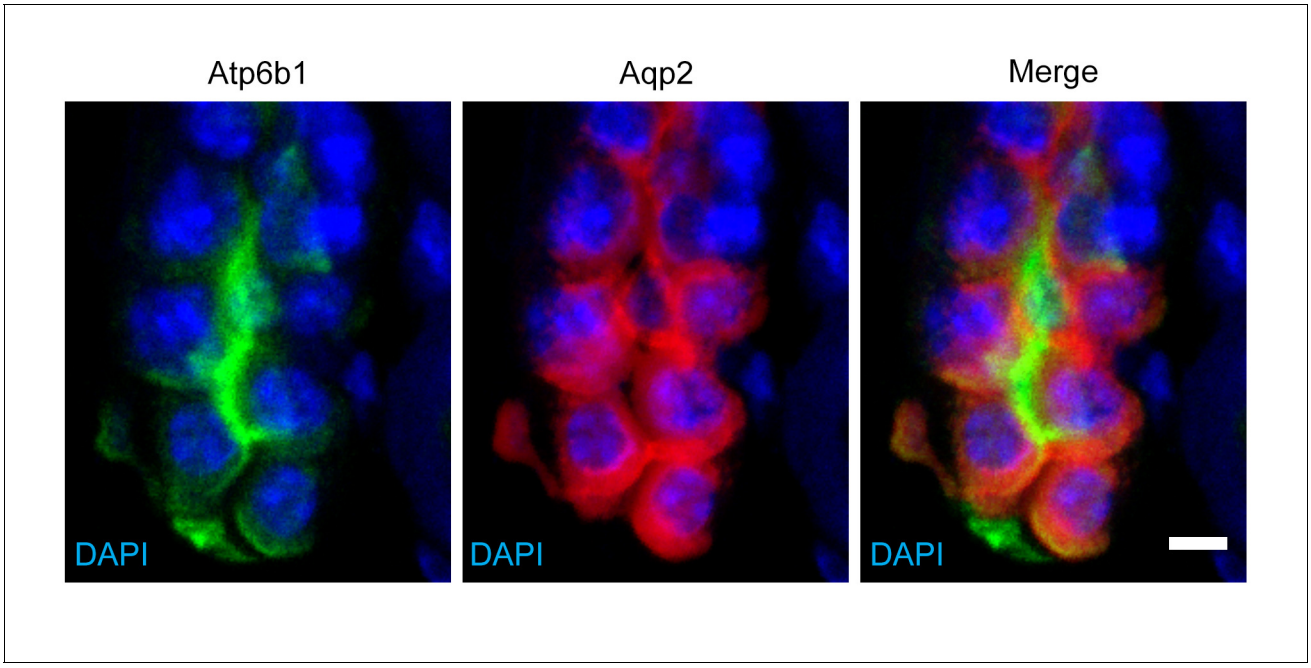

Figure 2—figure supplement 2. Detection of rare 'double positive' cells in adult collecting duct using marker proteins Atpb1 (IC cells) and *Aqp2* (PC cells). Bar = 5 μm.
DOI: 10.7554/eLife.24265.006

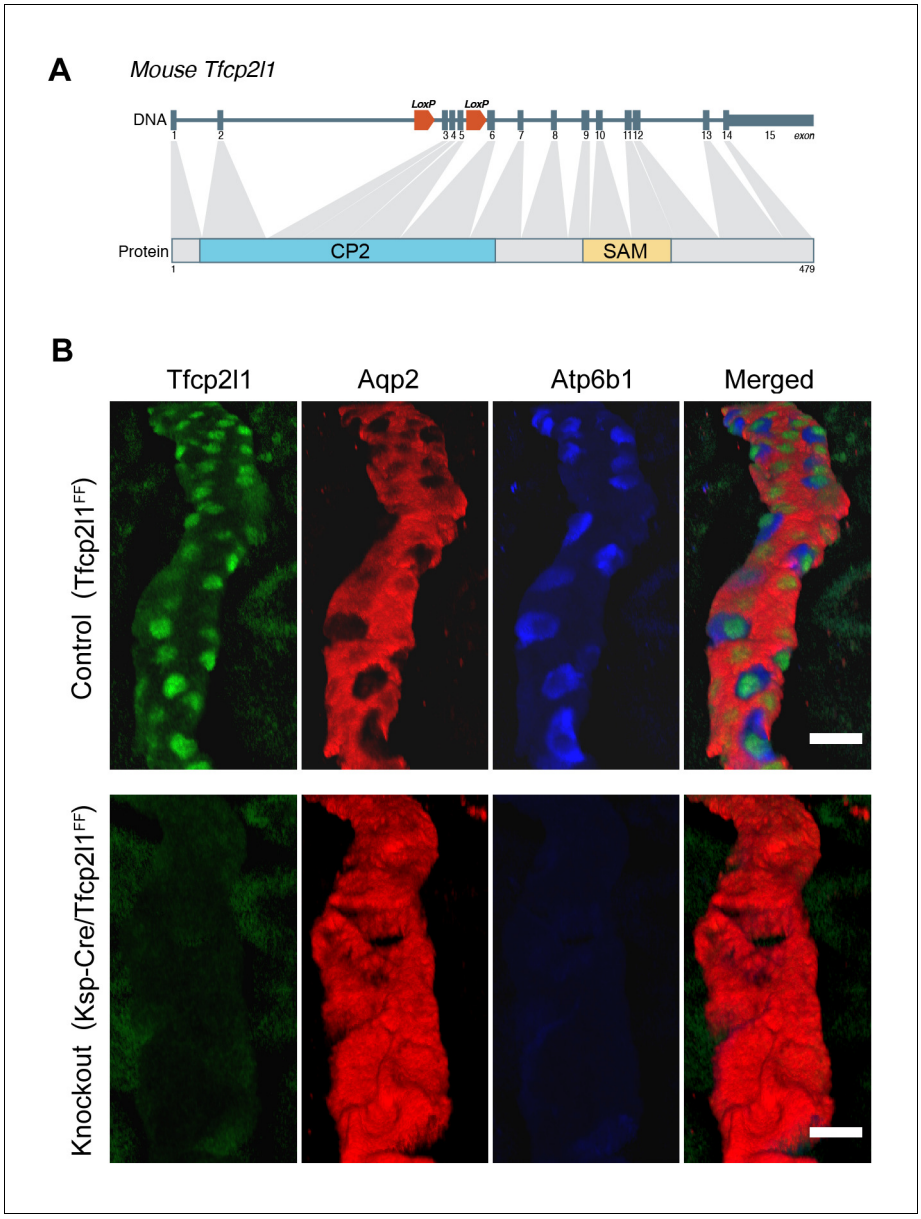

Figure 3. *Tfcp2l1* is necessary for the development of ICs. (**A**) Structure of mouse *Tfcp2l1* gene showing LoxP sites flanking the DNA-Binding CP2 domain (exon 3 and 4). (**B**) Control (*Tfcp2l1*$^{f/f}$) and *Tfcp2l1* knockout kidneys (*Cdh16-Cre;Tfcp2l1*$^{f/f}$) were analyzed for PC (*Aqp2* red) and IC (*Atp6b1* blue) proteins. Note that the deletion of *Tfcp2l1* replaced the normal patterning of IC and PC cells with a monotonous array of PC like cells (*Aqp2*$^{+}$). Z-stack reconstruction. Bars = 10 μm.

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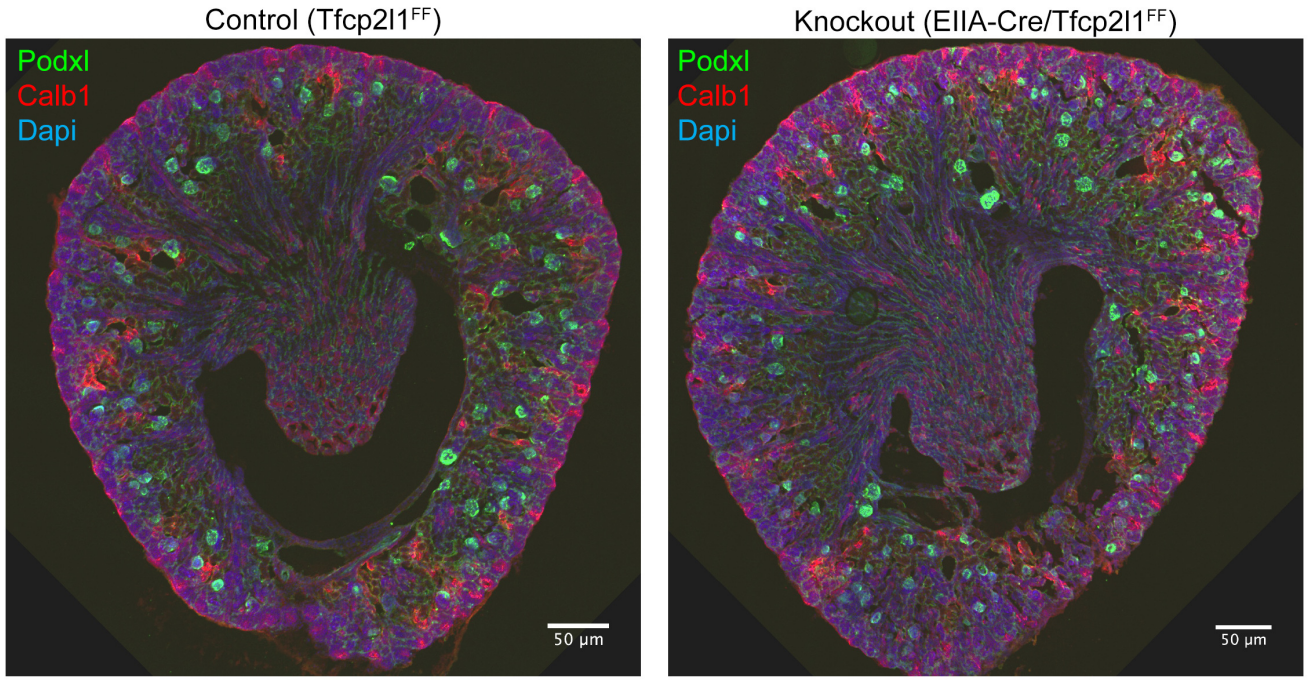

Figure 3—figure supplement 1. Gross kidney morphology was preserved after the global deletion of *Tfcp2l1* (*EIIA-Cre;Tfcp2l1*$^{f/f}$). Bars = 50 µM.
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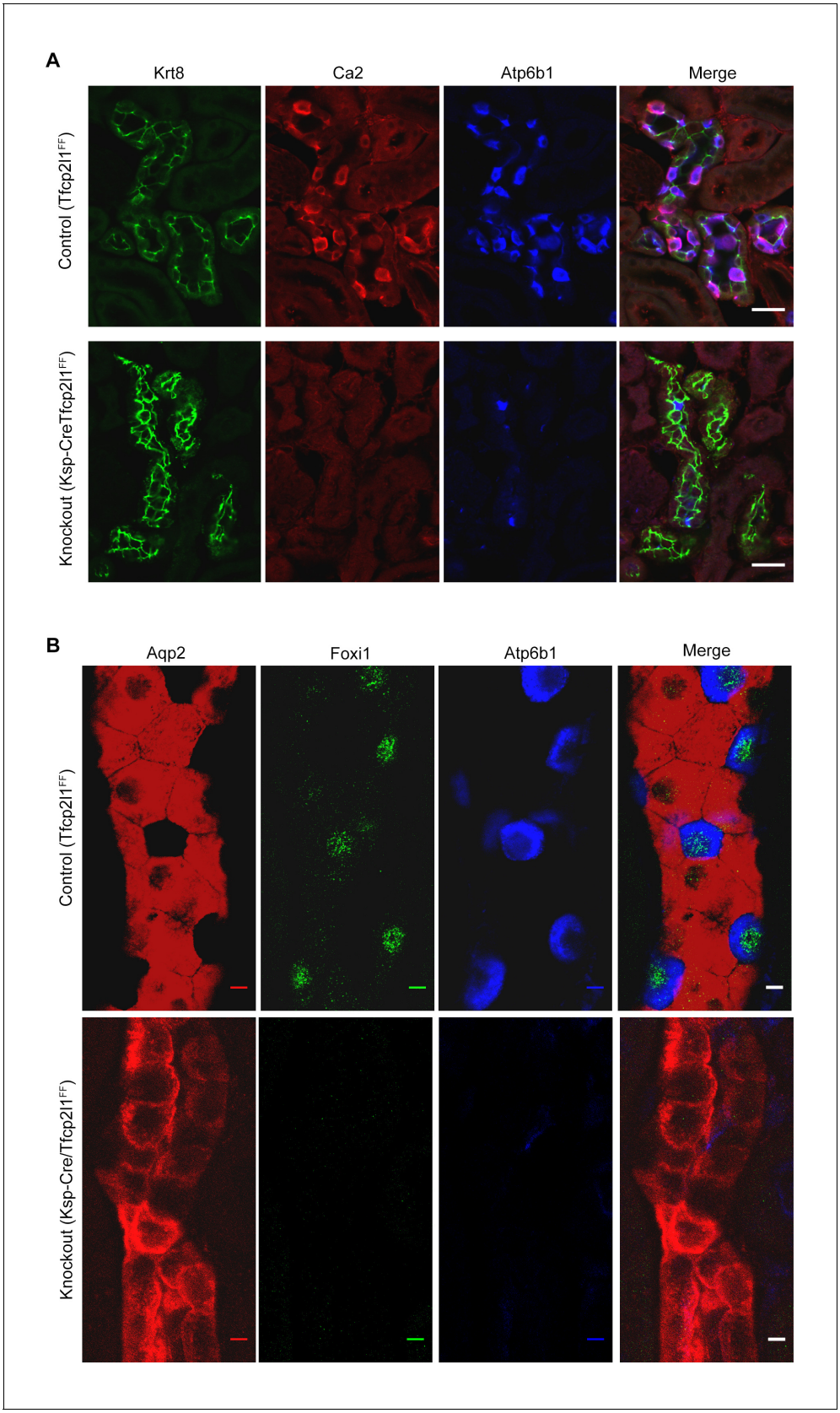

Figure 3—figure supplement 2. *Tfcp2l1* is necessary for the development of ICs. (**A,B**) The deletion of *Tfcp2l1* (*Cdh16-Cre;Tfcp2l1*$^{f/f}$) abolished the alternating pattern of IC and PC cells and deleted multiple IC specific proteins. *Tfcp2l1* knockout deleted the cellular enzymes *Ca2* (red), *Atp6b1* (blue),

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Figure 3—figure supplement 2 continued

and the transcription factor *Foxi1* (green), which are typically expressed by ICs. Conversely, *Tfcp2l1* knockout kidneys demonstrated prominent and uniform expression of PC markers *Aqp2* (red) and *Krt8* (green). (**A**) Bars = 10 μm; (**B**) Bars = 5 μm.

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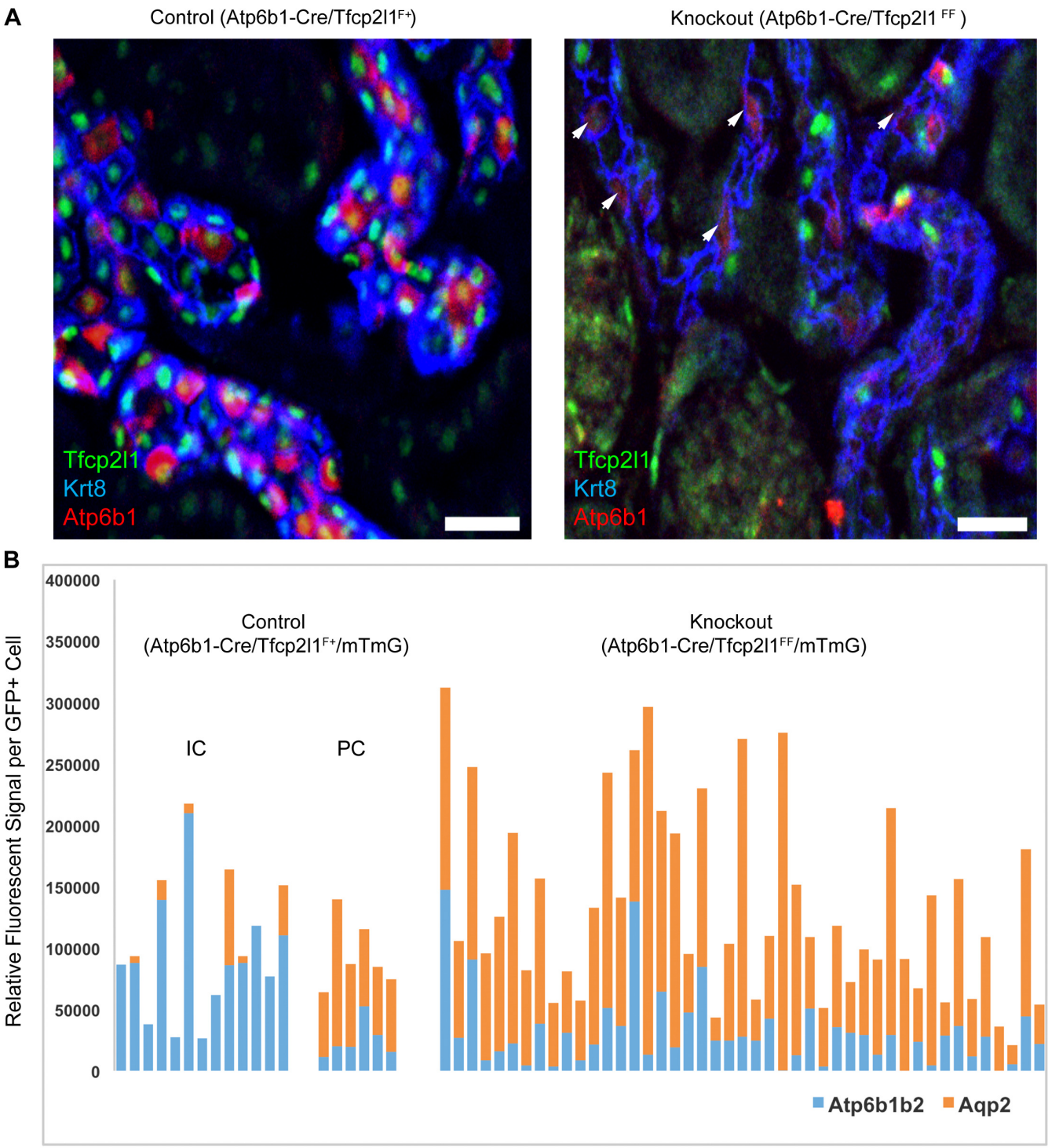

Figure 4. IC specific deletion of *Tfcp2l1* (green) by *Atp6b1*Cre results in loss of IC cells. (**A**) Deletion of *Tfcp2l1* resulted in the widespread loss of IC and PC patterning. Only residual expression of *Atp6b1* (red) in *Krt8*$^{+}$ (blue) PCs was detected in cortical collecting ducts (white arrows; n = 3 independent mice; Bars = 25 μm). (**B**) Cell fate analysis of *Tfcp2l1* knockout IC cells using genetic reporter (*Atp6b1-Cre;mTmG*). We analyzed single *GFP*$^{+}$ cells in Control (*Atp6b1-Cre;Tfcp2l1*$^{f/+}$*;mTmG*) and in Knockout (*Atp6b1-Cre;Tfcp2l1*$^{f/f}$*;mTmG*) collecting ducts by spot imaging. In control

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kidneys, *GFP*$^{+}$ cells were ICs or PCs (e.g. *GFP*$^{+}$ ICs: *Atp6b1>Aqp2* and *GFP*$^{+}$ PCs: *Aqp2>Atp6b1*), or expressed both markers in variable ratios (*Atp6b1* ≈ *Aqp2*). In contrast, in knockout kidneys, the majority of *GFP*$^{+}$ cells appeared to be PC-like or double positives cell types (compare knockout with wild type profiles). (n = 20 *GFP*$^{+}$ Control and n = 45 *Tfcp2l1* deleted *GFP*$^{+}$ cells from representative images; n = 4 independent kidneys).
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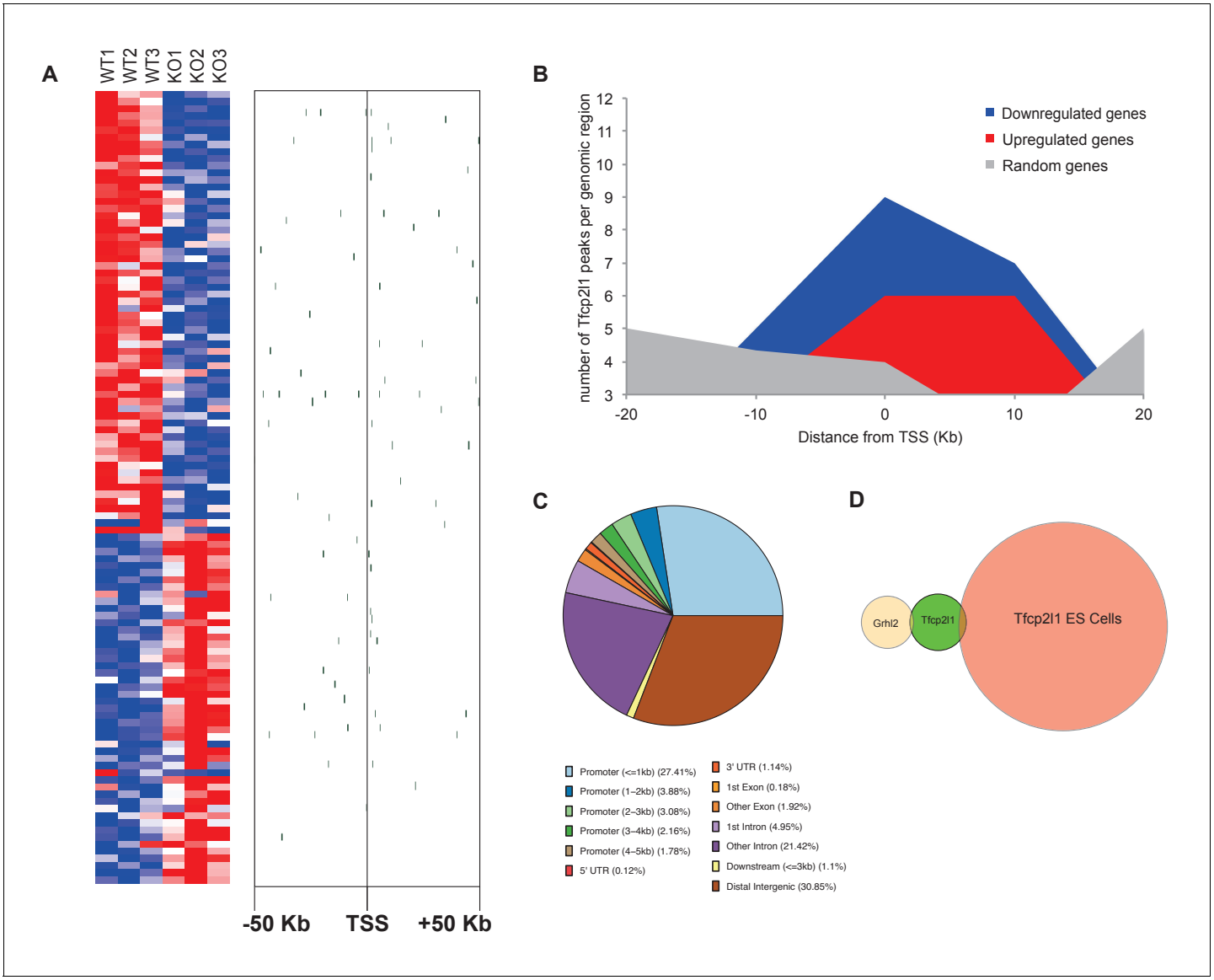

Figure 5. Identification of *Tfcp2l1* targets. (**A**) Identification of *Tfcp2l1* targets. Integration of knockout and *Tfcp2l1* ChIP-seq gene expression data obtained from P1 kidneys. Most of the genes significantly up or down regulated by *Tfcp2l1* (WT-*Tfcp2l1*$^{f/f}$ vs KO-EIIACre;*Tfcp2l1*$^{f/f}$) demonstrated binding peak(s) mapping between +50 KB to −50 KB relative to the TSS for each gene. (**B**) *Tfcp2l1* peaks of both up and down regulated genes were enriched at the TSS in comparison with a random set of *Tfcp2l1* independent genes. (**C**) Genome wide annotation of *Tfcp2l1* peaks revealed that ~27% of peaks were within 1 kb of the TSS and 38% located within 10 kb from TSS. (**D**) Cell type specificity of *Tfcp2l1* ChIP peaks. Comparison of *Tfcp2l1* ChIP peaks in different models. P1 kidney (our study, Green) is compared with *Tfcp2l1* peaks identified in ES Cells (***Chen et al., 2008***, Red) and with *Grhl2* peaks identified in E18 kidney (***Werth et al., 2010***), Tan color). Note the limited overlap between these datasets.

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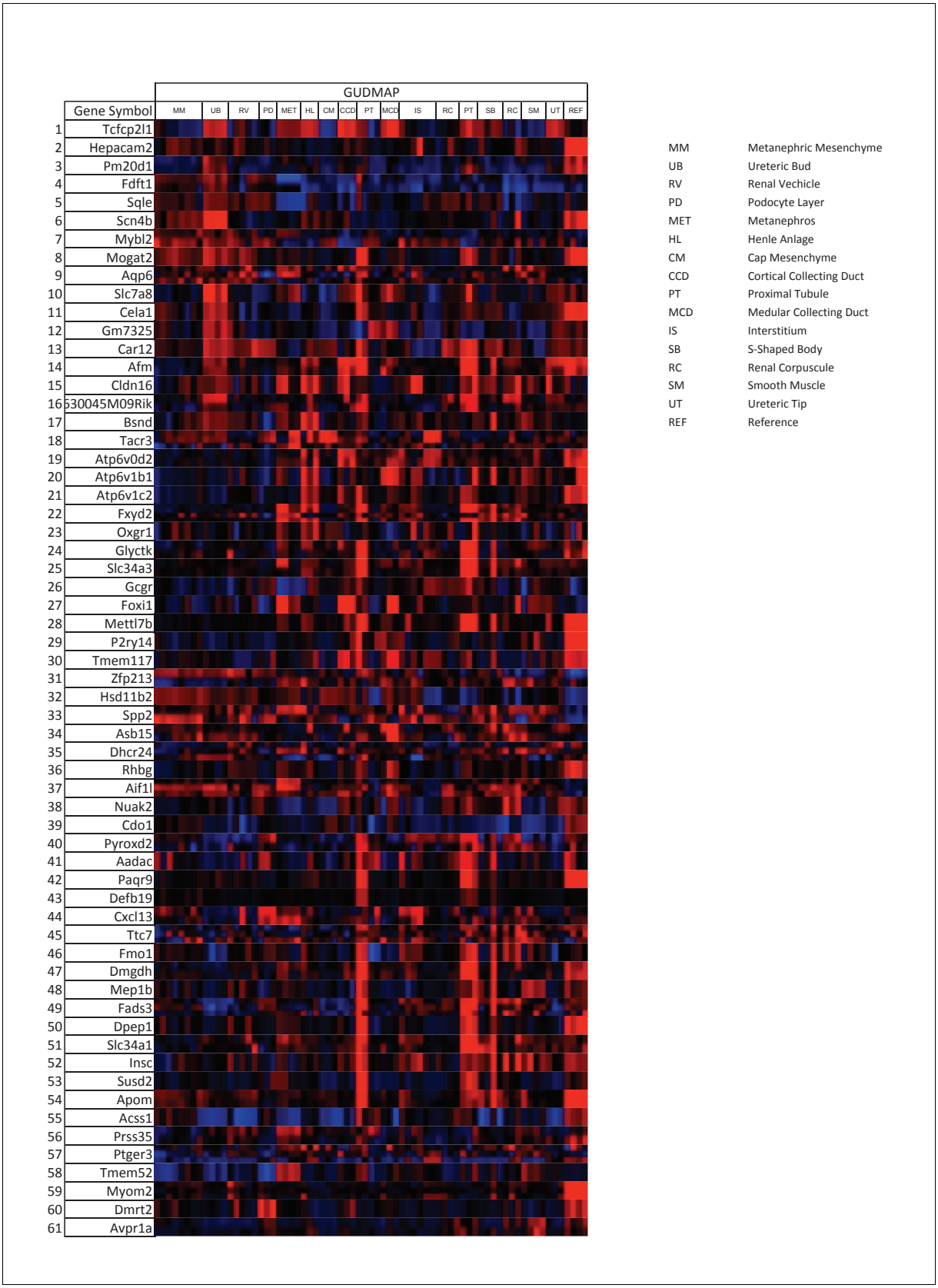

Figure 5—figure supplement 1. *Tfcp2l1* dependent genes localized to the collecting duct. Genes that were significantly downregulated by the knockout of *Tfcp2l1* (*EIIA-Cre;Tfcp2l1*$^{f/f}$;>1.25 fold downregulation; p<0.05; n = 3 independent knockout and wild type mice) were localized with the

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help of GUDMAP (http://www.gudmap.org/). Most of these genes appeared to be highly expressed by the Ureteric Bud (UB), the Loop of Henle (HL), the cortical and medullary collecting ducts (CCD and MCD, respectively), and to a lesser extent by the proximal tubule, parallel with the expression pattern of endogenous *Tfcp2l1* (top row). Red color indicates expression; Blue color indicates no expression.

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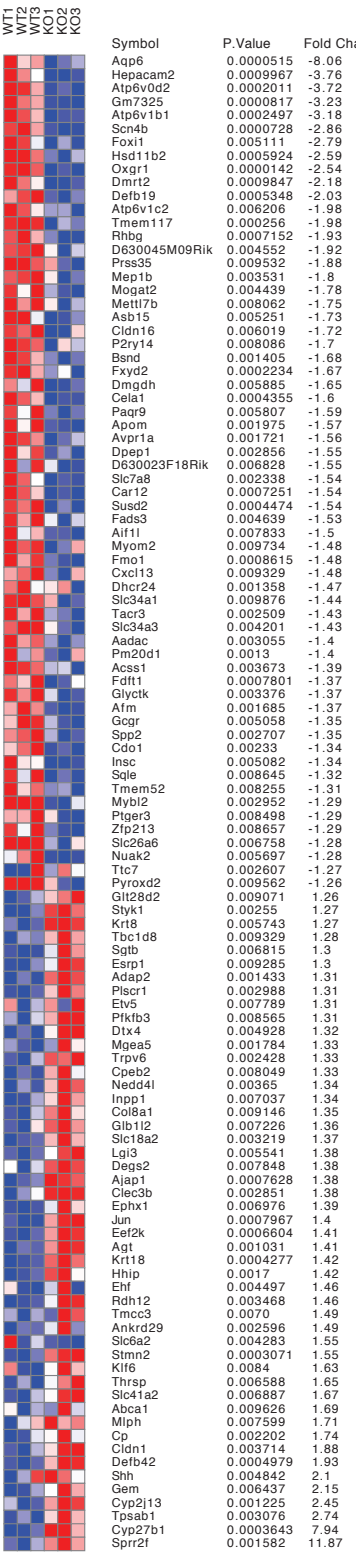

Symbol	P.Value	Fold Change
Aqp6	0.0000515	-8.06
Hepacam2	0.0009967	-3.76
Atp6v0d2	0.0002011	-3.72
Gm7325	0.0000817	-3.23
Atp6v1b1	0.0002497	-3.18
Scn4b	0.0000728	-2.86
Foxi1	0.005111	-2.79
Hsd11b2	0.0005924	-2.59
Oxgr1	0.0000142	-2.54
Dmrt2	0.0009847	-2.18
Defb19	0.0005348	-2.03
Atp6v1c2	0.006206	-1.98
Tmem117	0.000256	-1.98
Rhbg	0.0007152	-1.93
D630045M09Rik	0.004552	-1.92
Prss35	0.009532	-1.88
Mep1b	0.003531	-1.8
Mogat2	0.004439	-1.78
Mettl7b	0.008062	-1.75
Asb15	0.005251	-1.73
Cldn16	0.006019	-1.72
P2ry14	0.008086	-1.7
Bsnd	0.001405	-1.68
Fxyd2	0.0002234	-1.67
Dmgdh	0.005885	-1.65
Cela1	0.0004355	-1.6
Paqr9	0.005807	-1.59
Apom	0.001975	-1.57
Avpr1a	0.001721	-1.56
Dpep1	0.002856	-1.55
D630023F18Rik	0.006828	-1.55
Slc7a8	0.002338	-1.54
Car12	0.0007251	-1.54
Susd2	0.0004474	-1.54
Fads3	0.004639	-1.53
Aif1l	0.007833	-1.5
Myom2	0.009734	-1.48
Fmo1	0.0008615	-1.48
Cxcl13	0.009329	-1.48
Dhcr24	0.001358	-1.47
Slc34a1	0.009876	-1.44
Taor3	0.002509	-1.43
Slc34a3	0.004201	-1.43
Aadac	0.003055	-1.4
Pm20d1	0.0013	-1.4
Acss1	0.003673	-1.39
Fdft1	0.0007801	-1.37
Glyctk	0.003376	-1.37
Afm	0.001685	-1.37
Gcgr	0.005058	-1.35
Spp2	0.002707	-1.35
Cdo1	0.00233	-1.34
Insc	0.005082	-1.34
Sqle	0.008645	-1.32
Tmem52	0.008255	-1.31
Mybl2	0.002952	-1.29
Ptger3	0.008498	-1.29
Zfp213	0.008657	-1.29
Slc26a6	0.006758	-1.28
Nuak2	0.005697	-1.28
Ttc7	0.002607	-1.27
Pyroxd2	0.009562	-1.26
Glt28d2	0.009071	1.26
Styk1	0.00255	1.27
Krt8	0.005743	1.27
Tbc1d8	0.009329	1.28
Sgtb	0.006815	1.3
Esrp1	0.009285	1.3
Adap2	0.001433	1.31
Plscr1	0.002988	1.31
Etv5	0.007789	1.31
Pfkfb3	0.008565	1.31
Dtx4	0.004928	1.32
Mgea5	0.001784	1.33
Trpv6	0.002428	1.33
Cpeb2	0.008049	1.33
Nedd4l	0.00365	1.34
Inpp1	0.007037	1.34
Col8a1	0.009146	1.35
Glb1l2	0.007226	1.36
Slc18a2	0.003219	1.37
Lgi3	0.005541	1.38
Degs2	0.007848	1.38
Ajap1	0.0007628	1.38
Clec3b	0.002851	1.38
Ephx1	0.006976	1.39
Jun	0.0007967	1.4
Eef2k	0.0006604	1.41
Agt	0.001031	1.41
Krt18	0.0004277	1.42
Hhip	0.0017	1.42
Ehf	0.004497	1.46
Rdh12	0.003468	1.46
Tmcc3	0.0070	1.49
Ankrd29	0.002596	1.49
Slc6a2	0.004283	1.55
Stmn2	0.0003071	1.55
Klf6	0.0084	1.63
Thrsp	0.006588	1.65
Slc41a2	0.006887	1.67
Abca1	0.009626	1.69
Mlph	0.007599	1.71
Cp	0.002202	1.74
Cldn1	0.003714	1.88
Defb42	0.0004979	1.93
Shh	0.004842	2.1
Gem	0.006437	2.15
Cyp2j13	0.001225	2.45
Tpsab1	0.003076	2.74
Cyp27b1	0.0003643	7.94
Sprr2f	0.001582	11.87

Figure 5—figure supplement 2. Differentially expressed genes from kidneys of *Tfcp2l1* knockouts (*EIIA-Cre; Tfcp2l1*$^{f/f}$;>1.25 fold up- or down-regulated; p<0.05; n = 3 independent knockout and wild type mice).

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Figure 5—figure supplement 2 continued

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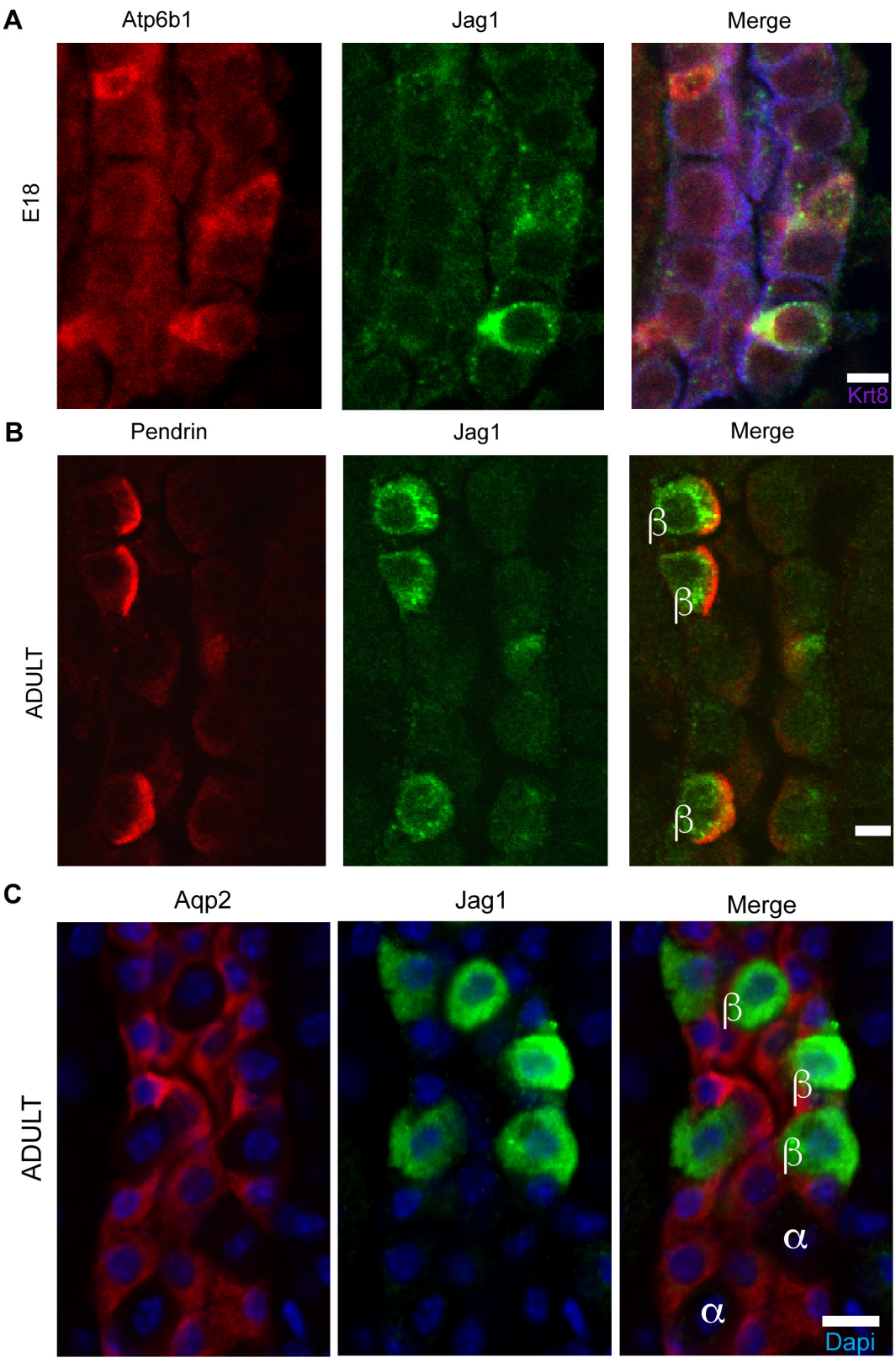
A
Atp6b1
Jag1
Merge
E18
Krt8
B
Pendrin
Jag1
Merge
ADULT
β
β
β
C
Aqp2
Jag1
Merge
ADULT
β
β
β
α
α
Dapi

Figure 6. *Jag1* is a novel early marker of developing (E18) and adult (P60) IC cells and activated Notch is found in PCs (**A**) *Jag1* (green) co-expressed with *Atp6b1* (red) at the first appearance of 'double positive' cells. *Krt8* (purple) is expressed by all cells at this stage. (**B**) In the adult kidney, *Jag1* (green) is specifically expressed in a subset of IC cells called Pendrin^{+} β-ICs (red) (P60), but **C** not in other collecting duct cell types including *Aqp2*$^{+}$ PC and *Aqp2*$^{-}$ α-ICs. Nuclei, blue (**A**,**B**) Bars = 5 μm. (**C**) Bar = 10 μm.

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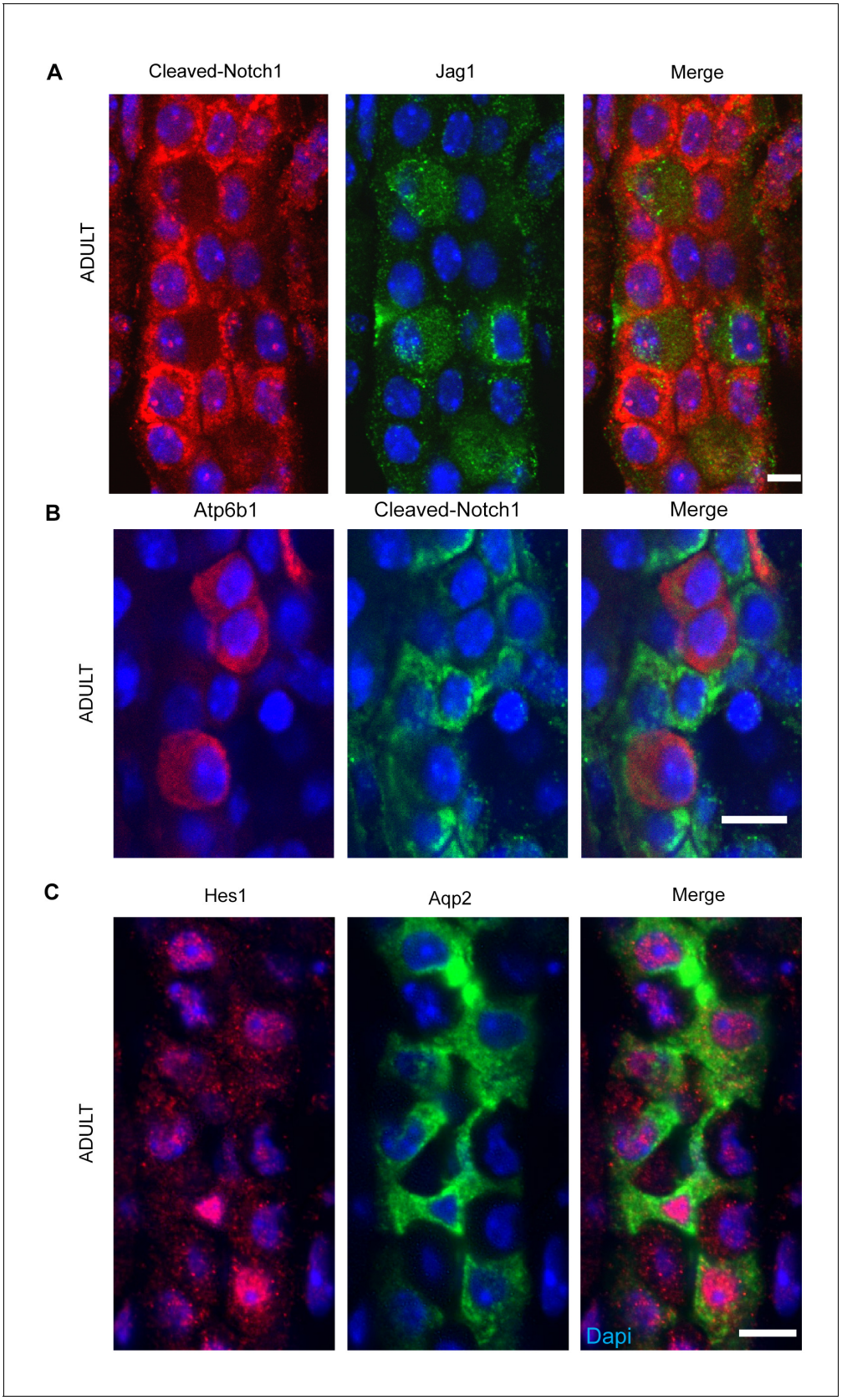

Figure 7. (**A**,**B**) *Jag1-Notch1* signaling in the collecting duct. Activated Notch is found in PC cells adjacent to *Jag1*$^{+}$*Atp6b1*$^{+}$*β*-ICs. (**C**) Consistently, Notch target gene *Hes1* (red) was detected in *Aqp2*$^{+}$ PC (green). **A, C** Bar = 10 µm, **B** Bar = 5 µm.

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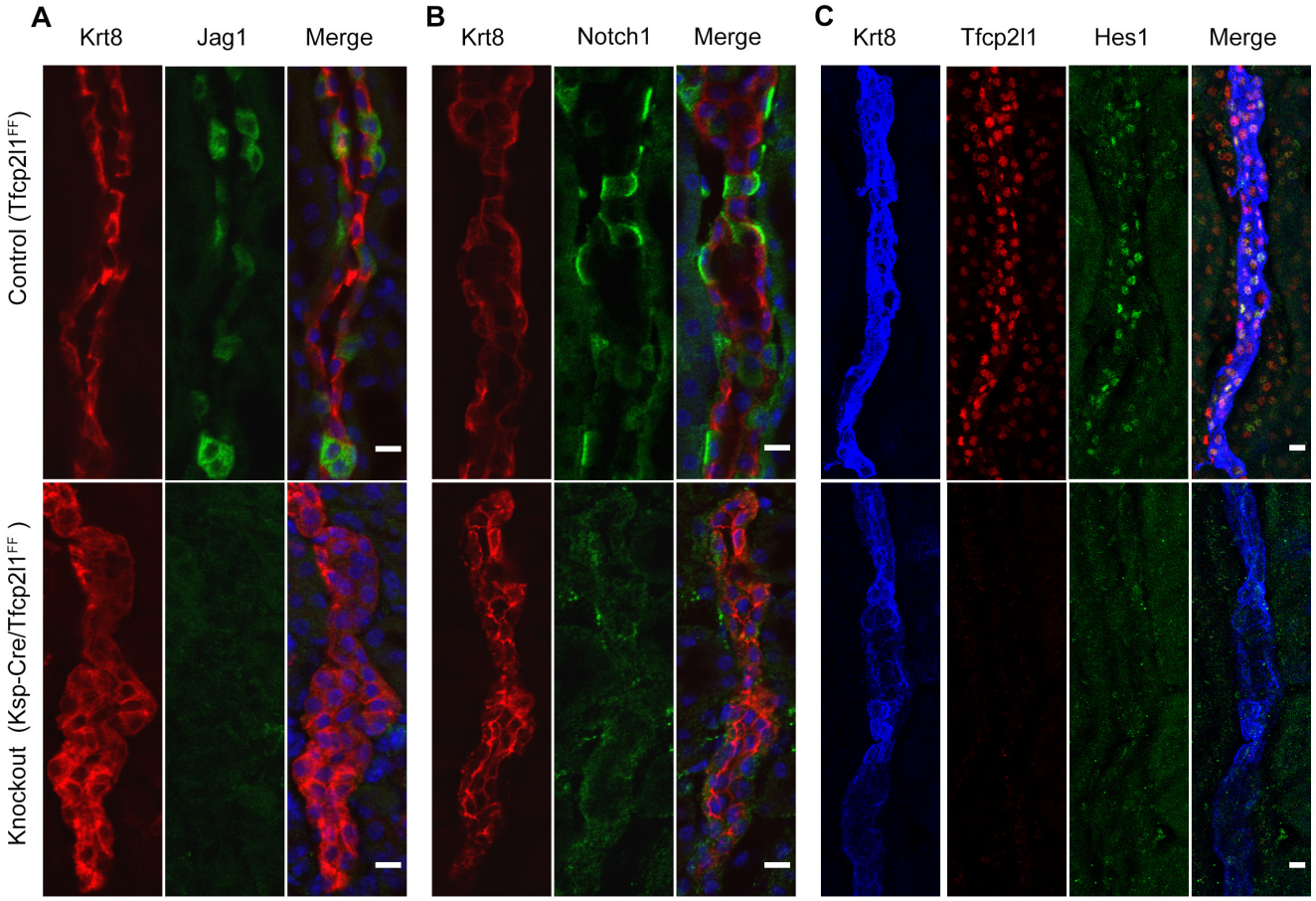

Figure 8. *Tfcp2l1* is required for Notch signaling in collecting ducts. *Tfcp2l1* knockout (*Cdh16-Cre;Tfcp2l1*$^{f/f}$) results in **A** depletion of *Jag1* (green) from *Krt8*$^{-}$ β-ICs (red) **B**. depletion of cell surface (inactive) *Notch1* (green) from *Krt8*$^{-}$ α-ICs (red) **C** depletion of nuclear Hes (green) from *Krt8*$^{+}$ PCs. (n = 4 independent mice for each immunodetection; Bars = 10 μm).

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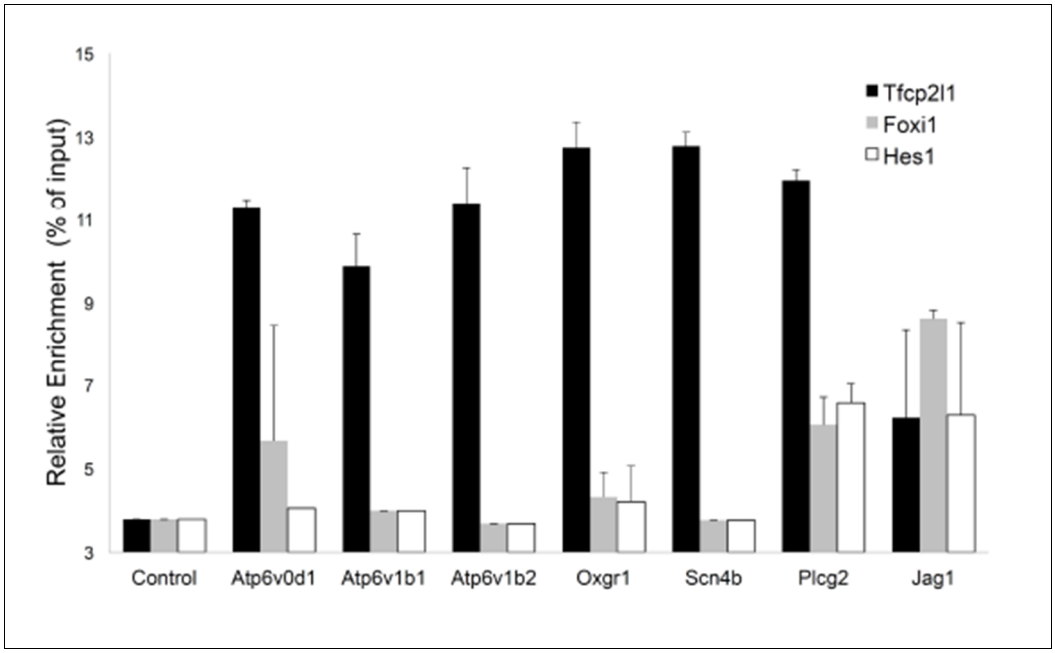

Figure 8—figure supplement 1. Sequential Chromatin IP. *Tfcp2l1* ChIP was followed by *Foxi1* or *Hes1* ChIP. Promoters of IC specific genes were analyzed in each ChIP by PCR. Three types of interactions were found in the selected promoters: *Tfcp2l1* alone, *Tfcp2l1+Foxi1* and *Tfcp2l1+Foxi1+Hes1* (n = 3 independent mice and ChIP).
DOI: 10.7554/eLife.24265.017

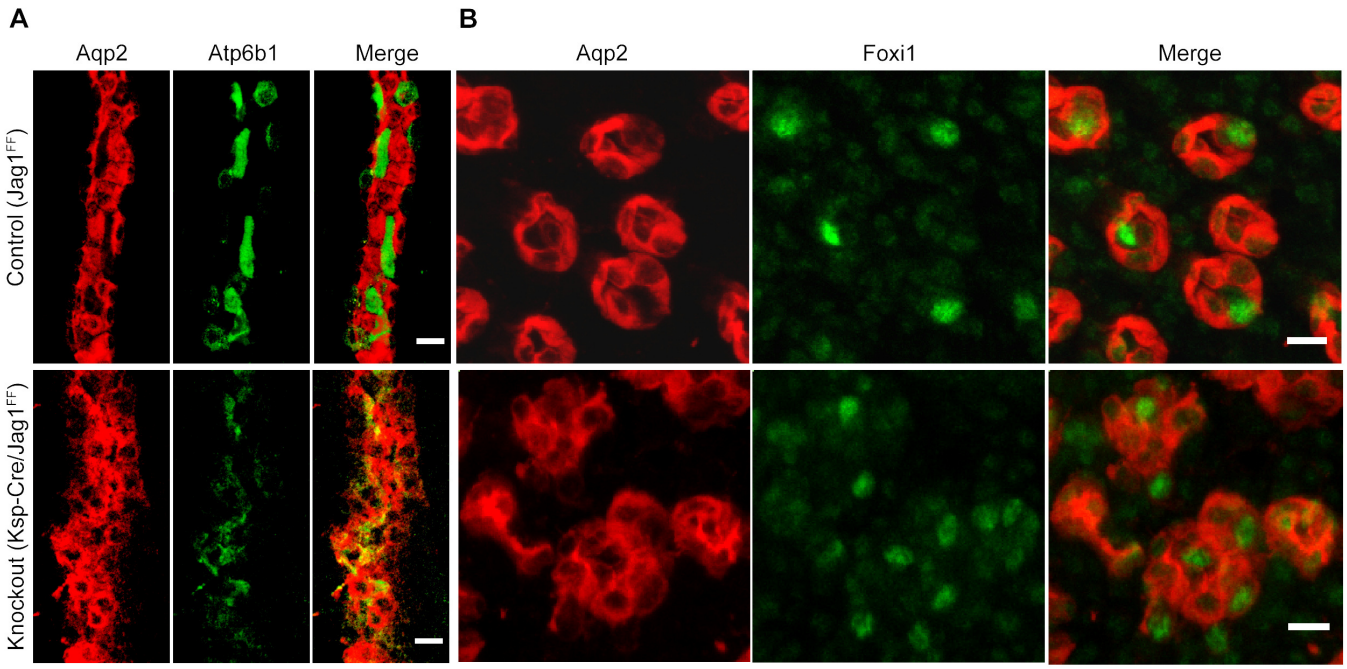

Figure 9. *Jag1* regulates the distribution of IC proteins in the collecting ducts. Knockout of *Jag1* (*Jag1*$^{f/f}$*;Cdh16-Cre*) resulted in diffuse expression of IC proteins **A** *Atp6b1* and **B** *Foxi1*. Expression of these proteins overlapped with *Aqp2* creating 'double positive' cells. (n = 4 independent mice; Bars = 10 μm).

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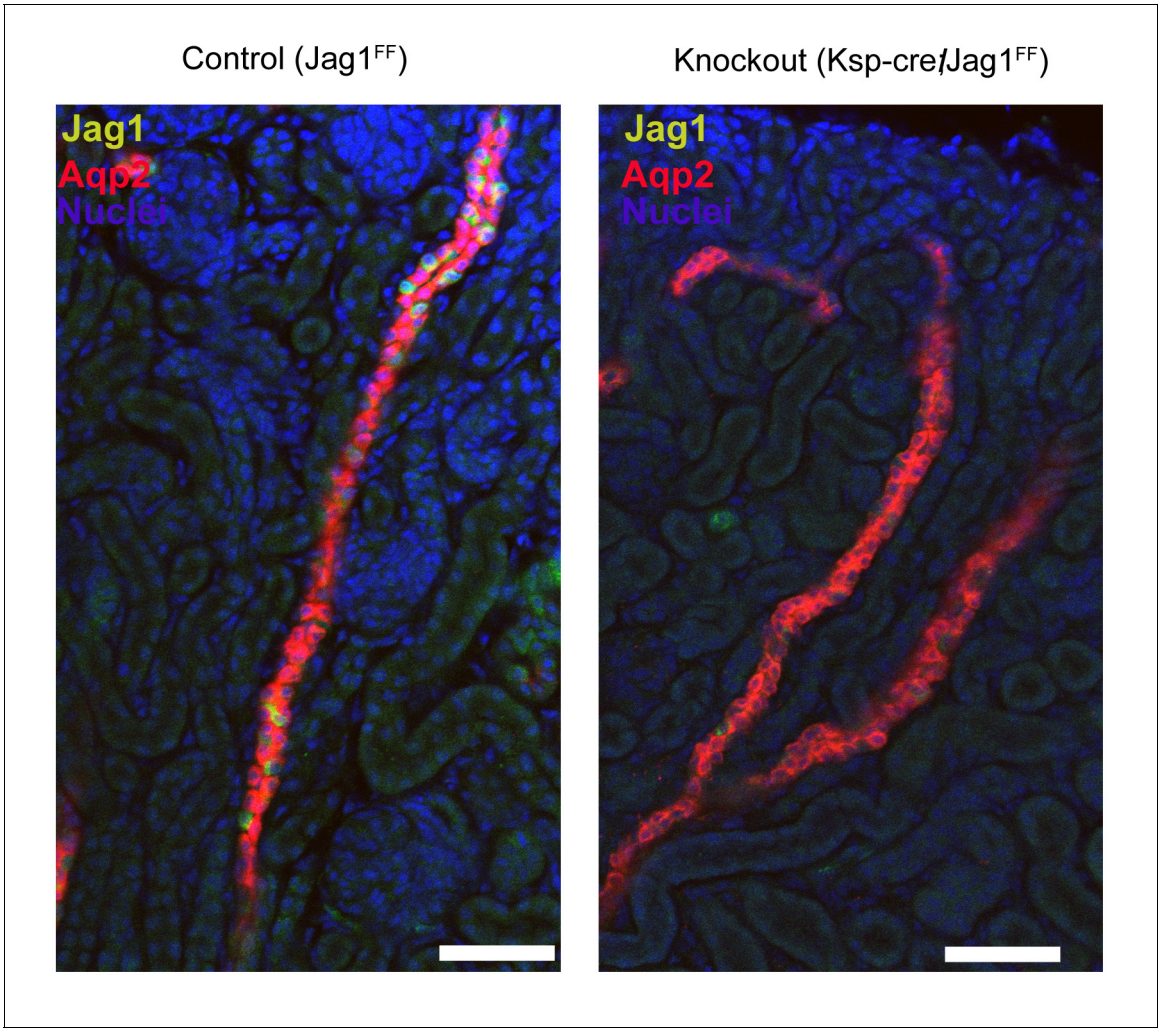

Figure 9—figure supplement 1. Deletion of *Jag1* by *Cdh16-Cre*. Note the loss of *Jag1* staining. Examined at P1. Bars = 50 μm.
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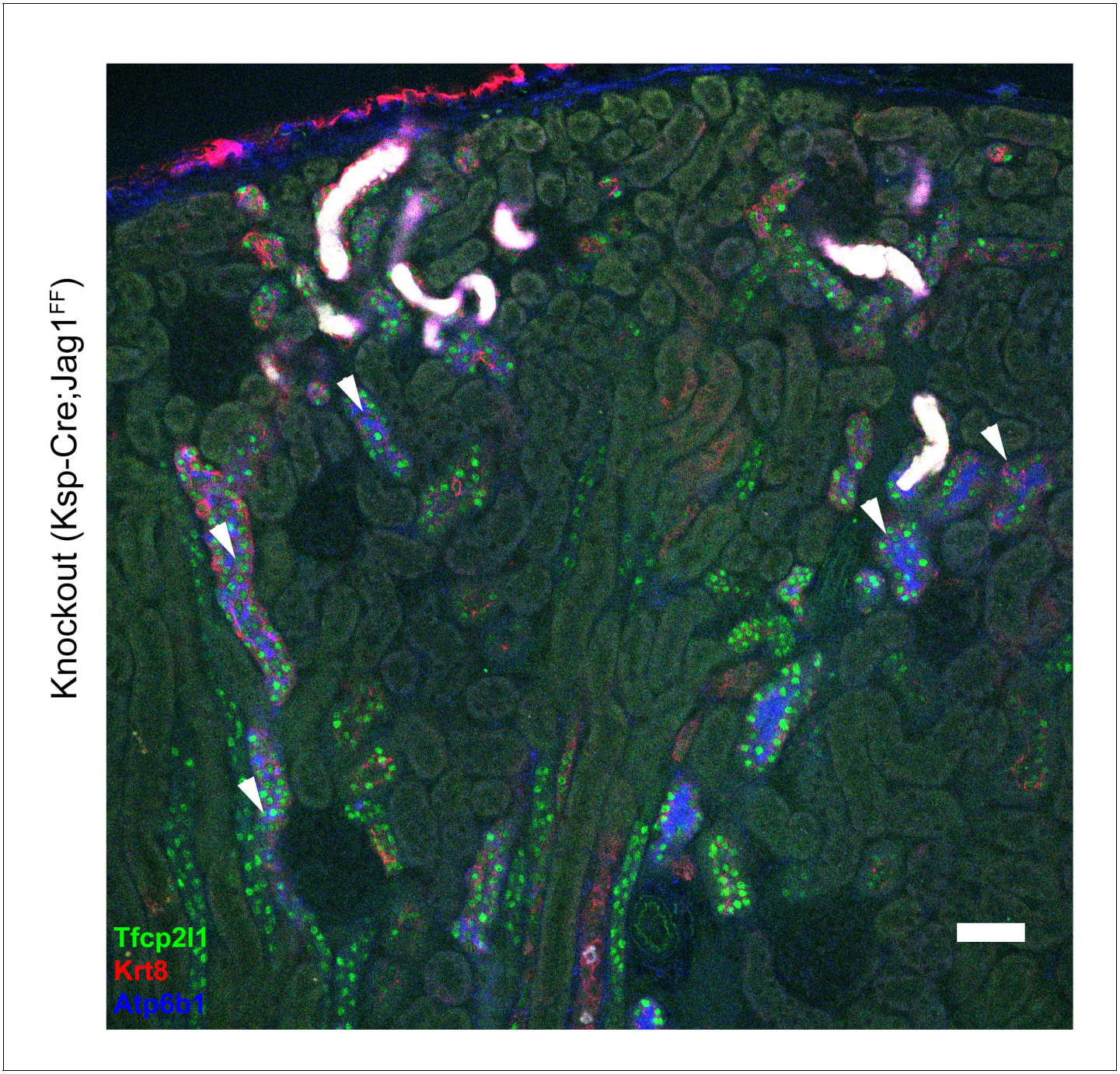

Figure 9—figure supplement 2. Overview of the gross morphology of *Jag1* knockout kidneys. Knockout of *Jag1* (*Cdh16-Cre;Jag1*$^{f/f}$) resulted in the uniform and overlapping expression pattern of *Atp6b1* (IC marker, blue) and *Krt8*$^{+}$ (PC marker, red) in cortical collecting ducts. However, the deletion of *Jag1* did not affect the expression of *Tfcp2l1*. 'White' color tubules result from luminal debris. Bar = 50 μm.
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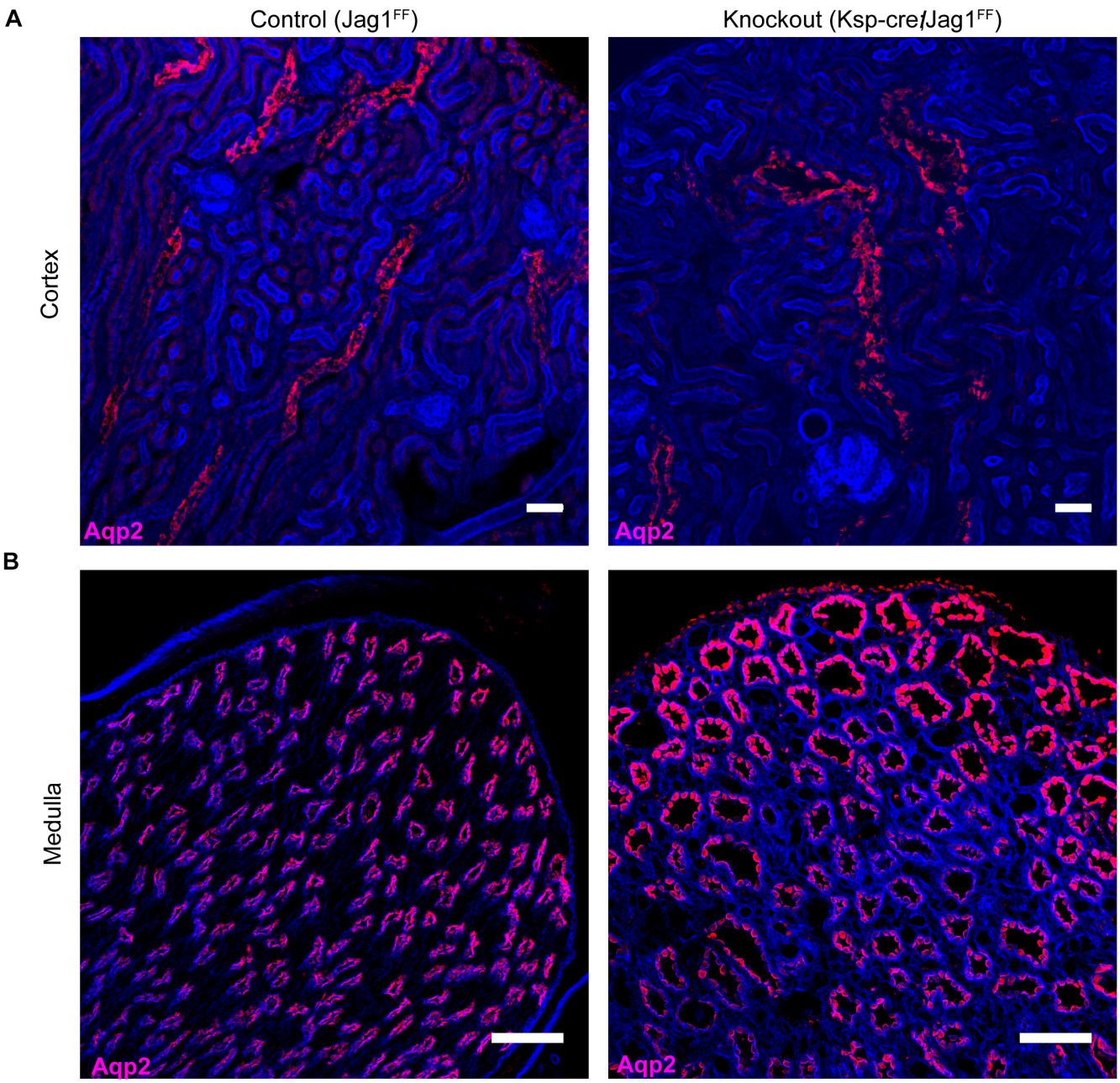

Figure 9—figure supplement 3. Jag1 is required for structural integrity of the collecting duct. (**A,B**) Deletion of *Jag1* (*Jag1*$^{f/f}$;*Cdh16-Cre*) resulted in dilation of *Aqp2*$^{+}$ (red) cortical and medullary collecting ducts. Phalloidin (blue) (n = 4 independent mice; Examined at P60. (**A**) Bars = 25 μm; (**B**) Bars = 50 μm).

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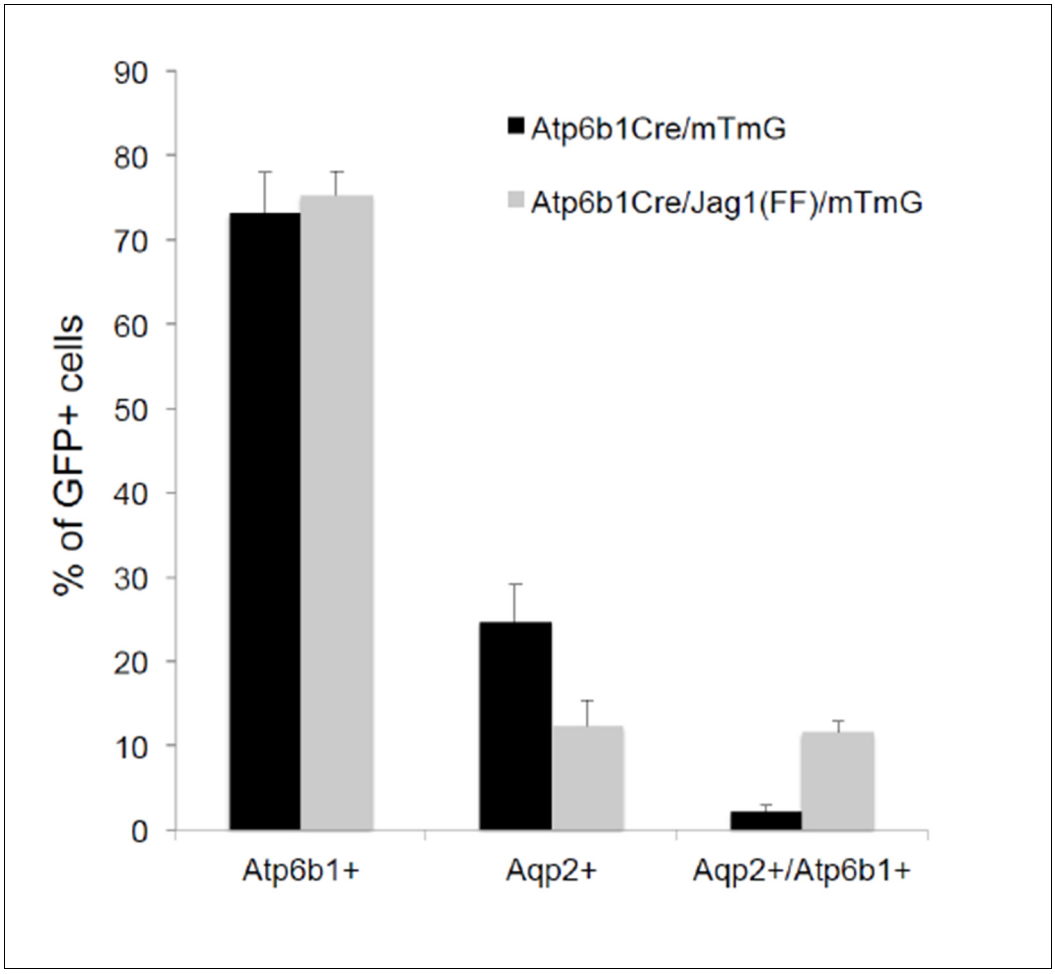

Figure 9—figure supplement 4. Deletion of *Jag1* in IC (*Jag1$^{f/f}$;Atp6b1-Cre;mTmG*) resulted in a six fold increase in 'double positive' cells (from 2% to 12%; n = 3 independent mice). Only *Atp6b1*Cre driven *GFP^{+}* cells were assayed.

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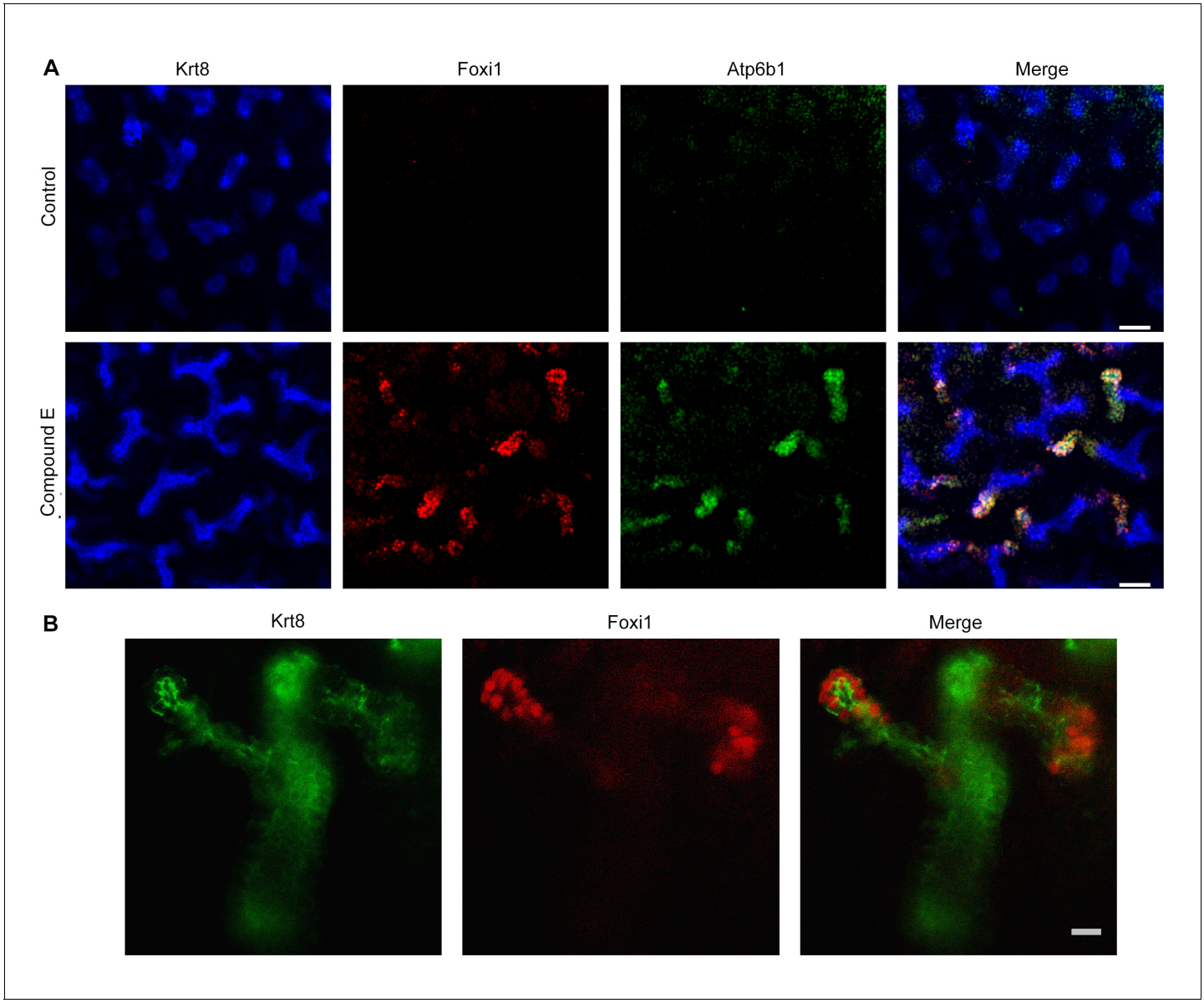

Figure 10. Manipulation of Notch signaling in vivo. (**A**) Inhibition of Notch signaling upregulates *Foxi1* and promotes IC cell differentiation. Acute inhibition of Notch signaling in E15 kidneys with Compound E (48 hr) resulted in the differentiation of IC cells at the tips of the UB/Collecting Ducts. Note that the IC cells demonstrated a 'double-positive' phenotype with the co-expression of PC (*Krt8*$^{+}$ blue) and IC (*Foxi1*$^{+}$ red, *Atp6b1*$^{+}$ green) proteins. (**B**) High power of Compound E treated kidneys (n = 6 independent mouse kidney cultures). (**A**) Bars = 50 μm and (**B**) Bar = 10 μm.
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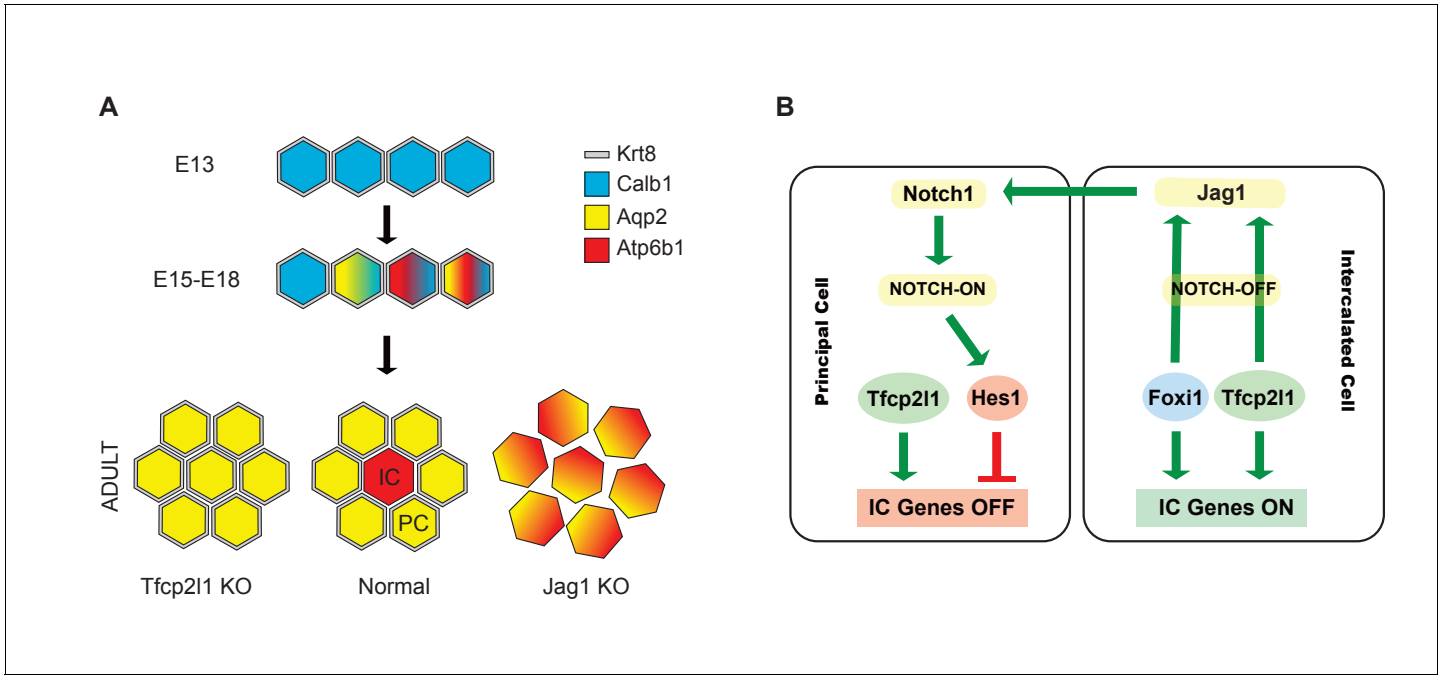

Figure 11. Models. (**A**) Development of cellular diversity in the collecting duct. Initially, we found a monotonous expression of PC proteins at E13, and then a transitional stage (E15–E18) characterized by the appearance of 'double-positive' cells. After birth, these cells achieved distinct identities and assumed rosette-like patterning. Deletion of *Tfcp2l1* resulted in a monotonic cell type expressing PC cell, but not IC cell markers. Inactivation of *Jag1* in contrast resulted in the loss of cell identity and patterning, increasing the number of 'double-positive' cells typified by E18 collecting ducts. (**B**) Proposed model of cell-autonomous and non-cell-autonomous actions of *Tfcp2l1*. *Tfcp2l1* induces the expression of IC genes, including *Jag1*. Expression of *Jag1* triggers Notch signaling in adjacent PC cells. *Jag1* signaling results in activation of *Hes1* (Notch-ON) in PCs and conversely *Foxi1* expression in ICs (Notch-OFF). We envision that the combination of *Tfcp2l1* with either *Foxi1* or *Hes1* drives cell identity. Maintenance of this circuit may depend on an excess of *Tfcp2l1* in ICs (***Figure 1***) as well as the expression of *Foxi1* which is known to induce *Jag1*. Conversely, *Jag1* suppression of *Foxi1* (***Figures 8*** and ***9***) demonstrates a negative feedback loop in neighboring PCs.

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