Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Electronic Supplementary Information

Biocompatible Cellulose Nanocrystal-Based Trojan Horse Enables Targeted Delivery of Nano Au Radiosensitizers to Triple Negative Breast Cancer Cells

Giacomo Biagiotti,[a,§] Riccardo Cazzoli,[b,c,§] Patrizia Andreozzi,[a] Giusi Aresta,[a] Francesco Mattii,[a] Chiara Mangini,[a] Paolo di Gianvincenzo,[d] Chiara Tobia,[e] Sandro Recchia,[f] Laura Polito,[g] Mirko Severi,[a] Orazio Vittorio,[c] Stefano Cicchi,[a] Sergio E. Moya,[d] Roberto Ronca,[e] Adriana Albini,[h] Debora Berti,[a] Roberto Orecchia,[b,h] Cristina Garibaldi,[i,*] Saverio Minucci,[b,j,#] Barbara Richichi[a,#,*]

[a] Department of Chemistry 'Ugo Schiff', University of Firenze, Via della Lastruccia 13, 50019 Sesto Fiorentino (Firenze, Italy)

[b] Department of Experimental Oncology, IEO European Institute of Oncology, IRCCS, 20141 Milan, (Italy)

[c] School of biomedical sciences, UNSW Sydney, Kensington, NSW (Australia).

[d] Soft Matter Nanotechnology, Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Paseo de Miramon 194, 20014, Donostia-San Sebastián (Spain)

[e] Experimental Oncology and Immunology, Department of Molecular and Translational Medicine, Viale Europa 11, 25123 Brescia (Italy).

[f] Department of Science and High Technology, University of Insubria, Via Valleggio 11, 22100 Como, (Italy).

[g] National Research Council, CNR-SCITEC, Via G. Fantoli 16/15, 20138 Milan, (Italy).

[h] Scientific Director, IEO European Institute of Oncology IRCCS, 20141 Milan, (Italy).

[i] Unit of Radiation Research, IEO European Institute of Oncology, IRCCS, 20141 Milan, (Italy)

[j] Department of Oncology and Hemato-Oncology, University of Milan, Milan, (Italy).

[§]G.B. and R.C. equally contributed to this work

[#]S.M. and B.R. are co-senior authors

*C.G. and B.R. are co-corresponding authors, cristina.garibaldi@ieo.it, barbara.richichi@unifi.it,

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Table S1. Characterization of the CNC*-AuNPs conjugates **4-7** by elemental analysis (EA), inductively coupled plasma atomic emission spectroscopy (ICP-AES) and UV-Visible (UV-vis) analysis.

EA				ICP-AES	UV-Vis	
CNC-AuNPs conjugates	**Nitrogen content (% w/w)**	**Loading**		**Au content (% w/w)**	**Loading**	
		mmol/100 mg	**(% w/w)**		**mmol/100 mg**	**(% w/w)**
CNC*	0.03	NM	NM	NM	NM	NM
CNC*-Au-LA 4	0.73	0.051 ± 0.003	12.4 ± 0.8	9.9	NM	NM
CNC*-Au-Glc 5	1.28	0.015 ± 0.001	5.1 ± 0.4	10.6	NM	NM
CNC*-Au-Man 6	1.25	0.015 ± 0.001	5.2 ± 0.3	9.9	NM	NM
CNC*-Au-Glc-BODIPY 7	NM	NM	NM	NM	0.0014	0.5

NM: not measured

Table S2. Characterization of CNC, CNC*, and CNC*-AuNPs conjugates **4-6** H_2O dispersions in terms of ζ potential.

CNC-AuNPs conjugates	ζ potential (mV)	Sulfatation degree
CNC	− 38 ± 1	0.028
CNC*	− 44 ± 1	0.12
CNC*-Au-LA 4	− 34 ± 1	NM
CNC*-Au-Glc 5	− 35 ± 1	NM
CNC*-Au-Man 6	− 31 ± 1	NM

NM: not measured

Evaluation of the sulfation degree

The degree of sulfation was calculated according to the literature[1] as the ratio between the % of sulfur and carbon from the elemental analysis using the formula:

$$DS = \frac{(S\,\% \times 72.066)}{(C\,\% \times 32.06)}$$

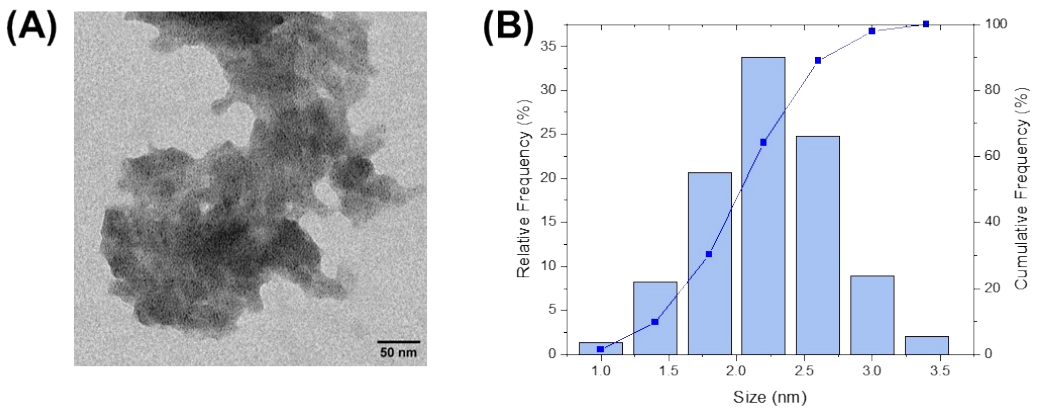

Figure S1. (A) Representative Transmission Electron Microscopy (TEM) micrograph of the **CNC*-Au-LA 4** and **(B)** related size distribution of AuNPs.

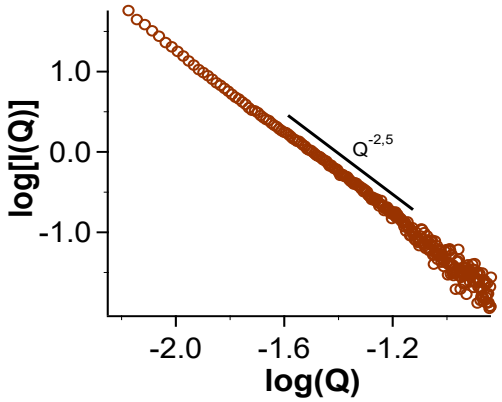

Figure S2. Porod's graph for SAXS profile obtained for **CNC*-Au-LA 4** H_2O dispersion (brown), and linear fit (black).

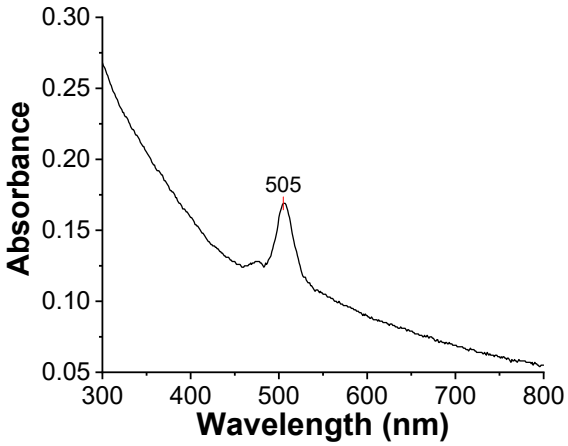

Figure S3. UV-Vis absorption spectrum of **CNC*-Au-Glc-BODIPY 7** (0.1 mg/mL in dimethyl sulfoxide).

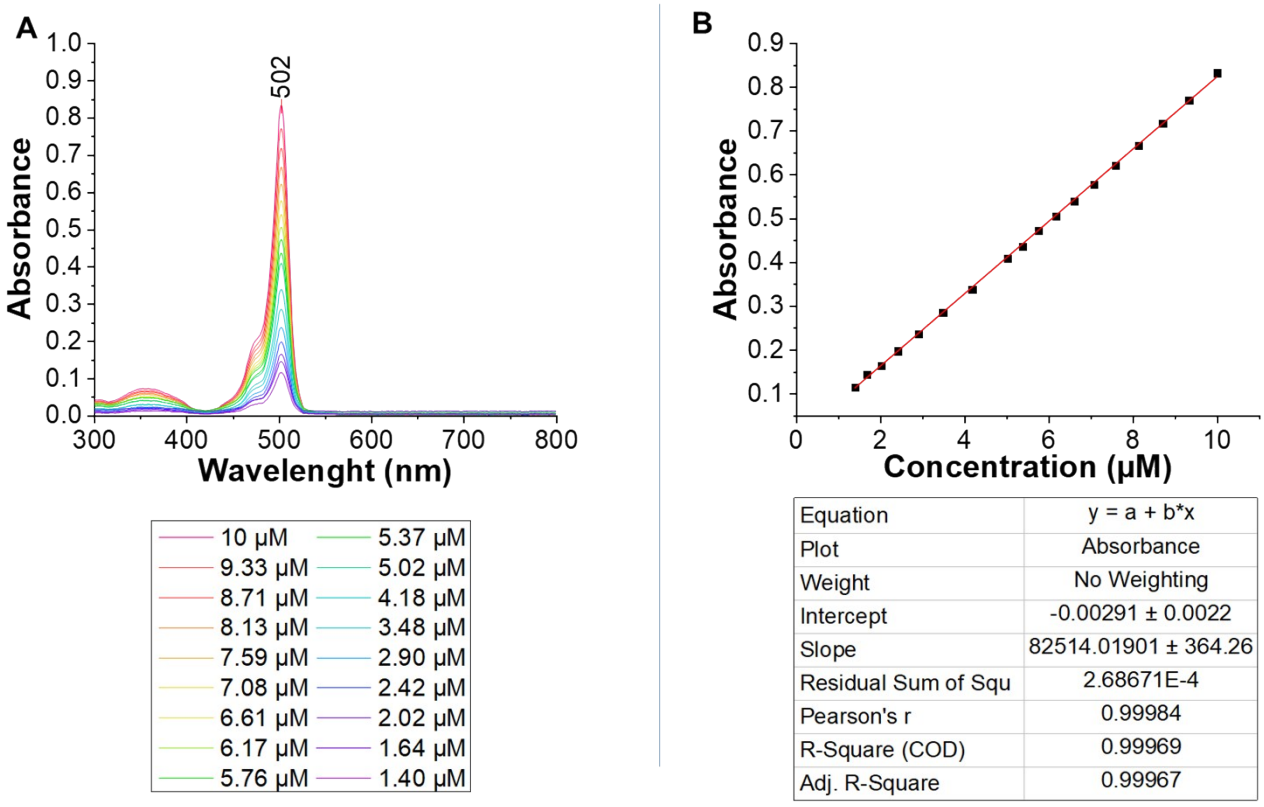

Figure S4. UV-Vis calibration curve for BODIPY **11** in dimethyl sulfoxide: **A**. Absorption spectra, **B**. Absorbance at λ_{max} = 502 nm *vs* different concentrations, Molar extinction coefficient calculated according to the Lambert-Beer Law = 82514 ± 364 $M^{-1}cm^{-1}$.

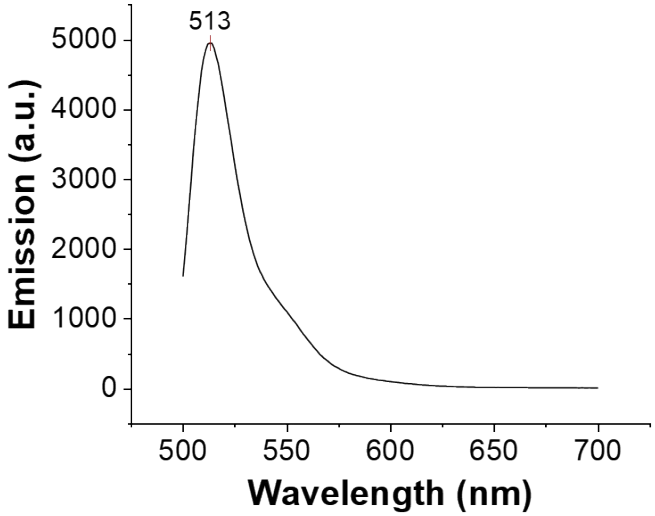

Figure S5. Fluorescence spectra of **CNC*-Au-Glc-BODIPY 7** (0.2 mg/mL in DMSO) after excitation at λ_{exc} = 480 nm.

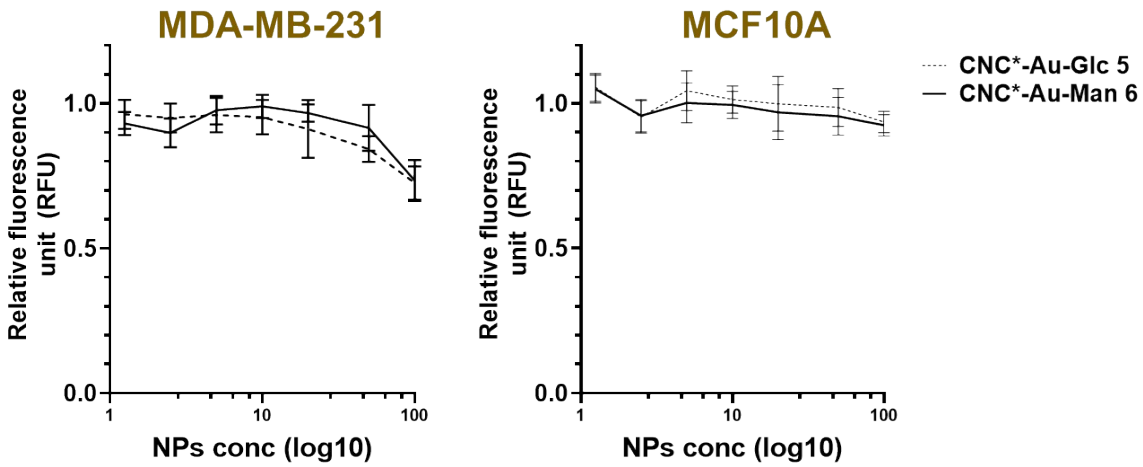

Figure S6. Proliferation analysis of MDA-MB-231 (left panel) and MCF10A (right panel) cells, after 24hrs treatment with **CNC*-Au-Glc 5** or **CNC*-Au-Man 6** at increasing concentrations. Measures have been normalized against untreated control cells.

Table S3. Gold content in MDA-MB-231 and MCF-10A cell lines (ICP-MS of cell lysates) after incubation with **CNC*-Au-LA 4**, **CNC*-Au-Glc 5** and **CNC*-Au-Man 6** at different time points.

CNC*-Au Conjugates	[CNC*-Au Conjugates][a] µg/mL	Cell line	Incubation time (hrs)	Au content in cell lysates		
				µg/L	pg/Cell	AuNPs/Cell[b]
4	2.5	MDA-MB-231	3.5	2.59 ± 0.84	0.09 ± 0.03	74 ± 24
4	2.5	MDA-MB-231	8	2.11 ± 1.18	0.07 ± 0.04	56 ± 31
4	2.5	MDA-MB-231	24	6.18 ± 1.97	0.16 ± 0.05	129 ± 41
4	5	MDA-MB-231	3.5	2.18 ± 0.21	0.08 ± 0.01	62 ± 6
4	5	MDA-MB-231	8	3.29 ± 0.44	0.11 ± 0.1	88 ± 12
4	5	MDA-MB-231	24	16.15 ± 2.21	0.42 ± 0.06	337 ± 46
4	10	MDA-MB-231	3.5	2.35 ± 0.26	0.08 ± 0.01	67 ± 7
4	10	MDA-MB-231	8	3.84 ± 1.56	0.13 ± 0.05	103 ± 42
4	10	MDA-MB-231	24	22.40 ± 9.48	0.58 ± 0.25	468 ± 122
4	20	MDA-MB-231	3.5	3.19 ± 0.68	0.11± 0.06	91 ± 48
4	20	MDA-MB-231	8	6.98 ± 1.55	0.23 ± 0.05	187 ± 41
4	20	MDA-MB-231	24	21.19 ± 5.77	0.55 ± 0.15	442 ± 120
5	2.5	MDA-MB-231	3.5	3.72 ± 0.95	0.14 ± 0.03	110 ± 28
5	2.5	MDA-MB-231	8	3.73 ± 1.23	0.13 ± 0.04	103 ± 34
5	2.5	MDA-MB-231	24	25.79 ± 0.54	0.68 ± 0.01	550 ± 7
5	5	MDA-MB-231	3.5	4.71 ± 1.77	0.17 ± 0.07	139 ± 52

5	5	MDA-MB-231	8	4.90 ± 0.56	0.17 ± 0.02	136 ± 15
5	5	MDA-MB-231	24	36.01 ± 1.86	0.95 ± 0.05	767 ± 53
5	10	MDA-MB-231	3.5	6.59 ± 3.53	0.24 ± 0.13	194 ± 104
5	10	MDA-MB-231	8	6.65 ± 1.17	0.23 ± 0.04	184 ± 32
5	10	MDA-MB-231	24	39.60 ± 3.83	1.04 ± 0.10	836 ± 81
5	20	MDA-MB-231	3.5	9.00 ± 2.85	0.33 ± 0.10	265 ± 84
5	20	MDA-MB-231	8	7.51 ± 3.35	0.26 ± 0.12	208 ± 93
5	20	MDA-MB-231	24	32.67 ± 2.62	0.86 ± 0.07	678 ± 73
6	2.5	MDA-MB-231	3.5	4.25 ± 2.24	0.15 ± 0.08	84 ± 1
6	2.5	MDA-MB-231	8	1.27 ± 0.50	0.04 ± 0.02	34 ± 13
6	2.5	MDA-MB-231	24	8.43 ± 2.37	0.22 ± 0.06	177 ± 50
6	5	MDA-MB-231	3.5	1.99 ± 0.13	0.07 ± 0.005	57 ± 4
6	5	MDA-MB-231	8	1.87 ± 0.48	0.06 ± 0.02	50 ± 13
6	5	MDA-MB-231	24	15.13 ± 1.62	0.40 ± 0.04	318 ± 34
6	10	MDA-MB-231	3.5	3.58 ± 0.97	0.13 ± 0.03	102 ± 28
6	10	MDA-MB-231	8	4.79 ± 2.05	0.16 ± 0.07	129 ± 55
6	10	MDA-MB-231	24	26.76 ± 3.44	0.70 ± 0.01	563 ± 72
6	20	MDA-MB-231	3.5	3.24 ± 0.65	0.12 ± 0.02	92 ± 19
6	20	MDA-MB-231	8	5.27 ± 1.39	0.18 ± 0.05	142 ± 37
6	20	MDA-MB231	24	31.66 ± 3.94	0.83 ± 0.10	666 ± 83
5	2.5	MCF-10A	24	6.92 ± 1.62	0.19 ± 0.01	153 ± 12
5	5	MCF-10A	24	11.00 ± 0.89	0.30 ± 0.01	243 ± 8
5	10	MCF-10A	24	15.35 ± 7.76	0.42 ± 0.21	339 ± 171
5	20	MCF-10A	24	16.04 ± 4.92	0.44 ± 0.13	354 ± 102
6	2.5	MCF-10A	24	6.49 ± 0.41	0.18 ± 0.1	144 ± 9
6	5	MCF-10A	24	8.33 ± 1.15	0.23 ± 0.03	184 ± 25
6	10	MCF-10A	24	10.01 ± 1.08	0.28 ± 0.03	221 ± 24
6	20	MCF-10A	24	10.36 ± 2.46	0.29 ± 0.07	229 ± 39

[a] The CNC*-Au- conjugates contain 10% w/w of AuNPs content; [b] the number of NPs per cell is reported divided by 10^3, and it was calculated according to the literature. [2]

The number of nanoparticles was calculated from gold content measured by ICP-MS, according to the literature[2] following the formula:

$$U = \frac{2}{3}\pi\left(\frac{D}{a}\right)^3$$

$$N = \frac{M}{U}$$

In which *D* is the NPs diameter expressed in angstrom *a* refers to the edge of a unit cell, which has a value of 4.0786 Å on the edge; *M* is the measured number of gold atoms from ICP-MS.

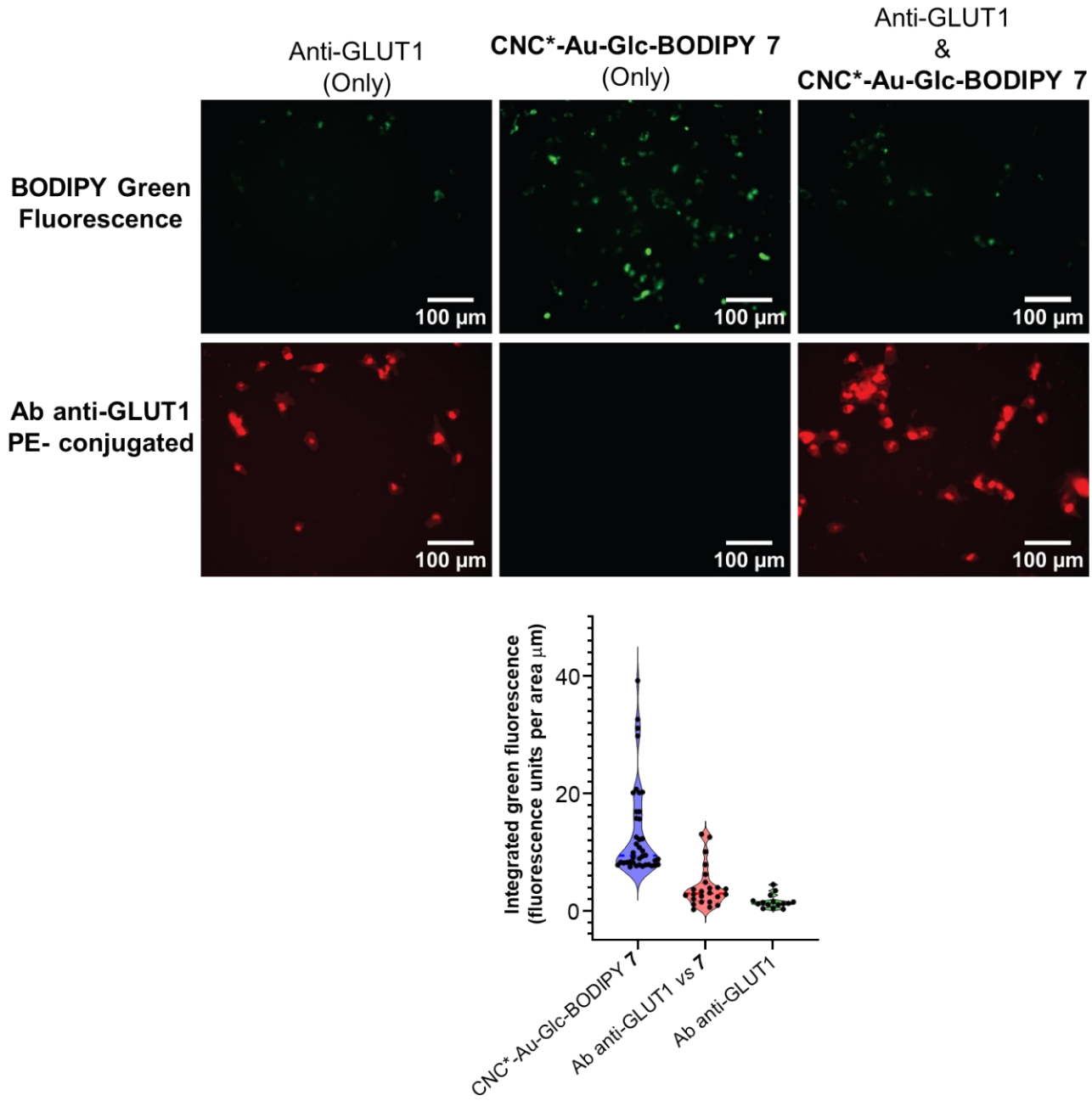

Figure S7. Violin plot of integrated fluorescence (fluorescence per µmeter) of MDA-MB-231 treated for 18 hrs with the Human Antibody (Ab) anti-GLUT1 PE-conjugated (left panels) with **CNC*-Au-Glc-BODIPY 7** at concentration of 2.5 µg/mL (central panels), or with both at the same time (right panels). Each dot represents a single ROI (containing at least 1 cell).

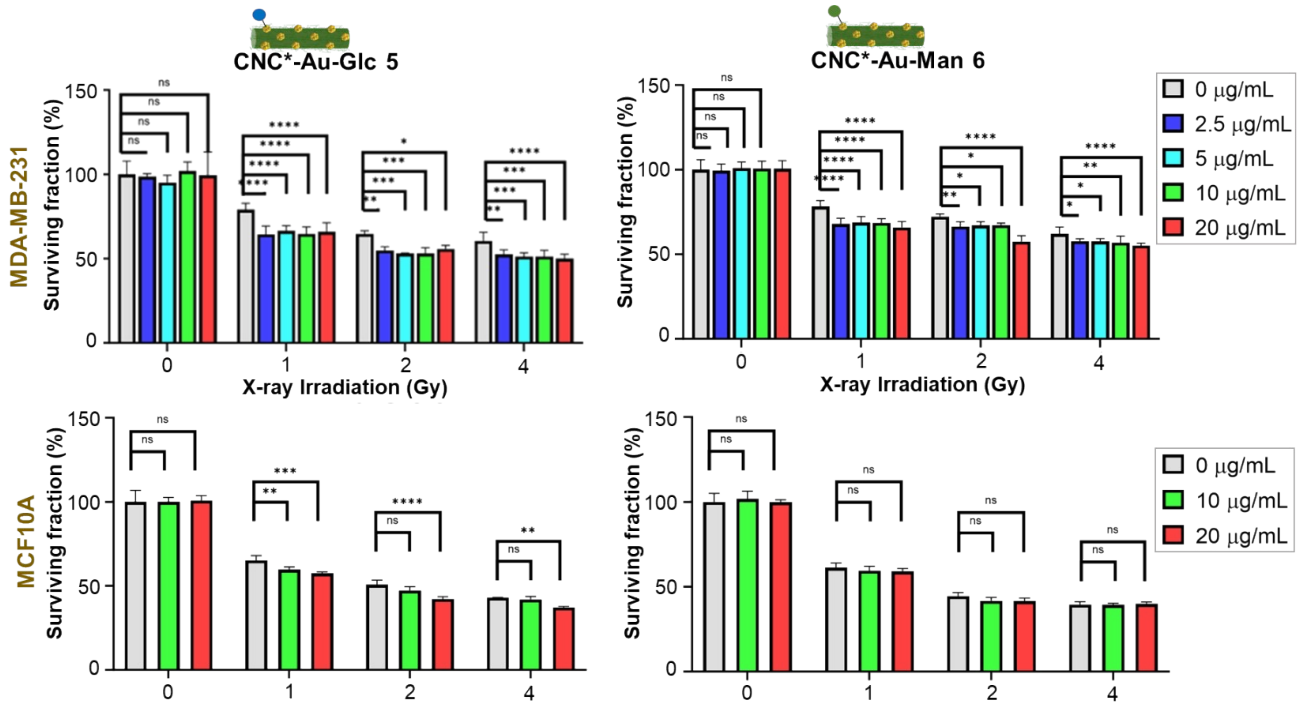

Figure S8. Short-term assay. Surviving fraction of MDA-MB-231 (upper panels) and MCF10A (lower panels) cells, after combinatory treatment of X-ray and **CNC*-Au-Glc 5** (left) or **CNC*-Au-Man 6** (right), counted 24 hrs after X-rays irradiation. Data from 3 independent experiments with 3 replicates each (n=9). Values are normalized on no-NPs no-radiation control cells. Significance is calculated comparing AuNPs treated points against radiation only controls within same level of Xray irradiation. (* P<0.5; ** P<0,01; *** P<0,001; **** P<0,0001).

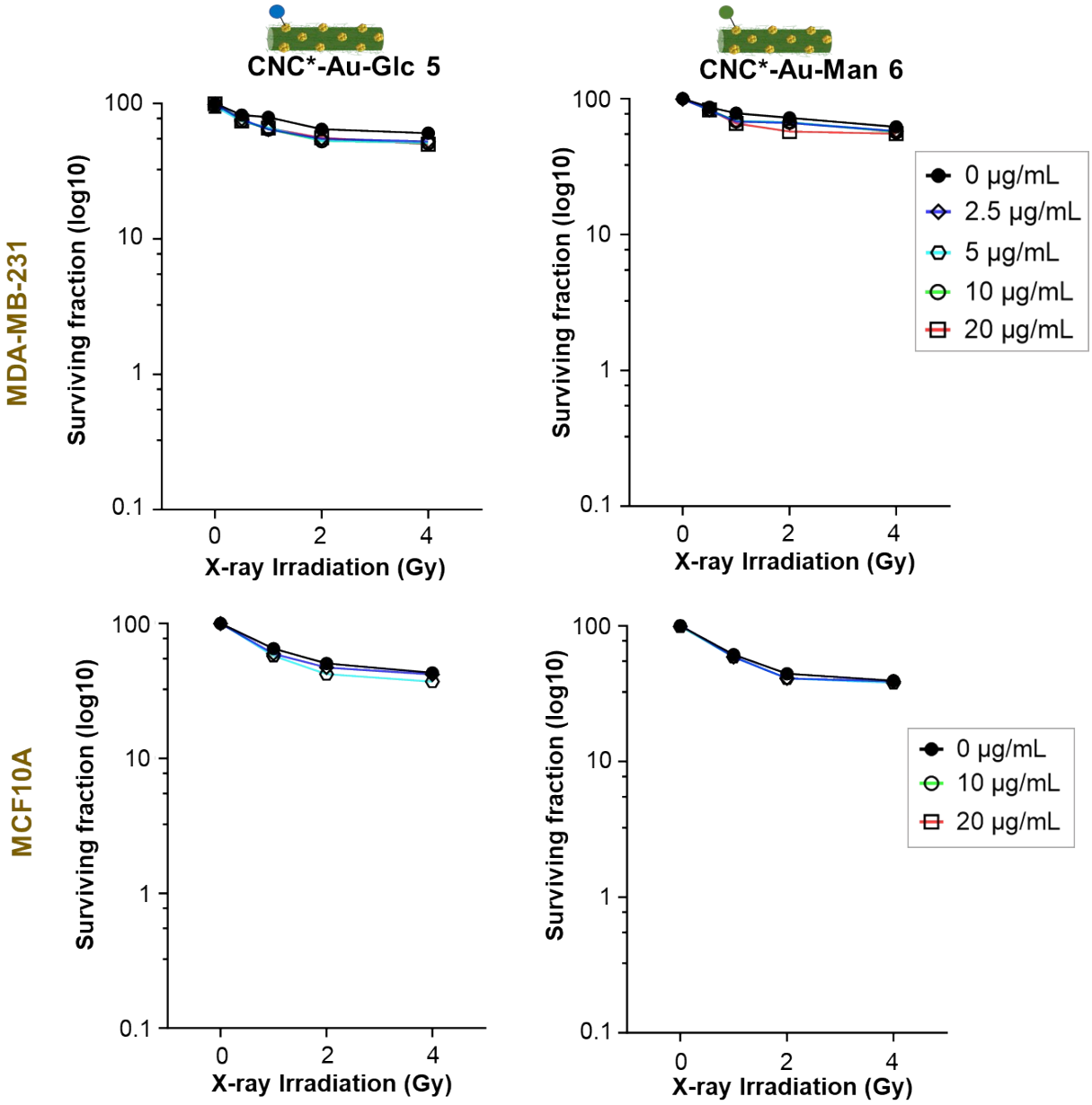

Figure S9. Short-term assay. Surviving fraction of MDA-MB-231 (upper panels) and MCF10A (lower panels) cells, after combinatory treatment of X-ray and **CNC*-Au-Glc 5** (left) or **CNC*-Au-Man 6** (right), counted 24 hrs after X-rays irradiation. Data from 3 independent experiments with 3 replicates each (n=9). Values are normalized on no-NPs no-radiation control cells.

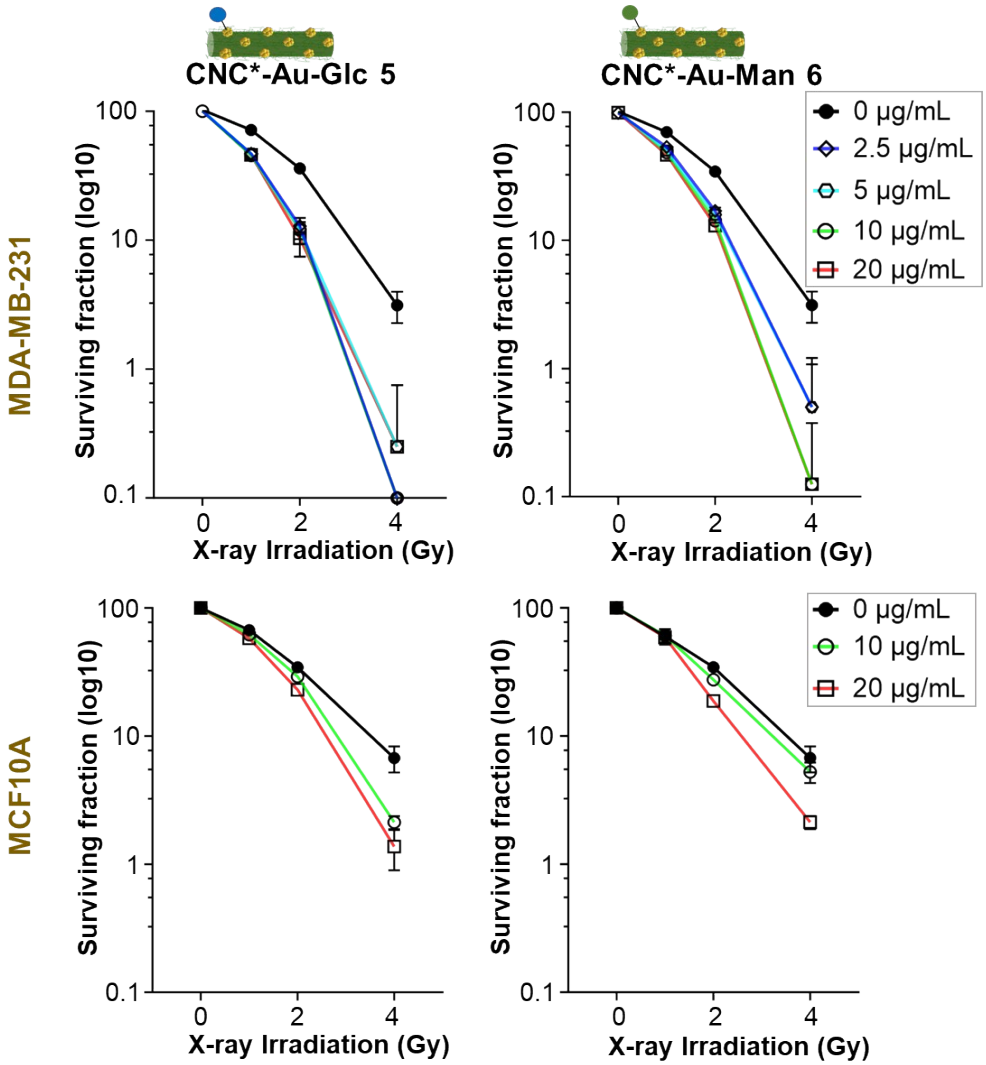

Figure S10. Long-term assay. Surviving fraction of MDA-MB-231 (upper panels) and MCF10A (lower panels) colonies, after combinatory treatment of X-ray and **CNC*-Au-Glc 5** (left) or **CNC*-Au-Man 6** (right). Cells have been exposed to X-rays with or without the glyconanomaterials and replated in low density immediately after. Colonies have been counted 14 days after irradiation. Data from 2 independent experiments 2 replicates each (n=4).Values are normalized on no-glyconanomaterials and no-radiation control cells.

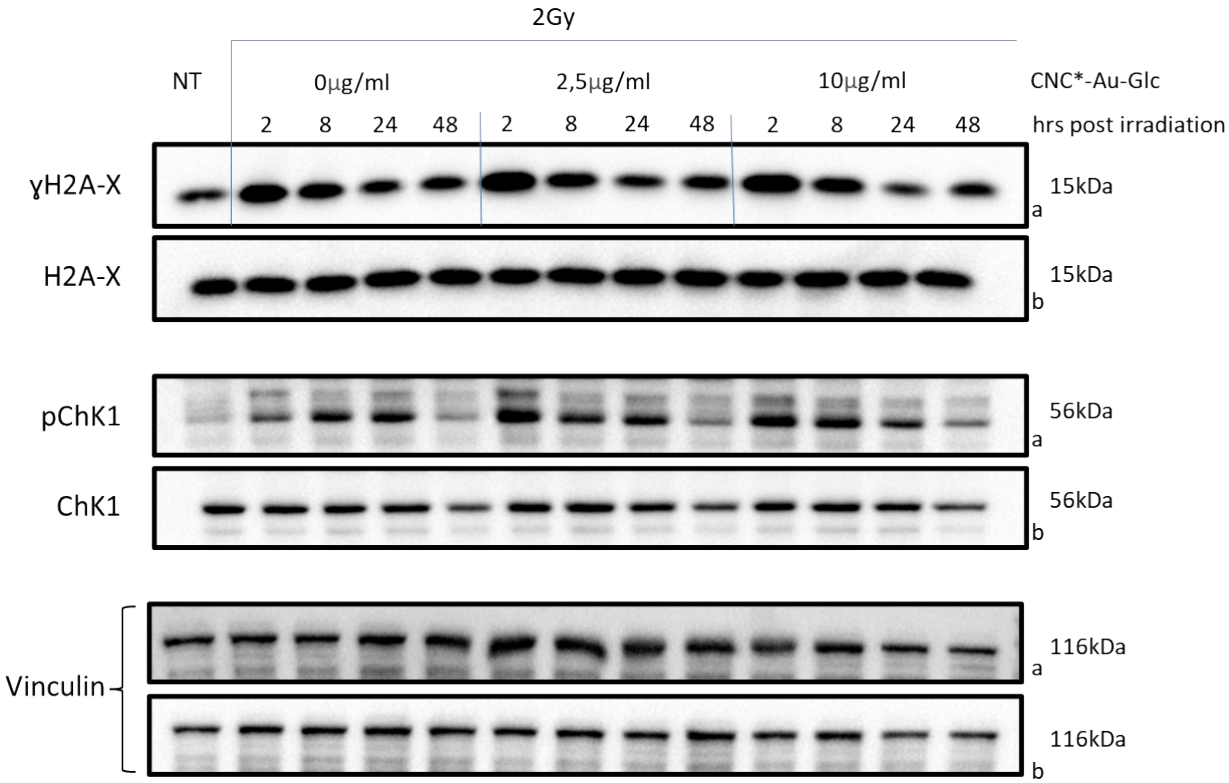

Figure S11. DNA damage response evaluation. Immunoblotting analysis to measure the level of γH2AX and pCHK1 on MDA-MB-231 lysates, after combinatory treatment of X-rays and **CNC*-Au-Glc 5**. Cells have been exposed to X-rays (2 Gy) with or without the glyconanomaterial at different concentrations and lysed at different time points, 2, 8, 24, 48 hrs. Vinculin has been used as a loading control. Full gel a and gel b are in **Figure S12**.

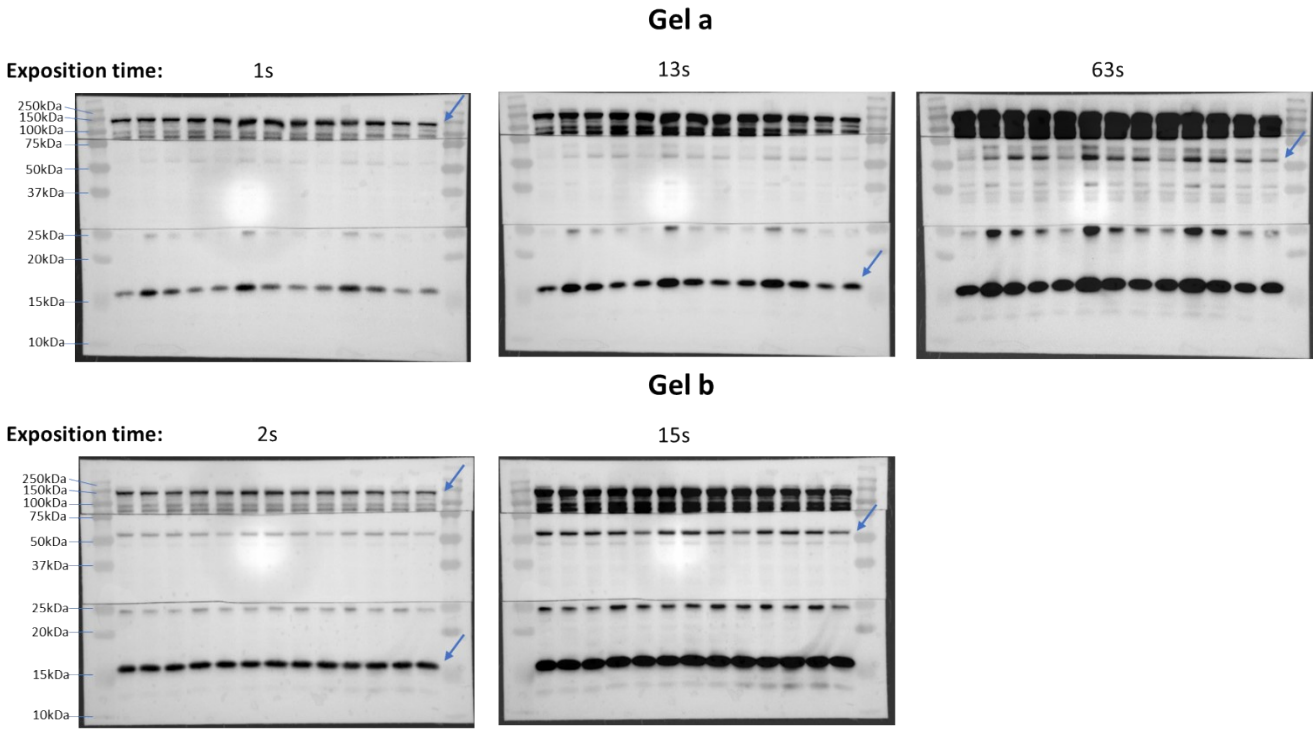

Figure S12. Immunoblot raw data. The gel a and b refer to the **Figure S11** in ESI; in gel a ɣH2A-X and pChk1 were analyzed Vinculin as a loading control. In gel b total H2A-X, total Chk1 and vinculin as a loading control.

Materials and methods

All reagents, whose synthesis is not described, were commercially available and were used without any further purification, if not specified otherwise. Cellulose nanocrystals (CNC) were purchased from CelluForce® (product name CelluForce NCC™). NMR spectra were recorded on Varian Inova 400, Mercury plus 400 and Gemini 200 instruments. ESI-MS were recorded on LC-MS LCQ Fleet ThermoFisher Scientific. UV–vis spectra were recorded on a BMG Labtech SPECTROstar Nano UV–vis spectrophotometer using a 1 cm cell. Fluorescence spectra were registered on a HORIBA FluorMax® Plus spectrofluorimeter using 1.0 cm cell. Elemental analyses were performed on a Vario MICRO cube instrument (Elementar). Varian 720-ES Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) was used to quantify the gold amount on CNC derivatives (see ESI).

Cell viability assay

MDA-MB-231 and MCF10A cells have been plated at 2k/well in 96 well plates and allowed to attach overnight. At t = 0 cells have been treated with **CNC*-Au-Glc 5** and **CNC*-Au-Man 6** at

different concentrations (2.5, 5, 10, 20, 50 and 100 μg/mL or left untreated) for 24 hrs, in a final volume of 100 μL. After 24 hrs viability has been assessed by CellTiter-Glo luminescent cell viability assay (Promega) as manufacturer's protocol, luminescence has been measured by GloMAX explorer plate reader (Promega). Figure S6, ESI.

Evaluation of nanoparticles uptake on MDA-MB-231 and MCF-10A cells.

MDA-MB-231 and MCF10A cells have been plated at 40k/well in 24 well plates, allowed to attach overnight. At t = 0 cells have been treated with **CNC*-Au-Glc 5** and **CNC*-Au-Man 6** at different concentrations (2.5, 5, 10 and 20 μg/mL or left untreated) for 24 hrs, in a final volume of 300 μL. After, **CNC*-Au-Glc 5** and **CNC*-Au-Man 6** incubation, cells have been washed 3 times with 100 μL of PBS, then detached in 100 μL of trypsin EDTA solution and collected. MilliQ distilled water has been added to detached cells in trypsin with vigorous pipetting to cause cell lysis due to ipotonic buffer. Lysate can be stored at -20°C. The digestion of cellular lysates has been done using aqua regia (a mixture of nitric acid and hydrochloric acid in molar ratio 3:1). Briefly, 400 μL of aqua regia are added on the lysates and left 72hrs under chemical hood. After the incubation 1500 μL of milliQ distilled water has been added and mixed by gently rocking the vials.

Short term evaluation of cell viability after X-rays irradiation.

MDA-MB-231 and MCF10A cells have been plated at 200k/plate in 6 cm plates, allowed to attach overnight. At t = 0 cells have been treated with **CNC*-Au-Glc 5** and **CNC*-Au-Man 6** at different concentrations (2.5, 5, 10, and 20 μg/mL or left untreated) for 24 hrs, in a final volume of 2.0 ml. After incubation with the glyconanomaterials, cells have been X-rays irradiated with Faxitron CP-160 (Faxitron Xray corp) at 100kV and 10mA to obtain a final irradiation of 0, 1, 2, and 4 Gy. Then, 24 hrs post irradiation, cells have been collected in trypsin and PI exclusion assay have been performed to count and assess viability, using MACSQuant X flow cytometer (Miltenyi Biotech). Debris have been excluded by size (forward scattering) and granularity (side scattering) evaluation. Final data are collected from 3 independent experiments with 3 replicates each (n=9). All the counts have been normalized against the untreated controls (no glyconanomaterials and no radiation), to obtain the percentage of cells surviving the treatments (surviving fraction %). Figures S7-S8, ESI.

Colony forming assay after X-rays irradiation.

MDA-MB-231 and MCF10A cells have been plated at 200k/plate in 6 cm plates, allowed to adhere overnight. At t = 0 cells have been treated with either **CNC*-Au-Glc 5** or **CNC*-Au-Man 6** at different concentrations (2.5, 5, 10 and 20 μg/mL or left untreated) for 24 hrs, in a final volume of 2.0 mL. Afterwards, glyconanomaterials incubation cells have been X-rays irradiated with Faxitron CP-160 (Faxitron Xray corp) at 100kV and 10mA to obtain a final irradiation of 0, 1, 2 and 4 Gy. Soon after the irradiation, cells have been counted and re-plated at 2 densities in 6 wells plates (100 and 200 cells-well) and left to grow up to 14 days. Afterwards, colonies have been stained with crystal violet and counted under microscope, considering valid colonies with more than 50 cells. Surviving fraction has been calculated as the ratio between counted colonies and plated cells. **Figure S10**.

Comet assay.

MDA-MB-231 have been plated 200k/plate in 6 cm plates, allowed to adhere overnight. At t = 0 cells have been treated with either **CNC*-Au-Glc 5** at different concentrations (2.5 and 10 μg/mL or left untreated) for 24 hrs, in a final volume of 2.0 mL. Afterwards, cells have been X-ray irradiated with Faxitron CP-160 (Faxitron Xray corp) at 100kV and 10mA to obtain a final irradiation of 0, 1 and 2 Gy. Cells have been counted and re-plated on agarose coated slides to perform comet assay. Upon lysis, DNA is denatured under alkaline conditions and run through electrophoresis. Images have been acquired using Leica DM6 B and analyzed using Cometanalyser.[3]

Immunoblotting

MDA-MB231 cells were plated at 200k cells/plate in 6cm diameter plates. At t=0 cells were treated with **CNC*-Au-Glc 5** at different concentrations (2.5, 10 μg/ml) or left untreated for 24 hrs, and then exposed to X-ray irradiation using the Faxitron CP-160 (Faxitron Xray corp, USA) at 100kV and 10mA, to obtain a final irradiation of 1 Gy and 2 Gy. Cells were collected at different timepoints as indicated and lysed with total lysis buffer complemented with benzonase (EMD Millipore Corp 70746-6) (6,25U in 100 µl lysis buffer). Lysates were quantified with DC Protein Assay Reagents (Bio-Rad). We used the following primary antibodies, pChk1 (#2348), Chk1 (#2360), ɣH2AX (#9718), H2AX (#7631) from Cell Signaling, Danvers USA diluted 1:1000, and Vinculin (#V9131) from Sigma Aldrich, Danvers USA diluted 1:10000. Images were collected with the ChemiDoc XRS+ (Bio-Rad, USA).

Statistical analysis

DEF has been calculated from clonogenic assay, using PRISM software to calculate non-linear regression – linear quadratic model with equation Y = 100 * exp(-1*(A*X + B*X^2)). DEF is defined as the ratio of doses required to give the same surviving fraction (40% reduction) as that of the radiation only control cells. Correlation analysis has been done with PRISM software using Pearson correlation one-tailed test, confidence interval 95%, table shows r-squared value. All data are expressed as mean ± s.d. and subjected to a two-way ANOVA test with (* p<0,1; ** p<0,05; *** p<0,01; **** p<0,005; ns non-significant.).

Sulfation of CNC*

CNC (500 mg, 2.8 mmol of glucose units) was added to vigorously stirred dimethyl sulfoxide (10.5 mL) at 25°C, the dispersion was stirred for 30 minutes then sulfur trioxide pyridine complex (200 mg, 1.25 mmol) was added, and the dispersion diluted with dimethyl sulfoxide (5.25 mL) to avoid gelification. After 1h and 2hrs a second and a third aliquot of sulfur trioxide pyridine complex (200 mg, 1.25 mmol and 250 mg, 1.57 mmol respectively) were added. The reaction mixture was stirred for other 2 hrs (total reaction time 4h) then it was diluted with acetone (50 mL) and the product precipitated in centrifuge (8000 rpm, 10 minutes), the supernatant was removed and the solid dispersed with acetone (50 mL) and precipitated in centrifuge (8000 rpm, 10 minutes). The solid was recovered and dissolved in aqueous sodium hydroxide 0.1 M (20 mL) and stirred for 30 minutes. The dispersion was diluted with milliQ water (30 mL) and dialyzed for 72 hrs and freeze dried to obtain the **CNC*** as a fluffy white solid (516 mg). Elemental analysis C 38.82 %, H 5.99 %, N 0.03 % and S 1.96 %. Degree of sulfation 0.12.

Synthesis of CNC*-Au-LA 4

A stirred solution of tetrachloroauric acid trihydrate 80 mg (0.20 mmol) in water milliQ (23 mL) was diluted with methanol (207 mL), then 230 mg of CNC* was added, and the mixture vigorously stirred for 30 minutes. Then, a solution of the LA spacer **8**[4] (172 mg, 0.71 mmol) in methanol (1.5 mL) and sodium borohydride (196 mg, 37.8 mmol) were added sequentially and the mixture stirred for additional 2 hrs. Finally, the crude dispersion of **4** was dialyzed for 24 hrs *versus* milliQ water and freeze dried to afford 385 mg of **4**. ICP-AES: Au content 9.9 % w/w. Elemental analysis C 35.44 %, H 5.69 %, N 0.73 % and S 3.16 %.

The reaction was repeated in triplicate affording a loading for **8** of 0.051 ± 0.003 mmol/100 mg (12.4 % ± 0.8 w/w) calculated according with the nitrogen content in the elemental analysis.

Synthesis of CNC*-Au-Glc 5

To a stirred solution of **CNC*-Au-LA 4** (60 mg, 0.03 mmol of **8**) in milliQ water (6 mL), **9**[5,6] (20 mg, 0.06 mmol) was added, and the mixture stirred for 30 minutes, then copper sulfate (1.5 mg) and sodium ascorbate (1.0 mg) were added, and the mixture stirred at room temperature for 2 hrs. After that, a second aliquot of catalysts was added (1.5 mg of copper sulfate and 1.0 mg of sodium ascorbate) and the mixture stirred at room temperature for 12 hrs. Then, the crude dispersion of **5** was dialyzed *versus* milliQ water for 24 hrs and freeze dried to afford 56 mg of **5**. ICP-AES: Au content 10.6 % w/w. Elemental analysis C 36.45 %, H 5.59 %, N 1.28 % and S 4.74 %. The reaction was repeated in triplicate affording a loading for **9** of 0.015 ± 0.001 mmol/100 mg (5.1% ± 0.4 w/w) calculated according with the nitrogen content in the elemental analysis.

Synthesis of CNC*-Au-Man 6

To a stirred solution of **CNC*-Au-LA 4** (60 mg, 0.03 mmol of **8**) in milliQ water (6 mL), **10**[4](20 mg, 0.06 mmol) was added and the mixture stirred for 30 minutes, then copper sulfate (1.5 mg) and sodium ascorbate (1.0 mg) were added, and the mixture stirred at room temperature for 2 hrs. After that, a second aliquot of catalysts was added (1.5 mg of copper sulfate and 1.0 mg of sodium ascorbate) and the mixture stirred at room temperature for 12 hrs. Then the crude dispersion of **6** was dialyzed *versus* milliQ water for 24 hrs and freeze dried to afford 58 mg of **6**. ICP-AES: Au content 9.9 % w/w. Elemental analysis C 36.61 %, H 6.04 %, N 1.28 % and S 3.29 %. The reaction was repeated in triplicate affording a loading for **10** of 0.015 ± 0.001 mmol/100 mg (5.2% ± 0.3 w/w) calculated according with the nitrogen content in the elemental analysis.

Synthesis of CNC*-Au-Glc-BODIPY 7

To a stirred solution of obtained **CNC*-Au-Glc 5** (27 mg) in dimethylformamide (2,7 mL), BODIPY **11** (2.5 mg, $7x10^{-3}$ mmol) was added, and the mixture stirred for 30 minutes, then copper sulfate (0.5 mg) and sodium ascorbate (0.4 mg) were added, and the mixture stirred at room temperature for 2 hrs. After that, a second aliquot of catalysts was added (0.5 mg of copper sulfate and 0.4 mg of sodium ascorbate) and the mixture stirred at room temperature for 12 hrs. The mixture was filtered over a 0.2 μm nylon membrane and the solid washed with

methanol (25 mL), dichloromethane (25 mL) and methanol (3 x 25 mL) respectively, until colorless solution was obtained, then the solid was dispersed in water (1 mg/mL) and dialyzed *versus* milliQ water (250 mL) for 24 hrs changing the water three times and freeze dried to afford 19 mg of **7**. The probe loading was measure by UV-Vis absorption analysis of a 0.1 mg/mL dispersion in dimethyl sulfoxide and calculating the amount of dye using a calibration curve on the maximum absorption peak at λ_{max} = 505 nm. The reaction afforded a loading of BODIPY **11** of 0.0014 mmol/100 mg, 0.5 % w/w with a glucose:BODIPY molar ratio 9:1.

Transmission Electron Microscopy (TEM)

CNC*-Au-LA 4 was dissolved in 48 µL of milliQ water (5 mg/mL). **CNC*-Au-LA 4** dispersion was sonicated for 30 min at 20-25°C in a sonication bath (59 kHz) and left overnight to stabilize. Then, the dispersion was diluted to 0.1 mg/mL with milliQ water and further sonicated for 30 min at 20-25°C in a sonication bath (59 kHz). Carbon films TEM grids (Aname, CF400-CU) were pre-treated in a glow discharged chamber Emitek K100X (2 minutes, 35 mA). A volume of 0.5 µL of the **CNC*-Au-LA 4** dispersion was applied on the grid and left dry overnight. TEM images were taken using a JEOL JEM 1010 microscope operating at an acceleration voltage of 100 kV.

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) analysis (cell lysates)

Measurements were performed on an iCAP-Q ICPMS (Thermo Scientific, Bremen, Germany), with autosampler ASX-520 (Cetac Technologies Inc., NE, USA). Data were analyzed with QtegraTM v2.6 (Themo Scientific, Bremen, Germany). Cell lysates were digested as described in the experimental section: Evaluation of nanoparticles uptake on MDA-MB-231 and MCF10A cells.

Inductive Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

Before ICP-AES analysis CNC-AuNPs derivatives have been digested using microwave assisted mineralization (MARS-Xpress, CEM) with a mixture of suprapure nitric acid obtained by sub-boiling distillation and hydrochloric acid (37%) in molar ratio 1:3. The determination of Au concentrations was done in triplicate using Ge at a concentration of 1.0 mg/L as internal standard. Wavelengths used for Au determination were 242.794, and 267.594 nm, whereas for Ge was used the line at 209.426 nm. The ICP operating conditions were optimized to obtain

maximum signal intensity, and between each sample, a rinse solution constituted by 2% v/v of HNO_3 was used.

Competition assay.

MDA-MB-231 cells have been plated at 15k/well in special µ-Slide 8 Well plate (IBIDI), left to attach overnight. Cells have been with **CNC*-Au-Glc-BODIPY 7** at concentration of 2.5 µg/mL, with the Human Glut1 PE-conjugated Antibody (FAB1418P) ratio 1:50 (Biotechne, Minneapolis, US), combination of **7** and antibody or left untreated for 18 hrs, in a final volume of 300 µL. Imagine acquisitions have been done at the end point using Cell Discoverer 7 (Zeiss, Oberkochen, Germany), data have been processed with ImageJ software (NHI, US). Brightness has been normalized using the untreated and unstained experimental point to exclude cells own autofluorescence, then threshold has been applied to all the acquisitions. ROIs have been designed using either the red signal where available, or using the cells own autofluorescence in the nanoparticles only condition, obtaining cells silhouette. Lastly, ROIs have been applied on overlay in both channels and integrated fluorescence have been calculated as intensity divided by area of each single ROI.

Dynamic of nanoparticles uptake.

MDA-MB-231 cells have been plated at 15k/well in special µ-Slide 8 Well plate (IBIDI), left to attach overnight. Cells have been stained with Celltracker deep red (Thermofisher) and Hoechst to define cytoplasms and nuclei prior to the treatment with **CNC*-Au-Glc-BODIPY 7** at concentration of 2.5 µg/mL or left untreated for 24 hrs, in a final volume of 300 µL. Imagine acquisitions have been done from 8 to 24 hrs, 1 image every 60 minutes with spinning disk confocal microscopy (SDCM) (Nikon Eclipse Ti2, CSU W1 Confocal scanner Unit) using NisElements 3.2, data have been processed with ImageJ software (NHI, US) to create time lapse videos, rendering of video has been performed using Premiere Pro CC (Adobe).

Protocols for the preparation of the CNC*-Au-Glc 5 and CNC*-Au-Man 6 dispersions for cellular assays.

The day prior to experiment, powder preparation of CNC*-AuNPs conjugates is first centrifuged 2 minutes, milliQ water has been added dropwise while gently vortexing to reach 5 mg/mL final concentration. The 5 mg/mL stock solution was then sonicated 30 minutes (60 sec on, 5 sec off) at 4°C in a Bioruptor sonication bath (Biosense), after sonication, vials are left overnight to

stabilize. On the day of the experiment, 5 mg/mL stocks are diluted to 1 mg/mL adding milliQ water and further sonicated 30 cycles (60 sec on, 5 sec off) at 4°C. 1 mg/ml vials have been added to cells growth medium to reach desired final concentrations.

Atomic Force Microscopy

A total of 30 µL of **CNC*** and **CNC*-Au-LA 4** 0.1 mg/mL H_2O dispersions were deposited on a mica surface, thereafter the solvent is evaporated with nitrogen flow. Samples are analysed, in non-contact mode with a Park XE7 atomic force microscope on which is mounted a PPP-NCHR tip (Nanosensors) with a nominal thickness of 4 nm, a nominal force constant of 42 N/m and a nominal resonance frequency of 330 kHz. Obtained images were analysed with the help of the Gwyddion 2.57 software program.

Dynamic Light Scattering and ζ-potential

DLS measurements at θ = 90°were performed on 0.1 mg/mL nanohybrid H_2O dispersions with a Brookhaven instrument (BI 9000AT correlator and BI200 SM goniometer) equipped with a Peltier temperature control system. A laser source emitting at a wavelength λ = 532 nm was used for the measurements. Scattered radiation is detected by a BI-APD detector. Obtained autocorrelation functions were analysed through Contin or double exponential algorithms, resulting in population distributions of the hydrodynamic diameter of the nanohybrids.

ζ-potential determination was performed using a Brookhaven Instrument 90 Plus (Brookhaven, Holtsville, NY). According to Helmholtz- Smoluchowski theory, the ζ-potential values were obtained from the electrophoretic mobility. Reported ζ-potential results are the average of 5 measurements each of 10 runs.

Small-Angle X-ray Scattering

CNC*-Au-LA 4 0.1 mg/mL dispersions were analysed at the SAXS beamline of synchrotron radiation Elettra (Trieste, Italy), which was operated at 2 GeV and a 300 mA ring current. The experiments were carried out with λ = 1.5 Å, and the SAXS signal was detected with a Pilatus 3 1M detector in the q-range from 0.009 to 0.7 $Å^{-1}$. The SAXS curves were recorded in a borosilicate glass capillary (1.5 mm diameter). The obtained profiles were analysed with the help of the IgorPro 6.10A software.

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