Supplemental Figures

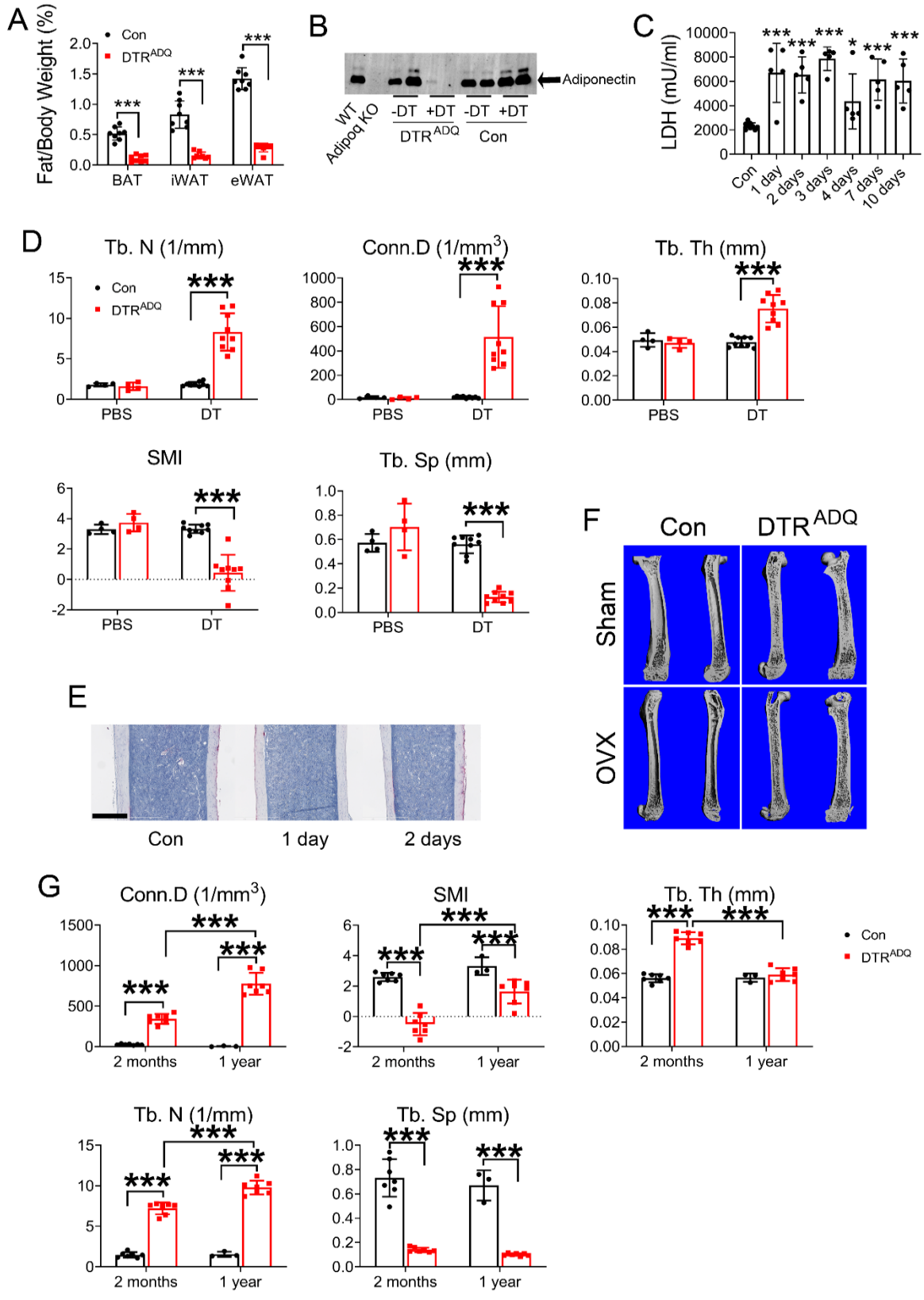


Figure S1. Related to Fig 1. Post-natal generated fat ablated mice are osteosclerotic.

(A) Weight of various fat depots in DTR^{ADQ} or Con mice following 10 days of daily DT administration. *n* = 8/group.

(B) Serum adiponectin of DTR^{ADQ} or Con mice following 7 days of DT (+DT) or PBS (-DT) administration. WT and adiponectin KO mice serve as respective positive and negative controls. *n* = 2 independent experiments.

(C) Serum LDH level of DTR^{ADQ} mice following DT administration as indicated time. WT mice with 3 days of DT administration serve as control (Con). *n* = 5-9/group.

(D) μCT quantitation of structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp) and connection density (Conn.D) of 2 month old DTR^{ADQ} and Con mice following 10 days of DT administration. *n* = 4-9/group.

(E) Representative histological image of femoral diaphysis of DTR^{ADQ} mice following DT administration with time. NO DT treatment serves as control. *n* = 3/group. Scale bar: 400 μm.

(F) Representative μCT image of femurs of DTR^{ADQ} and Con mice treated with DT for 10 days 3 weeks after ovariectomy or sham operation. n = 2–6/group.

(G) μCT quantitation of structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp) and connection density (Conn.D) of 2 month and 1 year old DTR^{ADQ} and Con mice following 10 days of DT administration. *n* = 3-7/group.

Data are presented as mean ± SD. * $P < 0.05$, *** $P < 0.001$ as determined by 1way (c) or 2 way (A, D, G) ANOVA with Holm-Sidak's post hoc analysis for multiple comparisons test.

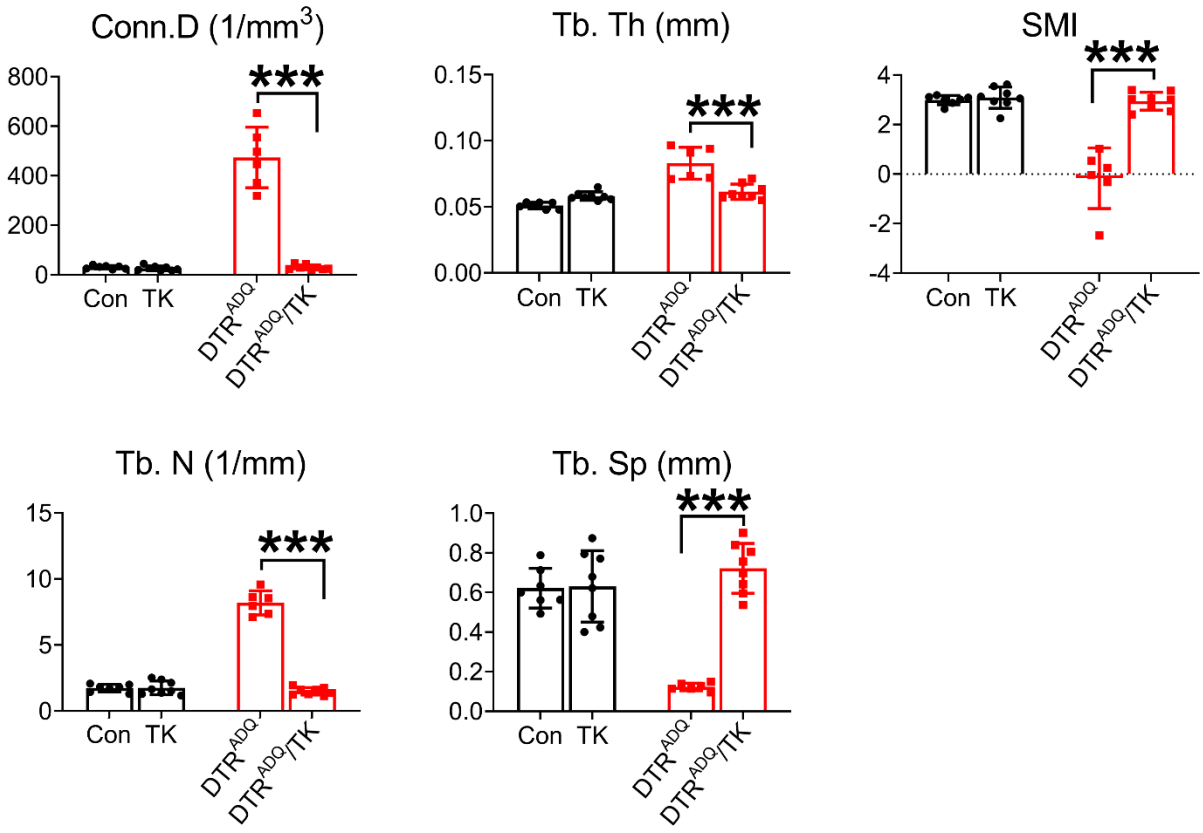


Figure S2. Related to Fig 3. DT/DTR^{ADQ} osteosclerosis is due to enhanced osteoblast recruitment and differentiation.

Con or DTR^{ADQ} mice were mated with Col1a1*3.6 -TK mice (TK). At 2 months of age all 4 genotypes were treated with DT and Ganciclovir for 10 days. μCT quantitation of structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp) and connection density (Conn.D). *n* = 6-8/group.

Data are presented as mean ± SD. *** $P < 0.001$ as determined by 2 way ANOVA with Holm-Sidak's post hoc analysis for multiple comparisons test.

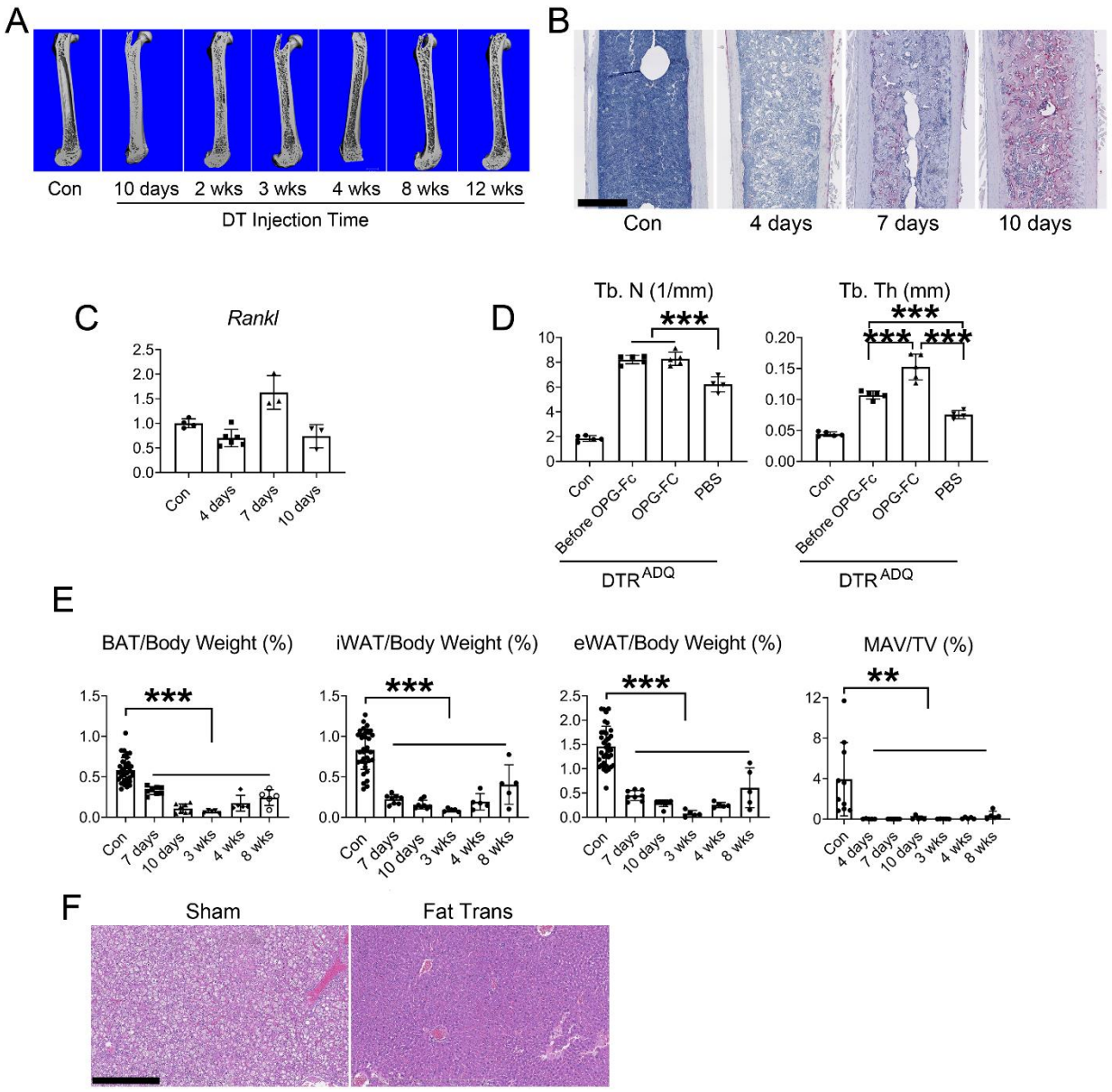


Figure S3. Related to Fig 4. Anti-resorptive therapy maintains DT/DTRADQ osteosclerosis.

(A) Representative μCT images of femur of Con and DTR^{ADQ} mice administered DT daily with time. *n* = 3-8/group.

(B) Histological images of femoral diaphysis of DTR^{ADQ} mice administered with DT daily for 4, 7 or 10 days. No DT treatment as control. *n* = 3-4/group. Scale bar: 500 μm.

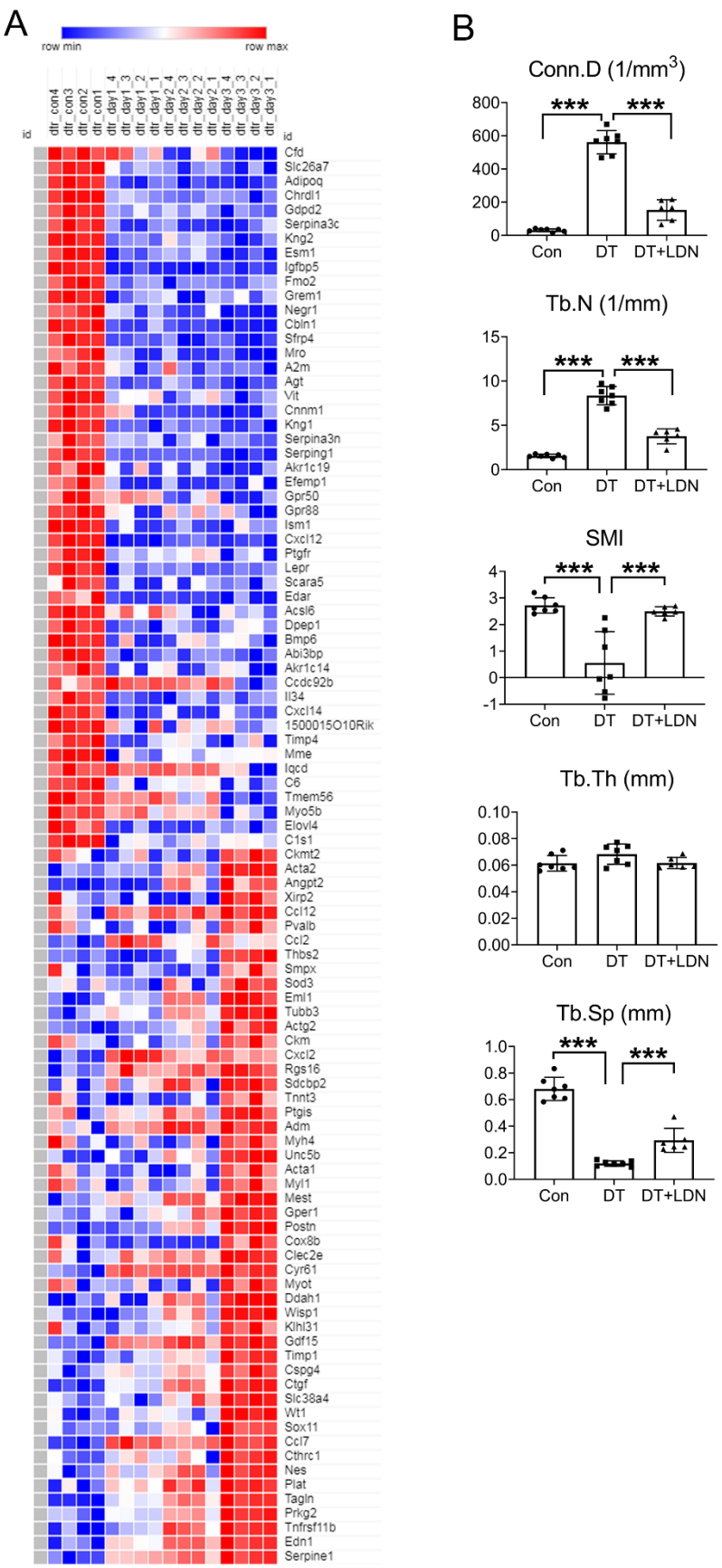
(C) qPCR analysis of femoral bone Rankl mRNA of 2month old DTR^{ADQ} mice treated with DT with time. No DT treatment serves as control. *n* = 3-6/group.

(D) μCT quantitation of trabecular number (Tb.N), and trabecular thickness (Tb.Th) of Con or DTR^{ADQ} mice treated with DT for 10 days (Con and Before OPG-FC), or DTR^{ADQ} mice after continued DT treatment, with or without (PBS) OPG-Fc, for an additional 20 days. *n* = 4-5/group.

(E) Weight of various fat depots in DTR^{ADQ} mice following indicated days of DT administration (left 3 panels), n = 5-35/group; right panel: femur marrow adipocytes of DTR^{ATQ} mice were stained with osmium after indicated time with DT. Marrow adipocytes volume per total volume (MAV/TV) was analyzed in osmium tetroxide stained femurs by µCT. No DT treatment serves as control. *n* = 5-10/group.

(F) Histological images of liver of DTR^{ADQ} mice treated with DT for 10 days 2 months after WAT transplantation or sham surgery. *n* = 3/group. Scale bar: 400µm.

Data are presented as mean ± SD. ** $P < 0.01$ and *** $P < 0.001$ as determined by 1 way ANOVA with Holm-Sidak's post hoc analysis for multiple comparisons test.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Mest
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0.0
Con
DT
DT+LDN

Figure S4. Related to Fig 6. BMPR signaling mediates adipocyte ablation-induced osteosclerosis.

(A) Heatmap of RNA sequencing of bone marrow mRNAs of DTR^{ADQ} mice treated with or without (Con) DT with time. Top 50 downregulated and upregulated genes are listed according to the Log FC value.

(B) μCT quantitation of structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp) and connection density (Conn.D) of 2 month old DTR^{ADQ} mice treated with DT in the presence or absence of LDN193189 for 10 days. No DT treatment serves as control. n = 6-7/group.

Data are presented as mean ± SD. *** $P < 0.001$ as determined by 1 way ANOVA with Holm-Sidak's post hoc analysis for multiple comparisons test.

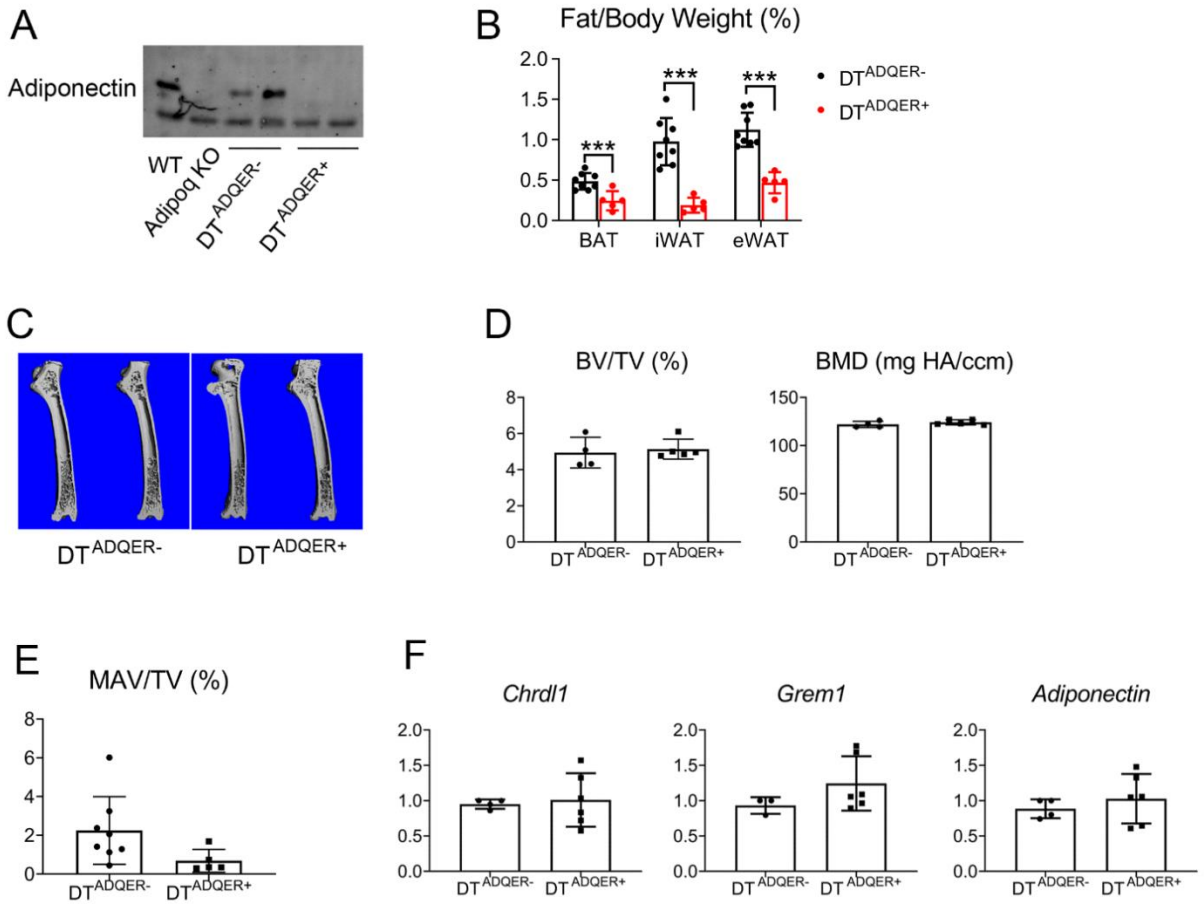

Figure S5. Related to Fig 6. Loss of marrow adipocytes is required for the promotion of osteogenesis.

(A - F) 2 – 3 months old Con and DT^{ADQER} mice were injected IP with Tamoxifen for 5 consecutive days. After 2 weeks,

(A) Serum adiponectin was assessed by immunoblot. Adiponectin-/- mice serve as negative control. *n* = 2 independent experiments.

(B) Weight of various fat depots in Con (DT^{ADQER-}) and DT^{ADQER+} mice. *n* = 5-8/group.

(C) Representative µCT images of femurs of DT^{ADQER} and Cre- control mice. *n* = 4-5/group.

(D) Quantitative µCT analysis of whole femur of DT^{ADQER} and Cre- control mice. *n* = 4-5/group.

(E) Femur marrow adipocytes were stained with osmium. Marrow adipocytes volume per total volume (MAV/TV) was analyzed by µCT. *n* = 5-8/group.

(F) qPCR analysis of Chrdl1, Grem1, Adiponectin femoral marrow mRNA of 2 month old DT^{ADQER} and Cre- control mice 2 weeks after Tamoxifen injection. *n* = 4-6/group.

Data are presented as mean ± SD. *** $P < 0.001$ as determined by 1 way ANOVA with Holm-Sidak's post hoc analysis for multiple comparisons test (B).

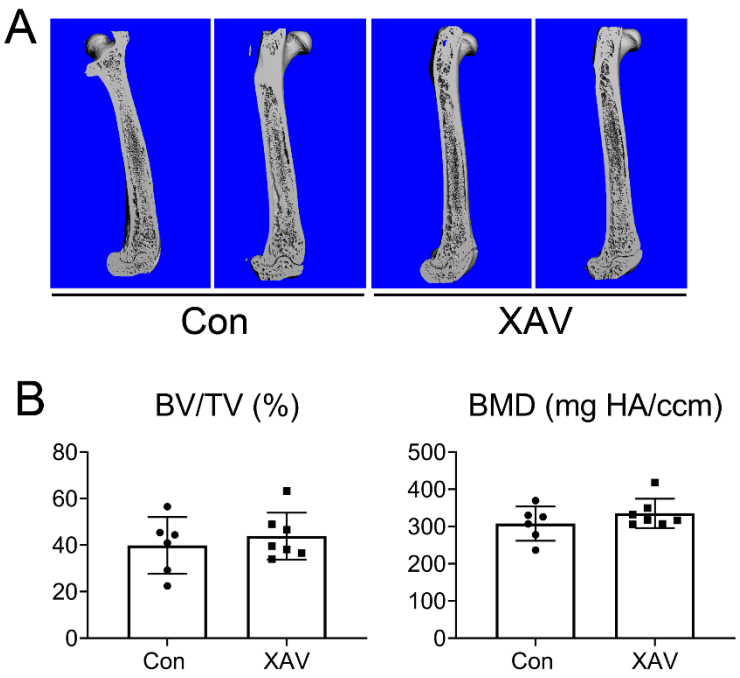

Figure S6. Related to Fig 6. Wnt signaling does not mediate adipocyte ablation-induced osteosclerosis.

(A) Representative µCT images of femur of DTR^{ADQ} mice administered DT daily, for 10 days, in the presence or absence of XAV-939 (10mg/Kg/day). *n* = 6-7/group.

(B) µCT quantitation of BV/TV and BMD of (A). *n* = 6-7/group.

Data are presented as mean ± SD.

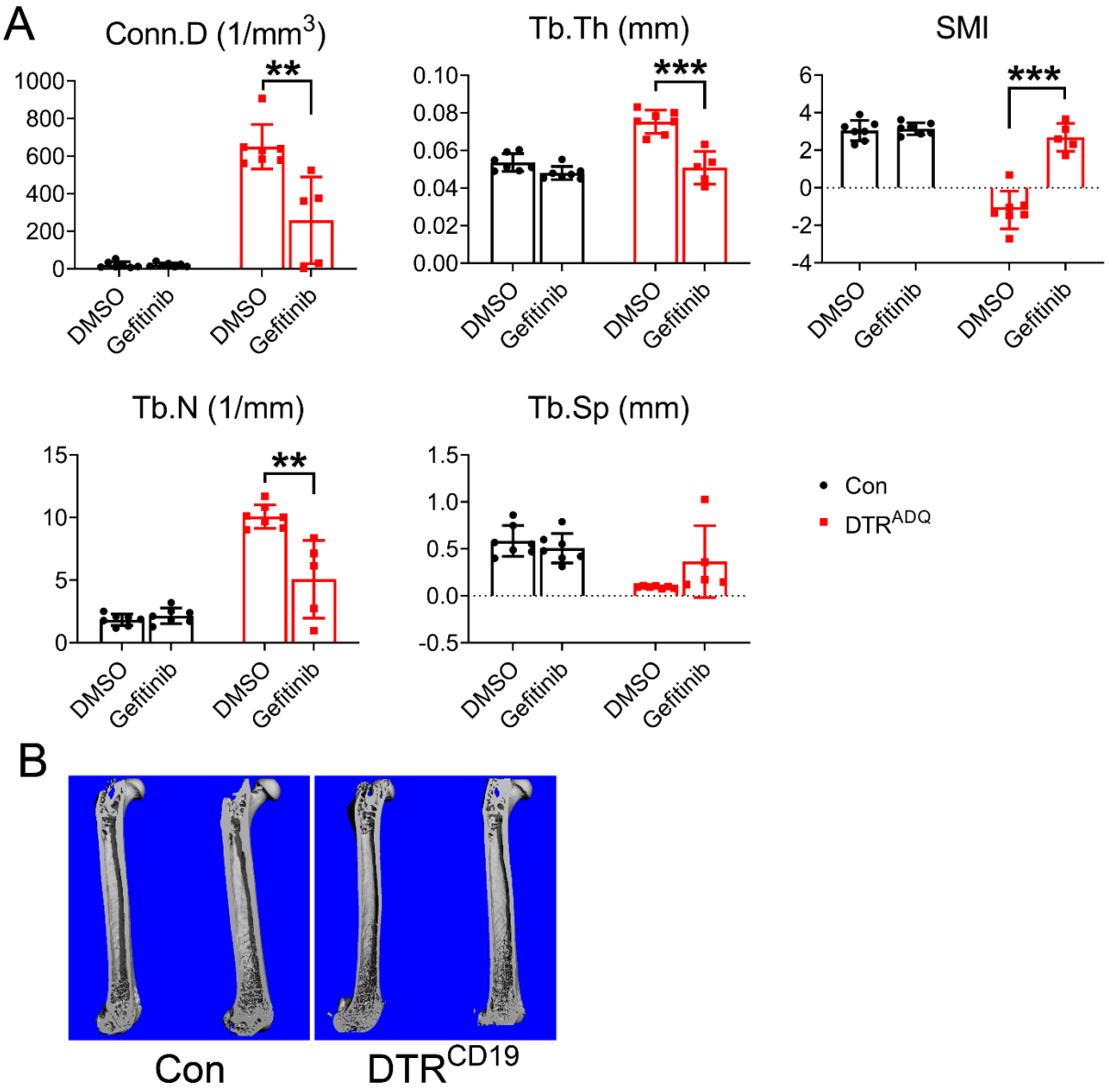

Figure S7. Related to Fig 7. EGFR and BMPR signaling partner to promote osteosclerosis.

(A) Whole femur µCT quantitation of structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp) and connection density (Conn.D) of Con or DTR^{ADQ} mice treated with DT in the presence or absence of Gefitinib (100mg/Kg) daily for 10 days. *n* = 5-7/group.
(B) Representative µCT images of femur of DTR^{CD19} and their Cre- littermate mice following 10 days of DT administration. *n* = 6-9/group.

Data are presented as mean ± SD. ** $P < 0.01$, *** $P < 0.001$ as determined by 2 way ANOVA with Holm-Sidak's post hoc analysis for multiple comparisons test.