Supporting Information for:

Carbon Dots Conjugated to SN38 for Improved Colorectal Anticancer Therapy

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Table S1. pH of the HT29 culture medium (T = 37 °C) at different time intervals.

#	pH		
	24 h	48 h	72 h
1	7.42	7.31	7.39
2	7.48	7.34	7.4
3	7.50	7.37	7.39

Table S2. Primer list.

Human Gene	Forward Primers 5' → 3'	Reverse Primers 5' → 3'
CDK7	GGC CGG ACG TCG TCC TT AT	CAT TTT CAG TGC CTG TGT GG
KI-67	TAA CAC CAT CAG CAG GGA AAG	CTG CACT GGA GTT CCC ATA AA
COLIαI	GGA TAC TCT ATA TCG CGC CTT G	CCT CAT CAT CTC CCT TCC ATT C
ECM1	GGG AGG ATA CCC TTG ACA AAT AC	CAA AGC ACT CAT CCC GAG TAG
TGFβ1	GGG AAT GCA GGG AGC TTA AT	CAC AGG CTT TGG ACT CAG ATA G
CASP3	ACT GGA CTG TGG CAT TGAGA	GCACAAAGCGACTGGATGAA
TLR4	CCA GCC TCC TCA GAA ACA GA	TCC CTC CAG CAG TGA AGA AG
RPL4	CGA GCA CCA CGC AAG AAG ATC CA	AAT GGT GTT CCG GCG CAT GGT

Supporting Figures

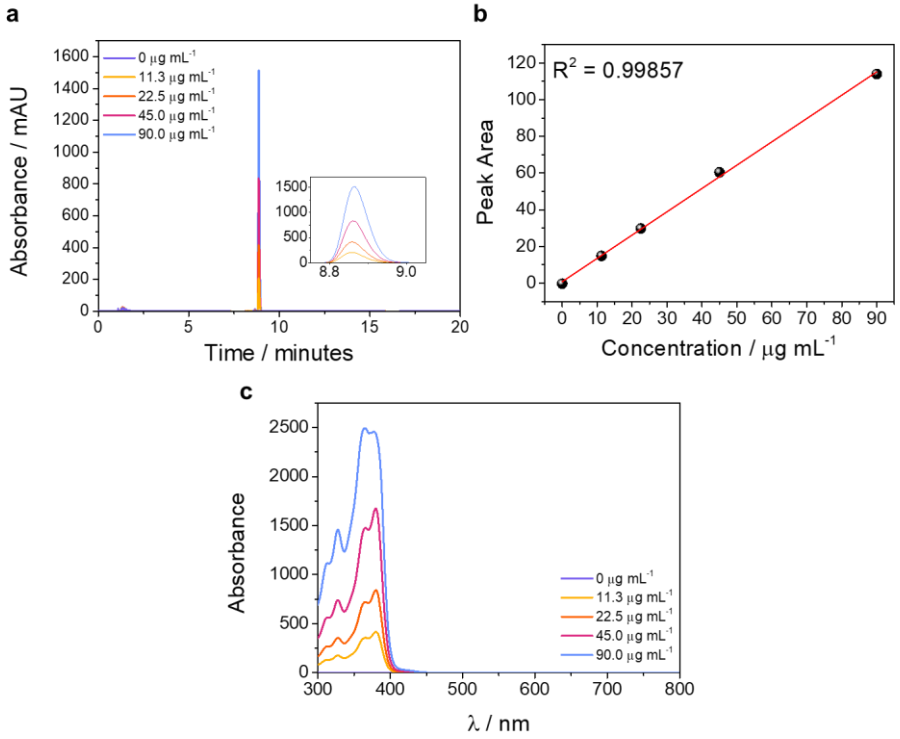


Figure S1. (a) Standard overlayed chromatograms of SN38 at different concentrations, inset shows the zoom of the interested peak; (b) standard calibration curve (red line) calculated from the area of the SN38 peak at different concentrations; (c) UV-Vis spectra corresponding to the SN38 chromatographic peak at different concentrations.

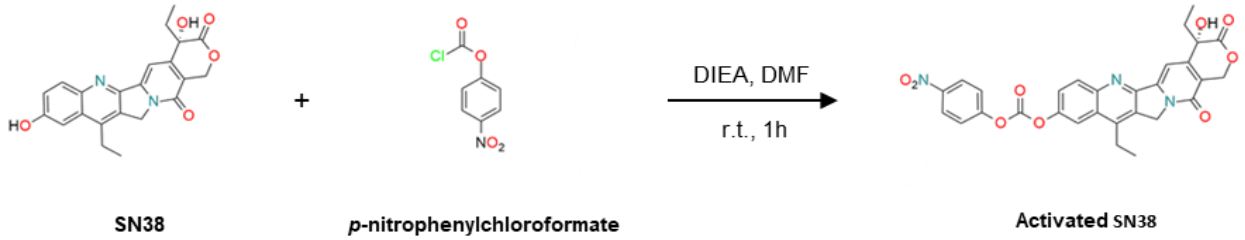


Figure S2. Synthetic scheme for SN38 activation [1].

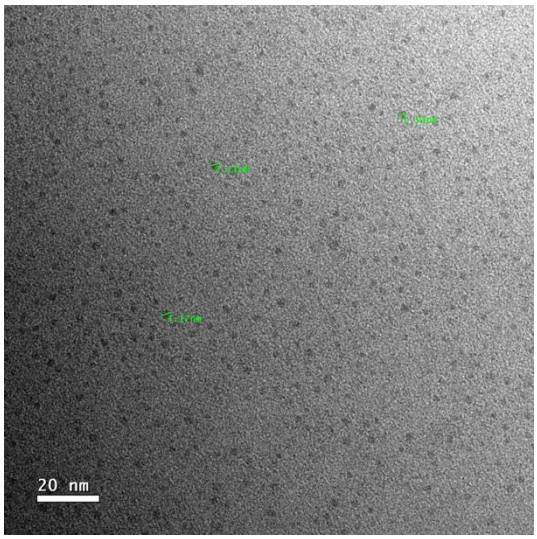


Figure S3. Representative TEM micrograph of CDs [2].

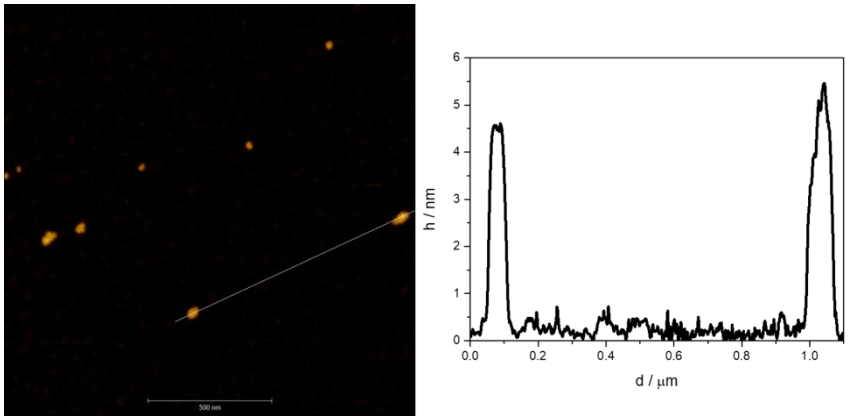


Figure S4. Representative tapping mode AFM images of CD-SN38 conjugate (1.7 x 1.7 µm) on a mica substrate (left) and height profile (right).

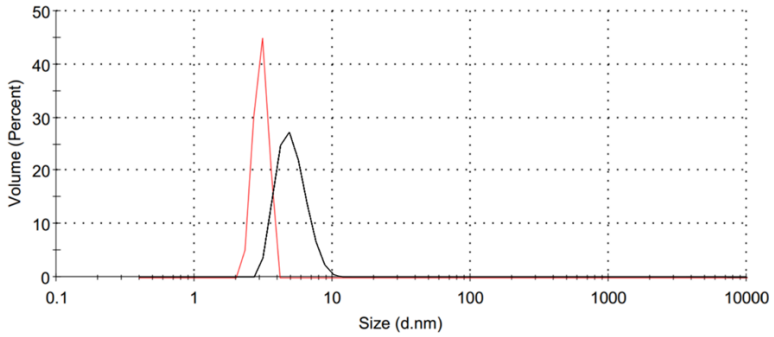


Figure S5. DLS reporting the size (nm) as hydrodynamic of CDs (red line) and of CD-SN38(black line).

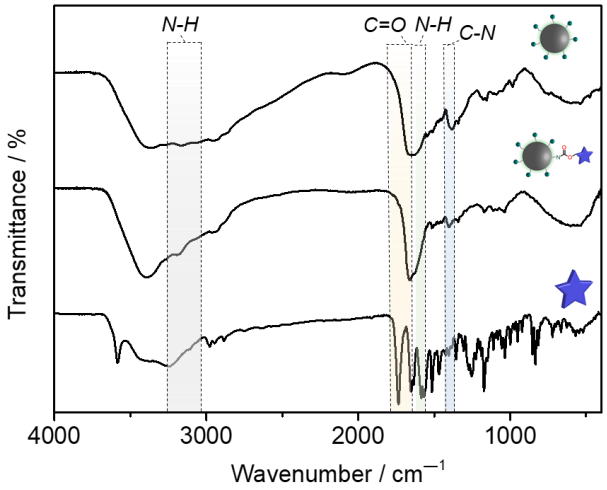


Figure S6. FT-IR (KBr) spectra of CDs (top) [2], CD-SN38 (middle), SN38 (bottom).

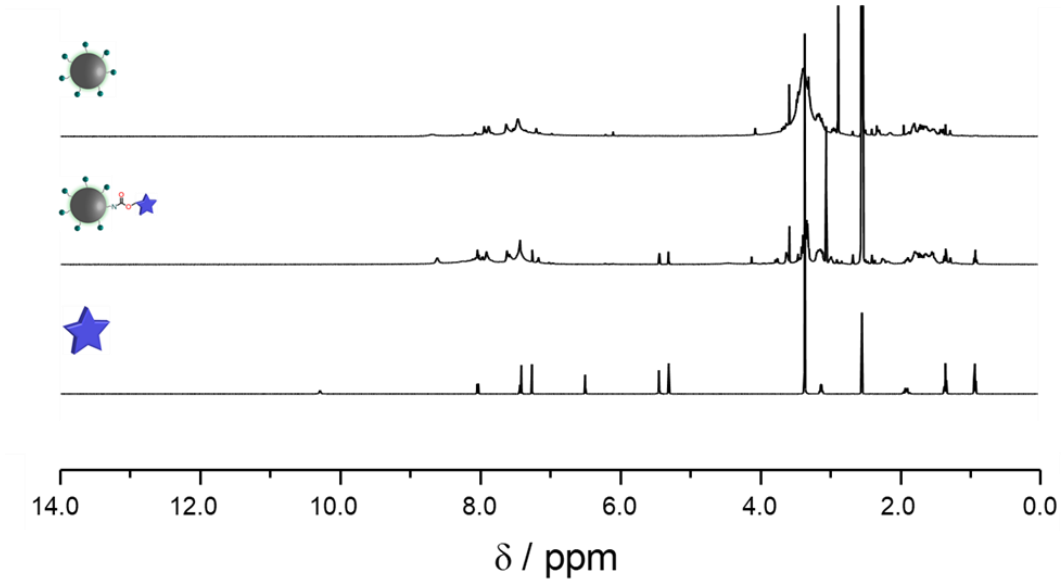


Figure S7. ^{1}H-NMR (DMSO-d_6, 298 K, 500 MHz) spectra of CDs, SN38 and CD-SN38.

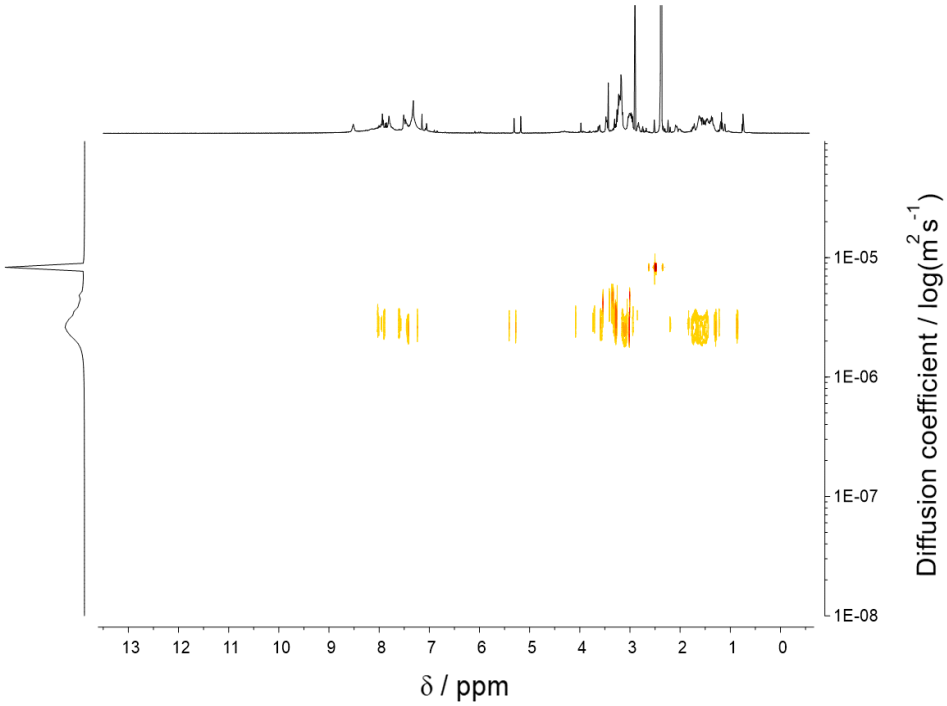


Figure S8. Bayesian DOSY transform spectra of CD-SN38 (DMSO-d_6, 298 K, 500 MHz).

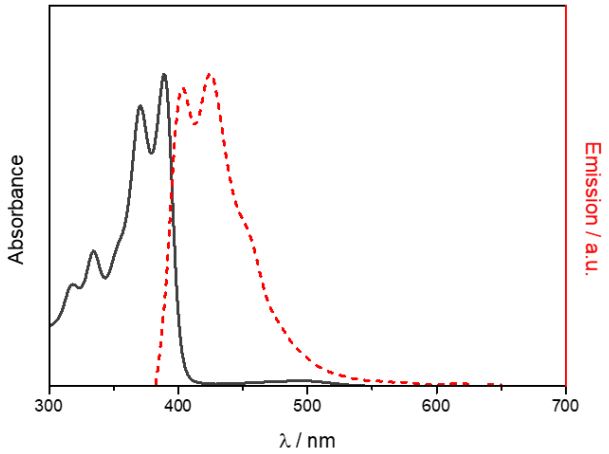


Figure S9. UV-Vis (black solid line), and FL (excitation at 334 nm, red dotted line) spectra of SN38 (DMF, 298 K).

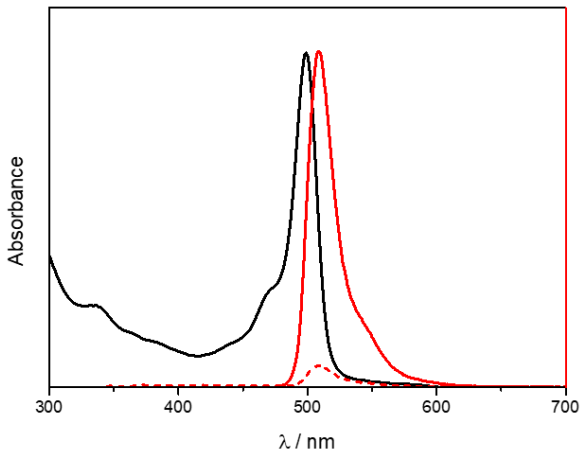


Figure S10. UV-Vis (black solid line), and FL (red dotted line for excitation at 334 nm, red solid line for excitation at 500 nm) spectra of CDs (DMF, 298 K).

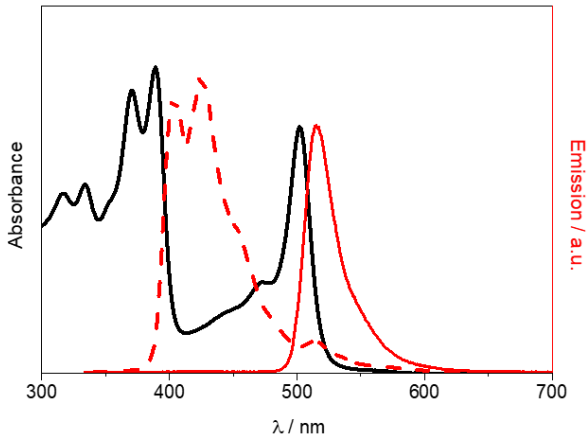


Figure S11. UV-Vis (black solid line), and FL (red dotted line for excitation at 334 nm, red solid line for excitation at 500 nm) spectra of CD-SN38 (DMF, 298 K).

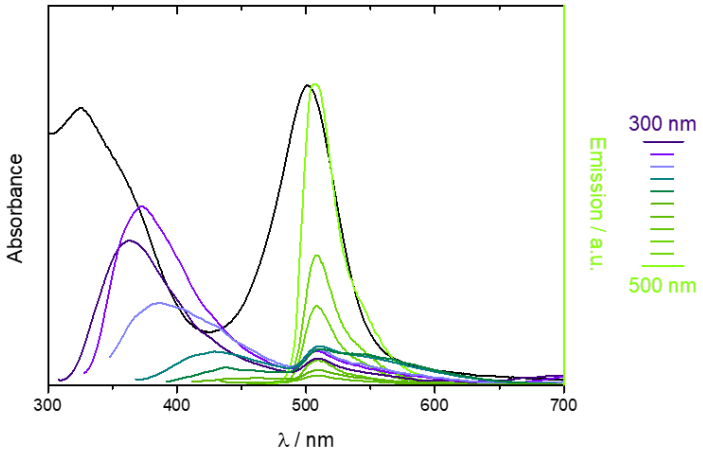


Figure S12. UV-Vis (black solid line), and FL (purple to green lines corresponding to excitation wavelengths ranging from 300 to 500 nm with increments of 20 nm) of CD-SN38 (water, 298 K). FLQYs are 5.4 ± 0.4% and 4.9 ± 1.2% at 300 and 466 nm excitation, respectively.

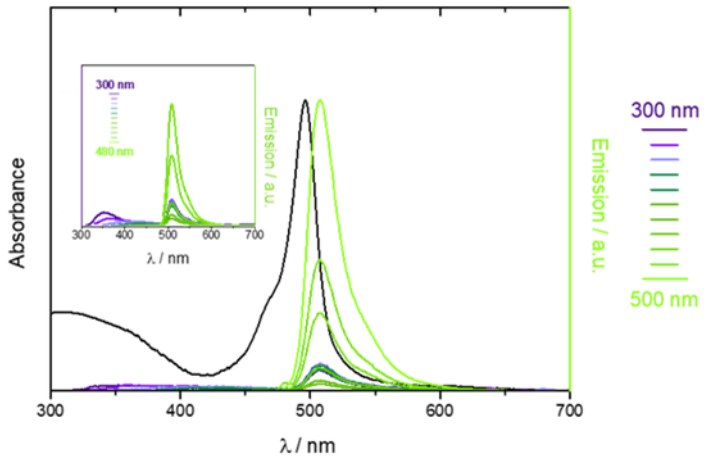


Figure S13. UV-Vis (black solid line), and FL (purple to green lines corresponding to excitation wavelengths ranging from 300 to 500 nm with increments of 20 nm) of CDs (water, 298 K). Inset graph shows the enlarged fluorescence emission scan in the range of 300 – 480 nm. FLQYs are 6.0 ± 0.4% and 26.7 ± 0.6% at 300 and 466 nm excitation, respectively [2].

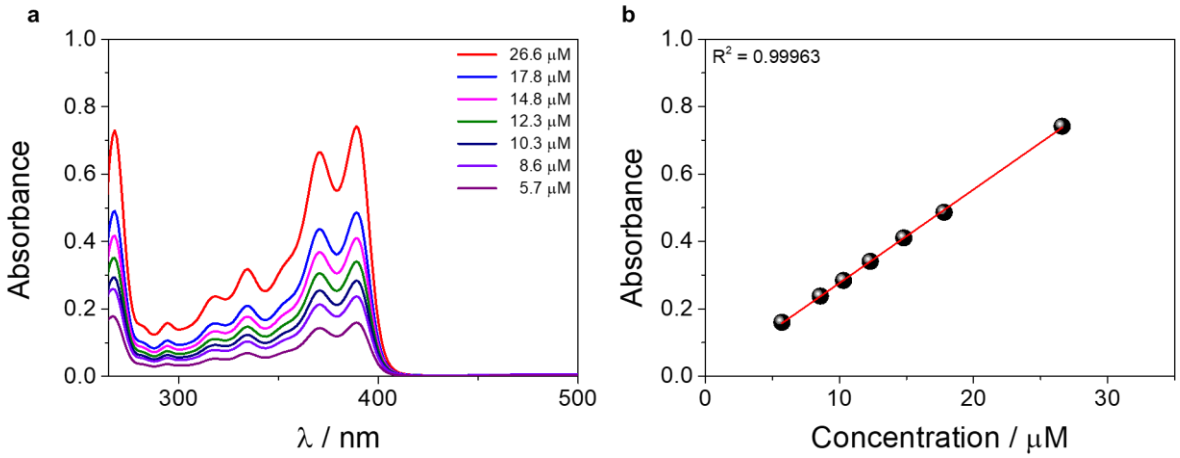


Figure S14. UV-Vis spectra (DMF, 298 K) of SN38 (a) at different concentrations, and (b) linear fit of absorbance values at 388 nm as a function of the molar concentration.

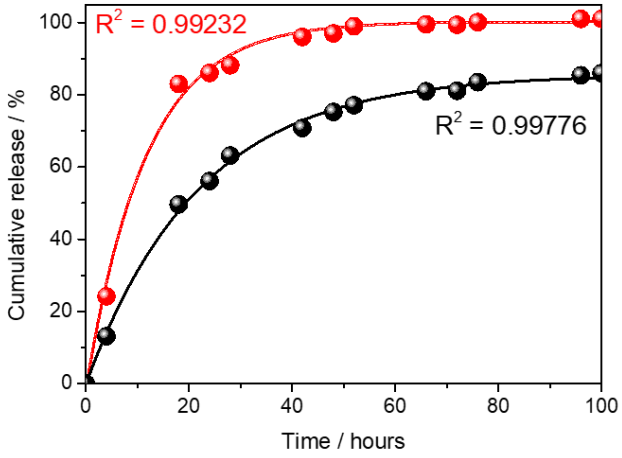


Figure S15. Quantitative analysis of the SN38 release from CD-SN38 at 37 °C and incubated at pH 7.4 (black trace) or pH 5.3 (red trace); the curves indicate the fit to experimental data using a first-order release model.

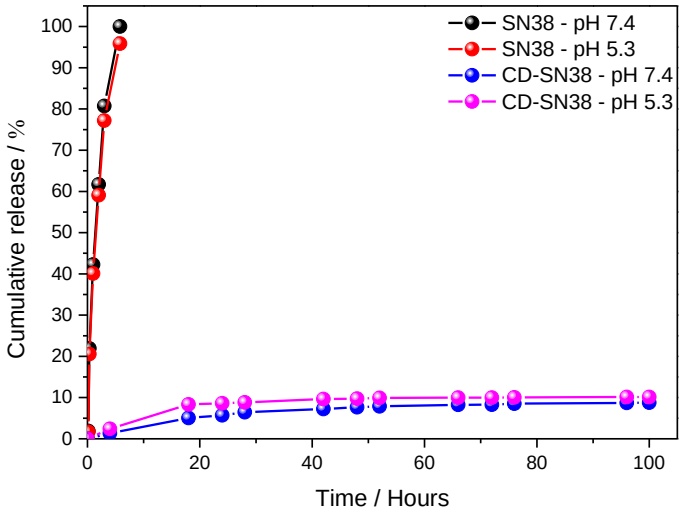
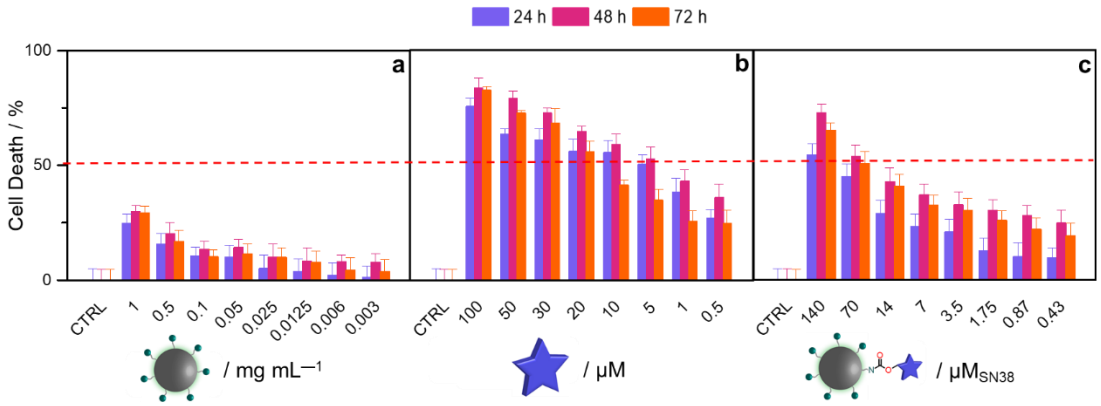


Figure S16. Quantitative analysis of the SN38 (starting concentration 1400 µg mL^{-1}) release as free drug and from CD-SN38 (starting concentration of the hybrid 570 µg mL^{-1}) at 37 °C and incubated at pH 7.4 or pH 5.3. The maximum concentration reached by free SN38 diffusion is ca. 15.40 µg mL^{-1}, while the maximum release from the hybrid corresponds to 1.35 and 1.56 µg mL^{-1} at pH 7.4 and 5.3, respectively. The cumulative release data of the CDSN38 is reported as relative percentage with respect to the maximum concentration reached by the free drug dissolution profile.



CDs mg/ml	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72 h
1	***	***	***
0,5	***	**	***
0,1	**	***	***
0,05	*	***	***
0,025	NS	***	***
0,0125	NS	***	***
0,006	NS	***	NS
0,003	NS	***	NS

SN38 µM	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72 h
100	***	***	***
50	***	***	***
30	***	***	***
20	***	***	***
10	***	***	***
5	***	***	***
1	***	***	***
0,5	***	***	***

CD-SN38 µM$_{SN38}$	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72 h
140	***	***	***
70	***	***	***
14	***	***	***
7	***	***	***
3,5	***	***	***
1,75	***	***	***
0,87	***	***	***
0,043	**	***	***

Figure S17. HT29 cells viability determined by MTT assay after 24, 48, and 72 h in the CTRL and after the treatment with different dosage of CDs (a), SN38 (b) and CD-SN38 (c). Red dotted line= LD50; n=6/group. **=p<0.05, **=p<0.01, ***=p<0.001.

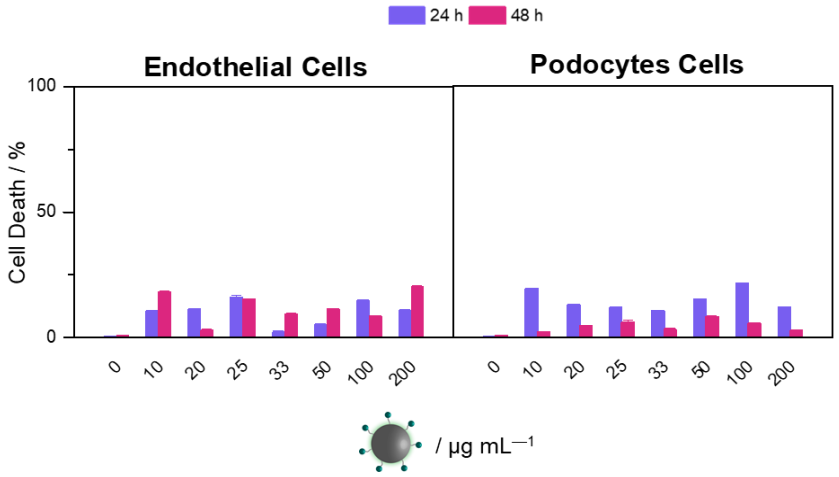
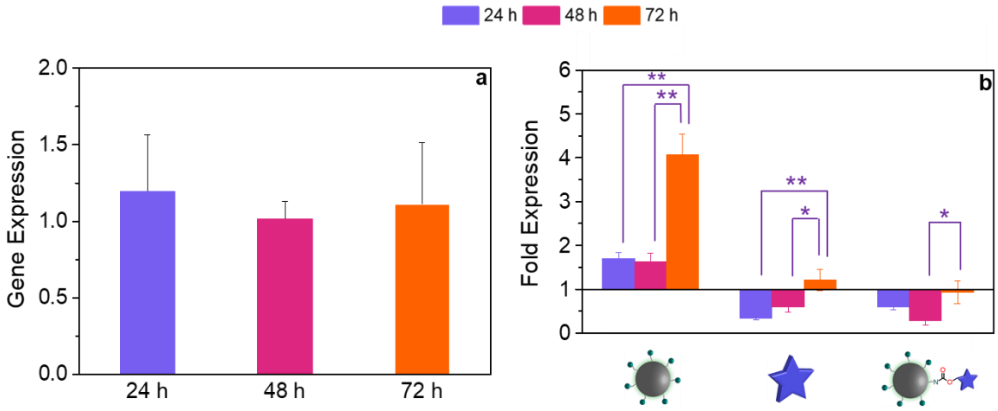
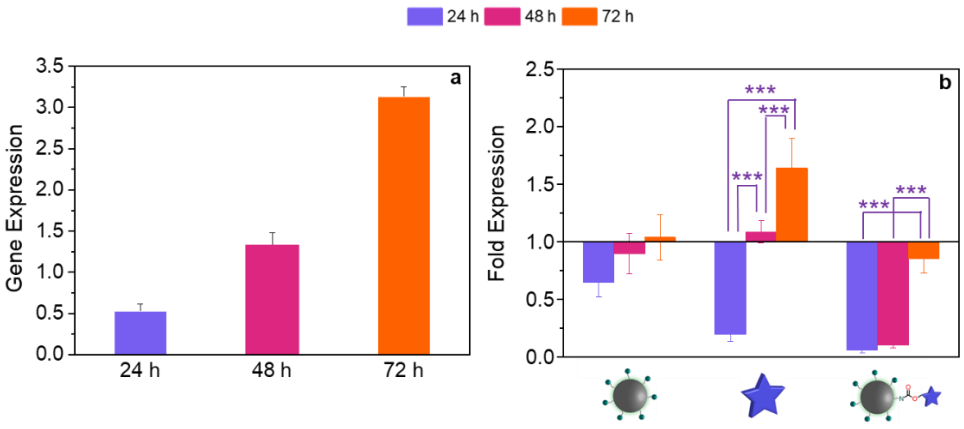


Figure S18. Endothelial and podocytes cells viability determined by MTT assay, after 24 and 48 h, at different dosages of CDs. Red dotted line= LD50 ± SD; n=6/group. **=p<0.05, **=p<0.01, ***=p<0.001.



Sample	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72 h
CDs	**	**	***
SN38	**	**	NS
CD-SN38	NS	***	NS

Figure S19. qRT-PCR analysis of CDK7 in HT29 cells CTRL (a) and after of 0.1 mg mL^{-1} of CDs, 1 µM of SN38 or 0.1 mg mL^{-1} of CD-SN38 at 24, 48 and 72 h (b). Black line = CTRL, n=3 /group. **=p<0.05, **=p<0.01, ***=p<0.001.



Sample	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72h
CDs	**	NS	NS
SN38	***	NS	***
CD-SN38	***	***	NS

Figure S20. qRT-PCR analysis of Ki-67 in HT29 cells CTRL (a) and after 0.1 mg mL^{-1} of CDs, 1 µM of SN38 or 0.1 mg mL^{-1} of CD-SN38 at 24, 48 and 72 h (b). Dotted line=CTRL, n=3 /group. **=p<0.05, **=p<0.01, ***=p<0.001

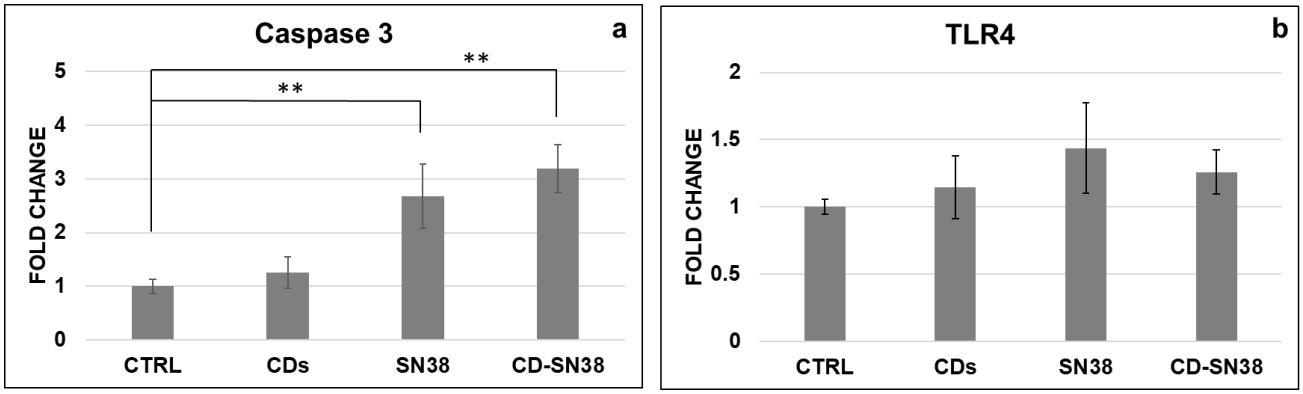
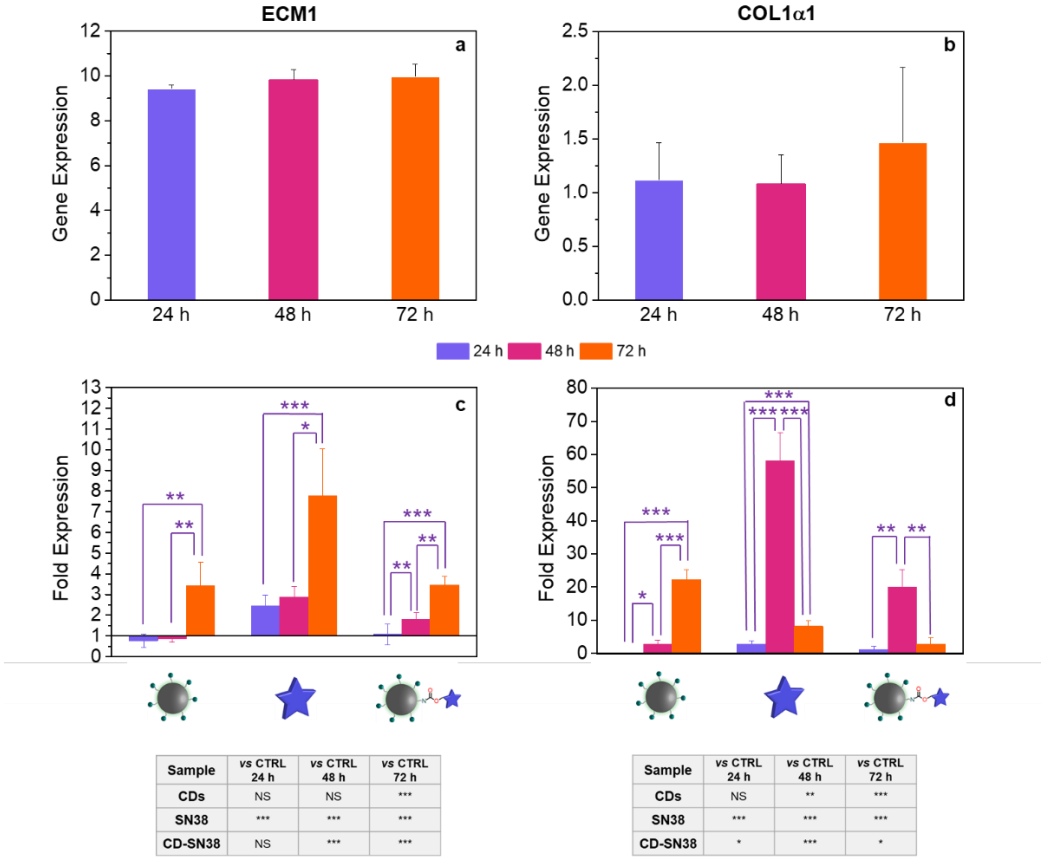


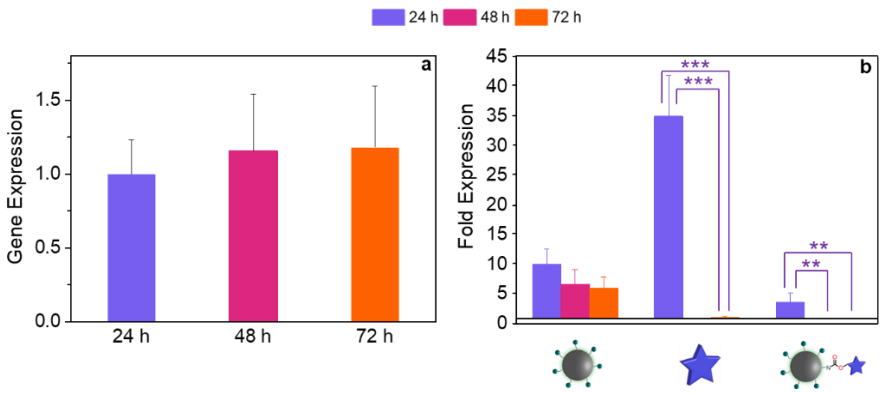
Figure S21. qRT-PCR analysis of a) Caspase 3 and b) Toll-like receptor 4 (TLR4) in HT29 cells and after CDs, SN38, and CD-SN38 at 48 h . n=3 /group. **=p<0.05, **=p<0.01, ***=p<0.001.



Sample	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72 h
CDs	NS	NS	***
SN38	***	***	***
CD-SN38	NS	***	***

Sample	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72 h
CDs	NS	**	***
SN38	***	***	***
CD-SN38	*	***	*

Figure S22. qRT-PCR analysis of ECM1 and COLI1α1 in HT29 cells CTRL (a, b) and after 0.1 mg mL^{-1} of CDs, 1 µM of SN38 and 0.1 mg mL^{-1} of CD-SN38 at 24, 48 and 72 h (c, d). Dotted line = CTRL, n=3 /group. **=p<0.05, **=p<0.01, ***=p<0.001



Sample	*vs* CTRL 24 h	*vs* CTRL 48 h	*vs* CTRL 72 h
CDs	***	***	***
SN38	***	NS	NS
CD-SN38	**	*	*

Figure S23. qRT-PCR analysis of TGFβ1 in HT29 cells CTRL (a) and after 0.1 mg mL^{-1} of CDs, 1 µM of SN38 and 0.1 mg mL^{-1} of CD-SN38 at 24, 48 and 72 h (b). Dotted line = CTRL, n=3 /group. **=p<0.05, **=p<0.01, ***=p<0.001.

Supplementary References

[1] S. V. Govindan, D.M. Goldenberg, Dosages of Immunoconjugates of Antibodies and SN-38 for Improved Efficacy and Decreased Toxicity. US20170014403A1, 2017

[2] F. Arcudi, L. Đorđević, S. Rebeccani, M. Cacioppo, A. Zanut, G. Valenti, F. Paolucci, M. Prato, Lighting up the Electrochemiluminescence of Carbon Dots through Pre- and Post-Synthetic Design, Adv. Sci. 8 (2021) 2100125. doi:10.1002/advs.202100125.