

Supplementary Information

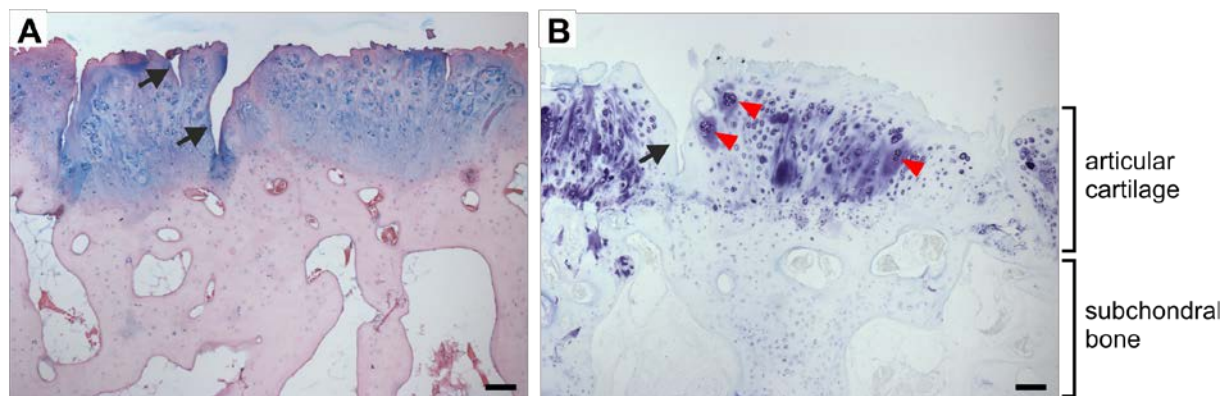


Fig. S1. Histology analysis of an MLIII gamma patient's femur head reveals cartilage degeneration. (A) Alcian blue/Haematoxylin/Eosin and (B) toluidine blue staining of paraffin-embedded sections from a femur head of Patient 9 (at the age of 43 years) diagnosed with MLIII gamma. Sites of cartilage erosion (clefts) are indicated with black arrows. Red arrowheads indicate clusters of chondrocytes. Scale bar: 100 μ m.

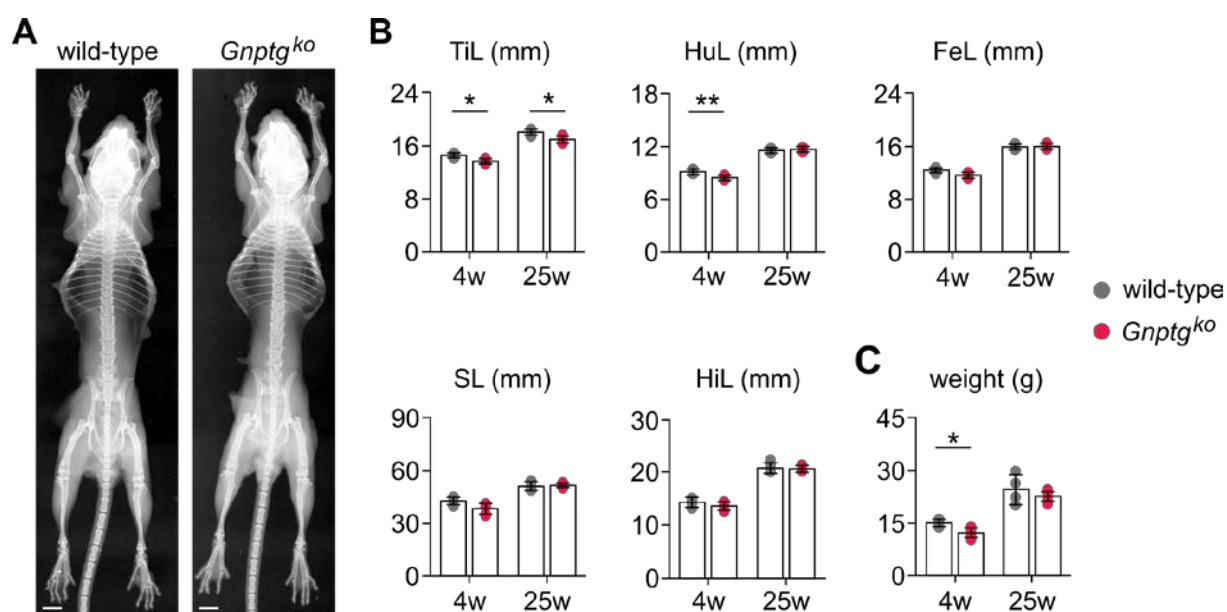


Fig. S2. *Gnptg^{ko}* mice display moderate growth retardation. (A) Representative contact radiographs of whole skeletons of 25-week-old wild-type and *Gnptg^{ko}* mice. Scale bar: 5 mm. (B) Quantification of tibia length (TiL), humerus length (HuL), femur length (FeL), spine length (SL) and hip length (HiL) of 4- and 25-weeks-old wild-type and *Gnptg^{ko}* mice. (C) Body weight of 4- and 25-weeks-old wild-type and *Gnptg^{ko}* mice. All data are shown as means ± SD (n ≥ 4). **P* ≤ 0.05, ***P* ≤ 0.01 (unpaired two-tailed *t*-test).

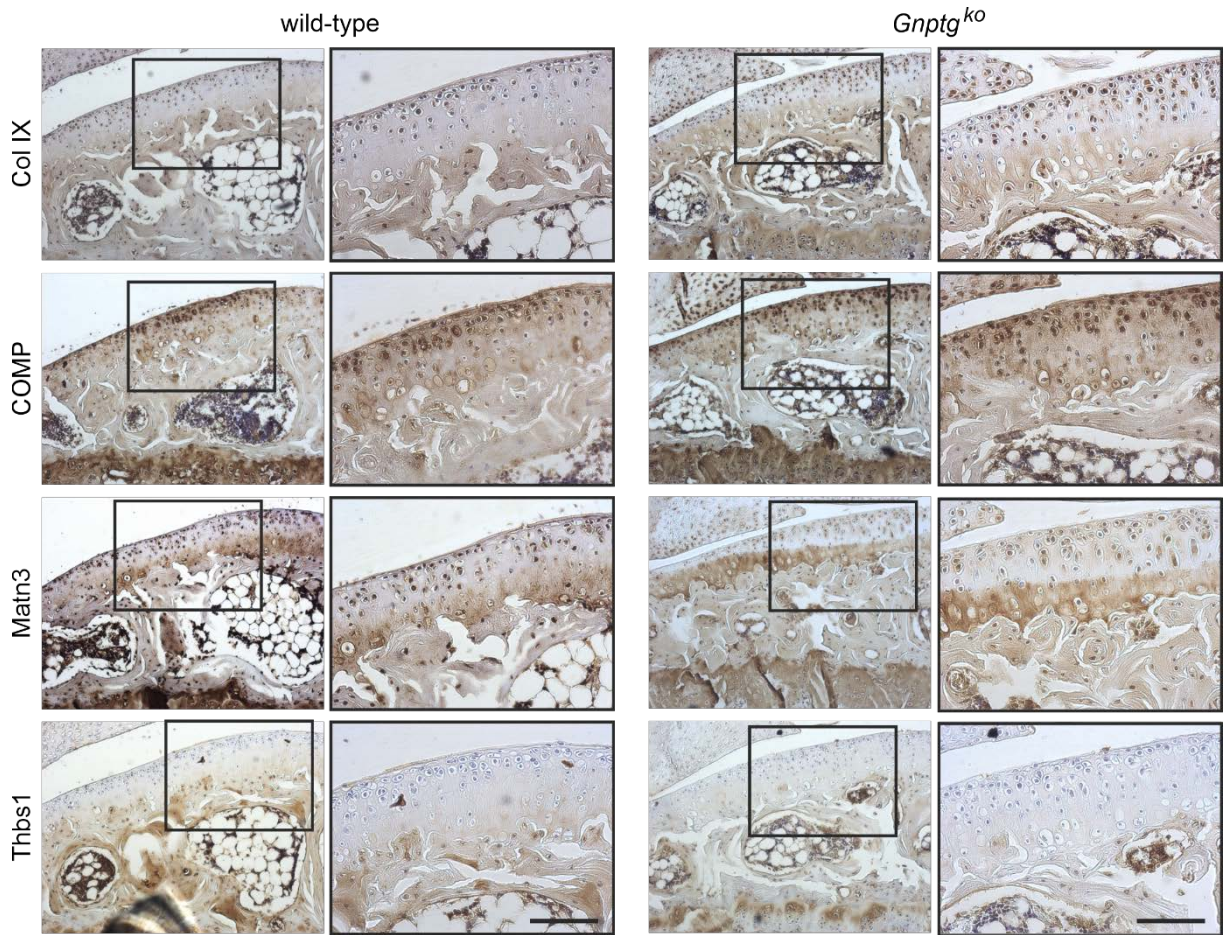


Fig. S3. Abundance of the cartilage ECM proteins is not altered in *Gnptg^{ko}* mice. Immunostaining of sagittal paraffin-embedded sections of the knee joint articular cartilage from 60-weeks-old wild-type and *Gnptg^{ko}* mice against collagen type IX (Col IX), cartilage oligomeric matrix protein/thrombospondin-5 (COMP), matrilin-3 (Matn3) and thrombospondin-1 (Thbs1). Representative images of three mice per genotype are shown. Scale bar: 100 μ m.

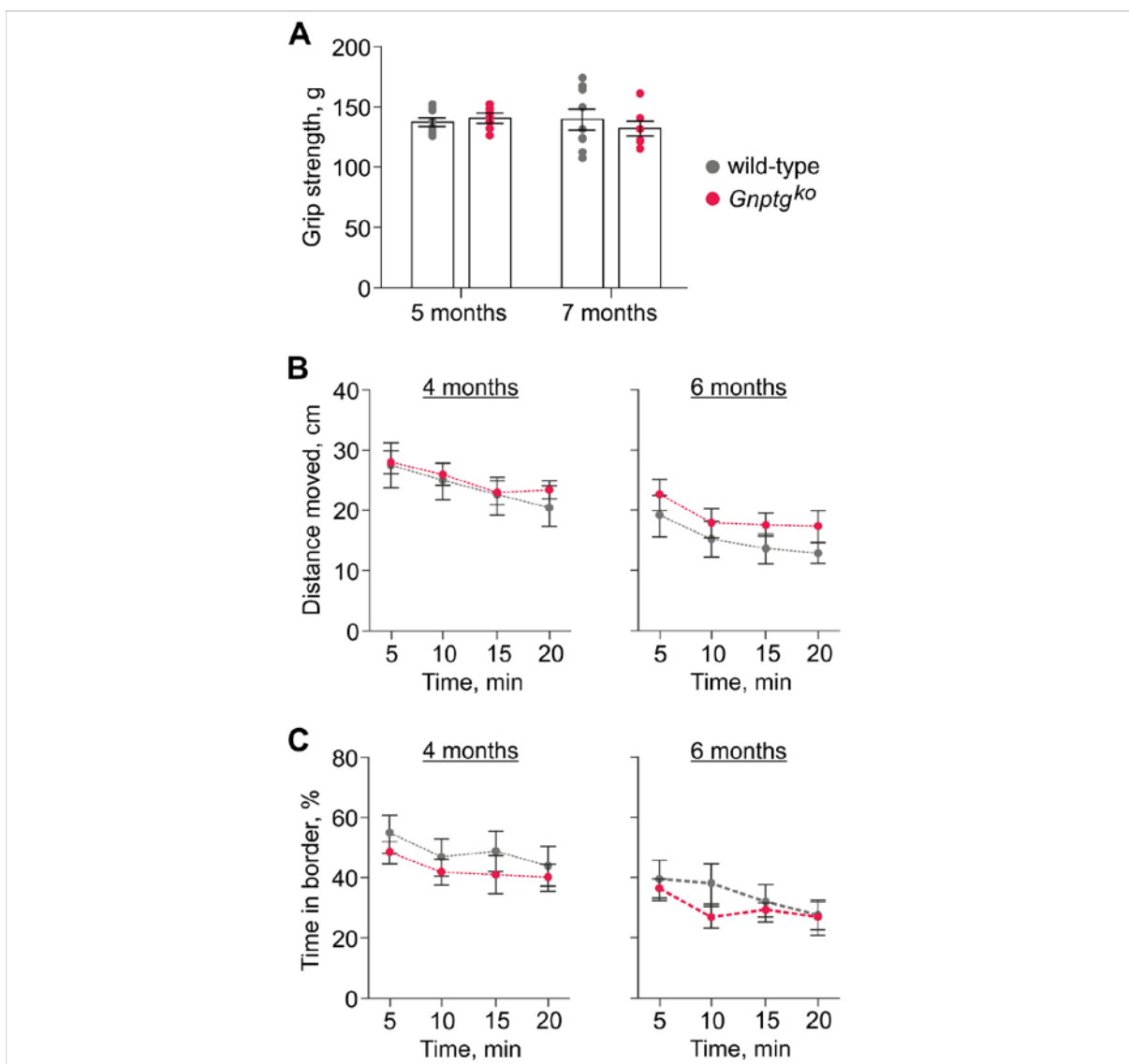


Fig. S4. *Gnptg^{ko}* mice display normal grip strength and behavior in the open field test. (A) Strength of the forelimbs was measured in 5- and 7-months-old *Gnptg^{ko}* and wild-type mice. (B, C) An open field test performed in 4- and 6-months-old *Gnptg^{ko}* and wild-type mice to assess locomotor activity and novelty-induced behavior. Values are shown as means \pm SEM (n = 10 for wild-type, n = 7 for *Gnptg^{ko}*).

Table S1. Patients with MLII, MLIII alpha/beta and MLIII gamma analyzed in this study

Patient	Age	Sex	Clinical diagnosis	Molecular diagnosis	References
1	33 mo	F	MLII	<i>GNPTAB</i> : ?/? *	this study
2	14 mo	M		<i>GNPTAB</i> : c.3503_3504del/ c.3503_3504del	[1], [2] (patient 12 in both)
3	61 mo	M		<i>GNPTAB</i> : c.3503_3504del/c.2808A>G	[1], [2] (patient 7 in both)
4	48 mo	F		<i>GNPTAB</i> : c.3503_3504del/c.1154C>T	[1] (patient 4), [3] (patient 6), [2] (patient 4)
5	19 yrs	M	MLIII alpha/beta	<i>GNPTAB</i> : c.3503_3504del/c.1196C>T	[1], [2] (patient 8 in both)
6	14 yrs	M		<i>GNPTAB</i> : c.3503_3504del/? *	this study
7	17 yrs	F	MLIII gamma	<i>GNPTG</i> : c.244_247dup/c.328G>T	[4] (patient 1), [5] (patient 1), [2] (patient 21)
8	41 yrs	M		<i>GNPTG</i> : c.328G>T/ c.328G>T	[6] (patient A), [2] (patient 19)
9	38 yrs	M		<i>GNPTG</i> : c.328G>T/c.328G>T	[6] (patient B), [2] (patient 20)

mo, months; yrs, years;

* Patients 1 and 6 were diagnosed clinically and biochemically as MLII and MLIII alpha/beta, respectively.

Table S2. Joint range of motions (°) measurements in patients with MLII, MLIII alpha/beta and MLIII gamma

Disease	Patient	MLII				MLIII alpha/beta		MLIII gamma			Reference values
		1	2	3	4	5	6	7	8	9	
Shoulder	flexion	117	101	137	137	104	123	160	137	126	150 - 180
	extension	58	71	60	61	68	33	66	67	68	50 - 60
	abduction	93	81	104	105	88	167	110	106	101	180
	internal rotation	31	50	40	70	52	80	50	35	32	70 - 90
	external rotation	81	79	75	88	58	77	70	53	40	90
Elbow	flexion	130	131	128	122	130	133	134	126	121	140 - 150
	extension	20	10	19	25	15	6	0	39	49	0 - 10
Wrist	flexion	70	67	48	39	89	48	60	64	32	60 - 80
	extension	31	55	41	36	66	49	40	20	54	60 - 70
Hip	flexion	127	131	122	114	131	106	125	70	99	100 - 120
	extension	11	19	11	18	14	5	22	16	16	20 - 30
	abduction	37	46	39	39	37	18	22	18	23	40
	adduction	23	23	22	29	27	21	28	22	24	20
	internal rotation	54	71	33	75	58	31	40	23	39	40 - 45
	external rotation	43	37	22	22	32	37	30	15	21	45 - 50
Knee	flexion	136	132	118	131	137	ND	140	130	126	150
	extension	27	21	11	37	10	ND	0	11	10	0 - 10
Ankle	flexion	37	19	30	43	10	11	ND	11	12	40 - 50
	dorsal extension	28	30	10	21	64	41	ND	23	25	20

ND, not determined

Table S3 (related to Fig. 2D). Expression of genes encoding lysosomal enzymes in wild-type primary chondrocytes relative to *Gapdh*

Gene	Protein encoded	ΔC_t Gene - <i>Gapdh</i>
<i>Ctsb</i>	Cathepsin B	2.62
<i>Ctsc</i>	Cathepsin C	3.01
<i>Ctsl</i>	Cathepsin L	3.03
<i>Ctsd</i>	Cathepsin D	3.23
<i>Ctsz</i>	Cathepsin Z	3.24
<i>Ctsk</i>	Cathepsin K	5.65
<i>Cln5</i>	Ceroid-lipofuscinosis neuronal protein 5	5.66
<i>Tpp1</i>	Tripeptidyl-peptidase 1	6.03
<i>Aga</i>	β -N-acetylglucosaminyl-L-asparaginase	6.08
<i>Gba</i>	Lysosomal acid glucosylceramidase	6.20
<i>Ctss</i>	Cathepsin S	6.56
<i>Neu1</i>	Sialidase-1	6.78
<i>Asah1</i>	Acid ceramidase	6.90
<i>Arsa</i>	Arylsulfatase A	7.06
<i>Plbd2</i>	Putative phospholipase B-like 2	7.24
<i>Gusb</i>	β -Glucuronidase	7.34
<i>Fuca1</i>	α -L-fucosidase	8.21
<i>Arsb</i>	Arylsulfatase B	8.23
<i>Ppt2</i>	Lysosomal thioesterase PPT2	8.56
<i>Ppt1</i>	Palmitoyl-protein thioesterase 1	8.67
<i>Pla2g15</i>	Phospholipase A2 group XV	9.12
<i>Hexb</i>	β -Hexosaminidase	9.33
<i>Glb1</i>	β -Galactosidase	9.41
<i>Lipa</i>	Lysosomal acid lipase	9.52
<i>Man2b1</i>	Lysosomal α -mannosidase	9.67
<i>Acp2</i>	Lysosomal acid phosphatase	9.81
<i>Galns</i>	N-acetylgalactosamine-6-sulfatase	10.01
<i>Prep</i>	Lysosomal Pro-X carboxypeptidase	10.21
<i>Hyal1</i>	Hyaluronidase-1	10.43
<i>Idua</i>	α -L-Iduronidase	10.55
<i>Dpp7</i>	Dipeptidyl peptidase 2	10.68
<i>Ggh</i>	γ -Glutamyl hydrolase	10.83
<i>Siae</i>	Sialate O-acetyltransferase	10.93
<i>Manba</i>	β -Mannosidase	10.99
<i>Acp5</i>	Lysosomal acid phosphatase 5	11.07
<i>Arsk</i>	Arylsulfatase K	11.20

Table S4 (related to Fig. 2H). Relative gene expression of chondrocyte markers in wild-type and *Gnptg*^{ko} primary chondrocytes.

Gene	Protein encoded	Wild-type	<i>Gnptg</i> ^{ko}
<i>Acan</i>	Aggrecan	1.01 ± 0.03	0.49 ± 0.19
<i>Bgn</i>	Biglycan	1.03 ± 0.05	0.58 ± 0.02
<i>Dcn</i>	Decorin	1.05 ± 0.03	0.62 ± 0.06
<i>Fnl</i>	Fibronectin	1.01 ± 0.04	0.34 ± 0.05
<i>Col2a1</i>	Collagen α1(II) chain	1.02 ± 0.06	0.20 ± 0.03
<i>Col9a1</i>	Collagen α1(IX) chain	1.02 ± 0.09	0.26 ± 0.04
<i>Col10a1</i>	Collagen α1(X) chain	1.01 ± 0.09	0.35 ± 0.01
<i>Gnptg</i>	γ-subunit GlcNAc-1-phosphotransferase	1.02 ± 0.04	0.14 ± 0.01
<i>Gnptab</i>	α/β-subunit GlcNAc-1-phosphotransferase	1.01 ± 0.05	1.01 ± 0.03

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