Supporting Information

Yashiro-Ohtani et al. 10.1073/pnas.1407079111

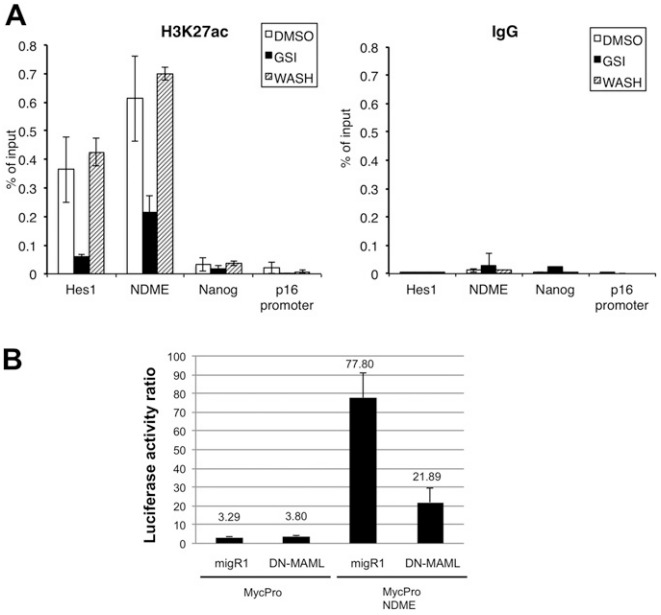

Fig. S1. Notch1 signaling regulates H3K27 acetylation of the murine *Myc* 3′ enhancer element (NDME). (*A*) T6E T-ALL cells treated with DMSO or 1 μM compound E for 16 h, or with compound E for 16 h followed by GSI washout and 4 h of additional incubation, were fixed and analyzed by local ChIP assay. ChIPs in *Left* were prepared with antibody specific for histone H3 acetylated at K27, whereas those in *Right* were prepared with a nonspecific Ig, as described (1). Data were obtained in triplicate in independent experiments; error bars correspond to the SEM. (*B*) Notch specifically regulates *NDME* activity. T6E cells were transfected with *Renilla* luciferase control (pRLTK), *Myc* promoter construct (*Myc*Pro) or the murine *Myc* enhancer reporter construct (*Myc*ProNDME) and either empty vector or a vector driving expression of the dominant-negative form of mastermind-like1 (DN-MAML).

1. Wang H, et al. (2014) NOTCH1-RBPJ complexes drive target gene expression through dynamic interactions with superenhancers. *Proc Natl Acad Sci USA* 111(2):705–710.

Myc 3′ enhancer alignment

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mouse AAAGCTACTCTTAGAGGCAATTTGACAGAGCTGTCCTTTGAAACTGGCATTTTCTAAGTG 60
human AAAGGTGTTCTTAGTGGCAATTTAACAGAGCTGTCCTTTGAAAGTGGCATTTTCTAAGTA 60
      **** *  ****** ******** ****************** ***************

mouse TTCTTCTAAATTACAGTTGGGCCATAAATATGGCAGGTTAAACGGAGACAGCTGAGAAAT 120
human TTCTCCTAAATTGCAGTTGGGCCATAAATACAACAGGTTAAACTAAGGCAGCTGGGAAAT 120
      **** ******* ***************** ********** ** ****** *****

mouse GGTGTAGGTGAAAAATTACAAGGATGGGATCTTTCTCCTCTTCCACATGAAGATTTAATC 180
human TATATAGGTGAAAAATTACAAGGACGAGATCTTTCCACTCAAGCATGTAAAGATTTAATC 180
      * ******************* * ******** *** ** * ***********

                                                    RBPJ site B
mouse TGCCAGATTAAAAAACCCTGAACCTGGTGATTGTGTCAAGATAACAGCTTGGAGGATGCT 240
human TCCCAGGTTAAAAA-CCCAGAACACAGTGATTACTTTGAGATAACAGCTCAGAGGATGCT 239
      * **** ******* *** ****   ******   *  ***********  *********

          RBPJ site A
mouse CAGAGATGGGGTTCCCAGGGTGTTT-CAAGGGAT-GGGTCTGTGGCCTACAGAGGCAGGT 298
human CAGAGATGGGGTTCCCATGGTATTTTCTGGGGACCGGGTCTGTGGCCTGCAGAGGCAGGT 299
      ***************** *** *** *  ****  ************* ***********

mouse GTTTCTCAGTTGG-AGCACAGAGGAGTTCTTGGCACCAGCACTGGGCCAGCTGTGAGTTT 357
human GTTTCCCAGTGGGGAGCACAGAGGAGCCCTTTTCACCCGTATCAGGCCAGCTGTGAGCTT 359
      ***** **** ** ************  ***  **** * *   ************* **

mouse ATCTGTGGTATCTGGCTTTCAAAGTAGTGTTCCCTGCAGTCTGCCTAAAAGAAAGAGATG 417
human ATCTGTGGCATCTCGCTTTCAAGGAACGGTTCCCCAGGGTCTGCCTAGGAGAAAG----- 414
      ******** **** ******** * *  ******    *********  ******

mouse ATCAAGATGAACGAAGAGGTAATTGCACTGTACTCTACTCTGATGGGAAAAAGGGGGGAG 477
human -TT--GATGAACCACATGGGAACTGTACAGCATATTA-----ACAGGAAAAAAATGTAAA 466
       *   ******* *   ** ** ** ** * *   **     *  *******   *  *

mouse AATTATGAAAGAAAAATATATAT 500
human AATCATGAAACA---------- 478
      *** ****** *
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Fig. S2. Sequence alignment of murine and human NDME elements.

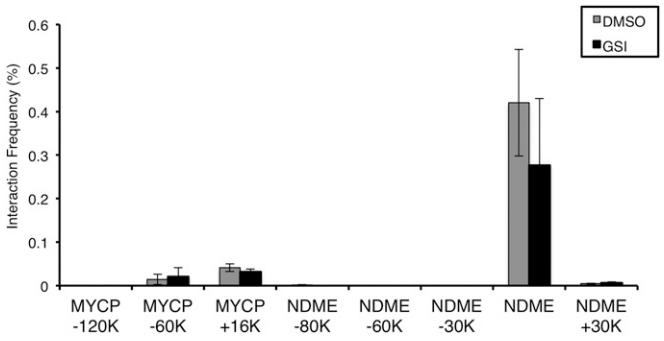
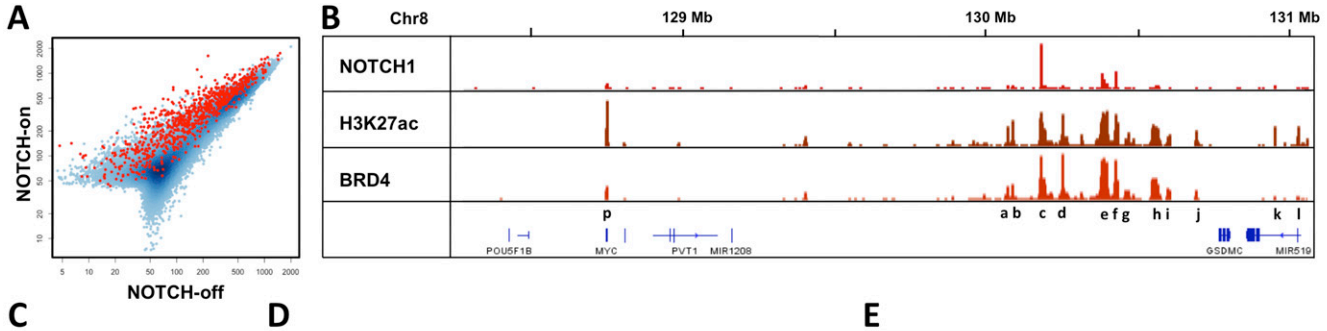


Fig. S3. Short-term Notch inhibition does not affect the interaction of the human *Myc* 3′ enhancer element (NDME) with the *Myc* promoter. Human CUTLL1 cells treated for 3 d with DMSO or 1 μM compound E were fixed and analyzed by 3C assay. The "probe" primer was located at the *Myc* promoter. Positions of other primers are shown in Fig. 5*C*. The 3C quantitative PCR (qPCR) products were quantified relative to the amounts of products generated with the same primer pairs, using a DNA substrate consisting of HindIII-digested, randomly ligated BAC DNA encompassing this region of the human genome. Data were obtained in triplicate in independent experiments; error bars correspond to the SEM.



C

Annotation (chromosome 8)		
	start (kb)	end (kb)
p	128743.6	128754.8
a	130061.4	130076.8
b	130077.6	130104.0
c	130171.8	130220.4
d	130236.6	130275.0
e	130361.4	130410.8
f	130414.2	130438.0
g	130457.6	130482.2
h	130538	130574.6
i	130593	130609.4
j	130687.6	130710.8
k	130950.2	130953.4
l	131024.4	131030.8

D

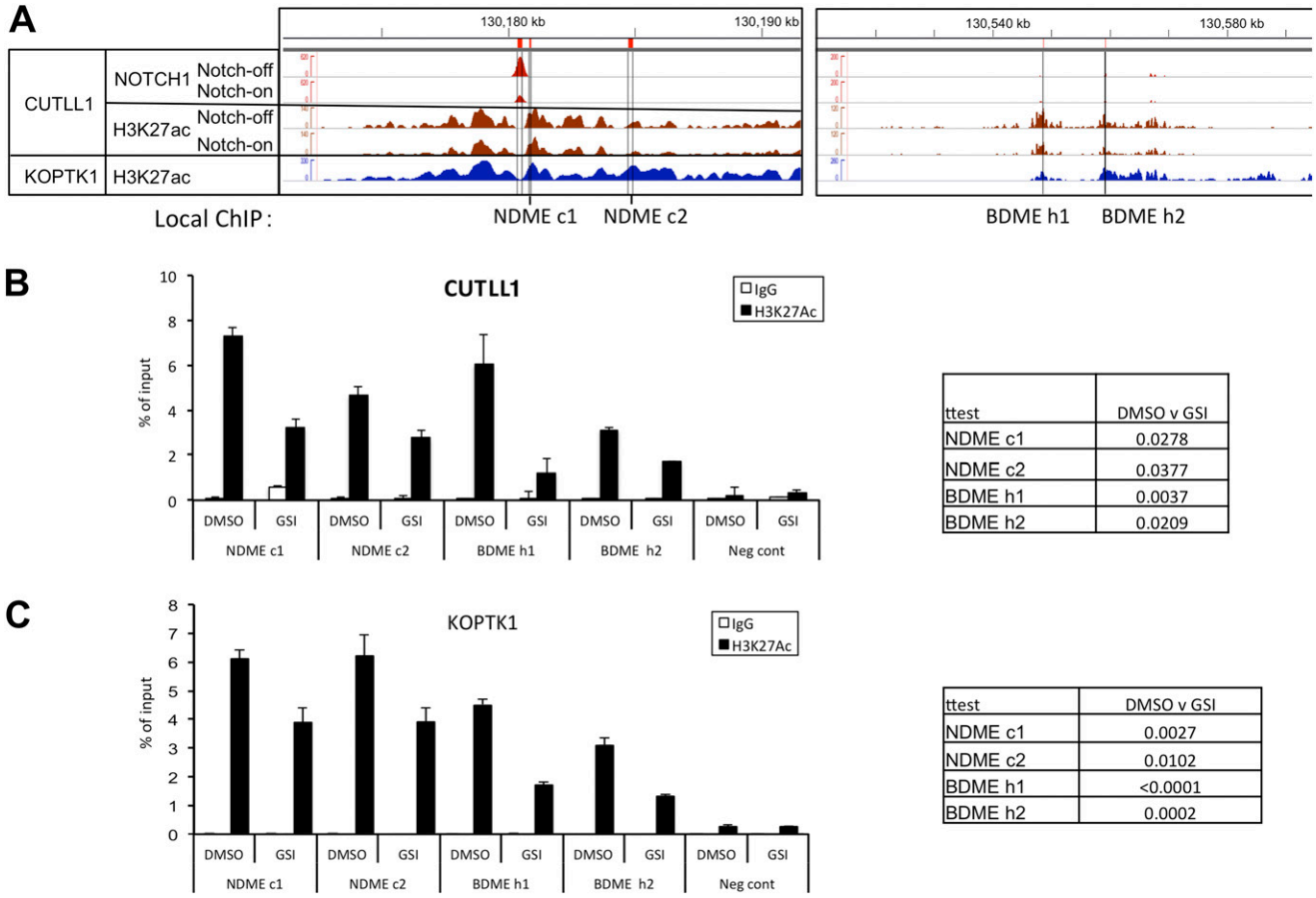
H3K27ac						
	CUTLL		DND41		KOPTK-1	
	W4h/ GSI	P-value	GSI naïve/ resistant	P-value	GSI naïve/ resistant	P-value
p	1.44	9.0E-97	0.80	1	0.93	1
a	1.70	7.5E-106	5.27	0	4.59	0
b	1.61	1.1E-129	2.49	0	1.70	1E-147
c	1.71	0	8.63	0	6.93	0
d	1.78	0	7.26	0	8.92	0
e	1.91	0	2.10	5.5E-269	3.98	0
f	1.98	0	1.01	0.74	2.80	0
g	1.62	1.5E-98	0.77	1	1.35	7E-08
h	2.02	0	0.49	1	0.85	1
i	1.89	3.1E-128	0.35	1	0.66	1
j	2.45	0	0.33	1	0.76	1
k	0.96	1	0.63	1	0.95	1
l	1.01	0.6	0.82	1	0.69	1

E

BRD4				
	DND41		KOPTK-1	
	GSI naïve / resistant	P-value	GSI naïve/ resistant	P-value
p	0.89	1	1.27	5.5E-07
a	2.70	5.6E-29	7.90	2.4E-275
b	2.41	2.0E-34	10.60	0
c	5.96	0	22.63	0
d	3.90	2.6E-220	18.21	0
e	2.09	3.9E-50	4.98	0
f	1.30	7.9E-08	4.29	0
g	0.91	1	1.90	5.4E-19
h	0.71	1	1.22	4.6E-18
i	0.49	1	0.60	1
j	0.44	1	0.89	1
k	0.72	1	1.06	0.5
l	0.75	1	1.22	0.1

Fig. S4. Quantification of the effects of acute and chronic changes in Notch activation on chromatin landscapes in the Myc 3′ superenhancer region. (*A*) Scatterplot of H3K27ac ChIP-Seq read counts. Each dot represents an H3K27ac peak identified in CUTLL1 cells following GSI washout. ChIP-Seq reads within 600 bp of a peak summit were counted in the Notch-on and -off states. Red dots indicate peaks with dynamic Notch1 binding sites (described in ref. 1), and blue dots indicate peaks without dynamic Notch1 binding sties. (*B*) Chromatin landscapes in human CUTLL1 cells for Notch1, H3K27ac, and Brd4 are shown in the Notch-on state. (*C*) Genomic location of labeled peaks in *A*. (*D*) Quantification of changes in H3K27Ac peaks in CUTLL1 cells after 3 d of GSI treatment and 4 h after Notch1 reactivation by GSI washout (expressed as W4h/GSI) and in Notch-dependent (GSI naïve) and Notch-independent (GSI resistant) DND-41 and KOPT-K1 cells (expressed as GSI naïve/resistant). (*E*) Quantification of changes in Brd4 peaks in Notch-dependent (GSI naïve) and Notch-independent (GSI resistant) DND-41 and KOPT-K1 cells (expressed as GSI naïve/resistant).

1. Wang H, et al. (2014) NOTCH1-RBPJ complexes drive target gene expression through dynamic interactions with superenhancers. *Proc Natl Acad Sci USA* 111(2):705–710.



ttest	DMSO v GSI
NDME c1	0.0278
NDME c2	0.0377
BDME h1	0.0037
BDME h2	0.0209

ttest	DMSO v GSI
NDME c1	0.0027
NDME c2	0.0102
BDME h1	<0.0001
BDME h2	0.0002

Fig. S5. Notch1 signaling regulates H3K27 acetylation of the human NDME and BDME. (*A*) Position of primers used to perform H3K27ac local ChIP superimposed over Notch1 and H3K27ac ChIP-Seq data obtained from human CUTLL1 and KOPT-K1 T-ALL cells. (*B* and *C*) NDME c1 and NDME c2 denote two regions in peak c, and BDME h1 and BDME h2 denote two regions in peak h that were amplified using specific primer pairs. CUTLL1 and KOPT-K1 T-ALL cells treated with vehicle (DMSO) or 1 μM compound E (GSI) for 3 d were fixed and analyzed by local ChIP assays prepared with nonspecific IgG or antibody specific for K27-acetylated histone H3. Data were obtained in triplicate; error bars correspond to SD. *P* values were determined with Student *t* test.

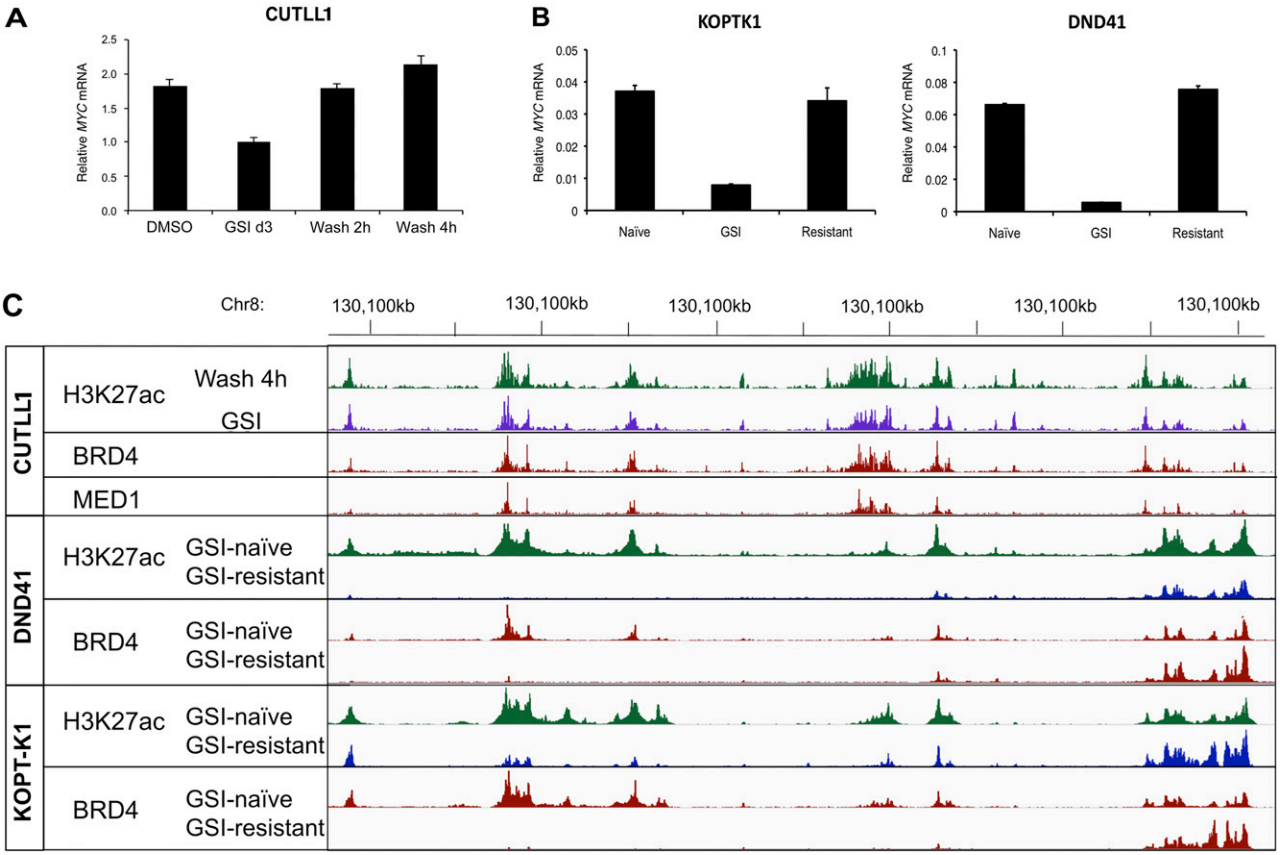


Fig. S6. Relative *Myc* mRNA expression in CUTLL1 cells following acute GSI blockade and washout and in GSI-sensitive and -resistant human KOPT-K1 and DND-41 cells. (*A*) *Myc* mRNA was quantified by qPCR in CUTLL1 cells incubated with vehicle, 1 μM compound E (GSI) for 3 d, or 1 μM compound E (GSI) for 3 d followed by 2 or 4 h of additional incubation after GSI washout. *Myc* expression was normalized to expression of the *Gapdh* housekeeping gene. (*B*) *Myc* expression in KOPT-K1 and DND-41 cells treated with vehicle for 3 d, GSI for 3 d, or selected for GSI resistant by long-term culture in GSI. *Myc* expression was normalized to expression of the *Gapdh* housekeeping gene. Data were obtained in triplicate in independent experiments; error bars correspond to the SEM. (*C*) Enlargement of the *Myc* 3′ enhancer cluster. The b–l peaks are defined in Fig. 5. See Fig. 5 for additional details.

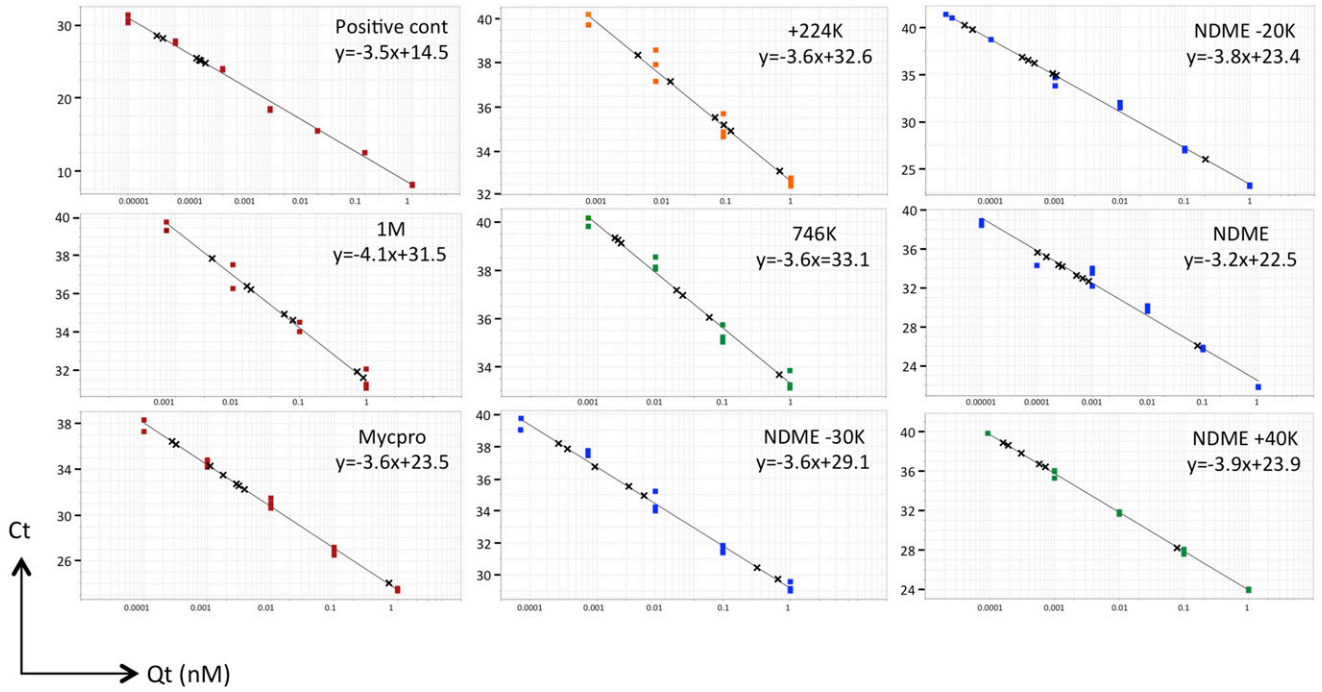


Fig. S7. Standard curves of qPCR for murine 3C experiments. Standard curves were prepared with serially diluted PCR DNA fragments (colored box). X shows Ct values from each PCR using 3C samples.

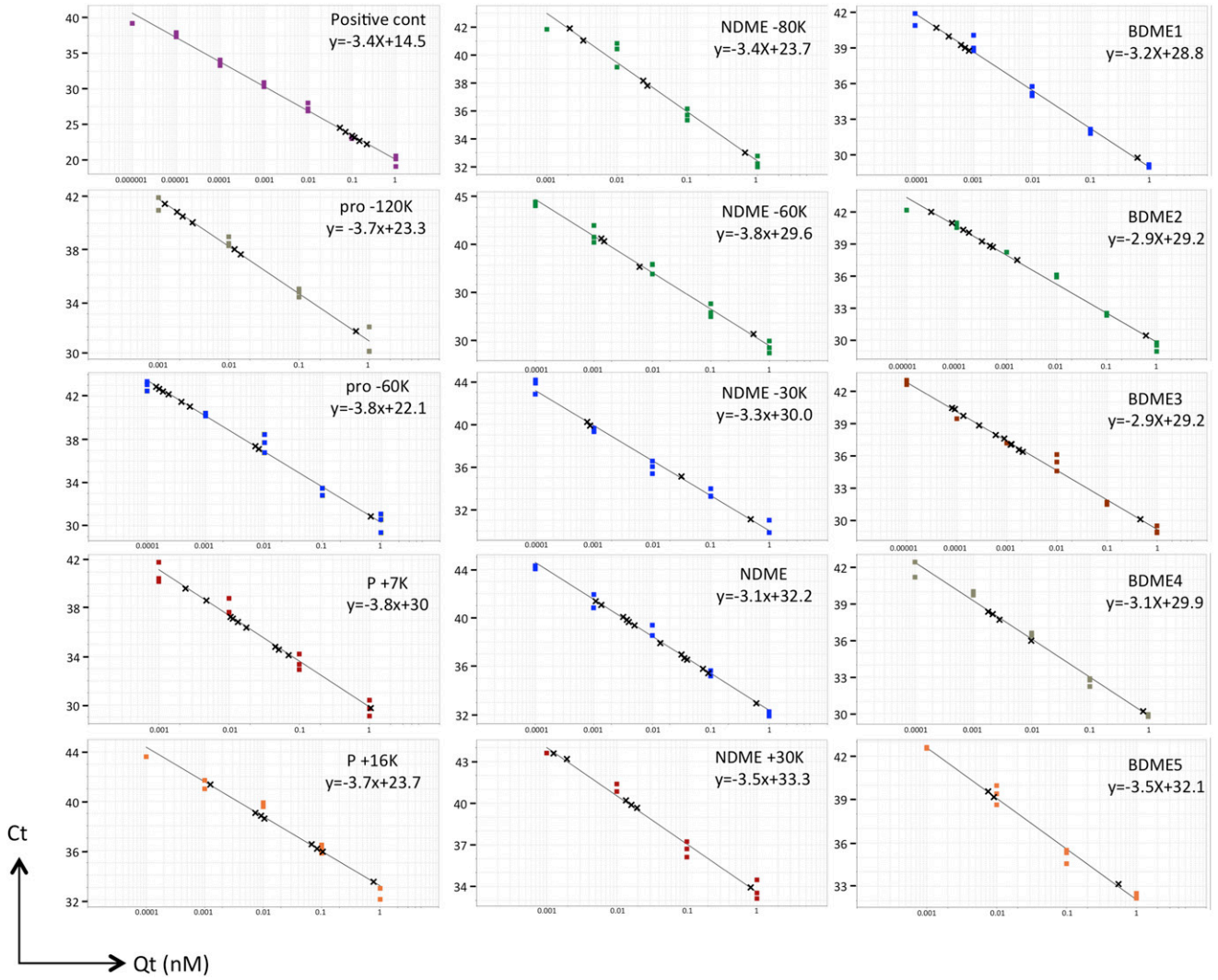


Fig. S8. Standard curves of qPCR for human 3C experiments. Standard curves were prepared with serially diluted PCR DNA fragments (colored box). X shows Ct values from each PCR using 3C samples.

Table S1. Primers used in these studies

	Forward	Reverse
ChIP primers		
Mouse Hes1 promoter	5′-TGCAGCGTCTCTGGGTTGTA-3′	5′-CCAGGACCAAGGAGAGAGGT-3′
Mouse Dtx1 intron3	5′-CGTGTCTCTTCCTCCCATTG-3′	5′-CCTGAGAGGAAGCGGTGT-3′
Mouse Myc enhancer	5′-ACCCTGAACCTGGTGATTGTG-3′	5′-CCACAGACCCATCCCTTGAA-3′
Mouse Nanog	5′-GGCTGCCTCTCCTCGCCCT-3′	5′-GTGCACACAGCTGGGCCTGA-3′
Human NDME c1	5′-GAGGCCCCCATTCATTACCC-3′	5′-CCAGGTAGGGGCATTACGTC-3′
Human NDME c2	5′-GCTGCCACATGCTGATGAAC-3′	5′-GCAGTTCTTCCTACGCTGGT-3′
Human BDME h1	5′-AGGAGCCCACCTTCTCATTT-3′	5′-ACATTGCAAGAGTGGCTGTG-3′
Human BDME h2	5′-AGGAAGTGGCTTTCACATGC-3′	5′-GCGTGCAAAAGAGAGAAACC-3′
human neg cont (+1M of *MYC*)	5′-AATGCTGGGCTTCCAAGGA-3′	5′-GACCTTGGTGACTGTTGAGGAAAC-3′
Cloning primers for pGL3		
Mouse Myc promoter	5′-GGTACCACGGTTTTCTTTATTCTAGGGTCT-3′	5′-GCTAGCCCCCAAATAGACAAAAATTCCCT-3′
Mouse Myc enhancer	5′-GGATCCACTGTTTGATTCTACCTCCCAAA-3′	5′-GGATCCTAGGCAGACTGCAGGGAAC-3′
Human MYC enhancer	5′-GGTACCGCTGTCCTTTGAAAGTGGCATT-3′	5′-GGTACCGCTGTACAGTTCCCATGTGGT-3′
Primers for 3C		
Mouse		
Myc probe	5′-FAM/AAGCCCTGCCCTTCAGGAGGC/TAMRA-3′	
Myc promoter F	5′-GTCCGACTCGCCTCACTCA-3′	
Myc control	5′-CCGCTCACTCCCTCTGTCTC-3′	
Myc promoter R	5′-CAAGGTTAGTGCCAAAGTCCATCT-3′	
Myc +224 K	5′-TGGTCTCCTGGTGCAGTTTG-3′	
Myc +746 K	5′-CAGCATGAATTATTTGACTTCTTGAAT-3′	
Human		
MYC probe	5′-FAM/CCCAGAAATGCTGGCTTTGCCA/TAMRA-3′	
MYC promoter F	5′-CTGCTACCTACCTCCAAAGCCTTA-3′	
MYC control R	5′-CCGCTCACTCCCTCTGTCTC-3′	
MYC promoter R	5′-GAGTGCATTCTCTCCACCACAGT-3′	
NDME -80K	5′-TCTCCAGAATGCTGTAAAGTAGACCA-3′	
NDME -60K	5′-GAATTCTATGTGCTCAGTGCCTTAAC-3′	
NDME -30K	5′-CTCCATTCCCTTCTGTATCTGCTATT-3′	
Mouse		
Myc+1M R	5′-AAGGCTCCCAGGTCAATGTG-3′	
NDME -30K	5′-TGTTTGCTTGCTTGCTTGATTT-3′	
NDME -20K	5′-CAAAGGCAGGATGGTTACTTTAGAA-3′	
NDME	5′-GTGTGTCACCGTGATTGTTCACT-3′	
NDME +40K	5′-TAAGGTAGACTTTCCATCTGACACAAA-3′	
Human		
NDME	5′-CTCCAGAGACAACAAGAGTGAGAAGAA-3′	
NDME +30K	5′-CAGCATTATCCATAGTAGCTCCAAACT-3′	
BDE E2 -23K	5′-ACCCCATAAGCACAGGCAAC-3′	
BDE E2	5′-GGTTCCAAAATCCGAGCTGA-3′	
BDE E3	5′-GCATGGCAGTGGTCACAGTT-3′	
BDE E4	5′-AAGAAGGTGGTTCTACCAAGAAAGG-3′	
BDE E4 +25K	5′-CCTGAAACCTGATTGCTCATGTAA-3′	
Myc+1M R	5′-AAGGCTCCCAGGTCAATGTG-3′	