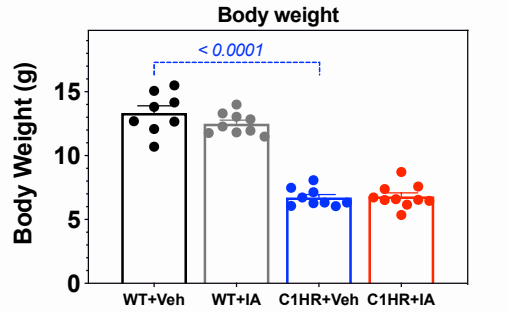
A



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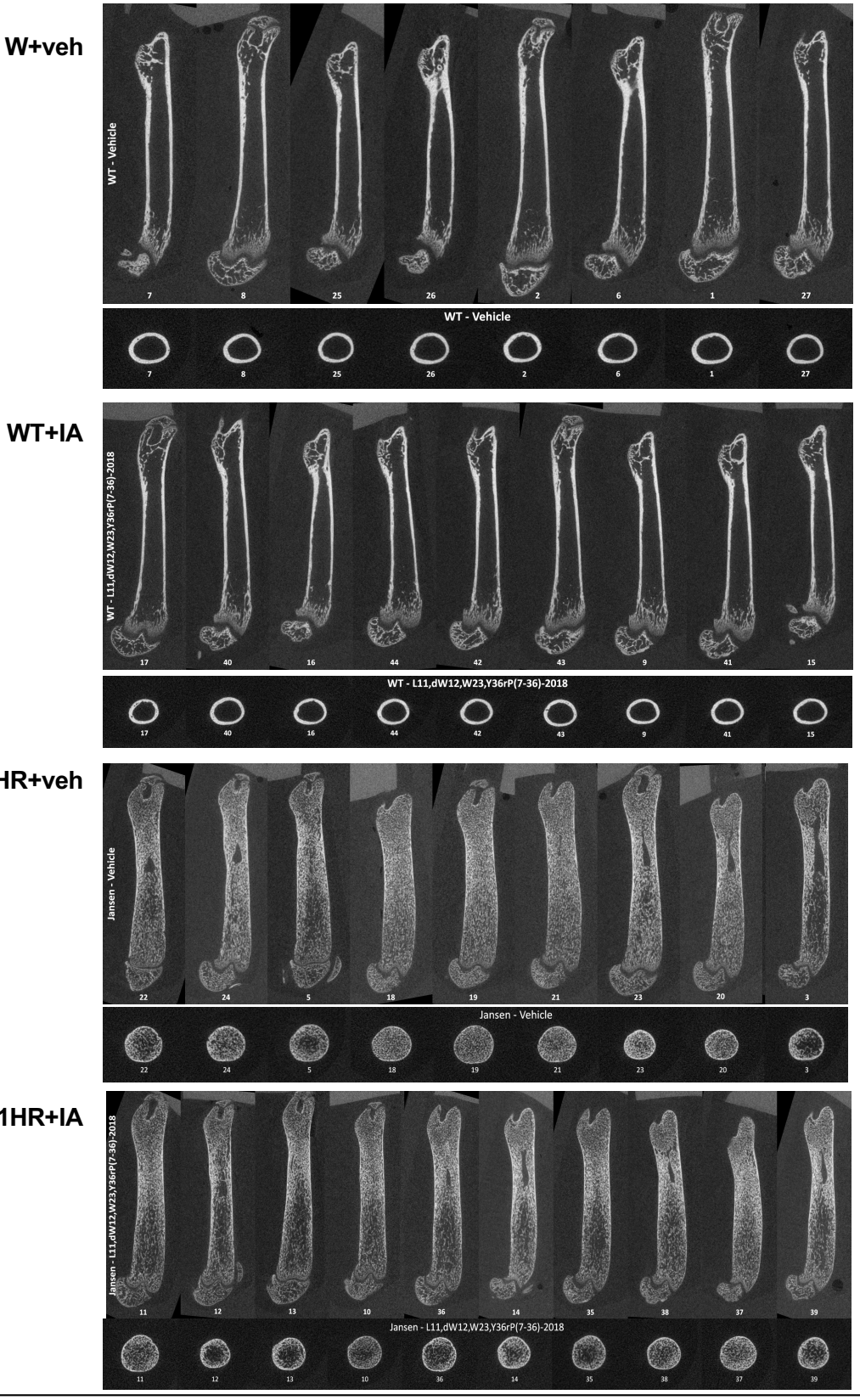


Fig. S-1 Body weights and Micro-CT Images of femurs. A) Body weights at the study end-point of wild-type (WT) or Col1-PTHR1-H223R (C1HR) mice injected twice-daily with either vehicle (veh) or inverse agonist (IA) for 17 days. Data are means±SD; P value derived by ANOVA with Tukey's ad hoc comparison. **B**) Micro-CT images: sagittal views of the total femur and corresponding transverse views of the mid-shaft of femurs obtained from wild-type (WT) or Col1-PTHR1-H223R (C1HR) mice injected twice-daily with vehicle (veh) or inverse agonist (IA) for 17 days

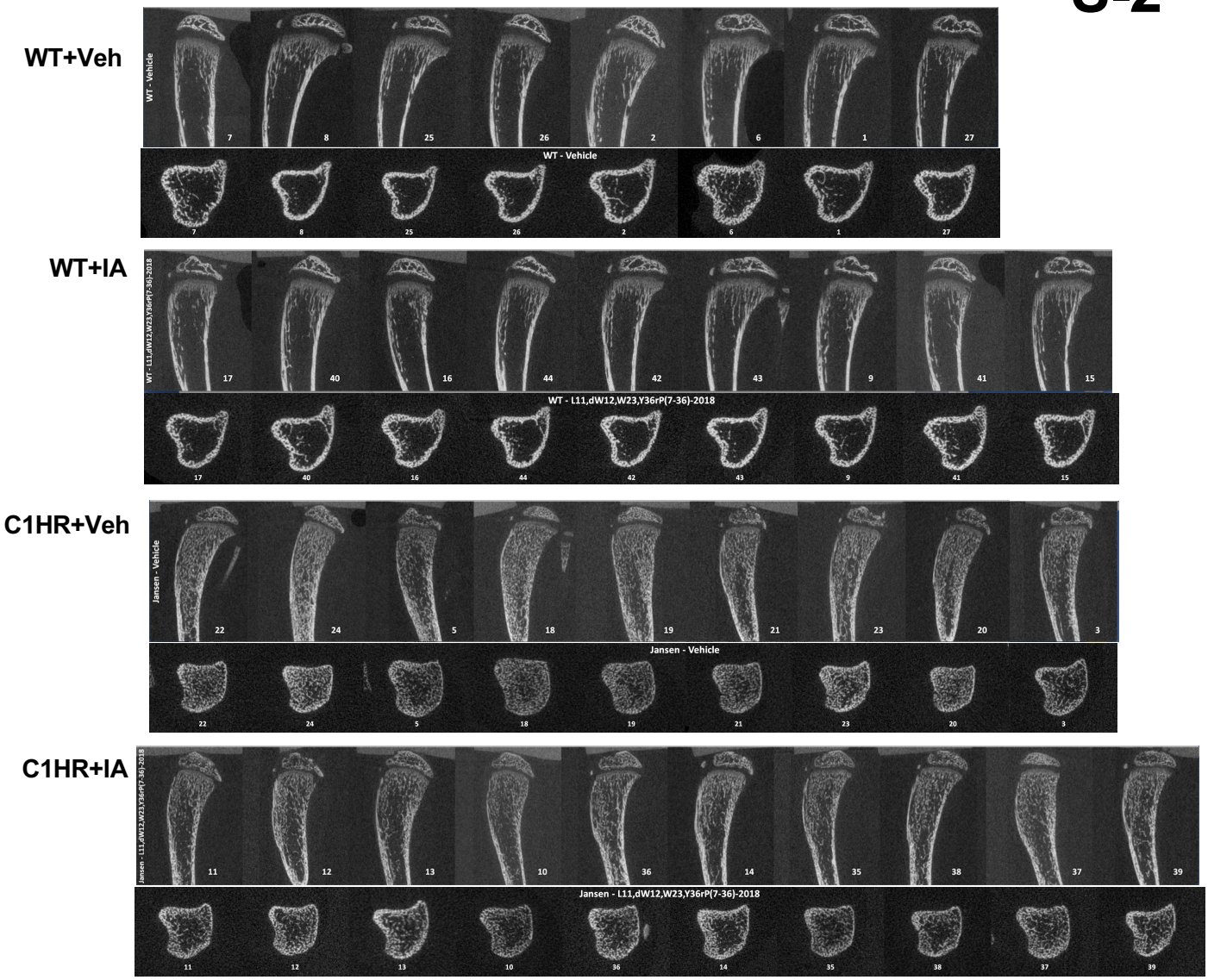
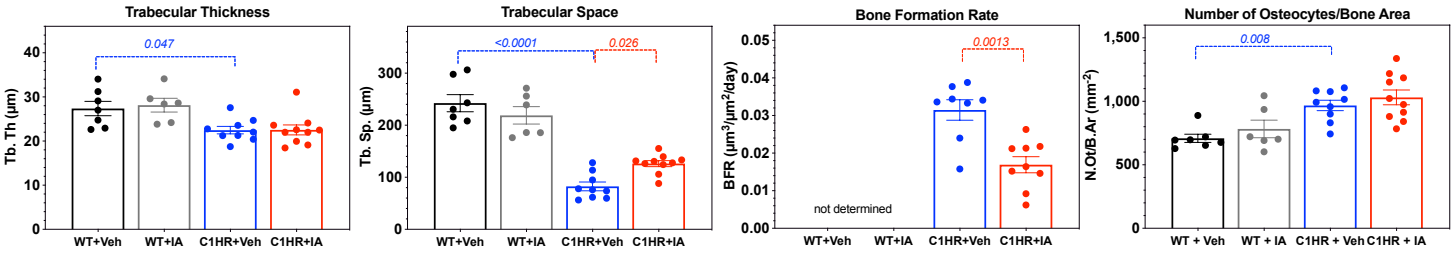


Fig. S2 Micro-CT of tibiae. Sagittal and corresponding transverse views of the proximal regions of tibiae obtained from wild-type (WT) or Col1-PTHR1-H223R (C1HR) mice injected twice-daily with vehicle (Veh) or inverse agonist (IA) for 17 days: transverse views show a region approximately 1 mm below the growth plates.

A



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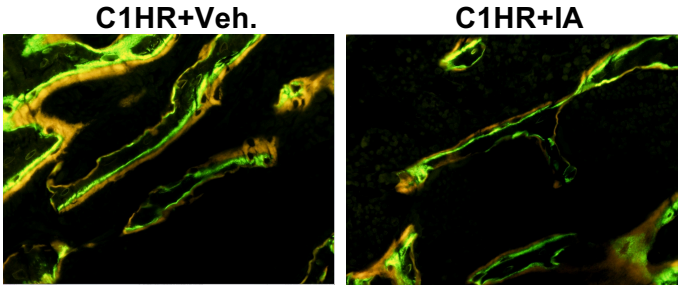

Fig. S-3 Histomorphometry of tibiae. Tibiae from wild-type (WT) or Col1-PTHR1-H223R (C1HR) mice after 17 days of twice-daily injection with either vehicle (Veh) or inverse agonist (IA) were analyzed histomorphometrically in a region just below the proximal growth plate. **A**) Quantification of histomorphometric parameters. Data are means±SD; P values (Tukey's multiple comparison test after ANOVA) are shown for paired groups marked by brackets. Bone formation rate was not determined in bones of WT mice due to inadequate separation of calcein and demeclocycline fluorescent labels. **B**) Fluorescent microscopy images of bone trabeculae in tibia from C1HR mice showing incorporated calcein (green) and demeclocycline (orange) labels administered two days and one day before sacrifice, respectively (400X). Histomorphometric data are also reported in **Figure 3 and Table S1.**

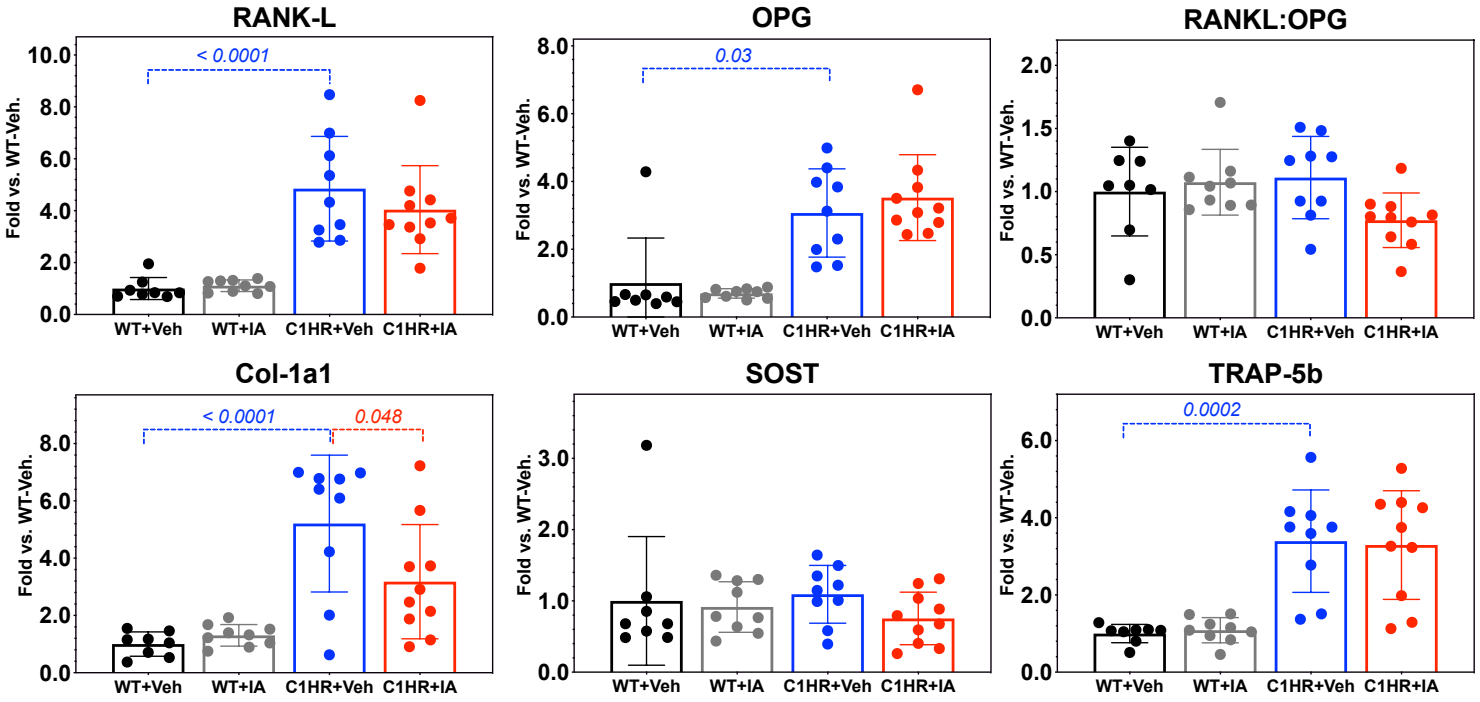

Fig. S-4 Effect of inverse agonist treatment on mRNA levels in femurs of WT or C1HR mice. Total RNA isolated from femurs obtained from wild-type (WT) or Col1-PTHR1-H223R (C1HR) mice after 17 days of twice-daily injection with either vehicle (Veh) or inverse agonist (IA) was analyzed by real-time (RT) PCR for mRNAs corresponding to the indicated genes. RT-PCR values for each gene were normalized to the value for 18sRNA and then by the corresponding 18S-normalized value obtained in WT-vehicle controls. Data are means±SD; P values (Tukey's multiple comparison test after ANOVA) are shown for paired groups marked by brackets.

TaqMan PCR probes (FAM) Thermofisher	
RANKL/Tnfsf11	Mm00441906_m1
OPG/Tnfrsf11b	Mm00435454_m1
TRAP5b/Acp5	Mm00475698_m1
Sclerostin/Sost	Mm00470479_m1
Col1a1	Mm00801666_g1
ALP	Mm00475834_m1

VIC®/TAMRA™ probe	Cat #
Eukaryotic 18S rRNA	4310893E

Fig. S-5 Taqman probes used for mRNA analysis.

Table S1 Histomorphometry of tibiae from mice injected with vehicle or inverse agonist.

	WT					Col1-H223R					
	Vehicle	n	Inverse Agonist	*P vs. WT-Veh*	n	Vehicle	n	*P vs. WT-veh.*	Inverse Agonist	n	*P vs. C1HR-Veh*
B.Ar/T.Ar (%)	**10.3 ± 0.8**	7	**11.8 ± 3.1**	0.91	6	**22.4 ± 5.8**	9	<0.0001	**15.3 ± 3.0**	10	0.00
N.Ob/T.Ar (mm^{-2})	**158 ± 8**	7	**179 ± 21**	0.96	6	**511 ± 129**	9	<0.0001	**290 ± 61**	10	<0.0001
N.Oc/T.Ar (mm^{-2})	**17.0 ± 2.0**	7	**20.8 ± 4.0**	0.90	6	**44.9 ± 11.9**	9	<0.0001	**47.7 ± 11.6**	10	0.93
N.Ob/B.Pm (mm^{-1})	**21.1 ± 1.2**	7	**21.8 ± 1.7**	0.97	6	**25.4 ± 2.1**	9	0.039	**21.0 ± 4.0**	10	0.018
N.Oc/B.Pm (mm^{-1})	**2.26 ± 0.32**	7	**2.57 ± 0.68**	0.89	6	**2.30 ± 0.64**	9	>0.99	**3.50 ± 0.87**	10	0.011
Ob.Pm/B.Pm (%)	**15.5 ± 1.2**	7	**15.3 ± 1.3**	>0.99	6	**18.0 ± 3.5**	9	0.263	**14.2 ± 1.8**	10	0.020
Oc.Pm/B.Pm (%)	**3.50 ± 0.40**	7	**3.92 ± 0.97**	0.95	6	**4.30 ± 1.37**	9	0.67	**6.91 ± 1.77**	10	0.0019
N.Ot/B.Ar (mm^{-2})	**708 ± 32**	7	**783 ± 169**	0.80	6	**968 ± 123**	9	0.0085	**1031 ± 184**	10	0.79
Fb.Ar./T.Ar/ (%)	**0.0017 ± 0.0017**	7	**0.0041 ± 0.0101**	>0.99	6	**0.22 ± 0.16**	9	0.0002	**0.11 ± 0.04**	10	0.048
MAR (um/day)	**N.D.**		**N.D.**			**1.47 ± 0.53**	8		**0.76 ± 0.18**	9	0.0050
BFR/BS (um^3/um^2/day)	**N.D.**		**N.D.**			**0.031 ± 0.008**	8		**0.017 ± 0.006**	9	0.0013

Proximal tibiae were analyzed in a ~1.0x1.0 mm area located ~1.0 mm below the center of the growth plate. Data are means ±SD; P values were determined by Tukey's multiple comparison test after ANOVA. N.D., not determined -- inadequate separation of demeclocycline and calcein labels in bones of WT mice precluded assessment of mineral apposition and bone formation rates.

B.Ar/T.Ar(%)	Bone area/tissue area
N.Ob/T.Ar (mm^{-2})	number of osteoblasts/tissue area
N.Oc/T.Ar (mm^{-2})	number of osteoclasts/tissue area
N.Ob/B.Pm (mm^{-1})	number of osteoblast/bone perimeter
N.Oc/B.Pm (mm^{-1})	number of osteoclast/bone perimeter
Ob.Pm/B.PM (%)	osteoblast perimeter/bone perimeter
Oc.Pm/B.PM (%)	osteoclast perimeter/bone perimeter
N.Ot/B.Ar (mm^{-2})	number of osteocytes/bone area
Fb.Ar./T.Ar. (%)	fibrosis area/tissue area
MAR (um/day)	mineral apposition rate
BFR/BS (um^3/um^2/day)	Bone formation rate

Table S2 Effect of inverse agonist treatment on bone length

	WT					Col1-H223R				
	Vehicle	n	Inverse Agonist	*P vs.WT-Veh*	n	Vehicle	*P vs.WT-Veh*	n	Inverse Agonist	*P* vs. C1HR- Veh	n
Femur length (mm)	**10.7 ± 0.4**	8	**10.6 + 0.4**	*0.95*	9	**9.0 ± 0.4**	*<0.0001*	9	**9.4 ± 0.4**	*0.080*	10
Tibia length (mm)	**13.4 ± 0.6**	6	**13.4 ± 0.3**	*0.99*	9	**11.6 ± 0.5**	*<0.0001*	8	**12.1 ± 0.5**	*0.195*	10

Bone length determined by micro CT. Data are means ±SD; P values determined by Tukey's multiple comparison test after ANOVA.