Supplementary Information

Regulation of STING activation and innate immune responses by IRF8 independent of its transcriptional role in monocytic cells

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Supplementary Figures 1 to 6 Supplementary Table 1 to 4



Supplementary Figure 1. Irf8-deficiency does not inhibit SeV-triggered signaling in monocytes.

a, Gene Set Enrichment Analysis (GSEA) enrichment score curves of cytosolic DNA-sensing pathway in WT vs. *Irf8*^{-/-} BMDMs.

b, Immunoblotting analysis of the indicated proteins in WT, $Irf8^{-/-}$ or $Irf3^{-/-}$ BMDMs uninfected or infected with SeV for the indicated times.

c, Immunoblot analysis of the indicated proteins in WT and *Irf8*^{-/-} BMDCs un-infected or infected with HSV-1(left) or SeV (right) for the indicated times.

d, Immunoblotting analysis of the indicated proteins in WT and $Irf8^{-/-}$ BMDMs un-treated or treated with cGAMP for the indicated times.

Source data are provided as a Source data file.

Supplementary Figure 2



Supplementary Figure 2. Irf8 regulates Sting-mediated innate immune responses independent of its role in monocytic cell differentiation.

a, qPCR analysis of *Ifnb1*, *Il6*, *Ifit1* and *Cxcl10* mRNA levels in Flt3L-BMDCs infected with HSV-1 or treated with DMXAA (50 μg/mL) for the indicated times.

b, Gating strategy to sort pDC (Bst2⁻B220⁺), cDC1 (Bst2⁻B220⁻CD11c⁺ MHCII⁺ CD24⁺

CD172a⁻) and cDC2 (Bst2⁻ B220⁻ CD11c⁺ MHCII⁺ CD172a⁺) cells from C57BL/6 WT mice.

c, qPCR analysis of Ifnb1, II6, Ifit1 and Cxcl10 mRNA levels in the indicated cells infected with HSV-1 or treated with DMXAA (50 μ g/mL) for the indicated times.

d, ELISA analysis of Ifn- β and IP-10 secretion in the indicated cells infected with HSV-1 or treated with DMXAA (50 μ g/mL) for 6 hours.

e, qPCR analysis of *Irf8* mRNA levels in the indicated cells.

Data in a, c-e are presented as mean, n = 2 biological replicates. Data were analyzed by unpaired two-tailed Student' s t-test. Source data are provided as a Source data file.



Supplementary Figure 3. Irf8 acts at the Sting level.

a, Immunoblot analysis of the expression of cGas in WT and $Irf8^{-/-}$ BMDMs un-infected or infected with HSV-1 for the indicated times.

b, HSV-1 infection induces complex formation of Irf8, Sting and Tbk1. BMDMs were left uninfected or infected with HSV-1 for 4 hours. Cell lysates were then fractionated by gel filtration chromatography, and the individual fractions were analyzed by immunoblotting with the indicated antibodies. Fraction sizes were calibrated with the gel filtration standard (Bio-Rad 151-1901).

Source data are provided as a Source data file.

Supplementary Figure 4



Supplementary Figure 4. IRF8 exists in an auto-inhibitory status.

a, Effects of IRF8 on auto-inhibition of STING. HEK293T cells were transfected with the indicated plasmids for 24 hours before co-immunoprecipitation and immunoblotting analysis with the indicated antibodies.

b, Domain mapping of IRF8 and STING interaction. HEK293 cells were transfected with the indicated truncations before coimmunoprecipitation and immunoblotting analysis with the indicated antibodies. The results were schematically presented (top panel).

c, Interaction of IRF8 IAD with its N- and C-terminal domains. HEK293 cells were transfected with the indicated plasmids for 24 hours before coimmunoprecipitation and immunoblotting analysis with the indicated antibodies.

d, Effects of N- and C-terminus of IRF8 on its association with STING. HEK293T cells were transfected with the indicated plasmids for 24 hours before coimmunoprecipitation and immunoblotting analysis with the indicated antibodies.

Source data are provided as a Source data file.



Supplementary Figure 5. The IAD of Irf8 is necessary for Sting-mediated signaling.

a and **b**, qPCR analysis of Ifn- β and IP10 mRNA levels (**a**) or ELISA analysis of Ifn- β and IP-10 secretions (**b**) in wild-type and *Irf8*^{-/-} BMDMs reconstituted with the indicated plasmids and infected with HSV-1 for 4 hours (**a**) or the indicated times

c, Reporter assays for *IFNB1* promoter activity in HEK293 cells transfected with the indicated plasmids for 24 hours and then treated with 2'3'-cGAMP for 10 hours. EV, empty vector. Data are presented as mean, (a-b) n = 2 biological replicates, (c) n = 2 technical replicates. Data were analyzed by unpaired two-tailed Student's t-test. Source data are provided as a Source data file.

Supplementary Figure 6



Supplementary Figure 6. IRF8 is involved in DNA damage-induced senescence and tumorigenesis.

a, SA- β -Gal assays for WT and *Irf8^{-/-}* BMDMs treated with hydroxyurea (HU, 10 mM) or mitomycin C (MMC, 1 μ M) for 5 days. β -galactosidase staining positive cells (blue) in at least three randomly selected fields were counted under a microscope (right). Scale bars, 200 μ m. Graph shows mean \pm SEM, n=3 independent samples.

b, qPCR analysis of the SASP genes in WT and *Irf* $8^{-/-}$ BMDMs treated with hydroxyurea (HU, 10 mM) or mitomycin C (MMC, 1 μ M) for 6 days. NT, untreated.

c, qPCR analysis of *ll1b* and *ll6* mRNA levels in wild-type and *Irf8*^{-/-} BMDMs reconstituted with the indicated plasmids and treated with mitomycin C (MMC, 1 μ M) for 6 days.

d, IRF8 and STING levels were correlated with beneficial prognosis of cancer patients. Kaplan– Meier survival analysis of IRF8 (top) and STING/TMEM173 (low) in the indicated patients with lung adenocarcinoma, liver cancer or sarcoma. (<u>http://kmplot.com/analysis/</u>).

Data in b-c are presented as mean, n = 2 biological replicates. Data were analyzed by unpaired two-tailed Student' s t-test. Source data are provided as a Source data file.

Supplementary Table 1. A list of DNA oligonucleotides.

VACV70	5'-CCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGAAGAGA
	GAAAGAGATAAAACTTTTTTACGACT-3'
HSV120	5'-AGACGGTATATTTTTGCGTTATCACTGTCCCGGATTGGACACGG
	TCTTGTGGGATAGGCATGCCCAGAAGGCATATTGGGTTAACCCCTT
	TTTATTTGTGGCGGGTTTTTTGGAGGACTT-3'
DNA90	5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTAC
	ATACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACA
	-3'
ISD:	5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTAC
	A-3'.

Antibody Supplier Catalog No. Appl.* Usage Mouse Flag M2 antibody Sigma-Aldrich #F3165 Lot: WB/IP 1:2000/1 μ g Mouse HA. 11 Epitope Tag BioLegend #901515 WB/IF/IP 1:2000/1:200/ antibody clone 16B12 Lot: B29401 1 μ g Mouse Myc-Tag antibody Sigma-Aldrich #A2276S WB 1:2000 clone 9B11 Lot: 24					
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Rabbit phosphor-IKK α CST 2078S WB 1:1000 (S176)/ β (S177) antibody Lot: 9 Lot: 9 1:1000 Rabbit LC3B antibody Abcam ab192890 Lot: WB 1:1000 GR-3338049-25 GR-3338049-25 1:1000 Rabbit STAT1 antibody(E- Santa Cruz SC-346 WB 1:1000 23) Lot: #K1115 Lot: #K1115 1:1000 Rabbit IRF3 antibody Proteintech 11312-1-AP WB 1:1000 Lot: 00082872 Lot: 00082872 Lot: 00082872 Lot: 00082872	Rabbit IKK β antibody	CST	8943S Lot: 4	WB	1:1000
$\begin{array}{c c} (S176)/\beta (S177) \text{ antibody} & \text{Lot: 9} \\ \hline \text{Rabbit LC3B antibody} & \text{Abcam} & ab192890 \text{ Lot: } WB & 1:1000 \\ \hline \text{GR-3338049-25} \\ \hline \text{Rabbit STAT1 antibody(E-} & \text{Santa Cruz} & \text{SC-346} & WB & 1:1000 \\ \hline \text{Lot: } \#\text{K1115} \\ \hline \text{Rabbit IRF3 antibody} & \text{Proteintech} & 11312-1-\text{AP} & WB & 1:1000 \\ \hline \text{Lot: } 00082872 \\ \hline \end{array}$	Rabbit phosphor-IKKα	CST	2078S	WB	1:1000
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GR-3338049-25Rabbit STAT1 antibody(E- 23)Santa Cruz Lot: #K1115WB1:1000Rabbit IRF3 antibodyProteintech11312-1-AP Lot:00082872WB1:1000	Rabbit LC3B antibody	Abcam	ab192890 Lot:	WB	1:1000
Rabbit STATT antibody(E- 23)Santa Cruz Lot: #K1115SC-346 Lot: #K1115WB1:1000Rabbit IRF3 antibodyProteintech11312-1-AP Lot:00082872WB1:1000			<u>GR-3338049-25</u>	N/D	1 1000
23)Lot: #K1115Rabbit IRF3 antibodyProteintech11312-1-APWB1:1000Lot:00082872Lot:00082872Lot:00082872Lot:00082872	Rabbit STATT antibody(E-	Santa Cruz	SC-346	wВ	1:1000
Lot:00082872	<u>23)</u> Babbit IDE2 antibada	Duataintaah	LOT: #K1113	WD	1.1000
	Raubit IRF5 antidouy	riotenneen	Lot:00082872	VV D	1.1000

Supplementary Table 2. A list of primary antibodies used in the study.

Supplementary Table 3. A list of gRNA sequences.

Human IRF8-gRNA-1	5'-GTACAGTGCGACAGCTAGAAT-3'
Human IRF8-gRNA-2	5'-GTGACCGGAATGGTGGTCGG-3'

Supplementary Table 4. A list of qPCR sequences.

Actin Forward	5'-CATTGCTGACAGGATGCAGAAGG-3'
Actin Reverse	5'-TGCTGGAAGGTGGACAGTGAGG-3'
Ifnb1 Forward	5'-TCCTGCTGTGCTTCTCCACCACA-3'
Ifnb1 Reverse	5'-AAGTCCGCCCTGTAGGTGAGGTT-3'
Cxcl10 Forward	5'-ATCATCCCTGCGAGCCTATCCT-3'
Cxcl10 Reverse	5'-GACCTTTTTTGGCTAAACGCTTTC-3'
<i>Il1b</i> Forward	5'-ACGGACCCCAAAAGATGAAG-3'
<i>Il1b</i> Reverse	5'-TTCTCCACAGCCACAATGAG-3'
<i>Il8</i> Forward	5'-GTCCTTAACCTAGGCATCTTCG-3'
118 Reverse	5'-TCTGTTGCAGTAAATGGTCTCG-3'
<i>116</i> Forward	5'-TCTGCAAGAGACTTCCATCCAGTTGC-3'
116 Reverse	5'-AGCCTCCGACTTGTGAAGTGGT-3'
p21waf Forward	5'-CAGATCCACAGCGATATCCAG-3'
p21waf Reverse	5'-AGAGACAACGGCACACTTTG-3'
GAPDH Forward	5'-GACAAGCTTCCCGTTCTCAG-3'
GAPDH Reverse	5'-GAGTCAACGGATTTGGTGGT-3'
IFNB1 Forward	5'-TTGTTGAGAACCTCCTGGCT-3'
IFNB1 Reverse	5'-TGACTATGGTCCAGGCACAG-3'
IFIT1 Forward	5'-TCATCAGGTCAAGGATAGTC-3'
IFIT1 Reverse	5'-CCACACTGTATTTGGTGTCTACG-3'
CXCL10 Forward	5'-GGTGAGAAGAGATGTCTGAATCC-3'
CXCL10 Reverse	5'-GTCCATCCTTGGAAGCACTGCA-3'
Il12b Forward	5'-ATGGCCATGTGGGAGCTGGAG-3'
<i>Il12b</i> Reverse	5'-TTTGGTGCTTCACACTTCAGG-3'