

Supplementary Material

NUP62 alleviates senescence and promotes the stemness of human dental pulp stem cells via NSD2-dependent epigenetic reprogramming

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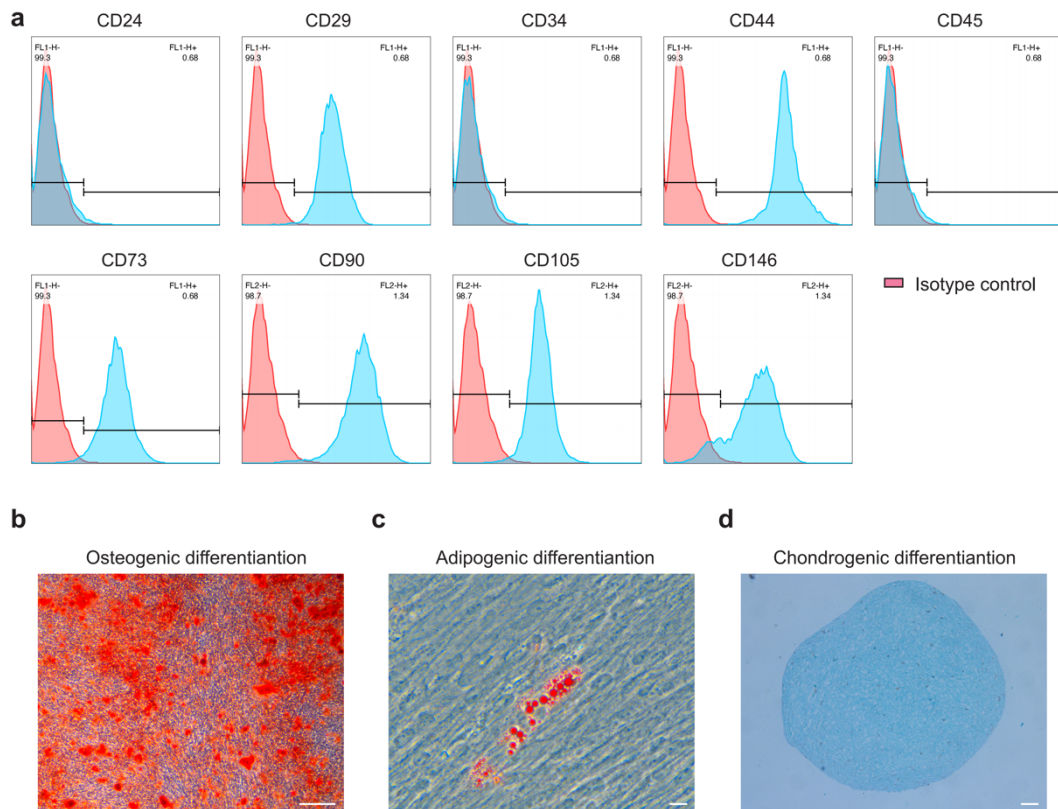


Fig. S1 Immunophenotyping and three-line differentiation of human dental pulp stem cells (HDPSCs). **a** Representative images of flow cytometric analysis of the surface marker expression. **b** Representative image of Alizarin Red S (ARS) staining

of HDPSCs induced in osteogenic induction medium for up to 21 days. Scale bar, 200 μm . **c** Representative image of Oil red O staining of HDPSCs induced in an adipogenic induction medium for up to 21 days. Scale bar, 20 μm . **d** Representative image of Alcian blue-stained HDPSCs induced in chondrogenic induction medium for up to 21 days. Scale bar, 20 μm .

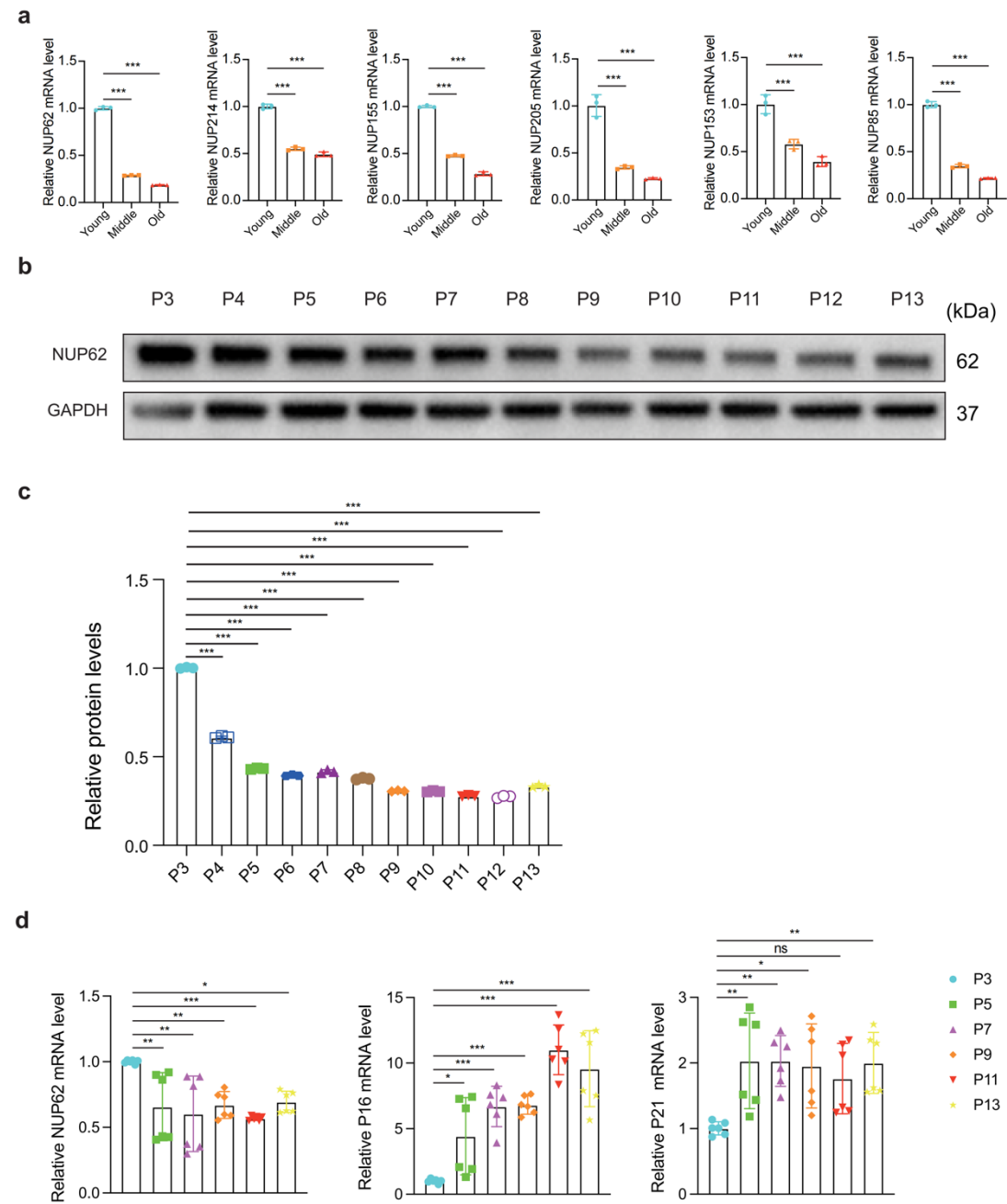


Fig. S2 NUP62 expression decreased in senescent human dental pulp stem cells

(HDPSCs). a Quantitative RT–PCR analysis of Nups expression in HDPSCs isolated from different donors (n=3). **b, c** Western blot analysis of NUP62 expression in HDPSCs with increasing passage numbers. **d** Quantitative RT–PCR analysis of the expression of NUP62 and senescence-associated genes (P16 and P21) with increasing HDPSC passage numbers (n=6). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

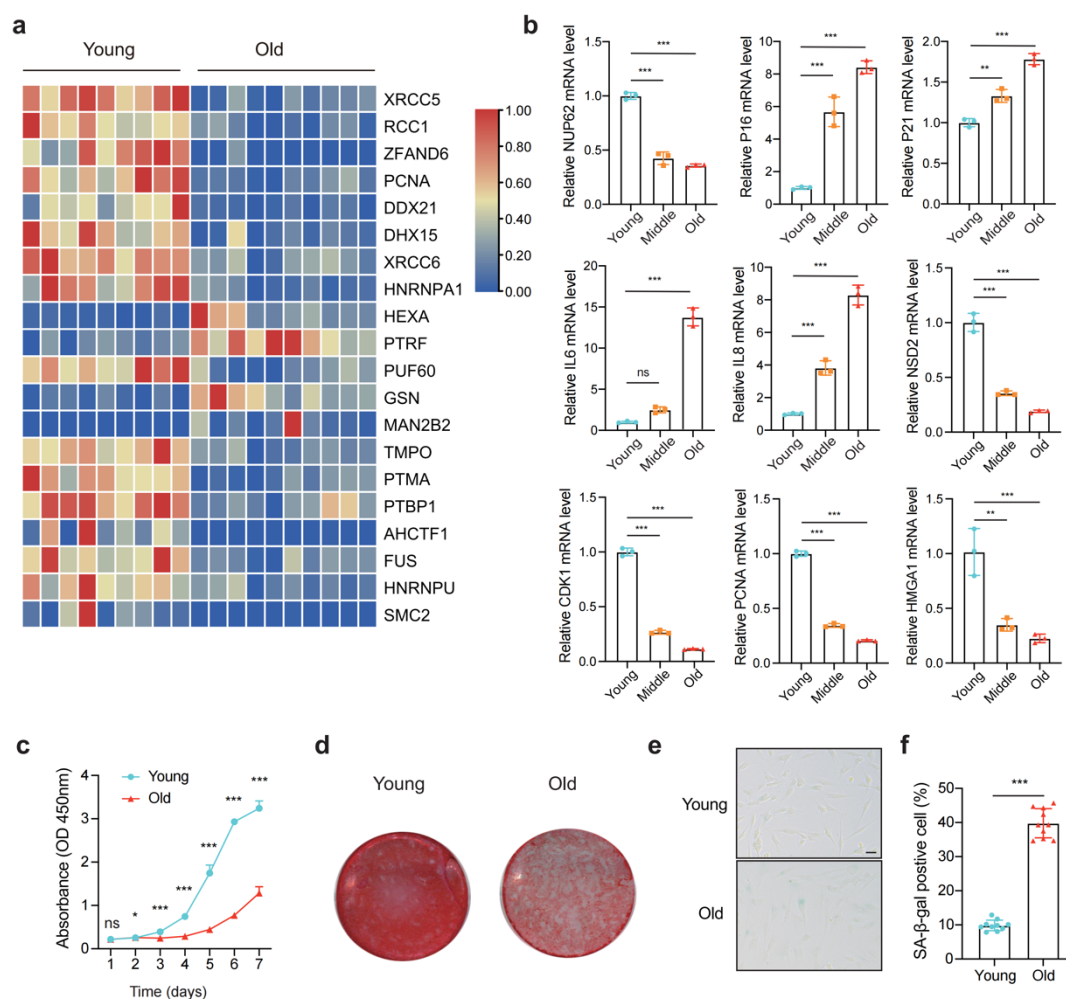


Fig. S3 Human dental pulp stem cells (HDPSCs) showed age-related phenotypes

with aging. a. Heatmap of Top 20 proteins that were differentially expressed in HDPSCs isolated from different donors (> 1.5 -fold, t test, $p < 0.05$). **b.** Quantitative

RT-PCR results of NUP62, P16, P21, IL6, IL8, NSD2, CDK1, PCNA and HMGA1 in HDPSCs with age (n=3). **c** CCK-8 assay results (n = 5). **d** Representative images of Alizarin red S staining of HDPSCs induced in an osteogenic induction medium for up to 21 days. **e, f** Representative images of senescence-associated β -galactosidase (SA β -gal) staining of HDPSCs and quantification of positive cells (n = 10). Scale bar, 50 μ m.

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

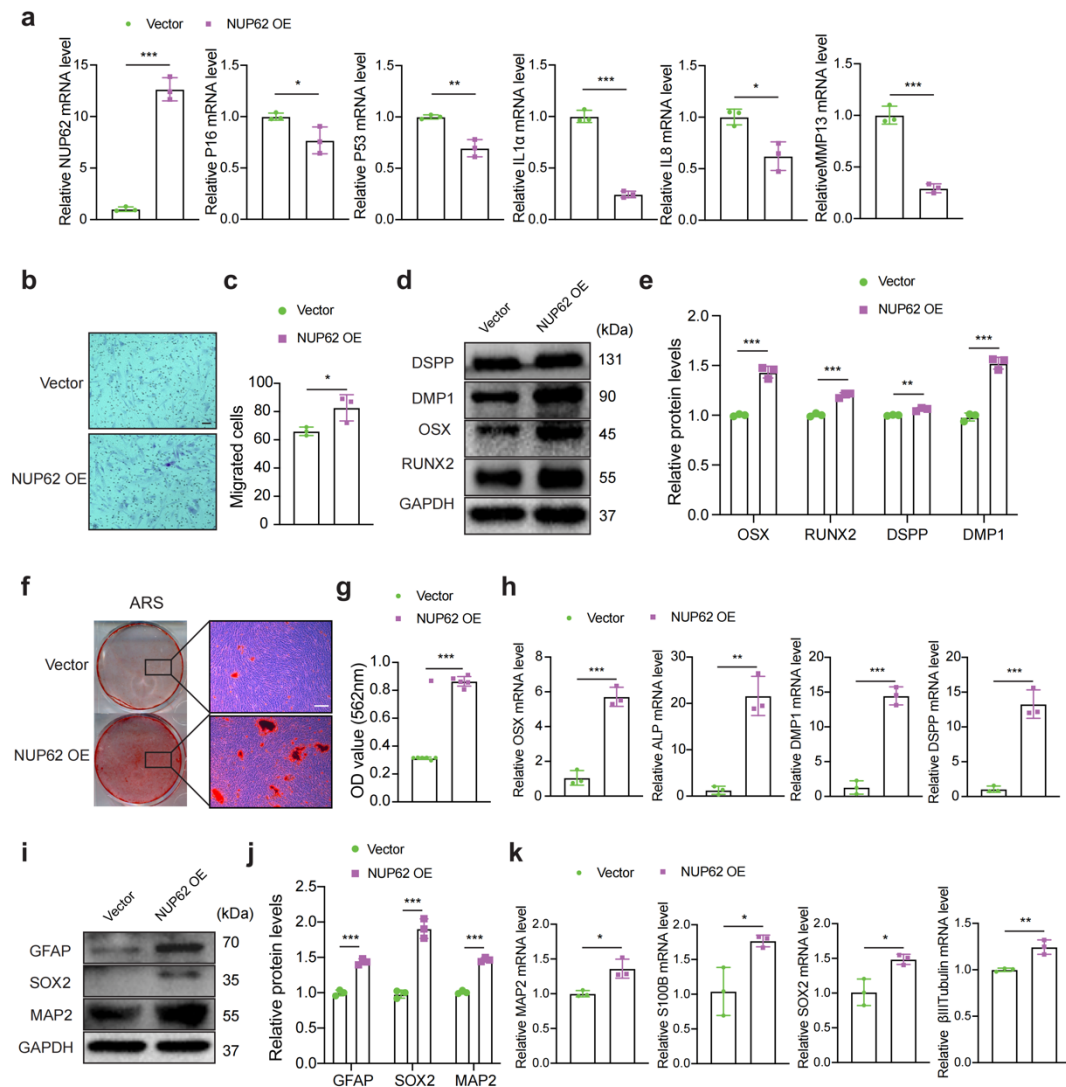


Fig. S4 Overexpression of NUP62 alleviates human dental pulp stem cell (HDPSC) senescence and promotes the differentiation potential of O-HDPSCs. **a** Quantitative RT–PCR results of NUP62 and senescence-associated genes (P16, P53, IL8, IL1 α and MMP13) (n = 3). **b, c** Crystal violet staining of migrated HDPSCs from the upper chamber to the lower bottom of the membrane and quantification of the results (n = 3). Scale bar, 50 μ m. **d,e** Western blot analyses of RUNX2, OSX, DMP1, DSPP and GAPDH in lysates of HDPSCs induced in an osteogenic induction medium for up to 14 days. **f** Representative images of Alizarin Red S (ARS) staining of HDPSCs induced in osteogenic induction medium for up to 14 days. Scale bar, 200 μ m. **g** Semiquantitative analysis of the ARS signal (n = 6). **h** Quantitative RT–PCR results showing the expression levels of ALP, OSX, DMP1 and DSPP mRNA in HDPSCs induced in an osteogenic induction medium for up to 7 days (n = 3). **i, j** Western blotting for GFAP, SOX2, MAP2 and GAPDH was performed using lysates from HDPSCs induced in a neural induction medium for up to 9 days. **k** Quantitative RT–PCR results showing the expression levels of MAP2, SOX2, S100B and β III tubulin mRNA in HDPSCs induced in a neural induction medium for up to 7 days (n = 3). * P <0.05, ** P <0.01 and *** P <0.001.

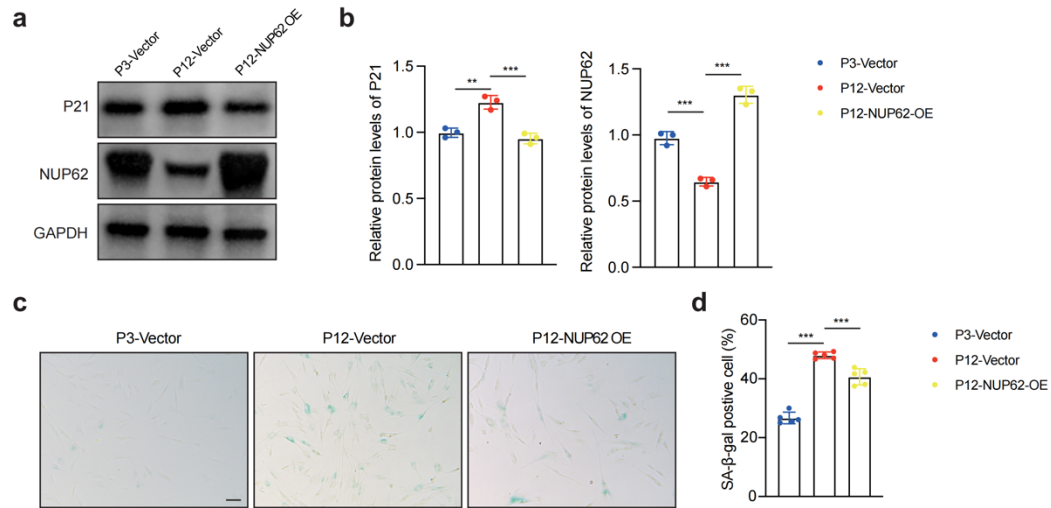


Fig. S5 Overexpression of NUP62 alleviates human dental pulp stem cell (HDPSC) senescence induced by cell passaging. a,b Western blot analyses of NUP62, P21 and GAPDH expression in HDPSCs. **c, d** Representative images of senescence-associated β -galactosidase (SA β -gal) staining of O-HDPSCs and quantification of positive cells (n=5). Scale bar, 50 μ m. ** P <0.01 and *** P <0.001.

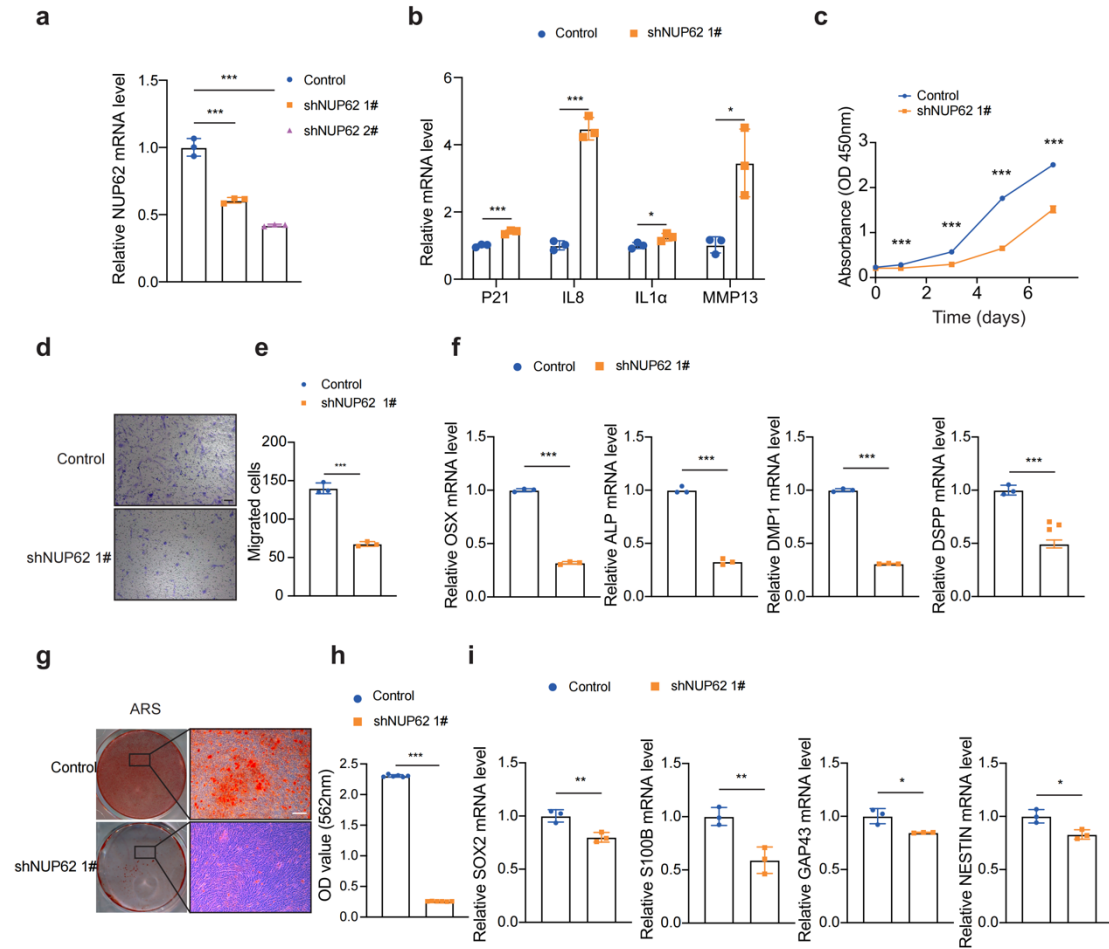


Fig. S6 NUP62 knockdown promotes Y-HDPSC senescence and impairs the differentiation potential of Y-HDPSCs. **a** Quantitative RT-PCR results showing NUP62 expression (n = 3). **b** Quantitative RT-PCR results showing the expression of senescence-associated genes (P21, IL8, IL1 α and MMP13) in HDPSCs (n = 3). **c** CCK-8 assay results (n = 5). **d**, **e** Crystal violet staining of migrated HDPSCs from the upper chamber to the lower bottom of the membrane and quantification of the results (n = 3). Scale bar, 50 μ m. **f** Quantitative RT-PCR results showing the mRNA expression levels of OSX, ALP, DMP1 and DSPP in HDPSCs induced in an osteogenic induction medium for up to 7 days (n = 3). **g** Representative images of Alizarin Red S (ARS)

staining of HDPSCs induced in an osteogenic induction medium for up to 14 days. Scale bar, 200 μ m. **h** Semiquantitative analysis of the ARS signal ($n = 6$). **i** Quantitative RT-PCR results showing the expression levels of SOX2, S100B, GAP43 and NSETIN mRNA in HDPSCs induced in a neural induction medium for up to 7 days ($n = 3$). $*P<0.05$, $**P<0.01$ and $***P<0.001$.

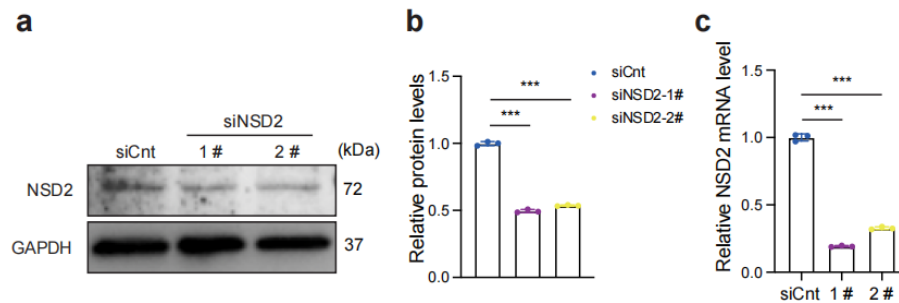


Fig. S7 Knockdown of NSD2 in HDPSCs transfected with siRNA. **a, b** Western blot analysis of NSD2 in HDPSCs transfected with control siRNA (siCnt) or NSD2 siRNA targeting 2 different sequences (#1, #2). **c** Quantitative RT-PCR analysis of the expression of NSD2 in HDPSCs transfected with control siRNA or NSD2 siRNA ($n=3$). $***P<0.001$.

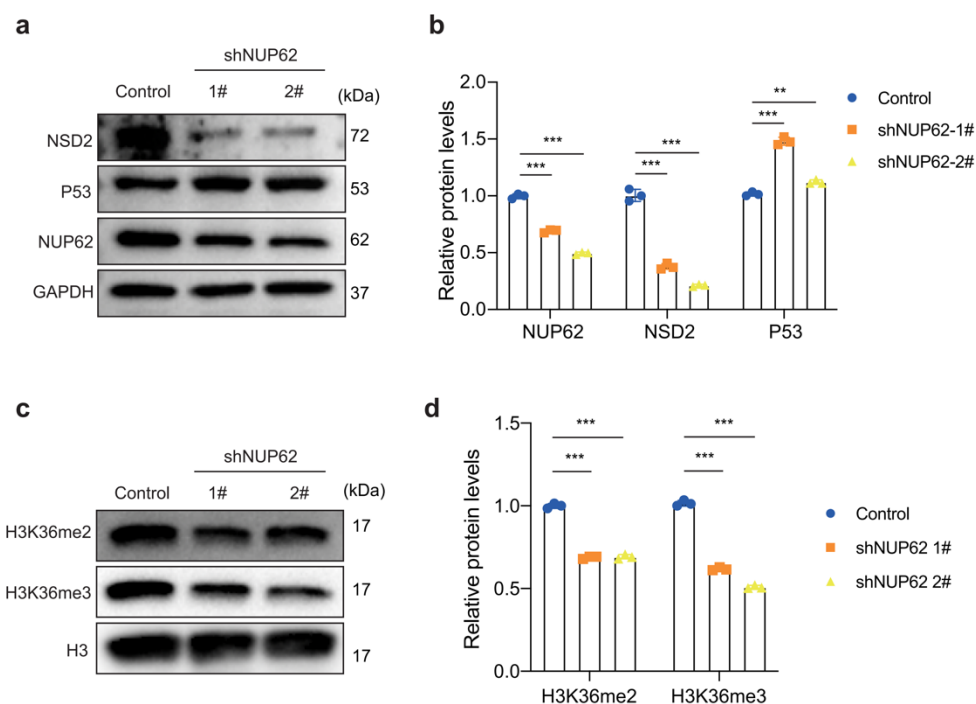


Fig. S8 Knockdown of NUP62 downregulates the expression of NSD2 , H3K36me2, and H3K36me3 levels. a,b Western blot analyses of NSD2, P53, NUP62 and GAPDH expression in HDPSCs with and without NUP62 knockdown. **c,d** Western blot analyses of H3K36me2, H3K36me3 and H3 expression in HDPSCs with and without NUP62 knockdown. ** $P < 0.01$ and *** $P < 0.001$.

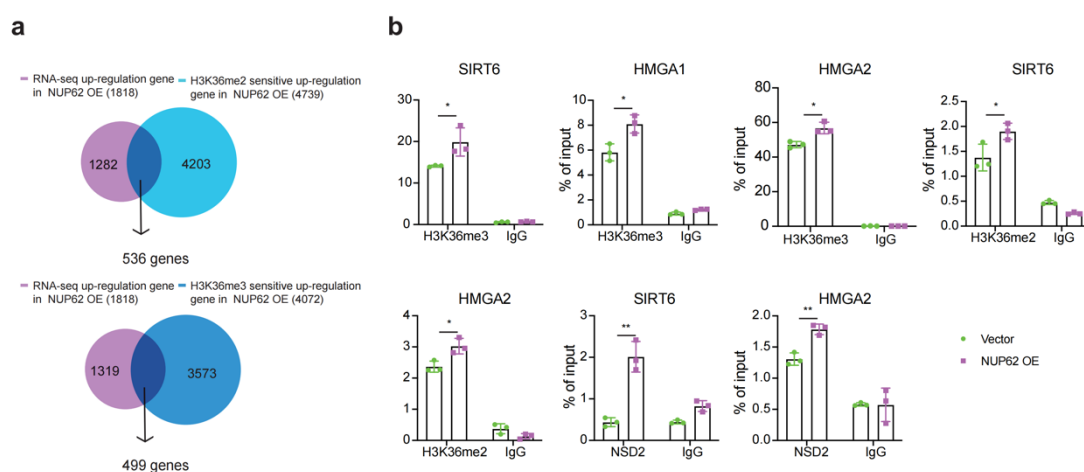


Fig. S9 NUP62-dependent genomic histone modification changes. **a** Venn diagrams depicting NUP62-dependent upregulated genes (1818) and H3K36me2-sensitive (4739) or H3K36me3-sensitive (4072) upregulated bound peaks at NUP62 overexpression. **b** ChIP-qPCR revealed that overexpression of NUP62 increased the occupancy of H3K36me3, H3K36me2 and NSD2 in the promoter regions of SIRT6, HMGA1 and HMGA2 (n = 3). * $P < 0.05$ and ** $P < 0.01$

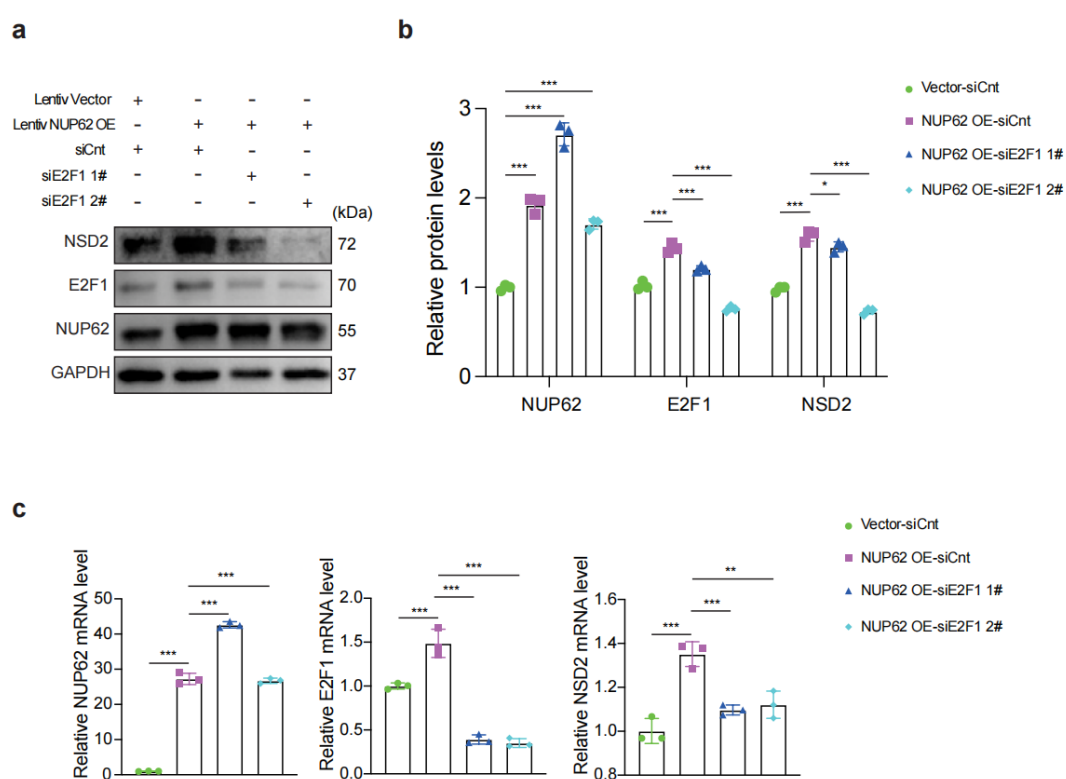


Fig. S10 NUP62 affects NSD2 transcription by regulating E2F1. **a, b** Western blotting for E2F1, NSD2, NUP62 and GAPDH was performed using lysates from HDPSCs transfected with lentiviral vector or overexpressing NUP62 and siRNA control or E2F1 siRNA. **c** Relative mRNA levels of NUP62, E2F1 and NSD2 in

HDPSCs transfected with the lentiviral vector or overexpressing NUP62 and siRNA control or E2F1 siRNA (n = 3). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Supplemental Table S1. Antibodies for flow cytometry

Reagent or Resource	Source	Identifier
Anti-Isotype control antibody	Abcam	ab91357
Anti-CD73 antibody	Abcam	ab133582
Anti-CD146 antibody	Abcam	ab75769
Anti-CD105 antibody	Abcam	ab231774
Anti-human CD90 antibody	Biolegend	328109
Anti-human CD24 antibody	Biolegend	311103
Anti-human CD34 antibody	Life	12-0349-42
Anti-CD45 antibody	Biolegend	103112
Anti-CD29 antibody	Abcam	ab179471
Anti-CD44 antibody	Abcam	ab23396

Supplemental Table S2. The information of sh/siRNA sequences

Name	Source	The target sequence of shRNA
sh-scramble-NUP62	OBIo	CCTAAGGTTAAGTCGCCCTCG
shNUP62-1	OBIo	GTTACGTTTGGCACTGCAAA
shNUP62-2	OBIo	ACCGGCTTTGCCTTGAATTTA
Name	Source	The target sequence of siRNA
siRNA Control	RIBOBIO	TTCTCCGAACGTGTCACGT
siRNA NSD2 1#	RIBOBIO	GGTCCAAAGTGTGCGGGTTA
siRNA NSD2 2#	RIBOBIO	GGGCATTGTTCAAGCAGAA
siRNA Control	RIBOBIO	TTCTCCGAACGTGTCACGT
siRNA E2F1 1#	RIBOBIO	GAGACCTCTCGACTGTGA
siRNA E2F1 2#	RIBOBIO	CTATGAGACCTCACTGAAT

Supplemental Table S3. RT-qPCR primers

Gene	Sequence 5' to 3'
GAPDH-F1	GGAGCGAGATCCCTCCAAAAT
GAPDH-R1	GGCTGTTGTCATACTTCTCATGG
P16-F1	TGCCCCAACGCACCGAATAGT
P16-R1	CAGCAGCTCCGCCACTCG
P21-F1	TGTCCGTCAGAACCCATGC
P21-R1	AAAGTCGAAGTTCCATCGCTC

P53-F1	AATCTACTGGGACGGAACAGCTTTGAGG
P53-R1	GGAGAGGAGCTGGTGTGTGTTGGG
IL6-F1	GCCCAGCTATGAACTCCTTCT
IL6-R1	GAAGGCAGCAGGCAACAC
IL8-F1	ACTGAGAGTGATTGAGAGTGGAC
IL8-R1	AACCCTCTGCACCCAGTTTTTC
MMP13-F1	ACTGAGAGGCTCCGAGAAATG
MMP13-R1	GAACCCCGCATCTTGGCTT
ALP-F1	GTGAACCGCAACTGGTACTC
ALP-R1	GAGCTGCGTAGCGATGTCC
RUNX2-F1	CAGACCAGCAGCACTCCATAT
RUNX2-R1	CAGCGTCAACACCATCATTC
BMP2-F1	ACTACCAGAAACGAGTGGGAA
BMP2-R1	GCATCTGTTCTCGGAAAACCT
DSPP-F1	GCATTACAGGGACAAGTAAGCA
DSPP-R1	CTTGGACAACAGCGACATCCT
DMP1-F1	AGCCATTCTGAGGAAGACGA
DMP1-R1	TGTTGTGATAGGCATCAACTGTTA
SOX2-F1	GCCGAGTGGAACTTTTGTCG
SOX2-R1	GGCAGCGTGTACTTATCCTTCT
S100B-F1	TGGCCCTCATCGACGTTTTTC
S100B-R1	ATGTTCAAAGAACTCGTGGCA
MAP2-F1	TCGTAGAGGGACGGAAAATTGA
MAP2-R1	GCCGTTGGTGTAGGGGTTC
TUBB3-F1	GGCCAAGGGTCACTACACG
TUBB3-R1	GCAGTCGCAGTTTTCACACTC
CDK1-F1	AAACTACAGGTCAAAGTGGTAGCC
CDK1-R1	TCCTGCATAAGCACATCCTGA
PCNA-F1	CCTGCTGGGATATTAGCTCCA
PCNA-R1	CAGCGGTAGGTCGAAGC
FOXMI-F1	GGAGCAGCGACAGGTTAAGG
FOXMI-R1	GTTGATGGCGAATTGTATCATGG
HMGA1-F1	GCTGGTAGGGAGTCAGAAGGA
HMGA1-R1	TGGTGGTTTTCCGGGTCTTG
HMGA2-F1	ACCCAGGGGAAGACCCAAA
HMGA2-R1	CCTCTTGGCCGTTTTTCTCCA
NSD2-F1	ACCGCGAGTGTTCTGTGTTT
NSD2-R1	GTCGTGGCCGTTAACTTCTG
E2F1-F1	ACGCTATGAGACCTCACTGAA
E2F1-R1	TCCTGGGTCAACCCCTCAAG

Supplemental Table S4. The promoter region sequences of NSD2

GCTAGAGTGCAGTGGCATGATCTCGGCTCACTGCAACCTCCACTTCCCGGGTTCAAGTGATTCTCC
TGCCTCAGCCTCCTGAATAGTTGCGATTACAGATGCCCGTCACTATGCCTGGCTAATTTTTGTATTT
TTTTTTAGTAGAGACGGGGTTTACCATTGTTAGCCAGGCTGGTCTCGAACTCCTGACCTCAGGCGA
TCCACCTGTCTCGGCCCTTCCAAAGTGCTTGATTAAAGGTGTGAGCCACCATGCCTGGCACTGGCC
GTAATTTTAAAAATTCATTTATTTGTTTGGTGATAGAGACGAGGTCTTGCTATGTTGCCCAGTCTG
GTCTCCAACCTCCTGGGCTCAAGCAATCCTTTCACTTTGGCCTCCCACAGTGCTGGCTCACACTTCTC
ACAGGTGTGAGCCACTGGCCTTGCTCCATTTTGAATTAATTTTGTAAAGGGTGAAAGTCTGTG
TCTAGCCTGATTTTTTTTTTGCATGTGGACATCCAGTTGTTCCAGCACCATTGTGCGAAAAGACTGTC
TTTTCTCCATTCCATTTCCCTAGAGTCAAGCTCAGCTGACTCCATTATGTGGTCTATATCCGGGTTCT
CTATTCTGTTTCATGGATCTATTTGTGATTTTTCCACCAGTACCACAATGTCTCAATTACTGTAGCC
TTATAGTAAGTCTTGCAGCAACCCCTGTTTTTTTTGGGGGGGCCACAACCTCAACCCATACAGGGGCC
CAGCACCTGTGAAGGGACAGCAGCGGAGGCGGCGCGGGAGAGGAAGAGGAGGTGAACTGTGGC
CACTCCTTGGTGGCTGGAGGTGGGTCCGGGGAGGCAGGACCTGGGCTGAGGTGTCGTTCTATAGC
CCGGGTAGCGCACTTTCACAAAGCTCTCCTTGACCAAACTCTCGCCAGGCTCCCCCTGGGCCCGCTT
CTCAGCGAGGGCTCAAGCTGGGCCTGTAAAGCTCGAGCAGACACTAAGGCTCAAGGCCGAAGGT
GTTCCCGGCTCCCTGAACCTGCGTGACAGAAAACCCAAGGCCGCCGGCTCCGGCAGCACCTGGAG
GCAGGAGCCGAACCTTCATGGGCGGCGGGCAGCAAATCACACCGGCTTCAACCGCCCCGGCCCGCC
CACTTTTTGCAGCGTCTGACTTCCCCGCTCGCCCGCGCCGCTTCCTCCTCCTCCCTGTGCCGCACGC
AGTCGCCCCGCGCCACCCCTGAAGCCCGGCTCTACCGCAGCGGCGCTGGGGTAGCGCCCCCTGGAC
CAGGCGTCCTCCCCCTCGCGAGCGGTCTCCCGCAGCCGCGCCAGCTCCGGGTGCGACCCTCGCC
CGGGGGACTCGTTCACGCCCTGCGGCCGTTAGGCGGGAAGAGCACGACGCCCTGCCCTGCCCCGT
CGTGCGTCTGACCCCTCTCCGCCACGTCTGTTCTGAGGCTCCGGGGCCGCCACGGCGGGGCTCTG
CGGCGGTTTCCCCACCCGTCCCCGCACTGCCGCTCCGCGAGGCGGACGCCGCTAGCAGCCTCCCG
CGGGCCCCAGGCCCCGCGCCCCTCTCACGGGACAGGAGCTTGGGGACCCGCGCGGGCGGGCCGC
GCGCCCAACGCCAGCCTCCGCGGCCGCGCGCCACGGCTCGGGGGCGCCGGCTCCGGACGGTGAT
TGGGCGCGGATCGTGACGTCACTGAGCGCGGCGCCCGCGGGCGCCGGGTCTGGGCCGGGTGCGC
GCCGCCGAGTAGCGGGAGCAGGGCGCGCGCCCGGCGCTACTCTGAGGCGGCTCGCGGGGGCCGC
GGCCCCGGCCCTCCCGGCGCTGCCAGCCCCGCCCCGCGCGGCCCCCACCAGCCGCGTGCCTGC
CCGCCCCGCCCCGTGCGCGGCCCGGCGCGGATTTGAAAAGCCCGGTCCGCGGGCCCCCGCGAGCGC
GGCAGCCAATCAGCGGCGCGCACTTTTCCCGCGGCTTCTGCGAGGCGGCGGCGGCCCCGGCTGCG
GCGGCGGCGGCGGGCGGGAGGCGCGGGGGCGGGGTGCGCGCCGCGCGCGAGAGCCTCGGCCTGG
CCGCGCTGCGCCCCGCGCCGCCGCCGCCCTCCCCGCTGGGCCCTACCGCCGCACGGCCCCGG
CCCCCTCCCAGCCTGCCGCTCCGAGAGCCGCCGCCGAGGATGCGACGCACCGCAGGTCACTGG
GGCGCCCCGCCAACAGTCGCGGGCCGCCAACCGCCGGGGCTG

Supplemental Table S5. Primers for Chip qPCR

Gene	Sequence 5' to 3'
SIRT6-F1	CCGACAGGGTGACCACAGA
SIRT6-R1	GGCTAGACAATGGGAGGGAC

HMGA1-F1	GGCGGGTAGGCAAAGTGTCG
HMGA1-R1	CACAGCAAGTTGGCGAGCAG
HMGA2-F1	GCCCTCCCGACAAAGAACG
HMGA2-R1	CCCTGGCTACCCTGCACTCT
NSD2-F1	GGACAGGAGCTTGGGGACC
NSD2-R1	AGTGACCTGCGGTGCGTC