

Supplementary Information for:

## **Mechanically activated ion channel Piezo1 modulates macrophage polarization and stiffness sensing**

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1 **Supplementary Tables**

2 **Supplementary table 1: List of antibodies with dilutions and vendors used for this study.**

3 Abbreviations: WB – Western blot, IF – Immunofluorescence, IHC – immunohistochemistry.

<b>Antibody</b>	<b>Dilution (Application)</b>	<b>Vendor (Cat. #)</b>
iNOS	1:1000 (WB), 1:100 (IHC)	Abcam (ab15323)
ARG1	1:1000 (WB), 1:50 (IHC)	Abcam (ab60176)
GAPDH	1:2000 (WB)	BioLegend (607902)
PIEZO1	1:1000 (WB)	ProteinTech (15939-1-AP)
RFP	1:1000 (WB), 1:400 (IF)	Rockland (600-401-379)
NFκB	1:1000 (WB), 1:800 (IHC)	Cell Signaling (8242S)
p- NFκB	1:1000 (WB)	Cell Signaling (3033S)
NFκB	1:100 (IF)	Santa Cruz Biotech (sc-8008)
STAT6	1:1000 (WB)	Cell Signaling (5397S)
p-STAT6	1:1000 (WB)	Cell Signaling (56554S)
STAT1	1:1000 (WB)	Cell Signaling (14994)
p-STAT1	1:1000 (WB)	Cell Signaling (9167)
STAT3	1:1000 (WB)	Cell Signaling (9139)
p-STAT3	1:1000 (WB)	Cell Signaling (9145)
F4/80	1:200 (IHC)	Fisher Scientific (50-112-9624)

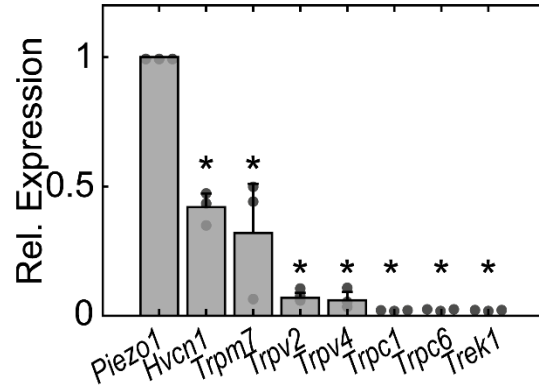
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5 **Supplementary table 2: List of primers used for this study.**

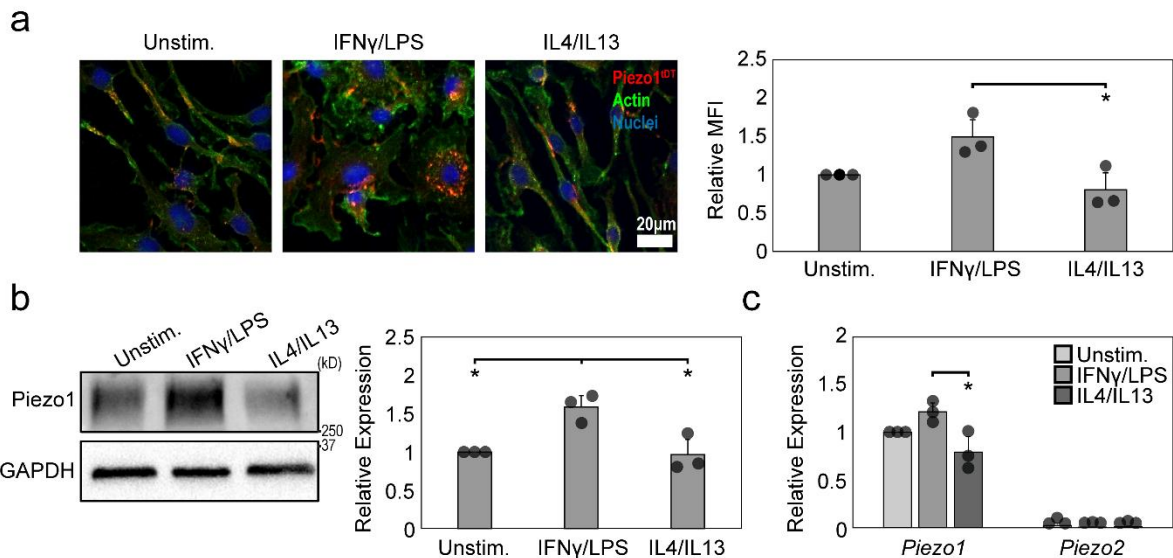
<b>Gene Name</b>	<b>Forward Primer (5'-3')</b>	<b>Reverse Primer (5'-3')</b>
<i>Piezo1</i>	GTTACCCCCTGGGAACATCT	TTCAGGAGAGAGGTGGCTGT
<i>Piezo2</i>	CTCACCTTTCCTGGCGTCAT	CCTCTTGAAACTCAGGCAGT
<i>Hvcn1</i>	CATCTGACCCAACACCACAG	CAGCTTCTTCTTCCCGTTTG
<i>Trpm7</i>	CCTCATGAAGACCATTTTCTAA	ACAAGTAAACCTTCCTCACAG
<i>Trpv2</i>	TTAAATGACTTGTGAGGGAGATAGC	CAAGTAACACAATCTACCCAAGGTC
<i>Trpv4</i>	TCACCTTCGTGCTCCTGTTG	AGATGTGCTTGCTCTCCTTG
<i>Trpc1</i>	GCCATCTTTGTCACCAGGTT	GCTCGAGCAAACCTTCCATTC
<i>Trpc6</i>	GCAGGATTTTCGTTGTTGGT	TGCTGACAGTTGGATGAGC
<i>Trek1</i>	ATTGTGCATGGTGACCTCAA	TGCTGACAGTTTGGATGAGC
<i>Il6</i>	CTGCAAGAGACTTCCATCCAGTT	GAAGTAGGGAAGGCCGTGG
<i>Nos2</i>	GAATCTTGAGCGAGTTGTGG	TTGTACTCTGAGGGCTGACAC
<i>Mcp1</i>	CAGCCAGATGCAGTTAACGC	GCCTACTCATTGGGATCATCTTG
<i>Il1b</i>	GATCCCAAGCAATACCCAAAG	CTTGTGCTCTGCTTGTGAGG
<i>Retnla</i>	GCCAATCCAGCTAACTATCCC	AGTCAACGAGTAAGCACAGG
<i>Mrc1</i>	TGTTTTGGTTGGGACTGACC	TGCAGTAACTGGTGGATTGTC
<i>Arg1</i>	CTCTGTCTTTTAGGGTTACGG	CTCGAGGCTGTCCTTTTGAG
<i>Gapdh</i>	GTCAAGCTCATTTCCTGGTAT	TCTCTTGCTCAGTGCCTTGC

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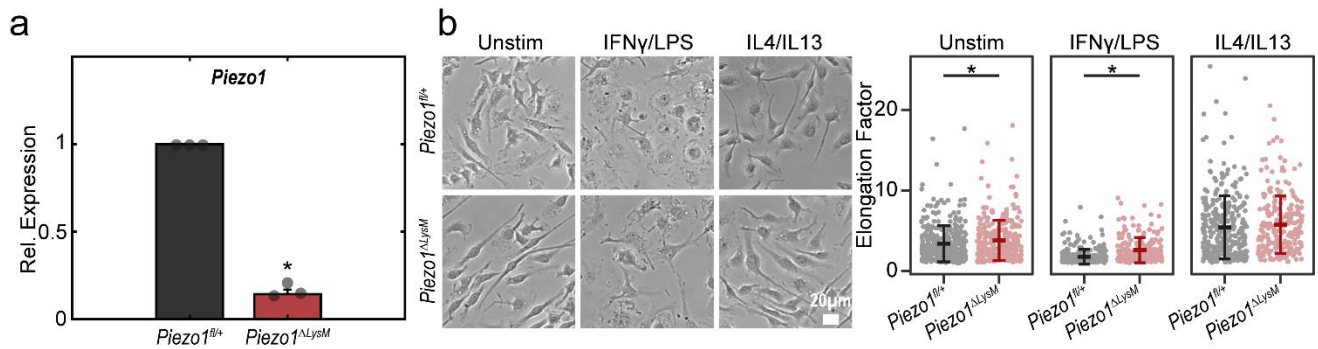
## 7 Supplementary Figures



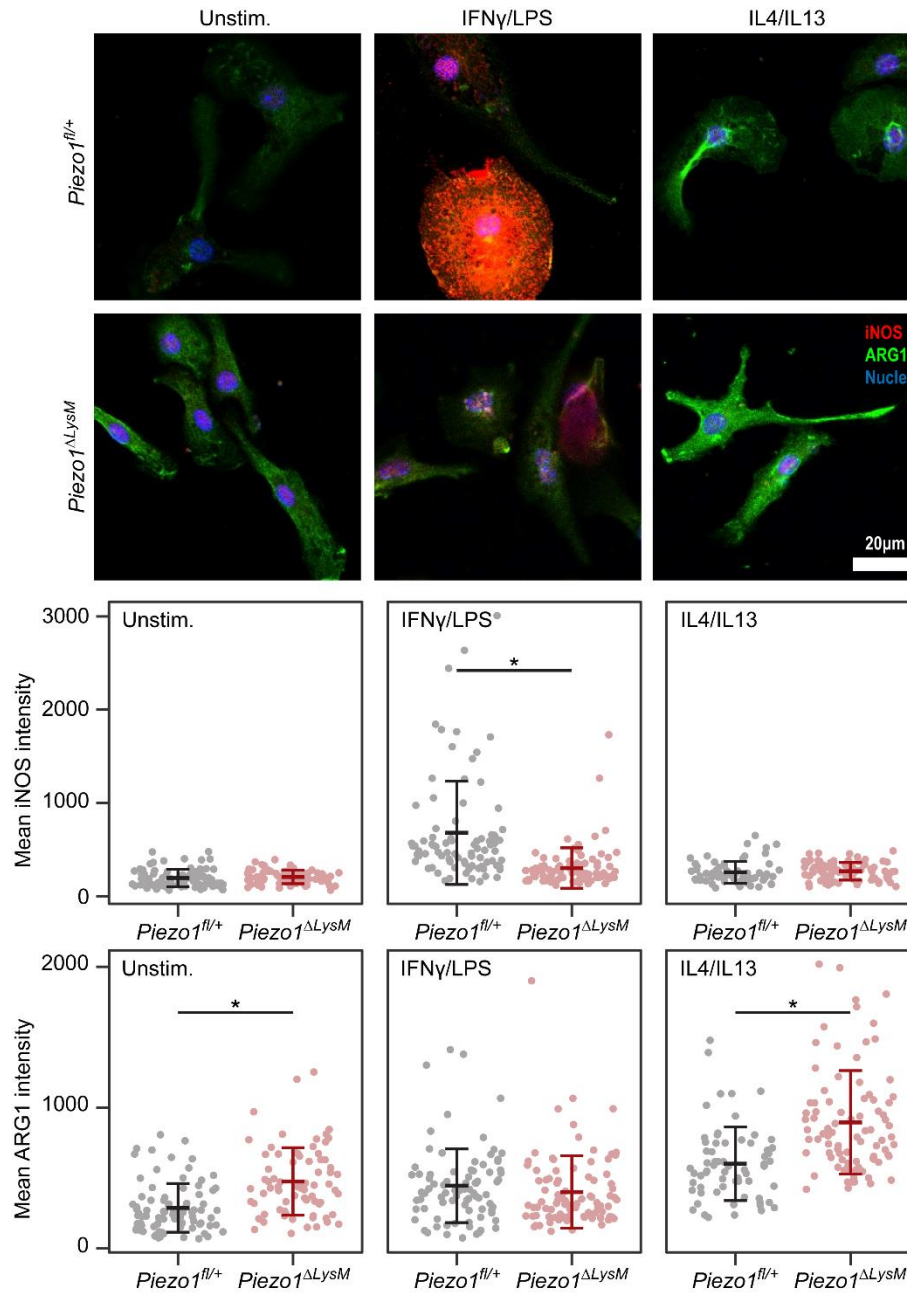
8 **Supplementary Figure 1: Piezo1 is highly expressed in murine BMDMs.** Relative gene expression  
9 of known mechanosensitive ion channels in BMDMs as measured by qPCR. Gene expression is  
10 normalized to the expression of *Piezo1*. Error bars indicate standard deviation for three independent  
11 experiments and \*  $p < 0.05$  when compared only to *Piezo1* expression as determined by a two-tailed  
12 paired t-test with Bonferroni correction used for multiple comparisons. Source data including exact p-  
13 values are provided as a Source Data file.



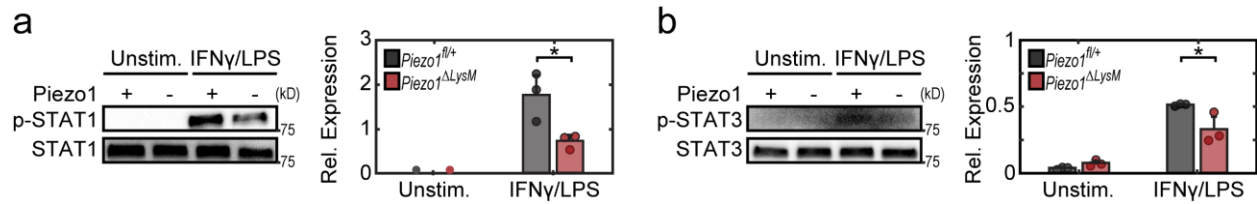
14 **Supplementary Figure 2: Macrophage activation influences Piezo1 expression.** (a) Representative  
 15 immunofluorescence images (left) and quantification of relative PIEZO1 mean fluorescence intensity  
 16 (MFI) of BMDMs isolated from Piezo1<sup>P1-tdT</sup> mice and incubated with media (Unstim.), 0.3ng/mL  
 17 IFN $\gamma$ /LPS, or 0.1ng/mL IL4/IL13. MFI is normalized to Unstim. condition. Error bars indicate standard  
 18 deviation for three independent experiments and \*  $p < 0.05$  when compared to the corresponding  
 19 condition by a two-tailed Student's t-test. (b) Representative Western blots (left) and quantification  
 20 (right) of Piezo1<sup>P1-tdT</sup> expression. Data normalized to Unstim. condition. Blots were obtained from  
 21 loading equal amounts of protein in separate gels (4% and 4-15% polyacrylamide gels for PIEZO1 and  
 22 GAPDH blots, respectively) and the resulting blots were processed in parallel. Error bars indicate  
 23 standard deviation for three independent experiments and \*  $p < 0.05$  when compared to the  
 24 corresponding condition by a two-tailed paired t-test. (c) Relative gene expression of Piezo1 and Piezo2  
 25 in Unstim., IFN $\gamma$ /LPS, and IL4/IL13 stimulated BMDMs. Gene expression is made relative to Gapdh  
 26 and normalized to the expression of Piezo1 in Unstim. macrophages. Error bars indicate standard  
 27 deviation for three independent experiments and \*  $p < 0.05$  when compared to the corresponding  
 28 condition by a two-tailed Student's t-test. Source data including exact p-values are provided as a Source  
 29 Data file.



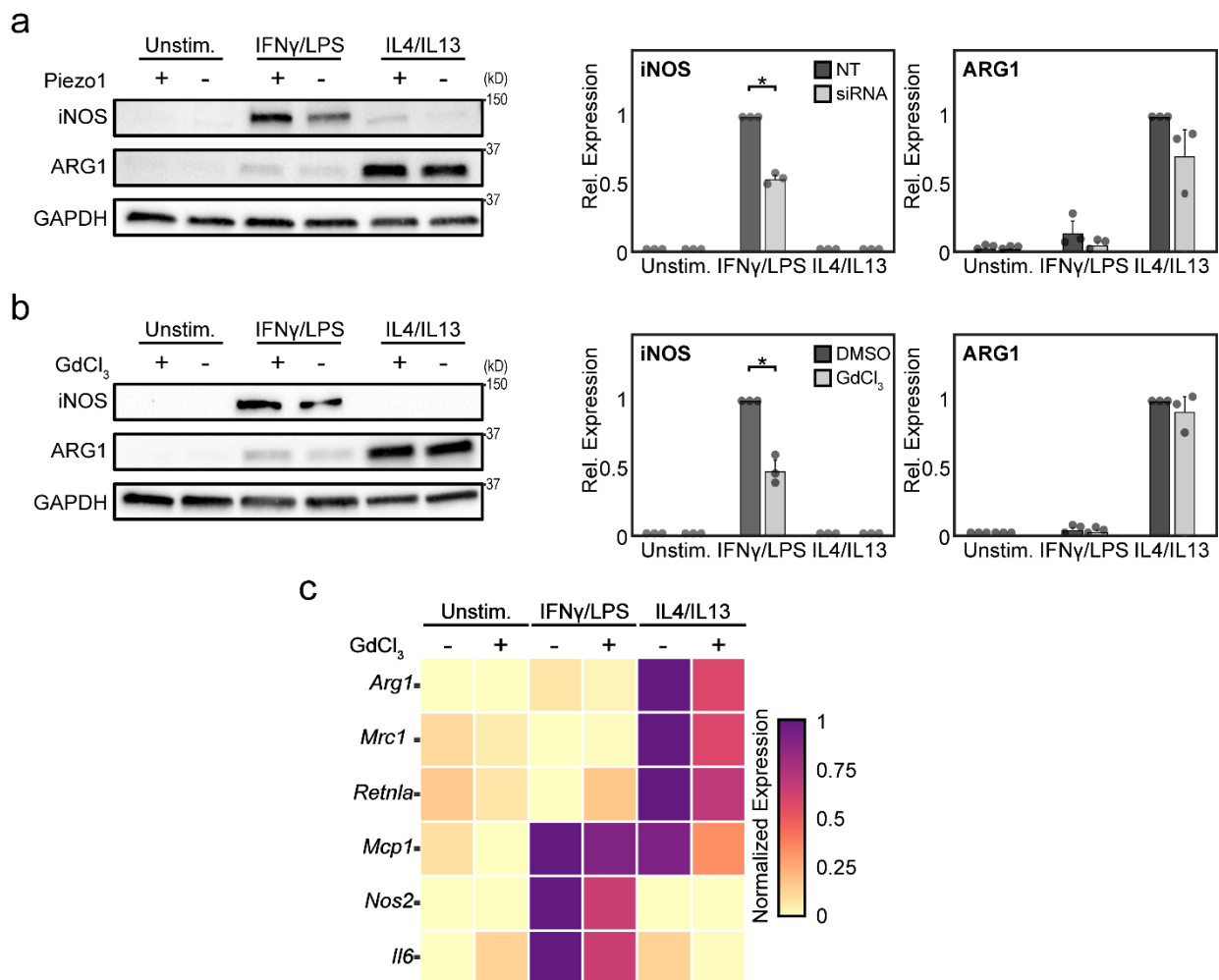
30 **Supplementary Figure 3: Validation of Piezo1 knockout in BMDMs.** (a) Relative gene expression  
 31 of *Piezo1* in unstimulated BMDMs isolated from *Piezo1<sup>fl/+</sup>* and *Piezo1<sup>ALysM</sup>* mice, n = 3. Gene  
 32 expression is normalized to the expression of *Piezo1* in BMDMs isolated from *Piezo1<sup>fl/+</sup>* mice. (b)  
 33 Representative phase contrast images (left) and quantification of cellular elongation (right) of *Piezo1<sup>fl/+</sup>*  
 34 and *Piezo1<sup>ALysM</sup>* BMDMs treated with media (Unstim.), IFN $\gamma$ /LPS, and IL4/IL13. N = 363, 306 cells  
 35 for Unstim., n = 290, 209 cells for IFN $\gamma$ /LPS, and n = 307, 231 cells for IL4/IL13 *Piezo1<sup>fl/+</sup>* and  
 36 *Piezo1<sup>ALysM</sup>* BMDMs, respectively. Error bars indicate standard deviation for BMDMs harvested from  
 37 a minimum of three *Piezo1<sup>fl/+</sup>* and *Piezo1<sup>ALysM</sup>* mice and \*  $p < 0.05$  when compared to the *Piezo1<sup>fl/+</sup>*  
 38 condition by a two-tailed paired t-test. Source data including exact p-values are provided as a Source  
 39 Data file.



40 **Supplementary Figure 4: Piezo1 deletion suppresses inflammatory and enhances healing**  
 41 **activation in peritoneal macrophages.** Representative images (top) and quantification of mean iNOS  
 42 and ARG1 intensity in *Piezo1<sup>fl/+</sup>* and *Piezo1<sup>ΔLysM</sup>* peritoneal macrophages stimulated with media  
 43 (Unstim.), IFN $\gamma$ /LPS, and IL4/IL13. N = 85, 75 cells for Unstim., n = 86, 92 cells for IFN $\gamma$ /LPS, and n  
 44 = 65, 87 cells for IL4/IL13 treated *Piezo1<sup>fl/+</sup>* and *Piezo1<sup>ΔLysM</sup>* peritoneal macrophages, respectively.  
 45 Error bars indicate standard deviation for BMDMs harvested from two mice and \*  $p < 0.05$  when  
 46 compared to indicated condition by two-tailed Mann-Whitney U test. Source data including exact p-  
 47 values are provided as a Source Data file.

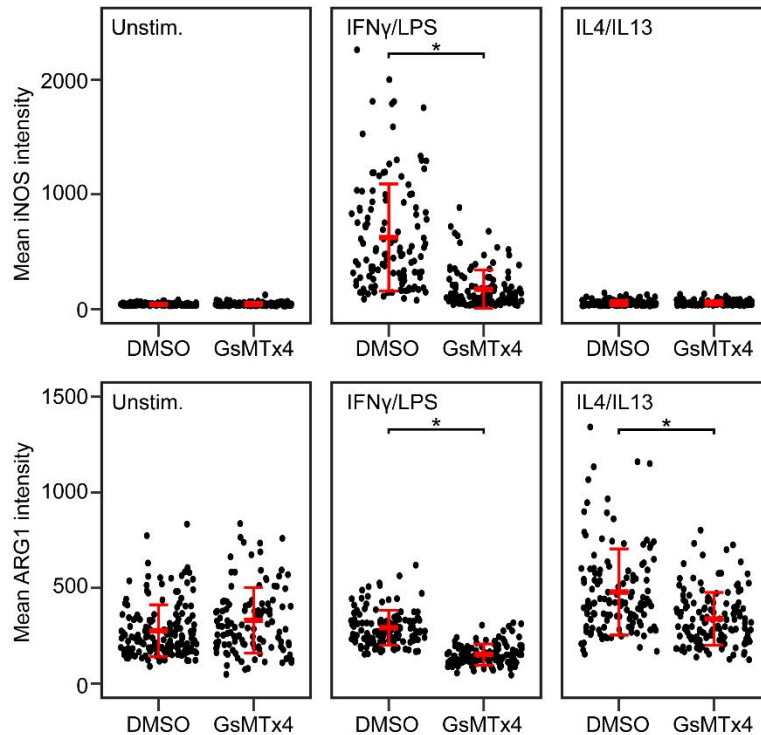
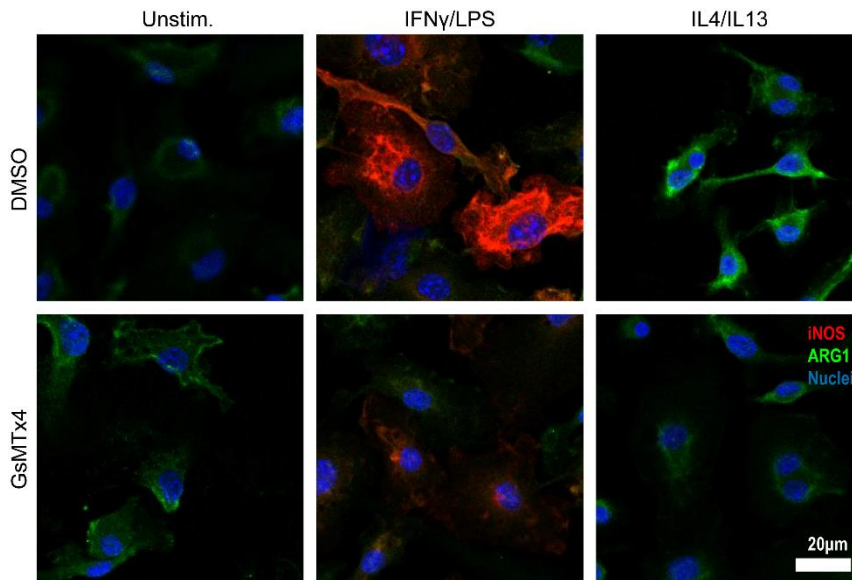


48 **Supplementary Figure 5: Piezo1 regulates activation of STAT1 and STAT3.** (a) Representative  
 49 Western blots (top) and quantification (bottom) of p-STAT1/STAT1 of *Piezo1<sup>fl/+</sup>* and *Piezo1<sup>ΔLysM</sup>*  
 50 BMDMs incubated with media (Unstim.) or IFN $\gamma$ /LPS for one hour. (b) Representative Western blots  
 51 (top) and quantification (bottom) of p-STAT3/STAT3 of *Piezo1<sup>fl/+</sup>* and *Piezo1<sup>ΔLysM</sup>* BMDMs incubated  
 52 with media (Unstim.) or IFN $\gamma$ /LPS for one hour. Error bars indicate standard deviation for BMDMs  
 53 harvested from n = 3 and \*  $p < 0.05$  when compared to indicated condition by a two-tailed Student's t-  
 54 test. Phosphorylated and total forms of transcription factors were obtained from loading equal amounts  
 55 of protein in separate gels and the resulting blots were processed in parallel. Source data including  
 56 exact p-values are provided as a Source Data file.

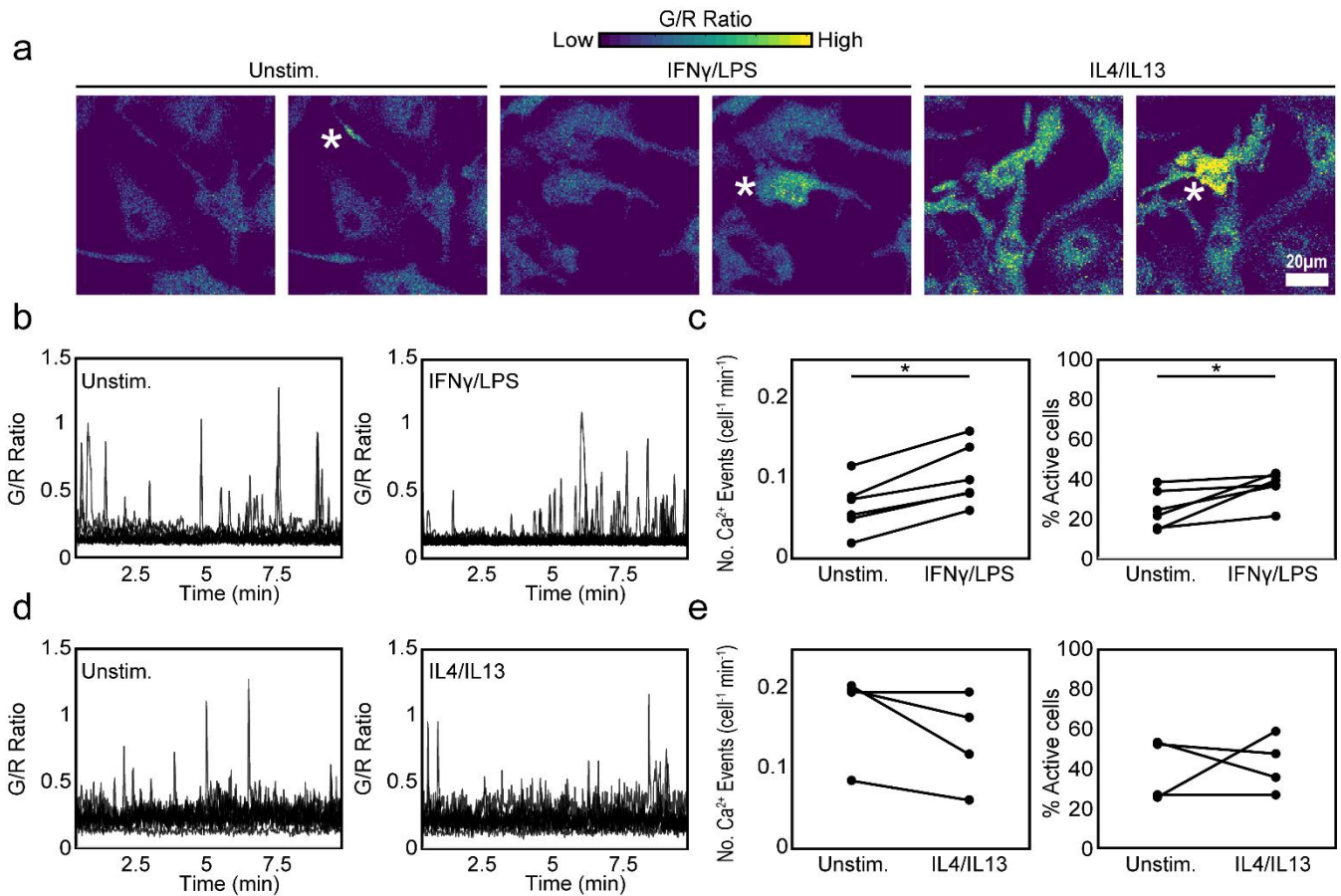


57 **Supplementary Figure 6: Piezo1 inhibition regulates macrophage activation.** (a) Representative  
 58 Western blots (left) and quantification (right) of iNOS, ARG1, and GAPDH of BMDMs treated with  
 59 control non-target (NT) or Piezo1 siRNA (siRNA) and incubated with media (Unstim.), 0.3ng/mL  
 60 IFN $\gamma$ /LPS, or 0.1ng/mL IL4/IL13. (b) Representative Western blots (left) and quantification (right) of  
 61 iNOS, Arg1, and GAPDH of BMDMs treated with DMSO or 25 $\mu$ M GdCl<sub>3</sub> and incubated with media  
 62 (Unstim.), 0.3ng/mL IFN $\gamma$ /LPS, or 0.1ng/mL IL4/IL13. (c) Relative gene expression of inflammatory  
 63 and healing markers in DMSO and GdCl<sub>3</sub> treated BMDMs incubated with the indicated conditions, as  
 64 measured by qPCR. Gene expression is shown relative to the highest expressing condition. Error bars  
 65 indicate standard deviation for three independent experiments and \*  $p < 0.05$  when compared to the  
 66 corresponding condition by a paired t-test. Source data including exact p-values are provided as a  
 67 Source Data file.

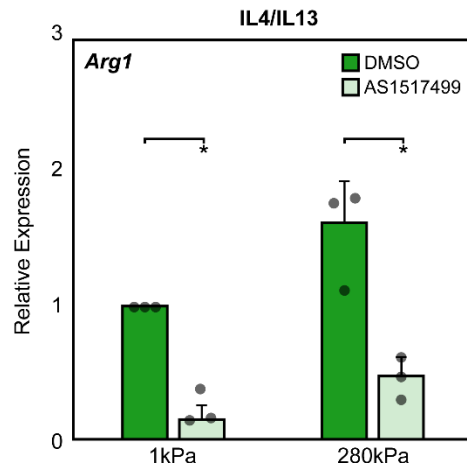




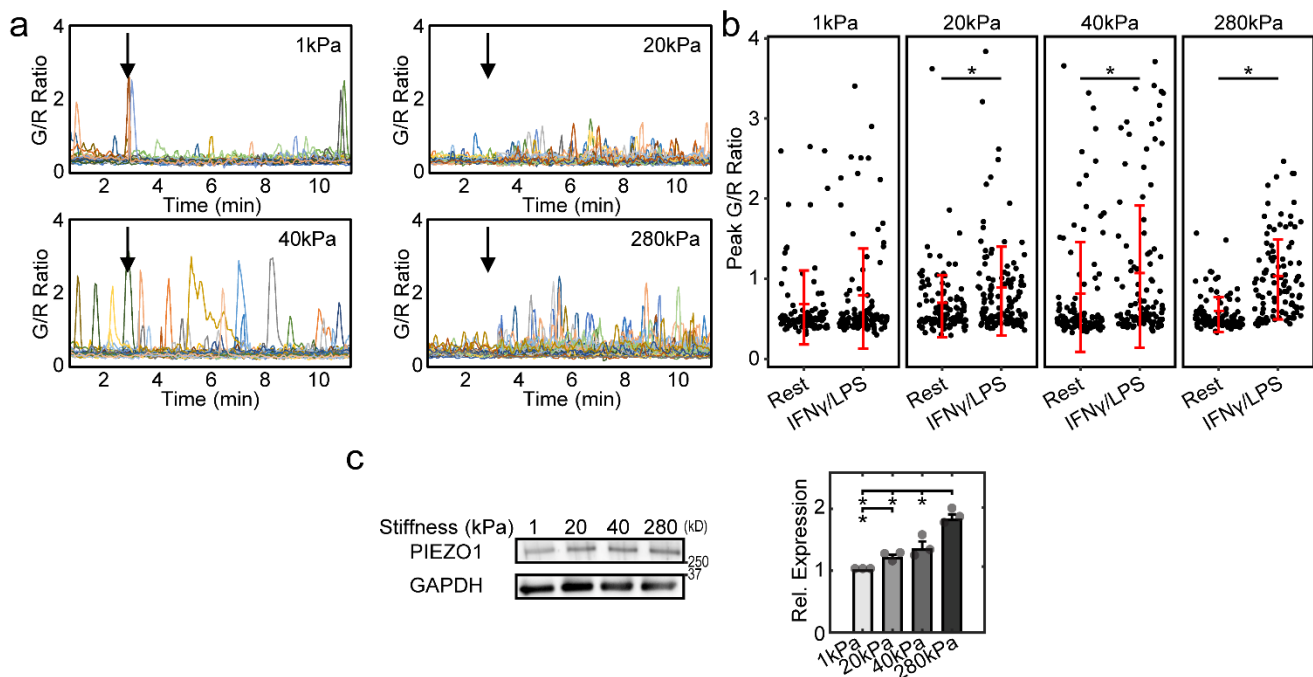
68 **Supplementary Figure 7: GsMTx4 treatment suppresses macrophage inflammatory activation.**  
 69 Representative images (top) and quantification of mean iNOS and ARG1 intensity in wild-type  
 70 BMDMs treated with 100µM GsMTx-4 in media and stimulated with media (Unstim.), IFNγ/LPS, and  
 71 IL4/IL13. N= 150-200 cells, error bars indicate standard deviation for BMDMs harvested from three  
 72 mice and \*  $p < 0.05$  when compared to indicated condition by two-tailed Mann-Whitney U test. Source  
 73 data including exact p-values are provided as a Source Data file.



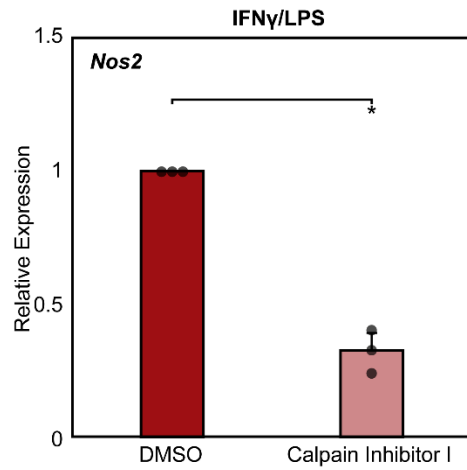
74 **Supplementary Figure 8: Acute addition of IFN $\gamma$ /LPS but not IL4/IL13 promotes Ca<sup>2+</sup> activity**  
 75 **in Salsa6f+ BMDMs.** (a) Representative G/R ratio images taken from a time-lapse video of  
 76 unstimulated (Unstim.), IFN $\gamma$ /LPS, or IL4/IL13 stimulated Salsa6f+ BMDMs. Asterisks denote the  
 77 occurrence of a Ca<sup>2+</sup> event. (b) Representative Ca<sup>2+</sup> traces following acute addition of Ringer solution  
 78 (Unstim.) or Ringer solution containing 100ng/mL IFN $\gamma$ /LPS in Salsa6f+ BMDMs. (c) Quantification  
 79 of number of Ca<sup>2+</sup> events (normalized for cell number and time) and fraction of cells showing Ca<sup>2+</sup>  
 80 elevations in BMDMs following acute addition of Ringer solution (Unstim.) or Ringer solution  
 81 containing 100ng/mL IFN $\gamma$ /LPS, captured by confocal microscopy. Each paired data point is calculated  
 82 from a 10-minute time-lapse video (N = 6 videos each) and \*  $p < 0.05$  as determined by a paired t-test.  
 83 (d) Representative Ca<sup>2+</sup> traces following acute addition of Ringer solution (Unstim.) or Ringer solution  
 84 containing 10ng/mL IL4/IL13 in Salsa6f+ BMDMs. (e) Quantification of number of Ca<sup>2+</sup> events  
 85 (normalized for cell number and time) and fraction of cells showing Ca<sup>2+</sup> elevations in BMDMs  
 86 following acute addition of Ringer solution (Unstim.) or Ringer solution containing 10ng/mL IL4/IL13,  
 87 captured by confocal microscopy. Each paired data point is calculated from a 10-minute time-lapse  
 88 video (N = 4 videos each) and \*  $p < 0.05$  as determined by a two-tailed paired Student's t-test. Source  
 89 data including exact p-values are provided as a Source Data file.



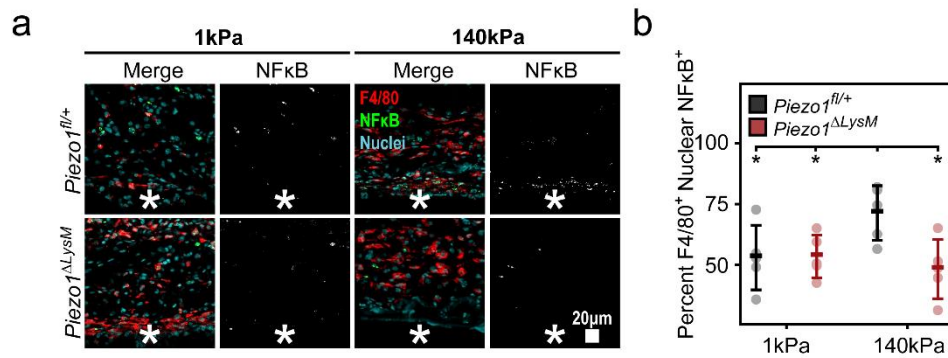
90 **Supplementary Figure 9: Pharmacological inhibition of STAT6 reduces healing activation in**  
 91 **IL4/IL13 stimulated BMDMs.** Relative *Arg1* gene expression in wild-type BMDMs seeded on 1 kPa  
 92 and 280 kPa surfaces, exposed to DMSO or 300 nM AS1517499 to inhibit STAT6, and stimulated with  
 93 IL4/IL13. Data normalized to *Gapdh* and expressed relative to 1kPa DMSO control. Error bars denote  
 94 Mean  $\pm$  SD for three separate experiments, \*  $p < 0.05$  as determined by a two-tailed paired t-test.  
 95 Source data including exact p-values are provided as a Source Data file.



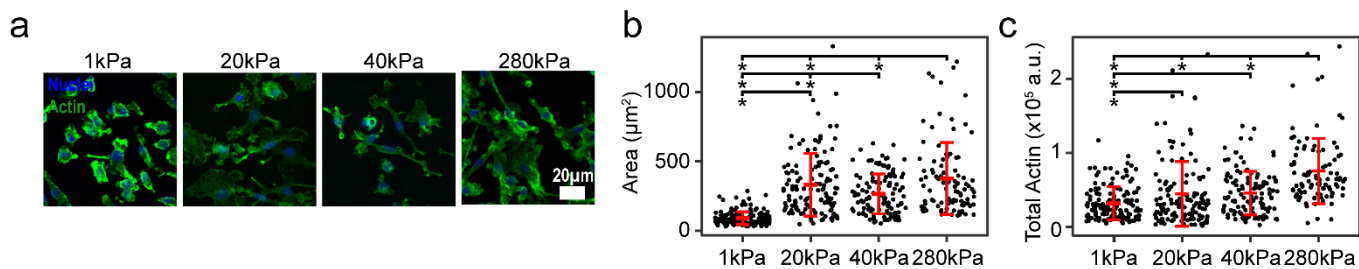
96 **Supplementary Figure 10: Substrate rigidity promotes Ca<sup>2+</sup> activity and Piezo1 expression.** (a)  
 97 Traces of individual Ca<sup>2+</sup> events in BMDMs seeded on polyacrylamide gels of 1, 20, 40, and 280kPa  
 98 stiffness and exposed to Ringer solutions containing 100ng/mL IFN $\gamma$ /LPS. Arrows indicate addition of  
 99 cytokines. (b) Quantification of peak G/R intensities of BMDMs on polyacrylamide gels at rest and  
 100 stimulated with 100ng/mL IFN $\gamma$ /LPS. N = 102, 118, 113, and 106 for 1, 20, 40, and 280kPa,  
 101 respectively. Error bars denote standard deviation and \*  $p < 0.05$  when compared to the corresponding  
 102 condition as determined by two-tailed Mann-Whitney U test. (c) Representative Western blots (left)  
 103 and quantification (right) of Piezo1 expression in wild-type BMDMs seeded on 1, 20, 40, and 280kPa  
 104 polyacrylamide gels. Blots were obtained from loading equal amounts of protein in separate gels (4%  
 105 and 4-15% polyacrylamide gels for PIEZO1 and GAPDH blots, respectively) and the resulting blots  
 106 were processed in parallel. Error bars indicate standard deviation for BMDMs harvested from three  
 107 mice and \*  $p < 0.05$  when compared to indicated condition by a paired t-test with Bonferroni correction  
 108 for multiple comparisons. Source data including exact p-values are provided as a Source Data file.



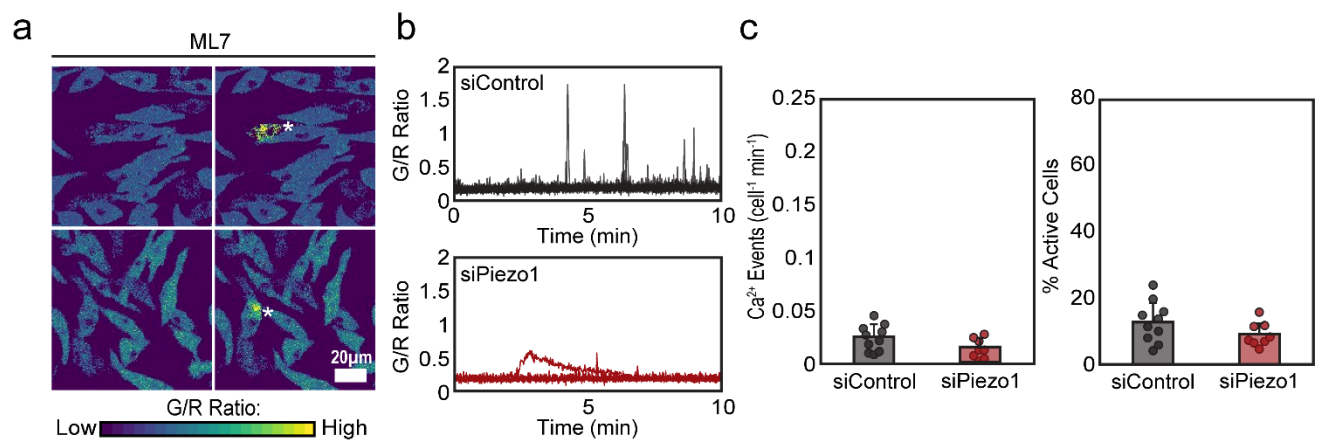
109 **Supplementary Figure 11: Calpains are involved in IFN $\gamma$ /LPS-mediated inflammation.** Relative  
110 *Nos2* gene expression in wild-type BMDMs exposed to DMSO or 5 $\mu$ M Calpain Inhibitor I and  
111 stimulated with IFN $\gamma$ /LPS. Data normalized to *Gapdh* and expressed relative to DMSO control. Error  
112 bars denote Mean  $\pm$  SD for three separate experiments, \*  $p < 0.05$  as determined by a two-tailed paired  
113 t-test. Source data including exact p-values are provided as a Source Data file.



114 **Supplementary Figure 12: Stiffness-mediated Piezo1 activation regulates NFκB translocation *in***  
 115 ***vivo*.** (a) Representative immunohistochemistry images of tissue surrounding soft (1kDa) and stiff  
 116 (140kDa) PEGDA material implanted in *Piezo1*<sup>fl/+</sup> and *Piezo1*<sup>ΔLysM</sup> mice and stained for F4/80, NFκB,  
 117 and Hoechst (left). Material location indicated with asterisk. (b) Quantification of percent cells that  
 118 stained positive for both F4/80 and nuclear localization of NFκB (right) for n = 5. Error bars denote  
 119 Mean ± SD, \* *p* < 0.05 as determined by a two-tailed Student's t-test. Source data including exact p-  
 120 values are provided as a Source Data file.



121 **Supplementary Figure 13: Substrate rigidity promotes cell spreading and actin formation.** (a-c)  
 122 Representative images (a) and quantification of area (b) or total actin integrated across cell area (c) in  
 123 wild-type BMDMs seeded on 1, 20, 40, 280kPa fibronectin conjugated polyacrylamide gels. N = 157,  
 124 128, 116, and 121 cells cultured on 1, 20, 40, and 280kPa substrates, respectively. Error bars indicate  
 125 standard deviation for BMDMs harvested from three mice and \*  $p < 0.05$  when compared to indicated  
 126 condition by two-tailed Mann-Whitney U test. Source data including exact p-values are provided as a  
 127 Source Data file.



128 **Supplementary Figure 14: Myosin light-chain kinase regulates Ca<sup>2+</sup> influx in BMDMs.** (a-c)  
 129 Representative G/R ratio images of cell at rest (left) or (a), traces of individual Ca<sup>2+</sup> events (b), and  
 130 quantification of number of Ca<sup>2+</sup> events (normalized for cell number and time, left) and fraction of cells  
 131 showing Ca<sup>2+</sup> elevations (right), (c) taken from a time-lapse video of siControl and siPiezo1 treated  
 132 Salsa6f+ BMDMs following addition of DMSO or 25μM ML7 in Ringer solution containing 100ng/mL  
 133 IFNγ/LPS. Asterisks denote the occurrence of a Ca<sup>2+</sup> event. Each data point in (c) denotes a single  
 134 video (N= 12 videos). Source data including exact p-values are provided as a Source Data file.