Supplementary Information

Excessive TGFβ signaling is a common mechanism in Osteogenesis Imperfecta

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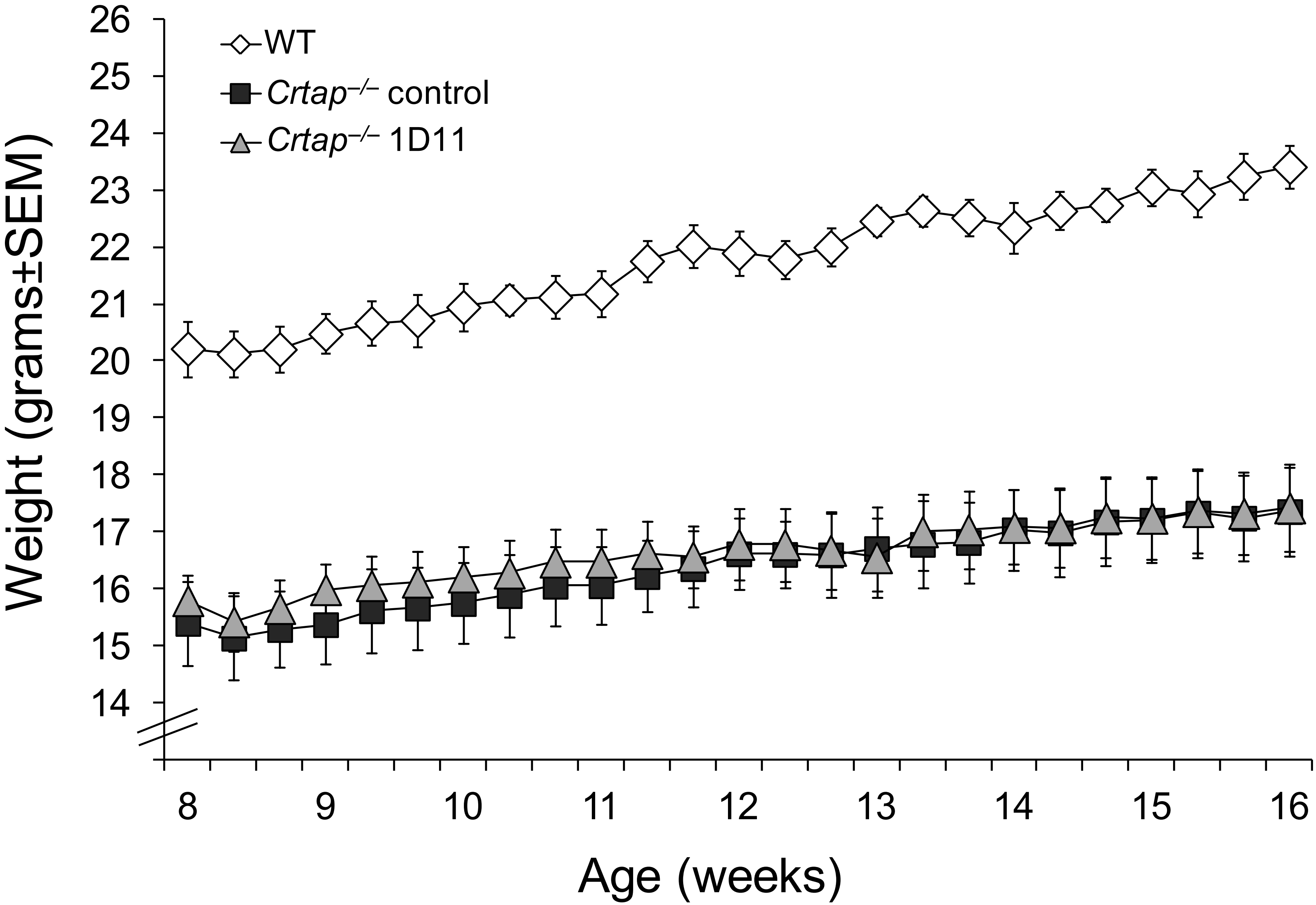
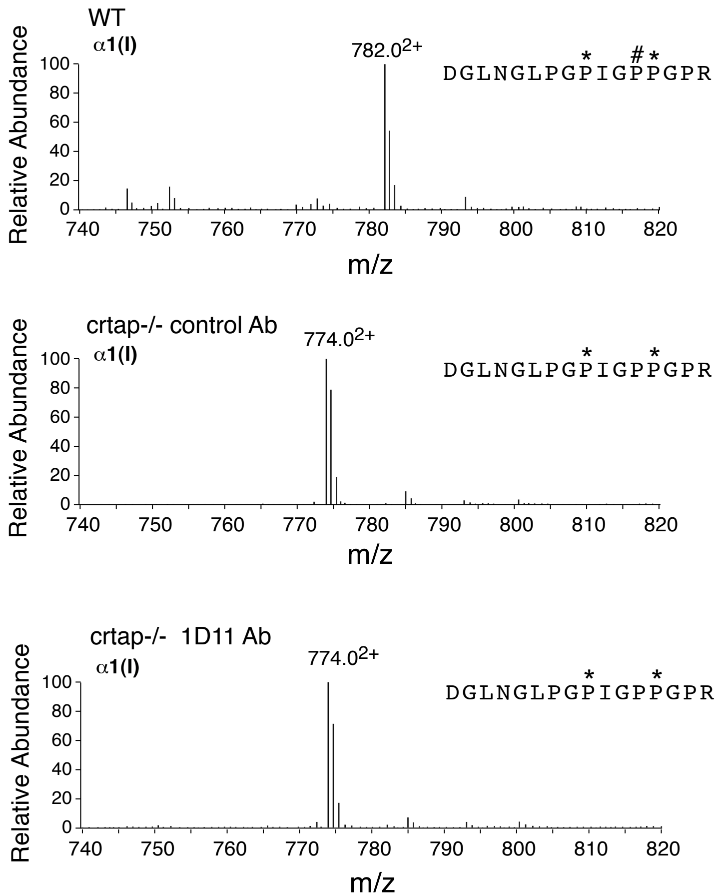
Supplementary Figure 1



Supplementary Figure 1. Weight curves showing weight of WT, control *Crtap* $^{-/-}$ mice and 1D11-treated *Crtap* $^{-/-}$ mice during the study period. N=8 per group.

Supplementary Figure 2

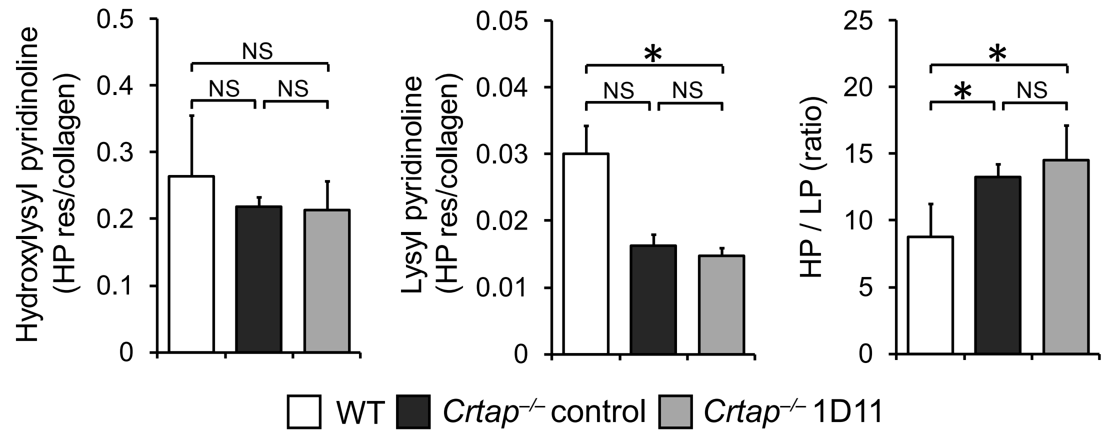
a



b

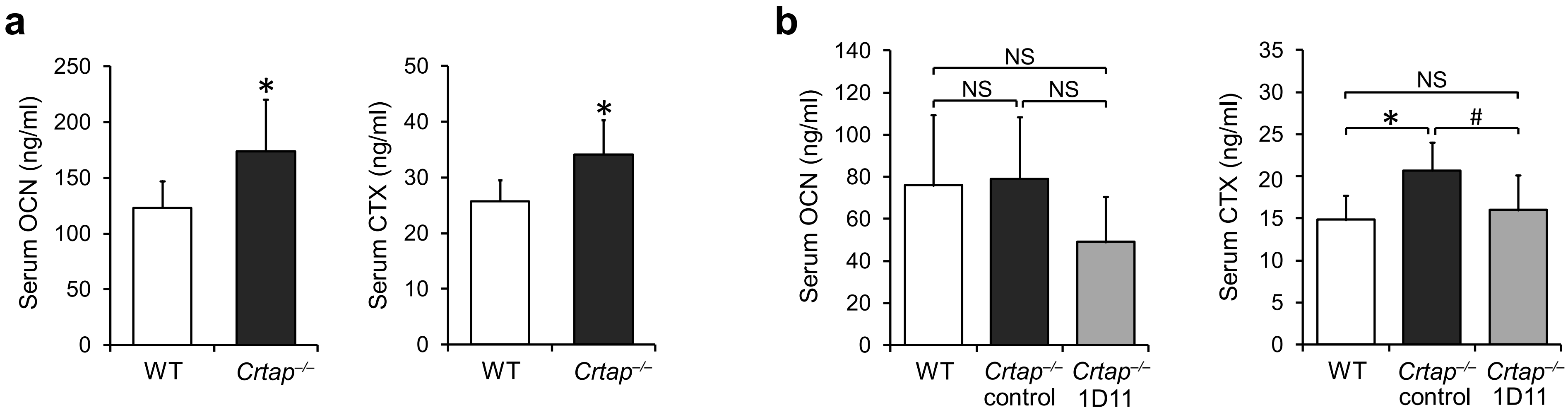
3Hyp	WT	*Crtap*$^{-/-}$ control	*Crtap*$^{-/-}$ 1D11
P986 alpha 1(I)	96.6% (2.1)	5.8% (3.3)	2.0% (1.2)

c



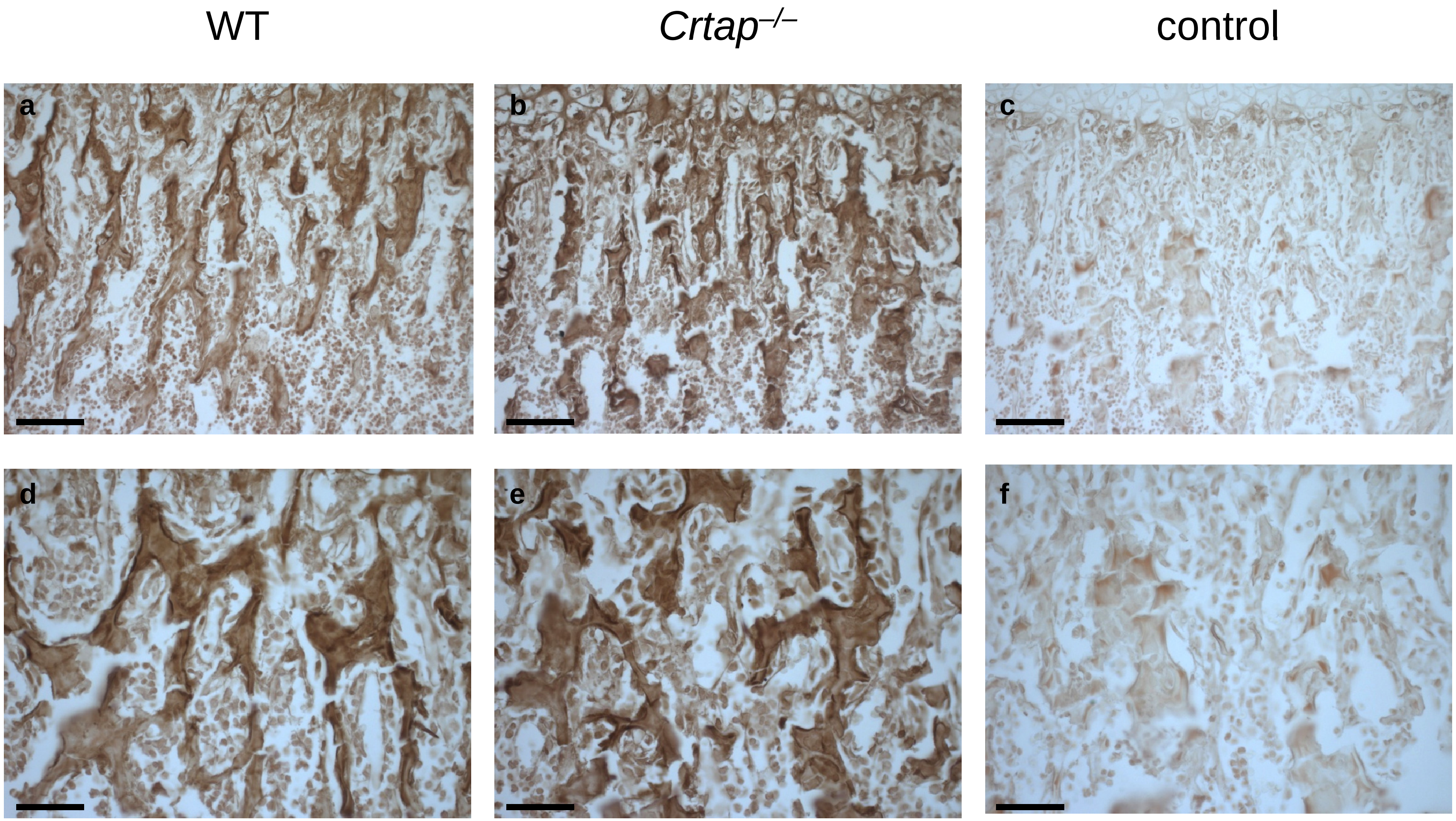
Supplementary Figure 2. No effect of TGFβ inhibition on the abnormal type I collagen post-translational modification in Crtap –/– mice. (a) Tandem mass spectra of extracted type I collagen from tibia of WT, control Crtap –/–, and 1D11- treated Crtap –/– mice (16 week old mice, after treatment for 8 weeks). (b) Status of collagen residue Pro986 alpha 1(I) 3-hydroxylation in bone samples of bone samples of WT, control Crtap –/– and 1D11- treated Crtap –/– mice assessed by tandem mass spectra analyses. Mean of percentage of 3-hydroxylated residues (±SD) is shown, n=5 per group. (c). Hydroxylysyl pyridinoline (HP), lysyl pyridinoline crosslinks (LP) levels and HP/LP ratio of bone type I collagen of WT, control Crtap –/– and 1D11- treated Crtap –/– mice. Results are given as means±SDs, n=4 mice per group, *P<0.05 for Crtap –/– vs. WT. NS, not significant.

Supplementary Figure 3



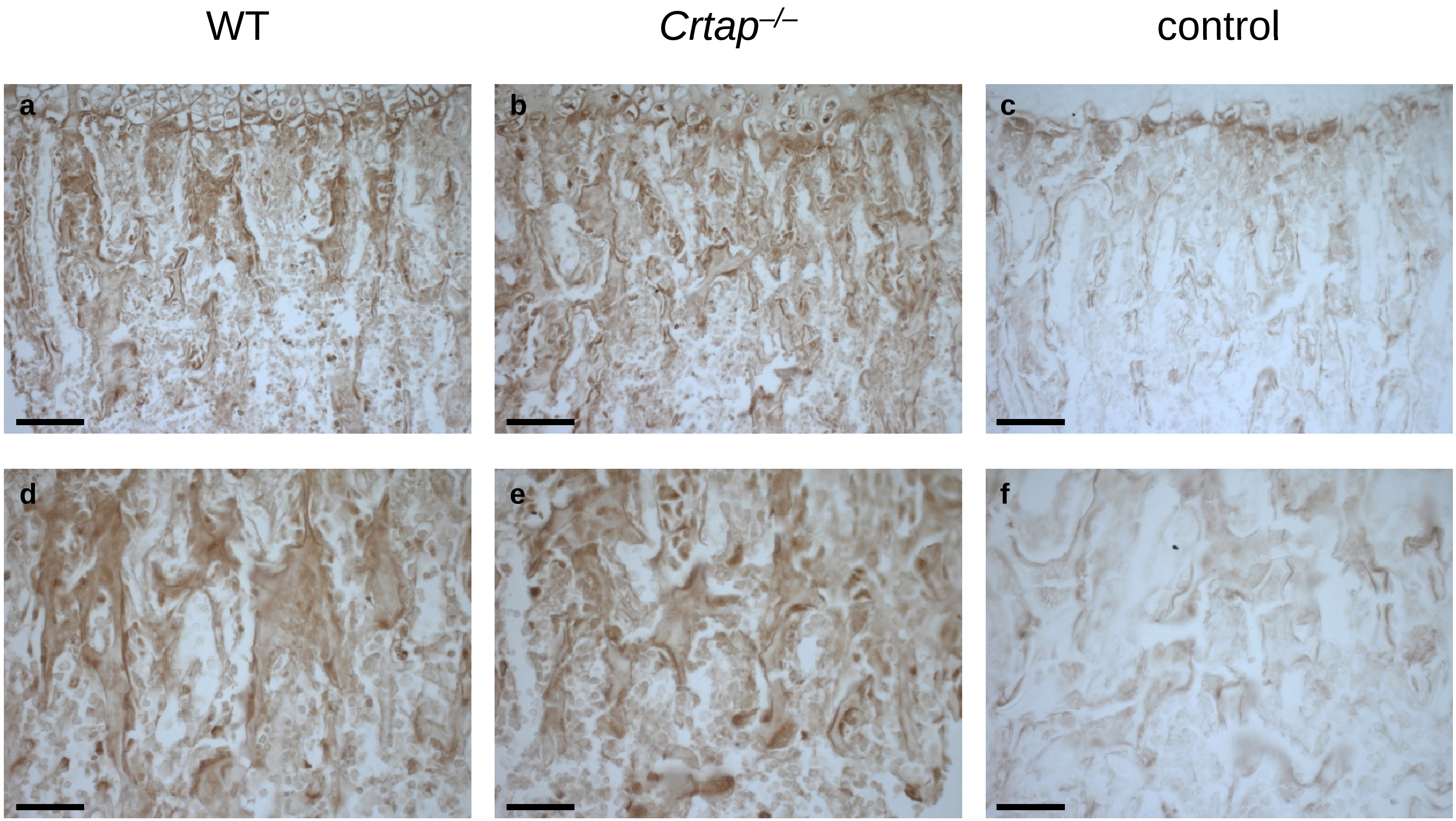
Supplementary Figure 3. Serum bone-turnover markers osteocalcin (OCN) and C-terminal cross-linked telopeptide of bone collagen (CTX) at start (a. 8 weeks of age) and end of the treatment study (b. 16 weeks of age). (a) Results are given as means±SDs, n=8 for WT, n=14 for Crtap –/– mice. (b) Results are given as means±SDs, n=8 for WT, n=7 per Crtap –/– group. *P<0.05 for Crtap –/– vs. WT, #P<0.05 for Crtap –/– 1D11 vs. Crtap –/– control. NS, not significant.

Supplementary Figure 4



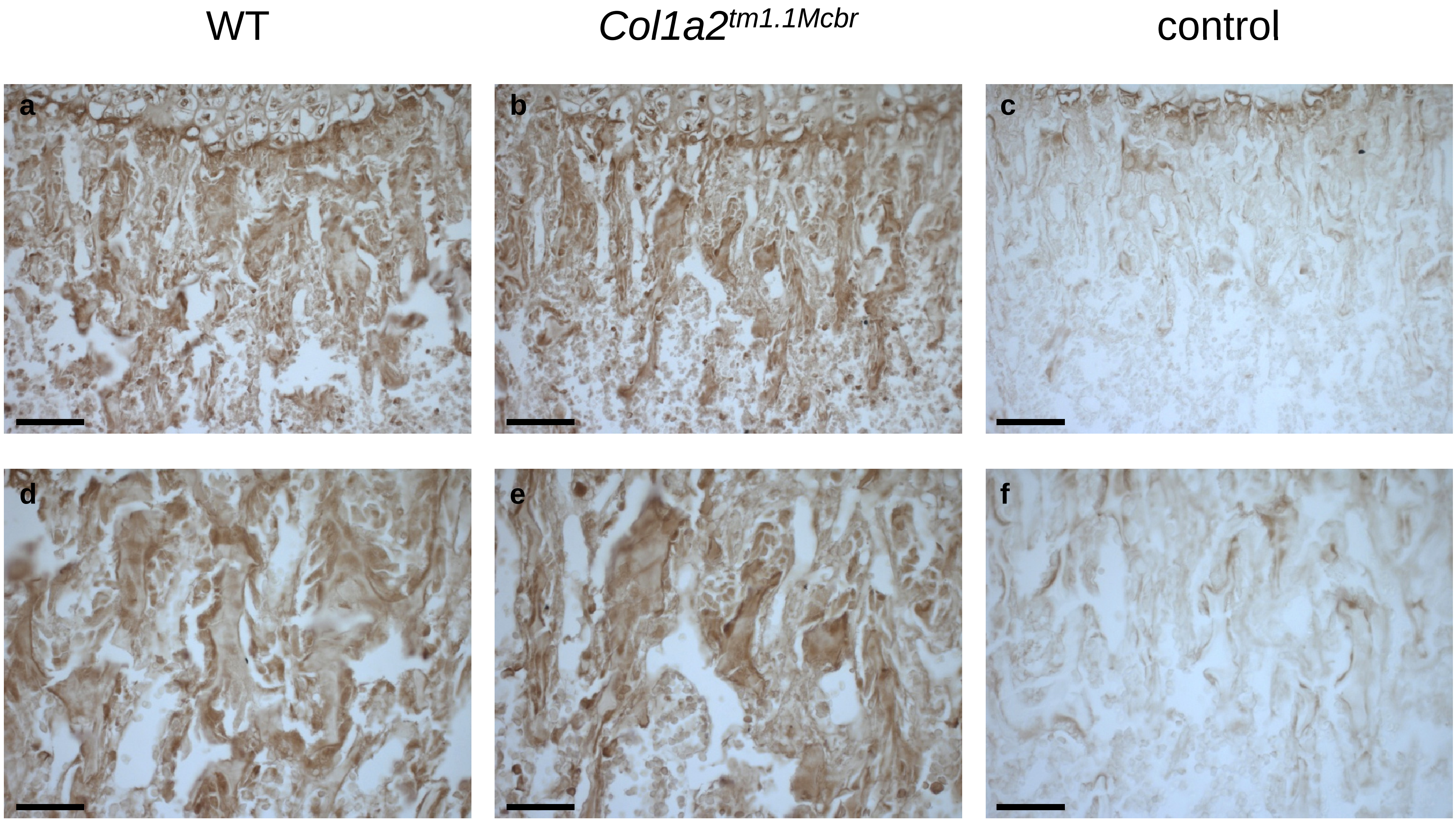
Supplementary Figure 4. Immunostaining for decorin in the distal femur metaphysis of WT and *Crtap* $^{-/-}$ mice is shown in 20X (**a**–**c**) and 40X magnification (**d**–**f**). Control femurs were incubated in secondary antibody only. (n=3 per genotype; scale bars=100µm (**a**–**c**), 50µm (**d**–**f**)).

Supplementary Figure 5



Supplementary Figure 5. Immunostaining for TGFβ1 in the distal femur metaphysis of WT and *Crtap* $^{-/-}$ mice is shown in 20X (**a**–**c**) and 40X magnification (**d**–**f**). Control femurs were incubated in secondary antibody only (n=3 per genotype; scale bars=100μm (**a**–**c**), 50μm (**d**–**f**)).

Supplementary Figure 6



Supplementary Figure 6. Immunostaining for TGFβ1 in the distal femur metaphysis of WT and *Col1a2*$^{tm1.1Mcbr}$ mice is shown in 20X (**a**–**c**) and 40X magnification (**d**–**f**). Control femurs were incubated in secondary antibody only. (n=3 per genotype; scale bars=100µm (**a**–**c**), 50µm (**d**–**f**)).

Supplementary Table 1

	BV/TV (%)	Tb.N (1/mm)	Tb.Th (µm)	Tb.Sp (mm)	BMD BV (mg HA/ccm)
Wild type	**31.173**	**4.845**	**63.988**	**0.145**	**703.189**
SD	6.543	0.583	9.482	0.034	19.014
***Crtap*$^{-/-}$ control**	**8.354**	**2.130**	**38.888**	**0.450**	**677.416**
SD	2.045	0.409	2.931	0.118	16.138
***Crtap*$^{-/-}$ 1D11**	**27.953**	**4.290**	**64.650**	**0.171**	**746.862**
SD	5.645	0.435	7.407	0.030	26.564
ANOVA *P* value	**<0.001**	**<0.001**	**<0.001^{+}**	**<0.001^{+}**	**<0.001**
Pairwise *P* values					
Wild type vs. *Crtap*$^{-/-}$ control	<0.001	<0.001	<0.05	<0.05	0.023
Wild type vs. *Crtap*$^{-/-}$ 1D11	n.s.	0.031	n.s.	n.s.	<0.001
Crtap$^{-/-}$ control vs. *Crtap*$^{-/-}$ 1D11	<0.001	<0.001	<0.05	<0.05	<0.001

Supplementary Table 1. MicroCT analyses of vertebral body L4 of WT, control *Crtap*$^{-/-}$ and 1D11 treated *Crtap*$^{-/-}$ mice (16 week old mice, after treatment for 8 weeks). Means±SDs are shown for bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone mineral density of bone volume (BMD BV); n=8 per group, + indicates Kruskal-Wallis one-way ANOVA on ranks where the equal variance test failed. n.s.=not statistically significant.

Supplementary Table 2

	BV/TV (%)	Tb.N (1/mm)	Tb.Th (µm)	Tb.Sp (mm)	BMD BV (mg HA/ccm)
Wild type	**10.698**	**2.808**	**37.413**	**0.341**	**733.864**
SD	3.558	0.684	5.071	0.108	25.997
***Crtap*$^{-/-}$ control**	**2.873**	**0.861**	**32.863**	**1.209**	**727.151**
SD	1.082	0.237	3.428	0.345	28.765
***Crtap*$^{-/-}$ 1D11**	**12.343**	**2.953**	**40.663**	**0.323**	**756.539**
SD	4.523	0.767	5.842	0.121	36.913
ANOVA *P* value	**<0.001^{+}**	**<0.001^{+}**	**0.015**	**<0.001**	**0.162**
Pairwise *P* values					
Wild type vs. *Crtap*$^{-/-}$ control	<0.05	<0.05	n.s.	<0.001	
Wild type vs. *Crtap*$^{-/-}$ 1D11	n.s.	n.s.	n.s.	n.s.	
Crtap$^{-/-}$ control vs. *Crtap*$^{-/-}$ 1D11	<0.05	<0.05	0,004	<0.001	

Supplementary Table 2. MicroCT analyses of trabecular bone in proximal femurs for WT, control *Crtap*$^{-/-}$, and 1D11- treated *Crtap*$^{-/-}$ mice (16 week old mice, after treatment for 8 weeks). Means±SDs are shown for bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and bone mineral density of bone volume (BMD BV); n=8 per group. + indicates Kruskal-Wallis one-way ANOVA on ranks where the equal variance test failed. n.s.=not statistically significant.

Supplementary Table 3

	Cortical thickness (mm)	BMD BV (mg HA/ccm)	Diameter a.p. (mm)	CSA (mm^2)	CSMI m.l. (mm^4)	CSMI a.p. (mm^4)
Wild type	**0.242**	**1084.726**	**1.239**	**0.905**	**0.134**	**0.221**
SD	0.014	28.375	0.063	0.079	0.023	0.042
***Crtap*$^{-/-}$ control**	**0.203**	**1084.885**	**1.142**	**0.731**	**0.101**	**0.162**
SD	0.020	34.256	0.065	0.082	0.021	0.034
***Crtap*$^{-/-}$ 1D11**	**0.221**	**1096.127**	**1.186**	**0.808**	**0.111**	**0.186**
SD	0.026	39.754	0.080	0.118	0.028	0.038
ANOVA *P* value	**0.003**	**0.753**	**0.039**	**0.005**	**0.032**	**0.021**
Pairwise *P* values						
Wild type vs. *Crtap*$^{-/-}$ control	<0.001		0.012	0.001	0.011	0.006
Wild type vs. *Crtap*$^{-/-}$ 1D11	n.s.		n.s.	n.s.	n.s.	n.s.
Crtap$^{-/-}$ control vs. *Crtap*$^{-/-}$ 1D11	n.s.		n.s.	n.s.	n.s.	n.s.

Supplementary Table 3. MicroCT analysis of cortical bone at the femur midshaft for WT, control *Crtap*$^{-/-}$, and 1D11-treated *Crtap*$^{-/-}$ mice (16 week old mice, after treatment for 8 weeks). Means±SDs are shown for cortical thickness, bone mineral density of bone volume (BMD BV), anterior-posterior (a.p.) diameter, cross-sectional area (CSA), and cross-sectional moments of inertia (CSMI) for medio-lateral (m.l.) and anterior-posterior (a.p.) axis; n=8 per group. n.s.=not statistically significant.

Supplementary Table 4

	Maximum load (N)	Stiffness (N/mm)	Energy to failure (N*mm)	Ultimate strength (MPa)	Toughness to failure (MPa)	Elastic modulus (GPa)	Total displacement (mm)	Elastic displacement (mm)	Post-yield displacement (mm)
Wild type	**22.831**	**230.578**	**7.846**	**154.704**	**11.912**	**6.960**	**0.444**	**0.068**	**0.376**
SD	2.860	37.053	3.985	7.478	5.695	0.731	0.207	0.014	0.203
***Crtap*$^{-/-}$ control**	**12.943**	**151.689**	**1.228**	**114.496**	**2.208**	**6.663**	**0.127**	**0.079**	**0.048**
SD	2.402	27.384	0.913	18.240	1.677	1.154	0.057	0.006	0.058
***Crtap*$^{-/-}$ 1D11**	**18.818**	**200.804**	**1.991**	**145.633**	**3.408**	**7.248**	**0.156**	**0.073**	**0.083**
SD	2.337	15.644	0.834	19.074	1.851	0.271	0.055	0.013	0.055
ANOVA *P* value	**<0.001**	**0.009**	**0.009**	**0.004**	**0.009**	**0.658**	**0.015**	**0.360**	**0.012**
Pairwise *P* values									
Wild type vs. *Crtap*$^{-/-}$ control	<0.001	0.003	0.005	0.001	0.005		0.009		0.007
Wild type vs. *Crtap*$^{-/-}$ 1D11	n.s.	n.s.	0.017	n.s.	0.017		0.023		0.019
Crtap$^{-/-}$ control vs. *Crtap*$^{-/-}$ 1D11	0.015	n.s.	n.s.	0.016	n.s.		n.s.		n.s.

Supplementary Table 4. Results of biomechanical testing of femurs from WT, control *Crtap*$^{-/-}$ and 1D11 treated *Crtap*$^{-/-}$ mice by 3-point bending (16 week old mice, after treatment for 8 weeks). N=6 for WT, n=4 for control *Crtap*$^{-/-}$ and n=3 for 1D11 treated *Crtap*$^{-/-}$ mice. Results are shown as means±SDs. n.s.=not statistically significant.

Supplementary Table 5

	BV/TV (%)	Tb.N (1/mm)	Tb.Th (µm)	Tb.Sp (µm)	N.Oc/BS (1/mm)	Oc.S/BS (%)	N.Ob/BS (1/mm)	Ob.S/BS (%)	N.Ot/B.Ar (1/mm2)
Wild type	**11.956**	**4.003**	**29.554**	**226.736**	**3.226**	**15.051**	**18.142**	**20.827**	**549.002**
SD	3.067	0.656	3.536	50.077	0.812	3.089	2.368	2.754	83.665
***Crtap*$^{-/-}$ control**	**3.817**	**1.909**	**19.502**	**587.008**	**4.768**	**19.885**	**24.487**	**26.888**	**735.561**
SD	1.656	0.692	2.560	289.835	0.914	3.407	5.421	6.983	72.617
***Crtap*$^{-/-}$ 1D11**	**10.203**	**3.411**	**30.182**	**277.886**	**1.772**	**6.829**	**8.638**	**9.748**	**565.044**
SD	3.097	0.878	6.982	69.118	0.673	2.235	2.855	4.718	51.014
ANOVA *P* value	**<0.001**	**<0.001**	**0.002**	**0.003^{+}**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Pairwise *P* values									
Wild type vs. *Crtap*$^{-/-}$ control	<0.001	<0.001	0.002	<0.05	0.005	0.012	0.011	n.s.	<0.001
Wild type vs. *Crtap*$^{-/-}$ 1D11	n.s.	n.s.	n.s.	n.s.	0.007	<0.001	<0.001	0.002	n.s.
Crtap$^{-/-}$ control vs. *Crtap*$^{-/-}$ 1D11	<0.001	0.003	0.001	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001

Supplementary Table 5. Histomorphometry analyses of L4 vertebral bodies of WT, control *Crtap* $^{-/-}$, and 1D11- treated *Crtap* $^{-/-}$ mice (16 week old mice, after treatment for 8 weeks). Means±SDs are shown for bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), number of osteoclasts/bone surface (N.Oc/BS), osteoclast surface/bone surface (Oc.S/BS), number of osteoblasts/bone surface (N.Ob/BS), osteoblast surface/bone surface (Oc.S/BS), and number of osteocytes/bone area (N.Ot/B.Ar); n=6 per group. + indicates Kruskal-Wallis one way ANOVA on ranks where equal variance test failed. n.s.=not statistically significant.

Supplementary Table 6

	BV/TV (%)	Tb.N (1/mm)	Tb.Th (µm)	Tb.Sp (mm)	BMD BV (mg HA/ccm)
Wild type	**24.708**	**3.939**	**62.533**	**0.193**	**716.669**
SD	3.282	0.330	3.497	0.025	7.626
Col1α2 $^{tm1.1Mcbr}$ **control**	**10.608**	**2.071**	**51.067**	**0.435**	**731.436**
SD	1.487	0.195	2.999	0.047	11.792
Col1α2 $^{tm1.1Mcbr}$ **1D11**	**29.715**	**4.244**	**69.983**	**0.166**	**754.115**
SD	1.984	0.199	2.329	0.013	7.844
ANOVA *P* value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Pairwise *P* values					
Wild type vs. *Col1α2* $^{tm1.1Mcbr}$ control	<0.001	<0.001	<0.001	<0.001	0.015
Wild type vs. *Col1α2* $^{tm1.1Mcbr}$ 1D11	0.002	n.s.	<0.001	n.s.	<0.001
Col1α2 $^{tm1.1Mcbr}$ control vs. *Col1α2* $^{tm1.1Mcbr}$ 1D11	<0.001	<0.001	<0.001	<0.001	<0.001

Supplementary Table 6. MicroCT analyses of vertebral body L4 of WT, control *Col1a2* $^{tm1.1Mcbr}$ and 1D11 treated *Col1a2* $^{tm1.1Mcbr}$ mice (16 week old mice, after treatment for 8 weeks). Means±SDs are shown for bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone mineral density of bone volume (BMD BV); n=6 per group, n.s.=not statistically significant.