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Supplemental Information

Hedgehog Signaling Regulates

Nociceptive Sensitization

Daniel T. Babcock, Shanping Shi, Juyeon Jo, Michael Shaw, Howard B. Gutstein, and Michael J. Galko

Supplemental Inventory

1. Supplemental Figures and Tables

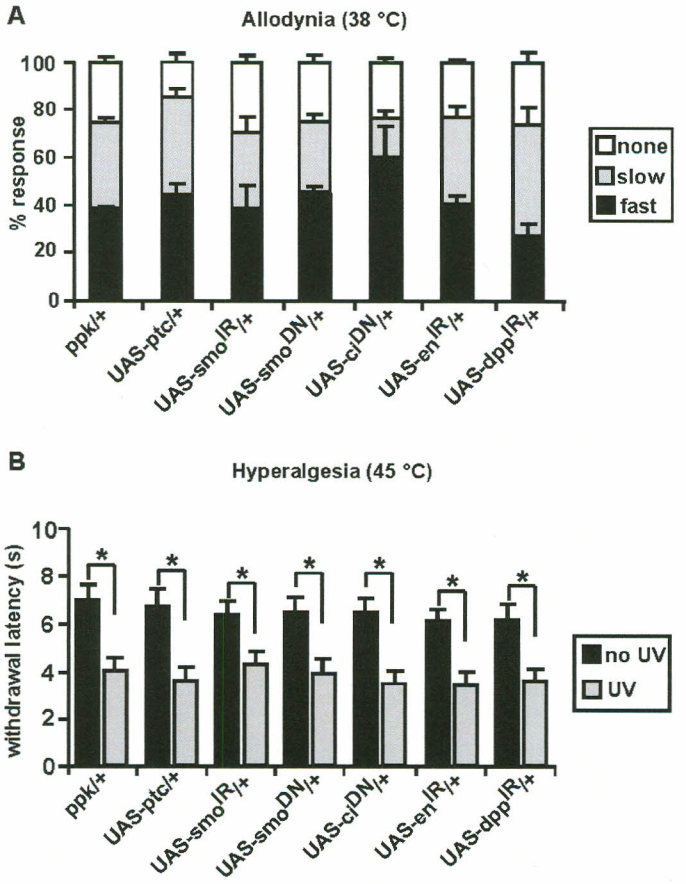

Figure S1. Thermal Allodynia and Hyperalgesia of UAS-Alone Controls, Related to Figure 2

Third instar larvae heterozygous for *ppk1.9-Gal4 (ppk1.9-Gal4* x w^{1118} = *ppk/+*) or the various UAS transgenes tested behaviorally in Figure 2 (*UAS-transgene x* w^{1118} = *UAS-transgene/+*) were tested for development of thermal allodynia at 38 °C 24 h after UV treatment (A) or thermal hyperalgesia at 45 °C 8 h after UV or mock treatment (B). Asterisk, statistical significance ($p < .05$) versus *ppk/+* control by student's t-test. All UAS alone controls developed normal allodynia and hyperalgesia suggesting that the insertion sites of these transgenes in the absence of the Gal4 driver do not affect either type of nociceptive sensitization. N = triplicate sets of 30 larvae per condition for allodynia and N = 50 larvae for hyperalgesia. Error bars represent Standard Error of the Mean (S.E.M). Bracket/asterisk, statistically significant comparison by student's t-test.

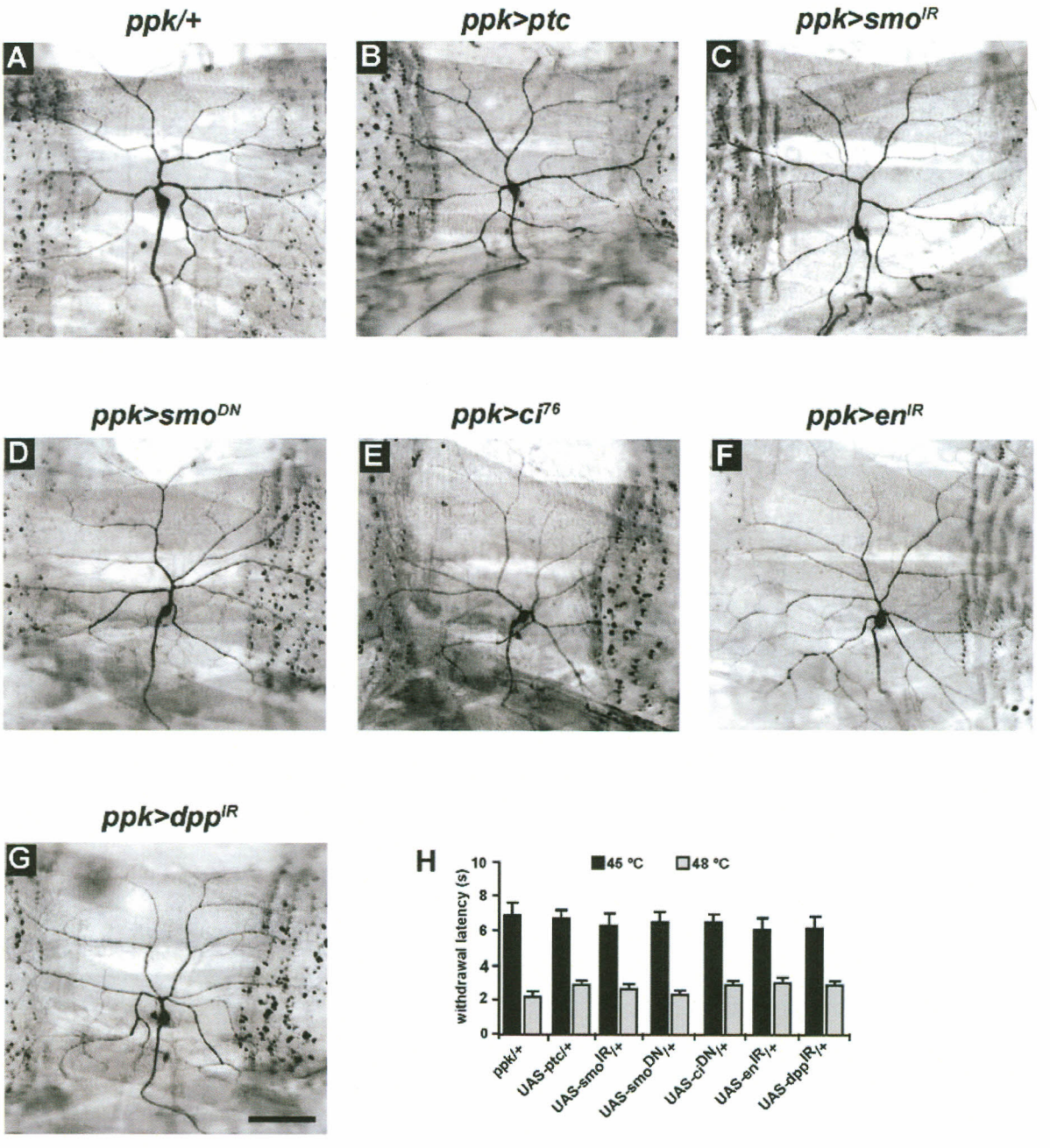
ppk/+
ppk>ptc
ppk>smo^IR
A
B
C
ppk>smo^DN
ppk>ci^76
ppk>en^IR
D
E
F
ppk>dpp^IR
G
H
45 °C
48 °C
withdrawal latency (s)
10
8
6
4
2
0
ppk/+
UAS-ptc/+
UAS-smo^IR/+
UAS-smo^DN/+
UAS-ci^DN/+
UAS-en^IR/+
UAS-dpp^IR/+

Figure S2. Sensory Neuron Morphology on Sensory Neuron Expression of UAS Transgenes that Block Hh Signaling and Baseline Nociception of UAS-Alone Controls, Related to Figure 3

Wholemounts of dissected L3 larvae of the indicated genotypes. *ppk1.9Gal4* was used to drive the indicated UAS transgene expression in nociceptive sensory neurons.

(A) Control.

(B) *UAS-patched.*

(C) *UAS-smoothened*^{IR}.

(D) *UAS-smoothened*^{DN}.

(E) *UAS-ci*^{76} = *UAS-ci*^{DN}.

(F) *UAS-engrailed*^{IR}.

(G) *UAS-dpp*^{IR}. Sensory neuron morphology is normal in all cases, as quantified in Figure 3. Scale bar, 100 μm for all panels.

(H) Baseline nociception of UAS-alone controls relevant to Figure 3d. Third instar larvae heterozygous for *ppk1.9-Gal4 (ppk1.9-Gal4* x w^{1118} = *ppk/+*) or the various UAS transgenes tested behaviorally in Figure 3d (*UAS-transgene x* w^{1118} = *UAS-transgene/+*) were tested for baseline nociception responses in the absence of UV at 45 °C and 48 °C. All UAS alone controls exhibited normal baseline nociception at both the middle and high end of their response range suggesting that the insertion sites of these transgenes in the absence of the Gal4 driver do not affect baseline nociception. N = 50 larvae. Error bars represent Standard Error of the Mean (S.E.M).

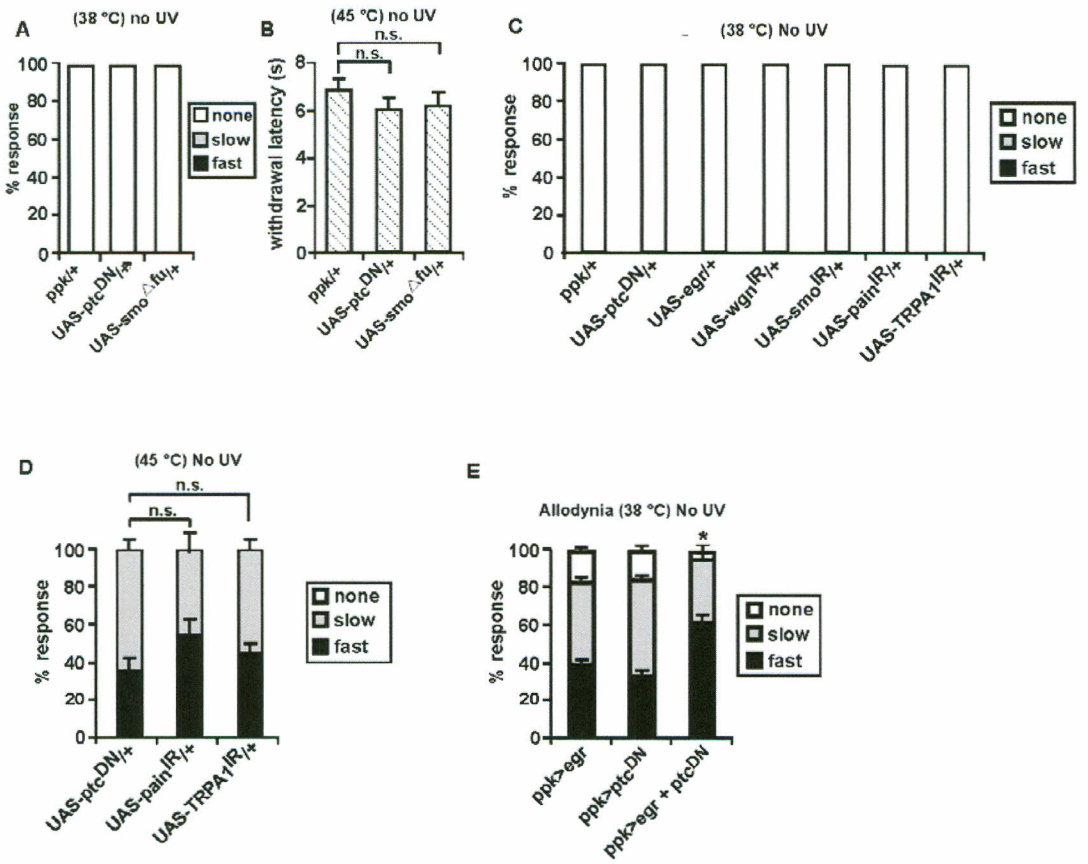

Figure S3. Baseline Nociception and Nociceptive Sensitization of UAS-Alone Controls, Relevant to Figure 4

Third instar larvae heterozygous for *ppk1.9-Gal4 (ppk1.9-Gal4* x w^{1118} = *ppk/+*) or the various UAS transgenes tested behaviorally in Figure 4 (*UAS-transgene x* w^{1118} = *UAS-transgene/+*) were tested for baseline nociception at 38 ° C (A, C) or 45 ° C (B, D) responses in the absence of UV. Genotypes in (A) are relevant to Fig. 4A, genotypes in (B) are relevant to Fig. 4b, genotypes in (C) are relevant to Fig. 4C-E and g, and genotypes in (D) are relevant to Fig. 4F and H. N = triplicate sets of 30 larvae per condition. Error bars represent Standard Error of the Mean (S.E.M).

(E) Enhanced allodynia on co-activation of TNF/Eiger and Hh signaling in nociceptive sensory neurons. *ppk1.9-Gal4* drives expression of the indicated UAS transgenes in nociceptive sensory neurons. Third instar larvae were tested for thermal allodynia at 38 ° C in the absence of UV-induced tissue damage. Co-expression of TNF/Eiger and a dominant-negative form of Patched leads to a behavioral response that is more severe than that observed on activation of each pathway in isolation (compare right bar to the two left bars). N = triplicate sets of 30 larvae per condition. Error bars represent Standard Error of the Mean (S.E.M).