

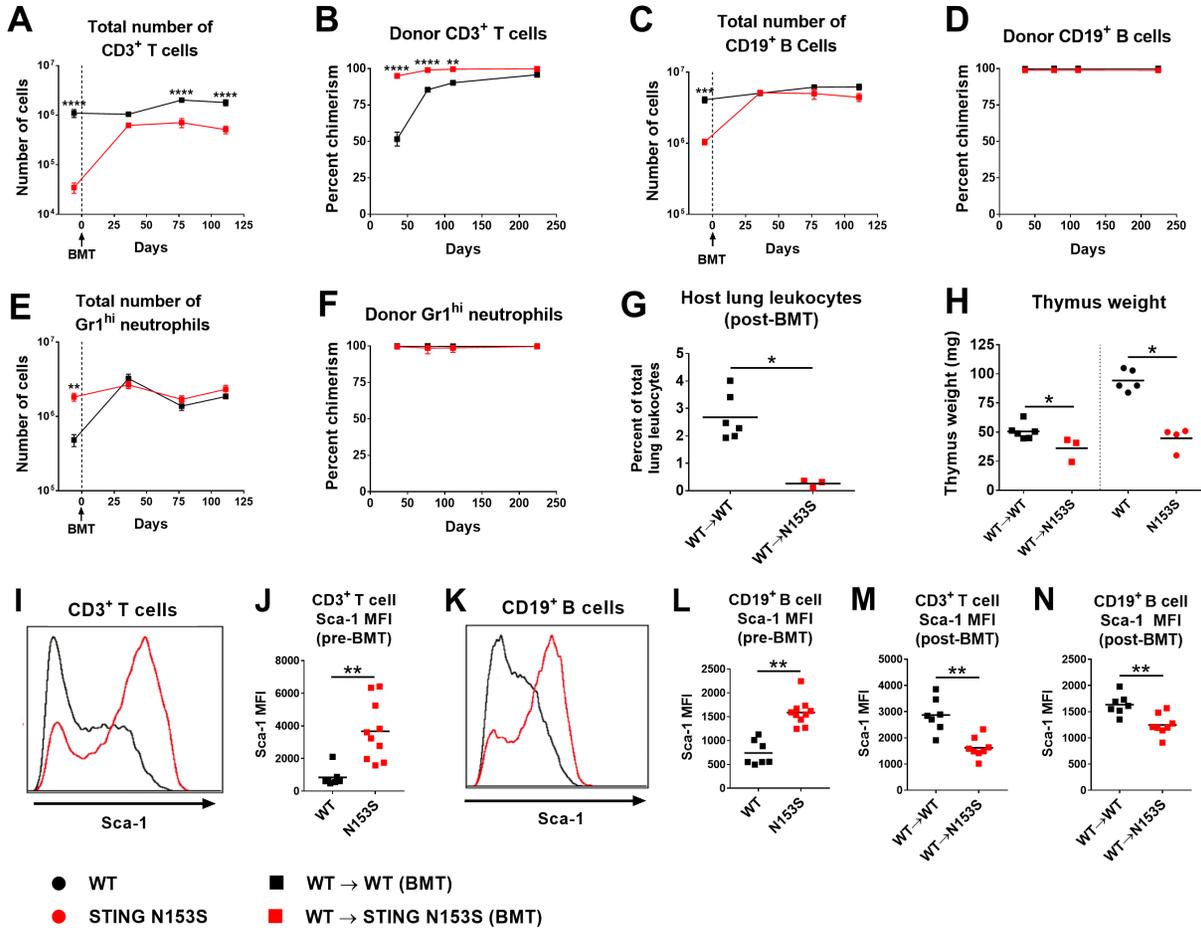
<i>Epitope</i>	<i>Clone</i>	<i>Manufacturer</i>
CD117	2B8	eBioscience 2
CD11b	M1/70	eBioscience, Biolegend 3
CD19	eBio1D3	eBioscience, Biolegend 4
CD3e	145-2C11, eBio500A2	eBioscience, Biolegend 5 6
CD4	GK1.5	eBioscience, Biolegend 7
CD45.1	A20	eBioscience, Biolegend 8
CD45.2	104	eBioscience, Biolegend 9
CD45R (B220)	RA3-6B2	eBioscience, Biolegend 10
CD8a	53-6.7	eBioscience, Biolegend 11
Ly6C	AL-21	Biolegend 12
Ly6G	1A8	Biolegend 13
NK1.1	PK136	eBioscience, Biolegend 14
Sca-1 (Ly-6A/E)	D7	eBioscience, Biolegend 15
Ter119	TER-119	eBioscience 16
CD44	IM7	eBioscience, Biolegend 17
CD25	PC61.5	eBioscience, Biolegend 18
F4/80	BM8	eBioscience, Biolegend 19 20

21 **Table E1.** Antibodies used in flow cytometry experiments.

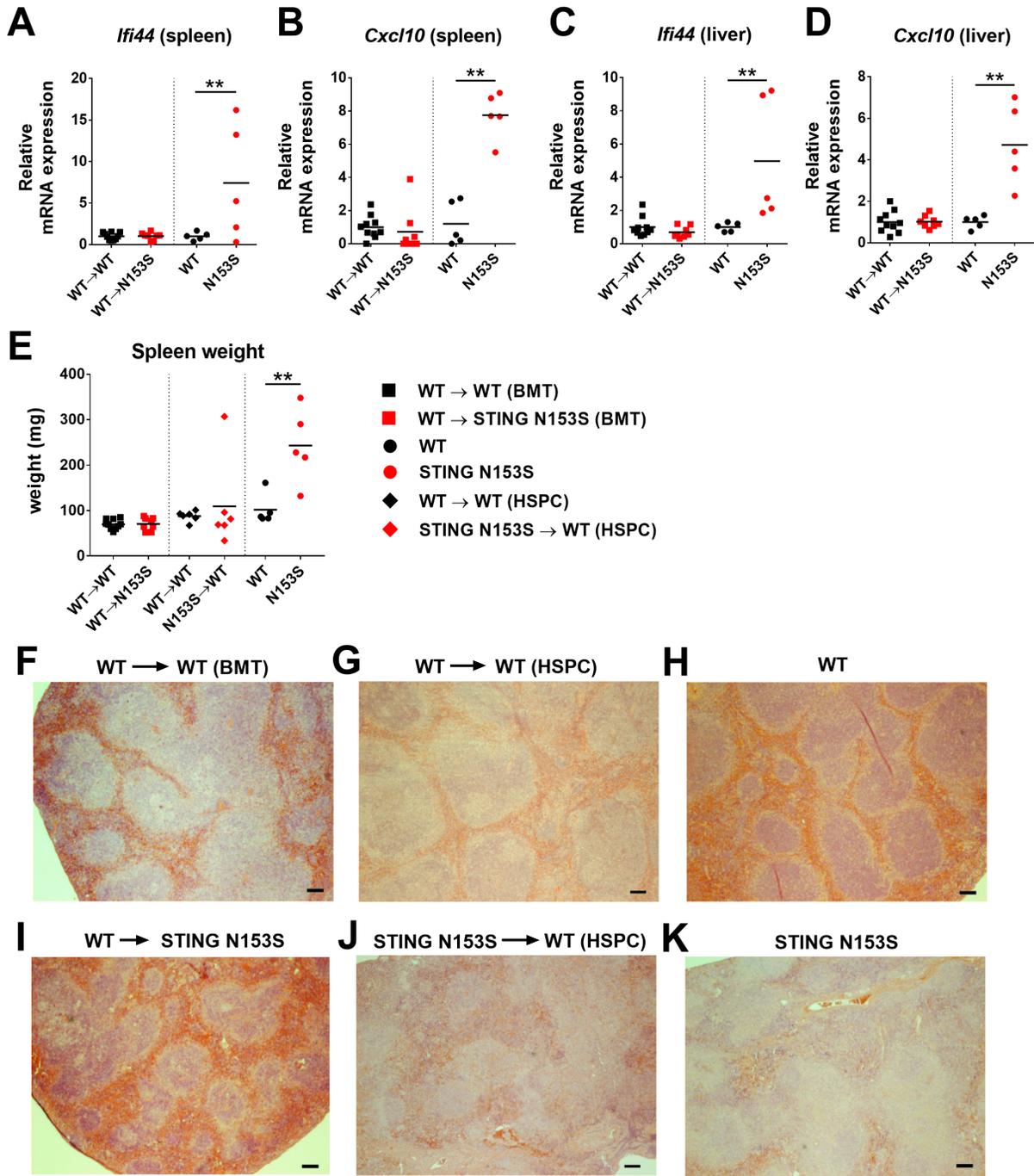
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<i>Primer</i>	<i>PrimerBank ID</i>	<i>Sequence</i>
excl10-for	10946576a1	CCAAGTGCTGCCGTCATTTTC
excl10-rev	10946576a1	GGCTCGCAGGGATGATTTCAA
ifi44-for	19527086a1	AACTGACTGCTCGCAATAATGT
ifi44-rev	19527086a1	GTAACACAGCAATGCCTCTTGT
hprt-for	7305155a1	TCAGTCAACGGGGGACATAAA
hprt-rev	7305155a1	GGGGCTGTACTGCTTAACCAG
rpl13a-for	334688867c2	AGCCTACCAGAAAGTTTGCTTAC
rpl13a-rev	334688867c2	GCTTCTTCTTCCGATAGTGCATC
eef2-for	237858599c1	CCGACTCCCTTGTGTGCAA
eef2-rev	237858599c1	AGTTCAGGTCGTTCTCAGAGAG

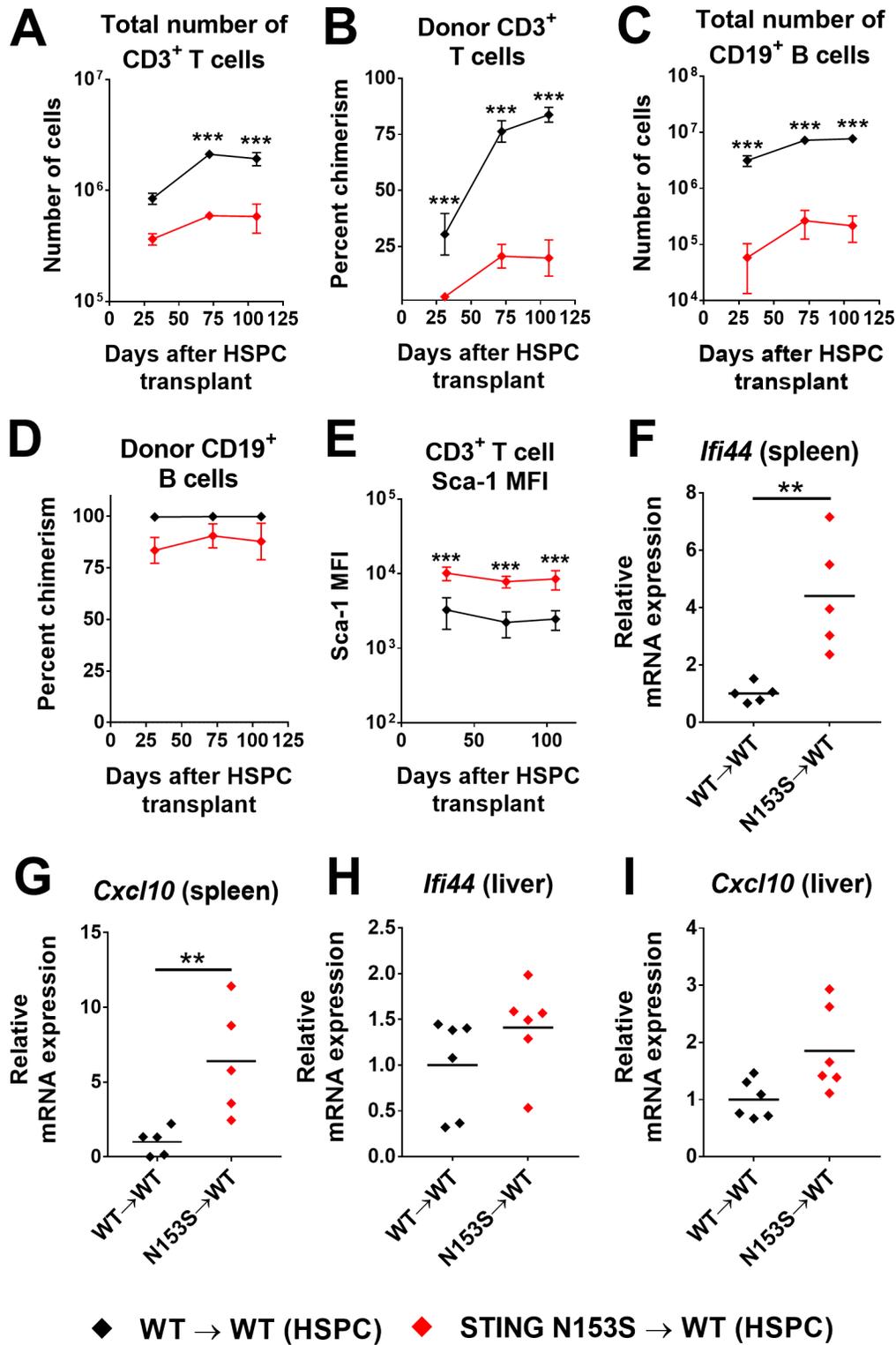
Table E2. Primers used in qRT-PCR experiments.



Supplemental Figure E1

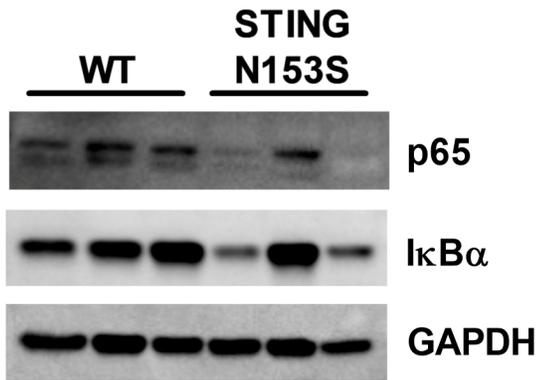


Supplemental Figure E2

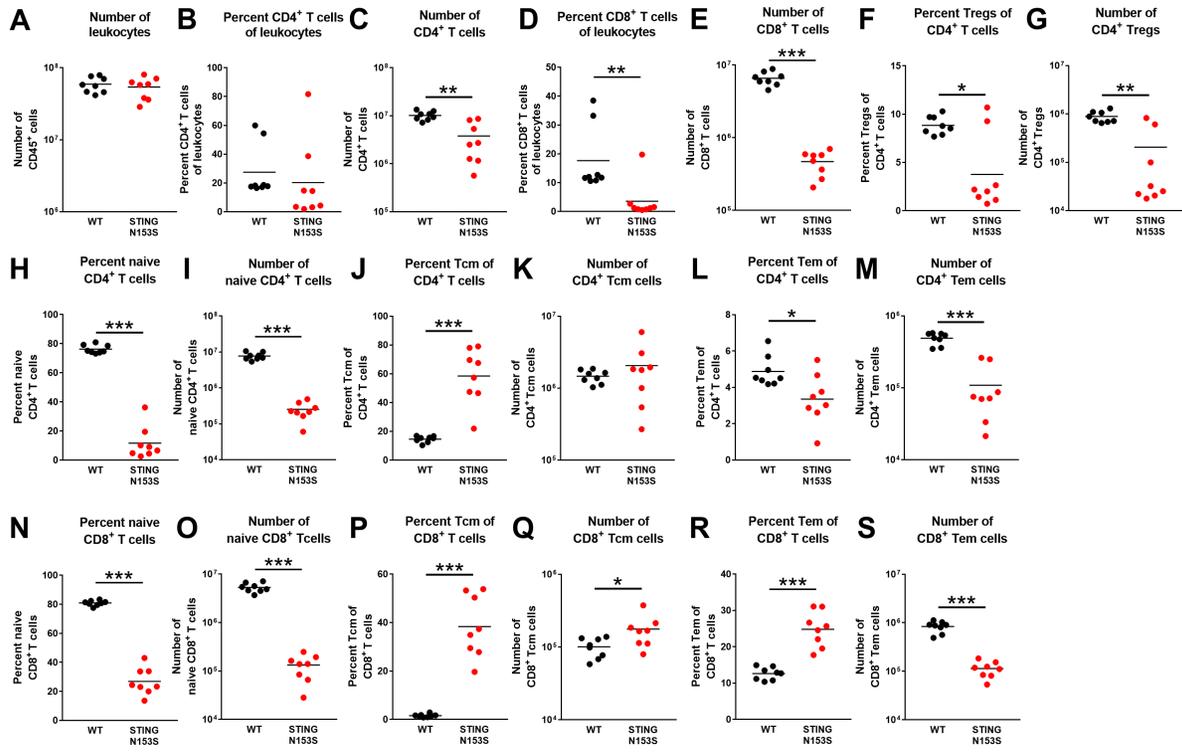


Supplemental Figure E3

A



Supplemental Figure E4



Supplemental Figure E6

1 **ONLINE REPOSITORY**

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3 **STING-associated lung disease in mice relies primarily on T cells but not type I interferon**

4

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26 **FIGURE LEGENDS**

27 **FIG E1. Mixed chimerism after WT bone marrow transplantation (BMT).** 9-13 week-old adult WT
28 and STING N153S mice were lethally irradiated and followed by transplantation of WT bone marrow. **A-F**,
29 Peripheral blood was longitudinally analyzed for total numbers of CD3⁺ T cells (A), donor CD3⁺ T cells
30 (B), total number of CD19⁺ T cells (C), donor CD19⁺ B cells (D), total number of CD11b⁺ Gr-1^{hi}
31 neutrophils (E) and donor neutrophils. Data in (A-F) represent the mean ± SEM of *n* = 6-10 samples. **G**,
32 Total number of CD45.2⁺ leukocytes in the lung of WT and STING N153S recipient mice as quantitated by
33 flow cytometry 224 days after transplantation. **H**, Thymus weight of transplanted WT and STING N153S
34 mice as well as age-matched non-transplanted WT and STING N153S control animals. **I**, Representative
35 histogram showing Sca-1 expression on CD3⁺ splenocytes of non-transplanted WT and STING N153S
36 mice. **J**, MFI of Sca-1 expression on CD3⁺ T cells before BMT. **K**, Representative histogram showing Sca-
37 1 expression on CD3⁺ splenocytes of non-transplanted WT and STING N153S mice. **L**, MFI of Sca-1
38 expression on CD19⁺ B cells before BMT. **M**, MFI of Sca-1 expression on CD3⁺ T cells after BMT. **N**,
39 MFI of Sca-1 expression on CD19⁺ B cells after BMT. with *n* = 3 - 6 mice per group. Data in (A-F) were
40 analyzed by 2-way ANOVA. **, *P* < 0.01; ***, *P* < 0.001; ****, *P* < 0.0001. Data in (G-N) were analyzed
41 by Mann-Whitney test. *, *P* < 0.05; **, *P* < 0.01.

42

43 **FIG E2. WT bone marrow transplantation (BMT) reduces ISG expression and improves splenic**
44 **architecture in STING N153S mice.** **A-D**, ISG expression levels in spleens and livers of indicated groups
45 of mice were assessed by qRT-PCR and reported as the fold change relative to the average of 3
46 housekeeping genes (*hprt1*, *rpl13a*, and *eef2*). Data represent the mean of *n* = 5-10 tissues per group.
47 Results were analyzed by Mann-Whitney test. *, *P* < 0.05; **, *P* < 0.01. **E**, Weights of spleen from
48 irradiated WT and STING N153S recipients of WT bone marrow, WT recipients of either STING N153S or
49 WT HSPCs and WT and STING N153S non-irradiated control animals. **F-K**, Representative images of

50 H&E-stained spleen sections of indicated genotypes. Scale bar = 200 μ m. Data represent the mean of $n = 5$ -
51 10 mice.

52 **FIG E3. STING N153S HSPC transplantation into WT mice causes ISG upregulation.** Flow
53 cytometric characterization of PBMCs and tissue expression of ISGs in WT mice transplanted with WT and
54 STING N153S HSPCs. **A-E**, Peripheral blood was longitudinally examined for total numbers of CD3⁺ T
55 cells (A), donor CD3⁺ T cells (B), total number of CD19⁺ B cells (C), donor CD19⁺ B cells (D), and Sca-1
56 MFI on CD3⁺ T cells (E). Data in (A-E) represent mean \pm SEM of two independent experiments and were
57 analyzed by two-way ANOVA test. $n = 6$ mice per group. ***, $P < 0.001$. **F-I**, ISG expression levels in
58 spleens and livers of indicated groups of mice were assessed by qRT-PCR and reported as the fold change
59 relative to the average of 3 housekeeping genes (*hprt1*, *rpl13a*, and *eef2*). Results were analyzed by Mann-
60 Whitney test. $n = 6$ per group. **, $P < 0.01$.

61

62 **FIG E4. NF- κ B activation in the lungs of WT and STING N153S mice.** Western blot analysis from WT
63 and STING N153S lung homogenates taken from $n = 3$ mice per genotype for p65, I κ B α , and housekeeping
64 control GAPDH protein expression in lung homogenate from age-matched 13-17 week-old WT and STING
65 N153S mice. Results are representative of 7 biological replicates analyzed in 2 independent experiments.

66

67 **FIG E5. Chemokine receptor expression on CD4⁺ and CD8⁺ splenic T cells WT and STING N153S**
68 **spleens were harvested for flow cytometric characterization of chemokine receptors on CD4⁺ and CD8⁺ T**
69 **cells. A-F, Percent positivity of CXCR3 (A,B), CX3CR1 (C,D), and CCR4 (E,F) on CD4⁺ and CD8⁺ splenic**
70 **T cells. Data in (A-F) represent mean of two independent experiments and were analyzed by Mann-Whitney**
71 **test. $n = 5$ mice per group. *, $P < 0.05$; **, $P < 0.01$.**

72

73 **FIG E6. T cell subset analysis in WT and STING N153S mice.** Splenocytes from 4-month-old WT and
74 STING N153S mice were harvested and T cell subsets examined by flow cytometry. **A**, Total number of
75 CD45⁺ cells. **B-C**, Percent and number of CD4⁺ T cells. **D-E**, Percent and number of CD8⁺ T cells. **F-G**,
76 Percent and number of CD25⁺ Foxp3⁺ CD4⁺ T cells. **H-I**, Percent and number of CD44^{lo} CD62L^{hi} naïve
77 CD4⁺ T cells. **J-K**, Percent and number of CD44^{hi} CD62L^{hi} CD4⁺ central memory T cells (Tcm). **L-M**,
78 Percent and number of CD44^{hi} CD62L^{lo} CD4⁺ effector memory T cells (Tem). **N-O**, Percent and number of
79 CD44^{lo} CD62L^{hi} CD8⁺ naïve T cells. **P-Q**, Percent and number of CD44^{hi} CD62L^{hi} CD8⁺ Tcm cells. **R-S**,
80 Percent and number of CD44^{hi} CD62L^{lo} CD8⁺ Tem cells. Data in A-S represent the mean of $n = 8$ mice per
81 genotype from two independent experiments. Data were analyzed by Mann-Whitney test. *, $P < 0.05$; **, P
82 < 0.01 .