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Supplemental Information

In Vivo RNAi Screen Unveils PPARγ as a Regulator of Hematopoietic Stem Cell Homeostasis

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Suplemental Material and methods

Antibodies used for Flow cytometry labelling

Antibodies used for labelling were: anti-mouse- CD45.1-Pe-Cy7 or -Fitc (Clone A20, Ebioscience), CD45.2-PE or -Fitc (Clone 104, Ebioscience), Blood Lineage cocktail biotinylated Abs kit (BD Biosciences), Sca1-PeCy7 (Clone D7, Ebioscience), cKit-APC (Clone 2B8, BD Biosciences), CD48-PE (Clone HM48-1, Ebioscience), CD150-Pacific Blue (Clone TC15-12F12.2, Bio Legend), CD3ε-Fitc (Clone 145-2C11, BD biosciences), CD11b-PE (Clone M1/70, BD biosciences), CD45R/B220-Alexa700 (Clone RA3-6B2, BD biosciences), and Ly6G/Ly-6C-APC (Clone RB6-8C5, BD biosciences). Streptavidin-Percp-Cy5.5 (BD Biosciences) was used for detection of biotinylated Abs labelled cells.

SFFVΔEcoR1-Egfp-ShRNA construction

In order to use the same vector for single ShRNA cloning, the SFFV-eGFP-ShRNA vector used in the library was digested by AsisI and PshAI (New England Biolabs) to remove a second EcoRI restriction site and allowed cloning after EcoRI/XhoI digestion. Digested vector was purified after agarose gel migration and self-ligated using T4 ligase (New England Biolabs) to create the SFFVΔEcoR1-Egfp-ShRNA. Single genes specific sequences were designed with the RNAi codex website (http://cancan.cshl.edu/cgi-bin/Codex/Codex.cgi) using the ShRNA sequence with the best enrichment score from screening. Single genes ShRNA targeting sequences used in this study are depicted in Table S1.

Lentivirus production

All lentiviruses were produced by the CCHMC viral vector with ShRNA lentiviral vector, pCMVΔR8.91 and pMD.G. Viruses were concentrated by ultracentrifugation and re-suspended in PBS. Virus titer was determined cytometry detection of GFP^+ cells 48h after transduction of 293T cells.

ShRNA sequences recovery and sequencing

ShRNA integrated sequences were recovered by PCR with a forward primer (link to the P7 Illumina sequence and index sequence = P7+Loop) matching the constant loop in 5' of the antisense ShRNA sequence and a reverse primer (link to the P5 Illumina sequence = P5+Mir30) matching the constant part of the vectors in 3'... PCR was performed using AmpliTaq gold polymerase (Life Technology) in a final reaction volume of 100uL. After 5 minutes denaturation of gDNA at 95°C, PCR was conducted with 25 cycles as follow: Denaturation = 95°C, 30s – Annealing = 54°C, 45s – Extension = 75°C, 30s. PCR products were purified and concentrated after agarose gel migration using a Wizard® SV Gel and PCR Clean-Up System (Promega). Quantification, library preparation and sequencing reaction were conducted by the CCHMC DNA Sequencing core using an Illumina Hi-Seq sequencer with the following sequencing primer: TAGCCCCTTGAATTCCGAGGCAGTAGGCA.

Supplemental Data

FigureS1: Repopulation and colony forming unit deficiencies of different FA deficient mice. (A) Donors BM cells from *Fanca*$^{-/-}$, *Fancc*$^{-/-}$, *Fancd2*$^{-/-}$ or WT mice were transplanted with recipient BM cells (Ratio 1:1) into lethally irradiated Boy/J mice (n=6 mice/genotypes). (B) BM cells from *Fanca*$^{-/-}$, *Fancc*$^{-/-}$, *Fancd2*$^{-/-}$ or WT mice were plated for 2 rounds of CFU assay. The histograms depict the colony number counted at 7 days of each plating (3 mice/genotype).

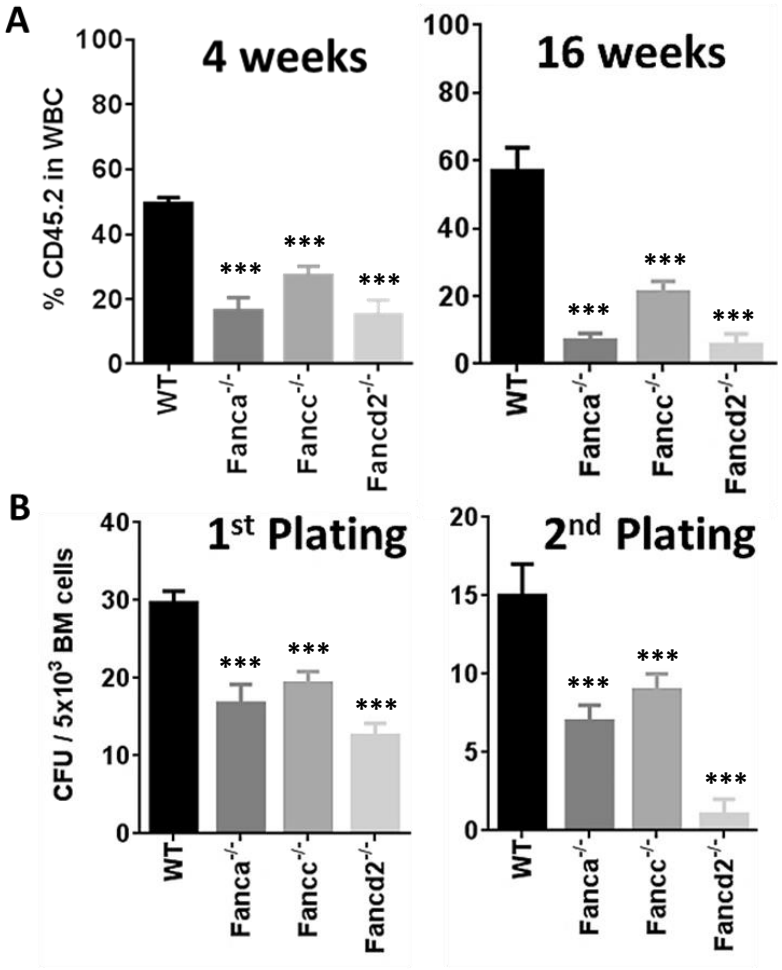
FigureS2: In-vivo ShRNA screening strategy and analyze details. Representation of the strategy used to conduct the in-vivo ShRNA screening on WT and *Fancd2* deficient LSK cells (genotyping on top of figures). LSK cells were sorted and transduced with the 1000 Cancer pool of shRNAs. After 48h, GFP expression was monitored in transduced LSKs, which were transplanted into lethally irradiated Boy/J mice. (B) Step by step pipeline of ShRNA screening analysis used in this paper (derived from Sim et al., 2011). (C) Schematic representation of the PCR and primers used for ShRNA sequence recovery before deep sequencing.

FigureS3: Figure 2, 3 and 4 supportive data. (A) Graphic showing the enrichment score profile (mean of 3 independent experiments) of all shRNA detected in Lin$^-$ BM cells, highlighting the 3 genes linked to TGF-Beta. (B) The table depicts for each TGF-Beta linked candidate genes the number of targeting enriched shRNA (enrichment score >2) and the enrichment score of the best shRNA (according to mean value) into the 3 replicates. (C) Validation of Sh*Pparg2* as described in Fig.2. (E) RT-qPCR validation of Sh*Pparg1* efficiency on sorted GFP$^+$Lin$^-$ cells at 4 and 16weeks after transplantation (n=4mice/group). (F) Wt or Fancd2$^{-/-}$ LSKs were sorted and transduced in vitro with ShScramble or sh*Pparg1*. GFP$^+$ cells were sorted 2 days post transduction and transplanted into lethally irradiated mice. Mice were followed for survival during 360 days. Data expressed as percentage of surviving mice. (G) Hemavet white blood cell count at 360 days post transplantation from the mice described in (F).

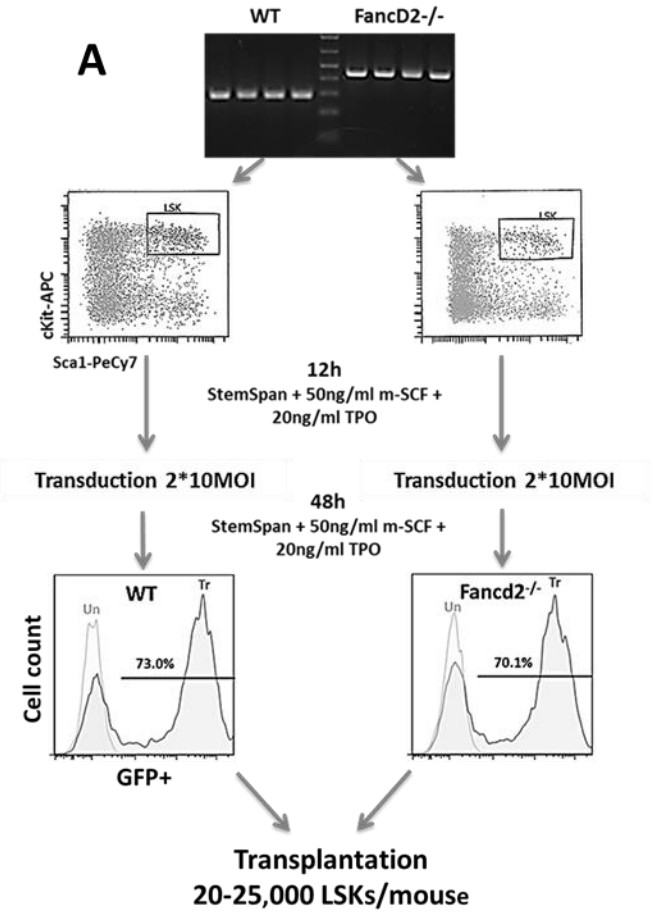
FigureS4: List of ShRNA sequences and Primer sequences used in qPCR experiments. (A) Table showing ShRNA sequences of the different genes targeted in this study and Scrambled ShRNA. (B) Table showing specific forward and reverse primer sequences used for each gene expression evaluated in this study.

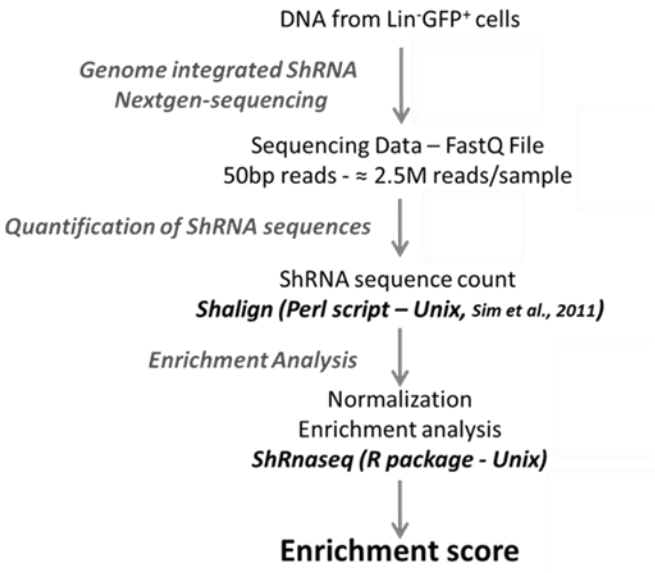
Table S1: Enrichment score of detected ShRNA targeted genes. Values represent the highest mean enrichment score obtained for each targeted genes.

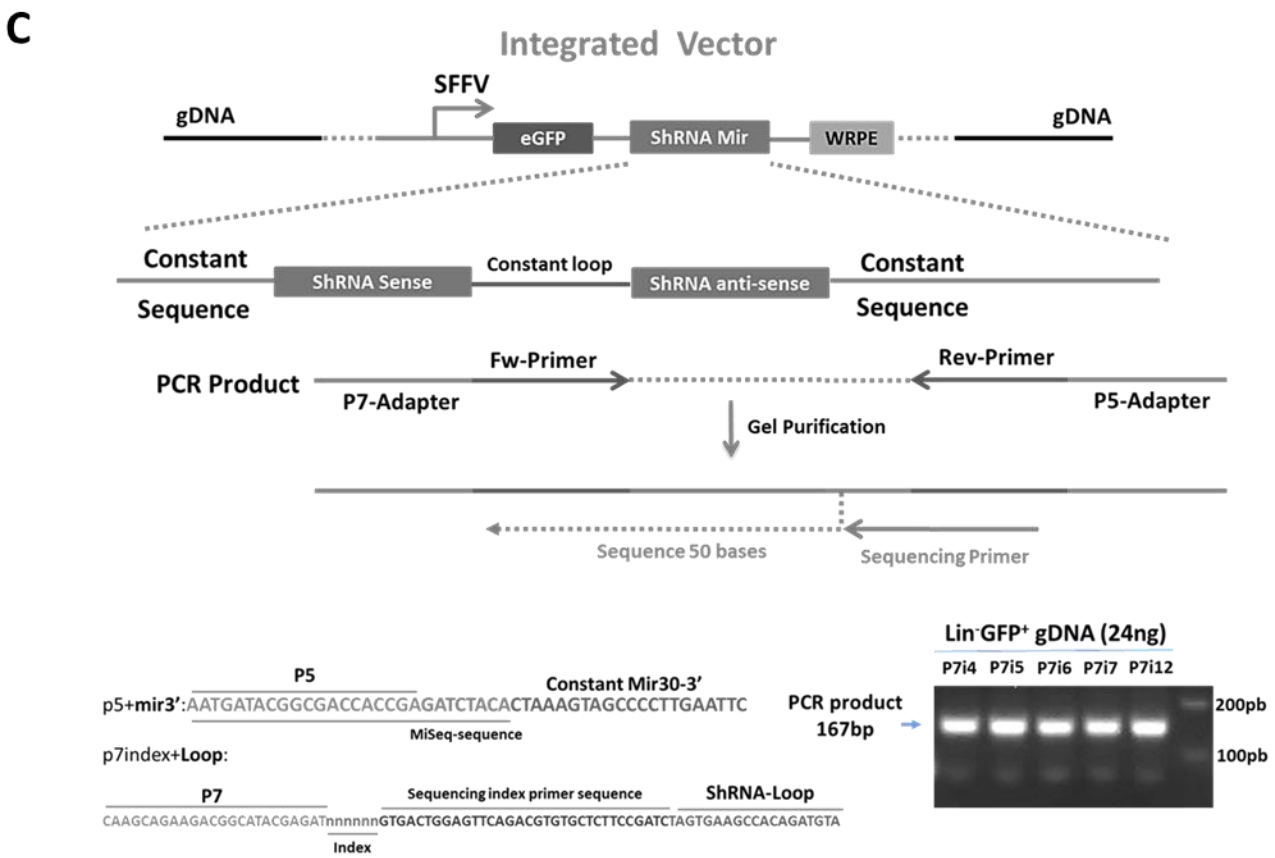
Supplemental Figure 1



Supplemental Figure 2

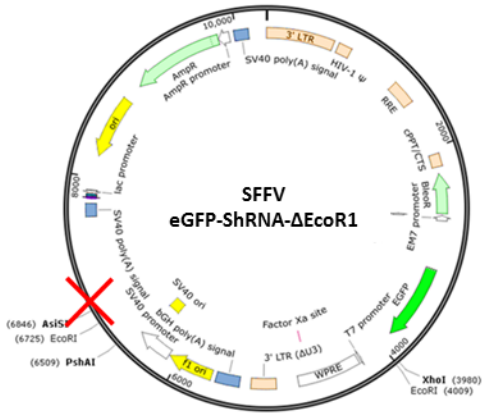






Supplemental Figure 3

A



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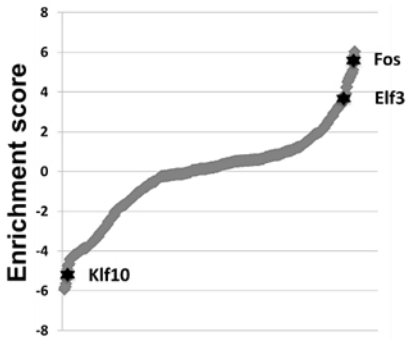
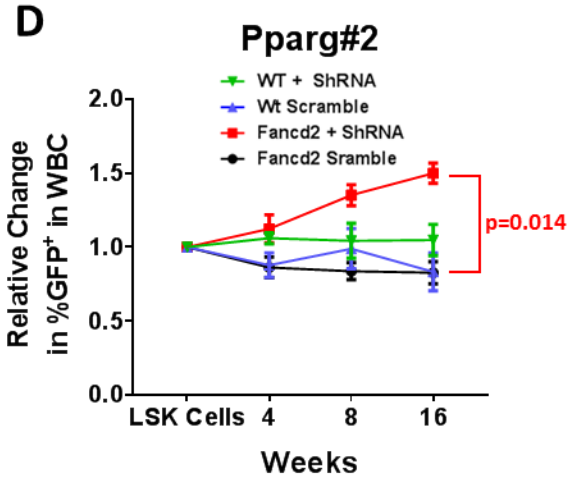


C

Target	Enriched ShRNA	Rep#1	Rep#2	Rep#3
Fos	2/3	5.8	6.5	5.3
Elf3	1/3	4.2	5.1	2.3
Klf10	2/3	-7.4	-4.3	-5.1

D



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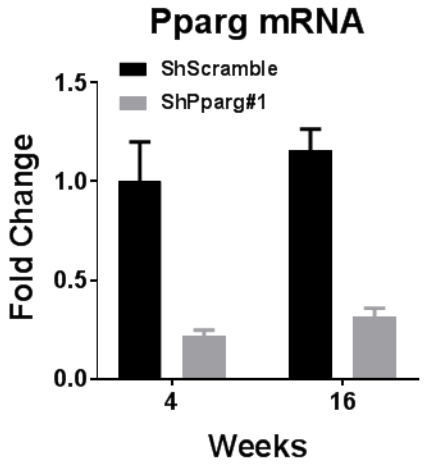

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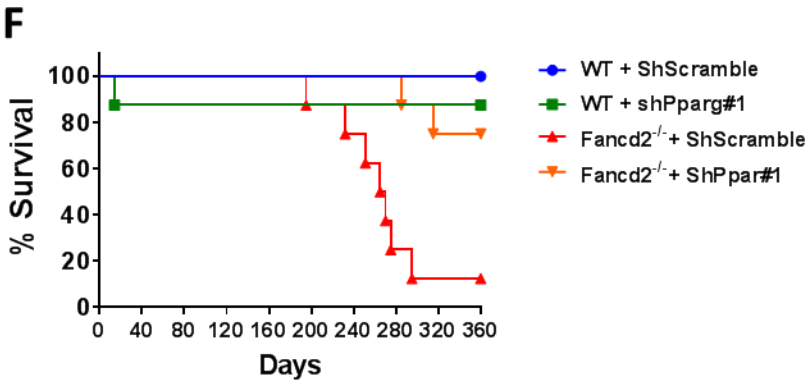

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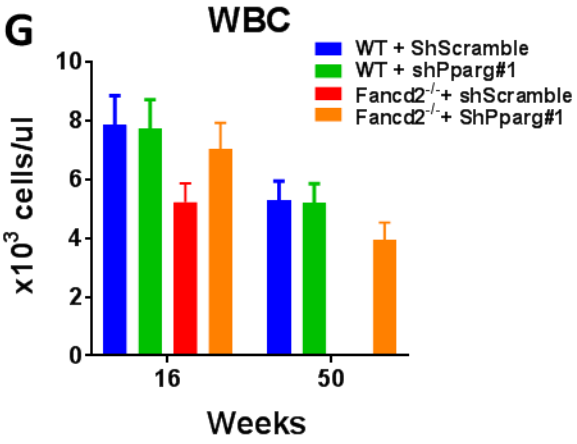

Supplemental Figure4

A

ShRNA	ShRNA sense sequence
sh*Pparg1*	CAATGGTTGCTGATTACAA
sh*Pparg2*	GATTGAAGCTTATTTATGA
sh*Ncoa4-1*	GAATGCCTATGGAACCTAA
sh*Ncoa4-2*	GGCAATCTGAAATGCCTAA
sh*Klf10*	GACTGGAAGTCTCATTTCA
sh*Fos*	GCAATAGCGTGTTCCAATT
Sh*Nr2c1*	GCGTCATTACGGAGCAATA
Sh*Scramble*	CTCGCTTGGGCGAGAGTAA

B

	Primer 5' -> 3'	
Gene	**FW**	**Rev**
mPParg_v1	TTTTCCGAAGAACCATCCGATT	ATGGCATTGTGAGACATCCCC
mPparg_v2	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
mNcoa4	GAACCATCAGGACACATGGAAA	AGGAGCCATAGCCTTGGGT
mPpargc1a	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
mPpargc1b	TCCTGTAAAAGCCCGGAGTAT	GCTCTGGTAGGGGCAGTGA
mCdkn1a	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
mKlf10	ATGCTCAACTTCGGCGCTT	CGCTTCCACCGCTTCAAAG
mFos	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
mNr2c1	ATGGCGACCATAGAAGAAATTGC	CAAGTGCTGTCACGATCTGGA
mHprt1	CGTCGTGATTAGCGATGATG	ACAGAGGGCCACAATGTGAT-
mActb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
hPPARG	AGAAGCCTGCATTTCTGCAT	TCAAAGGAGTGGGAGTGGTC
hNCOA4	ACAGTTGCATAAGCCGTCACC	TGAGCCTGCTGTTGAAGTGTC
hPPARGC1a	TCTGAGTCTGTATGGAGTGACAT	CCAAGTCGTTCACATCTAGTTCA
hPPARGC1b	GATGCCAGCGACTTTGACTC	ACCCACGTCATCTTCAGGGA
hCDKN1A	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
hHPRT1	GAAAAGGACCCCACGAAGTGT	AGTCAAGGGCATATCCTACAA
hACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT