SUPPLEMENTAL DATASETS

Data S1. Related to Figures 1-7.

A, Distribution of *TBL1XR1*^{MUT} cases into GCB and ABC subtypes, for the three DLBCL cohorts in **Figure 1A**. Numbers represent cases. *P-values* correspond to the comparison between *TBL1XR1* mutation incidences in ABC vs. GCB, for each cohort, using Fisher's exact test.

B, Distribution of *TBL1XR1*^{MUT} cases form the Scott & Morin cohort (Arthur et al., 2018) into novel DLBCL subtypes, following the classification criteria in (Schmitz et al., 2018).

C, *TBL1XR1* mutation co-occurrence in ABC-DLBCL cases, for the cohorts in **AA**, calculated using a hypergeometric test.

D, Complete FC gating strategy for **Figures 1C-D**.

E, Absolute FC-based quantification of (left) total splenic B-cells or (right) GCB from *Tbl1xr1*$^{D370Y/WT}$ or *Tbl1xr1*$^{WT/WT}$ mice, 9 days after SRBC immunization.

F, Representative FC plots (left) and quantification (right) of splenic GCB, using an alternative gating strategy to that presented in **Figure 1D**.

G, Representative FC plots (left) and quantification (right) of CXCR4 and CD86 expression in splenic GCB. Quantification is expressed as a ratio of "DZ" ($B220^+FAS^+GL7^+CXCR4^+CD86^-$) and "LZ" ($B220^+FAS^+GL7^+CXCR4^-CD86^+$) populations, per animal.

H, Representative FC plots (left) and quantification (right) of the relative contribution of *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ cells to the total B-cell and GCB compartments, based on CD45 allelic frequencies. Scheme on top shows the different ratios of *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ BM cells used for transplants.

I, Representative FC plots (left) and quantification (right) of the relative contribution of *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ cells to the total B-cell and GCB compartments, based on CD45 allelic frequencies.

J, Representative FC plots (left) and quantification (right) of the relative contribution of *Tbl1xr1*$^{D370Y/WT}$ and *Tbl1xr1*$^{WT/WT}$ cells to the total B-cell and GCB compartments, based

on CD45 allelic frequencies. Use of CD45 allelic variants in BM donors was inverted, as compared to results in **Figure 1I**.

K, Complete FC gating strategy for **Figure 1K**. A representative plot for NB cells is included to illustrate that YFP expression, under the *Cγ1Cre-Rosa26YFPstop* system, is limited to activated B-cells (see scheme in **Figure 1J**).

L, FC analysis of splenic YFP^+ GCB frequency, using an alternative GC gating strategy to that in **Figure 1K**. Left to right: n = 4, 5, 5, 4 (*Tbl1xr1*$^{WT/WT}$) or 4, 5, 5, 4 mice (*Tbl1xr1*$^{D370Y/WT}$).

M, Schematic representation of *TBL1XR1* focal deletions observed in DLBCL patients, from the cohort in Schmitz *et al*.(Schmitz et al., 2018).

N, Representative FC plots (left) and quantification (right) of splenic GCB, using an alternative gating strategy to that presented in **Figure S1K**.

O, Representative FC plots (left) and quantification (right) of CXCR4 and CD86 expression in splenic GCB. Quantification is expressed as a ratio of “DZ” and “LZ” populations, per animal.

P, Complete FC gating strategy for **Figures 2A** and **S2B**.

Q, Complete FC gating strategy for **Figures 2G-H**. A representative plot for NB cells is included to illustrate the *R26-Fucci2aR* system function in a non-proliferating cell population.

R, FC analysis of (left) $EFNB1^+$ or (right) preMB populations in splenic GCB, at (top) 4 days or (bottom) 15 days post SRBC immunization.

S, Representative FC plots (left) and quantification (right) of preMB populations in splenic GCB.

T, Representative FC plots (left) and quantification (right) of EdU incorporation by splenic GCB.

U, Complete FC gating strategy for **Figures 4D-G**.

V, Complete FC gating strategy for **Figure 4H**.

W, FC analysis of total splenic B-cells in *Tbl1xr1*$^{WT/WT}$, *IμBcl6-Tbl1xr1*$^{WT/WT}$ and *IμBcl6-Tbl1xr1*$^{D370Y/WT}$ mice, 9 days after SRBC immunization.

X, GSEA of gene signatures upregulated and downregulated in *IμBcl6-Tbl1xr1*$^{D370Y/WT}$ vs *IμBcl6-Tbl1xr1*$^{WT/WT}$ GCB, ranked against the preMB (EFNB1^{+}S1PR2^{lo}) GCB signature from GSE89897.

Y, FC analysis of (top) EFNB1^{+} or (bottom) preMB populations in splenic GCB, for animals in **Figure 5F**.

Z, Scheme depicting key transcription factors involved in post GCB fate.

AA, Schematic depiction of the proposed mechanism of action of *TBL1XR1* mutations or loss. Top: In a normal scenario, TBL1XR1 and its close homolog TBL1X form tetramers that can directly bind SMRT via their N-terminal domains (Oberoi et al., 2011). TBL1XR1 enables the association between the SMRT-HDAC3 complex and BCL6 (data herein). This requires the interaction between TBL1XR1's WD40 domain and an unknown putative partner. Middle: DLBCL mutations of TBL1XR1 allow TBL1XR1^{MUT} recruitment to the complex, but impair the function of the WD40 domain, specifically reducing the formation of BCL6-SMRT complexes, so that the complex now binds preferentially to BACH2. Bottom: Upon complete loss of TBL1XR1, the complex can still assemble due to the presence of the close homolog protein TBL1X, but it not cannot form a productive interaction with BCL6, thus favoring the association with BACH2 instead. Importantly, we cannot rule out that this "TBL1X only" version of the SMRT complex might also differ from the WT complex in other ways that are not captured in our assays.

BB, Complete FC gating strategy for **Figure 6A**.

CC, Complete FC gating strategy for **Figure 6B**.

DD, Complete FC gating strategy for YFP^{+} MB in **Figure 6D**.

EE, Complete FC gating strategy for **B** and **C**.

FF, Representative FC plots (bottom) and quantification (top) of B-cell lineage populations in the BM of 4m old *CD19Cre-Tbl1xr1*$^{D370Y/WT}$ or *CD19Cre-Tbl1xr1*$^{WT/WT}$ mice.

GG, Representative FC plots (bottom) and quantification (top) of B-cell lineage populations in the BM of 4m old *CD19Cre-Tbl1xr1*$^{KO/KO}$ or *CD19Cre-Tbl1xr1*$^{WT/WT}$ mice.

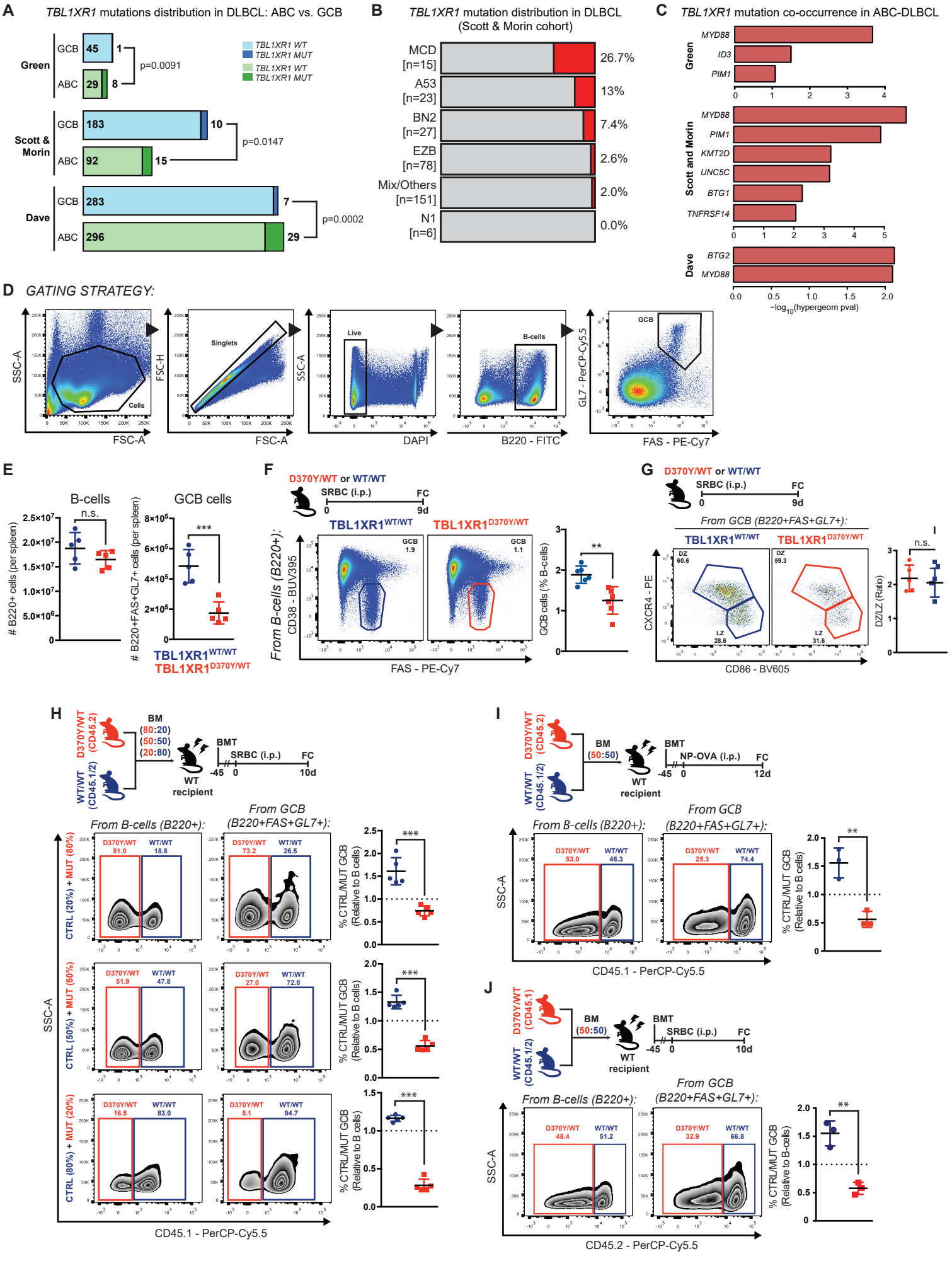
HH, Representative FC plots for PreMB quantification in **Figure 7F**.

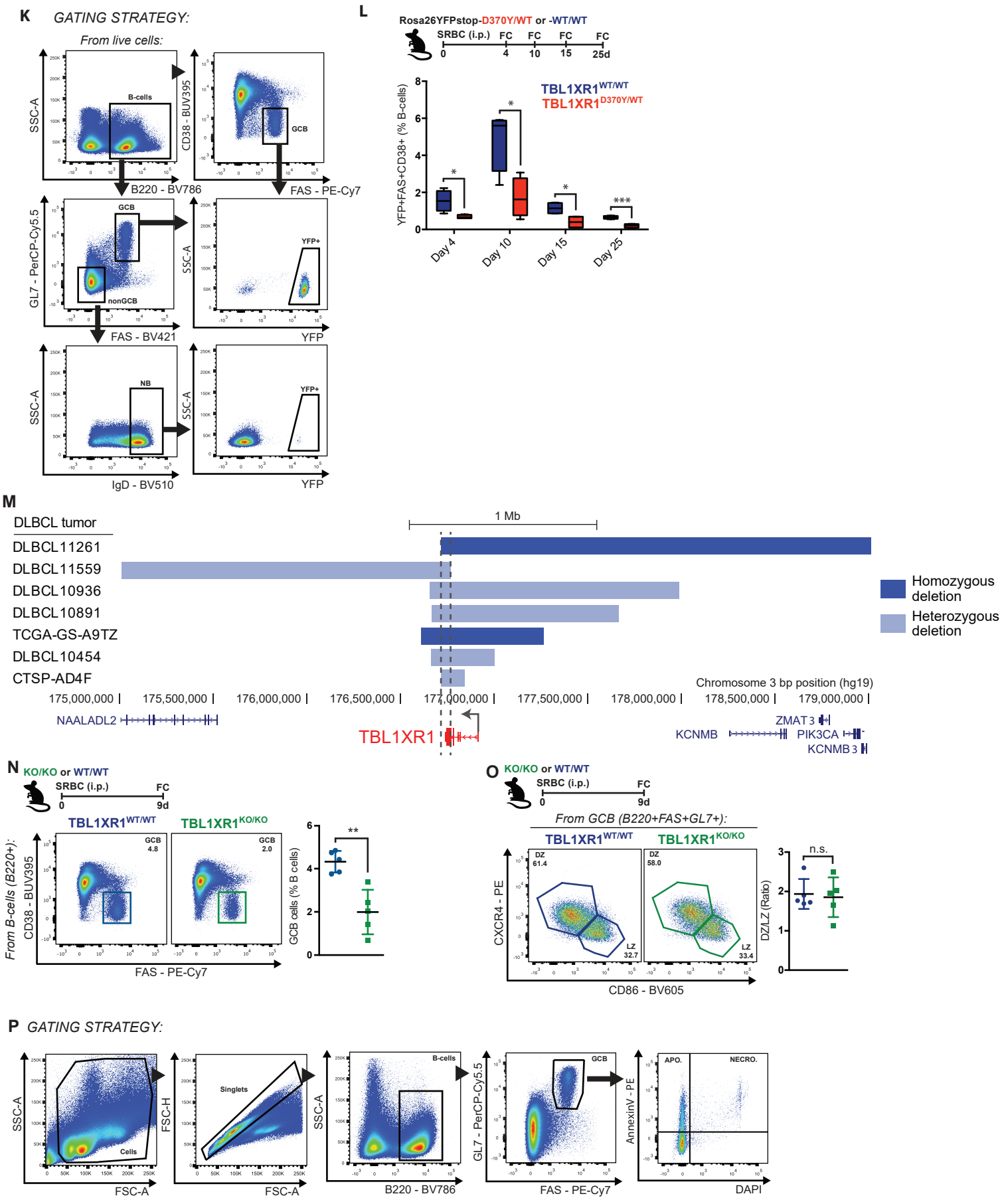
II, Representative FC plots for MB quantification in **Figure 7G**.

JJ-KK, Representative FC plots (left) and quantification (right) of (**JJ**) total B-cells or (**K**) mature and immature B-cells, in the BM of animals in **Figure 7A**.

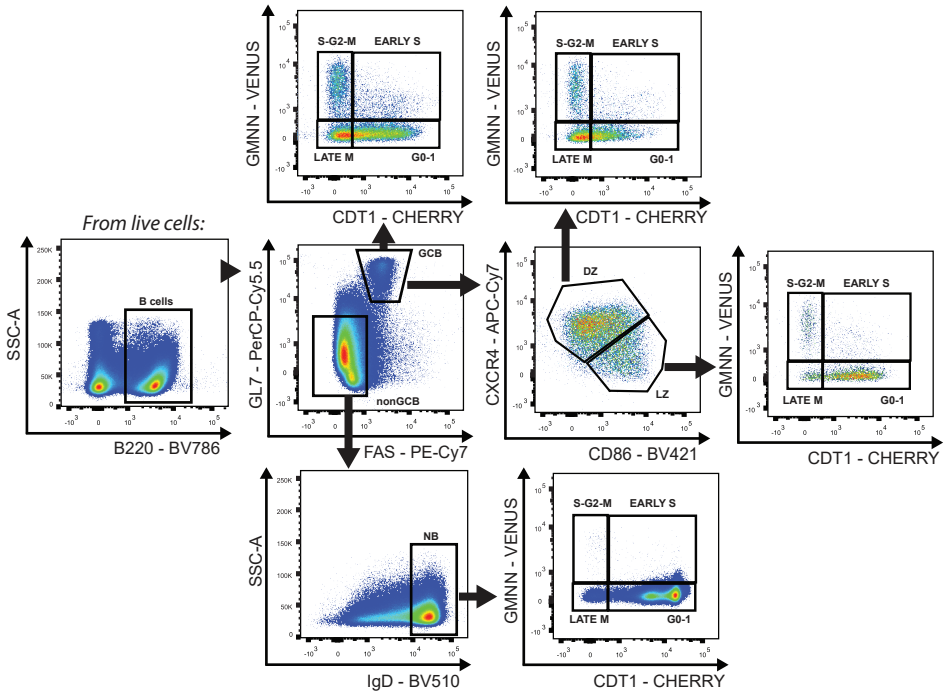
Values represent mean ± SEM. All data were reproducible with at least two repeats. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001, using unpaired (**E-G,L,N,O,R,FF-GG, JJ-KK**) or paired (**H-J**) two-tailed Student's t-test; or one-way ANOVA (**S-T,W,Y**) with Tukey's post-test.

DATA S1

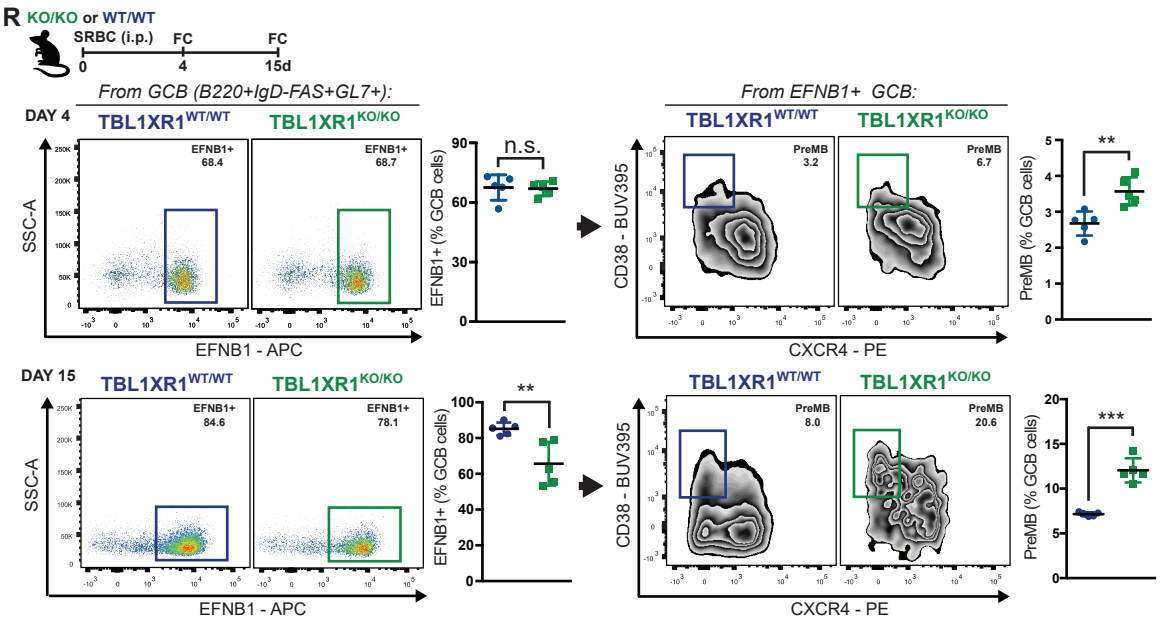

A
TBL1XR1 mutations distribution in DLBCL: ABC vs. GCB
Green
GCB 45 1
ABC 29 8
p=0.0091
TBL1XR1 WT
TBL1XR1 MUT
Scott & Morin
GCB 183 10
ABC 92 15
p=0.0147
Dave
GCB 283 7
ABC 296 29
p=0.0002
B
TBL1XR1 mutation distribution in DLBCL (Scott & Morin cohort)
MCD [n=15] 26.7%
A53 [n=23] 13%
BN2 [n=27] 7.4%
EZB [n=78] 2.6%
Mix/Others [n=151] 2.0%
N1 [n=6] 0.0%
C
TBL1XR1 mutation co-occurrence in ABC-DLBCL
Green
MYD88
ID3
PIM1
Scott and Morin
MYD88
PIM1
KMT2D
UNC5C
BTG1
TNFRSF14
Dave
BTG2
MYD88
−log10(hypergeom pval)
D
GATING STRATEGY:
SSC-A
Cells
FSC-A
FSC-H
Singlets
Live
DAPI
B-cells
B220 - FITC
GL7 - PerCP-Cy5.5
GCB
FAS - PE-Cy7
E
B-cells
GCB cells
n.s.
***
B220+ cells (per spleen)
B220+FAS+GL7+ cells (per spleen)
TBL1XR1WT/WT
TBL1XR1D370Y/WT
F
D370Y/WT or WT/WT
SRBC (i.p.)
FC
0
9d
TBL1XR1WT/WT
TBL1XR1D370Y/WT
From B-cells (B220+):
CD38 - BUV395
FAS - PE-Cy7
GCB 1.9
GCB 1.1
GCB cells (% B-cells)
**
G
D370Y/WT or WT/WT
SRBC (i.p.)
FC
0
9d
From GCB (B220+FAS+GL7+):
TBL1XR1WT/WT
TBL1XR1D370Y/WT
CXCR4 - PE
CD86 - BV605
DZ 60.6
LZ 28.6
DZ 59.3
LZ 31.6
DZ/LZ (Ratio)
n.s.
H
D370Y/WT (CD45.2)
WT/WT (CD45.1/2)
BM
(80:20)
(50:50)
(20:80)
WT recipient
BMT
SRBC (i.p.)
FC
-45
0
10d
From B-cells (B220+):
From GCB (B220+FAS+GL7+):
CTRL (20%) + MUT (80%)
CTRL (50%) + MUT (50%)
CTRL (80%) + MUT (20%)
D370Y/WT
WT/WT
81.0
18.8
73.2
26.5
51.9
47.8
27.0
72.8
16.5
83.0
5.1
94.7
SSC-A
CD45.1 - PerCP-Cy5.5
% CTRL/MUT GCB (Relative to B cells)
***
I
D370Y/WT (CD45.2)
WT/WT (CD45.1/2)
BM
(50:50)
WT recipient
BMT
NP-OVA (i.p.)
FC
-45
0
12d
From B-cells (B220+):
From GCB (B220+FAS+GL7+):
D370Y/WT
WT/WT
53.0
46.3
25.3
74.4
SSC-A
CD45.1 - PerCP-Cy5.5
% CTRL/MUT GCB (Relative to B cells)
**
J
D370Y/WT (CD45.1)
WT/WT (CD45.1/2)
BM
(50:50)
WT recipient
BMT
SRBC (i.p.)
FC
-45
0
10d
From B-cells (B220+):
From GCB (B220+FAS+GL7+):
D370Y/WT
WT/WT
48.4
51.2
32.9
66.8
SSC-A
CD45.2 - PerCP-Cy5.5
% CTRL/MUT GCB (Relative to B-cells)
**


K
GATING STRATEGY:
From live cells:
SSC-A
B-cells
B220 - BV786
CD38 - BUV395
GCB
FAS - PE-Cy7
GL7 - PerCP-Cy5.5
nonGCB
FAS - BV421
YFP+
YFP
NB
IgD - BV510
L
Rosa26YFPstop-D370Y/WT or -WT/WT
SRBC (i.p.)
FC
0
4
10
15
25d
TBL1XR1WT/WT
TBL1XR1D370Y/WT
YFP+FAS+CD38+ (% B-cells)
Day 4
Day 10
Day 15
Day 25
M
DLBCL tumor
1 Mb
DLBCL11261
DLBCL11559
DLBCL10936
DLBCL10891
TCGA-GS-A9TZ
DLBCL10454
CTSP-AD4F
Homozygous deletion
Heterozygous deletion
Chromosome 3 bp position (hg19)
175,000,000
175,500,000
176,000,000
176,500,000
177,000,000
177,500,000
178,000,000
178,500,000
179,000,000
NAALADL2
TBL1XR1
ZMAT3
KCNMB
PIK3CA
KCNMB3
N
KO/KO or WT/WT
SRBC (i.p.)
FC
0
9d
TBL1XR1WT/WT
TBL1XR1KO/KO
From B-cells (B220+):
CD38 - BUV395
GCB
4.8
2.0
FAS - PE-Cy7
GCB cells (% B cells)
**
O
KO/KO or WT/WT
SRBC (i.p.)
FC
0
9d
From GCB (B220+FAS+GL7+):
TBL1XR1WT/WT
TBL1XR1KO/KO
CXCR4 - PE
DZ
61.4
58.0
LZ
32.7
33.4
CD86 - BV605
DZ/LZ (Ratio)
n.s.
P
GATING STRATEGY:
SSC-A
Cells
FSC-A
FSC-H
Singlets
B-cells
B220 - BV786
GL7 - PerCP-Cy5.5
GCB
FAS - PE-Cy7
AnnexinV - PE
APO.
NECRO.
DAPI

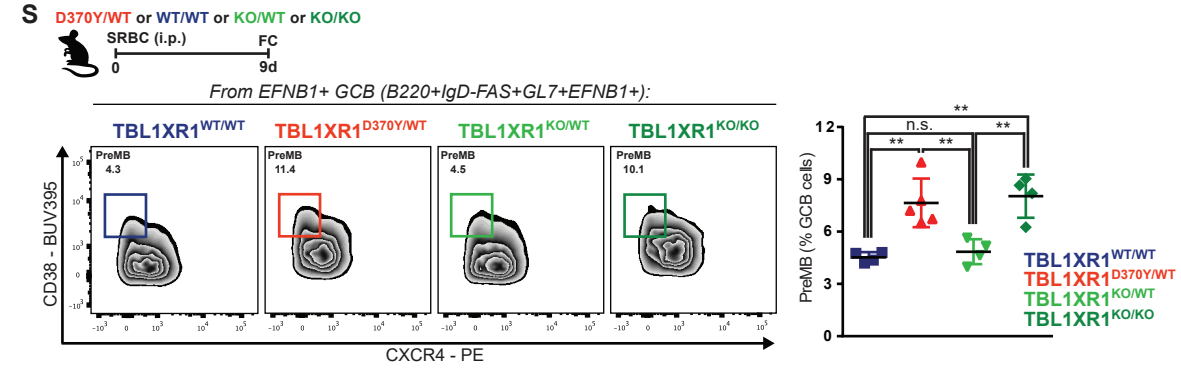
Q *GATING STRATEGY:*



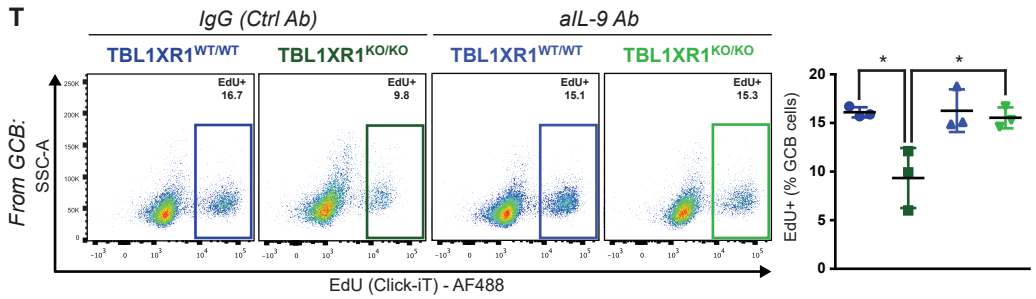
R KO/KO or WT/WT

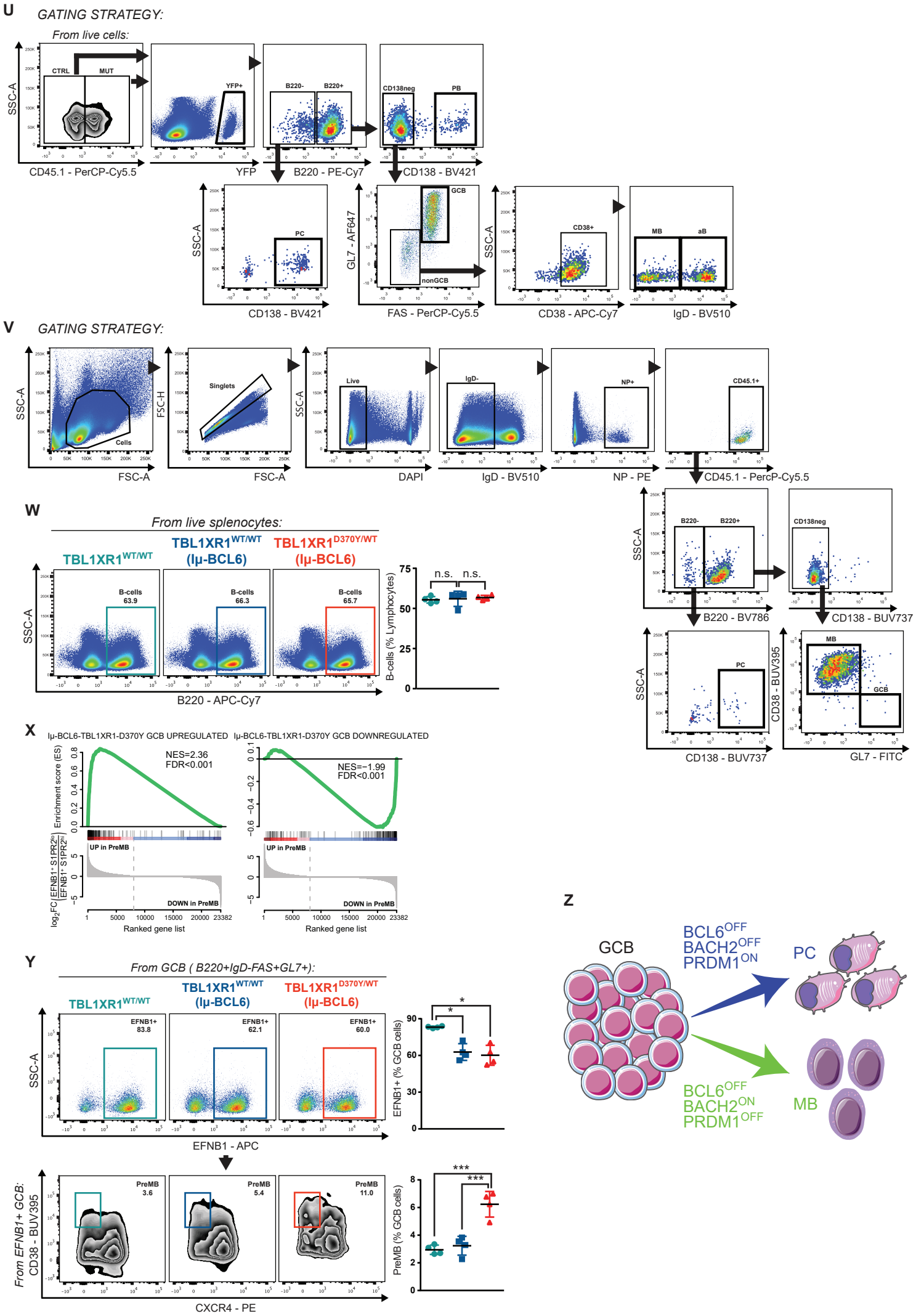


S D370Y/WT or WT/WT or KO/WT or KO/KO

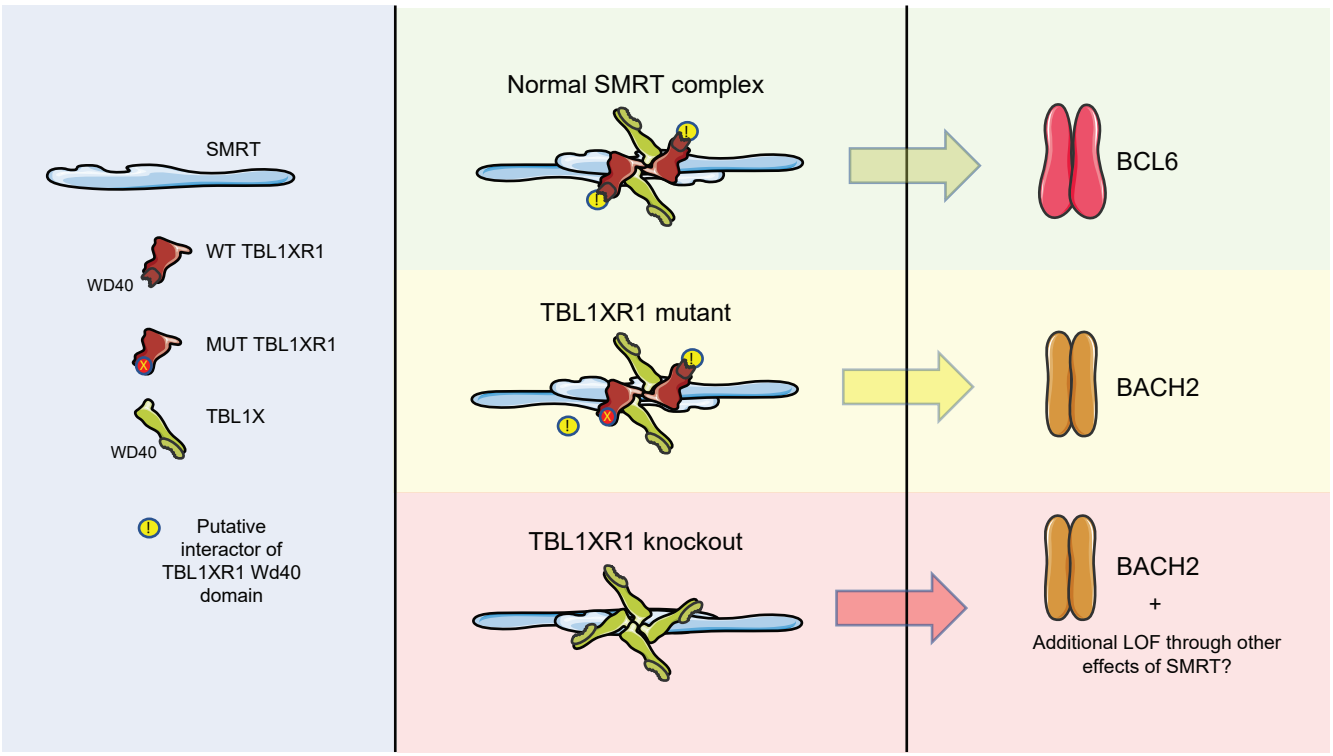


T



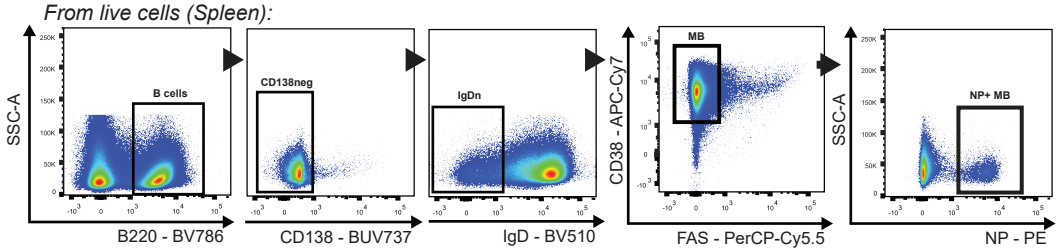

U
GATING STRATEGY:
From live cells:
CTRL
MUT
YFP+
B220-
B220+
CD138neg
PB
SSC-A
CD45.1 - PerCP-Cy5.5
YFP
B220 - PE-Cy7
CD138 - BV421
PC
GCB
nonGCB
GL7 - AF647
FAS - PerCP-Cy5.5
CD38+
CD38 - APC-Cy7
MB
aB
IgD - BV510
V
GATING STRATEGY:
Cells
FSC-A
FSC-H
Singlets
Live
DAPI
IgD-
NP+
NP - PE
CD45.1+
W
From live splenocytes:
TBL1XR1WT/WT
TBL1XR1WT/WT (Iμ-BCL6)
TBL1XR1D370Y/WT (Iμ-BCL6)
B-cells 63.9
B-cells 66.3
B-cells 65.7
B220 - APC-Cy7
B-cells (% Lymphocytes)
n.s.
n.s.
B220 - BV786
CD138 - BUV737
PC
MB
GCB
CD38 - BUV395
GL7 - FITC
X
Iμ-BCL6-TBL1XR1-D370Y GCB UPREGULATED
Iμ-BCL6-TBL1XR1-D370Y GCB DOWNREGULATED
NES=2.36
FDR<0.001
NES=−1.99
FDR<0.001
Enrichment score (ES)
UP in PreMB
DOWN in PreMB
log2FC (EFNB1+ S1PR2hi / EFNB1+ S1PR2lo)
Ranked gene list
Y
From GCB (B220+IgD-FAS+GL7+):
TBL1XR1WT/WT
TBL1XR1WT/WT (Iμ-BCL6)
TBL1XR1D370Y/WT (Iμ-BCL6)
EFNB1+ 83.8
EFNB1+ 62.1
EFNB1+ 60.0
EFNB1 - APC
EFNB1+ (% GCB cells)
From EFNB1+ GCB:
CD38 - BUV395
PreMB 3.6
PreMB 5.4
PreMB 11.0
CXCR4 - PE
PreMB (% GCB cells)
Z
GCB
BCL6OFF BACH2OFF PRDM1ON
PC
BCL6OFF BACH2ON PRDM1OFF
MB

AA



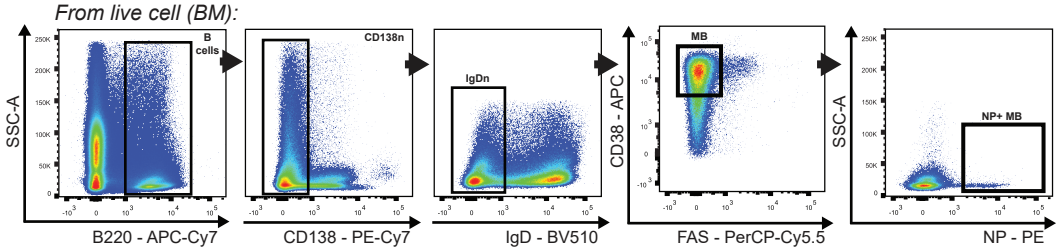
BB *GATING STRATEGY*

From live cells (Spleen):



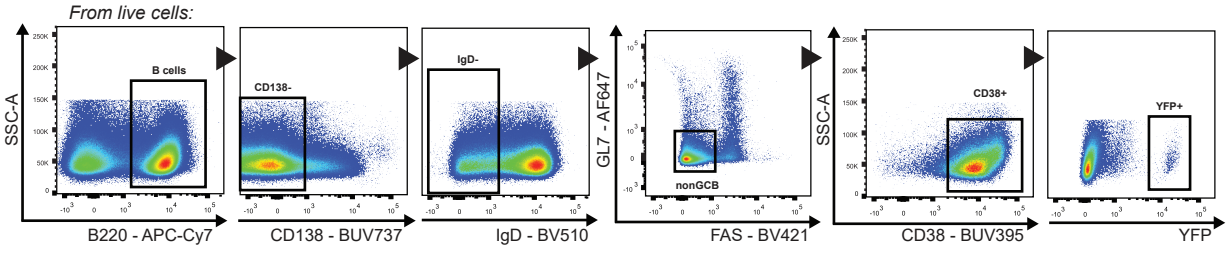
CC *GATING STRATEGY*

From live cell (BM):

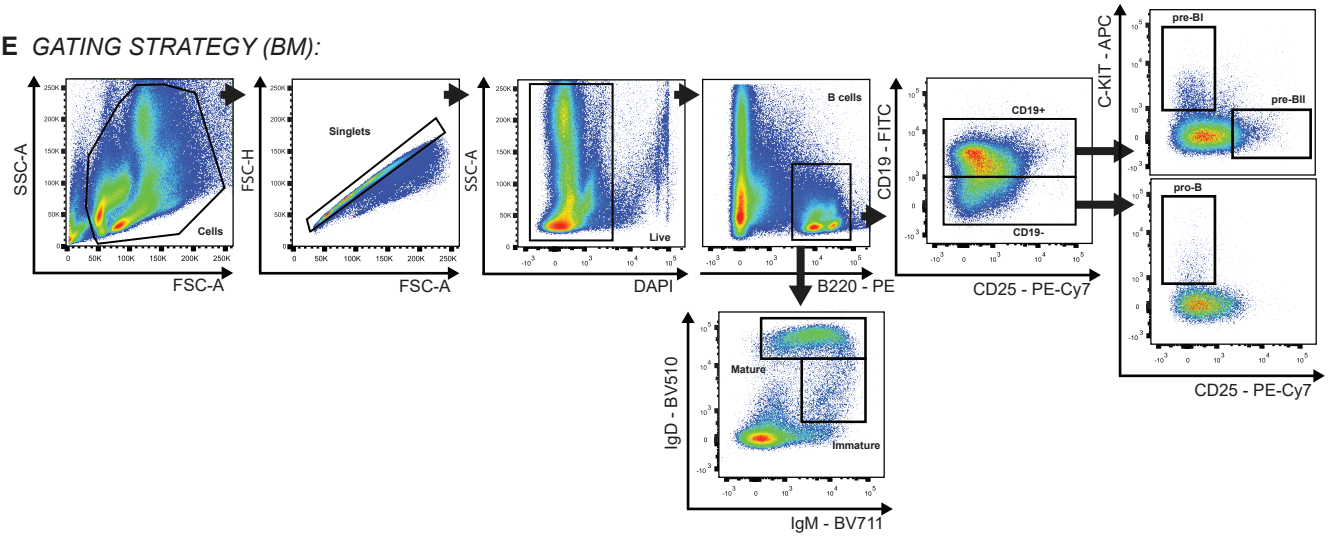


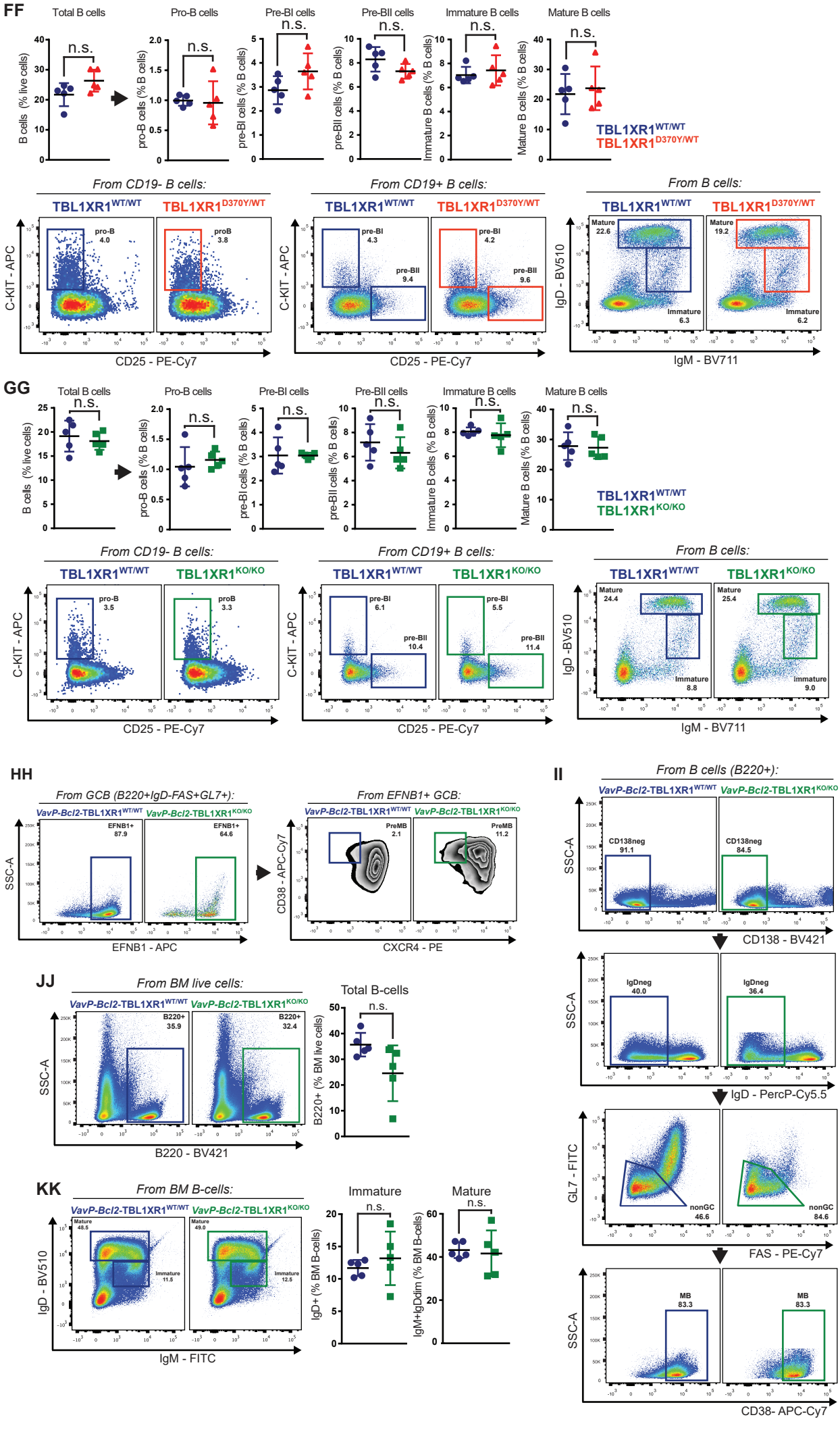
DD *GATING STRATEGY*

From live cells:



EE *GATING STRATEGY (BM):*




FF
Total B cells
Pro-B cells
Pre-BI cells
Pre-BII cells
Immature B cells
Mature B cells
B cells (% live cells)
pro-B cells (% B cells)
pre-BI cells (% B cells)
pre-BII cells (% B cells)
Immature B cells (% B cells)
Mature B cells (% B cells)
n.s.
TBL1XR1WT/WT
TBL1XR1D370Y/WT
From CD19- B cells:
From CD19+ B cells:
From B cells:
C-KIT - APC
CD25 - PE-Cy7
IgD - BV510
IgM - BV711
pro-B 4.0
proB 3.8
pre-BI 4.3
pre-BII 9.4
pre-BI 4.2
pre-BII 9.6
Mature 22.6
Immature 6.3
Mature 19.2
Immature 6.2
GG
TBL1XR1WT/WT
TBL1XR1KO/KO
pro-B 3.5
proB 3.3
pre-BI 6.1
pre-BII 10.4
pre-BI 5.5
pre-BII 11.4
Mature 24.4
Immature 8.8
Mature 25.4
Immature 9.0
HH
From GCB (B220+IgD-FAS+GL7+):
From EFNB1+ GCB:
VavP-Bcl2-TBL1XR1WT/WT
VavP-Bcl2-TBL1XR1KO/KO
SSC-A
EFNB1 - APC
CD38 - APC-Cy7
CXCR4 - PE
EFNB1+ 87.9
EFNB1+ 64.6
PreMB 2.1
PreMB 11.2
II
From B cells (B220+):
CD138neg 91.1
CD138neg 84.5
CD138 - BV421
IgDneg 40.0
IgDneg 36.4
IgD - PercP-Cy5.5
GL7 - FITC
FAS - PE-Cy7
nonGC 46.6
nonGC 84.6
MB 83.3
MB 83.3
CD38- APC-Cy7
JJ
From BM live cells:
Total B-cells
B220+ 35.9
B220+ 32.4
B220 - BV421
B220+ (% BM live cells)
KK
From BM B-cells:
Immature
Mature
IgD - BV510
IgM - FITC
IgD+ (% BM B-cells)
IgM+IgDdim (% BM B-cells)
Mature 48.5
Immature 11.5
Mature 49.0
Immature 12.5