Vol 458|16 April 2009|doi:10.1038/nature07815

LETTERS

IFNα activates dormant haematopoietic stem cells *in vivo*

Marieke A. G. Essers[1,2], Sandra Offner[3], William E. Blanco-Bose[3], Zoe Waibler[4], Ulrich Kalinke[4,5], Michel A. Duchosal[6] & Andreas Trumpp[1,2,3]

Maintenance of the blood system is dependent on dormant haematopoietic stem cells (HSCs) with long-term self-renewal capacity. After injury these cells are induced to proliferate to quickly re-establish homeostasis[1]. The signalling molecules promoting the exit of HSCs out of the dormant stage remain largely unknown. Here we show that in response to treatment of mice with interferon-α (IFNα), HSCs efficiently exit G_0 and enter an active cell cycle. HSCs respond to IFNα treatment by the increased phosphorylation of STAT1 and PKB/Akt (also known as AKT1), the expression of IFNα target genes, and the upregulation of stem cell antigen-1 (Sca-1, also known as LY6A). HSCs lacking the IFNα/β receptor (IFNAR)[2], STAT1 (ref. 3) or Sca-1 (ref. 4) are insensitive to IFNα stimulation, demonstrating that STAT1 and Sca-1 mediate IFNα-induced HSC proliferation. Although dormant HSCs are resistant to the anti-proliferative chemotherapeutic agent 5-fluoro-uracil[1,5], HSCs pre-treated (primed) with IFNα and thus induced to proliferate are efficiently eliminated by 5-fluoro-uracil exposure *in vivo*. Conversely, HSCs chronically activated by IFNα are functionally compromised and are rapidly out-competed by non-activatable *Ifnar*$^{-/-}$ cells in competitive repopulation assays. Whereas chronic activation of the IFNα pathway in HSCs impairs their function, acute IFNα treatment promotes the proliferation of dormant HSCs *in vivo*. These data may help to clarify the so far unexplained clinical effects of IFNα on leukaemic cells[6,7], and raise the possibility for new applications of type I interferons to target cancer stem cells[8].

Interferon (IFN) cytokines are produced by cells of the immune system in response to challenges by agents such as viruses, bacteria and tumour cells. IFNs suppress viral replication, have immunomodulatory activities and are used clinically to treat viral diseases, multiple sclerosis and malignancies such as chronic myeloid leukaemia (CML)[6,9,10]. Type I interferons (IFNα, IFNβ) bind and signal through the IFNα/β receptor (IFNAR)[11] and are strongly induced by the viral genome of many RNA viruses, which can be mimicked by the double-stranded RNA mimetic polyinosinic-polycytidylic acid (hereafter termed poly(I:C))[12]. Because Mx genes are strongly induced by IFNs, the *Mx1* promoter was used to generate the first inducible transgenic Cre line, *Mx1-Cre*, now one of the most commonly used mouse lines to eliminate genes flanked by *loxP* sites (floxed) in HSCs[13]. Although all functional mouse HSCs are contained within the Lin^{neg} $Sca1^{+}$ c-kit^{+} (LSK) population, less than 10% of LSK cells are repopulating HSCs. Significantly higher stem cell purities can be achieved by selecting LSK cells that are $CD34^{-}$, that show differential expression of SLAM receptors ($CD150^{+}$ $CD48^{-}$), or by selecting dormant HSCs using label-retaining assays[1,14,15].

Analysis of HSCs of 'control' animals lacking the *Mx1-Cre* or floxed genes, with or without poly(I:C) treatment, indicated that the proliferation of functional HSCs may be affected by poly(I:C) or IFNα. To systematically investigate this initial observation, C57Bl/6 (wild-type) mice were injected with poly(I:C) and the proliferation of the HSC/progenitor compartments was assessed by BrdU incorporation assays. As shown in Fig. 1a, proliferation of $LSKCD150^{+}$ cells is strongly induced in wild-type mice, but not in mice lacking the IFNAR, showing that poly(I:C)-mediated HSC proliferation requires IFNAR signalling. As expected, poly(I:C) treatment causes a significant increase in serum IFNα (Supplementary Fig. 1a). Moreover, poly(I:C)-induced proliferation is not restricted to $LSKCD150^{+}$ cells, but also occurs in populations further enriched in functional, predominantly quiescent HSCs such as $LSKCD150^{+}$ $CD34^{-}$ or $LSKCD135^{-}$ $CD34^{-}$ subsets (Supplementary Fig. 1b)[14–16]. The effects of poly(I:C) on proliferation of $LSKCD150^{+}$ cells is transient, peaking around 48 h after injection, and returning to normal after 4 days (Supplementary Fig. 1c). To examine whether IFNα has the same effect as poly(I:C), wild-type mice were injected with mouse recombinant IFNα4. Doses above 1,000 units per mouse significantly increased the proliferation of all tested HSC enriched populations, suggesting that not only HSCs but also early progenitors are stimulated to cycle in response to IFNα (Fig. 1b, c and Supplementary Fig. 1d, e).

As expected, $LSKCD150^{+}$ $CD48^{-}$ HSCs in untreated mice are predominantly in a quiescent, intracellular Ki67 negative ($icKi67^{neg}$ $Hoechst^{low}$) G_0 phase[15]. However, within 16 h of poly(I:C) injection most of these cells exit G_0 and enter an active cell cycle state ($icKi67^{+}$ $Hoechst^{med\text{-}hi}$) (Fig. 1d, e)[15,17]. To examine whether IFNα signalling also activates highly functional dormant HSCs, long-term label-retaining assays were performed[1]. DNA was labelled *in vivo* with BrdU for 10 days, followed by a 14-week BrdU-free 'chase' period, thus revealing long-lived dormant 'label retaining cells' (LRCs) within the $LSKCD150^{+}$ $CD48^{-}$ $CD34^{-}$ population, previously shown to contain the most highly potent HSCs[1]. After 12 weeks of chase, wild-type and *Ifnar*$^{-/-}$ mice were injected with either IFNα or poly(I:C), and the percentage of LRCs in various HSC populations was determined by fluorescence-activated cell sorting (FACS) at week 14. Notably, although the total number of cells remained unaltered (Supplementary Fig. 1f), the proportion of LRCs within the most quiescent HSC compartment ($LSKCD150^{+}$ $CD48^{-}$ $CD34^{-}$)[1,18] was greatly diminished in wild-type mice, suggesting that these cells have divided and diluted out the BrdU-label (Fig. 1f). As expected, although *Ifnar*$^{-/-}$ mice have a slightly reduced quiescent HSC compartment (Supplementary Fig. 1d), these cells do not proliferate in response to IFNα (Fig. 1h). These data indicate that even the most dormant HSC

[1]Division of Stem Cells and Cancer, Deutsches Krebsforschungszentrum (DKFZ), DKFZ-ZMBH Alliance, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. [2]Heidelberg Institute for Stem Cell Technologies and Experimental Medicine (HI-STEM), Im Neuenheimer Feld 280. D-69120 Heidelberg, Germany. [3]Ecole Polytechnique Fédérale de Lausanne (EPFL), ISREC—Swiss Institute for Experimental Cancer Research, School of Life Science, 1015 Lausanne, Switzerland. [4]Division of Immunology, Paul Ehrlich Institute, D-63225 Langen, Germany. [5]TWINCORE—Centre for Experimental and Clinical Infection Research Feodor-Lynen-Str. 7, 30625 Hannover, Germany. [6]Service and Central Laboratory of Hematology, CHUV, University Hospitals of Lausanne, CH-1011 Lausanne, Switzerland.

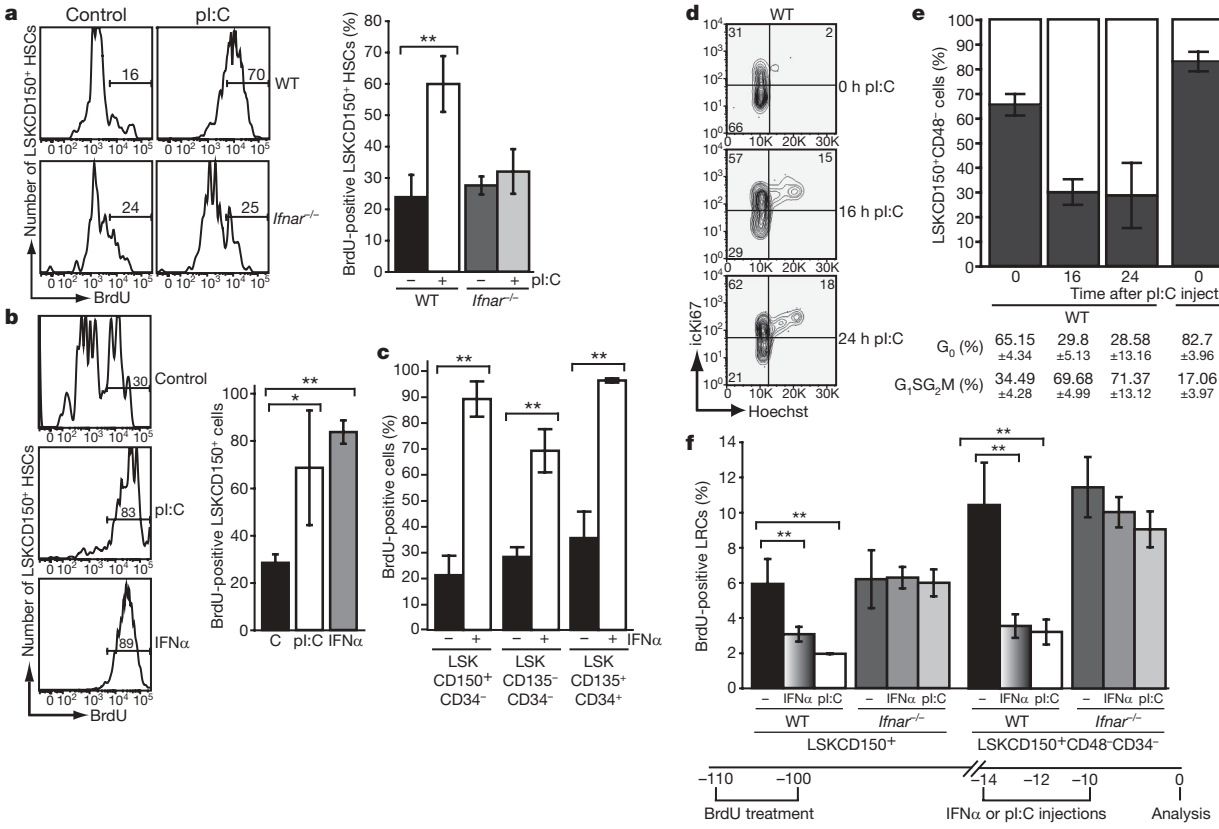


Figure 1 | IFNα induces efficient cell cycle entry of dormant HSCs *in vivo*. **a**, Sixteen-hours of BrdU incorporation in LSKCD150$^+$ cells from control or poly(I:C)-injected (pI:C; 10 μg g^{-1}; 0 and 48 h) wild-type (WT) and *Ifnar*$^{-/-}$ mice. FACS analysis at 72 h. Left, representative FACS profiles; right, quantitative and statistical analysis. **b**, Sixteen-hours of BrdU-uptake in LSKCD150$^+$ cells from wild-type mice injected with poly(I:C) (10 μg g^{-1}; 0 and 48 h), or with IFNα (10,000 units per mouse; 0, 24 and 48 h). Analysis at 72 h is shown. Left, representative FACS profiles; right, quantitative and statistical analysis. C, control. **c**, Analysis as in Supplementary Fig. 1b but using 10,000 units per mouse IFNα. **d**, Cell cycle analysis (Hoechst versus intracellular (ic) Ki67 to detect G_0 cells, icKi67^{neg} Hoechst^{low}) on LSKCD150$^+$ CD48$^-$ cells of wild-type mice, untreated or injected with poly(I:C) (5 μg g^{-1}; for 16 or 24 h). **e**, Quantitative analysis of **d**. Grey bars denote percentage G_0; white bars denote percentage $G_1/S/G_2/M$. **f**, BrdU LRC assay on wild-type and *Ifnar*$^{-/-}$ mice. The percentage of LRCs in each HSC-subpopulation plus poly(I:C) or mouse IFNα as indicated in the scheme. Data are the mean ± s.d. of four mice minimum per condition. These experiments were done in triplicate with similar results. *$P < 0.05$, **$P < 0.01$ (two-tailed *t*-test).

populations are induced to efficiently proliferate in response to IFNα stimulation *in vivo*.

It is thought that IFNα inhibits cellular proliferation, an observation which has been extensively demonstrated using *in vitro* culture systems[10]. Therefore our *in vivo* results are unexpected and raise the possibility that the observed HSC activation is caused by an IFNα-mediated effect on the stem cell niche[19]. However, wild-type HSCs present in chimaeric mice comprising wild-type haematopoietic cells in an *Ifnar*$^{-/-}$ stromal environment (reverse chimaeras) are still efficiently activated by IFNα, suggesting that IFNAR signalling in the stem cell niche itself is not required (Supplementary Fig. 2a). Although IFNα stimulation is sufficient to efficiently activate the proliferation of HSCs, IFNAR-mediated signalling is not required for HSC function, as *Ifnar*$^{-/-}$ HSCs can long-term reconstitute the entire blood system of lethally irradiated recipients (Supplementary Fig. 2b). Moreover, in mixed chimaeras generated by transplanting various input ratios of wild-type and *Ifnar*$^{-/-}$ bone marrow (5:95%, 50:50% and 95:5%) (Fig. 2a), the contribution of the *Ifnar*$^{-/-}$ bone marrow remains constant over a period of 3–5 months (6 ± 2%, 49 ± 3% and 94 ± 3% (means ± s.d.) *Ifnar*$^{-/-}$ cells, respectively; $n = 12$, $P < 0.01$), suggesting that *Ifnar*$^{-/-}$ HSCs have no competitive disadvantage over wild-type cells. To test whether IFNα directly or indirectly activates HSCs *in vivo*, 5:95% wild-type:*Ifnar*$^{-/-}$ chimaeras were stimulated with IFNα. This resulted in the efficient proliferation of the few wild-type (CD45.1$^+$) LSK-HSCs (Fig. 2b, left panel), even though 95% of the 'haematopoietic environment' is *Ifnar*$^{-/-}$ and thus

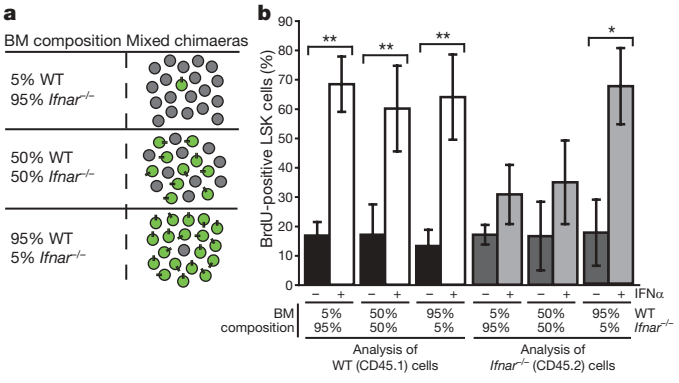


Figure 2 | A direct effect on HSCs, combined with an indirect mechanism, promotes HSC proliferation in response to IFNα *in vivo*. **a**, Schematic illustration of the generation and analysis of mixed bone marrow (BM) chimaeras. **b**, Proliferation of LSK cells in chimaeras comprised of different ratios of wild-type (WT; IFNα responsive) and *Ifnar*$^{-/-}$ (IFNα non-responsive) bone marrow. On the left, analysis gating on wild-type (CD45.1$^+$) cells is shown; on the right, analysis gating on *Ifnar*$^{-/-}$ (CD45.2$^+$) cells in the indicated chimaeras is shown. Each set represents six chimaeras, untreated or treated (24 and 16 h before analysis) with IFNα (10,000 units per mouse). BrdU was injected 16 h before analysis. Data are the mean ± s.d. of four mice minimum per condition. These experiments were done in triplicate with similar results. *$P < 0.05$, **$P < 0.01$ (two-tailed *t*-test).

unable to respond to IFNα. Furthermore, increasing the IFNα responsive wild-type haematopoietic environment from 5 to 95% did not further augment HSC proliferation (Fig. 2b, left panel) suggesting that IFNα directly promotes the proliferation of LSK-HSCs. In contrast, with high (but not low) numbers of wild-type cells present in the bone marrow, even *Ifnar*$^{-/-}$ LSK-HSCs (CD45.2^{+}) were induced to proliferate in response to IFNα treatment (95:5% wild-type:*Ifnar*$^{-/-}$ chimaeras) (Fig. 2b, right panel). This indicates that besides directly activating wild-type HSCs, IFNα can also promote the proliferation of cells lacking IFNAR, but only if a sufficient number of wild-type bone marrow cells are present. This indirect effect of IFNα is probably due to a positive feedback loop caused by the inhibitory effects of IFNα on wild-type differentiated blood cells, similar to what is observed after treatment with 5-fluoro-uracil (5-FU)[1,5,10].

To confirm the direct effect of IFNα on HSCs, complementary DNA microarray analysis was performed on sorted Lin^{neg}cKit^{+}CD150^{+}CD48^{-} HSCs from IFNα-treated (16 h) and untreated mice. This analysis showed the specific induction of a typical set of known IFN target genes in HSCs from IFNα treated mice, strongly supporting our data suggesting that IFNα directly activates dormant HSCs (Supplementary Table 1). Furthermore, the expression of several cell-cycle genes is altered in IFNα-stimulated HSCs, including upregulation of *Ccnb2* and *Cdk7*, as well as repression of *Pten* and *Elavl1*, known to stabilize p21^{Cip1} protein[20].

Type I IFN signalling is known to be mediated by activation of the JAK–STAT signalling pathway[9,11,21]. Indeed, not only are *Stat1* transcripts upregulated sixfold (Supplementary Table 1), a significant and transient increase in the phosphorylation of STAT1 (pSTAT1) is also observed in response to poly(I:C) in wild-type but not *Ifnar*$^{-/-}$ HSCs (Fig. 3a and Supplementary Fig. 3a, b). Interestingly, HSCs from poly(I:C)-treated *Stat1*$^{-/-}$ mice[3] showed no increase in BrdU-uptake, genetically demonstrating the requirement of STAT1 signalling for IFNα-mediated HSC activation (Fig. 3b and Supplementary Fig. 3c).

In agreement with the observed INFα-mediated repression of PTEN expression in HSCs (Supplementary Table 1), an increase of PKB/Akt phosphorylation was detected, suggesting that IFNα stimulation of HSCs results in activation of the PI3K signalling pathway (Fig. 3c). Part of this PI3K signalling seems to be downstream of STAT1, because the increase in PKB/Akt phosphorylation is reduced in *Stat1*$^{-/-}$ mice (Figs 3d and 4g).

Unexpectedly, IFNα-treated Lin^{neg}cKit^{+}CD150^{+} cells also show a significant increase in messenger RNA and cell surface expression of Sca-1 (Supplementary Table 1 and Fig. 3e). This glycosyl-phosphatidylinositol-linked cell surface receptor is highly expressed on all functional mouse HSCs, and *Sca-1*$^{-/-}$ HSCs have a competitive disadvantage in the presence of wild-type HSCs suggesting that this protein is important for HSC self-renewal[4,22]. To examine the role of Sca-1 in the IFNα-induced effect on HSC proliferation, poly(I:C)-treated *Sca-1*$^{-/-}$ mice[4] were analysed. Although the number of proliferating *Sca-1*$^{-/-}$ HSCs during homeostasis is slightly higher compared to control mice, treatment of poly(I:C) does not further augment HSC proliferation (Fig. 3f and Supplementary Fig. 3d). Furthermore, Sca-1 is not induced after IFNα simulation in the absence of STAT1 (Fig. 3g). These results show that Sca-1 is downstream of IFNAR-STAT1 signalling, and suggests that IFNα-induced HSC proliferation is not only mediated by STAT1, but also by Sca-1 (Fig. 4e).

Proliferating, but not dormant, HSCs are sensitive to treatment with anti-proliferative agents such as 5-FU[23]. Healthy mice survive even repeated treatment with 5-FU because dormant, and therefore drug-resistant HSCs are recruited into the cell cycle to rapidly produce new cells to quickly re-establish homeostasis[1]. Shifting the balance of the dormant HSC pool towards self-renewal has been shown to make mice exquisitely sensitive to repeated 5-FU treatment[24,25]. To test whether dormant, drug-resistant HSCs can be driven out of quiescence by IFNα, wild-type mice were primed at different times with poly(I:C), followed by two rounds of 5-FU

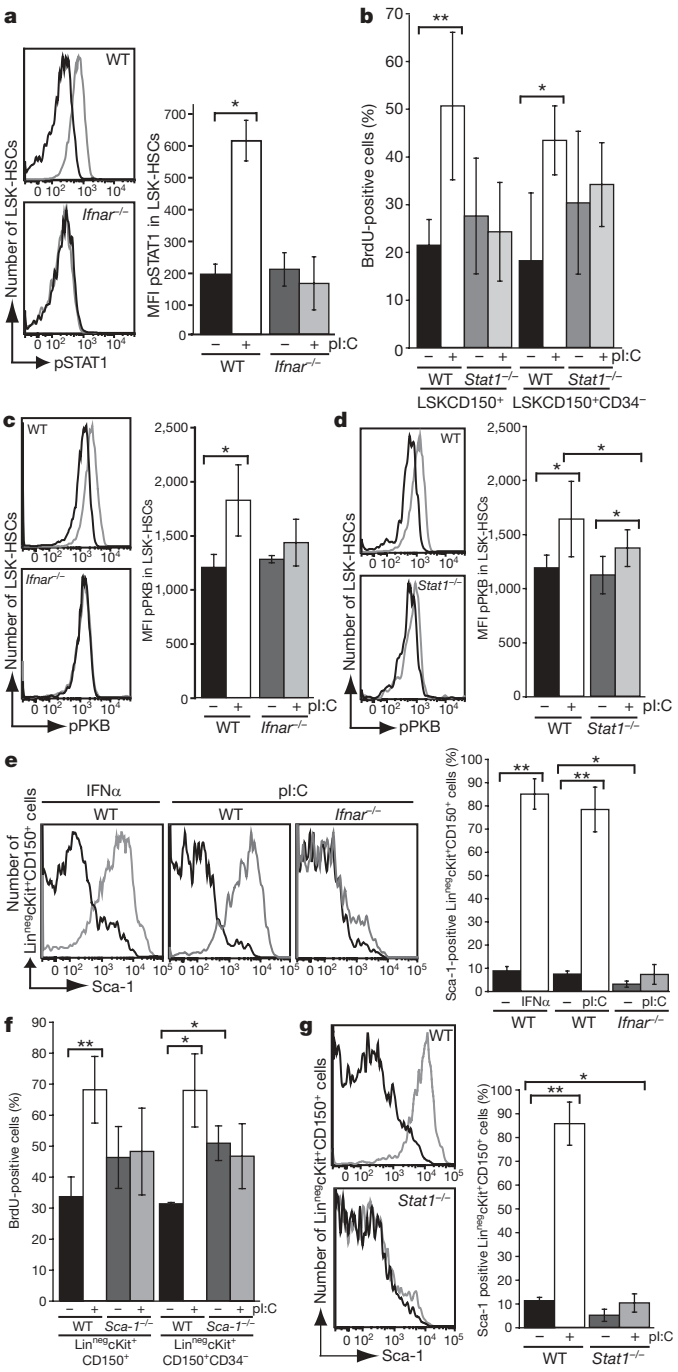


Figure 3 | IFNα-activated HSCs upregulate and are dependent on STAT1 and Sca-1 signalling. **a**, pSTAT1 expression in LSK cells of wild-type and *Ifnar*$^{-/-}$ mice, 16 h after i.p. injection of 5 μg g^{-1} poly(I:C) (denoted as pI:C). Left, representative FACS profiles; right, quantitative and statistical analysis; MFI, median fluorescence intensity. **b**, The 12 h BrdU-uptake of HSCs from wild-type and *Stat1*$^{-/-}$ mice 72 h after poly(I:C) (5 μg g^{-1}; at 0 and 48 h). **c**, Phosphorylated PKB/Akt (pPKB) expression in LSK cells of wild-type (WT) and *Ifnar*$^{-/-}$ mice 16 h after 5 μg g^{-1} poly(I:C) i.p. Left, representative FACS profiles; right, quantitative and statistical analysis. **d**, pPKB expression in LSK cells of wild-type and *Stat1*$^{-/-}$ mice; treatment as in **c**. **e**, Sca-1 expression in Lin^{neg}cKit^{+}CD150^{+} cells of wild-type and *Ifnar*$^{-/-}$ mice after IFNα and poly(I:C) as in Fig. 1d. Left, representative FACS profiles; right, quantitative and statistical analysis. **f**, Proliferation of indicated HSC populations isolated from wild-type or *Sca-1*$^{-/-}$ mice. Poly(I:C) injections as in **b**. **g**, The percentage of Sca-1^{+} cells within the Lin^{neg}cKit^{+}CD150^{+} population in wild-type and *Stat1*$^{-/-}$ mice, poly(I:C) injection as in **b**. Data are the mean ± s.d. of four mice minimum per condition. *$P < 0.05$, **$P < 0.01$ (two-tailed *t*-test).

treatment 7 days apart (Fig. 4a). Using this strategy, a treatment schedule was identified, resulting in the death of mice due to severe anaemia, probably because of the total loss of HSCs (Fig. 4b). Treatment schedules leading to death of the animals correlated with the increased proliferative status of LSKCD150$^+$ HSCs at the start of treatment, thus sensitizing them to 5-FU-mediated killing (Supplementary Fig. 4a). Lethality observed under these treatment regimes was dependent on IFNAR signalling because none was observed in *Ifnar*$^{-/-}$ mice (Supplementary Fig. 4b). These data indicate that IFNα priming provides an efficient way to induce cell-cycle entry of dormant HSCs, thus making them susceptible to elimination by anti-proliferative chemotherapeutic drugs such as 5-FU.

Because extensive HSC proliferation can lead to the exhaustion of stem cell function[25], bone marrow isolated from mice treated three times with poly(I:C) (followed by 10 days recovery) was serially transplanted into irradiated recipients. Because no significant difference in the repopulation activity of poly(I:C) treated cells was observed, transient activation of IFNα signalling does not affect the number of functional HSCs (Supplementary Fig. 4c). To study whether long-term (chronic) activation leads to a decrease in HSC activity, the mixed chimaeras described in Fig. 2a were treated eight times with poly(I:C) (every second day) and the number of phenotypic HSCs was determined 8 days later. Notably, in 50:50% wild-type:*Ifnar*$^{-/-}$ chimaeras all wild-type HSCs were lost and the HSCs present were exclusively derived from *Ifnar*$^{-/-}$ donors (Fig. 4c). Moreover, chimaeras containing 95:5% wild-type:*Ifnar*$^{-/-}$ HSCs before poly(I:C) treatment comprised only 12% wild-type and 88% *Ifnar*$^{-/-}$ HSCs after chronic IFNα signalling suggesting a marked competitive disadvantage of IFNα-stimulated HSCs (Fig. 4d). Moreover, transplantation of bone marrow from these mice at day 22 confirmed the complete loss of functional wild-type HSCs, as recipient mice showed no bone marrow reconstitution derived from wild-type but only *Ifnar*$^{-/-}$ cells (Supplementary Fig. 4d). Interestingly, in contrast to wild-type:*Ifnar*$^{-/-}$ mixed chimaeras, chronic IFNα treatment of non-chimaeric wild-type mice did not show any obvious HSC phenotype (data not shown). These results indicate that long-term activation of the IFNα signalling pathway does not simply eliminate HSCs, but rather markedly compromises their function, explaining why they are rapidly out competed by *Ifnar*$^{-/-}$ HSCs in competitive repopulation assays.

IFNα used to be a first line treatment for CML with variable outcomes. In recent years, imatinib mesylate has replaced IFNα owing to its far higher response rate accompanied with fewer side effects[26,27]. Unfortunately, discontinuation of imatinib mesylate results in a very high relapse rate, apparently due to the imatinib-mesylate-resistance of CML stem cells[6,28]. However, and relevant to our findings here, six patients initially treated with IFNα but subsequently switched to imatinib mesylate in 2002 showed a surprisingly high rate of long-term complete remission[29]. The anti-CML mechanism exerted by IFNα has classically been linked to the effects on immune cells including cytotoxic T cells[6,30]. As a non-exclusive alternative our results raise

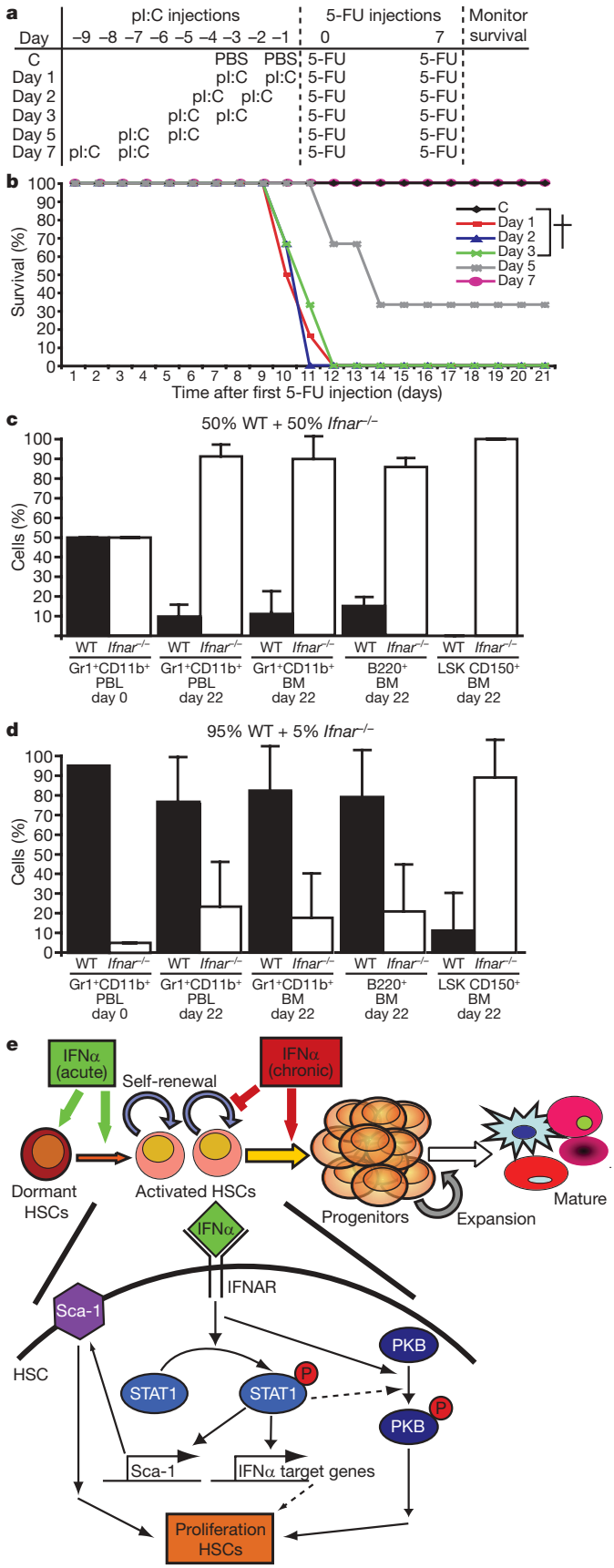


Figure 4 | Priming of IFNα followed by 5-FU treatment or chronic IFNα stimulation causes HSC loss. a, Schematic of various pre-treatments (priming) of wild-type (WT) mice with 10 μg g^{-1} poly(I:C) (pI:C) or PBS before injection with 5-FU. C, control. **b**, Kaplan–Meyer analysis of wild-type mice treated according to **a**. Each group comprises six wild-type mice. '†' represents death of the mice. **c**, **d**, 50:50% wild-type:*Ifnar*$^{-/-}$ (**c**) and 95:5% wild-type:*Ifnar*$^{-/-}$ (**d**) bone marrow chimaeras (day 0) treated eight times with poly(I:C) (5 μg g^{-1}) every second day, analysed at day 22. Chimaerism in myeloid cells, lymphoid cells and HSCs in peripheral blood (PBL) or bone marrow HSCs (as indicated); CD45.1$^+$ (filled bars), CD45.2$^+$ (open bars). All data normalized to 5, 50 or 95%. Six mice were analysed per condition. Data are the mean and s.d. **e**, Model showing the activating effects of short-term (acute) IFNα stimulation on dormant/quiescent HSCs, and the inhibitory effects of chronic IFNα treatment on HSC self-renewal. IFNα binds to and activates IFNAR, resulting in phosphorylation of STAT1 and PKB. Subsequently, several IFNα target genes are expressed (Supplementary Table 1). Sca-1 is upregulated at the mRNA and protein level, and Sca-1 protein is incorporated into the plasma membrane. Although the signalling pathway downstream of Sca-1 remains unknown, it is required for IFNα–IFNAR–STAT1-induced proliferation because *Sca-1*$^{-/-}$ mice do not respond to IFNα stimulation.

the possibility that IFNα pre-treatment in these patients might have activated and therefore sensitized the CML stem cells to imatinib mesylate. The so far unrecognized effects of acute and chronic IFNα signalling on stem cells may influence not only the future treatment of CML but potentially other diseases and malignancies as well.

METHODS SUMMARY

Mice. All mice were housed in individually ventilated cages in the EPFL/ISREC animal facility. Animal procedures were performed according to protocols approved by the Swiss Bundesamt für Veterinärwesen no. 1728. C57Bl/6 mice are referred to as wild-type mice. *Ifnar*$^{-/-}$, *Sca-1*$^{-/-}$ and *Stat1*$^{-/-}$ mice are all on a C57Bl/6 background[2–4]. CD45.1^{+} B6.SJL-Ptprca-Pep3b-/BoyJ donor mice were purchased from the Jackson Laboratory. Six–eight-week-old wild-type, *Ifnar*$^{-/-}$, *Sca-1*$^{-/-}$ or *Stat1*$^{-/-}$ mice were injected intraperitoneally (i.p.) with 5 μg g^{-1} poly(I:C) (Invivogen), or subcutaneously (s.c.) with 10,000 units per mouse of IFNα4 (gift from D. Tough), unless indicated differently. Control mice were injected with PBS. For the LRC assay, mice were BrdU-labelled for 10 days using BrdU water (0.8 mg ml^{-1}, glucose) followed by a 100-day chase. 5-FU studies were performed using i.p. injections of 150 mg kg^{-1} 5-fluoro-uracil (Sigma).

Preparation of bone marrow and PBL. To collect bone marrow cells, leg bones were crushed and cell suspensions were filtered before use. For FACS analysis of PBLs, six drops of blood were collected into a tube containing heparin, diluted in PBS, and centrifuged over a Histopaque-1083 (Sigma). PBLs were collected from the interface, washed and stained as described later.

FACS. Bone marrow cells were stained for haematopoietic subsets using lineage antibodies, cKit, Sca-1, CD135 (also known as Flt3), CD150 (Slamf1), CD34 and CD48. All cell suspensions were filtered through a nylon mesh filter (70 mm) before FACS analysis to prevent clumping. Statistical analysis was performed using a two-tailed *t*-test. Statistical significance is indicated by $*P < 0.05$ or $**P < 0.01$.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 25 September 2008; accepted 26 January 2009.
Published online 11 February 2009.

1. Wilson, A. *et al.* Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* **135,** 1118–1129 (2008).
2. Muller, U. *et al.* Functional role of type I and type II interferons in antiviral defense. *Science* **264,** 1918–1921 (1994).
3. Durbin, J. E., Hackenmiller, R., Simon, M. C. & Levy, D. E. Targeted disruption of the mouse *Stat1* gene results in compromised innate immunity to viral disease. *Cell* **84,** 443–450 (1996).
4. Ito, C. Y., Li, C. Y., Bernstein, A., Dick, J. E. & Stanford, W. L. Hematopoietic stem cell and progenitor defects in Sca-1/Ly-6A-null mice. *Blood* **101,** 517–523 (2003).
5. Randall, T. D. & Weissman, I. L. Phenotypic and functional changes induced at the clonal level in hematopoietic stem cells after 5-fluorouracil treatment. *Blood* **89,** 3596–3606 (1997).
6. Kujawski, L. A. & Talpaz, M. The role of interferon-α in the treatment of chronic myeloid leukemia. *Cytokine Growth Factor Rev.* **18,** 459–471 (2007).
7. Hehlmann, R., Hochhaus, A. & Baccarani, M. Chronic myeloid leukaemia. *Lancet* **370,** 342–350 (2007).
8. Trumpp, A. & Wiestler, O. D. Mechanisms of disease: cancer stem cells–targeting the evil twin. *Nature Clin. Pract. Oncol.* **5,** 337–347 (2008).
9. Borden, E. C. *et al.* Interferons at age 50: past, current and future impact on biomedicine. *Nature Rev. Drug Discov.* **6,** 975–990 (2007).
10. Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H. & Schreiber, R. D. How cells respond to interferons. *Annu. Rev. Biochem.* **67,** 227–264 (1998).
11. Darnell, J. E. Jr, Kerr, I. M. & Stark, G. R. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264,** 1415–1421 (1994).
12. Pichlmair, A. & Reis e Sousa, C. Innate recognition of viruses. *Immunity* **27,** 370–383 (2007).
13. Kuhn, R., Schwenk, F., Aguet, M. & Rajewsky, K. Inducible gene targeting in mice. *Science* **269,** 1427–1429 (1995).
14. Osawa, M., Hanada, K., Hamada, H. & Nakauchi, H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* **273,** 242–245 (1996).
15. Kiel, M. J. *et al.* SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121,** 1109–1121 (2005).
16. Adolfsson, J. *et al.* Upregulation of Flt3 expression within the bone marrow Lin^{-}Sca1^{+}c-kit^{+} stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity* **15,** 659–669 (2001).
17. Wilson, A. *et al.* c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev.* **18,** 2747–2763 (2004).
18. Wilson, A. *et al.* Dormant and self-renewing hematopoietic stem cells and their niches. *Ann. NY Acad. Sci.* **1106,** 64–75 (2007).
19. Wilson, A. & Trumpp, A. Bone-marrow haematopoietic-stem-cell niches. *Nature Rev. Immunol.* **6,** 93–106 (2006).
20. Yang, X. *et al.* Prostaglandin A_2-mediated stabilization of p21 mRNA through an ERK-dependent pathway requiring the RNA-binding protein HuR. *J. Biol. Chem.* **279,** 49298–49306 (2004).
21. van Boxel-Dezaire, A. H., Rani, M. R. & Stark, G. R. Complex modulation of cell type-specific signaling in response to type I interferons. *Immunity* **25,** 361–372 (2006).
22. Holmes, C. & Stanford, W. L. Concise review: stem cell antigen-1: expression, function, and enigma. *Stem Cells* **25,** 1339–1347 (2007).
23. Lerner, C. & Harrison, D. E. 5-Fluorouracil spares hemopoietic stem cells responsible for long-term repopulation. *Exp. Hematol.* **18,** 114–118 (1990).
24. Cheng, T. *et al.* Hematopoietic stem cell quiescence maintained by p21$^{cip1/waf1}$. *Science* **287,** 1804–1808 (2000).
25. Orford, K. W. & Scadden, D. T. Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. *Nature Rev. Genet.* **9,** 115–128 (2008).
26. O'Hare, T., Corbin, A. S. & Druker, B. J. Targeted CML therapy: controlling drug resistance, seeking cure. *Curr. Opin. Genet. Dev.* **16,** 92–99 (2006).
27. Atallah, E. & Cortes, J. Optimal initial therapy for patients with newly diagnosed chronic myeloid leukemia in chronic phase. *Curr. Opin. Hematol.* **14,** 138–144 (2007).
28. Heaney, N. B. & Holyoake, T. L. Therapeutic targets in chronic myeloid leukaemia. *Hematol. Oncol.* **25,** 66–75 (2007).
29. Rousselot, P. *et al.* Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood* **109,** 58–60 (2007).
30. Hochhaus, A. First-line management of CML: a state of the art review. *J. Natl. Compr. Canc. Netw.* **6** (suppl. 2), S1–S10 (2008).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We are grateful to M. Aguet for discussions and advice throughout the project, and for providing mouse strains. We thank D. Tough for providing mouse recombinant IFNα4, T. Pedrazzini, W. Stanford and M. Müller for mouse strains, K. Harshman and O. Hagenbüchle and the DAFL team for excellent service and help with the DNA microarrays. We thank C. Dubey and D. Aubry for animal husbandry, genetic screening and technical help, and J. Roberts for FACS sorting. We are grateful to A. Wilson for comments on the manuscript. M.A.G.E. is the recipient of an EMBO long-term fellowship. This work was supported by grants to A.T. from the Swiss National Science Foundation, the Swiss Cancer League, the EU- FP6 Program 'INTACT', the EU-FP7 Program 'EuroSyStem' and to UK from the Deutsche Forschungsgemeinschaft (SFB432.B15).

Author Contributions A.T., M.A.G.E. and U.K. designed the experiments and analysed the data. M.A.G.E., S.O. and Z.W. performed the experiments. W.E.B.-B. carried out the microarray analysis. A.T., M.A.G.E. and M.D. wrote the paper.

Author Information The microarray data have been deposited in the NCBI Gene Expression Omnibus (GEO) and are accessible through GEO series accession number GSE14361. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.T. (a.trumpp@dkfz-heidelberg.de).

METHODS

ELISA for IFNα. Wild-type mice were injected with the indicated amounts of poly(I:C). After 5 h, blood samples were collected and the concentration of mouse IFNα in the blood serum was determined by an ELISA kit according to the manufacturer's instructions (PBL Biomedical Laboratories).

Generation and analysis of chimaeras. CD45.1$^+$ B6.SJL-Ptprca-Pep3b-/BoyJ (the Jackson Laboratory) donor mice were purchased and maintained in the ISREC animal facility. To generate mixed chimaeras, transplantations were performed using mixtures of CD45.1$^+$ wild-type bone marrow and CD45.2$^+$ *Ifnar*$^{-/-}$ bone marrow with a total amount of 3×10^7 bone marrow cells (Fig. 2a). Bone marrow mixtures were injected intravenously (i.v.) into lethally irradiated CD45.1$^+$ wild-type recipient mice that had been pre-treated (48 h) with anti-NK1.1 monoclonal antibody. All chimaeric mice were maintained on antibiotic containing water (Bactrim, Roche). To generate reverse chimaeras, 3×10^7 CD45.1$^+$ wild-type bone marrow cells were injected i.v. into lethally irradiated CD45.2$^+$ *Ifnar*$^{-/-}$ mice, and bone marrow cells were analysed 1–2 months later. Three months after the bone marrow transplantation, mice were injected with IFNα and analysed for HSC proliferation.

Long-term reconstitution assay. Twenty-two days after the start of the chronic IFNα treatment, bone marrow from treated 50:50% wild-type:*Ifnar*$^{-/-}$ and 95:5%wild-type:*Ifnar*$^{-/-}$ chimaeras was isolated. Then, 3×10^7 bone marrow cells were injected i.v. into lethally irradiated CD45.1$^+$ wild-type recipient mice. Starting at week 6, reconstitution of PBL was analysed by tail vein bleeding every second week.

For the long-term reconstitution assay of wild-type versus *Ifnar*$^{-/-}$ bone marrow, and wild-type versus three-times poly(I:C)-injected bone marrow, 50,000 bone marrow cells from either wild-type (CD45.2$^+$) or *Ifnar*$^{-/-}$ (CD45.2$^+$) mice were together with 500,000 'rescue' bone marrow cells (Sca-1-depleted CD45.1$^+$ bone marrow cells) injected i.v. into lethally irradiated CD45.1$^+$ wild-type recipient mice.

Sorting of HSCs. To isolate HSCs, lineage magnetic depletion was performed to enrich for lineage-negative cells. Bone marrow cells were incubated with lineage antibodies (CD4, CD8, CD11b, Gr1, B220 and Ter119) and lineage-positive cells were removed using sheep anti-rat IgG-coated M450 Dynalbeads (Dynal Biotech). Lin^{neg} cells were stained for haematopoietic subsets and LSK-HSCs were sorted using a BD FACS Vantage-DIVA (Becton Dickinson).

Phosflow-staining FACS. For staining of pSTAT1 or pPKB, LSK-HSCs were sorted as described above, and stained according to the BD phosflow (Becton Dickinson) protocol for mouse cells using the STAT1 (pY701) (clone 4a)-Alexa647 or Akt (pS473)-Alexa647 antibody from BD phosflow.

BrdU staining and cell-cycle analysis. Proliferation analyses on HSCs were performed using cell surface staining in combination with BrdU. For BrdU analysis, mice were injected i.p. with BrdU (7.2 mg kg^{-1}, Sigma) 12 h before analysis, unless indicated differently. Mice were killed and bone marrow cells were isolated. Bone marrow cells were stained for haematopoietic subsets, and for BrdU staining a commercially available kit (BD Biosciences) was used.

Cell cycle analysis on HSCs was performed using cell surface staining in combination with intracellular Ki67 and Hoechst staining. In brief, bone marrow cells were labelled with monoclonal antibody conjugates to surface markers, fixed in 2% paraformaldehyde in PBS, washed and stained with anti-Ki67-FITC (BD Biosciences) for 30 min. During the last 10 min, cells were co-stained with Hoechst 33342 (Molecular Probes) at 20 μg ml^{-1}.

FACS. For flow cytometric analysis the 6 colour BD FACS Canto (BD Biosciences) equipped with a 488 nm and a 633 nm laser, the 8 colour BD FACS Vantage-DIVA (BD Biosciences) equipped with a 488 nm laser, a Multiline UV and a 647 nm laser, or the 9 colour analyser Cyan ADP Analyser (Beckman Coulter) equipped with a 488 nm, a 635 nm and 407 nm laser were used.

Antibodies. Gr-1 (Ly-6G, RB6-8C5)-FITC, -biotin and -Alexa647, Ter119-FITC and -biotin, B220 (RA3-6B2)-FITC and -biotin, CD11b-FITC and -biotin, CD4 (clone GK1.5)-FITC and -biotin, CD8α (53.6.7)-FITC and -biotin, CD45.1 (A20.1)-FITC, -biotin, -phycoerythrin (PE) or -Alexa647, CD45.2 (ALI-4A2)-FITC, -biotin or -Alexa647 were purified and conjugated in this laboratory following standard protocols. CD34 (RAM34)-FITC, CD135 (A2F10)-PE, cKit (2B8)-PE, -PeCy5, -PeCy7, -APC, and -APCCy7, Sca-1 (D7)-APC and (2B8)-biotin, CD48 (HM48-1)-PE, Sca1 (D7)-APC, and -Alexa700, CD4 (GK1.5)-PeCy7, CD8 (53-6.7)-PeCy7, CD11b (M1/70)-PeCy7, Gr1 (RB6-8C5)-PeCy7, B220 (RA3-6B2)-PeCy7, and Ter119-PeCy7 were purchased all from eBioscience. CD150 (TC15-12F12.2)-PeCy5 was purchased from Biolegend.

RNA isolation, microarray amplification and hybridization methods. Total RNA was isolated from sorted Lin^{neg}cKit$^+$ CD150$^+$ CD48$^-$ cells using the Qiagen RNA isolation kit (Qiagen). Per condition, three independent samples were analysed. Two rounds of amplification for each RNA sample were performed using the Nugen WT-Ovation Pico RNA Amplification System (Nugen). Biotin labelling of cRNA was performed using the Affymetrix GeneChip IVT labelling kit (Affymetrix). This biotinylated RNA was fragmented and hybridized to Affymetrix MOE430v.2 chips (Affymetrix) as per the manufacturer's protocol. Both raw image (.dat) and intensity (.cel) files were generated using the Affymetrix Gene Chip Operating Software.

Microarray analysis. Quality control tests were performed using the DNA Array Facility of Lausanne's Remote Analysis System (http://race.unil.ch). This is a web-based interface for various statistical analysis routines performed using the R language (http://www.r-project.org). These involved various quality tests such as the comparison of RNA 5′-end to 3′-end bias using RNA degradation plots to determine the quality of amplification, density of perfect match (PM) intensities, RMA normalization of the chips, sample clustering to control replicates, correlation matrix to observe correlation between samples and replicates, surface intensity in log scale to examine for chip and hybridization defects, scatter plots to compare samples and replicates. Chips that did not fit these quality checks were eliminated from the sample and not considered in the following statistical analysis. RMA-normalized data was entered into the Genespring program (Silicon Genetics) for data visualization as well as further filtering and examination of overlaps of various gene lists.

Samples having passed the quality check were then further statistically analysed using the Statistical Bayes Test Module on the RACE system. Genes showing a twofold difference with a minimum Baysian *P* value of 0.05 were then selected. These genes were then further analysed for significant genes and pathways using Ingenuity Pathways Analysis (Ingenuity Systems).

Supplementary Methods

ELISA for mIFNα

Wt mice were injected with indicated amounts of pI:C. 5 hours later, blood samples were collected and the concentration of mouse IFNα in the blood serum was determined by an ELISA kit according to the manufacturer's instructions (PBL Biomedical Laboratories).

Generation and analysis of chimeras

CD45.1$^+$ B6.SJL-Ptprca-Pep3b-/BoyJ (The Jackson Laboratory) donor mice were purchased and maintained in the ISREC animal facility. To generate mixed chimeras, transplantations were performed using mixtures of CD45.1$^+$ wt BM and CD45.2$^+$ IFNAR$^{-/-}$ BM with a total amount of 3x10^7 BM cells (Fig. 2a). BM mixtures were i.v. injected into lethally irradiated CD45.1$^+$ wt recipient mice that had been pre-treated (48h) with anti-NK1.1 mAb. All chimeric mice were maintained on antibiotic containing water (Bactrim, Roche). After 3 to 5 months the chimerism was determined in PBL and found in all cases to be as expected from the various input ratios. To generate reverse chimeras, 3x10^7 CD45.1$^+$ wt BM cells were i.v. injected into lethaly irradiated CD45.2$^+$ IFNAR$^{-/-}$ mice, and BM were analyzed 1-2 months later. 3 months after the BM transplantation mice were injected with IFNα and analyzed for HSC proliferation.

Long-term reconstitution assay

22 days after the start of the chronic IFNα treatment BM from treated 50%wt: 50% IFNAR$^{-/-}$ and 95%wt: 5% IFNAR$^{-/-}$ chimeras was isolated. 3x10^7 BM cells were i.v. injected into lethally irradiated CD45.1$^+$ wt recipient mice. Starting at week 6, reconstitution of PBL was analyzed by tail vein bleeding every second week.

For the long-term reconstitution assay of wt vs. IFNAR$^{-/-}$ BM, and wt vs. 3 times pI:C injected BM, 50000 BM cells from either wt (CD45.2$^+$) or IFNAR-/- (CD45.2$^+$) mice were together with 500000 'rescue' BM cells (Sca-1 depleted CD45.1$^+$ BM cells) i.v. injected into lethally irradiated CD45.1$^+$ wt recipient mice.

Sorting of HSCs

In order to isolate HSCs, lineage magnetic depletion was performed to enrich for lineage negative cells. BM cells were incubated with lineage antibodies (CD4, CD8, CD11b, Gr1, B220, Ter119) and lineage positive cells were removed using Sheep anti-Rat IgG coated M450 Dynalbeads (Dynal Biotech). Lineage negative cells were stained for haematopoietic

subsets and LSK-HSCs sorted using a BD FACS Vantage™ (Becton Dickinson, San Jose, CA).

Phosflow-stainings FACS

For staining of phosphorylated STAT1 (pSTAT1) or PKB/Akt (pPKB), LSK HSCs were sorted as described above, and stained according to the BD™ phosflow protocol for mouse cells using the STAT1 (pY701) (clone 4a)-Alexa647 or Akt (pS473)-Alexa647 antibody from BD™ phosflow.

BrdU stainings and cell cycle analysis

Proliferation analyses on HSCs were performed using cell surface staining in combination with BrdU. For BrdU analysis mice were injected i.p. with BrdU (7.2mg/kg, Sigma) 12h prior to analysis, unless indicated differently. Mice were sacrificed and BM cells were isolated. BM cells were stained for haematopoietic subsets, and for BrdU staining a commercially available kit (BD Biosciences) was used.

Cell cycle analysis on HSCs was performed using cell surface staining in combination with intracellular Ki67-Hoechst staining. Briefly, BM cells were labelled with mAb conjugates to surface markers, fixed in 2% paraformaldehyde in PBS, washed, and stained with anti-Ki67-FITC (BD Biosciences) for 30 min. During the last 10 min. cells were co-stained with Hoechst 33342 (Molecular Probes) at 20 μg/ml.

FACS

For flow cytometric analysis the 6 colour BD FACS Canto™ (BD Biosciences, San Jose, CA) equipped with a 488nm and a 633nm laser, the 8 colour BD Digital FACS Vantage™ (BD Biosciences, San Jose, CA) equipped with a 488nm, a Multiline UV and a 647nm laser, or the 9 colour analyzer Cyan™ ADP Analyzer (Beckman Coulter GmbH, Germany) equipped with a 488nm, a 635nm and 407nm laser were used.

Antibodies

Gr-1 (Ly-6G, RB6-8C5)-FITC, -biotin and -Alexa647, Ter119-FITC and -biotin, B220 (RA3-6B2)-FITC and -biotin, CD11b-FITC and -biotin, CD4 (clone GK1.5)-FITC and -biotin, CD8α (53.6.7)-FITC and -biotin, CD45.1 (A20.1)-FITC, -biotin, -PE or -Alexa647, CD45.2 (ALI-4A2)-FITC, -biotin or -Alexa647 were purified and conjugated in this laboratory following standard protocols. CD34 (RAM34)-FITC, CD135 (A2F10)-PE, cKit (2B8)-PE, -PeCy5, -PeCy7, -APC, and -APCCy7, Sca-1 (D7)-APC and (2B8)-biotin, CD48 (HM48-1)-PE, Sca1 (D7)-APC, and -Alexa700, CD4 (GK1.5)-PeCy7, CD8 (53-6.7)-PeCy7, CD11b (M1/70)-PeCy7, Gr1 (RB6-8C5)-PeCy7, B220 (RA3-6B2)-PeCy7, and Ter119-PeCy7 were

purchased all from eBioscience (San Diego, CA). CD150 (TC15-12F12.2)-PeCy5 was purchased from Biolegend (San Diego, CA).

RNA isolation, microarray amplification and hybridization methods

Total RNA was isolated from sorted lineage$^-$cKit$^+$CD150$^+$CD48$^-$ cells using the Qiagen RNA isolation kit (Qiagen AG, Basel,CH). Per condition 3 independent samples were analysed. Two rounds of amplification for each RNA sample were performed utilizing the Nugen WT-Ovation Pico RNA Amplification System (Nugen, San Carlos, CA, USA). Biotin labelling of cRNA was performed utilizing the Affymetrix GeneChip IVT labelling kit (Affymetrix, Santa Clara, CA, USA). This biotinylated RNA was fragmented and hybridized to Affymetrix MOE430v.2 chips (Affymetrix, Santa Clara, CA, USA) as per the manufacturers protocol. Both raw image (.dat) and intensity (.cel) files were generated utilizing the Affymetrix Gene Chip Operating Software (GCOS).

Microarray analysis

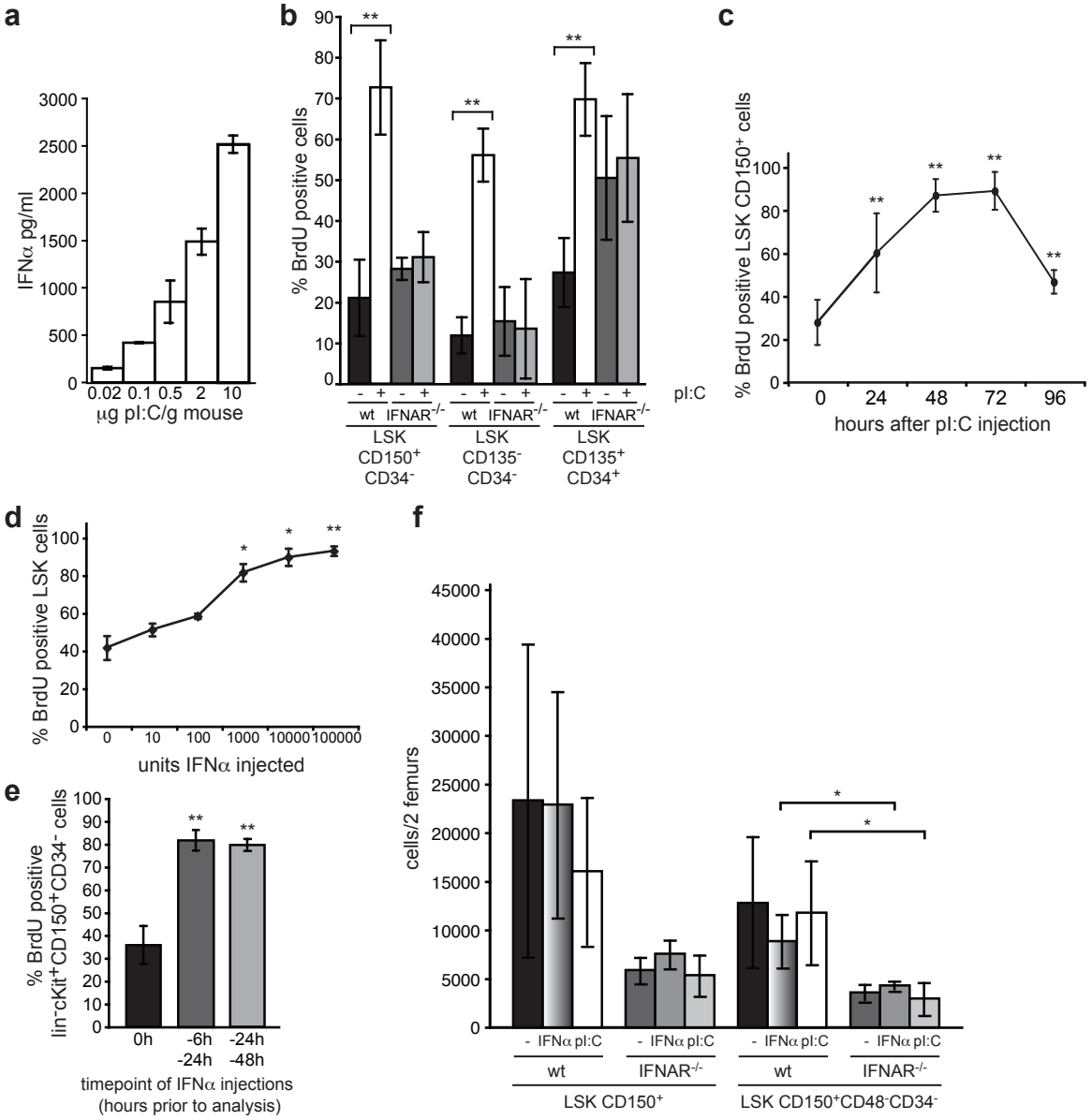
Quality control tests were performed utilizing the DNA Array Facility of Lausanne's Remote Analysis System (http://race.unil.ch)[4]. This is a web-based interface for various statistical analysis routines performed utilizing the R language (http://www.r-project.org). These involved various quality tests such as the comparison of RNA 5' end to 3'end bias utilizing RNA degradation plots to determine the quality of amplification; density of PM intensities; RMA normalization of the chips; sample clustering to control replicates; correlation matrix to observe correlation between samples and replicates; surface intensity in log scale to examine for chip and hybridization defects; scatter plots to compare samples and replicates. Chips that did not fit these quality checks were eliminated from the sample and not considered in the following statistical analysis. RMA normalized data was entered into the Genespring program (Silicon Genetics) for data visualization as well as further filtering and examination of overlaps of various gene lists.

Samples having passed the quality check were then further statistically analysed using the Statistical Bayes Test Module on the RACE system. Genes showing a 2-fold difference with a minimum Baysian p value of 0.05 were then selected. These genes were then further analysed for significant genes and pathways using Ingenuity Pathways Analysis (Ingenuity Systems, Redwood City, CA, USA).

References:

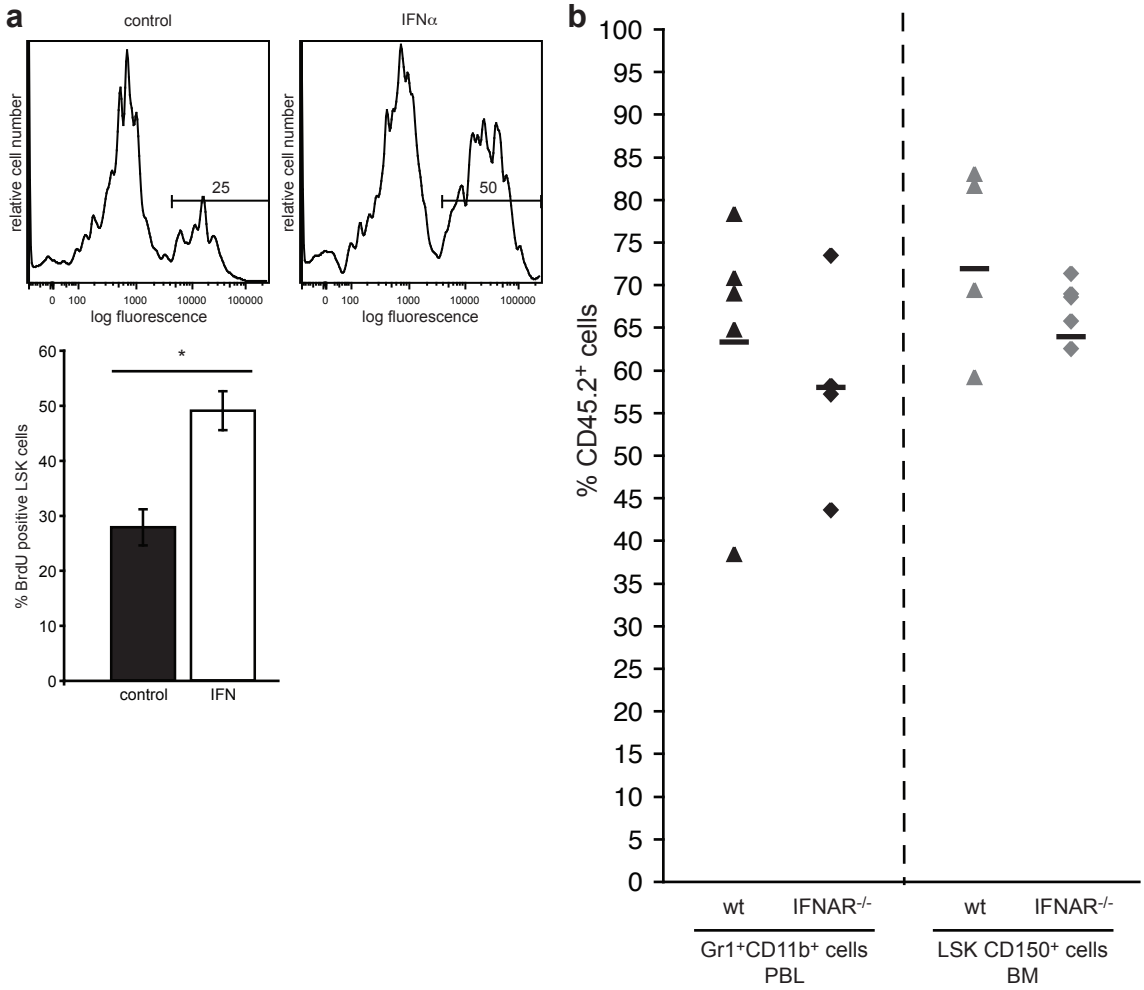
1 U. Muller, U. Steinhoff, L. F. Reis et al., *Science* **264** (5167), 1918 (1994).
2 W. L. Stanford, S. Haque, R. Alexander et al., *J Exp Med* **186** (5), 705 (1997).
3 J. E. Durbin, R. Hackenmiller, M. C. Simon et al., *Cell* **84** (3), 443 (1996).

4 M. Psarros, S. Heber, M. Sick et al., *Nucleic Acids Res* **33** (Web Server issue), W638 (2005).



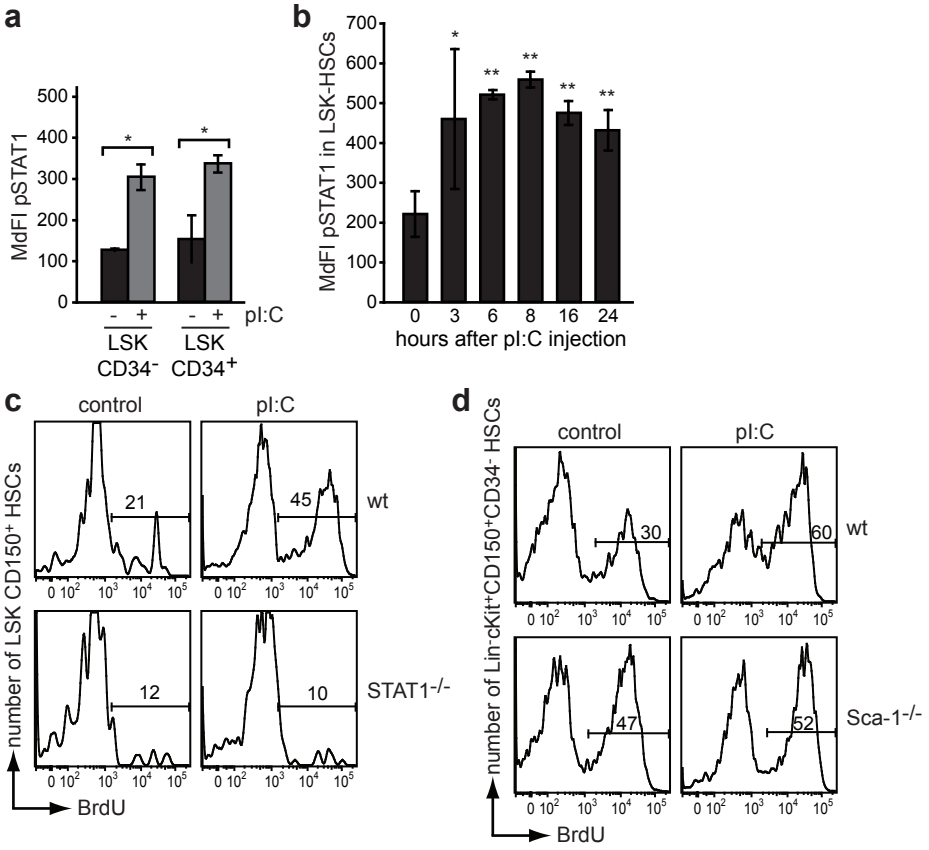
Supplementary Figure 1. Kinetic analysis of the effect of IFNα on the proliferation of HSCs.

a, IFNα levels (ELISA) in blood serum of wt mice injected once with indicated pI:C dose (24h). **b,** BrdU incorporation in HSC subsets. pI:C and BrdU injection as in Figure 1. **c,** Kinetic analysis of BrdU uptake of wt LSKCD150$^+$ cells after a single injection with pI:C. BrdU was injected 16h prior to analysis. **d,** LSK cell proliferation in response to a single injection of increasing amounts of mouse IFNα. BrdU was injected 16h prior to analysis. **e,** BrdU uptake of lin$^-$cKit$^+$CD150$^+$CD34$^-$ cells in response to two different IFNα injection schedules. BrdU was injected 16h prior to analysis. **f,** Total cell number/2 femurs of indicated HSC populations in LRC mice of Fig. 1h. n = 4 per condition. These experiments have been done in triplicate with similar results. Statistical analysis as in Figure 1.



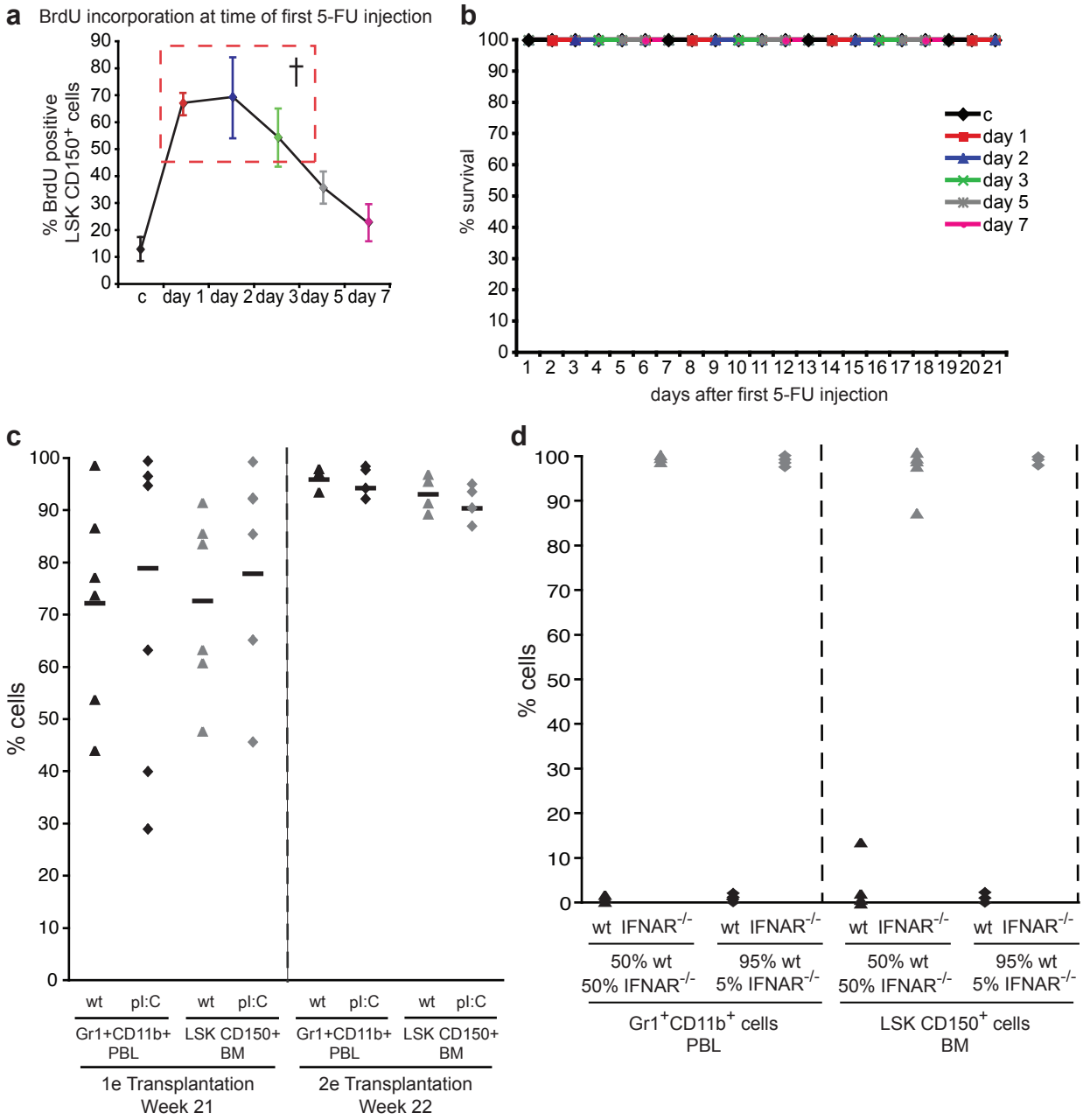
Supplementary Figure 2. No functional difference between wt and IFNAR$^{-/-}$ HSCs in long-term reconstitution assays

a, Proliferation of LSKCD150^{+} cells (16h BrdU) present in reverse chimeras comprised of a CD45.2^{+} IFNAR$^{-/-}$ (IFNα non-responsive) microenvironment and CD45.1^{+} wt BM. Each set represents 6 chimeras, untreated (control) or treated (24h and 16h before analysis) with 10.000 units/mouse IFNα . **b,** 100,000 BM cells from either wt (CD45.2^{+}) or IFNAR$^{-/-}$ (CD45.2^{+}) mice were injected into lethally irradiated wt recipient mice (CD45.1^{+}). 22 weeks post transplantation CD45.2^{+} Gr1+ myeloid chimerism was determined in PBL (left) and CD45.2^{+} LSKCD150^{+} cells in the BM (right). Individual mice are shown, mean is indicated by black bar. No significant differences were observed. Statistical analysis as in Figure 1.



Supplementary Figure 3. IFNα induced activation of HSCs is dependent on STAT1 and Sca-1 signalling.

a, Expression of phosphorylated STAT1 (pSTAT1) in HSCs of wt mice treated with 5μg/g pI:C (16h before analysis). **b,** Expression of pSTAT1 in LSK cells of wt mice at different time points after injection with 5μg/g pI:C. **c,** Representative FACS profiles of 12h BrdU uptake of LSKCD150^{+} cells isolated from wt or STAT1$^{-/-}$ mice. pI:C injections with 5μg/g (0h and 48h), analysis at 72h. **d,** Representative FACS profiles of BrdU uptake of LSKCD150^{+} cells isolated from wt or Sca-1$^{-/-}$ mice after pI:C injections as in (c). Statistical analysis as in Figure 1.



Supplementary Figure 4. Chronic IFNα stimulation causes a total loss of HSCs.

a, BrdU-uptake of LSKCD150$^+$ cells at day 0 (first 5-FU injection, see Figure 4a). 4 mice per condition. **b,** Kaplan-Meyer analysis of IFNAR$^{-/-}$ mice treated according to the scheme in Figure 4a. n = 6 per treatment. **c,** 100,000 BM cells from untreated wt (CD45.2$^+$) or pI:C treated (5μg/g pI:C i.p., day 0, 2 and 4) wt (CD45.2$^+$) mice were injected (day 10) into lethally irradiated wt recipient mice (CD45.1$^+$). 21 weeks post transplantation percentage CD45.2$^+$ Gr1$^+$CD11b$^+$ cells (PBL) and CD45.2$^+$ LSKCD150$^+$ cells (BM) were determined (left). 100,000 BM cells from these mice were injected into lethally irradiated wt recipient mice (CD45.1$^+$) for secondary transplants. 22 weeks post transplantation percentage CD45.2$^+$ Gr1$^+$CD11b$^+$ cells (PBL) and CD45.2$^+$ LSKCD150$^+$ cells (BM) were determined (right). Individual mice are shown, mean is indicated by black bar. No significant differences were observed. **d,** BM from 50%wt:50%IFNAR$^{-/-}$ (see Figure 4c) and 95%wt:5%IFNAR$^{-/-}$ (see Figure 4d) chimeras was isolated at day 22 (time of analysis in Figure 4c, 4d) and transplanted (3x10^7 BM cells) into lethally irradiated CD45.1$^+$ wt recipient mice. 22 weeks post transplantation wt (CD45.1$^+$) and IFNAR$^{-/-}$ (CD45.2$^+$) chimerism of Gr1$^+$CD11b$^+$ cells (PBL) and LSKCD150$^+$ cells (BM) was determined.

Table 1: IFN activated genes upregulated in lin$^-$cKit$^+$CD150$^+$CD48$^-$ HSCs in response to IFNα (16 hours) treated mice (microarray analysis)

gene ID	fold change	symbols	description
60440	54.80	Iigp1	interferon inducible GTPase1
15957	32.50	Ifit1	interferon-induced protein with tetratricopeptide repeats 1
15959	36.90	Ifit3	interferon-induced protein with tetratricopeptide repeats 3
246727	19.80	Oas3	2'-5' oligoadenylate synthetase 3
23962	13.50	Oasl2	2'-5' oligoadenylate synthetase-like 2
15951	8.63	Ifi204	interferon activated gene 204
76933	8.46	Ifi27	interferon-alpha inducible protein 27
16145	7.98	Igtp	interferon gamma induced GTPase
54123	7.06	Irf7	interferon regulatory factor 7
57444	7.03	Isg20	interferon stimulated protein
226695	6.53	Ifi205	interferon activated gene 205
15950	5.91	Ifi203	interferon activated gene 203
231655	5.86	Oasl1	2'-5' oligoadenylate synthetase-like 1
246730	5.33	Oas1a	2'-5' oligoadenylate synthetase 1G
15944	5.20	Ifi1	immunity-related GTPase family M, interferon inducible protein1, Irgm, LRG-47, Iigp3
246728	4.91	Oas2	2'-5' oligoadenylate synthetase 2
17857	4.74	Mx1	myxovirus (influenza virus) resistance protein 1, interferon inducible protein p78
16391	4.66	Isgf3g	interferon dependent positive acting transcription factor 3 gamma
15958	4.07	Ifit2	interferon-induced protein with tetratricopeptide repeats 2
54396	3.97	Iigp2	interferon inducible GTPase 2
17858	3.04	Mx2	myxovirus (influenza virus) resistance 2, interferon inducible GTP-binding protein Mx2
15953	3.03	Ifi47	interferon gamma inducible protein 47
71586	2.93	Ifih1	interferon induced with helicase C domain 1
70110	2.75	Ifi35	interferon induced protein 35
66141	2.63	Ifitm3	interferon induced transmembrane protein 3
110454	15.70	Ly6a/Sca-1	lymphocyte antigen 6 complex, locus A
20846	6.21	Stat1	signal transducer and activator of transcription 1
12572	4.06	Cdk7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)
12442	2.11	Ccnb2	cyclin B2
17246	2.19	Mdm2	transformed mouse 3T3 cell double minute 2
23797	1.81	Akt3	thymoma viral proto-oncogene 3
19211	-2.11	Pten	phosphatase and tensin homolog
15568	-6.99	Elavl1	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R), stabilizes p21

Supplementary Table 1: IFN responsive genes are up-regulated in sorted HSCs from mice treated with IFNα.

Microarray analysis of sorted lin$^-$cKit$^+$CD150$^+$CD48$^-$ HSCs from wt mice untreated or treated with 10.000 units/mouse IFNα for 16h. Per condition 3 independent samples were analysed.