Table S1: Primer and siRNA sequences

Gene	Sequence (5'-3')	
Bax	F: CCCGAGAGGTCTTTTTCCGAG	R: CCAGCCCATGATGGTTCTGAT
Bcl2	F: GGTGGGGTCATGTGTGTGG	R: CGGTTCAGGTACTCAGTCATCC
GXP4	F: GAGGCAAGACCGAAGTAAACTAC	R: CCGAACTGGTTACACGGGAA
HO-1	F: AAGACTGCGTTCCTGCTCAAC	R: AAAGCCCTACAGCAACTGTCG
Nrf2	F: GGTTGGCCCTTTCCTGCTTTATA	R: CCCTTGACAGCACAGAAGAGAAT
Piezo1	F: ACTCCGCACTCATCAAGTGG	R: CAGCAGGAGAAAGTCGCTGA
HLA-A	F: TTCTGGAGAGGAGCAGAGATACA	R: CTATCTGAGCTCTTCCTCCTCCA
β-actin	F: CATGTACGTTGCTATCCAGGC	R: CTCCTTAATGTCACGCACGAT
Piezo1-siRNA	S: CCAAGUACUGGAUCUAUGU	AS: ACAUAGAUCCAGUACUUGG

F: Forward; R: reverse; S: sense; AS: antisense

Figure S1

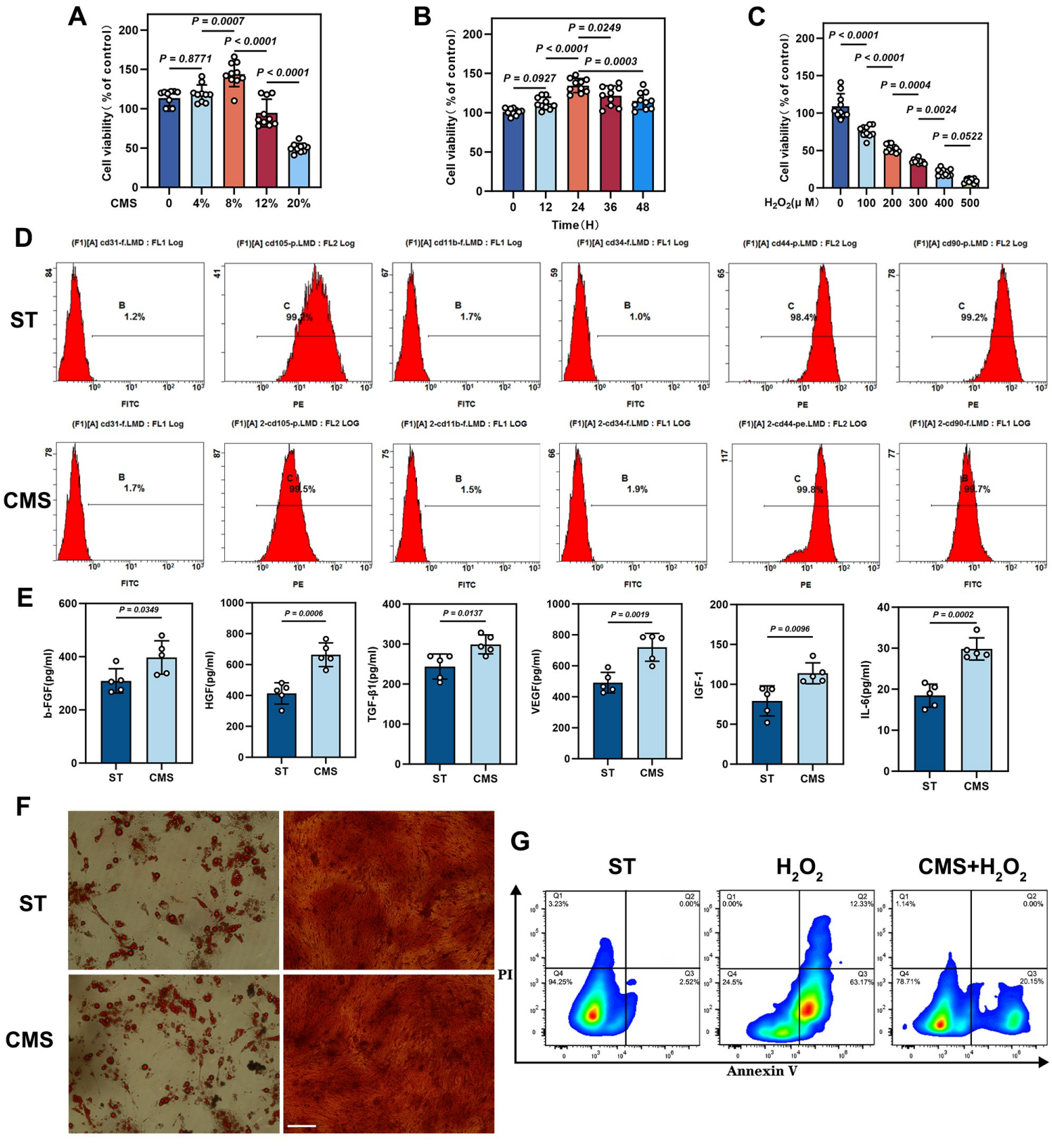
A
Cell viability(% of control)
P = 0.8771
P = 0.0007
P < 0.0001
P < 0.0001
CMS 0 4% 8% 12% 20%
B
Cell viability(% of control)
P = 0.0927
P < 0.0001
P = 0.0249
P = 0.0003
0 12 24 36 48
Time (H)
C
Cell viability(% of control)
P < 0.0001
P < 0.0001
P = 0.0004
P = 0.0024
P = 0.0522
H2O2(μ M) 0 100 200 300 400 500
D
ST
(F1)[A] cd31-f.LMD : FL1 Log
(F1)[A] cd105-p.LMD : FL2 Log
(F1)[A] cd11b-f.LMD : FL1 Log
(F1)[A] cd34-f.LMD : FL1 Log
(F1)[A] cd44-p.LMD : FL2 Log
(F1)[A] cd90-p.LMD : FL2 Log
B 1.2%
C 99.2%
B 1.7%
B 1.0%
C 98.4%
C 99.2%
FITC
PE
CMS
(F1)[A] cd31-f.LMD : FL1 Log
(F1)[A] 2-cd105-p.LMD : FL2 LOG
(F1)[A] 2-cd11b-f.LMD : FL1 LOG
(F1)[A] 2-cd34-f.LMD : FL1 LOG
(F1)[A] 2-cd44-pe.LMD : FL2 LOG
(F1)[A] 2-cd90-f.LMD : FL1 LOG
B 1.7%
C 99.5%
B 1.5%
B 1.9%
C 99.8%
B 99.7%
E
b-FGF(pg/ml)
P = 0.0349
HGF(pg/ml)
P = 0.0006
TGF-β1(pg/ml)
P = 0.0137
VEGF(pg/ml)
P = 0.0019
IGF-1
P = 0.0096
IL-6(pg/ml)
P = 0.0002
ST CMS
F
ST
CMS
G
ST
H2O2
CMS+H2O2
PI
Annexin V
Q1 3.23% Q2 0.00% Q4 94.25% Q3 2.52%
Q1 0.00% Q2 12.33% Q4 24.5% Q3 63.17%
Q1 1.14% Q2 0.00% Q4 78.71% Q3 20.15%

Fig. S1. Effects of Cyclic Mechanical Stretch (CMS) and oxidative stress on viability, phenotype, paracrine function, and apoptosis of adipose-derived stem cells (ADSCs).

(A) Cell Counting Kit-8 (CCK-8) proliferation assay of ADSCs subjected to varying mechanical strain amplitudes (0%, 4%, 8%, 12%, 20%) (n=10). (B) CCK-8 assay assessing ADSC proliferation after CMS for different durations (12 h, 24 h, 36 h, 48 h) (n=10). (C) H_2O_2 dose-response curve (0, 100, 200, 300, 400, 500 μM; 24 h) showing ADSC viability assessed via CCK-8 assay (n=10). (D) Flow cytometry identification of surface markers for ADSCs cultured under static conditions (ST) and after CMS. (E) Comparison of paracrine secretion profiles of ADSCs under CMS or ST conditions (n=5). (F) Representative images comparing adipogenic and osteogenic differentiation potential of ADSCs under CMS or ST conditions (Scale bar = 275 μm). (G) Apoptosis analysis of ADSCs assessed by flow cytometry using Annexin V/PI staining. (Data are presented as mean ± standard deviation).

Figure S2

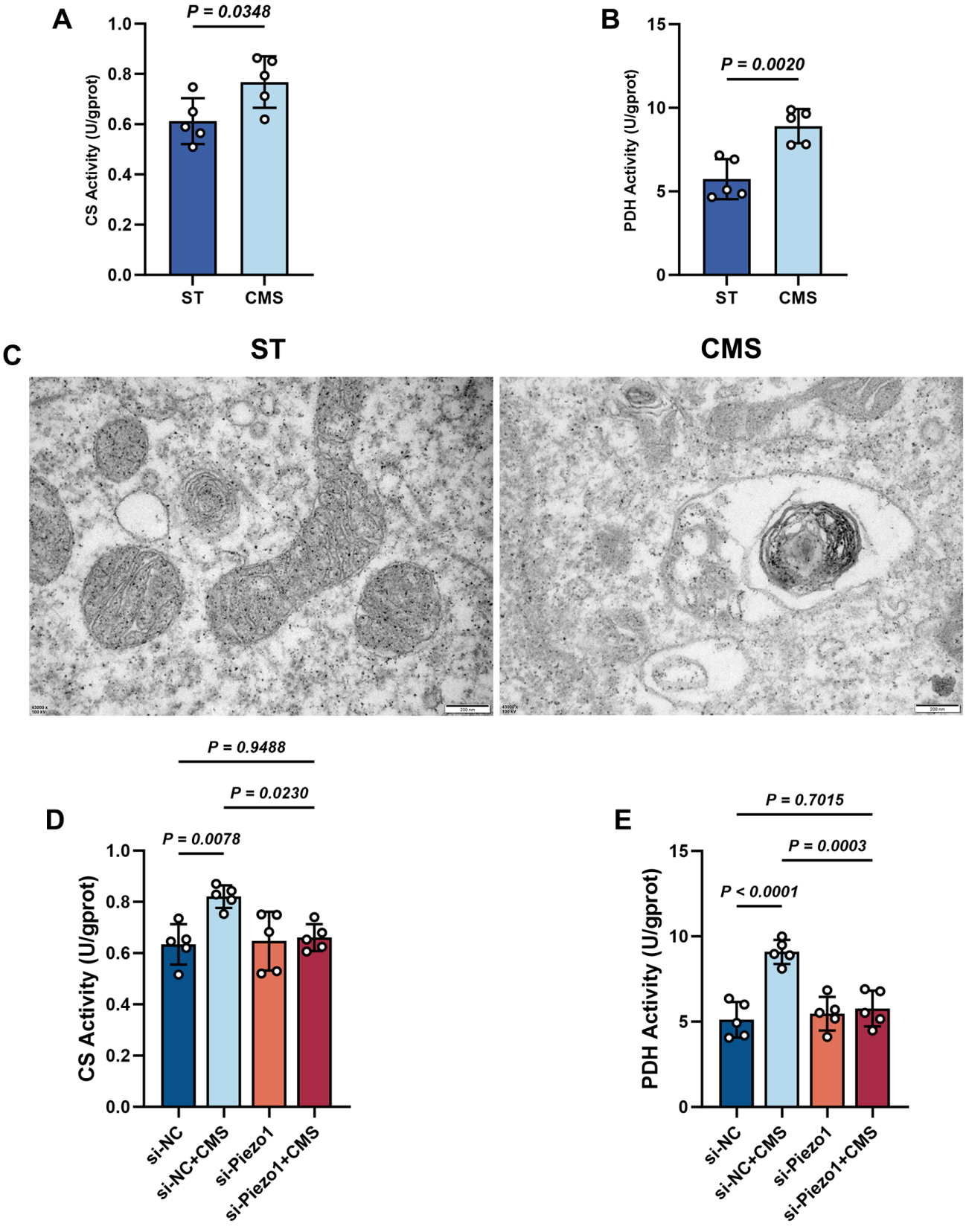
A
P = 0.0348
CS Activity (U/gprot)
1.0
0.8
0.6
0.4
0.2
0.0
ST
CMS
B
P = 0.0020
PDH Activity (U/gprot)
15
10
5
0
ST
CMS
C
ST
CMS
D
P = 0.9488
P = 0.0230
P = 0.0078
CS Activity (U/gprot)
si-NC
si-NC+CMS
si-Piezo1
si-Piezo1+CMS
E
P = 0.7015
P = 0.0003
P < 0.0001
PDH Activity (U/gprot)
si-NC
si-NC+CMS
si-Piezo1
si-Piezo1+CMS

Fig. S2. Effects of CMS and Piezo1 Knockdown on Citrate Synthase (CS) and Pyruvate Dehydrogenase (PDH) Levels in ADSCs

(A-B) CS and PDH levels in ADSCs cultured under CMS versus ST (n=5).
(C) Transmission electron microscopy images of mitochondrial ultrastructure (n=5). (D-E) CS and PDH content in ADSCs following Piezo1 knockdown (n=5). (Data are presented as mean ± standard deviation).

Figure S3

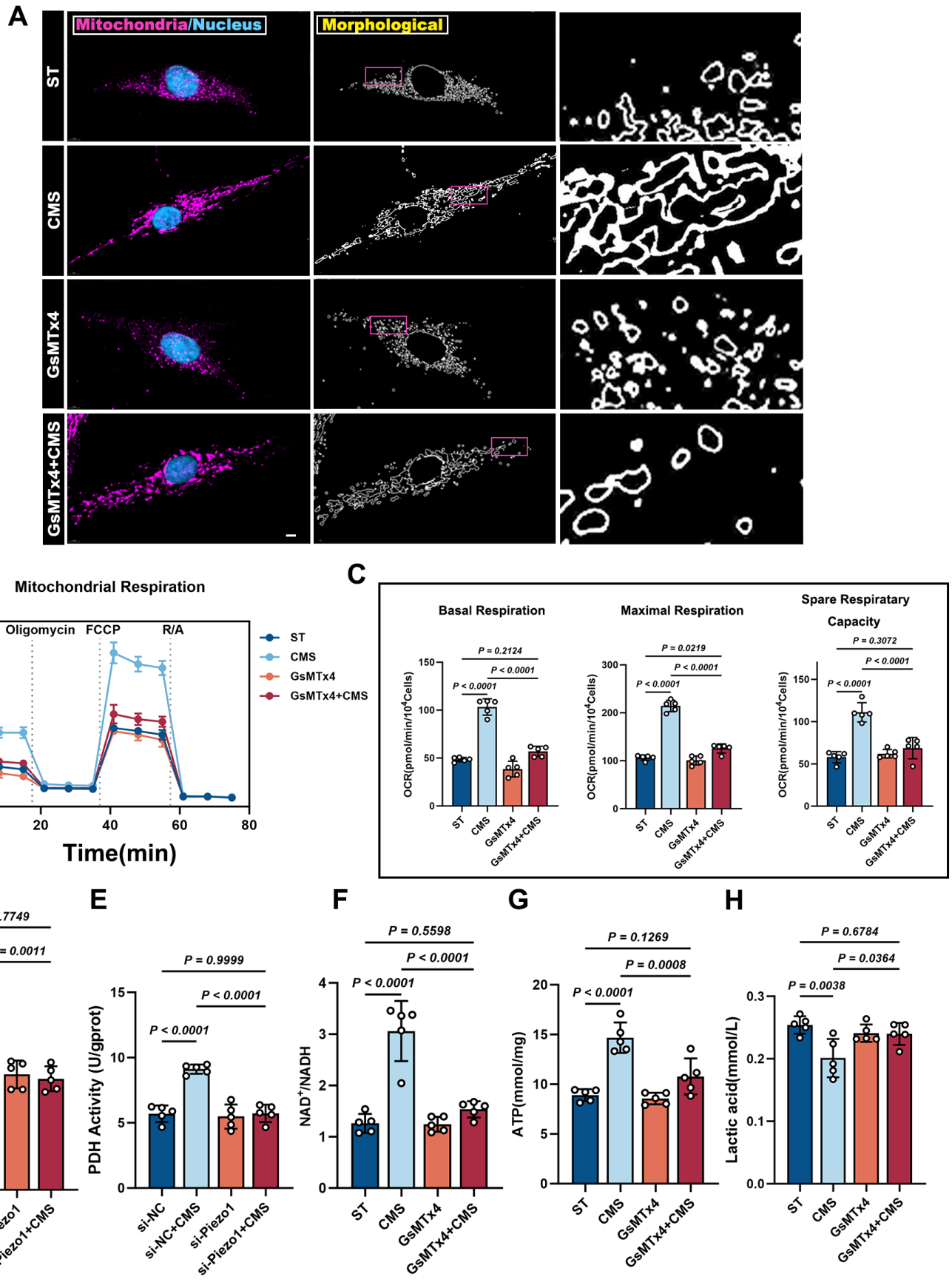
A
Mitochondria/Nucleus
Morphological
ST
CMS
GsMTx4
GsMTx4+CMS
B
Mitochondrial Respiration
Oligomycin
FCCP
R/A
OCR(pmol/min/10⁴Cells)
Time(min)
ST
CMS
GsMTx4
GsMTx4+CMS
C
Basal Respiration
Maximal Respiration
Spare Respiratary Capacity
P = 0.2124
P < 0.0001
P < 0.0001
P = 0.0219
P < 0.0001
P < 0.0001
P = 0.3072
P < 0.0001
P < 0.0001
D
CS Activity (U/gprot)
P = 0.7749
P = 0.0011
P = 0.0076
si-NC
si-NC+CMS
si-Piezo1
si-Piezo1+CMS
E
PDH Activity (U/gprot)
P = 0.9999
P < 0.0001
P < 0.0001
F
NAD⁺/NADH
P = 0.5598
P < 0.0001
P < 0.0001
G
ATP(mmol/mg)
P = 0.1269
P = 0.0008
P < 0.0001
H
Lactic acid(mmol/L)
P = 0.6784
P = 0.0364
P = 0.0038

Fig. S3. Effect of Piezo1 inhibitor GsMTx4 on mechanical stimulation-enhanced ADSCs mitochondrial dynamics and mitophagy.

(A) Cultured ADSCs were labeled with MitoTracker Red and Hoechst 33342, and mitochondrial morphological changes were observed using confocal microscopy in the presence of the Piezo1 inhibitor GsMTx4 (Scale bar = 5 μm). (B-C) Representative traces of OCR assay ADSCs in the presence of the Piezo1 inhibitor GsMTx4 (n=5). (D-H) CS, PDH, ATP, NAD^+NADH, MAD and FRAP levels in ADSCs in the presence of the Piezo1 inhibitor GsMTx4 (n=5). (Data are presented as mean ± standard deviation).

Figure S4

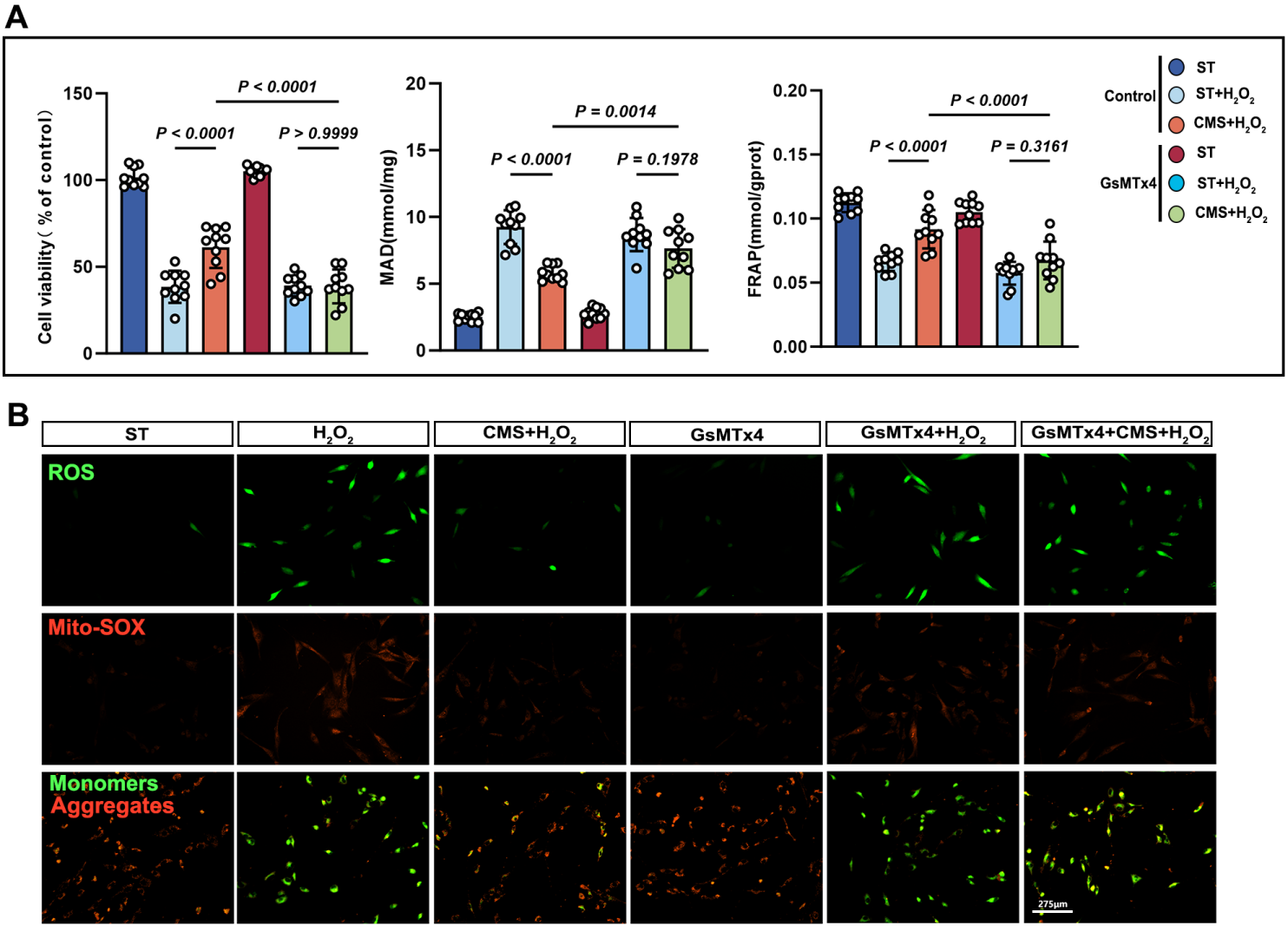

Fig. S4. Effect of Piezo1 inhibitor GsMTx4 on mechanical stimulation-enhanced survival and mitochondrial homeostasis of ADSCs under oxidative stress.

(A) CCK-8 assay and measurements of malondialdehyde and ferric reducing antioxidant power levels were conducted in the presence of the Piezo1 inhibitor GsMTx4 (n=10). (B) ROS levels (DCFH-DA/Mito-SOX) and mitochondrial membrane potential (JC-1, red: J-aggregates/green: monomers) were assessed by fluorescence imaging, with the red/green ratio quantifying ΔΨm, in the presence of the Piezo1 inhibitor GsMTx4(Scale bar = 275 μm). (Data are presented as mean ± standard deviation).