SUPPLEMENTARY APPENDIX

Signal-transducing adaptor protein-2 delays recovery of B-lineage lymphocytes during hematopoietic stress

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Supplemental Methods

Cultures

To evaluate B lineage development, the indicated populations were sorted and seeded onto monolayers of OP9 stromal cells. These co-cultures were maintained in aMEM (Gibco) containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 5 × 10^{-5} M 2-mercaptoethanol (ME), 100 U/ml penicillin, and 100 µg/ml streptomycin (PC/SM) in the presence of stem cell factor (SCF, 20 ng/ml), flt3-ligand (FL, 100 ng/ml), and IL-7 (1 ng/ml) for 2 weeks. For stromal cell-free cultures, cells were incubated in S-clone SF-03 medium (Iwai Chemicals) containing 1% lot selected FCS, 2-ME, PC/SM in the presence of SCF (20 ng/ml), FL (100 ng/ml), and IL-7 (1 ng/ml) for 10-14 days. For assessment of pre-B cell colony formation, MethoCult M3630 (StemCell Technologies) was used. To evaluate pre-B cell characteristics, $Mac1^-$ $B220^+$ $CD19^+$ $CD43^+$ IgM^- B progenitor cells were cultured in optiMEM (Gibco) with lot-selected 10% FCS , PC/SM and 2-ME in the presence of IL-7 (10 ng/ml) for 7 days. For some experiments, lipopolysaccharide (LPS, Sigma-Aldrich) or recombinant murine proteins of IFNg (0.1 ng/ml), IL-6 (50 ng/ml), IL-1b (5.0 ng/ml), and TNFa (50 ng/ml) were added. All recombinant proteins were purchased from R&D systems.

Supplemental table 1. Antibodies used in this study.

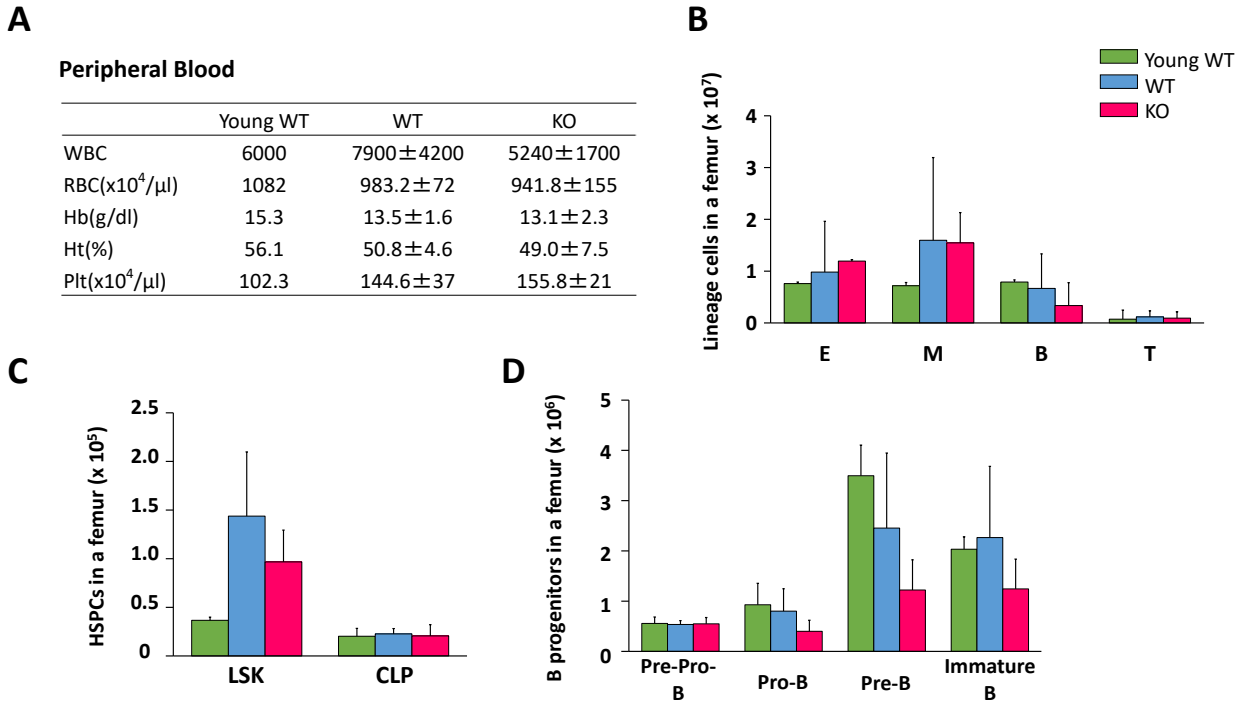
Antigen	Distributor	Clone
CD3	BioLegend	145-2C11
CD8	BioLegend	53-6.7
CD11b/Mac1	BioLegend	M1/70
CD11c	BD Biosciences	HL3
CD19	BioLegend	6D5
CD43	BioLegend	1B11
CD45R/B220	BioLegend	RA3-6B2
CD45.1	BD Biosciences	A20
CD45.2	BioLegend	104
CD150	BioLegend	TC15-12F12.2
c-Kit	BioLegend	2B8
Flk-2	BioLegend	A2F10
Gr1	BioLegend	RB6-8C5
IgM	BioLegend	RMM-1
IL7Ra	BD Biosciences	SB/199
Ly6C	BioLegend	HK1.4
NK1.1	BioLegend	PK136
Sca-1	BioLegend	E13-161.7
Ter119	BioLegend	TER-119
ERK1/2 (pT202/pY204)	BD Biosciences	20A
STAT5 (pY649)	BD Biosciences	47/Stat5(pY694)
P38MAPK (pT180/pY182)	BD Biosciences	36/p38 (pT180/pY182)

Supplemental Figure 1

A

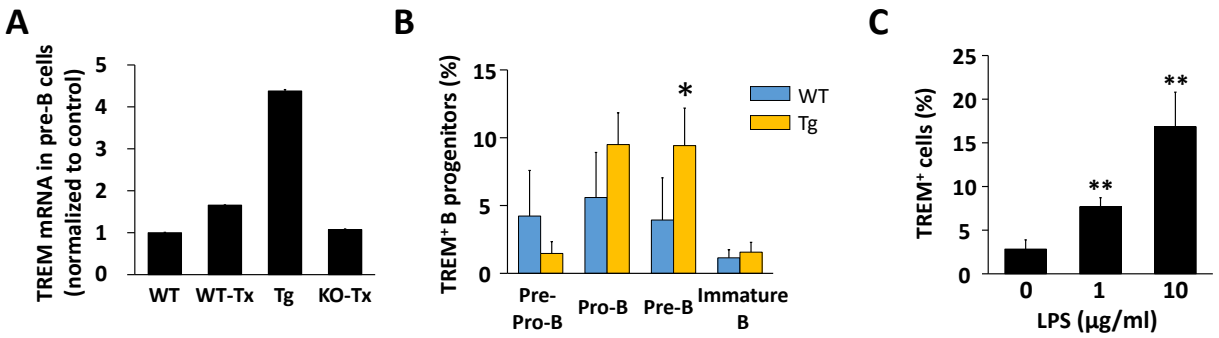
Peripheral Blood

	Young WT	WT	KO
WBC	6000	7900±4200	5240±1700
RBC(x10^4/μl)	1082	983.2±72	941.8±155
Hb(g/dl)	15.3	13.5±1.6	13.1±2.3
Ht(%)	56.1	50.8±4.6	49.0±7.5
Plt(x10^4/μl)	102.3	144.6±37	155.8±21



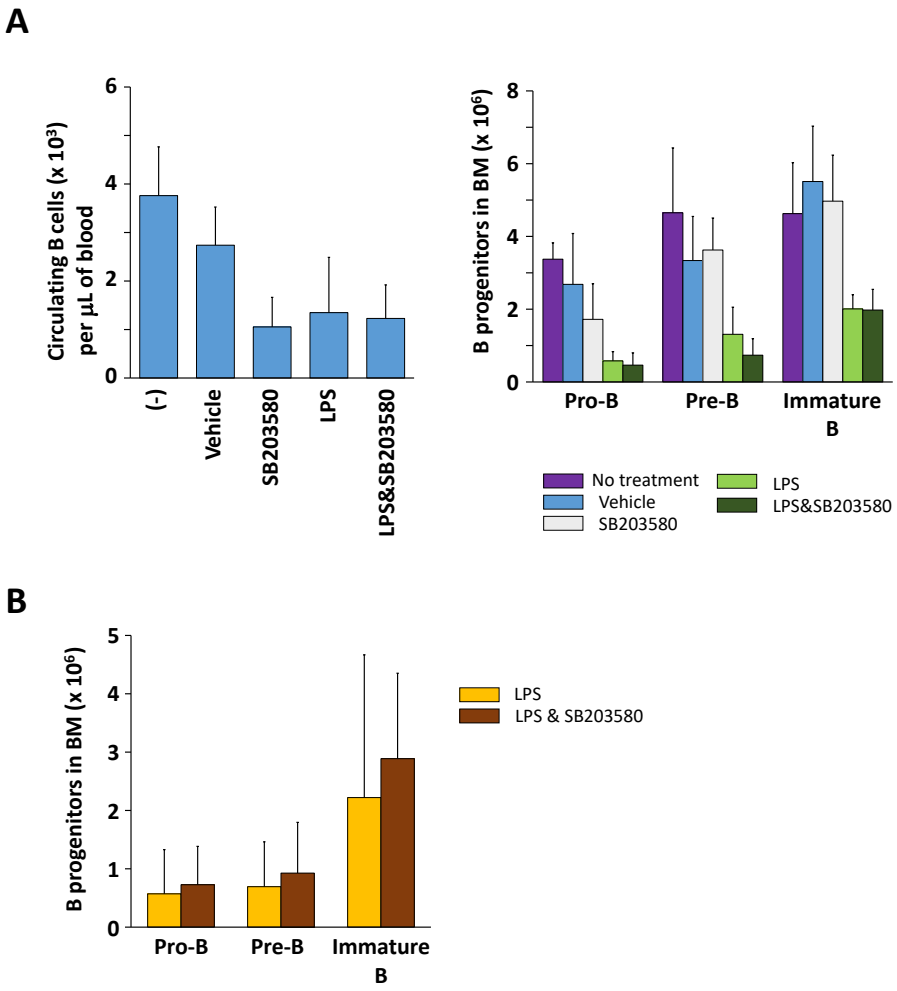
Supplemental Figure 1. Hematopoiesis in aged STAP-2 knockout (KO) mice.
Aged STAP-2 KO mice were used to evaluate the role of STAP-2 in hematopoiesis. Hematopoietic cells were isolated from 12 to 22 month-old KO mice (n = 5), and from 7-16 week-old and age-matched wild-type (WT) control mice (n = 5). (A) The number of peripheral blood cells was calculated using hemocytometer. (B) The percentages of $Ter119^+$ erythroid cells (E), $Gr1^+$ or $Mac1^+$ myeloid cells (M), $CD19^+$ B cells, or $CD3^+$ T cells in bone marrow were determined by flow cytometry, and absolute counts were calculated. (C-D) Without initial separation, the samples were stained for the lineage associated (Lin) marker, or other surface markers as indicated: Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^{Hi}$ (LSK) hematopoietic stem/progenitor cells; Lin^- $Sca1^+$ $cKit^{low}$ $Flk2^{high}$ IL7R*a^+ common lymphoid progenitors (CLPs); $B220^+$ $CD43^+$ $CD19^-$ IgM^- pre-pro-B; $B220^+$ $CD43^+$ $CD19^+$ IgM^- pro-B; $B220^+$ $CD43^-$ $CD19^+$ IgM^- pre-B; and $B220^+$ $CD43^-$ $CD19^+$ IgM^+ immature B cells. Similar results were obtained in three independent experiments. Pooled data are shown as mean ± SD. Statistical significances relative to WT controls were determined by unpaired two-tailed Mann-Whitney tests.

Supplemental Figure 2



Supplemental Figure 2. Regulation of TREM1 expression.
(A) RT-PCR was performed to analyze the expression of TREM-1 in pre-B cells with or without transplantation (Tx)(WT, n=6; WT-Tx, n=7; Tg, n=4; KO-Tx, n=4). The results are expressed as fold changes relative to wild-type (WT) samples. (B) The expression of TREM-1 on the indicated subset of B progenitors was analyzed by flowcytometry (n=8 in each). (C) TLR-4 transduced Baf-3 pre-B cells (kindly gifted from Dr. Miyake) were maintained in RPMI (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal calf serum (FCS) and PC/SM in the presence of IL-3 (10 ng/ml), and treated with the indicated concentration of lipopolysaccharide (LPS), and the expression of TREM-1 was analyzed. Similar results were obtained in three independent experiments. The results are shown as mean ± SD. Statistical significances, relative to WT control (A,B) or to a control without stimulation (C), were determined by unpaired two-tailed Mann-Whitney tests: *, $p < 0.05$ and **; $p < 0.01$.

Supplemental Figure 3



Supplemental Figure 3. Effects of p38 MAPK inhibitor on LPS-induced sepsis mouse model. WT (A, no treatment, n=3; vehicle, n=3; SB203580, n=4; LPS, n=4; LPS and SB203580, n=6) or Tg (B, n=5 in each) mice were treated with 1.0 mg /kg of lipopolysaccharide (LPS) and/or 20 mg /kg of p38 MAPK inhibitor SB203580, and flow cytometric analysis was conducted 2 days later. The number of $B220^+$ $CD19^+$ B cells in peripheral blood (A, left panel) as well as B lineage progenitor subsets (pro-B; $B220^+$ $CD43^+$ $CD19^+$ IgM^-, pre-B; $B220^+$ $CD43^-$ $CD19^+$ IgM^-, immature B; $B220^+$ $CD43^-$ $CD19^+$ IgM^+) in BM (A, right panel and B), are shown. While B cell number in peripheral blood was decreased in WT mice treated with SB203580, the inhibitor tended to attenuate the B progenitor suppression induced by STAP-2 overexpression. Similar results were obtained in two independent experiments. The results are shown as mean ± SD. Statistical significances were analyzed by unpaired two-tailed Mann-Whitney test, and there were no statistical differences.