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Supplementary Materials for

A MYC inhibitor selectively alters the MYC and MAX cistromes and modulates the epigenomic landscape to regulate target gene expression

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The PDF file includes:

Legends for tables S1 and S2
Figs. S1 to S4

Other Supplementary Material for this manuscript includes the following:

Tables S1 and S2

Supplementary Materials

Table S1. Related to Figure 1.
GSEA negative enrichment results for MYC-bound promoters comparing DMSO vs. 24-hr MYCi975 treatemnt and DMSO vs. 48-hr MYCi975 treatment. The table also contains the differential gene expression data from RNA-seq experiments on 22Rv1 cells treated with MYCi975 for 24 and 48 hrs.

“supplementalTable_1.xls”

Table S2. Related to Figure 2.
Differential gene expression and differential binding data of all MYCi975 types, as well as GO enrichment results for each MYCi975 type (http://geneontology.org).

“supplementalTable_2.xls”

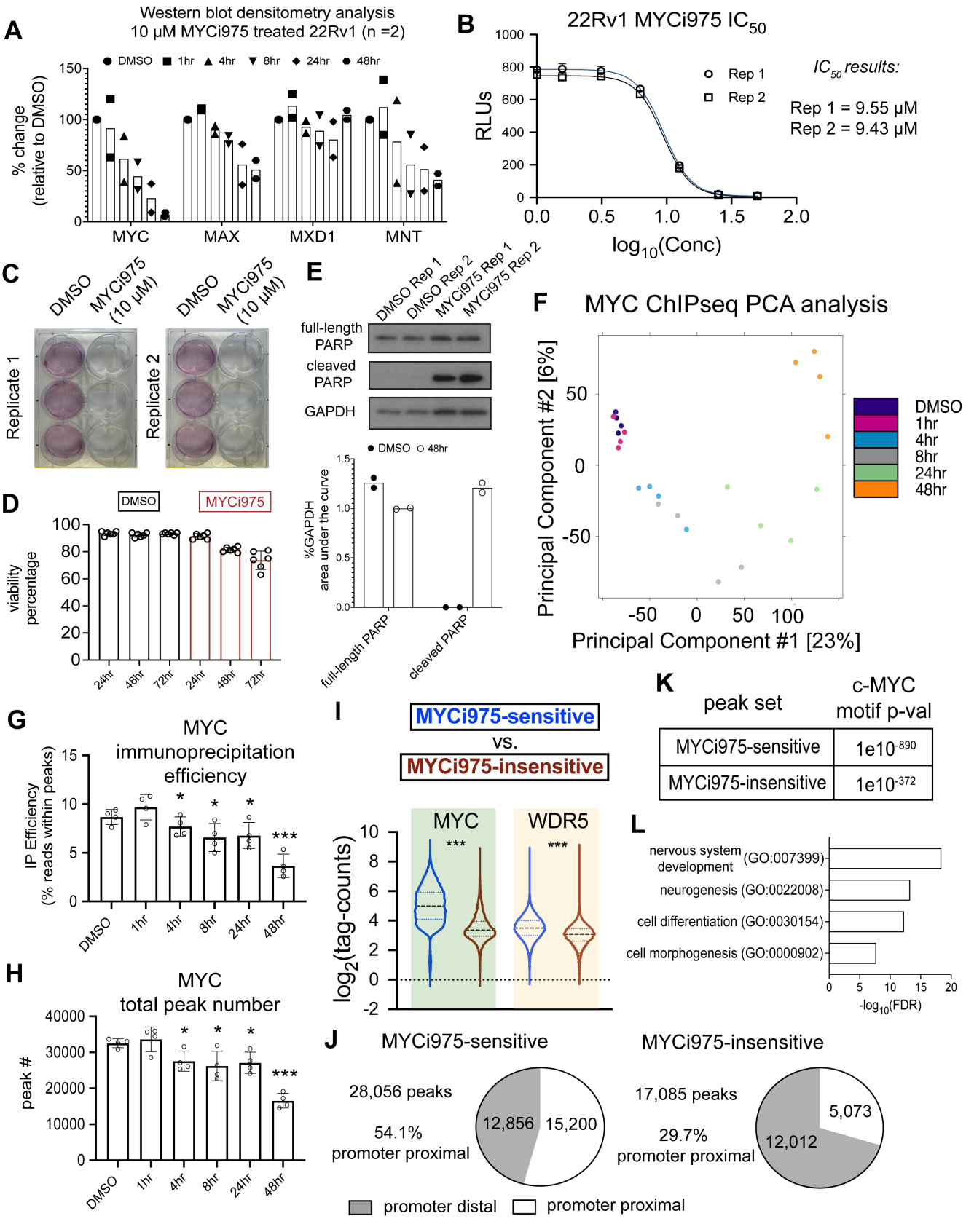
A
Western blot densitometry analysis
10 μM MYCi975 treated 22Rv1 (n =2)
DMSO
1hr
4hr
8hr
24hr
48hr
% change (relative to DMSO)
MYC
MAX
MXD1
MNT
B
22Rv1 MYCi975 IC50
RLUs
log10(Conc)
Rep 1
Rep 2
IC50 results:
Rep 1 = 9.55 μM
Rep 2 = 9.43 μM
C
DMSO
MYCi975 (10 μM)
Replicate 1
Replicate 2
D
DMSO
MYCi975
viability percentage
24hr
48hr
72hr
E
DMSO Rep 1
DMSO Rep 2
MYCi975 Rep 1
MYCi975 Rep 2
full-length PARP
cleaved PARP
GAPDH
%GAPDH area under the curve
F
MYC ChIPseq PCA analysis
Principal Component #2 [6%]
Principal Component #1 [23%]
DMSO
1hr
4hr
8hr
24hr
48hr
G
MYC immunoprecipitation efficiency
IP Efficiency (% reads within peaks)
H
MYC total peak number
peak #
I
MYCi975-sensitive
vs.
MYCi975-insensitive
MYC
WDR5
log2(tag-counts)
K
peak set
c-MYC motif p-val
MYCi975-sensitive
1e10-890
MYCi975-insensitive
1e10-372
L
nervous system development (GO:007399)
neurogenesis (GO:0022008)
cell differentiation (GO:0030154)
cell morphogenesis (GO:0000902)
-log10(FDR)
J
MYCi975-sensitive
28,056 peaks
54.1% promoter proximal
12,856
15,200
MYCi975-insensitive
17,085 peaks
29.7% promoter proximal
12,012
5,073
promoter distal
promoter proximal

Figure S1. Related to Figure 1. (A) Densitometry analysis of MYC family proteins (n=2) processed by ImageJ (*58*) with the area under the curve method repesenting the signal as a percentage of loading control protein GAPDH for two biological replicates. Loading control GAPDH was set to 100% and each timepoints was compared relative to GAPDH (individual values shown, bars represents sample mean). **(B)** IC_{50} curve analysis of 22Rv1 cells treated for 3 days. IC_{50} values were calculated using log_{10}(μM MYCi975) vs. response sigmoidal curves and nonlinear regression. **(C)** 22Rv1 cells were plated in 6-well plates at low density (25,000 cells/well), treated with MYCi975 (10 μM) for 4 days, and visualized by staining with 0.1% crystal violet. **(D)** Cell viability as determined by trypan blue exclusion assay of both vehicle control and 10 μM MYCi975-treated 22Rv1 cells (n = 4, individual values shown, bar represents sample mean, error bars = standard deviation). **(E)** 22Rv1 cells were treated with 10 μM MYCi975 for 48 hrs and 20 μg of whole cell protein extract was used to determine poly(ADP-ribose) polymerase (PARP) and cleaved-PARP protein levels (individual values shown, bar represents sample mean). **(F)** PCA analysis of differential peaks of all MYC ChIP-seq biological replicates for each timepoint (n = 4 for each timepoint). **(G)** Immunoprecipitaton (IP) efficiency (% of reads within peaks) of all MYC ChIP-seq biological replicates. **(H)** Total peak number in all biological replicates of MYC ChIP-seq samples. (individual values shown, bar represents sample mean, error bars represent mean ± SEM, *p < 0.05; ***p < 0.0001) **(I)** Log_2(tag-counts) of MYC (shaded green) and WDR5 (shaded brown) ChIP-seq signal at both MYCi975-sensitive (blue) and peaks where differential MYC binding analysis in MYCi975 treated 22Rv1 cells was insignificant (MYCi975-insensitive, red). A two-tailed unpaired parametric t-test was run on MYCi975-sensitive vs. MYCi975-insensitive log_2(tag-counts). (***p < 0.0001) **(J)** Both MYCi975-sensitve and MYCi975-insensitive peaks were split into promoter-proximal (±2kb from TSS) or promoter distal (> ±2kb from TSS). **(K)** Canonical c-Myc motif enrichment analysis of both MYCi975-sensitive and MYCi975-insensitive sites. **(L)** Gene ontology analysis (http://geneontology.org) of promoter proximal annotated MYC peaks where differential binding was insignificant (MYCi975-insensitive, 5,073 peaks from right panel (J)).

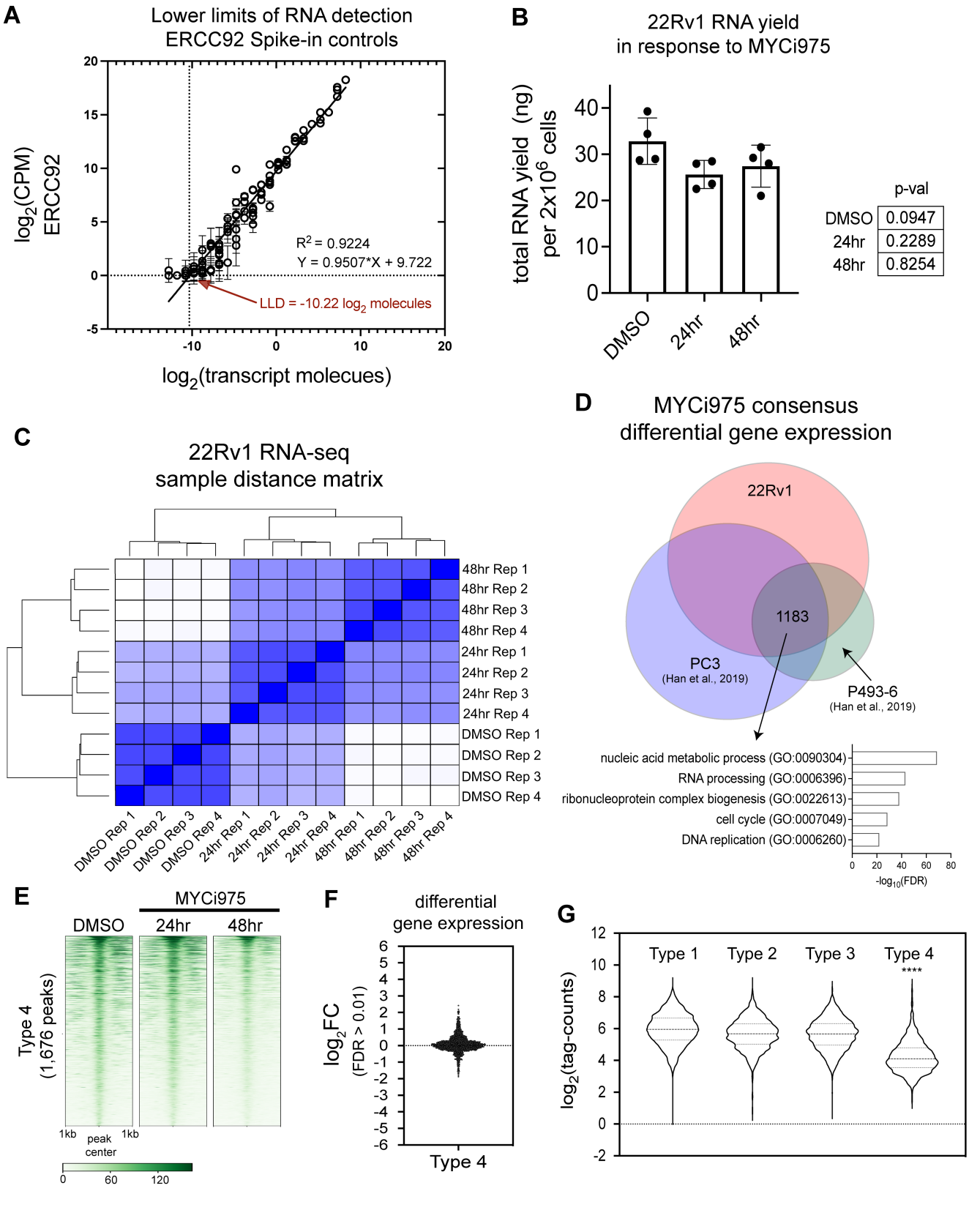
A
Lower limits of RNA detection
ERCC92 Spike-in controls
log2(CPM) ERCC92
log2(transcript molecues)
R2 = 0.9224
Y = 0.9507*X + 9.722
LLD = -10.22 log2 molecules
B
22Rv1 RNA yield
in response to MYCi975
total RNA yield (ng) per 2x10^6 cells
DMSO
24hr
48hr
p-val
DMSO 0.0947
24hr 0.2289
48hr 0.8254
C
22Rv1 RNA-seq
sample distance matrix
48hr Rep 1
48hr Rep 2
48hr Rep 3
48hr Rep 4
24hr Rep 1
24hr Rep 2
24hr Rep 3
24hr Rep 4
DMSO Rep 1
DMSO Rep 2
DMSO Rep 3
DMSO Rep 4
D
MYCi975 consensus
differential gene expression
22Rv1
1183
PC3
(Han et al., 2019)
P493-6
(Han et al., 2019)
nucleic acid metabolic process (GO:0090304)
RNA processing (GO:0006396)
ribonucleoprotein complex biogenesis (GO:0022613)
cell cycle (GO:0007049)
DNA replication (GO:0006260)
-log10(FDR)
E
MYCi975
DMSO
24hr
48hr
Type 4
(1,676 peaks)
1kb
peak
center
1kb
F
differential
gene expression
log2FC
(FDR > 0.01)
Type 4
G
log2(tag-counts)
Type 1
Type 2
Type 3
Type 4
****

Figure S2. Related to Figure 1 and 2. (A) Correlation plot for log_2(ERCC92 transcript molecules) detected within RNA-seq samples and the log_2(known transcripts molecules) to assess the lower limits of gene expression detection. **(B)** Total RNA yield from $2x10^6$ 22Rv1 cells treated with either DMSO or 10 μM MYCi975 for 24 or 48 hrs. One-way ANOVA analysis to test for significance of variance results are listed (right panel). (individual values shown, bar represents sample mean, errors bars = standard deviation). **(C)** Sample distance matrix calculations of log_2 normalized gene counts by DESeq2 with biological duplicates and all timepoints for RNA-seq in MYCi975-treated cells. **(D)** Publicly available RNA-seq results from MYCi975-treated PC3 and P493-6 cells ((*21*), GSE135877) was used to overlap consensus dysregulated genes. All differentially expressed genes were analyzed for overlap. In total, 1,183 genes were differentially expressed in all cell lines. Gene ontology enrichment analysis was run on the 1,183 consensus genes and the top enriched gene sets are reported (lower panel, http://geneontology.org). **(E)** MYCi975 Type 4 target gene heatmap representation demonstrating MYC promoter occupancy in cells treated with DMSO control or MYCi975 for 24 and 48 hrs. **(F)** Log_2(fold change) of MYC target genes in MYCi975 Type 4 sites demonstrating all genes with no significant change in RNA levels in DMSO vs. 48-hr MYCi975 treatment. **(G)** Log_2(tag-counts) for MYCi975 target gene Types 1-4. Using one-way ANOVA and Tukey's multiple comparison test with a single pooled variance, MYCi975 Type 4 target genes demonstrate a decrease in tag density compared to MYCi975 Type 1-3 target genes (****$p < 0.0001$).

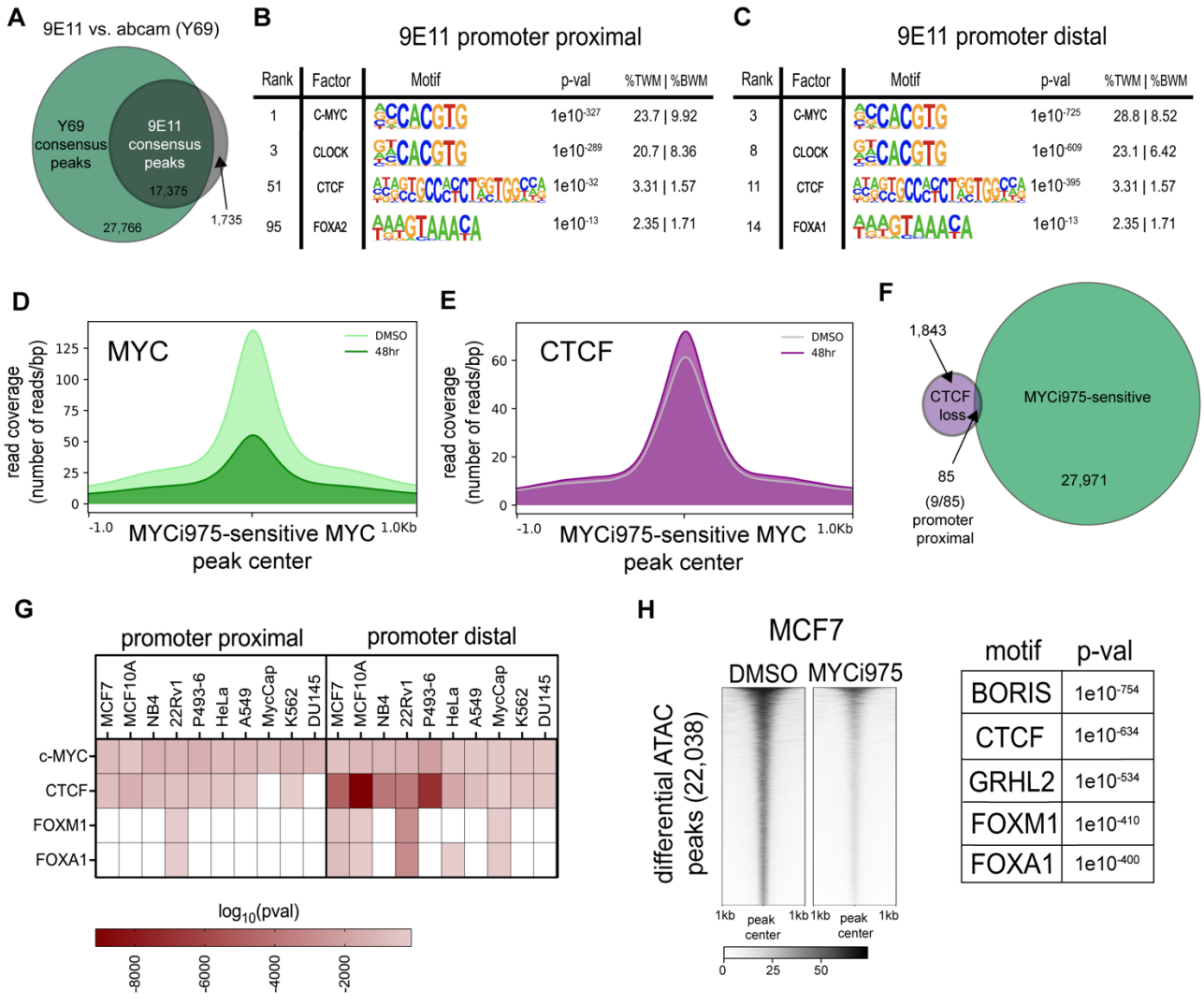
A
9E11 vs. abcam (Y69)
Y69 consensus peaks
9E11 consensus peaks
17,375
27,766
1,735
B
9E11 promoter proximal
Rank
Factor
Motif
p-val
%TWM | %BWM
1
C-MYC
1e10-327
23.7 | 9.92
3
CLOCK
1e10-289
20.7 | 8.36
51
CTCF
1e10-32
3.31 | 1.57
95
FOXA2
1e10-13
2.35 | 1.71
C
9E11 promoter distal
Rank
Factor
Motif
p-val
%TWM | %BWM
3
C-MYC
1e10-725
28.8 | 8.52
8
CLOCK
1e10-609
23.1 | 6.42
11
CTCF
1e10-395
3.31 | 1.57
14
FOXA1
1e10-13
2.35 | 1.71
D
MYC
DMSO
48hr
read coverage (number of reads/bp)
MYCi975-sensitive MYC peak center
-1.0
1.0Kb
E
CTCF
DMSO
48hr
read coverage (number of reads/bp)
MYCi975-sensitive MYC peak center
-1.0
1.0Kb
F
1,843
CTCF loss
MYCi975-sensitive
27,971
85
(9/85) promoter proximal
G
promoter proximal
promoter distal
MCF7
MCF10A
NB4
22Rv1
P493-6
HeLa
A549
MycCap
K562
DU145
c-MYC
CTCF
FOXM1
FOXA1
log10(pval)
-8000
-6000
-4000
-2000
H
MCF7
DMSO
MYCi975
differential ATAC peaks (22,038)
1kb
peak center
1kb
0
25
50
motif
p-val
BORIS
1e10-754
CTCF
1e10-634
GRHL2
1e10-534
FOXM1
1e10-410
FOXA1
1e10-400

Figure S3. Related to Figure 3. (A) Venn diagram of overlapping peaks in 22Rv1 cells using both Y69 and 9E11 (ab56) antibody clones (see Methods for details). Peak numbers are displayed. **(B,C)** Motif analysis of MYC peaks for the 9E11 antibody at promoter-proximal and promoter-distal sites (%TWM = percentage of targets with motif, %BWM = percentage of background with motif). **(D)** Mean MYC ChIP-seq read coverage plotted over sites with significant loss of MYC (DMSO vs. 48-hr MYCi975 treatment; 28,056 peaks). **(E)** Mean CTCF ChIP-seq read coverage plotted over MYC binding sites with significant loss of MYC (DMSO vs. 48-hr MYCi975 treatment; 28,056 peaks). **(F)** Venn diagram showing overlap of MYCi975-sensitive sites and differential CTCF lost sites in 22Rv1 cells treated with MYCi975 for 48 hrs. **(G)** Publicly available MYC ChIP-seq peaks from ENCODE and Cistrome Data Browser (https://www.encodeproject.org), http://cistrome.org/db/) were retrieved, converted to hg38 genome if applicable and split into promoter-proximal and promoter-distal sites. The heatmap plots the motif enrichment results as $\log_{10}$(p-value) for each motif (c-MYC, CTCF, FOXM1 and FOXA1) (HOMER v4.11.1). (H) Heatmap representation of differential ATAC-seq peaks in MYCi975-treated MCF7 cells (n= 2) and motif enrichment analysis results from the differential ATAC-seq peaks.

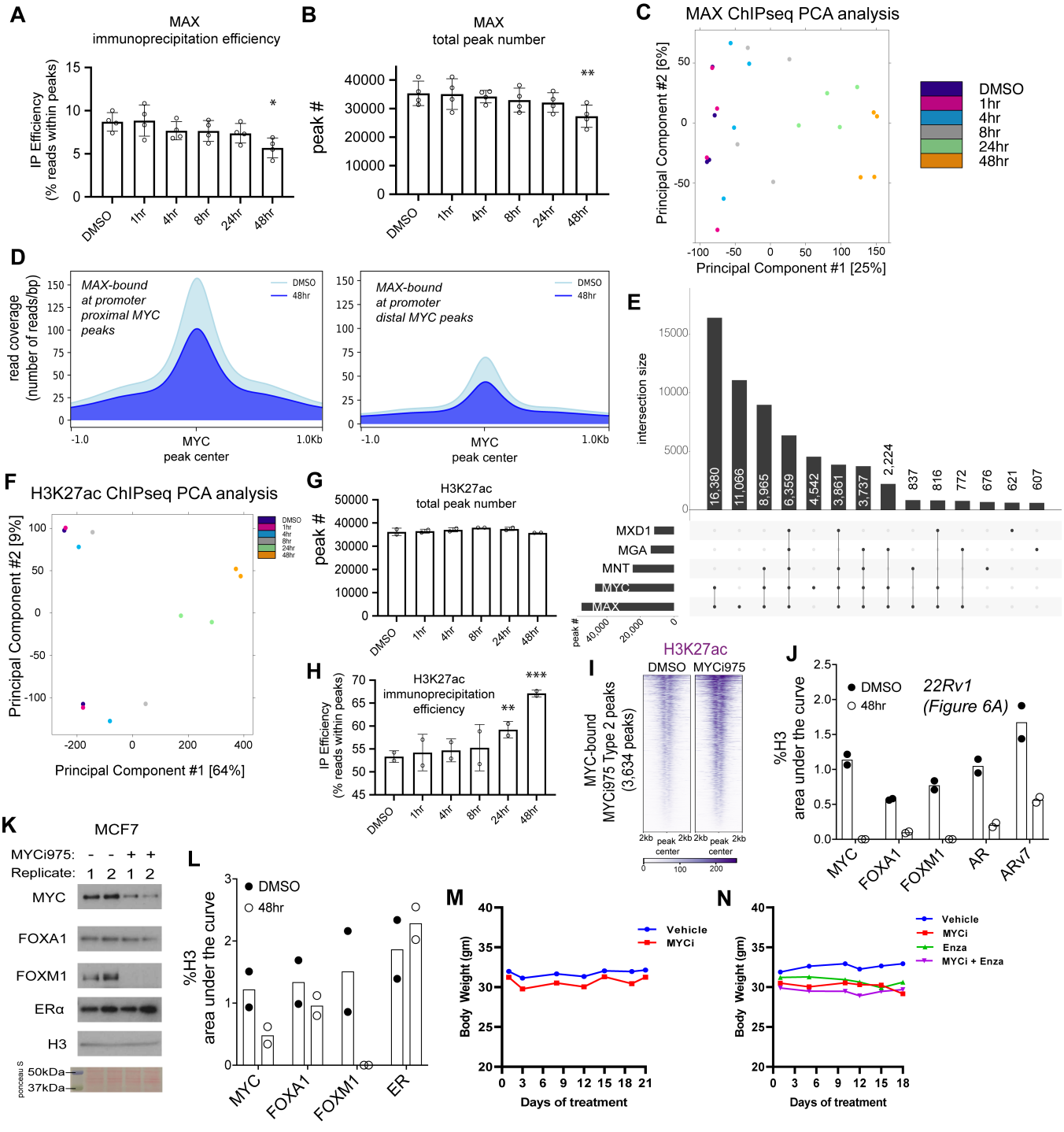
A
MAX
immunoprecipitation efficiency
IP Efficiency
(% reads within peaks)
DMSO
1hr
4hr
8hr
24hr
48hr
B
MAX
total peak number
peak #
C
MAX ChIPseq PCA analysis
Principal Component #2 [6%]
Principal Component #1 [25%]
D
MAX-bound
at promoter
proximal MYC
peaks
MAX-bound
at promoter
distal MYC peaks
read coverage
(number of reads/bp)
MYC
peak center
E
intersection size
16,380
11,066
8,965
6,359
4,542
3,861
3,737
2,224
837
816
772
676
621
607
MXD1
MGA
MNT
MYC
MAX
F
H3K27ac ChIPseq PCA analysis
Principal Component #2 [9%]
Principal Component #1 [64%]
G
H3K27ac
total peak number
H
H3K27ac
immunoprecipitation
efficiency
I
H3K27ac
DMSO
MYCi975
MYC-bound
MYCi975 Type 2 peaks
(3,634 peaks)
peak
center
J
DMSO
48hr
22Rv1
(Figure 6A)
%H3
area under the curve
MYC
FOXA1
FOXM1
AR
ARv7
K
MCF7
MYCi975: - - + +
Replicate: 1 2 1 2
MYC
FOXA1
FOXM1
ERα
H3
ponceau S
50kDa
37kDa
L
DMSO
48hr
%H3
area under the curve
MYC
FOXA1
FOXM1
ER
M
Vehicle
MYCi
Body Weight (gm)
Days of treatment
N
Vehicle
MYCi
Enza
MYCi + Enza
Body Weight (gm)
Days of treatment

Figure S4. Related to Figures 4, 5, and 6. (A) Immunoprecipitaton (IP) efficiency (% of reads within peaks) of all MAX ChIP-seq biological replicates. **(B)** Total peak number in all replicates of MAX ChIP-seq samples (*p < 0.05; **p < 0.001, bar represents sample mean, error bars represent mean ± SEM). **(C)** PCA analysis of differential peaks in all MAX ChIP-seq biological quadruplicates for each timepoint. **(D)** Mean MAX ChIP-seq read coverage at MYC-bound promoter proximal and distal sites. **(E)** Upset plot of peak overlap analysis of MYC, MAX, MNT, MGA, and MXD1 in 22Rv1 prostate cancer cells (*35*). **(F)** PCA analysis of differential peaks in all H3K27ac ChIP-seq biological duplicates for each timepoint. **(G)** Total peak number in all replicates of H3K27ac ChIP-seq samples. **H)** Immunoprecipitation (IP) efficiency (% of reads within peaks) of all H3K27ac ChIP-seq biological replicates (*p < 0.05; **p < 0.001, bar represents sample mean, error bars represent mean ± SEM). **(I)** Heatmap representation of H3K27ac occupancy at MYC-bound promoters in the MYCi975 Type 2 peak set demonstrating an increase. **(J)** Densitometry analysis processed by ImageJ (*58*) with the area under the curve method representing the signal as a percentage of loading control protein H3 for two biological replicates. Blots are from Figure 6A (individual values shown, bar represents sample mean). **(K)** Immunoblot of sonicated nuclear fractions of MCF7 cells treated with 10 μM MYCi975 for 48 hrs. Replicates (1, 2) represent biological replicates. **(L)** Densitometry analysis processed by ImageJ with the area under the curve method representing the signal as a percentage of loading control protein histone H3 for the two biological replicates of MCF7 cells. Blots are from (K) (individual values shown, bar represents sample mean). **(M)** Mouse average body weight (gm) from Figure 6D. (**N**) Mouse average body weight (gm) from Figure 6E.