Title: B-cell receptor-driven MALT1 activity regulates MYC signaling in mantle cell lymphoma.
Authors: Dai B, Grau M, Juilland M, Klener P, Höring E, Molinsky J, Schimmack G, Aukema SM, Hoster E, Vogt N, Staiger AM, Erdmann T, Xu W, Erdmann K, Dzyuba N, Madle H, Berdel WE, Trneny M, Dreyling M, Jöhrens K, Lenz P, Rosenwald A, Siebert R, Tzankov A, Klapper W, Anagnostopoulos I, Krappmann D, Ott G, Thome M, Lenz G
Journal: Blood
Year: 2017 Jan 19
Volume: 129
Issue: 3
Pages: 333-346
DOI: 10.1182/blood-2016-05-718775

B-cell receptor driven MALT1 activity regulates MYC signaling in mantle cell lymphoma

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Short title: MALT1 and MYC signaling in MCL

Scientific category: LYMPHOID NEOPLASIA
Word count manuscript: 3990
Abstract word count: 183
Number of figures: 7
Number of references: 44

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Key points

- MALT1 protease activity stabilizes MYC
- The MALT1-MYC network might represent a therapeutic target for MCL patients

Abstract

Mantle cell lymphoma (MCL) is a mature B-cell lymphoma characterized by poor clinical outcome. Recent studies revealed the importance of B-cell receptor (BCR) signaling in maintaining MCL survival. However, it remains unclear which role MALT1, an essential component of the CARD11-BCL10-MALT1 (CBM) complex that links BCR signaling to the nuclear factor kappa-B (NF-κB) pathway, plays in the biology of MCL. Here we show that a subset of MCLs is addicted to MALT1, as its inhibition by either RNA or pharmacologic interference induced cytotoxicity both in vitro and in vivo. Gene expression profiling following MALT1 inhibition demonstrated that MALT1 controls a MYC-driven gene expression network predominantly through increasing MYC protein stability. Thus, our analyses identify a previously unappreciated regulatory mechanism of MYC expression. Investigating primary mouse splenocytes, we could demonstrate that MALT1-induced MYC regulation is not restricted to MCL, but represents a common mechanism. MYC itself is pivotal for MCL survival as its downregulation and pharmacologic inhibition induced cytotoxicity in all MCL models. Collectively, these results provide a strong mechanistic rationale to investigate the therapeutic efficacy of targeting the MALT1-MYC axis in MCL patients.

Introduction

Mantle cell lymphoma (MCL) is characterized by an aggressive clinical course and short overall survival.[1] Different cytomorphological variants can be distinguished and especially, blastic variants are associated with poor overall survival.[2-4] Besides clinical factors summarized in the mantle cell lymphoma international prognostic index (MIPI), high cell proliferation has been identified as a major prognostic factor associated with adverse outcome.[5-7]

Pathogenetically MCL is characterized by cyclin D1 overexpression due to the chromosomal translocation t(11;14)(q13;q32).[8] In addition, various secondary genetic aberrations activating different pathways have been elucidated.[9,10] Recently, constitutive activation of B-cell receptor (BCR) signaling and downstream activation of the nuclear factor kappa-B (NF-κB) pathway have been identified to be critical for survival of MCL subsets.[11,12] Upon BCR stimulation MALT1 and BCL10 are recruited to CARD11 resulting in the formation of the CARD11-BCL10-MALT1 (CBM) complex and NF-κB activation.[13,14] Additionally, the protease activity of MALT1 is enhanced leading to cleavage of NF-κB inhibitors such as A20 and RelB.[15,16] Other known MALT1 substrates include BCL10, CYLD, Regnase-1, Roquin-1, Roquin-2, and HOIL1.[17-25]

Preliminary data suggested that MALT1 is constitutively activated in subsets of MCL.[11] However, its precise role in the pathogenesis of MCL remains unknown. Thus, we investigated the role of MALT1 in the biology of MCL in the current study.

Methods

Patient samples, immunohistochemistry and fluorescence in situ hybridization (FISH)

CD20+ MCL cells were separated from peripheral blood mononuclear cells (PBMCs; patient samples #1 and #2) or cell suspensions from lymph nodes (patient samples #3-5) of MCL patients by CD20 magnetic-activated cell sorting (Miltenyi Biotec, Bergisch Gladbach, Germany).

The immunohistochemical protocols are summarized in the Supplemental Material and Methods. FISH was performed as described.[26,27]

Cell culture, retroviral constructs and transductions

The experiments were performed as described.[28-30] Protocols are available in the Supplemental Material and Methods. The sequences of the utilized small hairpin RNAs (shRNAs) are summarized in Supplemental Table 1.

Viability assay, analysis of cell cycle, apoptosis and proliferation

The experiments were performed as described.[29,31,32] Protocols are available in the Supplemental Material and Methods.

Isolation and stimulation of mouse splenocytes

Protocols are available in the Supplemental Material and Methods.

In vivo xenograft mouse studies

The in vivo xenograft mouse studies were done as described.[33] Protocols are available in the Supplemental Material and Methods.

Gene expression profiling

Gene expression profiling was performed 24, 30, 36, 42, 48, and 54 hours following treatment with z-VRPR-fmk or DMSO in Mino and Rec-1 cells and analyzed as described in Supplemental Material and Methods.[32,34,35] The gene expression data has been deposited in the GEO database (http://www.ncbi.nlm.nih.gov/geo; accession number GSE81552).

Quantitative PCR

Quantitative PCR was performed as described using predesigned assays (Applied Biosystems, Carlsbad, CA, USA).[29]

Western blotting and analysis of MYC stability

Protocols are available in the Supplemental Material and Methods.

Results

MALT1 is expressed and activated in MCL

To assess if MALT1 is expressed in MCL, we determined its expression in 60 primary samples by immunohistochemistry. To establish the immunohistochemical assay, we stained five reactive lymph node and tonsil specimens. MALT1 was expressed in both B- and T-cell areas of the lymph node, albeit to varying degrees. The germinal center (GC) B-cells were strongly positive and staining was accentuated in the dark zone of the GC (Supplemental Figure 1). 56/60 (93%) of MCL cases stained positive for MALT1 and showed a diffuse cytoplasmic expression that was detectable in virtually all MCL cells (Figure 1A-B). We compared this expression pattern to other aggressive lymphomas by staining 81 primary DLBCL samples. We determined the

molecular DLBCL subtype by applying the Hans algorithm[36] and identified 34 GCB and 47 non-GCB DLBCLs. All 34 GCB as well as 46 out of the 47 (98%) non-GCB DLBCLs expressed MALT1 suggesting that different B-cell lymphoma subtypes express MALT1.

MALT1 functions as a protease and therefore its proteolytic activity is determining its biologic function. Thus, to determine MALT1 activity in primary MCL samples, we prepared cell lysates from CD20+ MCL cells that were isolated from either PBMCs or cell suspensions from affected lymph nodes. MALT1 expression was found in all five primary MCL samples, whereas CYLD cleavage as a direct marker of MALT1 proteolytic activity[22] was detectable in four out of five samples (Figure 1C).

To investigate MALT1 activity in additional MCLs, we analyzed ten established cell lines. MALT1 was expressed in all lines assessed by Western blotting (Figure 1D). Five cell lines (Mino, Jeko-1, Rec-1, SP49, and SP53) had detectable levels of cleaved forms of CYLD, RelB, A20, and BCL10 indicating constitutive MALT1 activity. In contrast, the other cell lines did not show cleavage of MALT1 targets suggesting absent MALT1 proteolytic activity (Figure 1E). Collectively, these data implicate that MALT1 is constitutively active in a substantial number of MCLs and that MCLs can be divided into two distinct subgroups based on their MALT1 activation status.

Activation of MALT1 in MCL is caused by constitutive BCR signaling

Next, we investigated the mechanisms leading to MALT1 activation in MCL. As BCR signaling is an activator of MALT1, we knocked down CD79A and CARD11 as central components of the BCR cascade to investigate the effects on MALT1 activity. Cleavage of the MALT1 targets CYLD, RelB, and BCL10 was significantly decreased in two MALT1-activated cell lines (Jeko-1 and Rec-1), but unaffected in two MALT1-

inactive MCL cell lines (Maver-1 and Z-138) after CD79A and CARD11 knockdown using specific shRNAs, respectively (Figure 2A). These results suggest that MALT1 is activated through constitutive BCR signaling in MCL. Interestingly, shRNA-mediated knockdown of CD79A, CARD11 or the CBM complex component BCL10 induced toxicity in Jeko-1 and Rec-1 cells, but not in Maver-1 and Z-138 cells, indicating dependency on BCR signaling only in MALT1-activated MCLs (Figure 2B and Supplemental Figure 2A-B). These results were further confirmed by experiments showing that all MALT1-activated MCL cell lines were sensitive to the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib, whereas all MALT1-inactive models did not respond (Figure 2C).

Downregulation of MALT1 is toxic to MALT1-activated MCLs in vitro and in vivo

To elucidate the functional significance of MALT1 in MCL, we knocked down its expression using different *MALT1*-specific shRNAs. Both shRNAs significantly decreased *MALT1* mRNA and protein levels after 48 hours (Figure 3A-B). Transduction of *MALT1* shRNAs induced cytotoxicity in MALT1-activated MCLs while it did not affect survival of any of the MALT1-inactive models (Figure 3C). To demonstrate that *MALT1* shRNA-mediated toxicity was specifically caused by MALT1 knockdown, we performed a rescue experiment by transducing Jeko-1 and Rec-1 cells with a vector carrying the *MALT1* cDNA that is not targetable by both *MALT1* shRNAs. Indeed, exogenous MALT1 expression restored growth of both MALT1-activated MCL models, indicating the specificity of our approach (Figure 3D).

We next determined if MALT1 dependency in MALT1-activated MCLs translates into an in vivo setting. To this end, we created MCL xenograft mouse models using Jeko-1 cells transduced with vectors encoding either *MALT1* shRNA #2 or a negative control shRNA. shRNA-mediated MALT1 knockdown was detectable by

Western blotting in different samples from sacrificed mice (Figure 3E). MALT1 knockdown significantly inhibited tumor growth over 12 days ($P = 1.9\times10^{-5}$ for *MALT1* shRNA vs. control shRNA on day 12; one-tailed two-sample t-test; Figure 3F), suggesting that MALT1 promotes lymphoma growth in MALT1-activated MCLs.

Next, we asked if signaling through MALT1 can be utilized therapeutically in MCL. Thus, we treated our MCL lines with the specific MALT1 inhibitor z-VRPR-fmk.[23] To confirm that z-VRPR-fmk indeed exerts its effect through inhibiting MALT1´s proteolytic activity, expression of the MALT1 targets CYLD, RelB, A20, and BCL10 were studied 48 hours after incubation with z-VRPR-fmk by immunoblotting. We detected a significant downregulation of their cleaved forms in MALT1-activated MCLs (Mino and SP53). In contrast, no changes in their expression levels were observed in the MALT1-inactive MCLs (Maver-1 and Z-138) (Figure 3G). These results confirm that z-VRPR-fmk inhibits the proteolytic function of MALT1. Subsequently, we determined cell viability seven days after z-VRPR-fmk treatment. In line with our MALT1 knockdown data, pharmacologic inhibition of MALT1 significantly reduced cell viability of MALT1-activated MCLs, whereas survival of MALT1-inactive MCLs was not affected (Figure 3H).

To obtain insights into the nature of the growth inhibitory effects of MALT1 inhibition through z-VRPR-fmk, we measured cell proliferation, the rate of apoptosis, and performed cell cycle analyses. We treated MALT1-activated (Mino) and MALT1-inactive (Z-138) MCLs with z-VRPR-fmk or DMSO. To assess proliferation, cellular divisions were determined by measuring CFSE dilutions in viable cells by flow cytometry. z-VRPR-fmk significantly downregulated proliferation of Mino cells ($P < 10^{-15}$ on day 6) , while Z-138 cells were not affected ($P = .4$ on day 6; Figure 3I). In addition, we quantified the number of cell divisions following treatment with z-VRPR-fmk. These analyses revealed that Mino cells divided 1.8 ± 0.17 or 0.9 ± 0.1 ×/d after

DMSO or z-VRPR-fmk treatment, respectively, whereas Z-138 cells divided 2.6 ± 0.03 x/d after DMSO treatment and 2.5 ± 0.04 ×/d following MALT1 inhibition. In contrast, neither changes in apoptosis (data not shown) nor cell cycle (Supplemental Figure 3) were detectable following MALT1 inhibition, indicating that z-VRPR-fmk exerts its growth inhibitory effect in MALT1-activated MCLs predominantly through reduction of cell proliferation (Figure 3I).

Inhibition of MALT1 overcomes ibrutinib resistance

The BTK inhibitor ibrutinib is effective in the treatment of relapsed/refractory MCL patients.[37] A recent study identified that the *BTK*^{C481S} mutation confers resistance to ibrutinib in MCL.[38] To investigate whether inhibition of MALT1 is able to overcome *BTK*^{C481S}-induced ibrutinib resistance, we expressed a *BTK*^{C481S} cDNA or an empty vector in all five MALT1-activated and two MALT1-inactive MCL lines (Figure 3J and Supplemental Figure 4A,C,E,G,I,K) and subsequently treated these cells with ibrutinib or z-VRPR-fmk. Introduction of the *BTK*^{C481S} mutation rescued all MALT1-activated lines from the toxic effect of ibrutinib (Figure 3K and Supplemental Figure 4B,D,F,H). In contrast, in MALT1-inactive MCLs transduction of the *BTK*^{C481S} mutant or an empty vector did not alter sensitivity to ibrutinib or z-VRPR-fmk (Figure 2C and 3H; Supplemental Figure 4J,L). These data indicate that inhibition of MALT1 might be effective in ibrutinib resistant MCLs.

MALT1 regulates MYC expression in MCL

To understand which biologic processes are regulated by MALT1 in MCL, we profiled gene expression changes after 24, 30, 36, 42, 48, and 54 hours of z-VRPR-fmk treatment in Mino cells. We identified 93 genes that were significantly downregulated ($P \leq 1\times10^{-5}$; paired t-tests over all time points) and 126 genes significantly

upregulated ($P \leq 1\times10^{-5}$) following pharmacologic MALT1 inhibition (Figure 4A; Supplemental Figure 5A; Supplemental Table 2).

To analyze the gene expression data in an unbiased manner, we performed a gene set enrichment analysis using a previously described gene expression signatures database consisting of 13,593 signatures (Supplemental Table 3). Our analysis revealed that the second most enriched downregulated signature was a previously described MYC target gene set (enrichment score = 0.841; $P \leq .001$; Figure 4B; Supplemental Figure 5B; Supplemental Table 3). In addition, various other independent MYC signatures were significantly enriched with downregulated genes and among the top downregulated signatures, suggesting that MYC expression and its gene expression network is regulated by MALT1 (Supplemental Table 3-4).

To confirm that MYC deregulation by MALT1 is a general mechanism in MCL, we further performed gene expression profiling in Rec-1 cells (Supplemental Figure 6 A-B; Supplemental Table 5). These analyses confirmed that various previously identified MYC target sets were downregulated following z-VRPR-fmk treatment (Supplemental Figure 6C-D; Supplemental Table 6-7). Additionally, the identified Mino target gene signature was significantly downregulated in Rec-1 following MALT1 inhibition (Supplemental Figure 6E) suggesting that very similar target genes are affected by MALT1 inhibition in Mino and Rec-1 cells. Finally, to confirm that the detected Mino target gene signature is also downregulated in other MCL models, we performed real-time PCR for eleven selected genes following MALT1 inhibition in both MALT1-activated and MALT1-inactive cell lines. Real-time PCR confirmed downregulation of ten out of eleven target genes in all MALT1-activated but not MALT-inactive models (Figure 4C and Supplemental Figure 7). These results suggest that the MYC target gene network seems to be controlled by MALT1 in

MCL. In contrast, previously identified NF-κB target gene signatures were not affected by MALT1 inhibition in both Mino and Rec-1 cells.

Due to the strong impact of MALT1 inhibition on the MYC expression profile, we asked whether MYC itself is regulated by MALT1. *MYC* mRNA expression was moderately suppressed by MALT1 inhibition ($\log_2$(ratio) = −.33 in Mino and $\log_2$(ratio) = −.23 in Rec-1). This result was confirmed by shRNA-mediated MALT1 knockdown that did not substantially alter *MYC* mRNA levels measured by quantitative PCR (Figure 4D). To elucidate if MYC is regulated posttranscriptionally by MALT1, we evaluated MYC protein expression after z-VRPR-fmk treatment. Immunoblotting revealed that MYC levels were reduced in MALT1-activated but not in MALT1-inactive MCLs following MALT1 inhibition (Figure 4E).

To corroborate these findings, we next investigated MYC expression levels following shRNA-mediated MALT1 knockdown in MALT1-activated and -inactive cells by Western blotting. MALT1 silencing induced a substantial decrease in MYC protein levels in MALT1-activated but not MALT1-inactive MCLs (Figure 4F). As MALT1 is activated by BCR signaling in MCL, we investigated whether inhibition of BCR signaling by ibrutinib alters MYC expression levels. To this end we treated MALT1-activated (Mino and Rec-1) and MALT1-inactive (Maver-1 and Z-138) cell lines with 5 and 10 nM ibrutinib for 12, 24, 36, and 48 hours. Ibrutinib treatment significantly decreased MYC expression in Mino and Rec-1 cells, whereas MYC levels in Maver-1 and Z-138 were unaffected (Supplemental Figure 8). Collectively, these data indicate that BCR-driven MALT1 activity regulates MYC expression.

MALT1 regulates MYC expression in primary mouse splenocytes

To elucidate if MALT1-induced regulation of MYC protein expression is relevant in other settings than MCL, we isolated primary mouse splenocytes expressing either

wild-type MALT1 (+/+) or a catalytically inactive *MALT1*^{C472A} mutant (ki/ki)[39] and subsequently stimulated the splenocytes with PMA and ionomycin for 30, 60, and 120 minutes (Figure 5A). Stimulation of MALT1 (+/+) and MALT1 (ki/ki) splenocytes was equally strong, as determined by monitoring the induction of ERK phosphorylation (Figure 5A). In contrast, CYLD cleavage, which served as a marker of MALT1 activity, was only detectable in MALT1 (+/+) splenocytes. Likewise, the stimulation-induced expression of MYC was considerably stronger in MALT1 (+/+) compared to MALT1 (ki/ki) splenocytes. Collectively, these findings suggest that MALT1 activity promotes MYC expression in activated primary lymphocytes (Figure 5A).

MALT1 stabilizes MYC expression

Next, we investigated whether MALT1 promotes MYC expression by controlling MYC protein stability. We treated Rec-1 and Mino cells with 50 µM z-VRPR-fmk or DMSO for 24 hours, followed by incubation with 10 µg/mL of the protein synthesis inhibitor cycloheximide. Immunoblotting revealed that MYC levels in Rec-1 cells declined faster following z-VRPR-fmk treatment (half-life 31.5 minutes) compared to DMSO (69.31 minutes). This was confirmed in Mino cells, in which the half-life of MYC decreased from 46.21 minutes in DMSO treated cells compared to 34.65 minutes in MALT1-inhibited cells (Figure 5B and Supplemental Figure 9A).

Next, to decipher if MALT1 affected proteasomal degradation of MYC, we assessed MYC levels in Mino, Rec-1, and SP53 cells that were incubated with z-VRPR-fmk or DMSO for 12 hours and 24 hours, respectively, followed by treatment with the proteasome inhibitor MG132 for two hours. Under these conditions, we detected a marked increase of MYC expression in z-VRPR-fmk and MG132 treated cells (Figure 5C and Supplemental Figure 9B). To confirm our MALT1 inhibitor data,

we transduced MALT1-activated (Rec-1 and SP53) and MALT1-inactive (Maver-1 and Z-138) MCLs with our two *MALT1* shRNAs and treated these cells with either DMSO or MG132. MG132 treatment significantly increased MYC expression levels following shRNA-mediated MALT1 knockdown in MALT1-activated MCLs (Figure 5D). Collectively, these results indicate that MALT1 increases MYC stability posttranslationally by preventing its proteasomal degradation.

MCLs depend on MYC signaling

Our analyses implicated that MALT1 regulates MYC expression. To validate these findings, we determined MYC expression in our cell lines and in MCL patient samples. All MCL cell lines expressed MYC protein by Western blotting irrespective of their MALT1 activation status (Figure 6A and Supplemental Figure 10), whereas the four MALT1-activated primary MCLs showed higher MYC expression levels compared to the MALT1-inactive specimen (Figure 1C).

Next, we determined MYC expression in 234 primary MCL samples. 104 (44.4%) samples did not show MYC expression. In contrast, 75 (32.1%) samples displayed an intermediate and 55 (23.5%) samples a high MYC positivity (Figure 6B-C). To compare the MYC staining pattern in MCL to other lymphomas, we stained 93 primary DLBCLs (10 MYC rearranged) and 7 BLs (all MYC rearranged). In BLs nuclear positivity was strong in >90% of cells. Eight out of ten MYC rearranged DLBCLs expressed MYC, while 52 out of 83 primary non-rearranged DLBCLs were MYC positive. In general, primary DLBCLs and MCLs were similar with respect to their staining intensity and more variable compared to the BL cases (Supplemental Figure 11).

To investigate if MYC expression correlates with the cytological subtype, we compared MYC expression in classical (n = 154), pleomorphic (n = 34), and blastoid

(n = 40) MCLs (Figure 6D). For six samples the cytological subtype was not available. MYC expression was significantly higher in pleomorphic ($P = 9.9\times10^{-4}$) and blastoid variants ($P = 2.6\times10^{-5}$) compared to classical MCLs. There was no difference in MYC expression between pleomorphic and blastoid MCLs ($P = .4$; Figure 6D).

To rule out that genetic aberrations involving the *MYC* locus are causative for increased MYC expression, we performed FISH in 80 MCLs with available MYC expression data. 55 (69%) of these cases were classical, 6 (8%) pleomorphic, and 13 (16%) blastoid MCLs (for six cases the cytological subtype was not available). 40 (50%) cases did not express MYC, 28 (35%) had intermediate MYC expression, whereas 12 (15%) samples had high MYC expression. Of the 80 cases, only one (1.3%) harbored a *MYC* translocation, whereas none of the cases showed a high-level *MYC* amplification, indicating that these genetic aberrations are extremely rare in MCL.

To elucidate the functional role of MYC expression in MCL, we transduced MCL cell lines with specific *MYC* shRNAs, which induced MYC downregulation 48 hours after induction (Supplemental Figure 12A). MYC knockdown was lethal to all MCL models (Figure 7A). To confirm the specificity of our approach, an exogenous *MYC* cDNA (which is not targeted by *MYC* shRNA #2) was introduced in Jeko-1, Rec-1, and SP53 cells prior to transduction with the *MYC* shRNA. Indeed, exogenous MYC expression rescued all MCL cells from shRNA-mediated toxicity (Figure 7B).

To evaluate the degree to which MYC downregulation contributes to the impaired viability of MALT1-silenced cells, we performed a rescue experiment introducing a *MYC* cDNA or an empty vector into *MALT1* shRNA-transduced Jeko-1, Rec-1, and SP53 cells. We detected a partial MYC-induced rescue in all three cell lines suggesting that MYC knockdown, at least partially, contributes to the lethal

effect of MALT1 silencing in these cells (Figure 7C; Supplemental Figure 12B). To confirm these results, we treated Jeko-1, Rec-1, SP53, and Mino cells that expressed either *MYC* cDNA or an empty vector with z-VRPR-fmk. In all cell lines we could detect a substantial MYC-induced rescue confirming that MYC downregulation is at least partially causative for the toxic effects of MALT1 inhibition in MALT1-activated MCLs (Figure 7D). To investigate whether this rescue effect is specific to z-VRPR-fmk, we determined cell viability of these exogenous MYC harboring cells after doxorubicin treatment. No resistance against doxorubicin was conferred by *MYC* cDNA expression, indicating the specificity of our findings (Supplemental Figure 12C).

To obtain insights into the nature of the growth inhibitory effects of MYC knockdown, we analyzed whether cell proliferation was negatively affected by MYC silencing. To this end, SNARF-1 staining was performed in Rec-1 and SP53 cells expressing *MYC* shRNA #1/#2. Cell divisions were compared after two days between cells with and without MYC knockdown. In both cell lines, the proliferation rate decreased substantially after MYC silencing (Figure 7E) indicating that MYC controls MCL proliferation.

To validate our in vitro findings, we stained our cohort of primary MCL samples for Ki-67 to assess proliferation. Indeed, overall Ki-67 and MYC expression correlated (r = .63; $P = 5\times10^{-27}$; Supplemental Figure 12D). Furthermore, samples with intermediate MYC expression had higher Ki-67 levels compared to MYC negative MCLs ($P = 1.1\times10^{-6}$; Figure 7F) whereas MYC positive MCLs had the highest Ki-67 levels ($P = 4.6\times10^{-24}$ vs. MYC negative MCLs and $P = 1.4\times10^{-12}$ vs. MYC intermediate MCLs; Figure 7F). This suggests that MYC regulates MCL proliferation in vivo.

Finally, we investigated if inhibiting MYC signaling can be exploited therapeutically. Cell viability was measured three days after treating MCL lines with the small molecule inhibitor 10058-F4 that inhibits MYC-MAX heterodimerization. U266 cells that do not express MYC were used as a negative control. Irrespective of MALT1 activity, three cell lines (Mino, Rec-1, and Z-138) were highly sensitive to 10058-F4. In contrast, U266 cells were virtually unaffected, whereas Maver-1 cells showed an intermediate sensitivity (Figure 7G). Taken together, these data suggest that MYC represents a promising target for future therapies of MCL patients.

Discussion

We detected a novel role of MALT1 in the biology of MCL. A substantial fraction of MCLs exhibit constitutive MALT1 activity and these MCLs are addicted to MALT1 function. In contrast, some MCLs do not show constitutive MALT1 proteolytic activity and these lymphomas do not depend on MALT1. Thus, our results indicate that MCLs can be divided into two distinct subgroups based on their MALT1 activation status.

MALT1 activity seems to be caused by constitutive BCR signaling. Recent work showed that activity of BCR signaling is correlated with increased MCL proliferation.[12] It seems conceivable that this could be caused by MALT1-induced upregulation of the oncogenic transcription factor MYC. MYC is crucial for the regulation of cell proliferation.[40] In line, pharmacologic MALT1 inhibition or shRNA-mediated MYC knockdown significantly decreased MCL proliferation. Moreover, Ki-67 expression as a marker for cell proliferation was significantly higher in primary MCL samples with MYC expression. MYC expression was detectable in more than 55% of primary MCLs indicating that MYC is frequently expressed in MCL. In our series of primary samples common genetic aberrations such as *MYC* locus

translocations or high-level amplifications that can cause upregulation of MYC were extremely rare, confirming results of previous studies.[41,42] However, given that MYC expression and MALT1 activation status did not correlate in all cell lines, additional molecular mechanisms regulate MYC expression in MCL besides MALT1 activity.

Recent work in chronic lymphocytic leukemia (CLL) has linked BCR signaling to upregulation of MYC expression, as anti-IgM-induced BCR signaling increased translation of *MYC* mRNA in primary CLL cells.[43] However, the exact molecular mechanisms how BCR signaling and MYC expression are linked were not elucidated. Our data using primary splenocytes suggest that MALT1-driven MYC expression is not restricted to MCL, but seems to be a common mechanism of MYC regulation. MALT1 seems to regulate MYC expression through different mechanisms. We detected a very moderate *MYC* downregulation on mRNA level following MALT1 knockdown or pharmacologic inhibition of MALT1. However, the predominant mechanism of MYC regulation involves control of MYC stability.

Interestingly, our gene expression data following MALT1 inhibition failed to show downregulation of previously identified NF-κB gene sets including signatures defined in MCL.[12] This finding is surprising given the role of MALT1 in activating NF-κB signaling. The protease activity of MALT1 is dispensable for initial NF-κB activation and instead promotes NF-κB signaling through cleavage of the negative NF-κB regulators RelB and A20.[15,16,44] The mechanisms why downregulation of NF-κB was not detectable by our transcriptome analyses are unclear and should be addressed in future studies.

Finally, our work revealed that the MALT1-MYC network could be exploited therapeutically in MCL patients. Despite improvements in therapy, MCL remains an incurable disease.[1] MALT1 inhibition was highly effective in all MALT1-activated models. These data warrant future clinical trials with MALT1 inhibitors in MCL

patients with constitutive MALT1 activity. Moreover, MALT1 inhibition was able to overcome *BTK*^{C481S}-induced ibrutinib resistance and might represent a novel therapeutic option for patients failing ibrutinib therapy. Similarly, MYC inhibition was lethal to MYC expressing models. These data suggest that MYC inhibition offers a promising target and a novel therapeutic strategy to overcome therapy resistance in MCL patients.

Acknowledgements

We thank André Weilemann for technical support. This work was supported by research grants to G.L. from the Deutsche Forschungsgemeinschaft (DFG), the Else Kröner-Fresenius-Stiftung, the Brigitte und Dr. Konstanze Wegener-Stiftung, the Deutsche Krebshilfe and the Swiss National Science Foundation (Sinergia grant). In addition, this work was also supported by the DFG EXC 1003 Cells in Motion - Cluster of Excellence, Münster, Germany. P.K. was supported by the Ministry of Health of the Czech Republic grant AZV 15-27757A. R.S. and W.K. were supported by the e:BIO project MMML-MYC-Sys, by the German Ministry for Education and Research, funding number 0316166B and 0316166I. A.S., E.H. and G.O. were supported by the Robert-Bosch-Foundation, Stuttgart, Germany. D.K. was supported by the Wilhelm Sander-Stiftung (2012.075.2).

Authorship

Contribution: B.D. designed research, performed experiments, analyzed data, and wrote the manuscript; M.G. performed bioinformatic and biophysical analyses; M.J., P.K., E.H., J.M., G.S., S.M.A., E.H., N.V., A.M.S., T.E., W.X., K.E., N.D., and H.M. performed and analyzed experiments; W.E.B., M.T., M.D., K.J., P.L., A.R., R.S., A.T.,

W.K., I.A., D.K., G.O., M.T. analyzed data; G.L. designed research, analyzed data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no conflict of interest.

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Figure Legends

Figure 1. MALT1 expression and activity in MCL. (A) Immunohistochemical MALT1 staining of a MALT1 positive MCL case (left picture; original magnification ×200) and a MALT1 negative MCL case (right picture; original magnification ×100). Images were captured using a Leitz DMRB microscope (Leica Microsystems, Wetzlar, Germany) equipped with Fluotar objective lenses (10×/0.30 NA, 20×/0.50 NA) and a KY-F75U digital camera (Victor, Yokohama, Japan) and were processed with the Diskus Program 4.20 (Hilgers Technical, Königswinter, Germany) that converts and exports images in jpeg file format. (B) MALT1 expression in MCLs determined by immunohistochemistry. (C) Western blot analysis of MALT1, full-length and cleaved forms of CYLD and MYC. MALT1 was highly expressed in CD20+ cells isolated from either PBMCs (patient samples #1 and #2) or lymph nodes (patient samples #3, #4, and #5) of five primary MCL patient samples. Cleaved CYLD indicating MALT1 proteolytic activity was detectable in four of five patient samples. MYC expression was higher in these four samples with activated MALT1. The MCL cell line Z-138 was used as a positive control for MALT1 expression and as a negative control for CYLD cleavage. Asterisk indicates non-specific band, which was not observed in any MALT1-activated MCL cell lines (Figure 1E). (D) Western blot analysis of MALT1 expression in MCL cell lines. MALT1 protein expression was detectable in all MCL cell lines. (E) Western blot analysis of different MALT1 targets. Cleaved forms of CYLD, RelB, A20, and BCL10 were detectable in Mino, Jeko-1, Rec-1, SP49, and SP53 cells. Asterisk indicates non-specific band.

Figure 2. Activation of MALT1 is caused by constitutive BCR signaling in MCL. (A) Western blot analysis of CD79A, CARD11, CYLD, RelB, and BCL10 following

shRNA-mediated knockdown of CD79A and CARD11, respectively. Cleavage of CYLD, RelB, and BCL10 was significantly downregulated following CD79A or CARD11 knockdown, respectively, in MALT1-activated cell lines (Jeko-1 and Rec-1), whereas none of these cleaved forms was detectable in MALT1-inactive cell lines (Maver-1 and Z-138) and the expression levels of the corresponding full-length forms were not affected. (B) shRNA-mediated knockdown of CD79A and CARD11 was toxic to the MALT1-activated cell lines Jeko-1 and Rec-1. In contrast, the MALT1-inactive cell lines Maver-1 and Z-138 were unaffected by CD79A and CARD11 knockdown. A previously described non-toxic shRNA against *MSMO1* did not induce toxicity in any cell line. Data are shown as means ± standard deviation of at least three independent experiments. (C) Cell viability of MCL cell lines after incubation with the BTK inhibitor ibrutinib. Representative results from at least three independent replicates are shown. Error bars indicate the standard deviation.

Figure 3. Subsets of MCLs are addicted to MALT1. (A) Effect of *MALT1* shRNA #1 and #2 on *MALT1* mRNA level in MALT1-activated (Jeko-1 and Rec-1) and MALT1-inactive (Maver-1 and Z-138) MCLs 48 hours after shRNA induction measured by quantitative PCR. *MALT1* mRNA levels were normalized to expression of *GAPDH*. Error bars indicate the standard deviation. (B) Effect of *MALT1* shRNA #1 and #2 on MALT1 protein in MALT1-activated (Jeko-1 and Rec-1) and MALT1-inactive (Maver-1 and Z-138) MCLs 48 hours after shRNA induction measured by Western blotting. (C) Effect of MALT1 knockdown by two independent shRNAs on viability of MCL cell lines. A previously described non-toxic shRNA against *MSMO1* did not induce toxicity in any cell line. Data are shown as means ± standard deviation of at least three independent experiments. (D) Rescue of Jeko-1 and Rec-1 cells from *MALT1* shRNA-induced toxicity by exogenous expression of a *MALT1* cDNA. Data are

shown as means ± standard deviation of at least three independent experiments. (E) Western blot analysis of MALT1 knockdown in Jeko-1 mouse xenograft tumor biopsies from cells transduced with *MALT1* shRNA #2 compared to control shRNA transduced cells (shRNA against *MSMO1*). (F) Tumor growth curve of Jeko-1 xenograft mouse models that inducibly express *MALT1* shRNA #2 (blue) or a control shRNA against *MSMO1* (red). MALT1 knockdown significantly reduced in vivo tumor growth ($P = 1.9\times10^{-5}$, *MALT1* shRNA vs. control shRNA on day 12; one-tailed two-sample t-test). Error bars indicate the standard deviation. (G) Western blot analysis of MCL cell lines, treated with z-VRPR-fmk for 48 hours, for cleavage of CYLD, RelB, A20, and BCL10 in MALT1-activated MCL cell lines (Mino and SP53) vs. MALT1-inactive MCLs (Maver-1 and Z-138). (H) Cell viability of MCL cell lines after incubation with the MALT1 inhibitor z-VRPR-fmk. Representative results from at least three independent replicates are shown. Error bars indicate the standard deviation. (I) CFSE staining after treatment with z-VRPR-fmk or DMSO was measured on day zero and after two, four, and six days. In Z-138 cells, no difference in cell proliferation was detectable ($P = .4$ on day 6). In contrast, Mino cells showed reduced proliferation after treatment with z-VRPR-fmk ($P < 10^{-15}$ on day 6). Representative results from at least three independent replicates are shown. (J) Western blotting for FLAG and BTK following transduction of Mino cells with either a BTK^{C481S} cDNA or an empty vector. (K) Determination of cell viability of Mino cells expressing either an empty vector (red) or a BTK^{C481S} cDNA (blue) following treatment with ibrutinib or z-VRPR-fmk. Representative results from at least three independent replicates are shown. Error bars indicate the standard deviation.

* $P < .05$, ** $P < .01$, *** $P < .001$.

Figure 4. MALT1 regulates the gene expression network of MYC in MCL. (A) Gene expression profiling following pharmacologic inhibition of the proteolytic MALT1 activity using z-VRPR-fmk vs. DMSO in Mino cells. Changes of gene expression were profiled at the indicated time points following treatment with z-VRPR-fmk. Each time point depicted the mean of log_2-transformed expression ratios for two replicates. Gene expression changes were depicted according to the color scale shown. Genes that are involved in critical biological processes are highlighted. (B) Gene set enrichment analysis of a previously described MYC gene expression signature. The MYC signature was significantly enriched with genes that are downregulated following pharmacologic MALT1 inhibition using z-VRPR-fmk in Mino cells. (C) Expression levels of MALT1 target genes in MALT1-activated and -inactive MCL cell lines determined by quantitative PCR. mRNA levels of *PFKM*, *CARD9*, and *MLKL* were normalized to expression of *GAPDH*. Error bars indicate the standard deviation. (D) *MYC* mRNA levels in Rec-1 and SP53 cells following shRNA-mediated knockdown of MALT1 as measured by quantitative PCR. *MYC* mRNA levels were normalized to expression of *GAPDH*. Error bars indicate the standard deviation. (E) Treatment with z-VRPR-fmk downregulated MYC protein in the MALT1-activated MCL cell lines Mino and Rec-1. In contrast, in the MALT1-inactive cell lines Maver-1 and Z-138 MYC was not affected by inhibition of MALT1 activity. Accumulation of full-length BCL10 in MALT1-activated MCL models after treatment with z-VRPR-fmk was used as a surrogate marker of MALT1 inhibition. (F) *MALT1* shRNA #1 and #2 downregulated MYC protein in MALT1-activated MCLs (Rec-1 and SP53), but not in MALT1-inactive MCLs (Maver-1 and Z-138) at the indicated time points after shRNA induction as measured by Western blotting.

N.D., not detectable, N.S., not significant, * $P < .05$, ** $P < .01$, *** $P < .001$.

Figure 5. MYC is stabilized by MALT1 function. (A) Primary mouse splenocytes expressing either wild-type MALT1 (+/+) or a catalytically inactive MALT1 mutant (ki/ki) were stimulated with PMA and ionomycin for the indicated time points. Stimulation efficiency and MALT1 activation were assessed by Western blotting using anti-p-ERK and anti-CYLD antibodies, respectively. (B) Rec-1 and Mino cells were first treated with z-VRPR-fmk or DMSO for 24 hours and subsequently with cycloheximide (CHX). MYC protein expression was assessed by Western blot using samples collected at the indicated time points. In both cell lines, MALT1 inhibition resulted in a reduced half-life of MYC protein. (C) Mino, Rec-1 and SP53 cells were treated with z-VRPR-fmk or DMSO and subsequently with MG132 or DMSO. MYC protein levels were increased by MG132 treatment as evaluated by Western blotting. (D) In Rec-1, SP53, Maver-1 and Z-138 cells either a control shRNA against *MSMO1* or one of the two *MALT1* shRNAs were induced with doxycycline for 24 hours. Subsequently cells were treated with MG132 or DMSO. MYC protein levels were increased by MG132 treatment in MALT1-activated MCLs (Rec-1 and SP53), but not in MALT1-inactive MCLs (Maver-1 and Z-138) as evaluated by Western blotting.

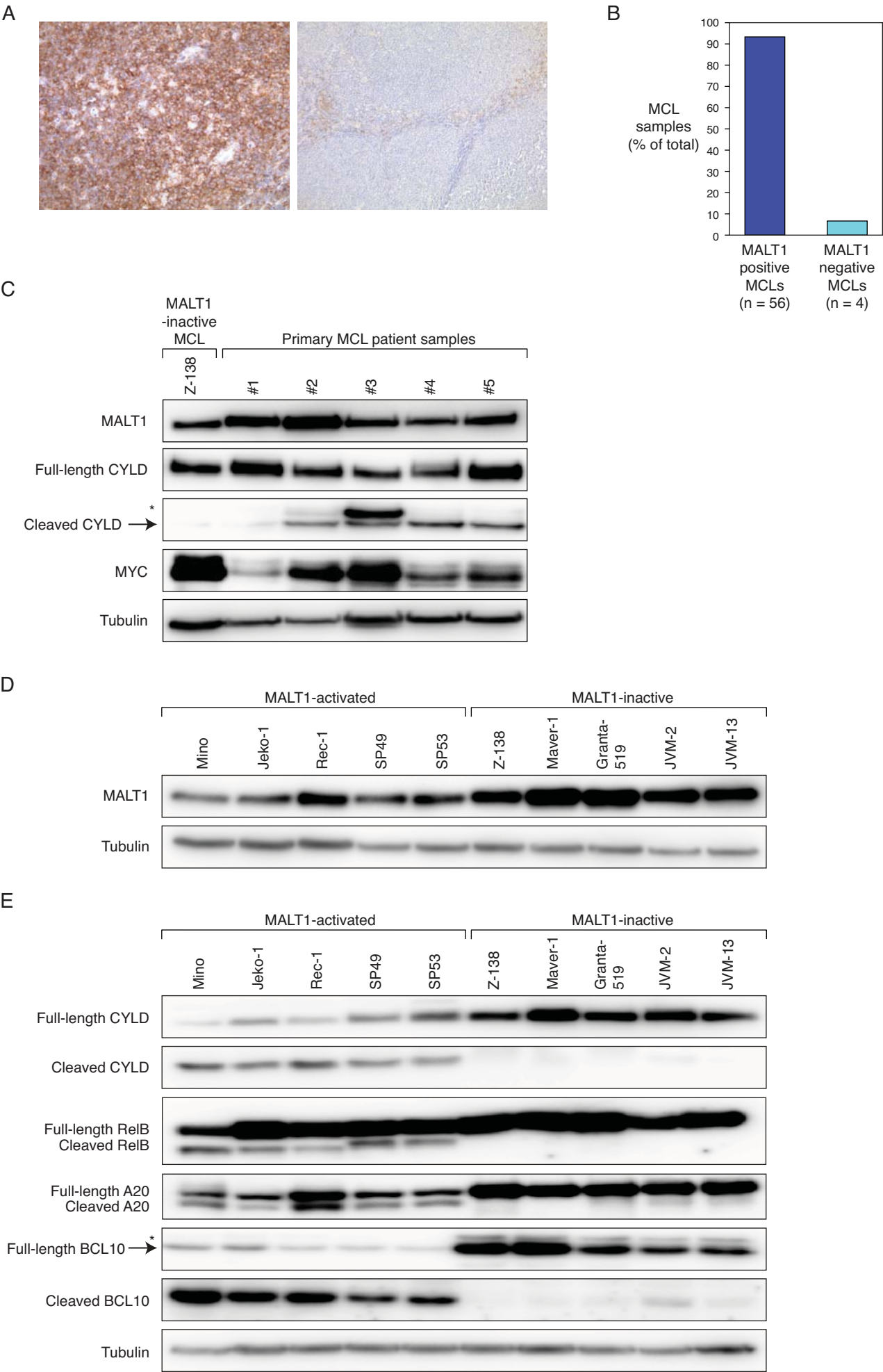
Figure 6. MYC expression in MCL. (A) Western blot analysis of MYC expression in ten MCL cell lines and in the MM cell line U266. All MCL cell lines had detectable MYC expression compared to the negative control U266. (B) Immunohistochemical MYC staining of a MYC positive MCL case (left picture; original magnification ×400) and a MYC negative MCL case (right picture; original magnification ×400). Images were captured using an Olympus BX51 microscope (Olympus, Tokio, Japan) equipped with an Olympus DP73 camera (Olympus) and were processed with Olympus cellSens software (Olympus). (C) Frequency of MYC expression in MCL. (D) Mean MYC expression in cytological MCL variants. MYC expression was

significantly higher in pleomorphic and blastoid variants compared to classical MCLs. Error bars indicate the standard errors of the mean.

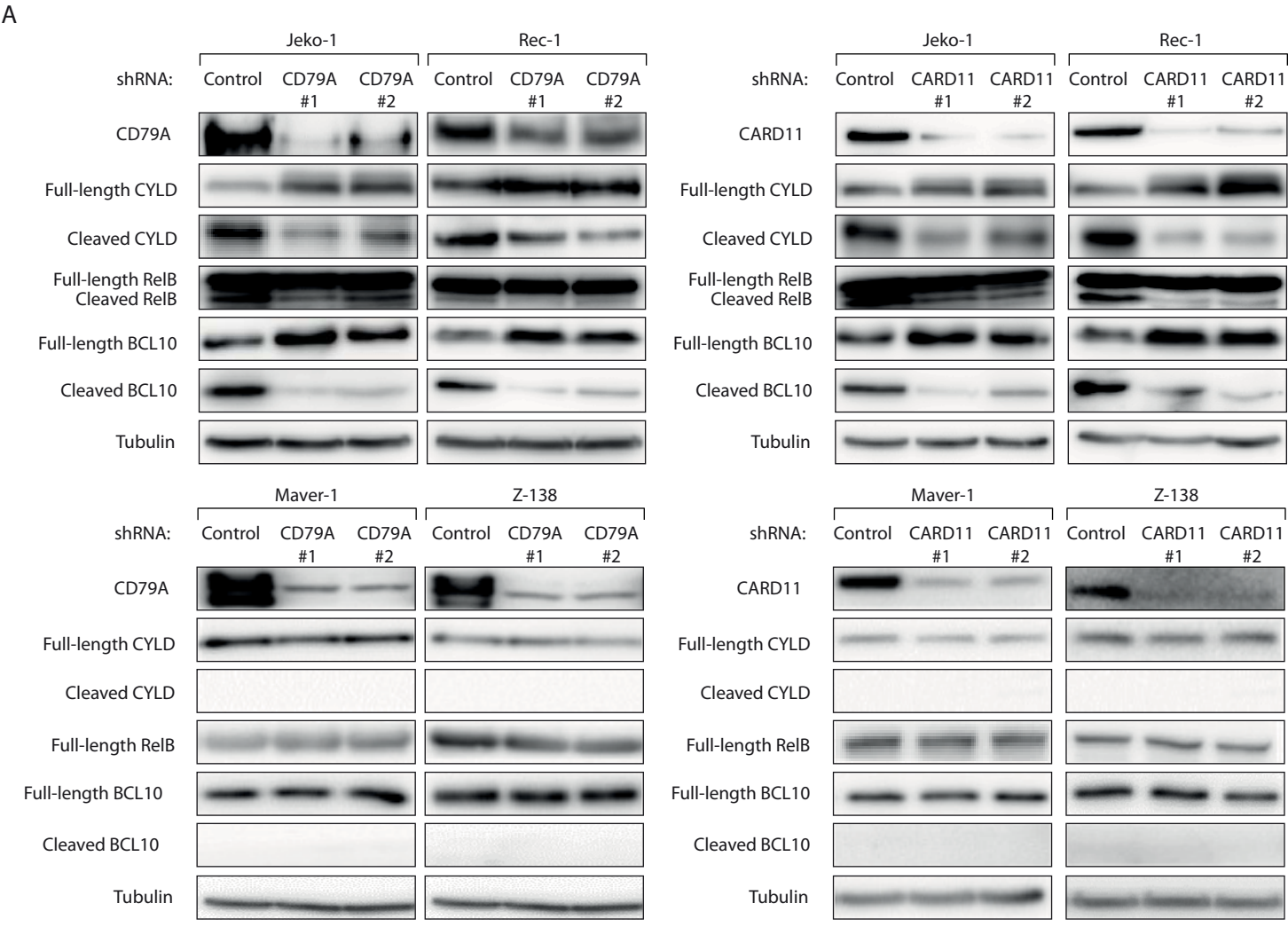
Figure 7. MCLs depend on MYC signaling. (A) shRNA-mediated MYC knockdown induced cytotoxicity in MCL cell lines. A previously described non-toxic shRNA against *MSMO1* did not induce toxicity in any cell line. Data are shown as means ± standard deviation of at least three independent experiments. (B) Expression of a *MYC* cDNA rescued Jeko-1, Rec-1, and SP53 cells transduced with *MYC* shRNA #2 (targeting the 3'UTR of MYC) from toxicity. Data are shown as means ± standard deviation of at least two independent experiments. (C) Expression of a *MYC* cDNA partially rescued Jeko-1, Rec-1, and SP53 cells transduced with *MALT1* shRNA #1 from toxicity. Data are shown as means ± standard deviation of at least two independent experiments. (D) Expression of a *MYC* cDNA partially rescued Jeko-1, Rec-1, SP53, and Mino cells treated with z-VRPR-fmk from toxicity. Data are shown as means ± standard deviation of at least two independent experiments. (E) shRNA-mediated knockdown of MYC significantly downregulated cell proliferation. Data are shown as means ± standard deviation of at least two independent experiments. (F) Correlation of Ki-67 and MYC expression determined by immunohistochemistry. Error bars indicate the standard errors of the mean. (G) Viability of MCL cell lines following MYC inhibition using the small molecule inhibitor 10058-F4 that inhibits MYC-MAX heterodimerization. Baseline MYC expression was assessed by Western blotting (Figure 6A). Representative results from at least three independent replicates are shown. Error bars indicate the standard deviation.

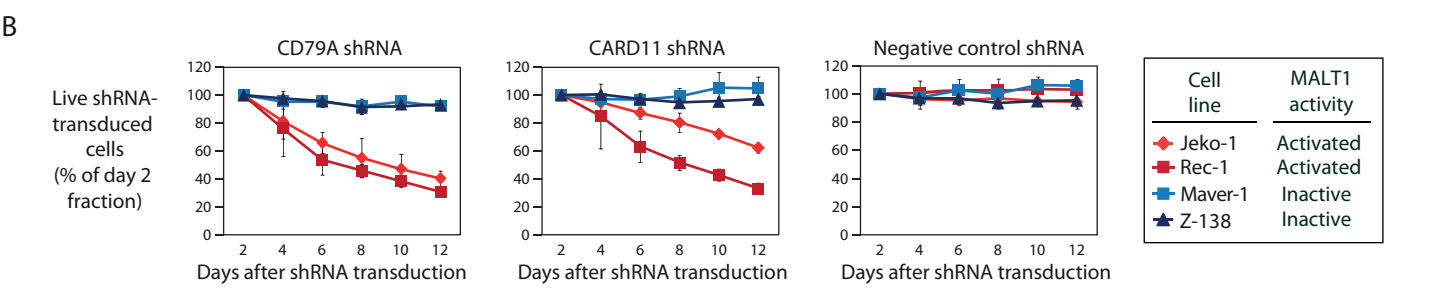
* $P < .05$, ** $P < .01$, *** $P < .001$.

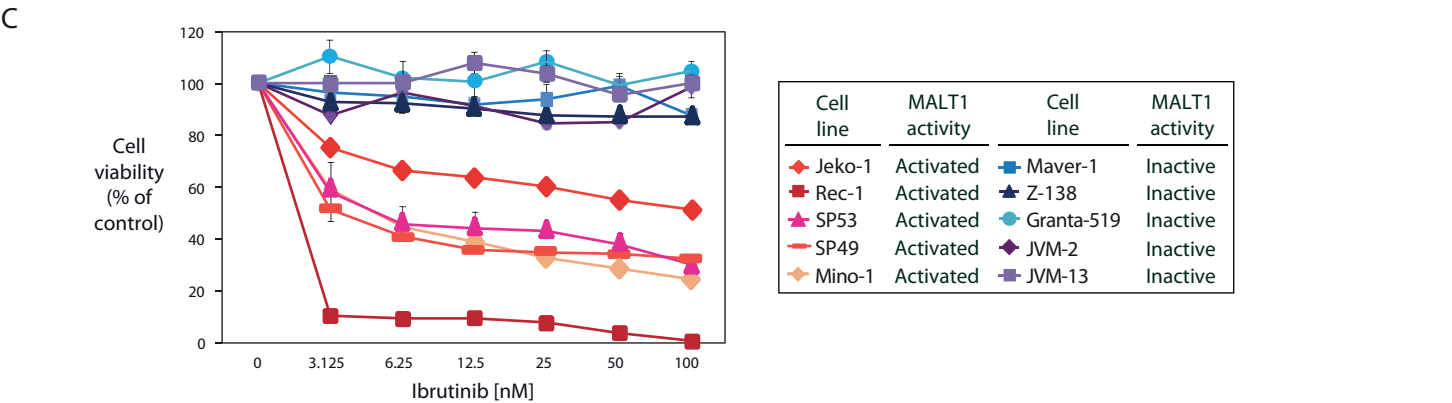
Dai et al. Figure 1



Dai et al. Figure 2







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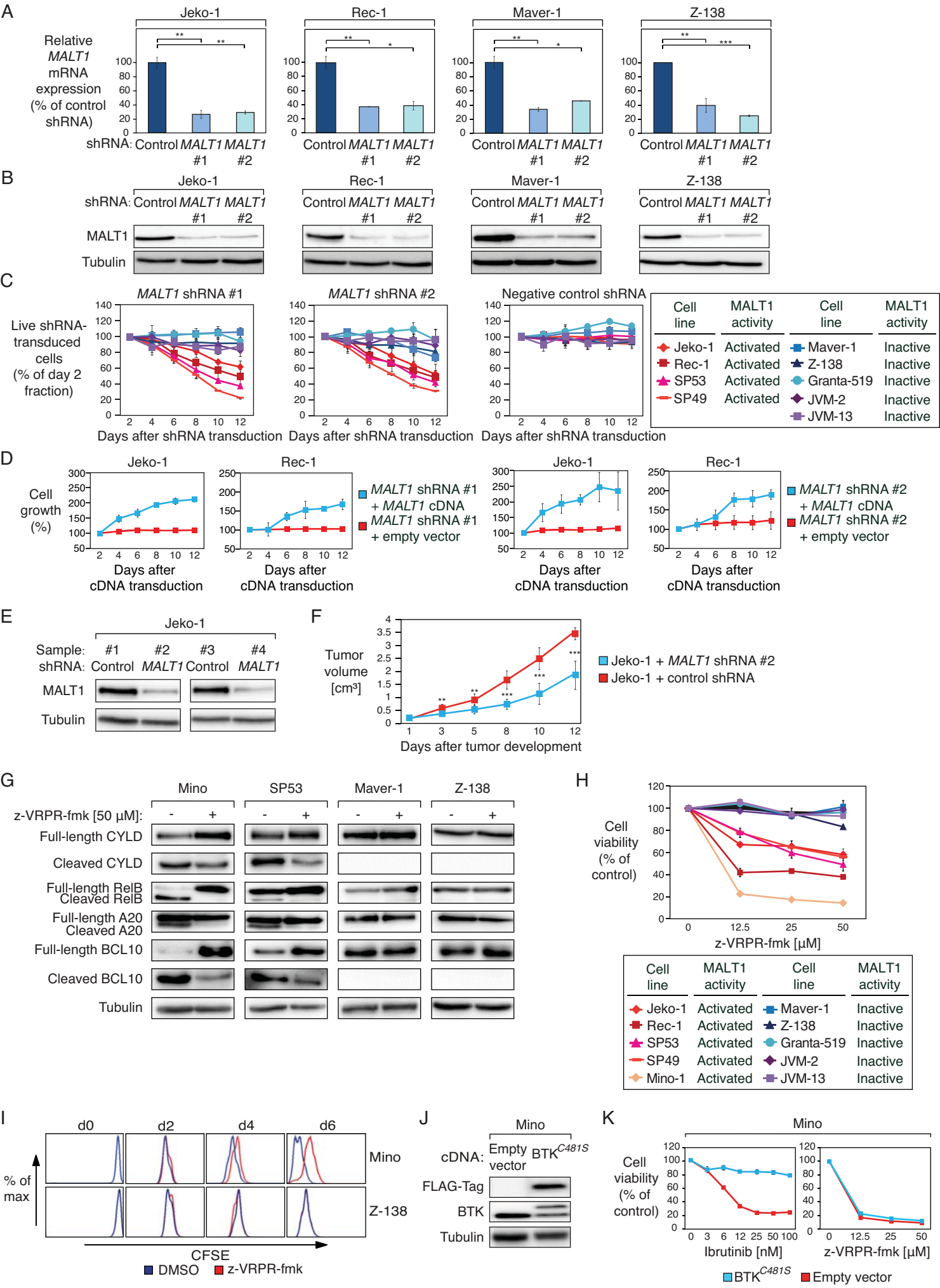

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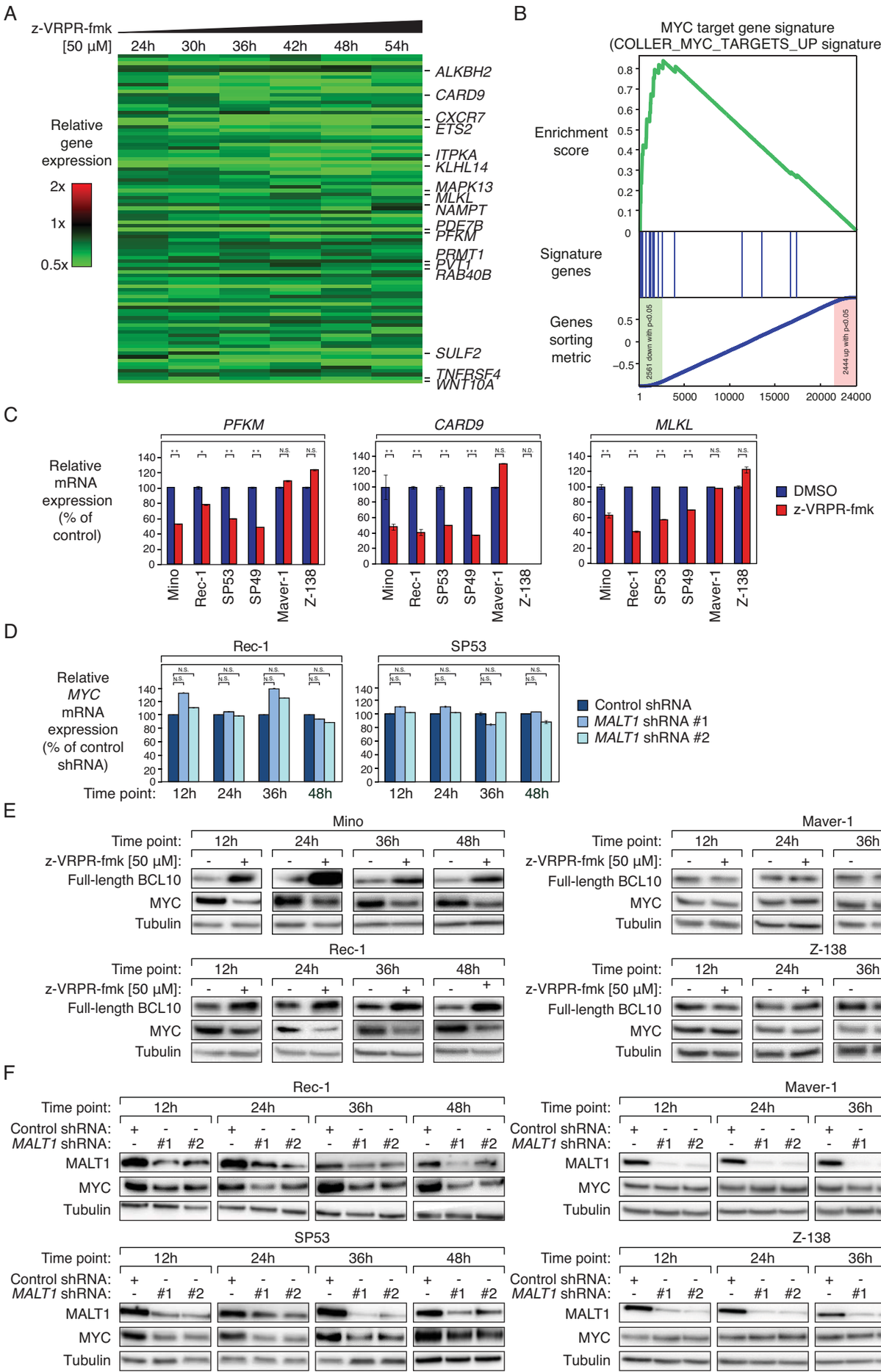

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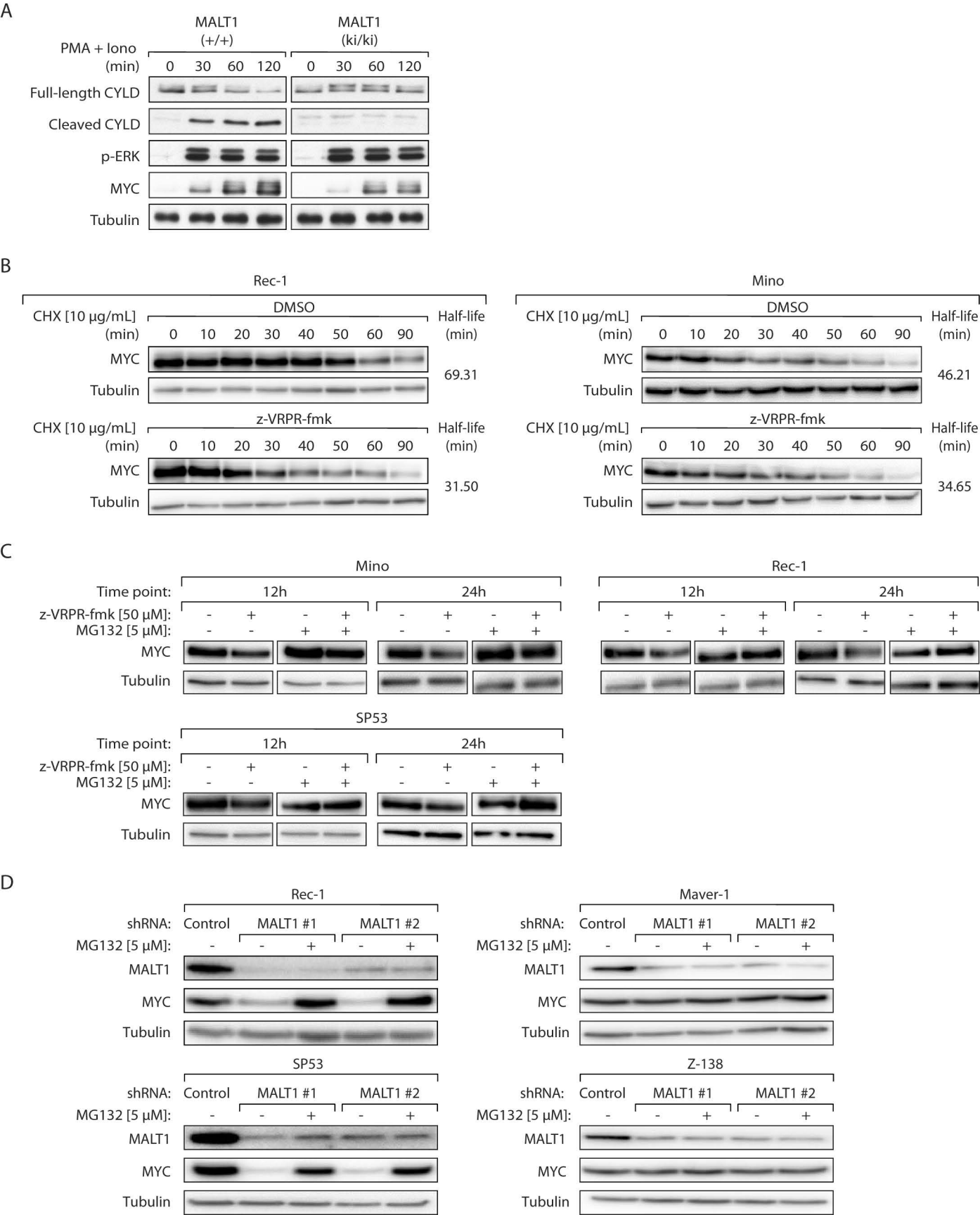

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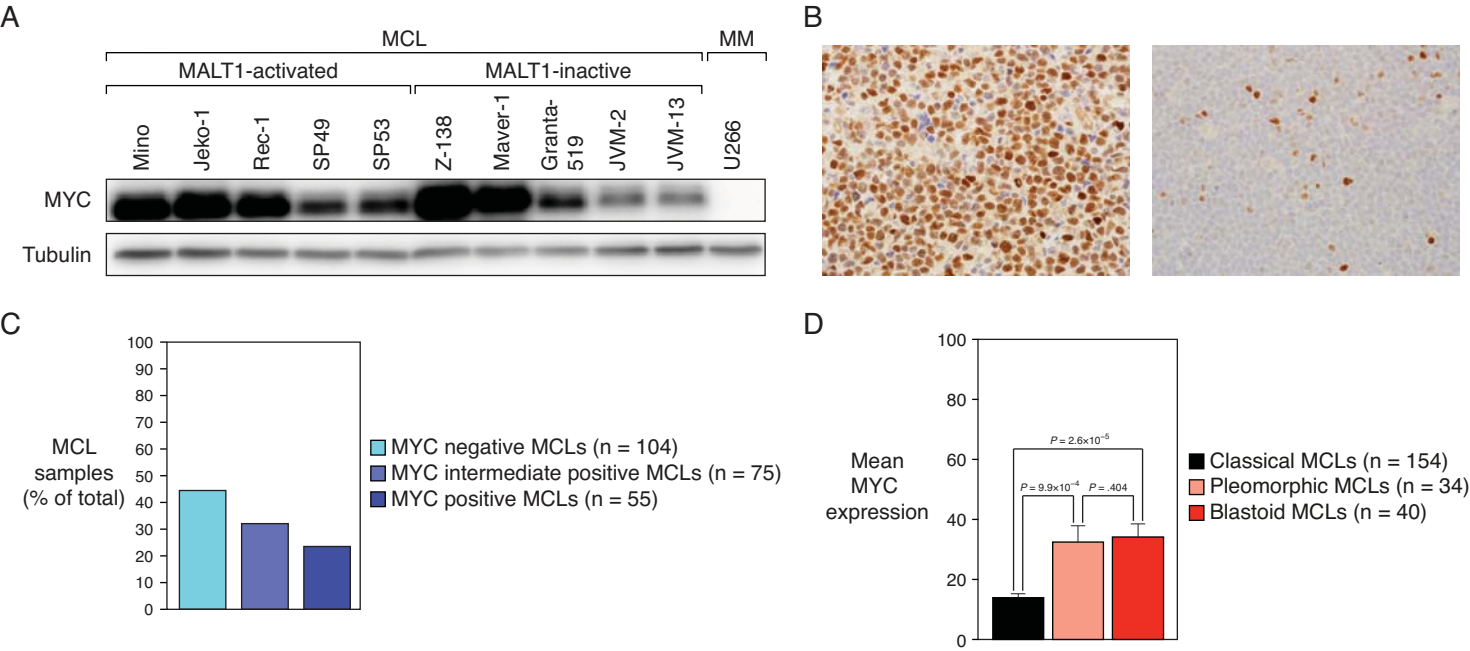

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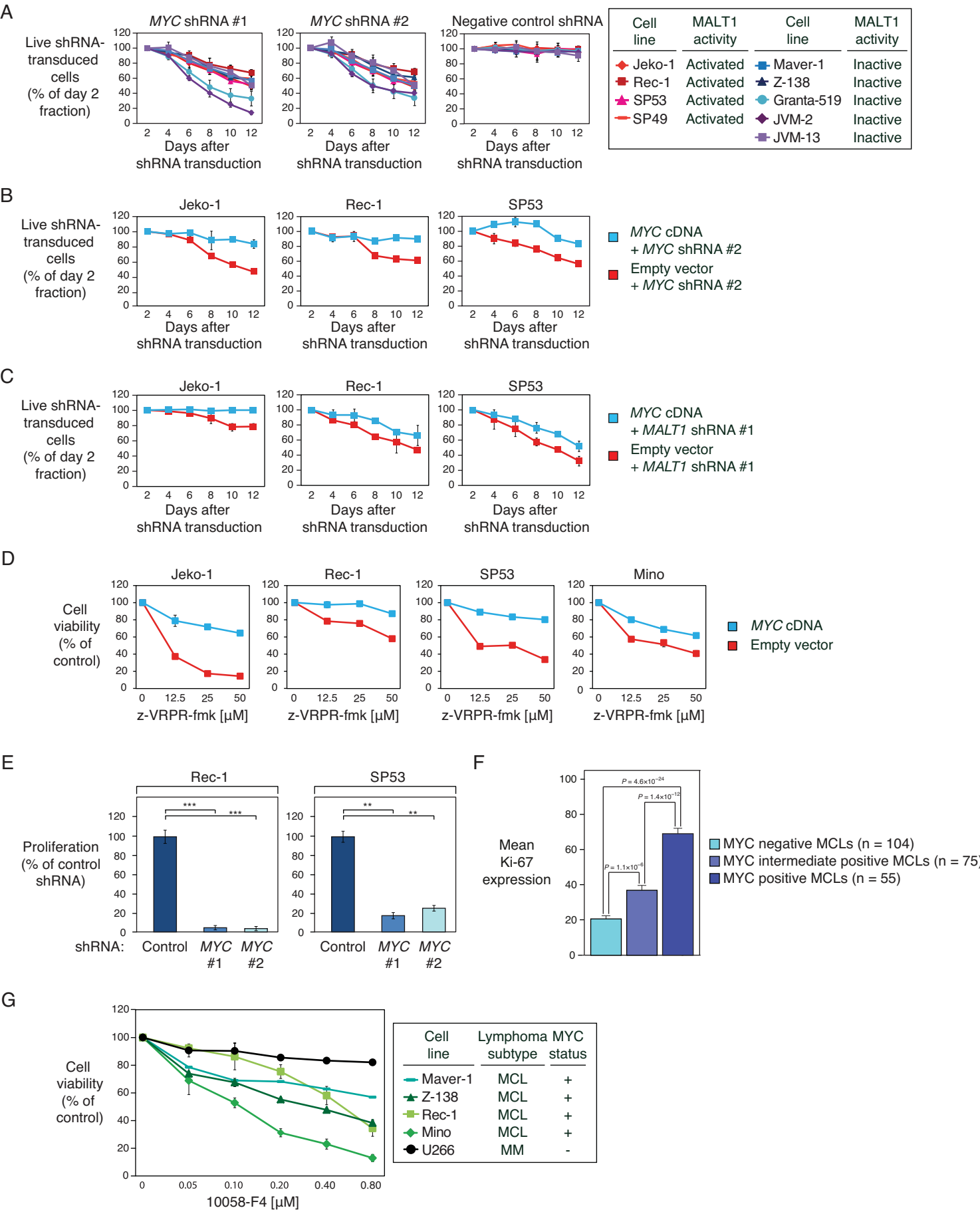

B-cell receptor driven MALT1 activity regulates MYC signaling in mantle cell lymphoma

Supplemental Material and Methods

Immunohistochemistry

Five reactive lymph node and tonsil specimens, 234 formalin-fixed, paraffin-embedded (FFPE) mantle cell lymphoma (MCL) samples, 93 diffuse large B-cell lymphoma (DLBCL) samples and seven Burkitt lymphoma (BL) samples were analyzed by immunohistochemistry. MYC staining was performed as described.[1] A cut-off level of ≥30% tumor cells with distinct nuclear staining was applied to define MYC positivity.[1,2] Samples with <10% positive cells were defined as MYC negative. Samples with ≥10% and <30% positive cells were determined as MYC intermediate positive. Ki-67 staining was done as described.[3] MALT1 immunohistochemistry was performed using the anti-human MALT1 antibody clone 50 (AbD Serotec, Puchheim, Germany) or the anti-human MALT1 antibody MT1/410 (Abcam, Cambridge, UK).

Cell culture, retroviral constructs and transductions

The human MCL cell lines Mino, Jeko-1, Rec-1, SP49, SP53, Z-138, Maver-1, and Granta-519 were cultured in RPMI 1640 with 10% or 20% fetal calf serum. Two MCL cell lines JVM-2 and JVM-13 as well as the multiple myeloma (MM) cell line U266 were cultured in Iscove´s modified Dulbecco´s medium supplemented with 20% human plasma. All cell lines were maintained at 37°C with 5% CO_2.

For efficient retroviral transductions, all cell lines were engineered to express the murine ecotropic receptor as previously described.[4,5] Additionally, these cell lines were engineered to express the bacterial tetracycline repressor allowing doxycycline-

inducible small hairpin RNA (shRNA) or complementary DNA (cDNA) expression.[4,5] The shRNA-mediated RNA interference and cytotoxicity assays were performed as described.[4,5] In brief, to assess toxicity of the shRNA, retroviruses that co-express green fluorescent protein (GFP) were used. Flow cytometry was performed two days after shRNA transduction to determine the initial GFP-positive proportion of live cells for each shRNA. Subsequently, cells were cultured with doxycycline (20 ng/mL) to induce shRNA expression and sampled over time. The GFP-positive proportion at each time point was normalized to that of the day two fraction. The targeting sequence of the utilized shRNAs directed against *BCL10, CARD11, CD79A, MALT1* and *MYC* are summarized in Supplemental Table 1. As a negative control shRNA, we used a previously described shRNA against *MSMO1* (Supplemental Table 1).[5] Each shRNA experiment was performed at least three times for each cell line. For the MALT1 and MYC rescue experiments a *MALT1* cDNA (NM_006785.3) and a *MYC* cDNA (NM_002467.2) were created and the experiment was performed as described.[5,6] The MALT1 rescue experiment was performed at least three times and the MYC rescue experiment at least two times. Expression of the *BTK*^{C481S} cDNA in Mino, Jeko-1, Rec-1, SP49, SP53, Z-138 and Maver-1 cells was performed as described.[7]

Isolation and stimulation of mouse splenocytes

Primary mouse splenocytes were isolated from C57/BL6 littermates expressing either wild-type MALT1 (+/+) or a catalytically inactive C472A mutant of MALT1 (ki/ki) as described.[8] Splenocyte stimulation was initiated by addition of phorbol myristate acetate (PMA; 80 ng/mL; Alexis, Enzo Life Sciences, Lausen, Switzerland) and ionomycin (1 µM; Calbiochem, Merck, Darmstadt, Germany) and cells were incubated for the indicated times at 37°C.

In vivo xenograft mouse studies

For in vivo testing of the Jeko-1 xenograft mouse models, six to eight week old female NOD.Cg-*Prkdc* severe combined immunodeficiency Il2rg^{tm1Wjl}/SzJ (NSG; Jackson Laboratory, Bar Harbor, ME, USA) mice were used. To induce either *MALT1* shRNA #2 or the non-toxic shRNA against *MSMO1*, mice received drinking water supplemented with 1 mg/mL doxycycline (Genaxxon, Ulm, Germany) and 5% sucrose immediately after they developed macroscopic signs of s.c. tumors. Tumor size was measured three times weekly in two dimensions for each mouse using caliper. Tumor volume was calculated according to the following formula: 1/2 × (length × width^2). All animal experiments were approved by the institutional Animal Care and Use Committee, as well as by the Research and Higher Education section of the Ministry of Education, Youth and Sports of the Czech Republic under the number 592/15 (MSMT-11255/2015-4).

Viability assay, analysis of cell cycle, apoptosis, proliferation

MCL cell lines were incubated with different concentrations of z-VRPR-fmk (Bachem, Bubendorf, Switzerland), 10058-F4 (Sigma-Aldrich, Schnelldorf, Germany), ibrutinib (Selleckchem, Houston, TX, USA), and doxorubicin (Sigma-Aldrich). Cell viability was measured after three days (doxorubicin and 10058-F4), five days (ibrutinib) or seven days (z-VRPR-fmk) of incubation using the Cell Titer Glo Assay (Promega, Dübendorf, Switzerland) as previously described.[5,9] Each experiment was reproduced at least two times for each cell line.

The number of cell divisions following treatment with z-VRPR-fmk or DMSO was quantified by using the following equation and stated as mean ± standard deviation of at least three independent experiments for each cell line.

$$N_t = N_0 2^{tf}$$

Variables:

N_t	Numbers of cells at time t
N_0	Numbers of cells initially
t	Time (days)
f	Frequency of cell division per day

Gene expression profiling

Gene expression profiling was performed 24, 30, 36, 42, 48, and 54 hours following treatment with z-VRPR-fmk or DMSO in cell lines Mino and Rec-1 as previously described.[10] Total RNA was isolated using the NucleoSpin RNA II Kit (Macherey & Nagel, Oensingen, Switzerland) according to the manufacturer's protocol. RNA was amplified and labeled with the TotalPrep RNA Amplification Kit (Illumina, Thermo Fisher Scientific, Waltham, MA, USA). Samples were subsequently hybridized on HumanHT-12 v4 Expression Bead Chips (Illumina) following the manufacturer's protocol as previously described.[10,11] Gene expression changes were measured in two independent biological replicates for each time point in Mino cells and in one experiment in Rec-1 cells. Gene expression changes in Mino and Rec-1 cells treated with z-VRPR-fmk were compared to cells treated with DMSO. The independent measurements were preprocessed and normalized in the following manner. Data were imported on raw bead level and subsequently a bead level spot filter was applied to each microarray experiment based on the fitted density mode for the background intensities. Afterwards, bead intensities of all measured microarrays were quantile-normalized and beads were grouped by measured sequence to form beadsets. Beadsets with more than 50% of their beads excluded by the spot filter were also excluded. Further analyses were performed on gene level using median aggregation and manufacturer’s annotations.

Differentially expressed genes were identified in the following manner. A one-tailed paired t-test was used to calculate *P*-values for each gene based on all the microarray pairs. Additionally, we used the Benjamini & Hochberg method to calculate a false discovery rate (FDR) for every significance threshold. In Rec-1 cells, we identified 113 genes that were significantly downregulated ($P \leq .0025$; FDR = .09) and 223 genes that were significantly upregulated ($P \leq .0025$; FDR = .05) across all time points following inhibition of MALT1 (paired t-tests over all time points of on one experiment; Supplemental Table 5). In Mino cells, we identified 93 genes that were significantly downregulated ($P \leq 1\times10^{-5}$; FDR = .0002) and 126 genes that were significantly upregulated ($P \leq 1\times10^{-5}$; FDR = .0001) across all time points following inhibition of MALT1 (paired t-tests over all time points of two independent replicates; Supplemental Table 2).

The gene set enrichment analysis (GSEA) was performed as previously described against an integrated database with 13,593 gene signatures, comprised of signatures from the Molecular Signatures Database v4, the GeneSigDB v4, HGCN gene families and the Staudt laboratory library.[12-16] Signatures with less than eight measured genes were excluded. GSEA *P*-values were computed by permutation tests and FDRs were computed relative to respective signature families. Top enriched signatures with an absolute enrichment score ≥ .7 in cell lines Mino and Rec-1 after treatment with z-VRPR-fmk are presented in Supplemental Table 3 and 6, respectively.

Western blotting and analysis of MYC stability

Western blotting was performed as described.[5] Image acquisition was performed using Amersham Imager 600 (GE Healthcare Life Sciences, Chicago, IL, USA). Band quantification was performed using the ImageQuant TL software (GE Healthcare Life

Sciences). All antibodies used in this study were obtained from Cell Signaling (Danvers, MA, USA) except of antibodies detecting A20, full-length BCL10, MYC (Abcam, Cambridge, UK), and α-Tubulin (Sigma-Aldrich). Antibodies directed against cleaved BCL10 and MALT1 have been previously described.[17,18] Full-length and cleaved isoforms of RelB, A20 and CYLD were detected with the same antibody, whereas full-length and cleaved forms of BCL10 were detected with different antibodies as stated above.

Non-transduced cells treated with z-VRPR-fmk (50 μM) or DMSO for the indicated time points or cells transduced with inducible *MALT1* shRNAs and treated with doxocycline for 24 hours were incubated with 5 μM MG132 (Selleckchem, Houston, TX, USA) or 10 μg/mL cycloheximide (Santa Cruz Biotechnology, Heidelberg, Germany) at 37°C, harvested, and subjected to immunoblotting.

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Supplemental Figures

Supplemental Figure 1. MALT1 expression in normal reactive tissue. Immunohistochemical MALT1 staining of a reactive tonsil specimen (original magnification ×200). Image was captured using an Olympus BX51 microscope (Olympus, Tokio, Japan) equipped with an Olympus DP73 camera (Olympus) and was processed with Olympus cellSens software (Olympus).

Supplemental Figure 2. Toxicity of shRNA-mediated knockdown of BCL10 in MCL cell lines. (A) A *BCL10* shRNA downregulates BCL10 protein in MALT1-activated (Jeko-1 and Rec-1) and MALT1-inactive (Maver-1 and Z-138) MCLs 48 hours after shRNA induction measured by Western blotting. (B) BCL10 knockdown induced toxicity in MALT1-activated MCLs (Jeko-1 and Rec-1), whereas it did not affect MALT1-inactive MCLs (Maver-1 and Z-138). A previously described non-toxic shRNA against *MSMO1* did not induce toxicity in any cell line. Data are shown as means ± standard deviation of at least three independent experiments.

Supplemental Figure 3. Cell cycle analysis following pharmacologic MALT1 inhibition. Cell cycle distribution of Mino and Z-138 cells after treatment with z-VRPR-fmk or DMSO alone was measured on day zero and after two, four, and six days. In both Mino and Z-138 cells no difference in cell cycle distribution was detectable. Data are expressed as means ± standard deviation of at least two independent experiments.

Supplemental Figure 4. Inhibition of MALT1 overcomes *BTK*^{C481S}-induced ibrutinib resistance in MALT1-activated MCLs. Western blotting for FLAG and

BTK following transduction of MCL cells with either a *BTK*^{C481S} cDNA or an empty vector (A. Jeko-1; C. Rec-1; E. SP53; G. SP49; I. Maver-1; K. Z-138). Determination of cell viability of MCL cells expressing either an empty vector (red) or a *BTK*^{C481S} cDNA (blue) following treatment with ibrutinib or z-VRPR-fmk (B. Jeko-1; D. Rec-1; F. SP53; H. SP49; J. Maver-1; L. Z-138). Representative results from at least two independent replicates are shown. Error bars indicate the standard deviation.

Supplemental Figure 5. Inhibition of MALT1 suppresses the gene expression network of MYC in Mino cells. (A) Gene expression profiling following MALT1 inhibition in Mino cells. Changes of gene expression were profiled at the indicated time points following treatment with z-VRPR-fmk relative to treatment with DMSO only. Each time point depicted the mean of log_2-transformed expression ratios for two replicates according to the color scale shown. (B) Inhibition of MALT1 significantly downregulated the previously identified MYC target gene signature "COLLER_MYC_TARGETS_UP". Changes of gene expression were profiled at the indicated time points following inhibition of MALT1. Each time point depicted the mean of log_2-transformed expression ratios for two replicates. Gene expression changes were depicted according to the color scale shown.

Supplemental Figure 6. Inhibition of MALT1 suppresses the gene expression network of MYC in Rec-1 cells. (A) Gene expression profiling following pharmacologic inhibition of the proteolytic MALT1 activity using z-VRPR-fmk vs. DMSO in Rec-1 cells. Changes of gene expression were profiled at the indicated time points following treatment with z-VRPR-fmk. Gene expression changes are depicted according to the color scale shown. Genes that are involved in critical biological processes are highlighted. (B) Gene expression profiling following MALT1 inhibition

in Rec-1 cells. Changes of gene expression were profiled at the indicated time points following treatment with z-VRPR-fmk relative to treatment with DMSO only. Gene expression changes are depicted according to the color scale shown. A fraction of genes that were also upregulated in Mino cells following MALT1 inhibition are highlighted. (C) Gene set enrichment analysis of a previously described MYC gene expression signature. The MYC signature was significantly enriched with genes that are downregulated following pharmacologic MALT1 inhibition using z-VRPR-fmk in Rec-1 cells. (D) Inhibition of MALT1 significantly downregulated the previously identified MYC target gene signature “COLLER_MYC_TARGETS_UP” in Rec-1 cells. Changes of gene expression were profiled at the indicated time points following inhibition of MALT1. Gene expression changes were depicted according to the color scale shown. (E) Downregulation of the Mino target gene signature (Figure 4A) in Rec-1 cells. Changes of gene expression were profiled at the indicated time points following treatment with z-VRPR-fmk relative to treatment with DMSO only. Gene expression changes are depicted according to the color scale shown. Genes that are highlighted in Figure 4A are also highlighted here.

Supplemental Figure 7. Expression levels of MALT1 target genes in MALT1-activated and -inactive MCL cell lines following MALT1 inhibition. mRNA levels of *ITPKA*, *MAPK13, PVT1, ETS2, NAMPT, KIF26B, C10orf10,* and *ALKBH2* in the indicated MCL cell lines following MALT1 inhibition were normalized to expression of *GAPDH*. Error bars indicate the standard deviation.

N.D., not detectable, N.S., not significant, * $P < .05$, ** $P < .01$, *** $P < .001$.

Supplemental Figure 8. Downregulation of MYC protein expression in MALT1-activated MCLs following ibrutinib treatment. MYC expression on protein level

was downregulated in MALT1-activated MCLs (Mino and Rec-1), but not in MALT1-inactive MCLs (Maver-1 and Z-138) following Bruton's tyrosine kinase (BTK) inhibition using the small molecule inhibitor ibrutinib.

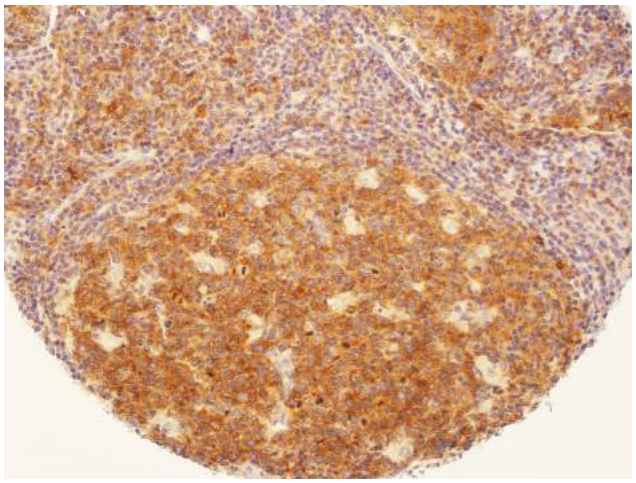
Supplemental Figure 9. Protein quantification of Western blot data depicted in Figure 5B and 5C. (A) Protein quantification of Western blot of Figure 5B. The MYC measurements of cells incubated with cycloheximide (CHX) were normalized to matched tubulin measurements and then depicted as a ratio of the normalized values of cells without CHX incubation. (B) Protein quantification of Western blot of Figure 5C. At indicated time points, the MYC measurements of cells treated with z-VRPR-fmk were normalized to matched tubulin measurements and then shown as a ratio of the normalized values of cells treated with DMSO.

Supplemental Figure 10. Protein quantification of Western blot data depicted in Figure 6A. For all cell lines MYC measurements were normalized to matched tubulin measurements and then depicted as fold change compared to the normalized values of U266 cells.

Supplemental Figure 11. MYC expression in MYC positive lymphomas. Immunohistochemical MYC staining of a BL case (left picture; original magnification ×400) and a DLBCL case (right picture; original magnification ×400). Images were captured using an Olympus BX51 microscope (Olympus) equipped with an Olympus DP73 camera (Olympus) and were processed with Olympus cellSens software (Olympus).

Supplemental Figure 12. Functional role of MYC in MCL. (A) *MYC* shRNA #1 and #2 significantly downregulated MYC protein measured by Western blotting in Rec-1 and SP53 cells. (B) Expression of a *MYC* cDNA partially rescued Jeko-1, Rec-1, and SP53 cells transduced with *MALT1* shRNA #2 from toxicity. Data are shown as means ± standard deviation of at least two independent experiments. (C) Expression of a *MYC* cDNA did not rescue Jeko-1, Rec-1, SP53, and Mino cells treated with doxorubicin for 72 hours from toxicity. Representative results from two independent replicates are shown. Error bars indicate the standard deviation. (D) Correlation of MYC and Ki-67 expression in MCL (r = .63; $P = 5\times10^{-27}$).

Dai et al. Supplemental Figure 1



Dai et al. Supplemental Figure 2

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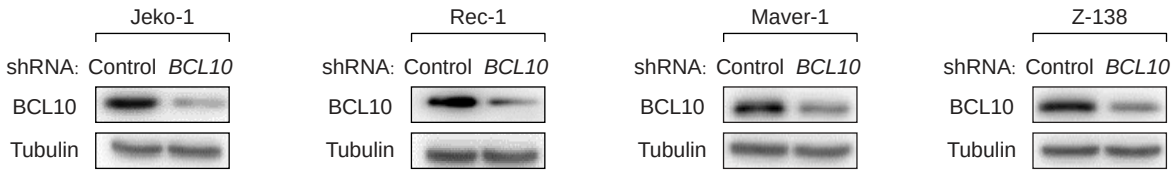

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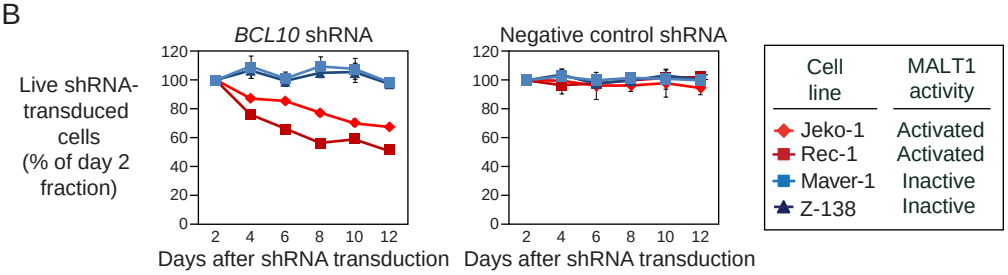
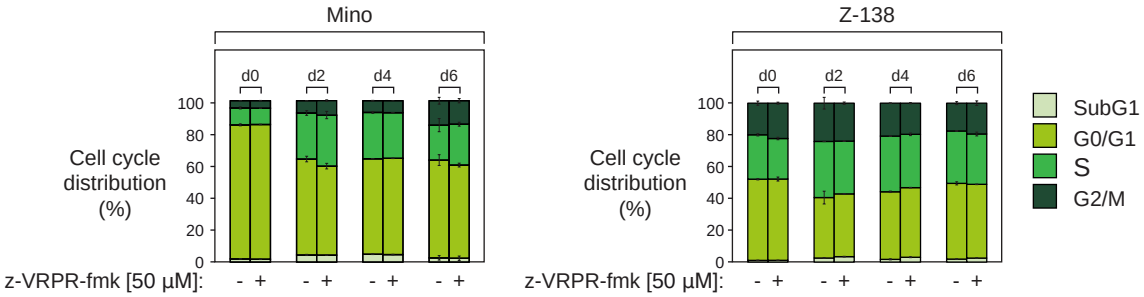


Dai et al. Supplemental Figure 3



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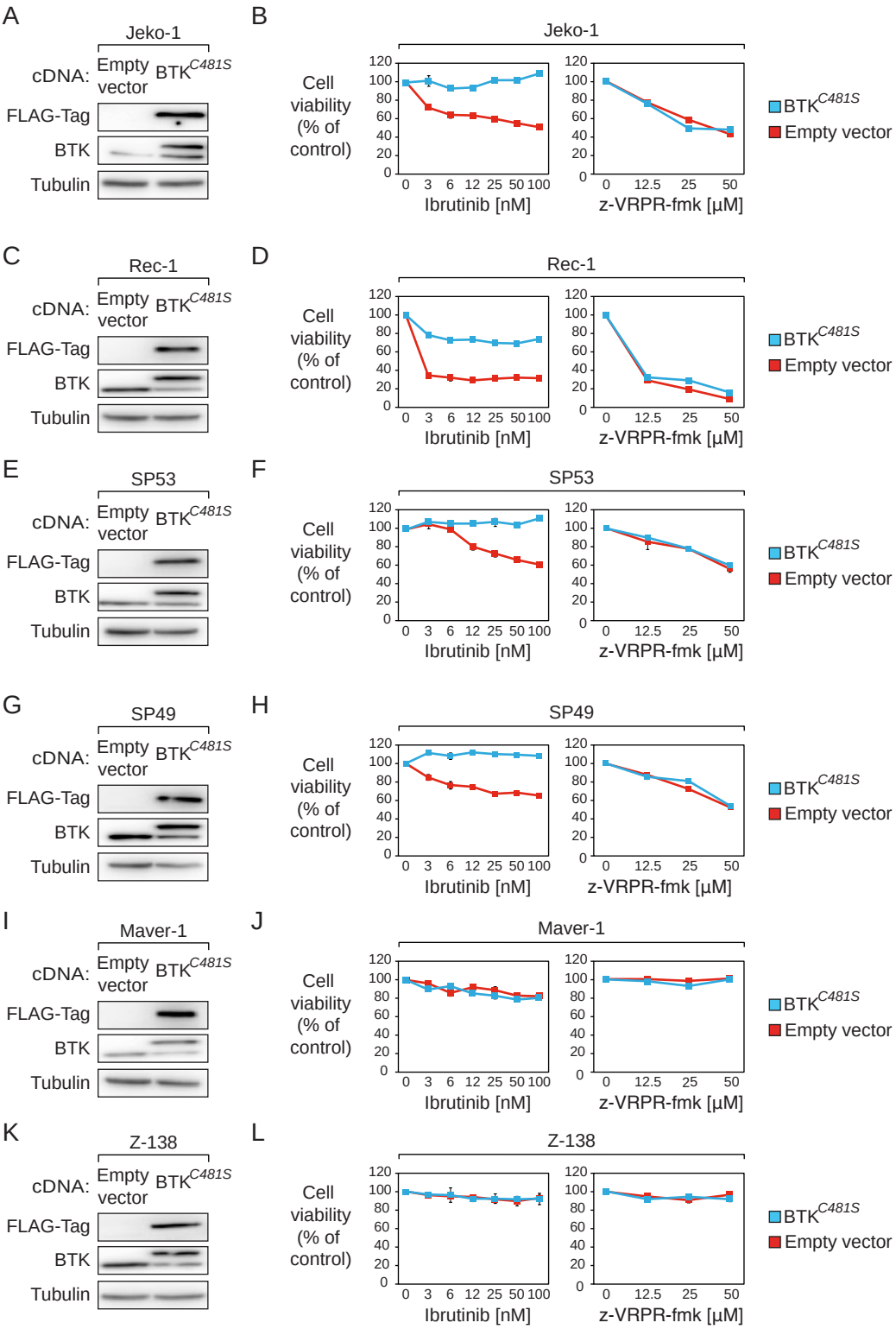

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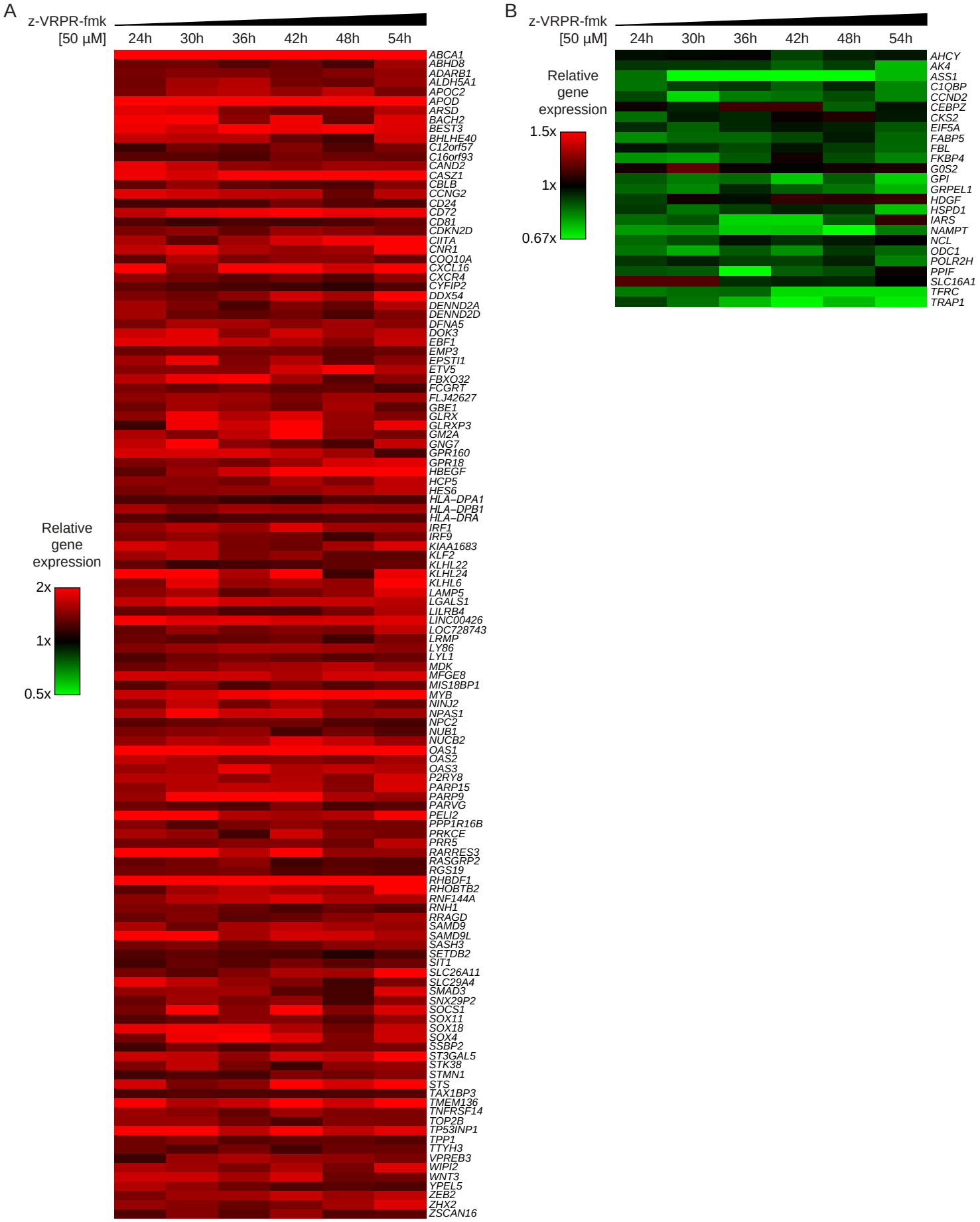

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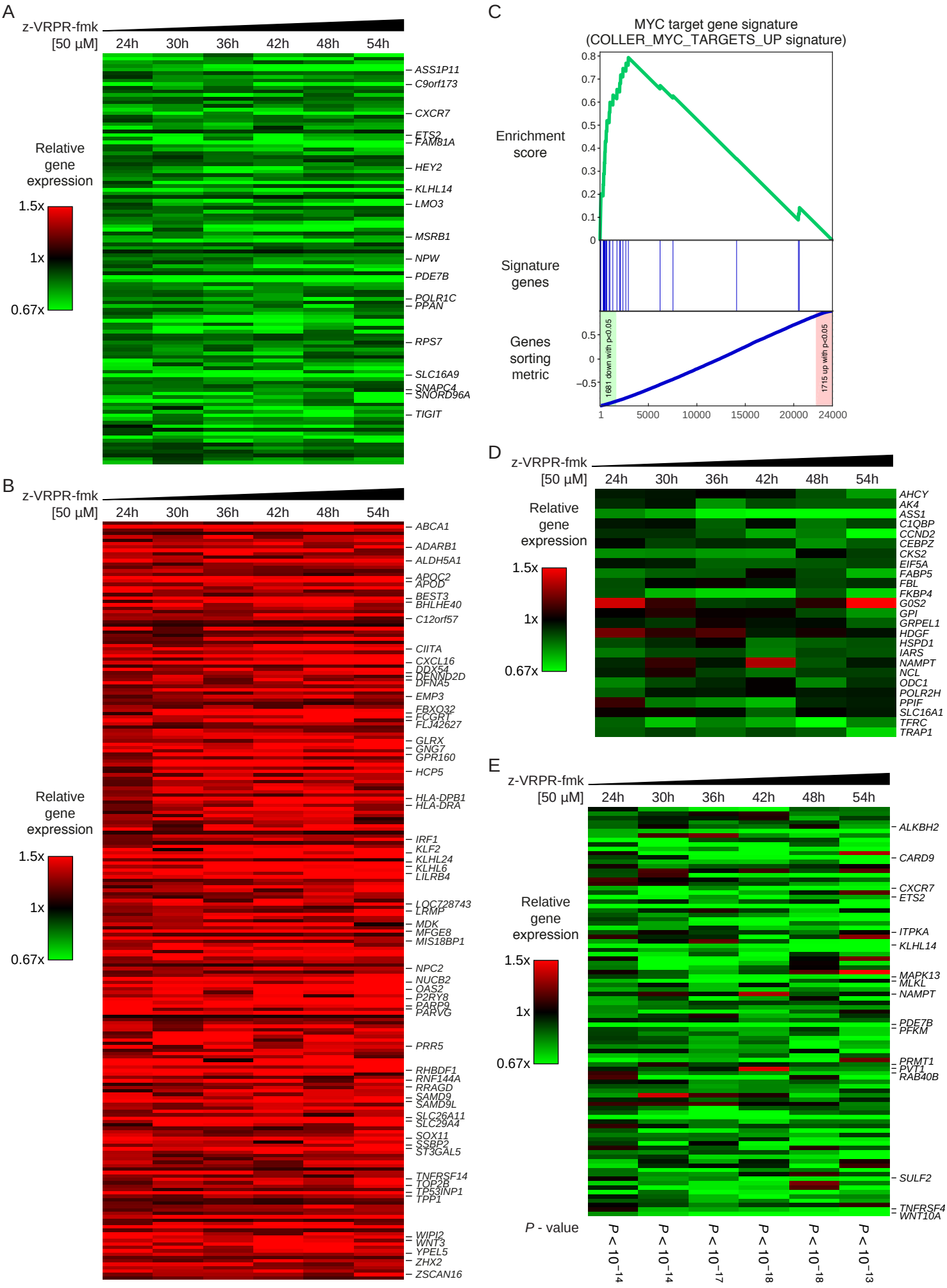

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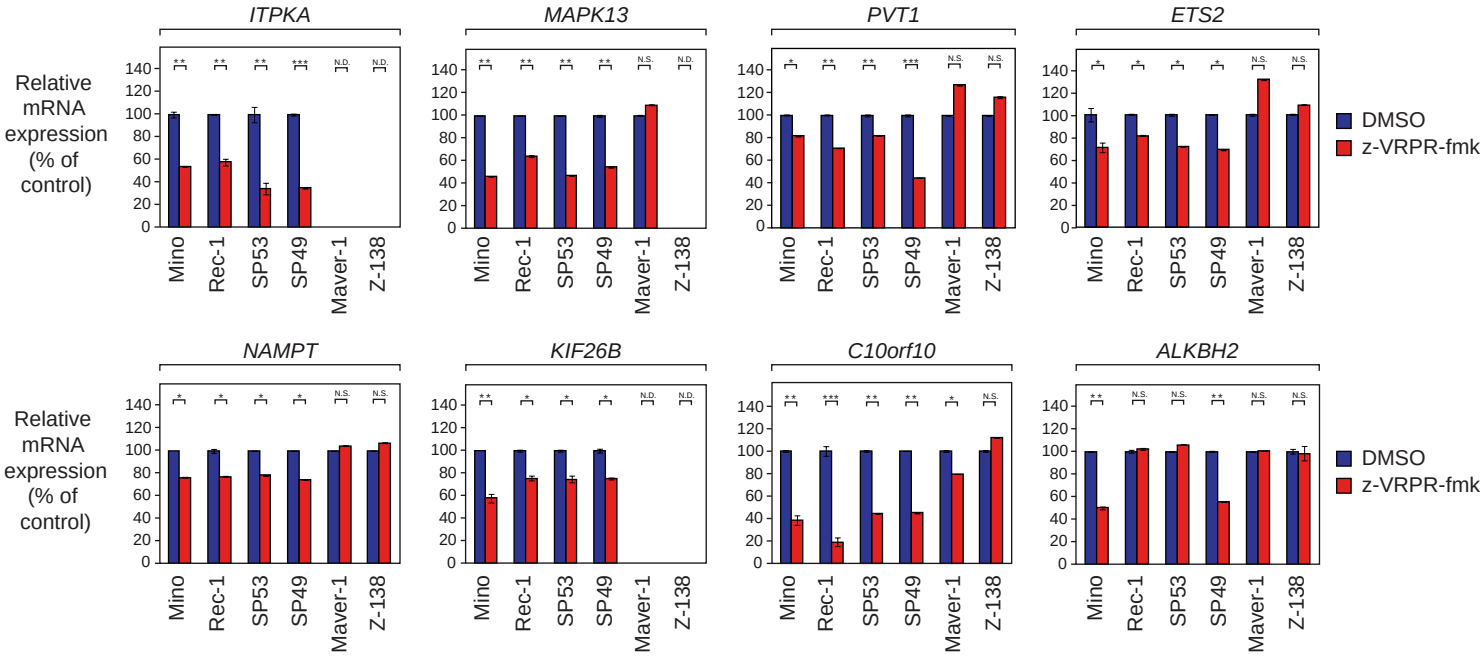

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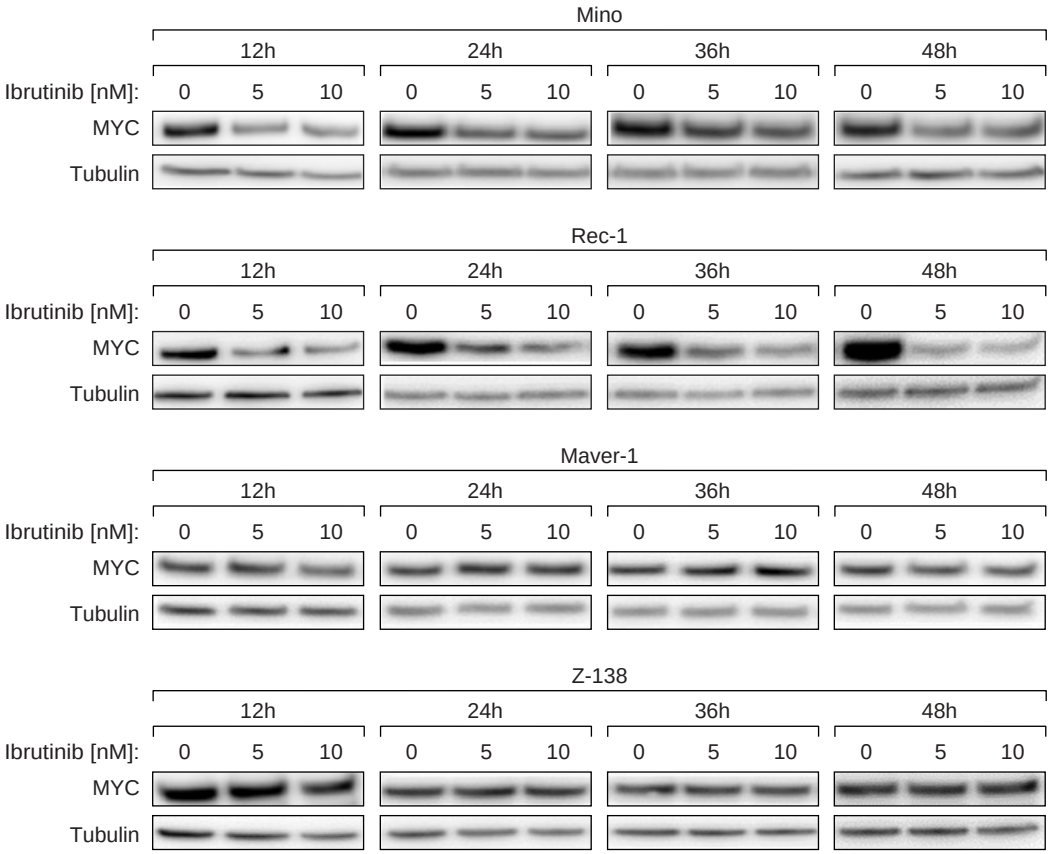

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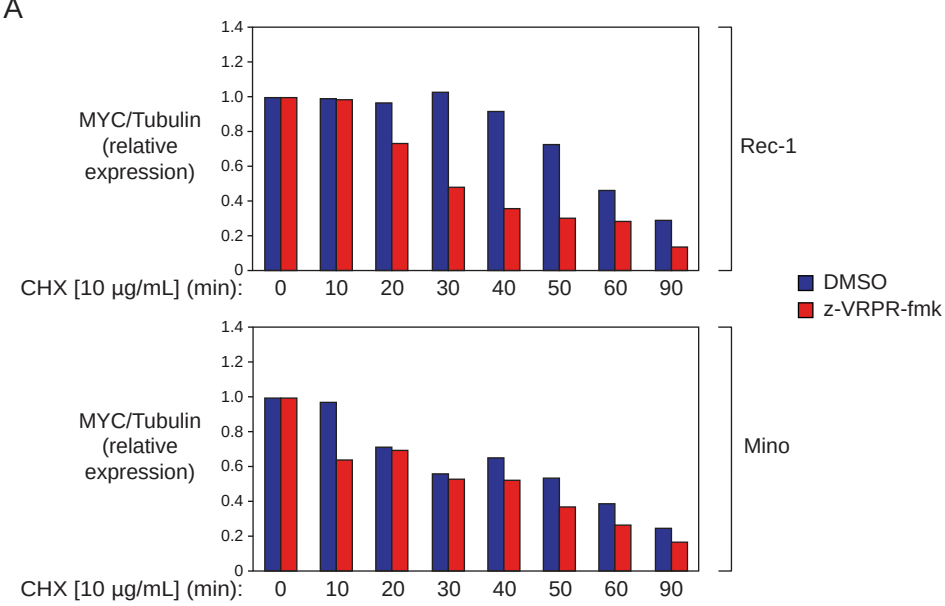

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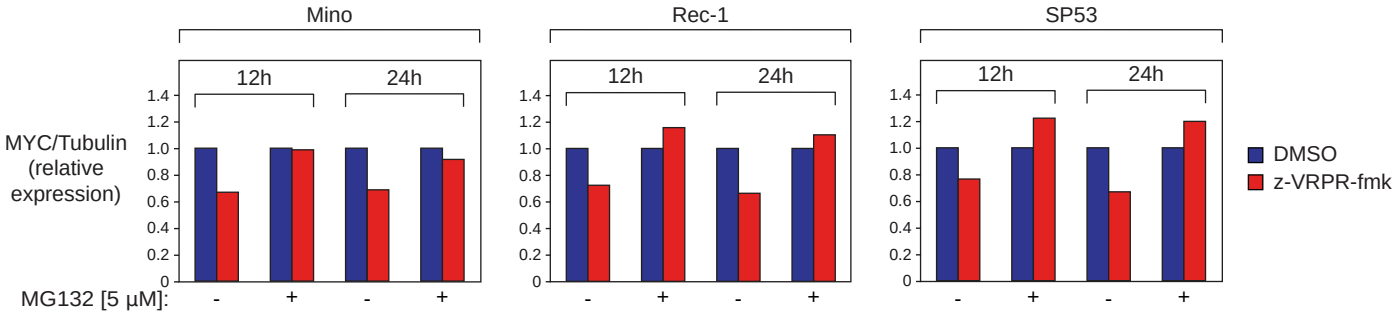

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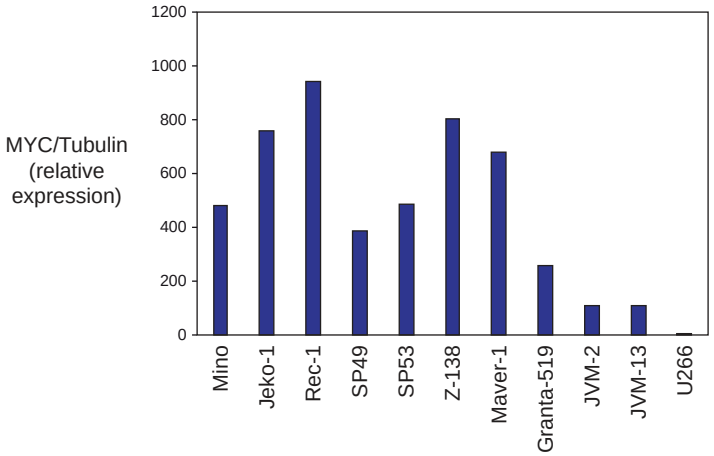

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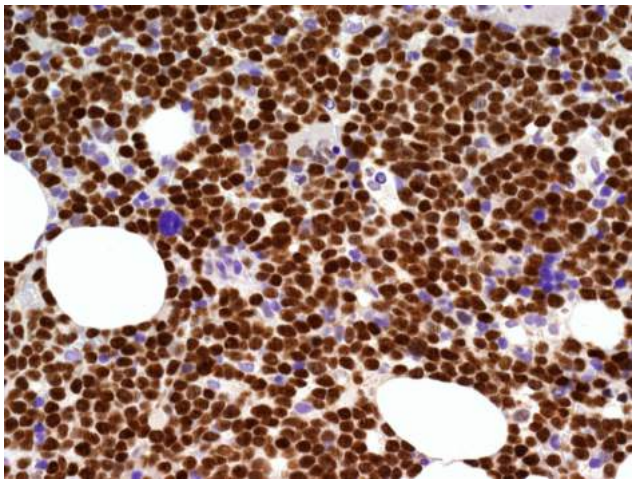

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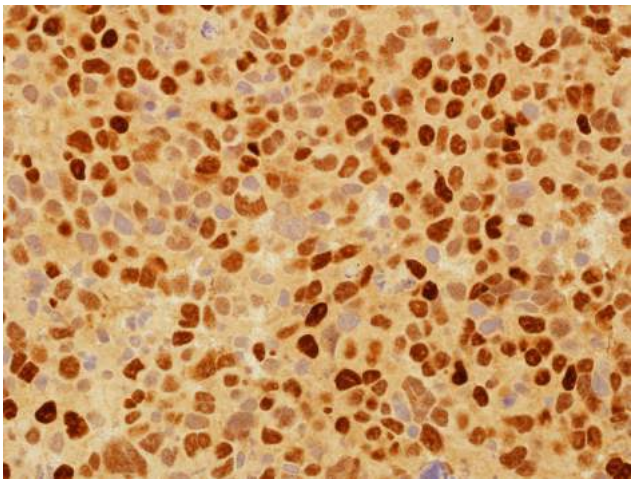

Dai et al. Supplemental Figure 12

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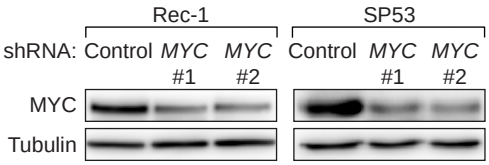

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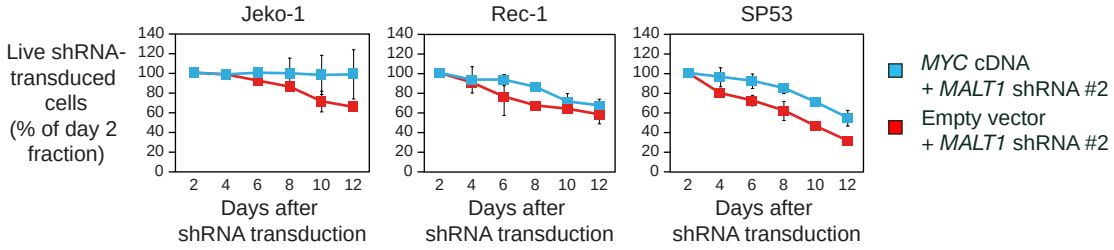

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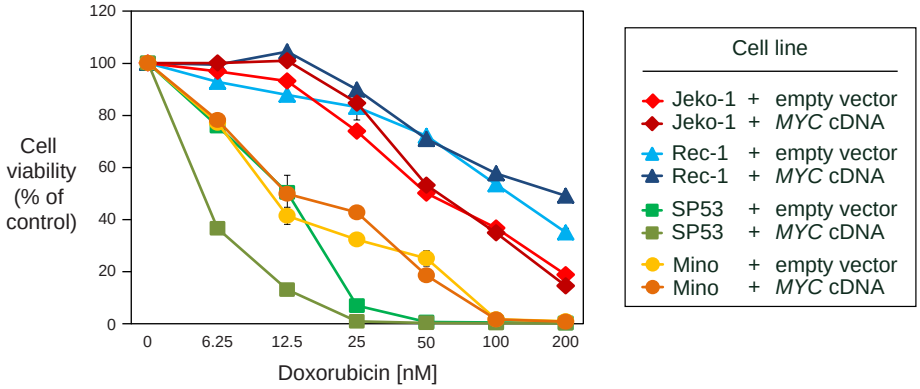

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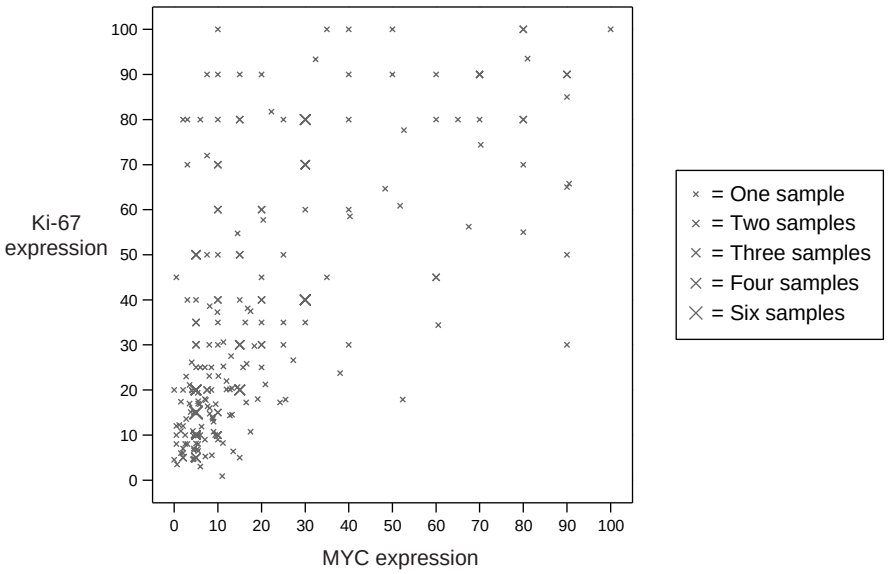

Supplemental Table 1. Sequences of utilized shRNAs.

Target gene	shRNA sequence
BCL10	shRNA #1: GATGAAGTGCTGAAACTTAGA
CARD11	shRNA #1: GGGGTGTGTACCAGGCTATGA
CARD11	shRNA #2: GGACGACAACTACAACTTAGC
CD79A	shRNA #1: GGGGCTTCCTTAGTCATATTC
CD79A	shRNA #2: CAGCGGGTAATGAGCCCTTAA
MALT1	shRNA #1: GGCAGCTACTTGGTATCAAAG
MALT1	shRNA #2: GTCACAGAATTGAGTGATTTC
MSMO1	shRNA #1: GGATAATGGTGATTGAGATGG
MYC	shRNA #1: CGATTCCTTCTAACAGAAATG
MYC	shRNA #2: CCTATGAACTTGTTTCAAATG

Supplemental Table 2. Downregulated and upregulated genes following z-VRPR-fmk treatment in Mino cells.

Signature name	Gene symbol	Gene ID	Gene description
Downregulated genes (alpha=1e-05)	ADAP2	55803	ArfGAP with dual PH domains 2
Downregulated genes (alpha=1e-05)	ADM	133	adrenomedullin
Downregulated genes (alpha=1e-05)	ADTRP	84830	androgen-dependent TFPI-regulating protein
Downregulated genes (alpha=1e-05)	AK2	204	adenylate kinase 2
Downregulated genes (alpha=1e-05)	ALKBH2	121642	alkB, alkylation repair homolog 2 (E. coli)
Downregulated genes (alpha=1e-05)	ASS1P11	340274	argininosuccinate synthetase 1 pseudogene 11
Downregulated genes (alpha=1e-05)	BSPRY	54836	B-box and SPRY domain containing
Downregulated genes (alpha=1e-05)	C10orf10	11067	chromosome 10 open reading frame 10
Downregulated genes (alpha=1e-05)	C1orf186	440712	chromosome 1 open reading frame 186
Downregulated genes (alpha=1e-05)	C9orf173	441476	chromosome 9 open reading frame 173
Downregulated genes (alpha=1e-05)	CAMP	820	cathelicidin antimicrobial peptide
Downregulated genes (alpha=1e-05)	CARD9	64170	caspase recruitment domain family, member 9
Downregulated genes (alpha=1e-05)	CD247	919	CD247 molecule
Downregulated genes (alpha=1e-05)	CHCHD10	400916	coiled-coil-helix-coiled-coil-helix domain containing 10
Downregulated genes (alpha=1e-05)	CHCHD6	84303	coiled-coil-helix-coiled-coil-helix domain containing 6
Downregulated genes (alpha=1e-05)	CHDH	55349	choline dehydrogenase
Downregulated genes (alpha=1e-05)	CHST7	56548	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7
Downregulated genes (alpha=1e-05)	COL9A2	1298	collagen, type IX, alpha 2
Downregulated genes (alpha=1e-05)	CXCR7	57007	chemokine (C-X-C motif) receptor 7
Downregulated genes (alpha=1e-05)	DNASE1L3	1776	deoxyribonuclease I-like 3
Downregulated genes (alpha=1e-05)	ETS2	2114	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)
Downregulated genes (alpha=1e-05)	FAM46C	54855	family with sequence similarity 46, member C
Downregulated genes (alpha=1e-05)	FAM81A	145773	family with sequence similarity 81, member A
Downregulated genes (alpha=1e-05)	FKBP11	51303	FK506 binding protein 11, 19 kDa
Downregulated genes (alpha=1e-05)	GCSH	2653	glycine cleavage system protein H (aminomethyl carrier)
Downregulated genes (alpha=1e-05)	GLT25D2	23127	glycosyltransferase 25 domain containing 2
Downregulated genes (alpha=1e-05)	HEY2	23493	hairy/enhancer-of-split related with YRPW motif 2

Downregulated genes (alpha=1e-05)	IL17RB	55540	interleukin 17 receptor B
Downregulated genes (alpha=1e-05)	ITPKA	3706	inositol-trisphosphate 3-kinase A
Downregulated genes (alpha=1e-05)	KIF26B	55083	kinesin family member 26B
Downregulated genes (alpha=1e-05)	KISS1R	84634	KISS1 receptor
Downregulated genes (alpha=1e-05)	KLHL14	57565	kelch-like 14 (Drosophila)
Downregulated genes (alpha=1e-05)	LMO3	55885	LIM domain only 3 (rhombotin-like 2)
Downregulated genes (alpha=1e-05)	LMO7	4008	LIM domain 7
Downregulated genes (alpha=1e-05)	LOC100132439	100132439	protein FAM27E3-like
Downregulated genes (alpha=1e-05)	LOC284837	284837	uncharacterized LOC284837
Downregulated genes (alpha=1e-05)	LOC642661	642661	translocase of outer mitochondrial membrane 40 homolog (yeast) pseudogene
Downregulated genes (alpha=1e-05)	LOC645638	645638	WDNM1-like pseudogene
Downregulated genes (alpha=1e-05)	MAPK13	5603	mitogen-activated protein kinase 13
Downregulated genes (alpha=1e-05)	MLKL	197259	mixed lineage kinase domain-like
Downregulated genes (alpha=1e-05)	MS4A6A	64231	membrane-spanning 4-domains, subfamily A, member 6A
Downregulated genes (alpha=1e-05)	MSRB1	51734	methionine sulfoxide reductase B1
Downregulated genes (alpha=1e-05)	NAMPT	10135	nicotinamide phosphoribosyltransferase
Downregulated genes (alpha=1e-05)	NOD2	64127	nucleotide-binding oligomerization domain containing 2
Downregulated genes (alpha=1e-05)	NPM3	10360	nucleophosmin/nucleoplasmin 3
Downregulated genes (alpha=1e-05)	NPW	283869	neuropeptide W
Downregulated genes (alpha=1e-05)	NRARP	441478	NOTCH-regulated ankyrin repeat protein
Downregulated genes (alpha=1e-05)	NRG4	145957	neuregulin 4
Downregulated genes (alpha=1e-05)	PDCD2L	84306	programmed cell death 2-like
Downregulated genes (alpha=1e-05)	PDE7B	27115	phosphodiesterase 7B
Downregulated genes (alpha=1e-05)	PFKM	5213	phosphofructokinase, muscle
Downregulated genes (alpha=1e-05)	PIEZO1	9780	piezo-type mechanosensitive ion channel component 1
Downregulated genes (alpha=1e-05)	PNMA3	29944	paraneoplastic Ma antigen 3
Downregulated genes (alpha=1e-05)	PNP	4860	purine nucleoside phosphorylase
Downregulated genes (alpha=1e-05)	POLR1C	9533	polymerase (RNA) I polypeptide C, 30kDa
Downregulated genes (alpha=1e-05)	POLR3G	10622	polymerase (RNA) III (DNA directed) polypeptide G (32kD)
Downregulated genes (alpha=1e-05)	PPAN	56342	peter pan homolog (Drosophila)
Downregulated genes (alpha=1e-05)	PPAN-P2RY11	692312	PPAN-P2RY11 readthrough
Downregulated genes (alpha=1e-05)	PRMT1	3276	protein arginine methyltransferase 1

Downregulated genes (alpha=1e-05)	PVT1	5820	Pvt1 oncogene (non-protein coding)
Downregulated genes (alpha=1e-05)	RAB40B	10966	RAB40B, member RAS oncogene family
Downregulated genes (alpha=1e-05)	RGS16	6004	regulator of G-protein signaling 16
Downregulated genes (alpha=1e-05)	RPF2P1	729608	ribosome production factor 2 homolog (S. cerevisiae) pseudogene 1
Downregulated genes (alpha=1e-05)	RPL29	6159	ribosomal protein L29
Downregulated genes (alpha=1e-05)	RPS7	6201	ribosomal protein S7
Downregulated genes (alpha=1e-05)	RSPH1	89765	radial spoke head 1 homolog (Chlamydomonas)
Downregulated genes (alpha=1e-05)	RXRA	6256	retinoid X receptor, alpha
Downregulated genes (alpha=1e-05)	SEPT3	55964	septin 3
Downregulated genes (alpha=1e-05)	SEPW1	6415	selenoprotein W, 1
Downregulated genes (alpha=1e-05)	SH3TC1	54436	SH3 domain and tetratricopeptide repeats 1
Downregulated genes (alpha=1e-05)	SLC16A9	220963	solute carrier family 16, member 9 (monocarboxylic acid transporter 9)
Downregulated genes (alpha=1e-05)	SLC25A12	8604	solute carrier family 25 (aspartate/glutamate carrier), member 12
Downregulated genes (alpha=1e-05)	SLC27A5	10998	solute carrier family 27 (fatty acid transporter), member 5
Downregulated genes (alpha=1e-05)	SLC2A5	6518	solute carrier family 2 (facilitated glucose/fructose transporter), member 5
Downregulated genes (alpha=1e-05)	SNAPC4	6621	small nuclear RNA activating complex, polypeptide 4, 190kDa
Downregulated genes (alpha=1e-05)	SNORA24	677809	small nucleolar RNA, H/ACA box 24
Downregulated genes (alpha=1e-05)	SNORA56	677835	small nucleolar RNA, H/ACA box 56
Downregulated genes (alpha=1e-05)	SNORA64	26784	small nucleolar RNA, H/ACA box 64
Downregulated genes (alpha=1e-05)	SNORD16	595097	small nucleolar RNA, C/D box 16
Downregulated genes (alpha=1e-05)	SNORD65	692106	small nucleolar RNA, C/D box 65
Downregulated genes (alpha=1e-05)	SNORD80	26774	small nucleolar RNA, C/D box 80
Downregulated genes (alpha=1e-05)	SNORD83B	116938	small nucleolar RNA, C/D box 83B
Downregulated genes (alpha=1e-05)	SNORD96A	619571	small nucleolar RNA, C/D box 96A
Downregulated genes (alpha=1e-05)	SPINK2	6691	serine peptidase inhibitor, Kazal type 2 (acrosin-trypsin inhibitor)
Downregulated genes (alpha=1e-05)	SULF2	55959	sulfatase 2
Downregulated genes (alpha=1e-05)	SUSD2	56241	sushi domain containing 2
Downregulated genes (alpha=1e-05)	SYTL3	94120	synaptotagmin-like 3
Downregulated genes (alpha=1e-05)	TIGIT	201633	T cell immunoreceptor with Ig and ITIM domains
Downregulated genes (alpha=1e-05)	TLCD1	116238	TLC domain containing 1
Downregulated genes (alpha=1e-05)	TM2D3	80213	TM2 domain containing 3
Downregulated genes (alpha=1e-05)	TNFRSF13B	23495	tumor necrosis factor receptor superfamily, member 13B

Downregulated genes (alpha=1e-05)	TNFRSF4	7293	tumor necrosis factor receptor superfamily, member 4
Downregulated genes (alpha=1e-05)	WNT10A	80326	wingless-type MMTV integration site family, member 10A
Upregulated genes (alpha=1e-05)	ABCA1	19	ATP-binding cassette, sub-family A (ABC1), member 1
Upregulated genes (alpha=1e-05)	ABHD8	79575	abhydrolase domain containing 8
Upregulated genes (alpha=1e-05)	ADARB1	104	adenosine deaminase, RNA-specific, B1
Upregulated genes (alpha=1e-05)	ALDH5A1	7915	aldehyde dehydrogenase 5 family, member A1
Upregulated genes (alpha=1e-05)	APOC2	344	apolipoprotein C-II
Upregulated genes (alpha=1e-05)	APOD	347	apolipoprotein D
Upregulated genes (alpha=1e-05)	ARSD	414	arylsulfatase D
Upregulated genes (alpha=1e-05)	BACH2	60468	BTB and CNC homology 1, basic leucine zipper transcription factor 2
Upregulated genes (alpha=1e-05)	BEST3	144453	bestrophin 3
Upregulated genes (alpha=1e-05)	BHLHE40	8553	basic helix-loop-helix family, member e40
Upregulated genes (alpha=1e-05)	C12orf57	113246	chromosome 12 open reading frame 57
Upregulated genes (alpha=1e-05)	C16orf93	90835	chromosome 16 open reading frame 93
Upregulated genes (alpha=1e-05)	CAND2	23066	cullin-associated and neddylation-dissociated 2 (putative)
Upregulated genes (alpha=1e-05)	CASZ1	54897	castor zinc finger 1
Upregulated genes (alpha=1e-05)	CBLB	868	Cbl proto-oncogene, E3 ubiquitin protein ligase B
Upregulated genes (alpha=1e-05)	CCNG2	901	cyclin G2
Upregulated genes (alpha=1e-05)	CD24	100133941	CD24 molecule
Upregulated genes (alpha=1e-05)	CD72	971	CD72 molecule
Upregulated genes (alpha=1e-05)	CD81	975	CD81 molecule
Upregulated genes (alpha=1e-05)	CDKN2D	1032	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)
Upregulated genes (alpha=1e-05)	CIITA	4261	class II, major histocompatibility complex, transactivator
Upregulated genes (alpha=1e-05)	CNR1	1268	cannabinoid receptor 1 (brain)
Upregulated genes (alpha=1e-05)	COQ10A	93058	coenzyme Q10 homolog A (S. cerevisiae)
Upregulated genes (alpha=1e-05)	CXCL16	58191	chemokine (C-X-C motif) ligand 16
Upregulated genes (alpha=1e-05)	CXCR4	7852	chemokine (C-X-C motif) receptor 4
Upregulated genes (alpha=1e-05)	CYFIP2	26999	cytoplasmic FMR1 interacting protein 2
Upregulated genes (alpha=1e-05)	DDX54	79039	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54
Upregulated genes (alpha=1e-05)	DENND2A	27147	DENN/MADD domain containing 2A
Upregulated genes (alpha=1e-05)	DENND2D	79961	DENN/MADD domain containing 2D

Upregulated genes (alpha=1e-05)	DFNA5	1687	deafness, autosomal dominant 5
Upregulated genes (alpha=1e-05)	DOK3	79930	docking protein 3
Upregulated genes (alpha=1e-05)	EBF1	1879	early B-cell factor 1
Upregulated genes (alpha=1e-05)	EMP3	2014	epithelial membrane protein 3
Upregulated genes (alpha=1e-05)	EPSTI1	94240	epithelial stromal interaction 1 (breast)
Upregulated genes (alpha=1e-05)	ETV5	2119	ets variant 5
Upregulated genes (alpha=1e-05)	FBXO32	114907	F-box protein 32
Upregulated genes (alpha=1e-05)	FCGRT	2217	Fc fragment of IgG, receptor, transporter, alpha
Upregulated genes (alpha=1e-05)	FLJ42627	645644	uncharacterized LOC645644
Upregulated genes (alpha=1e-05)	GBE1	2632	glucan (1,4-alpha-), branching enzyme 1
Upregulated genes (alpha=1e-05)	GLRX	2745	glutaredoxin (thioltransferase)
Upregulated genes (alpha=1e-05)	GLRXP3	100132510	glutaredoxin (thioltransferase) pseudogene 3
Upregulated genes (alpha=1e-05)	GM2A	2760	GM2 ganglioside activator
Upregulated genes (alpha=1e-05)	GNG7	2788	guanine nucleotide binding protein (G protein), gamma 7
Upregulated genes (alpha=1e-05)	GPR160	26996	G protein-coupled receptor 160
Upregulated genes (alpha=1e-05)	GPR18	2841	G protein-coupled receptor 18
Upregulated genes (alpha=1e-05)	HBEGF	1839	heparin-binding EGF-like growth factor
Upregulated genes (alpha=1e-05)	HCP5	10866	HLA complex P5 (non-protein coding)
Upregulated genes (alpha=1e-05)	HES6	55502	hairy and enhancer of split 6 (Drosophila)
Upregulated genes (alpha=1e-05)	HLA-DPA1	3113	major histocompatibility complex, class II, DP alpha 1
Upregulated genes (alpha=1e-05)	HLA-DPB1	3115	major histocompatibility complex, class II, DP beta 1
Upregulated genes (alpha=1e-05)	HLA-DRA	3122	major histocompatibility complex, class II, DR alpha
Upregulated genes (alpha=1e-05)	IRF1	3659	interferon regulatory factor 1
Upregulated genes (alpha=1e-05)	IRF9	10379	interferon regulatory factor 9
Upregulated genes (alpha=1e-05)	KIAA1683	80726	KIAA1683
Upregulated genes (alpha=1e-05)	KLF2	10365	Kruppel-like factor 2 (lung)
Upregulated genes (alpha=1e-05)	KLHL22	84861	kelch-like 22 (Drosophila)
Upregulated genes (alpha=1e-05)	KLHL24	54800	kelch-like 24 (Drosophila)
Upregulated genes (alpha=1e-05)	KLHL6	89857	kelch-like 6 (Drosophila)
Upregulated genes (alpha=1e-05)	LAMP5	24141	lysosomal-associated membrane protein family, member 5
Upregulated genes (alpha=1e-05)	LGALS1	3956	lectin, galactoside-binding, soluble, 1
Upregulated genes (alpha=1e-05)	LILRB4	11006	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), meml

Upregulated genes (alpha=1e-05)	LINC00426	100188949	long intergenic non-protein coding RNA 426
Upregulated genes (alpha=1e-05)	LOC728743	728743	zinc finger protein pseudogene
Upregulated genes (alpha=1e-05)	LRMP	4033	lymphoid-restricted membrane protein
Upregulated genes (alpha=1e-05)	LY86	9450	lymphocyte antigen 86
Upregulated genes (alpha=1e-05)	LYL1	4066	lymphoblastic leukemia derived sequence 1
Upregulated genes (alpha=1e-05)	MDK	4192	midkine (neurite growth-promoting factor 2)
Upregulated genes (alpha=1e-05)	MFGE8	4240	milk fat globule-EGF factor 8 protein
Upregulated genes (alpha=1e-05)	MIS18BP1	55320	MIS18 binding protein 1
Upregulated genes (alpha=1e-05)	MYB	4602	v-myb myeloblastosis viral oncogene homolog (avian)
Upregulated genes (alpha=1e-05)	NINJ2	4815	ninjurin 2
Upregulated genes (alpha=1e-05)	NPAS1	4861	neuronal PAS domain protein 1
Upregulated genes (alpha=1e-05)	NPC2	10577	Niemann-Pick disease, type C2
Upregulated genes (alpha=1e-05)	NUB1	51667	negative regulator of ubiquitin-like proteins 1
Upregulated genes (alpha=1e-05)	NUCB2	4925	nucleobindin 2
Upregulated genes (alpha=1e-05)	OAS1	4938	2'-5'-oligoadenylate synthetase 1, 40/46kDa
Upregulated genes (alpha=1e-05)	OAS2	4939	2'-5'-oligoadenylate synthetase 2, 69/71kDa
Upregulated genes (alpha=1e-05)	OAS3	4940	2'-5'-oligoadenylate synthetase 3, 100kDa
Upregulated genes (alpha=1e-05)	P2RY8	286530	purinergic receptor P2Y, G-protein coupled, 8
Upregulated genes (alpha=1e-05)	PARP15	165631	poly (ADP-ribose) polymerase family, member 15
Upregulated genes (alpha=1e-05)	PARP9	83666	poly (ADP-ribose) polymerase family, member 9
Upregulated genes (alpha=1e-05)	PARVG	64098	parvin, gamma
Upregulated genes (alpha=1e-05)	PELI2	57161	pellino E3 ubiquitin protein ligase family member 2
Upregulated genes (alpha=1e-05)	PPP1R16B	26051	protein phosphatase 1, regulatory subunit 16B
Upregulated genes (alpha=1e-05)	PRKCE	5581	protein kinase C, epsilon
Upregulated genes (alpha=1e-05)	PRR5	55615	proline rich 5 (renal)
Upregulated genes (alpha=1e-05)	RARRES3	5920	retinoic acid receptor responder (tazarotene induced) 3
Upregulated genes (alpha=1e-05)	RASGRP2	10235	RAS guanyl releasing protein 2 (calcium and DAG-regulated)
Upregulated genes (alpha=1e-05)	RGS19	10287	regulator of G-protein signaling 19
Upregulated genes (alpha=1e-05)	RHBDF1	64285	rhomboid 5 homolog 1 (Drosophila)
Upregulated genes (alpha=1e-05)	RHOBTB2	23221	Rho-related BTB domain containing 2
Upregulated genes (alpha=1e-05)	RNF144A	9781	ring finger protein 144A
Upregulated genes (alpha=1e-05)	RNH1	6050	ribonuclease/angiogenin inhibitor 1

Upregulated genes (alpha=1e-05)	RRAGD	58528	Ras-related GTP binding D
Upregulated genes (alpha=1e-05)	SAMD9	54809	sterile alpha motif domain containing 9
Upregulated genes (alpha=1e-05)	SAMD9L	219285	sterile alpha motif domain containing 9-like
Upregulated genes (alpha=1e-05)	SASH3	54440	SAM and SH3 domain containing 3
Upregulated genes (alpha=1e-05)	SETDB2	83852	SET domain, bifurcated 2
Upregulated genes (alpha=1e-05)	SIT1	27240	signaling threshold regulating transmembrane adaptor 1
Upregulated genes (alpha=1e-05)	SLC26A11	284129	solute carrier family 26, member 11
Upregulated genes (alpha=1e-05)	SLC29A4	222962	solute carrier family 29 (nucleoside transporters), member 4
Upregulated genes (alpha=1e-05)	SMAD3	4088	SMAD family member 3
Upregulated genes (alpha=1e-05)	SNX29P2	440352	sorting nexin 29 pseudogene 2
Upregulated genes (alpha=1e-05)	SOCS1	8651	suppressor of cytokine signaling 1
Upregulated genes (alpha=1e-05)	SOX11	6664	SRY (sex determining region Y)-box 11
Upregulated genes (alpha=1e-05)	SOX18	54345	SRY (sex determining region Y)-box 18
Upregulated genes (alpha=1e-05)	SOX4	6659	SRY (sex determining region Y)-box 4
Upregulated genes (alpha=1e-05)	SSBP2	23635	single-stranded DNA binding protein 2
Upregulated genes (alpha=1e-05)	ST3GAL5	8869	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
Upregulated genes (alpha=1e-05)	STK38	11329	serine/threonine kinase 38
Upregulated genes (alpha=1e-05)	STMN1	3925	stathmin 1
Upregulated genes (alpha=1e-05)	STS	412	steroid sulfatase (microsomal), isozyme S
Upregulated genes (alpha=1e-05)	TAX1BP3	30851	Tax1 (human T-cell leukemia virus type I) binding protein 3
Upregulated genes (alpha=1e-05)	TMEM136	219902	transmembrane protein 136
Upregulated genes (alpha=1e-05)	TNFRSF14	8764	tumor necrosis factor receptor superfamily, member 14
Upregulated genes (alpha=1e-05)	TOP2B	7155	topoisomerase (DNA) II beta 180kDa
Upregulated genes (alpha=1e-05)	TP53INP1	94241	tumor protein p53 inducible nuclear protein 1
Upregulated genes (alpha=1e-05)	TPP1	1200	tripeptidyl peptidase I
Upregulated genes (alpha=1e-05)	TTYH3	80727	tweety homolog 3 (Drosophila)
Upregulated genes (alpha=1e-05)	VPREB3	29802	pre-B lymphocyte 3
Upregulated genes (alpha=1e-05)	WIPI2	26100	WD repeat domain, phosphoinositide interacting 2
Upregulated genes (alpha=1e-05)	WNT3	7473	wingless-type MMTV integration site family, member 3
Upregulated genes (alpha=1e-05)	YPEL5	51646	yippee-like 5 (Drosophila)
Upregulated genes (alpha=1e-05)	ZEB2	9839	zinc finger E-box binding homeobox 2
Upregulated genes (alpha=1e-05)	ZHX2	22882	zinc fingers and homeoboxes 2

Upregulated genes (alpha=1e-05)	ZSCAN16	80345	zinc finger and SCAN domain containing 16

Supplemental Table 3. Signatures that are significantly enriched with top regulated genes following z-VRPR-fmk treatment in Mino cells.

Downregulated signatures:

Signatures DB	Category	Sub Category	Signature name	Signature links	Defined members	Enrichment score	*P* [GSEA] (by permutation test)	FDR [GSEA]
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_FORMATION_OF_ATP_BY_CHEMIOSM	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_FORMATION_OF_ATP_BY_CI	13	0.8416	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	COLLER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/COLLER_MYC_TARGETS_UP	25	0.8405	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	Mitochondrial ribosomal proteins	Mitochondrial ribosomal proteins / small subunits	http://www.genenames.org/genefamilies/MRP#MRPS	31	0.8392	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	FUNG_IL2_SIGNALING_1	http://www.broadinstitute.org/gsea/msigdb/cards/FUNG_IL2_SIGNALING_1	11	0.8324	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	Mitochondrial respiratory chain cc	Mitochondrial respiratory chain complex / Complex V	http://www.genenames.org/genefamilies/mitocomplex#FATP	16	0.8277	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	ATPases	ATPases / F-type	http://www.genenames.org/genefamilies/ATP#F1ATP	15	0.8254	0.0019	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	ORGANELLAR_SMALL_RIBOSOMAL_SUBUNIT	http://www.broadinstitute.org/gsea/msigdb/cards/ORGANELLAR_SMALL_RIBOSOMAL_SUB	11	0.8100	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	MITOCHONDRIAL_SMALL_RIBOSOMAL_SUBUNIT	http://www.broadinstitute.org/gsea/msigdb/cards/MITOCHONDRIAL_SMALL_RIBOSOMAL_S	11	0.8100	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	SMALL_RIBOSOMAL_SUBUNIT	http://www.broadinstitute.org/gsea/msigdb/cards/SMALL_RIBOSOMAL_SUBUNIT	11	0.8100	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	Mitochondrial respiratory chain cc	Mitochondrial respiratory chain complex / Complex III	http://www.genenames.org/genefamilies/mitocomplex#comIII	9	0.7990	0.0019	0.0093
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_103	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_103	12	0.7924	0.0010	0.0039
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	COENZYME_BIOSYNTHETIC_PROCESS	http://www.broadinstitute.org/gsea/msigdb/cards/COENZYME_BIOSYNTHETIC_PROCESS	10	0.7862	0.0103	0.0354
HGNCSigDB_dMay2014	gene families	General transcription factor IIH cc	General transcription factor IIH complex subunits	http://www.genenames.org/genefamilies/TFIIH	10	0.7861	0.0049	0.0086
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHLOSSER_MYC_AND_SERUM_RESPONSE_SYN	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_AND_SERUM_RESPO	32	0.7853	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	PROTEIN_TARGETING_TO_MITOCHONDRION	http://www.broadinstitute.org/gsea/msigdb/cards/PROTEIN_TARGETING_TO_MITOCHONDF	11	0.7803	0.0023	0.0212
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	XU_RESPONSE_TO_TRETINOIN_AND_NSC682994_	http://www.broadinstitute.org/gsea/msigdb/cards/XU_RESPONSE_TO_TRETINOIN_AND_NS	15	0.7791	0.0010	0.0010
StaudtSigDB_dNov2012	Signaling pathway	T cell cytokine signallng	Tcell_cytokine_induced_prolif	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=77	27	0.7749	0.0010	0.0010
GeneSigDB_v4_Sept2011	Mouse	Lung	homolog(Lung_Rangasamy09_10genes_DownRegulat	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19286929-SuppTable2b	9	0.7742	0.0071	0.0602
GeneSigDB_v4_Sept2011	Mouse	StemCell	homolog(StemCell_Parker05_27genes, from Mus mus	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15992799-table2	15	0.7702	0.0010	0.0052
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	RRNA_PROCESSING	http://www.broadinstitute.org/gsea/msigdb/cards/RRNA_PROCESSING	15	0.7674	0.0012	0.0083
GeneSigDB_v4_Sept2011	Human	Viral	Viral_Zafrakas07_14genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=18288381-Table1	8	0.7664	0.0218	0.0370
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_50	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_50	13	0.7641	0.0011	0.0049
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	RRNA_METABOLIC_PROCESS	http://www.broadinstitute.org/gsea/msigdb/cards/RRNA_METABOLIC_PROCESS	16	0.7630	0.0012	0.0059
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_471	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_471	10	0.7594	0.0094	0.0147
StaudtSigDB_dNov2012	Signaling pathway	MYC	Myc_overexpression_1.5x_up	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=16	86	0.7573	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	NUCLEOLAR_PART	http://www.broadinstitute.org/gsea/msigdb/cards/NUCLEOLAR_PART	18	0.7532	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_MRNA_DECAY_BY_3_TO_5_EXORIBO	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_MRNA_DECAY_BY_3_TO_5_I	11	0.7517	0.0058	0.0101
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	AMINO_ACID_DERIVATIVE_BIOSYNTHETIC_PROCI	http://www.broadinstitute.org/gsea/msigdb/cards/AMINO_ACID_DERIVATIVE_BIOSYNTHETI	10	0.7482	0.0108	0.0336
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / KEGG	KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_BIOS	http://www.broadinstitute.org/gsea/msigdb/cards/KEGG_VALINE_LEUCINE_AND_ISOLEUCI	11	0.7471	0.0040	0.0014
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	RIBOSOME_BIOGENESIS_AND_ASSEMBLY	http://www.broadinstitute.org/gsea/msigdb/cards/RIBOSOME_BIOGENESIS_AND_ASSEMBL	18	0.7467	0.0012	0.0032
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHUHMACHER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHUHMACHER_MYC_TARGETS_UP	80	0.7466	0.0010	0.0010
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_77	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_77	28	0.7441	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	OLIGOSACCHARYL_TRANSFERASE_COMPLEX	http://www.broadinstitute.org/gsea/msigdb/cards/OLIGOSACCHARYL_TRANSFERASE_COM	10	0.7370	0.0092	0.0056
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_25	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_25	13	0.7368	0.0027	0.0125
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	ORGANELLAR_RIBOSOME	http://www.broadinstitute.org/gsea/msigdb/cards/ORGANELLAR_RIBOSOME	22	0.7360	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	MITOCHONDRIAL_RIBOSOME	http://www.broadinstitute.org/gsea/msigdb/cards/MITOCHONDRIAL_RIBOSOME	22	0.7360	0.0010	0.0010
GeneSigDB_v4_Sept2011	Human	Lymphoma	Lymphoma_Cassinelli09_59genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19555670-Table3b	49	0.7356	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	TRNA_PROCESSING	http://www.broadinstitute.org/gsea/msigdb/cards/TRNA_PROCESSING	10	0.7343	0.0129	0.0470
StaudtSigDB_dNov2012	Signaling pathway	Notch	Notch_T-ALL_up_Palomero	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=23	47	0.7300	0.0011	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_RNA_POL_I_TRANSCRIPTION_TERMI	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_RNA_POL_I_TRANSCRIPTIO	21	0.7294	0.0010	0.0020
HGNCSigDB_dMay2014	gene families	N(alpha)-acetyltransferase subun	N(alpha)-acetyltransferase subunits	http://www.genenames.org/genefamilies/NAA	12	0.7290	0.0155	0.0111
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / KEGG	KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS	http://www.broadinstitute.org/gsea/msigdb/cards/KEGG_TERPENOID_BACKBONE_BIOSYN	15	0.7268	0.0010	0.0021
HGNCSigDB_dMay2014	gene families	Mitochondrial respiratory chain cc	Mitochondrial respiratory chain complex / Complex IV	http://www.genenames.org/genefamilies/mitocomplex#comIV	16	0.7265	0.0010	0.0010
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_528	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_528	11	0.7254	0.0136	0.0139
GeneSigDB_v4_Sept2011	Human	Breast	Breast_Larsson07_76genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=17638893-SuppTable7	45	0.7200	0.0010	0.0010
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer gene neighborhoods	GNF2_NS	http://www.broadinstitute.org/gsea/msigdb/cards/GNF2_NS	40	0.7162	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_BRANCHED_CHAIN_AMINO_ACID_CA	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_BRANCHED_CHAIN_AMINO_	17	0.7158	0.0014	0.0070
GeneSigDB_v4_Sept2011	Mouse	Bone	homolog(Bone_Kalajzic05_12genes, from Mus muscul	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15834136-Table3	10	0.7141	0.0340	0.0481
HGNCSigDB_dMay2014	gene families	Mitochondrial ribosomal proteins	Mitochondrial ribosomal proteins / large subunits	http://www.genenames.org/genefamilies/MRP#MRPL	49	0.7141	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHLOSSER_MYC_TARGETS_AND_SERUM_RESP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERU	47	0.7138	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHLOSSER_MYC_TARGETS_AND_SERUM_RESP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERU	47	0.7127	0.0010	0.0010
GeneSigDB_v4_Sept2011	Human	Breast	Breast_Lee10_23genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=20068086-ST1-3	23	0.7110	0.0010	0.0013
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_MITOCHONDRIAL_TRNA_AMINOACYL	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_MITOCHONDRIAL_TRNA_AM	21	0.7101	0.0015	0.0017
StaudtSigDB_dNov2012	Transcription factor target	PGC-1 alpha	PGC-1alpha_overepxression_up	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=21	27	0.7084	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	Aminoacyl tRNA synthetases	Aminoacyl tRNA synthetases / Class I	http://www.genenames.org/genefamilies/AARS#AARS1	19	0.7048	0.0010	0.0014
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_MITOCHONDRIAL_PROTEIN_IMPORT	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_MITOCHONDRIAL_PROTEIN_	52	0.7030	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	Short chain dehydrogenase	Short chain dehydrogenase/reductase superfamily / Ch	http://www.genenames.org/genefamilies/SDR#SDRC1	20	0.7024	0.0010	0.0011
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	RIBONUCLEOPROTEIN_BINDING	http://www.broadinstitute.org/gsea/msigdb/cards/RIBONUCLEOPROTEIN_BINDING	12	0.7018	0.0208	0.3597

Upregulated signatures:

Signatures DB	Category	Sub Category	Signature name	Signature links	Defined members	Enrichment score	*P* [GSEA] (by permutation test)	FDR [GSEA]
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_293	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_293	12	-0.8842	0.0010	0.0018
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_ENDOSOMAL_VACUOLAR_PATHWAY	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_ENDOSOMAL_VACUOLAR_P	8	-0.8811	0.0027	0.0086
HGNCSigDB_dMay2014	gene families	Histocompatibility complex	Histocompatibility complex	http://www.genenames.org/genefamilies/HLA	24	-0.8802	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	HOLLEMAN_ASPARAGINASE_RESISTANCE_B_ALL	http://www.broadinstitute.org/gsea/msigdb/cards/HOLLEMAN_ASPARAGINASE_RESISTANC	14	-0.8772	0.0010	0.0009
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_143	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_143	14	-0.8556	0.0010	0.0036
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	HOLLEMAN_DAUNORUBICIN_B_ALL_DN	http://www.broadinstitute.org/gsea/msigdb/cards/HOLLEMAN_DAUNORUBICIN_B_ALL_DN	12	-0.8443	0.0010	0.0009
GeneSigDB_v4_Sept2011	Mouse	Prostate	homolog(Prostate_Glinsky05_11genes, from Mus musi	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15931389-table3	8	-0.8367	0.0060	0.0111
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHMAHL_PDGF_SIGNALING	http://www.broadinstitute.org/gsea/msigdb/cards/SCHMAHL_PDGF_SIGNALING	9	-0.8335	0.0011	0.0073
GeneSigDB_v4_Sept2011	Human	Lymphoma	Lymphoma_Fernandez10_13genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=20124476-SuppTable3	12	-0.8277	0.0010	0.0075
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	G_PROTEIN_SIGNALING_ADENYLATE_CYCLASE_I	http://www.broadinstitute.org/gsea/msigdb/cards/G_PROTEIN_SIGNALING_ADENYLATE_CY	10	-0.8155	0.0011	0.0310

GeneSigDB_v4_Sept2011	Human	Lung	Lung_Nam10_10genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=20369051-Table1	9	-0.8094	0.0050	0.0122
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	MAINA_VHL_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/MAINA_VHL_TARGETS_UP	10	-0.7897	0.0022	0.0098
GeneSigDB_v4_Sept2011	Human	Colon	Colon_Protiva09_31genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19139017-Table3	23	-0.7806	0.0010	0.0009
GeneSigDB_v4_Sept2011	Human	Immune	Immune_Ristich05_13genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15770701-Table1	12	-0.7697	0.0033	0.0102
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	SMALL_CONJUGATING_PROTEIN_BINDING	http://www.broadinstitute.org/gsea/msigdb/cards/SMALL_CONJUGATING_PROTEIN_BINDIN	12	-0.7694	0.0010	0.0163
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	INTERLEUKIN_2_PRODUCTION	http://www.broadinstitute.org/gsea/msigdb/cards/INTERLEUKIN_2_PRODUCTION	11	-0.7680	0.0033	0.0251
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	UBIQUITIN_BINDING	http://www.broadinstitute.org/gsea/msigdb/cards/UBIQUITIN_BINDING	11	-0.7668	0.0036	0.0173
GeneSigDB_v4_Sept2011	Human	Liver	Liver_Liu03_11genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=14728809-Table3b	9	-0.7659	0.0034	0.0176
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_UNWINDING_OF_DNA	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_UNWINDING_OF_DNA	11	-0.7625	0.0052	0.0171
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	BOWIE_RESPONSE_TO_EXTRACELLULAR_MATRI	http://www.broadinstitute.org/gsea/msigdb/cards/BOWIE_RESPONSE_TO_EXTRACELLULA	17	-0.7620	0.0010	0.0022
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	ACTIN_FILAMENT_BASED_MOVEMENT	http://www.broadinstitute.org/gsea/msigdb/cards/ACTIN_FILAMENT_BASED_MOVEMENT	10	-0.7578	0.0109	0.0342
GeneSigDB_v4_Sept2011	Human	Viral	Viral_Stoff-Khalili06_9genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=16410819-Table5	9	-0.7553	0.0152	0.0222
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	VISALA_AGING_LYMPHOCYTE_UP	http://www.broadinstitute.org/gsea/msigdb/cards/VISALA_AGING_LYMPHOCYTE_UP	10	-0.7542	0.0105	0.0134
GeneSigDB_v4_Sept2011	Human	StemCell	StemCell_Novakova10_22genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19802007-Table1	14	-0.7514	0.0020	0.0109
GeneSigDB_v4_Sept2011	Human	StemCell	StemCell_Lambert09_12genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19946333-Table1	12	-0.7498	0.0010	0.0105
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	EGUCHI_CELL_CYCLE_RB1_TARGETS	http://www.broadinstitute.org/gsea/msigdb/cards/EGUCHI_CELL_CYCLE_RB1_TARGETS	23	-0.7483	0.0010	0.0009
GeneSigDB_v4_Sept2011	Human	Viral	Viral_Guo05_21genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=16254373-Table3	18	-0.7471	0.0016	0.0067
GeneSigDB_v4_Sept2011	Human	Leukemia	Leukemia_Wilson06_15genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=16597596-TableS6-2	14	-0.7461	0.0017	0.0075
GeneSigDB_v4_Sept2011	Human	Leukemia	Leukemia_Sanchez-Guijo08_14genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=18722011-SuppTable2a	13	-0.7453	0.0084	0.0112
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	GUTIERREZ_WALDENSTROEMS_MACROGLOBULI	http://www.broadinstitute.org/gsea/msigdb/cards/GUTIERREZ_WALDENSTROEMS_MACRO	13	-0.7433	0.0043	0.0080
GeneSigDB_v4_Sept2011	Human	Lymphoma	Lymphoma_Mahadevan05_11genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=16373702-Table3	9	-0.7371	0.0217	0.0213
GeneSigDB_v4_Sept2011	Human	Breast	Breast_Li09_24genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=18278552-Genes	13	-0.7366	0.0051	0.0103
HGNCSigDB_dMay2014	gene families	POTE ankyrin domain containing	POTE ankyrin domain containing	http://www.genenames.org/genefamilies/POTE	11	-0.7363	0.0091	0.0308
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / BioCarta	BIOCARTA_RANKL_PATHWAY	http://www.broadinstitute.org/gsea/msigdb/cards/BIOCARTA_RANKL_PATHWAY	14	-0.7359	0.0040	0.0392
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	UROSEVIC_RESPONSE_TO_IMIQUIMOD	http://www.broadinstitute.org/gsea/msigdb/cards/UROSEVIC_RESPONSE_TO_IMIQUIMOD	23	-0.7350	0.0010	0.0009
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	SEMBA_FHIT_TARGETS_DN	http://www.broadinstitute.org/gsea/msigdb/cards/SEMBA_FHIT_TARGETS_DN	10	-0.7344	0.0183	0.0239
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	BANDRES_RESPONSE_TO_CARMUSTIN_MGMT_2	http://www.broadinstitute.org/gsea/msigdb/cards/BANDRES_RESPONSE_TO_CARMUSTIN_	9	-0.7342	0.0171	0.0218
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	GRANDVAUX_IFN_RESPONSE_NOT_VIA_IRF3	http://www.broadinstitute.org/gsea/msigdb/cards/GRANDVAUX_IFN_RESPONSE_NOT_VIA_	14	-0.7340	0.0034	0.0082
HGNCSigDB_dMay2014	gene families	Chromatin-modifying enzymes	Chromatin-modifying enzymes / K-demethylases	http://www.genenames.org/genefamilies/KDM-KAT-KMT#KDM	20	-0.7338	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	MYLLYKANGAS_AMPLIFICATION_HOT_SPOT_12	http://www.broadinstitute.org/gsea/msigdb/cards/MYLLYKANGAS_AMPLIFICATION_HOT_SP	11	-0.7336	0.0085	0.0187
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	CASORELLI_APL_SECONDARY_VS_DE_NOVO_DN	http://www.broadinstitute.org/gsea/msigdb/cards/CASORELLI_APL_SECONDARY_VS_DE_N	9	-0.7335	0.0159	0.0245
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	CATION_TRANSPORTING_ATPASE_ACTIVITY	http://www.broadinstitute.org/gsea/msigdb/cards/CATION_TRANSPORTING_ATPASE_ACTIV	11	-0.7333	0.0125	0.0407
GeneSigDB_v4_Sept2011	Human	Breast	Breast_Feng07_21genesUpRegulated	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=17123152-Table3b	20	-0.7315	0.0010	0.0073
GeneSigDB_v4_Sept2011	Human	Lymphoma	Lymphoma_Blenk08_16genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=18416826-Table2	11	-0.7299	0.0143	0.0187
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_402	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_402	12	-0.7293	0.0040	0.0047
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	BOWIE_RESPONSE_TO_TAMOXIFEN	http://www.broadinstitute.org/gsea/msigdb/cards/BOWIE_RESPONSE_TO_TAMOXIFEN	18	-0.7271	0.0011	0.0050
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer gene neighborhoods	GNF2_VAV1	http://www.broadinstitute.org/gsea/msigdb/cards/GNF2_VAV1	36	-0.7267	0.0010	0.0010
GeneSigDB_v4_Sept2011	Human	StemCell	StemCell_Roth05_11genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15741219-Table2	9	-0.7266	0.0308	0.0253
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / KEGG	KEGG_ASTHMA	http://www.broadinstitute.org/gsea/msigdb/cards/KEGG_ASTHMA	30	-0.7264	0.0010	0.0009
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbation	GOLUB_ALL_VS_AML_UP	http://www.broadinstitute.org/gsea/msigdb/cards/GOLUB_ALL_VS_AML_UP	24	-0.7261	0.0010	0.0009
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_G1_S_SPECIFIC_TRANSCRIPTION	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_G1_S_SPECIFIC_TRANSCRII	18	-0.7201	0.0030	0.0084
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	THYROID_HORMONE_RECEPTOR_BINDING	http://www.broadinstitute.org/gsea/msigdb/cards/THYROID_HORMONE_RECEPTOR_BINDII	17	-0.7197	0.0012	0.0144
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	NUCLEAR_SPECK	http://www.broadinstitute.org/gsea/msigdb/cards/NUCLEAR_SPECK	11	-0.7159	0.0138	0.0253
GeneSigDB_v4_Sept2011	Human	Breast	Breast_Sgroi99_16genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=10582678-Table1	11	-0.7149	0.0152	0.0174
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / KEGG	KEGG_OTHER_GLYCAN_DEGRADATION	http://www.broadinstitute.org/gsea/msigdb/cards/KEGG_OTHER_GLYCAN_DEGRADATION	16	-0.7145	0.0040	0.0015
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	ESTABLISHMENT_OF_ORGANELLE_LOCALIZATIOI	http://www.broadinstitute.org/gsea/msigdb/cards/ESTABLISHMENT_OF_ORGANELLE_LOCA	18	-0.7100	0.0011	0.0207
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	INSULIN_LIKE_GROWTH_FACTOR_RECEPTOR_BII	http://www.broadinstitute.org/gsea/msigdb/cards/INSULIN_LIKE_GROWTH_FACTOR_RECEI	10	-0.7095	0.0252	0.0573
GeneSigDB_v4_Sept2011	Human	Skin	Skin_Zimmerer08_23genes_InVitrovsInVivo	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=18794103-Table3	20	-0.7046	0.0017	0.0070
StaudtSigDB_dNov2012	Transcription factor target	NFkB	NFkB_Up_OCILy3_only	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=87	10	-0.7041	0.0245	0.0152
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	VACUOLAR_MEMBRANE	http://www.broadinstitute.org/gsea/msigdb/cards/VACUOLAR_MEMBRANE	11	-0.7034	0.0203	0.0246
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer gene neighborhoods	GNF2_INPP5D	http://www.broadinstitute.org/gsea/msigdb/cards/GNF2_INPP5D	43	-0.7032	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	BETA_TUBULIN_BINDING	http://www.broadinstitute.org/gsea/msigdb/cards/BETA_TUBULIN_BINDING	10	-0.7019	0.0375	0.0477

Supplemental Table 4. MYC signatures enriched with top regulated genes following z-VRPR-fmk treatment in Mino cells.

Downregulated signatures:				
Signature name	Signature links	Defined members	Enrichment score	*P* [GSEA] (by permutation test)
COLLER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/COLLER_MYC_TARGETS_UP	25	0.8405	0.0010
SCHLOSSER_MYC_AND_SERUM_RESPONSE_SYNERGY	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_AND_SERUM_RESPONSE_SYNERGY	32	0.7853	0.0010
Myc_overexpression_1.5x_up	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=167	88	0.7573	0.0010
SCHUHMACHER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHUHMACHER_MYC_TARGETS_UP	80	0.7466	0.0010
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_DN	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_DN	47	0.7138	0.0010
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_UP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_UP	47	0.7127	0.0010
MENSSEN_MYC_TARGETS	http://www.broadinstitute.org/gsea/msigdb/cards/MENSSEN_MYC_TARGETS	53	0.6902	0.0010
Myc_overexpression_2x_up	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=168	36	0.6781	0.0010
CAIRO_PML_TARGETS_BOUND_BY_MYC_DN	http://www.broadinstitute.org/gsea/msigdb/cards/CAIRO_PML_TARGETS_BOUND_BY_MYC_DN	14	0.6580	0.0066

Supplemental Table 5. Downregulated and upregulated genes following z-VRPR-fmk treatment in Rec-1 cells.

Signature name	Gene symbol	Gene ID	Gene description
Downregulated genes (alpha=0.0025)	ANKRD27	84079	ankyrin repeat domain 27
Downregulated genes (alpha=0.0025)	AQP3	360	aquaporin 3 (Gill blood group)
Downregulated genes (alpha=0.0025)	ASH1L-AS1	645676	ASH1L antisense RNA 1
Downregulated genes (alpha=0.0025)	ASS1	445	argininosuccinate synthase 1
Downregulated genes (alpha=0.0025)	ASS1P11	340274	argininosuccinate synthetase 1 pseudogene 11
Downregulated genes (alpha=0.0025)	ATP5C1	509	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
Downregulated genes (alpha=0.0025)	BCS1L	617	BCS1 homolog, ubiquinol-cytochrome c reductase complex chaperone
Downregulated genes (alpha=0.0025)	C20orf27	54976	chromosome 20 open reading frame 27
Downregulated genes (alpha=0.0025)	C9orf173	441476	sperm-tail PG-rich repeat containing 3
Downregulated genes (alpha=0.0025)	CBX6	23466	chromobox 6
Downregulated genes (alpha=0.0025)	CCNY	219771	cyclin Y
Downregulated genes (alpha=0.0025)	CD3EAP	10849	CD3e molecule associated protein
Downregulated genes (alpha=0.0025)	CHCHD4	131474	coiled-coil-helix-coiled-coil-helix domain containing 4
Downregulated genes (alpha=0.0025)	CHEK2	11200	checkpoint kinase 2
Downregulated genes (alpha=0.0025)	CIZ1	25792	CDKN1A interacting zinc finger protein 1
Downregulated genes (alpha=0.0025)	CUX2	23316	cut like homeobox 2
Downregulated genes (alpha=0.0025)	CXCR7	57007	atypical chemokine receptor 3
Downregulated genes (alpha=0.0025)	CYSLTR1	10800	cysteinyl leukotriene receptor 1
Downregulated genes (alpha=0.0025)	DANCR	57291	differentiation antagonizing non-protein coding RNA
Downregulated genes (alpha=0.0025)	DEXI	28955	Dexi homolog
Downregulated genes (alpha=0.0025)	ERCC8	1161	ERCC excision repair 8, CSA ubiquitin ligase complex subunit
Downregulated genes (alpha=0.0025)	ERI3	79033	ERI1 exoribonuclease family member 3
Downregulated genes (alpha=0.0025)	ETS2	2114	ETS proto-oncogene 2, transcription factor
Downregulated genes (alpha=0.0025)	FAM203B	728071	
Downregulated genes (alpha=0.0025)	FAM81A	145773	family with sequence similarity 81 member A
Downregulated genes (alpha=0.0025)	FDXACB1	91893	ferredoxin-fold anticodon binding domain containing 1
Downregulated genes (alpha=0.0025)	FKBP4	2288	FK506 binding protein 4

Downregulated genes (alpha=0.0025)	FLJ39739	388685	long intergenic non-protein coding RNA 1138
Downregulated genes (alpha=0.0025)	GFM1	85476	G elongation factor mitochondrial 1
Downregulated genes (alpha=0.0025)	GMDS	2762	GDP-mannose 4,6-dehydratase
Downregulated genes (alpha=0.0025)	GRB14	2888	growth factor receptor bound protein 14
Downregulated genes (alpha=0.0025)	HEY2	23493	hes related family bHLH transcription factor with YRPW motif 2
Downregulated genes (alpha=0.0025)	HNRNPA1	3178	heterogeneous nuclear ribonucleoprotein A1
Downregulated genes (alpha=0.0025)	IARS	3376	isoleucyl-tRNA synthetase
Downregulated genes (alpha=0.0025)	IDH3A	3419	isocitrate dehydrogenase 3 (NAD(+)) alpha
Downregulated genes (alpha=0.0025)	IL2RB	3560	interleukin 2 receptor subunit beta
Downregulated genes (alpha=0.0025)	ITPK1	3705	inositol-tetrakisphosphate 1-kinase
Downregulated genes (alpha=0.0025)	KLHL14	57565	kelch like family member 14
Downregulated genes (alpha=0.0025)	KREMEN2	79412	kringle containing transmembrane protein 2
Downregulated genes (alpha=0.0025)	LDHA	3939	lactate dehydrogenase A
Downregulated genes (alpha=0.0025)	LFNG	3955	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase
Downregulated genes (alpha=0.0025)	LMO3	55885	LIM domain only 3
Downregulated genes (alpha=0.0025)	LOC146880	146880	Rho GTPase activating protein 27 pseudogene
Downregulated genes (alpha=0.0025)	LOC642909	642909	C-terminal binding protein 2 pseudogene 4
Downregulated genes (alpha=0.0025)	LOC644684	644684	BMS1, ribosome biogenesis factor pseudogene 12
Downregulated genes (alpha=0.0025)	LOC653557	653557	BMS1, ribosome biogenesis factor pseudogene 8
Downregulated genes (alpha=0.0025)	MARS2	92935	methionyl-tRNA synthetase 2, mitochondrial
Downregulated genes (alpha=0.0025)	MESP1	55897	mesoderm posterior bHLH transcription factor 1
Downregulated genes (alpha=0.0025)	MINA	84864	MYC induced nuclear antigen
Downregulated genes (alpha=0.0025)	MRPS23	51649	mitochondrial ribosomal protein S23
Downregulated genes (alpha=0.0025)	MSRB1	51734	methionine sulfoxide reductase B1
Downregulated genes (alpha=0.0025)	MYCBP	26292	MYC binding protein
Downregulated genes (alpha=0.0025)	NAA10	8260	N(alpha)-acetyltransferase 10, NatA catalytic subunit
Downregulated genes (alpha=0.0025)	NCLN	56926	nicalin
Downregulated genes (alpha=0.0025)	NDUFB8	4714	NADH:ubiquinone oxidoreductase subunit B8
Downregulated genes (alpha=0.0025)	NOP16	51491	NOP16 nucleolar protein
Downregulated genes (alpha=0.0025)	NPW	283869	neuropeptide W
Downregulated genes (alpha=0.0025)	NT5DC3	51559	5'-nucleotidase domain containing 3
Downregulated genes (alpha=0.0025)	NUFIP1	26747	NUFIP1, FMR1 interacting protein 1

Downregulated genes (alpha=0.0025)	NUP35	129401	nucleoporin 35
Downregulated genes (alpha=0.0025)	PDCL3	79031	phosducin like 3
Downregulated genes (alpha=0.0025)	PDE7B	27115	phosphodiesterase 7B
Downregulated genes (alpha=0.0025)	PDIA2	64714	protein disulfide isomerase family A member 2
Downregulated genes (alpha=0.0025)	PDIA5	10954	protein disulfide isomerase family A member 5
Downregulated genes (alpha=0.0025)	PHBP11	644214	prohibitin pseudogene 11
Downregulated genes (alpha=0.0025)	PNPO	55163	pyridoxamine 5'-phosphate oxidase
Downregulated genes (alpha=0.0025)	POLDIP2	26073	DNA polymerase delta interacting protein 2
Downregulated genes (alpha=0.0025)	POLR1C	9533	RNA polymerase I subunit C
Downregulated genes (alpha=0.0025)	POLR2F	5435	RNA polymerase II subunit F
Downregulated genes (alpha=0.0025)	PPAN	56342	peter pan homolog (Drosophila)
Downregulated genes (alpha=0.0025)	PSMG1	8624	proteasome assembly chaperone 1
Downregulated genes (alpha=0.0025)	PTCD2	79810	pentatricopeptide repeat domain 2
Downregulated genes (alpha=0.0025)	PTGES3P3	441050	prostaglandin E synthase 3 pseudogene 3
Downregulated genes (alpha=0.0025)	RASA4	10156	RAS p21 protein activator 4
Downregulated genes (alpha=0.0025)	RCBTB2	1102	RCC1 and BTB domain containing protein 2
Downregulated genes (alpha=0.0025)	RDH10	157506	retinol dehydrogenase 10 (all-trans)
Downregulated genes (alpha=0.0025)	RFESD	317671	Rieske Fe-S domain containing
Downregulated genes (alpha=0.0025)	RPL26P30	653147	ribosomal protein L26 pseudogene 30
Downregulated genes (alpha=0.0025)	RPL31P4	729646	ribosomal protein L31 pseudogene 4
Downregulated genes (alpha=0.0025)	RPS7	6201	ribosomal protein S7
Downregulated genes (alpha=0.0025)	RRP12	23223	ribosomal RNA processing 12 homolog
Downregulated genes (alpha=0.0025)	RRP15	51018	ribosomal RNA processing 15 homolog
Downregulated genes (alpha=0.0025)	RSAD1	55316	radical S-adenosyl methionine domain containing 1
Downregulated genes (alpha=0.0025)	RUVBL1	8607	RuvB like AAA ATPase 1
Downregulated genes (alpha=0.0025)	SART3	9733	squamous cell carcinoma antigen recognized by T-cells 3
Downregulated genes (alpha=0.0025)	SBDSP1	155370	Shwachman-Bodian-Diamond syndrome pseudogene 1
Downregulated genes (alpha=0.0025)	SCARNA13	677768	small Cajal body-specific RNA 13
Downregulated genes (alpha=0.0025)	SDC3	9672	syndecan 3
Downregulated genes (alpha=0.0025)	SLC16A9	220963	solute carrier family 16 member 9
Downregulated genes (alpha=0.0025)	SLC19A1	6573	solute carrier family 19 member 1
Downregulated genes (alpha=0.0025)	SLC25A19	60386	solute carrier family 25 member 19

Downregulated genes (alpha=0.0025)	SLC25A39	51629	solute carrier family 25 member 39
Downregulated genes (alpha=0.0025)	SNAPC4	6621	small nuclear RNA activating complex polypeptide 4
Downregulated genes (alpha=0.0025)	SNORD96A	619571	small nucleolar RNA, C/D box 96A
Downregulated genes (alpha=0.0025)	SPATA13	221178	spermatogenesis associated 13
Downregulated genes (alpha=0.0025)	SREK1IP1	285672	SREK1 interacting protein 1
Downregulated genes (alpha=0.0025)	STUB1	10273	STIP1 homology and U-box containing protein 1
Downregulated genes (alpha=0.0025)	TAF4B	6875	TATA-box binding protein associated factor 4b
Downregulated genes (alpha=0.0025)	TFRC	7037	transferrin receptor
Downregulated genes (alpha=0.0025)	TIGIT	201633	T-cell immunoreceptor with Ig and ITIM domains
Downregulated genes (alpha=0.0025)	TLE1P1	645381	transducin like enhancer of split 1 pseudogene 1
Downregulated genes (alpha=0.0025)	TSFM	10102	Ts translation elongation factor, mitochondrial
Downregulated genes (alpha=0.0025)	TUBA4A	7277	tubulin alpha 4a
Downregulated genes (alpha=0.0025)	TUT1	64852	terminal uridylyl transferase 1, U6 snRNA-specific
Downregulated genes (alpha=0.0025)	TYW3	127253	tRNA-yW synthesizing protein 3 homolog
Downregulated genes (alpha=0.0025)	UNC93B3	285296	unc-93 homolog B3 pseudogene (C. elegans)
Downregulated genes (alpha=0.0025)	UNC93B6	255620	unc-93 homolog B6 pseudogene (C. elegans)
Downregulated genes (alpha=0.0025)	VMA21	203547	VMA21 vacuolar H+-ATPase homolog (S. cerevisiae)
Downregulated genes (alpha=0.0025)	WDR4	10785	WD repeat domain 4
Downregulated genes (alpha=0.0025)	YIF1A	10897	Yip1 interacting factor homolog A, membrane trafficking protein
Downregulated genes (alpha=0.0025)	ZNF317	57693	zinc finger protein 317
Downregulated genes (alpha=0.0025)	ZNF589	51385	zinc finger protein 589
Downregulated genes (alpha=0.0025)	ZNRF3	84133	zinc and ring finger 3
Upregulated genes (alpha=0.0025)	AACS	65985	acetoacetyl-CoA synthetase
Upregulated genes (alpha=0.0025)	ABCA1	19	ATP binding cassette subfamily A member 1
Upregulated genes (alpha=0.0025)	ABCG1	9619	ATP binding cassette subfamily G member 1
Upregulated genes (alpha=0.0025)	ABI1	10006	abl interactor 1
Upregulated genes (alpha=0.0025)	ACSF2	80221	acyl-CoA synthetase family member 2
Upregulated genes (alpha=0.0025)	ACTA2	59	actin, alpha 2, smooth muscle, aorta
Upregulated genes (alpha=0.0025)	ADA	100	adenosine deaminase
Upregulated genes (alpha=0.0025)	ADARB1	104	adenosine deaminase, RNA specific B1
Upregulated genes (alpha=0.0025)	ADSSL1	122622	adenylosuccinate synthase like 1

Upregulated genes (alpha=0.0025)	AIM1	202	absent in melanoma 1
Upregulated genes (alpha=0.0025)	AKAP8L	26993	A-kinase anchoring protein 8 like
Upregulated genes (alpha=0.0025)	ALDH5A1	7915	aldehyde dehydrogenase 5 family member A1
Upregulated genes (alpha=0.0025)	ALPK1	80216	alpha kinase 1
Upregulated genes (alpha=0.0025)	AMY1B	277	amylase, alpha 1B (salivary)
Upregulated genes (alpha=0.0025)	ANKRD12	23253	ankyrin repeat domain 12
Upregulated genes (alpha=0.0025)	AP1G2	8906	adaptor related protein complex 1 gamma 2 subunit
Upregulated genes (alpha=0.0025)	APOC2	344	apolipoprotein C2
Upregulated genes (alpha=0.0025)	APOD	347	apolipoprotein D
Upregulated genes (alpha=0.0025)	ARID5B	84159	AT-rich interaction domain 5B
Upregulated genes (alpha=0.0025)	ARRB2	409	arrestin beta 2
Upregulated genes (alpha=0.0025)	BAZ2B	29994	bromodomain adjacent to zinc finger domain 2B
Upregulated genes (alpha=0.0025)	BCRP5	648980	breakpoint cluster region pseudogene 5
Upregulated genes (alpha=0.0025)	BEST3	144453	bestrophin 3
Upregulated genes (alpha=0.0025)	BHLHE40	8553	basic helix-loop-helix family member e40
Upregulated genes (alpha=0.0025)	BIRC7	79444	baculoviral IAP repeat containing 7
Upregulated genes (alpha=0.0025)	BRI3	25798	brain protein I3
Upregulated genes (alpha=0.0025)	BRI3P1	730010	brain protein I3 pseudogene 1
Upregulated genes (alpha=0.0025)	BTN2A2	10385	butyrophilin subfamily 2 member A2
Upregulated genes (alpha=0.0025)	C12orf57	113246	chromosome 12 open reading frame 57
Upregulated genes (alpha=0.0025)	C18orf8	29919	chromosome 18 open reading frame 8
Upregulated genes (alpha=0.0025)	C1orf85	112770	glycosylated lysosomal membrane protein
Upregulated genes (alpha=0.0025)	CAPN3	825	calpain 3
Upregulated genes (alpha=0.0025)	CARHSP1	23589	calcium regulated heat stable protein 1
Upregulated genes (alpha=0.0025)	CCDC109B	55013	mitochondrial calcium uniporter dominant negative beta subunit
Upregulated genes (alpha=0.0025)	CCDC42B	387885	cilia and flagella associated protein 73
Upregulated genes (alpha=0.0025)	CD52	1043	CD52 molecule
Upregulated genes (alpha=0.0025)	CECR1	51816	cat eye syndrome chromosome region, candidate 1
Upregulated genes (alpha=0.0025)	CIITA	4261	class II major histocompatibility complex transactivator
Upregulated genes (alpha=0.0025)	CLEC9A	283420	C-type lectin domain family 9 member A
Upregulated genes (alpha=0.0025)	CREBRF	153222	CREB3 regulatory factor
Upregulated genes (alpha=0.0025)	CRIP1	1396	cysteine rich protein 1

Upregulated genes (alpha=0.0025)	CXCL16	58191	C-X-C motif chemokine ligand 16
Upregulated genes (alpha=0.0025)	DCAF5	8816	DDB1 and CUL4 associated factor 5
Upregulated genes (alpha=0.0025)	DDAH2	23564	dimethylarginine dimethylaminohydrolase 2
Upregulated genes (alpha=0.0025)	DDX54	79039	DEAD-box helicase 54
Upregulated genes (alpha=0.0025)	DENND2D	79961	DENN domain containing 2D
Upregulated genes (alpha=0.0025)	DFNA5	1687	DFNA5, deafness associated tumor suppressor
Upregulated genes (alpha=0.0025)	DIP2C	22982	disco interacting protein 2 homolog C
Upregulated genes (alpha=0.0025)	DPYSL2	1808	dihydropyrimidinase like 2
Upregulated genes (alpha=0.0025)	DTX3L	151636	deltex E3 ubiquitin ligase 3L
Upregulated genes (alpha=0.0025)	DYRK1B	9149	dual specificity tyrosine phosphorylation regulated kinase 1B
Upregulated genes (alpha=0.0025)	EMP3	2014	epithelial membrane protein 3
Upregulated genes (alpha=0.0025)	FAIM3	9214	Fc fragment of IgM receptor
Upregulated genes (alpha=0.0025)	FAM60A	58516	family with sequence similarity 60 member A
Upregulated genes (alpha=0.0025)	FBLN2	2199	fibulin 2
Upregulated genes (alpha=0.0025)	FBXO15	201456	F-box protein 15
Upregulated genes (alpha=0.0025)	FBXO32	114907	F-box protein 32
Upregulated genes (alpha=0.0025)	FCGRT	2217	Fc fragment of IgG receptor and transporter
Upregulated genes (alpha=0.0025)	FLJ42627	645644	uncharacterized LOC645644
Upregulated genes (alpha=0.0025)	FOXN3	1112	forkhead box N3
Upregulated genes (alpha=0.0025)	GABARAPL2	11345	GABA type A receptor associated protein like 2
Upregulated genes (alpha=0.0025)	GATS	352954	GATS, stromal antigen 3 opposite strand
Upregulated genes (alpha=0.0025)	GFI1	2672	growth factor independent 1 transcriptional repressor
Upregulated genes (alpha=0.0025)	GIMAP8	155038	GTPase, IMAP family member 8
Upregulated genes (alpha=0.0025)	GLRX	2745	glutaredoxin
Upregulated genes (alpha=0.0025)	GM2A	2760	GM2 ganglioside activator
Upregulated genes (alpha=0.0025)	GNG7	2788	G protein subunit gamma 7
Upregulated genes (alpha=0.0025)	GPR137C	283554	G protein-coupled receptor 137C
Upregulated genes (alpha=0.0025)	GPR160	26996	G protein-coupled receptor 160
Upregulated genes (alpha=0.0025)	GYPC	2995	glycophorin C (Gerbich blood group)
Upregulated genes (alpha=0.0025)	H1FX	8971	H1 histone family member X
Upregulated genes (alpha=0.0025)	HAVCR2	84868	hepatitis A virus cellular receptor 2
Upregulated genes (alpha=0.0025)	HBA2	3040	hemoglobin subunit alpha 2

Upregulated genes (alpha=0.0025)	HCP5	10866	HLA complex P5 (non-protein coding)
Upregulated genes (alpha=0.0025)	HELQ	113510	helicase, POLQ-like
Upregulated genes (alpha=0.0025)	HILPDA	29923	hypoxia inducible lipid droplet associated
Upregulated genes (alpha=0.0025)	HIP1	3092	huntingtin interacting protein 1
Upregulated genes (alpha=0.0025)	HLA-DMA	3108	major histocompatibility complex, class II, DM alpha
Upregulated genes (alpha=0.0025)	HLA-DMB	3109	major histocompatibility complex, class II, DM beta
Upregulated genes (alpha=0.0025)	HLA-DOA	3111	major histocompatibility complex, class II, DO alpha
Upregulated genes (alpha=0.0025)	HLA-DOB	3112	major histocompatibility complex, class II, DO beta
Upregulated genes (alpha=0.0025)	HLA-DPB1	3115	major histocompatibility complex, class II, DP beta 1
Upregulated genes (alpha=0.0025)	HLA-DQA1	3117	major histocompatibility complex, class II, DQ alpha 1
Upregulated genes (alpha=0.0025)	HLA-DRA	3122	major histocompatibility complex, class II, DR alpha
Upregulated genes (alpha=0.0025)	HLA-DRB1	3123	major histocompatibility complex, class II, DR beta 1
Upregulated genes (alpha=0.0025)	HLA-DRB3	3125	major histocompatibility complex, class II, DR beta 3
Upregulated genes (alpha=0.0025)	HLA-DRB4	3126	major histocompatibility complex, class II, DR beta 4
Upregulated genes (alpha=0.0025)	HLA-E	3133	major histocompatibility complex, class I, E
Upregulated genes (alpha=0.0025)	HSPB1P1	653553	heat shock protein family B (small) member 1 pseudogene 1
Upregulated genes (alpha=0.0025)	IFIH1	64135	interferon induced with helicase C domain 1
Upregulated genes (alpha=0.0025)	IFIT2	3433	interferon induced protein with tetratricopeptide repeats 2
Upregulated genes (alpha=0.0025)	IL10RB	3588	interleukin 10 receptor subunit beta
Upregulated genes (alpha=0.0025)	IL4R	3566	interleukin 4 receptor
Upregulated genes (alpha=0.0025)	IRF1	3659	interferon regulatory factor 1
Upregulated genes (alpha=0.0025)	IRF2BPL	64207	interferon regulatory factor 2 binding protein like
Upregulated genes (alpha=0.0025)	IRS2	8660	insulin receptor substrate 2
Upregulated genes (alpha=0.0025)	KANSL1L	151050	KAT8 regulatory NSL complex subunit 1 like
Upregulated genes (alpha=0.0025)	KLF2	10365	Kruppel like factor 2
Upregulated genes (alpha=0.0025)	KLHDC8B	200942	kelch domain containing 8B
Upregulated genes (alpha=0.0025)	KLHL22	84861	kelch like family member 22
Upregulated genes (alpha=0.0025)	KLHL24	54800	kelch like family member 24
Upregulated genes (alpha=0.0025)	KLHL6	89857	kelch like family member 6
Upregulated genes (alpha=0.0025)	LGALS1	3956	galectin 1
Upregulated genes (alpha=0.0025)	LILRB4	11006	leukocyte immunoglobulin like receptor B4
Upregulated genes (alpha=0.0025)	LINC00426	100188949	long intergenic non-protein coding RNA 426

Upregulated genes (alpha=0.0025)	LITAF	9516	lipopolysaccharide induced TNF factor
Upregulated genes (alpha=0.0025)	LMOD3	56203	leiomodin 3
Upregulated genes (alpha=0.0025)	LOC10012955(	100129550	uncharacterized LOC100129550
Upregulated genes (alpha=0.0025)	LOC10013027(	100130276	uncharacterized LOC100130276
Upregulated genes (alpha=0.0025)	LOC440864	440864	uncharacterized LOC440864
Upregulated genes (alpha=0.0025)	LOC644173	644173	uncharacterized LOC644173
Upregulated genes (alpha=0.0025)	LOC644634	644634	family with sequence similarity 231 member D
Upregulated genes (alpha=0.0025)	LOC728743	728743	zinc finger protein pseudogene
Upregulated genes (alpha=0.0025)	LPIN1	23175	lipin 1
Upregulated genes (alpha=0.0025)	LRMP	4033	lymphoid restricted membrane protein
Upregulated genes (alpha=0.0025)	LY9	4063	lymphocyte antigen 9
Upregulated genes (alpha=0.0025)	LY96	23643	lymphocyte antigen 96
Upregulated genes (alpha=0.0025)	MACF1	23499	microtubule-actin crosslinking factor 1
Upregulated genes (alpha=0.0025)	MDK	4192	midkine (neurite growth-promoting factor 2)
Upregulated genes (alpha=0.0025)	MEF2D	4209	myocyte enhancer factor 2D
Upregulated genes (alpha=0.0025)	MFGE8	4240	milk fat globule-EGF factor 8 protein
Upregulated genes (alpha=0.0025)	MGC72080	389538	MGC72080 pseudogene
Upregulated genes (alpha=0.0025)	MICAL1	64780	microtubule associated monooxygenase, calponin and LIM domain containing 1
Upregulated genes (alpha=0.0025)	MIS18BP1	55320	MIS18 binding protein 1
Upregulated genes (alpha=0.0025)	MMP11	4320	matrix metallopeptidase 11
Upregulated genes (alpha=0.0025)	MVP	9961	major vault protein
Upregulated genes (alpha=0.0025)	MXD3	83463	MAX dimerization protein 3
Upregulated genes (alpha=0.0025)	MYB	4602	MYB proto-oncogene, transcription factor
Upregulated genes (alpha=0.0025)	MYO1G	64005	myosin IG
Upregulated genes (alpha=0.0025)	NEU1	4758	neuraminidase 1
Upregulated genes (alpha=0.0025)	NFATC2IP	84901	nuclear factor of activated T-cells 2 interacting protein
Upregulated genes (alpha=0.0025)	NPC2	10577	NPC intracellular cholesterol transporter 2
Upregulated genes (alpha=0.0025)	NPIPL3	23117	nuclear pore complex interacting protein family member B3
Upregulated genes (alpha=0.0025)	NREP	9315	neuronal regeneration related protein
Upregulated genes (alpha=0.0025)	NUAK2	81788	NUAK family kinase 2
Upregulated genes (alpha=0.0025)	NUCB2	4925	nucleobindin 2
Upregulated genes (alpha=0.0025)	OAS1	4938	2'-5'-oligoadenylate synthetase 1

Upregulated genes (alpha=0.0025)	OAS2	4939	2'-5'-oligoadenylate synthetase 2
Upregulated genes (alpha=0.0025)	OAS3	4940	2'-5'-oligoadenylate synthetase 3
Upregulated genes (alpha=0.0025)	P2RX4	5025	purinergic receptor P2X 4
Upregulated genes (alpha=0.0025)	P2RY8	286530	purinergic receptor P2Y8
Upregulated genes (alpha=0.0025)	PARP15	165631	poly(ADP-ribose) polymerase family member 15
Upregulated genes (alpha=0.0025)	PARP9	83666	poly(ADP-ribose) polymerase family member 9
Upregulated genes (alpha=0.0025)	PARVG	64098	parvin gamma
Upregulated genes (alpha=0.0025)	PATL2	197135	PAT1 homolog 2
Upregulated genes (alpha=0.0025)	PCNX	22990	pecanex homolog 1 (Drosophila)
Upregulated genes (alpha=0.0025)	PHEX	5251	phosphate regulating endopeptidase homolog, X-linked
Upregulated genes (alpha=0.0025)	PIK3C2B	5287	phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta
Upregulated genes (alpha=0.0025)	PIK3IP1	113791	phosphoinositide-3-kinase interacting protein 1
Upregulated genes (alpha=0.0025)	PINK1	65018	PTEN induced putative kinase 1
Upregulated genes (alpha=0.0025)	PLA2G2C	391013	phospholipase A2 group IIC
Upregulated genes (alpha=0.0025)	PLAU	5328	plasminogen activator, urokinase
Upregulated genes (alpha=0.0025)	PLXNB1	5364	plexin B1
Upregulated genes (alpha=0.0025)	PPP1R15A	23645	protein phosphatase 1 regulatory subunit 15A
Upregulated genes (alpha=0.0025)	PRR5	55615	proline rich 5
Upregulated genes (alpha=0.0025)	PYCARD	29108	PYD and CARD domain containing
Upregulated genes (alpha=0.0025)	PYROXD2	84795	pyridine nucleotide-disulphide oxidoreductase domain 2
Upregulated genes (alpha=0.0025)	RAB11FIP1	80223	RAB11 family interacting protein 1
Upregulated genes (alpha=0.0025)	RAB37	326624	RAB37, member RAS oncogene family
Upregulated genes (alpha=0.0025)	RARRES2	5919	retinoic acid receptor responder 2
Upregulated genes (alpha=0.0025)	RENBP	5973	renin binding protein
Upregulated genes (alpha=0.0025)	RHBDF1	64285	rhomboid 5 homolog 1
Upregulated genes (alpha=0.0025)	RHOBTB2	23221	Rho related BTB domain containing 2
Upregulated genes (alpha=0.0025)	RHOQ	23433	ras homolog family member Q
Upregulated genes (alpha=0.0025)	RNF144A	9781	ring finger protein 144A
Upregulated genes (alpha=0.0025)	RNF44	22838	ring finger protein 44
Upregulated genes (alpha=0.0025)	RRAGD	58528	Ras related GTP binding D
Upregulated genes (alpha=0.0025)	RRN3P2	653390	RRN3 homolog, RNA polymerase I transcription factor pseudogene 2
Upregulated genes (alpha=0.0025)	S1PR4	8698	sphingosine-1-phosphate receptor 4

Upregulated genes (alpha=0.0025)	SAMD9	54809	sterile alpha motif domain containing 9
Upregulated genes (alpha=0.0025)	SAMD9L	219285	sterile alpha motif domain containing 9 like
Upregulated genes (alpha=0.0025)	SBK1	388228	SH3 domain binding kinase 1
Upregulated genes (alpha=0.0025)	SERPINB9	5272	serpin family B member 9
Upregulated genes (alpha=0.0025)	SGSH	6448	N-sulfoglucosamine sulfohydrolase
Upregulated genes (alpha=0.0025)	SLC15A4	121260	solute carrier family 15 member 4
Upregulated genes (alpha=0.0025)	SLC26A11	284129	solute carrier family 26 member 11
Upregulated genes (alpha=0.0025)	SLC29A4	222962	solute carrier family 29 member 4
Upregulated genes (alpha=0.0025)	SLC29A4P1	402509	solute carrier family 29 member 4 pseudogene 1
Upregulated genes (alpha=0.0025)	SMG1	23049	SMG1, nonsense mediated mRNA decay associated PI3K related kinase
Upregulated genes (alpha=0.0025)	SNORA72	26775	small nucleolar RNA, H/ACA box 72
Upregulated genes (alpha=0.0025)	SNX30	401548	sorting nexin family member 30
Upregulated genes (alpha=0.0025)	SOX11	6664	SRY-box 11
Upregulated genes (alpha=0.0025)	SOX8	30812	SRY-box 8
Upregulated genes (alpha=0.0025)	SSBP2	23635	single stranded DNA binding protein 2
Upregulated genes (alpha=0.0025)	ST3GAL5	8869	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
Upregulated genes (alpha=0.0025)	STAT2	6773	signal transducer and activator of transcription 2
Upregulated genes (alpha=0.0025)	SUN2	25777	Sad1 and UNC84 domain containing 2
Upregulated genes (alpha=0.0025)	SYS1	90196	SYS1, golgi trafficking protein
Upregulated genes (alpha=0.0025)	TCL1B	9623	T-cell leukemia/lymphoma 1B
Upregulated genes (alpha=0.0025)	TIAF1	9220	TGFB1-induced anti-apoptotic factor 1
Upregulated genes (alpha=0.0025)	TM2D1	83941	TM2 domain containing 1
Upregulated genes (alpha=0.0025)	TMEM91	641649	transmembrane protein 91
Upregulated genes (alpha=0.0025)	TMX4	56255	thioredoxin related transmembrane protein 4
Upregulated genes (alpha=0.0025)	TNFRSF14	8764	TNF receptor superfamily member 14
Upregulated genes (alpha=0.0025)	TNFRSF17	608	TNF receptor superfamily member 17
Upregulated genes (alpha=0.0025)	TOP2B	7155	topoisomerase (DNA) II beta
Upregulated genes (alpha=0.0025)	TOX2	84969	TOX high mobility group box family member 2
Upregulated genes (alpha=0.0025)	TP53INP1	94241	tumor protein p53 inducible nuclear protein 1
Upregulated genes (alpha=0.0025)	TPP1	1200	tripeptidyl peptidase 1
Upregulated genes (alpha=0.0025)	TRIB1	10221	tribbles pseudokinase 1
Upregulated genes (alpha=0.0025)	TRIM13	10206	tripartite motif containing 13

Upregulated genes (alpha=0.0025)	TRIM22	10346	tripartite motif containing 22
Upregulated genes (alpha=0.0025)	TSC22D1	8848	TSC22 domain family member 1
Upregulated genes (alpha=0.0025)	TSC22D3	1831	TSC22 domain family member 3
Upregulated genes (alpha=0.0025)	TSPAN32	10077	tetraspanin 32
Upregulated genes (alpha=0.0025)	TUG1	55000	taurine up-regulated 1 (non-protein coding)
Upregulated genes (alpha=0.0025)	TYROBP	7305	TYRO protein tyrosine kinase binding protein
Upregulated genes (alpha=0.0025)	UAP1L1	91373	UDP-N-acetylglucosamine pyrophosphorylase 1 like 1
Upregulated genes (alpha=0.0025)	UBE2L6	9246	ubiquitin conjugating enzyme E2 L6
Upregulated genes (alpha=0.0025)	VANGL2	57216	VANGL planar cell polarity protein 2
Upregulated genes (alpha=0.0025)	WIPI2	26100	WD repeat domain, phosphoinositide interacting 2
Upregulated genes (alpha=0.0025)	WNT3	7473	Wnt family member 3
Upregulated genes (alpha=0.0025)	YPEL1	29799	yippee like 1
Upregulated genes (alpha=0.0025)	YPEL2	388403	yippee like 2
Upregulated genes (alpha=0.0025)	YPEL3	83719	yippee like 3
Upregulated genes (alpha=0.0025)	YPEL5	51646	yippee like 5
Upregulated genes (alpha=0.0025)	ZC3H12B	340554	zinc finger CCCH-type containing 12B
Upregulated genes (alpha=0.0025)	ZHX2	22882	zinc fingers and homeoboxes 2
Upregulated genes (alpha=0.0025)	ZNF219	51222	zinc finger protein 219
Upregulated genes (alpha=0.0025)	ZNF260	339324	zinc finger protein 260
Upregulated genes (alpha=0.0025)	ZNF608	57507	zinc finger protein 608
Upregulated genes (alpha=0.0025)	ZSCAN16	80345	zinc finger and SCAN domain containing 16
Upregulated genes (alpha=0.0025)	ZSCAN2	54993	zinc finger and SCAN domain containing 2

Supplemental Table 6. Signatures that are significantly enriched with top regulated genes following z-VRPR-fmk treatment in Rec-1 cells.

Downregulated signatures:

Signatures DB	Category	Sub Category	Signature name	Signature links	Defined members	Enrichment score	*P* [GSEA] (by permutation test)	FDR [GSEA]
HGNCSigDB_dMay2014	gene families	Mitochondrial respiratory chain cc	Mitochondrial respiratory chain complex / Complex III	http://www.genenames.org/genefamilies/mitocomplex#comIII	9	0.8030	0.0036	0.0064
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	RESPONSE_TO_HEAT	http://www.broadinstitute.org/gsea/msigdb/cards/RESPONSE_TO_HEAT	10	0.8013	0.0051	0.0202
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	DNA_FRAGMENTATION_DURING_APOPTOSIS	http://www.broadinstitute.org/gsea/msigdb/cards/DNA_FRAGMENTATION_DURING_APOPT(	13	0.7959	0.0010	0.0146
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	COLLER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/COLLER_MYC_TARGETS_UP	25	0.7935	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / KEGG	KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_BIOS'	http://www.broadinstitute.org/gsea/msigdb/cards/KEGG_VALINE_LEUCINE_AND_ISOLEUCI	11	0.7877	0.0018	0.0019
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	MULLIGAN_NTF3_SIGNALING_VIA_INSR_AND_IGF	http://www.broadinstitute.org/gsea/msigdb/cards/MULLIGAN_NTF3_SIGNALING_VIA_INSR_	23	0.7860	0.0010	0.0010
GeneSigDB_v4_Sept2011	Human	Viral	Viral_Tsunedomi06_12genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=17088983-Table2a	12	0.7793	0.0016	0.0084
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_50	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_50	13	0.7783	0.0010	0.0107
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_514	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_514	10	0.7761	0.0019	0.0125
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_RNA_POL_III_CHAIN_ELONGATION	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_RNA_POL_III_CHAIN_ELONG	17	0.7758	0.0010	0.0017
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	MYLLYKANGAS_AMPLIFICATION_HOT_SPOT_29	http://www.broadinstitute.org/gsea/msigdb/cards/MYLLYKANGAS_AMPLIFICATION_HOT_SF	8	0.7744	0.0049	0.0134
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHUHMACHER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHUHMACHER_MYC_TARGETS_UP	80	0.7722	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	ORGANELLAR_SMALL_RIBOSOMAL_SUBUNIT	http://www.broadinstitute.org/gsea/msigdb/cards/ORGANELLAR_SMALL_RIBOSOMAL_SUB	11	0.7618	0.0040	0.0029
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	MITOCHONDRIAL_SMALL_RIBOSOMAL_SUBUNIT	http://www.broadinstitute.org/gsea/msigdb/cards/MITOCHONDRIAL_SMALL_RIBOSOMAL_S	11	0.7618	0.0040	0.0029
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	SMALL_RIBOSOMAL_SUBUNIT	http://www.broadinstitute.org/gsea/msigdb/cards/SMALL_RIBOSOMAL_SUBUNIT	11	0.7618	0.0040	0.0029
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHLOSSER_MYC_TARGETS_AND_SERUM_RESP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERU	47	0.7549	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	XU_RESPONSE_TO_TRETINOIN_AND_NSC682994_	http://www.broadinstitute.org/gsea/msigdb/cards/XU_RESPONSE_TO_TRETINOIN_AND_NS	15	0.7505	0.0010	0.0016
GeneSigDB_v4_Sept2011	Mouse	StemCell	homolog(StemCell_Parker05_27genes, from Mus mus	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15992799-table2	15	0.7483	0.0020	0.0088
StaudtSigDB_dNov2012	Signaling pathway	T cell cytokine signallng	Tcell_cytokine_induced_prolif	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=77	27	0.7472	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	FU_INTERACT_WITH_ALKBH8	http://www.broadinstitute.org/gsea/msigdb/cards/FU_INTERACT_WITH_ALKBH8	13	0.7457	0.0040	0.0048
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	FUNG_IL2_SIGNALING_1	http://www.broadinstitute.org/gsea/msigdb/cards/FUNG_IL2_SIGNALING_1	11	0.7446	0.0051	0.0076
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	PROTEIN_TARGETING_TO_MITOCHONDRION	http://www.broadinstitute.org/gsea/msigdb/cards/PROTEIN_TARGETING_TO_MITOCHONDF	11	0.7437	0.0017	0.0342
HGNCSigDB_dMay2014	gene families	Mitochondrial ribosomal proteins	Mitochondrial ribosomal proteins / large subunits	http://www.genenames.org/genefamilies/MRP#MRPL	49	0.7426	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	RRNA_METABOLIC_PROCESS	http://www.broadinstitute.org/gsea/msigdb/cards/RRNA_METABOLIC_PROCESS	16	0.7409	0.0010	0.0064
StaudtSigDB_dNov2012	Signaling pathway	Notch	Notch_T-ALL_up_Palomero	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=23	47	0.7353	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHLOSSER_MYC_TARGETS_AND_SERUM_RESP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERU	47	0.7346	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / KEGG	KEGG_CITRATE_CYCLE_TCA_CYCLE	http://www.broadinstitute.org/gsea/msigdb/cards/KEGG_CITRATE_CYCLE_TCA_CYCLE	32	0.7341	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	RNA polymerase subunits	RNA polymerase subunits	http://www.genenames.org/genefamilies/POLR	29	0.7336	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	RIBOSOMAL_SUBUNIT	http://www.broadinstitute.org/gsea/msigdb/cards/RIBOSOMAL_SUBUNIT	20	0.7331	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_RNA_POL_III_TRANSCRIPTION_TERM	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_RNA_POL_III_TRANSCRIPTI(	19	0.7313	0.0010	0.0017
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	RRNA_PROCESSING	http://www.broadinstitute.org/gsea/msigdb/cards/RRNA_PROCESSING	15	0.7307	0.0010	0.0138
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	CELLULAR_RESPONSE_TO_STIMULUS	http://www.broadinstitute.org/gsea/msigdb/cards/CELLULAR_RESPONSE_TO_STIMULUS	19	0.7304	0.0010	0.0096
StaudtSigDB_dNov2012	Signaling pathway	MYC	Myc_overexpression_1.5x_up	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=16	86	0.7271	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	RAHMAN_TP53_TARGETS_PHOSPHORYLATED	http://www.broadinstitute.org/gsea/msigdb/cards/RAHMAN_TP53_TARGETS_PHOSPHORYL	21	0.7218	0.0010	0.0010
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_25	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_25	13	0.7214	0.0059	0.0157
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_PURINE_RIBONUCLEOSIDE_MONOPH	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_PURINE_RIBONUCLEOSIDE_	11	0.7173	0.0182	0.0128
StaudtSigDB_dNov2012	Signaling pathway	IL6	IL6_Ly10_Up_group2	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=25	9	0.7166	0.0416	0.0083
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	ORGANELLAR_RIBOSOME	http://www.broadinstitute.org/gsea/msigdb/cards/ORGANELLAR_RIBOSOME	22	0.7160	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	MITOCHONDRIAL_RIBOSOME	http://www.broadinstitute.org/gsea/msigdb/cards/MITOCHONDRIAL_RIBOSOME	22	0.7160	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_VITAMIN_B5_PANTOTHENATE_METAE	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_VITAMIN_B5_PANTOTHENAT	11	0.7156	0.0141	0.0139
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer gene neighborhoods	GNF2_MSH6	http://www.broadinstitute.org/gsea/msigdb/cards/GNF2_MSH6	31	0.7125	0.0010	0.0010
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	NUCLEOLAR_PART	http://www.broadinstitute.org/gsea/msigdb/cards/NUCLEOLAR_PART	18	0.7103	0.0010	0.0019
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	NEUTRAL_AMINO_ACID_TRANSMEMBRANE_TRAN	http://www.broadinstitute.org/gsea/msigdb/cards/NEUTRAL_AMINO_ACID_TRANSMEMBRA	12	0.7098	0.0197	0.0445
GeneSigDB_v4_Sept2011	Mouse	Bone	homolog(Bone_Kalajzic05_12genes, from Mus muscul	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15834136-Table3	10	0.7085	0.0232	0.0533
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	COFACTOR_TRANSPORT	http://www.broadinstitute.org/gsea/msigdb/cards/COFACTOR_TRANSPORT	11	0.7071	0.0126	0.0709
HGNCSigDB_dMay2014	gene families	Mitochondrial respiratory chain cc	Mitochondrial respiratory chain complex / Complex V	http://www.genenames.org/genefamilies/mitocomplex#FATP	16	0.7046	0.0062	0.0057
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	RNA_3END_PROCESSING	http://www.broadinstitute.org/gsea/msigdb/cards/RNA_3END_PROCESSING	10	0.7036	0.0225	0.0767
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer gene neighborhoods	MORF_GSPT1	http://www.broadinstitute.org/gsea/msigdb/cards/MORF_GSPT1	49	0.7007	0.0010	0.0010

Upregulated signatures:

Signatures DB	Category	Sub Category	Signature name	Signature links	Defined members	Enrichment score	*P* [GSEA] (by permutation test)	FDR [GSEA]
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_293	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_293	12	-0.8649	0.0010	0.0010
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_143	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_143	14	-0.8577	0.0010	0.0010
HGNCSigDB_dMay2014	gene families	Histocompatibility complex	Histocompatibility complex	http://www.genenames.org/genefamilies/HLA	24	-0.8512	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	BOWIE_RESPONSE_TO_TAMOXIFEN	http://www.broadinstitute.org/gsea/msigdb/cards/BOWIE_RESPONSE_TO_TAMOXIFEN	18	-0.8231	0.0010	0.0009
GeneSigDB_v4_Sept2011	Human	Stomach	Stomach_Nakamura09_9genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19881313-Table1c	9	-0.8083	0.0010	0.0154
GeneSigDB_v4_Sept2011	Human	Colon	Colon_Protiva09_31genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19139017-Table3	23	-0.7961	0.0010	0.0010
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_CASPASE_MEDIATED_CLEAVAGE_OF	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_CASPASE_MEDIATED_CLEA'	12	-0.7938	0.0010	0.0302
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	UROSEVIC_RESPONSE_TO_IMIQUIMOD	http://www.broadinstitute.org/gsea/msigdb/cards/UROSEVIC_RESPONSE_TO_IMIQUIMOD	23	-0.7827	0.0010	0.0009
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	CASORELLI_APL_SECONDARY_VS_DE_NOVO_DN	http://www.broadinstitute.org/gsea/msigdb/cards/CASORELLI_APL_SECONDARY_VS_DE_N	9	-0.7816	0.0097	0.0198
GeneSigDB_v4_Sept2011	Human	StemCell	StemCell_Roth05_11genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15741219-Table2	9	-0.7733	0.0089	0.0263
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	CHASSOT_SKIN_WOUND	http://www.broadinstitute.org/gsea/msigdb/cards/CHASSOT_SKIN_WOUND	10	-0.7687	0.0098	0.0174
MolSigDBv4_0_dMay2014	c4: computational gene sets	cancer modules	MODULE_543	http://www.broadinstitute.org/gsea/msigdb/cards/MODULE_543	17	-0.7683	0.0010	0.0010
GeneSigDB_v4_Sept2011	Human	Leukemia	Leukemia_Wilson06_15genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=16597596-TableS6-2	14	-0.7669	0.0010	0.0081
HGNCSigDB_dMay2014	gene families	Metallothioneins	Metallothioneins	http://www.genenames.org/genefamilies/MT	18	-0.7653	0.0010	0.0010
StaudtSigDB_dNov2012	Transcription factor target	NFkB	NFkB_Up_OCILy3_only	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=87	10	-0.7627	0.0124	0.0035
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	ZHANG_INTERFERON_RESPONSE	http://www.broadinstitute.org/gsea/msigdb/cards/ZHANG_INTERFERON_RESPONSE	23	-0.7589	0.0010	0.0009
StaudtSigDB_dNov2012	Transcription factor target	NFkB	NFkB_ChIPCHIP_Young_5factors	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=27	15	-0.7519	0.0015	0.0023
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	biological processes	GLYCOPROTEIN_CATABOLIC_PROCESS	http://www.broadinstitute.org/gsea/msigdb/cards/GLYCOPROTEIN_CATABOLIC_PROCESS	12	-0.7515	0.0018	0.1156
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_ENDOSOMAL_VACUOLAR_PATHWAY	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_ENDOSOMAL_VACUOLAR_P	8	-0.7510	0.0342	0.0761
GeneSigDB_v4_Sept2011	Human	Kidney	Kidney_Struckmann04_13genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=14996721-Table1b	13	-0.7491	0.0016	0.0178

MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	MOSERLE_IFNA_RESPONSE	http://www.broadinstitute.org/gsea/msigdb/cards/MOSERLE_IFNA_RESPONSE	31	-0.7402	0.0010	0.0009
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	molecular functions	SIALYLTRANSFERASE_ACTIVITY	http://www.broadinstitute.org/gsea/msigdb/cards/SIALYLTRANSFERASE_ACTIVITY	10	-0.7367	0.0133	0.1538
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	IIZUKA_LIVER_CANCER_PROGRESSION_G1_G2_L	http://www.broadinstitute.org/gsea/msigdb/cards/IIZUKA_LIVER_CANCER_PROGRESSION_	12	-0.7334	0.0032	0.0161
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	SCHMAHL_PDGF_SIGNALING	http://www.broadinstitute.org/gsea/msigdb/cards/SCHMAHL_PDGF_SIGNALING	9	-0.7323	0.0456	0.0358
GeneSigDB_v4_Sept2011	Human	Liver	Liver_Liu03_11genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=14728809-Table3b	9	-0.7310	0.0324	0.0338
GeneSigDB_v4_Sept2011	Human	Lung	Lung_Magda08_21genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=18593933-Table1a	14	-0.7297	0.0049	0.0196
GeneSigDB_v4_Sept2011	Human	Lymphoma	Lymphoma_Fogel07_33genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=17092989-SuppTable1	29	-0.7289	0.0010	0.0010
GeneSigDB_v4_Sept2011	Human	Lung	Lung_Nam10_10genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=20369051-Table1	9	-0.7288	0.0212	0.0329
GeneSigDB_v4_Sept2011	Human	Thyroid	Thyroid_Amin09_10genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19047894-Table2a	10	-0.7285	0.0214	0.0309
GeneSigDB_v4_Sept2011	Human	Skin	Skin_Zimmerer08_23genes_InVitrovsInVivo	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=18794103-Table3	20	-0.7278	0.0010	0.0045
GeneSigDB_v4_Sept2011	Human	Prostate	Prostate_Rothermund05_15genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=15790403-Table1	11	-0.7271	0.0078	0.0278
MolSigDBv4_0_dMay2014	c2: curated gene sets	canonical pathways / Reactome	REACTOME_DIGESTION_OF_DIETARY_CARBOHYI	http://www.broadinstitute.org/gsea/msigdb/cards/REACTOME_DIGESTION_OF_DIETARY_C	9	-0.7187	0.0594	0.1042
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	BOWIE_RESPONSE_TO_EXTRACELLULAR_MATRI	http://www.broadinstitute.org/gsea/msigdb/cards/BOWIE_RESPONSE_TO_EXTRACELLULA	17	-0.7178	0.0029	0.0071
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	ZHENG_RESPONSE_TO_ARSENITE_UP	http://www.broadinstitute.org/gsea/msigdb/cards/ZHENG_RESPONSE_TO_ARSENITE_UP	18	-0.7172	0.0010	0.0060
StaudtSigDB_dNov2012	Signaling pathway	Notch	Notch_T-ALL_down_Sharma	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=22	18	-0.7156	0.0020	0.0027
GeneSigDB_v4_Sept2011	Human	Viral	Viral_Guo05_21genes	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=16254373-Table3	18	-0.7141	0.0010	0.0085
StaudtSigDB_dNov2012	Signaling pathway	Notch	Notch_T-ALL_down_Palomero	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=23	14	-0.7124	0.0050	0.0026
MolSigDBv4_0_dMay2014	c2: curated gene sets	chemical and genetic perturbatior	BOYAULT_LIVER_CANCER_SUBCLASS_G5_DN	http://www.broadinstitute.org/gsea/msigdb/cards/BOYAULT_LIVER_CANCER_SUBCLASS_G	27	-0.7099	0.0010	0.0009
GeneSigDB_v4_Sept2011	Human	Leukemia	Leukemia_Ueno09_30genes_HighestUpRegulation3D	http://www.genesigdb.org/genesigdb/signaturedetail.jsp?signatureId=19749795-SuppTable1	22	-0.7081	0.0010	0.0058
StaudtSigDB_dNov2012	Cancer differential	Diffuse large B cell lymphoma	PMBLhigh_HLlow	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=6	8	-0.7072	0.0625	0.0156
MolSigDBv4_0_dMay2014	c5: gene ontology (GO) gene sets	cellular components	ENDOCYTIC_VESICLE	http://www.broadinstitute.org/gsea/msigdb/cards/ENDOCYTIC_VESICLE	14	-0.7032	0.0098	0.0518

Supplemental Table 7. MYC signatures enriched with top regulated genes following z-VRPR-fmk treatment in Rec-1 cells.

Downregulated signatures:				
Signature name	Signature links	Defined members	Enrichment score	*P* [GSEA] (by permutation test)
COLLER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/COLLER_MYC_TARGETS_UP	25	0.7935	0.0010
SCHUHMACHER_MYC_TARGETS_UP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHUHMACHER_MYC_TARGETS_UP	80	0.7722	0.0010
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_DN	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_DN	47	0.7549	0.0010
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_UP	http://www.broadinstitute.org/gsea/msigdb/cards/SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_UP	47	0.7346	0.0010
Myc_overexpression_1.5x_up	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=167	86	0.7271	0.0010
Myc_overexpression_2x_up	http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=168	34	0.6827	0.0010