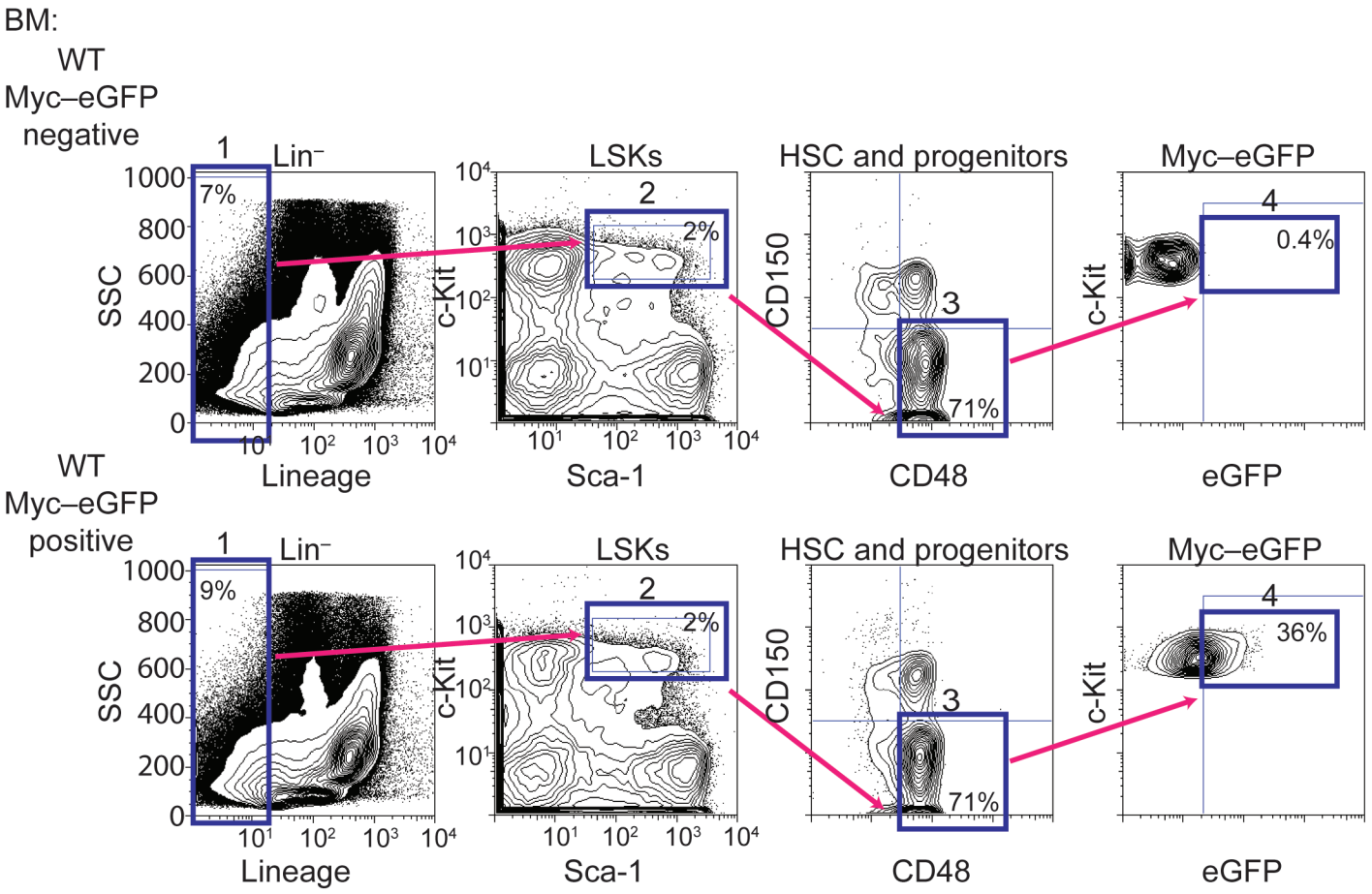
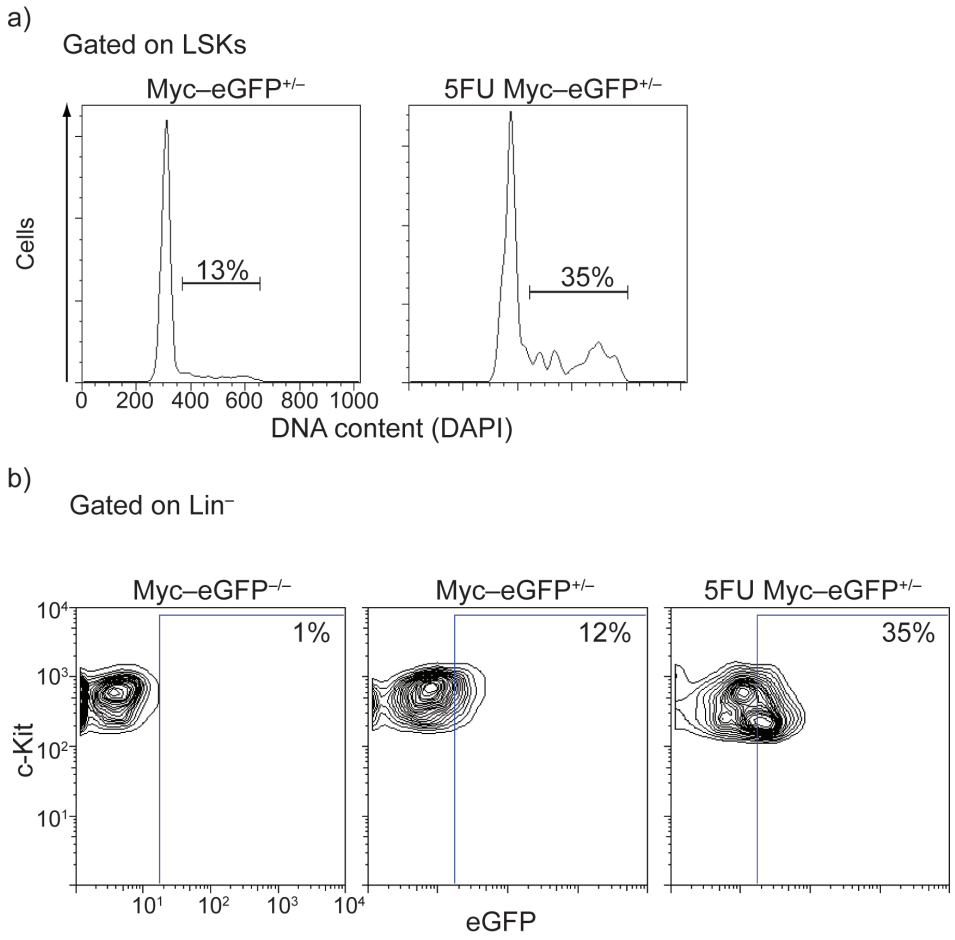
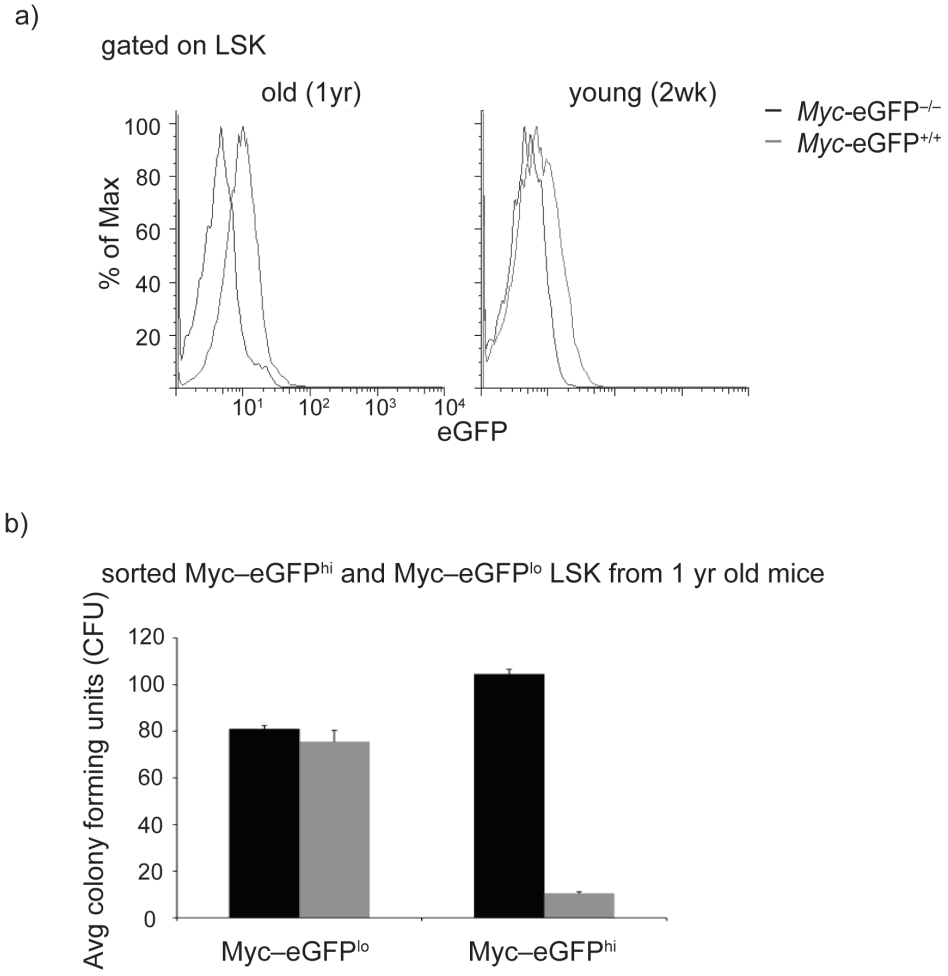


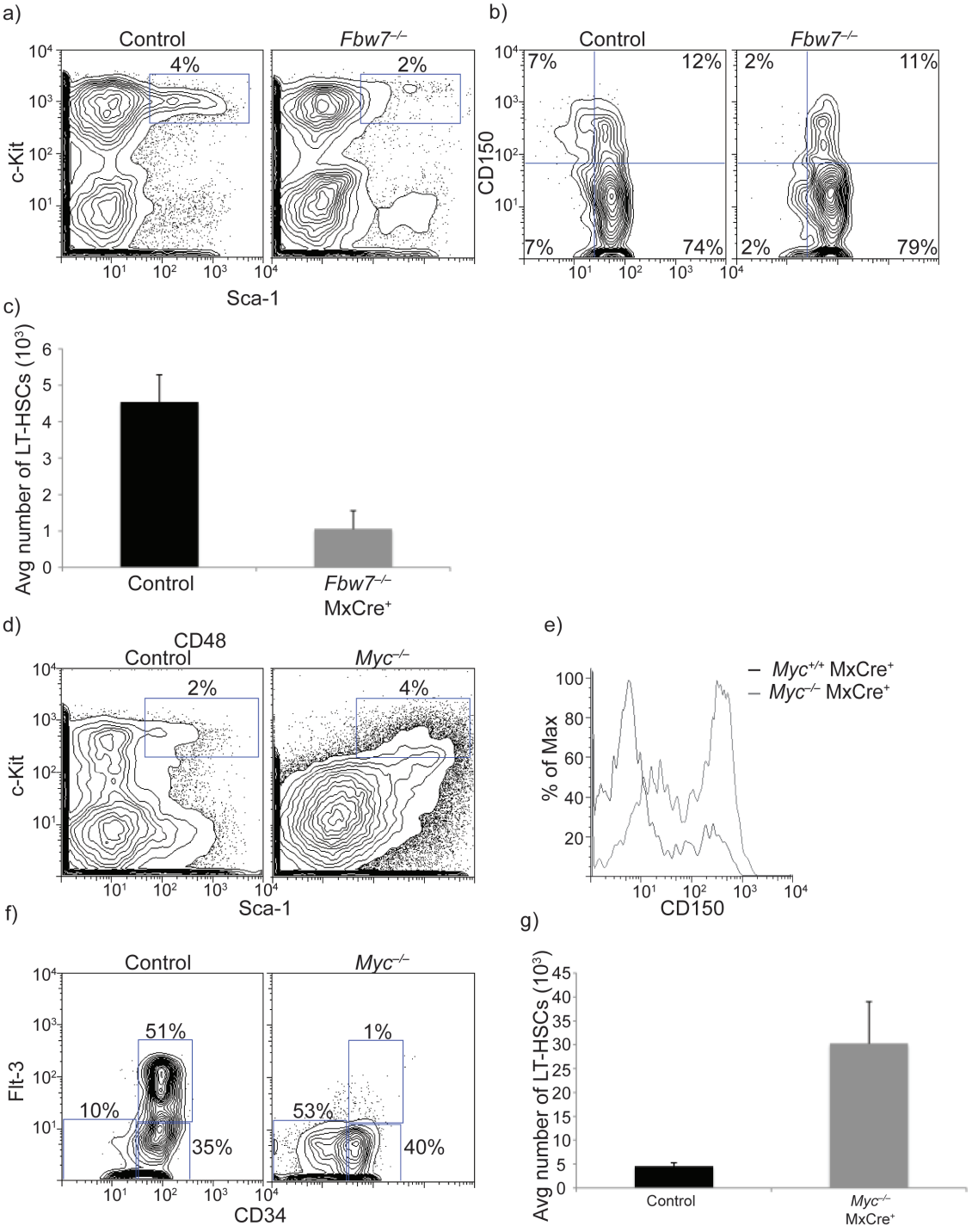
Supplemental Figure 1. Gating scheme and FMO control to identify true GFP positive populations. Diagram is a representation of gating scheme used to identify c-Myc-eGFP positive cells from the KI mouse. For each experiment evaluating the c-Myc-eGFP KI mouse, a c-Myc-eGFP negative littermate control was used to identify the appropriate GFP positive population (FMO control).



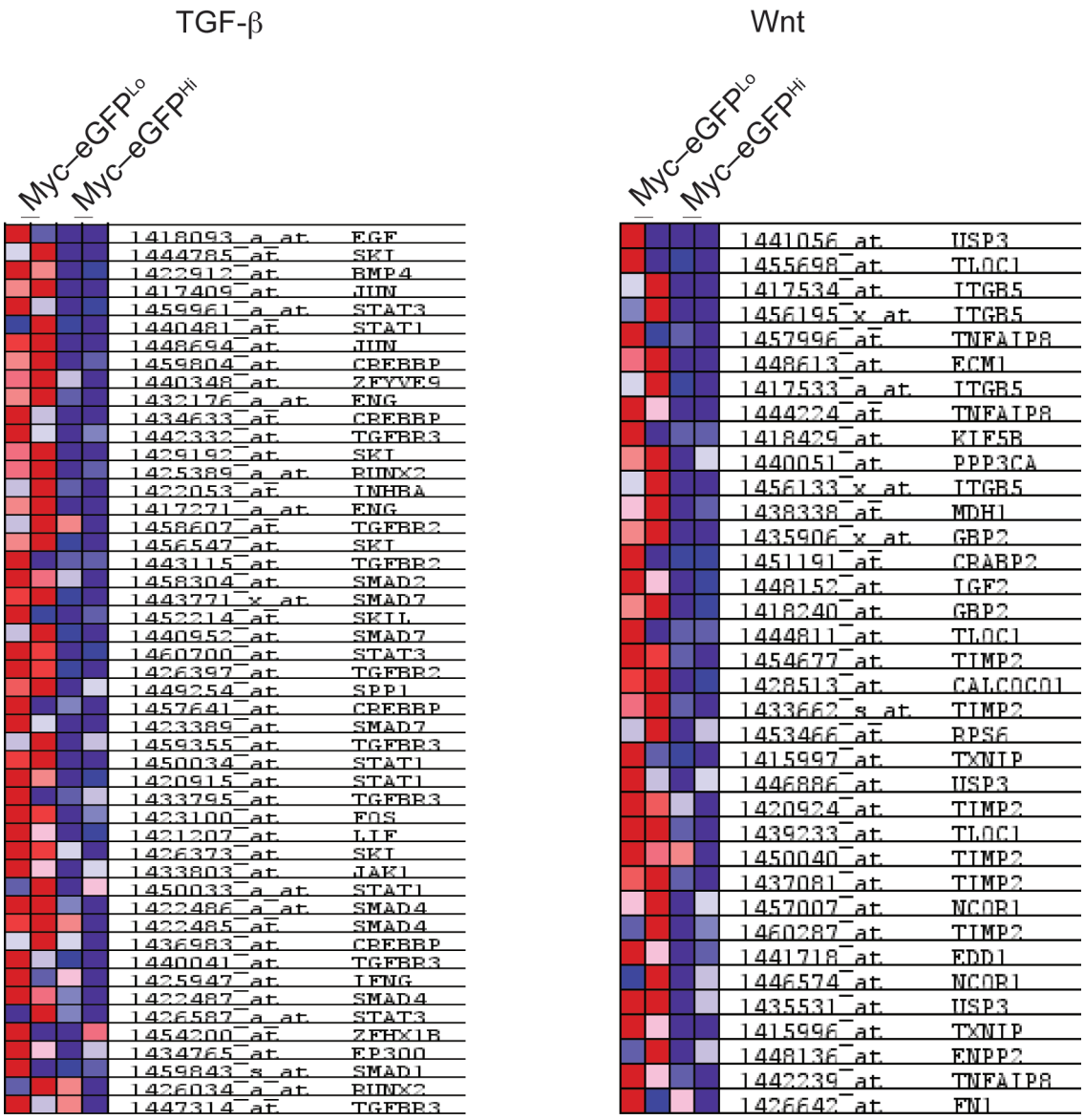
Supplemental Figure 2. 5-FU treatment increases the levels of c-Myc protein expression. a) 5-FU treatment leads to increased rates of cell cycle entry of LSK cells. DAPI staining of LSK cells 2 days after 5-FU treatment. b) Levels of c-Myc protein expression in LSK cells 2 days post 5-FU treatment. The gate was set using WT c-Myc-eGFP neagitve cells. Plots are representative of 3 independent experiments.



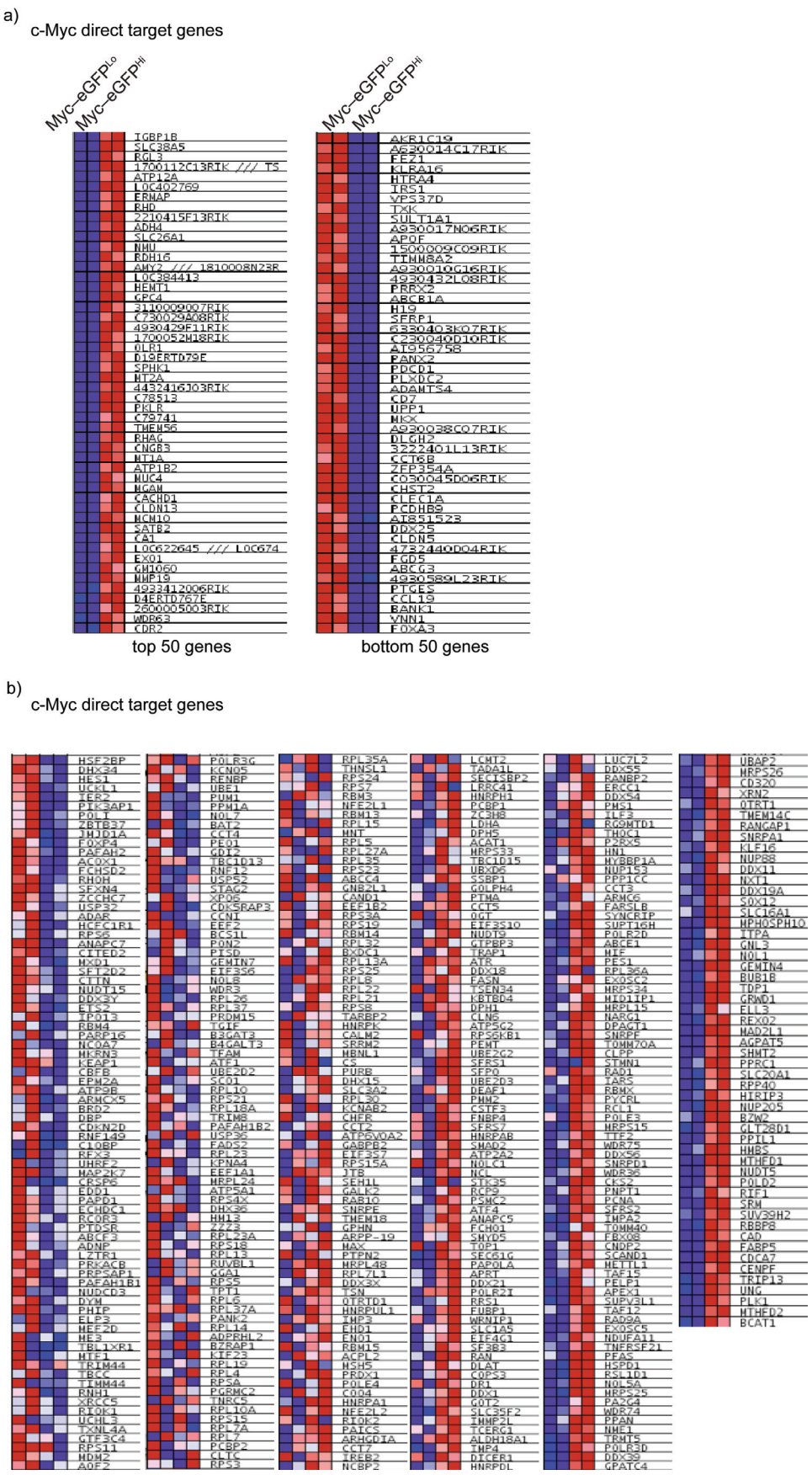
Supplemental Figure 3. Visualization of c-Myc protein abundance in "young" and "old" mice. a) Overlay histograms depicting levels of c-Myc-eGFP abundance in both young and old LSK subsets within the bone marrow. b) Methylcellulose in vitro cultures using sorted LSKs from "old" mice showing loss of self-renewal capacity in c-Myc-eGFPHi cells upon second plating yielding similar results as observed in 4-6wk old adult mice. Black: first plating, Grey: second plating Error bars indicate standard deviation (std) of n=3 mice.



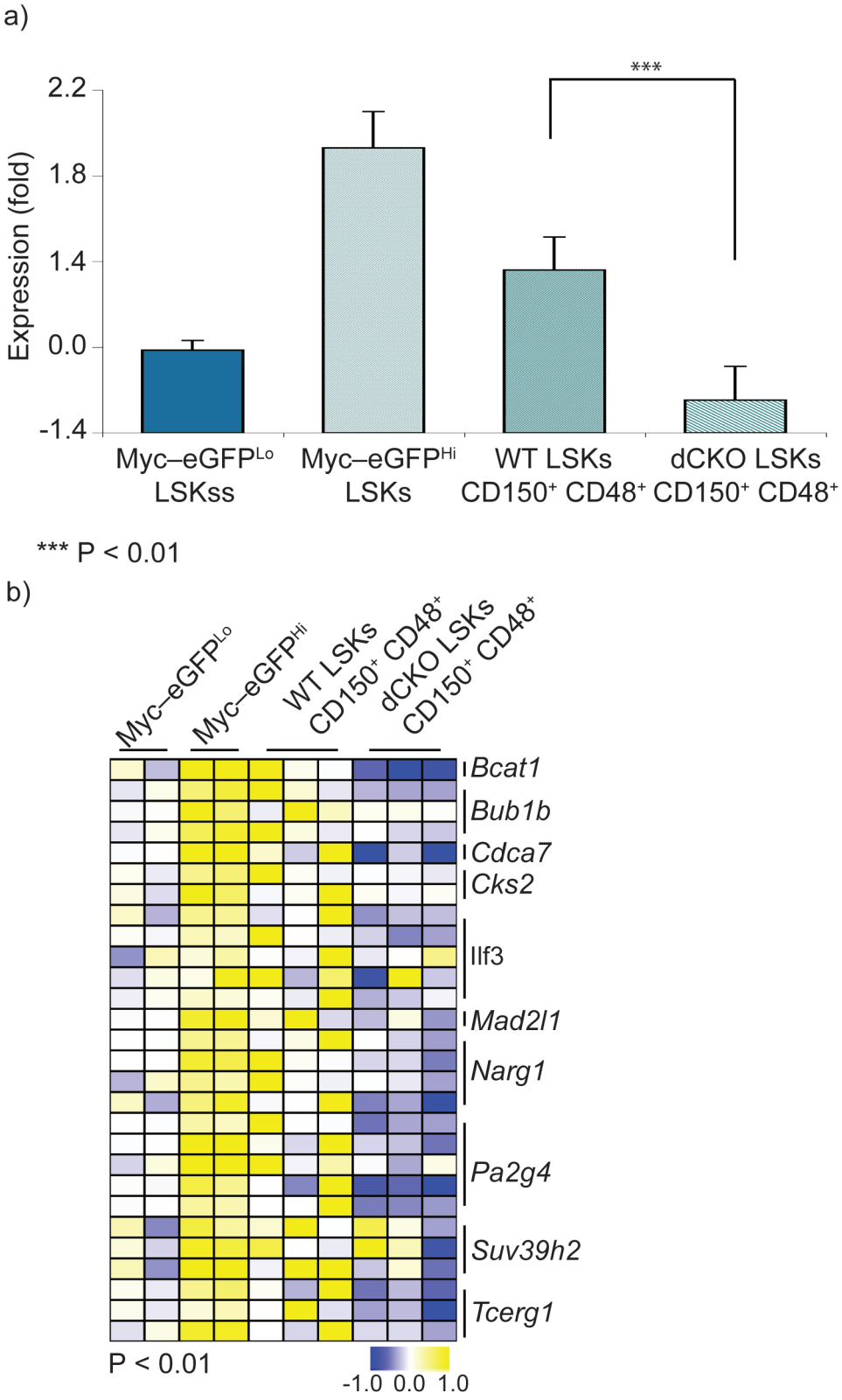
Supplemental Figure 4. Deletion of Fbw7 and Myc show opposing effects on HSC. a) FACS plot of LSK profile in control and Fbw7–/– mice. b-c) Fbw7 deletion leads to reduction in percentage (b) and total cell number (c) of LT-HSC population determined by FACS analysis using SLAM markers CD150 and CD48. Black: LT-HSC from control mice, in Grey: LT-HSCs from Fbw7–/– mice. d) FACS plot of control and Myc–/– LSK profiles. e-g) overlay and FACs plot of CD150+, CD34–, and FLT-3– LT-HSCs showing an increase the percentage (e-f) and total cell numbers (g) in Myc–/– mice. In Black: LT-HSC from control mice, in Grey: LT-HSCs from Myc–/– mice. Error bars indicate standard deviation (std) (n=5 mice). For all genotypes demonstrated n=5 mice. All analyses were performed two weeks post polyI-polyC-mediated gene deletion. Plots are representative of 3 independent experiments.



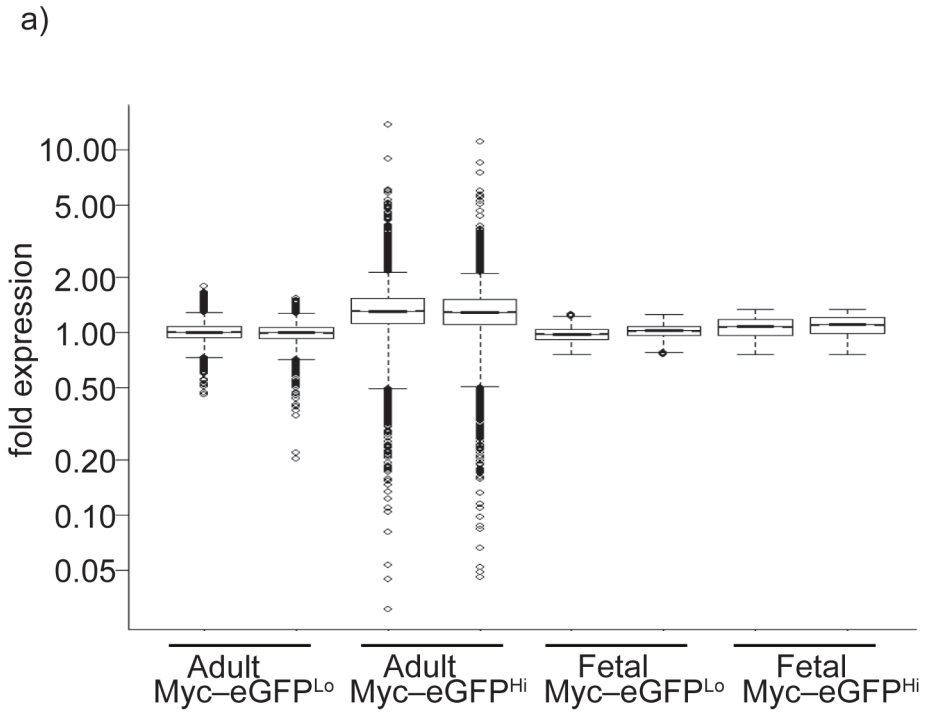
Supplemental Figure 5. Correlation between the c-Myc-eGFPLo fraction and the TGF-β and Wnt pathways. "Heat map" depicting upregulation of TGF-β and Wnt pathway members in the c-Myc-EGFPLo fraction. $P < 0.001$. Red: Up-regulation, Blue: Down-regulation.



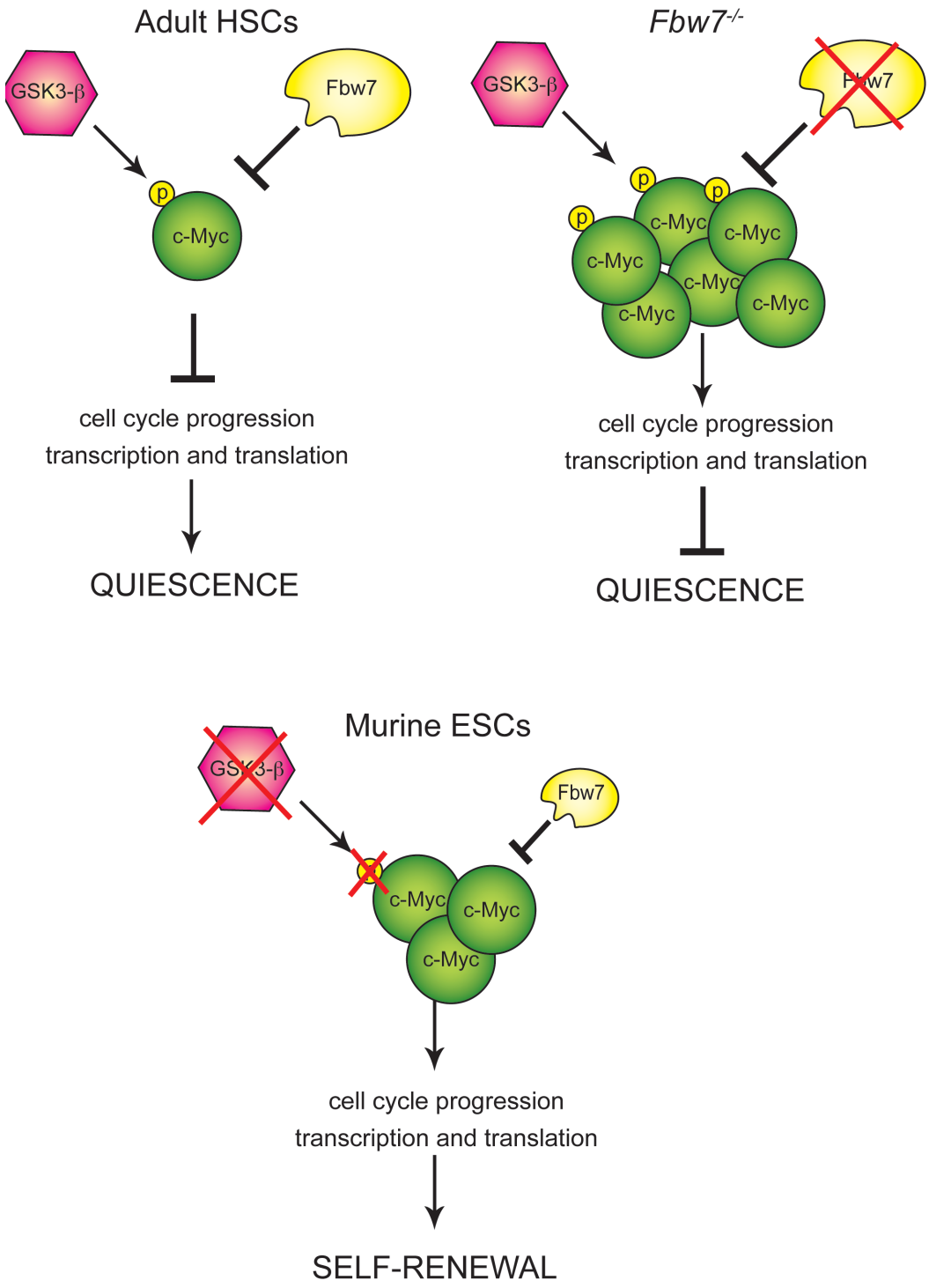
Supplemental Figure 6. Identification of direct c-Myc targets using ChIP2. a) "Heat map" depicting expression of the top and bottom 50 direct c-Myc target genes in the c-Myc-eGFPLo and c-Myc-eGFPHi subsets. b) "Heat map" showing gene expression profiles of c-Myc direct targets in the c-Myc-eGFPLo versus c-Myc-eGFPHi subsets (GSEA). A significant enrichment (P =0.05; NES=-1.98) of c-myc direct targets is present in the c-Myc-eGFPHi over-expressed genes.



Supplemental Figure 7. Direct regulation of HSC gene expression by c-Myc. a) Mean expression of all probe sets for the 10 "hand picked" validated genes in c-MyceGFPHi, c-Myc-eGFPLo, and Laurenti et al16. c- and N-Myc double knock out gene expression array. b) "Heat map" demonstrating gene expression of all probe sets for 10 "hand picked" validated genes in c-Myc-eGFPHi, c-Myc-eGFPLo, and Laurenti et al16 c- and N-Myc double knock out gene expression array.



Supplemental Figure 8. Absence of a distinct gene expression signature in fetal c-Myc-eGFPLo and c-Myc-eGFPHi LSKs. Box-Plot demonstrating the relative distribution of normalized gene expression (3865 genes) in the indicated bone marrow adult and fetal liver stem cell/progenitor populations separated based on levels of c-Myc protein expression. Black line: Median expression; Open Circle: outliers.



Supplemental Figure 9. Proposed schematic of the role of the Fbw7:c-Myc interaction in adult and embryonic stem cells.

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
Bcat1	GGGTGCAAATGTGAGTCTCC	AGTCGCTGCTGATGCAATCC
Cdca7	ACTCGCCGCTGGCCATCCT	AGGGGACGCAGAGCAAAAG
Dis3	AACGTCTTGGACCTGAGCATCTCGCCTCGC	GGGAGGAAACAACCAACACTCGCCCAATC
Ilf3	CCTGTCACGTGATGGAAGC	AGTAGGCGGGTGAGACGAA
Ipo7	CGGAGACTTGACAGGAAGTGGCCGGAGCAT	CCCGGAGGGCCTCGATGATGGTGTTGGGAT
Narg1	AAAAGAGGCGAAGGGAGTGT	GCATGGTTCCGGCTCCTC
Nme1	CAACAGCGGACTGGAAAGAGAATAAGGCAT	GGAGGGAGGTGGGCGTTCACTAAAG
Pa2g4	AAACCGGAGCAGTGGTCATA	TCCCCACTTCCTCTCCTACC
Rad9	CCTCCGCTTCCTTATTGGTT	GAGGAAGGACACAGGAGCAA
Rpl19	CGCGACTTAGCGTGACTTC	AAATGGTTCAGTCCGACTCC
Rps28	TTCTAAACACCCGCAGTCG	AAAGTGAGGCGTGGTCAGAG
Suv39h2	ACTCCACACCACCAGGACTC	TGCTACATGGCCACATCAAC
Tcerg1	TCTCGGCTTGCGCTATTAGT	GCTAGGTCCTCCCAAGTTCC
ActB	CCCTACAGTGCTGTGGGTTT	GCAAGGAGTGCAAGAACACA

Supplemental Table 1. Sequences of primers used in the ESC and mouse bone marrow progenitor ChIP experiments.

Genotyping primers:

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
Fbw7	ATTGATACAAACTGGAGACGAGG	ATAGTAATCCTCCTGCCTTGGC
Myc	TAAGAAGTTGCTATTTTGGC	TTTTCTTTCCGATTGCTGAC
Myc-eGFP	TTCAGGATTGGGGTACGC	GAAATTCTCTTCCTCGTCGC
MxCre	GCCTGCATTACCGGTCGATGCAACGA	GTGGCAGATGGCGCGGCAACACCATT

Quantitative real-time PCR primers:

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
Fbw7	GTGATAGAGCCCCAGTTCCA	CCTCAGCCAAAATTCTCCAG
Myc	CTTCTCTCCTTCCTCGGACTC	GGAGATGAGCCCGACTCCGACCTC
ActB	AGGTGACAGCATTGCTTCTG	GGGAGACCAAAGCCTTCATA

Supplemental Table 2: Primer sequences used for genotyping mice and quantitative real-time PCR experiments.

Fbw7.shRNA.mus.For1
CCGGCCAGTCATTAACGAGTGGAATCTCGAGATTCCACTCGTTAATGACTGGTTTTTG
Fbw7.shRNA.mus.Rev1
AATTCAAAAACCAGTCATTAACGAGTGGAATCTCGAGATTCCACTCGTTAATGACTGG
Fbw7.shRNA.mus.For2
CCGGCGGAGGATTACATCTGTCCAACTCGAGTTGGACAGATGTAATCCTCCGTTTTTG
Fbw7.shRNA.mus.Rev2
AATTCAAAAACGGAGGATTACATCTGTCCAACTCGAGTTGGACAGATGTAATCCT

Supplemental Table 3: Sequences of Fbw7 shRNAs.