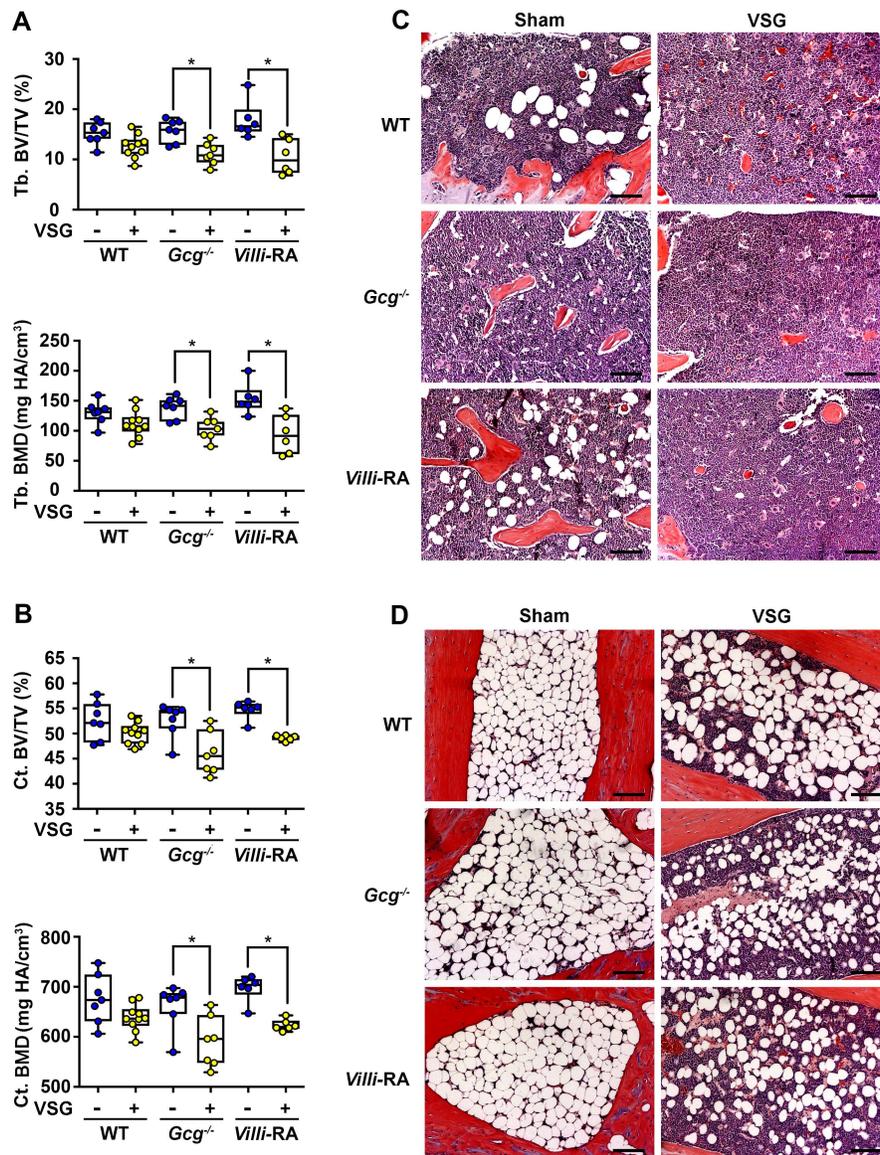
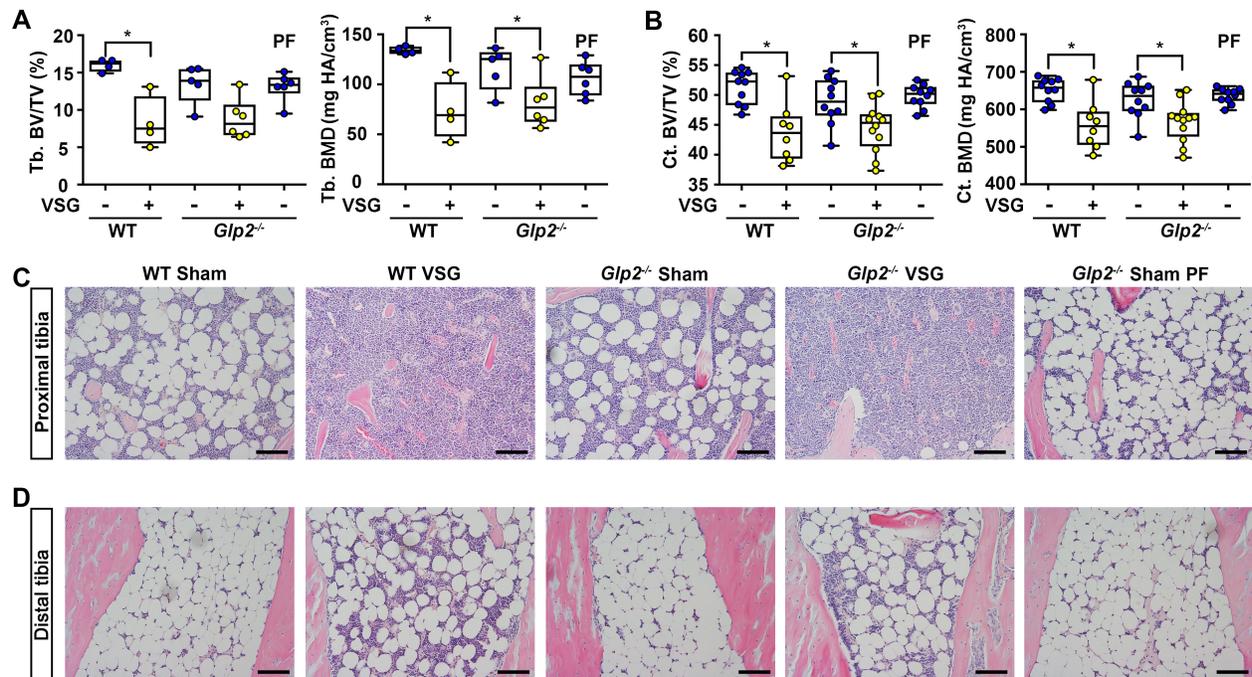


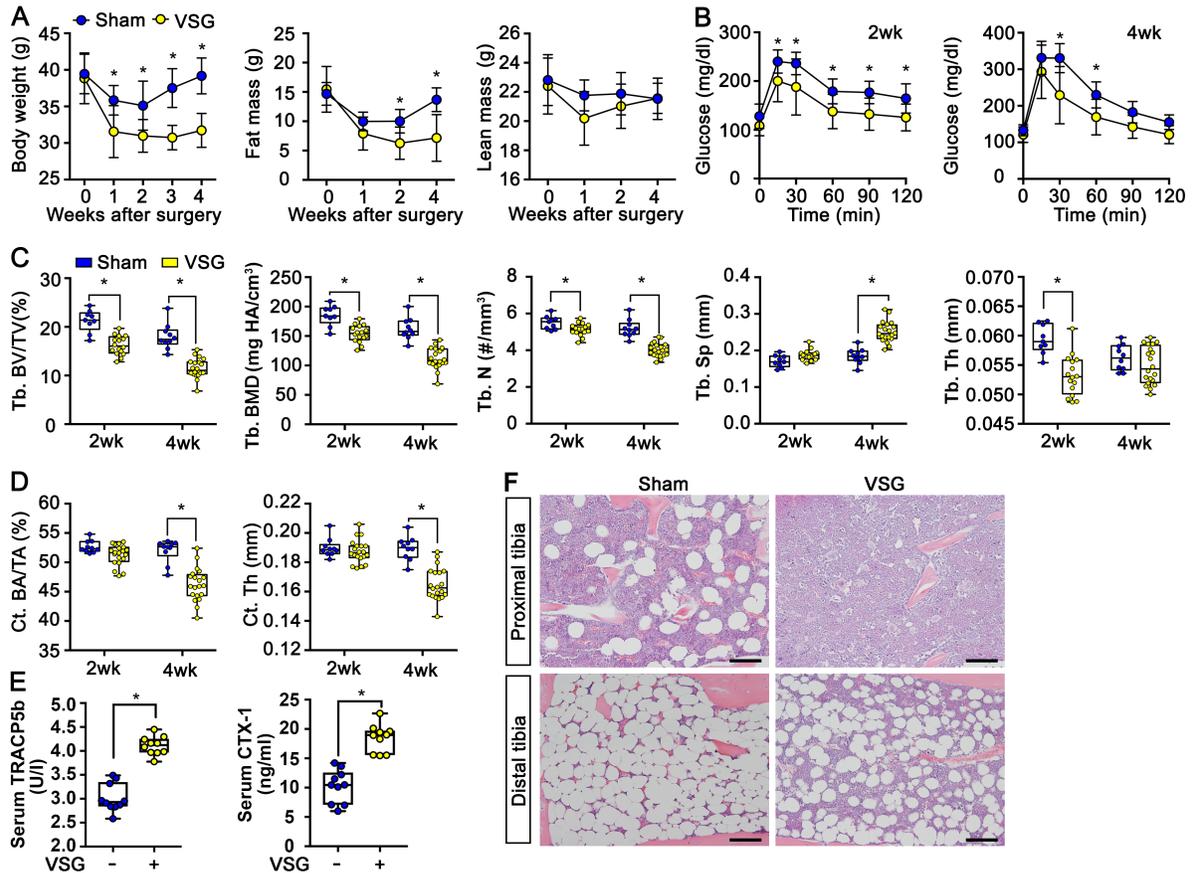
**Supplemental Figure 1. VSG induced bone and BMAT loss is independent of body weight, diet and sex.** Male and female C57BL/6J mice at 4 wk of age were fed a 60% HFD for 8 wk. Mice then received sham or VSG surgery and were placed on a NCD or HFD for a further 8 wk. Data in **A** and **B** are from male mice, whilst panels **C-J** are from female mice. **(A)** Linear regression of relationship between mid-cortical bone area and body weight, trabecular bone volume and body weight, and **(B)** distal tibial cortical bone area and BMAT, and between weights of gWAT and distal tibial BMAT were analyzed. **(C)** Body weight and fat mass of female mice were measured before euthanasia. **(D)** Mid-tibial cortical bone area (Ct. BA/TA) and thickness (Ct. Th) were measured with  $\mu$ CT. **(E-F)** Tibial trabecular bone volume fraction (Tb. BV/TV), bone mineral density (Tb. BMD), trabecular number (Tb. N), and thickness (Tb. Th) were estimated with  $\mu$ CT. **(G)** Decalcified tibiae were stained with osmium tetroxide and scanned by  $\mu$ CT. Quantification of BMAT relative to total bone volume at various regions was shown. **(H)** Linear regression of relationship between mid-cortical bone area and body weight in female mice was analyzed. **(I-J)** 4 wk old female mice were fed with 60% HFD for 7 wk, and then received ovariectomy (OVX). After recovering for 5 wk, VSG or sham surgery was performed on these mice. Each group was then fed NCD or HFD for a further 8 wk. **(I)** Mid-tibial cortical bone area (Ct. BA/TA) and thickness (Ct. Th). **(J)** Tibial trabecular bone volume fraction (Tb. BV/TV) and bone mineral density (Tb. BMD) were estimated with  $\mu$ CT. \* indicates statistical difference at  $P < 0.05$  by one-way ANOVA with Tukey's multiple comparisons test (**C-F** and **H-J**).



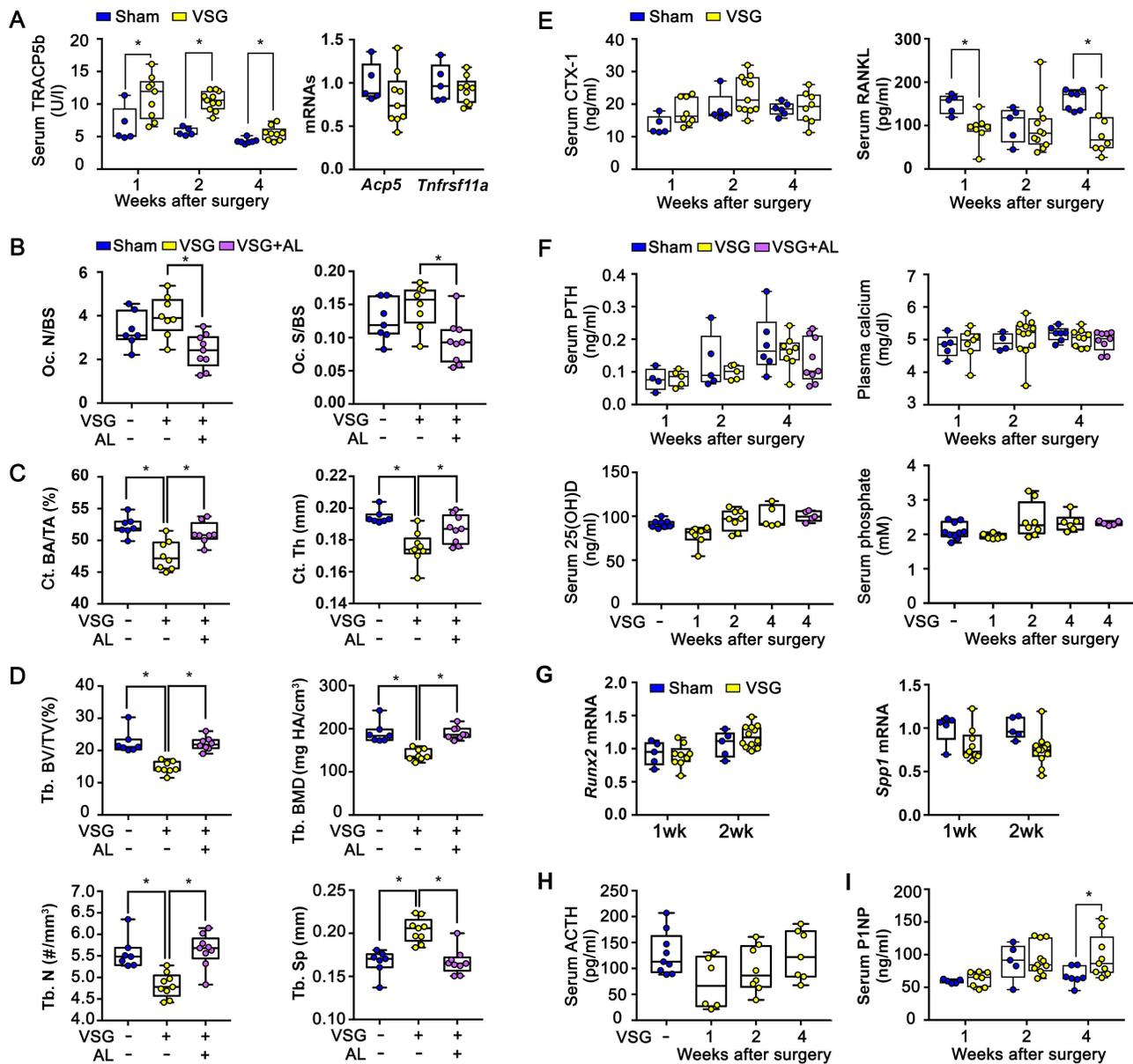
**Supplemental Figure 2. VSG induced bone and BMAT loss is independent of the proglucagon locus.** Animal models studied were global proglucagon (*Gcg*) knock-out mice (*Gcg*<sup>-/-</sup>) and global *Gcg*<sup>-/-</sup> knockout mice were *gcg* was specifically reactivated in intestine by crossing *Gcg*<sup>-/-</sup> mice with *villin 1-cre* mice (*Vill-RA*). These transgenic mice and their littermates received either sham or VSG after feeding 60% HFD for 6 wk and kept on HFD for another 8 wk until euthanasia. **(A)** Tibial trabecular bone volume fraction (Tb. BV/TV), bone mineral density (Tb. BMD), and **(B)** mid-tibial cortical bone area (Ct. BA/TA) and thickness (Ct. Th) were estimated with  $\mu$ CT. Representative sections from **(C)** proximal and **(D)** distal tibiae were stained with H&E and are shown at 200X magnification. Scale bar, 100  $\mu$ m. \* indicates statistical difference at  $P < 0.05$  by two-way ANOVA with Sidak's multiple comparisons test **(A and B)**.



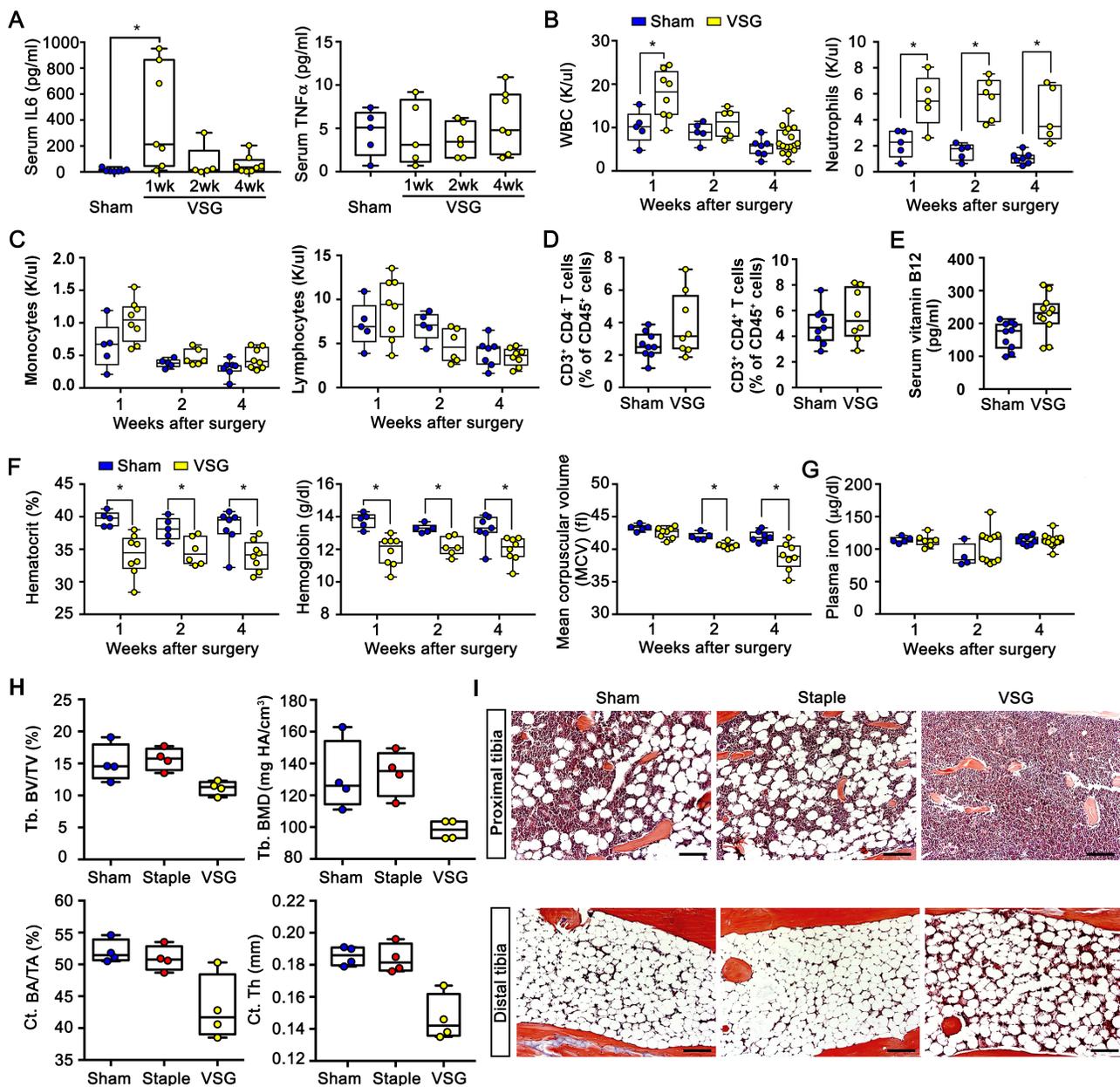
**Supplemental Figure 3. VSG induced bone and BMAT loss is independent of GLP2 receptor deficiency.** Male GLP2 receptor knockout (*Glp2<sup>-/-</sup>*) mice and wild-type littermates were placed on a 60% HFD at 4-6 wk of age and given ad libitum access to food. After 8 weeks, sham or VSG surgery were performed. Pair-feeding (PF) to food consumption of VSG mice continued until sacrifice at 10 wk after surgery. **(A)** Tibial trabecular bone volume fraction (Tb. BV/TV), bone mineral density (Tb. BMD), and **(B)** mid-tibial cortical bone area (Ct. BA/TA) and thickness (Ct. Th) were estimated with  $\mu$ CT. Representative sections from **(C)** proximal and **(D)** distal tibiae were stained with H&E and are shown at 200X magnification. Scale bar, 100  $\mu$ m. \* indicates statistical difference at  $P < 0.05$  by two-way ANOVA with Sidak's multiple comparisons test (**A** and **B**).



**Supplemental Figure 4. VSG causes rapid loss of bone and BMAT in obese mice.** Male C57BL/6J mice at 4 wk of age were fed a 60% HFD for 8 wk at which point they received either sham or VSG surgery. Mice remained on a HFD until euthanasia at 2 or 4 wk after surgery. **(A)** VSG causes loss of body weight and fat mass without altering lean mass. **(B)** Improved glucose tolerance by glucose tolerance test was observed 2 and 4 wk after surgery. **(C)** Trabecular bone volume fraction (Tb. BV/TV), bone mineral density (Tb. BMD), trabecular number (Tb. N), separation (Tb. Sp) and thickness (Tb. Th) were determined by  $\mu$ CT. **(D)** Cortical bone area (Ct. BA/TA) and thickness (Ct. Th) were also measured. **(E)** Circulating concentrations of TRACP5b and CTX-1 were determined at 4 wk after surgery. **(F)** Representative sections from proximal and distal tibiae 2 wk after surgery were stained with H&E and are shown at 200X magnification. Scale bar, 100  $\mu$ m. Data in **A** and **B** are mean  $\pm$  SD. All other data are in box and whisker plots. \* indicates statistical difference for indicated comparisons at  $P < 0.05$  by two-way ANOVA with Sidak's multiple comparisons test (**A-D**) and two-sample  $t$ -test (**E**).

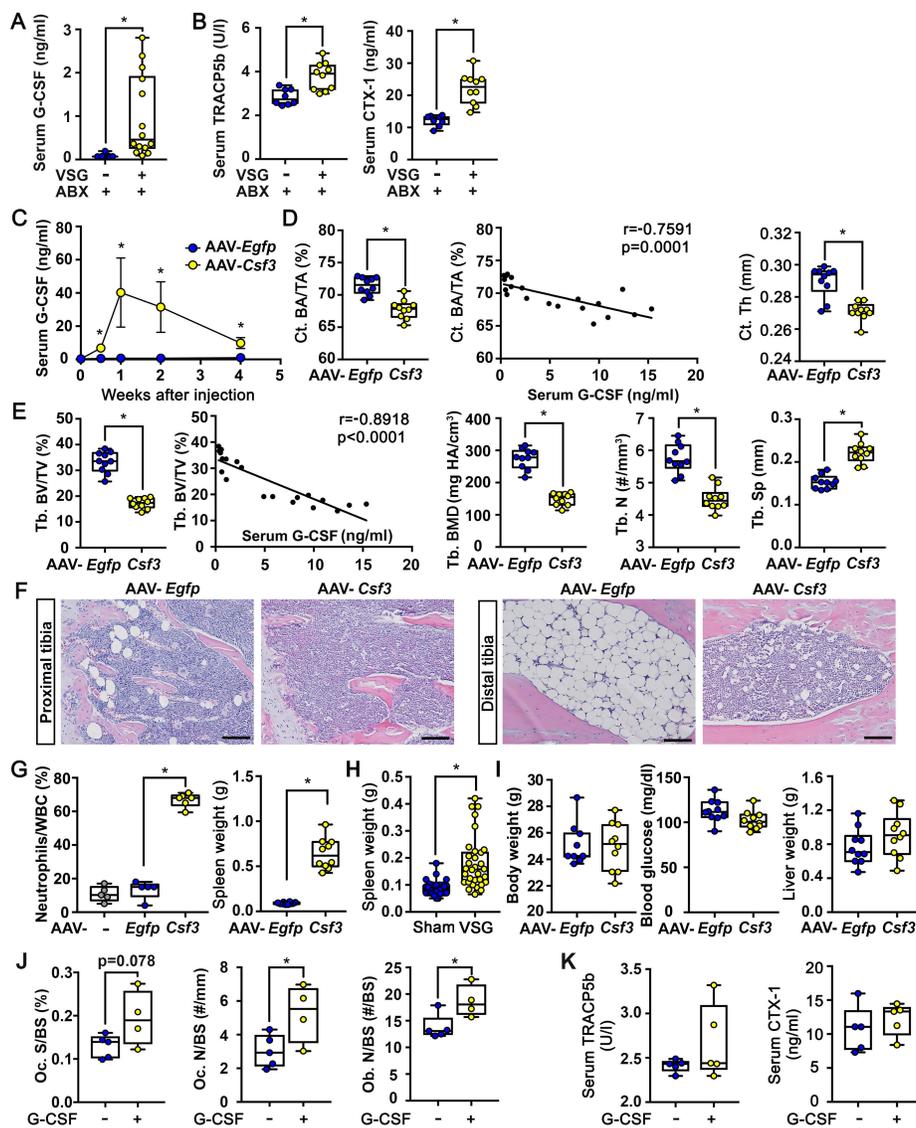


**Supplemental Figure 5. Loss of bone after VSG is blocked by alendronate and independent of calcium metabolism.** Male mice on a NCD received either sham or VSG surgery at 12 wk of age, and were euthanized 1, 2 or 4 wk later. A subgroup of VSG mice was treated with intraperitoneal alendronate (AL; 300  $\mu$ g/kg, twice a week) for 2 wk from the 2<sup>nd</sup> to the 4<sup>th</sup> wk. **(A)** Circulating concentrations of TRACP5b were determined at 1, 2 and 4 wk after surgery. Gene expression of *Acp5* and RANK (*Tnfrsf11a*) was measured by qPCR 1 wk after surgery. **(B)** Tibial bone surface (BS) was determined on H&E-stained decalcified sections and calculated by Bioquant software. Tibial osteoclast number (Oc. N) and surface (Oc. S) were determined from decalcified TRAP-stained sections and normalized by bone surface. **(C)** Mid-tibial cortical bone area (Ct. BA/TA) and thickness (Ct. Th), and **(D)** tibial trabecular bone volume fraction (Tb. BV/TV), bone mineral density (Tb. BMD), trabecular number (Tb. N) and separation (Tb. Sp) were determined with  $\mu$ CT. **(E)** Circulating concentrations of CTX-1 and RANKL were determined at 1, 2 and 4 wk after surgery. **(F)** Circulating PTH, calcium, 25(OH)-vitamin D, and phosphate, concentrations were measured at 1, 2 and 4 wk after surgery. **(G)** Expression of *Runx2* and osteopontin (*Spp1*) mRNAs were determined by qPCR. Circulating concentrations of **(H)** ACTH and **(I)** P1NP were measured at 1, 2 and 4 wk after surgery. \* indicates statistical difference for indicated comparisons at  $P < 0.05$  by two-way ANOVA with Sidak's multiple comparisons test (**A**, **E-F**, **H** and **J**), one-way ANOVA with Tukey's multiple comparisons test (**B-D** and **I**) and two-sample *t*-test (**G**).



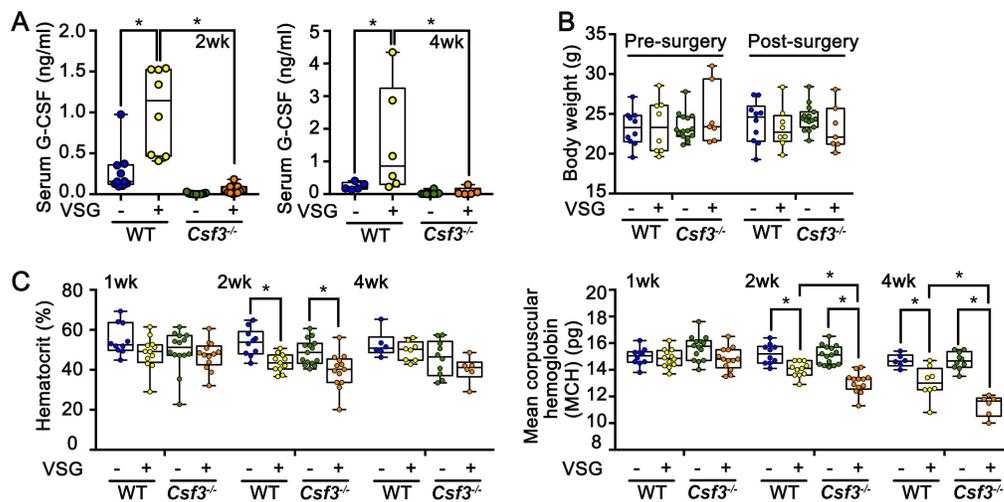
**Supplemental Figure 6. VSG increases circulating neutrophils, and causes microcytic anemia. Bone loss after VSG is dependent on reduced stomach volume rather than surgery, per se.**

(A-G). Male mice on a NCD received either sham or VSG surgery at 12 wk of age, and were euthanized 1, 2 or 4 wk later. (A) Multiplex assay was performed to detect changes in inflammatory factors, IL6 and TNF $\alpha$ . CBC was performed, and circulating (B) WBC, neutrophils, (C) monocytes and lymphocytes were determined. (D) CD3<sup>+</sup> and CD3<sup>+</sup> CD4<sup>+</sup> T cells were sorted by flow cytometry and normalized by CD45<sup>+</sup> cell counts. (E) Circulating concentrations of vitamin B12 were determined at 4 wk after surgery. (F) Hematocrit, hemoglobin and mean corpuscular volume (MCV) were determined by CBC. (G) Circulating iron concentrations. (H-I). Male mice at 4 wk of age were fed a 60% HFD for 8 wk at which point they received either sham or VSG surgery, as well as a Staple Sham group, in which the same surgical procedure was performed as VSG, including staple and suture on stomach, but without a decrease of stomach volume. Tibiae were collected for  $\mu$ CT analysis and decalcification 8 wk after surgery. (H) Trabecular bone volume fraction (Tb. BV/TV), bone mineral density (Tb. BMD), cortical bone area (Ct. BA/TA) and thickness (Ct. Th) were determined by  $\mu$ CT. (I) Decalcified tibiae were paraffin embedded, sectioned and stained with H&E. Representative images from proximal and distal tibiae at 200X magnification are shown. Scale bar, 100  $\mu$ m. \* indicates statistical difference for indicated comparisons at P < 0.05 by one-way ANOVA with Tukey's multiple comparisons test (A and H), two-way ANOVA with Sidak's multiple comparisons test (B-C and F-G) and two-sample *t*-test (D-E).



**Supplemental Figure 7. Elevation of circulating G-CSF after AAV infection causes loss of bone and BMAT.**

(A-B) C57BL/6J male mice were on 60% HFD for 10 wk before surgery. Antibiotics (ABX) were included in drinking water from the 1<sup>st</sup> wk after surgery until they were euthanized 4 wk after surgery. Circulating concentrations of (A) G-CSF, (B) TRACP5b and CTX-1 were determined by ELISA kits. \* indicates statistical difference for indicated comparisons at  $P < 0.05$ . (C-I) 12 wk old C3H/HeJ mice were administered AAV-Egfp or AAV-Csf3 via tail vein injection. Animals were euthanized 4 wk after injection. (C) Circulating G-CSF concentrations were determined each week. (D) Cortical bone area (Ct. BA/TA) and thickness (Ct. Th) were measured by  $\mu$ CT. Inverse correlation between cortical bone area (Ct. BA/TA) and circulating G-CSF concentrations determined by linear regression. (E) Trabecular bone volume fraction (Tb. BV/TV), bone mineral density (Tb. BMD), trabecular number (Tb. N) and separation (Tb. Sp) were calculated. Inverse correlation between trabecular bone volume fraction (Tb. BV/TV) and circulating G-CSF concentrations. (F) Decalcified tibiae were embedded with paraffin, sectioned and stained with H&E. Representative pictures from proximal and distal tibiae with 200X magnification are shown. Scale bar, 100  $\mu$ m. (G) Blood smears were Wright-Giemsa stained and cell types counted in a blinded manner. The proportion of neutrophils in total WBC was determined. Spleen weight was measured during dissection. (H) Weights of spleen were determined in the sham or VSG mice after euthanasia. (I) Body weight, random glucose concentrations before euthanasia and liver weights were determined. (J-K) C3H/HeJ mice at 12 wk of age were implanted with osmotic mini-pumps containing saline (-) or recombinant murine G-CSF (+) (3  $\mu$ g/mouse). Animals were sacrificed 4 wk after implantation. (J) Tibial osteoclast surface (Oc. S) and number (Oc. N) was determined from decalcified TRAP-stained sections. Tibial bone surface (BS) and osteoblast number (Ob. N) were determined on H&E-stained decalcified sections and calculated by Bioquant software. Oc. S, Oc. N and Ob. N were normalized by BS. (K) Circulating concentrations of TRACP5b and CTX-1 were determined. \* indicates statistical difference for indicated comparisons at  $P < 0.05$  by two-sample  $t$ -test (A-B, D-E, and G-K) and two-way ANOVA with Sidak's multiple comparisons test (C).



**Supplemental Figure 8. Circulating G-CSF, body weight, and anemia in WT and G-CSF deficiency mice with VSG.** Female mice at 12 wk of age received a sham (-) or VSG (+) surgery, and blood was collected at 1, 2 and 4 wk of surgery via tail vein for CBC. Animals were euthanized 4 wk after surgery. **(A)** Circulating G-CSF concentrations at 2 and 4 wk after surgery. **(B)** Body weight was measured before surgery and 4 wk after surgery. **(C)** Hematocrit and mean corpuscular hemoglobin were determined by CBC. \* indicate statistical difference for indicated comparisons at  $P < 0.05$  by one-way ANOVA with Tukey's multiple comparisons test **(A)**, two-way ANOVA with Sidak's multiple comparisons test **(B and C)**.