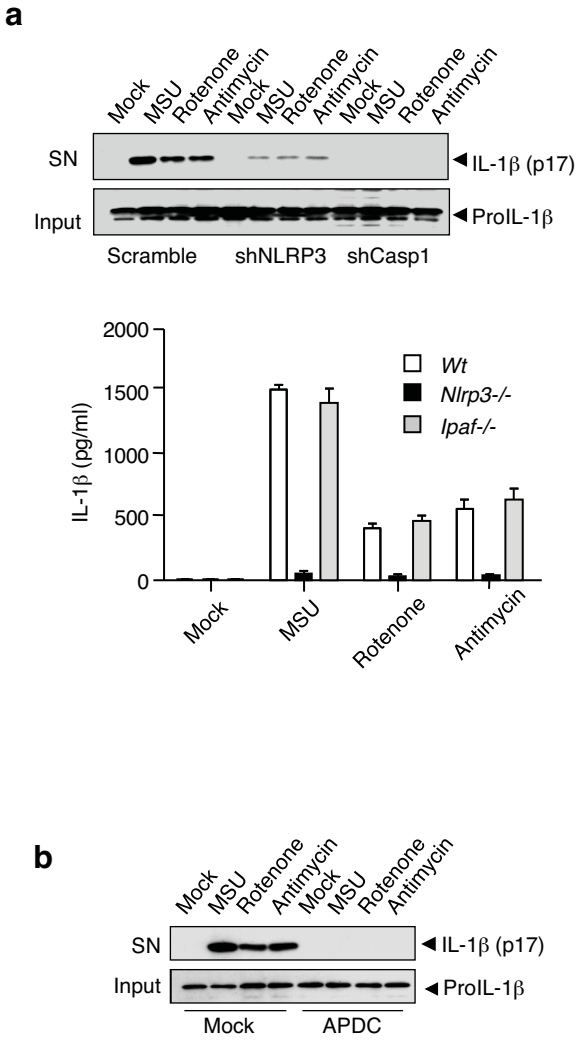
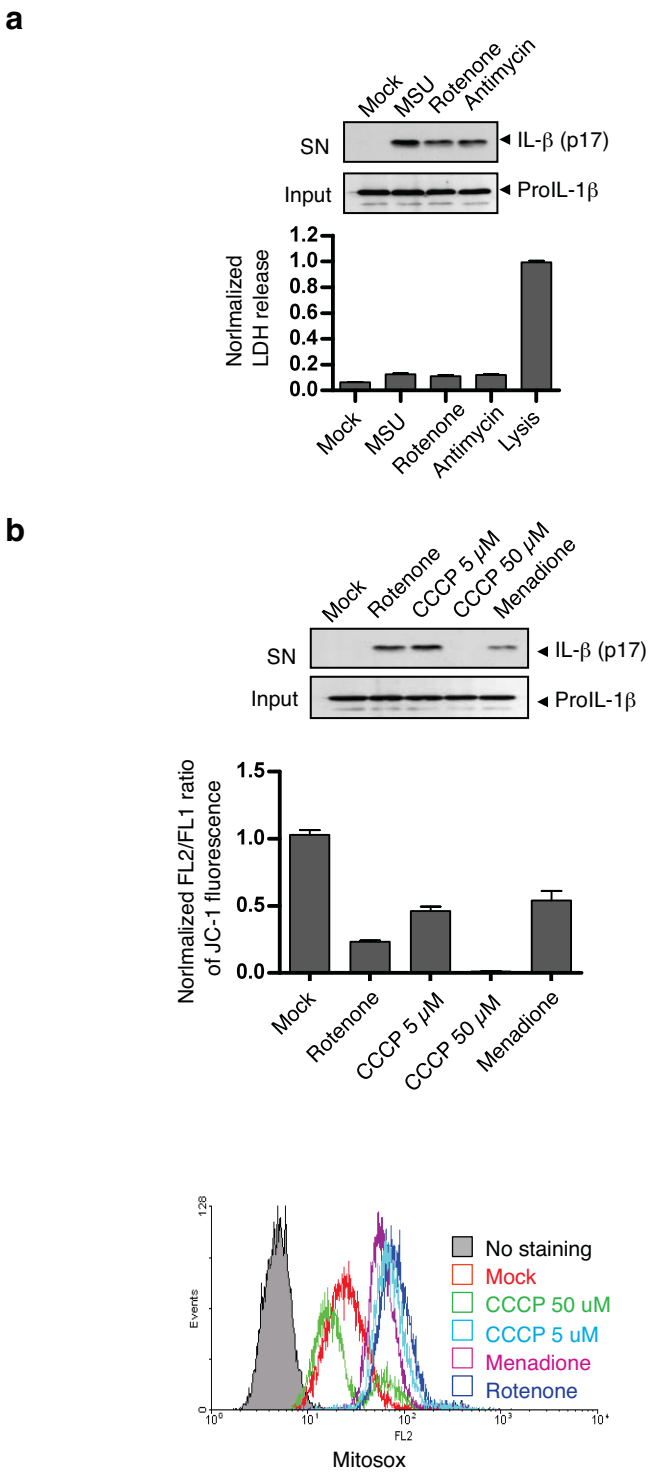
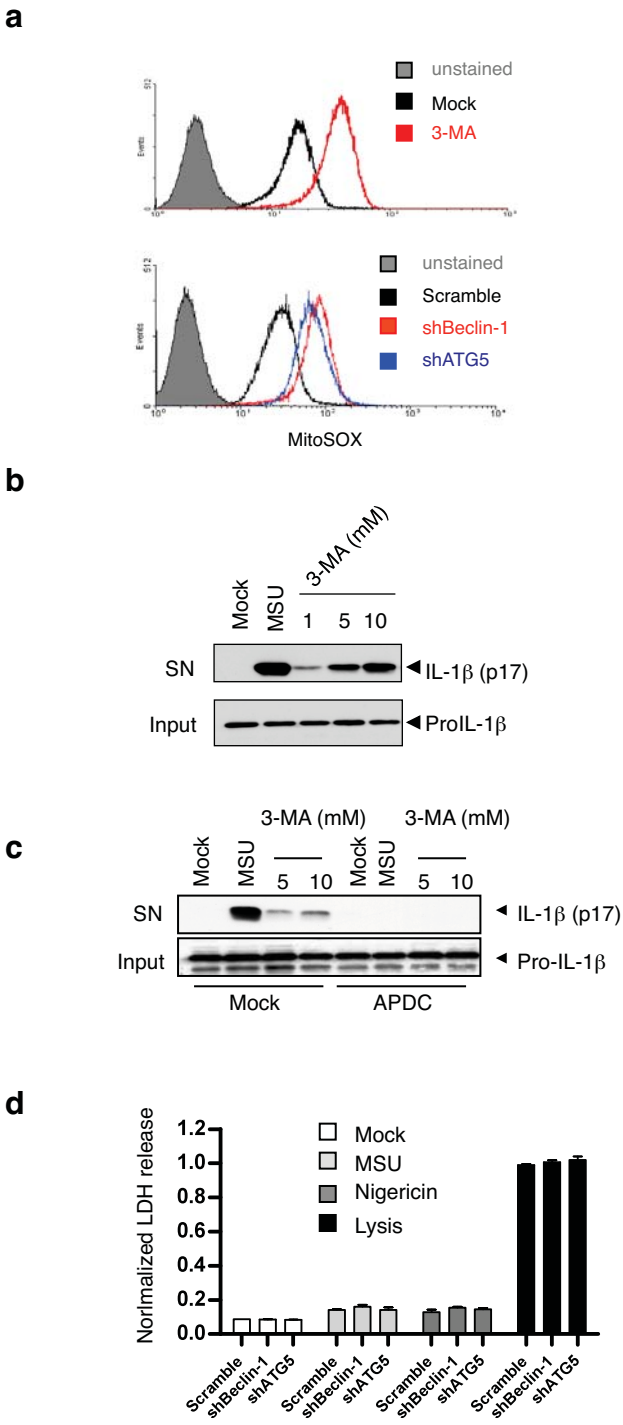
doi:10.1038/nature09663



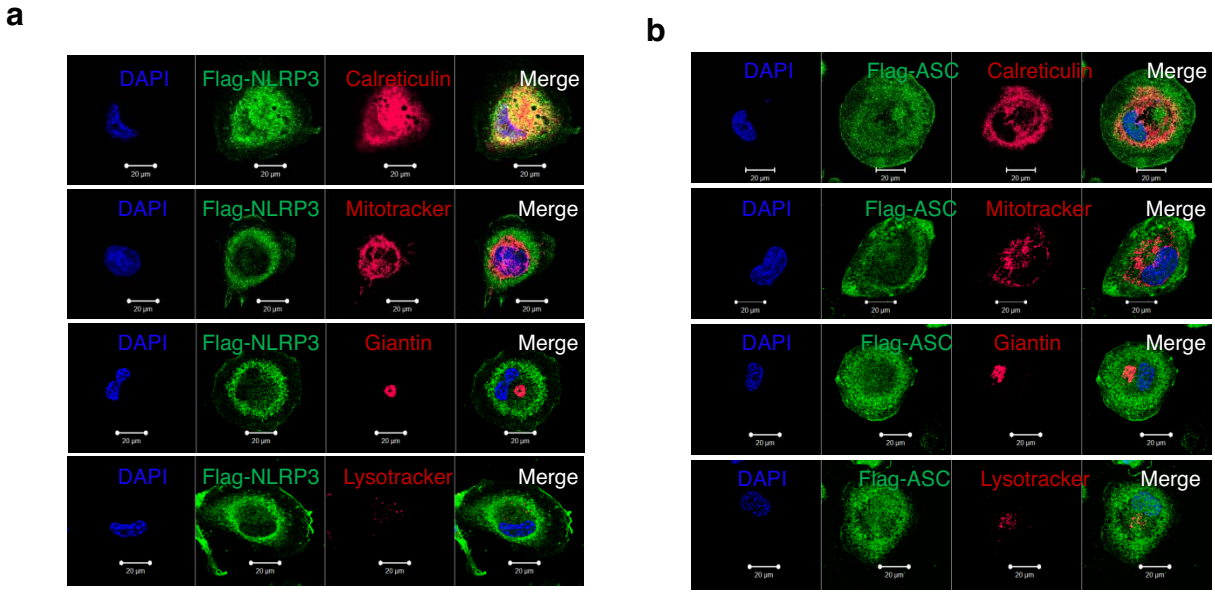
Supplemental Figure 1. Mitochondrial ROS can trigger NLRP3 inflammasome activation **a,** THP1 cells stably expressing shRNA against the indicated target genes or LPS-primed bone marrow-derived macrophages (BMDMs) from wild type (*wt*), *Nlrp3* or *Ipaf* deficient mice were stimulated 6 hrs with MSU (150 μg/ml), rotenone (10 μM for THP1 cells and 40 μM for BMDMs) or antimycin (40 μg/ml for THP1 cells and 10 μg/ml for BMDMs). The release Il-1 was then determined western blot (THP1) or ELISA (BMDMs). **b,** THP1 cells were stimulated for 6 hrs with MSU (150 μg), rotenone (10 μM) or antimycin (40 μg/ml) in the presence of APDC (100 μM) or its absence (Mock) and the release of IL-1 determined



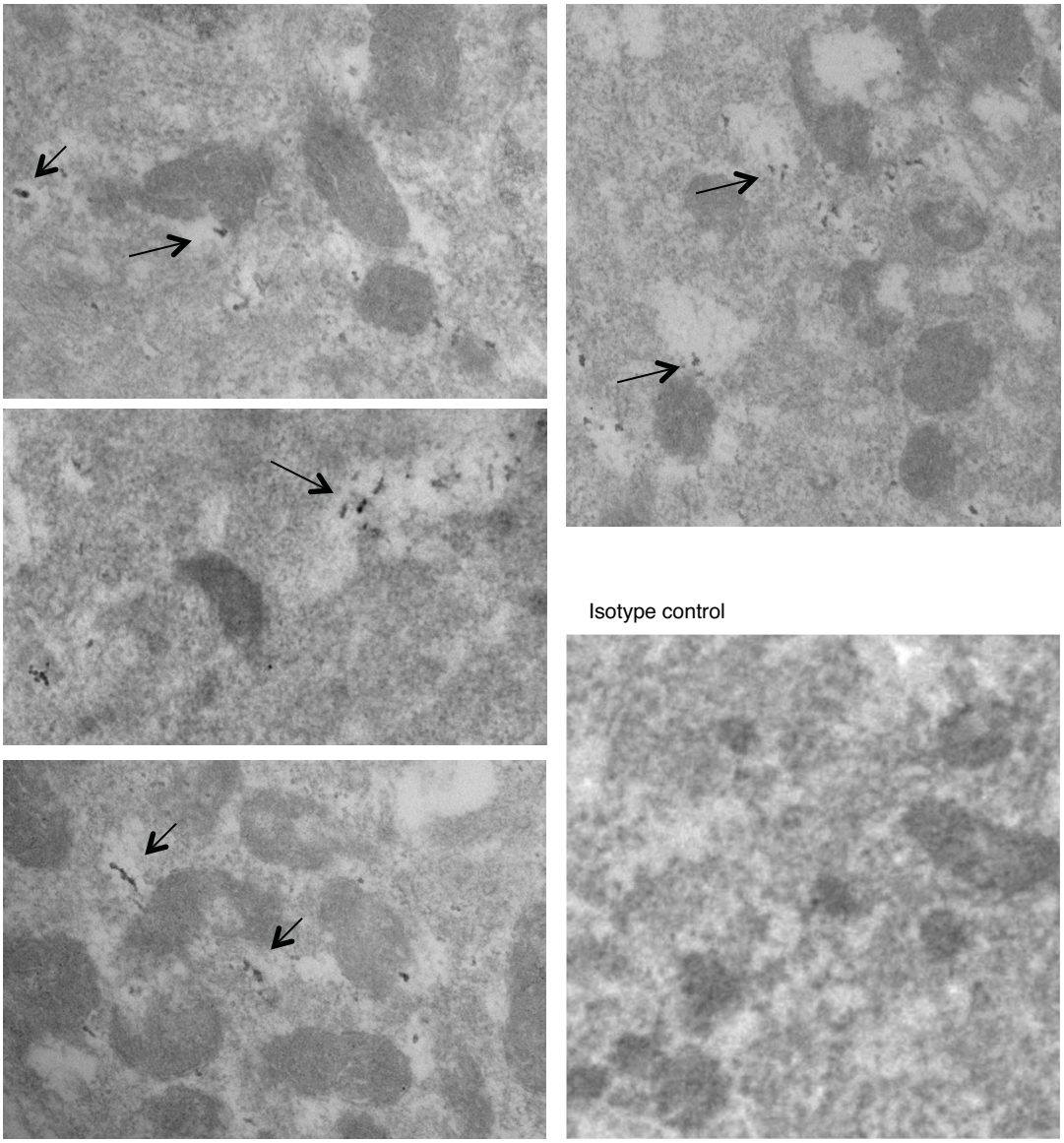
Supplemental Figure 2. Inhibition of mitochondrial complex I and complex 3 activity results in NLRP3 inflammasome but not in cell death. **a,** THP-1 cells were stimulated with MSU (150 μg/ml), rotenone (10 μM), antimycin (40 μg/ml) for 6 h. IL-1 and LDH release were determined. **b,** THP-1 cells were stimulated with the respiratory chain uncoupler CCCP or with the pro-oxidant menadione, rotenone or MSU as controls. The mitochondrial membrane potential and ROS generation were measured by FACS using the fluorescent probes JC-1 and mitosox, respectively



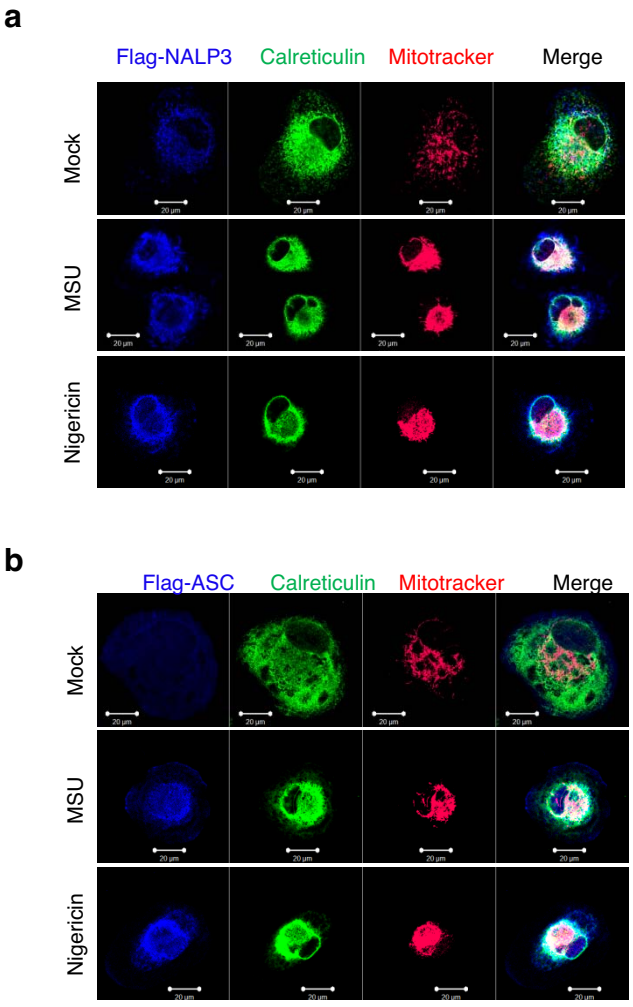
Supplemental Figure 3. Inhibition of autophagy/mitophagy generates ROS, activates the NLRP3 inflammasome which is inhibited by the anti-oxidant APDC but does not induce cell death. **a,** THP1 cells stimulated with 3-Methyladenine (3-MA, 10 mM) for 24 h or THP1 cells stably expressing shRNA against Beclin-1 or ATG5 were stained with MitoSOX for 30 min and analyzed by flow cytometry**. b,** THP1 cells were stimulated for 6 hrs with MSU (150 μg/ml) or the indicated amounts of 3-MA, supernatants and cell extracts were analyzed by western blotting. **c**, THP-1 cells were treated with 3-MA in the presence or absence of APDC. **d,** THP-1 cells stably expressing shRNA against Beclin-1 or ATG5 were stimulated with MSU, nigericin for 6 hrs and cell death determined by the measurement of LDH release.



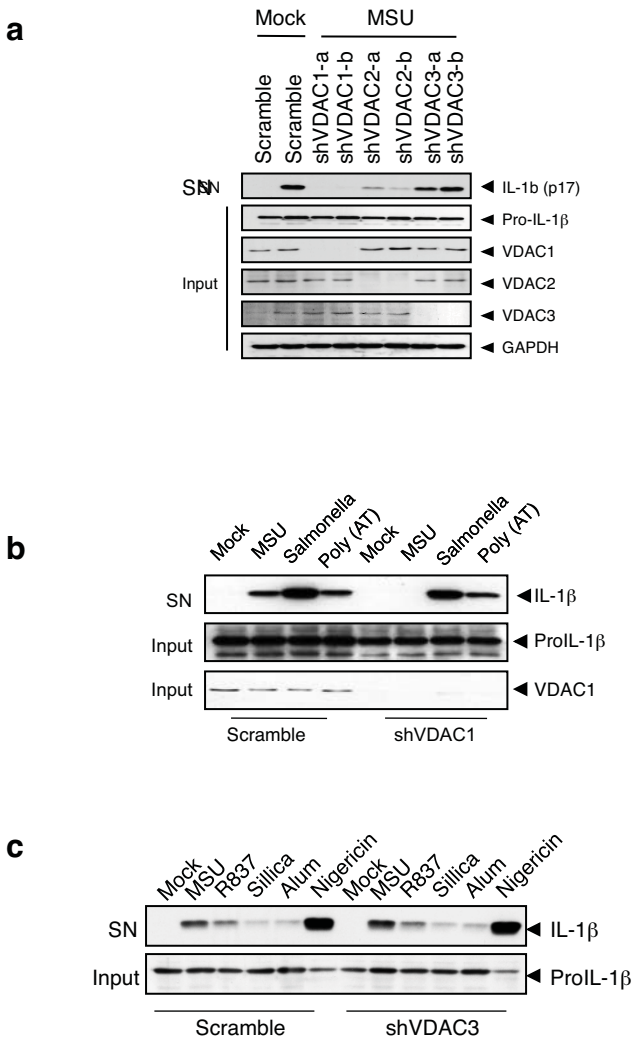
Supplemental Figure 4. Localization of NLRP3 **(a)** and ASC **(b)** in unstimulated THP-1 cells stably expressing Flag-NLRP3 or Flag-ASC. Organelle markers used are calreticulin (ER), mitotracker (mitochondria), Giantin (golgi) and lysotracker (lysosomes).



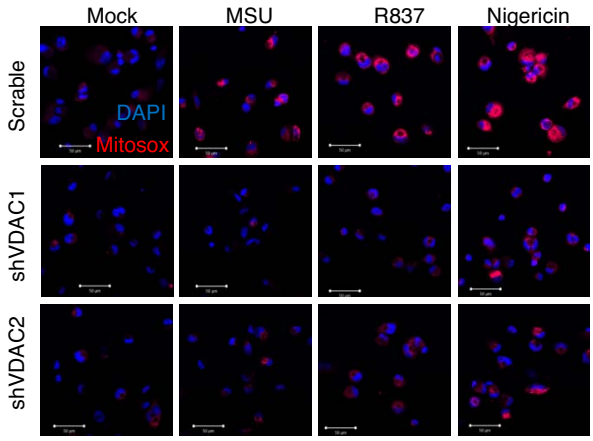
Supplemental Figure 5. Localization of NLRP3 in unstimulated THP-1 cells stably expressing Flag-NLRP3. Arrows point to immuno-gold labeled NLRP3. Isotype control is shown in the lower right panel



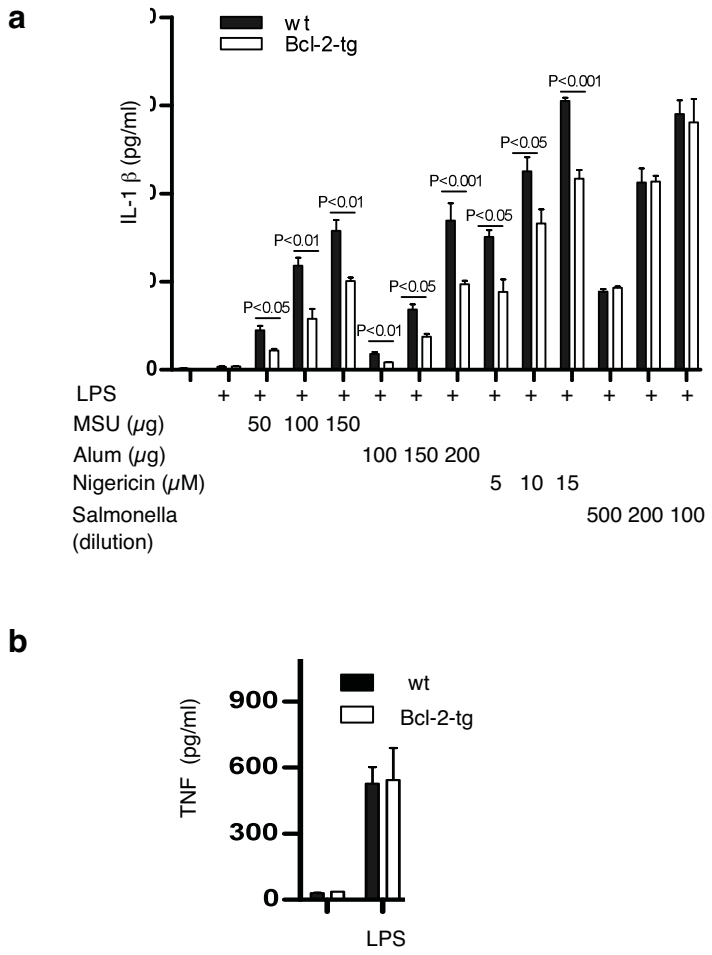
Supplemental Figure 6. Upon stimulation of the NLRP3 inflammasome, NLRP3 and ASC co-localize with perinuclear organelles that stain positively for both, the ER-marker calreticulin and mitochondria-associated mitotracker. THP-1 cells expressing Flag-NLRP3 (a) or Flag-ASC (b) were analyzed.



Supplemental Figure 7. VDACs are important for NLRP3- but not AIM2- or IPAF-inflammasome activation. **a,** Inhibition of NLRP3 inflammasome activation by MSU in various THP-1 clones expressing shRNA targeting one of the 3 VDAC isoforms. **b**, activation of the AIM2 and the IPAF proceeds normally in the absence of VDAC1. **c,** The absence VDAC3 has no effect on NLRP3 inflammasome activity.



Supplemental Figure 8. Inhibition of mitochondrial ROS in THP-1 cells stably expressing VDAC1 and VDAC2 upon stimulation wth MSU, R837 or nigericin.



Supplemental Figure 9. Bcl-2 modulates NLRP3 inflammasome activity. **a,** LPS-primed BMDMs from wt or bcl-2 transgenic mice were stimulated with NLRP3 (MSU, Alum, nigericin) or IPAF (Salmonella) inflammasome activators and IL-1β levels in the supernatants were determined. **b,** Absence of altered TNF release in BMDMs overexpressing Bcl-2. BMDMs isolated from wild-type and Bcl-2 transgenic mice were treated with LPS. TNF release was determined by ELISA. Data shown are representative of three independent experiments.