European Journal of Immunology

Supporting Information

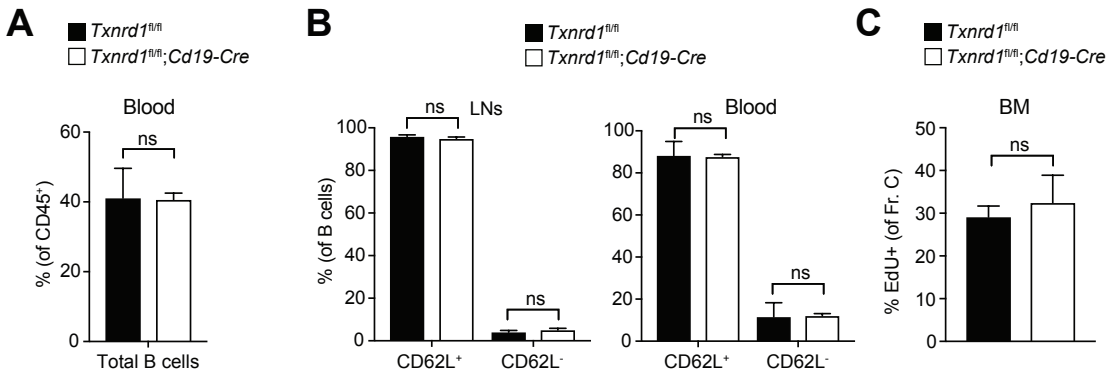
for

eji.201848044

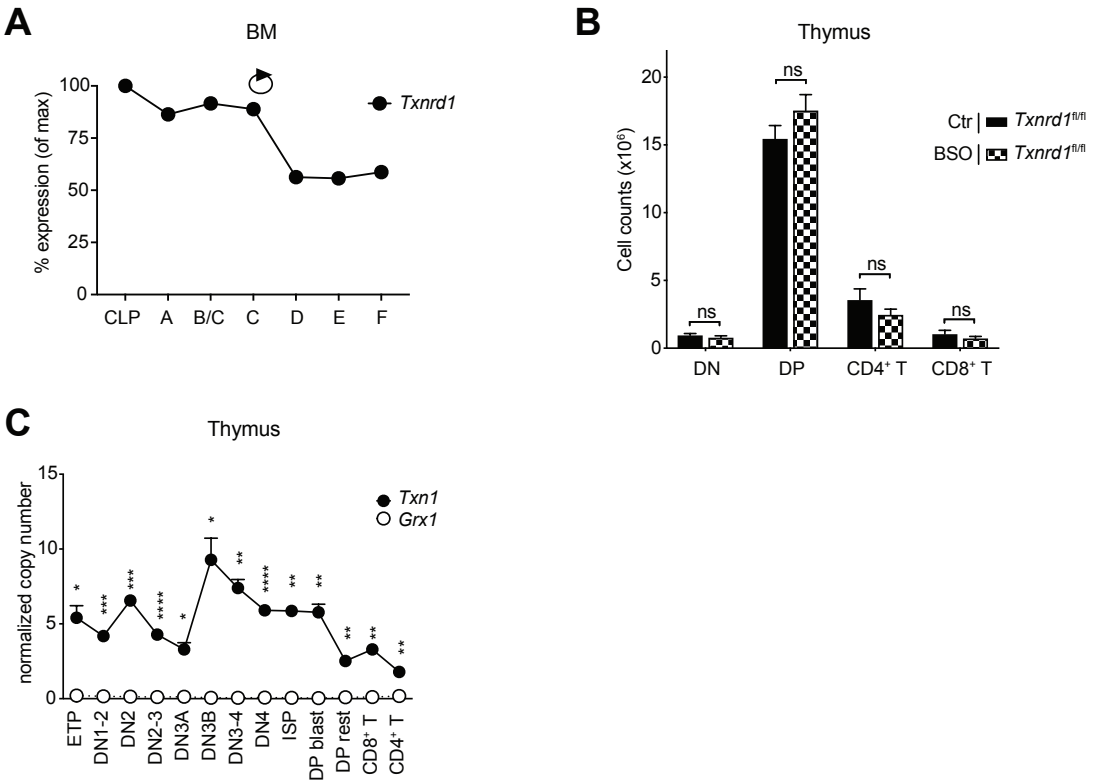
Jonathan Muri, Helen Thut, Sebastian Heer, Caroline C. Krueger, Georg W. Bornkamm, Martin F. Bachmann, Manfred Kopf

The thioredoxin-1 and glutathione/glutaredoxin-1 systems redundantly fuel B-cell development and responses

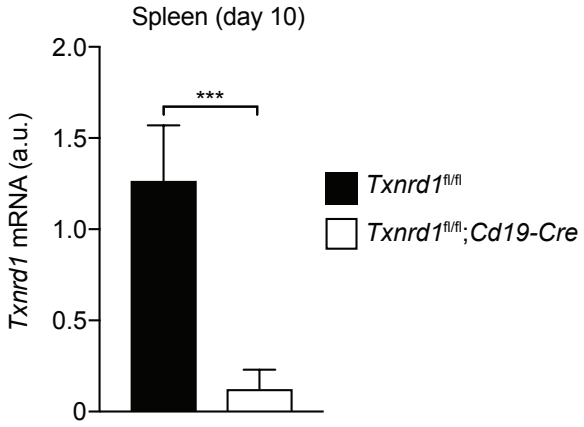
Supporting Information Figures



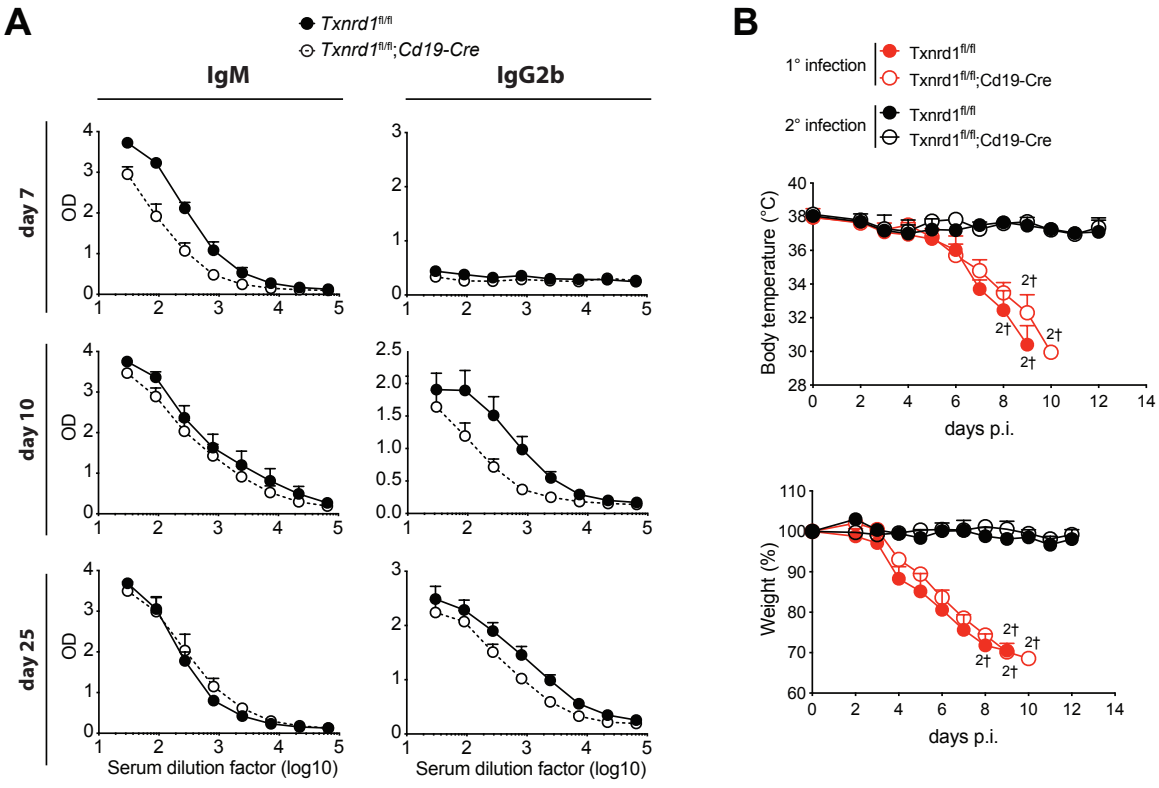
Supporting Information Figure 1. *Txnrd1* is dispensable for the homeostatic proliferation of B cells, Related to Figure 2. B cell populations in naïve *Txnrd1*$^{fl/fl}$*;Cd19-Cre* and *Txnrd1*$^{fl/fl}$ littermate control mice were analyzed by flow cytometry. (**A**) Percentage of total CD19^{+} B cells in the blood (n = 4-6). (**B**) Expression of CD62L on CD19^{+} B cells in the LNs (left) and in the blood (right; n = 4-6). (**C**) Proliferation of the Hardy fraction C in the bone marrow was assessed by EdU incorporation (n = 5-6). Bar graphs show mean + standard deviation. Data are representative of two independent experiments. Student's *t* test (two-tailed, unpaired) was used to compare *Txnrd1*$^{fl/fl}$ and *Txnrd1*$^{fl/fl}$*;Cd19-Cre* groups (**A-C**): ns, not significant.


A
BM
% expression (of max)
100
75
50
25
0
CLP
A
B/C
C
D
E
F
Txnrd1
B
Thymus
Cell counts (x10⁶)
20
15
10
5
0
ns
ns
ns
ns
DN
DP
CD4⁺ T
CD8⁺ T
Ctr | Txnrd1fl/fl
BSO | Txnrd1fl/fl
C
Thymus
normalized copy number
15
10
5
0
Txn1
Grx1
ETP
DN1-2
DN2
DN2-3
DN3A
DN3B
DN3-4
DN4
ISP
DP blast
DP rest
CD8⁺ T
CD4⁺ T

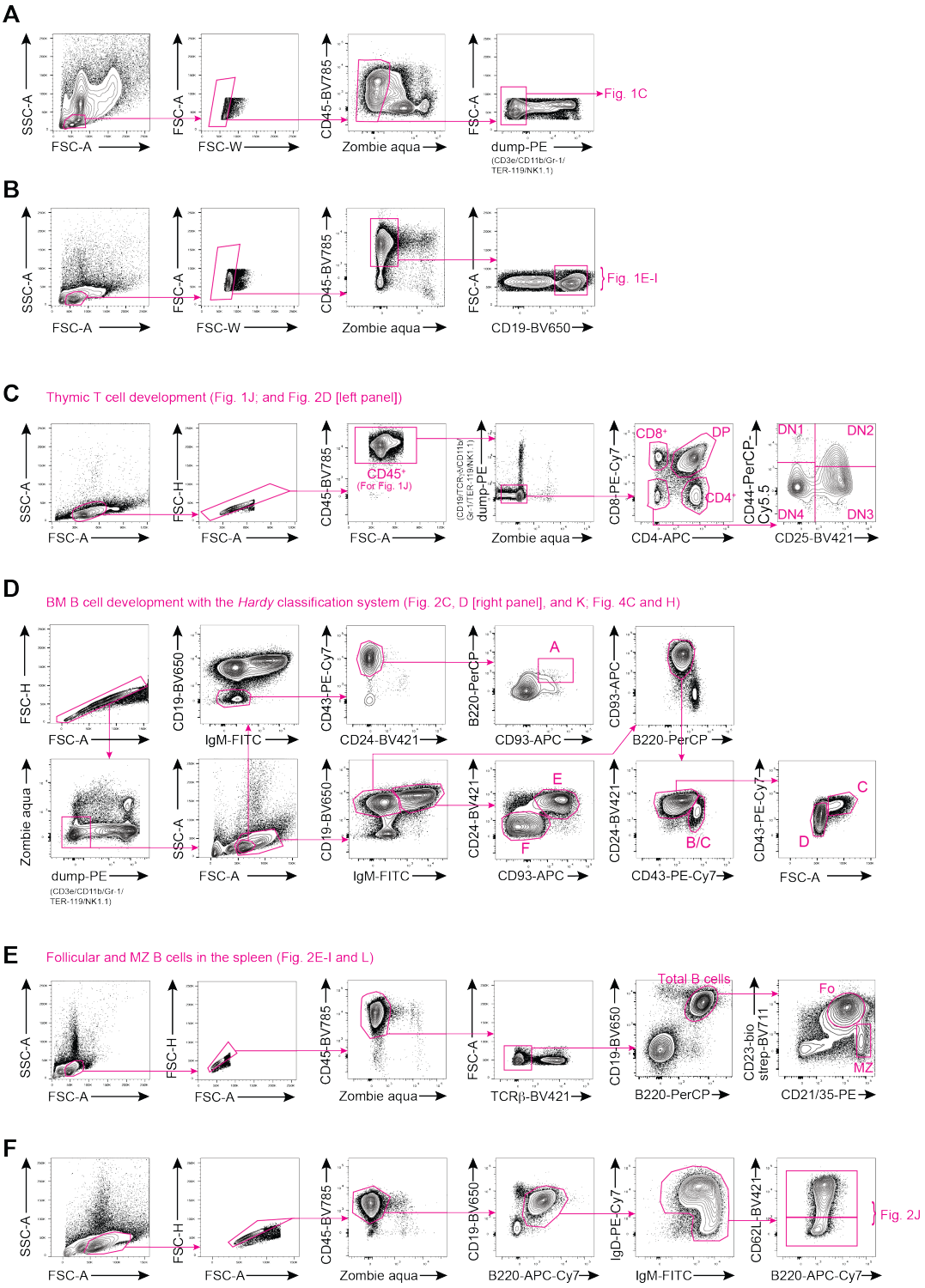
Supporting Information Figure 2. *Grx1* is barely expressed in thymocytes during development, Related to Figure 4. (**A**) Shown are the mean expression values of *Txnrd1* determined by microarray for different FACS-sorted populations obtained from the Immgen database. *CLP* stands for *common lymphoid progenitor*, and the letters A-F indicate the different B cell stages according to the Hardy's classification system. Circular arrow indicates the highly proliferative population (Fraction C). (**B**) *Txnrd1*$^{fl/fl}$ mice were treated with L-buthionine-sulfoximine BSO to deplete glutathione (GSH) in vivo. The absolute cell counts of CD4$^-$CD8$^-$ (DN), CD4$^+$CD8$^+$ (DP), CD4$^+$ and CD8$^+$ thymic T cell stages are shown (n = 4-6). (**C**) Normalized copy numbers of *Txn1* and *Grx1* for the indicated thymic populations determined real-time PCR. The obtained Ct values were converted to DNA concentration using a standard curve (n = 3). The following markers were used for FACS-sorting: lin$^-$CD44^{hi}c-Kit^{hi}CD25$^-$ (ETP), lin$^-$CD44^{hi}c-Kit^{hi}CD25^{int} (DN1-2), lin$^-$CD44^{hi}c-Kit$^{int/hi}$CD25^{hi} (DN2), lin$^-$CD44^{int}CD25^{hi} (DN2-3), lin$^-$CD44$^-$CD28$^-$CD25^{hi} (DN3A), lin$^-$CD44$^-$CD28$^+$CD25^{hi} (DN3B), lin$^-$CD44$^-$CD28$^+$CD25^{int} (DN3-4), lin$^-$CD44$^-$CD28$^+$CD25$^-$ (DN4), CD8$^+$CD24$^+$TCRβ^- (ISP), CD4$^+$CD8$^+$FSC^{hi} (DP blast), CD4$^+$CD8$^+$FSC^{lo} (DP rest), CD8$^+$ (CD8$^+$ T) and CD4$^+$ (CD4$^+$ T). Bar graphs or dots show mean + standard deviation. Data are representative of two independent experiments. Student's *t* test (two-tailed, unpaired) was used to compare control and BSO-treated mice (**B**) and to compare *Txn1* and *Grx1* expression (**C**): *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001; ****, P ≤ 0.0001; ns, not significant.



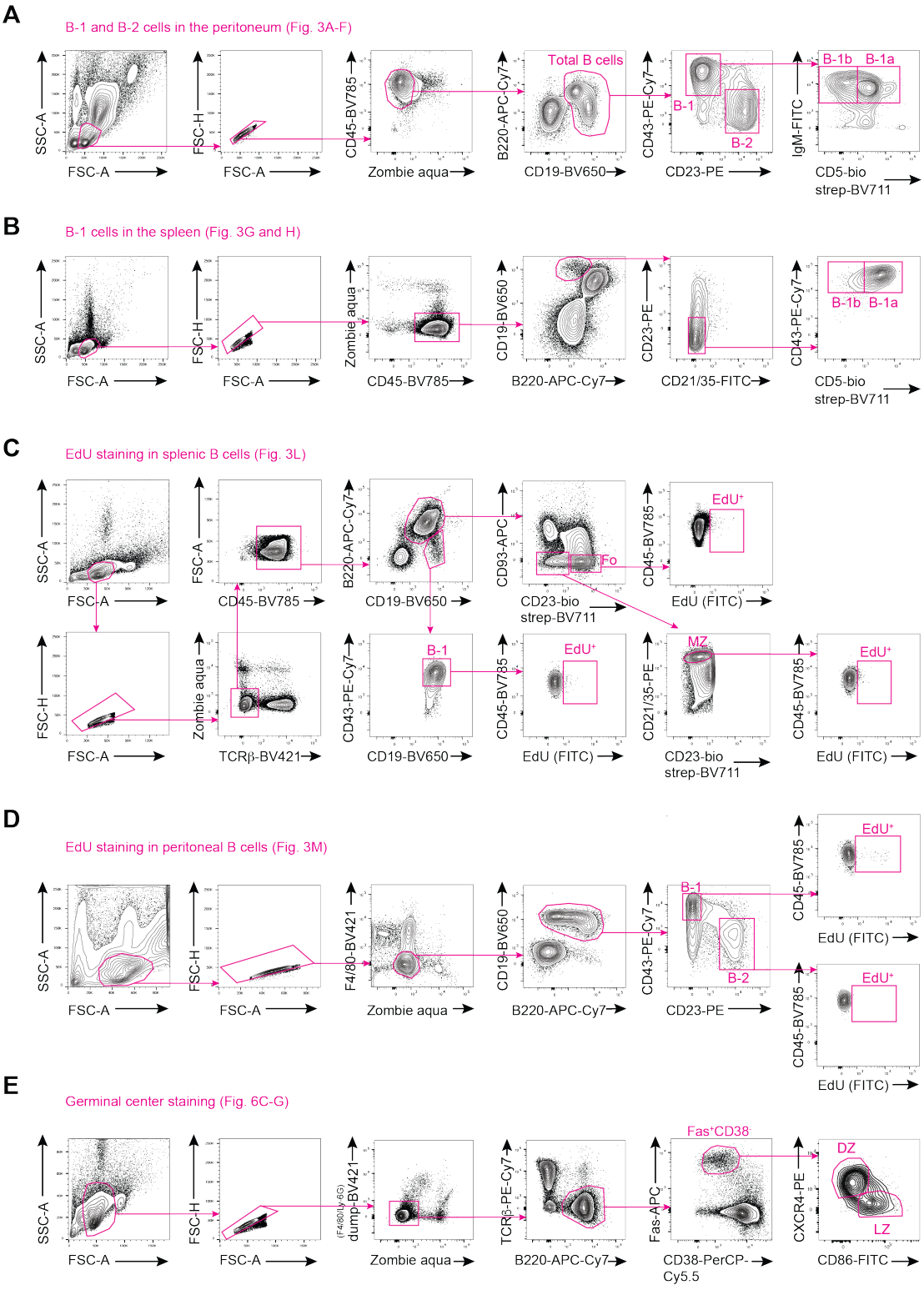
Supporting Information Figure 3. *Txnrd1* deletion was complete in GC B cells, Related to Figure 6. *Txnrd1$^{fl/fl}$;Cd19-Cre* and control *Txnrd1$^{fl/fl}$* littermates were intraperitoneally immunized with Qβ-VLPs containing *E. coli* ssRNA. At day 10 upon immunization, germinal center (GC) B cells were FACS-sorted as B220^{+}CD38^{low}Fas^{+}. For FACS-sorting, the gating strategy described for Fig. 6C was used. Depicted is the analysis of *Txnrd1* mRNA from GC B cells detected by real-time PCR (n = 3-4). For statistical analysis, student's *t* test (two-tailed, unpaired) was used: ***, P ≤ 0.001.



Supporting Information Figure 4. The antibody generated during a primary infection from *Txnrd1*$^{fl/fl}$-deficient B cells provide protection to a rechallenge with a lethal dose of influenza virus, Related to Figure 6. (**A**) *Txnrd1*$^{fl/fl}$*;Cd19-Cre* and *Txnrd1*$^{fl/fl}$ littermate control mice were infected with 10 pfu PR8 influenza A virus. Shown are the virus-specific IgM (left) and IgG2b (right) responses in the blood at the indicated days after immunization (n = 4-5). (**B**) Mice were primed with 10 pfu PR8 influenza A virus and then rechallenged with 500 pfu (lethal antigen dose) PR8 influenza A virus (depicted as *2° infection*). As a control for antigen dose lethality, naïve mice were infected with 500 pfu (lethal antigen dose) PR8 influenza A virus (depicted as *1° infection*). Shown are the body temperature (upper panel) and the weight (lower panel) relative to the day of infection (n = 4-5). Symbol † represents the mice, which were killed because they fulfilled the used severity criteria. Dot plots represent mean + standard error of mean (**A, B**). Data are representative of two independent experiments.



Supporting Information Figure 5. Gating strategies for Fig. 1, 2 and 4. (A-F) Representative FACS plots showing gating strategies for Fig. 1C **(A)**; for Fig. 1E-I **(B)**; for Fig. 1J and the left panel of Fig. 2D **(C)**; for Fig. 2C, D (right panel) and K, and for Fig. 4C and H **(D)**; for Fig. 2E-I and L **(E)**; and for Fig. 2J **(F)**.



Supporting Information Figure 6. Gating strategies for Fig. 3 and 6. (A-E) Representative FACS plots showing gating strategies for Fig. 3A-F **(A)**; for Fig. 3G and H **(B)**; for Fig. 3L **(C)**; for Fig. 3M **(D)**; and for Fig. 6C-G **(E)**.

Supporting Information Tables

Supporting Information Table 1. Antibodies and staining reagents for flow cytometry.

Specificity	Conjugation	Clone	Supplier	Dilution
B220	APC-Cy7	RA3-6B2	Biolegend	1:800
B220	PerCP	RA3-6B2	Biolegend	1:400
CD117	Biotin	2B8	Biolegend	1:200
CD19	APC-Cy7	6D5	Biolegend	1:800
CD19	PE	eBio1D3	eBioscience	1:500
CD19	BV650	6D5	Biolegend	1:500
CD21/35	FITC	7E9	Biolegend	1:300
CD21/35	PE	7E9	Biolegend	1:400
CD23	Biotin	B3B4	Biolegend	1:400
CD23	PE	B3B4	eBioscience	1:300
CD24	BV421	M1/69	Biolegend	1:1000
CD3e	PE	145-2C11	eBioscience	1:300
CD4	APC	GK1.5	BD Bioscience	1:1000
CD4	APC-Cy7	GK1.5	Biolegend	1:1000
CD11b	PE	M1/70	BD Bioscience	1:1000
CD25	BV421	PC61	eBioscience	1:400
CD28	PE	E18	eBioscience	1:300
CD38	PerCP-Cy5.5	90	Biolegend	1:300
CD43	PE-Cy7	S11	Biolegend	1:500
CD44	Biotin	IM7	Biolegend	1:2000
CD44	PerCP-Cy5.5	IM7	eBioscience	1:500
CD45	BV785	30-F11	Biolegend	1:1000
CD45.1	APC	A20	Biolegend	1:100
CD45.1	Biotin	A20	BD Bioscience	1:200
CD45.2	FITC	104	eBioscience	1:300
CD45.2	BV785	104	Biolegend	1:200
CD5	Biotin	53-7.3	eBioscience	1:300
CD62L	BV421	MEL-14	Biolegend	1:300
CD69	FITC	H1.2F3	Biolegend	1:300
CD8	PE-Cy7	53-6.7	Biolegend	1:1000
CD8	PerCP	53-6.7	Biolegend	1:600
CD86	FITC	GL-1	BD Bioscience	1:300
CD93	APC	AA4.1	Biolegend	1:100
CD95 (Fas)	APC	SA367H8	Biolegend	1:1000
CXCR4	PE	2B11/CXCR4	BD Bioscience	1:300
F4/80	BV421	BM8	Biolegend	1:300
Fc Block CD16/32	-	2.4G2	Home-made	1:1000
Gr-1	PE	RB6-8C5	Biolegend	1:800
IgD	PE-Cy7	11-26c.2a	Biolegend	1:1000
IgM	FITC	II/41	eBioscience	1:300
Ly-6G	BV421	1A8	Biolegend	1:800
NK1.1	PE	PK136	eBioscience	1:300
Streptavidin	BV711	-	BD Bioscience	1:1000
TCRβ	BV421	H57-597	Biolegend	1:300
TCRβ	PE-Cy7	H57-597	BD Bioscience	1:1000
TCR$\gamma\delta$	PE	GL3	eBioscience	1:300
TER-119	PE	TER-119	eBioscience	1:2000
ThiolTracker Violet	-	-	ThermoFischer Scientific	1:2000
Zombie Aqua	-	-	Biolegend	1:400

Supporting Information Table 2. Sequences of primers used for qRT-PCR.

Target gene	Primer sequences
Grx1	Forward: 5'-TGCAGAAAGACCCAAGAAATCCTCAGTCA-3' Reverse: 5'-TGGAGATTAGATCACTGCATCCGCCTATG-3'
Grx2	Forward: 5'-AAGGCTGTGGAGTTGGATATG-3' Reverse: 5'-TATCCTGGGAACGGTTCTTTC-3'
Grx3	Forward: 5'-ACGCTGTGGTTTCAGCAAG-3' Reverse: 5'-GGATAGGTGGGCCAATTAGAG-3'
Grx5	Forward: 5'-GAGCTGAGGCAAGGTATTAAAG-3' Reverse: 5'-CTCGCCGTTGAGGTACAC-3'
Txn1	Forward: 5'-ATGACTGCCAGGATGTTGC-3' Reverse: 5'-CCTTGTTAGCACCGGAGAAC-3'
Txnrd1 DNA	Forward: 5'-ACAGGAGTGATCCCCACAGACC-3' Reverse: 5'-CTGGAACCGCCCTGAATATCACC-3'
Txnrd1 mRNA in KO	Forward: 5'-GCTGACTAAGCAGCAGCTGG-3' Reverse: 5'-AACCTCAGCAGCCAGACTGG-3'
Tbp (housekeeping for mRNA)	Forward: 5'-TTGACCTAAAGACCATTGCACTTC-3' Reverse: 5'-TTCTCATGATGACTGCAGCAAA-3'
Txnrd1 (housekeeping for DNA)	Forward: 5'-ACAGATCGAAGCAGGAACAC-3' Reverse: 5'-TTCAGAGAGGAAAGTCACCC-3'