Supplemental information:

Suppl. materials and methods

Cell death assays

The percentages of viable cells was determined by staining single cell suspensions with 10 μg/ml 7AAD plus Annexin-V coupled either with Pacific Blue or APC, alone or in combination with additional cell surface marker specific antibodies. Samples were analyzed by flow cytometry.

CPD labelling

Cells were labelled with CPD eFluor 670 according to the manual (eBioscience). After three days cells were stained with anti-IgM and anti-IgD or anti-CD4 and anti-CD8 antibodies.

Cell culture and reagents

Cells were cultured at 37°C in a humidified atmosphere containing 5% CO2. 293T cells were cultured in the DME medium supplemented with 10% FCS (PAA), 250μM L-glu (Invitrogen) and penicillin/streptomycin (1U/ml, Sigma-Aldrich). Transfections were performed using Lipofectamine 2000 (Invitrogen). Primary hematopoietic cells and WEHI231 cells were cultured in RPMI 1640 medium (PAA), supplemented with 250μM L-glutamine (Gibco), 50μM 2-mercaptoethanol, 1mM sodium pyruvate (Gibco), 100μM liquid non-essential amino acids (Gibco), penicillin/streptomycine (Sigma) and 10% FCS (Gibco). For the induction of cell death the following reagents were used: 1 μg/ml Ionomycin (Sigma), 10ng/ml PMA (Sigma); and the 10^{-7}M dexamethasone; goat anti-mouse IgM $F(ab')_2$ fragments (Jackson Immuno-

Research), hamster anti-mouse CD3 mAb (clone 3C11) and anti-mouse CD28 mAb (clone 37.51).

Viral transduction procedures & vector information

For viral titre determination we used 1:10, 1:30, 1:100, 1:300, 1:1000,1:3000 of the viral concentrate to transduce 10 000 HEK 293T cells. On day 3 GFP expression was analysed by FACS and the viral titres determined by correlating the percent GFP^+ positive cells to the number of viral particles.The lentiviral construct used for transgenesis is based on the FUGW vector described by Lois et al (Science. 2002 Feb 1;295(5556):868-72. Epub 2002 Jan 10). Upstream of the Ubiquitin (U) promoter we incorporated the TRE driving the shA1 in the context of the miR30 background TmiR-A1 (Proc Natl Acad Sci USA. 2005 Sep 13;102(37):13212-7. Epub 2005 Sep 1.). F = HIV flap-fragment, W = woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).

Supplemental Figures:

Suppl. Figure 1: (A) 293T cells expressing FLAG-A1a were transiently transfected with lentiviral constructs harbouring one of two different shRNAs targeting all A1 isoforms. Knockdown efficiency was assessed 48h after transfection by Western blotting using anti-FLAG-M2 antibody. (B) Biogenesis of a siRNA targeting A1 mRNA in the context of the miR30 backbone was confirmed by Northern blot analysis of RNA derived from 293T cells transfected transiently with the pEGFP-miR30-A1 vector. Equal loading of the gel was confirmed by ethidium bromide staining. (C) The WEHI-231 mouse immature B cell line stably expressing FLAG-A1 was transduced with a retrovirus carrying one of two different sequences targeting A1. Knockdown was confirmed by Western blot analysis using anti-FLAG-M2 antibody. (D) Biogenesis of a siRNA targeting A1 mRNA in the context of the miR30 backbone was confirmed by Northern blot analysis of RNA derived from 293T cells transfected transiently with the TMP-miR30-A1 retroviral vector. **(E)** cDNA was generated from splenocytes of wt, sorted Venus^{+} cells from VVA1.1 or VVA1.2 mice. Knockdown efficiency was evaluated by qRT-PCR on cDNA using primers amplifying all *A1* isoforms yielding a 743bp product (upper panel). PCR products were purified and digested by restriction enzymes: *Bgl*II and *Nsi*I. A1-a, expressed mainly in myeloid cells and hence underrepresented in spleen, is digested by both enzymes yielding fragments 471bp, 131bp and 141bp in size (below detection limit) while A1-b is not digested; A1-d is digested only by *Bgl*II yielding 602bp and 141bp fragments. **(F)** cDNA was generated from spleen cells from wt as well as from eGFP^{++} cells from DT mice before and after 32 days of doxycycline treatment. Restriction polymorphism was assessed as in E.

Suppl. Figure 2: (A) Evaluation of eGFP expression levels in peripheral blood leukocytes from DT mice treated with doxycycline in the drinking water by flow cytometry over time. Data points represent means ± SEM (n=4). **(B)** eGFP expression in leukocytes from different hematopoietic organs from untreated or dox-treated mice. Representative dot blots from n=3 animals are shown. **(C)** Thymocyte subset distribution in the $eGFP^+$ fraction of TREA1 single transgenic mice and DT mice that received doxycycline for 32 days. Data points represent means ± SEM (n=3/genotype). **(D)** Distribution of splenic B cells as in C. **(E)** Distribution of myelocytes in the eGFP fractions in peripheral blood of DT mice treated with doxycycline in the drinking water. Bone marrow and spleen DT mice kept on doxycycline for 32 days was analysed for the presence of myelocytes and compared to TREA1 single transgenic littermates. Data points represent means ± SEM (n=3/genotype). **(F)** Spontaneous cell death of bone marrow-derived $eGFP^+$ granulocytes from TREA1 single transgenic or DT mice kept on doxycycline was assessed by 7AAD exclusion and flow cytometry after 24h in culture. Data represent means ± SD of 2 mice per group.

Suppl. Fig. 3: Thymocytes of different developmental stages, i.e. DN3, DN4 or DP, from **(A)** wt or VVA1.2 mice or **(B)** wt or DT mice were isolated by cell sorting, cultured in simple medium or treated with the indicated reagents. Viability was monitored over time by 7AAD-staining and flow cytometric analysis. Specific killing was calculated using the following equation (induced apoptosis/spontaneous cell death) x 100. Data represent means ± SE of 3 mice per genotype. **(C)** Pre-B cells from DT mice were isolated by cell sorting ($CD19^+CD25^+$) and cultured in simple medium. Viability was assessed after 24h as in A. **(D)** T2 transitional (IgM^+D^+) and

follicular B cells ($IgM^{low}D^{+}$) were isolated from the spleen by cell sorting from wt, DT or VVA1.2 mice and cultured in simple medium. Viability was assessed over time as in A. Data points represent means ± SE of 3 mice per genotype.

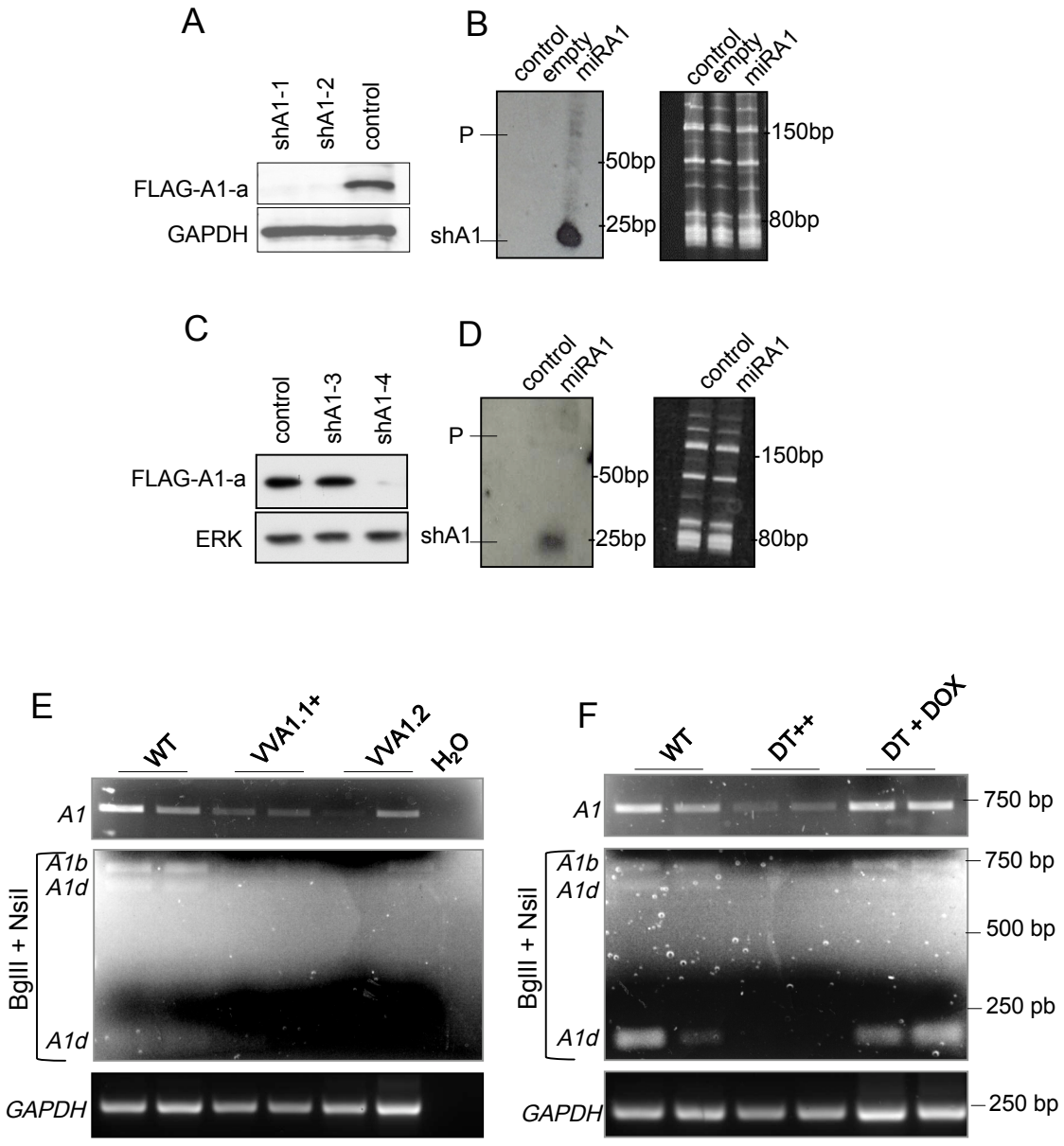
Suppl. Fig. 4: (A) Representative dot blots of flow cytometric analysis performed on bone marrow-derived single cell suspensions isolated from mice of the indicated genotypes using antibodies against CD25 and CD19 **(B)** Representative dot blots of flow cytometric analysis performed on splenic single cell suspensions isolated from mice of the indicated genotypes using antibodies against IgM and IgD.

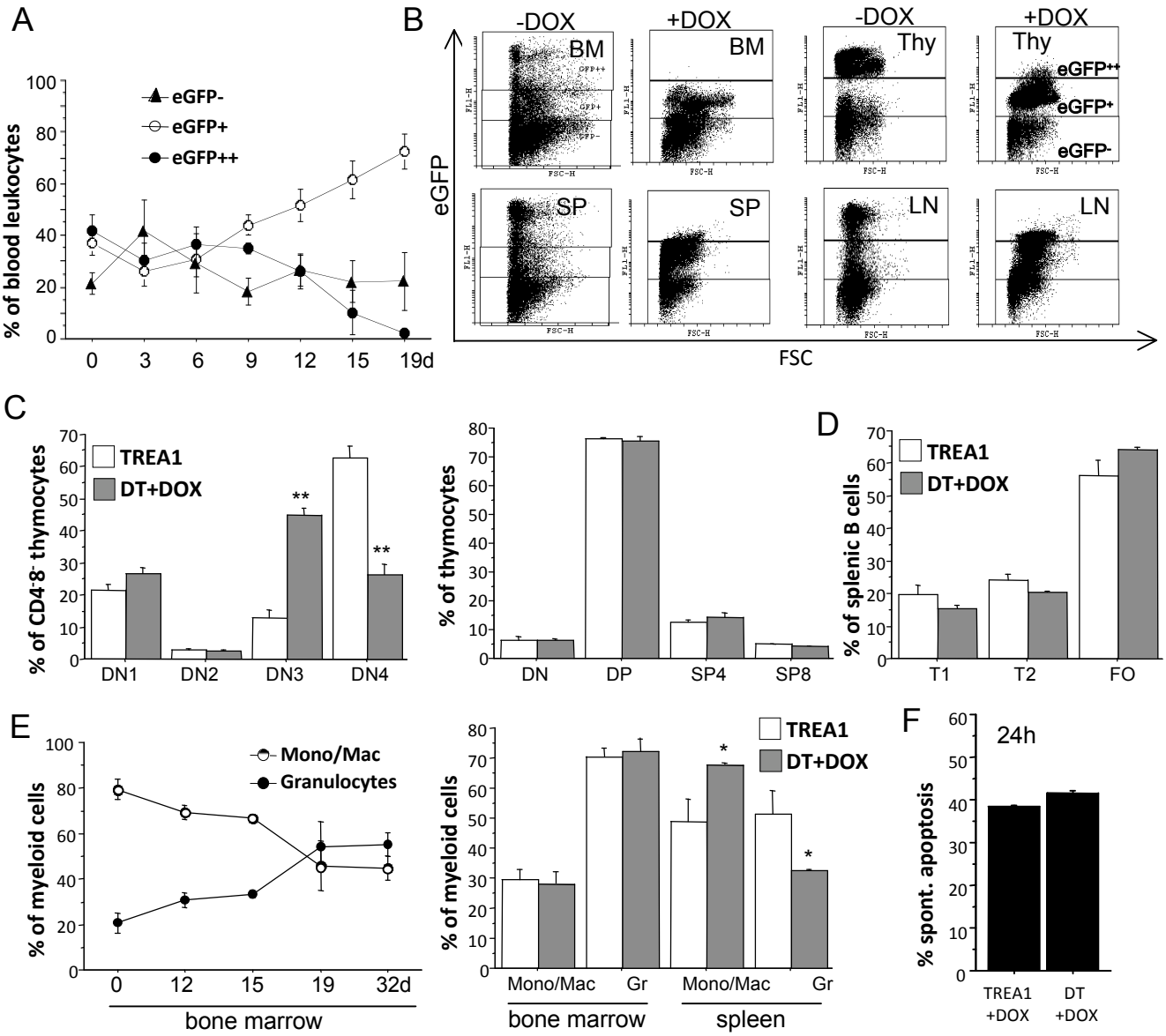
Suppl. Fig. 5: (A) Mitogen-induced proliferation of splenic $CD19^{+}$ B lymphocytes from wt, TREA1, DT and VVA1.2 mice was assessed by CPD-labelling and flow cytometric analysis of dilution of the fluorescence signal 72 h after stimulation in the different eGFP subsets (-, +, ++). Open histograms represent treated cells of different genotypes and reporter fractions; filled histograms represent un-stimulated control cells. Histogram overlays of one representative experiment out of three yielding similar results are shown. **(B)** Mitogen-induced proliferation of splenic T lymphocytes from DT or wt control mice was assessed by CPD-labelling and flow cytometric analysis of dilution of the fluorescence signal 72 h after stimulation in the different eGFP subsets (-, +, ++) or control cells. Histogram overlays represent treated cells (thin line) vs. un-stimulated control cells (bold line). One representative experiment out of two yielding similar results is shown.

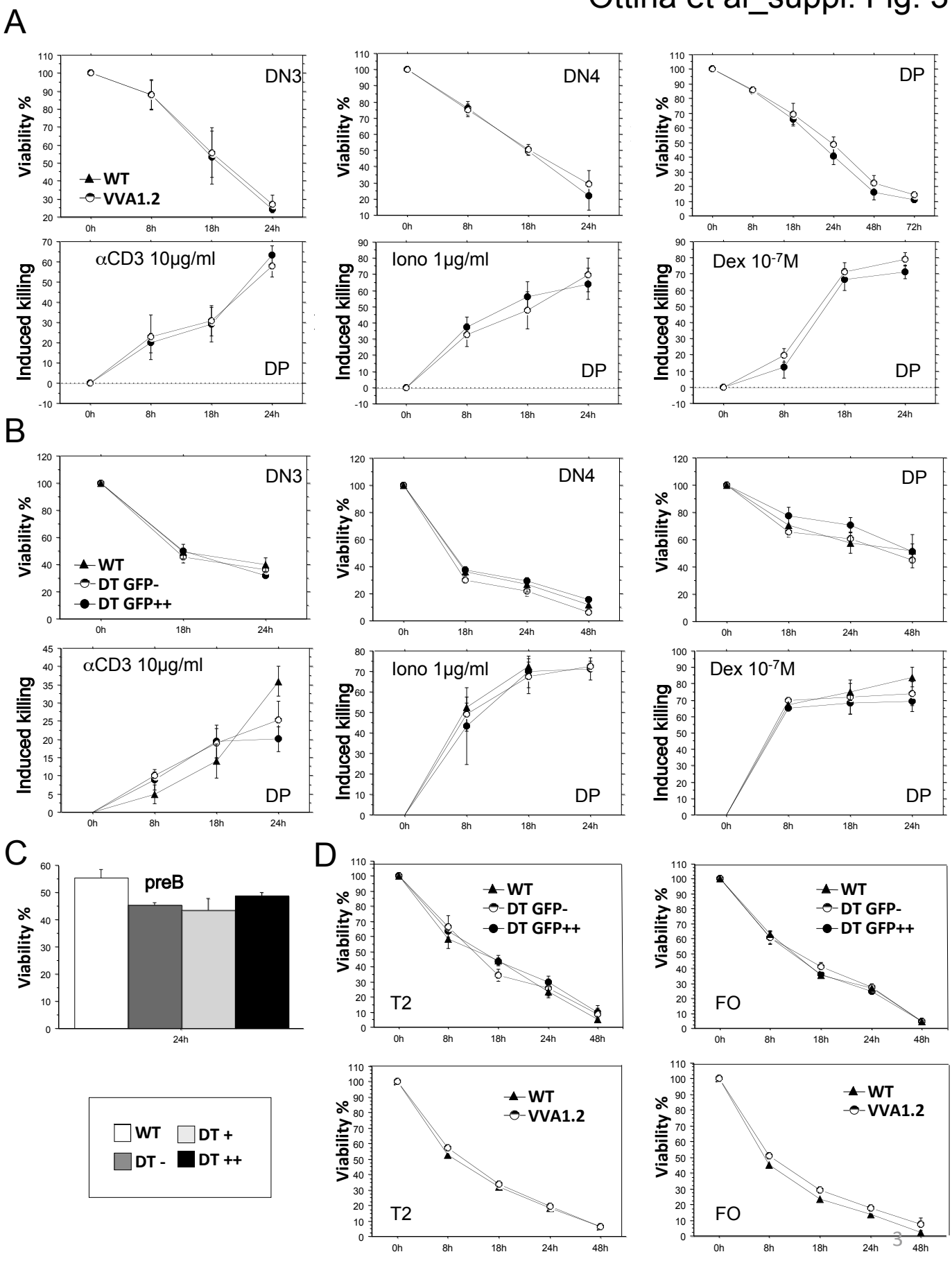
Suppl. Fig. 6: Representative dot blots of flow cytometric analysis of **(A)** bone marrow and **(B)** spleen of mice of the indicated genotypes using antibodies to identify

Mac-1^{+}Gr-1^{-} monocytes/macrophages and Mac-1^{+}Gr-1^{+} granulocytes, respectively, are shown. Numbers refer to percentages of region analysis.

Ottina et al_suppl. Fig. 1

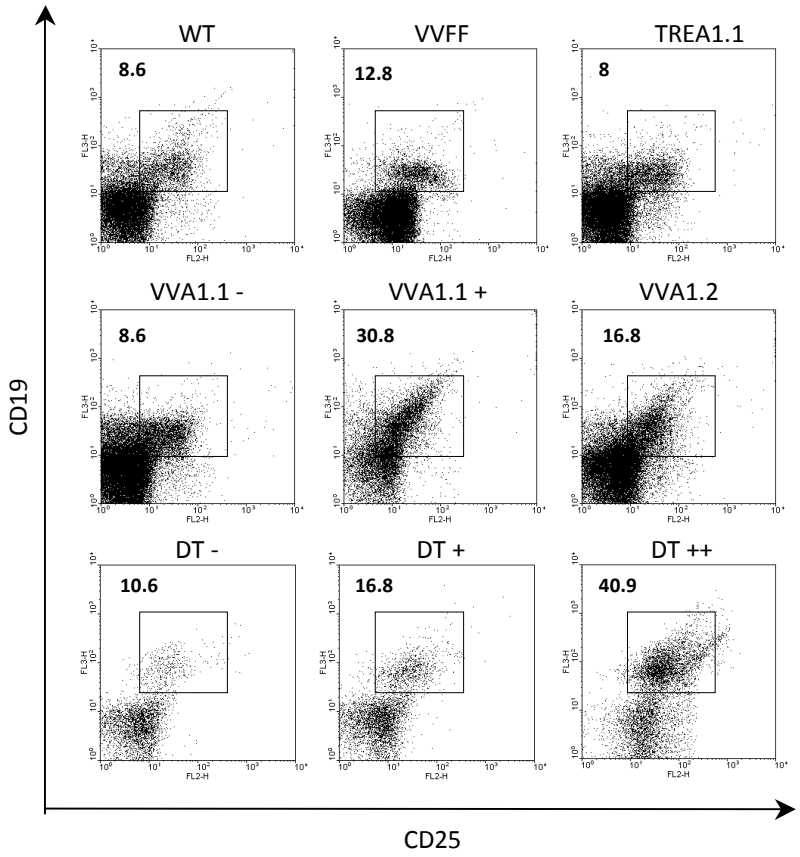



A
% of blood leukocytes
eGFP-
eGFP+
eGFP++
0 3 6 9 12 15 19d
B
-DOX
+DOX
-DOX
+DOX
BM
BM
Thy
Thy
eGFP++
eGFP+
eGFP-
SP
SP
LN
LN
eGFP
FSC
C
% of CD4-8- thymocytes
TREA1
DT+DOX
DN1 DN2 DN3 DN4
% of thymocytes
DN DP SP4 SP8
D
% of splenic B cells
TREA1
DT+DOX
T1 T2 FO
E
% of myeloid cells
Mono/Mac
Granulocytes
0 12 15 19 32d
bone marrow
% of myeloid cells
TREA1
DT+DOX
Mono/Mac Gr Mono/Mac Gr
bone marrow
spleen
F
24h
% spont. apoptosis
TREA1 +DOX
DT +DOX

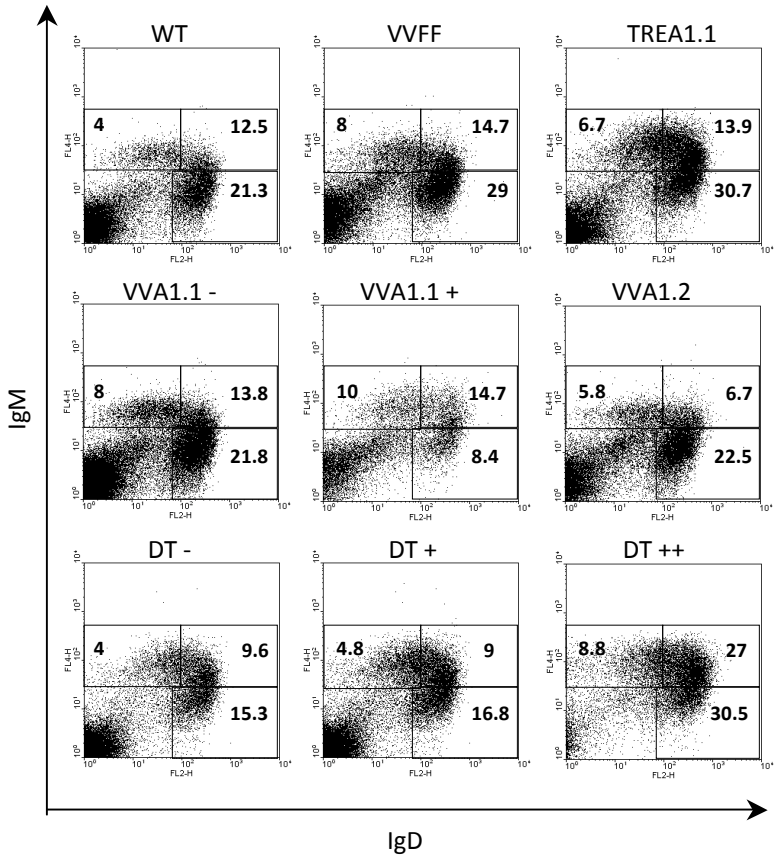

A
DN3
DN4
DP
Viability %
WT
VVA1.2
αCD3 10µg/ml
Iono 1µg/ml
Dex 10-7M
Induced killing
B
DT GFP-
DT GFP++
C
preB
WT
DT +
DT -
DT ++
D
T2
FO
0h
8h
18h
24h
48h
72h

Ottina et al_suppl. Fig. 4

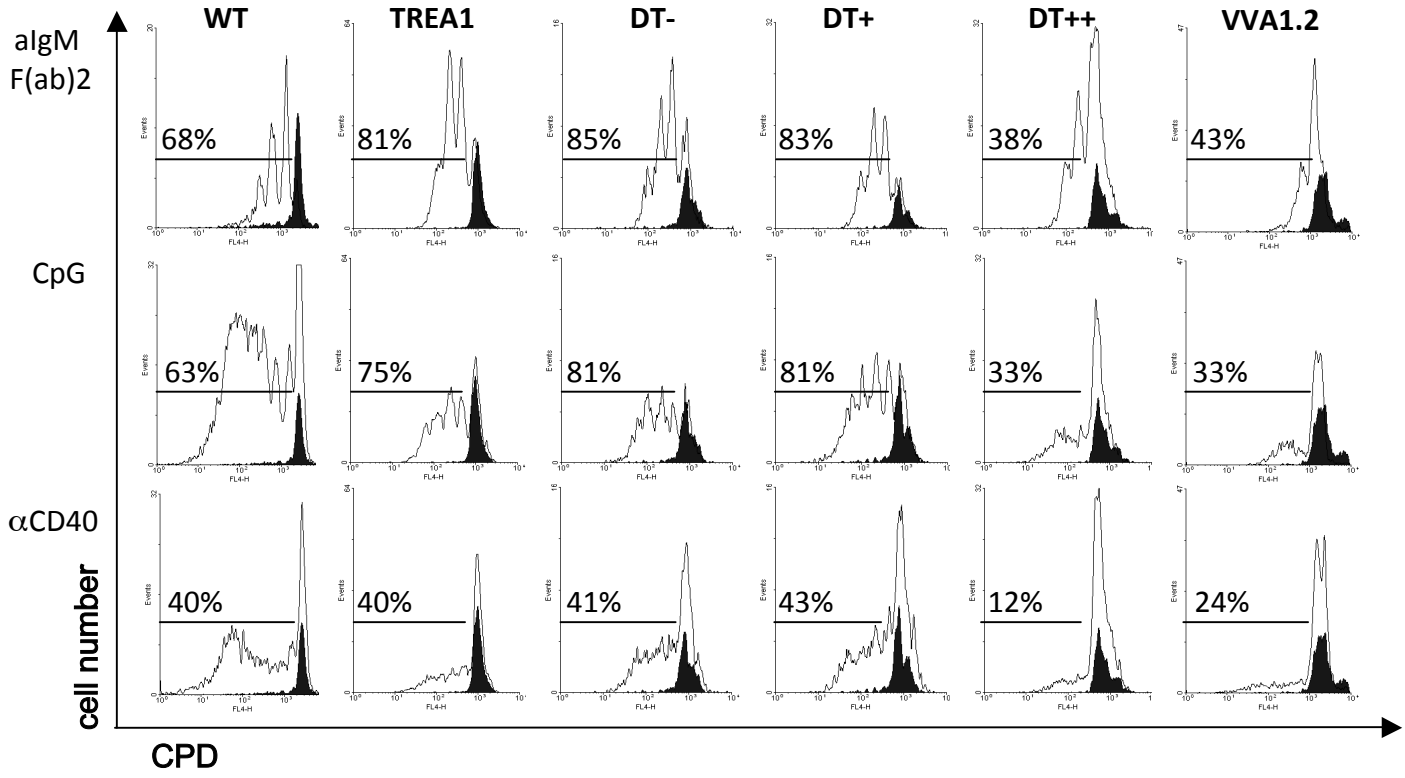
A



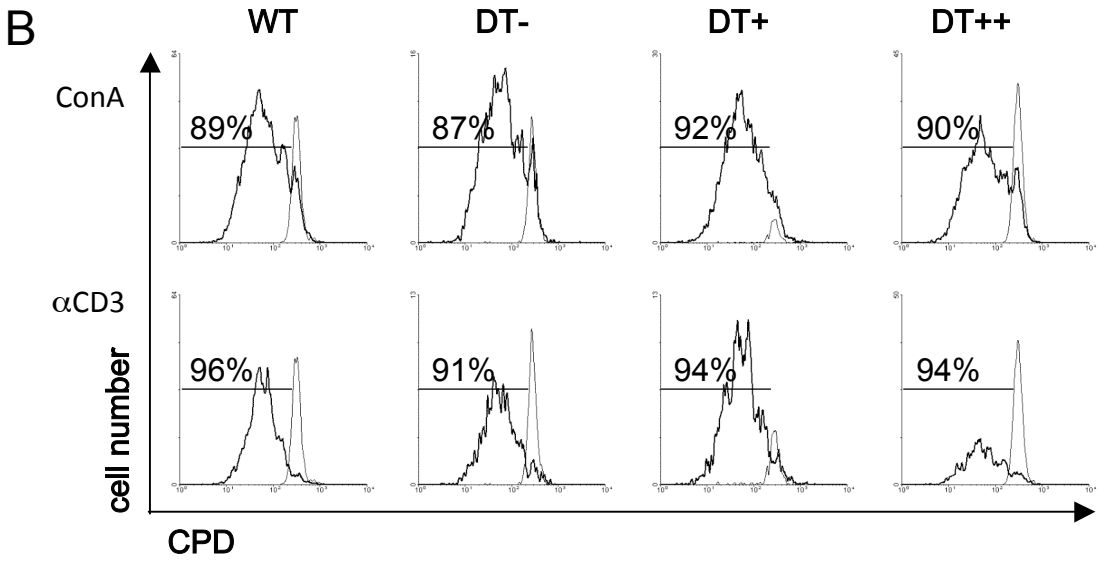
B

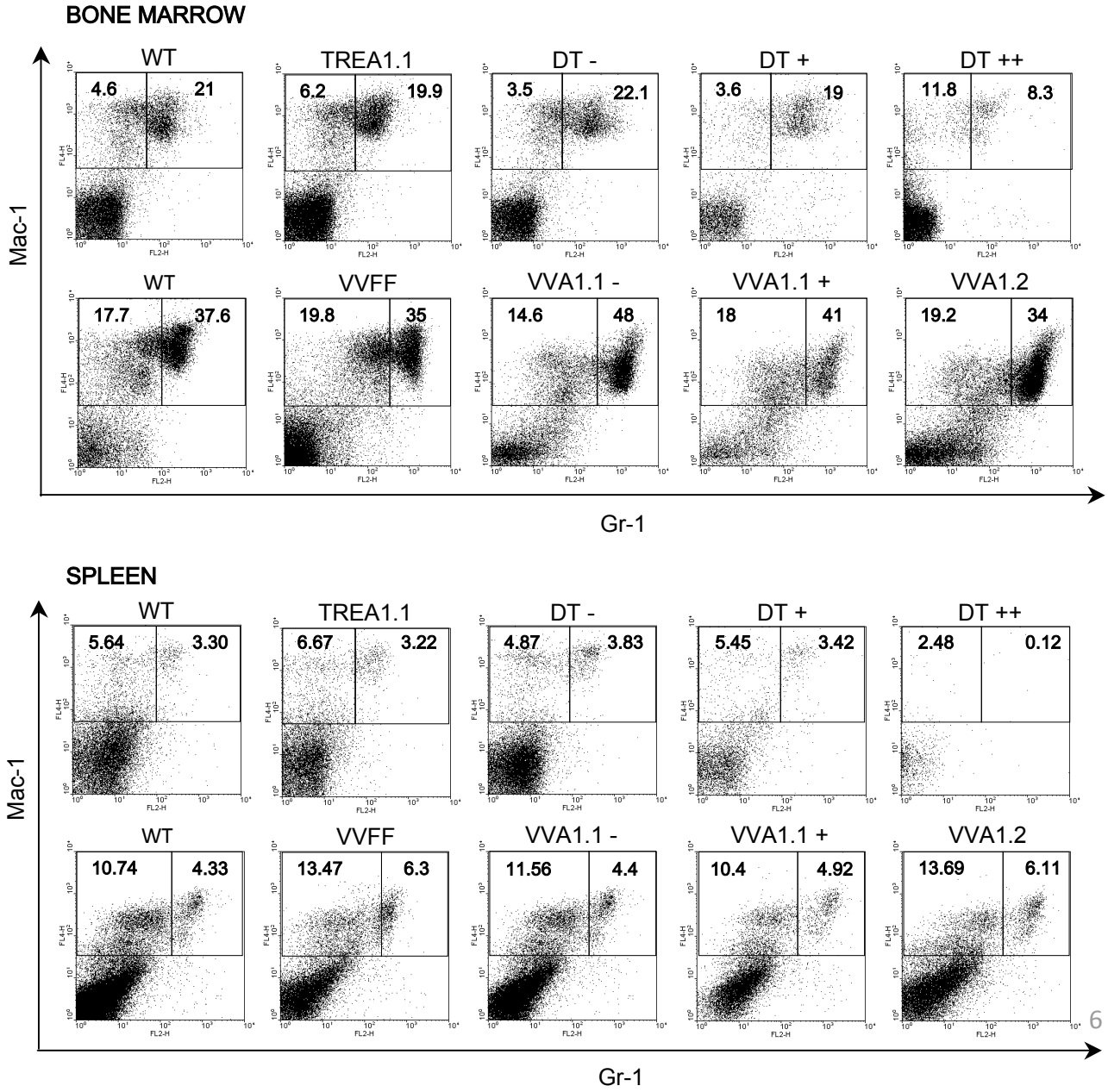


A


WT
TREA1
DT-
DT+
DT++
VVA1.2
aIgM F(ab)2
CpG
αCD40
68%
81%
85%
83%
38%
43%
63%
75%
81%
81%
33%
33%
40%
40%
41%
43%
12%
24%
cell number
CPD

B


WT
DT-
DT+
DT++
ConA
αCD3
89%
87%
92%
90%
96%
91%
94%
94%
cell number
CPD


BONE MARROW
WT
TREA1.1
DT -
DT +
DT ++
4.6
21
6.2
19.9
3.5
22.1
3.6
19
11.8
8.3
WT
VVFF
VVA1.1 -
VVA1.1 +
VVA1.2
17.7
37.6
19.8
35
14.6
48
18
41
19.2
34
Mac-1
Gr-1
SPLEEN
WT
TREA1.1
DT -
DT +
DT ++
5.64
3.30
6.67
3.22
4.87
3.83
5.45
3.42
2.48
0.12
WT
VVFF
VVA1.1 -
VVA1.1 +
VVA1.2
10.74
4.33
13.47
6.3
11.56
4.4
10.4
4.92
13.69
6.11
Mac-1
Gr-1