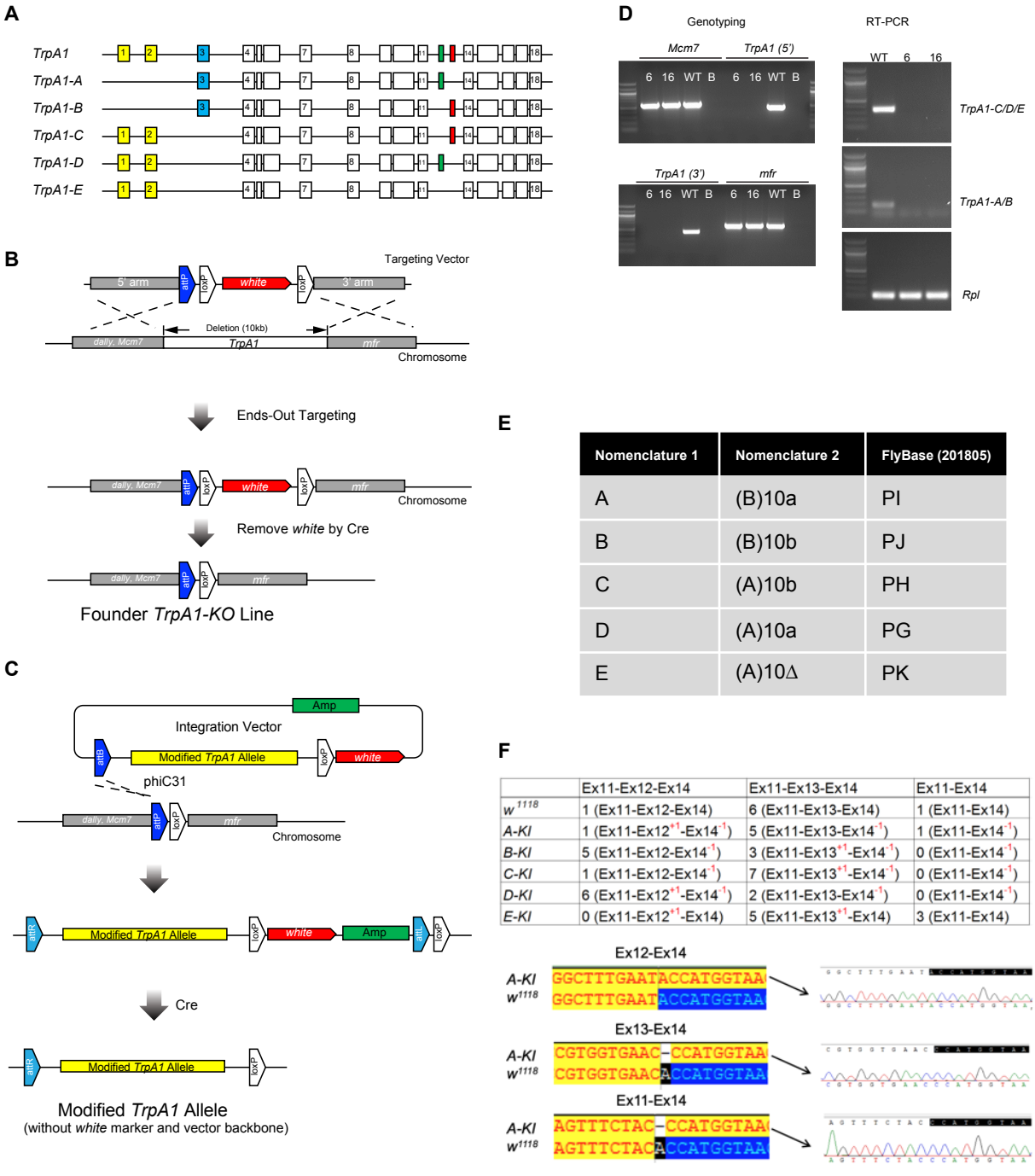


E

Nomenclature 1	Nomenclature 2	FlyBase (201805)
A	(B)10a	PI
B	(B)10b	PJ
C	(A)10b	PH
D	(A)10a	PG
E	(A)10Δ	PK

F

	Ex11-Ex12-Ex14	Ex11-Ex13-Ex14	Ex11-Ex14
w^{1118}	1 (Ex11-Ex12-Ex14)	6 (Ex11-Ex13-Ex14)	1 (Ex11-Ex14)
A-KI	1 (Ex11-Ex12^{+1}-Ex14^{-1})	5 (Ex11-Ex13-Ex14^{-1})	1 (Ex11-Ex14^{-1})
B-KI	5 (Ex11-Ex12-Ex14^{-1})	3 (Ex11-Ex13^{+1}-Ex14^{-1})	0 (Ex11-Ex14^{-1})
C-KI	1 (Ex11-Ex12-Ex14^{-1})	7 (Ex11-Ex13^{+1}-Ex14^{-1})	0 (Ex11-Ex14^{-1})
D-KI	6 (Ex11-Ex12^{+1}-Ex14^{-1})	2 (Ex11-Ex13-Ex14^{-1})	0 (Ex11-Ex14^{-1})
E-KI	0 (Ex11-Ex12^{+1}-Ex14)	5 (Ex11-Ex13^{+1}-Ex14)	3 (Ex11-Ex14)

Figure. S1. Two-step Genomic Engineering for Generation of *TrpA1* Isoform Alleles. Related to Figure 1.

(**A**) Exon-intron organization of *Drosophila TrpA1* gene and its five alternatively transcripts. TrpA1 protein contains 13 ankyrin repeats and six transmembrane domains, which are shared by all TrpA1 isoforms, and are encoded by exons 4-11 and 14-18, respectively. Color-coded exons are alternatively spliced. Exons 1-2 (yellow) and 3 (blue), encoding the N-terminus preceding ankyrin repeats, are spliced by alternative first exon. Exons 12 (green) and 13 (red), encoding the linker domain which connects ankyrin repeats and transmembrane domains, are spliced by a combination of mutually exclusive exon and cassette exon. (**B**) Schematics of step 1 to generate *TrpA1* knockout founder line by ends-out gene targeting. (**C**) Schematics of step 2 to generate *TrpA1* isoform-specific alleles by phiC31 integrase-mediated insertion of modified *TrpA1* genomic sequence to *TrpA1* native locus. (**D**) *TrpA1-KO* founder line was verified by genotyping and RT-PCR. Two independent *TrpA1-KO* alleles, 6 and 16, were obtained by end-out targeting. Two pairs of primers targeting the 5' and 3' *TrpA1* were used for genotyping. Two pairs of primers were used in RT-PCR to detect *TrpA1-A*/*B* and *TrpA1-C*/*D*/*E* transcripts. See methods for details of primer sequences. WT and B refer to wild-type and blank controls, respectively. (**E**) Comparison of different *TrpA1* isoform nomenclature systems. The alphabetic orders shown in nomenclature 1 refers to names used in the present study. Nomenclature 2 refers to alternative names of *TrpA1* isoforms proposed by Dr. Paul Garrity and his collogues. The right lane refers to nomenclature by the current version of FlyBase. **(F)** isoEXPRESS did not alter TrpA1 RNA splicing patterns. Alternative splicing patterns of *wt*, *TrpA1-A-KI*, *TrpA1-B-KI*, *TrpA1-C-KI, TrpA1-D-KI,* and *TrpA1-E-KI* were determined using a pair of primer flanking between exon 11 and exon 14. See methods for primer information. RT-PCR was performed from RNA extracted from the whole larvae. Top: Sequencing results of RT-PCR products. For each genotype, we picked 7-8 clones of RT-PCR products for sequencing. Alternative splicing patterns, including Ex11-Ex12-Ex14 (TrpA1-A and TrpA1-D), Ex11-Ex13-Ex14 (TrpA1-B and TrpA1-C), and Ex11-Ex14 (TrpA1-E), were listed and number of clones found for each splicing pattern was shown. No abberant alternative splicing mode was found. Nucleotide addition or deletion (+1 or -1, shown in red in brackets) events were confirmed by sequencing results. Bottom: Example sequencing traces of *TrpA1-A-KI* larvae, with *wt* sequence listed for reference. Boundary of exon 14 is highlighted.

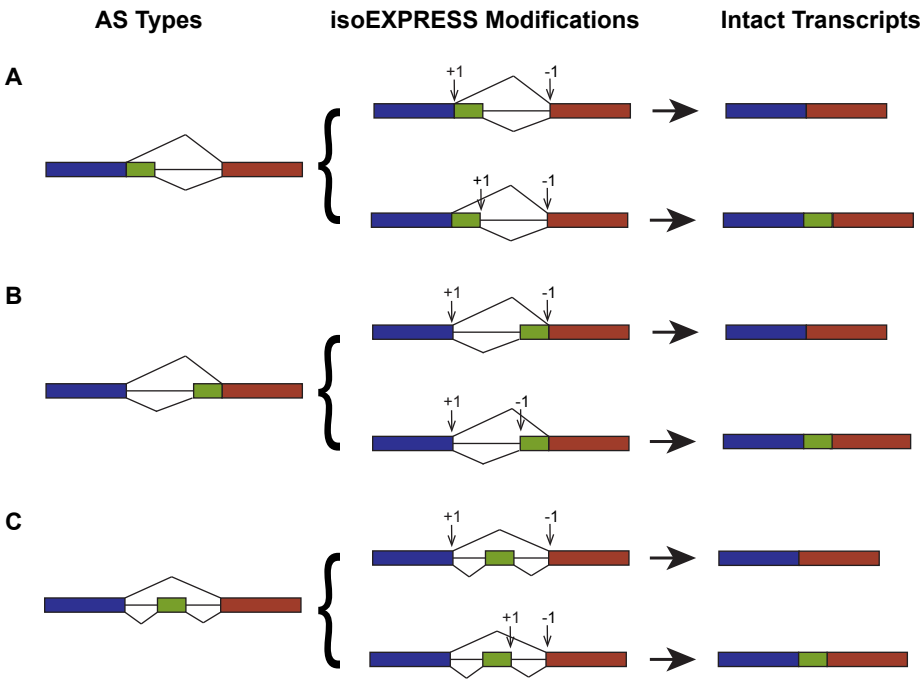

AS Types
isoEXPRESS Modifications
Intact Transcripts
A
B
C
+1
-1

Figure. S2. Application of isoEXPRESS on Other Alternative Splicing Types. Related to Figure 1.

By relocation of the first nucleotide of one exon (shown as '-1' in red or green exons) to the end of another exon (shown as '+1" in blue or green exons), isoEXPRESS-based genome engineering can be applied to knock in a single alternative isoform derived from alternative 5' splice site choice (**A**), alternative 3' splice site choice (**B**), and cassette exon inclusion/skipping (**C**). Only intact transcripts are shown.

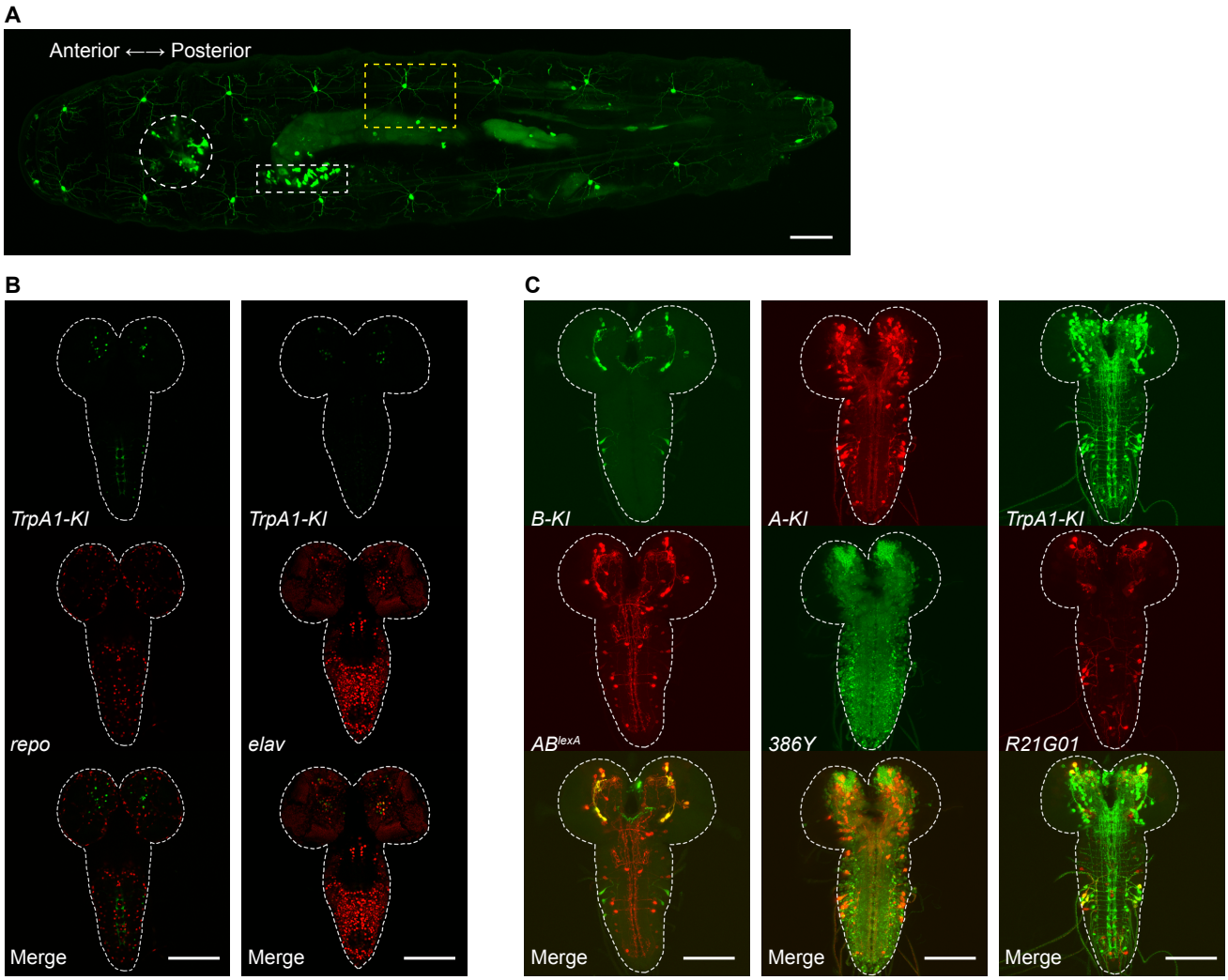
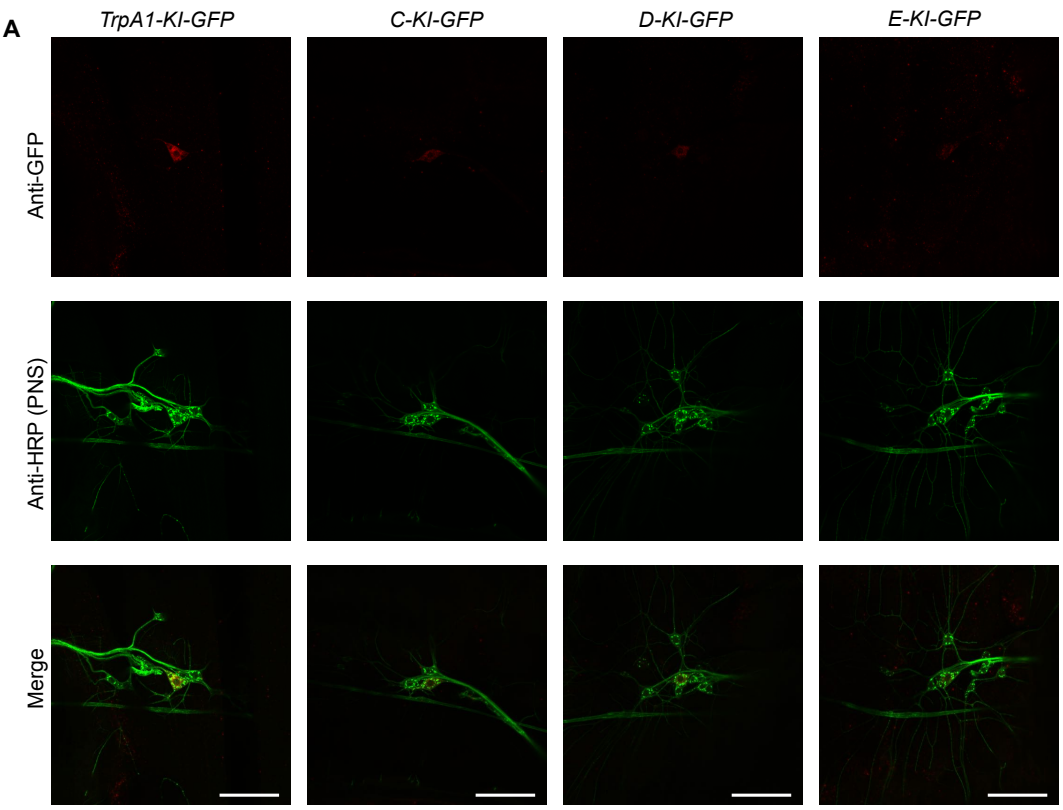
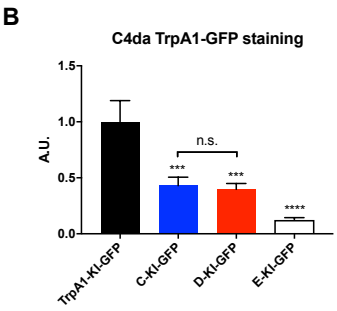

A
Anterior ⟷ Posterior
B
TrpA1-KI
repo
Merge
TrpA1-KI
elav
Merge
C
B-KI
A-KI
TrpA1-KI
AB^lexA
386Y
R21G01
Merge
Merge
Merge
D
TrpA1-KI-T2A-GAL4
>UAS-6XGFP
Anti-TrpA1
Merge
E
C-KI-GFP
D-KI-GFP

Figure. S3. TrpA1 Expression Patterns in *Drosophila* Larvae. Related to Figure 2.
(**A**) Representative images of TrpA1-positive cells in a larva harboring *TrpA1-KI-T2A-GAL4>UAS-mCD8GFP*. White dashed circle outlines expression in CC cells and brain. White dashed square indicates expression in the midgut. Yellow dashed square shows a single C4da nociceptor. Scale bar, 100 μm.
(**B**) Representative images of TrpA1-positive cells in larval brain lobes and VNC in relationship to neuronal and glial markers. Left column, confocal images of a single focal plane showing TrpA1-positive cells in green and glial cells in red in trans-heterozygotes harboring *TrpA1-T2A-lexA>lexAop2-GFP::NLS* and *repo-GAL4>UAS-mCherry::NLS*. Right column, confocal images of a single focal plane showing TrpA1-positive cells in green and neurons in red in trans-heterozygotes harboring *TrpA1-T2A-lexA>lexAop2-GFP::NLS* and *elav-GAL4>UAS-mCherry::NLS*. NLS refers to nuclear localization sequence. Scale bar, 100 μm.
(**C**) Representative images showing the expression of *TrpA1* translational driver lines generated in the present study, with respect to other driver lines in larval CNS, in larvae harboring *TrpA1-B-KI-T2A-GAL4>UAS-6XGFP* and
TrpA1-AB^{lexA}>lexAop2-6XmCherry (left panel);
TrpA1-A-KI-T2A-lexA>lexAop2-6XmCherry and *386Y-Gal4>UAS-6XGFP* (middle panel); *TrpA1-KI-T2A-GAL4>UAS-6XGFP* and *R21G01-lexA>lexAop2-6XmCherry* (right panel). Scale bar, 100 μm.
In B-C, dashed lines outline larval brain and VNC.
(**D**) Representative images of TrpA1 protein expression in larval CNS determined by the TrpA1 antibody. TrpA1 immunostaining was performed in larvae bearing *TrpA1-KI-T2A-GAL4>UAS-6XGFP*. White and yellow arrows point to larval brain cells and CC cells, respectively. Scale bar: 100 μm.
(**E**) Representative images of TrpA1-C and TrpA1-D protein expression in larval CNS determined by the GFP antibody. GFP immunostaining was performed in larvae bearing *TrpA1-C-KI-GFP* or *TrpA1-D-KI-GFP*. White and yellow arrows point to larval brain cells and CC cells, respectively. Scale bar: 50 μm.


A
TrpA1-KI-GFP
C-KI-GFP
D-KI-GFP
E-KI-GFP
Anti-GFP
Anti-HRP (PNS)
Merge


B
C4da TrpA1-GFP staining
A.U.
1.5
1.0
0.5
0.0
n.s.
***
***
****
TrpA1-KI-GFP
C-KI-GFP
D-KI-GFP
E-KI-GFP

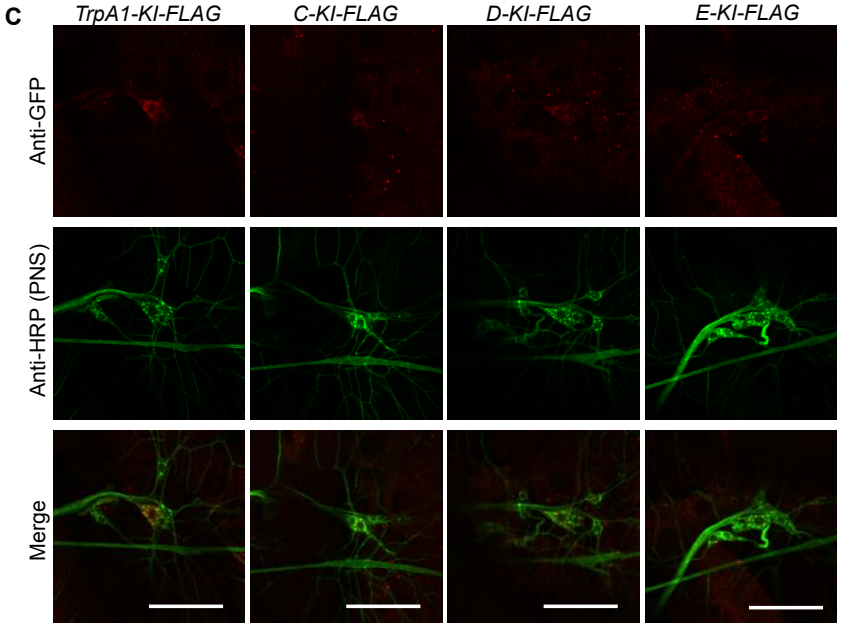

C
TrpA1-KI-FLAG
C-KI-FLAG
D-KI-FLAG
E-KI-FLAG
Anti-GFP
Anti-HRP (PNS)
Merge

Figure. S4. TrpA1 Protein Expression in Larval CNS and C4da Nociceptors. Related to Figure 2.

(**A**) Representative images of TrpA1-GFP expression in larval C4da nociceptors. Upper panels, antibody staining against GFP in larvae bearing *TrpA1-KI-GFP*, *TrpA1-C-KI-GFP*, *TrpA1-D-KI-GFP*, and *TrpA1-E-KI-GFP* alleles. Middle panels, sensory neurons in larval PNS labeled by the antibody against horseradish peroxidase (Anti-HRP). C4da nociceptors are identified by their stereotyped anatomical position. Scale bar: 50 μm.

(**B**) Quantification of expression levels of TrpA1-GFP in larval C4da nociceptors. Data are represented as normalized fluorescence intensities of GFP immunostaining as performed in (A). n=8 for *TrpA1-KI-GFP* and n≥14 for other genotype groups. Data are represented as mean ± SEM. One-way ANOVA with Tukey's post comparison test. ***$p<0.001$, ****$p<0.0001$, n.s. no significant difference.

(**C**) Representative images of TrpA1-FLAG expression in larval C4da nociceptors. Upper panels, antibody staining against FLAG in larvae bearing *TrpA1-KI-FLAG*, *TrpA1-C-KI-FLAG*, *TrpA1-D-KI-FLAG*, and *TrpA1-E-KI-FLAG* alleles. Middle panels, sensory neurons in larval PNS labeled by the antibody against horseradish peroxidase (Anti-HRP). Scale bar: 50 μm.

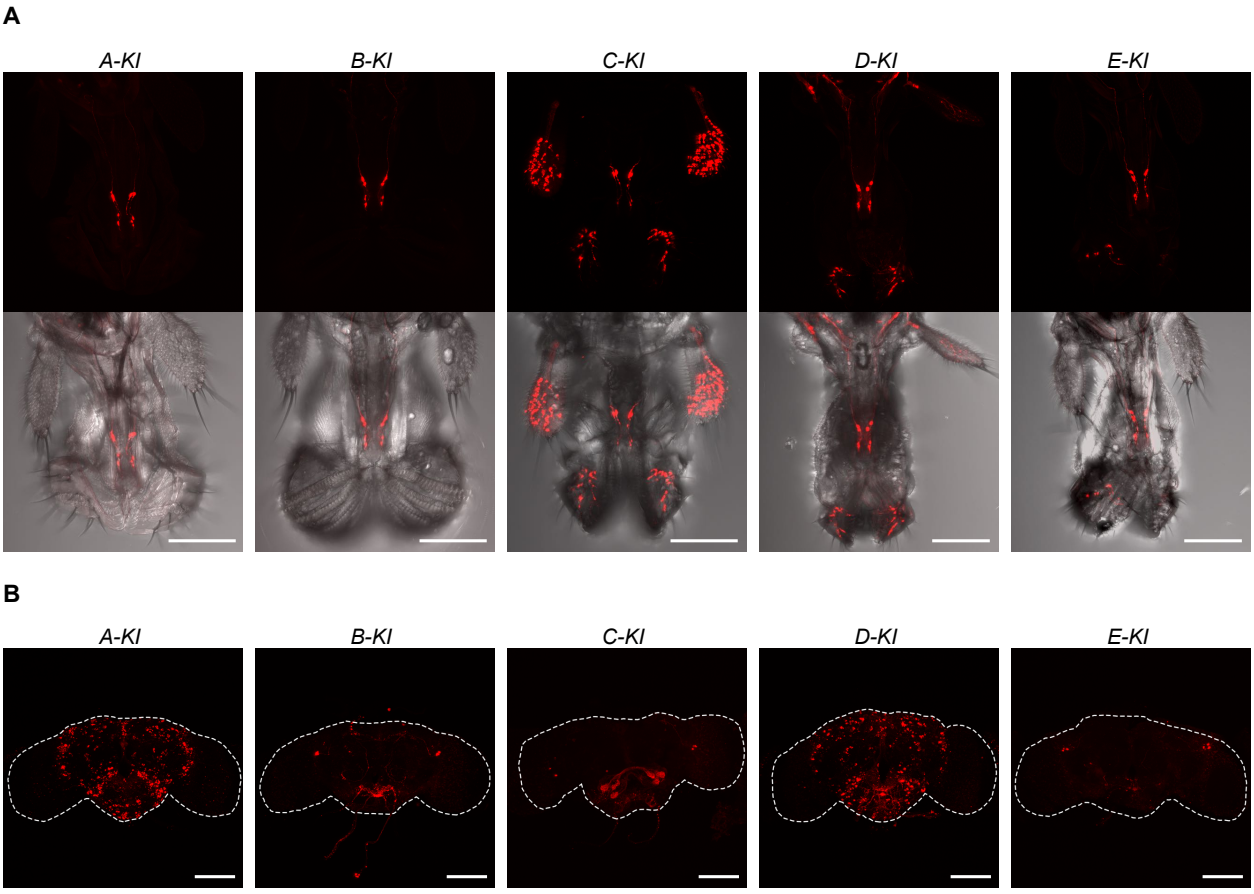
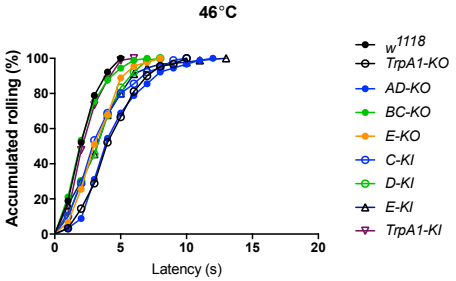

A
A-KI
B-KI
C-KI
D-KI
E-KI
B
A-KI
B-KI
C-KI
D-KI
E-KI

Figure. S5. TrpA1 Isoform-specific Expression in Adult *Drosophila*. Related to Figure 2.

(**A**) Representative images showing *TrpA1-isoform-KI-T2A-GAL4* activities in adult proboscis and maxillary palps, reported by *UAS-CD4TdTomato*. Upper panels show fluorescence signals only. Lower panels show fluorescence and bright field images. Scale bar, 100 μm.

(**B**) Representative images showing *TrpA1-isoform-KI-T2A-lexA* activities in adult fly brain reported by *lexAop2-6XmCherry*. Dashed lines outline adult brain. Scale bar, 100 μm.


46°C
Accumulated rolling (%)
100
80
60
40
20
0
0
5
10
15
20
Latency (s)
w1118
TrpA1-KO
AD-KO
BC-KO
E-KO
C-KI
D-KI
E-KI
TrpA1-KI

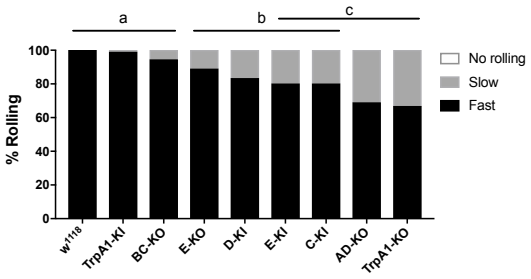

a
b
c
% Rolling
100
80
60
40
20
0
No rolling
Slow
Fast
w1118
TrpA1-KI
BC-KO
E-KO
D-KI
E-KI
C-KI
AD-KO
TrpA1-KO

Figure. S6. Rolling Behavior of Larvae Bearing *TrpA1* Isoform Alleles to a 46^{o}C Probe. Related to Figure 4.

Behavior responses of w^{1118} control and larvae bearing *TrpA1* isoform alleles to a 46^{o}C heat probe. 90 larvae were tested for each genotype. Log-rank test, corrected by total comparing group numbers, was used for statistical analysis of accumulated response curves. For clarity, results of statistical analysis are marked in the bar graphs. Columns grouped by the same horizontal line are not significantly different from each other ($p>0.05$).

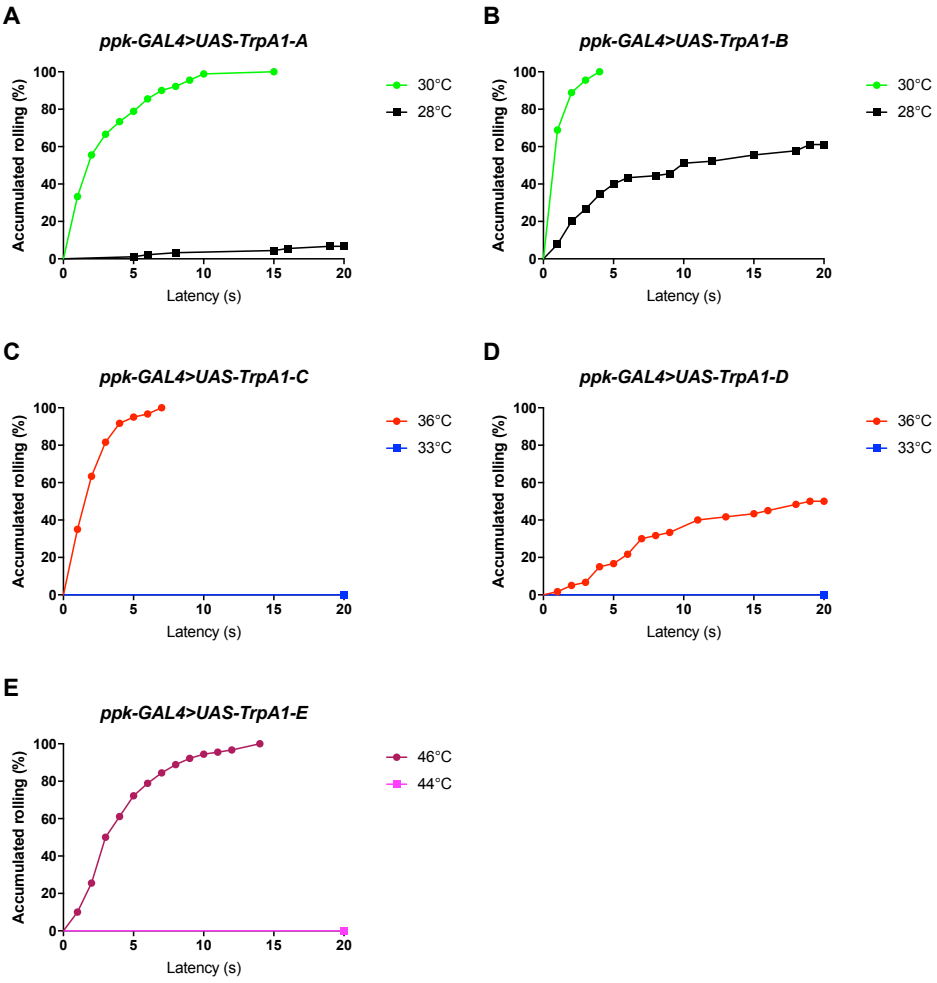

A
ppk-GAL4>UAS-TrpA1-A
Accumulated rolling (%)
Latency (s)
30°C
28°C
B
ppk-GAL4>UAS-TrpA1-B
Accumulated rolling (%)
Latency (s)
30°C
28°C
C
ppk-GAL4>UAS-TrpA1-C
Accumulated rolling (%)
Latency (s)
36°C
33°C
D
ppk-GAL4>UAS-TrpA1-D
Accumulated rolling (%)
Latency (s)
36°C
33°C
E
ppk-GAL4>UAS-TrpA1-E
Accumulated rolling (%)
Latency (s)
46°C
44°C

Figure. S7. Heat-induced Rolling Behavior of Larvae Bearing Overexpressed *TrpA1* Isoforms in C4da Nociceptors. Related to Figure 6.

(**A** to **E**) Rolling behavioral of larvae harboring *UAS-TrpA1*, under the control of *ppk-GAL4*, to the heat probe with different preset temperature. At least 60 larvae were tested for each group.