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Piezo1, a mechanically activated ion channel, is required for vascular development in mice

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Mechanosensation is perhaps the last sensory modality not understood at the molecular level. Ion channels that sense mechanical force are postulated to play critical roles in a variety of biological processes including sensing touch/pain (somatosensation), sound (hearing), and shear stress (cardiovascular physiology); however, the identity of these ion channels has remained elusive. We previously identified Piezo1 and Piezo2 as mechanically activated cation channels that are expressed in many mechanosensitive cell types. Here, we show that Piezo1 is expressed in endothelial cells of developing blood vessels in mice. Piezo1-deficient embryos die at midgestation with defects in vascular remodeling, a process critically influenced by blood flow. We demonstrate that Piezo1 is activated by shear stress, the major type of mechanical force experienced by endothelial cells in response to blood flow. Furthermore, loss of Piezo1 in endothelial cells leads to deficits in stress fiber and cellular orientation in response to shear stress, linking Piezo1 mechanotransduction to regulation of cell morphology. These findings highlight an essential role of mammalian Piezo1 in vascular development during embryonic development.

Mechanotransduction, the conversion of physical forces into a biochemical response, is an essential signaling mechanism used by all organisms (1). Diverse physiological processes including hearing, touch, and blood pressure regulation require the ability of cells to sense and respond to mechanical stimuli (2–4). Impairment in mechanotransduction can lead to a wide range of pathologies including deafness and atherosclerosis (5, 6). The cellular response to mechanical force requires the coordinated action of multiple proteins; however, the identity of mechanotransducers in mammals remains poorly understood (7).

Classical studies from auditory sensory cells first implicated ion channels to be inherently mechanically activated (8). Since then, stretch-activated cation channels have been recorded from various tissues (9), but their identity has remained unknown (10, 11). For an ion channel to be considered a physiologically relevant transducer of mechanical stimuli, several criteria must be met (12). The ion channel must be expressed in the appropriate mechanosensitive cells, its expression should be required for mechanotransduction in an in vivo setting, and ideally the expression of the channel in a naive cell must render that cell mechanosensitive.

Progress toward identification of mechanically activated (MA) ion channels has been limited to a subset of ion channel families. In the invertebrates Caenorhabditis elegans and Drosophila melanogaster, members of the DEG/ENaC family and the TRP ion channel NOMPC have been conclusively shown to act as mechanotransduction ion channels (13–16). Mammals, however, do not have an ortholog of NOMPC, and the physiological relevance of the mammalian ENaC orthologs in mechanotransduction remains unclear. Many other TRP channels including TRPPs, TRPA1, and TRPV4 have been implicated in sensing mechanical stimuli; however, none has been shown to be required in vivo for sensing mechanical forces, nor to be necessary and sufficient for mechanotransduction (12). The two-pore potassium channels TREK-1, TREK-2, and TRAAK are mechanically activated in heterologous expression systems, and TREK-1 plays a role in regulating the mechanical sensitivity of dorsal root ganglia neurons (17). Overall, the identification of relatively few candidate genes has been a major impediment to studying mechanotransduction processes in vivo.

We recently identified Piezo proteins as pore-forming subunits of an evolutionarily conserved MA cation channel family (18). Mouse Piezo1 forms a homo-multimeric complex of ∼1.2 mDa, with each monomer containing over 35 transmembrane domains (19). Piezo1 is necessary for mechanically activated currents in the Neuro2A cell line and is sufficient for conferring stretch-activated currents in heterologous cell expression. Purified mouse Piezo1 retains channel activity when reconstituted without accessory subunits into artificial lipid bilayers. The single Piezo member in Drosophila plays a role in sensing noxious mechanical stimuli (20), and mouse Piezo2 has been shown to be critically required for mechanotransduction in mammalian Merkel cells (21–23). Gain-of-function PIEZO1 mutations have recently been shown to cause hereditary xerocytosis in humans (24–26); however, it is still unclear whether the mammalian Piezo1 plays a role in mechanotransduction.
in vivo. Here we used a genetic strategy to ablate the function of Piezo1 in vivo and show that Piezo1 is a critical component of endothelial cell mechanotransduction.

**Results**

**Piezo1**

To characterize the physiological function of Piezo1, we used a gene trap transgenic line in which a promoter-less β-geo (β-gal and neomycin phosphotransferase) cassette had been randomly integrated in the intron between exons 18 and 19 of the mouse Piezo1 locus (Fig. L4). The gene trap cassette contains a splice acceptor that co-opts the endogenous splicing of the Piezo1 transcript, leading to an in-frame fusion protein containing the first 823 residues of Piezo1 and β-gal, a predicted nonfunctional protein. This gene trap allele, Piezo1<sup>gt</sup>, also provides two reporters of Piezo1 expression: β-gal fused to Piezo1 and human placental alkaline phosphatase (PLAP) expressed through an internal ribosome entry site (IRES) (27).

We confirmed proper splicing of the Piezo1<sup>gt</sup> allele by performing RT-PCR with primers targeting the Piezo1 transcript surrounding the integration site and the start of the gene trap cassette (Fig. S1A). We then cloned and expressed the entire Piezo1<sup>gt</sup> transcript in human embryonic kidney (HEK293T) cells (Fig. S1B) and found no cellular toxicity in three different assays (Fig. S1C). Furthermore, we showed that an N-terminal fragment of Piezo1, Piezo1 (1–823), fused to eGFP did not lead to any MA currents (Fig. S1D). These results indicate that Piezo1<sup>gt</sup> would lead to loss of Piezo1 ion channel activity while serving as a reporter of endogenous Piezo1 expression.

**Piezo1**<sup>gt</sup> mice were viable and fertile, but intercrossing Piezo1<sup>gt</sup> mice generated no viable homozygous offspring (Fig. 1B). Quantitative PCR (qPCR) analysis using a probe downstream of the gene trap integration site confirmed loss of wild-type Piezo1 transcript (Fig. 1C). Piezo1<sup>gt</sup> embryos seemed normal at embryonic day (E) 9, showing a regular heartbeat and blood flow in central vessels, and, at E9.5 major blood vessels such as the dorsal aorta and cardinal veins were present in Piezo1<sup>gt</sup> embryos, suggesting that the initial stages of vasculogenesis occurred normally in the absence of Piezo1 function (Fig. S2A). However, starting from E9.5, mutant embryos were growth-retarded (Fig. 1D and E), and many exhibited pericardial effusion at E10.5, indicative of poor cardiovascular function (28). A majority of Piezo1<sup>gt</sup> embryos died at midgestation before E14.5 (Fig. 1B).

**Piezo1 Expression in Embryonic Endothelial Cells.** Embryonic lethality beginning at E9.5 coincides with the development of the cardiovascular system, whose function is required for the survival of the embryo (28). We investigated where Piezo1 is expressed at this time. Piezo1 in situ hybridization probes, an antibody that we had previously generated, as well as commercially available antibodies were not sensitive enough to detect expression of endogenous Piezo1 (18, 29). We therefore used the Piezo1 promoter-driven β-gal and PLAP reporter genes to analyze the expression pattern of Piezo1. Weak β-gal activity was initially detected at E9 (Fig. S2F), when regular heartbeats and blood flow are first established (29). Between E9 and E10.5, β-gal activity increased and was restricted to the developing cardiovascular system, in particular to the vascular endothelium and the inner endocardium surrounding the heart lumen (Fig. 2A). Staining of PLAP, a GPI-linked cell surface marker, and platelet/endothelial cell adhesion molecule 1 (PECAM1), an endothelial marker, also confirmed Piezo1 expression in the vascular endothelium (Fig. 2B). In the heart, the highest level of X-gal staining at E11.5 was observed in the endocardium lining ventricular outflow tract and atrioventricular canal (Fig. 2C), areas predicted to experience strong fluid shear forces generated by blood flow (30, 31).

To provide an even more sensitive method to detect Piezo1 expression, we designed a gene-targeting strategy that would lead to a C-term fusion protein of Piezo1 with the fluorescent reporter tdTomato (Fig. S3A). Germ-line transmission and successful deletion of the neomycin cassette led to mice that expressed the fluorescent reporter allele Piezo1<sup>P1-tdT</sup> (Fig. S3B). RT-PCR analysis of Piezo1<sup>P1-tdT</sup> mice confirmed the presence of a fusion allele with the expected size (Fig. S3C). Western blot analysis confirmed the presence of a Piezo1 fusion protein in Piezo1<sup>P1-tdT</sup> mice (Fig. S3D). These mice were viable and expressed Piezo1 in levels similar to Piezo1<sup>gt</sup> controls (Fig. S3E).

We then used flow cytometry to detect the epifluorescence of tdTomato with single-cell resolution. We show that PECAM1<sup>+</sup> endothelial cells in the embryonic heart and yolk sac of midgestation Piezo1<sup>P1-tdT</sup> embryos display a prominent shift in fluorescence peak compared with Piezo1<sup>gt</sup> littermates, indicating expression of the Piezo1–tdTomato fusion allele (Fig. 2D). We further analyzed cells within the yolk sac that were PECAM1<sup>+</sup> as a control (Fig. 2E). The majority of yolk sac-derived nonendothelial cells did not show fluorescence above background, confirming our lacZ staining results that Piezo1 is preferentially expressed in endothelial cells. Interestingly, expression of Piezo1 persists in endothelial cells in adult heart, lung, and aorta, as well as in the human umbilical vein endothelial cells (HUVECs) (Fig. S4 A and B).

**Vascular Impairments in Piezo1<sup>gt</sup> Embryos.** Blood flow in mouse embryos begins at E8, and hemodynamic forces from blood flow have been shown to be a critical factor to the development of the
distribution of small- and large-diameter vessels, whereas Piezo1+/gyolk sacs lack major vessels (Fig. 3 C and D). Vascular remodeling in the Piezo1+/gyo yolk sacs also lishes major vessels compared with Piezo1+/gyo littermate controls (Fig. 5B). Histological analysis of the heart at E8.5 did not reveal obvious morphological differences between Piezo1+/gyo embryos and littermate controls (Fig. 5C). These data indicate a role for Piezo1 in the remodeling of the embryonic vasculature and suggest that a loss of functional Piezo1 leads to an impairment of mechanotransduction processes in vivo.

**Piezo1 Is Activated by Shear Stress.** We have previously shown that Piezo1 is activated by two types of distinct mechanical stimulations: by the indentation of a cell with a glass probe and by stretching the cell membrane (18). Although membrane stretch can affect endothelial cells, fluid shear stress represents the most relevant force experienced by endothelial cells (35, 36). We tested shear stress activation of Piezo1 using electrophysiology. We used a perfusion tube and subjected cells to laminar flow shear stress by applying 600-ms pulses of solution flow directed on to the recording cell (Fig. 4A). Whole-cell inward currents (Imax, <533 ± 64 pA) could be recorded from Piezo1-IRES-eGFP transfected HER293T cells when stimulated by shear stress in the intensity range of 52 ± 3-64 ± 2 dynes/cm², but not from IRES-eGFP transfected cells (Imax, <8.7 ± 1.3 pA) even up to 70 dynes/cm² (Fig. 4B). The pressure applied to the solution was correlated to shear stress by using the particle image velocimetry method to estimate the shear stress intensity at the cell surface (37). For pressure pulses ranging from 10 mmHg to 60 mmHg, the average bead velocity ranged from 6.9 ± 0.6 μm/ms to 21.0 ± 1.3 μm/ms, and the estimated wall shear stress intensity ranged from 23.3 ± 2.2 dynes/cm² to 70.2 ± 4.2 dynes/cm² (Fig. 4C). The half-maximal activation intensity for Piezo1 was measured to be 57 ± 3 dynes/cm² (Fig. 4D). Similar to MA currents, shear stress-activated Piezo1 channel currents inactivate in continued presence of stimulus, but with a slower time constant.

refined vascular network (32, 33). The initial stage of vascular remodeling relies entirely on endothelial cells because mural cells consisting of smooth muscle cells and pericytes present in mature vessels are not recruited until after the primary vessel structure has been established (34). Having shown that Piezo1 is expressed in embryonic endothelial cells, we characterized the morphology of the developing vascular system of Piezo1+/gyo mice.

We performed H&E staining and PECAM1 immunostaining to observe vessel morphology in the yolk sac and the embryo proper from E8.5 to E10.5, when vascular remodeling and network formation occur. PECAM1 staining of E8.5 yolk sac shows that the organization of the early vascular plexus is significantly altered in Piezo1+/gyo yolk sacs (Fig. 3 A and B). The layer of endothelial cells that cover the mesodermal cells of the yolk sac is wider in Piezo1+/gyo embryos, and the vasculature is delayed in its organization of major vessels. By E9.5, the yolk sac vasculature is less defined and the number of major vessels is fewer relative to littermate controls (Fig. S5A). H&E staining of a cross-section of the E9.5 yolk sac shows that wild-type yolk sacs have a wide

![Fig. 2.](image1)

**Fig. 2.** Piezo1 is expressed in embryonic vascular endothelial cells. (A) Representative images of X-gal-stained whole-mount Piezo1+/+ and Piezo1−/− embryos at E9.5 and E10.5. (B) Representative image of E9.5 whole-mount Piezo1+/+ and Piezo1−/− embryos stained for PLAP and PECAM1 and magnification image of embryonic head showing expression of PLAP and PECAM1 from the same embryos. (C) Representative images of X-gal-stained E11.5 whole-mount hearts and sections of Piezo1−/− embryos. (D) Representative histograms of tdTomato fluorescence in PECAM1+ cells from Piezo1+/+ (gray shaded) or Piezo1−/− (red line) embryos. E13.5 embryos and E11.5 embryos were used for heart and yolk sac analysis, respectively, owing to technical consideration for tissue preparation. (E) Histograms of tdTomato fluorescence in PECAM1+ cells from embryos in D. Most PECAM1+ cells from Piezo1−/− yolk sac do not express Piezo1; some PECAM1+ cells in Piezo1−/− yolk sac also express Piezo1-tdTomato. Stainings in A-C were performed in at least four embryos per genotype per time point. Histograms in E and E are representative of at least two embryos per genotype. [Scale bars: 400 μm (A), 500 μm and 200 μm, respectively (B), and 200 μm (C)].
Piezo1 is activated by fluid shear stress. (A) Pictorial representation of perfusion tube setup. Whole-cell currents were recorded from HEK293T cells while the cell was stimulated by shear stress induced by directing pulses of fluid flow through a perfusion tube placed relative to the cell. The flow rate is regulated by varying the pressure applied to the fluid inside the tube. (B) Representative traces of whole-cell currents recorded at –80 mV from HEK293T cells expressing mPiezo1 (Left) or IRES-eGFP (Right) in response to 600-ms pulses of increasing laminar shear stress. The stimulus trace above the current indicates the pressure pulse applied to the solution in the perfusion tube (which was later back-calculated to the applied shear stress intensities) and corresponds to the whole-cell current recorded for that stimulus. (Inset) Shear stress-induced average maximum whole-cell peak currents from mPiezo1 (n = 8) and IRES-eGFP (n = 6) transfected cells. Bars indicate mean ± SEM. (C) Pressure–wall shear stress relationship. Particle image velocimetry was used to estimate the wall shear stress intensity at the indicated tube velocity. Each data point is the mean ± SEM from values of five or six independent trials. The measurement was done for the range of pressure (0–60 mmHg) used to activate the cells during the experiment. (D) Wall shear stress to normalized peak current relationship plotted for six individual cells expressing mPiezo1, depicting range of activation. For each cell, currents were normalized to the peak current recorded at maximum shear stress, before losing the seal. The stimulus–response curve was fit with a Boltzmann function to determine the half-maximal response.

Fig. 4. Piezo1 is activated by fluid shear stress. (A) Pictorial representation of perfusion tube setup. Whole-cell currents were recorded from HEK293T cells while the cell was stimulated by shear stress induced by directing pulses of fluid flow through a perfusion tube placed relative to the cell. The flow rate is regulated by varying the pressure applied to the fluid inside the tube. (B) Representative traces of whole-cell currents recorded at –80 mV from HEK293T cells expressing mPiezo1 (Left) or IRES-eGFP (Right) in response to 600-ms pulses of increasing laminar shear stress. The stimulus trace above the current indicates the pressure pulse applied to the solution in the perfusion tube (which was later back-calculated to the applied shear stress intensities) and corresponds to the whole-cell current recorded for that stimulus. (Inset) Shear stress-induced average maximum whole-cell peak currents from mPiezo1 (n = 8) and IRES-eGFP (n = 6) transfected cells. Bars indicate mean ± SEM. (C) Pressure–wall shear stress relationship. Particle image velocimetry was used to estimate the wall shear stress intensity at the indicated tube velocity. Each data point is the mean ± SEM from values of five or six independent trials. The measurement was done for the range of pressure (0–60 mmHg) used to activate the cells during the experiment. (D) Wall shear stress to normalized peak current relationship plotted for six individual cells expressing mPiezo1, depicting range of activation. For each cell, currents were normalized to the peak current recorded at maximum shear stress, before losing the seal. The stimulus–response curve was fit with a Boltzmann function to determine the half-maximal response.

Piezo1 Is Involved in Endothelial Cell Alignment Under Flow. The ability of endothelial cells to align in the direction of fluid flow is one of the best-studied cellular consequences of mechanotransduction (39). To determine whether loss of PIEZO1 leads to defects in morphology of vascular endothelial cells and its responsiveness to shear flow, we compared the LS-induced remodeling in HUVECs transfected with siRNA for PIEZO1 and scrambled siRNA. HUVECs were subjected to shear stress (12 dynes/cm²) for 24 h and examined for changes in cellular morphology. qPCR analysis revealed ~80% knockdown of PIEZO1 transcript in PIEZO1 siRNA cells compared with scrambled siRNA (Fig. S6A). The relatively long duration and the level of shear stress were chosen based on previous studies on shear-induced remodeling of HUVECs (40).

Stress fibers visualized by F-actin immunostaining and their orientation angles show impaired alignment in PIEZO1 siRNA cells compared with scrambled siRNA (Fig. 5A). For HUVECs transfected with scrambled siRNA (Fig. 5B and C, Left), neither the stress fibers nor the cells showed any preferential orientation under static condition (ST), but both showed dominant orientations parallel to the flow direction (0°) under LS, with 41 ± 0.58% of the stress fibers and 54 ± 0.38% of the cells having orientations within the range of ±15°. Transfection of HUVECs with siRNA for PIEZO1 (Fig. 5B and C, Right) caused significant decreases in the LS-induced orientations of stress fibers (25 ± 0.71%) and cells (31 ± 1.30%) in comparison with scrambled siRNA under LS. Morphological determination of cell elongation in scrambled siRNA cells showed that LS caused an increase in aspect ratio to 3.3 ± 0.07 from the ST value of 2.0 ± 0.04 (Fig. S6B, Left). Cells transfected with PIEZO1 siRNA showed a reduction in the LS-induced elongation, with an aspect ratio (2.6 ± 0.08) significantly lower than that with scrambled siRNA, whereas the ST value of 2.1 ± 0.04 is not significantly different (Fig. S6B, Right). It is worth noting that we observe a role for Piezo1 in alignment and elongation in response to lower forces of shear stress compared with forces needed to activate Piezo1 in studies of electrophysiology. This is not surprising, because the 24-h time frame of the experiments involving altered cell morphology allows accumulation of low-level cation influx. These observations indicate that knocking down PIEZO1 results in significant decreases in the mechanosensitivity of the endothelial cells in terms of alignment and elongation in response to LS. However, the dependence on PIEZO1 is partial, and it is likely that other factors also contribute to the shear stress-dependent alignment and elongation.

Discussion

Vascular development and cardiogenesis in the mouse embryo is influenced by hemodynamic forces owing to blood flow. For example, vascular remodeling in the yolk sac is compromised when cardiac function/output is reduced or absent (41). Surgical ligation of the left arch arteries in mouse embryos prevents normal blood flow and leads to abnormal regression and an accompanying enlargement of the right arteries (42). In addition, local hemodynamic shear stress forces imparted on vascular endothelial cells of developing blood vessels are necessary and sufficient to induce remodeling in the mouse yolk sac (43). These data suggest that disruption of mechanotransduction in the cardiovascular system during the time when blood flow initiates around E8–E9 is expected to cause a defect in vascular development.

The direct effect of hemodynamic force on endothelial cells during vascular remodeling is not well understood. In response to fluid shear stress in vitro, endothelial cells change their gene expression profiles and can realign their cytoskeletal structure (35). Putative mechanotransducers have been identified from cultured endothelial cells or in the mature vasculature, including ion channels (44, 45), the apical glycocalyx (46), cytoskeleton (47), primary cilia (48), and a protein complex comprising PECAM1, vascular endothelial cadherin, and vascular endothelial growth factor receptor 2 (VEGFR2) (49). Ion channels such as TREK-1 have been shown to be activated by stretch and shear stress (50). However, the identity of the shear stress sensor or sensors during embryogenesis has been unknown. Our data reveal that Piezo1 is expressed in embryonic endothelial cells and that Piezo1 deficiency leads to disruption of vascular development in the mouse embryo and yolk sac. In addition, our results provide evidence that Piezo1 is a sensor of shear stress, and that endothelial cell alignment in response to shear stress is
compromised in the absence of Piezo1. It is possible that incomplete endothelial cell alignment in response to shear stress during remodeling of arteries leads to the vascular remodeling defect observed in Piezo1-deficient embryos. Alternatively, Piezo1-dependent calcium influx could modulate multiple downstream signaling pathways and influence vascular remodeling through pleiotropic mechanisms. Future studies with conditional knockouts will address whether endothelium and/or other cell types are dependent on Piezo1-signaling in the embryo and in the mature cardiovascular system.

Materials and Methods

All animal procedures were approved by The Scripps Research Institute's Institutional Animal Care and Use Committee. Detailed methods are provided in SI Materials and Methods.

X-gal and PLAP Stainings. Stainings were performed following standard procedures. For X-gal staining, embryos were washed in X-gal washing buffer (PBS with 2 mM MgCl₂, 0.02% Nonidet P-40, and 0.01% sodium deoxycholate), incubated in X-gal staining buffer (X-gal washing buffer with 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 1 mg/mL X-gal) overnight with gentle shaking at 37 °C, and then postfixed in neutral buffer. Whole-mount embryos and tissues labeled with X-gal were cleared in a 1:1 mixture of benzyl alcohol:benzyl benzoate clearing and imaged under Nikon SMZ1500 stereomicroscope. Images were obtained under Olympus AX70 microscope.

For PLAP staining, fixed embryos were heat-inactivated in PBS for 1.5 h at 65 °C and washed in buffer B1 [0.15 M NaCl and 0.1 M Tris-HCl (pH 7.5)] and buffer B3 [0.1 M NaCl, 50 mM MgCl₂, and 0.1 M Tris HCl (pH 9.5)] sequentially. Embryos were then incubated in NBT/BCIP solution (Roche) at room temperature overnight and postfixed. Images were obtained under Nikon SMZ1500 stereomicroscope.

Flow Cytometry Analysis. Hearts and yolk sacs were isolated from embryos generated from timed matings between Piezo1<sup>−/−</sup>tdT/+ mice. Yolk sacs and hearts were minced and incubated with 0.5 mg/mL collagenase type IV, 0.1 mg/mL DNaseI, and 1 mg/mL trypsin inhibitor at 37 °C for 30 min, being pipetted repeatedly several times throughout the incubation. The samples were washed with FACS buffer (PBS containing 2% (vol/vol) FBS and 2 mM EDTA) and centrifuged at 500 × g for 5 min. Erythrocytes were then lysed using a multispecies RBC lysis buffer (eBioscience) for 5 min at room temperature, then washed again with FACS buffer. The cells were then resuspended in FACS buffer, filtered through 100-μm-diameter nylon mesh, and incubated with FcBlock (BD) for 5 min at room temperature. They were then stained with an APC anti-PECAM1 antibody (BD) for 15 min at 4 °C in the dark. The cells were washed once then resuspended in FACS buffer for data acquisition using a BD LSRII flow cytometer. Data were analyzed using FlowJo (TreeStar).

LS Stimulation. Shear stress was applied using a perfusion tube (borosilicate glass capillary, pulled to an inner diameter 17 μm at the mouth) that was filled with extracellular solution (same as bath solution) and was positioned at an angle of 30° (sensapex SMX-series micromanipulation system; Warner Instruments) relative to the cell. The micromanipulator was used to control the position of the perfusion tube and maintain consistency between each trial. A Clampex 10.2 controlled pressure clamp HSPC-1 devise (ALA Scientific) was connected to the perfusion tube and was used to apply pressure on the solution to generate 600-ms pulses of flow. Starting from 0 mmHg, increasing increments of 20 mmHg pressure was applied every 20 s.

Fig. 5. Knockdown of PIEZO1 leads to defects in cellular alignment upon laminar shear stress. (A) F-actin images stained with TRITC-phalloidin (Upper) and angles of stress fiber orientation (Lower). Angle measurements were assigned a color code (right) where 0° is parallel to the flow chamber and −90° and 90° are perpendicular. (B) Histograms of stress fiber orientation of cells. (C) Histograms of cell orientation. The mean ± SEM values refer to percentage of orientations between ±15° for three independent biological experiments. Total numbers of cells analyzed are 348 (ST) and 236 (LS) for scrambled siRNA and 290 (ST) and 170 (LS) for PIEZO1 siRNA. ST, static; LS, laminar shear for 24 h. Asterisk indicates significant difference by Watson’s U2 test.
to raise the flow rate with each progressive pulse. Once a whole-cell inward current response was detected at a certain pressure level, the increments were reduced to 2 mmHg and the protocol was run again, starting from the lowest stimulus that elicited the initial response, to obtain a more accurate and sensitive stimulus–response curve for each cell. This data were then used to obtain activation range and half-maximal activation values.

Laminar Flow Experiments for Cellular Alignment. HUVECs were seeded on glass slides coated with 50 μg/mL collagen-1 1 d before the experiment. The slide was assembled into the flow chamber and exposed to laminar shear at a wall shear stress of 12 dynes/cm². The cells obtained under static condition were used as concurrent control groups. During the flow experiment, the system was kept at 37 °C and pH 7.5.

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