Supplementary Figure 1. Phagocytosis ability, LM phagosomal escape efficiency, and cell death are comparable between LM-infected gal3\(^{+/+}\) and gal3\(^{-/-}\) BMMs.

(A) Resting gal3\(^{+/+}\) (●) or gal3\(^{-/-}\) (○) BMMs were infected with wild-type LM 10403S (LM WT) at an MOI of 10 at 4°C. Synchronized phagocytosis was initiated by transferring the cells to 37°C. At 30 min, 1 h, and 2 h post-infection, unbound bacteria were washed off, and cells were fixed and processed for immunofluorescence staining for LM and F-actin. For quantification of phagocytic function, the number of internalized LM in each cell was counted in at least 200 macrophages, and the phagocytic index was calculated. Data are representative of three independent experiments. (B) A representative image of a gal3\(^{+/+}\) BMM with intracellular LM. Gal3\(^{+/+}\) BMMs were infected with LM WT at an MOI of 10. At 2 h post-infection, cells were stained for LM (red), F-actin (green), and nuclei (blue). Bacteria not stained with phalloidin-Alexa 488 were scored as “phagosomal”, while double-stained bacteria (appearing yellow) indicated “cytosolic” location. (C) At indicated time points, greater than 100 cells were examined and the number of phagosomal or cytosolic LM in each cell was recorded. The results represent the percentages of cytosolic LM among those counted. Data are
representative of three independent experiments. (D) Gal3+/+ (■) or gal3−/− (□) BMMs were left uninfected (untreated) or infected with LM WT at an MOI of 0.25. Culture supernatants were collected at 24 h post-infection and assayed for lactate dehydrogenase (LDH) release. Data represent the means ± SD of the results obtained from triplicate wells, and are representative of three independent experiments.
Supplementary Figure 2. LC3 puncta count per cell or total LC3-II levels in cell lysates are increased in gal3-deficient macrophages.

(A) Kinetics of the number of LC3 puncta per cell in WT or G3-KO RAW264.7 cells. Data were obtained by high-content imaging analysis. Each point represents the total value derived from nine image fields. (B) Immunoblot results showing the expression level of tubulin, p62, galectin-3, and LC3 in WT and G3-KO 264.7 cells at indicated time points after infection with \textit{LM} \Delta \textit{actA} at an MOI of 5. For the chloroquine (CQ)-treated group, cells were cultured in R10 medium supplemented with 10 \textmu M CQ at 30 min post-infection.
Supplementary Figure 3. Validation of galectin-8 KO (G8-KO) and galectin-3/galectin-8-double KO (G3/8-DKO) RAW264.7 cells by immunofluorescence staining.

G8-KO and G3/8-DKO RAW264.7 cells were generated as described in Materials and Methods. The cells were infected with LM \( \Delta \text{actA} \) for 1 h and immuno-stained with anti-galectin-3, anti-galectin-8, and anti-LM antibodies for validation of target protein expression.
Supplementary Figure 4. Recruitment of p62 or ubiquitin to LM is not altered in galectin-3-deficient or N-glycan-depleted macrophages.

(A and B) Kinetics of the percentage of p62-positive (A) or ubiquitin-positive (B) LM WT or LM ΔactA in WT or G3-KO RAW264.7 cells, as determined by high-content imaging analysis. Data points represent results quantified from at least 100 cells for each sample. (C and D) Confocal micrographs of control or KIF-treated J774 cells infected with LM WT for 1 h, then stained with DAPI, anti-LM, anti-galectin-3, anti-p62 (C), or anti-ubiquitin (D) antibodies. (E and F) Quantification of the percentages of LM co-localized with p62 (E) or ubiquitin (F). Data points represent the means ± SD of the results obtained from triplicate fields; greater than 100 LM were counted per field. *p < 0.05; **p < 0.01; ***p < 0.001 by Student’s t-test. Data are representative of three independent experiments.