

1 **SUPPLEMENTARY INFORMATION**

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4 **TITLE: Vascular Endothelial Growth Factor in Cartilage Development and**

5 **Osteoarthritis**

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7 **AUTHORS:**

8 Masashi Nagao, M.D., Ph.D., John L Hamilton, M.S., Ranjan Kc, Ph.D. , Agnes D

9 Berendsen, Ph.D. , Xuchen Duan, Ph.D. , Chan Wook Cheong, D.M.D, D.M.Sc, Xin Li, M.D.,

10 Ph.D., Hee-Jeong Im, Ph.D., Bjorn R. Olsen, M.D., Ph.D.

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2 **Supplementary Fig. 1. Body weights of *Col2-CreER;Vegf^{fl/fl}* mice.**

3 Corn oil (Ctrl) or tamoxifen (TAM) were administered to *Col2-CreER;Vegf^{fl/fl}* mice at 2
4 weeks of age. Body weights in male (**A**) and female (**B**) mice from 3 to 8 weeks were
5 similar in mutants and controls. Lengths of male (**C**) and female (**D**) mice at 8 weeks were
6 similar in the mutant and control femur, but slightly different in the tibia ($n=4-5$). $*P < 0.05$.
7 Data represent mean $\pm$ SD. 2-way ANOVA with Sidak's multiple comparison tests (**A-D**)
8 were used.

10 **Supplementary Fig. 2. Chondrocytes in normoxic and hypoxic culture conditions.**

11 Levels of LDH release in culture supernatants of chondrocytes isolated from *Vegf^{fl/fl}* (A) and
12 *Col2-CreER; Vegf^{fl/fl}* (B) mice (n=3). Levels are increased in hypoxia (1%) compared to
13 normoxia (20%). No differences between tamoxifen-treated (reduced VEGF) or tamoxifen-
14 untreated (control) cultures are seen. **P*< 0.05; ***P*< 0.01. Data represent mean ±SD.
15 Unpaired Student's t test was used.

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18 **Supplementary Fig. 3. Targeting cells in *Flk1-Cre* and *Col2-Cre* mice.**

19 Sections of tibial epiphysis in *Flk1-Cre;TdTomato* (A) and *Col2-Cre;TdTomato* (B) mice
20 were stained with antibodies against endomucin (Emcn, green); red represents Tomato-
21 expressing cells; green represents Emcn; blue represents DAPI; scale bars, 250µm.

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23 **Supplementary Fig. 4. Body weights and limb lengths in *Flk1-Cre;Vegf^{fl/fl}* mice.**

24 Body weights in male (**A**) and female (**B**) *Flk1-Cre* and *Flk1-Cre; Vegf^{fl/fl}* (*CKO^{Flk1}*) mice
25 from 4 to 8 weeks old ($n=4-10$). Hind limb lengths in males (**C**) and females (**D**) at 4 and 8
26 weeks of age ($n=4-8$). * $P < 0.05$. Data represent mean $\pm$ SD. 2-way ANOVA with Sidak's
27 multiple comparison tests (**A-D**) were used.

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29 **Supplementary Fig. 5. Growth plate and articular cartilage in *Flk1-Cre;Vegf fl/fl* mice.**

30 Sections of tibial growth plate (**A**) and knee joint (**B**) in *Flk1-Cre* and *Flk1-Cre;Vegf^{fl/fl}*

31 (*CKO^{Flk1}*) mice at 9 weeks of age stained with Safranin O-fast green. Scale bars, 250µm.

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33 **Supplementary Fig. 6. Body weights before and 12 weeks after DMM surgery.**

34 Body weights of 8 and 20 weeks old male (A) and female (B) *Col2-CreER;Vegf^{fl/+}* mice and
35 male (C) and female (D) *Col2-CreER;Vegf^{fl/fl}* (*CKO^{Col2ER}*) mice administered corn oil (Ctrl)
36 or tamoxifen (TAM) (*n*=3-5). Body weights of 8 and 20 weeks old male (E) and female (F)
37 *Flk-Cre* or *Flk1-Cre;Vegf^{fl/fl}* (*CKO^{Flk1}*) mice, *n*=3-5. **P*< 0.05. Data represent mean ±SD. 2-
38 way ANOVA with Sidak's multiple comparison tests were used (A-F).

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40 **Supplementary Table 1. Number of pups from different genotype combinations**

Male	Female	Total N of pups	N of <i>CKO</i> ^{<i>Col2</i>} mice (expected)
<i>Vegf</i> ^{<i>fl/fl</i>}	<i>Col2-Cre;Vegf</i> ^{<i>fl/+</i>}	69	14 (17)
<i>Vegf</i> ^{<i>fl/+</i>}	<i>Col2-Cre;Vegf</i> ^{<i>fl/+</i>}	23	3 (2.9)
<i>Col2-Cre;veg</i> ^{<i>fl/+</i>}	<i>Vegf</i> ^{<i>fl/fl</i>}	13	1 (3.3)
<i>Col2-Cre;veg</i> ^{<i>fl/+</i>}	<i>Vegf</i> ^{<i>fl/+</i>}	121	0 (15.1)

42 **Supplementary Table 2. Bone parameters in *Col2-Cre* and *Col2-Cre;Vegf^{fl/+}* mice.**

	Male			Female		
	<i>Col2-Cre</i> (95% CI)	<i>Col2-Cre;Vegf^{fl/+}</i> (95%CI)	<i>P</i> value	<i>Col2-Cre</i> (95%CI)	<i>Col2-Cre;Vegf^{fl/+}</i> (95%CI)	<i>P</i> value
BV/TV	0.14 (0.11-0.17)	0.09 (0.06-0.12)	*0.03	0.07 (0.06-0.09)	0.055 (0.035-0.074)	0.14
Tb.N	4.63 (4.31-4.95)	4.08 (2.64-5.53)	0.10	3.00 (2.49-3.52)	2.40 (1.89-2.91)	0.14
Tb.Th	0.043 (0.037-0.050)	0.040 (0.032-0.049)	0.52	0.038 (0.034-0.041)	0.040 (0.038-0.042)	0.32
Tb.Sp	0.21 (0.19-0.23)	0.25 (0.16-0.35)	0.07	0.35 (0.29-0.42)	0.43 (0.34-0.51)	0.16
C. Th	0.21 (0.20-0.22)	0.22 (0.19-0.24)	0.46	0.19 (0.18-0.21)	0.20 (0.18-0.21)	0.77

43 Quantification of Trabecular Bone Volume /Tissue Volume (BV/TV), Trabecular Number
44 (Tb.N), Trabecular Spacing (Tb.Sp), Trabecular Thickness (Tb.Th) and Cortical Thickness
45 (C.Th) in tibia of *Col2-Cre* and *Col2-Cre;veg^{fl/+}* mice (*n*=4). 95% confidence interval,
46 95%CI, **P*< 0.05. Unpaired student's t-test was used.

47 **Supplementary Table 3. Association between genotype and subchondral bone**
 48 **thickening 12 weeks after DMM surgery**

	Coefficient (95% CI)	<i>P</i> value
<i>Col2-Cre;Vegf^{fl/+}</i>	1.33 (-0.35 to 3.01)	0.09
<i>Col2-Cre</i>	1.45 (1.10 to 1.82)	<0.01*
<i>CKO^{Col2}</i>	1.51 (0.45 to 2.56)	0.01*
<i>CKO^{Col2} with no tamoxifen</i>	1.15 (0.22 to 2.07)	0.02*
<i>CKO^{Flk1}</i>	1.00 (0.03 to 1.97)	0.04*
<i>Flk1-Cre</i>	1.00 (0.50 to 1.49)	<0.01*

49 **P* < 0.05. Univariate regression analyses were used. Dependent variables were the OARSI
 50 grade of subchondral bone thickening and independent variables were genotypes.

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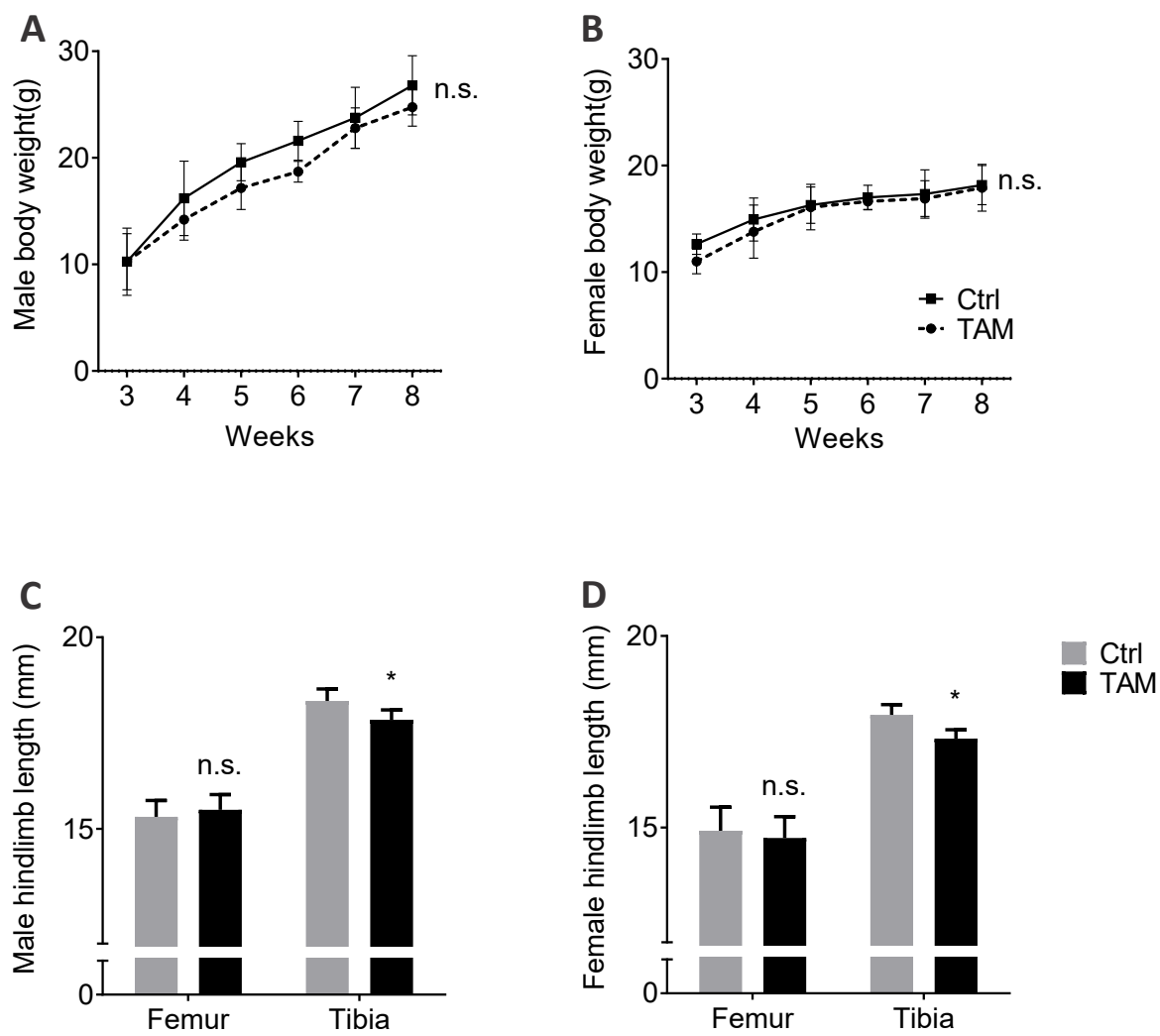
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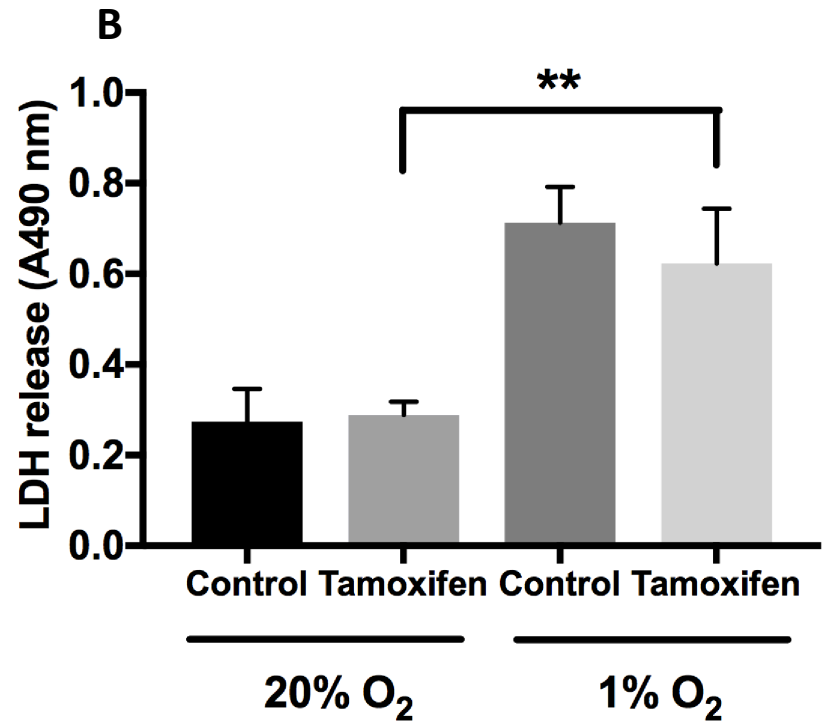
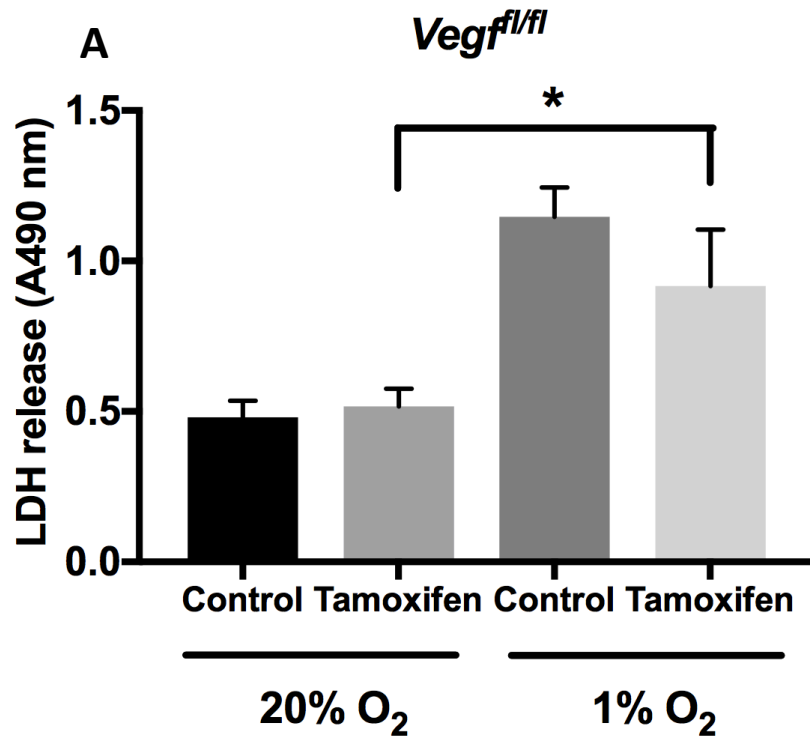
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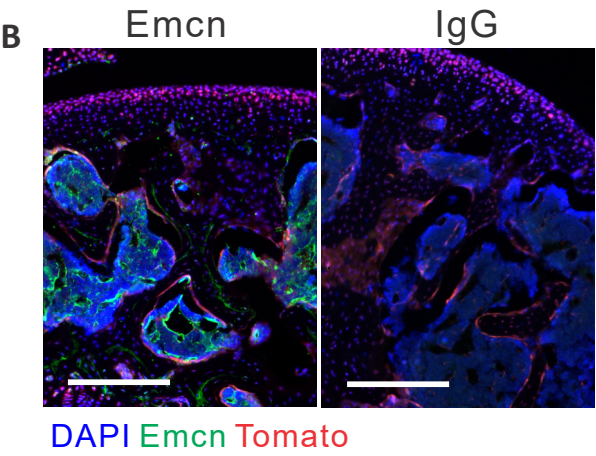
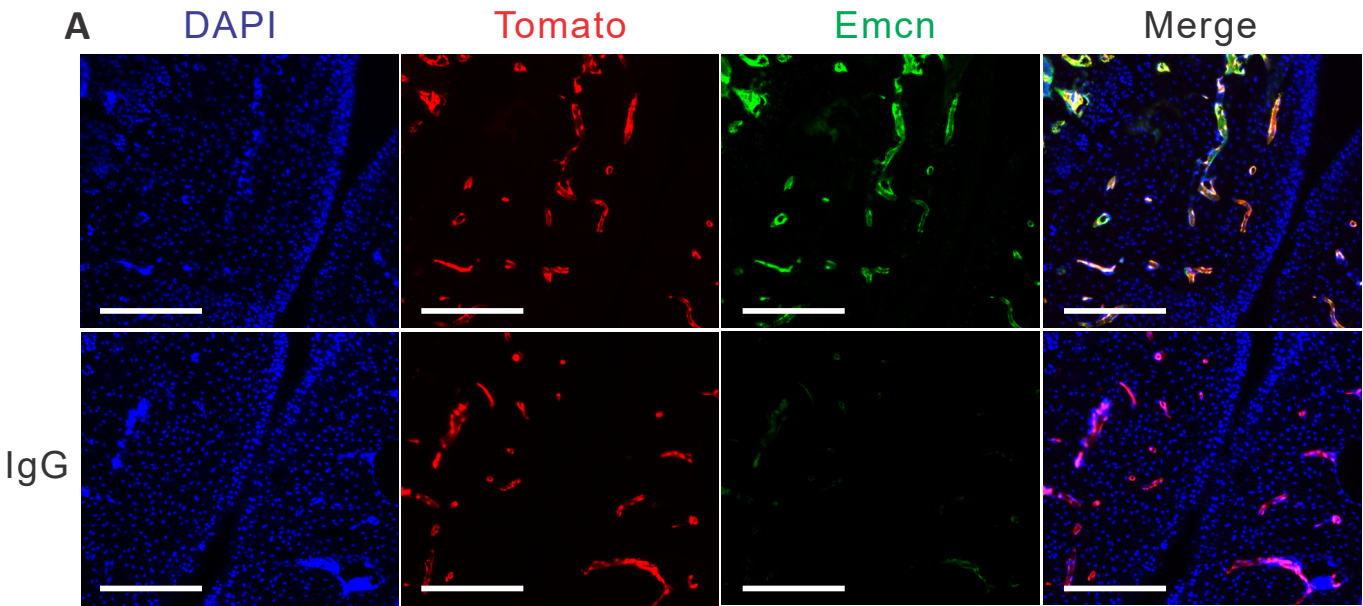
Supplementary figure 1



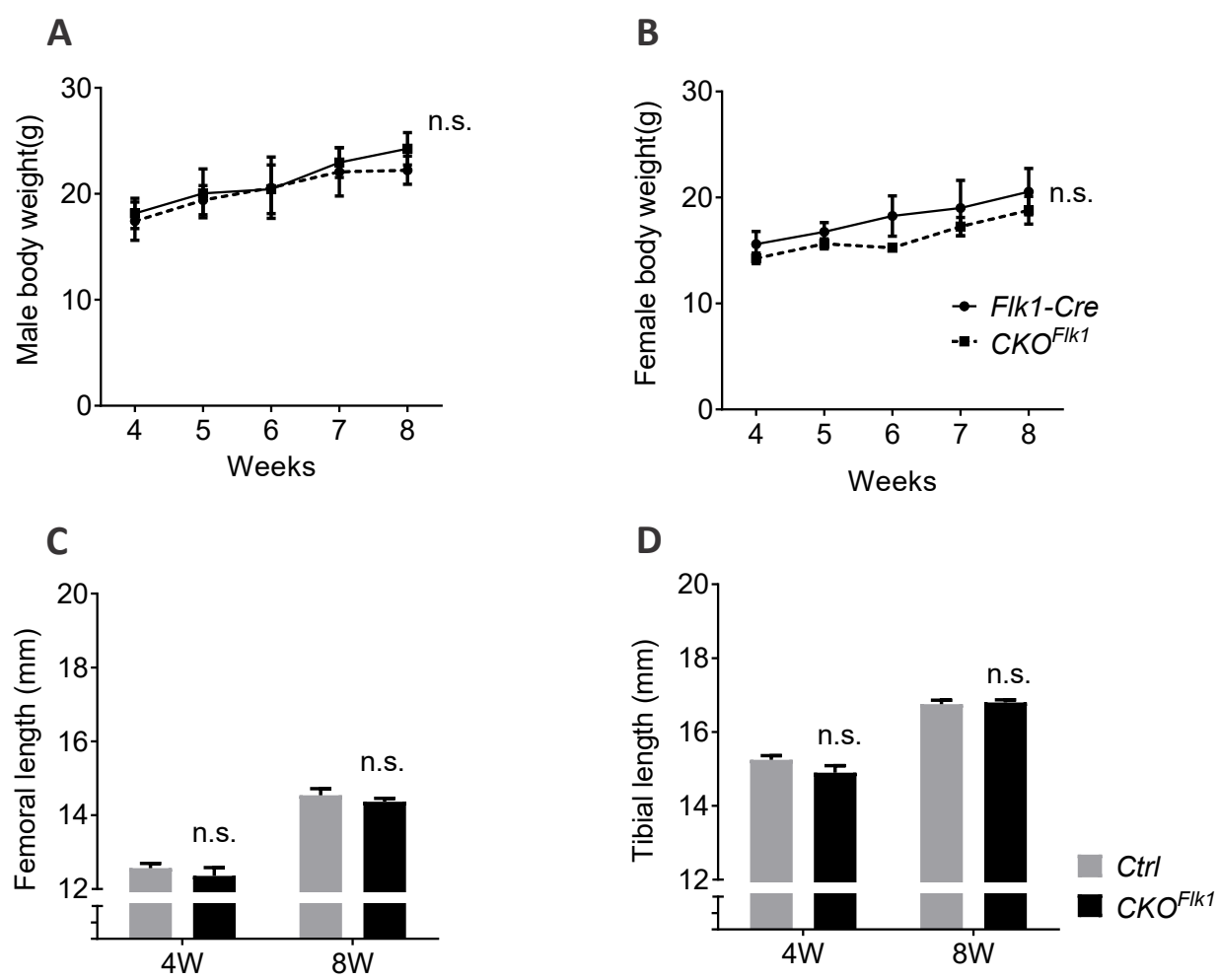
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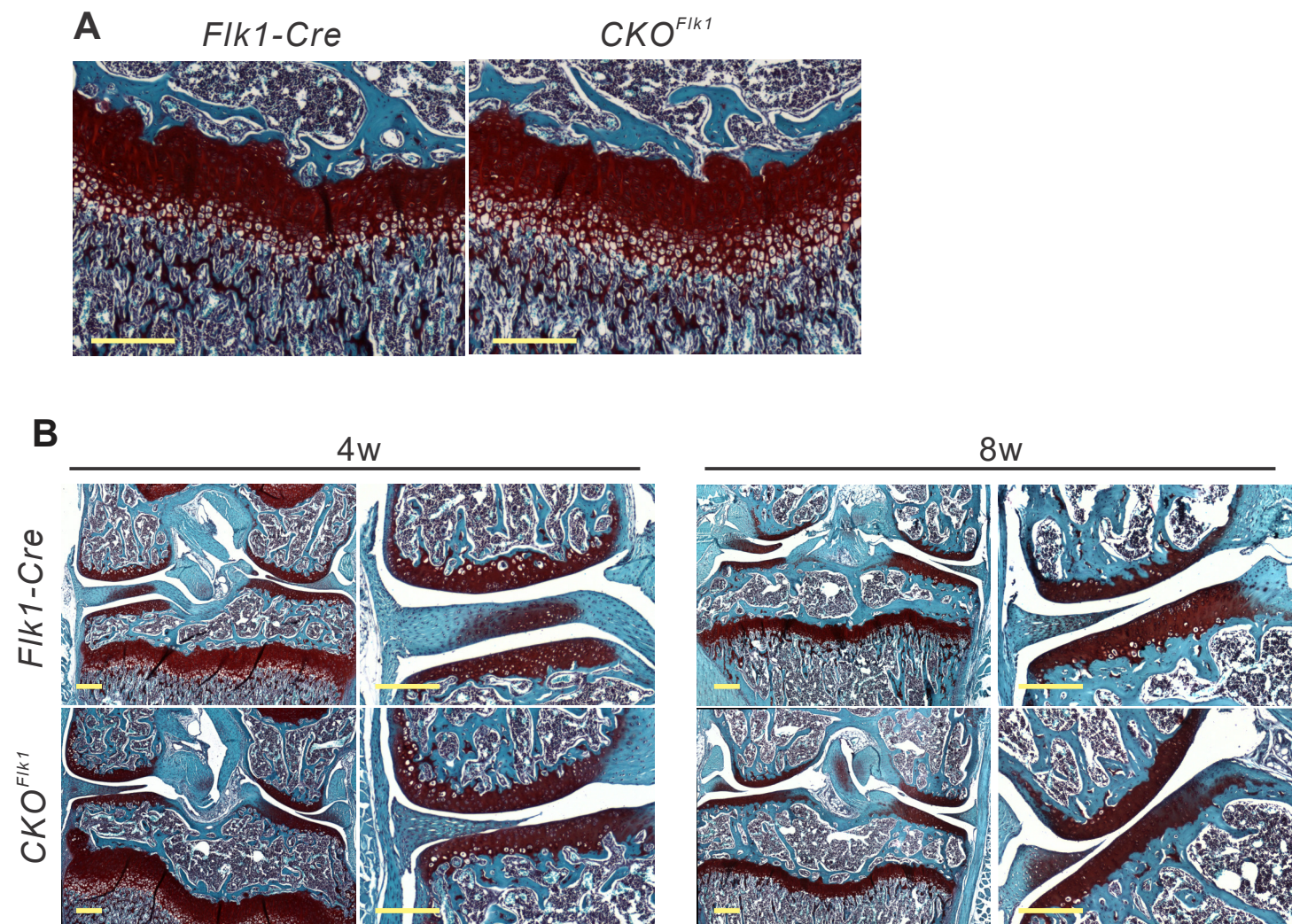
Supplementary figure 3



Supplementary figure 4



Supplementary figure 5



Supplementary figure 6

