Fig. S1. Generation of novel mutants in CUBAM genes. (A) Domain composition of vertebrate (grey background) and Drosophila CUBAM members. Regions used for the generation of anti-Cubn and anti-Cubn2 polyclonal antibodies are indicated. (B-E) Schemes showing the selected regions for guide-RNA targeting (orange and green arrowheads above the transcripts depicted in grey) on Cubn2 (B), Cubn (C) and Amnionless (D, E), the lesions generated on the DNA sequence and the resulting predicted truncated proteins, with mutant amino acids shown in red.
Fig. S2. Endocytic dextran uptake in cubn-2<sup>E31</sup>nephrocytes and quantification of acentric nuclei in CUBAM mutants. (A, B) Endocytic uptake of dextran (red) by nephrocytes is severely compromised in cubn-2 mutants (B), compare with the wild-type (A). (C) Quantification of acentric nuclei present in larval garland nephrocytes of the indicated genotypes, represented as percentage of the total number of nuclei. OR (n=107), Cubn<sup>E31</sup> (n=119), Cubn<sup>B2</sup> (n=80), Amnionless<sup>3</sup> (n=68), Amnionless<sup>3Sri</sup> (n=74), psG4>UAS-mgl; Amnionless<sup>3</sup> (n=156), prosG4>UAS-Ri-Cubn2; UAS-Ri-sec15 (n=67). Scale bars: 10 µm.
Fig. S3. Subcellular localisation of ectopic human AMN in wild-type and CUBAM mutant nephrocytes.

(A,B) In a wild-type genetic background (Amnionless+, Cubn+, Cubn2+) hAMN overexpressed with pros-G4 (red) is retained in the ER (A), whereas in the absence of endogenous Amnionless (B) it can traffic to the LCh membrane in the subcortical region (arrows). (C,D) In Cubn2 E3-1 mutants, hAMN traffics to the LCh membrane irrespectively of Amnionless presence. Asterisks point to the accumulation of hAMN close to ingressions of the external membrane labelled with anti-Duf. (E,F) In the absence of Cubn, hAMN is retained in the ER, even when there is no endoge-nous Amnionless (F). (G) There is amino acid conservation between vertebrate and Drosophila Cubn, but not with Cubn2, at the interface region of interaction with Amnionless (underlined in red). Similarities based on BLOSUM62 scores. Scale bars: 10µm.
Fig. S4. Cladogram showing relationships of cubilin proteins from selected organisms. In flies there are two well-defined clusters of Cubilin paralogues, Cubn and Cubn2, highlighted with green and light blue backgrounds respectively. Interestingly, some fly species have three Cubilin paralogues resulting from a more recent duplication of cubn or of Cubn2 genes. Drosophila elegans is highlighted in red as an example of a species with three Cubilin paralogues. A branch containing three aphid species is highlighted in green to point out that these species underwent a duplication of an ancestral cubilin gene similarly to flies. Aphis gossypii is highlighted in pink. Additional examples of cubin duplications are also included in the cladogram.
Fig. S5. SD positioning in several trafficking mutants. *dor*8 mutants display deep ingressions of SDs similar to CUBAM LOF alleles (A). In contrast, depletion of *Rabl* (B) or *It* (C) in nephrocytes does not induce internal accumulation of SD proteins. A decrease in the density of SDs can be observed in A and B (arrowheads). Compare with the wild-type in Fig.1A and CUBAM LOF mutants in 1B and 5E. Scale bars: 10µm.
Fig. S6. Endocytic uptake of dextran in Mgl-rescued Amnionless<sup>3</sup> nephrocytes. (A-C) 10 KDa ex vivo dextran uptake in the indicated genotypes (green). (A) Amnionless<sup>3</sup>; p s-GAL4 control nephrocytes show no dextran uptake. (B) Expression of Mgl (mgI<sup>X06105</sup>) with p s-GAL4 increases uptake of 10 KDa dextran in Amnionless<sup>3</sup> nephrocytes. (C) Dextran uptake in wild-type nephrocytes. The image display range for the dextran channel was optimized for better visualization. Inset shows the same image without optimization to compare with A,B. (D). Box-plot displaying quantification of dextran uptake (n=102N/16S). Scale bar: 10 µm, inset shown at a 50% reduction.
**Movie 1.** Segmentation and 3D reconstructions of SDs, LCh and cortical tubules from wild-type and *Cubn*<sup>E3-1</sup> larval garland nephrocytes, derived from FIB-SEM stacks.
Movie 2. Segmentation and 3D reconstructions of SDs, LCh and cortical tubules from wild-type and Cubn<sup>2<sup>E3-i</sup> larval garland nephrocytes, derived from FIB-SEM stacks.