

Supporting Information

Decorin Regulates Cartilage Pericellular Matrix Micromechanobiology

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Table S1. Summary of averaged values and statistical analysis outcomes of the thickness of PCM, as well as the area proportions of cell, PCM and T/IT-ECM domains, between wild-type (WT) and decorin-null (*Dcn*^{-/-}) cartilage, shown as mean ± 95% CI from values averaged by each animal

	PCM Thickness (µm)			Cell Area (%)		
	3 days	2 weeks	3 months	3 days	2 weeks	3 months
WT	3.05 ± 0.18	3.10 ± 1.01	2.04 ± 0.20	32.1 ± 1.8	26.2 ± 2.1	20.1 ± 1.6
<i>Dcn</i> ^{-/-}	2.96 ± 0.36	2.99 ± 0.57	2.12 ± 0.52	31.1 ± 1.9	27.0 ± 1.9	20.2 ± 0.6
<i>p</i> -value (genotype)	1.000	0.774	1.000	1.000	1.000	0.796
	PCM Area (%)			T/IT-ECM Area (%)		
	3 days	2 weeks	3 months	3 days	2 weeks	3 months
WT	32.3 ± 1.8	28.5 ± 1.2	21.1 ± 1.2	35.6 ± 1.2	45.3 ± 3.1	58.9 ± 1.4
<i>Dcn</i> ^{-/-}	33.9 ± 1.1	28.7 ± 1.2	22.0 ± 0.8	35.0 ± 3.0	44.3 ± 2.5	57.8 ± 1.2
<i>p</i> -value (genotype)	1.000	0.764	0.435	0.662	1.000	0.534
<i>p</i> -value (age)	PCM Thickness (µm)			Cell Area (%)		
	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months
WT	0.981	0.010	0.008	< 0.001	< 0.0001	< 0.001
<i>Dcn</i> ^{-/-}	0.990	0.009	0.008	0.003	< 0.0001	< 0.0001
<i>p</i> -value (age)	PCM Area (%)			T/IT-ECM Area (%)		
	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months
WT	0.002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<i>Dcn</i> ^{-/-}	< 0.001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001

Table S2. Summary of collagen fibril diameter distributions of 3-month-old wild-type (WT) and decorin-null (*Dcn*^{-/-}) cartilage, measured by TEM (data for the territorial and interterritorial domains are adapted from [32])

unit (nm)	Pericellular		Territorial		Interterritorial	
	WT	<i>Dcn</i> ^{-/-}	WT	<i>Dcn</i> ^{-/-}	WT	<i>Dcn</i> ^{-/-}
mean	26.1	29.0	59.7	60.9	67.5	69.7
std	5.8	8.0	9.8	11.4	13.3	18.5
<i>Q</i> ₁	22.0	23.2	52.5	53.3	58.4	56.7
<i>Q</i> ₂	25.8	28.3	60.1	60.1	65.6	67.7
<i>Q</i> ₃	29.7	34.0	67.2	69.5	74.8	81.7
min	10.7	6.5	32.5	30.1	32.7	32.3
max	49.9	59.6	85.0	87.9	119.0	124.1
<i>n</i> _{fibrils}	1,420	1,402	213	385	218	253
<i>p</i> -value (mean)	< 0.0001		0.225		0.145	
<i>p</i> -value (variance)	< 0.0001		0.015		< 0.0001	

Table S3. Summary of averaged values and statistical analysis outcomes of the micromodulus, E_{ind} , between wild-type (WT) and decorin-null ($Dcn^{-/-}$) cartilage, shown as mean \pm 95% CI from values averaged by each animal

E_{ind} (MPa)	PCM			T/IT-ECM		
	3 days	2 weeks	3 months	3 days	2 weeks	3 months
WT	0.058 \pm 0.005	0.285 \pm 0.019	0.907 \pm 0.057	0.105 \pm 0.038	0.438 \pm 0.033	1.544 \pm 0.097
$Dcn^{-/-}$	0.059 \pm 0.018	0.212 \pm 0.048	0.252 \pm 0.072	0.096 \pm 0.018	0.338 \pm 0.039	0.438 \pm 0.036
<i>p</i> -value (genotype)	0.974	0.001	< 0.0001	0.737	< 0.001	< 0.0001
<i>p</i> -value (age)	PCM			T/IT-ECM		
	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months
WT	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
$Dcn^{-/-}$	< 0.0001	< 0.0001	0.167	< 0.0001	< 0.0001	< 0.001
<i>p</i> -value (region)	WT			$Dcn^{-/-}$		
	3 days	2 weeks	3 months	3 days	2 weeks	3 months
PCM vs T/IT-ECM	0.020	< 0.0001	< 0.001	0.005	0.002	0.006

Table S4. Summary of averaged values and statistical analysis outcomes of $[Ca^{2+}]_i$ parameters between wild-type (WT) and decorin-null ($Dcn^{-/-}$) cartilage chondrocytes at all three tested ages, shown as mean \pm 95% CI from the values averaged by cells

% R_{cell}	Hypotonic			Isotonic			Hypertonic		
	3 days	2 weeks	3 months	3 days	2 weeks	3 months	3 days	2 weeks	3 months
WT	81 \pm 10	81 \pm 10	75 \pm 9	71 \pm 10	70 \pm 12	59 \pm 10	51 \pm 11	50 \pm 11	44 \pm 10
$Dcn^{-/-}$	80 \pm 9	68 \pm 11	42 \pm 8	71 \pm 11	48 \pm 10	33 \pm 7	51 \pm 10	32 \pm 8	22 \pm 6
<i>p</i> -value	0.993	0.330	< 0.001	1.000	0.029	0.001	1.000	0.030	0.002
n_{peak}	Hypotonic			Isotonic			Hypertonic		
	3 days	2 weeks	3 months	3 days	2 weeks	3 months	3 days	2 weeks	3 months
WT	7.78 \pm 0.29	6.66 \pm 0.24	6.15 \pm 0.32	5.25 \pm 0.20	4.53 \pm 0.28	3.60 \pm 0.19	3.11 \pm 0.21	2.42 \pm 0.19	2.05 \pm 0.22
$Dcn^{-/-}$	7.80 \pm 0.24	4.06 \pm 0.25	2.54 \pm 0.13	5.22 \pm 0.19	2.91 \pm 0.18	1.78 \pm 0.19	3.04 \pm 0.18	1.98 \pm 0.22	1.19 \pm 0.12
<i>p</i> -value	0.977	< 0.0001	< 0.0001	1.000	0.001	< 0.0001	1.000	0.651	0.009
t_{peak} (sec)	Hypotonic			Isotonic			Hypertonic		
	3 days	2 weeks	3 months	3 days	2 weeks	3 months	3 days	2 weeks	3 months
WT	18.2 \pm 0.6	20.9 \pm 0.8	21.7 \pm 0.5	25.9 \pm 0.7	27.3 \pm 0.8	29.0 \pm 0.9	50.4 \pm 1.5	50.2 \pm 1.6	54.3 \pm 1.5
$Dcn^{-/-}$	18.4 \pm 0.6	30.7 \pm 1.0	49.9 \pm 1.6	26.4 \pm 0.6	34.9 \pm 1.4	68.1 \pm 1.7	50.9 \pm 1.3	66.3 \pm 2.0	84.7 \pm 2.5
<i>p</i> -value	0.740	< 0.001	< 0.0001	1.000	0.002	< 0.0001	1.000	< 0.0001	< 0.0001

Table S5. Summary of statistical analysis outcomes of $[Ca^{2+}]_i$ parameters among the three tested ages for wild-type (WT) and decorin-null ($Dcn^{-/-}$) cartilage chondrocytes

% R_{cell}	Hypotonic			Isotonic			Hypertonic		
	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months
WT	0.599 (not sig.)			0.551 (not sig.)			1.000 (not sig.)		
$Dcn^{-/-}$	0.163	< 0.0001	0.001	0.009	< 0.0001	0.043	0.009	< 0.0001	0.091
n_{peak}	Hypotonic			Isotonic			Hypertonic		
	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months
WT	0.084	0.002	0.530	0.240	< 0.001	0.058	0.127	0.006	0.475
$Dcn^{-/-}$	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.001	0.004	< 0.0001	0.008
t_{peak}	Hypotonic			Isotonic			Hypertonic		
	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months	3 days vs 2 weeks	3 days vs 3 months	2 weeks vs 3 months
WT	0.001	< 0.001	0.267	0.191	0.006	0.111	0.975	0.018	0.013
$Dcn^{-/-}$	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001

Table S6. Summary of statistical analysis outcomes of $[Ca^{2+}]_i$ parameters among osmolarity conditions for wild-type (WT) and decorin-null ($Dcn^{-/-}$) cartilage chondrocytes at all three tested ages

% R_{cell}	3 days			2 weeks			3 months		
	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper
WT	0.384	< 0.001	0.024	0.374	< 0.001	0.036	0.067	< 0.001	0.099
$Dcn^{-/-}$	0.342	< 0.001	0.022	0.030	< 0.0001	0.034	0.227	< 0.001	0.043
n_{peak}	3 days			2 weeks			3 months		
	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper
WT	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
$Dcn^{-/-}$	< 0.0001	< 0.0001	< 0.0001	0.008	< 0.0001	0.014	< 0.0001	< 0.0001	< 0.0001
t_{peak}	3 days			2 weeks			3 months		
	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper
WT	< 0.001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001
$Dcn^{-/-}$	< 0.001	< 0.0001	< 0.0001	0.057	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001

Table S7. Summary of averaged values and statistical analysis outcomes of $[Ca^{2+}]_i$ parameters between genotype and treatment conditions (untreated versus chondroitinase ABC, or C'ABC) for 2-week-old wild-type (WT) and decorin-null ($Dcn^{-/-}$) cartilage chondrocytes, shown as mean \pm 95% CI from the values averaged by cells

%R _{cell}	Hypotonic			Isotonic			Hypertonic		
	Untreated	C'ABC	p-value	Untreated	C'ABC	p-value	Untreated	C'ABC	p-value
WT	64 \pm 11	51 \pm 10	0.343	51 \pm 11	36 \pm 8	0.138	39 \pm 9	27 \pm 6	0.127
$Dcn^{-/-}$	50 \pm 10	41 \pm 9	0.517	39 \pm 9	31 \pm 8	0.373	25 \pm 6	20 \pm 5	0.260
p-value	0.305	0.266		0.327	0.378		0.040	0.341	
n _{peak}	Hypotonic			Isotonic			Hypertonic		
	Untreated	C'ABC	p-value	Untreated	C'ABC	p-value	Untreated	C'ABC	p-value
WT	5.06 \pm 0.22	3.87 \pm 0.19	0.019	4.02 \pm 0.24	2.51 \pm 0.18	0.001	2.07 \pm 0.24	1.57 \pm 0.18	0.075
$Dcn^{-/-}$	4.04 \pm 0.17	2.67 \pm 0.20	0.002	3.17 \pm 0.18	1.78 \pm 0.20	< 0.001	1.68 \pm 0.16	1.18 \pm 0.13	0.086
p-value	0.078	0.010		0.093	0.076		0.176	0.207	
t _{peak} (sec)	Hypotonic			Isotonic			Hypertonic		
	Untreated	C'ABC	p-value	Untreated	C'ABC	p-value	Untreated	C'ABC	p-value
WT	24.5 \pm 0.7	36.8 \pm 0.9	< 0.001	35.4 \pm 1.2	52.4 \pm 1.6	< 0.0001	50.9 \pm 1.5	65.4 \pm 1.3	< 0.0001
$Dcn^{-/-}$	35.8 \pm 0.8	46.2 \pm 1.6	0.002	44.3 \pm 1.3	65.9 \pm 1.4	< 0.0001	65.1 \pm 1.5	81.4 \pm 1.6	0.002
p-value	< 0.001	< 0.001		< 0.001	< 0.0001		< 0.001	< 0.0001	

Table S8. Summary of statistical analysis outcomes of $[Ca^{2+}]_i$ parameters among osmolarity conditions for 2-week-old wild-type (WT) and decorin-null ($Dcn^{-/-}$) cartilage chondrocytes at each genotype and each treatment condition (untreated versus chondroitinase ABC, or C'ABC)

%R _{cell}	Untreated			C'ABC		
	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper
WT	0.183	0.003	0.257	0.057	< 0.001	0.186
$Dcn^{-/-}$	0.208	< 0.001	0.025	0.240	< 0.001	0.048
n _{peak}	Untreated			C'ABC		
	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper
WT	0.051	< 0.0001	< 0.0001	< 0.001	< 0.0001	0.004
$Dcn^{-/-}$	0.066	< 0.0001	< 0.0001	0.014	< 0.0001	0.052
t _{peak}	Untreated			C'ABC		
	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper	Hypo vs Iso	Hypo vs Hyper	Iso vs Hyper
WT	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
$Dcn^{-/-}$	0.003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001