



Article

Supplementary Materials: Mechano-sensing channel PIEZO2 enhances invasive phenotype in triple-negative breast cancer

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Table S1. qPCR primer sequence.

Gene	F	R
<i>PIEZO2</i>	TGCACAGTCAAAGGCTATCAA	ATGCTGTCCCAAATGATTCC
<i>CTGF</i>	AGGAGTGGGTGTGTGACGA	CCAGGCAGTTGGCTCTAATC
<i>ABCB1</i>	TCACTATTGTTTCTAGCCCTTGG	ATGTATCGGAGCCGCTTG
<i>ANKRD1</i>	CGAATTCCGTGATATGCTTGA	AAACATCCAGGTTTCCTCCA
<i>CAT</i>	GGATCACATACTTTCAAGCTGGT	TTTTTGATGCCCTGGTCAGT
<i>CYR61</i>	ACCCTTCTCCACTTGACCAG	CTTGGCGCAGACCTTACAG
<i>GPATCH4</i>	CCAAGCCCAACTTGCTGTAT	TGCAGCTCTCCAAGTCTTTGT
<i>LMNB2</i>	CCCACTCCTCATCTCACCAT	GCCCAAGCATCTTCTAAACCT
<i>PTGS2</i>	TGGGAAGCCTTCTCTAACCTC	TCAGGAAGCTGCTTTTACCTT
<i>TXN</i>	TTACAGCCGCTCGTCAGA	AAGGCTTCTGAAAAGCAGTC
<i>WSB2</i>	TGTGATTATGTGGGACCCCTA	CAGATCTCAGTGAGCTAATGTGG
<i>GAPDH</i>	AAGGTGAAGGTCGGAGTCAAC	GGGGTCATTGATGGCAACAATA

Table S2. List of antibodies for western blot.

Primary antibodies	Company	species	type	dilution	reference
SNAIL	CST	rabbit	monoclonal	1:300	3879S
Vimentin	CST	rabbit	monoclonal	1:3,000	5741S
E-cadherin	CST	rabbit	monoclonal	1:1,000	3195S
YAP/TAZ	CST	rabbit	monoclonal	1:1,000	8418S
p-TAZ	CST	rabbit	monoclonal	1:300	59971S
p-YAP	CST	rabbit	monoclonal	1:1,000	13008S
PI3K	CST	rabbit	monoclonal	1:1,000	4249S
Akt	CST	rabbit	monoclonal	1:1,000	9272S
p-Akt	CST	rabbit	monoclonal	1:1,000	4060S
GSK-3 β	CST	rabbit	monoclonal	1:1,000	12456S
p-GSK-3 β	CST	rabbit	monoclonal	1:500	9336S
GAPDH	CST	rabbit	monoclonal	1:10,000	5174S
YAP (for nuclear fraction)	CST	rabbit	monoclonal	1:1,000	14074T
β -Tubulin	CST	rabbit	monoclonal	1:1,000	2128S
Histone H3	CST	rabbit	monoclonal	1:1,000	4499S

CST: Cell Signaling Technologies

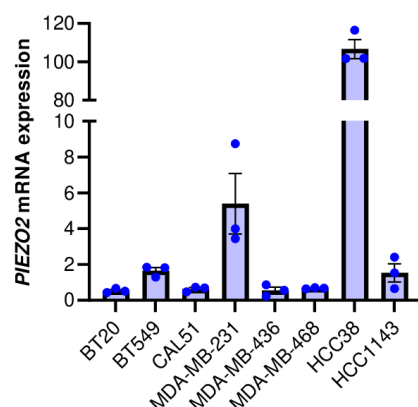


Figure S1. *PIEZO2* mRNA expression in triple-negative breast cancer (TNBC). *PIEZO2* mRNA expression in TNBC cell lines. Data was normalized with normal breast epithelial cell line, MCF10A as 1.

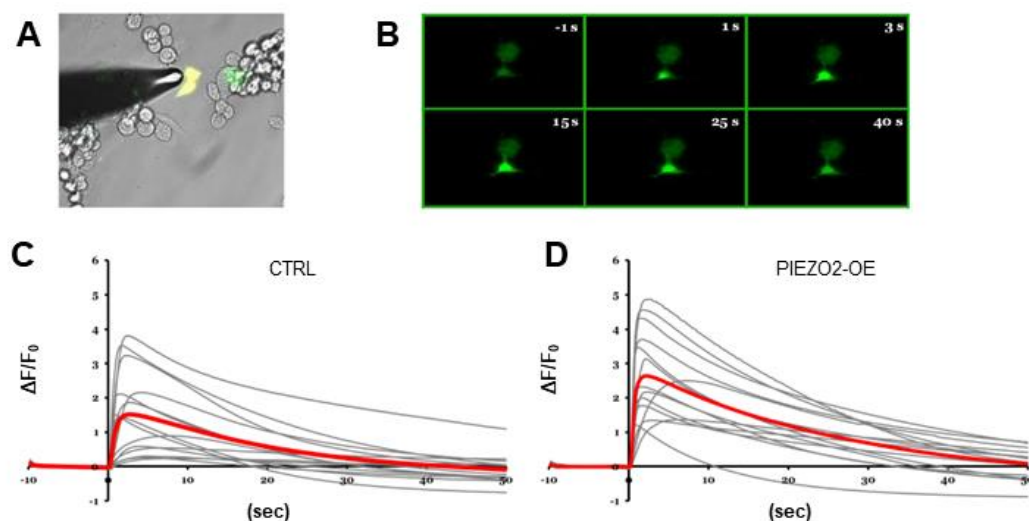


Figure S2. *PIEZO2* overexpression increases mechanically evoked calcium transients in MDA-MB-231 cells. MDA-MB-231 control (CTRL) or *PIEZO2* overexpressing cells (*PIEZO2*-OE) were transfected tdTomato for identification and GCaMP5, a genetically encoded indicator of cytoplasmic Ca^{2+} concentration. (A) Overlaid difference interference contrast (DIC)/epifluorescence image showing cells co-transfected with the red tdTomato and the green GCaMP5 (yellow). To the left, an electrically-driven blunted glass pipette is visualized. It delivers a 50 ms mechanical stimulus to the cells. Image magnification is 40x. (B) Stills from a green fluorescence time-lapse showing a representative experiment. The blunted pipette stimulates the cell at 0 sec. Typical fluorescence before mechanical stimulation (baseline, $t < 0$ s), followed by stimulation ($t > 0$ s), as the fluorescence sharply increases in response to the mechanical stimulus and returns to the pre-stimulation baseline. Image magnification is 40x. Normalized traces of GCaMP5 fluorescence for (C) CTRL and (D) *PIEZO2*-OE cells. Gray traces represent individual cells. Red traces represent the cumulative average. Negative time values are prior to mechanical stimulation. Positive time values are after mechanical stimulation. $\Delta F/F_0$ = difference in fluorescence from baseline normalized to baseline.

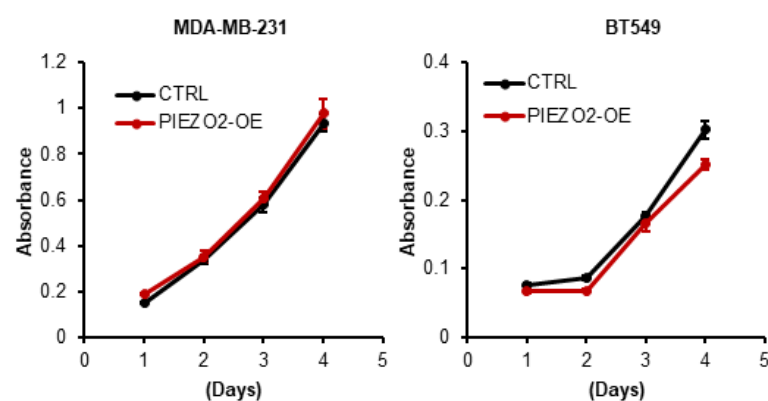


Figure S3. Proliferation of cells quantified by CCK8 in control (CTRL) and *PIEZO2* overexpressed (PIEZO2-OE) MDA-MB-231 and BT549 cells. $n=6$, each.

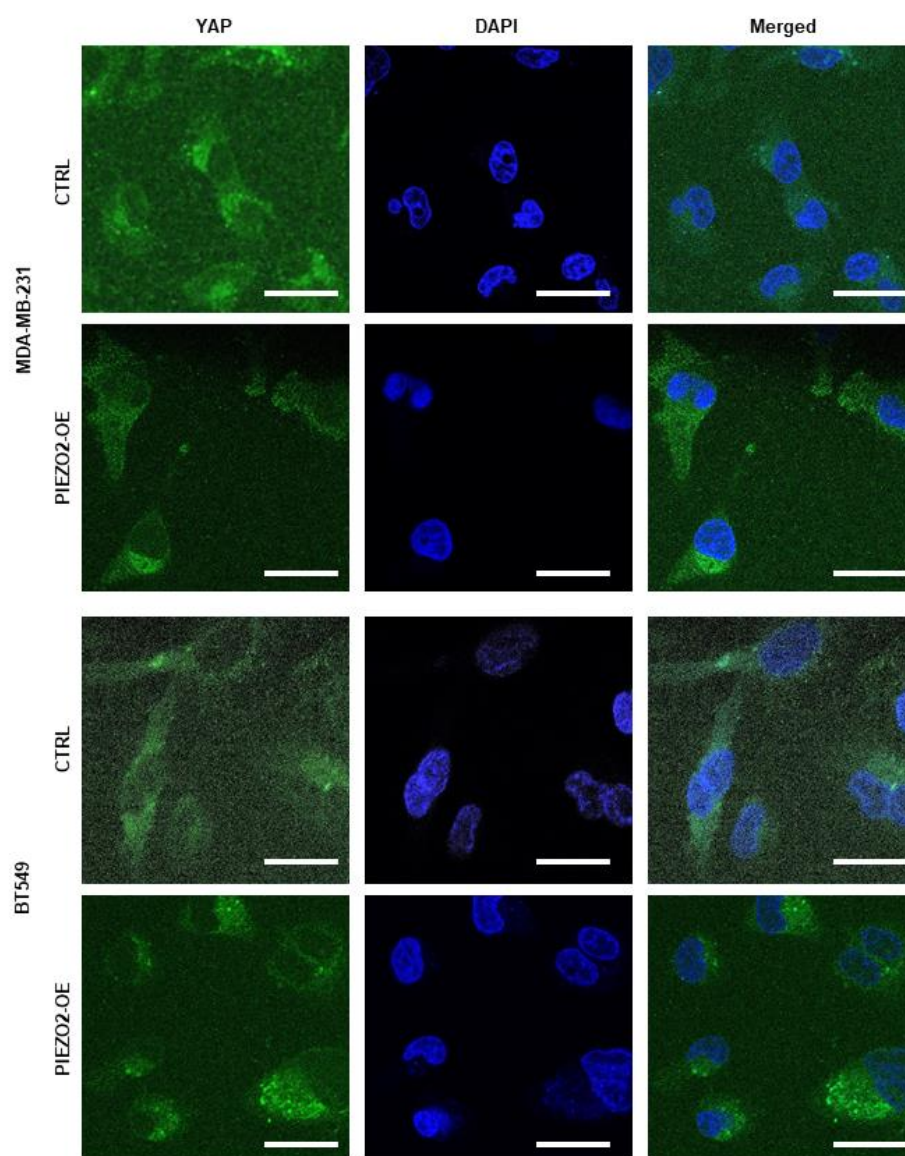


Figure S4. Representative images of YAP staining. YAP staining in control (CTRL) and *PIEZO2* overexpressed (PIEZO2-OE) cells of MDA-MB-231 and BT549. Scale bar indicates 25 μm .