Supplementary Material and Methods, Tables, Figures and Figure Legends

Material and Methods

Analysis of methylated DNA

Cells were seeded at a density of 40.000 cell/ml and transfected with siCEBPB-AS or siControl as described in the M&M. DNA was extracted after 48 h transfection using the QIAamp DNA Mini Kit (Qiagen). NEBuffer (New England BioLabs), supplemented with GTP and BSA, was added to 200 ng of each DNA sample. Samples were either treated with MrcBC enzyme (New England BioLabs) or mock treated, and incubated overnight at 37C. Next day, the enzyme was heat inactivated at 65C° for 1 hour. The enzyme MrcBC is a methylation-dependent endonuclease that cleaves methylated DNA at Pu^{m}CG sequence elements. Samples were subjected to RT-qPCR and standardized to uncut input. Delta CT values were converted to fold-change values and the ratio between siCEBPB-AS/siControl were calculated.

Tables

Table I: List of primers used in the study

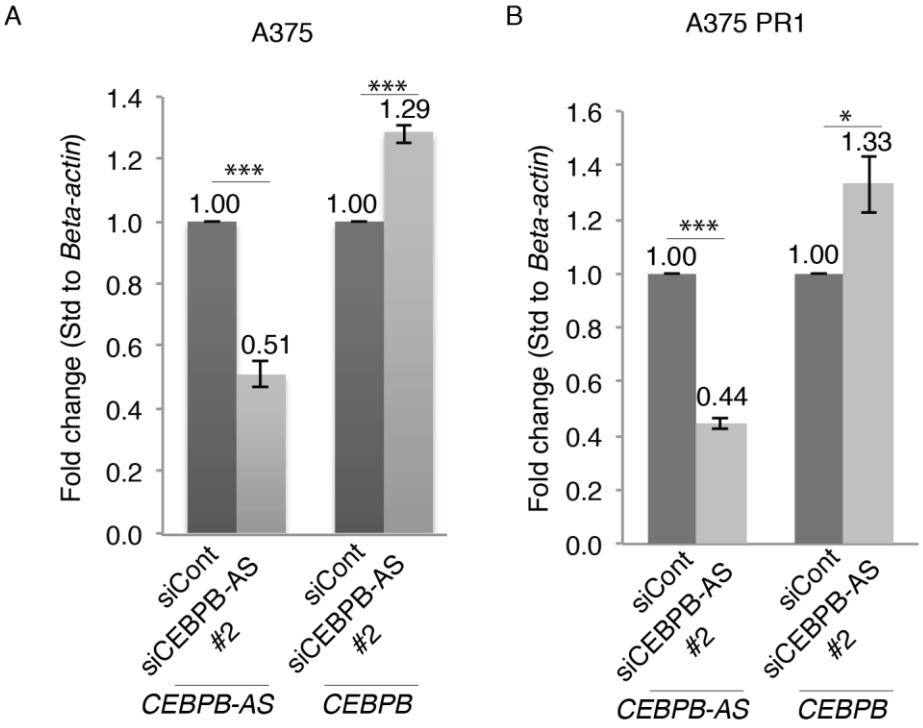
Primer target	*Primer sequence (5´-3´)*	*Method*
CEBPB	R0: GCTGCTCCACCTTCTTCTG F0: CGCGACAAGGCCAAGAT F1: CTTTAGCGAGTCAGAGC F2: CTTCTCCTGGAGCTAGA F3: CAAGTCTTCTTTCTAAAGCC	Primer walk
CEBPB-AS	F: ACTGAGGCGATTTGCCAAG R: CTGGCTGATTTCTAAGCCCTTT	RT-qPCR; Fractionation; semi-qRT-PCR; Poly(A) depletion; RNA stability
CEBPB	F: GGAGCCCGTCGGTAATTT R: TCTGCATGTGCGGTTGG	RT-qPCR; Fractionation; semi-qRT-PCR; RNA stability
CEBPB-AS unspliced	F: GAGGAGGCGGAGGTTTC R: GTCCAAGCCTCGAGCAG	Fractionation
Beta-actin	F: AGGTCATCACCATTGGCAATGAG R: CTTTGCGGATGTCCACGTCA	RT-qPCR; Poly(A) depletion
7SK	F: AATGAGGACCAGCTGAGTAGA R: GGAGGGATGAGAATGCATGAG	Fractionation
U48	F: AGTGATGATGACCCCAGGTA R: GGTCAGAGCGCTGCGGTGAT	Poly(A) depletion
CEBPB-AS promoter	F: TAAACTCTCTGCTTCTCCCTCT R: CGATTGCATCAACTTCGAAACC	ChIP
CEBPB promoter	F: CGTAAGCCTTAGGTTTGGGA R: TGCAATCCATGAAGGGTGT	ChIP
CEBPB-AS promoter Set1	F: CTTAGGTTTGGGAGTCTGGTG R: GGGTGAGTCACTTCACTTCTC	Methylation digestion

Table II: List of siRNAs used in the study

siRNA target	*siRNA sequence*	*supplier*
CEBPB	5´- AGUUGAUGCAAUCGGUUUAAACATG-3´ 3´- CUUCAACUACGUUAGCCAAAUUUGUAC-5´	IDT
CEBPB-AS1	5´- CGGCUUUAGAAAGAAGACUUGACGC-3´ 3´- GGGCCGAAAUCUUUCUUCUGAACUGCG- 5´	IDT
CEBPB-AS1 #2	5´- GGUACAUAGAAGGUGCUCGAUGAAT- 3´ 3´-GACCAUGUAUCUUCCACGAGCUACUUA-5´	IDT
siControl	catalog# 51-01-14-03	IDT

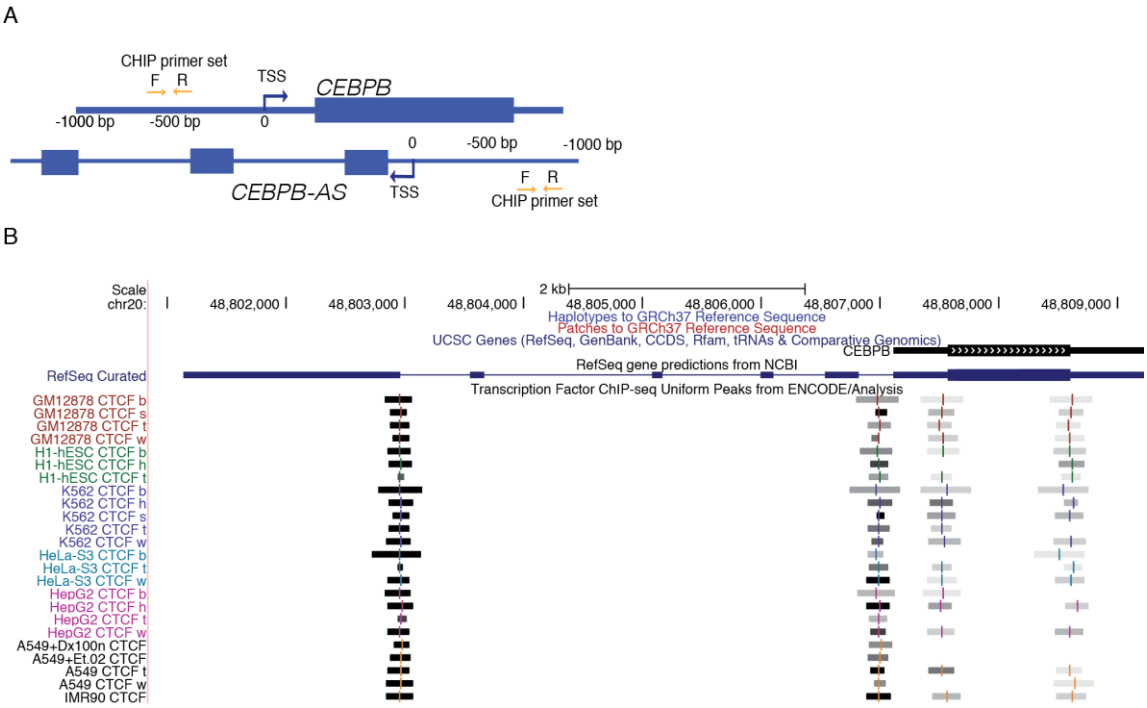
Figures

Supplementary Figure 1



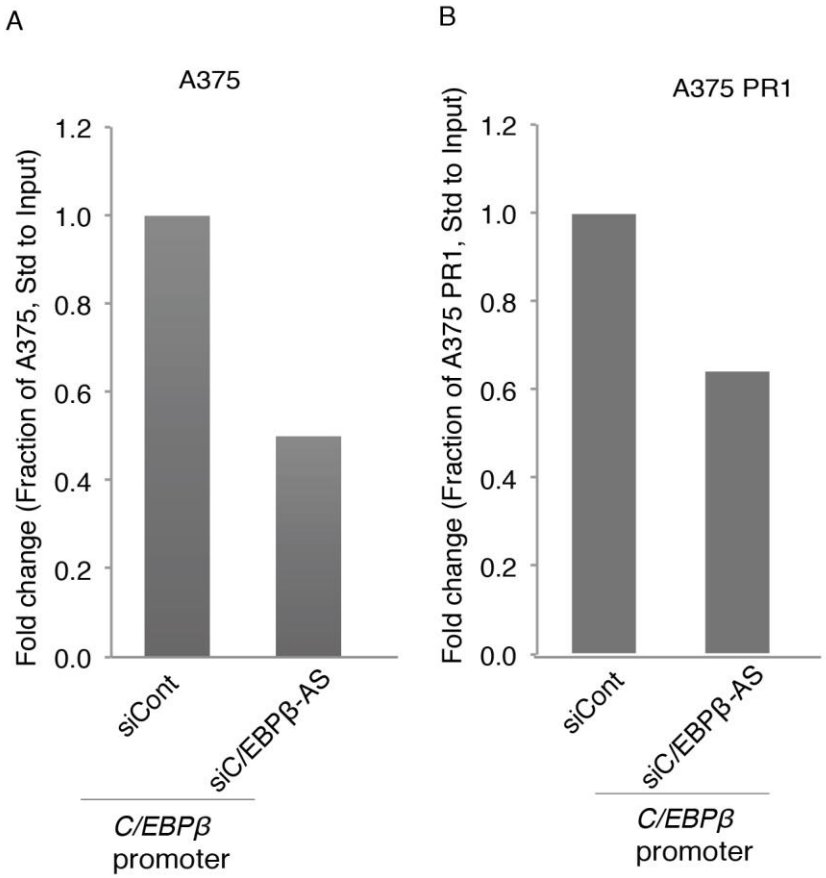
Supplementary Figure 1. *CEBPB* and *CEBPB-AS* expression as measured by RT-qPCR after knockdown of *CEBPB-AS* in A375 (A) or A375 PR1 (B) cell lines with a second siRNA targeting the *CEBPB-AS*. Independent experiments n=3. ***p<0.005, *p<0.05

Supplementary Figure 2



Supplementary Figure 2. (A). Schematic representation of the CEBPB locus with the indication of the location of primer sets used for ChIP. (B) CEBPB locus depiction from the UCSC genome browser with ChIP-seq data for the binding of CTCF in this locus and at the CEBPB regulatory region. Gray boxes - peak clusters of transcription factor occupancy. Darkness of the box is proportional to the signal strength.

Supplementery Figure 3



Supplementary Figure 3. Effect of *CEBPB-AS* silencing on the DNA methylation at the CEBPB regulatory region. A375 (A) and A375 PR1 (B) cells were transfected with either siControl or siCEBPB-AS; DNA was isolated and treated with the methyl-cytosine restriction enzyme MrcBc that cleaves only DNA containing methylcytosine followed by qPCR of the *CEBPB* regulatory region. Data represent two independent experiments. The decreased values correspond to less of the PCR product due to cutting by the restriction enzyme and therefore reflect an increased methylation in the region.