

Supplementary materials

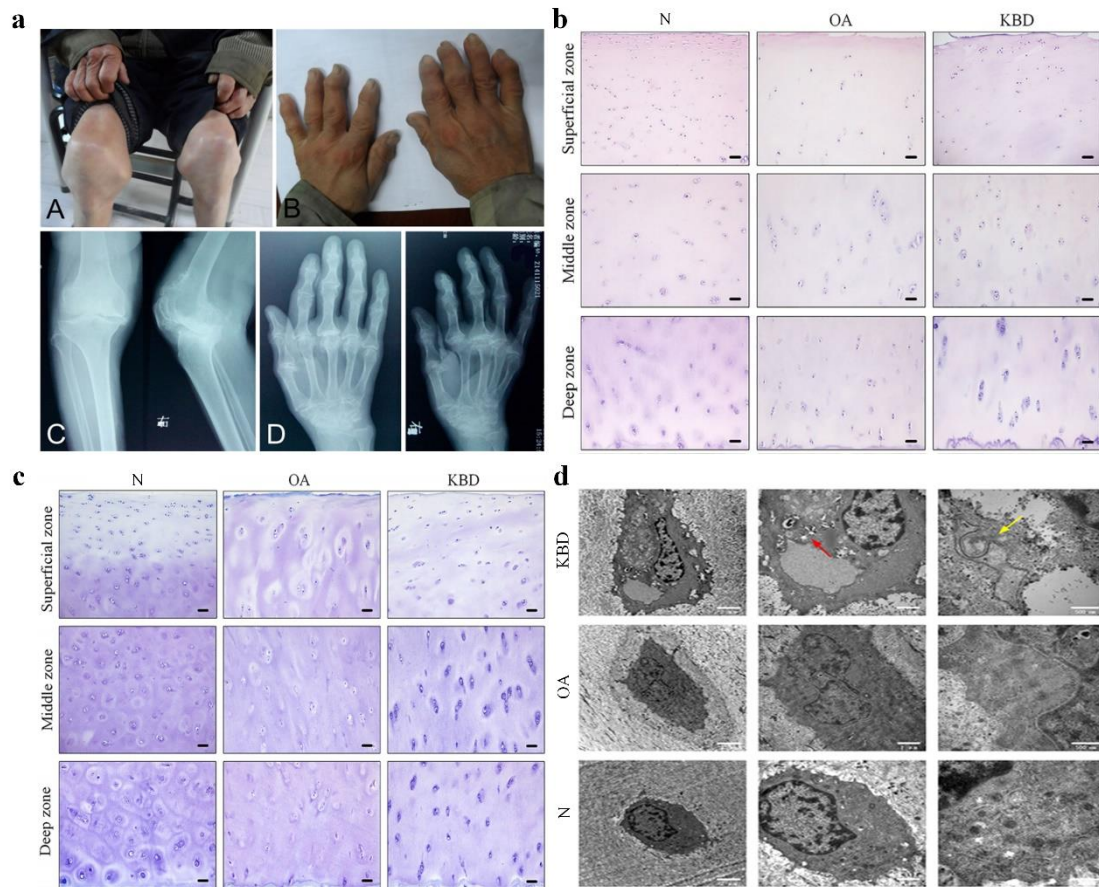


Figure S1. Clinical symptoms and pathological features of Kashin-beck disease. **a.** The main pathological features of Kashin-Beck disease (KBD) patients. Typical clinical manifestations of KBD patients [54]. **(A)** Deform joints in two knees and **(B)** flexion of the terminal finger joints or deformed fingers of grade III KBD adult. Radiographic findings in the right knee and right hand of a patient with grade II KBD. **(C)** Obvious joint space narrowing and loosened bodies in knee and D wrist joint crowded and space narrowing, phalanx thickening, and osteophyte formation. **b.** Hematoxylin and eosin (HE) staining of articular cartilage in normal (N), KBD, and osteoarthritis (OA) groups [55]. In KBD and OA groups, the cell numbers were much lower in all 3 layers compared with the N group. scale bar 20 μm . **c.** Toluidine blue (TB) staining of articular cartilage in N, KBD, and OA groups [55]. In KBD and OA groups, the rates of intensive staining areas were much lower in all 3 layers compared with the N group. scale bar 20 μm . **d.** TEM images of chondrocytes from KBD, OA patients compared with N (This part of the results has not yet been published). Compared with the N group, the KBD chondrocytes were observed mitochondrial damage (red box) and endoplasmic reticulum autophagy (yellow box).

Table S1. Characteristics of experiment subjects. OA: Osteoarthritis; KBD: Kashin-Beck-Disease; N: Normal adult; F: Female; M: Male. $\chi^2_{\text{Gender}}=0.9$, $F_{\text{Age}}=0.389$, both P value > 0.05 .

Sample set	KBD				OA				N			
	Sample	Subject	Age	Gender	Sample	Subject	Age	Gender	Sample	Subject	Age	Gender
1	KBD1	①	50	F	OA1	①	51	M	N1	①	65	F
		②	58	F		②	56	F		②	61	M
		③	50	M		③	68	M		③	59	M
		④	55	M		④	58	M		④	62	F
2	KBD2	⑤	61	M	OA2	⑤	60	F	N2	⑤	61	M
		⑥	66	F		⑥	57	F		⑥	57	M
		⑦	54	F		⑦	67	F		⑦	60	M
		⑧	68	M		⑧	55	M		⑧	63	F
3	KBD3	⑨	67	F	OA3	⑨	61	M	N3	⑨	60	M
		Mean	58.778	-		Mean	59.222	-		Mean	60.889	-

Table S2. The N-glycoproteins quantification results of the sample after pyrolysis. OA: Osteoarthritis; KBD: Kashin-Beck-Disease; N: Normal adult.

Sample	Weight (μg)	Volume(μL)	Concentration (μg/μL)
KBD1	900	120.5	7.47
KBD2	900	140.46	6.41
KBD3	900	134.63	6.68
OA1	900	161.23	5.58
OA2	900	128.71	6.99
OA3	900	122.25	7.36
N1	900	87.66	10.27
N2	900	82.87	10.86
N3	900	85.17	10.57

Table S3. The results of quantification of enzymatic hydrolysis peptide concentration. OA: osteoarthritis; KBD: Kashin-Beck-Disease; N: normal adult.

Sample	Volume(μ L)	Concentration(μ g/ μ L)	Weight (μ g)
KBD1	200	1.609	321.8
KBD2	220	1.725	379.5
KBD3	220	1.848	406.56
OA1	170	2.014	342.38
OA2	180	1.743	313.74
OA3	170	2.109	358.53
N1	160	2.223	355.68
N2	160	2.104	336.64
N3	160	2.165	368.05

Table S4. The list of identified N-glycoproteins.

Table S5. The list of identified N-glycopeptides.

Table S6. The list of identified N-glycosylation sites.

Table S7. The list of significant N-glycoproteins for analysis.

Table S8. TOP relative proteins or N-glycoproteins ranked by the involved signaling pathway.

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Uniprot Entry ID	Protein or N-glycoprotein	KBD vs OA/KBD vs N/OA vs N	Pathway name
P05556	ITGB1/Integrin beta-1	up/up/up	Phagosome; ECM-receptor interaction; Focal adhesion; Human papillomavirus infection; Cell adhesion molecules; PI3K-Akt signaling pathway; Shigellosis; Bacterial invasion of epithelial cells; Pertussis; Leishmaniasis; Arrhythmogenic right ventricular cardiomyopathy (ARVC); Hypertrophic cardiomyopathy (HCM); Small cell lung cancer; Dilated cardiomyopathy (DCM); Leukocyte transendothelial migration; Toxoplasmosis; Platelet activation; Tight junction; Axon guidance; Pathogenic Escherichia coli infection; Rap1 signaling pathway; Regulation of actin cytoskeleton; Pathways in cancer; Proteoglycans in cancer
P35442	THBS2/Thrombospondin 2	down/up/up	Phagosome; ECM-receptor interaction; Focal adhesion; Human papillomavirus infection; PI3K-Akt signaling pathway
P08236	GUSB/Beta-glucuronidase	-/up/-	Lysosome; Drug metabolism – other enzymes; Glycosaminoglycan degradation; Pentose and glucuronate interconversions Porphyrin and chlorophyll metabolism; Metabolic pathways
Q16853	AOC3/Membrane primary amine oxidase	-/-/up	Phenylalanine metabolism; beta-Alanine metabolism; Tyrosine metabolism; Glycine, serine, and threonine metabolism; Metabolic pathways
P24821	TNC/Tenascin-C	down/up/up	ECM-receptor interaction; Focal adhesion; Human papillomavirus infection; PI3K-Akt signaling pathway; MicroRNAs in cancer
P02458	COL2A1/Collagen alpha-1 (II) chain	-/up/up	ECM-receptor interaction; Protein digestion and absorption; Focal adhesion; Human papillomavirus infection; PI3K-Akt signaling pathway
P13473	LAMP2/Lysosome-associated membrane glycoprotein 2	down/up/up	Phagosome; Lysosome; Tuberculosis; Autophagy
Q7Z5Y6	BMP8A/Bone morphogenetic protein 8A	up/-/-	TGF-beta signaling pathway; Cytokine-cytokine receptor interaction; Hippo signaling pathway; Thermogenesis
P05164	MPO/Myeloperoxidase	down/down/down/	Phagosome; Transcriptional misregulation in cancer; Acute myeloid leukemia; Drug metabolism - other enzymes
P27487	DPP4/Dipeptidyl peptidase 4	up/up/-	Protein digestion and absorption
Q02388	COL7A1/Collagen alpha-1 (VII) chain	up/up/-	Protein digestion and absorption
Q7LGC8	CHST-3/Carbohydrate sulfotransferase 3	down/down/-	Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate
Q8NCG5	CHST-4/Carbohydrate sulfotransferase 4	down/down/-	Glycosaminoglycan biosynthesis - keratan sulfate

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