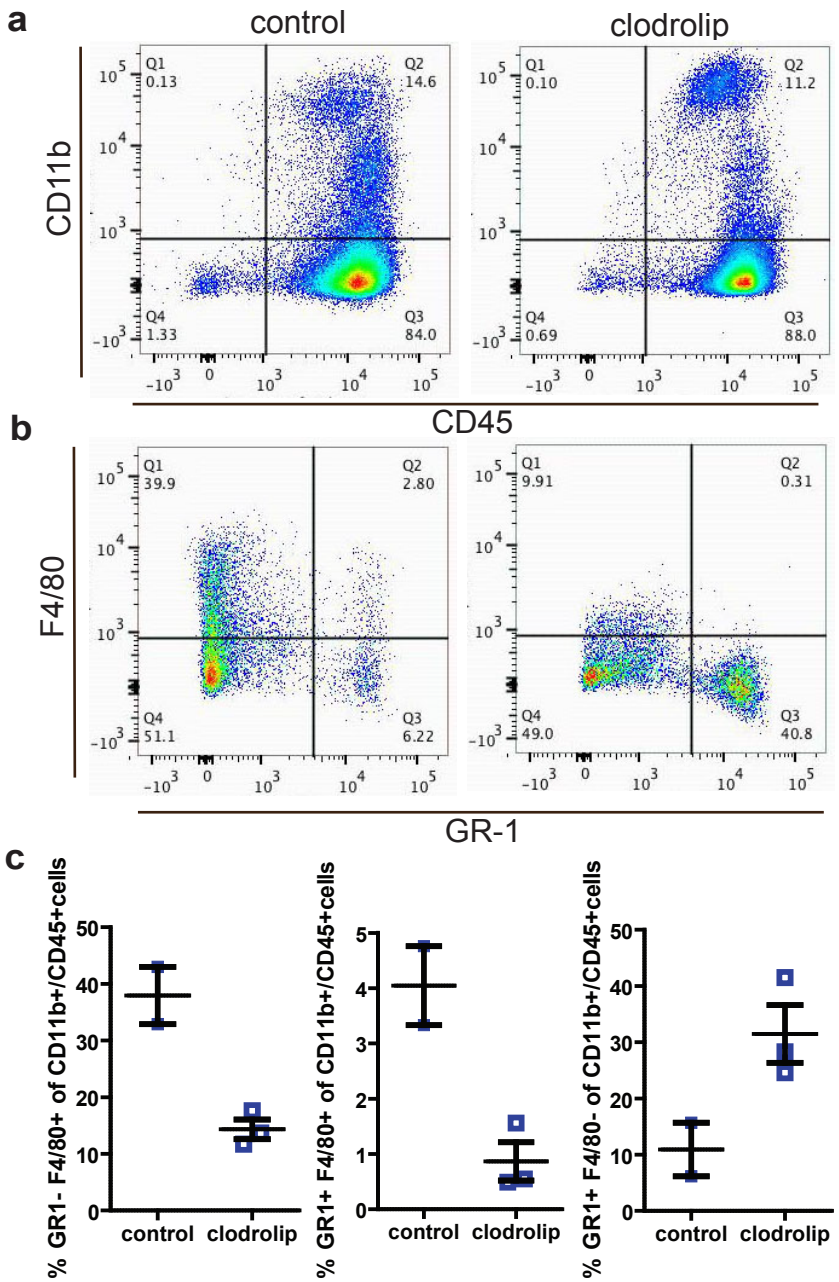
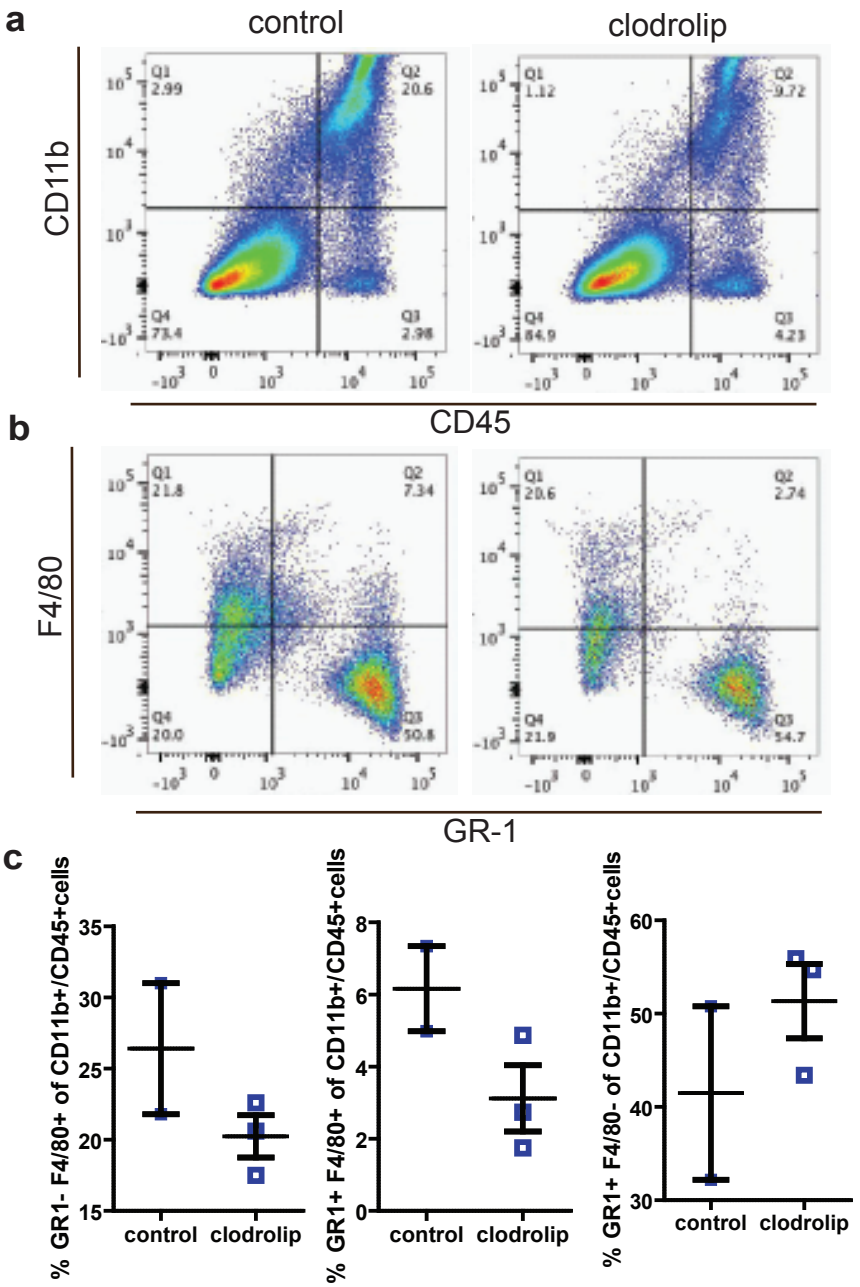
Supplementary Figure 1. Clodrolip depletes macrophages and monocytes from Atg16L1^{HM} mice.



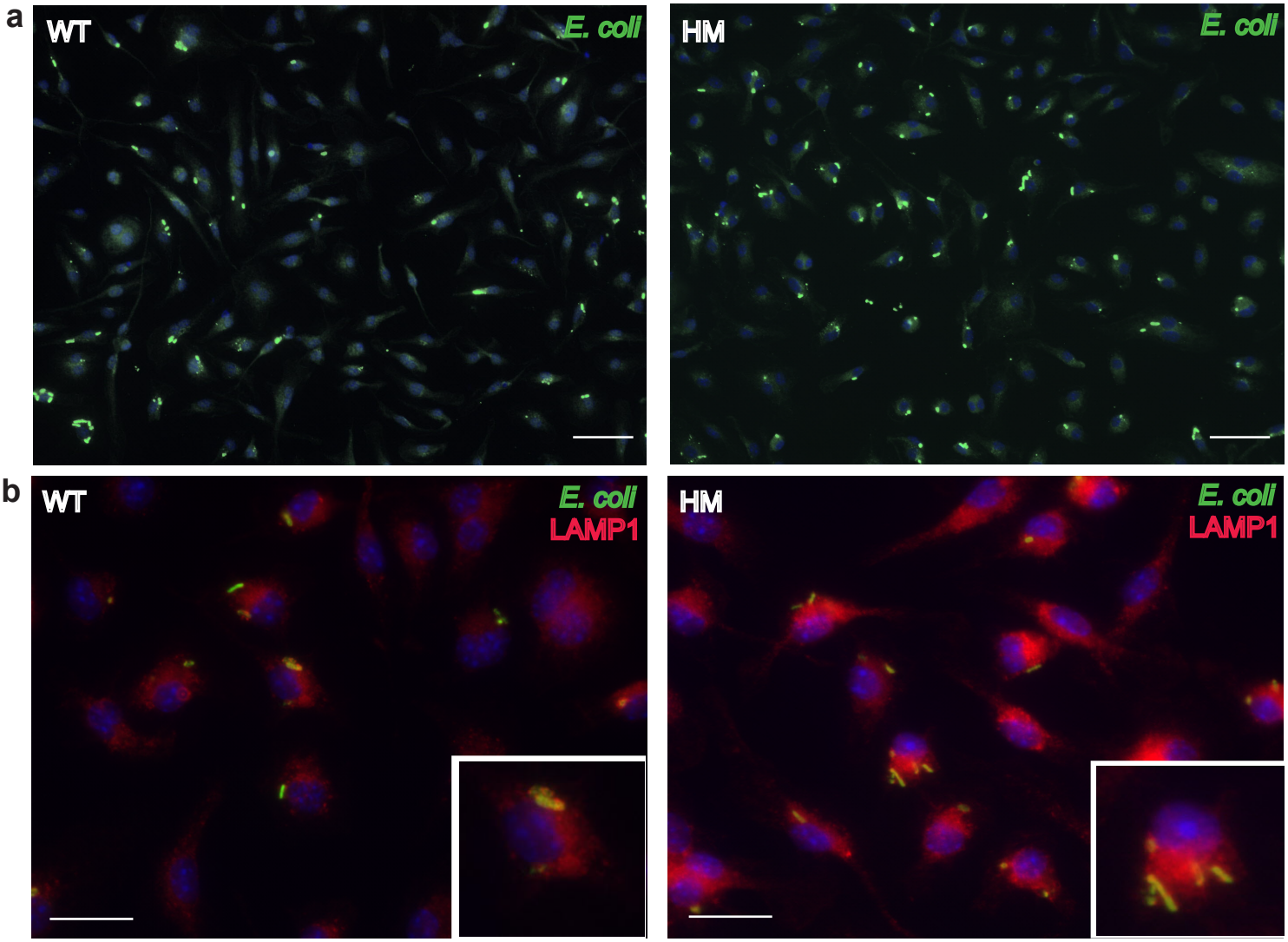
(a) Depletion of CD11b and CD45 double positive cells from the spleen of clodrolip-treated mice (b) Depletion of F4/80 positive cells (both GR1 low, macrophages, and GR1 medium, monocytes) in spleens (c) Quantification of macrophage (GR1 low, F4/80 positive), monocytes (GR1 medium, F4/80 positive), and neutrophil (GR1 hi, F4/80 negative) populations in spleens from multiple control or clodrolip treated animals.

Supplementary Figure 2. Clodrolip depletes macrophages and monocytes from the bladders of Atg16L1^{HM} mice 24hrs post infection with UPEC.



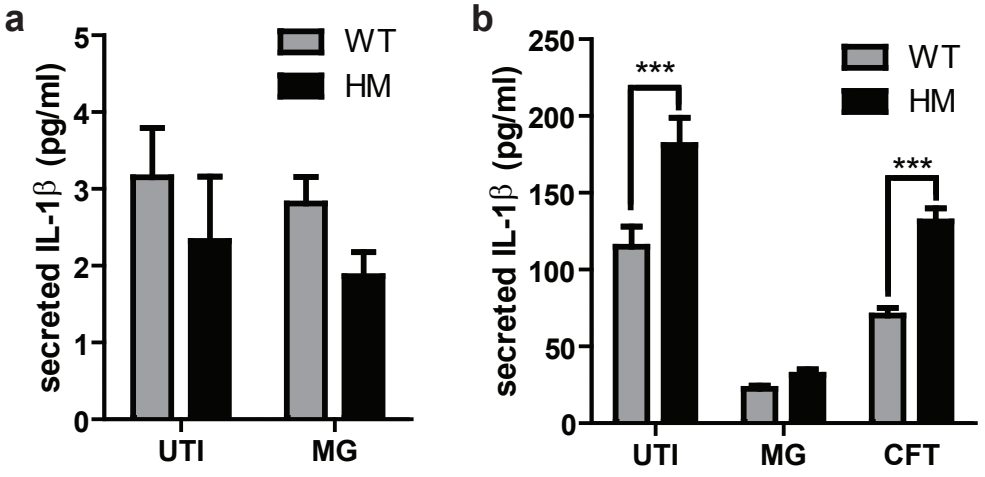
(a) Depletion of CD11b and CD45 double positive cells from the bladder of clodrolip-treated mice (b) Depletion of F4/80 positive cells (both GR1 low, macrophages, and GR1 medium, monocytes) in bladders (c) Quantification of macrophage (GR1 low, F4/80 positive), monocytes (GR1 medium, F4/80 positive), and neutrophil (GR1 hi, F4/80 negative) populations in bladders from multiple control or clodrolip treated animals.

Supplementary Figure 3. Increased uptake of UPEC into LAMP1 positive compartments in ATG16L1-deficient macrophages



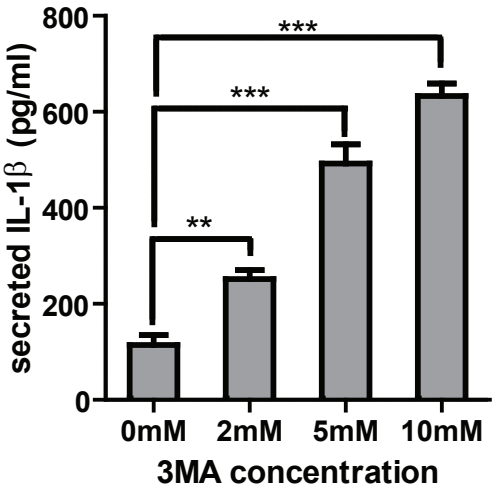
(a) Representative immunoflourescence images of UPEC within wild type (WT, left) and ATG16L1-deficient (HM, right) macrophages at 3 hours post challenge. E.coli (green) and nuclei are blue with biz-benzimide, quantification is presented in Fig. 2b. Scale bar = 40 μm. (b) Images showing co-localization of LAMP1 (red) with UPEC (green) in WT (left) and ATG16L1-deficient (right) BMDMs. Scale bar = 20μm.

Supplementary Figure 4. More IL-1β is secreted by ATG16L1-deficient macrophages in response to cystitis and pyelonephritis strains, after 24 hours



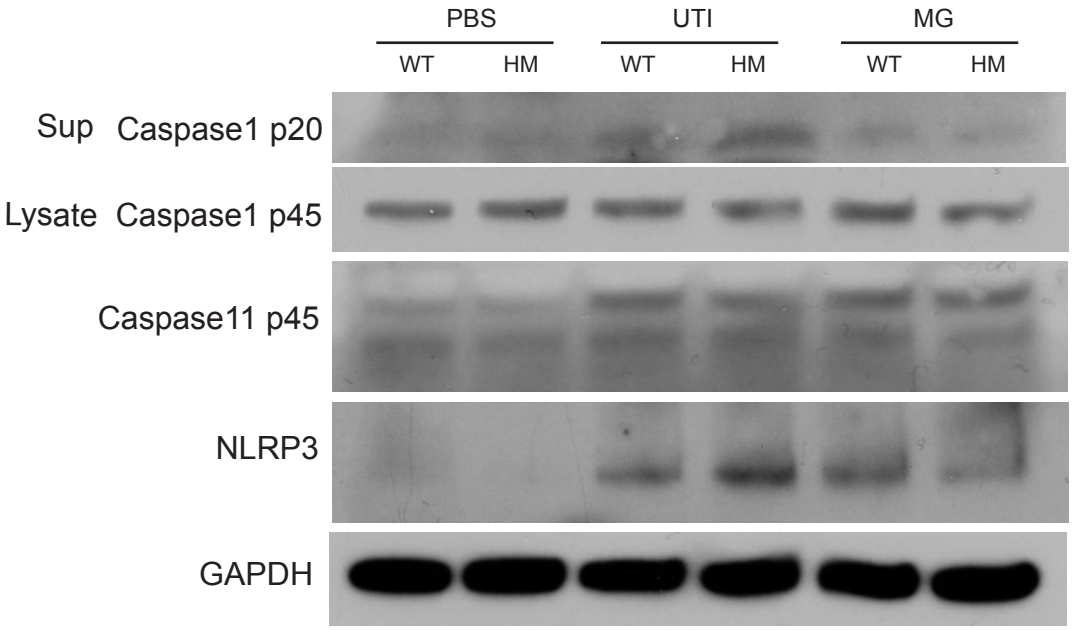
(a) Levels of IL-1β secretion by WT and HM macrophages at 2 hours in response to UTI89 and MG1655. (b) Levels of IL-1β secretion by WT and HM macrophages at 24 hours in response to UTI89 (cystitis strain), MG1655 (K-12 strain), or CFT073 (pyelonephritis strain). Two-way ANOVA with bonferroni post-tests. *** $P<0.001$.

Supplementary Figure 5. Inhibiting autophagy in WT macrophages leads to increased IL-1β secretion



Level of IL-1β secretion by WT macrophages at 24 hours when treated with increasing concentrations of the VPS34 inhibitor 3-MA and challenged with UTI89. Two-way ANOVA with bonferroni post-tests. ** $P<0.01$, *** $P<0.001$.

Supplementary Figure 6. More cleaved caspase-1 is found in the suprenatants of ATG16L1 deficient macrophages than WT macrophages



Western blot of cleaved caspase-1 (p20) in supernatants (24hrs) and uncleaved caspase-1 (p45), caspase-11 (p45), and NLRP3 (~117) in cell lysates (3hrs) of PBS, UTI89 and MG1655 challenged WT and ATG16L1-deficient macrophages.