Science Advances
AAAS

Supplementary Materials for

Stimulator of interferon gene facilitates recruitment of effector CD8 T cells that drive neurofibromatosis type 1 nerve tumor initiation and maintenance

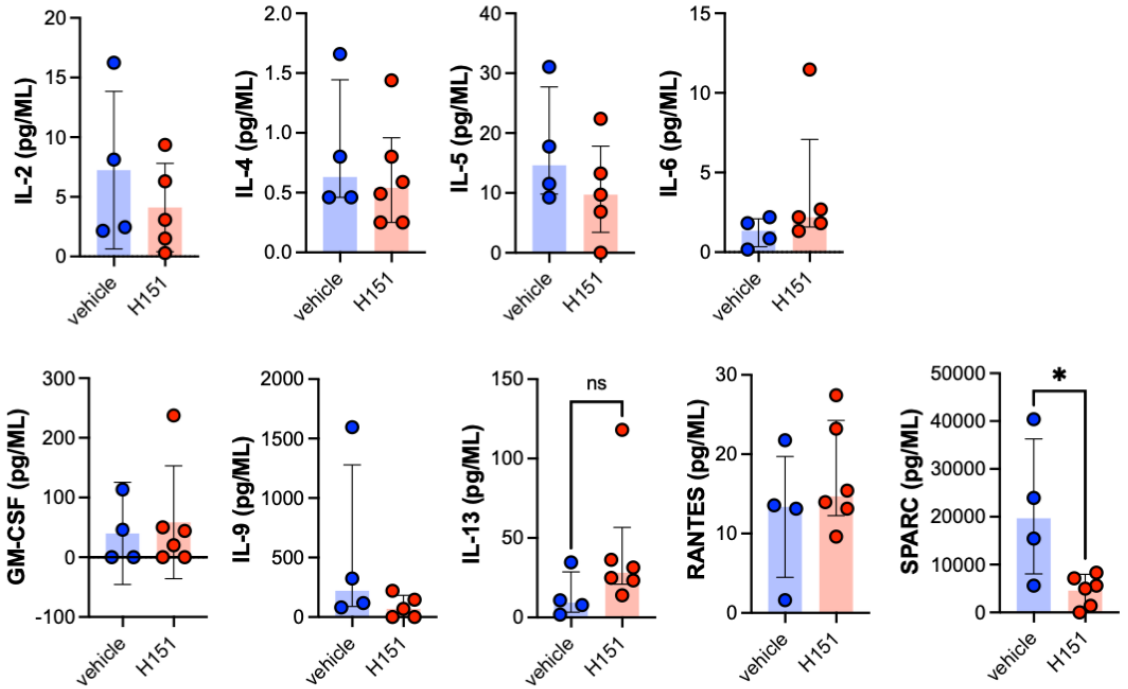
Jay Pundavela *et al.*

Corresponding author: Nancy Ratner, nancy.ratner@cchmc.org

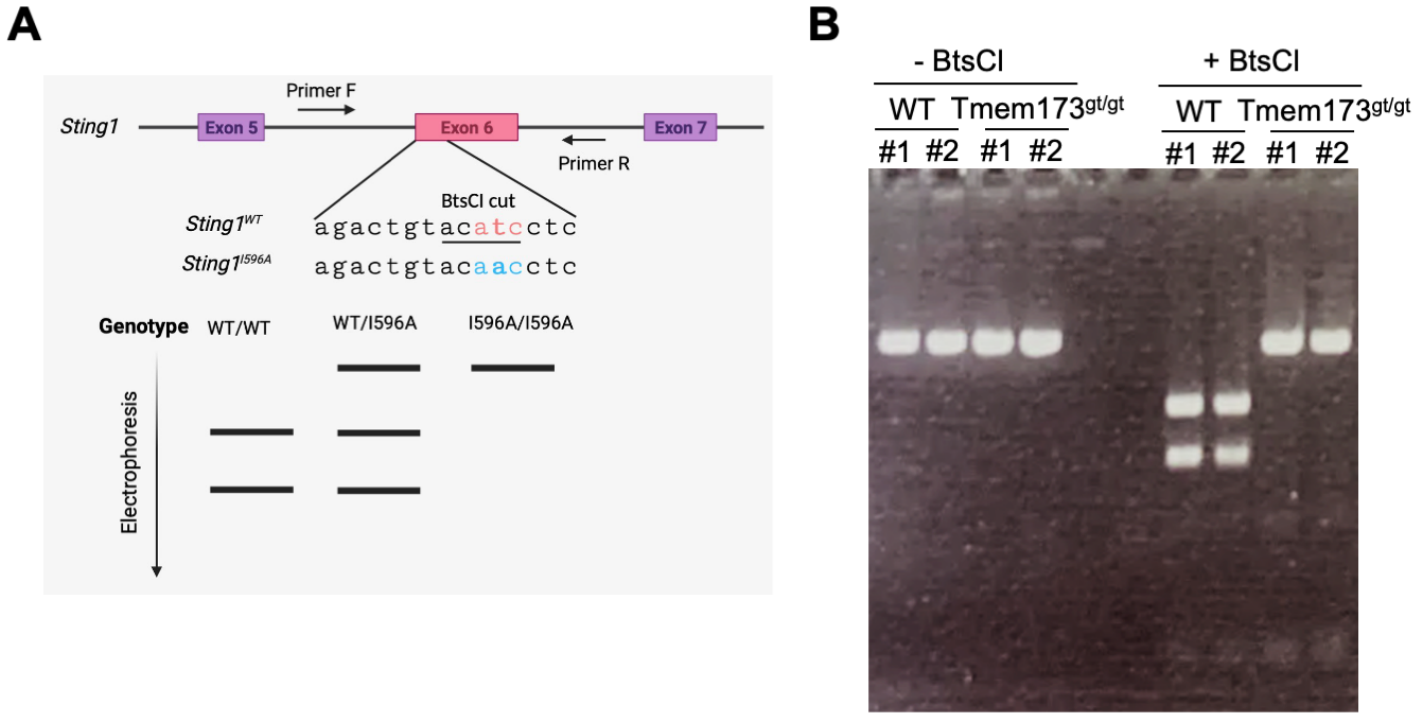
Sci. Adv. **10**, eado6342 (2024)
DOI: 10.1126/sciadv.ado6342

This PDF file includes:

Figs. S1 to S6

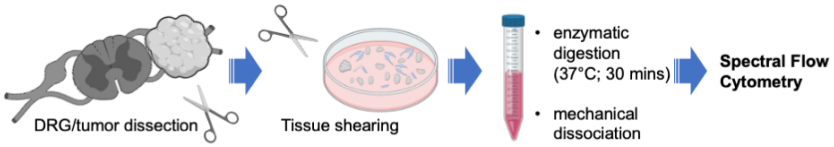


Supplemental Figure 1



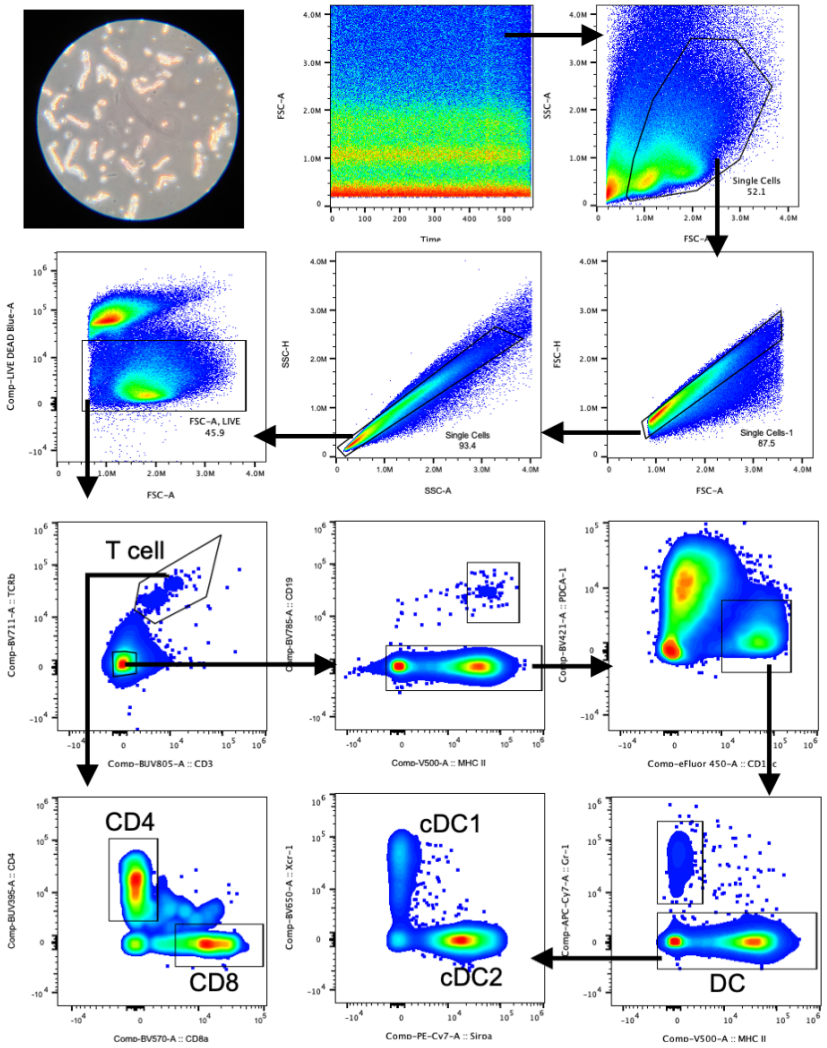
Supplemental Figure 2

A.

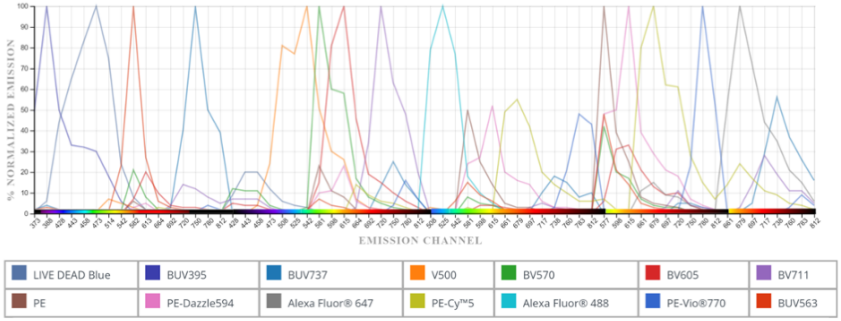


B.

C.



D.



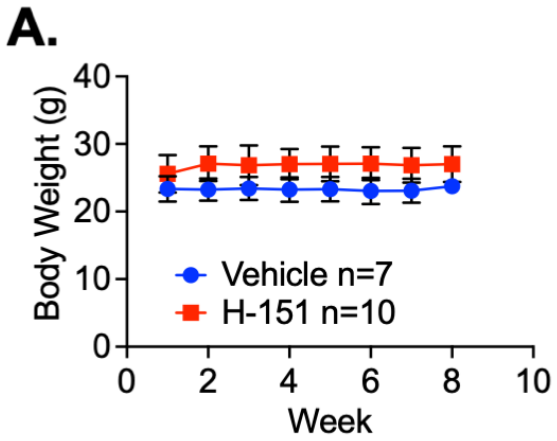
E.

Similarity™ Indices

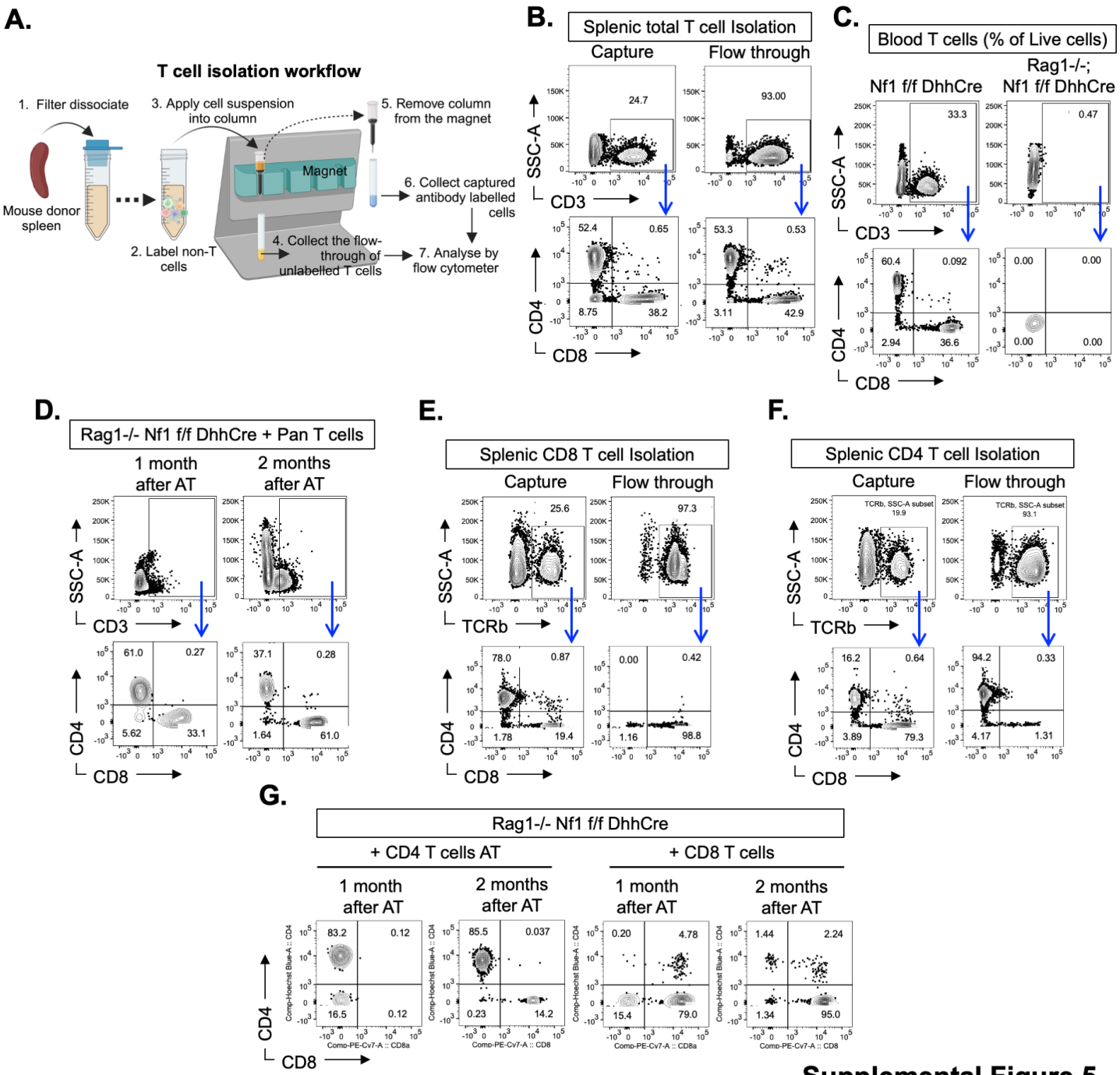
Configuration:5L 16UV-16V-14B-10YG-8R

	LIVE DEAD Blue	BUV395	BUV737	V500	BV570	BV605	BV711	PE	PE-Dazzle594	Alexa Fluor 647	PE-Cy5	Alexa Fluor 488	PE-Vio770	BUV563
LIVE DEAD Blue	1	0.51	0.02	0.08	0.04	0.02	0.02	0	0	0.01	0	0	0	0.09
BUV395	0.51	1	0.03	0.01	0.01	0.01	0	0.01	0.01	0	0	0.01	0	0.05
BUV737	0.02	0.03	1	0	0.02	0.05	0.41	0	0.02	0.22	0.1	0	0.14	0.01
V500	0.08	0.01	0	1	0.39	0.25	0.03	0.08	0.06	0	0.01	0.03	0	0.06
BV570	0.04	0.01	0.02	0.39	1	0.7	0.07	0.52	0.41	0.01	0.09	0.01	0.01	0.37
BV605	0.02	0.01	0.05	0.25	0.7	1	0.17	0.28	0.47	0.03	0.21	0	0.03	0.2
BV711	0.02	0	0.41	0.03	0.07	0.17	1	0.01	0.04	0.18	0.14	0	0.11	0.01
PE	0	0.01	0	0.08	0.52	0.28	0.01	1	0.67	0.01	0.14	0.07	0.02	0.51
PE-Dazzle594	0	0.01	0.02	0.06	0.41	0.47	0.04	0.67	1	0.06	0.43	0.05	0.05	0.36
Alexa Fluor 647	0.01	0	0.22	0	0.01	0.03	0.18	0.01	0.06	1	0.32	0	0.03	0.01
PE-Cy5	0	0	0.1	0.01	0.09	0.21	0.14	0.14	0.43	0.32	1	0.01	0.14	0.07
Alexa Fluor 488	0	0.01	0	0.03	0.01	0	0	0.07	0.05	0	0.01	1	0	0.05
PE-Vio770	0	0	0.14	0	0.01	0.03	0.11	0.02	0.05	0.03	0.14	0	1	0.01
BUV563	0.09	0.05	0.01	0.06	0.37	0.2	0.01	0.51	0.36	0.01	0.07	0.05	0.01	1

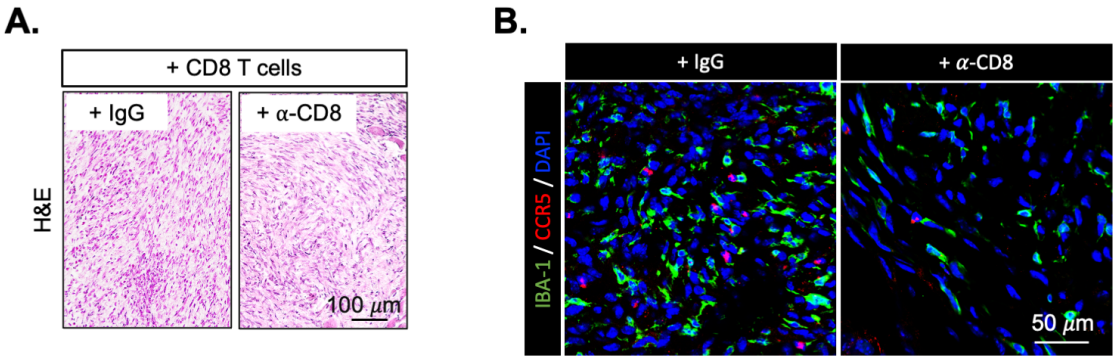
Supplemental Figure 3



Supplemental Figure 4.



Supplemental Figure 5



Supplemental Figure 6

Supplemental Figure Legends

Supplemental Figure 1. Plasma cytokine level. Enzyme-linked immunosorbent assay (ELISA) detection of plasma cytokines in mice treated with vehicle or STING antagonist H-151.

Supplemental Figure 2. Genotyping of STING deficient (*Tmem173*$^{gt/gt}$) mice. A) Primer design for genotyping *Tmem173*$^{gt/gt}$ mice. B) Representative image of an agarose gel electrophoresis loaded with PCR products for genotyping *Tmem173*$^{gt/gt}$ mice.

Supplemental Figure 3. Tumor processing and flow cytometer analysis. A) Schematic diagram of processing nerve tumors by enzymatic and mechanical dissociation method. B) Representative image under a light microscope of dissociated nerve tumor. C) Gating strategy for analysing immune cell types present in the nerve tumor by flow cytometry. D) Spectral representation of fluorophores used for flow cytometry. E. Similarity index of fluorophore panel used for flow cytometry.

Supplemental Figure 4. Body weight of mice treated with vehicle of H-151. A) Body weight of mice treated with vehicle or STING antagonist H-151 was monitored to assess toxicity of the compound.

Supplemental Figure 5. T cell isolation and adoptive transfer. A) Schematic image of isolating T cells from spleen of mice. B) Purity of isolation T cells. C) Absence of circulatory T cells in Rag1-/-; Nf1 f/f; DhhCre mice was confirmed by flow cytometer. D) Assessment of the efficiency of adoptive T cell transfer as indicated by the presence of circulatory T cells by flow cytometry. E) Purity of isolated CD8 or F) CD4 T cells. G) Efficiency of CD8 or CD4 T cells adoptive transfer.

Supplemental Figure 6. Histology and immunofluorescence of CCR5 expression. A) H&E stain and B) immunolabeling of CCR5 (red) and Iba1+ macrophages (green) of tumor section after anti-CD8 depletion versus IgG controls.