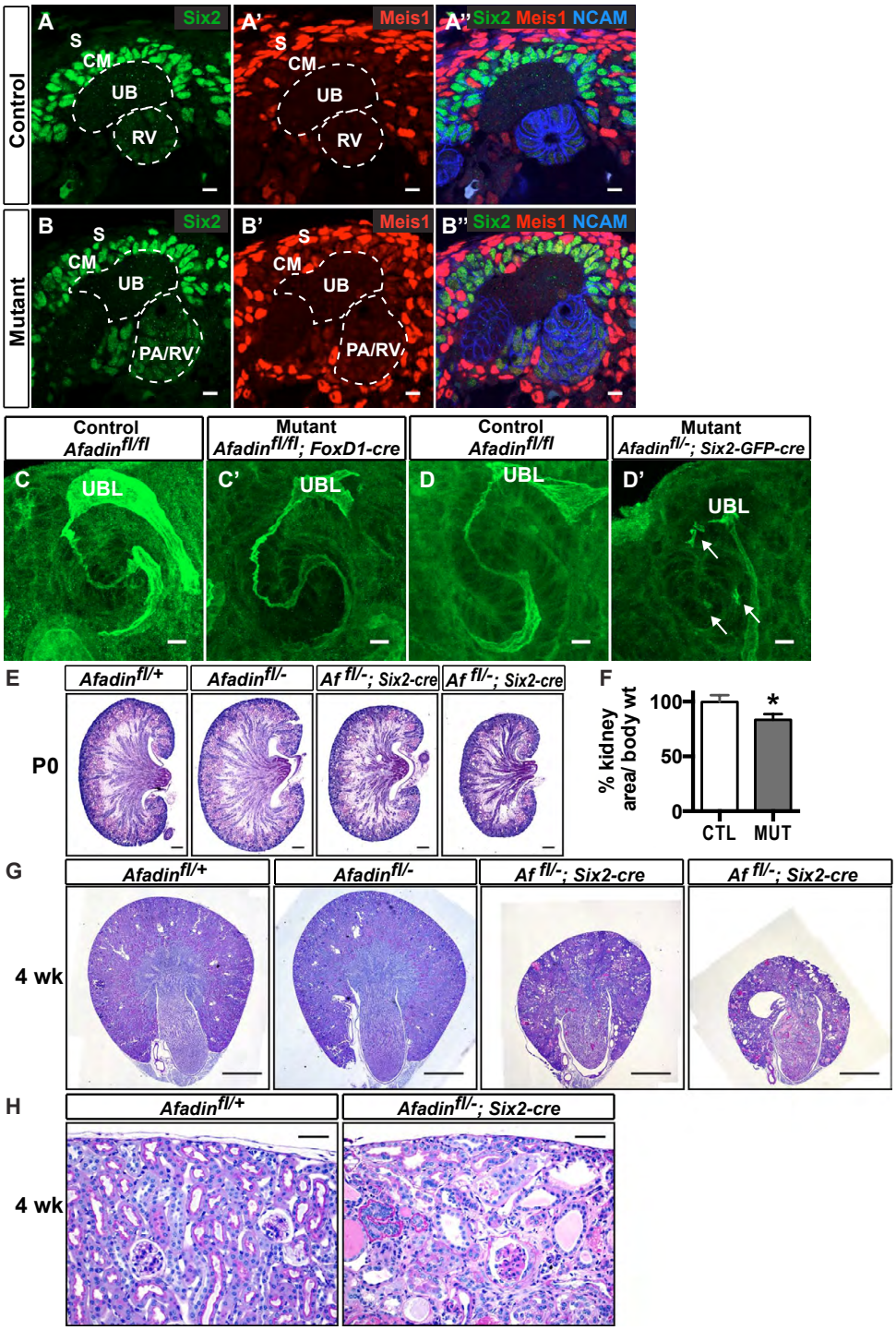

Fig. S1. Afadin is required for cell-autonomous lumen formation in nephrons. (**A-B″**) E14.5 kidneys from controls (*Afadin*$^{fl/fl}$) and mutants (*Afadin*$^{fl/fl}$; *Pax3-cre*) were immunostained with markers of nephron progenitors (Six2, green) and stroma (Meis1, red). NCAM (blue) marks the condensed mesenchyme (CM) and renal vesicle (RV). Stroma (S) surrounds CM. Six2 and Meis1 localize to the CM and stroma, respectively, in both control (A-A″) and mutant (B-B″). (**C-D′**) Three dimensional reconstructions of confocal *z*-stacks immunostained with antibody to aPKC, an apical marker that demarcates the lumen. Controls (*Afadin*$^{fl/fl}$; C,D) show continuous lumens in s-shaped bodies, as do mutants with conditional deletion of *Afadin* from the renal stroma (*Afadin*$^{fl/fl}$; *FoxD1-cre*, C′). Deletion of *Afadin* from the nephron progenitors (*Afadin*$^{fl/-}$; *Six2-GFP-cre*) shows multiple small, discontinuous lumens (D′, arrows indicate discrete lumens). This is similar to what was observed in *Afadin*$^{fl/fl}$; *Pax3-cre* mice (Fig 4L,M, compare with 4K). UBL denotes ureteric bud lumen. Results are representative of sections from two mice. (**E**) Hematoxylin and Eosin staining of P0 control (*Afadin*$^{fl/+}$), heterozygous (*Afadin*$^{fl/-}$), mutant (*Afadin*$^{fl/-}$; *Six2-GFP-cre*) kidneys. (**F**) Quantification of kidney size at P0 shows mutant (*Afadin*$^{fl/-}$; *Six2-GFP-cre*) has mildly reduced size (data are mean ± s.d. from normalized measurements of kidney area per body weight; kidney areas were calculated with ImageJ; n=6 *Afadin*$^{fl/+}$ and n=8 *Afadin*$^{fl/-}$; *Six2-GFP-cre*; *P<0.002, unpaired two-tailed Student's t-test). (**G**,**H**) Periodic acid Schiff-staining of 4-week-old control (*Afadin*$^{fl/+}$), heterozygous (*Afadin*$^{fl/-}$) and mutant (*Afadin*$^{fl/-}$; *Six2-GFP-cre*) kidneys. Mutants have evidence of dysplasia, glomerulosclerosis and tubule dilation compared with controls. Results are representative of four mutants and four controls. Scale bars: 10 μm in A-D′; 200 μm in E; 1 mm in G; 50 μm in H.

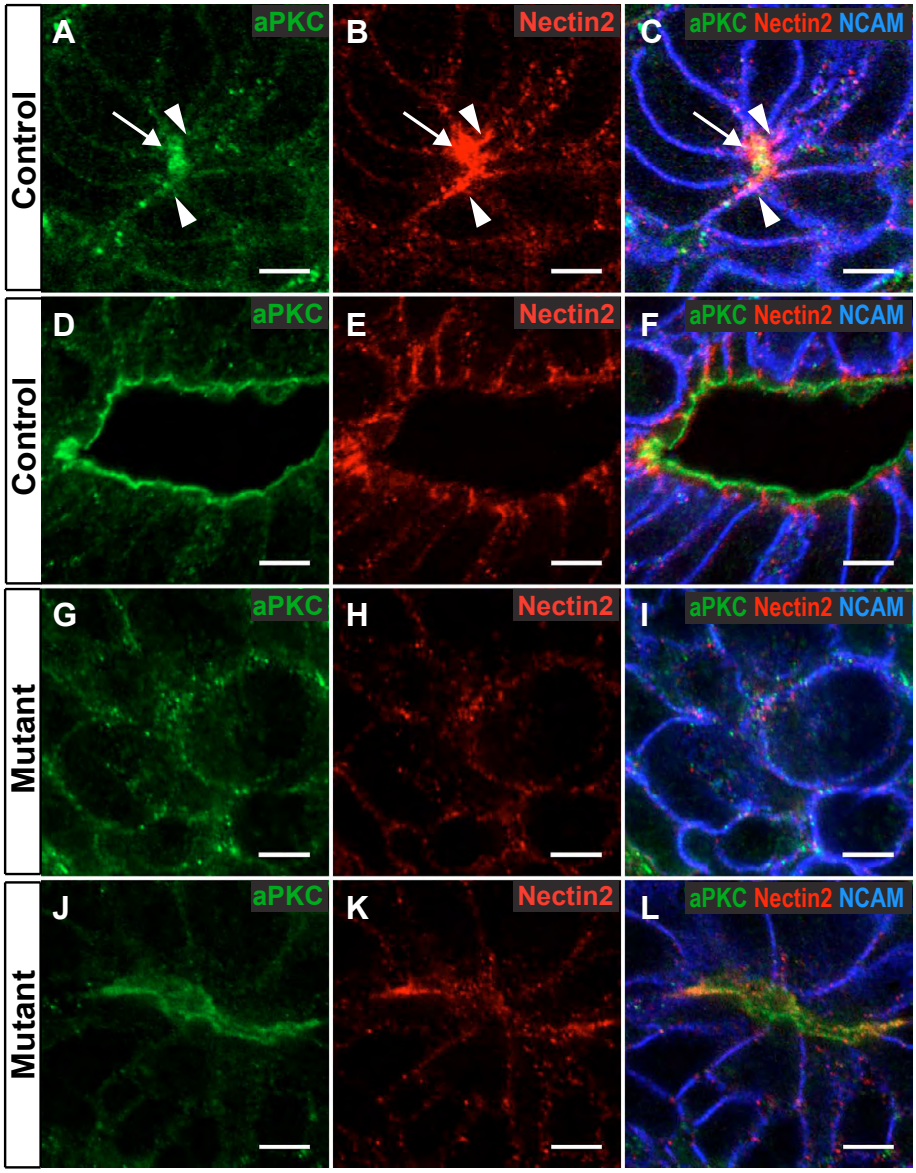

Fig. S2. Afadin is required for nectin segregation from aPKC during lumen formation. Immunolocalization of aPKC (green), nectin 2 (red) and NCAM (blue) during lumen initiation and expansion in control (*Afadin*$^{fl/fl}$, A-F) and mutant (*Afadin*$^{fl/fl}$; *Pax3-cre*, G-L) E14.5 kidneys. (**A-C**) Lumen initiation in a primitive RV shows apical accumulation of aPKC with colocalization of nectin 2 (arrow) and absence of apical NCAM. Some areas of nectin 2 basal to the apical surface are also noted (arrowheads). (**D-F**) During lumen expansion in the mature RV stage, aPKC is apical whereas nectin 2 is located basal to aPKC at apical intercellular junctions. NCAM is basal to nectin 2. (**G-I**) In mutants, evidence of lumen initiation is lacking in the PA/RV stage. aPKC is not recruited to an apical location that excludes NCAM and is not colocalized with nectin-2. (**J-L**) In the SB stage of mutants, small lumens elongate rather than widen significantly. Some aPKC and nectin 2 are recruited and colocalize at these lumens, which lack apical NCAM, but segregation of aPKC and nectin 2 does not occur (L). Scale bars: 5 μm.

Table S1. Nephron stages defined

Nephron precursor shape/name	Abbreviation	Lumen stage by IF	Molecular signature by polarity proteins*	Continuous nephron-UB tubule‡
Pretubular aggregate	PA	No lumen	Par-3-containing membrane microdomains lacking NCAM +/– F-actin recruitment	No
Primitive renal vesicle	PRV	Lumen initiation, microlumen	Small focus/foci of apical Par-3, aPKC, and F-actin Basolateral NCAM	No
Mature RV	MRV	Expanded, open ovoid lumen	Apical aPKC and F-actin Apical junctional Par-3 Basolateral NCAM	No
Extended RV (teardrop-shaped body)	ERV	Lumen extends toward UB, discontinuous with UB lumen	Apical aPKC and F-actin Apical junctional Par-3 Basolateral NCAM	Yes
Early S-shaped body	Early SB	Lumen is continuous, connects with UB, proximal region begins to fan out	Apical aPKC and F-actin Apical junctional Par-3 Basolateral NCAM	Yes
Late S-shaped body	Late SB	Continuous with UB, mid-distal region elongates, proximal region fans out in a cup shape	Apical aPKC and F-actin Apical junctional Par-3 Basolateral NCAM	Yes

*Apical F-actin refers to the cortical ring of F-actin located basal to aPKC.

‡Continuous nephron-UB tubule is defined by the absence of intervening laminin between nephron and UB.