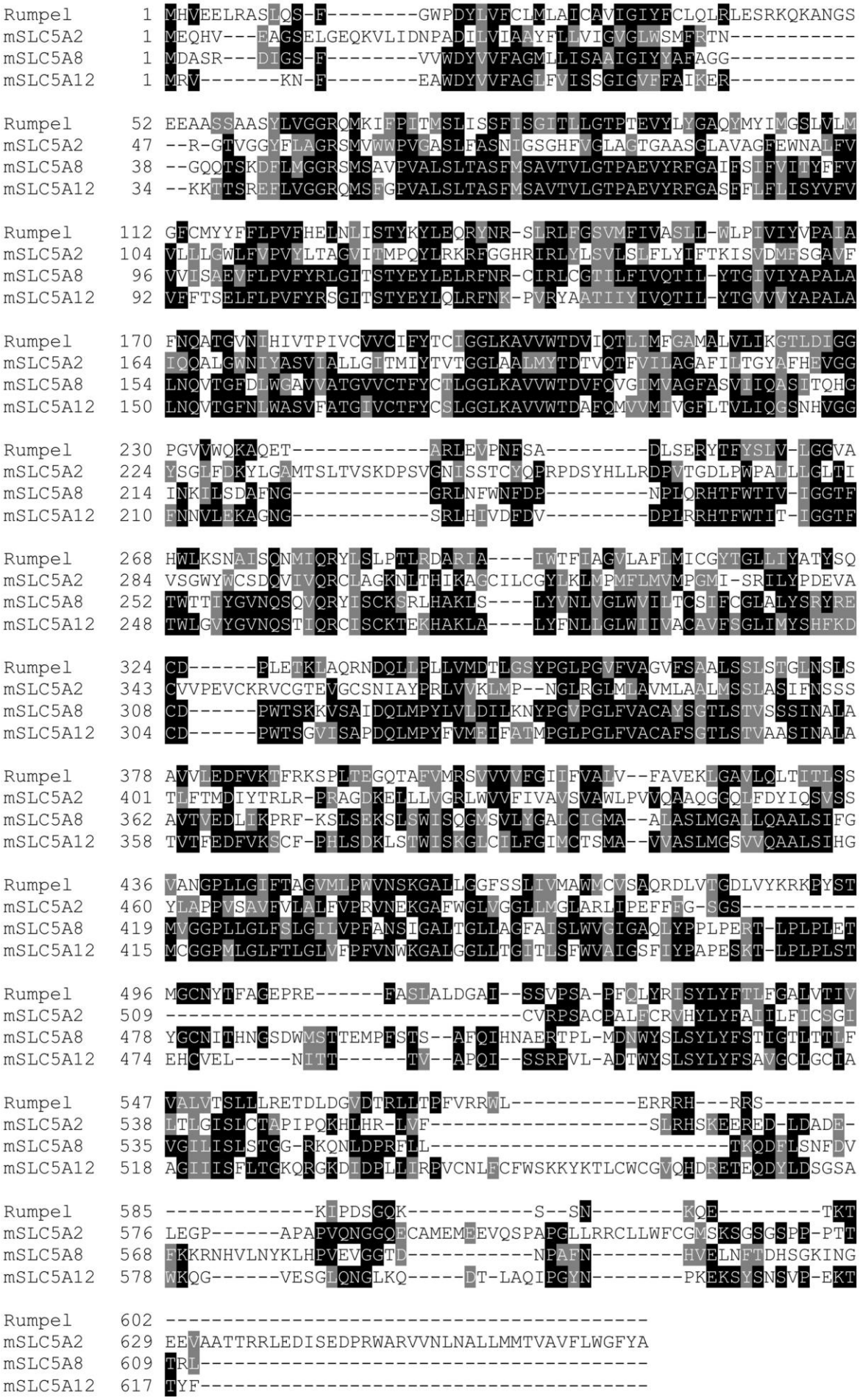

Fig. S1. Rumpel is predicted to be a member of the Sodium Solute symporter family (SLC5)

BLAST detects highest homology with SLC5A8 (35.77 % identity with 90% coverage, p=5e-112) and SLC5A12 (33.33 % identity with 92% coverage p= 3e-110). Less significant homology detected is SLC5A2 (23.21% with 67% coverage identity, p=3e-12). Identical and similar amino acids among proteins are indicated as black and grey fills, respectively. "-" indicates missing amino acids in the respective sequence.

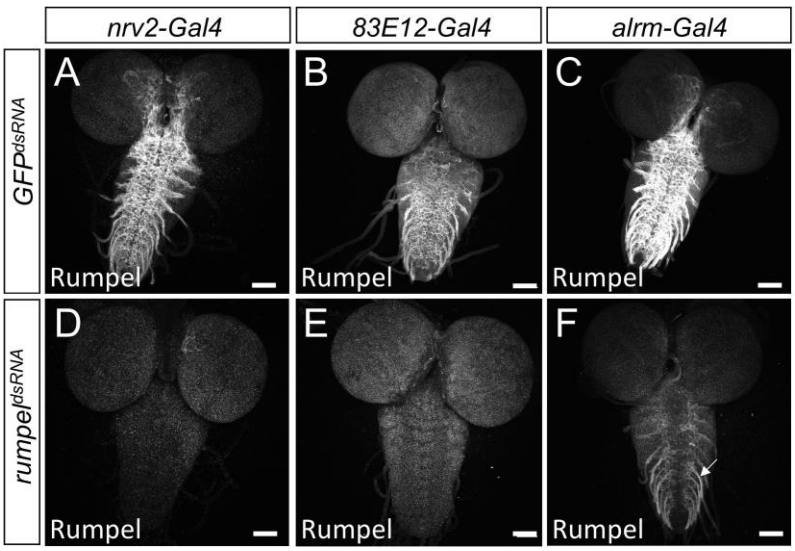

Fig. S2. Rumpel is expressed predominantly by ensheathing glial cells.

All specimens are stained for Rumpel (grey). (A-F) Confocal maximum projections of a third instar larval brain stained for Rumpel. (A-C) Rumpel expression is not affected by expressing dsRNA directed against *GFP* (*nrv2-Gal4>GFP*^{dsRNABL9330}; A), in ensheathing glia (*83E12-Gal4> GFP*^{dsRNABL9331}; B) or astrocyte-like glial cells (*alrm-Gal4>GFP*^{dsRNABL9330}; C). (D-F) No Rumpel expression is detected upon expression of dsRNA directed against *rumpel* in cortex glia and ensheathing glia (*nrv2-Gal4> rumpel*^{dsRNAv43922}*;* D). Following suppression of rumpel expression in ensheathing glial cells faint expression can be noted in the cortex and in the neuropil (*83E12-Gal4>rumpel*^{dsRNAv107361}; E). Upon silencing of rumpel only in astrocyte-like glial cells (*alrm-Gal4>rumpel*^{dsRNAv43922}; F) weak Rumpel expression can be detected in ensheathing glial cells (arrow) and in the cortex. Scale bars are 50 µm.

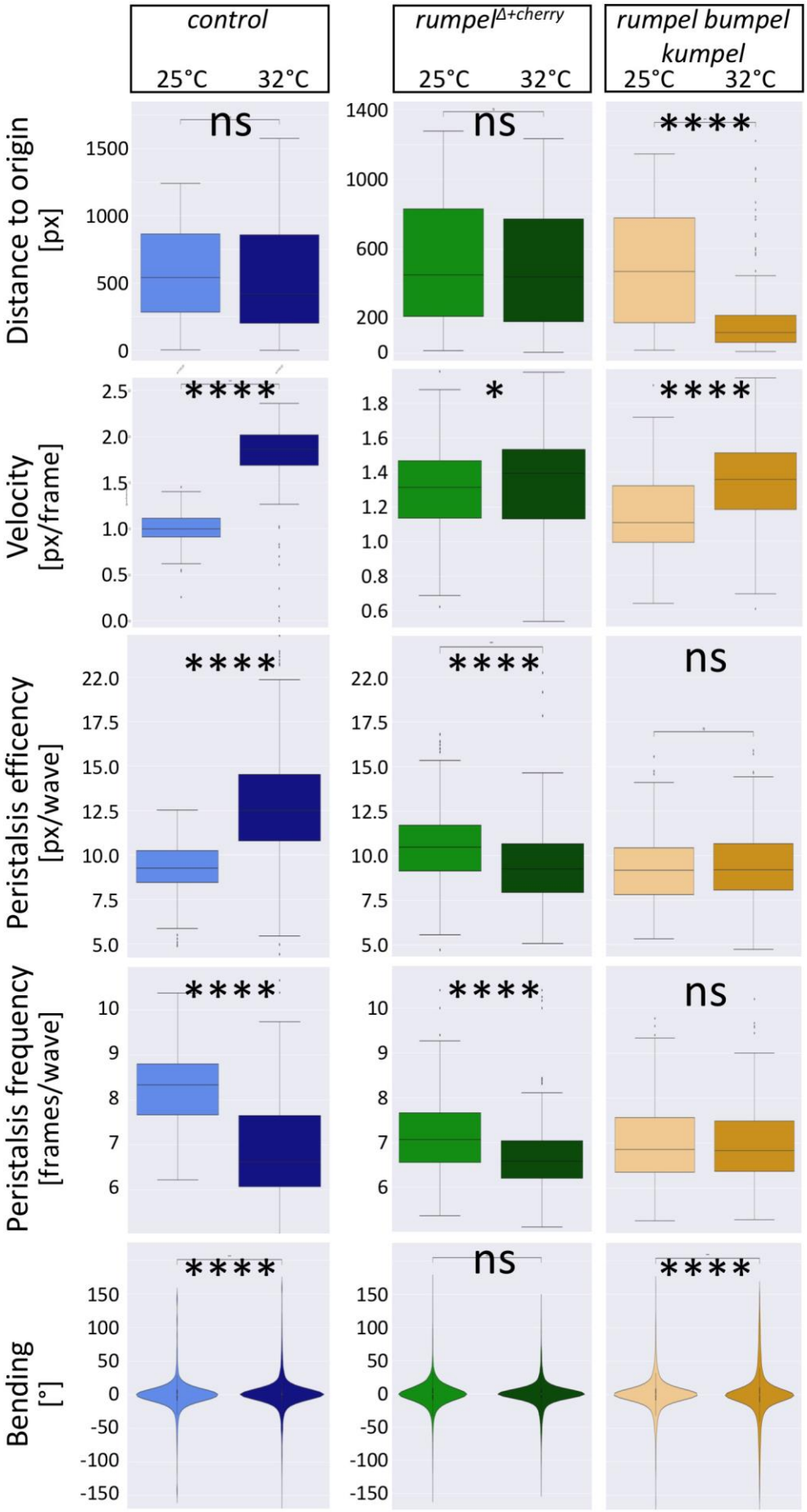

Fig. S3. *rumpel bumpel kumpel* triple mutant show a subtle temperature dependent locomotion phenotype

Free locomotion of 3-4 groups of 15 third instar larvae of the following genotypes [$rumpel^{\Delta+cherry}$, $rumpel^{\Delta+cherry}$ $bumpel^{2}$ $kumpel^{2}$, w^{1118}]. Although crawling velocity increases, control larvae [w^{1118}] show no reduced distance to origin at 32°C compared to 25°C, which is likely due to increased bending behavior. In *rumpel* mutant larvae, no difference in distance to origin can be determined. Locomotion speed increases very slightly which is due to faster not as efficient peristaltic contractions. In *rumpel bumpel kumpel* triple mutants distance to origin is dramatically affected, despite the fact that velocity increases by 20% due to an increased bending behavior.

A Percent amino acid identity

Proteins	CG9657 Rumpel	CG6723 Bumpel	CG42235 Kumpel^{PA}	CG42235 Kumpel^{PB}	CG42235 Kumpel^{PC}	CG42235 Kumpel^{PD}	CG42235 Kumpel^{PE}
Rumpel	-	51.61%	47.94%	47.94%	46.40%	43.04%	46.54%
Bumpel	52.55%	-	48.03%	46.68%	43.26%	43.43%	43.64%
Kumpel^{PA}	49.48%	48.03%	-	85.88%	63.83%	62.30%	64.01%
Kumpel^{PB}	49.14%	46.68%	85.88%	-	64.58%	63.15%	64.77%
Kumpel^{PC}	46.98%	43.26%	63.83%	64.58%	-	62.70%	67.69
Kumpel^{PD}	44.41%	43.43%	62.59%	63.15%	62.70%	-	65.09%
Kumpel^{PE}	46.99%	43.57%	64.01%	64.77%	67.69%	64.81%	-

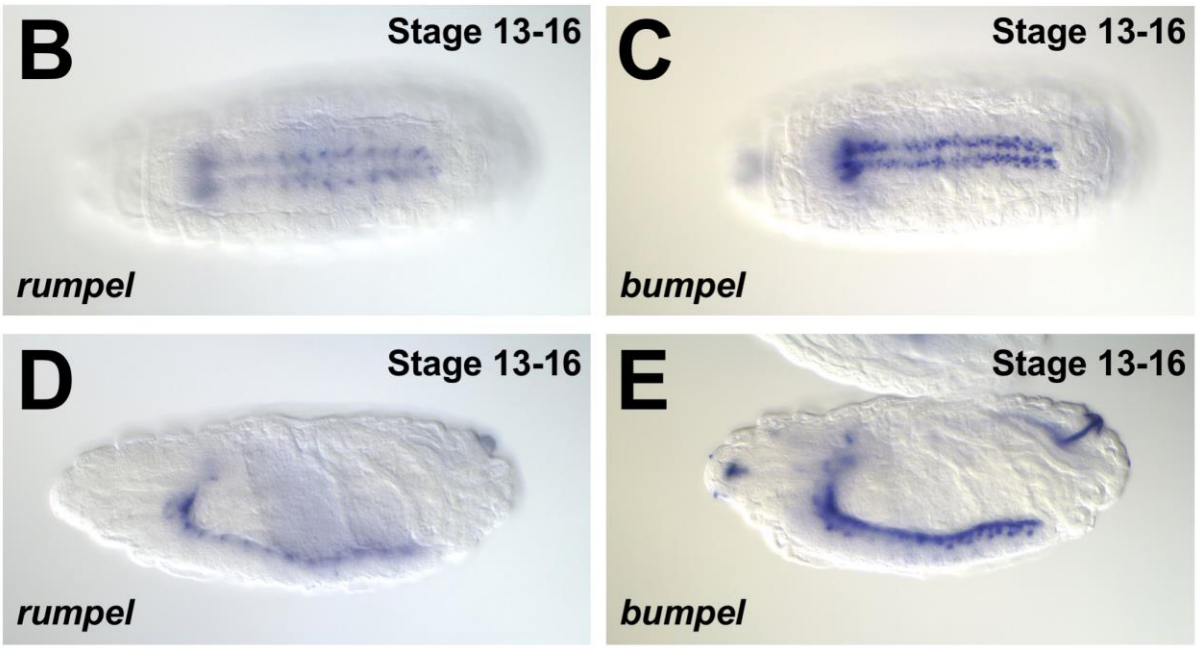

Fig. S4. Amino acid sequence comparison of Rumpel, Bumpel and Kumpel

(A) Pairwise amino acid sequence comparison between Rumpel, Bumpel and Kumpel using BLAST (ALTSCHUL et al., 1990). The homology of the different protein forms is indicated and ranges from 43% to 85% identity. The five different Kumpel isoforms are more similar on

amino acid level to each other (62-85%) than compared to Rumpel and Bumpel. (B-E) RNA *in situ* hybridizations of Drosophila embryos showing expression of *rumpel* (B,D) and *bumpel* (C,E) expression at embryonic stage 16 (images from BDGP). Both genes are expressed by the longitudinal glial cells, comprising ensheathing glia and astrocyte-like glia.

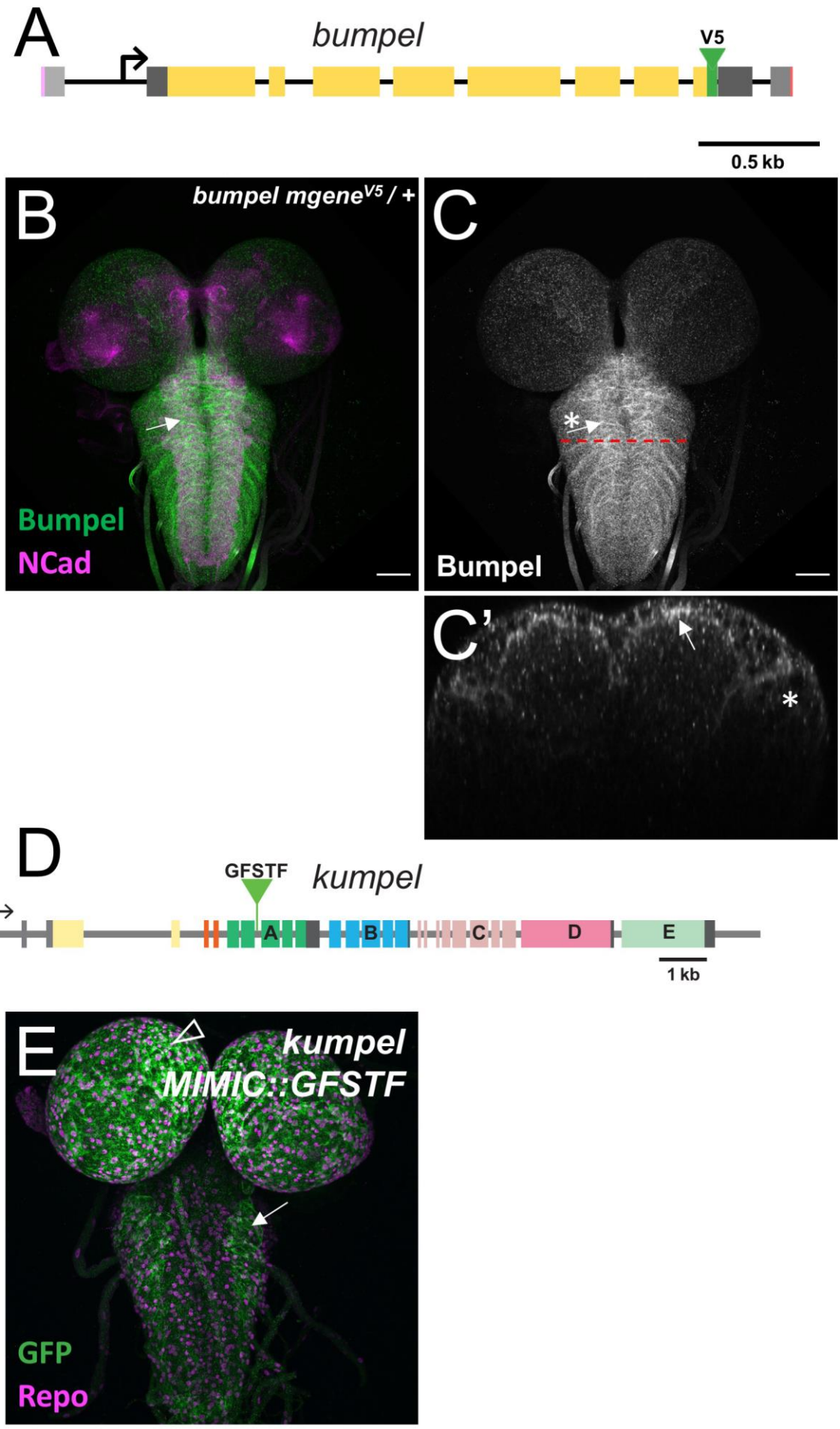

Fig. S5. *bumpel*^{mgeneV5} and *kumpel*$^{MiMIC::GFSTF}$ show expression in CNS glial cells.

All specimens are stained for Bumpel (V5; green/grey), Kumpel (GFP; green), Repo protein localization to define glial nuclei (magenta).

(A) Schematic representation of the *bumpel* minigene carrying 500 bp upstream sequence. A V5-tag was inserted at the C-terminus just before the stop-codon. (B-C) Following anti-V5 staining, Bumpel::V5 localization (green, grey) is predominantly detected in the ensheathing (arrow) and cortex glial cells (asterisk). Anti-N-Cadherin staining is used to visualize the neuropil (magenta). The red dotted line indicates the position of orthogonal plane shown in (C'). (D) Schematic representation of the MiMIC^{GFSTF} insertion into the *kumpel* gene. (E) Kumpel protein can be detected in cortex of the brain lobes (arrowhead) and the ventral nerve cord (arrow) of the larval CNS.