

Supplementary Materials: Correlation between Gene Expression and Osteoarthritis Progression in Human

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Collagen type II

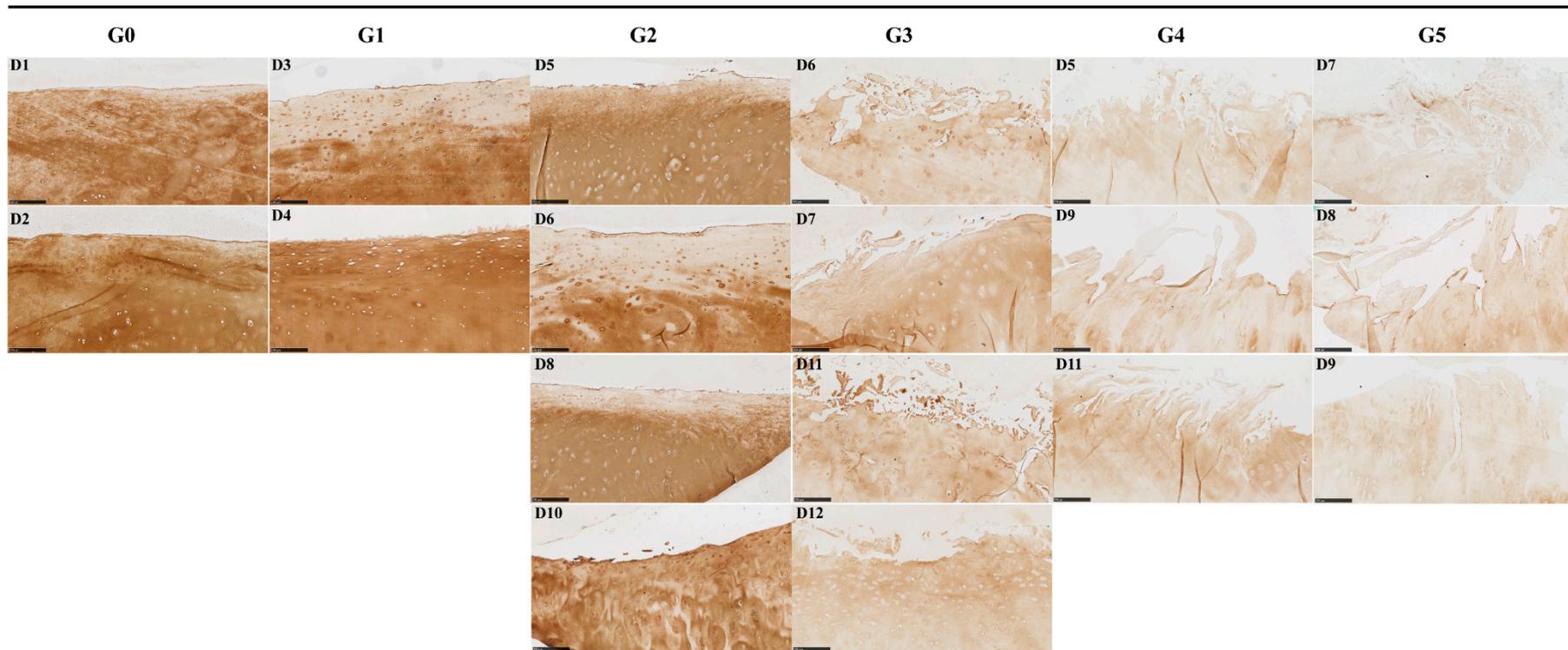


Figure S1. The protein expression of collagen type II was detected by IHC in each donor (scale bar: 250 μ m). Images were taken using the Nanozoomer. G0, G1, G2, G3, G4, G5 = Grade 0, Grade 1, Grade 2, Grade 3, Grade 4, Grade 5; D1–D12 = Donors 1–12.

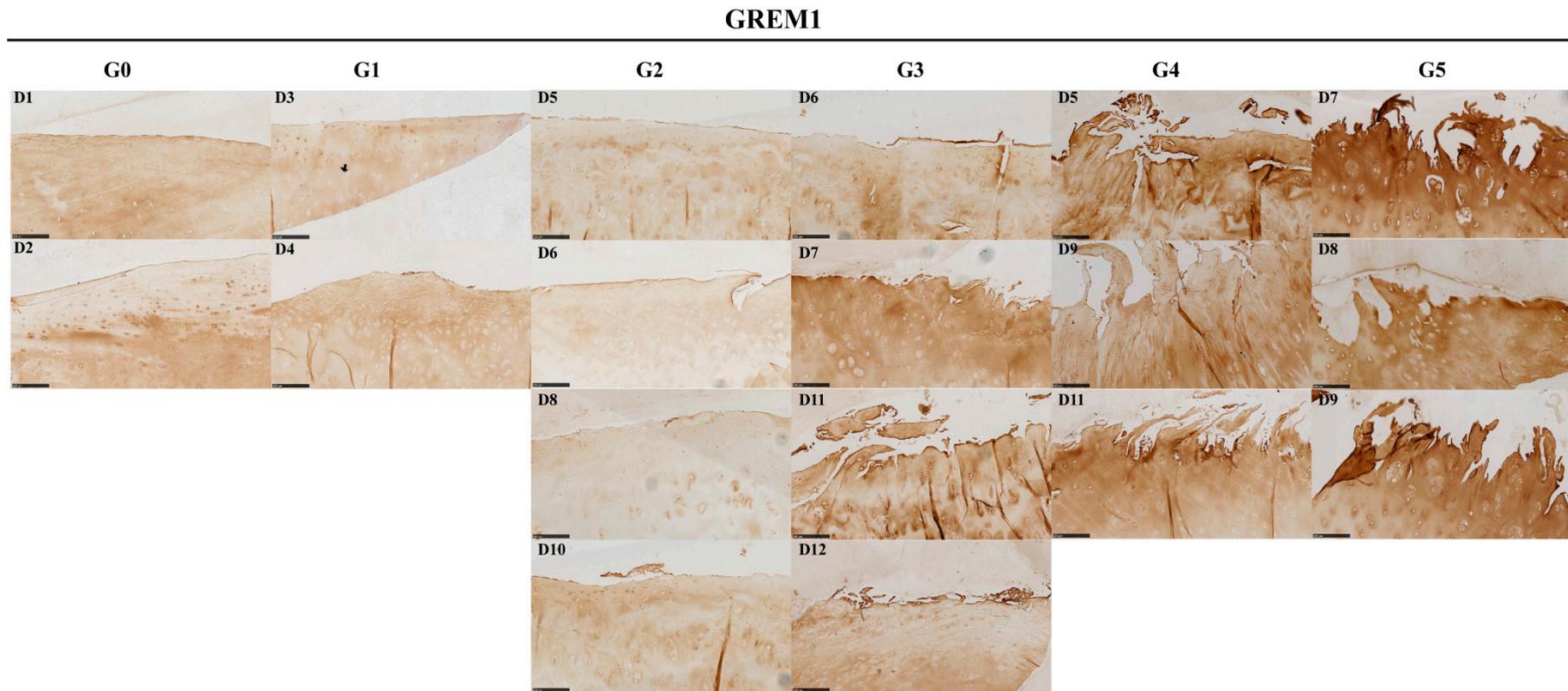


Figure S2. The protein expression of GREM1 was detected by IHC in each donor (scale bar: 250 μ m). Images were taken using the Nanozoomer. G0, G1, G2, G3, G4, G5 = Grade 0, Grade 1, Grade 2, Grade 3, Grade 4, Grade 5; D1–D12 = Donors 1–12.

Collage type X

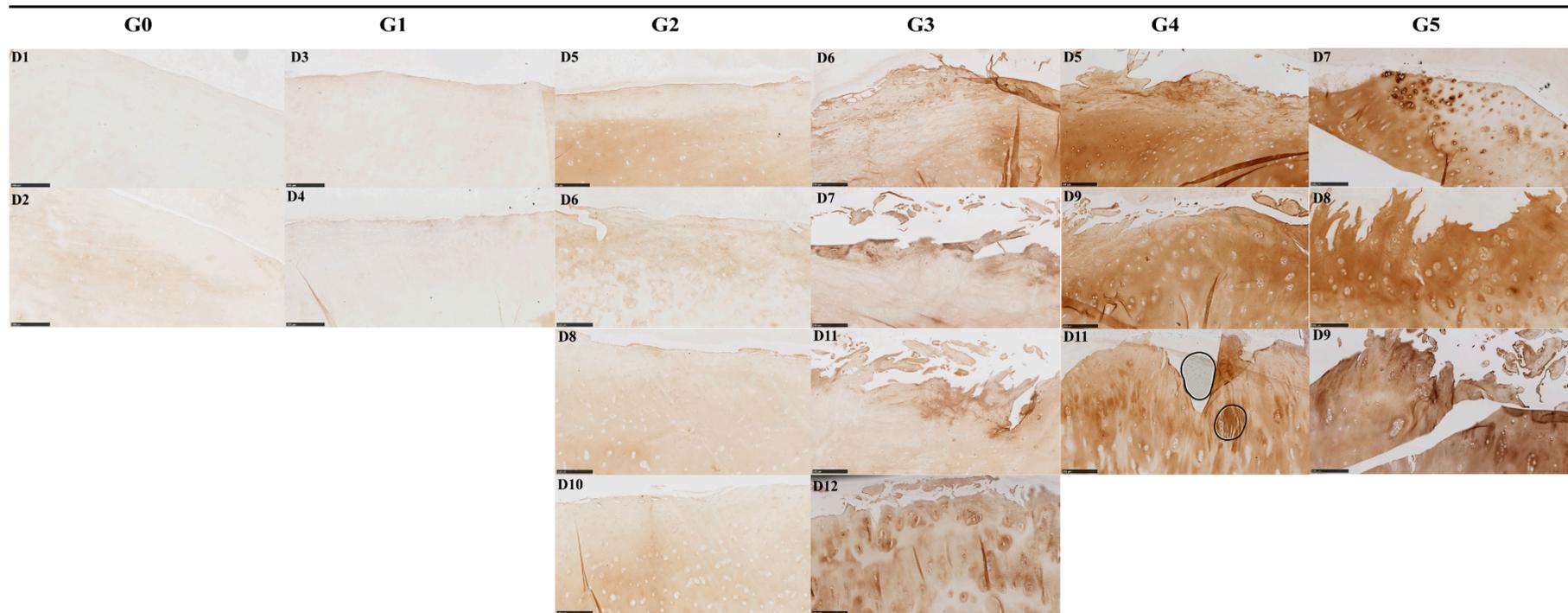


Figure S3. The protein expression of collagen type X was detected by IHC in each donor (scale bar: 250 μ m). Images were taken using the Nanozoomer. G0, G1, G2, G3, G4, G5 = Grade 0, Grade 1, Grade 2, Grade 3, Grade 4, Grade 5; D1–D12 = Donors 1–12.

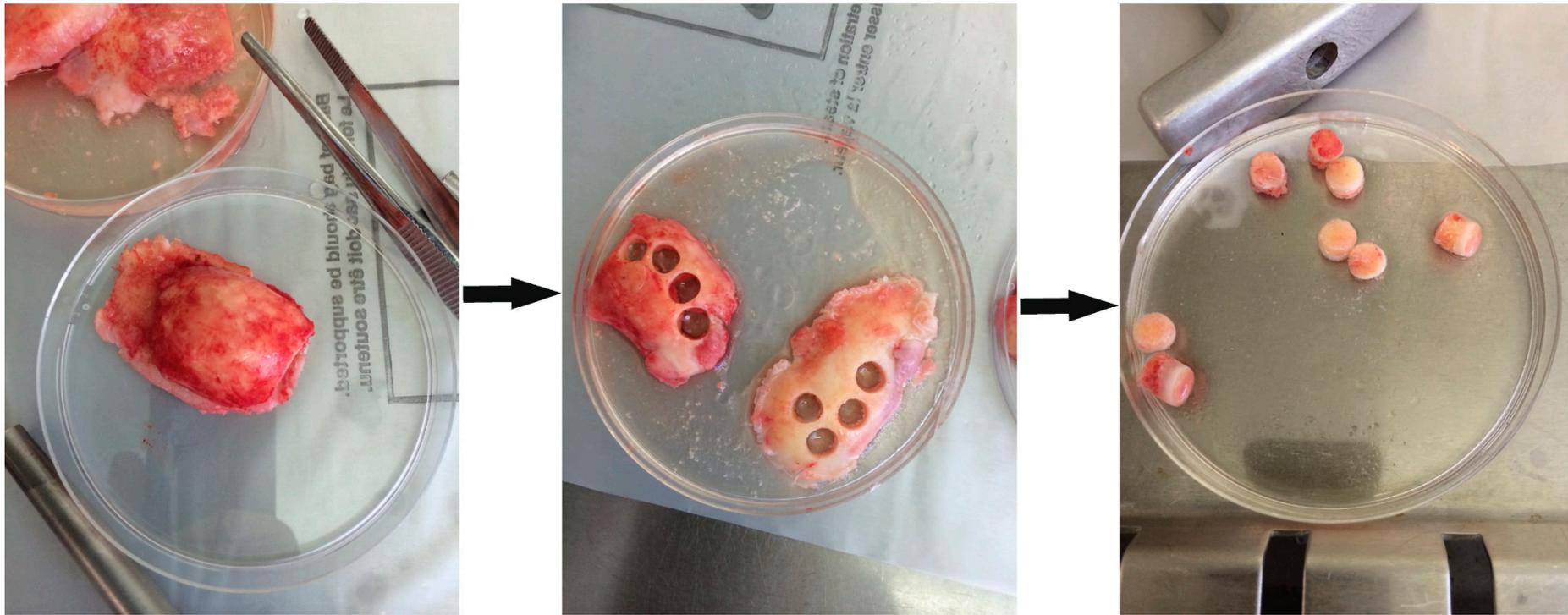


Figure S4. Articular cartilage was obtained from patients. The cartilage samples with subchondral bone were punched aseptically using a steel punch. Doughnut-shaped cartilage cylinders were obtained. Subsequently, the subchondral bone was removed.

Table S1. Pearson correlations of all tested genes. Correlation coefficient determined by Pearson's correlation analysis * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

<i>R</i> ²	<i>SOX9</i>	<i>ACAN</i>	<i>COL2A1</i>	<i>DKK1</i>	<i>FRZB</i>	<i>GREM1</i>	<i>COL10A1</i>	<i>IHH</i>	<i>RUNX2</i>	<i>AXIN2</i>
<i>SOX9</i>	–	–	–	–	–	–	–	–	–	–
<i>ACAN</i>	0.87 *	–	–	–	–	–	–	–	–	–
<i>COL2A1</i>	0.89 *	0.96	–	–	–	–	–	–	–	–
<i>DKK1</i>	0.61	0.76	0.82	–	–	–	–	–	–	–
<i>FRZB</i>	0.72	0.94 **	0.86 *	0.82 *	–	–	–	–	–	–
<i>GREM1</i>	–0.75	–0.57	–0.57	–0.34	–0.41	–	–	–	–	–
<i>COL10A1</i>	–0.72	–0.55	–0.55	–0.36	–0.42	0.99 ***	–	–	–	–
<i>IHH</i>	–0.97 **	–0.91 *	–0.91 *	–0.63	–0.78	0.83 *	0.81	–	–	–
<i>RUNX2</i>	–0.78	–0.65	–0.65	–0.77	–0.60	0.99 **	0.99 *	0.91	–	–
<i>AXIN2</i>	–0.91 *	–0.68	–0.68	–0.46	–0.54	0.93 **	0.93 *	0.91 *	0.95 *	–
<i>BMP2</i>	0.34	–0.16	–0.16	–0.28	–0.38	–0.39	–0.37	–0.19	–0.70	–0.48

Table S2. Patient's information. Cartilage was obtained from 12 patients (68 ± 6 years) with OA undergoing total knee replacement surgery. Patient's information, including age and gender (male (M) and female (F)), is indicated in this table. In addition, some cartilage specimens with different grades were collected from the same patient and cartilage specimens with the same grade were pooled together for RNA isolation. Information is provided for which samples were pooled for RNA extraction.

Grade			0	1	2	3	4	5
Donor Information								
Donor Number	Age	Gender	Pooled RNA Sample ($n = 2$)	Pooled RNA Sample ($n = 2$)	Pooled RNA Sample ($n = 4$)	Pooled RNA Sample ($n = 4$)	Pooled RNA Sample ($n = 3$)	Pooled RNA Sample ($n = 3$)
1	60	F	×	–	–	–	–	–
2	62	M	×	–	–	–	–	–
3	79	M	–	×	–	–	–	–
4	65	F	–	×	–	–	–	–
5	67	M	–	–	×	–	×	–
6	66	F	–	–	×	×	–	–
7	78	M	–	–	–	×	–	×
8	64	F	–	–	×	–	–	×
9	69	F	–	–	–	–	×	×
10	65	M	–	–	×	–	–	–
11	67	F	–	–	–	×	×	–
12	71	M	–	–	–	×	–	–

Table S3. Forward and reverse primers used for quantitative RT-PCR.

Gene Name	Primer Sequence	Product Size	Annealing Temperature
<i>GAPDH</i>	Forward primer: 5' CGCTCTCTGCTCCTCCTGTT 3' Reverse primer: 5' CCATGGTGTCTGAGCGATGT 3'	81	60
<i>SOX9</i>	Forward primer: 5' TGGGCAAGCTCTGGAGACTTC 3' Reverse primer: 5' ATCCGGGTGGTCCTTCTTGTG 3'	98	60
<i>ACAN</i>	Forward primer: 5' TTCCCATCGTGCCTTTCCA 3' Reverse primer: 5' AACCAACGATTGCACTGCTCTT 3'	121	60
<i>COL2A1</i>	Forward primer: 5' GGCGGGGAGAAGACGCAGAG 3' Reverse primer: 5' CGCAGCGAAACGGCAGGA 3'	129	60
<i>RUNX2</i>	Forward primer: 5' GGAGTGGACGAGGCAAGAGTTT 3' Reverse primer: 5' AGCTTCTGTCTGTGCCTTCTGG 3'	133	60
<i>COL10A1</i>	Forward primer: 5' GAACTCCCAGCACGCAGAAT 3' Reverse primer: 5' CCTGTGGGCATTTGGTATCG 3'	121	60
<i>IHH</i>	Forward primer: 5' GCCATCTCCTCTGCCATGAA 3' Reverse primer: 5' TGCCAGCCTCAAGGTCTCTA 3'	153	60
<i>DKK1</i>	Forward primer: 5' AGTACTGCGTAGTCCCACC 3' Reverse primer: 5' TCCTCAATTTCTCCTCGGAA 3'	172	60
<i>FRZB</i>	Forward primer: 5' ACGGGACACTGTCAACCTCT 3' Reverse primer: 5' CGAGTCGATCCTTCCACTTC 3'	155	60
<i>GREM1</i>	Forward primer: 5' GTCACACTCAACTGCCCTGA 3' Reverse primer: 5' GGTGAGGTGGGTTTCTGGTA 3'	375	60
<i>AIXN2</i>	Forward primer: 5' AGTGTGAGGTCCACGGAAAC 3' Reverse primer: 5' CTGGTGCAAAGACATAGCCA 3'	103	60
<i>BMP2</i>	Forward primer: 5' GCTAGACCTGTATCGCAGGC 3' Reverse primer: 5' TTTTCCCACTCGTTTCTGGT 3'	74	60
<i>COL1A1</i>	Forward primer: 5' GTCACCCACCGACCAAGAAACC 3' Reverse primer: 5' AAGTCCAGGCTGTCCAGGGATG 3'	121	60