

1 **DDX1 Methylation Mediated MATR3 Splicing Regulates Intervertebral Disc**
2 **Degeneration by Initiating Chromatin Reprogramming**

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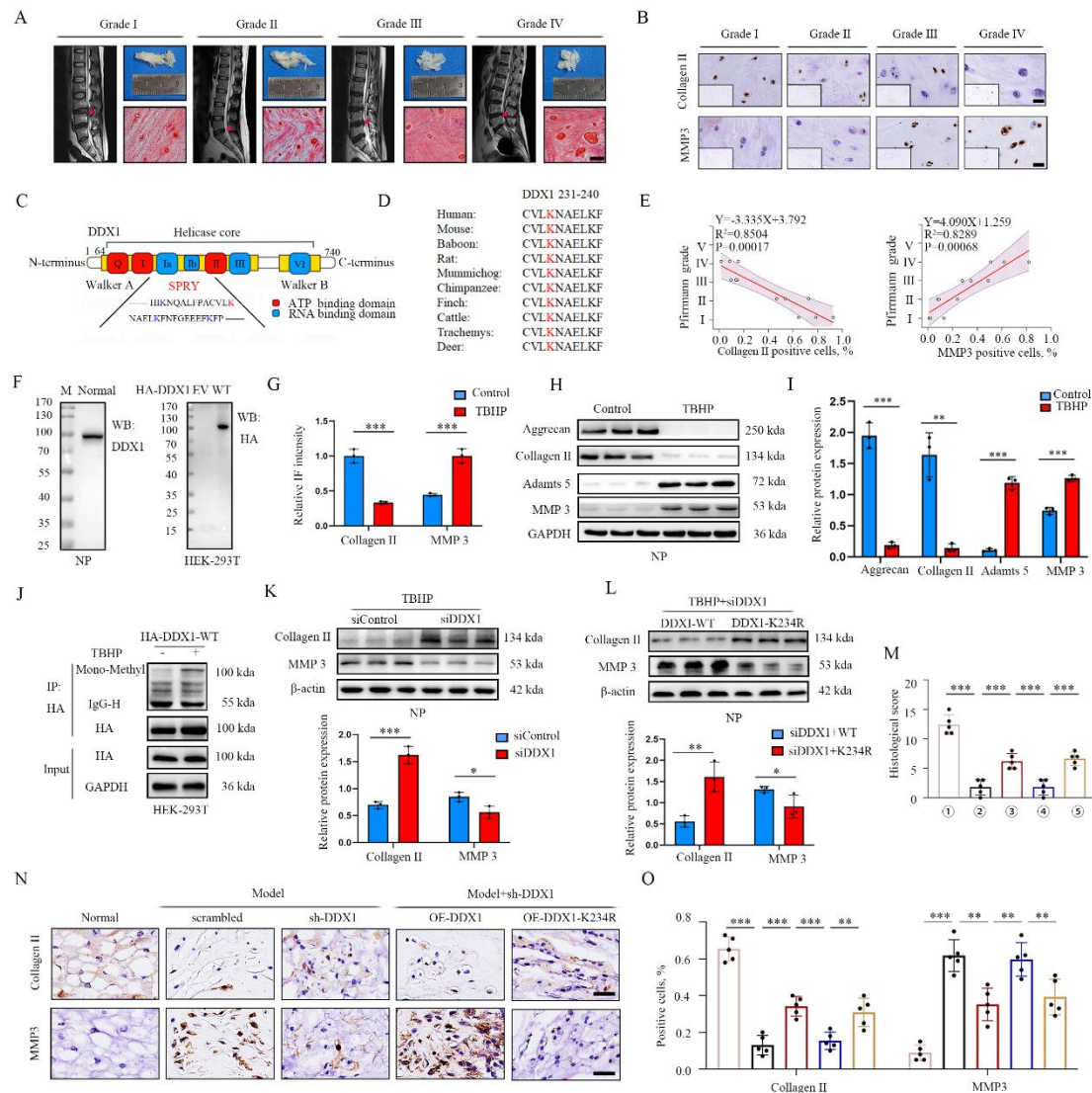
11 Supplementary Figure 1-7

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13 Uncropped Scans of Blots and Gels

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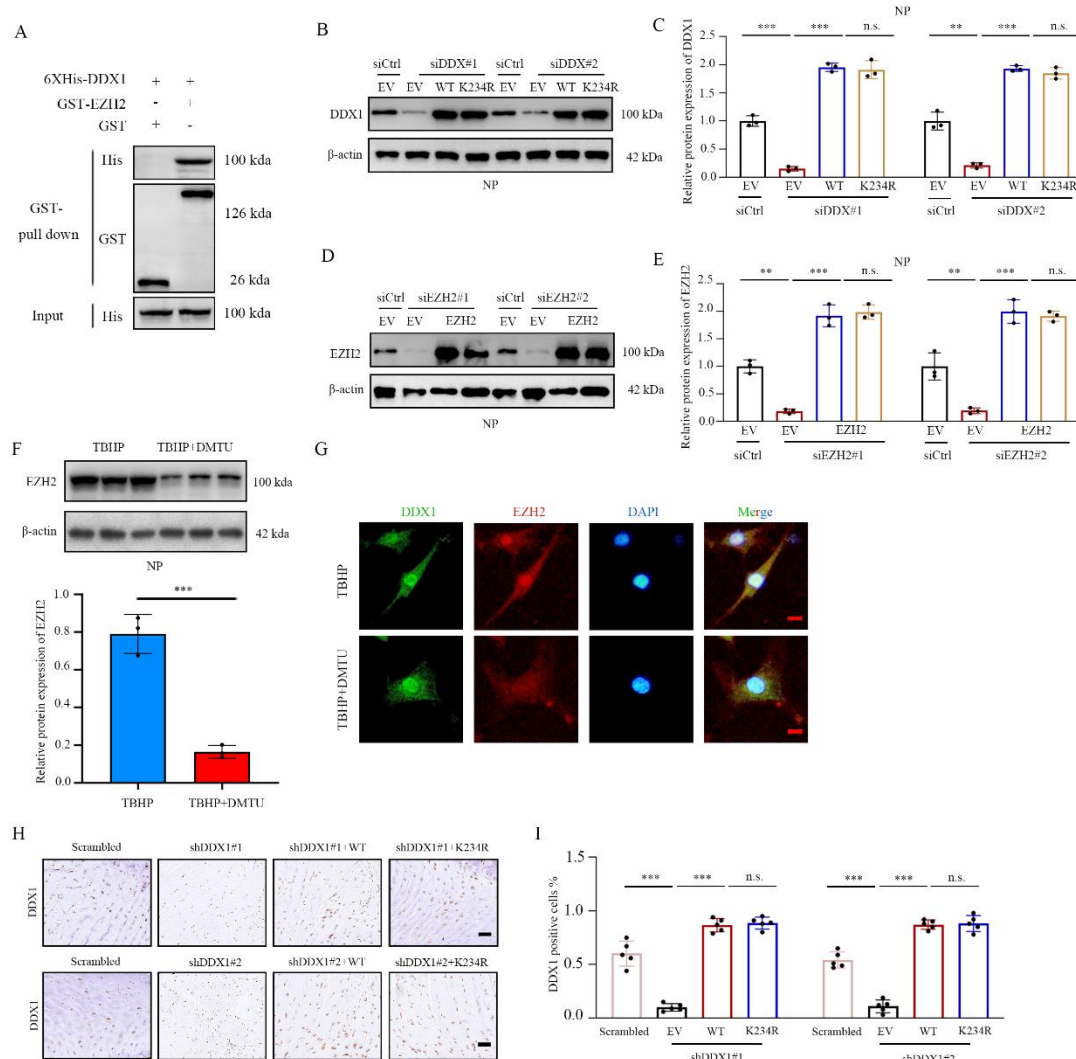
15 **Supplementary Figure 1-7**



16 **Supplementary Figure 1. DDX1 methylation promotes NP cells synthetic and**
 17 **catabolic disorders in IVDD**

18 A) Representative MRI images, general views and SO & FG staining of NP tissues with
 19 different Pfirrmann degenerative grades. Scale bar: 100μm. B) IHC staining of
 20 synthesis-catabolism related markers collagen II and MMP3 in human NP samples.
 21 Scale bar: 100μm (n=3). C) Schematic representation of the structure of DDX1,
 22 depicting the Walker binding domain, SPRY binding domain and their amino acid
 23 sequences, with all lysine residues highlighted in red or blue. D) Alignment of amino
 24 acid sequences of DDX1 across all species showing Lys234 highlighted in red. E)
 25 Correlation analysis between the collagen II ($P=0.00017$) and MMP3 ($P=0.00068$) and
 26 Pfirrmann degenerative grades (n=3). F) Western blot showing the molecular weight of

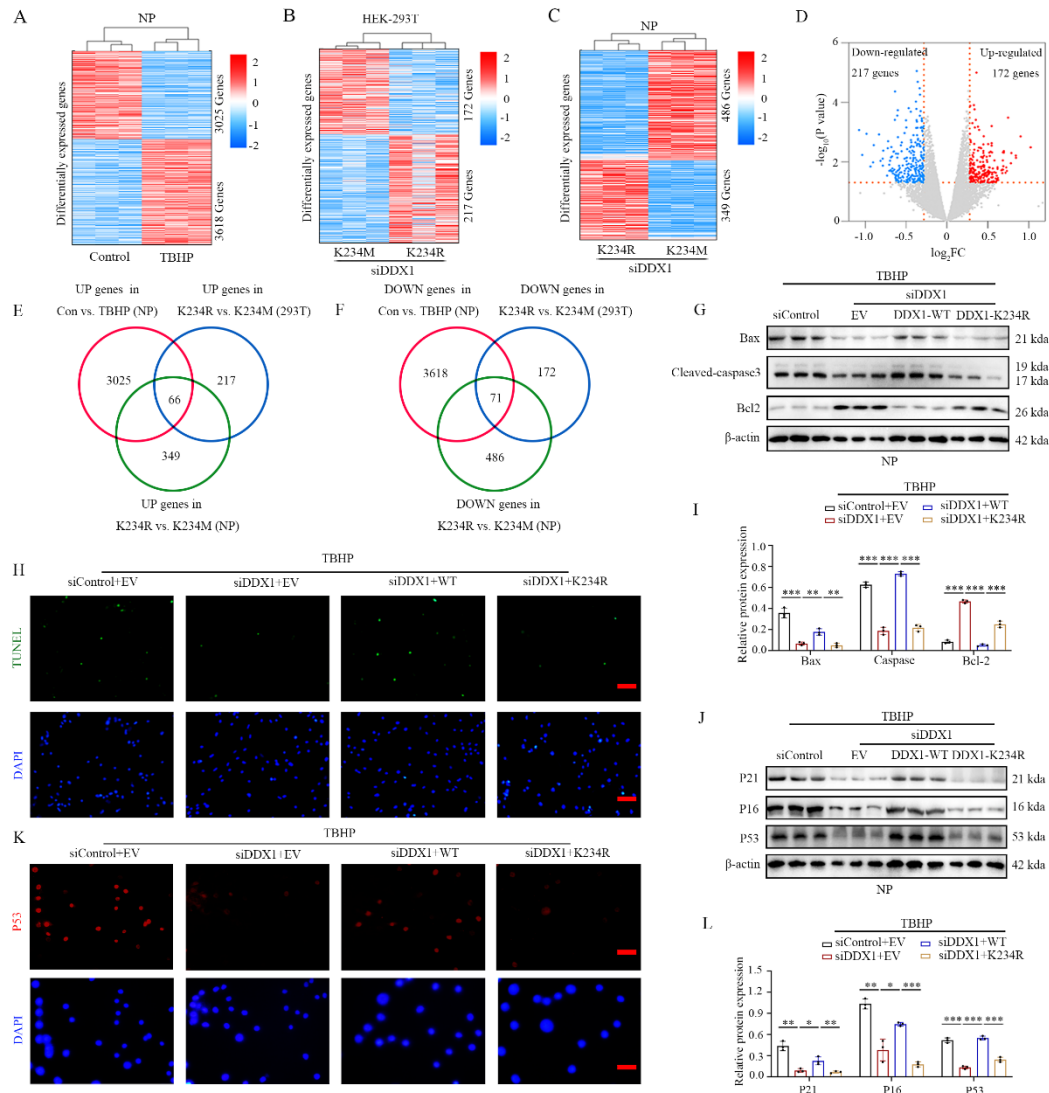
DDX1 in NP cells and HEK-293T cells as 100kda (n=3). G) IF level analysis of collagen II (P=0.000336) and MMP3 (P=0.000772) in normal and TBHP treated NP cells (n=3). H-I) Protein level analysis of aggrecan (P=0.000137), collagen II (P=0.002013), Adamts5 (P<0.0001), and MMP3 (P=0.000298) in normal and TBHP treated NP cells by western blot, GAPDH was used as the loading control (n=3). J) Expression of exogenous DDX1 lysine methylation was detected by CO-IP in normal and TBHP-treated HEK-293T cells (n=3). K) The siControl and siDDX1 plasmids were transfected into TBHP-treated NP cells, and the expression of Collagen II (P=0.000763) and MMP3 (P=0.023703) was determined by western blot (n=3). L) Cells derived from NP/siDDX1 cells re-overexpressed with indicated DDX1-WT or DDX1-K234R were used to detect Collagen II (P=0.008389) and MMP3 (P=0.029136) expression by western blot (n=3). M) histological score of coccygeal IVDs from rats with indicated treatment (n=10, P<0.0001, P=0.000697, P=0.000697, P=0.000260). N-O) IHC staining of collagen II (P<0.0001, P=0.000227, P=0.000333, P=0.005045) and MMP3 (P<0.0001, P=0.001379, P=0.002535, P=0.008526) in coccygeal IVDs from rats with the indicated treatment. Scale bar: 150µm (n=5). Data information: *P<0.05, **P<0.01, ***P<0.001. Data are represented as mean ± SEM. A significant P value was determined by Student's t-test (G, I, K-L) and two-way ANOVA (E, M, O). Panels (A, B, F-L) show one experiment representative of three independent experiments with similar results. Panels (N) show one experiment representative of five independent experiments with similar results.



Supplementary Figure 2. Additional si/shDDX1 and siEZH2 sequences were used to exclude off-target effects in NP cells

A) GST pull-down with purified GST-EZH2 followed by immunoblotting with anti-His and GST antibodies (n=3). B) Western blot analysis and C) quantification of the knockdown efficiency of siDDX1#1 ($P=0.000136$, $P<0.0001$, $P=0.69266$) and siDDX1#2 ($P=0.001233$, $P<0.0001$, $P=0.278346$) in NP cells, and the overexpression efficiency of WT and KR mutant DDX1 in NP cells. D) Western blot analysis and E) quantification of the knockdown efficiency of siEZH2#1 ($P=0.001195$, $P=0.00012$, $P=0.693334$) and siEZH2#2 ($P=0.005344$, $P=0.000145$, $P=0.564588$) in NP cells, and the overexpression efficiency of EZH2 in NP cells. F) Protein level analysis of EZH2 in human NP cells with indicated treatment by western blot, β -actin was used as the

loading control (n=3, P=0.000574) aining of EZH2 and DDX1 in human NP cells with indicated treatment. Scale bar: 10µm (n=3). H) IHC staining analysis and I) quantification of the knockdown efficiency of siDDX1#1 (P<0.0001, P<0.0001, P=0.603933) and siDDX1#2 (P<0.0001, P<0.0001, P=0.802643) and NP tissues, and the overexpression efficiency of WT and KR mutant DDX1 in NP tissues. Data information: *P<0.05, **P<0.01, ***P<0.001. Data are represented as mean ± SEM. A significant P value was determined by Student's t-test (F) and two-way ANOVA (C, E, I). Panels (A, B, D, F-G) show one experiment representative of three independent experiments with similar results. Panels (H) show one experiment representative of five independent experiments with similar results.

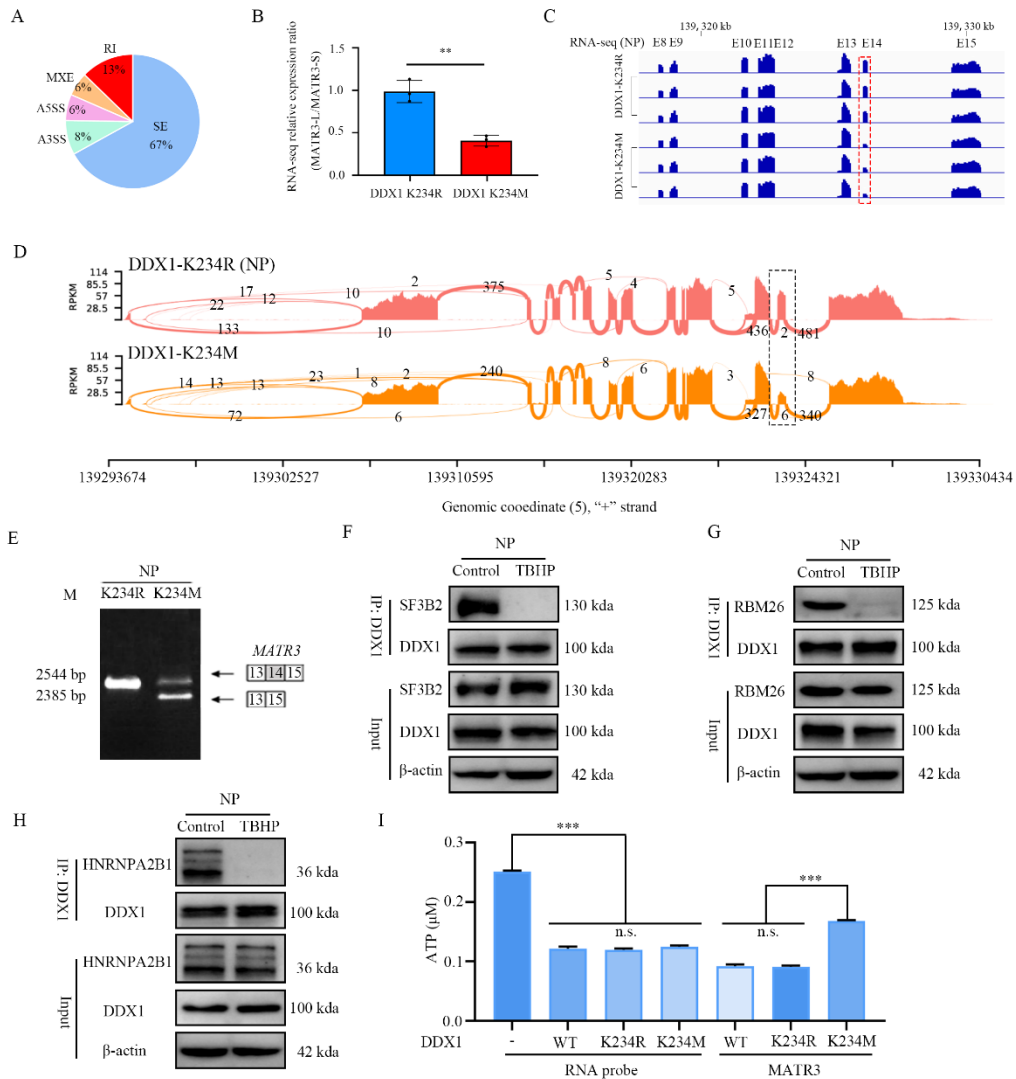


Supplementary Figure 3. DDX1 methylation promotes the senescence and apoptosis phenotype of NP cells

A) Heatmap of RNA-Seq analysis of DEGs in normal or TBHP treated NP cells (n=3). B) Heatmap of RNA-Seq analysis of DEGs in siDDX1/HEK-293T cells infected with siRNA-resistant DDX1 K234M and DDX1 K234R mutants (n=3). C) Heatmap of RNA-Seq analysis of DEGs in siDDX1/NP cells infected with siRNA-resistant DDX1 K234M and DDX1 K234R mutants (n=3). D) Volcano plot showing down-regulated and up-regulated genes of transcript profiles in siDDX1/HEK-293T cells infected with siRNA-resistant DDX1 K234M and DDX1 K234R mutants. E) Venn diagram of 3025 up-regulated genes in NP cell RNA-seq data (Control vs. TBHP), 349 up-regulated genes in NP cell RNA-seq data (K234R vs. K234M), 217 up-regulated genes with

HEK-293T cell RNA-seq data (K234R vs. K234M). F) Venn diagram of 3618 down-regulated genes in NP cell RNA-seq data (Control vs. TBHP), 486 down-regulated genes in NP cell RNA-seq data (K234R vs. K234M), 172 down-regulated genes with HEK-293T cell RNA-seq data (K234R vs. K234M). G, I) Representative images G) and quantitative analysis I) of Bax ($P=0.000538$, $P=0.005064$, $P=0.003863$), Caspase3 ($P<0.0001$, $P<0.0001$, $P<0.0001$) and Bcl-2 ($P<0.0001$, $P<0.0001$, $P=0.000394$) expression in the NP cells with the indicated treatment were detected by western blot ($n=3$). H) Representative images of TUNEL staining to quantify the apoptotic cells in human NP cells with the indicated treatment. Scale bar: $100\mu\text{m}$ ($n=3$). J, L) Representative images J) and quantitative analysis L) of P21 ($P=0.001109$, $P=0.019531$, $P=0.009262$), P16 ($P=0.002617$, $P=0.015207$, ($P<0.0001$)) and P53 ($P<0.0001$, $P<0.0001$, $P=0.000269$) expression in the NP cells with the indicated treatment were detected by western blot ($n=3$). K) Representative images of P53 staining to quantify the senescence cells in human NP cells with the indicated treatment. Scale bar: $100\mu\text{m}$ ($n=3$). Data information: $*P<0.05$, $**P<0.01$, $***P<0.001$. Data are represented as mean $\pm$ SEM. A significant P value was determined by Student's t-test (D) and two-way ANOVA (I, L). Panels (G-H, J-K) show one experiment representative of three independent experiments with similar results.

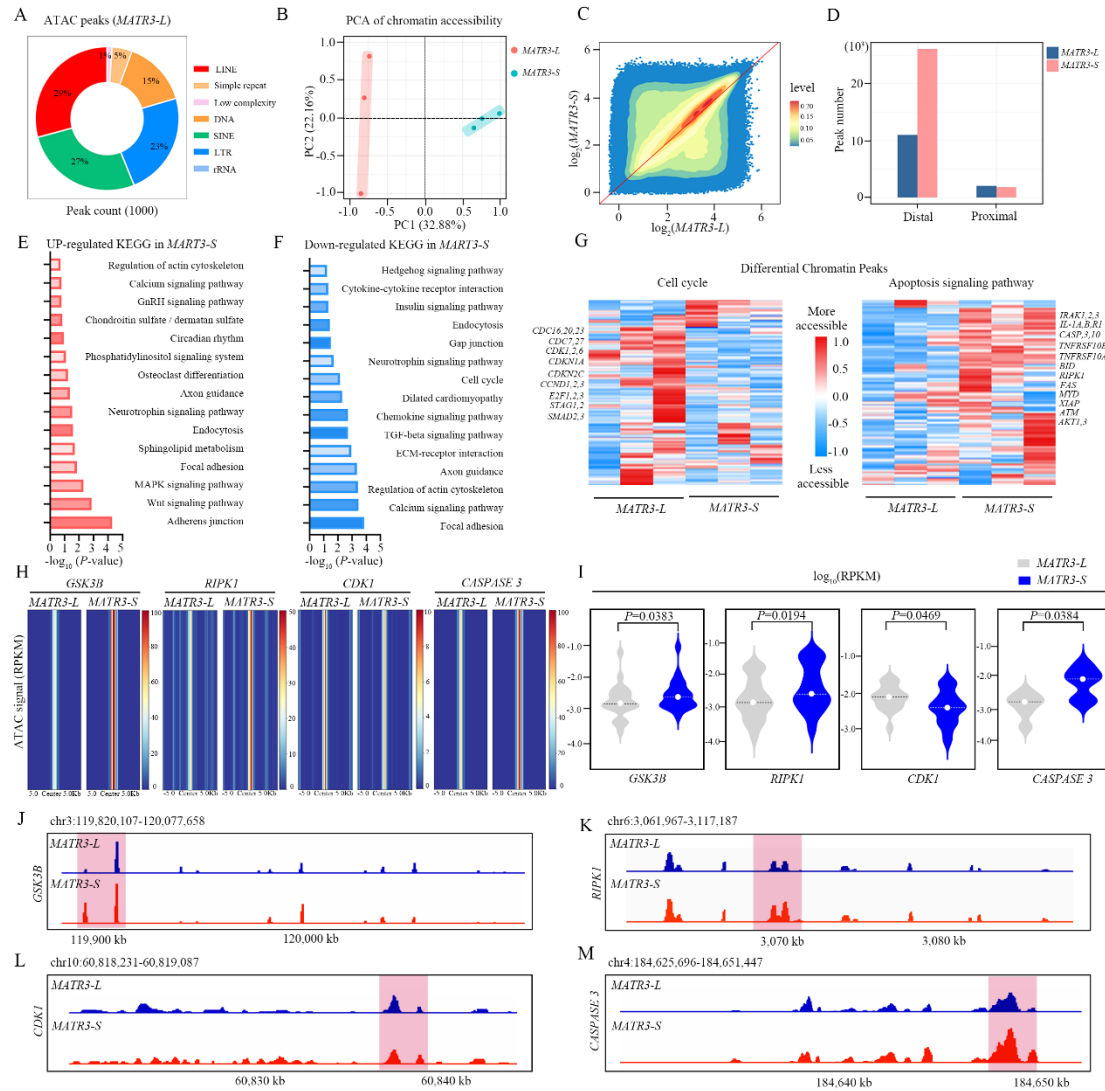
NP cells were highlighted in bright color (n=3). F) Quantitative analysis of SA- β -gal expression in the NP cells with the indicated treatment (n=3, P=000185, P=000264). G) Flow cytometry gating strategy: Cells were first gated to exclude debris and aggregates, selecting the main population based on forward and side scatter. Quadrant gating was then applied to a FITC-A vs. PI-A dot plot: Q4 (lower left), Annexin V⁻/PI⁻ (viable cells); Q3 (lower right), Annexin V⁺/PI⁻ (early apoptotic cells); Q2 (upper right), Annexin V⁺/PI⁺ (late apoptotic or necrotic cells); Q1 (upper left), Annexin V⁻/PI⁺ (primarily necrotic cells). The percentage of cells in each quadrant was calculated. Data information: *P<0.05, **P<0.01, ***P<0.001. Data are represented as mean $\pm$ SEM. A significant P value was determined by Student's t-test (A-D) and two-way ANOVA (F).



Supplementary Figure 5. The interaction of DDX1 with splicing factors and MATR3 is independent of its helicase activity

A) Pie chart depicting the proportions of different types of AS events in the RNA-seq data from NP cells with indicated treatment. B) The relative expression ratio of MATR3-L/MATR3-S was analyzed in NP cells with indicated treatment (n=3, P=0.002363). C) The AS pattern in MATR3 were visualized with IGV using RNA-seq of siDDX1/NP cells infected with DDX1 K234M and DDX1 K234R mutants (n=3). D) The Sashimi plots of exon 14 skipping in MATR3 in siDDX1/NP cell re-expressing DDX1 K234M (orange), and corresponding control cells (pink) (n=3). E) Semi-quantitative RT-PCR were performed to validate AS events of MATR3 from NP cells with indicated treatment (n=3). F) CO-IP analysis of the interaction between DDX1 and

SF3B2 in NP cells with indicated treatment (n=3). G) CO-IP analysis of the interaction between DDX1 and RBM26 in NP cells with indicated treatment (n=3). H) CO-IP analysis of the interaction between DDX1 and HNRNPA2B1 in NP cells with indicated treatment (n=3). I) Bar graphs quantify in vitro ATP consumption in different groups with the indicated treatment (n=3, $P<0.0001$, $P=0.177$, $P=0.599356$, $P<0.0001$). Data information: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Data are represented as mean $\pm$ SEM. A significant P value was determined by Student's t-test (B) and two-way ANOVA (I). Panels (E-H) show one experiment representative of three independent experiments with similar results.



Supplementary Figure 6. Excessive chromatin accessibility activates the senescence-apoptosis related signaling pathway

A) Visualization of distribution elements of ATAC peak in *MATR3-L* human NP cells.

B) PCA on all chromatin peaks from *MATR3-L* human NP cells and *MATR3-S* human NP cells. Each dot represents a single ATAC-seq library.

C) Scatter plot showing differential ATAC-seq peaks in *MATR3-L* human NP cells versus *MATR3-S* human NP cells.

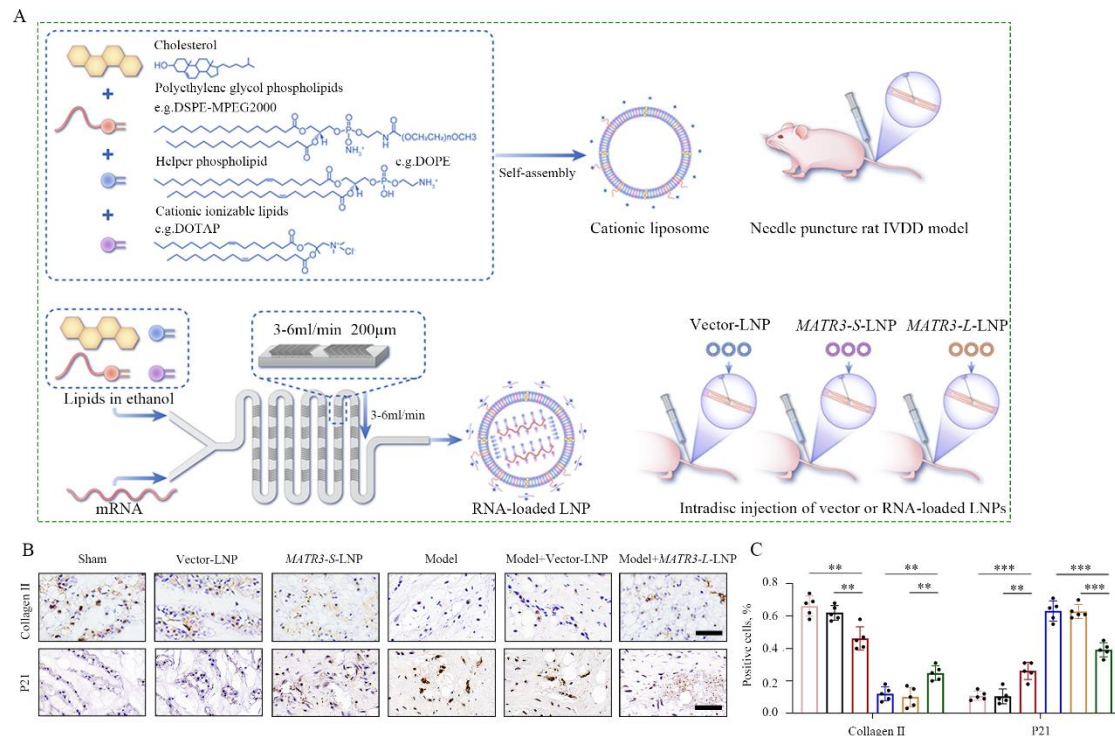
D) ATAC-seq peaks were annotated with ChIPseeker in *MATR3-L* human NP cells versus *MATR3-S* human NP cells. distal indicates the possible presence of noncoding regulatory elements.

E) KEGG analysis of up-regulated ATAC peak in *MATR3-S* human NP cells.

F) KEGG analysis of down-regulated ATAC peak in *MATR3-S* human NP cells.

G) Heat maps showing accessibility levels of differential

ATAC-seq peaks associated with the “Cell cycle” (left) and “Apoptosis signaling pathway” (right) that change with different MATR3 isoforms. Selected gene names with associated differentially accessible peaks are displayed. TMM-normalized read counts (by EdgeR), scaled row-wise. H) Heat map showing differential ATAC signal of GSK3B, RIPK1, CDK1 and CASPASE3 in MATR3-L human NP cells versus MATR3-S human NP cells. I) Violin plots were used to quantify the differential ATAC signals of GSK3B (P=0.0383), RIPK1 (P=0.0194), CDK1 (P=0.0469) and CASPASE3 (P=0.0384) in MATR3-L and MATR3-S human nucleus pulposus cells. J-M) IGV visualization of differentially accessible ATAC-seq peaks of J) GSK3B, K) RIPK1, L) CDK1 and M) CASPASE3 that change with different MATR3 isoforms. Data information: *P<0.05, **P<0.01, ***P<0.001. Data are represented as mean ± SEM. A significant P value was determined by Student's t-test (E-F, I).



Supplementary Figure 7. MATR3-L overexpression effectively promotes the anabolism and mitigates senescence in NP cells

A) Experimental design schematic. Composition of cationic LNPs synthesis components (top left). Microfluidic technology allows aqueous and lipid phases to flow independently into microfluidic channels (bottom left). Injection of engineered LNPs into NP region of SD rat coccygeal IVDs (right). B-C) IHC (Scale bar: 150μm) of collagen II (P=0.00156, P=0.003112, P=0.001753, P=0.002362) and P21 (P=0.00045, P=0.001058, P=0.00011, P<0.0001) in coccygeal IVDs from rats with the indicated treatment (n=5). Data information: *P<0.05, **P<0.01, ***P<0.001. Data are represented as mean ± SEM. A significant P value was determined by two-way ANOVA (C). Panels (B) show one experiment representative of five independent experiments with similar results.

Supplementary Table 1-3

Supplementary Table 1. Patient demographics

Case	Gender	Age	Grade	Diagnosis
Case 1	Female	13	I	Idiopathic scoliosis
Case 2	Female	16	I	Idiopathic scoliosis
Case 3	male	15	I	Idiopathic scoliosis
Case 4	male	12	I	Idiopathic scoliosis
Case 5	Female	17	I	Idiopathic scoliosis
Case 6	Female	25	II	Idiopathic scoliosis
Case 7	male	13	I	Idiopathic scoliosis
Case 8	Female	39	III	Lumbar disc herniation
Case 9	Female	40	IV	Lumbar disc herniation
Case 10	male	30	III	Lumbar disc herniation
Case 11	Female	28	III	Lumbar disc herniation
Case 12	male	21	III	Lumbar disc herniation
Case 13	Female	31	II	Lumbar disc herniation
Case 14	Female	37	II	Lumbar disc herniation
Case 15	male	39	II	Lumbar disc herniation
Case 16	Female	40	III	Lumbar disc herniation
Case 17	male	32	II	Lumbar disc herniation
Case 18	male	29	II	Lumbar disc herniation
Case 19	Female	44	III	Lumbar disc herniation
Case 20	Female	61	IV	Lumbar spinal stenosis
Case 21	male	55	IV	Lumbar spinal stenosis
Case 22	male	49	III	Lumbar spinal stenosis
Case 23	male	59	IV	Lumbar spinal stenosis
Case 24	male	50	IV	Lumbar spinal stenosis

Supplementary Table 2. Antibody information

Antibody	Company	Catalogue	Application/ Dilution
anti-Aggrecan	Affinity	DF7561	WB (1: 500)
anti-Collagen II	Affinity	AF5456	IHC (1:100); IF (1:100); WB (1:500)
anti-MMP3	Affinity	AF0217	IHC (1:100); IF (1:100); WB (1:500)
anti-Adamts5	Affinity	DF13268	WB (1: 500)
anti-DDX1	Proteintech	11357-1-AP	WB (1: 1000); IF (1:300); IP (4µg/1000µg)
anti-Mono Methyl Lysine	CST	14679	WB (1: 1000)
anti-EZH2	Abcam	ab307646	IHC (1:200); IF (1:400); WB (1: 500); IP (4µg/1000µg)
anti-GAPDH	Proteintech	60004-1-Ig	WB (1: 2000)
anti-β-actin	Proteintech	66009-1-Ig	WB (1: 2000)
anti-Bax	Affinity	AF0120	WB (1: 500);
anti-Cleaved-caspase3	CST	9661	WB (1: 500);
anti-Bcl-2	CST	3498	WB (1: 500)
anti-p21	CST	2947	IHC (1:150); IF (1:300); WB (1:500)
anti-P53	CST	2527	WB (1: 500)
anti-P16	CST	80772	WB (1:500)
anti-Flag tag	Proteintech	66008-4-Ig	WB (1: 1000); IP (4µg/1000µg)
anti-His tag	Proteintech	66005-1-Ig	WB (1: 1000); IP (4µg/1000µg)
anti-GST tag	Proteintech	66001-2-Ig	WB (1: 1000); IP (4µg/1000µg)
anti-HA tag	Proteintech	66006-2-Ig	WB (1: 1000); IP (4µg/1000µg)

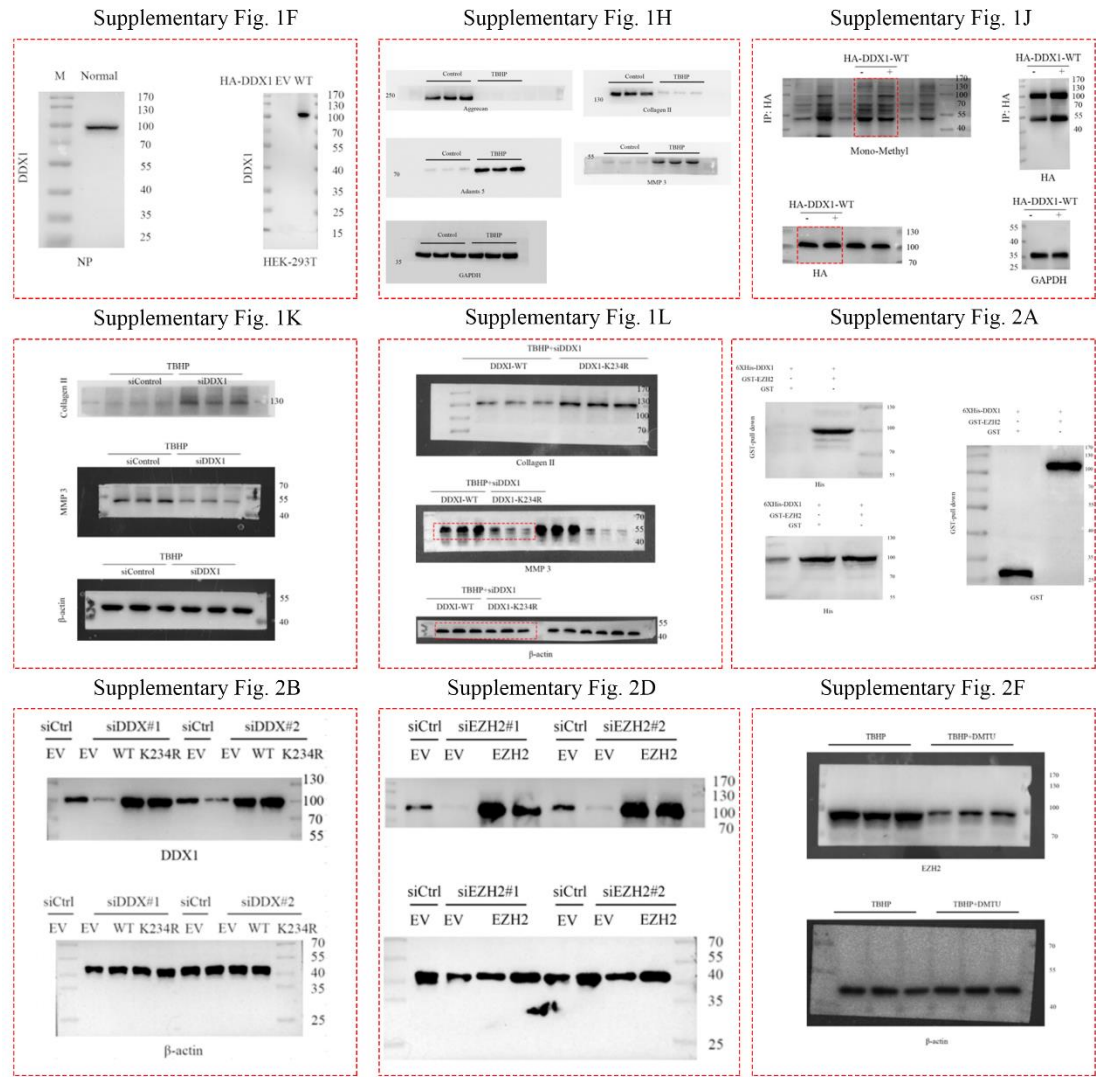
185 **Supplementary Table 3. Oligonucleotide sequences used in this study**

Oligonucleotide	Forward (5'-3')	Reverse (5'-3')
siRNA and shRNA sequence		
Homo-siDDX1#1	GGAUGAAUUUGAUGGGAAA	UUUCCCAUCAAUUCAUCC
Homo-siDDX1#2	GAUGGUCUUUGUUGUCAAGCTT	GCUUUGACAACAAAGACCAUCTT
Rat-shDDX1#1	GGAUGGUUACAUUGUCAAAUC	GAUUUGACA AUGUAACCAUCC
Rat -shDDX1#2	GCAAUUAUCUUCUGUAGAATT	UUCUACAGAAGAUAAUUGCTT
Homo-siEZH2#1	AUACAUGUGCAGCUUUCUG	CAGAAAGCUGCACAUGUAU
Homo-siEZH2#2	CCUCAUGUUUCCAGAUAAAGGTT	CCUUAUCUGGAAACA UUGAGGTT
Rat-shEZH2#1	GGAUGGUACUUUCAUUGAAGA	UCUUCAAUGAAAGUACCAUCC
Primers for Cloning plasmid		
Homo DDX1	GAATTGTGAGCGGATAACAATTCCCC TCTAGAATGGCGGCCTTCTCCGAGAT G	AGCCGGATCTCAGTGGTGGTGGTGGT GGTGCTCGAGGAAGGTTCTGAACAGC TGGT
Homo DDX1 K234R	TAAACTTAAGCTTGGTACCGAGCTCG GATCCGCCACCATGGCGGCCTTCTCC GA	CCAGAGGTTGATTGTCGAGATATCGG GCCCTCACTATGCGTAGTCTGGGACA TCA
Homo DDX1 K234M	TAAACTTAAGCTTGGTACCGAGCTCG GATCCGCCACCATGGCGGCCTTCTCC GA	CCAGAGGTTGATTGTCGAGATATCGG GCCCTCACTATGCGTAGTCTGGGACA TCA
Homo G9A	GACTCACTATAGGGAGACCCAAGCTG GCTAGCGCCACCATGGCGGCGGCGGC GGGAGCT	CCCACTGGACTAGTGGATCCGAGCT CGGTACCTCACTACTTGTTCATCGTCAT CCTTGTA
Homo SMYD3	TAAACTTAAGCTTGGTACCGAGCTCG GATCCGCCACCATGCGATGCTCTCAG TGCCGCG	CACTGTGCTGGATATCTGCAGAATTC TTATTTATCGTCATCATCTTTGTAGTC CTTGTCAT
Homo EZH2	GACTCACTATAGGGAGACCCAAGCTG GCTAGCGCCACCATGGGCCAGACTGG GAA	CCCACTGGACTAGTGGATCCGAGCT CGGTACCTCACTTGTTCATCGTCATCCT TG TAGTCGATGTC
Homo SETD7	CTCACTATAGGGAGACCCAAGCTGGC TAGCGCCACCATGGATAGCGACGAC	CAGAATTCCACCACACTGGACTAGTG GATCCTCACTTGTTCATCGTCATCCTT
Homo KMT5A	ACTTAAGCTTGGTACCGAGCTCGGAT CCGCCACCATGGCTAGAGGCAGG	CCGCCACTGTGCTGGATATCTGCAGA ATTCTTATTTATCGTCATCATCTT
Primers for PCR		
MATR3-L	GTTCAGTGGGAGACGAGACC	TGCCATGCCCCGATTTACGAA
MATR3-S	GACCAGACAGAGCAGGAACC	TCGATCTTGTCCACCTTTTAAAGC
EZH2	TCATGCAACACCCAACAC	CACAACCGGTGTTTCCTC
GAPDH	CAAGAAGGTGAAGCAGG	TCAAAGGTGGAGGAGTGGGT

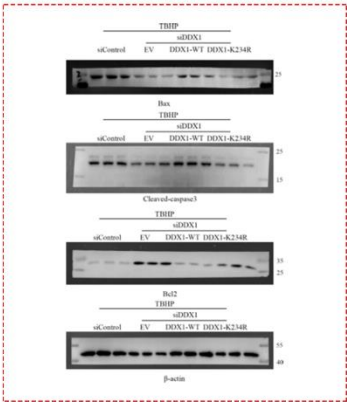
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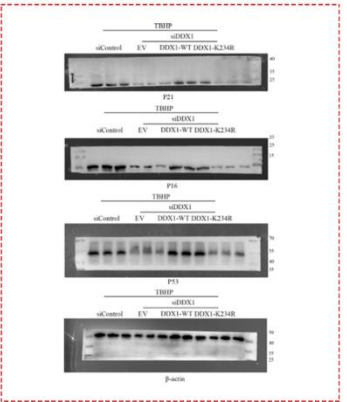
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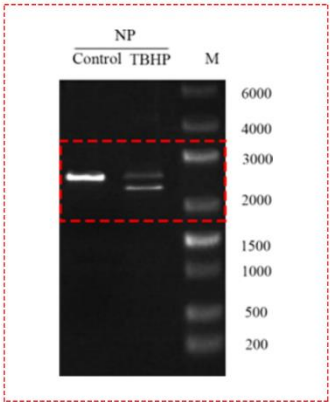
Supplementary Fig. 3G



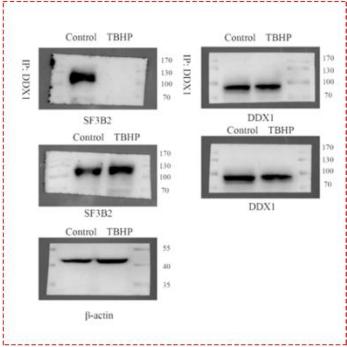
Supplementary Fig. 3J



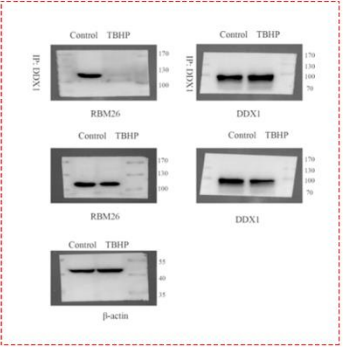
Supplementary Fig. 5E



Supplementary Fig. 5F



Supplementary Fig. 5G



Supplementary Fig. 5H

