# Ultra-rapid activation of TRPV4 ion channels by mechanical forces applied to cell surface $\beta$ 1 integrins

Benjamin D. Matthews<sup>1†</sup>, Charles K. Thodeti<sup>1,2†</sup>, Jessica D. Tytell<sup>1†</sup>, Akiko Mammoto<sup>1</sup>, Darryl R. Overby<sup>3</sup> and Donald E. Ingber<sup>1,4,5</sup>\*

#### **Electronic Supporting Information Figure Legends**

Fig. S1. Calcium response detected within milliseconds of mechanical strain. A-B) Representative scatter plots illustrating the temporal relationship between intracellular calcium (F/Fo) (left Y-axis), camera exposure (50 ms On, 20 ms Off), and force application (500 ms On/2 s Off) (right Y-axis) before, during and after a single integrindependent mechanical force pulse from a magnetic bead pulling experiment described in Fig. 1. Note that the timing between camera exposure and onset of mechanical force application is not linked in our system (camera and force data are offset vertically for purposes of clarity), and that the same data are shown in A and B with different ranges on the X-axis. In this recording, the camera exposure begins at 4863 ms and completes at 4914 ms, which is 13 ms after the initiation (at 4901 ms) of mechanical force application. A rise in F/Fo above baseline (see i and ii in A and B) detected beginning at 4863 ms therefore indicates the detection of increased intracellular calcium within 13 ms of force application. C) Temporal resolution of localized integrin-dependent forceinduced calcium transients. Scatter plot of relative rise in calcium (% of Peak levels) versus time from 6 separate calcium transients induced by force applied for only the last

4, 11, 13, 21, 24 or 50 ms of a 50 ms camera exposure, which displayed a similar rapid initial response as shown in A and B. Calcium (F/Fo) data from each transient were normalized relative to the peak and baseline F/Fo values [i.e. Calcium rise = (F/Fo – Baseline F/Fo)/(Peak F/Fo – F/Fo) x 100], where baseline F/Fo is defined as this value measured during the last frame prior to onset of force. Data are assigned temporal values in relation to the time of force application that occurs at time zero. Frames are numbered negatively or positively according to whether they were recorded before or after force initiation, and data from each frame are shaded gray within individual boxes for clarity. The calcium ratios in Frame 1 are significantly greater than baseline Frames -3 through -1 (Kruskall-Wallis Test, p < 0.003); post-hoc analysis using Dunn's Multiple Comparisons Test also confirmed that Frame 1 is greater than each of Frames -3, -2 and -1 (p < 0.05). These data indicate that calcium signaling can be detected as early as 4 msec after force application to integrins.

Fig. S2. Recruitment of zyxin, but not fodrin, to focal adhesions 15 min after BCE cell binding to microbeads coated with  $\beta$ 1 integrin ligands. (A) Fluorescence images of cells expressing GFP-zyxin and stained for fodrin focused at the base or apex of the same cell with a bound magnetic bead (4.5 µm) coated with anti- $\beta$ 1 integrin antibody (12G10). Top insets show higher magnification views in the vicinity of the white arrowhead showing that zyxin localizes within focal adhesions at the base of the cell and at the apex adjacent to the bead, whereas fodrin is absent from these regions. Inset at the bottom of the lower right image is a higher magnification view of an unattached bead showing low level of autofluorescence in the FITC channel used to identify fodrin.

(B, C) Measurements of fluorescence intensity for zyxin and fodrin in linear regions of interest (white lines from insets in (A) traversing a focal adhesion at the base of the cell (B) or at the apex adjacent to the ligand-coated microbead (C). (D) Averages of normalized maximal fluorescence intensity for zyxin and fodrin from linear regions of interest drawn through basal or apical focal adhesions.

Fig. S3. Partial disruption of actin cytoskeleton by cytochalasin D leaves existing focal adhesions intact. Phase contrast and immunofluorescence images of BCE cells with bound 12G10 anti- $\beta$ 1 integrin antibody-coated microbeads (4.5  $\mu$ m) in the absence or presence of cytochalasin D (CytoD, 2  $\mu$ g/ml, 20 min). Immunofluorescence photomicrographs show cells stained for actin or paxillin focused at the base or apex of the cell. Note that cytochalasin D induced disassembly of actin stress fibers, but left focal adhesions containing actin and paxillin at the base of the cell and at sites of bead binding intact.

**Fig. S4. Integrin chimera deletion strategy.** Schematic diagram of  $\beta$ 1 integrin chimeras containing an extracellular (EC) carbonic anhydrase IV (CA, grey) domain connected to the transmembrane (TM) domain of  $\beta$ 1-integrin ( $\beta$ 1, purple) or LDL (green), and the  $\beta$ 1-integrin intracellular (IC) domain (purple). Note sequential deletions ( $\Delta$ 1– $\Delta$ 5) of  $\beta$ 1-IC domains. "AA" represents replacement of segment with alanine, thin line denotes deletion of indicated region.

#### Fig. S5. Adhesion properties of BCE cells transfected with $\beta$ 1-integrin chimeras.

(A, B) Differential interference contrast (DIC) (A) and fluorescence (B) images showing cell spreading and focal adhesion formation in a BCE cell co-transfected with CA- $\beta$ 1- $\beta$ 1 and GFP-paxillin and plated on a substrate coated with anti-CA antibody. Inset in B shows higher magnification view of focal adhesions. (C, D) Merged phase contrast and immunofluorescence photomicrographs confirming that only cells transfected with the CA-LDL (C) or the CA–LDL- $\beta$ 1 (D) bind beads coated with anti-CA antibody (white nuclei indicate cells transfected with chimera and histone 2B Ds-red; adjacent non-transfected cells that do not exhibit bead binding are outlined in white).

**Fig. S6. Different integrin cytoplasmic tail deletions weaken BCE cell adhesions to beads to differing degrees.** Graph shows the percentages of cell-bound magnetic beads coated with anti-CA antibody that were dislodged by force (2 nN) application in cells expressing indicated integrin chimera deletion mutants (see Fig. S4).

Fig. S7. BCE cells transfected with the CA-LDL- $\beta 1\Delta 5$  deletion chimera still form focal adhesions when they bind anti-CA-coated beads. (A) Percentage of bound anti-CA antibody-coated beads displaying recruitment of paxillin in cells expressing CA-LDL- $\beta 1$  with the intact integrin cytoplasmic tail versus CA-LDL- $\beta 1\Delta 5$  that lacks the last 6 amino acids. (B) Representative fluorescence image demonstrating paxillin recruitment to focal adhesions adjacent to anti-CA antibody coated beads in cell expressing CA-LDL- $\beta 1\Delta 5$ . Fig. S8. BCE cells transfected with the CA-LDL- $\beta$ 1Δ5 deletion chimera fail to recruit CD98 to focal adhesions induced by binding to anti-CA-coated beads. Graph indicates the percent of cell-bound anti-CA antibody-coated beads that display recruitment of CD98 protein as determined by immunofluorescence in cells expressing CA-LDL- $\beta$ 1 versus CA-LDL- $\beta$ 1Δ5 (\*, p < 0.005).

**Fig. S9. CD98 is an integral focal adhesion protein.** (A) BCE cell binding to beads coated with anti-CD98 antibodies is sufficient to induce formation of focal adhesions containing paxillin and vinculin, as measured by immunofluorescence microscopy. (B) Representative graph of calcium influx in response to pulling on an anti-CD98 coated bead with 500 msec pulses of increasing force (850, 2000 pN) in BCE cell as indicated by measuring Fluo-4 signal intensity using microfluorimetry. (C,D) Quantitation (D) of Western blot (C) confirming CD98 protein levels were suppressed by more than 75% compared to those in wild type cells.

Fig. S10 Effect of siRNA treatment on TRPV4 mRNA and protein levels in BCE and HMVE cells. (A, B) RT-PCR analysis showing suppression of TRPV2 and TRPV4 mRNA in BCE (A) and HMVE cells (B) following treatment with respective siRNAs. (C-D) Western blots of proteins isolated from BCE (C) and HMVE cells (D) confirming that siRNA treatment knocked down TRPV4 protein levels by more than 60% compared to levels observed in control cells.

**Movie S1.** Ultra-rapid integrin-mediated mechanochemical conversion localized at the site of force application. Fluorescence time lapse images (captured every 70 ms, shown at 3X speed) of a BCE cell (from Fig. 1) loaded with the fluorescent calcium indicator Fluo-4 during application of four 500 msec force pulses of increasing magnitude (0.1, 0.45, 0.85 and 2 nN) spaced 2 seconds apart applied to BCE cells through beads coated with anti-β1 integrin antibody (12G10) using magnetic pulling cytometry. See Fig. 1C for phase contrast image of cell and position of bead. Note calcium influx is localized precisely to the site of force application at the bead-membrane interface. To best visualize the localized response, be sure to employ the "loop" function during Movie playback.

**Movie S2**. A pseudocolored ratiometric fluorescence time lapse of the same movie as shown in Movie S1 visualizing ultra-rapid integrin-mediated mechanochemical conversion localized to the site of force application. Fluorescence time lapse images (captured every 70 ms, shown at 3X speed) of a BCE cell (from Fig. 1) loaded with the fluorescent calcium indicator Fluo-4 during application of four 500 msec force pulses of increasing magnitude (0.1, 0.45, 0.85 and 2 nN) spaced 2 seconds apart applied to cell through bead coated with anti- $\beta$ 1 integrin antibody (12G10) using magnetic pulling cytometry. See Fig. 1C for phase contrast image of cell and position of bead. Calcium intensities are shown in colors, as indicated in the color bar. To best visualize the localized response, be sure to employ the "loop" function during Movie playback.

**Movies S3-S5.** Maps of cytoplasmic displacement caused by mechanical strain within focal adhesions as recorded in time lapse images of cells during exposure to 2 nN force pulses (500 ms) applied to cells using magnetic pulling cytometry with beads coated with anti- $\beta$ 1 integrin antibody (12G10) (**Movie S3**), HDL (**Movie S4**) or anti- $\beta$ 1 integrin antibody (12G10) in the presence of cytochalasin D (2 µg/ml) (**Movie S5**). Size and color of arrows are scaled with degree of local deformation relative to time 0, as indicated in color bar. Note intracellular deformation when force is applied to integrins that increases significantly in the presence of cytochalasin D, and that there is minimal intracellular displacement when force is applied to HDL receptors. To best visualize the localized response, be sure to employ the "loop" function during Movie playback.



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Time (msecs)



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Fig. S1 C



## Fig. S2



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# Fig. S3



Base

Apex











Α

α-CD98 Bead

С

Western

Fig. S9



RNAi

