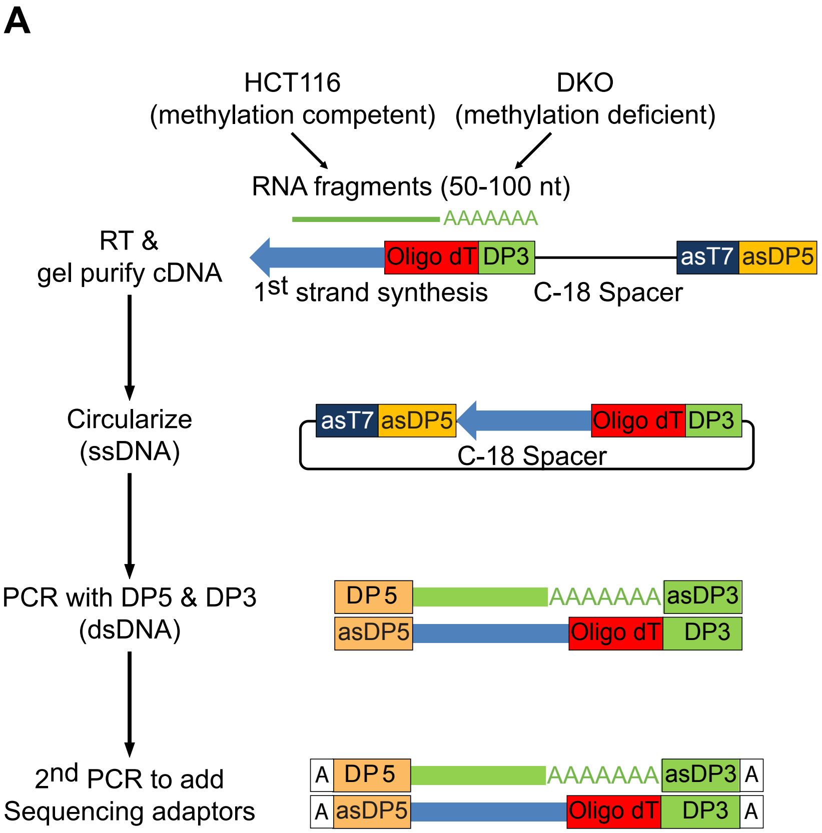
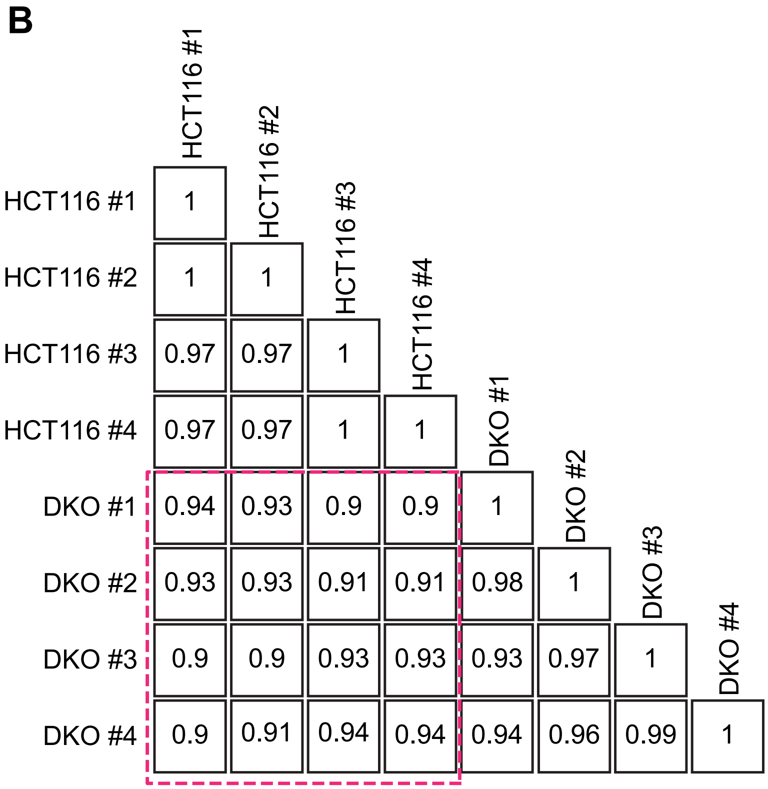
Supplementary Fig. 1





	HCT116 #1	HCT116 #2	HCT116 #3	HCT116 #4	DKO #1	DKO #2	DKO #3	DKO #4
HCT116 #1	1							
HCT116 #2	1	1						
HCT116 #3	0.97	0.97	1					
HCT116 #4	0.97	0.97	1	1				
DKO #1	0.94	0.93	0.9	0.9	1			
DKO #2	0.93	0.93	0.91	0.91	0.98	1		
DKO #3	0.9	0.9	0.93	0.93	0.93	0.97	1	
DKO #4	0.9	0.91	0.94	0.94	0.94	0.96	0.99	1

C

	HCT116		DKO		Union	
	pA sites	Genes	pA sites	Genes	pA sites	Genes
All pA sites	26,124	12,562	25,905	13,359	30,670	14,099
Unambig. Genes					29,573	11,219
Genes with more than 1 pA site					25,194	6,840
p.adj < 0.0001					2,015	1,173
Sites used > 5% in ≥ 1 cell line					1,819	1,106
Site changes > 1.5 fold					1,163	902
> 10% shift in pA site usage					718	546

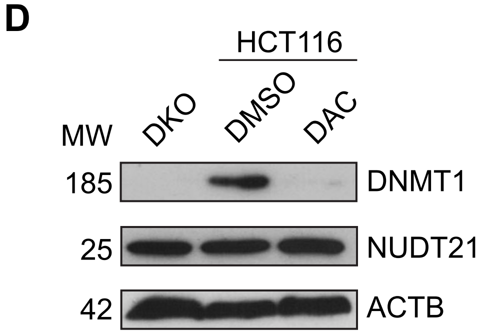


Figure S1: Protocol and data QC of poly(A)-seq (Related to Figure 1). a, Schematic of the poly(A) sequencing strategy. DP3 and DP5 are Illumina sequencing machine adapters. **b,** Correlations of poly(A) site usage ratios between poly(A)-seq replicates. **c,** Reanalysis of poly(A)-seq using only the two high depths replicates from HCT116 (Table S1) to confirm sequencing depths did not impact the accuracy of detection and quantification of APA candidates. **d,** Western blot analysis of NUDT21 and DNMT1 proteins in HCT116 cells treated with DMSO for 72 hours, DAC for 72 hours, and untreated DKO cells. ACTB is a loading control. MW, molecular weights (kD).

Supplementary Fig. 2

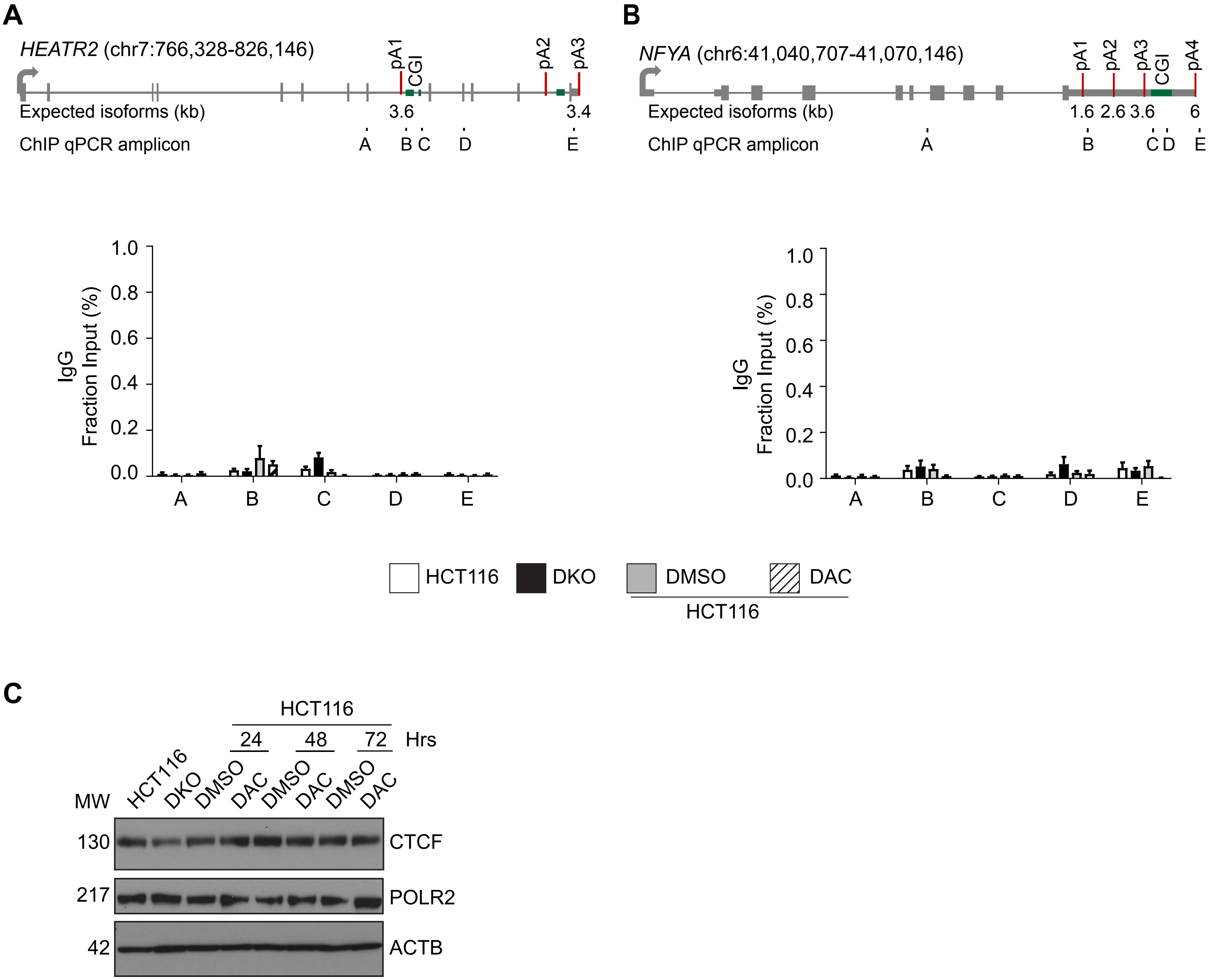
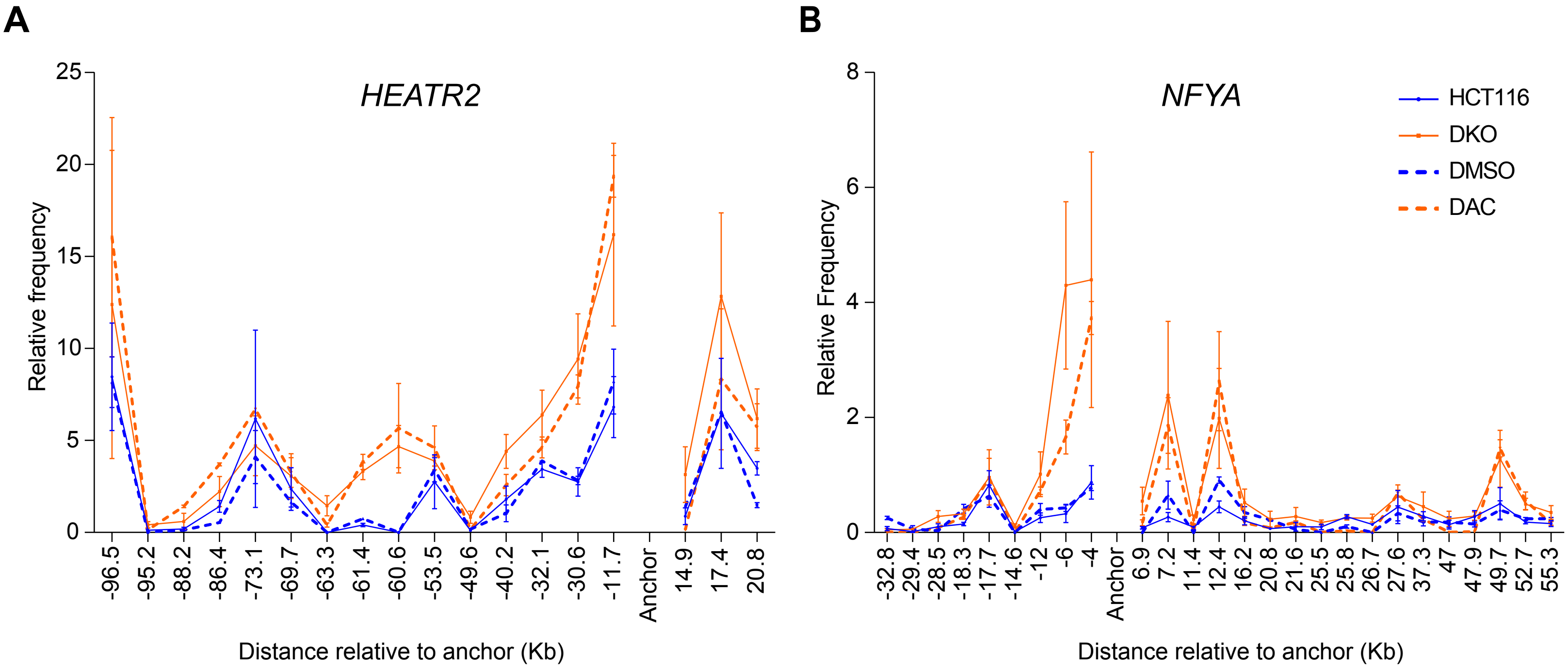


Figure S2: Removal of DNA methylation does not affect CTCF and POLR2 expression (Related to Figure 2). a, **b**, ChIP-qPCR for IgG control in HCT116, DKO, DMSO and DAC treated HCT116 cells in *HEATR2* **(a)** and *NFYA* **(b)**. **c**, Western blot analysis for CTCF and POLR2 in HCT116, DKO, DMSO and DAC treated HCT116 cells at 24, 48, and 72 hrs of treatment. ACTB is a loading control. MW, molecular weights (kD).

Supplementary Fig. 3



HCT116 vs DKO				HCT116 (DMSO vs DAC)			
Kb	HCT116	DKO	p-value	Kb	DMSO	DAC	P-value
-96.5	8.46E+00	1.24E+01	0.6806	-96.5	8.17E+00	1.61E+01	0.3016
-95.2	1.18E-01	4.13E-01	0.1788	-95.2	1.03E-02	1.41E-01	<0.0001
-88.2	1.86E-01	5.93E-01	0.4218	-88.2	1.28E-01	1.42E+00	<0.0001
-86.4	1.42E+00	2.22E+00	0.4173	-86.4	5.37E-01	3.70E+00	<0.0001
-73.1	6.17E+00	4.72E+00	0.7888	-73.1	4.10E+00	6.70E+00	0.1464
-69.7	2.35E+00	3.06E+00	0.695	-69.7	1.65E+00	3.26E+00	0.1321
-63.3	1.38E-06	1.45E+00	0.0584	-63.3	5.38E-04	3.98E-01	0.0311
-61.4	4.86E-01	3.32E+00	0.0039	-61.4	7.39E-01	3.84E+00	0.0017
-60.6	8.24E-03	4.67E+00	0.0157	-60.6	7.95E-04	5.67E+00	0.0404
-53.5	2.75E+00	3.88E+00	0.4915	-53.5	3.41E+00	4.60E+00	0.4329
-49.6	1.08E-01	8.21E-01	0.0988	-49.6	1.78E-01	1.65E-01	0.7946
-40.2	1.80E+00	4.40E+00	0.0902	-40.2	1.04E+00	2.58E+00	0.1056
-32.1	3.43E+00	6.38E+00	0.1072	-32.1	3.88E+00	4.62E+00	0.2652
-30.6	2.75E+00	9.43E+00	0.0303	-30.6	2.82E+00	7.94E+00	0.0016
-11.7	6.81E+00	2.04E+01	0.0125	-11.7	8.20E+00	1.94E+01	0.006
14.9	8.83E-01	3.14E+00	0.2275	14.9	1.40E+00	1.38E-01	<0.0001
17.4	6.47E+00	1.28E+01	0.3068	17.4	6.54E+00	8.32E+00	0.665
20.8	3.49E+00	6.19E+00	0.1785	20.8	1.49E+00	5.72E+00	0.0304

HCT116 vs DKO				HCT116 (DMSO vs DAC)			
Kb	HCT116	DKO	p-value	Kb	DMSO	DAC	P-value
-32.8	6.77E-02	4.41E-02	0.6097	-32.8	2.53E-01	3.75E-05	0.0011
-29.4	1.66E-02	5.70E-02	0.0699	-29.4	6.15E-02	2.20E-05	0.3737
-28.5	9.84E-02	2.79E-01	0.1937	-28.5	2.96E-02	8.57E-02	0.0781
-18.3	1.47E-01	3.25E-01	0.1057	-18.3	4.12E-01	2.68E-01	0.181
-17.7	8.29E-01	9.59E-01	0.8218	-17.7	6.31E-01	9.44E-01	0.4743
-14.6	1.33E-02	1.15E-01	0.0595	-14.6	1.28E-03	6.77E-03	0.0254
-12	2.55E-01	1.01E+00	0.1316	-12	4.02E-01	7.27E-01	0.0561
-6	3.25E-01	4.29E+00	0.0276	-6	4.26E-01	1.66E+00	0.015
-4	8.70E-01	4.39E+00	0.0382	-4	7.85E-01	3.73E+00	0.0006
6.9	8.31E-02	5.47E-01	0.1261	6.9	1.24E-04	1.68E-01	0.0316
7.2	2.68E-01	2.39E+00	0.0456	7.2	6.50E-01	1.86E+00	0.0895
11.4	9.23E-02	2.25E-01	0.1788	11.4	1.04E-03	N/A	
12.4	4.46E-01	1.98E+00	0.0077	12.4	9.09E-01	2.63E+00	0.1173
16.2	2.01E-01	5.19E-01	0.2684	16.2	3.49E-01	1.65E-01	0.1805
20.8	6.86E-02	2.31E-01	0.3048	20.8	2.20E-01	8.04E-02	0.0066
21.6	9.95E-02	2.80E-01	0.36	21.6	5.22E-02	1.78E-01	0.0109
25.5	9.76E-02	1.74E-01	0.3159	25.5	1.44E-03	4.51E-03	0.0792
25.8	2.76E-01	2.51E-01	0.6721	25.8	1.03E-01	2.64E-02	0.0608
26.7	1.42E-01	2.50E-01	0.2184	26.7	3.11E-03	1.97E-04	0.1259
27.6	4.43E-01	6.27E-01	0.6292	27.6	3.32E-01	6.82E-01	0.0715
37.3	2.82E-01	4.51E-01	0.5631	37.3	1.84E-01	2.32E-01	0.5825
47	1.38E-01	2.37E-01	0.5526	47	1.80E-01	5.37E-03	0.1253
47.9	2.73E-01	2.84E-01	0.9349	47.9	1.47E-01	1.30E-02	0.0622
49.7	5.00E-01	1.28E+00	0.2429	49.7	3.91E-01	1.46E+00	0.0083
52.7	1.79E-01	4.93E-01	0.0436	52.7	2.36E-01	5.50E-01	0.1129
55.3	1.53E-01	3.49E-01	0.2113	55.3	2.37E-01	1.57E-01	0.2374

Figure S3: 3C-qPCR analysis reveals interactions between APA control regions and distal genomic locations (Related to Figure 3). a, b, Top, 3C-qPCR analysis of HEATR2 (**a**) and NFYA (**b**) with respect to the anchor fragments containing the APA control regions in each gene. HCT116 (solid blue), DKO (solid orange), and HCT116 after 72 hours of treatment with DMSO (DMSO, dotted blue) or DAC (DAC, dotted orange) are plotted. Distance (kb) from the anchor fragment is noted on the x-axis. Bottom, mean relative interaction frequencies are summarized in the tables. Distance (Kb) relative to anchor is provided, and p-values are indicated for each tested interaction.

Supplementary Fig. 4

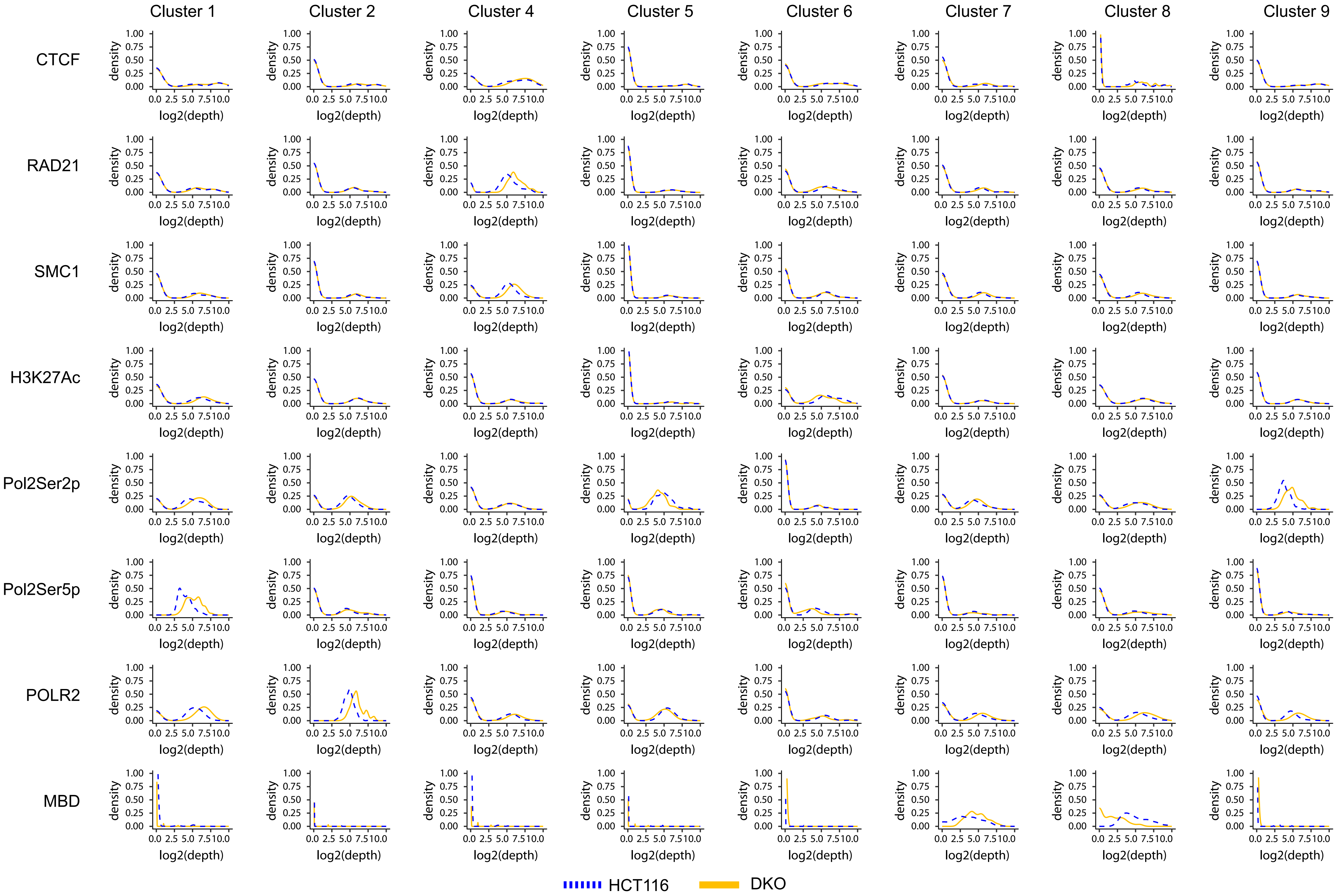


Figure S4: ChIP-seq cluster characteristics (Related to Figure 5). ChIP-seq signal characteristics for HCT116 and DKO in all other integrative consensus clusters. Blue dotted lines, HCT116 signals; yellow solid lines, DKO signals.

Supplementary Fig. 5

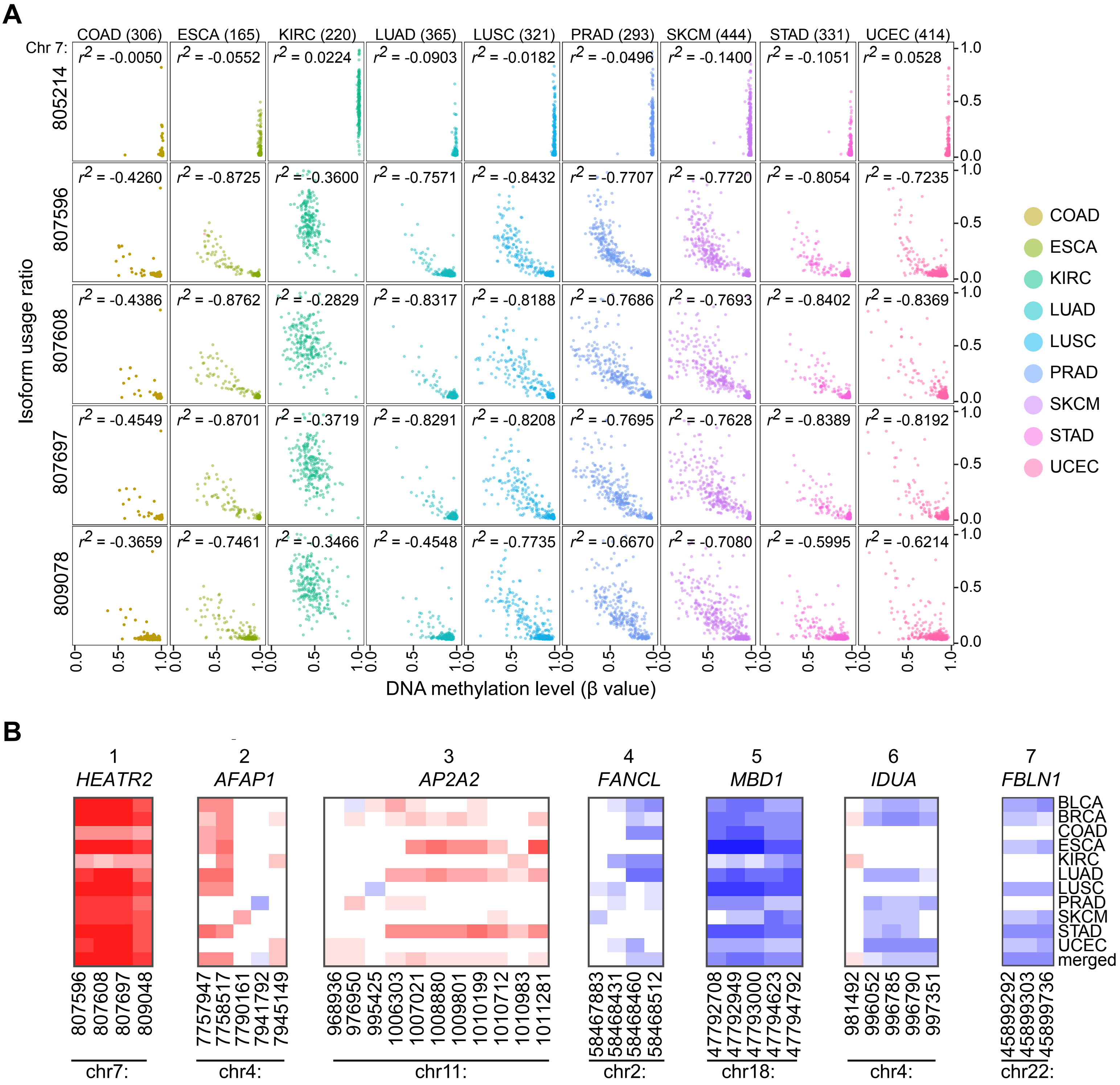


Figure S5: Additional TCGA correlation results (Related to Figure 5). a, Correlation between calculated isoform usage ratio and DNA methylation level at 5 CpG sites in HEATR2. COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; r2, Pearson correlation coefficient. The number of samples in each cohort is indicated in the parentheses. **b,** Enlarged view of the 7 best correlations from **Figure 5D**. CpG probe positions are noted below each heat map.

Supplementary Table 7. PCR primers used in the study (Related to STAR Methods)

Primer name	Primer sequence
Bisulfite sequencing	
HEATR2 BSF	TATTTATGGAGATTTATAGGGAGG
HEATR2 BSR	AAAATCAAACAATAATTAAAACAAC
NFYA BSF	GGTAGGTTTTTAATAGAGTTTGTTAGG
NFYA BSR	AAACCTCCAAAAACTTAAACCC
ChIP	
NFYA-A F	CACTACCAGTGGCAGGCAAT
NFYA-A R	AGGCATCAAGTTGCATGTTC
NFYA-B F	GATGCCTGGCAAATTGAAGT
NFYA-B R	TGTGTCTCCTTGCATCATCC
NFYA-C F	GCCAGTGGAACCTGTCCTTA
NFYA-C R	TCCTACGATAAAGCCGTTGG
NFYA-D F	GCCCTGTTTCCGGTACCTA
NFYA-D R	GGCTTCCGAAAGACCAGTAG
NFYA-E F	CCTGTGAGGAAGGAATAGGTGT
NFYA-E R	CAGTCCATGTCACAGGGTAGG
HEATR2-A F	AGCCCTGAGTGTTGGAATTG
HEATR2-A R	ATGTGCTTGCGGTAGAGGTC
HEATR2-B F	ATGGTCCGGTGGAAGTGTC
HEATR2-B R	ACGTGTCTACCTTCCAAGCA
HEATR2-C F	GTCCTTGCCCTGTCATTCAT
HEATR2-C R	ACTCAGGGCCAAGCTCCT
HEATR2-D F	GTTTCTTCCGAGCACCTGTC
HEATR2-D R	CCAGGATGTCCTTTGTCACC
HEATR2-E F	CTCTGTGCCAGCAGTGAGAC
HEATR2-E R	AGCCCTCTCGATGAACAGAA
Northern probe (for cloning constructs for in vitro transcription)	
289	TGTGATGGAGCTGATCAAGG
527	TCATCCGAATCCATGAAATG
505	GGAGACGATGGACTCACTGG
526	GTGCGACGATGACACTGAAC
316	TCCTACGAGCACCAAGACAA
549	CTAACCTCGCCCTTCTCCTT
qRT-PCR	
HEATR2 qRT proximal F	TGCATTTACTGCAGCTCTGG
HEATR2 qRT proximal R	GGCCCAAAGAGATGTGTGTT
HEATR2 qRT distal F	CTCTGTGCCAGCAGTGAGAC
HEATR qRT distal R	AGCCCTCTCGATGAACAGAA
NFYA qRT total F	GATGCCTGGCAAATTGAAGT
NFYA qRT total R	TGTGTCTCCTTGCATCATCC
NFYA qRT distal F	GCCAGTGGAACCTGTCCTTA
NFYA qRT distal R	TCCTACGATAAAGCCGTTGG

Methylation specific PCR	
NFYA_Methylated_MSP_F	ATTTATTGGTTTTTCGGAAGTTTC
NFYA_Methylated_MSP_R	TAAATACAAATACCGACCGTCG
NFYA_Unmethylated_MSP_F	ATTTATTGGTTTTTTGGAAGTTTTG
NFYA_Unmethylated_MSP_R	TTAAATACAAATACCAACCATCACC
CRISPR guide RNA	
NFYA_Guide_A	CGGCGTCGCATGCCGCTCGCCGG
NFYA_Guide_B	GGCGCTGCTTCCCGACCTACTGG
3C primers	
NFYA_3C_Primers	
-32.8	GCGTTTGAGCCTTGATTCTT
-29.4	GCTTGAGTTGAATGAATCAGGA
-28.5	CAAGCCTGATCTGGATGACA
-18.3	CCCAAGCATAAAGCATCAGAA
-17.7	CAATGCTCTCATGTCCCAAA
-14.6	AAAGATTTCTTAAAAGCACTTTCTGG
-12	TTGGCAAAAATATGCCAGTG
-6	CCATCATGGGCAATACTACTTTC
-4	GGTTTCACTCTGACACCCTTG
Anchor	AGCTTTTTCTTGGCTAGAGTCC
6.9	TTGTCCATTTGCTCACTCCA
7.2	GGATCCTTTGAGGCACAGAC
11.4	TTAGGCAGAATTCACTGATGAAA
12.4	ACCAGAAAAACATTCTTTATGCTTTCA
16.2	TCCTGAAGAAATATGCATGGAA
20.8	TGATTGTTGCAGGCTTTTTG
21.6	CACAGGTGAAAAAGCTATGACTCAG
25.5	TTGCACCTAGAAGCCAATAAAAGA
25.8	TGTTCAAATATAGGGCCCAAACA
26.7	AAGGCAGCTGCAATTTTTCTG
27.6	CTTCAAAGGAGAATCTTCACTGACAT
37.3	TGGTTTCTGAGTGAGGTCCAAA
47	AGGCCCAGATGGCTTCACT
47.9	AAAACACTCCACCCAACAGC
49.7	TGCCATTCAAAATTTTAGAGTAAGAAA
52.7	GGTCCAATCCCCCTACAAAT
55.3	AGCTTCCAGTGTTTTATTCCTGT
HEATR2_3C_Primers	
-96.5	CATTCTTGAGCCCTGGTCAC
-95.2	TGATTTCCATTTGCAGAAAGC
-88.2	CTGGCTCAAATATTCCCACA
-86.4	GGGCATGGCTGGAGTATCTA
-73.1	TCTGTGGCCTGATGTCTTTAAT
-69.7	AGGATTTGCAGCGGACTG

-63.3	CGCCTCTCAGAAAACCCTTT
-61.4	CTTCCTGCTTTGGGACTCAG
-60.6	TGTTTTGCTTGCAGCTCAGT
-53.5	CTTTGAAGTTGCATGCGTGT
-49.6	ACCGTCCCTTGTTTGGTCTC
-40.2	AGCAACACCATCAACCAACA
-32.1	TGGGTAGGGAAGTGGTTGAG
-30.6	ATGGATGGGAGCACTAAGGA
-11.7	TTTCCACATGGTGTGTACCTT
Anchor	TCACAGTGGATGAGGCTGG
14.9	ACTTGGTGCCCAACAAATTC
17.4	CCAAATGCGCTCAGATGC
20.8	GCTGGGAGAACAGTCTGGAG