SUPPLEMENTARY APPENDIX

Transient inhibition of NF-κB signaling enhances *ex vivo* propagation of human hematopoietic stem cells

Mehrnaz Safaee Talkhoncheh, Agatheeswaran Subramaniam, Mattias Magnusson, Praveen Kumar, Jonas Larsson and Aurélie Baudet

Division of Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, Sweden

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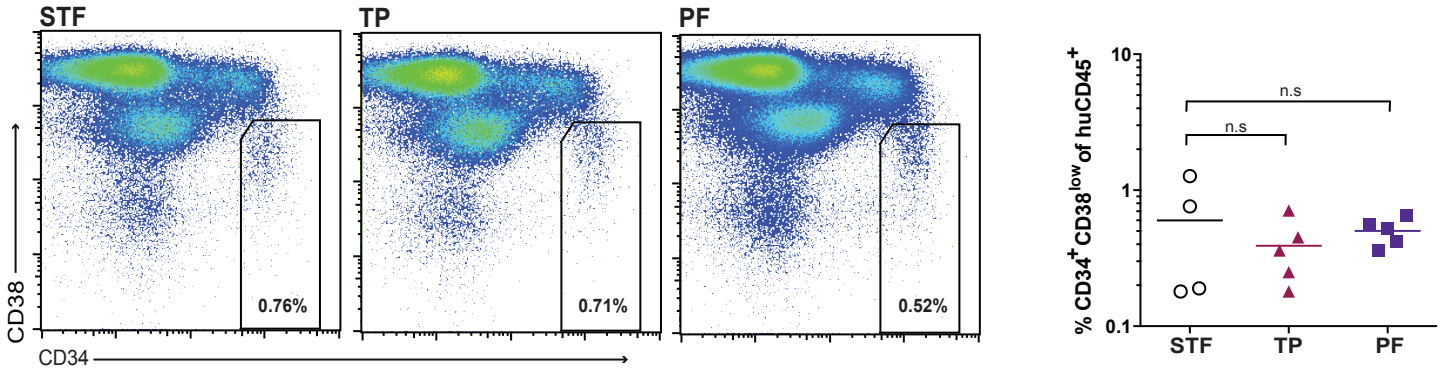
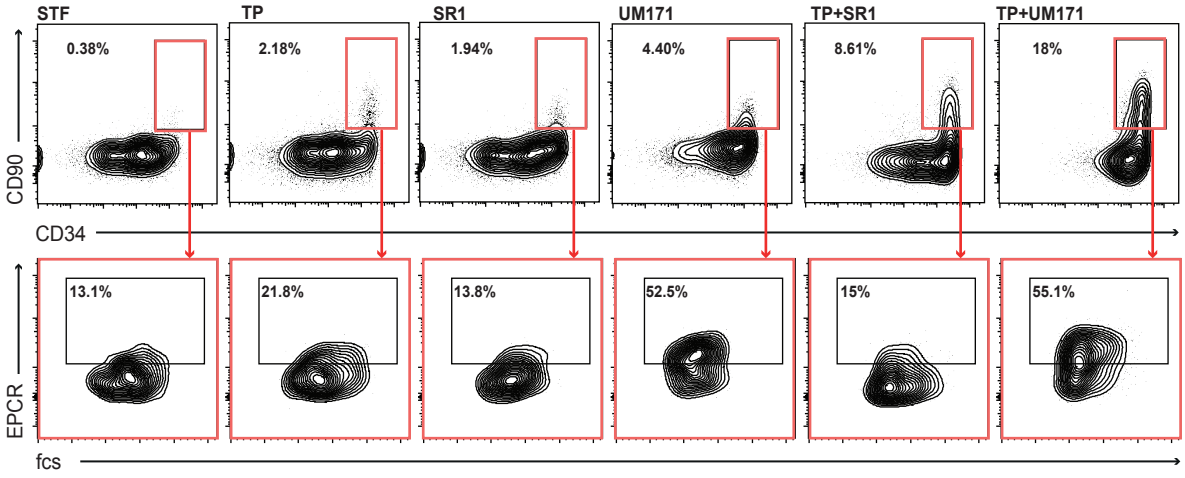
Correspondence: jonas.larsson@med.lu.se

Supplemental figure 1



Supplemental figure 1. Level of HSC engraftment ($CD34^{+}CD38^{low}$cells) was measured 16 weeks post-transplantation in the bone marrow. Representative FACS plots gated from human $CD45^{+}$cells and the accumulated data are shown. TP, TPCA1 and PF, PF184.

Supplemental figure 2



Supplemental figure 2. CD34^{+} cells were treated with indicated compounds for 6 days and analyzed by flow cytometry for expression of CD34, CD90, and EPCR markers. TP,TPCA1

Supplemental figure 3

Supplemental figure 3. CD34⁺ cells were treated with or without TP for 6 hours and subjected to transcriptome analysis by microarrays. Volcano plot and GO classification of two-fold modulated genes are shown for each condition.

Supplemental experimental procedure

FACS antibodies

Human engraftment was assessed by anti-human CD45 antibody (#304037) (BioLegend) and lineage distribution was evaluated with anti-CD33 (#55540, BD Biosciences), -CD15 (#301906, BioLegend), -CD3 (#4292971, eBioscience), and -CD19 (#562653, BD Biosciences) antibodies.

Microarray analysis

CB-derived $CD34^+CD38^-CD90^+CD45RA^-$ cells were transduced in triplicate with specified shRNA-expressing lentiviral vectors achieving around 60% transduction efficiency. Seven days after transduction, GFP^+CD34^+ cells still expressing CD90 were sorted into RLT buffer and RNA was extracted with the RNeasy Micro kit following manufacturer's instructions (Qiagen), including a DNAse incubation step. Microarrays (Affymetrix, U133 2.0) were hybridized and analyzed at the UCLA clinical microarray core (Los Angeles, USA). For the analysis of IKKβ-mediated transcriptional changes, CB-derived $CD34^+$ cells were treated with TPCA1 or STF in triplicate. After 6 hours, $CD34^+$or sorted $CD34^+CD90^+$ were lysed into RLT buffer. Subsequent sample processing and analysis on Affymetrix human genome U133+ array were performed at KFB (Regensburg, Germany). For both experiments, genes that showed a minimum of 2-fold modulation were considered for further analysis.

CFC assay

After 7 days in culture, frequencies of colony-forming cells (CFC) were determined by plating 300 enriched $CD34^+$ cells in Methylcellulose (Methocult H4230, Stemcell technologies) supplemented with SCF (25 ng/mL), GM-CSF (50 ng/mL), IL-3 (25 ng/mL) (Peprotech) and EPO (5U/mL, Janssen). Plates were incubated at 37°C and

mature hematopoietic colonies were scored after 14 days.

Western blot

Cells were collected and washed with PBS, lysed in Laemmli buffer, separated on NuPAGE^{TM} 4–12% Bis-Tris gel (#17033171, Invitrogen), and transferred to nitrocellulose using iBlot device. After 1 hour at room temperature in blocking solution (ECL advance kit, GE Healthcare, #9720875), membranes were incubated overnight at 4^{o}C with primary antibodies raised against IKKβ (D30C6, Cell Signaling, 1:2000), phosphorylated-IkBα (9246S, Cell Signaling, 1:2000) or total IkBα (4D4, Cell Signaling 1:2000), diluted in blocking solution. After several washes in PBS-1% Tween (Sigma), membranes were incubated with HRP-conjugated secondary antibody (anti-rabbit: 1/10,000, anti-mouse: 1/10,000). Equal loading was evaluated by establishing expression of actin (goat polyclonal IgG, Santa Cruz Biotechnology 1/500000). Luminescence signal was generated with ECL advance kit (GE Healthcare, #9740745), acquired using Chemidoc XRC apparel (Biorad), and images were processed with Image Capture software.

Cell cycle analysis

CB CD34^{+} cells were cultured with STF, TPCA1 or PF184 for 4 days and then analyzed for cell cycle status. Briefly, cells were stained for CD34 and CD90 markers prior to fixation with 0.4% paraformaldehyde (Sigma-Aldrich) in PBS (Invitrogen) for 30min. Next cells were incubated with Triton-X (0.2%, Sigma) for one hour at 4°C. Cells were washed and resuspended in PBS and stained with PE-Ki67 (BD Biosciences) for 2 hours at room temperature, and then DAPI (Invitrogen) was added for 30 minutes. Gated CD34^{+}CD90^{+} cells were analyzed for Ki67 expression and DAPI incorporation using FACS Canto II.

Supplemental Table 1:

Supplemental table 1, related to figure 4: List of annotated genes (51) that were modulated in the $CD34^{+}CD90^{+}$ population upon exposure to the IKKb inhibitor.

coding/non coding	Gene	FC	P	Corrected p Value	DAVID, Pubmed, ENSEMBL	Notes
C	CCL2	-9,04	0,000059	0,284272	inflammation	
C	CCL1	-6,84	0,000762	0,360118	immune function	monocyte chemoattraction
C	IL1A	-3,99	0,000236	0,301297	inflammation	
C	CCL4L2	-3,08	0,001057	0,390991	inflammation	
C	EBI3	-2,77	0,000087	0,284272	inflammation	
C	CSF2	-2,68	0,000477	0,311157	immune function	myeloid differentiation
C	IL2RA	-2,66	0,000178	0,301297	inflammation	
C	HAS2	-2,65	0,000259	0,301297	inflammation	
C	CCL3L3	-2,64	0,001448	0,430378	inflammation	
C	KYNU	-2,51	0,000363	0,301297	inflammation	NAD co-factor synthesis
C	EBF1	-2,41	0,001796	0,430378	immune function	Lymphocyte-specific gene expression
C	RXFP1	-2,35	0,039589	0,842789	inflammation	GTP-dependent signalling
C	FEZ1	-2,31	0,000123	0,284272	immune function	
C	BCL2A1	-2,3	0,000003	0,151196	Apoptosis	
C	LAMP3	-2,21	0,001231	0,42146	immune function	DC differentiation
C	CCL4	-2,2	0,002678	0,512495	inflammation	
C	TNF	-2,16	0,001694	0,430378	inflammation	
C	RGS1	-2,15	0,001013	0,389206	inflammation	GTP-dependent signalling
C	EDN1	-2,11	0,002538	0,497748	inflammation	
C	IL18RAP	-2,07	0,003267	0,529023	inflammation	
C	SAMHD1	-2,06	0,000945	0,389206	inflammation	
C	LAMC2	-2,01	0,00045	0,311157	immune function	neutrophil chemo attraction
C	GNRHR	2,08	0,032202	0,831791	immune function	Lymphocyte-specific gene expression
NC	RP11-212I21.2	-2,99	0,00125	0,42146	lincRNA	MMP2 gene
NC	RNU7-133P	-2,88	0,044941	0,854952	snRNA	
NC	RNU7-123P	-2,3	0,004666	0,578925	snRNA	EFCAB7 gene
NC	AC067945.4	-2,25	0,041683	0,846426	antisense	STAT4 gene
NC	GBP1P1	-2,19	0,006727	0,64184	pseudogen to GBP1, a ginterferon-induced GTPase	
NC	MIR155HG	-2,17	0,000359	0,301297	lincRNA. miRNA	undescribed locus
NC	AP001615.9	-2,16	0,049438	0,863853	antisense	RIPK4 gene
NC	RP11-388P9.2	-2,12	0,003109	0,51866	antisense	ANK3 gene
NC	RP11-288L9.1	-2,03	0,008705	0,661112	lincRNA	upstream of interferon-induced gene (IFI6)
NC	SNORD113-1	2,02	0,030866	0,828577	snoRNA	MAPK/ERK and TGFb inhibition

NC	RNU4-9P	2,09	0,027845	0,822106	snRNA-pseudogene	LATS2 gene
NC	RNU6-971P	2,1	0,011963	0,69766	snRNA-pseudogene	
NC	SNORD1A	2,17	0,021198	0,785433	snoRNA	
NC	SNORD113-2	2,18	0,033563	0,835474	snoRNA	
NC	RP11-49O14.3	2,25	0,008305	0,659428	sense intronic	undescribed ORF(s)
NC	MIR548L	2,26	0,02996	0,825677	miRNA	
NC	SNORD117	2,31	0,004317	0,561135	snoRNA	decreased in cancer (prostate)
NC	RNU6-1112P	2,34	0,012133	0,698686	snRNA-pseudogene	N4BP2 gene
NC	SNORD114-31	2,37	0,040179	0,843064	snoRNA	promote cell growth (APL)
NC	RNA5SP416	2,38	0,045237	0,85823	rRNA	
NC	RNU6-645P	2,4	0,002283	0,471259	snRNA-pseudogene	JARID2 gene
NC	RNU6-1316P	2,41	0,026968	0,815395	snRNA-pseudogene	TRAF3 gene
NC	RP11-862P13.1	2,51	0,029641	0,825677	antisense	GPHN gene
NC	RNU6-1318P	2,84	0,031442	0,83085	snRNA-pseudogene	SLC35A3 gene
NC	RNU6-292P	2,97	0,026488	0,812484	snRNA-pseudogene	
NC	SCARNA15	3,02	0,024683	0,80483	Splicing	Guide for RNU2
NC	RP11-90D4.3	3,44	0,010887	0,692446	lincRNA	
NC	RNU6-767P	3,53	0,005678	0,617247	snRNA-pseudogene	MRIP gene