# Supporting Information

## Cabrero et al. 10.1073/pnas.1412706111

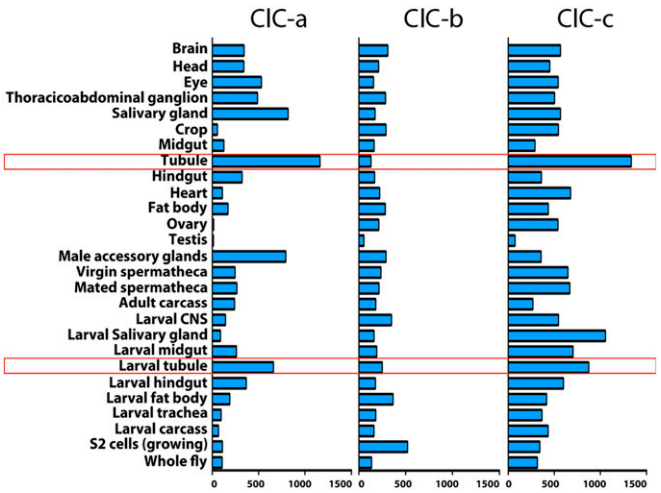


**Fig. S1.** *ClC* chloride channel family expression in *Drosophila melanogaster*. Data mining of FlyAtlas identified three *CLC* genes, but only two of the three show highly abundant expression in adult Malpighian tubules.

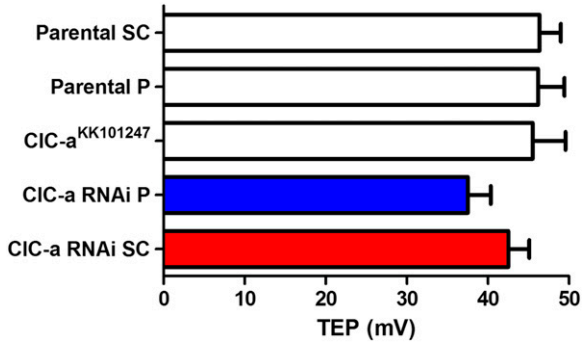


**Fig. S2.** Disruption of *ClC-a* expression has no impact on the resting transepithelial potential. Transepithelial potentials of GAL4 driver parental lines and the *ClC-a*$^{KK101247}$ insertional allele are compared with principal cell-specific and stellate-cell–specific *ClC-a* knockdowns. There is no significant difference between any of the lines. Data are shown as mean ± SEM ($n$ = 8).

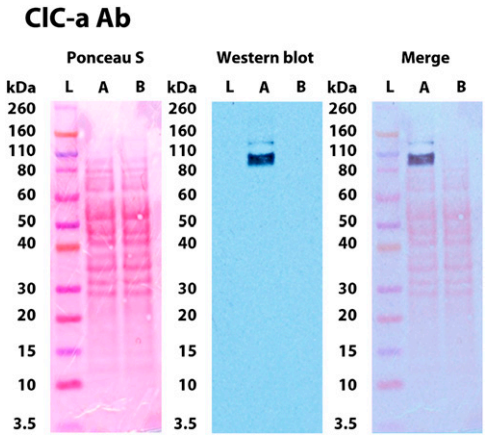


**Fig. S3.** Western blot analysis of Malpighian tubules using CLC-a antibody. (*A*) Ponceau S staining of control sample (*A*) and peptide block sample (*B*). (*B*) Western blot analysis of CLC-a antibody before (*A*) and after (*B*) blocking with the antigenic peptide. (*C*) Merge picture of *A* and *B*.

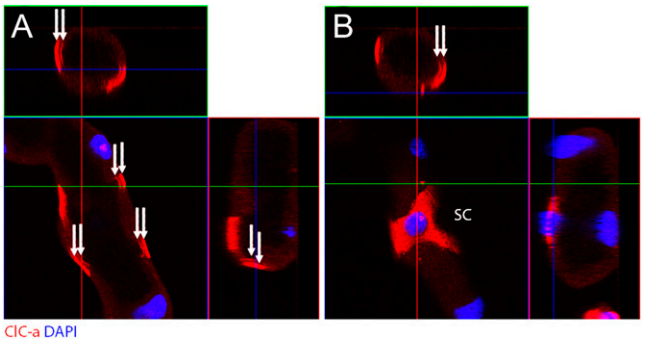


**Fig. S4.** Immunocytochemistry of CLC-a. Confocal microscopy sections of *D. melanogaster* Malpighian tubules showed membrane localization of CLC-a (arrows, red). *A* and *B* show two different transverse (*Upper*), coronal (*Lower*), and sagittal (*Right*) planes of the same Malpighian tubule after immunocytochemistry using CLC-a antibody.

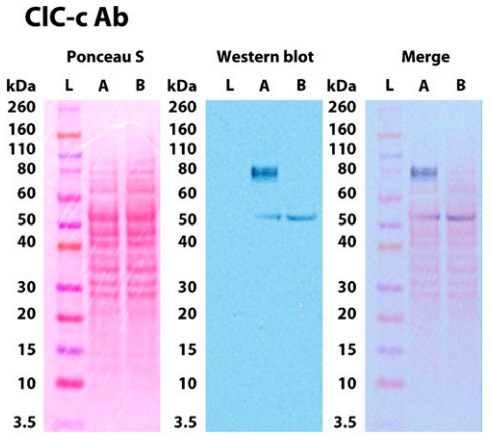


**Fig. S5.** Western blot analysis of Malpighian tubules using CLC-c antibody. (*A*) Ponceau S staining of control sample (*A*) and peptide block sample (*B*). (*B*) Western blot analysis of CLC-c antibody before (*A*) and after (*B*) blocking with the antigenic peptide. (*C*) Merge picture of *A* and *B*.

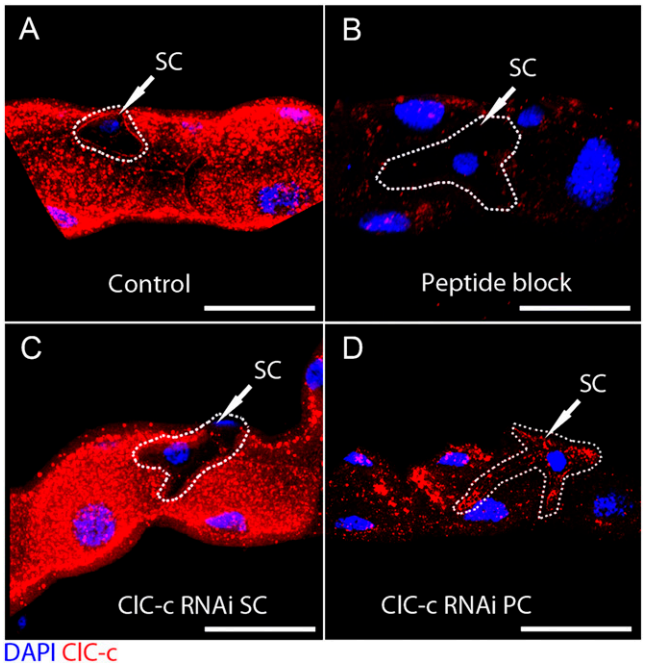


**Fig. S6.** Immunocytochemistry of CLC-c. (*A*) Control line showing principal and stellate cell (dashed lines) localization of CLC-c. (*B*) Specificity was confirmed by blocking CLC-c antibody with the antigenic peptide. (*C*) Down-regulation of ClC-a in stellate cells abolished the staining of CLC-c in the stellate cell and increased the staining in the principal cell. (*D*) Knockdown of ClC-c in principal cells decreased the staining in principal cells and increased staining in the stellate cells. (Scale bars: 25 μm.)