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**Supporting Information**

**for**

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**Differential impact of the transcriptional repressor Gfi1 on mature CD4<sup>+</sup>  
and CD8<sup>+</sup> T lymphocyte function**

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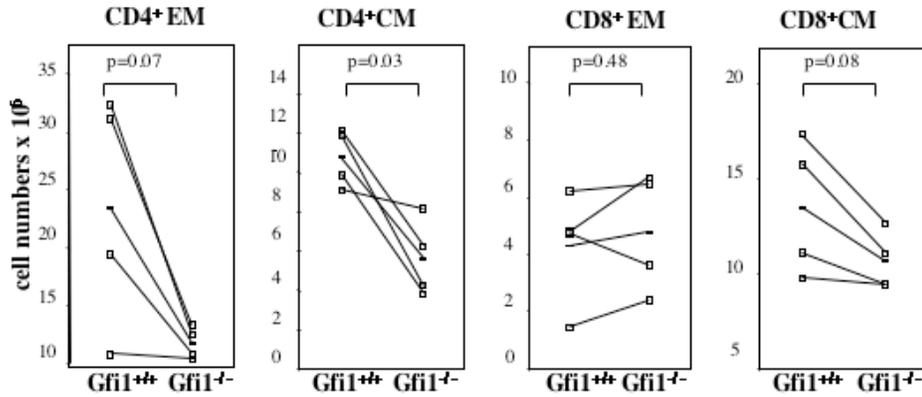
## **SUPPLEMENTARY TEXT**

### **Gene expression profiles for Gfi1<sup>-/-</sup> and wt T-cells**

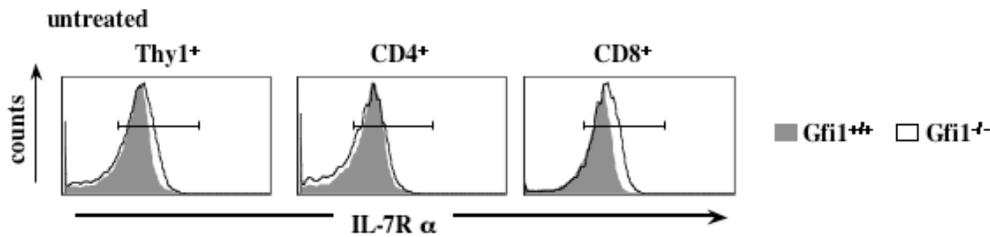
Splenic T-cells were MACS-purified and stimulated with anti-CD3/anti-CD28 antibodies for 4, 8 and 24 h. RNA was isolated at these different time points and subjected to DNA micro-array analysis using Affymetrix MOE430A\_2.0 murine chips representing 22,690 probesets. Differentially expressed genes were identified by comparison analysis (MAS5 algorithm) using p-detection value <0.001 and a fold change > 2.5. To reduce the number of false positive probesets only probesets were considered that showed at least one present detection call within the two arrays to be compared. 2,394 genes showed a significant divergence in their expression levels between Gfi1<sup>-/-</sup> versus Gfi1<sup>+/+</sup> T-cells at each time point. Spotfire software was used to organize these genes into groups according to their gene functions annotated in the GeneOntology (GO) database. The calculated p-value demonstrated the significance for the relative enrichment of genes relevant for a given biological process, and only processes, where a p-value <0.001 was obtained, were considered significant. The categories most highly represented among the 2,394 genes comprised processes as cell proliferation, -activation or -death (PCD) but also genes implicated in cellular defense and in stimulus response were activated in Gfi1<sup>-/-</sup> T-cells. From current GO annotations, we identified 25 genes involved in „positive regulation of apoptosis“ that showed higher than two fold changes of expression between Gfi1<sup>-/-</sup> and wt T-cells at each of those time points, at which the majority of these genes were highly upregulated in Gfi1<sup>-/-</sup> T cells after stimulation, i.e. showed positive values for the signal/log ratio.

SUPPLEMENTARY FIGURES

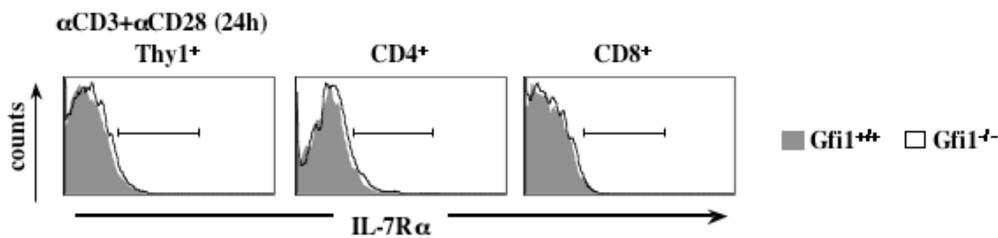
**a**



**b**



**c**

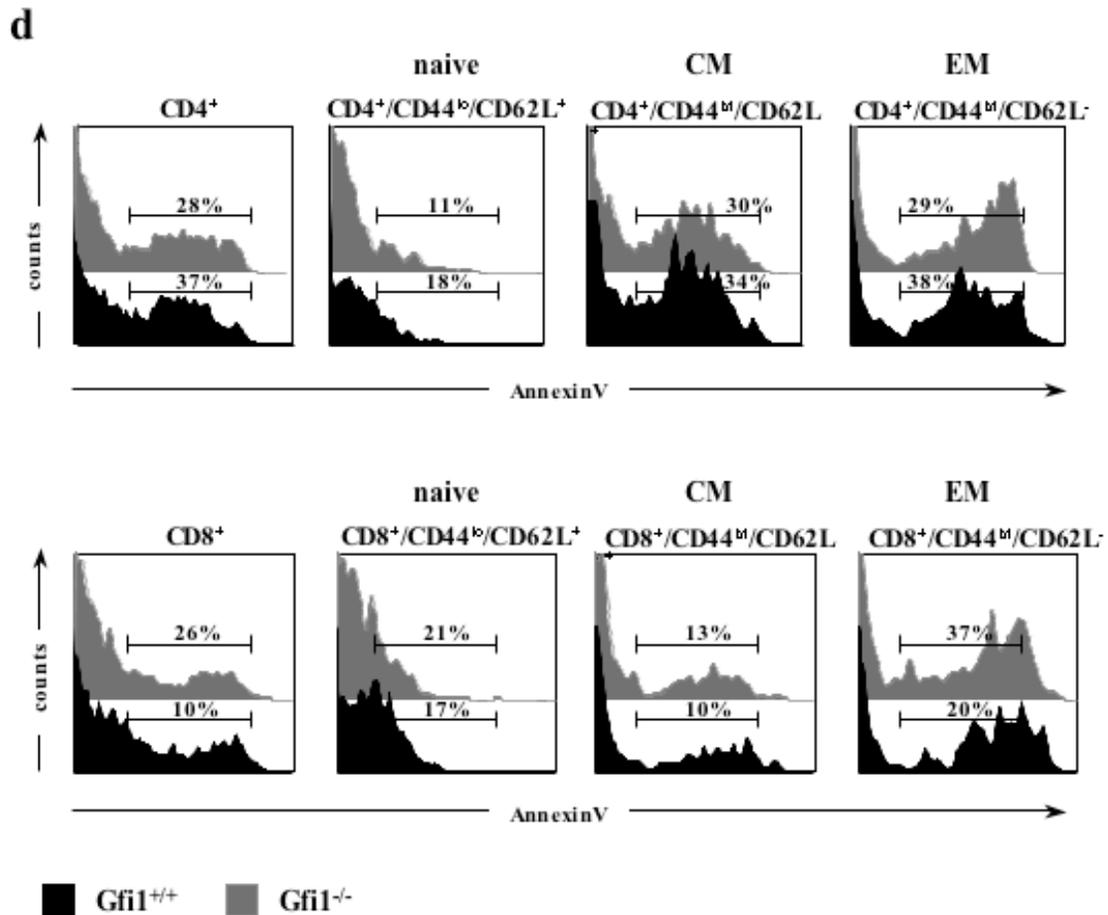


**Supp. Fig. S1**

Absolute cell numbers and IL-7 receptor  $\alpha$  expression on naive and activated T-cells

- Absolute cell numbers of the indicated memory cell subsets in wt and *Gfi1*<sup>-/-</sup> mice relative to a single spleen. EM, effector memory cells, CM central memory cells.
- Splenocytes derived from wt and *Gfi1*<sup>-/-</sup> mice were stained with anti Thy1.2, anti CD4, anti CD8 and anti IL-7 R $\alpha$  antibodies and the entire T-cell population or the CD4<sup>+</sup> and CD8<sup>+</sup> subsets were analyzed for IL-7 R $\alpha$  expression.
- As in b.) but after 24 stimulation with anti-CD3/anti-CD28. Note that IL-7R $\alpha$  is downregulated after antigenic activation in both CD4<sup>+</sup> and CD8<sup>+</sup> subsets regardless whether *Gfi1* was present or not.

Depicted are diagrams representative for several independent mouse pairs (wt/*Gfi1*<sup>-/-</sup>)

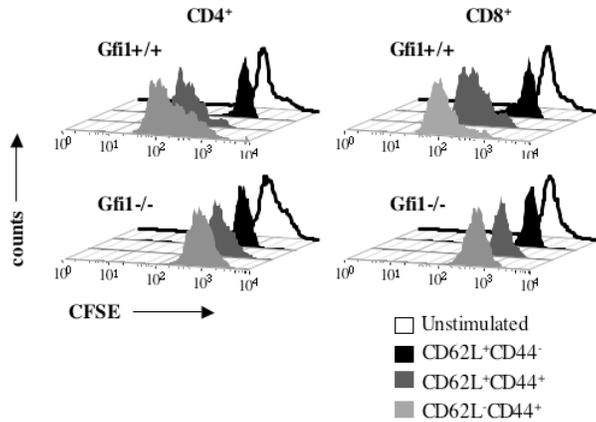


**Supp. Fig. S1 cont'd**

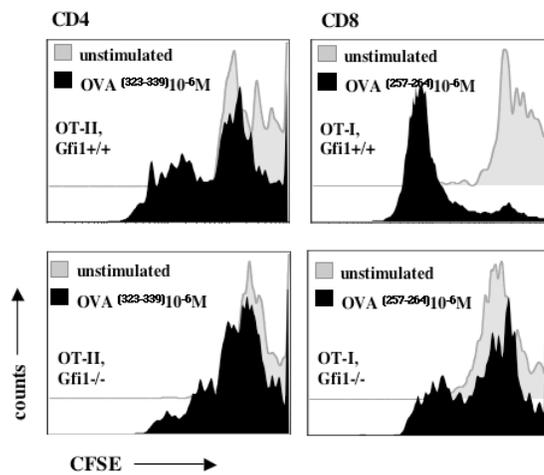
d.) AnnexinV staining of naive and memory T-cells from wt and *Gfi1*<sup>-/-</sup> mice. Splenocytes from wt and *Gfi1*<sup>-/-</sup> mice were stained with anti- CD4<sup>+</sup> (upper panel) or anti- CD8<sup>+</sup> (lower panel) and with anti- CD62L, anti- CD44 antibodies and AnnexinV and were analyzed by four-colour-flow cytometry. CD4<sup>+</sup> and CD8<sup>+</sup> T-cells were separated into naive (CD44<sup>lo</sup>, CD62L<sup>hi</sup>), central memory (CD44<sup>hi</sup>, CD62L<sup>hi</sup>) and effector memory T-cells (CD44<sup>hi</sup>, CD62L<sup>lo</sup>) and analyzed for AnnexinV binding.

Depicted are diagrams representative for several independent mouse pairs (wt/*Gfi1*<sup>-/-</sup>)

a



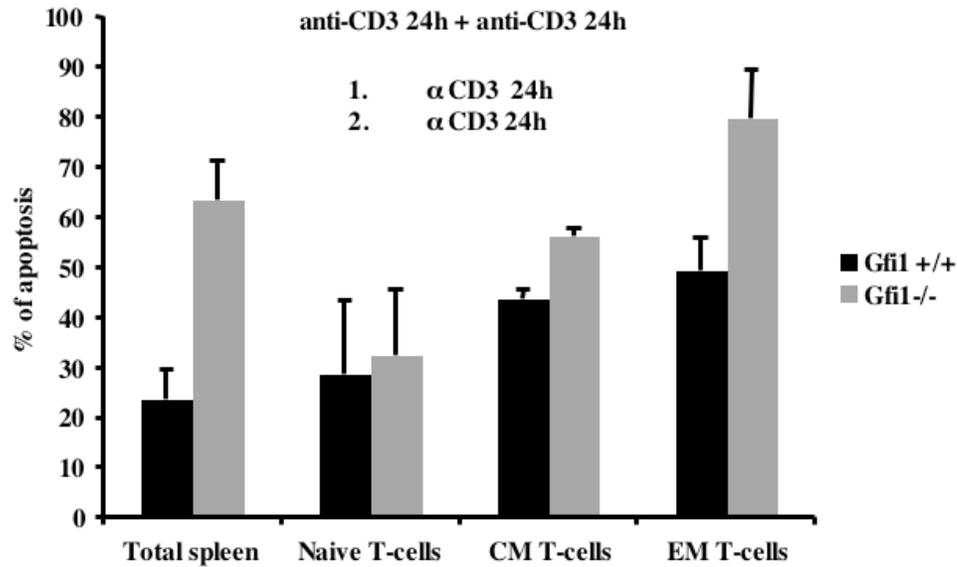
b



**Supp. Fig. S2**

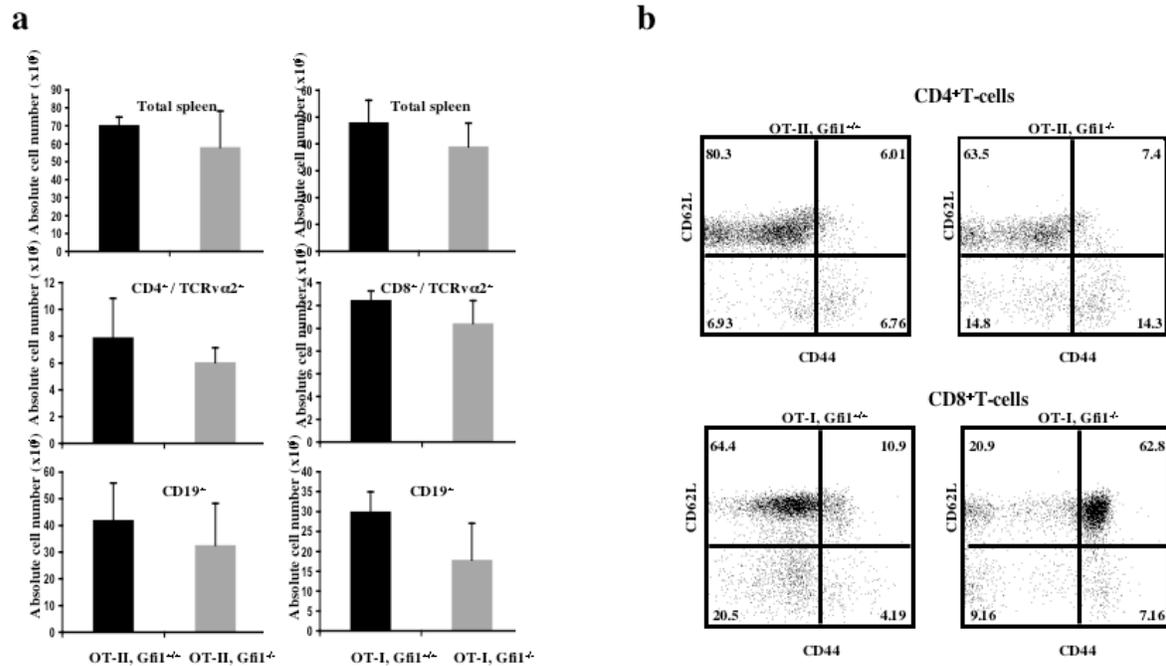
*In vitro* proliferation of splenocytes from *Gfi1*<sup>+/+</sup> and *Gfi1*<sup>-/-</sup> mice

- a.) Total splenocytes from *Gfi1*<sup>+/+</sup> and *Gfi1*<sup>-/-</sup> mice were stained with CFSE (1 $\mu$ M) and stimulated for 72h in 24 well plates (2x10<sup>6</sup> cells/ml) with anti-CD3 (0.5 $\mu$ g/ml, coated) and anti-CD28 (5 $\mu$ g/ml). Histograms show CD4<sup>+</sup> cells (left panel) or CD8<sup>+</sup> cells (right panel), gated on CD62L<sup>+</sup>CD44<sup>-</sup> (naive cells), CD62L<sup>+</sup>CD44<sup>+</sup> (central memory cells) and on CD62L<sup>-</sup>CD44<sup>+</sup> (effector memory cells). All subsets from *Gfi1*<sup>-/-</sup> mice showed a defect in their proliferation after  $\alpha$ CD3/ $\alpha$ CD28 stimulation *in vitro*. Shown is a result representative of several independent experiments.
- b.) Total splenocytes from OTI Tg x *Gfi1*<sup>+/+</sup> and OTI Tg x *Gfi1*<sup>-/-</sup>, and from OTII Tg x *Gfi1*<sup>+/+</sup> and OTII Tg x *Gfi1*<sup>-/-</sup> were stained with CFSE (1 $\mu$ M) and stimulated for 72h in 96-well plates (2x10<sup>6</sup> cells/ml) with 10<sup>-6</sup>M OVA<sub>(257-264)</sub> or OVA<sub>(323-339)</sub> peptides. CFSE fluorescence measured is representative of gated CD4/TCR $\nu$  $\alpha$ 2 positive cells for OTII Tg mice pairs (*Gfi1*<sup>+/+</sup> and *Gfi1*<sup>-/-</sup>), and gated CD8/TCR $\nu$  $\alpha$ 2 positive cells for OTI Tg mice pairs (*Gfi1*<sup>+/+</sup> and *Gfi1*<sup>-/-</sup>). Both OTI and OTII *Gfi1*<sup>-/-</sup> splenic T-cells showed a defect in their proliferation after antigenic stimulation *in vitro*. Shown is a result representative of three independent experiments.



**Supp Fig. S3**

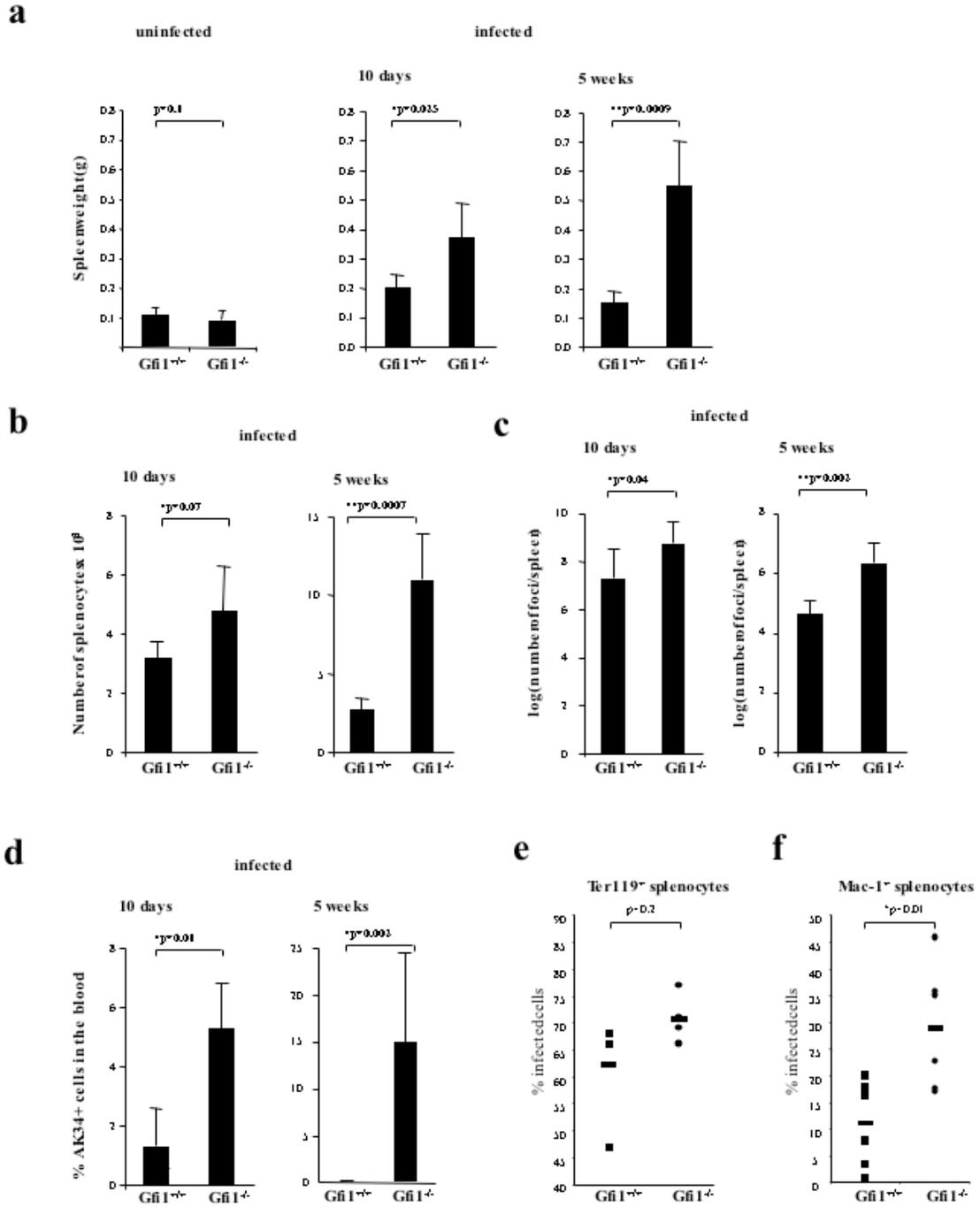
AICD is enhanced in total splenic T-cells and memory T-cells in *Gfi1* deficient mice  
Total spleen and sorted naive T-cells ( $CD4^+$ ,  $CD8^+$ ,  $CD44^{low}$ ,  $CD62L^{high}$ ), central memory T-cells (CM:  $CD4^+$ ,  $CD8^+$ ,  $CD44^{high}$ ,  $CD62L^{high}$ ) and effector memory T-cells (EM:  $CD4^+$ ,  $CD8^+$ ,  $CD44^{high}$ ,  $CD62L^{low}$ ) from *Gfi1*<sup>+/+</sup> (black bars) and *Gfi1*<sup>-/-</sup> (grey bars) were analyzed. The cells were pre-stimulated for 24h with anti-CD3 antibodies and apoptosis was induced with additional incubation with anti-CD3 antibodies for 24h. The percentage of Annexin-V positive, PI negative and  $CD4^+$ ,  $CD8^+$  T-cells were detected by flow cytometry. Gated T-cells from total spleen had an increased apoptotic rate in *Gfi1*<sup>-/-</sup> mice. Sorted central memory T-cells showed a mild increase while effector memory T-cells showed also an increase in anti-CD3 induced apoptosis. Data are representative of four independent experiments.



**Supp. Fig. S4**

A mild splenic lymphopenia and increased frequencies of T-cells with memory phenotype are observed in OTI Tg x *Gfi1*<sup>-/-</sup> and OTII Tg x *Gfi1*<sup>-/-</sup> mice

- Absolute numbers of total spleen, T-lymphocytes and B-lymphocytes for 3 pairs of OTI Tg x *Gfi1*<sup>+/+</sup> and OTI Tg x *Gfi1*<sup>-/-</sup>, and for 3 pairs of OTII Tg x *Gfi1*<sup>+/+</sup> and OTII Tg x *Gfi1*<sup>-/-</sup> (n=3) are shown. Splenocytes from each mouse line were stained with anti-CD4 and anti-TCR $\alpha$ 2 or anti-CD8 anti-TCR $\alpha$ 2 for T-cells and with anti-CD19 for B-cells. Absolute numbers are shown as average numbers and standard deviation calculated from percentage of frequencies in the spleen.
- Splenocytes from OTI Tg x *Gfi1*<sup>+/+</sup>, OTI Tg x *Gfi1*<sup>-/-</sup>, and from OTII Tg x *Gfi1*<sup>+/+</sup> and OTII Tg x *Gfi1*<sup>-/-</sup> were stained with anti-CD4, anti-CD8, anti-TCR $\alpha$ 2, anti-CD44 and anti-CD62L before analysis by flow cytometry. Percentages of naive (CD44<sup>low</sup>, CD62L<sup>high</sup>), central memory (CD44<sup>high</sup>, CD62L<sup>high</sup>) and effector memory (CD44<sup>high</sup>, CD62L<sup>low</sup>) T-cells are indicated for gated CD4<sup>+</sup> or CD8<sup>+</sup> T-cell subsets. Data are representative of 3 independent experiments.



**Supp. Fig. S5**

FV infection of Gfi1<sup>-/-</sup> and wt mice.

- a.) FV infected wt and Gfi1<sup>-/-</sup> mice were sacrificed ten days, five weeks post infection or without infection and the spleen weight was measured. Uninfected wt and Gfi1<sup>-/-</sup> mice had nearly the same spleen weight. After FV infection the spleen weight was significantly increased in the Gfi1<sup>-/-</sup> mice compared to wt mice.
- b.) Single cell suspensions of splenocytes from FV infected wt and Gfi1<sup>-/-</sup> mice were analyzed by flow cytometry and the number of live splenocytes was calculated. The number of splenocytes in Gfi1<sup>-/-</sup> mice compared to wt littermates was increased noticeably five weeks post FV infection.
- c.) By using the infectious center assay (see supplementary methods) a significantly higher count of virus specific foci could be detected in Gfi1<sup>-/-</sup> splenocytes ten days and more clearly five weeks post FV infection when compared to wt splenocytes.
- d.) Staining whole blood cells with the FV gag specific monoclonal antibody 34 (see supplementary methods) revealed a noticeable higher percentage of infected AK34<sup>+</sup> cells in the blood of Gfi1 deficient mice at ten days post FV infection. Five weeks post FV infection only a small percentage of AK34<sup>+</sup> cells were left in the blood of wt mice whereas the percentage of AK34<sup>+</sup> cells in the blood of FV infected Gfi1<sup>-/-</sup> mice was highly increased.
- e.) The higher virus load in Gfi1 deficient mice is due to both, an expansion of FV infected erythroid precursor cells and a virus replication in Mac1<sup>+</sup> cells. Splenocytes from wt and Gfi1<sup>-/-</sup> mice were stained with anti-Ter119 and AK34 antibodies and were cytometrically analyzed. The percentage of FV infected (i.e. AK34<sup>+</sup>) erythroid precursors relative to all Ter119<sup>+</sup> cells was similar in wt and Gfi1<sup>-/-</sup> mice.
- f.) Splenocytes from wt and Gfi1<sup>-/-</sup> mice were stained with anti-Mac-1 and AK34 antibodies and were cytometrically analyzed. The percentage of FV infected (i.e. AK34<sup>+</sup>) Mac-1<sup>+</sup> cells relative to all Mac-1<sup>+</sup> cells was significantly increased in Gfi1<sup>-/-</sup> mice compared to wt controls. Documented are the percentages of four individual sets of mice.

## SUPPLEMENTARY METHODS

### **Infectious center assays.**

Titration of single cell suspensions from infected mouse spleens were plated onto susceptible *Mus dunni* cells [25], co-cultivated for 3 days, fixed with ethanol, stained with F-MuLV envelope specific mAb 720 [36], and developed with peroxidase-conjugated goat anti-mouse antibodies and AEC to detect foci.

### **Flow cytometric analysis for F-MuLV antigen expression.**

Single cell suspensions were analyzed using a Becton Dickinson FACSCalibur flow cytometer. Cells were stained with hybridoma cell supernatant containing monoclonal antibody 34 [8], which is specific for F-MuLV glycoprotein expressed on the surfaces of infected cells. Development was done with FITC-labeled goat anti-mouse IgG2b-specific antiserum (Caltag Laboratories, Burlingame, CA) pre-absorbed with mouse cells to remove background activity ( $10^9$  nucleated spleen cells/ml serum, 40 minutes on ice).

### **T-cell culture and stimulation**

Splenic T-cells were purified from single cell suspension of Gfi1 knock-out and wild type mice with the Pan T-Cell Isolation kit by using the autoMACS system from Miltenyi Biotec. Isolation of the T-cells was performed according to the manufacturer's protocol. Cell cultures were set up with  $2 \times 10^6$  cells in complete RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine, penicillin G, streptomycin, and 2-mercaptoethanol. Purified splenic T-cells were cultured in the presence of 2  $\mu\text{g/ml}$  anti-CD3 (2C11) and anti-CD28 (37.51). For apoptosis assays splenic T-cells were stimulated 24h in the presence of 2  $\mu\text{g/ml}$  anti-CD3 (2C11) and IL-2 (20ng/ml) (TEBU), cells were washed and stimulated either with 2  $\mu\text{g/ml}$  anti-CD3 or 10  $\mu\text{g/ml}$  anti-CD95 antibody (PharMingen) in the presence of IL-2 (10ng/ml) for 12h. For detection of apoptotic cells the "apoptotic detection kit" from PharMingen was used as instructed by the manufacturer.

### **Antibody staining procedures and cell cycle analysis:**

Single cell suspensions were prepared as described [27] at the time of autopsy from spleen in PBS supplemented with 0.5% BSA (staining solution). Cells were washed in this solution and incubated on ice for 30 minutes with either antibodies directly conjugated with fluorochromes or with biotin labeled antibodies. Cells were washed in staining solution after the incubation and if necessary incubated with FITC or Phycoerythrin (PE) labeled streptavidin and examined with a FACSCalibur (Becton-Dickinson). For propidium iodide labelling and cell cycle analysis cells were washed once with cold PBS and fixed in Cytoperm/Cytofix (Becton Dickinson) 30 minutes or overnight at 4°C. The cells were washed with Cytofix washbuffer and then incubated with 20  $\mu\text{g/ml}$  propidium iodide (Sigma) and 250  $\mu\text{g/ml}$  RnaseA (Roche) in PBS for 15 min. FACS analysis was done in a FACSCalibur (Becton Dickinson). For CFSE staining  $10^7$  cells/ml in PBS without FCS were incubated with 5  $\mu\text{M}$  CFSE (Molecular Probes) for 5 minutes. Reaction was stopped by adding 10 vol. PBS<sup>+</sup> 5% FCS and cells were washed twice with this solution. For thymidine incorporation assay splenocytes from wt and Gfi1 deficient mice were isolated, and T-cells were purified by mouse CD90.1<sup>+</sup> magnetic beads that are based on positive selection for T-cells (autoMACS, Miltenyi). T cells were plated at the concentration of  $1 \times 10^6$ /ml and cultured with varying substances (anti-CD3/-CD28/IL-2). The cultures were incubated for the indicated time and pulsed with 1  $\mu\text{Ci}$  of [<sup>3</sup>H]thymidine for the last 8 h.