Supplementary Figure legend

Figure S1. Late-passage hMSCs exhibit enhanced senescence phenotype compared to early-passage cells

(A) Increased SA-β-gal activity in late-passage MSCs. The percentage of SA-β-gal-positive cells was quantified in passage 4 (P4) and passage 8 (P8) hMSCs. Data represent mean ± SEM; n = 3 biological replicates.

(B) Reduced cell proliferation in late-passage MSCs. Cell viability of P4 and P8 hMSCs was assessed by CCK-8 assay measuring absorbance at 450 nm. Data represent mean ± SEM; n = 3 biological replicates.

(C) Elevated senescence marker expression in late-passage MSCs. Relative mRNA levels of *CDKN1A* (p21) were quantified by quantitative RT-PCR in P4 and P8 hMSCs. Data represent mean ± SEM; n = 3 biological replicates.

Figure S2. Analysis of hMSCs treated with various concentrations of DOXO

(A) Dose-dependent cytotoxicity of doxorubicin (DOXO). Cell viability of hMSCs treated with 0, 50, 100, or 200 nM DOXO for 6 days was assessed by CCK-8 assay measuring absorbance at 450 nm. Untreated cells (Blank) served as control. Data represent mean ± SEM; n = 3 biological replicates.

(B) Time-course induction of senescence by DOXO. Representative SA-β-gal staining images of hMSCs treated with 100 nM DOXO or left untreated (Blank) at days 2, 4, and 6. Increased blue staining indicates progressive senescence induction.

(C) Time-dependent upregulation of senescence markers. Relative mRNA levels of *CDKN1A* (p21) were quantified by quantitative RT-PCR in hMSCs treated with 100 nM DOXO at days 2, 4, and 6. Data represent mean ± SEM; n = 3 biological replicates.

(D) Passage-dependent senescence inducibility. Relative mRNA levels of *CDKN1A* (p21) were measured by quantitative RT-PCR in passage 4 (P4) and passage 8 (P8) hMSCs following 100 nM DOXO treatment for 6 days. Data represent mean ± SEM; n = 3 biological replicates.

(E) Purity assessment of recombinant proteins. Purified EGFP, GAPDH, S100A8, and S100A9 proteins expressed in *E. coli* were resolved by SDS-PAGE and visualized by

Coomassie brilliant blue staining.

(F) Immunoblot validation of His-tagged recombinant proteins. Western blot analysis using anti-His tag antibody confirmed the presence of His-tagged EGFP, GAPDH, S100A8, and S100A9. Note the dimeric form of S100A8/S100A9 (indicated by asterisk).

Figure S3: GAPDH/S100A8/S100A9 protein combination attenuates rplicative senescence in human foreskin fbroblasts (HFF-1)

(A-B) Time-dependent suppression of senescence markers in HFF-1 cells. Relative mRNA levels of *CDKN2A* (p16), *CDKN1A* (p21), and *IL6* were quantified by quantitative RT-PCR at day 6 (A) and day 12 (B) following treatment with EGFP (control) or Pros (GAPDH/S100A8/S100A9, 200 nM). Data represent mean ± SEM; n = 3 biological replicates.

(C) Reduced SA-β-gal activity in Pros-treated HFF-1 cells. The percentage of SA-β-gal-positive cells was quantified in HFF-1 cells treated with EGFP or Pros (200 nM) for 12 days. Data represent mean ± SEM; n = 3 biological replicates.

(D) Enhanced cell viability in Pros-treated HFF-1 cells. Cell viability was assessed by CCK-8 assay measuring absorbance at 450 nm in HFF-1 cells treated with EGFP or Pros (200 nM) for 12 days. Data represent mean ± SEM; n = 3 biological replicates.

(E) Increased proliferative capacity in Pros-treated HFF-1 cells. Representative EdU incorporation images (left) and quantification of EdU-positive cells (right) in HFF-1 cells treated with EGFP or Pros (200 nM) for 12 days. Data represent mean ± SEM; n = 3 biological replicates.

Figure S4. Transcriptional features of Pros-treated hMSCs based on the RNA-seq analysis

(A) Differential gene expression in Pros-treated versus untreated hMSCs. Volcano plot showing differentially expressed genes (DEGs) in hMSCs treated with the protein combination (Pros: GAPDH/S100A8/S100A9, 200 nM) versus untreated control (Blank) for 20 days. Significantly upregulated genes ($\log_2$ fold change > 1, adjusted $P < 0.05$) are shown in red; significantly downregulated genes ($\log_2$ fold change < −1, adjusted $P < 0.05$) are shown in blue.

(B) Minimal transcriptional impact of EGFP treatment. Volcano plot showing DEGs in EGFP-treated (200 nM) versus untreated (Blank) hMSCs after 20 days. Note the substantially fewer DEGs compared to Pros treatment (panel A), confirming EGFP as an appropriate negative control.

(C) Transcriptome clustering analysis. Pearson correlation heatmap depicting transcriptional similarity among hMSCs treated with Pros, EGFP-treated hMSCs, and senescent hMSCs at passage 9 (P9) and passage 12 (P12). Distinct clustering of Pros-treated samples indicates substantial transcriptomic reprogramming compared to controls and senescent cells.