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SUPPLEMENTARY MATERIAL

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Antigen retrieval pre-treatment causes a different expression pattern of $Ca_v3.2$ in rat and mouse spinal dorsal horn

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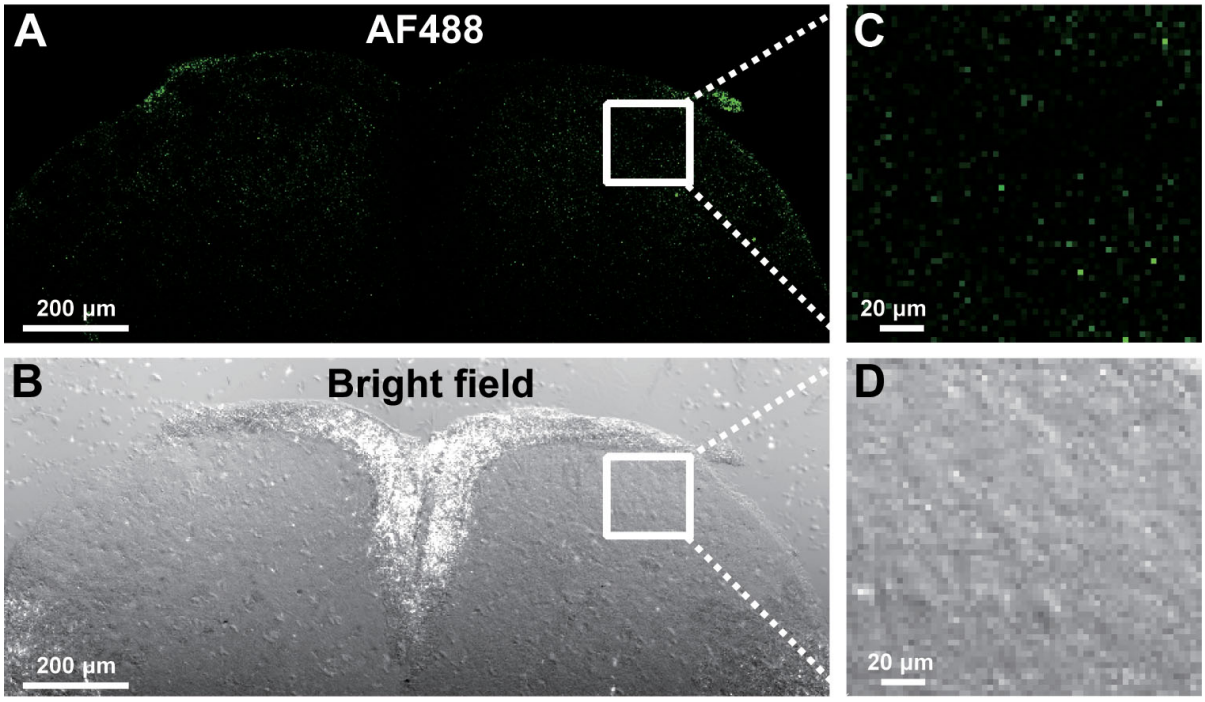
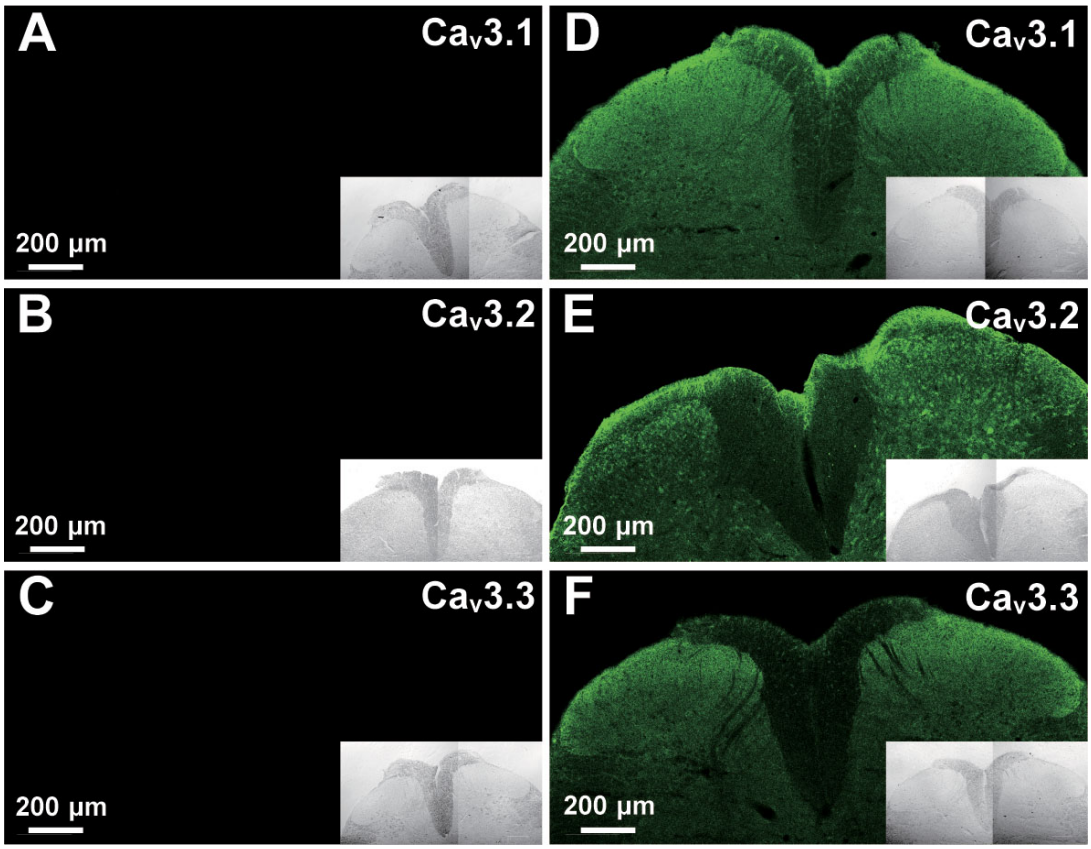
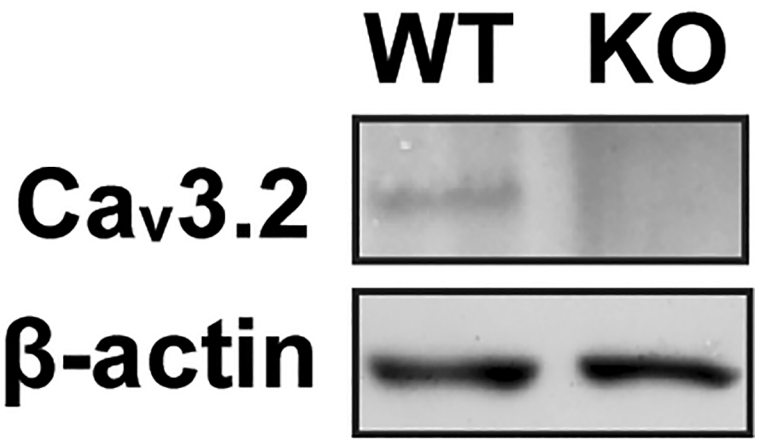
Key words: $Ca_v3.2$; immunohistochemistry; antigen retrieval; spinal dorsal horn.



Supplementary Figure 1.
Control experimental results showing the omission of primary antibodies. A) Representative images of sections stained with a secondary antibody Alexa Fluor 488 at a dilution of 1:400. B) Bright field image of the same section in A. C-D) Enlargement part of the boxes in A and B.



Supplementary Figure 2.
Control experimental results showing the pre-absorption of primary antibodies with peptide antigens. Representative images of sections stained with anti-$Ca_v3.1$ (A), anti-$Ca_v3.2$ (B), and anti-$Ca_v3.3$ (C) antibodies (Alomone) by pre-absorbing the antibodies with their antigen peptides, respectively. Representative images of anti-$Ca_v3.1$ (D), anti-$Ca_v3.2$ (E), and anti-$Ca_v3.3$ (F) antibodies (Alomone) without the treatment of antigens peptides. The insets in panels A-F are the corresponding sections under the observation of bright field microscope.



Supplementary Figure 3.

Western blotting showing Cav3.2 in the SDH of adult mice. β-actin served as an internal control. Cav3.2 is expressed in WT mouse rather than KO mouse. Mouse spinal dorsal horn (SDH) tissues were homogenized and lysed in lysis buffer containing 0.5% Lubrol-PX, 50 mM KCl, 2 mM CaCl2, 20% glycerol, 50 mM Tris-HCl (PH=7.4), 1 mM NaN3, and a mixture of 1% protease inhibitor (P8340, Sigma–Aldrich, St. Louis, MO) on ice. The equivalent denatured proteins (200 μg for Cav3.2 and 40 μg for β-actin) were loaded onto 6% to 10% SDS-polyacrylamide electrophoresis (P1200, Solarbio, China), and then were transferred to nitrocellulose membranes (HATF00010, Millipore, USA). The membranes were incubated with anti-Cav3.2 (1:200; Alomone) and anti-β-actin (1:1000; Proteintech, IL, USA) overnight at 4°C, respectively, followed with goat anti-rabbit IgG secondary antibody (1:1000; Thermo Fisher Scientific, USA) for 6 h at 4°C. Immunoblotting with anti-β-actin was used to indicate protein loading.