SCL is required for c-KIT-dependent hematopoietic cell survival

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Supplemental Methods and Figures

Supplemental Methods

All antibodies were purchased from Pharmingen and e-Biosciences, Annexin V (FITC or Cychrome) from Biodesign and used at concentrations determined by titration. The monoclonal anti-SCL antibody (BTL73) was a generous gift from Dr. Danièle Mathieu-Mahul (INSERM, France). Flow cytometry analysis was performed on the FACStar or FACScalibur flow cytometers (Becton-Dickinson) using double laser excitation.

c-Kit$^+$Sca1$^+$Lin$^-$ (KSL) and c-Kit$^+$Sca1$^-$Lin$^-$ (KL) were stained as previously described. Briefly, freshly isolated bone marrow cells were stained with c-Kit-APC, Sca1-PE and biotinylated antibodies for the following lineage markers: IL7-Rα, CD3, B220, FcRαI, Gr1, CD11b and TER119. Lin-biotin cells were revealed by PeCy5 coupled streptavidin. Dead cells were excluded using propidium iodide (PI). When apoptosis was investigated, lineage markers were coupled with PeCy7 and Annexin V-biotin revealed by streptavidin-PeCy5.

For KSL, CMP, GMP, MEP cell sorting, total bone marrow was depleted of lineage positive cells using anti-rat magnetic beads (Qiagen) and the remaining cells were stained with CD34-FITC, Sca1-PE, c-kit-APC, FcγRII/III-PeCy7 and biotinylated antibodies for the following lineage markers: IL7-Rα, CD3, B220, FcRαI, Gr1, CD11b and TER119. Lineage-positive cells were detected with PeCy5-conjugated streptavidin. For CLP purification, lineage depleted bone marrow cells were stained with Sca1-PE, c-kit-APC, IL-7Rα-PeCy5, Flt3-biotin (detected by streptavidin PeCy7) and FITC-conjugated antibodies for the lineage markers (Thy1.2, CD3, B220, Gr1, CD11b and TER119). Dead cells were excluded by PI staining. .

Purified myeloid progenitors were cultured in untreated Terazaki plates in IMDM 10% FBS. Cells were stimulated either with mGM-CSF and mIL-3 (20 ng/mL) or with mSteel Factor and 100 ng/mL hIL-11 (100 ng/mL). Cells were passaged every other day and kept at a concentration of 10^6 cells per mL. .

Following infection with MSCV neo^r virus, cells were plated at different concentrations ranging from 1500 to 6000 cells/ml in methyl cellulose culture consisting of 1% methyl cellulose (Fluka Chemie), 10% FBS, 200 μg/ml transferrin, 0.2% bovine serum albumin (BSA), $5x10^{-5}$ M α-Monothioglycerol, 50 ng/mL SF, 5 ng/mL IL-3, 10 ng/mL IL-6, and 1 U/mL Erythropoietin (Epo) in IMDM, in the presence or absence of 0.8 mg/mL G418 (Gibco) to assess the infection efficiency. Unfractionned bone marrow cells from *wt*, $W^{41/41}$

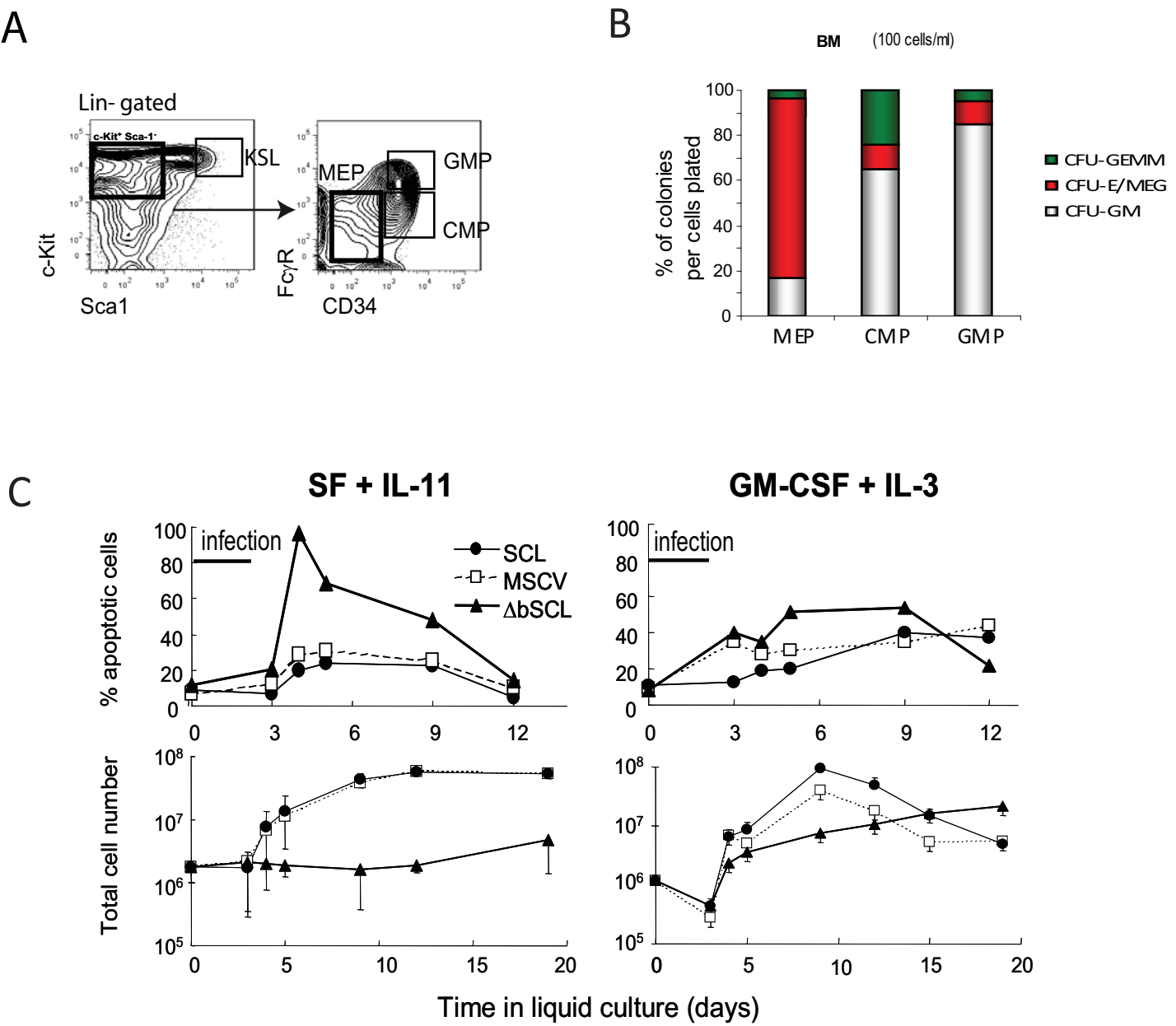
and $W^{41/41}$-Scl^{tg} mice were plated at concentration varying from 10 000 to 50 000 cells/mL in methyl-cellulose culture media as described above. Colonies were scored 9 days later.

Cytokine dose-response curves

Bone marrow cells from heterozygote SCL^{tg} mice (line A(5)3SCL) and wild type littermates were plated in methyl cellulose culture with increasing concentrations of SF (0-100 ng/mL) or IL-3 (0-5 ng/mL), in the presence of Epo (1 U/mL). Colonies were scored 9 days later.

Chromatin immunoprecipitation

TF-1 cell chromatin extracts were subjected to immunoprecipitations with SCL antibody or species-matched control IgG. After cross-linking reversal, DNA from immunoprecipitated chromatin was analysed by quantitative PCR to detect the presence of the h*Api5*, *Bag2*, *Birc2*, *Smnd1*, *Tnfaip8* and h*Hprt* promoter sequences, as well as the 3'UTR region of hAPI-5 mRNA. The occupancy of region X by protein p relative to input chromatin was obtained as described: p(X) = 2[Ct(IP) – Ct(input)]. Data are shown as fold enrichments over the values obtained for species-matched control Ig. Primer sequences are available upon request.

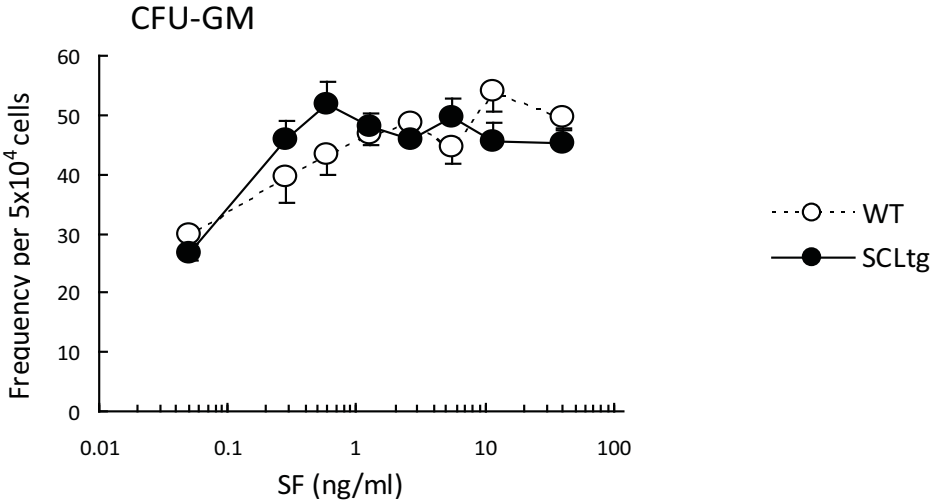


Supplemental Figure 1:

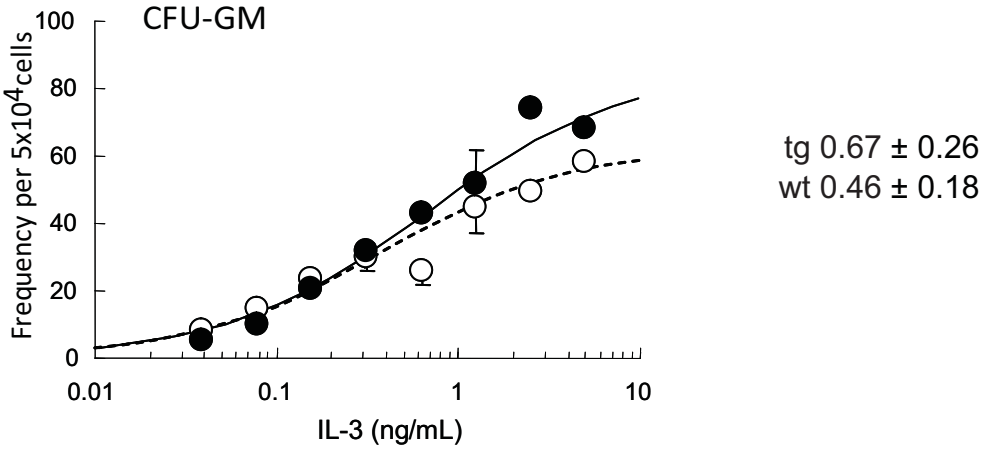
A-B) Purified MEP, CMP and GMP were plated in methylcellulose to determine their contents in colony forming cells. Cells were plated at a concentration of 100 cells/ml.

C) Bone marrow cells harboring wild type SCL, ΔbSCL or the empty vector were cultured with either early acting cytokines (SF and IL-11) or with myeloid cytokines (GM-CSF and IL-3). Apoptotic cell death and total cell numbers were monitored from day 1 to day 20.

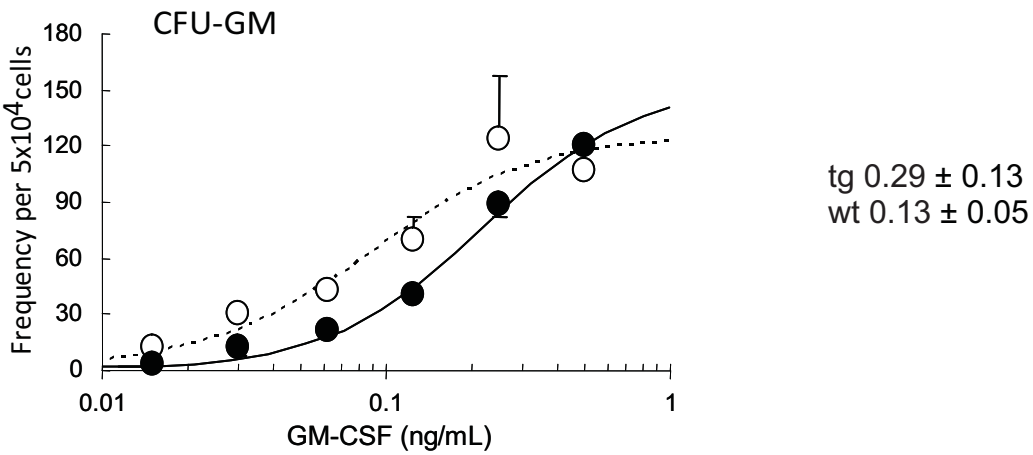
A



B

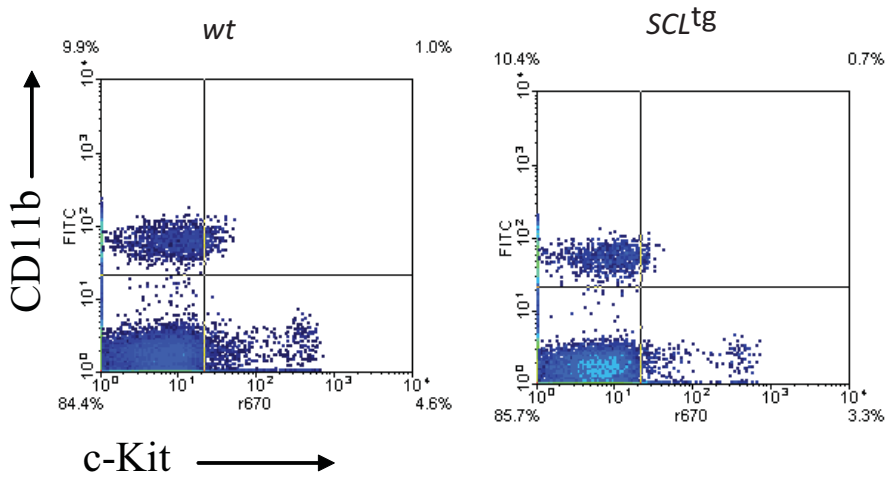


C



Supplemental Figure 2:

Myeloid colonies (CFU-GM) from heterozygous *SCL*^{tg} mice and *WT* bone marrow cells were plated in methylcellulose cultures in the presence of increasing concentrations of SF (A), IL-3 (B) or GM-CSF (C). Data were obtained from three independent experiments performed in duplicates or quadruplicates. Data were analyzed using a non-linear regression curve fitting routine (ALLFIT). Shown in each panel are estimates of the half effective concentrations (EC50) of the different ligands in bone marrow cultures from *SCL*^{tg} mice or their *WT* littermates. Data obtained with CFU-GM in cultures stimulated with SF did not fit the linear regression model (panel A) suggesting that colony growth in this case was not directly dependent on SF concentration whereas myeloid colony growth was directly-dependent on IL-3 or GM-CSF concentrations (panels B, C).

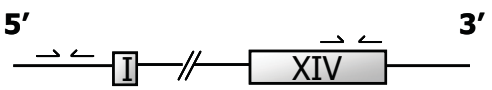


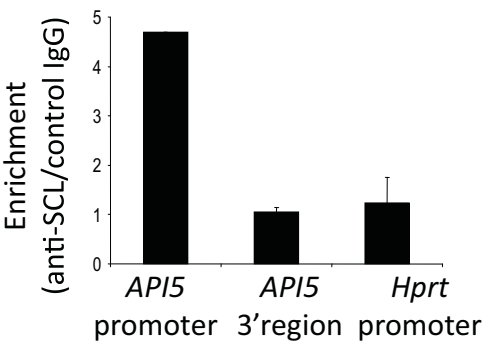
Supplemental Figure 3. The SCL transgene does not upregulate c-Kit expression in the bone marrow.

A

	Genes mapped to ChIP-Seq peaks							
	Wilson et al, 2010							Kassouf et al, 2010
	SCL	LMO2	GATA2	promoter 5'	exon	intron	3' region	SCL
Son	+			+	+			
Api5	+		+	+	+	+		
Tnfaip8	+	+	+	+		+		+
Cul5	+				+	+		+
Luc7L3								
Maea			+	+		+		+
Atg12								
Ankhd1								+
Ppp2cb		+	+			+	+	+
Bag2	+			+	+			
Birc2	+			+				
Rad21		+		+				
Smndc1	+	+		+				+
Cul2								
Bclaf1	+			+	+			+
Hspd1	+			+	+			
Ptpn6	+			+	+	+		
Cul3	+		+	+		+		
Tgfb1			+	+		+		+

B

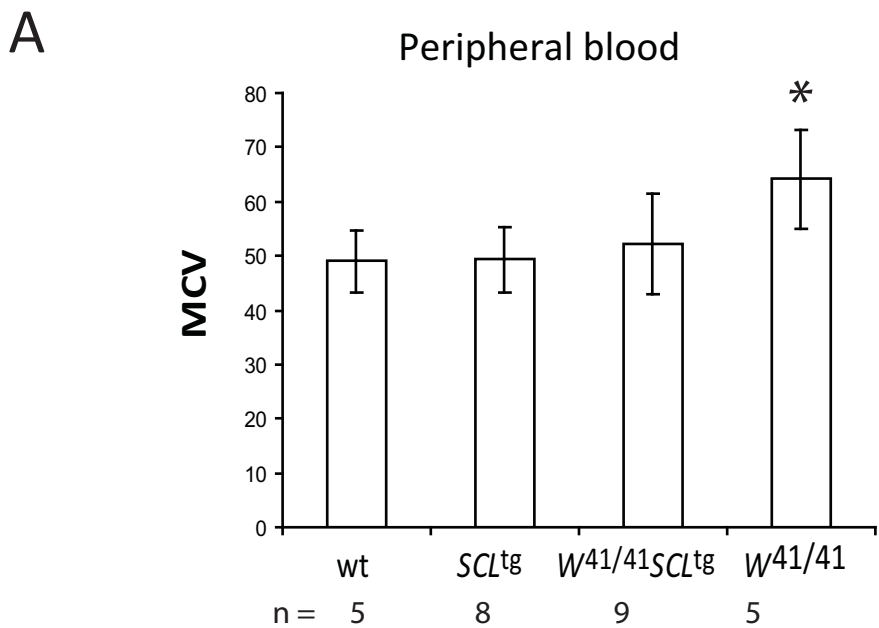


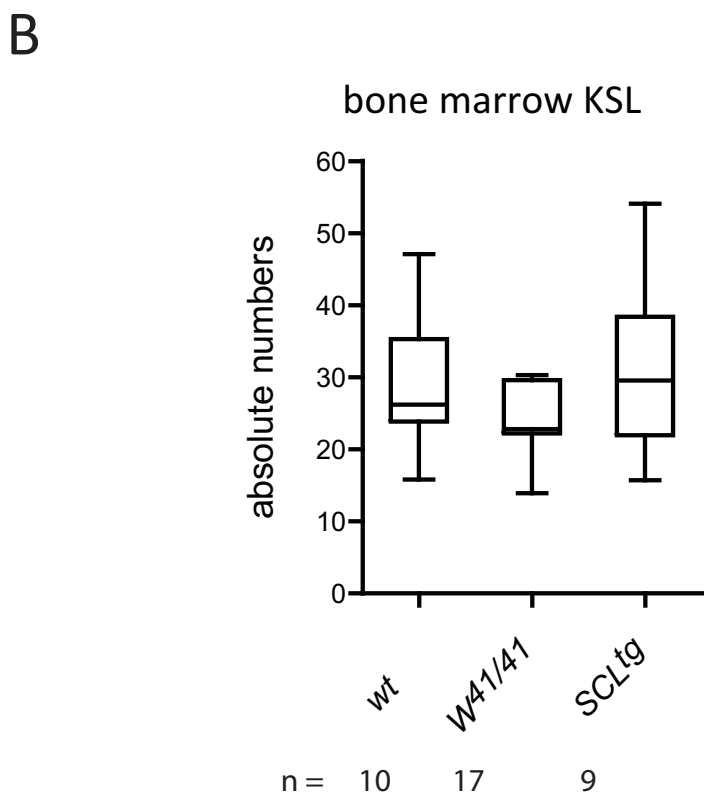


Supplemental Figure 4. Chromatin immunoprecipitation

A. 15 of these 19 genes were mapped to Chip-Seq peaks (+) identified by Wilson et al with anti-SCL, LMO2 and/or GATA-2 antibodies in murine hematopoietic progenitor cell line; 7 of those were independently reported by Kassouf et al in primary erythroid fetal liver cells with anti-SCL.

B. SCL occupies the *Api5* locus by chromatin immunoprecipitation. Chromatin extracts from TF-1 cell were subjected to immunoprecipitation with SCL antibody or with species-matched control IgG. Fold enrichments were calculated as described in Materials and Methods.

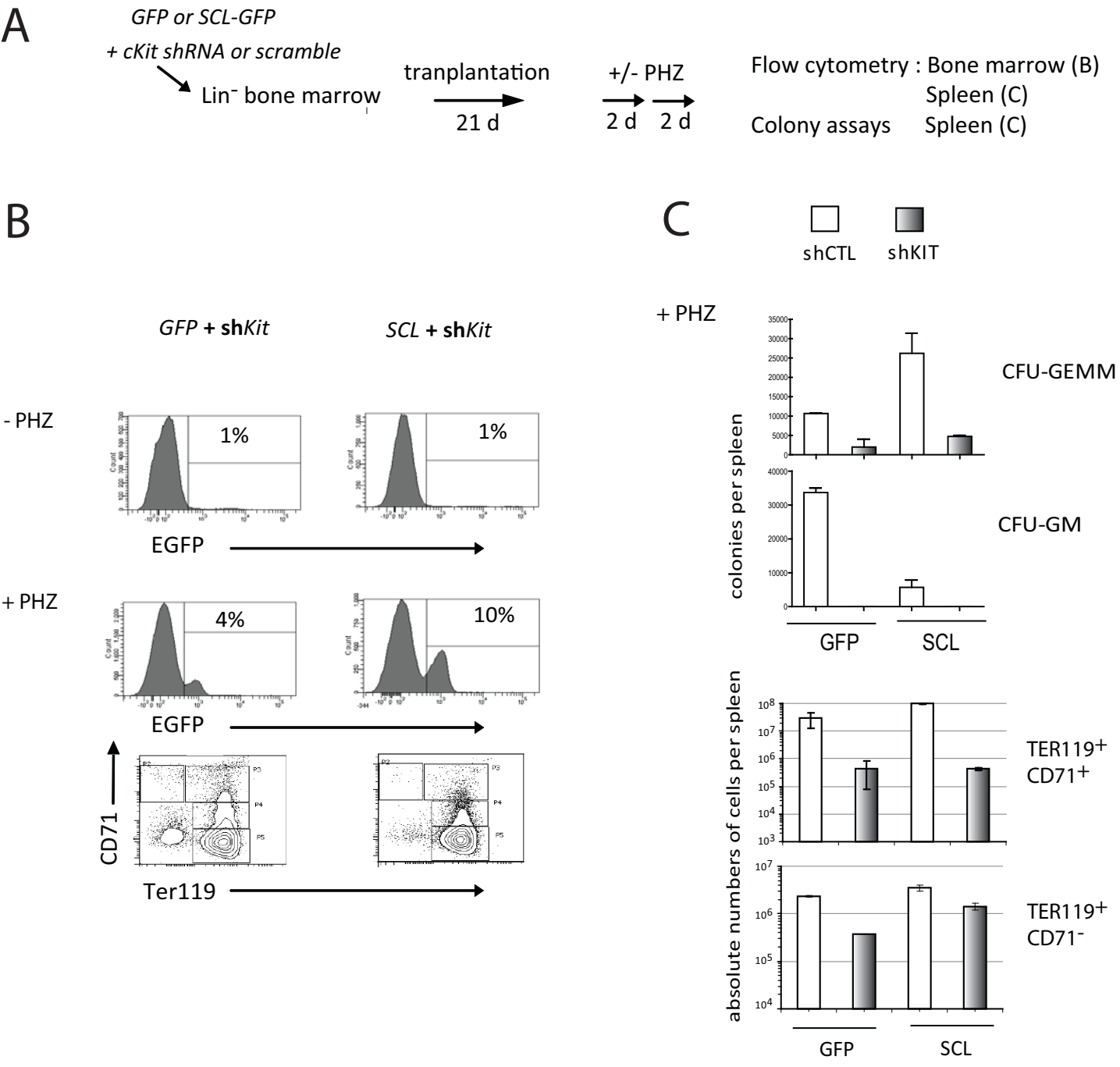




Supplemental Figure 5 . Analysis of $W^{41/41}$ and *SCL* ^{tg} mice

A) Analysis of the mean cell volume in the peripheral blood . Data are shown as the mean +/- SD of n mice per genotype (* $p<0.05$ compared to wt controls).

B) Analysis of the Kit+Sca+Lin- population in the bone marrow by flow cytometry. The absolute numbers of KSL cells per mice are shown as box plots for n mice of each genotype.



Supplemental Figure 6 : Stress erythropoiesis

A) Experimental strategy

B) c-Kit is required for bone marrow engraftment and stress response. Bone marrow cells from mice transplanted with cells expressing sh*Kit*, with or without *SCL* were analysed for engraftment 25 days later by the percentage of GFP-transduced cells. Mice were also treated with phenylhrazine (2x48h) prior to sacrifice to analyze erythropoietic stress. Compared to control cells expressing a non-directed sh*CTL* (Figure 6), cells lacking c-Kit show blunted PHZ response.

C) c-Kit is required for splenic stress response to PHZ. Spleen cells from mice transplanted with the indicated cells were analyzed after PHZ treatment by colony assays (upper panel) and by flow cytometry (lower panel). PHZ treatment induced a 10- to 50-fold increase in erythroid cells compared to untreated control mice (not shown). Illustrated are the total numbers of GFP^+ progenitors and of erythroid cells per spleens of PHZ-treated mice.