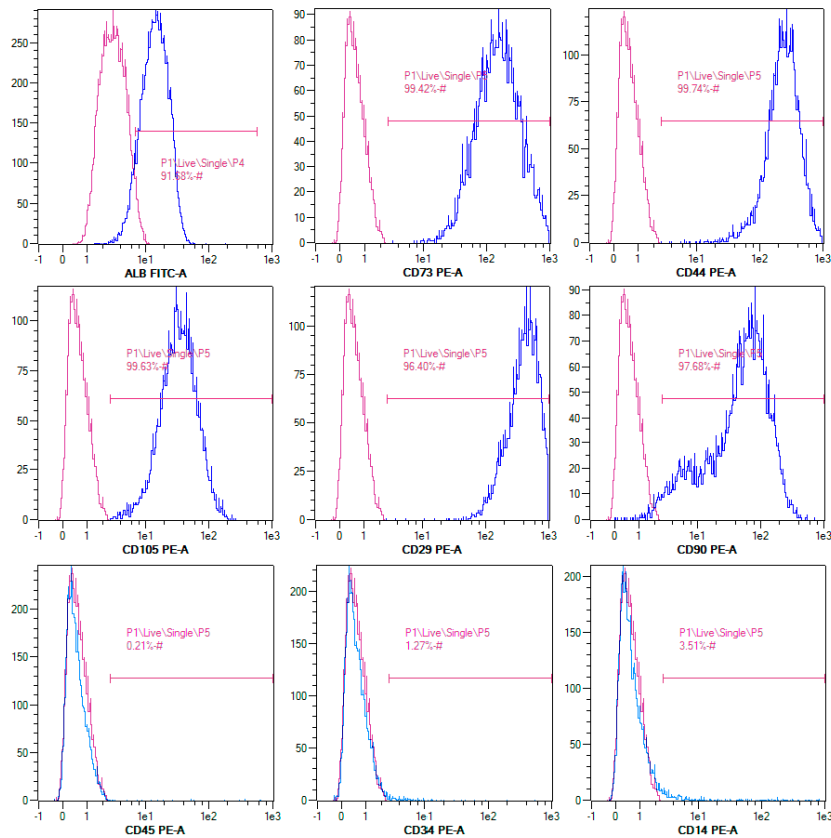


Supplementary Materials

Good manufacturing practice-derived human liver stem cell extracellular vesicles attenuate *in vivo* liver fibrosis



Supplementary Figure 1. Representative FACS analyses of HLSCs, after overnight starvation, showing the expression of albumin (ALB), CD73, CD44, CD105, CD29, and CD90 (blue histograms). CD45, CD34 and CD14 are not expressed. Pink histograms represent isotypic control.

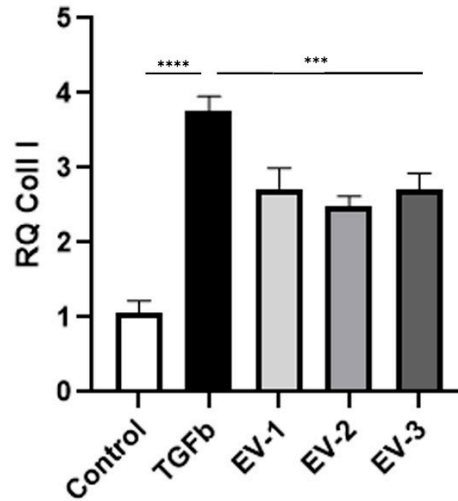
Supplementary Table 1. Result of quality control tests on DP for stability study.

Test	Acceptance criteria	Batch		
		nEV HLSC-CLF-05/23	nEV HLSC-CLF-06/23	nEV HLSC-CLF-07/23
EV concentration	$\geq 3.5 \times 10^9$ EV/mL	5.2×10^9 EV/mL	3.6×10^9 EV/mL	3.8×10^9 EV/mL
Size distribution	60-150 nm mean size 40-100 nm mode size	Mean = 71.1 nm Mode = 44.6 nm	Mean = 86.0 nm Mode = 48.8 nm	Mean = 96.1 nm Mode = 51.8 nm
Immunophenotype	Positive for CD63, CD81, CD9, CD29, CD44, CD105	CD63= 238.7% CD81= 46.4% CD9= 15.5% CD105 = 7.3% CD29 = 23.7% CD44 = 24.7%	CD63 = 230.4% CD81 = 19.4% CD9 = 16.0% CD105 = 10.2% CD29 = 32.8% CD44 = 43.5%	CD63=187.8% CD81=86.4% CD9=25.8% CD105=9.3% CD29= 42.5% CD44=58.3%
Appearance	Absence of extraneous particles	Absence of extraneous particles	Absence of extraneous particles	Absence of extraneous particles
Sterility	Culture negative	Culture negative at LOD	Culture negative at LOD	Culture negative at LOD
Endotoxin	< 0.5 EU/mL	< 0.0500 EU/mL	< 0.0500 EU/mL	< 0.0500 EU/mL
TEM	Intact membrane	Intact membrane	Intact membrane	Intact membrane

Supplementary Table 2. EV miRNA content.

non-GMP HLSC-EVs		
miRNA	CT mean	SD
hsa-miR-222	27.138	2.296
hsa-miR-24-3p	27.358	2.366
hsa-miR-31-5p	28.587	2.454
hsa-miR-29a-3p	28.763	2.857
hsa-miR-191	30.537	2.225
hsa-miR-146a-5p	31.912	2.922

Data are expressed as mean CT value $\pm$ SD (n = 3).



Supplementary Figure 2. Evaluation of EV effect on activated hepatic stellate cells. qRT-PCR analysis of the expression of Collagen I (Coll I) in activated LX-2 cells after 24 hours of incubation with EVs (50,000 EVs/cell). LX-2 cells activated with TGF- β (10 ng/mL), not incubated with EVs, were used as reference control (TGFb). LX-2 cells cultured without TGF- β were used as negative control (control). Gene expression levels were normalized to those of the housekeeping gene TBP. Results are shown as mean $\pm$ SD of three independent experiments performed in triplicate. *** $p \leq 0.001$ and **** $p \leq 0.0001$.