Cell Reports, Volume *38*

Supplemental information

Visualization of trigeminal ganglion sensory neuronal signaling regulated by Cdk5

Minghan Hu, Andrew D. Doyle, Kenneth M. Yamada, and Ashok B. Kulkarni

Supplemental Figure 1

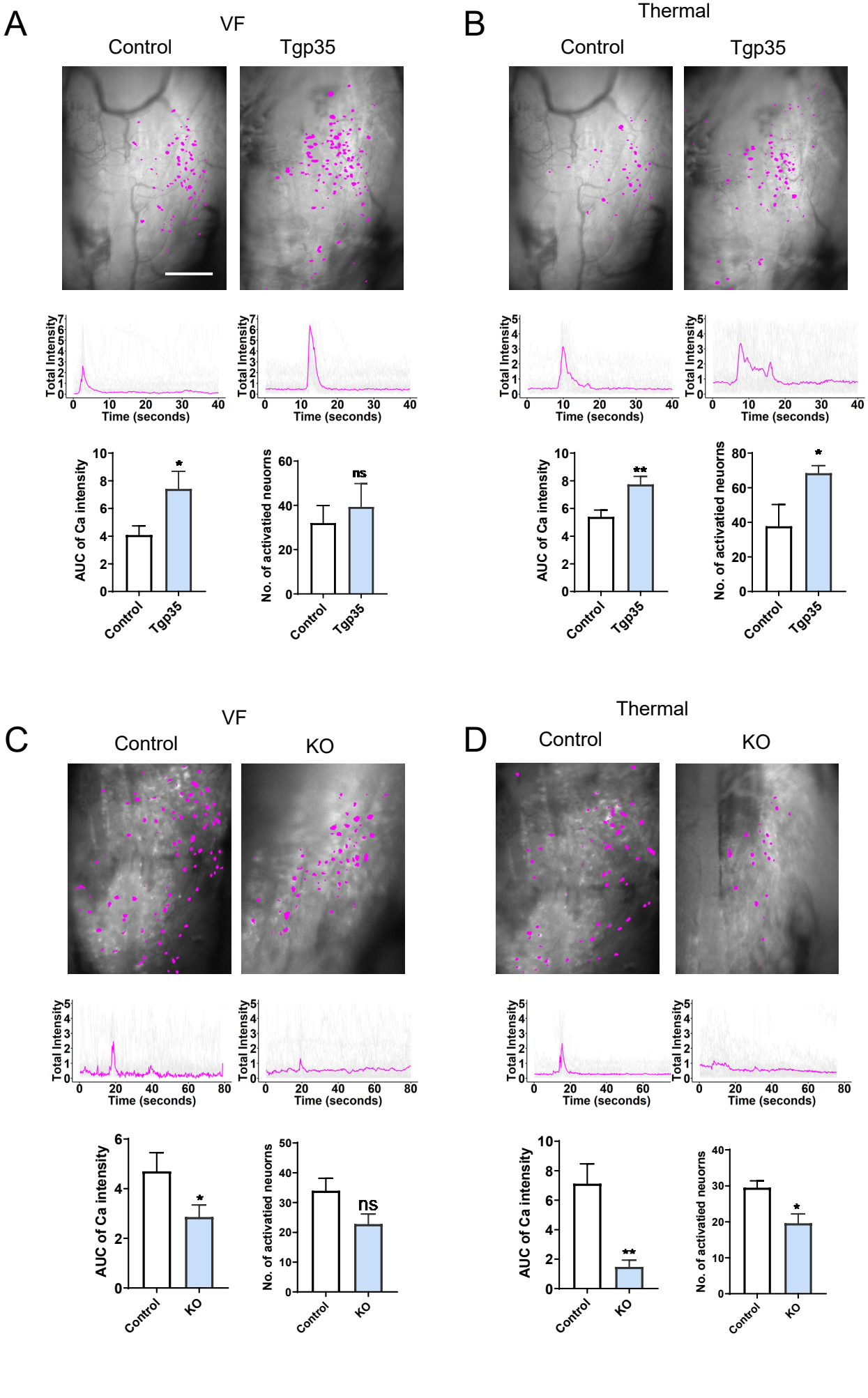


Fig. S1. Trigeminal ganglion sensory neuronal responses to von Frey hair or thermal facial stimulation in Tgp35 and p35 knockout mice. Related to Figure 2.

A. Top panels: representative imaging fields of trigeminal ganglion TRPV1-GCaMP6-expressing neurons in response to von Frey hair (VF) stimulation (2g) in control and Tgp35 mice, respectively. Middle panels: representative calcium traces in response to VF stimulation in individual mice, representing typical response profiles for this stimulation. Gray lines are traces from each trigeminal ganglion neuron; magenta line shows the mean values for these traces. Lower left panel: comparison of AUC quantifications of calcium traces from control and Tgp35 mice in response to VF, showing a significant increase in responses to this stimulation. Lower right panel: numbers of neurons activated by this stimulation, showing there is no significant difference in responses of Tgp35 compared to control mice (n= 6 mice). AUC, area under the curve; the data are presented as mean ± SEM, *$p<0.05$, **$p<0.001$, Scale bar, 500 μm. B. For thermal stimulation, the AUCs of total calcium intensity and numbers of activated neurons increase significantly in Tgp35 mice. C. For von Frey hair (VF) stimulation (2g), the AUCs of total calcium intensity decrease significantly in p35 knockout mice, but with no significant differences in numbers of neurons responding. D. For thermal (47°C) stimulation both AUCs and numbers of activated neurons dramatically decrease in p35 knockout mice compared to wildtype mice.

Supplemental Figure 2

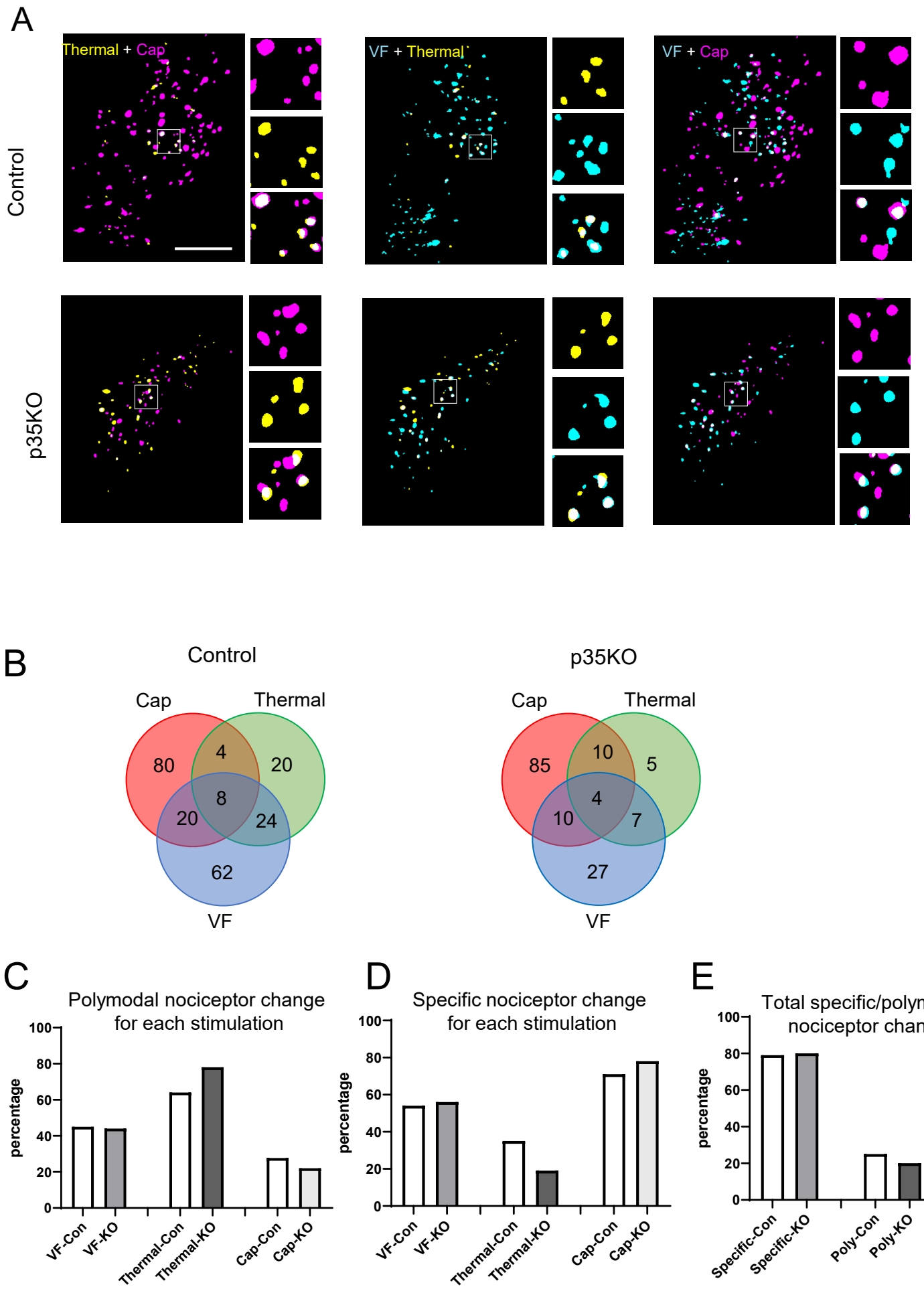
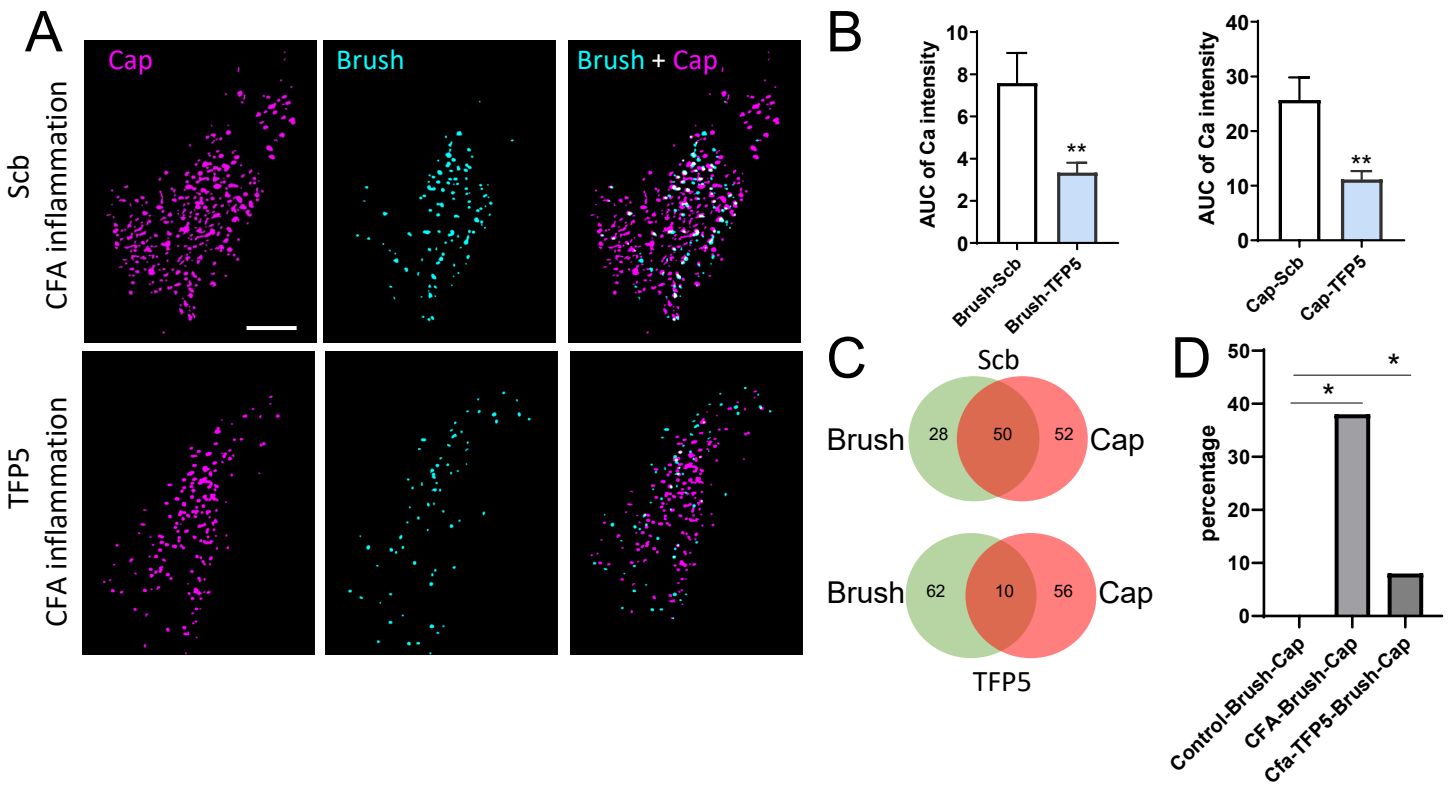
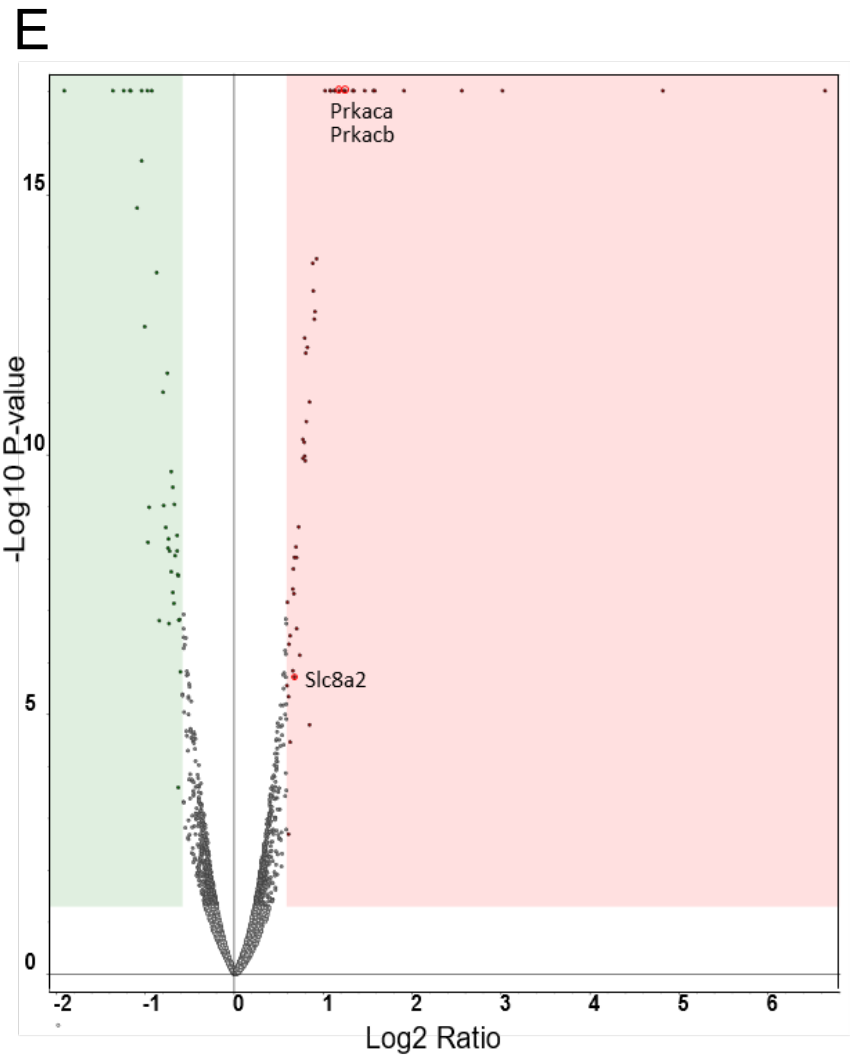


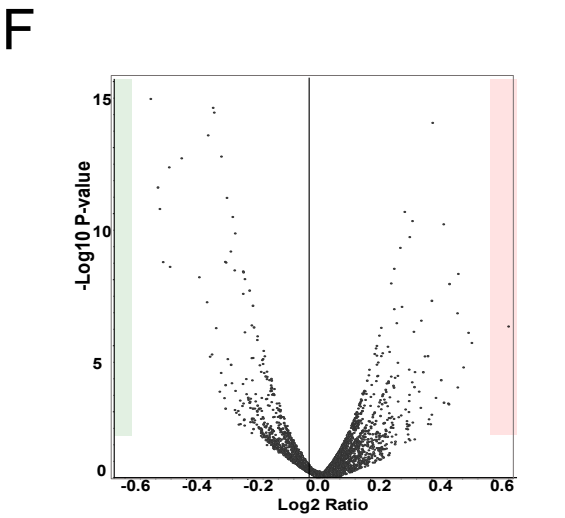
Fig.S2. Altered distribution of polymodal sensory neuron signaling in trigeminal ganglia of p35 knockout mice. Related to Figure 3

A. Representation of specific and polymodal neuron activation in Tgp35 and p35 knockout mice. Left panels: Color-coded maps of neurons activated by either capsaicin (magenta) or thermal (yellow), or by both stimuli (white) in control vs. p35 knockout mice. On the right side of each set of images are zoomed-in (magnified) versions of white-square areas illustrating thermal or capsaicin stimulation and the merge of the two stimulations. Middle and right sets of panels are color-coded maps showing the distribution of neurons responding to VF and thermal and then VF and capsaicin stimulations respectively. Scale bar, 500 μm. B. Venn diagrams illustrating the numbers of specific and polymodal of TRPV1-linage trigeminal ganglion neurons responding to von Frey mechanical (blue), thermal (green), or capsaicin (red) stimulation in control vs. p35 knockout mice. n=3 mice, 366 neurons. C. The percentage change of polymodal nociceptor neurons for each stimulation. D. The percentage distribution of specific nociceptor neurons responding to von Frey, thermal, or capsaicin stimulation. E. Summary of the percentages of specific and polymodal nociceptors responding to von Frey, thermal, or capsaicin stimulation, indicating no significant changes in the proportions of signaling from specific compared to polymodal nociceptor neurons.

Supplemental Figure 3







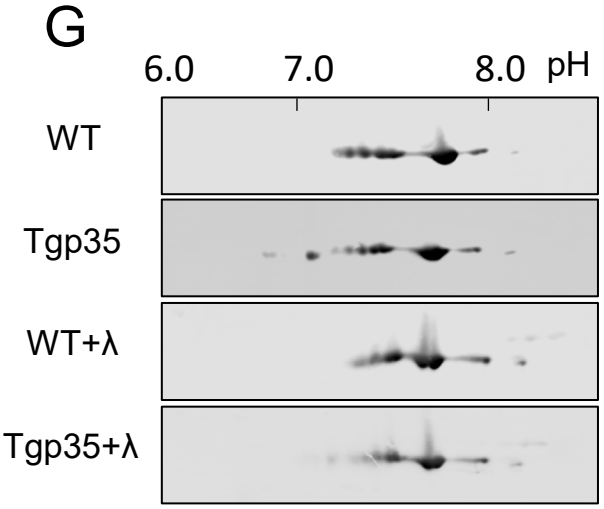


Fig. 3S A-D. Effects of Intraperitoneal injection of the Cdk5 inhibitor TFP5 on neuronal responses of trigeminal ganglia to orofacial brush and capsaicin in mice with facial inflammation. Related to Figure 4.

A. Representative color-coded maps illustrating neuronal responses to capsaicin (magenta) and brush (cyan) stimulation in mice treated with scrambled (Scb) control peptide or TFP5 peptide after CFA injection to induce inflammation. The righthand panels shows the merge of two stimulations, where white indicates overlap. Scale bar, 500 μm. B. Quantification comparing AUCs for each stimulation in Scb versus TFP5 mice showing a substantial decrease after TFP5 treatment compared to the Scb (scrambled) peptide control. C. Venn diagrams showing the distribution of specific and polymodal neuronal responses to brush and capsaicin stimulation. n=3 mice, 258 neurons. D. Altered percentages of polymodal nociceptor neurons responding to both brush and capsaicin stimulation. There is a robust percentage increase of polymodal nociceptors after CFA-induced inflammation in the orofacial region of mice, and a significant decrease back toward control levels after TFP5 treatment.

Fig. 3 E-G. Increased phosphorylation activity in Tgp35 mice. Related to Figure 3.

E. Volcano plot representing the global phosphoproteome dataset from trigeminal ganglion phosphopeptide-enriched samples according to log2 fold change (x axis) and p value (y axis). High-sensitivity LC-MS/MS-based proteomics and phosphoproteomics approaches were used to search for differentially expressed proteins and phosphoproteins in trigeminal ganglia from wildtype versus Tgp35 mice with elevated Cdk5 activity. Five biological replicates of the proteomics and five biological replicates of the phosphoproteomics were analyzed. The biological process comparison revealed 611 phosphorylated proteins. A phosphoprotein was considered differentially expressed when the protein had both a fold-change of more than 1.5 and a p value of less than 0.05. Red dots show increased levels of phosphorylated cAMP-dependent protein kinase (PKA) catalytic subunits α and β (Prkaca, Prkacb) and of the sodium/calcium exchanger Slc8a2 in the volcano plot.

F. Fractioned proteomics volcano plot to show the overall distribution of protein fold changes between wildtype and Tgp35 mouse trigeminal ganglion samples. The biological process comparison revealed 5426 proteins. There is no increased expression of the proteins prkaca, prkacb, and Slc8a2 according to the proteomics analysis.

G. Phosphorylation of TRPV1 detected by 2D gel electrophoresis. TRPV1 was detected by western blotting with a specific polyclonal antibody (Alomone labs). Acidic and basic ends of the horizontal gel are marked. Samples in order from top to bottom were (1) pooled lysates prepared from wildtype mouse trigeminal ganglion; (2) lysates from Tgp35 mouse trigeminal ganglia; (3) lysates from wildtype mouse trigeminal ganglia that had been treated with λ-phosphatase prior to 2D gel electrophoresis; (4) lysates prepared from Tgp35 mouse trigeminal ganglia, treated with λ-phosphatase. For the Tgp35 mice with elevated Cdk5 activity, increased phosphorylated residues of TRPV1 with a shift towards a more acidic isoelectric point was observed compared to both control mice and λ-phosphatase treated Tgp35 TRPV1.