

Supplementary Information

Non-invasive profiling of exosomal miRNA and protein biomarkers from vaginal discharge for the early detection of preterm labor

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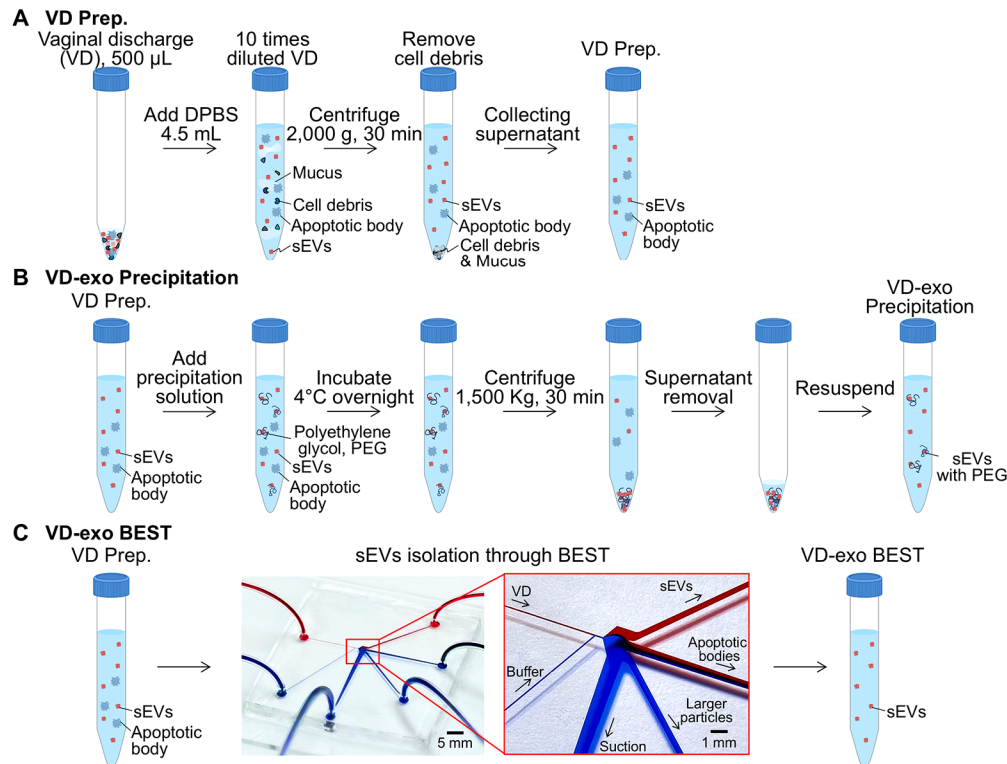
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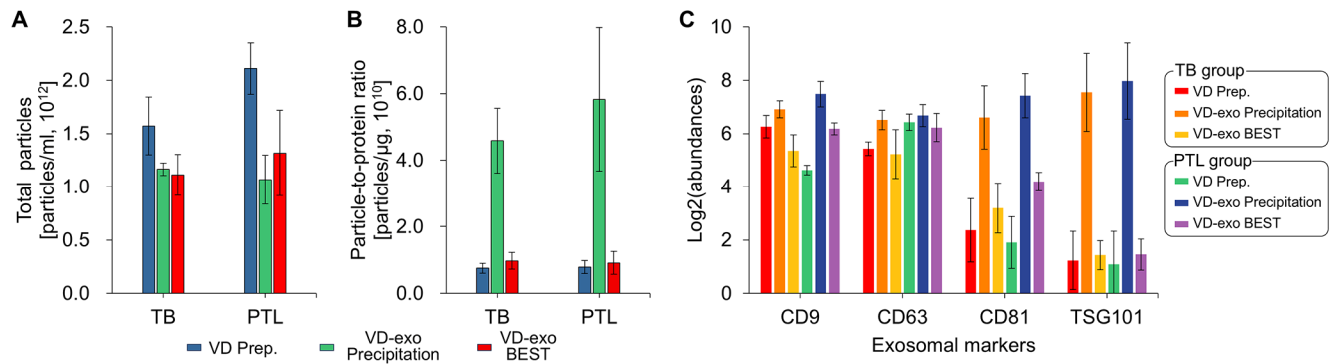
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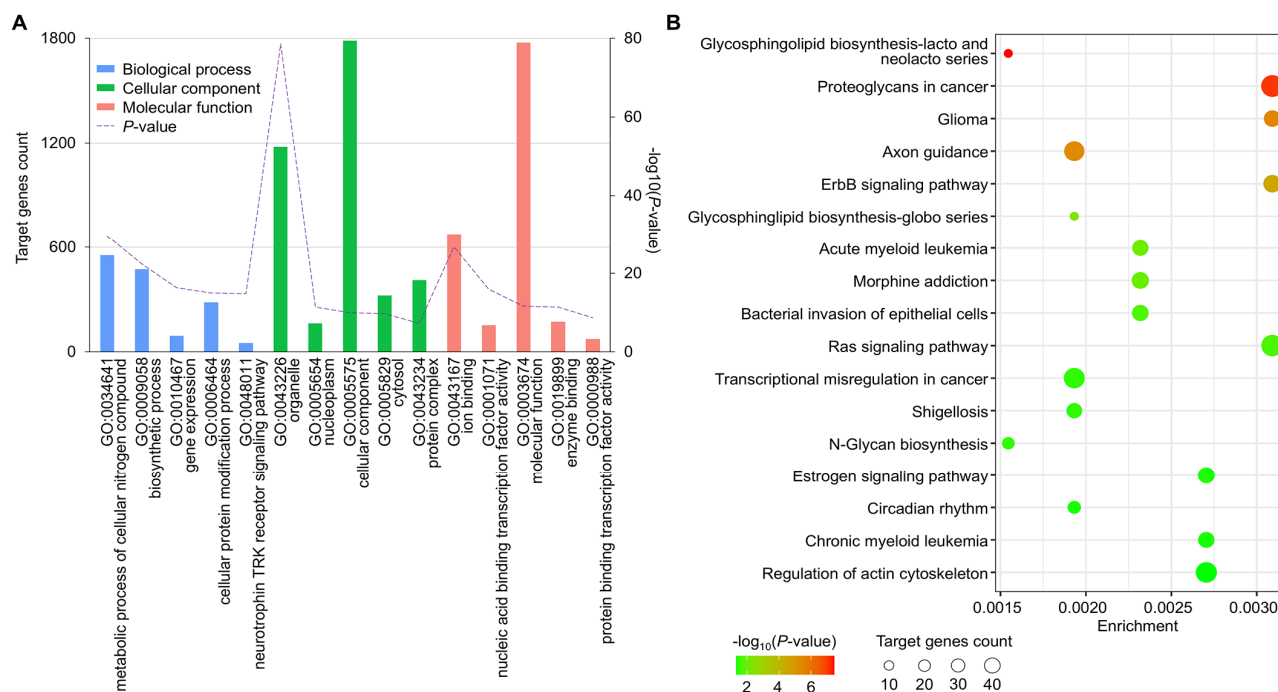
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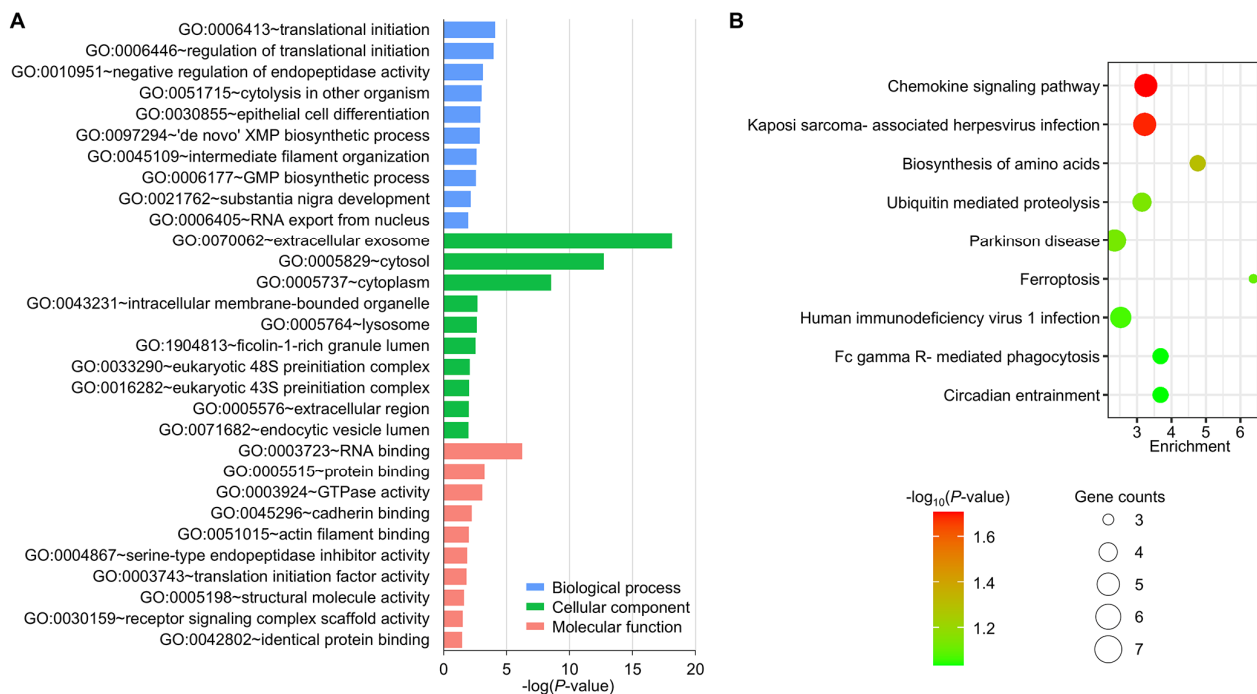
Supplementary Fig. 1 Sample preparation and small extracellular vesicles (sEVs) isolation from vaginal discharge. (A) Schematic of the preparation of vaginal discharge samples (VD Prep.). The viscosity of vaginal discharge was reduced by performing a 10-fold dilution in Dulbecco's Phosphate-Buffered Saline (DPBS). Subsequently, the diluted sample was centrifuged at $2,000 \times g$ for 30 minutes to remove whole cells and cell debris. (B) sEVs are isolated by adding polyethylene glycol (PEG) solution, resulting in the sample labeled VD-exo Precipitation. (C) sEV isolation using the BEST (Biologically intact exosome separation technology). The isolated sEV is referred to as VD-exo BEST.



Supplementary Fig. 2 Characterization of sEVs isolated from vaginal discharge using different isolation methods. (A) Total particle concentration measured by nanoparticle tracking analysis (NTA) in vaginal discharge samples. (B) Particle-to-protein ratio sEV preparations obtained using different isolation methods. (C) Relative abundances of representative sEV-associated proteins (CD9, CD63, CD81, and TSG101) quantified by proteomics in samples from term birth (TB) and preterm labor (PTL) groups. VD Prep., pretreated vaginal discharge; VD-exo Precipitation, sEVs isolated using the precipitation method from VD Prep.; VD-exo BEST, sEVs isolated using the BEST method from VD Prep.



Supplementary Fig. 3 Functional enrichment analysis of differentially expressed miRNAs in PTL compared to TB. (A) Gene Ontology (GO) enrichment analysis of differentially expressed miRNA from VD-derived sEVs of TB and PTL. The top 5 most significant GO terms of miRNA target genes were listed in three categories: biological process, cellular component, and molecular function, respectively. (B) Scatter plot for KEGG pathway enrichment of miRNA target genes. Enrichment is calculated as the ratio of differentially expressed miRNAs to all miRNAs annotated within a specific pathway term. Larger dots represent a higher count of target genes, and the red coloration indicates a more notable significance in terms of P-values. KEGG, Kyoto encyclopedia of genes and genomes.



Supplementary Fig. 4 Functional enrichment analysis of differentially expressed proteins in PTL compared to TB. (A) The bar plot shows the top significantly enriched GO terms associated with differentially expressed proteins in PTL compared to TB. The GO terms are categorized into three domains: biological processes (blue), cellular components (green), and molecular functions (red). (B) The bubble plot displays the KEGG pathway enrichment analysis of the differentially expressed proteins. The size of each bubble corresponds to the number of genes involved in the pathway, while the color gradient represents the $-\log(P\text{-value})$, with red indicating higher significance. The most significantly enriched terms include those related to chemokine signaling, viral infections, and biosynthesis of amino acids.