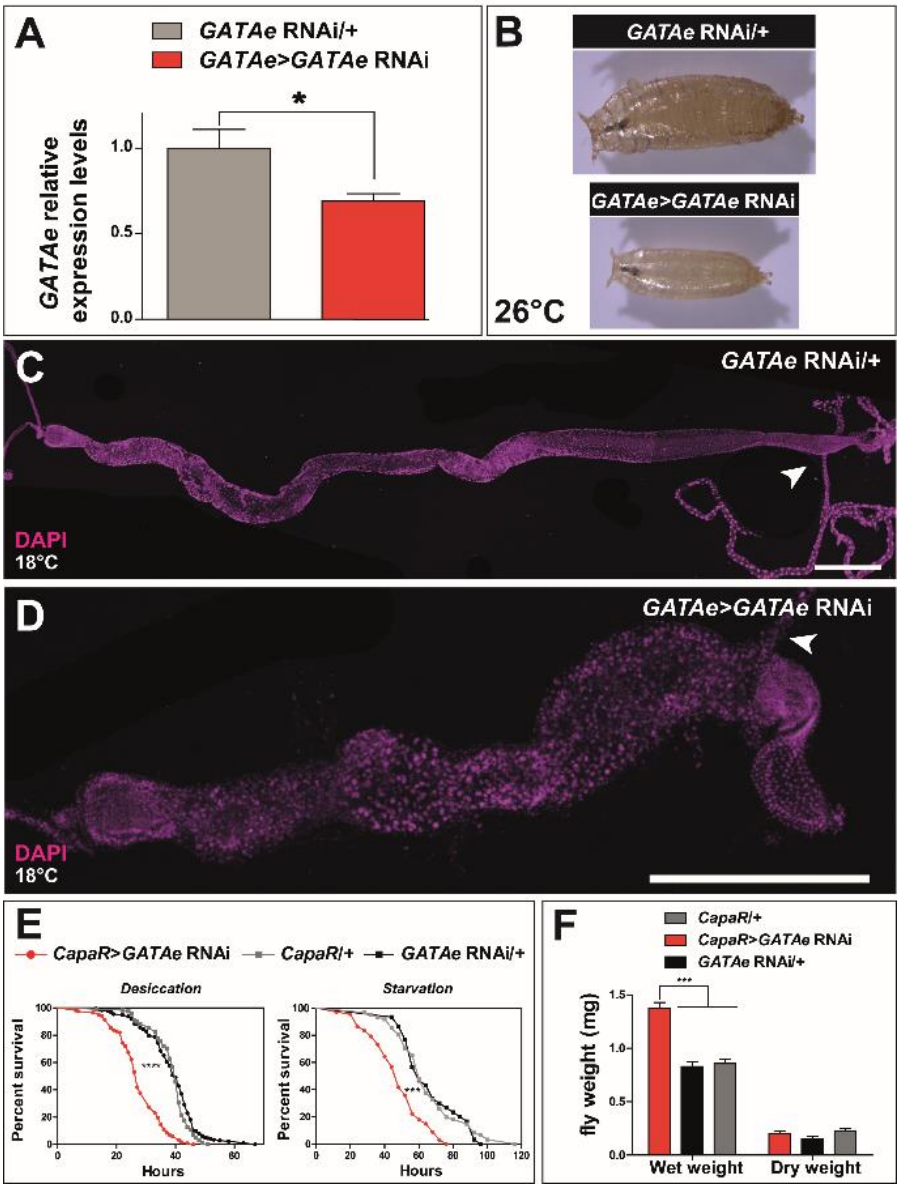

Figure S1. *GATAe>GATAe* RNAi characterisation and *CapaR>GATAe* RNAi stress assays.

(A) *GATAe* expression of *GATAe>GATAe* RNAi embryos compared to the control (15h after egg laying). The bar with * indicates P<0.05, Student's *t-test*, two tailed. **(B)** Size comparisons of *GATAe>GATAe* RNAi pupae (below) control (top) raised at 26°C. Both images are at same scale. **(C and D)** Adult MGs stained with DAPI (magenta) of *GATAe>GATAe* RNAi (C) and control (D, *GATAe* RNAi/+). Arrowheads indicate ureter regions. Scale bars are 500µm. **(E)** Desiccation (left) and starvation (right) assays of *CapaR>GATAe* RNAi adult female flies. Median survivals (hours) are: desiccation, *CapaR>GATAe* RNAi = 27, *CapaR*/+ = 40, *GATAe* RNAi/+ = 40 , n>100 flies, and starvation, *CapaR>GATAe* RNAi = 48, *CapaR*/+ = 60, *GATAe* RNAi/+ = 60. In both assays, *** indicates P<0.0001, Log-rank test. **(F)** Wet and dry weights of *GATAe* knockdown compared to both parental controls. *** indicates P<0.0001, n>90.

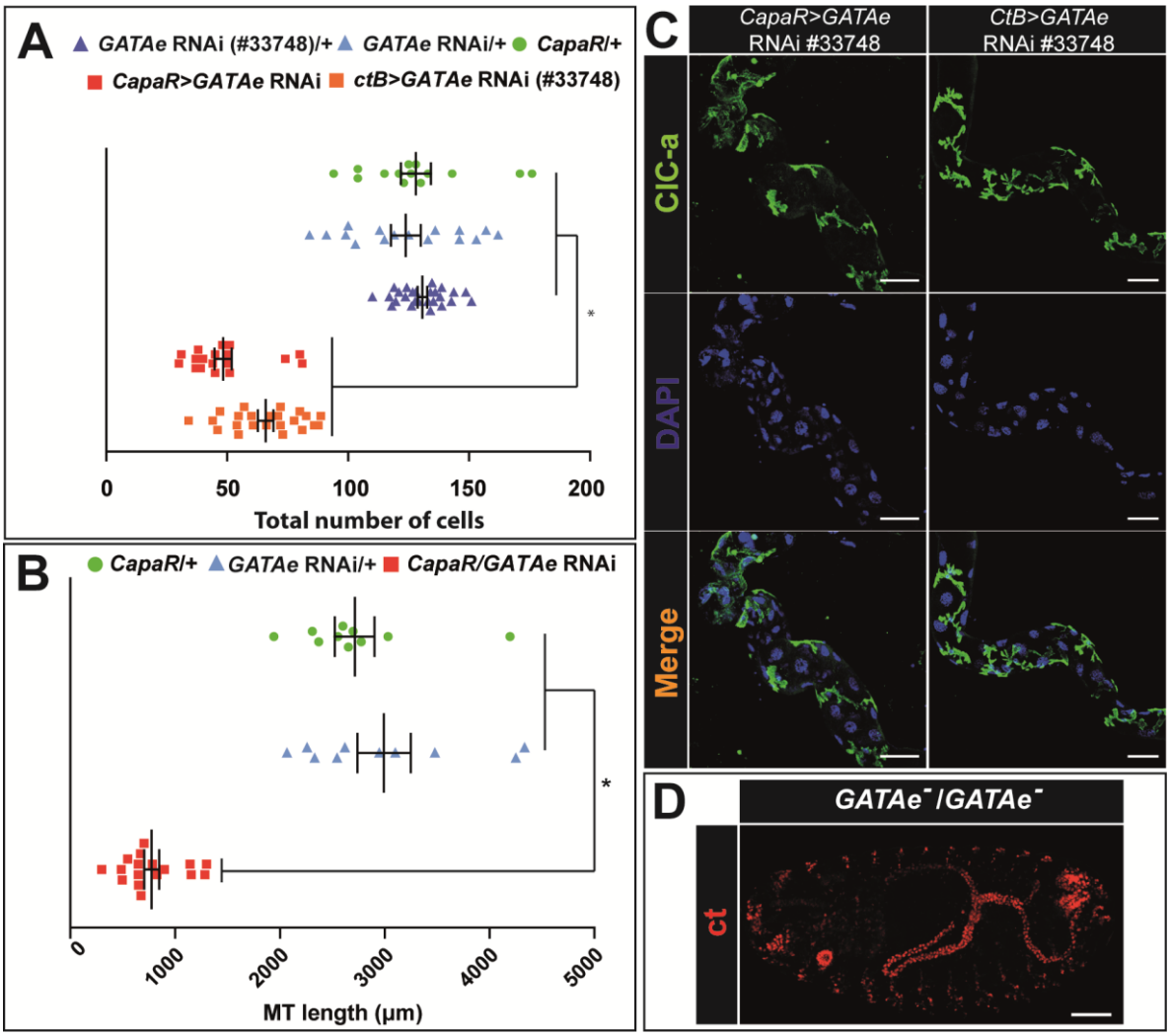

Figure S2. Length comparison of *GATAe* knockdown MTs.

(A) Cell number quantifications of both PCs and SCs in different control conditions (*GATAe* RNAi/+, *GATAe* RNAi (#33748)/+ and *CapaR*/+) and PC-specific knockdown conditions (*CapaR*>*GATAe* RNAi and ctB>*GATAe* RNAi (#33748)). Means of cells ± SEM are: *GATAe* RNAi/+ = 123.9 ± 6.1, n=18, *GATAe* RNAi (#33748)/+ = 130.7 ± 1.9, n=26, *CapaR*/+ = 128.1 ± 6.1, n= 16, *CapaR*>*GATAe* RNAi = 48.4 ± 3.6, n=18, *CtB*>*GATAe* RNAi (#33478) = 65.97 ± 3.2, n=23. * indicates P<0.05, Student's *t-test*, two-tailed. **(B)** MT length measurements of parental controls and *CapaR*>*GATAe* RNAi adult MTs. Means of lengths (µm) ± SEM are: *CapaR*>*GATAe* RNAi = 777.5 ± 70.2, n=17, *GATAe* RNAi/+ = 2994 ± 253.8, n=10, *CapaR*/+ = 2714 ± 189.2, n=10. * indicates P<0.05, Student's *t-test*, two-tailed. **(C)** *GATAe* knockdown adult tubule stained with ClC-a (green, stellate cell marker) and DAPI (blue), using an alternative RNAi line (#33748) and two different PC-specific Gal4 drivers (*CapaR*-Gal4 and *ctB*-Gal4). **(C)** Example of a *GATAe* mutant embryo stained with ct. Scale bars are 50µm.

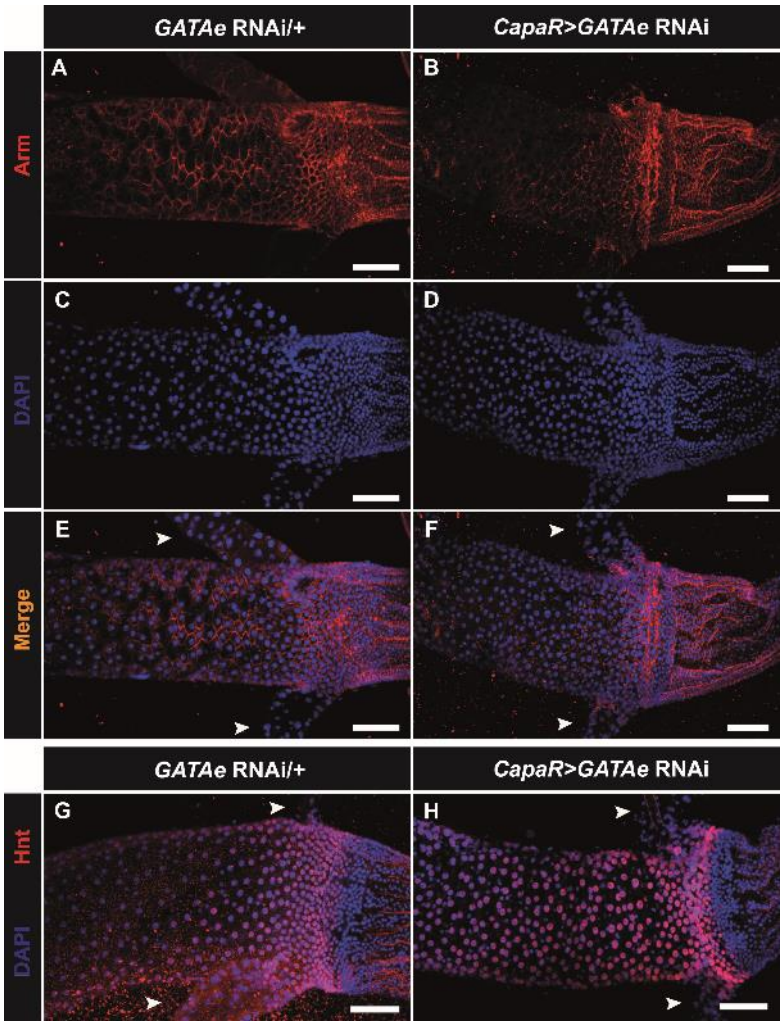

Figure S3. Silencing *GATAe* in PCs does not affect MG morphology.

Maximum projections of adult *CapaR>GATAe* RNAi posterior MGs, including a portion of the hindgut, stained with Arm (red) and DAPI (blue) **(A-F)** or with Hnt and DAPI **(G and H)**. White arrowheads indicate the ureters. Scale bars are 50µm.

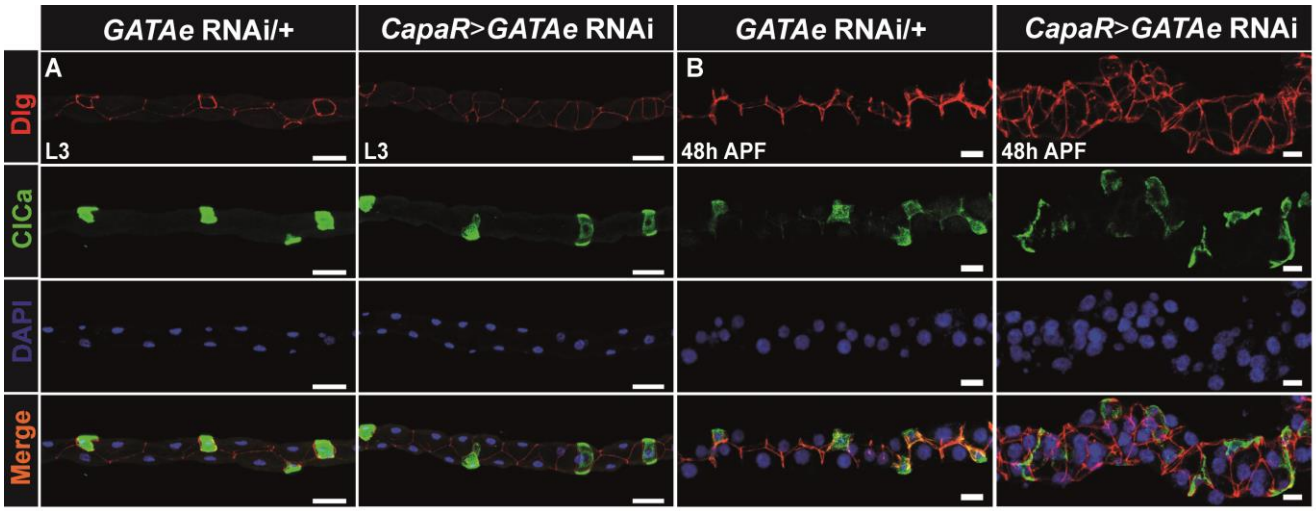

Figure S4. *CapaR>GATAe* RNAi tubules present structural defects from pupal stage.

(A) Comparison of L3 control and *CapaR>GATAe* RNAi knockdown MTs. Merge images show overlay of Dlg (red), ClC-a (green) and DAPI (blue). **(B)** Comparison of 48h APF control and *CapaR>GATAe* RNAi MTs. Scale bars are 50µm.

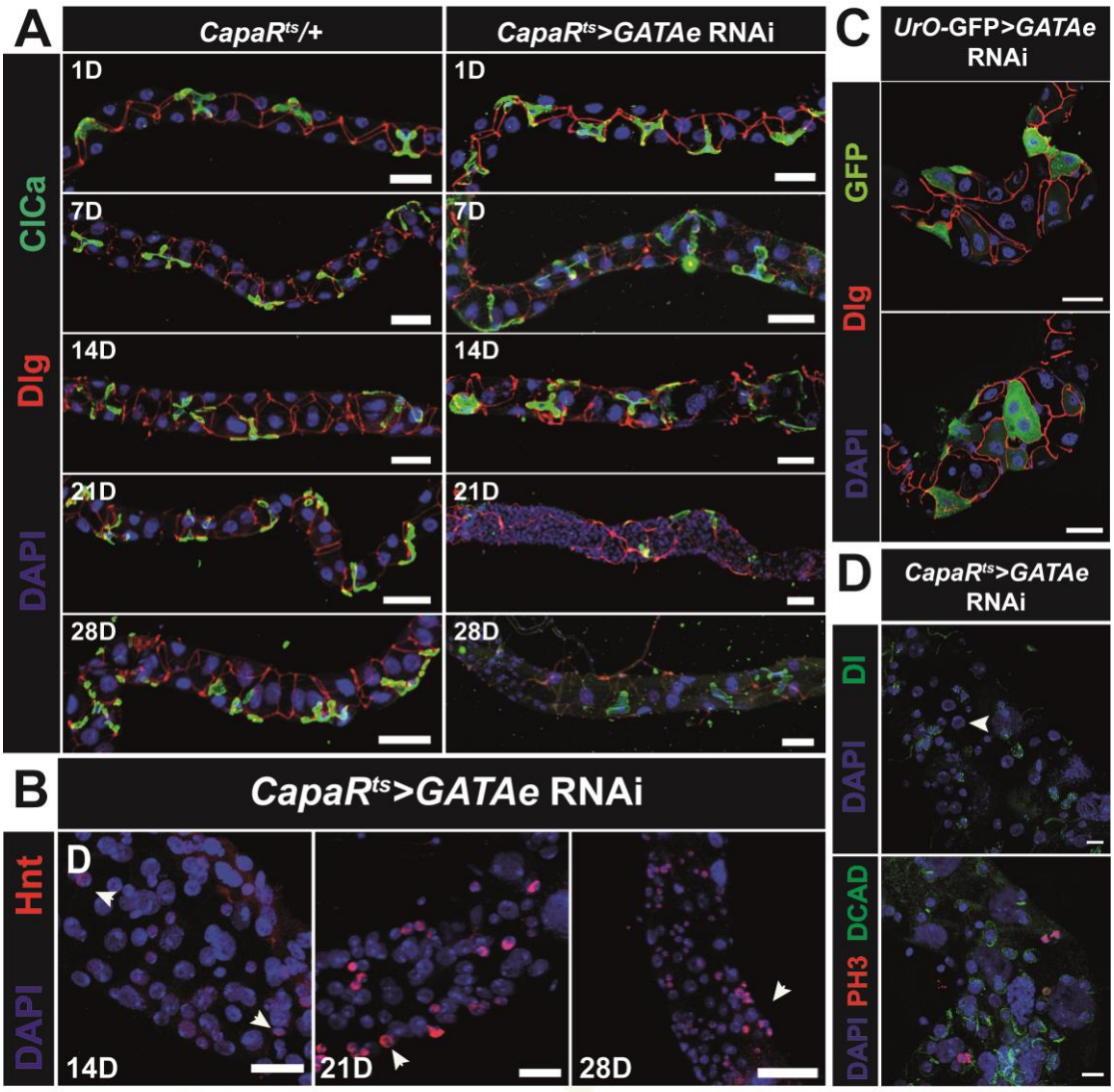

Figure S5. Tissue and time-specific downregulation of *GATAe*.

(A) Main segments of adult *CapaR*^{ts}>*GATAe* RNAi flies that were raised at 18°C until eclosion and then switched to 29°C from day 1 to day 28 and immunostained every 7 days with Dlg (red), ClC-a (green), and DAPI (blue). Scale bars are 50µm. **(B)** Regions of MTs from *CapaR*^{ts}>*GATAe* RNAi flies that were raised at 18°C until eclosion and then switched to 29°C and stained at 14 days, 21 days and 28 days with Hindsight (red) and DAPI (blue). Scale bars are 20µm in 14D and 21D and 50µm in 28D. **(C)** Main segments of *UrO*-GFP>*GATAe* RNAi MTs stained with Dlg (red) and GFP (green) and DAPI (blue). Cells with two nuclei are indicated with white arrows. Scale bars are 50µm. **(D, top)** Dl (green) staining for adult main segment, together with DAPI (blue). Dl^{-} cells correspond to RBs (white arrowhead). **(D, bottom)** Presence of proliferating cells in the main segment of *GATAe* knockdown tubule, stained with PH3, indicated with white arrowhead. Scale bars are 10µm.

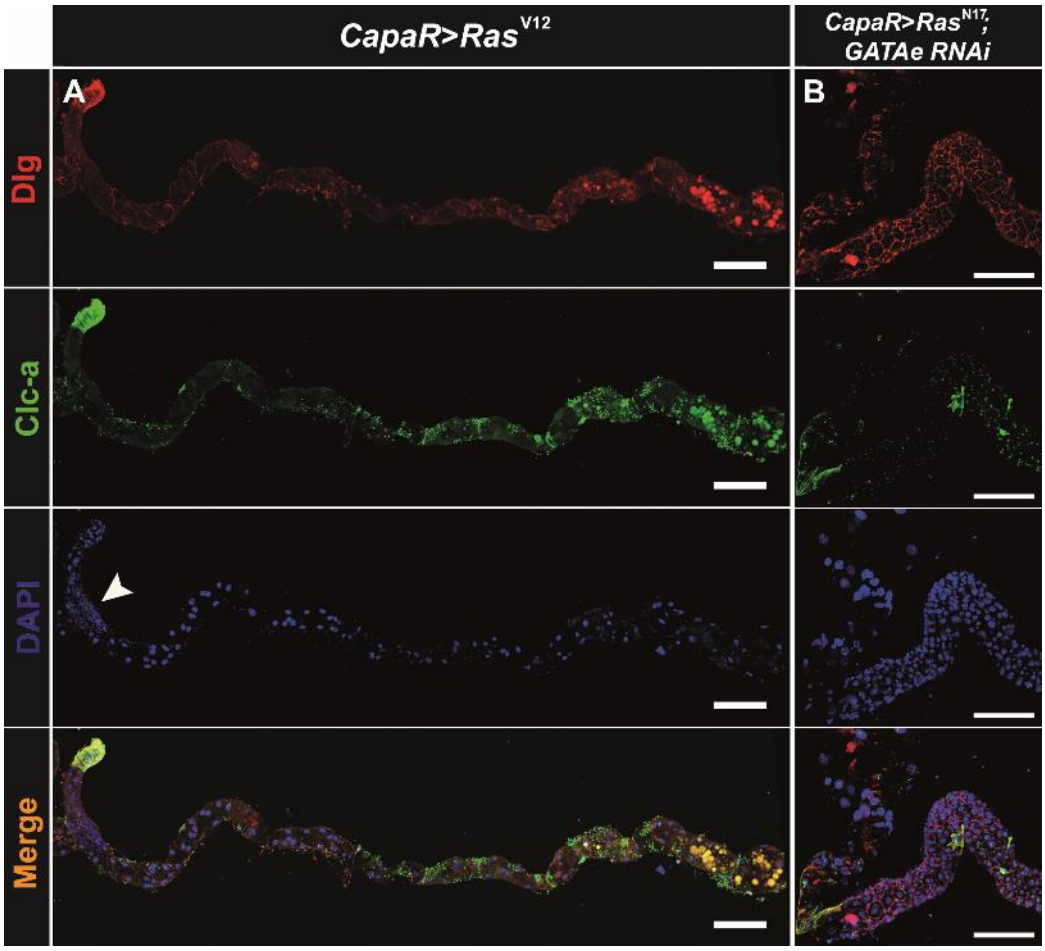

Figure S6. Overactivation of *Ras* signalling induces similar phenotypes to *GATAe* knockdown MTs.

(A) Immunocytochemistry of adult *CapaR>Ras^{V12}* MTs stained with Dlg (red), ClC-a (green) and DAPI (blue). Accumulation of potential RNSCs is indicated with white arrowhead. **(B)** Expression of a dominant negative form of *Ras85d* (*Ras^{N17}*) does not rescue the morphological defects caused by *GATAe* knockdown. Scale bars are 200µm in A and 100µm in B.

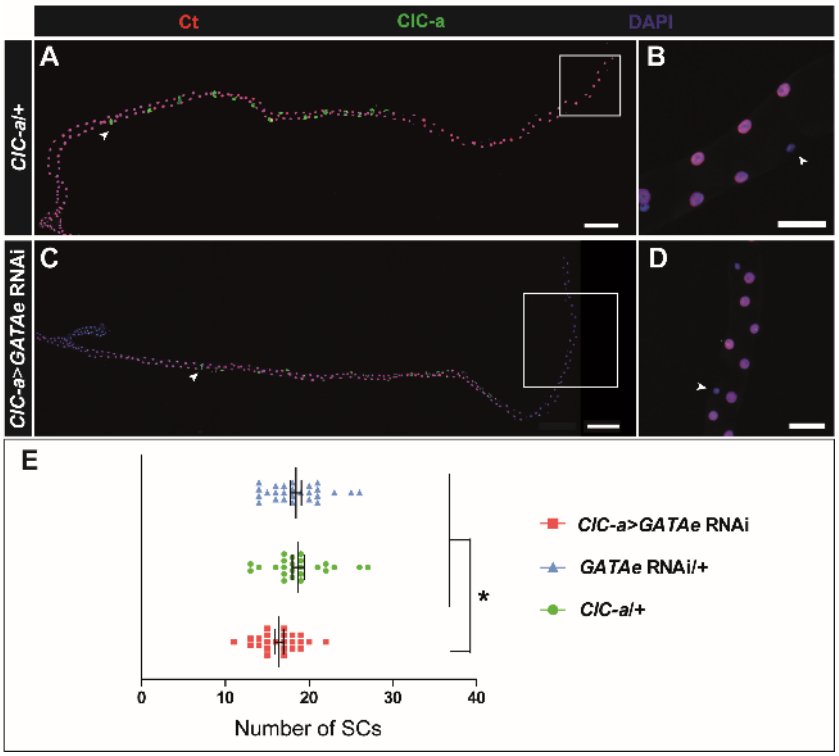

Figure S7. *GATAe* knockdown in SCs show normal localisation of SCs.

(A, D) Immunocytochemistry of L3 stage *ClC-a*/+ (A and B) and *ClC-a>GATAe RNAi* (C and D) MTs. White arrowheads in A and C indicate the most proximal SC. B and D are magnifications of white squares in A and C, respectively, showing SCs negative for ClC-a and ct (white arrowheads), which are bar cells (small cells located in the initial segment). Scale bars are 200µm (A and C) and 50µm (B and D). **(E)** SCs quantifications of adult tubules from different genotypes. Means ± SEM are: *ClC-a*/+ = 18.71 ± 3.48, n=24, *GATAe* RNAi/+ = 18.42 ± 3.28, n= 24, *ClC-a>GATAe* RNAi = 16.42 ± 2.5, n=26. * indicates $P < 0.05$, student's *t-test*, two-tailed.