Figure S1

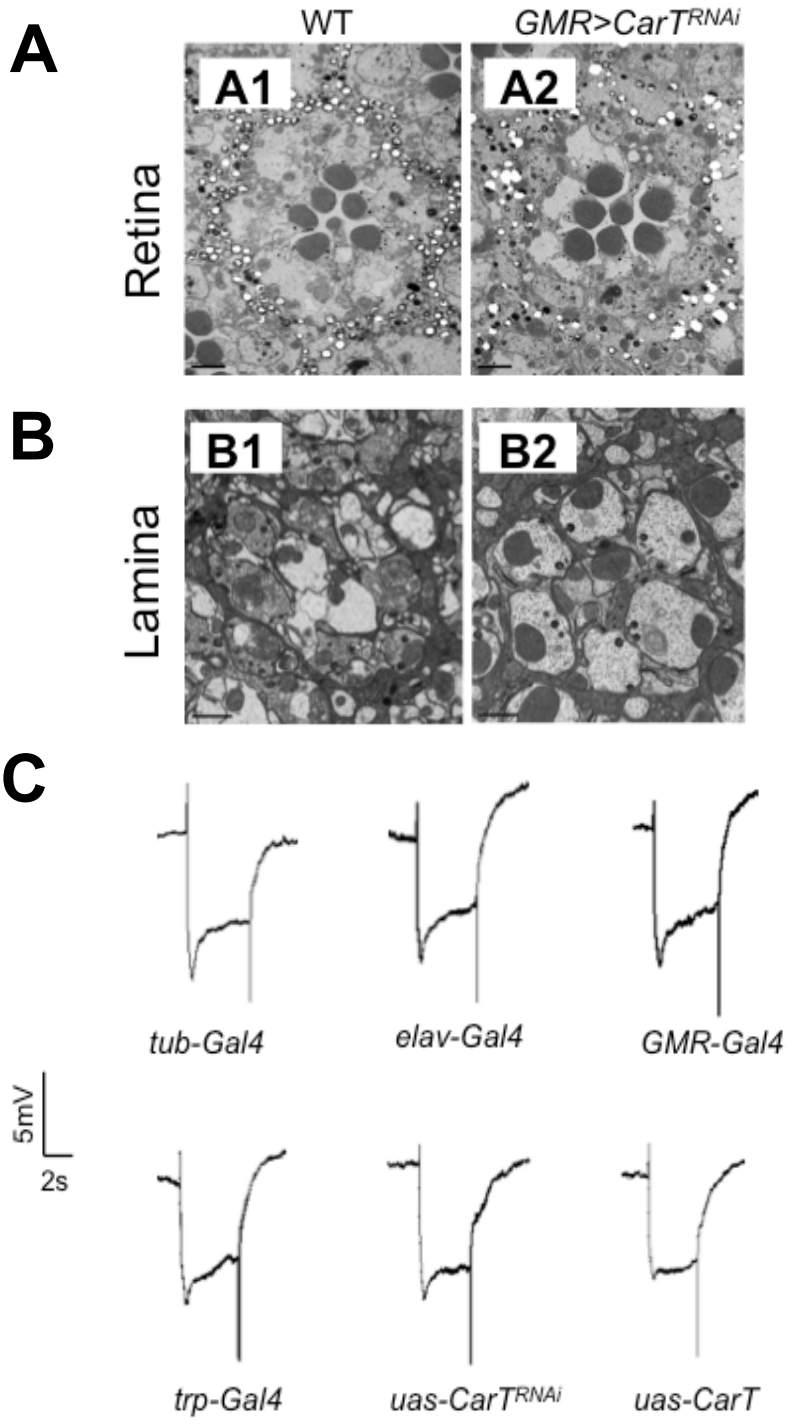

A
WT
GMR>CarT^RNAi
A1
A2
Retina
B
B1
B2
Lamina
C
tub-Gal4
elav-Gal4
GMR-Gal4
5mV
2s
trp-Gal4
uas-CarT^RNAi
uas-CarT

Figure S2

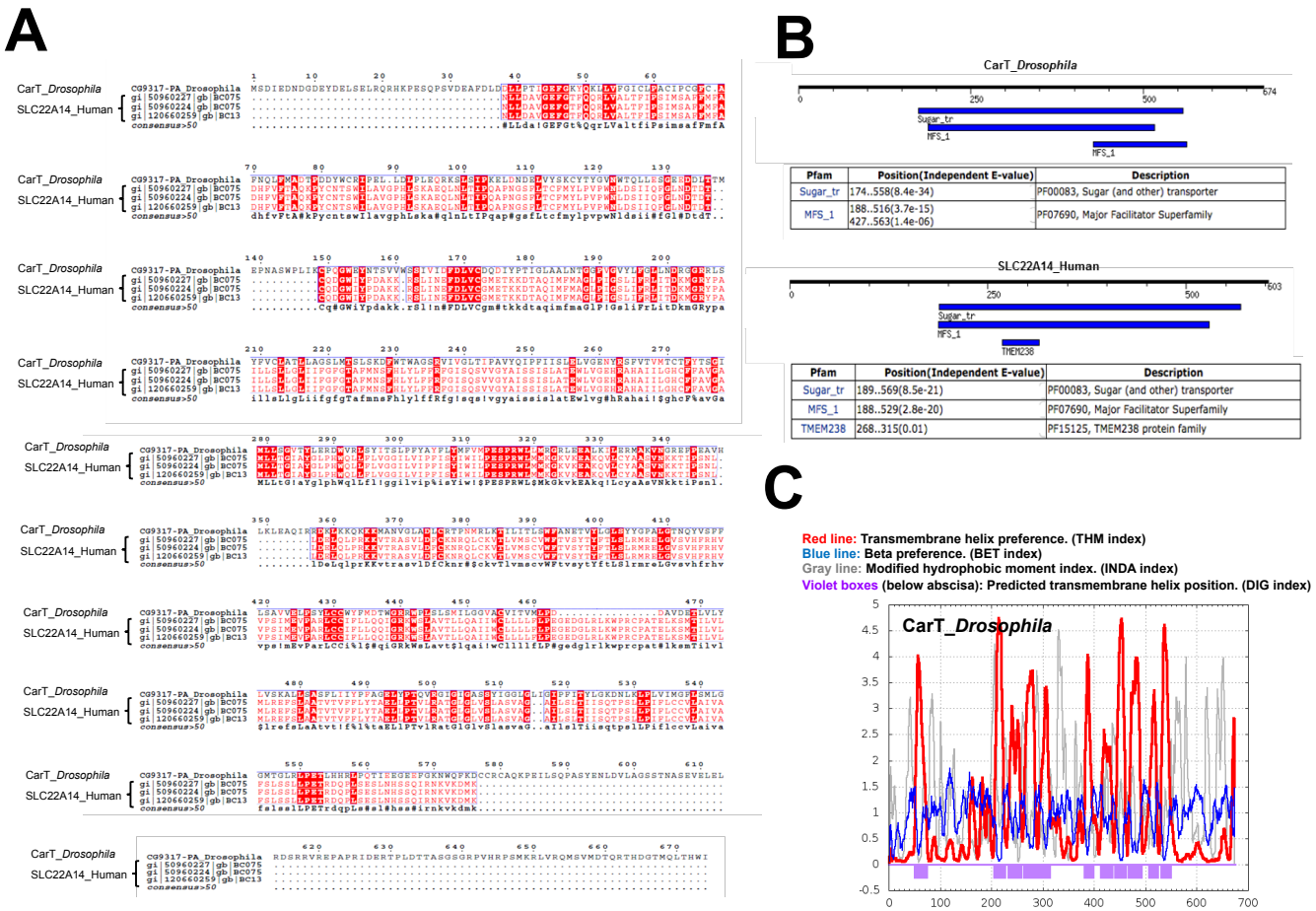

A
CarT_Drosophila
SLC22A14_Human
B
CarT_Drosophila
Sugar_tr
MFS_1
Pfam | Position(Independent E-value) | Description
Sugar_tr | 174..558(8.4e-34) | PF00083, Sugar (and other) transporter
MFS_1 | 188..516(3.7e-15) 427..563(1.4e-06) | PF07690, Major Facilitator Superfamily
SLC22A14_Human
TMEM238
Pfam | Position(Independent E-value) | Description
Sugar_tr | 189..569(8.5e-21) | PF00083, Sugar (and other) transporter
MFS_1 | 188..529(2.8e-20) | PF07690, Major Facilitator Superfamily
TMEM238 | 268..315(0.01) | PF15125, TMEM238 protein family
C
Red line: Transmembrane helix preference. (THM index)
Blue line: Beta preference. (BET index)
Gray line: Modified hydrophobic moment index. (INDA index)
Violet boxes (below abscisa): Predicted transmembrane helix position. (DIG index)
CarT_Drosophila

Figure S3

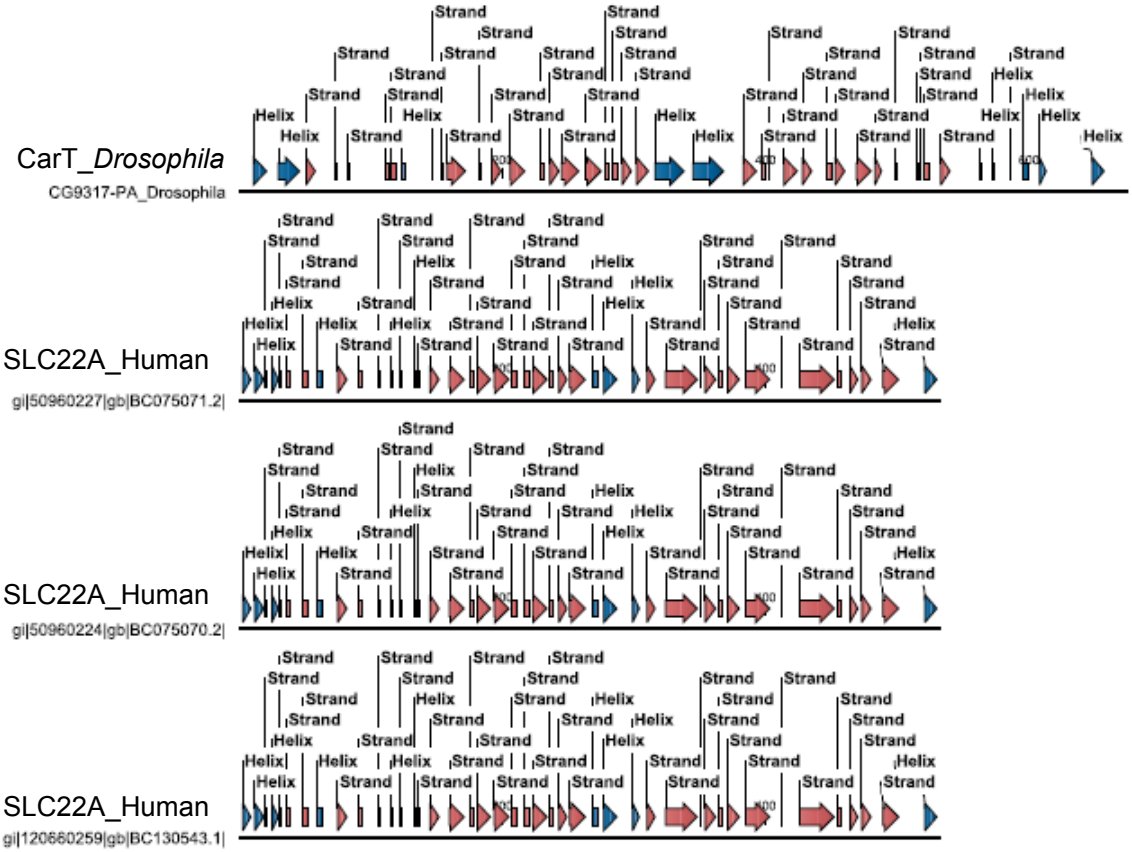
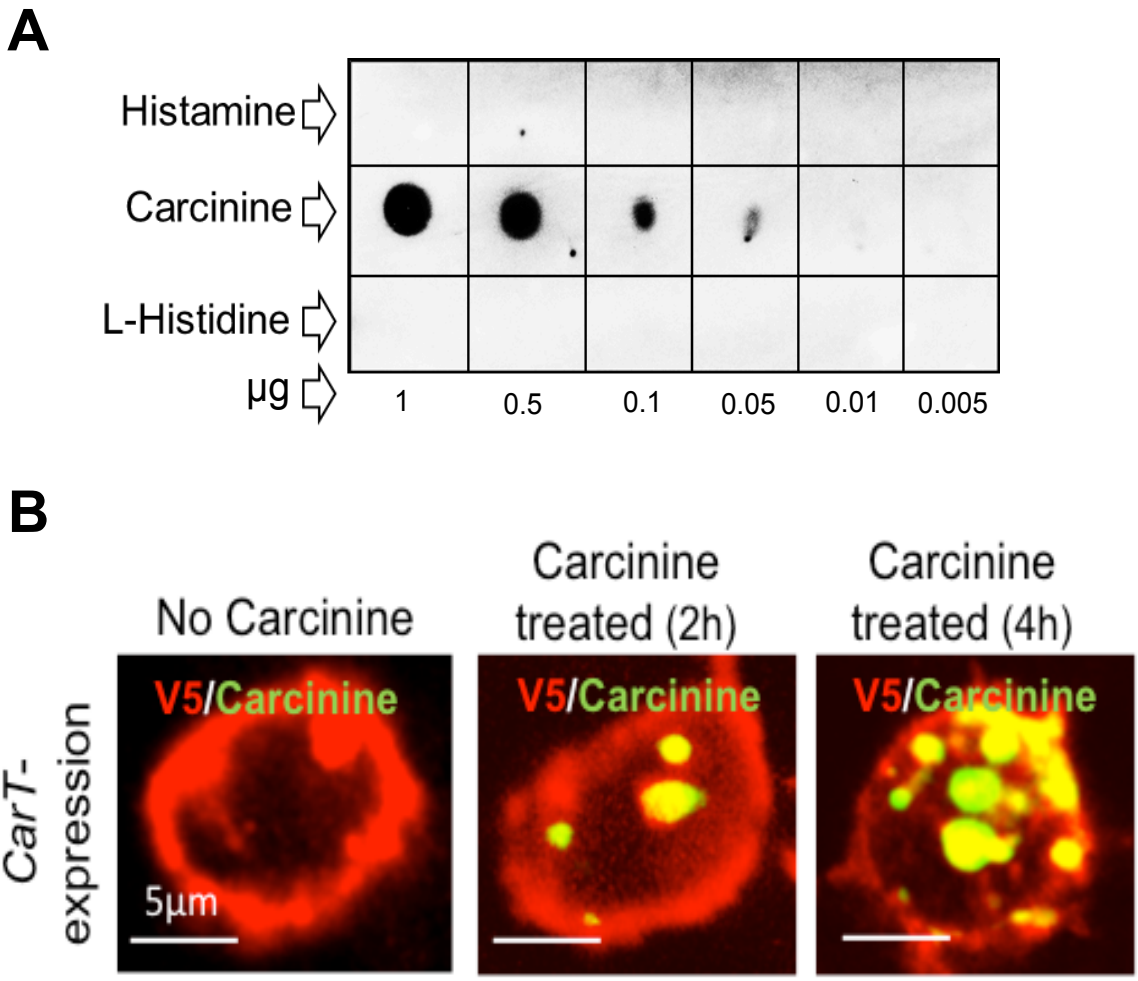

CarT_*Drosophila*
CG9317-PA_Drosophila
SLC22A_Human
gi|50960227|gb|BC075071.2|
SLC22A_Human
gi|50960224|gb|BC075070.2|
SLC22A_Human
gi|120660259|gb|BC130543.1|

Figure S4


A
Histamine
Carcinine
L-Histidine
µg
1
0.5
0.1
0.05
0.01
0.005
B
No Carcinine
Carcinine treated (2h)
Carcinine treated (4h)
CarT-expression
V5/Carcinine
V5/Carcinine
V5/Carcinine
5µm

Legends for Supplementary Figures:

Figure S1. Additional control experiments for Figure 1.

(A & B) KD of CarT in photoreceptors did not cause any degeneration or morphology change in the retina and lamina. EM pictures of single retinal ommatidium **(A)** and laminar cartridge **(B)** are shown for WT **(A1** and **B1)** and *GMR-CarT*^{RNAi} flies **(A2** and **B2)**. The retinal sections were cut at the level of the peripheral photoreceptor nucleus, and the laminar ones at level of epithelial glia. The scale bars in (**A)** and (**B)** are 1 and 0.5 μm, respectively.

(C) Control flies that separately contain tissue specific Gal4 drivers and UAS transgenes had intact ON and OFF transients in ERG, and thus normal synaptic transmission between photoreceptor and laminar neurons.

Figure S2. Sequence similarities between CarT and members of the human solute carrier family 22 (SLC22A proteins), i.e., polyspecific cation transporters, which form the rationale for experiments in Figure 2.

(A) Here we use ClustalW program (http://www.ebi.ac.uk/clustalw) to reveal the similarity between CarT and SLC22A-14 transporters. **(B)** CarT and human SLCs share the same PFAM Domains (MFS Domains, solute transporter domains, etc.) and thus may have similar functionality. **(C)** Transmembrane helix preferences and hydrophobic index predicted in CarT.

Figure S3. The secondary structure similarity between CarT and SLC22 further supports the rationale for experiments in Figure 2 and 3.

Based on Pfam, Transmembrane Domains and UniProt, CarT is orthologous to human Organic Cationic Transporters (OCTs). The human sequences [1] gi|120660259|gb|BC130543.1|, [2] gi|50960224|gb|BC075070.2| and [3] gi|50960227|gb|BC075071.2| have similar alpha-helix and beta-strand configurations as CarT.

Figure S4. Additional control experiments for Figure 3.

(A) In dot-blot assay, the carcinine antibody specifically recognizes carcinine.

(B) Staining with antibodies against the V5 peptide is conducted to confirm the expression of the tagged CarT protein in transfected S2 cells. The positive V5 staining was used as a marker to select cells for the uptake analysis.

Additional methods

Fly stocks:

Drosophila lines Canton-S (as wild type/WT), *tubulin-Gal4*, *repo-Gal4*, *elav-Gal4, GMR-Gal4,* and hdc^{jk910} were obtained from the Bloomington *Drosophila* Stock Center. The two *UAS-CarT*-RNAi lines were from the Vienna *Drosophila* RNAi Center and the Transgenic RNAi Project. The *trp-Gal4* construct was generated by cloning the *trp* gene promoter (−400–+226) from pCaSpeR-trp **(Han et al., 2006)** into the BglII/EcoRI site of a pG4PN2 vector (a gift from Dr. Marc Freeman), and the photoreceptor expression pattern was confirmed in transgenic flies. To generate the rescue construct, *UAS-CarT-FLAG*, wild type CarT (CG9317) cDNA was obtained by reverse transcription PCR (Primers: Forward 5'-CCGGAAT CGGCAAATACCAGAAGCTCTTGGTT-3' with EcoR1 restriction site, and Reverse 5'-CCGC TCGAGTTACTTATCGTCGTCATCCTTGTAATCGATCCAATGAGTCAACTGCATGGT-3' with a FLAG tag and Xho1 restriction site) and cloned into a pUAST vector (Addgene).

CRISPR/CAS9-mediated deletion of the *CarT* gene:

Two CRISPR derived gRNAs (TCAGATATTGAGGACAA|CGA**TGG** and AATGGTATCTG GTAGAC|AGC**TGG**, where '|' indicates the cutting site and the nucleotides in bold is the Protospacer Adjacent Motif) were designed for this purpose: one near the ATG of *CarT* and the other within Exon 4. Both sequences were cloned into a pCFD4 vector with dual U6 promoters and a vermilion marker (v+). The transgenic flies bearing the construct were selected based on

the vermilion marker, and crossed to Vasa-Cas9 flies (*w; +/+; PBac{y+ 3xP3-GFP vas-Cas9}attP9A*). Male *w;+/+;P{CaryP, gRNA,v+}attP2/PBac{y+,3xP3-GFP,vas-Cas9}attP9A* progenies from this cross were further crossed with *Cyo* virgins. Again, male flies were collected from the cross and screened for deletion using PCR and loss of the restriction sites Cgl13032II and PvuII. The deletion was further confirmed by sequencing, and stable $CarT^{Del}$ mutant lines were established after removing the gRNAs, Cas9 and *Cyo* through crosses.

Electroretinogram (ERG) recordings:

For ERG recordings, a recording glass microelectrode was gently placed on the eye surface of immobilized flies and a reference electrode was placed on the thorax, with both electrodes filled with Ringer's solution. The flies were first allowed to adapt to the dark conditions for 1 min before being stimulated by 5-sec white light pulses (3,000 lux, producing light responses of >90% of the maximum amplitude). The signals were recorded using a Warner IE210 Intracellular Electrometer. At least three responses were recorded for each fly, and 20 flies were examined for each genotype.

Immunofluorescence staining:

The brains of adult flies were dissected and fixed for 45–60 min on ice, in a fixation solution containing 2% (w/v) paraformaldehyde, 0.01 M $NaIO_4$, 75 mM lysine, 37 mM sodium phosphate buffer, pH 7.4. After blocking for 4 h in Phosphate-buffered saline (PBS) containing 5% (v/v) Fetal bovine serum and 0.3% Triton X- 100, brains were incubated overnight at 4°C with primary antibodies. After three washes in PBST (PBS with 0.3% Triton X-100), the brains were incubated with FITC/ TRITC conjugated secondary antibodies (1:200) for 4 h at room temperature followed by washing in PBST and then in PBS. After mounting, images were captured using a confocal microscope. Quantification of fluorescence intensities was performed using ImageJ software (NIH, Bethesda) by selecting the area and measuring the integrated densities and mean values of three successive optic slices in an image.

Rabbit anti-carcinine, anti-β-alanine and anti-histamine antibodies were obtained from ImmuoStar (Cat # 22939), Abcam (ab6453) and Sigma (H7403), respectively. The specificity of these antibodies was assured and confirmed by pre-absorption and mutant (*tan, hdc* and *ebony*) staining, as described previously (Chaturvedi et al., 2014). Additionally, the specificity of carcinine antibody was further confirmed by dot-blot assay (**Figure S4A**). A mouse anti-DLG (discs-large) antibody was purchased from the Developmental Studies Hybridoma Bank (4F3, Iowa, USA) and used to demarcate the photoreceptor axon region, with the glia-occupied region unstained.

Uptake assay with S2 cells:

Drosophila S2 cells were cultured in SFX growth medium (HyClone, USA) with 10% fetal bovine serum and 1% penicillin-streptomycin (Invitrogen, USA) in 6-well plates. After reaching 70-90% confluence, the cells were transfected with a *pAc5.1-CarT-V5-His* plasmid or the empty *pAc5.1/V5-His B* vector (2µg DNA/well) using Cellfectin II (Invitrogen, USA). Two days after transfection, the cells were incubated with 5 µM carcinine, histamine and β-alanine in SFX medium for 0, 2, 4 and 8 h and then washed with PBS, fixed with 4% paraformaldehyde in PBS on glass coverslips for 15 min, permeabilized and blocked with 0.1% Triton X-100 and 3% Bovine Serum Albumin in PBS, and immunostained for 4 h at room temperature with anti-carcinine (1:500), anti-histamine (1:500) and anti-β-alanine (1:500) antibodies, respectively, followed by incubation with secondary antibody (1:1000) for 2 h at room temperature. After three washes with PBS, the stained cells were imaged using a confocal microscope. Cells were selected for analysis based on the co-stained signal of V5, which confirms the expression of V5-His-tagged CarT **(Figure S4 B)**.