Supplemental Figures – Jin and Zhao et al.



**Figure S1.** Identification of BMMSCs used in the present study was in accordance with currently accepted MSC definitions. **(A)**: Colony formation of BMMSCs stained with crystal violet. BMMSCs expanded in a fashion that started from a single cell to a colony with over 50 cells. Bars: 500 μm. **(B)**: Undifferentiated BMMSCs expanded readily in a spindle shape. Bars: 500 μm. **(C)**: Osteogenic differentiation of BMMSCs stained with alizarin red after induction for 14 d. Bars: 200 μm. **(D)**: Adipogenic differentiation of BMMSCs stained with oil red O after induction for 14 d. Bars: 200 μm. **(E)**: Flow cytometry was performed to identify surface marker expression on BMMSCs. Data represents mean ± SEM. n=3 per marker. Abbreviations: BMMSC, bone marrow-derived mesenchymal stem cell; MSC, mesenchymal stem cell; Sca-1, stem cell antigen-1; SEM, standard error of the mean; VCAM1, vascular cell adhesion molecule 1.



**Figure S2.** Cortical bone mass and mechanical tests for bone quality. **(A-D)**: Representative micro-CT images illustrating the cortical bone mass in the midshaft of femora. Bars: 500  $\mu$ m. **(E-H)**: Corresponding parameters showing partial prevention of glucocorticoid-induced cortical bone loss by MSC therapy. **(I, J)**: Mechanical parameters of tibiae analyzed by 3-point bending tests demonstrating maintenance of bone quality by MSC therapy in glucocorticoid-treated mice. Data represents mean ± SEM. n=4 per group. \**P* < .05, \*\**P* < .01 and \*\*\**P* < .001. Abbreviations: BMMSC, bone marrow-derived mesenchymal stem cell; Cont, control; Ct.Ar, cortical bone area; Ct.Th, cortical bone thickness; GIOP, glucocorticoid-induced osteoporosis; MSC, mesenchymal stem cell; PBS, phosphate buffer saline; SEM, standard error of the mean; Tt.Ar, total area.



Figure S3. Study design of the Experiment 2 and levels of serological markers. (A): Schema indicating the study design of the Experiment 2 for bone modeling investigation. Calcein labeling and the blood and the bone sampling were performed at indicated time points. (B, C): ELISA analyses of bone formation (B) and bone resorption (C) markers in serum at 1 w post glucocorticoid treatment (24 h post infusion) showing inhibition of bone formation and elevation of bone resorption rates. BMMSC infusion partially rescued bone formation but did not prevent the stimulation of bone resorption. (D, E): ELISA analyses of bone formation (D) and bone resorption (E) markers in serum at 4 w post infusion showing maintenance of bone formation underlies therapeutic effects of infused BMMSCs in GIOP. (F, G): ELISA analyses of inflammatory markers in serum showing inhibited TNF- $\alpha$  level in GIOP with paralleled inflammation after BMMSC infusion. Data represents mean  $\pm$  SEM. n=4 per group. \*P < .05, \*\*P < .01 and \*\*\*P < .001. Abbreviations: BMMSC, bone marrow-derived mesenchymal stem cell; Cont, control; CTX-1, cross linked C-telopeptide of type 1 collagen; DEX, dexamethasone; ELISA, enzyme-linked immunosorbent assay; GFP, green fluorescent protein; GIOP, glucocorticoid-induced osteoporosis; IFN-y, inteferon-gamma; i.p., intraperitoneally; i.v., intravenously; MSC, mesenchymal stem cell; NS, not significant; P1NP, procollagen 1 Nterminal peptide; PBS, phosphate buffer saline; SEM, standard error of the mean; TNF- $\alpha$ , tumor necrosis factor-alpha.