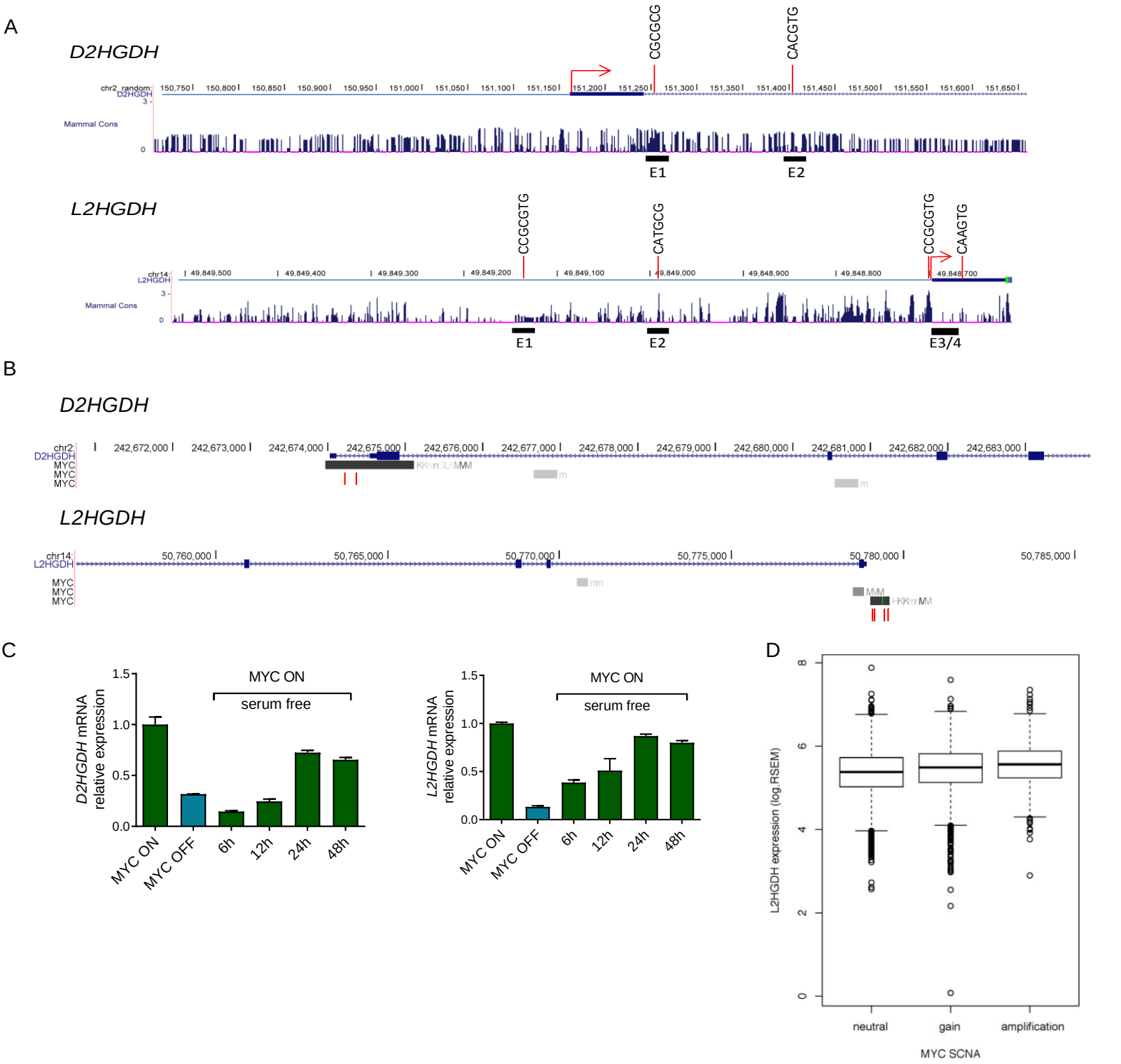


Figure S1, related to Figure 1. Characterization of *D2HGDH* and *L2HGDH* promoter regions in mammals. **A.** Conservation analysis using PhyloP data, a basewise measure of evolutionary conservation, from the comparative genomics function of the UCSC genomic browser is shown for the *D2HGDH* promoter (top panel, 50 bp intervals) and for the *L2HGDH* promoter region (bottom panel, 100bp intervals). Red arrows are TSSs; vertical red bars indicate the location of the putative E box with their sequence annotated; horizontal black bars – span of ChIP-PCR. E2 in D2HGDH, and the E3/4 area in L2HGDH were found to bind to MYC by ChIP-PCR. E2 in D2HGDH and E4 in L2HGDH are both canonical CANNTG sequences. **B.** MYC ChIP-seq signal from ENCODE as displayed in the UCSC genome browser. The gray boxes under the genes tracks (D2HGDH – top; L2HGDH – bottom) are peak clusters of transcription factor occupancy, with the darkness of the box being proportional to the maximum signal strength observed in cell lines in the cluster. Within a cluster, a green highlight indicates the highest scoring site of a Factorbook-identified canonical motif for the corresponding transcription factor. The red vertical lines represent the location of the E-boxes that we tested with ChIP-PCR. Letters represent different cell lines: K=K562, M=MCF-7, L=HepG2, G=GM12878, H=HeLa-S3; m=MCF10A-Er-Src; n=NB4. **C.** *D2HGDH* (left) and *L2HGDH* (right) mRNA expression in MYC-regulated P493-6 cells grown in serum free conditions; q-RT-PCR data are mean ± SD of samples analyzed in triplicate. **D.** Correlation between and MYC copy number across the TCGA pan-cancer sample set. The somatic copy number alterations (SCNA) of the MYC locus were defined in 10,713 human tumors, and discretized into neutral, gain (low level), and amplification (high level) using the GISTIC2.0 algorithm. Correlation with *L2HGDH* mRNA expression was examined with the Spearman test. A significant positive correlation ($p<2.2e-16$) between MYC amplification and *L2HGDH* expression was found.

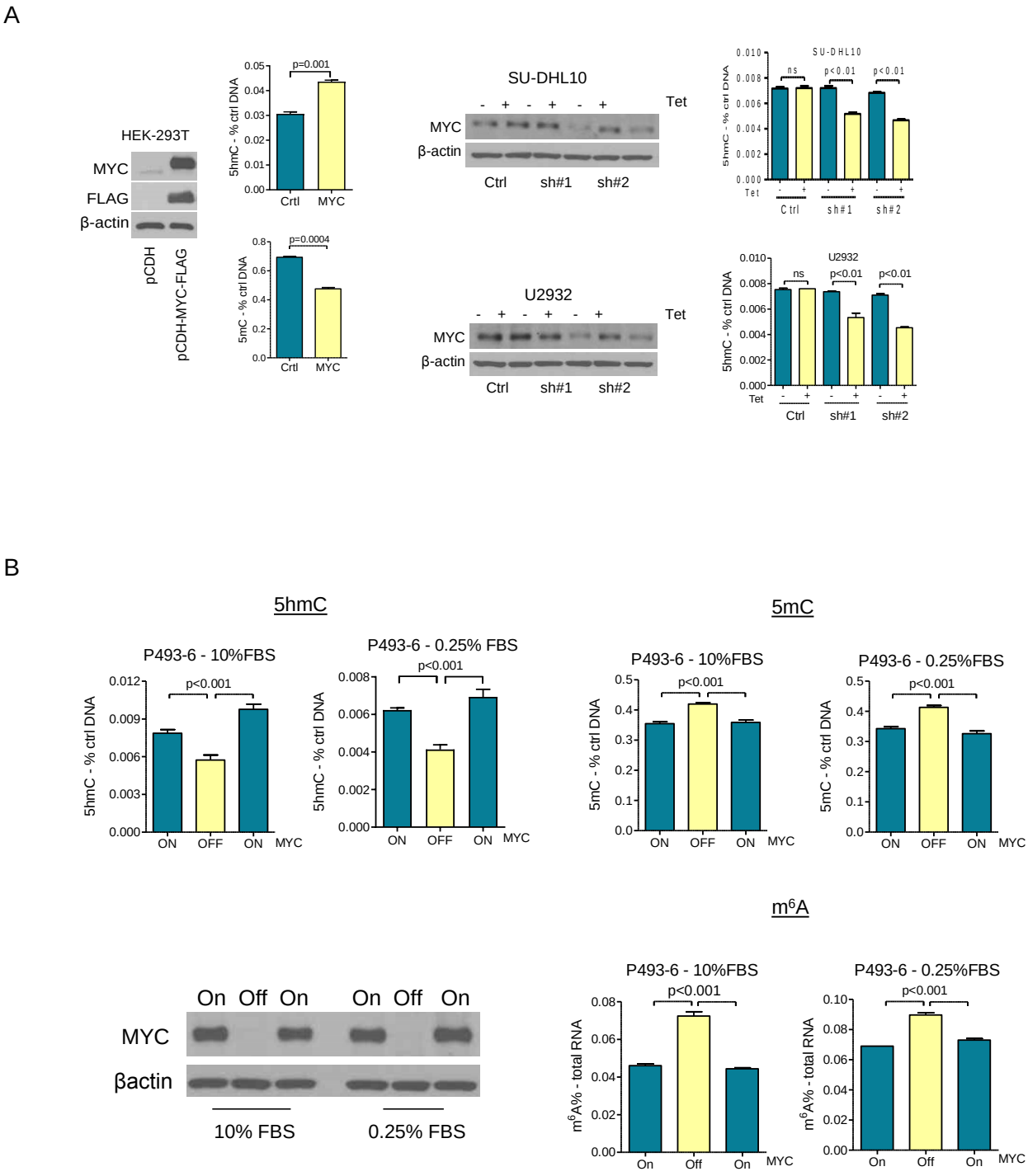


Figure S2, related to Figure 2. MYC expression and modulation of DNA and RNA methylation. A. Left panels. Immunoblot of HEK-293T cells stably expressing a FLAG-tagged MYC construct. Quantification of 5hmC and 5mC in this genetically modified cell model. Right panels. MYC immunoblots in the DLBCL cell lines SU-DHL10 and U2932 stably expressing inducible shRNA sequences directed at MYC. Quantification of 5hmC in these cell models is shown on the bar-graphs to the right. Control cells express the empty Tet-pLKO-puromycin plasmid; Tet = tetracycline exposure for 48h. All data shown are mean -/+ SD of assays performed in triplicate. P value is from two-tailed Student's t-test. **B.** Clockwise from the top left: Quantification of 5hmC, 5mC and m^6A levels in MYC inducible P493-6 cells grown in fetal bovine serum replete (10%) or deprived (0.25%) conditions (left and right panels, respectively). MYC immunoblot is shown on the bottom left. MYC suppression (OFF) was achieved by exposure to tetracycline for 36h. MYC re-expression (ON) All data shown are mean -/+ SD of assays performed in triplicate. P value is from two-tailed Student's t-test.

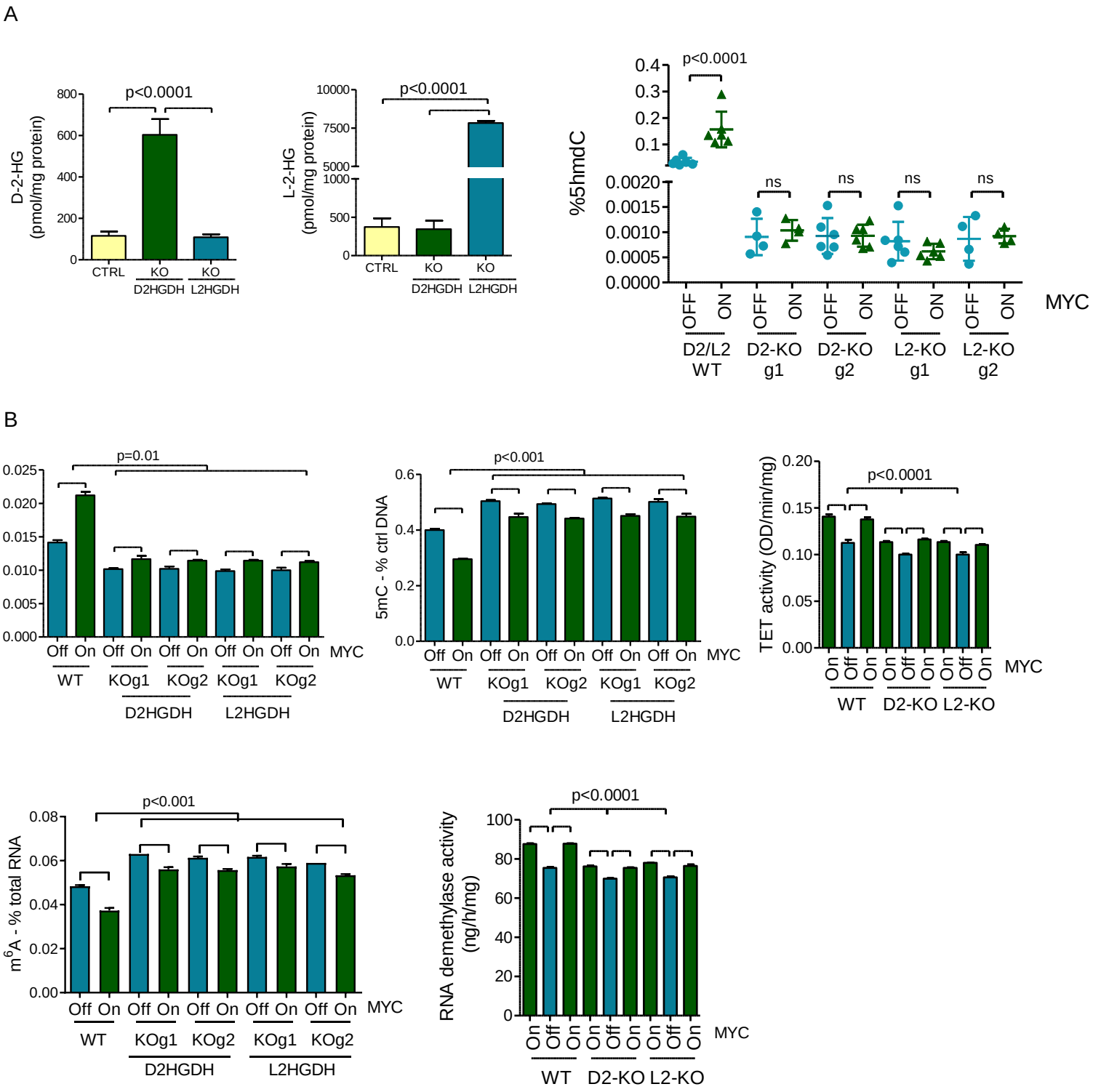


Figure S3, related to Figure 3. Effects of D2HGDH and L2HGDH KO on 2-HG levels and on DNA/RNA methylation and demethylases activity. A. Left and center graphs. Quantification of D-2-HG and L-2-HG by LC-MS/MS in P493-6 cells WT for D2HGDH and L2HGDH, or with KO of each gene. Data are mean -/+ SD of measurements completed in triplicate. Right graph. Quantification of 5hmC by mass-spectrometry in WT and D2HGDH or L2HGDH KO P493-6 cells, with or without MYC expression. Data are mean -/+ SD of two independent biological replicates each measured with 2 or 3 technical replicates; g1 and g2 represent two different guideRNAs for each targeted gene. Statistical significance determined with two-tailed Student's t-test. **B.** Top panel, left to right. Quantification of 5hmC, 5mC and TET activity in WT and D2HGDH or L2HGDH KO P493-6 cells upon modulation of MYC expression (MYC OFF-ON)**.** Bottom panels, left to right**.** Quantification of m^6A levels and RNA demethylase activity in WT and D2HGDH or L2HGDH KO P493-6 cells upon modulation of MYC expression (MYC OFF-ON). Data are mean -/+ SD of measurements in triplicate; g1 and g2 represent two different guideRNAs for each targeted gene. Statistical significance was tested with ANOVA and Bonferroni post-test.

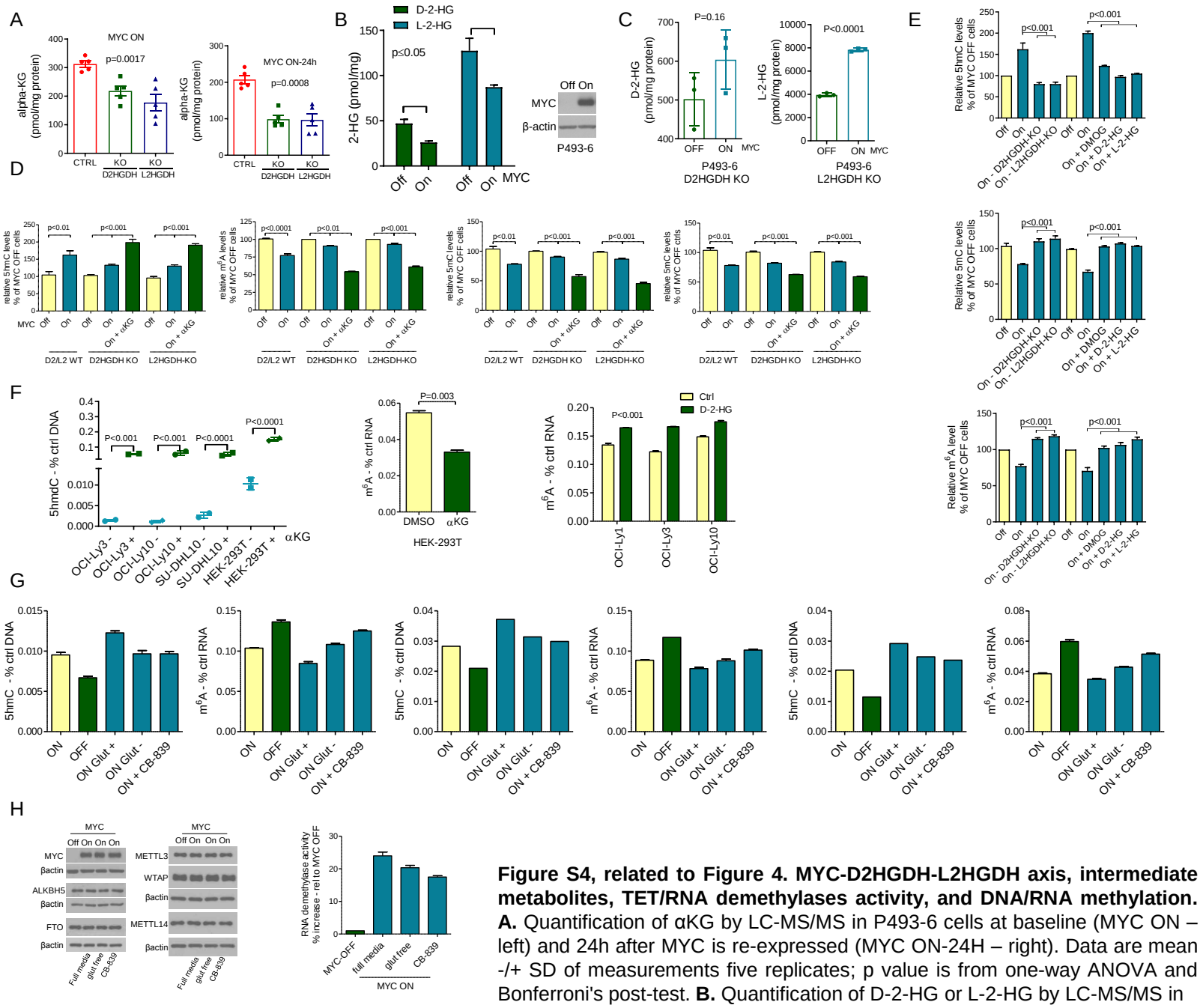


Figure S4, related to Figure 4. MYC-D2HGDH-L2HGDH axis, intermediate metabolites, TET/RNA demethylases activity, and DNA/RNA methylation. A. Quantification of αKG by LC-MS/MS in P493-6 cells at baseline (MYC ON – left) and 24h after MYC is re-expressed (MYC ON-24H – right). Data are mean -/+ SD of measurements five replicates; p value is from one-way ANOVA and Bonferroni's post-test. **B.** Quantification of D-2-HG or L-2-HG by LC-MS/MS in P493-6 cells with MYC expression abolished (OFF) or following its re-expression (ON). Data are mean -/+ SD of measurements completed in duplicate. MYC WB is also shown. P value is from a two-side Student's t-test. **C.** Quantification of D-2-HG and L-2-HG by LC-MS/MS in P493-6 cells with MYC expression abolished (OFF) or following its re-expression (ON) in D2HGDH KO cells (left) and L2HGDH KO cells (right). Data are mean -/+ SD of measurements completed in biological triplicates. P value is from a two-side Student's t-test. **D.** Quantification of 5hmC, m^6A and 5mC, in a MYC inducible cell model with WT or KO of D2HGDH and L2HGDH, with or without exposure to DMαKG (5mM). The 5hmC and m^6A data (left to right panels) are additional biological replicates to the assays shown in Figure 4B – main text; 5mC data, two independent assays, are shown in the two right most panels. Data are mean -/+ SD of triplicate measurements displayed as relative abundance (normalized to MYC OFF cells). P value is from one-way ANOVA and Bonferroni's post-test. **E.** Top to bottom **-** Quantification of 5hmC, 5mC and m^6A in a MYC inducible cell model with WT or KO of D2HGDH and L2HGDH, or in a MYC inducible cell model exposed to DMOG (1mM), octyl-D-2-HG or L-2-HG (100μM). Data are mean -/+ SD of triplicate measurements displayed as relative abundance (normalized to MYC OFF cells); p value is from one-way ANOVA and Bonferroni's post-test. **F.** Left to right - Quantification of 5hmC (mass spectrometry) and m^6A (antibody-based) in DLBCL cell lines and HEK-293T following exposure to DMαKG (5mM) or octyl-D-2-HG (100μM). Data are mean -/+ SD of duplicate or triplicate measurements and parallels the effects of these metabolites in the activity of TET or RNA demethylases (Figure 4D, main text). P value is from ANOVA with Bonferroni post-test, or two-tailed Student's t-test. **G.** Quantification of 5hmC (left panels) and m^6A (right panels) following induction of MYC expression in cells grown in full media, in glutamine free media or in the presence of CB-839 (1μM). Data shown are mean -/+ SD – each bar-graph is a independent biological replicate, which combined represent the data shown in Figure 4E – main text. **H.** WB analysis of RNA demethylases and components of the RNA methyltransferase complex in the MYC-inducible P493-6 cells grown in full media, glutamine free media or in the presence of the glutamynolisis inhibitor CB-839 (1μM). A MYC WB is also shown (same display as in Figure 4E, main text). Right panel: Quantification of RNA demethylase activity in MYC OFF and MYC ON P493-6 cells grown in full media, glutamine free media or in the presence of CB-839. Data are mean -/+ SD of triplicate measurements, displayed as % increase in activity relative to MYC OFF.

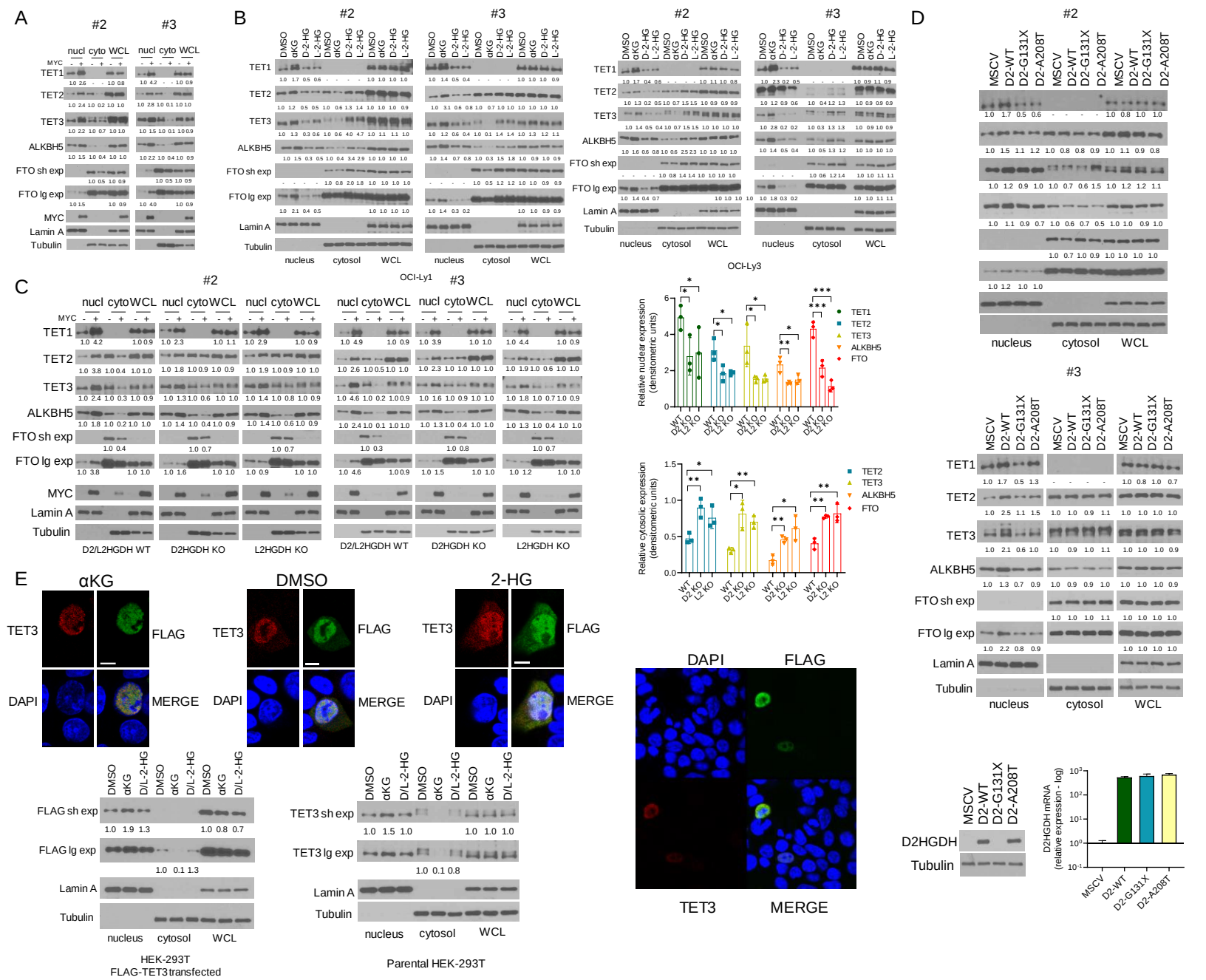
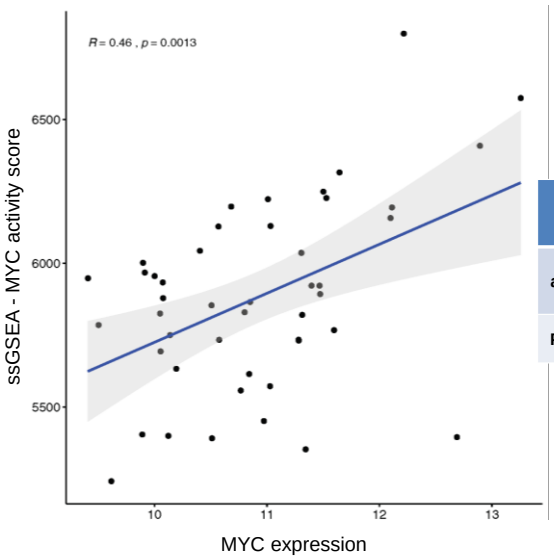


Figure S5, related to Figure 5. Effects of MYC on TET-1-3, FTO and ALKBH5 subcellular localization. A. WB analysis of nuclear (5µg) and cytoplasmic (15µg) fractions and whole cell lysate (WCL; 15µg) of P493-6 cells upon MYC suppression and its re-expression – replicates #2 and # 3 are shown. Densitometry of protein levels are shown at the bottom of the WB displays – all values are relative to controls (MYC-off, DMSO or MSCV) and corrected by WCL level, which is shown for reference. **B.** WB analysis of TET-1-3, FTO and ALKBH5 in the subcellular fractions and WCL of the DLBCL cell lines OCI-Ly1 (top) and OCI-Ly3 (bottom) exposed to dimethyl-αKG (5mM), octyl-D-2-HG or octyl-L-2-HG (100µM) for 8h - replicates #2 and # 3 are shown. Densitometry of protein levels are shown at the bottom of the WB displays – all values are relative to controls (MYC-off, DMSO or MSCV) and corrected by WCL level, which is shown for reference. **C.** Left: WB analysis of TET1-3, FTO and ALKBH5 in subcellular fractions and WCL of P493-6 cells expressing D2HGDH and L2HGDH, or with KO of either gene, upon MYC suppression and its re-expression - replicates #2 and # 3 are shown. Right: Graphic display and statistical analyses of the densitometrical quantification of MYC-induced nuclear accumulation (top panel) and cytoplasmic exclusion (bottom panel) in D2HGDH/L2HGDH WT or KO cells. TET1 is not detected in the cytosol and thus is not represented in the right panel. Data shown are from three biological replicates – one from Figure 5C – main text – and two shown above. Error bars are S.D. of the mean. *p<0.05, **p<0.01, ***p<0.001, Student's t-test. **D.** Top. WB analysis of TET-1-3, FTO and ALKBH5 in HEK-293 cells expressing an empty vector (MSCV), D2HGDH WT or the G131X and A208T mutants - replicates #2 and # 3 are shown. Bottom. D2HGDH is detected in HEK-293 expressing the WT gene or the A208T mutant; G131X truncates D2HGDH before the antibody epitope and thus is not detectable by WB. Q-RT-PCR (right most panel) confirms the mRNA expression of the G131X mutant.). Data are mean -/+ SD. **E.** Immunofluorescence and WB analysis. Top - staining with anti-TET3 or anti-FLAG antibodies yielded overlapping signals in cells exposed to dimethyl-αKG (5mM), 2-HG (octyl-D-2 and octyl-L-2-HG - 100µM/each) or DMSO for 6h; scale bar is 10 µm. Right - staining with anti-TET3 or anti-FLAG antibodies in a field with ~ 30 cells demonstrates that the ant-TET3 antibody detects only the ectopically expressed protein. Bottom - WB analysis of transiently transfected FLAG-TET3 (center) and of endogenous TET3 in parental HEK-293T cells (right) following exposure to dimethyl-αKG (5mM), 2-HG (octyl-D-2 and octyl-L-2-HG - 100µM/each) or DMSO for 6h. Densitometry of protein levels are shown at the bottom of the WB displays – all values are relative to DMSO and corrected by WCL, which is shown for reference.

A



	MYC activity score	MYC	TET1	TET2	TET3
Myc activity score	r=1.00	r=0.46	r=0.16	r=-0.16	r=-0.08
P value		P=0.0013	P=0.3	P=0.27	P=0.61

r = Pearson correlation coefficient

B

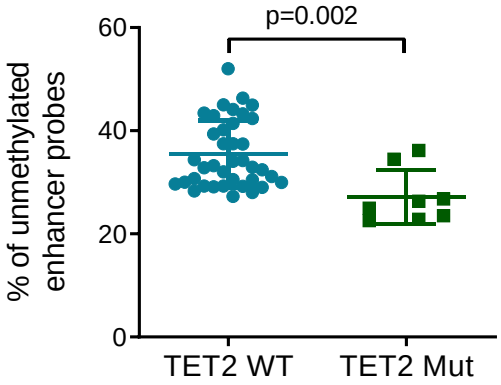


Figure S6, related to Figure 6. MYC activity and expression and TET2 genetic integrity regulate enhancer methylation. **A.** Pearson correlation between MYC expression and activity in 46 primary DLBCL. The Y axis depicts a single sample GSEA-generated MYC activity score, reflecting the aggregated expression and ranks of 17 genes that are known as direct MYC targets. Right panel. Correlation between MYC activity and MYC, TET1, TET2 and TET3 expression is shown in a tabular format. **B.** Comparison of the percentage of unmethylated enhancer probes in primary DLBCL dichotomized by TET2 status – wild-type vs. mutant; p value is from a two-tailed Student's t-test.

Gene	corr coefficient	p value	FDR
GRHPR	-0.750138445	1.96E-09	0.00000293
ATF5	-0.699500711	6.36E-08	0.0000948
KLHL14	-0.690230611	0.000000111	0.000165976
BRD2	-0.677760857	0.000000229	0.000341457
EEPD1	-0.663751939	0.000000495	0.000737091
PRDM15	-0.654407278	0.00000081	0.001204845
TCFL5	-0.654134991	0.000000822	0.001221113
MGAT3	-0.649237759	0.00000106	0.001568339
KLHDC5	-0.645637111	0.00000127	0.001879483
ASCL2	-0.640007015	0.00000167	0.002483486
RAPGEF5	-0.636576747	0.00000198	0.002934204
SIPA1L3	-0.630877293	0.0000026	0.003855486
CAMK4	-0.626709447	0.00000411	0.006079922
QSOX2	-0.622599147	0.00000384	0.005678793
BEND5	-0.619505102	0.00000442	0.006537265
KCNJ1	-0.612587212	0.0000126	0.018605723
PLCG2	-0.612162297	0.00000616	0.009098634
PIM1	-0.607215433	0.00000766	0.011305543
SERTAD2	-0.600001001	0.0000105	0.015437333
POU3F1	-0.598712444	0.0000111	0.016298455
FOXP1	-0.597124831	0.0000118	0.017422807
ANXA11	-0.594241273	0.0000134	0.01964659
EPHA4	-0.590430737	0.0000157	0.02301104
BICD2	-0.588987002	0.0000166	0.024408128
ZNF860	-0.58224319	0.0000272	0.039880359
ZNF516	-0.578733741	0.0000252	0.036950327
MGC12916	0.575433742	0.0000287	0.04205316
IDO2	0.619431479	0.00000736	0.010860783
SELPLG	0.666924182	0.000000418	0.000621665

Table S1, related to Figure 6c. Correlation between enhancer methylation and target gene expression

Name	Forward	Reverse
D2HGDH q-RT-PCR-human	GATATGGCTCACTGCATGGGAC	GTCTGCAGAACCTCAGCAAAGC
L2HGDH q-RT-PCR-human	TGGTGCAACAGTGAAGTATC	CCAGATTTCCATCTCTATCC
D2HGDH q-RT-PCR mouse	TGGTGACAGATGGCACRATG	CTTGAACACATAGCCATCTCG
L2HGDH q-RT-PCR mouse	GGAATTCCCTACAGGCAATG	CCTGCAATACGGCTCCTTCT
TBP - human	TATAATCCCAAGCGGTTTGCTGCG	AATTGTTGGTGGGTGAGCACAAGG
β-actin - human	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
TBP - mouse	CTGGAATTGTACCGCAGCTT	CAGTTGTCCGTGGCTCTCTT
D2HGDH promoter – cloning	ATTGCTAGCTAAGCTAAGGCTGGGGAC	CGGAAGCTTTGCACGTTGTACAAATTG
L2HDGH promoter – cloning	AAAGCTAGCACAGCCCATCGCCCTCATTT	ATGCTCGAGCTCAGAAGAAGCCACTTGAC
D2HGDH promoter E-box1 ChIP-PCR	GGAGCTCGGGACGTGATCGT	ACAGGCACCGACTGGCTGAC
D2HGDH promoter E-box2 ChIP-PCR	GTCAGCCAGTCGGTGCCTGT	ACGCAGCAGGCCTGGACTTA
L2HGDH promoter E-box1 ChIP-PCR	TTCTAGTGGACGCTCGACTG	GAAACTCTGGGCGGGATTAC
L2HGDH promoter E-box2 ChIP-PCR	GTAATCCCGCCCAGAGTTTC	CTAGGGTGGTGGTTGTGTTC
L2HGDH promoter E-box3/4 ChIP-PCR	TATTGGCCACCGCAGCCTCTC	GCAGGCACCAACCAAATAAC
D2HGDH promoter neg. ctrl ChIP-PCR	GGTTAAGGAGGACAGGAATG	GTCCTGGTAGTCACACAGAA
L2HGDH promoter neg. ctrl ChIP-PCR	GGCTGCAAAGGTAAGTTGTG	TATCCACCACCACAGTCAAG
LIN28B promoter pos. ctrl ChIP-PCR	GCAAATAACGCTGGATTCAGTG	GCACGAGGAGGAAAGAGAAATC
shRNA and guide RNAs		
D2HGDH-g1-RNA	CACCGGTTCTTGGGGTCGGCCGGT	
D2HGDH-g2-RNA	CACCGAGCCACGCGGGCCACGCCAG	
L2HGDH-g1-RNA	CACCGCGTGCGGGTTCGCGTCTGGG	
L2HGDH-g2-RNA	CACCGCCCGCGGGCTTTTCGCCGG	
MYC shRNA#1	CCTGAGACAGATCAGCAACAA	
MYC shRNA#2	CAGTTGAAACACAAACTTGAA	

Table S2, related to STAR Methods – PCR primers, shRNA and guide RNAs sequences.