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Supplementary Information for

Nociception and Hypersensitivity involve distinct neurons and molecular transducers in *Drosophila*

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This PDF file includes:

Figures S1 to S8
Legends for Movies S1 to S2

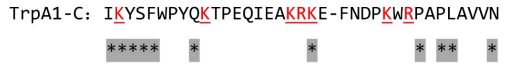
Other supplementary materials for this manuscript include the following:

Movies S1 to S2

A

TrpA1 alleles	Intact isoforms	Mutant isoforms
A-KI	A	B, C, D, E
B-KI	B	A, C, D, E
C-KI	C	A, B, D, E
D-KI	D	A, B, C, E
E-KI	E	A, B, C, D
AD-KO	B, C, E	A, D
BC-KO	A, D, E	B, C
E-KO	A, B, C, D	E

B



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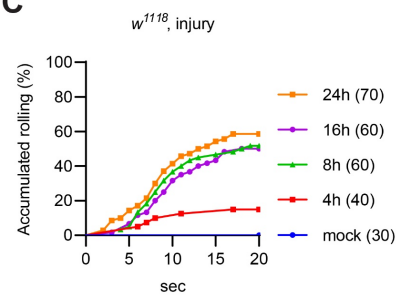

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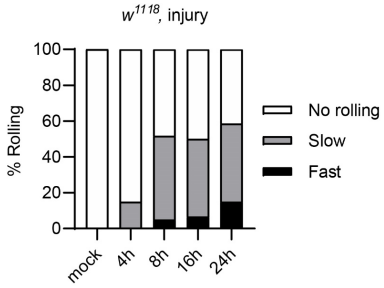

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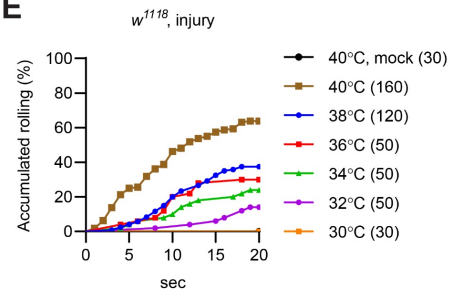

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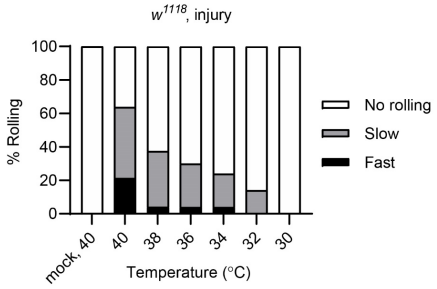

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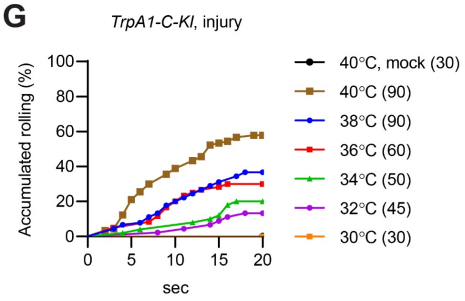

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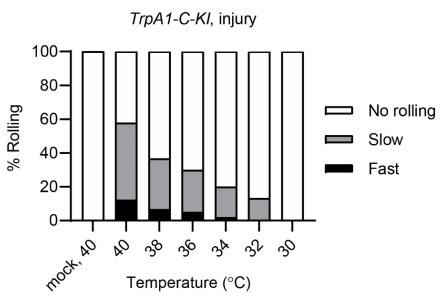

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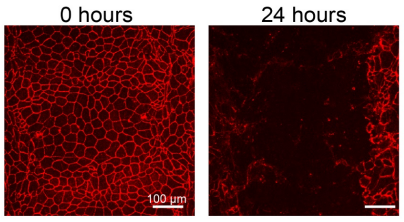

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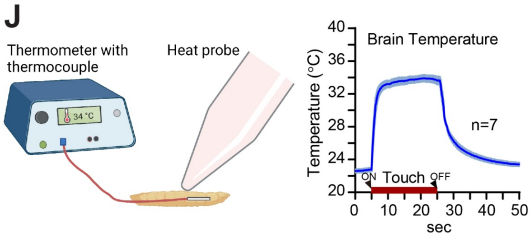

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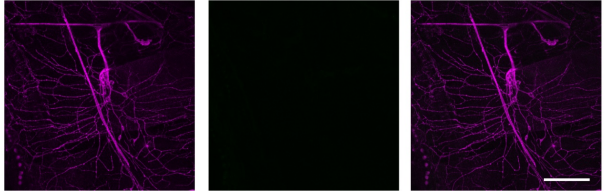

Figure S1. Thermal allodynia induced by UVC.

(A) Summary of *TrpA1* isoform-specific alleles used for behavioral assay. (B) Alignment of the linker domain encoded by alternative exon 13 (*TrpA1-C*) and exon 12 (*TrpA1-D*). Basic residues lysine (K) and arginine (R) are marked in red. Identical residues are marked with *. (C and D) Heat responses of w^{1118} larvae to 40℃ at various time points after UVC treatment. No rolling was found in mock group, which were tested 24h after mock treatment. (E and F) Heat responses of w^{1118} larvae to different temperatures at 24h after UVC treatment. (G and H) Heat responses of *TrpA1-C-KI* larvae to different temperatures at 24h after UVC treatment. For (C-H), sample numbers are indicated in the bracket. (I) Larval epidermal cell morphology was determined at 24h after UVC treatment, by immunostaining with the anti-Fasciclin-3 antibody to mark epidermal cell boundary. (J) Measurement of larval brain temperature in response to a 40℃-heat probe touching the A4 segment of larvae for a 20 s duration. As shown in the diagram, a tiny thermocouple was inserted into larval brain lobe location (around T3-A1 segment) through an excision at the posterior end. (K) *R21G01-GAL4* did not label larval PNS neurons. Larval PNS neurons were stained using the anti-HRP-Cy3 antibody in *R21G01-GAL4>UAS-6xGFP* larvae. Scale bar (I and K), 100 μm.

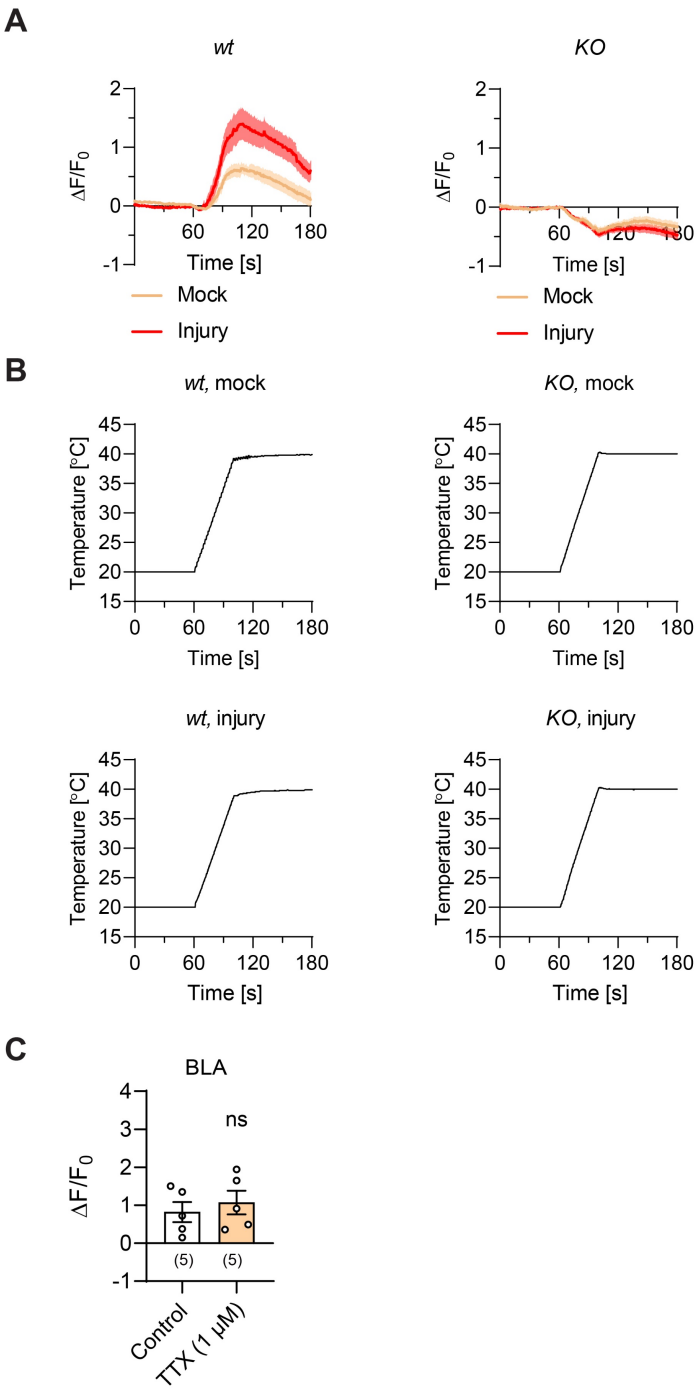

Figure S2. Heat responses of BLA neurons under mock and tissue injury conditions. (A) Averaged heat responses of BLA neurons from wild-type and *TrpA1-KO* larvae are shown over time. Larvae were subject to mock or UVC treatment 24 hrs before imaging. Heat responses of BLA neurons were measured by GCaMP fluorescent signals. Curves were presented in mean ± SEM. n = 7 for each condition. Genotypes used are: *wt* (*w; UAS-*

GCaMP6s/+; R21G01-GAL4/+). *KO* (*w*; *UAS-GCaMP6s/+; R21G01-GAL4, TrpA1-KO/TrpA1-KO)*. (B) Samples of temperature ramps are shown over time for each genotype and condition. The ramp was from 20℃ to 40℃ at about 0.5℃/sec and showed little variation. (C) Peak heat responses of BLA neurons in the absence or presence of tetrodotoxin (TTX, 1 μM). TTX was used here to isolate intrinsic heat responses of BLA neurons by blocking network activity. The sample numbers are indicated in the brackets. Error bars, SEM. Unpaired Student's *t*-tests were performed, ns, no significant difference.

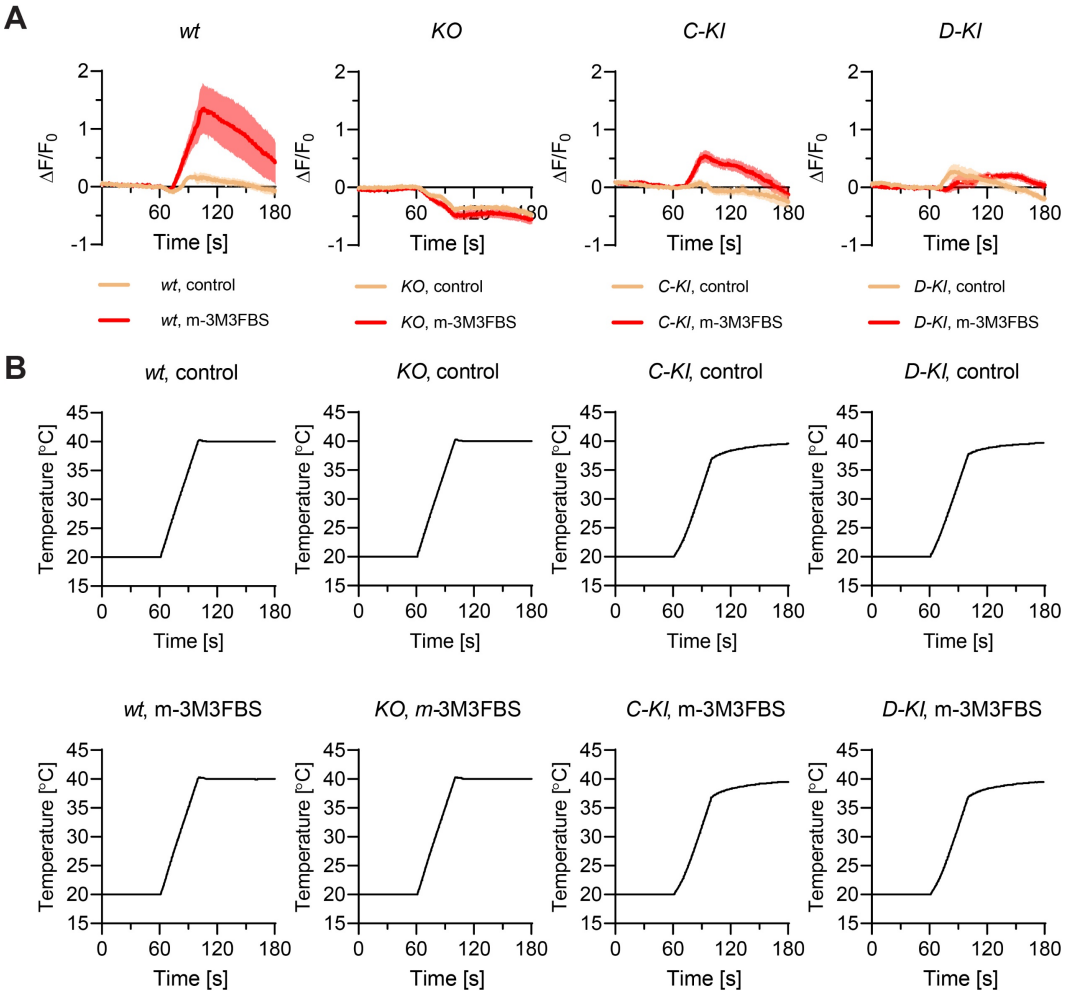

Figure S3. Heat responses of BLA neurons upon PLC activator m-3M3FBS.

(A) Averaged heat responses of BLA neurons from wild-type, *TrpA1-KO*, *TrpA1-C-KI,* and *TrpA1-D-KI* larvae are shown over time. Their genotypes are: *wt* (*w; UAS-GCaMP6s/+; R21G01-GAL4/+)*, *KO* (w; *UAS-GCaMP6s/+; R21G01-GAL4, TrpA1-KO/TrpA1-KO)*, *C-KI* (*w; R21G01-lexA/lexAop2-myr-GCaMP6s; TrpA1-C-KI/TrpA1-C-KI)*, and *D-KI* (*w; R21G01-lexA/lexAop2-myr-GCaMP6s; TrpA1-D-KI/TrpA1-D-KI*). Heat responses were measured by GCaMP fluorescent signals, and curves were presented in mean ± SEM. n = 7 or 8 for each condition. 20 μM m-3M3FBS or DMSO as control was bath applied and incubated for 15 mins before imaging. (B) Samples of temperature ramps are shown over time for each condition. The ramp speed was about 0.5℃/sec.

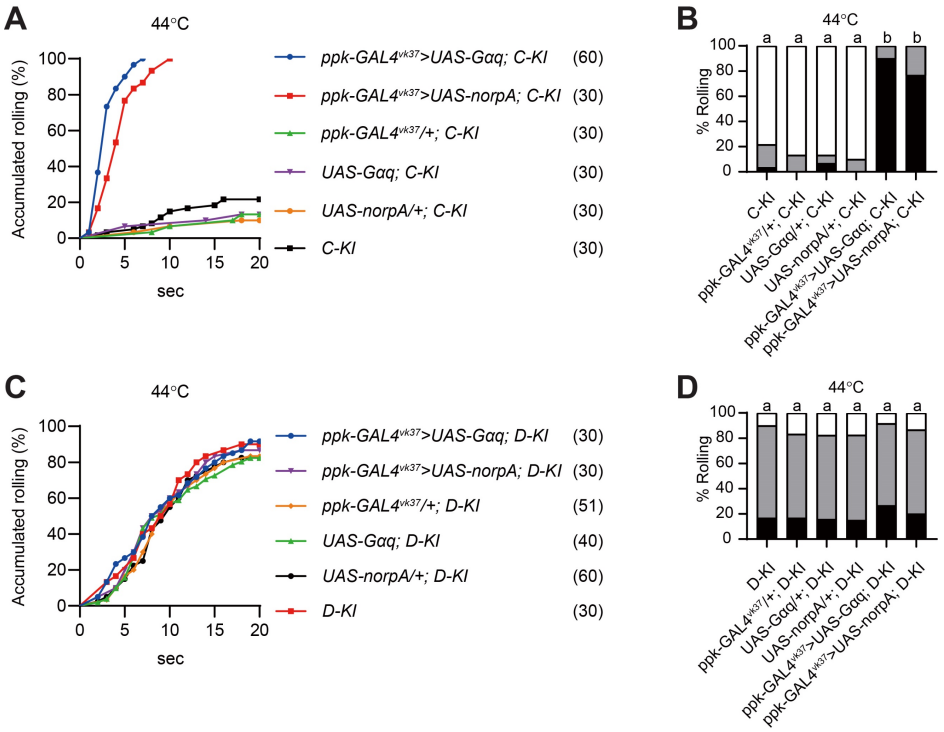

Figure S4. Gq-PLC modulates heat responses of TrpA1-C but not TrpA1-D in C4da neurons.

Responses of *TrpA1-C-KI* (A and B) and *TrpA1-D-KI* (C and D) larvae in which G protein (*Gαq*) and PLC (*norpA*) were overexpressed in C4da neurons under *ppk-GAL4^{vk37}* control, to a 44°C-heat probe in the absence of tissue injury. Sample numbers are indicated in the bracket. Log-rank tests were performed, corrected by total comparing group numbers. Columns with different superscripts (a and b) are significantly different from each other (p<0.05).

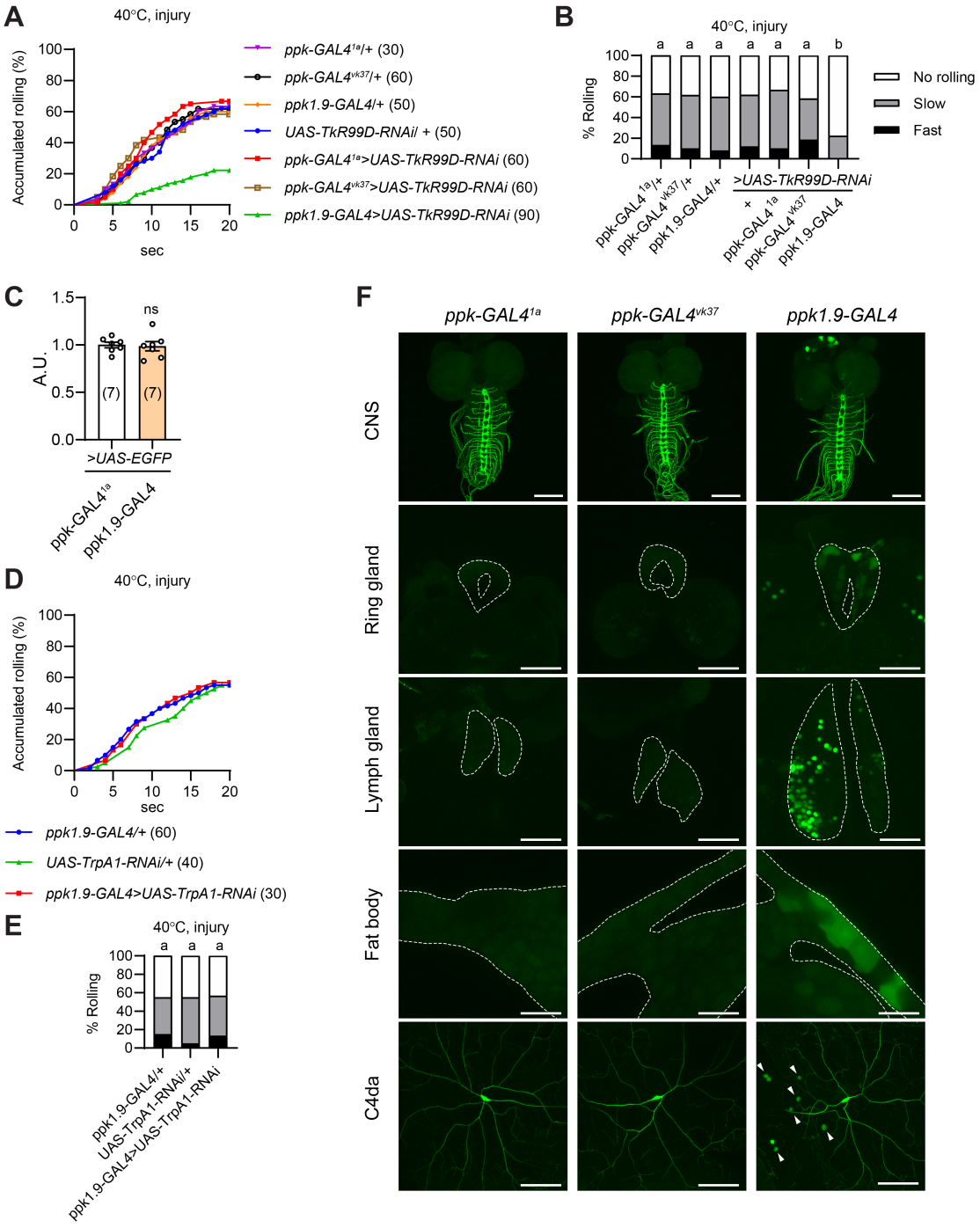

Figure S5. TkR99D knockdown in C4da neurons does not affect allodynia.

(A and B) Heat allodynia was impaired in *ppk1.9-GAL4>UAS-TkR99D-RNAi* larvae, but not in larvae in which *UAS-TkR99D-RNAi* was driven by *ppk-GAL4^{1a}* or *ppk-GAL4^{vk37}*. (C) GFP intensity in C4da neurons was comparable between *ppk-GAL4^{1a}>UAS-EGFP* and *ppk1.9-GAL4>UAS-EGFP* larvae. The sample numbers are indicated in the bracket. Error

bars, SEM. Unpaired Student's *t*-tests, ns, no significant difference. (D and E) Heat allodynia was unaffected in *ppk1.9-GAL4>UAS-TrpA1-RNAi* larvae. (F) Larval expression of *ppk-GAL4*^{1a}, *ppk-GAL4*^{vk37}, and *ppk1.9-GAL4*. In addition to C4da neurons, *ppk1.9-GAL4* was expressed in several other tissues. *UAS-6XGFP* was used for all expression pattern except for C4da neurons. *UAS-EGFP* was used for C4da neurons for better dendrite morphology. Arrowheads indicate hemocytes surrounding C4da neurons. For behavioral assay (A, B, D and E), The sample numbers are indicated in the bracket. Log-rank tests were performed, corrected by total comparing group numbers. Columns with different superscripts (a and b) are significantly different from each other (p<0.05).

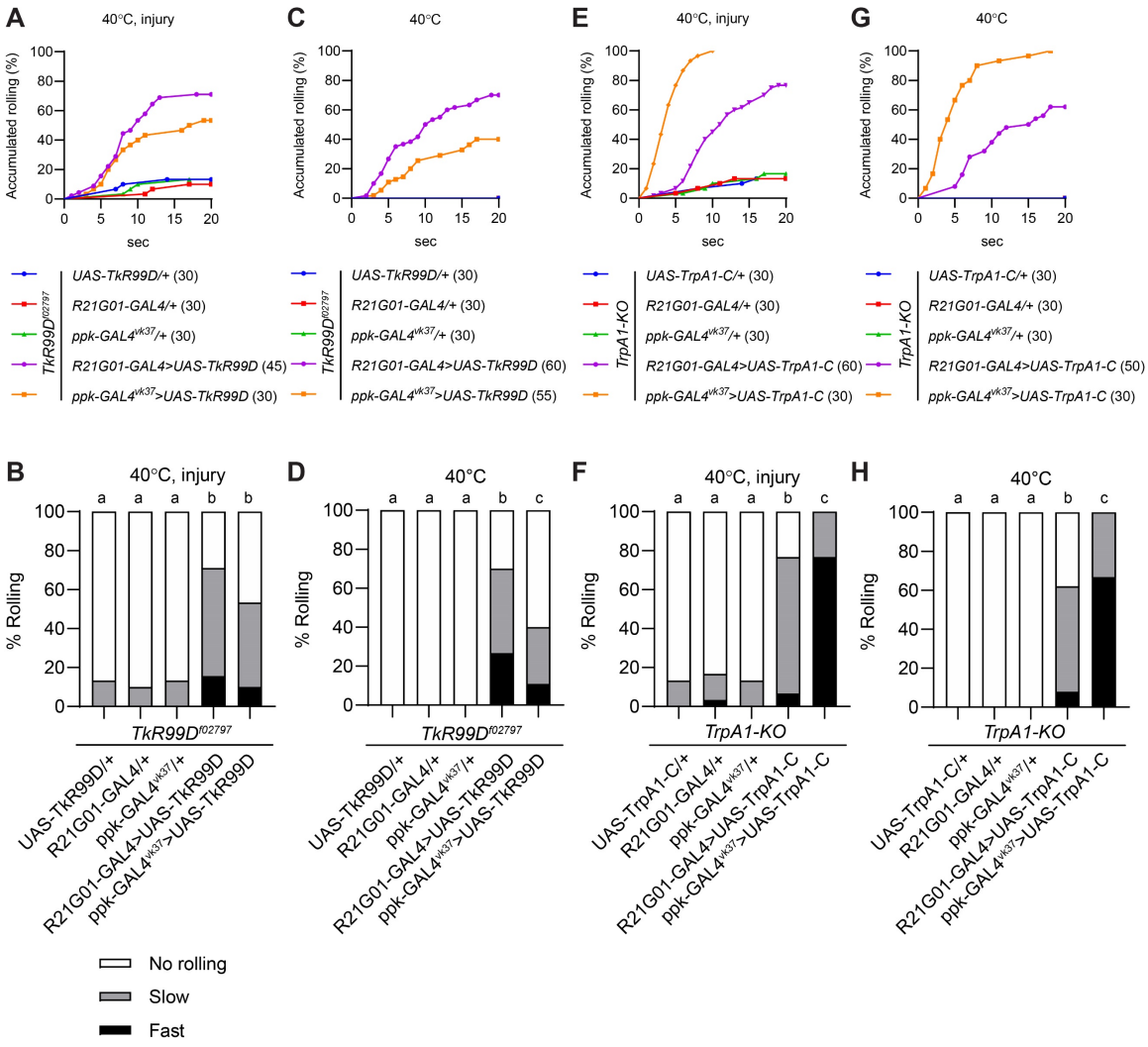

Figure S6. Genetic rescue studies.

(A and B) Rolling responses of TkR99D mutant larvae expressing *UAS-TkR99D* under control of either *ppk-GAL4^{vk37}* or *R21G01-GAL4*, to a 40℃ probe at 24 hrs after UVC treatment. (C and D) Rolling responses of TkR99D mutant larvae expressing *UAS-TkR99D* under control of either *ppk-GAL4^{vk37}* or *R21G01-GAL4* to a 40℃ probe, without UVC treatment. (E and F) Rolling responses of TrpA1 mutant larvae expressing *UAS-TrpA1-C* under control of either *ppk-GAL4^{vk37}* or *R21G01-GAL4*, to a 40℃ probe at 24 hrs after UVC treatment. (G and H) Rolling responses of TrpA1 mutant larvae expressing *UAS-TrpA1-C* under control of either *ppk-GAL4^{vk37}* or *R21G01-GAL4* to a 40℃ probe, without UVC treatment. The sample numbers are indicated in the bracket. Log-rank tests were performed, corrected by total comparing group numbers. Columns with different superscripts (a, b and c) are significantly different from each other (p<0.05).

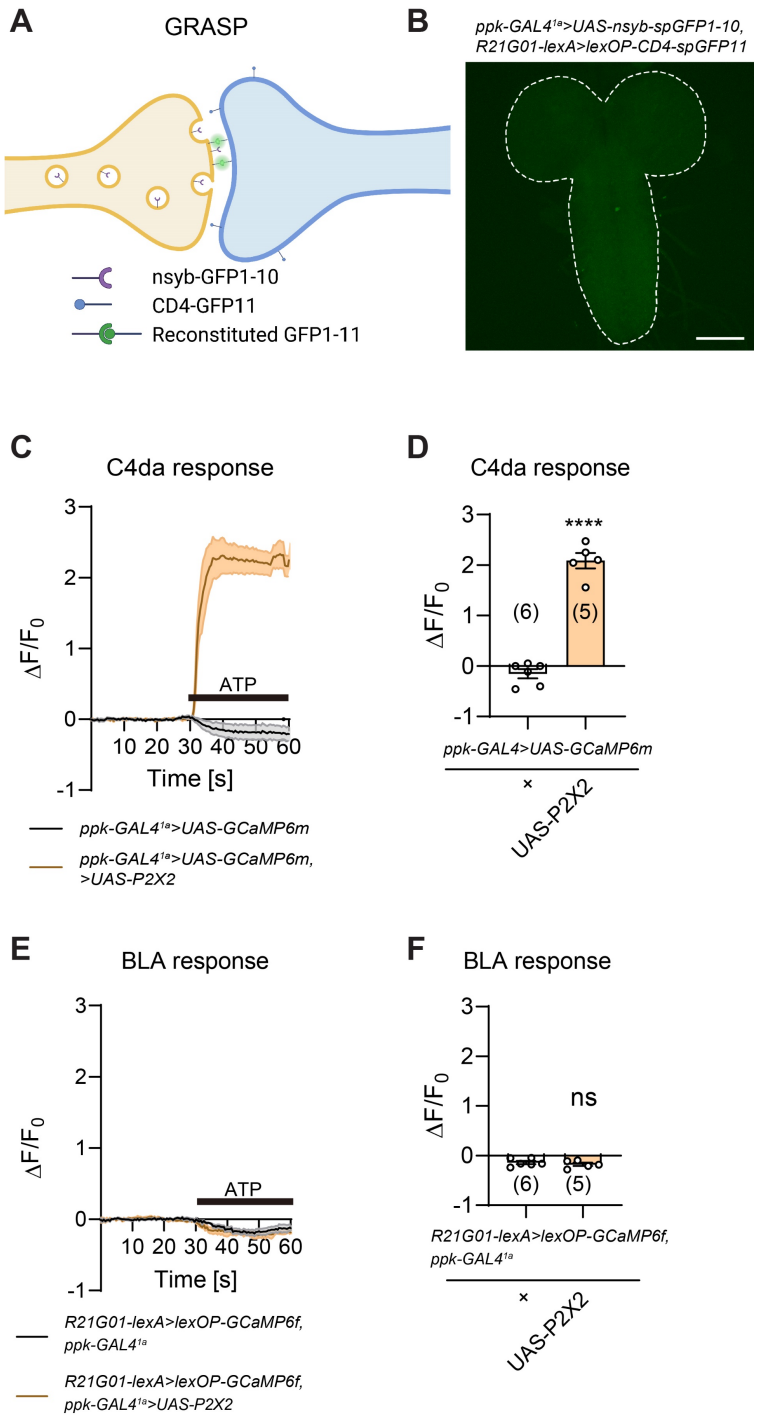

Figure S7. BLA neurons do not respond to C4da neuron activity.

(A) Diagram of GRASP. (B) No reconstituted florescent signals were detected in the CNS of larvae bearing *ppk-GAL4^{1a}>UAS-nsyb-GFP1-10* and *R21G01-lexA>lexOP-CD4-GFP11*. (C and D) acute application of 1mM ATP activated P2X2-expressing, but not

control, C4da neurons. (E and F) No responses were detected in BLA neurons after C4da neuron activation by ATP. (D and F) the sample numbers are indicated in the brackets. Error bars, SEM. Unpaired Student's *t*-tests, ****, $p<0.0001$, ns, no significant difference.

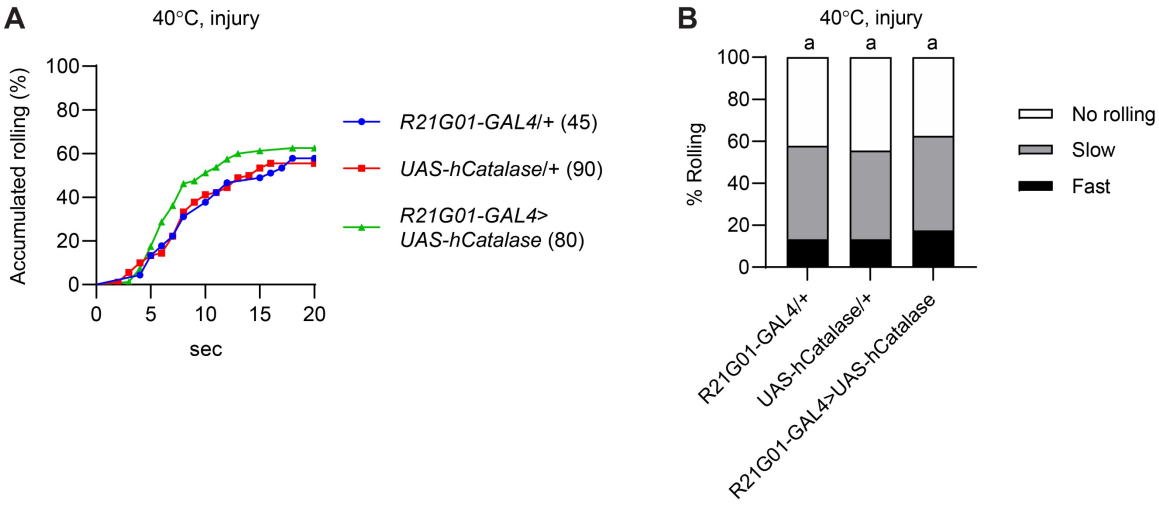

Figure S8. Catalase expression BLA neurons does not affect allodynia.

(A and B) Larvae expressing the ROS scavenger, hCatalase, under *R21G01-GAL4* control showed normal allodynia. The sample numbers are indicated in the bracket. Log-rank tests were performed, corrected by total comparing group numbers. Columns with the same superscript (a) indicated no significantly difference between groups (p>0.05).

Other Supplementary Materials

Movie S1 (separate file). Optogenetic stimulation of *UAS-CsChrimson* control larvae.

Movie S2 (separate file). Optogenetic stimulation of *R21G01-GAL4>UAS-CsChrimson* larvae.