Electronic Supplementary Material (ESI) for RSC Advances.


**Supplementary information**

# Contents

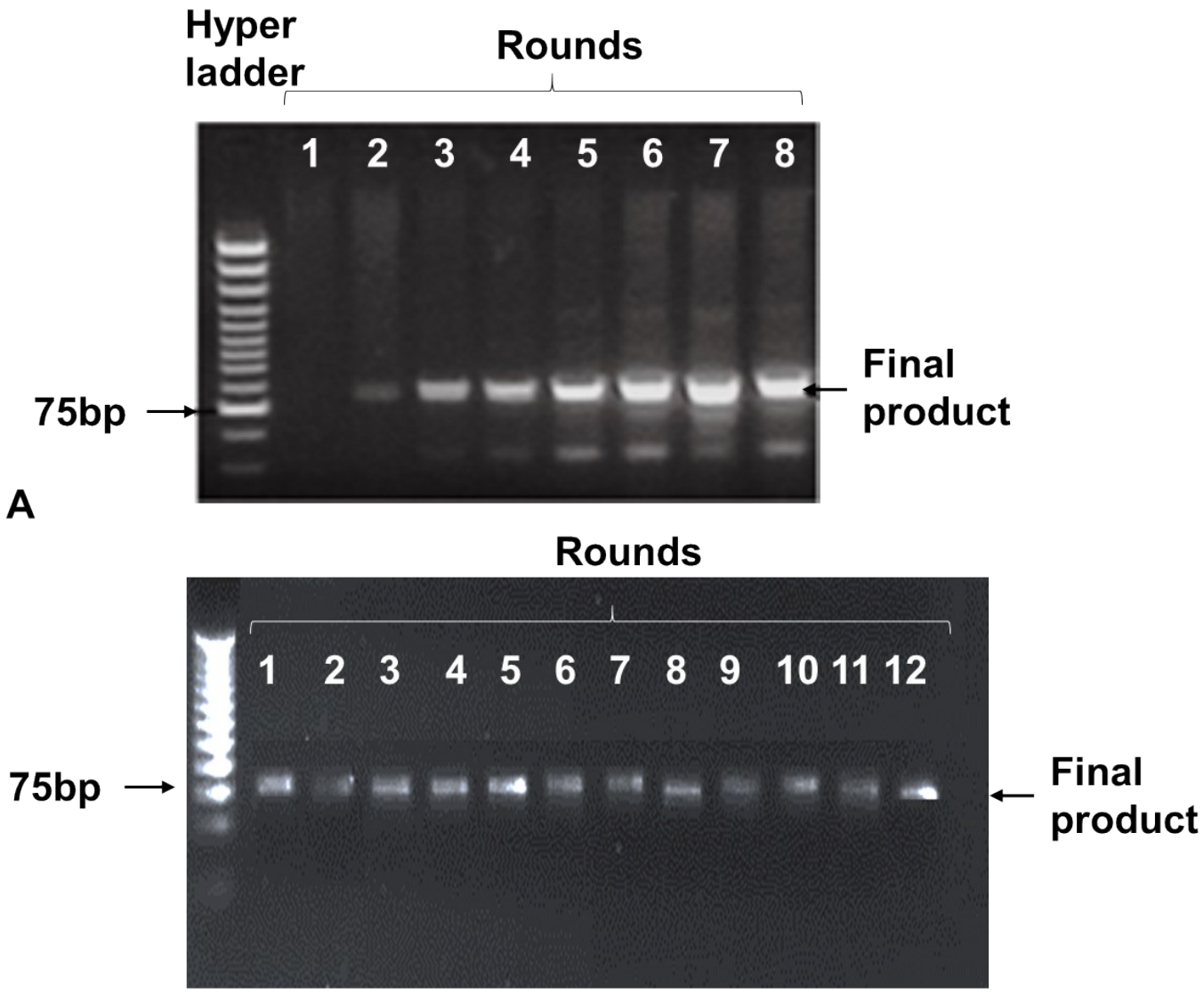



Figure 1: Enrichment of selected aptamers shown in 10% acrylamide gel. **A:** Analysis of PCR products during 8 rounds of selection: Lane 1, HyperLadder 25bp; Lanes 2 to 8, PCR product of the 1st to 8th rounds. **B:** Analysis of PCR products during 12 rounds of selection: Lane 1, HyperLadder 25bp; Lanes 2 to 8, PCR product of the 1st to 12th rounds.

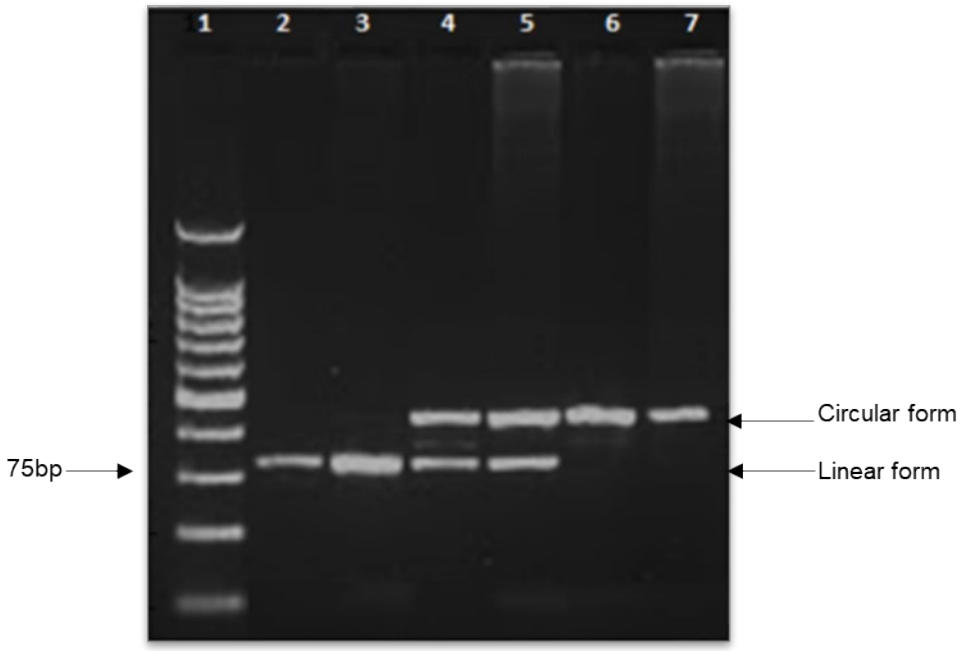



Figure 2: Circularisation of BAS Ligase converts linear BAS into closed circular ssDNA. A 77-nucleotide BAS oligo was converted to a circular ssDNA. Lane 1: Hyperladder 25bp; lanes 2 and 3: 77-nucleotide linear BAS oligo; lanes 4 and 5 closed-circular BAS reaction product; lanes 6 and 7: circular BAS after incubation with Exo I.

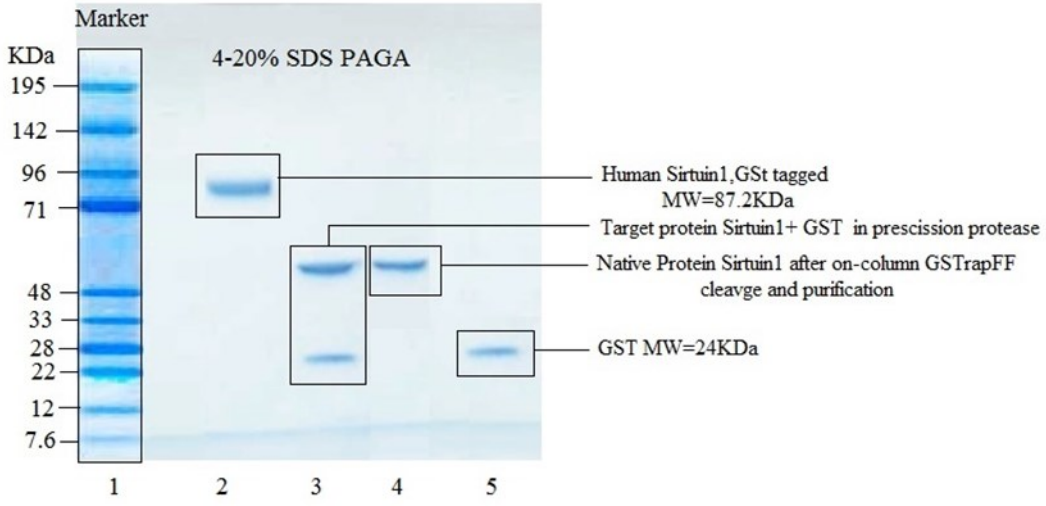



Figure 3: SDS-PAGE analysis of cleavage GST tag from SIRT1 enzyme. Lane1 marker protein; Lane2 recombinant SIRT1, GST tagged Purity> 80%; Lane3 target enzyme in PreScission protease; Lane4 Native enzyme after cleavage GST; Lane5 Eluted GST from column.

Table 1: aptamer sequences including the primers (in red) and their denominations. C: circular, L: linear, -P: with primer.

| Aptamers (clone) | Sequences |
|---|---|
| C1 | TTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAAC |
| C1-P | TTCGGAAGAGATGGCGAC TTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAAC CGAGCTGATCCTGATGGAA |
| C3 (AC3) | CGAGTGGGTTACATCGAAACTGGATCTCAACAGCGGTAAC |
| C3-P | TTCGGAAGAGATGGCGAC CGAGTGGGTTACATCGAAACTGGATCTCAACAGCGGTAAC CGAGCTGATCCTGATGGAA |
| C5 | CACTCCCTCTGCGTGCGAATTTTGCCTATGGCGCATATTC |
| C5-P | TTCGGAAGAGATGGCGAC CACTCCCTCTGCGTGCGAATTTTGCCTATGGCGCATATTC CGAGCTGATCCTGATGGAA |
| L1 | CGGACTGCAACCTATGCTATCGTTGATGTCTGTCCAAGCA |
| L1-P | TTCGGAAGAGATGGCGAC CGGACTGCAACCTATGCTATCGTTGATGTCTGTCCAAGCA CGAGCTGATCCTGATGGAA |
| L3 | CACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTA |
| L3-P | TTCGGAAGAGATGGCGAC CACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTA CGAGCTGATCCTGATGGAA |

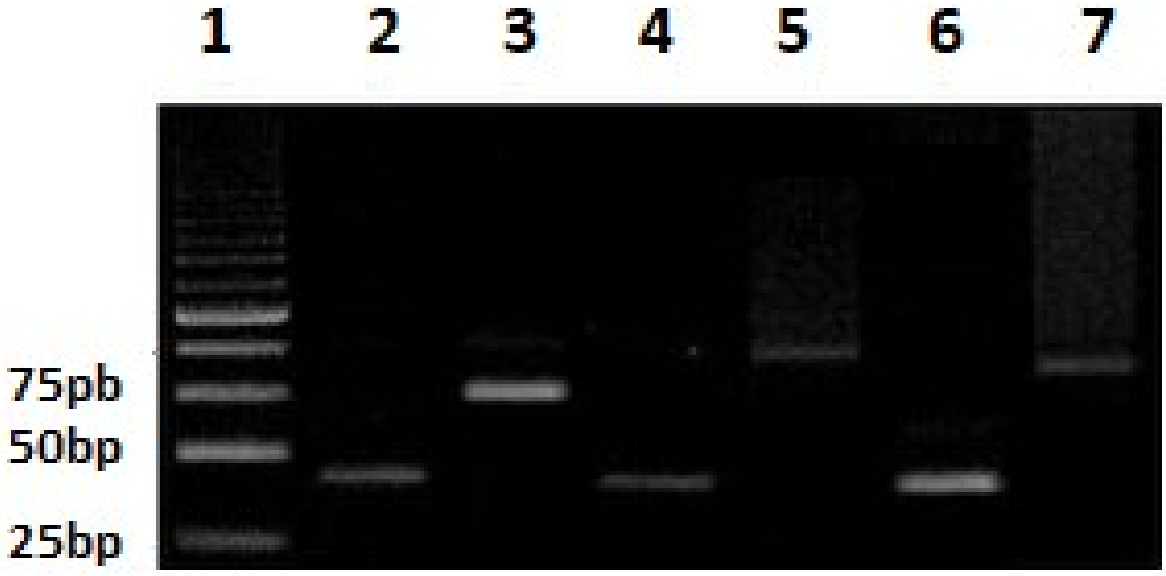



Figure 4: Circularisation was confirmed for the circular sequences through incubation with Exonuclease I. Lane 1: Hyperladder, lane 2: C1, lane 3: C1-P, lane 4: AC3, lane 5: C3-P, lane 6: C5, lane 7: C5-P. Circulars 1, 3 and 5 are 40bp as primers were removed. Circulars 1-P, 3-P AND 5-P are 77bp are primers are included.

Table 2: *in vitro* SIRT1 activity of all sequences the sequences presented in Table 1, using Fluor de Lys assessment kit, the fold activity is presented here as data normalised in respect to buffer. The sequences C1-P, C3-P and C5-P had to be tested at a different concentration because the synthesis, circularisation and purification process provided us with extremely low yield of circularised aptamer.

| µM | L1 | L1-P | L3 | L3-P | C1 | C1-P | AC3 | C3-P | C5 | C5-P | resveratrol |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.8 | 1.7 | 1.7 | 2.7 | 2.7 | 2.1 | | 2.9 | | 1.3 | | |
| 0.4 | 1.6 | 1.6 | 2.6 | 2.6 | 1.9 | | 2.7 | | 1.4 | | |
| 0.3 | | | | | | 1.9 | | 2.6 | | 1.7 | |
| 0.2 | 1.5 | 1.6 | 2.4 | 2.6 | 1.9 | | 2.6 | | 1.4 | | |
| 0.15 | | | | | | 1.8 | | 2.6 | | 1.7 | |
| 0.1 | 1.5 | 1.6 | 2.2 | 2.3 | 2.3 | | 2.5 | | 1.5 | | |
| 0.075 | | | | | | 1.7 | | 2.3 | | 1.6 | |
| 0.038 | | | | | | 1.5 | | 2.1 | | 1.6 | |
| 200 | | | | | | | | | | | 2.8 |

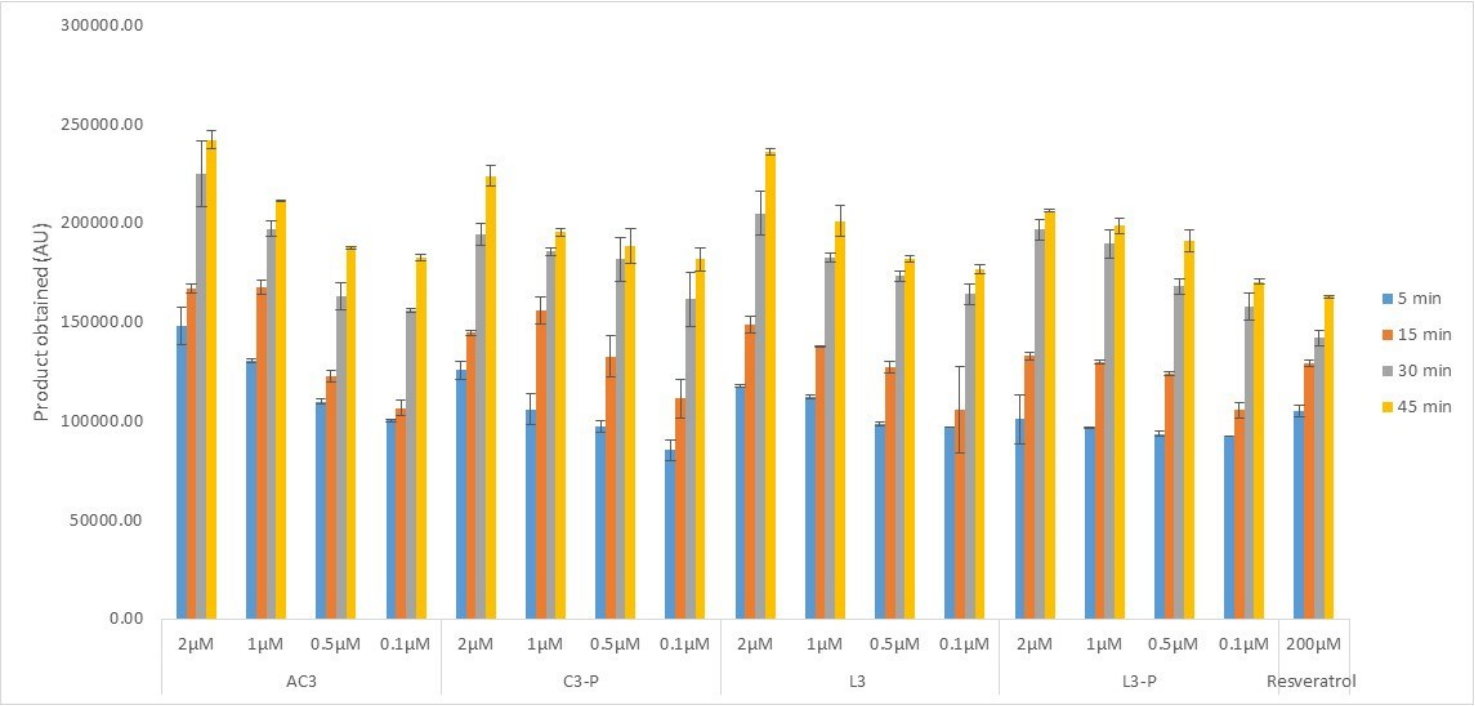



Figure 5: Product obtained for Fluor de Lys SIRT1 enzymatic assay for AC3, C3-P, L3 and L3-P at different concentrations and different time points.

## Surface Plasmon Resonance

To determine the dissociation constants ($K_D$) of C3, C3-P, L3 and L3-P aptamers and SIRT1 enzyme binding, we performed a SPR kinetic analysis using the ProteOn™ XPR36 system (http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_5390.pdf). The sensorgram must be fitted to a kinetic model using a mathematical algorithm. In ProteOn software, the most commonly used binding model for SPR biosensors is the Langmuir model. It describes a 1:1 interaction in which one ligand molecule (aptamer) interacts with one analyte molecule (SIRT1). In theory, the formation of the complex follows second-order kinetics. As majority of SPR biosensors are fluidics-based and capable of maintaining a constant aptamers concentration in a continuous liquid flow, complex formation actually follows pseudo–first-order kinetics. This model assumes that the binding reactions are equivalent and independent at all binding sites. This interaction is described by the simple equation shown below, where B represents the ligand (aptamer) and A is the analyte (SIRT1 enzyme). The rate of complex formation is represented by the association constant ($k_a$=$k_{on}$) and the rate of complex decay is represented by the dissociation constant ($K_d$=$k_{off}$), as given as:

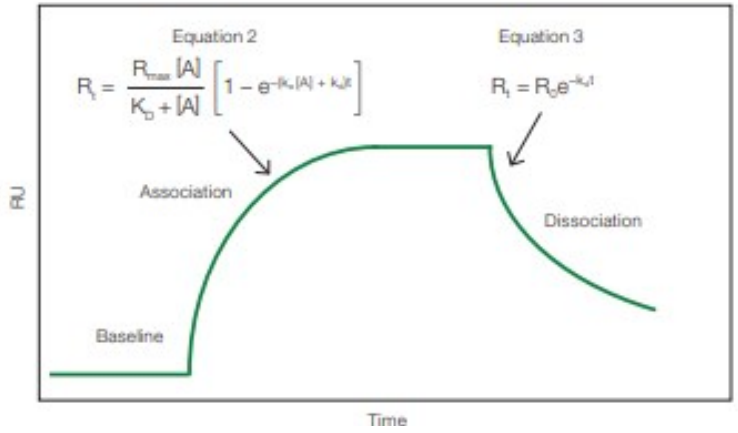
$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

Eq 1

In a kinetic analysis, the equilibrium constant ($K_D$) is calculated from the two kinetic constants through the defining relation $K_D = k_d/k_a$ ($K_D$=$k_{off}$/$k_{on}$). Relating the interaction state to the SPR sensorgram is accomplished by applying specific equations relevant to the different sensorgram phases, as shown below in an idealised sensorgram:

**Association:** $$R_t = \frac{R_{max}[A]}{K_D + [A]}\left[1 - e^{-(k_a[A] + k_d)t}\right]$$ **Equation 2**

**Dissociation:** $$R_t = R_0 e^{-k_d t}$$ **Equation 3**



Rmax is the maximal response that is reached when all ligand molecules are occupied by an analyte. Rt (response at time t) is the response at the end of analyte injection.

When various concentrations of SIRT1 were applied to 1 μM of AC3, C3-P, L3 and L3-P aptamers immobilised on a sensor chip, a specific real-time binding between the SIRT1 enzyme and aptamers was observed and quantified. Binding curves were globally fitted to various binding models, provided by the ProteOn™ XPR36 evaluation software. Sensorgrams of the binding response to aptamers measured for concentrations of SIRT1 at 12.5, 25, 50, 100, 200, 400 and 800 nM were determined. An aptamer level of 1000–1200 RU on the sensor surface was maintained in this experiment over the injection time during the immobilisation of the aptamer.

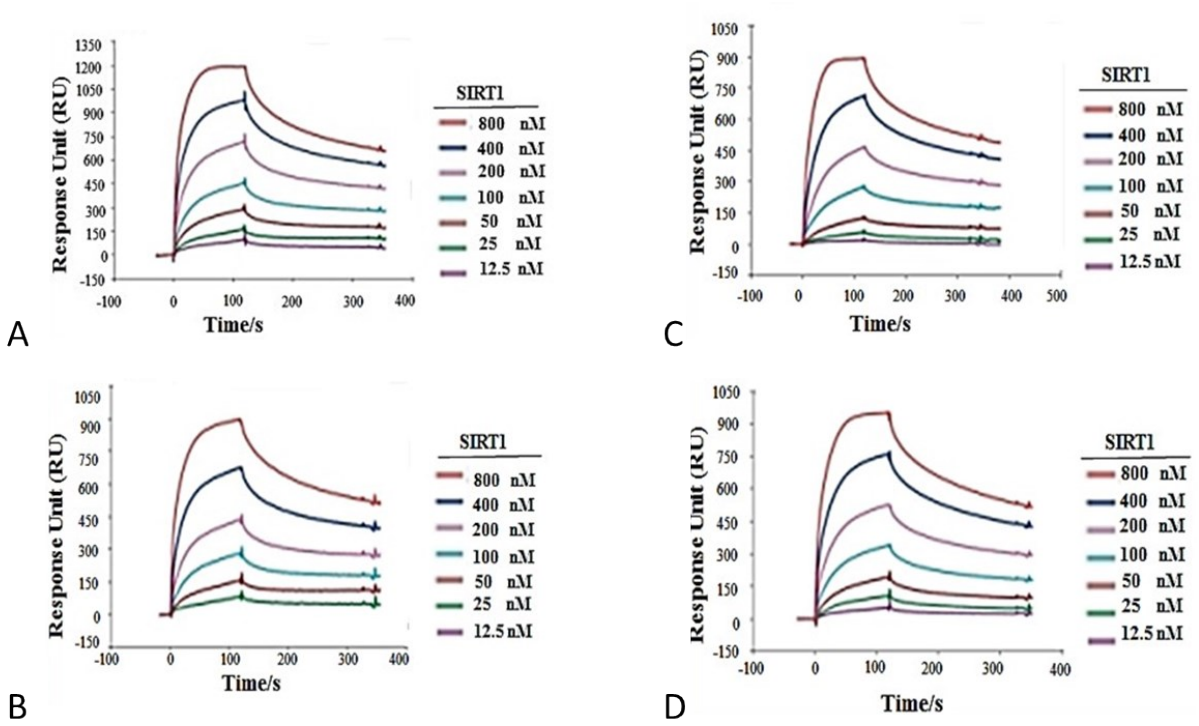



Figure 6: Determination of the affinities of aptamers for SIRT1 using surface plasmon resonance for **A:** AC3, $k_{on}$ 1.48 x $10^7$ $M^{-1}s^{-1}$ and $k_{off}$ 0.00401 $s^{-1}$. **B:** C3-P, $k_{on}$ 0.237 x $10^7$ $M^{-1}s^{-1}$ and $k_{off}$ 0.00158 $s^{-1}$. **C:** L3, $k_{on}$ 0.48 x $10^7$ $M^{-1}s^{-1}$ and $k_{off}$ 0.00232 $s^{-1}$. **D:** L3-P, $k_{on}$ 0.340 x $10^7$ $M^{-1}s^{-1}$ and $k_{off}$ 0.0035 $s^{-1}$.

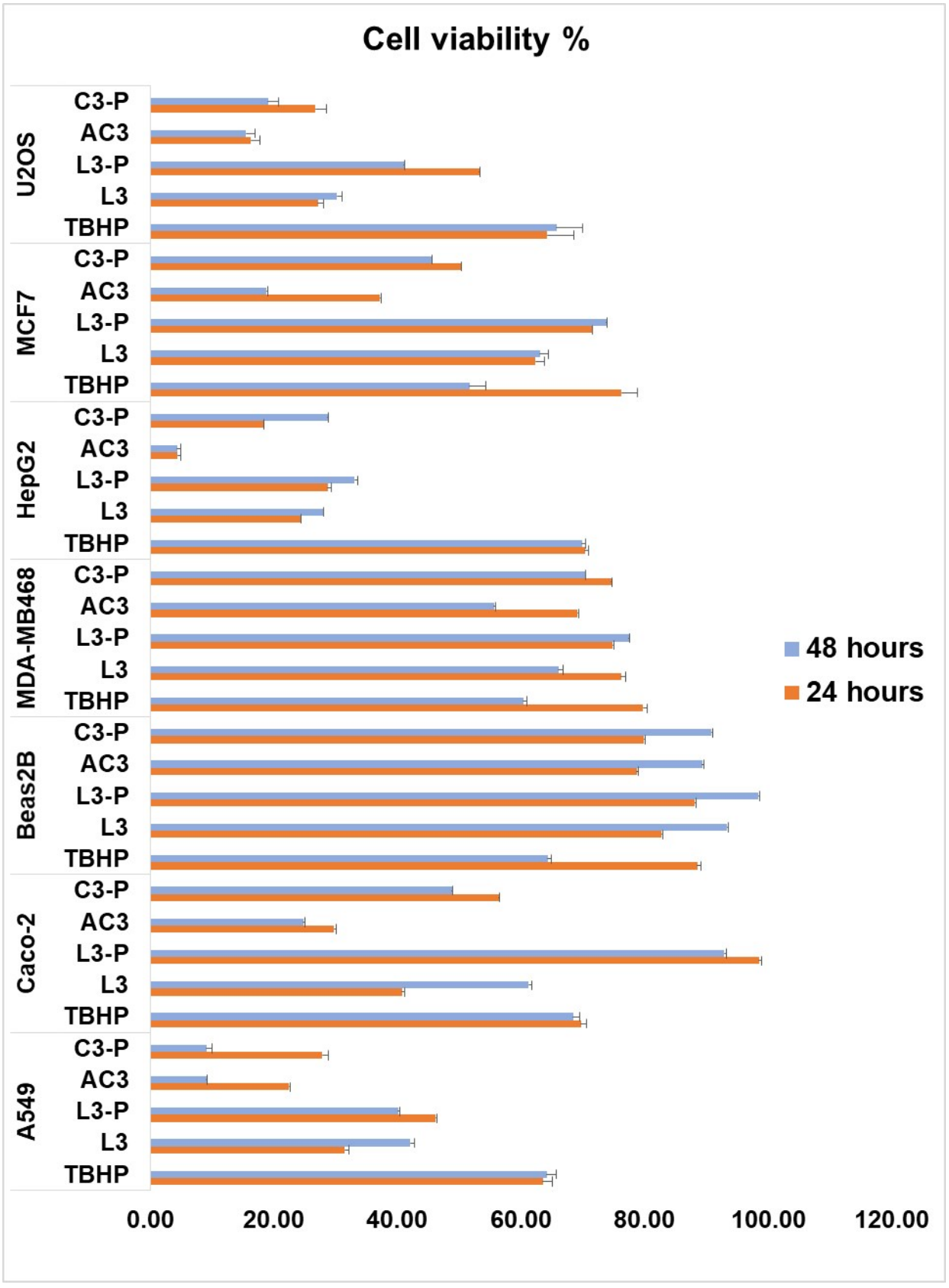



Figure 7: Cell viability results at 24 and 48 hours of incubation with aptamers at 2.5 µM final concentration. The results represent the mean ± SEM of 2 independent experiments. A549: $p < 0.005$ and 0.001 at 24h and 48h respectively. Hepg2: $p < 0.0005$ at 24h and 48h. MCF7: $p < 0.0001$ at 24h and 48h. MDA-MB468: $p < 0.005$ at 24h and 48h. U2OS: $p < 0.0005$ at 24h and 48h. Caco-2: $p < 0.05$, 0.005 at 24h and 48h respectively. Beas2B: $p > 0.05$ at 24h and 48 h.

Table 3: Evaluation of cell viability on Caco-2 cells for other forms of aptamers as negative control. The aptamers were tested at concentration of 2.5 μM in triplicates, standard deviation is presented as (). Data was normalised to control (media).

| **Aptamer type** | **Cell viability at 24 hours %** |
|---|---|
| BAS Library | 101.120 (0.009) |
| AC3 scramble | 96.069 (0.002) |
| AC3 linear | 79.398 (0.021) |
| AC3 circular | 29.833 (0.353) |

Dose-response plots to obtain $IC_{50}$

Cells were treated for 72h with 0.0078, 0.0156, 0.0312, 0.625, 0.125, 0.25, 0.5 and 1μM dose ranges of AC3. The normalised dose response was plotted over log transformed aptamer concentrations. $IC_{50}$ values were determined using nonlinear regression analysis (Origin 9.1 Microcal). Error bars represent the standard error of the mean (SEM) for triplicate data.

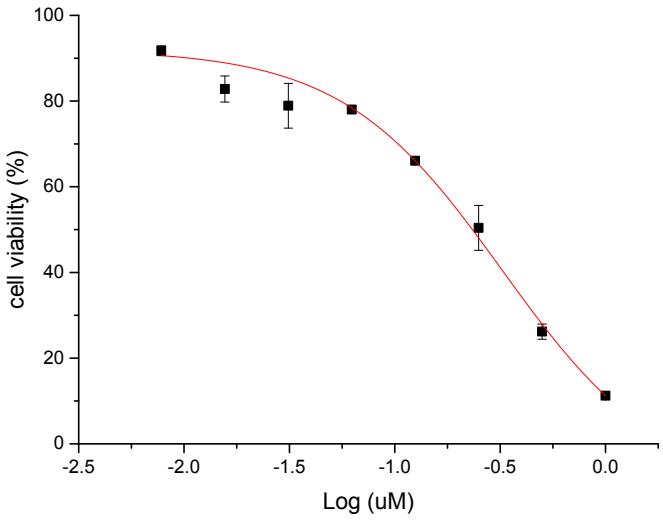



Figure 8: Dose-response plot for $IC_{50}$ A549 cells.

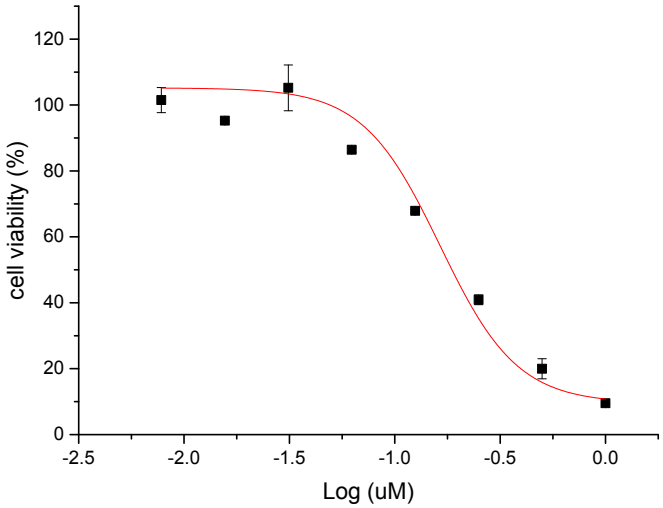



Figure 9: Dose-response plots for of $IC_{50}$ for HepG2 cells.

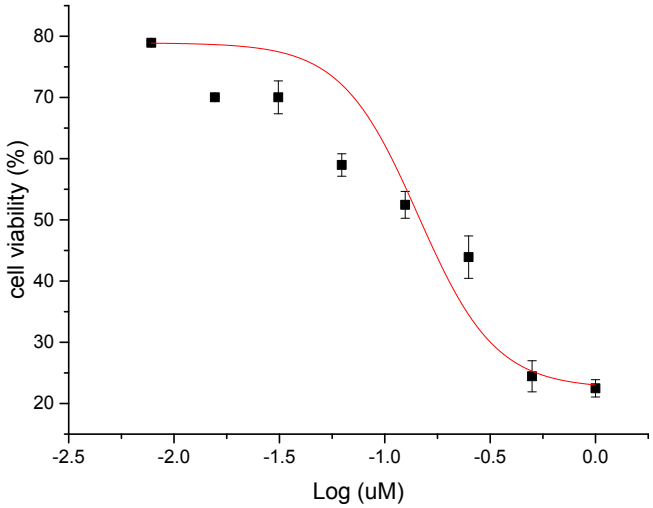



Figure 10: Dose-response plot for $IC_{50}$ for MCF-7 cells.

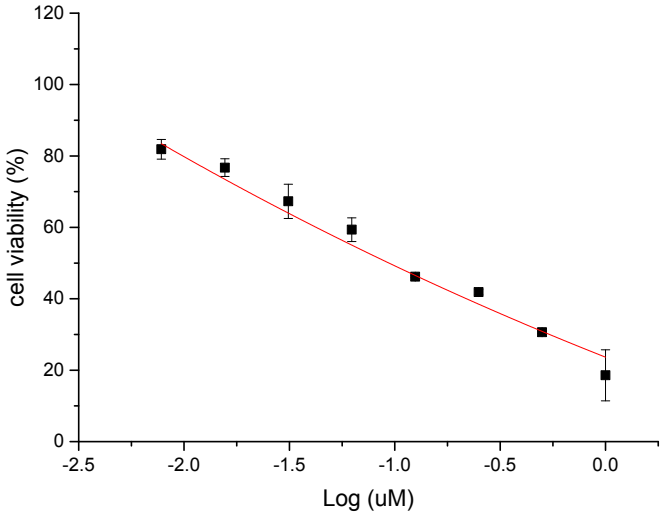



Figure 11: Dose-response plot for $IC_{50}$ MDA-MB-468 cells; as the fitting is not appropriate we evaluated the concentration needed to produce 50% cell death from the data in the excel file, this is 0.10 μM so we use this concentration in further studies.

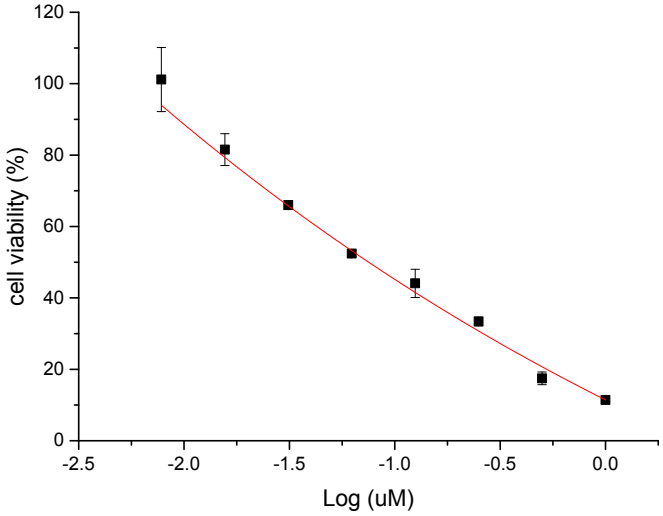



Figure 12: Dose-response plot for $IC_{50}$ for U2OS cells; as the fitting is not appropriate we evaluated the concentration needed to produce 50% cell death from the data in the excel file, this is 0.06 μM so we use this concentration in further studies.

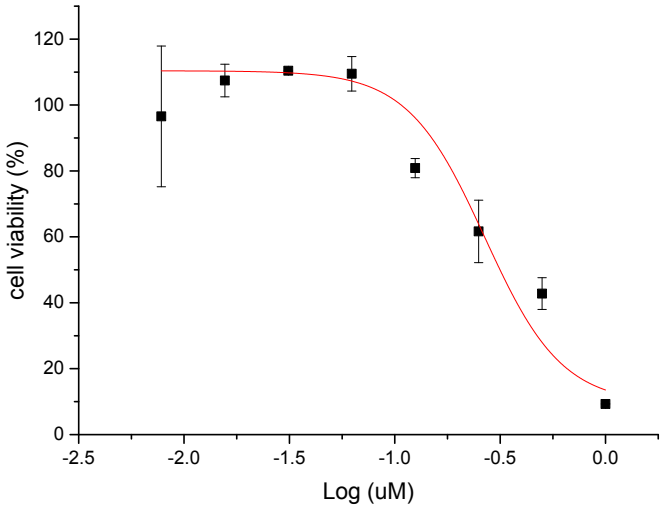



Figure 13: Dose-response plot for $IC_{50}$ for Caco-2 cells.

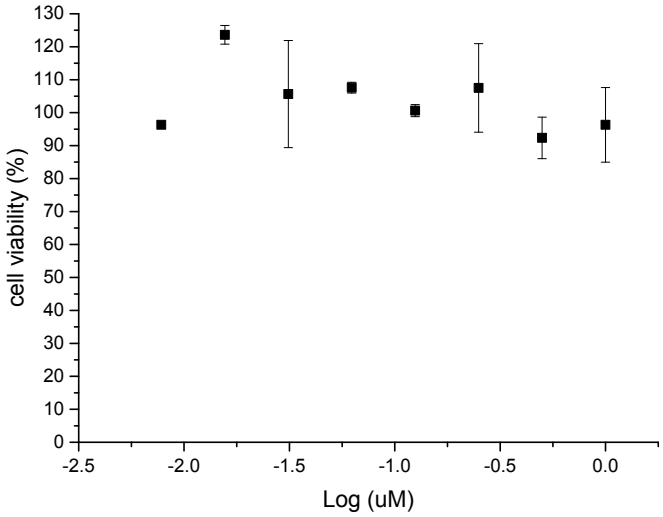



Figure 14: Dose-response plot for Caco-2 cells.

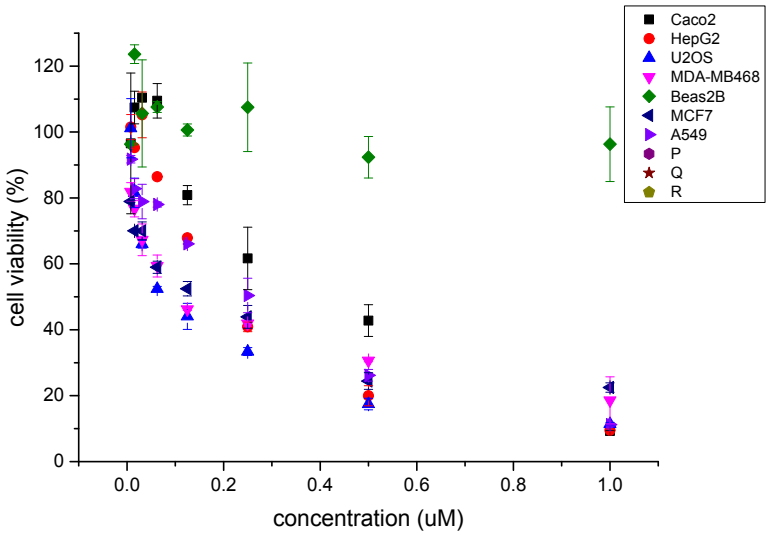



Figure 15: Dose-response plot for all cells.

Fluorescence microscopy

The nuclei has been stained with DAPI and results are shown as blue. SIRT1 has been stained with Texas Red and the results are red. AC3 has been stained with FAM and the results are green. Overlap of staining can produce variations in colour, yellow is the results of red and green (SIRT1 plus AC3), cyan is the result of green and blue (AC3 plus nuclei), magenta is the results of red and blue (nuclei plus SIRT1).

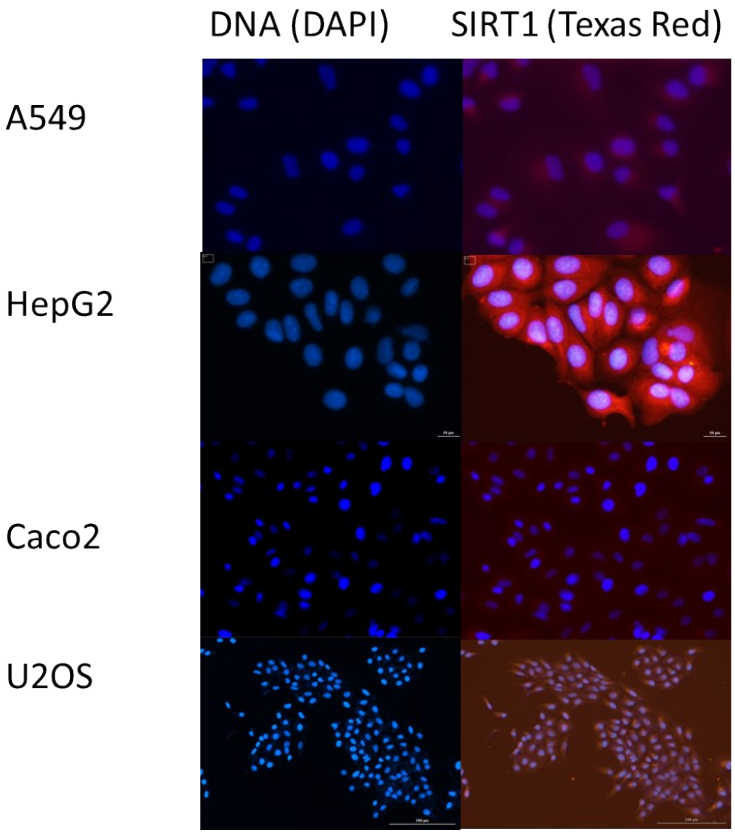



Figure 16: Fluorescence microscopy analysis of nuclei and SIRT1 enzyme localisation without addition of AC3, for A549, HepG2, Caco2 and U2OS cell lines. Only blue (DAPI) and red/magenta (SIRT1) are expected. The scales in µm are: 200 for A549, 20 for HepG2, 200 for U2OS.

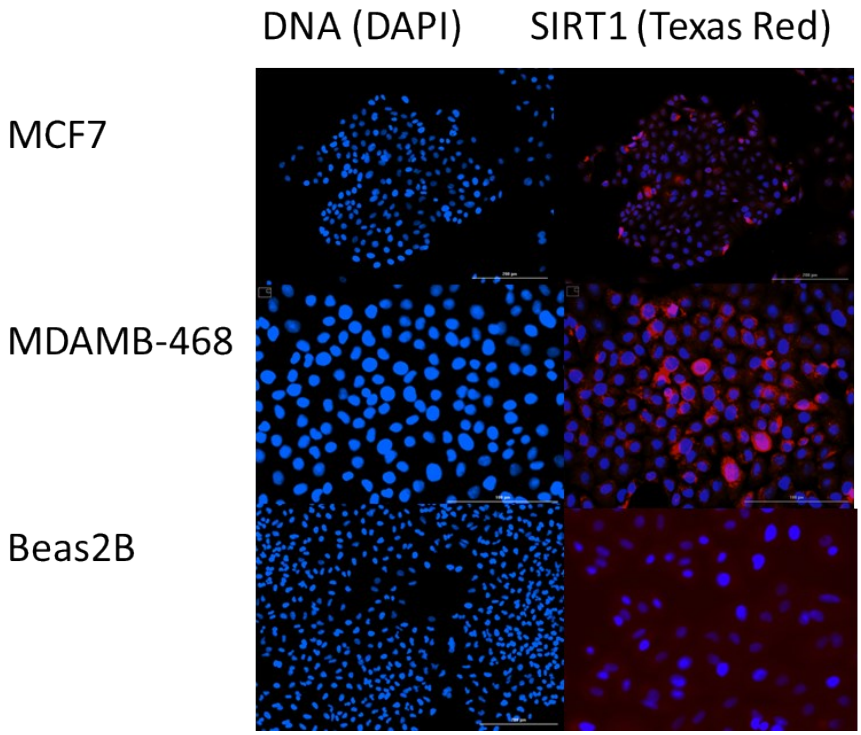



Figure 17: Fluorescence microscopy analysis of nuclei and SIRT1 enzyme localisation without addition of AC3, for MCF7, MBAMB-468 and Beas2B cell lines. Only blue (DAPI) and red/magenta (SIRT1) are expected. The scales in µm are: 200 for MCF7, 100 for MDA-MB468, 20 for Beas2B.

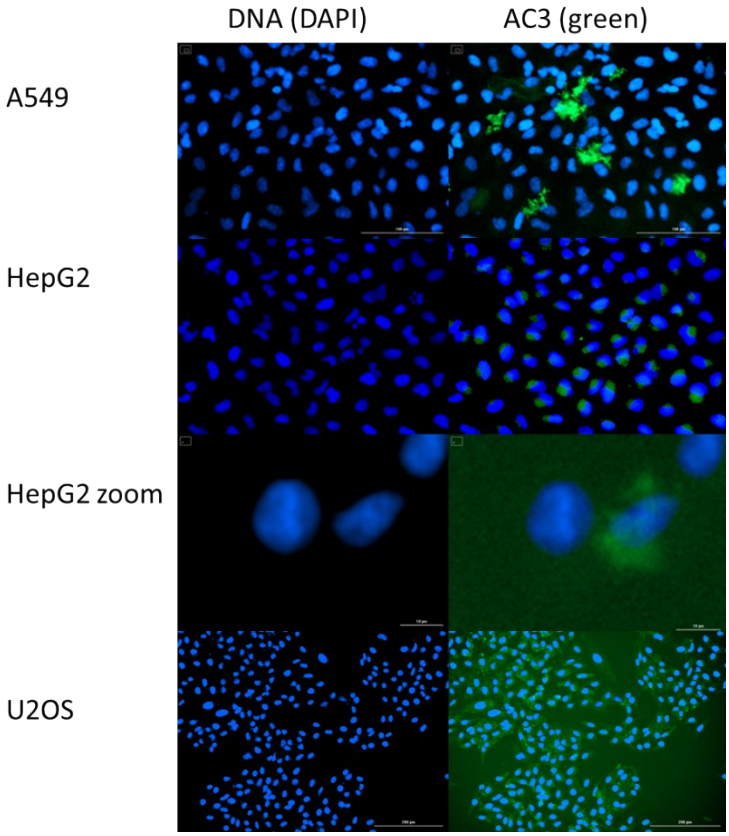



Figure 18: Fluorescence microscopy analysis of nuclei and AC33 localisation for A549, HepG2, and U2OS cell lines. The scales in µm are: 100 for A549, 10 for HepG2 zoom, 200 for U2OS.

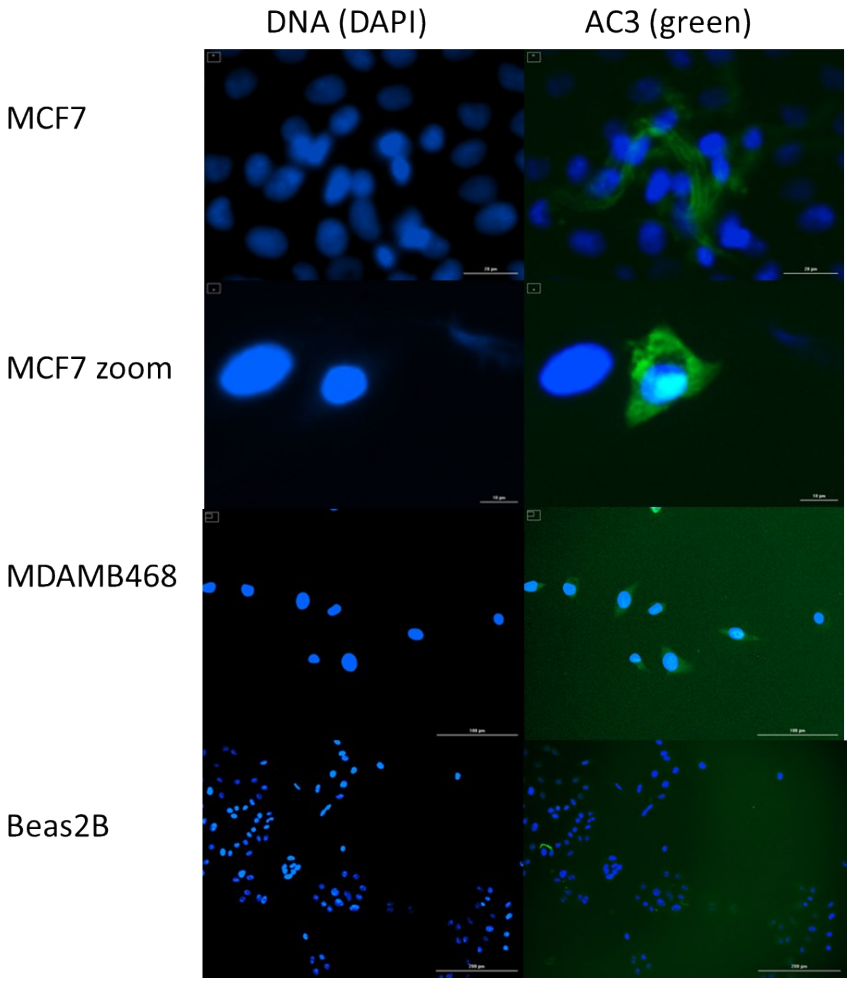



Figure 19**:** Fluorescence microscopy analysis of nuclei and AC3 localisation for MCF7, MDAMB468 and Beas2B cell lines. The scales in µm are: 20 for MCF7 zoom, 100 for MDA-MB468, 200 for Beas2B.

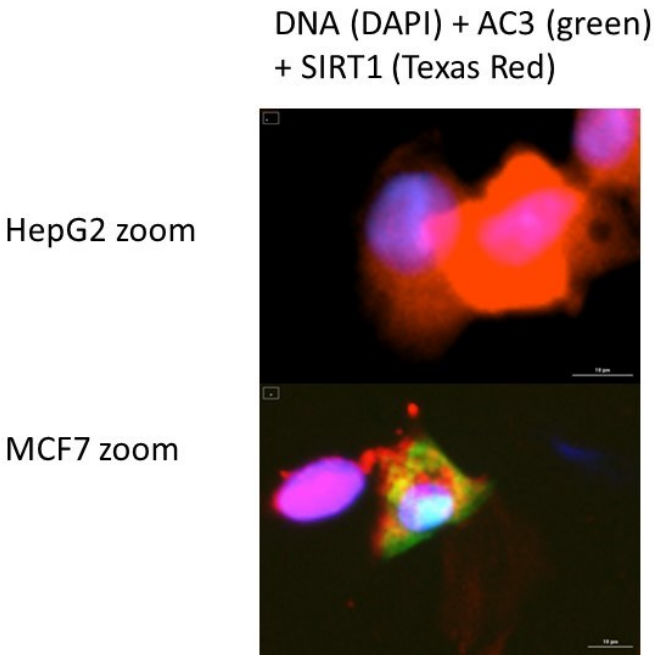



Figure 20: Fluorescence microscopy analysis of nuclei and SIRT1 enzyme localisation and AC3 zoomed HepG2 and MCF7.

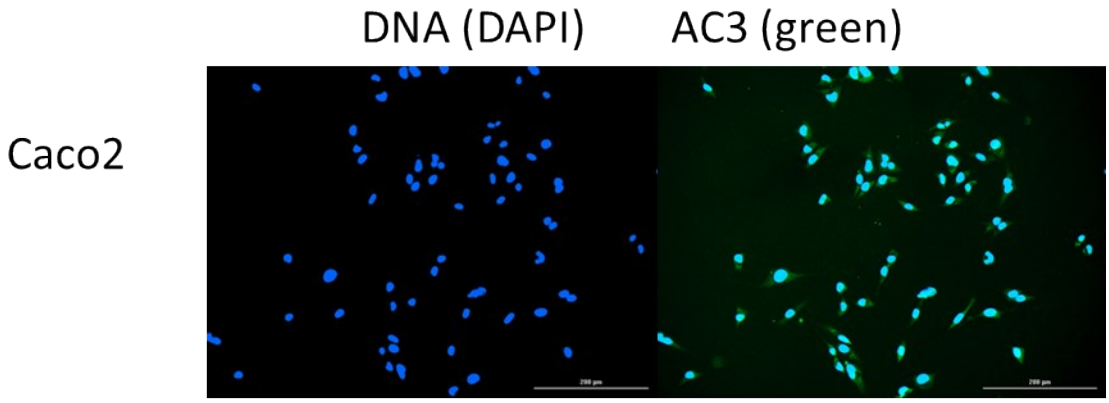



Figure 21: Fluorescence microscopy analysis of nuclei and AC3 localisation for Caco-2. The scales in µm are 200 for Caco2.