SUPPLEMENTAL MATERIAL
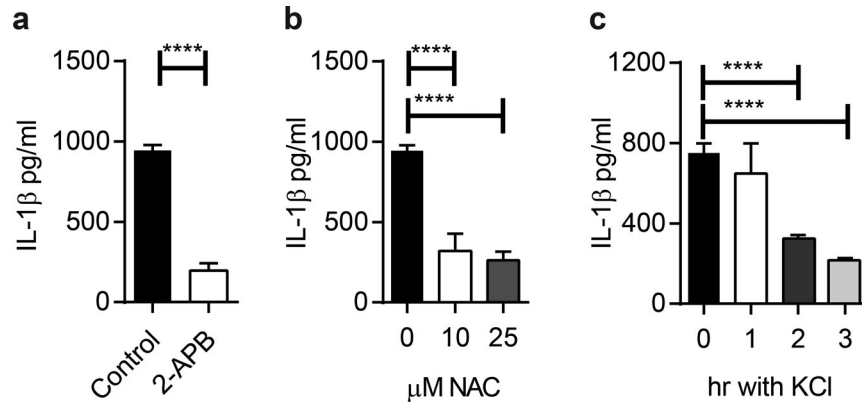
Figure S1.   **cGAMP induces K⁺/Ca²⁺– and ROS–dependent inflammasomes.** (a and b) IL-1β ELISAs from supernatants of BMDMs primed with LPS and treated with vehicle or 2-APB (a) or *n*-acetyl-cysteine (NAC; b) at the indicated concentrations followed by transfection with 2′3′-cGAMP for 6 h. (c) IL-1β ELISAs from BMDMs primed with LPS, followed by transfection with 2′3′-cGAMP. Extracellular KCl added for the last 1, 2, or 3 h of induction. *n* = 3 independent experiments. Error bars, SD; ****, P < 0.0001.
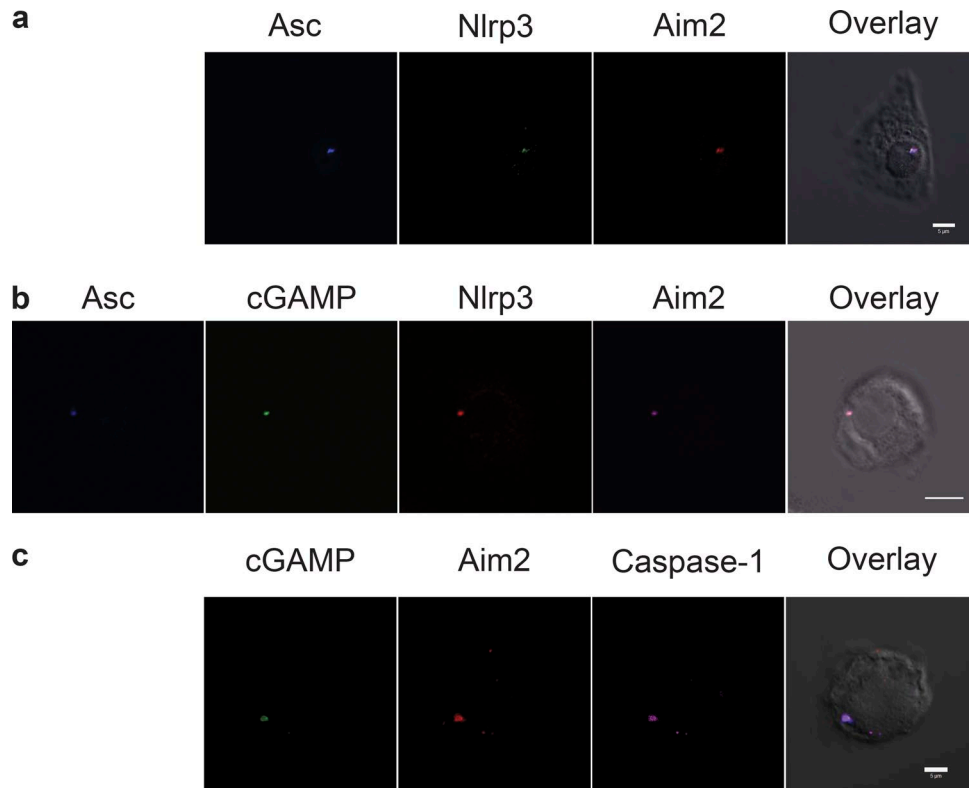
Figure S2. cGAMP induces complexes containing both NLRP3 and AIM2. Confocal microscopy of JAWSII cells expressing AIM2-Flag pulsed with doxycycline during LPS priming. (a–c) Cells were transfected with 2'3'-cGAMP (a), a 30:1 mixture of 2'3'-cGAMP and fluorescein-labeled 2'3'-cGAMP (b), or rhodamine-dAdT followed by FLICA-660 (c). Cells were labeled with primary antibodies to Asc, Nlrp3, and Flag (for AIM2-Flag) followed by Alexa Fluor–labeled secondary antibodies. Shown are pseudocolored images. Bars, 5 µm.
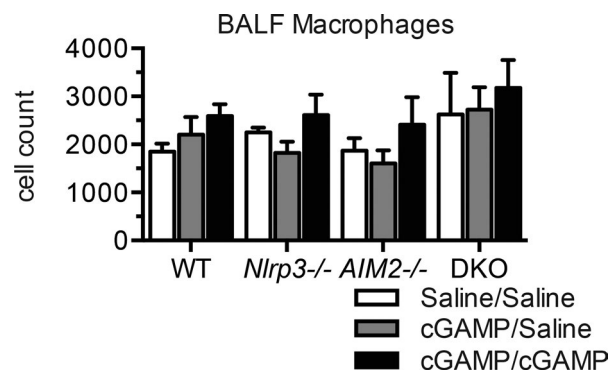

Figure S3. Bronchiolar macrophage cell count after cGAMP administration. WT, $Aim2^{-/-}$, $Nlrp3^{-/-}$, and $Aim2^{-/-}$ $Nlrp3^{-/-}$ DKO mice were intranasally dosed with 2'3'-cGAMP followed by a second dose 24 h later. 4 h after the second dose, BALF was collected, and cells were cytospun onto slides and stained with Diff-Quick solution for cell differentiation. Shown are total counts of bronchial macrophages. WT, $n = 17$; $Aim2^{-/-}$, $n = 10$; $Nlrp3^{-/-}$, $n = 8$; $Aim2^{-/-}$ $Nlrp3^{-/-}$ DKO, $n = 8$. Results are presented as the mean ± SEM.