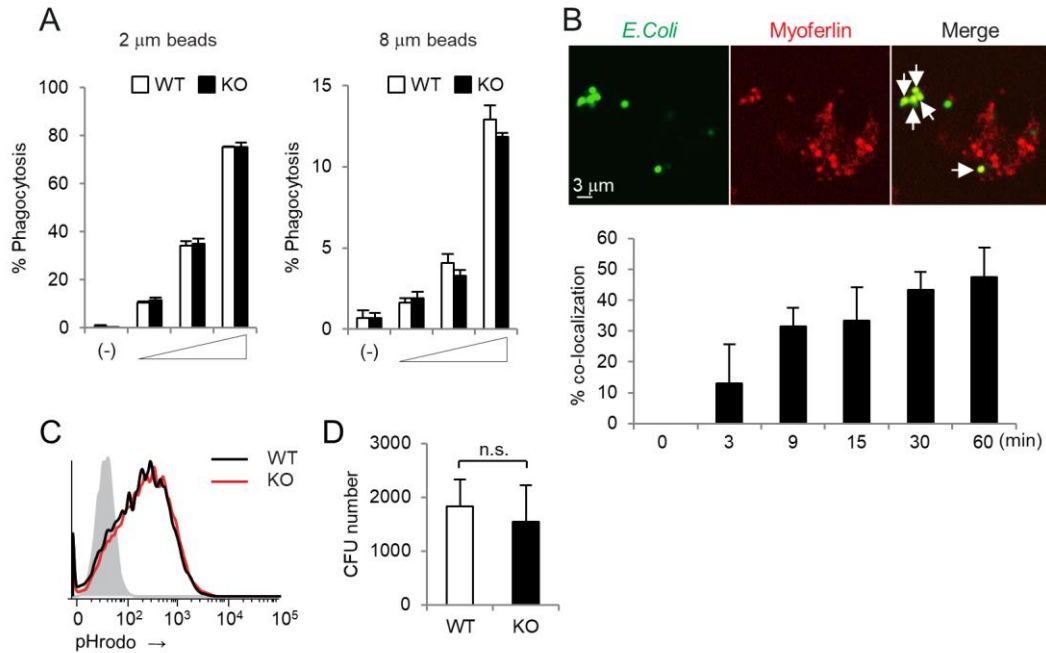


Supplemental Figure 1. Analyses of myoferlin-knockdown and -deficient cells.

(A) NIH3T3 cells expressing control or GFP-myoferlin were observed by confocal microscopy in the green or red channel. (B) NIH3T3 cells expressing control, myoferlin shRNA 1, 2, or human MYOFERLIN plasmid were subjected to western blot analysis against myoferlin. The arrow and asterisk indicate myoferlin and a non-specific band, respectively. (C) To detect autophagy, BMDMs from Myoferlin WT (white bars) or KO (black bars) mice were incubated in 0.5 μ M DAPGreen solution (Dojindo, Japan) for 30 min and autophagy was induced by incubation in HHBSS for 1 hour (starved). The occurrence of autophagy was quantified by the number of fluorescent puncta per cell (more than 10 cells). n.s., not significant, Student's *t*-test.



Supplemental Figure 2. Myoferlin plays a minimal role for phagocytosis.

(A) BMDMs from Myoferlin WT (white bars) or KO (black bars) mice were cultured with increasing amounts of 2 μm or 8 μm FITC-conjugated beads (Bay Bioscience, Japan) for 60 min. The percentage of BMDMs that engulfed the beads was quantified by flow cytometry. The experiment was performed in triplicate and the average values are plotted with the standard deviations. (B) BMDMs expressing RFP-myoferlin (red) were co-cultured with FITC-labeled *E. coli* (green) for up to 60 min and observed by confocal microscopy. Arrows indicate co-localization and lower graph indicates the percentage of *E. coli* in phagosomes which co-localized with myoferlin. (C) BMDMs from Myoferlin WT (black line) or KO (red line) mice were co-cultured with pHrodo Red *E. coli* BioParticles for 40 min, and the acidification of phagosomes due to phagosome-lysosome fusion was analyzed by flow cytometry. (D) BMDMs (1×10^5 cells) from Myoferlin WT (white bar) or KO (black bar) mice were cultured with *E. coli* (MOI = 10) for 30 min and treated for 10 min with 1 mg/mL gentamicin to kill extracellular bacteria. After the sterilization and washing three times with PBS, the BMDMs were lysed with double distilled water and the living intracellular bacteria were quantified with CFU assay by counting the colony number. Experiments were performed 5 times and the average number was plotted with the standard deviations. n.s., not significant, Student's *t*-test.