

Rumpel 1 MHVEELRASLQS-F-----GWPDYLVFCIMLAICAVIGIYFCIQLRLESRKQKANGS  
mSLC5A2 1 MEQHV---EAGSELGEQKVLIDNPADILVTAAYFLVIGVGLWSMFRFN-----  
mSLC5A8 1 MDASR---DIGS-F-----VVWDYVVFAGMLLSAATGIYAFAGG-----  
mSLC5A12 1 MRV-----KN-F-----EAWDYVVFAGLFLVLSGIGVFFAIKER-----

Rumpel 52 EEAASSAASYLVGGROMKIFPITMSLISSFISGITLLGTPTEVYLYGAQYMYIMGSLVLM  
mSLC5A2 47 --R-GTVGGYFLAGRSMVWVPGASLEASNI GSGHFVGLACTGAASGLAVAGFEWNALFV  
mSLC5A8 38 --GQOTSKDFLMGGRSMSAVPVALSLTASFMSAVTVLGTPAEVYRFGAIFSTIFVITYFFV  
mSLC5A12 34 --KKTTSREFLVGGROMSEFGPVALSLTASFMSAVTVLGTPAEVYRFGASEFLFLISYVVF

Rumpel 112 GFCMYFFFLPVFHELNLIISTYKYLEQRYNR-SLRLEFGSVMFIVASII-WLPIVIYVPAIA  
mSLC5A2 104 VLLLGWLFVVPVYLTAGVITMPOYLKRFGGHRIRLYLSVLSLFLYIFTKISVDMFSGAVF  
mSLC5A8 96 VVISAEVFLPVFYRLGITSTYEYLELRFNR-CIRLCGTILFVQITIL-YTGIVYAPALA  
mSLC5A12 92 VFFTSELFLPVFYRSGITSTYEYLOLRFNK-PVRYAATIIYIVQITIL-YTGIVVYAPALA

Rumpel 170 FNQATGVNIHIVTPIVCVCFYTCIGGLKAVVWTDVIQTLIMFGAMALVLIKGTLDIGG  
mSLC5A2 164 IQQALGNIIYASVIALLGITMIYTVTGGIAALMYTDIVQTFVILLAGAFIITGYAFHEVGG  
mSLC5A8 154 LNQVTGFDLWCAVAVATGVVCTFYCII GGLKAVVWTDVFCVGMVAGFASVLIQASITQHG  
mSLC5A12 150 LNQVTGFNLWASVFATGIVCTFYCSL GGLKAVVWTDVFCVGMVMI VGLTTLVIQGSNHVGG

Rumpel 230 PGVWVOKAQET-----ARLEVPNFS-----DLSERYTFYSLV-LGGVA  
mSLC5A2 224 YSGLEFDKYLGA MTSLTVSKDPSVGNISSTCYQRPDSYHLLRDPVTGDLFPALLLGLTI  
mSLC5A8 214 INKILSDAFNG-----GRLNFWNFD-----NPLQRHTFWTIV-IGGTF  
mSLC5A12 210 FNNVLEKAGNG-----SRLHIVDFDV-----DPLRRHTFWTIT-IGGTF

Rumpel 268 HWLKSNAISONMIQRYLSLPTLRDARIA----IWTFLAGVLAFLMICGYTGLLIYATYSQ  
mSLC5A2 284 VSGWYWCSDQVIVQRCLAGKNLTHIKAGCILCGYTKLMPMFLMVMPGMI-SRIYLPDEVA  
mSLC5A8 252 TWTTIYGVNQSQVQRYISCKSRLHAKLS----LYVNLVGLWVILTCSI FCGLALYSRYRE  
mSLC5A12 248 TWLG VYGVNQSTIQRCISCKTEKHAKLA----LYENLGLWII VACAVFSGLIMYSHFKD

Rumpel 324 CD-----PLETKLAQRNDQLPPLVMDTLGSPGLPGVFVAGVFS AALSSISTGINSLS  
mSLC5A2 343 CVVPEVCKRVC GTEVGC SNIAYPRLVVKLMP--NGLRGLM LAVMLAALMSSIASIFNSSS  
mSLC5A8 308 CD-----PWT SKKVS AIDQLMPYLVDILKNYPGV PGLFVACAYSGTLSTVSSSINALA  
mSLC5A12 304 CD-----PWTSGVISA PDQLMPYFVMEIFATMPGLPGLFVACAFSGTLSTVAASINALA

Rumpel 378 AVVLEDFVKTFRKSPLTEGQTA FVMRSVVVFGIIFVALV--FAVEKLGAVLQLTITISS  
mSLC5A2 401 TLFTMDIYTRLR-PRAGDKELLVGRLWVVFIVAVSWAWLPVVOAAQGGQIFDYIQSVSS  
mSLC5A8 362 AVTVEDLIKPRF-KSLSEKLSWISQGM SVLYGALCTGMA--ALASLMGALLQAALSIFG  
mSLC5A12 358 TVTFEDFVKSCF-PHLSDKLSTWLSKGLCILFGIMCT SMA--VVASLMG SVVQAALS IHG

Rumpel 436 VANGPLLGI FTAGVMLPWVNSKGAL LGFSSLIVMAWCVSAQRDLVTGDLVYKRKPYST  
mSLC5A2 460 YLAPPVSAVFLALFVPRVNEKGA FWGLVGGLLMGLARLIPEFFHG-SGS-----  
mSLC5A8 419 MVGGPLLGLFSLGII VPFANSIGALTGLLAGFALS LWWGIGAOIYPPLPERT-LPLPIET  
mSLC5A12 415 MCGGPMLGLFTLGLVFPFVNWKGALGGLLTGTLSEFWVAIGSFIYPAPESKT-LPLPLST

Rumpel 496 MGCNYTFAGEPRE-----FASLALDGA I--SSVPSA-PFOLYRISYLYFTLFGALVTIV  
mSLC5A2 509 -----CVRPSACPALECRVHYLYFAIILFICSGI  
mSLC5A8 478 YGCNIITHNGSDWMSTTEMPFSTS--AFQIHNAERTPL-MDNWYLSYLYFSTIGTITLIF  
mSLC5A12 474 EHCVEL-----NITT-----TV--APQI--SSRVL-ADTWYLSYLYFSAVGLG CIA

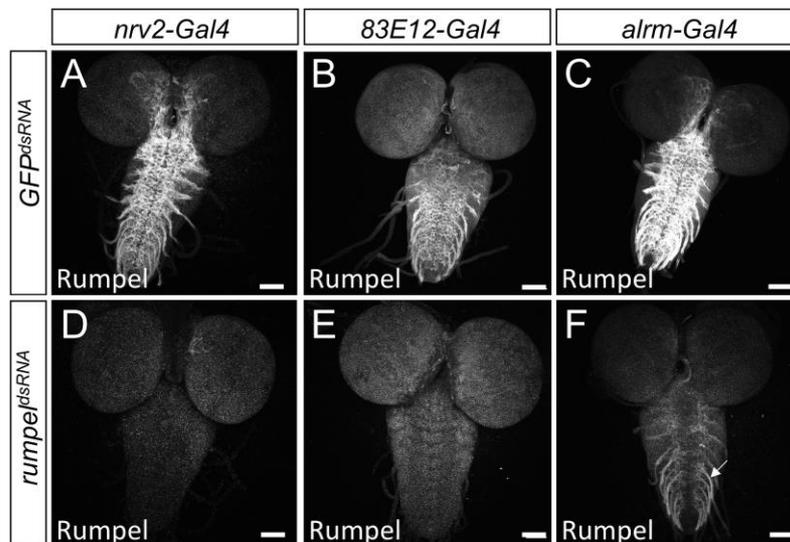
Rumpel 547 VALVTSLLRETDLDGVDTRLLTPFVRRWL-----ERRRH---RRS-----  
mSLC5A2 538 LTLGISLCTAPIPOKHLHR-LVF-----SLRHSKEERED-LDADE--  
mSLC5A8 535 VGILISLSTGG-RKQNLDPFLL-----TKODFLSNFDV  
mSLC5A12 518 AGI IISFLTGKQRGKDIDPLLRVPCNLFCFWSKKYKTLWCWGVQHDRETEODYLD SGSA

Rumpel 585 -----KIPDSGOK-----S--SN-----KQE-----TKT  
mSLC5A2 576 LEGP-----APAPVQGGQECAMEME EVQSPAPG LLRRCLLWFCGMSKSGSGSPPE-PTT  
mSLC5A8 568 FKKRNHVLN YKLHPVEVGGTD-----NPAFN-----HVELNFTDHS G KING  
mSLC5A12 578 WKQG-----VESGLQNG LKQ-----DT-LAQIPGYN-----PKEKSYNSVPE-EKT

Rumpel 602 -----  
mSLC5A2 629 EEVAATTRLEDISEDPRWARVVN LNALLMMTVA VFLWGFYA  
mSLC5A8 609 TRL-----  
mSLC5A12 617 TYF-----

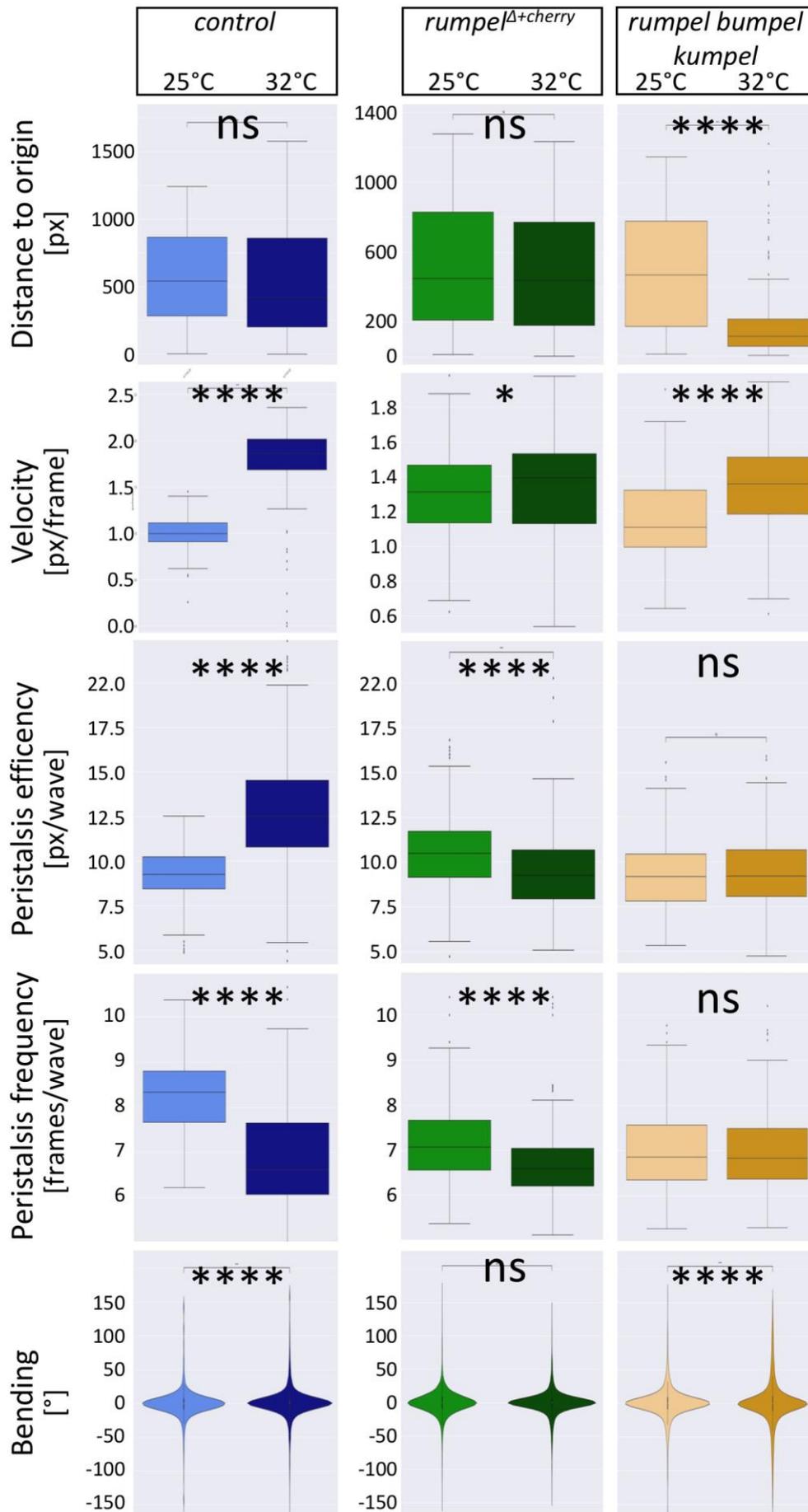
**Fig. S1. Rumpel is predicted to be a member of the Sodium Solute symporter family (SLC5)**

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<sup>dsRNABL9330</sup>*; A), in ensheathing glia (*83E12-Gal4>GFP<sup>dsRNABL9331</sup>*; B) or astrocyte-like glial cells (*alrm-Gal4>GFP<sup>dsRNABL9330</sup>*; C). (D-F) No Rumpel expression is detected upon expression of dsRNA directed against *rumpel* in cortex glia and ensheathing glia (*nrv2-Gal4>rumpel<sup>dsRNAv43922</sup>*; 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<sup>dsRNAv107361</sup>*; E). Upon silencing of *rumpel* only in astrocyte-like glial cells (*alrm-Gal4>rumpel<sup>dsRNAv43922</sup>*; F) weak Rumpel expression can be detected in ensheathing glial cells (arrow) and in the cortex. Scale bars are 50  $\mu$ m.



**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* <sup>$\Delta$ +cherry</sup>, *rumpel* <sup>$\Delta$ +cherry</sup> *bumpel*<sup>2</sup> *kumpel*<sup>2</sup>, *w*<sup>1118</sup>]. Although crawling velocity increases, control larvae [*w*<sup>1118</sup>] 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 <sup>PA</sup>	CG42235 Kumpel <sup>PB</sup>	CG42235 Kumpel <sup>PC</sup>	CG42235 Kumpel <sup>PD</sup>	CG42235 Kumpel <sup>PE</sup>
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 <sup>PA</sup>	49.48%	48.03%	-	85.88%	63.83%	62.30%	64.01%
Kumpel <sup>PB</sup>	49.14%	46.68%	85.88%	-	64.58%	63.15%	64.77%
Kumpel <sup>PC</sup>	46.98%	43.26%	63.83%	64.58%	-	62.70%	67.69
Kumpel <sup>PD</sup>	44.41%	43.43%	62.59%	63.15%	62.70%	-	65.09%
Kumpel <sup>PE</sup>	46.99%	43.57%	64.01%	64.77%	67.69%	64.81%	-

**B**

Stage 13-16

*rumpel***C**

Stage 13-16

*bumpel***D**

Stage 13-16

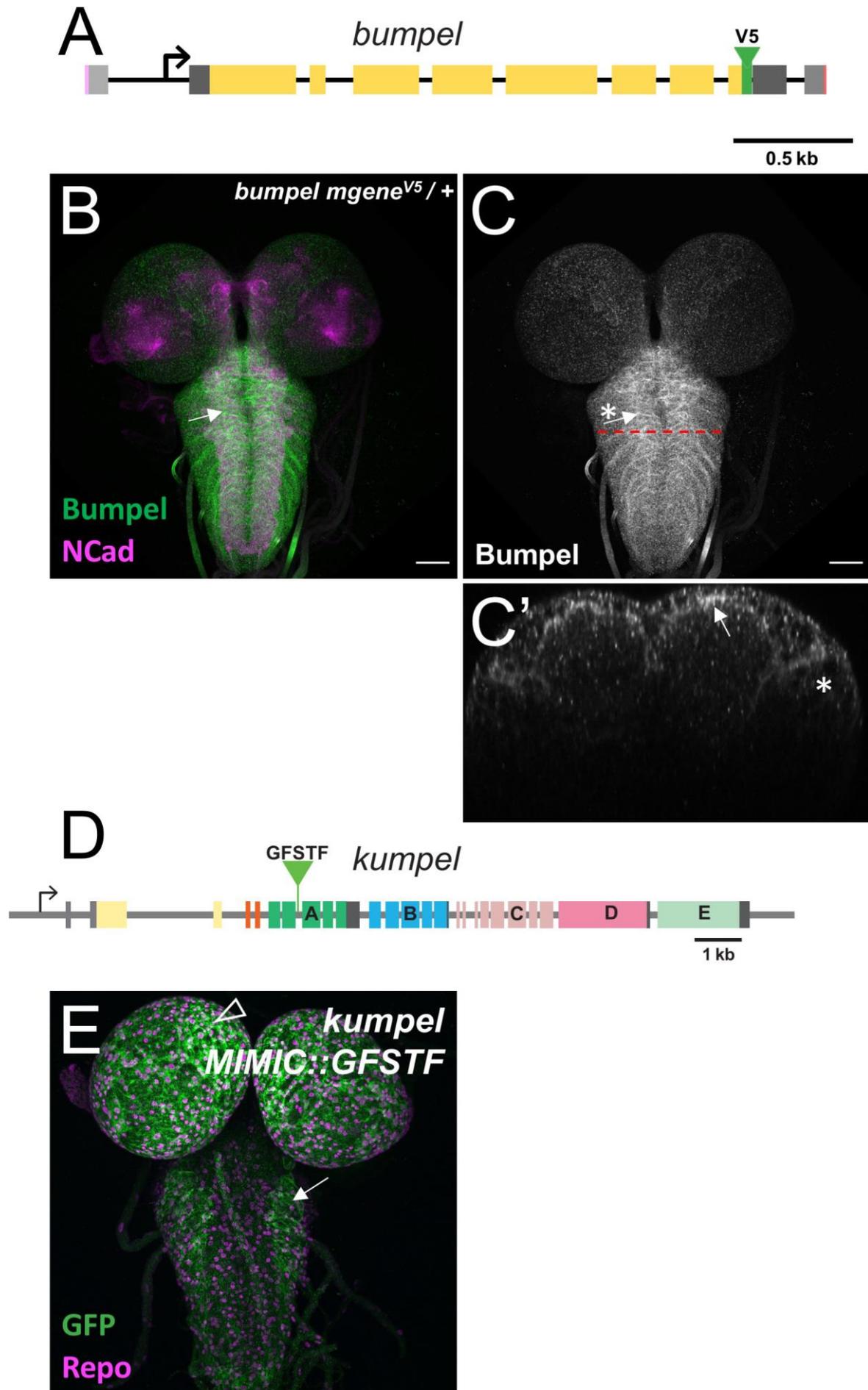
*rumpel***E**

Stage 13-16

*bumpel***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*<sup>mgeneV5</sup> and *kumpel*<sup>MiMIC::GFSTF</sup> 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<sup>GFSTF</sup> 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.