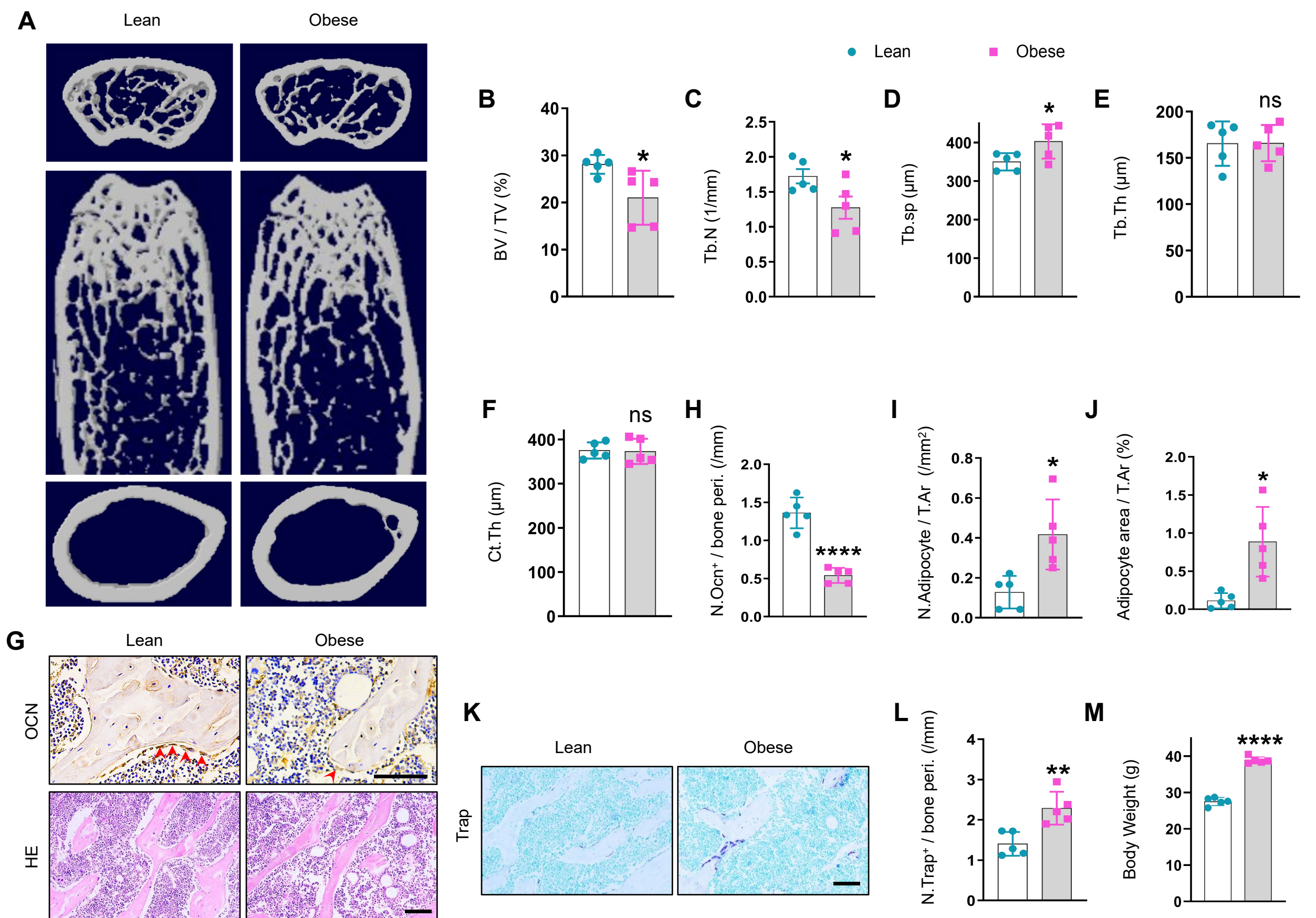


# Supplementary Figure 1



## Supplementary Figure Legends

### Figure S1. Obese mice exhibit bone deterioration and SSPC differentiation towards adipocytes than osteoblasts.

(A) Representative microcomputed tomography (μCT) images from lean and obese C57 BL/6J mice. n=5 per group.

(B-F) Quantitative μCT analysis of trabecular bone (B-E) and cortical bone (F) from lean and obese mice.

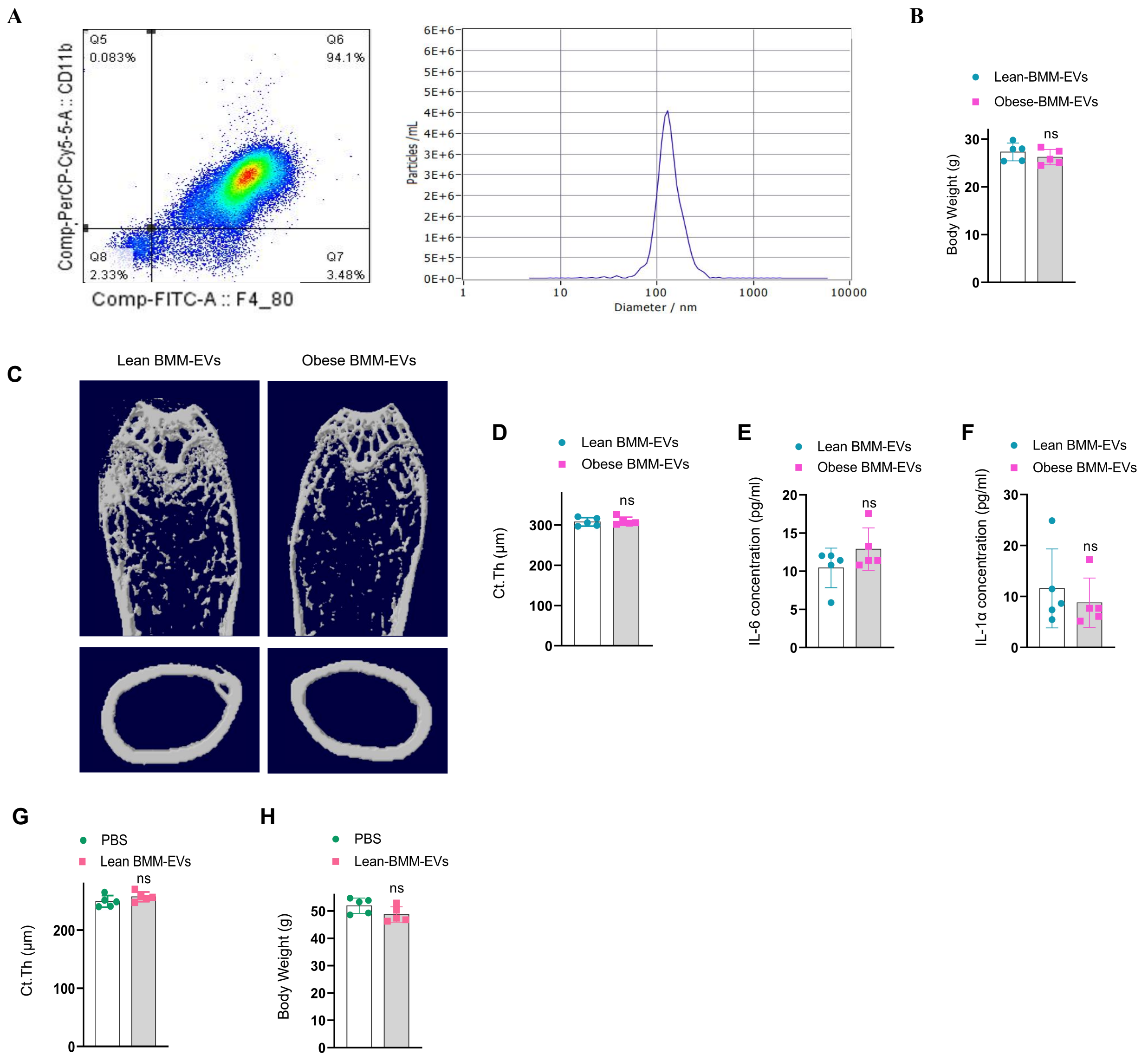
(G) Representative images of osteocalcin immunohistochemical staining (G, top) and H&E staining (G, bottom) in distal femora. Red arrows mark osteoblasts. Scale bar: 100 μm.

(H-J) Quantification of number of osteocalcin-positive osteoblasts (H); area and number of adipocytes (I-J). Scale bar: 100 μm.

(K-L) Representative images of tartaric acid resistant (Trap) staining and number of Trap-positive osteoclasts. Scale bar: 100 μm.

(M) The body weight of lean and obese mice.

# Supplementary Figure 2



**Figure S2. Characteristic and function of BMM-EVs. Related to Figure 1-2.**

(A) Flow cytometry analysis of F4/80 and CD11b expression in isolated macrophages (Left); Nanoparticle Tracking Analysis of BMM-EVs (Right).

(B) The body weight of mice with Lean-BMM-EVs and Obese-BMM-EVs intervention. n=5 per group.

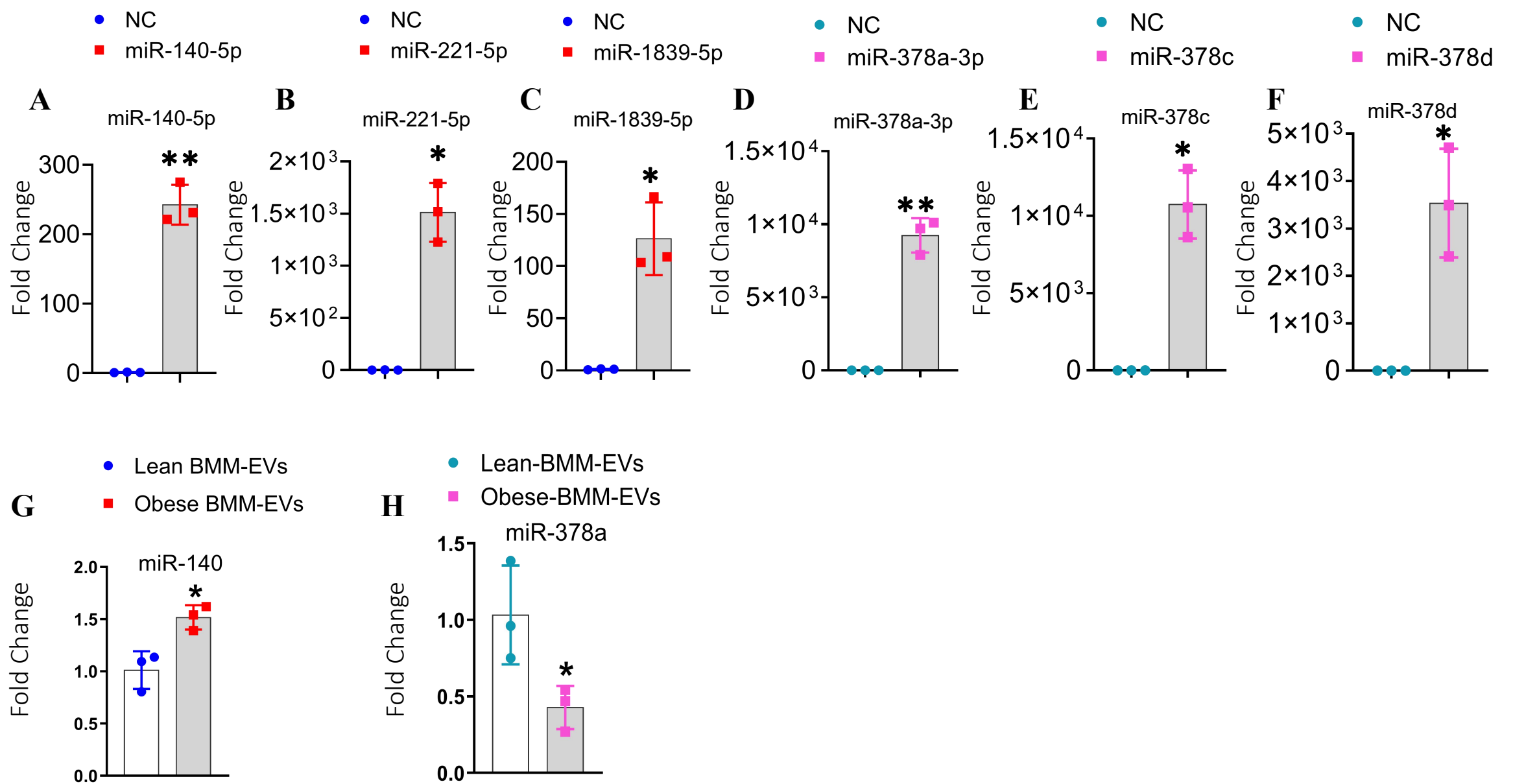
(C-D) Representative  $\mu$ CT images and quantitative  $\mu$ CT analysis of cortical bone from lean mice with lean/obese-BMM-EVs intervention. n=5 per group.

(E-F) IL-6 and IL-1 $\alpha$  Elisa assay of lean mice with lean/obese-BMM-EVs intervention.

(G) Quantitative  $\mu$ CT analysis of cortical bone from obese mice with PBS and/or lean-BMM-EVs intervention. n=5 per group.

(H) The body weight of obese mice with PBS and/or lean-BMM-EVs intervention. n=5 per group.

# Supplementary Figure 3



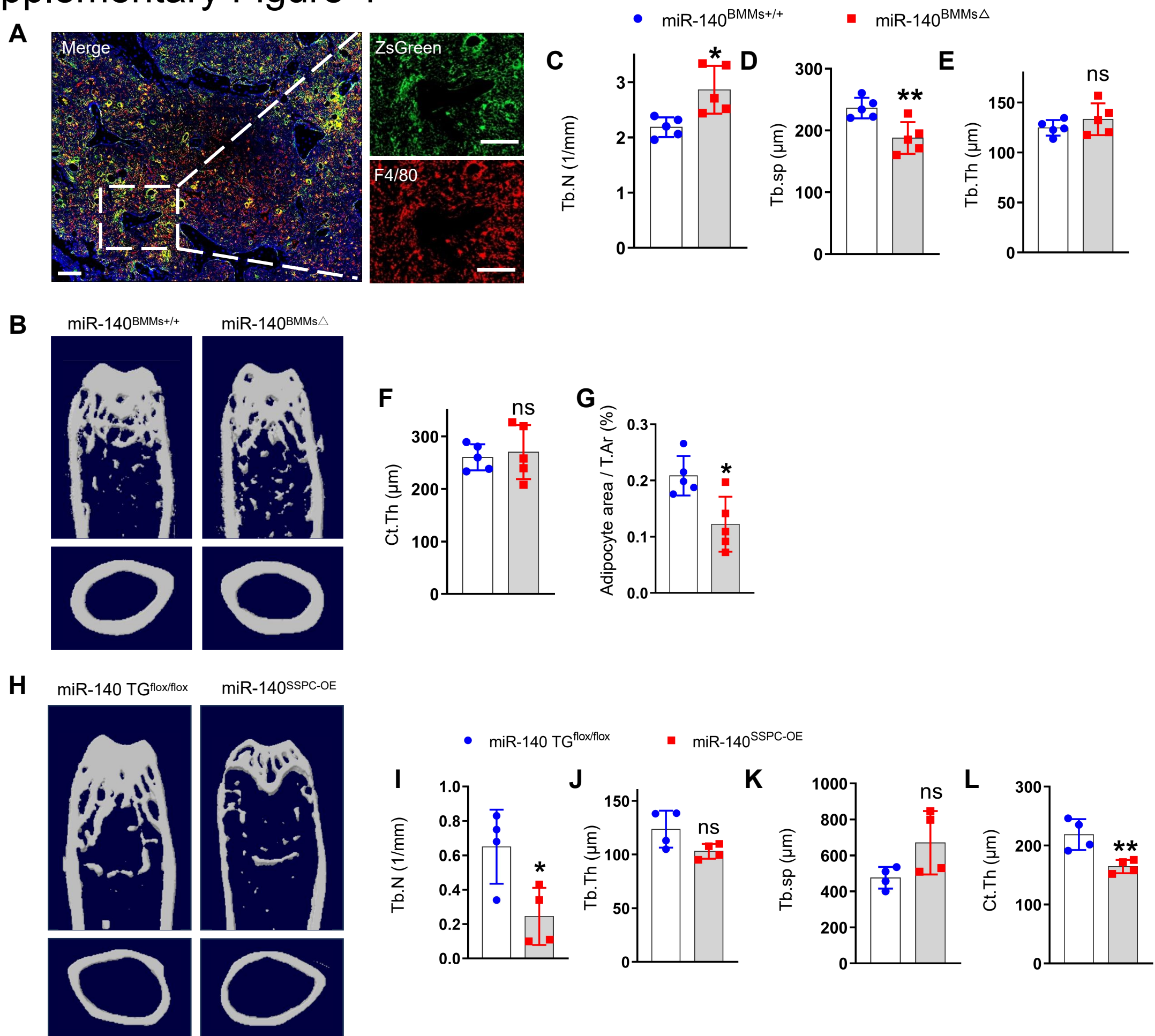
**Figure S3. BMM-EV miR-378a and miR-140 may coordinately regulate SSPC lineage fate. Related to Figure 3.**

(A-C) qRT-PCR analysis of the relative expression levels of microRNAs which be transfected into SSPCs. n=3 per group.

(G) qRT-PCR analysis of the relative expression levels of miR-140 in lean and obese BMM-derived extracellular vesicles. n=3 per group.

(H) qRT-PCR analysis of the relative expression levels of miR-378a in lean and obese BMM-derived extracellular vesicles. n=3 per group.

# Supplementary Figure 4



**Figure S4. miR-140 regulate bone deterioration and SSPC differentiation switch between osteoblasts and adipocytes *in vivo*. Related to Figure 4.**

(A) Successful AAV-F4/80-Cre-ZsGreen transfection in BMMs. Red fluorescence represents F4/80 labeled macrophages; green fluorescence indicates successful AAV transfection; yellow fluorescence indicates successful AAV transfection in BMMs. Scale bar: 100 μm.

(B) Representative μCT images of obese miR-140<sup>flox/flox</sup> mice with AAV-NC-ZsGreen and/or AAV-F4/80-Cre-ZsGreen intervention.

(C-F) Quantitative μCT analysis of trabecular bone (C-E), including Tb. N (C), Tb. sp (D) and Tb. Th (E); quantitative μCT analysis of cortical bone thickness (F).

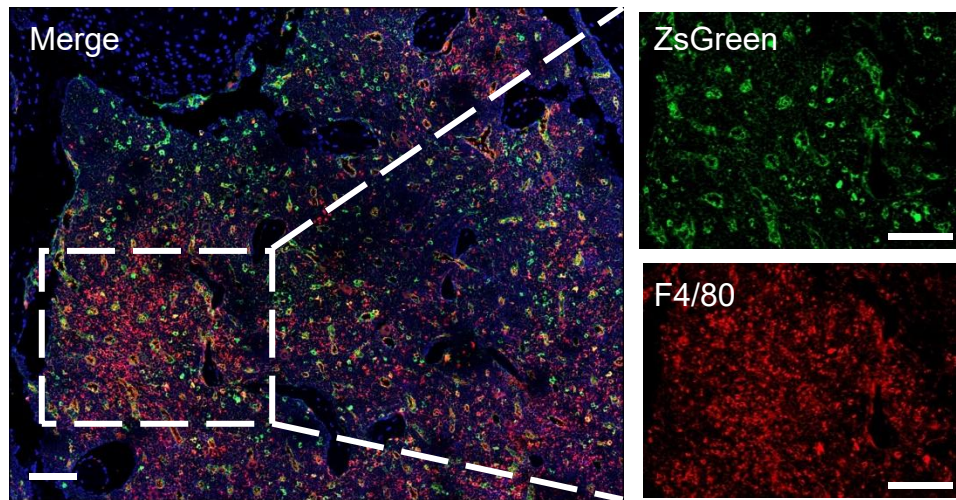
(G) Quantification of area of adipocytes from bone section of obese miR-140<sup>flox/flox</sup> mice with AAV-NC-ZsGreen and/or AAV-F4/80-Cre-ZsGreen intervention.

(H) Representative μCT of miR-140<sup>SSPC-OE</sup> and miR-140<sup>TG<sup>flox/flox</sup></sup> mice.

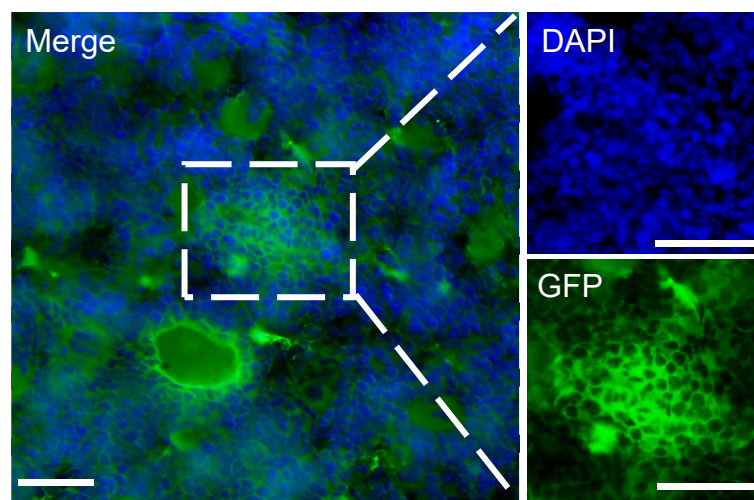
(I-L) Quantitative μCT analysis of trabecular (I-K), including Tb. N (I), Tb. Th (J) and Tb. sp (K); quantitative μCT analysis of cortical bone thickness (L) from miR-140<sup>SSPC-OE</sup> and miR-140<sup>TG<sup>flox/flox</sup></sup> mice.

## Supplementary Figure 5

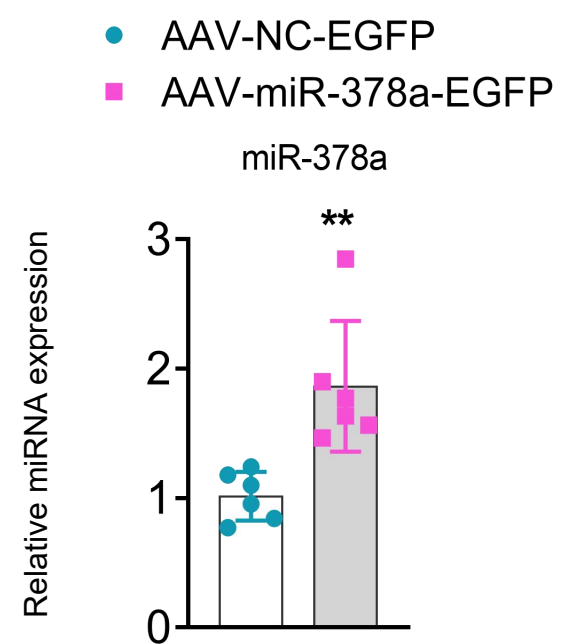
A



B



C



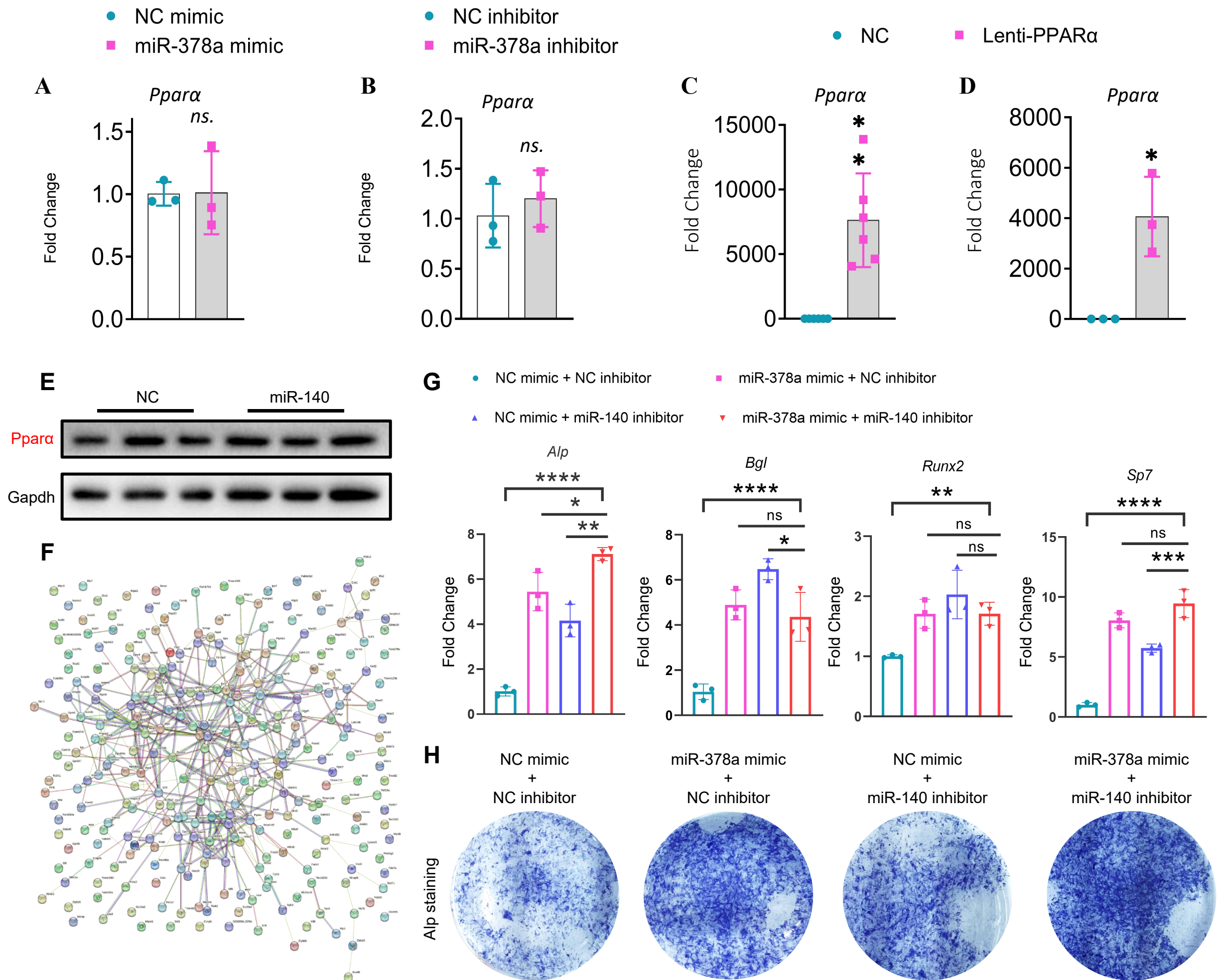
**Figure S5. Efficiency of adeno-associated virus intervention. Related to Figure 5.**

(A) Successful AAV-F4/80-Cre-ZsGreen transfection in BMMs. Red fluorescence represents F4/80 labeled macrophages; green fluorescence indicates successful AAV transfection; yellow fluorescence indicates successful AAV transfection in BMMs. Scale bar: 100  $\mu\text{m}$ .

(B) Representative images of successful AAV transfection. Scale bar: 50  $\mu\text{m}$ .

(C) Quantification of miR-378a expression level of femur that treated with AAV-miR-378a-EGFP and AAV-NC-EGFP. NC, negative control.

# Supplementary Figure 6



**Figure S6. miR-378a and miR-140 synergistically regulated SSPC lineage fate via targeting *Ppara*-*Abca1* axis. Related to Figure 6.**

(A-B) Relative mRNA expression levels of *Ppara* in SSPCs transfected with miR-378a mimic (A) and/or miR-378a inhibitor (B), respectively. n=3 per group.

(C) qRT-PCR analysis of the relative expression levels of *Ppara* in SSPCs transfected with *Ppara*, which cultured in osteogenic medium. n=6 per group.

(D) qRT-PCR analysis of the relative expression levels of *Ppara* in SSPCs transfected with *Ppara*, which cultured in adipogenic medium. n=3 per group.

(E) Representative western blotting images of *Ppara* in SSPCs transfected with miR-140 and its negative controls. n = 3 per group.

(F) Schematic of protein-protein interaction network for *Ppara* and miR-140 predicted targets.

(G) qRT-PCR analysis of the relative expression levels of *Alpl*, *Bgl*, *Runx2*, *Sp7* in SSPCs transfected with miR-378a mimic plus miR-140 inhibitor and those transfected with microRNA-378a mimics or microRNA-140 inhibitors alone. n = 3 per group.

(H) Alp staining of SSPCs transfected with miR-378a mimic plus miR-140 inhibitor and those transfected with microRNA-378a mimics or microRNA-140 inhibitors alone.