

## Supplemental material

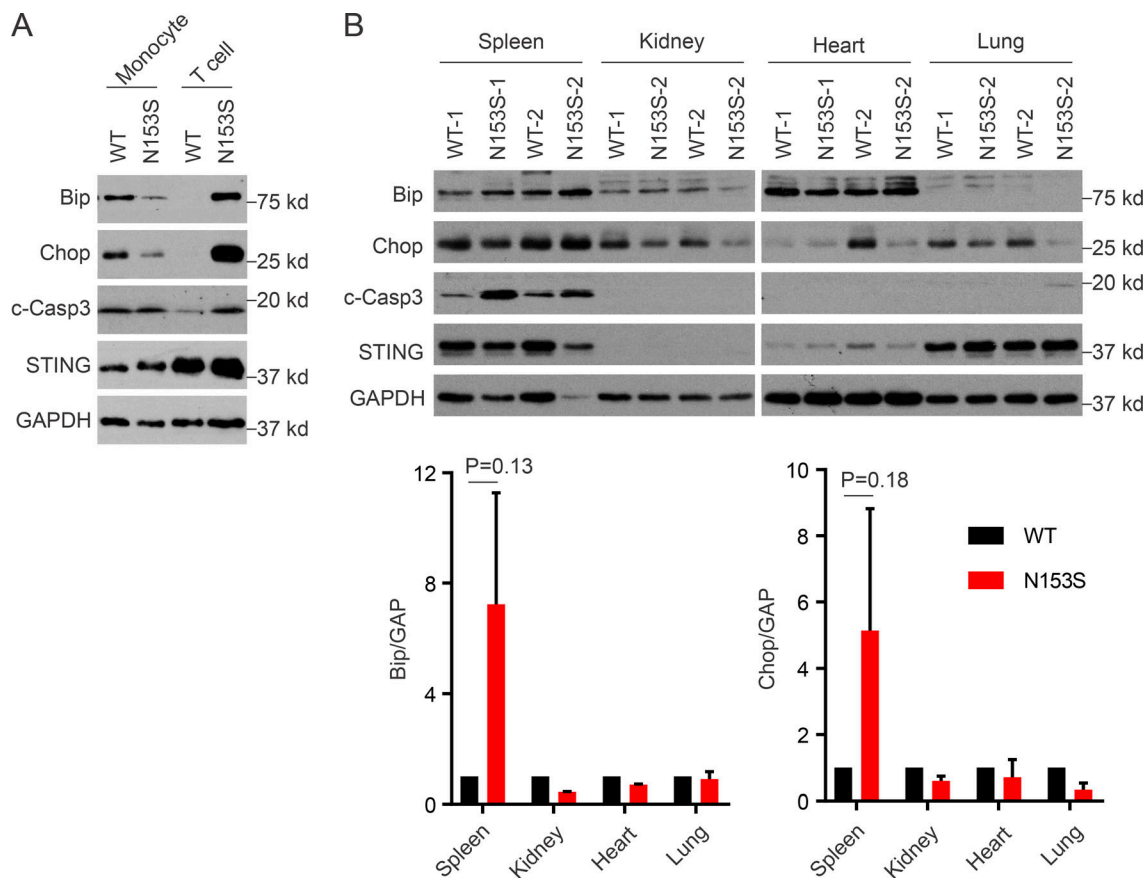
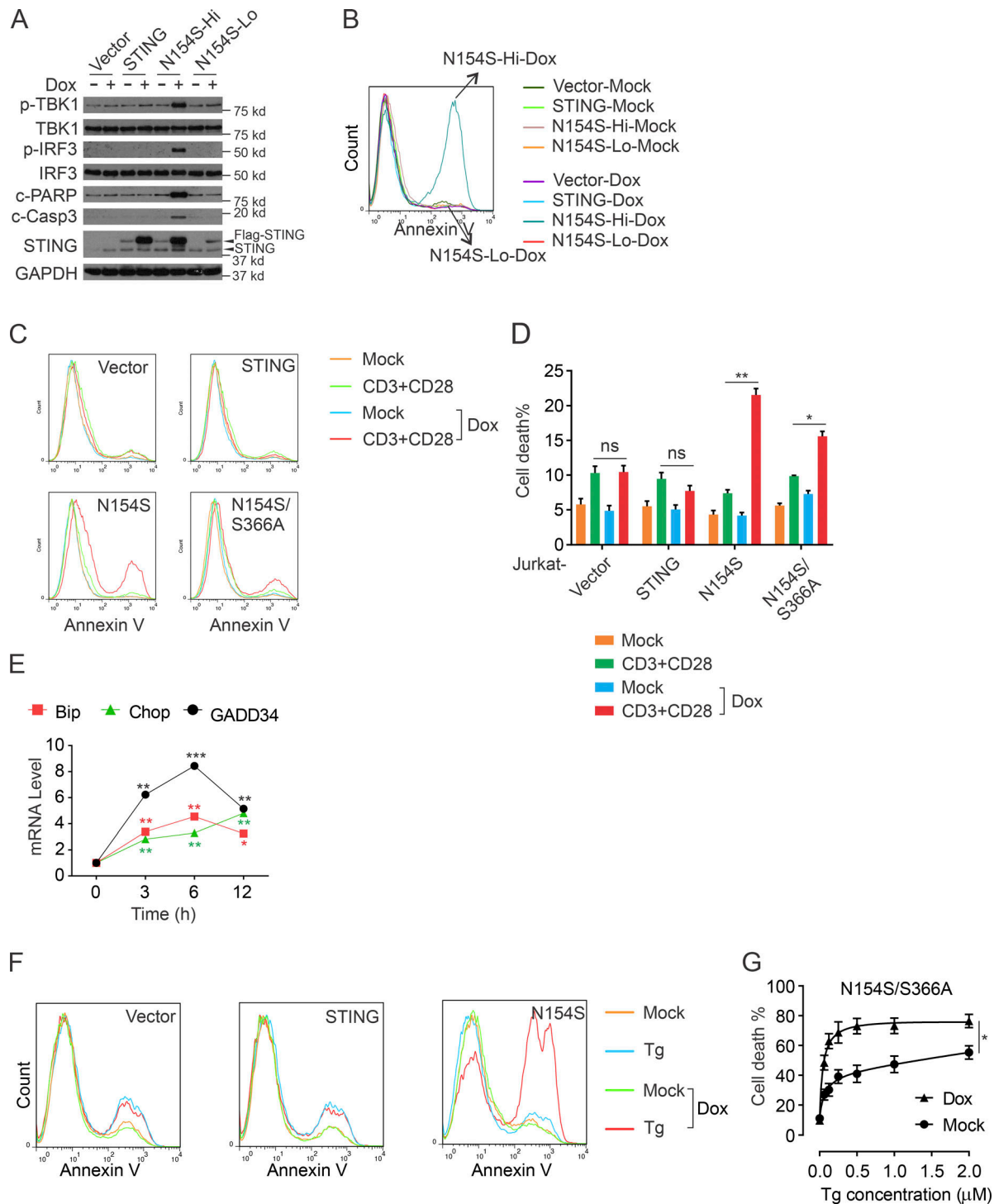
Wu et al., <https://doi.org/10.1084/jem.20182192>

Figure S1. **Chronic activation of ER stress and the UPR in the *Sting*<sup>N153S/+</sup> mouse.** (A) Immunoblot analysis of UPR proteins in WT and *Sting*<sup>N153S/+</sup> monocytes and T cells. Monocytes and T cells were isolated from spleens of littermate WT and *Sting*<sup>N153S/+</sup> mice, and lysates from fresh isolated cells were used for immunoblot analysis of indicated proteins and phosphor-proteins. (B) Immunoblot analysis of UPR proteins and apoptotic markers in whole tissues from WT and *Sting*<sup>N153S/+</sup> mice. Indicated tissues isolated from WT and *Sting*<sup>N153S/+</sup> mice were lysed and analyzed by Western blots. Top: Representative Western blot images. Bottom: Densitometry quantification of Bip and Chop normalized to Gapdh (GAP). The P values are shown on the bar graph. Student's *t* test. Data shown are representative of at least three independent experiments.



**Figure S2. Reconstitution of STING-N154S-mediated T cell death in human Jurkat T cells. (A and B)** Immunoblot (A) and FACS (B) analysis of Jurkat T cells expressing WT and mutant STING. Jurkat T cells reconstituted with vector, WT STING, N154S (Hi; high expression), N154S (Lo; low expression) were treated with (+) or without (-) Dox. Cells were analyzed by immunoblots for IFN signaling (pTBK1, pIRF3) and apoptotic signaling (cleaved PARP [c-PARP], cleaved caspase 3 [c-Casp3]; A). Cells were also stained for annexin V and analyzed by FACS (B). **(C and D)** Cell death FACS analysis of reconstituted Jurkat T cells treated with CD3/CD28 antibodies. Jurkat T cells reconstituted with vector or indicated STING variants were mock treated or treated with CD3/CD28 antibodies in the presence or absence of Dox for 2 d. The cells were then stained by annexin V and analyzed by FACS. A set of representative FACS plots are shown in C, and summary data are shown in D. **(E)** qRT-PCR analysis of UPR gene expression in WT cells after TCR activation. Jurkat T cells were treated with PMA+ionomycin for the indicated time (x axis). UPR gene expression was measured by qRT-PCR. **(F and G)** FACS analysis of reconstituted Jurkat T cells treated with Tg. Jurkat T cells expressing indicated constructs (top) were mock treated or treated with indicated conditions on the right (F). A dose curve of Tg was done in N154S/S366A cells (G). Error bars: SEM; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . Student's *t* test. Data shown are representative of at least three independent experiments.

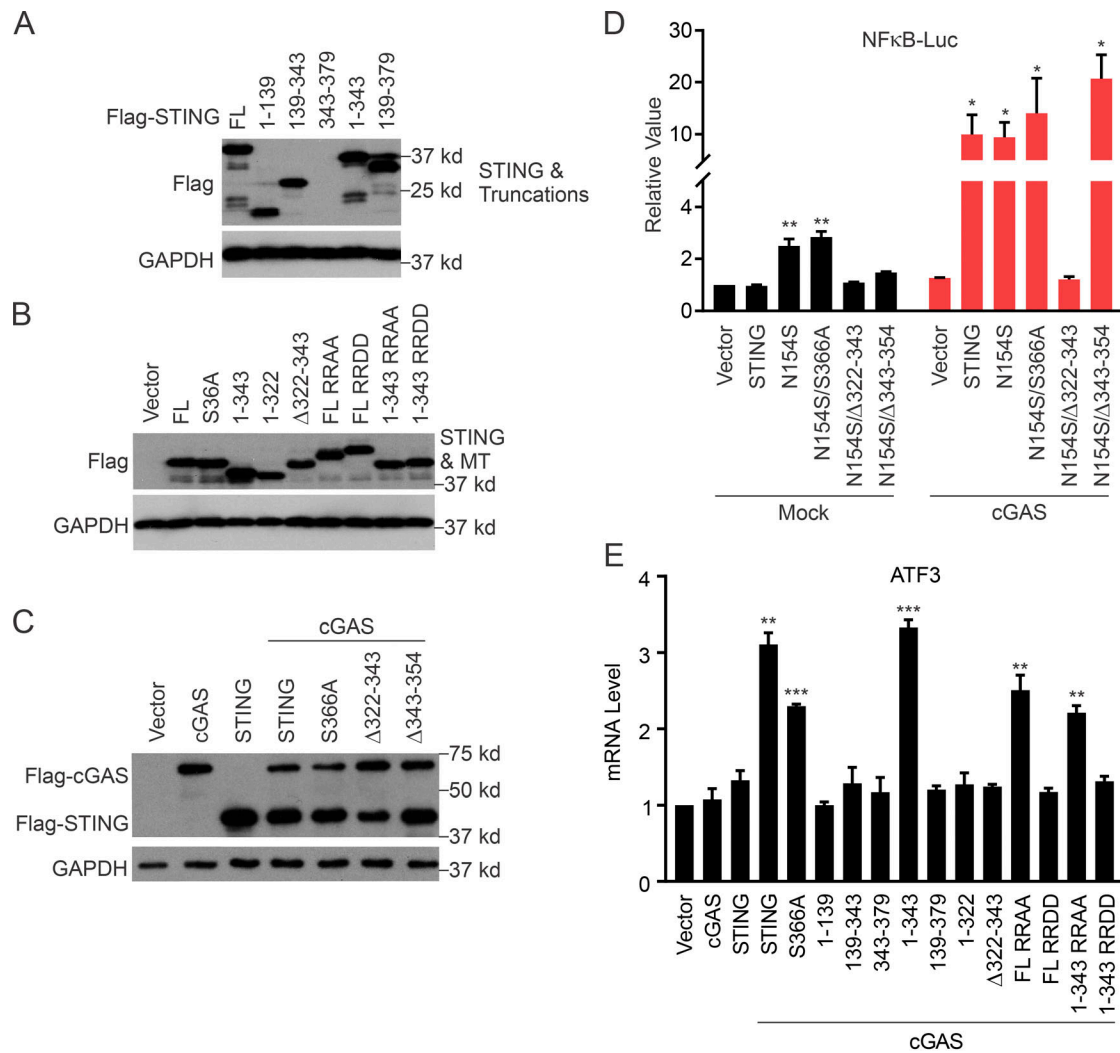


Figure S3. **Mapping the critical motif and residues for STING-mediated UPR.** (A–C) Immunoblots of STING mutant expression that are used in the Fig. 4. (D) Luciferase reporter assay of STING-mediated NF-κB signaling. HEK293T cells were transfected with NF-κB luciferase reporter and indicated STING WT or mutant expressing plasmids in the absence (left, black bars) or presence (right, red bars) of cGAS. Luciferase activity was measured 24 h after transfection. The relative luciferase activity was expressed as arbitrary units by normalizing firefly luciferase activity to Renilla luciferase activity. (E) ATF3 gene expression in cells transfected with indicated plasmids. Data shown are representative of at least three independent experiments. The P values were calculated by comparing the indicated group with vector control group. Error bars: SEM; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . Student's *t* test.

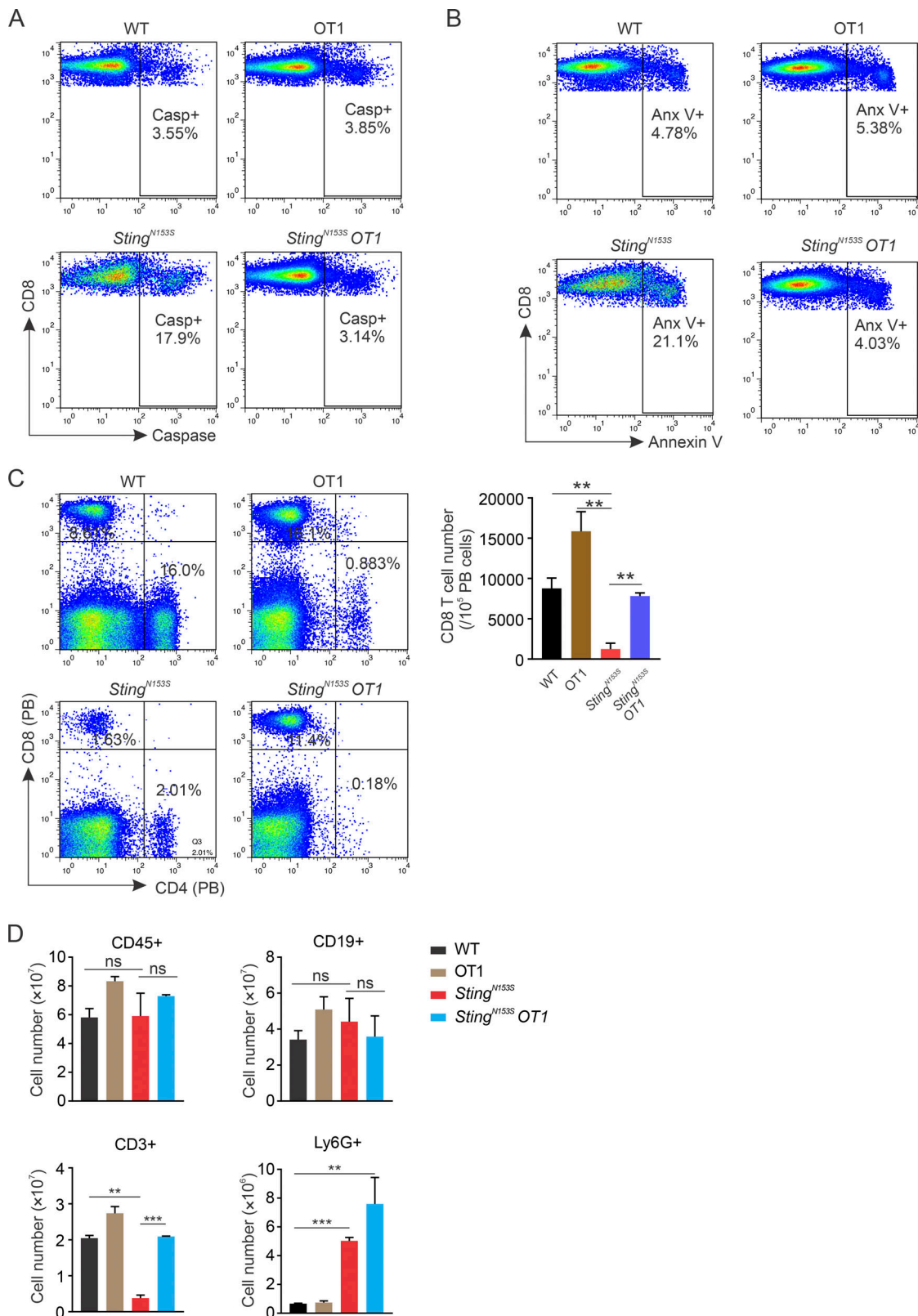


Figure S4. **OT-Irescues CD8<sup>+</sup> T cells in *Sting*<sup>N153S/+</sup> mice.** (A and B) FACS analysis of CD8<sup>+</sup> T cells from indicated mouse strains. Total splenocytes were stained as in Fig. 6 B. Representative FACS plots are shown here; the data summary is shown in Fig. 6 B. (C) FACS analysis of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in peripheral blood (PB) from indicated mouse strains. Peripheral blood mononuclear cells from indicated mouse strains were stained for CD4 and CD8. Representative FACS plots are shown on the left, and a data summary is shown on the right. (D) FACS analysis of lymphoid and myeloid cells in the spleen. Splenocytes from the indicated mouse strains were stained for CD45, CD19, CD3 and Ly6G, and indicated cell populations are shown.  $n = 4$ . Error bars: SEM; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.005$ . Student's  $t$  test. Anx V, annexin V; Casp, caspase.

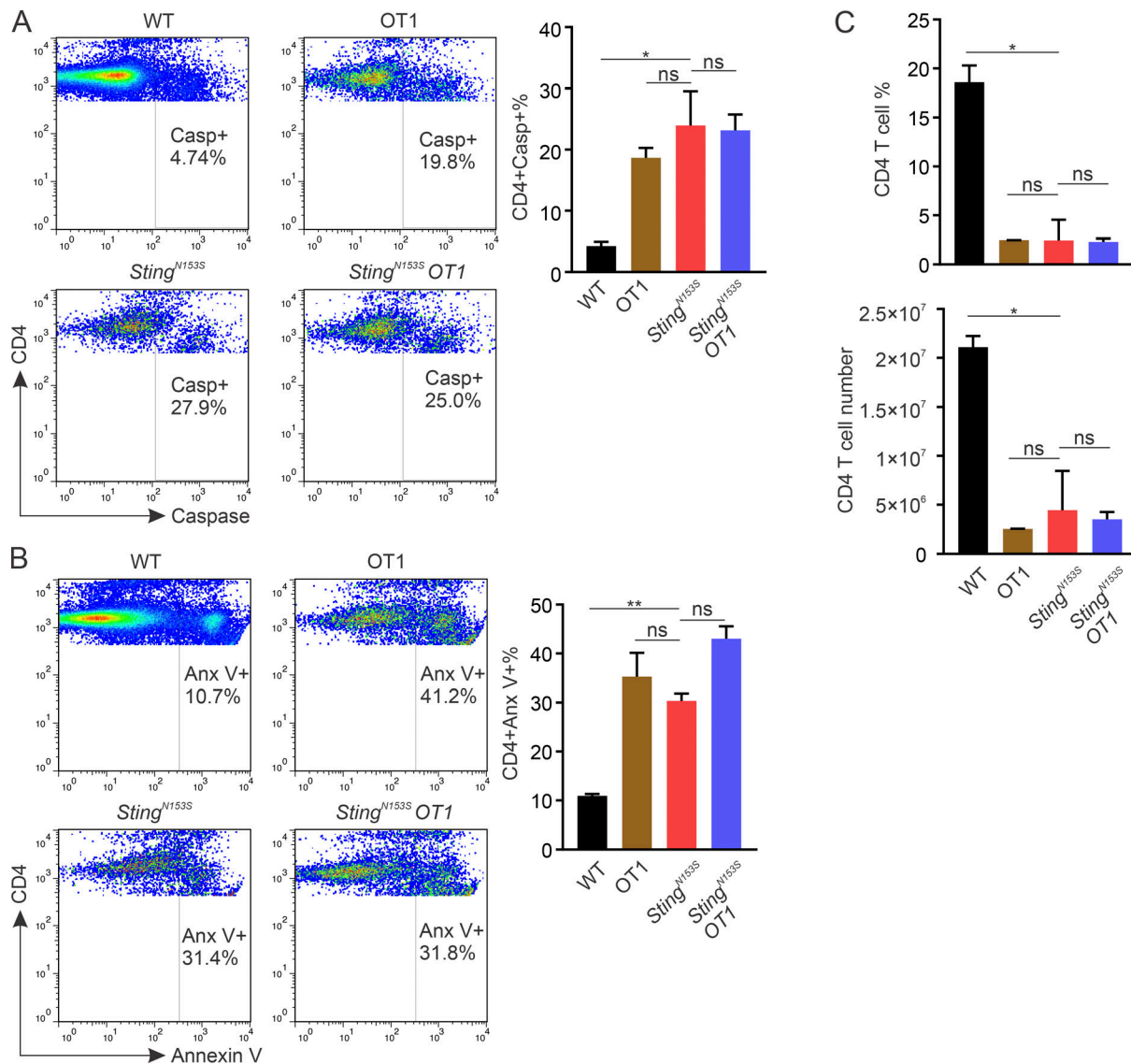


Figure S5. **CD4<sup>+</sup> T cells are not rescued by OT-1 in *Sting*<sup>N153S/+</sup> mice.** (A and B) FACS cell death analysis of splenic CD4<sup>+</sup> T cells. Total splenocytes from indicated mouse strains were stained for CD4 and cell death markers, CaspACE FITC-VAD-FMK (A) or annexin V (B). Representative FACS plots are shown on the left, and a data summary is shown on the right. (C) FACS analysis of CD4<sup>+</sup> T cell percentage and cell numbers from indicated mouse strains. *n* = 4. Error bars: SEM; \*, *P* < 0.05; \*\*, *P* < 0.01; ns, not significant. Student's *t* test. Anx V, annexin V; Casp, caspase.

Table S1. **Cloning primers**

| <b>Constructs</b>     | <b>Primers</b>   |
|-----------------------|--|
| hSTING-PCMV           | NNNNAAGCTTATATGCCCCACTCCAGCCTGCAT<br>NNNNCTCGAGTCAAGAGAAATCCGTGCGGAGAGG  |
| hSTING-TM-PCMV        | NNNNAAGCTTATATGCCCCACTCCAGCCTGCAT<br>NNNNCTCGAG TTACAGGCCCTTGAGGCCAGGAG  |
| hSTING-CBD-PCMV       | NNNNAAGCTTATGCCCCAGCTGAGATCTCTGCAGTG<br>NNNN CTCGAGTTACACAGTAACCTCTTCCTTTCTCC                                  |
| hSTING-CTT-PCMV       | NNNNAAGCTTATGTGGGCAGCTTGAAGACCTCAGC<br>NNNNCTCGAGTCAAGAGAAATCCGTGCGGAGAGG                                      |
| hSTING-TM-CBD-PCMV    | NNNNAAGCTTATATGCCCCACTCCAGCCTGCAT<br>NNNNCTCGAGTTACACAGTAACCTCTTCCTTTCTCC                                      |
| hSTING-CBD-CTT-PCMV   | NNNNAAGCTTATGCCCCAGCTGAGATCTCTGCAGTG<br>NNNNCTCGAGTCAAGAGAAATCCGTGCGGAGAGG                                     |
| hSTING-1-322-PCMV     | GCAGATGACAGCAGCTAATGATTCTCGCTGTCCAG<br>CTGGGACAGCGAGAATCATTAGCTGCTGTCATCTGC                                    |
| hSTING-D322-343-PCMV  | GCAGATGACAGCAGCGGCAGCTTGAAGACC<br>GGTCTTCAAGCTGCCGCTGCTGTCATCTGC   |
| hSTING-RRAA-PCMV      | TCCAGGAGGTTCTGCCCCACCTG GCCCAGGAGGAAAAGGAA<br>TTCCTTTTCTCCTGGGCCAGGTGGGCGAGAACCTCCTGGGA                        |
| hSTING-S366A-PCMV     | CCTGAGCTCCTCATCGCCGGAATGGAAAAGCCC<br>GGGCTTTTCCATTCCGGCGATGAGGAGCTCAGG   |
| hSTING-pEasiLV        | AATTCGAGCTCGGTACCCGGGGATCCATGGATTACAAGGATGACGACGA<br>AGCACAGGCTGCAGATGCATCTCGAGTCAAGAGAAATCCGTGCGGA            |
| hSTIM1(1-249)-pEasiLV | AATTCGAGCT CGGTACCCGGGGATCCGCCACCATGGATGTATGCGTCCGTCT<br>AGCACAGGCTGCAGATGCATCTCGAGCTACTCCAAGTCCTTCATCATCTTCTT |

Table S2. qPCR primers

| Genes        | Primers                   |
|--------------|---------------------------|
| Human Chop   | GAACCAGGAAACGGAAACAG      |
|              | ACCATTTCGGTCAATCAGAGC     |
| Human ATF3   | CACTGGTGTTTGAGGATTTTGC    |
|              | CCTTTCATCTTCTTCAGGGGCT    |
| Human BIP    | ATCATCAACGAGCCTACGG       |
|              | ACACGCTGGTCAAAGTCTTCT     |
| Human GADD34 | ACCTCTACTTCTGCCTTGCTCC    |
|              | TGGCTCCTTACTTCTTTCTGTT    |
| Mouse Chop   | AAAACCTTCACTACTCTTGACCCTG |
|              | CTTCTCCTTCATGCGTTGCTT     |
| Mouse GADD34 | GGGAGGCAGAACATCAAGC       |
|              | AGCATTCCGACAAGGGTGA       |
| Mouse BIP    | ATTGTTCTGGTTGGTGGATC      |
|              | TCTGAGACTTCTTGGTGGGT      |
| Mouse BCL2   | CTCTCGTCGCTACCGTCGT       |
|              | ATCCTCCCCCAGTTCACCC       |
| Mouse BIK    | CACCAACCTCAGGGAAAACA      |
|              | AGCAGCACCATCGGAAACA       |
| Mouse NOXA   | AAAAGAGCAGGATGAGGAGCC     |
|              | CTGCCGTAAATTCACTTTGTCTC   |