Supplemental Materials

Molecular Biology of the Cell

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Supplementary figures

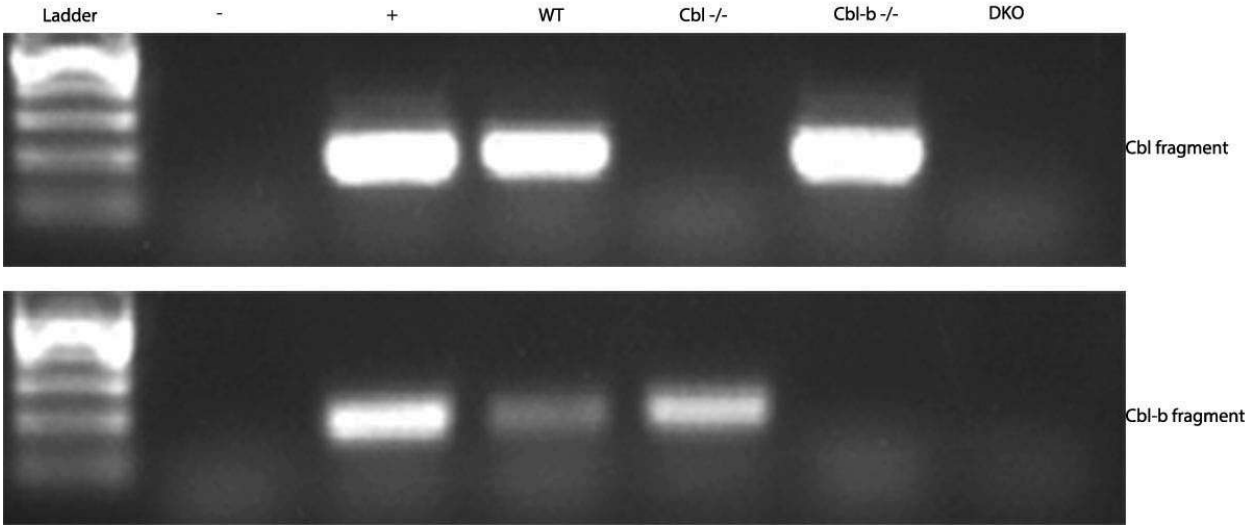

Figure S1. Gene knock out validation of macrophages by PCR. Gnomic DNA from different genotypes of macrophages were used as template in PCR to verify knocked out genotypes. Cbl fragment is amplified from WT, Cbl-b$^{-/-}$, but not in Cbl$^{-/-}$, and Cbl-b fragment is amplified from WT and Cbl$^{-/-}$, not in Cbl-b$^{-/-}$.

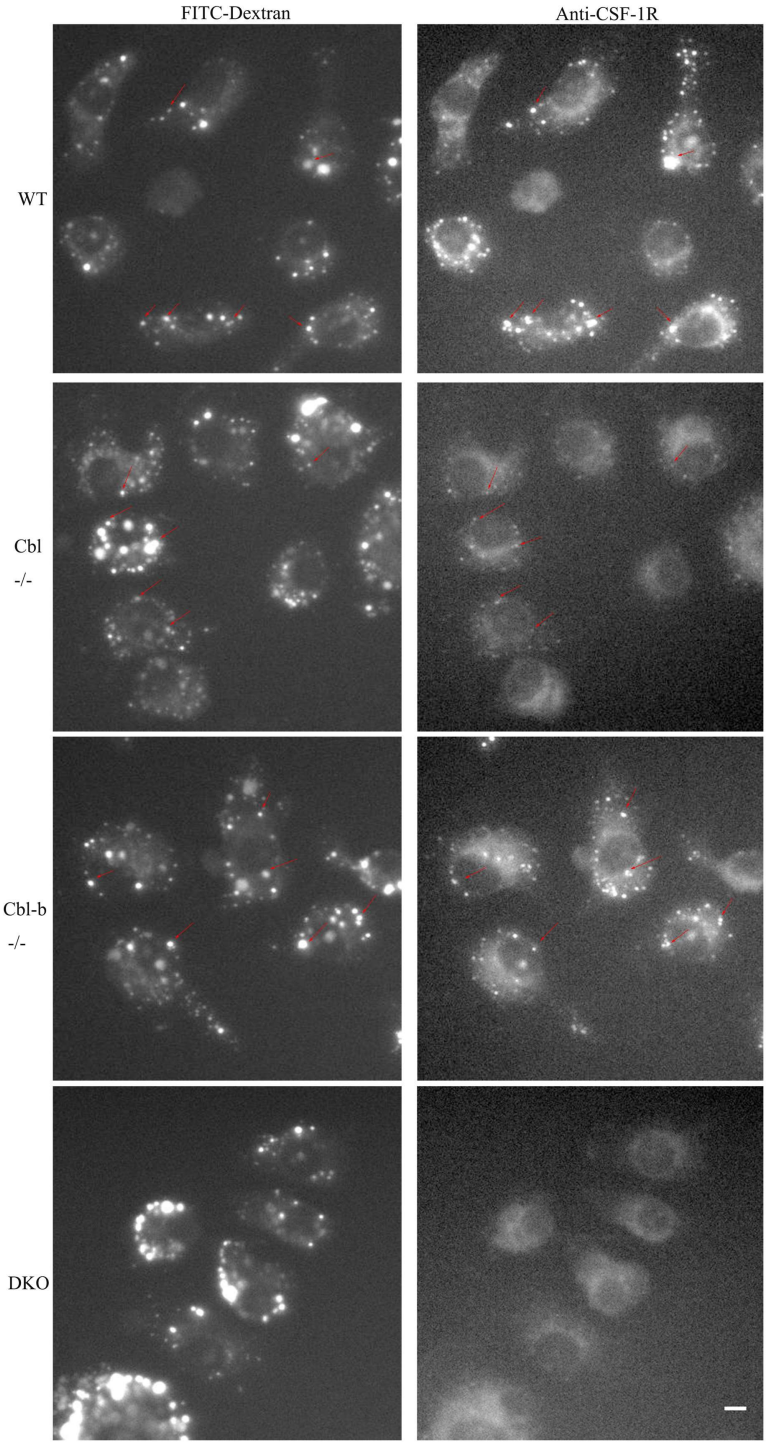

Figure S2. Defect of CSF-1R transport to macropinosomes in DKO macrophages. FITC-dextran (150 kD) labeled macropinosomes were sequentially permeabilized and then were probed for CSF-1R using anti-CSF-1R antibody-Dylight 594. Arrows indicate that macropinosomes (left) contain CSF-1R(Right). Scale bar = 5um.

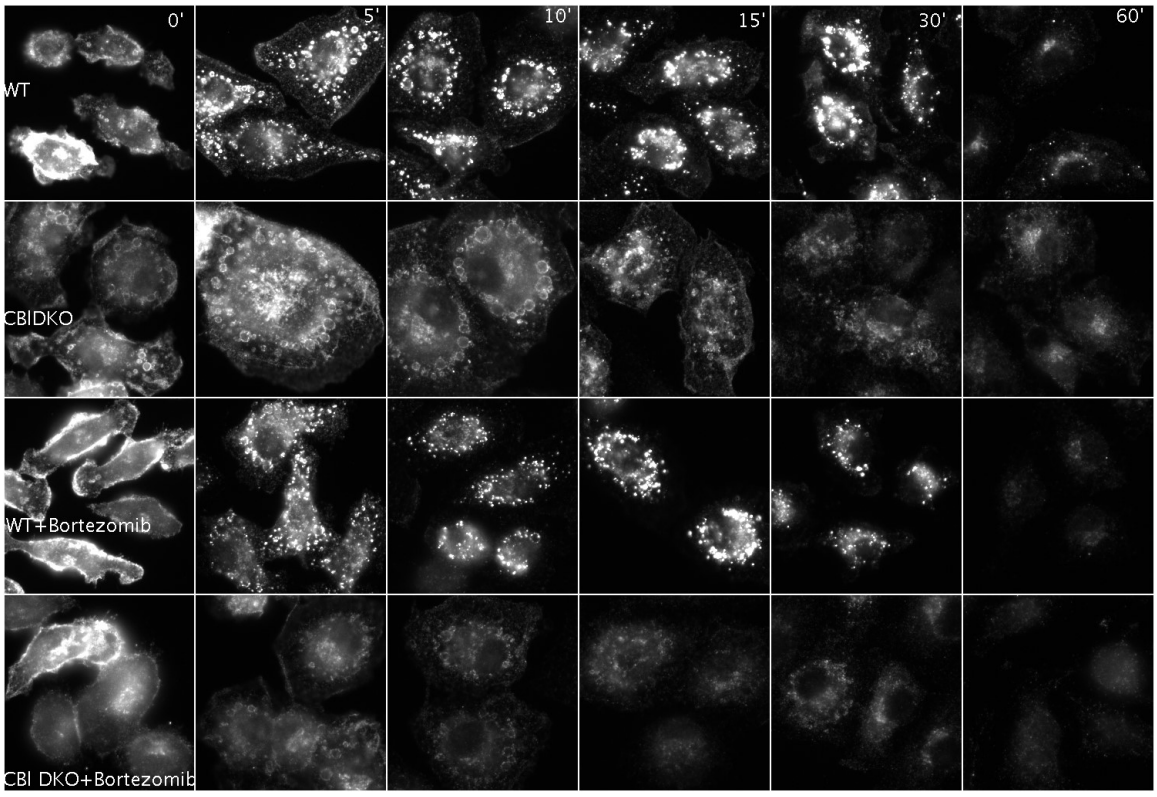

Figure S3. CSF-1R degradation in DKO macrophages was not a proteasome dependent pathway.

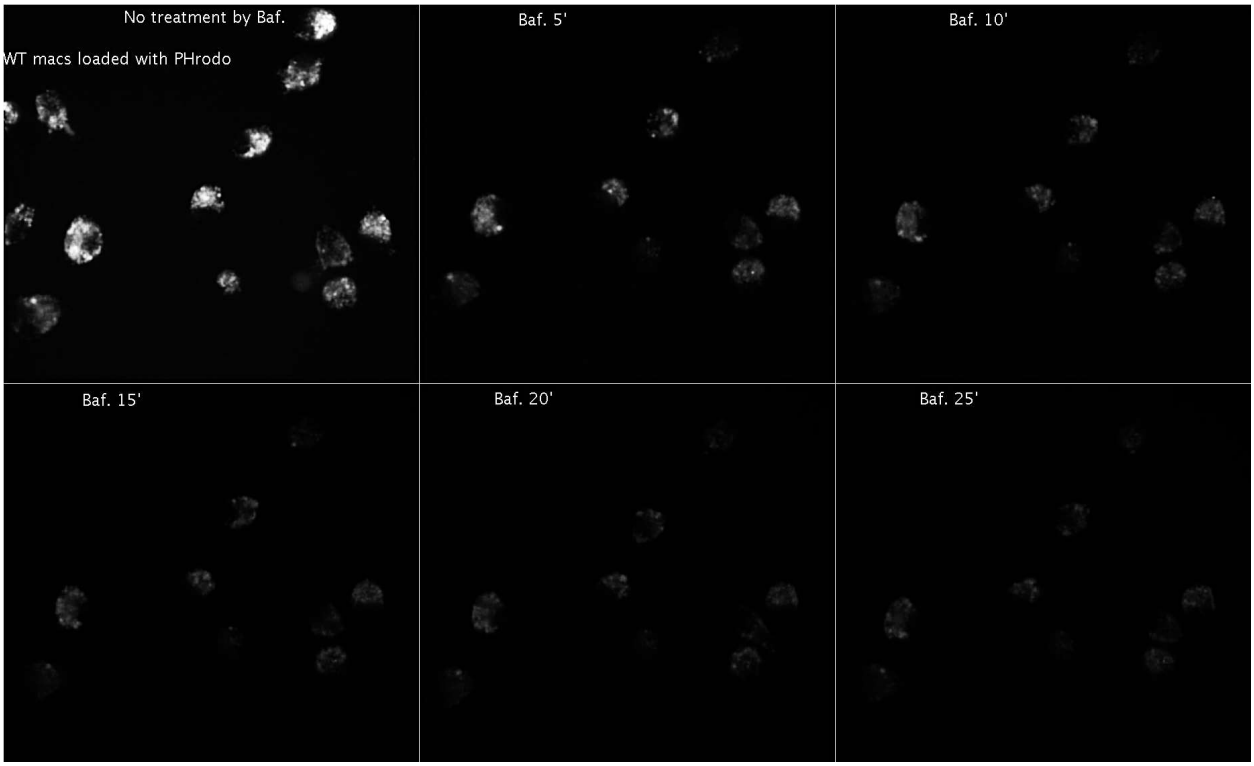

Figure S4. Neutralization of lysosomes by Bafilomycin A1. Macrophages were loaded with pHrodoRed-Dextran for 2h and then exposed to BafilomycinA1 at 100nM. Loss of pHrodoRed by about 15 min indicates neutralization of the lysosome.

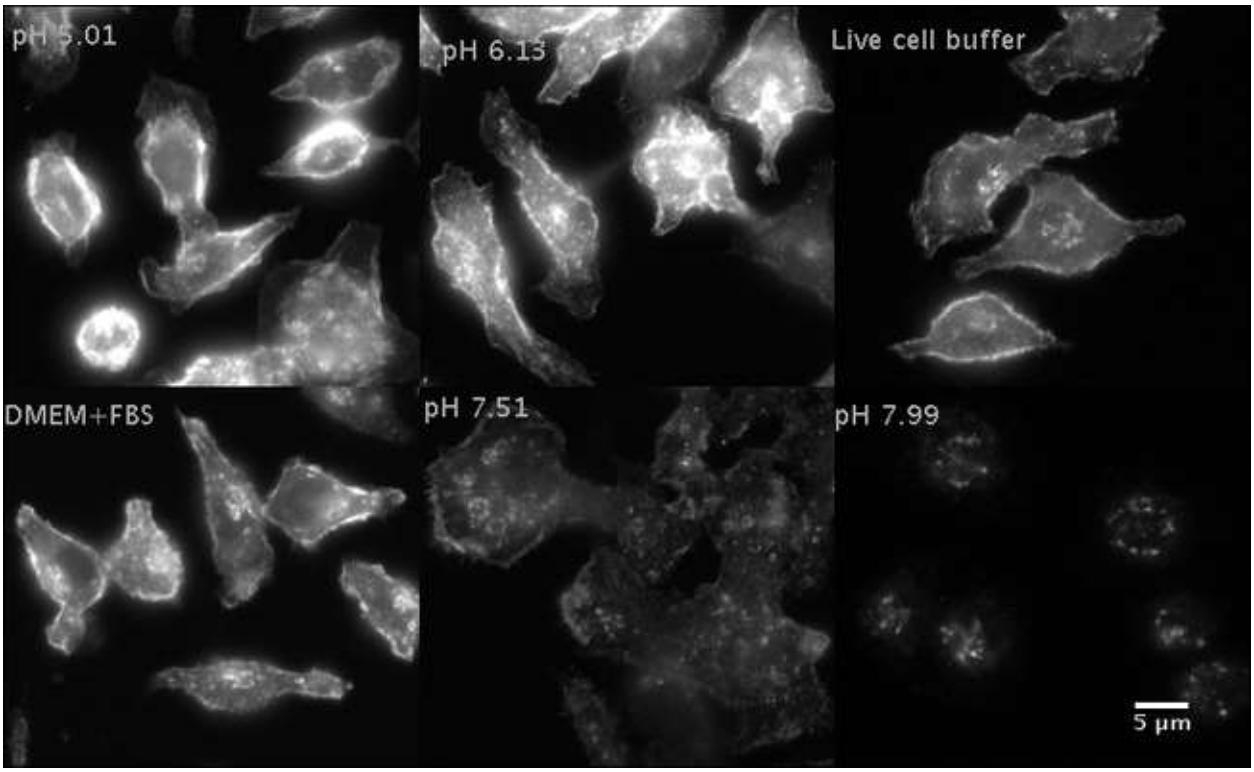

Figure S5. Media pH modulates CSF-1R traffic. Bone marrow derived macrophages were cultured for 15 min in live cell imaging buffer in air at 37 C with pH adjusted by adding HCl or NaOH as indicated or cultured in DMEM+10%FBS in a CO2 incubator. pH of live cell buffer is 7.2.