Supporting Information

A Highly Stable RNA Aptamer Probe for the Retinoblastoma Protein in Live Cells

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S1: The peptide sequence (LSKR[Y]EEIYLKN) derived from RB used for aptamer selection

The peptide corresponding to residues 317-328 of the RB protein. The figure below shows its structure in the N-terminal domain of the RB protein and as predicted by 'Pep Fold'.

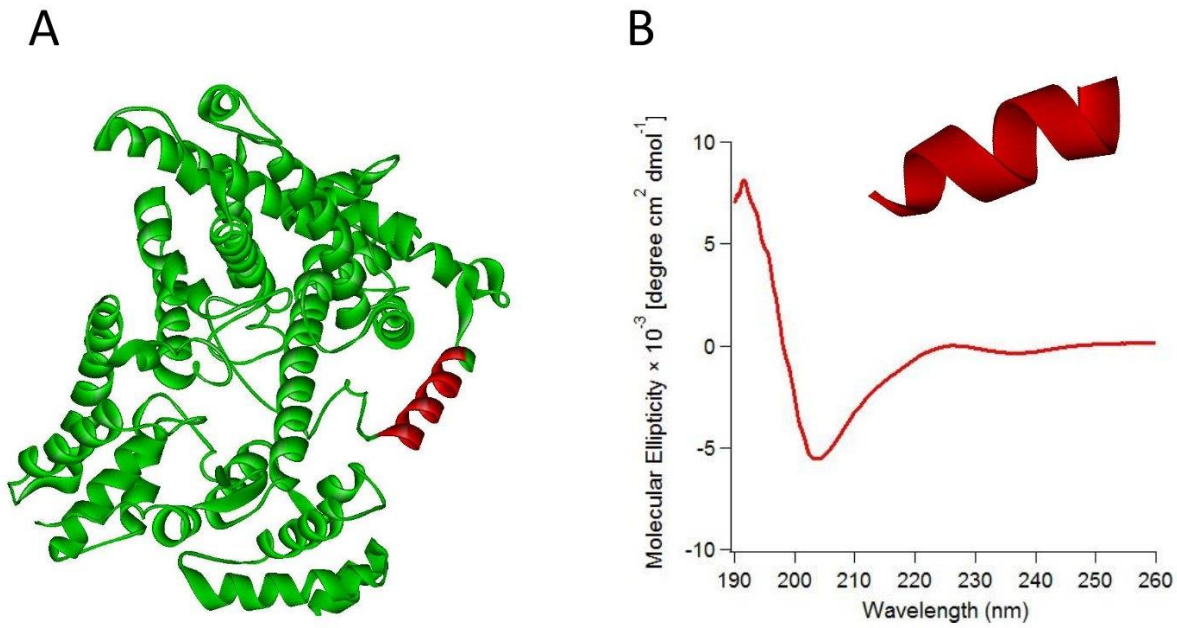

Fig. S1 The secondary structure of the peptide sequence. (A) X-ray Crystal Structure of the RB protein (PDB ID: 4ELJ). The peptide sequence that was used in selection had its amino acids shown in red. (B) CD (circular dichroism) spectrum of the 0.5 mg/mL of the peptide in the binding buffer showing the characteristic α-helical peaks (negative band at 208 nm and a positive band at 193 nm)[1-2]. The CD spectrum was obtained using a J-715 spectropolarimeter (Jasco). Three scans were recorded from 190 to 260 nm with the buffer spectrum subtracted and zero correction at 260 nm. The spectrum presents an average of the three scans. B's inset: predicted peptide structure using PEP-FOLD6[3-4].

S2: Sequencing data and analysis of the 12th round enriched library revealed the E16 sequence

Fig. S2 Sequence analysis of the 12th round enriched library. Sequence analysis was performed using Vector NTI software (Thermo Scientific). It showed sequence E16 occurs 4 times (as E1, E27, E7) and it also shares consensuses with E45.

S3: Binding isotherms of full length 80E16 aptamer to the phosphorylated and unphosphorylated peptide derivatives and to RB

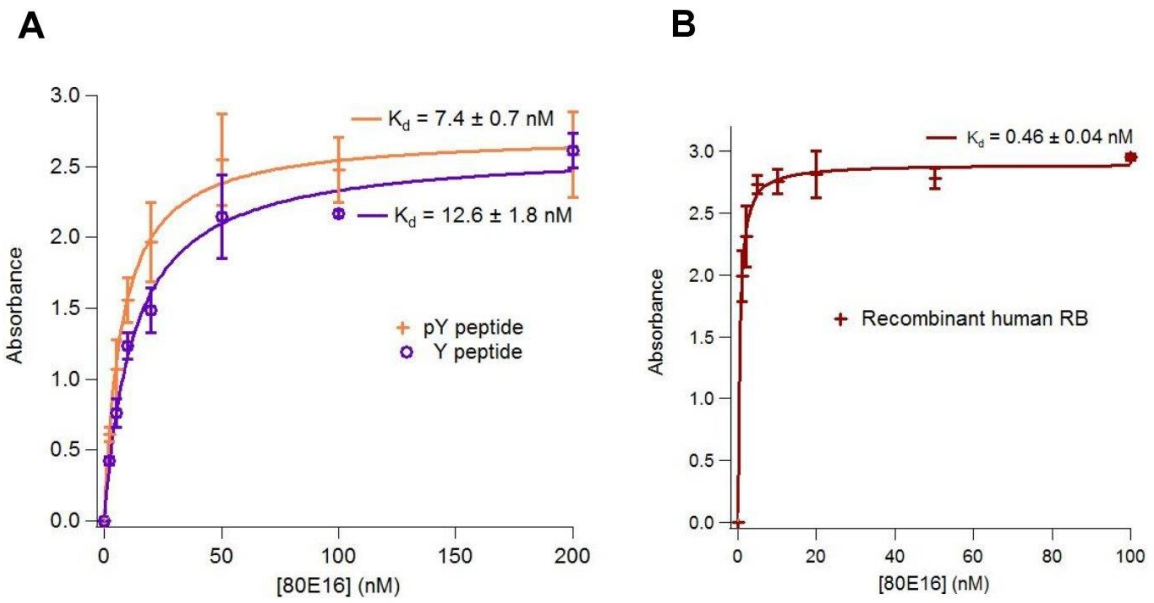

Fig. S3 ELONA binding assays. (A) Binding of the full length aptamer 80E16to the phosphorylated and unphosphorylated peptide sequences by ELONA. The peptides with a free amino at the N-terminal were covalently attached on a BSA-coated microtiter plate using EDC/NHS coupling. (B) Binding of 80E16 to recombinant human RB (expressed in insect cells), where the protein was adsorbed on a microtiter plate. The ELONA binding assays used HRP-labelled streptavidin, which binds to the biotin incorporated at the 3'end of the aptamer, for detection. The assays were run using the binding buffer containing 5 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 150 mM NaCl, 20 mM Hepes, pH 7.4. Changing Hepes to Tris in the buffer did not showed any effects on biding affinity between the aptamers and RB.

S4 Binding isotherms of the truncated aptamer 18E16 to the peptides and to RB

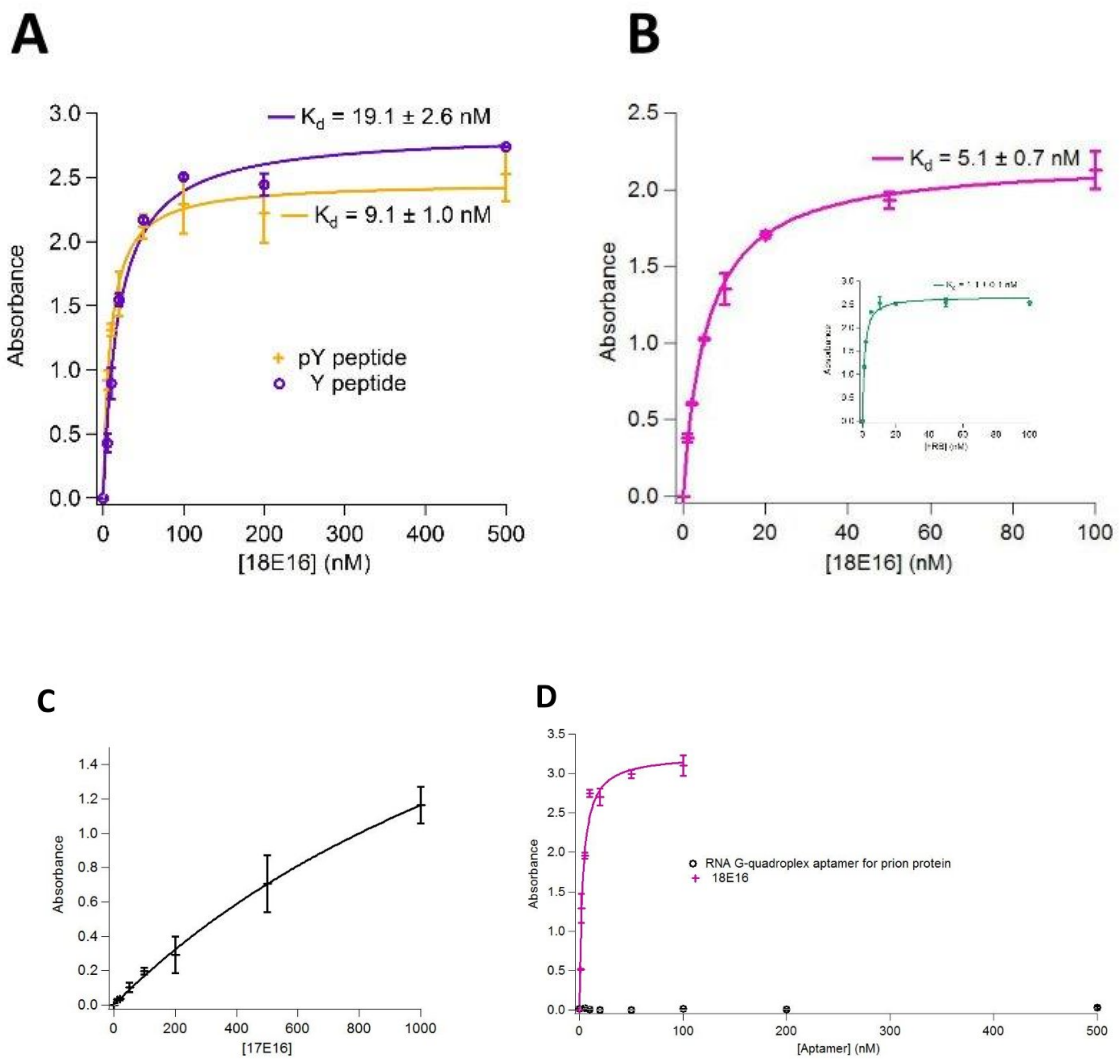

Fig. S4 Binding isotherms (A). Binding of the aptamer 18E16 to the phosphorylated and unphosphorylated peptide sequences using ELONA. (B) Binding of aptamer 18E16 to RB using ELONA. (C) Binding of 17E16 (an over-truncated sequence) to RB using ELONA. (D) Binding the RNA G-quadruplex aptamer for bovine prion protein to RB using ELONA. For ELONA assays, RB used was recombinant human RB expressed in insect cells. The RB was adsorbed on a microtiter plate and the detection was with HRP-labelled streptavidin which binds to the biotin incorporated at the 3'end of the aptamer. Inset: binding of RB to the RNA aptamer using ELISA (enzyme linked immunosorbent assay) where the aptamer had a PEG-biotin modification at its 3'-end and was immobilized on a streptavidin coated microtiter plate and the detection was with a HRP-labelled anti-RB antibody. Both ELISA and ELONA assays used the binding buffer containing 5 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 150 mM NaCl, 20 mM Hepes, pH 7.4. Changing Hepes to Tris in the buffer did not showed any effects on binding affinity between the aptamers and RB.

S5: Molecular recognition of 18E16/24E16 by an anti G-quadruplex antibody and NMM, a dye specific for parallel G-quadruplex structures

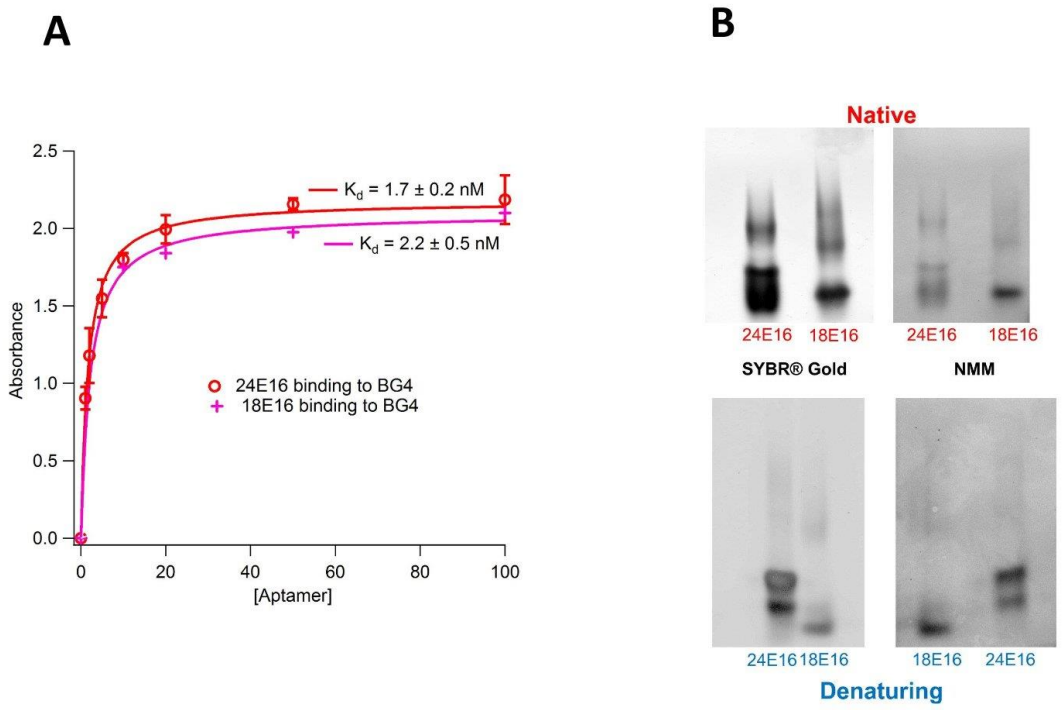

Fig. S5 Binding isotherms of the RNA aptamers, 18E16 (magenta) and 24E16 (red), to the anti- G-quadruplex antibody BG4 by ELONA. BG4 was adsorbed on a microtiter plate and the detection was with HRP-labelled streptavidin which binds to the biotin incorporated at the 3'end of the aptamer. The binding assays used the binding buffer containing 5 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 150 mM NaCl, 20 mM Hepes, pH 7.4. (B) Staining of 18E16 and 24E16 using SYBR®Gold and NMM (a parallel G-quadruplex specific dye) on native (TBE) and denaturing (TBE-urea) gels.

S6: Thermal profiles of 18E16 and 24E16

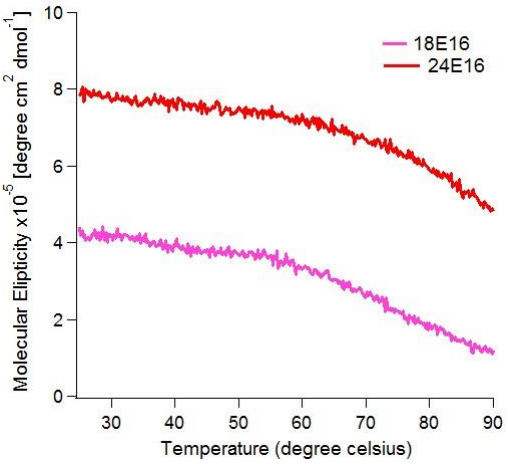

Fig. 6 18E16/24E16 melting profiles by CD. The measurements were performed on 10 µM in the binding buffer and at a concentration of 10 µM 18E16/24E16 in the binding buffer (5 mM KCl, 5mM $MgCl_2$, 1 mM $CaCl_2$, 150 mM NaCl, 20 mM Tris, pH 7.4). Melting curves were obtained by plotting molar ellipticity recorded at 262 nm using Igor (Wavemetrics Inc.). The CD data were obtained using a Chirascan (Applied Photo-Physics).

S7: Another truncated version with 24 nucleotides, 24E16 (GGA GGG UGG AGG GAA GGG UUU GGG)

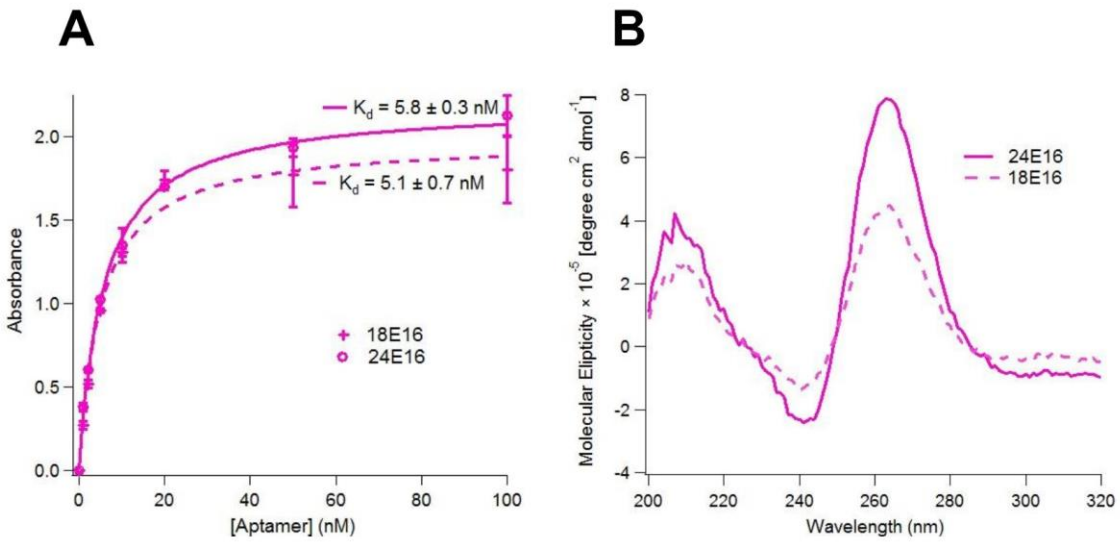

Fig. S7 (A) A ELONAs (enzyme linked oligonucleotide assays) for binding of the RNA G-quadruplex aptamers 24E16 (solid) compared to 18E16 (dash) to RB. (B) CD spectra of 24E16 (solid) compared to 18E16 (dash) at 10 µM in the binding buffer (5 mM KCl, 5mM $MgCl_2$, 1 mM $CaCl_2$, 150 mM NaCl, 20 mM Tris, pH 7.4). It showed 24E16 has almost double in intensity of the signature peak for the parallel G-quadruplex structure at 262 nm. The CD spectra were collected as an average of 10 scans using a Chirascan.

S8: Western blot analysis of RB captured by 18E16 from lysates of H460 and RB-knockout H460 cells

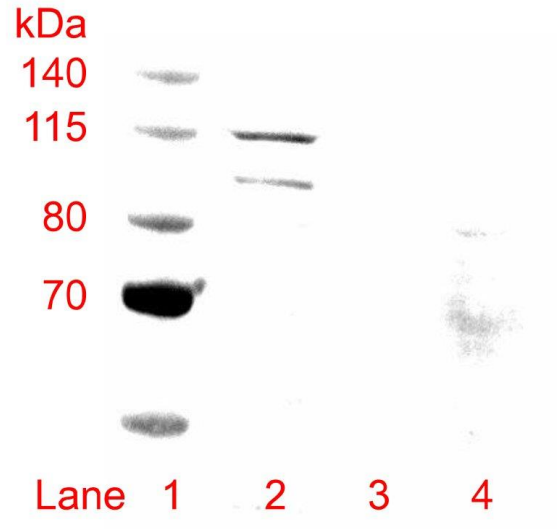

Fig. S8 Western blot of the aptamer pull-down of H460 and RB-knockout H460 cell lysates. Lane 1: Protein weight marker. Lane 2: Captured by 18E18 immobilized magnetic beads of H460 cell lysate. Lane 3: blank. Lane 4: Captured by 18E18 immobilized magnetic beads of RB-knockout H460 cell lysate. The cap-tured contents were then eluted from beads for gel electrophoresis and then transferred to a PDVF membrane for a Western blot. An anti-RB antibody labelled with HRP (sc-73598, Santa Cruz Biotechnology) was used to detect the protein.

S9 Confocal Microscopy and Deconvolution showing the aptamer in live cells

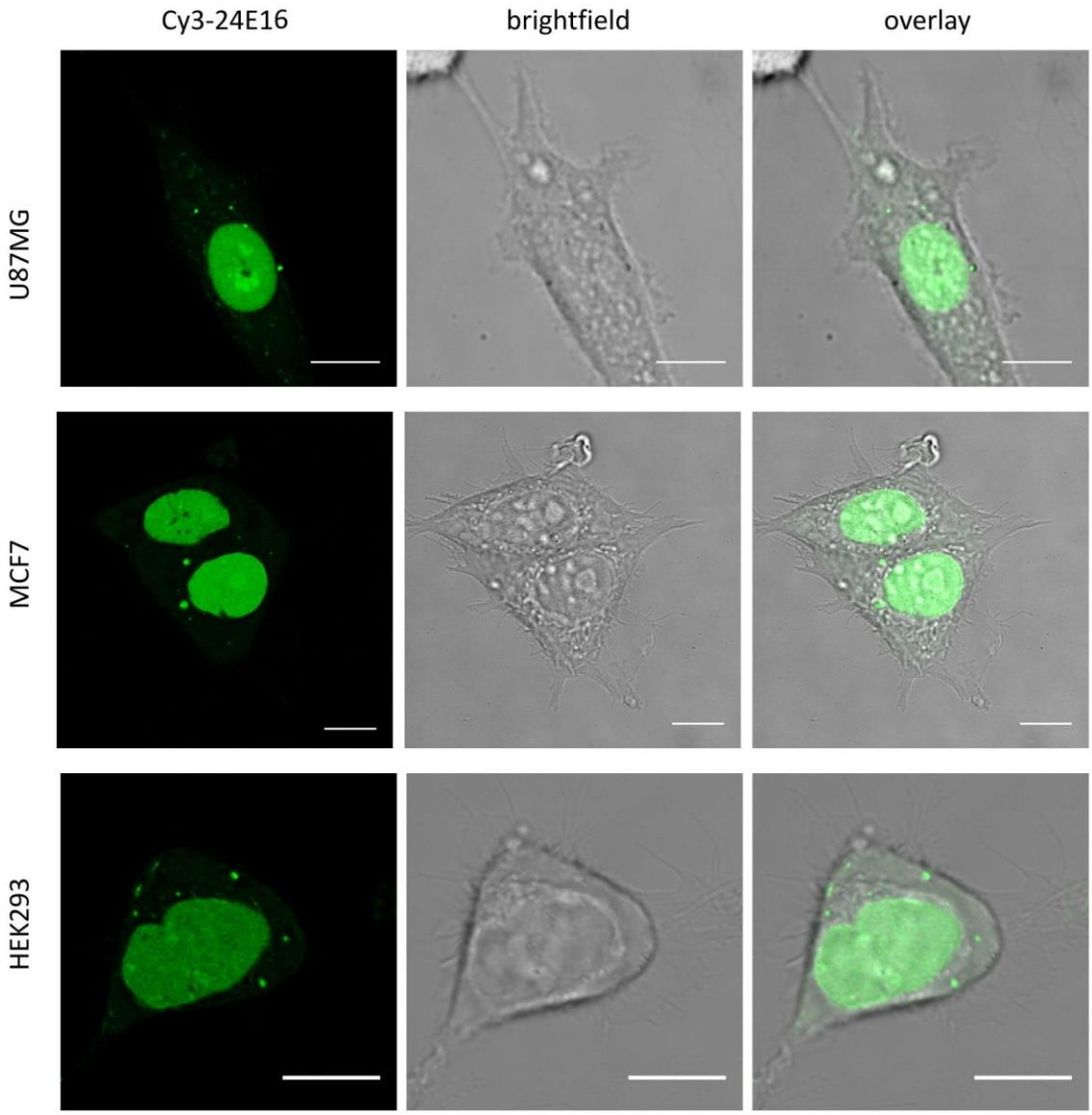

Fig. S9 Visualization of RB in live cells incubated with the fluorescently labelled aptamer using confocal microscopy and deconvolution together with transmitted light microscopy on three cell lines U87MG; MCF7 and HEK293. Scale bar 10 µm.

S10 Super Resolution Image by Structural Illumination Microscopy

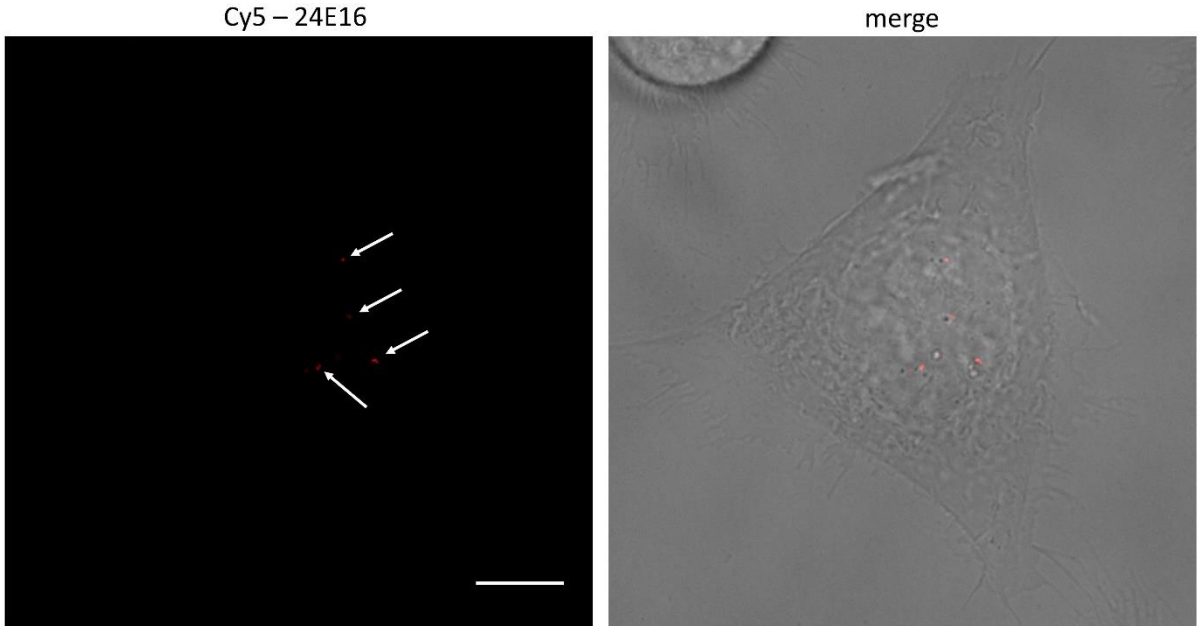

Fig. S10 Super-resolution image of an HEK293 live cell transfected with Cy5-labelled 24E16 (arrows) using structured illumination microscopy for more precise localization of RB in the nucleus. Scale bar 10 µm.

S11: U87MG cell transfected with aptamer *versus* a 15-nucleotide oligo

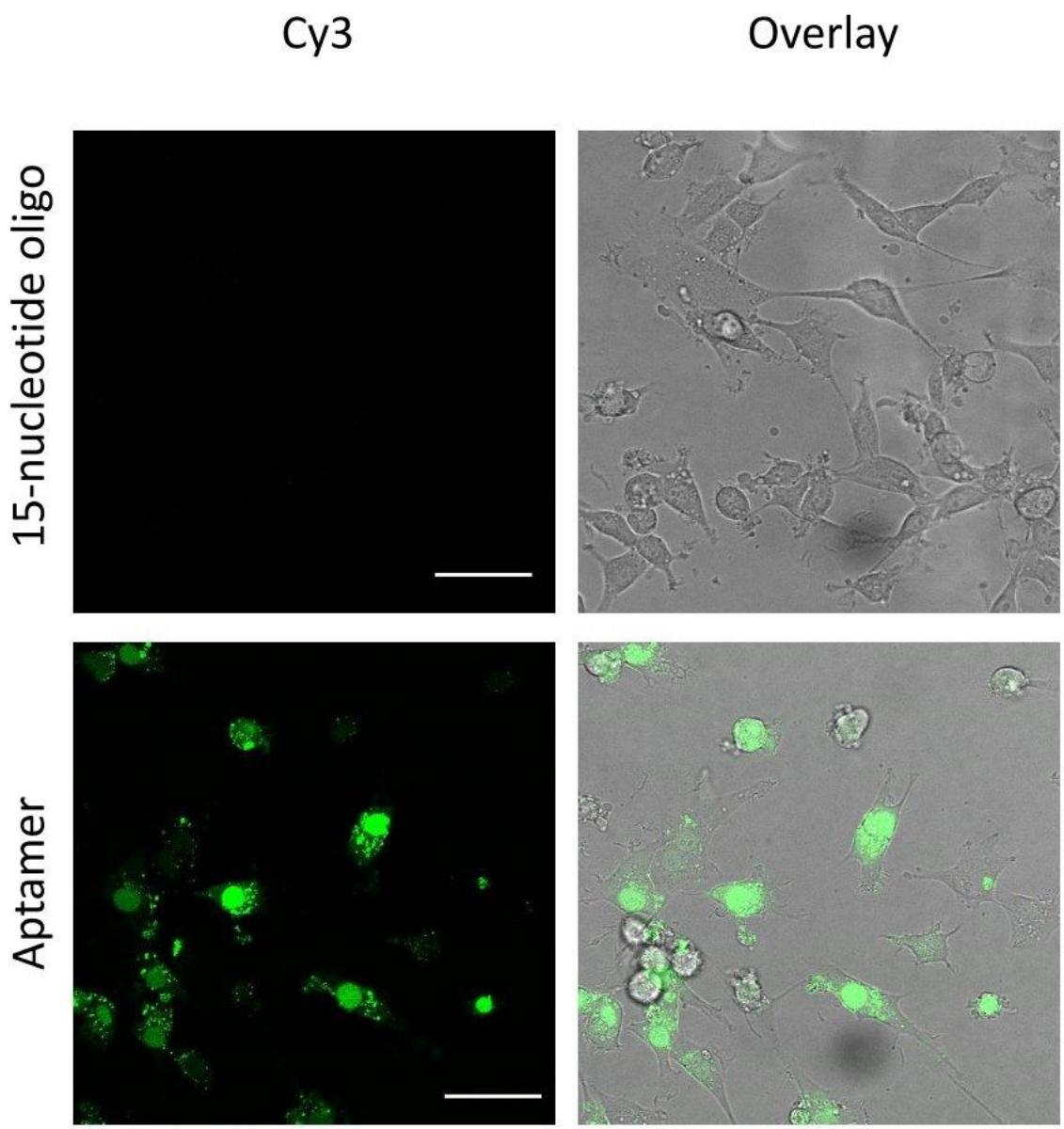

Fig. S11 U87MG cells transfected with aptamer *versus* a 15 nucleotide oligo. U87MG cells incubated with the RNA G-quadruplex aptamer showed fluorescent signal whiles U87MG cells incubated with the 15-nucleotide oligo did not. Scale bar 50 µm.

S12: Aptamer in RB-knockout H460 cells versus H46 cells

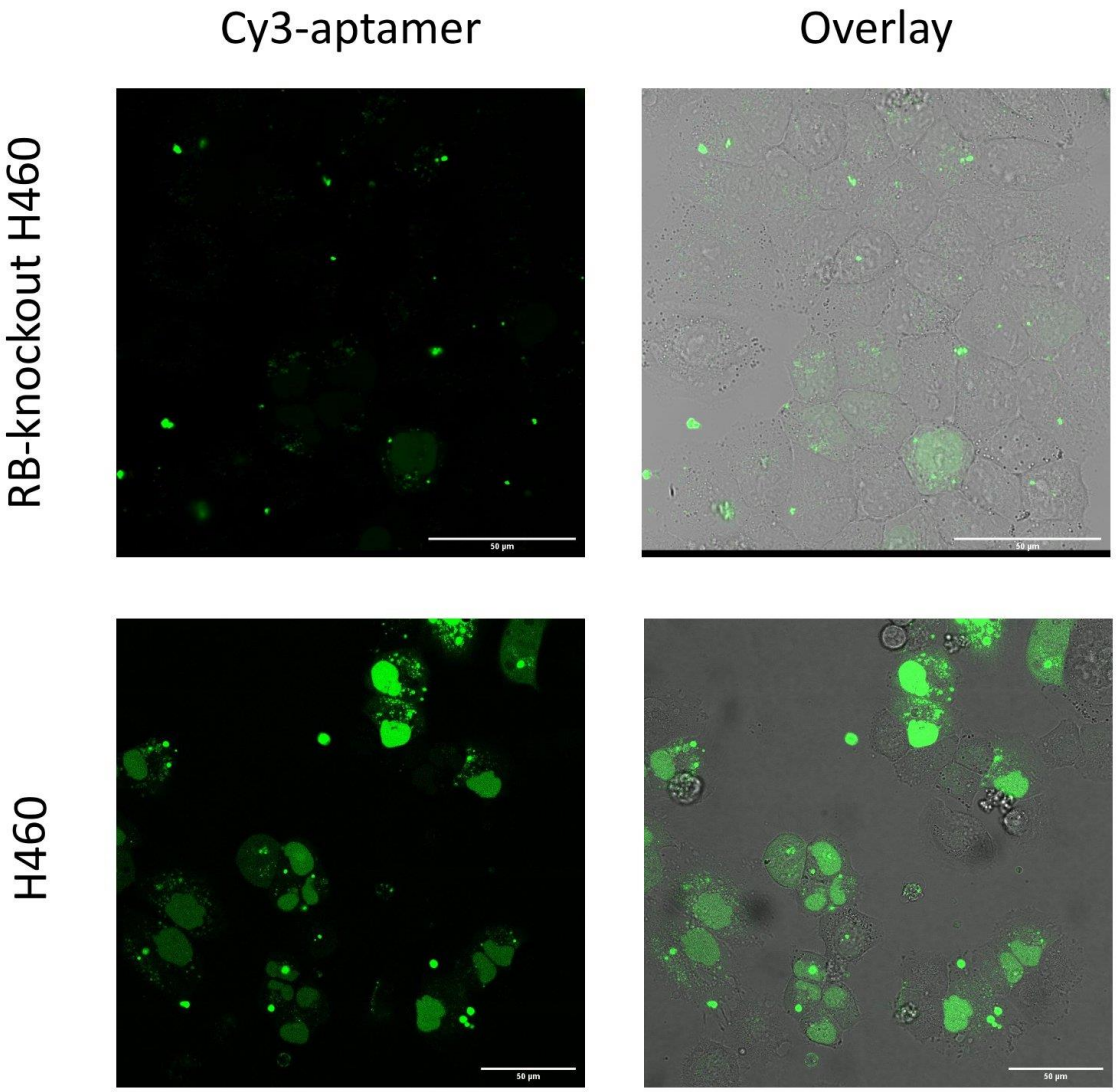

Fig. S12 Confocal images of RB-knockout H460 and H460 cells transfected with the fluorescently labelled aptamer. Scale bar 50 µm.

S13: Transfection of 5'end-Cy3-aptamer and 5'end-Cy5- aptamer (donor and acceptor on different molecules).

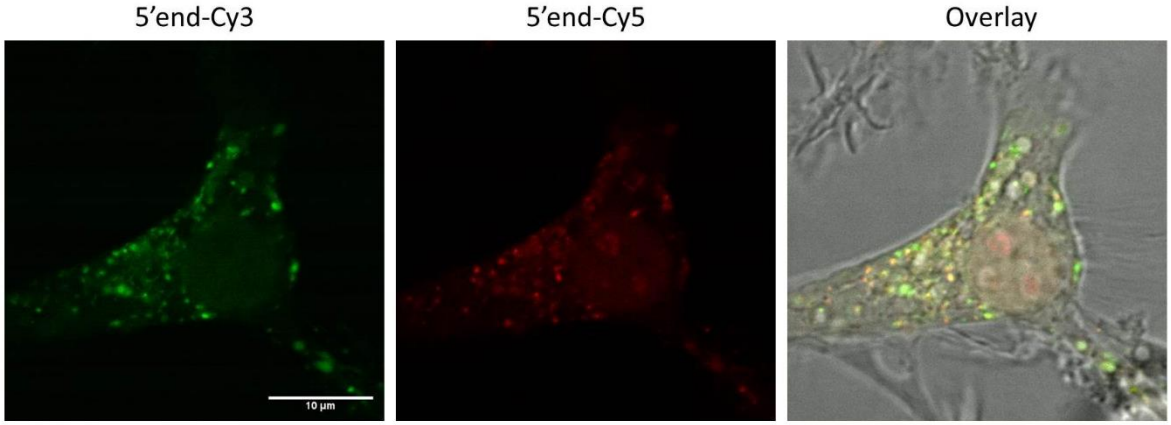

Fig. S13 Co-localisation of an equal molarity of 5'end-Cy3-aptamer and 5'end-Cy5- aptamer (donor and acceptor on different molecules) transfected U87MG live cells by confocal microscopy. The image shows co-localisation. The fluorescent signal of the Cy3 was not diminished as shown in Fig. 4 indicating no significant FRET occurs. This is consistent with each of the fluorescent molecules was not close enough to have FRET as each attached to a different aptamer molecule and that would be the case if the aptamer labelled with the dye pair, each at its ends, being degraded. The images were taken using a Leica SP8 with excitation at 561 nm for Cy3 and 633 nm for Cy5. Scale bar 10 µm.

S14: Panorama view of cells transfected with aptamer Cy3-labelled aptamer and Alexa647-labelled anti-RB antibody for co-localisation study

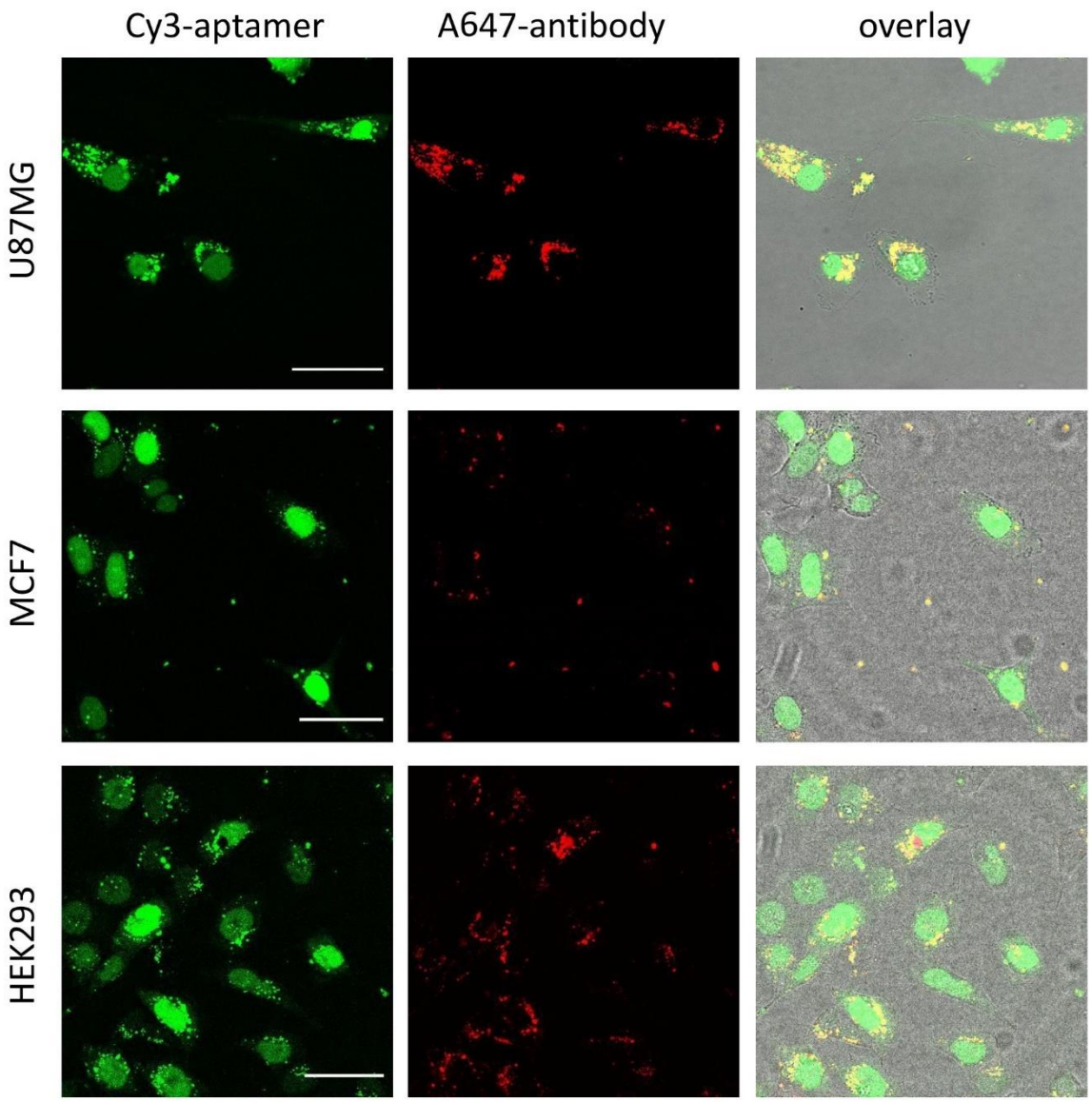

Fig. S14 Panorama view of co-localisation of aptamer Cy3-labelled aptamer and an Alexa647-labelled anti-RB antibody transfected into live cells, U87MG, MCF7 and HEK293, showing high efficiency of the aptamer entering nuclei. The bright-field images were processed to remove fringes by subtracting an averaged, low pass filtered image. Scale bar 50 µm.

References

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