CellPress

MYC through *miR-17-92* Suppresses Specific Target Genes to Maintain Survival, Autonomous Proliferation, and a Neoplastic State

Yulin Li,[1] Peter S. Choi,[1] Stephanie C. Casey,[1] David L. Dill,[2] and Dean W. Felsher[1,*]
[1]Division of Oncology, Department of Medicine and Pathology
[2]Department of Computer Science
Stanford University, Stanford, CA 94305, USA
*Correspondence: dfelsher@stanford.edu
http://dx.doi.org/10.1016/j.ccr.2014.06.014

SUMMARY

The *MYC* oncogene regulates gene expression through multiple mechanisms, and its overexpression culminates in tumorigenesis. MYC inactivation reverses turmorigenesis through the loss of distinguishing features of cancer, including autonomous proliferation and survival. Here we report that MYC via *miR-17-92* maintains a neoplastic state through the suppression of chromatin regulatory genes *Sin3b*, *Hbp1*, *Suv420h1*, and *Btg1*, as well as the apoptosis regulator *Bim*. The enforced expression of *miR-17-92* prevents MYC suppression from inducing proliferative arrest, senescence, and apoptosis and abrogates sustained tumor regression. Knockdown of the five *miR-17-92* target genes blocks senescence and apoptosis while it modestly delays proliferative arrest, thus partially recapitulating *miR-17-92* function. We conclude that MYC, via *miR-17-92*, maintains a neoplastic state by suppressing specific target genes.

INTRODUCTION

Cancers are often dependent on or addicted to the initiating oncogenes for the maintenance of the malignant phenotype (Chin et al., 1999; Felsher and Bishop, 1999; Huettner et al., 2000; Weinstein, 2002). The inactivation of a single driver oncogene can result in rapid and sustained tumor regression. Oncogene addiction has been exploited clinically in targeted therapies, such as imatinib for BCR-ABL-driven chronic myelogenous leukemia, gefitinib for lung adenocarcinoma with *EGFR* mutations, and vemurafenib for melanomas with *B-RAF* mutations (Chapman et al., 2011; Druker et al., 1996; Ladanyi and Pao, 2008). Hence, the targeted inactivation of oncogenes appears to be a generalizable approach for the treatment of many cancers.

The *MYC* oncogene is overexpressed in over half of human cancers (Dang, 2012). To study the role of MYC in the initiation and maintenance of tumorigenesis, some investigators have

used the tetracycline regulatory system (Tet system) to generate reversible models of cancer (Gossen and Bujard, 1992). In these mouse models, the overexpression of a conditional *MYC* transgene initiates tumorigenesis, and its inactivation results in rapid, complete, and sustained tumor regression. MYC inactivation is associated with the loss of many of the distinguishing features of tumorigenesis and results in proliferative arrest, apoptosis, differentiation, and senescence, as well as the shutdown of angiogenesis (D'Cruz et al., 2001; Felsher and Bishop, 1999; Hanahan and Weinberg, 2011; Shachaf et al., 2004; Wu et al., 2007).

MYC is a transcriptional regulator of a multitude of genes, but it is unclear if any of these genes are responsible for MYC maintaining a neoplastic state (Dang, 2012). Recently, it has been shown that MYC may regulate gene expression as a general transcriptional amplifier (Lin et al., 2012; Nie et al., 2012). However, it has been pointed out that this would not explain how MYC can suppress gene expression or regulate gene expression in a specific manner (Walz et al., 2013). MYC has also been

---

Significance

The MYC oncogene is frequently overexpressed in human cancers. MYC overexpression coordinates the expression of thousands of genes that could potentially contribute to its neoplastic properties. However, it was unclear which specific genes are responsible for MYC maintaining a neoplastic state. Here we show that MYC maintains a neoplastic state through the regulation of the microRNA cluster miR-17-92, which controls specific chromatin regulatory and survival programs. MYC inactivation, through the downregulation of miR-17-92, induces a loss of neoplastic features as a consequence of restoration of proliferative arrest, apoptosis, and senescence. Our results highlight how MYC maintains tumorigenesis through the regulation of miR-17-92-dependent epigenetic and survival programs and provide a mechanistic explanation of the phenomenon of oncogene addiction.

CrossMark

shown to regulate the expression of several microRNAs, including the polycistronic *miR-17-92* cluster (Bui and Mendell, 2010; O'Donnell et al., 2005; Sander et al., 2008). The *miR-17-92* cluster is overexpressed in human lymphomas (He et al., 2005). Notably, overexpression of *miR-17-92* cooperates with MYC to induce lymphomagenesis, while deletion of *miR-17-92* induces the death of lymphoma cells (He et al., 2005; Mu et al., 2009).

We hypothesized that *miR-17-92* is causally responsible for at least part of the mechanism by which MYC maintains a neoplastic state (Figure 1A). Here we found that MYC, through *miR-17-92*, regulates the expression of specific chromatin regulatory genes, such as *Sin3b*, *Hbp1*, *Suv420h1*, and *Btg1*, as well as the apoptosis regulator *Bim*. Upon MYC inactivation, the downregulation of *miR-17-92* and the corresponding induction of these target genes are causally required for the activation of the apoptosis and senescence programs and sustained tumor regression. Hence, MYC suppression of these genes is one of the required mechanisms for maintaining a neoplastic state.

RESULTS

Sustained *miR-17-92* Expression Rescues MYC Addiction In Vitro and In Vivo

We examined the level of several microRNAs known to be regulated by MYC by using real-time quantitative PCR in three lymphoma cell lines from *Eμ-tTA/tet-O-MYC* mice (O'Donnell et al., 2005; Sander et al., 2008). Upon MYC inactivation with doxycycline treatment, all members of the *miR-17-92* cluster were downregulated, while *miR-15/16* and *miR-26* were upregulated in a time-dependent manner (Figure S1A available online). Similarly, in MYC-driven hepatocellular carcinoma derived from *LAP-tTA/tet-O-MYC* transgenic mice, *miR-17-92*, but not *miR-15/16* and *miR-26*, was downregulated upon MYC inactivation (Kota et al., 2009; Shachaf et al., 2004) (Figure S1B). Thus, MYC generally regulates the expression of *miR-17-92* in tumor cells.

We speculated that *miR-17-92* was responsible for maintaining at least some of the distinguishing features of cancer in MYC-induced tumors (Figure 1A). To determine whether constitutive expression of *miR-17-92* could rescue any of the effects of MYC inactivation, we retrovirally infected MYC-induced lymphomas with the murine stem cell virus containing *miR-17-92* (MSCV-*miR-17-92*) and then confirmed that expression of *miR-17-92* was maintained even after MYC inactivation (Figures 1B and S1C). Retroviral *miR-17-92* expression abrogated the induction of proliferative arrest, apoptosis, and senescence, which we previously described as consequences of MYC suppression (Felsher and Bishop, 1999; Wu et al., 2007). The proliferation was sustained over a 5-day time course in *miR-17-92*-expressing cells after MYC inactivation, as shown by the S/G2/M population in the flow cytometric analysis of cell cycle distributions (Figures 1C and S1D). The induction of apoptosis by MYC inactivation was blocked by *miR-17-92* as shown by the subG1 population and the 7-AAD/Annexin V double positive population (Figures 1C, 1D, and S1D). Furthermore, retroviral *miR-17-92* expression blocked the induction of cellular senescence in a sustained manner as measured by senescence-associated β-galactosidase (SA-β-gal) staining (Figures 1E and S1E), histone H4 lysine 20 trimethylation (H4K20me3) staining (Figures

1E and S1F), and quantification of trimethylated histone H3 lysine 9 (H3K9me3) (Figure S1G). Similarly, in MYC-induced hepatocellular carcinoma and osteosarcoma (Jain et al., 2002; Shachaf et al., 2004), retroviral expression of *miR-17-92* abrogated the induction of cellular senescence upon MYC suppression as shown by SA-β-gal staining (control versus *miR-17-92*: 19-fold versus 3-fold induction in hepatocellular carcinoma and 10-fold versus 3-fold induction in osteosarcoma; Figures S1H and S1I). In contrast, *miR-17-92* expression in three BCR-ABL-driven B cell leukemia cell lines failed to rescue proliferative arrest or apoptosis upon BCR-ABL inactivation (Figure S1J). Hence, *miR-17-92* specifically rescues the proliferative arrest, apoptosis, and senescence upon MYC inactivation.

Next, we examined in vivo whether *miR-17-92* expression rescues the phenotypes of MYC inactivation. MYC-induced lymphoma cells expressing either an empty control vector or MSCV-*miR-17-92* were subcutaneously transplanted into syngeneic FVB/N hosts. Tumor cells were allowed to grow in vivo for about 2 weeks before MYC inactivation by doxycycline administration in the drinking water. Tumors were collected before and after MYC inactivation for examination of apoptosis, proliferation, and senescence (Figures 2A–2D). Phospho-histone H3 and Ki67 staining was used to measure mitotic and proliferative cells, respectively. Cleaved-caspase-3 staining was used for apoptotic cells and SA-β-gal and H4K20me3 staining was used for senescent cells. Upon MYC inactivation, in the control lymphoma versus lymphomas with *miR-17-92* expression, there was a 67% versus 15% decrease in phospho-histone H3 staining and an 85% versus 25% decrease in Ki67 staining (Figures 2A and S2), a 60% increase versus no change in cleaved-caspase-3 staining (Figure 2B), and a 3-fold increase versus no change in SA-β-gal and H4K20me3 staining (Figures 2C and 2D). Thus, *miR-17-92* expression prevented MYC inactivation from inducing proliferative arrest, apoptosis, and senescence in vivo.

MYC via *miR-17-92* Regulates Specific Target Genes

We reasoned that MYC, through *miR-17-92*, regulates a specific subset of genes responsible for maintaining autonomous proliferation and survival. Since the expression of *MYC* or *miR-17-92* can be turned off independently in our conditional system, this allows for the screening of genes regulated by MYC or *miR-17-92* (Figures 1B and S3). The genes that were differentially expressed before and after MYC inactivation in the control lymphoma were categorized as MYC regulated. The genes that were differentially expressed between the control lymphoma and retroviral *miR-17-92*-expressing lymphoma when MYC was turned off in both populations were defined as *miR-17-92*-regulated (Figure S3). The specific subset of MYC target genes regulated through *miR-17-92* would appear to be coregulated by both MYC and *miR-17-92* (Figures S3 and 3A). By comparing the microarray gene expression profiles of the control lymphoma versus *miR-17-92*-expressing lymphoma upon MYC inactivation, we found that 70% of *miR-17-92*-regulated genes were also regulated by MYC (Figure 3A). The 401 overlapping genes coregulated by MYC and *miR-17-92* were further separated into the upregulated and downregulated groups (Figure 3B). Among the genes upregulated by both MYC and *miR-17-92*, there was an enrichment of genes involved in DNA replication, repair, and cell cycle (Figure 3C). Notably, the genes
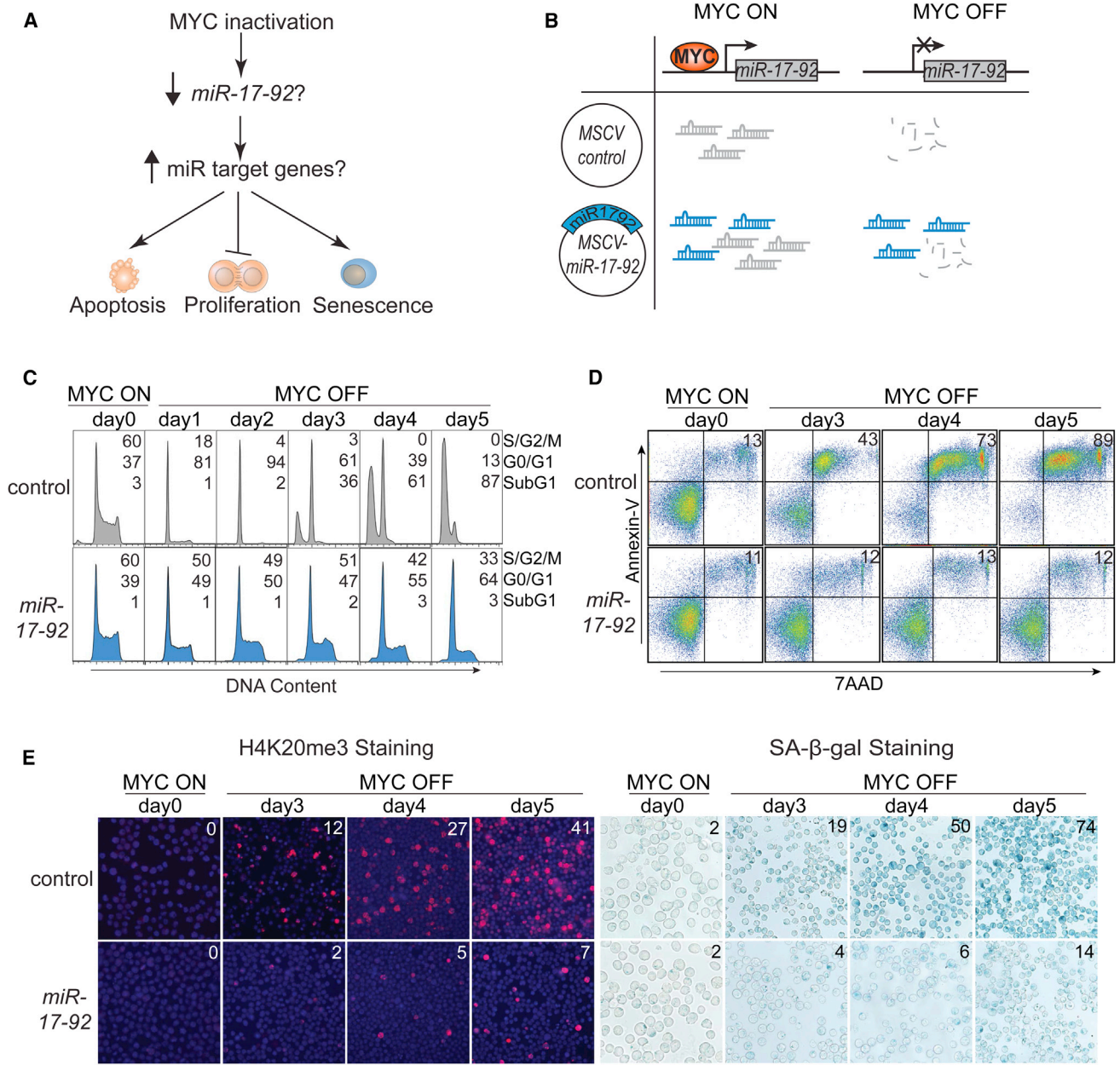
Figure 1. *miR-17-92* Expression In Vitro Rescues MYC Oncogene Addiction by Sustaining Proliferation and Blocking Apoptosis and Senescence

(A) Diagram of cellular changes upon MYC inactivation in MYC-driven tumors.

(B) Experimental strategy to sustain *miR-17-92* with retroviral expression. The endogenous *miR-17-92* is shown in gray, while the exogenous MSCV-*miR-17-92* is shown in blue.

(C) Cell cycle analysis of tumor cells over a 5-day time course with flow cytometry after propidium iodide staining. The numbers indicate the percentage of cells in different phases of the cell cycle. The experiments were repeated three times with similar results.

(D) Annexin V/7-AAD staining showing apoptotic cells over a 5-day time course of MYC inactivation. The numbers in the upper right quadrant indicate the percentage of apoptotic cells. The experiments were repeated three times with similar results.

(E) H4K20me3 and SA-β-gal staining of tumor cells after MYC inactivation for 5 days. The control cells are kept alive with *Bim* shRNA knockdown and *Bcl-xL* overexpression. The numbers in the upper right quadrant indicate the percentage of cells stained positive. Cell nuclei in the H4K20me3 panel were stained with DAPI. The experiments were repeated twice with similar results.

Scale bars, 50 μm. See also Figure S1.
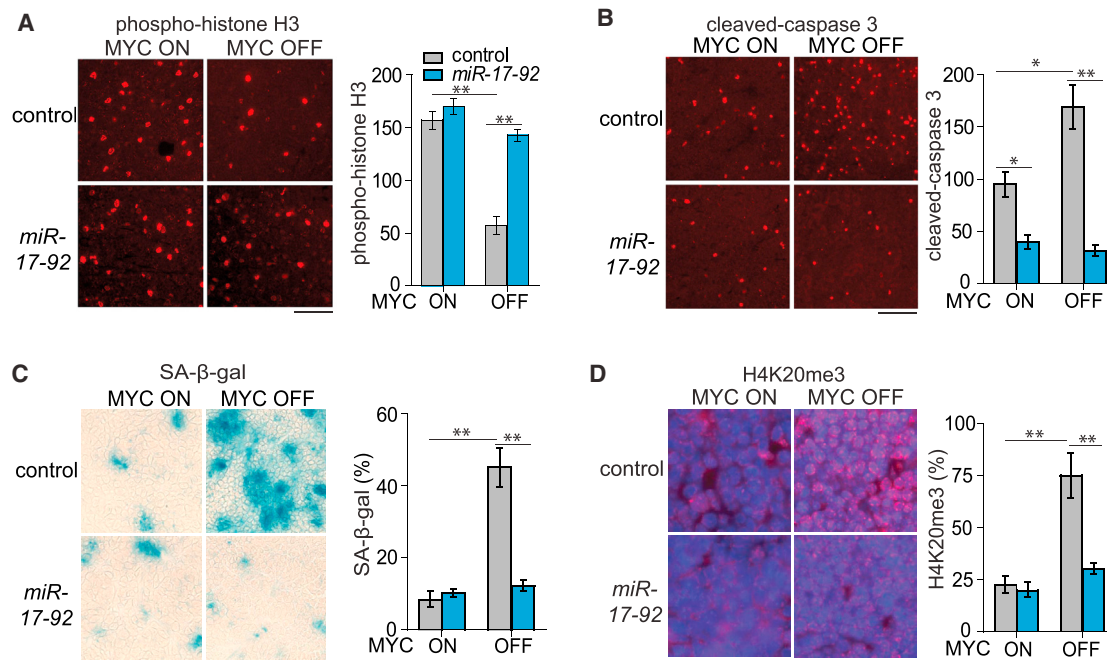
Figure 2. *miR-17-92* Expression In Vivo Mediates MYC Oncogene Addiction by Sustaining Proliferation and Blocking Apoptosis and Senescence

(A) Phospho-histone H3 staining showing cells in the metaphase of the cell cycle 4 days after MYC inactivation. The y axis denotes the number of positive staining cells per 20× magnification field.

(B) Cleaved-caspase 3 showing apoptotic cells 4 days after MYC inactivation. The y axis denotes the number of positive staining cells per 20× magnification field.

(C) SA-β-gal staining four days after MYC inactivation. The y axis denotes the percentage of area with positive SA-β-gal staining.

(D) H4K20me3 staining 4 days after MYC inactivation. The y axis denotes the percentage of cells with positive staining.

Results are presented as mean ± SEM, and n = 4 (A–D). Student's t test: *$p < 0.05$; **$p < 0.01$. Scale bars, 50 μm. See also Figure S2.
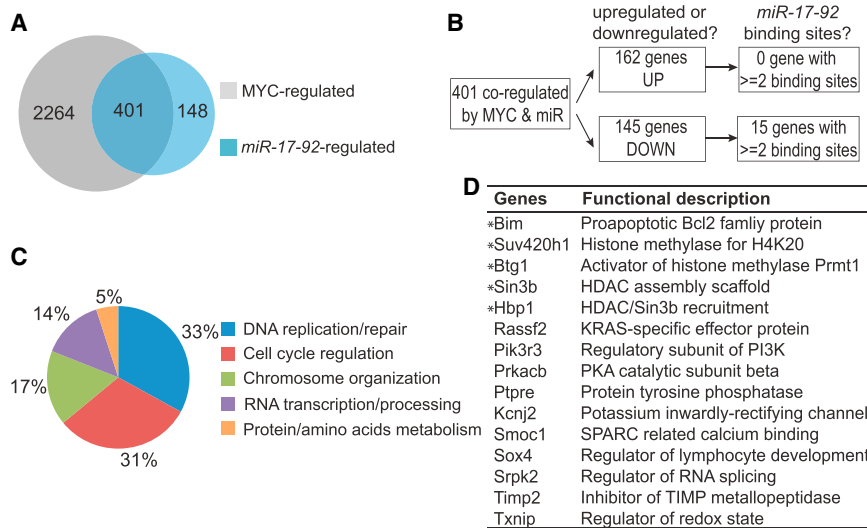
downregulated by both MYC and *miR-17-92* had 4.6-fold more *miR-17-92* binding sites in their 3′ UTR compared with upregulated genes (32% in downregulated versus 7% in upregulated genes; Tables S1 and S2). We inferred that these downregulated genes are directly regulated by *miR-17-92* binding.

Our gene list was further refined by only including genes with at least two *miR-17-92* binding sites in their 3′ UTR, as predicted by each of three microRNA target scanning programs (miRanda, Targetscan, and miRWalk) (Figures 3B and 3D; Table S3). Among these 15 genes were four chromatin modifiers that have not been previously reported as MYC or *miR-17-92* targets (*Sin3b*, *Hbp1*, *Suv420h1*, and *Btg1*). Also identified was the apoptosis regulator, *Bim*, which has been reported previously to be a *miR-17-92* target (Ventura et al., 2008; Xiao et al., 2008) (Figure 3D). Notably, all of these genes have been associated with proliferative control, senescence, and/or apoptosis (Berthet et al., 2002; David et al., 2008; Roninson, 2003; Swanson et al., 2004; van Oevelen et al., 2010). Thus, we focused our subsequent efforts on assessing whether these MYC-*miR-17-92* target genes could contribute to the consequences of MYC suppression in tumors.

We examined if these *miR-17-92* targets were directly regulated by MYC and *miR-17-92*. First, MYC inactivation induced the protein expression of Sin3b, Hbp1, Suv420h1, Btg1, and all three Bim isoforms in control, but not *miR-17-92*-expressing cells as measured by western blot analysis (Figures 4A and

S4). Second, to validate whether these genes are direct targets of *miR-17-92*, a dual luciferase assay was performed by cloning 3′ UTR fragments of all five genes, with either wild-type or mutant *miR-17-92* sites, downstream of the firefly luciferase coding region (Figure 4B, upper panel). Compared with mutant 3′ UTRs lacking *miR-17-92* binding sites, the wild-type 3′ UTRs conferred significant repression as shown by the 20%–60% lower firefly/renilla ratio (Figure 4B, lower panel). Hence, Sin3b, Hbp1, Suv420h1, Btg1, and Bim are regulated by MYC in a *miR-17-92*-dependent manner.

Next, we examined if MYC via *miR-17-92* was regulating chromatin through each of these gene products. *Sin3b* and *Hbp1* have been shown to be candidate target genes of *miR-19* (Mu et al., 2009). Sin3b interacts with Hbp1 and recruits histone deacetylases (HDACs) to repress the transcription of genes related to proliferation, such as *Aurkb*, *Mybl2*, *Cdc6*, and *Bub1b* (David et al., 2008; Swanson et al., 2004; van Oevelen et al., 2010). Indeed, these genes were upregulated by *miR-17-92* and MYC (Figure 3C; Table S1). Upon MYC inactivation, there was a 3- to 8-fold increase versus a 2-fold increase in Sin3b binding to these promoters in the control lymphoma versus *miR-17-92*-expressing lymphoma according to a chromatin immunoprecipitation assay (Figure 4C). Thus, the induction of Sin3b and Hbp1 upon MYC inactivation may contribute to proliferative arrest and cellular senescence by silencing genes related to proliferation and cell cycle.

Figure 3. Identification of *miR-17-92* Target Genes by Comparative Analysis of Genes Regulated by MYC and *miR-17-92*

(A) Venn diagram of genes regulated by both MYC and *miR-17-92*.

(B) Flowchart showing the analysis of the genes coregulated by MYC and *miR-17-92*. Genes were separated into either upregulated or down-regulated groups and analyzed for functional annotation and enrichment of *miR-17-92* binding sites.

(C) Functional categories of genes upregulated by both MYC and *miR-17-92* according to DAVID bioinformatic resources. The percentage refers to the number of genes within a particular category in relation to the total number of genes that have a Gene Ontology annotation.

(D) Candidate target genes with multiple *miR-17-92* binding sites. The histone modifiers and *Bim* were indicated with asterisks.

See also Figure S3 and Tables S1, S2, and S3.

Notably, Suv420h1 is a histone methyltransferase that catalyzes dimethylation and trimethylation of histone H4 lysine 20 (H4K20me2 and H4K20me3) (Fraga et al., 2005; Greer and Shi, 2012). H4K20me3 is a marker of heterochromatin formation and senescence, and its loss is a common hallmark of human cancer (Fraga et al., 2005; Greer and Shi, 2012; Nelson, 2012). Upon MYC inactivation, there was an induction of both H4K20me2 and H4K20me3 in control lymphoma cells, but not *miR-17-92*-expressing lymphoma cells (Figure 4D). In contrast, the monomethylated H4K20me1, catalyzed by Setd8 (Greer and Shi, 2012), did not increase in either control or *miR-17-92*-expressing lymphoma cells (Figure 4D), indicating that the induction of Suv420h1 specifically increases the dimethylation and trimethylation of H4K20. Finally, Btg1 is a tumor suppressor that can activate histone methyltransferase Prmt1 to dimethylate histone H4 arginine 3 (H4R3me2) (Berthet et al., 2002; Lin et al., 1996). Upon MYC inactivation, there was an accumulation of H4R3me2 in control cells, but not *miR-17-92*-expressing cells (Figure 4D). Therefore, MYC inactivation via *miR-17-92* regulates the biological function of the chromatin regulatory genes *Sin3b*, *Hbp1*, *Suv420h1*, and *Btg1*.

Suppression of Sin3b, Hbp1, Suv420h1, Btg1, and Bim Significantly Recapitulates *miR-17-92* Function

Our results suggest that suppression of the four epigenetic regulators (*Sin3b*, *H*bp1, *S*uv420h1, and *B*tg1, hereafter referred to as SHSB) and the proapoptotic protein Bim may contribute to MYC's ability to maintain tumorigenesis. To examine this, the *miR-30*-based retroviral short hairpin RNAs (shRNAs) were used to knock down the target genes individually or collectively in MYC-induced lymphoma cells. The target mRNA was knocked down to levels between 6% and 20% of the scrambled control (Figure S5A). Knocking down the expression of *Suv420h1* and *Btg1* reduced the levels of H4K20me2/3 and H4R3me2, respectively (Figures 4E and 4F). After 24 hr of MYC inactivation, lymphoma cells with individual knockdown of the chromatin modifiers exhibited a modest block in proliferative arrest, with 19%–30% of cells still remaining in S/G2/M phases

compared to only 11% for control cells (*Sin3b*: 25%; *Hbp1*: 25%; *Suv420h1*: 35%; *Btg1*: 19%; Figure S5B). Concurrently knocking down all five *miR-17-92* target genes (*Bim* and SHSB), modestly delayed the proliferative arrest after MYC inactivation as shown by flow cytometric analysis of the cell cycle distribution (Figures 5A and 5B). The knockdown of *Bim* alone reduced the induction of apoptosis following MYC inactivation, whereas the combined knockdown of *Bim* and SHSB further decreased the rate of apoptosis (Figures 5C and S5C). Hence, these data suggest that *miR-17-92* regulation of its target genes *Sin3b*, *Hbp1*, *Suv420h1*, *Btg1*, and *Bim* is required for proliferative arrest and apoptosis upon MYC inactivation.

Cellular senescence is characterized by a state of permanent cell cycle arrest (Guney and Sedivy, 2006; Nardella et al., 2011). We have shown previously that even brief suppression of MYC can induce senescence and result in sustained tumor regression (Jain et al., 2002). We tested if cell cycle arrest induced by MYC inactivation is reversible (Figure 5D, ON, OFF, and BACK ON). Upon MYC inactivation, control lymphoma cells progress from cell cycle arrest to high levels of apoptosis, with few viable cells remaining by 4–5 days after oncogene withdrawal (Figures 1C and 1D). To specifically examine the effect of MYC inactivation on cell cycle arrest, independently from apoptosis, we utilized lymphoma cells with the shRNA-mediated knockdown of *Bim*. Upon MYC inactivation, lymphoma cells with *Bim* knockdown persisted and underwent proliferative arrest. Moreover, they remained arrested even after reactivation of MYC (Figure 5D, upper panel). This irreversible cell cycle arrest indicated the induction of senescence by MYC inactivation. In contrast, lymphoma cells expressing *miR-17-92* or with the knockdown of *Bim* and SHSB resumed proliferation upon MYC reactivation (Figure 5D, middle and lower panels). Therefore, the expression of *miR-17-92* or the suppression of the *miR-17-92* target genes *Sin3b*, *Hbp1*, *Sub420h1*, and *Btg1* prevents the induction of senescence upon MYC inactivation.

The shRNA-mediated suppression of *Bim* and SHSB also impeded the in vivo proliferative arrest, apoptosis, and senescence upon MYC inactivation. Tumor cells were transplanted
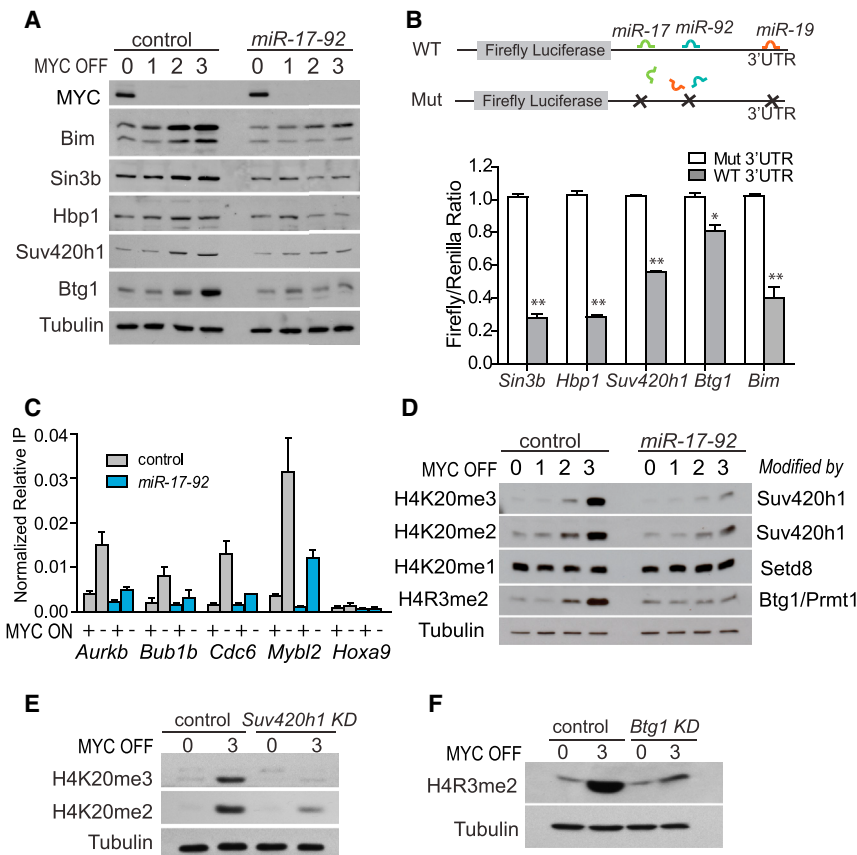
Figure 4. Validation of Target Genes of *miR-17-92*

(A) Changes in the expression of Bim and histone modifiers as detected by western blot 3 days after MYC inactivation.

(B) Top: wild-type (WT) and mutant (Mut) 3′ UTR reporter constructs. Bottom: dual luciferase assay using 3′ UTR reporters. The firefly luciferase signals were normalized with the internal control renilla luciferase. Results are presented as mean ± SEM. Student's t test: *p < 0.05; **p < 0.01.

(C) Chromatin immunoprecipitation with Sin3b antibody showing binding of Sin3b to promoters of four proliferation-related genes upon MYC inactivation. MYC OFF samples were taken at 2 days after MYC inactivation. PCR with *Hoxa9* promoter specific primers was included as a negative control. Data shown are averages of two experiments and are presented as mean ± SEM.

(D) Changes in histone H4 lysine 20 and arginine 3 methylation status upon MYC inactivation. The mono-, di-, and trimethylation of H4K20 and dimethylation of H4R3 are shown. The MYC OFF time course includes days 0, 1, 2, and 3. The respective enzymes catalyzing each of the modifications are shown on the left side of the lanes.

(E) Western blot analysis of di- and trimethylation of H4K20 in the presence of *Suv420h1* knockdown (KD).

(F) Western blot analysis of dimethylation of H4R3 in the presence of *Btg1* knockdown.

See also Figure S4.

subcutaneously into syngeneic FVB/N hosts and grown for about 2 weeks before MYC inactivation by doxycycline administration in the drinking water. In control lymphoma versus lymphomas with *Bim* and *SHSB* knockdown, there was a 4-fold decrease versus 1-fold decrease in phospho-histone H3 staining, a 1.5-fold increase versus no change in cleaved-caspase-3 staining, and a 3-fold versus less than 1-fold increase in SA-β-gal staining (Figures 6A–6C), similar to what was observed for *miR-17-92* expression (Figures 2 and 6D). Thus, *miR-17-92* target genes Sin3b, Hbp1, Suv420h1, Btg1, and Bim are required for the induction of proliferative arrest, apoptosis, and senescence in vivo upon MYC inactivation.

Suppression of *Sin3b*, *Hbp1*, *Suv420h1*, *Btg1*, and *Bim* Abrogates Sustained Tumor Regression upon MYC Inactivation

Since *miR-17-92* expression and knockdown of *miR-17-92* target genes significantly block the induction of proliferative arrest, apoptosis, and senescence both in vitro and in vivo, we examined their impact on sustained tumor regression upon MYC inactivation. MYC inactivation in lymphoma induced rapid tumor regression within 6 days (Figure 6E), without evidence of lymphoma recurrence even after 6 months of continuous observation (Figure 6F). In contrast, the lymphomas with enforced retroviral *miR-17-92* expression regressed only after 14 days and 80% of tumors reoccurred within 6 weeks (Figures 6E and 6F). Compared with the dramatic delay in tumor regression with *miR-17-92* expression, the knockdown of either *Bim* or

Bim combined with *SHSB* modestly delayed the kinetics of tumor regression (Figure 6E). Interestingly, although *Bim* knockdown was not associated with any tumor recurrence, the combined knockdown of *Bim* and *SHSB* was associated with the recurrence of 50% of tumors (Figure 6F). After prolonged MYC inactivation, the recurrent tumors eventually regained high levels of MYC expression, similar to what we have described previously (Figure S6) (Choi et al., 2011). Hence, the expression of *miR-17-92* or the suppression of *miR-17-92* target genes Sin3b, Hbp1, Suv420h1, Btg1, and Bim prevented sustained tumor regression upon MYC inactivation.

DISCUSSION

We have found that MYC through *miR-17-92* directly suppresses the expression of chromatin regulatory genes Sin3b, Hbp1, Suv420h1, and Btg1 and proapoptotic gene Bim. The suppression of these defined factors is causally required to maintain survival, autonomous proliferation, and self-renewal. Our results have general implications for how MYC maintains a neoplastic state.

MYC is known to globally regulate gene and protein expression (Dang, 2012). Many studies have identified hundreds of genes associated with MYC overexpression and tumorigenesis (Kim et al., 2008; Schlosser et al., 2005; Zeller et al., 2006). The expectation is that a similar multitude of genes would be required by MYC to initiate and maintain a neoplastic state. Surprisingly, we found that a single microRNA cluster, *miR-17-92*, among
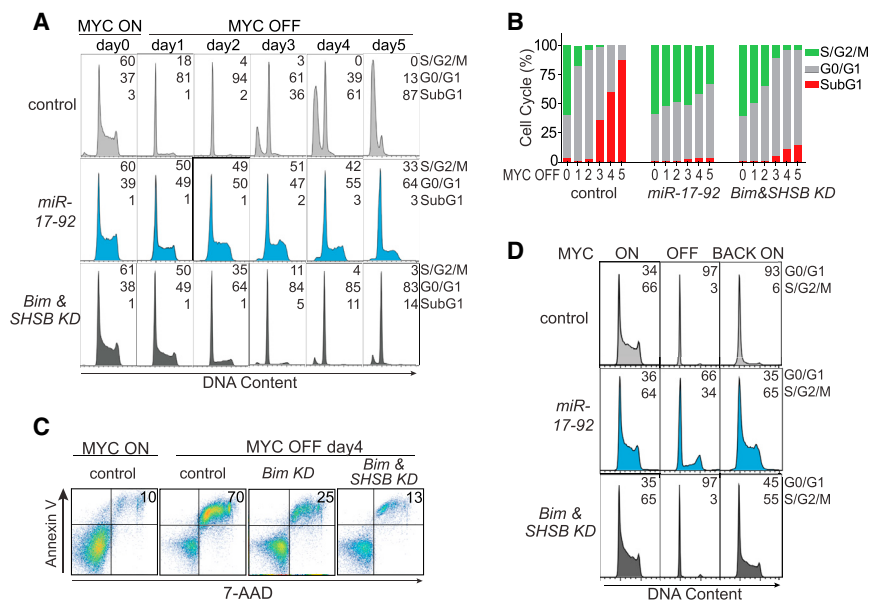
Figure 5. Suppression of *Bim* and the Chromatin Modifiers Delays Proliferative Arrest and Blocks Apoptosis and Senescence upon MYC Inactivation In Vitro

(A) Cell cycle distribution of cells with combined knockdowns of *SHSB* and *Bim* upon MYC inactivation for 5 days. The numbers indicate the percentage of cells in different phases of the cell cycle.

(B) Bar graph summarizing data shown in Figure 5A. Different phases of the cell cycle were color coded.

(C) Apoptosis of tumor cells with knockdown of *Bim* and *SHSB* upon MYC inactivation for 4 days. The numbers in the upper right quadrant indicate the percentage of apoptotic cells. A detailed 5-day time course is shown in Figure S5.

(D) Cell cycle distribution of tumor cells upon MYC inactivation and reactivation. MYC OFF samples were taken at 4 days of MYC inactivation with doxycycline treatment. MYC BACK ON samples were taken at 3 days after MYC reactivation by removing doxycycline. The events shown are gated on live cells. The numbers indicate the percentage of cells in either the G1/G0 or the S/G2/M phase of the cell cycle.

The experiments were repeated three times with similar results. See also Figure S5.

thousands of genes controlled by MYC can maintain a neoplastic state in MYC-induced tumors by sustaining autonomous proliferation and survival. We found that this mechanism was specific to MYC and unique to *miR-17-92*. Furthermore, the function of *miR-17-92* can be partially attributed to the suppression of a small number of target genes, such as the chromatin regulatory genes *Sin3b*, *Hbp1*, *Suv420h1*, and *Btg1*, as well as the proapoptotic gene *Bim*. Our results highlight how MYC maintains tumorigenesis through the regulation of *miR-17-92*-dependent epigenetic and survival programs.

Oncogene-induced tumorigenesis is generally suppressed through intrinsic barriers, such as apoptosis and senescence (Braig et al., 2005; Lowe et al., 2004; Nardella et al., 2011). The inactivation of a driver oncogene can restore these tumor suppressor mechanisms, even in a tumor that is genetically complex (Karlsson et al., 2003), thereby eliciting the phenomenon of oncogene addiction (Felsher, 2008; Weinstein, 2002). Hence, our results suggest that MYC via *miR-17-92* maintains autonomous proliferation, self-renewal, and survival. Correspondingly, MYC inactivation induces a loss of distinguishing features of tumors as a consequence of restoration of proliferative arrest, apoptosis, and senescence (Figure 7).

We have identified specific *miR-17-92* target genes that are essential for the reversal of neoplasia upon MYC inactivation. These genes drive proliferation arrest, senescence, and apoptosis by regulating chromatin modification and apoptosis. Sin3b interacts with Hbp1 and recruits HDACs to silence proliferation-related genes and mediate cell cycle exit and senescence (David et al., 2008; Grandinetti et al., 2009; Swanson et al., 2004). Suv420h1 is a histone methyltransferase that dimethylates and trimethylates H4K20 (Fraga et al., 2005; Greer and Shi, 2012). H4K20me3 is known to direct chromatin compaction and is a marker of heterochromatin formation and senescence (Greer and Shi, 2012; Lu et al., 2008; Nelson, 2012). Loss of H4K20me3 is a common feature in human cancer (Fraga

et al., 2005). Btg1 is a tumor suppressor that is frequently lost in acute lymphoblastic leukemia (Lundin et al., 2012; Waanders et al., 2012). It is also a biomarker of chemotherapy-induced cellular senescence (Roninson, 2003). Finally, Bim is a major tumor suppressor in MYC-induced lymphomagenesis (Egle et al., 2004). Bim is also frequently lost in human B cell lymphomas, and its loss can cause chemoresistance in patients (Richter-Larrea et al., 2010). Thus, these *miR-17-92* targets are some of the critical players in the maintenance of the neoplastic state by MYC.

Recently, it has been shown that MYC can function as a transcriptional amplifier of the already expressed genes within the cells without specificity (Lin et al., 2012; Nie et al., 2012). It is a remarkable finding and explains one aspect of MYC function that is consistent with prior studies (Dang, 2012; Guccione et al., 2006; Guney and Sedivy, 2006; Varlakhanova and Knoepfler, 2009). However, whether the transcriptional amplifier mechanism is required for MYC to maintain the neoplastic state is not known. Moreover, MYC's function as an amplifier is likely only one of its many functions and does not account for the ability of MYC to suppress gene expression, nor does it provide an explanation for gene-specific effects on expression (Walz et al., 2013). Our results highlight an additional mechanism by which MYC controls several essential features of a neoplastic state (Figure 7). MYC, through *miR-17-92*, controls a general ON and OFF switch of chromatin state and thereby regulates the decision between survival versus death and self-renewal versus senescence. Our findings are also complementary to MYC's role in transcriptional amplification. High levels of MYC can keep the chromatin transcriptionally accessible and allow for transcriptional amplification. When MYC is turned off, many genes are downregulated by the amplifier mechanism and this likely reduces the ability of tumor cells to grow and proliferate. However, the suppression of *miR-17-92* upon MYC inactivation allows the induction of many genes, including chromatin
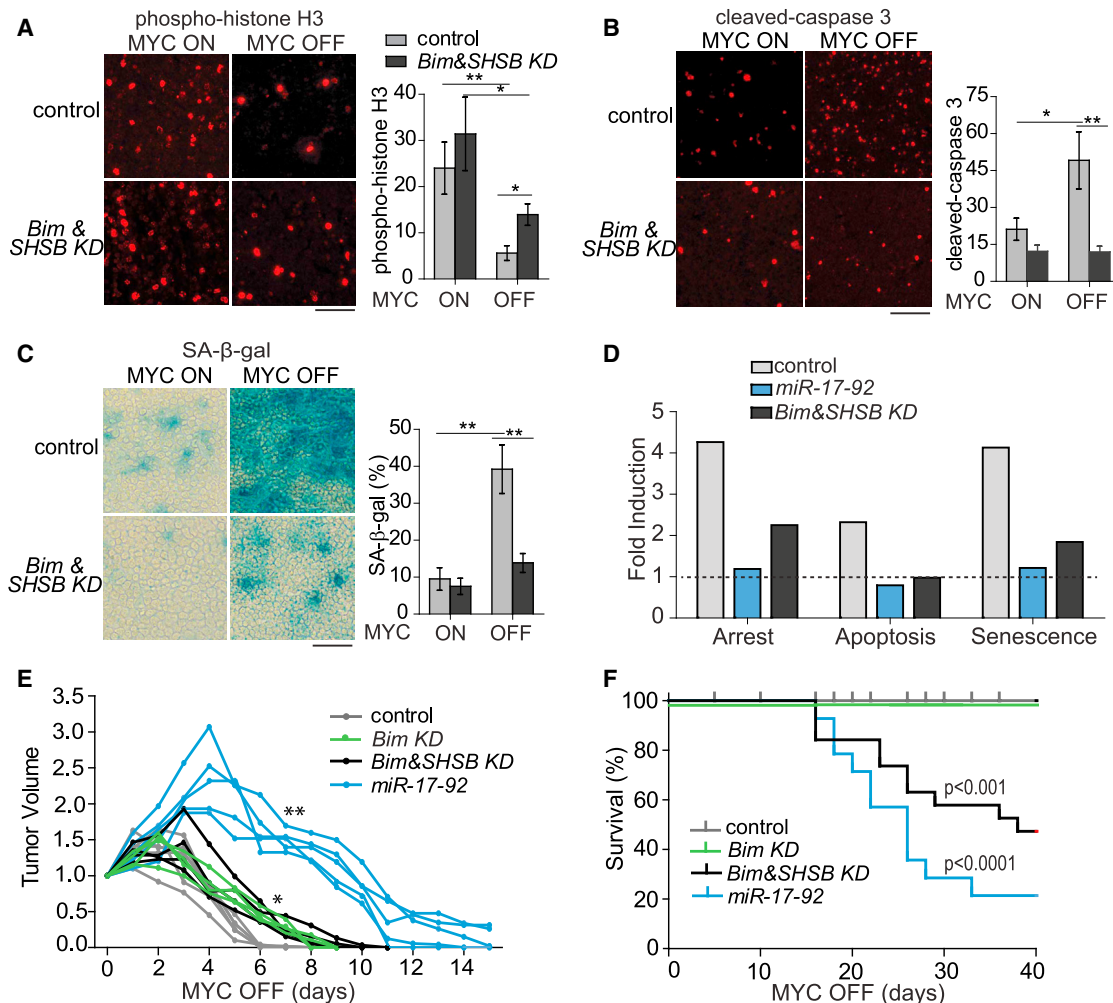
Figure 6. Suppression of *Bim* and the Chromatin Modifiers Delays Proliferative Arrest, Blocks Apoptosis and Senescence, and Abrogates Sustained Tumor Regression upon MYC Inactivation In Vivo

(A) Phospho-histone H3 staining showing cells in the metaphase of the cell cycle 4 days after MYC inactivation. The y axis denotes the number of positive staining cells per 40× magnification field.

(B) Cleaved-caspase 3 showing apoptotic cells 4 days after MYC inactivation. The y axis denotes the number of positive staining cells per 40× magnification field.

(C) SA-β-gal staining at 4 days after MYC inactivation. The y axis denotes the percentage of area with positive SA-β-gal staining, and n = 3. For panels (A)–(C), the results are presented as mean ± SEM. Student's t test: *$p < 0.05$; **$p < 0.01$. Scale bars, 50 μm.

(D) Comparison of in vivo induction of proliferative arrest, apoptosis, and senescence upon MYC inactivation in control lymphomas, lymphomas with retroviral *miR-17-92*, and lymphomas with knockdown of *Bim* and *SHSB*. To calculate the fold induction of proliferative arrest, we used the MYC ON/MYC OFF ratio for phospho-histone H3 staining. To calculate the fold induction of apoptosis and senescence, we used the MYC OFF/MYC ON ratio of cleaved-caspase-3 and SA-β-gal staining, respectively. The fold induction was computed by combining data presented in Figures 2 and 6A–6C. The dashed line indicates no induction.

(E) In vivo regression of transplanted tumors in severe combined immunodeficiency mouse host; n = 4–6. For *miR-17-92* versus control, **$p < 0.05$ for days 3–14. For *Bim* KD or *Bim&SHSB* KD versus control, *$p < 0.05$ for days 5–8. The comparisons were made with the two-tailed Student's t test.

(F) In vivo tumor reoccurrence upon MYC inactivation in syngeneic wild-type FVB/N mice. Tumor bearing mice were monitored for 6 weeks of doxycycline administration in drinking water. The mice were euthanized when the diameters of the relapsed tumors reach 1 cm; n = 10–16.

Log rank test: $p < 0.001$ for tumors with *Bim&SHSB* KD, and $p < 0.0001$ for tumors with *miR-17-92* expression. See also Figure S6.

modifiers and apoptosis regulators involved in apoptosis and senescence (Figure 7). Hence, MYC inactivation leads to a change in the neoplastic state.

MYC has been shown before to modulate global euchromatin structure that may contribute to self-renewal and pluripotency, but the specific mechanism has been elusive (Knoepfler et al., 2006; Varlakhanova and Knoepfler, 2009). Our finding that

MYC, through *miR-17-92*, regulates the heterochromatin formation may provide an explanation. Hence, MYC suppression in cancer cells results in irreversible changes in gene expression and the permanent loss of a neoplastic phenotype (Jain et al., 2002). We infer that MYC's ability to sustain autonomous proliferation, self-renewal, and survival is mediated through a *miR-17-92*-dependent chromatin regulatory and survival switch. The
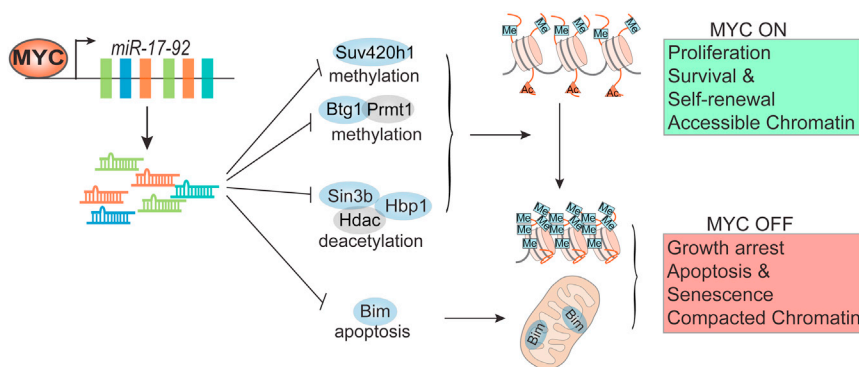
Figure 7. Model Illustrating that MYC, through *miR-17-92* and Its Target Genes, Controls a Chromatin Regulatory and Survival Switch that Is Required to Sustain a Neoplastic State

MYC through *miR-17-92* suppresses specific genes to maintain autonomous proliferation, self-renewal, and survival. Correspondingly, MYC inactivation induces a loss of neoplastic features as a consequence of restoration of proliferative arrest, apoptosis, and senescence.

shutoff of this epigenetic switch contributes to the mechanism of MYC-associated oncogene addiction.

EXPERIMENTAL PROCEDURES

Cell Lines, DNA Constructs, and Viruses

Conditional lymphoma and leukemia cell lines were derived from $E\mu$-tTA/tet-O-MYC mice. MYC inactivation was achieved with doxycycline treatment. The *miR-17-92* was cloned into pMSCV retroviral vectors. Virus production and infection of tumor cells was performed as previously reported (Wu et al., 2007). Construction of the 3′ UTR luciferase reporters and the shRNAs can be found in the Supplemental Experimental Procedures.

Tumor Transplantation

All animal experiments were approved by Stanford University's Administrative Panel on Laboratory Animal Care (APLAC) and in accordance with national guidelines. The conditional MYC lymphoma cell line was transplanted into host mice and allowed to grow to 1.5 cm in diameter before MYC inactivation with doxycycline. Tumor diameters were measured with a caliper. Tumor volume (V) was calculated as: $V = ab^2/2$, where a indicates length (millimeters) and b indicates breadth (millimeters). Further details can be found in the Supplemental Experimental Procedures.

Flow Cytometry, MicroRNA Quantification, Western Blot, Immunohistochemistry, SA-β-gal Staining, and Chromatin Immunoprecipitation

The microRNAs were quantified with TaqMan microRNA assay kits (Applied Biosystems). Western blotting, immunofluorescence, SA-β-gal staining, and chromatin immunoprecipitation were performed as described (van Oevelen et al., 2010; Wu et al., 2007). Details can be found in the Supplemental Experimental Procedures.

Microarray Analysis

Control and *miR-17-92*-expressing cell lines were used for the microarray analysis. MYC ON and MYC OFF samples were collected at 0 hr and 48 hr after MYC inactivation. Details of the microarray analysis can be found in the Supplemental Experimental Procedures.

Multiple Knockdown with shRNAs

The individual knockdown of *miR-17-92* target genes was accomplished using MSCV-LTRmiR30-PIG (LMP) shRNAs (OpenBiosystems). For multiple knockdown, the shRNAs were cloned into vectors with different drug selection markers to allow for simultaneous knockdown of multiple genes in the same cell. Further details can be found in the Supplemental Experimental Procedures.

ACCESSION NUMBERS

The Gene Expression Omnibus database accession number for the microarray analysis reported in this paper is GSE57507.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.ccr.2014.06.014.

REFERENCES

Berthet, C., Guéhenneux, F., Revol, V., Samarut, C., Lukaszewicz, A., Dehay, C., Dumontet, C., Magaud, J.P., and Rouault, J.P. (2002). Interaction of PRMT1 with BTG/TOB proteins in cell signalling: molecular analysis and functional aspects. Genes Cells *7*, 29–39.

Braig, M., Lee, S., Loddenkemper, C., Rudolph, C., Peters, A.H., Schlegelberger, B., Stein, H., Dörken, B., Jenuwein, T., and Schmitt, C.A. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. Nature *436*, 660–665.

Bui, T.V., and Mendell, J.T. (2010). Myc: Maestro of MicroRNAs. Genes Cancer *1*, 568–575.

Chapman, P.B., Hauschild, A., Robert, C., Haanen, J.B., Ascierto, P., Larkin, J., Dummer, R., Garbe, C., Testori, A., Maio, M., et al.; BRIM-3 Study Group (2011). Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N. Engl. J. Med. *364*, 2507–2516.

Chin, L., Tam, A., Pomerantz, J., Wong, M., Holash, J., Bardeesy, N., Shen, Q., O'Hagan, R., Pantginis, J., Zhou, H., et al. (1999). Essential role for oncogenic Ras in tumour maintenance. Nature *400*, 468–472.

Choi, P.S., van Riggelen, J., Gentles, A.J., Bachireddy, P., Rakhra, K., Adam, S.J., Plevritis, S.K., and Felsher, D.W. (2011). Lymphomas that recur after MYC suppression continue to exhibit oncogene addiction. Proc. Natl. Acad. Sci. USA *108*, 17432–17437.

D'Cruz, C.M., Gunther, E.J., Boxer, R.B., Hartman, J.L., Sintasath, L., Moody, S.E., Cox, J.D., Ha, S.I., Belka, G.K., Golant, A., et al. (2001). c-MYC induces mammary tumorigenesis by means of a preferred pathway involving spontaneous Kras2 mutations. Nat. Med. *7*, 235–239.

Dang, C.V. (2012). MYC on the path to cancer. Cell *149*, 22–35.

David, G., Grandinetti, K.B., Finnerty, P.M., Simpson, N., Chu, G.C., and Depinho, R.A. (2008). Specific requirement of the chromatin modifier mSin3B in cell cycle exit and cellular differentiation. Proc. Natl. Acad. Sci. USA *105*, 4168–4172.

Druker, B.J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G.M., Fanning, S., Zimmermann, J., and Lydon, N.B. (1996). Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat. Med. *2*, 561–566.

Egle, A., Harris, A.W., Bouillet, P., and Cory, S. (2004). Bim is a suppressor of Myc-induced mouse B cell leukemia. Proc. Natl. Acad. Sci. USA *101*, 6164–6169.

Felsher, D.W. (2008). Oncogene addiction versus oncogene amnesia: perhaps more than just a bad habit? Cancer Res. *68*, 3081–3086, discussion 3086.

Felsher, D.W., and Bishop, J.M. (1999). Reversible tumorigenesis by MYC in hematopoietic lineages. Mol. Cell *4*, 199–207.

Fraga, M.F., Ballestar, E., Villar-Garea, A., Boix-Chornet, M., Espada, J., Schotta, G., Bonaldi, T., Haydon, C., Ropero, S., Petrie, K., et al. (2005). Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat. Genet. *37*, 391–400.

Gossen, M., and Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc. Natl. Acad. Sci. USA *89*, 5547–5551.

Grandinetti, K.B., Jelinic, P., DiMauro, T., Pellegrino, J., Fernández Rodríguez, R., Finnerty, P.M., Ruoff, R., Bardeesy, N., Logan, S.K., and David, G. (2009). Sin3B expression is required for cellular senescence and is up-regulated upon oncogenic stress. Cancer Res. *69*, 6430–6437.

Greer, E.L., and Shi, Y. (2012). Histone methylation: a dynamic mark in health, disease and inheritance. Nat. Rev. Genet. *13*, 343–357.

Guccione, E., Martinato, F., Finocchiaro, G., Luzi, L., Tizzoni, L., Dall' Olio, V., Zardo, G., Nervi, C., Bernard, L., and Amati, B. (2006). Myc-binding-site recognition in the human genome is determined by chromatin context. Nat. Cell Biol. *8*, 764–770.

Guney, I., and Sedivy, J.M. (2006). Cellular senescence, epigenetic switches and c-Myc. Cell Cycle *5*, 2319–2323.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646–674.

He, L., Thomson, J.M., Hemann, M.T., Hernando-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S.W., Hannon, G.J., and Hammond, S.M. (2005). A microRNA polycistron as a potential human oncogene. Nature *435*, 828–833.

Huettner, C.S., Zhang, P., Van Etten, R.A., and Tenen, D.G. (2000). Reversibility of acute B-cell leukaemia induced by BCR-ABL1. Nat. Genet. *24*, 57–60.

Jain, M., Arvanitis, C., Chu, K., Dewey, W., Leonhardt, E., Trinh, M., Sundberg, C.D., Bishop, J.M., and Felsher, D.W. (2002). Sustained loss of a neoplastic phenotype by brief inactivation of MYC. Science *297*, 102–104.

Karlsson, A., Giuriato, S., Tang, F., Fung-Weier, J., Levan, G., and Felsher, D.W. (2003). Genomically complex lymphomas undergo sustained tumor regression upon MYC inactivation unless they acquire novel chromosomal translocations. Blood *101*, 2797–2803.

Kim, J., Lee, J.H., and Iyer, V.R. (2008). Global identification of Myc target genes reveals its direct role in mitochondrial biogenesis and its E-box usage in vivo. PLoS ONE *3*, e1798.

Knoepfler, P.S., Zhang, X.Y., Cheng, P.F., Gafken, P.R., McMahon, S.B., and Eisenman, R.N. (2006). Myc influences global chromatin structure. EMBO J. *25*, 2723–2734.

Kota, J., Chivukula, R.R., O'Donnell, K.A., Wentzel, E.A., Montgomery, C.L., Hwang, H.W., Chang, T.C., Vivekanandan, P., Torbenson, M., Clark, K.R.,

et al. (2009). Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell *137*, 1005–1017.

Ladanyi, M., and Pao, W. (2008). Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Mod. Pathol. *21* (*Suppl 2*), S16–S22.

Lin, W.J., Gary, J.D., Yang, M.C., Clarke, S., and Herschman, H.R. (1996). The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. J. Biol. Chem. *271*, 15034–15044.

Lin, C.Y., Lovén, J., Rahl, P.B., Paranal, R.M., Burge, C.B., Bradner, J.E., Lee, T.I., and Young, R.A. (2012). Transcriptional amplification in tumor cells with elevated c-Myc. Cell *151*, 56–67.

Lowe, S.W., Cepero, E., and Evan, G. (2004). Intrinsic tumour suppression. Nature *432*, 307–315.

Lu, X., Simon, M.D., Chodaparambil, J.V., Hansen, J.C., Shokat, K.M., and Luger, K. (2008). The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. Nat. Struct. Mol. Biol. *15*, 1122–1124.

Lundin, C., Hjorth, L., Behrendtz, M., Nordgren, A., Palmqvist, L., Andersen, M.K., Biloglav, A., Forestier, E., Paulsson, K., and Johansson, B. (2012). High frequency of BTG1 deletions in acute lymphoblastic leukemia in children with down syndrome. Genes Chromosomes Cancer *51*, 196–206.

Mu, P., Han, Y.C., Betel, D., Yao, E., Squatrito, M., Ogrodowski, P., de Stanchina, E., D'Andrea, A., Sander, C., and Ventura, A. (2009). Genetic dissection the miR-17~92 cluster of microRNAs in Myc-induced B-cell lymphomas. Genes Dev. *23*, 2806–2811.

Nardella, C., Clohessy, J.G., Alimonti, A., and Pandolfi, P.P. (2011). Pro-senescence therapy for cancer treatment. Nat. Rev. Cancer *11*, 503–511.

Nelson, D.M. (2012). Chromatin modification contributes to senescence associated proliferation arrest. (Glasgow: University of Glasgow).

Nie, Z., Hu, G., Wei, G., Cui, K., Yamane, A., Resch, W., Wang, R., Green, D.R., Tessarollo, L., Casellas, R., et al. (2012). c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell *151*, 68–79.

O'Donnell, K.A., Wentzel, E.A., Zeller, K.I., Dang, C.V., and Mendell, J.T. (2005). c-Myc-regulated microRNAs modulate E2F1 expression. Nature *435*, 839–843.

Richter-Larrea, J.A., Robles, E.F., Fresquet, V., Beltran, E., Rullan, A.J., Agirre, X., Calasanz, M.J., Panizo, C., Richter, J.A., Hernandez, J.M., et al. (2010). Reversion of epigenetically mediated BIM silencing overcomes chemoresistance in Burkitt lymphoma. Blood *116*, 2531–2542.

Roninson, I.B. (2003). Tumor cell senescence in cancer treatment. Cancer Res. *63*, 2705–2715.

Sander, S., Bullinger, L., Klapproth, K., Fiedler, K., Kestler, H.A., Barth, T.F., Möller, P., Stilgenbauer, S., Pollack, J.R., and Wirth, T. (2008). MYC stimulates EZH2 expression by repression of its negative regulator miR-26a. Blood *112*, 4202–4212.

Schlosser, I., Hölzel, M., Hoffmann, R., Burtscher, H., Kohlhuber, F., Schuhmacher, M., Chapman, R., Weidle, U.H., and Eick, D. (2005). Dissection of transcriptional programmes in response to serum and c-Myc in a human B-cell line. Oncogene *24*, 520–524.

Shachaf, C.M., Kopelman, A.M., Arvanitis, C., Karlsson, A., Beer, S., Mandl, S., Bachmann, M.H., Borowsky, A.D., Ruebner, B., Cardiff, R.D., et al. (2004). MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. Nature *431*, 1112–1117.

Swanson, K.A., Knoepfler, P.S., Huang, K., Kang, R.S., Cowley, S.M., Laherty, C.D., Eisenman, R.N., and Radhakrishnan, I. (2004). HBP1 and Mad1 repressors bind the Sin3 corepressor PAH2 domain with opposite helical orientations. Nat. Struct. Mol. Biol. *11*, 738–746.

van Oevelen, C., Bowman, C., Pellegrino, J., Asp, P., Cheng, J., Parisi, F., Micsinai, M., Kluger, Y., Chu, A., Blais, A., et al. (2010). The mammalian Sin3 proteins are required for muscle development and sarcomere specification. Mol. Cell. Biol. *30*, 5686–5697.

Varlakhanova, N.V., and Knoepfler, P.S. (2009). Acting locally and globally: Myc's ever-expanding roles on chromatin. Cancer Res. *69*, 7487–7490.

Ventura, A., Young, A.G., Winslow, M.M., Lintault, L., Meissner, A., Erkeland, S.J., Newman, J., Bronson, R.T., Crowley, D., Stone, J.R., et al. (2008).

Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. Cell *132*, 875–886.

Waanders, E., Scheijen, B., van der Meer, L.T., van Reijmersdal, S.V., van Emst, L., Kroeze, Y., Sonneveld, E., Hoogerbrugge, P.M., van Kessel, A.G., van Leeuwen, F.N., and Kuiper, R.P. (2012). The origin and nature of tightly clustered BTG1 deletions in precursor B-cell acute lymphoblastic leukemia support a model of multiclonal evolution. PLoS Genet. *8*, e1002533.

Walz, S., Eilers, M., Eisenman, R.N., and Dang, C.V. (2013). Unlocking the mysterious mechanisms of Myc. Nat. Med. *19*, 26–27.

Weinstein, I.B. (2002). Cancer. Addiction to oncogenes—the Achilles heal of cancer. Science *297*, 63–64.

Wu, C.H., van Riggelen, J., Yetil, A., Fan, A.C., Bachireddy, P., and Felsher, D.W. (2007). Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. Proc. Natl. Acad. Sci. USA *104*, 13028–13033.

Xiao, C., Srinivasan, L., Calado, D.P., Patterson, H.C., Zhang, B., Wang, J., Henderson, J.M., Kutok, J.L., and Rajewsky, K. (2008). Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat. Immunol. *9*, 405–414.

Zeller, K.I., Zhao, X., Lee, C.W., Chiu, K.P., Yao, F., Yustein, J.T., Ooi, H.S., Orlov, Y.L., Shahab, A., Yong, H.C., et al. (2006). Global mapping of c-Myc binding sites and target gene networks in human B cells. Proc. Natl. Acad. Sci. USA *103*, 17834–17839.