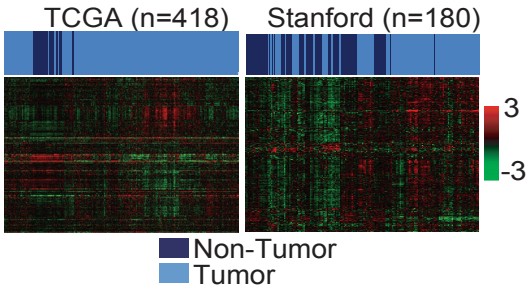
A



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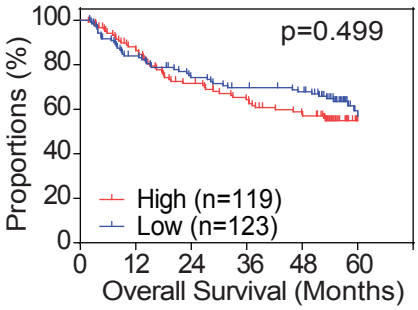
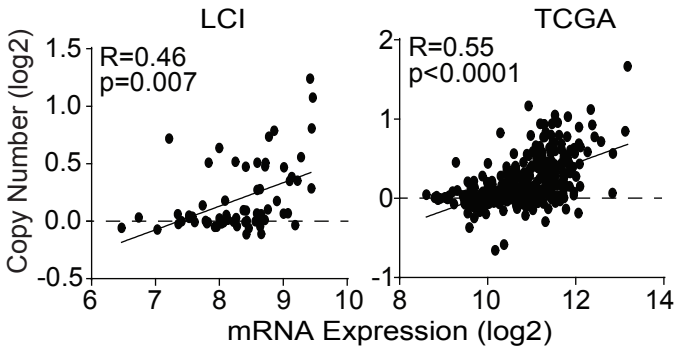


C

Set	mRNA	CNV	R	FDR
LCI	2.5	28.1%	0.46	0.007
TCGA	2.4	26.8%	0.55	<0.0001

D



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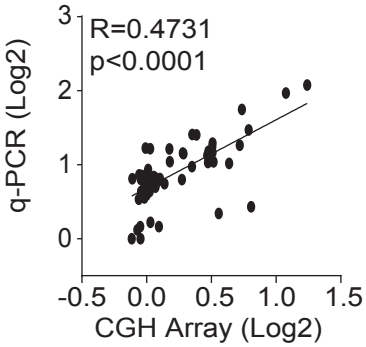
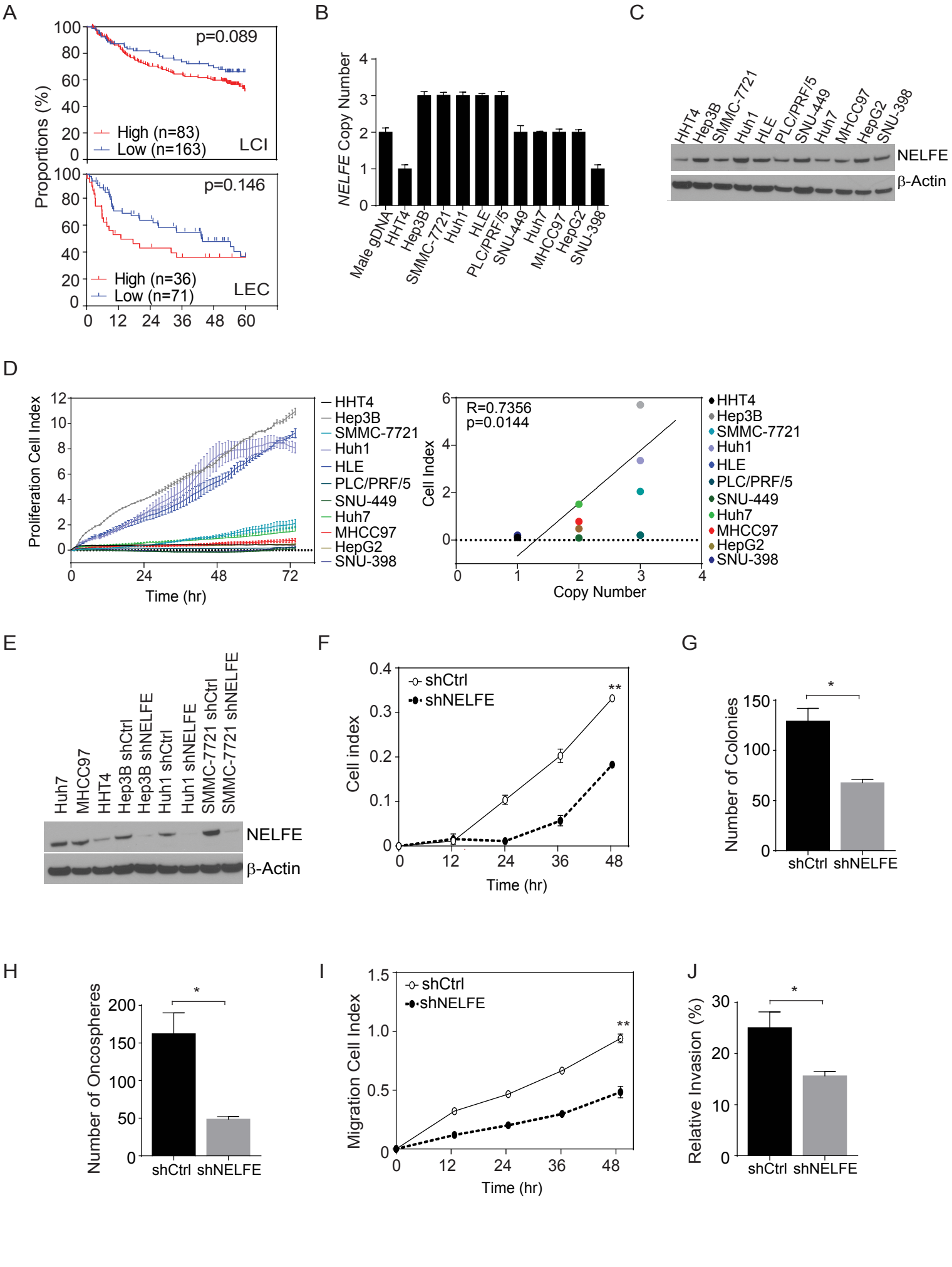
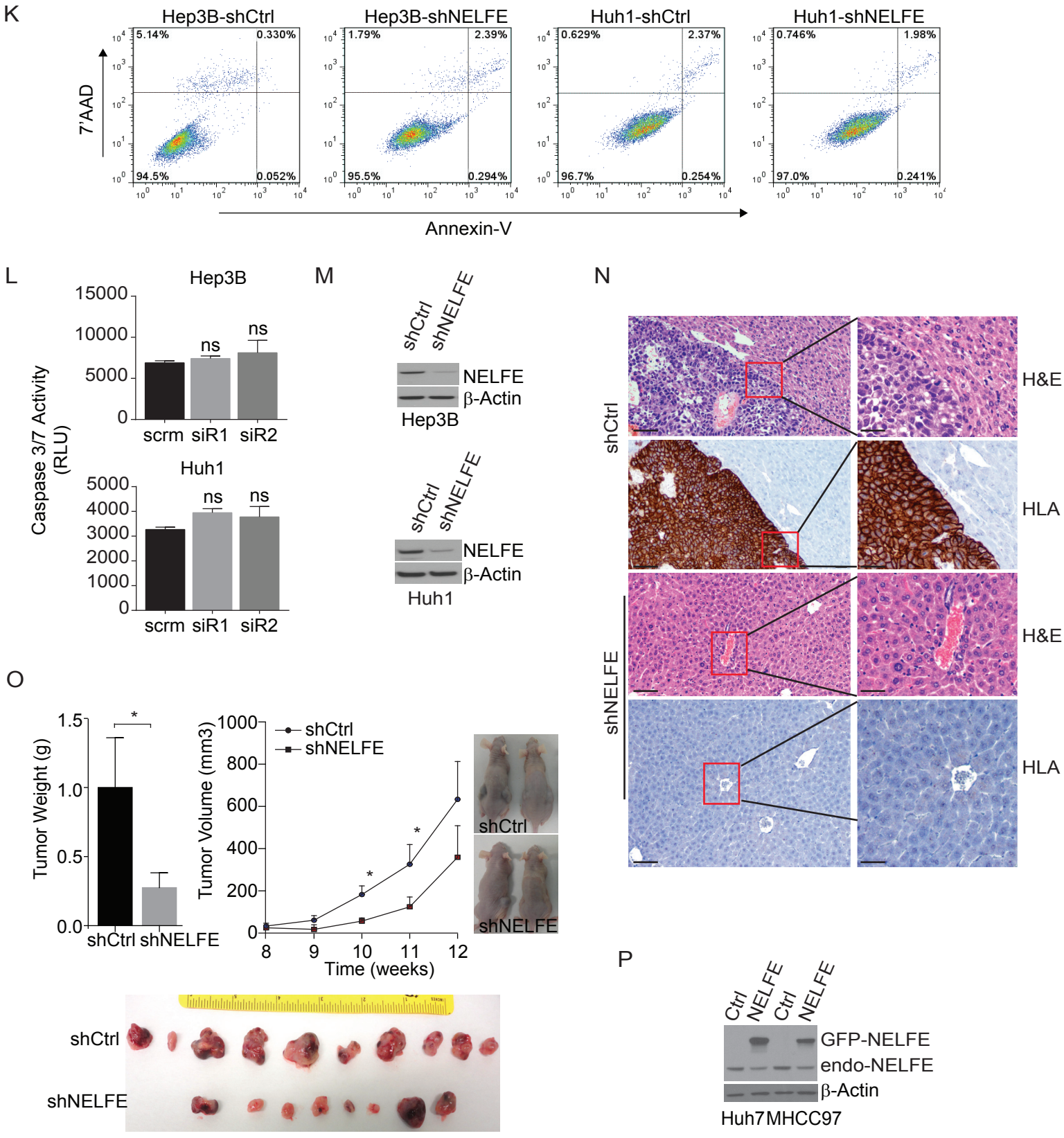
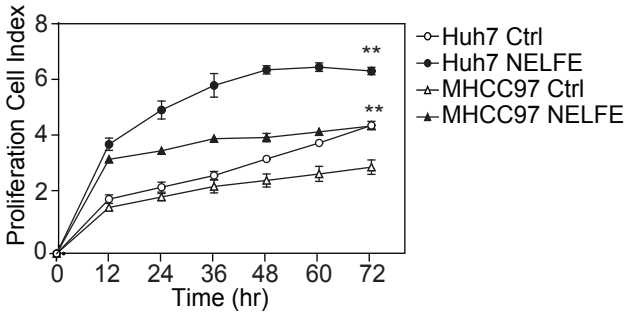


Figure S1, related to Figure 1. RBPs are ubiquitously altered in HCC. (A) Heatmap of the TCGA-LIHC and Stanford dataset from hierarchal clustering analysis of the 474 RBPs that are tumor specific. Pearson correlation with complete linkage was performed. (B) Kaplan-Meier curves of LCI cohort based on predictive survival analysis using the 629 transcription factors that were differentially expressed between tumor and non-tumor (p values are presented from Cox Log rank test). (C) Summary of NELFE expression, percentage of samples with amplification (calculated based on segment log2 values>0.32), Pearson correlation coefficient (R value) and FDR in two different datasets; LCI and TCGA. (D) Correlation of NELFE copy number from the LCI aCGH and microarray data from 64 HCC samples and TCGA-LIHC (SNP array compared to RNASeqV2, n=366). Pearson correlation (R) value and p values are depicted. (E) Correlation of NELFE copy number from aCGH and real-time polymerase chain reaction (RT-PCR) data from 64 HCC samples normalized to male gDNA. Pearson correlation (R) value and p values are depicted.

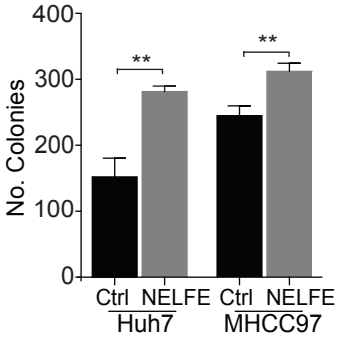

A
p=0.089
High (n=83)
Low (n=163)
LCI
p=0.146
High (n=36)
Low (n=71)
LEC
Proportions (%)
B
NELFE Copy Number
Male gDNA
HHT4
Hep3B
SMMC-7721
Huh1
HLE
PLC/PRF/5
SNU-449
Huh7
MHCC97
HepG2
SNU-398
C
NELFE
β-Actin
D
Proliferation Cell Index
Time (hr)
R=0.7356
p=0.0144
Cell Index
Copy Number
E
Huh7
MHCC97
HHT4
Hep3B shCtrl
Hep3B shNELFE
Huh1 shCtrl
Huh1 shNELFE
SMMC-7721 shCtrl
SMMC-7721 shNELFE
NELFE
β-Actin
F
shCtrl
shNELFE
Cell index
Time (hr)
G
Number of Colonies
shCtrl
shNELFE
H
Number of Oncospheres
shCtrl
shNELFE
I
shCtrl
shNELFE
Migration Cell Index
Time (hr)
J
Relative Invasion (%)
shCtrl
shNELFE


K
Hep3B-shCtrl
Hep3B-shNELFE
Huh1-shCtrl
Huh1-shNELFE
5.14%
0.330%
94.5%
0.052%
1.79%
2.39%
95.5%
0.294%
0.629%
2.37%
96.7%
0.254%
0.746%
1.98%
97.0%
0.241%
7'AAD
Annexin-V
L
Hep3B
Caspase 3/7 Activity (RLU)
15000
10000
5000
0
ns
ns
scrm siR1 siR2
Huh1
5000
4000
3000
2000
1000
0
ns
ns
scrm siR1 siR2
M
shCtrl shNELFE
NELFE
β-Actin
Hep3B
shCtrl shNELFE
NELFE
β-Actin
Huh1
N
shCtrl
shNELFE
H&E
HLA
H&E
HLA
O
Tumor Weight (g)
1.5
1.0
0.5
0.0
*
shCtrl shNELFE
Tumor Volume (mm3)
1000
800
600
400
200
0
shCtrl
shNELFE
*
*
8 9 10 11 12
Time (weeks)
shCtrl
shNELFE
shCtrl
shNELFE
P
Ctrl NELFE Ctrl NELFE
GFP-NELFE
endo-NELFE
β-Actin
Huh7 MHCC97

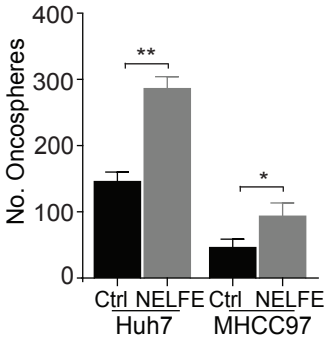
Q


Proliferation Cell Index
Time (hr)
Huh7 Ctrl
Huh7 NELFE
MHCC97 Ctrl
MHCC97 NELFE
**
**

R


No. Colonies
Ctrl
NELFE
Ctrl
NELFE
Huh7
MHCC97
**
**

S


No. Oncospheres
Ctrl
NELFE
Ctrl
NELFE
Huh7
MHCC97
**
*

T

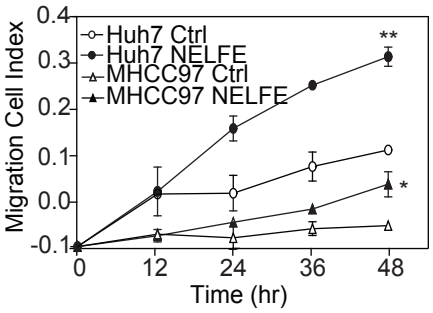
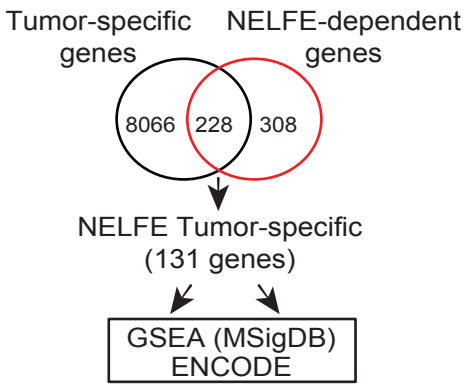

Migration Cell Index
Time (hr)
Huh7 Ctrl
Huh7 NELFE
MHCC97 Ctrl
MHCC97 NELFE
**
*

Figure S2, related to Figure 2. Alterations of NELFE in HCC cells. (A) Kaplan-Meier survival analysis of LCI and LEC cohort based on top 2/3 compared to bottom 1/3 values of NELFE (p values are presented from Cox Log rank test). (B) RT-PCR of male genomic DNA, eleven HCC cell lines, including the immortalized HHT4 normal hepatocyte cell line for NELFE gene copy number. (C) Immunoblot of NELFE and β–Actin of HCC cell lines and the hepatocyte HHT4 cell line. (D) Cell proliferation analysis of 11 cell lines via xCELLigence up to 72 hr. Correlation (Spearman rank test) plot of copy number as determined in compared to the cell index at 72 hr via xCELLigence. (E) Representative blot of HCC cells after 72 hr of NELFE shRNA and NELFE levels of HHT4, Huh7 and MHCC97 cells. (F) Cell proliferation analysis as measured by xCELLigence of SMMC-7721 cells with shCtrl or shNELFE knockdown after 48 hr (**p< 0.01). (G) Colony formation analysis after 10 days (**p< 0.01). (H) Oncosphere formation via Algimatrix after 10 days. $1x10^5$ cells were plated for 10 days followed by crystal violent staining and manual counting (**p<0.01). (I-J) Cell migration and relative invasion analysis as measured by xCelligence after 48 hr (*p<0.01). (K) Flow cytometry analysis of Hep3B and Huh1 cells after 48 hr of NELFE siRNA compared to scrm control. Cells were stained with 7'AAD and Annexin-V. (L) Caspase 3/7 activity assay after 48 hr of siRNA mediated knockdown of NELFE compared to scrambled (scrm). (M) Immunoblot of pLKO.1 shNELFE or shCtrl Huh1 or Hep3B cells before injected into animals. (N) Representative microscopic images of mice livers after eight weeks of injection. H&E staining and immunohistochemistry of HLA-A, specific for human Huh1 HCC cells are shown. Scale bar, 100 μM. (O) Hep3B cells were transfected with pLKO.1 shNELFE or shCtrl followed by 7 days of puromycin selection. Five million cells were subcutaneously injected bilaterally into the flanks of athymic nude mice. After 12 weeks, mice were sacrificed and all tumors formed are shown on the bottom panel. Tumor weight (right panel) after 12 weeks. Tumor volume (left panel) was measured using caliper weekly (*p<0.05) (shCtrl=10, shNELFE=8). (P) Immunoblot of NELFE in Huh7 and MHCC97 cells that have been stably transfected with lenti-mGFP-NELFE ORF (NELFE) or lenti-mGFP ORF (Ctrl). (Q) Cell proliferation as measured by xCELLigence after 72 hr (**p<0.01). (R) Colony formation of HCC cells (**p<0.01). (S) AlgiMatrixTM 3D Culture System was used for the oncospheroid formation assay for tumorigenic abilities of NELFE overexpression in HCC cells (*p<0.05, **p<0.01). (T) Cell migration assay determined by xCELLigence after 48 hr (*p<0.05, **p<0.01). All data are mean ± SD.

A



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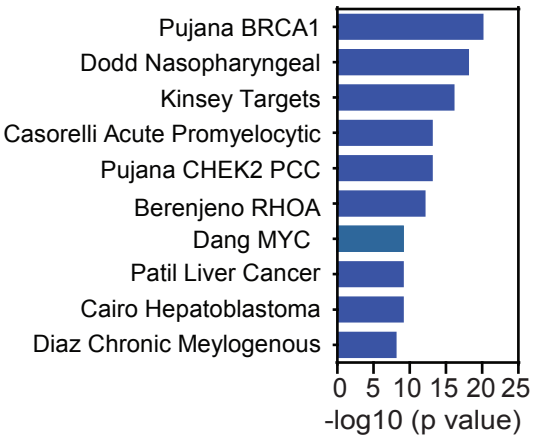
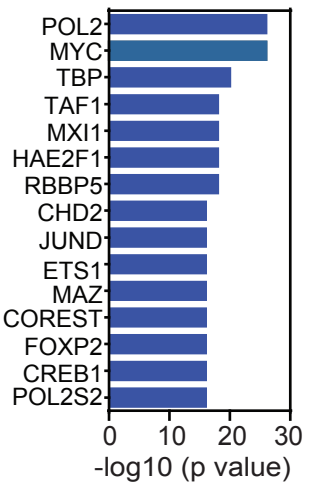
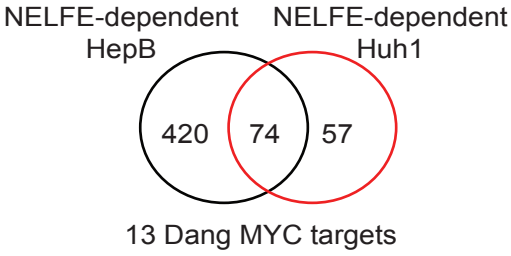


C



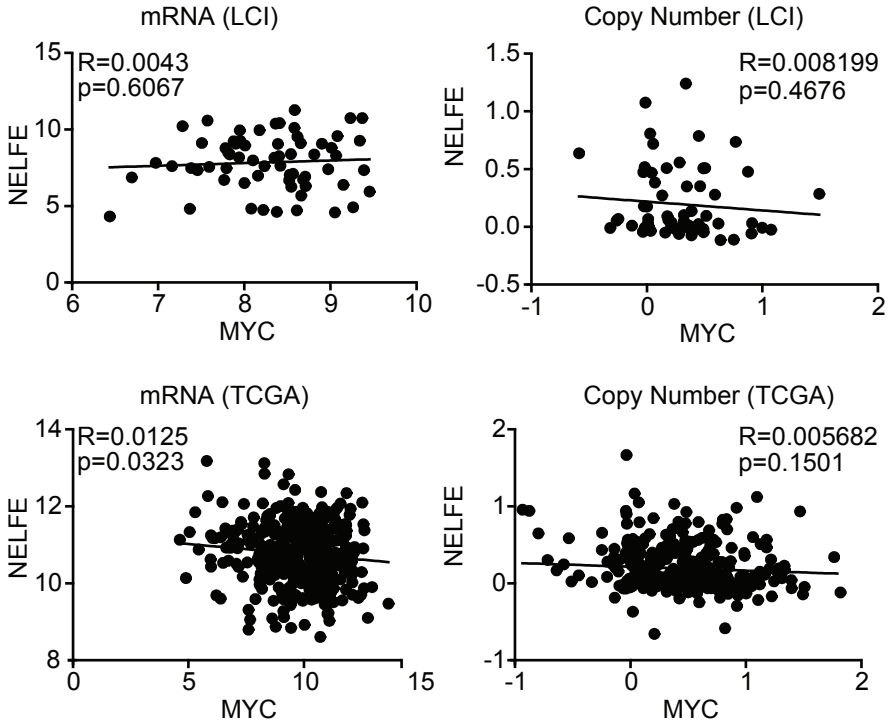
D



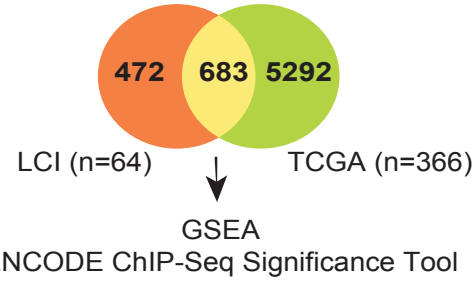
E

Gene	LCI	TCGA
Both	8/64(12.5%)	55/366(15%)
MYC	21/64(32.8%)	140/366(38%)
NELFE	11/64(17%)	39/366(10.8%)

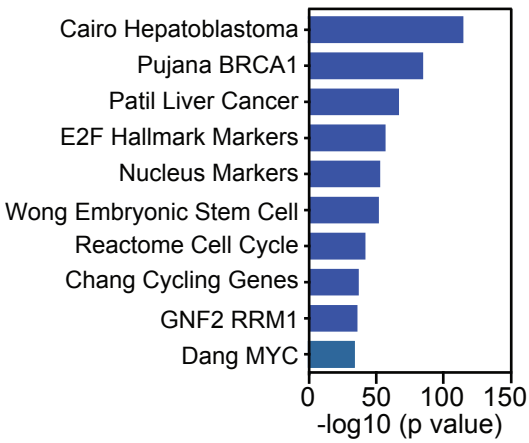
F



G



H



I

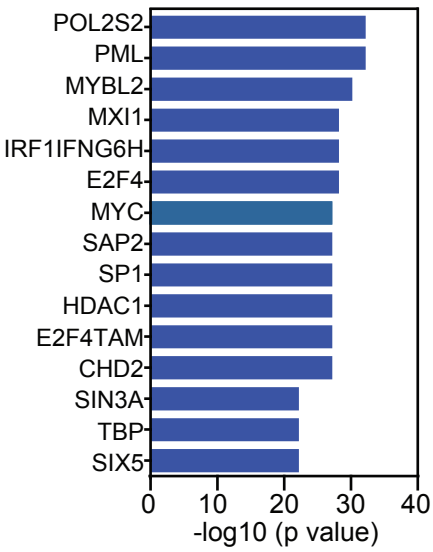


Figure S3, related to Figure 3. NELFE-dependent genes enhance MYC related genes. (A) Schematic of overview of microarray analysis using the Affymetrix HTA 2.0 genechip. The overlap of tumor specific genes from HCC clinical samples were compared to Huh1 NELFE siRNA treated cells. 131 genes were high in tumors but low after NELFE knockdown and vice versa were used for downstream analysis. (B) NELFE-dependent genes were enriched in MYC related genes and other HCC cancer specific signatures via GSEA analysis. (C) ENCODE analysis of 131 NELFE-dependent genes showing only the top 15 most enriched proteins including MYC. 90/131 genes are MYC related genes within 500 bp from the transcription start site. (D) 74 NELFE-dependent tumor-specific genes overlapped Hep3B and Huh1 cells, of which, 13 genes are also MYC target genes found in the Dang MYC target gene list. (E) Summary analysis of NELFE, MYC, or both NELFE/MYC gene copy alterations in HCC samples in the LIHC and TCGA-LIHC dataset. (F) Correlation analysis between NELFE and MYC mRNA levels and gene copy number alterations in the LCI and TCGA-LIHC dataset. R values are calculated using Pearson correlation analysis of log 2 values. (G) Schematic of overview of microarray analysis using the Affymetrix HTA 2.0 genechip looking at LCI cohort and TCGA-LIHC cohort. (H) GSEA analysis of 683 NELFE-dependent genes showing only the top 10 most enriched genesets. (I) ENCODE analysis of 683 NELFE-dependent genes showing only the top 15 most enriched proteins including MYC. Genes are MYC related genes within 500 bp from the transcription start site.

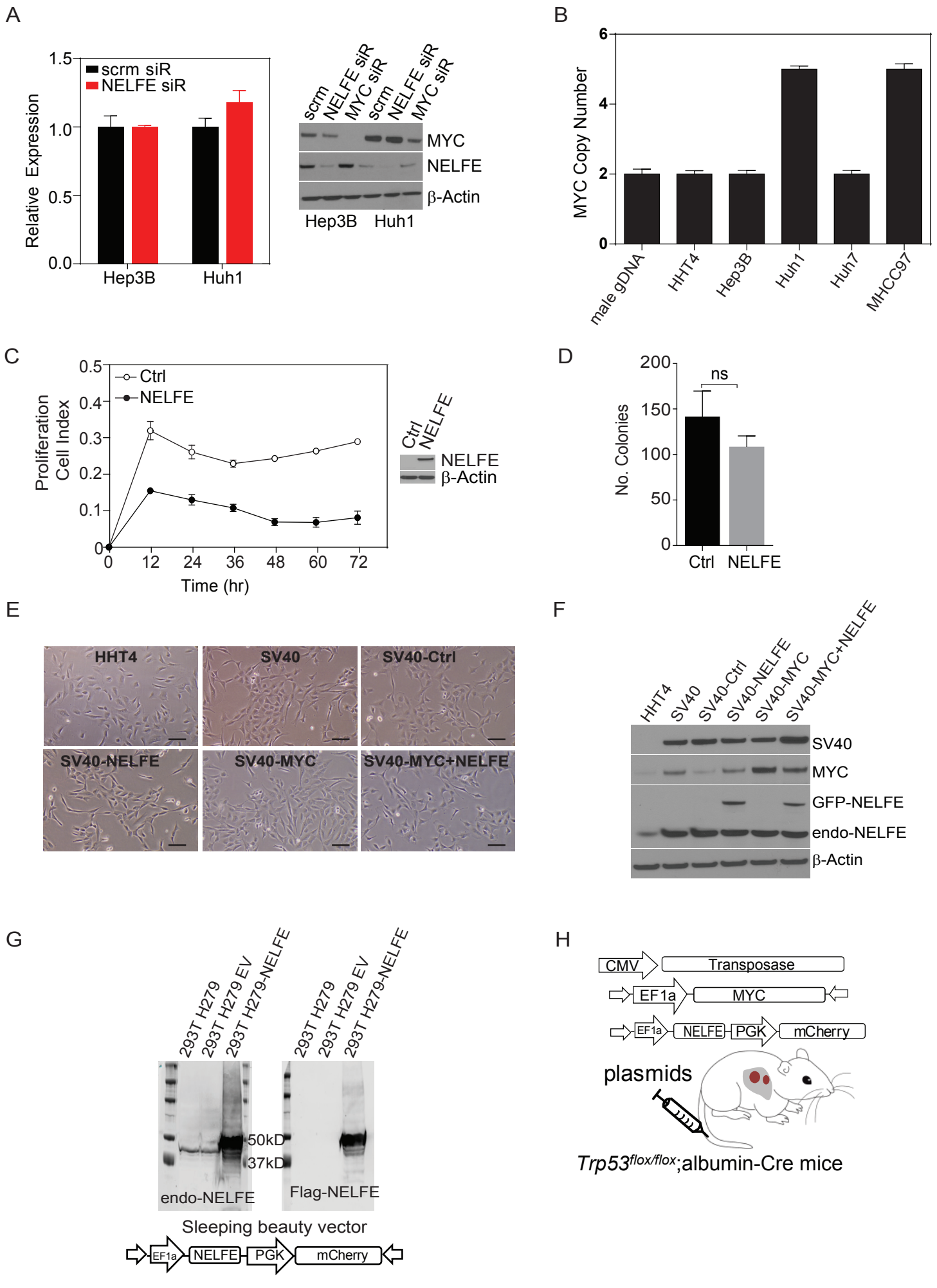
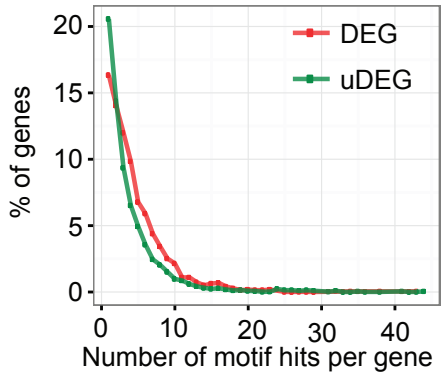

A
scrm siR
NELFE siR
Relative Expression
1.5
1.0
0.5
0.0
Hep3B
Huh1
scrm
NELFE siR
MYC siR
scrm
NELFE siR
MYC siR
MYC
NELFE
β-Actin
Hep3B
Huh1
B
MYC Copy Number
6
4
2
0
male gDNA
HHT4
Hep3B
Huh1
Huh7
MHCC97
C
Ctrl
NELFE
Proliferation Cell Index
0.5
0.4
0.3
0.2
0.1
0
0
12
24
36
48
60
72
Time (hr)
Ctrl
NELFE
NELFE
β-Actin
D
No. Colonies
200
150
100
50
0
ns
Ctrl
NELFE
E
HHT4
SV40
SV40-Ctrl
SV40-NELFE
SV40-MYC
SV40-MYC+NELFE
F
HHT4
SV40
SV40-Ctrl
SV40-NELFE
SV40-MYC
SV40-MYC+NELFE
SV40
MYC
GFP-NELFE
endo-NELFE
β-Actin
G
293T H279
293T H279 EV
293T H279-NELFE
293T H279
293T H279 EV
293T H279-NELFE
50kD
37kD
endo-NELFE
Flag-NELFE
Sleeping beauty vector
EF1a
NELFE
PGK
mCherry
H
CMV
Transposase
EF1a
MYC
EF1a
NELFE
PGK
mCherry
plasmids
Trp53flox/flox;albumin-Cre mice

Figure S4, related to Figure 4. NELFE enhances MYC tumorigenic capabilities. (A) RT-PCR (left panel) and representative immunoblot (right panel) of MYC after 48 hr of NELFE siRNA compared to scrambled (scrm) control in HCC. (B) RT-PCR of MYC copy number in HCC cells compared to male gDNA. (C) Cell proliferation as measured by xCELLigence up to 72 hr (left panel) of HHT4 cells overexpressed with NELFE. Immunoblot of NELFE in HHT4 cells transfected with lenti-mGFP-NELFE ORF (NELFE) or lenti-mGFP ORF (Ctrl) (right panel). (D) Colony formation analysis after 10 days. (E) Phase contrast images of HHT4 transduced with SV40, SV40-Ctrl, SV40-NELFE, SV40-MYC, or SV40-MYC+NELFE. Scale bar, 200 μM. (F) Immunoblot of NELFE in HHT4 cells transduced as specified. Representative Immunoblot of SV40, MYC, NELFE and β–Actin (endo-NELFE represents endogenous levels of NELFE). (G) Immunoblot of NELFE for confirmation of Sleeping beauty vectors with MYC or NELFE in HEK293T cells after transduction. (H) Cartoon of plasmid DNA which were tail-vein injected into 8 weeks old mice. All data are mean ± SD.

A

	hit	nohit
DEG	1839	346
uDEG	13746	5914

B



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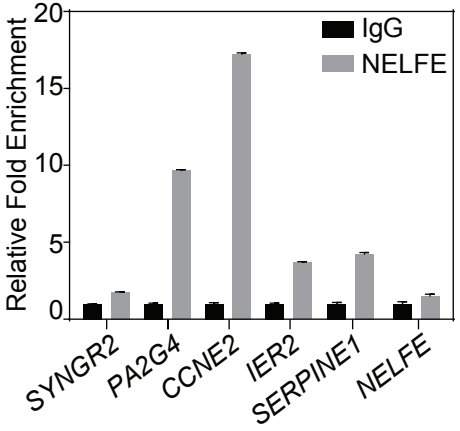

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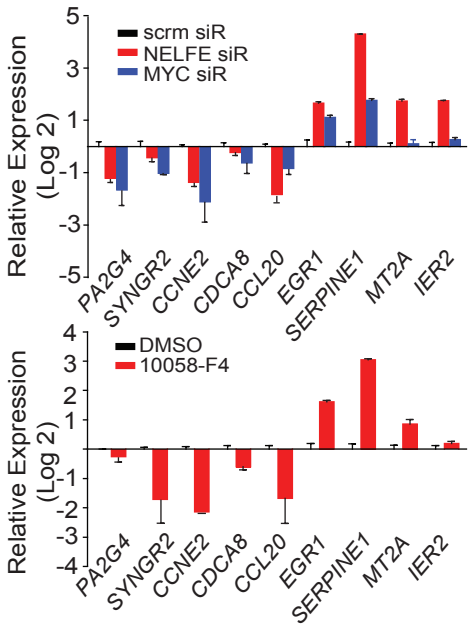


E

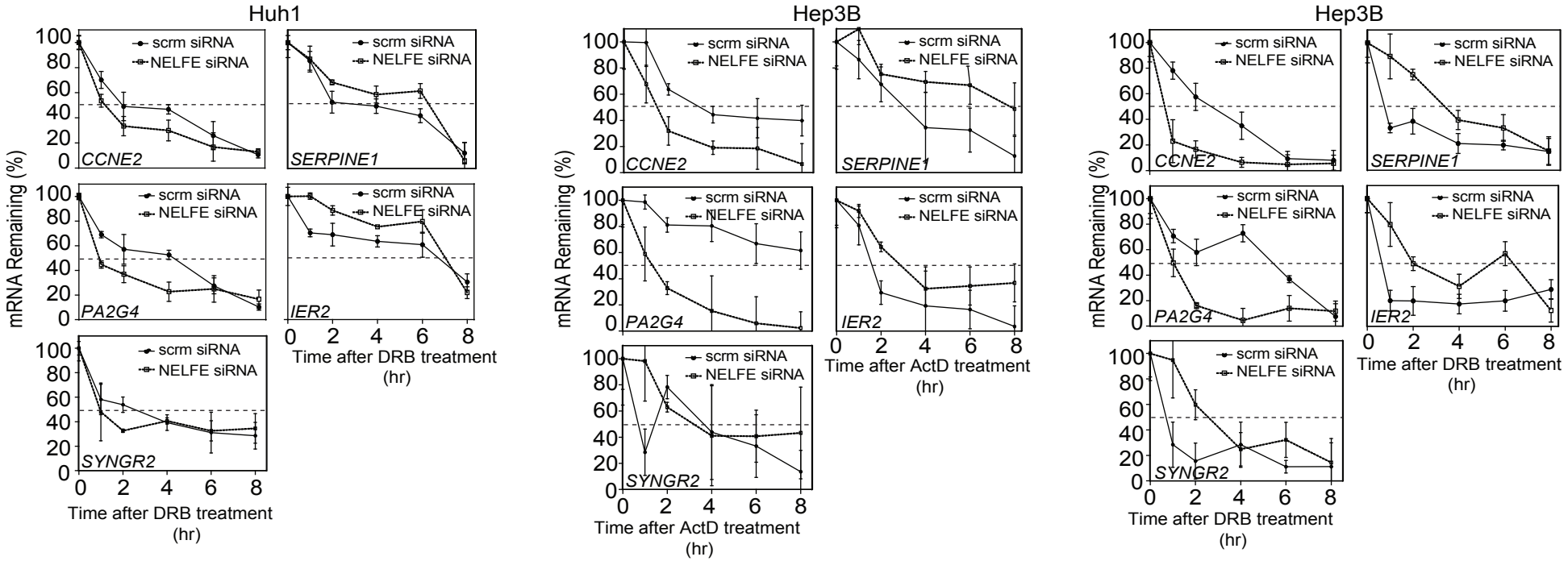
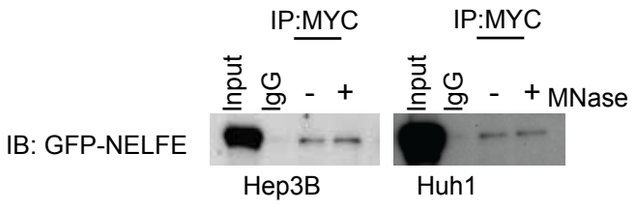


Figure S5, related to Figure 5. NELFE preferentially interacts with MYC related genes. (A) RNACompete analysis of differentially expressed genes (DEGs) and undifferentially expressed genes (uDEGs), which were determined previously by microarray analysis of Hep3B NELFE siRNA treatment vs. scrambled control. 2X2 table used to calculate Fishers exact test were performed to calculate p value. (B) The distribution of the number of motif hits per gene in DEGs or uDEGs. (C) Hep3B RNA immunoprecipitation followed by RT-PCR analysis of MYC related genes and NELFE predicted targets (*CCL20, PA2G4, CCNE2, IER2, SERPINE1, SYNGR2*). (D) Real-time PCR analysis (RT-PCR) of Huh1 cells after 72 hr of siRNA mediated knockdown of NELFE, MYC, or scramble control (scrm). Student's t-test was performed where treatments were compared to scrambled control (top panel). RT-PCR analysis of Huh1 cells after 16 hr of 10058-F4 (200 μM) treatment compared to DMSO (bottom panel). Student's t-test was performed where treatments were compared to scrambled control (scrm). (E) RT-PCR of MYC related genes after NELFE siRNA compared to scrambled siRNA (scrm) followed by Actinomycin D treatment (10 μg/ml) or DRB (50 μM) treatment at different time points (0, 1, 2, 4, 6, 8 hr) in Huh1 and Hep3B cells. Dotted lines are at 50% mRNA remaining. All data are mean ± SD.

A


IP:MYC
IP:MYC
Input
IgG
-
+
Input
IgG
-
+
MNase
IB: GFP-NELFE
Hep3B
Huh1

B

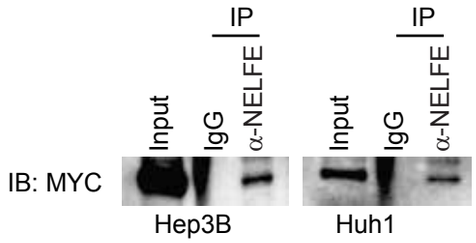

IP
IP
Input
IgG
α-NELFE
Input
IgG
α-NELFE
IB: MYC
Hep3B
Huh1

Figure S6, related to Figure 6. (A) Represented immunoblot of co-IP assay in Hep3B-mGFP-NELFE cells and Huh1-mGFP-NELFE cells with and without micrococcal nuclease treatment (MNase). MYC or Rabbit IgG was used for IP and mouse anti-NELFE was used for western blot. (B) Co-IP assay in Hep3B or Huh1 cells with NELFE or Mouse IgG to detect endogenous NELFE/MYC interaction. Rabbit anti-MYC was used for immunoblotting.