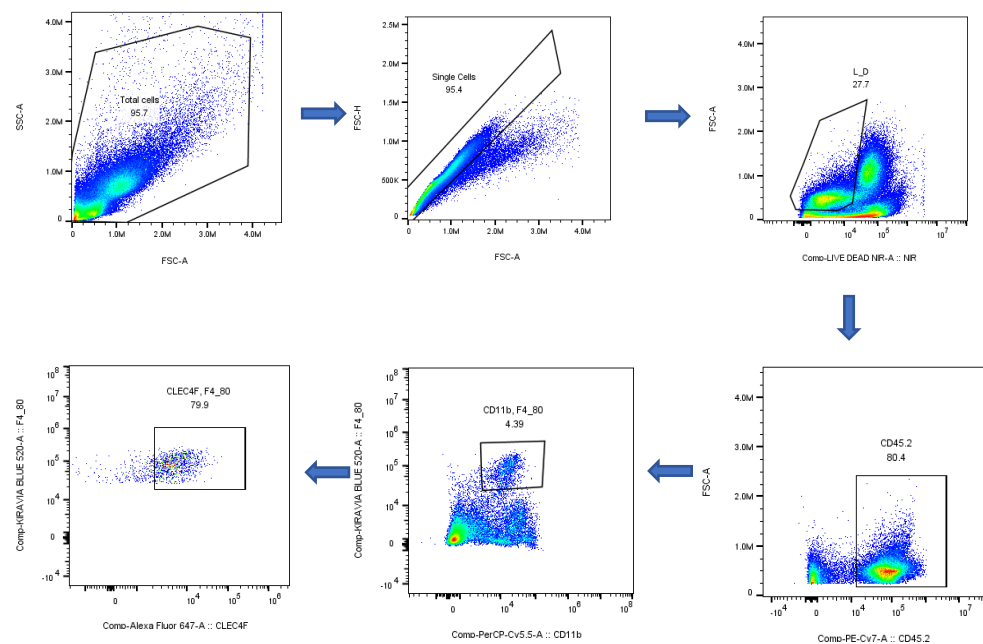
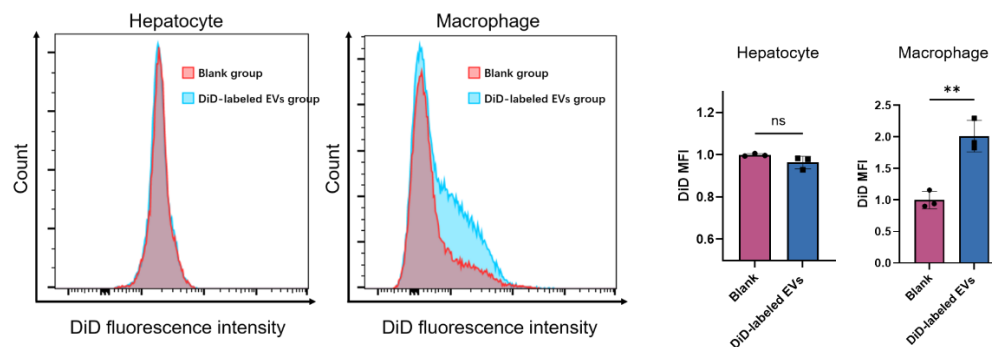


Supplementary Figure 1. Flow Cytometry Gating Strategy for Kupffer Cell Identification



Gating strategy for hepatic Kupffer cells: Single cells were first gated, followed by exclusion of dead cells. CD45⁺ immune cells were then selected, and the F4/80^{hi} CD11b^{int} population was further gated. Finally, CLEC4F⁺ cells within this population were defined as hepatic Kupffer cells. Subsequent analyses were performed on the F4/80^{hi} CD11b^{int} CLEC4F⁺ cell population.

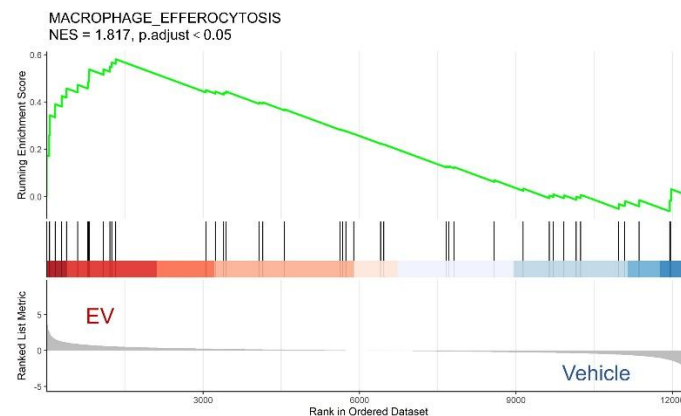
Supplementary Figure 2. Uptake of DiD-labeled AMSC-EVs by hepatocytes and macrophages



AMSC-EVs were labeled with DiD fluorescent dye (Beyotime, C1039) at a final concentration of 10 μ M for 15 min according to the manufacturer's instructions. Excess dye was removed by washing with PBS. DiD-labeled AMSC-EVs were administered to ALF mice. At 2 h after model establishment, primary hepatocytes and macrophages were

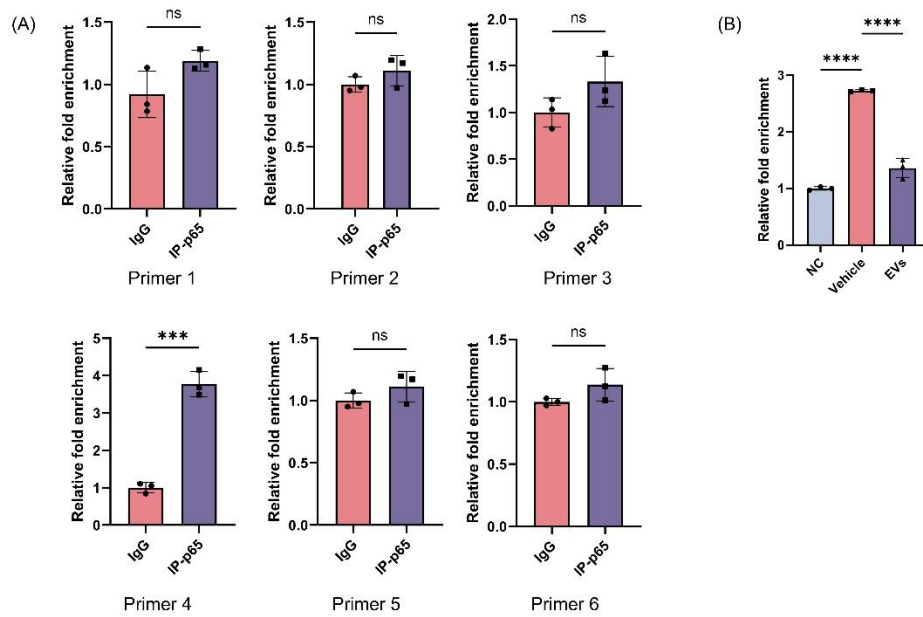
isolated from the liver, and their fluorescence intensity was analyzed by flow cytometry. The results showed that DiD-labeled AMSC-EVs were predominantly taken up by macrophages.

Supplementary Figure 3. GSEA of a custom efferocytosis-related gene set.



Gene set enrichment analysis (GSEA) was performed using a customized efferocytosis-related gene set comprising genes from GO:0006911, GO:0043277, and GO:0050766, together with additional genes reported in the literature to be involved in macrophage recognition, engulfment, processing and degradation of apoptotic cells. GSEA was performed in R using the clusterProfiler package. Genes were ranked according to log2 fold change, and enrichment of the predefined custom efferocytosis-related gene set was assessed using the fgsea algorithm with Benjamini–Hochberg correction for multiple testing.

Supplementary Figure 4. ChIP-qPCR analysis of p65 enrichment at the Adam17 promoter.



(A) ChIP-qPCR analysis was performed using six primer pairs targeting different regions of the Adam17 promoter. Among them, primer pair 4 showed clear enrichment of p65 compared with the IgG control and was used in subsequent experiments.

(B) ChIP-qPCR was performed to assess p65 binding to the Adam17 promoter in the NC, Vehicle, and EVs groups. For each group, p65 enrichment was first compared with the corresponding IgG control. The relative fold enrichment was then normalized to the NC group, which was set to 1.

NC, untreated cells; Vehicle, LPS-treated cells receiving PBS treatment; EVs, LPS-treated cells receiving treatment with AMSC-EVs. Data are presented as mean $\pm$ SD. *** $p < 0.001$, **** $p < 0.0001$, ns: not statistically significant.

Supplementary Tables

Supplementary Table 1. Antibodies used in flow cytometry experiments.

Antibody	Fluorochrome	Catalog No.	Manufacturer	Dilution
NIR	APC-Cy7	L34976	Invitrogen	1:400
CD45	APC	109814	BioLegend	1:200
CD45	PE-Cy7	109829	BioLegend	1:200

CD11b	PerCP-Cy5.5	101227	BioLegend	1:200
F4/80	KIRAVIA Blue 520	123162	BioLegend	1:200
F4/80	FITC	123108	BioLegend	1:200
CLEC4F	Alexa Fluor 647	156804	BioLegend	1:200
Ly6G	Alexa Fluor 700	127622	BioLegend	1:200
TREM2	PE	FAB17291P	R&D Systems	1:200

Supplementary Table 2. Antibodies used for western blotting.

Antibody	Catalog No.	Manufacturer
GM130	HA721282	HUABIO
CD63	ab193349	Abcam
Alix	92880S	CST
CD9	ab307085	Abcam
NLRP3	15101S	CST
TNF- α	ab183218	Abcam
IL-1 β	12426	CST
AXL	13196-1-AP	Proteintech
MerTK	ab300136	Abcam
TREM2	75756	CST
ADAM17	61048	CST
Phospho-NF-kappaB p65	3033S	CST
NF-kappaB p65	8242S	CST
HIF-1 α	36169	CST
SP1	21962-1-AP	Proteintech
TLR4	RT1666	HUABIO
β -ACTIN	66009-1-Ig	Proteintech

Supplementary Table 3. Primer sequences used for qPCR.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
<i>Il1b</i>	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
<i>Tnf</i>	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
<i>Nlrp3</i>	ATCAACAGGCGAGACCTCTG	GTCCTCCTGGCATAACCATAGA
<i>Il6</i>	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
<i>Ccl2</i>	TAAAAACCTGGATCGGAACCAAA	GCATTAGCTTCAGATTACGGGT
<i>Il10</i>	GCTGGACAACATACTGCTAACC	ATTTCGATAAGGCTTGGCAA
<i>Vegfa</i>	CTGCTGTAACGATGAAGCCCTG	GCTGTAGGAAGCTCATCTCTCC
<i>Trem2</i>	CTGGAACCGTCACCATCACTC	CGAAACTCGATGACTCCTCGG
<i>Adam17</i>	GGAACACGTCGTGGGATAATG	GGCAGACTTTGGATGCTTCTT
<i>Actb</i>	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC

Supplementary Table 4. ChIP-qPCR primer information for the mouse Adam17 promoter

Primer No.	Primer	Product size
Primer 1	F: TTACATTACCGTTTATAGCCGTAGC R: TCAGTGGCACTCAGCCTTCC	132 bp
Primer 2	F: CCAGACTTCACAGACCCTTCAA R: GGATGGGCAGGTCCTTAGAC	86 bp
Primer 3	F: CATAGCTCAGCTGCACAAAAGC R: TCCATGTCCCCAGTTTGTA	81 bp
Primer 4	F: TGGCCCTCTGAGATGTTTCCT R: GGCTGGCTTCACTGTGCTAA	226 bp
Primer 5	F: GAAGGAACAGAGCCCAGAAGA R: AGAAGGGTGCCGTAGAGCAT	91bp
Primer 6	F: ACTAGTTTAGCTAACATGCCCC R: TTCCCTACTCGACCCCTTGT	220 bp