

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.

a)



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.

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		EGF
	1444785_at	SKI
	1422912 at	
	1417409_At	STATS
	1440481 at	STAT1
	1448694 at	JUN
	1459804 at.	CREBBP
	1440348_at	<u>2 FYVE9</u>
	<u>1432176_a_at</u>	ENG
	1434633_at	CREBBP
	1442332_at	TGFBR3
	1429192 at	
	1422053 at	INHBA
	1417271 a at	ENG
	1458607 at	TGFBB2
	1456547 ⁻ at	SKI
	1443115 at.	TGFBB2
	1458304_at	SMAD2
	<u>1443771 x_at</u>	SMAD7
	1452214_at	SKIL
	1440952_at	SMAD7 CTATO
	1426207_at	TCEPPD2
	1449254 at	SPP1
	1457641 at	CREBBP
	1423389 at	SMAD7
	1459355 at	TGFBR3
	1450034_at.	STAT1
	1420915_at	STAT1
	1433795_at	TGFBR3
	1423100 at	
	1426272 at	SUT
	1433803_at	JARI
	1450033 a at	STAT1
	1422486 a at	SMAD4
	1422485 at	SMAD4
	1436983_at	CREBBP
	1440041_at.	TGFBR3
	1425947_at	I FNG
	1422487_at	SMAD4
	1454200 at	
	1434765_at	<u></u>
	1459843 s at	SMAD1
	1426034 a at	BUINX2
	1447314 at	TGFBB3

NNC NNC ECTPH 1441056 at USP3 1455698_at. 1417534_at TLOC1 ITGB5 <u>1456195 x at</u> ITGB5 1457996 at TNFAIPS 1448613 at ECM1 1417533_a_at ITGB5 1444224_at 1418429_at TNFAIPS KIF5B 1440051 at PPP3CA <u>1456133 x_at</u> ITGB5 1438338 at. MDH1 <u>1435906_x_at</u> GBP2 1451191_at. 1448152_at. CRABP2 IGF2 1418240⁻at GBP2 1444811_at. TLOC1 <u>1454677_at</u> TIMP2 <u>1428513_at</u> CALCOCOL 1433662_s_at 1453466_at 1415997_at TIMP2 RPS6 TXNIP <u>1446886</u>at USP3 <u>1420924_at</u> TIMP2 <u>1439233 at</u> TLOC1 TIMP2 <u>1450040_at</u> <u>1437081_at</u> TIMP2 1457007⁻at NCOR1 1460287⁻at TIMP2 <u>1441718_at</u> EDD1 1446574 at NCOR1 1435531_at USP3 1415996_at TXNIP 1448136_at ENPP2 1442239⁻at TNFAIPS 1426642 at. FN1

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.

Wnt



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
llf3	CCTGTCACGTGATGGAAGC	AGTAGGCGGGTGAGACGAA
lpo7	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
Мус	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	CTTCTCCTTCCTCGGACTC	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.