Supplemental Figure 1-7

A FOXO1-dependent transcription network is a targetable vulnerability of mantle cell lymphomas
Supplemental Figure 1. CRISPR/Cas9 screening identifies core survival transcription factors of MCL cells. (A) Ranking of the log2 fold-change (log2FC) of sgRNA abundance in CCMCL1, JEKO1, UPN1, MAVER1, and HEL cells (Ratio of start to end point) after 14 population doublings. (B) Scatterplot analysis of TF dependencies in JEKO1, UPN1, MAVER, and SEFA1 (Y axis) comparing to HEL (X axis) by the average sgRNA log2FC of each genes in the pooled CRISPR screen. (C) Dependency scores of four TFs extracted from Project Achilles (22Q2, ref 35). The box plots indicate the distribution of dependency score (CERES, a normalized metric of gene essentiality) of four TFs across all 1,086 cell lines or in the subset of 42 cell lines of B-lymphocyte origin. Data represent mean ± SEM. P value was calculated using a two-tailed unpaired Student’s t-test. (D) Competition-based proliferation assays to validate the results from the pooled screen. Experiments were conducted by transduction of Cas9-expressing CCMCL1. Data represent mean ± SEM (n=3). Results are representative of 2 independent experiments. Statistical analysis was performed using 1-way ANOVA with Tukey’s multiple-comparison test. ****p<0.0001, ***p<0.0005, **p<0.005, *p<0.05. (E) The design of the indicated CRISPR-resistant synonymous mutant. (F) Immunoblot performed at day 3 upon transduction of indicated sgRNAs. SE and LE denote short exposure and long exposure, respectively.
Supplemental Figure 2. IRF4, PAX5, EBF1 and FOXO1 are MCL lineage-survival TFs. (A) Immunoblot performed at day 7 upon transduction of indicated sgRNAs. (B-L) Competition-based proliferation assays to validate the results from the pooled screen. Experiments were conducted by transduction of Cas9-expressing CCMCL1 (B), JEO1 (C), MAVER (D), OCI-LY1 (E), BJAB (F), DG75 (G), HEL (H), U2OS (I), H1299 (J), and HeLa (K) cells with indicated lentivirus sgRNAs that co-express a GFP reporter. (L) PCNA positive control in all cell lines. Plotted are the GFP% cells (normalized to the day 3 measurement) at the indicated time-points during culturing. In B, C, E, F, G, H, and L, data represent mean ± SEM (n=3). Results are representative of 2 or 3 independent experiments. Statistical analysis was performed using 1-way ANOVA with Turkey’s multiple-comparison test. ****p<0.0001, ***p<0.0005, **p<0.005, *p<0.05.
### Supplemental Figure 3

#### A

Comparison of FOXO1 peaks and shuffled gene distances for various groups:

- **FOXO1 peaks**
- **Shuffled**

**Gene distance (kb):**

#### B

Comparison of FOXO1 peaks and gene distances for different genes:

- **FOXO1**
- **EBF1**
- **PAX5**
- **IRF4**

Gene distance (kb) vs. Gene intensity.

#### C

Distribution of genes for each group:

- **FOXO1**
- **EBF1**
- **PAX5**
- **IRF4**

Bar chart showing the number of genes for each group.

<table>
<thead>
<tr>
<th>Group</th>
<th># of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOXO1</td>
<td>5261</td>
</tr>
<tr>
<td>EBF1</td>
<td>1949</td>
</tr>
<tr>
<td>PAX5</td>
<td>3377</td>
</tr>
<tr>
<td>IRF4</td>
<td>1085</td>
</tr>
</tbody>
</table>

| Group | 100% | 24.596% | 42.444% | 15.586% | 66.393% | 100% | 43.766% | 19.805% | 66.123% | 25.259% | 100% | 16.02% | 66.123% | 25.259% | 100% | 16.02% | 75.576% | 35.576% | 49.861% | 100% | 75.576% | 35.576% | 49.861% | 100% | 75.576% | 35.576% | 49.861% | 100% |
Supplemental Figure 3. Analysis of co-occupancy of 4 TFs and Gene Ontology analysis of RNA-seq data from FOXO1-depleted CCMCL1. (A, B) Validation of ChIP-seq analysis. Peak enrichment was confirmed against shuffled peaks. (C) The UpSet plot depicting the intersection of genes occupied by the four factors. Percent overlap is indicated in the table. (D) Gene Ontology enrichment analysis of peak-associated genes. Gene ratios of pathway annotated genes in peak-associated genes are indicated by the size of the dot, and adjusted p-values are indicated by color according to the key to the right (a hypergeometric test followed by a Benjamini-Hochberg procedure). (E) RNA-seq analysis of CCMCL1 cells expressing specific sgRNA for each TF (96 h) was performed. RNA-seq data were analyzed for enrichment levels of selected gene sets from the MSigDB hallmarks collection. Significance and enrichment score are represented by circle size (-log10 of p-value) and color gradient (normalized enrichment score).
A

B

C

D

E

F

G

Supplemental Figure 4
Supplemental Figure 4. Ectopic expression of EBF1, IRF4, or PAX5 does not activate MCL lineage-survival TF program in HEL or THP1 cells. (A) Immunoblot analysis of EBF1, IRF4, and PAX5 expression in the indicated cell lines. (B, C) Immunoblot (B) and RT-qPCR analysis (C) of FOXO1, IRF4, and PAX5 expression in EBF1-transduced HEL or THP1 cells. In C, data represent mean ± SEM (n=3). (D, E) Immunoblot (D) and RT-qPCR analysis (E) of FOXO1, EBF1, and PAX5 expression in IRF4-transduced HEL or THP1 cells. In E, data represent mean ± SEM (n=3). (F, G) Immunoblot (F) and RT-qPCR analysis (G) of FOXO1, EBF1, and IRF4 expression in PAX5-transduced HEL or THP1 cells. In G, data represent mean ± SEM (n=3). (H, I) Immunoblot (H) and RT-qPCR analysis (I) of EBF1, IRF4, and PAX5 expression in FOXO1-transduced HelA or HEK293T cells. Lysates and total RNA were prepared at day 20 post-transduction of indicated genes. In I, data represent mean ± SEM (n=3). (C, E, G, I) Results are representative of 3 independent experiments. Statistical analysis in C, E, and G was performed using two-tailed unpaired Student’s t-test and I using 1-way ANOVA with Tukey’s multiple-comparison test. ns, no significant.
Supplemental Figure 5. FOXO1 but not FOXO isoforms is required for MCL maintenance. (A) Heatmap depicts log2FC of sgRNA abundance of FOXO1, FOX3, FOX4, and FOX6 (averaging each independent sgRNA targeting a gene). (B) Immunoblot analysis of MCL and AML cell lines for the indicated gene expression. (c-e) RT-qPCR analysis of FOXO1 (C), FOXO3 (D), and FOXO4 (E) mRNA expression in the indicated MCL or AML cell lines. Data represent mean ± SEM (n=3). Results are representative of 3 independent experiments. Statistical analysis was performed using 1-way ANOVA with Tukey’s multiple-comparison test. Each MCL cell line was compared with the mean values of the three AML cell lines. ***p<0.0001, **p<0.005, *p<0.05. ns, no significant. (F) Violin plots of RNA expression levels in transcripts per million (TPM) of FOXO isoforms in patient MCL cells (n=37). RNA-seq data were reanalyzed from GSE141336 of Zhao et al.
Supplemental Figure 6. TAD motif of FOXO1 is crucial for its MCL supporting activity. (A) Immunoblot analysis of control and FOXO3 expressing CCMCL1 cells with indicated sgRNA. (B) Sequence alignment between FOXO1 and FOXO3 proteins. Blue and pink box indicate DBD and TAD, respectively. (C, D) Immunoblot analysis of control and 3xFlag-tagged FOXO1r#1-3 (left) or FOXO3-1 (right) transduced CCMCL1 (C) and UPN1 (D) cells at day 3 following transduction of indicated sgRNAs. (E) Competition-based proliferation assays in FOXO1r#1-3 (left) or FOXO3-1 (right) transduced UPN1 cells. Data represent mean ± SEM (n=3). (F, G) Immunoblot (F) and RT-qPCR (G) analysis of EBF1, IRF4 and PAX5 induction in FOXO1r-3- (left) or FOXO3-1-transduced (right) HEL cells. Cell lysates and total RNA were prepared at day 7 post-infection of lentivirus encoding indicated variants. In G, data represent mean ± SEM (n=3). Results are representative of 3 independent experiments. (H) FOXO1-driven 4XIRE-luciferase reporter analysis of HEK293T cells co-transfected with indicated FOXO variants. Data represent mean ± SEM (n=3). (I, J) Immunoblot analysis of control and 3xFlag-tagged FOXO1r#1-3TAD (left) or FOXO3-1TAD (right) transduced CCMCL1 (I) and UPN1 (J) cells at day 3 following transduction of indicated sgRNAs. (K) Competition-based proliferation assays in FOXO1r#1-3TAD (left) or FOXO3-1TAD (right) transduced UPN1 cells. Data represent mean ± SEM (n=3). (L, M) Immunoblot (L) and RT-qPCR (M) analysis of EBF1, IRF4 and PAX5 induction in FOXO1r#1-3TAD- (left) or FOXO3-1TAD-transduced (right) HEL cells. Cell lysates and total RNA were prepared at day 7 post-infection of lentivirus encoding indicated variants. In M, data represent mean ± SEM (n=3). Results are representative of 3 independent experiments. (E, G, H, K, M) Statistical analysis in E, K was performing using 1-way ANOVA with Tukey’s multiple-comparison test and G, H, M using two-tailed unpaired Student’s t-test. ****p<0.0001, ***p<0.0005, **p<0.005, *p<0.05. ns, no significant.
Supplemental Figure 7. Depletion of FOXO1 suppresses MCL tumorigenesis in vivo. (A) cpd10 (B) 4XIRE-luciferase reporter assay in FOXO1 or FOXA2-transfected HEK293T cells at 48 h post cpd10 treatment. Mean ± SEM (n=3). (C) FOXO1 ChIP-qPCR of CCMCL1 cells with or without 2 μM cpd10 treatment for 4 h. Mean ± SEM (n=3). (D) Co-immunoprecipitation of FOXO1 with p300. Flag-FOXO1-expressing CCMCL1 cells were treated with 2 μM cpd10 or not for 24 h. (E) Left, heatmap of signals from ChIP-seq from CCMCL1 cells extending 5 kb in each direction from the FOXO1 peak center. Right, a histogram view of the data. Cells were treated for 48 h with 2 μM cpd10 or FOXO1 depletion by sgFOXO1. (F) ChIP-seq tracks for PTPRC locus. (G) Heatmap for the expression of 184 differentially regulated (FC>1.5), FOXO1 peak-associated genes in JEG01 or CCMCL1 treated with cpd10 or sgFOXO1 for 96 h. The expression values were scaled in the row direction. (H) Enrichment levels of selected gene sets from the hallmarks collection from MSigDB. Significance and enrichment score are represented by circle size (-log10 of p-value) and color gradient (normalized enrichment score). (I) Schematic of experimental design. (J) BLI of mice inoculated with sgRNA-transduced CCMCL1 cells at day 21 post-transplantation. (K) Quantification of BLI as in (J). Mean ± SEM (n=3). (L) A representative image of spleens from mice inoculated with sgROSA- or sgFOXO1-transduced CCMCL1 cells at day 21 post-transplantation. (M, N) B cell content analysis in 10-day vehicle or cpd10 (100 mg/kg/day i.p.) treated C57BL/6J mouse spleen. Representative flowcytometry analysis (M) or mean± s.d. (n=4) values of vehicle- or cpd10-treated mice (N). Statistical analysis in B was performed by 1-way ANOVA with Tukey’s multiple-comparison test and C, K, N by two-tailed unpaired Student’s t-test. ****p<0.0001, ***p<0.0005, **p<0.005, *p<0.05. ns, no significant.