SUPPLEMENTARY INFORMATION

Functional Characterizations of Interactive Recombinant PTEN-Silica Nanoparticles for Potential Biomedical Applications

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METHODS

Cloning of Human PTEN

Human PTEN was amplified from pANT_cGST vector using gene specific primers with restriction enzyme overhangs. The PCR amplification was performed using Bioline master mix and the reaction conditions consisted of 30 cycles of 95 ˚C for 30 seconds, 50 ˚C for 1 min and 72 ˚C for 1 min 20 seconds using gene specific forward primer 5'-CGGGATCCGACATGACAGCCATCATCAAAG-3' with a *BamHI* overhang and reverse primer 5'-CCCTCGAGGACTTTTGTAATTTGTGTATGC-3' with a *XhoI* overhang. The PCR products were purified and cloned into TA vector followed by cloning into bacterial expression vector PGEX-4T-2.

Expression and Purification of Recombinant PTEN

PGEX-4T-2-PTEN construct was transformed into E.coli BL21 (DE3) for expression and purification. The transformed bacteria were grown on LB agar plates containing ampicillin. Primary culture of 5 ml grown from isolated single colony overnight at 37 ˚C was used as an inoculum for secondary culture of 500 ml LB media. The expression of recombinant PTEN was induced at an OD_{600} of 0.6 by addition of IPTG at a final concentration of 0.5 mM. Culture was then shifted to 26 ˚C and allowed to further grow for 4 h. The cells were harvested by centrifugation at 7000 rpm for 8 min and stored at -20 ˚C until further use.

The frozen bacterial pellet was re-suspended in chilled lysis buffer of 20 mM Tris pH 7.4, 1 mM EDTA, 1 mM PMSF and 150 mM NaCl. The bacteria were lysed by probe sonication on ice at 30 % amplitude for 30 cycles of 2 seconds ON and 25 seconds OFF. Supernatant collected after centrifugation at 12000 rpm for 20 min was diluted with an equal amount of HBS (50 mM Hepes and 150 mM NaCl, pH 7.4) and filtered through 0.45µm syringe filter. The lysate was allowed to bind to glutathione-agarose beads under slow rocking conditions on ice for 45 min followed by several washes with HBS. Bound recombinant PTEN was eluted using 10 mM L-reduced glutathione in 50 mM Hepes pH 8.0. Eluted fractions were subjected to buffer exchange by dialysis against 25 mM Hepes pH 7.4 at 4 ˚C for 4 h. The dialyzed protein was concentrated by centrifugation at 5000 rpm for about 20 min using (30kDa MWCO) Spin-X UF concentrator and verified by 12 % SDS-PAGE analysis.

Characterization of Purified Protein

Western Blot

Purified GST-PTEN was separated on 12 % SDS-PAGE followed by transfer onto a PVDF membrane. After verifying the transfer of protein using ponceauS stain, the membrane was washed with PBST several times to remove the stain. Blocking was performed at room temperature for 2 h using 3 % BSA in PBST. Subsequently, the membrane was incubated overnight at 4 ˚C with anti-PTEN primary antibody raised in rabbit. The membrane was washed with PBST four times of 20 min each followed by incubation with HRP conjugated anti-rabbit secondary antibody at room temperature for 2 h and then the washing step was repeated. Blot was developed using chemiluminescent peroxidase substrate. GST was also probed using anti-GST antibody (raised in rat) and anti-rat as the primary and secondary antibody, respectively.

Circular Dichroism Spectroscopy Study

Protein was dialyzed against 10 mM Tris pH 7.4, and further concentrated by centrifugation at 5000 rpm for desired time using (30 kDa MWCO) Spin-X UF concentrator. Samples in 10 mM Tris pH 7.4, taken in cuvette with 5 mm pathlength were analyzed using JASCO-815 spectrometer. The CD spectra were recorded from 190 nm to 240 nm in 0.5 nm steps under constant nitrogen gas purging at a flow rate of 5 L/min and 25 ˚C temperatures. Only sample buffer was subtracted from the experimental data.

MALDI TOF-TOF Analysis

Purified GST-PTEN protein band was excised from SDS-PAGE gel after colloidal coomassie G-250 staining. In gel trypsin digestion was performed, using trypsin profile IGD kit (Sigma), according to manufacturer's protocol. Peptide sample desalted using ziptip C18 column was mixed with the matrix α-Cyano-4-Hydroxycinnamic acid in the ratio of 3:1 and spotted onto analyzer plate. MS-MS analysis was done using 4800 proteomics analyzer with TOF-TOF optics, Applied Biosystems. The results were analyzed using Expasy proteomics Findpept program.[1]

Synthesis and Characterization of Silica Nanoparticles

Synthesis of silica nanoparticles were carried out by modification of Stober's process, ethanol was mixed with NH_4OH at room temperature and the mixture was stirred for about 5 min to attain homogeneity. TEOS was then added to the solution under stirring condition and allowed to stir for 3 h. Nanoparticles formed were collected by centrifugation at 12000 rpm for 10 min and re-suspended in EtOH using water bath sonicator for 4 min. This process was repeated three times to remove any unreacted reagents and the nanoparticles were stored at 4 ˚C until further use. Stored nanoparticles were sonicated prior to any applications. Synthesized nanoparticles were characterized by FESEM, TEM, DLS and Zeta measurements. For FESEM, synthesized silica NPs were drop coated on aluminum foil, air dried overnight, double coated with gold (SC7620"Mini", Polaron Sputter Coater, Quorum Technologies, Newhaven, England) and analyzed in a Carl Zeiss, SIGMA VP, instrument. For TEM, 7 µl of sample was drop coated on carbon coated copper grid, air dried overnight and observed in JEM 2100 (Jeol, Peabody, MA; accelerating voltage of 200 keV). For DLS and zeta potential analysis, silica nanoparticles diluted in Milli-Q was analyzed using Malvern Zeta sizer Nano ZS to determine the hydrodynamic diameter and surface charge, respectively.

Immobilization of GST-PTEN onto Silica NPs

FTIR analyses of the lyophilized samples were carried out by mixing the samples with KBr to form pellet and the spectra was recorded in the frequency range of 4000-500 cm^{-1} using Perkin Elmer Spectrum One machine. The peaks obtained were analyzed to study binding of the recombinant protein to the silica nanoparticles. For DLS measurement, diluted and thoroughly mixed samples were analyzed using Malvern Zeta sizer Nano ZS.

3. Results

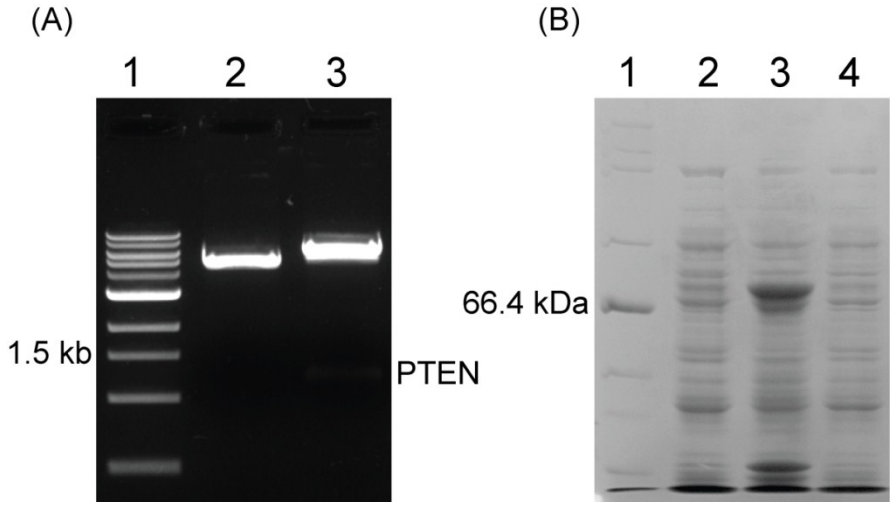


Fig S1. Cloning and Induction of GST-PTEN (A) Lane 1 shows 1 kb DNA ladder, Lane 2 shows the uncut PGEX-4T-2 plasmid and Lane 3 shows restriction digestion of PGEX-4T-2-PTEN by *BamHI* and *XhoI* (B) Induction of GST-PTEN expression, Lane 1 shows 2-212 kDa protein ladder, Lane 2 displays supernatant fraction of induced cell lysate, Lane 3 displays expression of GST-PTEN in pellet fraction of induced cell lysate (around 81 kDa) and Land 4 shows uninduced cell lysate

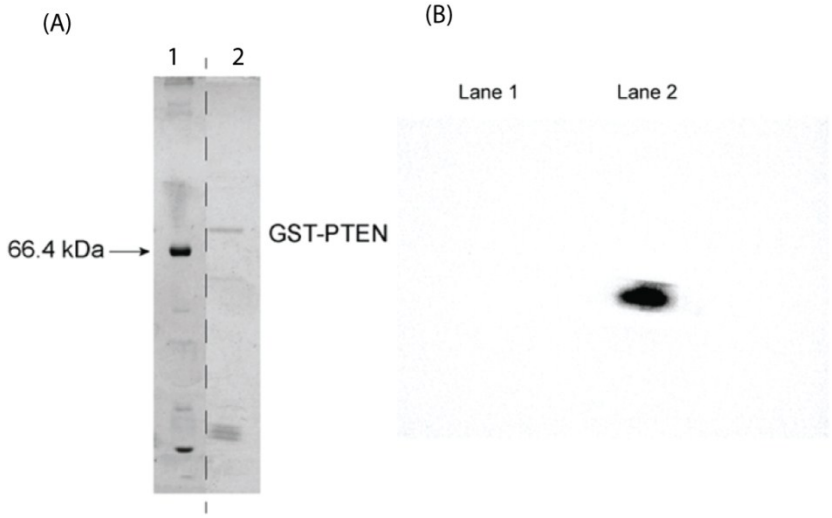


Fig S2. (A) Purification of GST-PTEN by Glutathione-agarose affinity chromatography where Lane 1 shows 2-212 kDa protein ladder and Lane 2 shows the purified band at 81 kDa (B) Western blot analysis of purified recombinant PTEN, Lane 1 shows negative control, Lane 2 shows band development for purified GST-PTEN.

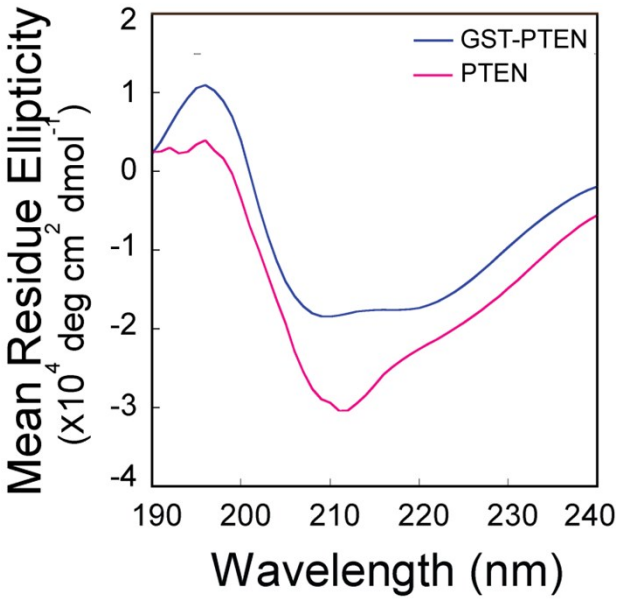


Fig S3. CD spectra of GST tagged and untagged PTEN

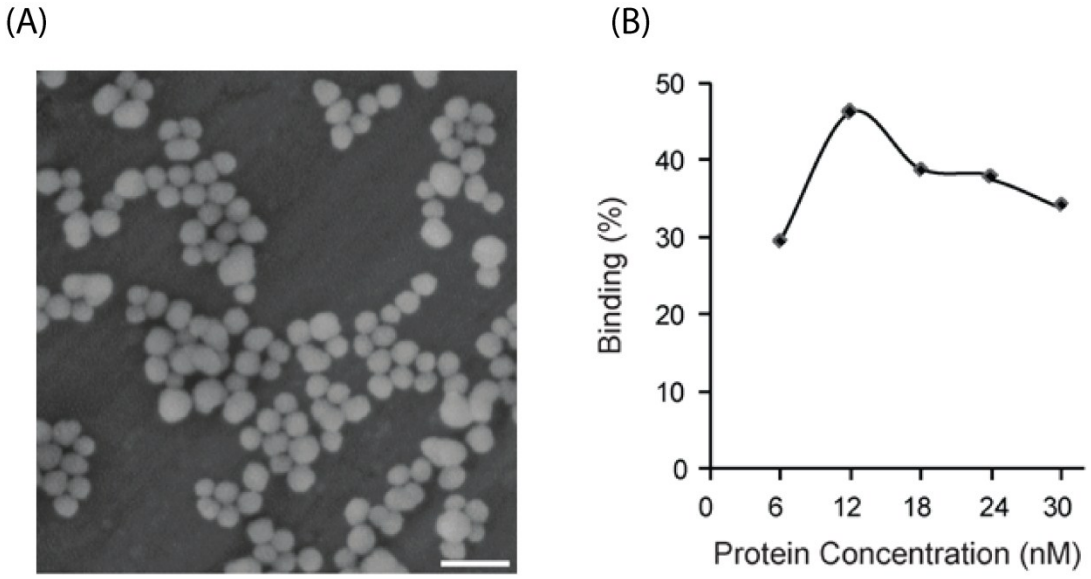


Fig S4 (A) FESEM image of silica nanoparticles (Scale Bar 100 nm) (B) Percentage binding of GST-PTEN onto silica nanoparticles at varying concentration of proteins, maximum binding obtained was 49 % at a protein concentration of 12 nM

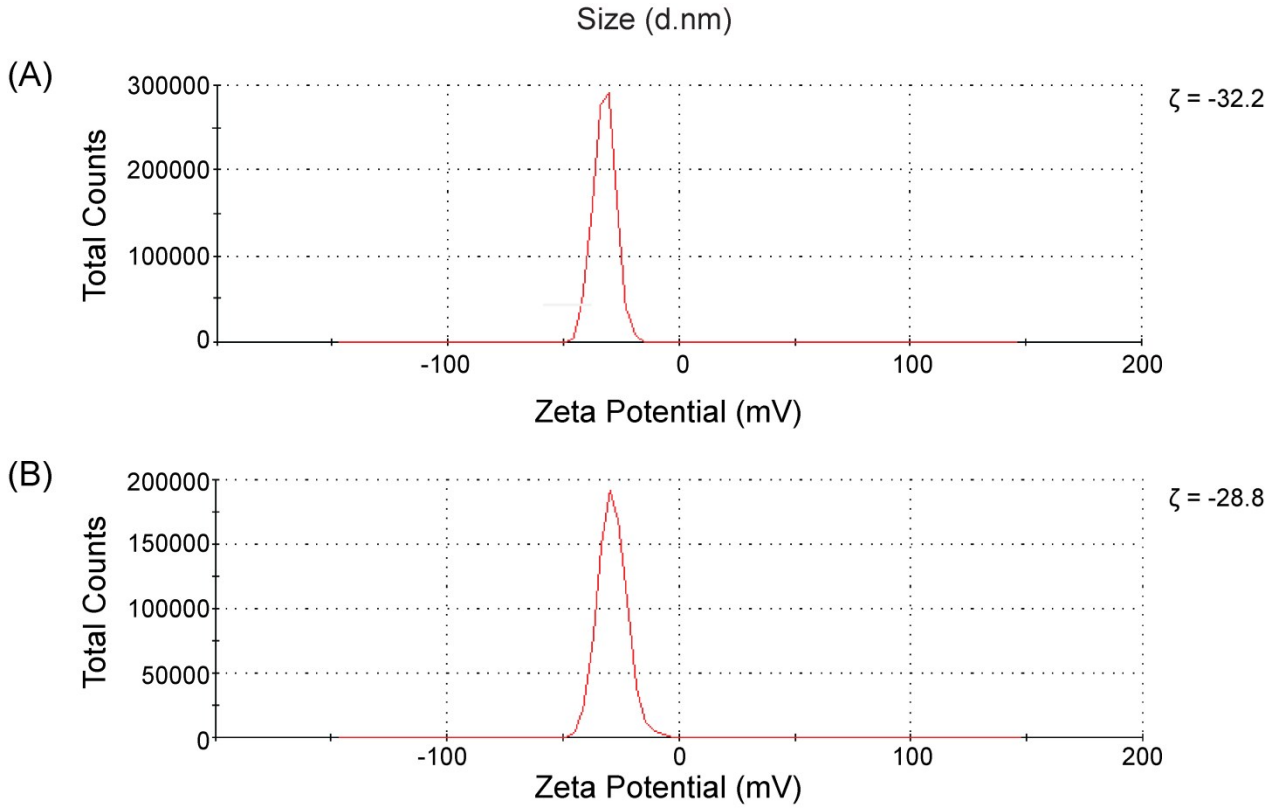


Fig S5 Zeta potential of (A) Silica nanoparticles (B) Recombinant PTEN bound silica nanoparticles

Protein adsorption onto nanoparticles is known to induce change in zeta potential of the nanoparticles.[2] Thus, measuring surface charge can be used a simple tool to characterize protein-nanoparticle interaction. Zeta potential of silica nanoparticles and GST-PTEN immobilized onto silica nanoparticle was recorded using Malvern Zeta sizer Nano ZS at pH 7.4. The results show a slight shift in the zeta potential of the nanoparticles indicating protein-nanoparticle interaction.

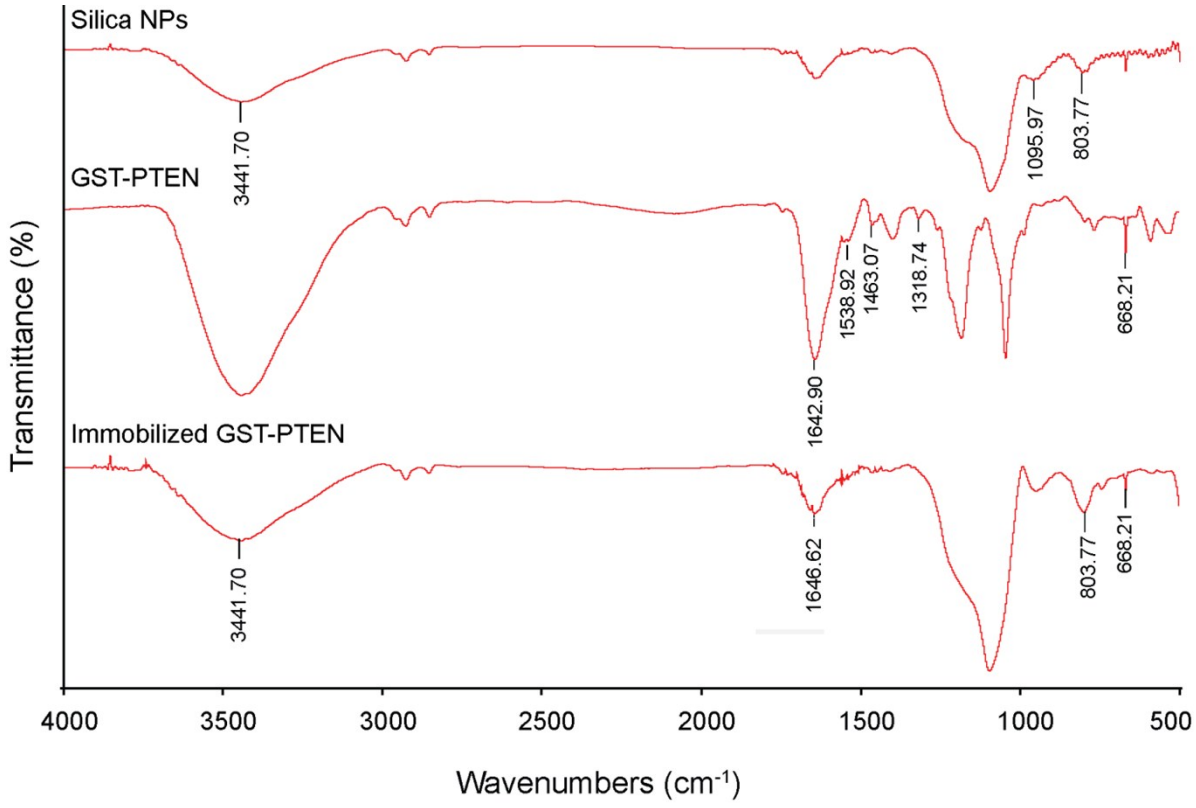
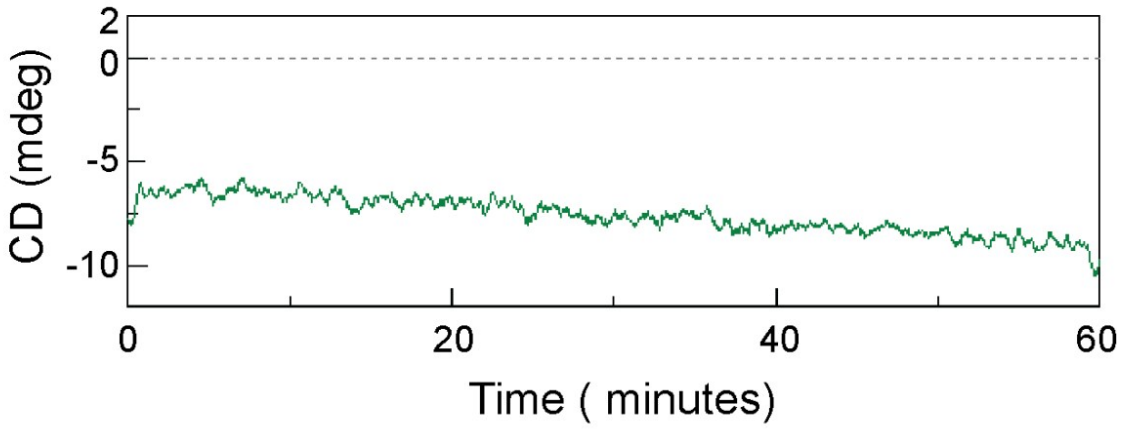


Fig S6. FTIR spectra of silica nanoparticles, GST-PTEN and GST-PTEN SNP indicating protein loading

(A)



(B)

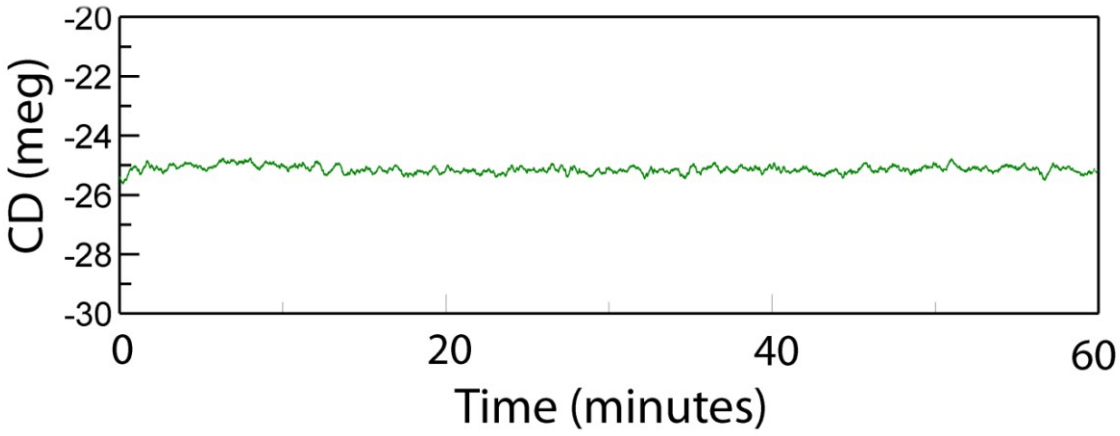


Fig S7 Monitoring shift at 222 nm by circular dichroism (A) GST-PTEN immobilized onto silica nanoparticles (B) Only GST-PTEN

Monitoring the shift at 222 nm by circular dichroism reveals the conformational change that takes place in protein upon adsorption onto nanoparticle surface.[3] Kinetic experiment was conducted with recombinant protein to nanoparticles ratio displaying maximum binding. Silica nanoparticles and protein solutions were rapidly mixed and taken in to cuvette with 5mm pathlength. The ellipticity was measured at 222 nm as a function of time for 60 min using JASCO-815 spectrometer.This measurement helps to monitor the structural change taking place upon protein nanoparticle interaction. It is interesting to observe that the initial conformational change is slow indicating that the interaction between the protein

and silica nanoparticles does not rapidly perturb the folding and stability of the protein, which is very crucial to achieve successful binding.

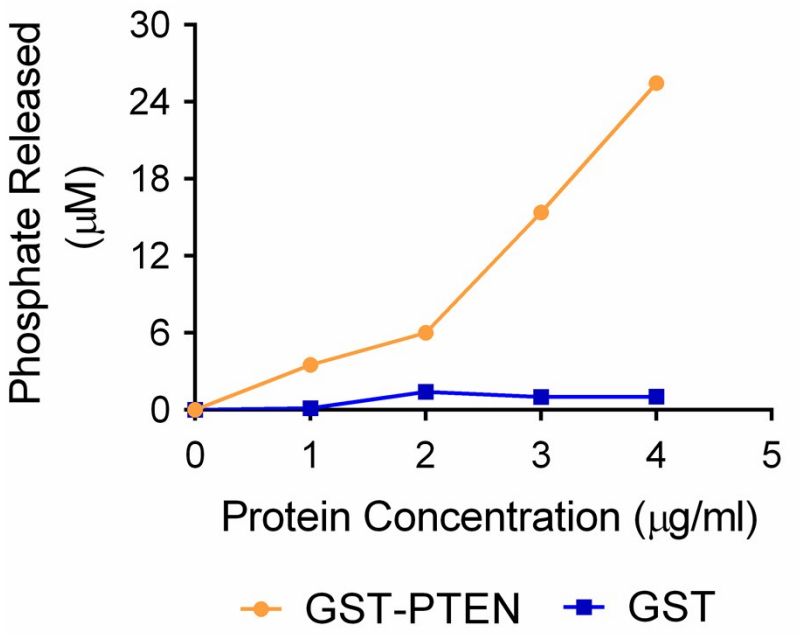


Fig S8 Phosphatase activity of GST protein

The phosphatase activity of GST alone was determined using PNPP as substrate. The reaction mixture consisted of 25 mM Hepes pH 7.4, 50 mM PNPP, 10 mM DTT and varying concentration of the protein. The results indicate no significant phosphatase activity of GST.

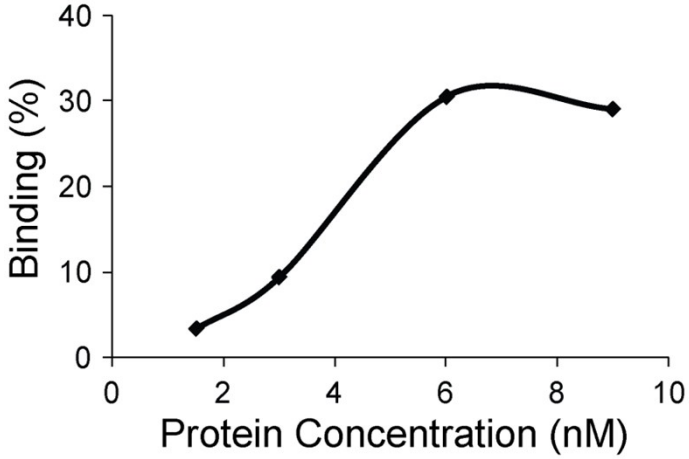


Fig S9 Immobilization of PTEN onto silica nanoparticles

To investigate adsorption of PTEN protein on the surface of silica nanoparticles varying concentrations of protein (1.5 nM to 9 nM) was incubated with constant concentration of silica nanoparticles (0.4 mg/ml) in 25 mM Hepes, pH 7.4 for 2 h at room temperature. Binding was determined by probing the inherent fluorescence of the protein. The maximum binding percentage was calculated to be 30%, at a protein concentration of 6 nm. Binding percentage was calculated using the following formula-

$$Binding(\%) = \frac{Emission\ of\ Bound\ Protein}{Emission\ of\ Total\ Protein} X\ 100$$

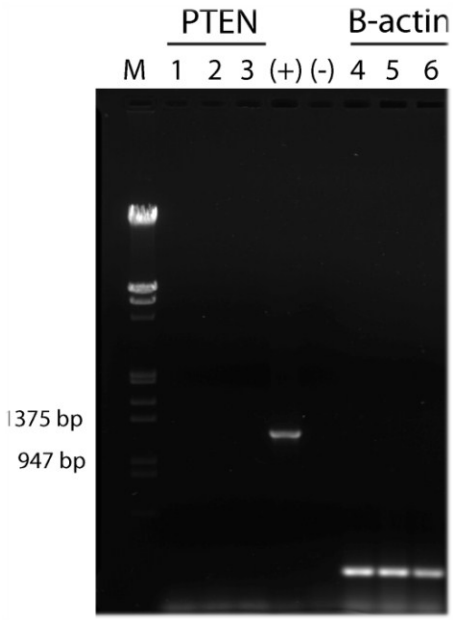


Fig S10 Expression study of PTEN in U-87 MG cells, where M is the DNA marker (Lambda DNA/ EcoRI + HindIII Marker), Lanes 1,2 and 3 represent amplification of cDNA using PTEN gene specific primers at 50 ˚C, 55 ˚C and 60 ˚C annealing temperatures respectively, (+) Positive control showing 1.2 kbp PTEN band, (-) Negative Control, Lanes 4,5 and 6 represent amplification of cDNA using β-actin primers as control at 50 ˚C, 55 ˚C and 60 ˚C annealing temperatures respectively.

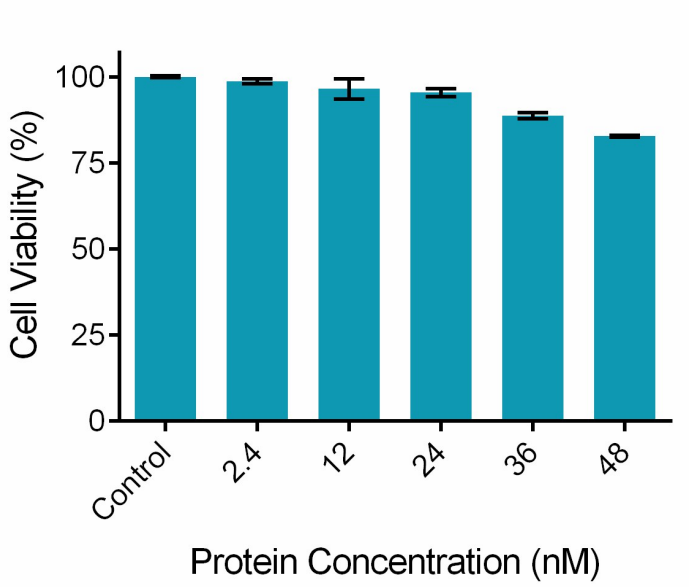


Fig S11 Assessment of viability of U-87 MG cells upon treatment with varying concentration of GST-PTEN protein for 48 h by MTT assay

References

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2 K. Rezwan, A. R. Studart, J. Voros and L. J. Gauckler, *The journal of physical chemistry B*, 2005, **109**, 14469-74.

3 P. Billsten, M. Wahlgren, T. Arnebrant, J. McGuire and H. Elwing, *Journal of Colloid and Interface Science*, 1995, **175**, 77-82.