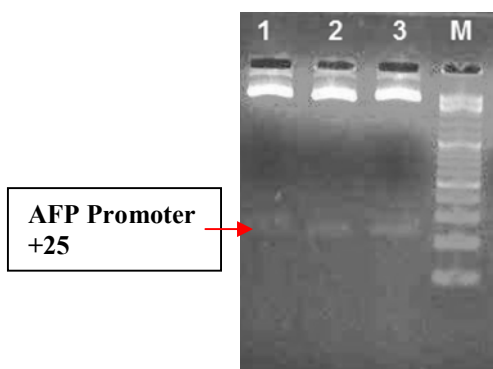
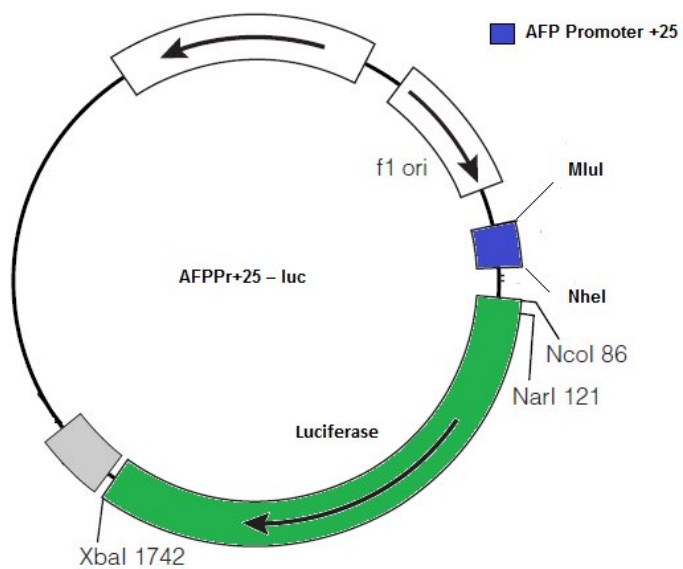
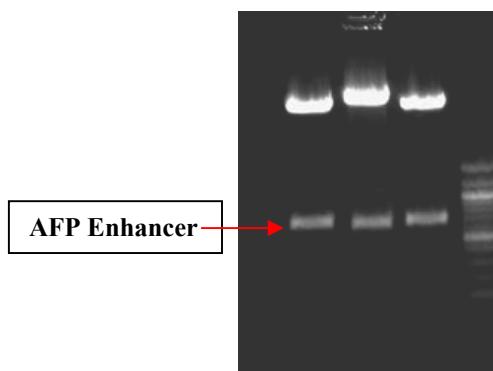
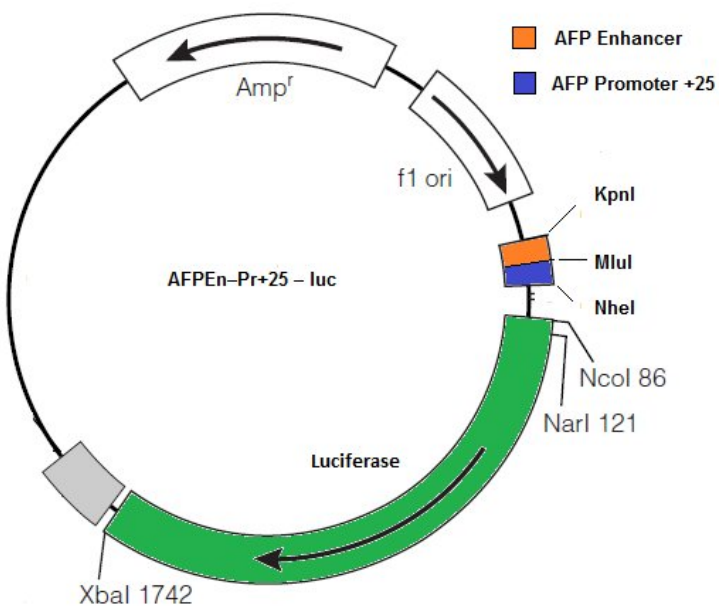
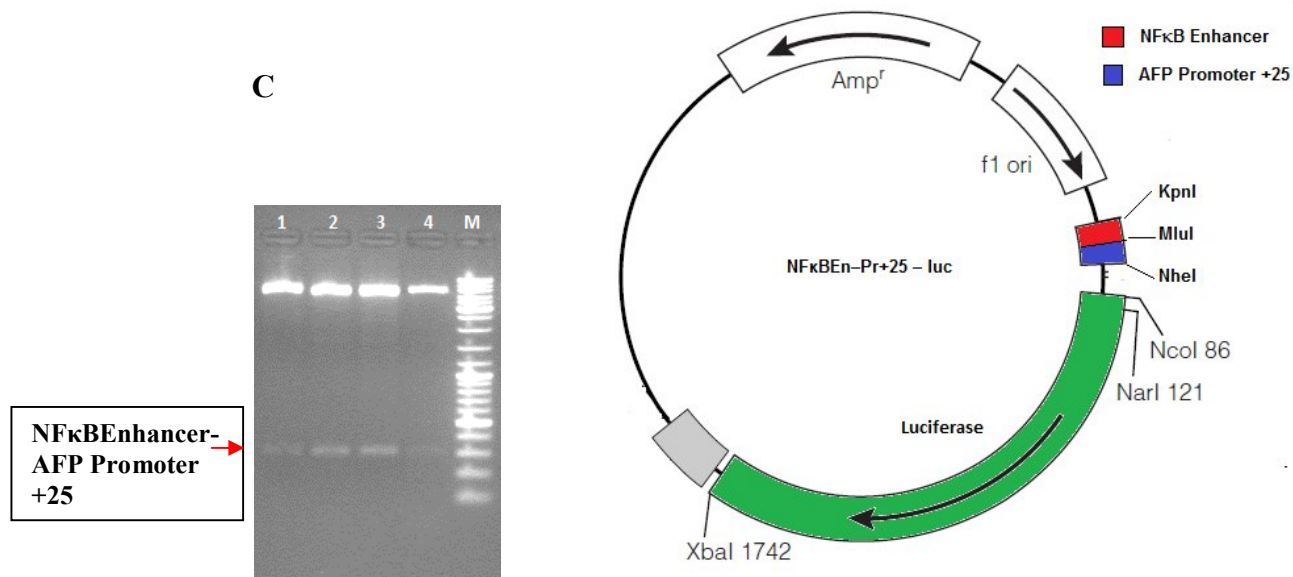


A



B





Additional file 2: Figure S2. Clones of various AFP promoter/enhancer driven luciferase constructs. (A) AFPPr+25 – luc clone was confirmed by restriction digestion with MluI and NheI restriction enzymes. **(B)** AFPEn-Pr+25 - luc was digested with KpnI and MluI restriction endonucleases and **(C)** NFκBEn-Pr+25 - luc with KpnI and NheI.

CpG1

C CGCCAC CGCCGGGCC CGGC CGTCCCTGGCTCCCCTCCTGCCT CGAGAAGGGCAGGGCTTCTCAGAGGCTTGGC
GGGAAAAAGAAC CGGAGGGAGGGAT CGCG CTGAG TATAAAGC CGGTTTT CGGGCTTTATCTAACT CGCTGTAGT
AATTCCAG CGAGAGGCAGAGGGAG CGAG CGGG CGGCC CGGCTAGGGTGGAAGAGC CGGG CGA

CpG20

Additional file 2: Figure S3. Sequence of *c-Myc* P2 promoter with siRNA target site and CpG islands. GAA CGGAGGGAGGGAT CGCG CT is the siRNA target site for P2 promoter of *c-myc* and contain CpG sites 8, 9 and 10. CpG sites are highlighted in red. TATAAAG represents the TATA box.

The diagram illustrates the cloning strategy for the AFPPr+2-myc construct. It shows a circular plasmid with a Neomycin resistance gene and an AFP Promoter +2 region. The plasmid is linearized using BamHI and HindIII. A c-myc shRNA expression cassette, containing a c-myc shRNA gene, a c-myc promoter, and a c-myc tag, is inserted into the plasmid. The resulting construct is labeled AFPPr+2-myc.

The image shows a gel electrophoresis result with two lanes. The left lane is labeled 'cmyc Annealed' and the right lane is labeled 'cmyc Un-Annealed'. A red arrow points to a band in the 'cmyc Annealed' lane. The 'cmyc Un-Annealed' lane shows a different banding pattern.

**c-myc annealed
oligos**

**c-myc un-annealed
oligos**

AFP Promoter-cmyc M

A gel electrophoresis image showing five lanes. The first four lanes are labeled 'AFP Promoter-cmyc' and the fifth is labeled 'M'. The 'M' lane contains a DNA ladder with multiple bands of varying sizes. The first four lanes show a single prominent band at approximately the same position. A red arrow points to a band in the fourth lane from the left, which is slightly lower than the main band in that lane.

**AFP Promoter +2
– cmyc shRNA**

Additional file 2: Figure S4. AFP promoter – c-myc shRNA (AFPPr+2 – myc) clone.

(A) Schematic representation for cloning of AFPP_r+2 – myc construct. **(B)** Annealed *c-myc* sense and antisense oligos. **(C)** AFPP_r+2 – myc clone confirmation by digestion with EcoRI and HindIII restriction enzymes.

EcoRI

BamHI

5' gatccgaacggaggaggaggatogogctaacaaagagaagcgcgatccctccctcggtca3'

3' gcttgctccctccctagcgcgatgtttctcttcgogctaggaggaggaggaagtcca 5'

HindIII

c-myc shRNA

HindIII

BamHI

EcoRI

Neomycin

NFκB En-Pr+2 - myc

NFκB Enhancer

AFP Promoter +2

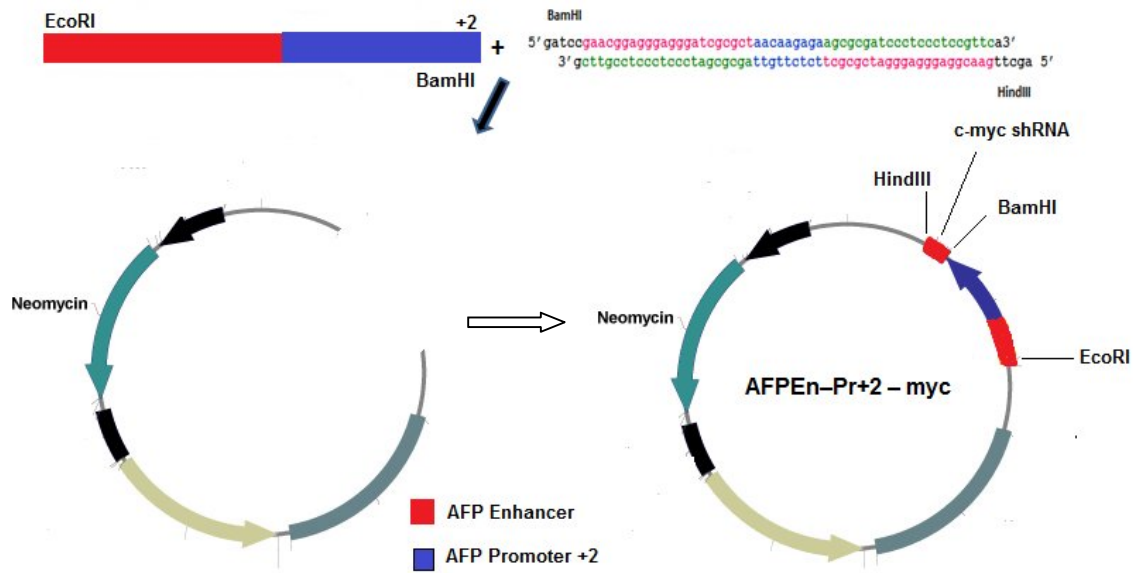
B

EMSA gel image showing NFκB binding to the AFP promoter. The gel has four lanes labeled NFκB, AFP, M, and AFP Uncut. A red arrow points to a band in the M lane.

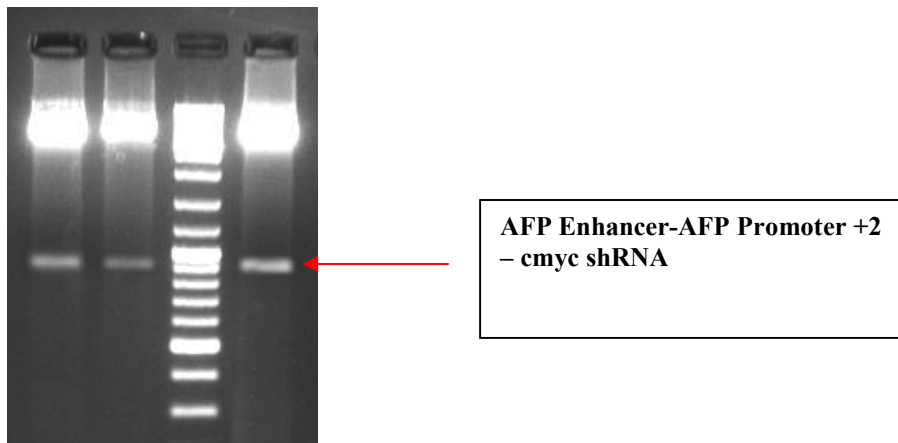
**NFκB Enhancer-AFP Promoter
+2 – cmyc shRNA**

Additional file 2: Figure S5. NFκB – c-myc shRNA (NFκBEn–Pr+2 – myc) clone. (A) Cloning strategy followed for the generation of this construct. **(B)** NFκB conjugated c-myc shRNA was confirmed by EcoRI and HindIII digestion.

A



B



Additional file 2: Figure S6. AFP enhancer – AFP promoter – *c-myc* shRNA (AFPEn-Pr+2 – myc) clone. (A) Cloning strategy followed for the generation of this construct. (B) AFP enhancer and promoter conjugated *c-myc* shRNA was confirmed by EcoRI and HindIII digestion.

(All clones were verified by DNA sequencing from professional agencies)