

Figure S1: Piezo1 distribution in mouse primary airway smooth muscle cells (pASMCs) from spontaneous breathing (Control) or mechanical ventilation (MV). **(A)** Representative images of Piezo1 expression and distribution in primary airway smooth muscle cells (pASMCs) isolated from mice under either Control or MV at high vital volume (V_T , 18 ml/kg, 3 h), as evaluated with immunofluorescence assay. Bar = 20 μ M. **(B)** The fluorescence intensity analysis of Piezo1 in pASMCs with ImageJ. **(C)** The ratio of cytosol intensity (left panel) or nucleus intensity (right panel) to cell membrane intensity. $n = 3$. * $P < 0.05$; ** $P < 0.01$; n.s., not significant.

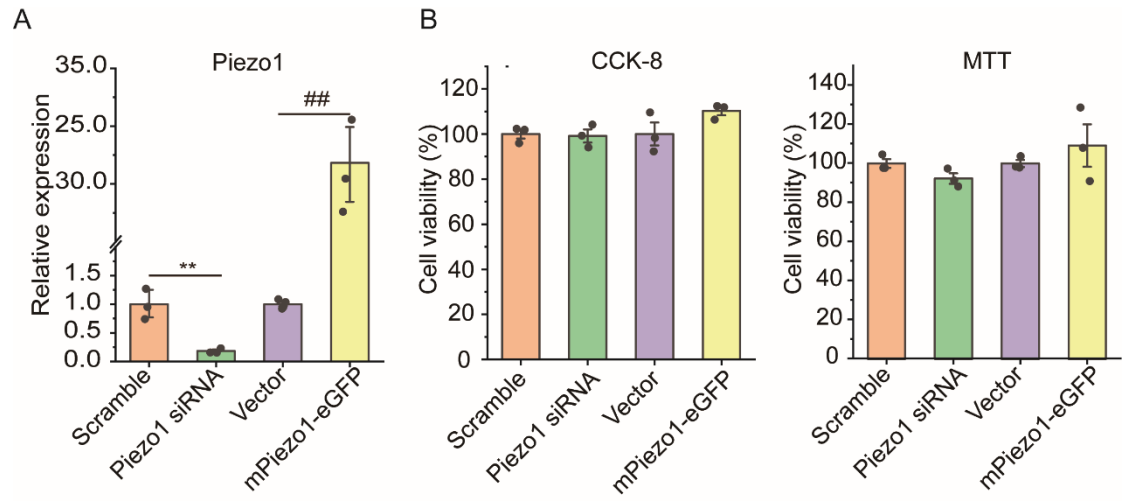


Figure S2: Piezo1 mRNA expression quantified by quantitative PCR (qPCR) (A) and cellular activity evaluated by CCK-8 or MTT assay (B) in cell line mouse ASMCs (mASMCs) transfected with either Piezo1 siRNA, mPiezo1-eGFP, Scramble, or Vector. $n = 3$. ** $P < 0.05$; ## $P < 0.01$.

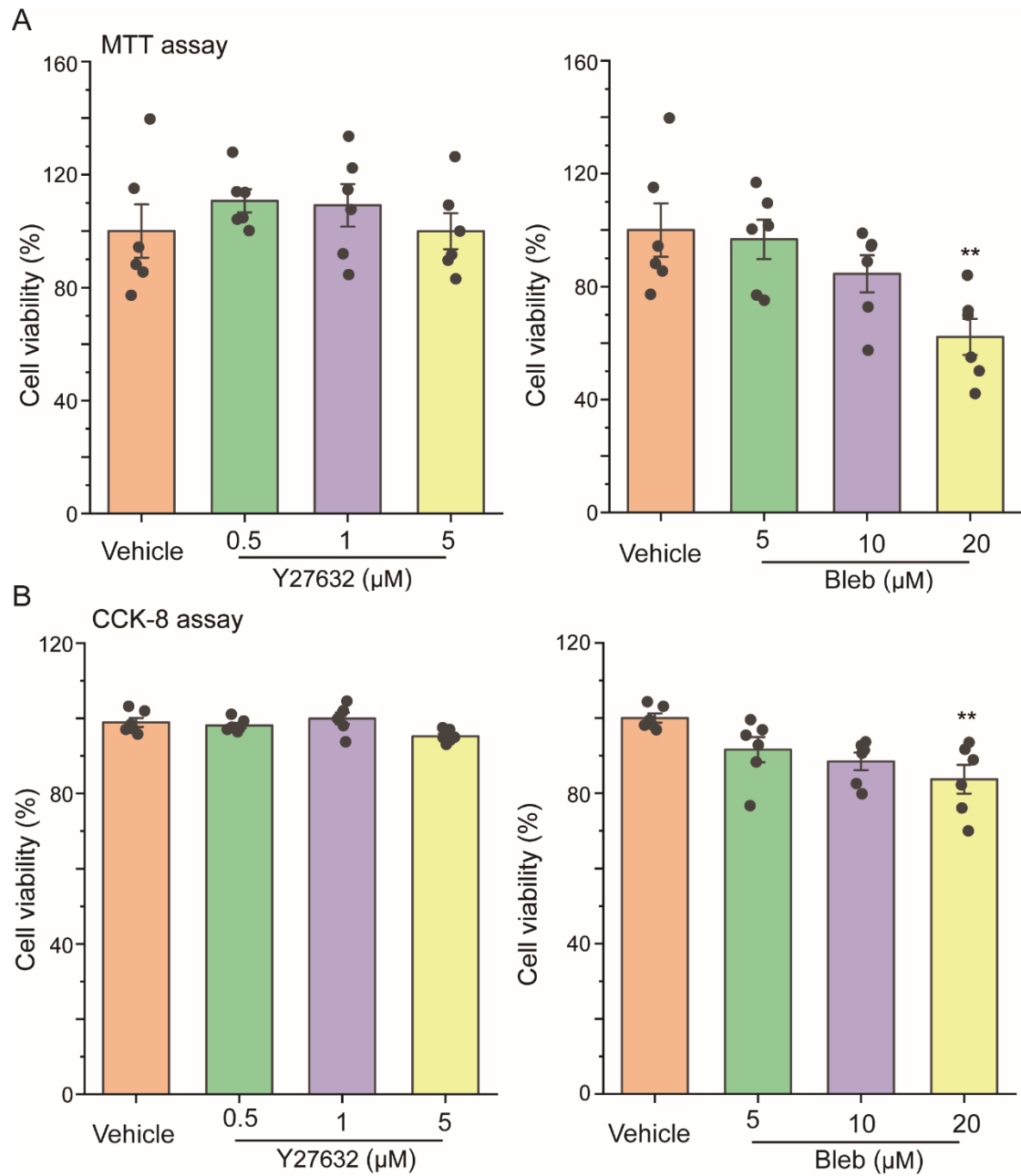


Figure S3: The effect of ROCK signaling inhibitor Y27632 (0.5, 1, and 5 μM , dissolved with DMSO) and myosin II inhibitor blebbistatin (Bleb, 5, 10, and 20 μM , dissolved with DMSO) on the cellular activity of mASMCs evaluated with MTT (**A**) and CCK-8 (**B**). $n = 6$. ** $P < 0.01$.

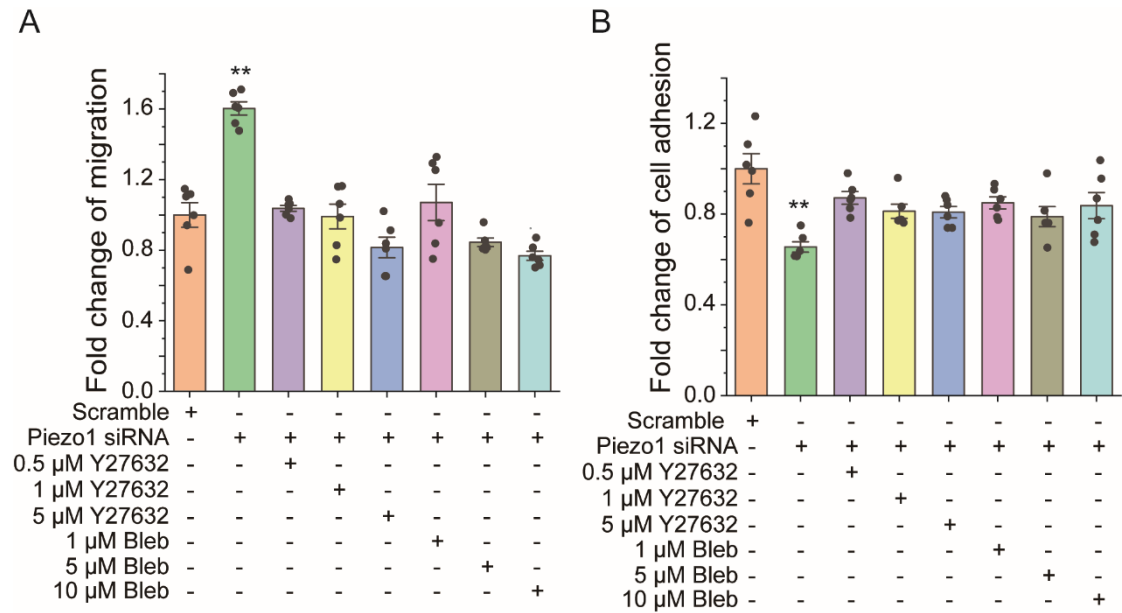


Figure S4: The effect of ROCK signaling inhibitor Y27632 (0.5, 1, and 5 μM, dissolved with DMSO) and myosin II inhibitor blebbistatin (Bleb, 1, 5, and 10 μM, dissolved with DMSO) on the loss of Piezo1-enhanced cell migration and -decreased cell adhesion evaluated with transwell assay (A) and cell adhesion assay (B). n = 6. ** $P < 0.01$.

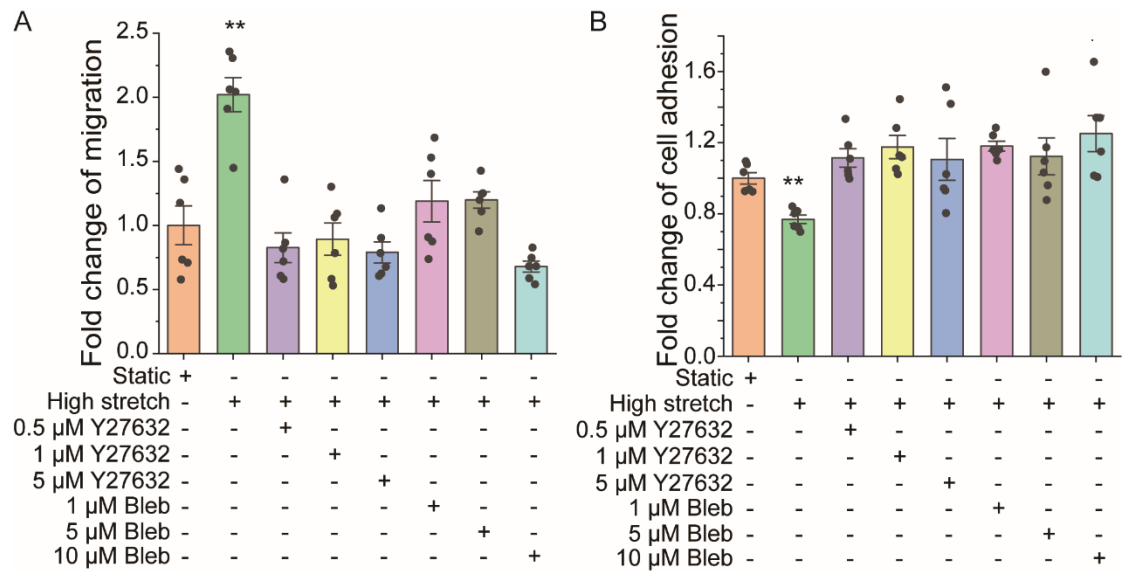


Figure S5: The effect of ROCK signaling inhibitor Y27632 (0.5, 1, and 5 μM, dissolved with DMSO) and myosin II inhibitor blebbistatin (Bleb, 1, 5, 10 μM, dissolved with DMSO) on high stretch-enhanced cell migration and -decreased cell adhesion evaluated with transwell assay (A) and cell adhesion assay (B). n = 6. ** $P < 0.01$.

Table S1: Primer sequence of mRNA for mouse airway smooth muscle cells

No.	Genes	Forward primer	Reverse primer
1	GADPH	AGGTCGGTGTGAACGGATT TG	GGGGTCGTTGATGGCAACA
2	Integrin α V	CGGGTCCCGAGGGAAGTTA	TGGATGAGCATTACATTTG AGA
3	Integrin β 1	TGGTCAGCAACGCATATCT GG	GATCCACAAACCGCAACCT
4	Piezo1	TCATCATCCTTAACCACATG GTG	TGAAGACGATAGCTGTCAT CCA
7	Piezo1 siRNA	CCG GCAUCUACGUCAAUATT	UAUUUGACGUAGAUGCCG GUG
8	Piezo1 scramble controls	UUCUCCGAACGUGUCACG UTT;	ACGUGACACGUUCGGAGA ATT