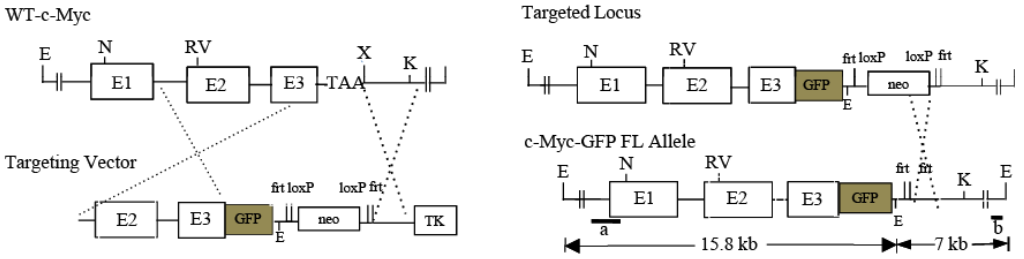
Supplemental Figure

Supplementary Figure S1

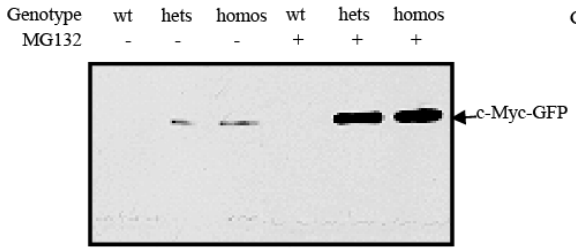
A



B

Constructs	Colonies formation
w/o DNA	0
pBw1549 vector for vRas	0
pEGFP	0
pd1-EGFP	0
pBW 1423 (vRas)	2
c-Myc-EGFP	0
c-Myc-pd1-EGFP	0
c-Myc-Stop-dp1-EGFP	1
pBW1423+pEGFP	0
pBW1423+pd1-EGFP	0
pBW1423+c-Myc-EGFP	16
pBW1423+c-Myc-pd1-EGFP	42
pBW1423+c-Myc-stop-pd1-EGFP	34

C



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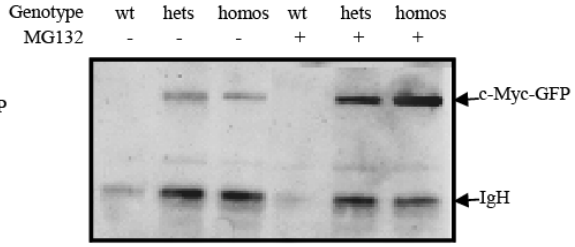

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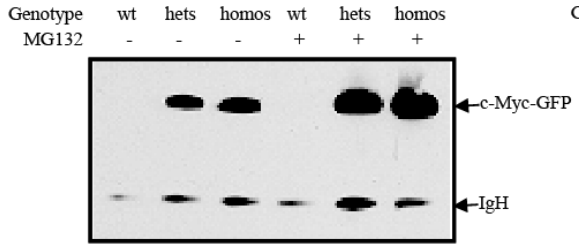

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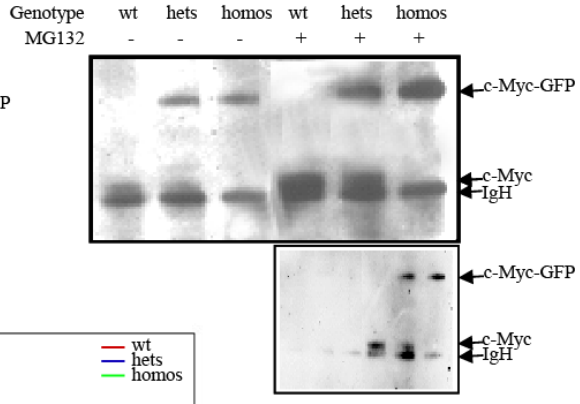

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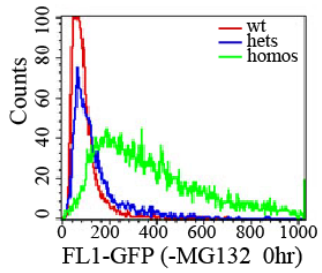

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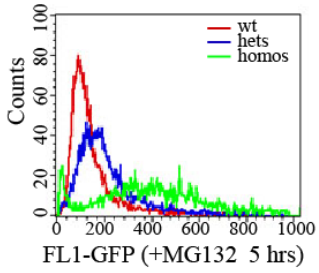

Supplementary Figure S1: Myc-EGFP knock-in and native Myc have similar biological properties

(A) Schematic map of wild-type c-Myc locus (left top), targeting vector (left bottom), targeted locus (right top) and c-Myc-GFP full length allele (right bottom). Boxes E1, E2 and E3 indicate c-Myc exons; boxes neo, GFP and TK represent the neomycin resistance cassette, the pd4-EGFP expression cassette and Herpes simplex virus thymidine kinase cassettes, respectively. The letters E, K, N, RV, X are the EcoRI, KpnI, NotI, EcoRV and xhoI restriction enzyme sites. frt, and *lox*p sites, the 5' and 3' probes a and b are also indicated.

(B) Chimeric c-Myc-EGFP construct mimics c-Myc to cooperate with RAS to transform cells Myc-EGFP, Myc-pd1EGFP, Myc-pd1-stop-EGFP are human c-Myc fused with EGFP, destabilized-EGFP (one-hour half-life) and Myc with pd1-EGFP inserted after the former's stop codon. pBW1423 is a vRAS expression vector pBw1549 is the empty vector into which RAS was inserted.

(C-F) Expression of c-Myc –EGFP vs. c-Myc protein in mouse embryo fibroblasts (MEFs) from c-Myc-EGFP knock-in homo- and heterozygous mice. Immunoprecipitation (IP) and Western blots of endogenous c-Myc and c-Myc-EGFP from wild-type (wt), heterozygous (hets) and homozygous (homos) MEF cell lysates. Cells were untreated (left three lanes in each panel) or treated with MG132 to inhibit degradation (right three lanes). For IP, anti-GFP was used in C and D, and anti-c-Myc was used in E and F. For immunoblot detection, anti GFP was used in C and E and anti c-Myc was used in D and F.

(G and H) Flow cytometric analysis of c-Myc-EGFP in wild-type (wt), (red), heterozygous (hets), (blue), and homozygous (homos), (green) MEF cells. Serum starved and re-stimulated cells were untreated with MG132 (E), and treated with MG132 for 5 hours (F). Because the het peak is unimodal between the wt and the homos, c-Myc expression is bi-allelic.

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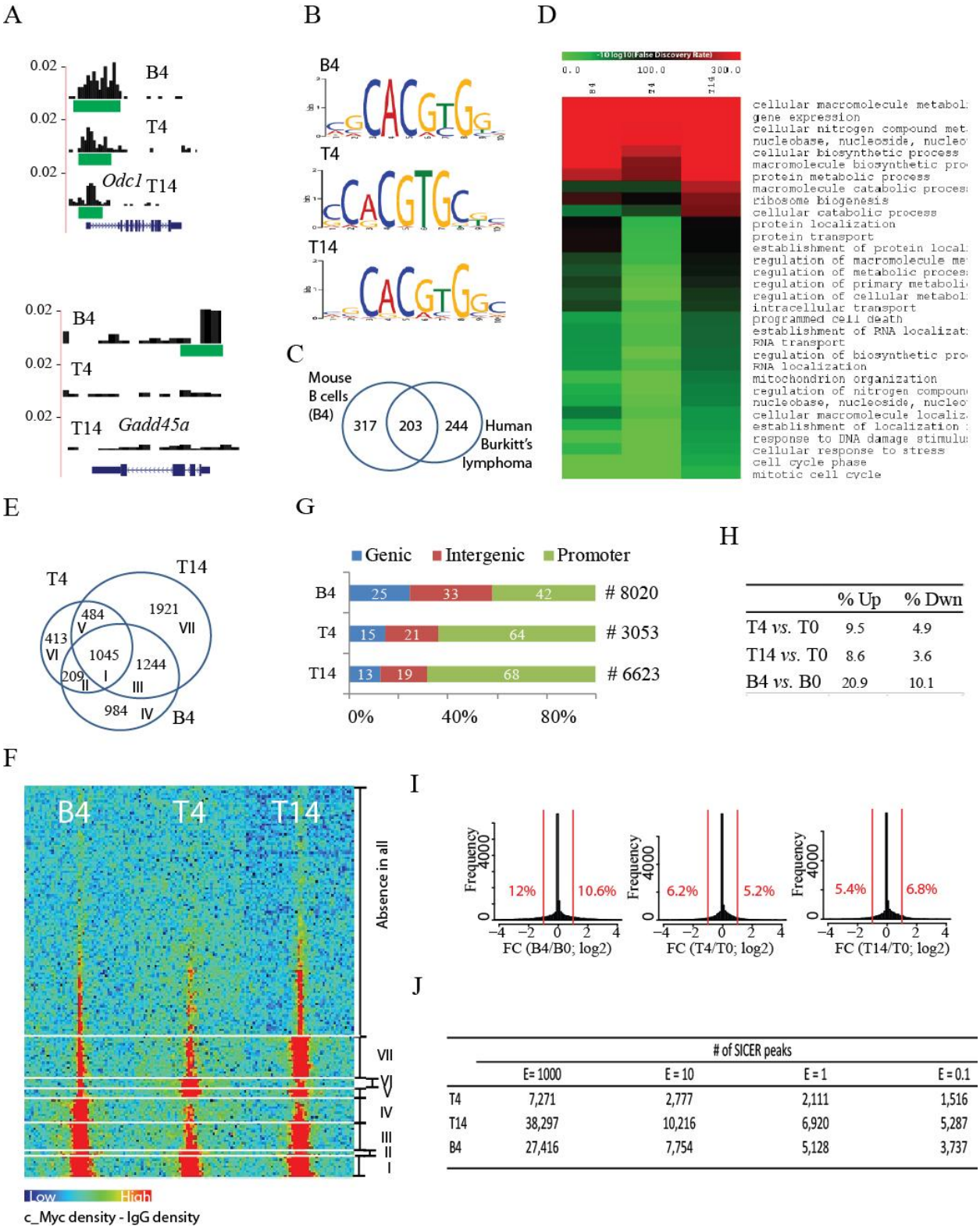
Supplemental Figure

Supplementary Figure S2



Supplementary Figure S2: Myc target genes

(A) Examples of Myc binding for genes expressed in activated B cells (4 hrs; B4) and T cells (4 hrs & 14 hrs; T4 & T14). The regions with Myc tag enrichments are highlighted by green bars. Y-axis shows the normalized ChIP-seq tag densities for Myc.

(B) E-box motifs found at c-Myc tag enriched peaks by MEME (Bailey et al. 2006).

(C) Li et al. (2003) identified 15% of ~4000 genes in human Burkitt lymphoma cells Myc targets ChIP-ChIP. Filtering for homologous mouse-human gene pairs by Gene Symbols yielded 2309 of the 4000 genes. With stringent parameters for peak calling, out of the 2309 genes, our study and Li et al. (2003) identified 447 targets and 520 targets for mouse B4 cells and human lymphoma cells, respectively. The Venn diagram shows that the two sets share 203 targets. A GO analysis shows that the common targets are enriched in house-keeping functions such as translation (13.8%; FDR=2E-11).

(D) GO term analysis of Myc targets in activated B cells (4 hrs; B4) and T cells (4 hrs & 14 hrs; T4 & T14). The false discovery rates (FDRs) of the enrichment are indicated by color (red: low, green: high). Myc targets are defined by genes with Myc peaks at promoters.

(E) Venn diagram of Myc targeted genes among B cells and T cells. The targets are defined by the enrichment of Myc binding tags at promoters (± 2K bps of TSS).

(F) Heatmaps for normalized c-Myc tag densities (against IgG) in a region +/- 5K bps around TSS (100 windows of 50-bp) for B4, T4 and T14 cells. Genes are first sorted by the sectors specified in the venn diagram (panel E) and within each sector are then sorted into bins of 100 genes based on average expression level from B4, T4 and T14. Shown are the average tag densities within each bin. Genes that are not identified as c-Myc target in any condition are included for completeness (denoted as "absence in all").

(G) Genomic distribution of Myc binding sites. The % of Myc binding sites in promoters, intragenic and intergenic regions are shown in accumulated bar graphs for B4, T4 and T14 cells, respectively. The total numbers of Myc peaks are shown on the right.

(H) The % of up-regulated (up) and down-regulated (dwn) genes among Myc targets during B cell and T cell activations normalized for RNA (p-value < 10-5 & FC > 2).

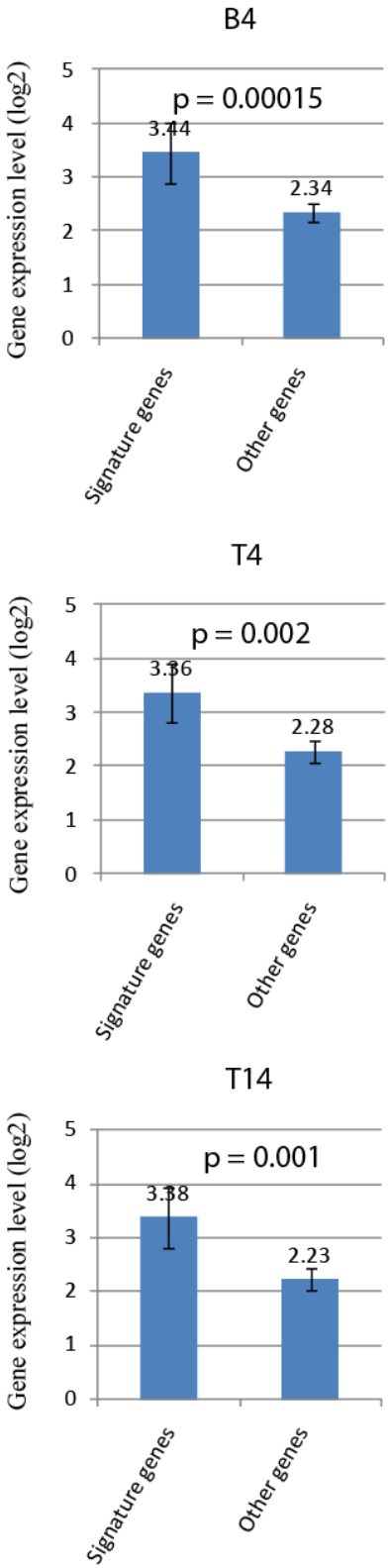
(I) Histogram of fold change of gene expression over all genes during B/T cell activation. Changes over 2-fold are indicated red vertical lines.

(J) Number of Myc tag enriched regions returned by SICER (window size = 200, window gap disabled) under different settings of E-value.

Supplemental Figure

Supplementary Figure S3

A



B

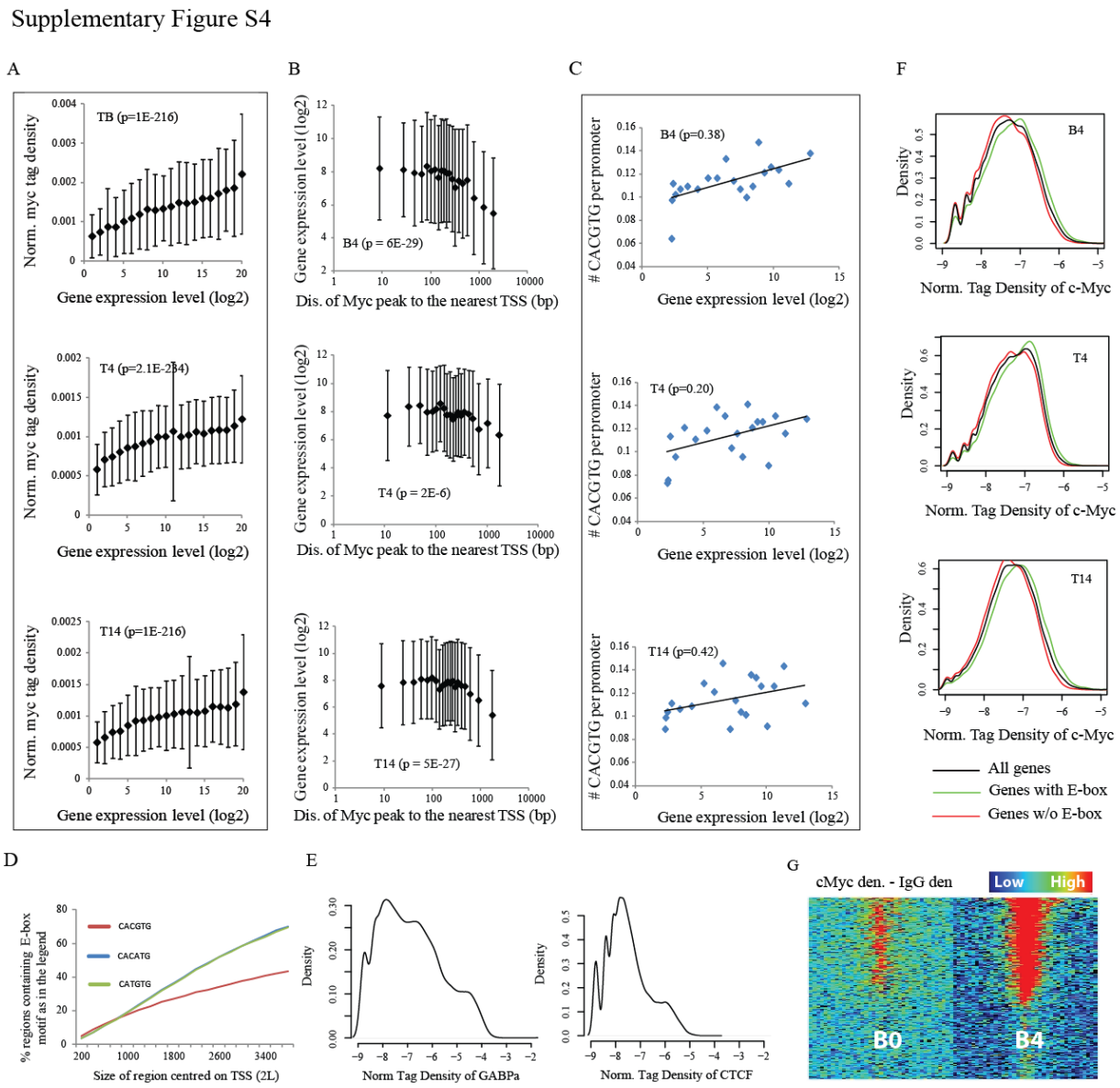
	B4	T4	T14
ADSL	1	1	1
CDK4	1	1	1
DHX15	1	1	1
EIF3B	1	1	1
ETNK1	1	1	1
GNL3	1	1	1
HSPA9	1	1	1
NOLC1	1	1	1
NOP56	1	1	1
PHB2	1	1	1
POLD2	1	1	1
PSME3	1	1	1
PUF60	1	1	1
SLC38A2	1	1	1
SNW1	1	1	1
SRPK1	1	1	1
ST13	1	1	1
UCK2	1	1	1
UNG	1	1	1
CSTF3	0	1	1
DNAJC2	0	1	1
ENO1	0	1	1
GSPT1	0	1	1
IMPDH2	0	1	1
TRAP1	0	1	1
ADSS	1	0	1
AKAP10	1	0	1
BAG1	1	0	1
CD164	1	0	1
CDC123	1	0	1
EIF4G1	1	0	1
FBL	1	0	1
FBXO45	1	0	1
GLS	1	0	1
HMGB1	1	0	1
HSP90B1	1	0	1
NPM1	1	0	1
NPM3	1	0	1
ROCK1	1	0	1
SCARB1	1	0	1
TFAM	1	0	1
WHSC1	1	0	1
GMPS	0	0	1
SRP72	0	0	1
THOP1	0	1	0
CYCS	1	0	0
MFSD8	0	0	0
MNAT1	0	0	0
SKIV2L2	0	0	0
TUBA1A	0	0	0

Supplementary Figure S3: Myc signature genes

(A) Microarray gene expressions levels of Myc signatures are generally higher than that of overall genes. P-values are calculated by *t*-test.

(B) Presence (1) and absence (0) of Myc peaks at the promoters of Myc signature genes.

Supplemental Figure



Supplementary Figure S4: Association of Myc binding with gene expression level.

(A) Myc binding levels at promoters are strongly correlated with gene expression levels. Genes were sorted into 20 equal size bins based on expression level. The average of c-Myc tag densities at promoters and that of gene expression levels are shown for each bin. To better show the standard deviation, the x-axis is attributed as category, rather than the actual expression level as in Fig H4. The significance of the correlation is quantified by one-way ANOVA**.**

(B) MYC is most closely associated with high promoter output if bound within ~250 bp of the TSS. Myc Targets were sorted into 20 equal-size bins based on the distance of TSS to the nearest peak of Myc binding. Shown is the average of expression level of genes for each bin. The significance of the correlation is quantified by one-way ANOVA.

(C) Highly expressed MYC target genes also tended to be associated with MYC-bound E-boxes. Myc Targets were sorted into 20 equal-size bins based on expression level. Shown are the average expression levels of genes and # of E-box per gene for each bin. Linear regression is shown with a line. The significance of the correlation is quantified by one-way ANOVA.

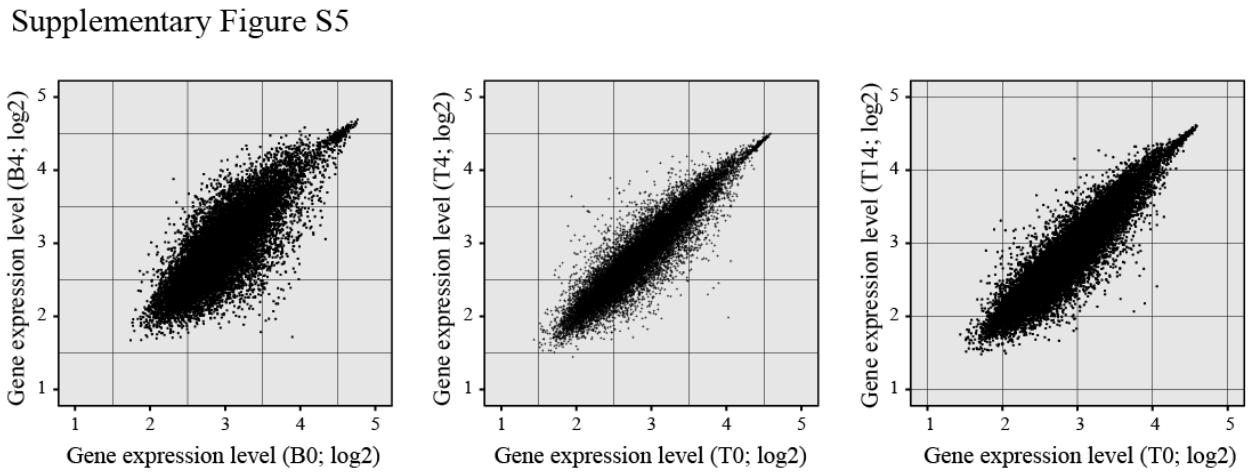
(D) % of genomic regions that contain canonical E-box (CACGTG) or non-canonical E-box (CATGTG or CACATG). The regions are centered at TSS, spanning *L* bp upstream and *L* bp downstream. The % is calculated for each *L*, ranging from 100bp to 2000 bp, at a step of 100 bp.

(E) Normalized ChIP-seq tag densities of GABP-α (human hematopoietic stem cells) and CTCF (mouse ES cells) were calculated from promoter regions of all genes (TSS+/-2Kbp). Kernel density estimates for each point of tag density are calculated by the density function R.

(F) Normalized tag densities of Myc are calculated for the promoters (TSS+/-2Kbp) of all genes, genes with or without canonical E-box (CACGTG) within 2K bps of TSS. Then kernel density estimates for each point of tag density were calculated by the density function from R. The comparisons were made independently for cells of B4 (left panel), T4 (middle panel) and T14 (right panel).

(G) Heatmap for normalized c-Myc tag densities (against IgG) in a region +/- 2K bps around TSS (40 windows of 50-bp) in resting B cells (B0). Genes are sorted into 200 bins based on gene expression levels in B0 from high to low. Shown are the average tag densities within each bin. The average c-Myc densities from B4 cells for the same bins are shown side-by-side for comparison.

Supplemental Figure

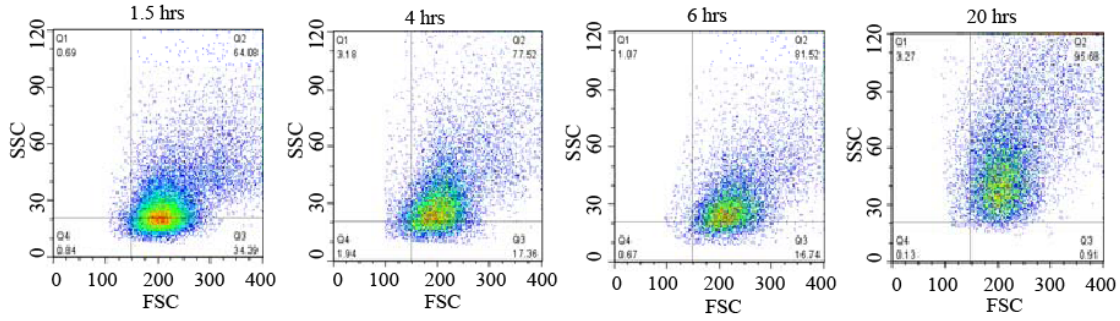


Supplementary Figure S5: Scatter plot of gene expression values for cells of B0 vs B4 (left panel), T0 (middle panel) vs T4 and T0 vs T14 (right panel)

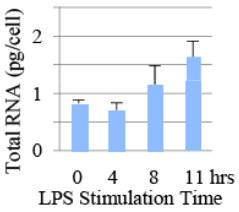
Supplemental Figure

Supplementary Figure S6

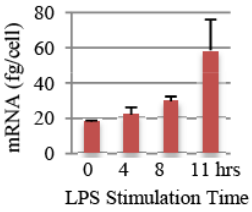
A



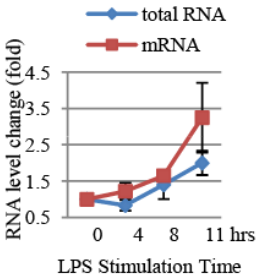
B



C



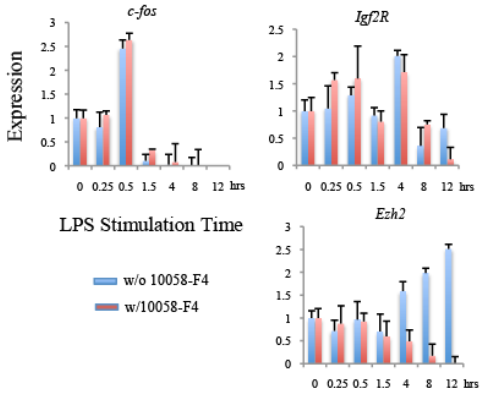
D



E

Gene name	Primer sets	Probe # and cat. No.
Loh12cr1	5'-CAACCTTCCAGCCCCTTT-3'	#60
	5'-CTAGCTTCTCCAGTTTGGCATT-3'	04688589001
Mecr	5'-GATCCAGCCAAGGTCGTC-3'	#1
	5'-TGGACGTCAGATCCTTCCA-3'	04684974001
SPg21	5'-ATGCCATTGATTTCATGGTG-3'	#19
	5'-GCCTTGAAGCCAGTTCACTT-3'	04686926001
Tsc2	5'-CGCAGCATCAGTGTATCTGAA-3'	#84
	5'-CGCTCGTAAGGGATGTCTGT-3'	04689089001
Qrich1	5'-AACAGATTGGCCCCCATT-3'	#82
	5'-AAAGGCCATAATTCAATTCAGC-3'	04689054001
Kif22	5'-TGAAGAAAGGCCCCAAAAG-3'	#78
	5'-TCCTCATCCTTTTCCACAGC-3'	04689011001
cdk7	5'-CACACATCAAGTTGTGACCAGA-3'	#92
	5'-CCCACATGTCTACTCCCACA-3'	04692098001
Rtn4	5'-TTTGCTCTTCCTGCTGCAT-3'	#82
	5'-TCCAGTACAGGAGGTCAACAAC-3'	04689054001
Lmf1	5'-CGCTGGCTGATCTTCAGAAT-3'	#20
	5'-GGGCTGAGTCTCATAGTGGAA-3'	04686934001
Hsd11b1	5'-TCTACAAATGAAGAGTTCAGACCAG-3'	#1
	5'-GCCCCAGTGACAATCACTTT-3'	04684974001
Bex1	5'-AGGAGAAGGCAAGGATAGGC-3'	#63
	5'-TTCTGATGGTATCTTGTGGCTTT-3'	04688627001
Igf2R	5'-CCTTCTCTAGTGGATTGTCAAGTG-3'	#33
	5'-AGGGCGCTCAAGTCATACTC-3'	04687663001
Bend3	5'-TCTCGTCCCAGTGGAATAGAA-3'	#13
	5'-GGGAGCAGTCCAAGGTAGC-3'	04685121001
Ezh2	5'-TGGAAGCAGCGGAGGATA-3'	#100
	5'-GTCACTGGTGACTGAACACTCC-3'	04692187001

F



Supplementary Figure S6: Activated cells increase in size and RNA content after Myc is activated. Cell size, total RNA and mRNA levels were followed during a time course of B-cell activation

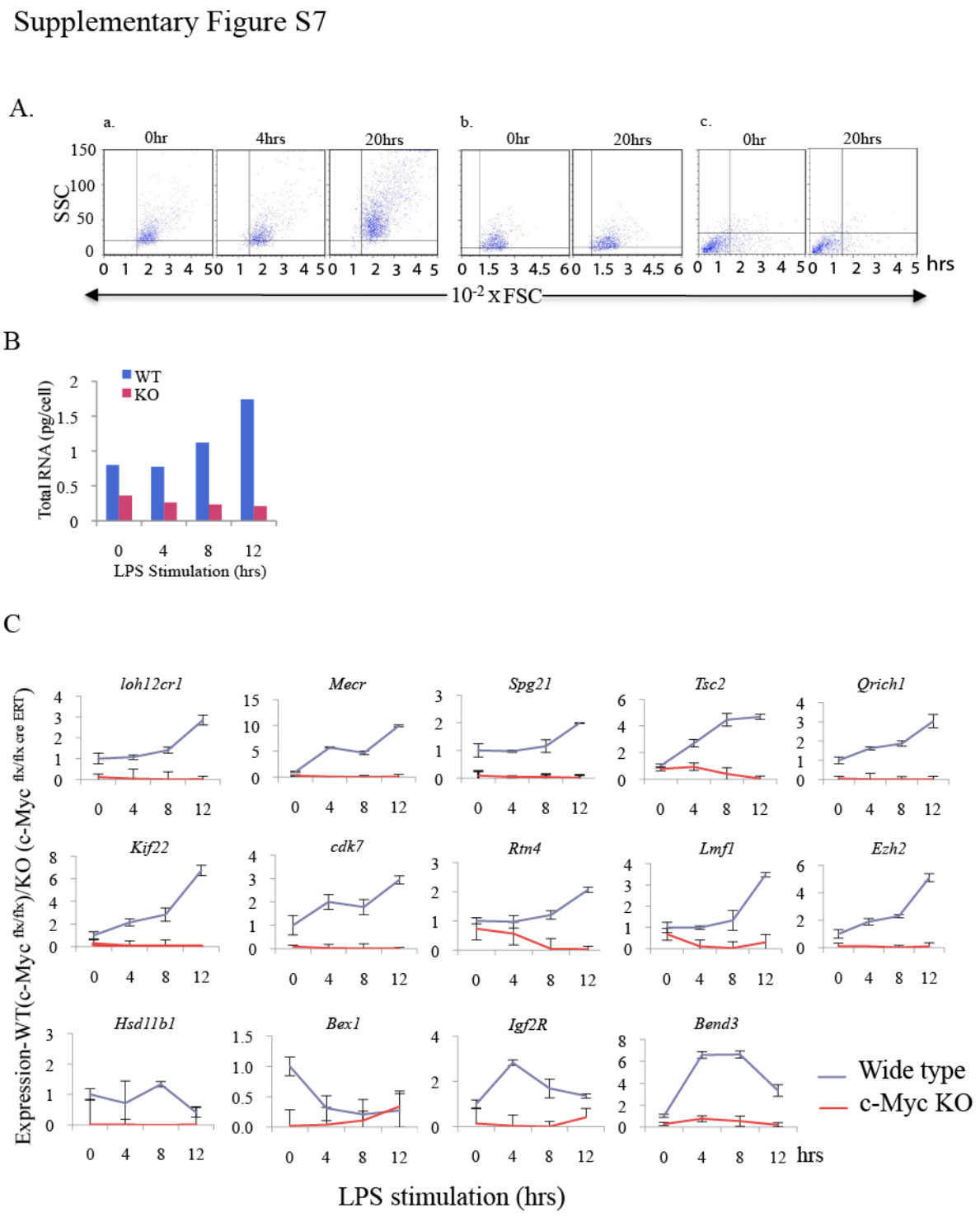
(**A**) Flow cytometric analysis of the differences of the cell size after the LPS stimulation for 1.5, 4, 6 and 20hrs, respectively.

(**B**) and (**C**) indicated the changes of total RNA per cell (pg/cell) (B) and mRNA per cell (fg/cell) (C) after the stimulation of LPS for 0, 4, 8 and 11 hrs, respectively.

(**D**) The fold-change of total RNA (blue) and mRNA (red)

(**E)** The primers and probes used for the Q-RT-PCR quantifications of mRNA expression levels of genes shown in Figure 6D.

(**F)** q-RT-PCR expression analysis of *c-fos* (an immediate early gene) during LPS stimulation with and without 10058-F4 treatment. An active gene *EZH2* and another immediate early gene *Igf2R* are included for comparison.



Supplementary Figure S7: c-Myc knockout B-cells are defective for the universal amplification of gene expression – related to Figure 6.

(A) Flow cytometric analysis of the size-change of B-splenocytes after LPS stimulation for 20hrs. a. c-Myc-EGFP knock-in mice (similar to Figure S6A). b. c-Myc -EGFP knock-in B

cells treated without or with 50 μM 10058-F4 for (right panel). c. c-Myc deficient B-cells from naïve c-Myc $^{flx/flx, Cre ERT}$ treated in vivo with tamoxifen for 3 days, purified (right panel) and then stimulated with LPS (left panel). For all samples, propidium iodide positive cells were gated away. The gain for FSC/SSC in a, b, and c, were 130/180, 130/160 and 165/200 respectively.

(B) Myc dependent total RNA accumulation. Known numbers of B-cells from untreated c-Myc$^{flx/flx}$ mice (WT) or from c-Myc$^{flx/flx, cre ERT}$ mice treated with tamoxifen in vivo for three days to knockout Myc (KO) were stimulated with LPS for the indicated times. Total RNA was recovered and normalized according to input cell number to yield RNA per cell (pg/cell).

(C) c-Myc deficient B-cells do not amplify mRNA expression. The same panel of RNAs were examined by qPCR as in Figure 6D.The more complete deprivation of Myc for a longer time by conditional knockout with CreERT2 compared with inhibition by 10058-F4 yielded smaller naïve B-cells (panel A/c/left) with lower starting levels of mRNAs than wild-type.