Supplementary material and methods

RNA sequencing

To characterize the changes in L02 cells subjected to H/R injury after pretreatment with hucMSC-exos, we performed transcriptome sequencing on L02 cells from the control, H/R, and exos1 groups (n = 3). First, total RNA was extracted from L02 cells *via* TRIzol reagent (Invitrogen). The RNA quality was then assessed *via* NanoDrop technology and an Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA, USA), with an RNA integrity number ≥ 7 set as the minimum threshold. Subsequently, mRNA libraries were constructed and amplified, and sequencing was conducted by Ic-bio Co., Ltd. (Hangzhou, China) *via* the Illumina HiSeq 2000 system (Illumina, San Diego, CA, USA). The transcriptome sequencing data were subjected to quality control and aligned to the reference genome. The thresholds for screening differentially expressed genes were set at $|\log2(\text{FoldChange})| \geq 1$ and q value < 0.05. Finally, differentially expressed genes were subjected to analysis using the kyoto encyclopedia of genes and genomes (KEGG).

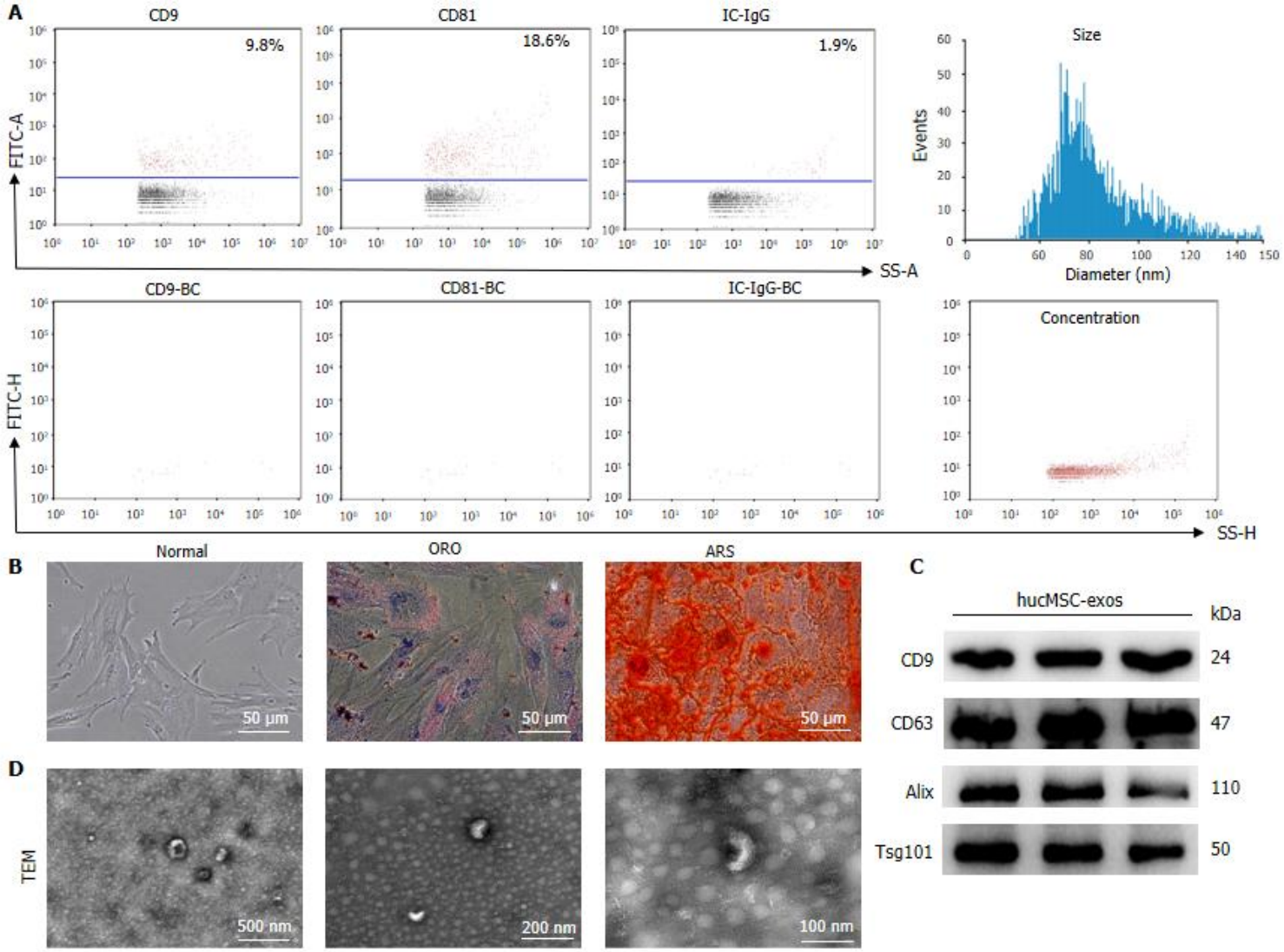
MiRNA sequencing

To identify which miRNAs in hucMSC-exos are involved in ameliorating H/R injury in L02 cells, we selected L02 cells from the normal, H/R, and exos1 groups, as well as exosomes from the hucMSC-exos group, for miRNA expression profiling. Three samples from each group were chosen for miRNA sequencing. The miRNA sequencing library was prepared *via* the TruSeq Small RNA Sample Prep Kit (Illumina, San Diego, USA). After library preparation, sequencing was performed *via* the Illumina HiSeq 2500 platform. The raw sequencing data were provided by Ic-bio Co., Ltd. Differentially expressed miRNAs were screened with a

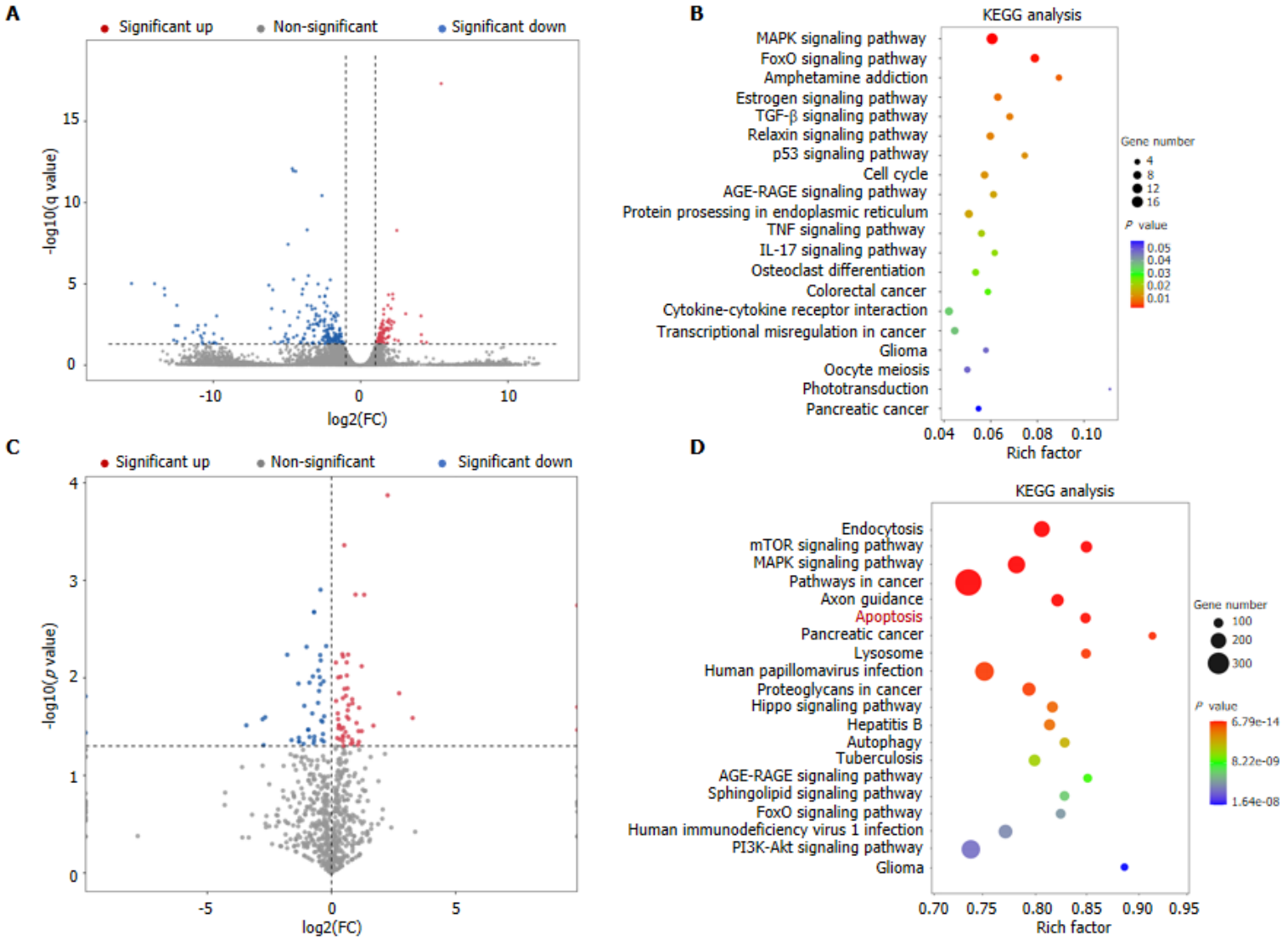
threshold of a *P* value < 0.05. Target gene prediction for significantly different miRNAs was conducted *via* two software tools, TargetScan (v5.0, MIT, Cambridge, MA, USA) and miRanda (v3.3a, MSK, Manhattan, NY, USA). The intersection of the target genes predicted by both software programs was considered the final target gene set for the differentially expressed miRNAs. The predicted target genes were subsequently subjected to KEGG analysis.

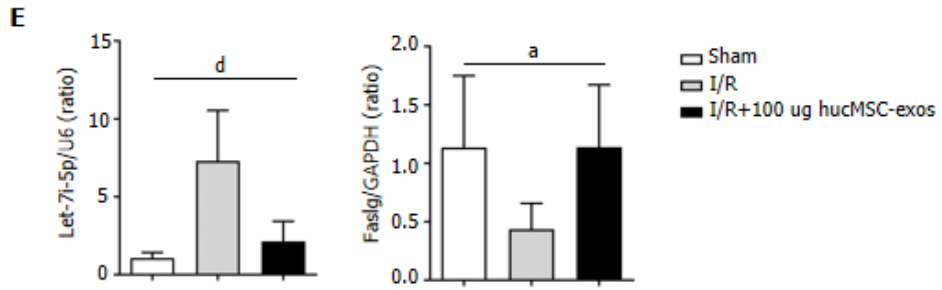
MiRNA-mRNA integrated analysis

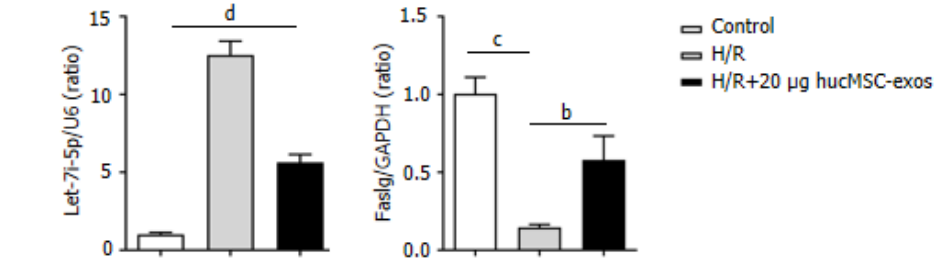
We conducted a Venn analysis on the differential miRNAs between the normal and H/R groups, the exos1 and H/R groups, and the exos1 and hucMSC-exos groups to identify miRNAs in hucMSC-exos that may be involved in ameliorating H/R-induced liver injury. These miRNAs were subsequently correlated with differentially expressed mRNAs according to transcriptome sequencing, with a focus on the expression levels of miRNAs and mRNAs and the negative regulatory relationships between their upregulation and downregulation. Finally, an enrichment analysis of the relevant target genes was carried out using the KEGG.

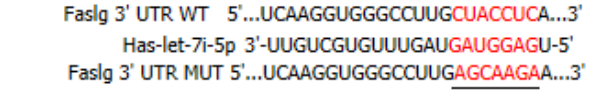

A
CD9
9.8%
CD81
18.6%
IC-IgG
1.9%
FITC-A
SS-A
Size
Events
Diameter (nm)
CD9-BC
CD81-BC
IC-IgG-BC
FITC-H
SS-H
Concentration
B
Normal
ORO
ARS
50 μm
C
hucMSC-exos
kDa
CD9
24
CD63
47
Alix
110
Tsg101
50
D
TEM
500 nm
200 nm
100 nm

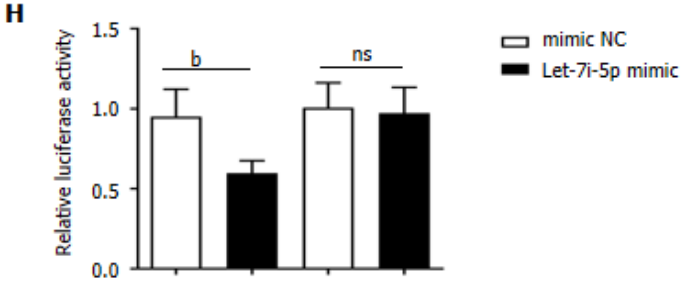
Supplementary Figure 1 Characterization of hucMSC-exos. A: Analyze the expression levels of CD9 and CD81 in hucMSC-exos using flow cytometry, as well as determine the particle size and concentration of the exosomes; B: Observe the normal morphology of human umbilical cord mesenchymal stem cells and perform Alizarin Red and Oil Red O staining; C: Examine the expression levels of CD9, CD63, Alix, and Tsg101 in hucMSC-exos using Western blot analysis; D: Observe the morphology of hucMSC-exos under different magnifications using transmission electron microscopy; SS-A: Side Scatter-Area; SS-H: Side Scatter-Height; FITC-H: Fluorescein 5-isothiocyanate-Height; hucMSC-exos: Human umbilical cord mesenchymal stem cells-derived exosomes; IC: Isotype control; BC: Blank control; ORO: Oil red o staining; ARS: Alizarin red staining; Alix: ALG-2-interacting protein x; Tsg101: Tumour susceptibility 101; TEM: Transmission electron microscopy; KD: Kilodalton.


A
Significant up
Non-significant
Significant down
-log10(q value)
log2(FC)
B
KEGG analysis
MAPK signaling pathway
FoxO signaling pathway
Amphetamine addiction
Estrogen signaling pathway
TGF-β signaling pathway
Relaxin signaling pathway
p53 signaling pathway
Cell cycle
AGE-RAGE signaling pathway
Protein prosessing in endoplasmic reticulum
TNF signaling pathway
IL-17 signaling pathway
Osteoclast differentiation
Colorectal cancer
Cytokine-cytokine receptor interaction
Transcriptional misregulation in cancer
Glioma
Oocyte meiosis
Phototransduction
Pancreatic cancer
Rich factor
Gene number
P value
C
Significant up
Non-significant
Significant down
-log10(p value)
log2(FC)
D
KEGG analysis
Endocytosis
mTOR signaling pathway
MAPK signaling pathway
Pathways in cancer
Axon guidance
Apoptosis
Pancreatic cancer
Lysosome
Human papillomavirus infection
Proteoglycans in cancer
Hippo signaling pathway
Hepatitis B
Autophagy
Tuberculosis
AGE-RAGE signaling pathway
Sphingolipid signaling pathway
FoxO signaling pathway
Human immunodeficiency virus 1 infection
PI3K-Akt signaling pathway
Glioma
Rich factor
Gene number
P value


E
Let-7i-5p/U6 (ratio)
15
10
5
0
d
Faslg/GAPDH (ratio)
2.0
1.5
1.0
0.5
0.0
a
Sham
I/R
I/R+100 ug hucMSC-exos


F
Let-7i-5p/U6 (ratio)
15
10
5
0
d
Faslg/GAPDH (ratio)
1.5
1.0
0.5
0.0
c
b
Control
H/R
H/R+20 μg hucMSC-exos


G
Faslg 3' UTR WT 5'...UCAAGGUGGGCCUUGCUACCUCA...3'
Has-let-7i-5p 3'-UUGUCGUGUUUGAUGAUGGAGU-5'
Faslg 3' UTR MUT 5'...UCAAGGUGGGCCUUGAGCAAGAA...3'


H
Relative luciferase activity
1.5
1.0
0.5
0.0
b
ns
mimic NC
Let-7i-5p mimic

Supplementary Figure 2 The influence of hucMSC-exos on the mRNA and miRNA expression profiles of L02 cells after H/R injury. A: The volcano plot displays the genes with significant differential expression between the H/R group and the exos1 group; B: Differential genes enriched in 20 pathways; C:The volcano plot for the differential miRNA; D:KEGG analysis revealed that the differential miRNAs between the H/R group and the exos1 group were significantly enriched in 20 pathways; E: In *in vivo* experiments, this negative regulatory relationship was further validated using qRT-PCR; F:In *in vitro* experiments, it was discovered that let-7i-5p has a negative regulatory effect on Faslg mRNA; G: Schematic diagram showing the wild-type and mutant 3' UTRs of Faslg targeted by let-7i-5p, with the mutant sequence underlined; H: A dual-luciferase reporter assay confirmed that let-7i-5p targets the 3'-UTR of Faslg mRNA. ns, no significance. $^{a}P < 0.05$. $^{b}P < 0.01$. $^{c}P < 0.001$. $^{d}P < 0.0001$.

H/R: Hypoxia/reoxygenation; hucMSC-exos: Human umbilical cord mesenchymal stem cells-derived exosomes; exos1, exos1 group: 20 μg hucMSC-exos incubated with H/R L02 cells; NC: Negative control; WT: Wild-type; MUT: Mutant; DEGS: Differentially expressed gene; KEGG: Kyoto Encyclopedia of Genes and Genomes; qRT-PCR: Quantitative real-time polymerase chain reaction; Faslg:Factor-related apoptosis ligand; I/R: Ischaemia/reperfusion; mRNA: messenger RNA; miRNA: microRNA; Has: Homo sapiens. UTR: Untranslated regions.

Supplementary Table 1 Primer sequences used in qRT-PCR

Primer name	Sequence (5′ to 3′)
GAPDH forward	CCCATCACCATCTTCCAGG
GAPDH reverse	CATCACGCCACAGTTTCCC
let-7i-5p forward	TGAGGTAGTAGTTTGTGCTGTT
let-7i-5p reverse	GTCGTATCCAGTGCAGGGT
Faslg forward	TCTTCCCTGTCCAACCTCTGT
Faslg reverse	GTGGCCTATTTGCTTCTCCAA
U6 forward	CTCGCTTCGGCAGCACATATACT
U6 reverse	ACGCTTCACGAATTTGCGTGTC

Supplementary Table 2 miRNA-mRNA correlation analysis

miR_name	up/down	Expression level	gene_name	KEGG
rno-miR-3559-5p	down	middle	NA	NA
mmu-miR-3968_L-2_1ss14AT	down	middle	Emp2	NA
mdo-miR-210-3p_L-1R+1	down	middle	NA	NA
rno-miR-1843b-5p_R-2	down	middle	Zmynd19	NA
			Map1a	NA
			Bnc1	NA
			Sfrp4	04310 (Wnt pathway)
mmu-miR-362-5p	up	middle	Nptx1	NA
			Ccdc141	NA
			Map1a	NA
mdo-miR-26-5p_R+3	up	middle	NA	NA

mmu-let-7j_1ss8TG	up	middle	Faslg	04010(MAPK pathway)、04068(FoxO pathway)、04151(PI3K-Akt pathway)、04210(Apoptosis)
			Depp1	NA
			Slpil2	NA
hsa-miR-1290_R-1_1ss13TG	up	low	Socs3	04630(Jak-STAT pathway)、04668(TNF pathway)
			Slc25a25	NA
hsa-let-7i-5p	up	high	Faslg	04010(MAPK pathway)、04068(FoxO pathway)、04151(PI3K-Akt pathway)、04210(Apoptosis)
			Depp1	NA
			Slpil2	NA
rno-miR-3558-5p_R-1	up	middle	Ccdc141	NA
			Map1a	NA