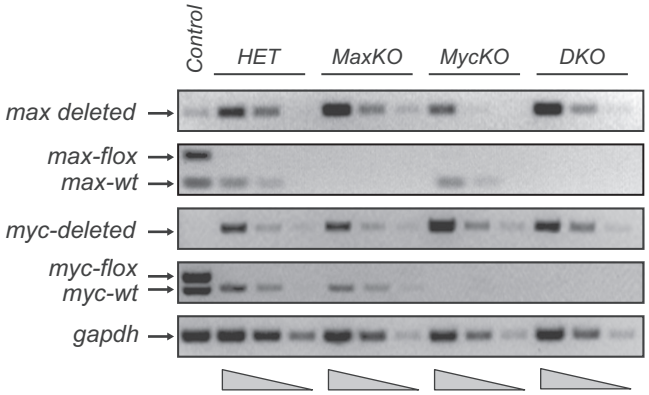
Expanded View Figures

A

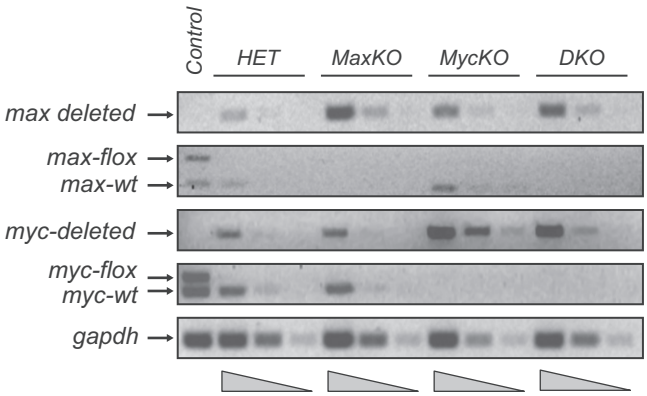
PCR strategy

B

Sorted B220⁺GFP⁺ Bone Marrow cells



Sorted B220⁺GFP⁺ Spleen cells



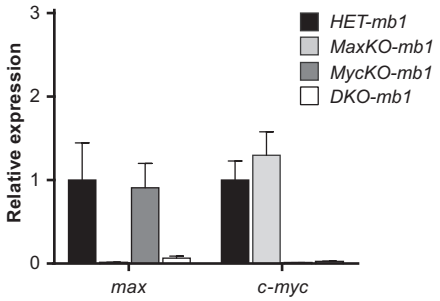
Mouse Genotypes

HET-mb1: $myc^{fl/+};max^{fl/+};mb1^{cre/+};rosa26^{gfp/gfp}$
MaxKO-mb1: $myc^{fl/+};max^{fl/fl};mb1^{cre/+};rosa26^{gfp/gfp}$
MycKO-mb1: $myc^{fl/fl};max^{fl/+};mb1^{cre/+};rosa26^{gfp/gfp}$
DKO-mb1: $myc^{fl/fl};max^{fl/fl};mb1^{cre/+};rosa26^{gfp/gfp}$

Mouse Genotypes

HET-cd19: $myc^{fl/+};max^{fl/+};cd19^{cre/+};rosa26^{gfp/gfp}$
MaxKO-cd19: $myc^{fl/+};max^{fl/fl};cd19^{cre/+};rosa26^{gfp/gfp}$
MycKO-cd19: $myc^{fl/fl};max^{fl/+};cd19^{cre/+};rosa26^{gfp/gfp}$
DKO-cd19: $myc^{fl/fl};max^{fl/fl};cd19^{cre/+};rosa26^{gfp/gfp}$

C



D

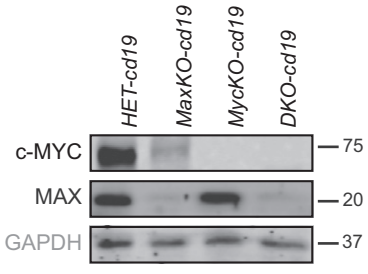


Figure EV1

Figure EV1. Efficient deletion of *c-myc* and *max* in B lymphocytes from bone marrow and spleen of conditional mice.

A PCR strategy to amplify flox, deleted, and wt alleles of *c-myc* or *max*. For *c-myc*, exons 2 and 3 were flanked by loxP sites and, for *max*, exons 4 and 5.

B Genomic PCR analysis of *wt*, *deleted*, and *flox* alleles of *c-myc* and *max* from sorted B220$^+$GFP$^+$ bone marrow or spleen lymphocytes of *mb1-cre*- or *cd19-cre*-bearing mice, respectively. *gapdh* was used for normalization. Experiment representative of three independent experiments.

C RNA expression of *max* and *c-myc* genes from sorted bone marrow GFP$^+$B220$^+$ IgM$^+$ cells of *HET-mb1*, *MaxKO-mb1*, *MycKO-mb*, and *DKO-mb1* mice. $n = 3$. Error bars are standard deviations.

D Western blotting of c-Myc and Max in sorted B220$^+$IgM$^+$GFP$^+$ lymphocytes. Spleen cells from the indicated mice were sorted and activated with LPS and IL-4 for 3 days before analysis. Experiment representative of three independent experiments.

Source data are available online for this figure.

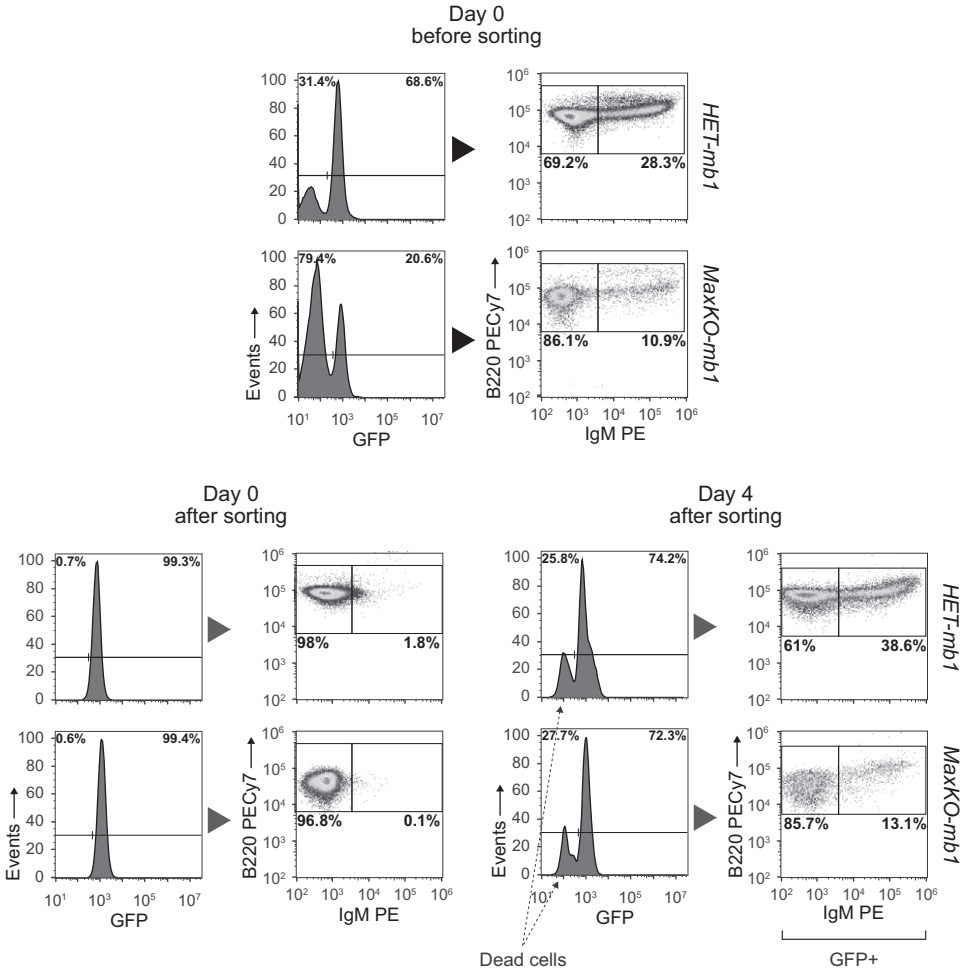


Figure EV2. Max-deficient cells generate B220$^+$IgM$^+$ lymphocytes *in vitro*.

Sorted GFP$^+$B220$^+$IgM$^-$ BM cells from *HET-mb1* and *MaxKO-mb1* mice were cultured with IL-7, rSCF, and rFlt3L and analyzed by flow cytometry 4 days later. For BM analysis, cells were isolated from femora and tibiae and sorted by flow cytometry (GFP$^+$cells, purity > 99%) before culture. After 4 days, the generation of GFP$^+$B220$^+$IgM$^+$ cells was analyzed. A GFP$^-$ population corresponding to dead cells is indicated. Experiment representative of at least three independent experiments.

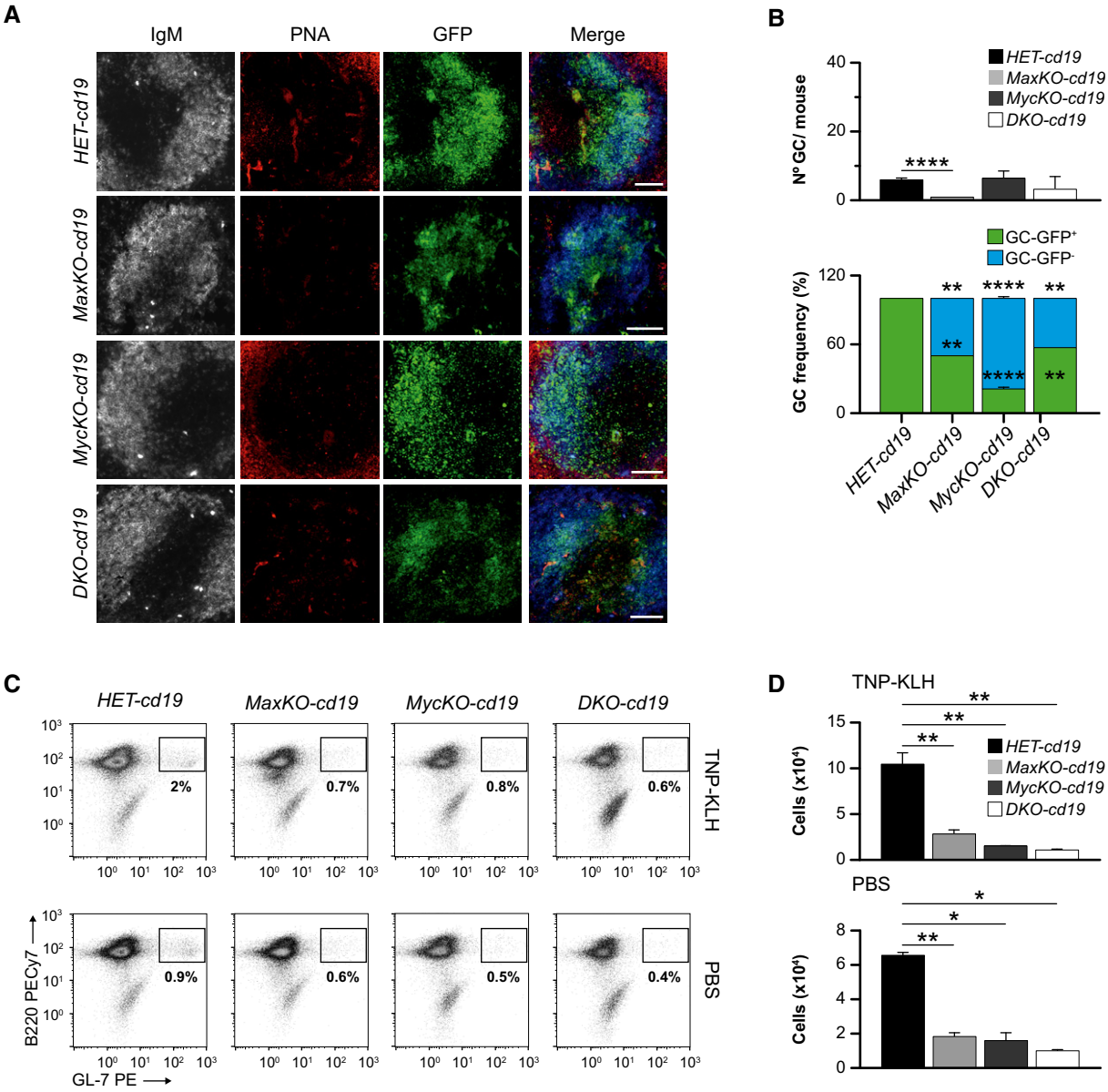


Figure EV3. Analysis of GC formation in the spleen of *MaxKO-cd19*, *MycKO-cd19*, *DKO-cd19*, and heterozygous control mice upon TNP-KLH and PBS immunization.

A Representative images of frozen spleen sections stained for IgM (gray/blue), PNA (GC marker; red), and GFP (deleted B cells; green) of mice immunized with PBS. Analysis was performed at 12 days post-immunization. Scale bar, 80 μm.

B Upper, number of GCs per mouse in spleen sections of PBS-immunized *MaxKO-cd19* (n = 2 mice, n = 84 follicles analyzed), *MycKO-cd19* (n = 2 mice, n = 163 follicles analyzed), *DKO-cd19* (n = 2 mice, n = 157 follicles analyzed), and heterozygous control mice (n = 2 mice, n = 182 follicles analyzed). Lower, frequencies of GC (PNA^+) GFP^+ (deleted cells) or GFP^- (non-deleted cells) in PBS-immunized *MaxKO-cd19* (n = 2 GCs), *MycKO-cd19* (n = 14 GCs), *DKO-cd19* (n = 7 GCs), and heterozygous control (n = 13 GCs) mice at 13 days post-immunization. Error bars are standard deviations.

C Flow cytometry analyses of GC B lymphocytes ($GFP^+B220^+GL\text{-}7^+$) in the spleen of TNP-KLH or PBS-immunized mice. Single-cell suspensions from *MaxKO-cd19*, *MycKO-cd19*, *DKO-cd19*, and heterozygous control mice were prepared, stained, and analyzed (n = 3).

D Absolute numbers of GC B cells ($GFP^+B220^+GL\text{-}7^+$) in *MaxKO-cd19*, *MycKO-cd19*, *DKO-cd19*, and heterozygous control mice from (C). Error bars are standard deviations.

Data information: Statistical analyses were performed using Student's two-tailed unpaired t-test. *P < 0.05, **P < 0.01, ****P < 0.0001.

Figure EV4. RNA-sequencing analyses.

A Heatmaps of DEGs (adj. *P*-value < 0.01) of cell cycle pathways and genes involved in B lymphocyte differentiation. Genes highlighted in red were previously tested by qPCR [22].

B GO classification of biological process and molecular functions of downregulated (blue) and upregulated (red) genes for all three conditions (±1.5-FC and $P < 0.01$). The 20 most significantly (FDR < 0.05, Dataset EV1) affected GO categories are represented.

C GO and pathways enrichment analysis of 45 downregulated and 49 upregulated genes in MycKO only indicated in Fig 4D. Statistically significant GO categories and KEGG pathways (FDR < 0.05) are shown.

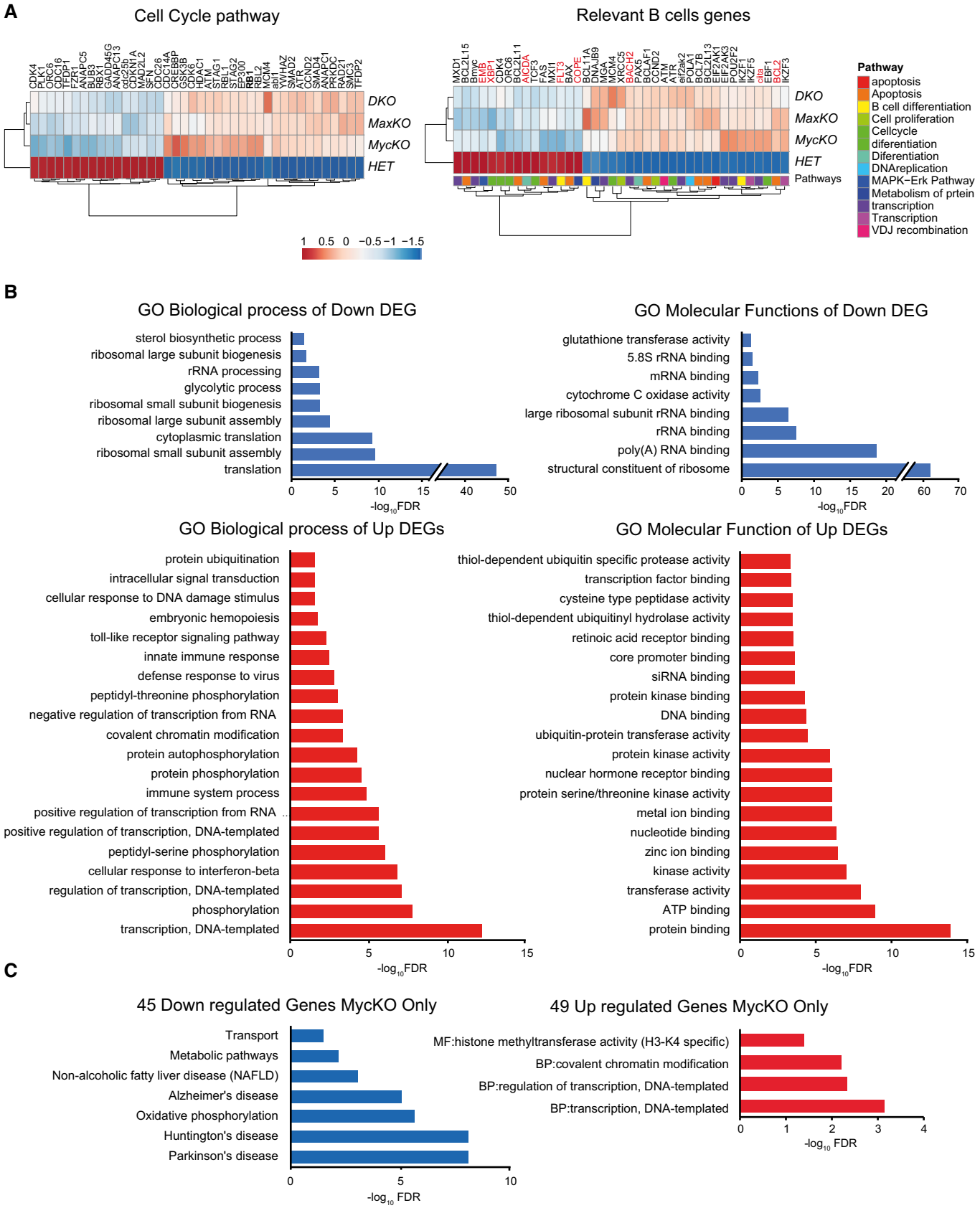


Figure EV4.

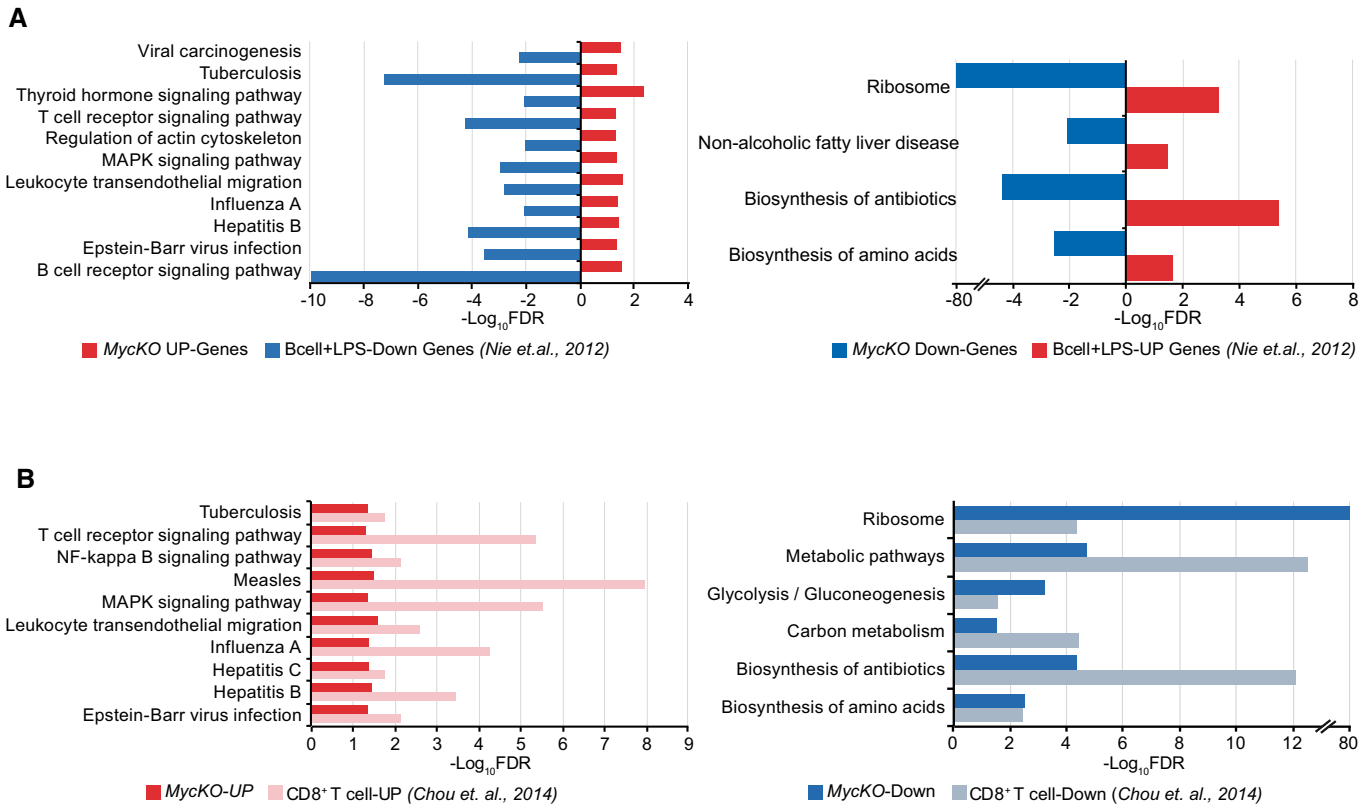


Figure EV5. Comparison of c-Myc transcriptome.

A, B Common KEGG pathways between primary normal B lymphocytes (A) [52] or primary $CD8^+$ T cells lacking *c-myc* (B) [53] and the findings of this study.