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Supporting Information

for *Adv. Sci.*, DOI 10.1002/adv.202206814

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Tenogenic Differentiation of Mesenchymal Stem Cells

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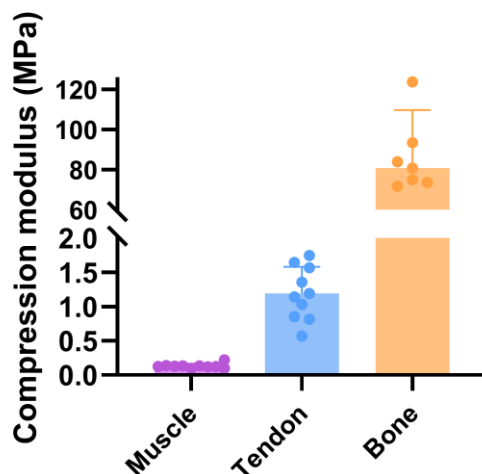


Figure S1. Compressive modulus of the muscle (soleus), tendon (Achilles' tendon), and bone (tibia) of SD rat. The results are shown in mean \pm SD.

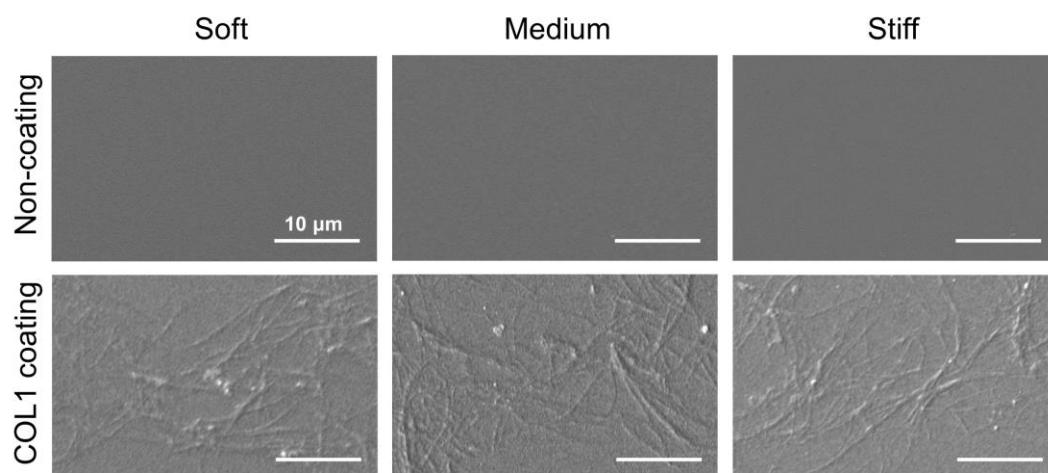


Figure S2. SEM images of three PDMS substrates before and after COL1 coating. Scale bar = 10 μm.

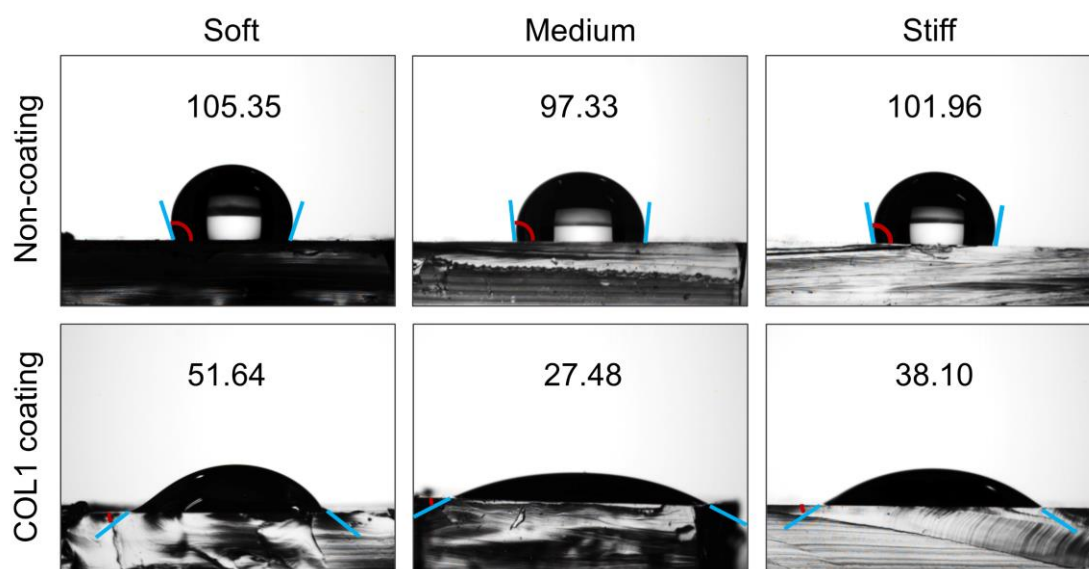


Figure S3. Water contact angles of three PDMS substrates before and after COL1 coating.

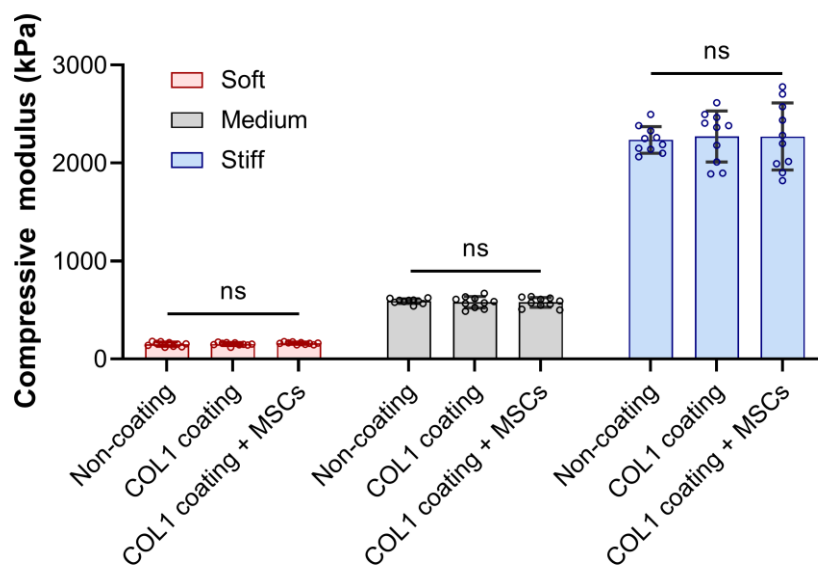


Figure S4. Compressive modulus of three PDMS substrates before and after COL1 coating and MSCs seeding (n = 10). The results are shown in mean \pm SD. ns ($P > 0.05$) determined using one-way ANOVA with Tukey's post hoc test.

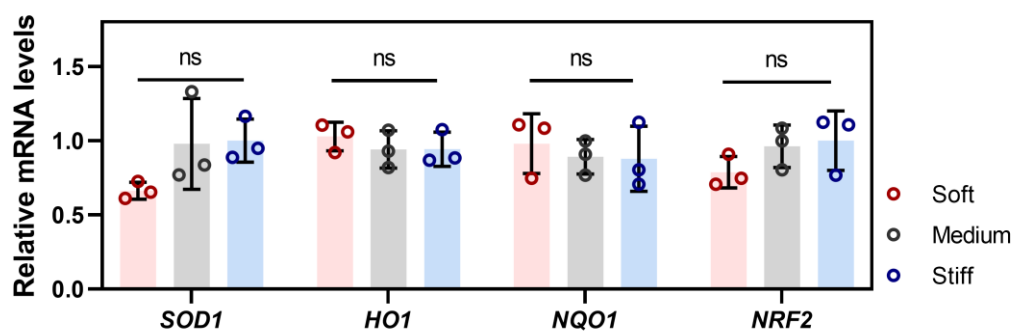


Figure S5. Gene expression of antioxidant mediators in macrophages on day 3 by qPCR (n = 3). The results were shown in mean \pm SD. ns ($P > 0.05$) determined using one-way ANOVA with Tukey's post hoc test.

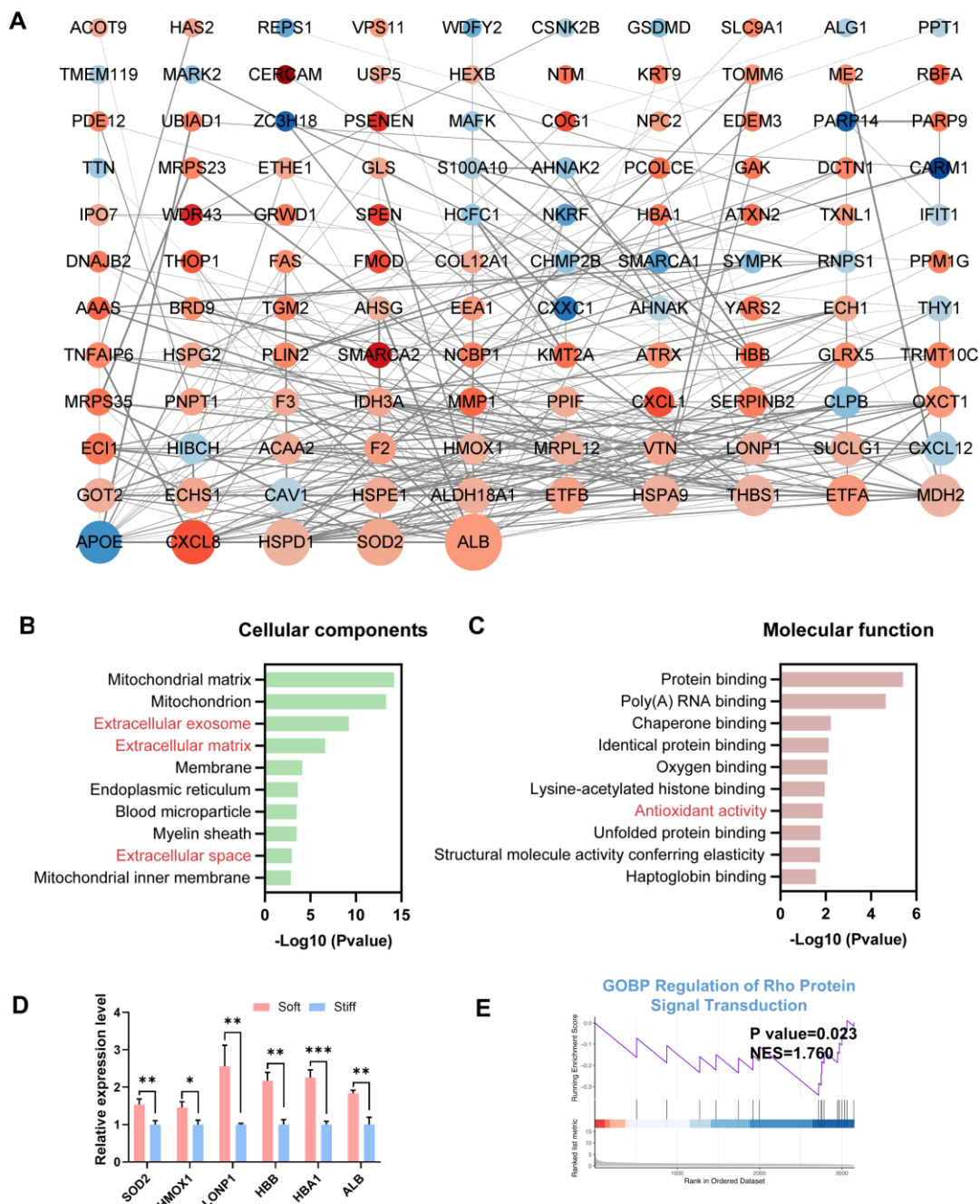


Figure S6. Functional insights of DEPs in Soft vs. Stiff. (A) The protein interaction network for all DEPs (Soft vs. Stiff). The larger size of the protein node indicates a higher frequency in the protein network. The up-regulated proteins are presented in red while the down-regulated are in blue. A deeper color of nodes represents a lower or higher fold change of DEPs. (B-C) Key GO terms significantly enriched in CC and MF (Soft vs. Stiff). (D) Protein expression of several antioxidative mediators from proteomic profiles (n = 3). (E) GSEA plot of regulation of Rho protein signal transduction (Soft vs. Stiff). The results are shown in mean \pm SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ determined using two-tailed student's t-test (D).

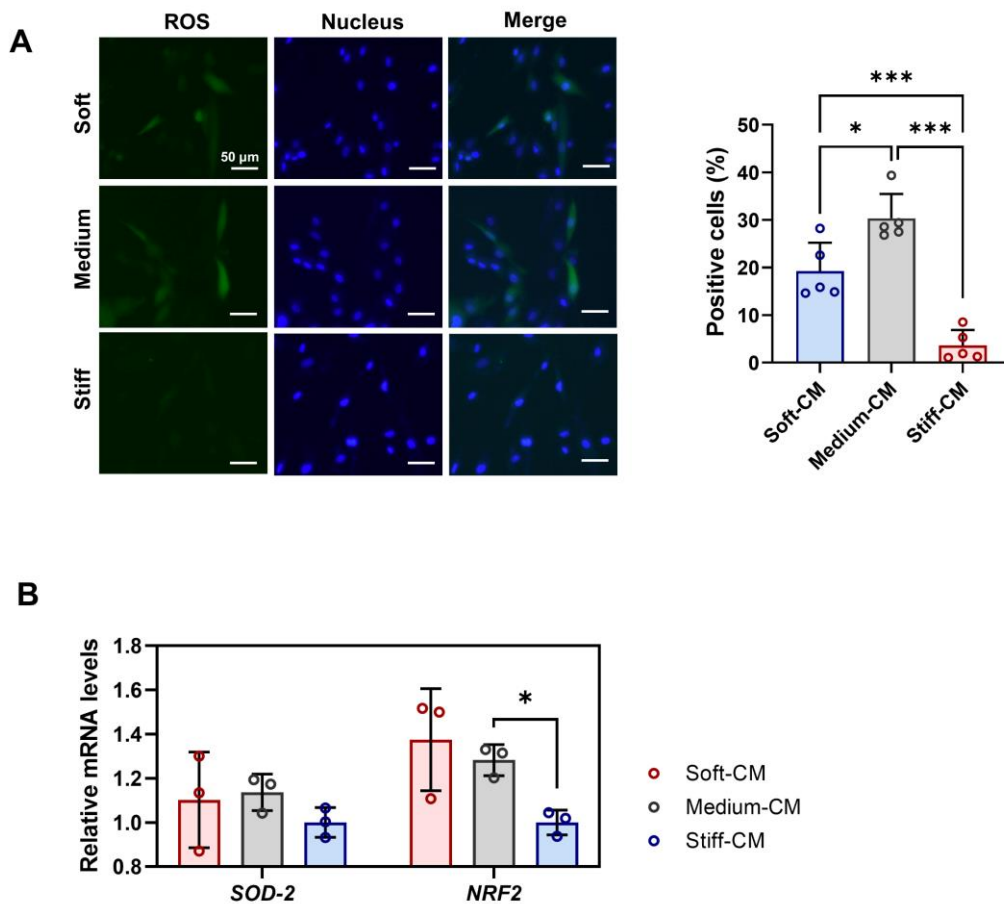


Figure S7. Influences of different CMs on the oxidative stress of MSCs. (A) Representative images and quantitative analysis of ROS staining in MSCs exposed to different CMs for 1 d (n = 5). Scale bar = 50 μ m. (B) Gene expression of *SOD2* and *NRF2* in MSCs exposed to different CMs by qPCR (n = 3). The results are shown in mean \pm SD. *p < 0.05; **p < 0.01; ***p < 0.001 determined using one-way ANOVA with Tukey's post hoc test.

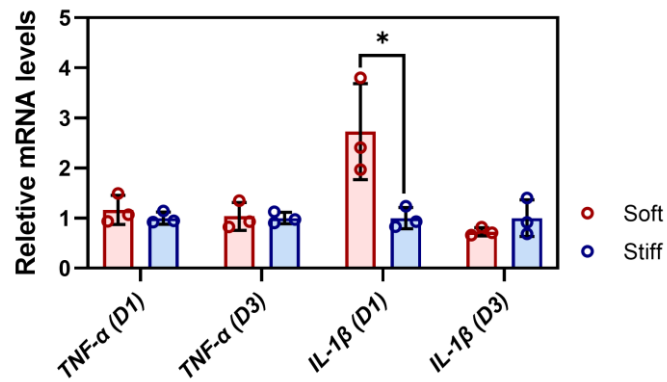


Figure S8. Gene expression of *TNF-α* and *IL-1β* in MSCs exposed to different stiffnesses and CMs on days 1 and 3 by qPCR (n = 3). The results are shown in mean ± SD. *p < 0.05 determined using two-tailed student's t-test.

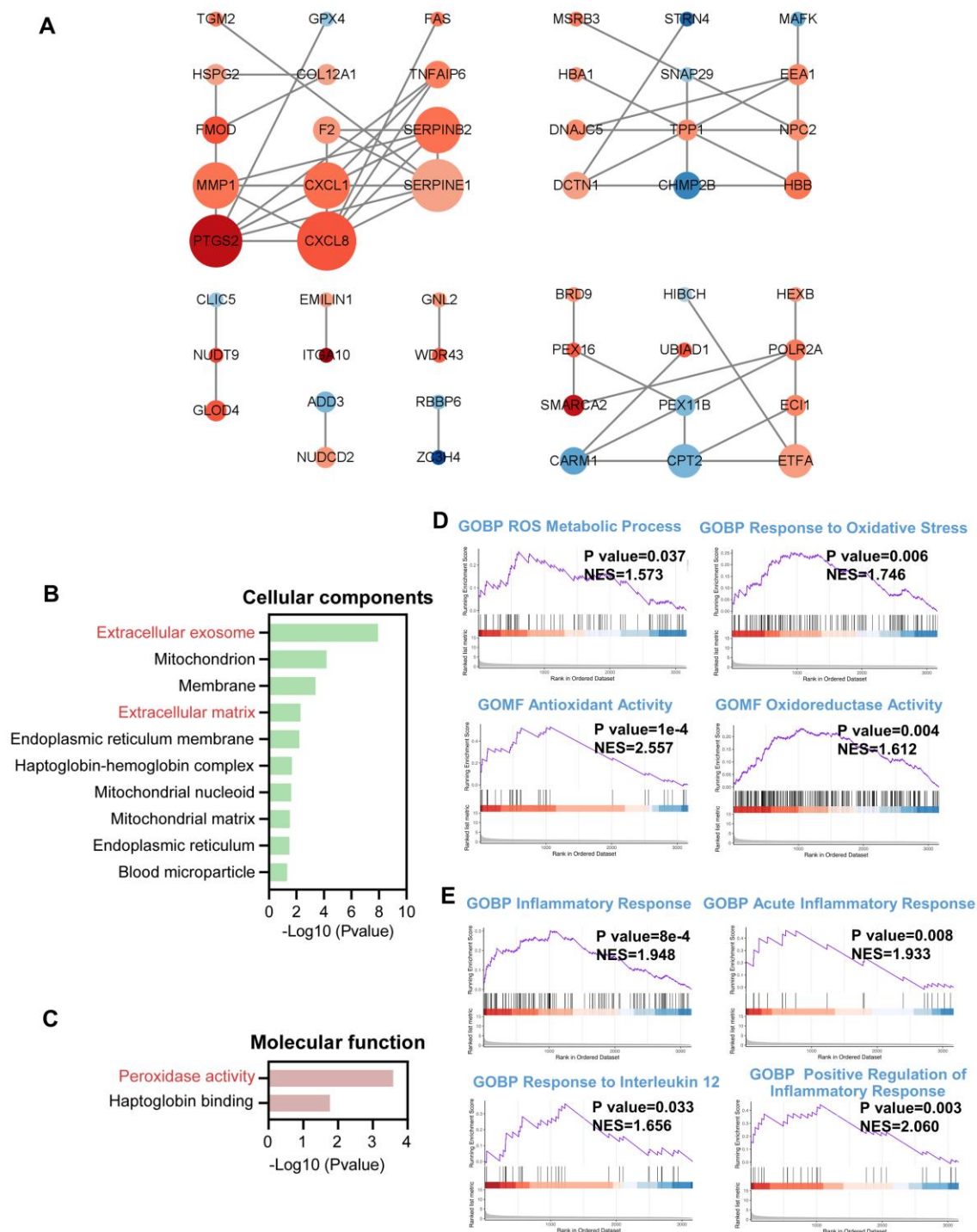


Figure S9. Functional insights of DEPs in Medium vs. Stiff. (A) The protein interaction network for all DEPs (Medium vs. Stiff). The larger size of the protein node indicated a higher frequency in the protein network. The up-regulated proteins are presented in red while the down-regulated are in blue. A deeper color of nodes represents a lower or higher fold change of DEPs. (B-C) Key GO terms significantly enriched in CC and MF (Medium vs. Stiff). (D) GSEA plot of GO terms associated with oxidative stress (Medium vs. Stiff). (E) GSEA plot of GO

terms associated with inflammation (Medium vs. Stiff).

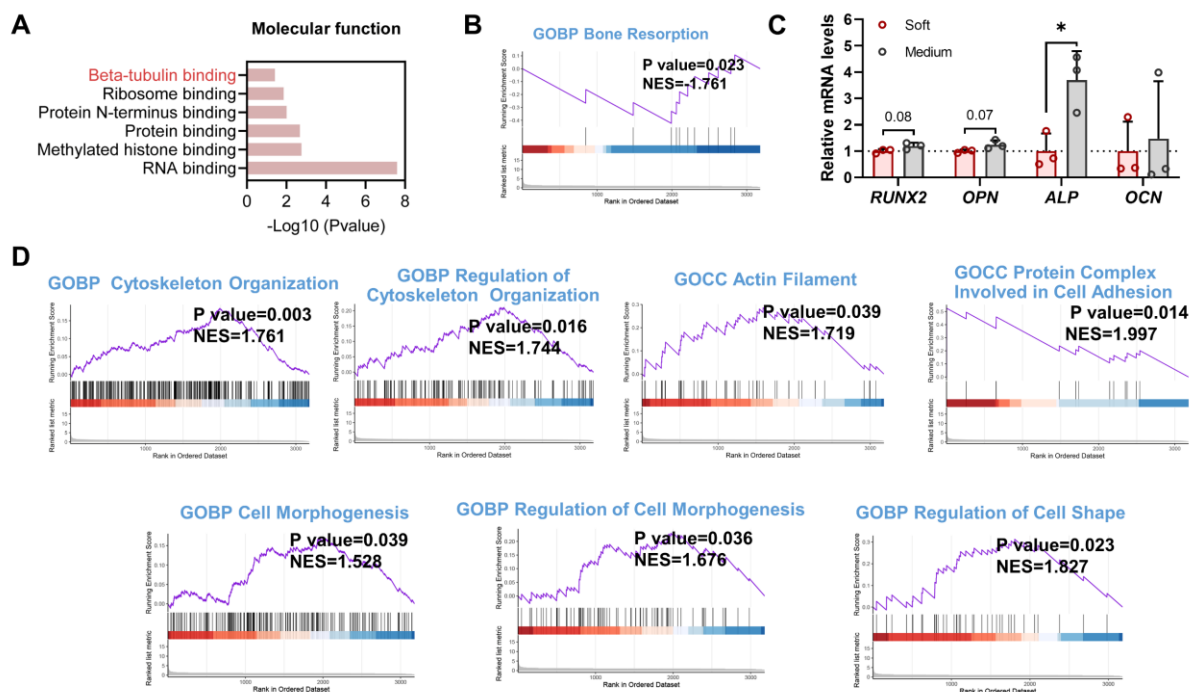


Figure S10. Functional insights of DEPs in Medium vs. Soft. (A) Key GO terms significantly enriched in MF (Medium vs. Soft). (B) GSEA plot of bone resorption (Medium vs. Soft). (C) Gene expression of osteogenic markers in MSCs on day 1 by qPCR (n = 3). (D) GSEA plot of GO terms associated with focal adhesion, cytoskeletal organization, and cell morphogenesis (Medium vs. Soft). The results are shown in mean ± SD. *p < 0.05 determined using two-tailed student's t-test (C).

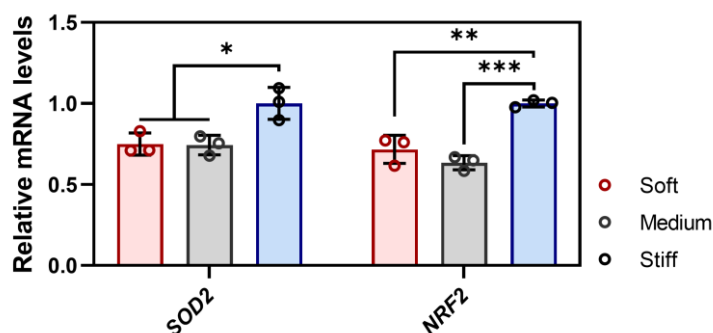


Figure S11. Gene expression of *SOD2* and *NRF2* in MSCs cultured on different substrates on day 3 by qPCR (n = 3). The results are shown in mean \pm SD. *p < 0.05; **p < 0.01; ***p < 0.001 determined using one-way ANOVA with Tukey's post hoc test.

Table S1. Primer sequences are specific to MSCs and Raw264.7 for qPCR.

Species	Gene	Forward & Reverse	Sequence (5'- 3')
Human	<i>GAPDH</i>	Forward	TGACGCTGGGGCTGGCATTG
		Reverse	GGCTGGTGGTCCAGGGGTCT
Human	<i>SCX</i>	Forward	CGAGAACACCCAGCCCAAAC
		Reverse	CTCCGAATCGCAGTCTTTCTGTC
Human	<i>MKX</i>	Forward	GAAGGCAACTTTGTCTATCGCA
		Reverse	TGATCTCCTTCCAATACGTGTC
Human	<i>TNMD</i>	Forward	CCATGCTGGATGAGAGAGGT
		Reverse	CTCGTCCTCCTGGTAGCAG
Human	<i>COL1</i>	Forward	CGATGGATTCCAGTTCGAGTAT
		Reverse	CATCGACAGTGACGCTGTAGG
Human	<i>COL3</i>	Forward	TTTTGCAGTGATATGTGATGTT
		Reverse	GGATGGTGGTTTTAGTTTA
Human	<i>COL14</i>	Forward	AAGGATTGCCCTCCGACTACAC
		Reverse	CTGATGCGTTCATTGCCTTCTC
Human	<i>SOD2</i>	Forward	CTGGACAAACCTCAGCCCTA
		Reverse	TGATGGCTTCCAGCAACTC
Human	<i>NRF2</i>	Forward	AGTGGATCTGCCAACTACTC
		Reverse	CATCTACAAACGGAATGTCTG
Human	<i>IL-1β</i>	Forward	GCAAGGGCTTCAGGCAGGCCGCG
		Reverse	GGTCATTCTCCTGGAAGGTCTGTGGGC
Human	<i>TNF-α</i>	Forward	ATGTTGTAGCAAACCCTCAAGC
		Reverse	TGATGGCAGAGAGGAGGTTG
Human	<i>MAP2K2</i>	Forward	CGGACCTGAAGATGCTCACA
		Reverse	TTCAGCCGCAGGGTTTTACA

Human	<i>MAP4K4</i>	Forward	GGGGAACGCTTCAGAGTGAG
		Reverse	GTGCGGTCAGATCAGCAGG
Human	<i>TAOK1</i>	Forward	CCTCCTCCTCCTCACTCCTC
		Reverse	GGCATAAACTACCTTTGCGCCG
Human	<i>FAS</i>	Forward	ATTATCGTCCAAAAGTGTTA
		Reverse	TCACACAATCTACATCTT CTG
Mouse	<i>GAPDH</i>	Forward	GCAAGTTCAACGGCACAG
		Reverse	CGCCAGTAGACTCCACGAC
Mouse	<i>CCR7</i>	Forward	ATGGACCCAGGTGTGCTTCT
		Reverse	TCAGTATCACCAGCCCGTTG
Mouse	<i>iNOS</i>	Forward	CCTGTGTTCCACCAGGAGAT
		Reverse	CCCTGGCTAGTGCTTCAGAC
Mouse	<i>IL-1β</i>	Forward	AAGGAGAACCAAGCAACGACAAAA
		Reverse	TGGGGAACCTCTGCAGACTCAAACCT
Mouse	<i>TNF-α</i>	Forward	CGTCAGCCGATTTGCTATCT
		Reverse	CGGACTCCGCAAAGTCTAAG
Mouse	<i>IL-10</i>	Forward	CCAAGCCTTATCGGAAATGA
		Reverse	TTTTACAGGGGAGAAATCG
Mouse	<i>CD206</i>	Forward	AGCTTCATCTTCGGGCCTTTG
		Reverse	GGTGACCACTCCTGCTGCTTTAG
Mouse	<i>SOD1</i>	Forward	AACCAGTTGTGTTGTCAGGAC
		Reverse	CCACCATGTTTCTTAGAGTGAGG
Mouse	<i>HO1</i>	Forward	GATAGAGCGCAACAAGCAGAA
		Reverse	CAGTGAGGCCCATACCAGAAG
Mouse	<i>NQO1</i>	Forward	AGGATGGGAGGTACTIONCGAATC
		Reverse	TGCTAGAGATGACTCGGAAGG
Mouse	<i>NRF2</i>	Forward	TAGATGACCATGAGTCGCTTGC
		Reverse	GCCAACTTGCTCCATGTCC