Table S1. Detailed patient data
Primary human leukemic cells were obtained from BM or PB of four patients with AML, two obtained at the time of diagnosis). Control samples were taken from AML patients in complete remission. The diagnoses were made according to FAB criteria.
* Transient increase in blasts shortly after chemotherapy.
Abbreviations used: **AML** acute myeloid leukemia; **sAML** secondary AML after myelodysplastic syndrome; **ALL** acute lymphoblastic leukemia; **Ph$^-$c-ALL**, Philadelphia negative common ALL; **Ph$^-$pre-B-ALL,** Philadelphia negative pre-B-ALL; **CML,** chronic myeloid leukemia; **CR,** complete remission; **CP,** chronic phase; **M2,** acute myeloblastic leukemia, with maturation; **M5,** acute monoblastic leukemia; **WBC,** white blood cell count; **PB,** peripheral blood; **BM**, bone marrow; **complex karyotype**, ≥ three chromosomal abnormalities.

Table S2. Infection rates of transduced bone marrow cells after 48 hours
The percentages of GFP$^+$ cells in donor BM are listed after 48 hrs of co-culture with the respective producer cells. The percentages of total GFP$^+$ BM cells are presented in the first column, those of GFP$^+$ cells of the lymphoid lineage in the second, and of the myeloid lineage in the third. In the fourth column, the percentages of GFP$^+$ lymphoid progenitors and in the fifth column the percentages of GFP$^+$ myeloid progenitors are displayed relative to all progenitor cells. The percentages of GFP$^+$ hematopoietic stem cells are shown in the last column. The data represent four individual experiments.

Table S3. Total numbers of lymphoid and myeloid cells in the peripheral blood of transplanted mice
The percentages of lymphoid and myeloid cells in the PB of all transplant groups are shown at 8 and 32 weeks as analyzed by flow cytometry. The number of myeloid cells is clearly increased in diseased cS5-transplanted mice (8 weeks), whereas it is unchanged in all serine mutants (n≥8).

Figure S1. Stat5 is constitutively phosphorylated on Ser725 and Ser779 when expressed in 293T cells
receptor. Cells were treated (+) with 50 U of Epo or left untreated (-). Whole cell extracts were prepared and subjected to Western blot analysis to evaluate tyrosine and Ser725/779 phosphorylation. GpE+86 cells expressing wt Stat5a were used as positive control.

Figure S2. Control animals used in the Stat5$^{null/null}$ fetal-liver transplant
Stat5ab$^{null/null}$ fetal livers (CD45.2) were retrovirally transduced with MSC vector and transplanted into lethally irradiated CD45.1 recipients. Fetal livers of GFP-transgenic mice harboring an intact Stat5 locus were used as controls. Mice were bled 8 weeks after transplantation and analyzed by FACS for donor engraftment (CD45.2), GFP expression, and lineage repopulation. Repopulating capacity was assessed for (A) B-cells (B220), (B) T-cells (CD8), (C) granulocytes (Gr-1), and (D) erythrocytes (Ter119). For each lineage, marker expression was plotted against GFP expression (left), GFP expression against CD45.2 expression of the donor (center), and marker against CD45.2 expression (right). Mice receiving vector-transduced Stat5ab$^{null/null}$ fetal liver cells displayed a lymphopenic and anemic phenotype. GFP^{tg} mice served as controls for normal lineage composition (n=2 per group).

Figure S3. Analysis of PB, BM, and spleens from transplanted mice at the time of sacrifice
(A) FACS analysis of BM and spleen samples from representative cS5-, cS5-S725A-, cS5-S7279A-, and cS5-SASA-transplanted mice at the time of sacrifice. In cS5-transplanted mice, there was a marked increase in GFP^+ myeloid cells, not only in PB but also in the bone marrow and spleen. In cS5 single-serine mutants, the increase in GFP^+ lymphoid cells, which was seen in the PB, was less pronounced in the bone marrow and spleen. In cS5-SASAtransplanted animals, there was significant, albeit low GFP expression in the bone marrow and spleen at the endpoint of analysis. (B) The spleens of transplanted mice were subjected to Western blot analysis to determine Stat5a serine phosphorylation. Strong Ser779 phosphorylation was detected in diseased cS5- and cS5-S725A-transplanted mice, whereas Ser725 was only weakly phosphorylated in cS5-reconstituted mice. A stronger signal for Ser779 phosphorylation was obtained in terminally diseased cS5 mice exhibiting high WBCs and expressing a high percentage of GFP^+ cells, while a weaker signal was seen in mice with lower WBCs and lower GFP expression. No serine phosphorylation signal was detected in diseased cS5-S779A-transplanted mice and all healthy animals tested. GpE+86 fibroblasts expressing exogenous S5 were used as positive control. All samples were exposed on the same blot but the membrane was cut to present the data more clearly. Similar results were obtained in three independent analyses.

No.	Sex	Age	Diagnosis	Subtype	WBC	[%] Blasts PB	cytogenetics	Sample preparation
#1	f	35	AML	M5	80,000	90	46XX	PB
#2	f	75	AML	sAML	53,300	20	46XX	PB
#3	m	63	AML	M2	65,000	96	46XY	PB
#4	m	73	AML	M2	70,108	90	n.d.	PB
#5	f	33	ALL	Ph^- B-ALL	117,880	84	46XX,del(9)(p13)	BM
#6	m	26	ALL	Ph^- c-ALL	29,860	41	complex	BM
#7	m	85	CML	CP	45,740	1	46XY,t(9;22)(q34;q11)	PB
#8	m	68	CML	CP	35,940	0	46XY,t(9;22)(q34;q11)	BM
#9	m	58	CR (AML)	-	4,220	0	46XY	BM
#10	m	73	CR (AML)	-	8,330	2*	46XY	BM

Table S1

GFP$^+$ cells [%] n=4	Total GFP$^+$ cells [48h]	Lymphoid lineage CD19$^+$; CD3$^+$; GFP$^+$	Myeloid lineage Gr-1$^+$; Mac-1$^+$; GFP$^+$	Lymphoid progenitors Lin$^-$; IL7Rα^+; ckit$^+$; Sca1$^-$; GFP$^+$	Myeloid progenitors Lin$^-$; IL7Rα^-; ckit$^+$; Sca1$^-$; GFP$^+$	LSKs Lin$^-$; Sca1$^+$; ckit$^+$; GFP$^+$
vector	15.0 ± 2.8	6.3 ± 2.4	24.9 ± 9.1	2.2 ± 0.3	11.7 ± 4.6	11.3 ± 0.3
cS5	15.1 ± 2.0	2.5 ± 0.3	19.1 ± 1.0	2.3 ± 0.3	9.5 ± 3.4	14.6 ± 3.0
cS5-S725A	12.5 ± 0.4	1.9 ± 0.7	14.2 ± 1.1	2.0 ± 0.5	5.3 ± 0.8	13.8 ± 1.7
cS5-S779A	15.1 ± 1.6	2.9 ± 0.1	17.1 ± 0.8	2.6 ± 0.2	10.2 ± 3.5	14.2 ± 3.6
cS5-SASA	15.0 ± 7.7	8.0 ± 3.0	35.8 ± 14.3	3.4 ± 2.8	29.1 ± 25.3	12.4 ± 3.0

Table S2

Total number of cells [% of living cells] n≥8	8 weeks post transplantation		32 weeks post transplantation	
	Lymphoid lineage CD19$^+$; CD3$^+$	Myeloid lineage Gr-1$^+$; Mac-1$^+$	Lymphoid lineage CD19$^+$; CD3$^+$	Myeloid lineage Gr-1$^+$; Mac-1$^+$
vector	65.3 ± 1.6	23.3 ± 1.1	50.1 ± 2.8	37.6 ± 3.6
cS5	6.5 ± 1.6	89.3 ± 10.3	no survivors	
cS5-S725A	64.6 ± 0.8	27.2 ± 4.6	46.9 ± 0.0	49.8 ± 0.0
cS5-S779A	58.6 ± 3.1	35.2 ± 4.1	38.5 ± 7.5	51.8± 9.2
cS5-SASA	63.3 ± 9.1	29.8 ± 10.2	54.3 ± 9.9	33.2± 12.0

Table S3

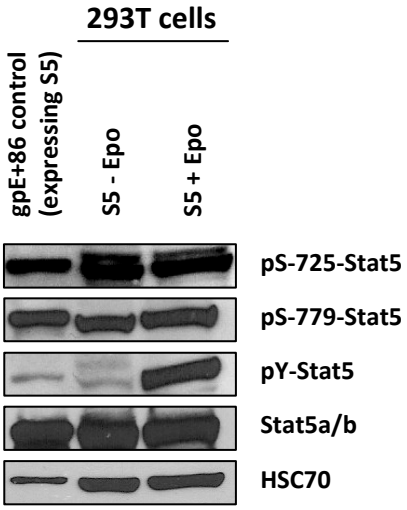
293T cells
gpE+86 control (expressing S5)
S5 - Epo
S5 + Epo
pS-725-Stat5
pS-779-Stat5
pY-Stat5
Stat5a/b
HSC70

Figure S1

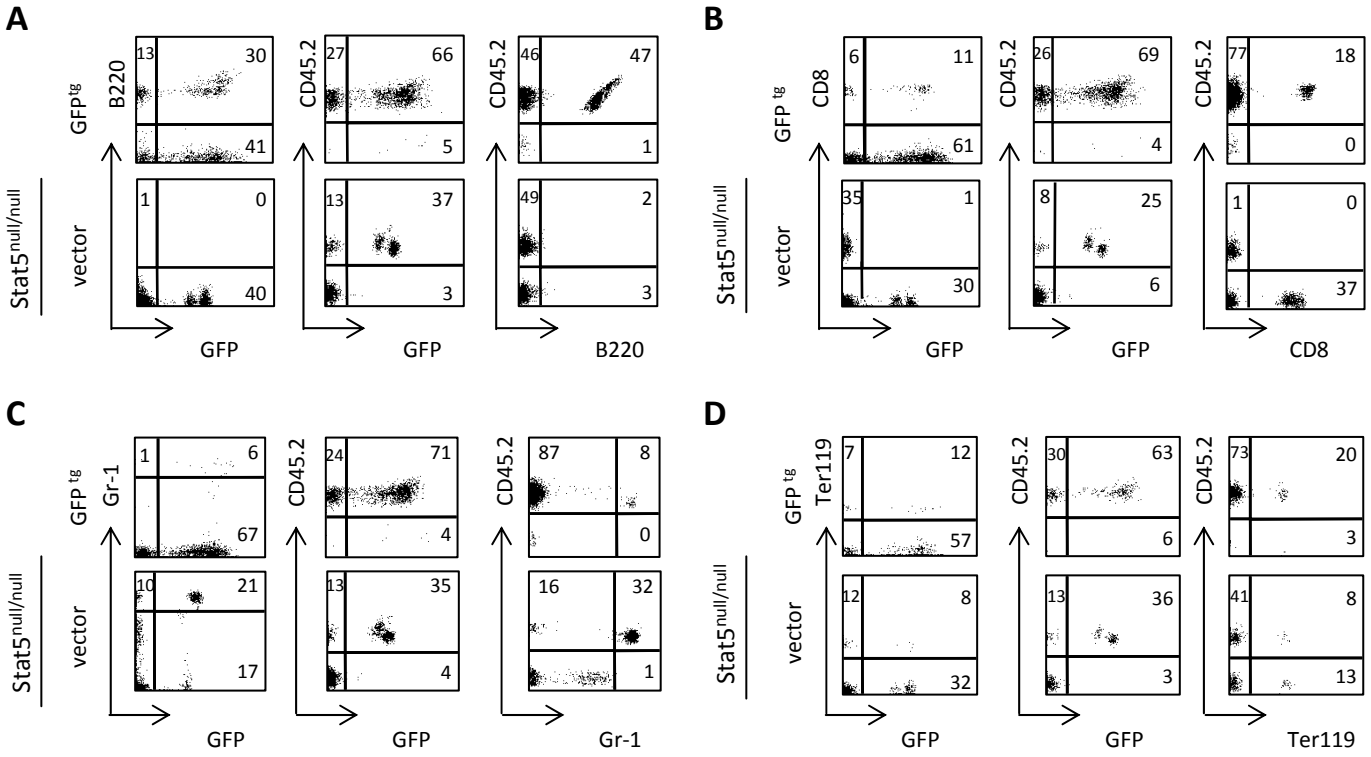
A
B
C
D
GFP tg
B220
CD45.2
CD8
Gr-1
Ter119
Stat5null/null
vector
GFP
B220
CD8
Gr-1
Ter119

Figure S2

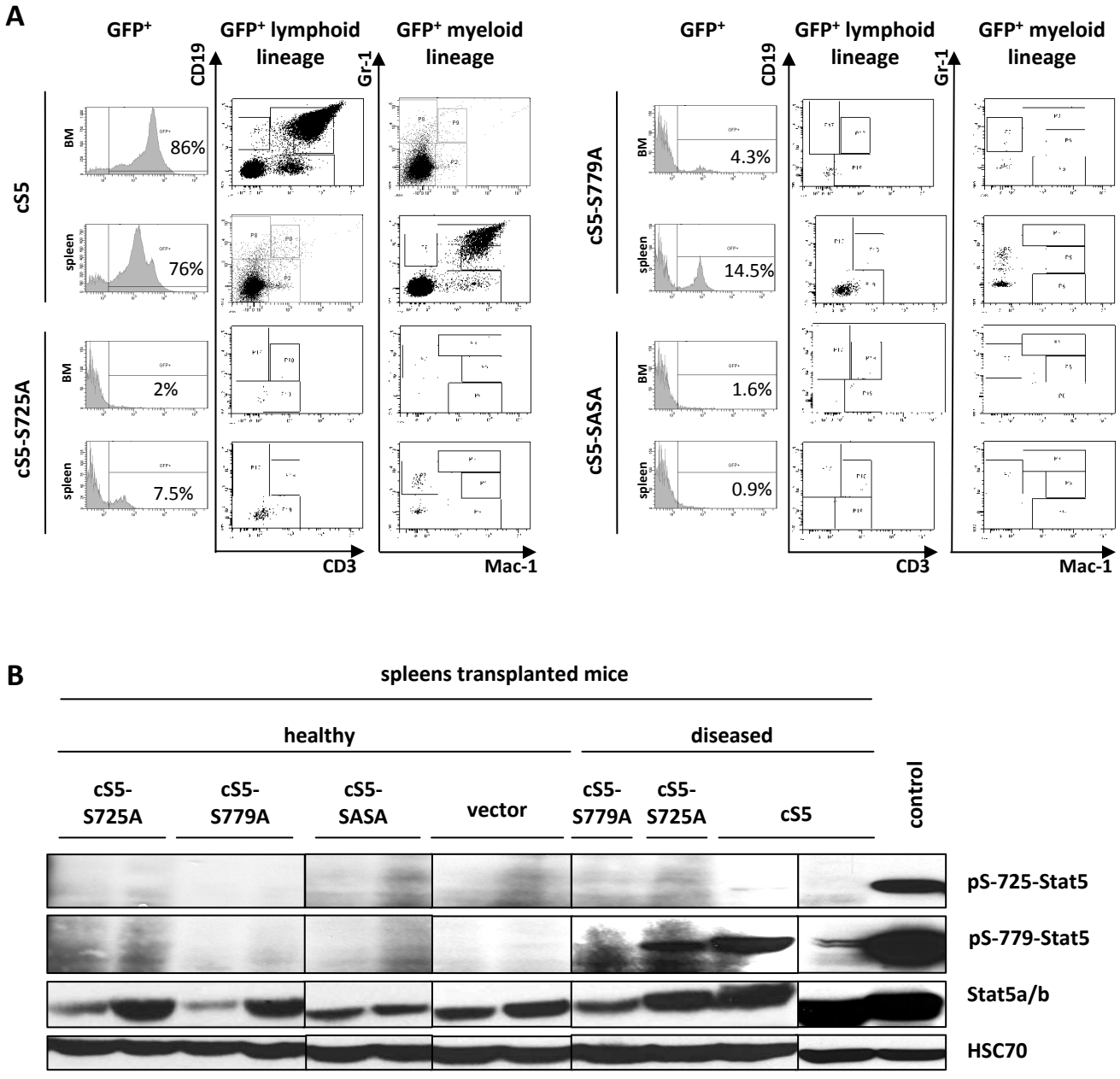
A
GFP+
GFP+ lymphoid lineage
GFP+ myeloid lineage
CD19
Gr-1
cS5
BM
86%
spleen
76%
cS5-S725A
BM
2%
spleen
7.5%
CD3
Mac-1
GFP+
GFP+ lymphoid lineage
GFP+ myeloid lineage
CD19
Gr-1
cS5-S779A
BM
4.3%
spleen
14.5%
cS5-SASA
BM
1.6%
spleen
0.9%
CD3
Mac-1
B
spleens transplanted mice
healthy
diseased
cS5-S725A
cS5-S779A
cS5-SASA
vector
cS5-S779A
cS5-S725A
cS5
control
pS-725-Stat5
pS-779-Stat5
Stat5a/b
HSC70

Figure S3