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Supplementary Materials for

Augmented ERAD (ER-associated degradation) activity in chondrocytes is necessary for cartilage development and maintenance

Hyo Jung Sim, Chanmi Cho, Ha Eun Kim, Ju Yeon Hong, Eun Kyung Song, Keun Yeong Kwon, Dong Gil Jang, Seok-Jung Kim, Hyun-Shik Lee, Changwook Lee, Taejoon Kwon, Siyoung Yang*, Tae Joo Park*

*Corresponding author. Email: parktj@unist.ac.kr (T.J.P.); yangsy@ajou.ac.kr (S.Y.)

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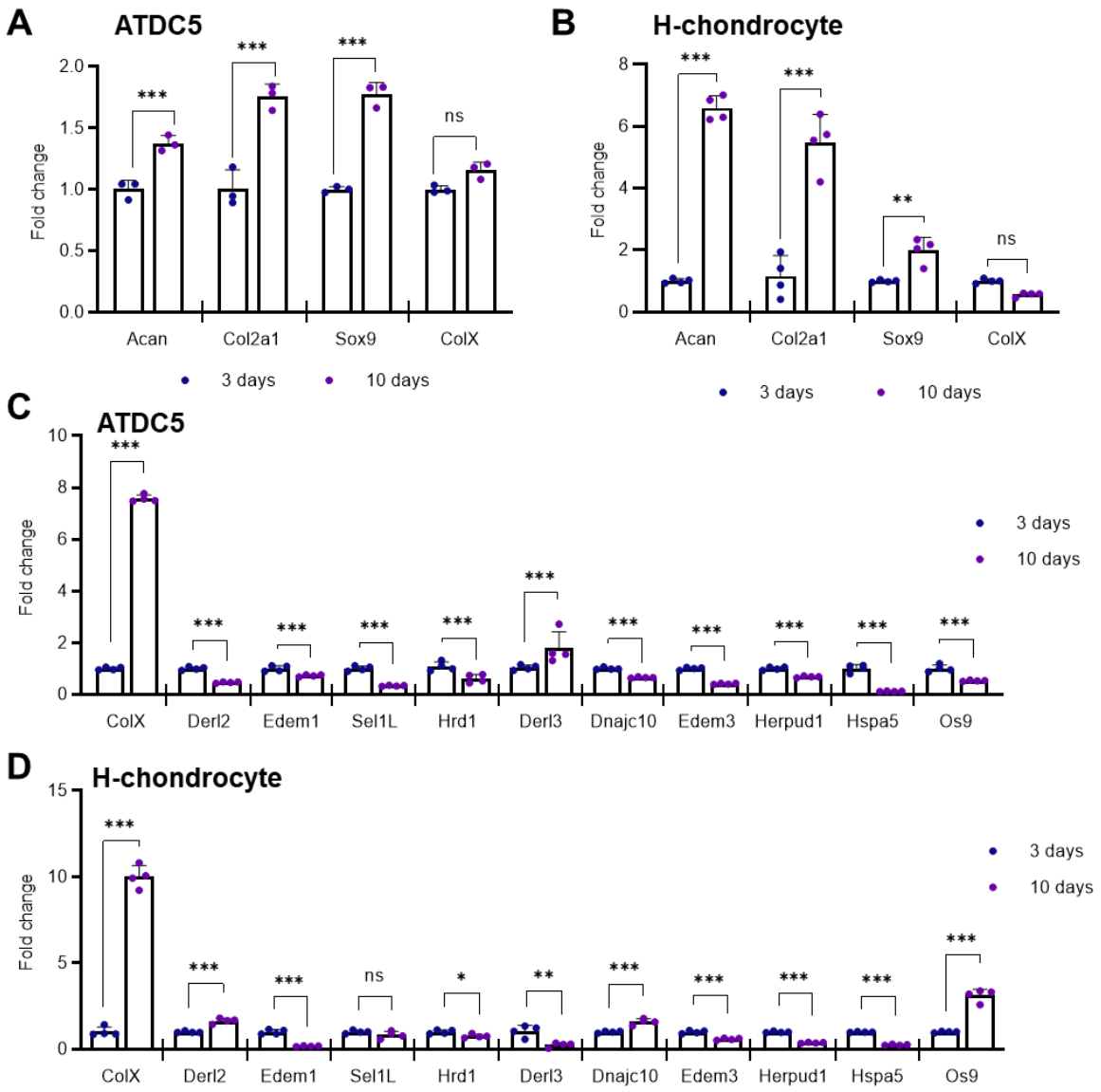
This PDF file includes:

Supplementary Figure S1



Supplementary Figure S1. Supplementary information for Fig 1. A-B.

A) Expression of cartilage anabolic factors Acan, Col2a1, Sox9, and hypertrophy marker ColX was measured by qPCR in differentiating ATDC5 micromasses (n = 3). Chondrogenic markers were increased while hypertrophic chondrocyte maker, ColX was not induced at 10 days after induction of chondrogenesis. Statistical analysis was performed using Student's t-test.

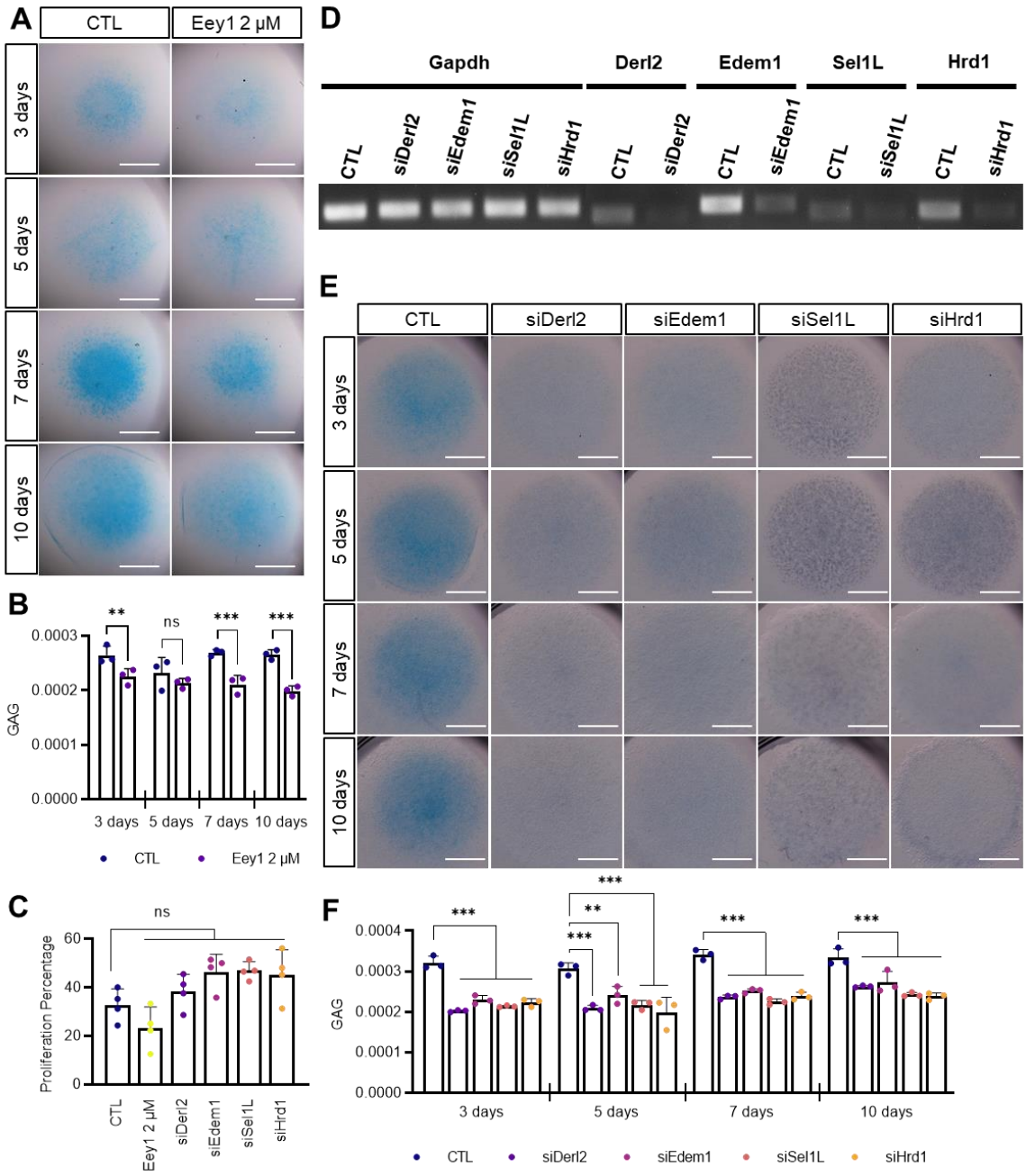
B) Expression of cartilage anabolic factors Acan, Col2a1, Sox9, and hypertrophy marker ColX was measured by qPCR in differentiating human chondrocyte micromasses (n = 3). Chondrogenic markers were increased while hypertrophic chondrocyte maker, ColX was not induced at 10 days after induction of chondrogenesis. Statistical analysis was performed using Student's t-test.

C) Expression of ERAD genes during hypertrophic chondrocyte differentiation was measured using quantitative (q)PCR in ATDC5 micromasses (n = 4). ERAD gene expression is not associated with the hypertrophic differentiation overall. Statistical analysis was performed using Student's t-test.

D) Expression of ERAD genes during hypertrophic chondrocyte differentiation was measured using quantitative (q)PCR in human chondrocyte micromasses (n = 4). ERAD gene expression is not associated with the Hypertrophic differentiation overall. Statistical analysis was performed using Student's t-test.

***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

Supplementary Figure S2



Supplementary Figure S2. Supplementary information for Fig 1. C-H, Malfunction and depletion of ERAD complex causes abnormal chondrogenesis

A) Differentiating ATDC5 cell micromasses were treated with Eey1 after chondrogenic induction, and Alcian blue staining was performed on the indicated days after chondrogenic

induction. Eey1-treated micromasses displayed sharp reduction in glycosaminoglycan (GAG) accumulation. Scale bar = 1 mm.

B) GAG levels in chondrogenic micromasses shown in (A) were measured using Alcian blue staining and normalized to the total protein level. Statistical analysis was performed using one-way ANOVA. All, $n = 3$.

C) Proliferation of siRNA or Eey1 treated ATDC cells was measured and plotted ($n = 4$). siRNA or Eey1 treated cells were stained using Edu, TUNEL, and DAPI to quantify only live cells. Statistical analysis was performed using one-way ANOVA.

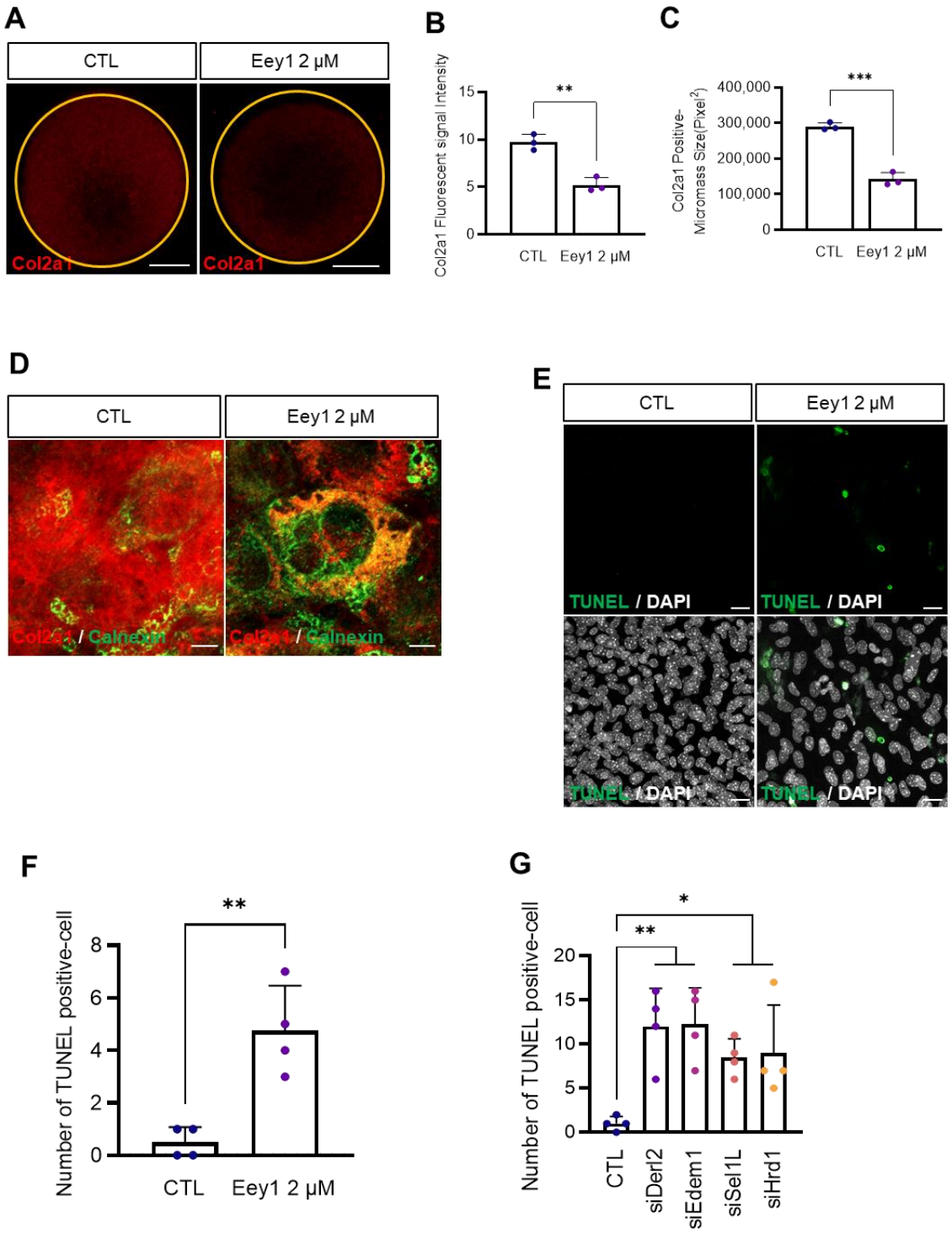
D) Efficacy of siRNAs targeting ERAD genes was tested using reverse transcription (RT)-PCR. RT-PCR data show siRNA efficiently depleted ERAD genes.

E) Expression of ERAD genes was depleted using siRNAs, and chondrogenesis was induced in the ATDC5 cell micromasses. The micromasses were stained with Alcian blue on the indicated days after chondrogenic induction. Depletion of any one ERAD gene strongly impaired chondrogenesis. Scale bar = 1 mm.

F) GAG level in chondrogenic micromasses shown in (E) was measured using Alcian blue and normalized to the total protein level. Depletion of any one of the ERAD genes significantly decreased GAG accumulation. Statistical analysis was performed using one-way ANOVA. n = 3.

***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

Supplementary Figure S3



Supplementary Figure S3. Malfunction of ERAD function caused abnormal accumulation of collagen in the ER lumen.

A) Collagen deposition in chondrogenic micromasses was detected by immunofluorescence staining and image analysis upon treated with Eey1. Malfunction of ERAD genes caused severe reduction in collagen deposition compared to control micromass. Scale bar = 1 mm.

B) Staining intensity of collagen-positive micromass cultures shown in (A) were measured and plotted (n = 3). Statistical analysis was performed using Student's t-test.

C) The areas of collagen-positive micromass cultures shown in (A) were measured and plotted (n = 3). Areas of collagen positivity for Eey1-treated micromasses were significantly reduced. Statistical analysis was performed using Student's t-test.

D) High-resolution images of micromasses shown in (A). (Top panels) Red represents Col2a1, blue represents ER marker calnexin. In the control micromass, collagen is secreted and deposited in the extracellular matrix (ECM). However, in Eey1-treated micromasses, most of the collagen signal was detected in the ER lumen, and not the ECM. Scale bar = 5 μm.

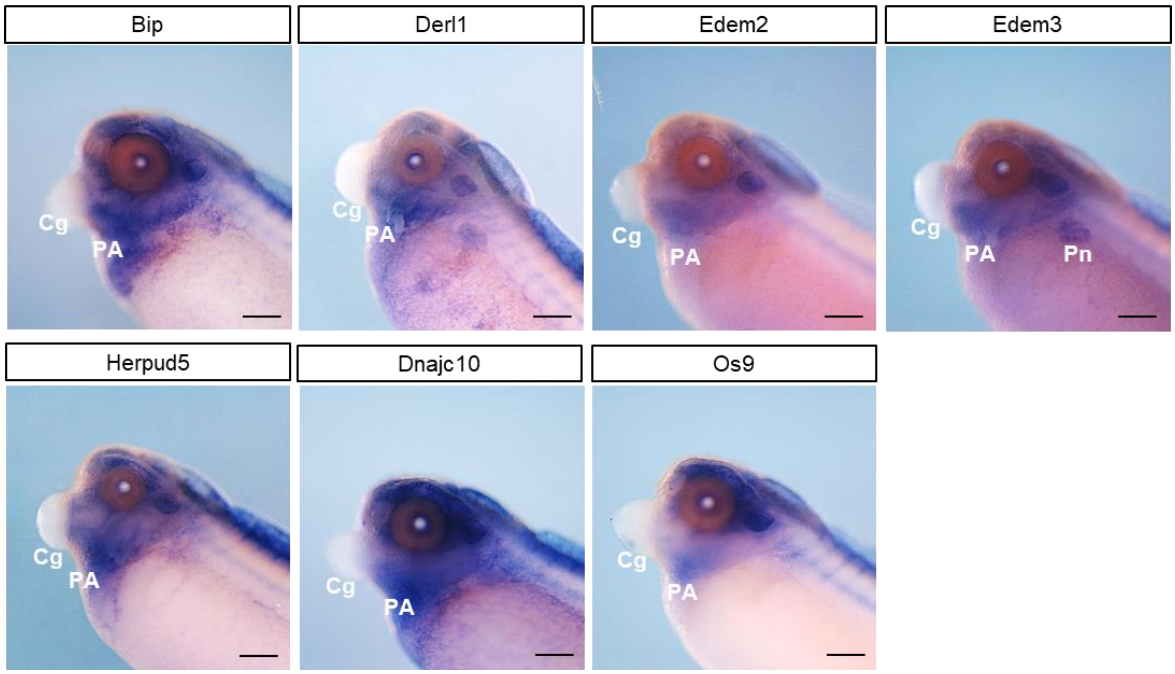
E) TUNEL staining revealed increased cell death in Eey1-treated micromasses. Scale bar = 20 μm.

F) The number of TUNEL-positive micromass cultures shown in (E) were measured and plotted (n = 4). Statistical analysis was performed using Student's t-test.

G) The number of TUNEL-positive cells shown in (Fig. 2E) were measured and plotted (n = 4). Statistical analysis was performed using one-way ANOVA.

***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

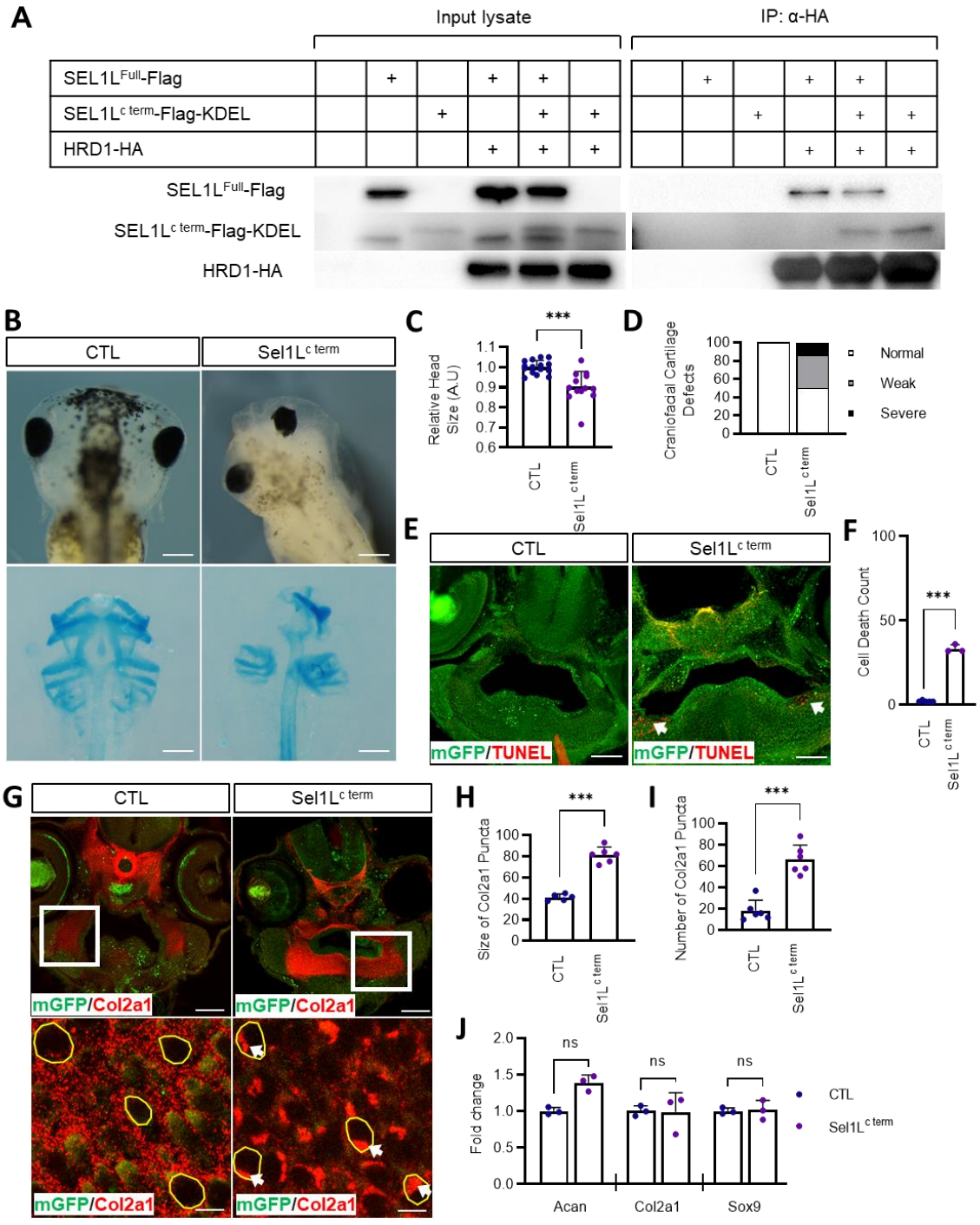
Supplementary Figure S4



Supplementary Figure S4. Supplementary information for Fig. 3A. ERAD genes are expressed in differentiating cartilage tissue

A) Whole mount *in situ* hybridization analysis showed ERAD genes are also highly expressed in developing facial cartilage tissue (PA) of *X. laevis* embryos. PA, pharyngeal arches; Cg, cement gland. Scale bar = 200 μm.

Supplementary Figure S5



Supplementary Figure S5. Sel1L$^{c\ term}$ expression inhibits chondrogenesis and results in intracellular accumulation of collagen in the craniofacial cartilages of *Xenopus laevis*.

A) Sel1L$^{c\ term}$ is co-immunoprecipitated with expression HRD1. Also, Sel1L$^{c\ term}$ inhibited interaction between Sel1L and HRD1 functioning as a dominant negative mutant.

B) Sel1L$^{c\ term}$ overexpression resulted in severe chondrodysplasia in craniofacial cartilage.The lower panels show Alcian blue staining of craniofacial cartilage. Scale bar = 200 μm.

C) Head size of *Xenopus* tadpoles shown in (B) was measured and plotted. Head size of Sel1L$^{c\ term}$ overexpression significantly reduced the size of Statistical analysis was performed using Student's *t*-test. From left to right, n = 15, 13.

D) Craniofacial-cartilage defects in *Xenopus* shown in (B) were counted and plotted. Sel1L$^{c\ term}$ overexpressed embryos showed craniofacial cartilage defects; From left to right, n = 16, 14.

E) Cell death were analyzed in Sel1L$^{c\ term}$ overexpressed craniofacial tissues of *Xenopus*. Green signal is membrane GFP and red signal is TUNEL-positive cells. Cell death is increased significantly in Sel1L$^{c\ term}$ overexpressed craniofacial tissues. Scale bar = 100 μm

F) TUNEL-positive cells in *Xenopus* shown in (E) were counted and plotted. Sel1L$^{c\ term}$ overexpression increased the number of TUNEL-positive cells. Statistical analysis was performed using Student's *t*-test. From left to right, $n = 3$.

G) Collagen deposition were analyzed in Sel1L$^{c\ term}$ overexpressed craniofacial tissues of *Xenopus*. Green represents membrane GFP, red represents Col2a1. Sel1L$^{c\ term}$ overexpressed craniofacial tissues showed intracellular accumulation of collagen. White arrow represents abnormal collagen puncta (middle, bottom). 100 μm (middle), 5 μm (bottom).

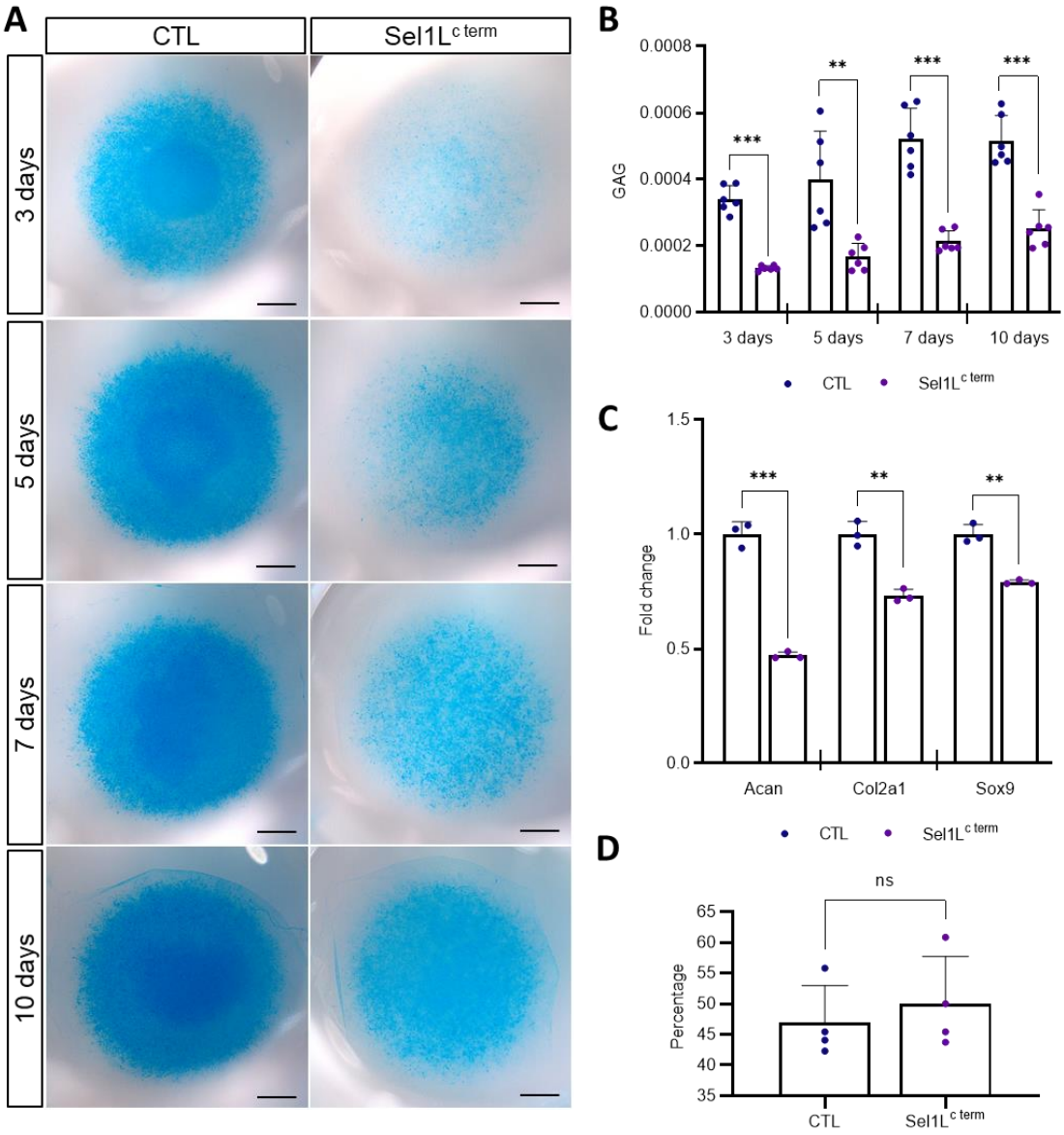
H) The size of collagen puncta shown in (G) was counted and plotted. $n = 6$.

I) The number of collagen puncta shown in (G) was counted and plotted. $n = 6$.

J) Expression of cartilage anabolic factors Acan, Col2a1, and Sox9 was analyzed using qPCR. Sel1L$^{c\ term}$-expression did not significantly changed the expression of the anabolic factors. Statistical analysis was performed using Student's *t*-test. $n = 3$.

***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

Supplementary Figure S6



Supplementary Figure S6. Sel1L$^{c\ term}$ expression inhibits chondrogenesis in the ATDC5 micromasses.

A) ATDC5 overexpressing Sel1L$^{c\ term}$ were induced for chondrogenic differentiation and alcian blue staining was performed at the indicated days after induction. Sel1L$^{c\ term}$

overexpressing micromasses displayed sharp reductions in glycosaminoglycan (GAG) accumulation. Scale bar = 1 mm.

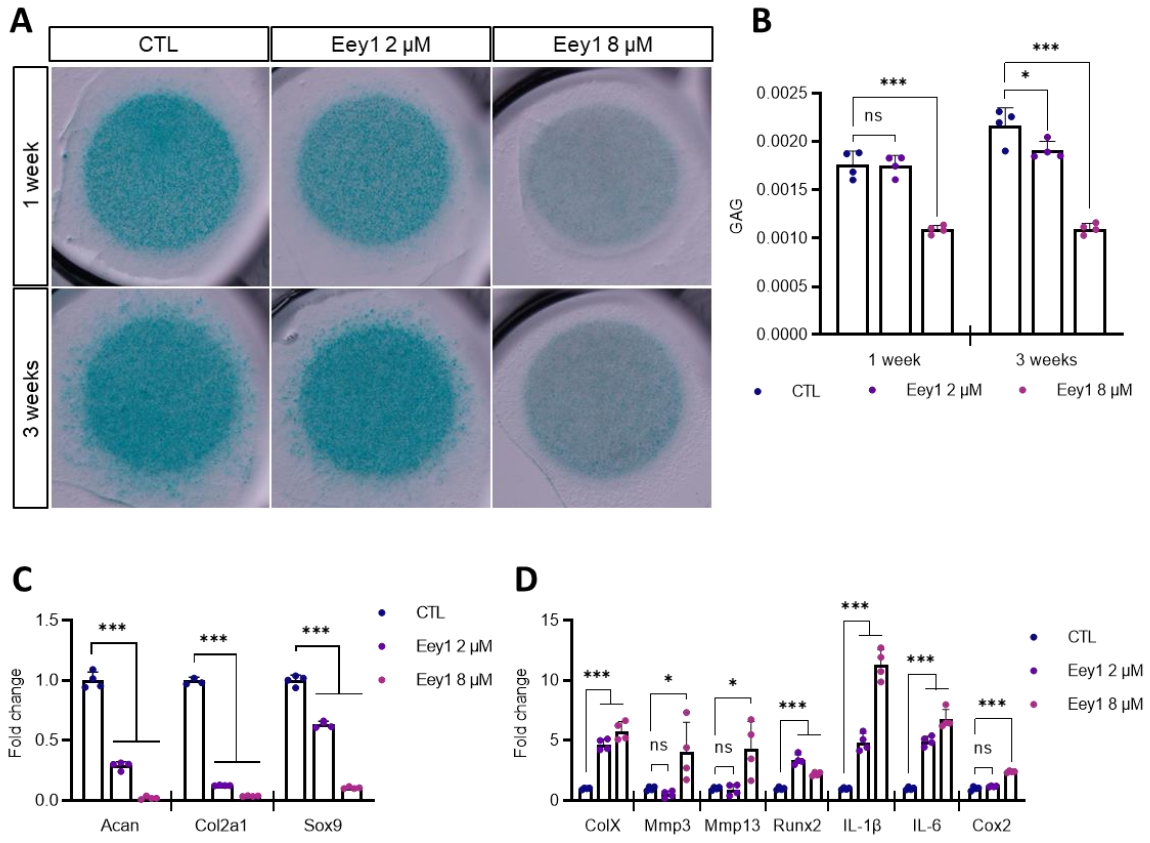
B) GAG level in chondrogenic micromasses shown in (B) was measured using Alcian blue staining and normalized to total protein level (n = 6). Statistical analysis was performed using Student's t-test.

C) Expression of cartilage anabolic factors, aggrecan (Acan), collagen type IIa1 (Col2a1), and Sox9 were measured by qPCR 10 days after chondrogenic induction (n = 3). Sel1L$^{c\ term}$ overexpression significantly reduced the expression of anabolic factors (n = 3). Statistical analysis was performed using Student's t-test. Proliferation of Sel1L$^{c\ term}$ overexpressed ATDC cells were measured and plotted (n = 4). Sel1L$^{c\ term}$ overexpressed cells were stained using Edu, TUNEL, and DAPI to quantify only live cells. Statistical analysis was performed using Student's t-test.

D) Proliferation of Sel1L$^{c\ term}$ overexpressed ATDC cells were measured and plotted (n = 4). Sel1L$^{c\ term}$ overexpressed cells were stained using Edu, TUNEL, and DAPI to quantify only live cells. Statistical analysis was performed using Student's t-test.

***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

Supplementary Figure S7



Supplementary Figure S7. Inhibition of ERAD function causes cartilage loss in fully differentiated micromasses.

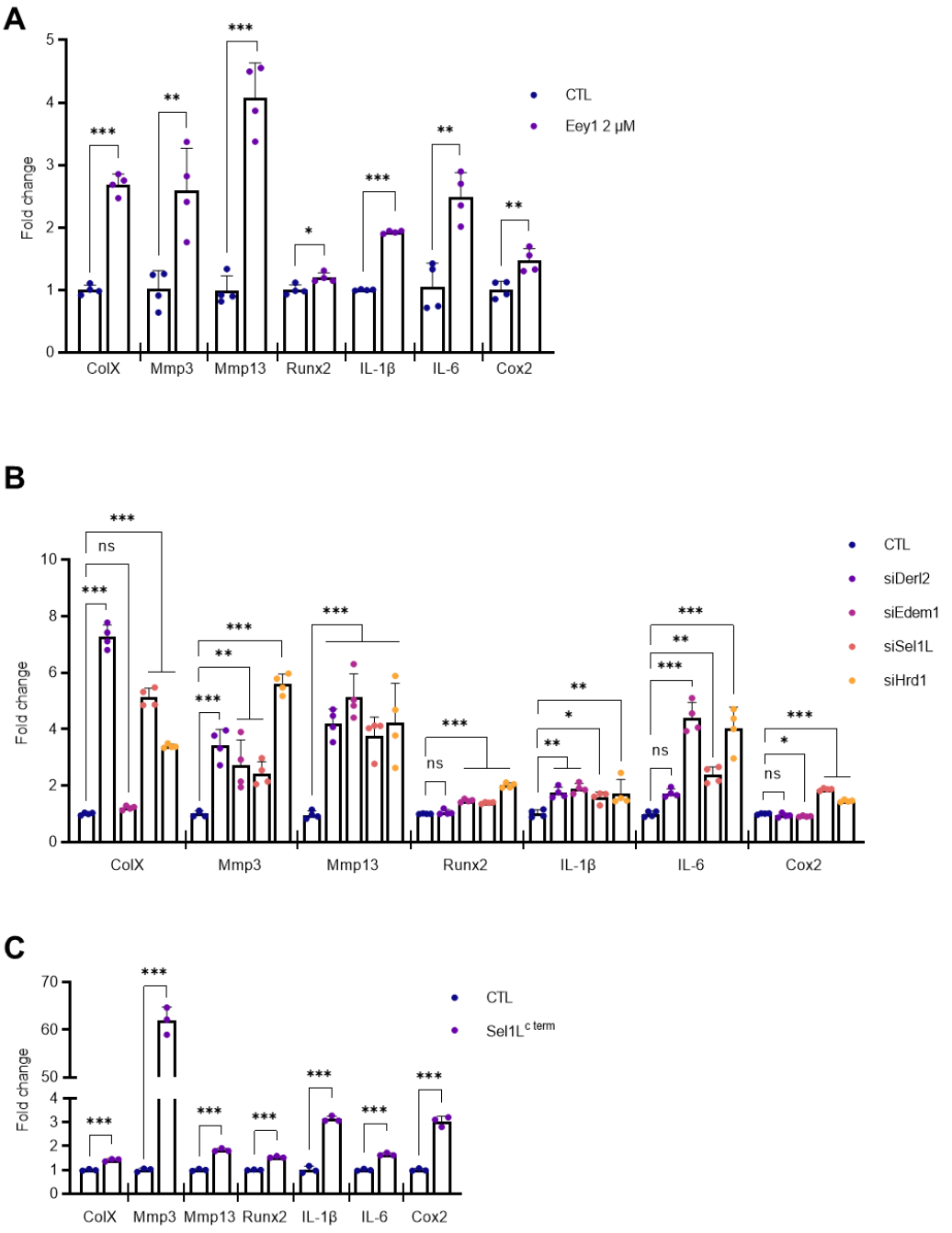
A) Differentiating ATDC5 cell micromasses were treated with Eey1 2 weeks after chondrogenic induction, and alcian blue staining was performed 3 weeks after Eey1 treatment. Eey1-treated micromasses displayed sharp reduction in GAG accumulation. Scale bar = 100 μm.

B) GAG levels in chondrogenic micromasses shown in (A) were measured using alcian blue and normalized to the total protein level. Statistical analysis was performed using one-way ANOVA. All, $n = 4$.

C) Cartilage anabolic gene expression in chondrogenic micromasses shown in (A) were measured using qPCR. Statistical analysis was performed using one-way ANOVA. All, $n = 4$.

D) Cartilage catabolic gene expression in chondrogenic micromasses shown in (A) were measured using qPCR. Statistical analysis was performed using one-way ANOVA. All, $n = 4$. ***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

Supplementary Figure S8



Supplementary Figure S8. Inhibition of ERAD function causes cartilage loss in micromasses

A) Expression of hypertrophy markers ColX, Runx2, cartilage catabolic factors MMP3, MMP13, Cox2, and proinflammatory cytokines IL-1β, and IL-6 was measured using

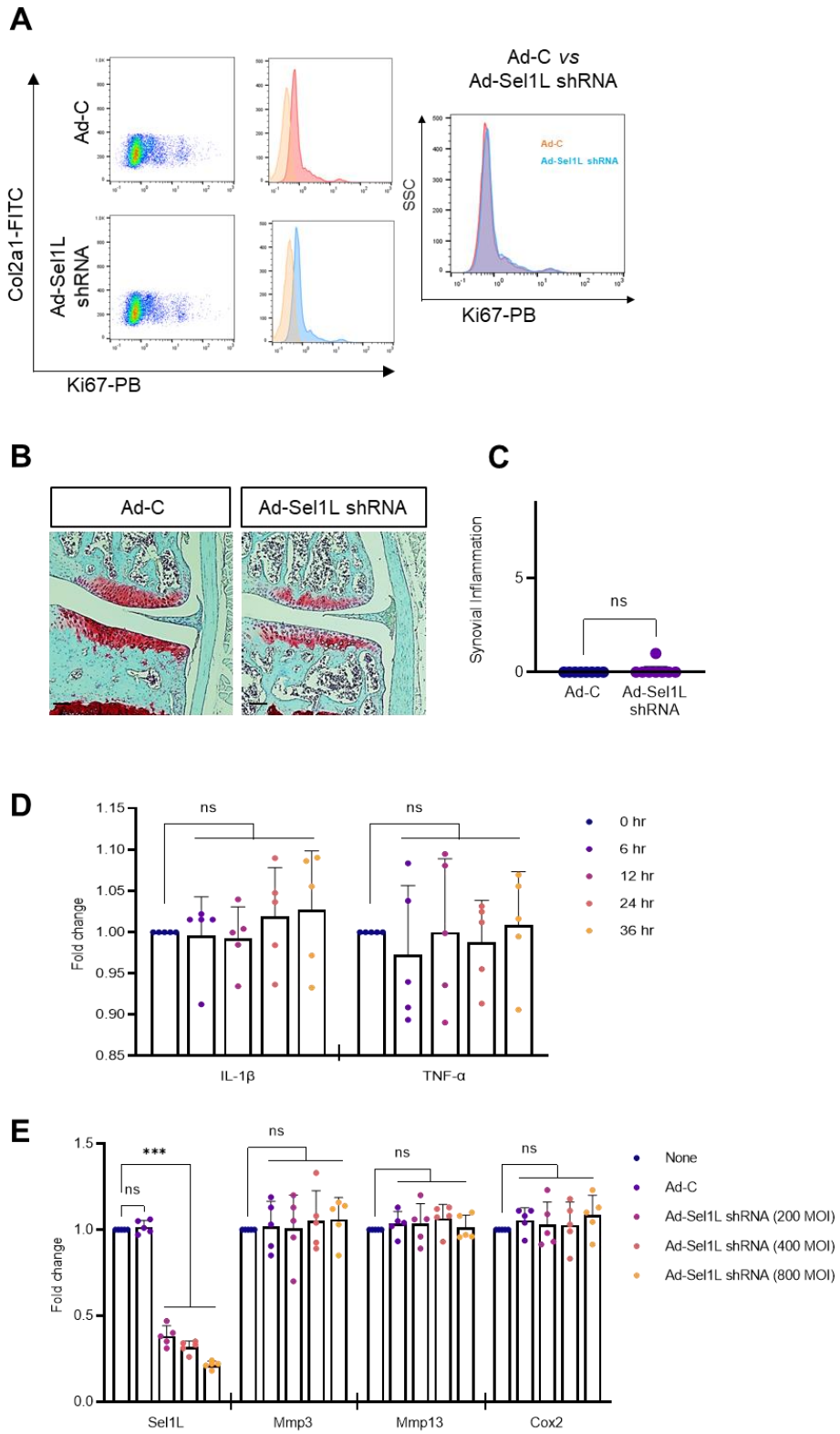
quantitative (q)PCR in Eey1 treated ATDC5 micromasses (n = 4). All of the gene expression was significantly increased at 10 days after induction of chondrogenesis in Eey1-treated micromasses. Statistical analysis was performed using Student's t-test.

B) Expression of hypertrophy markers ColX, Runx2, cartilage catabolic factors MMP3, MMP13, Cox2, and proinflammatory cytokines IL-1β, and IL-6 was measured using quantitative (q)PCR in ERAD siRNA treated ATDC5 micromasses (n = 4). Most of the gene expression was significantly increased at 10 days after induction of chondrogenesis in ERAD-depleted micromasses. Statistical analysis was performed using one-way ANOVA.

C) Expression of hypertrophy markers ColX, Runx2, cartilage catabolic factors MMP3, MMP13, Cox2, and proinflammatory cytokines IL-1β, and IL-6 was measured using quantitative (q)PCR in $Sel1L^{c\text{-}term}$ overexpressing ATDC5 micromasses (n = 4). All of the gene expression was significantly increased at 10 days after induction of chondrogenesis in $Sel1L^{c\ term}$- expressing micromasses. Statistical analysis was performed using Student's t-test.

***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

Supplementary Figure S9



Supplementary Figure S9. Sel1L depletion did not significantly affect cell proliferation or synovitis in mouse joints.

A) Cell proliferation of mouse chondrocyte was analyzed after Ad-Sel1L shRNA transduction using FACS analysis. Sel1L depletion did not significantly affect cell proliferation.

B) Scoring of synovial inflammation was determined by safranin-O staining and hematoxylin staining in Sel1L-depleted mouse articular tissues. Scale bar = 100 μm.

C) Synovial inflammation shown in (B) was quantified and plotted. Statistical analysis was performed using Student's t-test.

D) Mouse primary synovial fibroblast cells were treated with proinflammatory cytokines IL-1β or TNF-α, and Sel1L gene expression was analyzed by qPCR. Proinflammatory cytokines did not significantly reduced Sel1L gene expression (n = 5). Statistical analysis was performed using the Student's t-test.

E) Expression of cartilage catabolic factors MMP3, MMP13, and Cox2, was measured using quantitative (q)PCR in ad-Sel1L shRNA treated synovial fibroblast cells (n = 5). Sel1L expression was not significantly affected by Sel1L-depletion. Statistical analysis was performed using the Student's t-test.

***($p < 0.001$), **($0.001 < p < 0.01$), *($0.01 < p < 0.05$), ns ($0.05 < p$).

Table S1. Characteristics of the human tissue donors

No	Age (years)/ Gender	ICRS grade	Joint	Weight (kg)	Height (m)	BMI (kg/m^2)	Use
1	65/F	4	Knee	158	53	21.23	IHC
2	80/F	4	Knee	143.3	55.1	26.83	IHC
3	72/F	4	Knee	165	65	23.88	IHC
4	63/F	4	Knee	152	52	22.51	IHC
5	69/F	4	Knee	151	60	26.31	IHC
6	73/F	4	Knee	153.8	70.75	29.89	IHC
7	63/F	4	Knee	156	72.2	29.67	IHC
8	73/F	4	Knee	154	83	35	IHC
9	75/F	4	Knee	154	55	23.19	IHC
10	63/F	4	Knee	163	74.3	27.96	IHC

Table S2. Primer sequences for probe synthesis

Gene	Origin	Strand	Sequence	Size (bp)	AT[a] (°C)
Derl1	*Xenopus*	S	5’-ATGTCTGACCTCGGGGACTGG-3’	762	58
		As	5’-GCGATTTAGGTGACACTATAGCTAGTCTCCTAGACGGAAGCCTTGC-3’		
Derl2	*Xenopus*	S	5’-GTAAGTGGAGGAGTCGGTGGGAG-3’	999	58
		As			

			5’-GCGATTTAGGTGACACTATAGTATCGTTCTTTGTATCAAAGAAATTAAGAAAAATA-3’		
			5’-CCCCCCAGATAGTAGAAATGAGACC-3’		
Edem1	*Xenopus*	S	5’-GCGATTTAGGTGACACTATAGTGCGGCACTTTGCTTTTACG-3’	945	57
		As			
			5’-CCCTGACCTGTGATGGACAAGATAC-3’		
Edem2	*Xenopus*	S	5’-GCGATTTAGGTGACACTATAGGGAAAACTGGCATAGTGACCGTG-3’	753	57
		As			
			5’-AGCAGAGGGGATGTTGATGACG-3’		
Edem3	*Xenopus*	S	5’-GCGATTTAGGTGACACTATAGTGGAGGCTGGCTTATGTATCGC-3’	717	58
		As			
			5’-CCTGAGGCTGATGGCATTAGAC-3’		
Herpud1	*Xenopus*	S	5’-GCGATTTAGGTGACACTATAGGATTGTGCTTGGAGAGTGGCAG-3’	692	56
		As			
			5’-AACATCACAGGCTAAAGCGTTACC-3’		
Os9	*Xenopus*	S	5’-GCGATTTAGGTGACACTATAGTTCCTCCCCCGAATCAGTTG-3’	623	56
		As			
Hrd1	*Xenopus*	S	5’-GTTCCGTGATGATTTCAGCCC-3’	804	57
		As			

			5’-GCGATTTAGGTGACACTATAGGGAGTTGGTTGTTGTTGAGCCTG-3’		
Bip	*Xenopus*	S	5’-GGTAACCGTATCACACCCTCGTATG-3’	775	57
		As	5’-GCGATTTAGGTGACACTATAGCCTCCCCTTCAAAGAAAGATTCG-3’		
Dnajc10	*Xenopus*	S	5’-GCAGGATTGCTGGAAGGTCTTG-3’	763	57
		As	5’-GCGATTTAGGTGACACTATAGCGGTGGACTGGTTGAACACTACTG-3’		
Sel1L	*Xenopus*	S	5’-GTGGAAAATCCAGGCATGGC-3’	565	57
		As	5’-GCGATTTAGGTGACACTATAGCTGTGCCAGTCGCATGCATC-3’		

Table S3. **Primer sequences and qRT-PCR conditions**

Gene	Origin	Strand	Sequence	Size (bp)	AT[a] (°C)
Derl2	Human	S	5’-TTTTTGGGCCAGTTGGATTCA-3’	212	56
		As	5’-GCTCCACACATAGACGAGCATTA-3’		
Derl3	Human	S	5’-GGCGTCCTTATGACCCTGC-3’	206	56
		As	5’-AGGTCCACGAGGATGGAGTT-3’		

Dnajc10	Human	S	5’-TGTCCACCATGTCGAGCTTTA-3’	193	56
		As	5’-CAGCAGAGTGATGTCCTTCATAC-3’		
Edem1	Human	S	5’-ACAGGGATTCCATATCCTCGG-3’		
		As	5’-CTCCCGCTGTGCATGTCTC-3’		
Edem3	Human	S	5’-CATGCCTTTAACCTGTAGAGGTC-3’	250	55
		As	5’-GGAGTGCCCACCCAAAAGAC-3’		
Herpud1	Human	S	5’-CCGGTTACACACCCTATGGG-3’	220	56
		As	5’-TGAGGAGCAGCATTCTGATTG-3’		
Hspa5	Human	S	5’-CACGGTCTTTGACGCCAAG-3’	215	55
		As	5’-CCAAATAAGCCTCAGCGGTTT-3’		
Os9	Human	S	5’-CTGTCCAGTTTGTTAGGACTGC-3’	111	55
		As	5’-GATCCCATAACGCATCTCACTC-3’		
Hrd1	Human	S	5’-GCTCACGCCTACTACCTCAAA-3’	215	55
		As	5’-GCCAGACAAGTCTCTGTGACG-3’		
Sel1L	Human	S	5'-TCCCAGCAGGCAACTCAAAG-3'	210	55
		As	5'-AAGGCTCTGACATCCGACTTCC-3'		
Acan	Human	S	5'-ACCCTGGAAGTCGTGGTGAAAG-3'	116	57.5
		As	5'-GCAATGATGGCACTGTTCTGC-3'		
Col2a1	Human	S	5'-CAGCAAGAGCAAGGAGAAG-3'	126	54.1
		As	5'-AGGCGTAGGAAGGTCATC-3'		
Sox9	Human	S	5'-GCGGAGGAAGTCGGTGAAGA-3'	237	63.3
		As	5'-CCCTCTCGCTTCAGGTCAGC-3'		

ColX	Human	S	5'-GCTAAGGGTGAAAGGGGTTC-3'	118	55.9
		As	5'-CTCCAGGATCACCTTTTGGA-3'		
Gapdh	Human	S	5'-AAGGTGAAGGTCGGAGTCAACG-3'	227	55
		As	5'-TGGAAGATGGTGATGGGATTTC-3'		
Derl2	Mouse	S	5’-CAATAATGCTGGTCTACGTGTGGAG-3’	162	56
		As	5’-CAACTGCAATACCCAAAAGGTCC-3’		
Derl3	Mouse	S	5’-CGGTGGTGTTCTTATGACTCTGC-3’	152	57
		As	5’-GGAATGGTGCCTGGAAGTTGAG-3’		
Dnajc10	Mouse	S	5’-GGGAAAACTCACTGGGTGGTTG-3’	179	57
		As	5’-ACACTGGGGTAGGCTTTGATGC-3’		
Edem1	Mouse	S	5’-GAACACCTGGATTGACTCGCTG-3’	228	56
		As	5’-TTCTTGGTTGCCTGGTAGAGGAG-3’		
Edem3	Mouse	S	5’-GCAAAGATAGTGGAGTTGGAGCG-3’	158	56
		As	5’-CACATCAAGTAGGAGAGGTGGCTG-3’		
Herpud1	Mouse	S	5’-CCTTTACTTCTACTCCTCGCTGAGC-3’	200	56
		As	5’-GGTCTTCCATTTCTGGGTCCATAC-3’		
Hspa5	Mouse	S	5’-GCCACTAATGGAGATACTCACCTGG-3’	151	57
		As	5’-TAGCCTTTTCTACCTCACGCCG-3’		
Os9	Mouse	S	5’-TGTTGAGCCCAATGAGAGATGC-3’	171	56
		As	5’-TTTCGTCGTCCCAGTTGAAGG-3’		
Hrd1	Mouse	S	5’-CACATTCCCACTCTTTGCCATTAG-3’	192	56
		As	5’-GCACCAGTCACCATTTCTTCTCTG-3’		

Sel1L	Mouse	S	5’-CTTTGCCCACAGATGAGTCAGTG-3’	180	56
		As	5’-TCTTGCTGCTTGGATTCGGAG-3’		
Acan	Mouse	S	5‘-CTTTTATGCGACATCCCCAGAG-3’	236	55
		As	5‘-GGTTGGCGTGTAGATAGACAGTCC-3’		
Col2a	Mouse	S	5‘-GATGACTTTCCTCCGTCTACTGTCC-3’	172	56
		As	5‘-GTATGTGAACCTGCTGTTGCCC-3’		
Sox9	Mouse	S	5‘-AACGGCTCCAGCAAGAACAAG-3’	169	56
		As	5‘-TCTTCTCGCTCTCGTTCAGCAG-3’		
ColX	Mouse	S	5'-TGCTGCTAATGTTCTTGACCCTG-3'	158	56.2
		As	5'-GCCTTGTTCTCCTCTTACTGGAATC-3'		
Mmp3	Mouse	S	5'-CAAGGGATGATGATGCTGGTATG-3'	266	55.1
		As	5'-GGATTTCCTCCATTTTGGCG-3'		
Mmp13	Mouse	S	5'-CAGAATCTATGATGGCACTGCTGA-3'	379	57
		As	5'-TGTTTTGGGATGCTTAGGGTTG-3'		
IL-1β	Mouse	S	5'-TCACAAGCAGAGCACAAGCCTG-3'	107	54.7
		As	5'-GAAACAGTCCAGCCCATACTTTAGG-3'		
IL-6	Mouse	S	5'-CAAGAGACTTCCATCCAGTTGCC-3'	179	56.7
		As	5'-CATTTCCACGATTTCCCAGAGAAC-3'		
Runx2	Mouse	S	5'-GCACAAGTGATTGGTTGAACTGC-3'	200	57.2
		As	5'-TTCCCCTGAATGGCTGTATGG-3'		
Cox2	Mouse	S	5'-AAGACTTGCCAGGCTGAACT-3'	150	60
		As	5'-CTTCTGCAGTCCAGGTTCAA-3'		

		S	5‘-TGCACCACCAACTGCTTAG-3’		
Gapdh	Mouse			176	56
		As	5‘-GATGCAGGGATGATGTTC-3’		
		S	5‘-AACGCTTTGGATGGTGTGACTG-3’		
Acan	*Xenopus*			171	56
		As	5‘-AAGTGGGTAGGTGGGCATAGAGAC-3’		
		S	5‘-TCTGCCCAACTGAGCAATCTTC-3’		
Col2a	*Xenopus*			170	55
		As	5‘-TTTCTCGCCCTTATCTCCACG-3’		
		S	5‘-CCATAAACAGCGAGCAAAGCC-3’		
Sox9	*Xenopus*			210	56
		As	5‘-AATAGGAGTTGGAGCCCTGGTG-3’		
		S	5’-GCCGTGTATGTGGTGGAATCT-3’		
Gapdh	*Xenopus*			230	55
		As	5’-AAGTTGTCGTTGATGACCTTTGC-3’		

[a]AT, annealing temperature; [b]S, sense primer; [c]As, antisense primer

Table S4. **Morpholinos**

Gene	Origin	Sequence
Sel1L	*Xenopus*	5’-AAAGCGCACAGTCCTGCCCCCCATT-3’

Table S5. **siRNAs**

Gene	Origin	Strand	Sequence
Derl2	Mouse	S	5’-CCGUAUGUCCGCAUGAACUUU-3’

		As	5’-AGUUCAUGCGGACAUACGGUU-3’
Edem1	Mouse	S	5’-CCUUUCUGCUCACAGAAUAUU-3’
		As	5’-UAUUCUGUGAGCAGAAAGGUU-3’
Sel1L	Mouse	S	5’-CCAUUGUAGGUGAGAAUGAUU-3’
		As	5’-UCAUUCUCACCUACAAUGGUU-3’
Hrd1	Mouse	S	5’-GACAACAAGGCUGUAUACAUU-3’
		As	5’-UGUAUACAGCCUUGUUGUCUU-3’

Table S6. Primer sequences for plasmid construction

Gene	Origin	Strand	Sequence	Size (bp)	AT[a] (°C)
Sel1L-SP	Xenopus	S	5'-AATAGATCTGGACTGTCACACAGAATAGCACGG-3'	150	60
		AS	5'-AATGAATTCACTACCCTTGGGAACATCAGCG-3'		
Sel1L^{Full}	Xenopus	S	5'-AATAGATCTGGACTGTCACACAGAATAGCACGG-3'	2508	59.4
		AS	5'-AATACTAGTCTGTGGTTGCTGGACTTCCTGTT0-3'		
Sel1L$^{c\ term}$	Xenopus	S	5'-AATGAATTCGGTGATTATCATTTCTATGGCTACGG-3'	285	56.5
		AS	5'-AATTCTAGATTCTTTAACATTCACATCACGCAGAA-3'		
Sel1L^{Full}	Mouse	S	5'-AATGAATTCATGCAGGTCCGCGTAAGGC-3'	2370	60.6
		AS	5'-AATTCTAGACTGTGGTGGCTGCTGCTCTG-3'		
Sel1L$^{c\ term}$	Mouse	S	5'-AATGAATTCGGAGACTACCACTTCTATGGC-3'	285	56.3
		AS	5'-AATTCTAGAATCTCGAATGTTTGCTTCCCG-3'		
Hrd1	Mouse	S	5'-AATGAATTCATGTTCCGCACCGCAG-3'	1836	60.8
		AS	5'-AATTCTAGAGTGGGCAACAGGGGACTC-3'		