Supplemental Information

Inhibition of TRPV1 by SHP-1 in nociceptive primary sensory neurons is critical in PD-L1 analgesia

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Inventory of Supplemental Information

Supplemental Figures

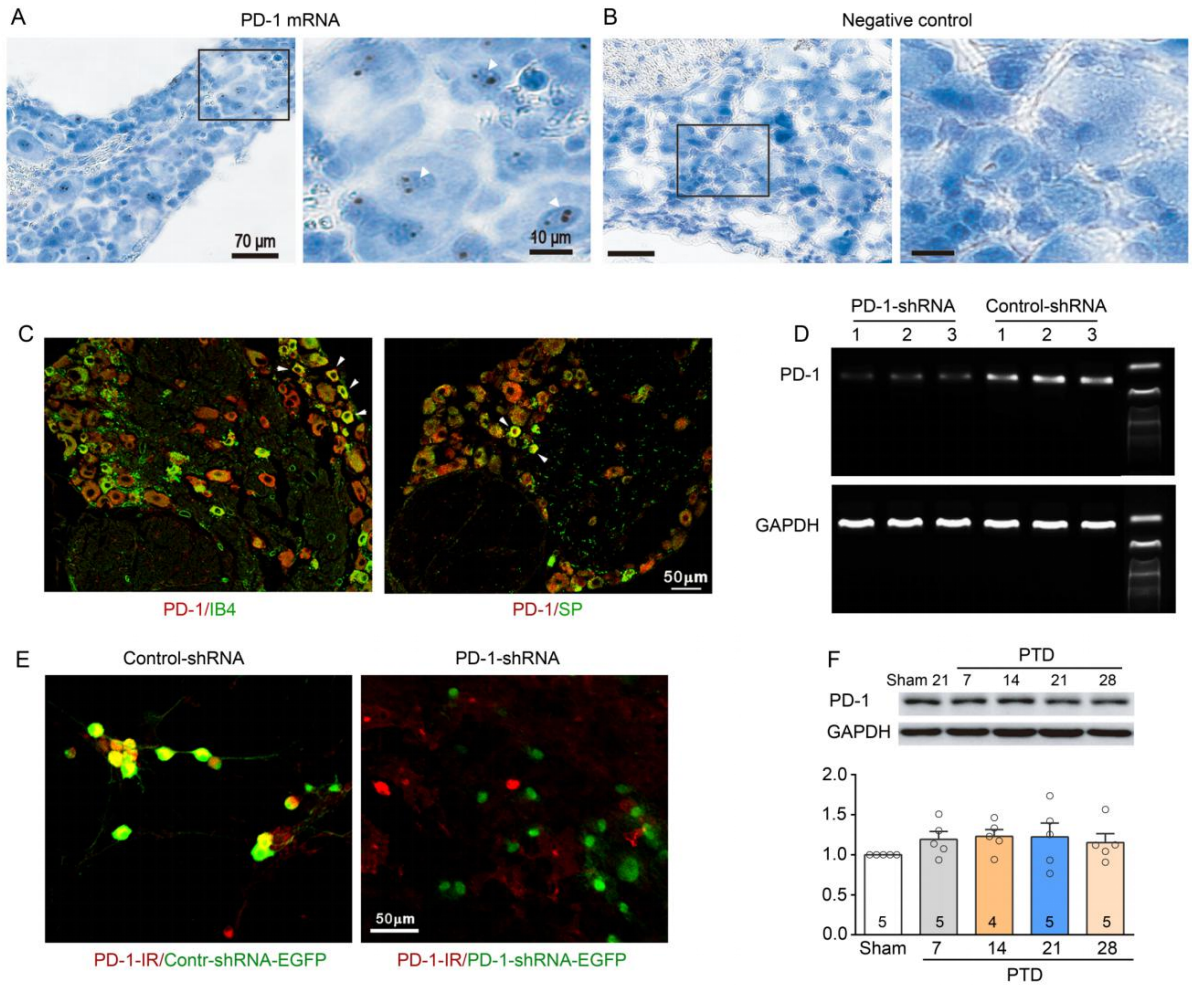

Figure. S1. Related to Figure 2, Expression of PD-1 in the mouse DRG. (A & B) RNAsope *in situ* hybridization (ISH) showing PD-1 mRNA expression in L_4 DRG of naive mice (A). B shows negative control. Scale bar: 70 μm (low magnification) and 10 μm (high magnification). (C) Double immunofluorescence staining reveals the expression of PD-1 in IB4 positive and SP positive neurons in L_4 DRG. Scale bar: 50 μm. (D) RT-PCR showing the efficiency of PD-1 knockdown. PD-1 mRNA level was decreased in cultured DRG neurons from three independent experiments at 3 days after transfection of PD-1 shRNA-lentivirus. GAPDH mRNA was used as internal control. (E) The specificity of PD-1 antibody was verified in PD-1 knockdown cells. Few PD-1 immunoreactivity (IR, red) colocalized with PD-1 shRNA-EGFP (right) in cultured DRG neurons. Scale bar: 50 μm. (F) Western blot analysis showing PD-1 level in the L3-L5 DRGs after tumor inoculation. n.s. no significant, one-way ANOVA; n=5, 5, 4, 5 and 5 (mice).

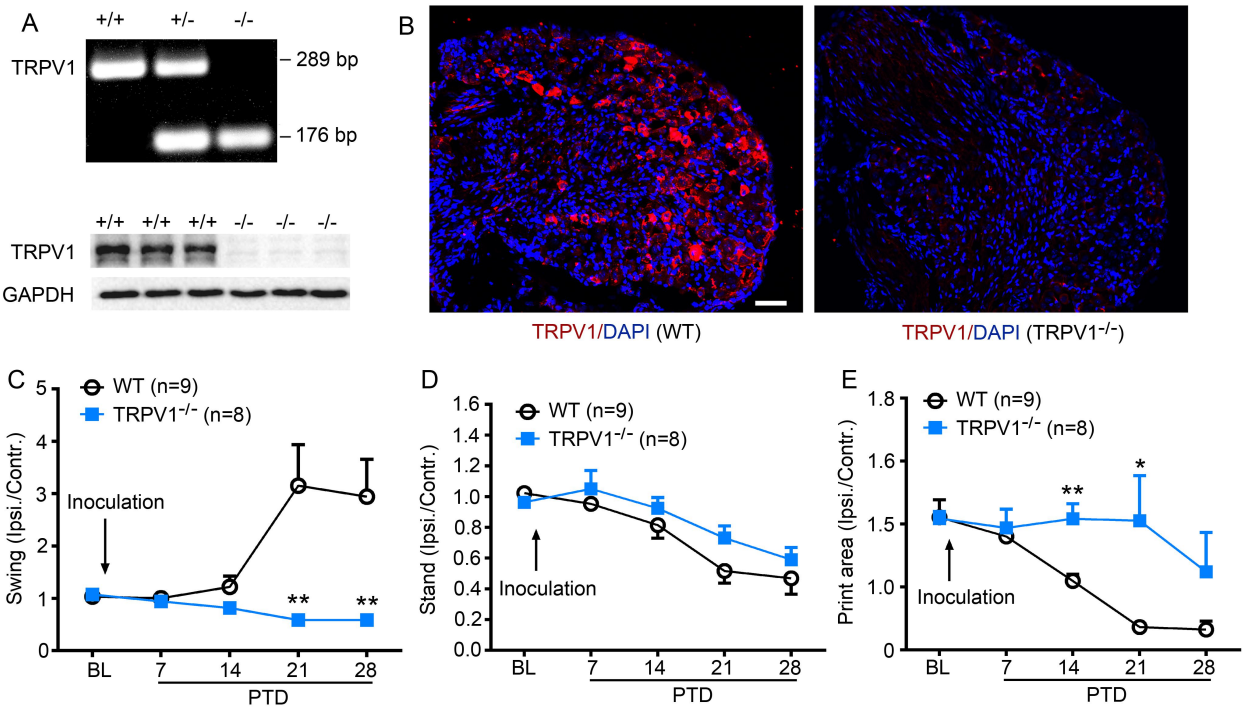

Figure S2. Related to Figure 3, Knockout of TRPV1 blocks bone cancer-induced pain-like behaviors. (A and B) TRPV1 knockout mice showing lost TRPV1 signal by RT-PCR, western blot and immunohistochemisty. (C–E) CatWalk gait analysis showing lack of pain-like behaviors in TRPV1 knockout mice after tumor inoculation. *p<0.05, **p<0.01 versus wild-type mice; two-way RM ANOVA followed by post hoc Student-Newmann-Keuls test; n=8 and 9 (mice).