

C-S Shi et al. Supplementary Figure 1

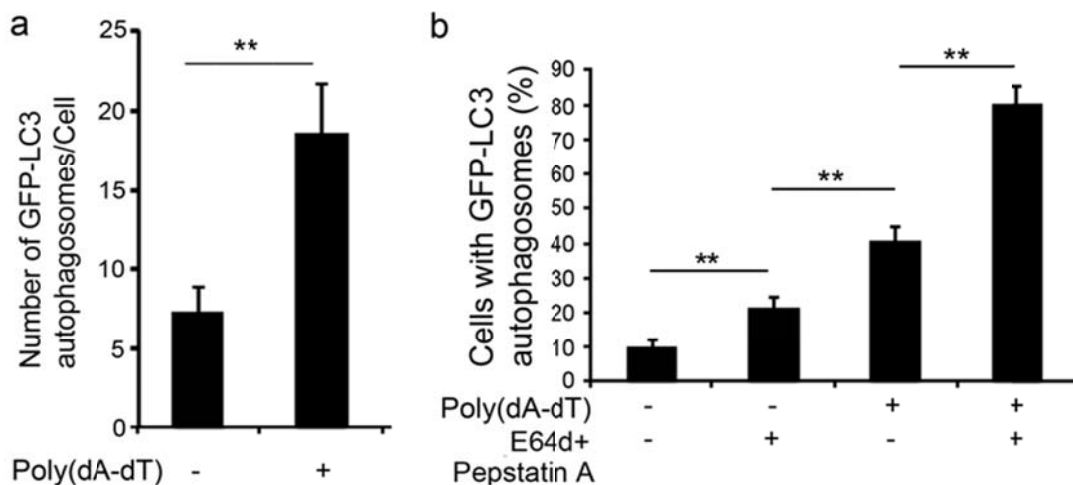


Figure 1 Poly(dA-dT) induces autophagy. (a) GFP dots in differentiated THP-1 cells expressing GFP-LC3 transfected with 1.5 $\mu\text{g/ml}$ poly(dA-dT) or sham transfected. Six hours later the numbers of GFP aggregates per GFP positive cell were enumerated. The results are from one of two experiments performed, p value < 0.01 . (b) GFP dots in differentiated THP-1 cells treated with lysosome inhibitors for 5h, or not, transfected with poly(dA-dT) 1h prior to the addition of the inhibitors. E64d+Pepstatin A were used at 5 $\mu\text{g/ml}$. Results are from three experiments, p values < 0.01 (**).

C-S Shi et al. Supplementary Figure 2

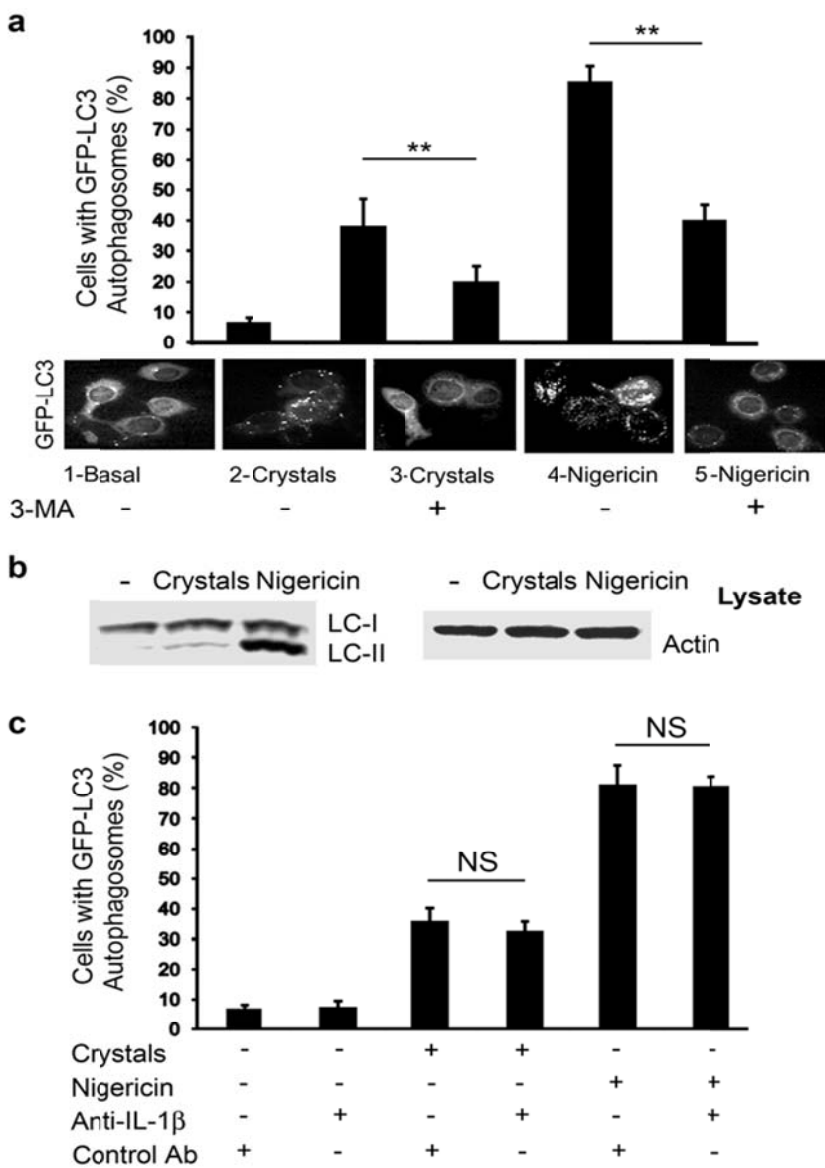


Figure 2 Activation of the NLRP3 inflammasome triggers autophagy in macrophages. **(a)** GFP dots in differentiated THP-1 cells stably expressing GFP-LC3 treated with uric acid crystals (50 $\mu\text{g/ml}$) or nigericin (4 μM) for 5h during which time the cells were exposed to 3-MA (5mM) or not. Representative images of individual cells are shown below. Results are mean and standard deviation of three experiments, $p < 0.01$ (**). **(b)** Immunoblot of LC3 processing following exposure of differentiated THP-1 treated with either uric acid crystals or nigericin as above. The levels of actin in the cell lysates are shown to right. **(c)** The percentage of GFP dots in GFP-LC3 expressing differentiated THP-1 cells treated with the uric acid crystals, nigericin, or not, in the presence of either neutralizing antibodies to IL-1 β or control antibodies. Results are mean and standard deviation of three experiments performed. NS- non significant.

C-S Shi et al. Supplementary Figure 3

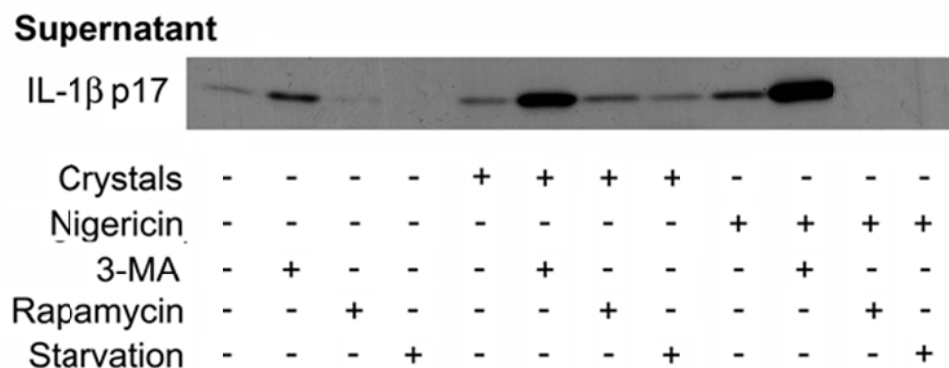


Figure 3 Autophagy affects the activity of NLRP3 inflammasomes. Differentiated THP-1 cells were treated with Uric acid crystals (50 μ g/ml), nigericin (4 μ M) or not for 5h in the presence or absence of 3-MA (5 mM), rapamycin (25 nM), or serum starved. The levels IL-1 β in the culture media were measured by immunoblotting. Results are representative of one of three experiments performed.

C-S Shi et al. Supplementary Figure 4

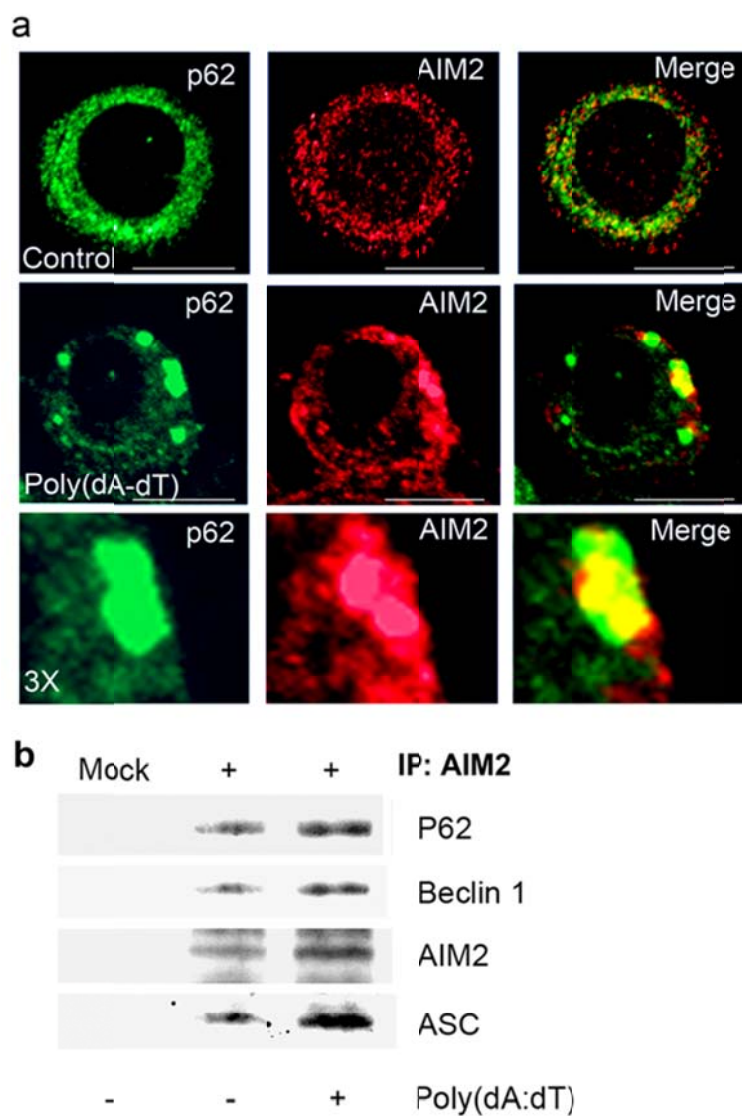


Figure 4 AIM2 partially co-localizes with p62. **(a)** Confocal microscopy of differentiation THP1 cells transfected with 1.5 $\mu\text{g/ml}$ poly(dA-dT) for 2h, or not, and immunostained for AIM2 and p62. Shown below is a 3X electronic zoom of the confocal image. **(b)** Immunoblot of AIM2 immunoprecipitates from differentiated THP-1 cells transfected with poly(dA-dT) for 2h, or not, to detect Beclin-1 and AIM2. The immunoblot was subsequently stripped and re-blotted for ASC and p62. Scale bars shown are 10 μm . Experiment performed twice.

C-S Shi et al. Supplementary Figure 5

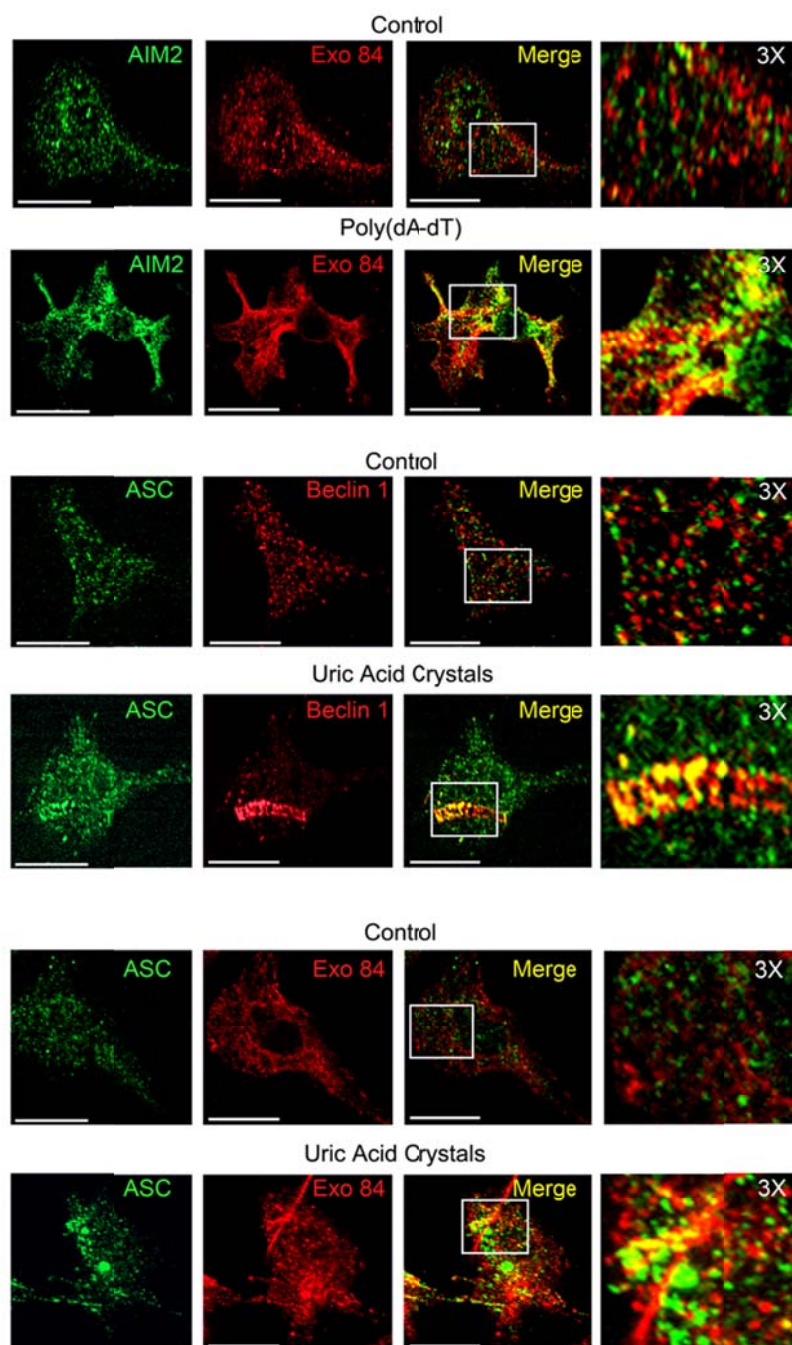


Figure 5 Poly(dA-dT) and crystals trigger co-localization of proteins involved in inflammasomes and autophagy. Confocal microscopy of mouse BMDM primed with LPS (20 ng/ml) for 2 hours and exposed to 1.5 μ g/ml poly(dA-dT) for 30 minutes, uric acid crystals (50 μ g/ml) for 2 hours, or not stimulated immunostained as indicated. The images on the right are a 3X electronic zoom of the area outlined by the white square. Scale bars shown are 10 μ m. Experiments performed three times.

C-S Shi et al. Supplementary Figure 6

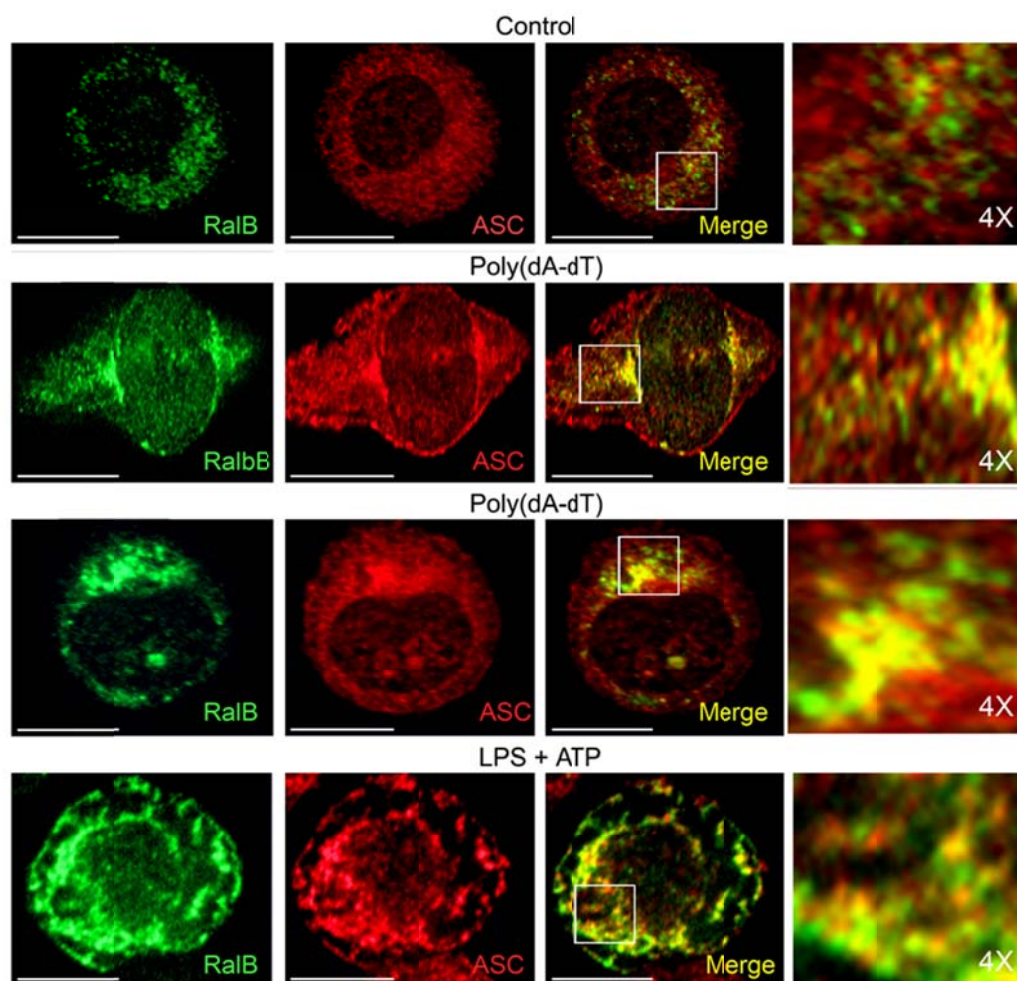


Figure 6 ASC and RalB partially co-localize following activation of either AIM2 or NLRP3 inflammasomes in THP-1 cells. Confocal microscopy of differentiated THP-1 cells primed with LPS (20 ng/ml) for two hours and then stimulated for 1h with either 1.5 μ g/ml poly(dA-dT) or 30 minutes with LPS (400 ng/ml) plus ATP (5 mM), or not stimulated and immunostained for RalB and ASC. Single and merged images from representative cells are shown. The images on the right are a 4X electronic zoom of the area outlined by the white square. Scale bars shown are 10 μ m.