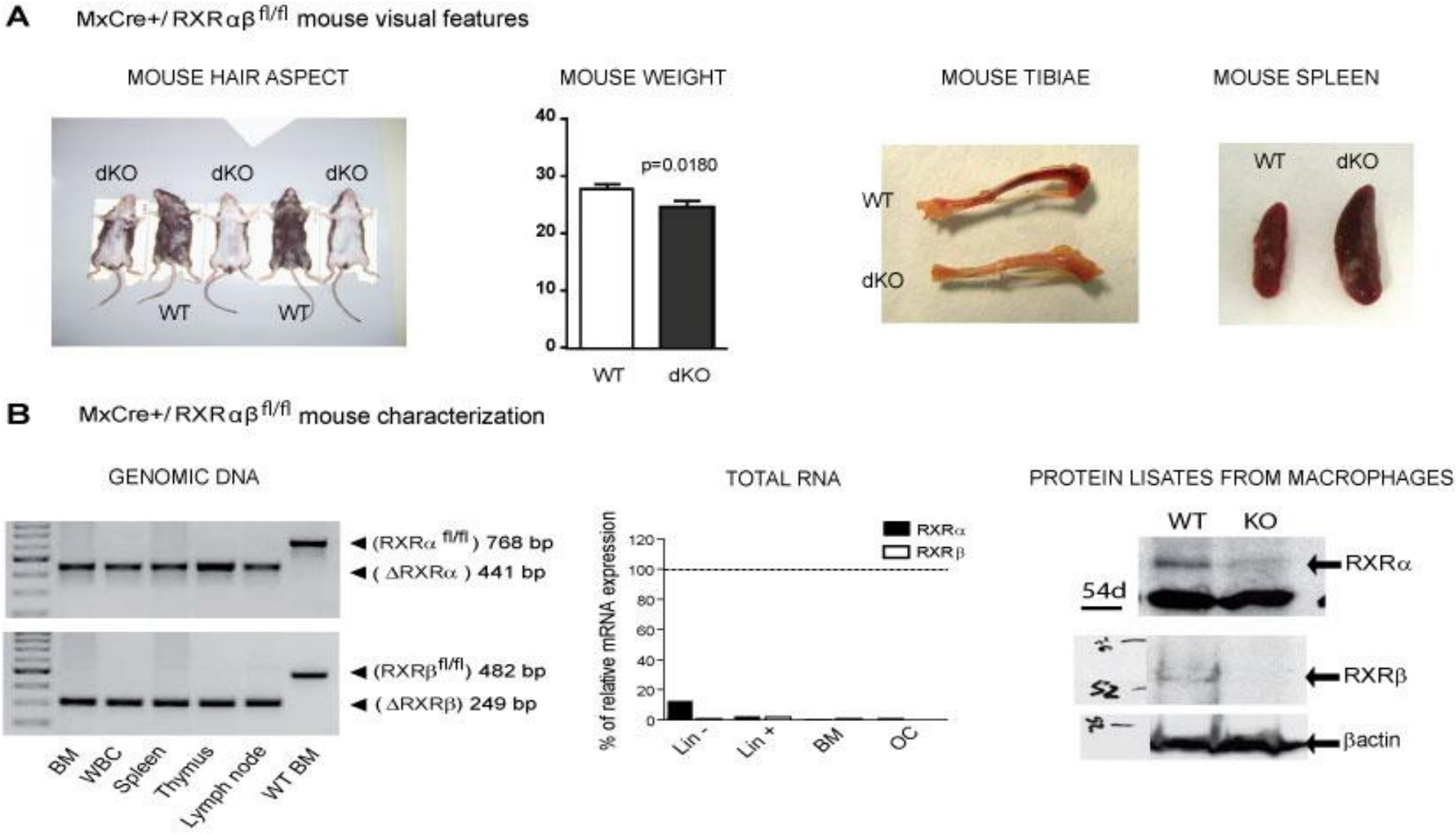

Figure 1. Phenotype presented by MxCre+/ RXR αβ fl/fl (dKO) mice. (A) Visual features observed in 5 month old dKO mice (B) Efficiency of Mx-Cre-mediated loxP recombination in hematopoietic tissues. After Cre recombination, exons 4 of RXRα and RXRβ are deleted (ΔRXRα and ΔRXRβ) resulting in smaller DNA amplicon sizes. Specific primers for qPCR were designed in the exons 4 regions and specific RXR antibodies for western blot were from Santa Cruz.

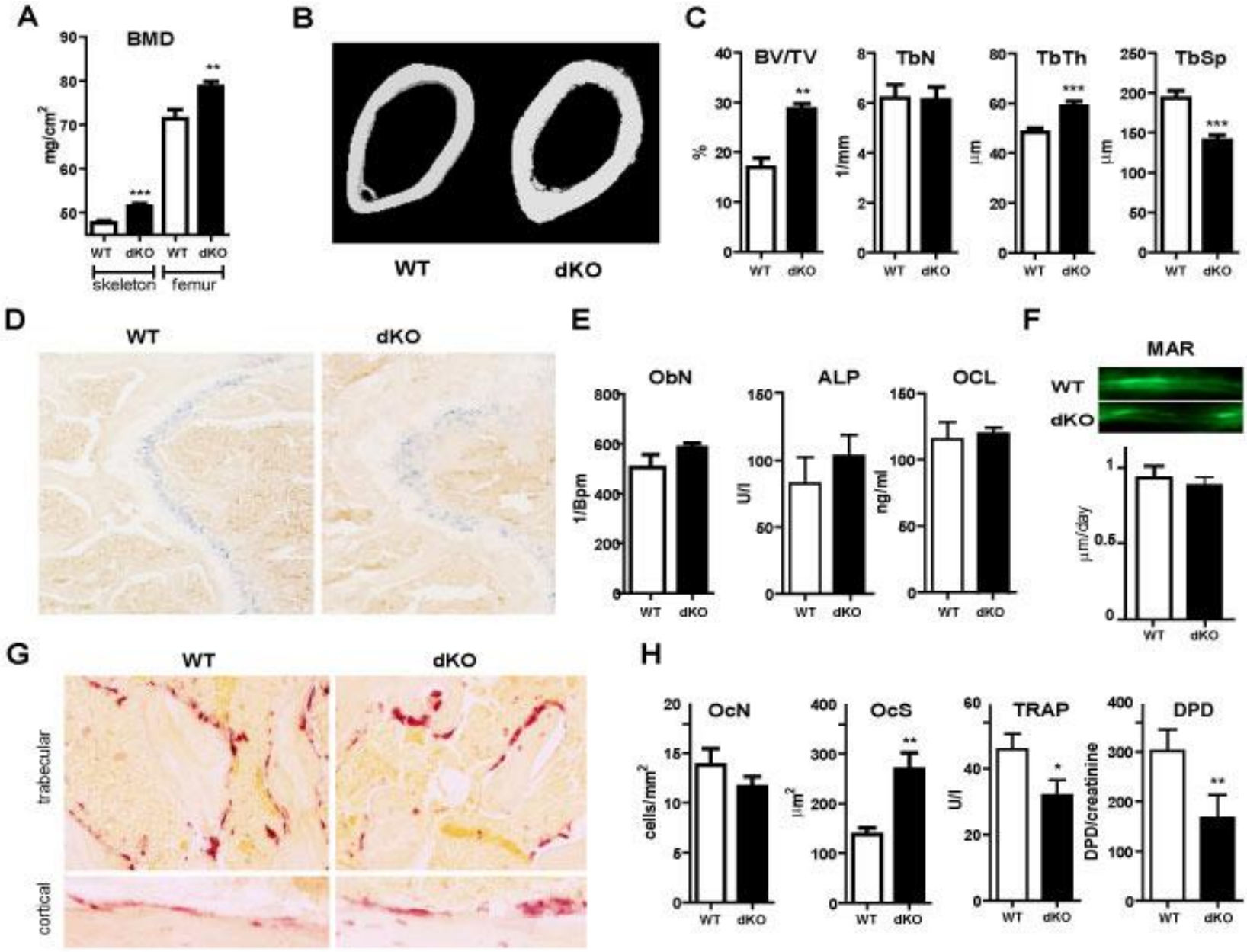

Figure 2. Summary of bone phenotype in dKO animals. Bone density determination by DEXA (A) µCT (B) and histomorphometric studies (C). Characterization of the osteoblast bone composition (D) and determination of different OB parameters (E and F). Characterization of the OC bone composition (G) and determination of different OC parameters (H). BV/TV: bone volume/Trabecule volume, TBN: trabecule number, TbTh: Trabecule thickness, TbS: Trabecule separation, ObN: Osteoblast number, OcN: Osteoclast number, OcS: Osteoclast surface

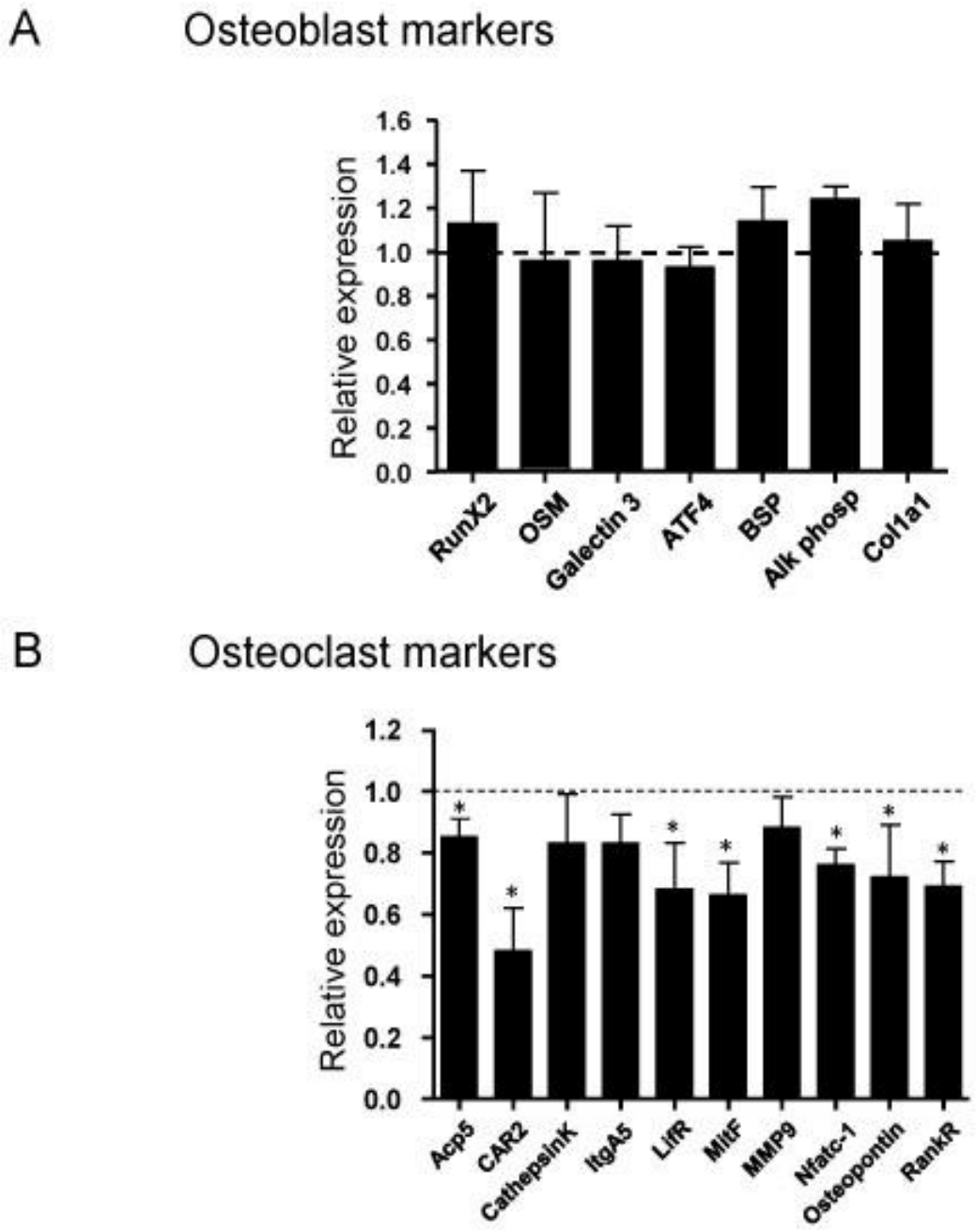

Figure 3. qPCR analysis of different osteoblast and osteoclast markers in total RNA from dKO tibiaes. The expression of each gene is represented as levels in dKO tibiaes compared to WT levels which are fixed to the relative value 1.

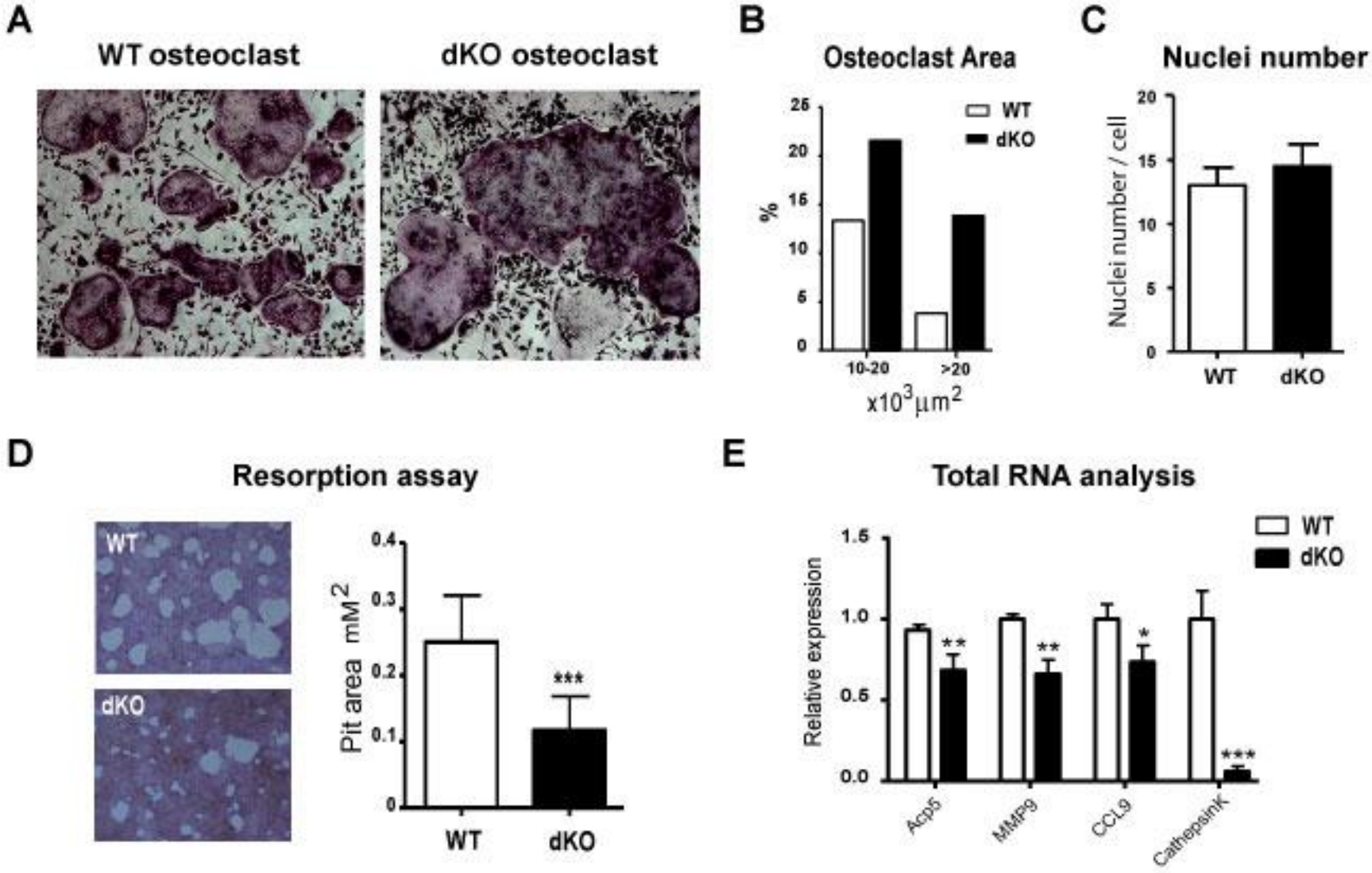

Figure 4. dKO OC characterization. (A) Adult WT and dKO OC recognized as multinucleated TRAP$^+$ cells. (B) Gigantic OC were classified in two groups depending on their area (OC with an area between 10 and 20x10^{3}um^2 and OC with a surface bigger than 20x10^{3}um^2). Cell percentage from the total number of multinucleated cells in the culture is represented for each group. (C) Nuclei number per cell was scored. (D) OC lytic activity dertermination after BM cell diferentiation into OC in a calcium phosphate (CaP)- coated well plate. (E) OC activity related genes determined by RT-qPCR.

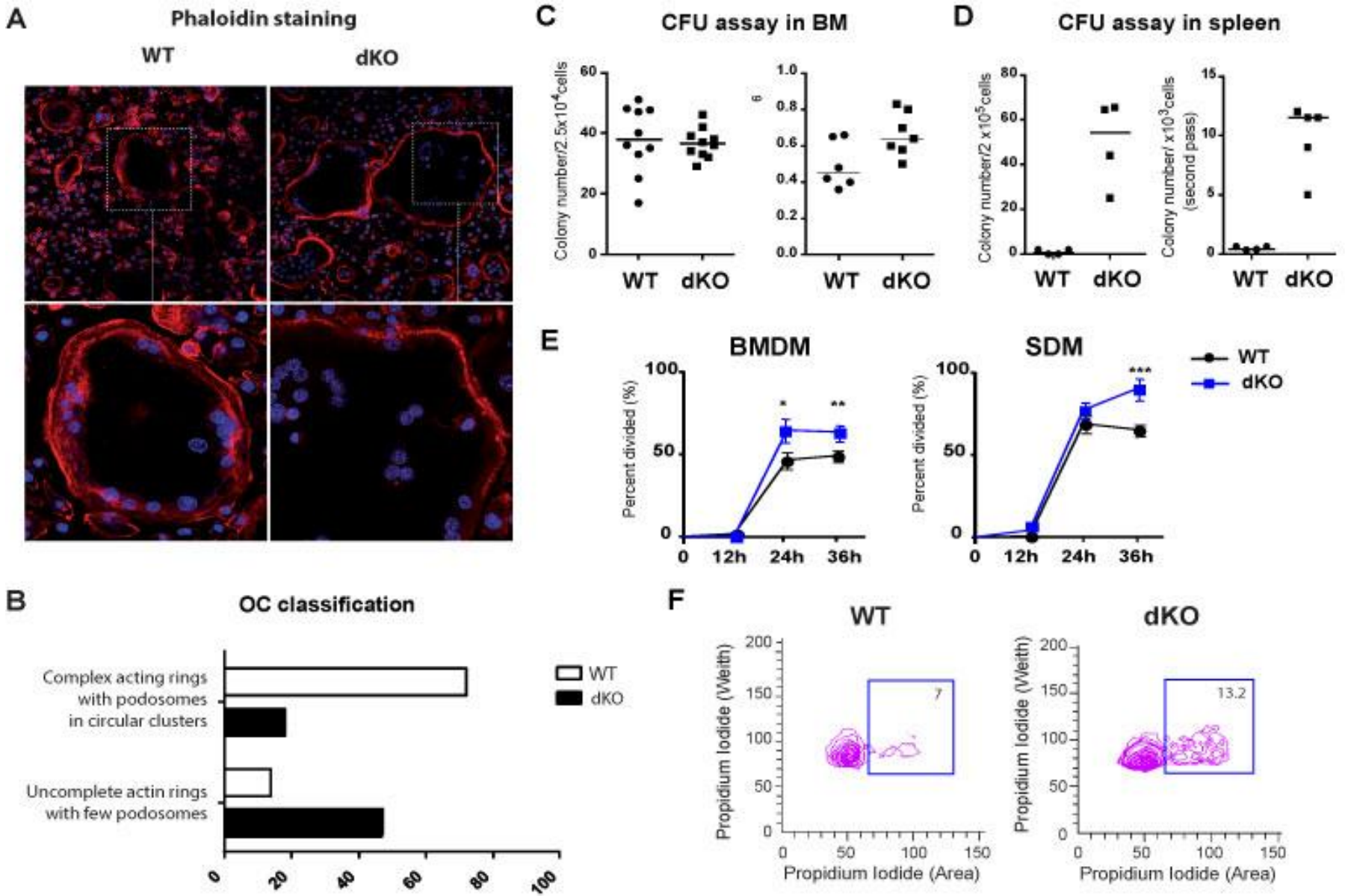

Figure 5. Myeloid cell response to M-CSF. Organization of actin cytoskeleton in dKO OC (A and B). Colony formation assays using preformulated methylcellulose medium containing M-CSF (C and D). Percentage of division calculated by cell proliferation analysis of bone marrow derived macrophages (BMDM) and spleen derived macrophages (SDM) (E). Flow cytometric analysis of Propidium Iodide incorporation and DNA content of peritoneal macrophages after 24h treatment with M-CSF

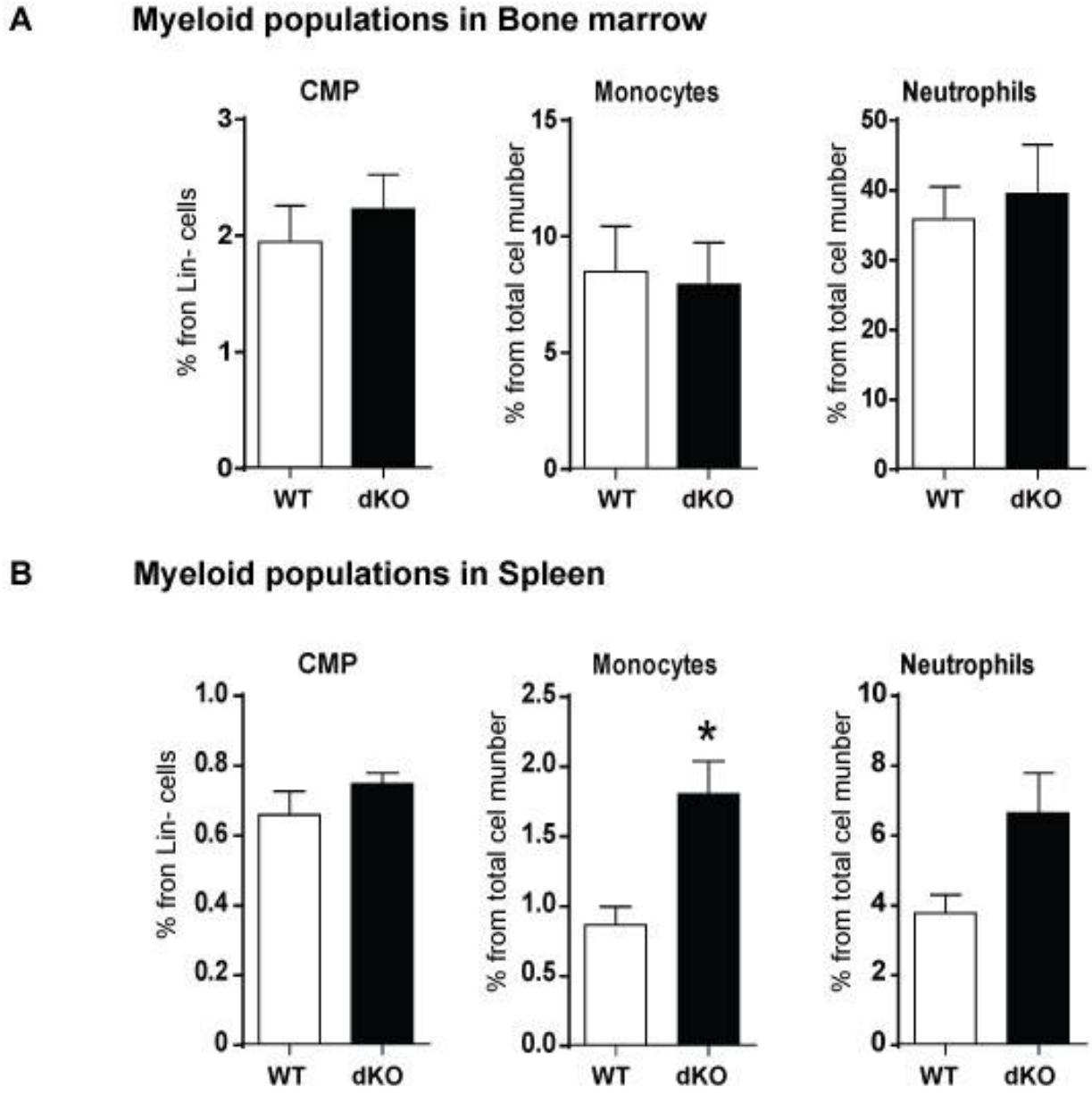

Figure 6. BM (A) and Spleen (B) myeloid composition in dKO mice determined by flow cytometic analysis. % of Common Myelid Progenitors (CMP) (Lin-, cKIT+ and sca-mid), monocytes (CD11b+ and CD115+) and neutrophils (CD11b+and GR1+) are represented.

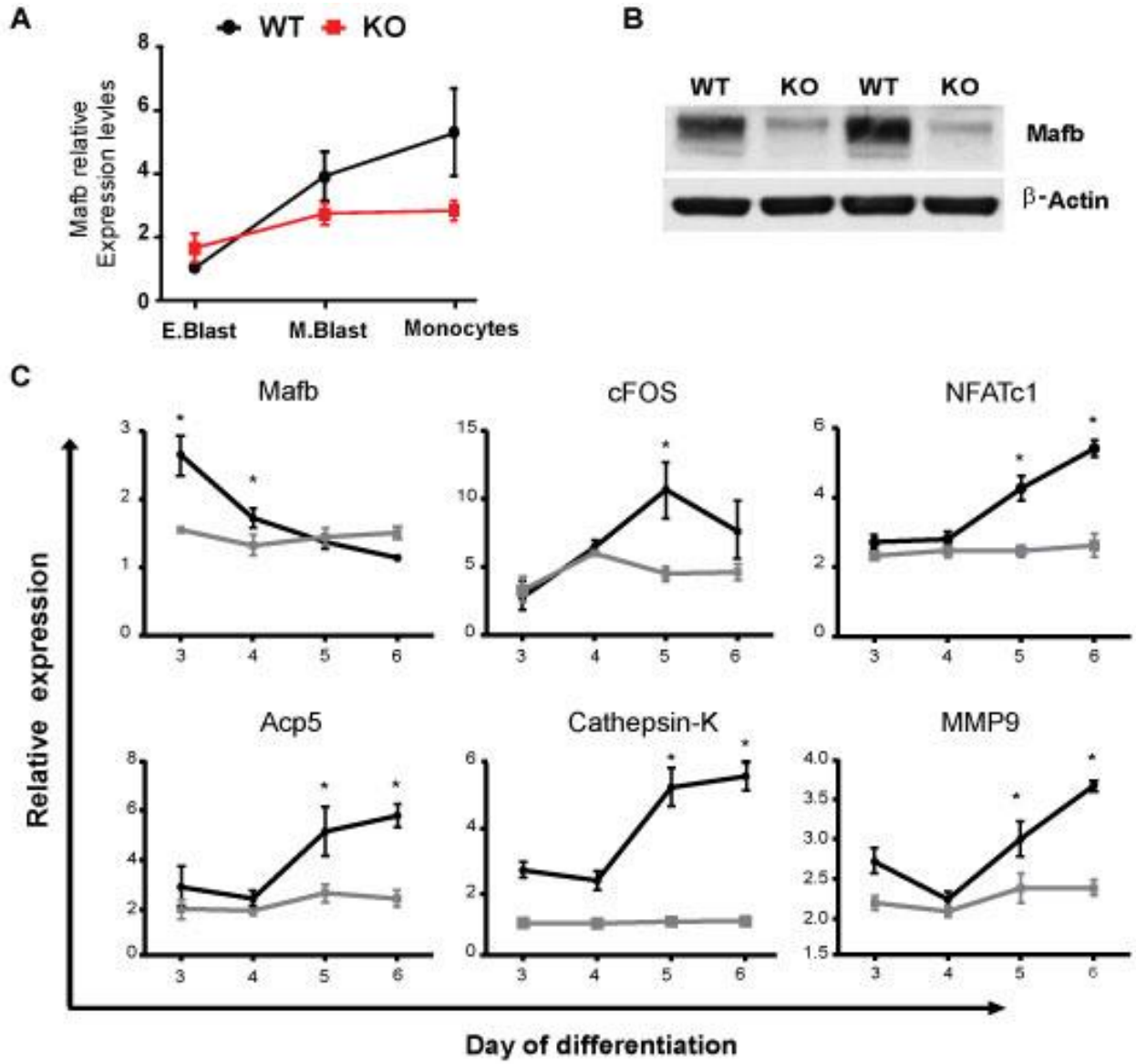

Figure 7. Mafb expression profile in dKO myeloid lineage. (A) Mafb relative levels were determined by qPCR from total RNA from Early blast (E. Blast), myeloid blast (M.Blast) and monocytes purified from BM. (B) Protein basal levels of Mafb were determined by western blot in BMDM. (C) RNA expression profile of Mafb, transcription factors driven osteoclstogenesis and OC-activity related genes during osteoclastogenesis

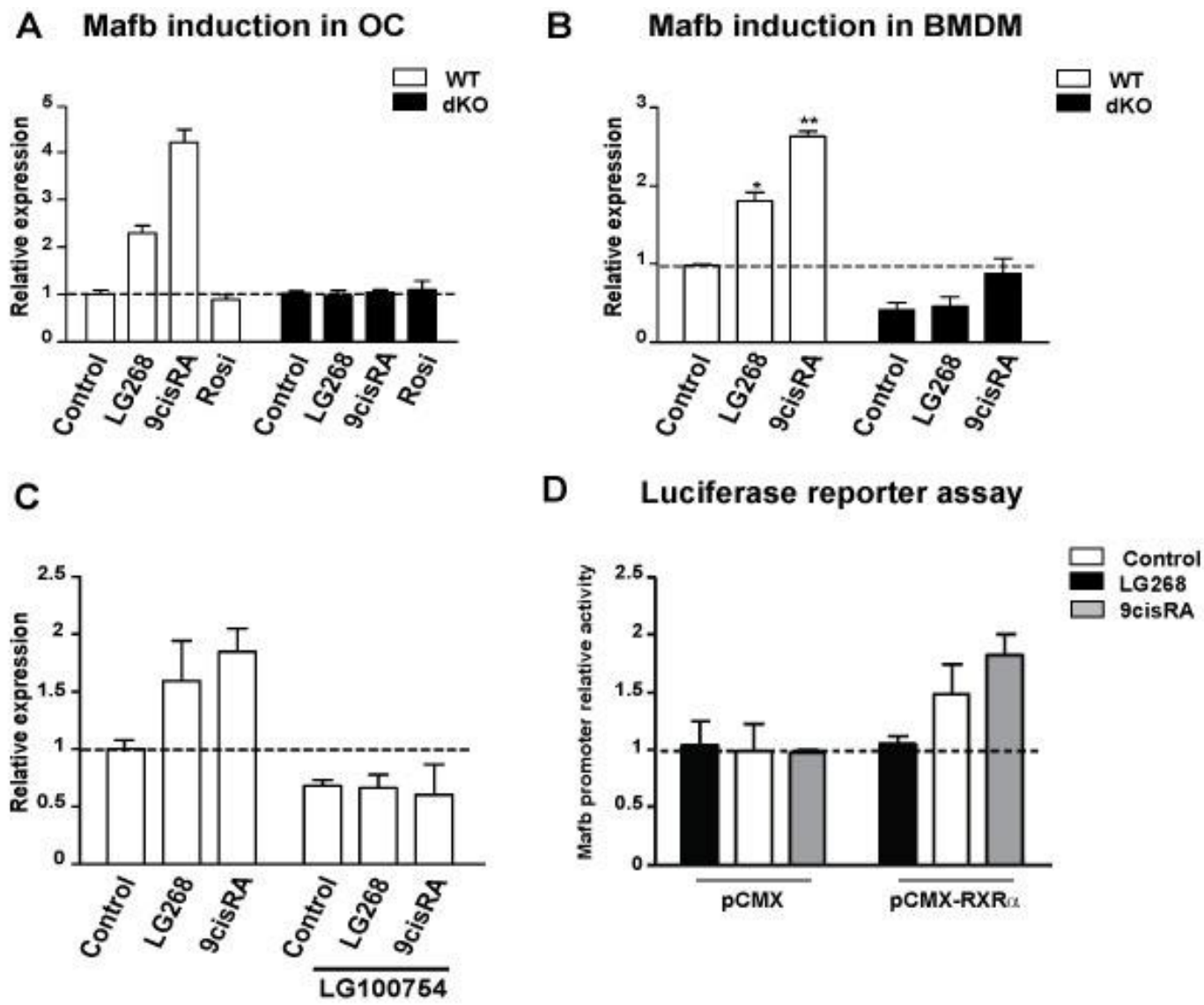

Figure 8. Mafb modulation by RXR activation. (A and B) q-PCR analysis of Mafb mRNA eexpression in dKO OC and dKO BMDM treated for 24h with the RXR ligands 9cisRA (1μM), LG100268 (50nM) or the PPARg ligand, Rosiglitazone (1μM). (C) q-PCR analysis of Mafb mRNA expression in WT BMDM pretreated with the RXR/RXR antagonist, LG100754 for 18h before RXR ligand treatment. (D) Raw 264.7 macrophages cotransfected with a luciferase reporter plasmid under the transcriptional control of Mafb together with an RXRa expression plasmid or empty vector. Cells were treated with RXR ligands and analized for reporter activity 24h after. LG268: LG100268, pCMX empty expression vector.

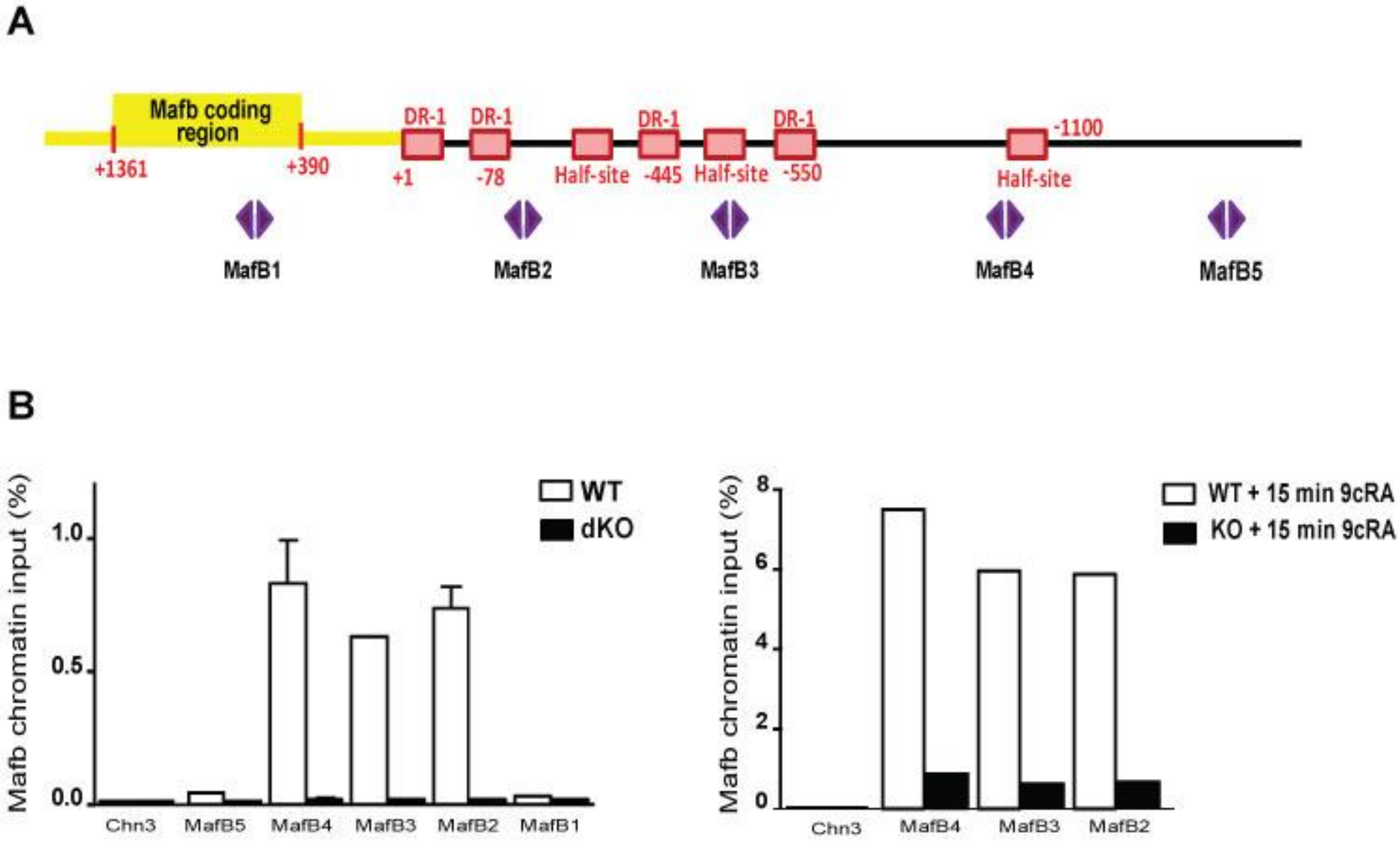

Figure 9. Mafb a possible direct RXR/RXR target gene. (A) Position in the proximal Mafb promoter of the DR1 motifs in silico recognize by the MULAN software and regions targeted by the different Mafb pair of primers (Mafb1 to Mafb5) designed for Chip studies. (B) Chip analysis of the binding of RXR to Mafb promoter regions in BMDM at basal or RXRligand stimulated conditions. Chn3: protein/DNA binding negative control