Supplementary Materials

SOD3 Suppresses the Expression of MMP-1 and Increases the Integrity of Extracellular Matrix in Fibroblasts

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Table S1. Primer sequences used for reverse transcription-polymerase chain reaction of mouse (m) *Sod* mRNAs and human (h) *COL1A1*, *COL1A2*, *MMP1*, *and GAPDH* mRNAs.

Gene Symbol	Nucleotide Sequence	Nucleotide Position	Annealing Temp. (°C)	GenBank #
m*SOD1*	5′- CAAGATGACTTGGGCAAAGGTGG -3′ 5′- ACTGCGCAATCCCAATCACTCC -3′	466–488 559–538	55	NM_011434.2
m*SOD2*	5′- CCTCACTCACGGCCACATTG -3′ 5′- ACAGCACCCCAGTCATAGTGC -3′	879–898 963–943	60	NM_013671.3
m*SOD2*	5′- CCAGGACACCTTAGTTAACCCAG -3′ 5′- GGGGTTCTCTGAGAGCAGACTC -3′	1260–1282 1411–1390	65	NM_011435.3
h*COL1A1*	5′- ACAGCGTCACTGTCGATGGCTG -3′ 5′- GGAGGGAGTTTACAGGAAGCAGACAG -3′	4341–4372 4522–4497	55	NM_000088
h*COL1A2*	5′- GAGGGCAACAGCAGGTTCACTTACAC -3′ 5′-GTCAGCACCACCGATGTCCAA AG -3′	4044–4069 4196–4174	55	NM_000089
h*MMP1*	5′- GTACTGATATAATTTAGTTC -3′ 5′- GTTATCCCTTGCCTATCTAG -3′	1656–1675 1908–1889	45	NM_002421
h*GAPDH*	5′- ACTGCTTAGCACCCCTGGCCA -3′ 5′- TTGGCAGTGGGGACACGGAAG -3′	488–508 740–720	55	BC023632

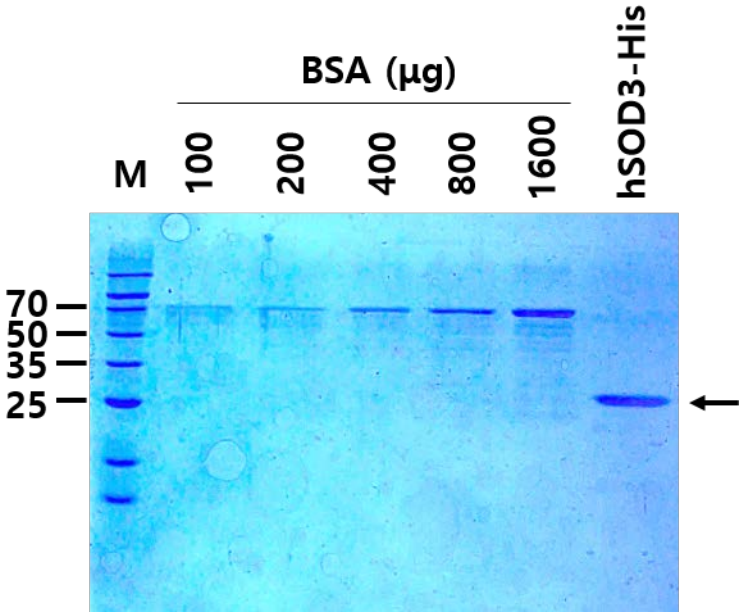

Figure S1. Purification of recombinant human SOD3-His.

Recombinant human (h) SOD3-His polypeptide was purified from the conditioned medium of HEK293 cells stably transfected with pcDNA3.1-hSOD3-His. The figure shows purified hSOD3-His polypeptide resolved by SDS-PAGE and stained with Coomassie brilliant blue. Various amounts of bovine serum albumin (BSA) were loaded to estimate the concentration of hSOD3-His polypeptide. Migration of molecular weight markers (M) is shown in the left lane of the gel.

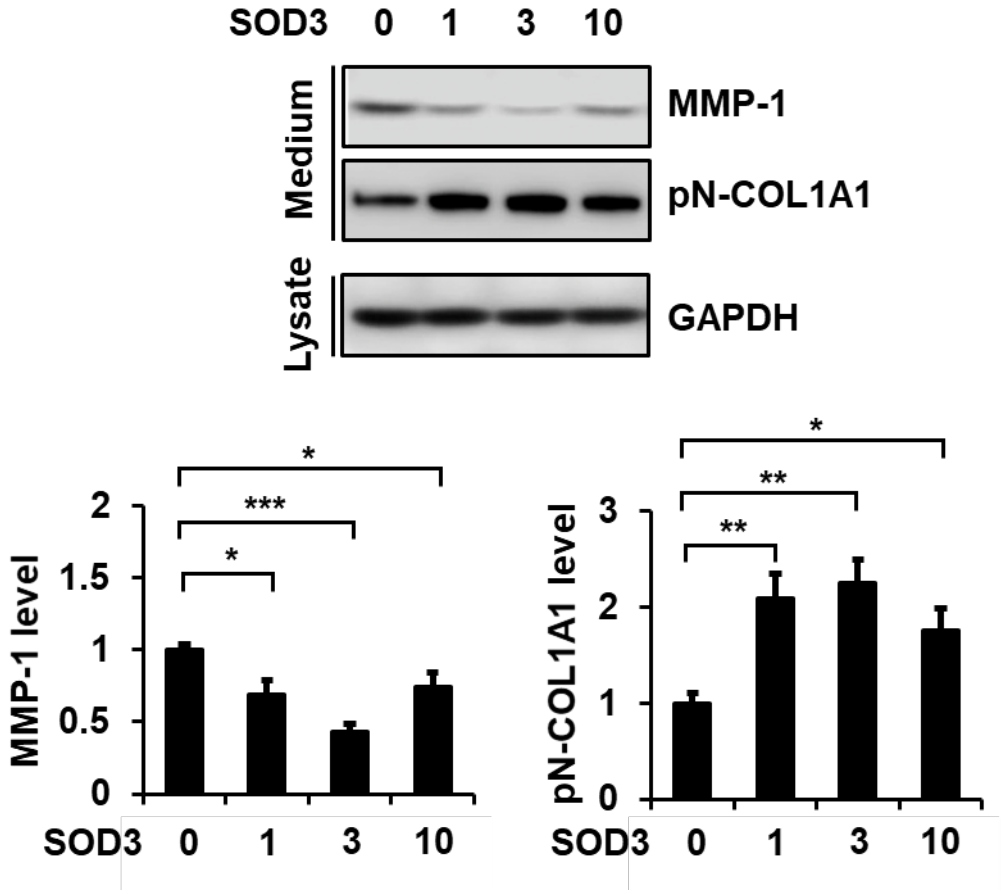

Figure S2. Titration of the optimal SOD3 concentration to decrease MMP-1 secretion and increase type I collagen secretion in fibroblasts.

Human foreskin fibroblasts were incubated in a serum-free medium in the presence of 0, 1, 3, and 10 μg/ml human SOD3-His for 24 h. The secreted levels of MMP-1 and type I collagen in conditioned media were assessed by western blot analysis using antibodies against MMP-1 and pN-COL1A1, respectively. The GAPDH level in cell lysates was monitored as a loading control. Graphs show levels of MMP-1 and type I collagen relative to the level in non-treated cells, which was quantified by image J software. Each value is the mean ± standard deviations of three independent experiments. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. control.