



**Supplementary Figure 3 Neutrophil-related EVs were enriched in fracture calluses.** Plasma EVs and fracture callus EVs were harvested from young (age: 4 months) and aged (age: 24 months) mice (n=6 per group) on day 7 post fracture, and profiled with the indicated surface markers using high resolution multicolor flow cytometry. The graphs present a summary of the percentages of EV subpopulations carrying each surface marker in the matched plasma and calluses of young<sup>fx</sup> (a) and aged<sup>fx</sup> (b) mice. CD11b<sup>+</sup>Ly6C<sup>intermediate</sup>Ly6G<sup>high</sup> neutrophil-associated EVs include CD11b<sup>+</sup>, Ly6C<sup>+</sup> and Ly6G<sup>+</sup> EVs. Comparisons between the matched plasma EVs and callus EVs by paired t test for each marker were performed with results indicated as \* p < 0.05, \*\*p < 0.01, \*\*\*p < 0.01.