Supplementary Methods

Flow cytometry: Single-cell suspensions were prepared from peripheral blood, bone marrow, spleen and thymus using erythrocyte lysis when required. Cells were then stained for 30 minutes at room temperature with antibodies as indicated and analysed by flow cytometry for GFP and cell surface marker expression. For SAP intracellular staining, following fixation and permeabilisation (IntraPrep, Beckman Coulter), cells were incubated either with a rat anti-human SAP antibody (Cell Signaling Technology) or with the corresponding control rat IgG1 (BD Pharmingen).

Methylcellulose Colony Forming Unit assay: 3,000 transduced or untransduced murine Lin-ve cells were seeded in MethoCult® (Stem Cell Technology) and incubated at 37°C. Colonies were scored after 2 weeks.

^{51}Cr release assay: NK cells were isolated from single-cell splenocyte suspensions after erythrocyte lysis using MACS® CD49b (DX5) MicroBeads (Miltenyi Biotec) and cultured for 7 days in the presence of mIL-2 (1000U/mL; Peprotech). Cells were then incubated for 4 hours with 5,000 RMA/S target cells labelled with 1mCi ^{51}Cr at decreasing effector: target ratios. The percentage of specific lysis was calculated according to the following formula: (experimental-spontaneous release)/ (maximal-spontaneous release) x100. Assays were done in triplicate.

Immunisation and antibody responses: All control and reconstituted animals were injected i.p with NP(65)-CGG (1mg/mL) (Biosearch Technologies) mixed with alum (Serva) in a 1:2 ratio. Serum was collected at various time points after immunisation and NP-specific immunoglobulins were detected and quantitated by enzyme-linked immunosorbent assays

(ELISAs) using NP(23)-BSA (Biosearch Technologies) as a capture antigen. The mouse immunoglobulin standard panel was obtained from Southern Biotechnology Associates.

Germinal centre formation: Immunised animals were sacrificed 10 days after NP-CGG injection and germinal centre B cells were detected by flow cytometry in single-cell suspensions of splenocytes after erythrocyte lysis using anti-CD19 and anti-Gl-7 antibodies (BD Biosciences). Spleens were then fixed in formalin, sectioned and stained with hematoxylin and eosin to allow visualisation of tissue structure. Germinal centre B cells were stained by immunohistochemistry using unconjugated Peanut agglutinin (Vector Laboratories).

Imaging : The images were captured by Olympus BX51 with Olympus Paln Apo objectives, and Nikon DigitalSight DS-L1 digital microscope camera with automatic exposure and on camera white balancing. Magnifications are as stated in the figures and figure legends.

Statistics: Statistical significance was determined by using unpaired, two-tailed Student's *t* tests

Viral copy number analysis. The number of integrated viral copies in genomic DNA from animal tissues or PBMCs was analysed by qPCR using the following primers and probes: WPRE Fwd TGGATTCTGCGCGGGA, WPRE Rev GAAGGAAGGTCCGCTGGAT, WPRE Probe FAM-ACGTCCTTTCCATGGCTGCTCGC-TAMRA, Titin Fwd AAAACGAGCAGTGTCGTGAGC, Titin Rev TTCAGTCATGCTGCTAGCGC, Titin Probe FAM- TGCACGGAAGCGTCTCGTCTCAGTC-TAMRA.

Supplementary Figure legends

Supplementary Figure 1: Transduction efficiency of lineage negative cells and viral copy number in reconstituted animals. Flow cytometry analysis of GFP expression in Lin-ve cells 72h after lentiviral infection. Data shown are the mean ± SEM of values from two independent experiments.

Supplementary Figure 2: Colony formation after vector transduction. Upper panel shows the total number of colonies observed after plating in semi-solid media for either untransduced cells or cells transduced with either the EFSeGFP or EFS-SAPeGFP vectors at increasing MOIs (multiplicities of infection – 10, 25 or 100). Lower panel shows the type of colonies observed after plating. The results shown are for lin-ve cells that had been transduced at an MOI of 100.

Supplementary Figure 3: SAP expression in PBMCs, bone marrow and thymus. A. Viral copy number analysis in PBMCs at 12 weeks post-injection. Unmanipulated wild-type animals are shown as negative controls. Data presented are the mean ± SEM of values from two independent experiments. **B.** Detection of human SAP expression in PBMCs, bone marrow cells and thymocytes by intracellular FACS staining (control IgG2b in grey, anti-SAP antibody in white) at the time of sacrifice. Wild-type and GFP-reconstituted animals were used as negative controls. **C.** SAP expression in control human PBMCs is shown as a reference.

Supplementary Figure 4: NKT cell development in thymus. A. Detection of NKT cells in the thymus of control and reconstituted animals by flow cytometry. Dot plots showing TCR versus NK1.1 expression are representative of each experimental group. **B.** FACS analysis of GFP expression within the thymic NKT cell population (TCR$^+$/NK1.1$^+$/GFP$^+$ cells) in the WT

and EFS-SAPeGFP reconstituted groups (left panel). FACS analysis of GFP expression in different peripheral cell lineages following reconstitution (right panel) **C.** FACS analysis of mature single-positive $CD4^+$ or $CD8^+$ thymocytes and $CD4^+$ /$CD8^+$ double-positive thymocytes in wild-type and EFSeGFP or EFS-SAPeGFP reconstituted mice at the time of sacrifice.

Supplementary Figure 5: Reconstitution of SAP-/- recipients with wild-type murine bone marrow. A. Flow cytometry analysis of hematopoietic lineages in control and experimental animals 12 weeks after reconstitution. **B.** NK cells cytotoxic activity measured in a ^{51}Chromium release assay against the radiolabelled murine T lymphoma target cells (RMA/S). Assays were done in triplicate and data shown are mean ± SEM of all values. **C.** Detection of NKT cells in the thymus of control and reconstituted animals by staining for the TCR receptor and NK1.1 surface marker. **D.** Quantification of baseline serum immunoglobulin levels by ELISA 12 weeks after reconstitution. Values for individual mice are shown as dots, and the mean of all values is represented by a horizontal line.

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Supplementary Figure 1

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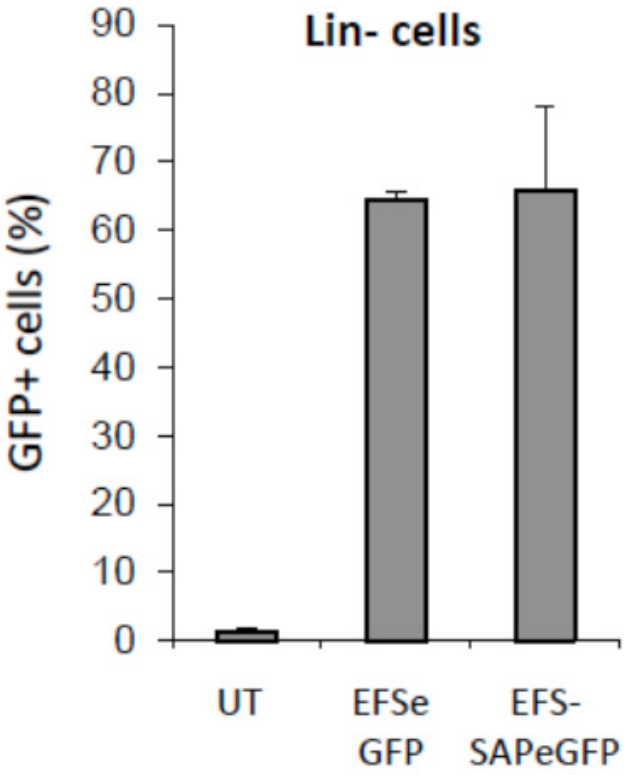

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Supplementary Figure 2

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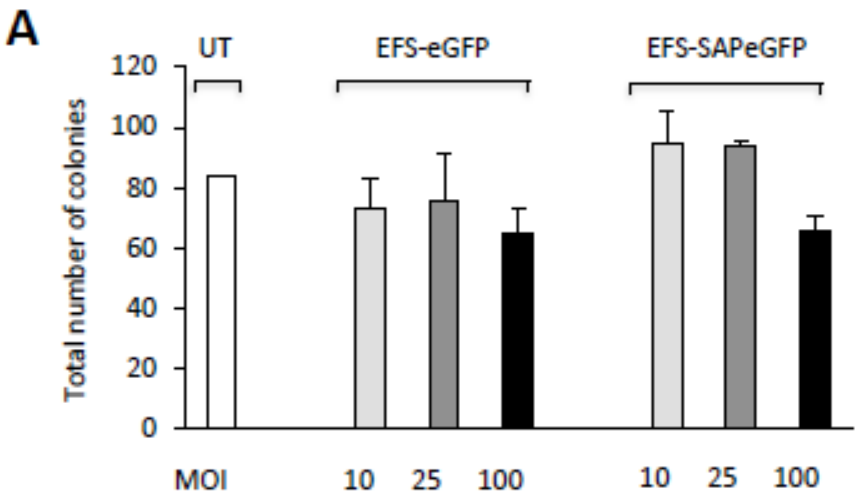

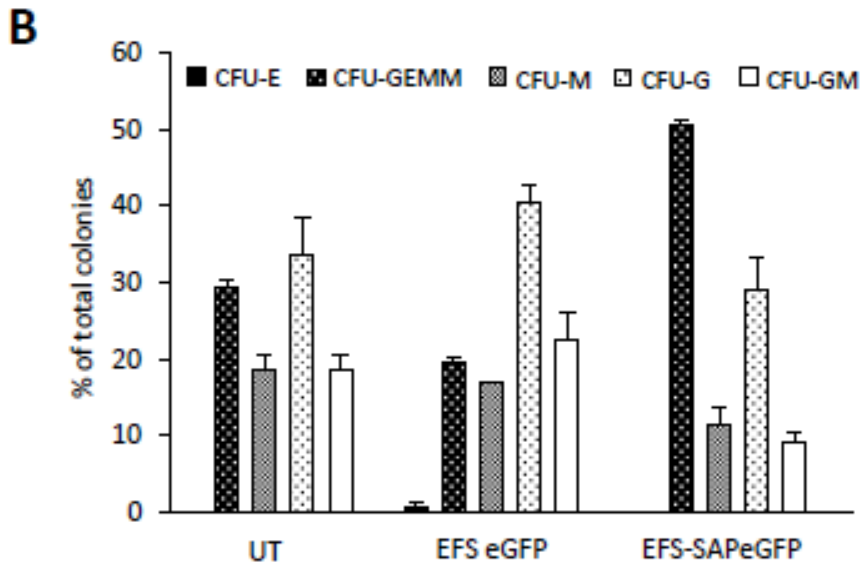

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Supplementary Figure 3#

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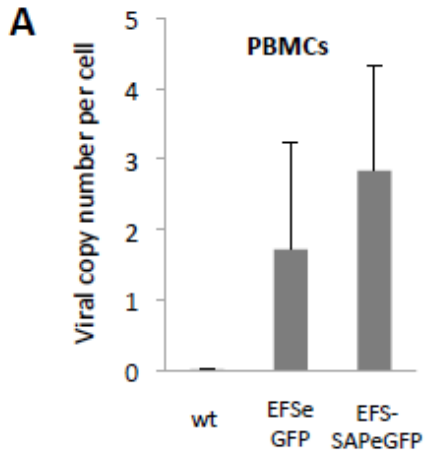

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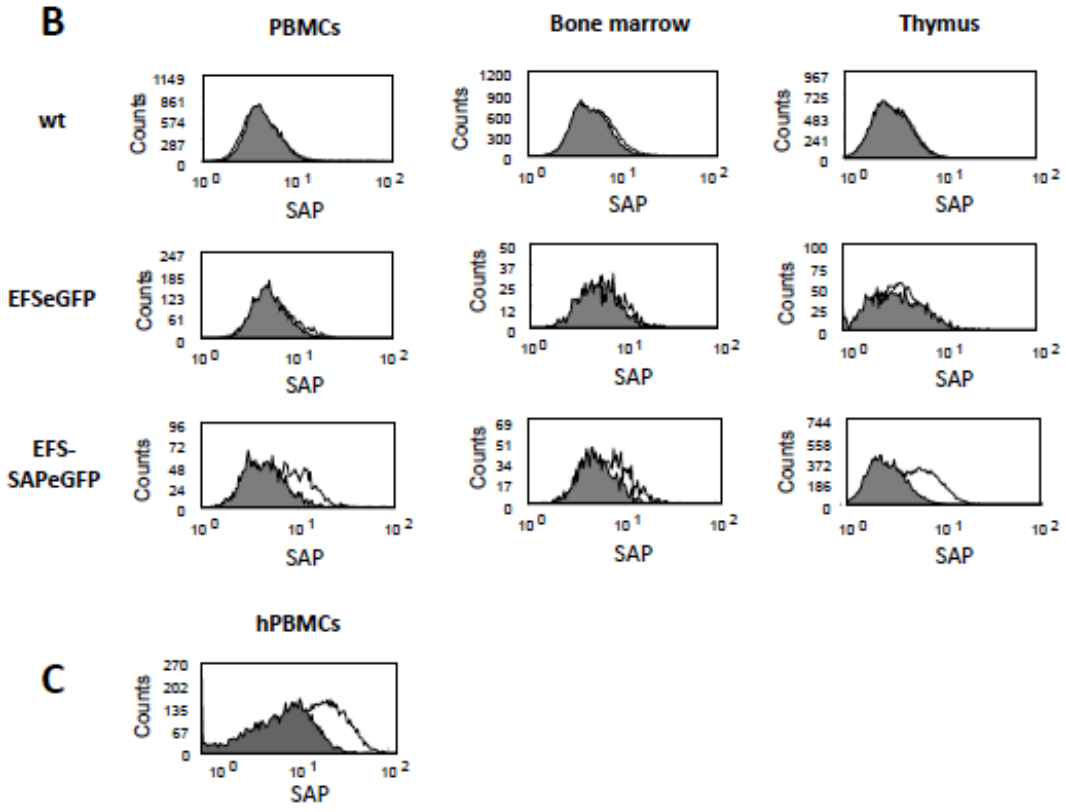

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Supplementary Figure 4#

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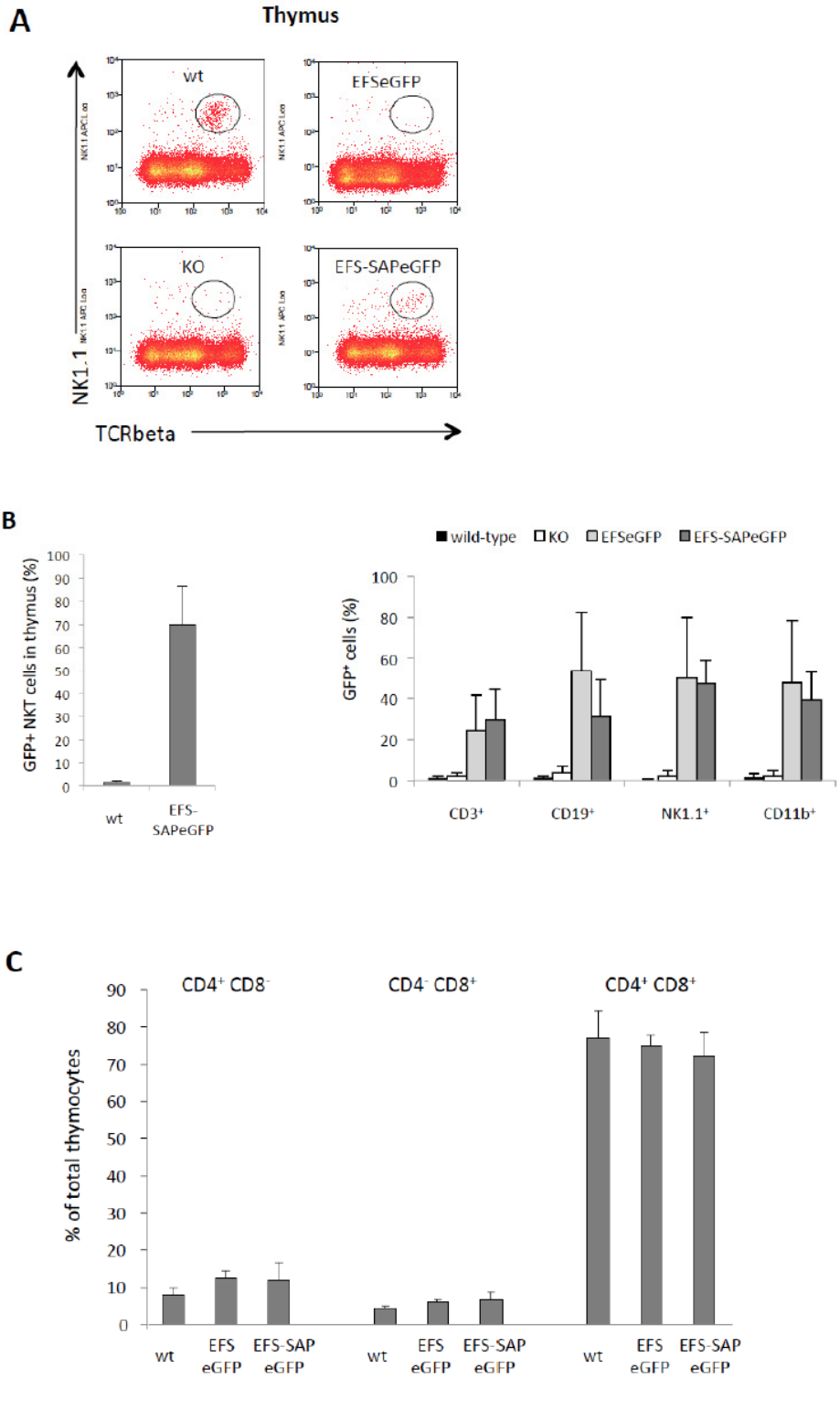

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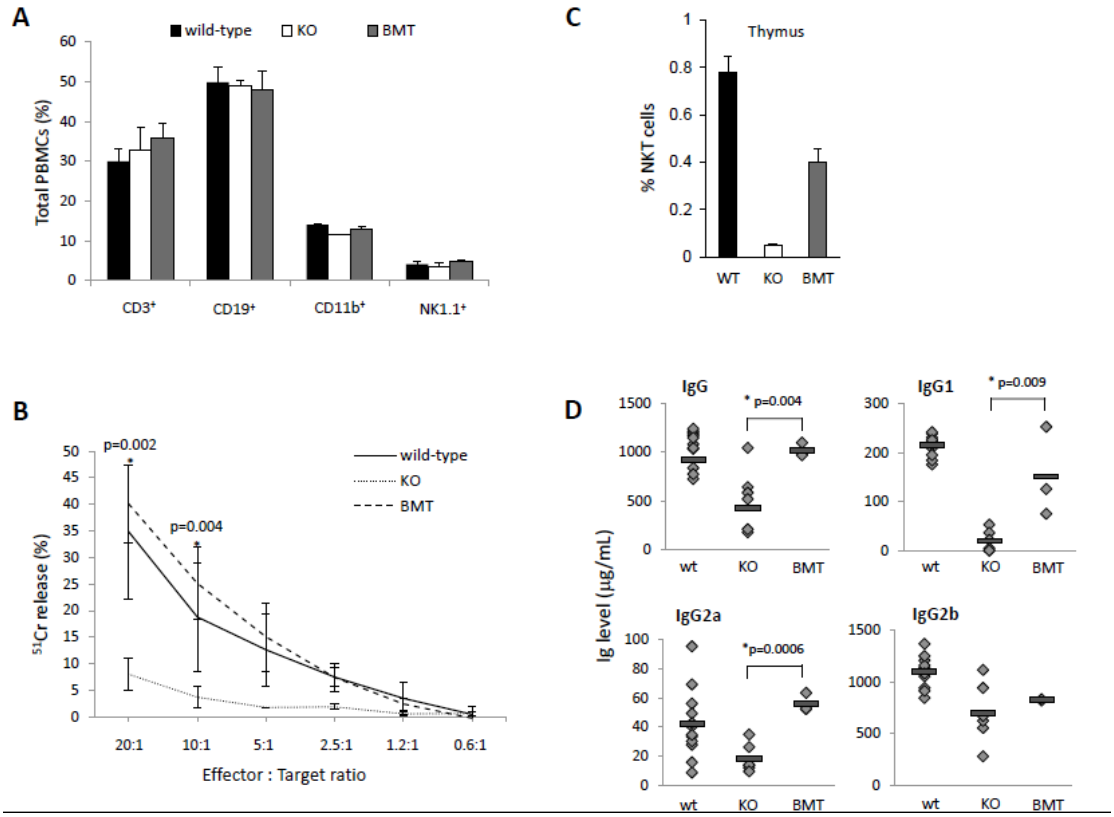
Supplementary Figure 5



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