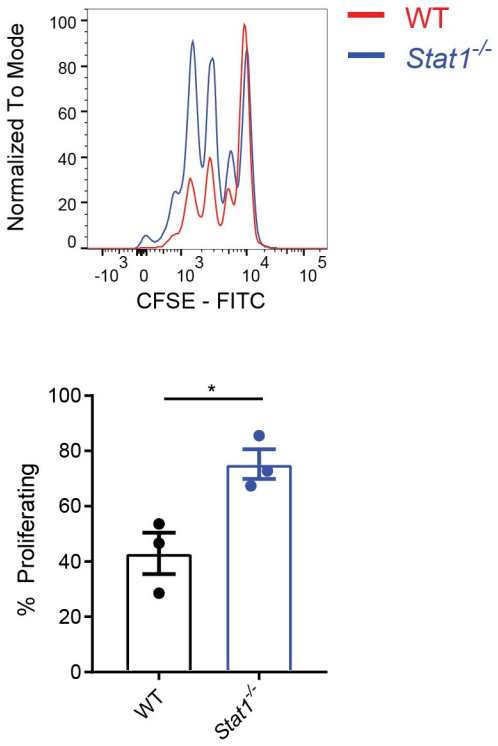
STAT1 signaling shields T cells from NK cell mediated cytotoxicity

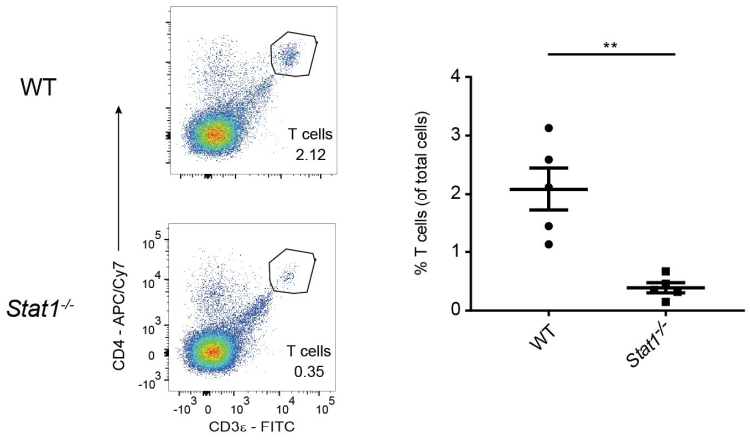
Kang et. al.

Supplementary Figures

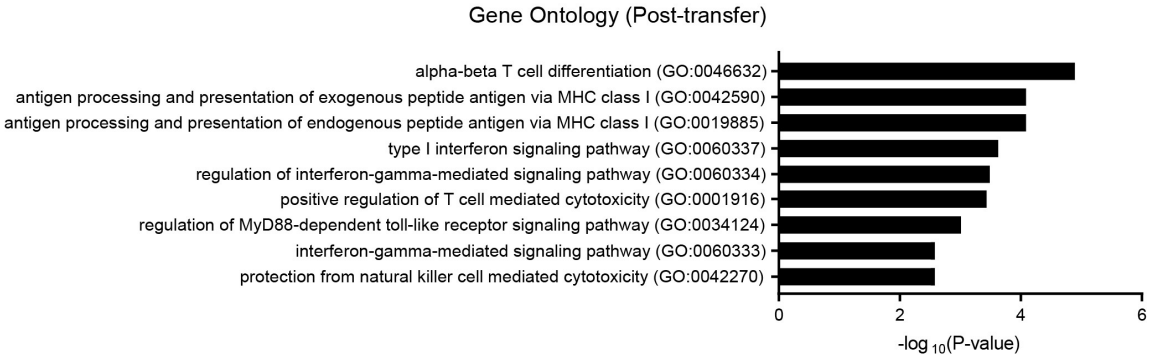


Supplementary Fig. 1 *Stat1*$^{-/-}$ T cells do not display defective expansion *in vitro*. WT or *Stat1*$^{-/-}$ unfractionated CD4^{+} T cells were stimulated *in vitro* with anti-CD3 (3μg/ml) and anti-CD28 (1μg/ml) for 3 days and cell proliferation analyzed. Representative flow cytometry plots are shown (gated on live CD4^{+} cells) with mean frequencies ± SEM. Data are pooled from 3 independent experiments, with each point representing the average across technical replicates in each experiment. *p<0.05 by unpaired two-tailed t-test. Refers to Fig. 4.

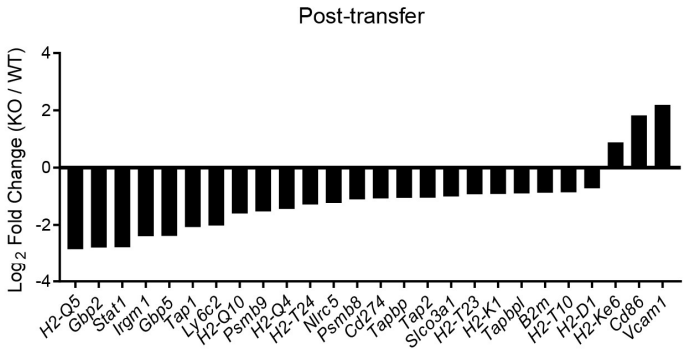
a


WT
Stat1-/-
T cells
2.12
T cells
0.35
CD4 - APC/Cy7
CD3ε - FITC
**
% T cells (of total cells)
WT
Stat1-/-

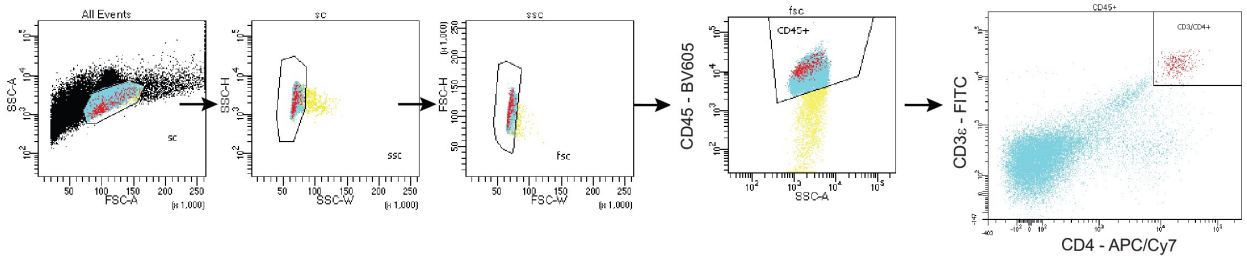
b


Gene Ontology (Post-transfer)
alpha-beta T cell differentiation (GO:0046632)
antigen processing and presentation of exogenous peptide antigen via MHC class I (GO:0042590)
antigen processing and presentation of endogenous peptide antigen via MHC class I (GO:0019885)
type I interferon signaling pathway (GO:0060337)
regulation of interferon-gamma-mediated signaling pathway (GO:0060334)
positive regulation of T cell mediated cytotoxicity (GO:0001916)
regulation of MyD88-dependent toll-like receptor signaling pathway (GO:0034124)
interferon-gamma-mediated signaling pathway (GO:0060333)
protection from natural killer cell mediated cytotoxicity (GO:0042270)
-log 10(P-value)

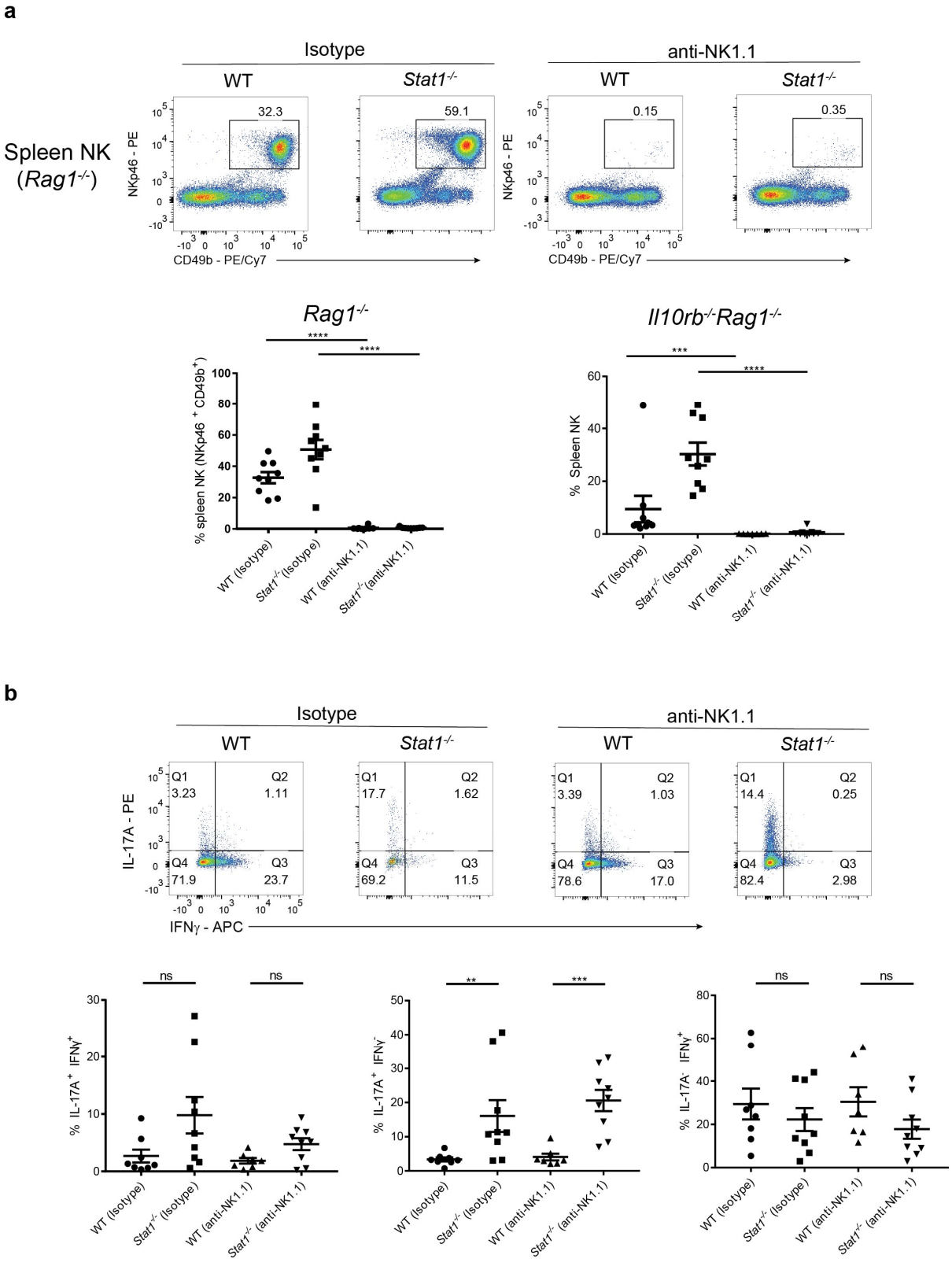
c


Post-transfer
Log2 Fold Change (KO / WT)
H2-Q5
Gbp2
Stat1
Irgm1
Gbp5
Tap1
Ly6c2
H2-Q10
Psmb9
H2-Q4
H2-T24
Nlrc5
Psmb8
Cd274
Tapbp
Tap2
Slco3a1
H2-T23
H2-K1
Tapbpl
B2m
H2-T10
H2-D1
H2-Ke6
Cd86
Vcam1

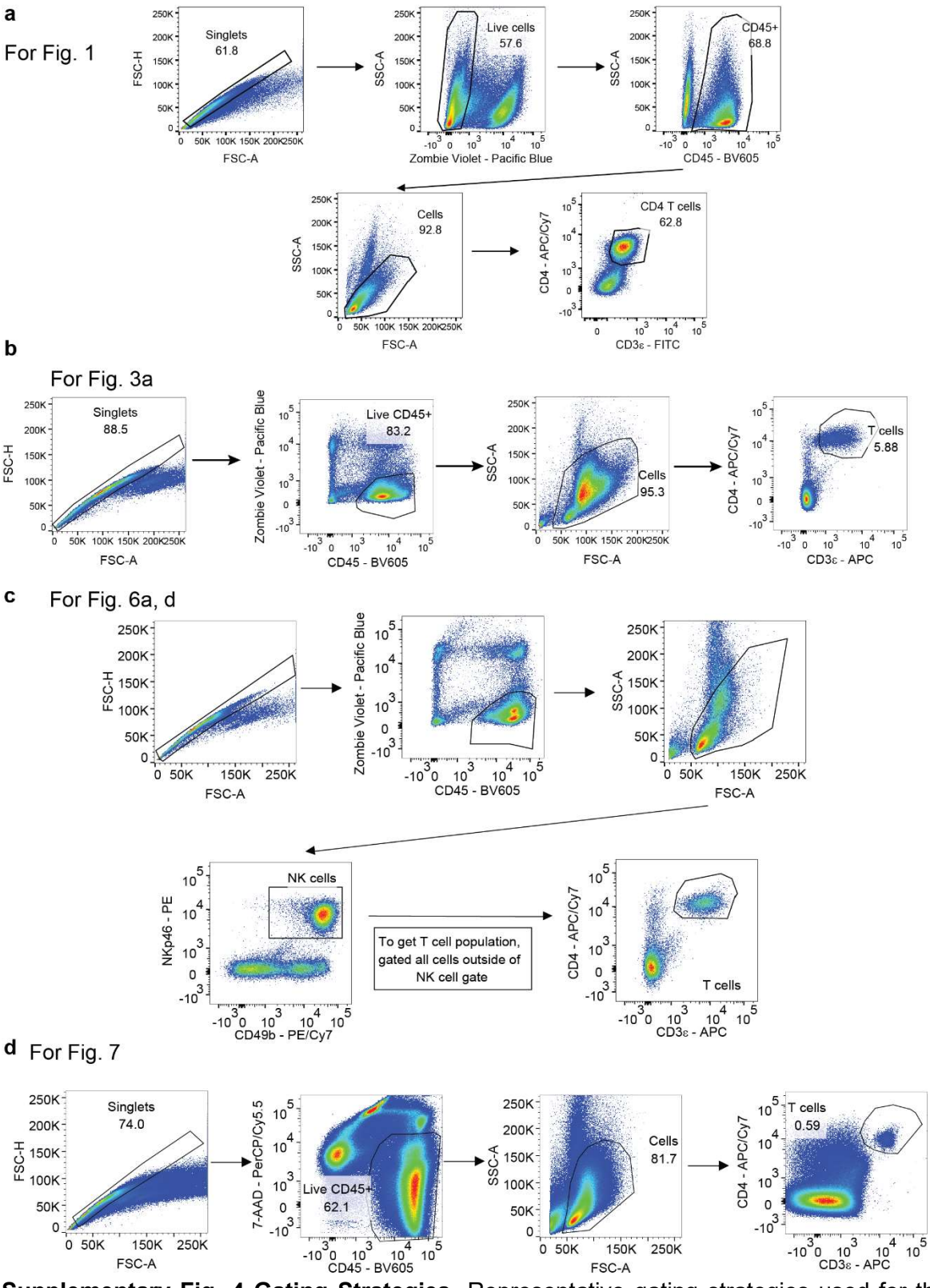
d


All Events
SSC-A
FSC-A
sc
SSC-H
SSC-W
ssc
FSC-H
FSC-W
Fsc
CD45 - BV605
SSC-A
CD45+
CD3ε - FITC
CD4 - APC/Cy7
CD3/CD4+

Supplementary Fig. 2 Downregulation of the MHC-I pathway in *Stat1*$^{-/-}$ T cells post-transfer. *Rag1*$^{-/-}$ mice were injected i.p. with 5 X 10^6 WT or *Stat1*$^{-/-}$ unfractionated CD4$^+$ T cells and analyzed 1 week post transfer. **(a)** Representative flow cytometry plots of CD4$^+$ T cells (spleen + lymph nodes) post-transfer, along with their mean frequencies ± SEM. Each point represents an individual mouse. **(b)** Selected Gene ontology terms showing differential expression of the MHC class I pathway in *Stat1*$^{-/-}$ T cells post-transfer. **(c)** Similar downregulation of various genes involved in MHC class I antigen presentation in *Stat1*$^{-/-}$ T cells compared to WT T cells in the post-transfer setting. **(d)** Gating strategy for sorting T cells for RNA-seq post transfer. **p<0.01 by two-tailed Mann-Whitney test. Refers to Fig. 5.


a
Spleen NK
(Rag1-/-)
Isotype
anti-NK1.1
WT
Stat1-/-
32.3
59.1
0.15
0.35
NKp46 - PE
CD49b - PE/Cy7
Rag1-/-
Il10rb-/-Rag1-/-
% spleen NK (NKp46+CD49b+)
% Spleen NK
WT (Isotype)
Stat1-/- (Isotype)
WT (anti-NK1.1)
Stat1-/- (anti-NK1.1)
b
Isotype
anti-NK1.1
WT
Stat1-/-
Q1 3.23
Q2 1.11
Q4 71.9
Q3 23.7
Q1 17.7
Q2 1.62
Q4 69.2
Q3 11.5
Q1 3.39
Q2 1.03
Q4 78.6
Q3 17.0
Q1 14.4
Q2 0.25
Q4 82.4
Q3 2.98
IL-17A - PE
IFNγ - APC
% IL-17A+ IFNγ+
% IL-17A+ IFNγ-
% IL-17A- IFNγ+
ns
**
***

Supplementary Fig. 3 NK cell depletion does not alter *Stat1*$^{-/-}$ T cell differentiation *in vivo*. **(a)** anti-NK1.1 depleting antibody effectively removes NK cells. Representative flow cytometry plots of splenic NK cells (NKp46^{+} CD49b^{+}) in *Rag1*$^{-/-}$ mice injected with control or NK cell depleting antibody at 3 weeks post transfer, as well as their mean frequencies ± SEM in both *Rag1*$^{-/-}$ and *Il10rb*$^{-/-}$*Rag1*$^{-/-}$ mice. **(b)** Colonic T cells (gated on live CD45^{+} TCRβ^{+} CD4^{+} T cells) from *Il10rb*$^{-/-}$ *Rag1*$^{-/-}$ mice at 3 weeks post transfer were analyzed for their differentiation profile by IL-17A and IFNγ staining. Representative plots are shown along with their mean frequencies ± SEM. Data pooled from 3 independent experiments, with each point representing an individual mouse. **p<0.01, ***p<0.001, ****p<0.0001 by two-tailed Mann-Whitney test. Refers to Fig. 6.



Supplementary Fig. 4 Gating Strategies. Representative gating strategies used for the flow cytometric analyses in **(a)** Fig. 1, **(b)** Fig. 3a, **(c)** Fig. 6a,d and **(d)** Fig. 7.