SUPPLEMENTAL MATERIAL

Warner et al., https://doi.org/10.1084/jem.20171351

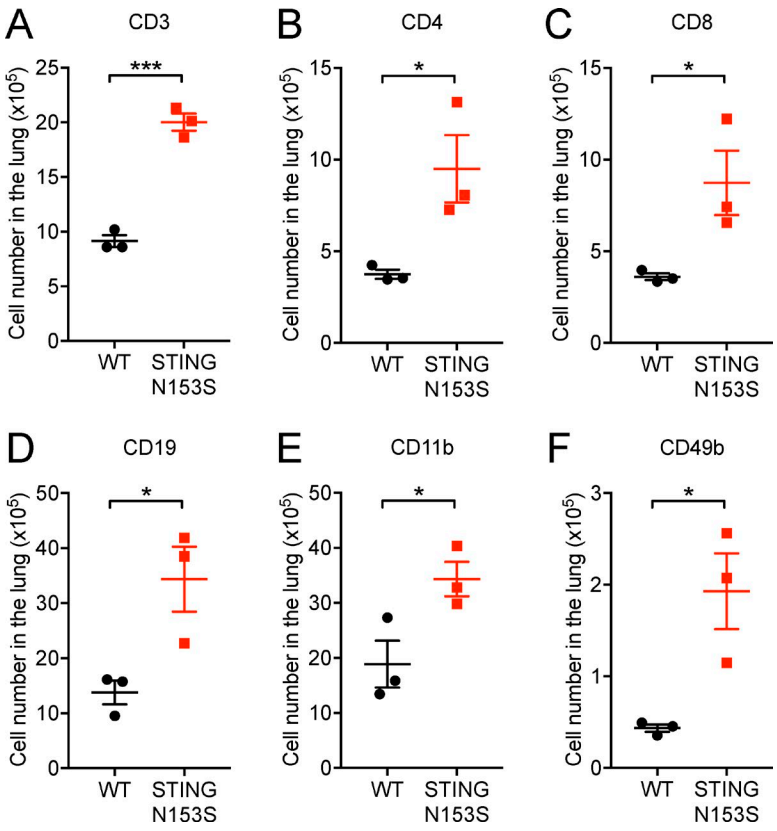

Figure S1. **Pulmonary immune cell analysis of WT and STING N153S mice.** (A–F) Flow cytometry analysis of immune cells isolated from the lungs of WT and STING N153S mice. Total numbers were determined for the following immune cell subsets: $CD3^+$ (A), $CD4^+$ (B), $CD8^+$ (C), $CD19^+$ (D), $CD11b^+$ (E), and $CD49b^+$ populations (F). Data represent the mean ± SEM of $n = 3$ mice per group. *, $P < 0.05$, ***, $P < 0.0005$ by unpaired t test.

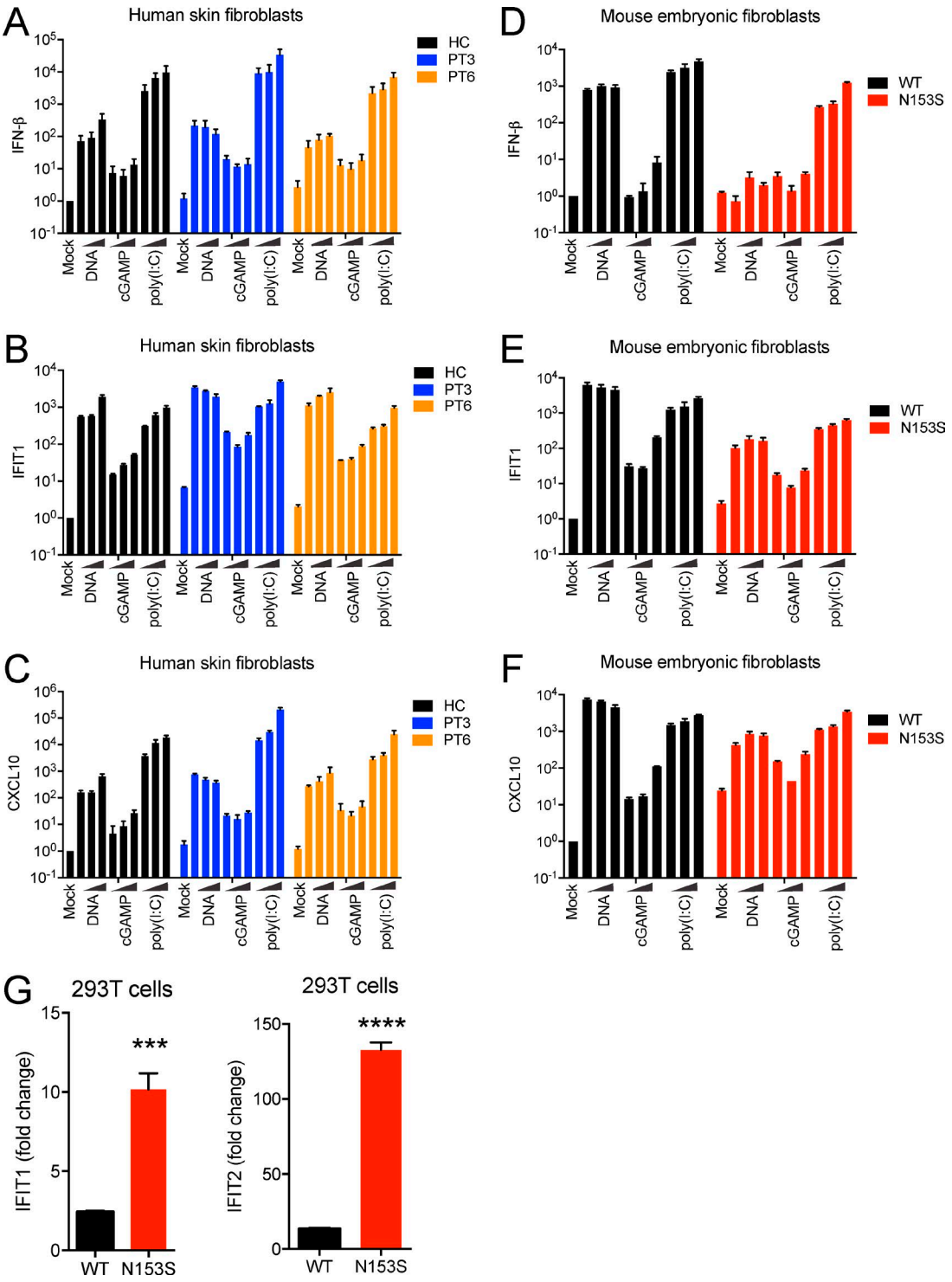

Figure S2. **IFN-β and ISG expression in primary STING N154S patient skin fibroblasts, STING N153S MEFs, and transfected 293T cells.** (A–F) Cells were stimulated with increasing amounts of 2′3′-cGAMP (2, 4, and 8 µg), herring testes DNA (htDNA; 0.1, 0.5, and 1 µg) and polyinosinic–polycytidylic acid (poly I:C; 0.1, 0.5, and 1 µg) for 6 h. There was no significant difference cGAMP-transfected WT and STING N153S MEFs or between healthy control and STING N154S skin fibroblasts. Data were analyzed by ANOVA. (G) ISG expression in 293T cells 24 h after overexpression of WT mouse or mutant STING N153S as previously described (Dobbs et al., 2015). ***, $P < 0005$; ****, $P < 0.0001$ by *t* test. Stimulations were performed in biological triplicates, and qRT-PCR was performed in technical triplicates. Data represent the mean ± SEM.

Table S1. **Antibodies used in CyTOF**

Tag	Target	Clone	Manufacturer	Catalog #
089Y	CD45	30-F11	Fluidigm	3089005B
141Pr	pSHP2	D66F10	Fluidigm	3141002A
142Nd	Caspase 3 (Cleaved)	D3E9	Fluidigm	3142004A
143Nd	TCRβ	H57-597	Fluidigm	3143010B
145Nd	CD4	RM4-5	Fluidigm	3145002B
146Nd	CD8α	53-6.7	Fluidigm	3146003B
147Sm	pSTAT5	47	Fluidigm	3147012A
148Nd	CD11B (Mac-1)	M1/70	Fluidigm	3148003B
149Sm	p4E-BP1	236B4	Fluidigm	3149005A
150Nd	CD25	3C7	Fluidigm	3150002B
151Eu	Ly-6G	1A8	Fluidigm	3151010B
153Eu	pSTAT1	58D6	Fluidigm	3153003A
154Sm	TER119	TER-119	Fluidigm	3154005B
155Gd	pTBK1/NAK	D52C2	CST	5483BF
158Gd	pSTAT3	4/P-Stat3	Fluidigm	3158005A
159Tb	TCRγδ	GL3	Fluidigm	3159012B
160Gd	CD45R (B220)	RA3-6B2	Fluidigm	3160012B
161Dy	pBAD	40A9	Fluidigm	3161006A
164Dy	IκBα	L35A5	Fluidigm	3164004A
165Ho	CD3ε	145-2C11	Fluidigm	3165020B
166Er	CD19	6D5	Fluidigm	3166015B
168Er	Ki67	B56	Fluidigm	3168007B
170Er	NK1.1	PK136	Fluidigm	3170002B
171Yb	CD44	IM7	Fluidigm	3171003B
172Yb	pS6	N7-548	Fluidigm	3172008A
173Yb	CD117 (c-kit)	2B8	Fluidigm	3173004B
174Yb	MHCII	M5/114.15.2	Fluidigm	3174003B
176Yb	pCREB	87G3	Fluidigm	3176005A

Table S2. **Definition of populations used in CyTOF analysis**

Cell populations	Marker expression
Fig. 5	
CD4 (CD4 T cells)	$CD45^+$ $TER119^-$ $CD3^+$ $CD4^+$ $TCR\beta^+$
CD8 (CD8 T cells)	$CD45^+$ $TER119^-$ $CD3^+$ $CD8^+$ $TCR\beta^+$
NK (NK cells)	$CD45^+$ $TER119^-$ $NK1.1^+$ $CD3^-$
CD19 (B cells)	$CD45^+$ $TER119^-$ $CD19^+$ $B220^+$ $MHCII^{+/int/lo}$
$Ly6G^+$ ($CD11b^{hi}$ $Ly6G^{hi}$)	$CD45^{+/lo}$ $TER119^-$ $CD11b^{hi}$ $Ly6G^{hi}$
Monocytes	$CD45^+$ $TER119^-$ $CD11b^+$ $Ly6G^-$ $MHCII^-$
Immature myeloid	$CD45^{lo}$ $TER119^-$ $CD117^{+/lo}$ $CD11b^+$ $Ly6G^{int/lo/-}$
CD3 double-negative (DN)	$CD45^+$ $TER119^-$ $CD3^+$ $CD4^-$ $CD8^-$
Fig. 6	
$CD4^+$ $CD8^-$	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD4^+$ $CD8^-$
$CD8^+$ $CD4^-$	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD8^+$ $CD4^-$
$CD4^+$ $CD8^+$	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD4^+$ $CD8^+$
$CD4^-$ $CD8^-$	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD4^-$ $CD8^-$
$CD19^+$ $B220^+$	$CD45^+$ $TER119^-$ $B220^+$ $CD19^+$
$NK1.1^+$	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^+$
DN1	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD4^+$ $CD8^+$ $CD44^+$ $CD25^-$
DN2	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD4^+$ $CD8^+$ $CD44^+$ $CD25^+$
DN3	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD4^+$ $CD8^+$ $CD25^+$ $CD44^{lo/-}$
DN4	$CD45^+$ $TER119^-$ $B220^-$ $CD19^-$ $NK1.1^-$ $CD4^+$ $CD8^+$ $CD44^{lo/-}$ $CD25^-$
Fig. 7	
B cells	$CD45^+$ $TER119^-$ $CD3^-$ $CD19^+$
T cells	$CD45^+$ $TER119^-$ $CD19^-$ $CD3^+$
Monocytes	$CD45^+$ $TER119^-$ $CD3^-$ $CD19^-$ $CD11b^+$ $Ly6G^-$ $MHCII^-$
NK cells	$CD45^+$ $TER119^-$ $CD19^-$ $CD3^-$ $NK1.1^+$
Immature myeloid	$CD45^+$ $TER119^-$ $CD3^-$ $CD19^-$ $CD11b^+$ $MHCII^-$

REFERENCE

Dobbs, N., N. Burnaevskiy, D. Chen, V.K. Gonugunta, N.M. Alto, and N. Yan. 2015. STING activation by translocation from the ER is associated with infection and autoinflammatory disease. *Cell Host Microbe.* 18:157–168. http://dx.doi.org/10.1016/j.chom.2015.07.001