YMTHE, Volume *30*

Supplemental Information

Apoptotic vesicles activate autophagy in recipient cells to induce angiogenesis and dental pulp regeneration

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Supplementary Figures

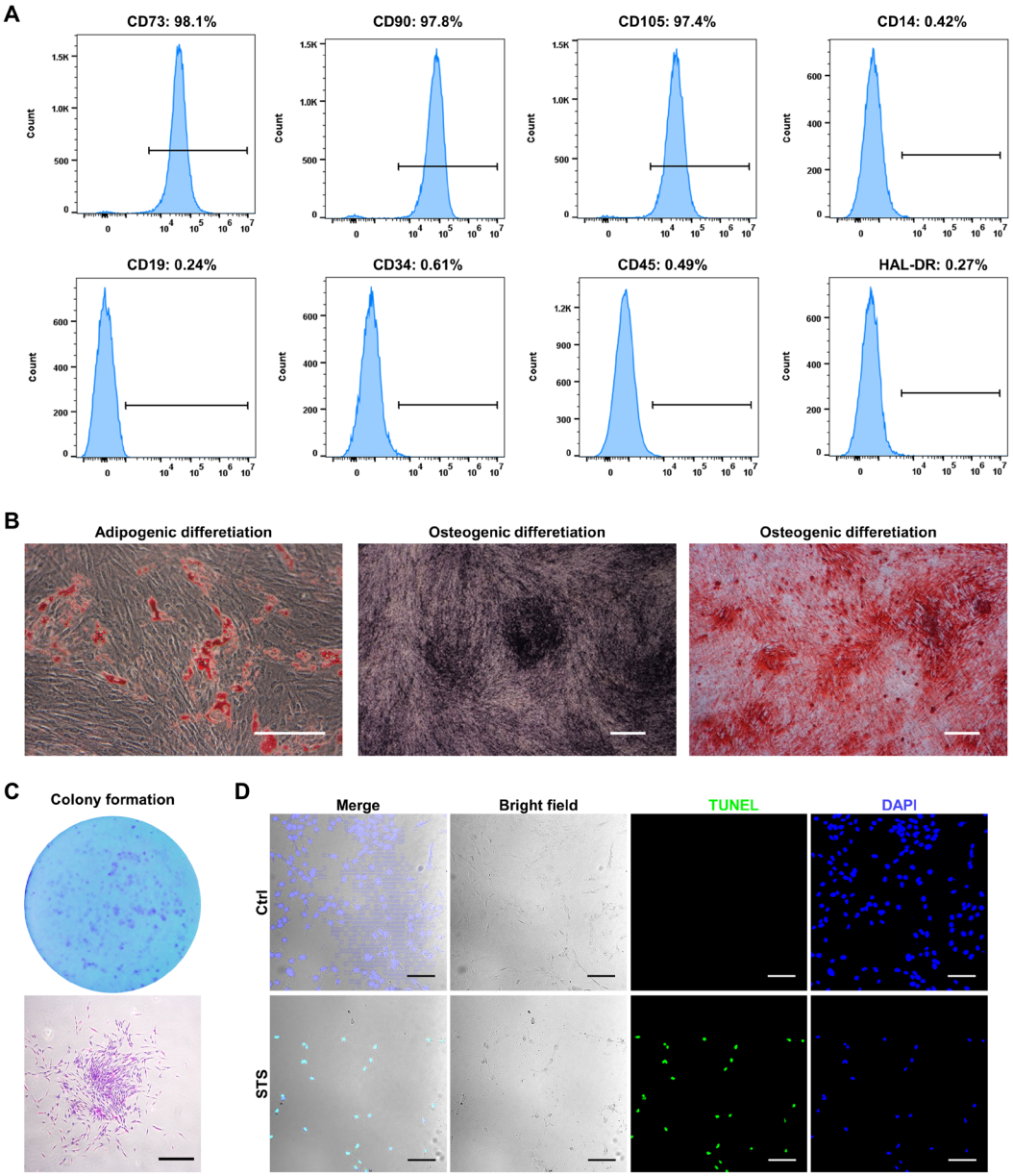

Figure S1. Characterization of human deciduous pulp stem cell (hDPSC).

(A) Flow cytometric analysis revealed positive expression of CD73, CD90, CD105, and negative expression of CD13, CD19, CD34, CD45 and HAL-DR. **(B)** Oil red O staining, alkaline phosphatase staining and Alizarin red S staining of hDPSCs after being induced in adipogenic or osteogenic medium. Scale bar, 250 μm. **(C)** Crystalline violet staining showed the colony formation ability of hDPSCs. Scale bar, 250 μm. **(D)** Representative bright field and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (green) staining images of hDPSCs and staurosporine (STS)-induced apoptotic hDPSCs, counterstained by DAPI (blue). Scale bars, 100 μm (TUNEL staining).

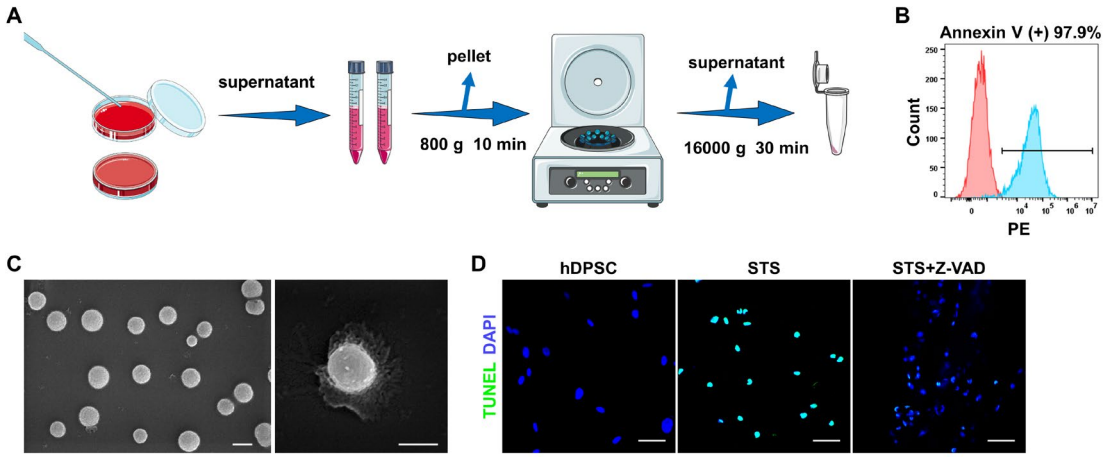

Figure S2. Isolation, identification of apoptotic vesicles derived from hDPSC (hDPSC-apoVs).

(A) Flow chart showed the protocol of isolate apoptotic extracellular vesicles from hDPSC. **(B)** Flow cytometric analysis of Annexin V staining in apoVs. **(C)** Representative wide scanning electron microscope (SEM) image showed the morphology of apoVs. Scale bar, 200 nm. **(D)** Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (green) staining images of hDPSCs pretreated with Z-VAD, counterstained by DAPI (blue). Scale bars, 50 μm. (TUNEL staining).

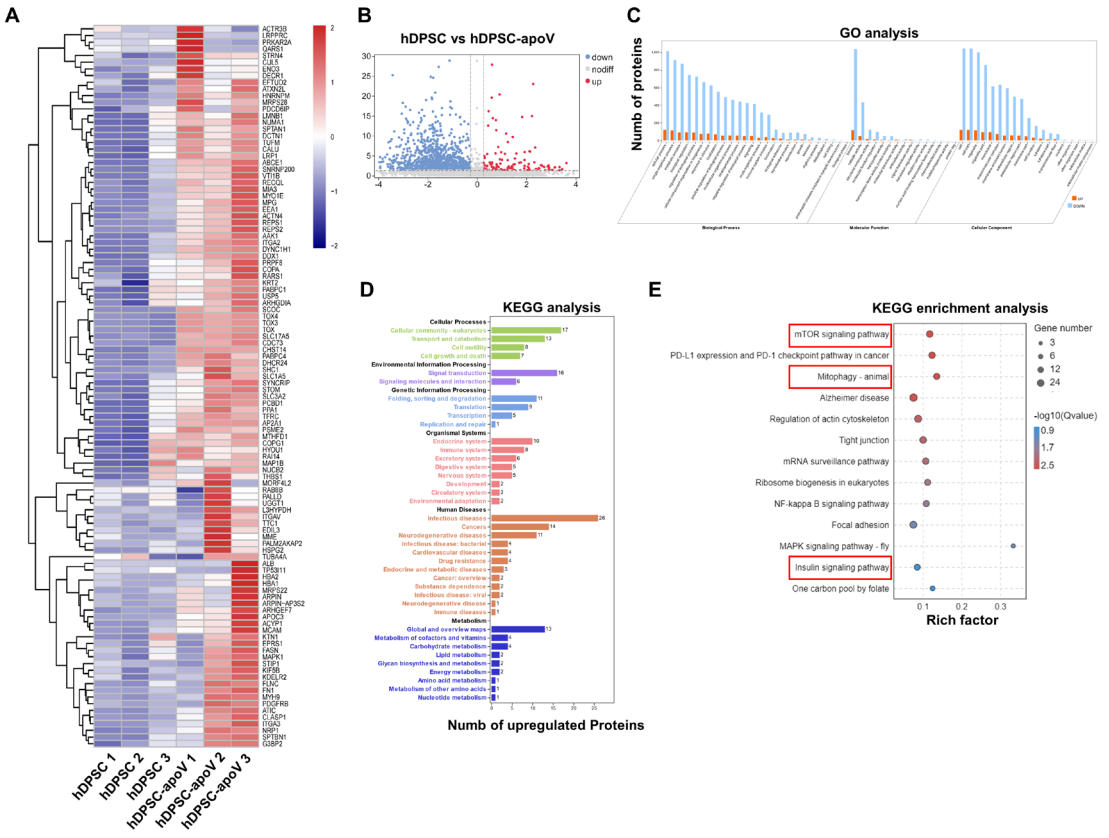

Figure S3. Proteomic analysis of hDPSC and hDPSC-apoVs.

(A) Hierarchical clustering of differentially expressed proteins (DEPs) (Fold change > 1.5 and P value < 0.05) between hDPSC and hDPSC-apoV, with protein abundance being Z-score normalized. Rows represent proteins and columns represent individual replicates. **(B)** Volcano plot showed significantly upregulated (red dots) and downregulated (blue dots) proteins in apoVs, compared to hDPSCs. **(C)** Gene ontology (GO) analysis of significantly differentially expressed proteins in apoVs, categorized into “Cellular component”, “Molecular function” and “Biological process”. **(D)** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of significantly upregulated proteins in apoVs. **(E)** KEGG pathway enrichment analysis of upregulated proteins in apoVs. The enriched KEGG pathways were presented as a bubble chart. The Y-axis represents KEGG pathways and the X-axis represents rich factor. The color of the bubble represented enrichment significance and the size of the bubble represented the number of upregulated proteins.