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Supplemental Material to:

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PGE2 induces interleukin-8 derepression in human astrocytoma through coordinated DNA demethylation and histone hyperacetylation

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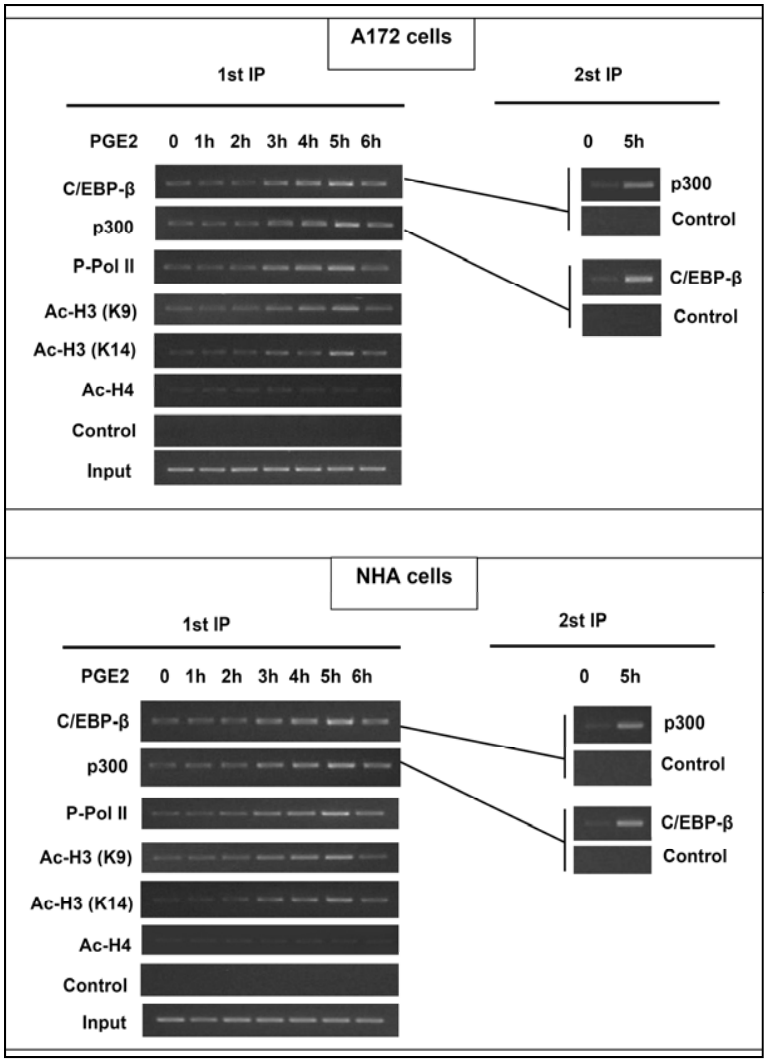
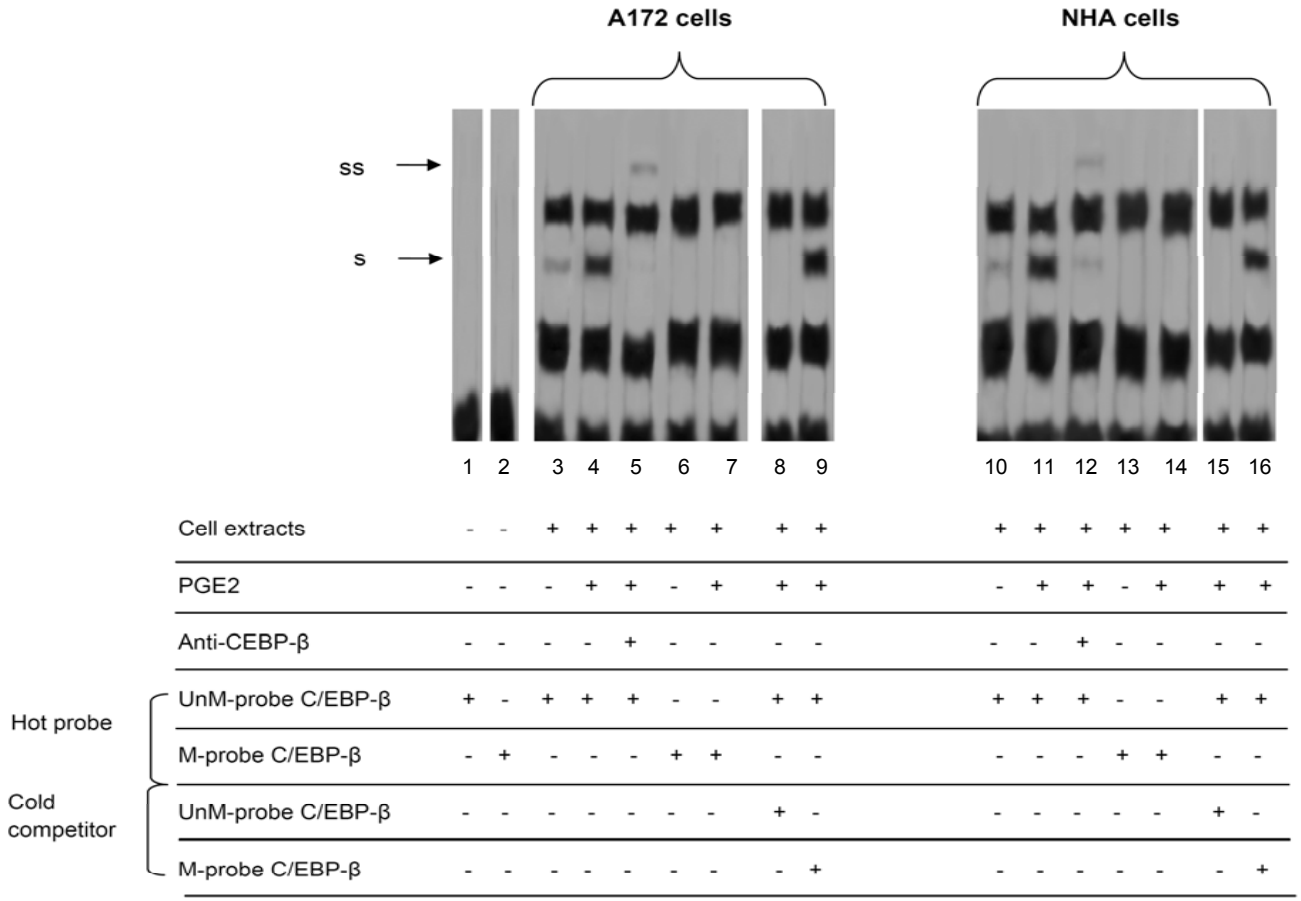


Supplemental figure 2 – PGE2 increases occupancy of endogenous C/EBP-β and p300 on the IL-8 promoter and H3 acetylation. A172 (*upper panel*) and NHA (*lower panel*) cells were treated with PGE2 for the indicated times. Primary ChIP was performed using anti-C/EBP-β, anti-p300, anti-P-Pol II, anti-Ac-H3 (K9), anti-Ac-H3 (K14), and anti-Ac-H4. The beads from the first IP were washed and eluted for the second IP. The eluate from the first IP with anti-C/EBP-β was used for the second IP with anti-p300 or no antibody as control (2nd IP, *top*). The eluate from the first IP with anti-p300 was used for the second IP with anti-C/EBP-β or no antibody as control (2nd IP, *bottom)*. *Brackets* indicate which of the first IP was used for second IP. Similar data were obtained in another experiment.



Supplemental figure 1 – CpG site 5 methylation inhibits the binding of C/EBP-β to IL-8 promoter. Nuclear extracts prepared from untreated (lanes 3,6,10,13) or 10 μM PGE2-treated (lanes 4,7,11,14) A172 and NHA cells were incubated with double-stranded oligonucleotide probe (-95/-52 bp) containing either unmethylated cytosine (*UnM-probe C/EBP-β*) or methylated cytosine (*M-probe C/EBP-β*) at CpG site 5 (nucleotide -83). In supershift analysis, 2 μg antibody against C/EBP-b (lanes 5,12) was incubated at room temperature (for 20 min) with the nuclear extracts prior to probe addition. Competition with a 200-fold excess of either unlabeled *UnM-probe C/EBP-β* (lanes 8,15) or unlabeled *M-probe C/EBP-β* (lanes 9,16) showed the specificity of protein binding.