

Supplementary Figures

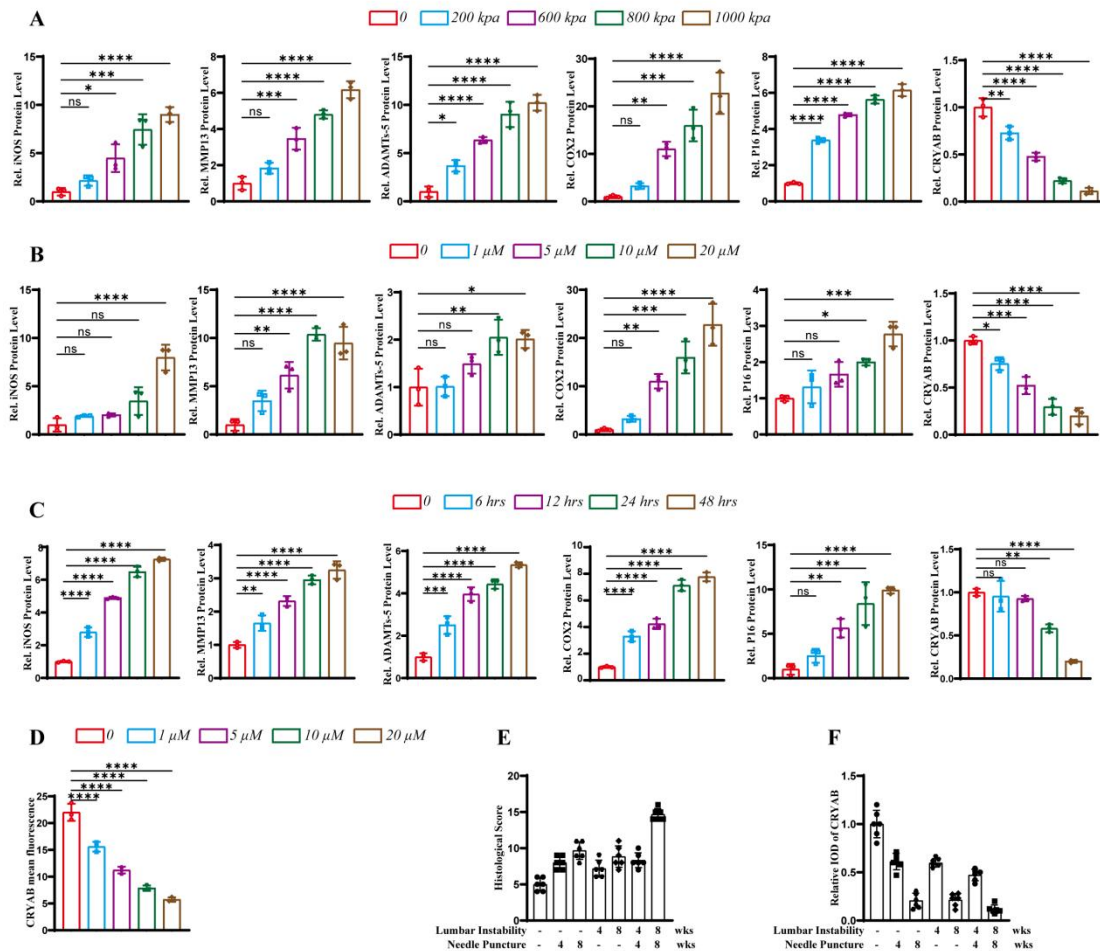


Figure S1. Pathological mechanical stress and Piezo1 activation drive dose- and time-dependent catabolism and CRYAB depletion.

(A) Quantitative densitometry of catabolic, inflammatory, senescence markers, and CRYAB protein levels in primary NP cells subjected to escalating hydrostatic pressure (0–1000 kPa). (B, C) Immunoblot quantification of corresponding marker proteins following Yoda1-mediated Piezo1 activation across varying concentrations (B) and temporal durations (C). (D) Quantitative analysis of CRYAB mean fluorescence intensity in NP cells treated with escalating Yoda1 doses. (E) Histological scores quantifying intervertebral disc structural deterioration in murine lumbar instability and needle puncture models at 4 and 8 weeks post-operation. (F) Quantitative relative IOD analysis of CRYAB immunohistochemical staining corresponding to the in vivo surgical models over time. Data are represented as mean $\pm$ SD. Statistical significance was determined utilizing one-way or two-way ANOVA followed by Tukey's post-hoc test (ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

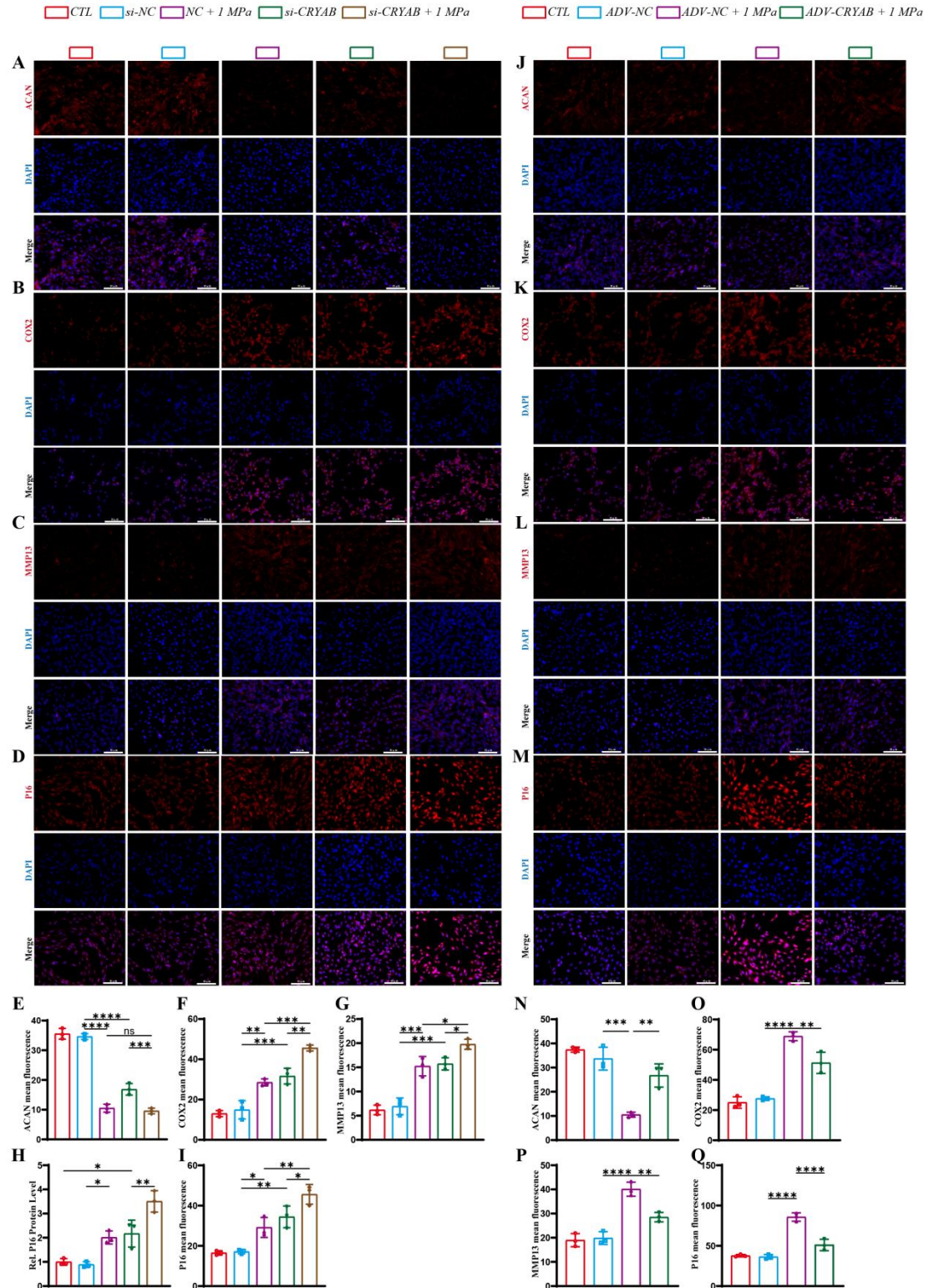


Figure S2. *CRYAB* modulation directly dictates the mechanosensitive accumulation of catabolic, inflammatory, and senescent markers in NP cells.

(A-D) Representative immunofluorescence images visualizing the spatial expression of ACAN, COX2, MMP13, and P16 in NP cells subjected to targeted *CRYAB* silencing, with or without concurrent 1.0 MPa mechanical stress. (E-I) Quantitative

mean fluorescence intensity and relative protein level analysis corresponding to the gene silencing cohorts. **(J-M)** Representative immunofluorescence images of ACAN, COX2, MMP13, and P16 illustrating the protective preservation of matrix integrity and suppression of catabolic executioners by ADV-CRYAB under 1.0 MPa loading. **(N-Q)** Quantitative mean fluorescence intensity corresponding to the protective viral overexpression cohorts. Scale bars as indicated. Data are represented as mean $\pm$ SD. Statistical significance was determined utilizing one-way or two-way ANOVA followed by Tukey's post-hoc test (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

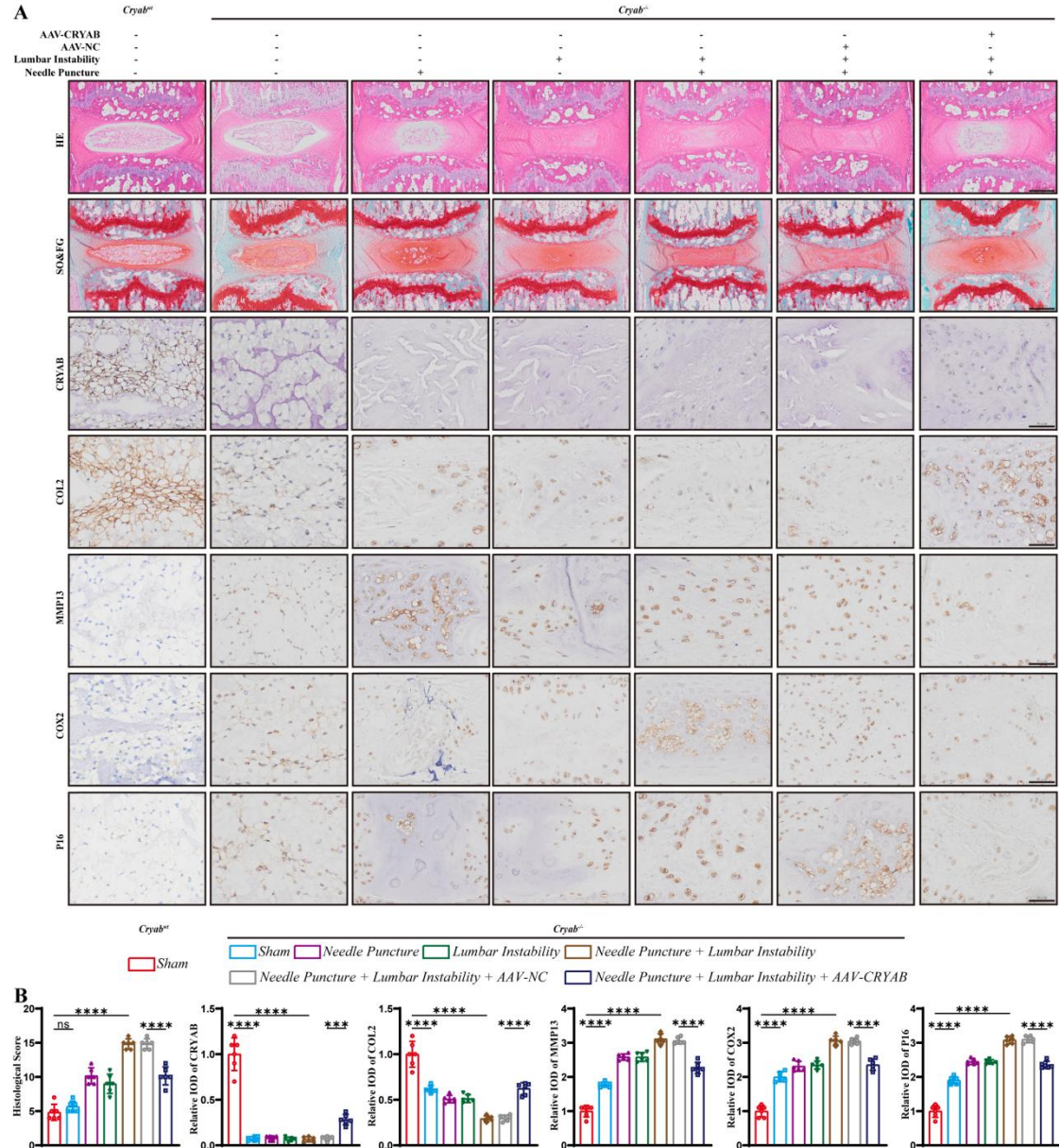


Figure S4. *Cryab* deficiency amplifies load-induced disc degeneration, which is effectively reversed by AAV-mediated targeted reconstitution.

(A) Representative H&E and SO&FG staining, alongside IHC profiling of CRYAB, COL2, MMP13, COX2, and P16 in intervertebral discs from *Acan-CreERT2*; *Cryab^{fl/fl}*, and *Cryab^{fl/fl}* mice. The panels encompass varying surgical loading models (Sham, Needle Puncture, Lumbar Instability) and localized AAV rescue interventions. (B) Comprehensive quantitative analysis encompassing histological scoring and relative IOD of IHC markers across all genetic and surgical cohorts. Scale bars as indicated. Data are represented as mean $\pm$ SD. Statistical significance was determined utilizing one-way or two-way ANOVA followed by Tukey's post-hoc test (ns $P > 0.05$, *** $P < 0.001$, **** $P < 0.0001$).

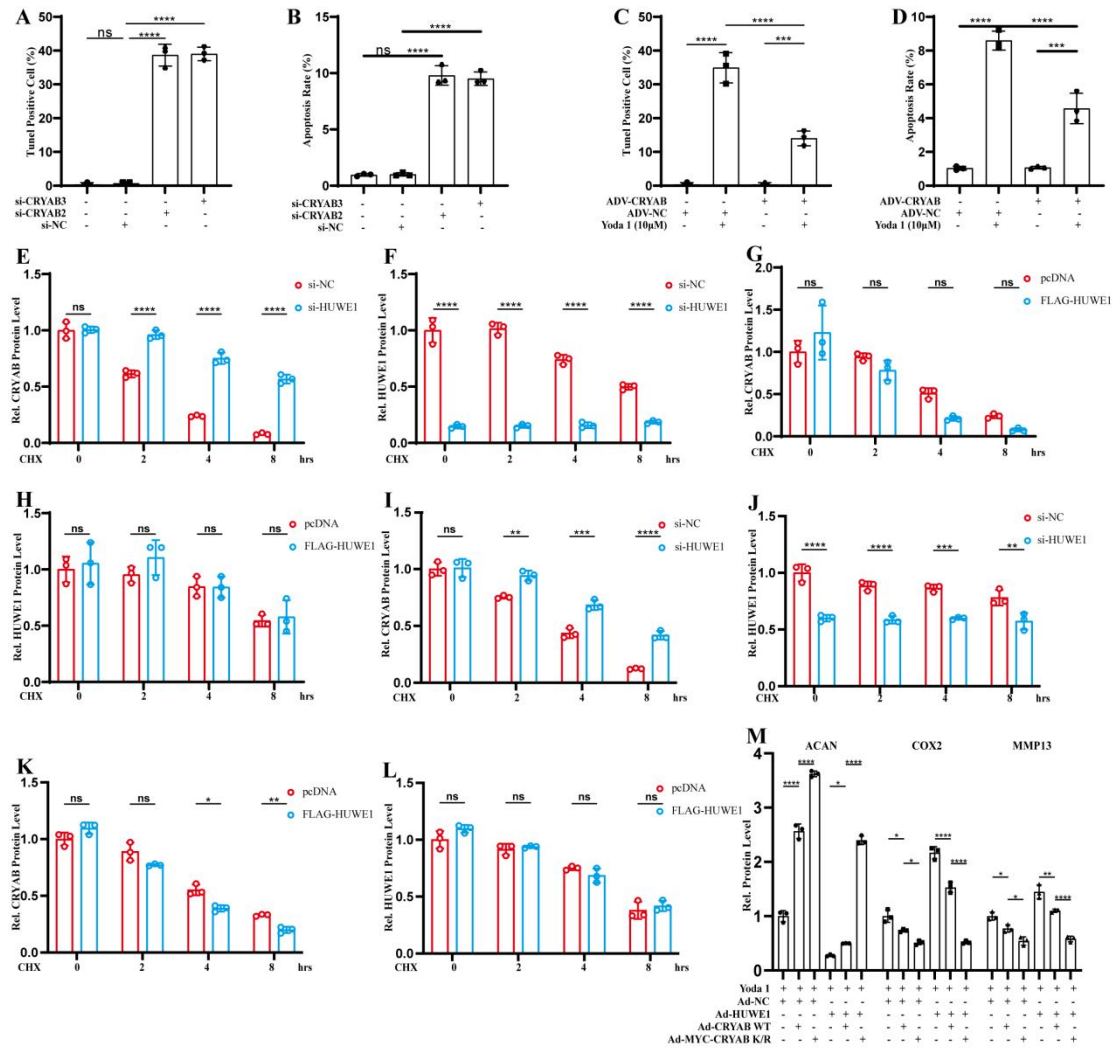


Figure S5. HUWE1 expression correlates with clinical IVDD severity and autonomously promotes catabolism while dictating CRYAB degradation kinetics.

(A, B) Quantitative flow cytometric (Annexin V/PI) and TUNEL assessments of apoptotic rates in primary NP cells following targeted *CRYAB* silencing. (C, D) Corresponding apoptotic quantifications demonstrating enhanced cellular viability by ADV-CRYAB under Yoda1-induced mechanical stress. (E-H) Quantitative densitometry of cycloheximide (CHX)-chase assays delineating CRYAB and HUWE1 half-life dynamics following *HUWE1* knockdown (E, F, I, J) or Flag-HUWE1 overexpression (G, H, K, L). (M) RT-qPCR transcript quantification of ACAN, COX2, and MMP13 confirming the sustained protective efficacy of the ubiquitination-resistant CRYAB K/R mutant against HUWE1-exacerbated mechanical injury. Scale bars as indicated. Data are represented as mean $\pm$ SD. Statistical significance was determined utilizing one-way or two-way ANOVA followed by Tukey's post-hoc test (ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

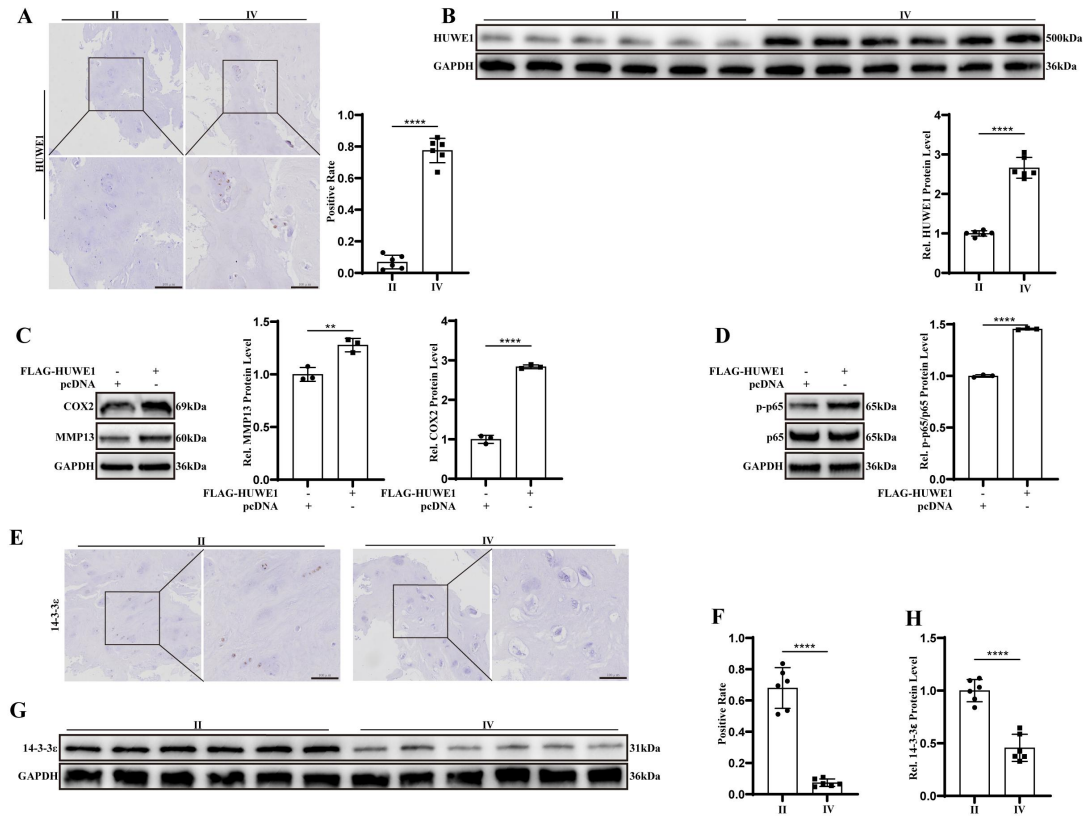
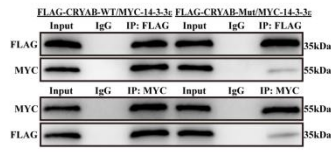


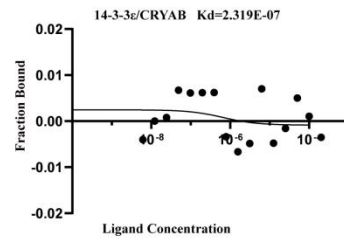
Figure S6. Expression of 14-3-3ε associated with IVDD.

(A, B) Representative IHC imaging, positive rate quantification, and immunoblotting revealing upregulated HUWE1 expression in severe (Grade IV) compared to mild (Grade II) clinical NP specimens. (C, D) Immunoblot analyses and corresponding quantifications demonstrating independent activation of the NF-κB pathway (p-p65) and accumulation of downstream effectors (COX2, MMP13) upon FLAG-HUWE1 overexpression. (E-H) Representative IHC, positive rate quantification, and immunoblotting demonstrating the significant decline of 14-3-3ε protein levels in advanced (Grade IV) versus early (Grade II) human IVDD clinical specimens. Scale bars as indicated. Data are represented as mean ± SD. Statistical significance was determined utilizing one-way or two-way ANOVA followed by Tukey's post-hoc test (**P < 0.01, ****P < 0.0001).

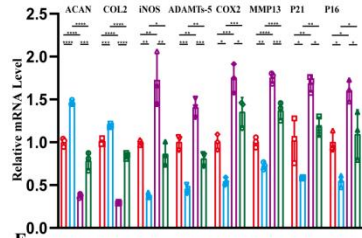
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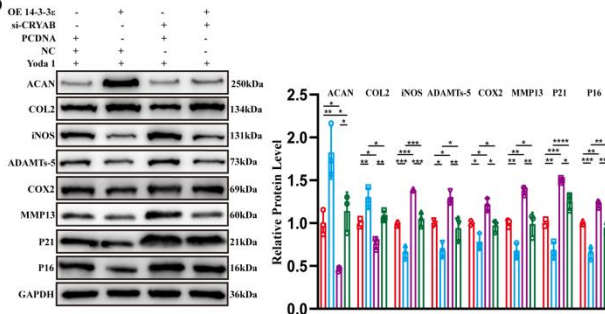
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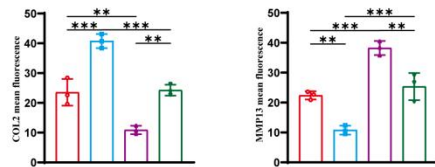
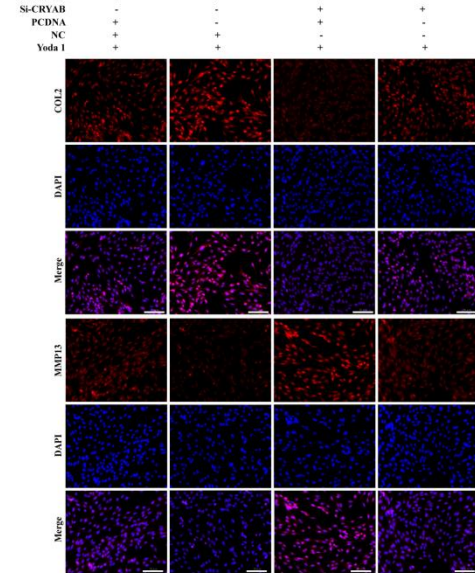
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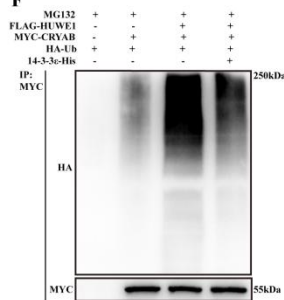
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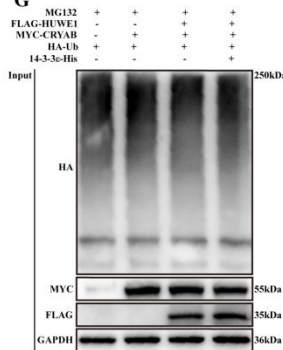


Figure S7. The chondroprotective function of 14-3-3 ϵ relies on the CRYAB K150/K161 interface to competitively restrict HUWE1-driven ubiquitination.

(A) Reciprocal Co-IP assay demonstrating impaired physical interaction between 14-3-3 ϵ and the CRYAB K150R/K161R ubiquitination-resistant mutant. (B) MST binding curve confirming the impaired physical engagement between 14-3-3 ϵ and CRYAB. (C, D) RT-qPCR and immunoblot profiling of matrix synthesis, catabolic, inflammatory, and senescence markers demonstrating that the protective effects of 14-3-3 ϵ overexpression against Yoda1-induced stress are completely abolished upon concurrent *CRYAB* silencing. (E) Immunofluorescence imaging (COL2, MMP13, COX2, P16) and corresponding mean fluorescence quantification corroborating the obligate dependency of 14-3-3 ϵ -mediated chondroprotection on CRYAB availability. (F) In vitro ubiquitination assay illustrating the competitive restriction of HUWE1-mediated CRYAB polyubiquitination by recombinant 14-3-3 ϵ -His. Scale bars as indicated. Data are represented as mean $\pm$ SD. Statistical significance was determined utilizing one-way or two-way ANOVA followed by Tukey's post-hoc test (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

Supplementary Table 1

Target (Human)	Forward primers	Reverse primers
PTGS2	TCAGCCATACAGCAAATCCTT G	GTCCGGGTACAATCGCGACTT
NOS2	CGTGGAGACGGGAAAGAAGT	GACCCCAGGCAAGATTTGGA
MMP13	ATTAAGGAGCATGGCGACTTC T	GCCCAGGAGGAAAAGCATGA
ADAMTS5	GAA ACA ACG GAC GCT ACT GC	ATG ATT TAC CAT TGG GTG GGC A
ACAN	GGGAAGGCTGCTATGGAGAC	ACCTCACCTCCATCTCCTC
COL2A1	CTGGAAAAGCTGGTGAAAGG	GGCCTGGATAACCTCTGTGA
CDKN2A	CGCAGGTTCTTGGTCACTGT	TGTTACGAAAGCCAGAGCG
CDKN1A	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
GAPDH	ACGGGAAGCTTGTCATCAAT	TGGA CTCTCGACGTACTCA

The primer sequences used for mouse genotyping were as follows:

Cryab forward: 5' - TCTGGTTTGCTTCAGCTCCT -3' ,

Cryab reverse: 5' - GTCAGGGTTTCCCGAGAGTG -3' ;

Acan transgene forward: 5' - TGTTAAAGCCTTCAGGTGGTTGG -3' ,

Acan transgene reverse: 5' - CATGTCCATCAGGTTCTTGCGAAC -3' ,

Acan internal positive control forward: 5' ,

-TGGGAGAACCAATGTACCAACCA-3' ,

Acan internal positive control reverse: 5' ,

-ACTTGTACCCTGTATTCGGAACCC-3' .