Chronic exposure to IFNα drives medullar lymphopoiesis towards T-cell differentiation in mice

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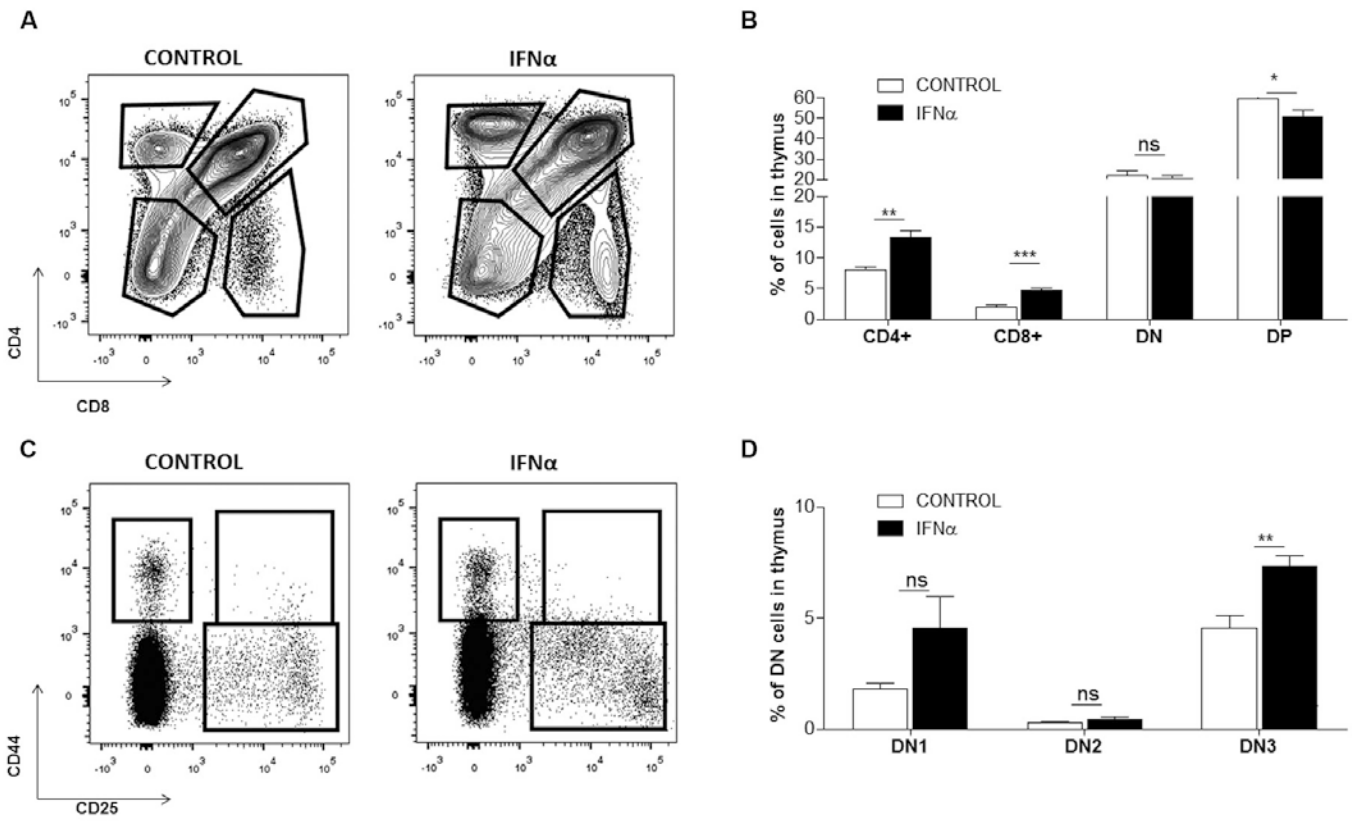
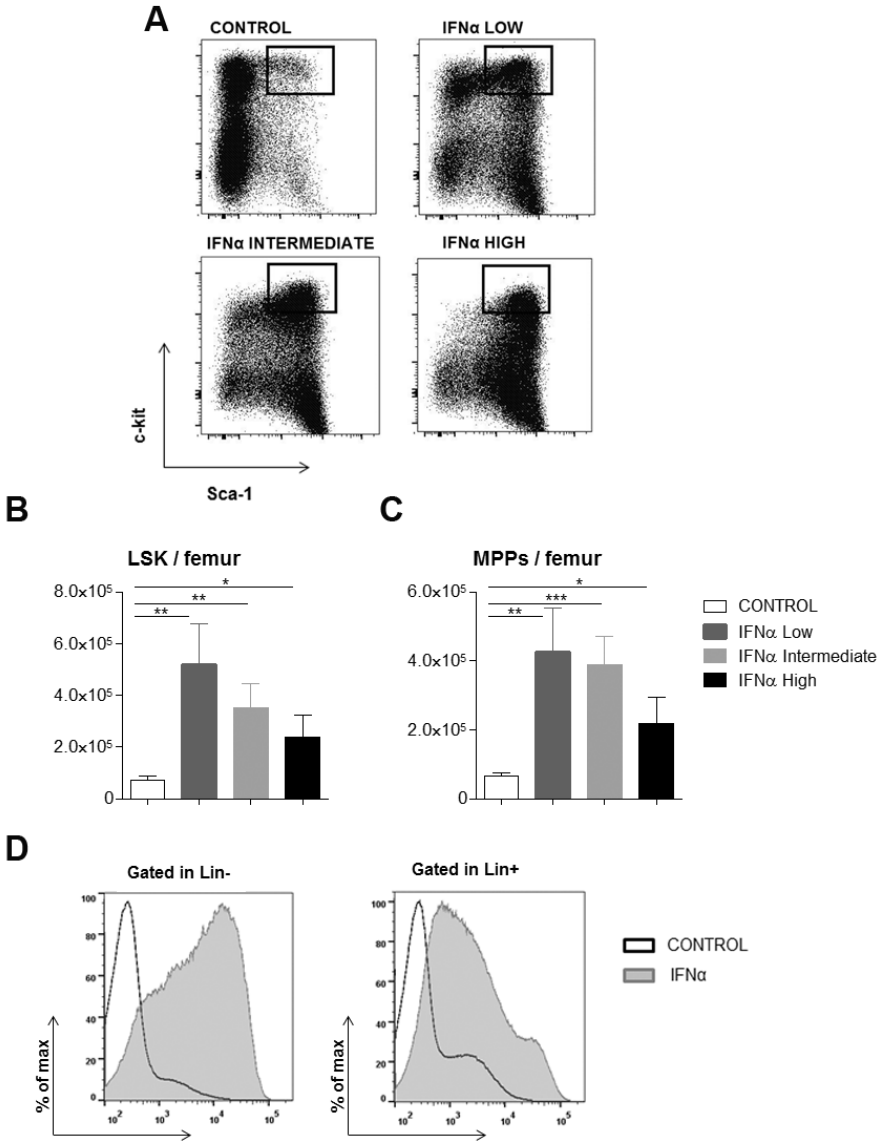
SUPPLEMENTARY FIGURE S1



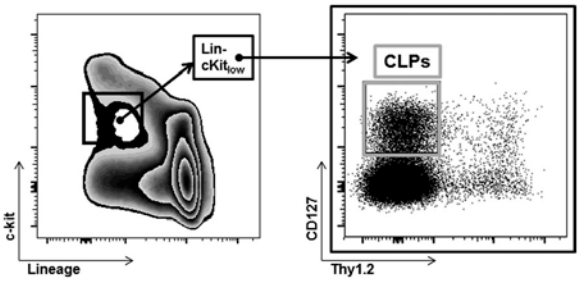
Chronic IFNα expression affects the cellular composition of the thymus. Three weeks after AAV-IFNα or AAV-Luc injection (5-6 mice/group) the cellular composition of the thymus was analysed by flow cytometry analysis. (A) Representative flow cytometry analysis of thymic cells labeled with anti-CD4 and anti-CD8 antibodies. (B) Percentage of CD4+, CD8+, double-negative (DN) or double-positive (DP) cells in thymi. (C) Representative flow cytometry analysis of thymus cells labeled with anti-CD44 and anti-CD25 cells gate on DN cells to differentiate among DN1, DN2 and DN3 subsets. (D) Percentage of DN1 (CD8-CD4-CD44+CD25-) or DN2 (CD8-CD4-CD44+CD25+) or DN3 (CD8-CD4-CD44-CD25+) in thymi. Results are expressed as the mean +/- SD. Statistical significance was determined by Student's t-test (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$).

SUPPLEMENTARY FIGURE S2



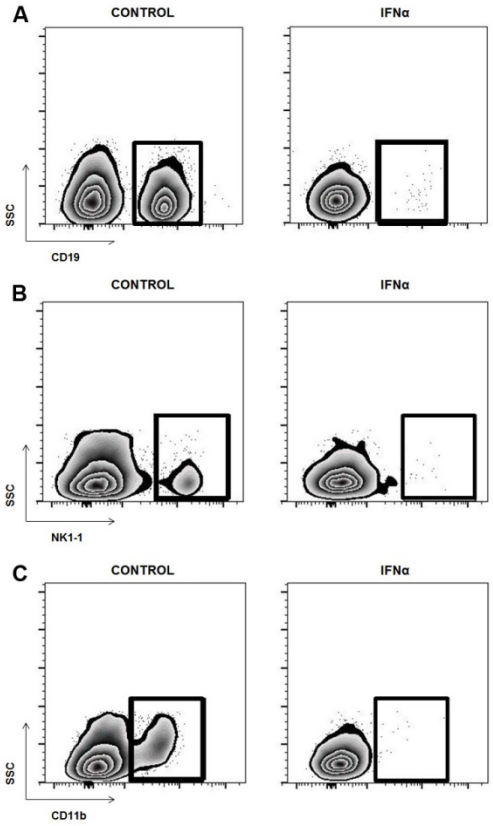
IFNα induces an aberrant expression of Sca-1 marker. (A) Representative flow cytometry analysis of Lineage negative (Lin-) cKit+ and Sca-1+ cells (LSK) of BM cells obtained from mice treated with different doses of AAV-IFNα or AAV-Luc (high dose). The analysis was performed three weeks after vector injection. (B-C) Bar graphs represent the numbers of LSK (B) and MPPs (C) per femur (5-6 mice/group). Results are expressed as the mean +/- SD. Statistical significance was determined by Student's t-test (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$). (D) Representative flow cytometry histogram analysis of Sca-1+ cells in the BM of AAV-treated mice gated in Lin- (left) or Lin+ (right) population.

SUPPLEMENTARY FIGURE S3



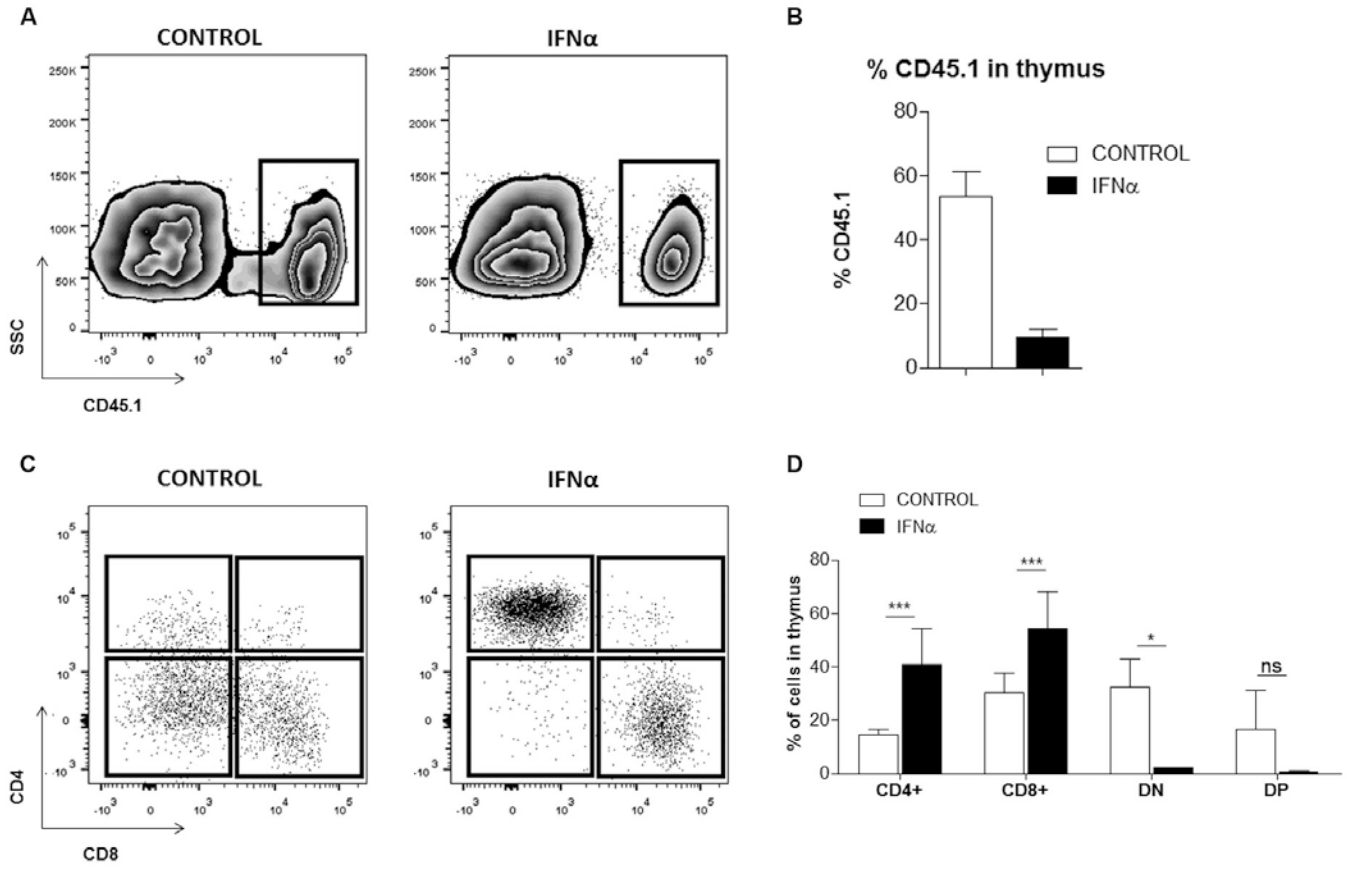
Gating strategy for the identification of CLPs population. CLPs were defined as Lin- ckitl^{low} CD127+ Thy- cells.

SUPPLEMENTARY FIGURE S4



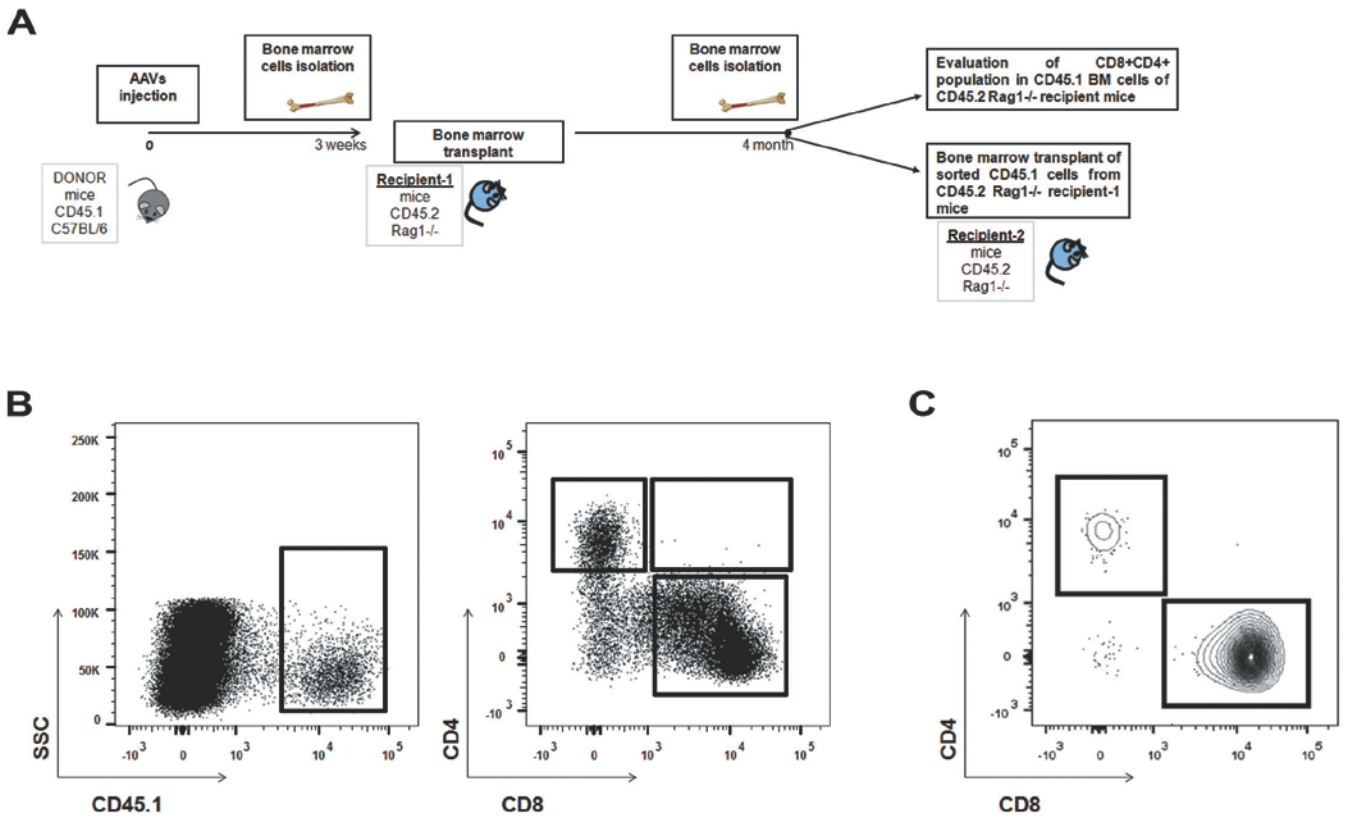
IFNα expression affects the repopulating ability of hematopoietic cells. (A-C) Representative flow cytometry analysis of CD45.1+ CD19+ (A), CD45.1+NK1.1+ (B), CD45.1+CD11b+ (C) donor-cells from AAV- IFNα or AAV-Luc treated mice in blood of CD45.2 recipient mice (4-5 mice/group).

SUPPLEMENTARY FIGURE S5



Analysis of cellular composition of the thymus in RAGB6 CD45.2+ recipient animal transplanted with CD45.1+ BM cells from AAV-IFNα or AAV-Luc treated mice. (A) Representative flow cytometry analysis of CD45.1 donor-cells obtained from the thymus of RAGB6 mice transplanted with bone marrow cells from AAV-IFNα or AAV-Luc treated mice (5 mice/group). (B) Mean percentage of CD45.1+ cells in thymus of recipient animals. (C) Representative flow cytometry analysis of the CD45.1+ CD4+ and/or CD8+ cells in thymi obtained from recipient mice 120 days after BM trasplantation. (D) Mean percentage of CD45.1+ CD4+ and/or CD8+ cells in thymi of recipient animals.

SUPPLEMENTARY FIGURE S6



Sustained IFNα expression drives medullar lymphopoiesis to T cell development and it is not due to BM-DP population present in the AAV-IFNα treated mice. (A) Schematic representation of the experiment. Three weeks after AAV-IFNα injection BM cells from C57/BL6 CD45.1 were isolated and transplanted by iv injection into RAG-1-deficient (RAG-/-) CD45.2 recipient mice (Recipient-1). Four months after transplantation CD45.1 were isolated from the BM of Recipient-1 and re-transplanted by iv injection into RAG-1-deficient (RAG-/-) CD45.2 (Recipient-2). (B) Representative flow cytometry analysis of CD45.1+ CD4+ and/or CD8+ cells in the bone marrow of Recipient-1 mice (5 mice/group). (C) Analysis of CD45.1+ CD4+ and/or CD8+ cells in the peripheral blood of Recipient-2 mice (5 mice/group).

Supplementary Table 1. List of antibodies used for flow cytometry of blood cells

Antibody	Conjugate	Clone	Conc.	Dilution	Supplier
CD8a	FITC	53-6.7	0.5 mg/ml	1/200	eBiosciences
CD8a	Pacific Blue	53-6.7	0.5 mg/ml	1/200	Biolegend
CD4	APC/Cy7	GK1.5	0.2 mg/ml	1/1000	Biolegend
CD19	APC/Cy7	PeCa1		1/100	ImmunoTools
CD3	PerCP/Cy5.5	17A2	0.2 mg/ml	1/200	Biolegend
NK1.1	APC	PK136	0.2 mg/ml	1/200	Biolegend
CD11b	FITC	M1/70.15		1/100	ImmunoTools
CD11c	APC	HL3		1/100	ImmunoTools
CD45.1	PE	A20	0.2 mg/ml	1/150	Biolegend
CD45R/B220	FITC	RA3-6B2	0.5 mg/ml	1/200	BD Pharmingen
CD44	APC/Cy7	IM7	0.2 mg/ml	1/400	Biolegend
CD25	BV421	PC61	12 μg/mL	1/200	Biolegend

Supplementary Table 2. List of antibodies used for flow cytometry of bone marrow cells

Antibody	Conjugate	Clone	Conc.	Dilution	Supplier
Lineage coktail	APC			1/150	BD Pharmingen
Lineage coktail	FITC			1/150	Biolegend
CD117(C-KIT)	PE	2B8	0.2 mg/ml	1/150	Biolegend
CD117(C-KIT)	APC/Cy7	2B8	0.2 mg/ml	1/200	Biolegend
Ly-6A/E (Sca-1)	Pacific Blue	D7	0.5 mg/ml	1/200	Biolegend
CD48	FITC	HM48-1	0.5 mg/ml	1/500	Biolegend
CD150	PE/Cy7	TC15-12F12.2	0.2 mg/ml	1/150	Biolegend
CD34	PE	RAM34	0.2 mg/ml	1/100	Biolegend
CD34	BV421	MEC14.7	0.1 mg/ml	1/100	Biolegend
CD34	FITC	RAM34	0.5 mg/ml	1/100	Biolegend
CD16/32	PE/Cy7	93	0.2 mg/ml	1/300	Biolegend
CD90.2	BV510	53-2.1	0.1 mg/ml	1/500	Biolegend
CD127	APC	SB/199	0.2 mg/ml	1/400	Biolegend
CD135	PE/Cy5	A2F10	0.2 mg/ml	1/100	Biolegend
CD8a	FITC	53-6.7	0.5 mg/ml	1/200	eBiosciences
CD8a	Pacific Blue	53-6.7	0.5 mg/ml	1/200	Biolegend
CD4	APC/Cy7	GK1.5	0.2 mg/ml	1/1000	Biolegend
CD19	APC/Cy7	PeCa1		1/100	ImmunoTools
CD3	PerCP/Cy5.5	17A2	0.2 mg/ml	1/200	Biolegend
NK1.1	APC	PK136	0.2 mg/ml	1/200	Biolegend
CD11b	FITC	M1/70.15		1/100	ImmunoTools
CD11c	APC	HL3		1/100	ImmunoTools
CD45.1	PE	A20	0.2 mg/ml	1/150	Biolegend
CD45R/B220	FITC	RA3-6B2	0.5 mg/ml	1/200	BD Pharmingen
Ter-119	APC	TER-119	0.2 mg/ml	1/200	BD Pharmingen
CD41	PE	MW/Reg30	0.2 mg/ml	1/100	BD Pharmingen

Supplementary Table 3: quantitative PCR primer list

Gene name	Forward	Reverse
IL-7 RECEPTOR ALPHA	CTGCAGTCCCAGTCATCATGA	GTGGCACTCAGATGATGTGACA
NOTCH-1	CGTGATGACCTAGGCAAGT	CAGTCTCATAGCTGCCCTCA
GATA 3	CTTATCAAGCCCAAGCGAAG	CCCATTAGCGTTCCTCCTC
DLL-4	TGTCTCCACGCCGGTATTG	AGGTCGTCTCCCGGTGTGT
DELTEX-1 (DTX-1)	GAGGATGTGGTTCGGAGGTA	CAGGCAGAGCAGGTGATACA
RUNX1	GCTTTCAAGGTGGTGGCAC	CTCGCTACCTGGTTCTTCATG
GATA1	CCAGTTTGTGGATTCTGCCC	GTAGGCCAGTGCTGATGCTGC
MEIS-1	GCATGCAGCCAGGTCCAT	TAAAGCGTCATTGACCGAGGA
SOCS1	CACCTTCTTGGTGCGCG	AAGCCATCTTCACGCTGAGC
E2A	CTTTGACCCTAGCCGGACATAC	GTGCCAACACTGGTGTCTCTC
EBF1	AGATTGAGAGGACGGCCTTTGT	TCTGTCCGTATCCCATTGCTG
PAX5	AATCGCTGAGTACAAACGCCAA	TCCGAATGATCCTGTTGATGGA
FOXO1	TCGTACGCCGACCTCATCA	TCCTTGAAGTAGGGCACGCTC

SUPPLEMENTARY METHODS

Determination of murine IFNα

Concentration of murine IFNα was determined by VeriKine Mouse Interferon-Alpha ELISA Kit (PBL interferon source).

Hemogram

Blood samples were analyzed using an automated veterinary hematological analyzer with a preprogrammed murine calibration mode (Hemavet 950FS; Drew Scientific, Waterbury, CT).

Cell isolation

For bone marrow cell isolation bones were flushed using a 23-gauge (femurs and tibias) or 26-gauge needle (iliac crests) and the bones discarded. The cells were obtained by mechanical disruption and washed by centrifuging at 1300rpm for 5 minutes at 4°C, resuspended in PBS, and then filtered through a 70-µm filter. Red blood cells were removed using a lysis Buffer. Cell concentrations were determined with an automatic cell counter (Z1 Coulter Particle Counter, Beckman Coulter).

For thymi cell isolation thymocytes were harvested by homogenizing thymic lobes with a syringe followed by centrifugation through a 40-µm Nylon mesh. Cells were washed in FACS-Buffer (PBS with 0.5% BSA and 0.05% NaN3), counted and stained using various antibody combinations.

Blood cells analysis

Blood single-cell suspensions were pretreated with FcR-Block (anti-CD16/32 clone 2.4G2; BD Bioscience-Pharmigen). Afterward, cells were stained with different combinations of antibodies. A list of antibodies is provided in supplemental Table 1.

Cell cycle analysis of CD8+ and CD4+ T cells.

Cell cycle analysis of CD8+ and CD4+ T cells were performed using Click-it EdU Flow cytometer assay kit according to the manufacturer's instructions (Invitrogen).

Hematopoietic Stem cell (HSC) staining

Whole bone marrow were isolated and stained on ice with various antibody cocktails to identify each progenitor compartment. A list of antibodies used is provided in supplemental Table 2.

Cell sorting

Different cell fractions were stained with antibodies and further purified on a FACSAria cell sorter (BD Biosciences, San Diego, CA).

In vitro treatment

3 x 10^5 LK cells cells were cultivated in StemSpa Serum-Free Expansion Medium (SFEM) medium (StemCell technologies) in presence or absence of 0,5U/µL of recombinant IFNα. LK were treated for 8h or 24h and harvested to analyse the TF by RT-PCR.

Bone marrow re-transplant

CD45.1+ cells derived from AAV-treated mice were purified on a FACSAria cell sorter from BM of CD45.2 Rag-/- (recipient-1) mice. Then these CD45.1+ cells were re-transferred by retroorbital injection into other group of RAG1-/- mice.

Statistical analysis of RT-PCR data

RT-PCR data were preprocessed and analyzed with R/Bioconductor.[23] The Delta Ct (DCt) method was used to quantify relative expression of each gene with respect to the housekeeping gene control GAPDH. The $2^{-\Delta\Delta Ct}$ formula was used to calculate the differential gene expression. Data are shown as log2 of the ratio between IFNα-exposed and control cells. LIMMA (Linear Models for Microarray Data)[24] was used to find out the

differentially expressed genes between the samples treated with IFNα and the controls. A False Discovery Rate (FDR) of 0.05 was established as selection criteria.[25]