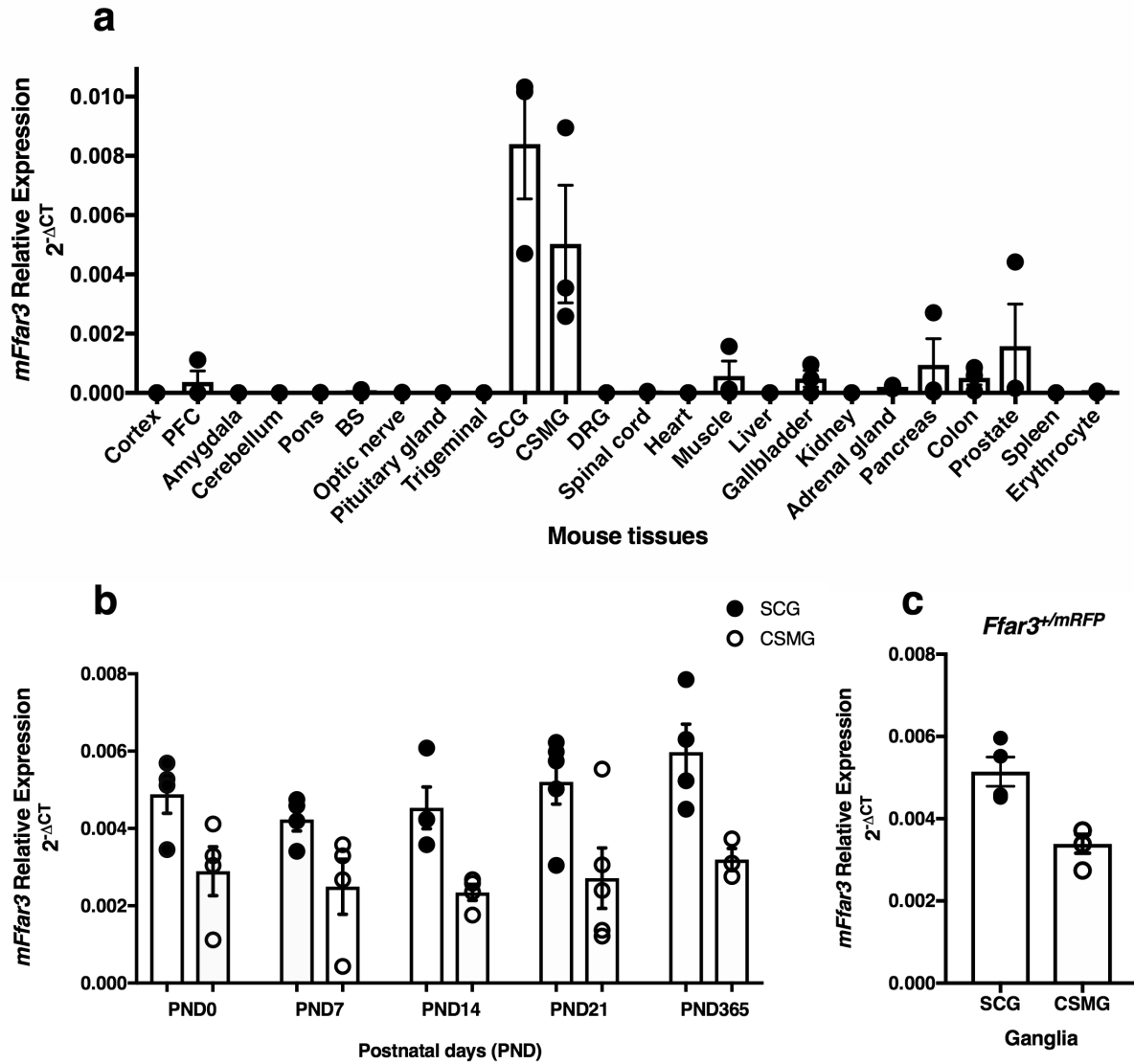


Selective tracking of FFAR3-expressing neurons supports receptor coupling to N-type calcium channels in mouse sympathetic neurons.

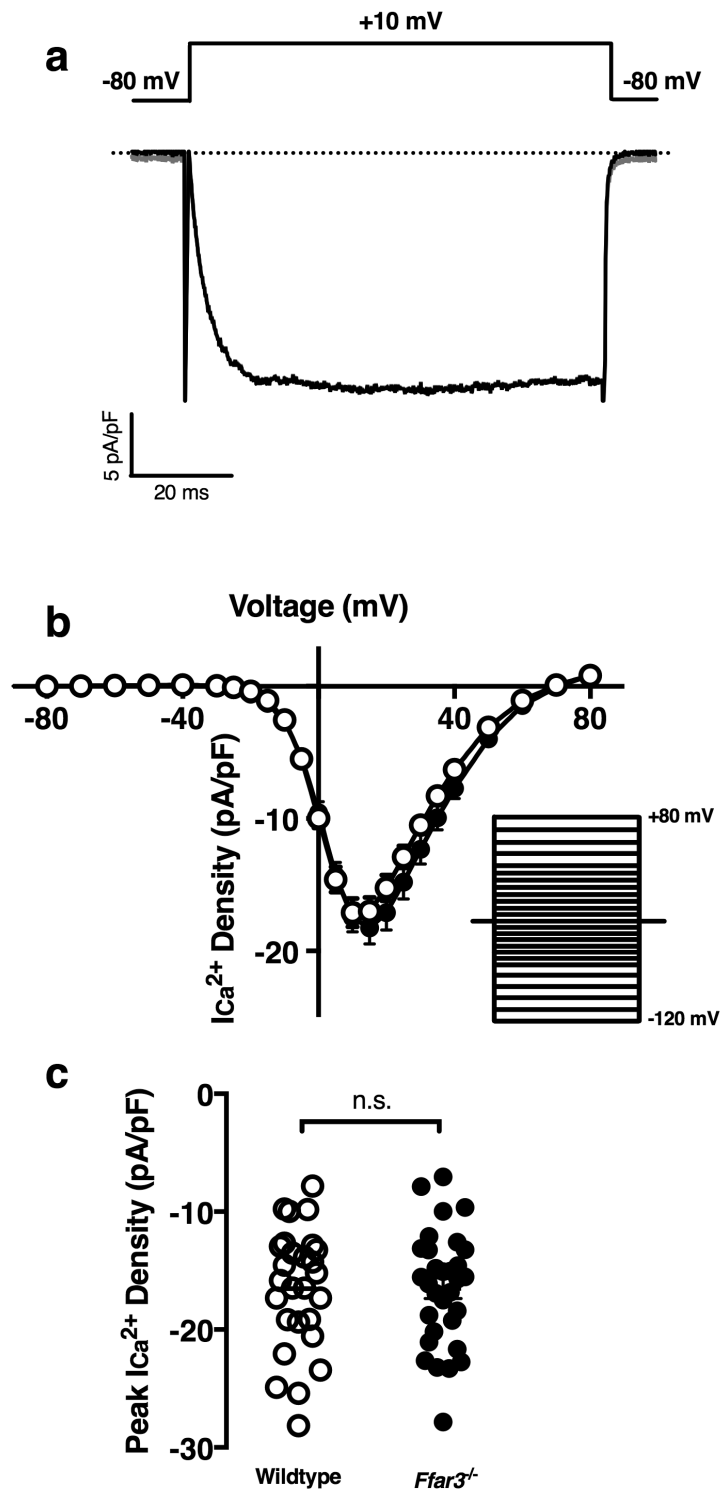
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Supplementary Figures:

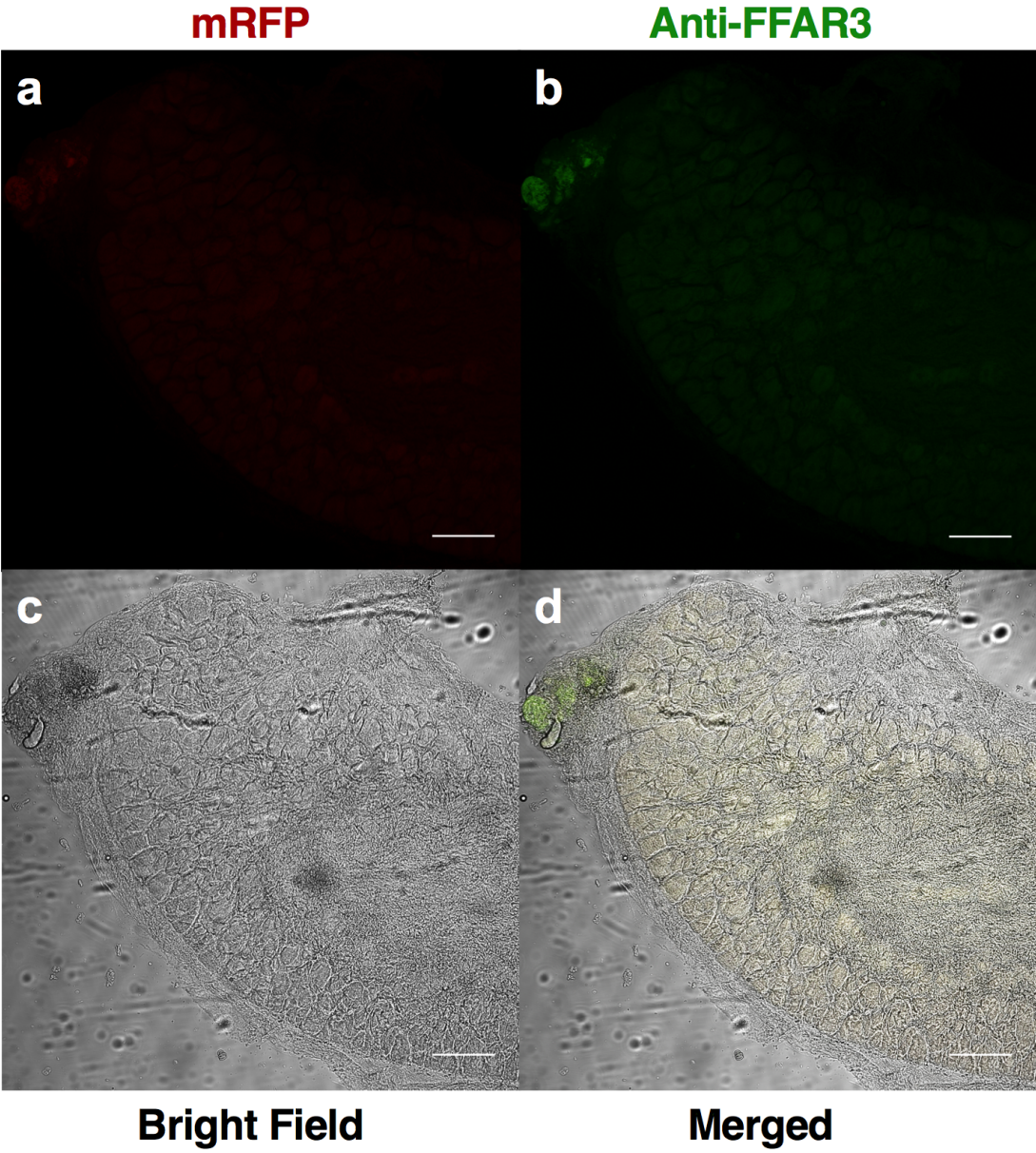
Figures 1



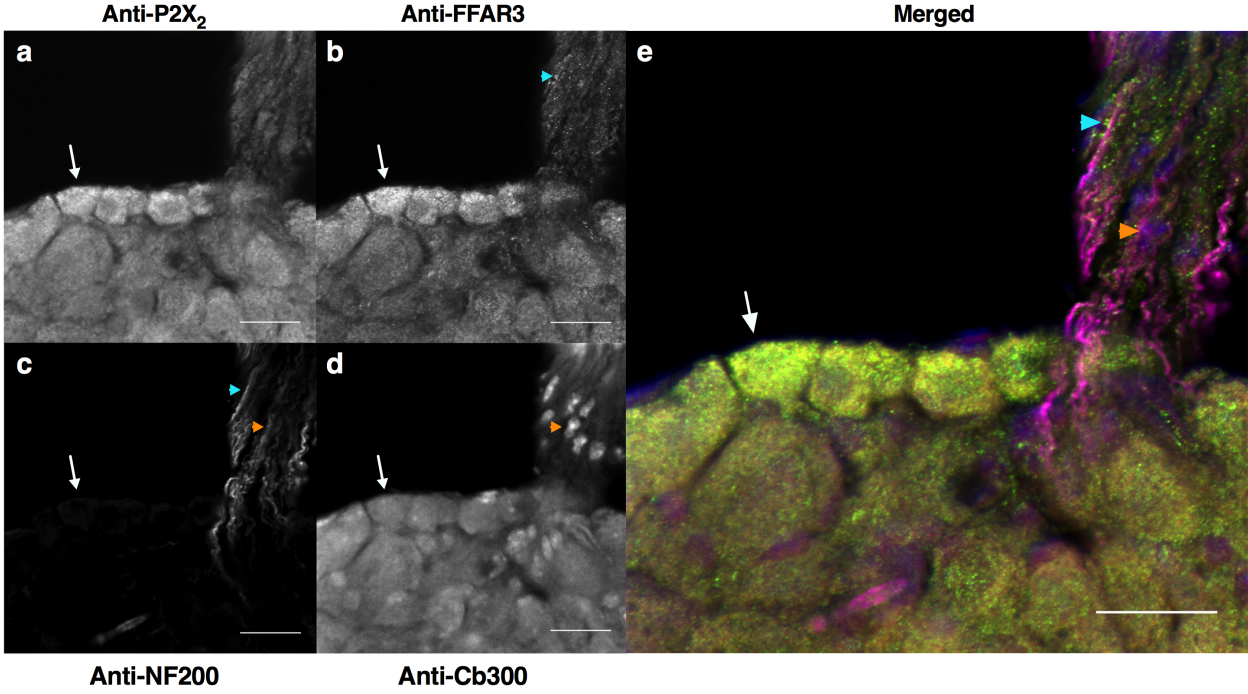
Figures 2



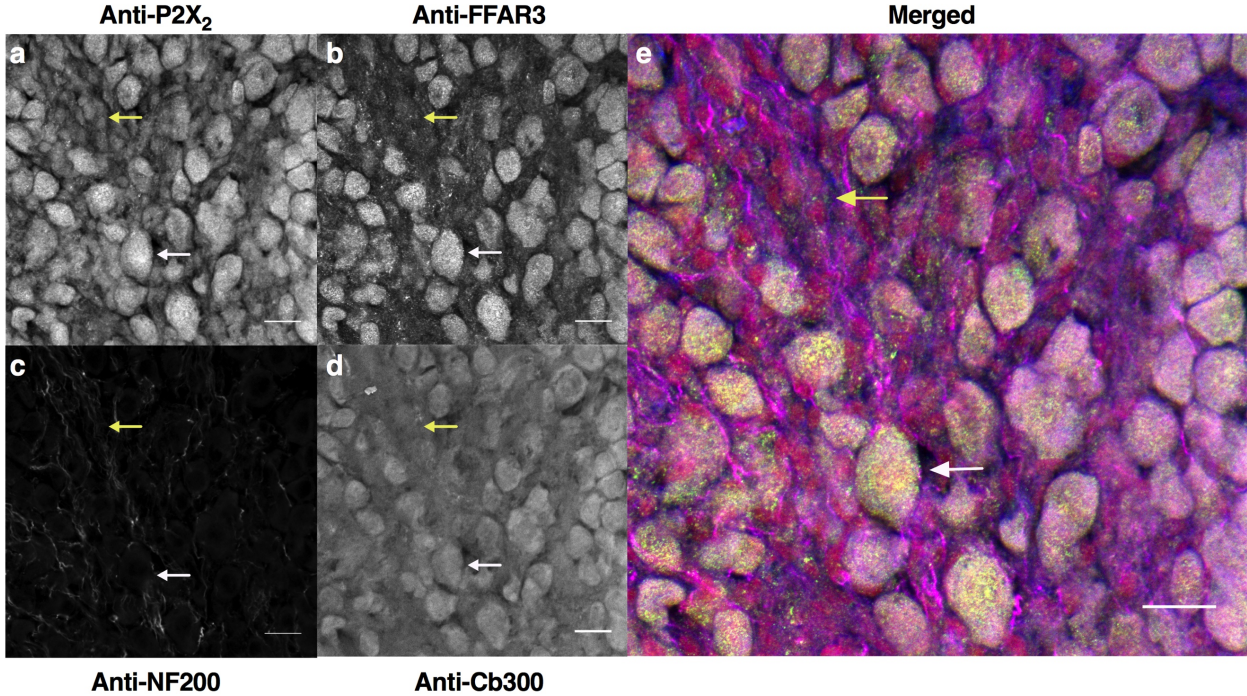
Figures 3



Figures 4



Figures 5



Supplementary Figures legends.

Supplementary Figure 1: *Ffar3* gene expression in mice tissues, developmental dynamic changes and *Ffar3* gene expression in mRFP hemizygous mice in pre/paravertebral ganglia.

a. Postnatal expression (PND 56-84) of *Ffar3* mRNA in different wild-type mouse tissues. PFC=prefrontal cortex, BS=Brainstem, SCG=superior cervical ganglion, CSMG=celiac-superior mesenteric ganglion, DRG=dorsal root ganglion. Total RNA was isolated from the indicated tissue/ganglia at postnatal day PND 84-168 (n = 3) from 8 mice and cDNA was reverse transcribed as indicated in materials and methods. A stochastic dominance was found on every tissue when compared to SCG *Ffar3* gene expression Kruskal-Wallis test followed by Dunn's multiple comparisons test (SCG vs different tissues ***, KW=55.6, $\alpha=0.05$ and $p=0.0002$). b. Postnatal expression of *Ffar3* mRNA in SCG (black circles) and CSMG (white circles). Total RNA was isolated from the indicated ganglia at postnatal day (PND) 0 (n = 4), 7 (n = 4), 14 (SCG n = 3, CSMG n=4), 21(n = 5), and 365 (n = 3). No evidence of stochastic dominance was found with the Kruskal-Wallis test followed by Dunn's multiple comparisons test (SCG KW= 5.16, $p=0.27$ and CSMG KW= 2.99, $p=0.56$). c. Postnatal expression of *Ffar3* mRNA in SCG (black circles) and CSMG (white circles) in *Ffar3*^{+mRFP} hemizygous mice. Total RNA was isolated from the indicated ganglia at postnatal day (PND) 182 (n = 4). In a, b and c *Ffar3* mRNA was quantified by qPCR using a custom made TagMan primers-probe set, targeted to the nucleotide 50 and probe was positioned to span an exon-exon junction. *Ffar3* gene expression was normalized to the geometric mean of *Actb* and *Gapdh* housekeeping genes and expressed as *Ffar3* relative expression.

Figure 2: Current densities are not altered by gene ablation of *Ffar3*.

a. Representative $I_{Ca^{2+}}$ density time courses for wild-type (black line) and *Ffar3*^{-/-} (red line). Voltage step is shown in the inset of panel a, 100 ms pulse from -120 mV to +80 mV. Only the $I_{Ca^{2+}}$ density generated by a +10 mV voltage step is showed for clarity. In order to obtain the $I_{Ca^{2+}}$ density, currents were normalized to whole-cell membrane capacitance (C_m). C_m was calculated by integration of an uncompensated cell capacitive transient current (charge, Q) elicited by 10mV depolarizing test pulse for 5 ms, from a holding potential of -80 mV and using

the equation $C_m=Q/V$ (3). b. Mean $I_{Ca^{2+}}$ density (pA/pF)-voltage (mV) relations. $I_{Ca^{2+}}$ was evoked from a holding potential of -80 mV to the test potential indicated in inset of panel b. $I_{Ca^{2+}}$ amplitudes and normalization was done as described in a. Each point represents mean \pm S.E.M. of wild-type neurons (open circles, n=25) and knockout mice (*Ffar3*^{-/-}, black circles, n=27). Points from -120 to -90 mV have been omitted for clarity. c. Peak current densities at +10 mV calculated from current recordings using acutely dissociated CSMG neurons from wild-type and *Ffar3*^{-/-}. Two-tailed unpaired *t*-test was used to determine whether the difference between the population means (wild-type vs *Ffar3*^{-/-}) were statistically significant. The difference between the means was not statistically significant, $t(56)=0.016$, with $\alpha=0.1$, 90% CI: -2.2-2.2, $n_1=27$, $n_2=31$ and $p=0.99$. Four wild-type animals and three knockout mice were used to generate the plots.

Figure 3: Cryosections of *Ffar3*-reporter hemizygous mice dorsal root ganglion (DRG) FFAR3-labelled used as a control.

Representative fluorescent IF images of three repetitions with DRG cryosections from hemizygous *Ffar3*^{+/*mRFP*} reporter mice. Left upper panel: Direct mRFP (red, a) fluorescence detection in DRG slices counterstained with Anti-FFAR3 antibody (green, b). c. Bright field image and d superimposed images. Note that mRFP and FFAR3 antibody fluorescence are not present in this ganglion, which correlates with *Ffar3* mRNA levels. Scale bars are 50 μ m and the same in all images.

Figure 4: Cryosections of mice celiac-mesenteric ganglia quadruple-labelled to show chemical coding of postganglionic neurons and FFAR3-expressing neurons.

Quadruple-staining of CSMG sections were performed with Anti- P2X₂ ionotropic purinergic receptor (Anti- P2X₂, a), Anti-FFAR3 (b), Anti-Neurofilament 200 kDa (Anti-NF200, c) and Calbindin D-28k (Anti-Cb300, d). Cyan arrowhead indicates A-fiber surrounded by FFAR3 punctuate immunoreactivity (b-c and e). Orange arrowhead indicates Schwann cells associated with A-fibers (c-e). Labeling was repeated twice and using four wild-type animals on each replicate. Scale bars in all images are 20 μ m.

Figure 5: Cryosections of mice superior cervical ganglia quadruple-labelled to show chemical coding of postganglionic neurons and FFAR3-expressing neurons.

Quadruple-staining of SCG sections were performed with Anti-P2X₂ ionotropic purinergic receptor (Anti-P2X₂, a), Anti-FFAR3 (b), Anti-Neurofilament 200 kDa (Anti-NF200, c) and Calbindin D-28k (Anti-Cb300, d). White arrow indicates representative neurons that is P2X₂-, FFAR3- and Cb300-IR. Yellow arrow indicates a representative group of SIF-like cells that are P2X₂-IR and Cb300-IN, FFAR3-IN. Labeling was repeated twice and using four wild-type animals on each replicate. Scale bars in all images are 20 μm.