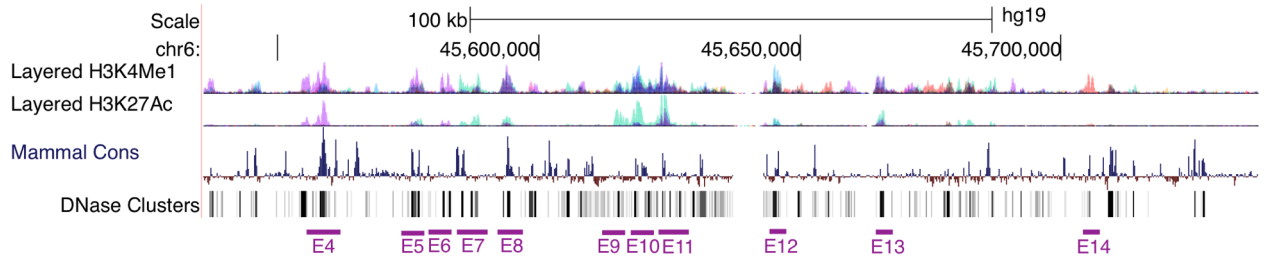
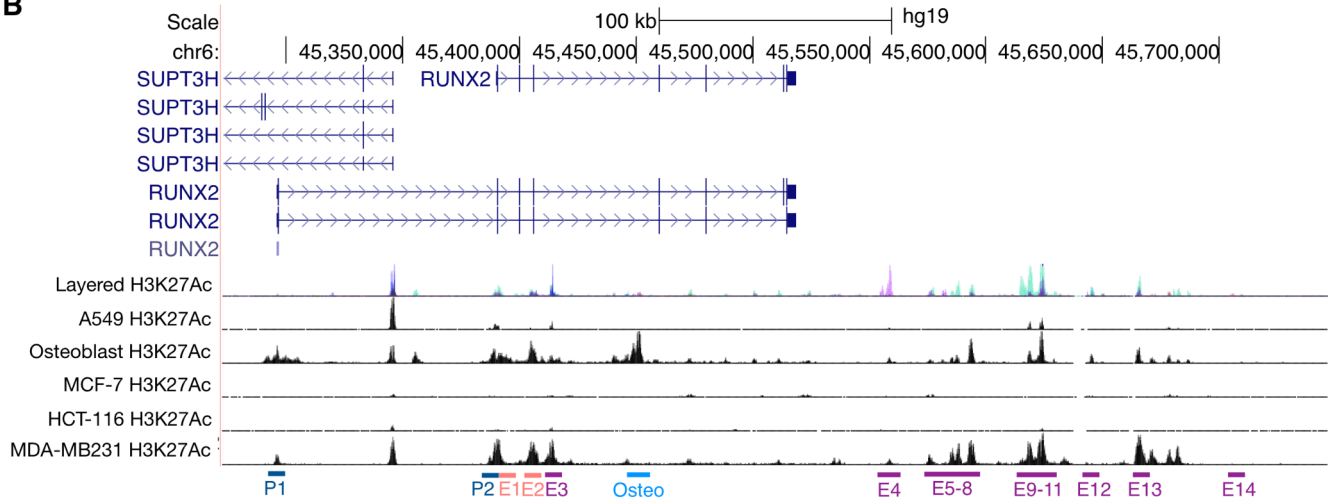


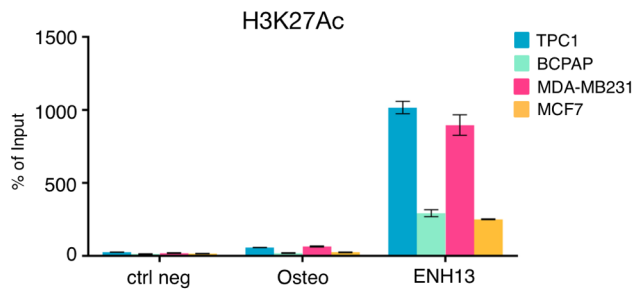
**A**

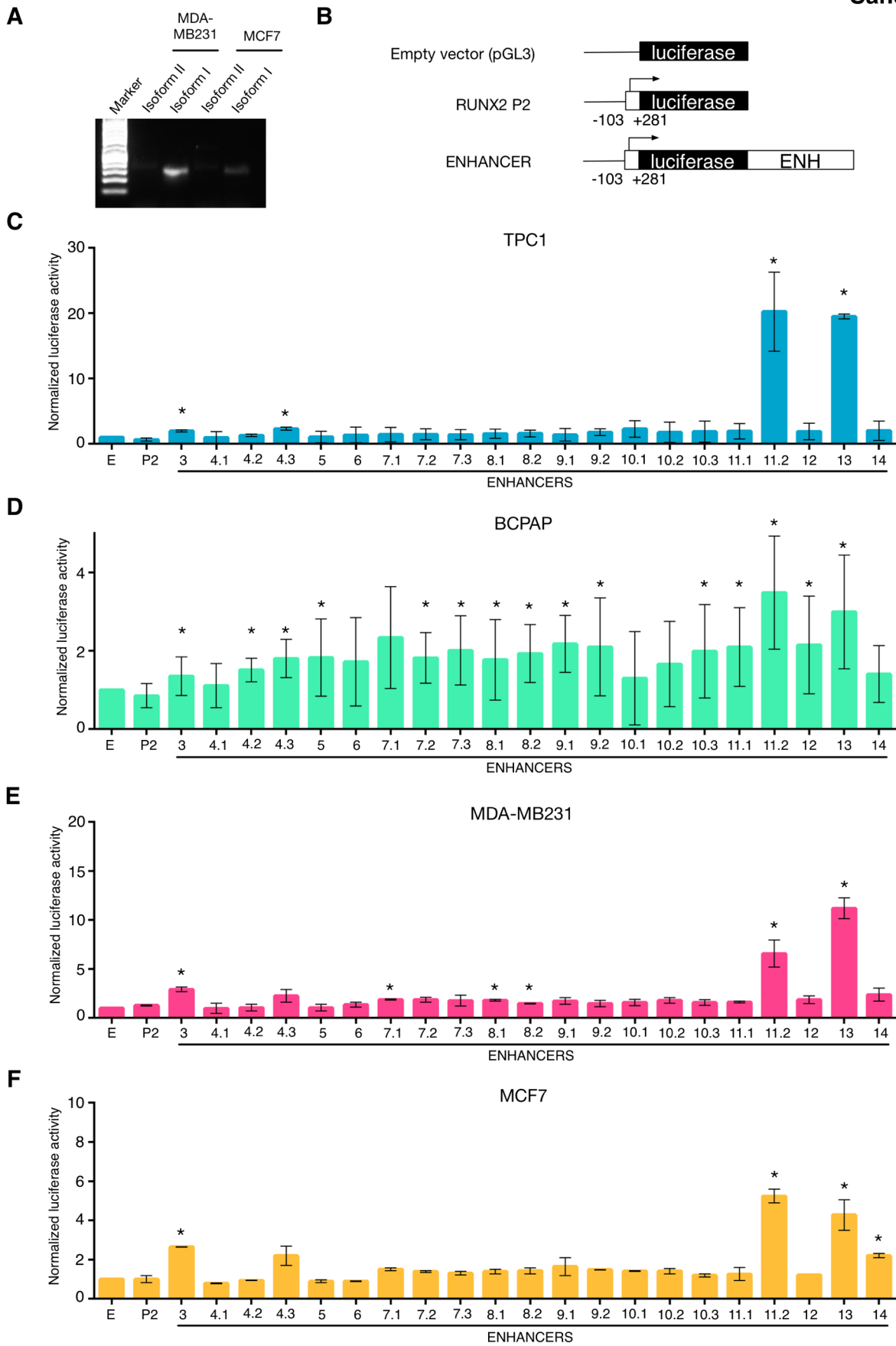


**B**



**C**





**A**

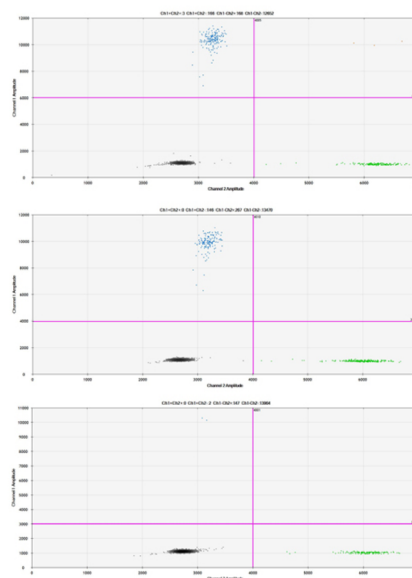
	Clone #	CNV
ENH3	3F	1.63
	9E	0.32
	7A	1.09
	8C	1.3
	8E	0.97
	1E	1.04
	1G	0.06
	7G	0.03
ENH11	8H	0.01
	2E	1.55
	11E	0.96
	2B	1.03
	2F	0.88
	1H	1.03
ENH13	3C	1.62
	5H	1.24
	4E	1.02
	8H	1.54
	8B	1.08
	12C	0.8
	4H	1.43
	6D	1.61
6B	0.55	
3F	1.24	
1D	0.91	

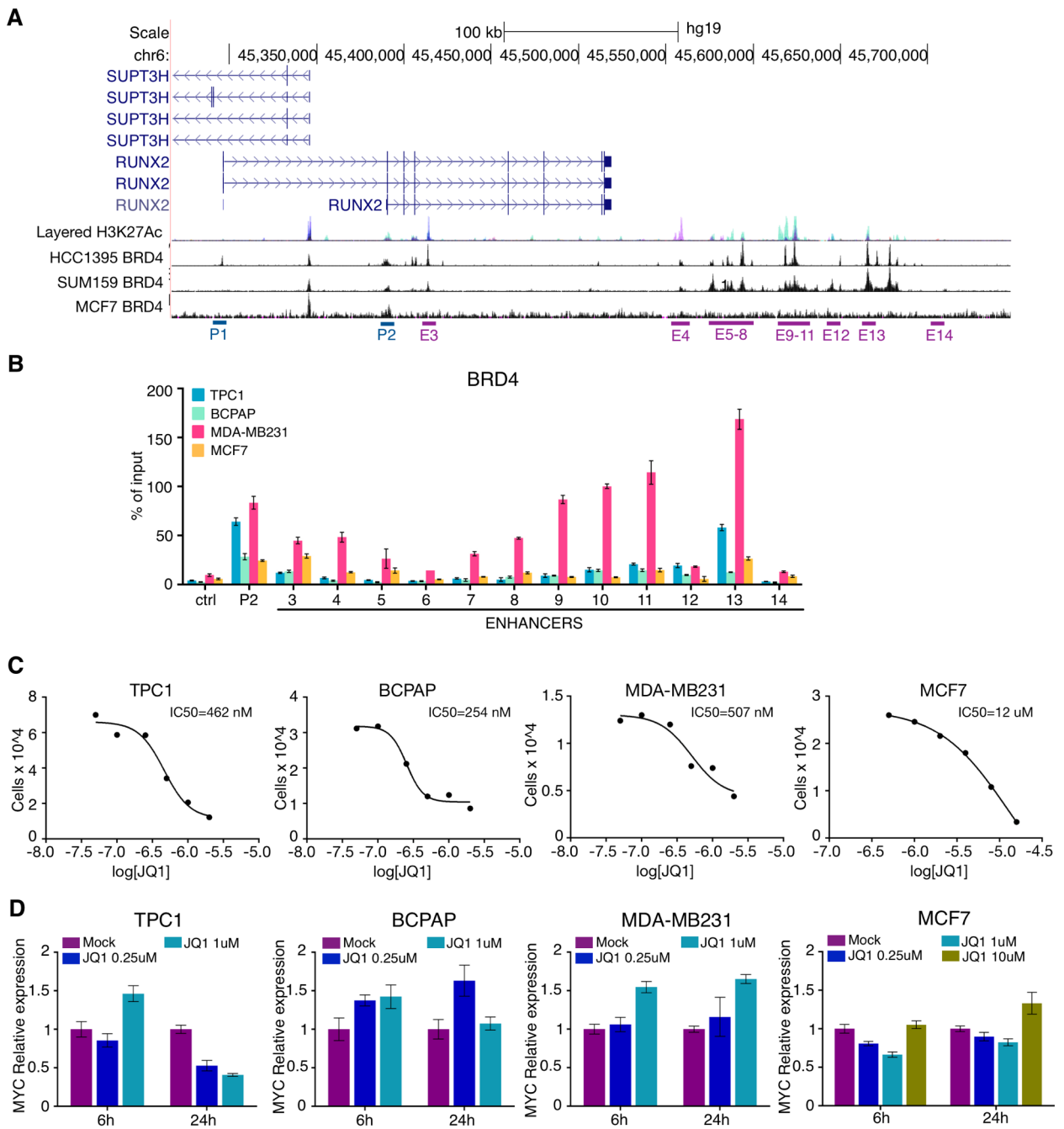
**B**

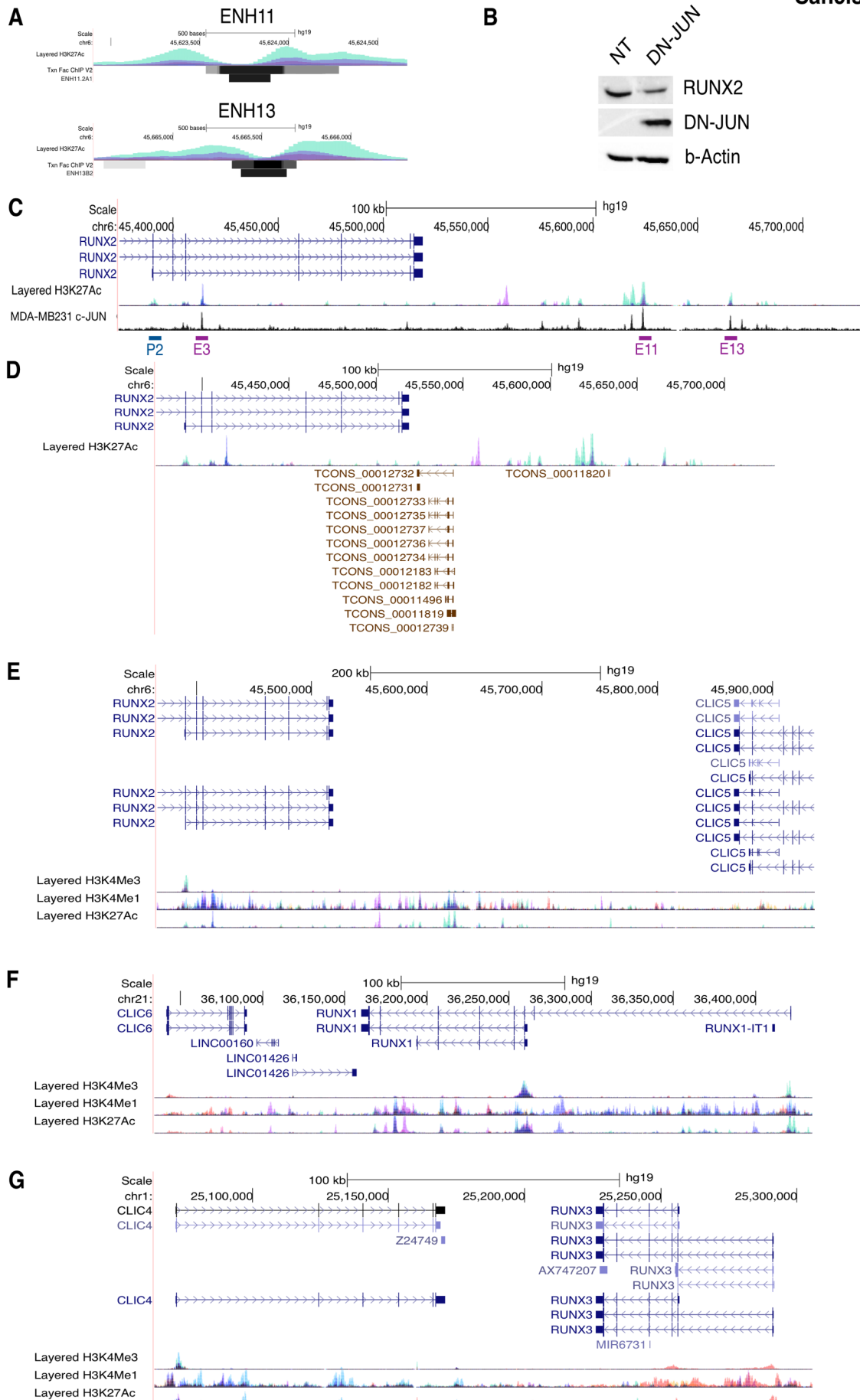
Wild-type

Deletion in heterozygosis

Deletion in homozygosis







## Supplementary Figures Legends

### *Supplementary Figure S1. Identification of putative RUNX2 ENHs in cancer cells*

A) Schematic representation of RUNX2 genomic locus showing the position of the putative ENHs. Tracks corresponding to the histone modifications, DNase clusters and mammals conservation used for the identification of the ENHs are displayed. The diagram was obtained by modification of the genome browser view (<http://genome.ucsc.edu>). B) Schematic representation of RUNX2 genomic locus showing the position of the two promoters (P1 and P2) and the putative ENHs we identified. An additional ENH is present only in osteoblast (Osteo). Tracks corresponding to H3K27ac enrichment in different cell lines are displayed. The diagram was obtained by modification of the genome browser view (<http://genome.ucsc.edu>). C) ChIP analysis of the osteoblasts-specific ENH (Osteo) in the indicated cell lines. A negative control region and ENH13 region are displayed for comparison.

### *Supplementary Figure S2. Luciferase analysis of ENHs fragments in the single cell lines*

A) End-point PCR analysis of RUNX2 Isoform I and RUNX2 Isoform II expression in MDA-MB231 and MCF7 cell lines. B) Schematic representation of the constructs used for luciferase analysis. C) Luciferase analysis of ENH3-14 regions activity in TPC1, BCPAP, MDA-MB231 and MCF7 cells. Cells were transfected with the indicated pGL3 constructs. The bars represent the average fold change of luciferase activity in cells transfected with the pGL3-P2 or pGL3-P2/ENHs vectors, normalized to Renilla luciferase activity for transfection efficiency control and to empty vector activity. All data are expressed as mean values  $\pm$  SEM, N=3 \*p<0.05.

### *Supplementary Figure S3. CRISPR/Cas9 deleted clones genotyping*

A) Table indicating for each CRISPR/Cas9 deleted clone the copy number for the region containing each ENH, measured by droplet digital PCR. B) A representative droplet digital PCR output for a wild-type clone, a clone carrying an ENH deletion in heterozygosis and a clone carrying an ENH deletion in homozygosis.

*Supplementary Figure S4. BRD4 binding to RUNX2 locus and JQ1 response*

A) Schematic representation of the RUNX2 locus showing BRD4 binding enrichment in a panel of breast cancer cell lines. The positions of the identified ENHs are indicated. B) ChIP analysis of BRD4 binding to the P2 and ENH4-14 regions in the indicated cell lines. C) JQ1 IC50 curves at 72h for the indicated cell lines. IC50 values were calculated using GraphPad Prism Software. D) qRT-PCR analysis of MYC expression in the indicated cell lines treated with the indicated concentrations of JQ1 or mock (DMSO). All data are expressed as mean values  $\pm$  SEM. \*  $P < 0.05$ . N=3

*Supplementary Figure S5. Additional analyses of the RUNX2, RUNX1 and RUNX3 loci*

A) Detail of H3K27ac enrichment and transcription factor binding density for the ENH11 and ENH13 regions. B) Western blot analysis of RUNX2 protein levels in TPC1 cells transfected with c-JUN DN construct or empty vector (NT). C) Schematic representation of the RUNX2 locus showing c-JUN binding in MDA-MB231 cell line. The positions of RUNX2 P2 promoter and ENHs are indicated. D) Non-coding RNAs identification in the RUNX2 locus. E-G) Comparison of the genomic organization of RUNX2, RUNX1 and RUNX3 loci.

**SUPPLEMENTARY TABLE I: PRIMERS**

<b>Cloning primers</b>	
<b>Name</b>	<b>Sequence</b>
ENH_4.1 Fw	gctacgGGATCC GCAACGGTGGTGATAATGAA
ENH_4.1 Rv	gctacgGTCGAC TTTTCATGCAAGGCACTGAG
ENH_4.2 Fw	gctacgGTCGACC CAAACAGGCAAAATGTGTG
ENH_4.2 Rv	gctacgGTCGAC CAAGGTGCAAATCATGCCTA
ENH_4.3 Fw	gctacgGGATCC CAAGAAGGCTTTGGCTTTCA
ENH_4.3 Rv	gctacgGTCGAC TTCCGAAGATAAGTTCGTGTGA
ENH_5 Fw	gctacgGGATCC CCCTTCAGTGAGGTTGGGAC
ENH_5 Rv	gctacgGTCGAC GGCTCTTGACCTCCACTCAG
ENH_6 Fw	gctacgGGATCC TGCCTAACCACAAAATCACAAG
ENH_6 Rv	gctacgGTCGAC ACTGGCTTGCAAAATTCCTG
ENH_7.1 Fw	gctacgGGATCC TGGGGATCTGTCTTGAGAGG
ENH_7.1 Rv	gctacgGTCGAC TAACTTTCTGGCCCTCAGCA
ENH_7.2 Fw	gctacgGGATCC GCAATAAGTTCTGCCGCAAT
ENH_7.2 Rv	gctacgGTCGAC GAGACGGGAATGATGTGGTC
ENH_7.3 Fw	gctacgGGATCC TGTGCTTGGCATTTCACAT
ENH_7.3 Rv	gctacgGTCGAC GTCCAAGGTCACACAGCAA
ENH_8.1 Fw	gctacgGGATCC AGAAGGCCATTTTCTCCTC
ENH_8.1 Rv	gctacgGTCGAC GGGAGTTGCTTGTTTTAGGG
ENH_8.2 Fw	gctacgGGATCC CCAAATCCACTTTTCTCCTG
ENH_8.2 Rv	gctacgGTCGAC GTGCAGTACCATGCTCATCC
ENH_9.1 Fw	gctacgGGATCC GTCTGTGGGGAGCAGAGAAG
ENH_9.1 Rv	gctacgGTCGAC TCCTGTA ACTCTTCCCCTGTG
ENH_9.2 Fw	gctacgGGATCC GGTAAGAAAGCAGGCCAAG
ENH_9.2 Rv	gctacgGTCGAC GAGCAGATTCTGGGAAGCAC
ENH_10.1 Fw	gctacgGGATCC CCCAGAATCTGCTCCATTA
ENH_10.1 Rv	gctacgGTCGAC AGCCTTGCCACTGTGTCTCT
ENH_10.2 Fw	gctacgGGATCC CCTGGCACAAGGGTTATCA
ENH_10.2 Rv	gctacgGTCGAC GGAACACACCCATTTGGAAG
ENH_10.3 Fw	gctacgGGATCC GCAGAACAGCCTTGCCTAAG
ENH_10.3 Rv	gctacgGTCGAC GTTGCCCAACTGTGTGAATG
ENH_11.1 Fw	gctacgGGATCC GGCAACCATCACCAGAACTT
ENH_11.1 Rv	gctacgGTCGAC CAGGAGAGCTGGAAGTACC
ENH_11.2 Fw	gctacgGGATCC CTGGTCAGTTCAGCTCTCC
ENH_11.2 Rv	gctacgGTCGAC CTATGACTTGCCCCAGGAAA
ENH_12 Fw	gctacgGGATCC CATGCATGTATGCACAGTGG
ENH_12 Rv	gctacgGTCGAC GGCCAAGGATACATGACCAA
ENH_13 Fw	gctacgGGATCC TCAGAAACCTTCAGGGCTTG
ENH_13 Rv	gctacgGTCGAC CAAGTGATTCTCCAGCCTCAG
ENH_14 Fw	gctacgCCCGGG CCCTCTCCTTCTTTCCCTTG
ENH_14 Rv	gctacgCCCGGG CTCCCAAAGTGCTGGGATTA
ENH11.2A Fw	gctacg GGATCC CTGGTCAGTTCAGCTCTCC
ENH11.2A Rv	gctacg GTCGAC AGAGATAGTTGGGATGTGGAAAC
ENH11.2B Fw	gctacg GGATCC GTTTCACATCCCAACTATCTCT
ENH11.2B Rv	gctacg GTCGAC TACATTTTCCAGCCTCTCTTTCA



ENH11.2C Fw	gctacg GGATCC TGAAAGAGAGGCTGGAAAATGTA
ENH11.2C Rv	gctacg GTCGAC CTATGACTTGCCCCAGGAAA
ENH11.2A1 Fw	gctacg GGATCC CTGGTCAGTTCCAGCTCTCC
ENH11.2A1 Rv	gctacg GTCGAC CTCTCTTCAGAAGCACCGG
ENH11.2A2 Fw	gctacg GGATCC TCATCAGACACTCAGCCCTC
ENH11.2A2 Rv	gctacg GTCGAC GGCAGCCCTCAGAAACATTT
ENH11.2A3 Fw	gctacg GGATCC CCTCTTCTCTTCCAGTCCCC
ENH11.2A3 Rv	gctacg GTCGAC AGAGATAGTTGGGATGTGGAAAC
ENH13A Fw	gctacg GGATCC TCAGAAACCTTCAGGGCTTG
ENH13A Rv	gctacg GTCGAC AAAGTGGCTAACATAACCCTGT
ENH13B Fw	gctacg GGATCC ACAGGGTTATGTTAGCCAGTTT
ENH13B Rv	gctacg GTCGAC AAGACCCAGAGAATCCCAGG
ENH13C Fw	gctacg GGATCC CCTGGGATTCTCTGGGTCTT
ENH13C Rv	gctacg GTCGAC CAAGTGATTCTCCAGCCTCAG
ENH13B1 Fw	gctacg GGATCC ACAGGGTTATGTTAGCCAGTTT
ENH13B1 Rv	gctacg GTCGAC TGTGTTAATTCAGGTAGGGCTG
ENH13B2 Fw	gctacg GGATCC GTCAGTTAATTACAGCCCTACCT
ENH13B2 Rv	gctacg GTCGAC GACCCCAAACAGAACACATGT
ENH13B3 Fw	gctacg GGATCC AAAACCATGGCCTTTTCACCC
ENH13B3 Rv	gctacg GTCGAC AAGACCCAGAGAATCCCAGG

<b>Real-Time PCR primers</b>		
	<b>Name</b>	<b>Sequence</b>
	RUNX2_for	GCTCTTCTTACTGAGAGTGGAAGG
	RUNX2_rev	GTGCCTAGGCGCATTTCA
	SUPT3H_for	GGGGAGCAAGGGTAATCACT
	SUPT3H_rev	TCATCGATGCCTTTGACAATCT
	MYC_for	TCCTCAAGAGGTGCCACG
	MYC_rev	GGCCTTTTTCATTGTTTTCCA
	c-JUN_for	TGACTGCAAAGATGGAAACG
	c-JUN_rev	CAGGTCATGCTCTGTTTCA
	TEAD1_for	CCACAAGCTCAAACACTTACCA
	TEAD1_rev	ACACAGGCCATGCAGAGTAG
	BRD4_for	ATGCCGTCAAGCTGAACCTC
	BRD4_rev	GATACATTCCTGAGCATTCCAGT
	RAIN_for	CGCTGATGCTACTGATCCAA
	RAIN_rev	GCTAAAGGGCAGATCACAGG
	CYPA_for	GACCCAACACAAATGGTTCC
	CYPA_rev	TTTCACTTTGCCAAACACCA
<b>CRISPRs/Cas9 sgRNA</b>		
	<b>Name</b>	<b>Sequence</b>
ENH3	sgRNA_A	TGTGCTAAGGACTAAAACCC
	sgRNA_B	AATAGTGTTAGGGACTTGAC
ENH11	sgRNA_C	AGGAGGGTCTGCTACACTCT

	sgRNA_D	GGGACCAAGAATTAGGCAA
ENH13	sgRNA_E	GTATGCTGTACAAGCCCTGA
	sgRNA_F	GTTACATCCATGACCTCACC
	<b>CRISPRs/Cas9 primers</b>	
	<b>Name</b>	<b>Sequence</b>
	ENH3_F	GGACACAGTTCTTGCATTCACA
	ENH3_R	GGACAGGTATGAATGGGAAAAGC
	ENH11_F	AAAACATGAAACTTGCTTTTATTGAG
	ENH11_R	TGACTTGCCCCAGGAAAACC
	ENH13_F	GAAAGGACATATACTGCAAGGGA
	ENH13_R	TTGGCCTAACAGTCAGCCAA
	<b>RACE primers</b>	
	<b>Name</b>	<b>Sequence</b>
	RAIN_RACE_F	<b>GATTACGCCAAGCTT</b> AGAAGTTCTGGAGTGGGGCCTGAGA
	RAIN_RACE_R	<b>GATTACGCCAAGCTT</b> GCCGGTTTTGCTGTTTCTTCCGCTAA

<b>ChIP primers</b>	
<b>Name</b>	<b>Sequence</b>
Neg Ctrl_for	TCTCAAGGTGCCTGTCTGC
Neg Ctrl_rev	TGAAGTTTGGCCTCTGGTCT
P2_for	ACCATGGTGGAGATCATCG
P2_rev	GGCAGGGTCTTTGTTGCAG
ENH3_for	GCTGGGAAGATAGCCAAGAA
ENH3_rev	CCTTGCATCAGTCCACAGA
ENH4_for	AAGTTGGCAGGGGAACAGAG
ENH4_rev	GTATTTTGACACGGTGCAGCA
ENH5_for	TCAAGCCGGAGTTCCTGATG
ENH5_rev	TTGTTGAAAGCCAGCCCTCA
ENH6_for	TGAGGAAGTCAGATGGCGTG
ENH6_rev	ACGATTGGGTGCACTGTGAA
ENH7_for	GAGGCCTTCAGAGACTGCAG
ENH7_rev	CACACCTTCTTGCCTCCTCC
ENH8_for	TGGCTCAGAGTCTTGGGAAC
ENH8_rev	TTTCTCTGAACCCTCGCCAG
ENH9_rev	GCTCACAATCTCCATGGCCT
ENH9_rev	GGCACACAGAAACACATCCC
ENH10_for	AGGAGGTGAGCTGCATAAACA
ENH10_rev	TCCCTCTGTACAGTCCCCAA
ENH11_for	CCCAAACCCAAAGCAGAGA
ENH11_rev	CCCAAGTTCTCACCAGGCAT
ENH12_for	GGTGAATTAGGGAGCCGAGG
ENH12_rev	ATTTGCAGGACACCTCAGGC
ENH13_for	GTGGAGTGGAGAGAGGAGAA
ENH13_rev	TGGCTTCATCTCACCCCTCAG
ENH14_for	GACTGGCTCTGCTCTTCTGG

ENH14_rev	CTGGTCGACTTTCCTCCCAG
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**SUPPLEMENTARY TABLE II: 3C FRAGMENTS AND PRIMERS**

**GENOMIC POSITIONS**

P2 ANCHOR	chr6:45,388,380-45,401,628
<b>P2 DOWNSTREAM</b>	
2	chr6:45,401,562-45,404,498
3	chr6:45,404,505-45,407,769
4	chr6:45,407,755-45,410,354
5	chr6:45,410,240-45,425,721
6	chr6:45,425,600-45,426,720
7	chr6:45,426,703-45,432,581
8	chr6:45,432,548-45,437,536
9	chr6:45,437,520-45,438,734
10	chr6:45,438,722-45,439,814
11	chr6:45,439,795-45,442,715
12	chr6:45,442,704-45,442,886
13	chr6:45,442,878-45,444,008
14	chr6:45,444,002-45,444,657
15	chr6:45,444,638-45,449,436
16	chr6:45,449,405-45,457,244
17	chr6:45,457,220-45,457,383
18	chr6:45,457,369-45,459,704
19	chr6:45,459,689-45,461,191
20	chr6:45,461,174-45,462,059
21	chr6:45,462,048-45,462,868
22	chr6:45,462,861-45,463,383
23	chr6:45,463,370-45,464,316
24	chr6:45,464,297-45,467,214
25	chr6:45,467,200-45,469,222
26	chr6:45,469,213-45,470,846
27	chr6:45,470,796-45,486,270
28	chr6:45,486,288-45,488,817
29	chr6:45,488,800-45,489,989
30	chr6:45,489,977-45,491,727
31	chr6:45,491,720-45,492,189
32	chr6:45,492,181-45,496,716
33	chr6:45,496,708-45,496,727
34	chr6:45,496,716-45,502,108
35	chr6:45,502,085-45,503,447
36	chr6:45,503,439-45,509,323
37	chr6:45,509,315-45,510,784
38	chr6:45,510,772-45,512,638
39	chr6:45,512,630-45,517,606
40	chr6:45,517,596-45,518,581

41	chr6:45,518,575-45,527,827
42	chr6:45,527,799-45,532,810
43	chr6:45,532,791-45,536,995
44	chr6:45,536,982-45,539,452
45	chr6:45,539,444-45,539,836
46	chr6:45,539,829-45,541,206
47	chr6:45,541,194-45,542,261
48	chr6:45,542,255-45,545,864
49	chr6:45,545,852-45,546,101
50	chr6:45,546,089-45,549,294
51	chr6:45,549,285-45,551,242
52	chr6:45,551,235-45,552,542
53	chr6:45,552,535-45,558,096
54	chr6:45,558,071-45,558,625
55	chr6:45,558,615-45,571,333
56	chr6:45,571,279-45,574,169
57	chr6:45,574,159-45,575,493
58	chr6:45,575,483-45,576,356
59	chr6:45,576,349-45,577,133
60	chr6:45,577,123-45,579,564
61	chr6:45,579,554-45,581,333
62	chr6:45,581,325-45,581,393
63	chr6:45,581,384-45,582,892
64	chr6:45,582,883-45,585,635
65	chr6:45,585,625-45,585,683
66	chr6:45,585,671-45,593,464
67	chr6:45,593,455-45,599,018
68	chr6:45,598,993-45,601,235
69	chr6:45,601,227-45,602,114
70	chr6:45,602,106-45,602,913
71	chr6:45,602,908-45,605,005
72	chr6:45,604,999-45,605,698
73	chr6:45,605,690-45,607,376
74	chr6:45,607,368-45,607,551
75	chr6:45,607,542-45,609,546
76	chr6:45,609,534-45,612,278
77	chr6:45,612,271-45,616,903
78	chr6:45,616,892-45,617,759
79	chr6:45,617,751-45,618,897
80	chr6:45,618,885-45,624,527
81	chr6:45,624,521-45,624,544
82	chr6:45,624,538-45,626,897
83	chr6:45,626,887-45,629,780
84	chr6:45,629,778-45,632,226
85	chr6:45,632,217-45,632,413
86	chr6:45,632,408-45,633,502

87	chr6:45,633,487-45,637,111
88	chr6:45,637,105-45,639,482
89	chr6:45,639,485-45,641,380
90	chr6:45,641,366-45,655,165
91	chr6:45,655,136-45,664,962
92	chr6:45,664,956-45,671,543
93	chr6:45,671,527-45,672,348
94	chr6:45,672,345-45,673,518
95	chr6:45,673,508-45,675,926
96	chr6:45,675,919-45,678,884
97	chr6:45,678,874-45,685,609
98	chr6:45,685,594-45,692,682
99	chr6:45,692,673-45,693,501
100	chr6:45,693,495-45,702,880
101	chr6:45,702,871-45,710,300
102	chr6:45,710,293-45,710,870
103	chr6:45,710,863-45,714,528
104	chr6:45,714,523-45,715,662
105	chr6:45,715,660-45,718,215
106	chr6:45,718,214-45,719,186
107	chr6:45,719,181-45,721,624
108	chr6:45,721,614-45,735,624
<b>P2 UPSTREAM</b>	
0	chr6:45,385,651-45,388,413
-1	chr6:45,385,507-45,385,805
-2	chr6:45,384,829-45,385,513
-3	chr6:45,379,610-45,384,843
-4	chr6:45,379,254-45,379,645
-5	chr6:45,377,180-45,379,262
-6	chr6:45,366,667-45,377,213
-7	chr6:45,363,827-45,366,733
-8	chr6:45,362,763-45,363,841
-9	chr6:45,362,612-45,362,772
-10	chr6:45,362,213-45,362,621
-11	chr6:45,361,569-45,362,220
-12	chr6:45,359,132-45,361,590
-13	chr6:45,358,792-45,359,145
-14	chr6:45,355,631-45,358,810
-15	chr6:45,346,052-45,355,671
-16	chr6:45,338,948-45,346,106
-17	chr6:45,338,209-45,338,974
-18	chr6:45,336,312-45,338,215
-19	chr6:45,334,837-45,336,324
-20	chr6:45,333,053-45,334,851
-21	chr6:45,332,961-45,333,065
-22	chr6:45,318,430-45,332,995

-23	chr6:45,313,544-45,318,454
-24	chr6:45,312,688-45,313,573
-25	chr6:45,311,424-45,312,702
-26	chr6:45,310,938-45,311,438
-27	chr6:45,310,489-45,310,945
-28	chr6:45,308,716-45,310,497
-29	chr6:45,307,203-45,308,734
-30	chr6:45,306,183-45,307,214
-31	chr6:45,305,041-45,306,191
-32	chr6:45,304,869-45,305,050
-33	chr6:45,304,001-45,304,879
-34	chr6:45,303,054-45,304,012
-35	chr6:45,301,045-45,303,071
-36	chr6:45,296,024-45,301,080
-37	chr6:45,294,987-45,296,039
-38	chr6:45,293,966-45,295,002
-39	chr6:45,292,743-45,293,985
-40	chr6:45,290,030-45,292,756
-41	chr6:45,279,084-45,290,132

## PRIMERS

P2 ANCHOR	TGAGGCTATTACACCCCAAGAAG
PROBE	AGTACACAACGCCGAGGGTAAAACGG
<b>P2 DOWNSTREAM</b>	
2	GGGGTCAGCTCTGTTTGGACA
3	TGACAATATGTACCAATGCAAAGTTT
4	GCCTACCACTTGCAGATCTGTATC
5	ACAGCAAGAGAATTTAAAGTATCCTATGT
6	AACCAGACAAATGCATTTTAGAGCC
7	GAAATTAATCAGGAGAGGGGAAGGA
8	CAATCCCACTGTGCATCAAGAGA
9	TTTCCTAGCTCATGTCATTGGAGAG
15	GGCTTCAAGTTCCTATGACTATGGA
18	ACACACATGCACACACATAGTATTG
24	ATCTAGGCTTTGCATGAGTTTCCTA
28	GGTTTCATTCTGAGCTTTAGTATCTCA
38	ATGAATATGATGTGGAGGAGAGTGC
43	ATACGTTGACACTTGAAGCAAAT
51	AGAGTTCTTTGTTTCTTGTATTGTTTTCAGA
52	AAAAGACCCTCCCCAACATAATACA
53	CAGGATGATTGTCTTCTAATTATGGCA
54	AAACAGGACAGTGAGGCAAGTCTA
55	GCCATAAATCAGACTTAGGCCTCAA
57	TTTTCTGCAGATATCCAGTAGTCTTT

58	TGTCTATGATCCATTTTCAGCAAGC
59	GGCTTGACTAGATAGGAGTGGAATT
60	GTGACTAAGGCAGACTCATACTC
61	TCAAAATGCTTCATGTCTAGAAAGGA
63	GGGCTATGACAGAGTAGGGTTC
66	CTACCAGTCTTAAAATGGGAATCTCTCA
68	AATATGTTTGCCACCCTGAAAAGTT
75	TAGTCCCTCCTTACCATCATTAAGC
76	GAAAGGGAATTAAGGAGCAAACAGG
77	CTGTCTCAGCCAAGCCAAGG
78	GGGAAGGAAAATCTCAACTTGAAGG
79	AAAACAAATGGAGTGCTACCATGAG
80	CCTTCTAATGATAACCCTTTGTGCC
82	TTGTCAGACTTGTTAGAGGTTTTGC
83	GCAGACATCCACCAGCTATAGTTAG
88	TGCACATGTACCCTAAAACCTAAAGT
91	GCACTGGGATTATTTGCTTAACCAT
92	CAAGCATTCTCCAGCTAACTCAAAT
95	TTTTGTTGAGTCAAGCATTACAGAAA
97	GAGGCGTATAAAATCAGAACAGCTC
100	ACCAGAACAAATATCACCAACCTA
101	TTTTACAAATCAGAAAACCGAGGCA
103	AGTGAACAAAACAAACAAGGTCCTT
104	TGGATCTATGGGTCCACTCTCTG
105	GAGCCCCTGAAGTTTACAACCAA
<b>P2 UPSTREAM</b>	
0	GGAAAACCAATGGAAAATTATGGGCA
-2	GCTTAGTAACTTAAGTGATCCCAAAGC
-3	AGTAAAGCACACTGAATGAGATGTTC
-5	TCATACTGGAATAAAGAAATGAGAAGCT
-6	CCCTAACTAGAAATTTATTTACAGCTTGC
-12	AAGTAATCATAGTACCAATATCTGCCCA
-14	CTTTCTCTTTCACAGGCCAATTTCTT
-15	CATTAAGATCCCGTTCCTCCAATTC
-16	TGTTTAATGCAGAATTCTCATAAGCT
-17	AAAATCTTGTGACCATGGCTTTTATTTT
-18	AGGGTGCAGAACTAGTAATAAGGTT
-20	TGCATTTACTTATAGCTAGGTACATTTTGT
-23	TCTAGAGAGAAACTGGGTCATAGAAA
-29	ACCTCTATTGGAAGAAAAGGAAGGAA
-34	GCAGTAGGTTGACATGTTGTAAAATG
-35	TGTATGGGAATGTATGGTCTGTTTCA
-36	CTTGTTGGTTCTGTGGTTGTTTGTG
-37	TGAGCTTAGAATAGAGTGGGTTAACA
-38	ATGTAGGTAGCTGCTGTAGTATGATC
-39	TTGGATTTGATGTATAGCTGTTTAAG



**SUPPLEMENTARY TABLE III: ENHs MUTANTS**

<b>ENH 11.2A1</b>		
<b>TF binding site</b>	<i>Sequence</i>	
	<b>Wild Type</b>	<b>Mutated</b>
AP-1 I	TTGAGTCA	TTGAGTCc
AP-1 II	TTGACTCA	TTGtCTCA
Tef-1	ACATTCCTGGAA	AgAcTCCTGGAA

<b>ENH 13B2</b>		
<b>TF binding site</b>	<i>Sequence</i>	
	<b>Wild Type</b>	<b>Mutated</b>
AP-1 I	TGACTAAA	TGACaAAA
AP-1 II	TGAGTCAT	TGAGcCAT
AP-1 III	TGACTACA	TGAgTACA
RUNX	GACCACAA	GACCaAA
Tef-1	GCATTCCAAAA	GCATTgCAAAA

*Mutated nucleotides are shown in lower case*

**SUPPLEMENTARY TABLE IV: CHIP ANTIBODIES**

<b>Name</b>	<b>Code</b>	<b>Manufacturer</b>
Anti - H3K27Ac	ab4729	Abcam
Anti - H3K4me1	ab8895	Abcam
Anti - H3K4me3	ab8580	Abcam
Anti - BRD4	A301-985A50	Bethyl
Anti - MED1 (anti-CRSP1/TRAP220)	A300-793A	Bethyl
Anti - c-Jun	60A8	Cell Signaling
Anti - Pol II PHOSPHO	ab5408	Abcam
Normal Rabbit Igg	2729S	Cell Signaling
Normal Mouse Igg	sc-2025	Santa Cruz

**SUPPLEMENTARY TABLE V: GENOMIC COORDINATES OF NGS CUSTOM PANEL**

<b>Region</b>	<b>chromosome</b>	<b>Start Position</b>	<b>End Position</b>
P2	chr6	45388682	45391269
ENH3	chr6	45413249	45414099
ENH11	chr6	45621903	45625564
ENH13	chr6	45664388	45666134

**SUPPLEMENTARY TABLE VI: LIST OF ENH11 AND ENH13 PREDICTED TF'S BINDING SITES**

**ENH11**

<b>Matrix</b>	<b>Factor name</b>	<b>Position (strand)</b>	<b>Core score</b>	<b>Matrix score</b>	<b>Sequence</b>
V\$RUSH1A_02	hltf	201 (+)	997	993	tccCTTTTgt
V\$XVENT1_01	Xvent-1	147 (-)	911	852	tggAAAAATAaaag
V\$CDX2_01	CDX group	146 (+)	1000	869	ctggaaaATAAAagaa
V\$FREAC3_01	FOXC	144 (+)	800	805	tcctgGAAAAataaaag
V\$BCL6_Q3_01	BCL-6 factors	144 (-)	950	952	tcctGGAAAa
V\$BCL6_Q3_01	BCL-6 factors	143 (-)	951	901	ttccTGAAAa
V\$STAT1_Q6	STAT factors	143 (+)	979	986	ttccTGAAAa
V\$BCL6_Q3_01	BCL-6 factors	142 (+)	984	928	aTTCCTggaa
V\$STAT1_Q6	STAT factors	142 (-)	991	989	aTTCCTggaa
V\$TEF1_Q6_04	Tef-1-related factors	140 (+)	1000	995	acATTCCtggaa
V\$P53_04	p53 related factors	133 (+)	965	766	tctCATGAcattcctggaaa
V\$CPBP_Q6	KLF6	128 (-)	997	997	gtGGGTC
V\$AP1_Q6_02	AP-1	93 (+)	1000	1000	TGACTcac
V\$NFAT1_Q4	NFAT-related factors	148 (+)	1000	1000	GGAAAa
V\$TATA_01	TBP-related factors	151 (+)	1000	890	aaATAAAagaagtga
V\$SREBP_Q6	SREBP factors	155 (-)	991	886	aaaagaAGTGAatat
V\$IK_Q5_01	Ikaros	199 (-)	952	953	ccTCCCT
V\$GKLF_Q4	KLF4 group	199 (+)	1000	1000	CCTCCct
V\$MAFA_Q4	MafA group	194 (+)	1000	960	TCAGCcc
V\$SMAD4_Q6_01	SMAD factors	186 (-)	1000	1000	tCAGACa
V\$MAFA_Q4	MafA group	174 (-)	1000	960	ggGCTGA
V\$ZNF333_01	ZNF333	168 (-)	1000	1000	ATTAT
V\$ZFP105_04	Zfp105	164 (-)	837	812	gaatattATTGGgctga
V\$ARID5A_03	Arid5a	163 (+)	1000	942	tgAATATtattggg
V\$PIT1_Q6_01	POU1F1	162 (-)	1000	939	gTGAATatta
V\$HMGIIY_Q3	HMG A factors	161 (+)	865	874	agtgaATATtattgg
V\$ARID5A_03	Arid5a	159 (-)	1000	955	gaagtgaATATTat
V\$NKX25_Q6	Nkx group	156 (-)	1000	950	aaagAAGTGaa
V\$AP1_Q6_02	AP-1	92 (-)	928	935	ttgACTCA
V\$TTF1_Q5_01	TTF-1 (Nkx2.1)	91 (+)	1000	990	CTTGAct
V\$P53_04	p53 related factors	44 (+)	891	778	tgcCTGGTgagaacttgga
V\$CP2_Q6	CP2-related factors	43 (+)	990	982	atgCCTGGtg
V\$BCL6_Q3_01	BCL-6 factors	32 (+)	984	980	gTTCCTagga
V\$RFX1_01	RFX-related factors	30 (-)	982	901	atGTTCCtaggagatgc
V\$YY1_Q6_03	YY1-like factors	28 (+)	996	996	CCATGtt
V\$MAFA_Q4	MafA group	20 (-)	1000	1000	ctGCTGA
V\$P53_04	p53 related factors	16 (-)	970	858	tctcctgctgagCCATGttc

V\$P53_04	p53 related factors	16 (+)	944	844	tctCCTGCtgagccatgttc
V\$RFX1_01	RFX-related factors	6 (-)	982	861	caGTTCCagctctcctg
V\$GEN_INI_B	general initiator	3 (+)	995	961	ggtCAGTT
V\$MYB_Q4	Myb-like factors	2 (+)	1000	987	tggtCAGTTcca
V\$ERALPHA_01	ER group	2 (-)	1000	707	tGGTCAgttccagct
V\$P53_04	p53 related factors	44 (-)	953	778	tgctggtgagaACTTGgga
V\$RBPJK_01	RBP-Jkappa	47 (+)	914	874	ctggTGAGAac
V\$TTF1_Q5_01	TTF-1 (Nkx2.1)	57 (+)	978	978	CTTGgga
V\$P53_04	p53 related factors	88 (-)	913	864	agacttgactcaCCAGGtct
V\$P53_04	p53 related factors	88 (+)	935	887	agaCTTGActcaccaggtct
V\$DR4_Q2	NR-DR	87 (+)	873	754	tagacttgacTCACCag
V\$P53_Q3	p53 related factors	87 (-)	931	920	tagACTTGact
V\$XVENT1_01	Xvent-1	77 (+)	1000	915	gaggtATTTGtag
V\$MEF2_03	Mef-2	75 (-)	1000	859	cagaggTATTTgtagacttgac
V\$AP1_Q6_02	AP-1	70 (+)	928	935	TGAGTcag
V\$AP1_Q6_02	AP-1	69 (-)	1000	1000	ttgAGTCA
V\$MAF_Q4	MAF group	69 (-)	937	917	tTGAGTcaga
V\$PBX_Q3	Pbx	67 (+)	1000	868	GATTGagtcaga
V\$GEN_INI_B	general initiator	67 (-)	995	964	GATTGagt
V\$IK_Q5_01	Ikaros	59 (+)	1000	971	TGGGAtc
V\$ERALPHA_Q6_01	ER group	1 (-)	1000	986	ctGGTCA

### ENH13

Matrix	Factor name	Position (strand)	Core score	Matrix score	Sequence
V\$CDX2_01	CDX group	2 (-)	862	843	tcagTTAATTacagcc
V\$FREAC3_01	FOXC	3 (-)	800	769	cagttaATTACagccc
V\$DRI1_01	DRI1	6 (-)	1000	1000	TTAATt
V\$DLX3_02	Dlx group	6 (+)	1000	998	tTAATTac
V\$IPF1_Q5	PDX group	6 (+)	962	958	tTAATTa
V\$DR4_Q2	NR-DR	18 (+)	708	716	ctacctgaatTAACaCa
V\$BBX_04	Bbx	20 (-)	1000	832	acctgaatTAACacact
V\$BBX_04	Bbx	21 (+)	798	809	cctgaATTAacacactt
V\$PIT1_Q6_01	POU1F1	22 (-)	1000	937	cTGAATtaac
V\$CDC5_01	CDC5L	24 (+)	1000	855	gaaTTAACacac
V\$DRI1_01	DRI1	25 (+)	1000	1000	aATTAA
V\$NKX25_Q6	Nkx group	31 (+)	1000	981	caCACTTgact
V\$EBOX_Q6_01	Ebox	32 (+)	980	973	aCACTTgact
V\$TTF1_Q5_01	TTF-1 (Nkx2.1)	35 (+)	1000	990	CTTGAct
V\$AP1_Q6_02	AP-1	37 (+)	1000	970	TGACTaaa
V\$FAC1_01	FAC1	41 (-)	897	879	taaatCTTTTgctt
V\$CEBPA_Q6	C/EBP group	47 (-)	997	953	ttTTGCTtatgga
V\$NF1A_Q6_01	NF-1A	61 (-)	991	985	cCTGGCg
V\$AP2ALPHA_03	AP-2	61 (+)	586	765	ccTGGCGgaggccgt
V\$AP2ALPHA_03	AP-2	61 (-)	954	765	cctggcggAGGCCgt

V\$GKLF_Q4	KLF4 group	65 (-)	1000	947	gcGGAGG
V\$BCL6_Q3_01	BCL-6 factors	76 (+)	886	909	cTTACAagaa
V\$BEN_01	BEN	86 (-)	863	871	gccTGCTG
V\$MEIS1_01	MEIS, PKNOX, TGIF	88 (-)	1000	997	ctgcTGTCAgaa
V\$GEN_INI_B	general initiator	98 (-)	991	961	AATTGagt
V\$AP1_Q6_02	AP-1	100 (-)	1000	1000	ttgAGTCA
V\$AP1_Q6_02	AP-1	101 (+)	928	935	TGAGTcat
V\$MAFA_Q4	MafA group	108 (+)	1000	960	TCAGCtt
V\$TTF1_Q5_01	TTF-1 (Nkx2.1)	112 (+)	968	968	CTTGCgt
V\$HIF1A_Q5	HIF1	113 (+)	965	958	ttGCGTGca
V\$AHR_Q6	AhR	114 (-)	1000	1000	tGCGTG
V\$SOX2_Q3_01	Sox-related factors	122 (+)	1000	946	tgaagaACAAAtgct
V\$SR_Y_Q6	Sox-related factors	126 (-)	1000	947	gAACAA
V\$XVENT1_01	Xvent-1	126 (-)	1000	848	gaaCAAATgctta
V\$GKLF_Q4	KLF4 group	141 (+)	1000	1000	CCTCctt
V\$RUSH1A_02	hltf	142 (+)	989	988	ctcCTTCTgc
V\$CEBPA_Q6	C/EBP group	143 (+)	1000	988	tccttcTGCAAtg
V\$CDPCR1_01	CDP	150 (-)	757	810	gcaaTGcATt
V\$TEF1_Q6_04	Tef-1-related factors	155 (+)	1000	963	gcATTCCaaaa
V\$CEBPA_Q6	C/EBP group	156 (-)	976	976	caTTCCAaaagaa
V\$FAC1_01	FAC1	158 (+)	897	874	ttccAAAAGaacga
V\$FAC1_01	FAC1	170 (+)	926	909	gaccACAAAaccat
V\$AML3_Q6	Runt-related factors	170 (+)	1000	985	gACCACaa
V\$HNF4A_Q3	HNF-4 group	182 (-)	869	889	atggcCTTTTcacc
V\$RUSH1A_02	hltf	184 (+)	997	990	ggcCTTTTca
V\$SREBP_Q6	SREBP factors	187 (+)	1000	991	ctttTCACCcagga
V\$GATA_Q6	GATA	210 (-)	1000	1000	cTTATct
V\$MECP2_02	MECP2	211 (-)	1000	977	ttatcTCCGG
V\$CEBPA_Q6	C/EBP group	220 (-)	997	996	ggTTGCTcaacc
V\$MAFA_Q4	MafA group	222 (-)	895	898	ttGCTCA
V\$AP1_Q6_02	AP-1	233 (+)	1000	917	TGACTaca
V\$EBOX_Q6_01	Ebox	236 (-)	989	942	ctacATGTGt
V\$FAC1_01	FAC1	239 (-)	934	929	catgtGTTCTgttt
V\$RREB1_01	RREB-1	243 (-)	1000	832	tgttctgtTTGGGg

**SUPPLEMENTARY TABLE VII: EXPRESSION OF ENH11 and ENH13 PREDICTED TFs FROM THE CANCER CELL LINE ENCYCLOPEDIA PROJECT**

**ENH11**

	<b>MDA-MB-231</b>	<b>MCF-7</b>	<b>BCPAP</b>
<b>ARID5A</b>	-0.46	0.38	1.09
<b>BCL6</b>	0.47	0.3	0.55
<b>CDX2</b>	-0.34	-0.38	0.47
<b>ESR1</b>	-0.26	13.75	2.38
<b>FOS</b>	0.57	0.43	0.64
<b>FOXC1</b>	0.40	0.38	0.33
<b>HMG1Y</b>	1.77	-1.34	1.37
<b>IKZF1</b>	0.42	-0.33	-0.07
<b>JUN</b>	0.64	-0.31	0.54
<b>KLF4</b>	0.71	0.27	0.069
<b>KLF6</b>	0.75	-1.04	-0.66
<b>MAF</b>	-0.40	-0.30	-0.14
<b>MAFA</b>	-	-	-
<b>MEF2A</b>	-0.17	0.63	0.18
<b>MYB</b>	-0.62	2.06	-0.78
<b>NFATC2</b>	0.24	-0.17	-1.76
<b>NKX2-5</b>	0.02	-0.48	-1.08
<b>NR1H3</b>	0.72	-0.26	0.40
<b>NR1I2</b>	1.09	-0.22	1.12
<b>NR1I3</b>	2.18	3.35	-2.13
<b>NR2F2</b>	0.93	0.64	-0.28
<b>PBX1</b>	0.26	1.25	0.55
<b>POU1F1</b>	-0.48	0.15	0.17
<b>RARS</b>	0.25	0.44	2.68
<b>RBPJ</b>	2.14	-1.01	0.93
<b>RFX1</b>	-0.17	2.05	2.01
<b>SMAD4</b>	-0.66	-1.56	0.87
<b>SREBF1</b>	-0.40	1.77	0.30
<b>STAT1</b>	-0.29	0.58	0.12
<b>TEAD1</b>	0.87	-0.96	1.09
<b>TEAD2</b>	0.07	0.65	0.58
<b>TEAD3</b>	1.10	0.03	0.23
<b>TEAD4</b>	-0.49	-0.52	-0.57
<b>TFCP2</b>	-1.80	-7.09	0.47
<b>TP53</b>	0.26	0.86	1.20
<b>TTF1</b>	0.15	0.46	0.83
<b>YY1</b>	-0.29	0.58	0.45
<b>ZNF333</b>	-0.11	-0.33	1.34

ZNF35	0.008	-1.08	-0.74
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### ENH13

	MDA-MB-231	MCF-7	BCPAP
AHR	0.59	0.46	-0.67
ARID3A	-1.03	0.51	0.20
BBX	1.54	-1.58	1.35
BCL6	0.47	0.30	0.55
BPTF	-1.36	2.78	0.62
CDC5L	1.32	0.26	-0.19
CDX2	-0.34	-0.38	0.47
CEBPA	-0.49	1.17	-0.58
CR1	0.06	0.15	-0.77
CUX1	-0.67	1.80	-0.61
DLX3	0.24	2.52	-0.33
FOS	0.57	0.43	0.64
FOXC1	0.40	0.38	0.33
GATA1	0.71	1.10	-1.90
HIF1A	0.32	-0.36	0.54
HNF4A	0.17	0.07	-0.69
JUN	0.64	-0.31	0.54
KLF4	0.71	0.27	0.07
MAFA	-	-	-
MECP2	1.11	0.98	2.11
MEIS1	-1.08	-0.26	2.95
NFIA	0.16	-1.05	-0.31
NKX2-5	0.02	-0.48	-1.08
NR1H3	0.72	-0.26	0.40
NR1I2	1.09	-0.22	1.12
NR1I3	2.18	3.35	-2.13
NR2F2	0.93	0.64	-0.28
NR5A2	-0.39	0.37	-0.59
POU1F1	-0.48	0.15	0.17
RARS	0.25	0.44	2.68
RREB1	-0.35	1.12	-0.42
RUNX2	2.57	0.58	2.58
SOX2	-0.22	1.53	-0.60
SREBF1	-0.40	1.77	0.30
TEAD1	0.87	-0.96	1.09
TEAD2	0.07	0.65	0.58
TEAD3	1.10	0.03	0.23

<b>TEAD4</b>	-0.49	-0.52	-0.57
<b>TFAP2A</b>	-0.32	1.04	-0.67
<b>TTF1</b>	0.15	0.46	0.83

Cell lines expression data were obtained from Cancer Cell Line Encyclopedia (CCLE) and examined using Integrative Genomics Viewer (IGV)

<https://portals.broadinstitute.org/ccle/data/browseAnalyses>

Values reported from CCLE are gene-centric RMA-normalized mRNA expression data.