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Supplemental information

Effect of mitophagy in the formation of osteomorphs derived from osteoclasts

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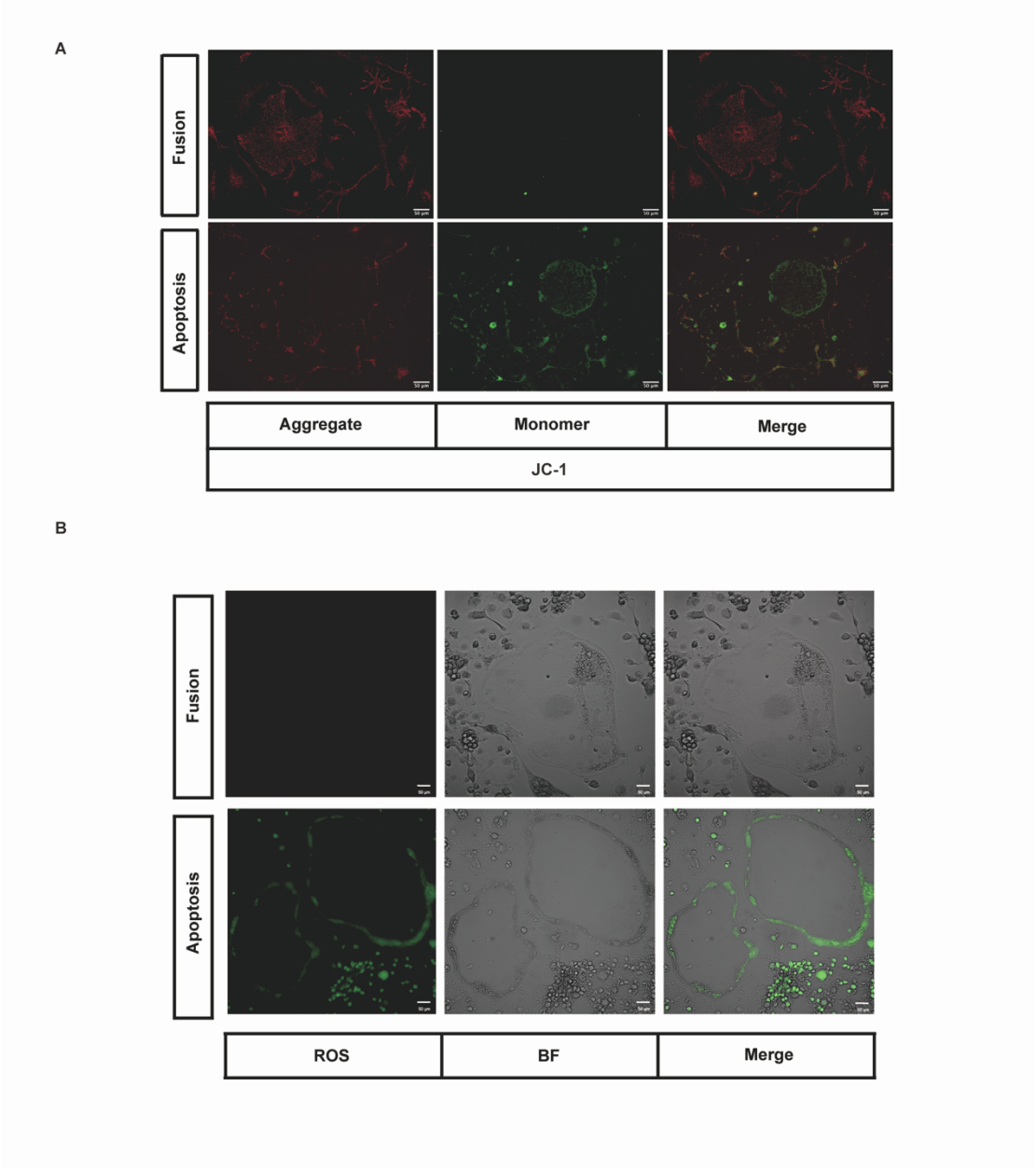

Figure S1. The alterations of mitochondrial membrane potential and ROS in apoptotic osteoclasts, Related to Figure 5.

(A) Representative JC-1 fluorescence images showing fused osteoclasts and apoptosis osteoclasts (J-aggregate, red; J-monomer, green) (n = 5). The mitochondrial membrane potential of apoptotic cells disappeared

(B) Representative ROS fluorescence images showing fused osteoclasts and apoptosis osteoclasts (ROS, green) (n = 5). ROS accumulated in apoptotic cells.

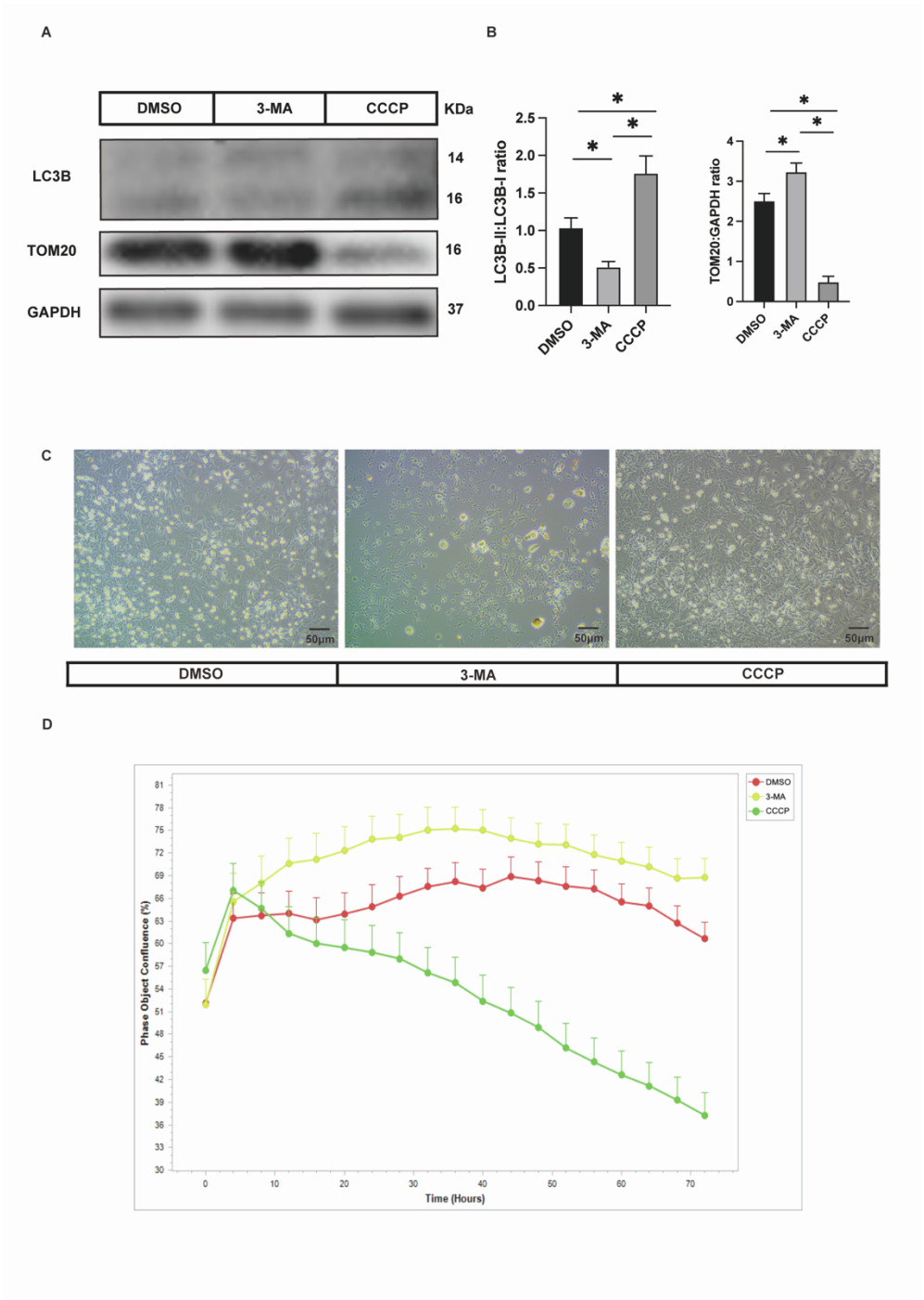

Figure S2. Inhibiting mitophagy induces osteoclast apoptosis, Related to Figure 6.

(A) Western blot analysis of the protein levels of LC3B, TOM20 and GAPDH in different osteoclast groups (n = 3). The osteoclasts were treated with DMSO, 3-MA and CCCP respectively after fused.

(B) Quantification of LC3B and TOM2o by immunoblotting.

(C) Representative images of osteoclasts after treated with drugs 3 days (n = 3).

(D) The cell confluence in different group (n = 5).

*$p < 0.05$ by one-way ANOVA (B).

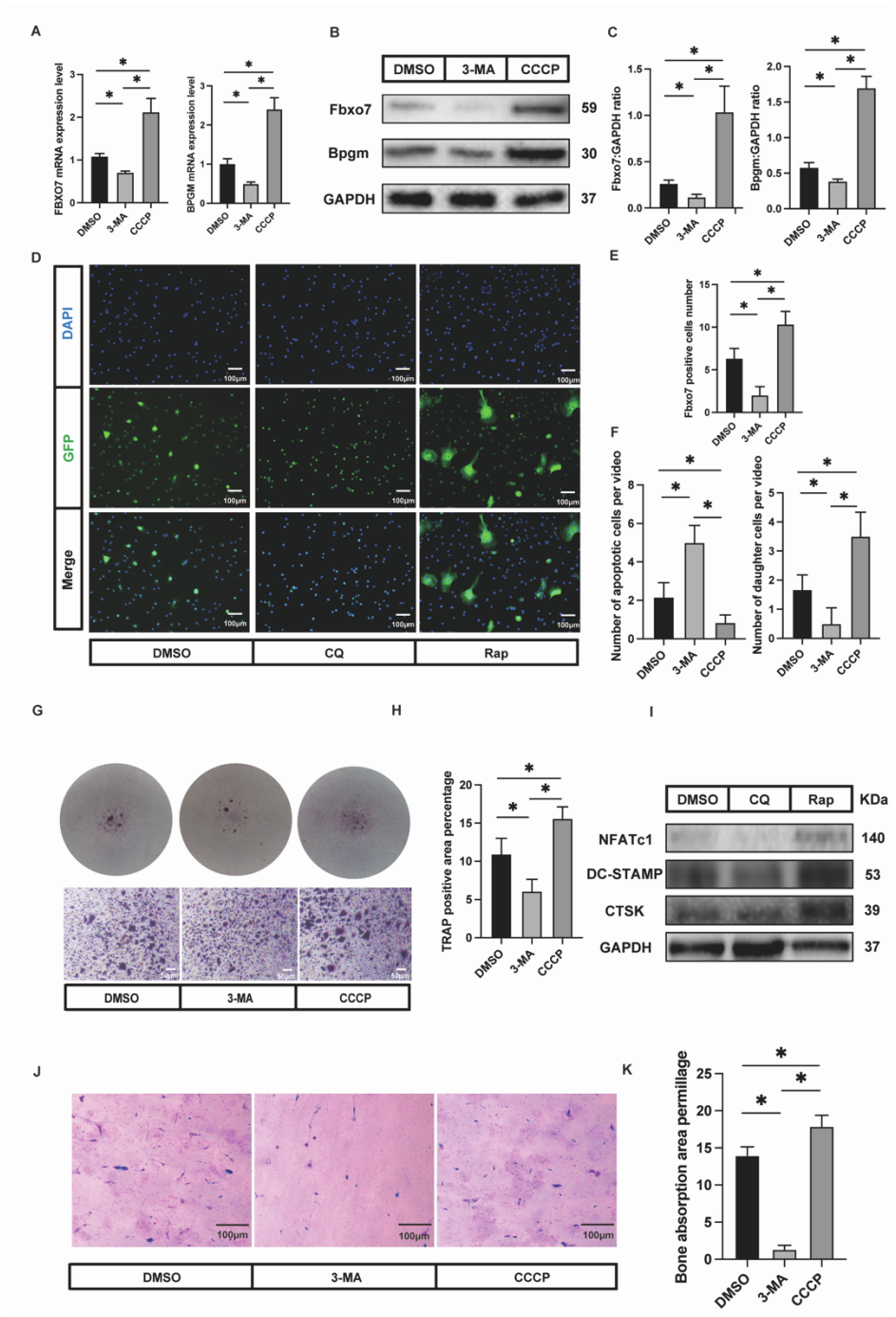

Figure S3. Enhancing mitophagy promotes osteoclast-osteomorph recycling, Related to Figure 7.

(A) The relative mRNA levels of Fbxo7 and Bpgm genes in DMSO, 3-MA and CCCP group (n = 3).

(B) Western blot analysis of the protein levels of Fbxo7, Bpgm and GAPDH in different osteoclast groups (n = 3).

(C) Quantification of Fbxo7 and Bpgm by immunoblotting.

(D) Representative images showing immunofluorescence staining (Fbxo7, green; DAPI, blue) in DMSO, 3-MA and CCCP group (n = 5).

(E) Number of Fbxo7 positive cells (n = 5).

(F) Number of daughter cells and apoptotic cells in different groups (n = 6).
(G) Representative images showing TRAP staining in DMSO, 3-MA and CCCP group (n = 3). The cells were treated with RANKL again for 12 h after large osteoclasts disappearing.
(H) Quantification of TRAP positive area by ImageJ.
(I) Western blot analysis of the protein levels of NFATc1, DC-STAMP, CTSK, and GAPDH in different osteoclast groups.
(J) Representative images showing bone resorption pits in DMSO, 3-MA and CCCP group (n = 3).
(K) Quantification of bone resorption pits area.
*$p < 0.05$ by one-way ANOVA (A, C, E, F, H, and K).