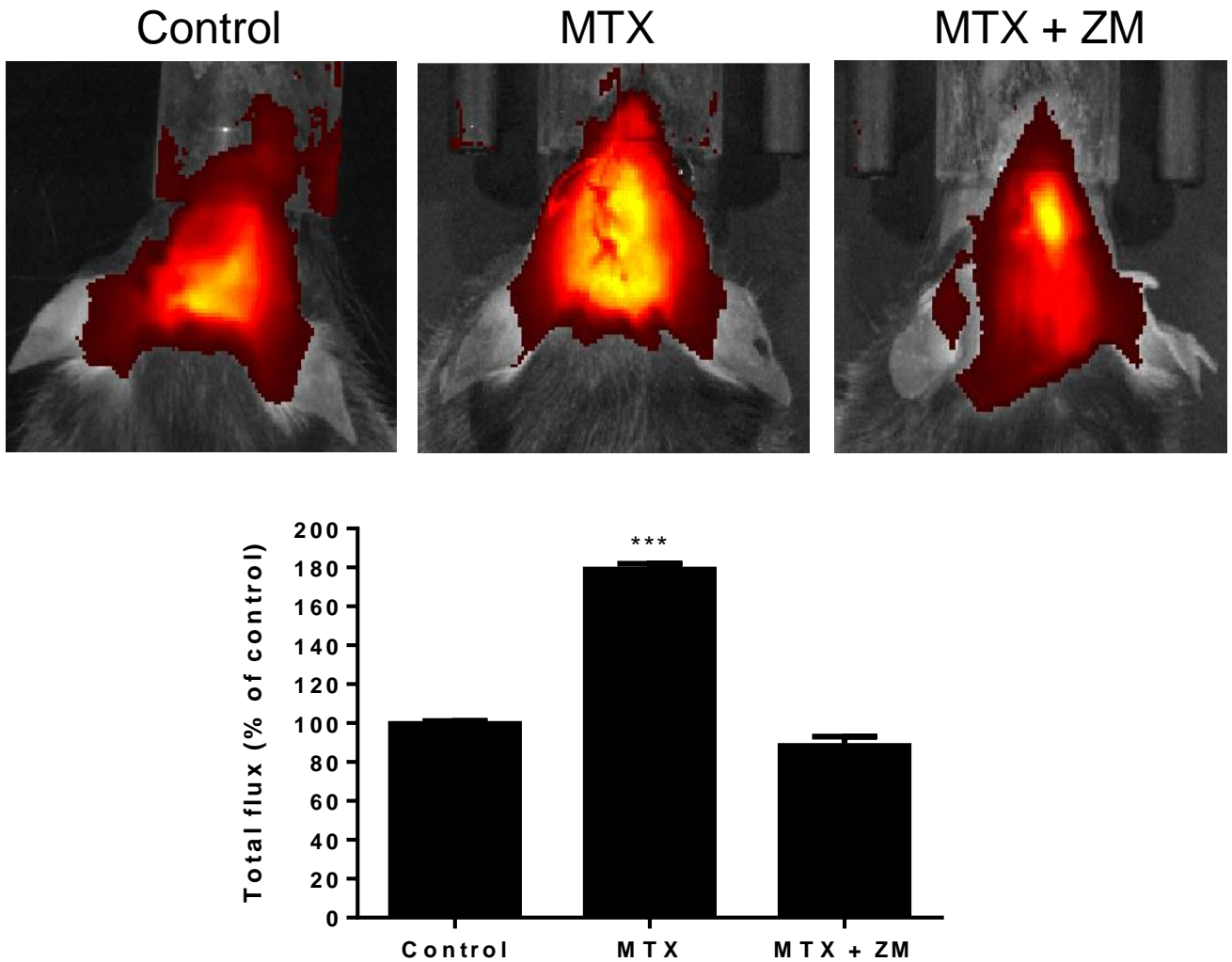
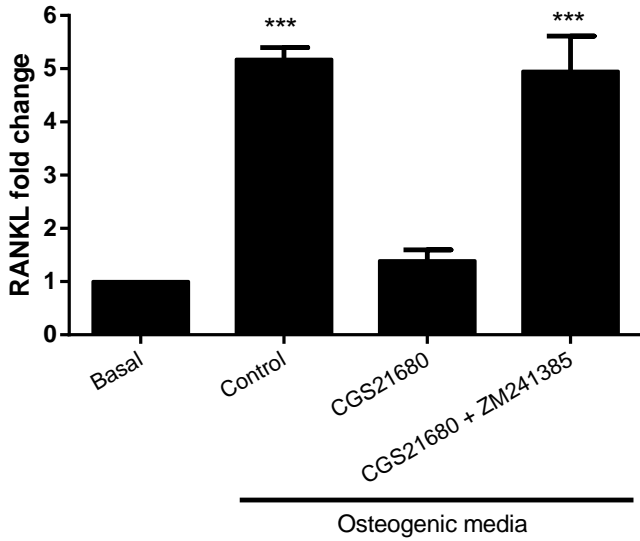


Supplemental Figure 1: Immunohistochemistry for markers of osteoclasts and osteoblast. Calvaria were processed and immunohistologic staining carried out. A) Shown are representative sections of calvaria (from n=5 mice per group) stained for RANK (green). B) Shown are representative sections of calvaria (from n=5 mice per group) stained for RANKL (green). Nucleus is shown in blue (Dapi). All images were taken at the same magnification 400X. Scale bar indicates 50 µm.

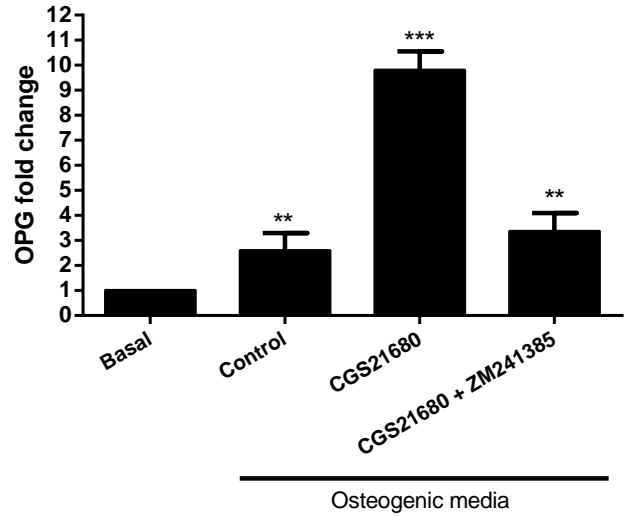


Supplemental Figure 2: MTX increases new bone formation. One week after surgery, XenoLight RediJect Bone Probe 680 conjugate was intravenous injected, and the fluorescence image captured. Total flux in photons/sec were normalized and expressed as percentage of control to avoid intrinsic changes among animals. Red indicates low signal intensity and low rates of new bone formation, while yellow indicates a high signal intensity and high rates of new bone formation. Data are expressed as means \pm SEM (n=5 per group). ***p<0.001 compared to control (ANOVA).

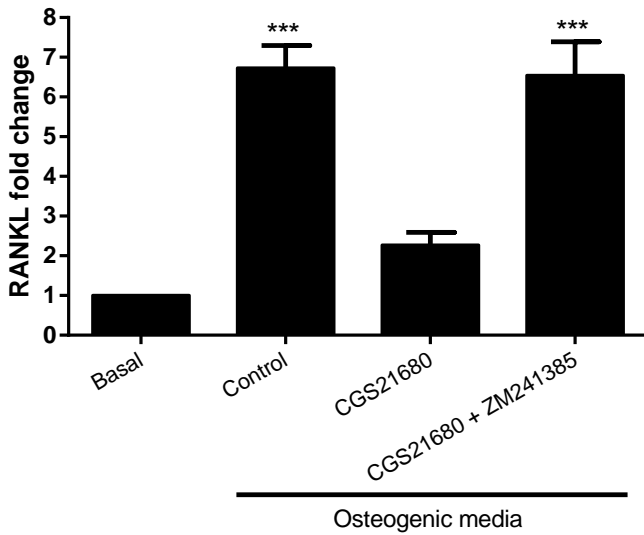
A



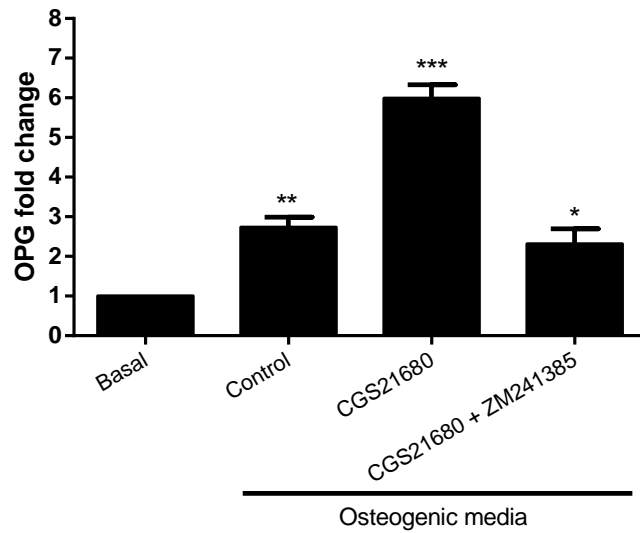
B



C



D



Supplemental Figure 3: Adenosine $A_{2A}R$ activation alters RANKL/OPG mRNA expression ratio in the osteoblast. RANKL and Osteoprotegerin (OPG) mRNA expression were analyzed in murine and human BMCs in the presence of CGS21680 in alone or with of ZM241385 1μ each. A) RANKL mRNA fold change in murine osteoblast precursors after 24 hours challenge with osteogenic media in the presence of CGS21680 alone or with ZM241385 compared to control (n=6). B) OPG mRNA fold change in murine osteoblast precursors after 24 hours challenge with osteogenic media in the presence of CGS21680 alone or with ZM241385 compared to control (n=6). C) RANKL mRNA fold change in human osteoblast precursors after 24 hours challenge with osteogenic media in the presence of CGS21680 alone or with ZM241385 compared to control (n=6). D) OPG mRNA fold change in human osteoblast precursors after 24 hours challenge with osteogenic media in the presence of CGS21680 alone or with ZM241385 compared to control (n=6). ***p<0.001 (ANOVA).