

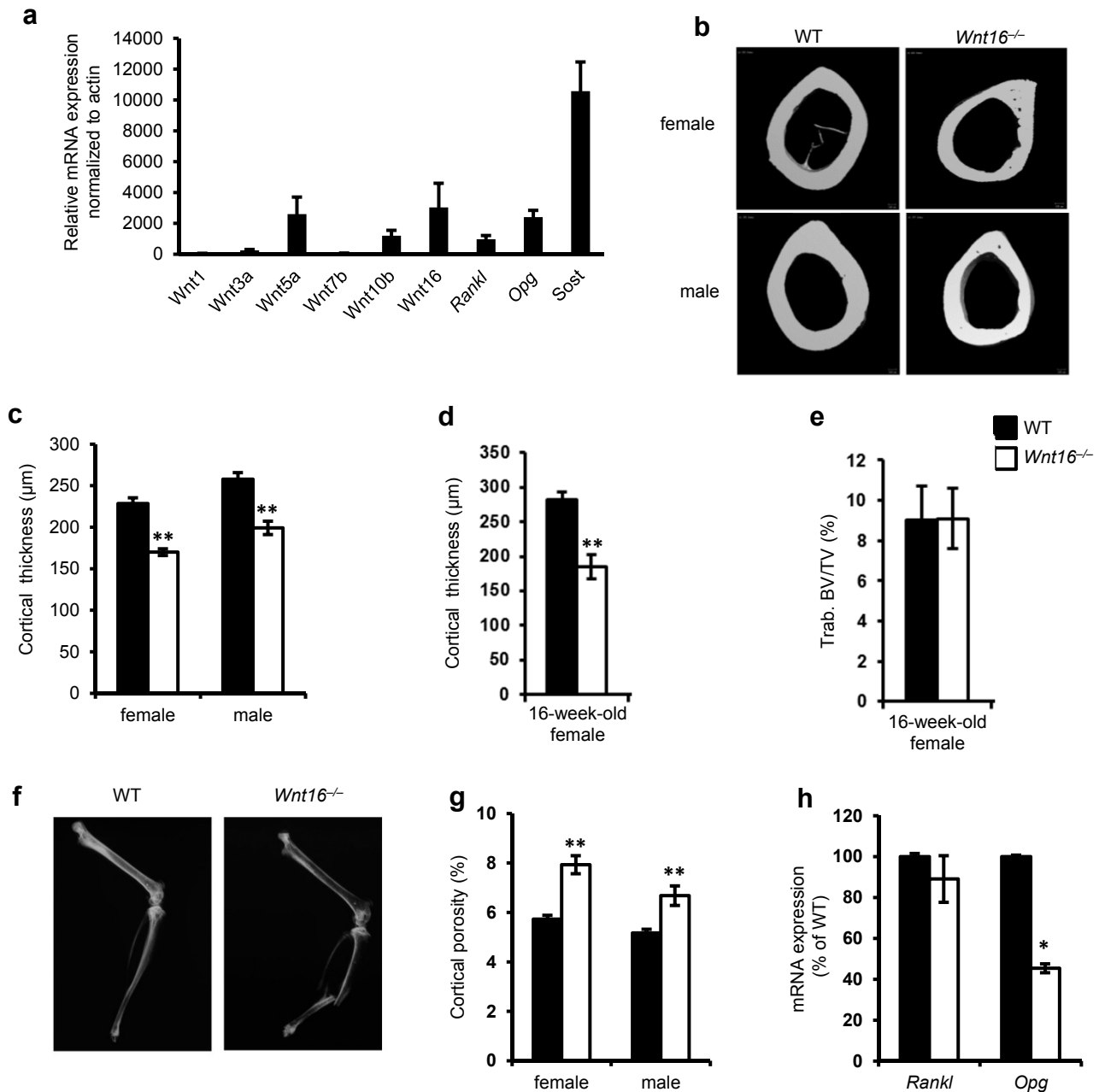
## **SUPPLEMENTARY INFORMATION**

### **Osteoblast-derived WNT16 represses osteoclastogenesis and prevents cortical bone fragility fractures**

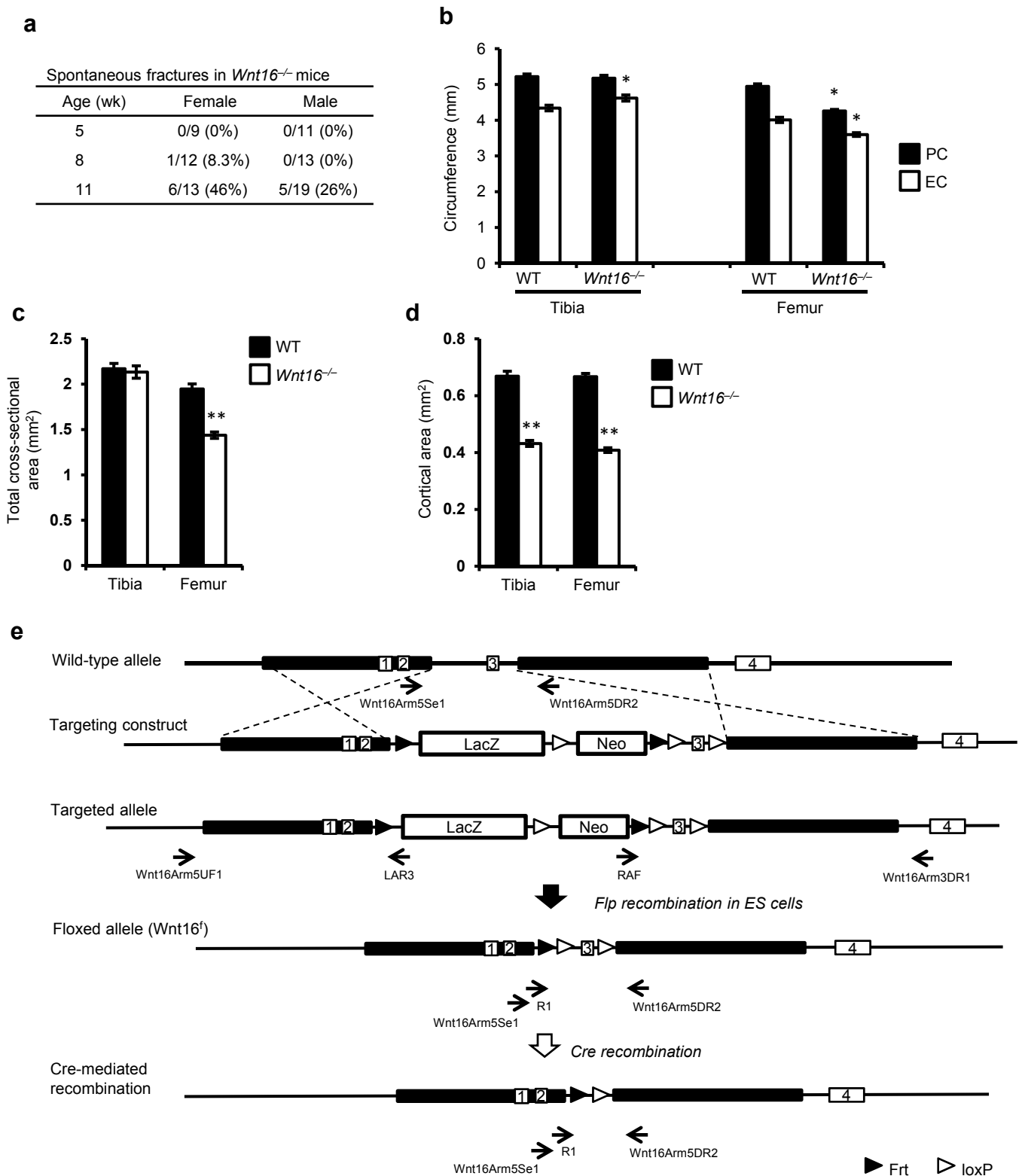
Sofia Movérare-Skrtic<sup>#</sup>, Petra Henning<sup>#</sup>, Xianwen Liu<sup>#</sup>, Kenichi Nagano, Hiroaki Saito, Anna E. Börjesson, Klara Sjögren, Sara H. Windahl, Helen Farman, Bert Kindlund, Cecilia Engdahl, Antti Koskela, Fu-Ping Zhang, Emma E. Eriksson, Farasat Zaman, Ann Hammarstedt, Hanna Isaksson, Marta Bally, Ali Kassem, Catharina Lindholm, Olof Sandberg, Per Aspenberg, Lars Sävendahl, Jian Q. Feng, Jan Tuckermann, Juha Tuukkanen, Matti Poutanen, Roland Baron\* Ulf H. Lerner\*, Francesca Gori\* and Claes Ohlsson\*

<sup>#</sup> Shared first authors

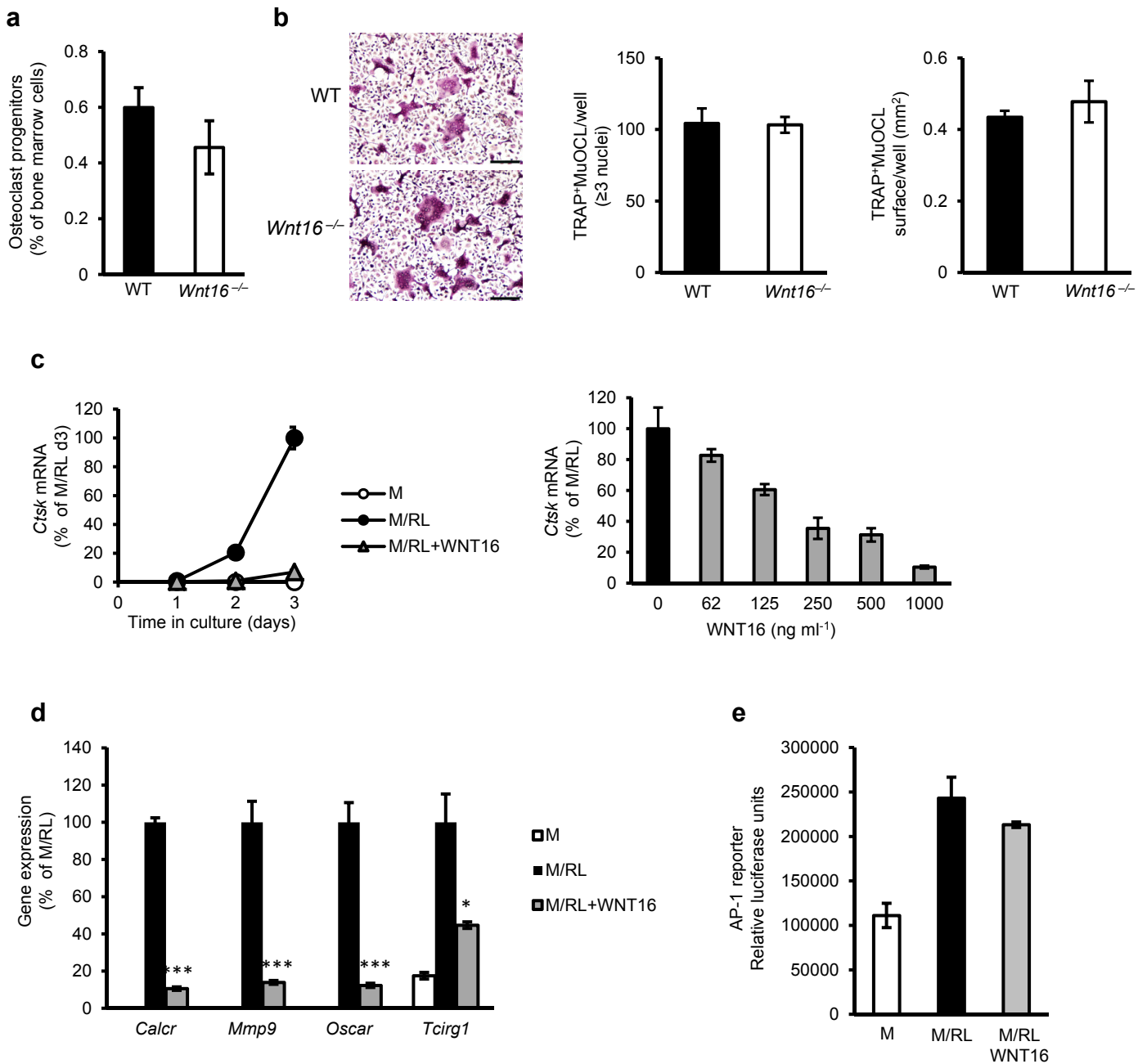
\* Shared last authors



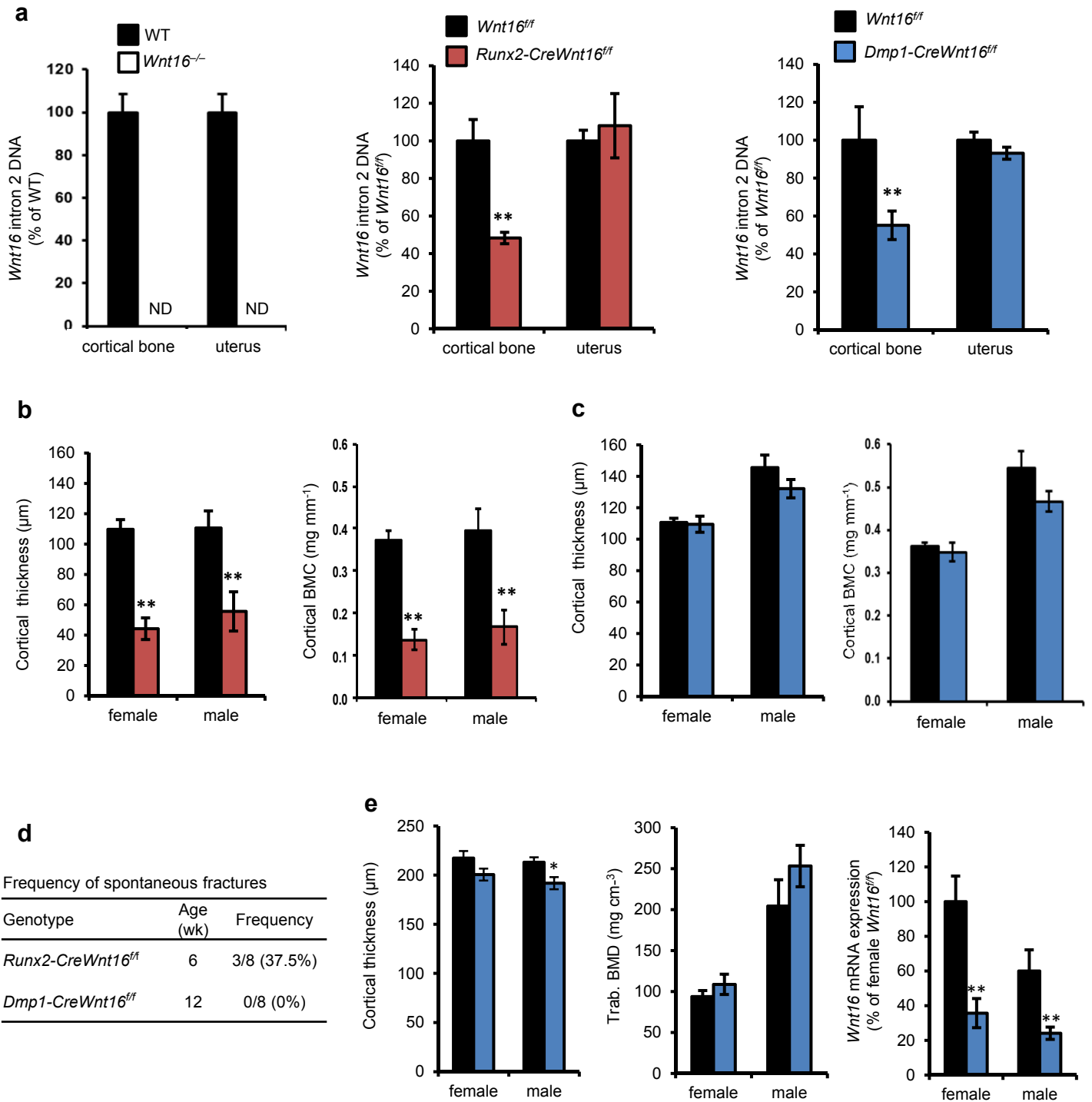
**Supplementary Fig. 1:** (a) mRNA expression of Wnt ligands in cortical bone. Values are expressed as relative expression of the normalized mRNAs levels ( $n=3$ ). (b-h) Skeletal phenotype of *Wnt16*<sup>-/-</sup>*exon1-3* mice. (b) Representative  $\mu$ CT images of cortical thickness of femur of 12-week-old WT and *Wnt16*<sup>-/-</sup>*exon1-3* mice. (c)  $\mu$ CT analysis of distal femur cortical bone thickness at 12 weeks. ( $n=5$ ). (d) Cortical thickness and (e) trabecular bone volume over total volume (BV/TV) of tibia in 16-week-old female WT and *Wnt16*<sup>-/-</sup>*exon1-3* mice as measured by histomorphometry. (WT  $n=4$ ; *Wnt16*<sup>-/-</sup>*exon1-3*  $n=6$ ). (f) Representative radiographic images of WT and *Wnt16*<sup>-/-</sup>*exon1-3* hindlimbs of 12 weeks of age males. (g)  $\mu$ CT analysis of cortical bone porosity in femur at 12 weeks. ( $n=5$ ). (h) Expression of *Rankl* and *Opg* (*Tnfrsf11* and *Tnfrsf11b*, respectively) in cortical bone ( $n=3$ ). Data are represented as mean  $\pm$  SEM. \*  $P < 0.05$ , \*\*  $P < 0.005$  by Student's t-test.



**Supplementary Fig. 2:** (a) Frequency of fractures in *Wnt16*<sup>-/-exon1-4</sup> mice. (b) Periosteal (PC), endosteal (EC) circumference, (c) total cross-sectional area, and (d) cortical area of tibia and femur of 11-week-old female *Wnt16*<sup>-/-exon1-4</sup> mice compared to WT mice. \**p* < 0.05, \*\**P* < 0.01, Student's t-test vs. littermate WT mice. (*n* = 6). Values are given as mean ± SEM. (e) Generation of *Wnt16* conditional knockout mice. Schematic presentation of wild-type allele, targeting vector, targeted allele and floxed allele of *Wnt16* gene. The approximate locations of Flp-sites (▶), loxP-sites (▷) and PCR primers used to screen for homologous recombination, floxed allele and genotypes are shown (arrows) with the original and predicted structures of the gene after homologous recombination, Flp- and Cre-recombination.



**Supplementary Fig. 3:** (a) Percentage osteoclast progenitors in bone marrow measured by FACS (WT  $n = 4$ , *Wnt16*<sup>-/-</sup>  $n = 5$ ). (b) RANKL stimulated osteoclastogenesis in bone marrow macrophages in cultures from *Wnt16*<sup>-/-</sup> and WT mice. (left) TRAP staining at day 5. Scale bar 100  $\mu\text{m}$ . Number (left graph) and surface (right graph) of TRAP<sup>+</sup>MuOCL. (c) Time (left) and dose (right) dependent inhibition of *Ctsk* (*Cathepsin K*) mRNA by WNT16 in RANKL stimulated bone marrow macrophage cultures. Gene expression was analyzed at day 3 of culture in the dose response. Data are expressed as % of M/RL treated group at day 3  $\pm$  SEM. (d) Gene expression in BMM cultured in M, M/RL or M/RL+WNT16 (1000 ng ml<sup>-1</sup>) for 3 days. Data are expressed as % of M/RL treated group  $\pm$  SEM. *Calcr* = *Calcitonin receptor*, *Mmp9* = *Matrix metalloproteinase 9*, *Oscar* = *Osteoclast associated receptor*, *Tcirg1* = *Atp6i*. M: M-CSF stimulation, M/RL: M-CSF and RANKL stimulation, M/RL+WNT16: M-CSF, RANKL and WNT16 stimulation. \*  $P < 0.05$ , \*\*\*  $P < 0.005$ , Student's t-test vs. M/RL treated group. (e) AP-1 luciferase gene reporter assay in BMM cultured in M, M/RL or M/RL+WNT16 (500 ng ml<sup>-1</sup>) for 48 h. Data are expressed as relative luciferase units  $\pm$  SEM.



**Supplementary Fig. 4:** (a) Degree of recombination/deletion as analyzed using a *Wnt16* intron 2 specific assay on purified DNA from *Wnt16<sup>-/-</sup> exon1-4* (left), *Runx2-CreWnt16<sup>ff</sup>* (middle) and *Dmp1-CreWnt16<sup>ff</sup>* (right) mice. \*\*  $P < 0.01$ , Student's *t*-test vs. control. ( $n = 8$ ). ND = not detectable. (b, c) Reduced tibia diaphyseal cortical thickness (b, left) and bone mineral content (BMC) (b, right) in 5-week-old *Runx2-CreWnt16<sup>ff</sup>* but not in 5-week old *Dmp1-CreWnt16<sup>ff</sup>* (c) mice compared to littermate controls (*Wnt16<sup>ff</sup>*;  $n = 8$ ; 5-week-old). Cortical thickness and cortical BMC were analyzed using peripheral quantitative computed tomography (pQCT). \*\*  $P < 0.01$ , Student's *t*-test vs. control. (d) Frequency of fractures in *Runx2-CreWnt16<sup>ff</sup>* and *Dmp1-CreWnt16<sup>ff</sup>* mice. (e) 52-week-old *Dmp1-CreWnt16<sup>ff</sup>* mice have slightly reduced cortical thickness compared to littermate control *Wnt16<sup>ff</sup>* mice. (e, left) Cortical thickness and (e, middle) trabecular bone mineral density (BMD) as measured by pQCT. (e, right) *Wnt16* mRNA expression in cortical bone. ( $n = 6-9$ ). \*  $P < 0.05$ , \*\*  $P < 0.01$ , Student's *t*-test vs. *Wnt16<sup>ff</sup>*. Values are given as mean  $\pm$  SEM.

**Supplementary Table 1. Body and bone characteristics in *Wnt16*<sup>-/-</sup> mice**

	Female		Male	
	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>
<b>Tissue weights</b>				
Body weight (g)	20.1 ± 0.35	18.9 ± 0.23 *	25.8 ± 0.69	24.5 ± 0.59
Liver (%)	5.07 ± 0.12	4.95 ± 0.11	4.95 ± 0.11	4.74 ± 0.09
Heart (%)	0.52 ± 0.01	0.51 ± 0.01	0.52 ± 0.01	0.53 ± 0.03
Kidney (%)	1.12 ± 0.02	1.12 ± 0.03	1.20 ± 0.02	1.23 ± 0.02
Spleen (%)	0.39 ± 0.01	0.39 ± 0.02	0.30 ± 0.01	0.32 ± 0.01
Thymus (%)	0.28 ± 0.01	0.29 ± 0.02	0.16 ± 0.01	0.17 ± 0.01
Uterus (%)	0.37 ± 0.07	0.40 ± 0.08	-	-
Ovaries (%)	0.09 ± 0.01	0.09 ± 0.01	-	-
Testicles (%)	-	-	0.58 ± 0.02	0.56 ± 0.02
Seminal vesicle (%)	-	-	0.69 ± 0.03	0.73 ± 0.03
Gonadal fat (%)	0.75 ± 0.06	0.81 ± 0.04	1.17 ± 0.11	1.23 ± 0.07
Retroperitoneal fat (%)	0.14 ± 0.02	0.16 ± 0.02	0.26 ± 0.03	0.24 ± 0.02
Brown fat (%)	0.24 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
<b>Bone parameters</b>				
Cortical thickness (µm)	140 ± 4	88 ± 2 **	156 ± 5	102 ± 4 **
<b>Trabecular</b>				
BV/TV (%)	21.5 ± 1.0	18.1 ± 1.6	30.8 ± 2.7	27.9 ± 2.1
Tb.Th (µm)	57 ± 1	54 ± 1	60 ± 2	59 ± 2
Tb.N (mm <sup>-1</sup> )	3.76 ± 0.14	3.30 ± 0.22	5.00 ± 0.30	4.70 ± 0.20
Tb.Sp (µm)	195 ± 5	206 ± 6	124 ± 6	130 ± 4

Body and bone characteristics of 11-week-old *Wnt16*<sup>-/- exon1-4</sup> mice and wild type (WT) mice.

Organ weights expressed as % of body weight. Bone parameters measured by µCT analyses of tibia. Tb.Th = Trabecular thickness, Tb.N = Trabecular number, Tb.Sp = Trabecular separation.

\**P* < 0.05, \*\* *P* < 0.01, Student's t-test (female WT, *n* = 9; female KO, *n* = 8; male WT, *n* = 11; male KO, *n* = 12). Values are given as mean ± SEM.

**Supplementary Table 2. Histomorphometry of cortical bone in *Wnt16*<sup>-/-</sup> mice**

	8 weeks		11 weeks	
	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>
Cortical thickness (μm)	166 ± 5	135 ± 3**	184 ± 3	149 ± 4**
<b><i>Endosteal</i></b>				
MAR (μm day <sup>-1</sup> )	1.86 ± 0.21	2.39 ± 0.14	1.43 ± 0.08	1.93 ± 0.25
OS/BS (%)	89.0 ± 5.2	49.4 ± 21.4	ND	ND
<b><i>Periosteal</i></b>				
MAR (μm day <sup>-1</sup> )	0.81 ± 0.06	0.78 ± 0.09	0.71 ± 0.10	0.72 ± 0.11

Static and dynamic and histomorphometry of cortical bone in the mid-diaphyseal region of tibia in female *Wnt16*<sup>-/- exon1-4</sup> and wild type (WT) mice. MAR = mineral apposition rate, OS/BS = osteoid/bone surface. ND = not determined. \*  $P < 0.05$ , \*\*  $P < 0.01$ , Student's t-test (8-weeks old WT and KO,  $n = 9$ ; 11-weeks old WT  $n = 7$ , KO  $n = 5$ ). Values are given as mean ± SEM.

**Supplementary Table 3. High resolution  $\mu$ CT and histomorphometry of trabecular bone in *Wnt16*<sup>-/-</sup> mice**

	5 weeks		11 weeks	
	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>
<b>High resolution <math>\mu</math>CT of trabecular bone in femur</b>				
BV/TV (%)	27.0 $\pm$ 0.8	28.3 $\pm$ 0.5	21.3 $\pm$ 1.0	18.2 $\pm$ 1.4
Tb.Th ( $\mu$ m)	34 $\pm$ 1	34 $\pm$ 1	44 $\pm$ 1	43 $\pm$ 2
Tb.N (mm <sup>-1</sup> )	7.88 $\pm$ 0.27	8.27 $\pm$ 0.18	4.73 $\pm$ 0.19	4.18 $\pm$ 0.24
Trabecular BMD (g cm <sup>-3</sup> )	0.024 $\pm$ 0.01	0.025 $\pm$ 0.01	0.032 $\pm$ 0.01	0.029 $\pm$ 0.014
<b>Histomorphometry of trabecular bone in vertebrae L4</b>				
<i>Static histomorphometry</i>				
BV/TV (%)	ND	ND	21.8 $\pm$ 1.04	19.4 $\pm$ 1.53
Tb.N (mm <sup>-1</sup> )	ND	ND	6.1 $\pm$ 0.22	5.5 $\pm$ 0.35
Tb.Th ( $\mu$ m)	ND	ND	35.8 $\pm$ 1.4	35.0 $\pm$ 1.4
Tb.Sp ( $\mu$ m)	ND	ND	129.4 $\pm$ 5.5	149.8 $\pm$ 13.2
Oc.S/BS (%)	ND	ND	2.85 $\pm$ 0.28	3.75 $\pm$ 0.41
<i>Dynamic parameters</i>				
MS/BS (%)	ND	ND	46.2 $\pm$ 1.26	47.8 $\pm$ 0.94
MAR ( $\mu$ m day <sup>-1</sup> )	ND	ND	1.24 $\pm$ 0.02	1.20 $\pm$ 0.04
BFR ( $\mu$ m <sup>2</sup> $\mu$ m <sup>-1</sup> day <sup>-1</sup> )	ND	ND	0.57 $\pm$ 0.02	0.57 $\pm$ 0.03

High resolution  $\mu$ CT analyses of trabecular bone in the distal metaphyseal region in femur from 5- and 11-week-old *Wnt16*<sup>-/-</sup> *exon1-4* mice and wild type (WT) mice. (5- and 11-weeks old WT, *n* = 9; 5- and 11-weeks old KO, *n* = 8). Histomorphometry of trabecular bone in vertebra L<sub>4</sub> of 11-week-old female *Wnt16*<sup>-/-</sup> *exon1-4* (*n* = 6) and wild type (WT) (*n* = 7) mice. BV/TV = trabecular bone volume/tissue volume, Tb.N = trabecular number, Tb.Th = trabecular thickness, Tb.Sp = trabecular separation, Oc.S/BS = osteoclast surface/bone surface. MS/BS = mineralizing surface/bone surface, MAR = mineral apposition rate, BFR = bone formation rate. ND = not determined. Values are given as mean  $\pm$  SEM.



**Supplementary Table 4. Serum/plasma markers of calcium homeostasis in *Wnt16*<sup>-/-</sup> mice**

	WT	<i>Wnt16</i> <sup>-/-</sup>
PTH (pg ml <sup>-1</sup> )	45.4 ± 2.3	46.4 ± 3.1
Calcium (mg dl <sup>-1</sup> )	10.6 ± 0.2	10.9 ± 0.2
1,25(OH) <sub>2</sub> D (nmol l <sup>-1</sup> )	1.85 ± 0.25	1.77 ± 0.18
Phosphate (mmol l <sup>-1</sup> )	5.6 ± 0.51	4.5 ± 0.42

Serum/plasma analysis of 5-week-old (PTH, calcium and 1,25(OH)<sub>2</sub>D) or 8-week-old (phosphate) female *Wnt16*<sup>-/-</sup> *exon1-4* and wild type (WT) mice (*n* = 8). Values are given as mean ± SEM.

**Supplementary Table 5.  $\mu$ CT analyses of trabecular and cortical bone in *Wnt16*<sup>-/-</sup> mice**

	6 weeks				12 weeks			
	Male		Female		Male		Female	
	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>
<b>Cortical bone</b>								
Cortical thickness ( $\mu$ m)	208 $\pm$ 4	156 $\pm$ 8**	186 $\pm$ 2	140 $\pm$ 8**	258 $\pm$ 8	199 $\pm$ 8**	228 $\pm$ 7	170 $\pm$ 4**
<b>Trabecular bone</b>								
BV/TV (%)	17.4 $\pm$ 1.4	17.7 $\pm$ 1.4	12.0 $\pm$ 1.0	16.5 $\pm$ 1.0*	15.5 $\pm$ 2.7	22.5 $\pm$ 0.6*	12.5 $\pm$ 1.4	14.2 $\pm$ 1.7
Tb.Th ( $\mu$ m)	42.1 $\pm$ 1.6	43.2 $\pm$ 4.9	36.5 $\pm$ 0.6	39.7 $\pm$ 0.6*	45.4 $\pm$ 1.2	52.4 $\pm$ 1.2**	42.7 $\pm$ 1.8	42.8 $\pm$ 2.0
Tb.N (mm <sup>-1</sup> )	5.55 $\pm$ 0.18	5.49 $\pm$ 0.33	4.58 $\pm$ 0.22	5.29 $\pm$ 0.10	4.78 $\pm$ 0.31	5.43 $\pm$ 0.14	4.12 $\pm$ 0.14	4.49 $\pm$ 0.20
Tb.Sp ( $\mu$ m)	174 $\pm$ 8	173 $\pm$ 14	217 $\pm$ 14	181 $\pm$ 5	204 $\pm$ 15	169 $\pm$ 4	239 $\pm$ 9	215 $\pm$ 11

$\mu$ CT analyses of femur from 6-week-old and 12-week-old *Wnt16*<sup>-/- exon1-3</sup> mice and wild type (WT) mice. Tb.Th = Trabecular thickness, Tb.N = Trabecular number, Tb.Sp = Trabecular separation. \*  $P < 0.05$ ; \*\*  $P < 0.005$ , Student's t-test. Values are given as mean  $\pm$  SEM. (6 weeks:  $n=6$  for WT and  $n=4$  for *Wnt16*<sup>-/-</sup> mice; 12 weeks:  $n=5$  for WT and  $n=5$  for *Wnt16*<sup>-/-</sup> mice)

**Supplementary Table 6. Histomorphometry of cortical and trabecular bone in *Wnt16*<sup>-/-</sup> mice**

	6 weeks				12 weeks			
	Male		Female		Male		Female	
	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>	WT	<i>Wnt16</i> <sup>-/-</sup>
<b>Cortical bone</b>								
Cortical thickness (μm)	254±7	203±43	234±9	176±17*	294±16	256±51	252±9	186±18*
Endo MAR (μm day <sup>-1</sup> )	3.82±0.25	3.15±1.52	3.28±0.41	2.92±0.83	0.56±0.14	0.93±0.24	0.61±0.21	1.22±0.36
Ec.Oc.s/BS (%)	4.73 ±1.56	5.03±1.03	4.12±0.86	6.89±1.56	2.38±0.79	4.79±0.61*	1.66±0.61	3.83±1.20
Ec.N.Oc/BS (mm <sup>-1</sup> )	2.10±0.59	2.72±0.92	2.06±0.42	3.89±0.71*	1.81±0.60	3.59±0.35*	1.15±0.36	2.28±0.69
Peri MAR (μm day <sup>-1</sup> )	1.78±0.20	1.10±0.15(*)	1.30±0.23	0.85±0.14	0.74±0.06	0.82±0.22	0.64±0.07	0.62±0.07
<b>Trabecular bone</b>								
BV/TV (%)	11.9±0.8	12.0 ±1.8	9.4±0.8	12.4±1.9	12.1±1.9	17.9±1.7*	7.7±1.9	12.8±1.2
Tb.N (mm <sup>-1</sup> )	4.0±0.3	4.0±0.4	2.9±0.2	3.7±0.4	3.2±0.3	4.3±0.3*	2.2±0.5	3.3±0.2
Tb.Th (μm)	30.2±0.5	30.1±2.1	32.3±1.3	33.1±2.7	37.1±3.0	41.8±2.4	34.1±1.9	38.9±1.7
Tb.Sp (μm)	230±19	226±24	324±25	246±29	292±39	195±16	551±168	270±17
Ob.S/BS (%)	8.83±0.76	9.19±1.17	16.74±2.48	15.03±1.43	9.50±1.69	11.06±0.94	17.54±4.78	15.12±1.20
N.Ob/BS (mm <sup>-1</sup> )	5.54±0.57	5.69±0.43	10.61±1.78	9.84±1.10	10.08±1.89	11.47±1.17	12.26±1.29	15.22±3.13
OS/BS (%)	3.30±0.40	3.70±1.25	3.86±0.67	4.23±0.73	3.53±0.77	3.02±0.56	8.7 ±3.82	5.06±1.03
Oc.S/BS (%)	2.62 ±0.27	2.30±0.39	4.17±0.25	3.11±0.41*	3.94±0.41	3.80±0.31	6.39±1.19	4.84±0.23
N.Oc/BS (mm <sup>-1</sup> )	0.81±0.09	0.82±0.13	1.42±0.07	1.15±0.14	1.81±0.20	1.70±0.14	2.75±0.54	2.20±0.13
ES/BS (%)	3.42 ±0.32	3.07±0.51	5.50±0.19	3.75±0.38*	1.20±0.16	1.43±0.16	2.60±0.79	1.93±0.55
MS/BS (%)	17.5±1.2	18.3±2.6	18.9±1.8	19.0±4.2	19.9±0.8	26.3±2.1*	23.4±1.7	28.2±1.9
MAR (μm day <sup>-1</sup> )	1.99±0.12	2.17±0.14	2.21±0.18	1.77±0.20	1.32±0.16	1.36±0.05	1.60±0.32	2.14±0.13
BFR/BV (% Year <sup>-1</sup> )	825±104	936±164	914±110	765±211	584±100	649±60	820±169	1229±118

Histomorphometry of cortical and trabecular bone in the tibia of 6-week-old and 12-week-old *Wnt16*<sup>-/-</sup> *exon1-3* mice and wild type (WT) mice. Endo MAR = endocortical mineral apposition rate, Ec.Oc.s/BS = endocortical osteoclast surface/bone surface, Ec.N.Oc/BS = Number of endocortical osteoclasts/bone surface. Peri MAR = periosteal mineral apposition rate, BV/TV = trabecular bone volume/tissue volume, Tb.N = trabecular number, Tb.Th = trabecular thickness, Tb.Sp = trabecular separation, Ob.S/BS = osteoblast surface/bone surface, N.Ob/BS = number of osteoblast/bone surface, OS/BS = osteoid surface/bone surface, Oc.S/BS = osteoclast surface/bone surface. MS/BS = mineralizing surface/bone surface, BFR = bone formation rate. Cortical bone; 6 weeks: *n* = 6 for WT and *n* = 3 and 4 for *Wnt16*<sup>-/-</sup> mice males and females, respectively; 12 weeks: *n* = 5 for WT and *n* = 5 for *Wnt16*<sup>-/-</sup> mice. Trabecular bone; 6 weeks: *n* = 6 for WT and *n* = 4 for *Wnt16*<sup>-/-</sup> *exon1-3* mice; 12 weeks: *n* = 5 for WT and *n* = 5 for *Wnt16*<sup>-/-</sup> *exon1-3* mice). Values are given as mean ± SEM. (\*)*P* = 0.06, \* *P* < 0.05, Student's t-test.

**Supplementary Table 7. Skeletal characteristics in *Wnt16<sup>ff</sup>* mice**

	WT	<i>Wnt16<sup>ff</sup></i>
Body weight (g)	18.5 ± 0.6	18.3 ± 1.3
Trabecular BMD (mg cm <sup>-3</sup> )	301 ± 7.1	276 ± 11.7
Cortical thickness (μm)	119 ± 8	111 ± 11

Skeletal characteristics of the tibia in 5-week-old *Wnt16<sup>ff</sup>* male mice compared with WT male mice as measured by pQCT. BMD = bone mineral density. *n* = 8-9. Values are given as mean ± SEM.

**Supplementary Table 8. Body characteristics in *Runx2-CreWnt16<sup>ff</sup>* mice**

	Female		Male	
	Control	<i>Runx2-CreWnt16<sup>ff</sup></i>	Control	<i>Runx2-CreWnt16<sup>ff</sup></i>
Body weight (g)	16.7 ± 1.0	15.9 ± 0.7	21.0 ± 0.9	20.5 ± 0.9
Liver (%)	4.96 ± 0.07	4.98 ± 0.16	5.20 ± 0.16	5.17 ± 0.14
Heart (%)	0.54 ± 0.02	0.53 ± 0.01	0.47 ± 0.02	0.48 ± 0.01
Kidney (%)	1.21 ± 0.03	1.24 ± 0.02	1.25 ± 0.03	1.28 ± 0.03
Spleen (%)	0.36 ± 0.02	0.34 ± 0.02	0.33 ± 0.03	0.32 ± 0.01
Thymus (%)	0.43 ± 0.03	0.41 ± 0.02	0.27 ± 0.03	0.32 ± 0.02
Uterus (%)	0.29 ± 0.07	0.31 ± 0.06	-	-
Ovaries (%)	0.06 ± 0.003	0.08 ± 0.01	-	-
Testicles (%)	-	-	0.76 ± 0.05	0.84 ± 0.02
Seminal vesicle (%)	-	-	0.40 ± 0.05	0.40 ± 0.05
Gonadal fat (%)	0.99 ± 0.13	0.98 ± 0.14	1.12 ± 0.10	1.21 ± 0.08
Retroperitoneal fat (%)	0.29 ± 0.04	0.35 ± 0.04	0.26 ± 0.03	0.27 ± 0.03
Brown fat (%)	0.33 ± 0.02	0.33 ± 0.03	0.28 ± 0.03	0.31 ± 0.01

Body characteristics in 7-week-old *Runx2-CreWnt16<sup>ff</sup>* and littermate *Wnt16<sup>lox/flox</sup>* (Control) mice. Organ weights expressed as % of body weight (female *Runx2-CreWnt16<sup>ff</sup>* and control,  $n = 10$ ; male *Runx2-CreWnt16<sup>ff</sup>*,  $n = 7$ ; male control,  $n = 10$ ). Values are given as mean ± SEM.

**Supplementary Table 9. Trabecular bone microstructure parameters in *Runx2-CreWnt16<sup>ff</sup>* and *Dmp1-CreWnt16<sup>ff</sup>* mice**

	<b>Control</b>	<b><i>Runx2Cre-Wnt16<sup>ff</sup></i></b>	<b>Control</b>	<b><i>Dmp1Cre-Wnt16<sup>ff</sup></i></b>
Tb. Th (μm)	34.1 ± 03	32.6 ± 0.7	33.7 ± 0.3	34.3 ± 0.4
Tb. N (mm <sup>-1</sup> )	6.1 ± 0.6	6.9 ± 0.5	6.4 ± 0.4	6.8 ± 0.6
Tb. Sp (μm)	81.0 ± 4.4	75.9 ± 4.8	76.1 ± 3.4	72.6 ± 2.7

Trabecular μCT analyses of distal femur from 5-week-old female *Runx2-CreWnt16<sup>ff</sup>* and *Dmp1-CreWnt16<sup>ff</sup>* mice compared to littermate controls (*Wnt16<sup>ff</sup>*). Tb.Th = Trabecular thickness, Tb.N = Trabecular number, Tb.Sp = Trabecular separation. Values are given as mean ± SEM (*n* = 7-8).

**Supplementary Table 10. Skeletal characteristics in *Runx2-Cre* mice**

	WT	<i>Runx2-Cre</i>
Body weight (g)	26.8 ± 1.5	26.1 ± 1.0
Trabecular BMD (mg cm <sup>-3</sup> )	348 ± 40	339 ± 62
Cortical thickness (µm)	204 ± 6	192 ± 9

Skeletal characteristics of the tibia in 9-week-old *Runx2-Cre* heterozygote male mice as compared with WT male mice as measured by pQCT. BMD = bone mineral density. *n* = 3-4. Values are given as mean ± SEM.