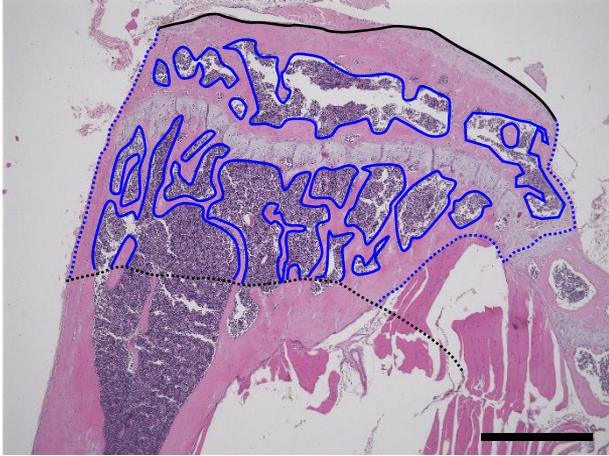
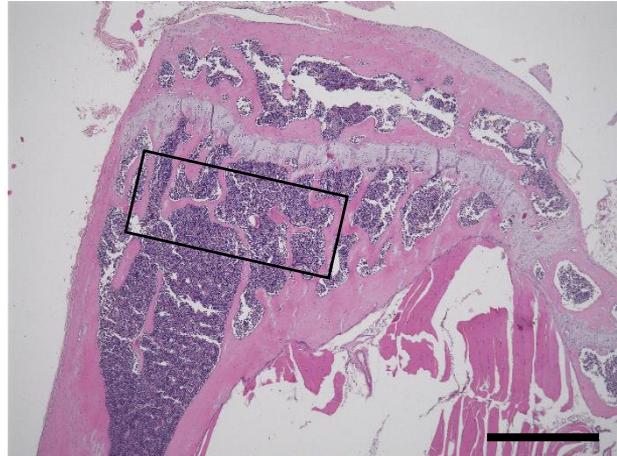
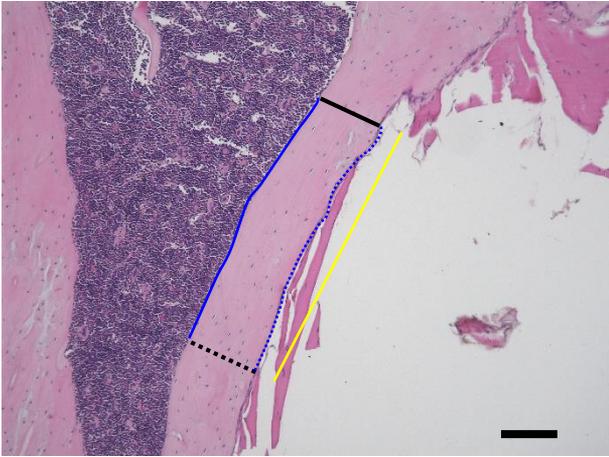




Title	Feature Article: Altered morpho-functional features of bones in autoimmune disease-prone BXSB/MpJ-Yaa mice
Author(s)	Namba, Takashi; Ichii, Osamu; Nakamura, Teppei; Masum, Md Abdul; Otani, Yuki; Otsuka-Kanazawa, Saori; Elewa, Yaser Hosny Ali; Kon, Yasuhiro
Citation	Experimental biology and medicine, 244(5), 333-343 https://doi.org/10.1177/1535370219832810
Issue Date	2019-04
Doc URL	http://hdl.handle.net/2115/75199
Rights	Takashi Namba, Osamu Ichii, Teppei Nakamura, Md Abdul Masum, Yuki Otani, Saori Otsuka-Kanazawa, Yaser Hosny Ali Elewa, and Yasuhiro Kon, Feature Article: Altered morpho-functional features of bones in autoimmune disease-prone BXSB/MpJ-Yaa mice, Experimental Biology and Medicine 244(5) pp. 333-343. Copyright © 2019 the Society for Experimental Biology and Medicine. DOI: 10.1177/1535370219832810.
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Supplemental Figure.pdf

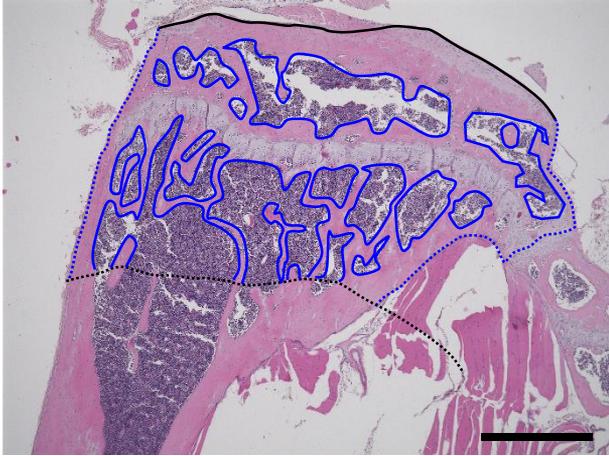
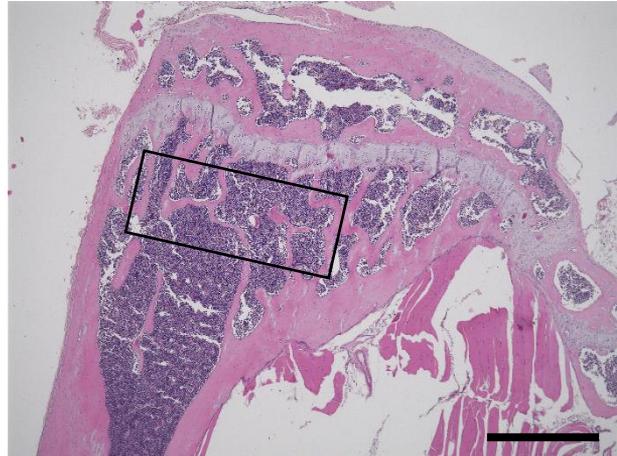
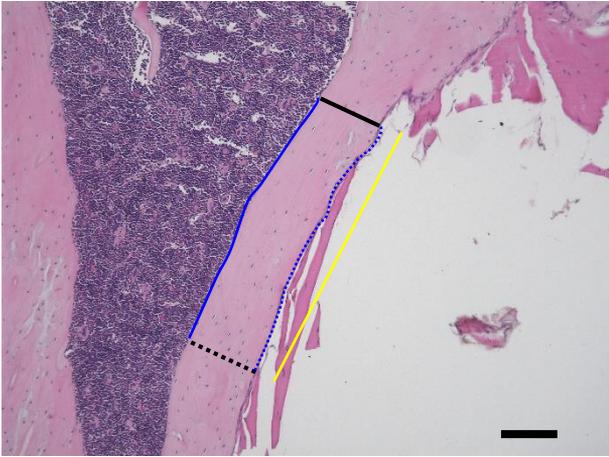


[Instructions for use](#)

(a)**(b)****(c)****(d)**

Supplementary Figure 1. The measurement of bone histoplanimetry.

- (a) The measurement of area ratio of bone to bone marrow (BM). Bars = 500 μm . Digital images of hematoxylin and eosin (HE) stained sections were taken by Nanozoomer 2.0 RS (Hamamatsu Photonics Co., Ltd.; Hamamatsu, Japan), and the ratio of bone to BM is measured after drawing lines using NDP.view2 (Hamamatsu Photonics Co., Ltd.). In the tibia, the line along the surface of articular cartilage at proximal epiphysis (line A, black), the same shaped line with line A at 2 mm distal from the line A (line B, black-dotted), and the lines along the medial and lateral surfaces of the bone (blue line C and blue-dotted line C') were drawn on the digital image using NDP.view2. The measurement was performed within the defined area surrounded by the lines A, B, C, and C'.
- (b) The measurements of trabecular bones. Bars = 500 μm . Using HE stained sections, the box, whose length along the long axis direction is 500 μm , was drawn 100 μm away from the growth plate and compact bone drawing square using NDP.view2. In the mentioned box, the tissue area (T.Ar), trabecular bone area (B.Ar), and bone perimeter (B.Pm) were measured, and then trabecular area (Tb.Ar/T.Ar), trabecular width (Tb.Wi), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were calculated according to a parallel plate model.^{25, 26} $\text{Tb.Wi} = 2/(\text{B.Pm}/\text{B.Ar}) \times 1000$; $\text{Tb.N} = (\text{BV}/\text{TV})/\text{Tb.Wi}$; $\text{Tb.Sp} = (1/\text{Tb.N}) - \text{Tb.Wi}$.
- (c) The measurement of number of osteocytes. Bars = 100 μm . To count the number of osteocytes in the HE stained sections, a line of length 500 μm was initially drawn along the long axis direction in the lateral compact bone of the tibia (line D, yellow). Subsequently, the two lines vertical to line D at its tips were drawn on the proximal and distal sides (black line E and black-dotted line E'). The lines along the medial and lateral surfaces of the compact bone were drawn (blue line F and blue-dotted line F'). The number of osteocytes in the area surrounded by the lines E, E', F, and F' was calculated and expressed as number/ mm^2 by NDP.view2.
- (d) The measurement of number of osteoclasts or osteoblasts. Bars = 500 μm . After being stained with the TRAP kit, TRAP⁺ osteoclasts were also counted using Nanozoomer 2.0 RS and NDP.view2. Briefly, similar to line A and B in the tibia, the line along the surface of the articular cartilage at proximal epiphysis (line G, black), the line with the same shape as line G present at 1 mm distal from the line G (line H, black-dotted), and the line along the medial surfaces of the bone between the growth plate and line H (line I, blue) were drawn on the digital image. In the defined area surrounded by line H and I, the number of TRAP⁺ osteoclasts along the line I was calculated and expressed as number/mm by NDP.view2. The same area surrounded by the line H and I was used to evaluate the osteocalcin⁺ osteoblasts. However, along the line I, the area of osteocalcin⁺ osteoblasts lining the medial side of the compact bone and spongy area in the tibia was measured by the BZ-X Analyzer of a fluorescence microscope BZ-X710 (Keyence; Osaka, Japan). The numerical values were expressed as osteocalcin⁺ area in unit area (mm^2/mm).

(a)**(b)****(c)****(d)**

Supplementary Figure 1. The measurement of bone histoplanimetry.

- (a) The measurement of area ratio of bone to bone marrow (BM). Bars = 500 μm . Digital images of hematoxylin and eosin (HE) stained sections were taken by Nanozoomer 2.0 RS (Hamamatsu Photonics Co., Ltd.; Hamamatsu, Japan), and the ratio of bone to BM is measured after drawing lines using NDP.view2 (Hamamatsu Photonics Co., Ltd.). In the tibia, the line along the surface of articular cartilage at proximal epiphysis (line A, black), the same shaped line with line A at 2 mm distal from the line A (line B, black-dotted), and the lines along the medial and lateral surfaces of the bone (blue line C and blue-dotted line C') were drawn on the digital image using NDP.view2. The measurement was performed within the defined area surrounded by the lines A, B, C, and C'.
- (b) The measurements of trabecular bones. Bars = 500 μm . Using HE stained sections, the box, whose length along the long axis direction is 500 μm , was drawn 100 μm away from the growth plate and compact bone drawing square using NDP.view2. In the mentioned box, the tissue area (T.Ar), trabecular bone area (B.Ar), and bone perimeter (B.Pm) were measured, and then trabecular area (Tb.Ar/T.Ar), trabecular width (Tb.Wi), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were calculated according to a parallel plate model.^{25,26} $\text{Tb.Wi} = 2/(\text{B.Pm}/\text{B.Ar}) \times 1000$; $\text{Tb.N} = (\text{BV}/\text{TV})/\text{Tb.Wi}$; $\text{Tb.Sp} = (1/\text{Tb.N}) - \text{Tb.Wi}$.
- (c) The measurement of number of osteocytes. Bars = 100 μm . To count the number of osteocytes in the HE stained sections, a line of length 500 μm was initially drawn along the long axis direction in the lateral compact bone of the tibia (line D, yellow). Subsequently, the two lines vertical to line D at its tips were drawn on the proximal and distal sides (black line E and black-dotted line E'). The lines along the medial and lateral surfaces of the compact bone were drawn (blue line F and blue-dotted line F'). The number of osteocytes in the area surrounded by the lines E, E', F, and F' was calculated and expressed as number/ mm^2 by NDP.view2.
- (d) The measurement of number of osteoclasts or osteoblasts. Bars = 500 μm . After being stained with the TRAP kit, TRAP⁺ osteoclasts were also counted using Nanozoomer 2.0 RS and NDP.view2. Briefly, similar to line A and B in the tibia, the line along the surface of the articular cartilage at proximal epiphysis (line G, black), the line with the same shape as line G present at 1 mm distal from the line G (line H, black-dotted), and the line along the medial surfaces of the bone between the growth plate and line H (line I, blue) were drawn on the digital image. In the defined area surrounded by line H and I, the number of TRAP⁺ osteoclasts along the line I was calculated and expressed as number/mm by NDP.view2. The same area surrounded by the line H and I was used to evaluate the osteocalcin⁺ osteoblasts. However, along the line I, the area of osteocalcin⁺ osteoblasts lining the medial side of the compact bone and spongy area in the tibia was measured by the BZ-X Analyzer of a fluorescence microscope BZ-X710 (Keyence; Osaka, Japan). The numerical values were expressed as osteocalcin⁺ area in unit area (mm^2/mm).