**Supporting information of**

# A Five-Part Pentameric NanoComplex Shows Improved Efficacy of Doxorubicin in CD44+ Cancer Cells

*Authors: Nathan Beals[1], Praveena S. Thiagarajan[2], Eric Soehnlen[1], Arijit Das[1], Ofer Reizes[2,3,4], Justin D. Lathia[2,3,4] and Soumitra Basu[1*]*

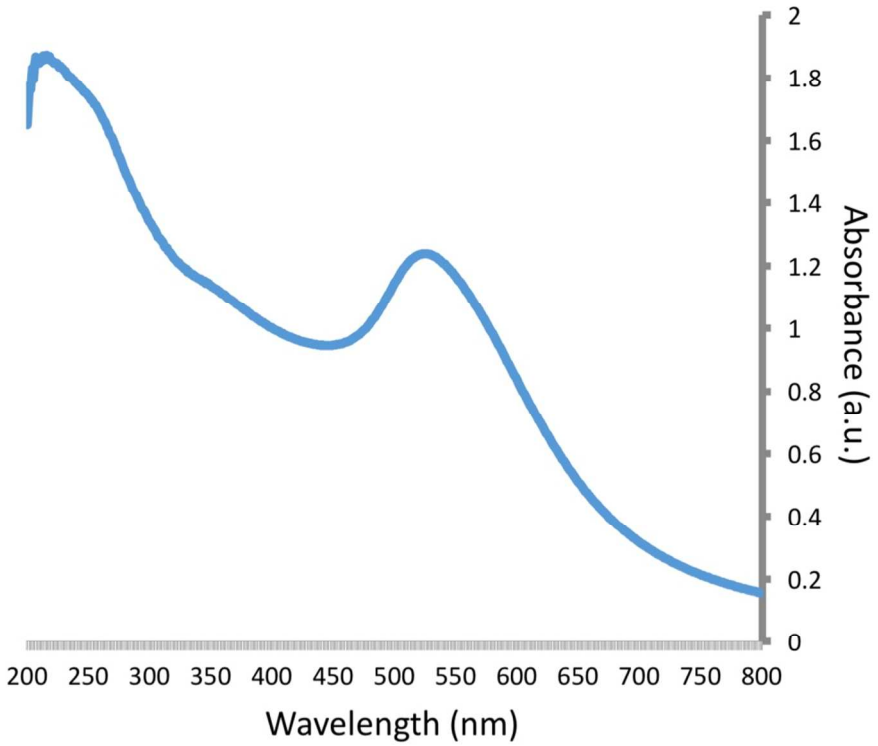



**Figure S1.** UV-Vis spectrophotometry was used to verify AuNP formation and concentration (Molar extinction coefficient $1.5x10^{7}$) [1].

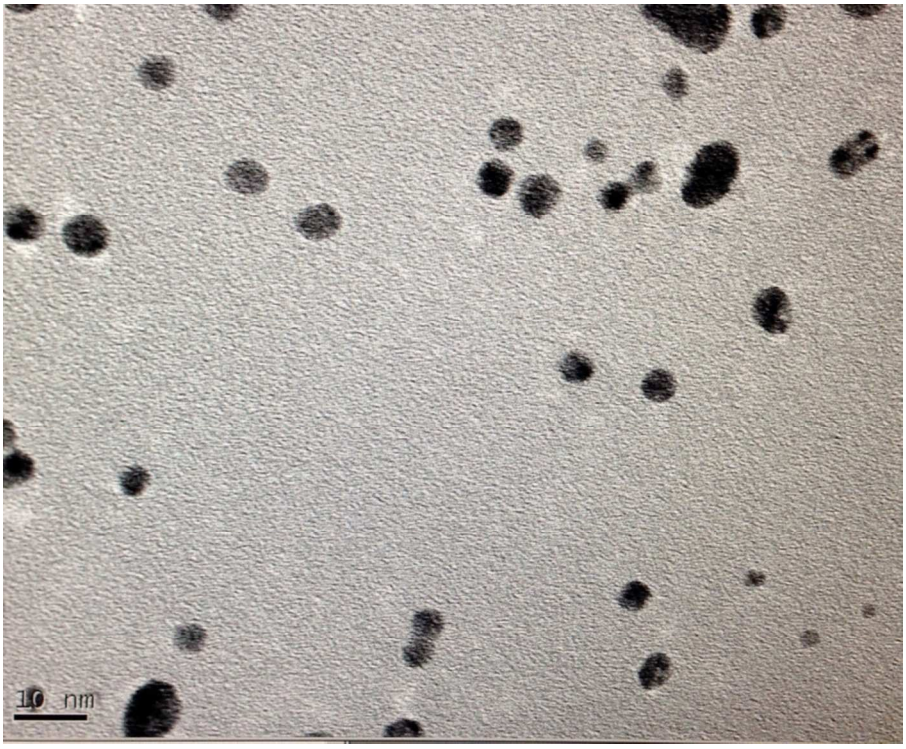



**Figure S2.** TEM images were used to determine AuNP size and morphology.

| AuNP # | AuNP Size (cm) | AuNP Size (nm) | |
|---|---|---|---|
| 1 | 0.4 | 2.5 | |
| 2 | 1.2 | 7.5 | |
| 3 | 0.7 | 4.375 | |
| 4 | 0.8 | 5 | |
| 5 | 0.6 | 3.75 | |
| 6 | 0.6 | 3.75 | |
| 7 | 0.6 | 3.75 | |
| 8 | 0.7 | 4.375 | |
| 9 | 0.4 | 2.5 | |
| 10 | 0.4 | 2.5 | |
| 11 | 0.3 | 1.875 | |
| 12 | 0.7 | 4.375 | |
| 13 | 0.6 | 3.75 | |
| 14 | 0.7 | 4.375 | |
| 15 | 0.7 | 4.375 | |
| 16 | 0.8 | 5 | |
| 17 | 0.7 | 4.375 | |
| 18 | 0.8 | 5 | |
| 19 | 0.7 | 4.375 | |
| 20 | 0.65 | 4.0625 | |
| 21 | 0.65 | 4.0625 | |
| 22 | 0.7 | 4.375 | |

| | | | |
|---|---|---|---|
| 23 | 0.8 | 5 | |
| 24 | 0.65 | 4.0625 | |
| 25 | 0.5 | 3.125 | |
| 26 | 0.6 | 3.75 | |
| 27 | 0.7 | 4.375 | |
| 28 | 1 | 6.25 | |
| 29 | 0.55 | 3.4375 | |
| 30 | 0.7 | 4.375 | |
| 31 | 0.7 | 4.375 | |
| 32 | 0.8 | 5 | |
| Total Size | 21.4 | 133.75 | |
| Average Size | 0.669 | 4.180 | 1.053 |
| | | | Standard Deviation |

**Table S1.** AuNP from TEM images were measured and averaged to find the nanoparticle size and range of sizes. The table corresponds with the TEM image seen in Supplementary Figure 2. (10 nm scale bar is 1.6 cm)

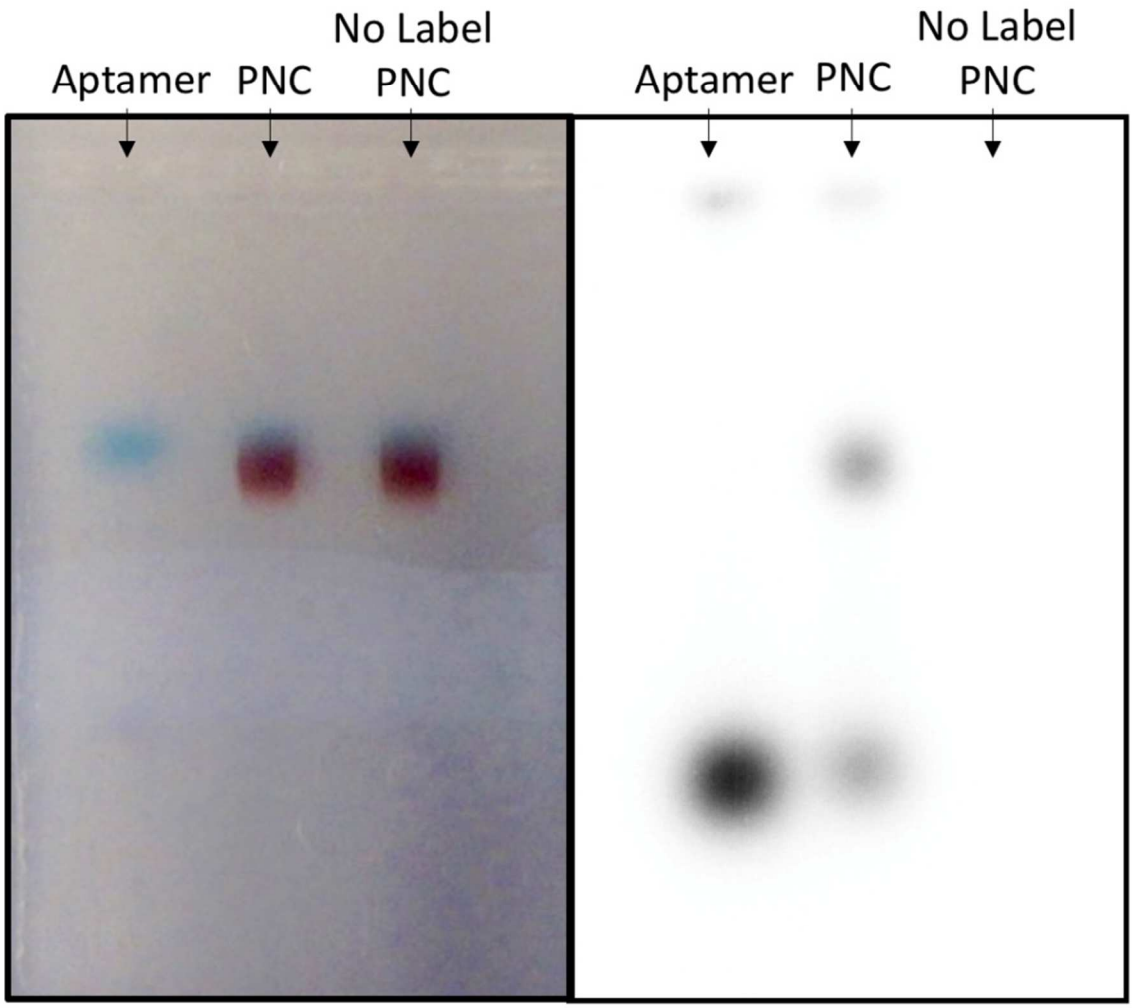



**Figure S3.** Yield of PEGylated CD44 aptamer to AuNP is less when added after HASH-Dox polymer (Left) Bright field image of radiolabeled PEGylated CD44 aptamer, the PNC complex with radiolabeled PEGylated CD44 aptamer, and a nonlableled PEGylated CD44 PNC complex ran on an 1% agarose gel. (Right) Phosphoimaged of the gel on the right panel, showing about 50% conjugation when compared to 90% in **Figure 2** when the PEGylated CD44 aptamer was added first.


**Figure S4.** A no aptamer PNC and the PNC were evaluated using a 1% agarose gel to view migration with and without the incorporation of the PEGylated CD44 aptamer.

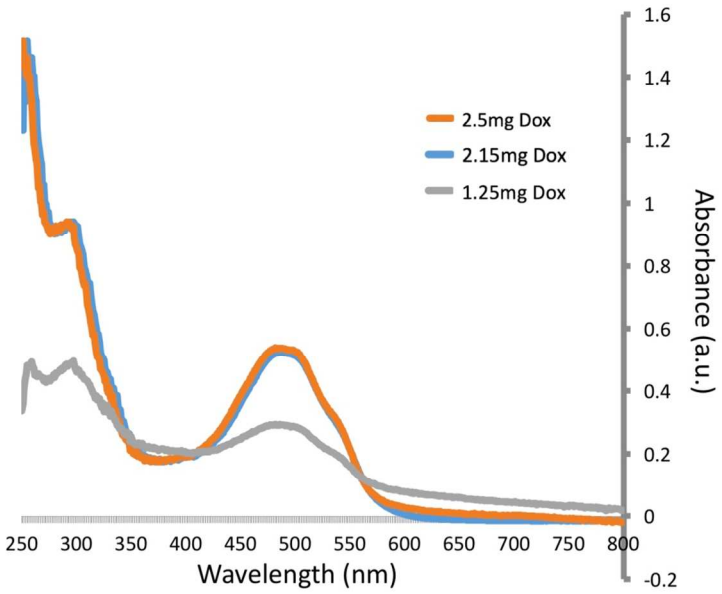



**Figure S5.** UV-Vis spectrophotometry was used to view HASH-Dox conjugation and find Dox concentration of the polymer (Molar extinction coefficient $1.15x10^4$)[1]. Increasing concentrations of Dox were used in the conjugation of HASH-DOX and showed that loading efficiency started to decrease after 2.15 mg of Dox used per 15mg of HASH-ADH.

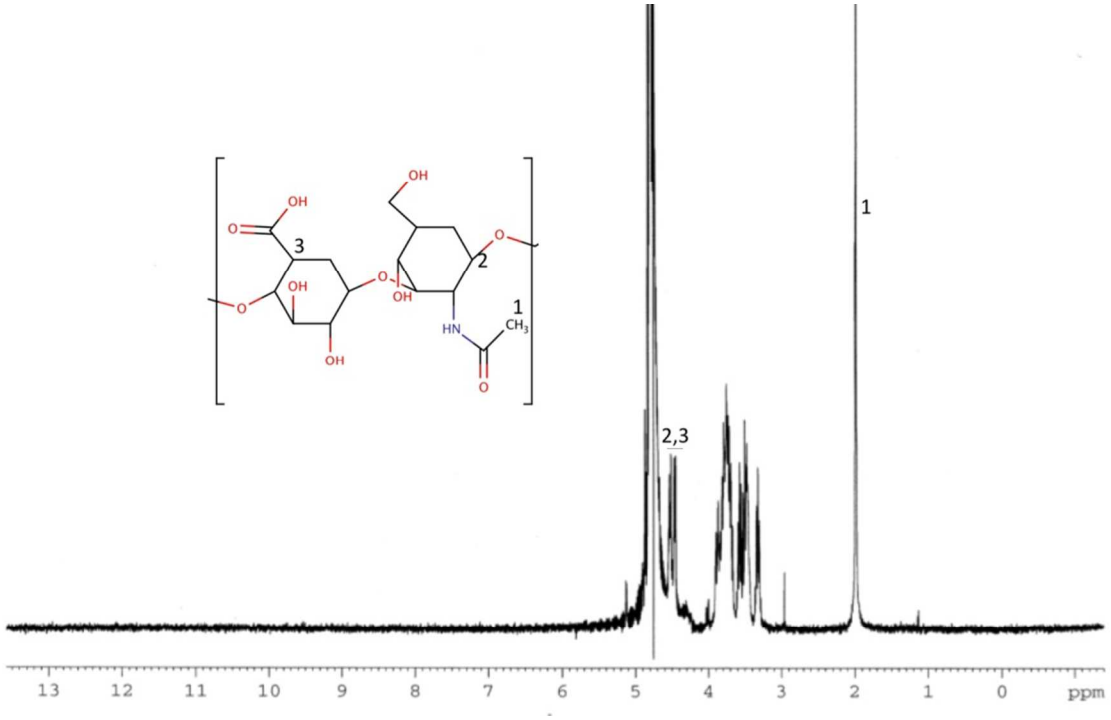



**Figure S6.** $H^1$ proton NMR spectrum of HASH (15 mg) in $D_20$ was acquired using a Bruker 400 NMR.

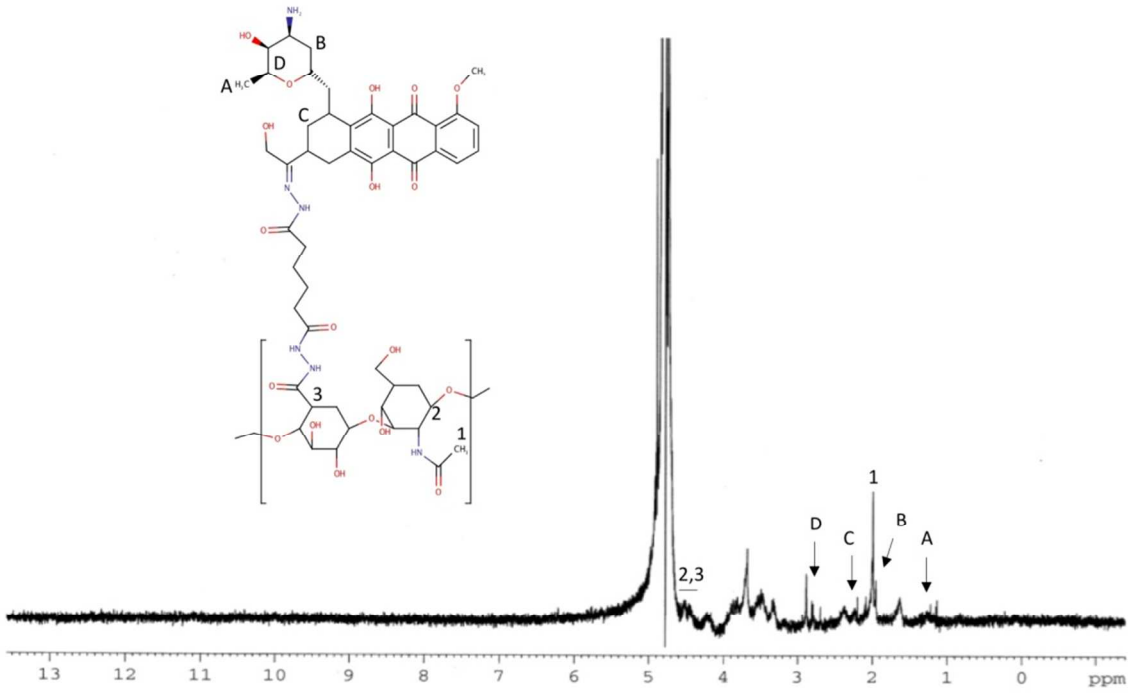



**Figure S7.** $H^1$ proton NMR spectrum of HASH-Dox (10 mg) in $D_20$ was acquired using a Bruker 400 NMR.

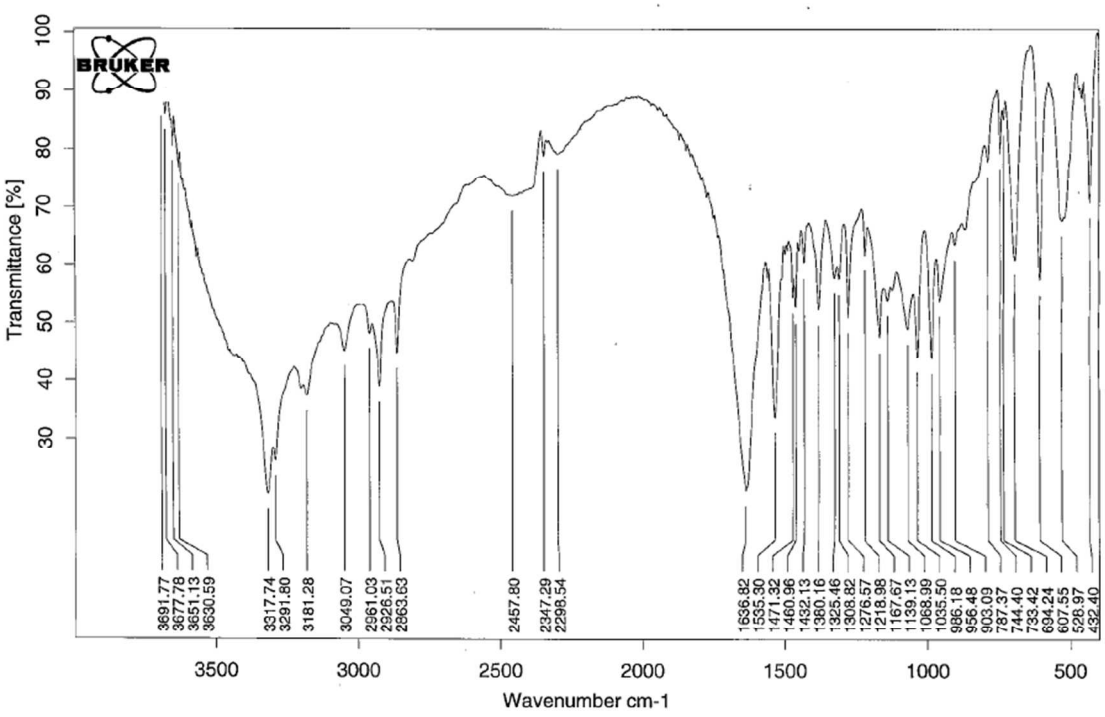



**Figure S8.** IR spectrum of HASH (15 mg) using KBr windows was acquired using a Bruker FT-IR.

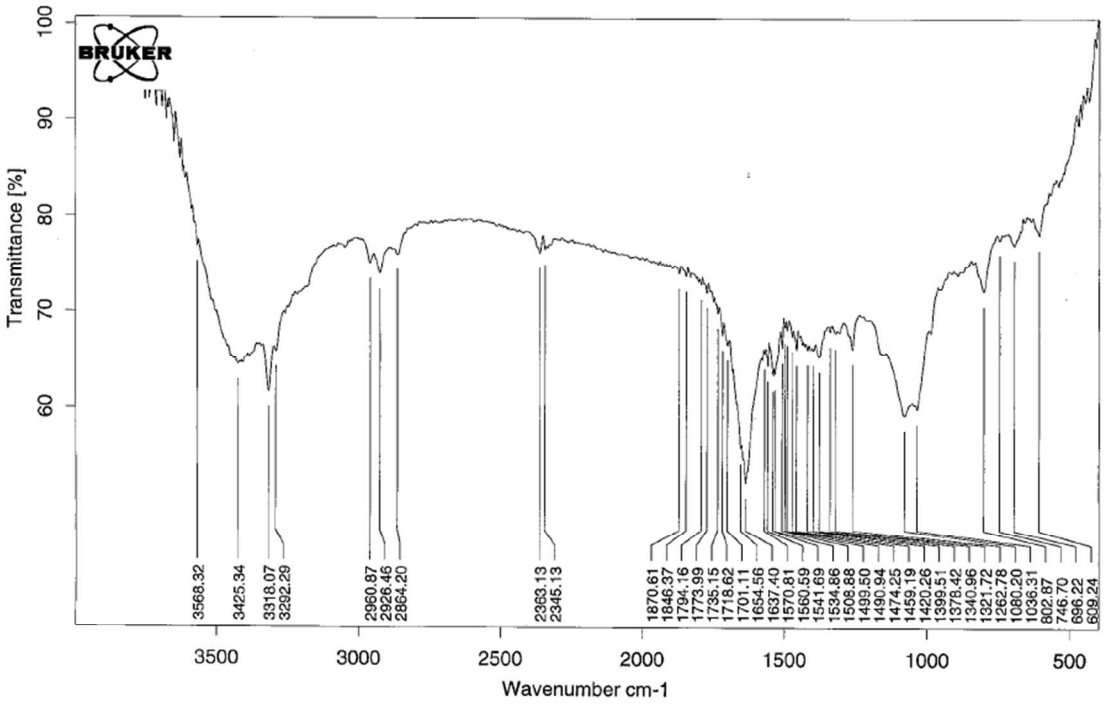



**Figure S9.** IR spectrum of HASH-Dox (10 mg) using KBr windows was acquired using a Bruker FT-IR. Past reports have shown the increase in peaks between 1600-1800, specifically the 1654 peak is representative of the bond presence of the imine[2, 3]. Imine bond formation occurs during the Schiff base conjugation of the HASH and Dox.

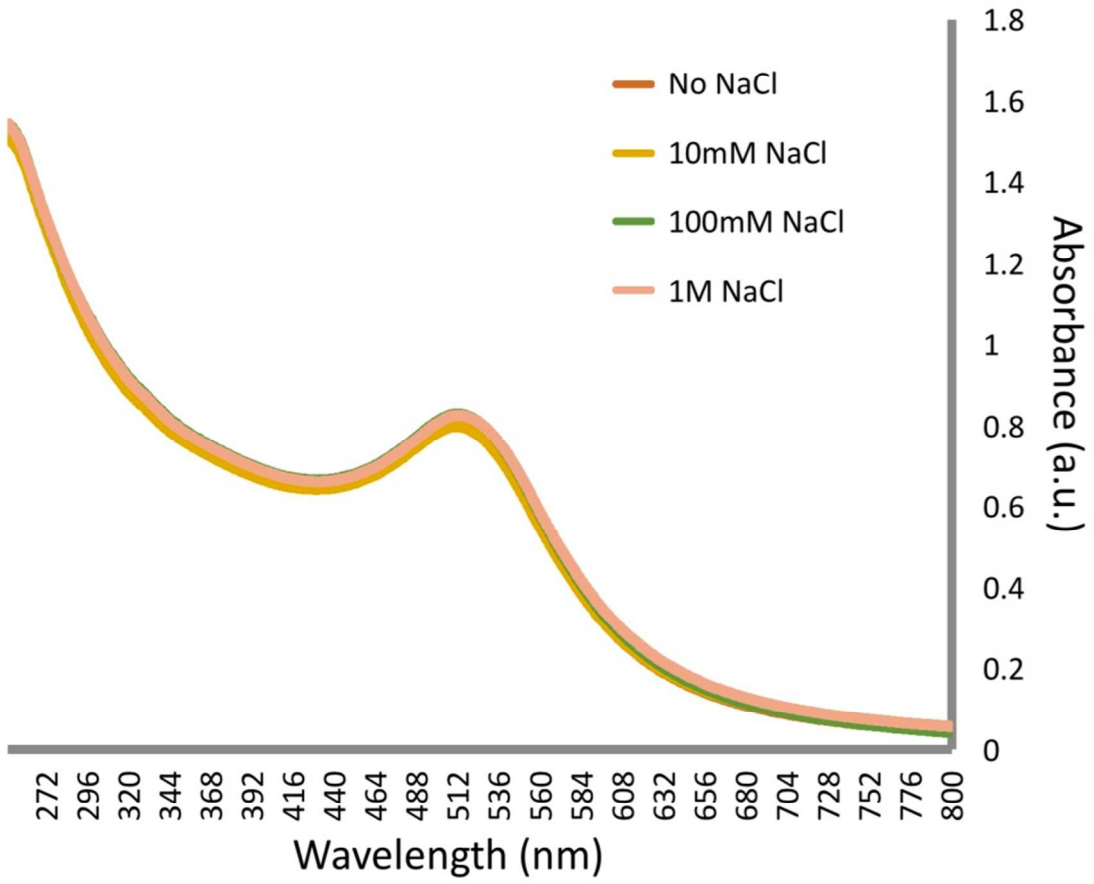



**Figure S10.** UV-Vis spectra of the PNC in increasing concentrations of NaCl to test nanoparticle stability. Samples were incubated for 12 hrs at different salt concentrations. AuNP aggregation would have resulted in broadening of the peak and a red shift from the 515 nm initial peak. Virtually no change in the spectra established that the PNC is stable even at 1M NaCl.

| Nanoparticle | Zeta Potential | S.D. |
|---|---|---|
| AuNP-PEG | -71.2 | 6.3 |
| AuNP-HASHDox | -18.5 | 2.6 |
| PNC | -29.0 | 3.1 |
| AuNP-HASH | -44.1 | 1.8 |

**Table S2.** Zeta potential measurements in water were performed of the PNC and AuNP with varying types of HASH derivatives. Three portions of each sample were individually measure in triplicates using a Nanopartica Nanoparticle Analyzer SZ-100.

| Complex | PDI |
|---|---|
| AuNP | 0.276 |
| AuNP-HASHDox | 0.004 |
| AuNP-PEG | 0.304 |
| PNC | 0.175 |
| AuNP-HASH | 0.086 |

**Table S3.** PDI of different nanoparticle conjugates based on DLS results. Polydispersity increased in the PNC is thought to be a factor of different number of aptamers per nanoparticle.

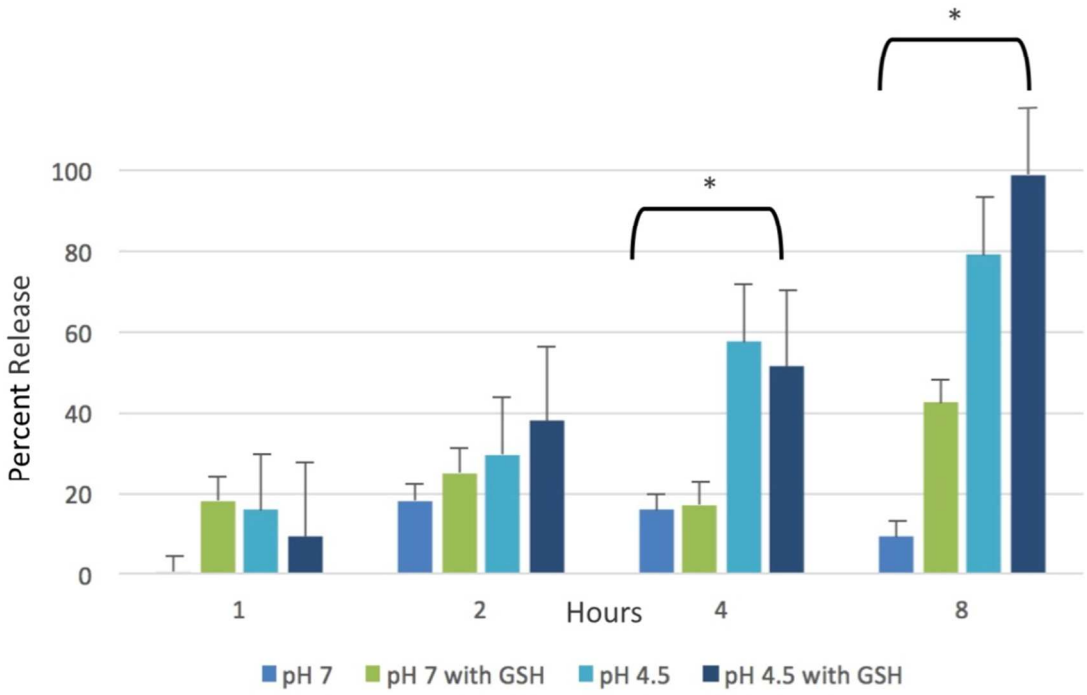
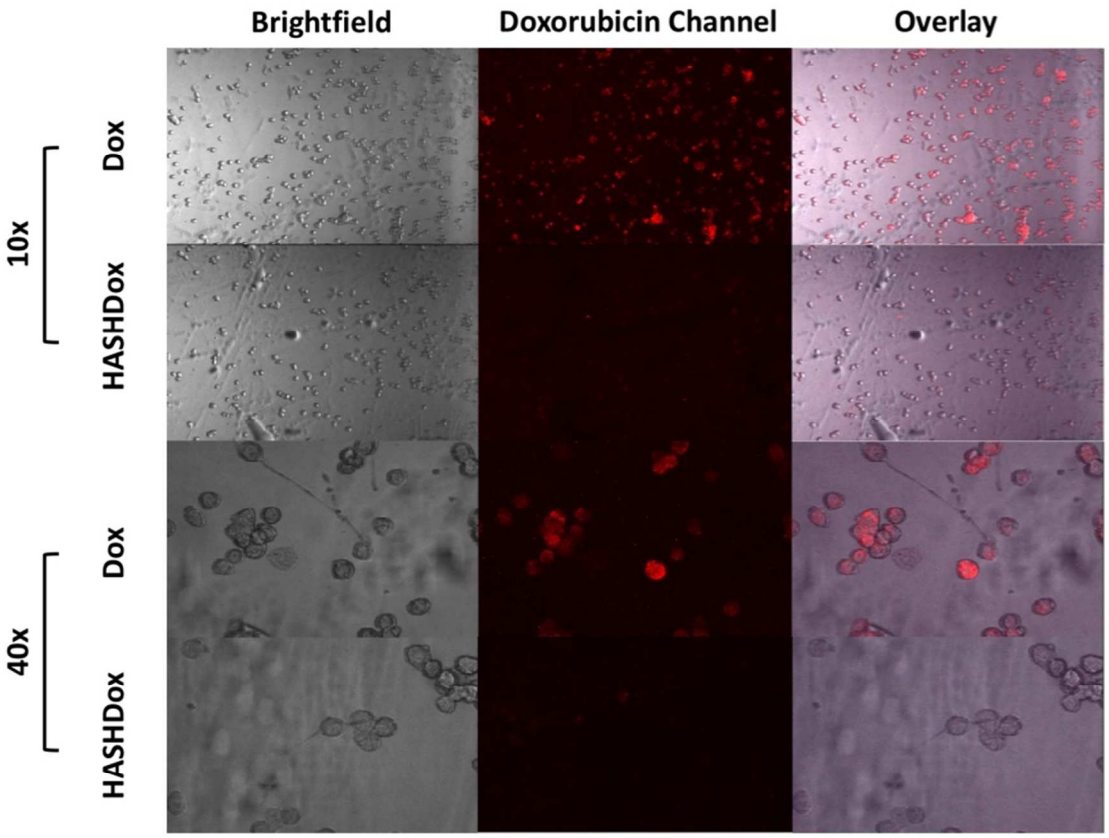



**Figure S11.** UV-Vis Spectroscopy was used to measure percent of free Dox released from PNC under physiological and lysosomal pH conditions with and without 10 mM GSH. Release at pH 7 slightly decreases over time, thought to be because of a plateauing of release and error within the extraction process as different samples were used at each time point. The changes in release is not statistically significant. *indicates statistical significance $p < 0.05$


Brightfield
Doxorubicin Channel
Overlay
10x
40x
Dox
HASHDox
Dox
HASHDox

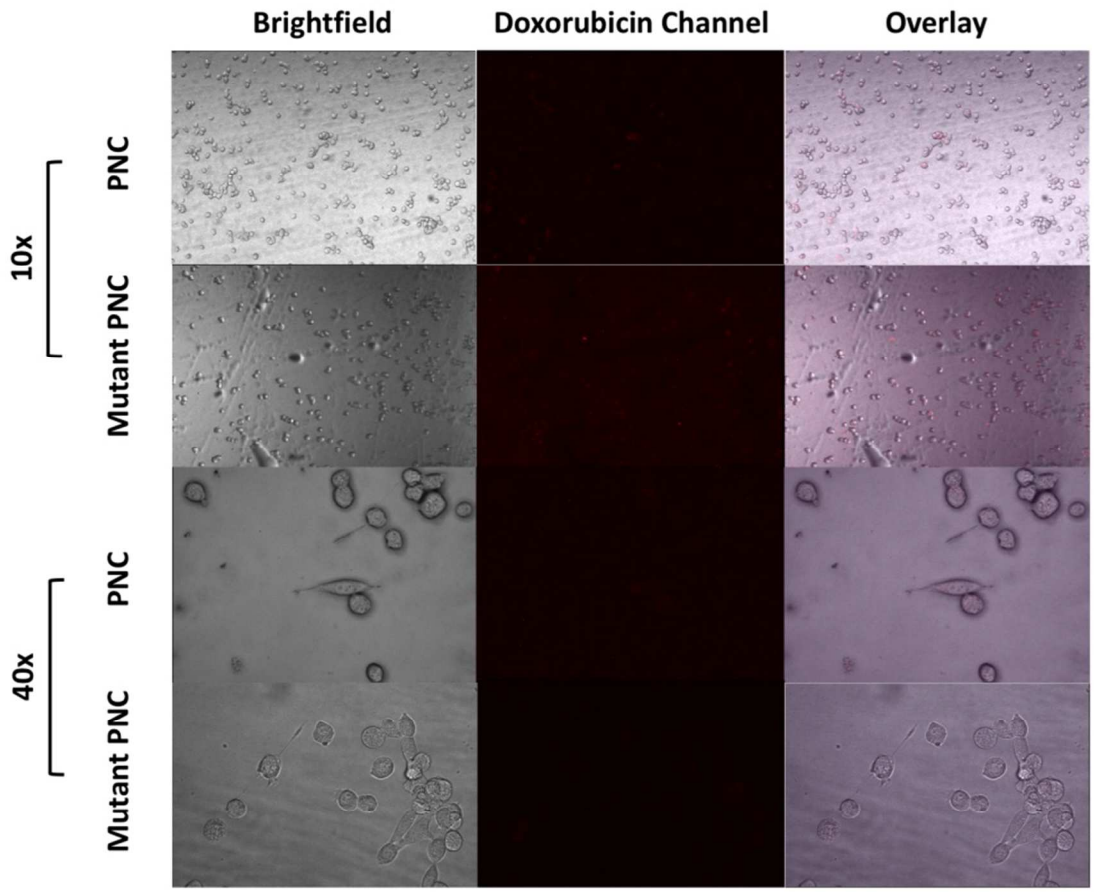



**Figure S12.** Confocal images of NIH3T3 cells after 12 hrs of treatment with (Up) Doxorubicin and HASH-Dox (Bottom) PNC and mutant PNC at 1 μM.

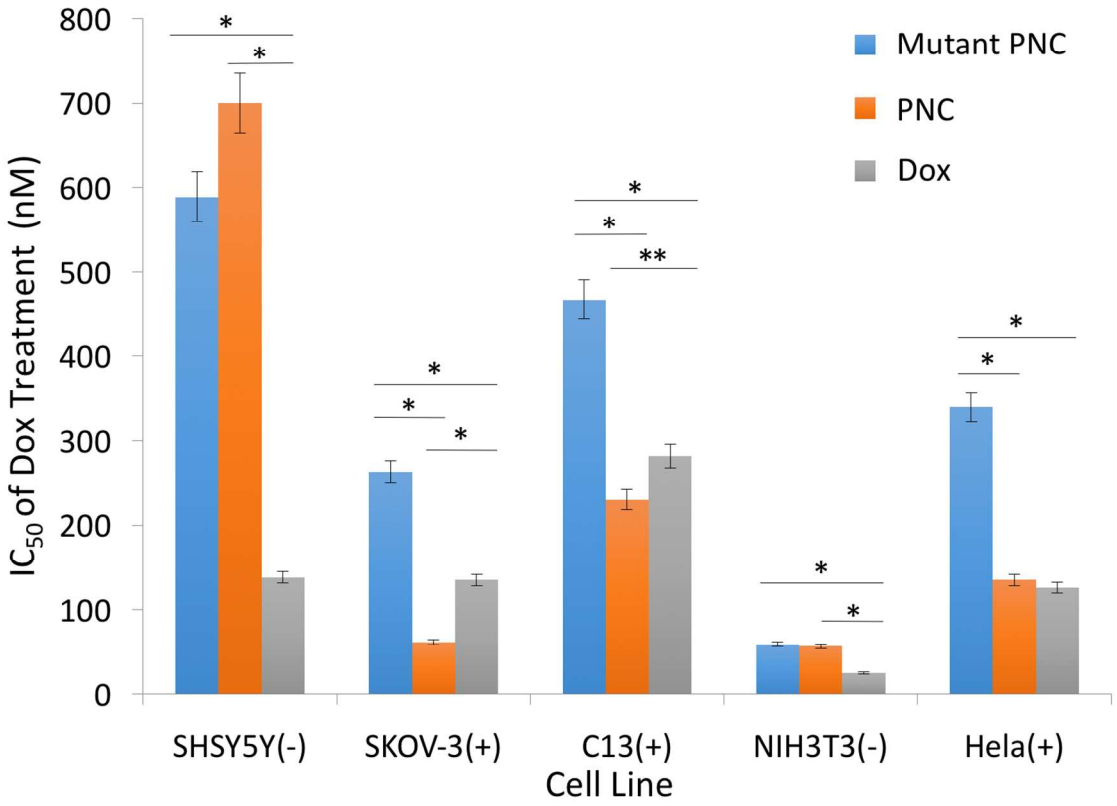



**Figure S13.** Histograms to show $IC_{50}$ values for the different cell lines. Cell lines with CD44 overexpression are indicated with a (+) and low expression of CD44 are (-). * = 0.001, ** = 0.005 *indicates statistical significance $p < 0.001$, ** indicates statistical significance $p < 0.005$

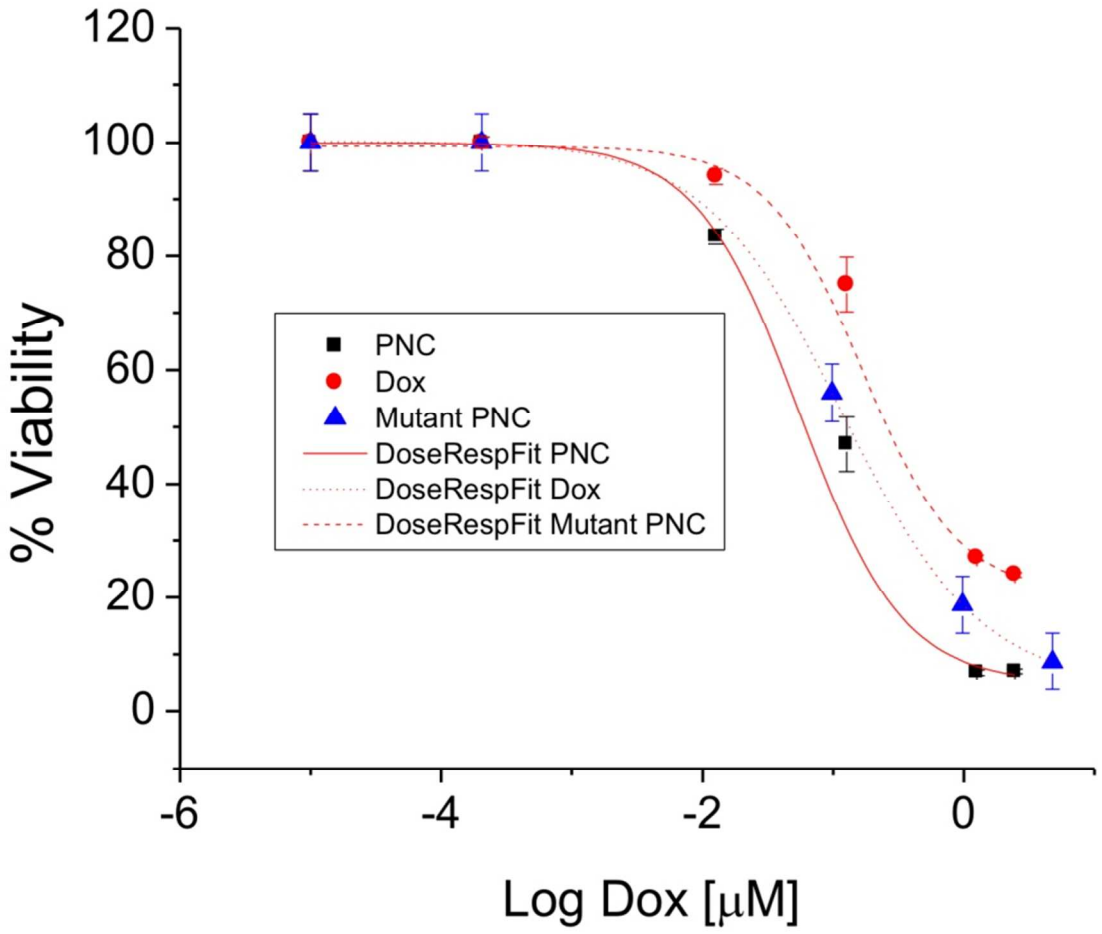



**Figure S14.** Cellular viability study of SKOV-3 cells treated with PNC, Mutant PNC and Dox. Experiments were performed in triplicates and $IC_{50}$ values were calculated using a MTS assay 72 hrs post treatment.

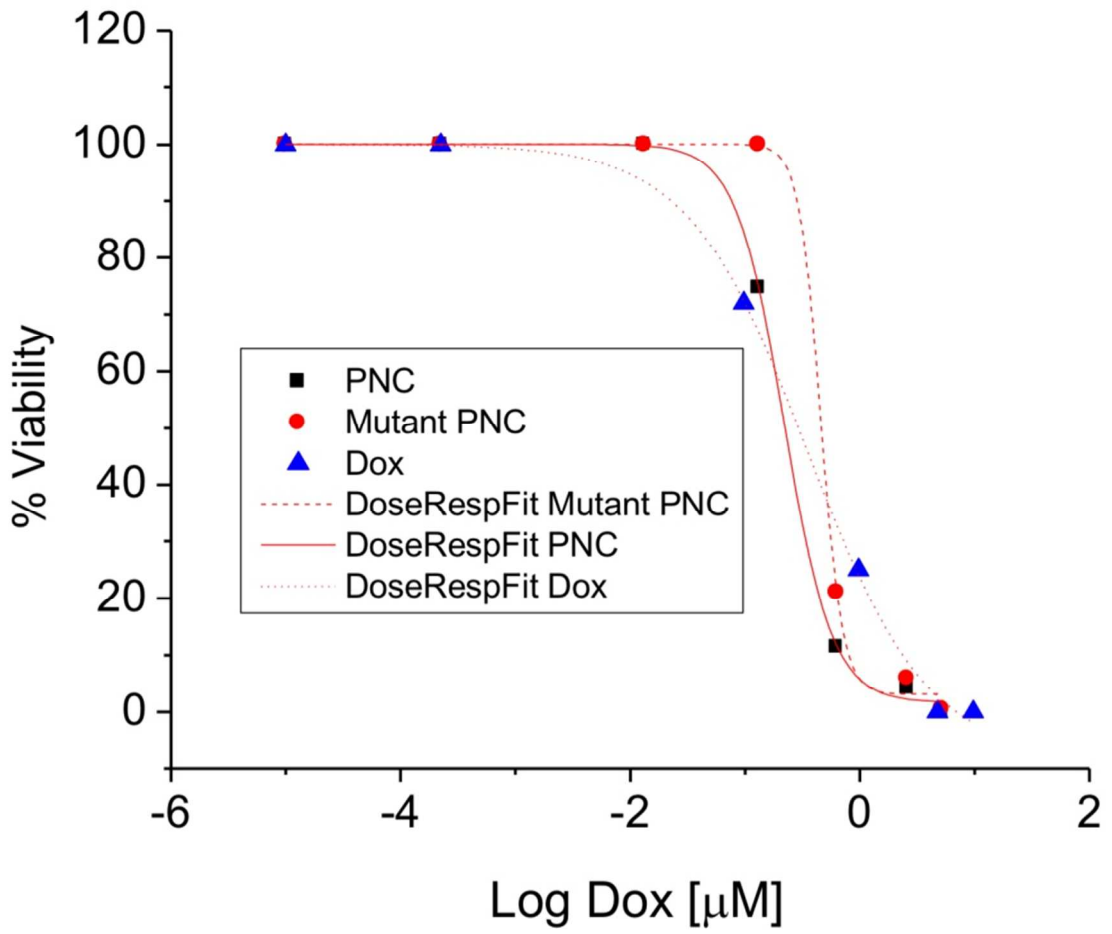



**Figure S15.** Cellular viability study of C13 (cisplatin resistant A2008 ovarian cancer cell line) treated with PNC, Mutant PNC and Dox. Experiments were performed in triplicates and $IC_{50}$ values were calculated using a MTS assay 72 hrs post treatment.

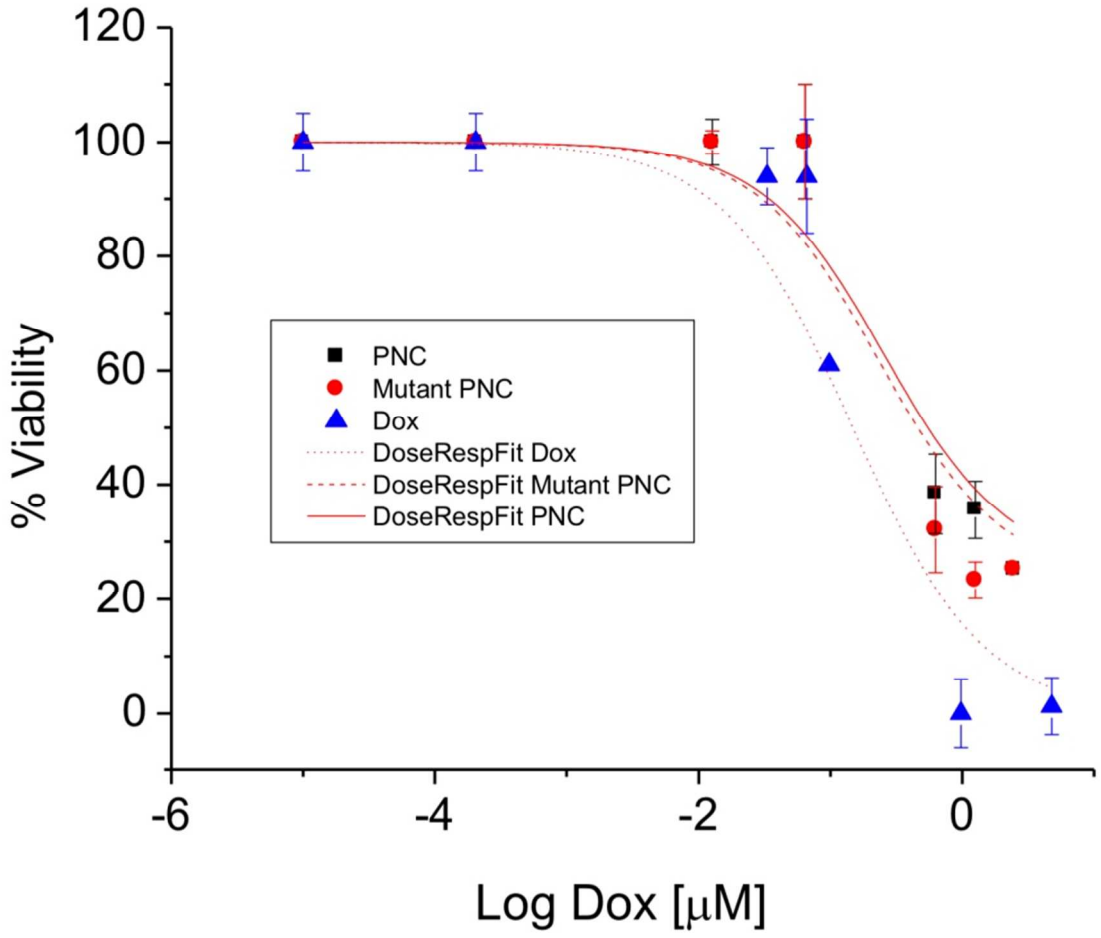



**Figure S16.** Cellular viability study of SH-SY5Y cells treated with PNC, Mutant PNC and Dox. Experiments were performed in triplicates and $IC_{50}$ values were calculated using a MTS assay 72 hrs post treatment.

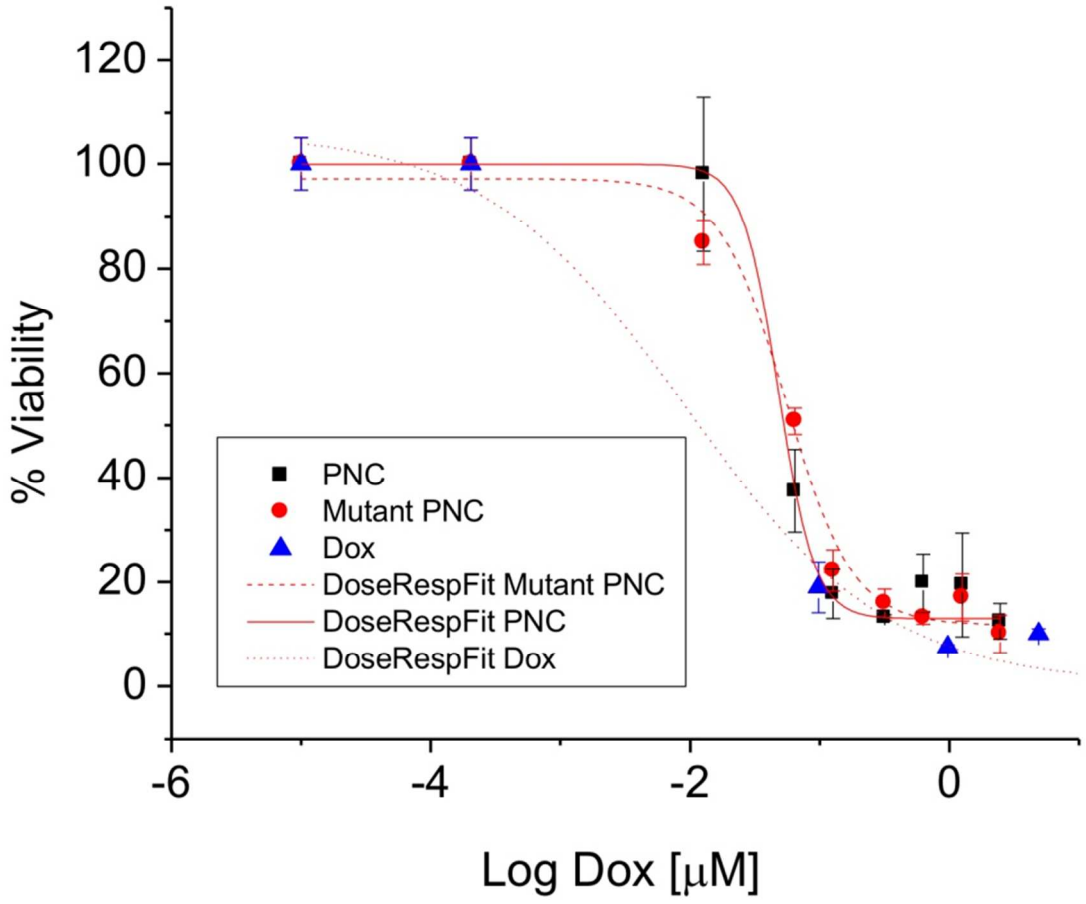



**Figure S17.** Cellular viability study of NIH3T3 cells treated with PNC, Mutant PNC and Dox. Experiments were performed in triplicates and $IC_{50}$ values were calculated using a MTS assay 72 hrs post treatment.

1. Cheng, Y.; Dai, Q.; Morshed, R. A.; Fan, X.; Wegscheid, M. L.; Wainwright, D. A.; Han, Y.; Zhang, L.; Auffinger, B.; Tobias, A. L.; Rincon, E.; Thaci, B.; Ahmed, A. U.; Warnke, P. C.; He, C.; Lesniak, M. S., Blood-brain barrier permeable gold nanoparticles: an efficient delivery platform for enhanced malignant glioma therapy and imaging. *Small* **2014,** *10* (24), 5137-50.

2. Acharya, S.; Dilnawaz, F.; Sahoo, S. K., Targeted epidermal growth factor receptor nanoparticle bioconjugates for breast cancer therapy. *Biomaterials* **2009,** *30* (29), 5737-50.

3. Hussain, Z.; Yousif, E.; Ahmed, A.; Altaie, A., Synthesis and characterization of Schiff's bases of sulfamethoxazole. *Org. Med. Chem. Lett.* **2014,** *4* (1), 1.