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Supplementary Materials for

Extracellular vesicle–encapsulated IL-10 as novel nanotherapeutics against ischemic AKI

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Other Supplementary Material for this manuscript includes the following:

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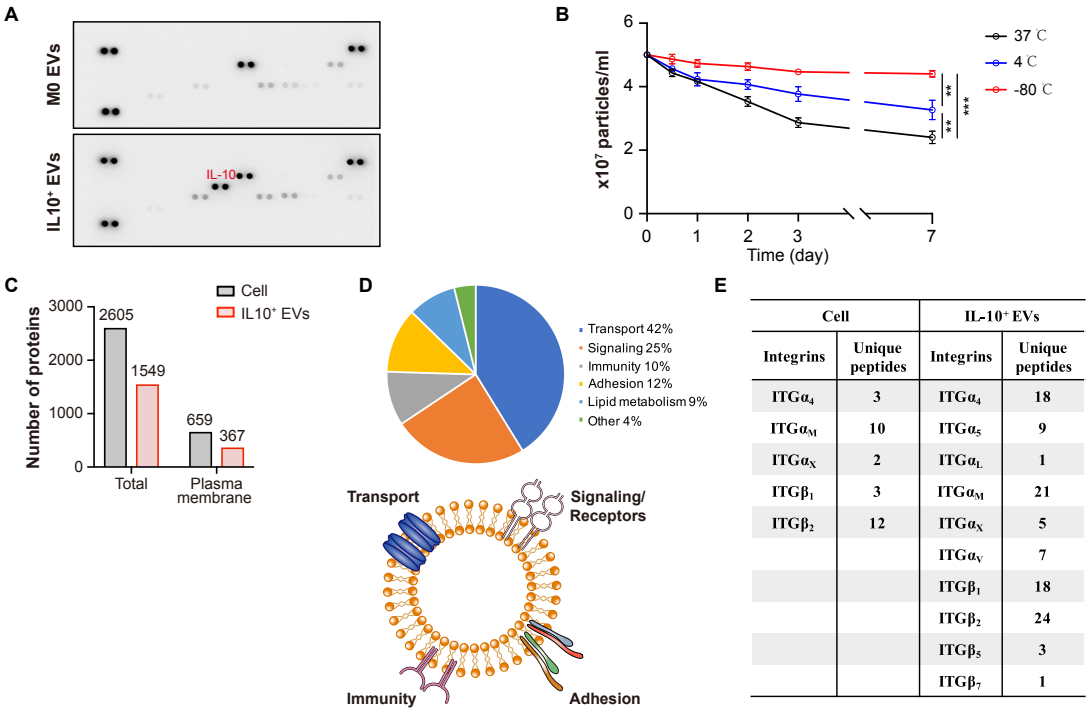

Cell		IL-10⁺ EVs	
Integrins	Unique peptides	Integrins	Unique peptides
$ITG\alpha_4$	3	$ITG\alpha_4$	18
$ITG\alpha_M$	10	$ITG\alpha_5$	9
$ITG\alpha_X$	2	$ITG\alpha_L$	1
$ITG\beta_1$	3	$ITG\alpha_M$	21
$ITG\beta_2$	12	$ITG\alpha_X$	5
		$ITG\alpha_V$	7
		$ITG\beta_1$	18
		$ITG\beta_2$	24
		$ITG\beta_5$	3
		$ITG\beta_7$	1

Figure S1 Characterization of the IL-10⁺ EVs.

(**A**) Cytokine antibody array analysis of IL-10⁺ EVs and M0 EVs (EVs from untreated RAW cells). (**B**) Assessment of the stability of IL-10⁺ EVs. IL-10⁺ EVs were preserved in PBS at 37 ℃, 4 ℃ or -80 ℃ for a week, and then EVs were recollected and suspended in 300 μL PBS for NTA analysis at different time points (n=3). (**C-E**) Proteomic profiling of the IL-10+ EVs. (**C**) The number of total and plasma membrane-associated proteins. (**D**) Functional characterization of the plasma membrane proteins identified in the IL-10⁺ EVs. (**E**) Integrin expression identified in IL-10⁺ EVs and parental RAW cells. Data are presented as mean ± SD. ** $p<0.01$, *** $p<0.001$, one-way ANOVA.

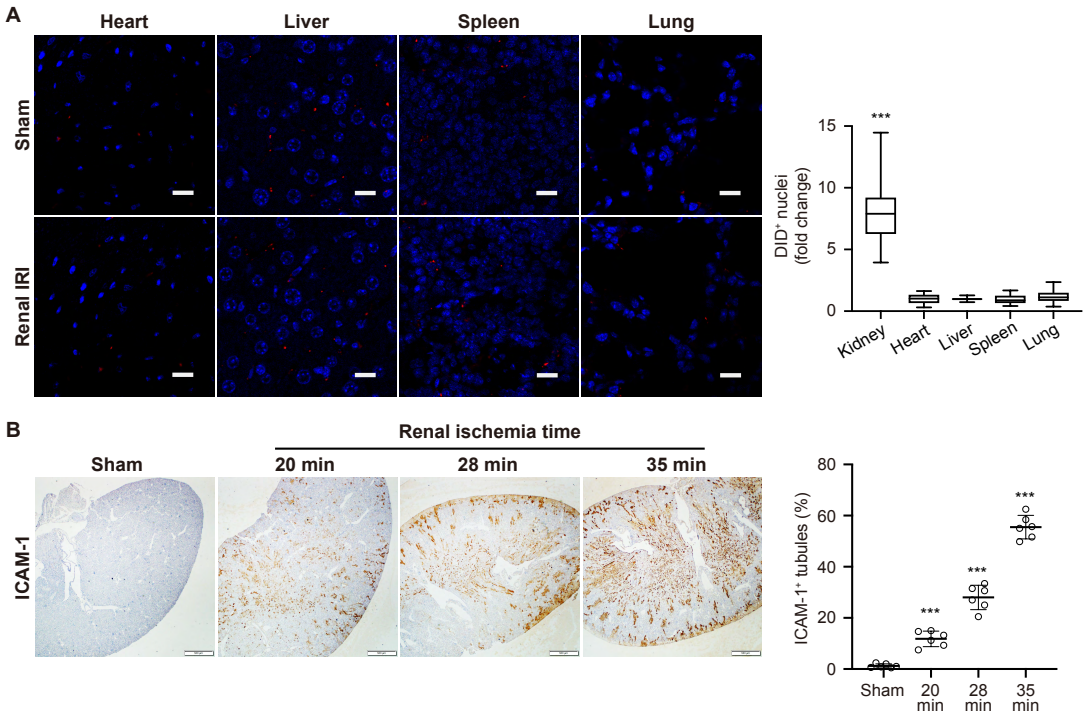

Figure S2 Tissue distribution of IL-10^{+} EVs.

(**A**) Representative micrographs of the indicated organs of mice injected with DID-labelled IL-10^{+} EVs (n=3). scale bar, 25 μm. (**B**) Immunohistochemical analysis of ICAM-1^{+} tubules (n=6). scale bar, 500 μm. Data are presented as mean ± SD. *** $p<0.001$, two-tailed t-test (A), one-way ANOVA (B).

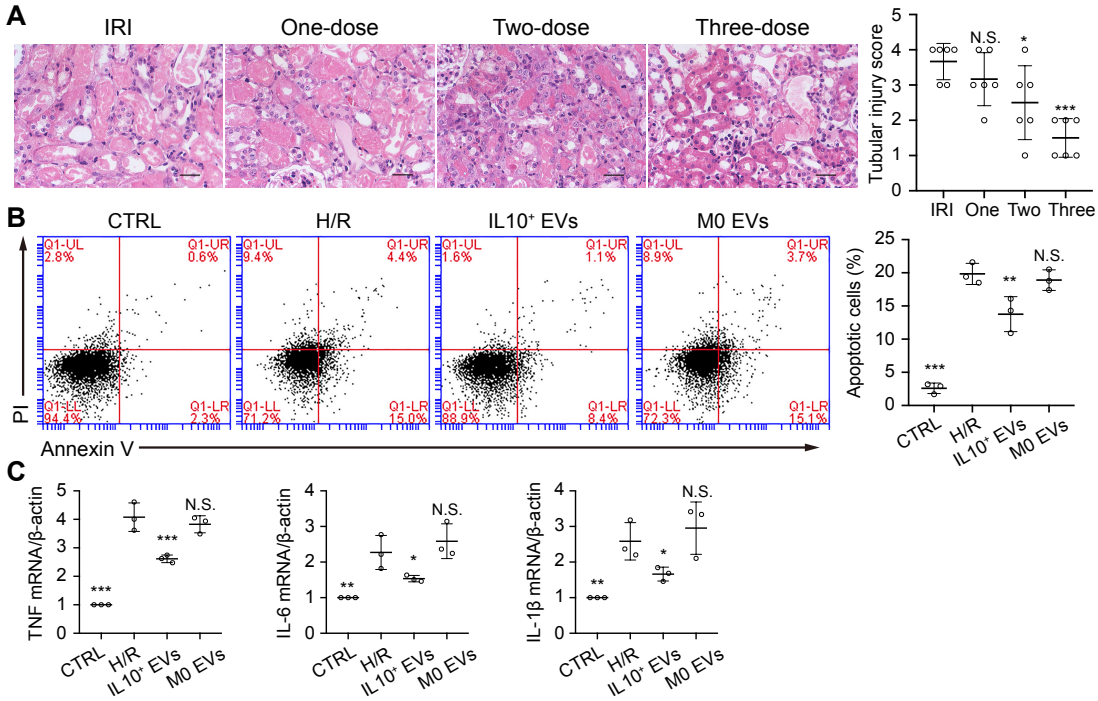

Figure S3 Therapeutic efficacy of IL10⁺ EVs.

(**A**) IRI mice were treated with IL-10⁺ EVs (200 μg) after reperfusion for different doses and were euthanized at 3 days post-reperfusion (n=6). scale bar, 50 μm. (**B,C**) Cultured TECs were stimulated with H/R and treated with IL-10⁺ EVs (15 μg) or M0 EVs (15 μg) for 12 hours (n=3). (A) Flow cytometry analysis of the apoptosis of TECs. (B) Real-time PCR analysis of inflammatory cytokine mRNA levels in TECs. Data are presented as mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. IRI or H/R group, one-way ANOVA.

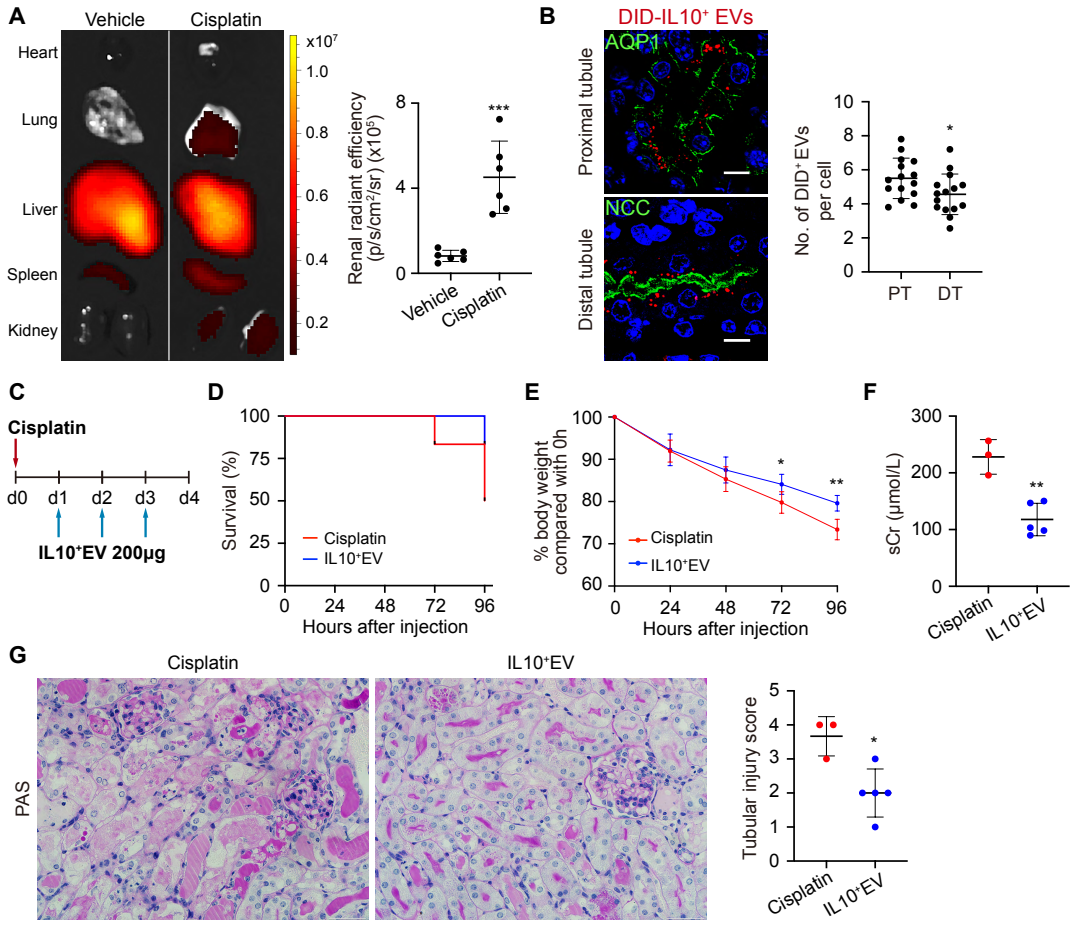

Figure S4 IL-10$^+$ EVs protect against cisplatin-induced AKI.

(**A and B**) Mice were injected intravenously with DID-labeled IL-10$^+$ EVs (100 μg) 24 hours after cisplatin administration (n=3). (**A**) Imaging of fluorescence intensity of indicated organs at 12 hours after injection. (**B**) Representative confocal images showed DID-labeled IL-10$^+$ EVs in proximal tubules and distal tubules. scale bar, 10 μm. (**C**) Schematic diagram of the experimental design. In brief, mice were injected intraperitoneally with cisplatin (20 mg/kg) at d0 and then were treated with IL-10$^+$ EVs (200 μg) every 24 h. (**D**) Survival and (**E**) body weight (vs. 0 hour) over time (n=6). (**F**) Serum creatinine and (**G**) representative images of PAS staining and quantification of tubular injury score at 96 h (cisplatin, n=3; IL-10$^+$ EVs, n=5). scale bar, 50 μm. Data are presented as mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, two-tailed t-test.

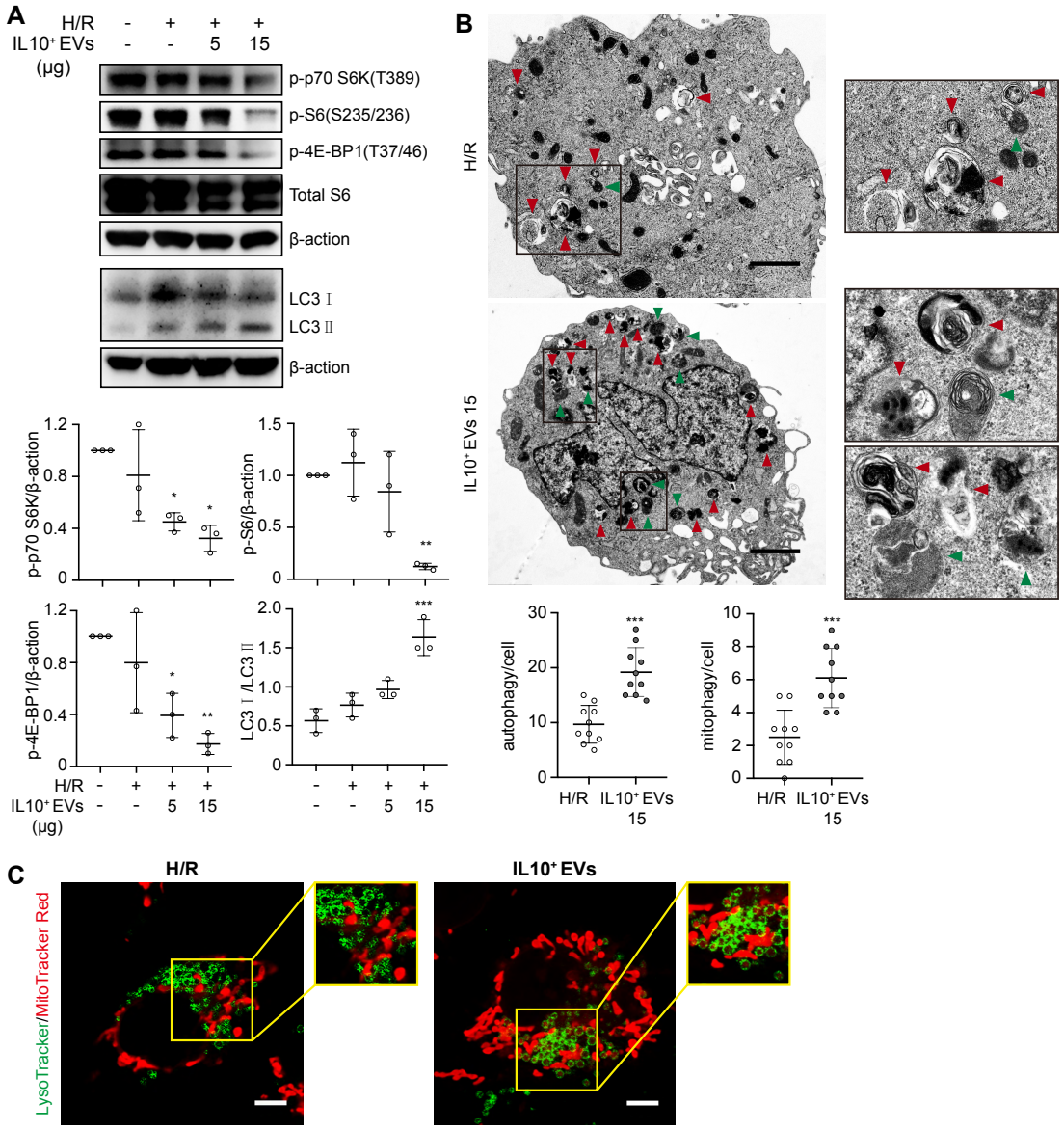

Figure S5 Induction of mitophagy after IL10$^+$ EVs treatment in vitro.

(**A**) Western blotting analysis of mTOR signaling and LC3 in TECs (n=3). (**B**) Representative TEM images of autophagic events in TECs. The number of autophagosomes and autolysosomes in each cell were quantified (n=3). Triangle, autophagy; Green triangle, mitophagy. Scale bar, 2 μm. Data are presented as mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, one-way ANOVA (A), two-tailed t-test (B). (**C**) Colocalization of lysosome and mitochondria in TECs was assessed using confocal microscopy. TECs were pre-stained with LysoTracker (Green) one day before H/R stimulation. Twelve hours later, TECs were stained with MitoTracker Red. Higher magnification of the indicated area (box) was shown in the right panels. Scale bar, 10 μm.

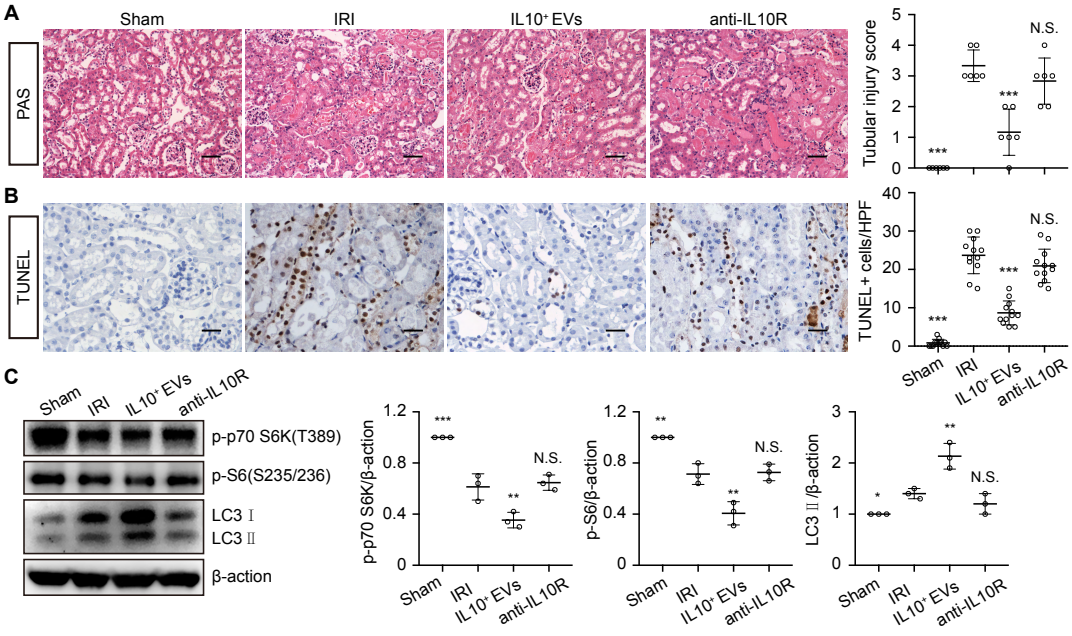

Figure S6 Blocking IL-10 signaling impairs the therapeutic effects of IL10$^+$ EVs. (**A**) Representative images of H&E staining and quantification of tubular injury (n=6). Scale bar, 100 μm. (**B**) Representative images of TUNEL staining and quantification of the apoptotic cells (n=4). Scale bar, 50 μm. (**C**) Western blotting analysis of mTOR signaling and LC3 in kidney tissues (n=3). Data are presented as mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. IRI group, one-way ANOVA.

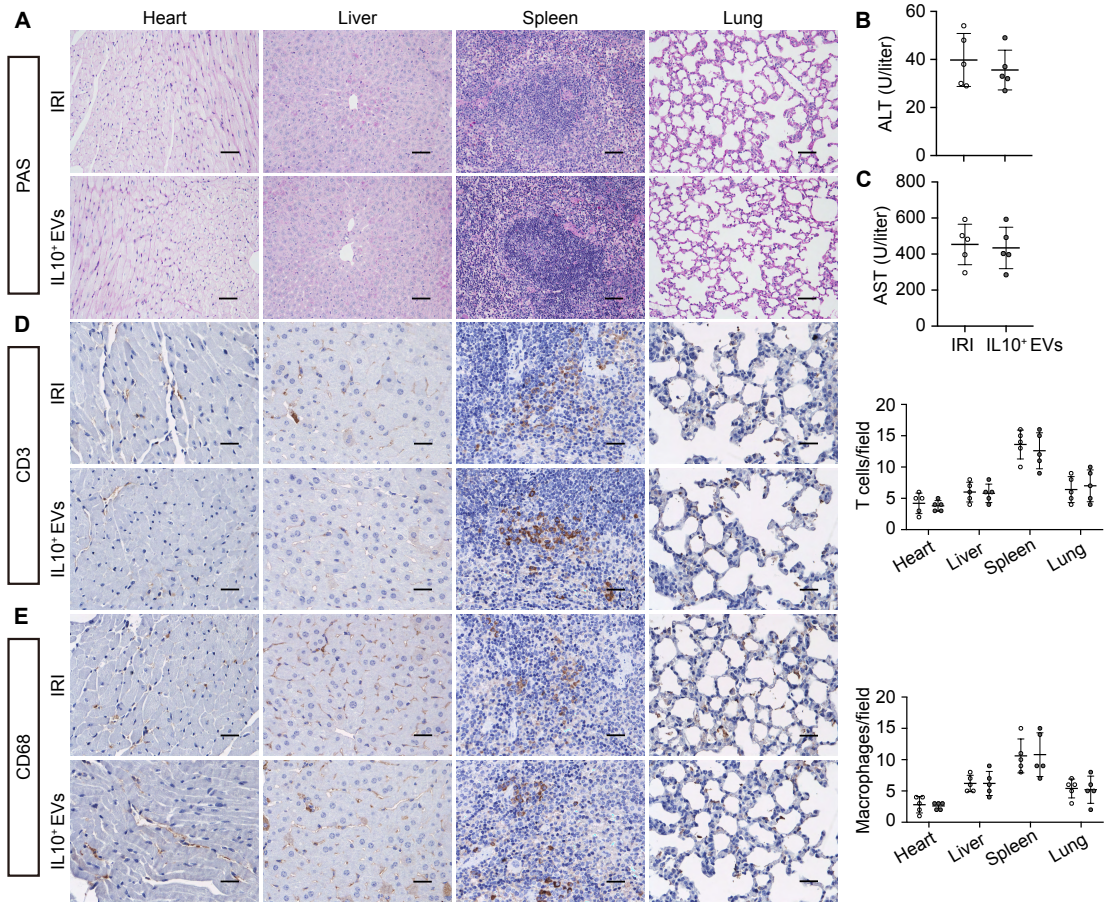

Figure S7 Assessment of toxicity and inflammation in IL-10⁺ EVs-treated mice.
(**A**) Representative images of PAS staining of major organs from untreated IRI mice or IL-10⁺ EVs-treated mice. Scale bar, 50 μm. Measurement of serum alanine aminotransferase (ALT) (**B**) and aspartate aminotransferase (AST) (**C**). Immunohistochemical analysis of CD3 T cells (**D**) and macrophages (**E**) in tissue sections. Scale bar, 50 μm. n=5. Data are presented as mean ± SD. two-tailed t-test.

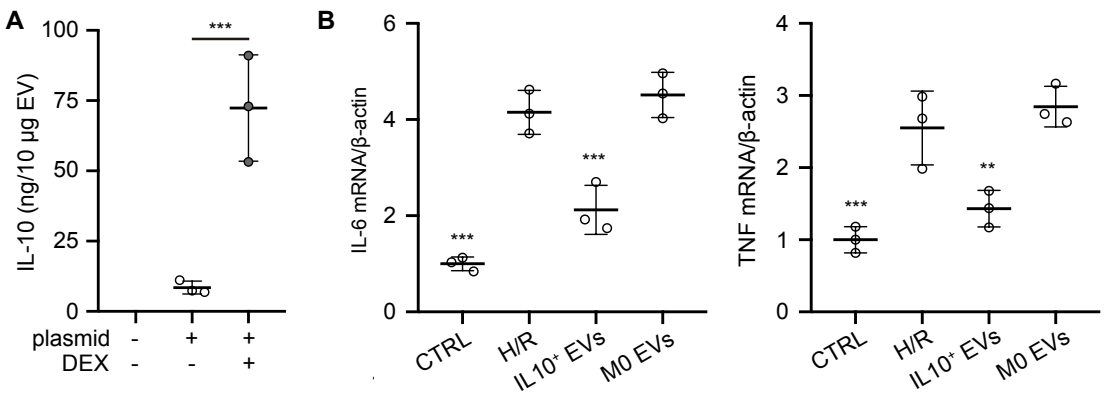

Figure S8 Manufacturing IL-10$^+$ EVs using primary cells (BMDM).

(**A**) ELISA analysis of IL-10 in BMDM-derived EVs. (**B**) Effects of BMDM-derived IL-10$^+$ EVs on the IL-6 and TNF mRNA levels in H/R-induced TECs. n=3. Data are presented as mean ± SD. ** $p<0.01$, *** $p<0.001$ vs. H/R group.

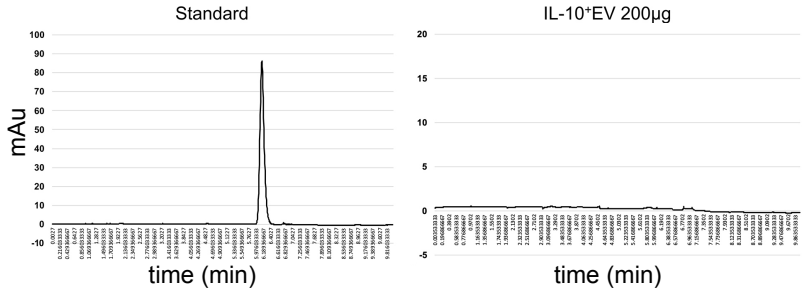

Figure S9 HPLC analysis of DEX in IL-10$^+$ EVs.

HPLC analysis showed there was no DEX encapsulated in IL-10$^+$ EVs.

Table 1 Primers used in this study.

Gene	Forward	Reverse
β-actin MUS	GGGAAATCGTGCGTGAC	AGGCTGGAAAAGAGCCT
CCL-2 MUS	TTGAGGTGGTTGTGGAAAAGG	GTGCTGACCCCAAGAAGGAAT
TNF-α MUS	AGACAGAGGCAACCTGACCAC	GCACCACCATCAAGGACTCAA
IL-1β MUS	GGTAAGTGGTTGCCCATCAGA	GTCGCTCAGGGTCACAAGAAA
IL-6 MUS	GTCACCAGCATCAGTCCCAAG	CCCACCAAGAACGATAGTCAA
CCL-5 MUS	CAGAATCAAGAAACCCTCTATCCTA	ACTCCCTGCTGCTTTGCCTAC
iNOS MUS	CAGATCGAGCCCTGGAAGAC	CTGGTCCATGCAGACAACCT
CD206 MUS	GTTCTGACTCTGGACACTTGC	TACTTGGACGGATAGATGGAG
Arg1 MUS	GCAGAGGTCCAGAAGAATGG	GGAGAAAGGACACAGGTTGC