

**Supplementary Figure S1**. Generation of a syngeneic MM model with specific deletion of Runx2 in immature OBs. **A,** Schematic depicting the generation of a syngeneic murine MM model with Runx2 knockout. **B,** Tibias were collected from newborn Col1a1-Cre and tdTomato double-transgenic littermates and sectioned. Representative bright field/fluorescence images reflect the overall Cre signal (red) in the developing bone. Col1a1-Cre activity (red) is limited to cortical (CB) and trabecular bone (TB), with no activity in muscle or chondrocytes (HC: hypertrophic chondrocytes). **C,** PCR confirmation of littermate genotypes**. D,** PCR amplification detected the Samsn1 gene in the original OB-Runx2+/- mice but not in the original C57BL6/KaLwRij or in the OB-Runx2+/+ and OB-Runx2-/- (crossed) mice. Primers flanking the Samsn1 deletion breakpoint amplified a product in C57BL6/KaLwRij, OB-Runx2+/+, and OB-Runx2-/- genomic DNA, confirming Samsn1 deletion in these mice. **E,** Proteins were isolated from OBs harvested from 5‐week‐old OB-Runx2+/+ and OB-Runx2-/- mouse long bones and subjected to SDS-PAGE. Blots were probed with antibodies against Runx2 and β-actin (loading control). Deletion of exon 8 results in a mutant Runx2 protein (Δ369) in OB-Runx2-/- mice that lacks 159 amino acids at the C-terminus. Full-length Runx2 (WT) and mutant proteins (Δ369) are shown.



**Supplementary Figure S2.** Comparison of the number of OBs, osteocytes, and osteoclasts in OB-Runx2+/+ and OB-Runx2-/- mice. **A,** H&E staining was performed on the femurs harvested from OB-Runx2+/+ and OB-Runx2 -/- mice (n=7 mice/group). OBs on the bone surface were counted. No significant change in OB number was observed in OB-Runx2-/- mice. **B,** The number of osteocytes embedded in bone tissue was determined. No significant change in the number of osteocytes was observed in OB-Runx2-/- mice. **C,** TRAP staining shows no significant difference in the number of osteoclasts between the genotyping groups. ns, not significant; osteocytes, Ocy; osteoclasts, OC.



**Supplementary Figure S3.** Depletion of MDSCs by 5-FU restores the BM immunity in OB-Runx2-deficient mice.Immune cells in the BM of non-tumor-bearing 5-week-old OB-Runx2+/+ and OB-Runx2-/- mice were profiled by FACS after i.p. injection of PBS or 5-FU (30 mg/kg body weight/injection) once every 3 days for 4 weeks (n=3 mice/group). **A,** The percentage of MDSCs (Gr1+CD11bhi) among all BM cells. **B,** The percentage of Tregs (CD25+CD127-) among CD4+ T cells. **C,** The percentage of Bregs (CD5+CD1d+) among B220+ cells. **D,** The expression of PD-1 and TIM-3 in CD8+ T cells of OB-Runx2-/- mice relative to that in OB-Runx2+/+ mice. Data are presented as mean ± SEM. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.



**Supplementary Figure S4.** 5-FU-mediated MDSC depletion enhances antitumor immunity in OB-Runx2-/- mice. **A-F,** OB-Runx2+/+ and OB-Runx2-/- mice were i.v. injected with 5TGM1 MM cells (106 per mouse). Five days after tumor cell injection, mice began treatment with PBS or 5-FU (30 mg/kg body weight/injection) i.p. injected once every 3 days for 4 weeks (n=5-7 mice/group). FACS analysis of BM cells in tumor-bearing bones was performed after 4 weeks of PBS or 5-FU treatment. **A,** The percentage of MDSCs among BM cells (left) and proportion of polymorphonuclear MDSCs (PMN-MDSCs, CD11b+LY6G+LY6Clow/-) and monocytic MDSCs (M-MDSCs, CD11b+LY6Glow/-LY6Chigh) among MDSCs. **B,** Expression of ARG1, iNOS, and IL-10 in MDSCs of each group relative to that in PBS-treated OB-Runx2+/+ mice. **C,** The percentage of Tregs among CD4+ T cells (left) and of Bregs among B220+ cells (right). **D,** The expression of PD-1 and TIM-3 in CD8+ T cells of each group relative to that in PBS-treated OB-Runx2+/+ mice. **E,** Percentage of activated (granzyme B+ perforin+) cells among CD8+ T cells. **F,** Percentage of MM cells among BM cells (left) and relative expression of Ki-67 among MM cells in each group relative to that in MM cells of PBS-treated OB-Runx2+/+ mice (right). **G,** To determine the effect of MDSC depletion on bone-homing of MM cells in OB-Runx2-/- mice, we treated mice with 5-FU or PBS for 2 weeks and then i.v. injected 5TGM1-GFP MM cells (2×106 cells per mouse, n=12 mice/group). BM cells were harvested 1, 2 and 6 hours after tumor cell injection (n=4 mice/time point/group), and GFP+ MM cells homing to bone were counted by FACS. Data are presented as mean ± SEM. ns, not significant, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.