miR-34c-5p functions as pronociceptive microRNA in cancer pain by targeting Cav2.3 containing calcium channels

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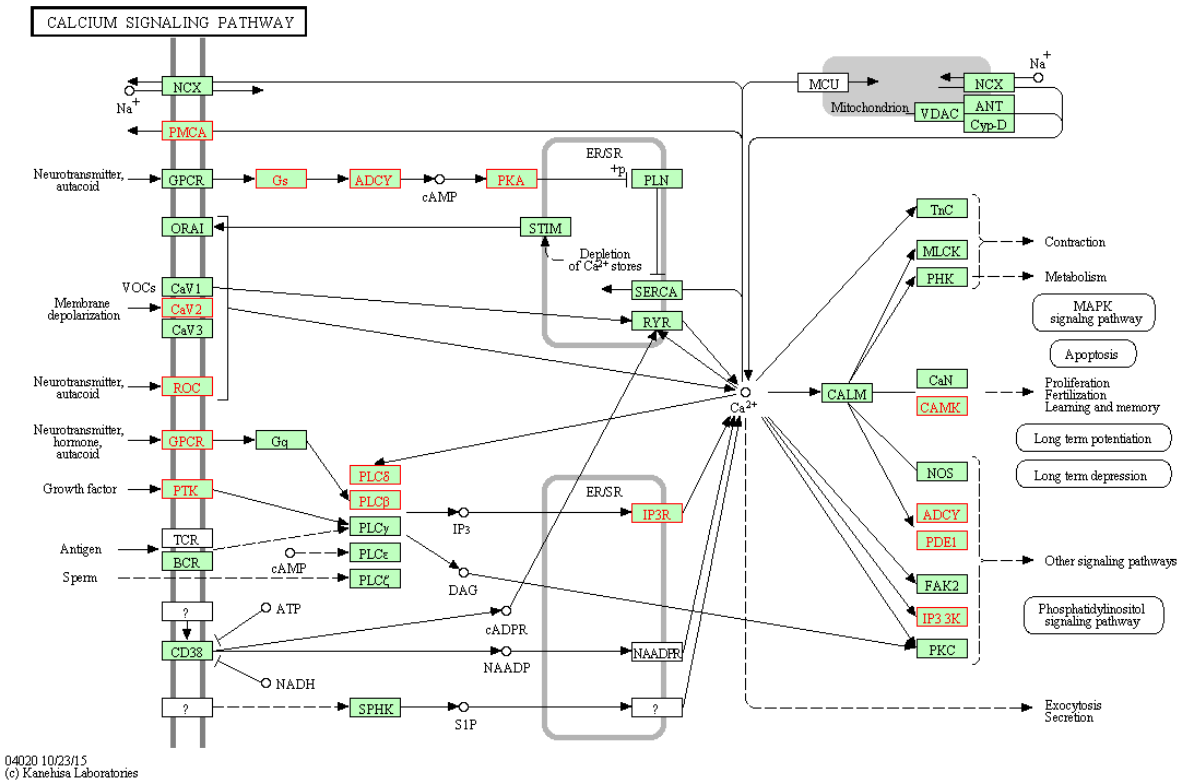
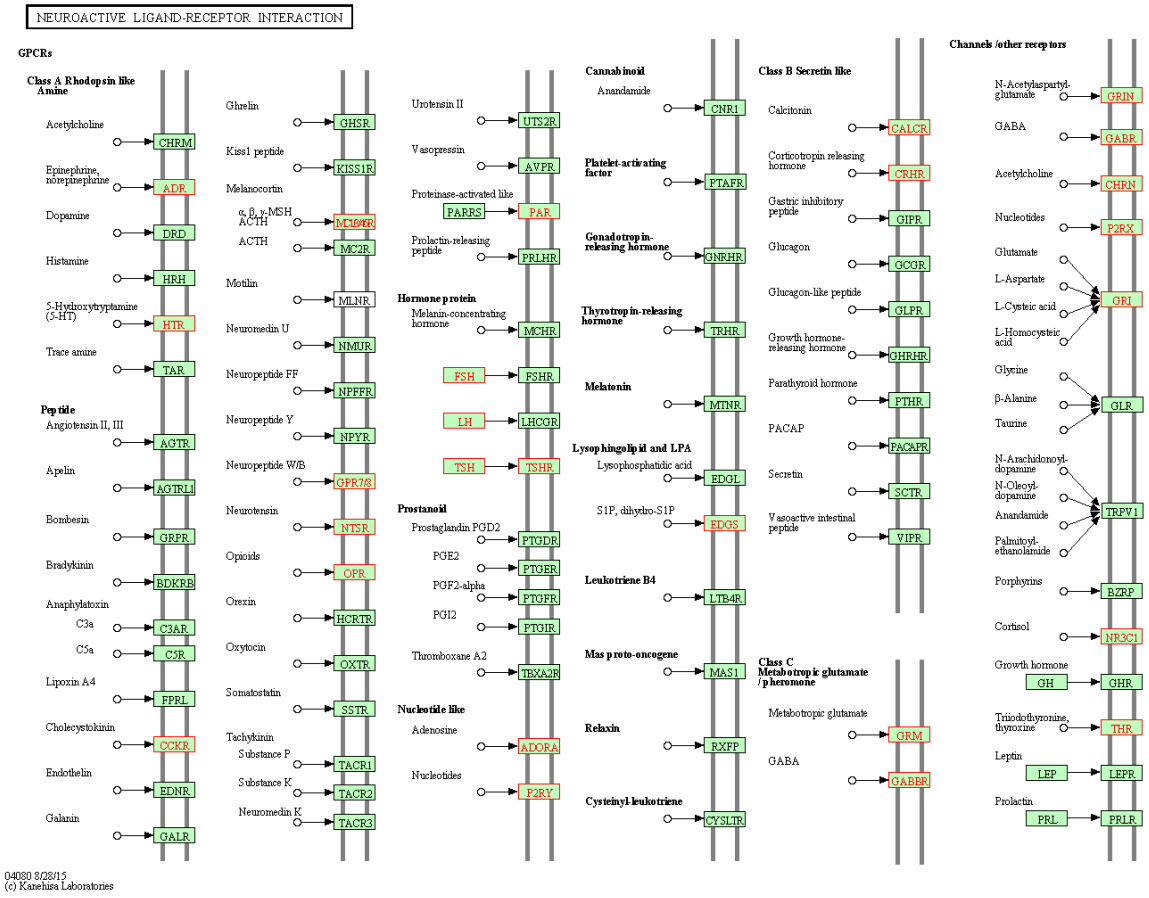
Supplementary Figures:

Supplementary Figure 1:



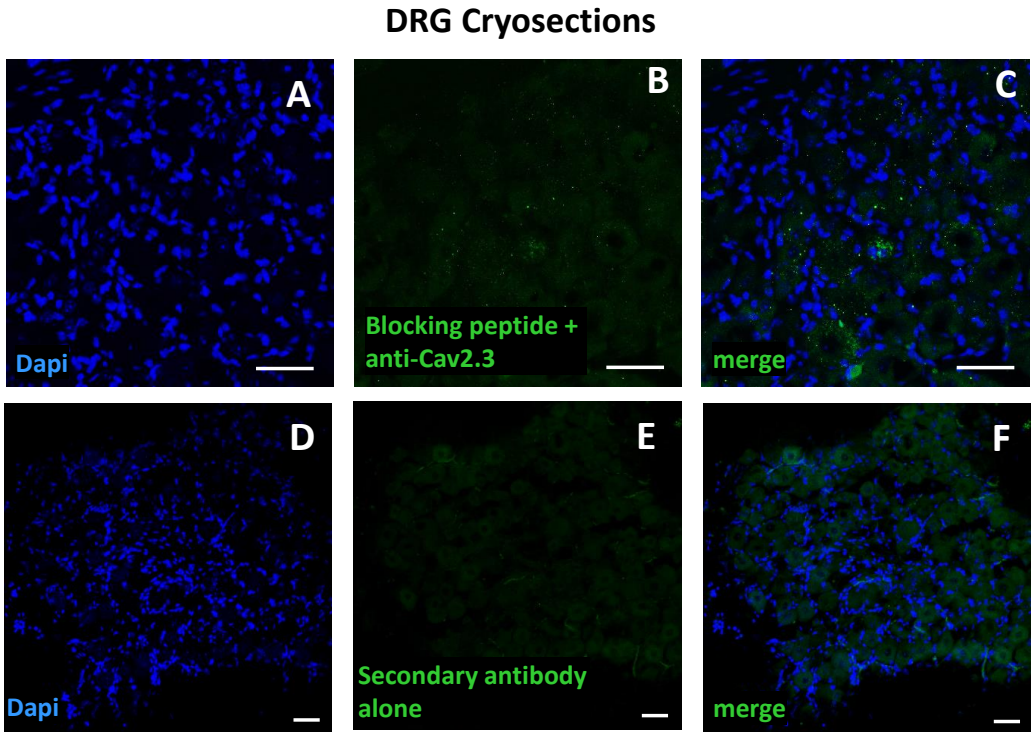
Suppl. Fig. 1. Enriched KEGG pathway among putative targets of mir-34c-5p. Graphical representation of *Calcium signaling pathway* which is identified as one of significantly enriched KEGG pathways (adj. $P \leq 0.05$ calculated by the multiple test adjustment method as compared to a number of reference genes in the category genome-wide) from the list of 1533 genes commonly predicted as targets for miR-34c-5p by 6 independent algorithms. Pathway components that are predicted as putative targets for miR-34c-5p are highlighted in the red color text.

Supplementary Figure 2:



Suppl. Fig. 2. Enriched KEGG pathway among putative targets of mir-34c-5p. Graphical representation of *neuroactive ligand-receptor interaction pathway* which is identified as one of significantly enriched KEGG pathways (adj. P ≤ 0.05 calculated by the multiple test adjustment method as compared to a number of reference genes in the category genome-wide) from the list of 1533 genes commonly predicted as targets for miR-34c-5p by 6 independent algorithms. Pathway components that are predicted as putative targets for miR-34c-5p are highlighted in the red color text.

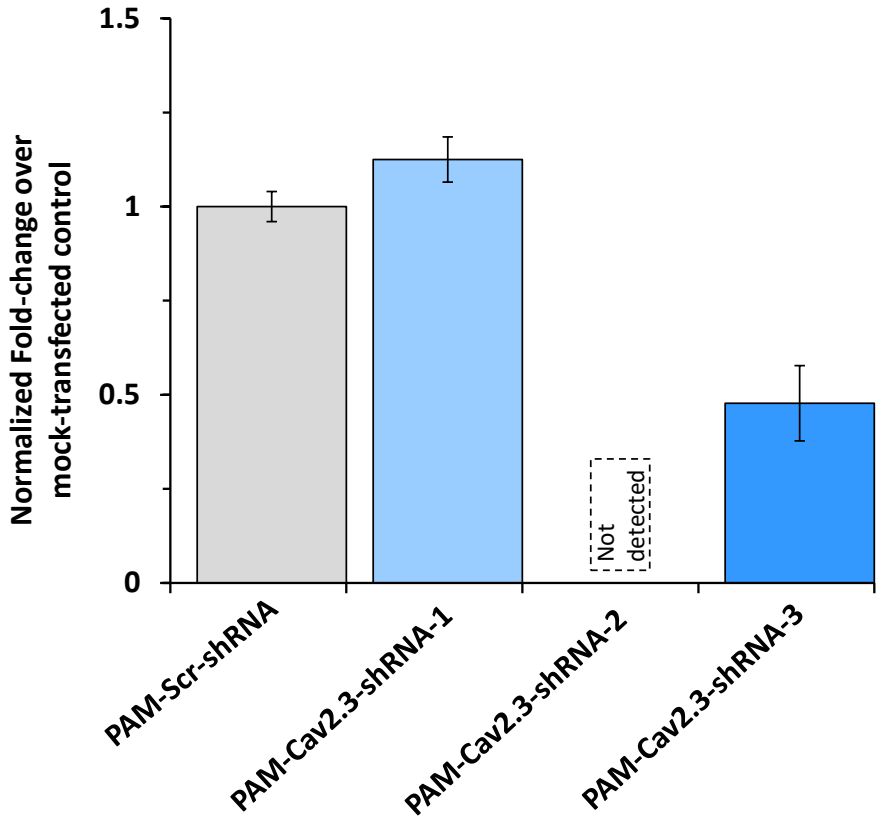
Supplementary Figure 3:



Suppl. Fig.3. Representative images from the cryosections of lumbar DRG to confirm the lack of unspecific staining for images shown in Fig.3 panel A, Fig. 4 and Fig.5.

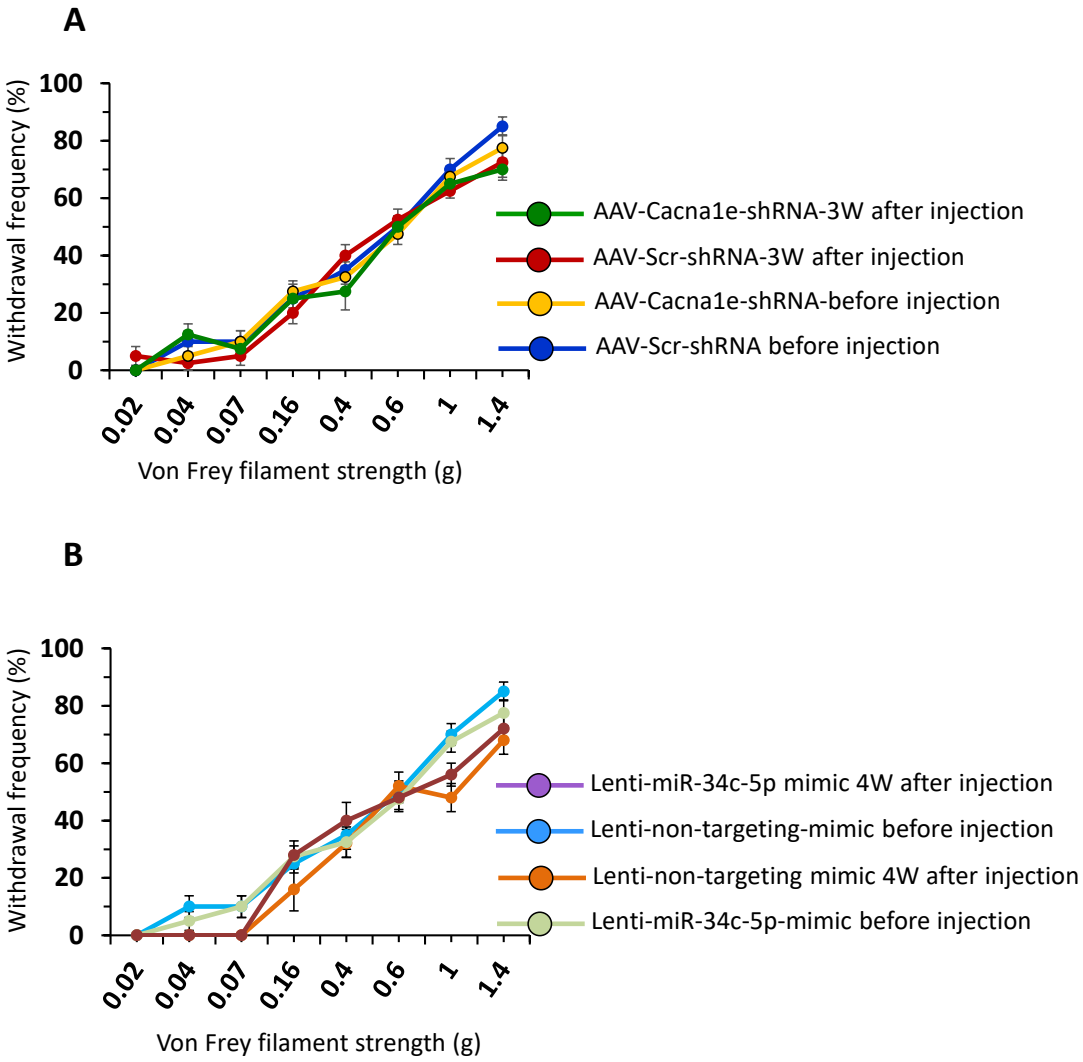
(A-C) Tissue sections were pre-incubated with blocking peptide prior to the addition of anti-Cav2.3 antibody. **(D-F)** Alexa- 488 conjugated anti rabbit secondary antibody was incubated in the absence of anti-Clcn3 antibody. Nuclei were visualized with Dapi. Scale bar equals to 50 µm.

Supplementary Figure 4:



Suppl. Fig.4. Identification of potent shRNA against *Cav2.3*. qRTPCR analysis of change in expression of *Cav2.3* following 3 different shRNAs directed against the coding region of *Cav2.3* in cultured sensory neurons.

Supplementary Figure 5:



Suppl. Fig. 5. Analyses of functional contribution of Cav2.3 and miR-34c-5p in the mediation of mechanical sensitivity in wild-type mice

A. Change in the frequency of paw withdrawal to the plantar application of graded von Frey filament forces of different strength in contralateral paws following intraganglionic injection of AAVs carrying either scrambled shRNA (AAV-Scr-shRNA) or shRNA directed against coding sequence of Cav2.3 (AAV-Cav2.3-shRNA), measured before and at 3W after viral injections. **B.** Change in the frequency of paw withdrawal to the plantar application of graded von Frey filament forces of different strength in contralateral paws following intraganglionic injection of lentivirus carrying either non-targeting miRNA mimic (Lenti-non-targeting-mimic) miR-34c-5p specific mimic (Lenti-miR-34c-mimic), measured before and at 4W after viral injections. In both panels, there was no statistical difference between the groups, n = 8 mice per group.