Supporting Information for

Original article

Potentiation of PIEZO2 mechanically-activated currents in sensory neurons mediates vincristine-induced mechanical hypersensitivity

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1. Supporting figures

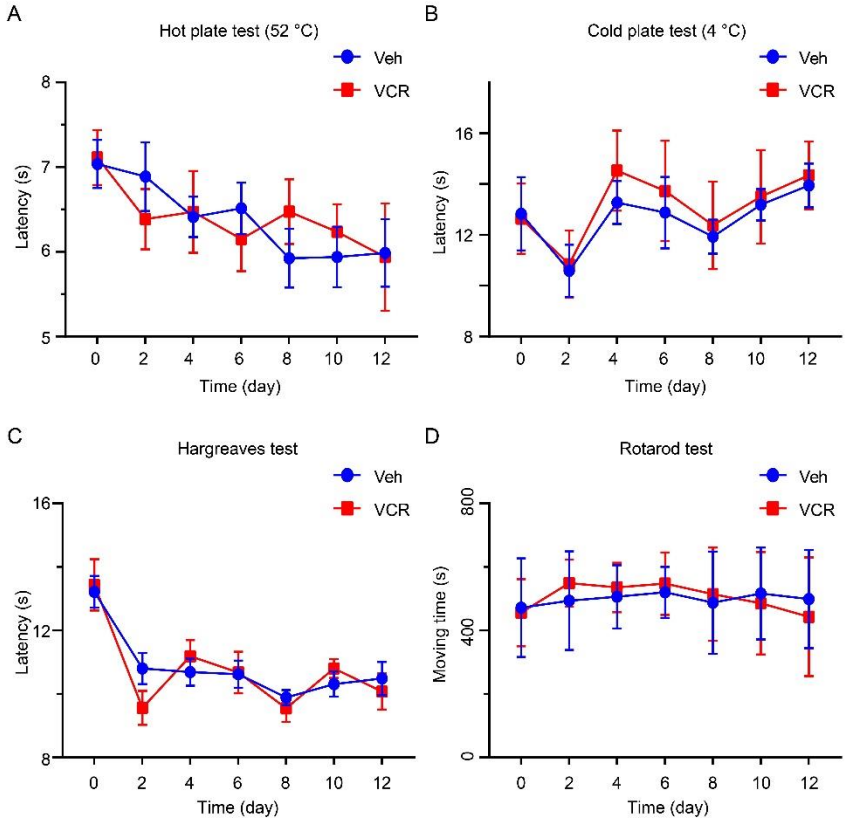

Figure S1 Behavioral test. Hot plate test **(A)**, cold plate test **(B)**, Hargreaves test **(C)** and Rota-rod test **(D)** show that the current pattern of VCR administration (0.05 mg/kg/day, i.p., 5-day-on and 2-day-off) did not significantly affect thermal sensations and motor functions of animals. Data are shown in mean ± SEM, *n* = 8 each group.

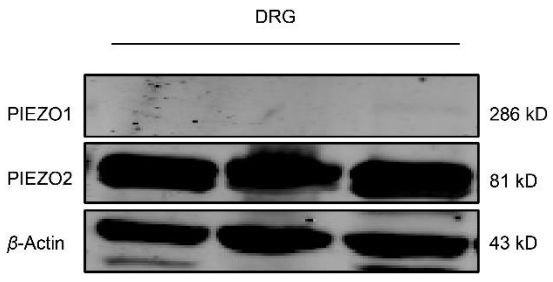

Figure S2 Western blot assay. Representative Western blot assay shows PIEZO2 was dominantly expressed while PIEZO1 was hard to be detected in rat DRG neurons.

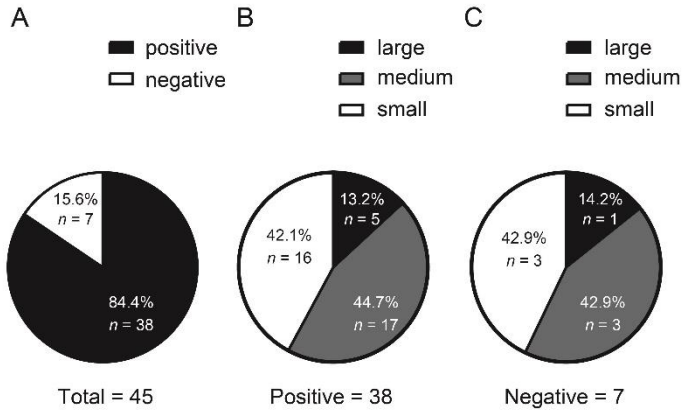

Figure S3 Percentage and cell size of DRG neurons responded to VCR. **(A)** Percentage of DRG neurons responded to VCR. **(B)** Percentage of different cell size of DRG neurons responded to VCR. **(C)** Percentage of different cell size of DRG neurons not responded to VCR.

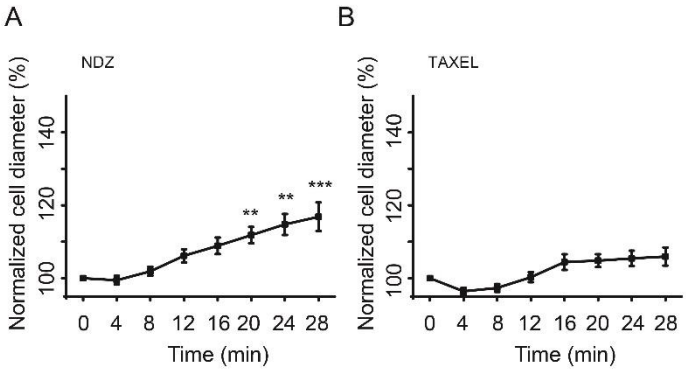

Figure S4 Changes on cell size. Pipette application of NDZ **(A)** but not TAXEL **(B)** induced cell swelling of rat DRG neurons. Cell swelling was represented by the relative changes in cell diameter. $^{**}P < 0.01$, $^{***}P < 0.001$. Data are shown in mean ± SEM, $n = 8$ and 9 in (A) and (B), respectively.

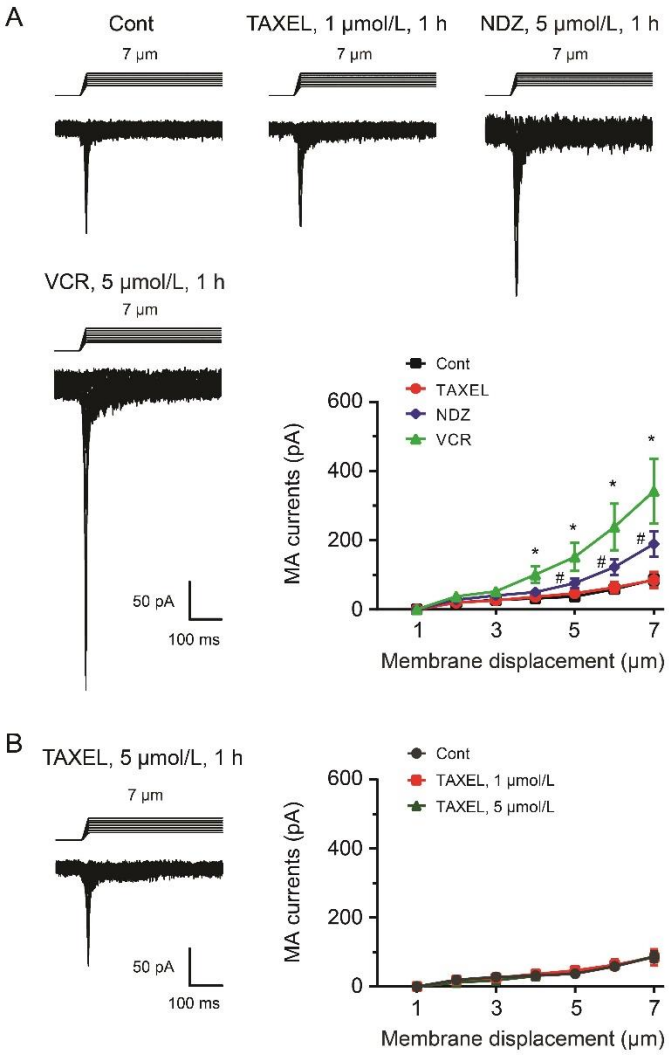

Figure S5 Effects of extracellular application of VCR, NDZ and TAXEL on PIEZO2 MA currents in rat DRG neurons. **(A)** Incubation of rat DRG neurons with VCR and NDZ but not TAXEL for 1 h significantly potentiated PIEZO2 MA currents in rat DRG neurons. Mechanical stimulation (top) is shown in the above current traces (bottom). The lower right panel shows the summary data of the effects of VCR (green triangle, n = 8), NDZ (blue diamond, n = 7) and TAXEL (red circle, n = 11) on PIEZO2 MA currents evoked with different membrane displacements in DRG neurons. **(B)** Incubation of rat DRG neurons with TAXEL at the concentrations of 1 μmol/L (red square, n = 11) and 5 μmol/L (green triangle, n = 9) for 1 h, respectively, shows no effects on PIEZO2 MA currents. n = 9 in Cont group. Data are shown in mean ± SEM; $^{*/\#}P < 0.05$.

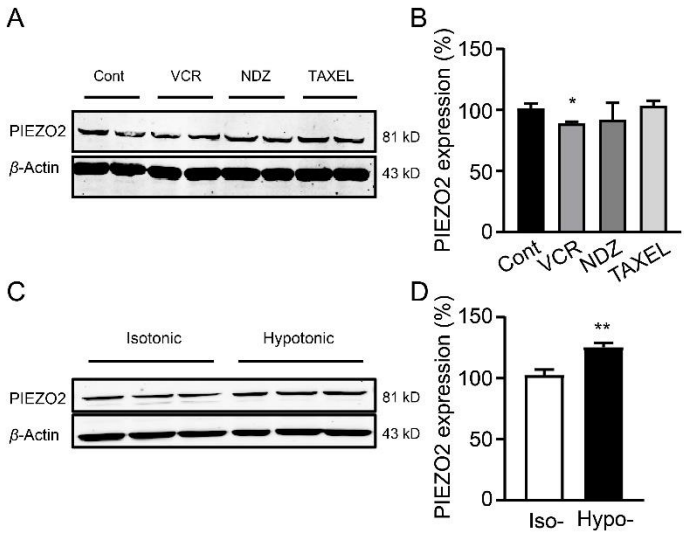

Figure S6 Western blot assay. Representative **(A)** and summary **(B)** data of Western blot assay show the effects of 1 h incubation with VCR, NDZ and TAXEL on the PIEZO2 expression in rat DRG neurons, respectively. n = 6 each group. Representative **(C)** and summary **(D)** data of Western blot assay show the effects of 30 min incubation with hypotonic solution on the expression of PIEZO2 in rat DRG neurons. n = 9 each group. β-Actin was used as control. Data are shown in mean ± SEM; *P < 0.05, **P < 0.01.

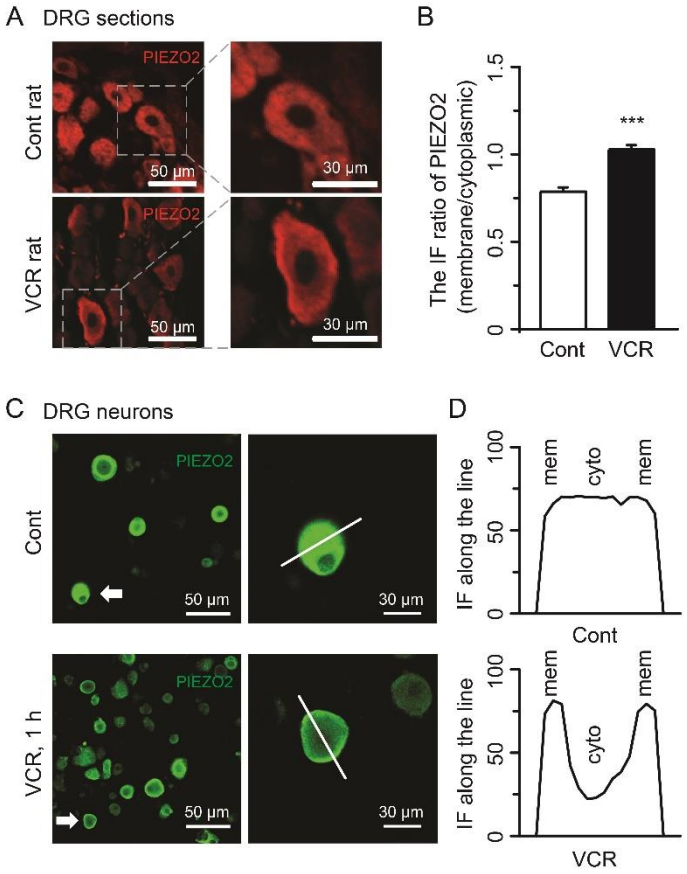

Figure S7 Immunostaining assay. Representative images **(A)** and PIEZO2 IF ratio of cell membrane to cytoplasm **(B)** of DRG sections in control rats (top) and VCR-pain model rats (bottom) are shown. Cells represented in right panels in (**A)** are extended from the cells (square area) in left panels. Representative images **(C)** and PIEZO2 IF alterations **(D)** along the line over cells in cultured DRG neurons in control group (CONT) and the group of incubation with VCR for 1 h (VCR, 1 h) are shown. Cells represented in right panels in (**C)** are extended from the cells (arrow) in left panels. The mem and cyto in (**D)** represent cell membrane and cytoplasm, respectively. Data are shown in mean ± SEM, n = 41 and 53 in Cont and VCR group, respectively; ***P < 0.001.