NCBI gi no.	Protein Name	Hits
169234614	immune-responsive gene 1 protein	70
260166704	ubiquitin-associated protein 2-like isoform 3	65
160333881	tripartite motif-containing protein 29 / TRIM29	60
11596855	transferrin receptor protein 1	35
21312352	superkiller viralicidic activity 2-like 2	30
6755919	polyubiquitin-B	27
31321959	inhibitor of kappaB kinase gamma / NEMO	25
6678493	ubiquitin carboxyl-terminal hydrolase 10	22
34328268	TGF-beta-activated kinase 1 and MAP3K7-binding protein 1	20
27881429	mitogen-activated protein kinase kinase kinase 7 isoform A	18
20149752	TGF-beta-activated kinase 1 and MAP3K7-binding protein 2	18
38348246	TNF receptor-associated factor 6	15
145587104	X-ray repair cross-complementing protein 6	10
87239996	influenza virus NS1A-binding protein homolog isoform 3	5

Supplementary Table 1 NEMO is identified in the TRIM29-binding protein complex.
MH-S cell lysate was prepared, followed by anti-TRIM29 immunoprecipitation and protein sequencing by liquid chromatography-mass spectrometry. NCBI gi no: unique protein identification number; Hits: the number of peptides ions matched that associated protein.

NEMO Ubiquitination Sites

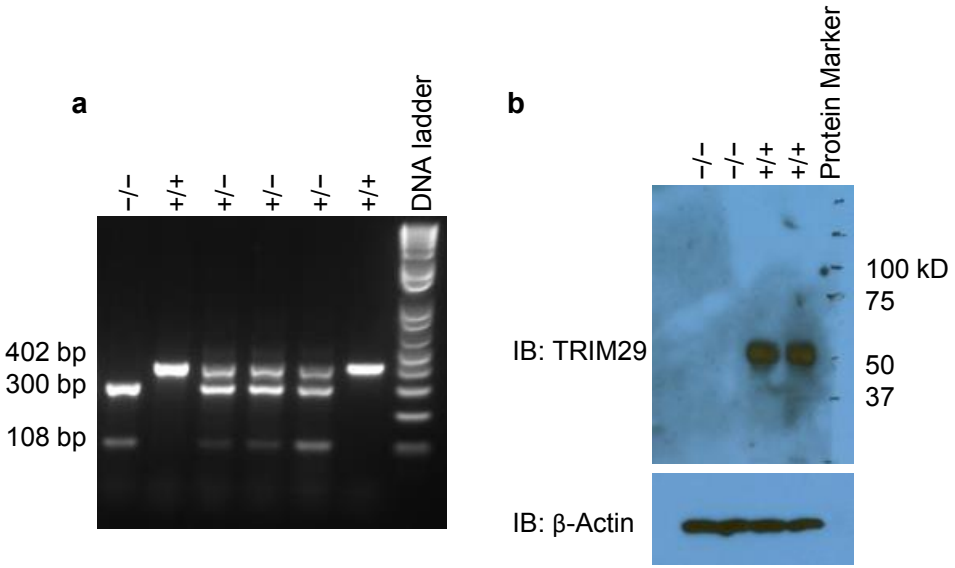
MYIRYCCDDQDTQTLSCWMNKHPWKNQLSEMVQPSGGPAEDQDMLGEESSLGKPAMLHLPSEQGTPETLQRCLEENQ
ELRDAIRQSNQMLRERCEELLHFQVSQREEKEFLMCKFQEARKLVERLSLEKLDLRSQREQALKELEQLKKCQQQMAED
KASVKAQVTSLLGELQESQSRLEAATKDRQALEGRIRAVSEQVRQLESEREVLQQQHSVQVDQLRMQNQSVEAALRMER
QAASEEKRKLAQLQAAYHQLFQDYDSHIKSSKGMQLEDLRQQLQQAEEALVAKQELIDKLKEEAEQHKIVMETVPVLKAQA
DIYKADFQAERHAREKLVEKKEYLQEQLEQLQREFNKLKVGCHESARIEDMRKRHVETPQPPLLPAPAHHSFHLALSNQR
RSPPEEPPDFCCPKCQYQAPDMDTLQIHVMECIE

Residue	Score	Ubiquitinated		Residue	Score	Ubiquitinated	
21	0.51	No		264	0.74	Yes	Medium confidence
25	0.64	Yes	Low confidence	267	0.80	Yes	Medium confidence
53	0.89	Yes	High confidence	288	0.75	Yes	Medium confidence
108	0.81	Yes	Medium confidence	294	0.77	Yes	Medium confidence
114	0.60	No		296	0.69	Yes	Medium confidence
120	0.53	No		303	0.64	Yes	Low confidence
129	0.66	Yes	Low confidence	313	0.67	Yes	Low confidence
141	0.75	Yes	Medium confidence	320	0.58	No	
147	0.64	Yes	Low confidence	332	0.61	No	
148	0.72	Yes	Medium confidence	336	0.65	Yes	Low confidence
157	0.80	Yes	Medium confidence	337	0.60	No	
161	0.88	Yes	High confidence	353	0.40	No	
183	0.99	Yes	High confidence	355	0.55	No	
242	0.67	Yes	Low confidence	369	0.78	Yes	Medium confidence
244	0.80	Yes	Medium confidence	410	0.62	Yes	Low confidence

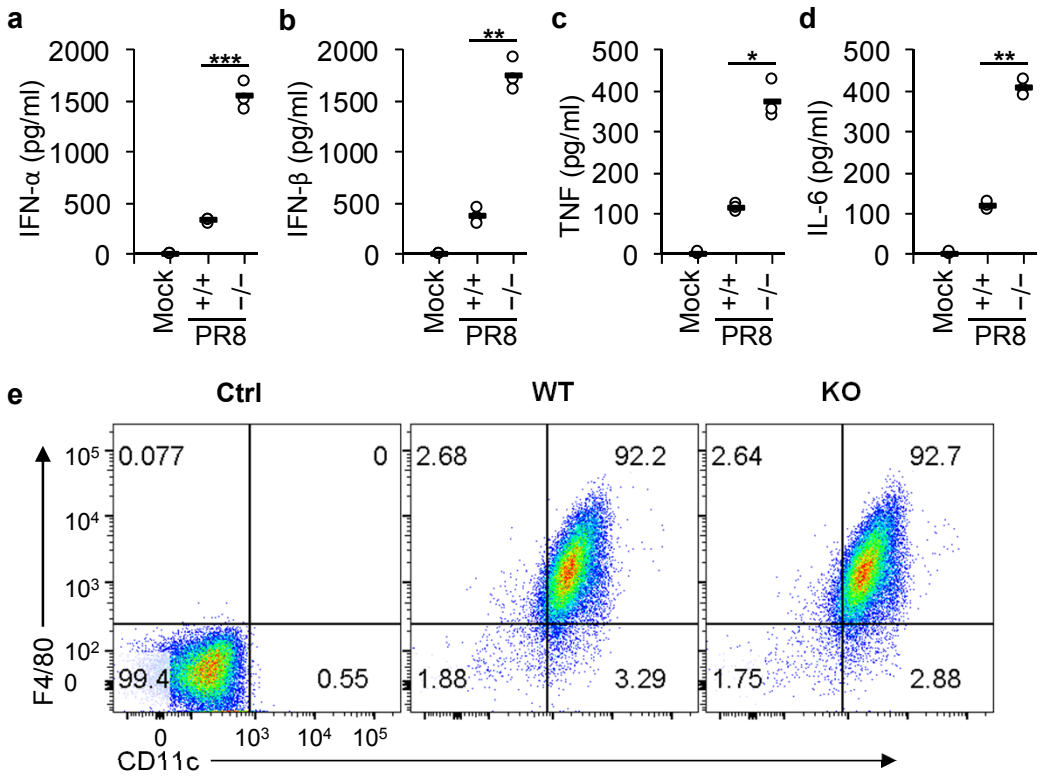
Label	Score range	Sensitivity	Specificity
Low confidence	$0.62 \leq s \leq 0.69$	0.464	0.903
Medium confidence	$0.69 \leq s \leq 0.84$	0.346	0.950
High confidence	$0.84 \leq s \leq 1.00$	0.197	0.989

Supplementary Table 2 The potential ubiquitination sites at the NEMO molecule.

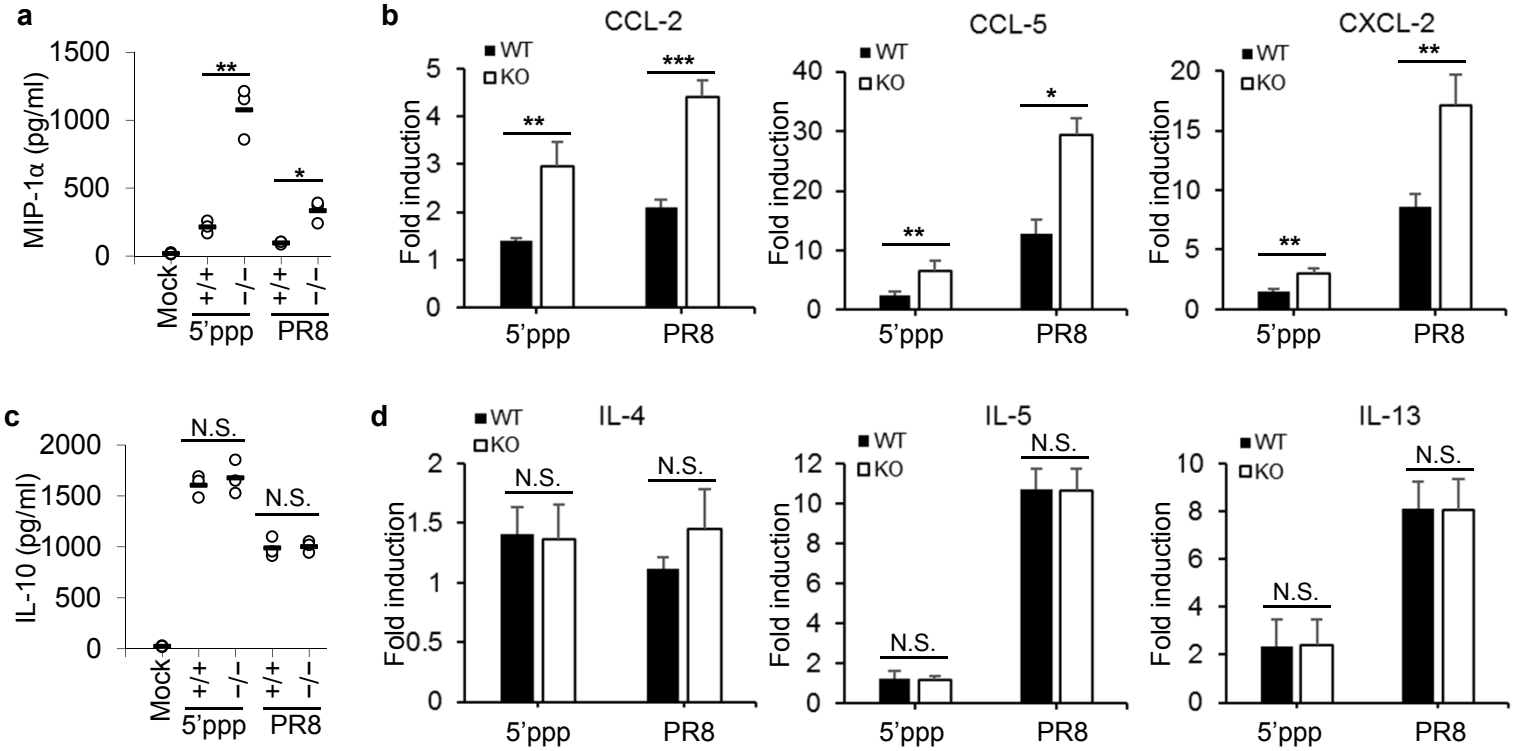
The possibility of ubiquitination of lysine residues in NEMO (middle panel) was predicted from the amino acid sequence of NEMO (top panel) by “UbPred: predictor of protein ubiquitination sites”.



Supplementary Figure 1.
***Trim29* gene targeting strategy.**
(**a**) Genotyping of wildtype mice (+/+), *Trim29* heterozygous mice (+/−) and homozygous mice (−/−). (**b**) Immunoblot analysis of TRIM29 in lung primary AMs from wildtype mice (+/+) or TRIM29 knockout mice (−/−). Data are representative of three independent experiments with similar results.



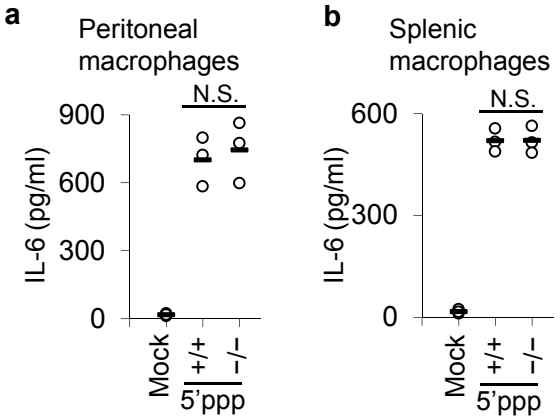
Supplementary Figure 2.
TRIM29 negatively regulates the production of type I IFN and proinflammatory cytokines in primary AMs.
(**a-d**) ELISA of IFN-α (**a**), IFN-β (**b**), TNF (**c**) and IL-6 (**d**) production by primary AMs from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) after 20 h infection with influenza PR8 virus. Virus was used at a multiplicity of infection (MOI) of 5. Individual circle represents the value from each independent experiment; small horizontal lines indicate the average of triplicates. Mock, wildtype cells without infection. (**e**) Flow cytometry analyzing CD11c and F4/80 expression in the primary AMs isolated from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) using isotype control antibodies (Control, Ctrl), CD11c-FITC and F4/80-APC antibodies. Flow cytometry data were acquired on a LSR-II flow cytometer (Beckton Dickinson) and analyzed using FlowJo v10 software (Tree Star). **P*<0.005, ***P*<0.0005, ****P*<0.0001 (unpaired *t* test). Data are representative of three independent experiments with similar results (**a**–**d**) or two experiments (**e**).



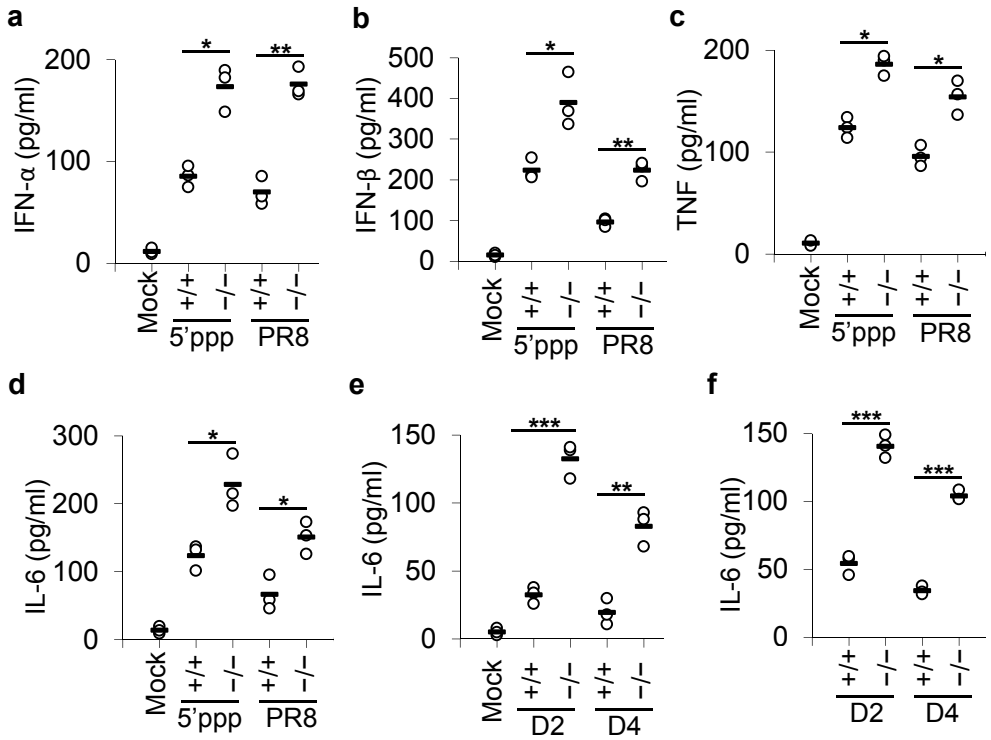
Supplementary Figure 3.

TRIM29 negatively regulates the production of chemokines, but not IL-10 and type II cytokines, in responses to 5'pppRNA and virus infection in primary AMs.

(**a,c**) ELISA of MIP-1α (**a**) and IL-10 (**c**) production by primary AMs from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) after 12 h of stimulation with 5'pppRNA (5'ppp, 1 μg/ml) delivered by Lipofectamine 3000 or infection with influenza PR8 virus. Virus was used at a MOI of 5. Individual circle represents the value from each independent experiment; small horizontal lines indicate the average of triplicates. Mock, wildtype AMs without stimulation or infection. N.S., not significant, **P*<0.005, ***P*<0.0005 (unpaired *t* test). (**b,d**) Quantification of the mRNA expression of chemokines including CCL-2, CCL-5 and CXCL-2 (**b**) and type II cytokines including IL-4, IL-5 and IL-13 (**d**) produced by primary AMs from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) after 12 h of stimulation with 5'pppRNA (5'ppp) or infection with influenza PR8 virus as shown in **a** and **c**. N.S., not significant, *P<0.05, **P<0.01, ***P<0.001 (unpaired *t* test). Data are representative three independent experiments with similar results (mean and s.d. in **b,d**).

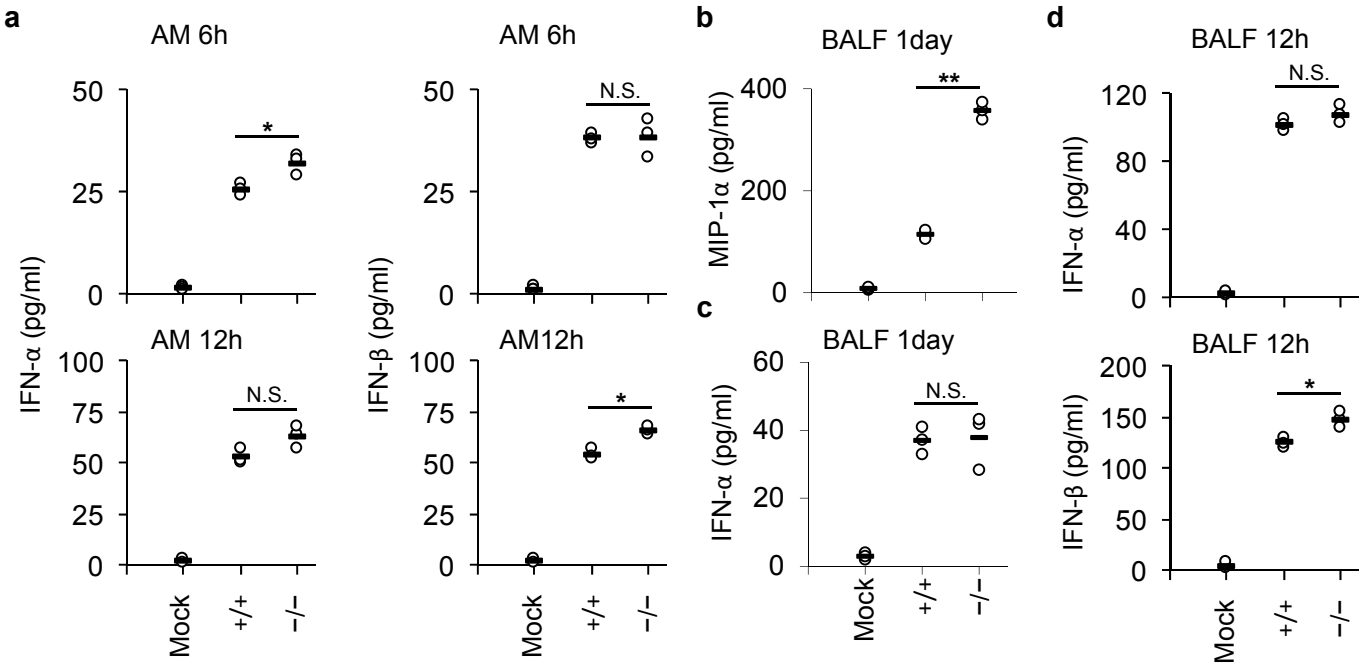


Supplementary Figure 4.
TRIM29 does not affect IL-6 production by peritoneal macrophages and splenic macrophages in response to 5'pppRNA.
(**a,b)** ELISA of IL-6 production in peritoneal macrophages (**a**) or splenic macrophages (**b**) from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) after 12 h of stimulation with 5'pppRNA (5'ppp, 1 μg/ml) delivered by Lipofectamine 3000. Individual circle represents the value from each independent experiment; small horizontal lines indicate the average of triplicates. Mock, wildtype cells without stimulation. N.S., not significant (unpaired *t* test). Data are representative three independent experiments with similar results.

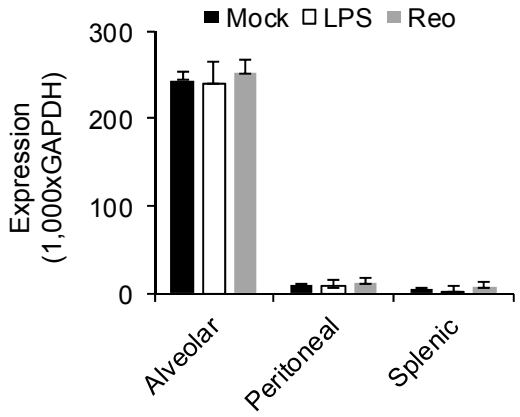


Supplementary Figure 5.
TRIM29 negatively regulates the cytokines production in response to 5'pppRNA and virus infection.

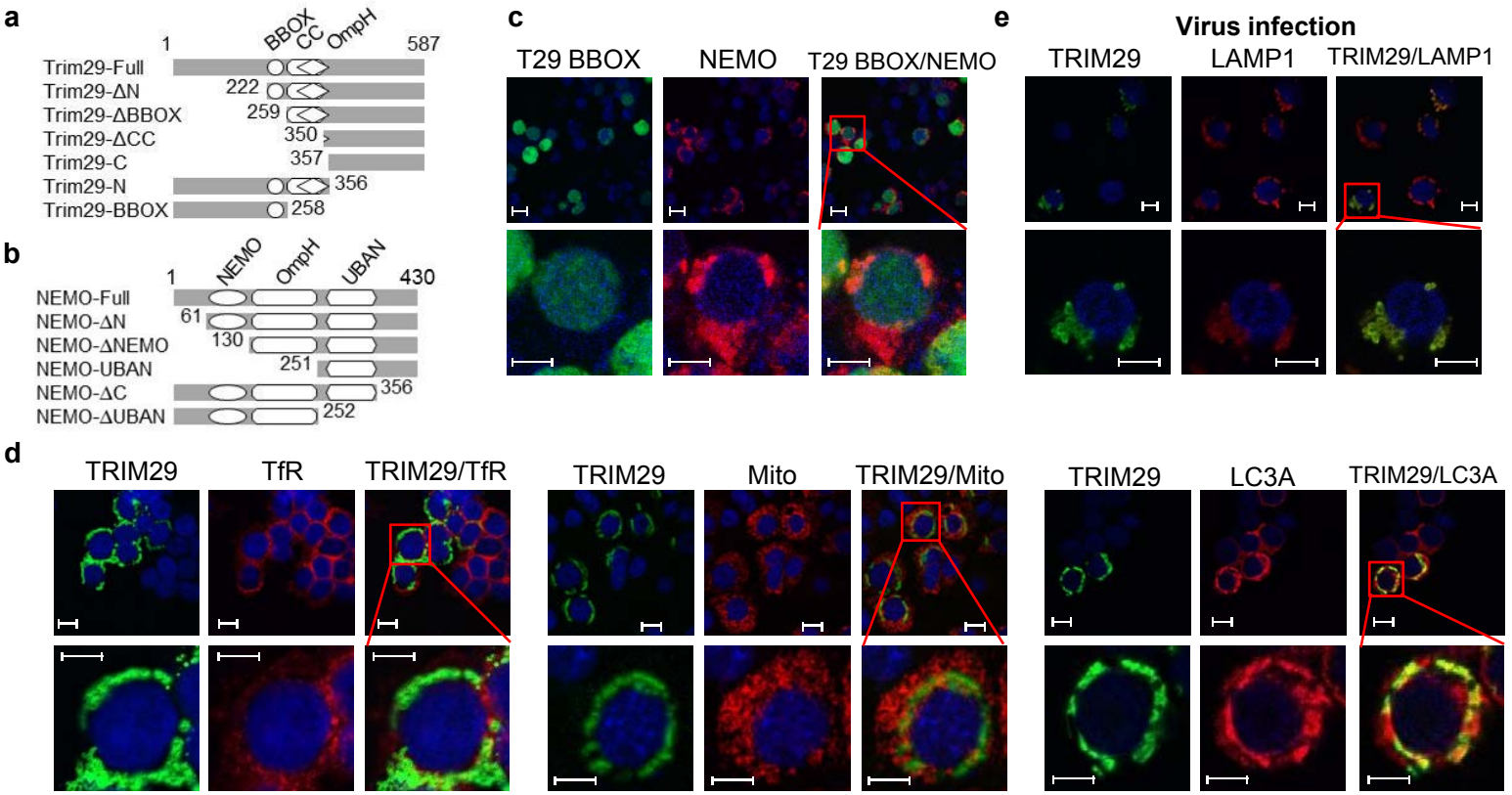
(**a,b**) ELISA of IFN-α (**a**), IFN-β (**b**), TNF (**c**) and IL-6 (**d**) production in primary CD11c$^+$ splenic dendritic cells from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) after 12 h of stimulation with 5'pppRNA (5'ppp, 1 μg/ml) delivered by Lipofectamine 3000 or infection with influenza PR8 virus. Virus was used at a MOI of 5. Mock, wildtype CD11c$^+$ splenic dendritic cells without stimulation or infection. (**e,f**) ELISA of IL-6 in BALF samples from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) at day 2 (D2) or day 4 (D4) of intranasal infection with high dose 1×10^5 PFU (**e**) or low dose 1×10^2 PFU (**f**) of influenza PR8 virus. Mock, wildtype mice without infection. Individual circle represents the value from each independent experiment; small horizontal lines indicate the average of triplicates. **P*<0.05, ***P*<0.01, ****P*<0.001 (unpaired *t* test). Data are representative two independent experiments with similar results.



Supplementary Figure 6.
TRIM29 plays a minimal role in type I IFN production in response to LPS or *H. influenzae* infection.
(**a**) ELISA of IFN-α and IFN-β production in primary AMs from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) after 6 h or 12 h stimulation with LPS (10 ng/ml). Mock, wildtype cells without stimulation. (**b**,**c**) ELISA of MIP-1α (**b**) and IFN-α (**c**) production in BALF samples from wildtype mice (+/+) a nd *Trim29*$^{-/-}$ mice (−/−) at day 1 of intratracheal inoculation with *H. influenzae* infection (1×10^7 CFU per mouse). Mock, wildtype mice without *H. influenzae* infection. (**d**) ELISA of IFN-α and IFN-β production in BALF samples from wildtype mice (+/+) and *Trim29*$^{-/-}$ mice (−/−) at 12 h of intranasal inoculation with LPS. Mock, wildtype mice without LPS challenge. Individual circle represents the value from each independent experiment; small horizontal lines indicate the average of triplicates. N.S., not significant, **P*<0.05, ***P*<0.0001 (unpaired *t* test). Data are representative two independent experiments with similar results.



Supplementary Figure 7.
TRIM29 expression is not affected by LPS or reovirus infection in macrophages.
Quantification of TRIM29 mRNA expression in alveolar macrophages, peritoneal macrophages and splenic macrophages mock treated or treated with LPS (20 ng/ml) or reovirus infection (Reo) for 6h. Virus was used at a MOI of 5. Mock, cells without stimulation. Data are representative two independent experiments with similar results.



Supplementary Figure 8.
TRIM29, but not TRIM29 BBOX, colocalizes with NEMO in the lysosome.
(**a**) Schematic diagram showing full-length TRIM29 and serial truncations of TRIM29 with deletion (Δ) of various domains (left margin). (**b**) Schematic diagram showing full-length NEMO and serial truncations of NEMO with deletion of various domains (left margin); numbers at ends indicate amino acid positions (top). (**c–e**) Confocal microscopy of HEK293T cells co-transfected with HA-TRIM29 or HA-TRIM29 BBOX (T29 BBOX, lacking interaction with NEMO) and Myc-NEMO expression plasmids and mock (**c** and **d**) or infected with influenza PR8 virus for 4 h (**e**). TRIM29 was strained with Alexa Fluor 488–anti-HA (green), MitoTracker was used to probe the mitochondrion (red). LAMP1, TfR and LC3A served as the markers of lysosome, endosome and autophagosome (red). DAPI served as the nuclei marker (blue). Scale bars represent 10 μm for original images and 5 μm for enlarged images. Data are representative of two independent experiments with similar results.