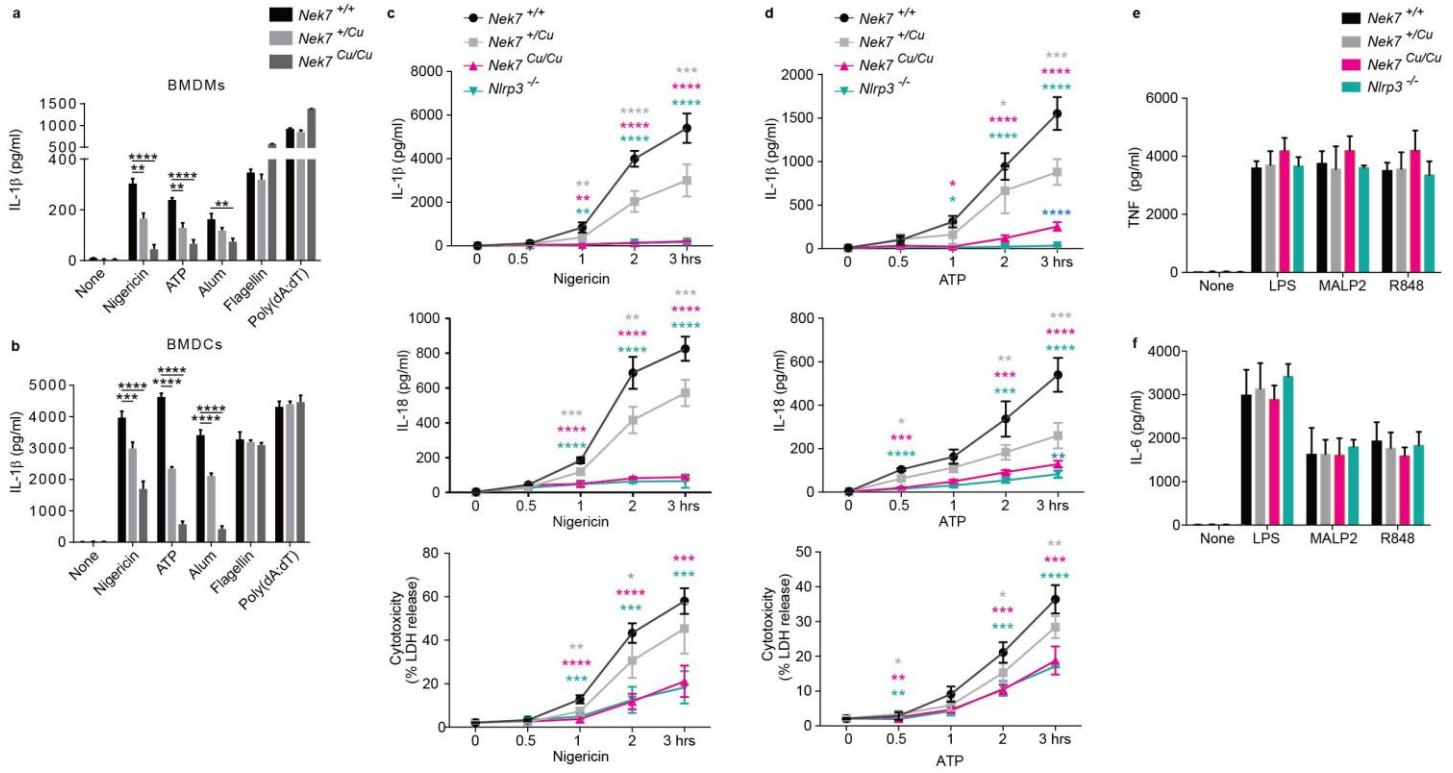
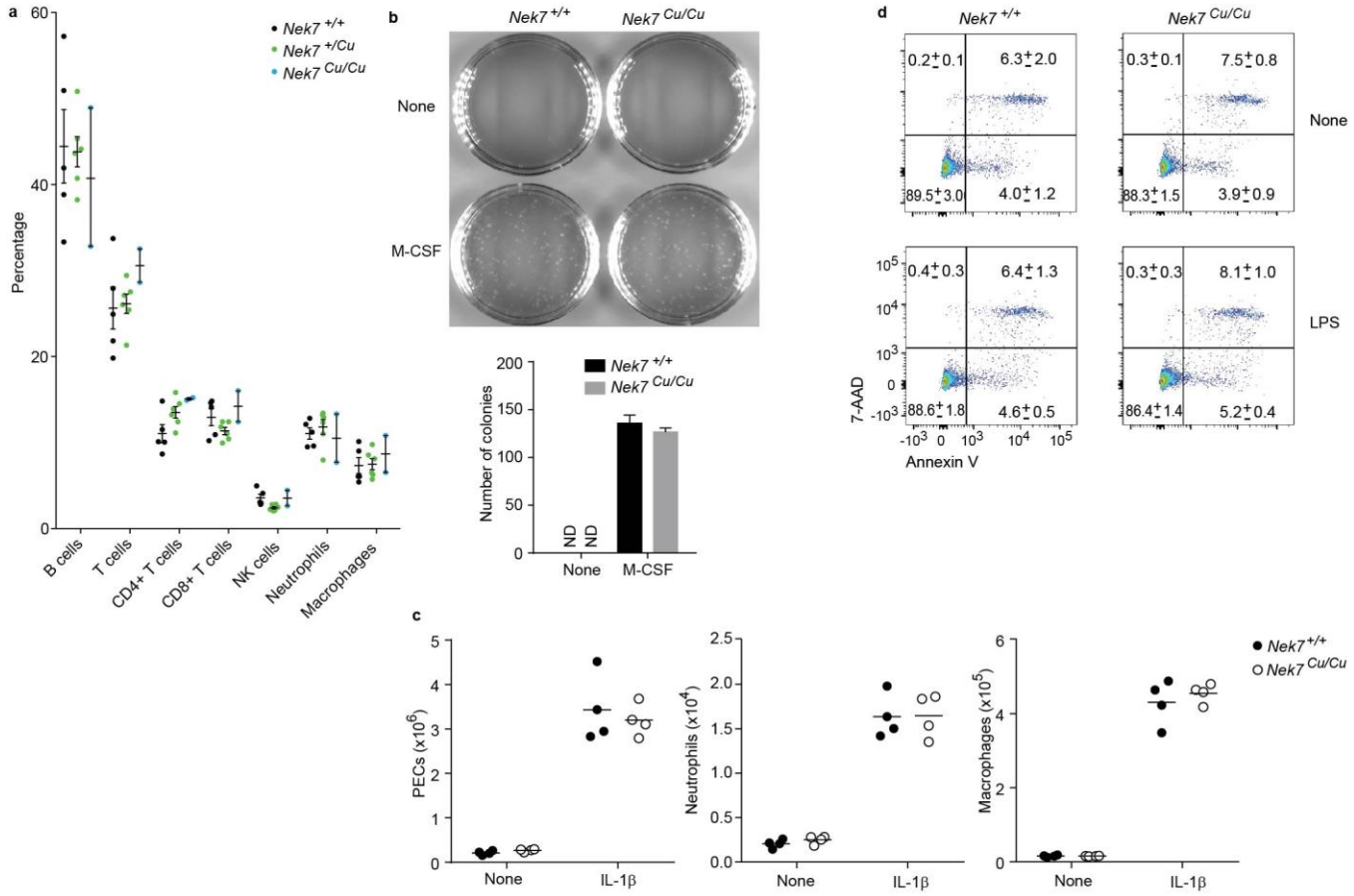


Supplementary Figure 1

Specific, functional defect in activation of the NLRP3 inflammasome in Cuties mice.

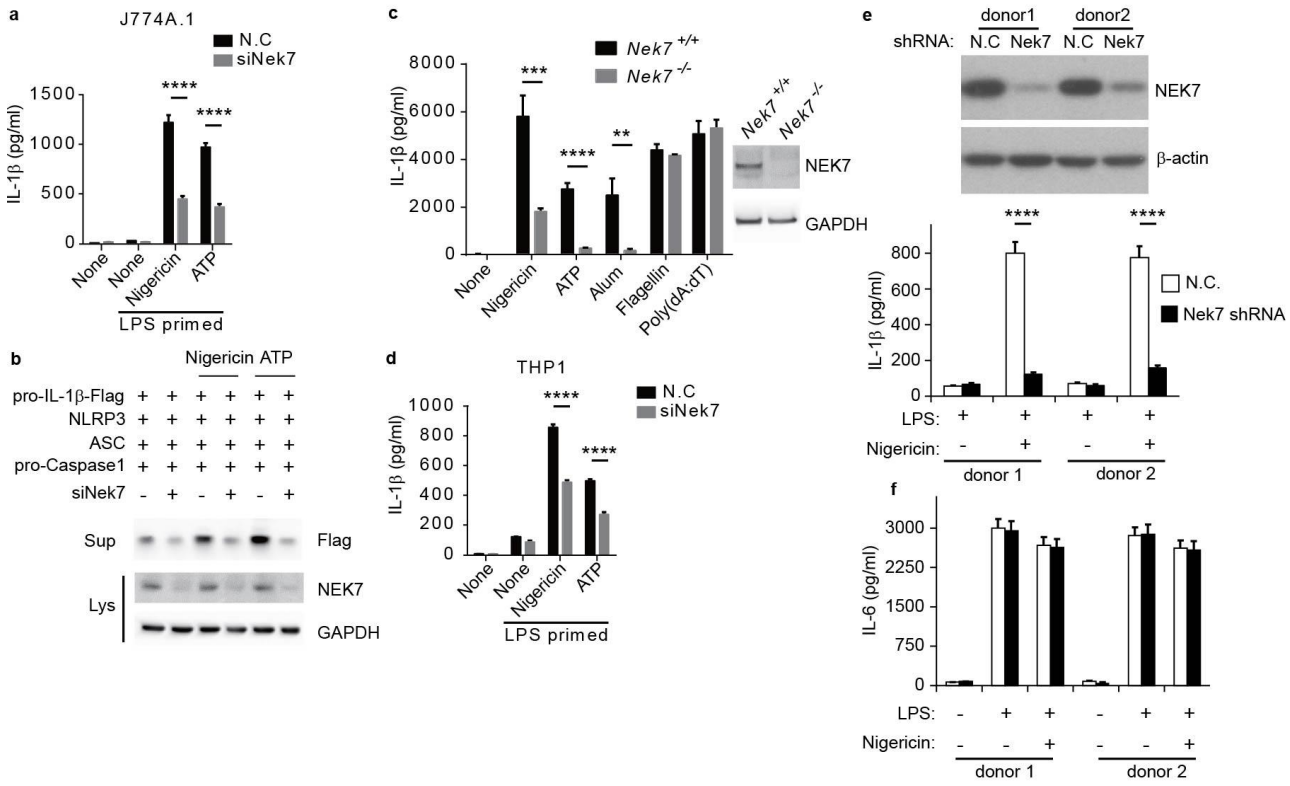
ELISA analysis of IL-1β secretion by (**a**) BMDM and (**b**) BMDC primed with LPS and treated with the indicated stimuli (n = 3 mice per genotype). (**c,d**) Time course of IL-1β secretion, IL-18 secretion, and pyroptosis of peritoneal macrophages primed with LPS and treated with (**c**) nigericin or (**d**) ATP (n = 4 mice per genotype). P values: *Nek7*$^{+/+}$ vs. *Nek7*$^{+/Cu}$ (gray); *Nek7*$^{+/+}$ vs. *Nek7*$^{Cu/Cu}$ (magenta); *Nek7*$^{+/+}$ vs. *Nlrp3*$^{-/-}$ (teal); *Nek7*$^{Cu/Cu}$ vs. *Nlrp3*$^{-/-}$ (blue). * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$ (unpaired, two-tailed Student's t test). (**e**) TNF and (**f**) IL-6 secretion by peritoneal macrophages treated with the indicated stimuli (n = 4 mice per genotype). Results are representative of two independent experiments (mean and s.d. shown).



Supplementary Figure 2

Cuties mice have normal blood cell populations and macrophage development, peritoneal recruitment and apoptosis.

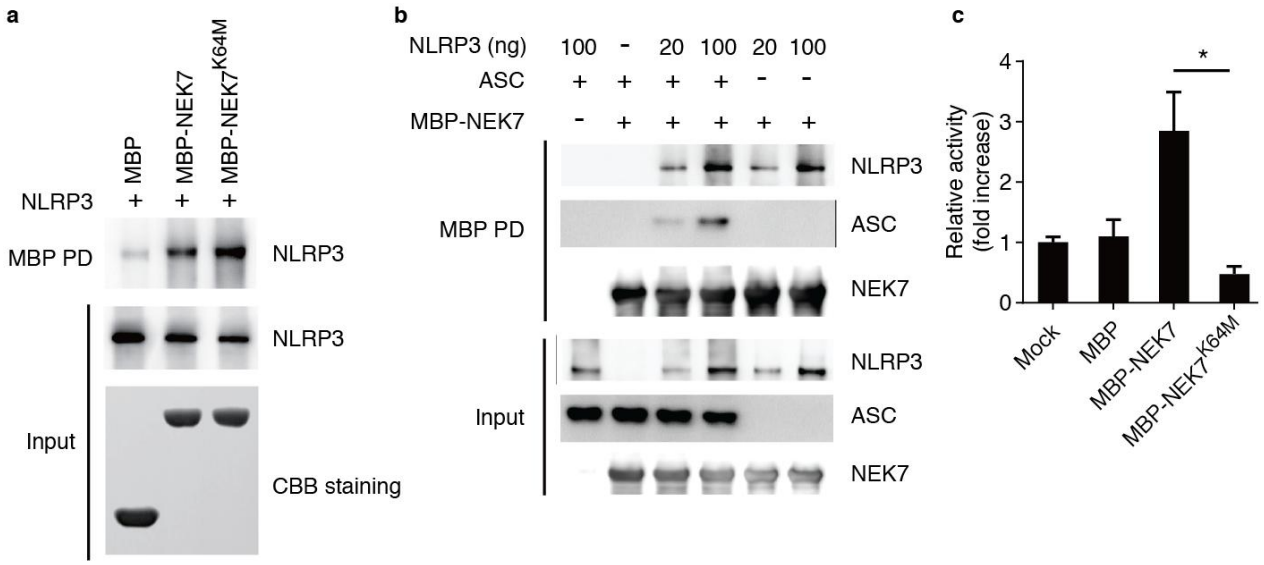
(**a**) Flow cytometric analysis of the frequencies of myeloid cells and lymphocytes in peripheral blood of Cuties mice. Data points represent individual mice. (**b**) Bone marrow progenitor colony forming unit assay. Colonies formed from bone marrow cells cultured in methylcellulose base media with M-CSF (upper panel) and quantitated in the lower panel (n = 4 mice per genotype). (**c**) Flow cytometric analysis of the number of peritoneal exudate cells (PECs), neutrophils ($Ly6G^+$ $F4/80^-$), and monocytes/macrophages ($F4/80^+$) in the peritoneum of $Nek7^{+/+}$ (filled circles) or $Nek7^{Cu/Cu}$ mice (open circles) 6 h after intraperitoneal injection of IL-1β. Data points represent individual mice. (**d**) Flow cytometric analysis of untreated or LPS-primed macrophages stained with Annexin V and the viability dye 7-AAD. Results are representative of two independent experiments (mean and s.d. shown in **a** and **b**; horizontal line represents mean in **c**).



Supplementary Figure 3

Confirmation of the Cuties phenotype in mouse macrophages and human monocytes.

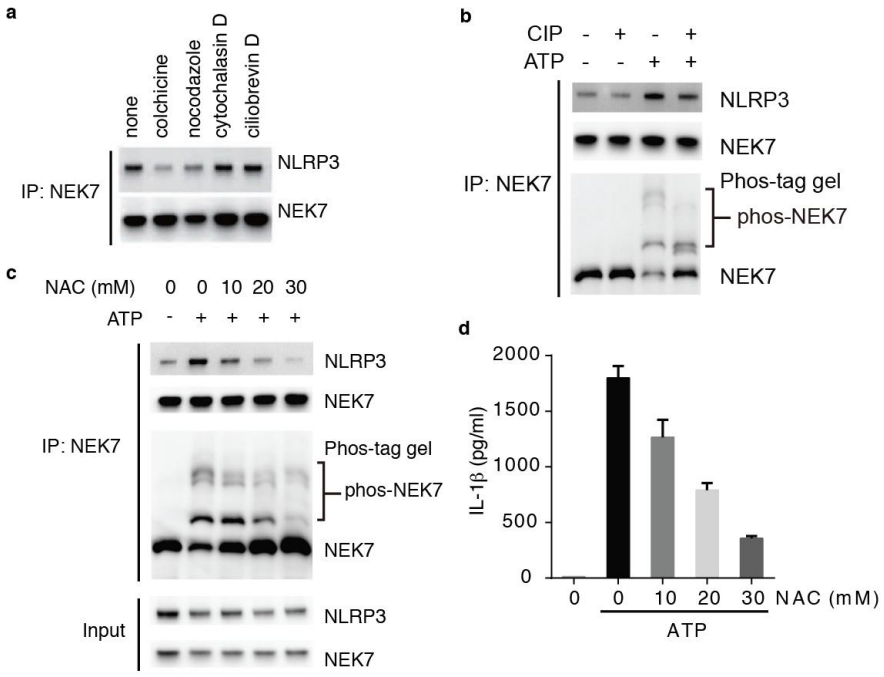
(**a,b,d**) Endogenous NEK7 expression was knocked down using siRNAs. (**a**) ELISA analysis of IL-1β secretion by J774A.1 cells primed with LPS and treated with nigericin or ATP. (**b**) HEK293T cells were transfected with NLRP3 inflammasome components and treated with nigericin or ATP. Immunoblots showing expression of the indicated proteins in cell lysates (Lys) and culture supernatants (Sup). IL-1β maturation was assessed by p17-Flag immunoblot of the supernatant. (**c**) *Nek7*$^{-/-}$ mice were generated by CRISPR/Cas9 targeting and peritoneal macrophages were assayed by ELISA for IL-1β secretion after priming with LPS and treatment with the indicated inflammasome stimuli (*n* = 3 mice per genotype). (Inset) Immunoblot showing NEK7 expression in peritoneal macrophages. (**d**) ELISA analysis of IL-1β secretion by THP1 cells primed with LPS and treated with nigericin or ATP. (**e,f**) Endogenous NEK7 expression was knocked down using shRNAs in primary human monocytes from healthy donor peripheral blood. ELISA analysis of (**e**) IL-1β and (**f**) IL-6 secretion in response to LPS priming and nigericin treatment. (**e**, top) Immunoblot showing NEK7 expression in primary human monocytes after shRNA knockdown. ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$ (unpaired, two-tailed Student's t test). Results are representative of two independent experiments in which treatments were performed in triplicate (**a,d**), two independent experiments (**b,c**) and three independent experiments in which treatments were performed in triplicate (**e,f**); data in **a** and **c**-**f** presented as mean and s.d.. N.C, non-targeting control siRNA or shRNA.



Supplementary Figure 4

Direct interaction between NEK7 and NLRP3 and *in vitro* NEK7 kinase assay.

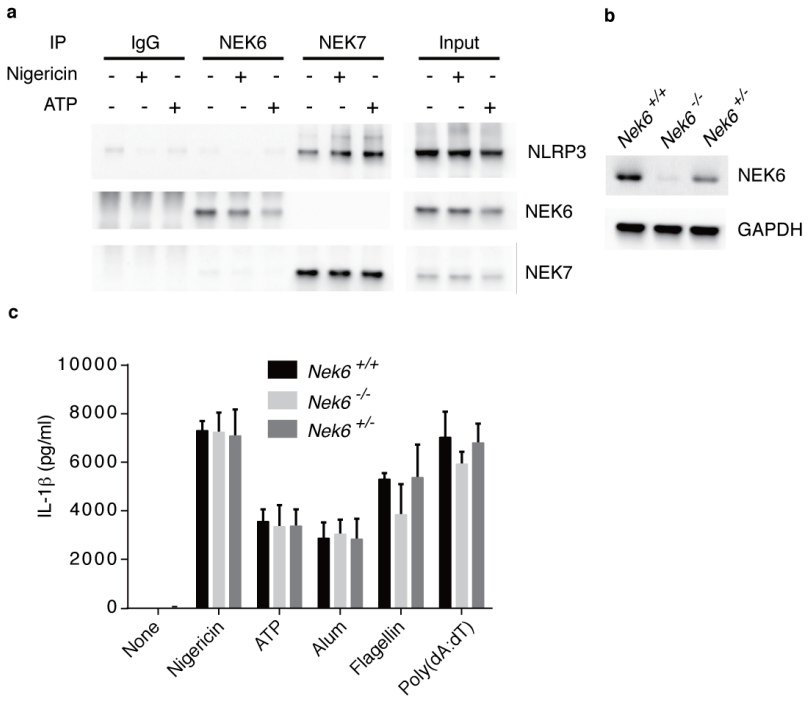
(**a,b**) The indicated recombinant proteins were incubated together and amylose magnetic beads were used to precipitate MBP or MBP tagged NEK7 proteins (MBP PD). Complexes were analyzed by immunoblotting with the indicated antibodies. CBB, Coomassie Brilliant Blue. (**c**) Recombinant MBP-NEK7 or MBP-NEK7(K64M) was incubated with β-casein and ATP. NEK7 kinase activity was measured using an ADP-based phosphatase coupled kinase assay and plotted relative to the activity measured without any MBP or MBP conjugated protein (Mock). * $P \leq 0.05$ (unpaired, two-tailed Student's t test). Results are representative of two independent experiments (**a** and **b**) or two independent experiments in which assays were performed in triplicate (**c**; mean and s.d. shown).



Supplementary Figure 5

NEK7 phosphorylation enhances its binding to NLRP3 and promotes inflammasome activation.

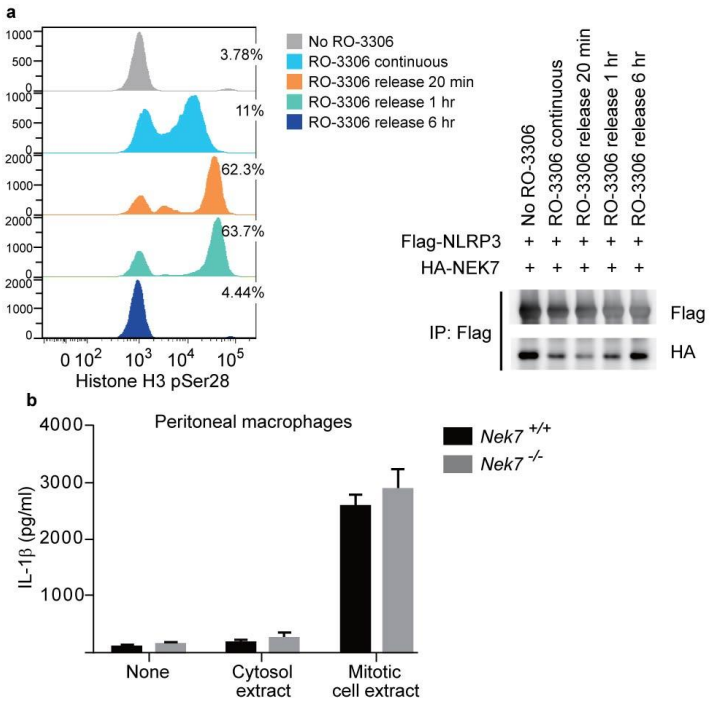
(**a**) Endogenous NEK7-NLRP3 association in LPS-primed J774A.1 cells treated with the indicated inhibitors, stimulated with nigericin, and analyzed by immunoprecipitation and immunoblot. (**b,c**) Endogenous NEK7-NLRP3 association was analyzed by immunoprecipitation and immunoblot. The phosphorylation state of NEK7 was analyzed using Phos-tag SDS-PAGE. (**b**) J774A.1 cells primed with LPS and stimulated with ATP were lysed and treated with calf intestinal alkaline phosphatase (CIP). (**c**) J774A.1 cells primed with LPS and stimulated with ATP together with *N*-acetylcysteine (NAC). (**d**) ELISA analysis of IL-1β in the culture supernatants of J774A.1 cells treated as in **c**. The means of triplicate samples are plotted. Results are representative of two independent experiments.



Supplementary Figure 6

NEK6 is not required for activation of the NLRP3 inflammasome.

(**a**) Wild type peritoneal macrophages were primed with LPS and stimulated with nigericin or ATP. The interaction between endogenous NEK6 or NEK7 and NLRP3 was analyzed by immunoprecipitation and immunoblot. (**b**) Immunoblot showing NEK6 expression in lysates of peritoneal macrophages from mice with a *Nek6* mutation, a T to C transition at 38569737 bp on Chr 2 affecting the invariant GU dinucleotide of the intron 7 donor splice site (+2 bp from the exon 7 boundary). (**c**) ELISA analysis of IL-1β secretion by peritoneal macrophages primed with LPS and treated with the indicated inflammasome stimuli (*n* = 3 mice per genotype). Results are representative of two independent experiments (mean and s.d. shown in **c**).



Supplementary Figure 7

The NLRP3 inflammasome is not activated by lysates of mitotic cells.

(**a**) NEK7-NLRP3 interaction during mitosis. HEK293T cells were transfected as indicated (above lanes in right panel) and then treated with RO-3306 for 20 h before release in fresh media for the indicated times. Flow cytometric analysis of phosphorylated histone H3-positive mitotic cells (left); the percentage of mitotic cells is indicated. The interaction between NEK7 and NLRP3 was analyzed by immunoprecipitation and immunoblot (right). (**b**) ELISA analysis of IL-1β secretion by peritoneal macrophages primed with LPS and treated with lysates of non-synchronized cycling HEK293T cells (cytosol extract), or lysates of mitotic HEK293T cells (mitotic cell extract) (mean and s.d. of *n* = 4 mice per genotype). Results are representative of two independent experiments.