Figures and figure supplements

The β-alanine transporter BalaT is required for visual neurotransmission in *Drosophila*

Yongchao Han *et al*

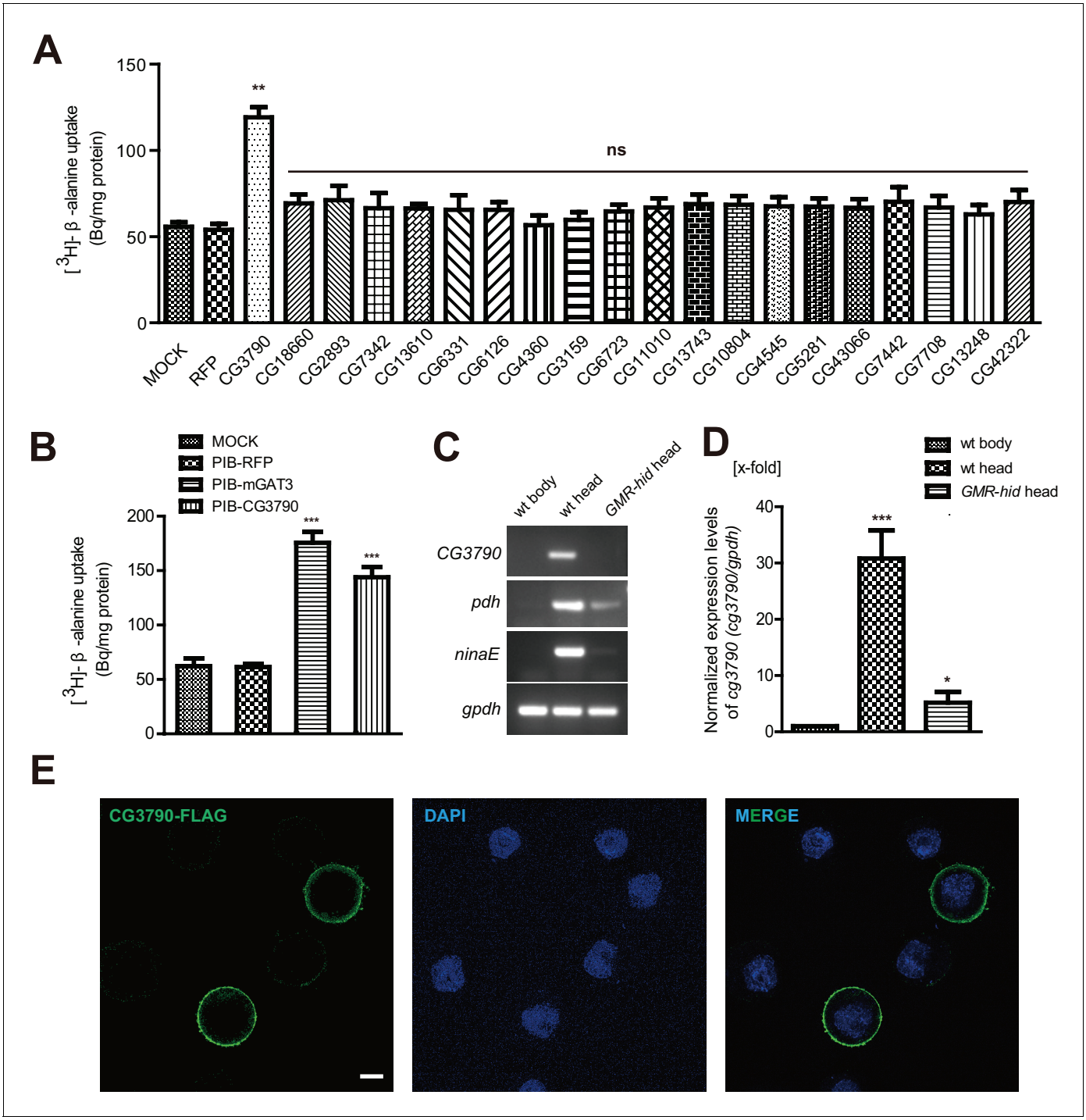

Figure 1. CG3790 is a retina-enriched β-alanine transporter. (**A**) Screening for a β-alanine transporter. HEK293T cells transiently expressing one of ~20 head-enriched potential transporters were exposed to [^{3}H]-β-alanine, which was added to the ECF buffer (final concentration 3.7 × 10^4 Bq). RFP (red fluorescent protein) was expressed as a negative control. (**B**) CG3790 transported β-alanine into S2 cells. Mouse GAT3 and RFP were used as positive and negative control, respectively. The results given are the mean values ± S.D. of four experiments. (**C–D**) CG3790 was expressed at high levels in the compound eye. (**C**) Relative RNA transcript levels (RT-PCR experiments) show that CG3790 expression is enriched in wild-type (w^{1118}) heads compared with wild-type bodies or *GMR-hid* heads. (**D**) Relative *CG3790* transcript levels from wild-type (wt) bodies, wt heads, and *GMR-hid* heads (*gpdh* served as an internal control). RNA levels were normalized to levels in wt bodies, which were set to 1. Error bars indicate standard deviations (SDs) from three replicate experiments. Significant differences between candidates and control were determined using unpaired t-tests (**p<0.01; ns, not significant). (**E**) S2 cells were transiently transfected with 3xFlag-tagged CG3790, and then labeled with Flag antibody (green) and DAPI (blue). Scale bar, 2 μm.

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Figure 1 continued on next page

Figure 1 continued

The following source data is available for figure 1:

Source data 1. Relates to ***Figure 1A and B***.
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Source data 2. Relates to ***Figure 1D***.
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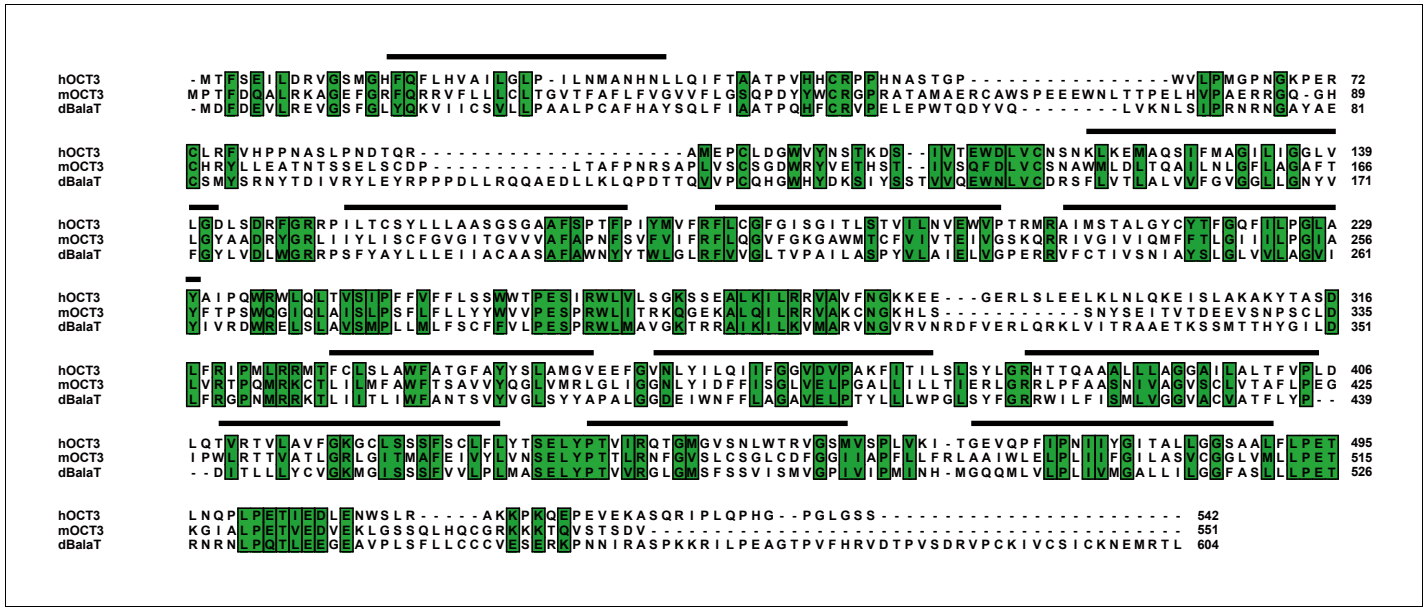

Figure 1—figure supplement 1. CG3790 is a SLC22 family protein. Alignment of the *Drosophila* CG3790 amino acid sequence with mouse OCT3 (mOCT3) and human OCT3 (hOCT3). Identical residues, found in at least two proteins, are enclosed in green boxes. CG3790 is 29% identical to mOCT3% and 30% identical to hOCT3. The transmembrane domains are indicated by solid lines above the sequences. The running tally of amino acids is indicated to the right.

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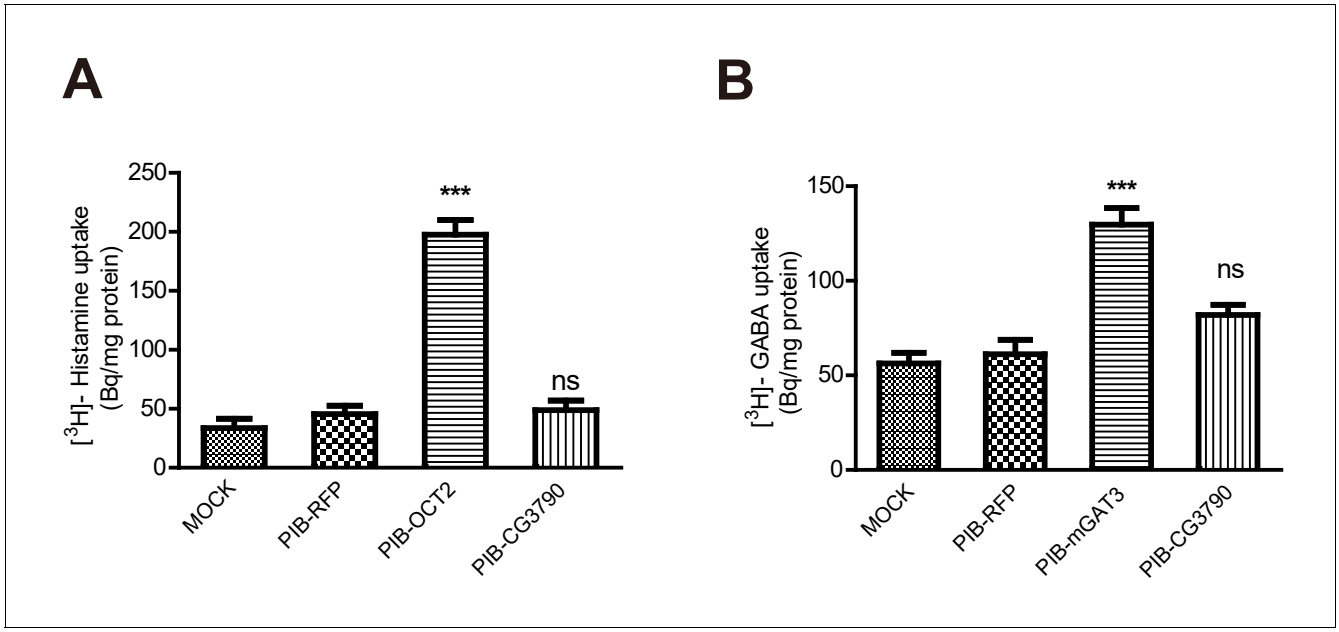

Figure 1—figure supplement 2. CG3790 is unable to transport histamine and GABA. (**A**) CG3790 did not transport GABA into S2 cells. Mouse GAT3 was used as a positive control, and RFP was used as a negative control. (**B**) CG3790 did not exhibit histamine transporting activity in S2 cells. Human OCT2 and RFP were used as positive and negative control, respectively. [^{3}H]-GABA or [^{3}H]-histamine was added to the ECF buffer (final concentration 3.7×10^4 Bq). The results given are the mean values ± S.D. of three experiments. Significant differences were determined using unpaired t-tests (***p<0.001; ns, not significant).

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The following source data is available for figure 1:

Figure supplement 2—Source data 1. Relates to ***Figure 1—figure supplement 2A and B***.

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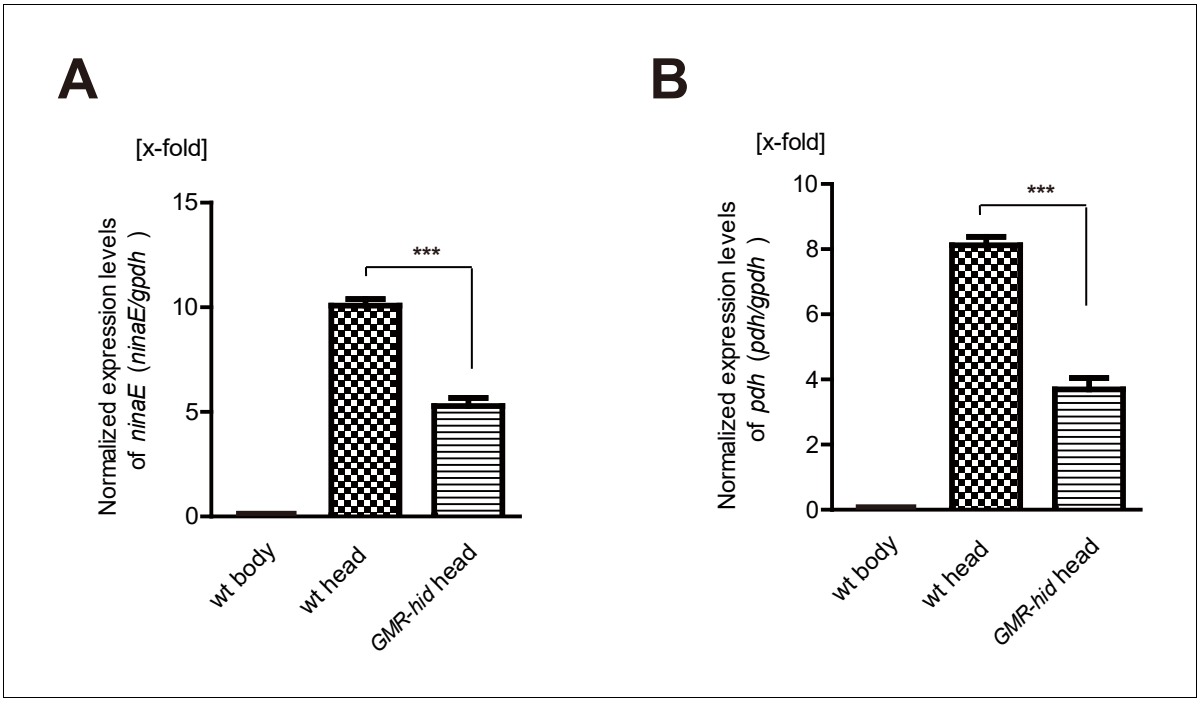

Figure 1—figure supplement 3. *GMR-hid* abolished expression of *ninaE* and *pdh*. (**A–B**) Wild-type heads contain more transcripts for (**A**) *ninaE* and (**B**) *pdh* than wild-type bodies and *GMR-hid* heads (*gpdh* served as an internal control, and mRNA levels were normalized to wild-type body). Data is processed by logarithmic 2 and averaged from three replicate experiments. Error bars indicate SDs. Significant differences between candidates and control were determined using unpaired t-tests (***p<0.001).

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The following source data is available for figure 1:

Figure supplement 3—Source data 1. Relates to ***Figure 1—figure supplement 3A and B***.

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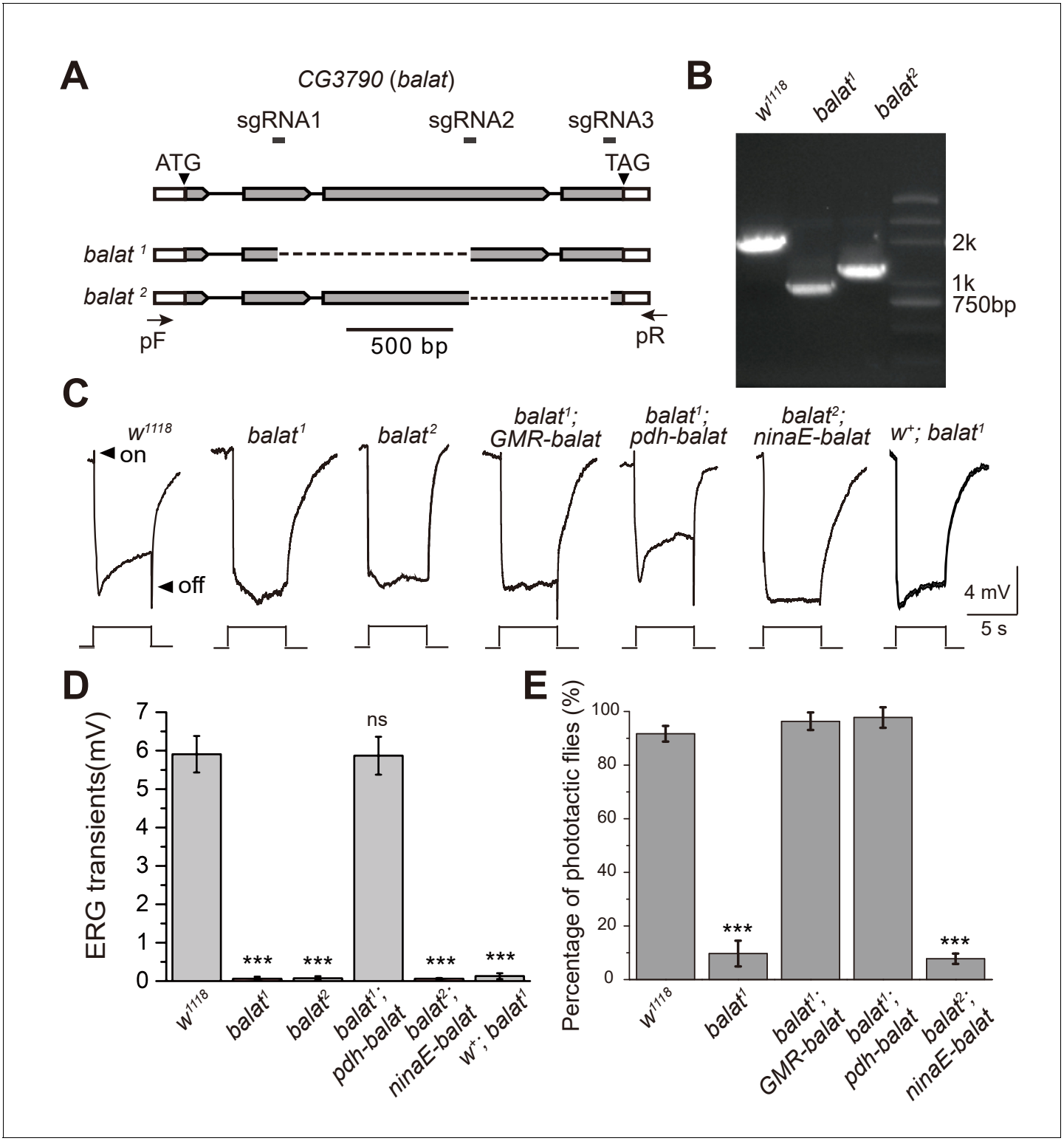

Figure 2. Mutations in *balat* disrupt photoreceptor cell synaptic transmission and visual behavior. (**A**) Schematic for *balat* knock-out by sgRNA targeting. The organization of the *balat* locus and the expected structures of *balta*^{1} and *balat*^{2} alleles are shown. Boxes represent exons with the coding region between ATG and TAG. The sgRNA1 and sgRNA2 primer pair was used to generate the *balat*^{1} allele; the sgRNA2 and sgRNA3 primer pair was used to generate the *balat*^{2} allele. Arrows indicate the primers used for genomic PCR. (**B**) PCR products obtained from *balat*^{1} and *balat*^{2} mutants show successful gene deletions. (**C**) ERG recordings from: wild-type (*w*^{1118}), *balat*^{1}, *balat*^{2}, *balat*^{1}*;GMR-balaT*, *balat*^{1}*;pdh-balaT*, *balat*^{2}*;ninaE-balaT* and *w*$^{+}$*; balat*^{1} flies. Young flies (<3 days after eclosion) were dark adapted for 1 min and subsequently exposed to a 5 s pulse of orange light.

Figure 2 continued on next page

Figure 2 continued

ON and OFF transients are indicated by arrows. All flies contained the w^{1118} mutation except for the w^{+}; $balat^{1}$ flies. (**D**) Quantitative analysis of the amplitudes of ERG OFF transients shown in C. (**E**) Phototaxis behaviors of wt, $balat^{1}$ $balat^{1}$;*GMR-balaT*, $balat^{1}$;*pdh-balaT* and $balat^{2}$;*ninaE-balaT* flies. Significant differences between mutant and wild-type flies were determined using unpaired t-tests (***p<0.001; ns, not significant).

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The following source data is available for figure 2:

Source data 1. Relates to ***Figure 2D and E***.

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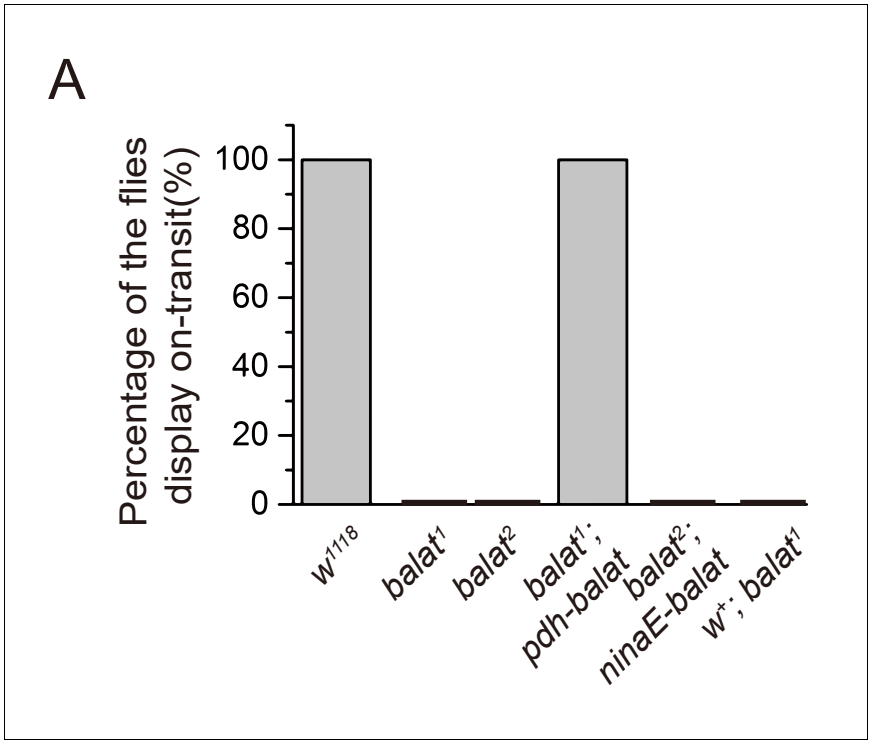

Figure 2—figure supplement 1. Quantification of the ERG ON transients. Percentage of flies of indicated genotypes that have ON transients were quantified. The ERG profiles from at least 7 flies of each genotype were scored.

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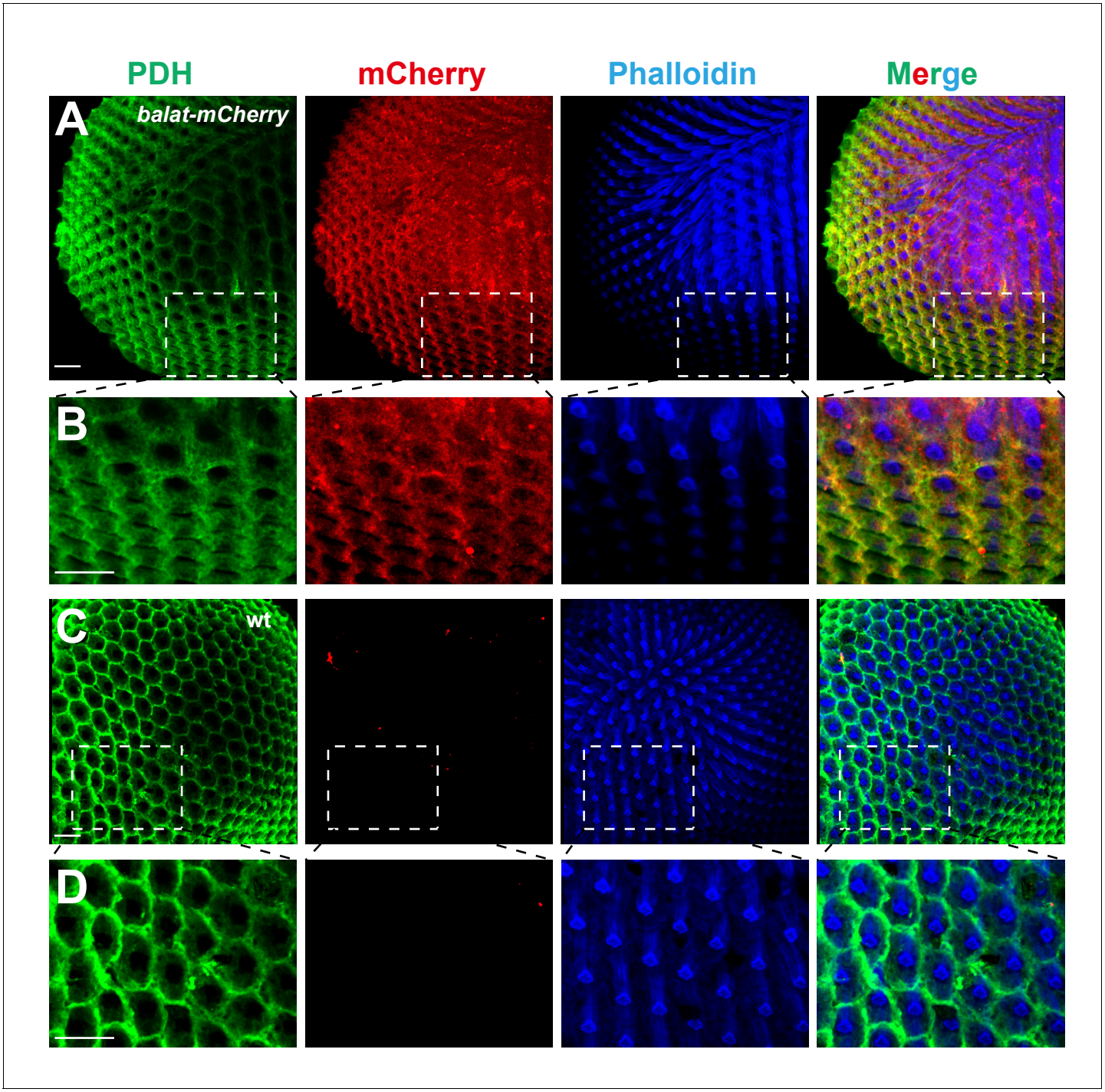

Figure 3. BalaT localizes to pigment cells. (**A–B**) Compound eyes from *balat-mcherry* flies that express mCherry (red) driven by the endogenous *balat* promoter were labeled for PDH (green), mCherry (red), and phalloidin (blue). High-magnification images are shown in (**B**). (**C–D**) mCherry signals were not detected in wild-type (wt) retinas. Scale bars represent 20 μm.

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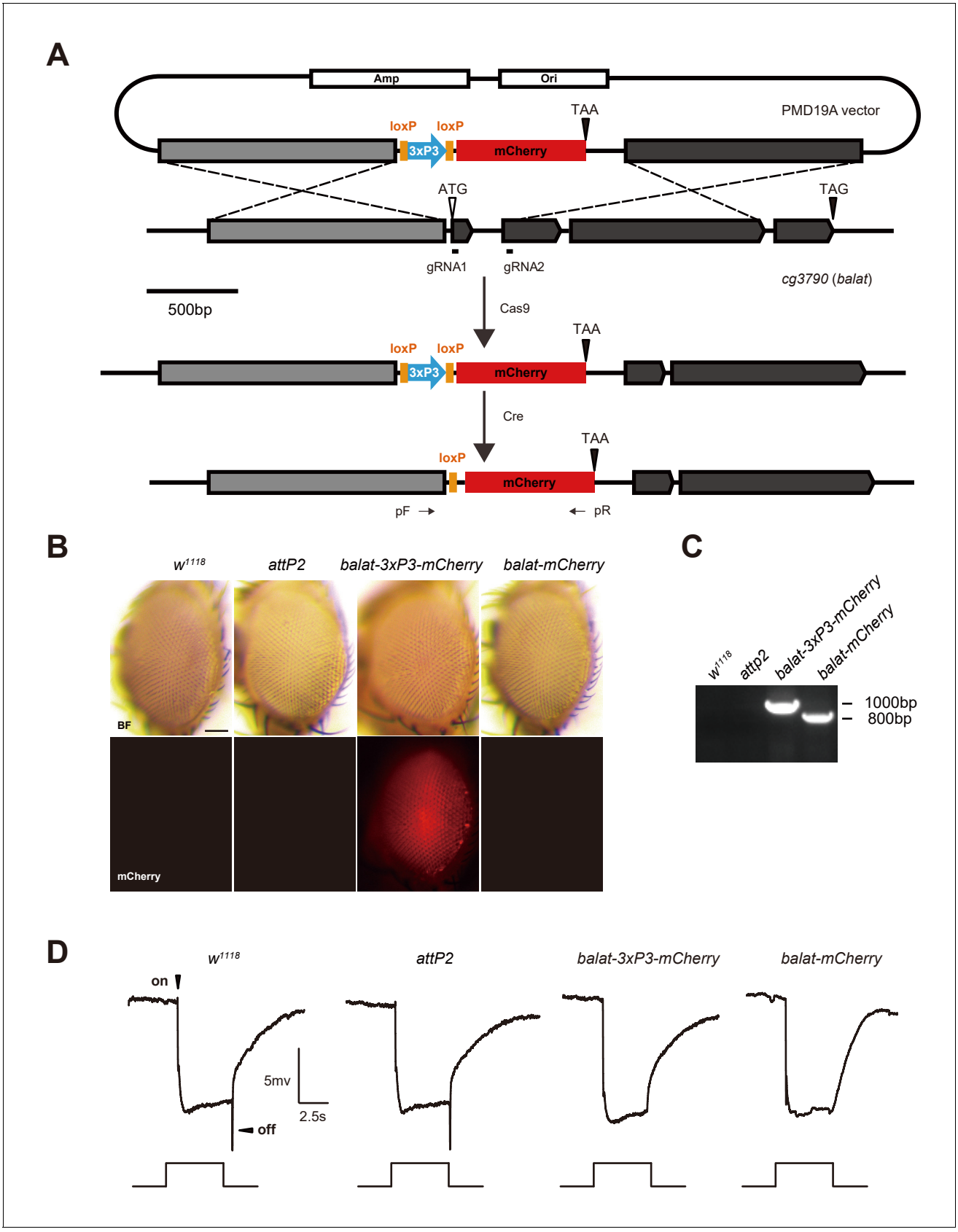
A
Amp
Ori
PMD19A vector
TAA
loxP
loxP
3xP3
mCherry
ATG
TAG
gRNA1
gRNA2
cg3790 (balat)
500bp
Cas9
TAA
loxP
loxP
3xP3
mCherry
Cre
TAA
loxP
mCherry
pF
pR
B
w1118
attP2
balat-3xP3-mCherry
balat-mCherry
BF
mCherry
C
w1118
attp2
balat-3xP3-mCherry
balat-mCherry
1000bp
800bp
D
w1118
attP2
balat-3xP3-mCherry
balat-mCherry
on
off
5mv
2.5s

Figure 3—figure supplement 1. Generation of *balat-mcherry* flies. (**A**) Schematics for generating *balat-mcherry* flies. The mcherry fluorescent protein, driven by an eye-specific 3XP3 promoter, was inserted into the *balat* genomic locus using CRISPR/Cas9-mediated homologous recombination. The 3XP3 promoter region was subsequently removed by Cre recombinase. PCR primers (arrows, pF and pR) were used to verify the *balat-mcherry* knock-in flies. (**B**) Images show flies with the 3XP3-mcherry reporter inserted into the *balat* locus (*balat-3XP3-mcherry*), and the *balat-mcherry* knock-in flies after Cre/LoxP-mediated recombination. Scale bar represents 150 μm. (**C**) Genomic PCR products from wild-type (w^{1118}), attP2, *balat-3XP3-mcherry*, and *balat-mcherry* flies show successful gene targeting. (**D**) ERG recordings from wild-type (w^{1118}), *attP2*, *balat-3XP3-mcherry*, and *balat-mcherry* flies. The *mcherry* insertion caused the loss of 'on' and 'off' transients.
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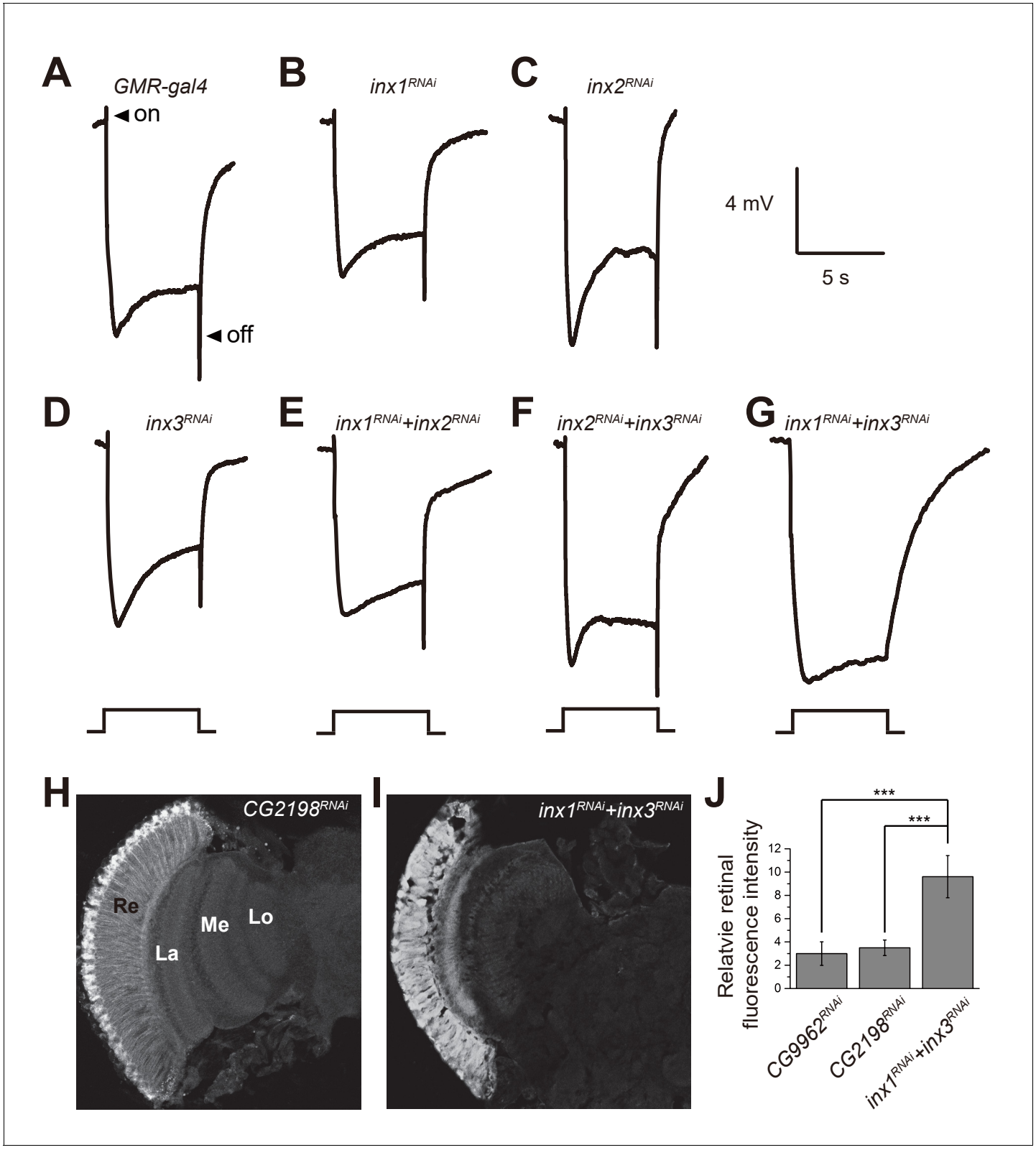

Figure 4. Specific knockdown of Inx1 and Inx3 in compound eyes blocks visual neurotransduction. ERG recordings of (**A**) control (*GMR-gal4*), (**B**) *inx1*^{RNAi} (*GMR-gal4/ UAS-inx1*^{RNAi}), (**C**) *inx2*^{RNAi} (*GMR-gal4/UAS-inx2*^{RNAi}), (**D**) *inx3*^{RNAi} (*GMR-gal4/ UAS-inx3*^{RNAi}), (**E**) *inx1*^{RNAi} + *inx2*^{RNAi} (*GMR-gal4/ UAS-inx1*^{RNAi} *UAS-inx1*^{RNAi}), (**F**) *inx2*^{RNAi} + *inx3*^{RNAi} (*GMR-gal4/UAS-inx2*^{RNAi} *UAS-inx3*^{RNAi}), and (**G**) *inx1*^{RNAi} + *inx3*^{RNAi} (*GMR-gal4/UAS-inx1*^{RNAi} *UAS-inx3*^{RNAi}) are shown. Arrows indicate ON and OFF transients in (**A**). Flies (~1 day after eclosion) were dark adapted for 1 min and subsequently exposed

Figure 4 continued on next page

Figure 4 continued

to a 5 s pulse of orange light. (**H–I**) β-alanine was immunolabeled in horizontal sections of heads from (**H**) control: *CG2198^{RNAi}* (*GMR-gal4/UAS-CG2198^{RNAi}*) and (**I**) *inx1^{RNAi} + inx3^{RNAi}* (*GMR-gal4/UAS-inx1^{RNAi} UAS-inx3^{RNAi}*) flies. Re, retina; La, lamina; Me, medulla; Lo, lobula. (**J**) Fluorescence intensity ratios of β-alanine signals between retina and lobula. Quantifications of all genotypes are averages of six replicate experiments. Significant differences between *inx1^{RNAi} + inx3^{RNAi}* and controls (*CG2198^{RNAi}* and *CG9962^{RNAi}*) were determined using unpaired t-tests (***p<0.001; ns: not significant).

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The following source data is available for figure 4:

Source data 1. Relates to ***Figure 4J***.

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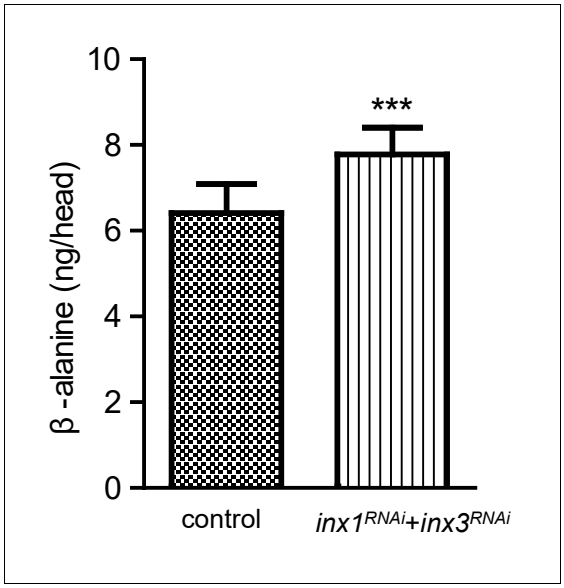

Figure 4—figure supplement 1. Accumulation of *β*-alanine in heads of *Inx1* and *Inx3* double RNAi flies. Levels of *β*-alanine in heads of control and $inx1^{RNAi}$ + $inx3^{RNAi}$ (*GMR-gal4/UAS-*$inx1^{RNAi}$ *UAS-*$inx3^{RNAi}$) flies. Error bars indicate SDs, and significant differences between mutant and wt flies were determined using unpaired t-tests (***p<0.001).

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The following source data is available for figure 4:

Figure supplement 1—Source data 1. Relates to ***Figure 4—figure supplement 1***.

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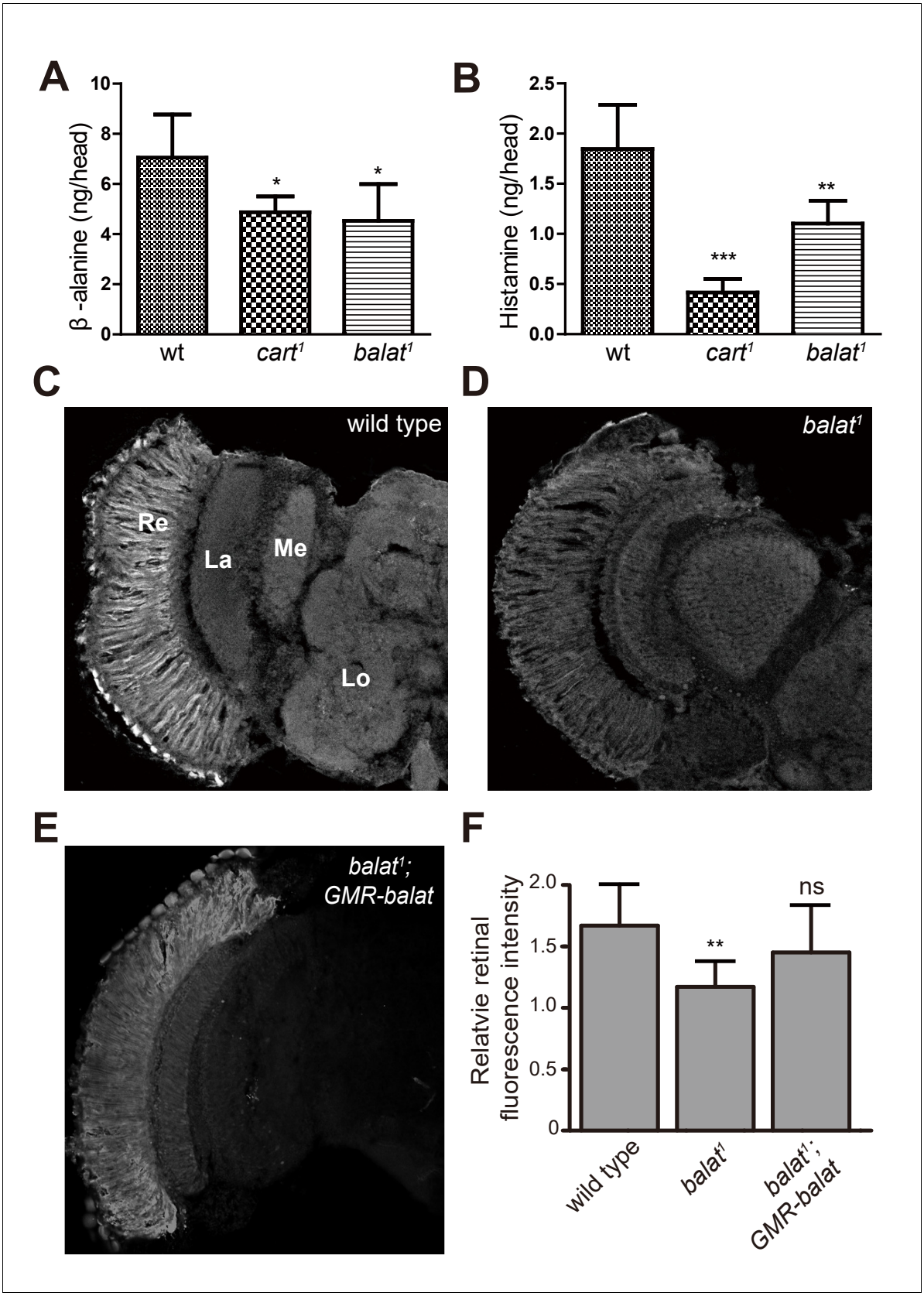

Figure 5. Loss of BalaT affects the level and distribution of β-alanine in vivo. (**A–B**) Levels of (**A**) β-alanine and (**B**) histamine in heads of wild-type (wt, w^{1118}), $cart^1$, and $balat^1$ flies are shown. Error bars indicate SDs, and significant differences between mutant and wt flies were determined using

Figure 5 continued on next page

Figure 5 continued

unpaired t-tests (*p<0.05; **p<0.01). (**C–E**) β-alanine was immunolabeled in horizontal sections of heads from (**C**) wild-type (w^{1118}), (**D**) *balat*^{1}, and (**E**) *balat*^{1}*;GMR-balat* flies. Re, retina; La, lamina; Me, medulla; Lo, lobula. (**F**) Fluorescence intensity ratios of β-alanine labeling between retina and lobula. Significant differences between mutant and wild-type flies were determined using unpaired t-tests (**p<0.01; ns: not significant).

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The following source data is available for figure 5:

Source data 1. Relates to ***Figure 5A and B***.

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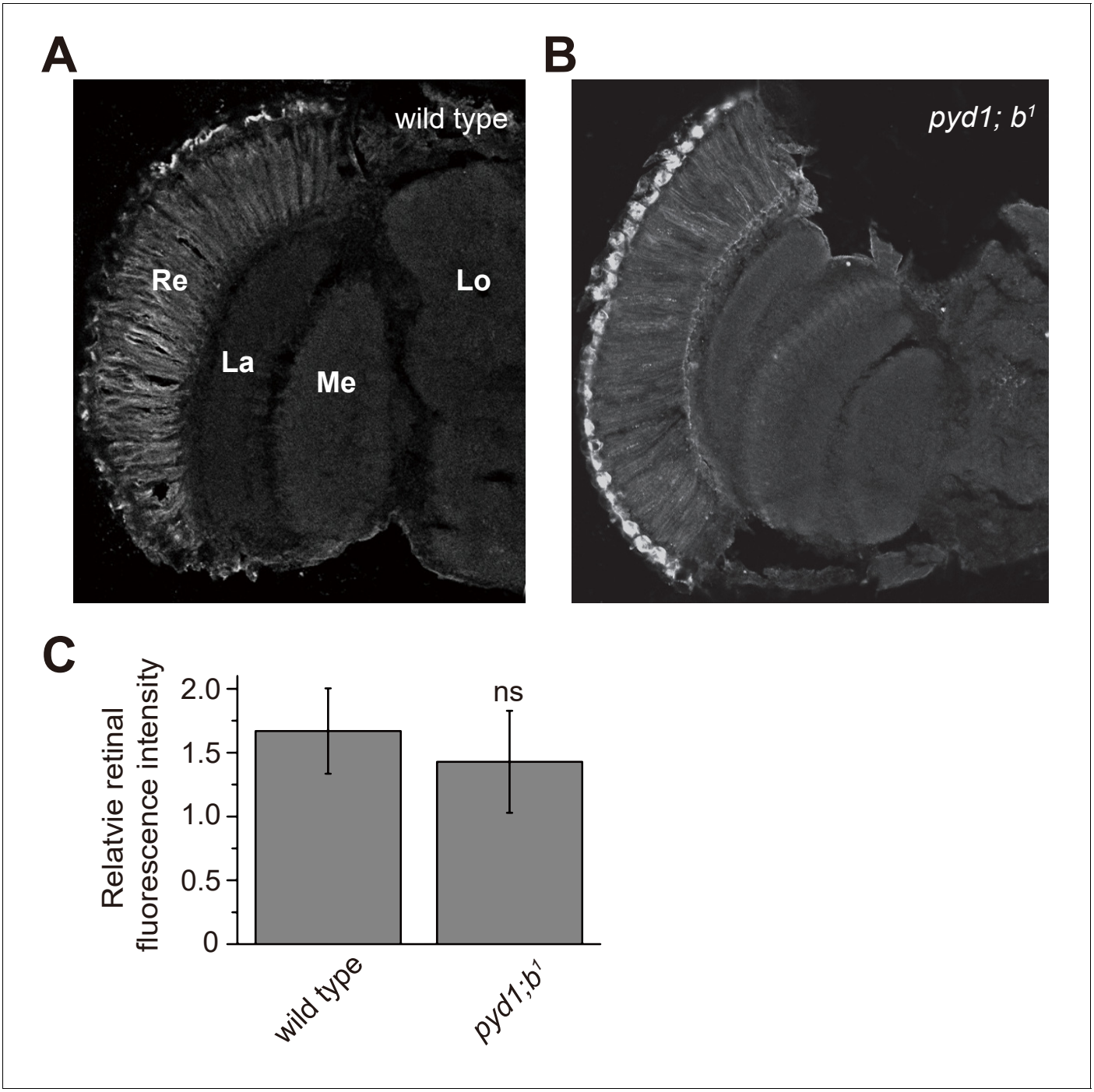

Figure 5—figure supplement 1. Distribution of β-alanine was unaffected in *pyd1* and *balck* double mutant flies. β-alanine was immunolabeled in horizontal sections of heads from (**A**) wild type (w^{1118}) and (**B**) *pyd1;b*^{1} flies. Re, retina; La, lamina; Me, medulla; Lo, lobula. (**C**) Fluorescence intensity ratios of β-alanine signals between retina and lobula. Quantifications of both genotypes are averages of six replicate experiments. Significant difference between wild type and *pyd1;b*^{1} was determined using unpaired t-tests (ns: not significant).

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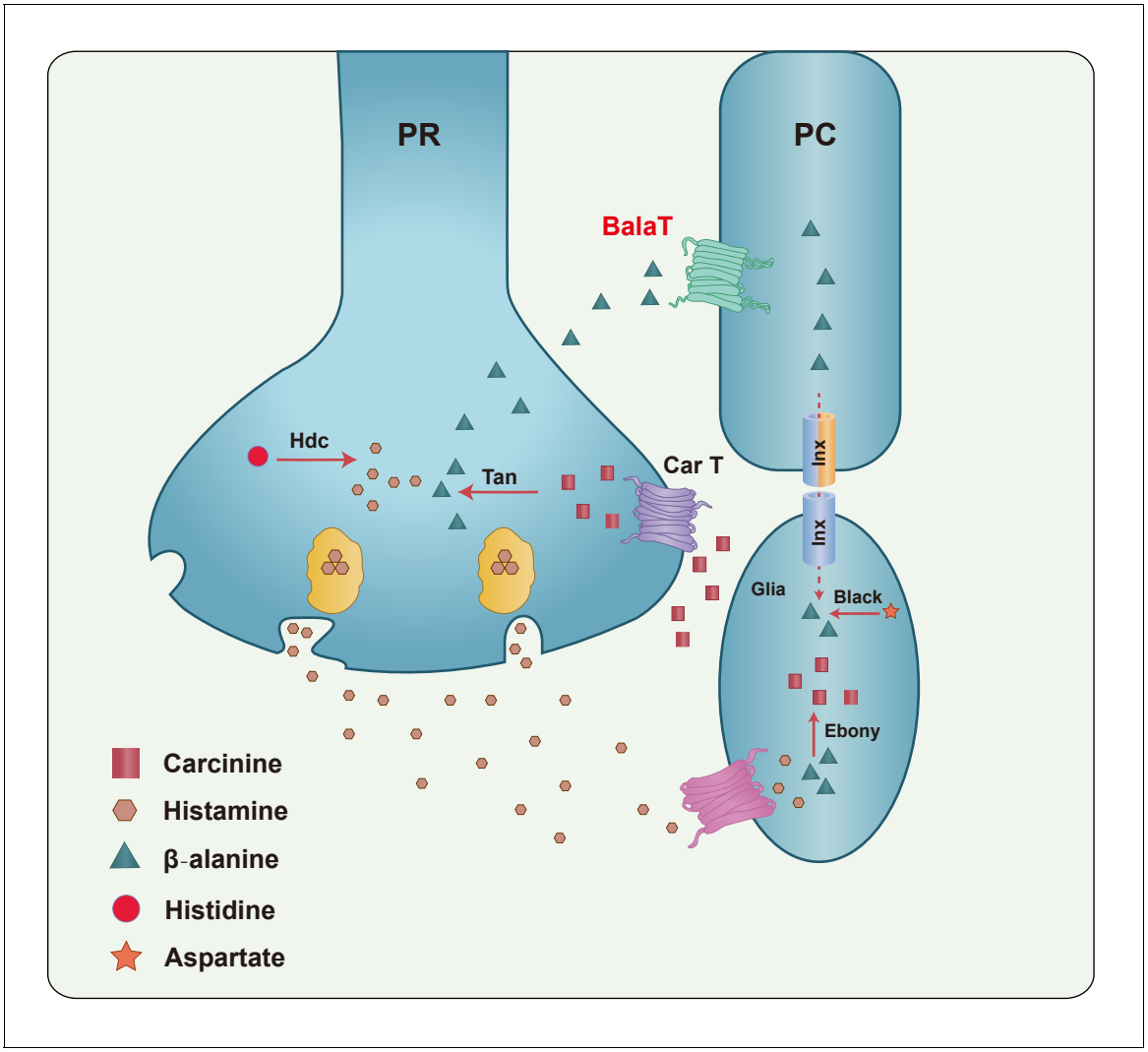

Figure 6. A model of the histamine recycling pathway. Histamine is initially synthesized by histidine decarboxylase (Hdc) in photoreceptor cells (PR). Upon light stimulation, PRs release histamine into the synaptic cleft. Released histamine is quickly taken up by an unknown histamine transporter into epithelial glial cells that express Ebony. In these glia, histamine is conjugated to β-alanine, which inactivates histamine and generates carcinine. Carcinine is released into the synaptic cleft and subsequently internalized, via CarT, by the PRs. After carcinine is hydrolyzed to histamine and β-alanine by Tan hydrolase in the PR, histamine is re-packaged into synaptic vesicles, whereas β-alanine is delivered to and subsequently internalized, via the BalaT transporter, by retinal pigment cells (PCs). PCs can store β-alanine or deliver β-alanine to the laminar glia cells through a gap junction network involving Inx1 and Inx3, which are expressed in PCs. In glial cells, β-alanine is conjugated to histamine, and the cycle repeats. Moreover, β-alanine can be synthesized by Black, which is an aspartate decarboxylase that is expressed together with Ebony in optic lobe glia.

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