Supplementary Information

Engineered fusion protein-loaded gold nanocarriers for targeted co-delivery of doxorubicin and erbB2 siRNA in human epidermal growth factor receptor-2 expressing ovarian cancer

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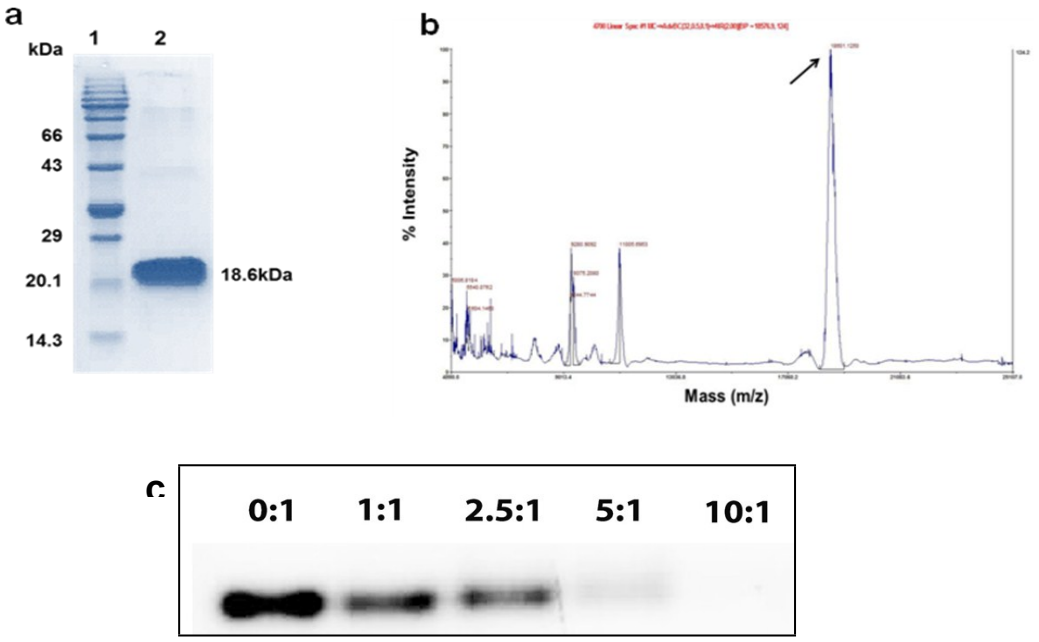

Figure S1: a) 12% SDS-PAGE analysis of purified TRAF(C) fusion protein as described in materials and methods. Lane 1: Molecular weight marker, Lane 2: Purified TRAF(C) fraction b) MALDI-TOF analysis of the purified TRAF(C) fusion protein of molecular weight 18.6 kDa. C) Gel binding assay of TRAF(C) protein with siRNA. Different mole ratios of TRAF(C) protein was used by keeping siRNA ratio constant.

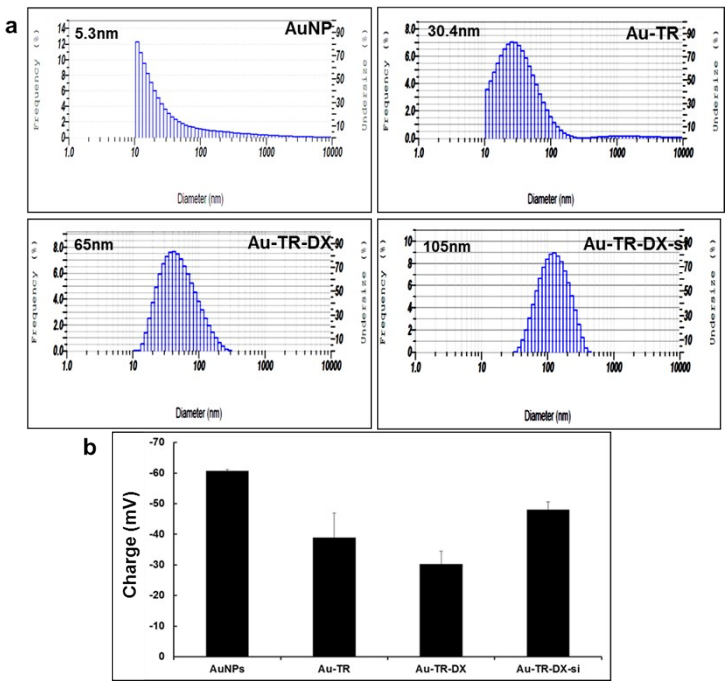

Figure S2: a) Determination of hydrodynamic diameter by dynamic light scattering of indicated formulations AuNPs, AuNPs with TR, DX and siRNA. The figure shows the gradual increase in the size of nanoparticles with the addition of respective biomolecules b) Determination of surface charge of AuNPs, AuNPs with TR, DX and siRNA formulations.

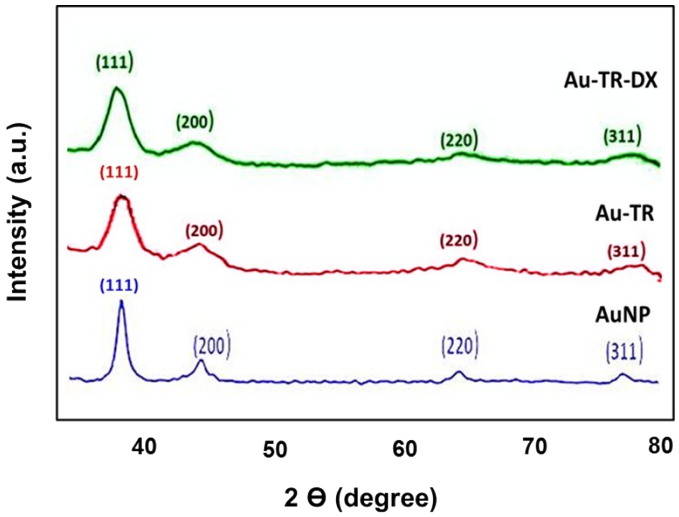

Figure S3. Determination of surface crystallinity of gold nanocomplex by X-Ray Diffraction analysis. Similarity in XRD patterns of Au-TR, Au-TR-DX and AuNP indicates that the surface crystallinity of AuNP is unchanged upon conjugation with TR and DX.

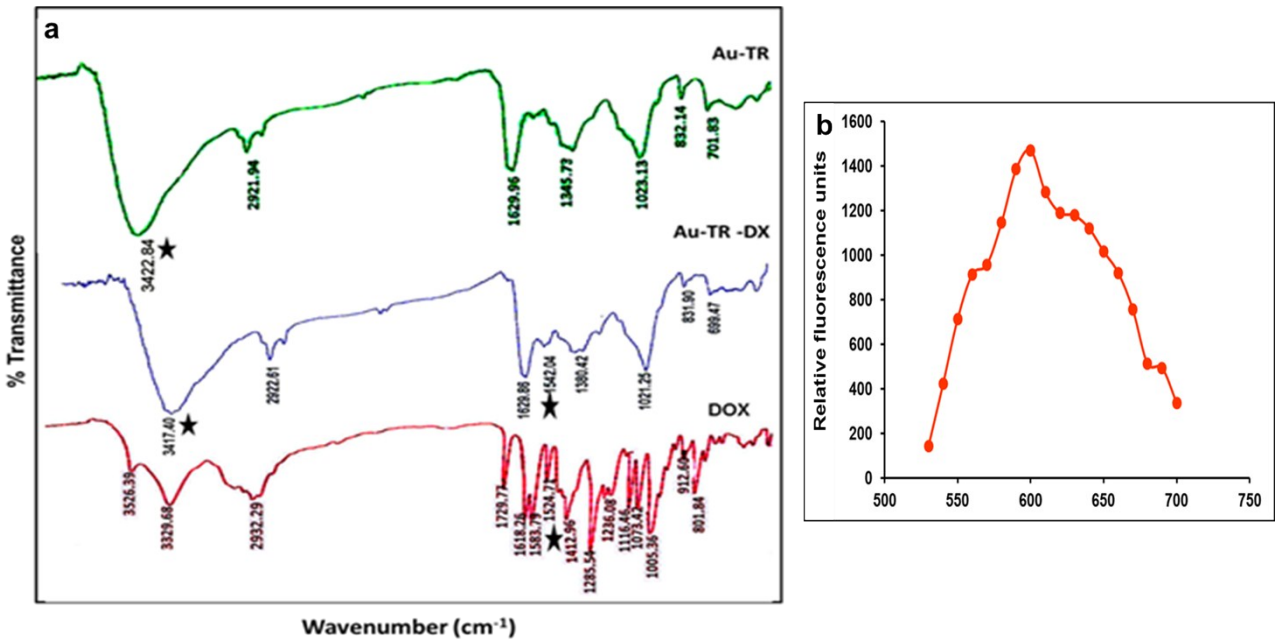

Figure S4. (a) Fourier transform infrared spectroscopy (FTIR). Functional group analysis of doxorubicin alone, Au-TR and Au-TR-DX formulations. Free doxorubicin shows a peak at 1524 cm^{-1} which is shifted to1542 cm^{-1} in Au-TR-DX which indicates the possible weak covalent bonding between Au-NH_2. Another peak in Au-TR at 3422cm^{-1} shifted to lower frequency (3417cm^{-1}) for Au-TR-DX indicating possible dative bonding (Au-OH) between -OH group of doxorubicin and gold nanosurface. (b) The graph depicts the fluorescence peak of doxorubicin originating from Au-TR-DX-si pellet.

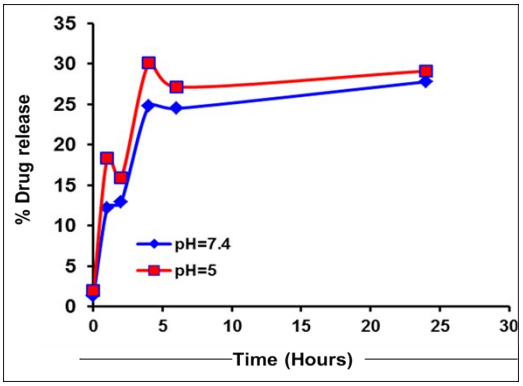

Figure S5. Spectroscopic evaluation of release of doxorubicin from Au-TR-DX-si complex. Graph depicts sustained time-dependent release of doxorubicin from Au-TR-DX-si complex at pH 5.0 and pH 7.4

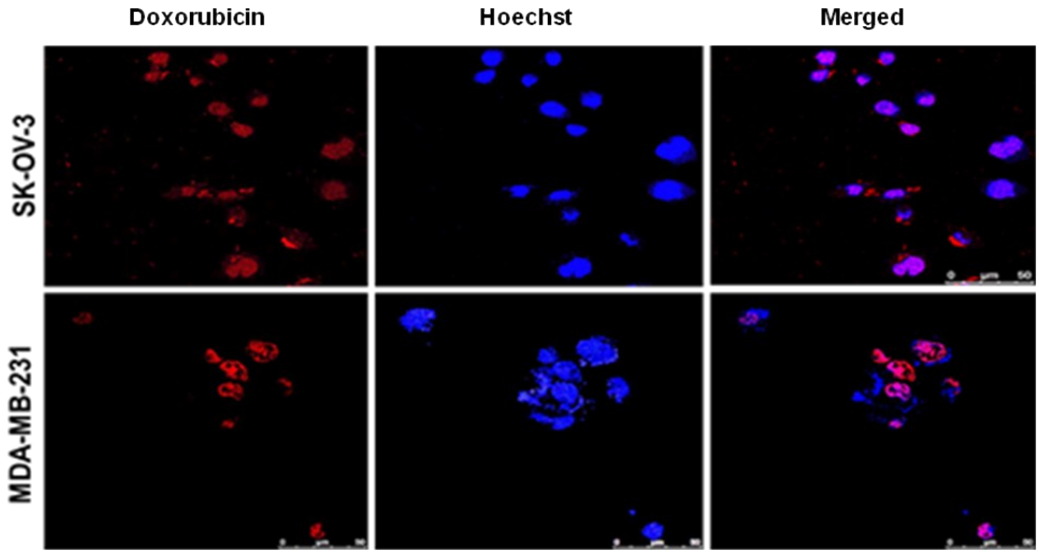

Figure S6. Comparative analysis of doxorubicin uptake in MDA-MB-231 and SK-OV-3 cells by confocal microscopy.

Nonspecific uptake of doxorubicin is evidenced by strong red fluorescence in the nuclei stained with Hoechst-33258 (Blue).

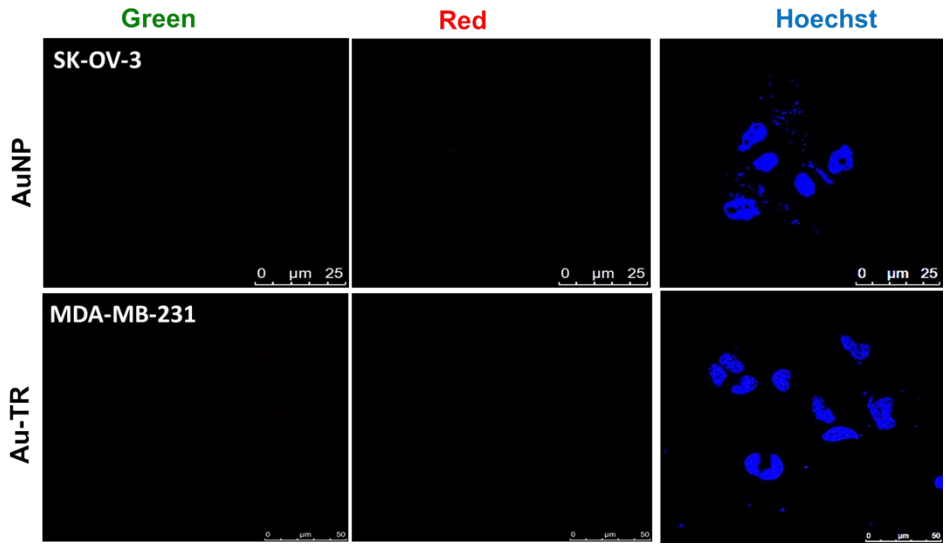

Figure S7. Confocal microscopy analysis of cell uptake of Au-TR and free AuNP in MDA-MB-231 and SK-OV-3 cells. The cells do not show any fluorescence from either Au-TR or free AuNPs uptake in this control experiment.

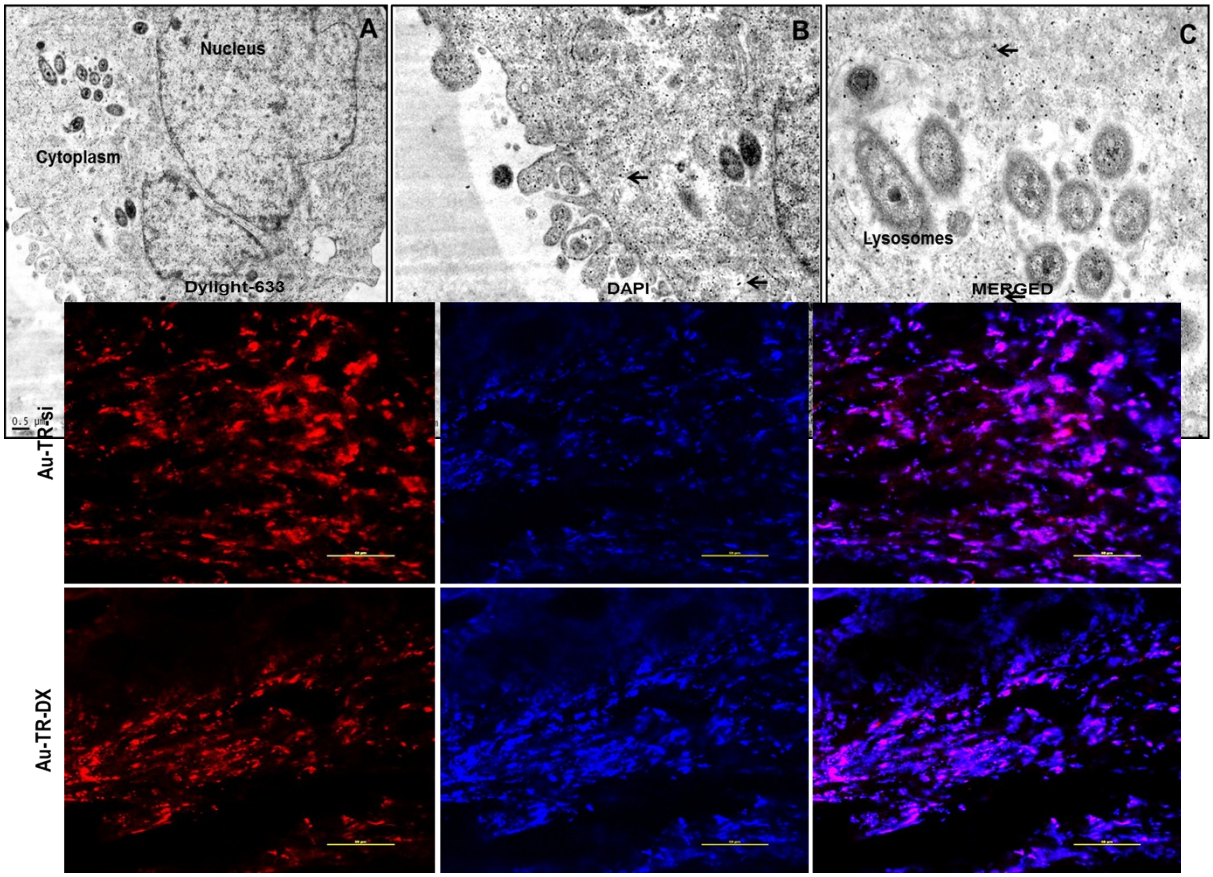

Figure S8. Internalization of TDDS in SK-OV-3 cells by TEM. TEM images depict spherical AuNPs. Panel A represents cells treated with TDDS. Panels B and C are the portions of magnified image of A to represent the cytoplasmic distribution of AuNPs as black dots.

Figure S9. Immunostaining of Au-TR-si and Au-TR-DX treated SK-OV-3 tumor sections by confocal microscopy using Ki-67 antibodies. The image represents tumor sections treated with Au-TR-si and Au-TR-DX treated SK-OV-3 tumor sections respectively following immunostaining.

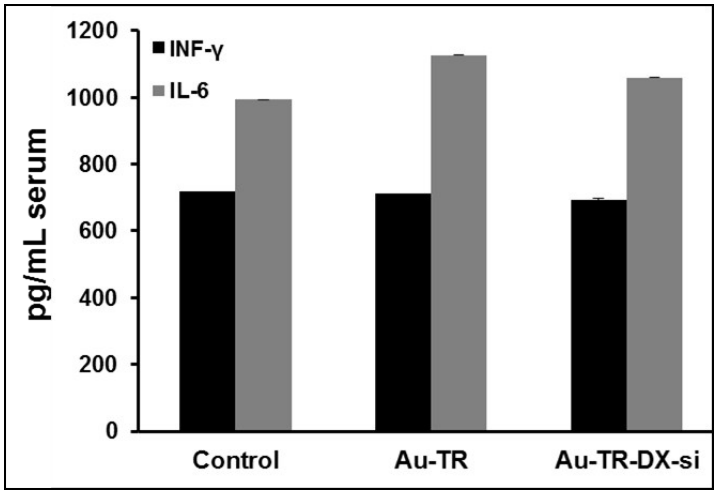

Figure S10. Determination of serum cytokine levels by ELISA. C57/BL/6 mice were treated with Au-TR and TDDS and compared with untreated groups. Graph depicts measurement of IFN-γ and IL-6 levels in serum, following intraperitoneal injection. No significant change in IFN-γ and IL-6 levels confirm the non-immunogenic nature of the complex.