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Supplemental Information

Checkpoint Receptor TIGIT Expressed on Tim-1^{+} B Cells Regulates Tissue Inflammation

Sheng Xiao, Lloyd Bod, Nathalie Pochet, Savithri Balasubramanian Kota, Dan Hu, Asaf Madi, Jessica Kilpatrick, Jingwen Shi, Allen Ho, Huiyuan Zhang, Raymond Sobel, Howard L. Weiner, Terry B. Strom, Francisco J. Quintana, Nicole Joller, and Vijay K. Kuchroo

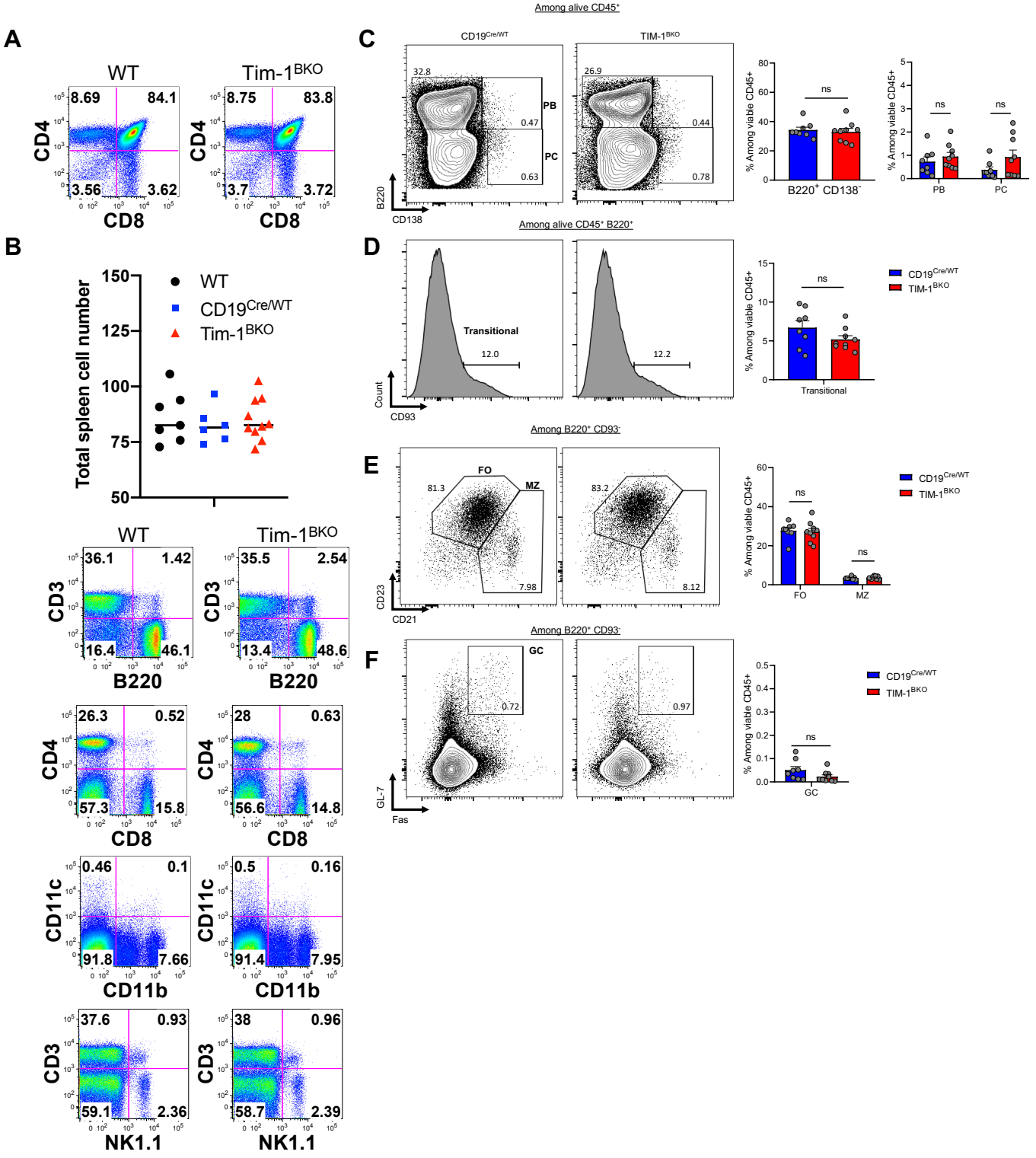

Figure S1. Normal development of T and B cells in Tim-1^{BKO} mice, Related to Figure 1. Representative FACS plots showing immune cell phenotypes in thymi (**A**) and spleens (**B**) in 6-8 weeks old mice (n=6-10). (**C-F**) Representative FACS plots and bar charts showing splenic B cell subsets from 8-10 weeks old CD19$^{Cre/WT}$ and Tim-1^{BKO} mice. Bar charts showing the frequencies of (**C**) CD138- B cells and Plasma cells (PC) and plasmablasts (PB), (**D**) Transitional, (**E**) Follicular (FO) and Marginal zone (MZ), (**F**) Germinal center (GC) B cells. Unpaired t –test. n.s., not significant. Data are represented as mean±SEM.

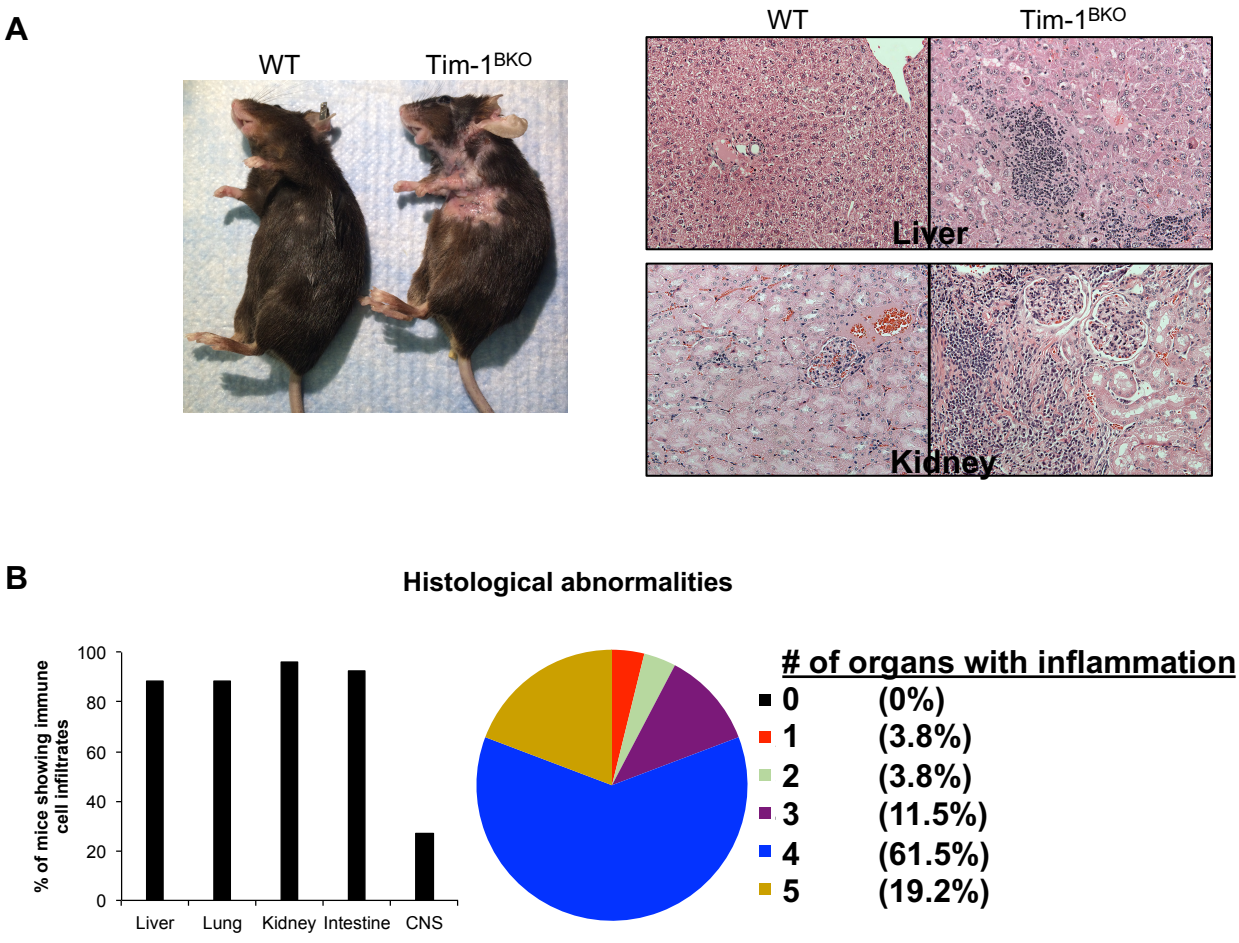

Figure S2. Ageing Tim-1^{BKO} mice develop spontaneous inflammation in multiple organs and tissues, Related to Figure 2. (**A**) Aged Tim-1^{BKO} mice showed dermatitis (left panel), and histologically aged Tim-1^{BKO} mice showed mixed lymphoid and granulocytic infiltrates in periportal areas of livers, and mononuclear cell infiltrates in the renal cortex interstitium (Right panel). (**B**) Bar graph and pie chart showing the frequency of Tim-1^{BKO} mice with spontaneous inflammation in the indicated organs and tissues.

A

Strain	Number of mice	Incedence of EAE	Mean onset date	Mean maximal score	Mean cumulative score[a]
WT	8	100%	14.4±2.07	2.5±0.5	34.2±11.3
Tim-1^{BKO}	8	100%	11.4±1.52*	3.3±0.27	50.6±8.59*
The values shown are the mean±SEM					
[a]The cumulative score was calculated by adding the disease score from the day of onset to day 30					
* P<0.01					

B

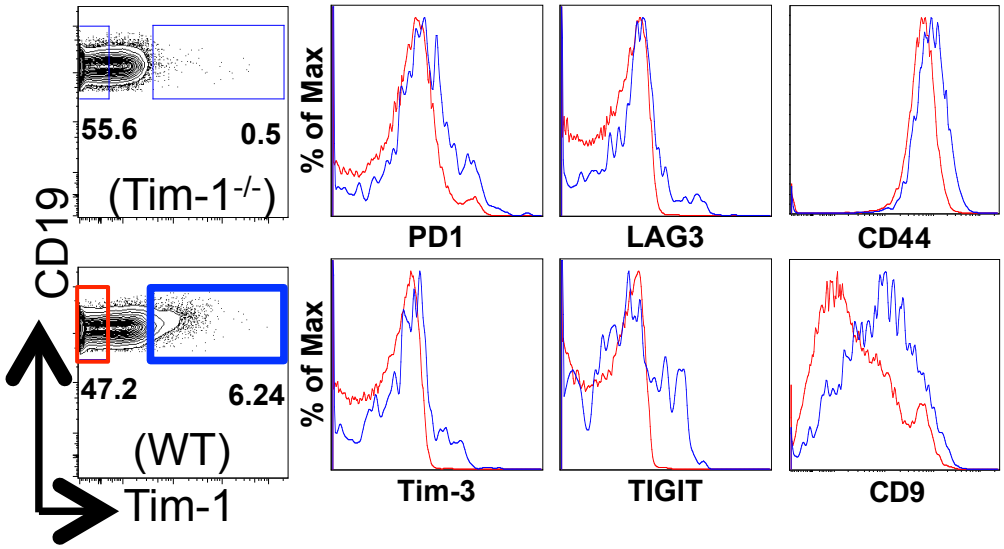

Figure S3. EAE development in young Tim-1^{BKO} mice and surface molecule expression on Tim-1^{+} B cells, Related to Figure 3. (A) Summary table of clinical EAE in Tim-1^{BKO} mice (6-8 weeks of age; n=8) immunized with MOG_{35-55}/CFA; (**B**) Representative FACS plots showing expression of indicated cell surface molecules in Tim-1^{+} vs Tim-1^{-} B cells from 8-week old WT mice (n=3).

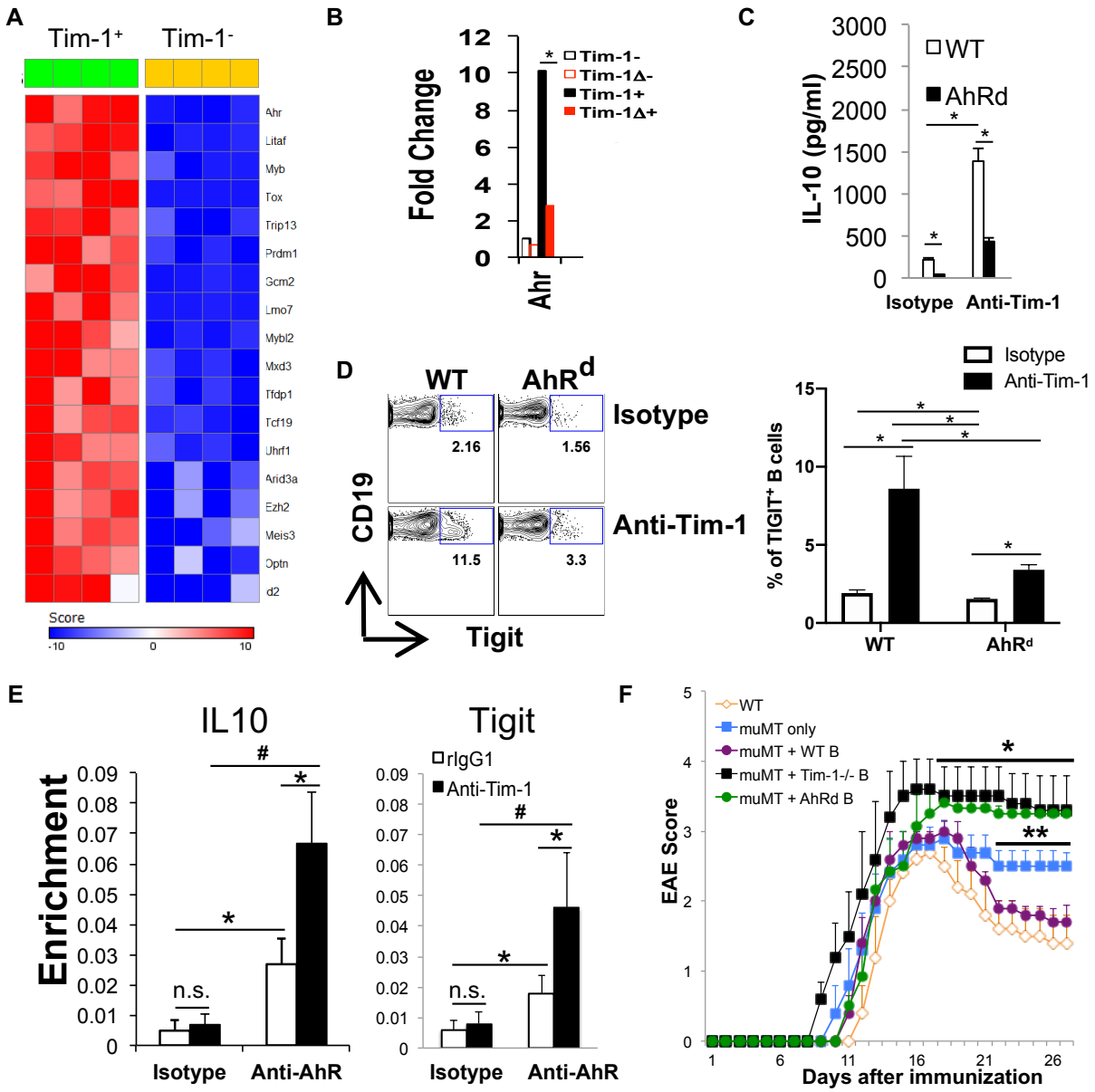

Figure S4. Role of AhR in B cell regulatory function, Related to Figure 4. (**A**) Heatmap of a selection of genes from **Figure 2E** showing expression of TFs in isolated Tim-1^{+} vs Tim-1^{-} B cells. (**B**) qPCR measurement of *AhR* mRNA expression in WT Tim-1^{-} and Tim-1^{+} B cells and Tim-1$^{\Delta mucin-}$ and Tim-1$^{\Delta mucin+}$ B cells, normalized to expression in WT Tim-1^{-} B cells. *P<0.01 (Tim-1^{+} vs. Tim-1$^{\Delta mucin+}$ cells; n=4). (**C-D**) B cells from WT and AhR^{d} mice (n=6) were treated with anti-Tim-1 or control rIgG1. Three days after, IL-10 production (**C**) in culture supernatants was measured by ELISA, and TIGIT expression (**D**) was determined by flow cytometry; * P<0.01. **E**) AhR ChIP-PCR in the XRE in the IL10 and Tigit promoters in WT B cells (n=5) treated for 24 h with anti-Tim-1 or rIgG1; *P<0.01; $^{\#}$P<0.001. **F**) WT, AhR^{d}, and Tim-1$^{-/-}$ B cells were transferred into B cell deficient muMT mice (n=8/group) and then induced EAE with MOG_{35-55}/CFA. Clinical EAE development was monitored daily. * P<0.01. ** P<0.05. Data are represented as mean±SEM.

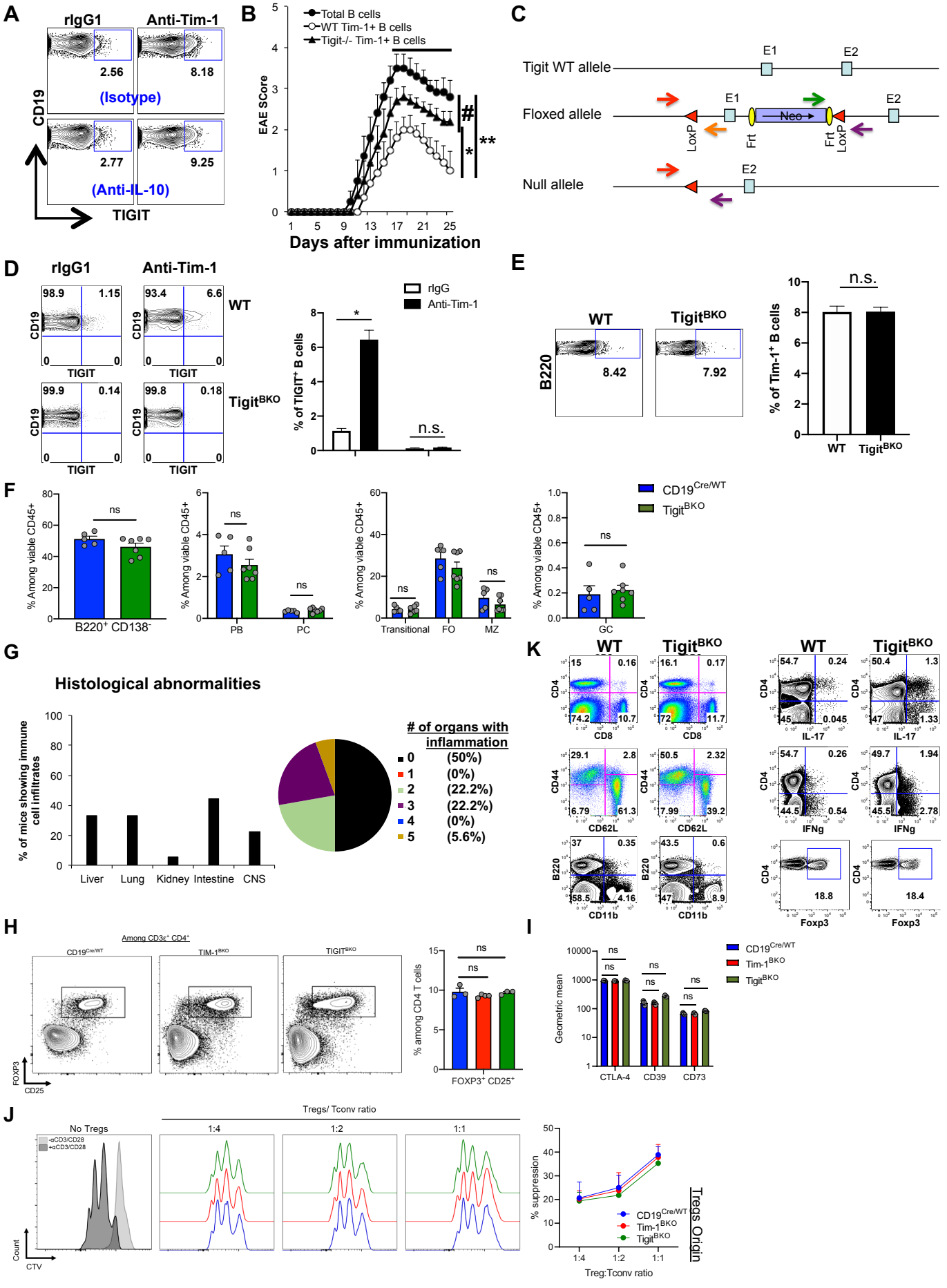
A
rIgG1
Anti-Tim-1
2.56
8.18
(Isotype)
2.77
9.25
(Anti-IL-10)
CD19
TIGIT
B
Total B cells
WT Tim-1+ B cells
Tigit-/- Tim-1+ B cells
EAE SCore
Days after immunization
#
*
**
C
Tigit WT allele
Floxed allele
Null allele
E1
E2
Neo
LoxP
Frt
D
rIgG1
Anti-Tim-1
WT
TigitBKO
98.9
1.15
93.4
6.6
99.9
0.14
99.8
0.18
rIgG
Anti-Tim-1
% of TIGIT+ B cells
n.s.
E
WT
TigitBKO
B220
8.42
7.92
% of Tim-1+ B cells
n.s.
F
CD19Cre/WT
TigitBKO
% Among viable CD45+
B220+ CD138-
PB
PC
Transitional
FO
MZ
GC
ns
G
Histological abnormalities
% of mice showing immune cell infiltrates
Liver
Lung
Kidney
Intestine
CNS
# of organs with inflammation
0 (50%)
1 (0%)
2 (22.2%)
3 (22.2%)
4 (0%)
5 (5.6%)
K
WT
TigitBKO
CD4
CD8
CD44
CD62L
B220
CD11b
IL-17
IFNg
Foxp3
H
Among CD3ε+ CD4+
CD19Cre/WT
TIM-1BKO
TIGITBKO
FOXP3
CD25
% among CD4 T cells
FOXP3+ CD25+
I
CD19Cre/WT
Tim-1BKO
TigitBKO
Geometric mean
CTLA-4
CD39
CD73
J
Tregs/ Tconv ratio
No Tregs
1:4
1:2
1:1
-αCD3/CD28
+αCD3/CD28
Count
CTV
% suppression
Treg:Tconv ratio
Tregs Origin

Figure S5. Immunoregulatory role of TIGIT in B cells, Related to Figure 4. (**A**) Representative FACS plots showing TIGIT expression in B cells from 8-week old WT mice (n=4) after the cells were treated with anti-Tim-1 or control rIgG1 in the presence or absence of anti-IL-10 blocking mAb for 3 days. (**B**) Total WT B cells, WT Tim-1^{+} B cells, or Tigit$^{-/-}$ Tim-1^{+} B cells from 8-week old mice were transferred into 8-week old WT mice; the recipients were then immunized with MOG_{35-55}/CFA to induce EAE. Mice were scored daily for clinical signs of EAE (n =8 per group). *, ** P<0.01; #P<0.05. (**C**) Strategy of generating Tigit floxed mice. (**D**) B cells isolated from 6-8 weeks old mice (n=4) were activated with anti-Tim-1 or control rIgG for three days and then examined for TIGIT expression by flow cytometry. (**E**) B cells isolated from 6-8 weeks old mice (n=3) were examined for Tim-1 expression by flow cytometry. (**F**) Bar charts showing the frequencies of CD138^{-} B cells, Plasma cells (PC) and plasmablasts (PB), Transitional, Follicular (FO) and Marginal zone (MZ), Germinal center (GC) B cells. (**G**) Bar graph and pie chart showing the frequency of Tigit^{BKO} mice with spontaneous inflammation in different organs and tissues. (**H**) Representative FACS plots and bar charts showing Tregs frequency and (**I**) bar charts showing the median fluorescent intensity (MFI) of CTLA-4, CD39 and CD73 on Tregs from peripheral lymph nodes from 8 weeks old CD19$^{Cre/WT}$ and Tim-1^{BKO} or Tigit^{BKO} mice. (**J**) CD3^{+}CD4^{+}CD25^{High} cells from CD19$^{Cre/WT}$ (n=3, Blue line), Tim-1^{BKO} (n=4, Red line) and TIGIT^{BKO} (n=3, Green line) mice were sorted using flow cytometry, and their suppressive abilities to inhibit the proliferation of Cell Trace Violet-labelled conventional CD4 T cells (CD3^{+}CD4^{+}CD25^{-} from CD19$^{Cre/WT}$ mice) in the presence of anti-CD3/anti-CD28 coated beads were tested. Proliferation was measured and percent suppression was calculated as described in Methods. (**K**) Representative FACS plots showing immune cell phenotypes in spleens of aged Tigit^{BKO} mice with paralysis (n=4). Unpaired t –test. n.s., not significant. Data are represented as mean±SEM.