Supplemental Data

Figure S1.A

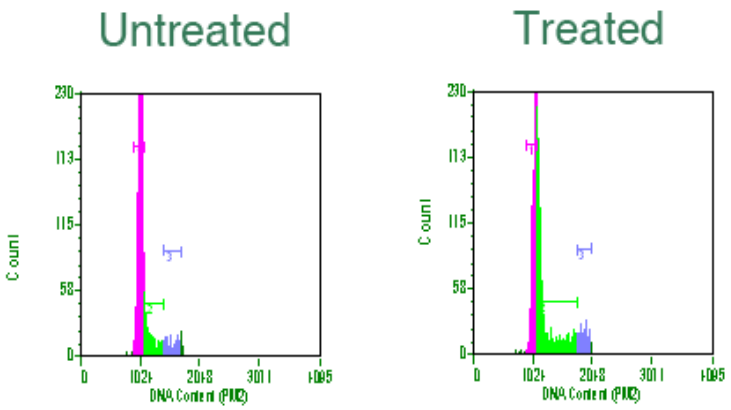

Figure S1.A: Flow cytometry analysis of DNA content of untreated and sodium butyrate treated Hela cells as described in materials and methods. The pin, green and blue peaks corresponds to cells in G1, S and G2/M phases of cell cycle respectively.

Figure S1.B

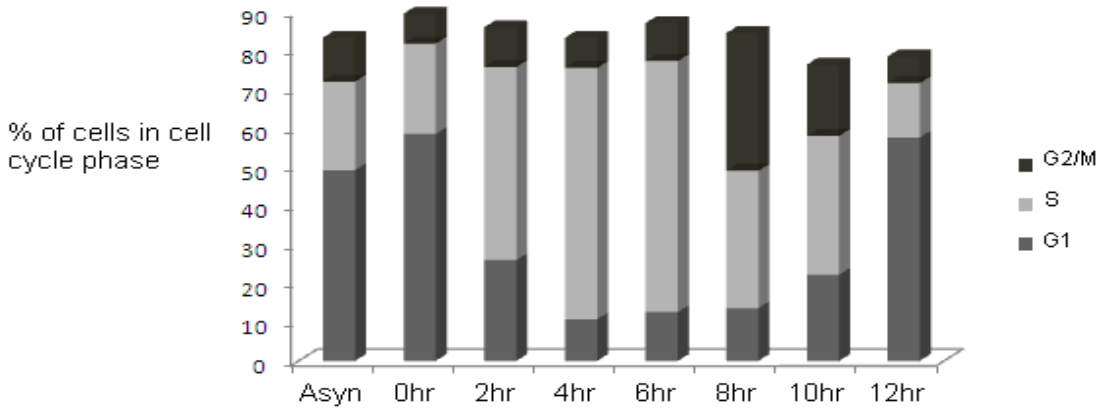

Figure S1.B: Cell cycle analysis. Hela cells were synchronized by double thymidine block and released to proceed through the cell cycle as described in 'Experimental Procedures'. Cells were collected after thymidine treatment (0 h) and at indicated time points after release and cell cycle progression was analyzed by monitoring DNA content employing flow cytometry as described in 'Experimental Procedures'.

Figure S1C

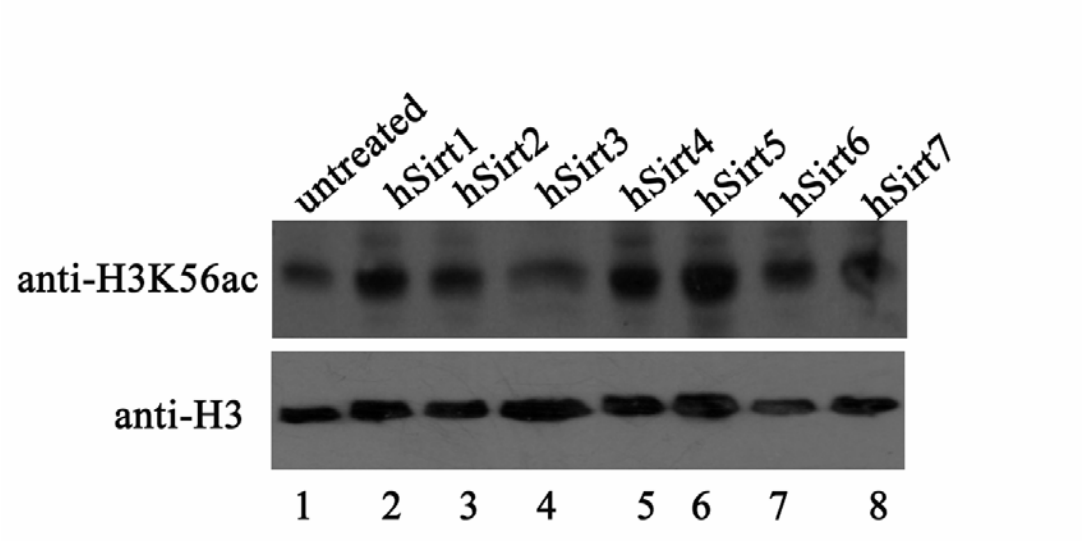

Figure S1.C: ***In vivo*** **deacetylation of H3K56 by sirtuins.** Sirtuins involved in the deacetylation of H3K56ac were tested by monitoring the reduction in level of H3K56 acetylation upon overexpression of seven human sirtuins, by transient transfection in HEK-293T cells as described in 'Experimental Procedures'. Immunoblot with anti-pan H3 was used as loading control.

Figure S1.D

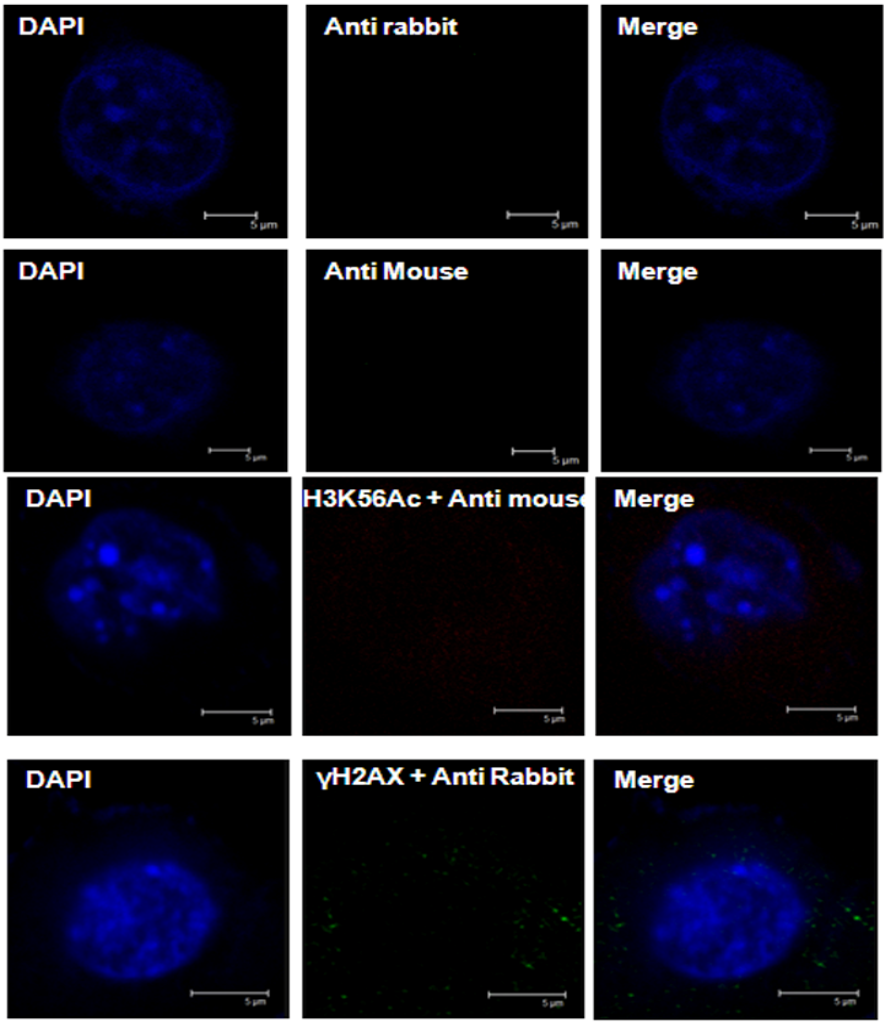

Figure S2.D: Control experiments for coimmunofluoresence (Co-IF). Top two panels depict a control experiment without primary antibodies showing there is no background staining caused by the secondary antibodies alone. HEK-293T cells were stained with Alexa Fluor 488-conjugated donkey anti-rabbit and Alexa Fluor 647-conjugated goat anti-mouse secondary antibodies without prior incubation with primary antibodies. Third and fourth rows show cells stained with anti-H3K56ac antibody in combination with AlexaFluor 647-coupled goat anti-mouse IgG and anti-γH2AX with AlexaFluor 488 coupled donkey anti-rabbit IgG, respectively, to show lack of cross reactivity.

Figure S1.E

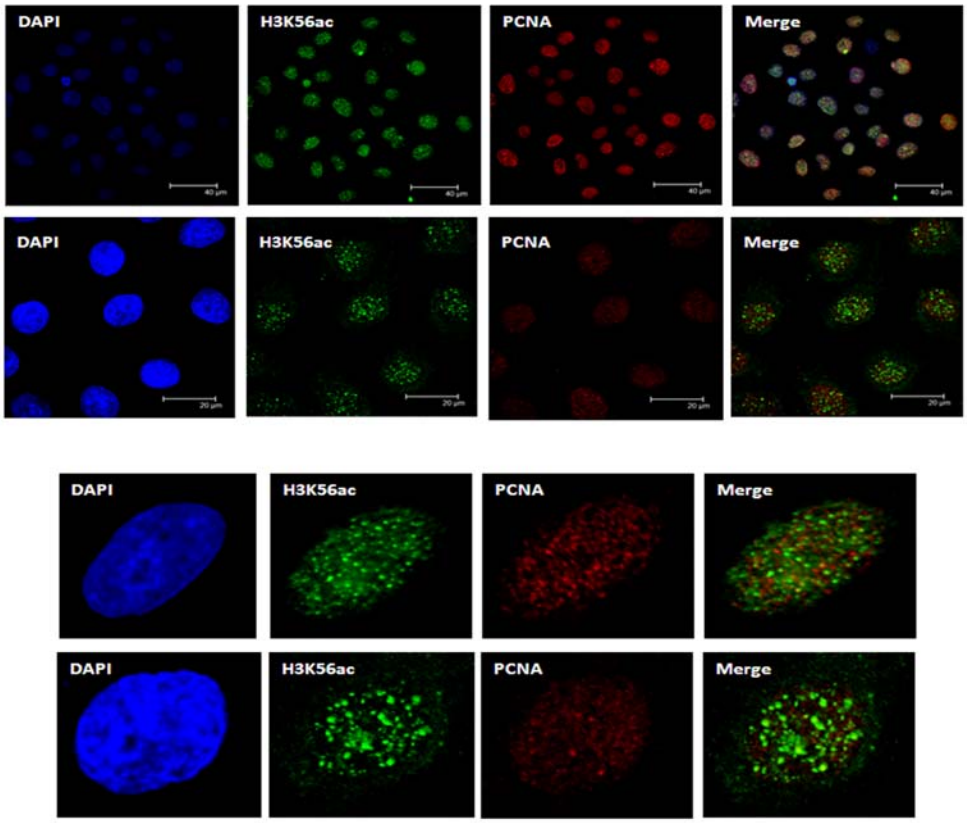

Figure S1.E: Coimmunofluoresence showing localization of H3K56ac and PCNA in Hela cells. Asynchronous HeLa cells were coimmunostained with anti-H3K56ac antibody in combination with anti-PCNA antibody. Nuclei were stained with DAPI.

Figure S2. A

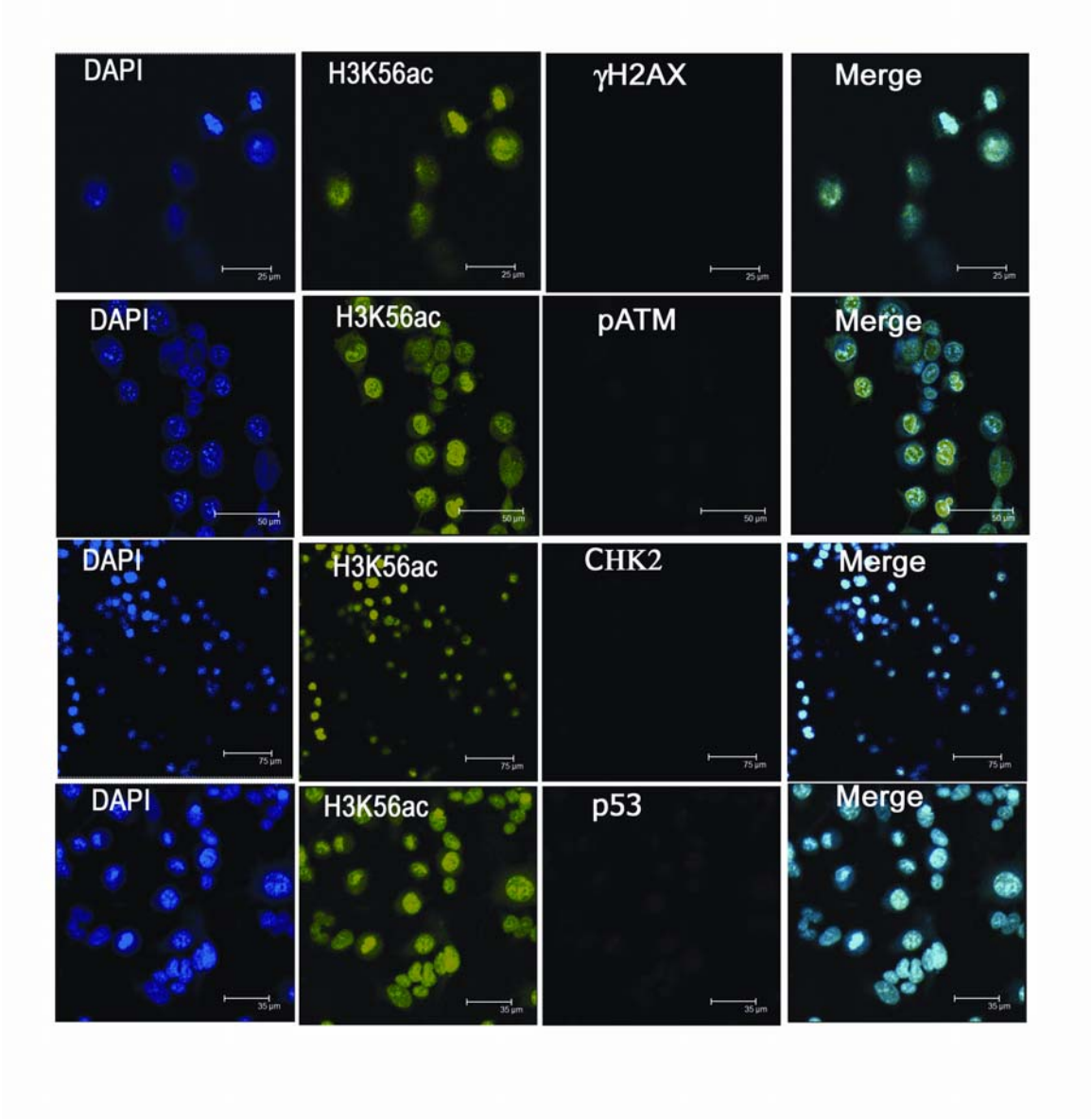

Figure S2.A: A field of untreated HEK293 cells showing diffused H3 K56ac staining: Untreated HEK293T cells immunostained with H3K56ac antibodies and antibodies against each of the indicated DNA DSB markers (γ-H2AX, pATM, Chk2 and p53).

Figure S2. B

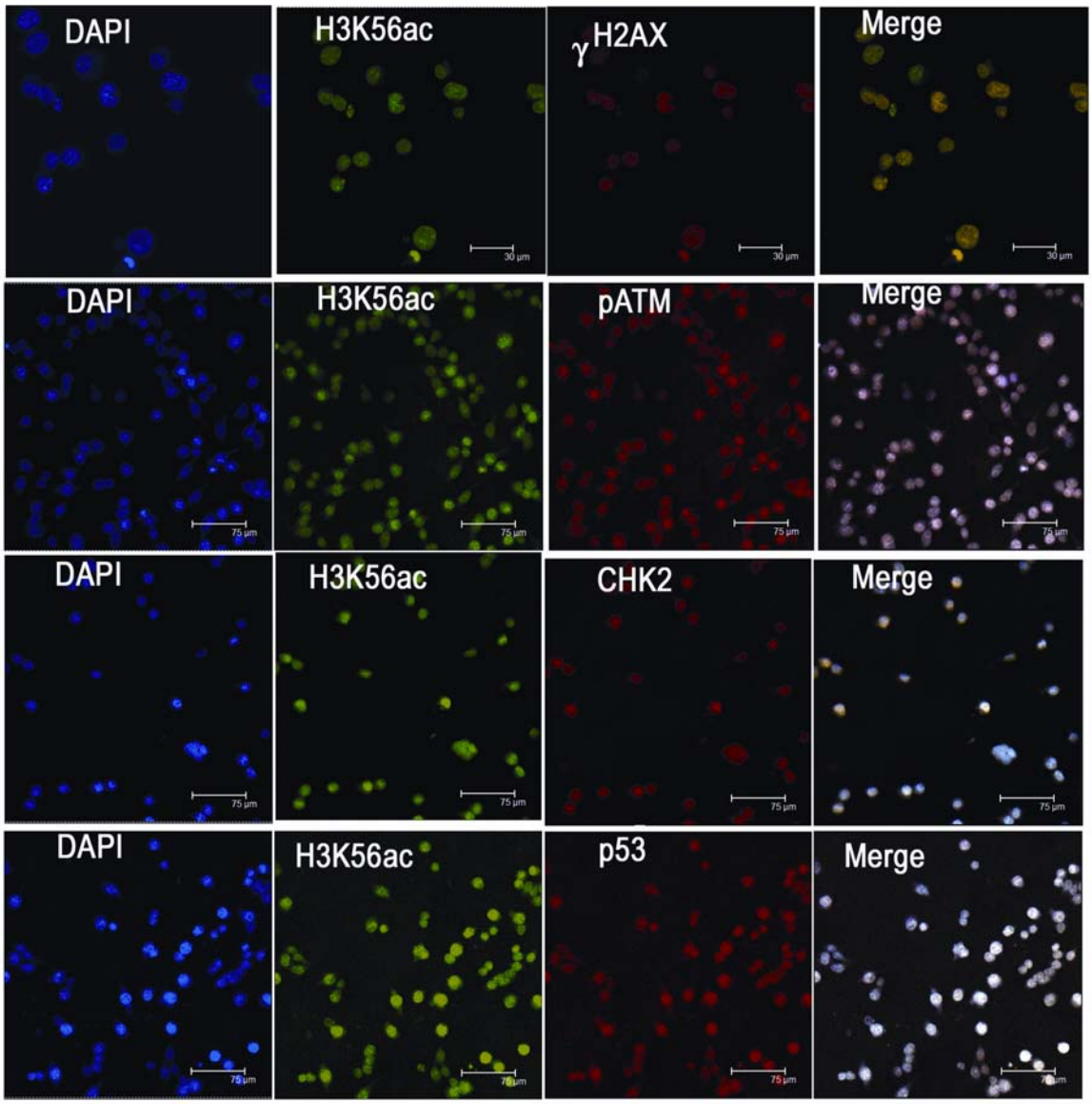

Figure S2.B: Colocalization of H3K56ac with γ-H2AX, pATM, Chk2 and p53. A field of MMS (0.02%) treated HEK293 cells immunostained with H3K56ac antibodies and antibodies against each of the indicated DNA DSB markers showing H3 K56ac.

Figure S2.C

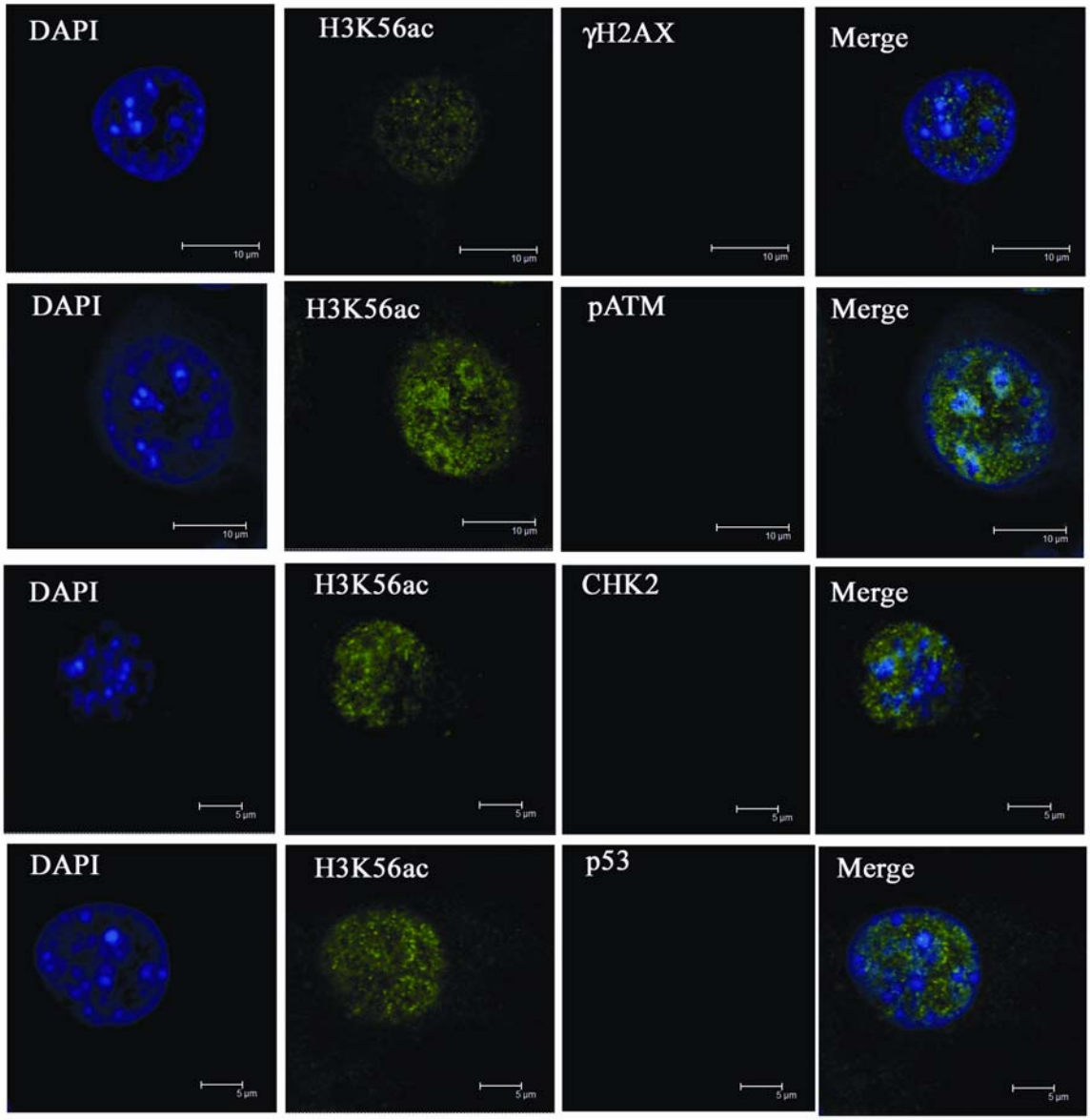

Figure S2.C: Coimmunofluorescence of H3K56ac and γ-H2AX, pATM, Chk2 and p53 in untreated HEK293T cell. A single untreated HEK293T cells immunostained with H3K56ac antibodies and antibodies against each of the indicated DNA DSB markers (γ-H2AX, pATM, Chk2 and p53).

Figure S3

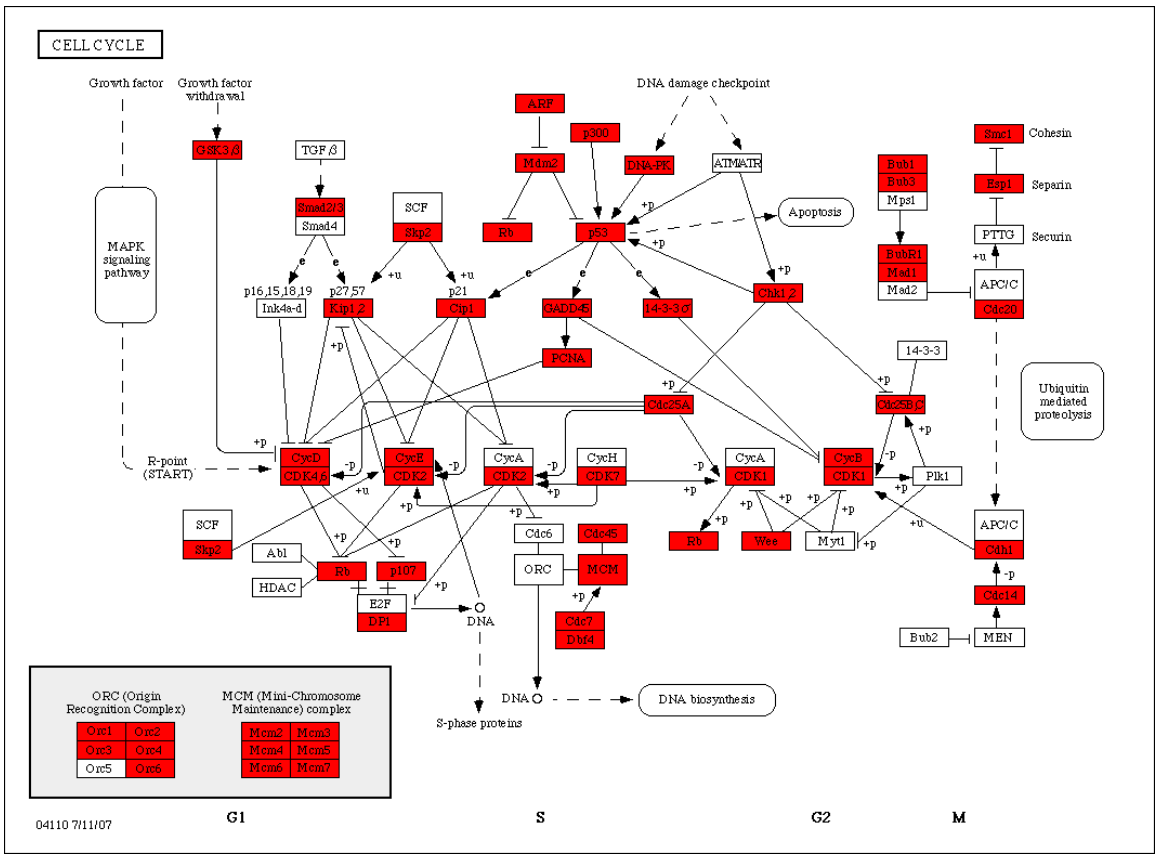

Figure S3. Pathway analysis showing promoters of several genes in cell cycle pathway have elevated levels of H3K56ac. ChIP on chip was carried out using anti-H3K56ac antibodies as described in experimental procedure. Pathway analysis of enriched gene promoters was carried out using gene spring software. Genes indicated in red have increased levels of H3K56ac at their promoters.

Figure S4

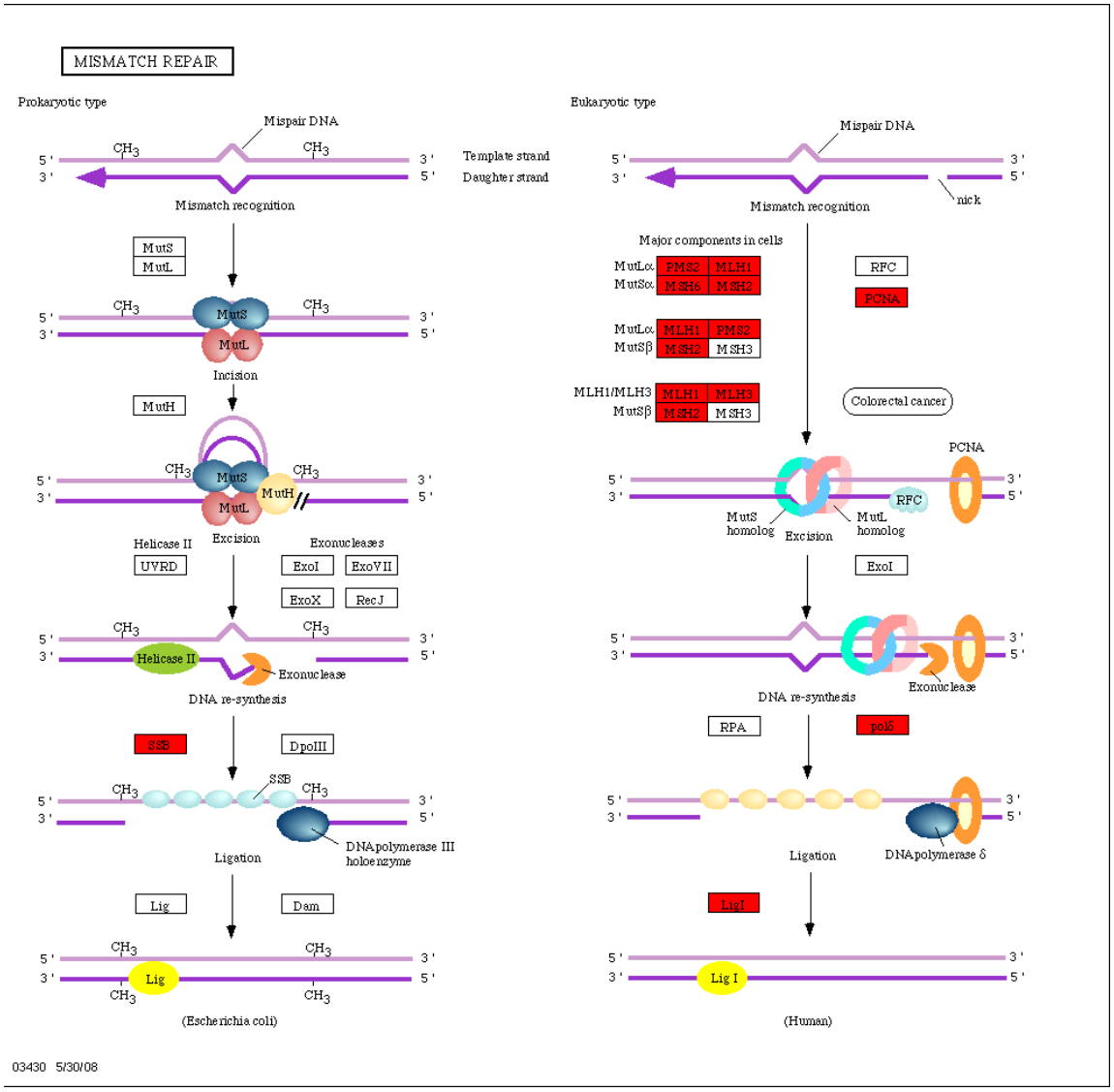

Figure S4. Pathway analysis showing promoters of several genes in mismatch repair pathway have elevated levels of H3K56ac. ChIP on chip was carried out using anti-H3K56ac antibodies as described in experimental procedure. Pathway analysis of enriched gene promoters was carried out using gene spring software. Genes indicated in red have increased levels of H3K56ac at their promoters.

Figure S5

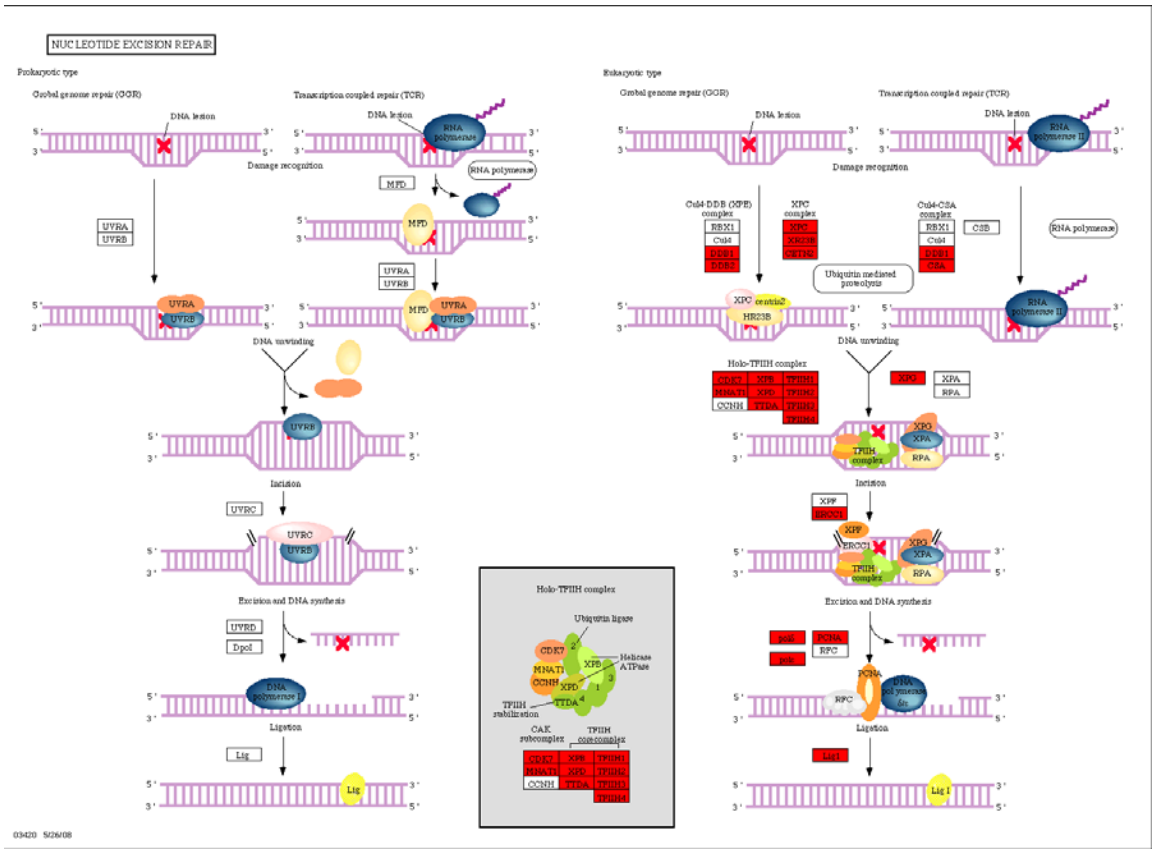

Figure S5. Pathway analysis showing promoters of several genes in nucleotide excision repair pathway have elevated levels of H3K56ac. ChIP on chip was carried out using anti-H3K56ac antibodies as described in experimental procedure. Pathway analysis of enriched gene promoters was carried out using gene spring software. Genes indicated in red have increased levels of H3K56ac at their promoters.

Figure S6

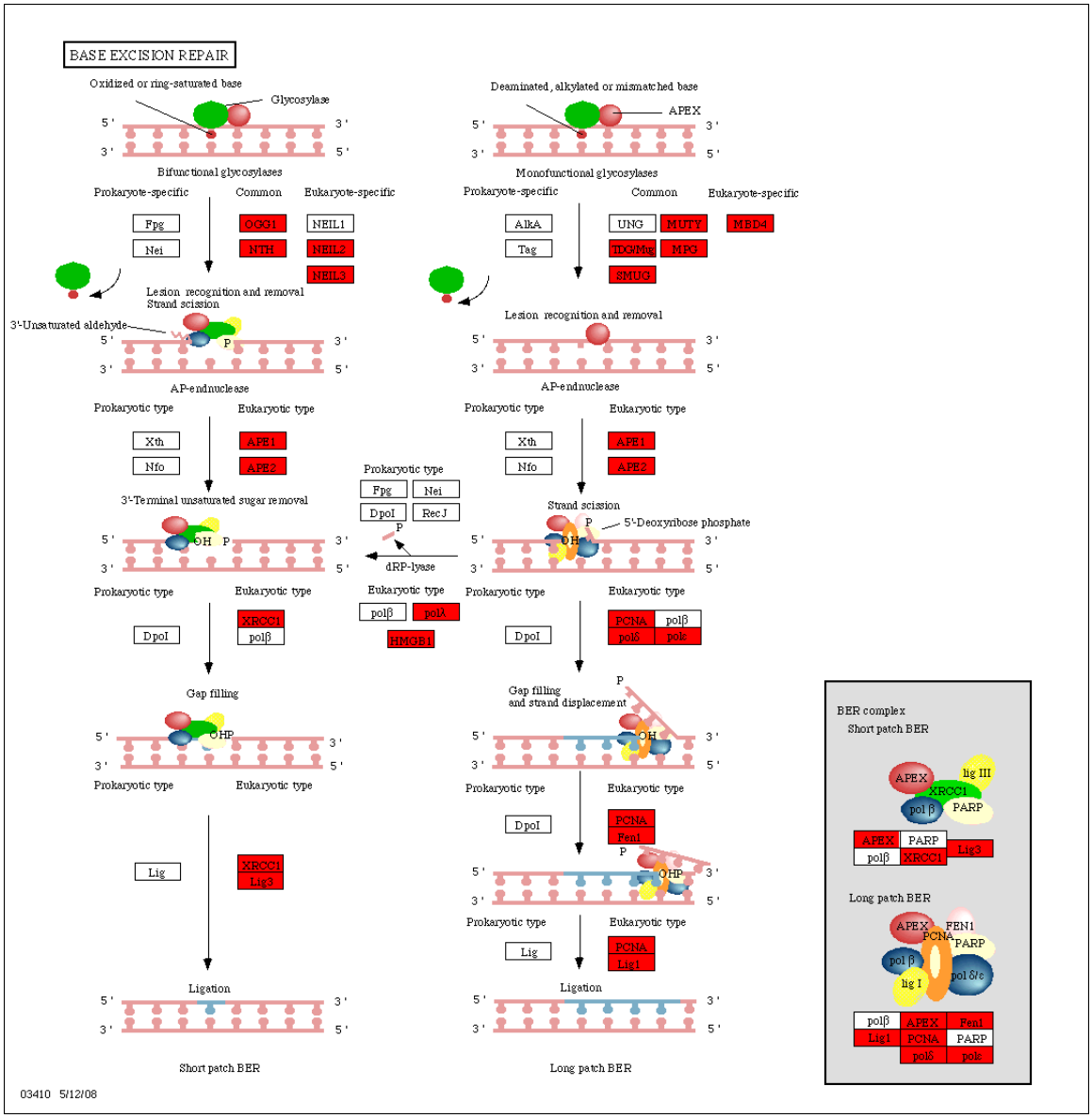

Figure S6. Pathway analysis showing promoters of several genes in base excision repair pathway have elevated levels of H3K56ac. ChIP on chip was carried out using anti-H3K56ac antibodies as described in experimental procedure. Pathway analysis of enriched gene promoters was carried out using gene spring software. Genes indicated in red have increased levels of H3K56ac at their promoters.

Figure S7

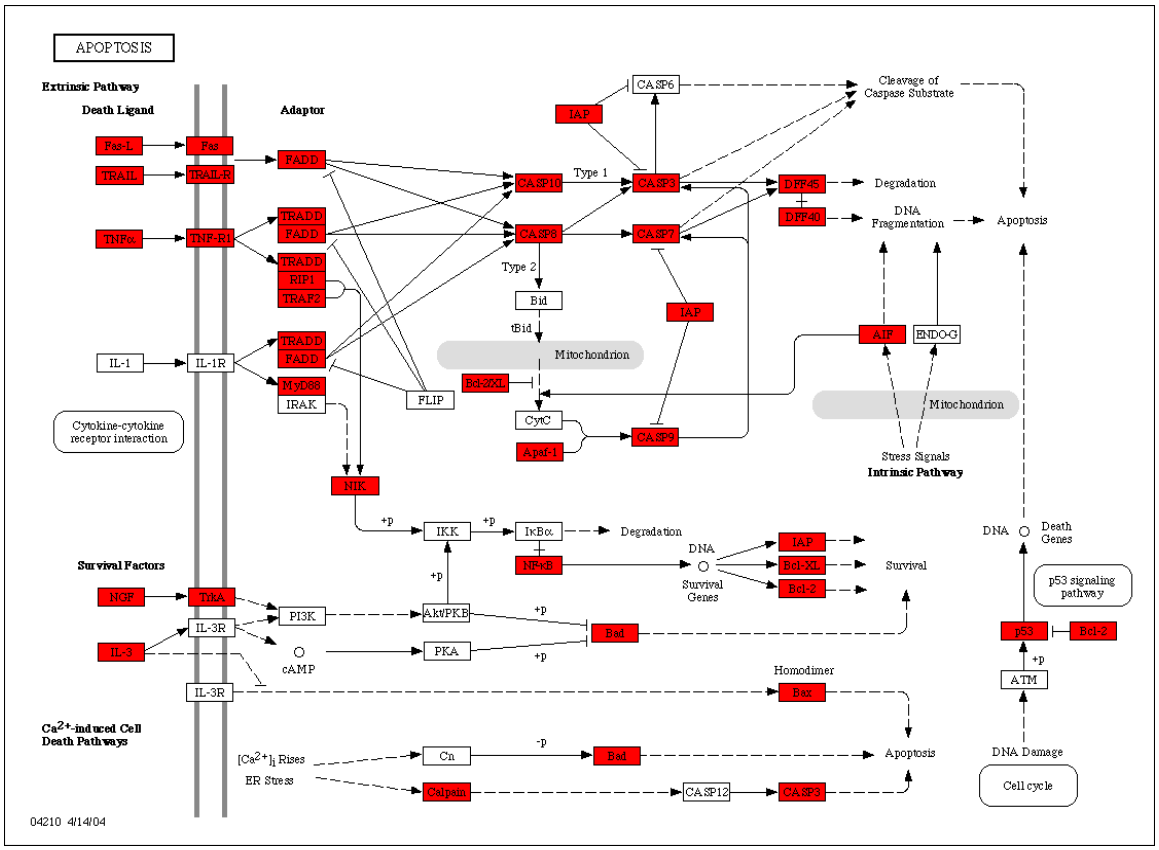

Figure S7. Pathway analysis showing promoters of several genes in apoptosis pathway have elevated levels of H3K56ac. ChIP on chip was carried out using anti-H3K56ac antibodies as described in experimental procedure. Pathway analysis of enriched gene promoters was carried out using gene spring software. Genes indicated in red have increased levels of H3K56ac at their promoters.

Figure S8

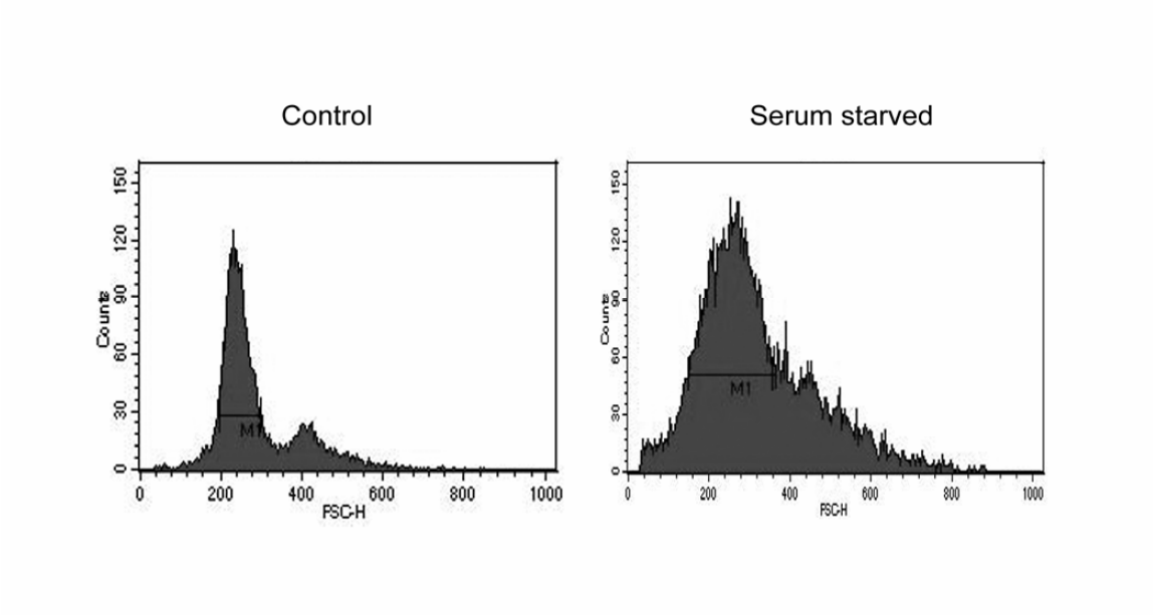

Figure S8: Flow cytometry analysis of DNA content of control and serum-starved HaCaT cells. Control and serum-syarved HacaT cells were subjected to flow cytometry analysis for total DNA content as described in 'Experimental Procedures'. M1 peak corresponds to cells in G1 phase of cell cycle and were 32 and 75% respectively.

Figure S9

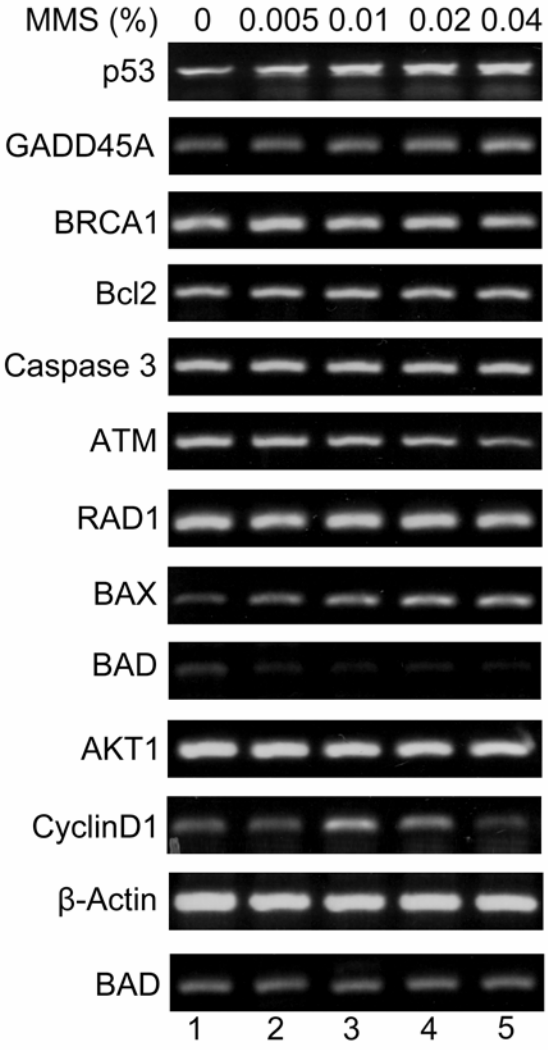

Figure S9: MMS treatment alters expression of some of the genes involved in DNA damage response and cell cycle. Jurkat cells were treated with .005 % (lane 1), .01 % (lane 2), .02 % (lane 3) and .04 % (lane 4) MMS for 2 h. RNA was then isolated and RT-PCR was performed for representative genes involved in cell cycle and DNA damage. Untreated cells (lane 1) represent the control. RT-PCR for -actin served as control.

Table S1.

S. No.	Gene/locus	Fold enrichment over IgG (ChIP in Jurkat)	Fold change upon serum starvation (in HaCaT)	Fold enrichment over IgG (ChIP in serum starved HaCaT)
1	GPR174	315.2	4.67	12.06
2	L1CAM	52.0	2.21	1.23
3	PFTK1	9.2	1.46	6.34
4	CDH2	4.9	0.91	1.56
5	LBP	24.3	2.42	8.14
6	SERPINB7	12.1	1.58	1.52
7	MYCBP	16.0	2.07	3.37
8	ST7-I	36.8	2.87	6.41
9	ST7-II	32.0	1.23	4.02
10	ASZ1	21.1	3.3	4.12
11	DPT	26.0	1.97	2.1
12	OR51G1	9.20	1.76	5.72
13	DVL1	36.8	2.13	3.56
14	CTGF	16.0	0.86	2.53
15	GCC1	48.5	2.34	10.4
16	CTAGE1	9.2	4.02	3.73
17	SSTR1	9.8	1.45	1.02
18	EPC2	90.5	0.99	3.46
19	TLR4	2.8	2.78	0.11
20	HIST1H4L	68.6	2.03	1.07
21	HDAC9	45.3	1.89	3.18
22	A	1.12		
23	B	0.97		
24	C	1.03		
25	D	0.97		
26	E	1.03		

Table S1: Pathway analysis for genomic regions enriched in H3K56ac. ChIP-on-Chip analysis was performed as described in 'Experimental Procedures'. Key pathways in which multiple genes were significantly hyperacetylated are listed.

Table S2

S. No.	Gene/locus	Fold enrichment over IgG (ChIP in Jurkat)	Fold change upon serum starvation (in HaCaT)	Fold enrichment over IgG (ChIP in serum starved HaCaT)
1	GPR174	315.2	4.67	12.06
2	L1CAM	52.0	2.21	1.23
3	PFTK1	9.2	1.46	6.34
4	CDH2	4.9	0.91	1.56
5	LBP	24.3	2.42	8.14
6	SERPINB7	12.1	1.58	1.52
7	MYCBP	16.0	2.07	3.37
8	ST7-I	36.8	2.87	6.41
9	ST7-II	32.0	1.23	4.02
10	ASZ1	21.1	3.3	4.12
11	DPT	26.0	1.97	2.1
12	OR51G1	9.20	1.76	5.72
13	DVL1	36.8	2.13	3.56
14	CTGF	16.0	0.86	2.53
15	GCC1	48.5	2.34	10.4
16	CTAGE1	9.2	4.02	3.73
17	SSTR1	9.8	1.45	1.02
18	EPC2	90.5	0.99	3.46
19	TLR4	2.8	2.78	0.11
20	HIST1H4L	68.6	2.03	1.07
21	HDAC9	45.3	1.89	3.18
22	A	1.12		
23	B	0.97		
24	C	1.03		
25	D	0.97		
26	E	1.03		

Table S2: Quantitation of fold enrichment of H3K56ac occupancy at upstream regulatory regions of multiple genes in Jurkat and G1-arrested HaCaT cells. Chromatin immunoprecipitation was performed as described in 'Experimental Procedures'. Purified immunoprecipitated chromatin was subjected to PCR amplification using specific primers for the promoters of genes enriched in H3K56 acetylation, along with input chromatin. Quantitative RT PCRs were performed, for DNA immunoprecipitated from Jurkat cells and G1-arrested HaCaT cells (Column 3 and 5

respectively). Fold enrichment over the IgG is depicted. cDNA was prepared from G1-arrested cells (serum starved) and control cells and quantitative RT-PCRs were performed. Fold enrichment in expression for G1-arrested (serum starved HaCaT cells) over control cells, for indicated genes, is depicted in (Column 4). All quantitative RT-PCRs were performed using iCycler (BioRad) using iQ SYBR Green mix (BioRad).

Table S3:

S. No.	Gene	Chromosomal location	Primer	Primer sequence	Amplicon size (bp)	Position	Nucleotide position relative to methionine
1	GPR174	Chr X	Forward primer	AAAAGCTGGAGGAAAATCCC	228	Promoter	-5033
			Reverse primer	TCTGGCTGGCTATCAGTGTC			
2	L1CAM	Chr X	Forward primer	AAGCTCAGGGAGGATGGAAT	200	Inside (intron)	+41829
			Reverse primer	CAGACCTGCCTGAGTCACTG			
3	PFTK1	Chr 7	Forward primer	GTTTGCTTGCTGGGATGTTT	340	Inside (intron)	+130353
			Reverse primer	CCGCAGTCTTTTTCACTCCT			
4	CDH2	Chr 18	Forward primer	GGGCTTTTTAAGTCTTGCTCC	210	Inside (intron)	+90049
			Reverse primer	ATGCAGTCGTAAGCTTGGCT			
5	LBP	Chr 20	Forward primer	CTGCATTGGTTCTTGGGATT	247	Promoter	-3824
			Reverse primer	ATGGCAGGAGGTCAGATGTC			
6	SERPINB7	Chr 18	Forward primer	GCTAAATTAGAGGCAGGCCC	327	Promoter	-18313
			Reverse primer	GGCCAAACCACTTTGTCACT			
7	MYCBP	Chr 1	Forward primer	TCTCTCTCTTTCAAAACCCTCA	247	Inside (intron)	+10342
			Reverse primer	TGGCTGGTGCCTCTTCTAGT			
8	ST7-I	Chr 7	Forward primer	AGGAATGGGGAAACAAAGGT	294	Promoter	-5831
			Reverse primer	AGGTTTGGAAGGGAATTTGC			
9	ST7-II	Chr 7	Forward primer	GCCTGGTCAGTGGAGCTTT	234	Inside (intron)	+24726
			Reverse primer	CCTGCAAGTCAGGAAGCAAC			
10	ASZ1	Chr 7	Forward primer	ACCAGACTCCAATGCCAATC	240	Inside (intron)	+62733
			Reverse primer	GAGAGAGGGTGGGAGAGGTC			
11	DPT	Chr 1	Forward primer	GTGGCCGCATAGAGAATGAT	259	Promoter	-3179

			Reverese primer	GATGGAAGGCATTGCAGAAT			
12	OR51G1	Chr 11	Forward primer	TGGCAAGGAAAGAGGAAGAA	287	Promoter	-1929
			Reverse primer	CAGCTTCACTATGCCTGCTG			
13	DVL1	Chr 1	Forward primer	GAACGGCTCAGGACCCC	241	Inside (exon)	+244
			Reverse primer	CTACCACATGGACGAGGAGG			
14	CTGF	Chr 6	Forward primer	TAAGCTGGCCACTGAGTCCT	213	Promoter	-3368
			Reverse primer	TCCTGCCAGGTTTTCTCTGT			
15	GCC1	Chr 7	Forward primer	CAAAAGTCAAAAGCACAGGGA	299	Inside (exon)	+5080
			Reverse primer	TTATGGGTTTAGTGGGCAGG			
16	CTAGE1	Chr 18	Forward primer	CTCACTGGAAGGAAGGCAAG	234	Inside (intron)	-3629
			Reverse primer	ATGCCACTCCAGTCTCTGCT			
17	SSTR1	Chr 14	Forward primer	TAGGAAAATCAGTGGGCCAG	225	Promoter	-5490
			Reverse primer	ATGGCCTACAGCAAAGGTTG			
18	EPC2	Chr 2	Forward primer	TGCATACGCCATTCACAAAT	231	Promoter	-5596
			Reverse primer	CAATCCCTGTGAAGGCAGTT			
19	TLR4	Chr 9	Forward primer	CATCATACCAGGCCTCCATT	253	Promoter	-5456
			Reverse primer	GTACTGCCCTGTGCATCTCA			
20	HIST1H4L	Chr 6	Forward primer	GCCTGATGATAGCTCCAAGG	265	Promoter	-483
			Reverse primer	AGGAACATGAGGGAGCTCAA			
21	HDAC9	Chr 7	Forward primer	TGGGAGAAGAGGTAACATCAA	271	Promoter	-5490
			Reverse primer	TGGAAACAAGCAAAGGAGGT			
22	A	116243332-116244154	Forward primer	GCCATCAGAATATTGAAAGAA TGACCTC	682	Promoter	N/A
			Reverse primer	CTGCTTGTTGCAGGTACTTGGCA AATAG			
23	B	59396319-59397663	Forward primer	GGTTTGCTTAGGTGAGGTGCGG CGGTGTGC	650	Promoter	N/A

			Reverse primer	GACCCTCCAGACCTCACGTTTG TTCCGGAG			
24	C	152133502-152134181	Forward primer	GGGCTGGGAGGAGTGGGAACAA CATGGGCTC	668	Promoter	N/A
			Reverse primer	CACTGGCCAGGCACTTTTCTGCC TGG			
25	D	4812338-4813100	Forward primer	GGAATTTAGAAGCTAACCCACAG AAGAAGG	512	Promoter	N/A
			Reverse primer	GAGGCTTAAGACTTCTCAGGGGT TGCCTAG			
26	E	117218625-117219107	Forward primer	GGATGGTTTTGGTGGCAGCTGAG GGGAGGATG	453	Promoter	N/A
			Reverse primer	CCCAAAGTCCTCCGTCAAGAGAT GTCTCGCC			

Supplemental Table 3. Oligonucleotides used for ChIP-PCR. The oligonucleotides used for ChIP-PCR of the genes studied (represented by their official symbols in Figure 7A and C) are listed in the table. Also included are the chromosomal locations of the respective genes, the amplicon size, position of the amplicon (in promoter or in the exon/intron of the gene) and the position of the amplicon relative to ATG encoding initiator methionine. The oligonucleotides were designed after retrieving the 300 bp sequences, using UCSC Table Browser (Karolchik et al., 2004), flanking either side of the probes (40-60 mer) used in the microarray analysis. "+" or "–" indicate the relative location of amplicon (upstream and downstream, respectively) from the initiation site. Amplicons A-E (last five panels in Figure 7A) used as genomic controls were taken from the promoter regions identified by Kim et al. (2005).

Table S4:

S. No.	Gene	Chromosomal location	Primer type	Primer sequence	Ampli-con size (bp)	Position	Full-length cDNA (nt)	Nucleotide position relative to methionine
1	GPR174	Chr X	Forward primer	GTGCCAGGTCTCATAGGGAA	285	Inside	1002	91
			Reverse primer	GCGAAAGGGGTACATGAGAA				
2	L1CAM	Chr X	Forward primer	GTGAGTGCCATCATCCTCCT	328	Inside	3774	3379
			Reverse primer	CCTTCTCCTTCTTGCCACTG				
3	PFTK1	Chr 7	Forward primer	CCAAGGAGTTGCTGCTTTTC	253	Inside	1356	924
			Reverse primer	TGCCGACAGTCTGTTCTTTG				
4	CDH2	Chr 18	Forward primer	CCATCACTCGGCTTAATGGT	242	Inside	2721	1958
			Reverse primer	GATGATGATGCAGAGCAGGA				
5	LBP	Chr 20	Forward primer	AACTTCAGCCCTGGGAATCT	324	Inside	1446	1048
			Reverse primer	CTGAACACGCTTCAGCAGAG				
6	SERPINB7	Chr 18	Forward primer	ACGTTCTGCTGCCTGAGAAT	345	Inside	1143	713
			Reverse primer	TGGACTGAGGGAGTTGCTTT				
7	MYCBP	Chr 1	Forward primer	GCCGCCGACTCGAAGCGTGAG	276	Inside	312	16

			Reverse primer	CTGAGGTGGTTCATACTGAGC				
8	ST7-I	Chr 7	Forward primer	TGCACACTGGAAGAGAGTGG	261	Inside	1665	1278
			Reverse primer	AACTGATGTGTCAGGAGGGC				
9	ST7-II	Chr 7	Forward primer	GAGGGGTTGACAACAACTCTTC	310	Inside	1758	317
			Reverse primer	GCTTCATGAGCTGCAGAAATCC				
10	ASZ1	Chr 7	Forward primer	GAGAGTAGCGAGAGCGAGGA	233	Inside	1428	46
			Reverse primer	TCTGCATTGGCAACACTAGC				
11	DPT	Chr 1	Forward primer	GGCTTCAGCTACCAGTGTCC	304	Inside	606	133
			Reverse primer	TTAGCCAGCAGGAATATGGG				
12	OR51G1	Chr 11	Forward primer	ACCTCACCATTCTCCACGTC	323	Inside	966	131
			Reverse primer	CACTGAGCTTAGCCCCATCT				
13	DVL1	Chr 1	Forward primer	GGAAGCAGCGCCTTCGGCAG	251	Inside	2013	671
			Reverse primer	CACGTCATTCACCTGCAGC				
14	CTGF	Chr 6	Forward primer	CCGTACTCCCAAAATCTCCA	237	Inside	1050	771
			Reverse primer	AAGATGTCATTGTCTCCGGG				
15	GCC1	Chr 7	Forward primer	GCACCTACGAGAAAGCCAAG	255	Inside	2328	1133
			Reverse primer	CCAGGTCACAGAGCTTCTCC				
16	CTAGE1	Chr 18	Forward primer	CCCTCAAATCACAAGTAGCTG	275	Inside	2238	455
			Reverse primer	CTTTAGCAAGCGTTCAGTCAG				
17	SSTR1	Chr 14	Forward primer	GGGCTATCTGCCTGTGCTAC	294	Inside	1176	710
			Reverse primer	GGAAAGAGCGCTTGAAGTTG				
18	EPC2	Chr 2	Forward primer	GGGGGTATCACAGAAGAGCA	287	Inside	2424	1726
			Reverse primer	GGCTGGACAACACCGTTTAT				
19	TLR4	Chr 9	Forward primer	CCAGGATGAGGACTGGGTAA	294	Inside	1920	1446
			Reverse primer	CTGAGCAGGGTCTTCTCCAC				

20	HIST1H4L	Chr 6	Forward primer	AGGGTCTGGGCAAAGGAG	230	Inside	312	26
			Reverse primer	CATGGCTGTGACTGTCTTGC				
21	HDAC9	Chr 7	Forward primer	CAGTTCTCCAGGCTCTGGTC	343	Inside	3201	804
			Reverse primer	AGTAACATGAGGGTGGCTGG				
22	β-**actin**	Chr 7	Forward primer	TGGAATCCTGTGGCATCCA	257	Inside	1128	806
			Reverse primer	TAACAGTCCGCCTAGAAGCA				

Supplemental Table 4. Oligonucleotides used for quantitative RT-PCR. The oligonucleotides used for quantitative RT-PCR (for the genes represented by their official symbols in Figure 7B) are listed it the table, along with the chromosomal location of the respective genes, the amplicon size, position of the amplicon (which is always inside the coding region), size of full-length cDNA and the position of the amplicon relative to ATG encoding initiator methionine.