**Supplementary Figure 1**

**a**

Figure S1. Bone marrow-derived macrophages and dendritic cells respond to alum in vitro by producing IL-1β. a, WT bone marrow-derived macrophages were cultured as in Fig. 3a and IL-1β was measured by ELISA from supernatants after 8 hours of Imject alum stimulation (mean and s.d.). b, BMDCs were left untreated (LPS -) or stimulated with 50 ng/mL LPS (+) for 18 hours and then 500 μg/mL Imject alum (“Alum1”) or aluminum hydroxide gel (Sigma-Aldrich) (“Alum2”) as indicated for 8 hours. IL-1β released into culture supernatants was measured by ELISA with a minimum detection level of 200 pg/mL (mean and s.d.).
Figure S2. TNF-α and IL-6 are induced by LPS but not by alum alone in vitro. Unprimed or LPS-primed peritoneal WT macrophages were cultured as in Fig. 3a and TNF-α (a) and IL-6 (b) were measured by ELISA from supernatants after 8 hours of Imject alum stimulation (mean and s.d.). Macrophages were treated with either Colchicine (28 μg/mL) or Cytochalasin B (10 μM) for 1 hour prior to the addition of Imject alum (500 μg/mL) where indicated.
Figure S3. Antibody production induced by aluminum adjuvants administered subcutaneously is reduced in the absence of Nalp3. WT (C57BL/6) or Nalp3⁻/⁻ mice (3-4 mice/group) were immunized subcutaneously with 50 μg of OVA in PBS (“-Alum”) or adsorbed to 2 mg Imject alum (+) on day 0 and 25 μg OVA with 2 mg Imject alum on day 10. Sera was collected on day 12 and analyzed for OVA-specific IgG1.
Figure S4. MyD88-deficient mice are able to mount an antibody response with an alum adjuvant. 6-8 week-old WT (C57BL/6) or MyD88 knockout mice were injected intraperitoneally with 50 ug of OVA adsorbed to 4 mg Imject alum on day 0 and 25 ug OVA with 4 mg Imject alum on day 10. Mice were then challenged intranasally with OVA on days 21, 22 and 23 and sera were collected from mice on day 25.
Figure S5. Separate injections of OVA and Alum do not induce a significant antibody response to OVA. a, WT mice were primed and challenged as in Fig. 4a (OVA adsorbed to alum and injected together in the left (L) side of the peritoneum). One group received the same dose of OVA and Imject alum but at different sites intraperitoneally (OVA on the right (R) and alum on the left (L)). All mice were sacrificed on day 25 and analyzed as described in Fig. 4a for OVA-specific IgG1 (a) and BAL (b). The group “WT” from Fig 4a and 4c is provided for comparison. P value obtained by Mann Whitney U-test.
Figure S6. MSU injected intraperitoneally with antigen induces a humoral immune response in WT but not Nalp3-deficient mice. WT (C57BL/6) or Nalp3-/- mice (4 mice/group) were primed with 50 μg human serum albumin (HSA) in either PBS without adjuvant (-), 2 mg Imject (Alum) or 2 mg MSU and challenged with intranasal HSA two weeks later. Sera was collected on day 21 and analyzed for HSA-specific IgG1 (p value obtained by Mann Whitney U-test).
Supplementary Figure 7

Figure S7. Expression of *IL-1β* mRNA is increased in peritoneal cells from mice immunized with OVA and alum. WT (C57BL/6) or Nalp3−/− mice (3 mice/group) were primed with 50 μg OVA in either PBS without adjuvant (-) or with 2 mg Imject (Alum; +). Four days later, cells were isolated by peritoneal lavage and pooled from each group. RNA was isolated from these cells and *IL-1β* gene expression was determined by RT-PCR. Graph shows relative fold induction as compared to WT mice immunized with OVA alone with all signals normalized to *Hprt*. 

![Graph showing fold induction of IL-1β mRNA with and without alum in WT and Nalp3−/− mice](image-url)