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Supplemental Information

Polycomb Group Protein YY1 Is an Essential Regulator of Hematopoietic Stem Cell Quiescence

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Supplemental Experimental Procedures

Colony formation assays

Primary bone marrow cells were plated in duplicate in Methocult M03434 complete medium (StemCell Technologies) at a density of 3×10^4 cells per 35 mm plate. Plates were incubated for 10 to 12 days before counting BFU-E, CFU-GM, and CFU-GEMM colonies. To evaluate the colony-forming ability of purified LT-HSCs, single LT-HSCs were sorted directly into individual wells of 96-well plates containing Methocult M03434 medium and incubated for 2 weeks prior to the quantification.

Bone marrow transplantations

Recipient C57BL/6 ($CD45.1^+$) mice were lethally irradiated with 2 doses of 6 Gy each, delivered 4 hours apart, and transplanted with 5×10^5 cells. For competitive bone marrow transplantation assay, bone marrow from *Mx1-cre* or *Yy1*$^{f/f}$ *Mx1-cre* mice ($CD45.2^+$) were mixed with $CD45.1^+$ competitor cells at a 1:1 or 9:1 ratio and total 2×10^6 BM cells were transplanted. At 4-week post-transplantation, recipient mice were treated with 5 doses of pI-pC to delete the endogenous *Yy1*. For secondary BMTs, 10×10^6 BM cells from two donor mice were mixed and transplanted to lethally irradiated $CD45.1^+$ congenic recipients at 16-20 weeks post primary BMT.

RNA sequencing and gene set enrichment analysis

Sequencing libraries were prepared by using Ovation RNA-Seq System V2 (NuGen) according to the manufacturer's specifications and were sequenced by an Illumina HiSeq 2000 at the University of Wisconsin Biotechnology Center Gene Expression Center. Sequencing reads were adapter and quality trimmed using the Skewer trimming program. Sequencing reads were mapped to the genome using Subjunc aligner from subread package (verion 1.5.0-p1). Quantification of expression for each gene was calculated using the featureCounts tool from subread. Genes with at least one condition where 66.6% of the replicates have a count of > 0 were used in downstream analysis. Gene set enrichment analysis (GSEA) was performed by calculating a ranked vector as sign (foldChange)* 1/p-value. GENE Symbols chip file and REACTOME (Croft et al., 2014; Fabregat et al., 2016) were used to run GSEA Ranked using GSEA version 2-2.2.2. GO analysis was performed using Gorilla (Eden et al., 2009). Analysis was done using R 3.3.1/Bioconductor 2.32.0 (Huber et al., 2015).

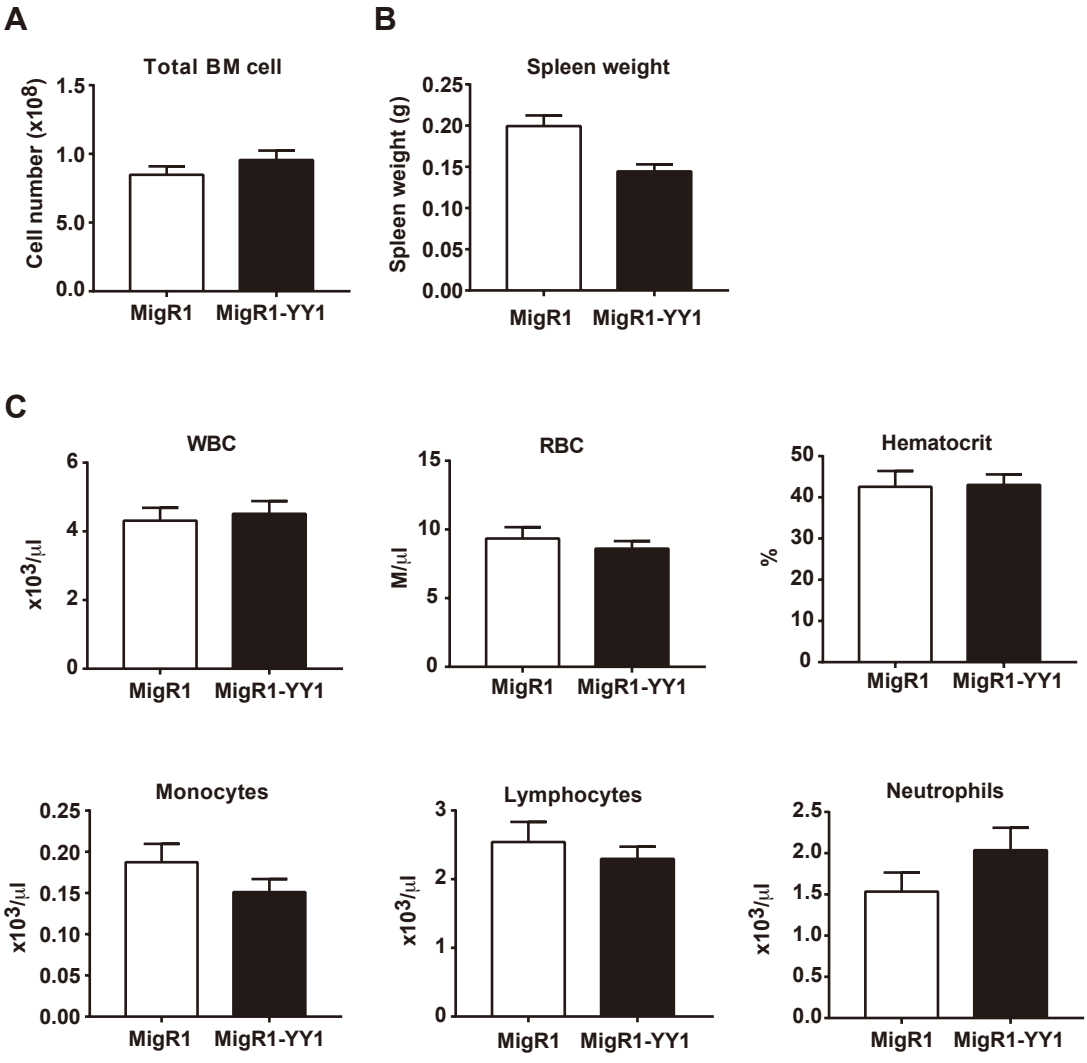

Figure S1, Related to Figure 1. (A) Total bone marrow count in mice with ectopic YY1 expression (MigR1-YY1) versus control (MigR1). (B) Spleen weight of mice with ectopic YY1 expression (MigR1-YY1) versus control (MigR1). (C) CBC of mice with ectopic YY1 expression (MigR1-YY1) versus control (MigR1).

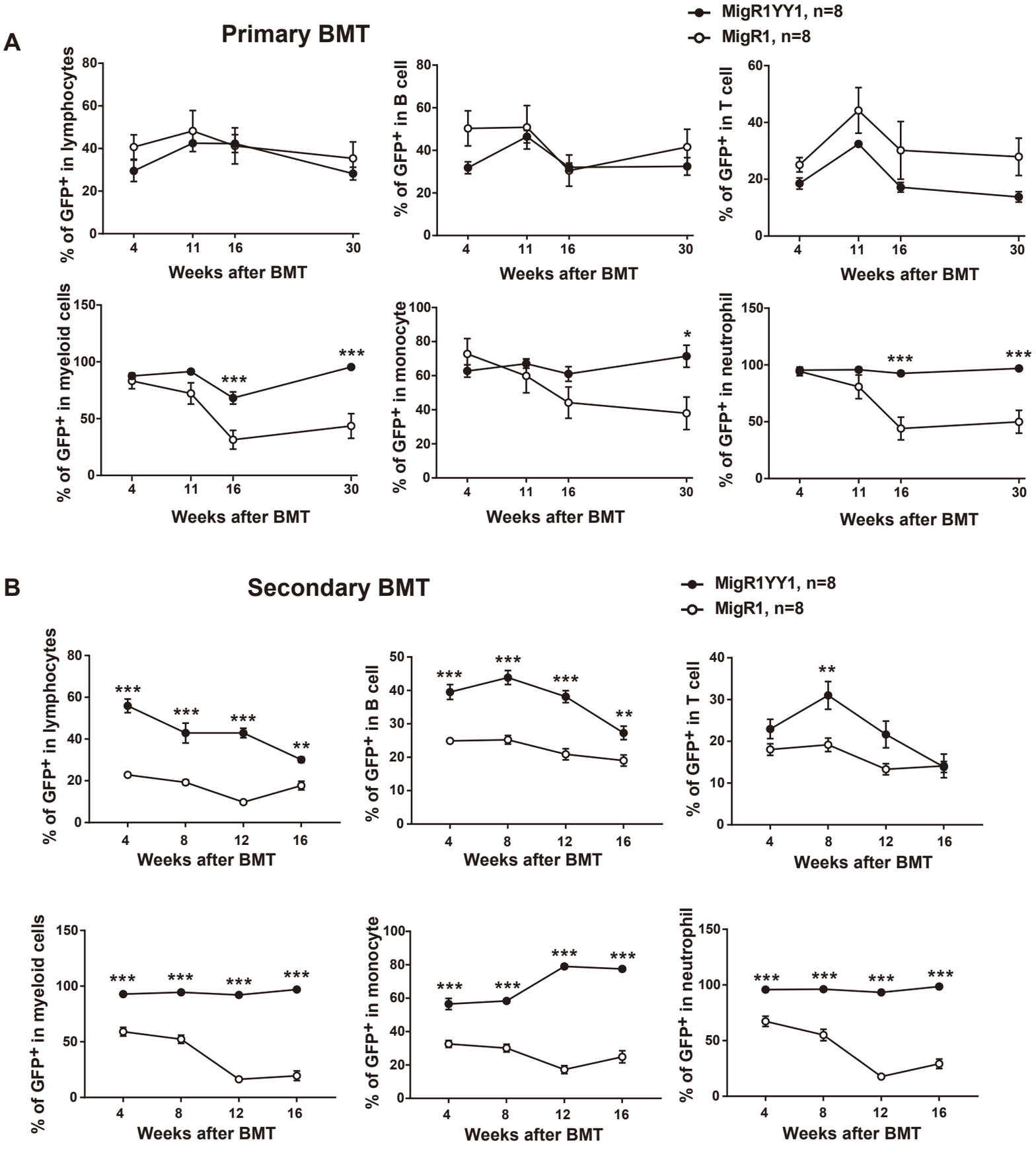

Figure S2, Related to Figure 1. (A) Primary BMT evaluations of donor-derived contribution in peripheral lymphocytes and myeloid cells of mice with ectopic YY1 expression (MigR1-YY1) versus control (MigR1). (B) Secondary BMT evaluations of donor-derived contribution in peripheral blood lymphocytes and myeloid cells of mice with ectopic YY1 expression (MigR1-YY1) versus control (MigR1). Graphs show means ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001.

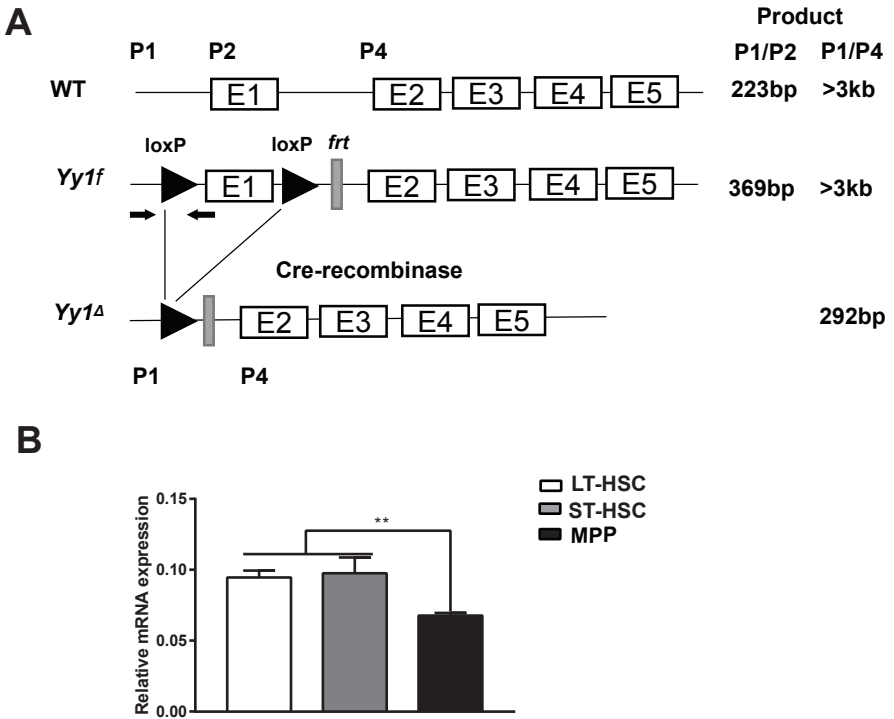

Figure S3, Related to Figure 2. (A) Schematic illustration of the *Yy1* locus. The conditional *Yy1* allele (*Yy1f*) was constructed by inserting a pair of loxP sites flanking exon 1 and the promoter region, which is excised in the presence of pI-pC induced Cre recombinase expression. The arrows indicate the location of the primers used for PCR detection of deletion efficiency. Primers 1 and 2 detect both $Yy1^{+}$ (223bp) and *Yy1f* (369bp). Primers 1 and 4 detect $Yy1^{\Delta}$ (292bp). (B) YY1 mRNA expression in LT-HSC, ST-HSC and MPP. LT-HSC (Lin-Sca1+c-Kit+ CD48-CD150+), ST-HSC (Lin-Sca1+c-kit+CD48-CD150-) and MPP (Lin-Sca1+c-kit+ CD48+CD150-) were sorted from B6 mice and YY1 mRNA expressions were detected by qRT-PCR. Graphs show means ± SEM; **P < 0.01.

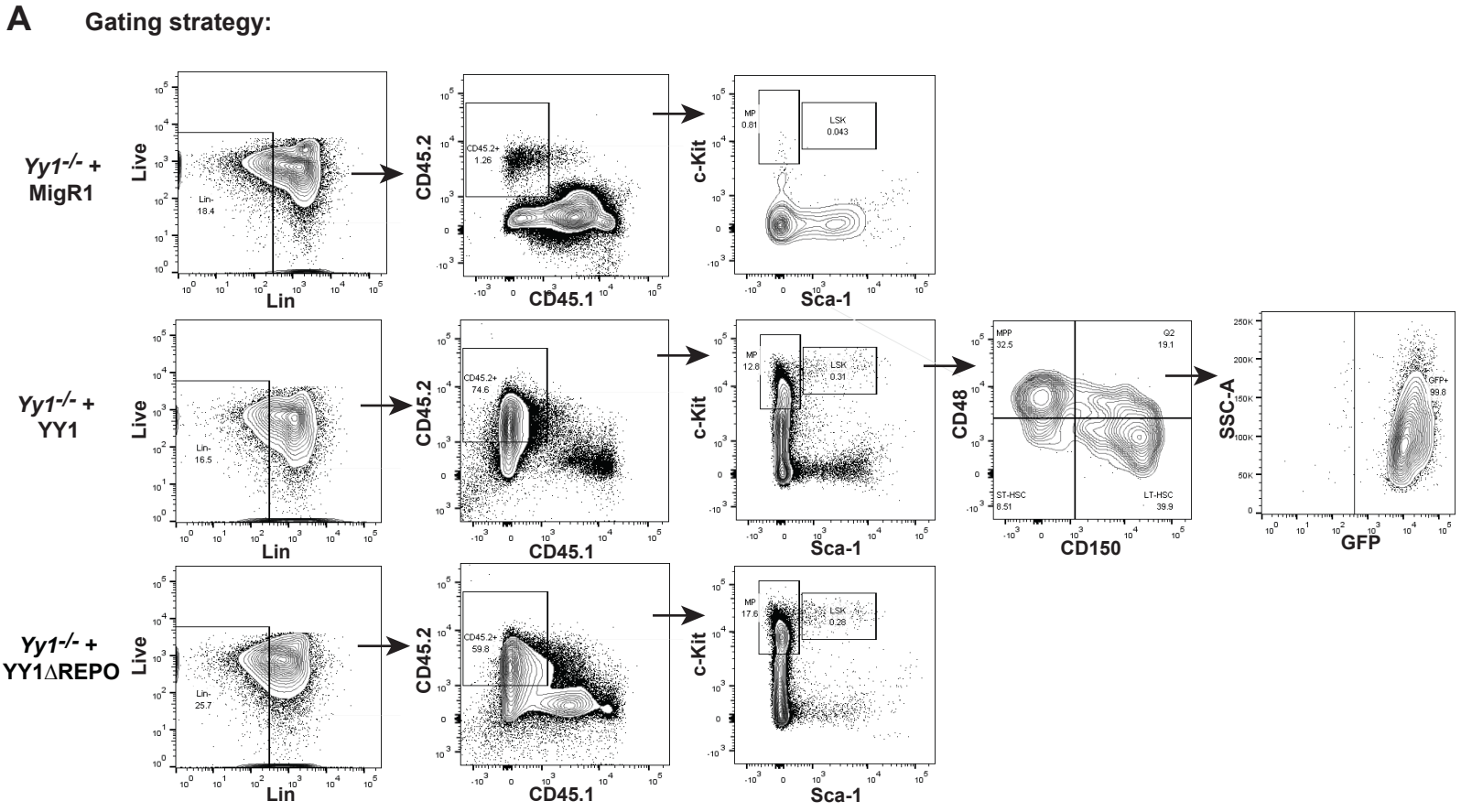

Figure S4, Related to Figure 4. (A) Gating Strategy of % GFP+ in donor derived BM LT-HSC (Lin-Sca1+c-Kit+ CD48-CD150+), ST-HSC (Lin-Sca1+c-kit+CD48-CD150-), MPP (Lin-Sca1+c-kit+ CD48+CD150-), LSK (Lin-Sca1+c-kit+), and MP (Lin-Sca1-c-kit+). (B) Colony formation assay. Total bone marrow cells harvested from MigR1- FlagYY1 versus MigR1-FlagYY1ΔREPO rescued primary BMT mice were plated in Methocult M03434 complete medium. CFU-GEMM, CFU-GM and BFU-E were counted 8 days afterwards. Graphs show means ± SEM; *P < 0.05, ***P < 0.001.

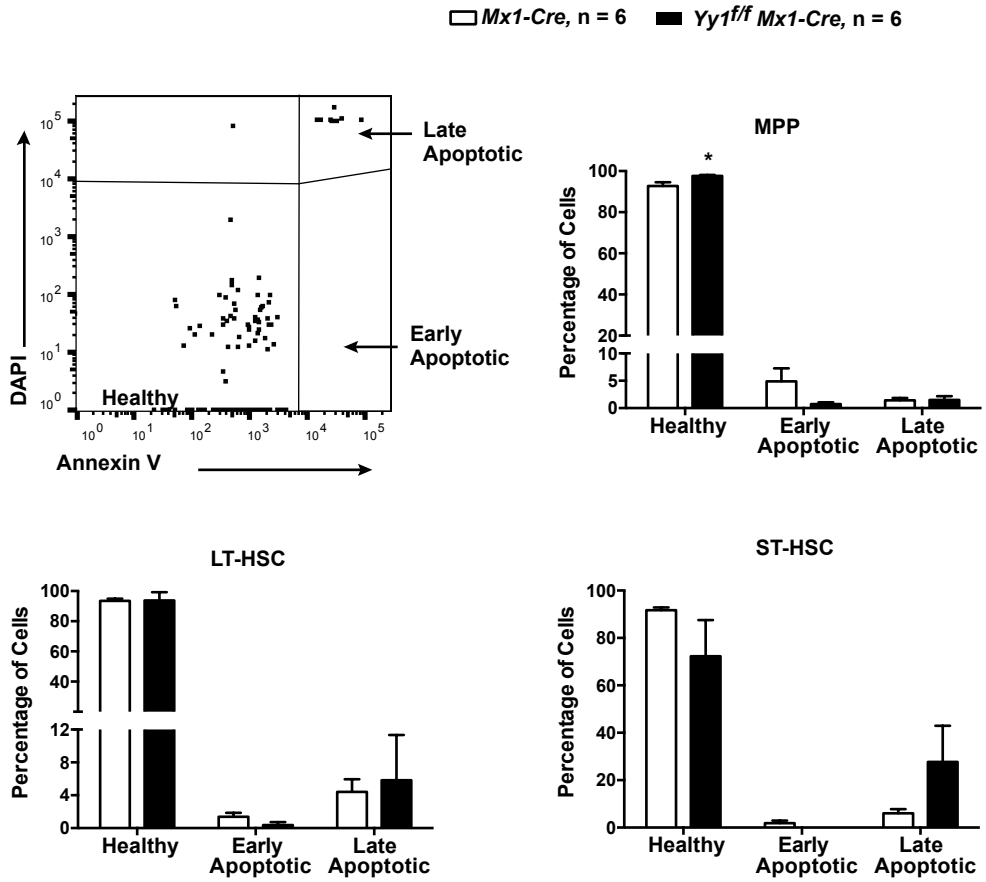

Figure S5, Related to Figure 6. YY1 deficiency does not induce apoptosis in HSCs. Healthy (AnnexinV- DAPI-), early apoptotic (AnnexinV+ DAPI-), and late apoptotic cells (AnnexinV+ DAPI+) were gated by flow cytometry in bone marrow LT-HSC (Lin-Sca1+c-Kit+ CD48-CD150+), ST-HSC (Lin-Sca1+c-Kit+CD48-CD150-), and MPP (Lin-Sca1+c-Kit+ CD48+CD150-). The representative dot plot shows LT-HSC population. Graphs show means ± SEM; *P < 0.05.

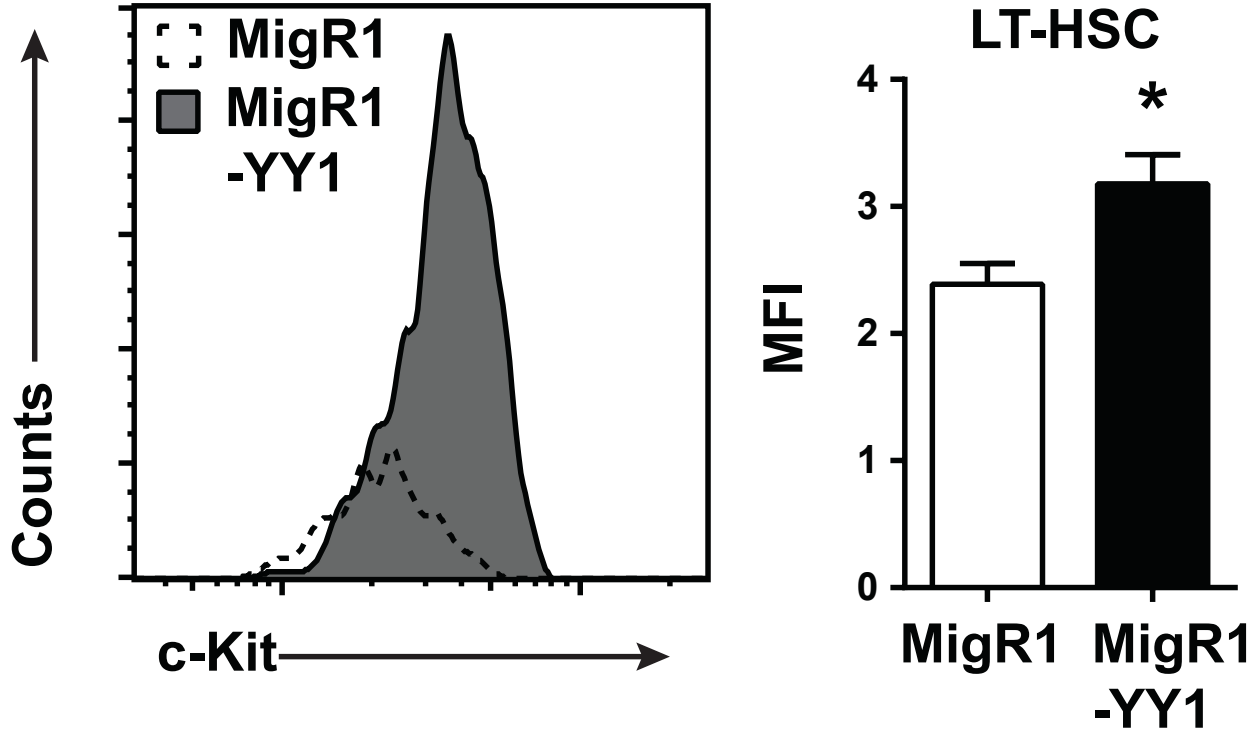

Figure S6, Related to Figure 7. Evaluation of c-Kit MFI in LT-HSC (Lin-Sca1+c-Kit+ CD48-CD150+) of mice with ectopic YY1 expression (MigR1-YY1) versus control (MigR1). Graphs show means ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001

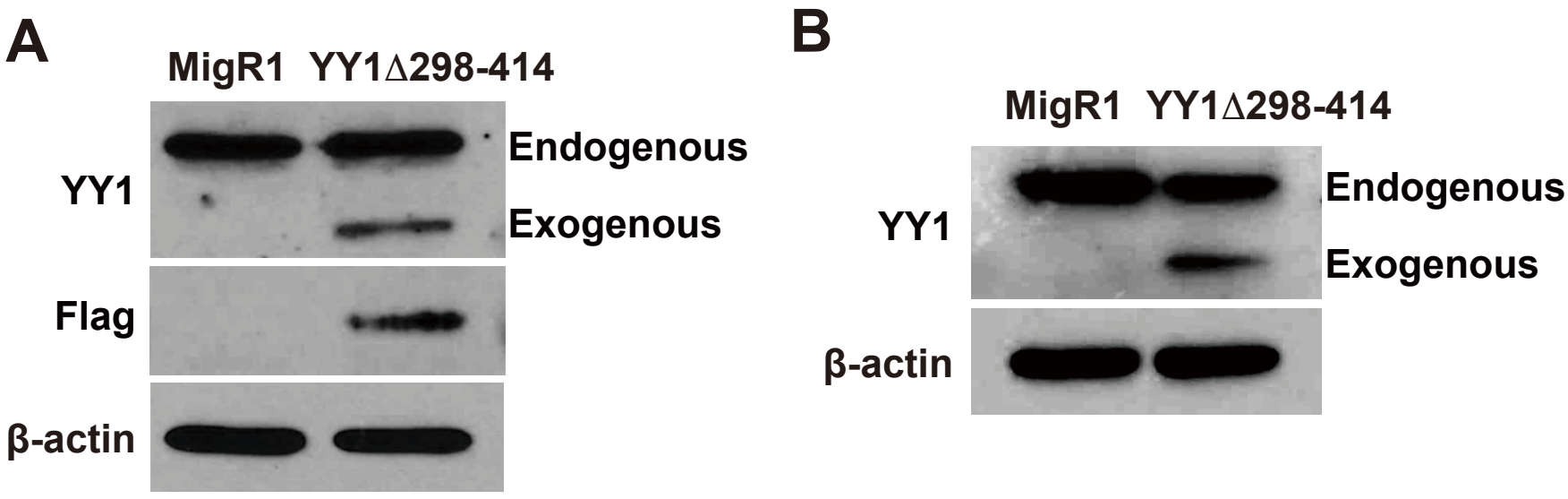

Figure S7, Related to Figure 7. YY1-Flag-YY1Δ298-414 was stably expressed in HPC7 cell and BMT mice. (A) Western blot of endogenous YY1 and exogenous Flag-YY1Δ298-414 expressions in HPC-7 cells infected with MigR1 or MigR1-Flag-YY1Δ298-414. (B) Western blot to detect endogenous YY1 and exogenous Flag-YY1Δ298-414 expressions in GFP+ peripheral blood lymphocytes. Before pI-pC injections, GFP+ peripheral blood were sorted from 4 mice transplanted with *Yy1*$^{+/+}$ + MigR1 or *Yy1*$^{+/+}$ + YY1Δ298-414 at 4 weeks post BMT.

Table S1: Primer List, Related to Experimental Procedures.

ChIP qRT-PCR analysis

	Forward	Reverse
-114.5	CTCCCTTCGATTCGCTCAAGA	CTGTTCTGAGATGCGGTTGC
-4	TCACAGATTGGTCTGCCTCTG	ATCCTCCAAAGGAGTTGGGGT
-0.06	CTGGGAGGAGGGCTGGAG	TGAGCTCCTCTCTCTGCTACA
+53.6	CTGCCTCTGAGCTGTGTTCTC	GAAACGCACTGGGGTTTTTCAA
+54	CGAGTTGTCCCTGAAGGCAG	GGCCATGTAGACTACTTCAGACC

qRT-PCR primers for mRNA expression

	Forward	Reverse
Yy1	TCAGACCCTAAGCAACTGGCAGAA	TTGAGCTCTCAACGAACGCTTTGC
Gata2	GCAGAGAAGCAAGGCTCGC	CAGTTGACACACTCCCGGC
Kit	AGTGGACGTACAGGTCCAGAA	GCCTGGATTTGCTCTTTGTTGT
Runx1	TCACTGGCGCTGCAACAA	TCTGCCGAGTAGTTTTCATCGTT
Smc1a	TTACCGCCATCATTGGACCC	GCTCCATGTATCAGGTCCCG
Smc3	ATCAACCAAATGGCAACGGC	TGCAGGCGCTCTTCAATGTA
Smc4	CAGGTACAATGAGTGGCGGT	GTCTCGTTCACTGTGCCGTA
Ncapg	GACTCCTGTGAGGGATGGAAA	TGTTTTGGCTCGTCTGGGTAA
Lcn2	TTTGTTCCAAGCTCCAGGGC	ACTGGTTGTAGTCCGTGGTG
Socs2	ACCGACTAACCTGCGGATTG	GCAGAGTGGGTGCTGATGTA
P53	GGCGTAAACGCTTCGAGATG	CAGTTTGGGCTTTCCTCCTTGA
Hlf	ATGACAAGTACTGGGCGAGG	TGTTCTTGCATTTGCCCAGC
Gapdh	TCCTGCACCACCAACTGCTT	GTCTTCTGGGTGGCAGTGAT

Supplemental References:

Croft, D., Mundo, A.F., Haw, R., Milacic, M., Weiser, J., Wu, G., Caudy, M., Garapati, P., Gillespie, M., Kamdar, M.R.*, et al.* (2014). The Reactome pathway knowledgebase. Nucleic acids research *42*, D472-477.
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