

## SUPPLEMENTARY MATERIAL

**Supplementary material****Table i.** The bone histomorphometric parameters of the same sham specimen of mandible and tibia at the five scanning resolutions

Parameters*	2D histology	Scanning resolutions ( $\mu\text{m}$ )				
		8.82	13.57	35.32	43.74	104.84
Mandible						
BV/TB (%)	62.11	63.38	71.68	57.41	55.26	Incalculable
BS/BV ( $\text{mm}^2/\text{mm}^3$ )	14.53	15.53	10.34	11.37	11.34	
Tb.Th (mm)	0.13 <sup>†</sup>	0.13	0.19	0.18	0.18	
Tb.N (1/mm)	4.07	4.92	3.71	3.26	3.13	
Tb.Sp (mm)	0.06	0.07	0.08	0.13	0.14	
Tibia						
BV/TB (%)	44.23	50.07	80.88	64.08	77.57	Incalculable
BS/BV ( $\text{mm}^2/\text{mm}^3$ )	21.35	27.84	10.05	10.36	7.68	
Tb.Th (mm)	0.08*	0.07	0.20	0.19	0.26	
Tb.N (1/mm)	4.05	6.97	4.06	3.32	2.98	
Tb.Sp (mm)	0.06	0.07	0.05	0.11	0.08	

BV/TB, bone volume density; BS/BV, bone surface density; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; OVX, ovariectomised rats; OVX-ZOL, zoledronate-injected ovariectomised rats

\*all parameters were calculated from the same rat in the sham group

†Tb.Th in two-dimensional histology is derived from Tb.Dm. It assumes that trabeculae are cylindrical rods; Tb.Dm = 4/(BS/BV). It assumes that trabeculae are thin plates; Tb.Th = 2/(BS/BV)

**Table ii.** Basic descriptive statistics of histological and micro-tomographic parameters of the mandible.

Parameters	Sham (n = 5)			OVX (n = 5)			OVX-ZOL (n = 5)		
	Mean	sd (CV %)	Range	Mean	sd (CV %)	Range	Mean	sd (CV %)	Range
2D histology									
BV/TB (%)	60.33	2.52 (4.17)	58.03 to 63.16	48.00	5.29 (11.02)	44.23 to 53.96	62.19	1.02 (1.64)	60.05 to 62.33
BS/BV ( $\text{mm}^2/\text{mm}^3$ )	16.69	0.15 (0.90)	16.53 to 16.83	19.33	1.28 (6.62)	18.37 to 20.78	16.24	0.08 (0.49)	16.17 to 16.32
Tb.Dm (mm)*	0.24	0.02 (6.45)	0.22 to 0.25	0.18	0.02 (11.11)	0.16 to 0.20	0.22	0.02 (7.05)	0.20 to 0.23
Tb.N (1/mm)	4.20	0.27 (6.41)	3.98 to 4.50	4.87	0.23 (4.78)	4.66 to 5.12	4.94	0.14 (2.89)	4.82 to 5.10
Tb.Sp (mm)	0.07	0.01 (14.29)	0.06 to 0.08	0.10	0.01 (5.97)	0.09 to 0.10	0.05	0.01 (20.00)	0.04 to 0.06
3D tomography									
BV/TB (%)	61.11	2.50 (4.09)	58.25 to 62.86	50.01	3.79 (7.58)	46.11 to 53.68	64.45	2.83 (4.38)	62.77 to 67.71
BS/BV ( $\text{mm}^2/\text{mm}^3$ )	17.29	0.80 (4.60)	16.44 to 18.01	23.18	1.74 (7.52)	21.24 to 24.61	17.65	0.83 (4.72)	16.69 to 18.22
Tb.Th (mm) <sup>†</sup>	0.12	0.01 (4.66)	0.11 to 0.12	0.09	0.01 (7.77)	0.08 to 0.09	0.11	0.01 (4.84)	0.11 to 0.12
Tb.N (1/mm)	5.28	0.18 (3.49)	5.11 to 5.48	5.78	0.37 (6.38)	5.46 to 6.18	5.68	0.03 (0.61)	5.65 to 5.72
Tb.Sp (mm)	0.07	0.01 (7.99)	0.07 to 0.08	0.09	0.01 (11.88)	0.08 to 0.10	0.06	0.005 (7.55)	0.06 to 0.07

BV/TB, bone volume density; BS/BV, bone surface density; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; OVX, ovariectomised rats; OVX-ZOL, zoledronate-injected ovariectomised rats

\*Assumes that trabeculae are cylindrical rods; Tb.Dm = 4/(BS/BV)

†Assumes that trabeculae are thin plates; Tb.Th = 2/(BS/BV)

The coefficient of variation (CV) is calculated as the standard deviation (sd) in percentage of the mean

**Table iii.** Bivariate linear regressions as well as mean actual and percentage differences between histological and micro-tomographic parameters of the mandible

Parameter (n=15)	Coefficient of correlation ( <i>r</i> )	Coefficient of determination ( <i>r</i> <sup>2</sup> ) (%)	Mean actual difference	Mean % difference
Mandible				
BV/TV	0.95	90	2.080	3.55
BS/BV	0.94	88	1.956 mm <sup>2</sup> /mm <sup>3</sup>	9.24
Tb.Dm* (Tb.Th) <sup>†</sup>	0.86	74	-0.106 mm (-0.0002 mm <sup>‡</sup> )	-101.08 (-0.54 <sup>‡</sup> )
Tb.N	0.90	81	0.909/mm	16.39
Tb.Sp	0.92	85	0.002 mm	4.59
Tibia				
BV/TV	0.97	94	9.683	24.70
BS/BV	0.99	98	4.325 mm <sup>2</sup> /mm <sup>3</sup>	16.84
Tb.Dm* (Tb.Th) <sup>†</sup>	0.85	72	-0.068 mm (0.006 mm <sup>‡</sup> )	-96.88 (1.56 <sup>‡</sup> )
Tb.N	0.87	76	1.773/mm	31.81
Tb.Sp	0.97	94	0.001 mm	10.37

BV/TV, bone volume density; BS/BV, bone surface density; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; OVX, ovariectomised rats; OVX-ZOL, zoledronate-injected ovariectomised rats

\*Assumes that trabeculae are cylindrical rods; Tb.Dm = 4/(BS/BV)

†Assumes that trabeculae are thin plates; Tb.Th = 2/(BS/BV)

‡The results are calculated when Tb.Dm is derived to Tb.Th according to above formula (Tb.Dm = 2\* Tb.Th)

Mean actual difference is the average of 3D parameters minus 2D parameters

Mean percentage difference is the average of the percentage differences between undecalcified histological sections and micro-CT

The formula of the percentage differences is (3D parameters – 2D parameters) / 2D parameters

**Table iv.** Basic descriptive statistics of histological and micro-tomographic parameters of the tibia

Parameters	Sham (n = 5)			OVX (n = 5)			OVX-ZOL (n = 5)		
	Mean	sd (CV %)	Range	Mean	sd (CV %)	Range	Mean	sd (CV %)	Range
2D histology									
BV/TV (%)	40.33	4.04 (10.02)	38.11 to 45.24	14.67	2.08 (14.19)	12.87 to 17.41	47.33	3.51 (7.42)	43.83 to 51.35
BS/BV (mm <sup>2</sup> /mm <sup>3</sup> )	24.68	1.38 (5.59)	23.35 to 26.11	32.65	1.04 (3.19)	31.38 to 33.33	14.09	2.38 (16.89)	11.85 to 17.83
Tb.Dm (mm)*	0.15	0.01 (6.67)	0.14 to 0.17	0.12	0.01 (8.33)	0.12 to 0.13	0.17	0.01 (5.88)	0.16 to 0.19
Tb.N (1/mm)	4.40	0.42 (9.65)	4.05 to 4.87	2.70	0.25 (9.25)	2.50 to 2.98	4.39	0.81 (18.45)	3.60 to 5.21
Tb.Sp (mm)	0.06	0.01 (9.12)	0.06 to 0.07	0.21	0.03 (13.53)	0.18 to 0.23	0.05	0.01 (12.37)	0.04 to 0.05
3D tomography									
BV/TV (%)	47.20	2.51 (5.31)	45.41 to 50.70	23.40	1.60 (6.82)	22.00 to 25.14	60.78	2.98 (4.91)	58.74 to 64.20
BS/BV (mm <sup>2</sup> /mm <sup>3</sup> )	28.95	1.37 (4.73)	27.84 to 30.48	36.62	1.17 (3.19)	35.72 to 37.94	18.82	3.63 (19.26)	16.19 to 22.96
Tb.Th (mm) <sup>†</sup>	0.07	0.003 (4.64)	0.066 to 0.072	0.055	0.001 (1.82)	0.053 to 0.056	0.12	0.004 (3.33)	0.11 to 0.12
Tb.N (1/mm)	6.82	0.21 (3.08)	6.58 to 6.97	4.28	0.27 (6.21)	3.98 to 4.49	5.70	0.97 (17.00)	5.09 to 6.82
Tb.Sp (mm)	0.08	0.01 (6.77)	0.07 to 0.08	0.18	0.01 (8.30)	0.17 to 0.20	0.07	0.01 (15.47)	0.06 to 0.08

BV/TV, bone volume density; BS/BV, bone surface density; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; OVX, ovariectomised rats; OVX-ZOL, zoledronate-injected ovariectomised rats

\*Assumes that trabeculae are cylindrical rods; Tb.Dm = 4/(BS/BV)

†Assumes that trabeculae are thin plates; Tb.Th = 2/(BS/BV)

The coefficient of variation (CV) is calculated as the standard deviation (sd) in percentage of the mean

**Table v.** Comparison of the same histomorphometric parameters of the tibia or mandible among sham, ovariectomised (OVX) and zoledronate-injected ovariectomised (OVX-ZOL) groups by histological indices or micro-CT analysis

<b>Parameters</b>	<b>Tibia</b>			<b>Mandible</b>		
	<b>Sham (n = 5)</b>	<b>OVX (n = 5)</b>	<b>OVX-ZOL (n = 5)</b>	<b>Sham (n = 5)</b>	<b>OVX (n = 5)</b>	<b>OVX-ZOL (n = 5)</b>
2D histology						
BV/BV (%)	40.33 SD 4.04	14.67 SD 2.08§	47.33 SD 3.51‡**	60.33 SD 2.52	48.00 SD 5.29§	62.19 SD 1.02**
BS/BV (mm <sup>2</sup> /mm <sup>3</sup> )	24.68 SD 1.38	32.65 SD 1.04§	14.09 SD 2.38**,**	16.69 SD 0.15	19.33 SD 1.28	16.24 SD 0.08
Tb.Dm (mm)*	0.15 SD 0.01	0.12 SD 0.01§	0.17 SD 0.01**	0.24 SD 0.02	0.18 SD 0.02§	0.22 SD 0.02¶
Tb.N (1/mm)	4.40 SD 0.42	2.70 SD 0.25§	4.39 SD 0.81**	4.20 SD 0.27	4.87 SD 0.23§	4.94 SD 0.14§
Tb.Sp (mm)	0.06 SD 0.01	0.21 SD 0.03‡	0.05 SD 0.01¶	0.07 SD 0.01	0.10 SD 0.01§	0.05 SD 0.01‡.**
3D tomography						
BV/BV (%)	47.20 SD 2.51	23.40 SD 1.60§	60.78 SD 2.98§,**	61.11 SD 2.50	50.01 SD 3.79§	64.45 SD 2.83**
BS/BV (mm <sup>2</sup> /mm <sup>3</sup> )	28.95 SD 1.37	36.62 SD 1.17§	18.82 SD 3.63§,**	17.29 SD 0.80	23.18 SD 1.74§	17.65 SD 0.83**
Tb.Th (mm)†	0.07 SD 0.003	0.05 SD 0.001§	0.12 SD 0.004§,**	0.12 SD 0.01	0.09 SD 0.01§	0.11 SD 0.01**
Tb.N (1/mm)	6.82 SD 0.21	4.28 SD 0.27§	5.70 SD 0.97	5.28 SD 0.18	5.78 SD 0.37‡	5.68 SD 0.03
Tb.Sp (mm)	0.08 SD 0.01	0.18 SD 0.01§	0.07 SD 0.01**	0.07 SD 0.01	0.09 SD 0.01	0.06 SD 0.005**

BV/BV bone volume density, BS/BV bone surface density, Tb.Th trabecular thickness, Tb.N trabecular number, Tb.Sp trabecular separation, OVX ovariectomised rats, OVX-ZOL zoledronate-injected ovariectomised rats

Data are expressed as mean and SD

\*Assumes that trabeculae are cylindrical rods; Tb.Dm = 4/(BS/BV)

†Assumes that trabeculae are thin plates; Tb.Th = 2/(BS/BV)

‡p < 0.05

§p < 0.01 vs sham group (one-way analysis of variance (ANOVA))

¶p < 0.05

\*\*p < 0.01 vs OVX group (one-way ANOVA)