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Article

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Distinct changes in endosomal composition promote NLRP3 inflammasome activation

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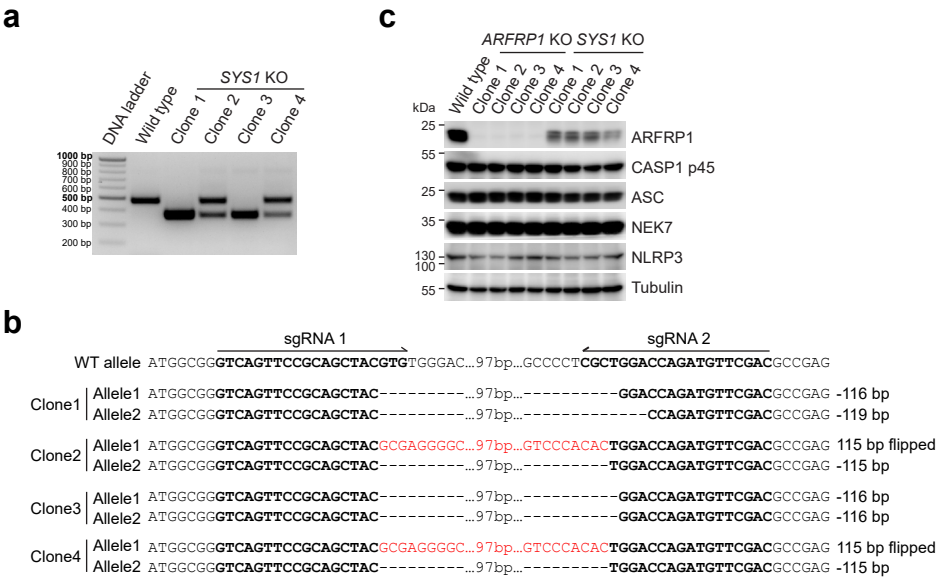
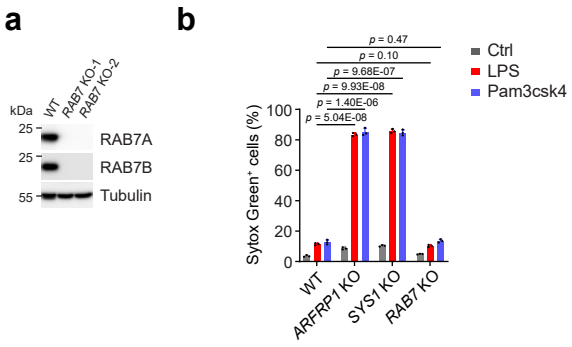
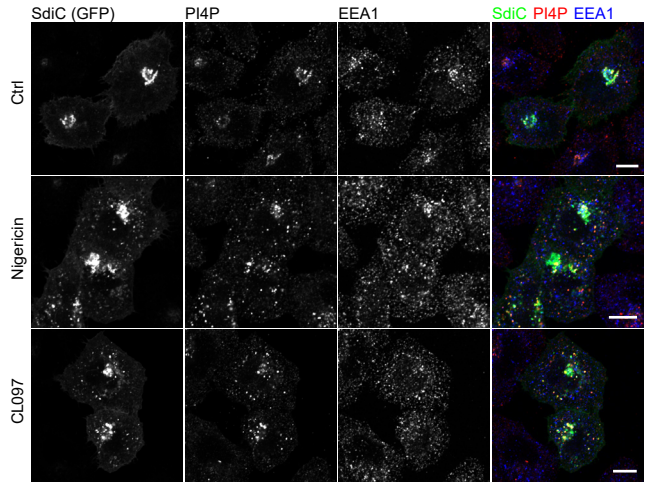


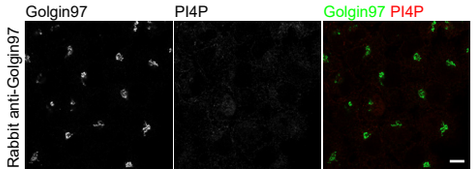
Supplementary Figure 1 Validation of *ARFRP1* KO and *SYS1* KO THP-1 cells generated by CRISPR/-Cas9-mediated gene editing. a, Genotyping of *SYS1* KO THP-1 cell clones by PCR. **b**, Validation of THP-1 *SYS1* KO clones by Sanger sequencing. **c**, Immunoblotting of cell lysates from wild type (WT), *ARFPR1* KO and *SYS1* KO THP-1 cells. Antibodies against ARFRP1, CASP1, ASC, NEK7 and NLRP3 were used. An antibody against Tubulin was used as a loading control. Data shown in panel a and c are representative of three independent experiments. Data shown in panel b was performed once.



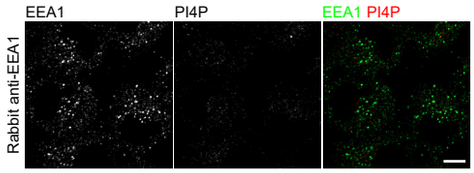
Supplementary Figure 2 Deletion of *RAB7* did not potentiate NLRP3 inflammasome activation. **a**, Immunoblotting of cell lysates from wild type (WT) and *RAB7* KO THP-1 cells. Antibodies against RAB7A and RAB7B were used. An antibody against Tubulin was used as a loading control. **b**, Cellular uptake of Sytox Green in wild type (WT), *ARFRP1* KO, *SYS1* KO and *RAB7* KO (*RAB7* KO-1) THP-1 cells treated with vehicle (Ctrl), 1 µg/ml LPS or 1 µg/ml Pam3csk4 for 2 hours. Means ± SD, N=3. Data were analyzed with an unpaired two-sided *t*-test (b). Data shown in a are representative of three independent experiments.



Supplementary Figure 3 Visualization of intracellular PI4P by indirect immunofluorescence and SidC-GFP. Indirect immunofluorescence using an anti-PI4P antibody and the PI4P probe SidC-GFP showed similar distribution pattern of intracellular PI4P. HeLa cells expressing SidC-GFP were treated with vehicle (Ctrl), 15 μM nigericin or 45μg/ml CL097 for 60 min. After fixation, cells were co-stained with antibodies against PI4P and EEA1. Scale bar: 10 μm. Data shown are representative of three independent experiments.



FOM control for Extended Data Fig.1a, 2c,



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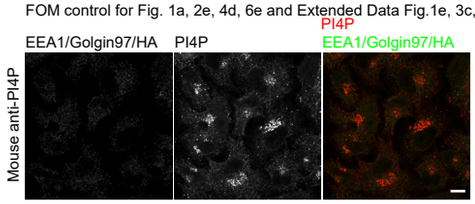

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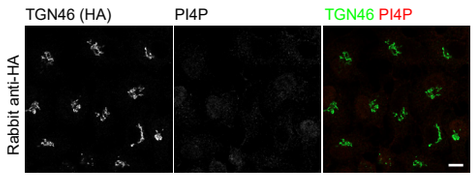
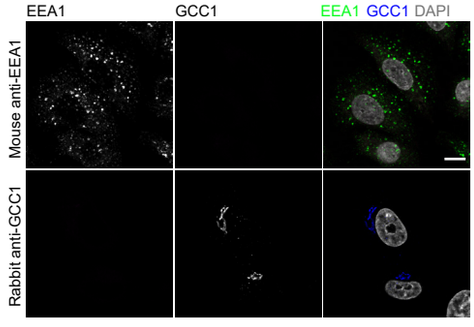
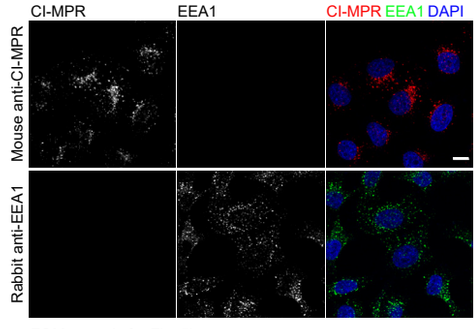


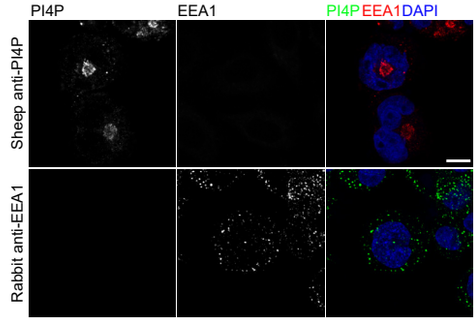
FOM control for Fig. 4d



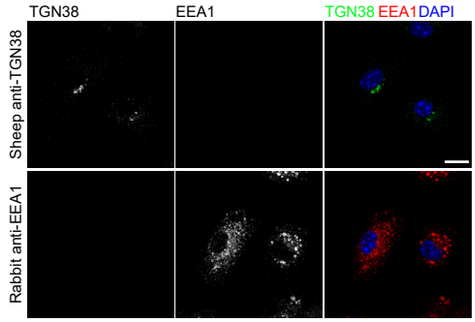
FOM contorls for Fig. 5a and Extended Data Fig. 9a



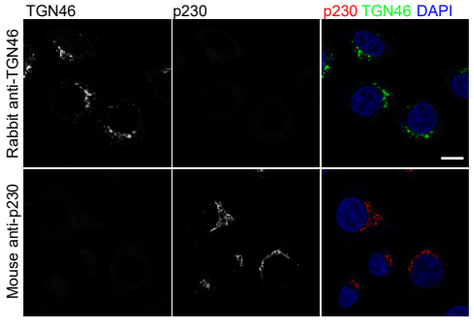
FOM controls for Fig. 5b



FOM controls for Extended Data Fig. 2a, 2b



FOM controls for Extended Data Fig. 7a



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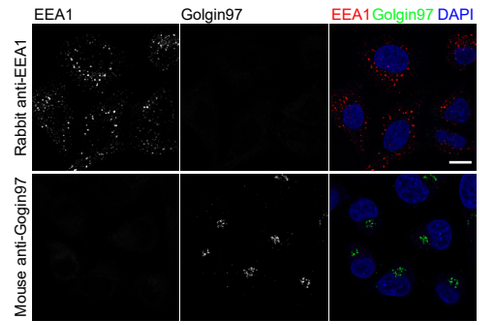

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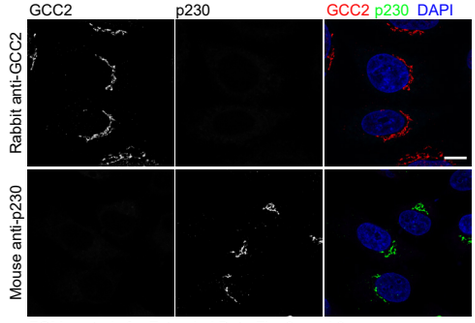
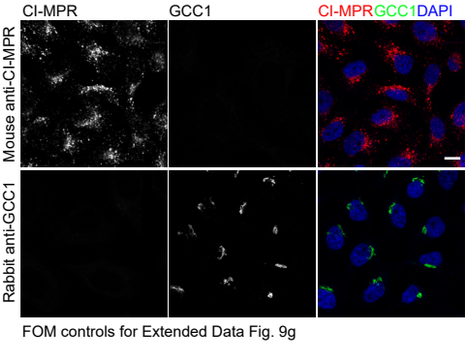


FOM contorls for Extended Data Fig. 8c and 9c



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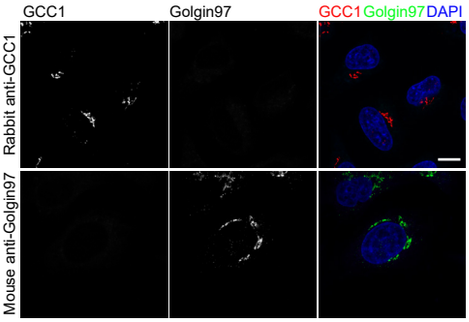

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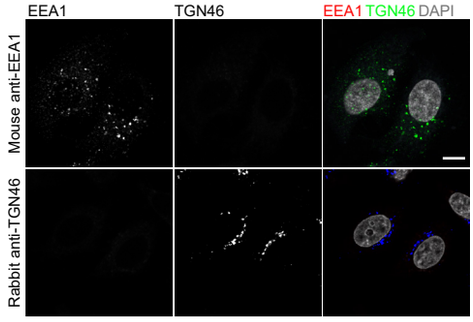

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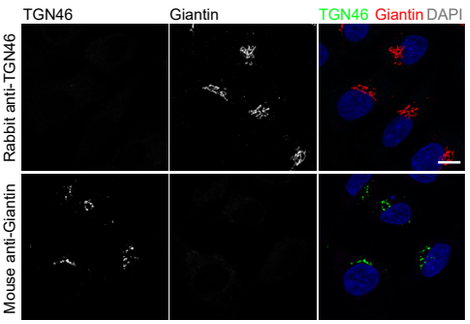

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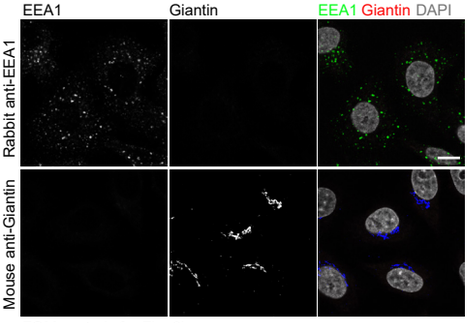
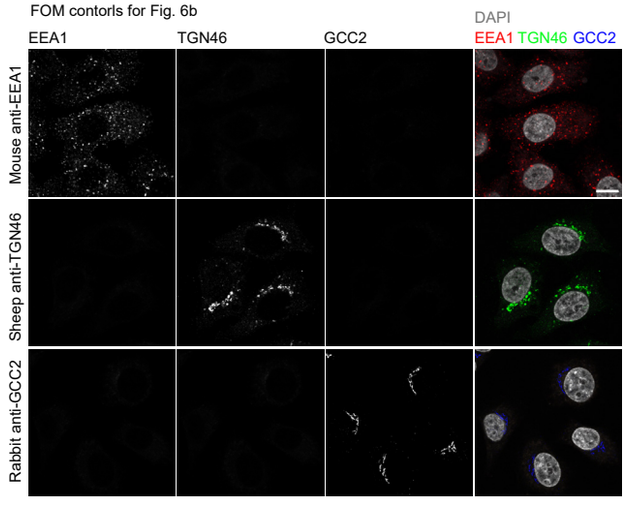
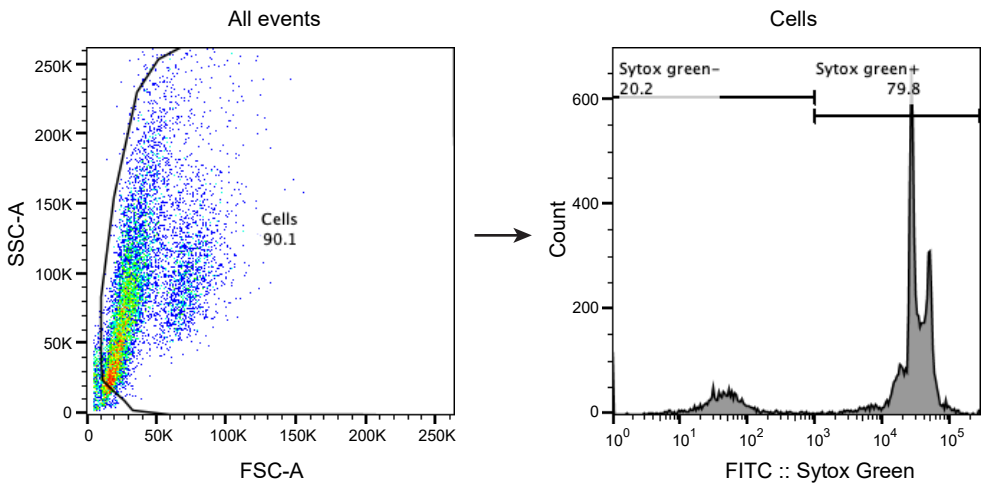


FOM contorls for Fig. 6b

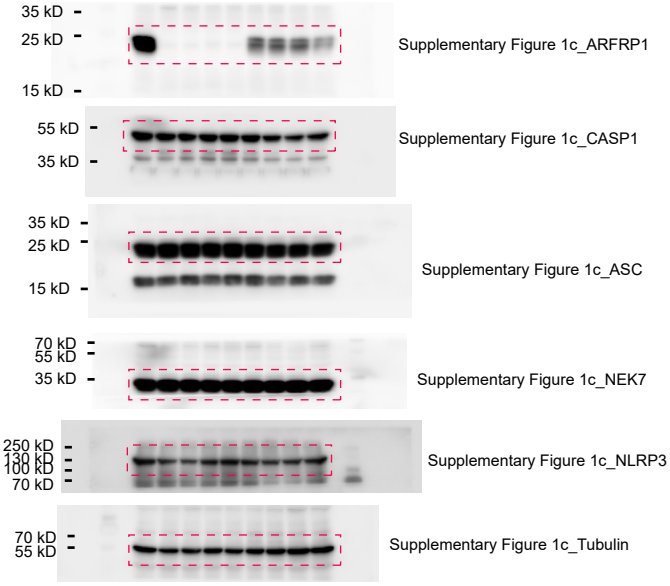


FOM contorls for Extended Data Fig. 7c

Supplementary Figue 4 Fluorescence-minus-one (FMO) controls for all fluorescently labelled antibodies. Cells were co-stained with indicated antibody and control IgG/IgM from the same specie of cognate antibody. Scale bar: 10 μm.



Supplementary Figue 5 The gating strategy of FACS analysis of pyroptotic cell death by Sytox green staining



Unprocessed Western Blots of Supplemenatry Figure 1c

Unprocessed Western Blots of Supplemenatry Figure 2a