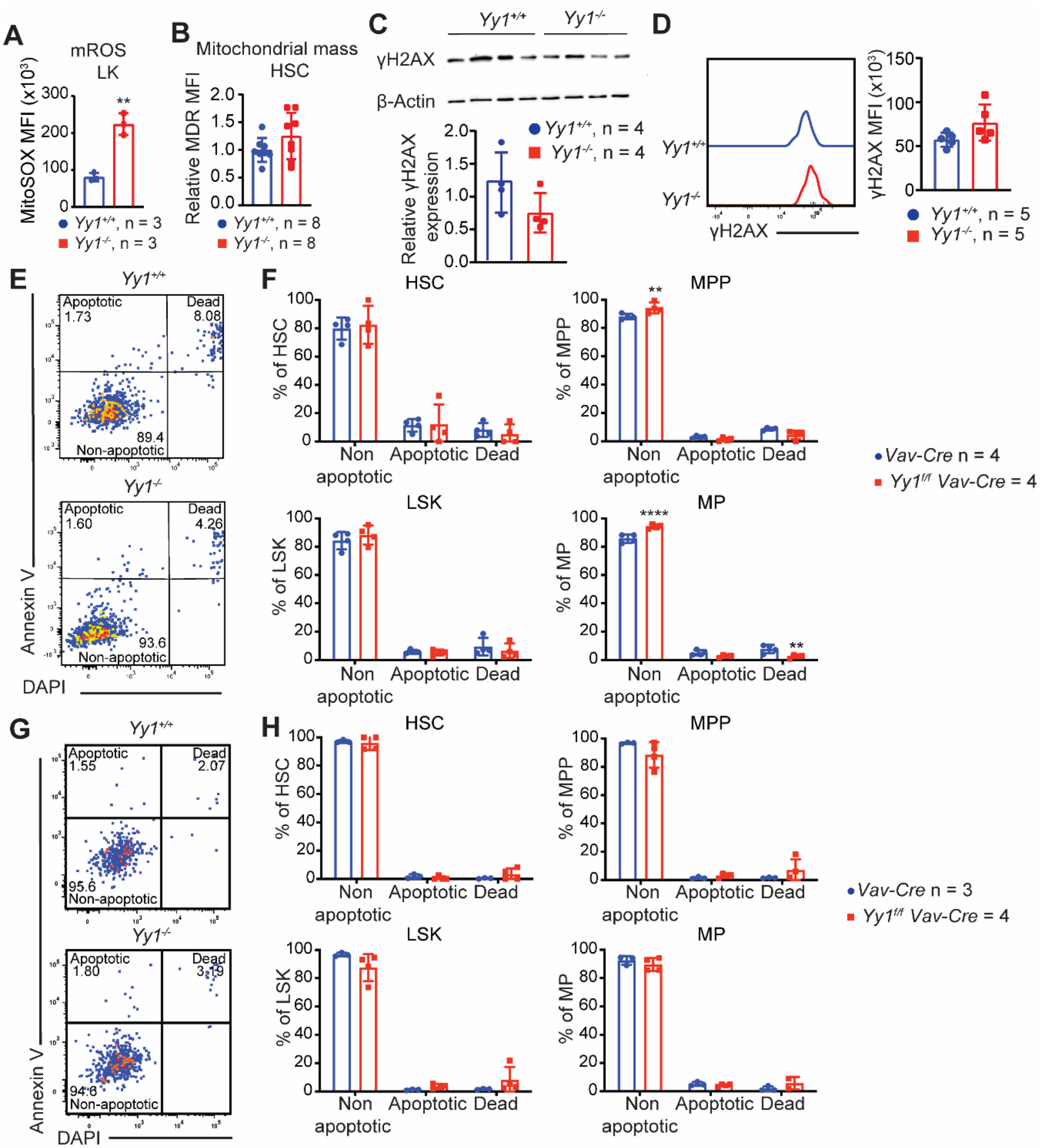
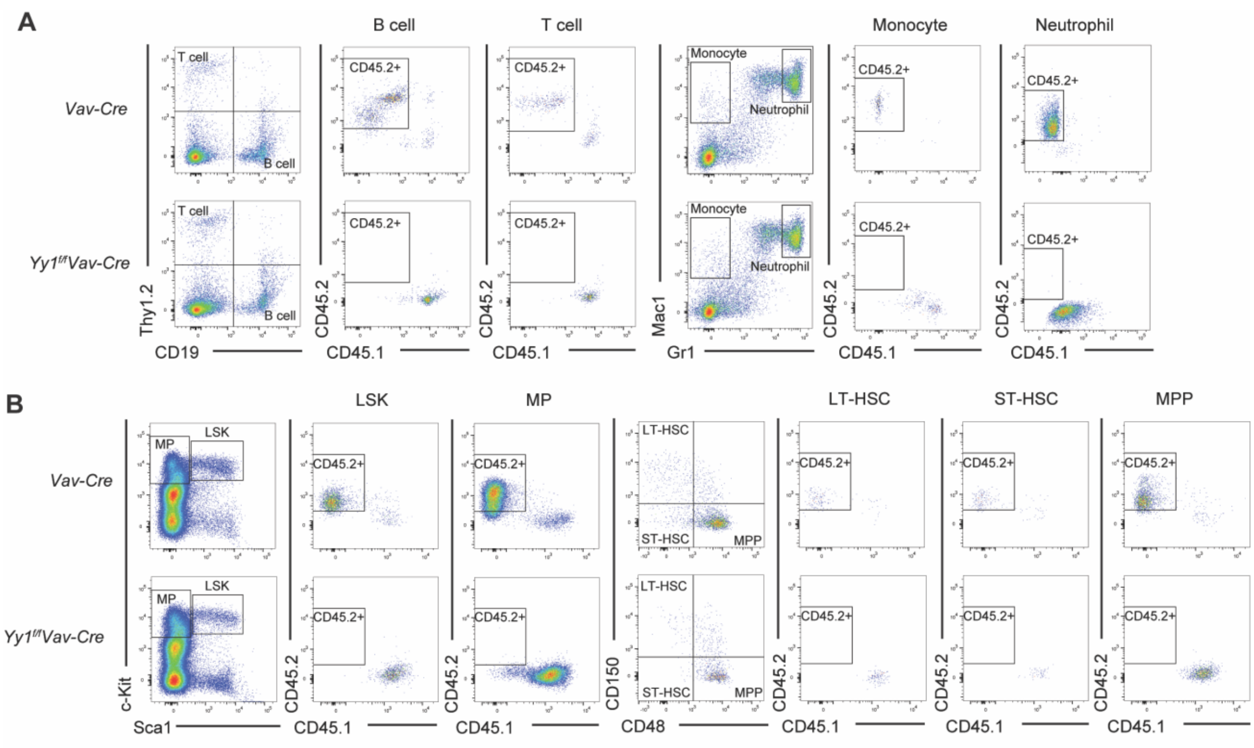
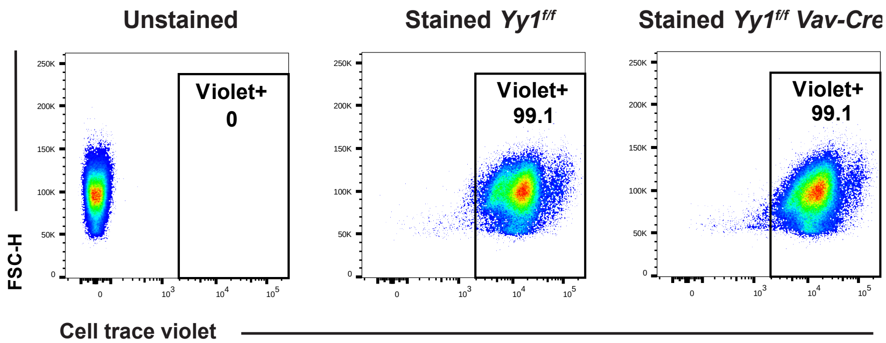
**Supplementary data**



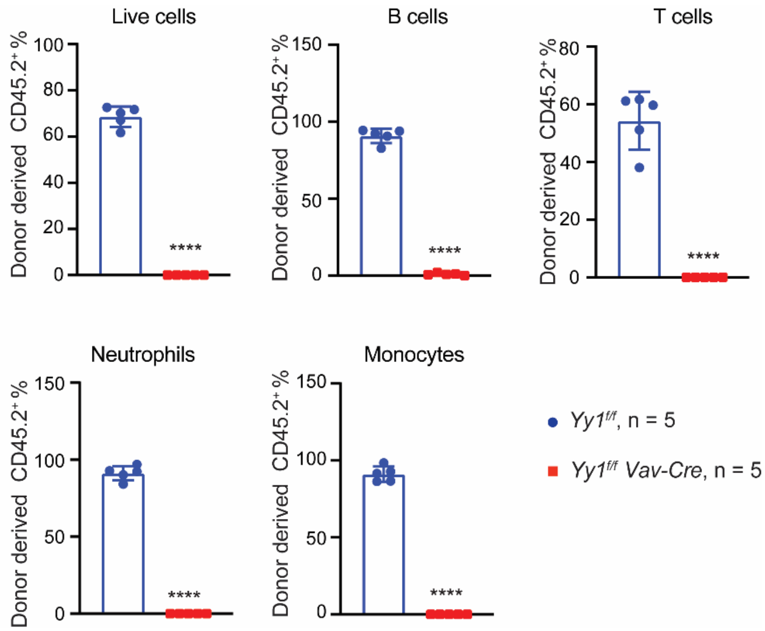

**Supplemental Figure 1.** Flow cytometry evaluation of (**A)** Mitochondrial ROS levels and (**B**) Mitochondrial mass in WT and YY1 KO HSCs. (**C**) WB and quantification of γH2AX in Lin$^{-}$ FL cells. (**D**) Flowcytometry evaluation of γH2AX expression in WT and YY1 KO HSCs. **(E-H)** In vivo and ex vivo survival assay of fetal HSPCs**. (E)** Representative flow gating strategy for non-apoptotic (Annexin $V^-$, $DAPI^-$), apoptotic (Annexin $V^+$, $DAPI^-$) and dead cells (Annexin $V^+$, $DAPI^+$) in the freshly isolated FL HSPC cells. **(F)** Quantification of percentage of non-apoptotic, apoptotic, and dead cells in FL HSC, MPP, LSK, and MP cells. **(G)** Representative flow gating strategy and (**H**) quantification of non-apoptotic, apoptotic, and dead FL HSPCs cultured with serum free media overnight. N represents the number of fetuses; graphs show means ± SD, **P <0.01, ****<0.0001 by unpaired t test (**A-D**), 2-way ANOVA (**F** and **H**).



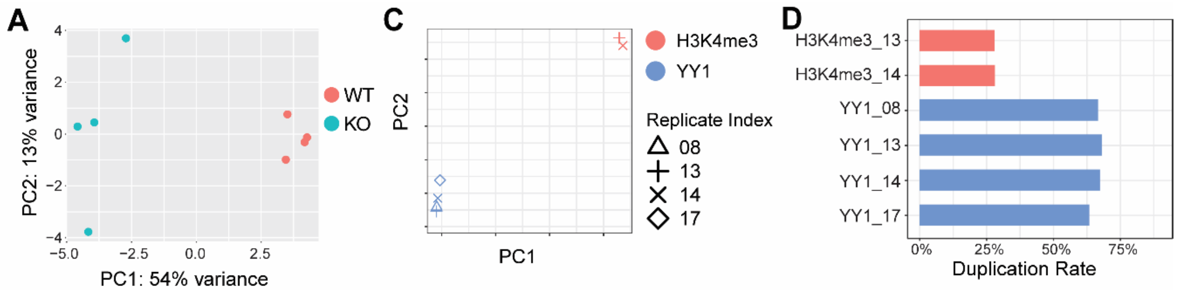

**Supplemental Figure 2. (A)** Representative flow gating strategy for donor derived BM B cells (CD45.2$^{+}$Thy1.2$^{-}$CD19$^{+}$), T cells (CD45.2$^{+}$Thy1.2$^{+}$CD19$^{-}$), neutrophils (CD45.2$^{+}$ Mac1$^{+}$Gr1$^{hi}$) and monocytes (CD45.2$^{+}$ Mac1$^{+}$Gr1$^{-}$) of FL transplant. (**B)** Representative flow gating strategy for donor derived BM LT-HSC (CD45.2$^{+}$Lin$^{-}$Sca1$^{+}$c-Kit$^{+}$CD48$^{-}$CD150$^{+}$), ST-HSC (CD45.2$^{+}$Lin$^{-}$Sca1$^{+}$c-Kit$^{+}$CD48$^{-}$CD150$^{-}$), MPP (CD45.2$^{+}$Lin$^{-}$Sca1$^{+}$c-Kit$^{+}$CD48$^{+}$CD150$^{-}$), LSK (CD45.2$^{+}$Lin$^{-}$Sca1$^{+}$c-Kit$^{+}$) and MP (CD45.2$^{+}$Lin$^{-}$Sca1$^{-}$c-Kit$^{+}$) populations.

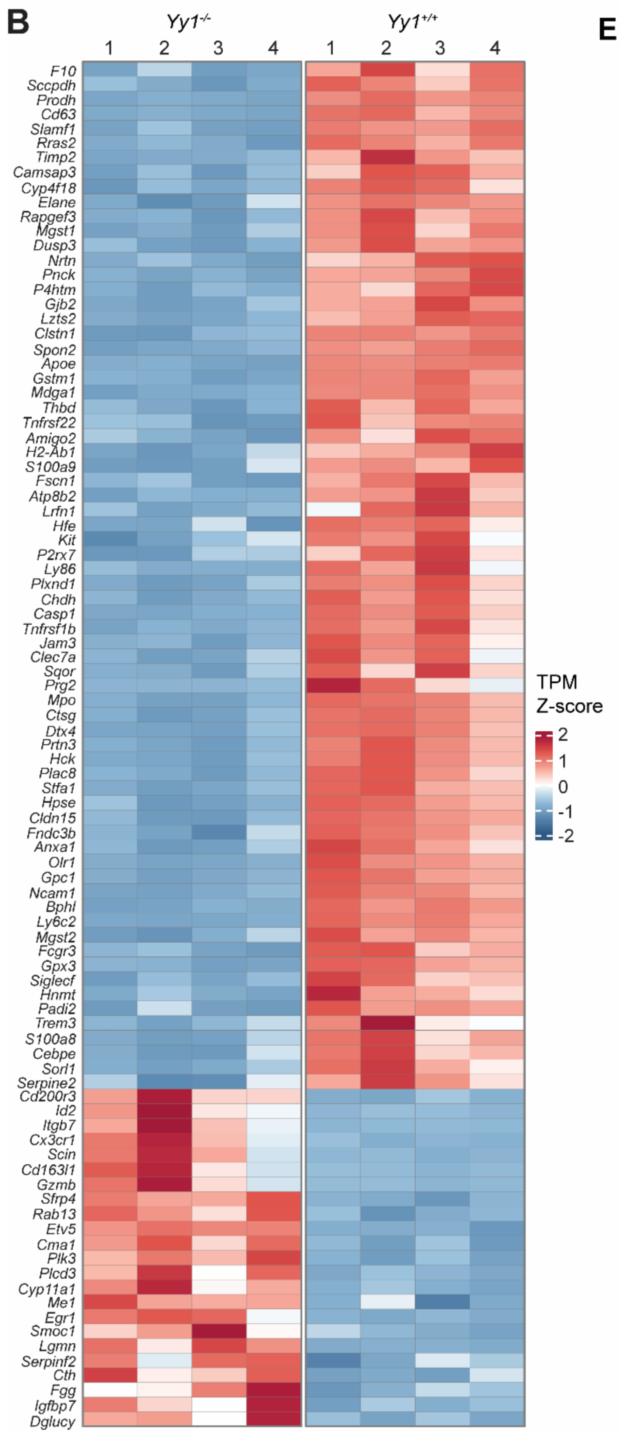



**Supplemental Figure 3.** CellTrace Violet dye labeled over 99% cells. Representative flow grating strategy for percentage of CellTrace Violet$^{+}$ *Yy1$^{f/f}$* and *Yy1$^{f/f}$ Vav-Cre* Lin- fetal liver cells after staining.




**Supplemental Figure 4.** Donor derived percentage of total live cells (CD45.2$^{+}$), B cells (CD45.2$^{+}$Thy1.2$^{-}$CD19$^{+}$), T cells (CD45.2$^{+}$Thy1.2$^{+}$CD19$^{-}$), neutrophils (CD45.2$^{+}$Mac1$^{+}$Gr1$^{hi}$) and monocytes (CD45.2$^{+}$ Mac1$^{+}$Gr1$^{-}$) in bone marrow of recipient mice that received intraosseous transplant of wild-type or YY1 deficient FL cells**.** N represents the number of mice; graphs show means ± SD, ****P<0.0001 by unpaired t test.

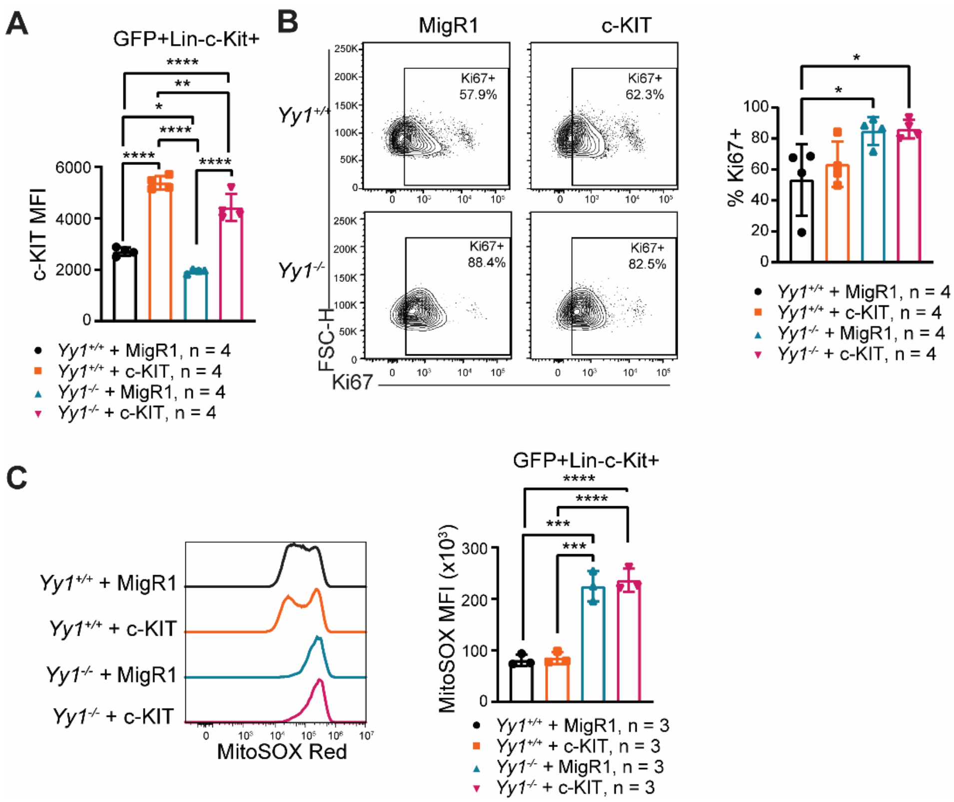


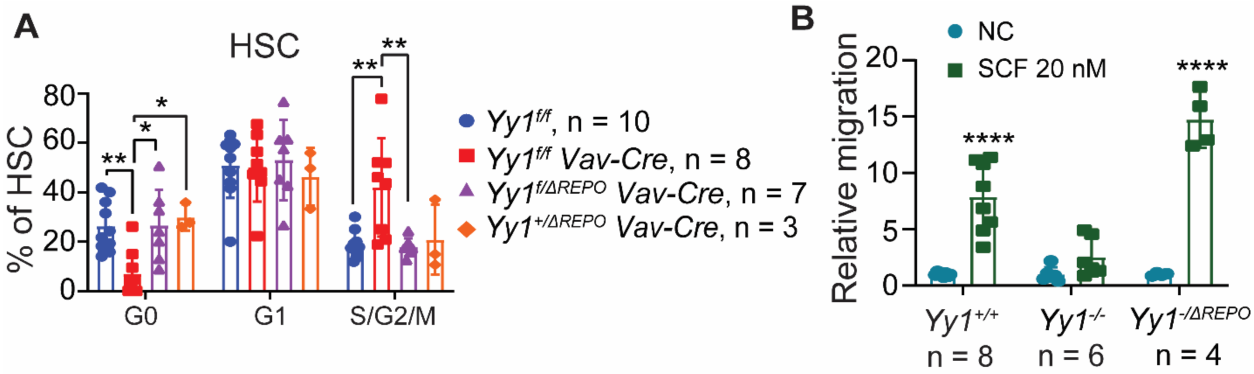

E

| Rank | TF | Adjusted P | Motif Sequence |
|---|---|---|---|
| 1 | KLF3 | 0 | GGRGVDGGGCGGGGCYGGG |
| 2 | E2F4 | 0 | RGGGGGCGGGRMNN |
| 3 | SP3 | 0 | SGVVGGGGGCGGGGCBRGSS |
| 4 | SP2 | 0 | GGSSVGGGGGCGGGGCCDGSGS |
| 5 | KLF1 | 2.4E-303 | DGGGYGKGGCYGGG |
| 6 | E2F1 | 3.8E-272 | GRGGGCGGGRMV |
| 7 | KLF5 | 2.1E-269 | RGGGSWGGGHGGGGC |
| 8 | SP4 | 1.5E-259 | SRGVARGRGGCGGRGCHDRR |
| 9 | KLF4 | 3.7E-228 | KRRRVWGGGTGKGGC |
| 10 | KLF6 | 2.9E-219 | GSRRGGGHGGGGMHGGGRV |
| 11 | FOXI1 | 2.7E-188 | YTBTGATTGGYY |
| 12 | SP1 | 1.1E-184 | GGVGGGGGVGGGGGMGGGGCSGGG |
| 13 | NRF1 | 3.1E-174 | CWSTGCGCATGCGCVRS |
| 14 | EGR1 | 1.4E-158 | GKGCGKGGGCGGGG |
| 15 | NFYB | 3.1E-154 | YRRCCAATSAGMR |
| 16 | KLF15 | 9.4E-147 | RGGGMGGRGVDGGGGGRGG |
| 17 | ZBT17 | 1.7E-140 | GGSGGHGGGGGAGGGGMGG |
| 18 | NFYC | 4.3E-140 | BYARCCAATCAGAR |
| 19 | EGR2 | 6.8E-119 | KGHGTGGGHGGGGG |
| 20 | E2F2 | 1.5E-109 | GGCGCGAAAC |
| 21 | ZFX | 3.5E-109 | GSCBVGGCCTGSSSSSCBS |
| 22 | E2F3 | 1.3E-98 | GGGCGGGARS |
| 23 | CTCFL | 4.4E-97 | GCCRSCAGGGGGCGCYVB |
| 24 | NFYA | 1.4E-96 | BYRRCCAATCAGAR |
| 25 | WT1 | 1.3E-94 | RGGVGGGGGAGGRGGVGGRG |
| 26 | E2F7 | 4.4E-93 | GDGGCGGGAARDR |
| 27 | SP5 | 1.6E-86 | RRGGRGGRGGRGRRGGGGGAGGRG |
| 28 | MBD2 | 4.0E-77 | SSGKCCGGMGR |
| 29 | FLI1 | 9.6E-74 | GGVRCCGGAAGYGS |
| 30 | E2F6 | 3.6E-71 | NKGGCGGGARRG |
| 31 | MECP2 | 6.0E-71 | SCCGGRR |
| 32 | GABPA | 1.2E-69 | GGVRCCGGAAGTGV |
| 33 | ELF1 | 1.4E-69 | SRACCCGGAAGTGS |
| 34 | CTCF | 2.5E-62 | YGGCCASCAGRGGGCGCYVB |
| 35 | SALL4 | 2.2E-60 | GSDGGGWGGG |
| 36 | TYY1 | 2.1E-59 | CAAVATGGCGGC |
| 37 | ZN281 | 1.9E-54 | GGGGTGGGGGAGGGG |
| 38 | AP2B | 1.3E-49 | GCCYGVGGGS |
| 39 | PBX3 | 1.7E-48 | YBYTGATTGRYTGR |
| 40 | ELK1 | 1.4E-47 | RCCGGAAGTGV |
| 41 | ARNT | 3.6E-46 | BYRCGTGC |
| 42 | AHR | 6.0E-46 | DTYGCGTGM |
| 43 | ELK4 | 3.0E-43 | SRRCCGGAAGYRG |
| 44 | FEV | 2.6E-41 | GCVGGAAGYG |
| 45 | TFEB | 2.9E-38 | RGTCACGTG |
| 46 | MAZ | 5.0E-37 | GGGGGAGGGGGDGRGRRRGRRG |
| 47 | ATF1 | 3.3E-36 | VTGACGTCAS |
| 48 | ZFP57 | 2.2E-34 | CTGCGGCARR |
| 49 | AP2C | 3.0E-34 | YRSCCYCAGGSYN |
| 50 | MYC | 3.3E-34 | MGCCACGTGSBC |
| 51 | ETS1 | 5.0E-34 | VRRRCMGGAAGTGG |
| 52 | KAISO | 4.5E-32 | SARRYCTCGCGAGAV |
| 53 | TAF1 | 4.5E-31 | VAARAWGGCGGMGG |
| 54 | MAX | 2.4E-30 | SVSCACGTGGC |
| 55 | AP2A | 6.1E-30 | VTRSCCTGRGGC |
| 56 | PKNX1 | 8.6E-30 | TGABTGRCAGS |
| 57 | USF2 | 9.3E-27 | GGGTCACGTGRBSVSSSSB |
| 58 | RFX1 | 5.2E-26 | SSSSCBGTTGCCAKGGVRACSS |
| 59 | PBX1 | 7.3E-26 | TGAKTGACAGS |
| 60 | KLF8 | 1.7E-24 | CAGGGKGTG |
| 61 | ZIC1 | 7.0E-23 | KGGGWGGTS |
| 62 | ERG | 5.9E-22 | VVRCMGGAAGYRVV |
| 63 | SRBP2 | 6.3E-20 | VSGTGGGGWGABG |
| 64 | SRBP1 | 1.9E-18 | GGTGGGGTGAB |
| 65 | USF1 | 3.7E-16 | SRGTCACGTGRCS |
| 66 | MYCN | 4.5E-13 | SCACGTGG |
| 67 | E2F5 | 1.5E-12 | SGCGCSAAAH |
| 68 | MXI1 | 1.8E-12 | VCACGTGGSNGSBGS |
| 69 | MTF1 | 2.2E-12 | MGKGCCGTGTGCAAARS |
| 70 | HINFP | 4.0E-12 | DMSHHMGCGGACGTTV |
| 71 | HEN1 | 8.8E-12 | BGGGKMKCAGCTGCGKCCYY |
| 72 | INSM1 | 8.0E-11 | TGTMAGGGGGCR |
| 73 | PAX5 | 6.8E-10 | VDRVKCAGYSRAGCGTGAC |
| 74 | ZFP42 | 1.2E-09 | GGCMGCCATTTT |
| 75 | ELF2 | 3.4E-09 | TDNCAGGAAGTRRVT |
| 76 | OVOL2 | 9.4E-09 | RTAACGGD |
| 77 | EPAS1 | 1.8E-07 | VTACGTGMC |
| 78 | NR1H4 | 3.8E-07 | RRGGKCAVTGRCCBSGVGG |
| 79 | HIF1A | 4.2E-07 | GDACGTGM |
| 80 | ZBT7A | 3.4E-06 | VRGGGKCKY |
| 81 | ZIC3 | 7.3E-06 | GGCYHCCTGCTGWGH |
| 82 | ZN143 | 1.2E-05 | KGCMTKCTGGGARWTGTAGTYY |
| 83 | GATA1 | 6.2E-05 | TGSNSSNNDSAGATAAGRV |
| 84 | TFE3 | 1.1E-04 | RGTCACGTGA |
| 85 | ZN322 | 9.1E-04 | GAGCCTGSTACWSWGCCTGR |
| 86 | ZIC2 | 1.1E-03 | DGCYHCCTGCTGWGH |
| 87 | COE1 | 3.4E-03 | RGYCCCWGGGGAS |
| 88 | RFX3 | 3.6E-03 | CSGTTGCCATGGMRAC |
| 89 | MITF | 5.3E-03 | YCWYGTGACY |
| 90 | TAL1 | 6.4E-03 | CTGSNNNNNRSAGATAAGRV |
| 91 | PRDM5 | 1.1E-02 | VMTGGAGADCMRGGW |
| 92 | EHF | 1.2E-02 | VWACSAGGAAGTDVS |
| 93 | ETV2 | 1.2E-02 | RRARRCAGGAARYRGS |
| 94 | MYBA | 1.8E-02 | VGYGGCVGTTRSVGV |

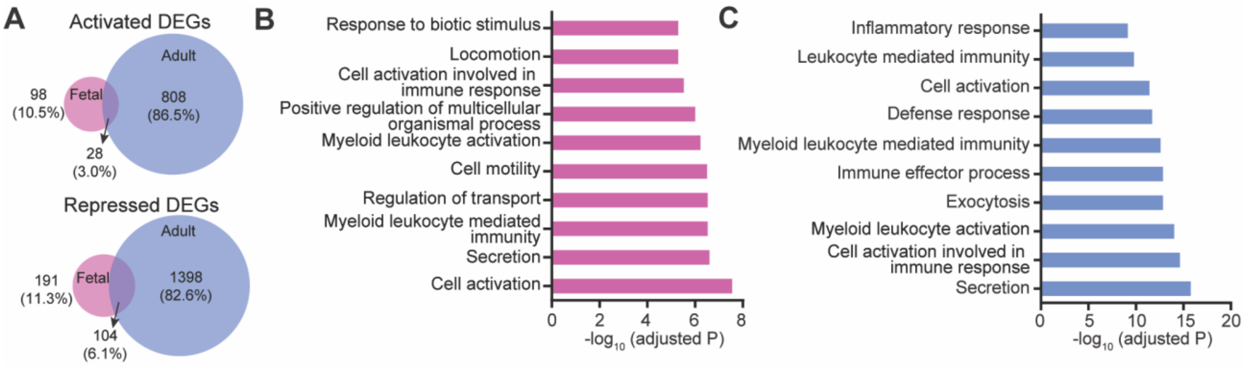
**Supplemental Figure 5**. **(A)** PCA plot of RNA-seq from sorted *Yy1*$^{+/+}$ and *Yy1*$^{-/-}$ Lin$^{-}$ cKit$^{+}$ E14.5 FL cells. **(B)** Heatmap depicting selected upregulated and down-regulated genes based on TPM. **(C)** PCA plot of CUT&Tag. **(D)** Duplication rate of each CUT&Tag sample. **(E)** Motif analysis identified 94 significantly enriched (adjusted P $< 0.05$) transcription factor motifs, ranked based on adjusted P. TF motifs known to play essential roles in hematopoiesis, chromatin structure regulation, cell stress response, and cellular proliferation are highlighted.




**Supplemental Figure 6. Ectopic expression of c-Kit did not rescue YY1-dependent cell cycle or mitochondrial ROS in FL LK cells. (A)** Ectopic expression of c-Kit rescued c-Kit MFI in YY1 deficient FL LK cells. **(B)** Representative gating strategy for Ki67 proliferation assay. E14.5 Lin⁻ FL cells were infected with either MigR1 (control) or MigR1-c-Kit vectors. The percentages of Ki67⁺ and Ki67⁻ cells were quantified within GFP⁺Lin⁻c-Kit⁺ populations. **(C)** Representative flow cytometry plots and quantification of mitochondrial ROS levels in GFP⁺Lin⁻c-Kit⁺ cells infected with either MigR1-c-Kit or MigR1 vector control. N represents the number of fetuses; graphs show means ± SD, *P <0.05, **P <0.01, ***P<0.001, ****P<0.0001 by one way ANOVA.




**Supplemental Figure 7. The YY1 REPO domain deletion did not impact HSC proliferation and SCF-dependent cell migration. (A)** Quantification of percentage of fetal HSC in G0 ($Ki67^-$ $DAPI^-$), G1 ($Ki67^+DAPI^-$) and S/G2/M ($Ki67^+DAPI^+$) phases. (**B**) Ex vivo migration assay of *$Yy1^{-/\Delta REPO}$*, *$Yy1^{-/-}$ and $Yy1^{+/+}$* $Lin^-$ FL cells in response to SCF stimulation. While YY1-deficient cells exhibit significantly impaired migration upon SCF stimulation, both wild type and *$Yy1^{-/\Delta REPO}$* cells are responsive to SCF stimulation. N represents the number of fetuses; graphs show means ± SD, *P <0.05, **P <0.01, ****P<0.0001 by 2-way ANOVA.




**Supplemental Figure 8.** (**A**) Venn diagram of activated and repressed DEGs associated with YY1 deletion in fetal versus adult HSPCs. (**B-C**) GSEA analysis of enriched GO pathways in fetal (**B**) and adult HSPCs (**C**).

**Table 1**

**qRT-PCR Primer list**

| | Forward | Reverse |
|---|---|---|
| *Yy1* | TCAGACCCTAAGCAACTGGCAGAA | TTGAGCTCTCAACGAACGCTTTGC |
| *Kit* | AGTGGACGTACAGGTCCAGAA | GCCTGGATTTGCTCTTTGTTGT |
| *S100a8* | ATCACCATGCCCTCTACAAGAATG | GTCCAATTCTCTGAACAAGTTTTCG |
| *S100a9* | AAGCTGCATGAGAACAACCCA | CCCAGAACAAAGGCCATTGA |
| *Gpc1* | GAATTTGGCCAACCACAGCC | GTAGTAGAGGCGCAGCTCAG |
| *Hck* | GCCAACCTGATGAAGTCGCT | TGTACTCATTGTCCTCGATGATTCG |
| *Csf1r* | CAGTTCAGAGTGATGTGTGGTC | CTTGTTGTTCACTAGGATGCCG |
| *Padi2* | CAGCCGCCTATACGGGAAA | CTTCACACCTTCCGAGTGCT |
| *Jam3* | TACCGCAATGATGTGCCACT | GATGAAGCAGCCTCGTCTGT |
| *Cd63* | CTGTGGGCTGTGGGAATGAT | CCTCCACAAAAGCAATGCCC |
| *Cxcr2* | CATCCCGTTTGAGGGTCGTA | GCAGGAAGACAAGGACGACA |
| *Mpo* | CCTCCAACCGTGCCTTTGTA | GTCAGCTGATCGTTGGGGAA |
| *Olr1* | CTGCGAATGACGAGCCTGA | CCAGAGTCATAGCAGCAGGG |
| *Pnck* | ATCGCGGTACTTCGCAGAAT | CCAAGGACCTGCCCTACAAG |
| *Cxcr4* | GACTGGCATAGTCGGCAATG | AGAAGGGGAGTGTGATGACAAA |
| *Gapdh* | TCCTGCACCACCAACTGCTT | GTCTTCTGGGTGGCAGTGAT |

**Table 2**

**Genotyping Primer list**

| | Forward | Reverse |
|---|---|---|
| *Yy1* P1, P2 | ACCTGGTCTATCGAAAGGAAGCAC | GCTTCGCCTATTCCTCGCTCATAA |
| *Yy1* P3, P4 | TAGAGAATAGGAACTTCGGCCGCCA | CCAAAGTTCGAAACCTGCTTTCCT |
| $Yy1^{\Delta REPO}$ | GACGGCTTCGAGGACCAGAT | GCGCCCATCACACACATAAAA |