Supplementary material (for online-publication)

Materials and Methods

Transfection of AP-1 decoy ODNs using other transfection reagents than Oligofectamine

Approximately 1.5 x 10^5 KB-3 cells/well were plated on sterile cover slips in 12-well plates one day before transfection. When 50-60% confluent, cells were transfected with 100 nM AP-1 ODNs using different reagents as indicated by each manufacturer's protocol. Thus, DNA was initially diluted in serum-free media. Then, the transfection reagent was mixed with DNA solution and incubated as following: Polyfect (Qiagen, Valencia, CA), 6 µl, followed by 10 min incubation; Transfectin (Biorad, Hercules, CA), 5 µl diluted in 100 µl serum-free media followed by 20 min incubation; and Fugene 6 (Roche Applied Science), 1.5 µl diluted in 50 µl serum –free media followed by 15 min incubation. The DNA-transfection reagent complexes were added to KB-3 cells containing 250 µl normal growth media (Polyfect), 300 µl serum-free media (Transfectin) or 450 µl serum–free media (Fugene). After 4 h, 500 µl normal media was added or the media was replaced with fresh normal media for the cells transfected with Transfectin or Fugene, respectively. Each experiment included untransfected cells as a negative control. The slides for fluorescence microscopy were prepared as described in the Material and Methods section.

Supplementary figure
Fig. S1.

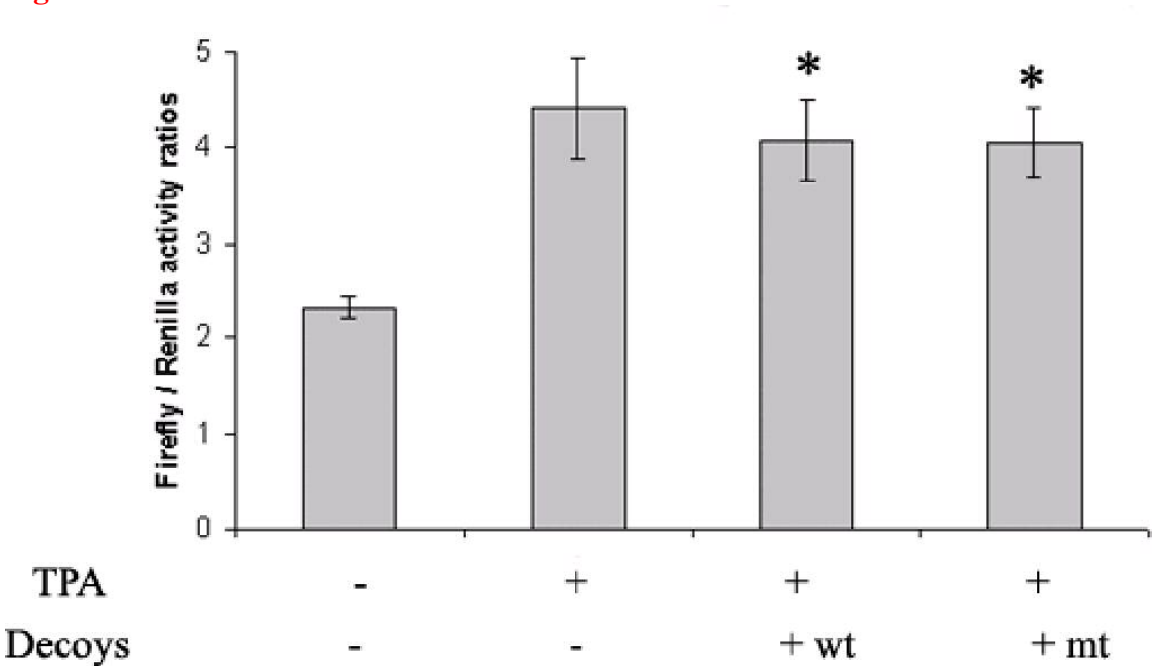

Fig.S1 Approximately 60% confluent KB-3 cells were cotransfected with pAP-1-Luc and pRL-TK plasmids in a 10:1 ratio using Lipofectamin Plus Reagent. After 24 h, the wild-type and mutated AP-1 ODN decoys were introduced into the cells following the protocol described in *Material and Methods*. To stimulate AP-1 activity, cells were treated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 16 h. To analyze luciferase expression cells were washed with PBS and lysed with 200 µl of 1X Reporter Lysis Buffer (Promega, Madison, WI). The results are expressed as AP-1 Firefly luciferase activity/ Renilla luciferase activity ratios and represent the means of measurements from triplicate wells ± SD from two independent experiments. There is no significant difference between wt or mt AP-1 decoy ODN-transfected cells, when compared with untransfected cells (* p value > 0.5) after treatment with TPA; wt, wild-type; mt, mutated.