Supplemental information

SAM68 directs STING signalling to apoptosis in macrophages

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Short title: STING engages SAM68 to induce apoptosis

Supplementary Table S1

Mass spectrometry dataset on proteins co-immunoprecipitating with STING

Supplementary Table S2

gRNA sequences used in the study

Supplementary Table S3

Antibodies used in the study

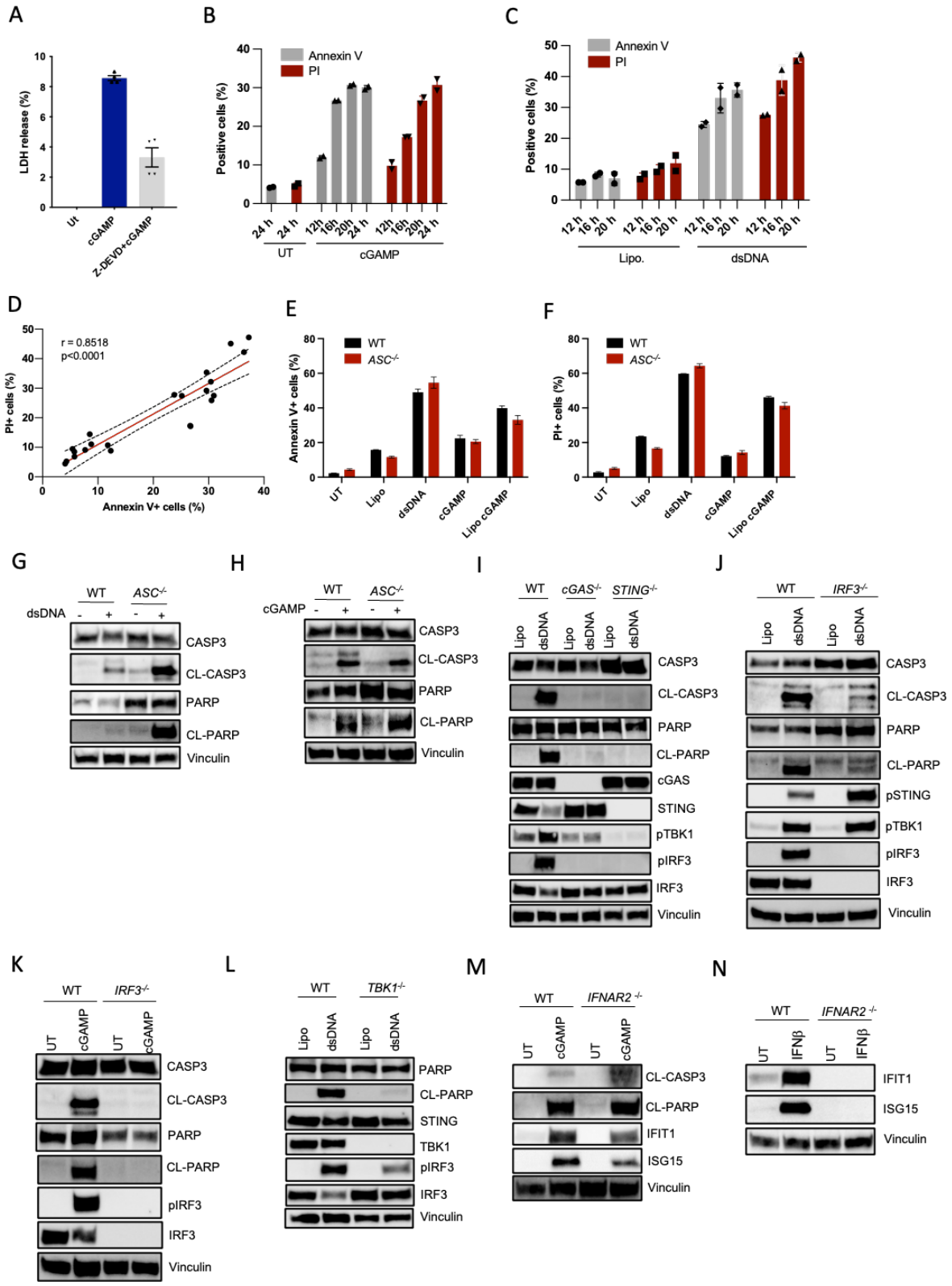

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Figure S1. STING induces cell death through the mitochondrial apoptosis pathway in monocytes.

(**A**) PMA-differentiated THP1 cells (PMA-THP1) were treated with Z-DEVD (25μM) 1 h prior to transfection with cGAMP (100 μg.mL^{-1}). LDH levels in the culture supernatants isolated 8h later were measured by LDH release assay (n=2 in biological duplicates).

(**B-C**) PMA-THP1 cells were treated with dsDNA (4μg.mL^{-1}) and cGAMP (100 μg.mL^{-1}) for the indicated time intervals and evaluated for staining for Annexin V and Propidium Iodide (PI) by flow cytometry. Data are from one representative experiment performed in biological duplicates. Experiment has been repeated twice with a similar trend.

(**D**) Illustration data presented in panel c and a as PI^{+} *versus* Annexin V^{+}.

(**E-F**) WT and *ASC*$^{-/-}$ THP1 cells were treated with dsDNA (4μg.mL^{-1}) or cGAMP (100 μg.mL^{-1}) for 20h, and evaluated for staining for Annexin V and Propidium Iodide (PI) by flow cytometry (n=2).

(**G-H**) WT and *ASC*$^{-/-}$ PMA-THP1 cells were treated with dsDNA (4μg.mL^{-1}) or cGAMP (100 μg.mL^{-1}) for 5h, and lysates were immunoblotted for Cleaved (CL) caspase 3, total caspase 3, cleaved PARP, total PARP and vinculin.

(I-M) WT*, cGAS*$^{-/-}$*, STING*$^{-/-}$*, IRF3*$^{-/-}$*, TBK1*$^{-/-}$, and *IFNAR2*$^{-/-}$ PMA-THP1 cells were treated with dsDNA (4μg.mL^{-1}) or cGAMP (100 μg.mL^{-1}) for 5h, and lysates were immunoblotted for the indicated protein (n=more than 3 for most of the KO cell lines tested). CL, cleaved. pSTING, phosphorylation at S366; pTBK1, phosphorylation as S172; pIRF3, phosphorylation at S396.

(**N**) WT and *IFNAR2*$^{-/-}$ PMA-THP1 cells were treated with IFNβ (100 units/mL) for 1h, and lysates were immunoblotted for the indicated protein (n=2).

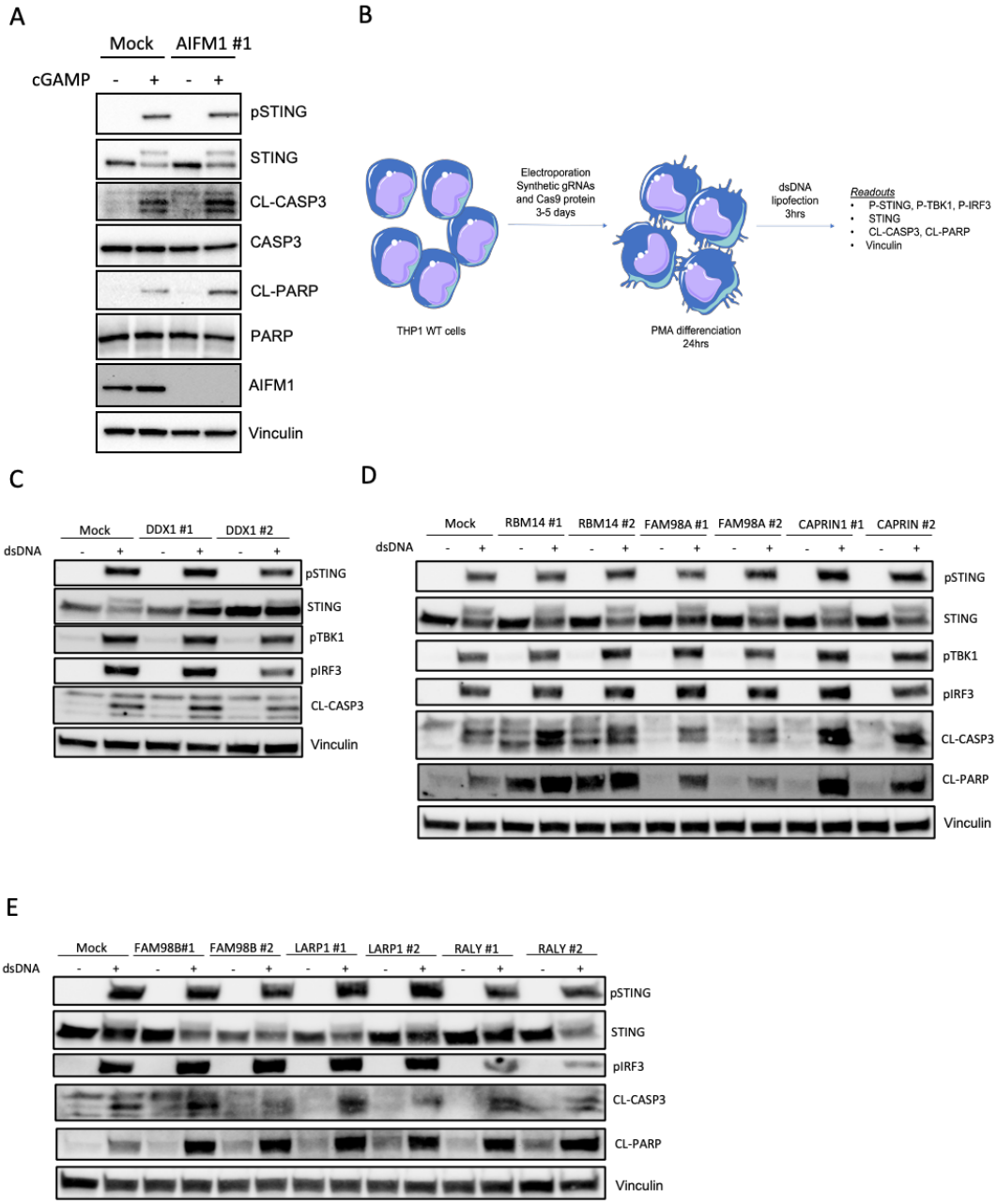

Figure S2. SAM68/KHDRBS1 is essential for induction of apoptosis downstream of STING

(A) THP1 cells were treated with gRNA/Cas9 RNP complexes targeting the iAIFM1 gene, and differentiated into PMA-THP1 macrophages. The cells were treated with cGAMP (100µg.mL^{-1}) for 5h and lysates were immunoblotted as shown (n=2). CL, cleaved. pSTING, phosphorylation at S366.

(**B**) Set-up for analysis of identified candidates.

(**C-E**) THP1 cells were treated with gRNA/Cas9 RNP complexes targeting the indicated genes (two gRNAs per gene), and differentiated into PMA-THP1 macrophages. The cells were treated with dsDNA (4µg.mL^{-1}) for 5h and lysates were immunoblotted as shown (n=2). CL, cleaved. pSTING, phosphorylation at S366; pTBK1, phosphorylation at S172; pIRF3, phosphorylation at S396.

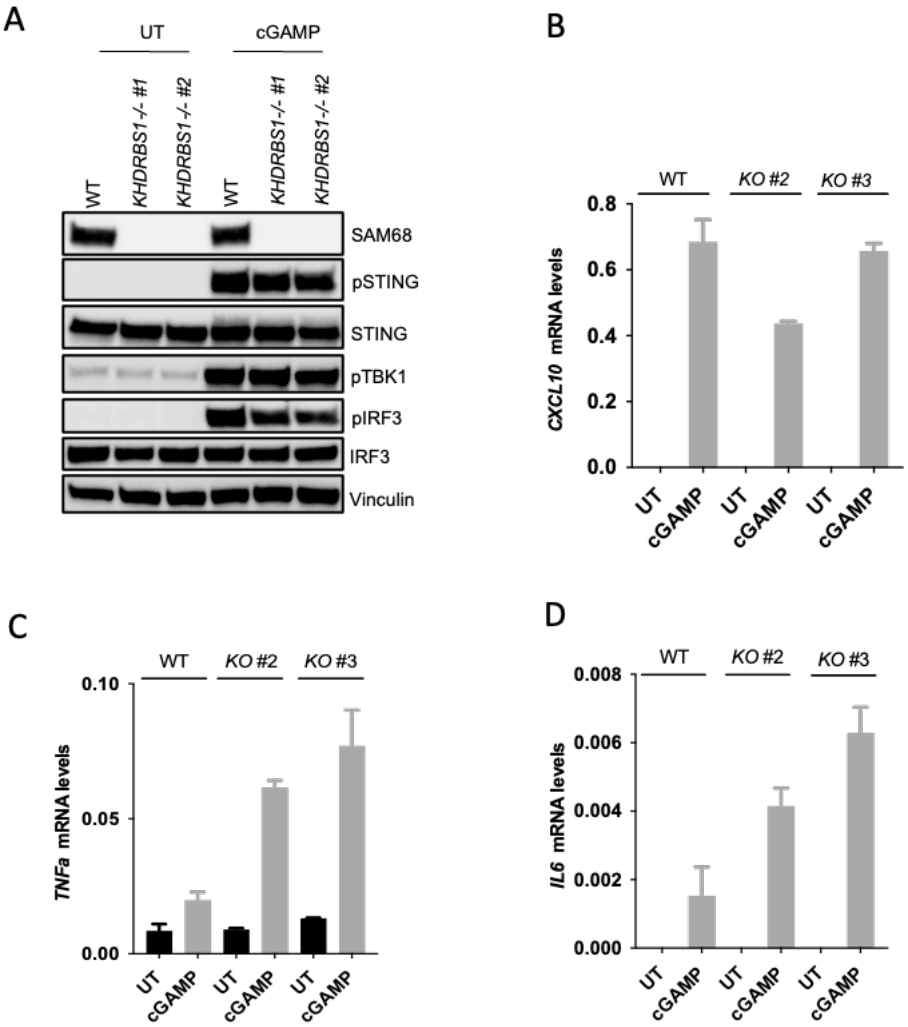

Figure S3. SAM68 is not involved in STING-mediated activation of the IFN and NF-κB responses.

(**A**) WT and *KHDRBS1*$^{-/-}$ PMA-THP1 cells were treated with cGAMP (100µg.mL^{-1}) for 5h, and lysates were immunoblotted for the indicated proteins (n=3). pSTING, phosphorylation at S366; pTBK1, phosphorylation as S172; pIRF3, phosphorylation at S396.

(**B-D**) WT and *KHDRBS1*$^{-/-}$ PMA-THP1 cells were treated with cGAMP (100µg.mL^{-1}) for 5h. RNA was isolated and examined for levels of *CXCL10*, *TNFA*, and *IL6*. Data are means for one experiment performed in biological duplicates.

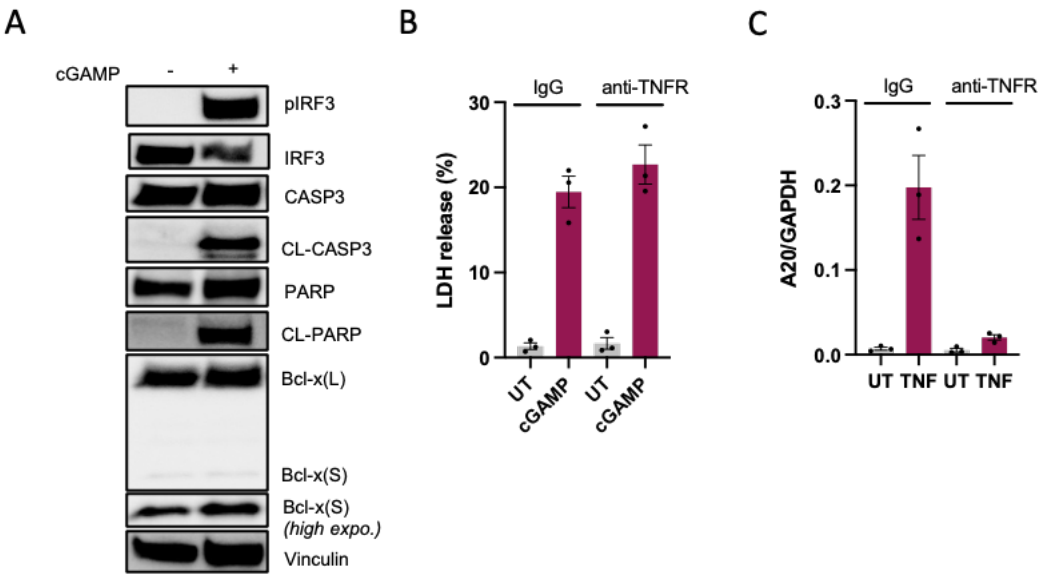

Figure S4. SAM68 does not promote STING-dependent apoptosis through known mechanisms

(**A**) PMA-THP1 cells were treated with cGAMP (100µg.mL^{-1}) for 5h, and lysates were immunoblotted for the indicated proteins (n=2). CL, cleaved. pIRF3, phosphorylation at S396.

(**B-C**) PMA-THP1 cells were treated with neutralizing Rabbit anti-human TNFα IgG or control Rabbit IgG (10 µg/ml), 30 min before stimulation with cGAMP (100µg.mL^{-1}) or TNFα (20 ng/ml). Culture supernatants were collected 16h after cGAMP stimulation, and total RNA was isolated 4h after TNFα stimulation and LDH release and A20 mRNA levels were measured, respectively (n=2).