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## Nociceptor-enriched genes required for normal thermal nociception

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### Summary

Here, we describe a targeted reverse genetic screen for thermal nociception genes in *Drosophila* larvae. Using laser capture microdissection and microarray analyses of nociceptive and non-nociceptive neurons we identified 275 nociceptor-enriched genes. We then tested the function of the enriched genes with nociceptor-specific RNAi and thermal nociception assays. Tissue-specific RNAi targeted against 14 genes caused insensitive thermal nociception while targeting of 22 genes caused hypersensitive thermal nociception. Previously uncategorized genes were named for heat resistance (ie. *boilerman*, *fire dancer*, *oven mitt*, *trivet*, *thawb* and *bunker gear*) or heat sensitivity (*firelighter*, *black match*, *eucalyptus*, *primacord*, *jet fuel*, *detonator*, *gasoline*, *smoke alarm*, and *jetboil*). Insensitive nociception phenotypes were often associated with severely reduced branching of nociceptor neurites and hyperbranched dendrites were seen in two of the hypersensitive cases. Many genes that we identified are conserved in mammals.

### Introduction

The pomace fly *Drosophila melanogaster* has been developed as a robust system to study nociception (Babcock et al., 2009; Babcock et al., 2011; Im et al., 2015; Neely et al., 2010; Tracey et al., 2003). *Drosophila*, with its unparalleled genetic tools, is an excellent model to uncover nociception genes. *Drosophila* larvae rotate along the long body axis in a corkscrew like fashion in response to noxious stimuli such as heat (>39°C) or harsh mechanical stimuli

#### Author Contributions

KH performed experiments, analyzed data and wrote the manuscript, SEM performed experiments, analyzed data and wrote the manuscript, YW performed LCM experiments, JHPS directed the LCM core for the NIH Microarray Consortium, WDT conceived the project, analyzed data, and wrote the manuscript.

#### Accession numbers:

The ArrayExpress (<https://www.ebi.ac.uk/arrayexpress/>) accession number for the microarray dataset reported in this study is E-MTAB-3863.

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(Tracey et al., 2003). This highly stereotyped response to harmful stimuli, named nocifensive escape locomotion (NEL) or rolling, serves as a robust behavioral readout of nociception since it is specifically triggered by noxious stimuli and it is clearly distinguishable from normal locomotion and other somatosensory responses.

Several lines of evidence indicate that Class IV multidendritic (md) neurons are polymodal nociceptive sensory neurons responsible for larval thermal and mechanical nociception. The *pickpocket* and *balboa/ppk-26* genes show highly specific expression in these neurons and are required for mechanical nociception (Gorczyca et al., 2014; Guo et al., 2014; Mauthner et al., 2014; Zhong et al., 2010). Similarly, reporter genes for specific *dTRPA1* transcripts are specifically expressed in the Class IV cells and *dTRPA1* is required for both mechanical and thermal nociception (Zhong et al., 2012). Genetic silencing of Class IV neurons severely impairs thermal and mechanical nociception behavior and optogenetic activation of these neurons is sufficient to evoke NEL (Hwang et al., 2007; Zhong et al., 2012).

## Results and Discussion

### Laser capture microdissection and microarray analyses identify 275 nociceptor-enriched genes

Genes involved in nociception are often preferentially expressed in nociceptors (Akopian et al., 1996; Caterina et al., 1997; Chen et al., 1995; Dib-Hajj et al., 1998; Mauthner et al., 2014; Nagata et al., 2005; Zhong et al., 2012; Zhong et al., 2010). Thus, to identify *Drosophila* nociceptor-enriched genes we performed laser capture microdissection to isolate RNAs from nociceptive and non-nociceptive neurons (Mauthner et al., 2014). We then performed microarray analyses on the isolated samples (Mauthner et al., 2014). We compared the gene expression profiles of nociceptive Class IV multidendritic (md) neurons to Class I md neuron profiles (Mauthner et al., 2014) as Class IV md neurons are polymodal nociceptors (their output is both necessary and sufficient for triggering larval nociception behaviors), and Class I md neurons are functionally dispensable for nociception (Hwang et al., 2007). Indeed, as internal validation of these methods, this microarray study successfully detected the enrichment of genes previously thought to be preferentially expressed in Class IV relative to Class I neurons, such as *cut*, *knot*, *Gr28b*, *ppk* and *balboa/ppk26* (Mauthner et al., 2014) (Table S1A).

To further identify nociceptor-enriched genes, we made a side-by-side comparison of the normalized hybridization intensity between Class IV and Class I neurons for all Affymetrix probe sets, and identified 278 probe sets corresponding to 275 genes that showed a greater than two-fold higher expression in Class IV neurons in comparison to Class I neurons (Class IV / Class I > 2;  $p < 0.05$  with Welch t-test) (Figure S1 and Table S1A).

### Nociceptor-specific RNAi screens uncover 36 genes required for larval thermal nociception

We subsequently tested the function of the nociceptor-enriched genes in thermal nociception responses. In order to test their requirement specifically in nociceptors, we used RNAi to knock down each gene in a tissue-specific pattern using the Class IV specific GAL4 driver

*ppk1.9-GAL4. UAS-dicer2* was also present in the driver strain in order to enhance RNAi knockdown (Dietzl et al., 2007). A total of 419 UAS-RNAi lines were obtained from the Vienna Drosophila RNAi library (Dietzl et al., 2007), the TRiP RNAi library (Ni et al., 2009), and the National Institute of Genetics RNAi library and were used to knock down 229 of the 275 (83.3%) nociceptor-enriched genes. In control experiments, we found that the baseline nociception responses differed among the genetic backgrounds that were used to generate the different collections of UAS-RNAi strains. Thus, the progeny of our GAL4 driver strain crossed to UAS-RNAi lines from the four different collections (VDRC 1st-generation, VDRC 2nd-generation, TRiP and NIG) were each statistically analyzed in comparison to the relevant genetic controls for parental isogenic background. Progeny of each *ppk-GAL4 UAS-dicer-2* × *UAS-RNAi* cross were tested in an established larval thermal nociception assay (Tracey et al., 2003). In order to identify either insensitive or hypersensitive phenotypes, independent tests were carried out at two different probe temperatures. A 46°C probe was used to screen for insensitive phenotypes (defined as a lengthened latency to respond to the 46°C stimulus) while a 42°C probe was used to assess hypersensitivity (defined as a shortened latency to respond to this stimulus). Average latency to 46°C and 42°C thermal probe stimulation were determined for each RNAi knockdown genotype (Figure 1 and Table S1B–E). We set our initial cut-off line at the +1σ (84.13th percentile) in the insensitivity screen and –1σ (15.87th percentile) in the hypersensitivity screen, and all *ppk-GAL4 UAS-dicer-2* × *UAS-RNAi* pairs that met these cut-offs were subjected to retesting (Figure 1 and Table S1B–E). Only pairs showing significant insensitivity or hypersensitivity in comparison to the appropriate control for genetic background ( $p < 0.05$  with Steel's test) during the retest were considered as positive hits. Sixteen RNAi lines targeting 14 genes were identified in the insensitivity screen and 24 RNAi lines targeting 22 genes were found in the hypersensitivity screen (Table 1, Figure 2 and S1F–M). We confirmed that these positive RNAi lines did not show the observed nociception phenotypes when crossed to *w<sup>1118</sup>* strain (no driver control), suggesting that the phenotypes observed in the screen were dependent on GAL4-driven expression of RNAi (Table S1N and O).

Neely et al. carried out a genome-wide RNAi screen for nociception genes using adult *Drosophila* (Neely et al., 2010). Neely and colleagues identified 3 genes of the 14 that we found with insensitive nociception phenotypes (*dpr11*, *lis-1* and *vha100-1*), and a single gene out of the 22 with hypersensitive phenotypes (*retinal degeneration C*). As this prior screen relied on thermally-induced paralysis of adult flies as a surrogate for studying larval nociception behavior, it is possible that molecular mechanisms of nociception in adult flies and larval flies may be distinct. In addition, Neely et al. used broadly expressed knockdown approaches. It is possible that pleiotropy caused by opposing effects in distinct tissues may have masked the effects that we are able to detect with nociceptor specific knockdown. In either case, it is likely that a large number of *bona fide* nociception signaling genes remain to be discovered using larval nociception assays.

### Uncharacterized Genes Identified in the Screens

Our screen identified genes that remain named according to Celera Gene (CG) numbers (Table 1). Knockdown of seven CGs caused insensitivity and nine CGs caused

hypersensitivity. Thus, given these loss of function phenotypes we have named each of the heat-insensitivity screen genes (*boilerman (boil)*, *fire dancer (fid)*, *oven mitt (ovm)* and *trivet (trv)*, *thawb (thw)*, *bunker gear (bug)*, *space blanket (spab)*) (Table 1). And we have also named uncharacterized genes that were identified in the hypersensitivity screen (*firelighter (fir)*, *black match (bma)*, *eucalyptus (euc)*, *primacord (prim)*, *jet fuel (jef)*, *detonator (dtn)* and *gasoline (gas)*, *smoke alarm (smal)*, *jetboil (jtb)*) (Table 1).

### Genes required for Class IV neuron morphogenesis

*Lis1*, which showed an insensitive nociception phenotype in our screen (Figure 2A and Table 1), is a component of a dynein-dependent motor complex that is known to play a role in dendrite and axonal morphogenesis in Class IV neurons (Sato et al., 2008). Similarly, a reduced dendrite phenotype in another study was associated with insensitive nociception behaviors (Stewart et al., 2012). These observations raised the possibility that nociception phenotypes detected in our screen might also be associated with defects in dendrite morphogenesis.

To test for this possibility, we used confocal microscopy to observe and quantify the dendritic coverage of CD8-GFP expressing Class IV neurons in all of the genotypes that showed insensitive or hypersensitive thermal nociception phenotypes (Figure 3 and 4). Consistent with the previous study, *Lis1* RNAi showed severe defects in dendrite morphogenesis (Figure 3). Significantly reduced nociceptor dendrites were also found with RNAi lines targeting *piezo*, *oven mitt*, *trivet*, *fire dancer*, *SECISBP2*, *pros-alpha1* and *NC2beta* (Figure 3). Among hypersensitive hits, *smoke alarm* and *G-alpha47A* RNAi resulted in significantly increased dendrite phenotypes, which potentially contributes to their hypersensitive nociception phenotypes (Figure 4).

In contrast, some hypersensitive hits actually showed a mild (but statistically significant) reduction in dendrite coverage (*Mnt*, *sop2*, *ppk*, *fire exit* and *Mctp*) (Figure 4). Thus, perhaps not surprisingly, the degree of dendrite branching cannot be perfectly correlated with noxious heat sensitivity. An interesting possibility is that targeting these genes with RNAi results in hypersensitivity due to effects in another compartment of the cell such as axons and/or synapses. Or alternatively, hypersensitivity in the dendrites masks a pleiotropic morphologically reduced dendrite phenotype.

*highwire*, one of hypersensitive candidates (Figure 2B and Table 1), has been shown to be important for dendrite and axonal morphogenesis of Class IV neurons (Wang et al., 2013). Interestingly *hiw* RNAi did not cause reduced dendritic arbors that have been reported with strong loss of function alleles for *hiw*. Our detailed analyses of *hiw* indicate that the hypersensitive nociception phenotype is more sensitive to *hiw* dosage than are the previously reported dendrite phenotypes (Honjo and Tracey, unpublished observations). As in the case of *hiw*, RNAi knockdown often results in phenotypes that are less severe than those that would be observed with null alleles.

Indeed, there are other caveats to be considered when using an RNAi screening methodology. The incomplete knockdown effect can also result in false negatives, which are estimated to occur in up to 40% of the UAS-RNAi strains in the major collection of strains

used in our screen. Thus, the lack of a phenotype in our screen cannot be used to conclusively infer a lack of function for a particular gene of interest. As well, false positives may occur, presumably due to off target effects. When the UAS-RNAi used in conjunction with *UAS-dicer-2* (as in our experiments) the effectiveness of knockdown is enhanced, and off-target effects are seen in approximately 6% of lines (when tested in the very sensitive crystalline lattice of the eye, or in the notum). Applying this estimate to the 36 genes implicated by our screen cautions that two or more of the candidates may represent false positives.

### **Nociception genes are evolutionarily conserved in mammals**

Twenty out of the thirty-six of the genes that are implicated here in nociception have clearly predicted mammalian orthologues (Table 1). Interestingly, published evidence supports roles for some of these orthologues in regulating mammalian nociception. Nociceptor and thermoreceptor-specific knock-out of *MYCBP2*, a mammalian homologue of *highwire*, shows prolonged thermal hypersensitivity with formalin-induced hyperalgesia (Holland et al., 2011). Knockdown of *highwire* caused an intriguingly similar hypersensitivity to heat (Figure 2B and Table 1).

RNAi targeting *Neprilysin-3*, encoding a neutral endopeptidase, showed hypersensitive nociception (Figure 2B and Table 1). Loss of ECE2, one of six predicted mammalian homologues of Neprilysin-3, has been also implicated in hypersensitive nociception as a knock-out mouse for ECE2 exhibits thermal hypersensitivity and rapid tolerance to morphine induced analgesia (Miller et al., 2011). In addition, knock-out of MME (aka NEP), another homologue of Neprilysin-3, causes thermal hyperalgesia (Fischer et al., 2002). These results thus raise the possibility that inhibitory nociceptive functions of Neprilysin-3 may be conserved between flies and mammals.

To our knowledge, the remaining conserved genes that our studies implicate in nociception remain to be functionally implicated in mammalian pathways. However, it is very intriguing that many of these conserved candidate genes are more highly expressed in nociceptors compared to non-nociceptive neurons or other tissues (Chiu et al., 2014; Goswami et al., 2014; Thakur et al., 2014). The orthologues of the seventeen out of twenty candidate nociception genes that came out from our study have been reported to show significantly enriched expression in nociceptive sensory neurons (Table 1). These genes will thus be particularly promising targets to identify previously uncharacterized molecular pathways involved in nociception.

Two ion channel genes from our screen have been implicated in mechanical nociception, *ppk* and *Piezo* (Figure 2, Table 1). Since studies of *Piezo* in Class IV neurons have observed defective phenotypes in mechanical nociception assays but not in thermal nociception assays (Kim et al., 2012) it is surprising that *Piezo* RNAi causes thermal insensitivity. The apparent discrepancy may be because the two *Piezo* RNAi strains used in this study specifically target low-abundance exons that were previously annotated as an independent gene, *fos28F* (Graveley et al., 2011). And in our microarray dataset we found enriched expression for “*fos28F*” in Class IV neurons but not for *piezo*. Our interpretation of these microarray data is that a nociceptor specific transcript for *piezo* exists and it contains sequences from the

previously annotated gene *fos28F*. Both of the RNAi constructs that target the *fos28F/piezo* exons caused gross abnormalities in dendritic and axonal gross morphology (Figure 3 and data not shown). RNAi lines targeting canonical *piezo* exons do not cause a similar thermal nociception phenotype (KH unpublished observations). Thus, the nociceptor-specific knockdown of the low-abundance transcriptional variant containing exons from *fos28F* appears to disrupt the morphology and thermal nociception capacity of Class IV neurons.

It was also unexpected that two independent *ppk* RNAi strains collected from different libraries showed hypersensitive thermal nociception phenotypes. This contrasts with the severely insensitive mechanical nociception phenotypes that occur with the loss of *ppk* (Zhong et al., 2010). Our previous studies did not detect thermal hypersensitivity due to the testing with a single higher probe temperature (46 °C). The finding that *ppk* RNAi causes thermal hypersensitivity highlights the importance of using the 42° C probe temperature in the search for hypersensitive phenotypes. In addition, the results indicate that modality specific phenotypes within the larval nociceptors can be of opposite sign. It is interesting to note that *ppk* mutants have been found to show a locomotion phenotype in which the animals crawl rapidly in a straight line across the substrate with very infrequent turning (Ainsley et al., 2003). A similar form of locomotion is also seen in a second phase of nociception behavior that immediately follows rolling behavior (NEL) (Ohyama et al., 2013). Thus, it is interesting to speculate that the locomotion phenotype of *ppk* mutants is actually a consequence of a hypersensitive process in the nociceptors. This in turn may be causing the larvae to continuously manifest the fast crawling phase of nociception escape.

In conclusion, we have carried out a large-scale screen that combines molecular approaches to identify cell type enriched nociceptor RNA with *in vivo* functional studies of the same identified RNAs in a phenotypic screen. This approach has led to the identification of a set of nociception genes. Many of these genes are evolutionarily conserved and also show enriched expression in mammalian nociceptors, future studies will reveal the physiological importance and molecular mechanisms that depend on these molecules.

## Experimental procedures

### Laser Capture Microdissection and Microarray analysis

Detailed methods for our Laser Capture Microdissection and Microarray analysis were previously described in Mauthner et al. (Mauthner et al., 2014).

### Thermal nociception screen

Three males of each RNAi strain were crossed to six virgin females of *ppk1.9-GAL4; UAS-dicer2* strain in a standard molasses cornmeal food vial, and incubated for 5 to 7 days at 25° C prior to harvest of the F1 wandering third instar larvae. Control crosses (a control strain crossed to *ppk1.9-GAL4; UAS-dicer2*) were performed side-by-side.

Thermal nociception assays were performed as described previously (Hwang et al., 2012; Tracey et al., 2003; Zhong et al., 2012). To detect insensitivity and hypersensitivity phenotype efficiently, each *UAS-RNAi* × *ppk1.9-GAL4; UAS-dicer2* pair was tested by

using two different probe temperatures: A custom-made thermal probe heated to 46° C was used to test insensitivity and the probe heated to 42° C was used for hypersensitivity.

### Quantifying dendrite coverage of Class IV neurons

Dendritic field coverage was quantified on composite images of maximum intensity projections of confocal micrographs and quantified as described previously (Stewart et al., 2012) with slight modifications. Images of ddaC neurons were overlaid with a grid of 32 × 32 pixel squares (14 × 14 μm), and squares containing dendritic branches were counted to calculate a dendritic field coverage score (i.e. the percentage of squares containing dendritic branches / the total number of squares). Counting dendrite-positive squares was done with a Matlab custom code and then manually curated to eliminate false-positives and false-negatives. One or two neurons from each imaged animal were analyzed.

### Statistical analyses

All pair wise comparisons were performed with Mann-Whitney's U-test. For multiple comparisons, Steel's test (non-parametric equivalent of Dunnet's test) was used. Statistical analyses were performed in the R software and Kyplot.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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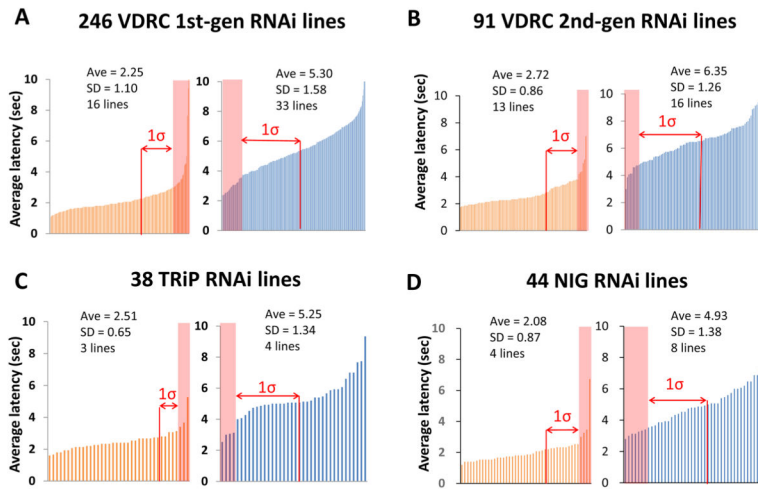
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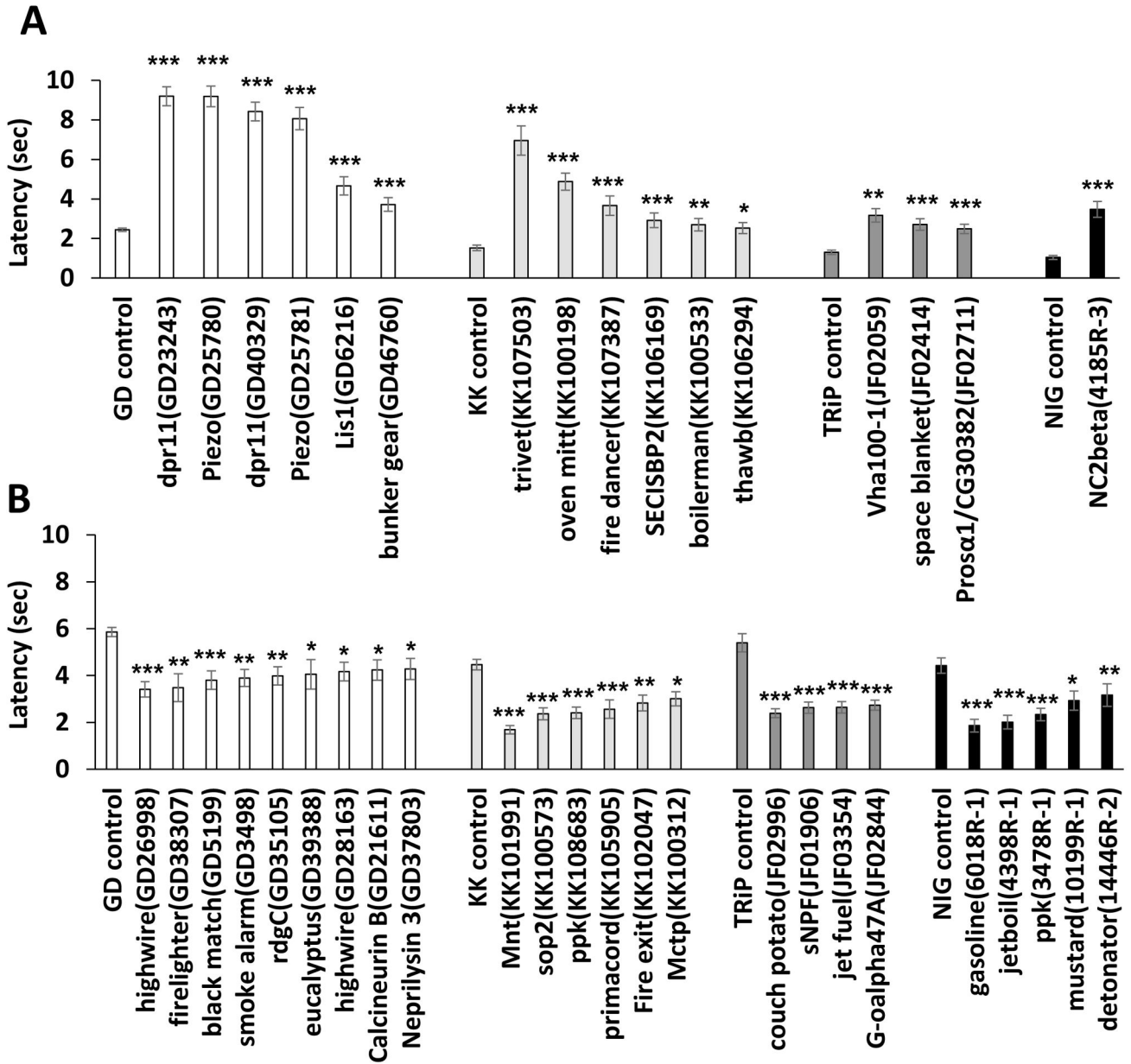


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**Figure 1. Summary of primary screen results**

Summary of the insensitivity and the hypersensitivity screen with (A) 1st-generation VDCR (GD) lines, (B) 2nd-generation VDCR (KK) lines, (C) TRiP lines, (D) and NIG lines. The left chart in each panel with the orange bars show the results of the insensitivity screen with a 46°C probe. The right chart in each panel, with blue bars, shows the hypersensitivity screen results with a 42°C probe. The average latency and standard deviation of all tested lines and the number of lines which survived the initial cut-off (+1σ for the insensitivity screen and -1σ for the hypersensitivity screen) are indicated with each graph. Shaded areas indicate lines that were selected for retesting. See also Table S1B–E.



**Figure 2. RNAi lines that showed significant insensitivity or hypersensitivity upon retesting with a larger sample size**

The behavioral responses of retested lines in the hypersensitivity and insensitivity screens. Each panel shows average latency on the Y axis and the targeted genes for each genotype are listed along the X axis. (A) *ppk*-GAL4 dependent insensitive behavioral responses seen with crossing to 1st-generation VDRC (GD) lines, 2nd-generation VDRC (KK) lines, TRiP lines and NIG lines. (B) *ppk*-GAL4 dependent hypersensitive behavioral responses seen with crossing to VDRC (GD and KK) lines, TRiP lines and NIG lines. Steel's test was used to statistically compare each genotype to its appropriate control except that Mann-Whitney's U-test was used to perform the pair-wise comparison of NC2beta versus NIG control (n >

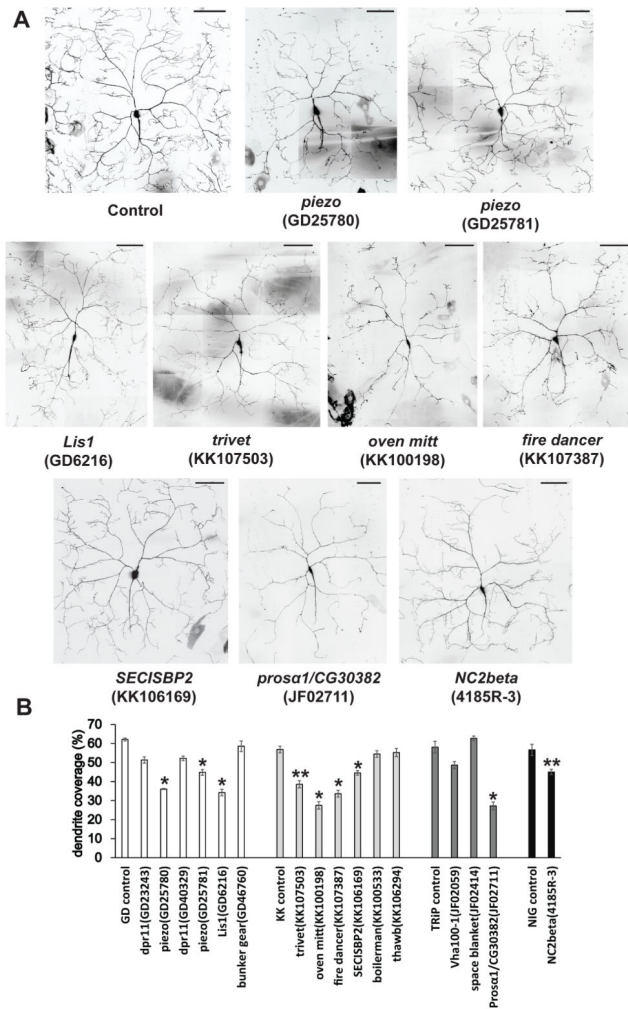
32; \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ ). Error bars represent S.E.M. See also Table S1F-O, Table S2 and Figure S1.

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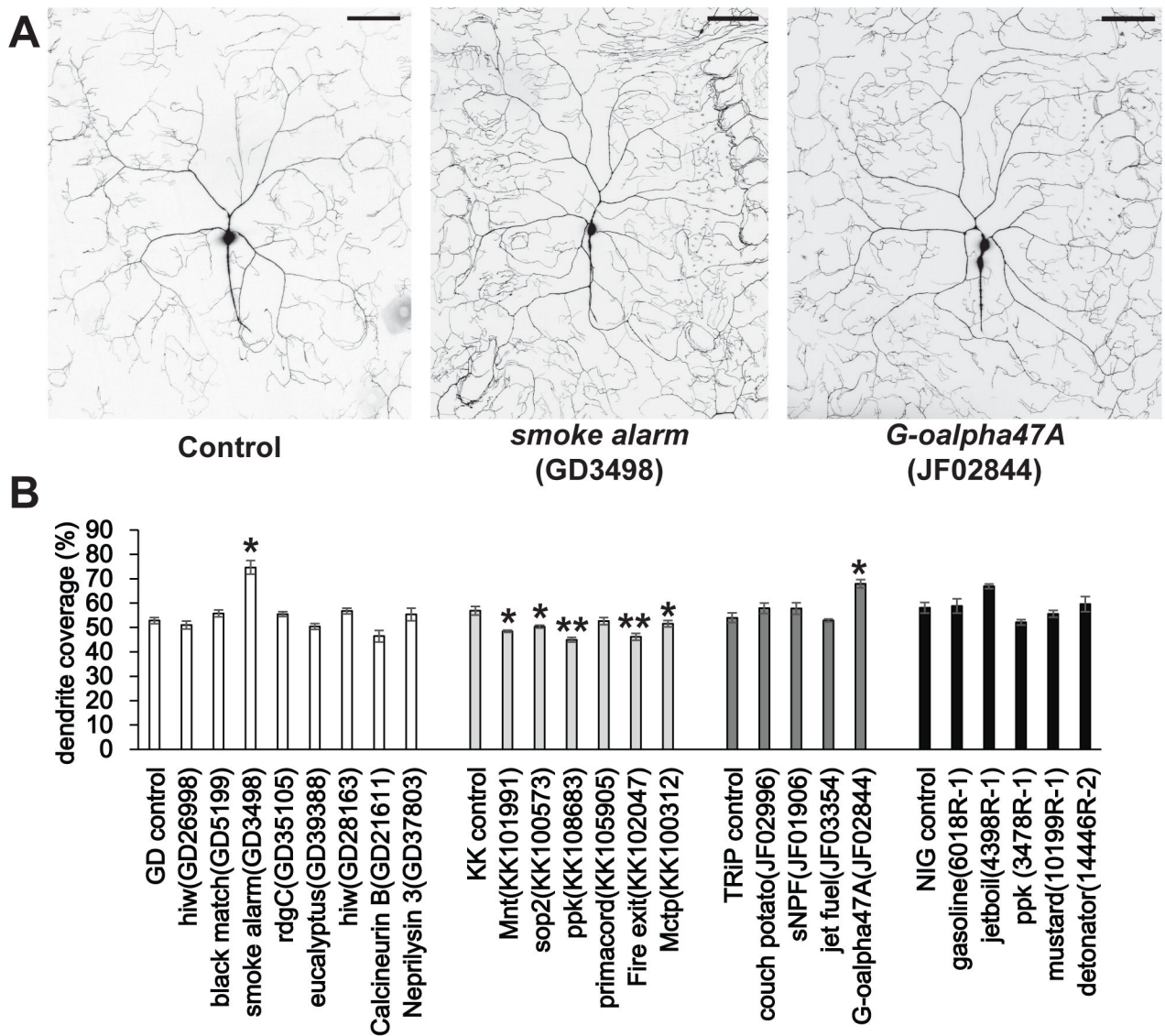
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**Figure 3. Genes targeted with RNAi that showed a reduced dendrite phenotype**  
 (A) Representative composite images of the dendritic structure of Class IV ddaC neurons in thermal nociception insensitive RNAi lines that also showed significantly reduced dendritic coverage. Scale bars represent 100  $\mu$ m. (B) Quantification of dendritic coverage for insensitive RNAi lines. Steel’s test was used for statistical comparisons between each genotype and controls, except that Mann-Whitney’s U-test was used to compare NC2beta and NIG control (n > 4; \* p < 0.05 and \*\* p < 0.01). Error bars represent S.E.M. For representative images of RNAi lines that did not show significantly reduced dendrite, see Figure S2.



**Figure 4. Genes targeted with RNAi that showed an increased dendrite coverage phenotype** (A) Representative composite images of the dendritic field of Class IV ddaC neurons in RNAi lines with hypersensitive thermal nociception animals that also showed significantly increased dendritic coverage. Scale bars represent 100  $\mu$ m. (B) Quantification of dendritic coverage for hypersensitive candidate RNAi lines. Steel's test was used for statistical comparisons between each genotype and controls, except that Mann-Whitney's U-test was used to compare NC2beta and NIG control ( $n > 4$ ; \*  $p < 0.05$  and \*\*  $p < 0.01$ ). Error bars represent S.E.M. For representative images of RNAi lines that did not show significantly increased dendrite coverage, see Figure S3. The effects seen in the KK collection was not a consequence of unintended Tio expression (See supplemental experimental procedures, Table S2 and Figure S2)

Table 1

Candidate genes

Insensitive Candidates										
CG number	Synonym	New name	RNAi line	IV/I	Latency	Significance	Gene Ontology	Human orthologs		
CG33202	<i>dpr11</i>		GD23243 GD40329	9.83	9.20 ± 0.48 8.42 ± 0.47	p < 0.001 p < 0.001	Ig family, Membrane protein			
CG18103	<i>Piezo</i>		GD25780 GD25781	6.12	9.19 ± 0.52 8.06 ± 0.56	p < 0.001 p < 0.001	mechanically-gated ion channel activity	<i>PIEZO1<sup>a</sup></i> , <i>PIEZO2<sup>a,b,c</sup></i>		
CG8297	<i>CG8297</i>	<i>bunker gear (bug)</i>	GD46760	2.82	3.72 ± 0.34	p < 0.001	Thioredoxin-like fold.	<i>TXNDC15</i>		
CG8440	<i>Lis1</i>		GD6216	2.81	4.66 ± 0.46	p < 0.001	Dynein-binding, Microtubule organization	<i>PAFAH1B1<sup>c</sup></i>		
CG12681	<i>CG12681</i>	<i>boilerman (boil)</i>	KK100533	12.48	2.70 ± 0.32	p < 0.01	Unknown			
CG14608	<i>CG14608</i>	<i>thawb (thw)</i>	KK106294	43.59	2.52 ± 0.28	p < 0.05	Chitin binding, Chitin metabolic process			
CG31976	<i>CG31976</i>	<i>oven mitt (ovm)</i>	KK100198	15.81	4.88 ± 0.43	p < 0.001	Unknown			
CG34362	<i>CG12870</i>	<i>trivet (trv)</i>	KK107503	10.06	6.96 ± 0.74	p < 0.001	Nucleotide binding, Alternative splicing	<i>TIA1<sup>b</sup></i> , <i>TIA1L</i>		
CG7066	<i>SECIS-binding protein2</i>		KK106169	3.37	2.92 ± 0.37	p < 0.001	Selenoprotein synthesis	<i>SECISBP2<sup>c</sup></i> , <i>SECISBP2L</i>		
CG42261	<i>CG8968</i>	<i>fire dancer (fid)</i>	KK107387	3.06	3.66 ± 0.49	p < 0.001	Methyltransferase activity			
CG18495	<i>Proteasome α.1 subunit</i>		JF02711	2.18	2.48 ± 0.33	p < 0.001	Endopeptidase activity, Proteasome core complex.	<i>PSMA6</i>		
CG1709	<i>Vha100-1</i>		JF02059	3.24	3.17 ± 0.33	p < 0.01	V-ATPase, Calmodulin binding, synaptic vesicle exocytosis	<i>ATP6V0A4</i> , <i>ATP6V0A1</i> , <i>ATP6V0A2<sup>c</sup></i>		
CG8216	<i>CG8216</i>	<i>space blanket (spab)</i>	JF02414	13.27	2.71 ± 0.29	p < 0.001	Unknown			
CG4185	<i>NC2 beta</i>		4185R-3	6.994	3.47 ± 0.40	p < 0.001	histone acetyltransferase activity	<i>Drf1<sup>a</sup></i>		

Hypersensitive Candidates

Hypersensitive Candidates										
CG number	Synonym	New name	RNAi line	IV/I	Latency	Significance	Gene Ontology	Human orthologs		
CG32592	<i>highwire</i>		GD26998 GD28163	6.04	3.41 ± 0.33 4.17 ± 0.40	p < 0.001 p < 0.05	ubiquitin-protein ligase activity	<i>MYCBP2<sup>c</sup></i>		
CG14946	<i>CG14946</i>	<i>firefighter (firf)</i>	GD38307	2.627	3.48 ± 0.59	p < 0.01	Short-chain dehydrogenase/reductase SDR; Glucose/ribitol dehydrogenase; NAD(P)-binding domain.	<i>HSD17B13</i> , <i>SDR16C5</i> , <i>RDH10</i> , <i>HSD17B11<sup>c</sup></i> , <i>DHR53</i>		
CG34356	<i>CG12524</i>	<i>black match (bma)</i>	GD5199	3.85	3.80 ± 0.39	p < 0.001	ATP binding, protein kinase activity			

Hypersensitive Candidates										
CG number	Synonym	New name	RNAi line	IV/I	Latency	Significance	Gene Ontology	Human orthologs		
CG34380	CG13988	<i>smoke alarm (small)</i>	GD3498	2.57	3.89 ± 0.37	p < 0.01	Receptor signaling	DDR1 <sup>a</sup> , DDR2		
CG4209	<i>Calcineurin B</i>		GD21611	3.79	4.24 ± 0.44	p < 0.05	calcium ion binding; cithreomine phosphatase activity	PPP3R1 <sup>a</sup> , PPP3R2, WDR92		
CG6571	<i>rdgC</i>		GD35105	3.74	3.98 ± 0.39	p < 0.01	Calmodulin binding, phototransduction	PPEF1 <sup>a,b</sup> , PPEF2		
CG12269	CG12269	<i>eucalyptus (euc)</i>	GD39388	11.57	4.05 ± 0.63	p < 0.05	sterol binding			
CG9565	<i>Neprilysin 3</i>		GD37803	2.33	4.28 ± 0.45	p < 0.05	Metalloendopeptidase	ECE1 <sup>a,c</sup> , MMEL1, ECEL1, ECE2 <sup>a,c</sup> , MME, PHEX		
CG1079	<i>fire exit</i>		KK102047	14.98	2.82 ± 0.33	p < 0.01	Unknown			
CG13316	<i>Mint</i>		KK101991	2.69	1.68 ± 0.18	p < 0.001	DNA binding, Transcriptional repressor, Negative regulator of cell growth, Phagocytosis	PHF15 <sup>a</sup> , PHF16 <sup>a</sup> , PHF17		
CG15078	<i>Mcp</i>		KK100312	34.97	3.00 ± 0.31	p < 0.05	calcium ion binding	MCTP1 <sup>a,c</sup> , MCTP2 <sup>c</sup>		
CG15704	CG15704	<i>primacord (prim)</i>	KK105905	4.35	2.56 ± 0.39	p < 0.001	Unknown			
CG3478	<i>pickpocket1</i>		KK108683	28.03	2.41 ± 0.24	p < 0.001	acid-sensing ion channel activity			
			3478R-1		2.33 ± 0.27	p < 0.001				
CG8978	<i>Suppressor of profilin 2</i>		KK100573	15.74	2.36 ± 0.26	p < 0.001	actin binding; structural constituent of cytoskeleton	ARPC1B <sup>a,b,c</sup>		
CG13968	<i>short neuropeptide F</i>		JF01906	62.4	2.63 ± 0.24	p < 0.001	hormone activity; neuropeptide hormone activity			
CG31243	<i>couch potato</i>		JF02996	2.57	2.39 ± 0.19	p < 0.001	mRNA binding; nucleic acid binding; nucleotide binding	RBPMS <sup>a</sup> , RBPMS2		
CG2204	<i>G protein o alpha 47A</i>		JF02844	2.66	2.73 ± 0.22	p < 0.001	G-protein beta/gamma-subunit complex binding; G-protein coupled receptor binding; GTPase activity; signal transducer activity	GNAT1, GNAI2 <sup>a,c</sup> , GNAO1 <sup>a,b,c</sup> , GNAI3 <sup>a</sup> , GNAZ <sup>a</sup> , GNAT2, GNAT3		
CG12858	CG12858	<i>jet fuel (jef)</i>	JF03354	7.1	2.64 ± 0.25	p < 0.001	transmembrane transport	MFSD6 <sup>c</sup>		
CG32464	<i>mustard</i>		10199R-1	7.276	2.93 ± 0.41	p < 0.05	Peptidoglycan-binding Lysin subgroup, immune response			
CG14446	CG14446	<i>detonator (dtn)</i>	14446R-2	4.027	3.17 ± 0.48	p < 0.01	Unknown			
CG4398	CG4398	<i>jetboil (jib)</i>	4398R-1	13.39	2.01 ± 0.29	p < 0.001	Insect allergen-related			
CG6018	CG6018	<i>gasoline (gas)</i>	6018R-1	96.18	1.86 ± 0.27	p < 0.001	carboxylesterase activity, Carboxylesterase, type B			

<sup>a</sup>Genes enriched in nociceptive lineage neurons compared to proprioceptive lineage neurons in Chiu et al. (2014).

<sup>b</sup>Genes enriched in nociceptors compared to unpurified DRG neurons in Thakur et al. (2014).



Genes enriched in nociceptors compared to cortical neurons in Thakur et al. (2014).

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