Select autophagy genes maintain quiescence of tissue-resident macrophages and increase susceptibility to Listeria monocytogenes

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Innate and adaptive immune responses that prime myeloid cells, such as macrophages, protect against pathogens16,17. However, if left uncontrolled, these responses may lead to detrimental inflammation1. Macrophages, particularly those resident in tissues, must therefore remain quiescent between infections despite chronic stimulation by commensal microorganisms. The genes required for quiescence of tissue-resident macrophages are not well understood. Autophagy, an evolutionarily conserved cellular process by which cytoplasmic contents are targeted for lysosomal digestion, has resident macrophages are not well understood. Autophagy, an evolutionarily conserved cellular process by which cytoplasmic contents are targeted for lysosomal digestion, has homeostatic functions including maintenance of protein and organelle integrity and regulation of metabolism18. Recent research has shown that degradative autophagy, as well as various combinations of autophagy genes, regulate immunity and inflammation19–22. Here, we delineate a function of the autophagy proteins Beclin 1 and FIP200—but not of other essential autophagy components ATG5, ATG16L1 or ATG7—in mediating quiescence of tissue-resident macrophages by limiting the effects of systemic interferon-γ. The perturbation of quiescence in mice that lack Beclin 1 or FIP200 in myeloid cells results in spontaneous immune activation and resistance to Listeria monocytogenes infection. While antibiotic-treated wild-type mice display diminished macrophage responses to inflammatory stimuli, this is not observed in mice that lack Beclin 1 in myeloid cells, establishing the dominance of this gene over effects of the bacterial microbiota. Thus, select autophagy genes, but not all genes essential for degradative autophagy, have a key function in maintaining immune quiescence of tissue-resident macrophages, resulting in genetically programmed susceptibility to bacterial infection.

L. monocytogenes is a bacterial pathogen that replicates intracellularly in macrophages until interferon-γ (IFN-γ) triggers cellular activation for effective bacterial killing13,14. The involvement of autophagy in this process has been largely supported by studies in cultured cells showing that autophagy markers LC3 and GABARAPs and p62 co-localize with structures that are either induced by or contain L. monocytogenes15–17, and by studies that noted modestly increased L. monocytogenes replication in mice lacking Atg5 in myeloid cells18. Moreover, L. monocytogenes possesses diverse strategies to avoid degradation by autophagy-lysosomal pathways that may circumvent the autophagy machinery to promote pathogenesis14,19. The precise roles of autophagy in restricting L. monocytogenes have therefore been challenging to reconcile. We used a genetic approach in matched littermate mice to elucidate the function of autophagy genes in resistance to L. monocytogenes infection. We first investigated the role of Beclin 1, a central component of the phosphatidylinositol-3-kinase (PI3K) complex that initiates autophagosome formation21. Compared with wild-type (WT; Becn1f/f) littermates, mice lacking Becn1 expression in myeloid cells (Becn1−/−Lyz2-cre+/− [Becn1mye−/−]) were resistant to L. monocytogenes and controlled bacterial dissemination early after infection (Fig.1a–c). Fip200mye−/− mice were similarly resistant to L. monocytogenes (Fig. 1b,e). Notably, a previous report was unable to detect a difference in survival of Fip200−/− mice when infecting with a lower dose of L. monocytogenes, because the non-littermate WT control mice were not susceptible to that dose19. These findings are distinct from the reported functions of another autophagy protein, ATG5, in controlling L. monocytogenes infection18,22. We confirmed these previous results—Atg5mye−/− mice exhibited a modest increase in L. monocytogenes susceptibility (Fig. 1d). By contrast, mice lacking other essential autophagy genes in myeloid cells, Atg7mye−/− and Atg16l1mye−/−, showed WT-level susceptibility to infection and bacteria dissemination (Fig. 1e,f, Extended Data Fig. 1a). Atg14mye−/− mice also showed WT-level susceptibility to L. monocytogenes-induced lethality but harboured a reduced burden of bacteria in the liver (Fig. 1g, Extended Data Fig. 1a). The autophagy genes were all effectively excised in the macrophages of these mice as measured by p62 degradation and LC3 lipidation (Extended Data Fig. 1b). These findings were particularly notable as Atg7mye−/− and Atg16l1mye−/− mice from the same facility are significantly more...
susceptible to T. gondii infection, whereas Atg5 is uniquely required to control Mycobacterium tuberculosis\(^{3,23}\). Resistance to L. monocytogenes is therefore genetically distinct from these other infections.

Previous studies found that L. monocytogenes vacuolar escape and intracellular growth is independent of Atg5 or Beclin\(^{19,24}\), this led us to consider the alternative hypothesis that macrophages lacking Beclin 1 and FIP200 are activated in vivo before infection. We therefore analysed naïve macrophages that are resident in the peritoneal cavity, as these cells provide a first line of defence against intraperitoneal (i.p.) challenge with L. monocytogenes. Peritoneal macrophages from Beclin\(^{mye}\) mice infected in vitro with L. monocytogenes showed enhanced control of the bacterial replication, demonstrating
cell-intrinsic resistance to *L. monocytogenes* (Fig. 1b, Extended Data Fig. 1c). We found that naive peritoneal resident macrophages, defined by surface markers as CSF1R+ICAM2+CD11b+ (ICAM2+ macrophages; Supplementary Fig. 1a), from *Becn1*Δmye mice showed marked upregulation of major histocompatibility complex (MHC) class II expression (Fig. 2a), whereas total peritoneal cell counts, as well as absolute numbers of ICAM2+ peritoneal macrophages and peripheral blood monocytes, were unaffected (Fig. 2b, Supplementary Fig. 2b). Explanted macrophages from *Becn1*Δmye mice expressed increased inducible nitric oxide synthase (iNOS) after stimulation with lipopolysaccharide (LPS) or IFN-γ (Fig. 2c), indicating that the cells were primed in vivo before infection. As *L. monocytogenes* disrupts genes in multiple cell lineages in addition to resident macrophages in the peritoneal cavity, including neutrophils, dendritic cells (DCs) and small peritoneal macrophages (SPMs)20–24, we next examined the effect of *Becn1* deletions in these other cell types. We infected *Becn1*ΔMye-Cre mice and *Becn1*ΔCD11c-Cre mice—with deletions for *Becn1* in neutrophils and in DCs/SPMs, respectively—with *L. monocytogenes*. Loss of *Becn1* from neutrophils or DCs and SPMs was not sufficient to result in the phenotypes observed in the *Becn1*Δmye mice (Extended Data Fig. 2), suggesting *L. monocytogenes* resistance and macrophage activation is specific to macrophage deletion of *Becn1*.

Similar to *Becn1*Δmye mice, MHC-II+ICAM2+ macrophages were observed in naive *Fip200*Δmye mice but were absent in *Atg5*mye, *Atg7*mye and *Atg16l1*mye mice (Fig. 2d,e, Extended Data Fig. 3a–c). In *Atg14*Δmye mice, we observed variation in macrophage MHC-II level (Extended Data Fig. 3d). However, on average, *Atg14*Δmye mice had lower levels of MHC-II+ICAM2+ macrophages compared with *Becn1*Δmye or *Fip200*Δmye mice (Extended Data Fig. 3e). Global knockout of *Rubicon*, a gene that mediates LC3-associated phagocytosis (LAP) and that is a repressor of degradative autophagy25, had no effect on MHC-II+ICAM2+ macrophages in naive mice (Fig. 2e, Extended Data Fig. 3b,c). Together, these data show that resistance to *L. monocytogenes* correlates with the level of MHC-II+ICAM2+ macrophages observed in uninfected mice.

We next investigated whether the macrophage activation that occurs in the absence of *Becn1*Δmye or *Fip200*Δmye is systemic. Blood from both *Becn1*Δmye and *Fip200*Δmye mice contained increased MHC-II+ monocytes (Fig. 2f,g). Tissue-resident macrophages from multiple tissues of *Becn1*Δmye mice expressed higher levels of MHC-II (Fig. 2h). To determine the changes in tissue pathology that were associated with the systemic macrophage activation, we examined H&E stained sections from mice (aged 8–12 weeks) and observed mild inflammation in multiple tissues (Supplementary Fig. 2). The lungs of *Becn1*Δmye mice showed mild, patchy bronchiolitis with perivascular lymphoid aggregates without associated bronchiectasis, interstitial fibrosis, acute lung injury or vasculitis. Very mild, widely spaced periportal aggregates of chronic inflammatory cells were present in the liver of these mice, without associated fibrosis or necrosis. The kidneys showed very mild patchy interstitial chronic inflammation without associated fibrosis or vasculitis, or overt evidence of glomerulitis or glomerulosclerosis in the *Becn1*Δmye mice. The spleens of *Becn1*Δmye mice showed increased extramedullary haemopoiesis.

To better define the activation state of macrophages before infection, we performed RNA sequencing (RNA-seq) on ICAM2+ macrophages from the peritoneum of naive WT and *Becn1*Δmye mice. The genes of which expression was increased in the absence of *Becn1* included interferon-stimulated genes (ISG) and inflammatory cytokines (Fig. 2i, Extended Data Fig. 4a, Supplementary Table 1). Gene set enrichment analysis demonstrated significant upregulation of IFN-γ and IFN-α/β-responsive genes (Fig. 2j, Extended Data Fig. 4b, Supplementary Fig. 3). Quantitative PCR with reverse transcription (RT–qPCR) analysis validated findings from the RNA-seq analysis (Fig. 2k, Extended Data Fig. 4c). Upregulation of inflammatory cytokine genes Cxc9, Cxcl10 and Ccl5 also suggested that Beclin 1-deficient macrophages were activated in naive mice (Fig. 2k). Similar upregulation of IFN response genes and inflammatory cytokines were observed in FIP200-deficient macrophages (Supplementary Fig. 4).

To understand the cellular events that are associated with pro-inflammatory macrophage activation, we analysed DNA damage responses, as accumulating evidence highlights direct links between the DNA damage response and innate immune response signalling that lead to the production of inflammatory cytokines26–29. However, we did not observe changes in the DNA damage response in resting or bleomycin-treated Beclin 1-deficient macrophages compared to WT cells (Extended Data Fig. 5a). As peritoneal resident macrophages are maintained in the peritoneal cavity through self-renewal, we wondered whether macrophage activation was associated with a replication defect in Beclin 1-deficient macrophages. Peritoneal resident ICAM2+ macrophages from both WT and *Becn1*Δmye mice undergo slow basal in situ proliferation (Extended Data Fig. 5b). This is consistent with previous reports of low levels of BrdU+ proliferating macrophages in steady state30.

To induce rapid local expansion of peritoneal resident macrophages, we injected mice with IL-4 complex (IL-4c)31. In WT mice, we observed increased proliferation and accumulation of ICAM2+ macrophages after injection with IL-4c (Extended Data Fig. 5c). By contrast, ICAM2+ macrophages from *Becn1*Δmye mice displayed limited replication in response to IL-4c, although they still expressed alternative activation marker RELM-α (Extended Data Fig. 5c). These results suggested that Beclin 1 regulates proliferation, but not polarization, of resident macrophages in response to IL-4c, which may be related to its function in controlling macrophage activation.

Given our observations that Beclin 1 influences activation state and proliferation of peritoneal macrophages, we performed a more global analysis of the peritoneal cell populations that rely on Beclin 1 expression in myeloid cells using single-cell transcriptomic analysis (single-cell RNA-seq). Cells from both *Becn1*Δmye and WT mice were partitioned into 20 clusters (C1–C20; Supplementary Fig. 5). Myeloid clusters (C1–C10) demonstrated a core transcriptional program of Csf1r (Fig. 3a,b). In WT mice, C1 and C2 represented tissue-resident peritoneal macrophages with high levels of Adgre1, Timd4 and Gata6 expression15–17 (Fig. 3b). We identified a macrophage subpopulation (C4) that bridges monocytes (C9) and SPMs (C8) with tissue-resident macrophages (C1 and C2; Fig. 3a). Reduced expression of genes that defined the core tissue-resident feature (Adgre1, Gata6 and Timd4) and increased Ccr2 levels implied that the C4 cells had a monocyte origin (Fig. 3b). This is in agreement with the idea that intermediates between newly arrived monocytes and fully mature tissue-resident macrophages are present in the peritoneum32.

Cells from *Becn1*Δmye mice showed distinct clustering with the major populations shifted away from the C1 and C2 clusters present in WT mice to C3 and C4, and two distinct clusters C5 and C6 (Fig. 3a). *Becn1*Δmye ICAM2+ macrophages showed increased expression of IFN-pathway genes (Fig. 3c), confirming the RNA-seq analysis (Fig. 2i). The accumulation of intermediate C4 and the appearance of C6—macrophages with low Adgre1, Gata6, and Timd4—expression suggested that Beclin 1 has a function in maintaining expression of tissue-specific genes in these macrophages (Fig. 3a,b). C4 and C6 macrophages showed varying transcriptional patterns that were an intermediate between tissue-resident macrophages and monocyte-derived cells observed in WT mice (Supplementary Fig. 5d). Furthermore, compared with the WT mice, *Becn1*Δmye mice showed accumulation of Ccr2+Itgax+ monocytes in C9 (Fig. 3a,b). Consistent with these findings, flow cytometry analysis identified an increase of CSF1R+MHC-II+ICAM2+ cells in *Becn1*Δmye mice, which included an accumulation of CD226+ monocyte-like cells and DCs, whereas the CD226+ SPM population was unaltered (Fig. 3d). Together these data indicate that the differentiation and activation state of resident peritoneal macrophages depends on the expression of Beclin 1.
Fig. 2 | Alterations of peritoneal tissue-resident macrophages in mice with select autophagy gene deficiency. a-d, Flow cytometry of macrophage subsets in the peritoneum of Beclin$^{+/+}$ and Beclin$^{+/−}$ (a, left), and Fip200$^{−/−}$ and Fip200$^{+/+}$ (d) adult mice; $n \geq 3$ independent experiments with $n \geq 3$ mice. The percentage of MHC-II$^{−/−}$ and MHC-II$^{+/+}$ macrophages in total ICAM2$^{+}$ macrophages (a, right). Beclin$^{+/+}$, $n = 13$; Beclin$^{+/−}$, $n = 15$. Data are mean ± s.e.m. Adjusted $P$ values were calculated using two-way ANOVA with Sidak’s multiple comparisons test on MHC-II$^{−/−}$ data. b, The number of the total cells, and total, MHC-II$^{−/−}$ and MHC-II$^{+/+}$ fractions of ICAM2$^{+}$ macrophages. Data are mean ± s.e.m., pooled from at least three experiments. Beclin$^{+/+}$, $n = 13$; Beclin$^{+/−}$, $n = 15$. Adjusted $P$ values were calculated using multiple $t$-tests. c, Ex vivo stimulated peritoneal macrophages were analysed for intracellular iNOS after stimulation. Data are mean ± s.e.m., representative of three experiments; $n = 4$. Adjusted $P$ values were calculated using multiple $t$-tests. e, The percentage of MHC-II$^{−/−}$ and MHC-II$^{+/+}$ ICAM2$^{+}$ macrophages; Fip200$^{−/−}$, $n = 14$; Fip200$^{+/+}$, $n = 15$; Atg5$^{+/−}$, $n = 10$; Atg5$^{+/+}$, $n = 7$; Atg7$^{+/−}$, $n = 4$; Atg7$^{+/+}$, $n = 4$; Atg16l1$^{+}$, $n = 6$; Atg16l1$^{−/−}$, $n = 6$; Rubicon$^{+/−}$, $n = 10$; Rubicon$^{+/+}$, $n = 9$ mice. Data are mean ± s.e.m. Adjusted $P$ values were calculated using two-way ANOVA with Sidak’s multiple comparisons test on MHC-II$^{−/−}$ data. f, Flow cytometry analysis of MHC-II level on monocytes; two experiments with $n \geq 3$ biological replicates per group. g, The percentage of MHC-II$^{−/−}$ monocytes; Beclin$^{+/+}$, $n = 9$; Beclin$^{+/−}$, $n = 8$; Fip200$^{−/−}$, $n = 8$; Fip200$^{+/+}$, $n = 8$ mice. Data are mean ± s.e.m. $P$ values were calculated using two-tailed Mann-Whitney U-test. h, Flow cytometry analysis of tissue-resident macrophages; two experiments with $n \geq 3$ biological replicates per group. i, Heat map of selected genes regulated by Beclin1 using RNA-seq; $n = 4$ samples per group. j, Gene set enrichment analysis of Beclin1-dependent signature; the green curve represents the density of the genes identified in the RNA-seq analysis with normalized enrichment score (NES). False discovery rate (FDR)-adjusted $P$ values are indicated. k, RT-qPCR measurements of transcript levels in naive peritoneal macrophages. Data are mean ± s.e.m. from three independent experiments; Beclin$^{+/+}$, $n = 9$; Beclin$^{+/−}$, $n = 8$. $P$ values were calculated using two-tailed Mann-Whitney U-tests. ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05.
Fig. 3 | Disrupted immune cell homeostasis associated with Becn1 myeloid deficiency. a–e.h. Myeloid SPMs (a), B-cell (e) and T- and NK-cell (h) clusters from single-cell profiles of peritoneal cavity cells visualized with t-distributed stochastic neighbour embedding (tSNE); n = 4 naive mice, aged 8 weeks. Gene expression (b) and the IFN-γ pathway (c) was projected onto the tSNE plots with two groups of mice overlaid in b. Colour scales for each gene with the highest log-transformed expression level are shown. d. Flow cytometry validation on naive mice. Data are mean ± s.e.m. from three experiments; Becn1+/−, n = 8; Becn1−/−, n = 9 mice. Adjusted P values were calculated using two-way ANOVA with Sidak’s multiple comparisons test. f.g.i. Flow cytometry analysis on naive mice. Three experiments; n ≥ 3 mice each). j.k. Ifng expression (violin plot (j); dot plots (k)) in T- and NK-cell clusters on the basis of single-cell RNA-seq analysis. Adjusted P values were calculated using model-based analysis of single-cell transcriptomics differential expression test. ***P < 0.001; **P < 0.01; *P < 0.05.

We next examined the effects of myeloid cell deficiency of Beclin 1 on other peritoneal cells. B cells were grouped into Zbtb32+ B1 cells (C11, C12 and C14)35, Ccr7+Sell+ B2 cells (C13) and Mki67+ proliferating B cells (C15; Fig. 3e, Extended Data Fig. 6a). Reduced B-cell numbers were observed in Becn1−/− mice (Extended Data Fig. 6a). Flow cytometry confirmed that total B cells and B1 cells were reduced in Becn1−/− mice (Fig. 3f,g, Extended Data Fig. 6b), correlating with a decrease in macrophage expression of Cxcl13 (Fig. 3b, Supplementary Fig. 5)35,38. T cells were divided into naive (C16) and activated T cells (C17) on the basis of levels of Sell...
previous reports have linked autophagy deficiency to inflammation. To better understand the role of autophagy in innate immune cells, we assessed the involvement of Beclin 1 in myeloid cells, which have been shown to be enriched due to overlapping gene sets with IFN-γ pathway. We focused on the role of Beclin 1 in macrophages, as they are key players in the immune system and play a critical role in the clearance of intracellular bacteria.

We examined the role of Beclin 1 in the regulation of the basal activation state of macrophages. We observed that Beclin 1 deficiency in myeloid cells led to increased macrophage activation, manifested by reduced B1 cells and activation of T cells. The role of Beclin 1 in immune quiescence is therefore distinct from its role in maintaining immune responses to pathogens.

In contrast to adult mice, neonatal Beclin 1−/− mice showed increased resistance to pulmonary infections. This was not due to inefficient clearance of the bacteria, as the bacterial microbiota did not play a role in this process. Instead, the role of Beclin 1 in maintaining immune quiescence in neonatal mice was due to the absence of commensal bacteria, which are known to play a critical role in establishing the microbiota.

In conclusion, our study highlights the importance of autophagy in maintaining immune quiescence and protecting against infections. These findings have implications for the development of autophagy-targeted therapies for the treatment of inflammation-related diseases.
Fig. 4  |  IFN-γ signalling is necessary for macrophage activation and has a dominant effect over antibiotic-mediated immune quiescence in Beclin1 deficiency. a. Flow cytometry analysis of peritoneal macrophages obtained from naive adult mice. Data represent three experiments, n ≥ 3 mice per group. b. Peritoneal macrophages from naive adult mice; Beclin1+/+Ifngr−/−, n = 8; Beclin1+/+Ifngr−/−, n = 9 mice. Data are mean ± s.e.m. Statistical analysis was performed using two-way ANOVA with Sidak’s multiple comparisons; no significant difference was found. c, d. The percentage of MHCIIm-1+ blood monocytes in naive adult mice; Beclin1+/+Ifngr−/−, n = 8; Beclin1+/+Ifngr−/−, n = 9 mice. Data are mean ± s.e.m. Statistical analysis was performed using two-way ANOVA with Sidak’s multiple comparisons. e. Percentage survival of mice with Ifngr−/− or iNOS of ex vivo stimulated peritoneal macrophages from mice of C57BL/6J (n = 278). Signalling is necessary for macrophage activation and has a dominant effect over antibiotic-mediated immune quiescence in Beclin1. f. The percentage of MHCII+ cells in naive adult mice; Beclin1+/+Ifngr−/−, n = 8; Beclin1+/+Ifngr−/−, n = 9 mice. Data are mean ± s.e.m. Statistical analysis was performed using two-tailed Mann-Whitney U-test; no significant difference was found. g. The percentage of MHCIIm-1+ blood monocytes in naive adult mice; Beclin1+/+Ifngr−/−, n = 6; Beclin1+/+Ifngr−/−, n = 6; Beclin1+/+Ifngr−/−, n = 8 mice. Data are mean ± s.e.m. P values were calculated using two-way ANOVA with Sidak’s multiple comparisons test.  

Statistical analysis was performed using log-rank Mantel-Cox tests. h. Flow cytometry analysis of peritoneal macrophages obtained from naive neonatal mice; Beclin1−/−, n = 5; Beclin1+/+; n = 5 mice. Data are mean ± s.e.m. Statistical analysis was performed using two-way ANOVA with Sidak’s multiple comparisons on MHCIIm-1+ data; no significant difference was found. i. Intracellular iNOS of ex vivo stimulated peritoneal macrophages from mice of C57BL/6J (I) or Ifngr−/− (I) background. Three independent experiments were performed; for I, Beclin1+/+ (Kool-Aid), n = 4; Beclin1−/− (antibiotics treated (abx)), n = 8; Beclin1+/+ (Kool-Aid), n = 12; Beclin1+/+ (abx), n = 8; for all of the groups in I, n = 4. Data are mean ± s.e.m. P values were calculated using two-tailed t-tests. j. The fractions of ICAM2+ macrophages. Data were pooled from at least three experiments; Beclin1+/+ (Kool-Aid), n = 14; Beclin1−/− (abx), n = 17; Beclin1−/− (Kool-Aid), n = 14; Beclin1+/+ (abx), n = 15. Adjusted P values were calculated using ANOVA with Tukey’s multiple comparison test on MHCIIm-1+ data. k. Quantification of CD226+ MHCIIm-1+ICAM2+ SPMs. Data were pooled from at least three experiments; Beclin1+/+ (Kool-Aid), n = 7; Beclin1−/− (abx), n = 9; Beclin1+/+ (abx), n = 9; Beclin1+/+ (Kool-Aid), n = 9; Beclin1+/+ (abx), n = 10; adjusted P values were calculated using two-way ANOVA with Sidak’s multiple comparisons test. ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05.
Methods
Mice. Atg5−/− and Atg5−/−Ly2-cre+/− mice were generated as described previously in an enhanced barrier facility.15-17 Becn1−/−Ly2-cre+/− (ref. 17), Fip200−/−Ly2-cre+/− (ref. 18), Atg8−/−Ly2-cre+/− (ref. 17) and Atg16l1−/−Ly2-cre+/− (ref. 17) mice were generated in the same way as the Atg5−/−Ly2-cre+/− mice. Becn1−/−CD11c−cre− and Becn1−/−Mrp8-cre mice were generated by breeding Becn1−/− mice with CD11c−cre+ mice (007567) and Mrp8-cre mice (021614). mice, respectively, from the Jackson Laboratory. Rag1−/− mice were obtained from the Jackson Laboratory.

Rubicon mice were generated in the same way as the Rubicon−/− mice.

L. monocytogenes and influenza virus infection. L. monocytogenes WT strain EGD was used for this study. Listeria glycerol stocks were stored at −80 °C, and thawed and diluted into PBS for i.p. injection into mice. To determine tissue burden, spleens, livers and lungs were homogenized in 1 mL PBS containing 0.05% Triton X-100 and serial dilutions were plated on brain–heart infusion (BHI) agar plates. Listeria c.f.u. were counted after overnight growth at 37 °C. The H1N1 influenza virus A strain used was A/Puerto Rico/8/1934 (PR8). Mice (aged 6–8 weeks) were infected with 250 TCID50 (50% tissue culture infectious dose) of PBS intranasally; weight loss and morbidity and mortality of the mice were monitored.

Ex vivo L. monocytogenes bactericidal assay. The protocol was adapted from previous reports23,24. In brief, peritoneal cells from naïve mice were plated in DMEM + 10% heat-inactivated fetal bovine serum (FBS) supplemented with non-essential amino acids, sodium pyruvate, HEPES, and 50 U ml−1 streptomycin and 50 μg ml−1 streptomycin in 96-well plates (Costar) at 1 × 106 cells per well, and were allowed to adhere at 37 °C. Non-adherent cells were removed by washing three times with warm antibiotic-free medium and 5 × 104 Listeria were put into each well. The plates were centrifuged at 600 × g for 5 min at room temperature to synchronize the infection of cells and were then incubated at 37 °C for 15 min (time 0). After an additional 15 min at 37 °C, the medium was again aspirated and changed to medium containing 5% FBS. At designated time points after infection, cells were washed five times in warm PBS, and were then lysed with cold sterile denatured water with 0.1% Triton X-100. Serial dilutions of lysates were plated on BHI agar plates to enumerate c.f.u.

Treatment of mice with IL-4c complexes, BrdU labelling and intracellular staining. IL-4c complexes were prepared fresh as described previously32,63. IL-4 complexes were administered to mice via i.p. injection. At 4 h before treatment at time 0, mice were i.p. injected with 0.1% BSA diluted in 1 µl PBS and injected i.p. in 300 µl volume per mouse. Control injections were 0.1% BSA diluent in 1x DPBS. Mice received injections on day 0 and day 2, followed by euthanasia on day 4. BrdU labelling was performed according to the manufacture’s protocol (BD, 552858). BrdU was i.p. injected into mice 3 h before collection of cells for BrdU staining. The cells were first stained for surface markers and then permeabilized using a fixation/permeabilization solution kit (BD, 554714). After blocking with mouse and rabbit serum, the samples were stained using anti-RELM-α antibodies (PeproTech, 500-P214) for 1 h at room temperature and subsequently with secondary antibodies for 20 min at room temperature before flow cytometry analysis.

Peritoneal cell isolation, tissue leukocyte collection and flow cytometric analysis. Peritoneal cells were collected from mice after injection of 5 ml of DMEM containing 2 mM EDTA and 2% FBS into the peritoneal space. Peritoneal cells of pups were collected by 500 µl injection. Total peritoneal leukocytes were counted using an automated cell counter (Invitrogen). Blood was collected by submandibular bleeding into EDTA or lithium heparin tubes. Lungs, liver and spleen were excised, placed in DMEM containing 10% FBS, minced finely and digested at 37 °C for 1 h with mechanical disruption using a stir bar and enzymatic digestion. Lung was digested with Liberase Blendzyme III (Roche), hyaluronidase (Sigma-Aldrich) and DNase I (Sigma); spleen with collagenase B (Roche) and DNase I (EMD); and liver with collagenase D and DNase I as described previously19. Small-intestinal lamina propria cells were isolated as described previously20. Cells were treated with ACK buffer to remove red blood cells and were passed through a 70 μm cell strainer to generate single-cell suspension.

Cells were suspended in PBS with 2 mM EDTA, 0.1% sodium azide and 3% FBS. Peritoneal cells were stained with anti-FcγRIIIa (BioLegend, 101302) and labelled with specific antibodies against CSF1R (eBioscience, 415-11820-12), I-Ab (BioLegend, 105060), F4/80 (eBioscience, 25-4801-82), CD226 (BioLegend, 128805), Ly-6G (BioLegend, 127624), I-A/1-E (BioLegend, 106761), CD11b (BioLegend, 101237), TCRβ (eBioscience, 11-9961-85), CD19 (BD, 523854), CD5 (BD, 553022), CD62L (BioLegend, 104432) and CD44 (BioLegend, 103012). The total cell number was multiplied by the percentage of specific cell type in total single cells, as analysed by flow cytometry. Gating of tissue/blood cell populations was performed as described previously23,24. In brief, blood monocytes were gated as CD45+CD11b+CD11c−/+. Lung alveolar macrophages were gated as CD45+Siglec-F+CD11c−. Liver Kupffer cells were gated as CD45+F4/80+CD11b+Ly6C+ Spleen red pulpc macrophages were gated as F4/80+/CD11b+CD11c++. Flow cytometric analysis was performed on an LSRFortessa (BD Biosciences) and data were analysed using Flowjo software (Tree Star).

Peritoneal macrophage western blot, immunofluorescence and ex vivo stimulation. Adherent macrophages were lysed using RIPA buffer (Sigma) and then diluted in 2x Laemmlli buffer, resolved using 4–20% polyacrylamide gels (BioRad), transferred to PVDF membranes (BioRad) and detected using the following antibodies: anti-LC3b (Sigma, L7543), anti-p62/SQSTM1 (Sigma, P0067) and anti-GAPDH–HRP (sigha, C9295) or secondary goat-anti- Rabbit–HRP (Jackson, 11-035-144). HRP was detected using ECL (Biorad). For immunofluorescence, adherent macrophages were stimulated with 1 μg ml−1 blomycin or were not stimulated. Cells were fixed and permeabilized before staining with antibodies against γ-H2AX (clone JBW301, Millipore) and p62/SQSTM1 (GP62-C, Progen). To analyse iNOS, adherent macrophages were stimulated with 20 μM recombinant IFN-γ (R&D Systems) and 10 ng ml−1 LPS (Sigma). After stimulation, the medium was removed and replaced with cold PBS with 2 μM EDTA and incubated on ice for 10 min to detach the cells. The cells were first stained for live cells (Live/Dead Fixable Aqua, Invitrogen) and surface staining with antibodies. After fixation and permeabilization using BD Cytofix/ Cypermab (BD Biosciences), the cells were stained for iNOS (eBioscience 17-9520-82) and analysed using flow cytometry.

RNA Isolation, RNA-seq and RT-qPCR. For RNA-seq, peritoneal macrophages from naïve mice were purified by sorting using a Aria II flow cytometer (BD Biosciences) with >95% purity. RNA was isolated from cells using the RNeasy mini kit (QIAGEN) and from peritoneal cells in accordance with the manufacturer’s instructions. An mRNA Illumina sequencing library was generated and run on an Illumina HiSeq as previously described25. Each group contained n = 4 samples, and each sample contained RNA extracted from three sorted biological replicates. DESeq2 was used for differential gene expression analysis26, and the data from which was used as a ranked list in pre-ranked gene set enrichment analyses to identify pathway enrichment as previously described25. For RT–qPCR, RNA was extracted from sort-purified or adherent macrophages using the RNeasy mini kit (QIAGEN) followed by cDNA synthesis using iScript (Promega), RT–qPCR using Taqman-based assays (IDT) and copy numbers were determined using a standard curve. Actb (IDT Assay ID: Mm.PT.58.33540333) H2-B (IDT Assay ID: Mm.PT.58.396768) H2-A (IDT Assay ID: Mm.PT.58.42625719) Gcta (IDT Assay ID: Mm.PT.58.41742553) Stat1 (IDT Assay ID: Mm.PT.58.27921521) Ifi191 (IDT Assay ID: Mm.PT.58.33554590) Cxcl9 (IDT Assay ID: Mm.PT.58.3276745) Cxcl10 (IDT Assay ID: Mm.PT.58.43557827) Cel-5 (IDT Assay ID: Mm.PT.58.43548565) Gbp4 (IDT Assay ID: Mm.PT.58.13414486) Gbp8 (IDT Assay ID: Mm.PT.58.31788892) Socs1 (IDT Assay ID: Mm.PT.58.11527306) Ifi191 (IDT Assay ID: Mm.PT.58.11087779). Genes of interest were normalized to Actb copy numbers.

Single-cell RNA-seq data generation. Peritoneal cells were partitioned into nanolitre-scale Gel Bead-in-Eмуlations to achieve single-cell resolution for a maximum of 10,000 individual cells per sample. Utilizing the v2 Chromium Single Cell 3’ Library Kit and Chromium instrument (10x Genomics), poly-adenylated mRNA from an individual cell was tagged with a unique 16bp barcode. Use of Chromium single-cell 3’ data files for each sample was processed using Cell Ranger to align reads to the mm10 genome.

Preprocessing analysis using the Seurat package. For the analysis, the Seurat package (v2.3.4) was used. Genes that were filtered by barcode expression...
matrices using Cell Ranger were used as analysis inputs. The samples were pooled together using the AddSample function. Expression measurements for each cell were normalized to total expression and then scaled to 10,000. Then, log normalization was performed.

**Dimensionality reduction and clustering.** The most variable genes were detected using FindVariableGenes function in Seurat. Principal component analysis was run using only these genes. Cells are represented with tSNE plots. We applied the RustTSNE function to normalized data, using first 10 principal components. For clustering, we used the function FindClusters, which implements a shared nearest neighbour modularity optimization-based clustering algorithm on 10 principal components with a resolution of 0.8. Twenty clusters were detected, one of which contained poorly covered cells (a lower number of unique molecular identifiers and detected genes); this cluster was excluded from further analysis.

**Heat maps.** All of the heat maps were generated using Phantasus web service (https://artyomovlab.wustl.edu/phantasus/). For bulk RNA-seq, scaled expression values for every gene were averaged per cluster and then log–normalized before heat maps were generated. For single-cell RNA-seq, scaled expression values for every gene were averaged per cluster and then log–normalized before heat maps were generated.

**Single-cell RNA-seq differential expression.** To obtain differential expression values between clusters and file expression conditions in T cells, a MAST test was performed and P values were adjusted using Bonferroni correction

**Antibiotic treatment of mice.** Adult C57BL/6 mice were treated orally with a combination of broad-spectrum antibiotics, as previously described: vancomycin (0.5 g l$^{-1}$; Sigma-Aldrich), neomycin (1 g l$^{-1}$; Sigma-Aldrich), ampicillin (1 g l$^{-1}$; Sigma-Aldrich) and metronidazole (1 g l$^{-1}$; MP Biomedicals) dissolved in grape Kool-Aid (20 g l$^{-1}$; Kraft Foods). This solution was substituted for drinking water for 2 weeks before euthanasia and cell analysis; control mice received the grape Kool-Aid without antibiotics.

**Quantification and statistical analysis.** Data were analysed using Prism 7 (GraphPad Software). For all of the plots, ***P<0.001, **P<0.01, *P<0.05 and ns indicates not significant (P>0.05) as determined by Mann–Whitney U-tests, Gehan–Breslow–Wilcoxon tests or two-way ANOVA with Tukey's multiple-comparison test, Kruskal–Wallis test or Sidak's multiple comparisons test, as indicated in the figure legends.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data that support the findings of this study are available from the corresponding authors on reasonable request. RNA-seq data are available at the European Nucleotide Archive (PRJEB29191). Single-cell RNA-seq data are available at the GEO database (GSE121521).

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Author contributions
Y.-T.W. designed the project, performed experiments, analysed the data and wrote the manuscript. H.W.V. supervised project design and edited the manuscript. C.L.S. assisted with project design and edited the manuscript. Q.I.L., S.L., W.T.S., L.D. and C.B.W. performed experiments. K.-W.K., D.R.B., R.C.O., A.O., S.P., D.K. and M.T.B. assisted with experiments or project design. C.D. and S.A.H. helped to design RNA-seq experiments and analyse the data. K.Z. and M.N.A. analysed RNA-seq and single-cell RNA-seq data. J.D.P. performed the histology. All of the authors read and edited the manuscript.

Competing interests
H.W.V. is a founder of Casma Therapeutics and PierianDx. The work reported here was not funded by either company.

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Extended Data Fig. 1 | Mice with deficiencies of certain autophagy genes in myeloid cells display enhanced resistance to L. monocytogenes.

a, L. monocytogenes CFU in spleen or liver 3 days after infection of mice harboring myeloid deficiency (myeΔ) in multiple autophagy genes (data pooled from 2 experiments, Atg7f/f, n=9; Atg7myeΔ, n=13; Atg16l1f/f, n=8; Atg16l1myeΔ, n=10; Atg14f/f, n=8; Atg14myeΔ, n=11 mice; mean ± s.e.m.; P by 2-tailed t test).

b, Western blot analysis of p62, LC3 and GAPDH in peritoneal macrophages from naïve mice (Representative of n≥3 replicates).

C, Ex vivo phagocytosis activity of peritoneal macrophages at 0 hour (data pooled from 2 experiments, Beclin1f/f, n=12; Beclin1myeΔ, n=11; mean ± SEM; ns=not significant by 2-tailed t test).
Extended Data Fig. 2 | Mice with Beclin 1 deletion in DCs or neutrophils do not display *L. monocytogenes* resistance or macrophage activation phenotype. **a**, Survival of mice harboring Beclin 1 deletion in CD11c⁺ and MRP8⁺ cells vs littermate controls, after i.p. inoculation with 4–5x10⁵ CFUs of *L. monocytogenes* (Data pooled from 3-4 experiments; not significantly different by Log-rank Mantel-Cox test). **b**, Flow cytometry of ICAM2⁺ macrophage subsets in peritoneum of adult naïve mice (Data represents 2 experiments, *Becn1<sup>f/f</sup>, n=3 vs *Becn1<sup>f/f</sup>-Mrp8-cre, n=3; *Becn1<sup>f/f</sup>, n=5 vs *Becn1<sup>f/f</sup>-CD11c-cre, n=4; mean ± SEM; not significant by 2way ANOVA Sidak's multiple comparisons).
Extended Data Fig. 3 | Alterations of peritoneal tissue resident macrophages in mice with central autophagy gene deficiency. a, b, c, d. Quantification of number of total cells, total ICAM2+ macrophages, and numbers of MHC-IIhigh and MHC-IIlow fractions of ICAM2+ macrophages from peritoneum lavage of mice harboring myeloid deficiency (myeΔ) in multiple autophagy genes and total deficiency (Δ) of Rubicon. (Data pooled from ≥3 independent experiments: Fip200fl/fl, n=14; Fip200myeΔ, n=16; Atg5fl/fl, n=14; Atg5myeΔ, n=12; Atg7fl/fl, n=8; Atg7myeΔ, n=8; Atg16l1fl/fl, n=18; Atg16l1myeΔ, n=13; Rubicon WT, n=10; Rubicon KO, n=9; Becn1fl/fl, n=12; Becn1myeΔ, n=16; Fip200fl/fl, n=15; mean ± SEM; P, or Padj for multiple comparison with 2-tailed t test). e. Violin plot showing percent of MHC-IIhighICAM2+ macrophages in total peritoneal immune cells (Atg14fl/fl, n=42; Atg14myeΔ, n=38; Becn1fl/fl, n=12; Becn1myeΔ, n=16; Fip200fl/fl, n=15; mean ± SEM; Padj by Kruskal–Wallis Dunn’s multiple comparison test).
Extended Data Fig. 4 | Beclin 1 deficiency augmented baseline macrophage IFN signaling. a, Volcano plot shows genes upregulated in macrophages from Beclin1<sup>−/−</sup> mice on the left and downregulated on the right in RNA-seq data set (Beclin<sup>+/+</sup>, n=4; Beclin<sup>−/−</sup>, n=4). b, Gene set enrichment analysis of Beclin1 dependent signature. (The green curve represents the density of the genes identified in the RNAseq with Normalized Enrichment Score (NES), P value and False Discovery Rate (FDR) listed.) c, Transcript levels of the indicated genes in naïve peritoneal macrophages. (3 independent experiments, Beclin<sup>+/+</sup>, n=9; Beclin<sup>−/−</sup>, n=8; mean ± SEM; P by 2-tailed t test.)
Extended Data Fig. 5 | DNA damage response and cell proliferation of Beclin 1 deficient peritoneal macrophages. a, The presence of DNA double-strand break were revealed by immunofluorescence for γ-H2AX (red) in peritoneal macrophages treated with Bleomycin for 6 hours or untreated p62 is stained in green and nuclei were labeled by DAPI (blue). Cells displaying ≥10 γ-H2AX foci were counted as positive. (Data represents 2 independent experiments; n=4, mean ± SEM; P by 2-tailed t test.). b, Flow cytometry of BrdU incorporation by WT and Beclin 1- deficient ICAM2+ macrophages. (2 independent experiments; Becn1fl/fl, n=6 vs. Becn1mye, n=8; mean ± SEM; not significant by 2-tailed t test.). c, WT and Beclin 1-deficient ICAM2+ macrophages were enumerated after IL-4c injections (3 independent experiments; Becn1fl/fl+PBS, n=11; Becn1mye+PBS, n=16; Becn1fl/fl+IL4c, n=11; Becn1mye+IL4c, n=19), and analyzed for frequency of BrdU+ and RELMα level (2 independent experiments; Becn1fl/fl+PBS, n=6; Becn1mye+PBS, n=9; Becn1fl/fl+IL4c, n=11; Becn1mye+IL4c, n=11; mean ± SEM; Padj by Tukey’s multiple comparisons test.).
Extended Data Fig. 6 | Peritoneal lymphocytes changes revealed by Single-cell RNA sequencing. 

**a** and **c**, Violin plots showing the expression of marker genes of B (**a**) and T (**c**) cells clusters by single cell RNAseq. Bar graph comparing fraction size of clusters.

**b** and **d**, Flow cytometry validation on naïve mice (Becn1f/f, n=9; Becn1myeΔ, n=10; mean ± SEM; P and Padj for multiple comparison, by 2-tailed t test).

**e**, Ifng transcript level among clusters revealed by single cell RNAseq.
Extended Data Fig. 7 | Peritoneal macrophage activation in Becn1mye mice is independent of inflammasome and adaptive immune response.

a and b. Peritoneal macrophages obtained from naïve mice were analyzed for total cells (a), total ICAM2+ macrophages, MHC-IIhigh and MHC-IIlow fractions of ICAM2+ macrophages (b) by flow cytometry (Becn1f/fCasp1/11, n=6; Becn1myeCasp1/11, n=6; Becn1f/fRag1, n=7; Becn1myeRag1, n = 8 mice, mean ± SEM, P and Padj by unpaired 2-tailed t test).

c and d. Survival of mice after i.p. inoculation of 5x10⁵ CFUs (c) or 5x10⁴ CFU (d) of L. monocytogenes (Data pooled from 3-4 experiments, P by Log-rank Mantel-Cox test).
Extended Data Fig. 8 | Ifngr \( ^{-} \) rescues peritoneal immune cell homeostasis in Beclin1 \( ^{-/-} \) mice. a–e, Flow cytometry analysis of total cells (a), B cells (b), T cells (c), SPM and monocytes (d), and peritoneal neutrophils (e) and obtained from naïve mice of the indicated genotypes. (Data are from 2 independent experiments; Beclin1\( ^{wt} \), \( n=8; \) Beclin1\( ^{-/-} \), \( n=9; \) mean ± SEM; not significant by 2-tailed Mann-Whitney test.). f, Blood neutrophils were analyzed by flow cytometry. (\( n=6; \) mean ± SEM; \( P \) by 2-tailed Mann-Whitney test.).
Extended Data Fig. 9 | Peritoneal macrophage activation in Becn1<sup>−−</sup> mice is independent of the presence of microbiota. **a and c.** Quantification of total peritoneal cells and ICAM2<sup>+</sup> macrophages (a) and numbers of ICAM2<sup>+</sup> macrophages and CD226 fraction of ICAM2<sup>+</sup> macrophages (c) (Padj by Dunn’s multiple comparisons test). Becn1<sup>−−</sup>(Kool-Aid), <i>n</i> = 7; Becn1<sup>−−</sup>(abx), <i>n</i> = 9; Becn1<sup>−−<sub>mye</sub></sup>(Kool-Aid), <i>n</i> = 9; Becn1<sup>−−<sub>mye</sub></sup>(abx), <i>n</i> = 10; mean ± SEM, Padj by one-way ANOVA with Dunn’s multiple comparisons test). **b.** Quantification of 16S copy number from stool samples of mice. (Becn1<sup>−−</sup>, <i>n</i> = 7 each for kool-aid and abx, ns. Becn1<sup>−−<sub>mye</sub></sup>, <i>n</i> = 5 each for kool-aid and abx; mean ± SEM, *<i>P</i>adj analyzed by Tukey’s multiple comparisons test).
Extended Data Fig. 10 | **Becn1**<sup>−/−</sup> mice did not exhibit enhanced resistance to pulmonary influenza infection. Mice were infected intranasally with 250 TCID<sub>50</sub> influenza A PR8 and monitored for weight loss. (Data pooled from 4 independent experiments, mean ± SEM, not significant by 2way ANOVO for the whole curve or by 2-tailed t test for each time point).
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- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | Description |
|-----------------|-------------|
| BD FACSDiva For scRNAseq. The Cell Ranger Single-Cell Software Suite (version 2.0.2) (https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger) was used to perform sample demultiplexing, barcode processing, and single-cell 3’ counting. |
| Data analysis | Methodology used |
| Flowjo and Prism 7 software. Heatmap sweere generated using Phantasus web service (https://artyomovlab.wustl.edu/phantasus/). For the analysis of scRNAseq data, the Seurat package (version 2.3.4) was used. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All other data are available from the corresponding authors on reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size is mentioned for each experiment. For western blot analyses and autophagy analyses, three mice per genotype were used for each analysis. This sample size is sufficient to determine whether there is a biologically meaningful difference between different genotypes. For Listeria susceptibility analysis, the maximum number of wild-type and mutant littermates born within a week period were used for each experiment and the experiments were repeated 3-4 times. For flow cytometric analyses of immune cells, ≥3 age-matched littermates of WT and mutant mice were sacrificed for each experiment and the experiments were repeated ≥2 times. No statistical methods were used to predetermine sample size. |
| --- | --- |
| Data exclusions | no data were excluded |
| Replication | All attempts at replication were successful. |
| Randomization | Groups were established based off of genotype and infection status. Experimental groups consisted of littermates of different genotypes. All other aspects were randomized. |
| Blinding | All data acquisition and analysis was performed by investigators blinded to experimental group. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
| --- | --- |
| n/a | Involved in the study |
| | Antibodies
| | Eukaryotic cell lines
| | Palaeontology
| | Animals and other organisms
| | Human research participants
| | Clinical data |
| n/a | Involved in the study |
| | Antibodies
| | Eukaryotic cell lines
| | Palaeontology
| | Animals and other organisms
| | Human research participants
| | Clinical data |
| Antibodies | For flowcytometry: anti-FcγRⅡⅢ (biolegend 101302) and labeled with specific antibodies against CSF1R (eBioscience 25-4801-82), CD226 (biolegend 128805), Ly-6G (BioLegend 127624), I-A/I-E (BioLegend 107631), CD11b (BioLegend 101327), TCRb (eBioscience 11-5961-85), CD19 (BD 552854), CDS (BD 553022), CD62L (BioLegend 104432), CD44 (BioLegend 103012). |
| Validation | All antibodies validation are available on the manufacturers’ websites. |

Antibodies

| Antibodies used | For flowcytometry: anti-FcγRⅡⅢ (biolegend 101302) and labeled with specific antibodies against CSF1R (eBioscience 25-4801-82), CD226 (biolegend 128805), Ly-6G (BioLegend 127624), I-A/I-E (BioLegend 107631), CD11b (BioLegend 101327), TCRb (eBioscience 11-5961-85), CD19 (BD 552854), CDS (BD 553022), CD62L (BioLegend 104432), CD44 (BioLegend 103012). |

Animals and other organisms

Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Atg5f/f and Atg5f/f-Lyz2cre+/- mice were generated as described previously in an enhanced barrier facility. Beclin1f/f- Lyz2cre+/-, Fip200f/f- Lyz2cre+/-, Atg7f/f-5-Lyz2cre+/-, and Atg16/f/-Ly22cre+/- were generated in the same way as Atg5f/f- Lyz2cre+/-, Beclin1f/f-CD11c-cre+/-, and Beclin1f/f-Mrp8-cre+/- were generated by breeding Beclin1f/f to CD11ccre+/- (R007567) and Mrp8-cre+/- (R021614) from the Jackson Laboratory. Rubicon-/- knockout mice were kindly provided by Doug Green and Jennifer Martinez. Rag1-/- (R02216), Ifngr-/- (R003288), and Casp11/-/- (R016621) mice were from the Jackson. |

| Validation | All antibodies validation are available on the manufacturers’ websites. |
Laboratory. All mice used for experimental procedures were 8–12 weeks of age and sex matched littermates unless specified otherwise.

**Wild animals**
Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

**Field-collected samples**
For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

**Ethics oversight**
Mice were housed and bred at Washington University in St. Louis in specific pathogen-free conditions in accordance with federal and university guidelines, and protocols were approved by the Animal Studies Committee of Washington University.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Flow Cytometry

**Plots**

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

| Sample preparation | sample preparation is listed in Methods |
|--------------------|----------------------------------------|
| Instrument          | LSRFortessa (BD Biosciences)           |
| Software            | FACSDiva for collection and FlowJo for analysis |
| Cell population abundance | Populations were validated for purity by a post-sort analysis by FACS |
| Gating strategy     | Every flow cytometry analysis was initiated as follows: FSC-A/H and SSC-A/H to gate the singlet population and then as described in supplemental figures. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.