

Supplementary Information for

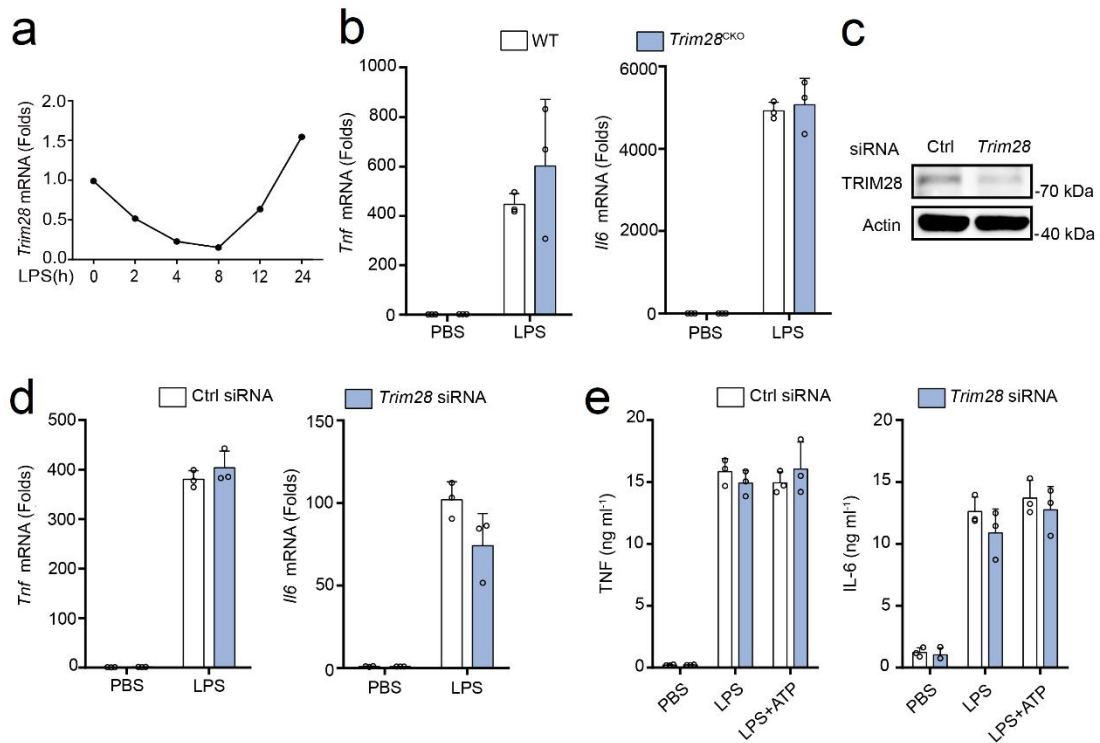
**TRIM28 SUMOylates and stabilizes NLRP3 to facilitate inflammasome activation**

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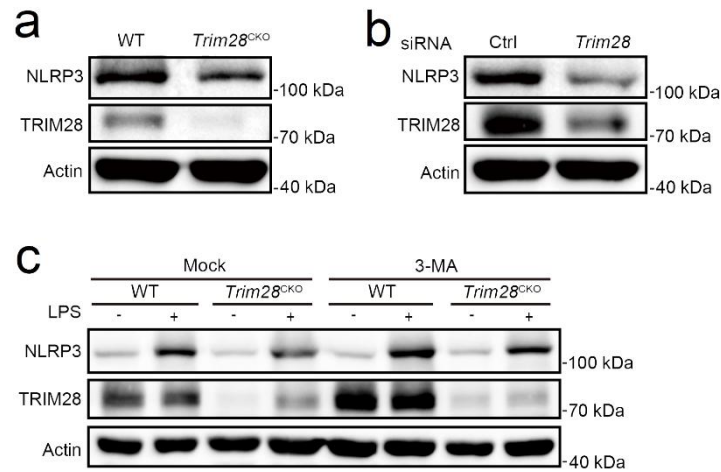
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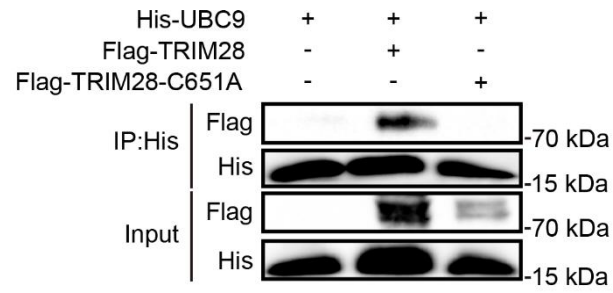
**Supplementary Figure. 1 *Trim28* knockdown has no effects on NLRP3 inflammasome priming.**

(a) RT-PCR analysis of *Trim28* mRNA in LPS-stimulated mouse PMs. (b, d) RT-PCR analysis of *Tnf* and *Il6* mRNA in LPS stimulated mouse PMs from WT or *Trim28*<sup>CKO</sup> mice (b) or PMs transfected with Ctrl siRNA or *Trim28* siRNA (d). (c) Immunoblot analysis of TRIM28 expression in mouse PMs transfected with control (Ctrl) siRNA or *Trim28* siRNA for 48h. (e) ELISA analysis of TNF and IL-6 in supernatants from mouse PMs transfected with Ctrl siRNA or *Trim28* siRNA for 48h, following priming with LPS for 7 h and subsequent stimulation with ATP for 1h. All data are represented as means  $\pm$  SD in b-d. Similar results were obtained from three independent experiments.



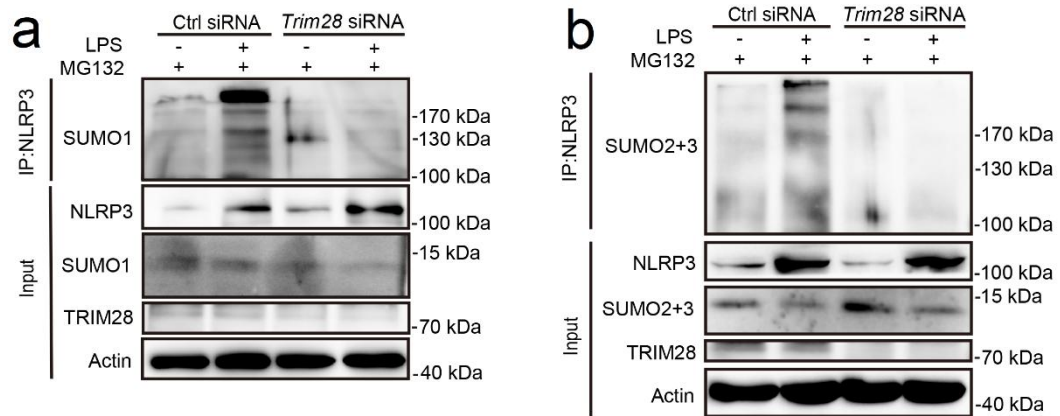
**Supplementary Figure. 2 TRIM28 enhances NLRP3 expression.**

(a, b) Immunoblot analysis of lysates of mouse PMs from WT or *Trim28<sup>CKO</sup>* mice, or PMs transfected with Ctrl siRNA or *Trim28* siRNA for 48h. (c) Immunoblot analysis of NLRP3 expression in mouse PMs from WT or *Trim28<sup>CKO</sup>* mice primed with LPS for 4h and then treated with 3-MA (10 mM) for 40min. Similar results were obtained from three independent experiments.



**Supplementary Figure. 3 TRIM28 interacts with UBC9.**

IP analysis of the interaction between UBC9 and TRIM28 in HEK293T cells transfected with His-UBC9, together with Flag-TRIM28 WT or C651A. Similar results were obtained from three independent experiments.



**Supplementary Figure. 4 *Trim28* knockdown inhibits NLRP3 SUMOylation.**

Immunoblot analysis of lysates from mouse peritoneal macrophages transfected with Ctrl siRNA or *Trim28* siRNAs for 48h, primed with LPS for 4h, together with MG132 (10  $\mu$ M) treatment for 4h, and followed by SUMOylation assays with NLRP3 antibody. Similar results were obtained from three independent experiments.

**Supplementary Table 1 Sequences of PCR primers used in this study**

<b>Name</b>	<b>Prime</b>	<b>Sequence</b>
<i>mActb</i>	Forward	5'-TGTTACCAACTGGGACGACA-3'
	Reverse	5'-CTGGGTCATCTTTTCACGGT-3'
<i>mNlrp3</i>	Forward	5'-TGGATGGGTTTGCTGGGAT-3'
	Reverse	5'-CTGCGTGTAGCGACTGTTGAG-3'
<i>mI1b</i>	Forward	5'-ACCTTCCAGGATGAGGACATGA-3'
	Reverse	5'-AACGTCACACACCAGCAGGTTA-3'
<i>mTnf</i>	Forward	5'-GCCACCACGCTCTTCTGTCT-3'
	Reverse	5'-TGAGGGTCTGGGCCATAGAAC-3'
<i>mI16</i>	Forward	5'-ACAACCACGGCCTTCCCTAC-3'
	Reverse	5'-CATTTCCACGATTTCCAG-3'
<i>mTrim28</i>	Forward	5'-CACACTCACCTGCCGCGA-3'
	Reverse	5'-TCAACCTGCACTCGCTTCTG-3'