Supplementary materials

Pyroptosis-preconditioned mesenchymal stromal cell-derived extracellular vesicles as advanced nanomedicines for treating inflammatory diseases

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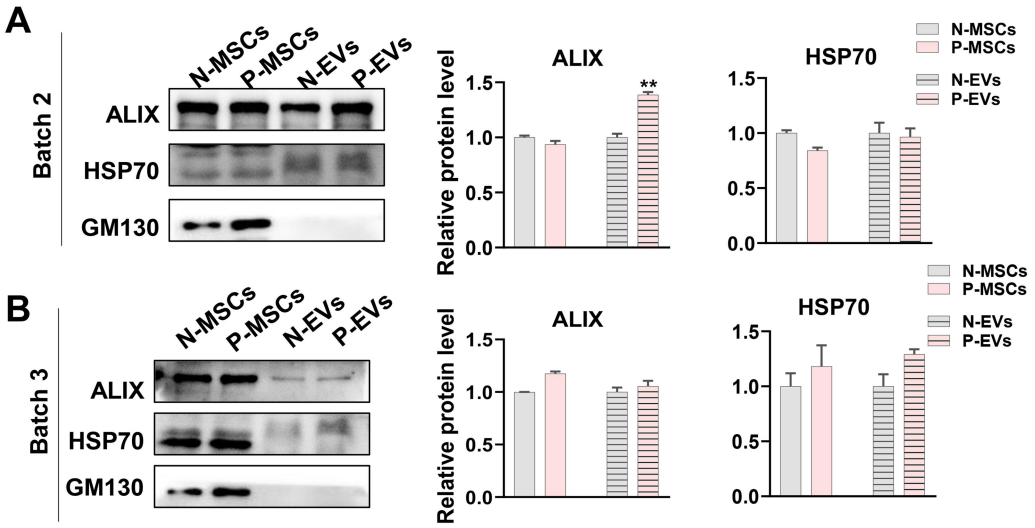

Figure S1 (A-B) Western blot analysis of EV-positive markers (ALIX and HSP70) and negative markers (GM130) in two batches cells and EVs (equal EV particle number, 4×10^9).

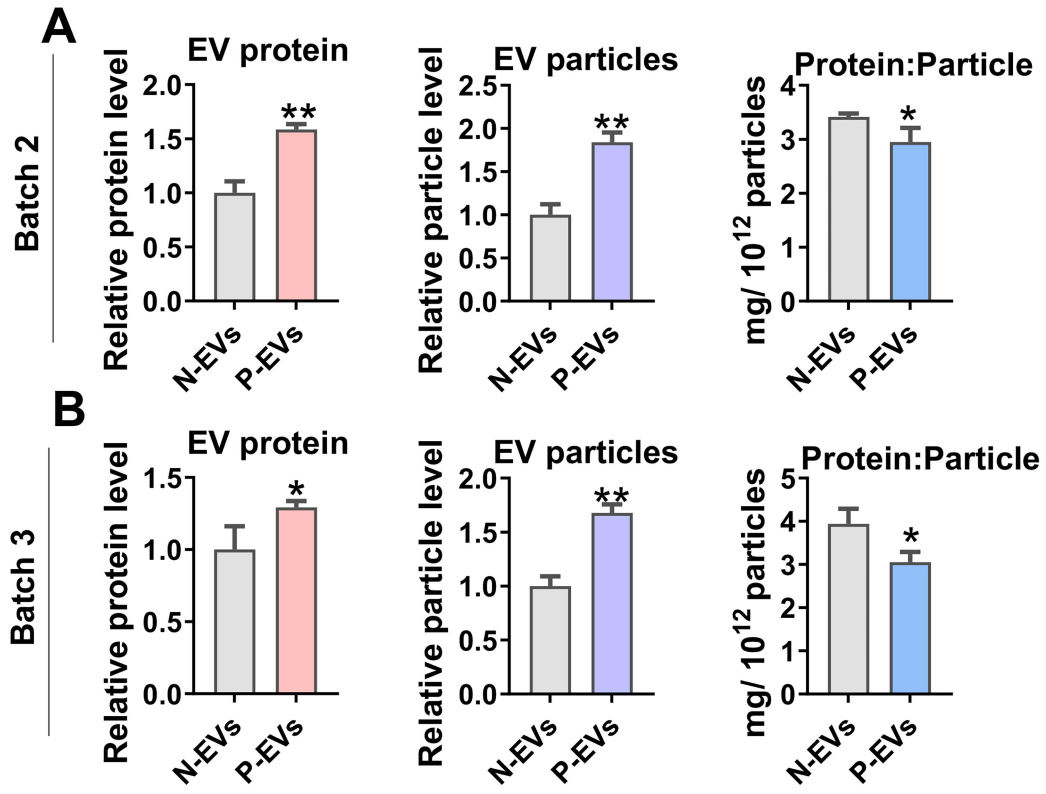

Figure S2 (A-B) Comparison of EV yield (quantified by protein amount or particles amount) and the protein-to-particle (P:P) ratio of N-EVs and P-EVs from two batches MSCs (n = 3 biological replicates, **p<0.01 vs the N-EV group).

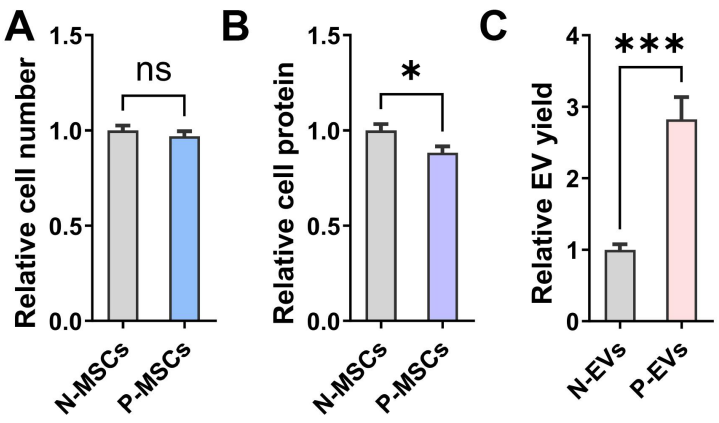

Figure S3 (A) Relative cell number of N-MSCs and p-MSCs at 48 h. (B) Relative cell protein mass of N-MSCs and p-MSCs at 48 h. (C) Relative EV yield (quantified by EV particle count per MSC) (n=3 biological replicates , *$p<0.05$ vs the N-MSCs group, ***$p < 0.001$ vs the N-EVs group)

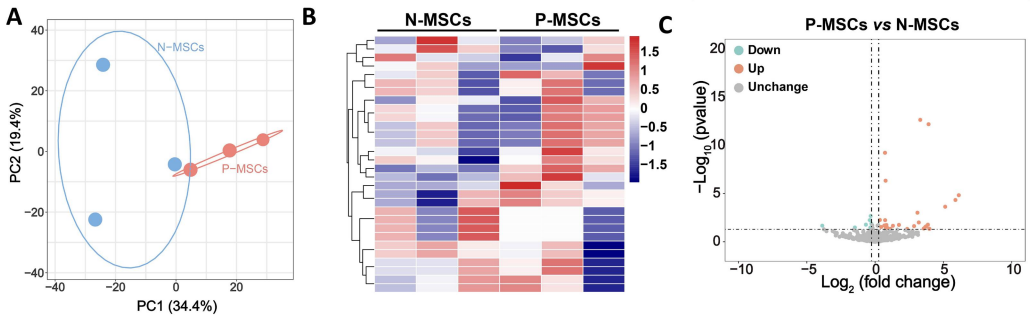

Figure S4 (A) PCA score plot of miRNAs in different groups representing discrepancies between N-MSCs and P-MSCs (n = 3 biological replicates). (B) Heatmap of miRNAs and the average expression curve above the heatmap (n = 3 biological replicates). (C) Volcano plots of miRNAs showing the significantly changed miRNAs (FC > 1.2 and p value < 0.05).

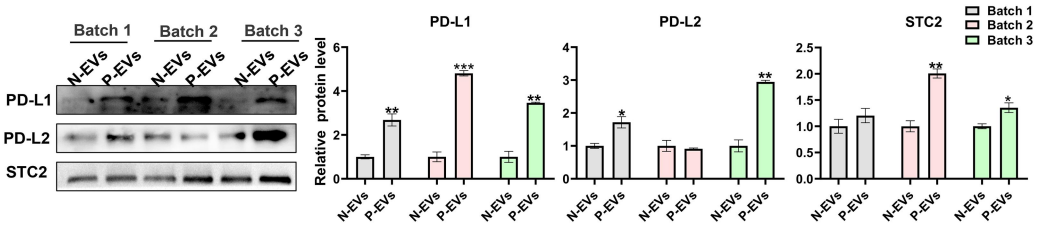

Figure S5 Western blot analysis of EV cargo (PD-L1, PD-L2 and STC2) in three batches cells derived EVs (equal particle number, 4×10^9, *p<0.05 vs the N-EVs group).

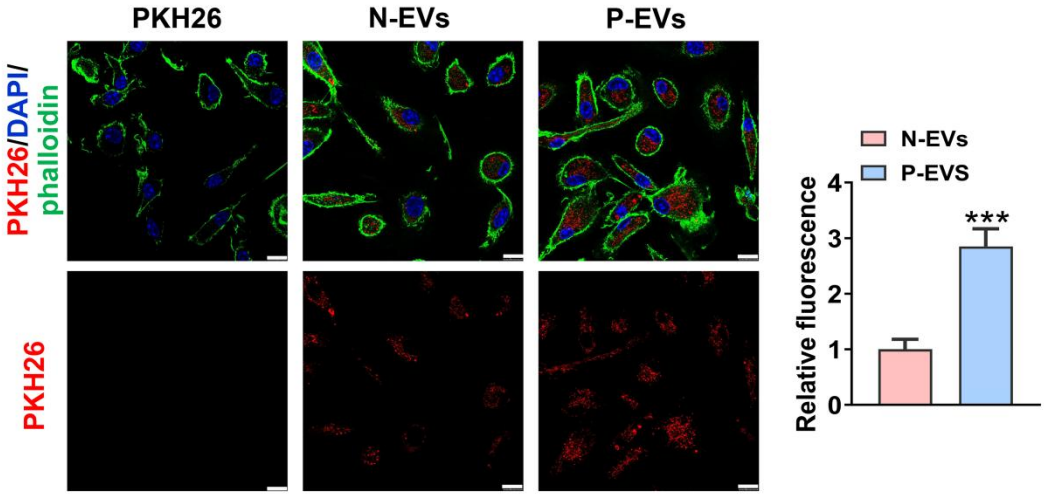

Figure S6 Representative micrographs of EV uptake by BMDMs. The cells were incubated with equal particle number (2×10^9 /mL) PKH26-labeled EVs (red) for 4 h and then stained with FITC-phalloidin (green) and DAPI (blue) (scale bar = 50 μm, n=3 biological replicates using the average of technical duplicates for each, 6 fields of view were used for biological replicate, *$p<0.05$ vs the N-EVs group).

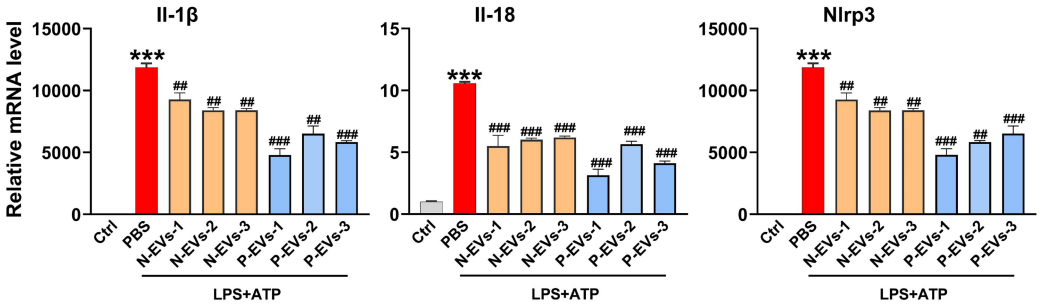

Figure S7 qRT-PCR analysis of mRNA (IL-1β, IL-18 and NLRP3) in BMDMs from different groups, BMDMs were pretreated with equal particle number (2×10^9 /mL) N-EVs or P-EVs from three batches MSCs for 30 min and then stimulated with LPS (100 ng/mL) for 6 h and ATP (5 mM) for 1 h. (n=3 biological replicates, *$p<0.05$ vs the Ctrl group, #$p < 0.05$ vs the LPS + ATP group).

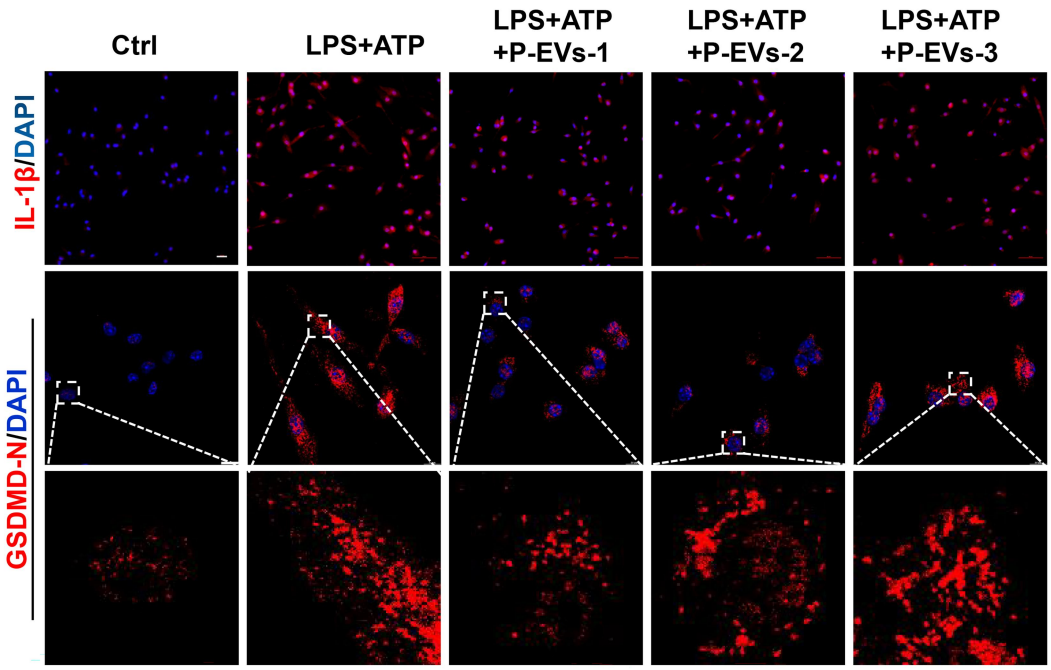

Figure S8 Immunofluorescence analysis of IL-1β and GSDMD-N expression in BMDMs, BMDMs were pretreated with equal particle number (2×10^9 /mL) P-EVs from three batches MSCs for 30 min and then stimulated with LPS (100 ng/mL) for 6 h and ATP (5 mM) for 1 h. (scale bar = 20 μm for IL-1β staining, scale bar = 10 μm for GSDMD-N staining).

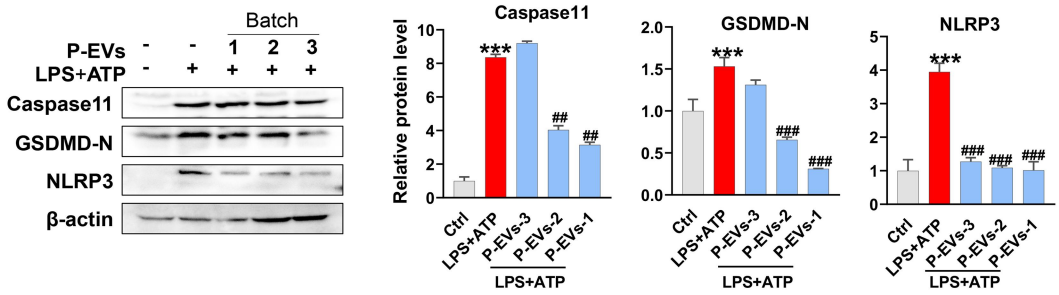

Figure S9 Western blot analysis and quantification of pyroptosis-related proteins (NLRP3, Caspase11 and GSDMD-N) in BMDMs from different groups, BMDMs were pretreated with equal particle number (2×10^9 /mL) P-EVs from three batches MSCs for 30 min and then stimulated with LPS (100 ng/mL) for 6 h and ATP (5 mM) for 1 h. (n=3 biological replicates , *p<0.05 vs the Ctrl group, #p < 0.05 vs the LPS + ATP group).

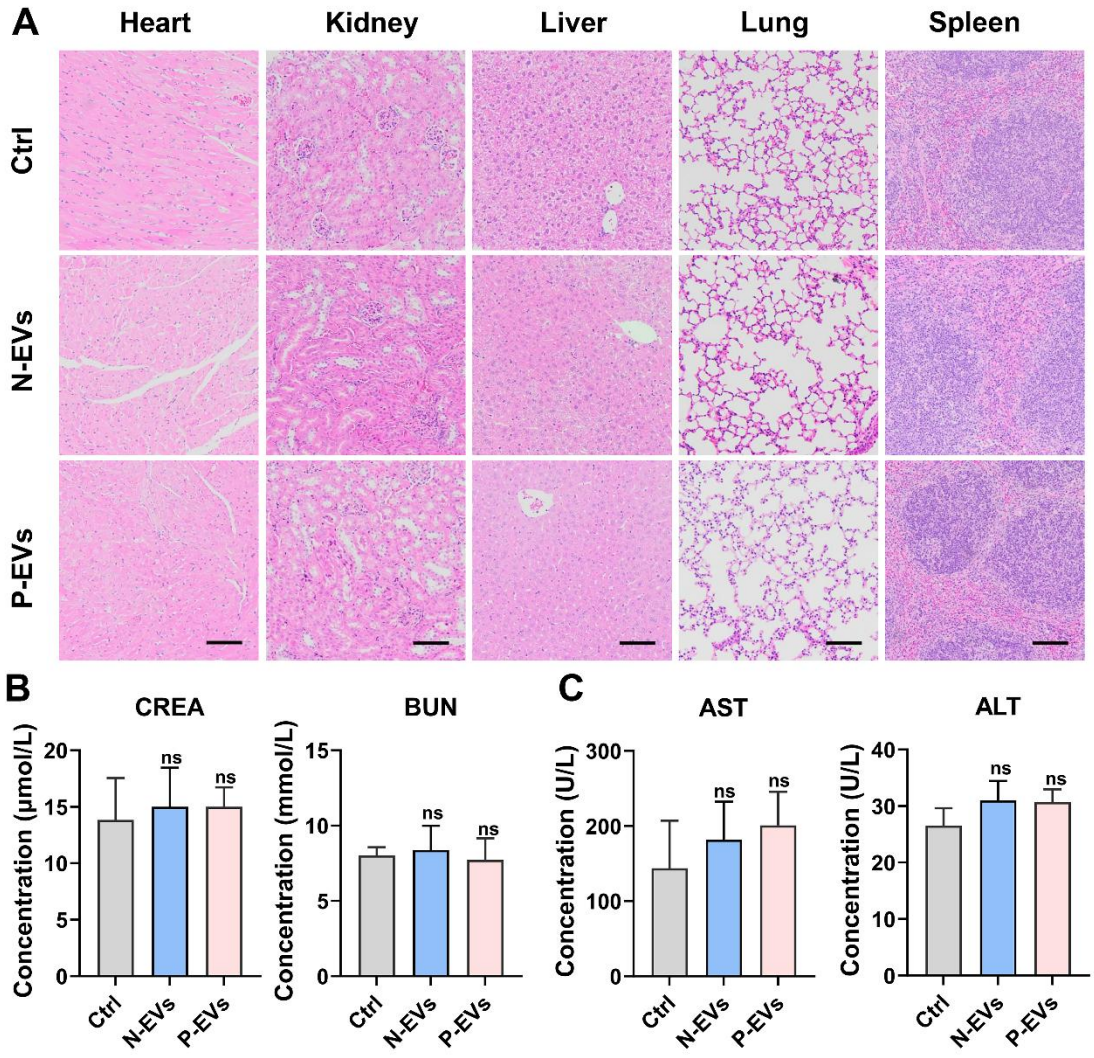

Figure S10 Biosafety assays of MSC-EVs *in vivo*. (A) Representative H&E staining micrographs of heart, kidney, lung, liver and spleen sections at 24 h after N-EV or P-EV injection (scale bar =100 μm). (B) Serum ALT, AST, CREA and BUN levels in mice at 24 h after injection of N-EVs or P-EVs (n = 6 mice).

Table S1 Real-time PCR primers used in the study

Gene	Sequence 5'-3'	Species
Il-1β	Forward: CACCTCTCAAGCAGAGCACAG	Mouse
	Reverse: GGGTTCCATGGTGAAGTCAAC	
Icam-1	Forward: ACCCAACTGGAAGCTGTTTG	Mouse
	Reverse: CACACTCTCCGGAAACGAAT	
Il-18	Forward: TGTTCTTACAGGAGAGGGTAGAC	Mouse
	Reverse: GACAGCCTGTGTTCGAGGATATG	
Tnf-α	Forward: ACGGCATGGATCTCAAAGAC	Mouse
	Reverse: AGATAGCAAATCGGCTGACG	
Gsdmd	Forward: CGATGGGAACATTCAGGGCAGAG	Mouse
	Reverse: ACACATTCATGGAGGCACTGGAAC	
Kim-1	Forward: AGGAAGTCAGCATCTCTAAGCG	Mouse
	Reverse: ACACAGAAAATCGCCTTGGC	
Nlrp3	Forward: TGTTCTTTATCCACTGCCGAG	Mouse
	Reverse: TCCCCAATGTGCTCGTCAA	
Rps18	Forward: TTCGCCATCACTGCCATTAAGGG	Mouse
	Reverse: ATCACTCGCTCCACCTCATCCTC	