

1 Supplementary materials

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3 *Selective autophagy receptor NBR1 retards nucleus pulposus cell senescence by directing the*
4 *clearance of SRBD1*

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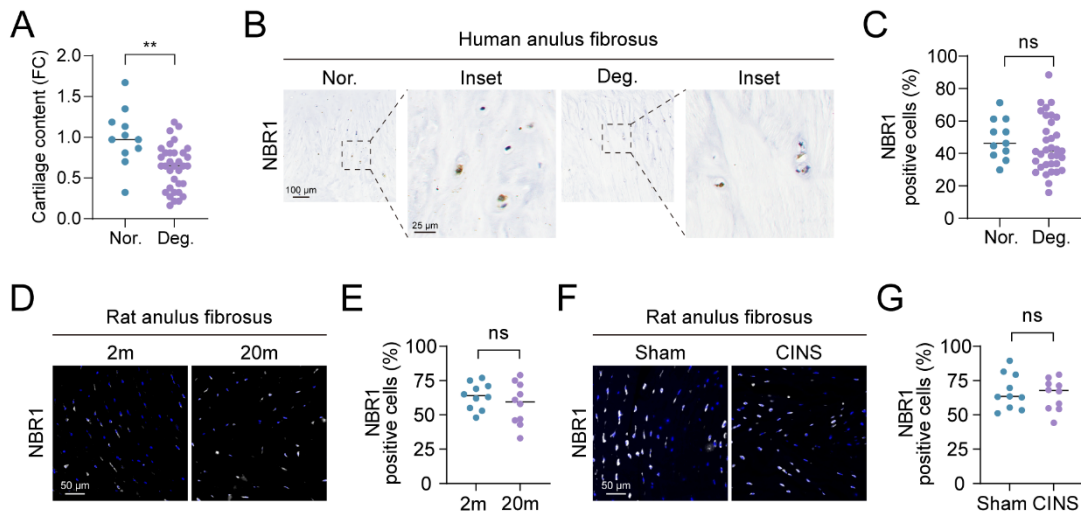
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20 **Supplementary Figures**

21 **Supplementary Fig. S1**



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23 **Supplementary Fig. S1 NBR1 is specifically downregulated in NPCs.** (A) Quantification of S&O

24 staining (red) in normal (n=11) and degenerated (n=34) groups. (B and C) Representative images

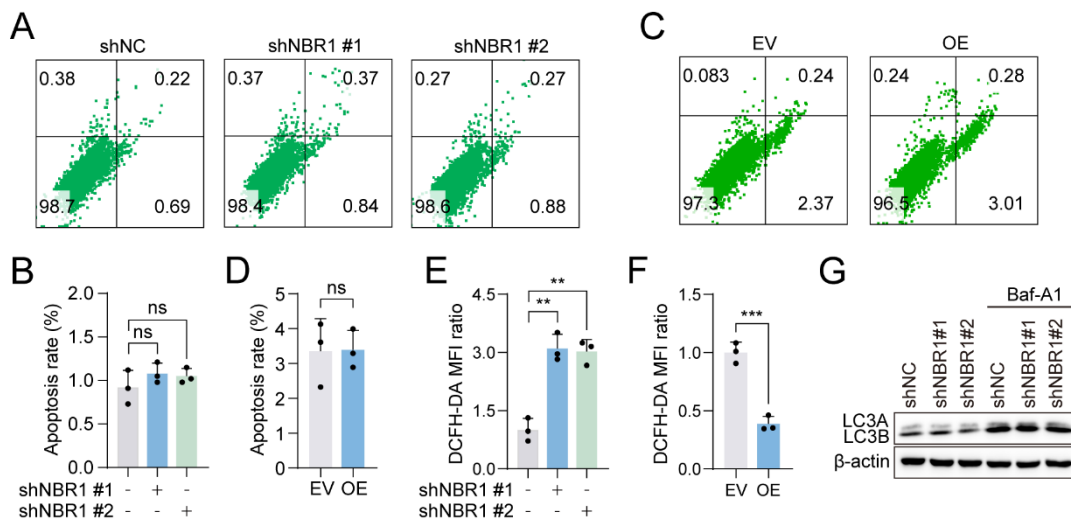
25 and quantification of human annulus fibrosus tissues from normal (n=11) or degenerated group. (D

26 and E) Representative images of annulus fibrosus from rats aged 2 months or 20 months, sections

27 were immunostained with NBR1. (F and G) Representative images of annulus fibrosus from rats in

28 sham or CINS group, sections were immunostained with NBR1.

29 **Supplementary Fig. S2**



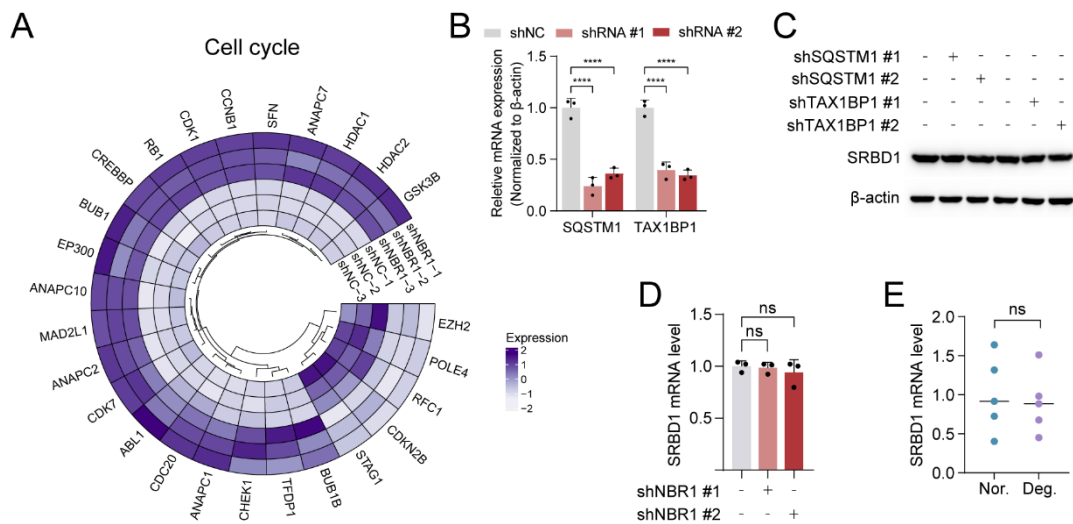
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31 **Supplementary Fig. S2 NBR1 regulates senescent phenotypes.** (A and B) Flow cytometry

32 analysis and quantification for cell apoptosis of NPCs infected with shNC or shNBR1 lentivirus. (C
 33 and D) Flow cytometry analysis and quantification for cell apoptosis of NPCs infected with vector
 34 or NBR1 lentivirus. Quantification of DCFH-DA staining for DCFH-DA staining of NBR1 silence
 35 (E) or overexpression (F) NPCs. (G) LC3A/B protein level in NBR1 silence NPCs treated with or
 36 without Baf-A1.

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38 **Supplementary Fig. S3**

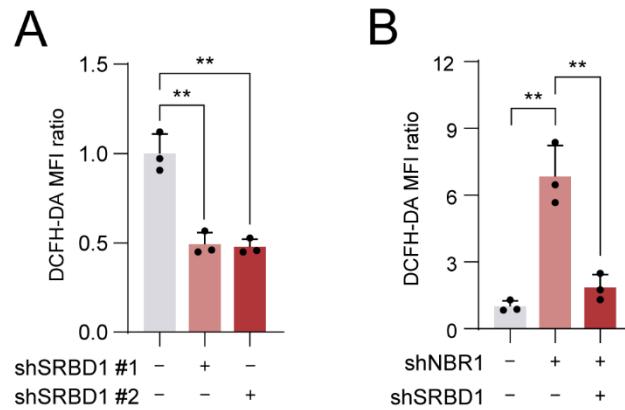


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40 **Supplementary Fig. S3 NBR1 retards NPCs senescence by regulating SRBD1.** (A) Circos plot
 41 comparing differentially expressed proteins in NPCs after transfected with shNBR1 lentivirus,
 42 highlighting proteins related to cell cycle. (B) SQSTM1, TAX1BP1 mRNA levels of NPCs
 43 transfected with corresponding shRNAs, as determined by qRT-PCR. (C) SQSTM1, TAX1BP1
 44 protein levels of NPCs transfected with corresponding shRNAs, as determined by western blot. (D)
 45 SRBD1 mRNA expression level in NBR1 silence NPCs. (E) SRBD1 mRNA level of NP tissues
 46 obtained from patients in normal (n=5) or degenerated group (n=5).

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48 **Supplementary Fig. S4**



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50 **Supplementary Fig. S4 NBR1 regulates the senescent phenotype of NPCs through modulation**

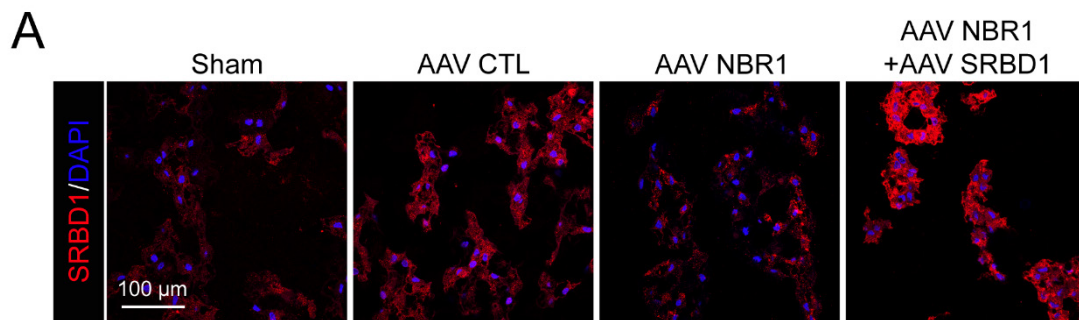
51 **of SRBD1. (A)** Quantification of flow cytometry for DCFH-DA staining of NPCs infected with

52 shNC or shSRBD1 lentivirus. **(B)** Quantification of flow cytometry for DCFH-DA staining of NPCs

53 in various experimental groups.

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55 **Supplementary Fig. S5**



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57 **Supplementary Fig. S5 NBR1/SRBD1 axis regulates NPC senescence and IDD progression in**

58 *in vivo*. **(A)** Representative images of rat coccygeal sections immunostained with SRBD1.

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61 Table S1 Primer sequences for mRNA detection

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Name	Forward (5'→3')	Reverse (5'→3')
<i>NBR1</i>	TTCCAGAACGCCCCGAGCCAA	GGGGCTAGTCAAGTCTGTC
<i>ACAN</i>	GTGCCTATCAGGACAAGGTCT	GATGCCTTTCACCACGACTTC
<i>ADAMTS4</i>	GTCCCATGTGCAACGTCAAG	ATGCGGCCATCTTGTCATCT
<i>ADAMTS5</i>	GGGCACTGGCTACTATGTGG	CGTCACAGCCAGTTCTCACA
<i>MMP13</i>	TCGGCCACTCCTTAGGTCTT	AAGTGGCTTTTGCCGGTGTA
<i>P16</i>	GATCCAGGTGGGTAGAAGGTC	CCCCTGCAAACCTTCGTCCT
<i>P21</i>	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
<i>IL-1β</i>	CCACAGACCTTCCAGGAGAAT	GTGCAGTTCAGTGATCGTACAGG
	G	
<i>IL-6</i>	AGACAGCCACTCACCTCTTCA	TTCTGCCAGTGCCTCTTTGCTG
	G	
<i>AKT1</i>	TGGACTACCTGCACTCGGAGA	GTGCCGCAAAGGTCTTCATGG
	A	
<i>CDK1</i>	GGAAACCAGGAAGCCTAGCAT	GGATGATTCAGTGCCATTTTGCC
	C	
<i>EP300</i>	GATGACCCTTCCCAGCCTCAA	GCCAGATCTCATGGTGAAGG
	A	
<i>HDAC1</i>	GGTCCAAATGCAGGCGATTCC	TCGGAGAACTCTTCCTCACAGG
	T	
<i>CCNB1</i>	GACCTGTGTCAGGCTTTCTCT	GGTATTTTGGTCTGACTGCTTGC
	G	
<i>CDC20</i>	CGGAAGACCTGCCGTTACATT	CAGAGCTTGCACTCCACAGGTA
	C	
<i>EZH2</i>	GACCTCTGTCTTACTTGTGGA	CGTCAGATGGTGCCAGCAATAG
	GC	
<i>β-actin</i>	CACCATTGGCAATGAGCGGTT	AGGTCTTTGCGGATGTCCACGT
	C	

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65 Table S2 Antibodies information

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Antibodies	Company	Catalog #	Application/Dilution
NBR1	Santa Cruz	sc-130380	WB (1:200); IHC (1:50); IF (1:50)
ACAN	Proteintech	13880-1-AP	WB (1:1000); IF (1:500)
ADAMTS4	Abcam	ab185722	WB (1:1000)
ADAMTS5	Abcam	ab41037	WB (1:1000)
MMP13	Abcam	Ab39012	WB (1:1000)
p21	Proteintech	10355-1-AP	WB (1:1000)
p16	Santa Cruz	sc-51243	WB (1:100); IF (1:50)
SRBD1	Sigma	SAB1407615	WB (1:1000)
p53	Abcam	ab26	WB (1:1000)
AKT1	CST	2938	WB (1:1000)
p-p65	CST	3033	WB (1:1000)
p65	CST	3034	WB (1:1000)
RB	Abcam	ab181616	WB (1:1000)
β -actin	HUABIO	M1210-2	WB (1:5000)

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69 Table S3. Detailed information for 32 proteins identified by 4D label-free proteomic and IP-MS
70 (ranked by fold change)
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Number	Accession	Description	Fold change
1	Q8N5C6	SRBD1_HUMAN S1 RNA-binding domain-containing protein 1 OS=Homo sapiens OX=9606 GN=SRBD1 PE=1 SV=2	50.63543978
2	A0A0J9YWL9	TX13C_HUMAN Putative testis-expressed protein 13C OS=Homo sapiens OX=9606 GN=TEX13C PE=5 SV=1	14.02979976
3	O95251	KAT7_HUMAN Histone acetyltransferase KAT7 OS=Homo sapiens OX=9606 GN=KAT7 PE=1 SV=1	5.224938763
4	Q9BRZ2	TRI56_HUMAN E3 ubiquitin-protein ligase TRIM56 OS=Homo sapiens OX=9606 GN=TRIM56 PE=1 SV=3	3.598650766
5	Q16384	SSX1_HUMAN Protein SSX1 OS=Homo sapiens OX=9606 GN=SSX1 PE=1 SV=2	3.399472561
6	O95425	SVIL_HUMAN Supervillin OS=Homo sapiens OX=9606 GN=SVIL PE=1 SV=2	2.793122259
7	Q9NQ29	LUC7L_HUMAN Putative RNA-binding protein Luc7-like 1 OS=Homo sapiens OX=9606 GN=LUC7L PE=1 SV=1	2.737533548
8	Q9UQ88	CD11A_HUMAN Cyclin-dependent kinase 11A OS=Homo sapiens OX=9606 GN=CDK11A PE=1 SV=4	2.684602714
9	Q96T88	UHRF1_HUMAN E3 ubiquitin-protein ligase UHRF1 OS=Homo sapiens OX=9606 GN=UHRF1 PE=1 SV=1	2.568367809
10	Q9HC07	TM165_HUMAN Transmembrane protein 165 OS=Homo sapiens OX=9606 GN=TMEM165 PE=1 SV=1	2.562645777
11	Q96NE9	FRMD6_HUMAN FERM domain-containing protein 6 OS=Homo sapiens OX=9606 GN=FRMD6 PE=1 SV=1	2.557427654
12	P46013	KI67_HUMAN Proliferation marker protein Ki-67 OS=Homo sapiens OX=9606 GN=MKI67 PE=1 SV=2	2.526341421
13	Q01804	OTUD4_HUMAN OTU domain-containing protein 4 OS=Homo sapiens OX=9606 GN=OTUD4 PE=1 SV=4	2.506026463

14	Q9HAN9	NMNA1_HUMAN Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1 OS=Homo sapiens OX=9606 GN=NMNAT1 PE=1 SV=1	2.462463804
15	P46778	RL21_HUMAN 60S ribosomal protein L21 OS=Homo sapiens OX=9606 GN=RPL21 PE=1 SV=2	2.447680516
16	Q9UH17	ABC3B_HUMAN DNA dC->dU-editing enzyme APOBEC-3B OS=Homo sapiens OX=9606 GN=APOBEC3B PE=1 SV=1	2.39955418
17	O43660	PLRG1_HUMAN Pleiotropic regulator 1 OS=Homo sapiens OX=9606 GN=PLRG1 PE=1 SV=1	2.391817094
18	Q9H9B1	EHMT1_HUMAN Histone-lysine N-methyltransferase EHMT1 OS=Homo sapiens OX=9606 GN=EHMT1 PE=1 SV=4	2.372888048
19	Q15800	MSMO1_HUMAN Methylsterol monooxygenase 1 OS=Homo sapiens OX=9606 GN=MSMO1 PE=1 SV=1	2.249218587
20	Q8N684	CPSF7_HUMAN Cleavage and polyadenylation specificity factor subunit 7 OS=Homo sapiens OX=9606 GN=CPSF7 PE=1 SV=1	2.220114633
21	O75533	SF3B1_HUMAN Splicing factor 3B subunit 1 OS=Homo sapiens OX=9606 GN=SF3B1 PE=1 SV=3	2.186060336
22	P82675	RT05_HUMAN 28S ribosomal protein S5, mitochondrial OS=Homo sapiens OX=9606 GN=MRPS5 PE=1 SV=2	2.161491677
23	O15226	NKRF_HUMAN NF-kappa-B-repressing factor OS=Homo sapiens OX=9606 GN=NKRF PE=1 SV=2	2.13815002
24	Q86YT6	MIB1_HUMAN E3 ubiquitin-protein ligase MIB1 OS=Homo sapiens OX=9606 GN=MIB1 PE=1 SV=1	2.124131404
25	Q15334	L2GL1_HUMAN Lethal(2) giant larvae protein homolog 1 OS=Homo sapiens OX=9606 GN=LLGL1 PE=1 SV=3	2.077573619
26	Q02543	RL18A_HUMAN 60S ribosomal protein L18a OS=Homo sapiens OX=9606 GN=RPL18A PE=1 SV=2	2.072391233
27	P50402	EMD_HUMAN Emerin OS=Homo sapiens OX=9606 GN=EMD PE=1 SV=1	2.060547814
28	P08621	RU17_HUMAN U1 small nuclear ribonucleoprotein 70 kDa OS=Homo sapiens	2.05489978

		OX=9606 GN=SNRNP70 PE=1 SV=2	
29	P43355	MAGA1_HUMAN Melanoma-associated antigen 1 OS=Homo sapiens OX=9606 GN=MAGEA1 PE=1 SV=1	2.046375095
30	Q9BQ39	DDX50_HUMAN ATP-dependent RNA helicase DDX50 OS=Homo sapiens OX=9606 GN=DDX50 PE=1 SV=1	2.042845041
31	Q9Y4P3	TBL2_HUMAN Transducin beta-like protein 2 OS=Homo sapiens OX=9606 GN=TBL2 PE=1 SV=1	2.026773541
32	P10301	RRAS_HUMAN Ras-related protein R-Ras OS=Homo sapiens OX=9606 GN=RRAS PE=1 SV=1	2.002732076

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