SUPPLEMENTARY INFORMATION

Direct Observation of Nanoparticle-Cancer Cell Nucleus Interactions

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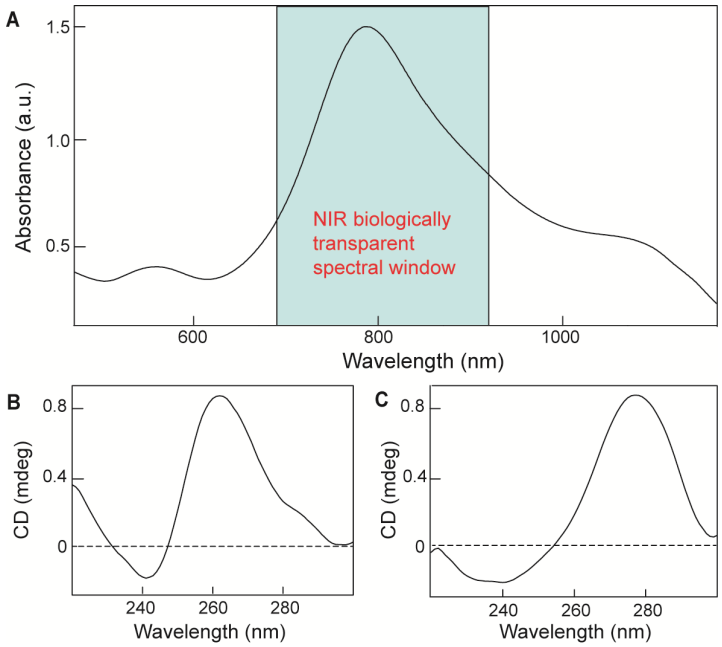

Figure S1: Optical properties of AuNSs and structure of the aptamers. (A) The extinction spectrum of AuNS shows a localized surface plasmon resonance centered at 780 nm, which is within the biologically transparent spectral window. This strong absorption at NIR wavelengths ensures that light can penetrate several mm into tissue. **(B)** The circular dichroism (CD) spectrum of AS1411 shows a positive peak at 265 nm and a negative peak at 241 nm, which is characteristic of a G-quartet structure and in agreement with the CD spectrum of AS1411 in previous work.[1] **(C)** CD spectrum of the control nanoconstruct (cApt) shows only a dominant peak at 280 nm, which indicates that there is no G-quartet structure.

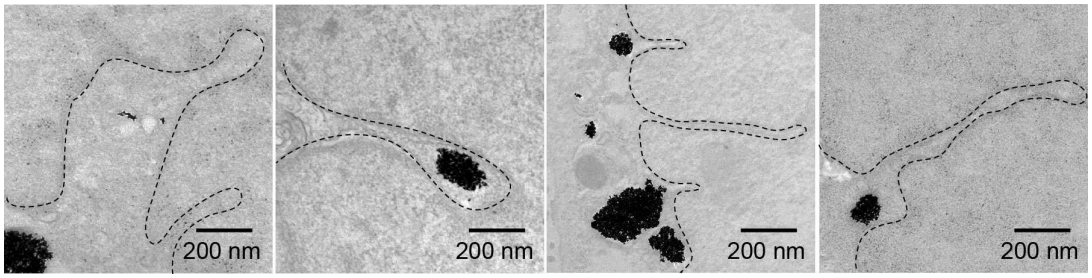

Figure S2: Location of Apt-AuNS closed to the NE folding. Zoom-in images of portions of different nuclei showing that the openings of the NE folds occurs near the Apt-AuNS clusters.

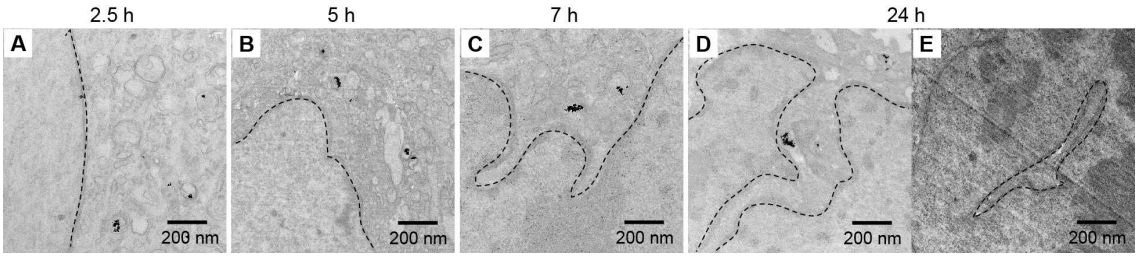

Figure S3: Increased incubation times of the nanoconstruct resulted in larger changes in nuclear phenotype. (A) No deformations of the nuclear envelopes (NE) were found after 2.5 h of incubation with Apt-AuNS. **(B)** Deeper invaginations and **(C)** multi-branched folds of the NE were observed at 5-h and 7-h incubations, respectively. After 24 h of incubation, **(D)** very deep NE folds intruded into the nucleoplasm and **(E)** cross-sections of the deep folds could be observed inside the nucleoplasm.

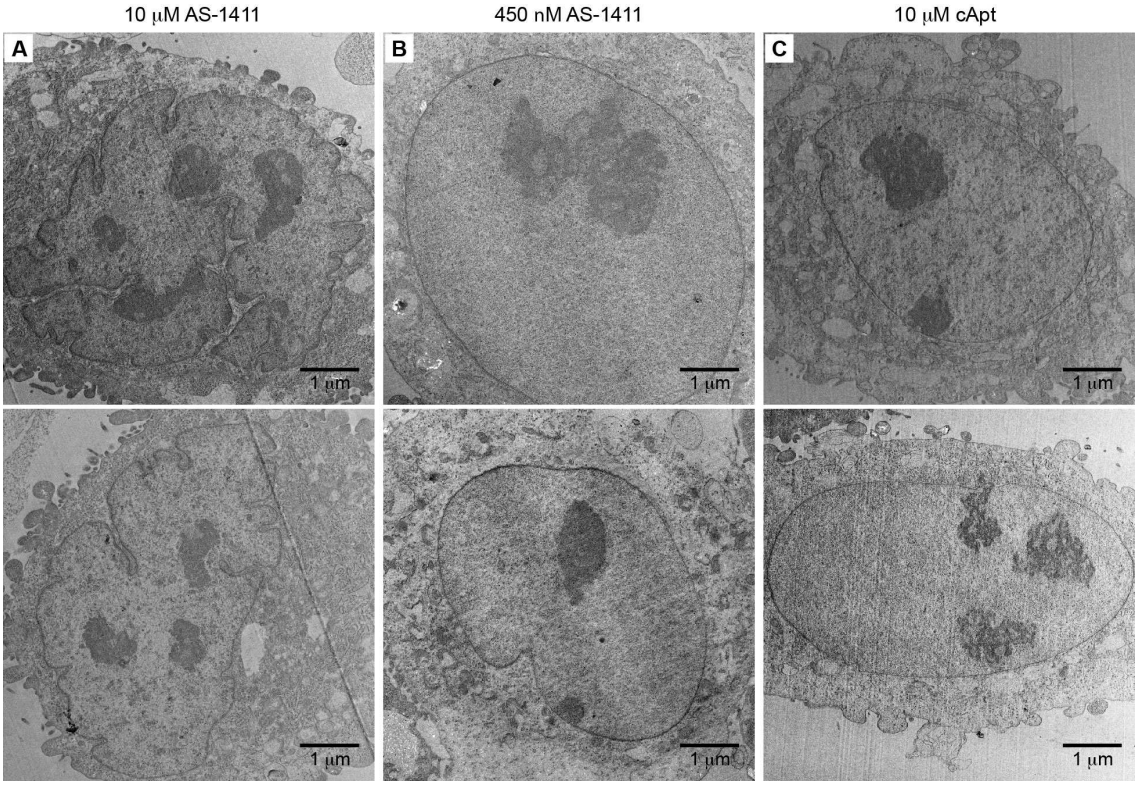

Figure S4: **Comparison of nuclei of HeLa cells after treatment with free AS1411 and the free control aptamers**. To test whether nuclear deformations originated from the interaction of AS1411 with the cell nucleus, HeLa cells were treated with **(A)** a therapeutic concentration (10 μM) of free AS1411[2] **(B)** 450 nM of free AS1411 which is equivalent to the concentration of AS1411 in 0.3 nM Apt-AuNS and **(C)** 10 μM of the free control aptamer. NE folding was observed in ca. 70% of the HeLa cell population after treatment with 10 μM AS1411 but no nuclear deformations were observed after treatment with the control aptamer or 450 nM AS1411.

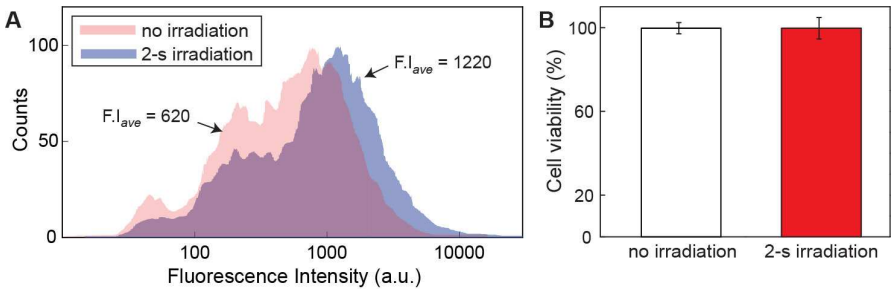

Figure S5: Light-triggered release of aptamers from nanoconstructs in cells. **(A)** HeLa cells treated with Cy5 labeled Apt-AuNS (Apt-Cy5-AuNS) were irradiated with NIR fs-pulses to monitor the release of aptamers from the nanoconstructs in a cellular environment. Fluorescence variation was monitored with flow cytometry. Irradiation conditions: pulse duration = 40 fs, power density = 4.8 W/cm^2, irradiation time = 2 s, frequency = 1 kHz, wavelength = 800 nm). A two-fold increase in average Cy5 fluorescence intensity (F.I$_{ave}$) was observed compared to non-irradiated samples, which shows that the aptamers are being released from the Apt-AuNSs. **(B)** Cell viability assays show minimal influence of the laser pulses on the HeLa cells in the absence of the Apt-AuNS.

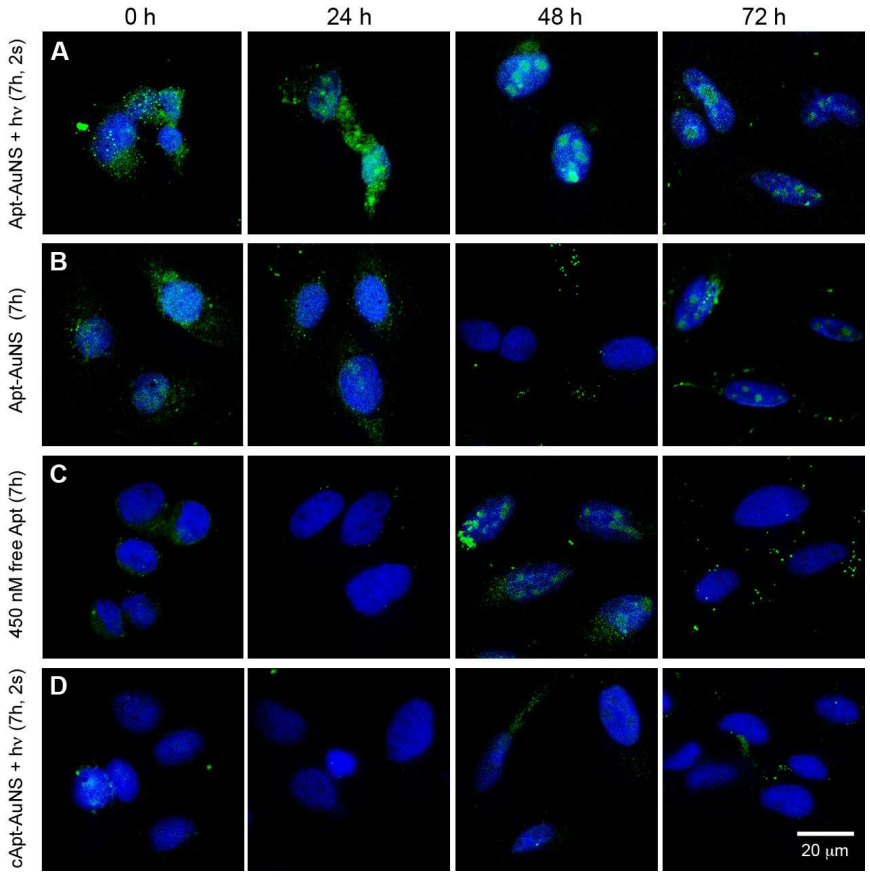

Figure S6: Double stranded DNA breaks (DSBs) of HeLa cells treated under various conditions. **(A-C)** DSBs occurred in the nuclei of cancer cells treated with Apt-AuNSs (Apt-AuNSs + *hν* (7 h, 2 s), Apt-AuNSs (7 h) and 450 nM free Apt (7 h)). Among these conditions, the highest quantity of DSBs foci were observed in the nuclei of cells treated with Apt-AuNSs for 7 h followed by irradiation with the same conditions as in Fig. S5 (Apt-AuNSs + *hν* (7h, 2s)). Although DSBs were found in HeLa cells after treatment with free aptamers at concentration similar to that of aptamers conjugated to the AuNS (450 nM, 7h), the quantity of DSBs foci was significantly less than those of cells treated with Apt-AuNSs + *hν*. **(D)** Only negligible amount of DSBs were observed on cells when they were treated with cApt-AuNSs + *hν* (7 h, 2 s).

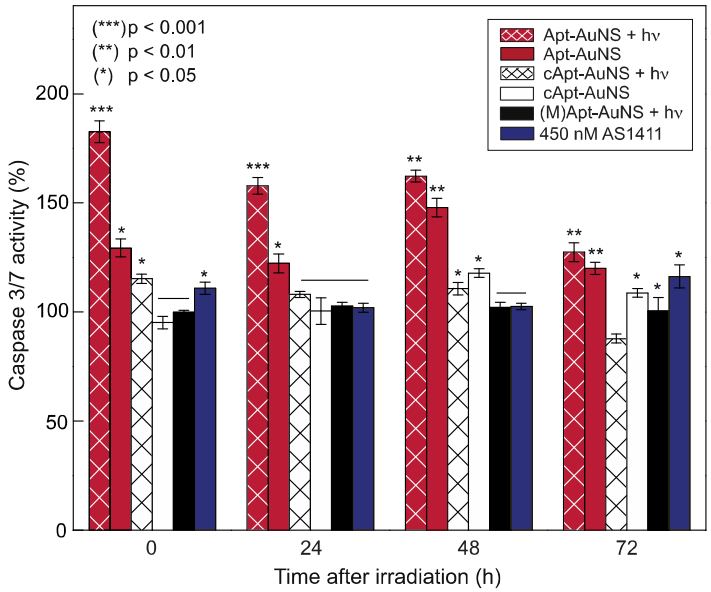

Figure S7: Caspase 3 and 7 activities of cells treated with various conditions over 72 h. Increases in the caspase activities of HeLa cells treated with Apt-AuNS were observed over 72 h regardless of whether the aptamer was released from the nanoparticles. The caspase activity was highest in samples treated with Apt-AuNSs + *hν*. Surprisingly, the caspase activity of cells treated with 450 nM of free aptamer (a concentration similar to that estimated by the number of aptamers on the AuNSs and the concentration of AuNS) was much lower than those treated with Apt-AuNSs. The caspase activities of HeLa cells treated with cApt-AuNSs were not noticeable even after laser irradiation to release cApt. In addition, the caspase activity of MCF-10A cells treated with Apt-AuNSs did not increase after laser irradiation. Lines over bars indicate groups that are not significantly different.

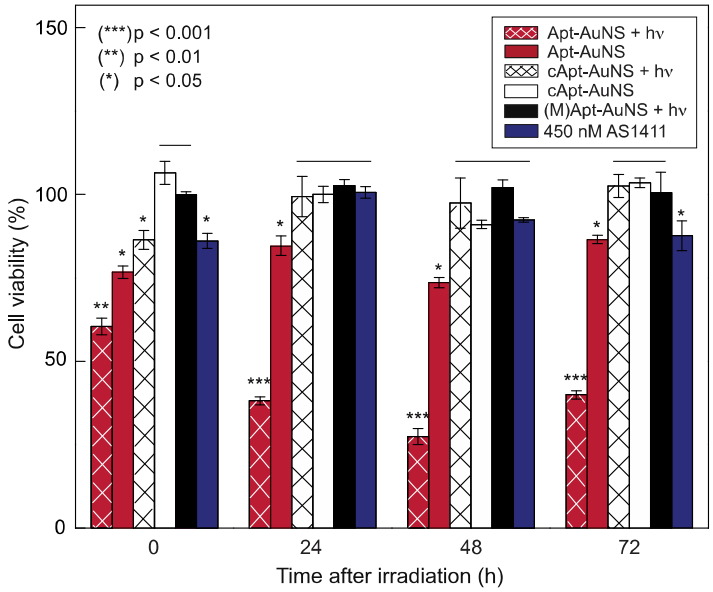

Figure S8: Cell viability assay on cells treated under various conditions. HeLa cells treated with Apt-AuNS show decreased cell viability over 72 h. Consistent with increased amounts of NE folding, the HeLa cell population treated with Apt-AuNSs + *hν* decreased over 70% between 48 and 72 h after release of the aptamer, which was much higher than that of Apt-AuNS only treated cells (ca. 25%). Similar to the trend observed in caspase activity (Fig. S7), the viability changes of free aptamer (450 nM) treated HeLa cells was minimal (ca.100%). The viability of cApt-AuNS treated HeLa cells did not decrease regardless of laser irradiation. Similarly, the viability of Apt-AuNS treated MCF-10A cells after light-irradiation was nearly 100%. Lines over bars indicate groups that are not significantly different.

Supplementary Information References

1. Bates, P. J.; Laber, D. A.; Miller, D. M.; Thomas, S. D.; Trent, J. O., Discovery and Development of the G-Rich Oligonucleotide AS1411 As a Novel Treatment for Cancer. *Exp and Mol Pathol* **2009,** 86, 151-164.

2. Soundararajan, S.; Chen, W. W.; Spicer, E. K.; Courtenay-Luck, N.; Fernandes, D. J., The Nucleolin Targeting Aptamer AS1411 Destabilizes Bcl-2 Messenger RNA in Human Breast Cancer Cells. *Cancer Res* **2008,** 68, 2358-2365.