Native Piezo2 Interactomics identifies Pericentrin as a Novel Regulator of Piezo2 in Somatosensory Neurons

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Running title: Native Piezo2 interactomics

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Supporting Information for Publication

- Table S-1: Results of quantitative mass spectrometry from all replicates of Piezo2-affinity-purfications compared to controls;
- Figure S-1: Specificity test of commercial Piezo2 antibodies
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- Figure S-4: Piezo2-currents upon co-expression with Pcnt in HEK293T cells
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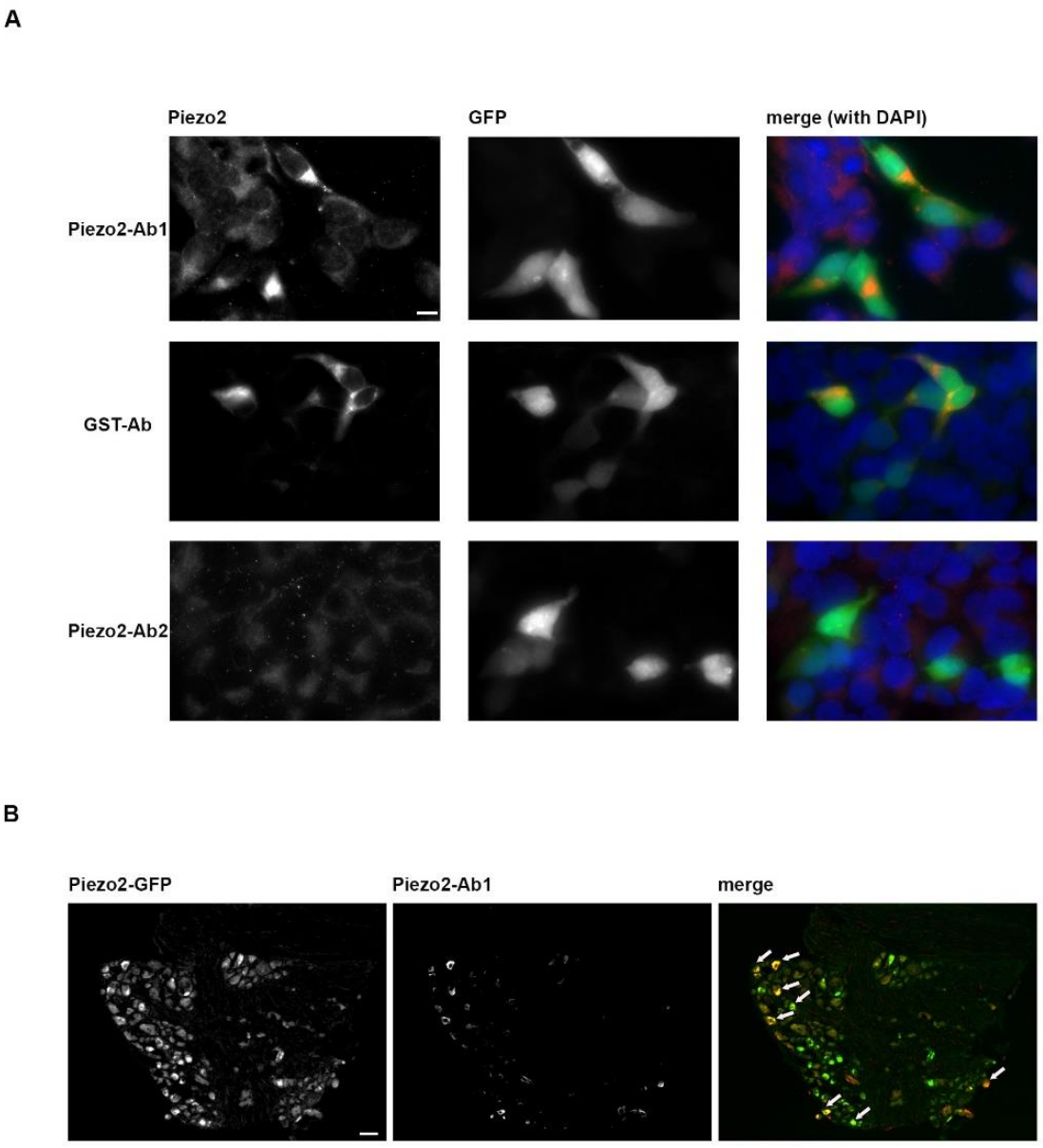

Figure S-1: Specificity test of commercial Piezo2 antibodies

(**A**) Representative images of immunolabeling of Piezo2 upon heterologous expression of Piezo2-GST-IRES-GFP in HEK293T cells. Antibodies used for Piezo2 immunolabel are indicated for each row (downwards: Piezo2-Ab1; GST, Piezo2-Ab2). Only Piezo2-Ab1 labels Piezo2 in a similar manner as GST. Piezo2-Ab2 did not yield any label. Images of GFP and DAPI are shown to visualize Piezo2-positive cells and cell nuclei, respectively. 3 independent culture preparations each. Scale bar: 10 µm.

(**B**) Representative images of immunohistochemistry on DRG cryosections from Piezo2^{GFP} mice [1] probed with Piezo2-Ab1 and GFP visualizing Piezo2-expressing neurons. Quantification of the percentage of positively-labeled neurons revealed that 12.7% of neurons were labeled by the Piezo2-Ab1 antibody of which 82.9% were co-labeled by GFP (GFP-positive neurons: 37.6%). Examples of co-labeled neurons are indicated by arrows. The non-labeled background of the Piezo2-GFP image is visualized on purpose so that boundaries and morphology of the DRG cross-section can be discerned. Piezo2-Ab1 labeling required heat-induced epitope retrieval (please see Methods for details). In total we analysed n = 1387 neurons from independent tissue preparations of 2 Piezo2^{GFP} mice.

Scale bar: 50 µm.

	Piezo2-Ab2 Replicate 1	Piezo2-Ab2 Replicate 2	Piezo2-Ab2 Replicate 3	IgG Replicate 1	IgG Replicate 2	IgG Replicate 3	Piezo2 Replicate 1	Piezo2 Replicate 2	Piezo2 Replicate 3
Piezo2 (Q8CD54) MS/MS Count	0	0	0	0	0	0	33	84	63

Figure S-2: Specific Piezo2-immunoaffinity-purification by the positively-tested Piezo2 antibody (Piezo2)

MS/MS counts represent the number of Piezo2 spectra identified in all replicates of immunoaffinity-purifications performed with Piezo2-Ab2, IgG and the positively-tested Piezo2 antibody (Piezo2). Piezo2-specific spectra could only be detected in samples in which the positively-tested Piezo2 antibody (Piezo2) was used.

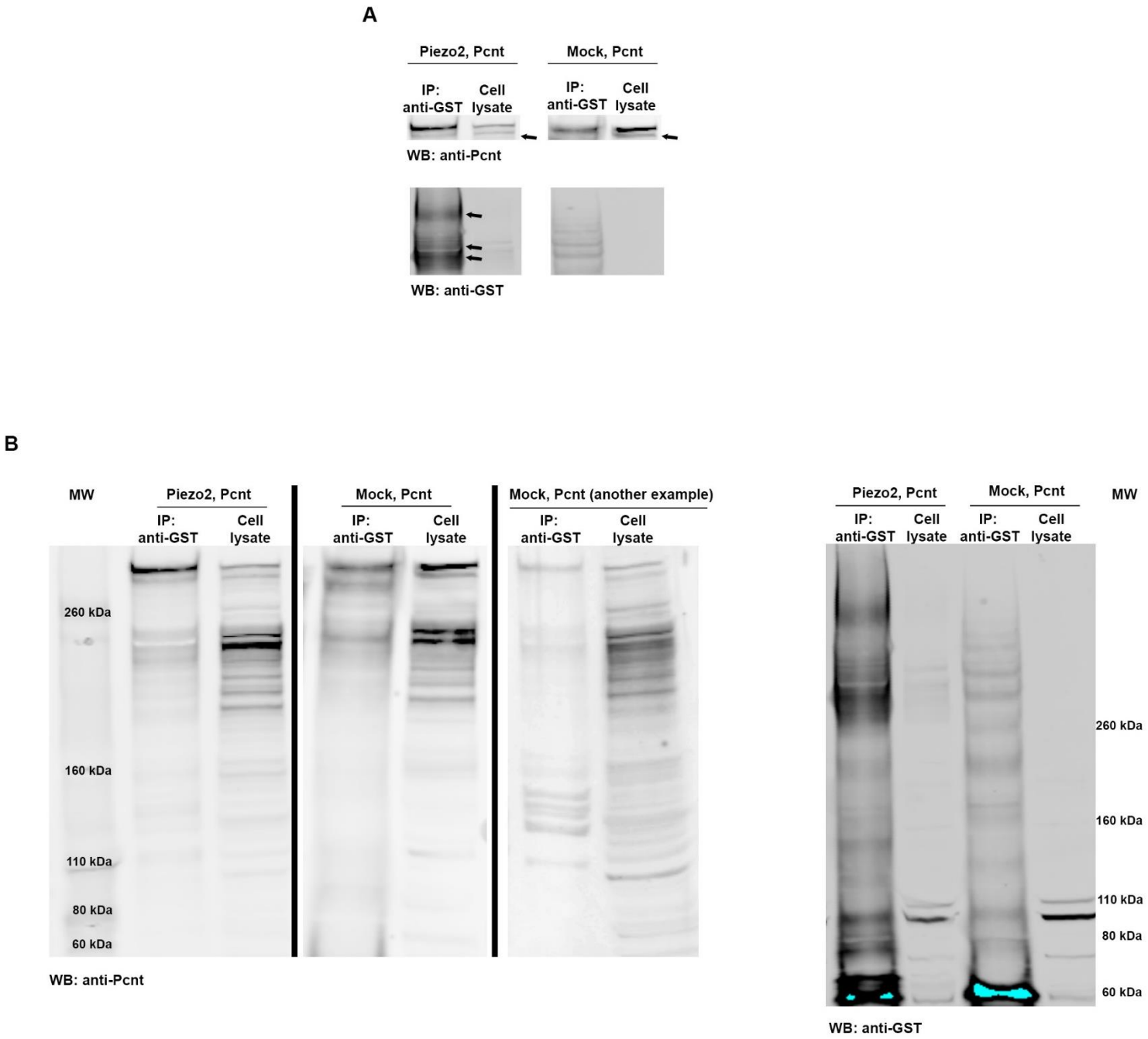

Figure S-3: Co-immunoprecipitation of Piezo2 and Pcnt

(**A**) Representative western blots (WB) upon co-immunoprecipitation (IP) of Pcnt with Piezo2-GST in HEK293T cells (Mock: GST-control plasmid). In the anti-Pcnt blot (upper panel), arrows highlight the specific Pcnt band: ca. 360kDa. Of note, the upper band in anti-Pcnt blots is of undefined origin and appears in all conditions.

In the anti-GST blot (lower panel), high molecular weight Piezo2 bands (ca. 300kDa, 360kDa and above 400kDa; highlighted by arrows) are detected after IP as reported elsewhere [2].

(**B**) Original blots referred to above and another example of the experiment in cells transfected with Mock + Pcnt (labeled with “another example”). Blobby bands around 60 kDa represent IgG heavy chains of the GST antibody used for IPs. MW, molecular weight marker. Black vertical bars indicate separate blots.

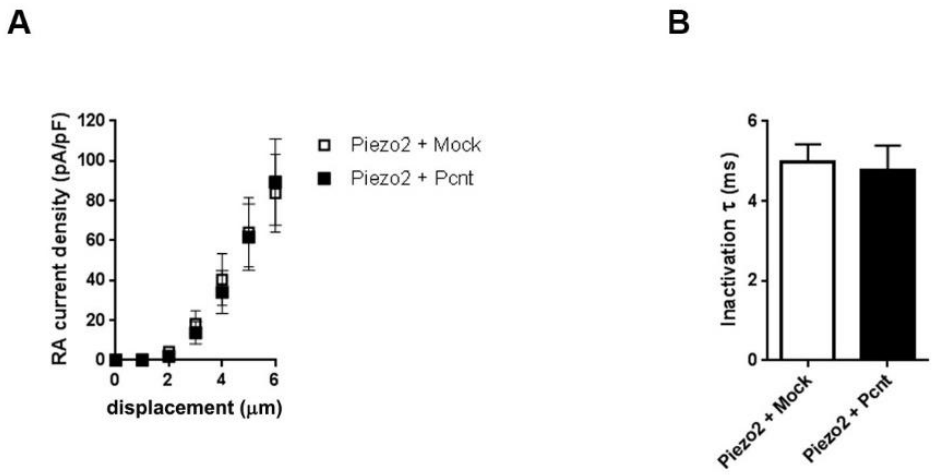

Figure S-4: Piezo2-currents upon co-expression with Pcnt in HEK293T cells
(**A**) Stimulus (displacement)-response curves show no difference in RA MA currents between co-expression of Piezo2 and Mock (pCMV-Sport6) or Piezo2 and Pcnt (Piezo2 + Mock: n=26 cells; Piezo2 + Pcnt: n=27 cells; N=3 independent cultures; ns; 2-way ANOVA). (**B**) Inactivation time constants were unchanged between conditions (Piezo2 + Mock: 4.98 ± 0.43 ms; Piezo2 + Pcnt: 4.76 ± 0.61 ms; ns; unpaired t-test).

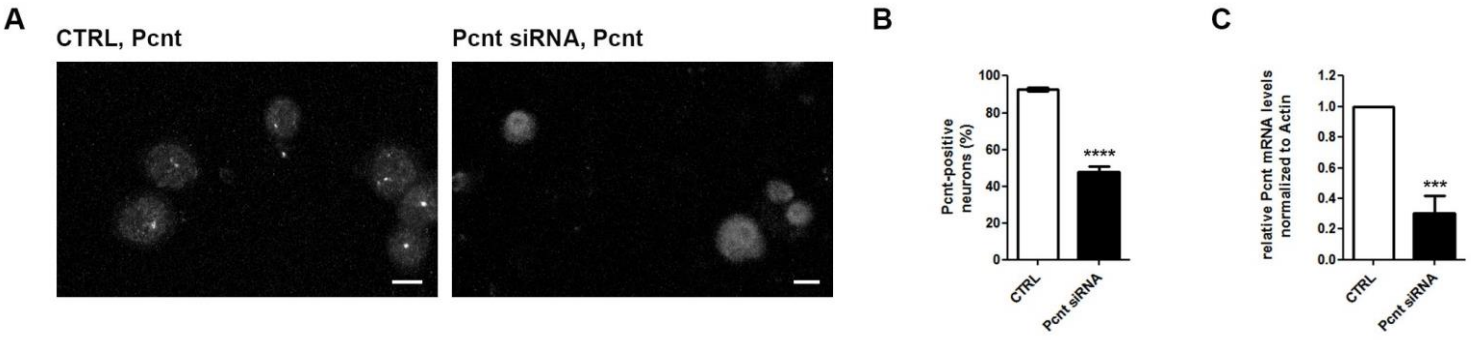

Figure S-5: Specificity of Pcnt antibodies

(A-B) Representative immunocytochemistry of Pcnt (**A**) and quantification of Pcnt-positive neurons (**B**) in CTRL (ON-Target*plus* Nontargeting siRNA) and Pcnt siRNA-treated DRG cultures. SiRNA treatment significantly reduced Pcnt positive cells (CTRL: 92.71 ± 0.94%; siRNA: 47.64 ± 2.87%, n > 1000 neurons, N=14, $p < 0.0001$; unpaired t-test). Two different Pcnt antibodies gave similar results (please see Methods for more detail). (**C**) Significant reduction of Pcnt mRNA by Pcnt siRNA (0.31 ± 0.11 compared to Actin used as reference, N=5, p=0.0036; one sample t-test). Using GAPDH as reference gave similar results (data not shown).

Scale bars: 15 µm.

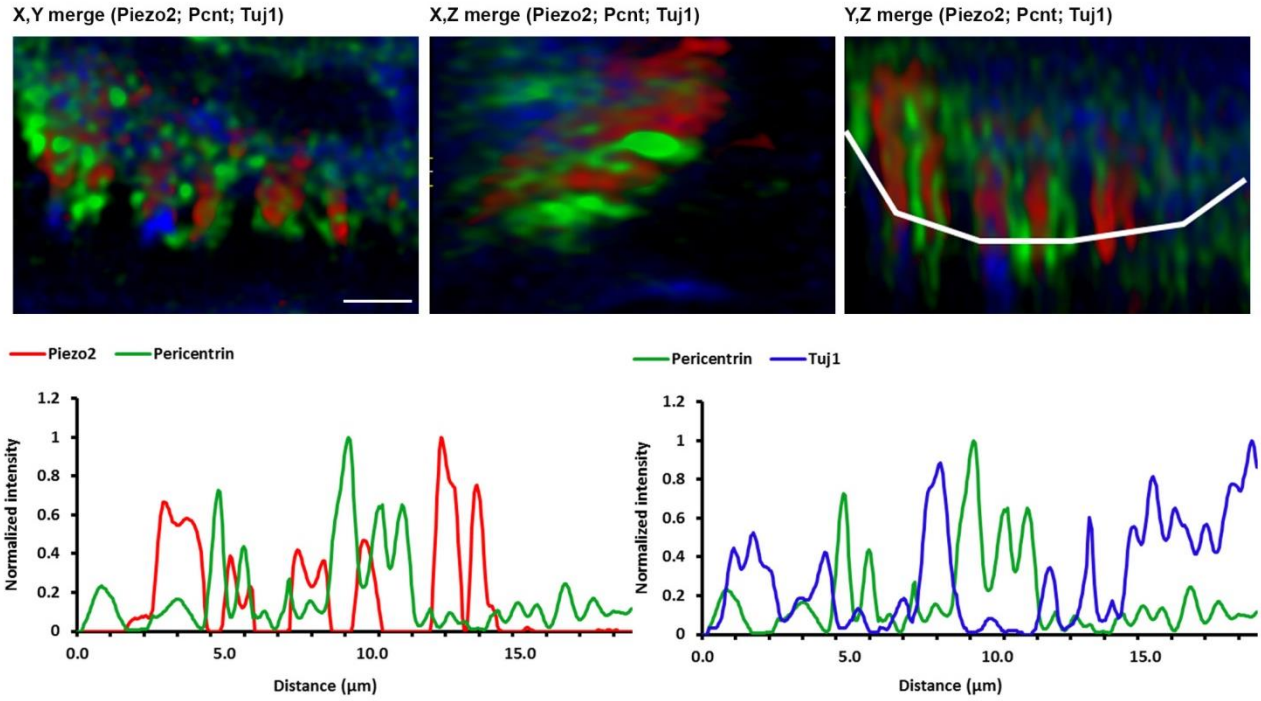

Figure S-6: Pcnt expression in lanceolate endings
Representative immunohistochemistry of lanceolate endings of back skin isolated from Piezo2^{GFP} mice and stained for GFP (red), Pcnt (green) and Tuj1 (blue) followed by deconvolution (please see materials and methods for details). Line in right image indicates the position of the line scan. Line scans show that Pcnt is localized within and more frequently in close apposition to label of Piezo2-GFP and Tuj1, respectively. Scale bar: 2 µm.

References

1. Woo, S.-H.; Ranade, S.; Weyer, A. D.; Dubin, A. E.; Baba, Y.; Qiu, Z.; Petrus, M.; Miyamoto, T.; Reddy, K.; Lumpkin, E. a.; Stucky, C. L.; Patapoutian, A., Piezo2 is required for Merkel-cell mechanotransduction. *Nature* **2014,** 509, 622-6.
2. Poole, K.; Herget, R.; Lapatsina, L.; Ngo, H.-D.; Lewin, G. R., Tuning Piezo ion channels to detect molecular-scale movements relevant for fine touch. *Nature communications* **2014,** 5, 3520.