

Supplemental information

Effects of long-term culture on the biological characteristics and RNA profiles of human bone-marrow-derived mesenchymal stem cells

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**Table S1. Quantitative Profiles of the Cell Cycle Study of MSCs at P4, P6, P8, P10 and P12
(n=9, means \pm SD)**

Percent of Cells in the Cell Cycle	P4	P6	P8	P10	P12
Percent of G1 cells	61.42 \pm 7.33	61.76 \pm 7.17	66.88 \pm 9.07	65.53 \pm 5.00	59.56 \pm 9.74
Percent of S+G2/M cells	26.72 \pm 3.54	19.39 \pm 1.82	14.16 \pm 2.51	10.86 \pm 2.19	7.51 \pm 1.58
Percent of S cells	20.9 \pm 2.52	15.54 \pm 1.82	12.08 \pm 3.05	9.28 \pm 2.29	6.68 \pm 1.78
Percent of G2/M cells	5.82 \pm 2.32	3.85 \pm 1.60	2.35 \pm 0.73	1.58 \pm 0.62	0.84 \pm 0.46

Table S2. Top 10 Terms with the Largest Significant Differences in the GO Biological Process**Analysis of DE mRNAs Related to Proliferation**

Term	Count	Rich Ratio	<i>p</i> Value	Gene
Positive regulation of cell proliferation	21	0.029577	3.90E-10	LEP, GREM1, S1PR1, LIF, EPGN, TNC, SOX4, S1PR3, FGF2, CCND1, THBS1, ADM, EDN1, PTN, STAT1, DMD, CXCL12, RUNX2, GPNMB, IGF2, GRK5
Positive regulation of apoptotic process	16	0.034858	3.68E-16	CLU, FGD4, FANK1, ARHGEF3, SOX4, MMP9, ANKRD1, EVI2A, ADM, PTN, BNIP3, NGF, PYCARD, PNMA2, LDHA, SFRP4
Heart development	10	0.031949	5.60E-10	SOX4, CRIP1, GLI3, EVI2A, ADM, EDN1, PTN, ZFP36L1, DVL2, HEG1
Response to hypoxia	9	0.038961	7.83E-10	LEP, EVI2A, CAV1, THBS1, ADM, EDN1, CXCL12, BNIP3, LDHA
Cellular response to transforming growth factor beta stimulus	6	0.075	1.29E-08	TINAGL1, ANKRD1, CAV1, CLEC3B, EDN1, ZFP36L1
Telomere organization	4	0.285714	1.42E-08	HIST1H4A, HIST1H3I, HIST1H3H, HIST1H3A
Vasculogenesis	6	0.073171	1.50E-08	TEAD2, CAV1, ADM, ZFP36L1, EPHA2, HEG1
Negative regulation of apoptotic process	12	0.015287	3.55E-08	LEP, FANK1, EPHB2, MMP9, GLI3, EVI2A, THBS1, COMP, BNIP3, NGF, SH3D21, EPHA2
Negative regulation of cell death	6	0.063158	3.66E-08	CLU, TEAD2, SOX4, FGF2, BNIP3, WISP2
DNA replication-dependent nucleosome assembly	4	0.181818	1.03E-07	HIST1H4A, HIST1H3I, HIST1H3H, HIST1H3A
Chromatin silencing at rDNA	4	0.166667	1.49E-07	HIST1H4A, HIST1H3I, HIST1H3H, HIST1H3A

Table S3. Top 10 Terms with the Largest Significant Differences in the GO Biological Process**Analysis of DE mRNAs Related to Differentiation**

Term	Count	Rich Ratio	<i>p</i> Value	Gene
Ossification	15	0.12605	1.63E-16	TMEM119, LRRC17, IFITM1, MMP9, STC1, ACP5, MMP16, TWIST1, CLEC3B, PTN, COMP, RUNX2, KAZALD1, MGP, IGF2
Angiogenesis	21	0.058496	8.62E-16	LEP, NDNF, GREM1, ANGPTL4, S1PR1, NRP2, SLC38A1, EPGN, CXCL8, EPHB2, EGFL7, ADGRA2, SERPINE1, JAG1, TGFBI, FZD8, CAV1, CCBE1, NRCAM, EPHA2, COL4A1
Cell differentiation	32	0.027515	3.14E-14	TMEM119, NOTCH3, HERC4, ANGPTL4, SH3PXD2B, GLIS2, EPHB2, EGFL7, SOX4, OSR1, TWIST1, CAV1, NR1H3, CAV2, DMD, BEX1, SERPINE2, TENM4, RUNX2, EFNA5, ZNF521, KAZALD1, MGP, CADM1, MDK, EPHA2, PTPRU, RARRES2, SFRP4, DCLK1, STMN1, PSMB8
Osteoblast differentiation	10	0.070423	6.96E-09	TMEM119, RRAS2, LRRC17, TNC, EVI2A, TWIST1, RUNX2, GPNMB, EPHA2, IGF2
Multicellular organism development	32	0.025157	3.59E-13	EYA1, NOTCH3, ANGPTL4, GLIS2, LIF, EPHB2, EGFL7, JAG1, TWIST1, GDF6, FZD8, DKK1, CCBE1, NR1H3, SERPINE2, ZFP36L1, DVL2, TENM4, KIAA1217, EYA2, EFNA5, ZNF521, SERPINF1, KAZALD1, MGP, CADM1, EPHA2, IGF2, HEG1, SFRP4, DCLK1, STMN1
Skeletal system development	14	0.075676	2.21E-12	SH3PXD2B, FAM20C, NPR3, SOX4, PAPSS2, MMP9, ANKH, EDN1, AEBP1, COMP, RUNX2, TNFRSF11B, EPHA2, IGF2
Vasculogenesis	6	0.073171	1.50E-08	TEAD2, CAV1, ADM, ZFP36L1, EPHA2, HEG1
Extracellular matrix organization	14	0.050909	4.42E-10	NDNF, RECK, TNC, FGF2, MMP9, SERPINE1, EVI2A, TGFBI, THBS1, COMP, TNFRSF11B, COL14A1, KAZALD1, COL4A1
Regulation of signalling receptor activity	17	0.037862	5.32E-10	LEP, NBL1, LIF, CXCL8, SERPINE1, STC1, JAG1, GDF6, DKK1, GDF5, ADM, EDN1, PTN, CXCL12, NGF, GPNMB, IGF2
Cell adhesion	21	0.027027	1.96E-09	S1PR1, RGMB, NRP2, TNC, EGFL7, JUP, THBS2, LOXL2, TGFBI, THBS1, CDH6, CXCL12, PERP, THBS3, COMP, COL14A1, CADM1, NRCAM, GPNMB, EPHA2, PTPRU

**Table S4. Top 10 Terms with the Largest Significant Differences in the GO Biological Process
Analysis of DE mRNAs Related to the Immune Response**

Term	Count	Rich Ratio	<i>p</i> Value	Gene
Immune system process	20	0.038685	1.24E-13	ULBP2, CLU, TINAGL1, GSDMD, CD74, SAMHD1, IFITM1, PSMB9, C1S, C1R, ECSIT, HLA-B, CD55, SPON2, CFI, SLC7A8, CFB, PYCARD, CADM1, PSMB8
Positive regulation of apoptotic process	16	0.034858	2.02E-10	CLU, FGD4, FANK1, ARHGEF3, SOX4, MMP9, ANKRD1, EVI2A, ADM, PTN, BNIP3, NGF, PYCARD, PNMA2, LDHA, SFRP4
Immune response	17	0.028428	1.18E-09	ULBP2, TINAGL1, LIF, CXCL8, CD74, SAMHD1, CRIP1, GBP2, SUSP2, C1R, THBS1, HLA-B, 105369230, CXCL12, FCGRT, TNFRSF11B, COLEC12
Inflammatory response	16	0.030075	1.71E-09	MAP2K3, GSDMD, GGT5, CXCL8, S1PR3, MGLL, PTX3, CHI3L1, PTGFR, THBS1, TNFRSF11B, PYCARD, SDC1, SELENOP, EPHA2, RARRES2
Response to glucocorticoid	8	0.096386	3.43E-09	IGFBP2, CCND1, ADM, TYMS, PAPP, SDC1, MDK, PTPRU
Response to hypoxia	11	0.047619	6.75E-09	LEP, ANGPTL4, EVI2A, CAV1, THBS1, ADM, EDN1, CXCL12, BNIP3, SOD3, LDHA
Innate immune response	17	0.025223	7.06E-09	CLU, TINAGL1, GSDMD, SAMHD1, IFITM1, PTX3, C1S, C1R, ECSIT, HLA-B, CD55, SPON2, CFI, CFB, PYCARD, COLEC12, RARRES2
Cellular response to hypoxia	8	0.055172	2.76E-07	NDNF, ANKRD1, TWIST1, STC2, EDN1, PTN, ZFP36L1, BNIP3
Regulation of complement activation	7	0.067961	3.86E-07	CLU, C1S, C1R, CD55, CFI, CFB, CPNE2
Cellular response to interleukin-1	7	0.06422	5.68E-07	TFPI, CXCL8, ANKRD1, GBP2, CHI3L1, EDN1, PYCARD

Table S5. Characteristics of the circRNAs Involved in the Prediction ceRNA Network

circBankID	circbaseID	Position	Stran SplicedSe			Gene Symbol
			d	qLength	BestTranscript	
hsa_circTHBS2_011	hsa_circ_0078715	chr6:169632074-169633112	-	500	NM_003247	THBS2
hsa_circTHBS2_016	hsa_circ_0078711	chr6:169626274-169629774	-	387	NM_003247	THBS2
hsa_circTHBS1_125	hsa_circ_0034540	chr15:39885597-39885869	+	272	NM_003246	THBS1
hsa_circTHBS1_055	hsa_circ_0034470	chr15:39877670-39879721	+	268	NM_003246	THBS1
hsa_circTHBS1_081	hsa_circ_0034496	chr15:39881159-39882224	+	500	NM_003246	THBS1
hsa_circTHBS1_108	hsa_circ_0034523	chr15:39883391-39885428	+	742	NM_003246	THBS1
hsa_circTHBS1_022	hsa_circ_0034437	chr15:39875817-39876388	+	276	NM_003246	THBS1
hsa_circTHBS1_086	hsa_circ_0034501	chr15:39881159-39886397	+	1720	NM_003246	THBS1
hsa_circTHBS1_058	hsa_circ_0034473	chr15:39879547-39880419	+	351	NM_003246	THBS1
hsa_circTHBS1_023	hsa_circ_0034438	chr15:39875817-39876623	+	399	NM_003246	THBS1
hsa_circTHBS1_093	hsa_circ_0034508	chr15:39881402-39886397	+	1592	NM_003246	THBS1
hsa_circTHBS1_075	hsa_circ_0034490	chr15:39880800-39882224	+	600	NM_003246	THBS1
hsa_circTHBS1_115	hsa_circ_0034530	chr15:39883705-39886397	+	952	NM_003246	THBS1
hsa_circTHBS1_001	hsa_circ_0034417	chr15:39873279-39874125	+	246	NM_003246	THBS1
hsa_circTHBS1_033	hsa_circ_0034448	chr15:39876188-39876623	+	323	NM_003246	THBS1
hsa_circTHBS1_012	hsa_circ_0034428	chr15:39874029-39882224	+	2174	NM_003246	THBS1
hsa_circTHBS1_096	hsa_circ_0034511	chr15:39882005-39883551	+	487	NM_003246	THBS1
hsa_circTHBS1_119	hsa_circ_0034534	chr15:39884768-39885869	+	735	NM_003246	THBS1
hsa_circTHBS1_048	hsa_circ_0034463	chr15:39876500-39879721	+	391	NM_003246	THBS1
hsa_circTHBS1_107	hsa_circ_0034522	chr15:39883391-39885003	+	514	NM_003246	THBS1
hsa_circTHBS1_089	hsa_circ_0034504	chr15:39881402-39882224	+	372	NM_003246	THBS1
hsa_circTHBS1_080	hsa_circ_0034495	chr15:39881159-39881555	+	281	NM_003246	THBS1
hsa_circTHBS1_034	hsa_circ_0034449	chr15:39876188-39877764	+	417	NM_003246	THBS1
hsa_circTHBS1_105	hsa_circ_0034520	chr15:39882716-39886397	+	1220	NM_003246	THBS1
hsa_circTHBS1_008	hsa_circ_0034424	chr15:39874029-39874953	+	656	NM_003246	THBS1
hsa_circTHBS1_050	hsa_circ_0034465	chr15:39876500-39882224	+	1242	NM_003246	THBS1
hsa_circTHBS1_109	hsa_circ_0034524	chr15:39883391-39885869	+	1014	NM_003246	THBS1
hsa_circTHBS1_099	hsa_circ_0034514	chr15:39882005-39886397	+	1439	NM_003246	THBS1
hsa_circTHBS1_065	hsa_circ_0034480	chr15:39880242-39880900	+	351	NM_003246	THBS1
hsa_circTHBS1_121	hsa_circ_0034536	chr15:39884768-39886641	+	973	NM_003246	THBS1
hsa_circTHBS1_117	hsa_circ_0034532	chr15:39884768-39885003	+	235	NM_003246	THBS1
hsa_circTHBS1_106	hsa_circ_0034521	chr15:39883391-39883824	+	279	NM_003246	THBS1
hsa_circTHBS1_123	hsa_circ_0034538	chr15:39885200-39885869	+	500	NM_003246	THBS1
hsa_circTHBS1_110	hsa_circ_0034525	chr15:39883391-39886641	+	1252	NM_003246	THBS1
hsa_circTHBS1_102	hsa_circ_0034517	chr15:39882716-39883824	+	387	NM_003246	THBS1
hsa_circTHBS1_118	hsa_circ_0034533	chr15:39884768-39885428	+	463	NM_003246	THBS1
hsa_circTHBS1_113	hsa_circ_0034528	chr15:39883705-39885003	+	354	NM_003246	THBS1
hsa_circTHBS1_120	hsa_circ_0034535	chr15:39884768-39886397	+	833	NM_003246	THBS1
hsa_circTHBS1_030	hsa_circ_0034445	chr15:39875817-39886397	+	2738	NM_003246	THBS1

hsa_circTHBS1_101	hsa_circ_0034516	chr15:39882716-39883551	+	268	NM_003246	THBS1
hsa_circTHBS1_126	hsa_circ_0034541	chr15:39885597-39886397	+	370	NM_003246	THBS1
hsa_circTGFB1_015	hsa_circ_0074046	chr5:135389631-135396625	+	780	NM_000358	TGFB1
hsa_circTGFB1_009	hsa_circ_0128030	chr5:135382539-135385269	+	454	NM_000358	TGFB1
hsa_circSERPINE2_006	hsa_circ_0058476	chr2:224849468-224856717	-	397	NM_006216	SERPINE2
hsa_circSERPINE2_004	hsa_circ_0005773	chr2:224856519-224863059	-	426	NM_006216	SERPINE2
hsa_circSERPINE1_013	hsa_circ_0081574	chr7:100778774-100780365	+	272	NM_000602	SERPINE1
hsa_circSERPINE1_010	hsa_circ_0081571	chr7:100776975-100780365	+	471	NM_000602	SERPINE1
hsa_circSERPINE1_007	hsa_circ_0081568	chr7:100773701-100775350	+	429	NM_000602	SERPINE1
hsa_circSERPINE1_001	hsa_circ_0081562	chr7:100770378-100771945	+	419	NM_000602	SERPINE1
hsa_circMT2A_002	hsa_circ_0039460	chr16:56642903-56643409	+	301	NM_005953	MT2A
hsa_circLOXL2_002	hsa_circ_0083670	chr8:23198504-23217778	-	388	NM_002318	LOXL2
hsa_circLOXL2_007	hsa_circ_0083666	chr8:23167180-23174627	-	410	NM_002318	LOXL2
hsa_circLOX_001	hsa_circ_0073686	chr5:121406188-121411236	-	391	NM_002317	LOX
hsa_circLDHA_006	hsa_circ_0006469	chr11:18422383-18425358	+	466	NM_001165414	LDHA
hsa_circLDHA_005	hsa_circ_0021450	chr11:18422383-18424560	+	348	NM_001165414	LDHA
hsa_circLDHA_009	hsa_circ_0021453	chr11:18424386-18425358	+	292	NM_001165414	LDHA
hsa_circLDHA_004	hsa_circ_0021449	chr11:18418365-18421095	+	268	NM_001165414	LDHA
hsa_circCD74_002	hsa_circ_0074531	chr5:149784242-149786887	-	500	NM_001025159	CD74
hsa_circCCND1_002	hsa_circ_0023304	chr11:69457798-69458759	+	376	NM_053056	CCND1

Table S6. Primers Used for qPCR in the Validation

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
GAPDH	GGATGCAGGGATGATGTTCC	TGCACCACCAACTGCTTAG
U6	CTCGCTTCGGCAGCACAA	AACGCTTCACGAATTTGCGT
miR-1908-5p	CGGCGGGGACGGCGATTGGTC	CAGTGCCTGTCGTGGAGT
miR-16-5p	GGGTAGCAGCACGTAAATA	CAGTGCCTGTCGTGGAGT
miR-122-5p	GGGTGGAGTGTGACAATGG	CAGTGCCTGTCGTGGAGT
miR-193a-3p	GGGAACTGGCCTACAAAGT	CAGTGCCTGTCGTGGAGT
miR-199b-5p	CCAGTGTTTAGACTATCTGTTC	CAGTGCCTGTCGTGGAGT
miR-339-5p	TCCCTGTCCTCCAGGAGCT	CAGTGCCTGTCGTGGAGT
miR-210-5p	GGGAGCCCCCTGCCACCCG	CAGTGCCTGTCGTGGAGT
miR-431-5p	GGGUGUCUUGCAGGCCGU	CAGTGCCTGTCGTGGAGT
miR-17-5p	GGGCAAAGUGCUUACAGUGC	CAGTGCCTGTCGTGGAGT
miR-451a	AAACCGTTACCATTACTGAGTT	CAGTGCCTGTCGTGGAGT
COMP	GGCCCTGCATAGGGAATTAGA	TGTCCTCTGACATCCAACCTC
STC1	CACGAGCTGACTTCAACAGGA	GGATGTGCGTTTGTATGTGGG
ADAMTS1	TGCGACGCTGCTTCTATTCTG	CCTCGGTAGCCAAAGGCTC
INKA2	AAAGAATTCTCCATTATGACGATGGAGAGCAG	AAACTCGAGTCAGACCCAAACAGCTGTGTTA
CDH6	AGAACTTACCGCTACTTCTTGC	TGCCACATACTGATAATCGGA
SPON2	GGAGAGTCCATCTGTTCCGC	ATGCTGTAGTCGGAGCTATGC
STC2	GCGTGCAGGTTCAAGTGTGA	GGCCAGTCTCCCTACTGCT
SERPINE2	GGAAGGAACCATGAACTGGC	GGGGAGAGATCACGATGTTGT
LIF	CCAACGTGACGGACTTCCC	TACACGACTATGCGGTACAGC
PTN	GGAGCTGAGTGCAAGCAAAC	CTCGCTTCAGACTTCCAGTTC
RUNX2	CCGCCTCAGTGATTTAGGGC	GGGTCTGTAATCTGACTCTGTCC
GREM1	CGGAGCGCAAATACCTGAAG	GGTTGATGATGGTGCAGCTGT
DKK1	CCTTGAACCTCGGTTCTCAATTCC	CAATGGTCTGGTACTTATTCCCG
SERPINF1	GGAAATTCCCAGATGAGATCAGC	AGTCAAACCTTTGTTACCCACTGC
ANGPTL4	GGCTCAGTGGACTTCAACCG	CCGTGATGCTATGCACCTTCT
PLTP	AAGAGCGGATGGTGTATGTGG	ATGGGGAGTCAATCACTGCTG
MMP9	TGTACCGCTATGGTTACTCTCG	GGCAGGGACAGTTGCTTCT
CCND1	GCTGCGAAGTGGAACCATC	CCTCCTTCTGCACACATTTGAA
SERPINE1	AGTGGACTTTTCAGAGGTGGA	GCCGTTGAAGTAGAGGGCATT
NDUFA4L2	CCTGAGCCCCAATGACCAATA	TCTGGCCGGTCTTCTTCA
TINAGL1	ATGGGACCCACTCAGTCAAGA	GTTGGCCGCAGTCCAGTATTT
COLEC12	AATCCTTCGGTTACAAGCGGT	ACTGTGATTGTTAGCAAGGCAC
APOE	GTTGCTGGTCACATTCCTGG	GCAGGTAATCCCAAAAGCGAC
MDK	CGCGGTTCGCCAAAAGAAAG	TACTTGCAGTCGGCTCCAAAC
AEBP1	ACCCACACTGGACTACAATGA	GTTGGGGATCACGTAACCATC
MIR99AHG	AGATTGCCTGCCACACACAT	AAGTTTTCTGCATCCGTGCG
LINC01503	CTTTCCCTGAGGACCATCTG	CAAAATCCGGTCTTTCTGGA
ITGA6-AS1	AGGTGGCAACATCCCTACAC	GACCCAACAGGCATGAAGGTA
INKA2-AS1	ACATTCAGCCTGGACACCC	TGAAGGTCCTTTTCAGCTCCC
STXBP5-AS1	GAGATTTAGGTGGGGACGCTGC	AGGGACTTGCCTTGTCTGCTGAT

LOC102723409	CAGGAGCCCAGCAAGTGTCT	TGATAGGGAGCAAGCAGTTCG
LOC105747689	AATATGGGGCACCAACGGAG	GCAAGCTCCTCCACTGAACA
LINC02407	GATGAAAACGACATGCGTGACA	TTCACTGTCGGTCTCCCTCT
LOC105377378	TTGGATGGATGACTATTTCTGC	AGGGGAAGTGGTACAAAAGCA
BCYRN1	ATAGCTTGAGCCCAGGAGTT	GCTTTGAGGGAAGTTACGCTTAT
LINC00968	AGATGATGTCGCAAGGGTGG	GGCTCCACCTGAAGTTACCC
LINC01423	CAAGGTCCTGCTGTCTTTCCA	GATGGTAACACCCCATCTGCT
LINC01013	CATCCAGCAGTGCCCAAGTA	GCTCTCCTTTGTTCCAGGCT
circ_0078715	GGGAAGCAGGGGTTGGATAA	CTCATCTGCGGGGAGGACT
circ_0078711	TCATCACAGGCATCGCCAATC	GACGGTGTGGGGGATCACT
circ_0058476	ACCCTTGAAATACACTGCGT	CAGCACCAAGACCATAGACA
circ_0005773	TACCAACTCCACACCGGAAC	AAGAAACGCACTTTCGTGGC
circ_0081571	GTCATAGTCTCAGCCCGCAT	GTGAACTCAGCTGTGGGGTT
circ_0081562	GCAACGTGGTTTTCTCACCC	ACAGCTGTGCTCCTCATCAA
circ_0041340	CAGCATTCTCCTTCTCGGTGT	TACCATGGATGTCTGGGCTG

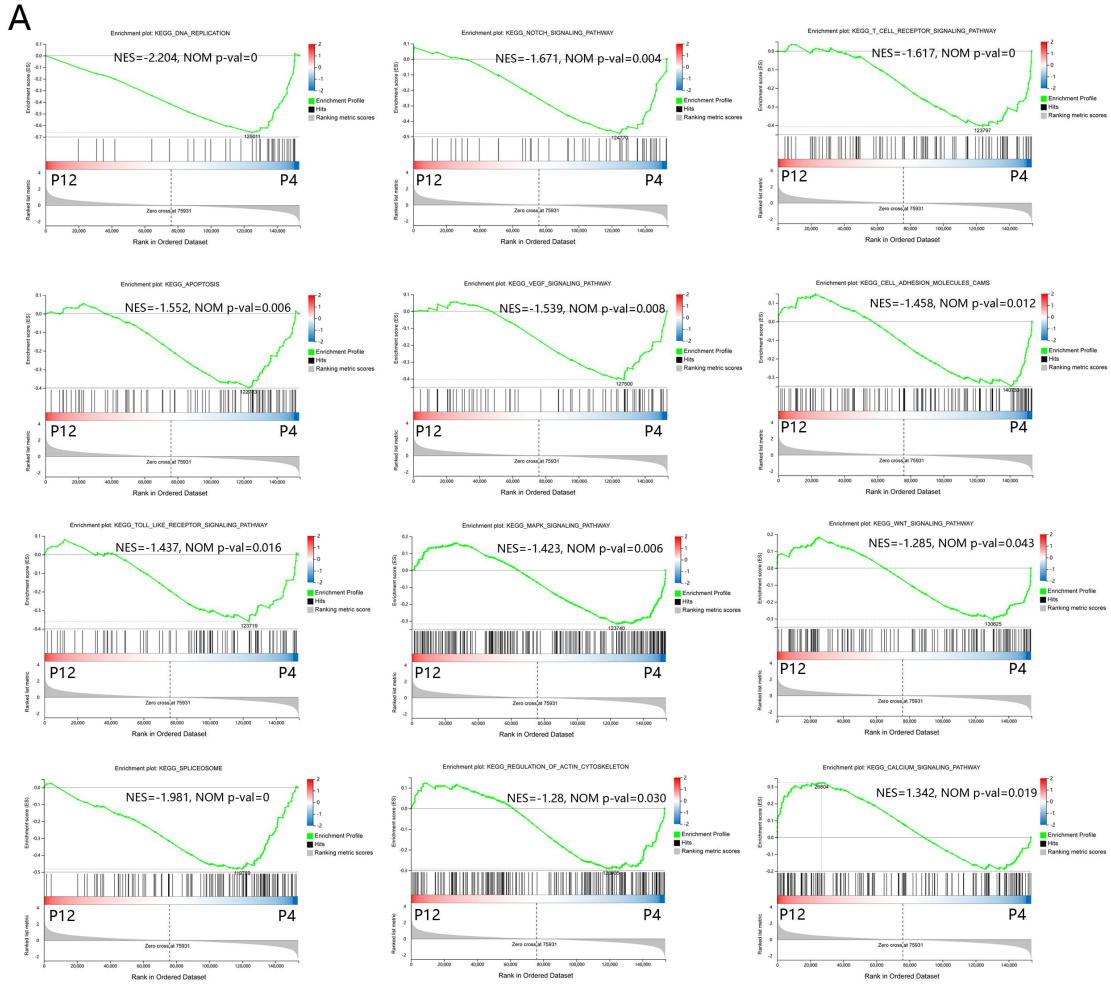


Figure S1. Results of KEGG from GSEA.

A.12 representative enriched senescence-related KEGG pathways from genes of P4 and P12. Normalized enrichment score (NES) and nominal p-value (NOM p-val) are shown.

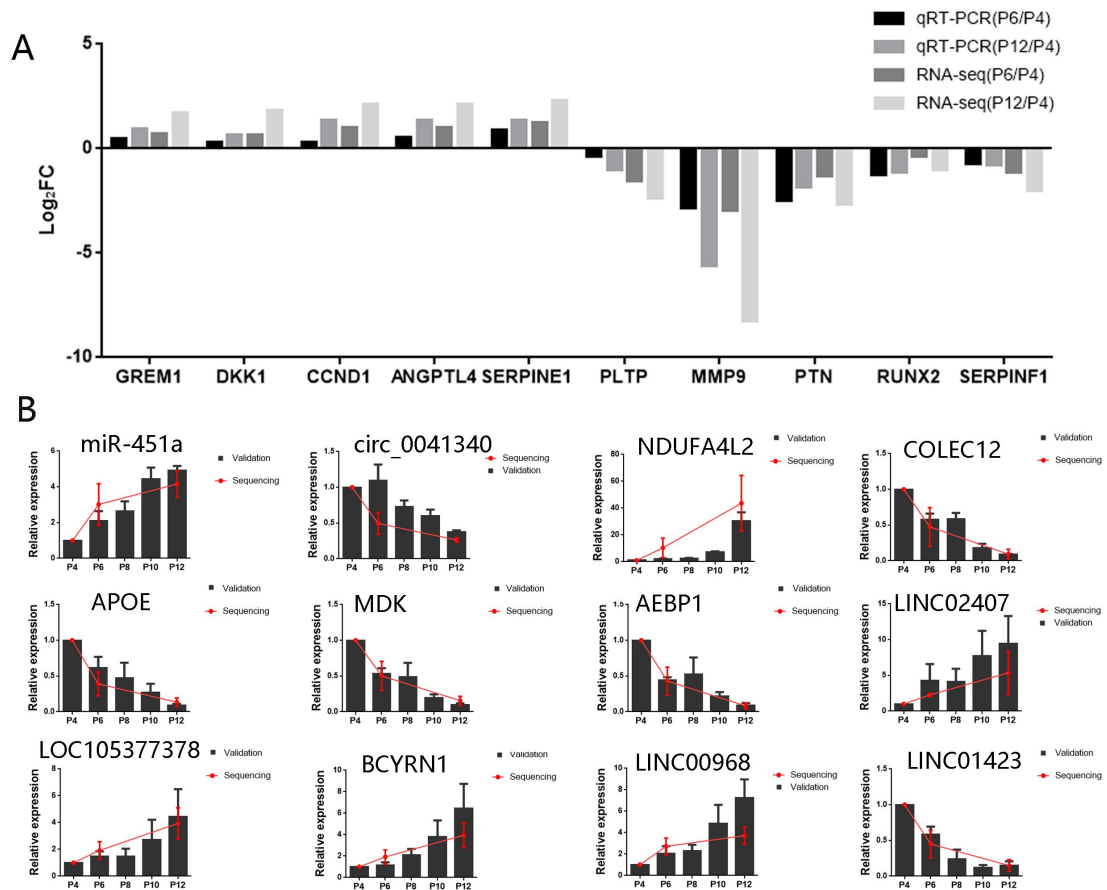


Figure S2. qPCR Validation

A. Comparison of \log_2FC of mRNAs involved in important process and pathway between RNA-Seq and qRT-PCR. B. qPCR validation of the expression levels of some RNAs in MSCs at P4, P6, P8, P10 and P12 merging the RNA sequencing (RNA-seq) results in P4, P6 and P12. Data are presented as the means \pm SEM. $n=3$ MSCs per passage in RNA-Seq. $n=10$ per passage in qRT-PCR.