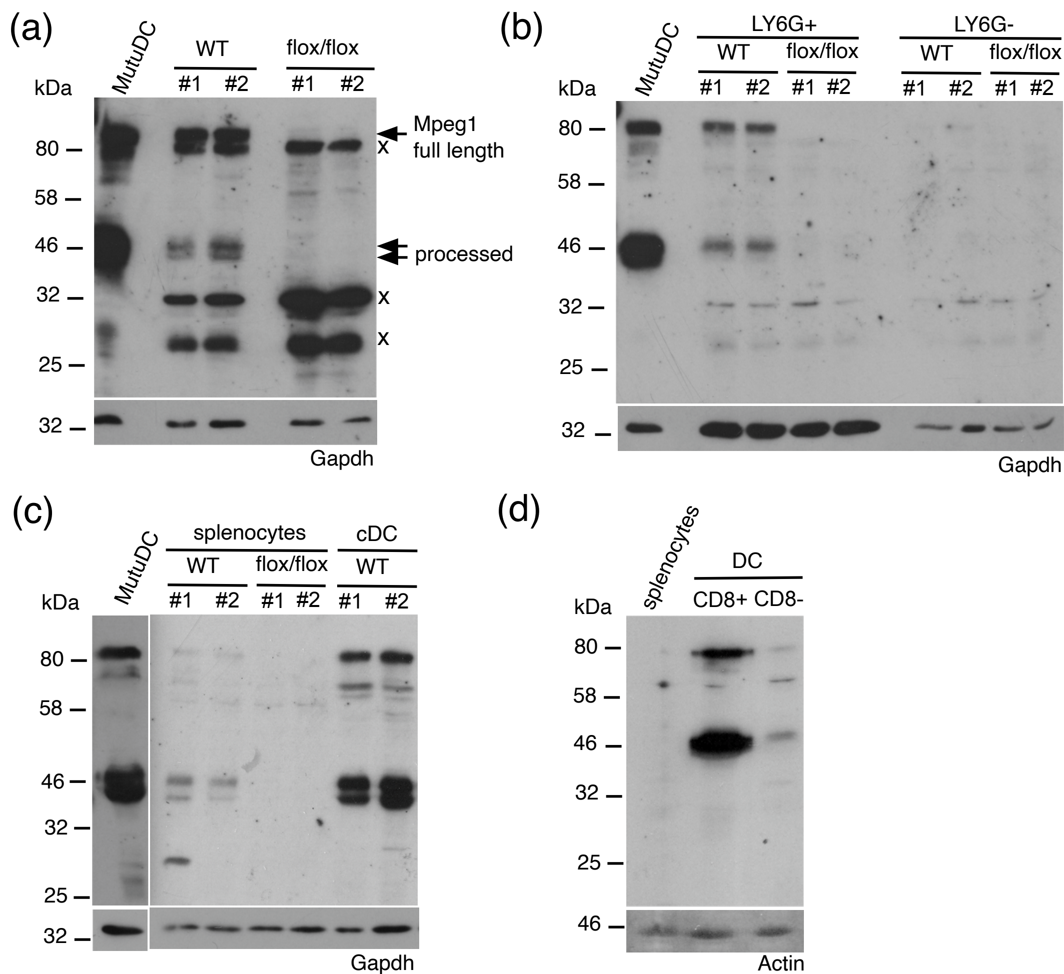
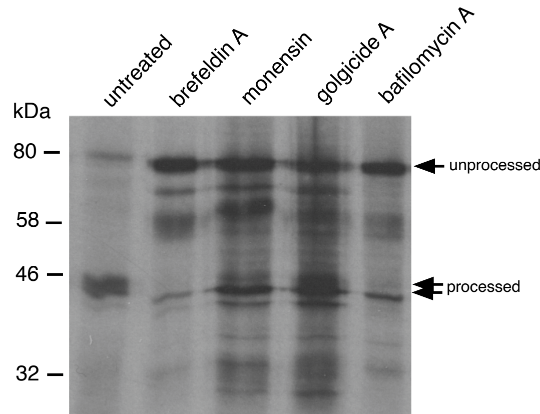
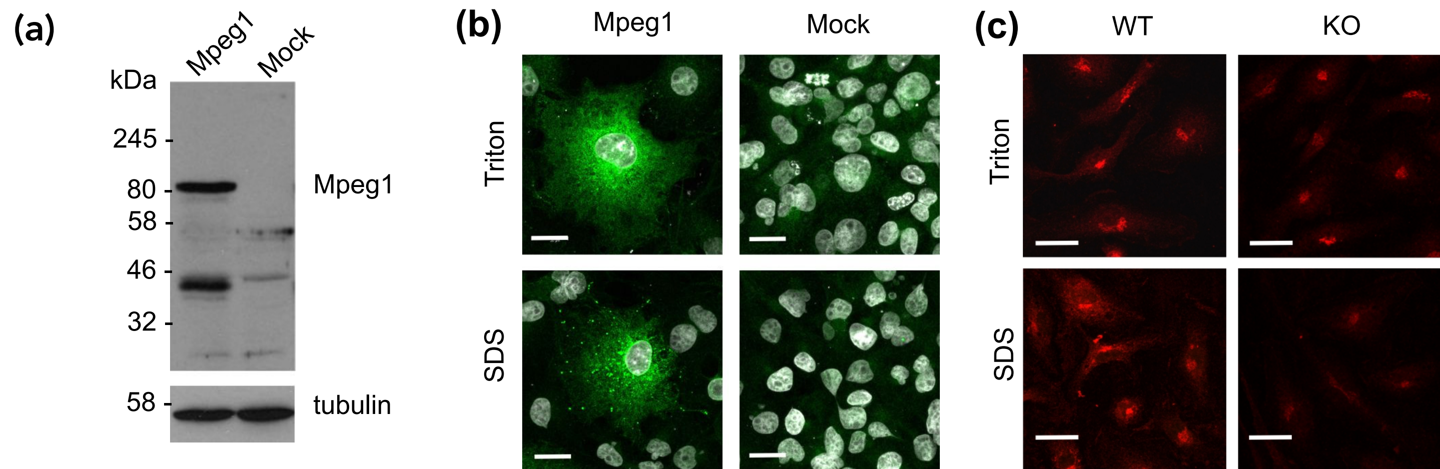


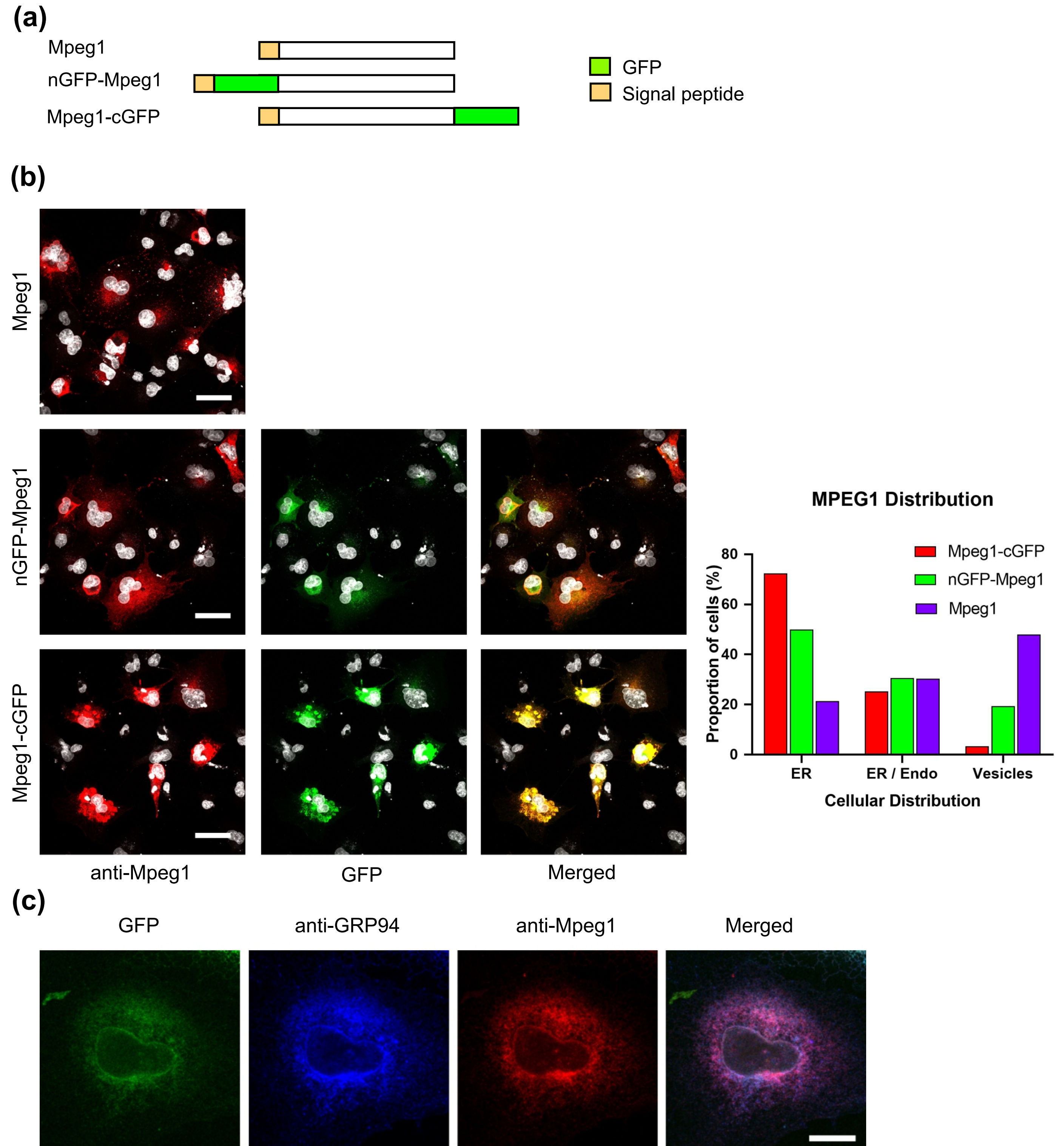
Supplementary figure 1. Mpeg1 is produced by professional phagocytes. **(a)** Splenic macrophages. **(b)** Bone marrow derived neutrophils. **(c)** Splenic conventional dendritic cells (cDC). **(d)** cDC subsets. Primary cells were isolated from tissues via cell sorting. Lymphocytes were isolated from splenocytes via FACS using CD19, CD3 and NK1.1 to identify B cells, T cells and NK cells respectively. Macrophages were isolated using CD11b and F4/80. Neutrophils were purified from bone marrow cells using Ly6G antibody. DC were isolated from splenocytes via negative selection using MACS. Lysates corresponding to 1×106 cells per lane were resolved by 10% SDS-PAGE and immunoblotted sequentially for Mpeg1 (1:1000 rabbit antiserum) and either GAPDH (1:5000) or actin (1:1000). Proteins cross-reacting with the polyclonal Mpeg1 antiserum are evident in panel **(a)** and are indicated by an x.



Supplementary figure 2. Mpeg1 processing occurs in a post-Golgi compartment. 2 x 10^6 MutuDC cells were plated in each of 4 x 6 cm dishes and cultured overnight. The next day 1 dish only was treated with 100 nM bafilomycin A overnight. The following day cells were treated with either 5 µg / ml brefeldin A, 2 µM monensin or 10 µm golgicide A (GCA) for 2 h at 37 C. Monolayers were washed once with PBS and cells 'starved' in RPMI-met$^-$ containing the indicated inhibitor for 30 min at 37 C. The medium was replaced with fresh RPMI-met$^-$ containing the indicated inhibitor and approximately 50 µCi ^{35}S-methionine for 15 min. The labelling medium was removed, monolayers were washed once with PBS, and cells chased in complete medium plus inhibitor for 2 h. Washed monolayers were lysed in 0.5% (w/v) SDS and immunoprecipitated using 1 µL of rabbit antibody to MPEG and analyzed via 10% SDS-PAGE, as described in the Methods.

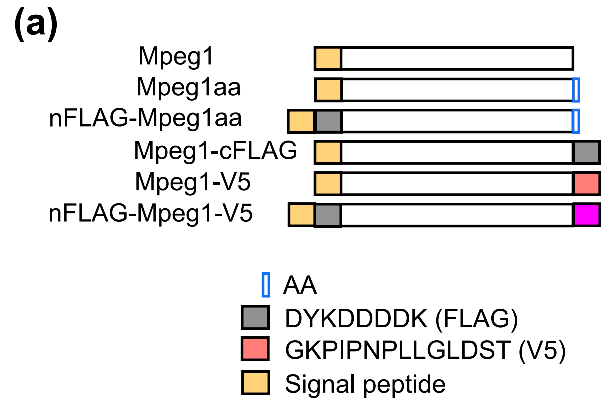


Supplementary figure 3. Abcam ab25146 anti-Mpeg1 antibody detects recombinant mouse Mpeg1 transiently expressed in COS-1 cells. **(a)** Immunoblotting. Cells were transfected with pSVTf/MPEG1 using dextran/chloroquine. Monolayers were washed with PBS and lysed in LSB. Following sonication to reduce viscosity, lysates were run on 10% SDS/PAGE gels and transferred to nitrocellulose. The membrane was probed with 1:1000 dilution of the anti-Mpeg1 Ab followed by anti-Rb Ig conjugated to HRP. The membrane was then stripped and probed for tubulin (mouse anti-tubulin, clone AA2 from Sigma, cat#05-661) followed by anti-mouse Ig conjugated to HRP. **(b)** Imaging. Cells on glass multi-well slides were fixed with 4% PFA for 20 min. Monolayers were permeabilised with either 0.5% Triton X-100 for 5 min or 1.0% SDS for 10 min. Cells were stained with 1:200 anti-Mpeg1 Ab followed by 1:800 anti-rabbit Af488 and DAPI. Scale bar is 20 μm. **(c)** Anti-Mpeg1 antibody cross-reacts with an unidentified protein in mouse BMDM. Bone marrow cells from WT and KO mice were differentiated by culturing in L929 culture supernatant which contains M-CSF. After 7 days, cells were fixed and permeabilised as for COS cells and stained with 1:200 anti-Mpeg1 Ab followed by 1:800 anti-Rb Af568. Images were collected on a Leica Sp5 scanning confocal microscope. Shown are maximum intensity projections (MIPs).

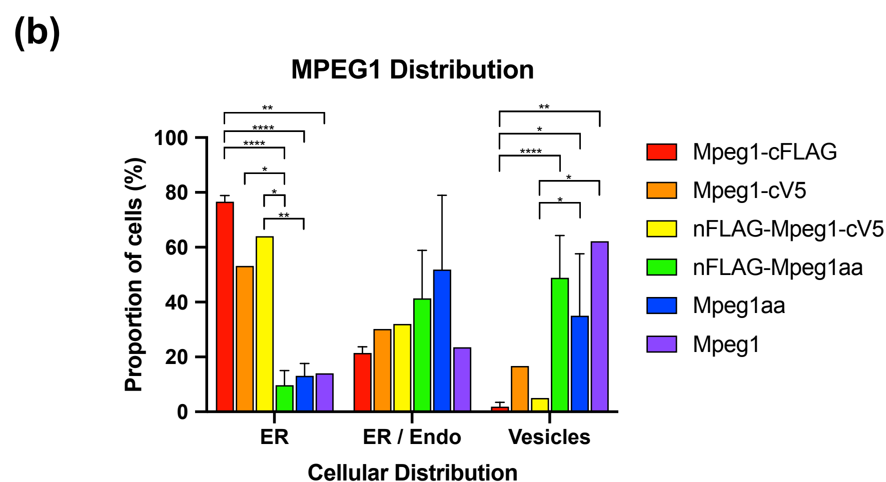


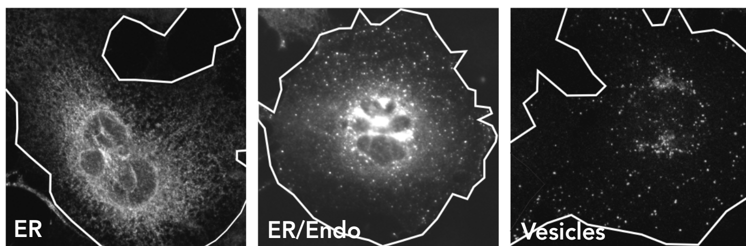
Supplementary figure 4. GFP-tagged Mpeg1 is mainly located in the ER. **(a)** Structure of GFP-tagged Mpeg1 expression constructs. **(b)** Localization of GFP-tagged Mpeg1 constructs in COS-1 cells. Cells were transfected with pSVTf/Mpeg1-cGFP or pSVTf/nGFP-Mpeg1 and prepared as described in Supplementary figure 1, and simultaneously examined for GFP natural fluorescence, Mpeg1 protein (1:200 rabbit anti-Mpeg1; anti-rabbit Ig af647) and GRP94 protein (1:200 rat anti GRP94; anti-rat Ig Af568). Images (MIPS) were captured on a Leica Sp5 scanning confocal microscope. Scale bar 20 µm. Distribution data are from a single experiment assessing approximately 100 cells per transfection using the criteria outlined in Supplementary figure 3. **(c)** C-terminally tagged Mpeg1 co-localizes with the ER marker GRP94 in COS-1 cells. Scale bar 40 µm.

(a)

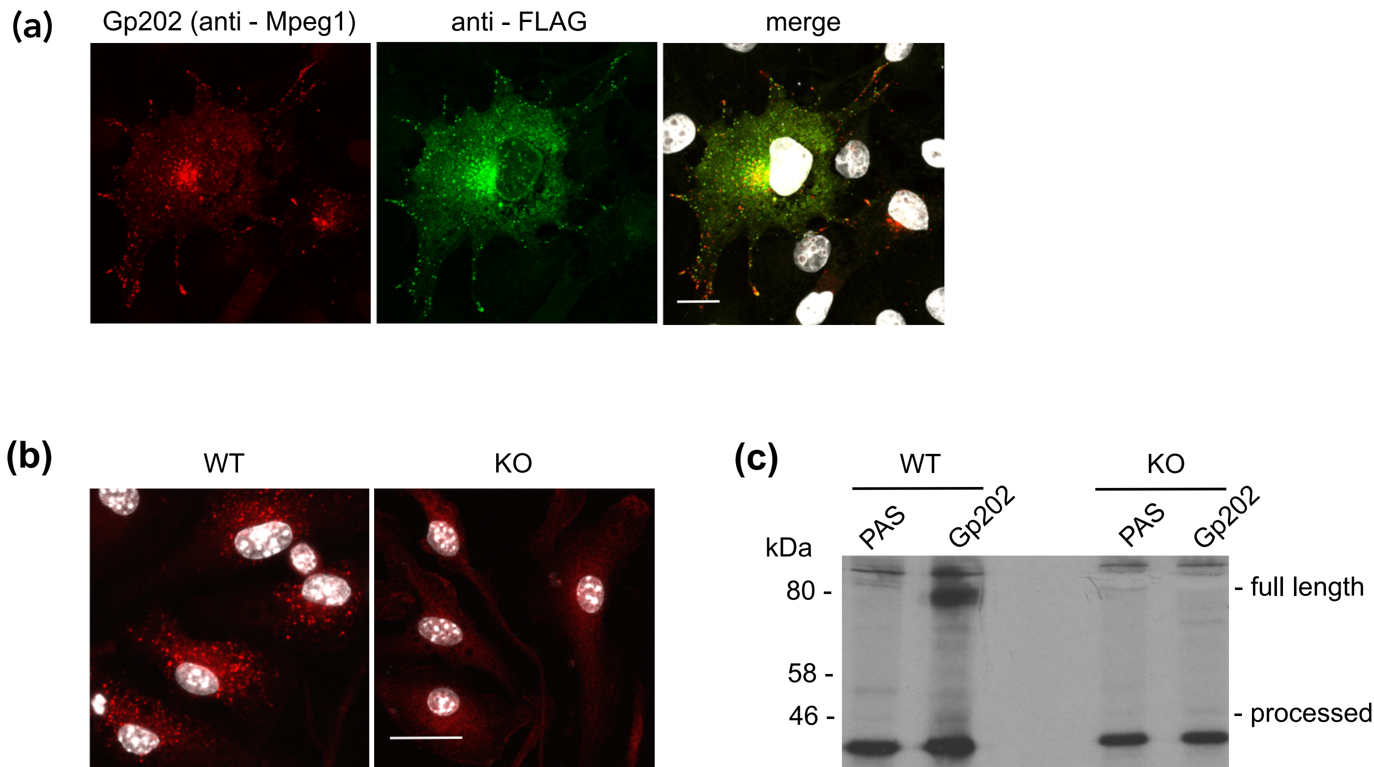


(b)

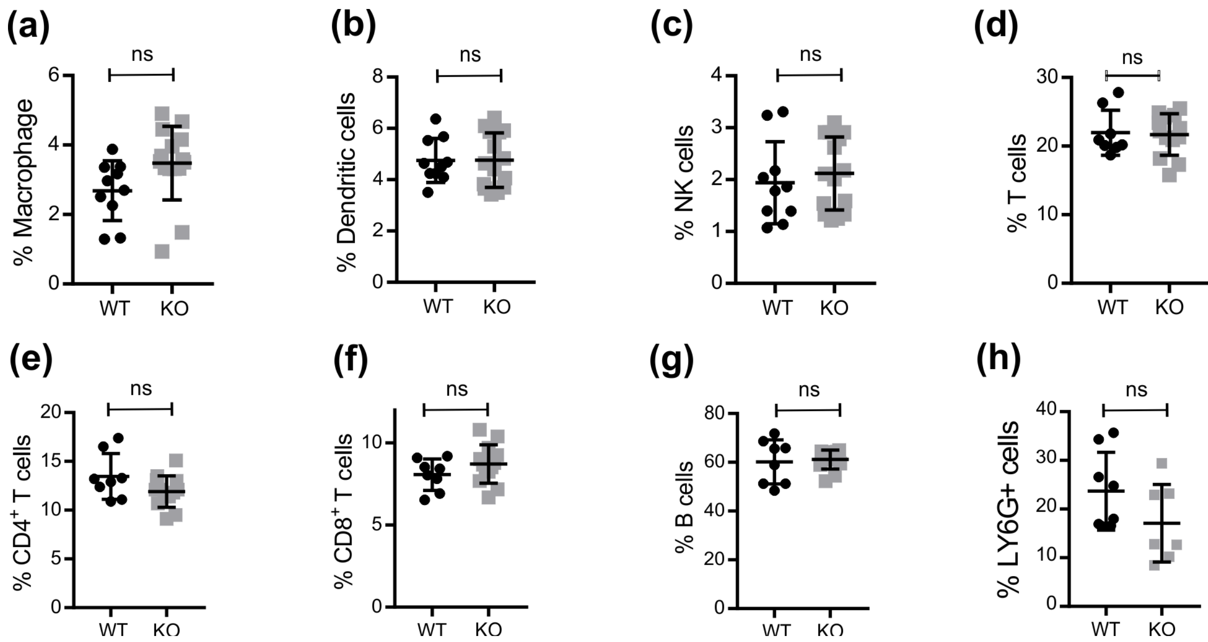




Supplementary figure 5. C-terminal epitope tag perturbs cellular localization of Mpeg1. **(a)** Structure of epitope-tagged Mpeg1 expression constructs. **(b)** Distribution of tagged Mpeg1 constructs in COS-1 cells. Shown below are examples of cells expressing Mpeg1 predominantly in the ER; the ER and endosomal network; or vesicles. Plasma membranes of cells are traced in white. The mean percentage distribution of Mpeg1 in the ER; the ER and endosomal network, or vesicles was determined for each construct: Mpeg1 (n=1 expt; 119 cells); Mpeg1aa (n=2 expt; 98/107 cells); nFLAG-Mpeg1aa (n=5 expt; 69/105/122/122/152/101 cells); Mpeg1-cFLAG (n=4 expt; 78/102/102/100 cells); Mpeg1-V5 (n=1 expt; 126 cells); nFLAG-Mpeg1-V5 (n=1 expt; 107 cells). Statistical analysis via multiple two-way ANOVA: $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$, $****P < 0.0001$.



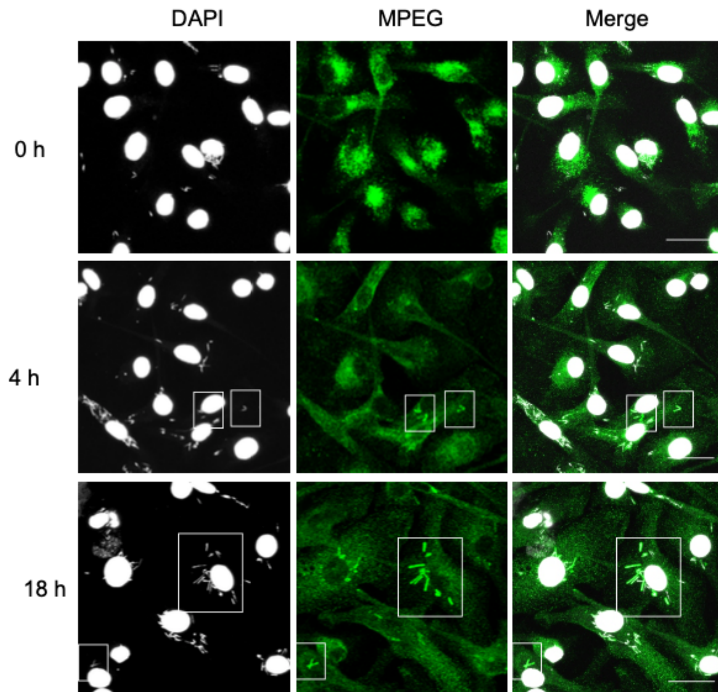
Supplementary figure 6. Guinea pig Gp202 anti-Mpeg1 antibody characterization. **(a)** Indirect immunofluorescence Cos-1 cells were transfected with pSVTf/nFLAG-MPEG1aa using dextran/ chloroquine. Cells on glass multi-well slides were fixed with 4% PFA for 20 min. Monolayers were permeabilised with 0.5% Triton X-100 for 5 min. Cells were co-stained with 1:200 Gp202 and 1:1000 anti-FLAG, followed by 1:800 anti-rabbit Ig Af647, 1:800 anti-mouse Ig Af488 and DAPI. Scale bar is 20 µm. **(b)** Gp202 antibody detects Mpeg1 in mouse BMDM. Bone marrow cells from WT or KO mice were differentiated by culturing in L929 culture supernatant which contains M-CSF. After 6 days, cells were fixed and permeabilised as for COS cells and stained with 1:200 anti-Mpeg1 Ab followed by 1:800 anti-Rb Af568. Images were collected on a Leica Sp5 scanning confocal microscope. Shown are maximum intensity projections (MIPs). Scale bar is 20 µm. **(c)** Gp202 antibody immunoprecipitates Mpeg1 in mouse BMDM. 8 day BMDM were 'starved' in serum-free RPMI medium lacking cysteine and methionine (RPMI-met-) for 30 min at 37 C. The 'starve' medium was replaced with fresh medium containing approximately 100 µCi 35-S-methionine. Cells were labelled for 2 h after which they were washed and lysed in NP40 lysis buffer containing protease inhibitors (Ap, Pep, Leu and AEBSF). Nuclei and cell debris were removed by centrifugation and Mpeg1 was immunoprecipitated by adding an equal volume of NETGEL, 100 µl of a 10% slurry of protein A-sepharose (PAS) beads and 2 µl of Gp202. As a control, PAS beads without antibody were added to a parallel sample. After overnight incubation at 4 C, the protein A-sepharose beads were washed twice with NETGEL and once with Wash III and resuspended in LSB/0.1 M DTT. Following separation by SDS/PAGE, the gel was fixed, treated with Amplify for 30 min at room temperature, dried and exposed to X-ray film at -80 C.



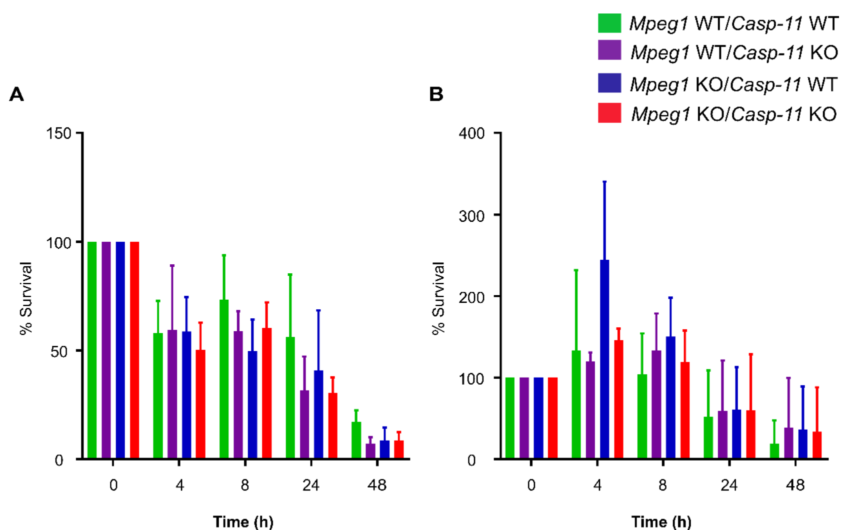
Supplementary figure 7. Mpeg1 does not influence the proportions of immune cells in the spleen or blood. Spleens were harvested from C57BL/6J WT (n=8-10) or Mpeg1-/- (n=13-15) mice. **(a)** Macrophages (CD11b$^+$, F4/80$^+$). **(b)** Dendritic cells (CD11c$^+$, MHC-II+). **(c)** NK cells (NK1.1$^+$, DX5$^+$). **(d)** total T cells (CD3$^+$). **(e)** CD4+ T cells (CD3+, CD4+). **(f)** CD8+ T cells (CD3$^+$, CD8$^+$). **(g)** B cells (CD19$^+$). **(h)** Neutrophils (Ly6G$^+$) in blood. Each data point represents a single mouse. Statistical significance was assessed using the Student's *t*-test with Holm-Sidak correction for multiple comparisons. ns, $P > 0.05$.

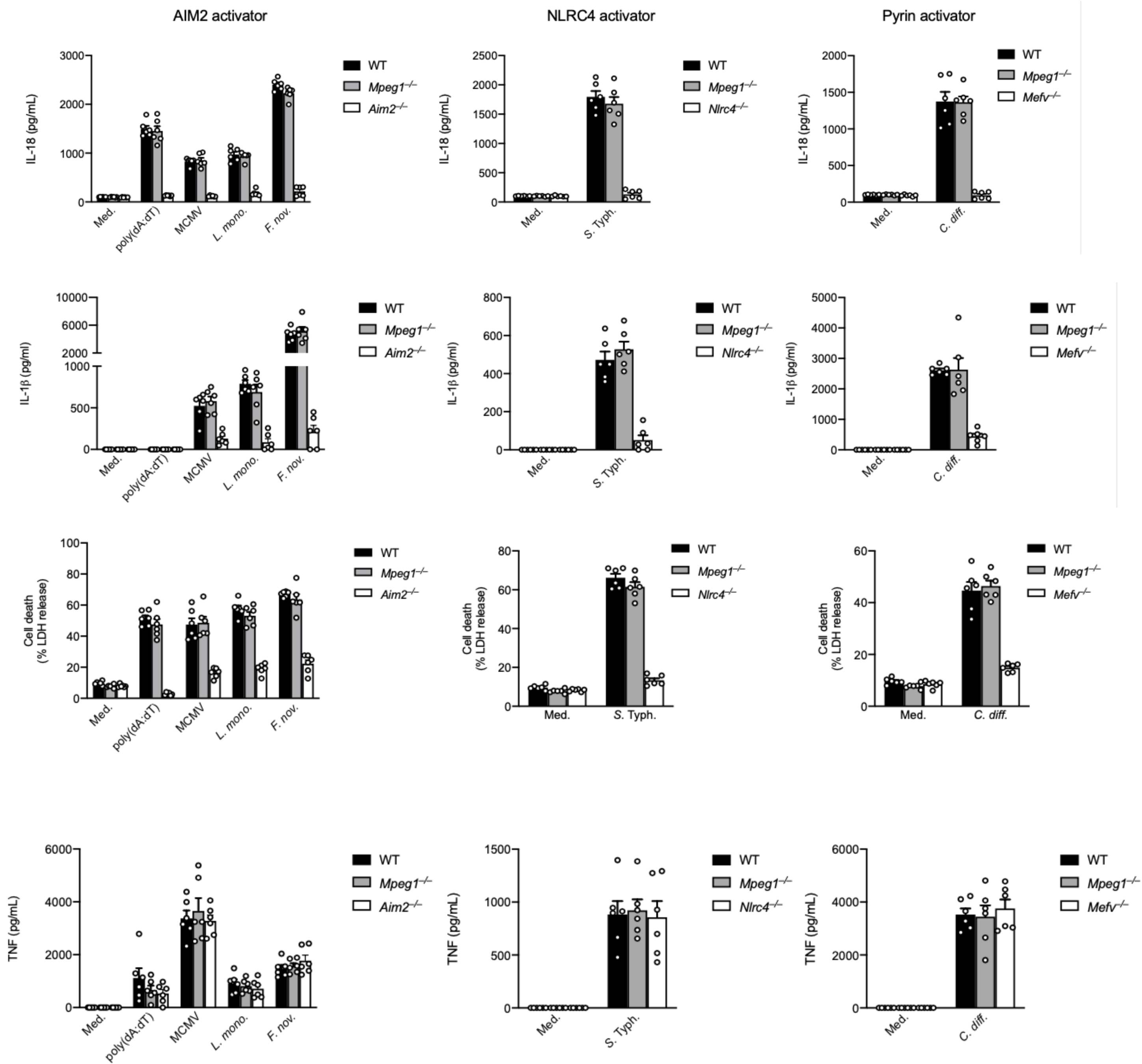
Representative gating strategies for Supplementary figure 7

Supplementary figure 8 (a). Mpeg1 and bacteria colocalize in infected macrophage. Day 8 BMDM were cultured on glass coverslips in 24-well trays in antibiotic-free medium. On day 11 cells were infected with 3 x 10^6 CFU M. smegmatis for 1 h at 37 °C at a moi ~ 50:1. Monolayers were washed three times with 1 mL PBS to remove non-adherent bacteria and fresh medium containing 50 ug / ml gentamicin was added for 1 h to kill remaining extracellular bacteria. Monolayers were either fixed immediately for staining or incubation was continued for the indicated times. Monolyaers were washed in PBS and °C. Cells were permeabilized and stained with 1:400 Gp202 Mpeg1 for 30 min at room temperature followeed by 1:800 anti-Gp AlexaFluor568 (false coloured green for ease of visualisation). Bacterial and nuclear DNA were stained with DAPI. Imaged on a Leica SP8 confocal microscope. Scale bars are 20 microns. Images are maximum intensity projections.

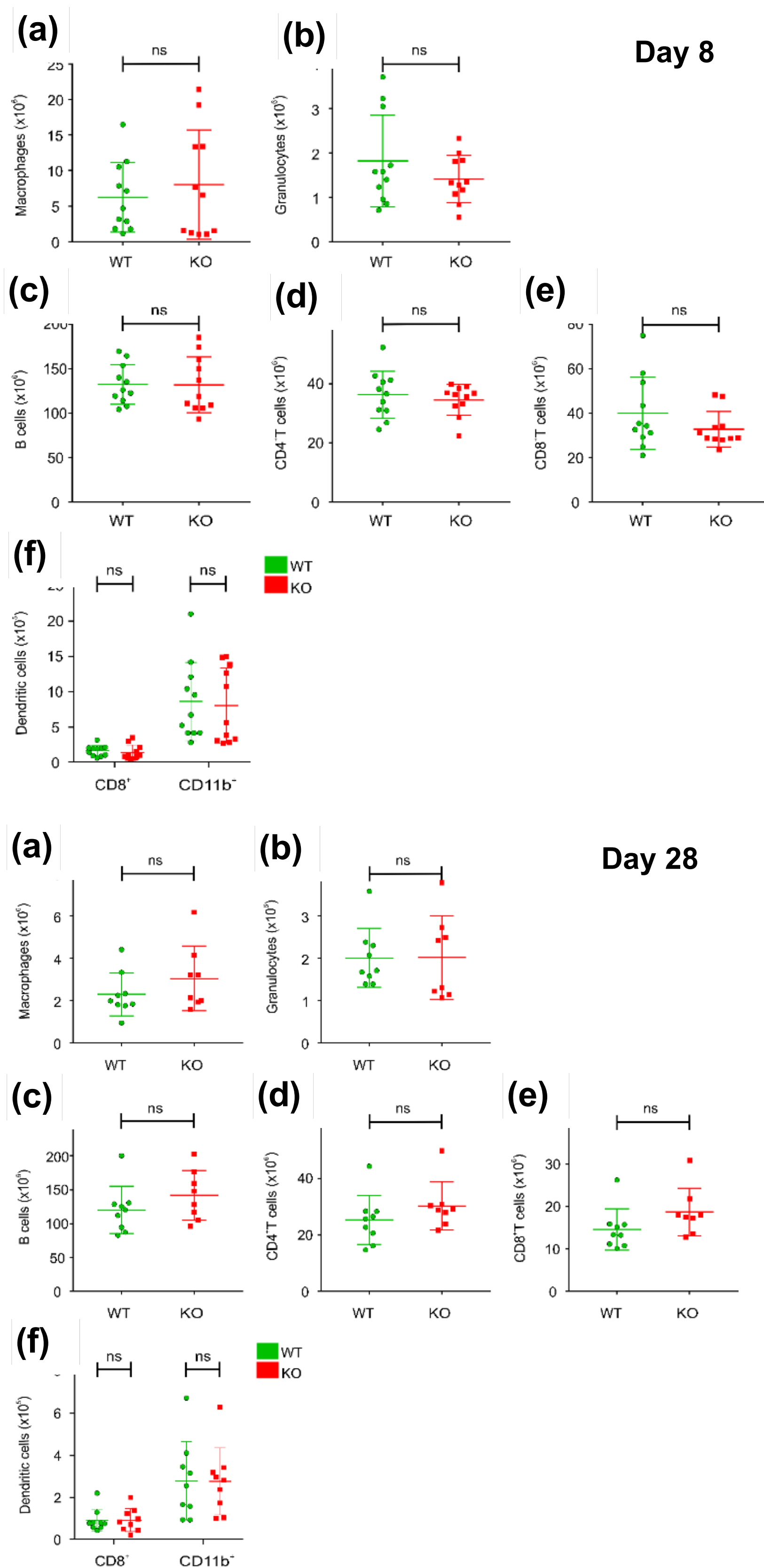


Supplementary figure 8 (b). MPEG1 is not essential for bacterial killing by BMDM from mixed background mice. Bone marrow cells from the four different genotypes of 129X1/SvJ:C75BL/6J (mixed background) mice were differentiated to macrophages. At day 10 after differentiation, BMDM were infected with **A.** *Staphylococcus aureus* or **B.** *Escherichia coli* K12 at the MOI 5-10 and 30-50 respectively. At different time points post infection, intracellular bacteria were released by lysing the cells in water. The lysates were serially diluted and plated in triplicate. CFU were determined, and data reported as a percentage of survival (where the number of bacteria recovered at 0 h post infection was taken to be 100% survival, and survival at each time point expressed as a fraction of the number of cells present at 0 h). Statistical analysis of the percentage of survival between groups at different time points was assessed using two-way ANOVA and Tukey's HSD test and showed no significant differences between groups at indicated time points. The experiments were done on three separate occasions and the % survival at each time point pooled. Hence, data are mean ± SD of percentage of survival from three independent experiments.

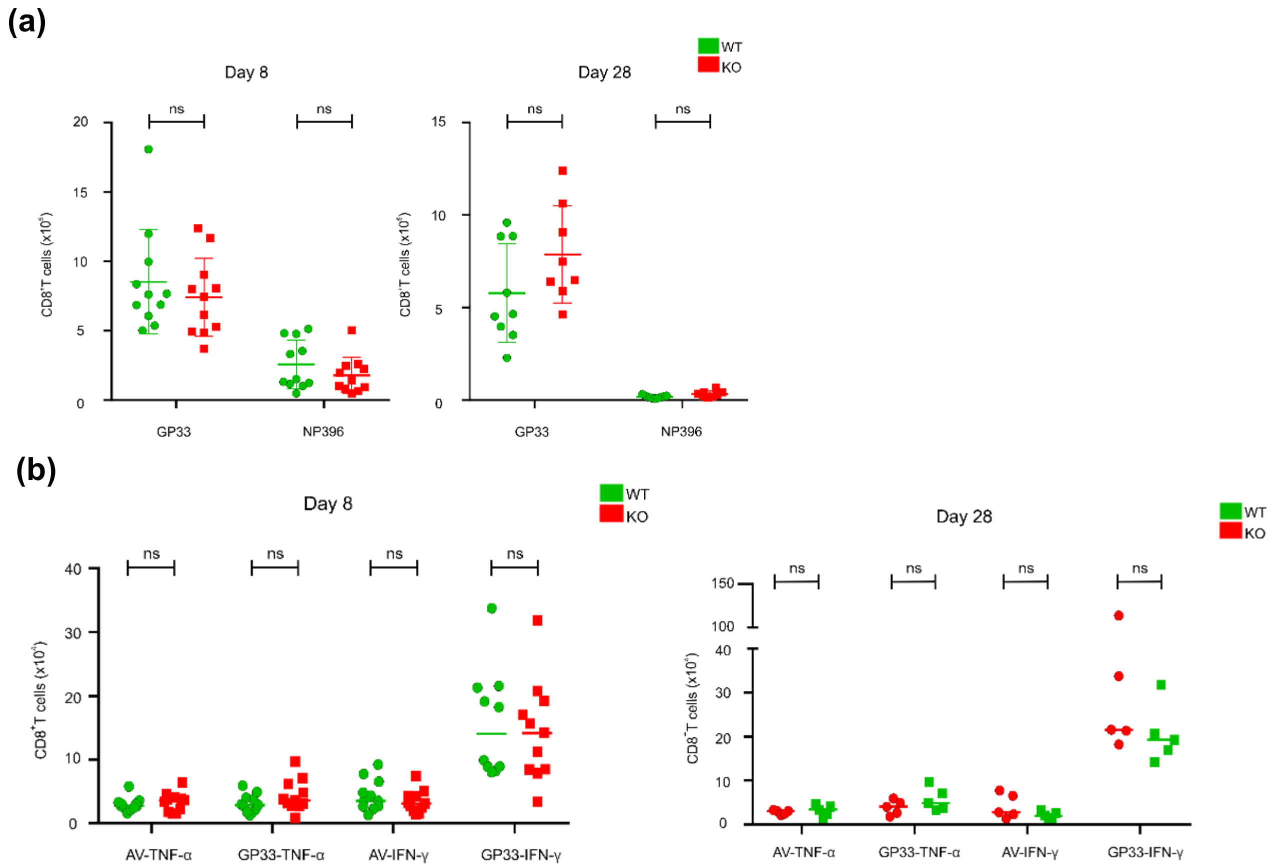




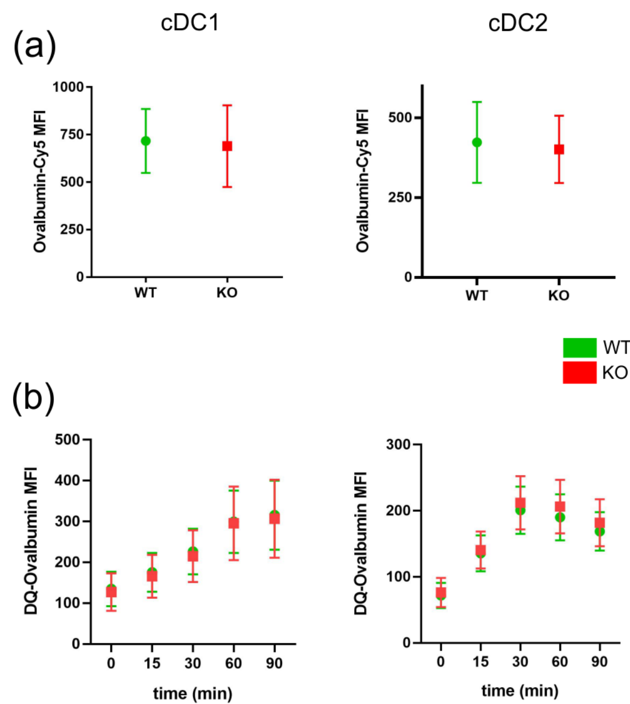
Supplementary figure 9. Mpeg1 is not required for inflammasome activation. BMDM were treated with the indicated activators and cytokine release was measured by ELISA. Pyroptosis was evaluated via measurement of LDH release. Med. - medium; LPS - lipopolysaccharide; MCMV - murine cytomegalovirus; C . rod - *Citrobacter rodentium;* L. mon - *Listeria monocytogenes*; F.nov - *Francisella novicida;* S. typh - *Salmonella typhimurium;* C. diff - *Clostridium difficile.*



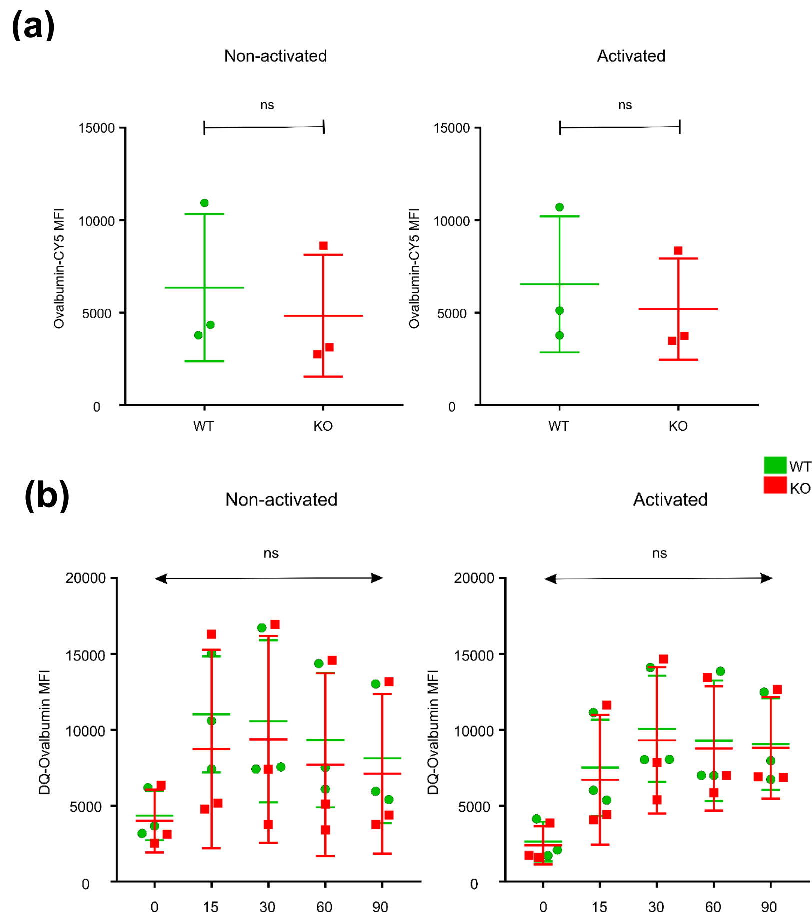
Supplementary figure 10. Immune cell distribution is not significantly affected in LCMV-infected *Mpeg1*$^{-/-}$ mice. Mice were infected with 2 × 10^6 focus-forming units of LCMV Docile strain via intravenous administration. 8- or 28 days post-infection immune cells in spleen were assessed and compared between WT and Mpeg1 KO groups of mice. **(a)** Macrophage (CD11b$^+$, F4/80$^+$). **(b)** Granulocytes (Gr-1). **(c)** B cells (CD19$^+$), **(d)** T cells (CD3+, CD4$^+$), **(e)** CD8$^+$ T cells (CD3$^+$, CD8$^+$) and **(f)** DC (CD8$^+$ and CD11b$^+$). Each data point represents a single mouse (n=11). Statistical significance was assessed using the Student's *t*-test with Holm-Sidak correction for multiple comparisons. ns, *P*>0.05.



Supplementary figure 11 (a) Generation of LCMV-specific cytolytic T cells is unimpaired in *Mpeg1*$^{-/-}$ mice. Mice were infected with 2 × 10^6 focus-forming units of LCMV Docile strain via intravenous injection. 8- or 28-days post infection, the number of GP33 or NP396 specific $CD8^+$ T cells was determined using tetramer complexed with either LCMV–GP33–41 (KAVYNFATM) or LCMV–NP396–404 (FQPQNGQFI) and flow cytometry. Each data point represents a single mouse (n = 11). Statistical significance was assessed using the Student's *t*-test with Holm-Sidak correction for multiple comparisons. ns, $P > 0.05$. **(b)** Generation of LCMV-GP33-specific cytotoxic T cells secreting TNF-α and IFN-γ is unimpaired in *Mpeg1*$^{-/-}$ mice. Splenocytes from mice 8 days or 28 days after LCMV infection were restimulated using irrelevant peptide as a negative control (AV) or $CD8^+$T cells-specific GP33 peptide (10^{-7}M) in the presence of GolgiStop (BD Biosciences) for 5 hours. After 5 h, cells were surface stained for CD8 and then fixed overnight with 2% paraformaldehyde. Cells were permeabilized with Perm/Wash solution and intracellularly stained for IFN-γ and TNF-α using fluorochrome-conjugated antibodies and evaluated with flow cytometry. Each data point represents a single mouse. Statistical significance was assessed using the Student's *t*-test with Holm-Sidak correction for multiple comparisons. ns, $P > 0.05$.

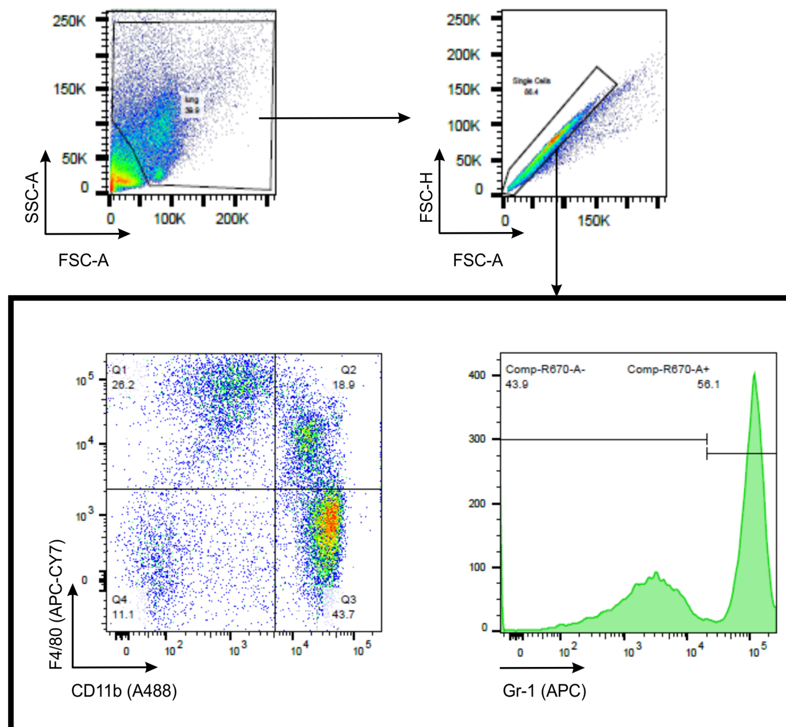


Supplementary figure 12 (a) MPEG1 is not involved in ovalbumin uptake by conventional DC. DC pulsed with ovalbumin-CY5, either at 37˚C or at 4˚C, were washed twice and kept on ice until analysis by flow cytometry to evaluate antigen uptake. Antigen uptake does not take place at 4˚C, so cells pulsed at this temperature acted as a negative control. For background correction, the MFI of the negative control was subtracted from the MFI of the test. Each data point represents pooled results from two independent experiments. **(b)** Absence of MPEG1 does not affect ovalbumin processing by DC. DC were pulsed with DQ-ovalbumin for 15 min and then further incubated at 37˚C or 4˚C for the indicated times. Cells were washed twice after being pulsed with antigen and also after each time point at 37˚C or 4˚C. The amount of proteolysis, corresponding to increased fluorescence, was determined by flow cytometry. Cells pulsed with antigen at 4˚C are considered a negative control and the MFI of the negative control was subtracted from the MFI of the test. Each data point represents pooled results from two independent experiments. Methods: For each experiment, DC were purified and pooled from 4 WT or Mpeg1$^{-/-}$ spleens. Cells were pulsed with ovalbumin-Cy5 (50 µg mL^{-1}) and DQ-ovalbumin (20 µg mL^{-1}) in medium for 15 min, either at 37˚C or at 4˚C, in triplicates. Cells were washed twice in EDTA-BSS FCS 2% and kept on ice. For the chase experiment, cells were re-incubated at 37˚C and collected at different time points (0 min, 15 min, 30 min, 60 min, 90 min). All the cells were then stained for flow cytometry analysis with anti-CD11c-PeCy7, anti-CD8a-BV421, anti-CD11b-PE for 20 min at 4˚C. Cells were washed 2x in EDTA-BSS FCS 2%, resuspended in EDTA-BSS FCS 2% + PI (as live/dead cell marker) and analysed by flow cytometry.

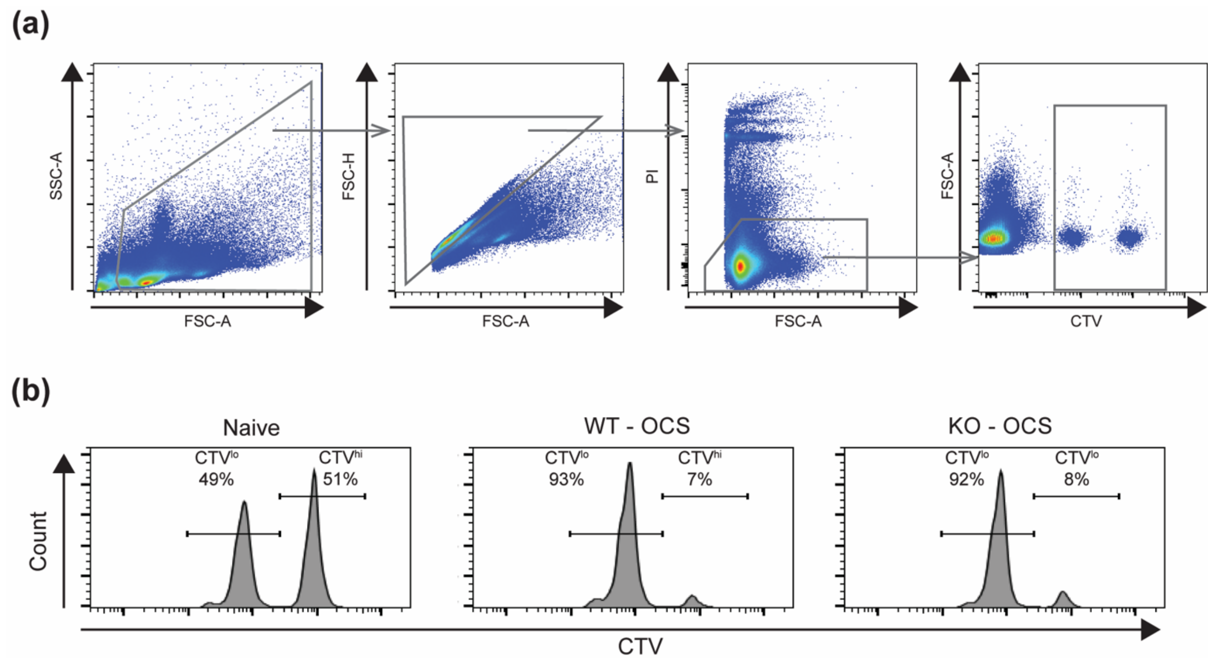


Supplementary figure 13. (a) MPEG1 is not involved in ovalbumin uptake by BMDM**.** At Day 7, BMDM from WT and *Mpeg1*$^{-/-}$ mice, non-activated or activated overnight with LPS (10 ng mL^{-1}) and IFN-γ (100 µg mL^{-1}), were pulsed with ovalbumin-CY5 for 15 min, either at 37˚C or at 4˚C. They were washed twice and kept on ice until analysis by flow cytometry to evaluate antigen uptake. Antigen uptake does not take place at 4˚C, so cells pulsed at this temperature acted as a negative control. For background correction, the MFI of the negative control was subtracted from the MFI of the test. Each data point represents a single mouse. Statistical significance was assessed using the Student's *t*-test. ns, $P > 0.05$.

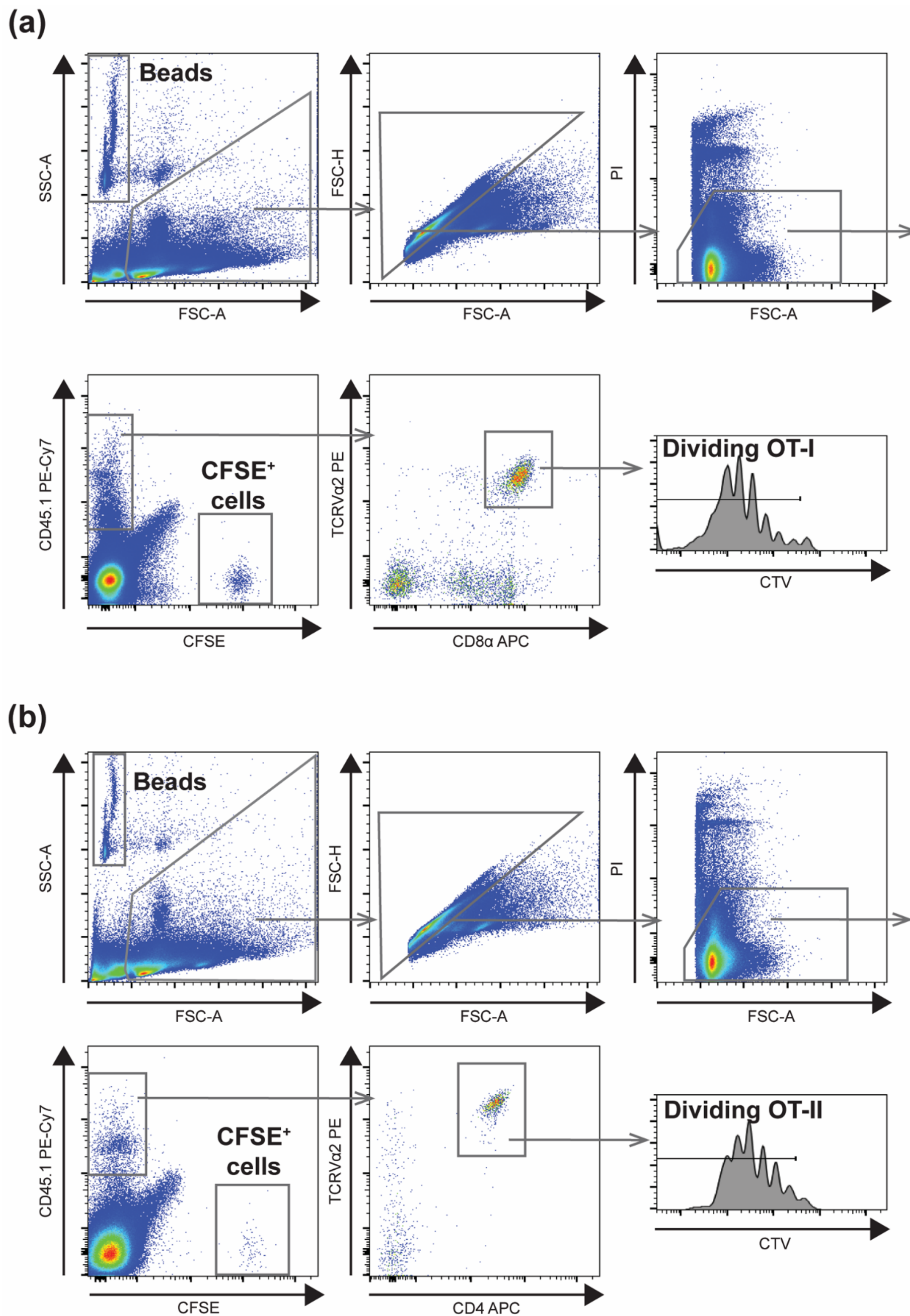
(b) Absence of MPEG1 does not affect ovalbumin processing. BMDM from WT and *Mpeg1*$^{-/-}$ mice, either non-activated or activated overnight with LPS (10 ng mL^{-1}) and IFN-γ (100 µg mL^{-1}), were pulsed with DQ-ovalbumin for 15 min and then further incubated at 37˚C or 4˚C for the indicated times. Cells were washed twice after being pulsed with antigen and also after each time point at 37˚C or 4˚C. The amount of proteolysis, corresponding to increased fluorescence, was determined by flow cytometry. Cells pulsed with antigen at 4˚C are considered a negative control and the MFI of the negative control was subtracted from the MFI of the test. Each data point represents a single mouse. Statistical significance was assessed using the Student's *t* -test. ns, $P > 0.05$.



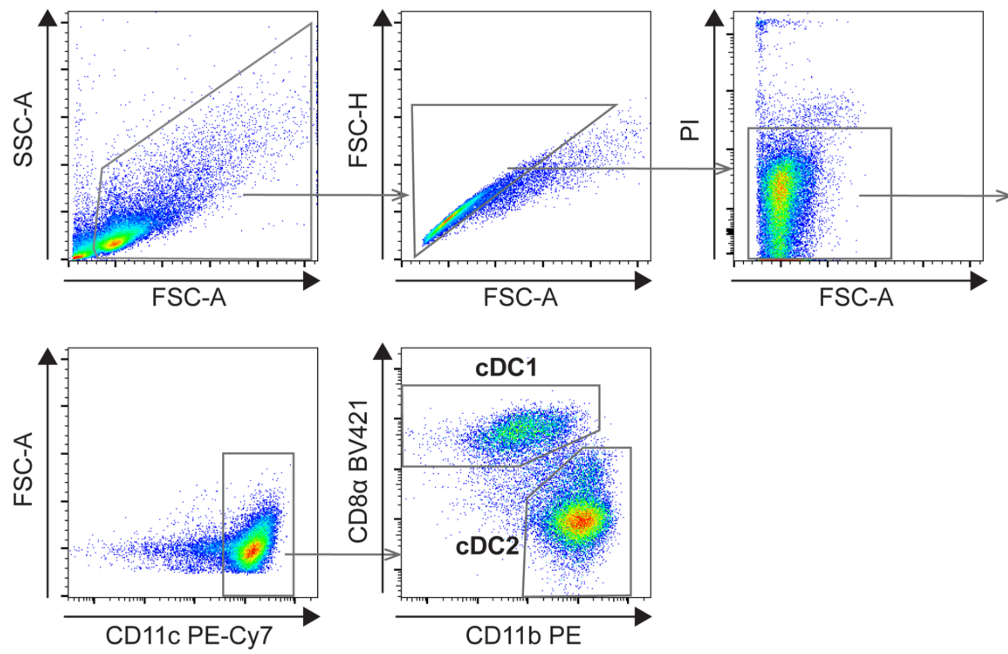
Supplementary figure 14. Representative gating strategy for flow cytometry analysis in the experiment shown in Figure 6b.



Supplementary figure 15. Analysis of WT and MPEG-KO CTL activity by flow cytometry. WT and MPEG1-KO mice were immunized i.v. with OCS in the presence of LPS. Six days later, mice were challenged with equal number of CTV^{hi}($OVA_{257-264}$) and CTV^{lo}(OVA^{-}) target cells. 36-42 h later, the spleens were harvested and analysed by flow cytometry. **(a)** Representative gating strategy of CTV^{hi} and CTV^{lo} target cells. **(b)** Representative histograms of CTV^{hi} target cell killing in each group, including naive negative control.



Supplementary figure 16. Gating strategy for the analysis of antigen presentation *in vivo* by flow cytometry. WT and MPEG1-KO were adoptively transferred with CTV-labeled OT-I or OT-II cells and CFSE-labeled C57BL/6 splenocytes. 24 h later, mice were immunized with OCS or soluble OVA and spleens were harvested 64 h later for flow cytometry analysis. The representative strategy for the gating of blank calibration beads, $CFSE^+$ splenocytes and dividing OT-I (A) and OT-II (B cells) is shown.



Supplementary figure 17. Representative flow cytometry gating strategy of mouse spleen cDC1 and cDC2.