Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Tables

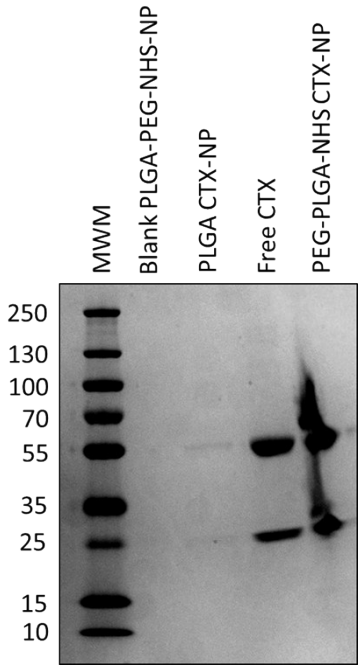
Supplementary Table 1. KRAS mutational status of selected EGFR-positive cell lines

Cell line	Cancer type	K-Ras mutational status
HCT116	colorectal carcinoma	G13D/WT
HKH-2	colorectal carcinoma	WT/-
A549	lung adenocarcinoma	G12S/G12S
HCC827	lung adenocarcinoma	WT/WT
PANC-1	pancreatic adenocarcinoma	G12D/WT
BxPC-3	pancreatic adenocarcinoma	WT/WT

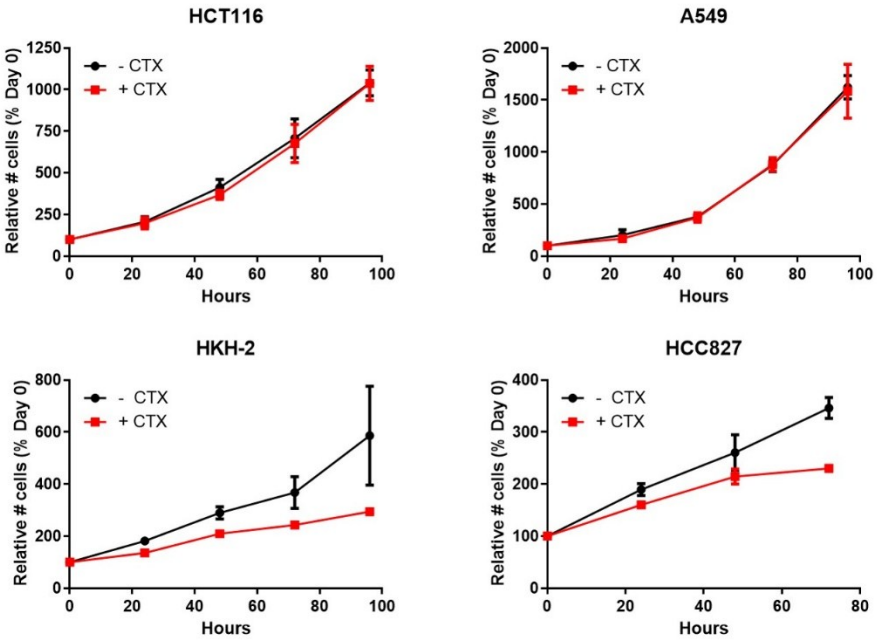
Supplementary Table 2. Characterisation of Rhodamine 6G encapsulated PLGA NPs before and after CTX conjugation. Mean ± s.e.m (n=3); measured in dH_2O.

NP formulation	Size (nm)	PDI	Zeta potential (mV)	CTX conjugation (µg/mg)
Rho-NP	219.9 ± 3.43	0.087 ± 0.02	-18.77 ± 0.86	NA
CTX-Rho-NP	223.1 ± 5.33	0.111 ± 0.02	-18.55 ± 0.63	6.66 ± 1.7

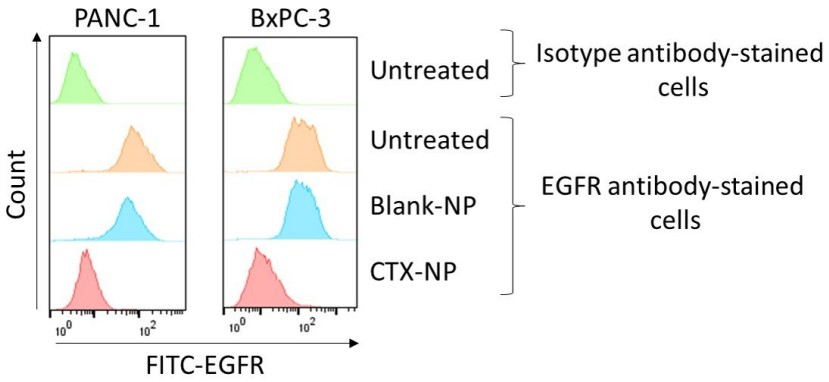
Supplementary Figures



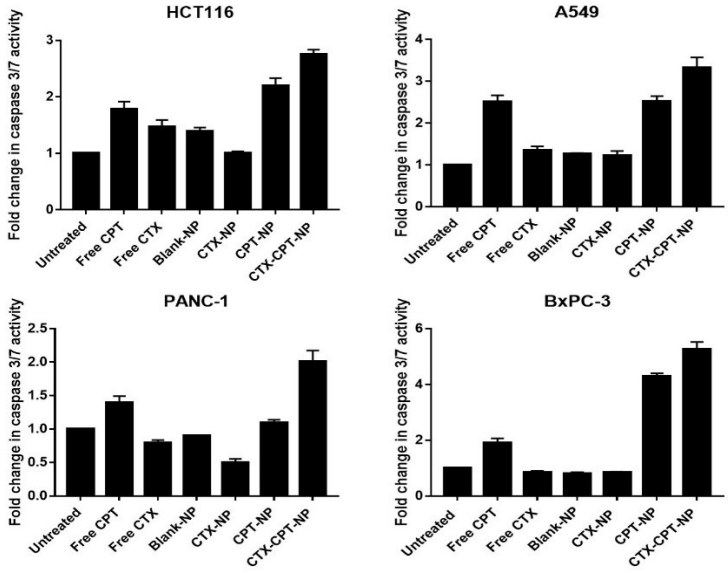
Supplementary figure 1. Covalent attachment of CTX to PEG-PLGA-NHS NPs. 1 mg of blank PLGA-PEG-NHS NPs, CTX-conjugated PLGA NPs, CTX-conjugated PEG-PLGA-NHS NPs and an equivalent amount of free CTX were run on an SDS-PAGE gel under reducing conditions and stained with Coomassie blue to detect CTX. CTX was reduced into heavy (55 kDa) and light chains (25 kDa). MWM: molecular weight marker (kDa).



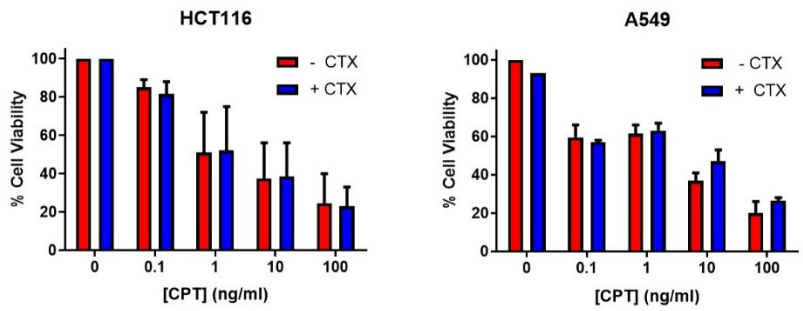
Supplementary figure 2. CTX sensitivity of cancer cell lines. Cells were treated with 20 µg/ml CTX, and every 24 hours, the percentage growth compared to day 0 was measured by MTT assay. Mean ± s.e.m. Data representative of three independent experiments.



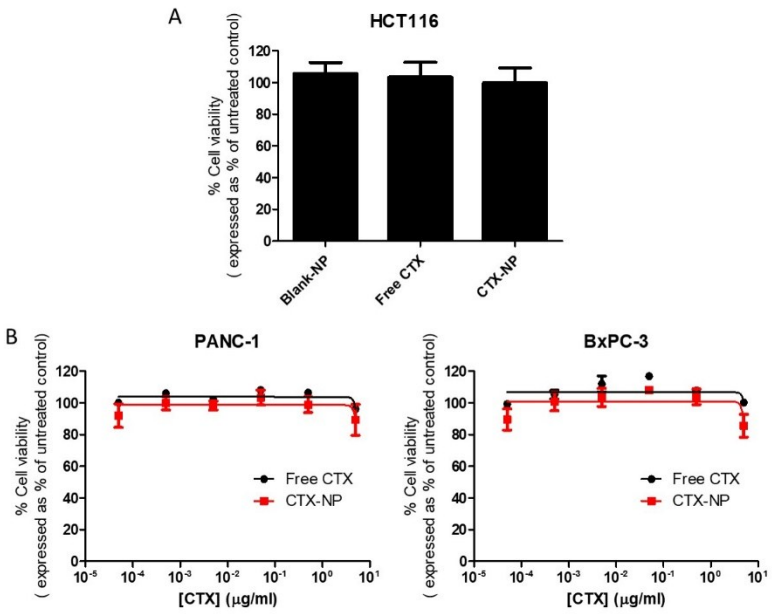
Supplementary figure 3. EGFR occupancy in the presence of CTX-NPs. Cells were treated with equal concentrations of CTX-conjugated to NPs and blank-NPs as a control for 20 minutes at 4 ˚C. After 20 minutes, the cells were stained with FITC-EGFR antibody or FITC-Isotype control antibody, and the amount of EGFR available for antibody binding after NP treatment was measured by FACS. Data representative of three independent experiments. Data analysed using the FlowJo 10 software.



Supplementary figure 4. Caspase-3/-7 response. Cells were treated with 40 ng/ml CPT within CTX-conjugated NPs, along with relevant controls. After 24 hours, caspase-3/-7 levels were measured by Caspase-Glo 3/7 assay. Mean ± s.e.m. Data representative of three independent repeats.



Supplementary figure 5. Effect of free CTX upon CPT growth inhibition effects. Cells were treated with increasing concentrations of CPT in the presence or absence of 10 µg/ml free CTX. After 48 hours, viability was measured by MTT assay. Mean ± s.e.m. Data representative of two independent experiments.



Supplementary figure 6. Nanoconjugated CTX does not reduce viability in KRAS mutant cell lines. (A) HCT116 cells were treated with NPs that had the same amount of CTX conjugated to the surface as the CTX-CPT-NPs in Figure 6A. After 72 hours, cell viability was measured by Cell Titer-Glo assay. Mean ± s.e.m (n=3). (B) PANC-1 and BxPC-3 cells were treated with increasing concentrations of CTX, either as a free antibody or conjugated to NPs, and after 72 hours, cell viability was measured by Cell Titer-Glo assay. Mean ± s.e.m. Data representative of three independent experiments.