SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transient and stable transfection

To examine the reciprocal regulation between MATα1 and c-Myc/MafG/c-Maf, $1X10^5$ KMCH, Huh-28 or HepG2 cells per well of 6-well plates were transfected with vectors containing overexpressing MATα1, c-Myc, MafG, c-Maf or empty vectors for 24 h using Lipofectamine 2000 (Life Technologies, Grand Island, NY) according to the manufacturer's protocol. For gene knockdown studies, 10nM siRNA against MAT1A, c-Myc, Maf or MafG and equivalent scramble control were delivered into KMCH, Huh-28 or HepG2 cells for 24 h by Lipofectamine RNAiMAX (Life Technologies) following the manufacturer's protocol. For promoter activity assays, transient transfection analysis of MAT1A, c-Myc, Maf and MafG promoter activities were performed in KMCH, Huh-28 or H69 cells for 16 h using Lipofectamine 2000 as described previously (2).

To establish stable expression, 10^5 KMCH cells were seeded in a 24-well plate 1 day prior to infection. To generate cells stably expressing less MATα1, c-Myc, MafG or c-Maf, cells were infected with lenti-viral vectors containing CRISPR/Cas9 (CRISPR) for MAT1A, c-Myc, MafG, c-Maf, or scramble control (Sigma-Aldrich). To generate cells stably overexpressing MATα1, c-Myc, MafG or c-Maf, cells were treated with empty vector (EV) or MAT1A overexpression vector (13), or overexpression vectors for c-Myc, MafG, or c-Maf (GeneCopoeia, Rockville, MD). Following selection with puromycin (Invitrogen), stable clonal cell lines were established and examined for the expression of these proteins and green fluorescent protein expression by real-time PCR.

RNA isolation and gene expression analysis

Total RNA was isolated by the TRIzol reagent (Life Technologies) or using commercially available kit (Qiagen, USA) from liver tissues, CCA specimens and CCA cells. Gene expression was assessed using real-time PCR. Total RNA was subjected to reverse transcription (RT) by using M-MLV Reverse transcriptase (Lucigen, Middleton, WI). TaqMan probes for human and murine MAT1A, c-Myc, MafG, c-Maf and the Universal PCR Master Mix were purchased from Bio-Rad (Hercules, CA). HPRT1 or BACT (for human CCA) was used as a housekeeping gene. The thermal profile consisted of an initial denaturation at 95°C for 3 minutes followed by 40 cycles at 95°C for 3 seconds and at 60°C for 30 seconds. The cycle threshold (Ct value) of the target genes was normalized to that of the housekeeping gene to obtain the delta Ct (DCt). The DCt was used to find the relative expression of target genes according to the formula: relative expression= 2^{-DDCt}, where DDCt= DCt of target genes in experimental condition – DCt of target gene under control condition.

ChIP and Sequential-ChIP (Seq-ChIP) assay

Briefly, DNA immunoprecipitated by Max antibody was processed for a second round of immunoprecipitation using anti-MATα1, anti-c-Myc, anti-MafG, anti-c-Maf, or anti-Mnt antibodies. The purified DNA was detected by PCR analysis. PCR of the mouse *Mat1a* promoter region containing E-box used forward primer 5'-GAGTCACCTGGGAGCAAGCTG-3' (bp- 248/-227) and reverse primer 5'-CGACTTCACTTCTCCAAAGTGAC-3' (bp -41/-19 relative to the ATG start site)

(GenBank® accession no. NM_133653.3). PCR of the mouse *c-Myc* promoter region containing E-box used forward primer 5'-GATAACTCATTCGTTCGTCCTTCC-3' (bp -1073/-1052) and reverse primer 5'-GGTGTAAACAGTAATAGCGCAGCATG-3' (bp -815/-790 relative to the ATG start site) (GenBank® accession no. NM_001177352). PCR of the mouse *MafG* promoter region containing E-box used forward primer 5'-GTCTCTGCAGGAGCACGGTCAAC-3' (bp –448 to -426) and reverse primer 5'-TCCTCAGACGTCACATGATGTTTG-3' (bp -247 to -224 relative to ATG start site) (GenBank® accession no. NM-010756). PCR of the mouse *c-Maf* promoter region containing E-box used forward primer 5'-GCAAGGGTTAAGCAAACCTGTCC-3' (bp -1173 to -1150) and reverse primer 5'- AAGCCTCTGATCCCAGCGAGAAG (bp -973 to -951 relative to ATG start site) (GenBank® accession no. NM_001025577). PCR for the human *MAT1A* promoter region containing E-box used forward primer 5'-ATGCCTGCCAGCCTTTTAGAGAAG-3' (bp -547 to -524) and reverse primer 5'-GCAACAGTGCCTTTGCTGTTGGG-3' (bp -345 to -323 relative to transcriptional start site) (GenBank® accession no. NM_000429.2). All PCR products were run on 2% agarose gels. The PCR conditions consisted of an initial denaturation at 94°C for 3 minutes followed by 25 cycles at 94°C for 30 seconds, the annealing and extension at 67°C for 90 seconds using the Advantage GC 2 PCR kit (Clontech), in accordance to their suggested protocol.

Western blot and Co-immunoprecipitation (Co-IP) analysis

Total and nuclear protein extracts from BDL, LCA liver tissues, murine CCA specimens were subjected to Western blot analysis as described (10). Equal amounts of total or

nuclear protein extracts (15 μg/well) were resolved on 12.5% SDS–polyacrylamide gels. Membranes were probed with antibodies to MATα1, c-Myc, MafG and c-Maf (Abcam). To ensure equal loading, membranes were stripped and re-probed with anti-Actin (for total) or Histone H3 (for nuclear) antibodies (Cell Signaling). Blots were developed by enhanced chemiluminescence (Millipore Corporation, Billerica, MA). For co-IP, nuclear proteins were immunoprecipitated by anti-Matα1, c-Myc, MafG or c-Maf antibodies and then subjected to Western blotting with anti-Matα1, anti-c-Myc, anti-MafG, anti-c-Maf, anti-Mnt, or anti-Max antibodies. They were normalized to anti-Histone H3 antibody.

IP and proteomics assays by mass spectrometry (MS)

LCA treated livers (14 days), murine CCA and their respective control liver tissues were weighed, diced into very small pieces using a clean razor blade, collected in a pre-chilled 5 ml conical tube, suspended in PBS buffer, and subsequently lysed with a 2 ml lysis buffer containing 1 × protease inhibitor cocktail and phosphatase inhibitor mixture (Active Motif, Carlsbad, CA). After centrifugation at 12,000Xg for one hour, the supernatant was collected. 1500 ug of protein extracts and 15ug antibody to c-Myc, c-Maf, MafG or MATα1 were combined and incubated for 4 hours at 4^0C on a rotator. After antibody treatment, 150 ul of protein G magnetic beads were added to the samples and incubated for 1 hour at 4^0C. The magnetic protein G beads were washed four times with IP/wash buffer and eluted following instructions in the universal magnetic IP kit (Active Motif). MS assays were described previously (18).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1 – MAT1A expression is down-regulated in human CCA. MAT1A mRNA level normalized to BACT was assayed in clinical tumor (n=13) and available adjacent benign (n=7) tissue samples by real time quantitative PCR. ***p<0.001

Supplemental Figure 2 – Interactions of Matα1, c-Myc, Mnt, MafG and c-Maf in murine CCA.

The liver protein lysates from control and CCA tumors previously described (3) were subjected to IP using anti-Matα1, anti-c-Myc, anti-c-Maf and MafG, followed by mass spectrometry. The table shows a small subset of scores representing potential interacting proteins of interest. NS=no score.

Supplemental Figure 3. MATα1, c-Myc, c-Maf and MafG binding dynamics to the E-box region of human *MAT1A* promoter in benign and malignant cell lines. H69 (benign bile duct epithelial cells), Hu28 and KMCH (CCA cells) and HepG2 (liver cancer cells) were subjected to ChIP analysis with Max followed by Seq-ChIP with c-Myc, Mnt, MATα1, MafG, or c-Maf spanning the E-box containing human MAT1A promoter (shown above) as described in Supplemental Methods.

Supplemental Figure 4. MATα1 positively auto-regulates its own expression. Effect of overexpressing MATα1 (MAT1A OV) on the promoter activity of MAT1A in the

H69 cell line was examined as described in Supplemental Methods. *p< 0.05 vs Con Vec. Results represent 3 independent experiments done in duplicate.

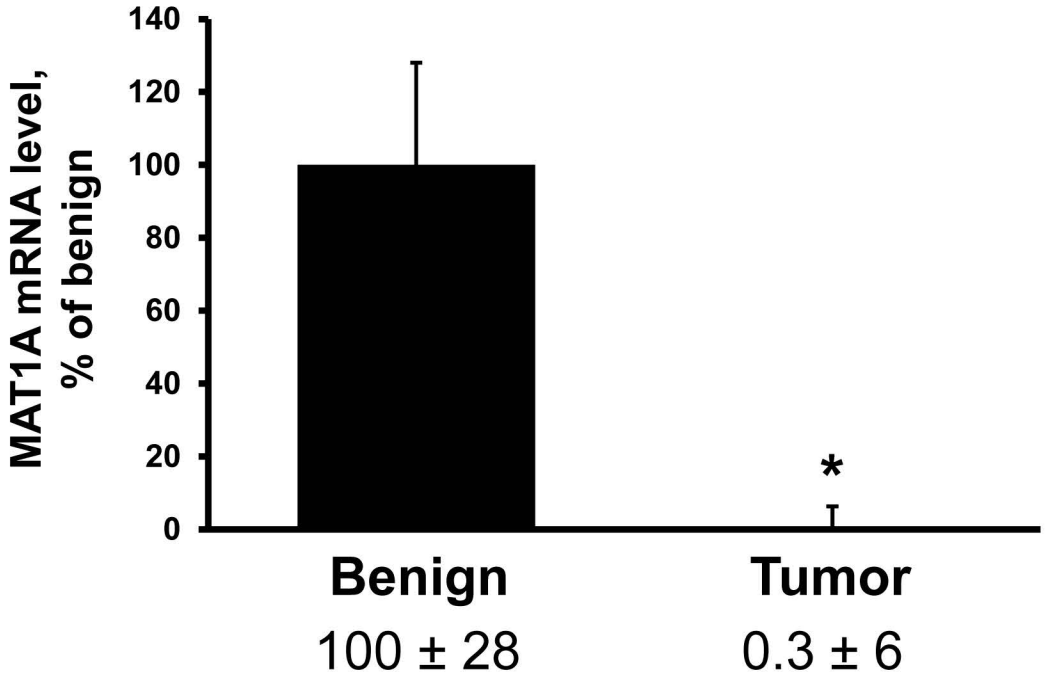
Supplemental Figure 5. Reciprocal regulation between MATα1 and c-Myc/MafG/c-Maf in HepG2 cells. HepG2 cells were treated with siRNA (si) against MAT1A, c-Maf, or MafG or c-Myc overexpression vector as described in Supplemental Methods. (**A**) Knocking down MAT1A raised c-Myc, c-Maf and MafG mRNA levels, while knocking down c-Maf or MafG lowered c-Myc, c-Maf or MafG but raised MAT1A mRNA levels. (**B**) c-Myc overexpression lowered MAT1A but raised c-Maf and MafG mRNA levels. *p< 0.05 vs scrambled siRNA control (**A**) or control empty vector (**B**). Results represent 3 independent experiments done in duplicate.

Supplemental Figure 6 - Effect of MATα1, c-Myc, c-Maf and MafG on cell growth. To determine the role of MATα1 induction and c-Myc, c-Maf and MafG knockdown on growth, BrdU incorporation was measured in KMCH cells that were transiently transfected with either Matα1 overexpression, or c-Myc, c-Maf or MafG knockdown for 24 hours. *p < 0.05 vs. Con vec (left panel); *P < 0.05 vs. scrambled (SC, right panel). n = 3 experiments, each with 5-6 determinations for BrdU.

Supplemental Figure 7. *Mat1a* promoter region is hypermethylated in mouse CCA as compared to normal liver. Diagram shows *Hpa*II and *Msp*I site between restriction enzyme sites (*Bgl*II and *Nde*I) in the mouse *Mat1a* promoter. TSS=Transcriptional start site. Also shown are expected sizes of the fragments after enzyme digestion. Below the

diagram is Southern blot analysis of *Mat1a* promoter using DNA from mCCA samples from our previous study (3) and normal mouse livers, performed as described in Methods. *Msp*I digestion results in mainly two bands, 235 and 173 bp in both mCCA and normal liver. *Hpa*II digestion in mCCA results in a much stronger 408 bp band in mCCA, while the lower molecular weight bands decreased in intensity as compared to normal liver. This is consistent with the *Mat1a* promoter being hypermethylated in mCCA as compared to normal liver.

Supplemental Figure 1



Supplemental Figure 2

Matα1 IP+MS scores

	Con	mCCA
c-Myc	NS	8.22
c-Maf	NS	11.64
MafG	NS	13.54
Mnt	22.57	NS

c-Myc IP+MS scores

	Con	mCCA
Matα1	6.41	NS
c-Maf	NS	13.12
MafG	NS	29.34
Mnt	17.85	NS

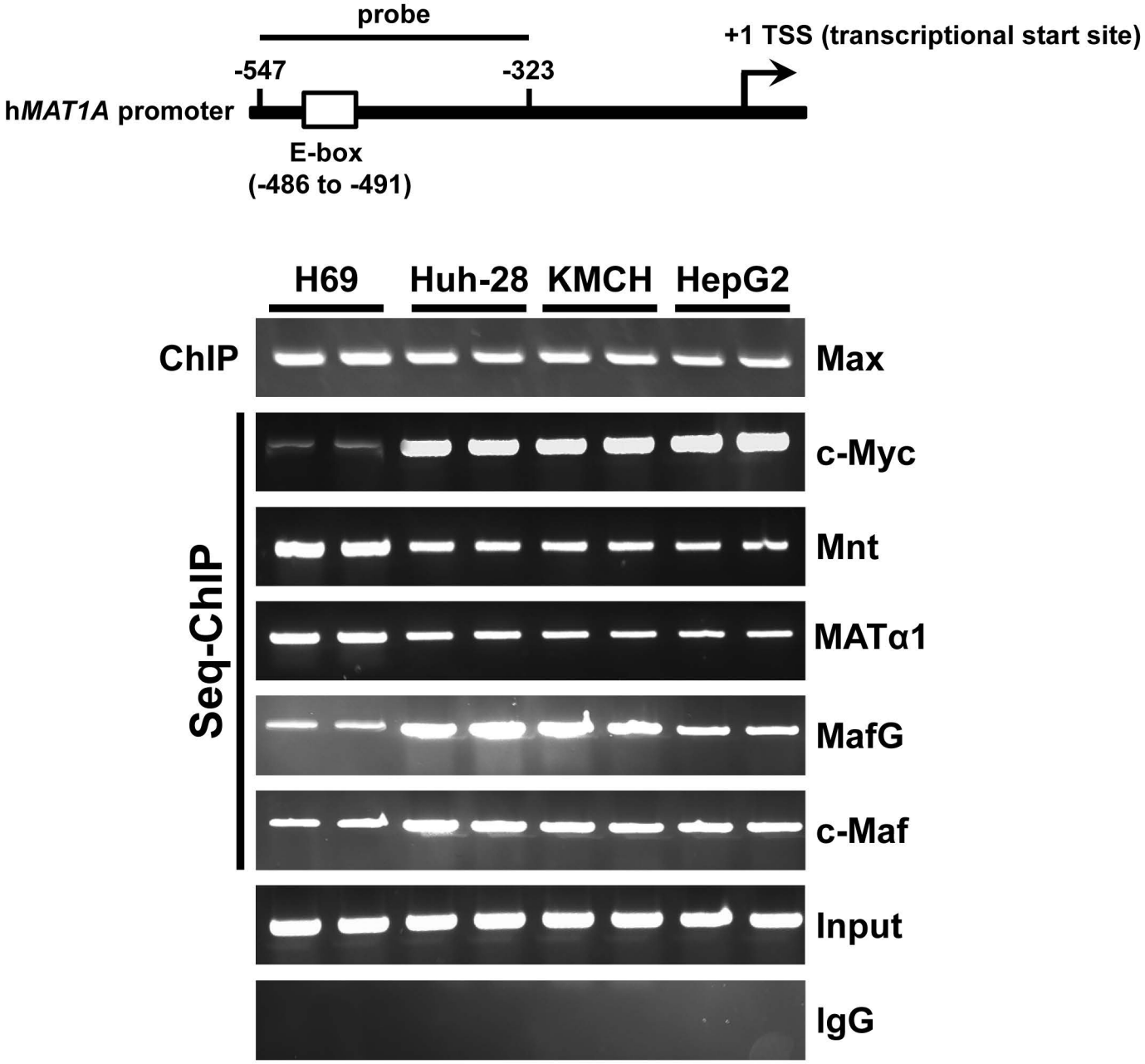
c-Maf IP+MS scores

	Con	mCCA
Matα1	3.75	2.01
c-Myc	NS	11.23
MafG	NS	8.28
Mnt	18.06	NS

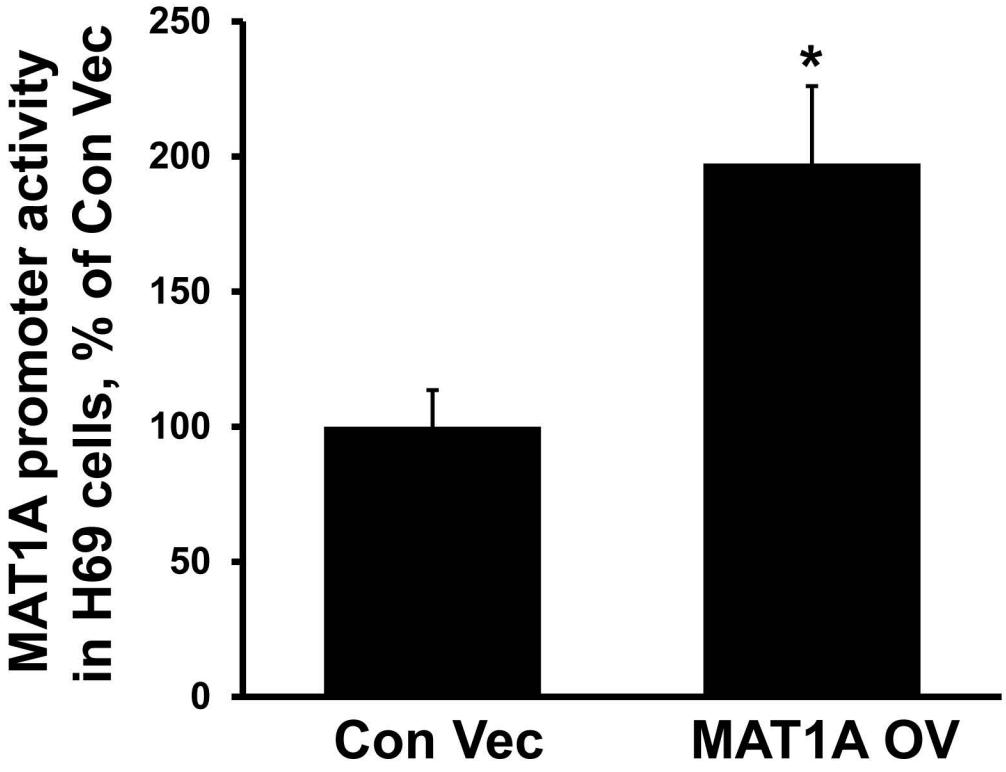
MafG IP+MS scores

	Con	mCCA
Matα1	25.58	1.81
c-Maf	NS	19.5
c-Myc	NS	14.8
Mnt	24.92	3.44

Supplemental Figure 3

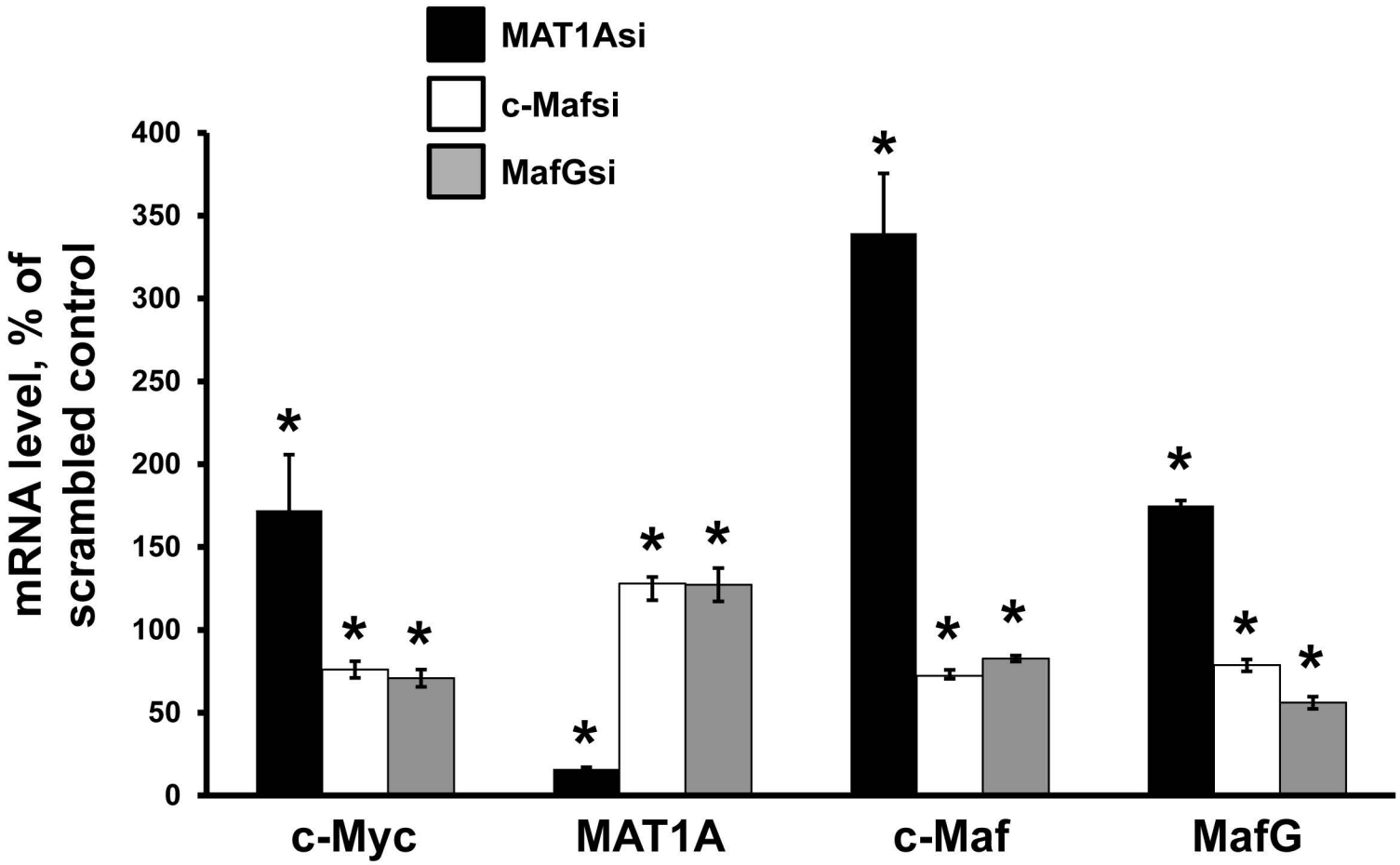


Supplemental Figure 4



Supplemental Figure 5

A



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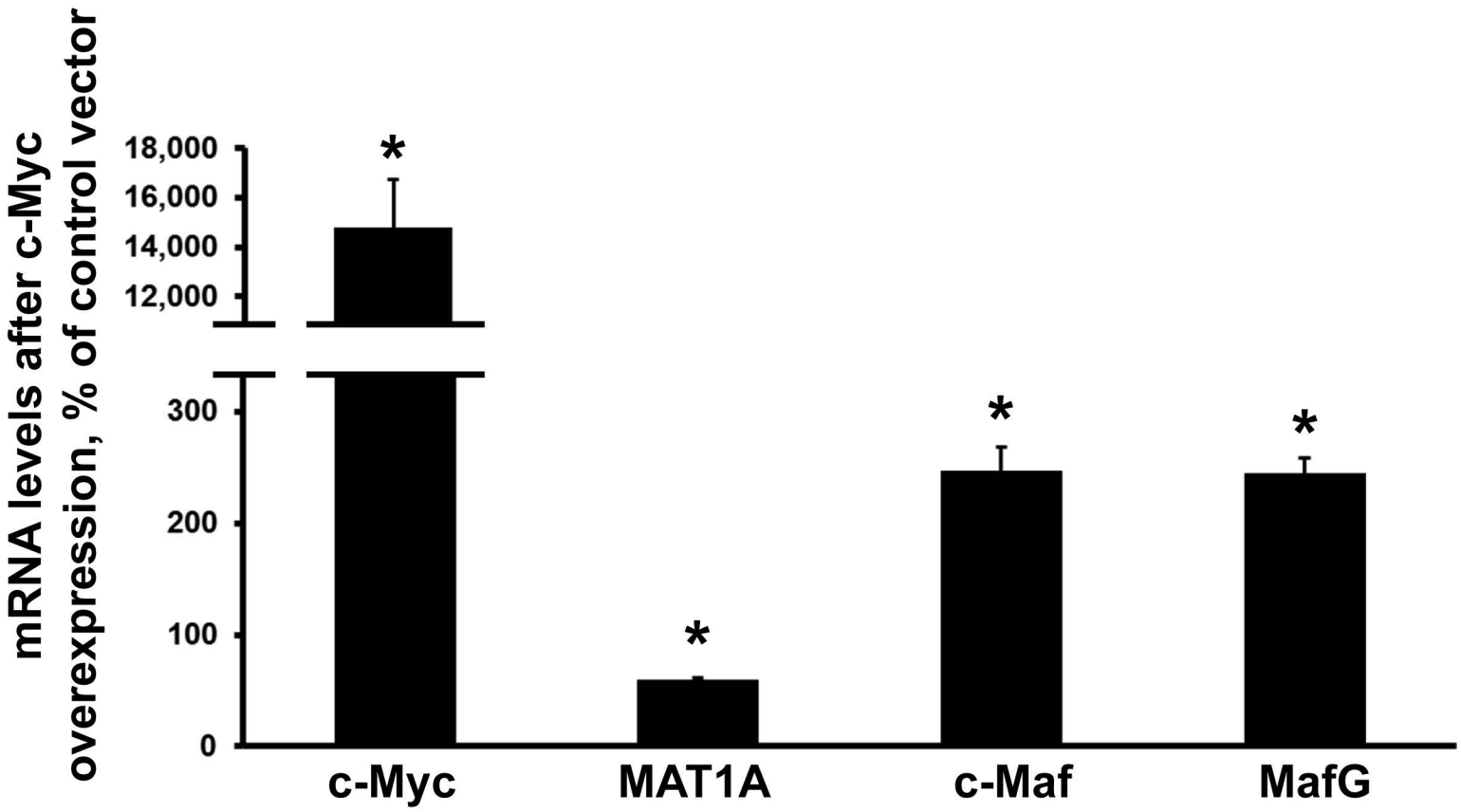
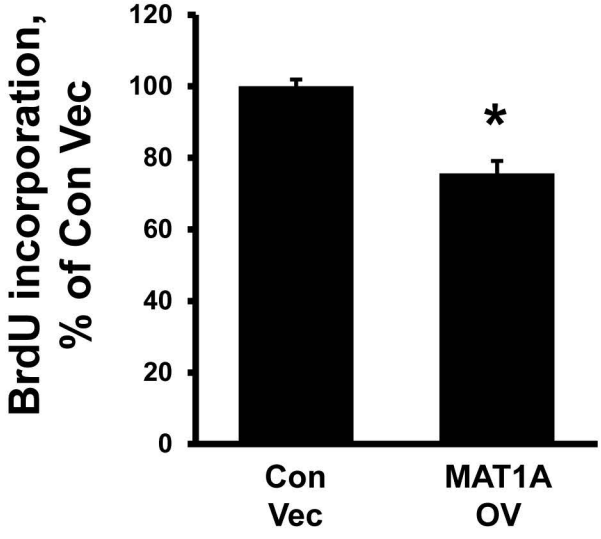
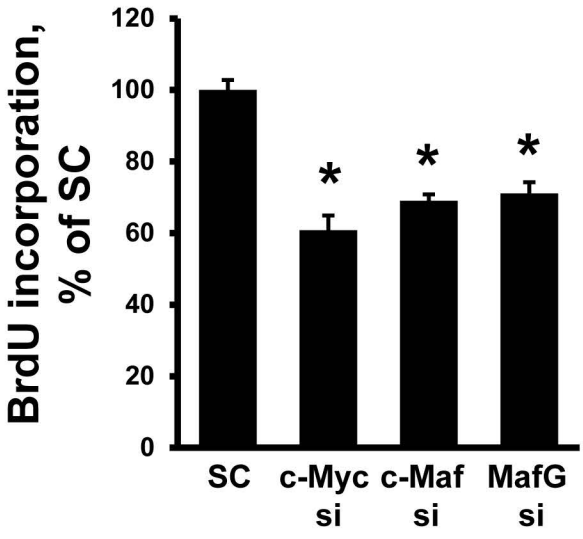


Supplemental Figure 6





Supplemental Figure 7