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# Supplementary Materials for

## Inhibition of somatosensory mechanotransduction by annexin A6

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**
(available at www.sciencesignaling.org/cgi/content/full/11/535/eaao2060/DC1)

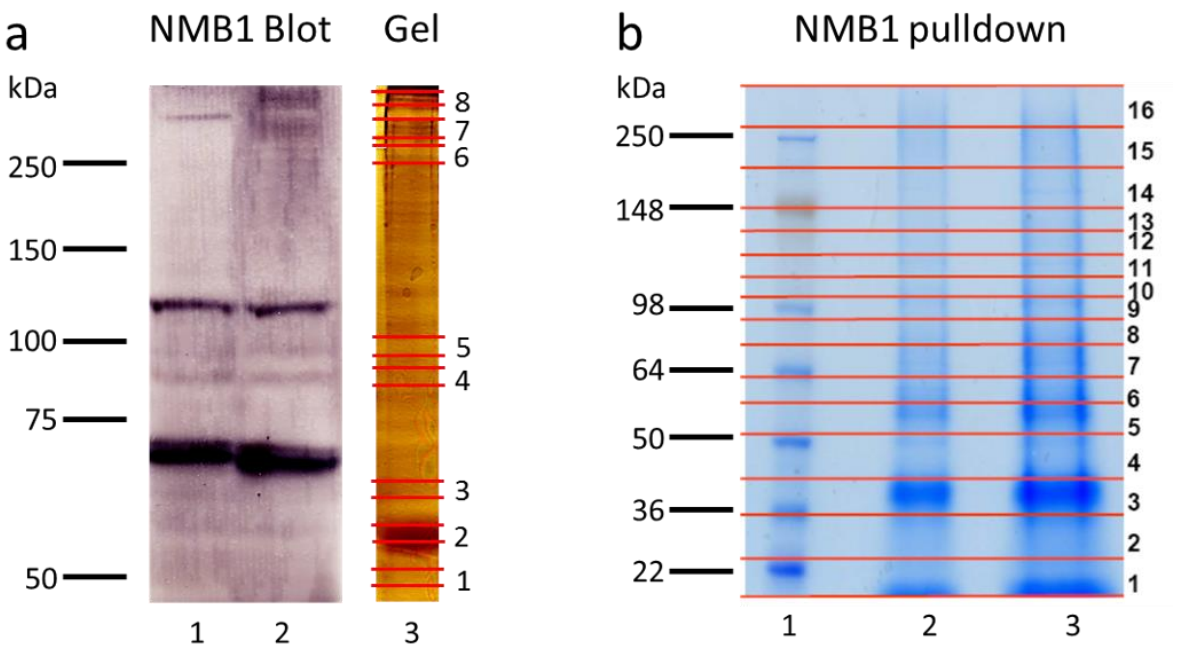


**Figure S1. Isolation of NMB-1 peptide-binding partners.** (a) Biotinylated NMB-1 was used to visualize binding silver stained SDS gel loaded with DRG protein extract processed under the same conditions is juxtaposed alongside the NMB-1 stained blot (3). Silver stained gel with reactive bands on the NMB-1 blot that were excised (location of the bands shown with red lines) and processed for LC MS/MS analysis. (b) NMB-1 pulled from differentiated ND-C cell protein extracts was treated with 2% (lane 2) or 4% (lane 3) beta-mercaptoethanol and resolved on 10% SDS gel. The gel was cut into 16 strips and processed for LC MS/MS analysis.

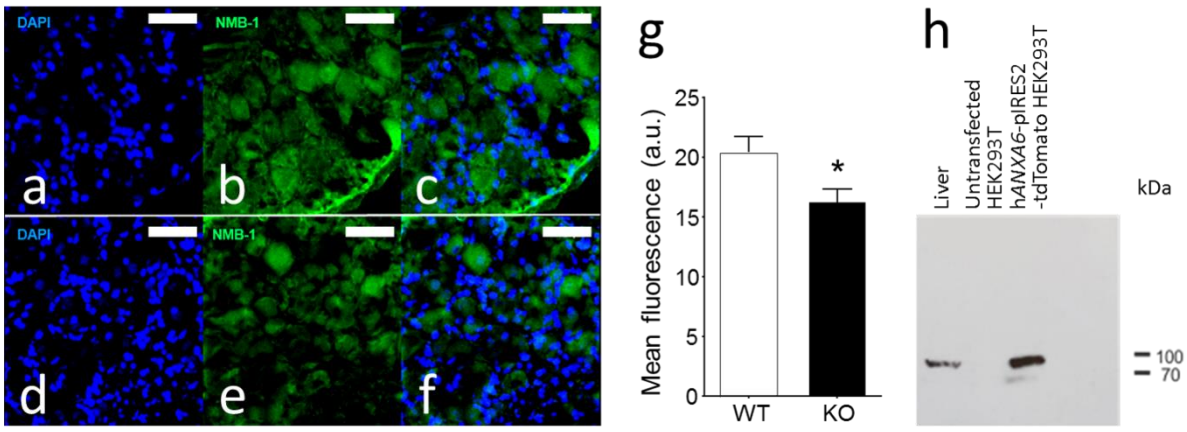


**Figure S2. Interaction between NMB-1 and annexin A6.** (a-g) WT (a-c) and *Anxa6* KO (d-f) DRG sections were incubated with bNMB-1 followed by FITC-Avidin (scale bars = 50 µm) and the fluorescence intensity was quantified (g) ($p$=0.0298, n=10 and represent sections obtained from 2 mice per group). (h) Direct interaction of annexin A6 and NMB-1 was probed using Western blot. The panel shows expression of annexin A6 in mouse liver, *hANXA6*-pIRES2-tdTomato expressing HEK293T cells, and untransfected HEK293T cells (negative control).

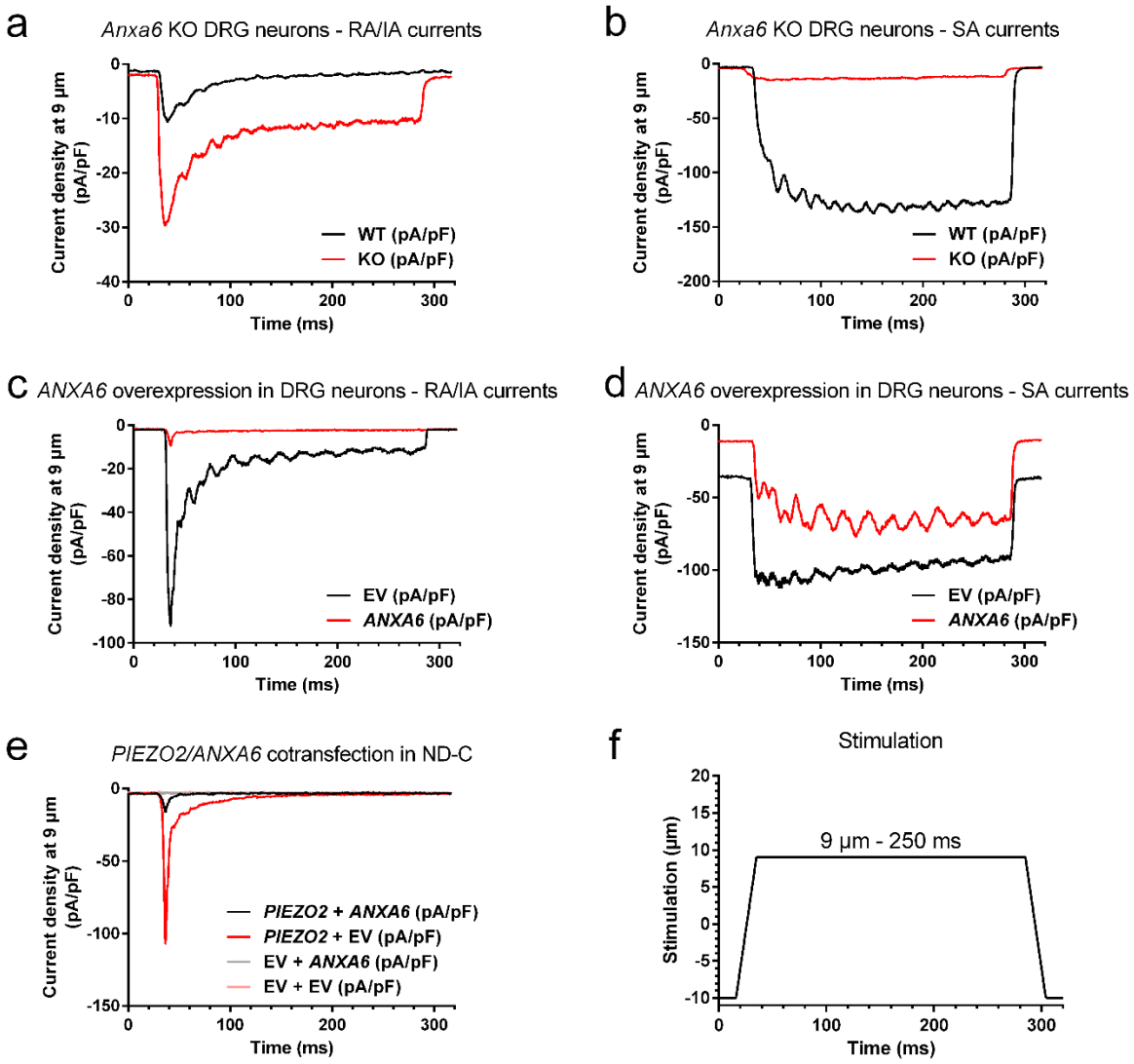


**Figure S3. Representative traces of MA currents in vitro.** (a-b) RA/IA and SA currents recorded in *Anxa6* KO DRG neurons, representative of the data given in Fig. 2. (c-d) RA/IA and SA currents recorded in WT DRG neurons overexpressing *hANXA6*, representative of the data given in Fig. 3. (e) RA currents recorded in ND-C cells transfected with *hPIEZO2* ± *hANXA6* or their respective empty vectors, representative of the data given in Fig. 4. (f) All current traces shown were recorded in response to a 9 µm deformation of the plasma membrane by a firepolished glass pipette, lasting for 250 ms.

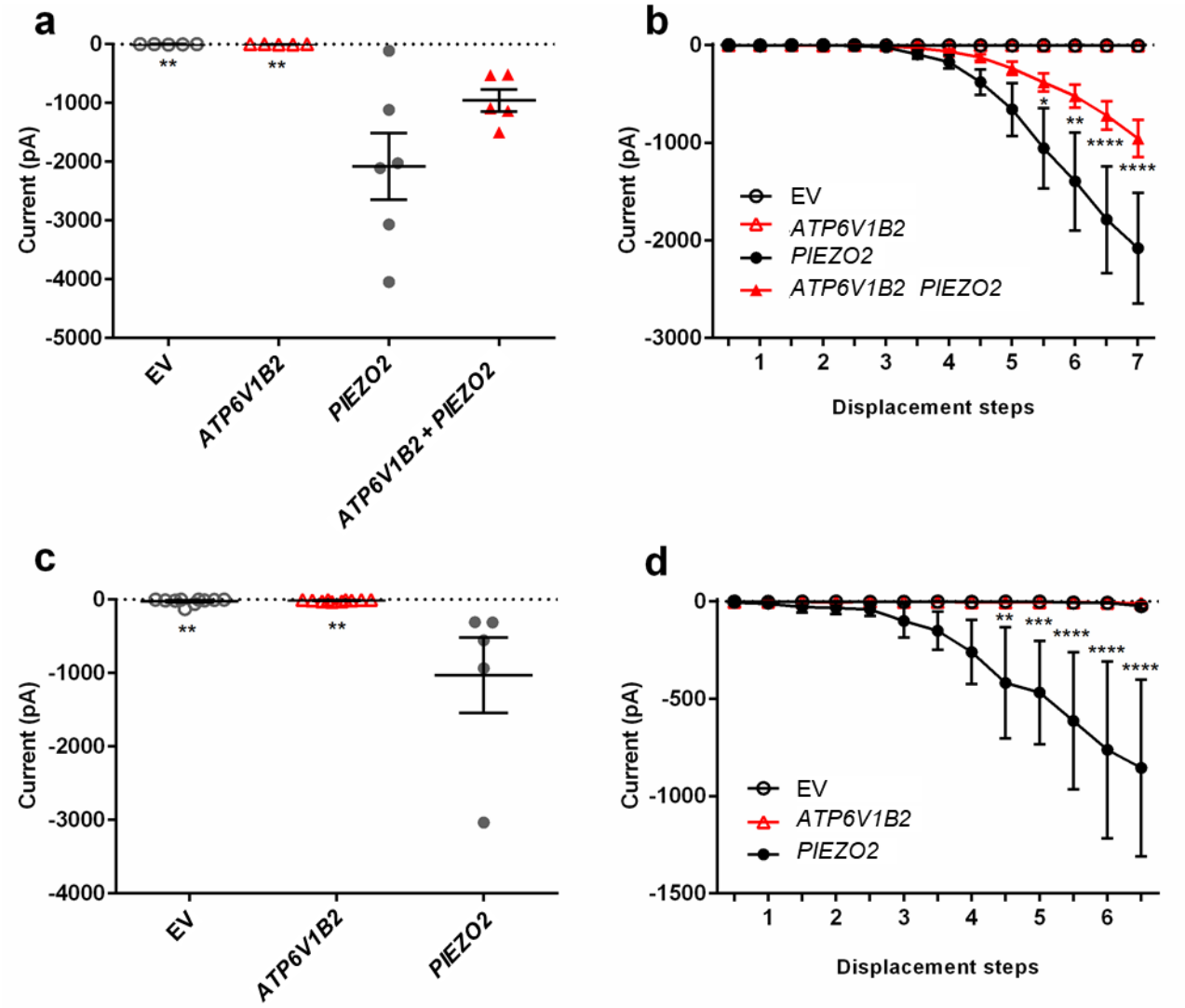


**Figure S4. ATP6V1B2 does not confer mechanosensitivity to HEK or ND-C cells but inhibits Piezo2 current.** Peak current (a) and current-displacement curve (b) for HEK cells transfected with h*ATP6V1B2*. Peak current (c) and current-displacement curve (b) for ND-C cells transfected with h*ATP6V1B2*. (HEK cells: *ATP6V1B2* n=10; EV n=10; *PIEZO2* n=5; *ATP6V1B2* + *PIEZO2* n=5; ND-C cells: *ATP6V1B2* n=10; EV n=10; *PIEZO2* n=5). Data are shown as Mean±SEM. One way ANOVA with Dunnett's post-hoc test (statistics shown on graph is each group compared to *PIEZO2*) (a,c); Two-way repeated measures ANOVA with Tukey post-hoc test (statistics shown on graph is *ATP6V1B2+PIEZO2* compared to *PIEZO2*) (b); Two-way repeated measure ANOVA with Tukey post-hoc test (statistics shown on graph is *ATP6V1B2* compared to *PIEZO2*) (d). *p<0.05, **p<0.01, ***p<0.001; ****p<0.0001.

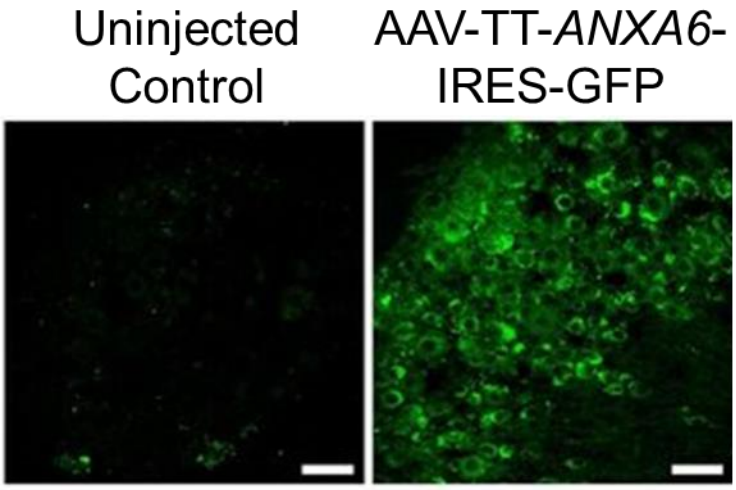


**Figure S5. Transduction of DRG neurons using AAV-TT serotype.** 15 weeks after intrathecal injection, robust GFP expression was observed in lumbar DRG of animals treated with AAV-TT-*hANXA6*-IRES-GFP compared to untreated animals. Similar results were obtained from 3 different animals per condition. Scale bar, 50 µm.

**Table S1. Top NMB-1–binding candidates in ND-C cells.**

| Protein | | UniprotKB | MW | NMB-1 Peptide hits |
|---|---|---|---|---|
| Anxa6 | Annexin A6 | P14824 | 75,838 | 24 |
| Atic | 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase | B0LAC5 | 64,117 | 6 |
| Atp6v1b2 | Vacuolar ATP synthase subunit B, brain isoform | P62814 | 56,515 | 4 |
| Abcb10 | ATP-binding cassette sub-family B member 10, mitochondrial | Q9JI39 | 73,972 | 4 |
| Anxa5 | Annexin A5 | P48036 | 35,730 | 4 |
| G6pdx | Glucose-6-phosphate 1-dehydrogenase X | Q00612 | 59,225 | 4 |
| Hspa9 | Stress-70 protein, mitochondrial precursor | P38647 | 44,145 | 3 |
| Rps3 | 40S ribosomal protein S3 | P62908 | 26,674 | 3 |
| Msn | Moesin | P26041 | 67,725 | 3 |
| Stxbp1 | Isoform 1 of Syntaxin-binding protein 1 | O08599 | 67,526 | 3 |
| Hspa1l | Heat shock 70 kDa protein 1L | P16627 | 70,593 | 3 |
| Ppp2r1a | Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform | Q76MZ3 | 65,281 | 3 |
| Vim | Vimentin | P20152 | 53,655 | 2 |

**Data File S1. NMB-1–binding candidates in DRG neurons.**

**Data File S2. NMB-1–binding candidates from differentiated ND-C cells.**