

Figure S1 Intratracheal injection of identified umbilical cord mesenchymal stem cells with a nebulizing syringe. (A) Bright-field images of umbilical cord MSCs with long fusiform and shoal-like distribution. (B) Flow analysis of the surface markers of umbilical cord MSCs showed that CD29, CD73, CD90, and CD105 were positive, while CD34, CD45, HLA-DR, and CD79a were negative (blue represents blank; red represents samples labeled with antibodies). (C) MSCs were nebulized with a nebulizing syringe. (D) The activity of MSCs before and after nebulization was compared by AOPI staining, scale bar: 25 μ m, n=6, ns, P>0.05. MSC, mesenchymal stem cell; AOPI, acridine orange propidium iodide.

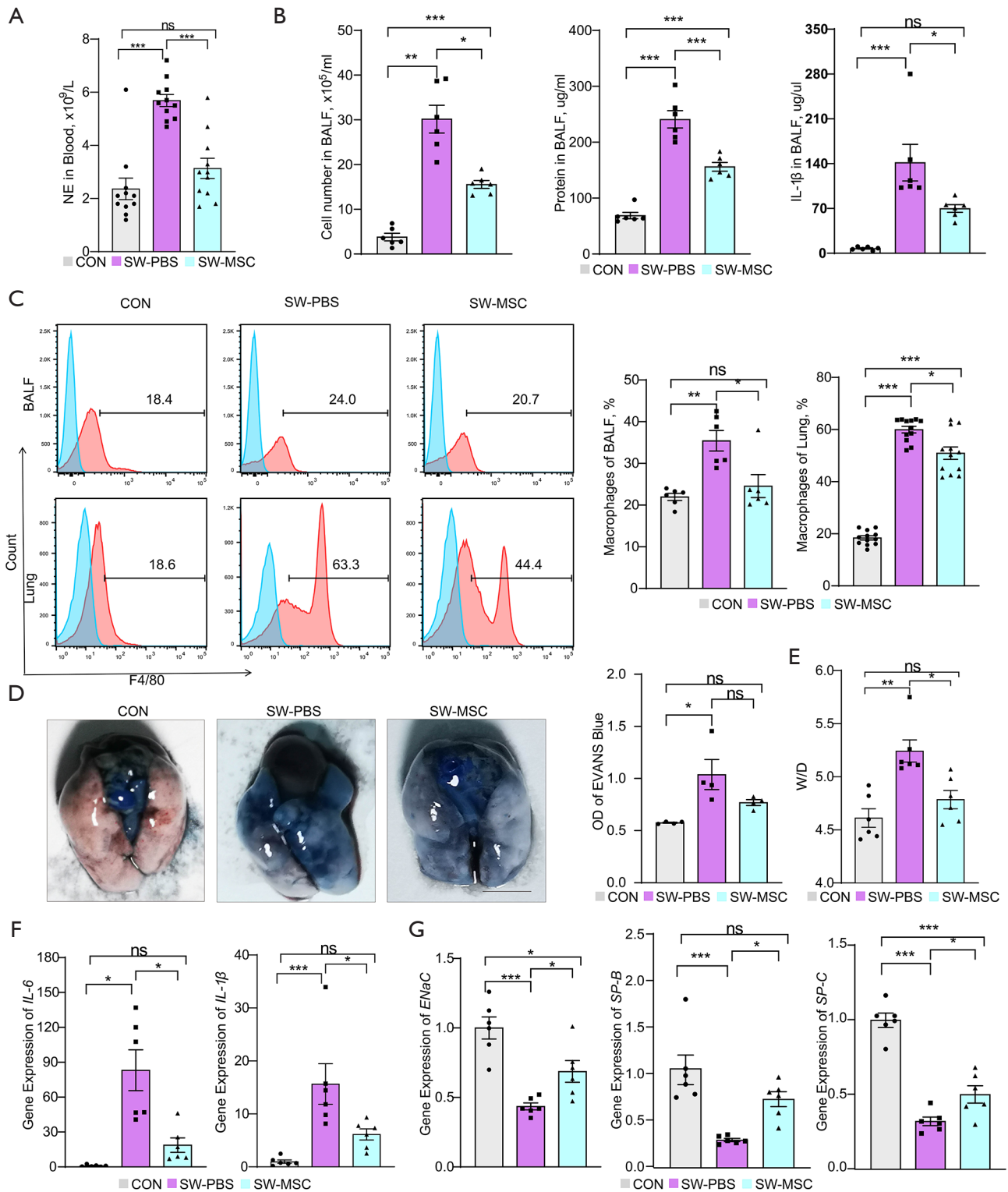


Figure S2 Mesenchymal stem cells effectively improved SWD-ALI. (A) Quantification of the neutrophils in the blood, $n=11$, ns, $P>0.05$; ***, $P<0.001$. (B) Quantification of total number of cells, total protein and IL-1 β in the BALF, $n=6$, ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. (C) The percentages of BALF and lungs macrophages in each group were analyzed by flow cytometry, and the macrophages in BALF and lungs were compared among the groups, $n=6$ (BALF), $n=12$ (lung), ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (blue represents blank; red represents samples labeled with antibodies). (D) Photographs showed lung vascular permeability, which was assessed by Evans blue accumulation in the lung tissues, and the levels of Evans blue dye were assessed by measuring absorbance at 620 nm, scale bar: 5 mm, $n=4$, ns, $P>0.05$; *, $P<0.05$. (E) Lung vascular permeability was assessed by the lung wet to dry (W/D) ratio, $n=6$, ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$. (F,G) The RNA levels of *IL-6*, *IL-1 β* , *ENaC*, *SP-B* and *SP-C* in lung tissues were detected by qPCR, $n=6$, ns, $P>0.05$; *, $P<0.05$; ***, $P<0.001$. CON, control group; SW-PBS, seawater drowning + phosphate buffer solution group; SW-MSC, seawater drowning + mesenchymal stem cells group; SWD-ALI, seawater drowning induced acute lung injury; BALF, bronchoalveolar lavage fluid.



Video S1 Mesenchymal stem cell treatment alleviates seawater drowning induced lung injury by inhibiting the TNF α /Snail/EMT pathway.

Table S1 Antibodies used for flow cytometry

Antigen	Source	Dilution	Identifier
HLA-DR (APC)	Invitrogen	1:100	47-9956-42
CD45 (APC)	Invitrogen	1:100	17-0459-42
CD73 (APC)	Invitrogen	1:100	17-0739-42
CD29 (APC)	Invitrogen	1:100	17-0299-42
CD79a (APC)	BioLegend	1:100	986502
CD105 (PE)	Invitrogen	1:100	12-1051-82
CD90 (PE)	Invitrogen	1:100	12-0909-42
CD34 (PE)	BioLegend	1:100	378603
CD11b (FITC)	BioLegend	1:50	101206
F4/80 (PerCP-Cy5.5)	BioLegend	1:50	123128
Iy6G (PE-Cy7)	BioLegend	1:50	127618

Table S2 Primary antibodies used for Western blotting

Antigen	Host	Source	Dilution	Identifier
Snail	Rabbit	Cell Signaling Technology	1:1000	3879
Slug	Rabbit	Cell Signaling Technology	1:1000	9585
E-cadherin	Rabbit	Cell Signaling Technology	1:1000	3195
N-cadherin	Rabbit	Cell Signaling Technology	1:1000	13116
Claudin-1	Rabbit	Cell Signaling Technology	1:1000	13255
TNF α	Rabbit	ABclone	1:1000	A0277
GAPDH	Rabbit	Cell Signaling Technology	1:20000	5174

Table S3 Secondary reagents used for Western blotting

Item	Source	Dilution
Anti-rabbit	Cell Signaling Technology	1:20000

Table S4 Primary antibodies used for immunofluorescence

Antigen	Host	Source	Dilution	Identifier
Snail	Rabbit	Servicebio	1:3000	GB11260
Slug	Rabbit	Servicebio	1:2000	GB115172
TNF α	Rabbit	Servicebio	1:2000	GB11188
SP-C	Rabbit	Servicebio	1:5000	GB114059
E-cadherin	Rabbit	Servicebio	1:1000	GB11082
N-cadherin	Rabbit	Servicebio	1:1000	GB12135
DAPI	—	Servicebio	2 μ g/mL	G1012

Table S5 Secondary reagents used for immunofluorescence

Items	Conjugate(s)	Source	Identifier
TSAPLus fluorescent double staining kit	Alexa Fluor 488/555	Servicebio	G1226
TSAPLus fluorescent 4-label 5-color staining kit	Anti-Rabbit IgG Alexa Fluor 440/488/594/647	Servicebio	G1255

Table S6 Other main reagents used in this experiment

Items	Source	Identifier
UC-MSC Adipogenic Induction Medium	OriCell	HUXUC-90021
UC-MSC Chondrogenic Induction Medium	OriCell	HUXUC-90031
UC-MSC Osteoblast Medium	OriCell	HUXUC-90041
Serum-free mesenchymal stem cell medium	Beijing Sanly Sci-Tech	1208
1640 medium	Gibco	C11875500BT
TNF α ELISA kit	Raybiotech	P06804
IL-1 β ELISA kit	Raybiotech	P10749

Table S7 Sequence of specific primers used for qPCR analysis

Species	Genes	Forward sequence (5'→3')	Reverse sequence (5'→3')
Mouse	<i>Gapdh</i>	ACTCCACTCACGGCAAATTC	TCTCCTATGGTGGTGACGACA
	<i>IL-1β</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
	<i>IL-6</i>	GGGAGCGTATCATCTGCGTT	AGTCAGGAGGTTTGTCTTGT
	<i>TNFα</i>	CCCCAAAGGGATGAGAAGTTCC	TTTGCTACGACGTGGGCTA
	<i>SP-B</i>	CTGCTTCCTACCCTCTGCTG	CTTGGCACAGGTCATTAGCTC
	<i>SP-C</i>	ATGGACATGAGTAGCAAAGAGGT	CACGATGAGAAGGCGTTTGAG
	<i>ENaC</i>	CCTTCTCCTTGGATAGCCTGG	CAGACGGCCATCTTGAGTAGC
Human	<i>GAPDH</i>	TCTCCTCTGACTTCAACAGCGAC	CCCTGTTGCTGTAGCCAAATTC
	<i>TNFα</i>	CTCCTCACCCACACCATCAGCCGCA	ATAGATGGGCTCATACCAGGGCTTG
	<i>Snail (SNAI 1)</i>	TGCTCATCTGGGACTCTGTC	AAGCCTGGGAAGGCAGCATA
	<i>Slug (SNAI 2)</i>	CAACGCCTCCAAAAGCCAA	ACTCACTCGCCCCAAAGATG