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Supplemental information

The Wnt/PCP formin Daam1 drives cell-cell adhesion during nephron development

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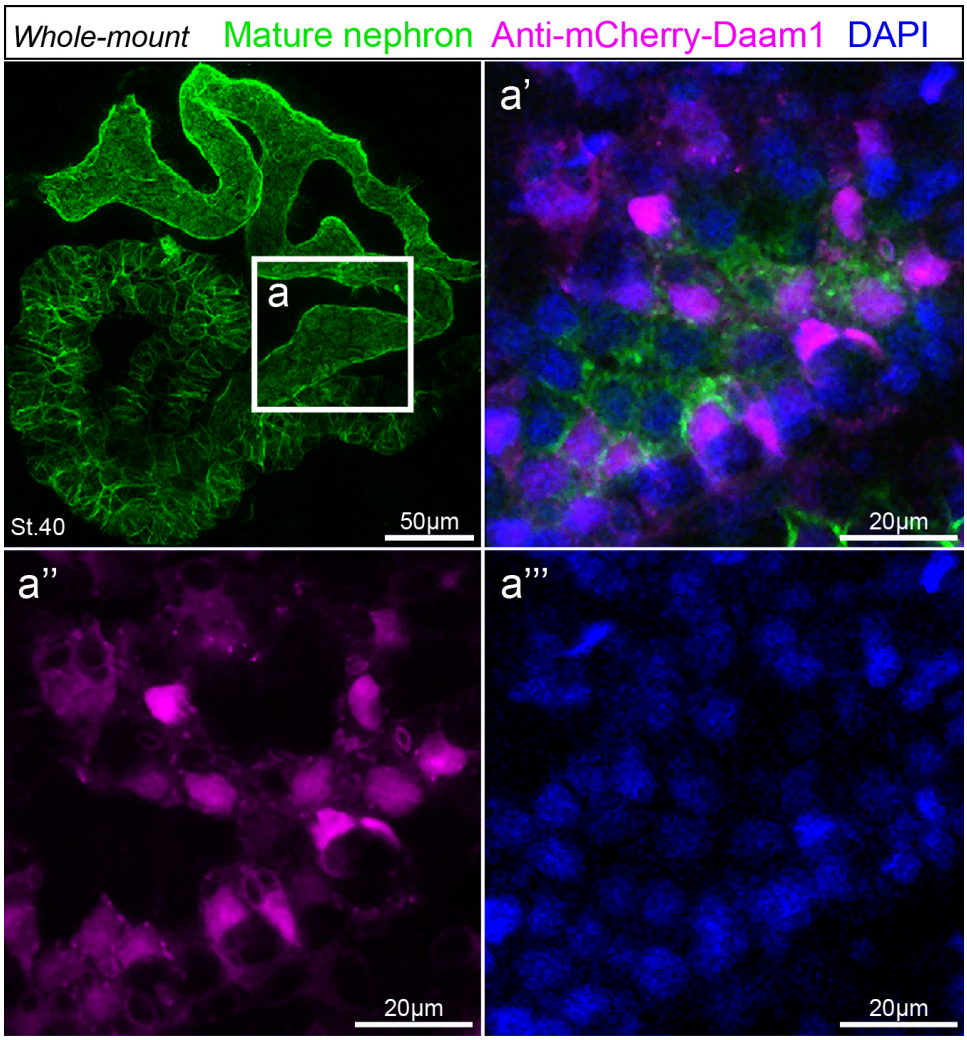


Figure S1. Daam1 is absent from cell-cell junctions in the fully developed embryonic nephron, Related to Figures 1 and 2.
Maximum confocal projections of the mature *Xenopus* embryonic nephron labeled with 3G8 and 4A6 antibodies (green), RFP antibody to visualize Daam1(magenta), and DAPI staining labeling nuclei (blue); scale bar equals to 50 microns. a'-a''' close-up images of the white box; scale bars equal to 20 microns.

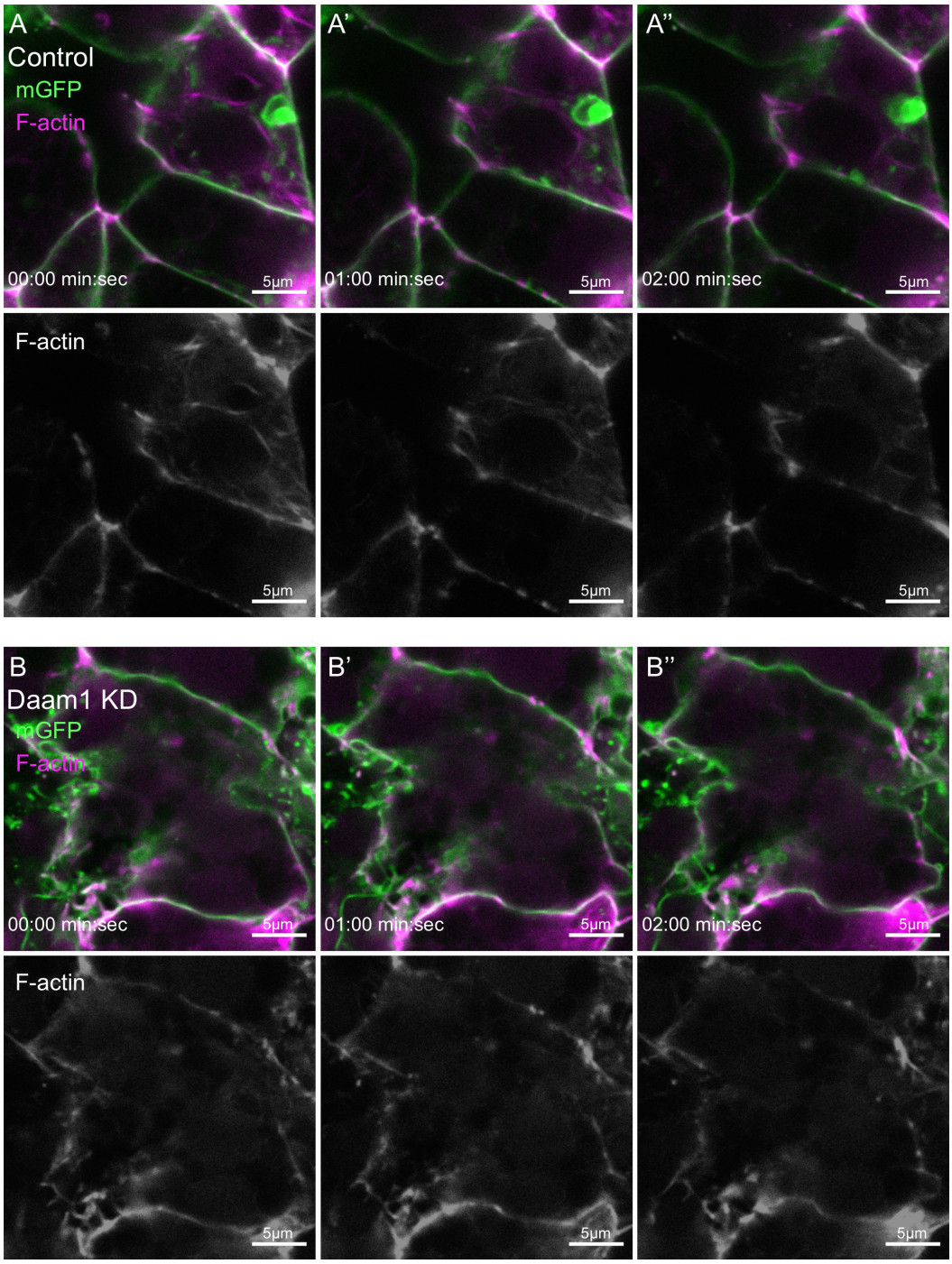
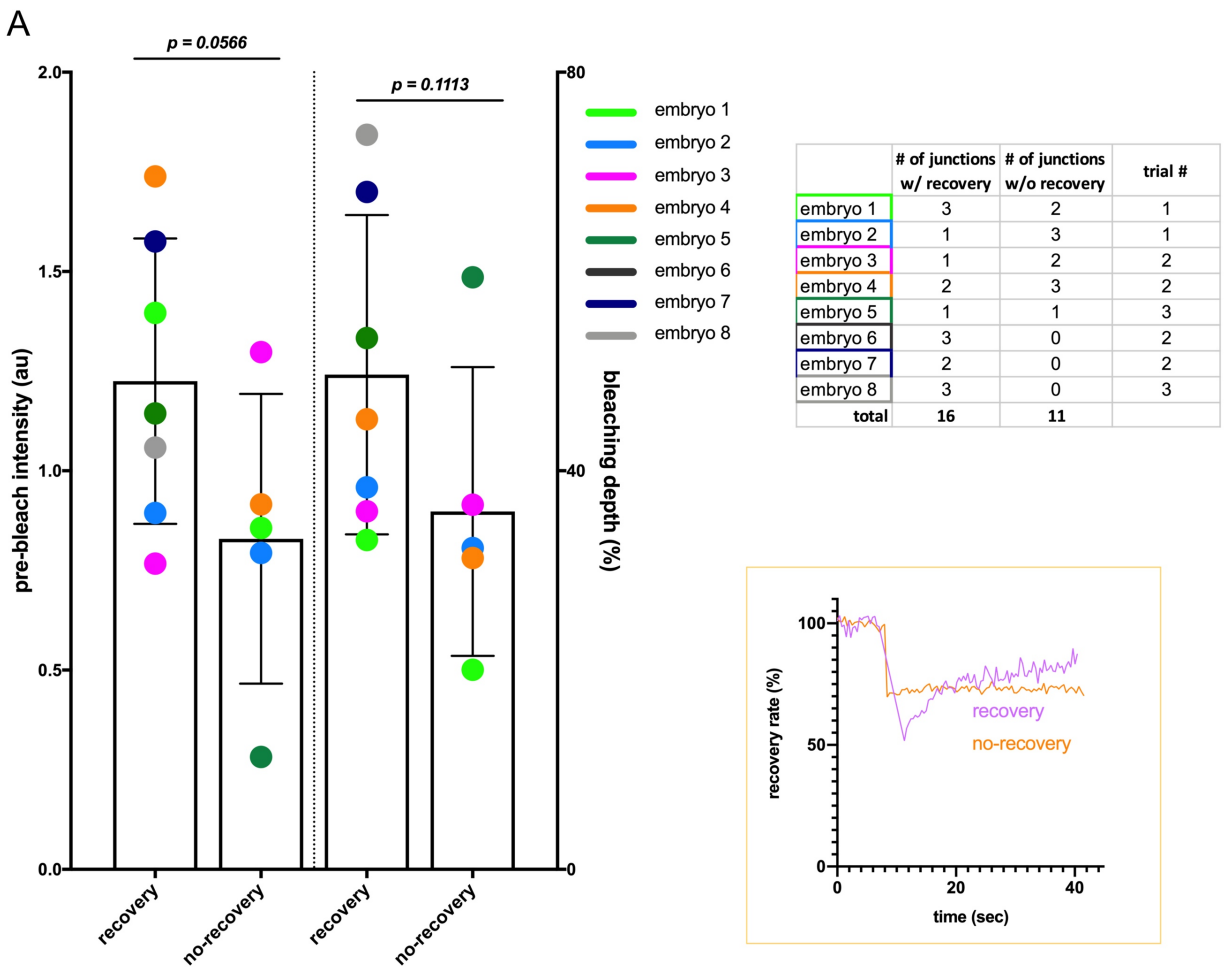


Figure S2. Daam1 regulates the morphology of intercellular boundaries in developing nephrons, Related to Figures 3 and 4.
Confocal time-lapse imaging of F-actin in Control and Daam1KD developing nephrons.
(A-B) Stills from time-lapse movies of "kidney-windowed" embryos. Elapsed time is indicated at the top. Scale bars equal to 5 microns. mCherry tagged Utrophin marks F-actin (magenta), and membrane tagged GFP (green) indicates cell boundaries in
(A) Control and
(B) Daam1 KD cells
in developing nephrons.



	# of junctions w/ recovery	# of junctions w/o recovery	trial #
embryo 1	3	2	1
embryo 2	1	3	1
embryo 3	1	2	2
embryo 4	2	3	2
embryo 5	1	1	3
embryo 6	3	0	2
embryo 7	2	0	2
embryo 8	3	0	3
total	16	11	

Figure S3. FRAP analysis of Daam1 KD nephric junctions, Related to Figure 4.
Daam1 KD nephrons exhibited both junctions with and without recovery of F-actin fluorescence signal following photobleaching. We compared the pre-bleach fluorescence levels of F-actin and the bleaching depths between Daam1 KD junctions with and without recovery of F-actin fluorescence signal (magenta and orange, respectively) in these nephrons. Measures normalized for background and general photobleaching [I_N] were used to estimate the mean fluorescence intensity of each junction before bleaching [$I_{N\ pre\text{-}bleach}$] and to plot the percentage of recovery after photobleaching, which was determined as follows: % recovery rate = (I_N / $I_{N\ pre\text{-}bleach}$) x 100. The bleaching depth for each junction was determined based on its % recovery rate plot as follows: % bleaching depth = %recovery rate $_{pre\text{-}bleach}$ - % recovery rate $_{post\text{-}bleach}$. Each dot represents one embryo with 1-3 junctions analyzed per nephron, per condition (n_{total}=8 embryos across 3 experiments; 27 junctions comprising 16 recovered, and 11 no-recovery junctions).
(A) The bar graph represents the quantification of F-actin pre-bleach fluorescence levels and bleaching depths for Daam1 KD junctions with recovery and no-recovery of fluorescence signal following photobleaching. Significance analyzed by Student's t-test; mean +/- S.D. The distribution of recovery and no-recovery junctions within individual embryos and across different trials is shown in a summary table to the right. Representative % recovery plot of Daam1 KD junctions with and without recovery of F-actin fluorescence signal following FRAP is shown within the yellow box.

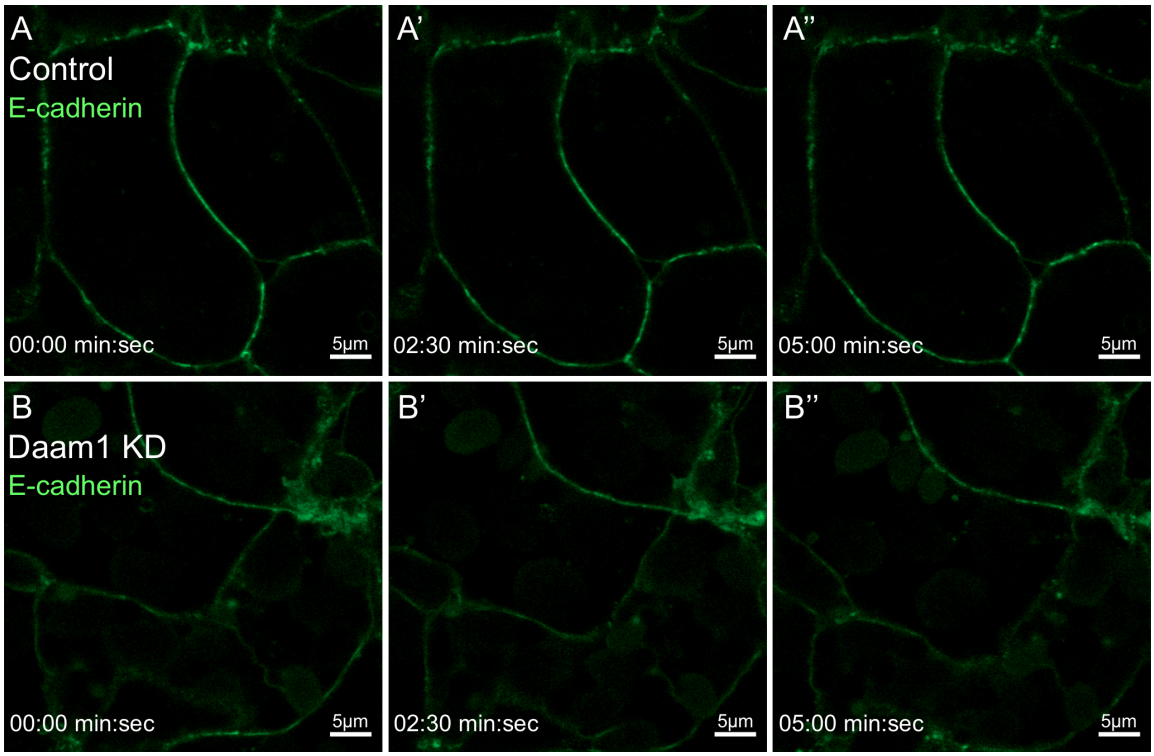


Figure S4. In-vivo imaging of E-cadherin localization in Daam1-depleted developing nephrons, Related to Figures 5 and 6.

Confocal time-lapse imaging of GFP-E-cadherin (green) in Control and Daam1KD developing nephrons. Stills from time-lapse movies of "kidney-windowed" embryos. Elapsed time is indicated at the top. Scale bars equal to 5 microns. GFP-E-cadherin in

(A) Control and

(B) Daam1 KD

pronephric progenitor cells.

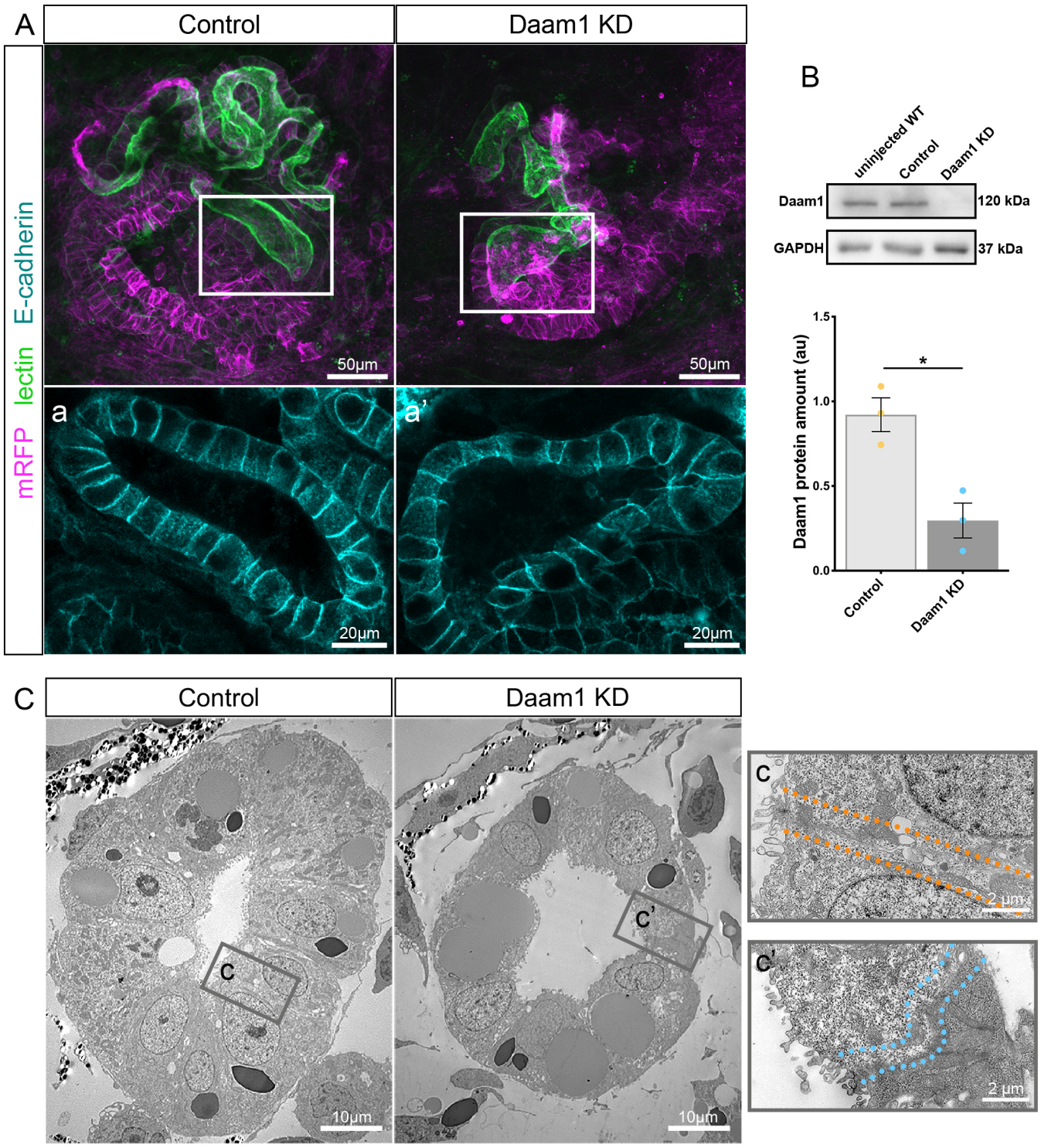


Figure S5. Loss of Daam1 results in cell shape and tissue architecture abnormalities in mature nephrons, Related to Figures 5 and 6.
Kidney-targeted morpholino microinjections were carried out to manipulate the expression levels of Daam1. Control (Standard) or Daam1 antisense morpholinos were co-injected with membrane tagged RFP (mRFP) mRNA as a linage tracer. Analyses of mature nephrons by confocal and Transmission Electron Microscope (TEM) imaging show that a decrease in Daam1 signaling levels affects the size and shape of nephric cells.

(A) Maximum projection confocal images of mature nephrons labeled with lectin (green) and antibodies against RFP (magenta) and E-cadherin (cyan); scale bars equal to 50 microns. a-a' - close-up images corresponding to regions in the white boxes; scale bars equal to 20 microns. The top panels consist of the entire z-stacks to show the overall morphology. a-a'' and b'-b'' represent close-up images of the corresponding regions in white boxes consisting of a subset of the z slices within the projections as the intense E-cadherin signal within the *Xenopus* skin, see Videos S5 and S6.
(B) Western blot and the graph of densitometry measures showing Daam1 and GAPDH protein levels for uninjected wild type (WT), Control (Standard morpholino), and Daam1 KD (Daam1 morpholino) injected embryos. Embryo lysates pooled from 10-20 NF stages 39/40 embryos (2 cell V2 embryo injections, approximately 2 embryos per lane). Individual band intensities plotted in arbitrary units (au) for Control (orange) and Daam1 KD (blue). The results are expressed as means ± S.E.M. from three independent experiments. $^{ns}P > 0.05$, $^{**}P \leq 0.01$, analyzed by one way ANOVA.
(C) TEM images show cross-section samples of the Control and Daam1-depleted nephrons; scale bars equal to 10 microns. b-b' - close-up images corresponding to regions in the gray boxes; scale bars equal to 2 microns. The orange and blue dotted lines outline the morphology of intercellular junctions.

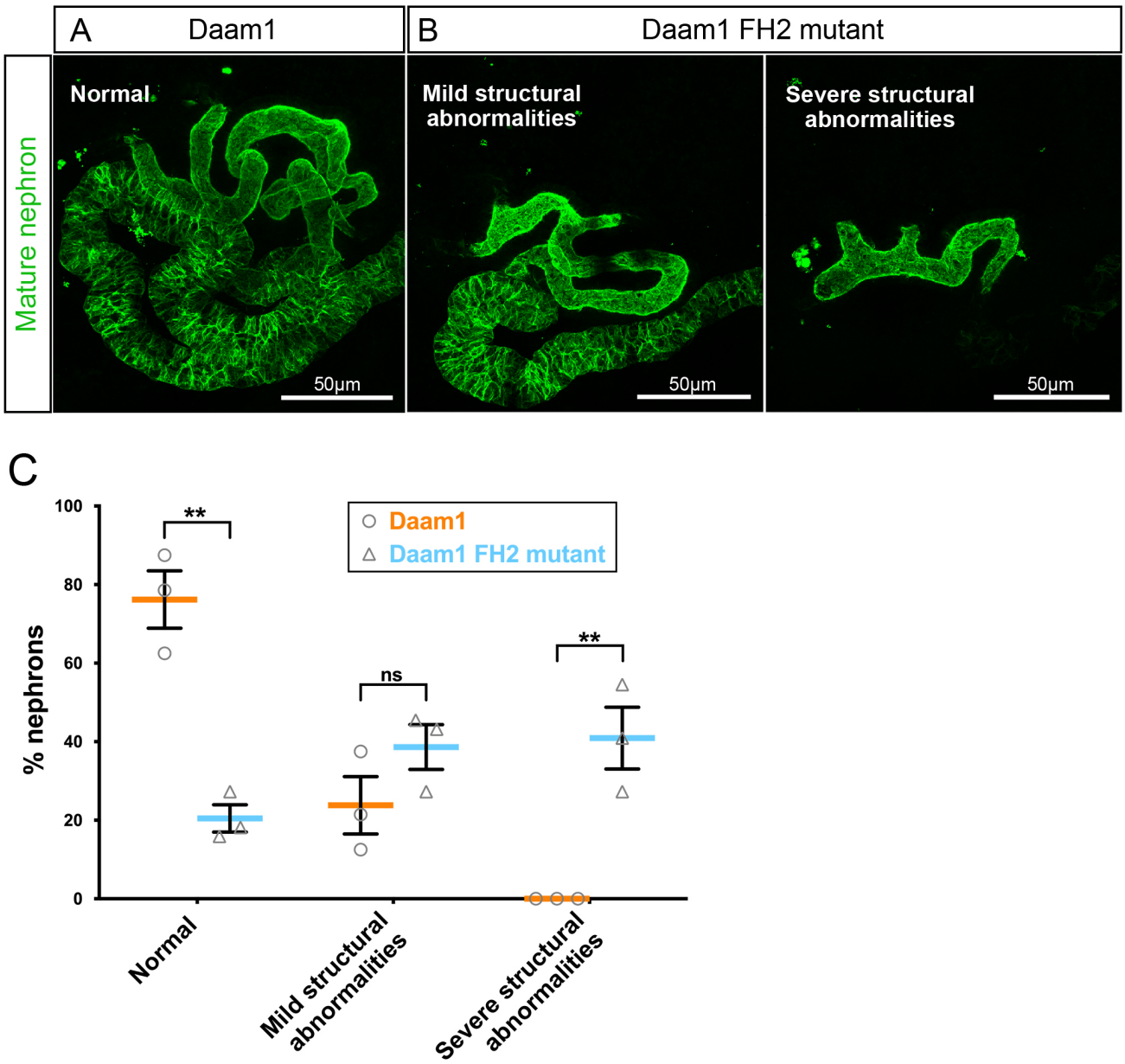


Figure S6. The FH2 domain of Daam1 mediates nephron morphology, Related to Figure 6.
Nephric progenitors expressing full-length Daam1 mRNA ultimately develop structurally normal nephrons compared to nephric progenitors expressing Daam1 FH2 mutant mRNA. Maximum projection confocal images of mature nephrons (NF stages 39/40) visualized by 3G8 and 4A6 antibodies (green) in embryos injected with 1ng Daam1 or 1ng Daam1FH2 mutant mRNA. Embryo microinjections were carried out at the 8 cell-stage into V2 blastomere fate-mapped to pronephric primordium. Scale bars equal to 50 microns.
(A) Representative image of mature nephron from embryos injected with 1ng Daam1 mRNA.
(B) Representative images of mature nephrons from embryos injected with 1ng Daam1 FH2 mutant mRNA.
(C) The graph represents the quantification of the phenotypic severity of mature nephrons in embryos from A and B. Bars represent the mean percentage of nephrons in each phenotypic category for Daam1 control injected (orange) and Daam1 FH2 mutant (blue) embryos. n_{Daam1}=54 embryos across 3 experiments and $n_{Daam1FH2mutant}$ = 66 embryos across 3 experiments. Error bars indicate S.E. of the mean. $^{ns}P > 0.05$, $^{**}P \leq 0.01$, analyzed by unpaired t-test.

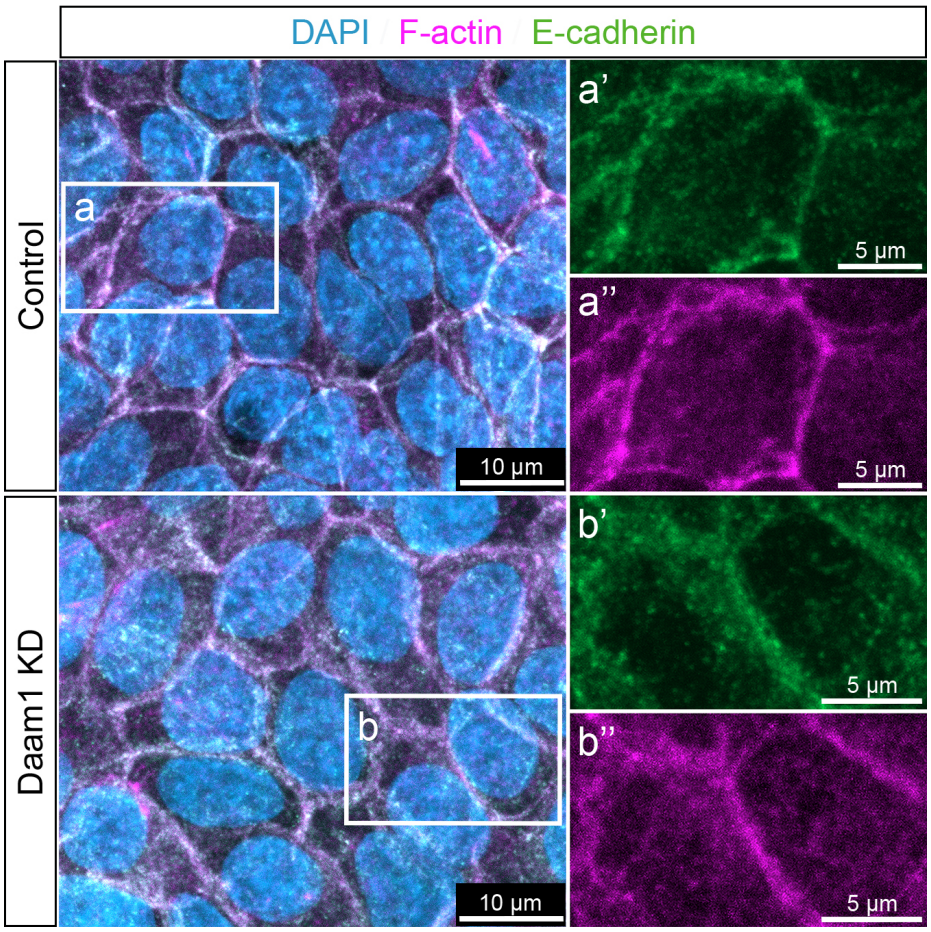


Figure S7. E-cadherin localizes to shDaam1 depleted mature cell-cell junctions, Related to Figure 7.
Maximum confocal projections showing E-cadherin (green), F-actin (magenta), and DAPI (cyan) in confluent the MDCK Control and shDaam1 KD cells. The first column shows merged images of individual color channels. Scale bars equal to 10 microns. The second column shows images of individual color channels for F-actin and E-cadherin. a'-a''' and b'-b''' are close-up images corresponding to regions shown in a and b, respectively. Scale bars equal to 5 microns.