Supplementary Figure 1: Diagrammatic representation of Cell-SELEX process used for selection of H1975-specific internalizing aptamer. The RNA pools transcribed from an initial DNA library were incubated with L132 normal lung cells (counter selection). After washing, the unbound RNA sequences were incubated with H1975 cells for their uptake. The internalized sequences were extracted by trizol. The collected sequences were then reverse transcribed and amplified by PCR. The PCR products were then again transcribed for next round of incubation. 12 such iterative cycles were performed before sequencing.
Supplementary Figure 2: Predictive secondary structures of internalized aptamer. The conserved regions that share similar structures in the box is the truncated aptamer used in the study. We selected the truncated from by using a method reported by Shangguan et al (Shangguan D, Tang Z, Mallikaratchy P, Xiao Z, Tan W, Chembiochem 2007, 8, (6), 603-606). We selected only the minimized length of the aptamer required for specific binding. Moreover,
it has been observed earlier that truncated aptamers show enhanced internalization specificity as the non-specific antigen binding site has been removed from these sequences.

**Supplementary Figure 3:**

**Supplementary Figure 3:** RNA library uptake was evaluated in H1975 and L132 cells: Texas red labelled RNA library was incubated with cells for 60 minutes under standard conditions. Thereafter the cells were observed under fluorescent microscope. In all the images, the nucleus is stained as blue (DAPI) and the red colour (Texas Red) signifies the presence of aptamer. No uptake of RNA library is observed in both the cell types.
Supplementary Figure 4: Quantification of Internalized Aptamer: Percentage of cells which internalized the initial RNA library used for aptamer selection was measured by Tali® Image-Based Cytometer. The cells were treated with texas red labelled RNA library for 60 minutes and then scrapped, washed and loaded onto Tali® Cellular Analysis Slide by pipetting the sample in half-moon shaped sample loading area. The slide was inserted in the cytometer and the reading was recorded. Three independent experiments were performed for each cell type. All values are represented as Mean ± SEM (n=3). No significant uptake of RNA library was observed in any of the cell type used.
Supplementary Figure 5: Comparative anti-cancer activity of conjugated and unconjugated aptamer and GNPs. Effect of conjugated and un-conjugated aptamer and GNP on the viability of cells was evaluated by using Tali® Image-Based Cytometer. The cells were plated in 100mm³ dishes and allowed to grow till the cells were 70% confluent. They were then treated with GNPs, conjugated Apt-GNPs and un-conjugated Apt-GNP for 48 hours. After treatment time the cells were scrapped and 1μl of SYTOX dye was added per 100μl of cell suspension and incubated for 1 minute in dark. The sample was then loaded on Tali® Cellular Analysis Slide and the slide was inserted in cytometer. The viability in percentage (%) was then noted. Similarly untreated cells were also assessed by similar method where the viability of cells was 100%. All values are represented as Mean ± SEM (n=3); ***p<0.001 Vs Control and ###p<0.001 Vs GNP.
Supplementary Figure 6: qRT-PCR of Ets family members after treatment with siRNA and Apt-GNP: H1975 cells were treated with siRNA and Apt-GNP bio-conjugate for 48 hours and thereafter the levels of Ets family members (Tumour suppressive: Ets2, Elf5, PDEF and Tumour promoters: PEA3, Elk and ESE) were checked. Herein we aimed to check the
specificity of inhibitory activity of siRNA and aptamer on various members of Ets family proteins. The qRT-PCR graphs are shown in the figure. As can be seen in the representative graphs, siRNAs decrease the mRNA levels of almost all the proteins without any discrimination. On the other hand, aptamers do not decrease the levels of these proteins but interestingly increases the mRNA levels of tumour suppressive proteins. Ets-2, Elf-5 and PDEF have been reported to exhibit tumour suppressive effects whereas PEA3, Elk and ESE are known oncogenic proteins. siRNA knocked down the levels of the tumour suppressive proteins, non-specifically, but aptamers increased the levels of these proteins. To understand this we need to highlight the difference between the mechanism of action of siRNA and aptamers. siRNA knocks down the expression of a protein by targeting its mRNA and degrading it. In the process, siRNA may non-specifically degrade mRNA of other Ets family proteins as well. But aptamers do not work through the same mechanism. We suggest that aptamers bind to the Ets1 protein and inhibits the binding of the co-activators and thus hindering the interaction between Ets1 and DNA. As Ets1 is unable to perform its assigned function, the cellular machinery shuts down the production of the Ets1 protein through feedback mechanism. Here comes in the compensatory mechanism which leads to an increase in the levels of Ets2, Elf5 and PDEF. Interestingly, aptamers did not increase the levels of tumour promoters PEA3, Elk and ESE. Further studies are required to explain this interesting behaviour.
Materials and Methods

Cell Lines
H1975, A549, A431, MDA-MB231, MCF-7 were obtained from American Type Cell Culture (ATCC, Manassas). L132, NCI-H460, NCI-H23 and DU-145 cells were from NCCS Pune, India. Cells were grown under standard conditions of 5% CO₂ and 37°C in controlled humidified incubator. They were cultured in DMEM (A549, A431, MDA-MB231, MCF-7, DU-145 cells), MEM (L132) and RPMI-1640 (H1975, NCI-H460, NCI-H23) media (Sigma Aldrich) supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin. All the cells were used prior to passage 20. Cells were routinely passaged using 0.25% trypsin/0.1% EDTA.

RNA Library and Primers
The DNA library 5'-C A T C G A T G C T A G T C G T A A C G A T C C-25N C G A G A C G T T T C T C T C C C T A T A G T G A G T C G T A T T A-3' (Sigma) was amplified by PCR (5 min at 95°C, followed by cycles of 0.5 min at 95°C, 0.5 min at 65°C, and 1 min at 72°C, followed by 2 min at 72°C), with Reverse-Primer 5'-CATCGATGCTAGTCGTAACGATCC-3' and Forward-Primer 5'-T A A T A C G A C T C T C A CTATAGGGAGAGAGAAACGTT CTCG-3'. The resultant dsDNA was converted into RNA library by in-vitro transcription by using epicentre in-vitro transcription kit. The purity of both DNA and RNA was checked on agarose gel.

Selection of Aptamers by Cell SELEX
The RNA library was briefly denatured at 90 °C in 20 ml of selection buffer (EBSS with 1 mM MgCl2), cooled slowly and then warmed up to 37 °C before incubating with counter-selection cell-line L132 cells. After each incubation (60 min each), the unbound RNAs were collected for the next incubation with positive-selection cell H1975 with varied incubation time: 60 min for rounds 1–2, 45 min for rounds 3–5 and 30 min for rounds 6–12. After every incubation the cells were extensively washed before collecting the internalized RNA sequences. The internalized RNA sequences were then extracted using Trizol Reagent (Invitrogen). Selected
sequences were treated with DNase (Sigma), before reverse-transcription and PCR amplification. To monitor the enrichment of internalizing aptamer candidates, quantitative PCR method was used to quantify the numbers of PCR cycles carried out to obtain the same amount of PCR products. Subsequently, the PCR products were purified, transcribed into RNA and treated with DNase before starting the next cycle. During the selection, the number of H1975 cells exposed to the RNA library progressively decreased, starting with $1 \times 10^7$ and diminishing by $1–2 \times 10^6$ cells every other round until reaching $1 \times 10^6$ for round 12. After 12 rounds of selection, the resultant samples were given for sequencing.

**Quantification of drug conjugated to aptamer**

The concentration of nanoparticles bound to aptamer was calculated by HPLC. Aptamer conjugated GNPs were digested overnight in methanol and centrifuged. The supernatant was analyzed for concentration of gefitinib using Shimadzu HPLC system. A C-18 column was used. The mobile phase consisted of 0.02M dipotassium hydrogen ortho phosphate and methanol in the ratio of 10:90 v/v delivered at a flow rate of 1ml/min. 20µl of methanol extract was injected and gefitinib was quantified by UV detection at 246nm.

**Partial knockdown of Ets-1 in H1975 cells**

Lipofectamine (Invitrogen) was used for transfection assay. Briefly, esiRNA and lipofectamine were individually added to 1.5 ml plain medium without serum and antibiotic each and incubated for 5 minutes at room temperature. Thereafter both esiRNA and lipofectamine solutions were mixed and vortexed. The mixed solution was kept at room temperature for 20 minutes for the formation of esiRNA complex with lipofectamine. After the incubation time, the esiRNA and lipofectamine complex was added to the cells. The medium of the cells was changed after 8 hours so as to avoid any cell death due to the presence of lipofectamine. The cells were then allowed to grow for 48 hours before analyzing them for percentage knockdown
and other western blotting studies. Percentage knockdown was calculated by quantitative RT-PCR where cells treated with scrambled esiRNA were considered as control.

**qRT-PCR analysis**

Total RNA was extracted from H1975 cells, using TRIzol reagent (Invitrogen) and purified using RNeasy kit (Auprep RNeasy mini kit, Life Technologies) according to the manufacturer’s instructions. RNA quantity was measured by A260/280 absorbance ratio using ‘Nanodrop’ and integrity of each sample was assured by agarose gel electrophoresis, respectively. Total RNA prepared from cells was used for cDNA synthesis.

cDNA was prepared from 20 µg of total RNA by using SuperScript III (Invitrogen Life Technologies) and oligo dT primer (Invitrogen Life Technologies). The quantitative real time PCR reaction was performed using Light Cycler 2.0 (Roche Diagnostics) in final volume of 20µl containing 50 ng of cDNA, 15µl of reaction buffer from SYBR® Premix Ex Taq™ (Takara Bio Inc, Japan) and the specific forward and reverse primers (Essesnce Lifescience), according to manufacturer’s protocol. The data analysis was performed using Light Cycler software (Roche Diagnostics). Relative fold change in gene expression of the selected genes was calculated using 18S rRNA as reference standard.

**Table 1: Sequence of primers used for mRNA quantitation by RT-PCR**

| S.No. | Gene name | Primer sequence |
|-------|-----------|----------------|
| 1     | ETS-1     | Fwd: GGTAAGCTTTCAGGGCTGG  
          Rev: AGGCACCTGCATCCAAGAGA |
| 2     | 18S rRNA  | Fwd: GCAATTATCCCCCATGAACG  
          Rev: AGGGCCTCCTAAACCAGTCC |
Transfection of Ets-1 in L132 cells

Briefly, 2 X 10^6 cells were seeded in cell culture dishes and were allowed to achieve 70% confluency before transfection. Transfection reagent lipofectamine was diluted in MEM medium without FBS or antibiotics. The tube was incubated for 5 minutes at room temperature. 5μg of plasmid DNA was diluted in MEM medium without FBS or antibiotics. Thereafter diluted lipofectamine as well as plasmid were combined and allowed to sit at room temperature for 30 minutes. The transfection mixture was then added dropwise to the culture plates in which the cells were grown. Cells were then incubated under standard conditions of 5% CO₂ and 37°C for 48 hours before testing the effects of over-expression by western blotting.

Protein isolation and Western blotting

H1975 cells were plated for western analysis. Cells were treated with 8μM gefitinib, GNPs and Apt-GNPs for 48 hours. Briefly, treated cells were washed with chilled PBS. The cells were then scraped in lysis buffer and sonicated. The proteins were separated by centrifugation at 10000 rpm for 8 minutes. Protein estimation was performed using Lowry’s method. The samples were then prepared for SDS-PAGE and then loaded on polyacrylamide gel. The proteins were transferred on a PVDF membrane which was incubated in presence of anti-p300, anti-MMP-13, anti-tubulin, anti-H3- acetylation, anti-Actin (1:1000 dilution; obtained from Santa Cruz Biotechnology) overnight. The antigen antibody complex was detected using HRP-coupled secondary antibodies (Santa Cruz Biotechnology). Specific bands were detected by chemiluminescence (NOVEX Invitrogen), and visualization was performed by exposure of the membranes to hyperfilm (GE Healthcare).

Statistical Analysis

Tukey’s test was performed to determine which groups in the sample differ. It defines a value termed as Honest Significance Difference (HSD) which basically represents the minimum distance between the groups that must be present for a result to be statistically significant.