SUPPLEMENTARY FIGURES

Supplementary Figure 1:

A. Truncating mutations and copy number alterations of *MGA* and *MYC* in lung adenocarcinomas.

B. Loss-of-function alterations (truncating mutations and copy number deletions) of *MGA* in diverse cancer types in the TCGA Pan-Cancer Analysis. UCEC: uterine corpus endometrial carcinoma, LUAD: lung adenocarcinoma, CRC: colorectal carcinoma, STAD: stomach adenocarcinoma, SKCM: skin cutaneous melanoma, BLCA: bladder carcinoma, KIRC: kidney renal clear cell carcinoma, PRAD: prostate adenocarcinoma, LUSC: lung squamous cell carcinoma, LIHC: liver hepatocellular carcinoma, KIRP: kidney renal papillary cell carcinoma, CESC: cervical squamous cell carcinoma, HNSC: head and neck squamous cell carcinoma, BRCA: breast adenocarcinoma, LGG: brain lower grade glioma.

Supplementary Figure 2:

A. David pathway analysis for proteins enriched by MGA immunoprecipitation.

B. Members of the PCGF6-PRC1 complex captured in the IP-Mass Spec assay.

Supplementary Figure 3:

A. Immunoprecipitation (IP) of MGA and IgG, followed by immunoblotting of MGA and HDAC4 in NCI-H23 cells.

B. Immunoprecipitation (IP) of E2F6, IgG and RNF2, followed by immunoblotting of MGA, E2F6 and RNF2 in NCI-H23 cells.

Supplementary Figure 4: Homer de novo motif analysis results for MGA binding sites identified from MGA ChIP-seq in A549 cells.

Supplementary Figure 5:

A. Pearson correlation between the ChIP-seq signal of MGA, MYC, MAX and E2F6 from A549 cells across MGA binding sites. The correlation r values are indicated.

B. ChIP-qPCR of MYC and MGA in NCI-H23 cells at promoter regions of MYC-target genes *AURKA* and *CDK4*. Genomic regions that are not bound by MYC and MGA serve as negative controls (NC1 and NC2).

Supplementary Figure 6: ChIP-seq signal of MGA at the *AURKA* and *NME1* (MYC target genes) loci in A549 cells with and without MGA overexpression. Positions of E-box DNA motif (CACGTG) or its variant (CACNTG) are indicated.

Supplementary Figure 7:

A. Gene Set Enrichment Analysis (GSEA) analysis of RNA-seq results in A549 cells showed that MGA-repressed genes (blue) and MYC-activated genes (red) are enriched in the same GSEA Hallmark gene sets.

B. Percentage of MGA-repressed/MYC-activated genes that are bound by MGA and E2F6 at their promoter regions.

Supplementary Figure 8:

A. Immunoblots showing the overexpression of *MGA* in the *MGA*-mutant LXF289 cells.

B. MGA overexpression decreases the proliferation of LXF289 cells. The percentage of GFP-positive cells was counted four and six days post seeding the cells and normalized to the empty-ZsGreen control (n=2).

C. MGA overexpression reduces expression of MYC-target genes *CDK4*, *AURKA* and *NME1* in LXF289 cells (n=2).

Supplementary Figure 9

A. Apoptosis assay using Annexin/PI staining (n=2).

B. Cell cycle assay using PI staining (n=2). P values are derived from *t* tests, error bars: s.d. NS: not significant; *P<0.05;

Figure S1

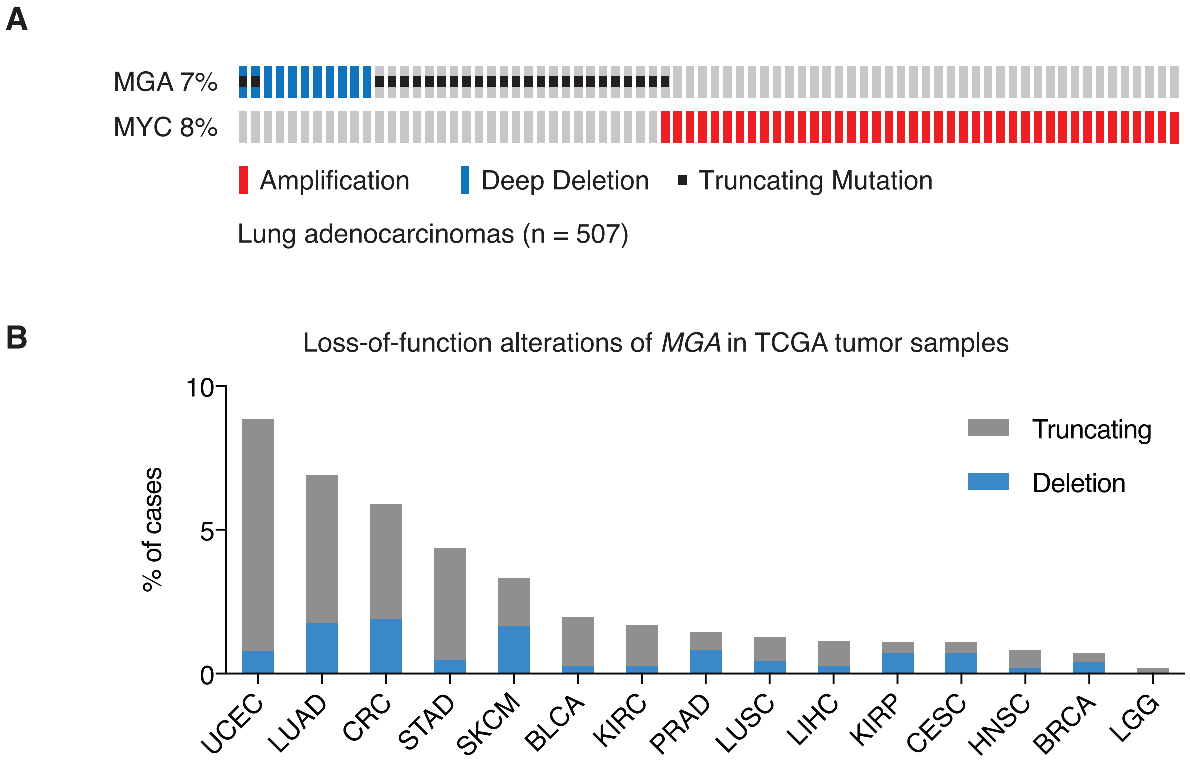

A
MGA 7%
MYC 8%
Amplification
Deep Deletion
Truncating Mutation
Lung adenocarcinomas (n = 507)
B
Loss-of-function alterations of *MGA* in TCGA tumor samples
% of cases
10
5
0
Truncating
Deletion
UCEC
LUAD
CRC
STAD
SKCM
BLCA
KIRC
PRAD
LUSC
LIHC
KIRP
CESC
HNSC
BRCA
LGG

Figure S2

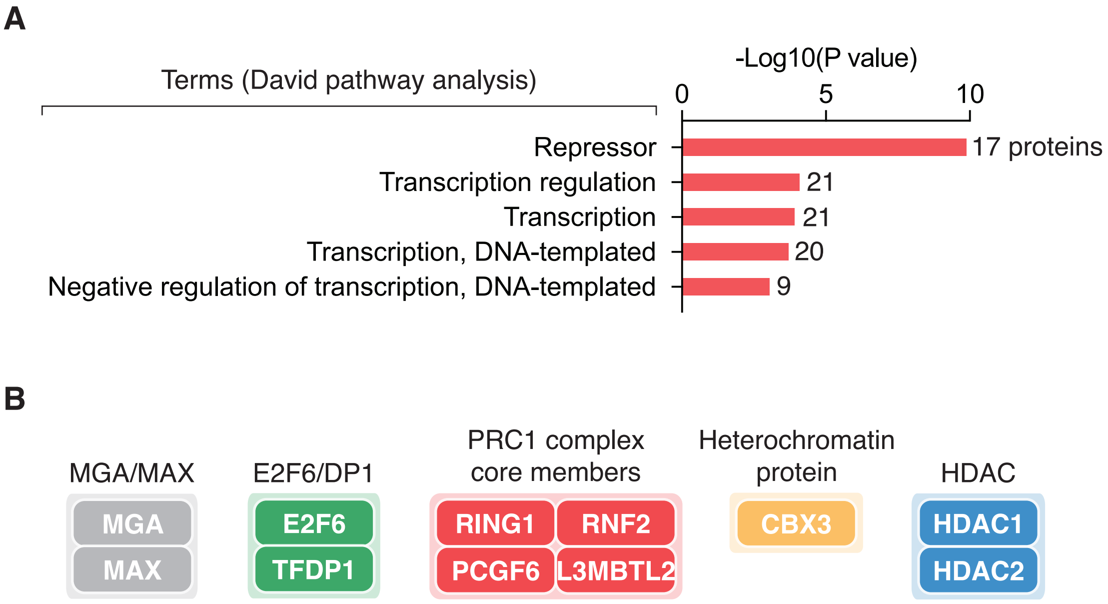

A
Terms (David pathway analysis)
-Log10(P value)
0
5
10
Repressor
Transcription regulation
Transcription
Transcription, DNA-templated
Negative regulation of transcription, DNA-templated
17 proteins
21
21
20
9
B
MGA/MAX
MGA
MAX
E2F6/DP1
E2F6
TFDP1
PRC1 complex core members
RING1
RNF2
PCGF6
L3MBTL2
Heterochromatin protein
CBX3
HDAC
HDAC1
HDAC2

Figure S3

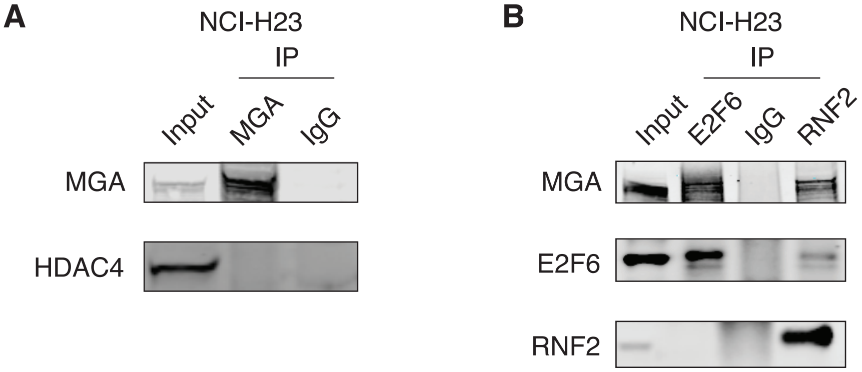

A
NCI-H23
IP
Input
MGA
IgG
MGA
HDAC4
B
NCI-H23
IP
Input
E2F6
IgG
RNF2
MGA
E2F6
RNF2

Figure S4

	Rank	P-value	log P-pvalue	% of Targets	% of Background	Best Match/Details
	1	1e-650	-1.50E+03	38.40%	14.87%	n-Myc(bHLH)/mES-nMyc-ChIP-Seq(GSE11431)/Homer(0.971)
	2	1e-458	-1.06E+03	2.06%	0.00%	STAT5(Stat)/mCD4+-Stat5-ChIP-Seq(GSE12346)/Homer(0.796)
	3	1e-446	-1.03E+03	2.63%	0.02%	MYBL2/MA0777.1/Jaspar(0.532)
	4	1e-428	-9.88E+02	2.17%	0.01%	PB0060.1_Smad3_1/Jaspar(0.662)
	5	1e-400	-9.22E+02	2.04%	0.01%	CHR(?)/Hela-CellCycle-Expression/Homer(0.724)
	6	1e-337	-7.77E+02	2.61%	0.04%	PB0126.1_Gata5_2/Jaspar(0.659)
	7	1.00E-296	-6.83E+02	1.71%	0.01%	PB0098.1_Zfp410_1/Jaspar(0.694)
	8	1.00E-286	-6.60E+02	2.18%	0.03%	HSF2/MA0770.1/Jaspar(0.625)
	9	1.00E-278	-6.41E+02	2.03%	0.03%	GATA3(Zf),DR4/iTreg-Gata3-ChIP-Seq(GSE20898)/Homer(0.690)
	10	1.00E-273	-6.30E+02	2.10%	0.03%	ZNF415(Zf)/HEK293-ZNF415.GFP-ChIP-Seq(GSE58341)/Homer(0.661)
	11	1.00E-260	-6.01E+02	2.34%	0.05%	SOX10/MA0442.1/Jaspar(0.667)
	12	1.00E-249	-5.74E+02	1.23%	0.00%	STAT6(Stat)/CD4-Stat6-ChIP-Seq(GSE22104)/Homer(0.716)
	13	1.00E-219	-5.05E+02	1.65%	0.02%	ZFX(Zf)/mES-Zfx-ChIP-Seq(GSE11431)/Homer(0.733)
	14	1.00E-157	-3.63E+02	4.96%	1.03%	AP-1(bZIP)/ThioMac-PU.1-ChIP-Seq(GSE21512)/Homer(0.975)
	15	1.00E-146	-3.37E+02	1.03%	0.01%	Oct2(POU,Homeobox)/Bcell-Oct2-ChIP-Seq(GSE21512)/Homer(0.638)
	16	1.00E-118	-2.73E+02	50.05%	38.04%	ZNF711(Zf)/SHSY5Y-ZNF711-ChIP-Seq(GSE20673)/Homer(0.805)
	17	1.00E-113	-2.61E+02	20.25%	11.88%	Elk1(ETS)/Hela-Elk1-ChIP-Seq(GSE31477)/Homer(0.872)
	18	1.00E-105	-2.42E+02	18.65%	10.88%	NRF(NRF)/Promoter/Homer(0.760)
	19	1.00E-95	-2.19E+02	0.99%	0.04%	TFCP2/MA0145.3/Jaspar(0.743)
	20	1.00E-70	-1.63E+02	8.69%	4.36%	BORIS(Zf)/K562-CTCFL-ChIP-Seq(GSE32465)/Homer(0.868)
	21	1.00E-67	-1.56E+02	41.33%	32.57%	PB0009.1_E2F3_1/Jaspar(0.652)
	22	1.00E-57	-1.32E+02	2.07%	0.49%	PB0146.1_Mafk_2/Jaspar(0.728)
	23	1.00E-57	-1.32E+02	0.36%	0.00%	Pax7(Paired,Homeobox)/Myoblast-Pax7-ChIP-Seq(GSE25064)/Homer(0.591)
	24	1.00E-46	-1.07E+02	0.48%	0.02%	PH0016.1_Cux1_1/Jaspar(0.608)
	25	1.00E-46	-1.06E+02	0.80%	0.08%	PH0037.1_Hdx/Jaspar(0.741)
	26	1.00E-18	-4.28E+01	0.18%	0.01%	Gfi1b(Zf)/HPC7-Gfi1b-ChIP-Seq(GSE22178)/Homer(0.641)
	27	1.00E-17	-3.93E+01	0.17%	0.01%	PB0046.1_Mybl1_1/Jaspar(0.585)
	28	1.00E+00	-3.24E-02	0.15%	0.24%	PB0097.1_Zfp281_1/Jaspar(0.899)

Figure S5

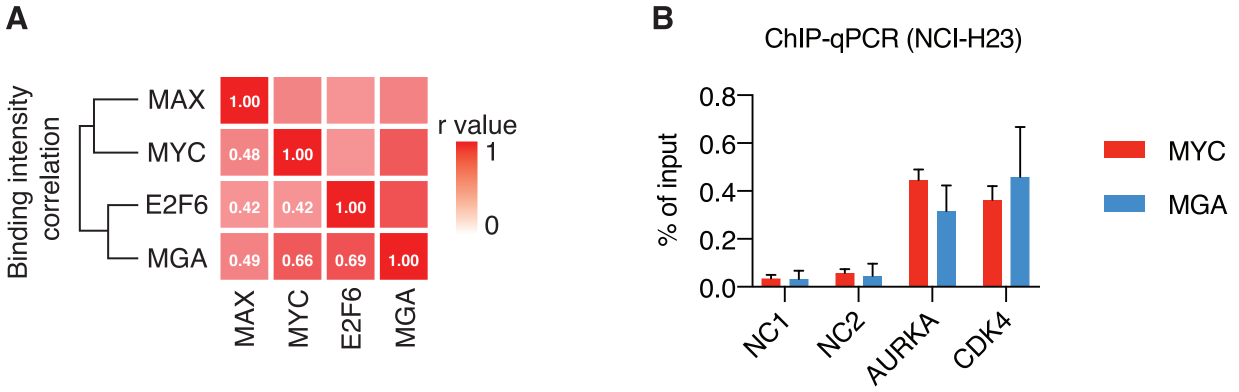
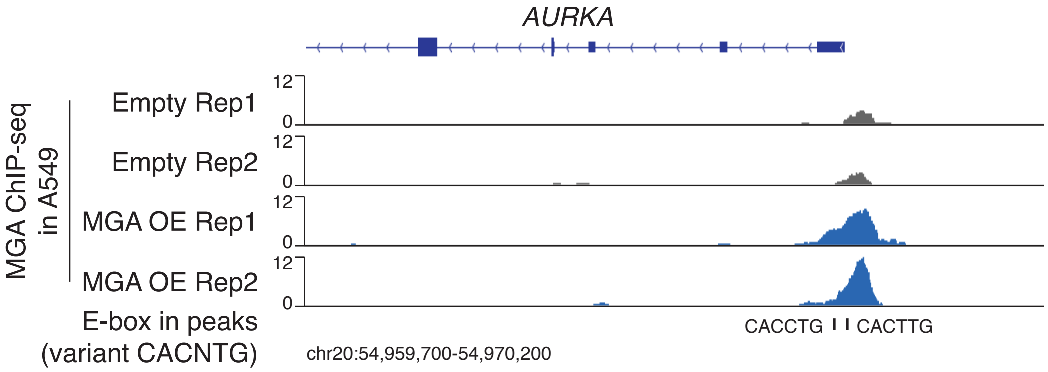

A
Binding intensity correlation
MAX
MYC
E2F6
MGA
1.00
0.48
1.00
0.42
0.42
1.00
0.49
0.66
0.69
1.00
MAX
MYC
E2F6
MGA
r value
1
0
B
ChIP-qPCR (NCI-H23)
% of input
0.8
0.6
0.4
0.2
0.0
NC1
NC2
AURKA
CDK4
MYC
MGA

Figure S6


AURKA
MGA ChIP-seq in A549
Empty Rep1
Empty Rep2
MGA OE Rep1
MGA OE Rep2
E-box in peaks (variant CACNTG)
12
0
12
0
12
0
12
0
CACCTG
CACTTG
chr20:54,959,700-54,970,200

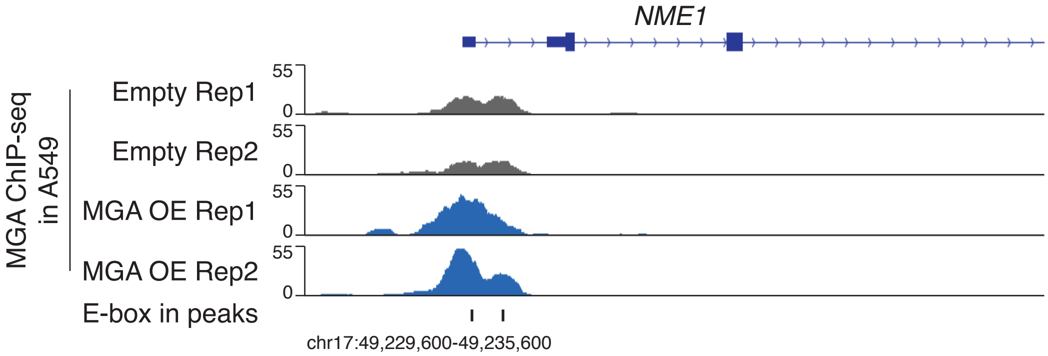

NME1
MGA ChIP-seq in A549
Empty Rep1
Empty Rep2
MGA OE Rep1
MGA OE Rep2
E-box in peaks
55
0
55
0
55
0
55
0
chr17:49,229,600-49,235,600

Figure S7

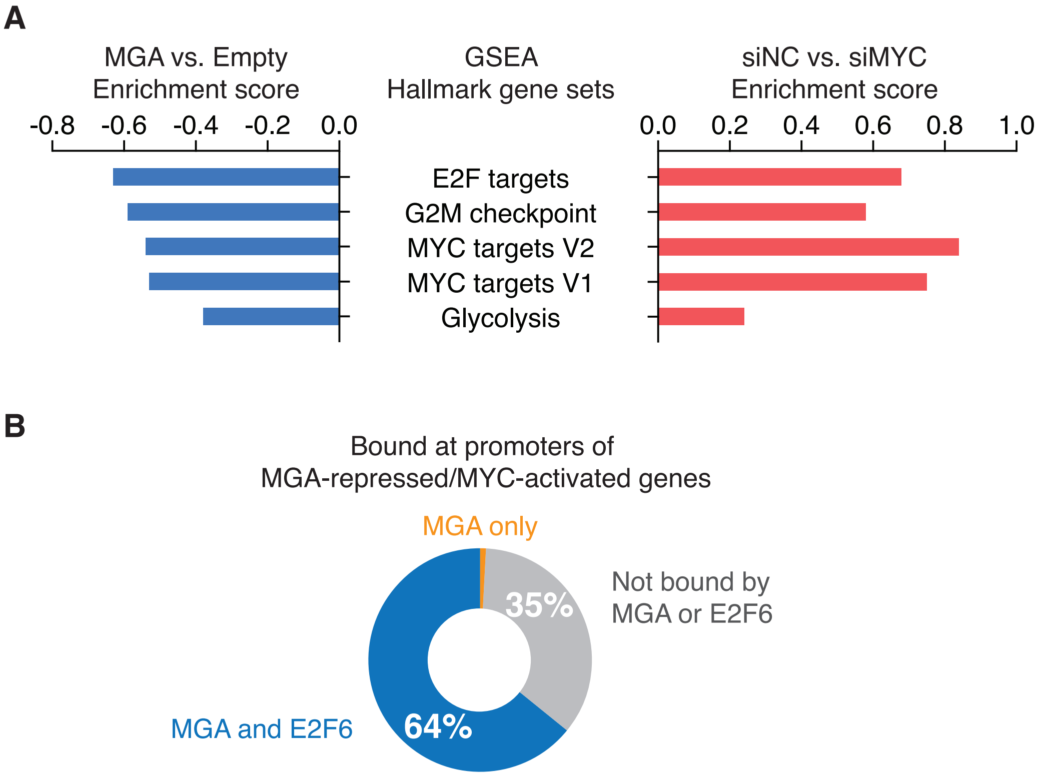
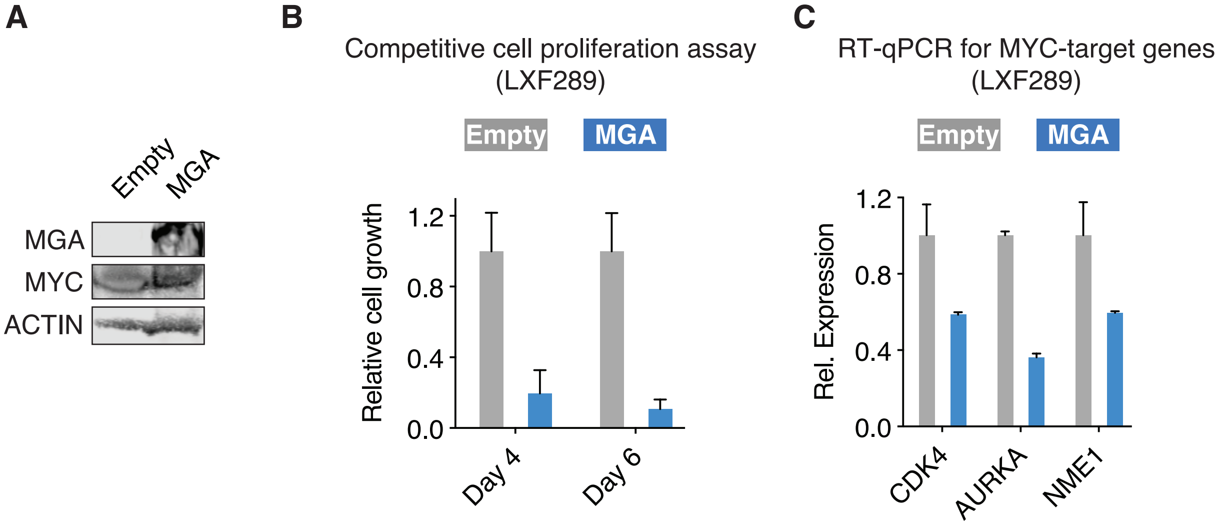

A
MGA vs. Empty
Enrichment score
-0.8
-0.6
-0.4
-0.2
0.0
GSEA
Hallmark gene sets
siNC vs. siMYC
Enrichment score
0.0
0.2
0.4
0.6
0.8
1.0
E2F targets
G2M checkpoint
MYC targets V2
MYC targets V1
Glycolysis
B
Bound at promoters of
MGA-repressed/MYC-activated genes
MGA only
Not bound by
MGA or E2F6
35%
MGA and E2F6
64%

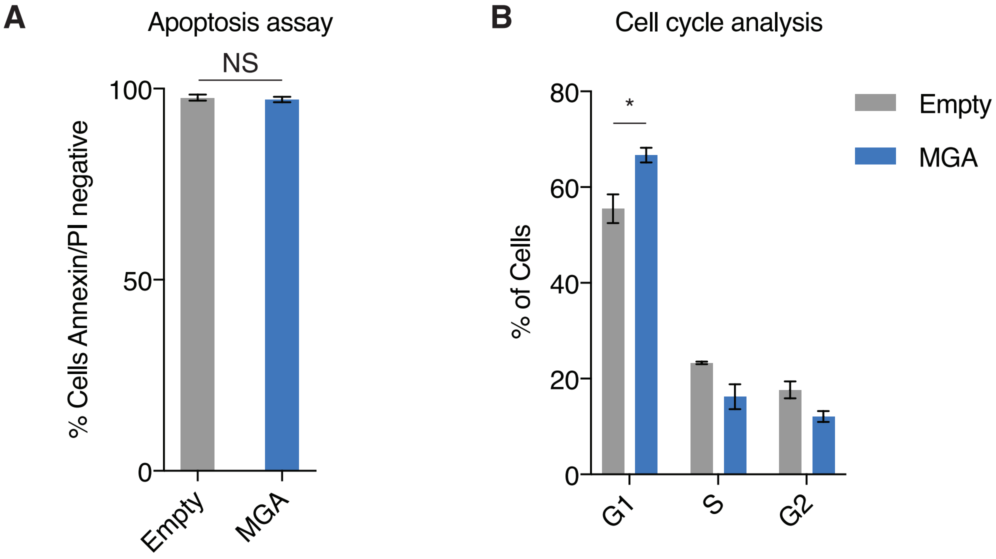
Figure S8



Competitive cell proliferation assay (LXF289)

RT-qPCR for MYC-target genes (LXF289)

Figure S9


A
Apoptosis assay
NS
% Cells Annexin/PI negative
100
50
0
Empty
MGA
B
Cell cycle analysis
*
% of Cells
80
60
40
20
0
G1
S
G2
Empty
MGA