

## Supplemental Figure Legends

**Figure S1. Cx43(M2) antibody enhances HC opening induced by mechanical loading in aged trabecular bone *in vivo*.** Representative images of EB dye uptake in metaphyseal trabecular bone for both loaded and contralateral unloaded tibias of vehicle- and Cx43(M2)-treated mice. White arrowheads indicate EB-positive osteocytes. Scale bar, 20  $\mu$ m.

**Figure S2. Experimental setup for hindlimb suspension to mimic mechanical unloading.** (A) Weekly monitoring of body weights in control and unloading mice of vehicle- and Cx43(M2)-treated mice. n=7-8 per group. (B) Schematic workflow of the experimental plan. (C) Copper wire used for HLS in a mouse tail. Data are expressed as mean  $\pm$  SD. Statistical analysis was performed using two-way ANOVA with Tukey test among different groups (C).

**Figure S3. Effect of enhanced activity of Cx43 HCs by Cx43(M2) on mechanical Mechanical properties during unloading and loading.** (A-D) The three-point bending assay was performed for mid-diaphyseal femur bone of vehicle- and Cx43(M2)-treated mice: (A) stiffness, (B) elastic modulus, (C) ultimate force, and (D) ultimate stress. n=8 per group. (E) Mechanical loading did not change tibial stiffness in either vehicle- or Cx43(M2)-treated mice. n=6-7 per group. Data are expressed as mean  $\pm$  SD. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001. Statistical analysis was performed using the paired t test for loaded and contralateral tibias (E), and two-way ANOVA with Tukey test for differences among groups (A-E).

**Figure S4. Effect of Cx43M2 antibody on osteogenesis in control and unloading mice.** (A) BFR/BS, (B) MAR, and (C) MS/BS were assessed along cortical surfaces of control and unloading

mice treated with either vehicle or Cx43(M2). **(D)** Representative images of double-calcein (green) labeling on tibias. n=3-6 per group. Scale bar: 50  $\mu$ m. Data are expressed as mean  $\pm$  SD. \*, P<0.05. Statistical analysis was performed using two-way ANOVA with Tukey test among different genotypes **(A-C)**.

**Figure S5. Experimental setup for tibia axial compressive loading.** **(A and B)** Schematic illustration of the experimental plan and tibia loading setup. **(C)** Diagram showing the left tibia positioned in the loading device and the direction of loading. **(D)** Weekly body weights of vehicle- and Cx43(M2)-treated groups. n=12 per group. Data are expressed as mean  $\pm$  SD. Statistical analysis was performed using two-way ANOVA with Tukey test among different genotypes **(D)**.

**Figure S6. Cx43(M2) has no additional effect on cortical bone during mechanical stimulation in young adult mice.** The cortical bone structure located 37% distal from the proximal end was analyzed in 16-week-old male mice loaded 5 days/week for 2 weeks. **(A)** Representative 3D models of the cortical bone in vehicle- and Cx43(M2)-treated mice. **(B-D)**  $\mu$ CT was used to assess structural parameters of cortical bone located 37% distal from the proximal end: **(B)** B.Ar, **(C)** B.Ar/T.Ar, and **(D)** Ct.Th. n=6-7 per group. Data are expressed as mean  $\pm$  SD. \*, P<0.05. Statistical analysis was performed using the paired t test for loaded and contralateral tibias **(B-D)**, and two-way ANOVA with Tukey test for differences among groups **(B-D)**.

**Figure S7. Cx43(M2) antibody has no additional effect on load-induced endosteal osteogenesis in young adult mice.** After 5 days/week loading for 2 weeks, bone histomorphometric analyses were performed on the tibias within cortical bone located 37% distal from the proximal end of 16-week-old vehicle- and Cx43(M2)-treated mice. **(A)** Representative

images of calcein (green) and alizarin (red) double labeling at the 37% diaphysis for all groups. Scale bar: 200  $\mu$ m. **(B and E)** MAR, **(C and F)** MS/BS, and **(D and G)** BFR/BS were assessed along periosteal and endosteal surfaces of all tibias. n=3 per group. Scale bar: 200  $\mu$ m. Data are expressed as mean  $\pm$  SD. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001. Statistical analysis was performed using the paired t test for loaded and contralateral tibias **(B-G)**, and two-way ANOVA with Tukey test for differences among groups **(B-G)**.

**Figure S8. Cx43(M2) inhibits osteoclasts in tibial metaphyseal trabecular bone during mechanical loading.** **(A)** Representative images of TRAP-positive osteoclasts (red) in tibial metaphyseal trabecular bone. Scale bar, 80  $\mu$ m. **(B)** Quantification of TRAP-positive osteoclast number per bone perimeter (N.Oc/BS) and **(C)** osteoclast surface per bone perimeter (Oc.S/BS) in tibial metaphyseal trabecular bone. n=5 per group. Data are expressed as mean  $\pm$  SD. \*, P<0.05. Statistical analysis was performed using the paired t test for loaded and contralateral tibias **(B and C)**, and two-way ANOVA with Tukey test for differences among groups **(B and C)**.

Fig. S1

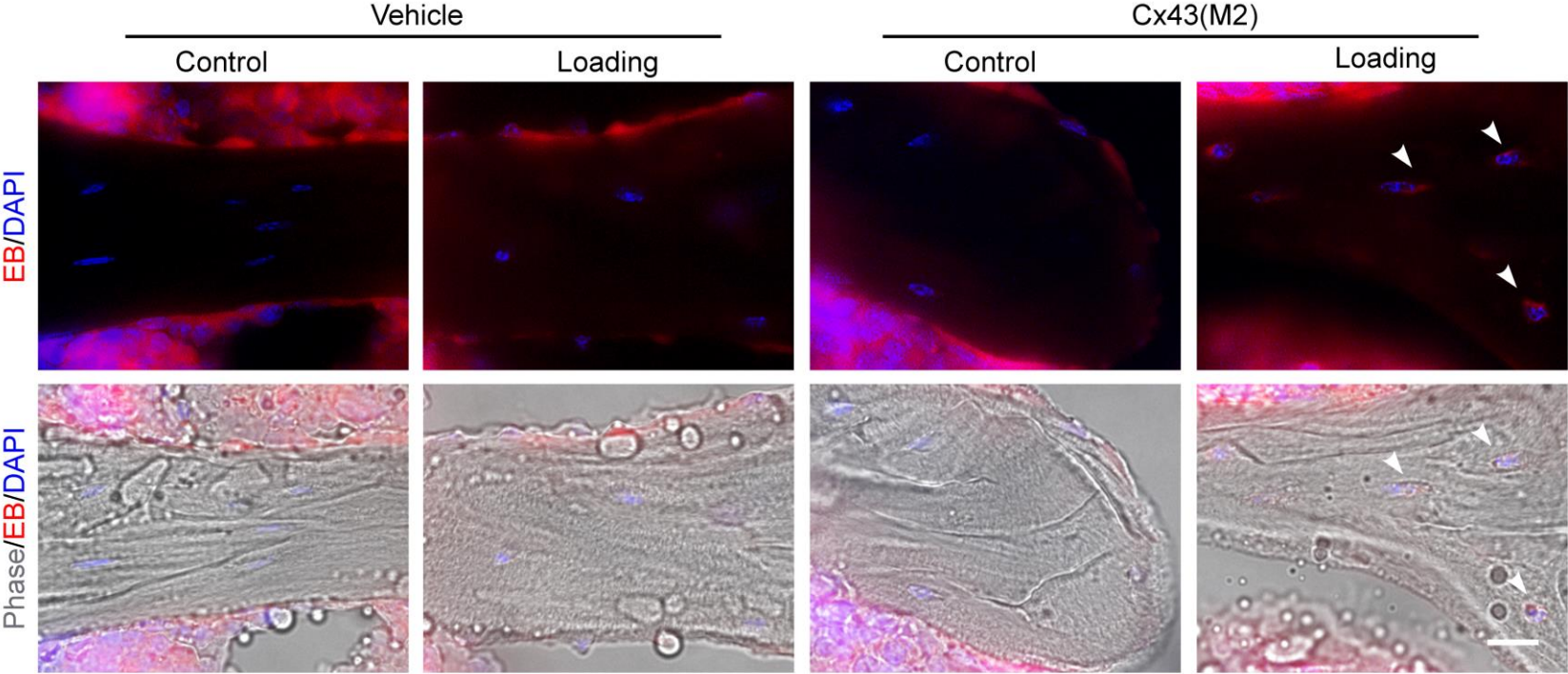


Fig. S2

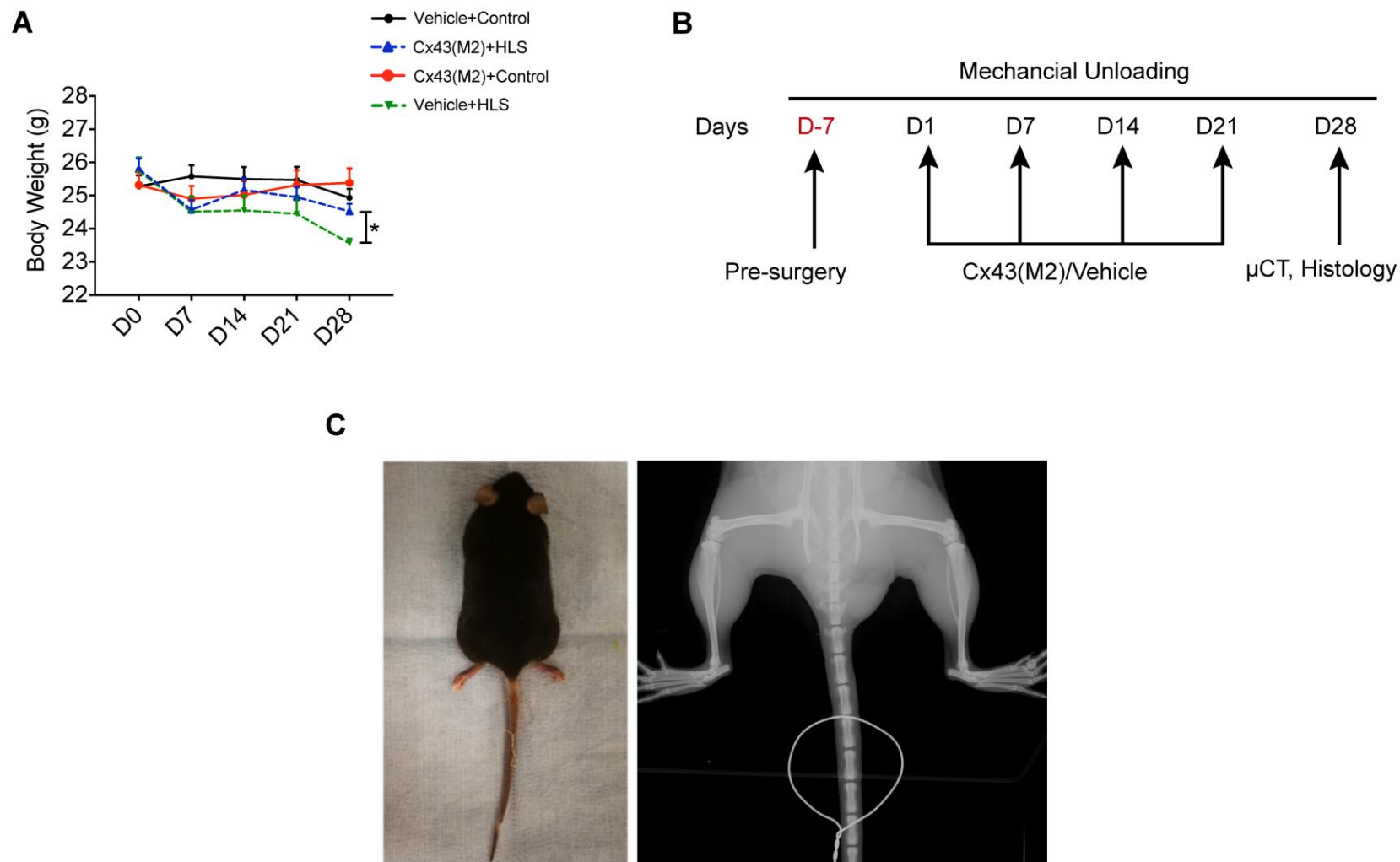


Fig. S3

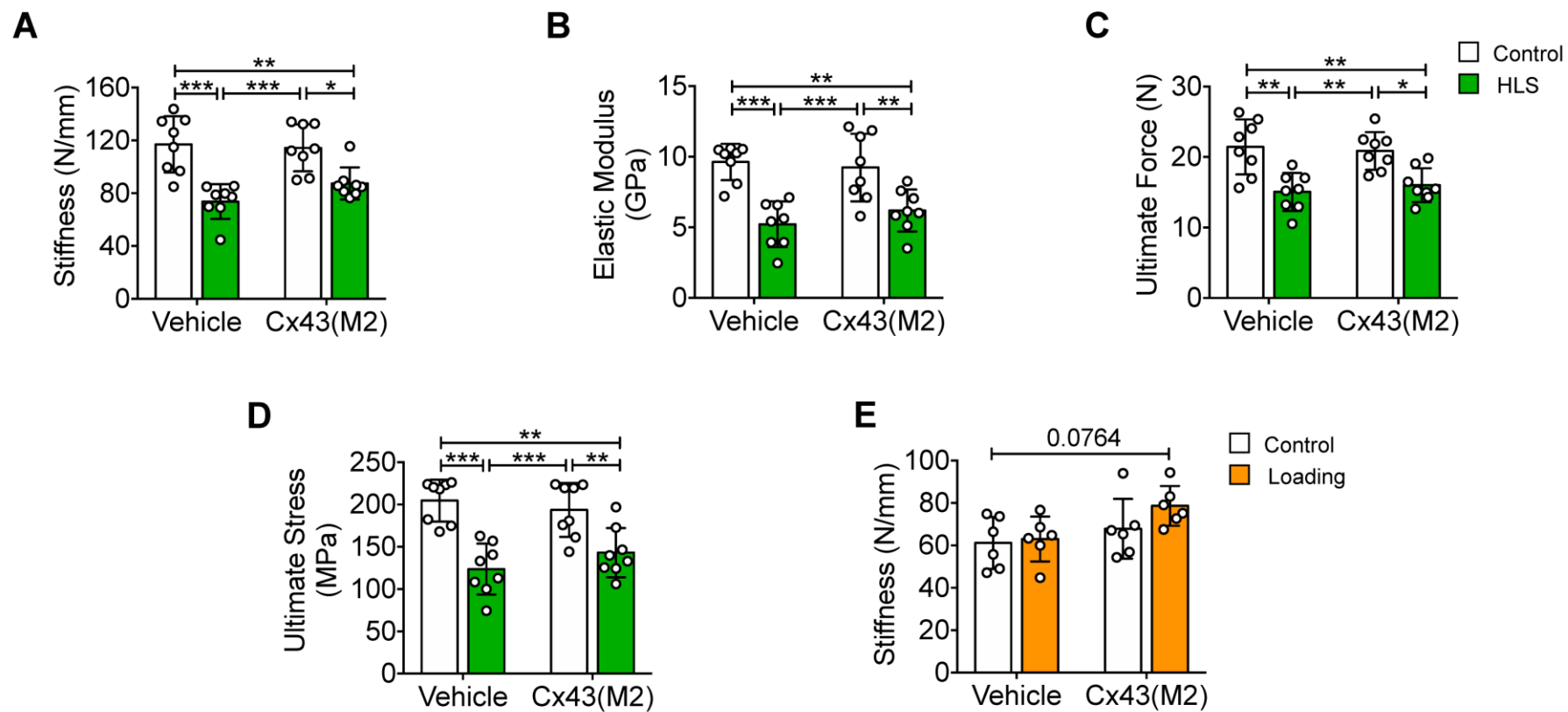


Fig. S4

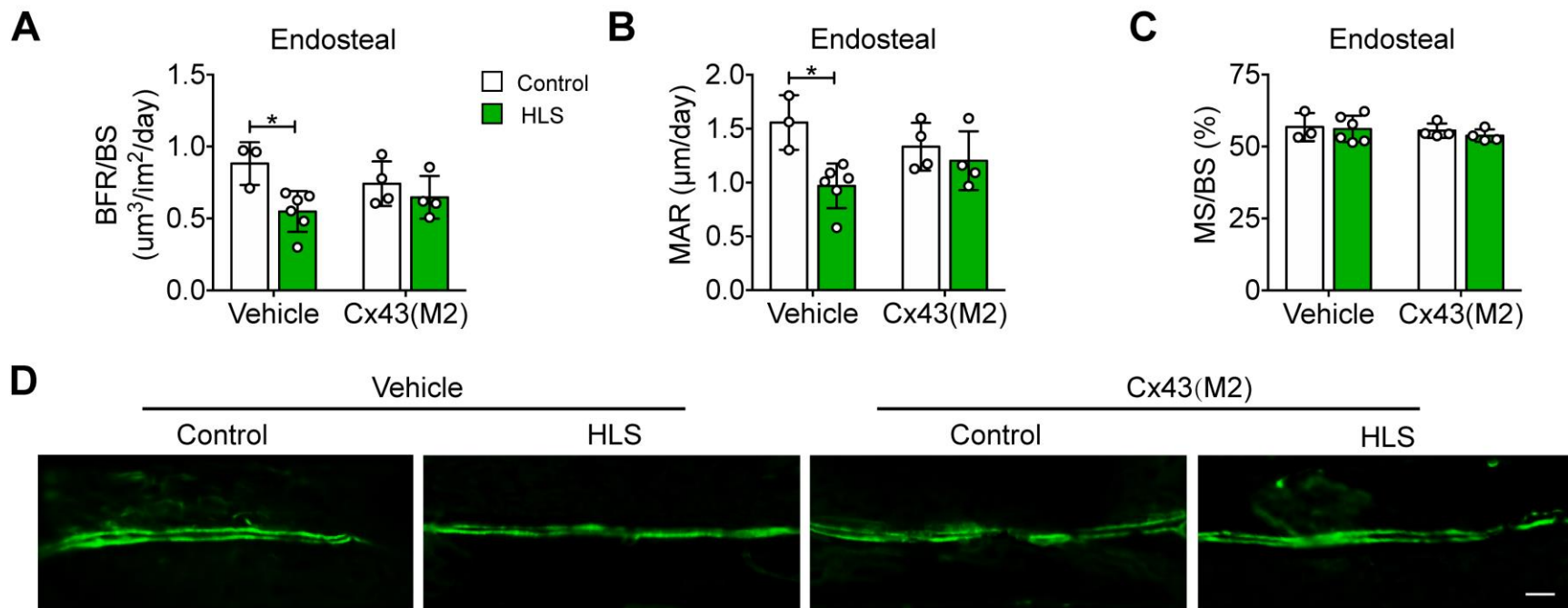


Fig. S5

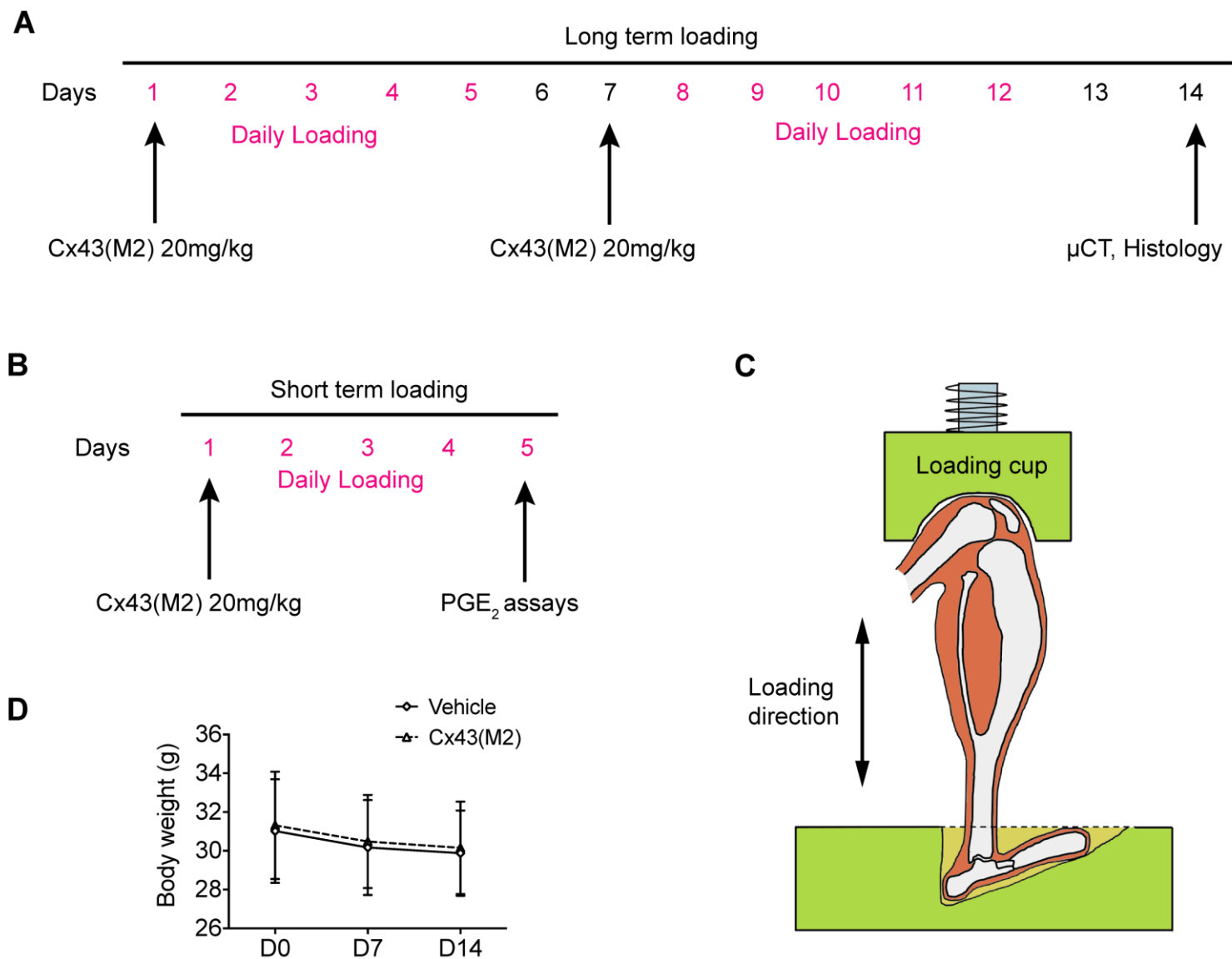


Fig. S6

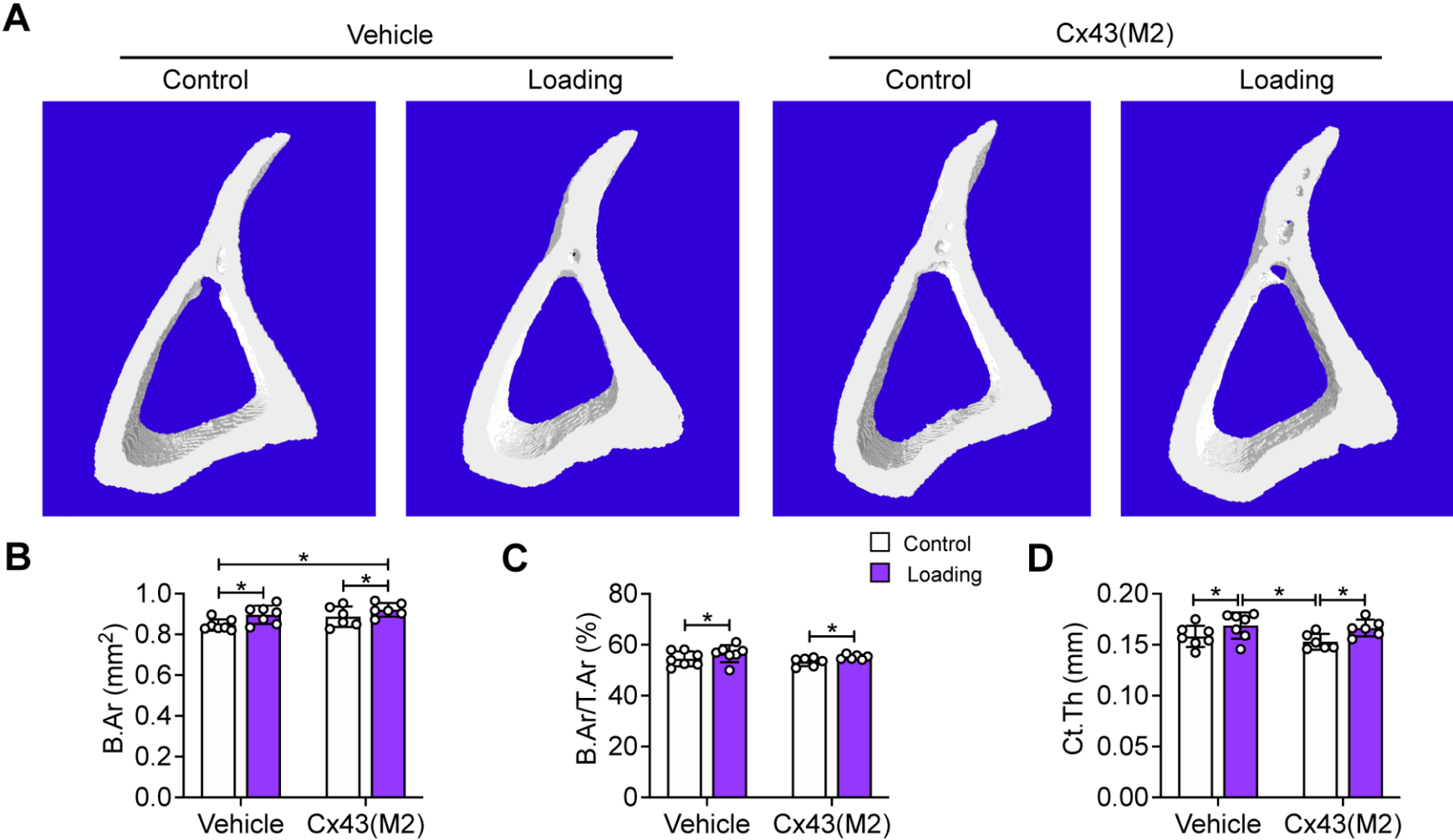
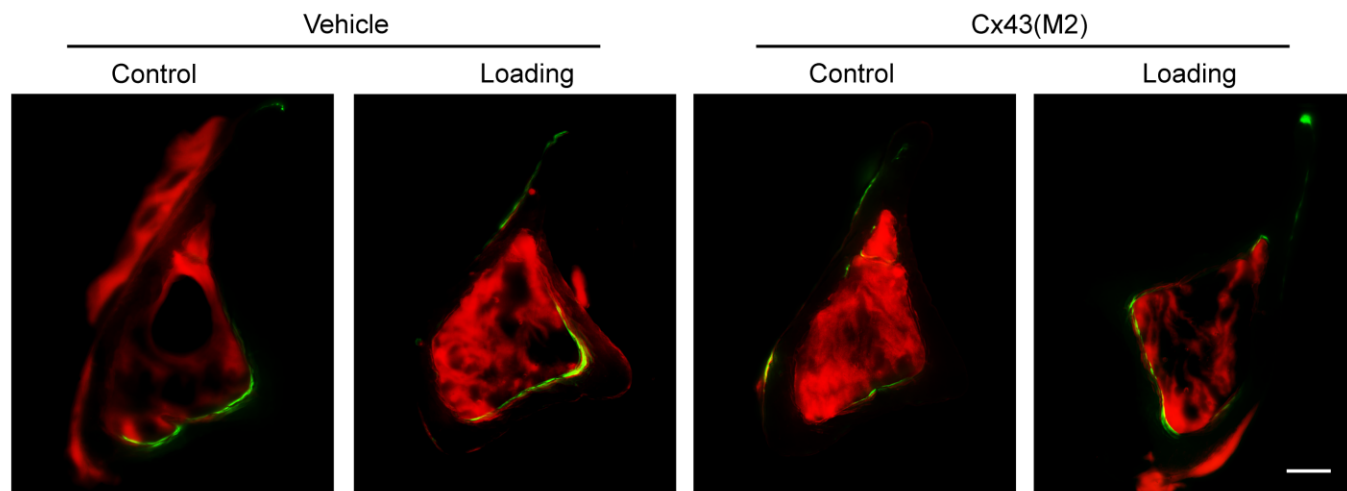
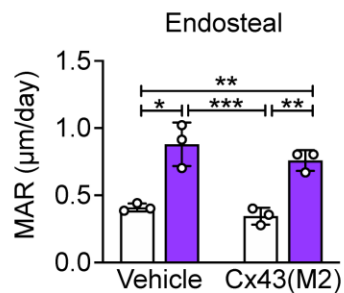


Fig. S7

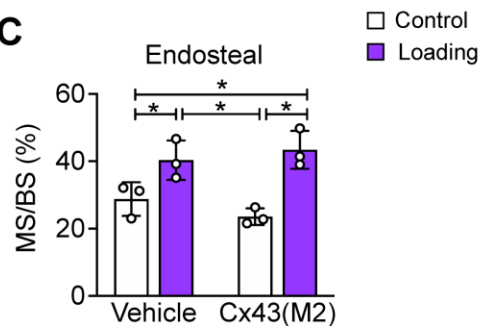
**A**



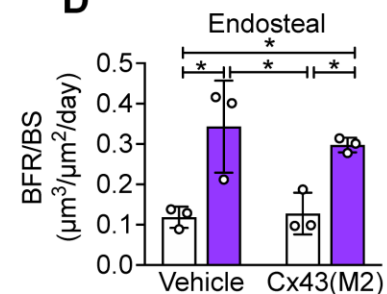
**B**



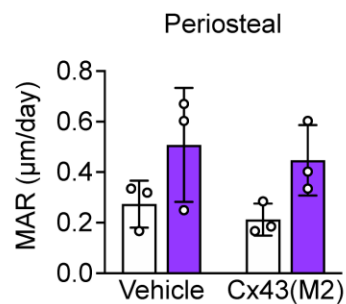
**C**



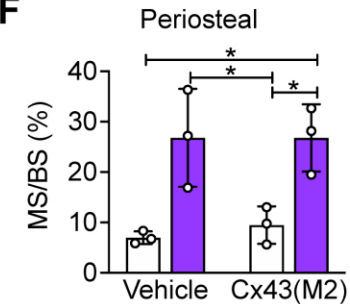
**D**



**E**



**F**



**G**

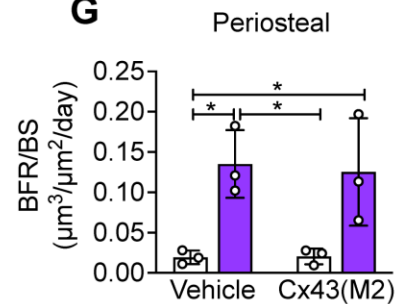


Fig. S8

