

Supplementary Materials

Multiorgan repair by MSC-derived extracellular vesicles in hepatorenal syndrome through necroptosis alleviation, immune reprogramming and fibrosis resolution

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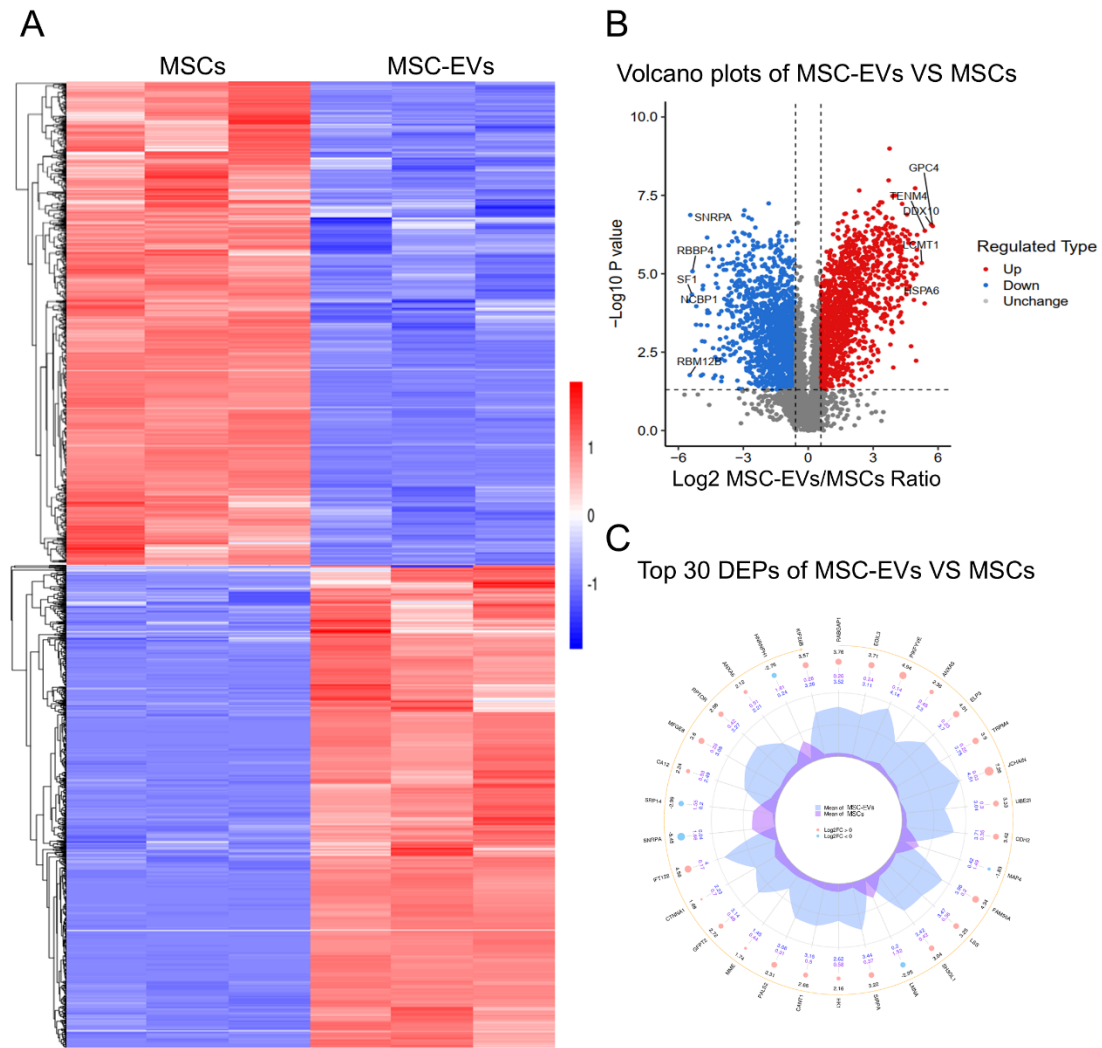
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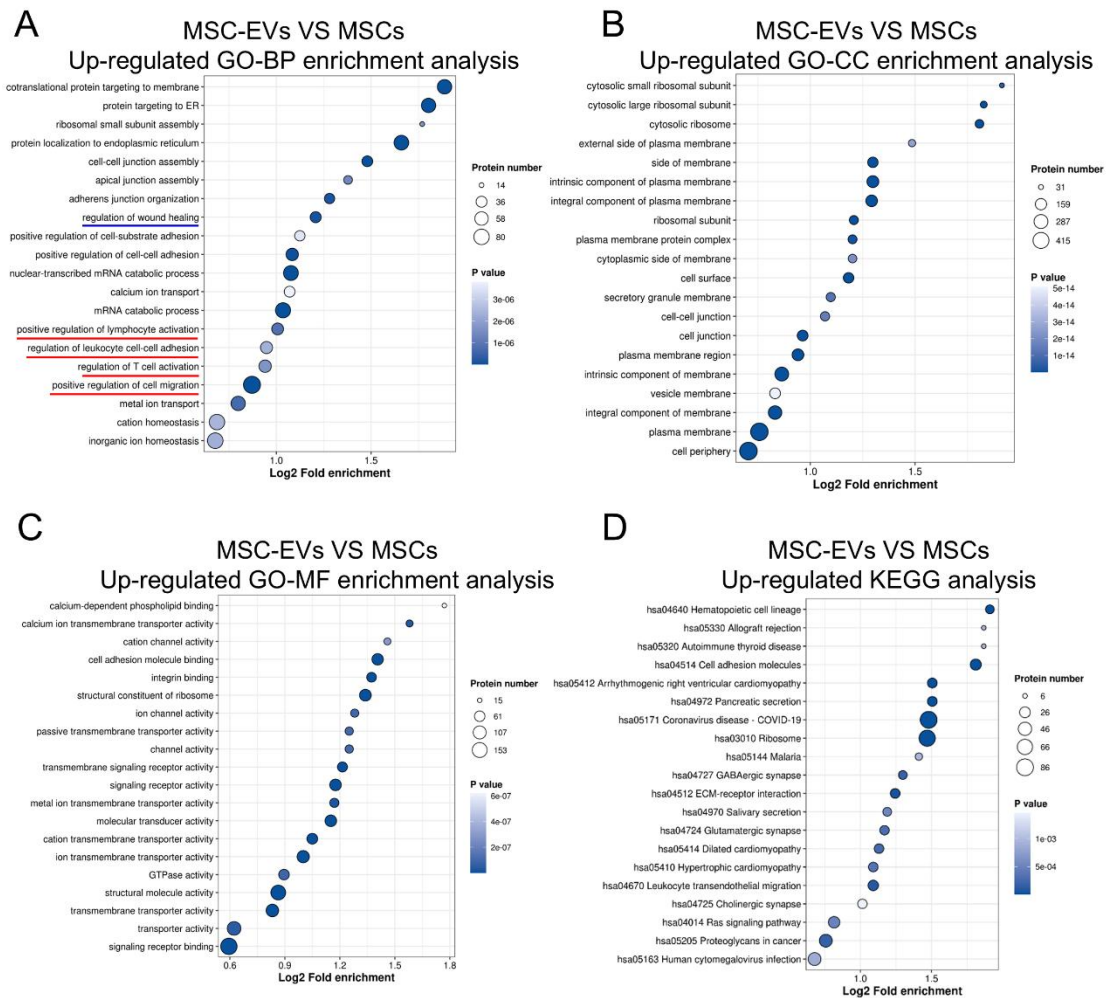
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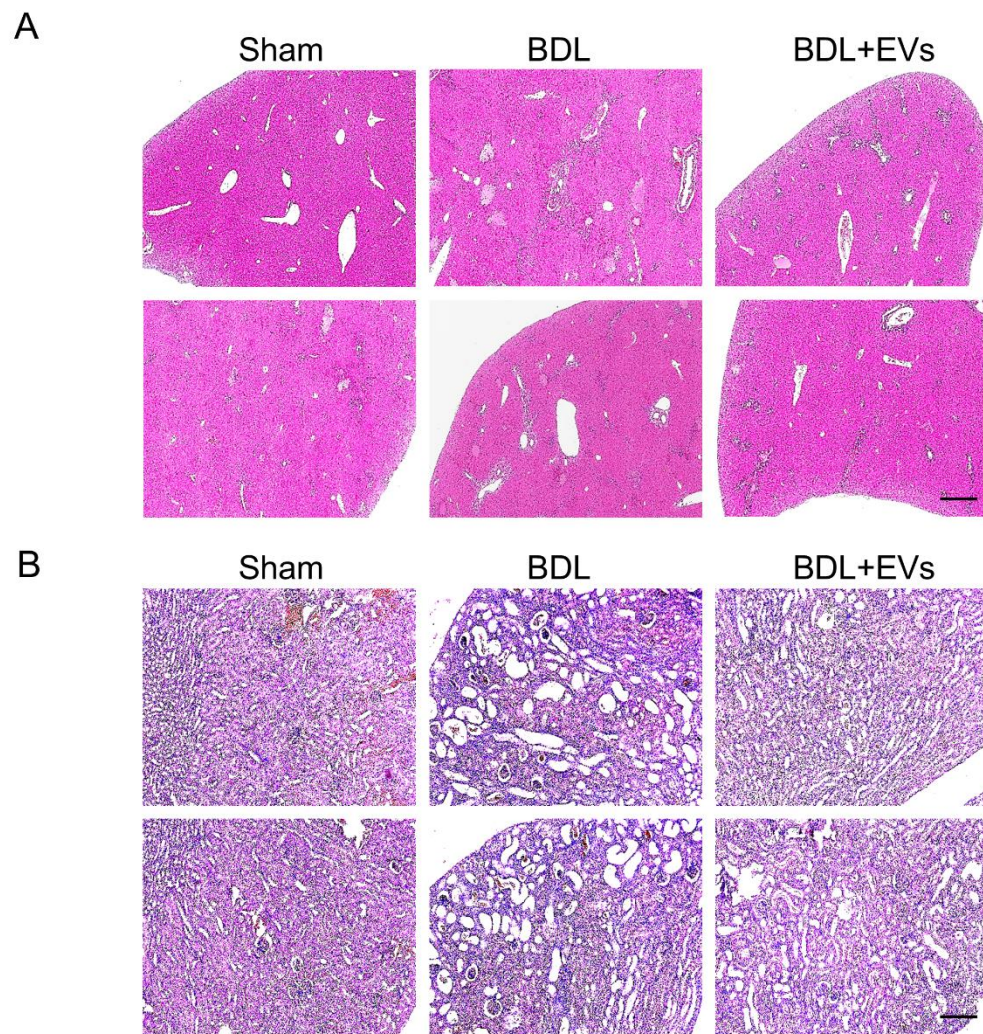
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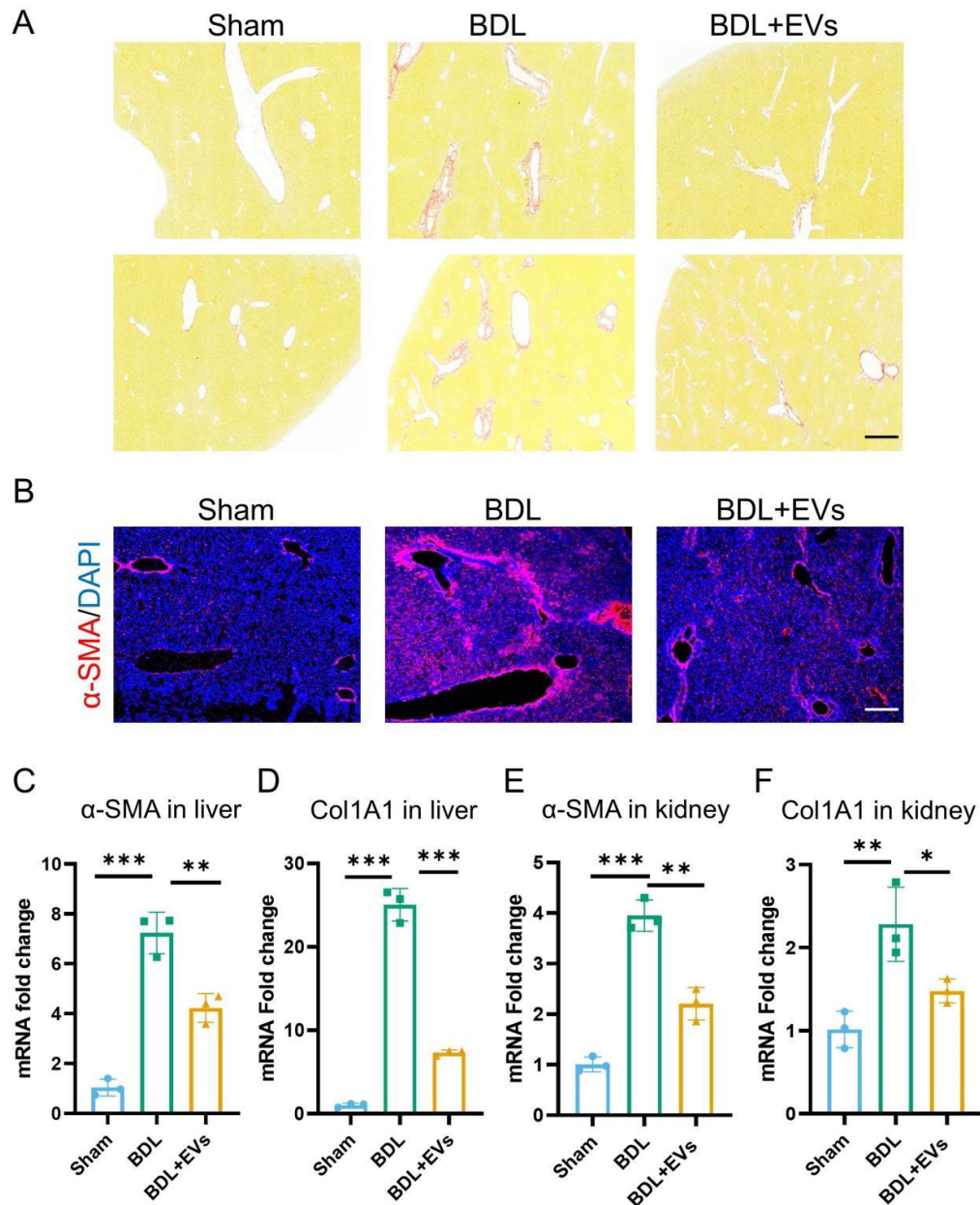
Supplementary Figure 1. Proteomic analysis of MSC-EVs vs. MSCs. (A) Heatmap showing significantly upregulated (red dots) and downregulated (blue dots) proteins in MSC-EVs, compared to MSCs; (B) Volcano plot showing significantly upregulated (red dots) and downregulated (blue dots) proteins in MSC-EVs, compared to MSCs; (C) Radar map showing top 30 DEPs of MSC-EVs vs. MSCs.



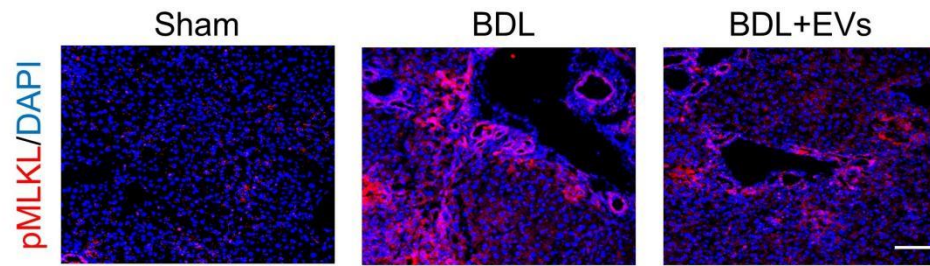
Supplementary Figure 2. Upregulated proteomic enrichment analysis of MSC-EVs vs. MSCs. (A) GO-BP enrichment analysis of top 20 up-regulated proteins in MSC-EVs; (B) GO-CC enrichment analysis of top 20 up-regulated proteins in MSC-EVs; (C) GO-MF enrichment analysis of top 20 up-regulated proteins in MSC-EVs; (D) KEGG enrichment analysis of top 20 up-regulated proteins in MSC-EVs.



Supplementary Figure 3. MSC-EVs attenuate histopathological damages in liver and kidney. (A) Histological examination of liver tissue morphology by H&E staining (scale bar = 50 μ m); (B) Histological examination of kidney tissue morphology by H&E staining (scale bar = 50 μ m).



Supplementary Figure 4. MSC-EVs reduce fibrosis in liver and kidney. (A) Histological examination of liver tissue morphology by Sirius red staining (scale bar = 50 μ m); (B) α -SMA (red) and DAPI (blue) co-immunostaining of Sham, BDL and EV treatment groups in liver (scale bar = 50 μ m); (C) qRT-PCR analysis of the mRNA expression levels of α -SMA in liver ($n = 3$); (D) qRT-PCR analysis of the mRNA expression levels of *COL1A1* in liver ($n = 3$); (E) qRT-PCR analysis of the mRNA expression levels of α -SMA in kidney ($n = 3$); (F) qRT-PCR analysis of the mRNA expression levels of *COL1A1* in kidney ($n = 3$). Data are presented as mean \pm SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.



Supplementary Figure 5. MSC-EVs ameliorate liver necroptosis. pMLKL (red) and DAPI (blue) co-immunostaining of Sham, BDL and EV treatment groups in liver (scale bar =100 μ m).

Supplementary Table 1. Primers for qRT-PCR

| Gene name | Gene primers |
|--------------------------------|--|
| <i>GAPDH</i> | F: 5'-TGACCTCAACTACATGGTCTACA-3' R: 5'-CTTCCCATTCTCGGCCTTG-3' |
| <i>IL-17</i> | F: 5'-CTCCAGAAGGCCCTCAGACTAC-3 R: 5'-GGGTCTTCATTGCGGTGG-3' |
| <i>α-SMA</i> | F:5'-GTACCACCATGTACCCAGGC-3' R:5'-GCTGGAAGGTAGACAGCGAA -3' |
| <i>COL1A1</i> | F:5'-CGACCTCAAGATGTGCCACT-3' R:5'-GCAGTAGACCTTGATGGCGT-3' |