Additional file 2:
Vector construction and oligonucleotide sequences

Construction of reporter gene plasmids

All human promoter constructs were obtained by PCR cloning from genomic DNA isolated from the human HEK293 cell line. Amplification of the highly GC-rich template was achieved by using the JumpStart REDAccuTaq LA polymerase mix (Sigma-Aldrich) and adding DMSO to the reaction mix at a final concentration of 9%. The reporter construct for the putative promoter in front of human exon 1M (TSS-M) contained bp –2726 to –1899. Promoter constructs for TSS A and TSS B contained fragments of variable length as indicated in the figure legends. Suitable restriction sites were introduced by the design of the PCR primers. PCR products were cloned between the *Nhe*I-site and the *Bgl*II-site (exon 1M) or between the *Nhe*I-site and the *Hin*dIII-site (all other human constructs) in front of the firefly luciferase cDNA in the vector pGL3-basic (Promega, Mannheim, Germany). Sequencing of several independent clones revealed the presence of an additional G between bp –200 and -201 in comparison with the human genome sequence accessible at the UCSC Genome Bioinformatics Site (genome.ucsc.edu; release hg18). This difference is a known insertion/deletion polymorphism (refSNP ID:rs34481457). In addition, the following differences from the database sequence were identified: construct –1453, T(-938)C, construct -904, C(-676)T, construct –418, G(-179)A and C(-13)T. Construct -1453 was used as a template to amplify the other deletion fragments.

Sequences of the oligonucleotides used for PCR and as EMSA probes

Oligonucleotides used as primers for RACE and for PCR in Fig. 2

Primer	**Sequence 5´ → 3´**	
Rev1	GGCTGGCGACGGTCACTGTACTGATG	Exon 3
Rev2	GTCCAGCCATCTGAAGGCCAGCAG	Exon 3
1Afor	AATTGCTAGCGTCCGTCGCCATTTTGTTG	Exon 1A
1Bfor	CCGAGGCTGAGACTCAC	Exon 1B

Oligonucleotide primers for cloning of promoter constructs

Position relative to TSS-B	**Sequence 5´ → 3´**	**Restriction site**
-2726for	AATTGCTAGCTTGGTTGTATAGGTGGAAGC	*Nhe*I
-1899rev	AATTAGATCTCAGAGTCTTAATTCGCCTGC	*Bgl*II
-1453for	AATTGCTAGCTTCGAAGAACCGGCTCCACG	*Nhe*I
-756rev	TTAAAAGCTTCAACAAAATGGCGAC	*Hind*III
-904for	AATTGCTAGCGAACGGAATCTGCCGTTACC	*Nhe*I
-839for	AATTGCTAGCGAGGACGTCAGCACGTCAGC	
-742for	AATTGCTAGCTAAACCCTTTCGCTTCCCGC	
-647for	AATTGCTAGCACTGCTGCTGTCGCTGCTGC	
-541for	AATTGCTAGCCCTGCTGCTGCTGTTGGTGC	
-418for	AATTGCTAGCGAGTGTCTGTCTGTCTGTGC	
-324for	AATTGCTAGCCGCTGCGCAGGTTCGGAGC	
-226for	AATTGCTAGCGCCGACGCCGCCCTCTGC	
-114for	AATTGCTAGCGCCGCTGGAACCGCGAGC	
+44rev	AATTAAGCTTTTCCTCCGGTGAGTCTCAGC	

Oligonucleotides used for site directed mutagenesis:

	Sequence 5´ → 3´	
Mutation of E2F1 site	GGACTGCGGTATTTCCATGGGAGGGGGCTGTC GACAGCCCCCTCCCATGGAAATACCGCAGTCC	*Nco*I
Mutation of Sp1 sites	GCGCCGGGGGTAGAGAGAGGCTGCCAGATCTGCGCTGGCTGCG CGCAGCCAGCGCAGATCTGGCAGCCTCTCTCTACCCCCGGCGC	*Bgl*II

Oligonucleotides used for EMSA probes:

Name of probe	**Sequence 5´ → 3´**
Consensus CRE	AGAGATTGCC**TGACGTCA**GAGAGCTAG CTAGCTCTC**TGACGTCA**GGCAATCTCT
Mutated CRE	CAGGTAGATC**TG**TG**GTCA**AGAGATGGC GCCATCTCT**TGAC**CA**CA**GATCTACCTG
CRE-like motif in promoter A	GGCGGGAGGAG**GACGTCA**GCACGTCAG CTGACGTGC**TGACGTC**CTCCTCCCGCC