Supporting Information

for

Antisense DNA loaded 2D nanosheets for combined photodynamic and antisense cancer therapy

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Experimental methods

β-Cyclodextrin was commercially purchased from Sigma Aldrich. The **A-DNA** and **DNA-FAM** were purchased from Sigma Aldrich and were used as obtained. Deionized water was used for all the experiments. AFM analyses were carried out on a Multimode BRUCKER AFM (Veeco Nanoscope V). TEM analyses were carried out on FEI Tecnai G2 F20 (120 kV) microscope. Confocal imaging was done using Nikon EclipseTi. MTT assay was used to study the cytotoxicity of the nanoformulation using MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), which was purchased from Sigma Aldrich. All the photophysical studies were carried out using a quartz cuvette of 10 mm path length on a Shimadzu UV-3600 Vis-NIR spectrophotometer. FACS analyses were carried out on a FACS LSR Fortessa flow cytometer (BD, USA). Cell lines were borrowed from NCCS, Pune (HeLa). The lysosomes and mitochondria were stained using lysotracker deep red and moto tracker deep red. The nucleus of the cell was stained using Hoechst. TMRM dye was used to analyse the integrity of mitochondria after the administration of nanoparticle formulation. Annexin V-FITC apoptosis detection kit was purchased from BD Bioscience. The live/dead assay was done using calcein-AM/PI co-staining following reported protocols.

Microscopic (TEM & AFM) analyses

For the TEM analyses, **1/β-CD$^+$/A-DNA** nanosheet [**β-CD$^+$** (10 μM), **1 (**10 μM**) A-DNA (**1 μM)] were drop casted onto a 400-mesh carbon-coated copper grid (Ted Pella, Inc.), the sample was kept on the grid for 2 min, and the excess sample was wiped off using tissue paper. After repeating the process 2-3 times, the samples were kept under a desiccator for drying and were used for the TEM analyses. For the AFM analyses, samples were drop cast onto a freshly cleaved mica surface, dried overnight and analyzed using Multimode BRUCKER AFM (Veeco Nanoscope V).

Confocal laser scanning microscopic (CLSM) analyses

HeLa cells were plated in μ-Slide 8 well slides at a seeding density of 1000 cells, kept at 5 % CO_2 incubator at 37 °C in DMEM culture media for 24 h. Once the cells have reached 70 % confluency the media was changed and the cells were treated with **1/β-CD$^+$/DNA-FAM** or **DNA-FAM** for 12 h. The lysosome was stained with lysotracker deep red, and mitochondria with Mito tracker deep red following the manufacture's protocol. TMRM was used for monitoring the mitochondrial

damage and acridine orange was used for evaluating lysosomal integrity and washed off three times with PBS and imaged under Nikon Eclipse Ti with a 20X objective.

Fluorescence-activated cell sorting (FACS) analyses

For cellular internalization studies of **1/β-CD$^+$/DNA-FAM**, HeLa cells were seeded at a cell density of 1×10^5 cells in 35 mm culture dish and grown in a 5 % CO_2 incubator at 37 °C in for 24 h. Once the cells has reached 90 % confluency, **1/β-CD$^+$/DNA-FAM** were diluted with cell culture media (DMEM) with **1/β-CD$^+$/DNA-FAM** (**β-CD$^+$** and **1**: 10 μM and **DNA-FAM**: 5 μM) as final concentration and kept for 12 h. Once the incubation time was over, the media was changed with fresh media and cells were collected by trypsinization and washed three times with 1X PBS. Cells were then collected in 300 μl PBS in a FACS tube and analysed using FACS cytometry.

Reactive oxygen species generation studies

HeLa cells were plated in μ-Slide 8 well slides at a seeding density of 1000 cells, kept at 5 % CO_2 incubator at 37 °C in DMEM culture media for 24 h. Subsequently, the cells were treated with **1/β-CD$^+$/A-DNA** sheet (20 μM with respect to **1/β-CD$^+$** and 10 μM with respect to **A-DNA**) and NIR light illumination was done after 2 hr of cellular internalization using 635 nm laser (0.75 W/cm^2) for 10 min. The cells were then stained with ROS-sensitive dye DCFHDA (10 μM) in an FBS-free medium for 45 min and washed off three times with PBS, and imaged under Nikon Eclipse Ti with a 20 X objective.

MTT assay

HeLa cells were seeded to 96-well with a density of 1×10^2 cells per well and were grown for 24 h. After confirming the cellular confluency of about 70 %, cells were incubated with different concentrations of **A-DNA** (50-1000 nM), and different concentrations of **1/β-CD$^+$**, **1/β-CD$^+$/A-DNA**(10 μM with respect to **1/β-CD$^+$** and 1 μM with respect to **A-DNA**) with and without NIR light illumination. NIR illumination was done after 2hr of cellular internalization using 635 nm laser (0.75 W/cm^2) for 10 min. Subsequently, the cytotoxicity was analysed by MTT assay. MTT ((3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)) reagent in fresh media was added by replacing the old media and kept for 3 h of incubation at 37 °C in 5 % CO_2 incubator. After 3 h, the media was aspirated and 100 μL DMSO was added to solubilize the formazan

crystals, and absorbance at 565 nm was measured using a microplate reader to evaluate the cytotoxicity.

Live/dead cell staining assay

The live/dead cell staining assay was performed on HeLa cells using Calcein AM/PI co-staining technique. The HeLa cells were plated in μ-Slide 8 Well slides at a seeding density of 1000 cells, kept at 5 % CO_2 incubator at 37 °C in DMEM culture media for 24 h. Subsequently, the cells were treated with **1/β-CD$^+$/A-DNA** (10 μM with respect to **1/β-CD$^+$** and 1 μM with respect to **A-DNA**) and incubated for 24 h. After 24 h of incubation, cells were washed with PBS. In fresh medium, Calcein AM (1 μM) and propidium iodide (10 μL from10 μg/mL) were added and incubated for 30 min. The plates were further washed with 1X PBS and after that, imaged under Nikon Eclipse Ti with a 20 X objective.

Annexin V-FITC apoptosis assay

The Annexin V-FITC apoptosis assay was performed on HeLa cells using an Annexin V-FITC apoptosis detection kit (BD Bioscience). The HeLa cells were plated in μ-Slide 8 well slides at a seeding density of 1000 cells, kept at 5 % CO_2 incubator at 37 °C in DMEM culture media for 24 h. The cells were treated with **1/β-CD$^+$/A-DNA** (10 μM with respect to **1/β-CD$^+$** and 1 μM with respect to **A-DNA**) and incubated for 24 h. After 24 h of incubation, cells were washed with PBS. The Annexin V-FITC (5 μL) in Annexin V-FITC binding buffer (200 μL), and a PI solution (10 μL from 10 μg/mL)were added for 20 min. Washed with PBS 1X and then imaged under Nikon Eclipse Ti with a 20 X objective. For the FACS analysis, HeLa cells were seeded at 24 well plates and grown in a 5 % CO_2 incubator at 37 °C in DMEM cell culture media for 24 h. Once the cells has reached 70 % confluency, **1/β-CD$^+$/A-DNA** (10 μM with respect to **1/β-CD$^+$** and 1 μM with respect to **A-DNA**) was diluted with DMEM and kept for 24 h. Once the incubation time was over, the media was changed with fresh media, and cells were collected by trypsinization and washed three times with 1X PBS. Cells were then collected in Annexin V-FITC binding buffer and treated with Annexin V-FITC (5 μL) in Annexin V-FITC binding buffer and a PI solution (10 μL from 10 μg/mL) which was kept at 15 minutes and centrifuged the cell suspension at 150 RCF for 3 minutes and supernatant were removed. The cell suspension was then analysed using a flow cytometer.

Western blotting

For western blot studies, HeLa cells were seeded at a cell density of 1×10^5 cells in 35 mm culture dish and grown in a 5 % CO_2 incubator at 37 °C in for 24 h. Once the cells has reached 90 % confluency, **A-DNA, 1/β-CD$^+$/A-DNA** were diluted with cell culture media (DMEM) with **1/β-CD$^+$/A-DNA** (**β-CD$^+$** and **1**: 10 μM and **A-DNA:** 1 μM) as final concentration and kept for 24 h. Once the incubation time was over, the media was changed with fresh media and cells were collected by trypsinization and washed three times with 1X PBS. Cells were harvested in ice cold lysis buffer containing 50mM Tris Cl, 150 mM NaCl, 1 mM EGTA, 1% (vol/vol) Nonidet™ P 40 Substitute (Sigma-Aldrich 74385) , 2mM MgCl2, 0.5% (wt/vol) Na+ deoxycholate and 0.1 % SDS (wt/vol) with 1X protease inhibitor cocktail (Sigma-Aldrich P8340) and 1mM PMSF (Sigma-Aldrich P7626) and placed in ice for 30 minutes with occasional vertexing. After clearance by centrifugation at 18,000×g for 10 minutes, supernatant was collected and boiled for 10min at 95°C after mixing with 5X Laemmli buffer (250 mM Tris-Cl, pH 6.8, 10% SDS, 30% glycerol, 5% 2-mercaptoethanol, and 0.02% bromophenol blue). Samples were then loaded to SDS PAGE and transferred to PVDF membrane (Merck, IPVH00010) at 100mA for 3 hours. Membranes were blocked with 3% (wt/vol) skim milk powder (HiMedia, GRM1254) in TBST and incubated with primary antibodies BCL2 (Proteintech, 12789-1-AP, 1:1000) and Tubulin (Sigma-Aldrich, T6199, 1:2000) in blocking solution either at 3 hour room temperature or 4^0C overnight. After washing in TBST, membranes were incubated with hrp conjugated secondary antibodies in blocking solution for 1hr room temperature. Membranes were developed using Immobilon Western Chemiluminescent HRP Substrate (Merck, WBKLS0500) and signals were captured using ChemiDoc XRS+ System (Bio-Rad, 1708265).

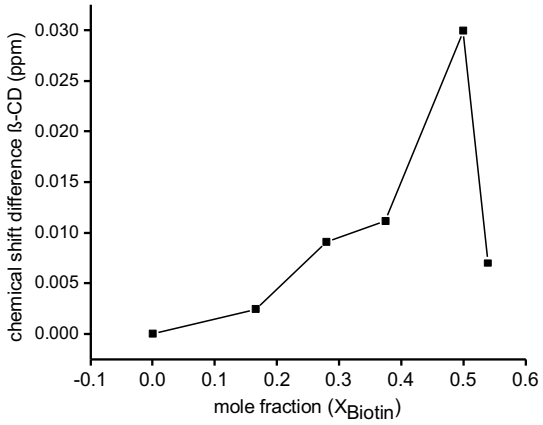

Figure S1. Job's plot derived from the changes of chemical shift of -OH group at 3.865 residing inside the hydrophobic cavity of **β-CD**$^+$ for the NMR titration of β-CD and biotin.

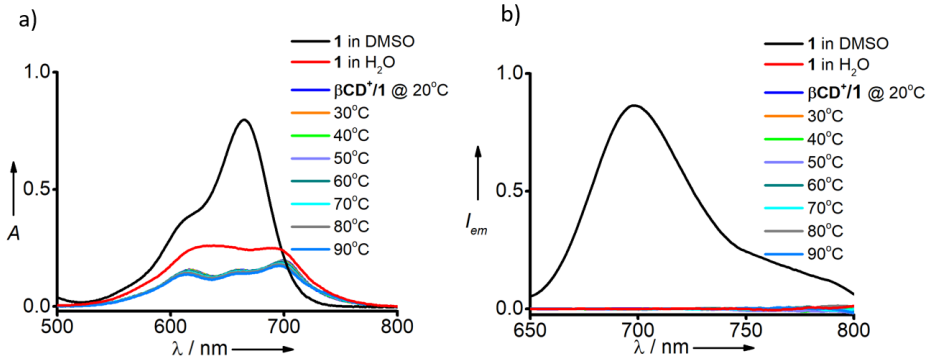

Figure S2. Temperature-dependent absorption (a) and emission (b) spectra of **1/β-CD**$^+$.

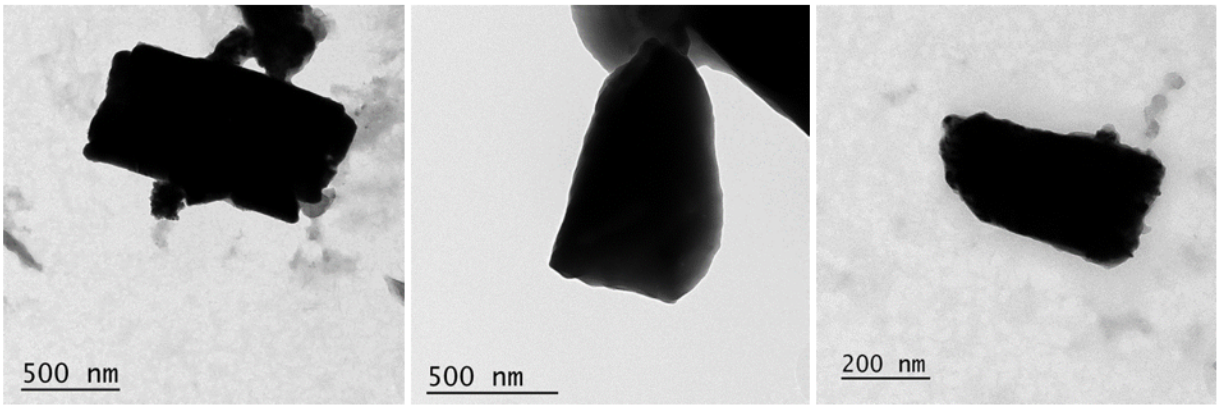

Figure S3. Additional TEM imges of **1/β-CD**$^+$.

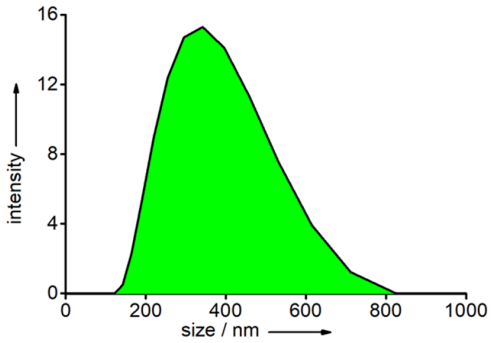

Figure S4. Dynamic light scattering size distribution curve of **1/β-CD$^+$** in water.

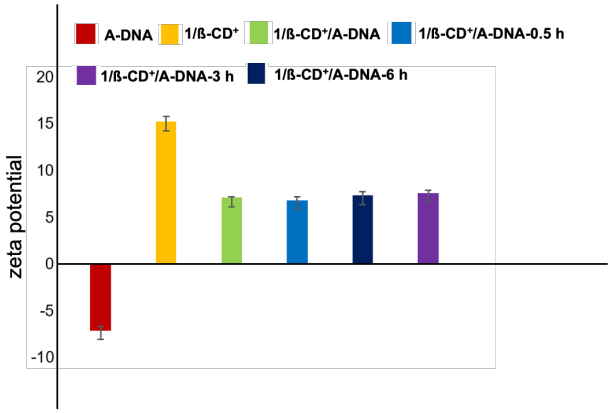

Figure S5. Time-dependent zeta potential analyses of **1/β-CD$^+$/A-DNA**.

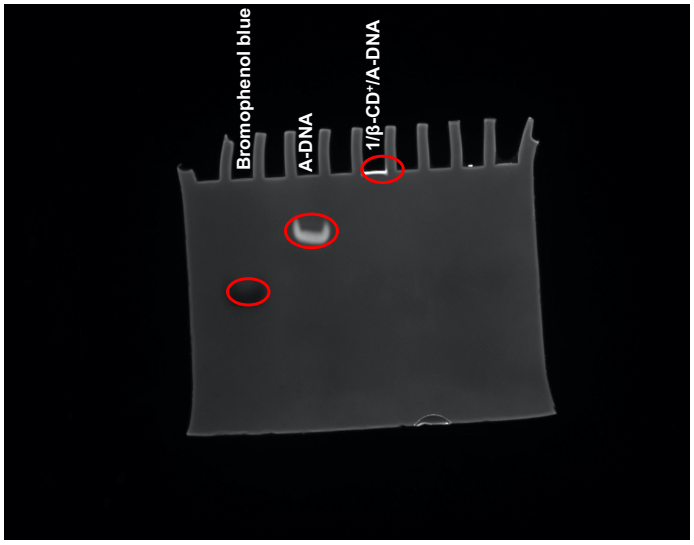

Figure S6. Native PAGE (20%) analyses of **1/β-CD$^+$/A-DNA**.

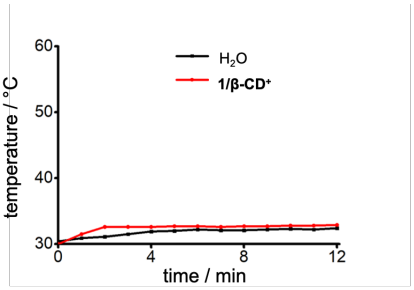

Figure S7. Time-dependent photothermal effect of **1/β-CD$^+$/A-DNA** sheets upon NIR light illumination (635 nm laser at 0.75 W/cm^2) for 12 min.

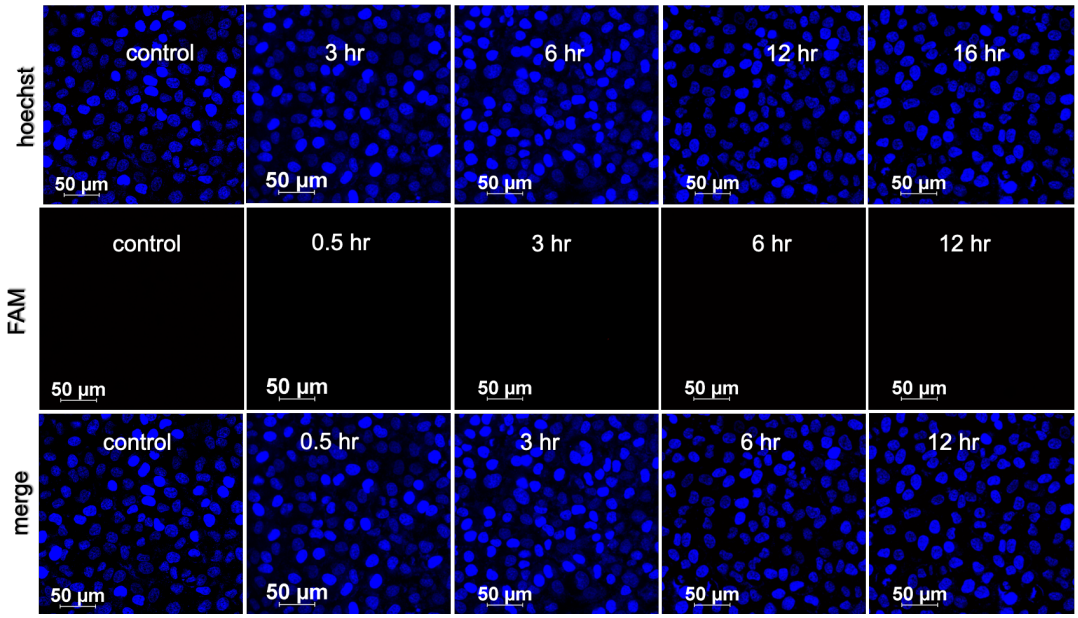

Figure S8. Time-dependent cellular internalization studies of **DNA-FAM**-treated HeLa cells.

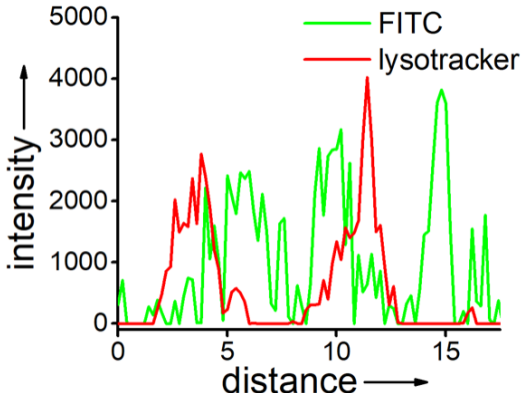

Figure S9. Line analysis of HeLa cells treated with **1/β-CD$^+$/DNA-FAM** sheets. The cells were stained with Lysotracker deep red to follow the colocalization of **1/β-CD$^+$/DNA-FAM** sheets.

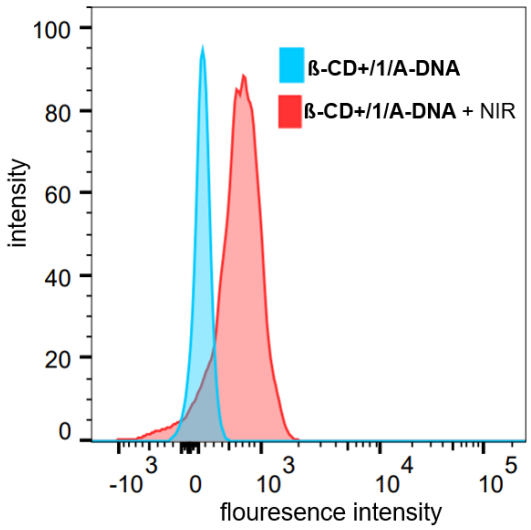

Figure S10. FACS analysis of HeLa cells treated with **1/β-CD$^+$/A-DNA** and DCFHDA under NIR light illumination to evaluate the singlet oxygen ($^1O_2$) generation capability of the sheet under NIR light illumination.

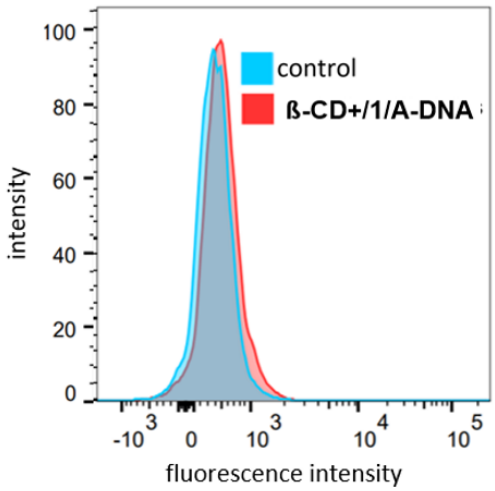

Figure S11. FACS analysis of HeLa cells treated with **1/β-CD$^+$/A-DNA** and AO under NIR light illumination to evaluate the lysosomal damage due to the in situ generation of singlet oxygen ($^1O_2$) inside HeLa cells.

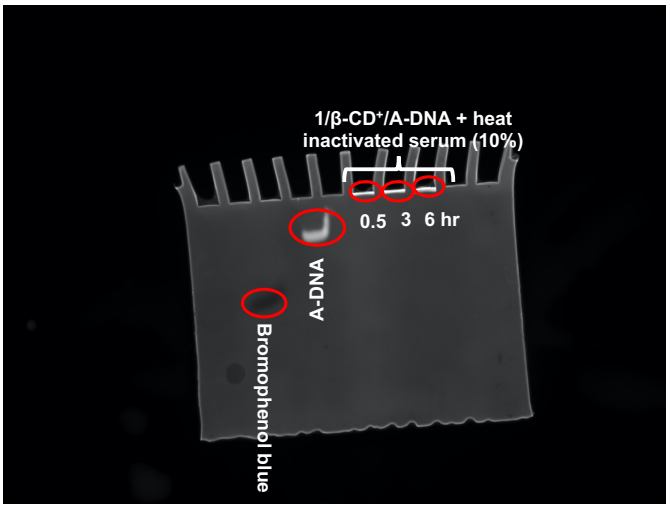

Figure S12. Native PAGE (20%) analysis of **1/β-CD$^+$/A-DNA** treated with 10 % heat-inactivated serum.

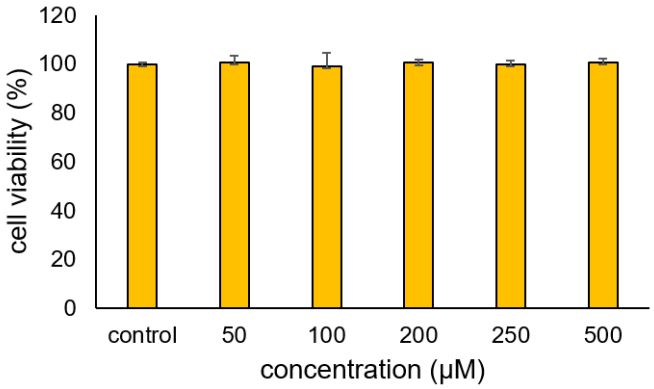

Figure S13. Concentration dependent MTT assay analysis of **β-CD$^+$** treated HeLa cells.

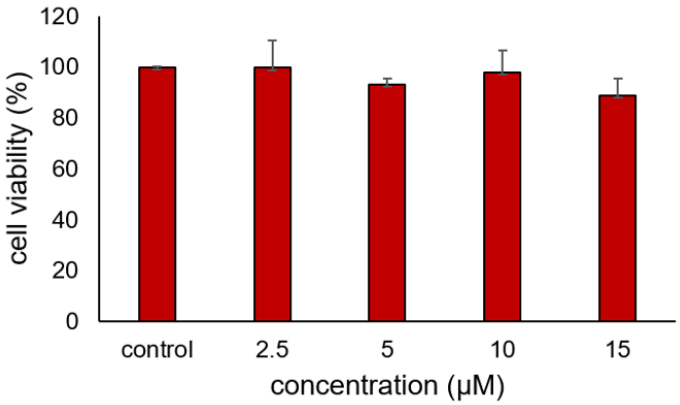

Figure S14. Concentration dependent MTT assay analysis of **1/β-CD$^+$** treated HeLa cells.

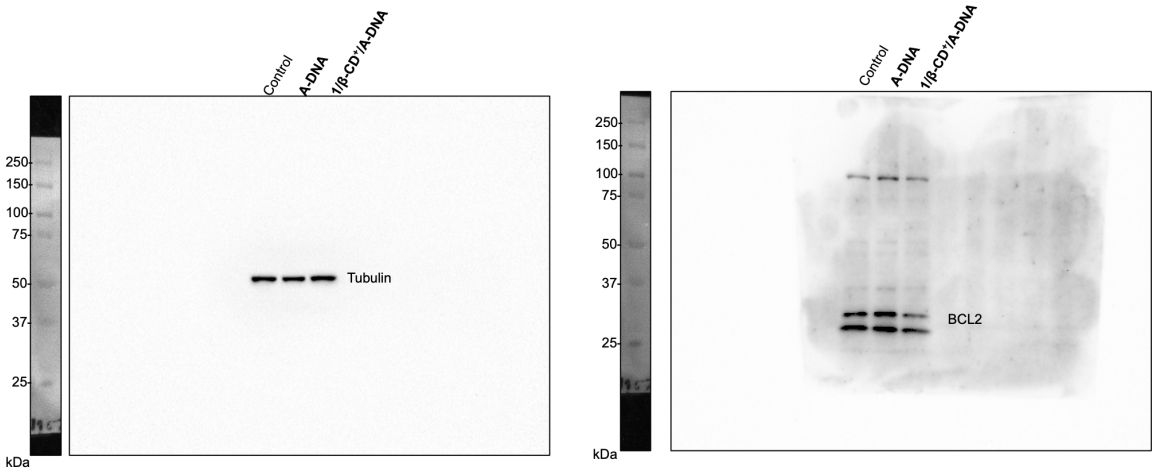

Figure S15. Raw data of Western blot analysis provided in Figure 6c.

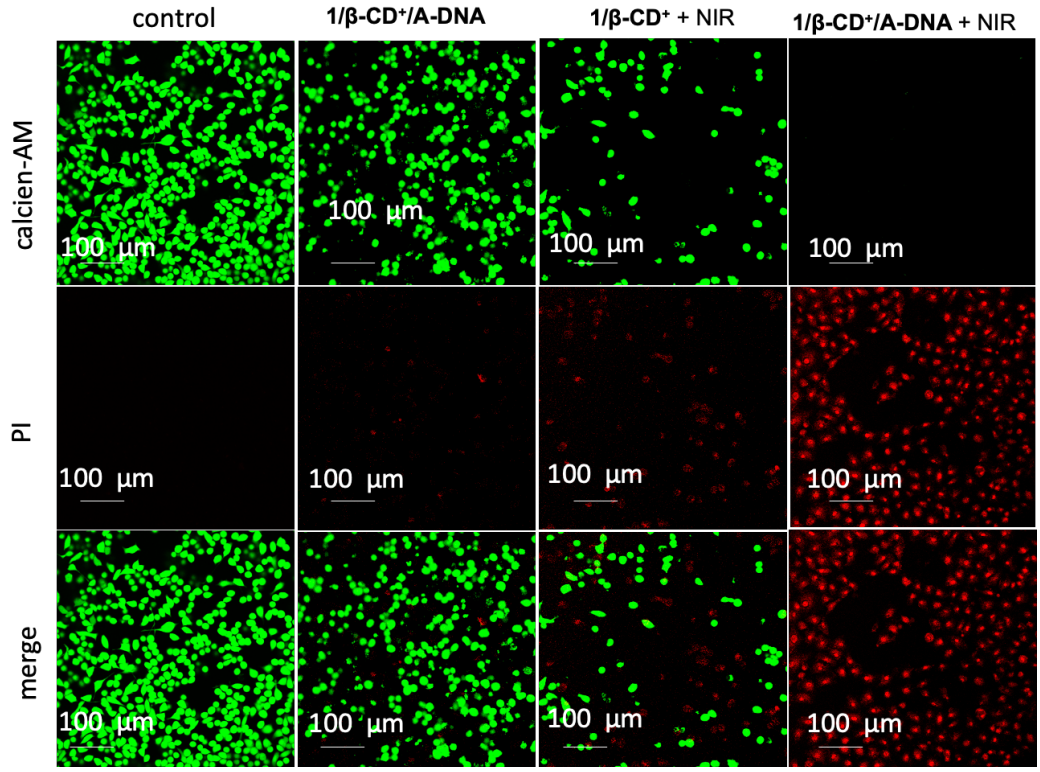

Figure S16. CLSM images for calcein-AM/PI live-dead cell assay of **1/β-CD$^+$/A-DNA**, **1/β-CD$^+$** + NIR and **1/β-CD$^+$/A-DNA** + NIR-treated HeLa cells.

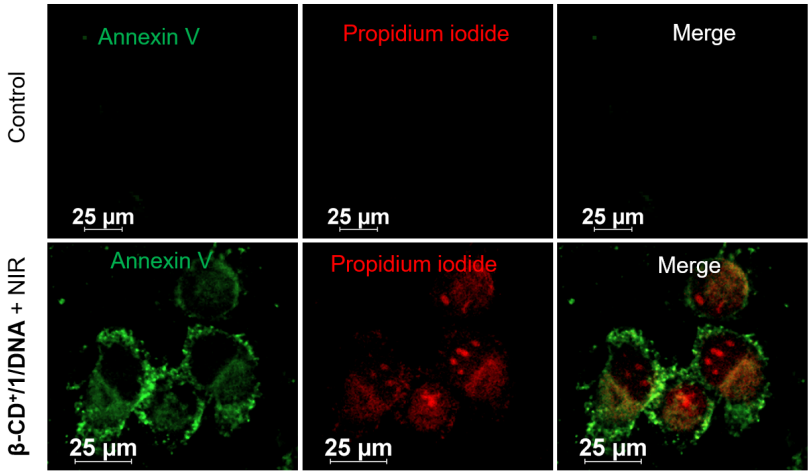

Figure S17. CLSM images for Annexin V-FITC/propidium iodide assay of **1/β-CD$^+$/A-DNA** treated HeLa cells.

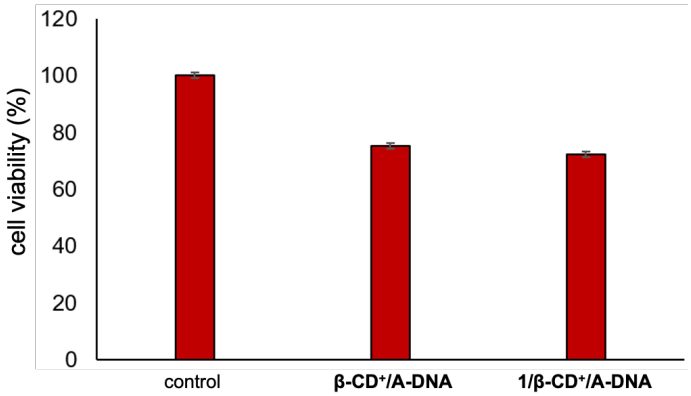

Figure S18. MTT assay of **β-CD$^+$/A-DNA** and **1/β-CD$^+$/A-DNA**-treated HeLa cells.

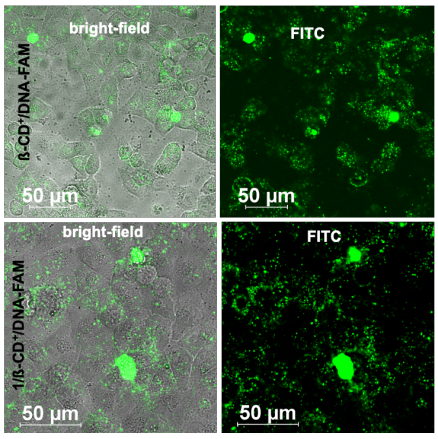

Figure S19. CLSM images of **β-CD$^+$/DNA-FAM** and **1/β-CD$^+$/DNA-FAM**-treated HeLa cells.

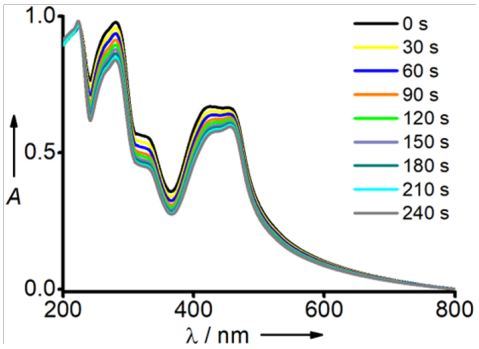

Figure S20. Time dependent UV-Vis absorption spectra of a solution of DPBF upon NIR light illumination (635 nm laser at 0.75 W/cm2) for 4 min.