Supplemental Experimental Procedures:

Molecular cloning and generation of ASC-citrine transgenic mice:

Conditional m*ASC-citrine* transgenic mice were generated by knocking in a cassette containing m*ASC-citrine* gene preceded by the CAG promoter and a transcriptional STOP site, into the Rosa26 locus, as previously described (Xiao et al., 2007). An frt-flanked IRES-hCD2 cassette was placed downstream of the m*ASC-citrine* gene before the polyadenylation signal (pA). Embryonic stem (ES) cells (E14.1, C57BL/6) were transfected with the linearized targeting vector. G418-selection yielded resistant colonies of which 8 out of 200 showed the correct targeting event as verified by Southern blot analysis. Knock-in mice were on a C57BL/6 genetic background. Homozygous transgenic mice were born at Mendelian ratios, were fertile, and had no obvious aberrant phenotype. Mice 6-8 weeks of age were used in all experiments. All mice were bred and maintained in specific pathogen-free conditions; mouse protocols were approved by the Institutional Animal Care and Use Committee at the University of Massachusetts Medical School.

Virus stimulation *in vitro*:

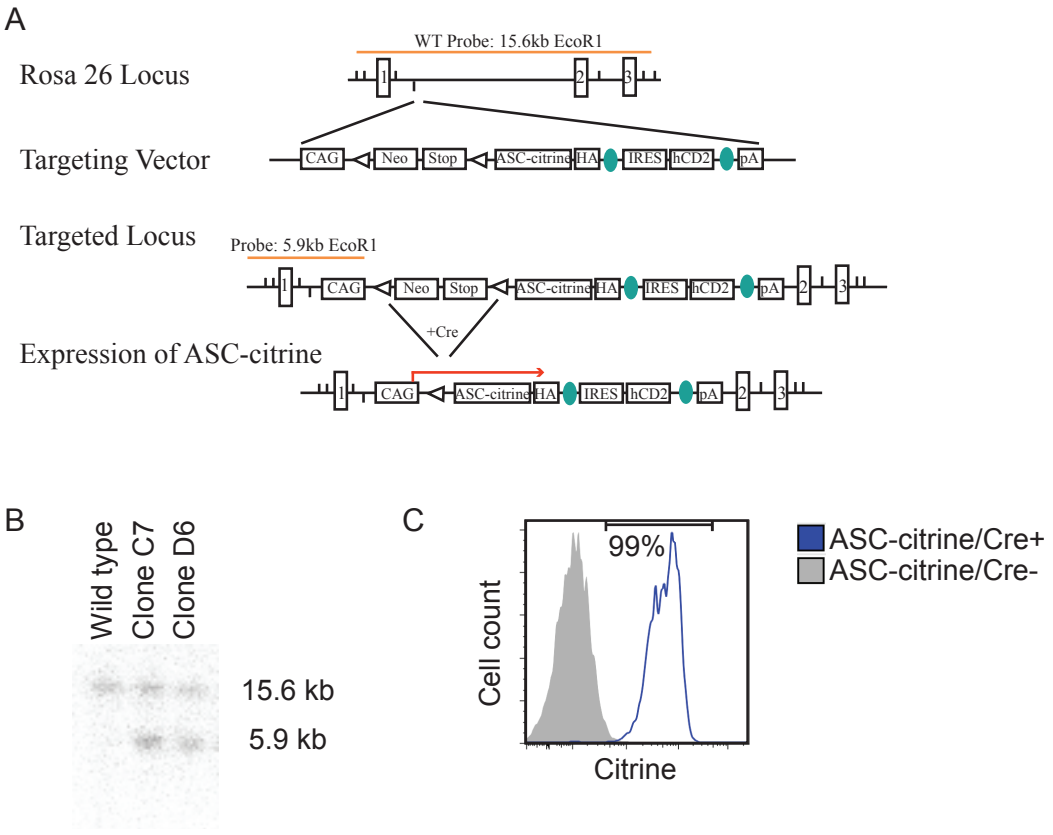
Bone marrow macrophages ($1x10^6$ cells/well in a 12-well plate) were stimulated with influenza A/PR8 (MOI=2), vesicular stomatitis virus (VSV, Indiana strain; MOI=20), mouse cytomegalovirus (mCMV; MOI=10), or herpes simplex virus

(HSV; MOI=2). Speck formation was assessed by flowsight after 4 or 12 hrs of stimulation.

Necrotic death stimulation *in vitro*

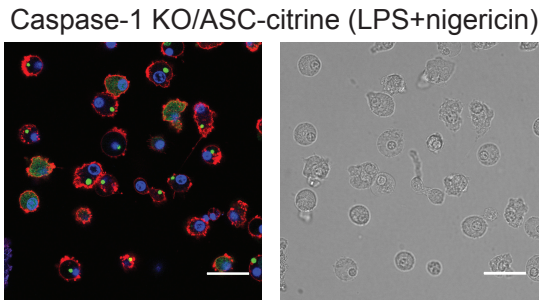
Bone marrow macrophages ($1x10^6$ cells/well in a 12-well plate) were stimulated with 100ng/ml of TNFα (R&D systems), 500nM of Birinapant (Apexbio) and/or 20μM of zVAD (R&D systems) for 24 hours. Control samples were primed with 100ng/ml LPS for 2 hours and then stimulated with nigericin (10μM) for 1 hr. Cells were harvested and stained for CD11b expression and 7AAD for flowsight analysis.

Supplemental Figures:



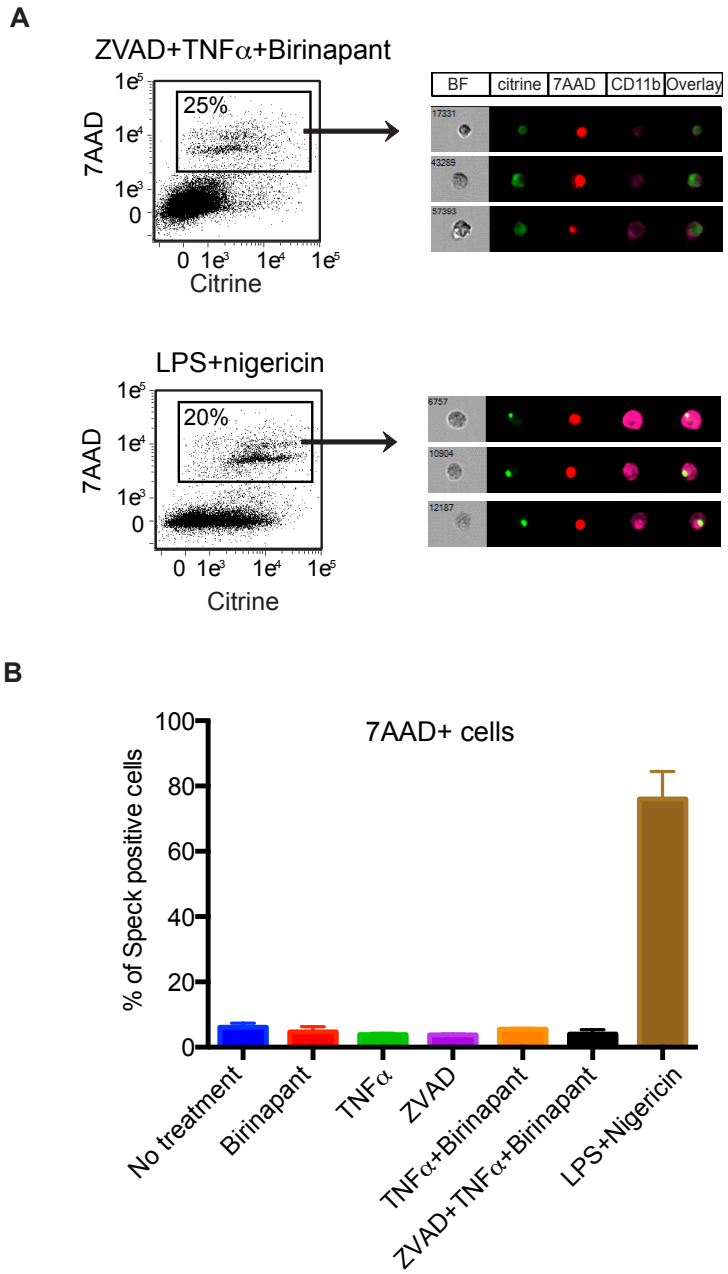
Supplemental Figure 1. Generation of a conditional m*ASC-citrine* allele in the ROSA26 locus, related to Figure 1A.

(A) Schematic representation of the targeting strategy. (B) Southern blot analyses of targeted ES clones. Genomic DNA from wild-type (lane 1) and two representative targeted ES clones (lanes 2 and 3) was digested with EcoRI and subjected to Southern blot test. The targeted ES clones contain a 5.9kb band. (C) *mASC-citrine* expression is detected in blood cells using flow cytometry.



Supplemental Figure 2. Speck formation occurs independently of caspase-1, related to Figure 1C.

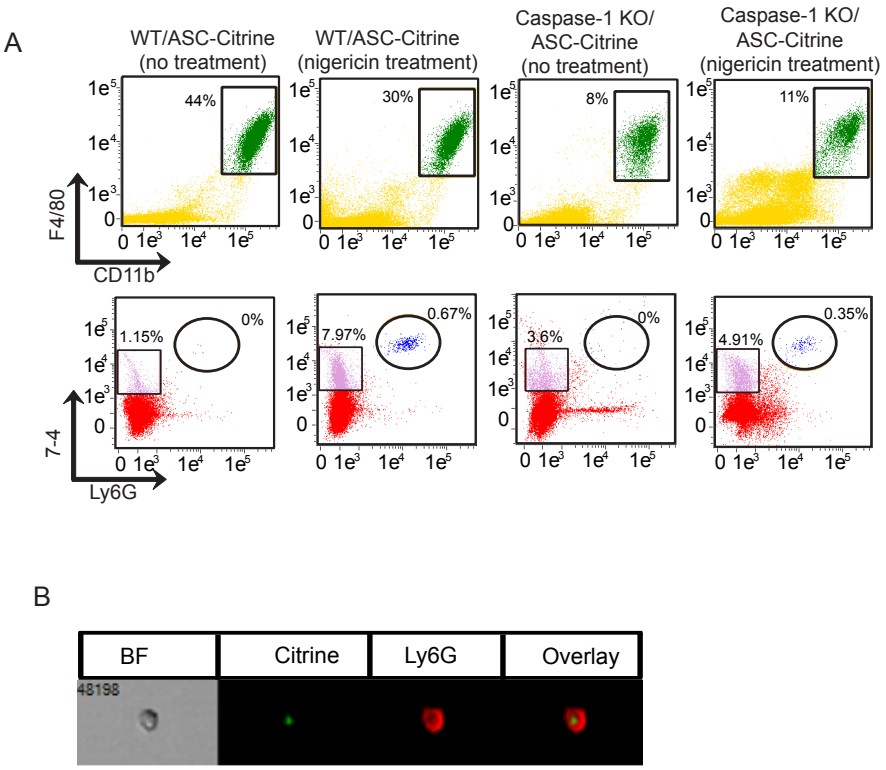
BMDMs from Caspase-1 KO/ASC-citrine mice were primed with 100 ng/mL LPS for 2 hr followed by stimulation with nigericin for 1 hr. Living cells were stained with Hoechst nuclear dye (blue) and CellMask Deep Red plasma membrane dye (red). ASC-citrine fusion proteins are shown in green. Speck formation was visualized by confocal microscopy. Scale bars represent 30 μm.



Supplemental Figure 3. Necrotic death stimulation does not induce speck formation, related to Figure 2F.

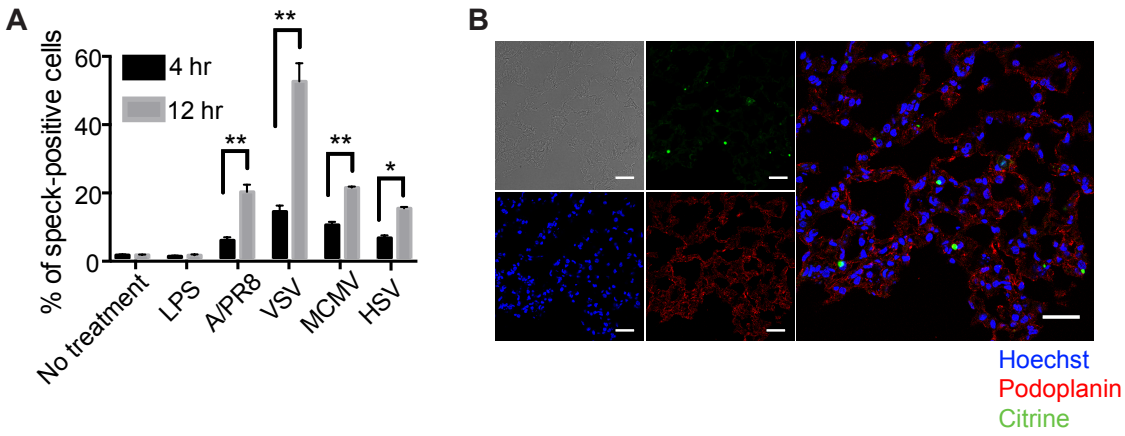
BMDMs from ASC-citrine mice were stimulated with 100ng/ml of TNFα, 500nM of Birinapant , and/or 20μM of zVAD for 24 hours. Control samples were primed

with 100 ng/mL LPS for 2 hr followed by stimulation with nigericin for 1 hr. Cells were stained with mAb to CD11b and 7AAD for image-based flow cytometry analysis. (A) Images from the flowsight analysis showing speck positive cells in 7AAD+ population. (B) Quantitative results showing the percentage of speck positive cells in 7AAD+ gated cells.



Supplemental Figure 4. Immune cell populations in nigericin induced peritonitis model, related to Figure 3.

(A) Gating strategies and the percentage for F4/80^{high}/CD11b+ residential macrophages (green), Ly6G+ neutrophils (blue) and 7-4+ recruited monocytes (pink) from each treatment conditions are shown in the dot plots. Data are representative of three independent experiments. (B) Image-based flow figures showing speck formed cells in Ly6G+ neutrophils.



Supplemental Figure 5. Both RNA and DNA virus induce speck formation *in vitro*, related to Figure 5.

(A) BMDMs from ASC-citrine/Cre+ mice were primed with LPS for 2 hr followed by stimulation with different viruses for 4 or 12 hr. Cells were then harvested and subjected to image-based flow cytometry analysis. CD11b+ cells were gated and the percentage of speck-positive cells was analyzed as described in Figure 3. MOI for each infection are as follow: influenza A/PR8 (MOI=2), VSV (MOI=20), MCMV (MOI=10), and HSV (MOI=20). Data are presented as mean ± SD of triplicates and are representative of two independent experiments. (B) Lung tissue from day 1 influenza A/PR8 infected ASC-citrine/Cre+ mice was fixed and stained with podoplanin (gp38) (red) and Hoechst nuclear dye (blue). Green represents ASC-citrine molecules. Scale bar represents 30 μm. Data are representative of three independent experiments. *p<0.05, **p<0.01 by student's *t*-test.