

## *Supporting information*

### ***Studies on Interaction Potency Model based Drug Synergy and Therapeutic Potential of Triple Stimuli Responsive Delivery of Doxorubicin and 5-Fluoro-2-Deoxyuridine against Lymphoma using Disulfide Bridged Cysteine over Mesoporous Silica Nanoparticles***

*Prateek Srivastava<sup>†‡</sup>, Sumit Kumar Hira<sup>‡</sup>, Ankush Paladhi<sup>‡</sup>, Ranjeet Singh<sup>†</sup>, Uttam Gupta<sup>†</sup>, Divesh Narayan Srivastava<sup>#</sup>, Ram Adhar Singh<sup>§</sup> and Partha Pratim Manna<sup>†\*</sup>*

<sup>†</sup> Immunobiology Laboratory, Department of Zoology, Department of Zoology, and <sup>§</sup>Department of Chemistry, Centre of Advanced Study, Institute of Science, Banaras Hindu University, Varanasi 221005, India

<sup>‡</sup> Cellular Immunology Laboratory, Department of Zoology, The University of Burdwan, Bardhaman 713104, India.

<sup>#</sup>CSIR-Central Salts and Marine Chemicals Research Institute, Bhavnagar 364002, Gujarat. India

Email: pp\_manna@yahoo.com

Key words: Mesoporous silica, Cysteine, Doxorubicin, 5-fluoro-2-deoxyuridine,  $\beta$ -cyclodextrin, Lymphoma

## *Supporting information*

### **Material and methods**

CTAB (cetyltrimethylammonium bromide), TEOS (tetraethyl orthosilicate), MPTMS (3-mercaptopropyltrimethoxysilane), APTMS (3-aminopropyl trimethoxysilane), cysteine and Aldrithiol-2 were procured from sigma. EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride), NHS (N-Hydroxysuccinamide) and Doxorubicin were purchased from the Alfa Aesar. DCC (dicyclohexylcarbodiimide), DMAP (4-Dimethylaminopyridine), HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium3-oxidhexafluorophosphate), DIPEA (N,N-diisopropylethylamine), 4- carboxyphenylboronicacid, triethanolamine and betacyclodextrin were procured form the Alfa Aesar. 5-fluoro-2-deoxyuridine and folic acid were also purchased from Alfa Aesar.

### **Synthesis of mesoporous silica nanoparticles**

In the typical synthesis procedure, 0.5 g of CTAB was dissolved in 200 mL of water. Later, 1.75 mL of sodium hydroxide (2M) was added and the resulting mixture was stirred at 80°C temperature. After an hour, 2.0 mL of TEOS was added drop wise and the resulting solution were vigorously stirred for another 3 hours to obtain as synthesized MSNP. The formed MSNP were isolated and washed with ethanol and de-ionized water many times. In the next step, 200 mg of as synthesized MSNP was added in methanol (25 mL) containing 200 µL of triethanolamine following the introduction of 3-mercaptopropyltrimethoxysilane (50 µL) and the final reaction was stirred overnight. The formed MSNP-SH was precipitated by centrifugation and dried in vacuum. For the removal of CTAB, MSNP-SH was heated in a mixture composed of HCl (37% 6 mL) and ethanol (54 mL) at 60°C for 10 hours. Afterwards, the calcinated MSNP-SH was washed with distilled water and ethanol. The resulting silica nanoparticles were dried under vacuum to remove the remaining solvent from the pores.

## *Supporting information*

### **Cysteine amino acid decoration over the silica nanoparticles**

MSNP-SH particles (400 mg) were introduced in a solution of Aldrithiol-2 (170 mg) in 100 mL PBS. Following stirring for 12 hours at room temperature (RT), the resulting material i.e., 2-pyridinyldisulfanylpropyl-functionalized MSNP was produced and washed with ethanol and cold PBS many times. Later, the purified material was again introduced to a 200 mL PBS solution consisting of cysteine amino acids (65 mg) to have the desired product MSNP-CYS. The amount of the cysteine molecules attached over the silica was determined by the decrease in the free -SH group via Ellman's reagent suggesting approximately 107  $\mu\text{g}$  of the cysteine was attached per mg of the silica nanoparticles.

### **Annexation of 5-fluoro-2-deoxyuridine over the silica surface**

For the typical reaction procedure, 50.0 mg of MSNP-CYS was added to 20 mL of dry DCM followed by the addition of the DCC (20.0 mg) and DMAP (17.0 mg) and the reaction was stirred at RT under N<sub>2</sub> conditions. After an hour, 5 mg of 5-fluoro-2-deoxyuridine was added (1mg/mL DMSO) to the reaction mixture and the final step proceeds for 24 hours to form an ester bond between -COOH group derived from cysteine and -OH group present in 5FU molecules. After completion of the reaction, the product was washed several times with DCM and methanol and dried in vacuum to get MSNP-CYS-5FU.

### **Addition of the targeting device folic acid over the nano-construct**

The folic acid carboxyl groups were activated via employing EDC/NHS prior to its fabrication over the silica surface through an amide bond. In the reaction procedure, approximately 8.0 mg of folic acid was dissolved in 2 mL of DMSO solution followed by the addition of the EDC (5.0mg) and NHS (4.0mg) and the reaction was allowed to stir for 30 minutes at RT. Later, the folic acid

## *Supporting information*

activated mixture was introduced to an ethanolic solution containing approximately 50.0 mg of MSNP-CYS-5FU and the resulting mixture was stirred for 8 hours to form MSNP-CYS-5FU-FA. In the proceeding steps, the particles were isolated with centrifugation and washed with distilled water and ethanol several times.

### **Aminopropyl group condensation over the orifices of silica**

After the CTAB flushing from the MSNP, the orifices were left for further functional group annexation. Thus, in order to employ the amine groups over the orifices, MSNP-CYS-5FU-FA (50.0 mg) was condensed with 10  $\mu$ L of APTMS in 15 mL of the ethanolic solution. The condensation process proceeds overnight and the final product was isolated and washed with distilled water many times to get MSNP-CYS-5FU-FA-NH<sub>2</sub>.

### **Addition of the boronic acid over the orifices**

The formed nano-construct MSNP-CYS-5FU-FA-NH<sub>2</sub> was reacted with 4-carboxyphenyl boronic acid to attain boronic acid decorated orifices. In a typical reaction process, 5.0 mg of 4-carboxyphenyl boronic acid was dissolved in 10 mL of DMF, followed by the addition of HATU (10.0 mg) and DIPEA (0.2 mL), and the reaction was stirred for an hour at room temperature. Later, 25 mg of the MSNP-CYS-5FU-FA-NH<sub>2</sub> was added and the reaction was allowed to stir for another 8 hours to have boronic acid functionalized silica (MSNP-CYS-5FU-FA-BA). Later, the particles were isolated, washed and dried in vacuum.

### **DOX loading and sealing of pores via beta cyclodextrin**

The as synthesized MSNP-CYS-5FU-FA-BA (20.0 mg) was added to the 5 mL of distilled water solution containing DOX molecules (2.0 mg) and the reaction was stirred for another 24 hours so that the DOX molecules were caged inside the porous silica. The DOX loaded particles were

## *Supporting information*

represented as MSNP-CYS-5FU-FA-BA@DOX and were centrifuged at 12,000 rpm for 10 minutes and the supernatant was collected for the spectroscopic analysis. In the next step, the beta cyclodextrin molecules were introduced to the nano-system to obtain a stimuli responsive cap system. Briefly, 4.0 mg of beta cyclodextrin was dissolved in 5 mL of DMF solution, followed by the addition of MSNP-CYS-5FU-FA-BA@DOX (10.0 mg) to the reaction mixture. The resulting mixture was stirred for 12 hours to have the efficient boronic ester bond formation between the vicinal diols inheriting the cyclodextrin and the boronic acid derived silica. The final product (MSNP-CYS-5FU-FA-BA@DOX-CD) was isolated, centrifuged and stored at 4°C.

### **Loading content of the 5-fluoro-2-deoxyuridine and the doxorubicin molecules**

Since the disulfide bond formation is usually favored at basic pH, so the amount of the 5FU molecules clenched to the MSNP-CYS was determined at basic pH which dislodges the ester bond. 2 mg of MSNP-CYS-5FU was suspended in distilled water at pH 9.0 and allowed to stir for 12 hours at 60°C. The resulting product was centrifuged at 10,000 rpm and the supernatant was collected for determining the amount of 5FU molecules attached to the cysteine molecules. The 5FU molecules inherited the pyrimidine structure so they tend to absorb strongly at 278 nm. Approximately 45.7 µg of 5FU was attached per mg of the MSNP-CYS. The amount of doxorubicin molecules entrapped inside the MSNP was determined by taking absorbance at 485 nm and approximately 35.5 µg of doxorubicin was entrapped inside the MSNP.

### **Characterization**

TEM images were taken on a JEOL JEM-2100 transmission electron microscope with an accelerating voltage of 200 kV. For the TEM measurements, the colloidal suspension were prepared in ethanol solution and allowed to dry over the copper grid. To monitor the functionalized nanoparticles in the proceeding steps, the Fourier Transforms Infrared (FTIR)

## *Supporting information*

Spectroscopy(Perkin Elmer), hydrodynamic size by DLS, and Zeta potential (Nano ZS90Zetasizer, Malvern Instruments, UK) were performed. FTIR spectroscopy was performed by creating a pellet of sample with KBr (Sigma-Aldrich) and analyzing the absorbance of the pellet. For DLS and Zeta studies, scattered light detection was measured at 90° to the incident beam at 660 nm wavelength. The amine group on MSNP was also determined by Ninhydrin staining. UV-Vis absorption was recorded utilizing the Shimadzu 1700 spectrophotometer. The BET specific surface area and the pore size distribution were measured with specific surface areas and pore size analyzer (V sorb 2800P, Gold APP, China). The TG curves were measured on an STA 409/PC simultaneous thermal analyzer (Netzsch, Germany) with a heating rate of 10°C/min.

### **Cell lines, cell culture and doxorubicin resistant tumor cells**

Culture of Dalton's lymphoma (DL), MCF-7 and K562 cells and normal human peripheral blood lymphocytes and monocytes were performed as described by us before. Doxorubicin (DOX) resistant DL cells were generated as described by us before.

### **Isolation of splenic dendritic cells and killer cells**

Splenic dendritic cells (DC) was isolated from normal, DL tumor bearing mice and treated mice as described by us before. DC obtained was characterized by FACS analysis for determining the purity and expression of DC specific marker viz CD40, CD86 and MHC class II. Killer CD8<sup>+</sup> T cells and natural killer (NK) cells were isolated from non-adherent splenocytes obtained after two rounds of glass plate adherence of total splenocytes at 37°C, 5% CO<sub>2</sub> for 3 hours each. In this process, nearly all macrophages and splenic dendritic cells were removed leaving only the non-adherent lymphocytes. CD8<sup>+</sup>T cells and CD49b<sup>+</sup> NK cells were enriched from the non-adherent splenocytes by negative selection using antibodies specific to CD4 (helper T cells), CD19 (B cells) plus Dynal beads. The bead bound cells were separated by magnet and the CD8<sup>+</sup> T cells and NK

## *Supporting information*

cells were enriched from the supernatant and were used as effector killer cells against the DL and YAC-1 cells. Enrichment of CD8<sup>+</sup> T cells and NK cells was demonstrated by staining with fluorescein conjugated anti CD8 and anti CD49b antibodies.

### **Cell Viability Assay**

Effect of free doxorubicin, 5FU or other constructs including MSNP-CYS-5FU-FA-BA@DOX-CD on the viability of tumor cells or normal human cells was evaluated by a colorimetric XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) assay (Roche, Indianapolis, IN). Tumor cells, lymphocytes or monocytes were plated ( $5 \times 10^3$  cells/well) in 96-well culture dish and incubated with different concentrations of the above mentioned compounds and incubated at 37°C, 5% CO<sub>2</sub> for 18 hours. OD was taken at 450 nm in a plate reader (Synergy HT, BioTek, USA). The percent viable cell was calculated employing the formula below:

$$\% \text{ Cell Viability} = \frac{\text{Experimental OD}_{450}}{\text{Control OD}_{450}} \times 100$$

### **Cell Proliferation Assay**

Growth inhibitory potential by the compounds against DL, MCF-7 or K 562 and their doxorubicin resistant counterparts was studied by MTT assay. Tumor target cells ( $5 \times 10^3$  cells /well) in a 96 well culture dish were treated with serial concentrations of the compounds. Following incubation at 37°C, 5% CO<sub>2</sub>, for 48 hours, proliferation of the tumor cells was assessed by MTT assay using CellTiter 96 kit (Promega, USA). The measurement of absorbance (OD value) was made at 570 nm in a plate reader (BioTek, USA). Percent inhibition of tumor cells was calculated using under mentioned formula:

$$\% \text{ Growth Inhibition} = \left[ 1 - \frac{\text{Experimental OD}_{570}}{\text{Target OD}_{570}} \right] \times 100$$

## *Supporting information*

Where Experimental OD value indicate the values of tumor cells in the presence of the indicated formulations and Target OD indicate the corresponding values of tumor cells alone, cultured in medium only.

### **Cytotoxicity Assay**

The lytic activity of MSNP-CYS-5FU-FA-BA@DOX-CD and other constructs or free drugs against tumor cells or normal human cells was measured by cytotoxicity assay using the CytoTox 96 Cytotoxicity assay kit from Promega, USA. Normal or tumor target cells ( $5 \times 10^3$ ) were co-cultured with varying concentrations of the indicated formulations in a 96 well culture dish. The culture dish was incubated for 18 hours at 37°C, 5% CO<sub>2</sub>. Percent-specific lysis was determined from the under mentioned formula:

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous})}{(\text{Target Maximum} - \text{Target Spontaneous})} \times 100$$

### **Combination Index Analysis**

The unified theory of using both linear and nonlinear regression analysis, introduced by Chou et al. was used to evaluate the synergism, additivity and antagonism of the combination drug treatment. Combination index (CI) values were calculated using the CompuSyn software (CompuSyn Inc., Paramus, NJ) using under mentioned equation.

$$CI = \frac{C_{A,X}}{IC_{X,A}} + \frac{C_{B,X}}{IC_{X,B}}$$

Where  $C_{A,X}$  and  $C_{B,X}$  are the concentrations of drug A and drug B in the combination to produce a certain effect X.  $IC_{X,A}$  and  $IC_{X,B}$  are the concentrations of drug A and drug B used as a single agent to produce that same effect.

### **Interpretation of drug-combination plots**

## *Supporting information*

CompuSyn also generates a plot of CI values at different fraction affected (Fa) levels referred to as Fa-CI plot or the Chou-Talalay plot, which are widely used to interpret drug combination effects. A CI value of <0.1 indicates very strong synergism, 0.1–0.3 strong synergism, 0.3–0.7 synergism, 0.7–0.9 moderate to slight synergism, 1 nearly additive, 1.1–1.45 slight to moderate antagonism, 1.45–3.3 antagonism, and >3.3 strong to very strong antagonism. To further study the dose-dependent interaction of two drugs' isobologram, particularly at Fa levels of 50%, 70%, and 80% inhibition were created. Since the single agents Fa value corresponds to IC<sub>50</sub> value, the 50% isobologram of the combination provided direct comparison with single agent treatment. The 70% and 80% isobologram represent combination at a high effect level and have practical implications in therapeutic oncology. Data points above or below the line of additivity indicate antagonism or synergism, respectively.

### **Evaluation of drug combination effect using Zero interaction potency (ZIP) dose-response surface model**

Most synergy analyses focus on the differences in isobologram shapes at fixed effects, and summary interaction scores such as the fractional inhibitory concentration ( $\Sigma$ FIC) index. The  $\Sigma$ FIC index, like the isobologram upon which it is based, is insufficient to effectively capture the combination effects that may occur across multiple dose regions. An inherent limitation of the  $\Sigma$ FIC index is the focus on a single interaction parameter. Yadav et al. have developed a score that enables the use of an interaction landscape over the full dose–response matrix to identify combination effects across multiple dosages and response levels. Rather than relying on a single parameter such as the IC<sub>50</sub> measurement, the delta-score was calculated by assessing changes in both the shape parameter and the midpoint of each dose–response curve for individual samples and combinations thereof. The delta scores were visualized using a response surface plot to visualize the combination effect landscape over all tested dosage combinations, enabling

## *Supporting information*

identification of potency changes and differences in combination effects even within the same sample pair. For evaluation of drug combination effect, Zero interaction potency (ZIP) dose-response surface model was adopted using R-statistics. The surface plot of the delta scores was utilized to visualize the interaction landscape for the drug combination, aiming to identify synergistic and antagonistic dose regions for further dose optimization in a validation screen.

### **Apoptosis Assay**

Evaluation of apoptotic cell death in parental and doxorubicin resistant DL, MCF-7 or K562 cells, treated with free doxorubicin, 5FU,MSNP-CYS-5FU-FA-BA@DOX-CD or other constructs for 12 hours. The cells were washed in PBS and were stained with FITC-conjugated Annexin V for 30 minutes. These cells were washed in Annexin buffer. The FITC-conjugated Annexin V positive cell & intracellular DOX localization was visualized under a fluorescence microscope (EVOS FL Cell Imaging System equipped with Plan Fluor, 40X, NA 0.75 objective, Life Technologies, USA) as describe earlier.

### **Hemolysis Assay**

For concentration dependent kinetics, the blood sample was incubated with varying concentrations (5-50 $\mu$ M) of free doxorubicin, 5FU,MSNP-CYS-5FU-FA-BA@DOX-CD or other constructs for 4 hours. Hemolysis assay was performed according to the standard protocol. In brief, an aliquot of each blood sample was centrifuged for 5 minutes. 25  $\mu$ L plasma aliquot was diluted with 225  $\mu$ L Drabkin's reagent (Sigma) in a 96-well plate and mixed for 2 minutes under lateral agitation (300 rpm). After 10 minutes equilibration at room temperature, optical density was recorded at 540 nm in Synergy HT Multi-Mode Microplate Reader BioTek, USA. Blood hemoglobin was determined by measuring the absorbance of 100-fold dilution of the whole blood in Drabkin's reagent at 540 nm. Saponin (2 mg/mL final blood concentration) and PBS were used as positive and negative

## *Supporting information*

control respectively. A sample of plasma without additives was considered as basal conditions. The standard calibration curve was obtained with the solution containing 0.07–3.8 mg/mL bovine hemoglobin (Sigma) treated with a Drabkin's reagent. The results were presented as percent hemolysis indicating the free plasma hemoglobin (mg/mL) and was measured as released hemoglobin divided by the total blood hemoglobin (mg/mL) multiplied by 100. All measurements were performed in triplicate.

### **Murine Lymphoma Model**

Female AKR/J mice were maintained and bred under pathogen-free conditions in the central animal house facility of the department. DL tumors were grown in mouse peritoneum via transplanting tumor cells in PBS ( $3 \times 10^4$  cells/mouse) intraperitoneally. Tumor growth and measurements were conducted in an unbiased and blinded fashion. Mice (n=6/group) were intraperitoneally transplanted with tumor cells and the treatment was started after 96 hours (Day 0). Mice with tumors were given injections containing indicated constructs including MSNP-CYS-5FU-FA-BA@DOX-CD or free doxorubicin or 5FU only in PBS. The injections were administered intraperitoneally as mentioned in the therapy schedule. Animals treated with PBS only were used as the control. Altogether, 5 doses were administered starting at 96 hour's post-tumor transplant, which was considered as 0 hours. The doses were administered every 96 hours for each formulation. The formulations were validated so that 100  $\mu$ L of MSNP-CYS-5FU-FA-BA@DOX-CD contained 3 mg/kg of body weight of DOX. PBS (100  $\mu$ L) was used as the vehicle control for drug treatment. Tumor volume (abdominal circumference) and body weights were determined daily. The animals were maintained up to day 50, when the final assessment of the body weight, abdominal circumference and days of survival was performed for the Kaplan-Meyer survival analysis. In an identical parallel experiment, the animals (n=3) were sacrificed when the average

## *Supporting information*

abdominal circumference of the control (PBS only) exceeded 15.5 cm. Before sacrifice, tumor cells in the peritoneum were collected as ascitic fluid. Blood was also collected for assessment of the doxorubicin concentration. Blood films were made on a slide, fixed with methanol and stained with Leishman stain to study the effects of DOX on leukocytes. Organs (spleen, liver, heart, lung, and kidney) were dissected and weighed. Organs were cut into parts. A portion of the liver, spleen and lung was preserved in 10% formalin for histopathological analysis, and the remaining parts were used for analysis of the DOX distribution. Mice were under observation for 50 days when the final data collection was conducted for Kaplan-Meyer survival analysis as well as the measurement of other parameters.

## *Supporting information*

**Table S1.** BJH pore size distributions of the MSNP, MSNP-CYS, MSNP-CYS-5FU-FA and MSNP-CYS-5FU-FA-BA-CD

MATERIAL	BET Surface area (m <sup>2</sup> /gm)	BET Pore Volume (Cm <sup>3</sup> /gm)	BJH pore diameter (nm)
<b>Bare MSNP</b>	801.673	0.585	3.0
<b>MSNP-CYS</b>	489.135	0.359	2.1
<b>MSNP-CYS-5FU-FA</b>	321.241	0.278	2.0
<b>MSNP-CYS-5FU-FA-BA-CD</b>	127.563	0.172	--

## *Supporting information*

**Table S2.** Comparison of abdominal circumference in mice treated with indicated formulations. Days indicate the time when treatment started and the number of animal used for treatment with each formulation was six. Levels of significance between the two treatment conditions are presented in bold. No entry on day 38 and 42 in **MSN-CYS-5FU-FA** indicates the death of all the animals.

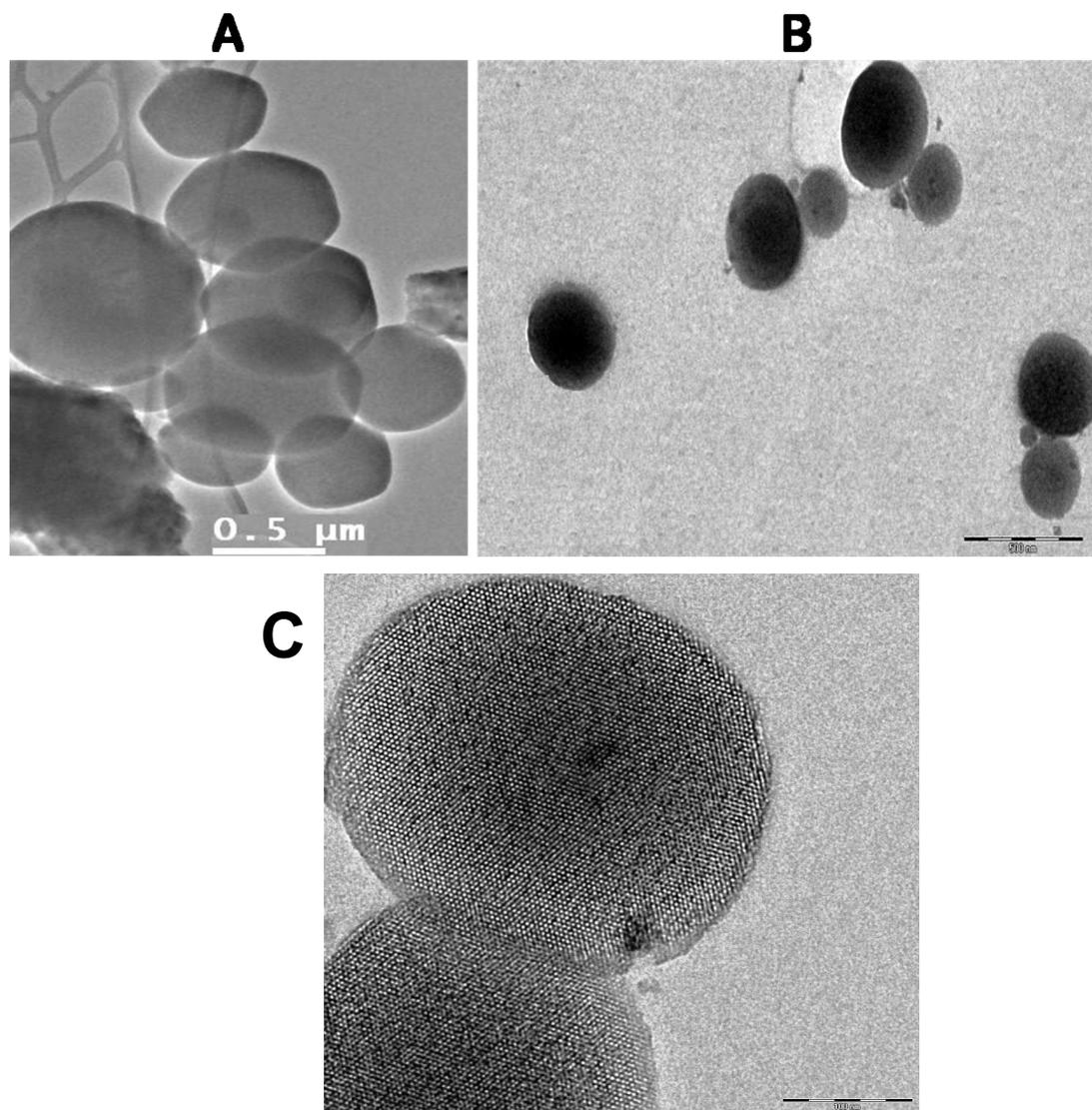
	<b>MSN-CYS-5FU-FA</b>		<b>MSNP-CYS-5FU-FA-BA@DOX-CD</b>		
Days	Mean	SD	Mean	SD	Two-tailed p values
0	7.85	0.729	7.85	0.295	ns
4	7.85	0.729	8.1	0.273861	ns
8	8.45	0.154	8.333333	0.661564	ns
12	9.183333	0.32	8.55	0.774597	0.4673
16	12.76667	0.462	11.25	1.219836	<b>0.0173</b>
20	13.63333	0.746	12.03333	1.491867	<b>0.0407</b>
24	14.45	0.119	13.15	0.233574	<b>0.0006</b>
28	16.5	0.042	15.43333	1.05314	<b>0.0326</b>
32	19.35333	0.26454489	18.36667	0.3034	<b>0.0001</b>
38	0	0	20.32	2.311528	
42	0	0	20.73667	1.528014	

## *Supporting information*

**Table S3.** Comparison of abdominal circumference in mice treated with indicated formulations. Days indicate the time when treatment started and the number of animal used for treatment with each formulation was six. Day 20 data is presented in bold. No entry on empty boxes indicates the death of all the animals. Data presented as Mean  $\pm$  SD

<b>Days Post Tumor Transplant</b>	<b>DL</b>	<b>DOX</b>	<b>5FU</b>	<b>MSNP-CYS-5FU-FA</b>	<b>MSNP-CYS-5FU-FA-BA@DOX-CD</b>
0	7.85 $\pm$ 0.73	7.85 $\pm$ 0.95	7.85 $\pm$ 1.02	7.85 $\pm$ 0.72	7.85 $\pm$ 0.72
4	8.55 $\pm$ 0.54	8.55 $\pm$ 0.54	7.85 $\pm$ 0.85	7.85 $\pm$ 0.79	8.11 $\pm$ 0.27
8	9.85 $\pm$ 0.55	9.4 $\pm$ 0.27	8.25 $\pm$ 0.27	8.45 $\pm$ 0.15	8.33 $\pm$ 0.66
12	10.80 $\pm$ 0.38	10.35 $\pm$ 0.47	9.2 $\pm$ 0.47	9.18 $\pm$ 0.32	8.55 $\pm$ 0.77
16	15.78 $\pm$ 0.66	11.15 $\pm$ 0.67	12.3 $\pm$ 0.67	12.76 $\pm$ 0.46	11.25 $\pm$ 1.21
<b>20</b>	<b>18.02 <math>\pm</math> 0.88</b>	<b>14.68 <math>\pm</math> 1.69</b>	<b>15.83 <math>\pm</math> 1.69</b>	<b>13.63 <math>\pm</math> 0.74</b>	<b>12.03 <math>\pm</math> 1.49</b>
24	20.35 $\pm$ 0.87	16.66 $\pm$ 1.16	17.81 $\pm$ 1.66	14.45 $\pm$ 1.01	13.15 $\pm$ 3.23
28		18.60 $\pm$ 1.23	19.75 $\pm$ 1.93	16.5 $\pm$ 1.04	15.43 $\pm$ 3.05
32				19.35 $\pm$ 2.08	18.36 $\pm$ 2.80
38					20.32 $\pm$ 2.31
42					20.73 $\pm$ 1.52

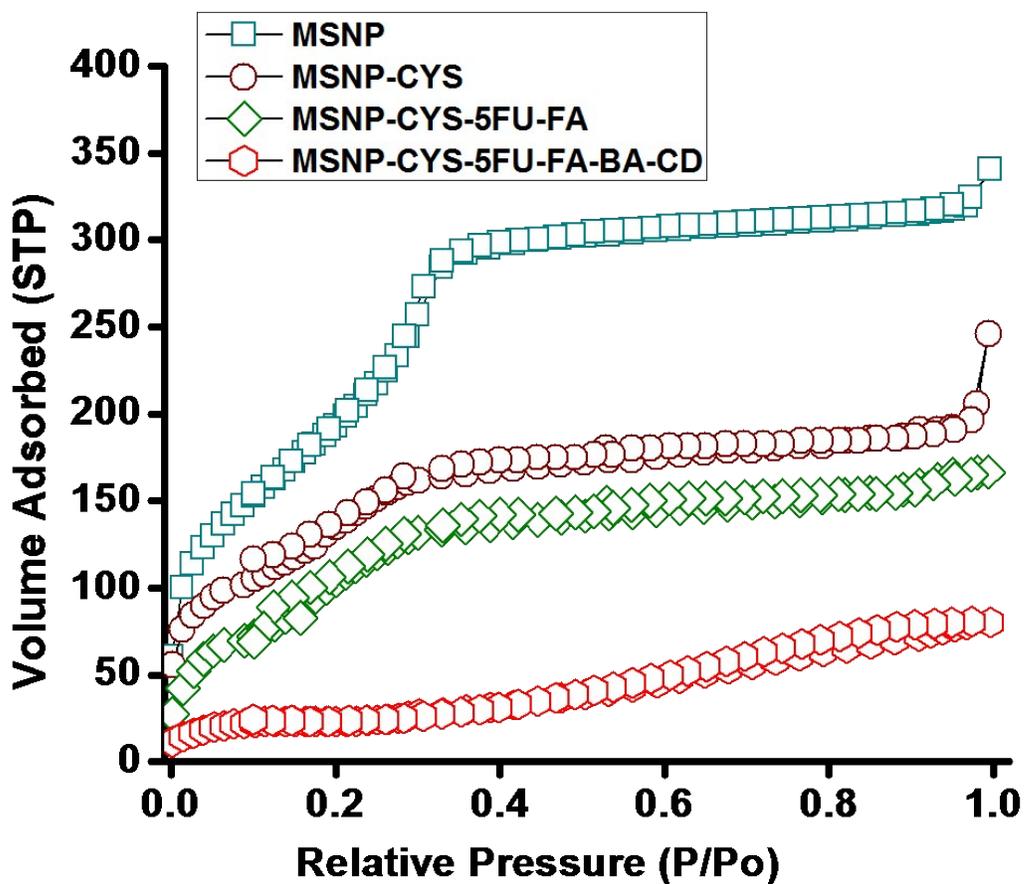
## Supporting information



**Figure S1.** TEM images of as synthesized bare MSNP (A) and MSNP-CYS-5FU-FA-BA@DOX-CD (B). Mesoporous structure of the

## Supporting information

synthesized MSNP (C) Scale bar is 500 nm.



**Figure S2.** Nitrogen Adsorption-Desorption isotherm of MSNP, MSNP-CYS and MSNP-CYS-5FU-FA-BA-CD

## Supporting information

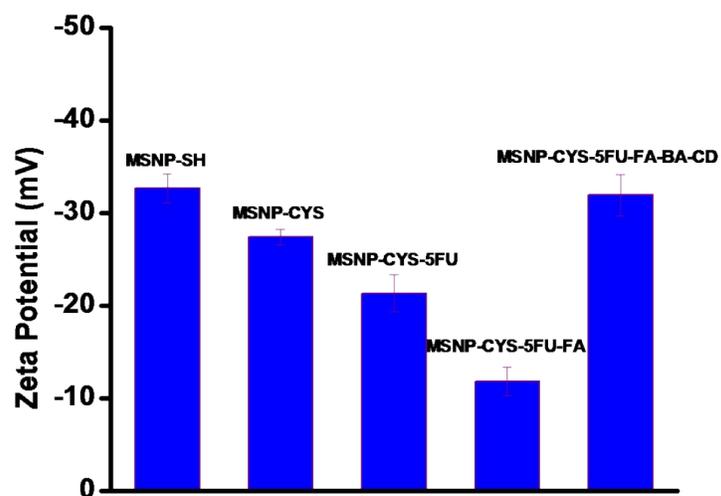


Figure S3. Zeta potential measurements of the nano constructs

*Supporting information*

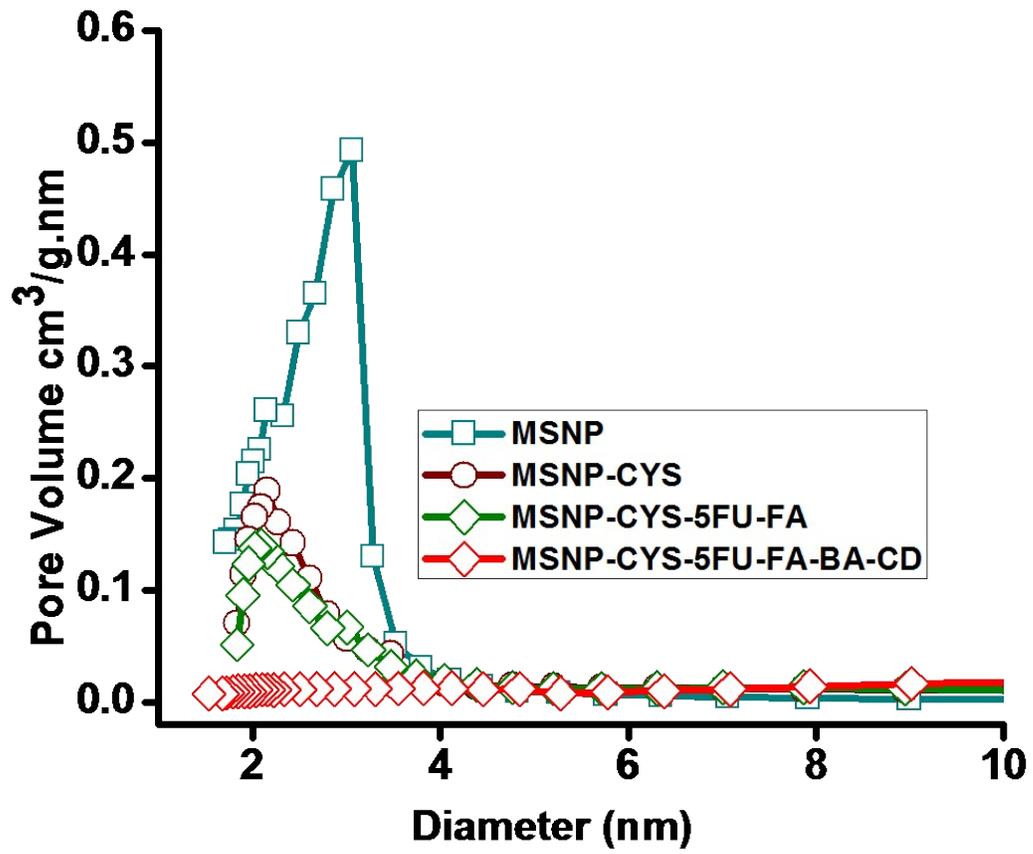
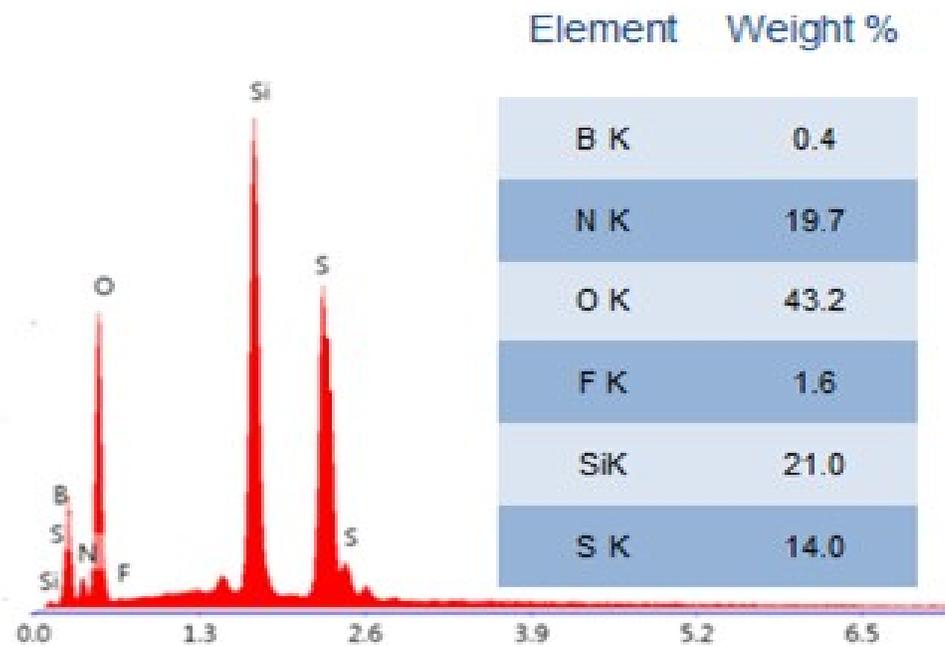


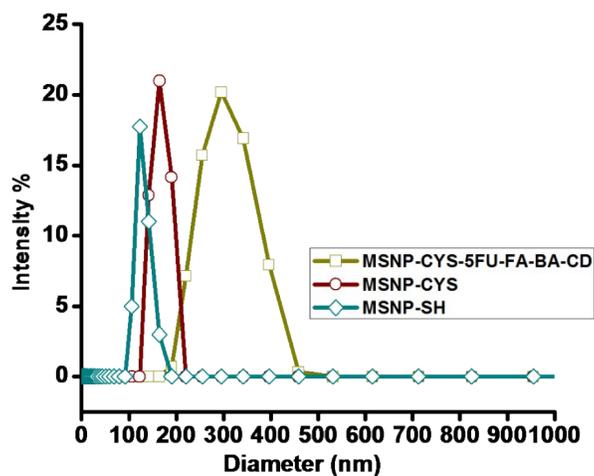
Figure S4. BJH pore size distribution of the MSNP, MSNP-CYS and MSNP-CYS-5FU-FA-BA-

CD

## Supporting information

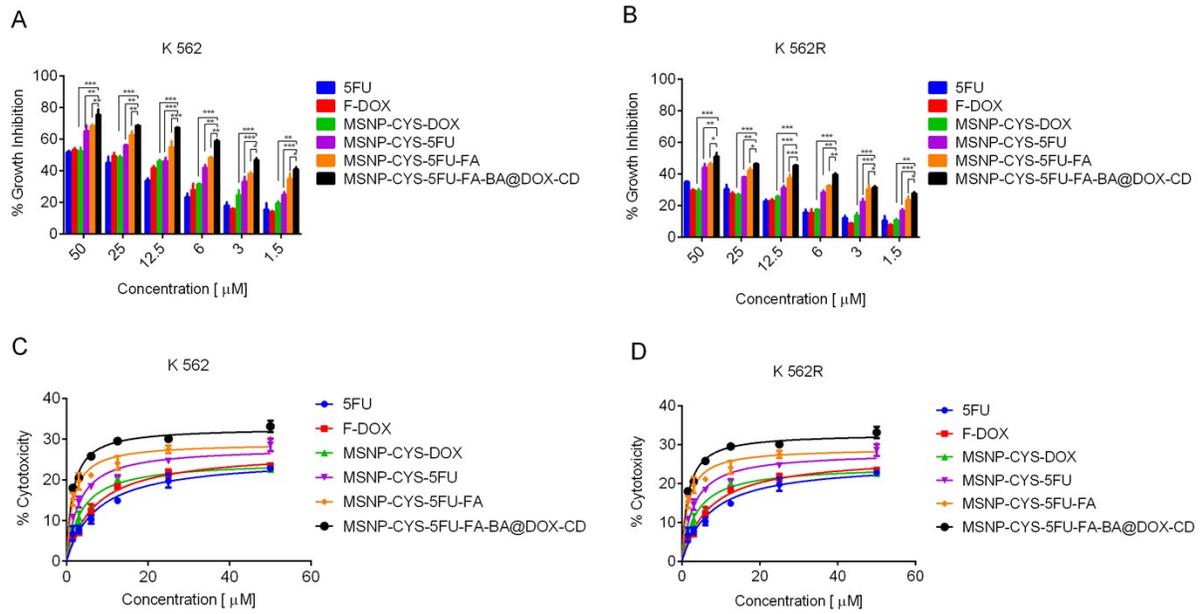


**Figure S5.** EDAX analysis for conjugation of the 5-carboxyphenylboronic acid over the MSNP



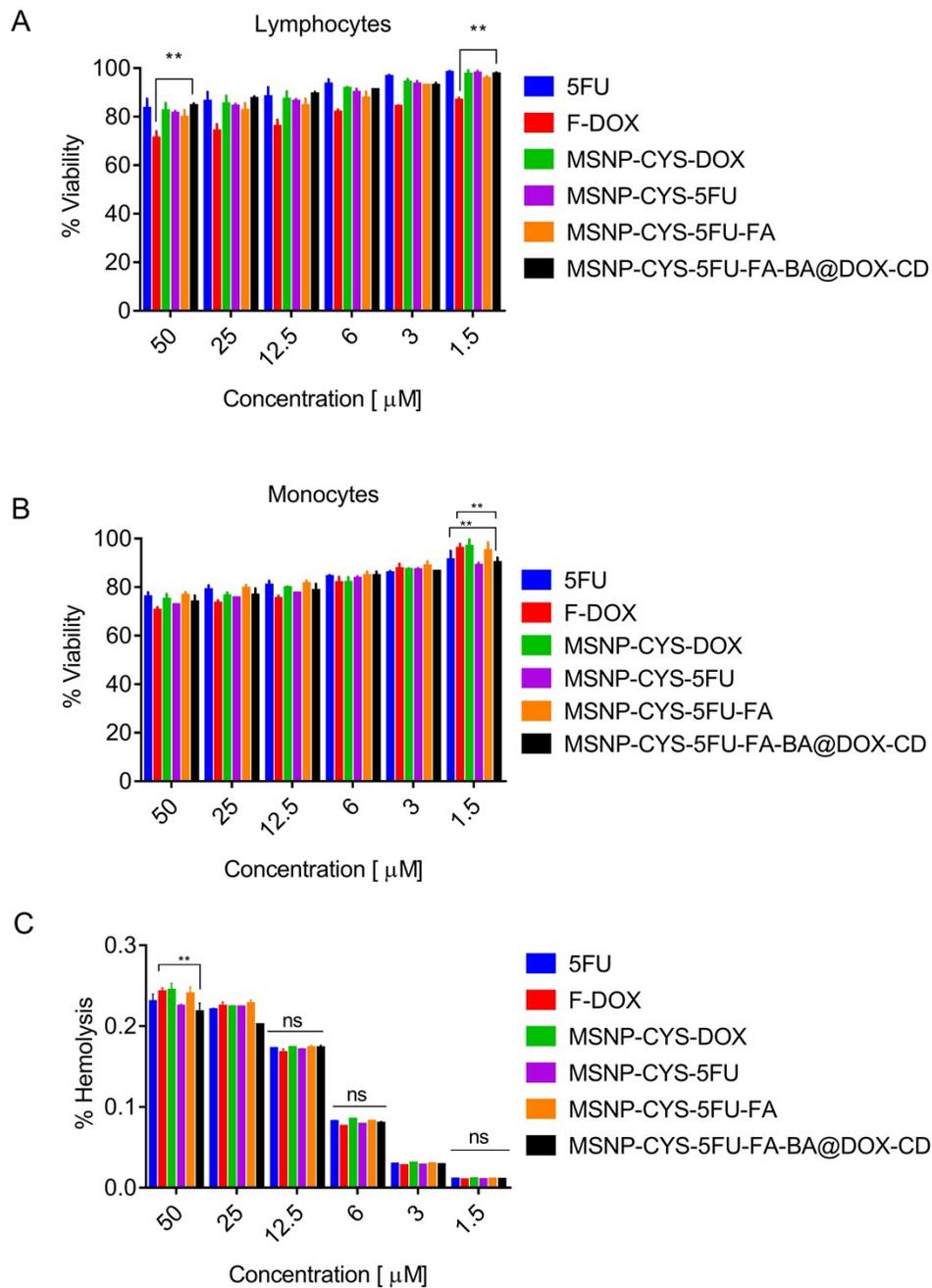
**Figure S6.** Hydrodynamic size of the MSNP-SH, MSNP-CYS and MSNP-CYS-5FU-FA-BA-CD in 10 mM PBS solution (pH 7.4)

# Supporting information



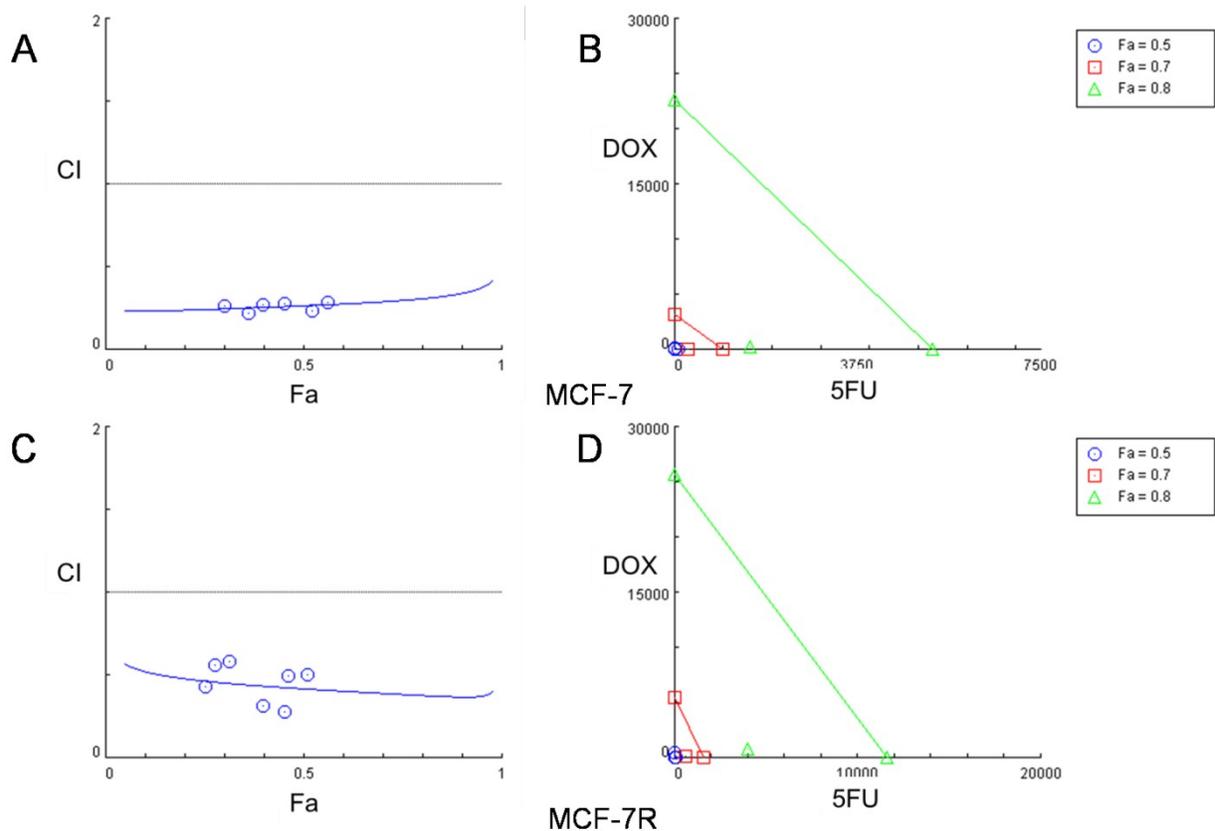
**Figure S7.** Growth inhibition of K562 and K562R (A & B) in the presence of the MSNP-CYS-5FU-FA-BA@DOX-CD and other formulations. Dose dependent cytotoxicity of MSNP-CYS-5FU-FA-BA@DOX-CD and other formulations against K562 and K562R cells (C & D)

## Supporting information



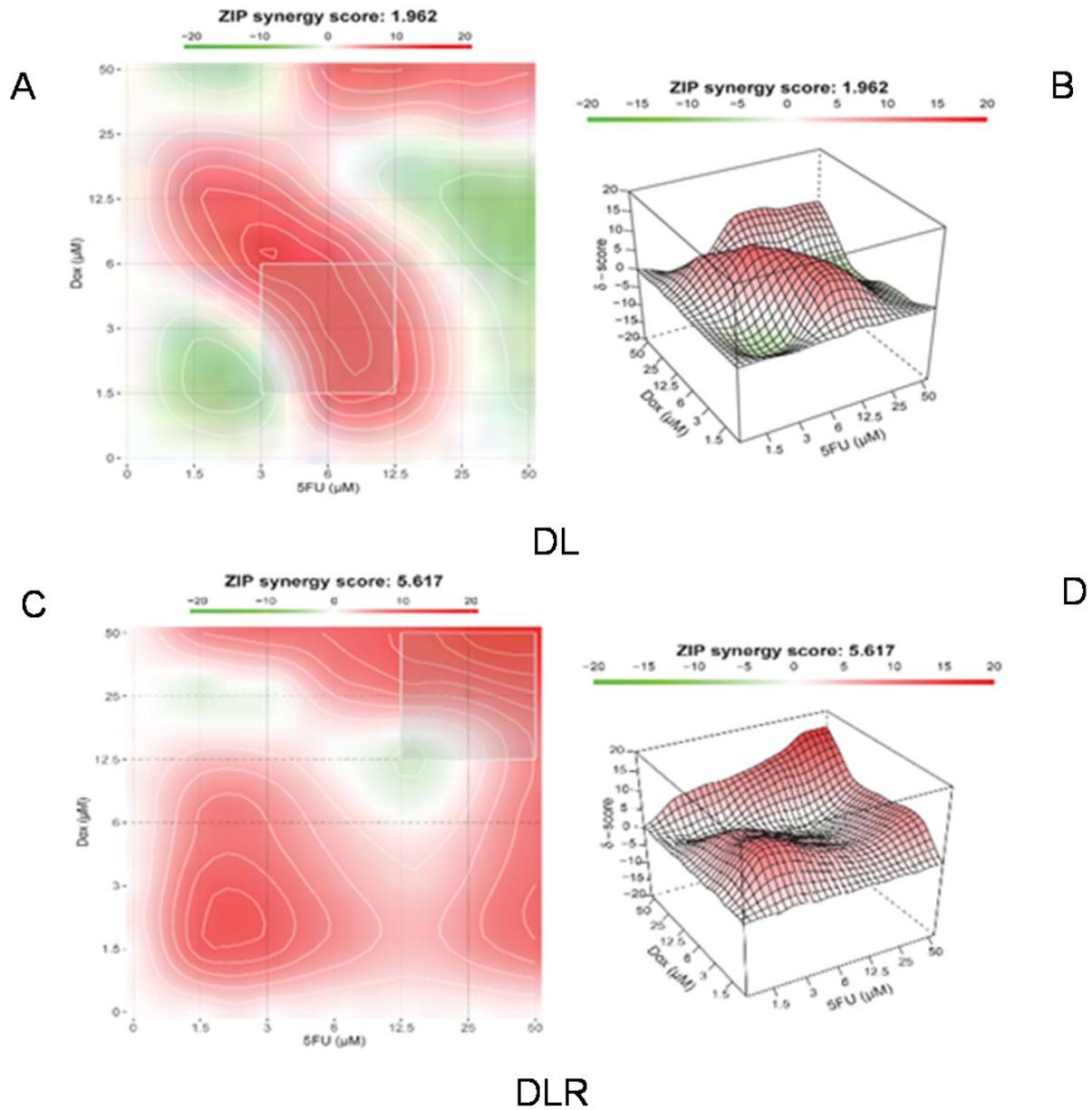
**Figure S8.** Viability of lymphocytes (A), monocytes (B) in the presence of indicated constructs, MSNP-CYS-5FU-FA-BA@DOX-CD and free doxorubicin or 5FU. Hemolysis of the red blood cells in the presence of above-mentioned formulations (C)

## Supporting information



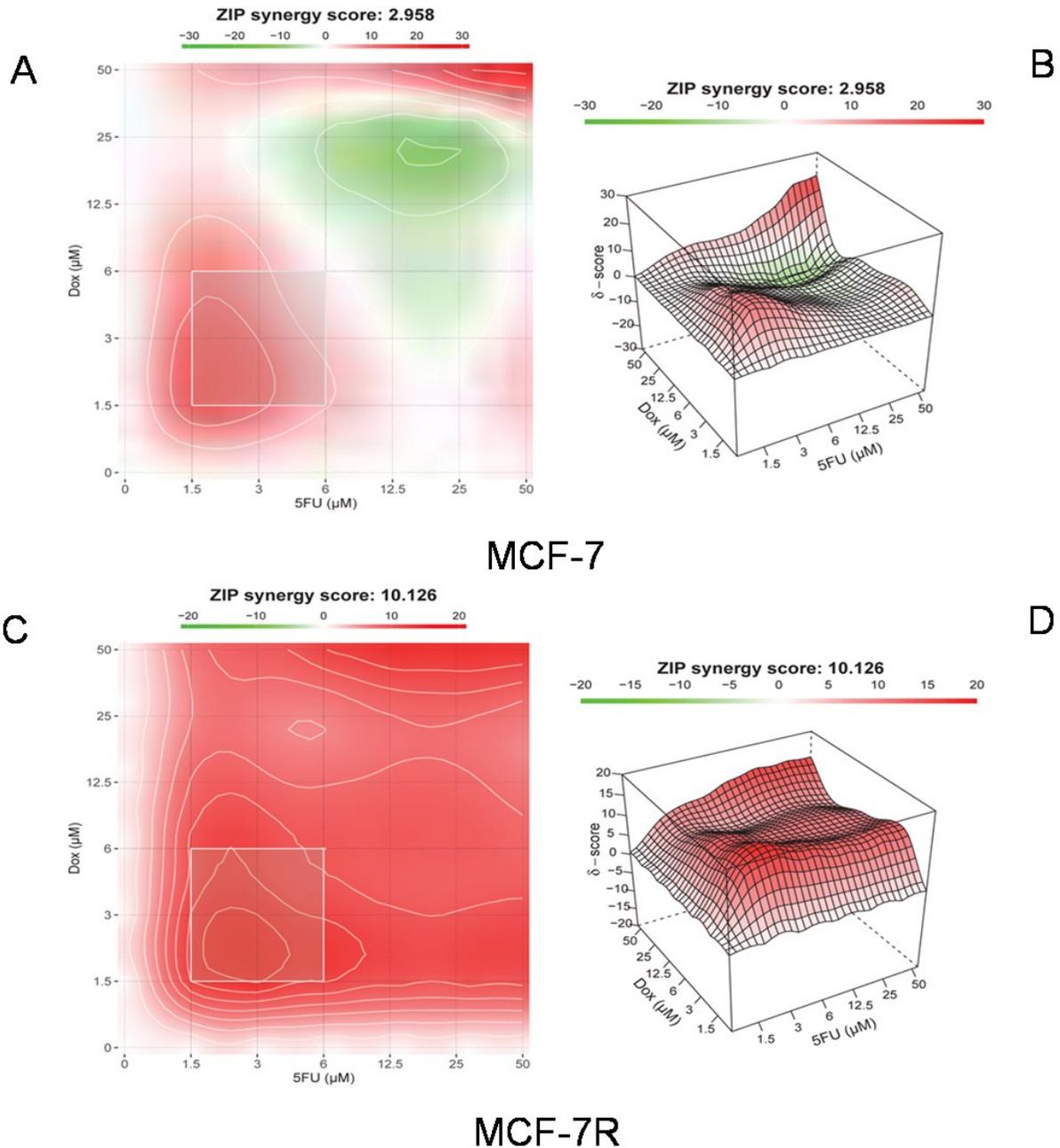
**Figure S9.** Combination effect of therapies with MSNP-CYS-5FU-FA-BA-CD. MCF-7 and MCF-7R cells were treated with the indicated combination of the drugs and analyzed as described in Methods. A and C represents the Fa-CI plot. Blue values above the gray horizontal ‘additive’ line indicate trends towards antagonism. Low CI values with increased Fa values suggest better compatibility and high synergism between the drugs. B and D shows the Isobolograms. The green, red and blue lines indicate where the theoretical additive line is for a particular Fa value, Fa 0.8 (Green), Fa 0.7 (Red) and Fa 0.5 (Blue) respectively. Calculated values closer to the origin indicates greater the synergy between the drugs.

## Supporting information



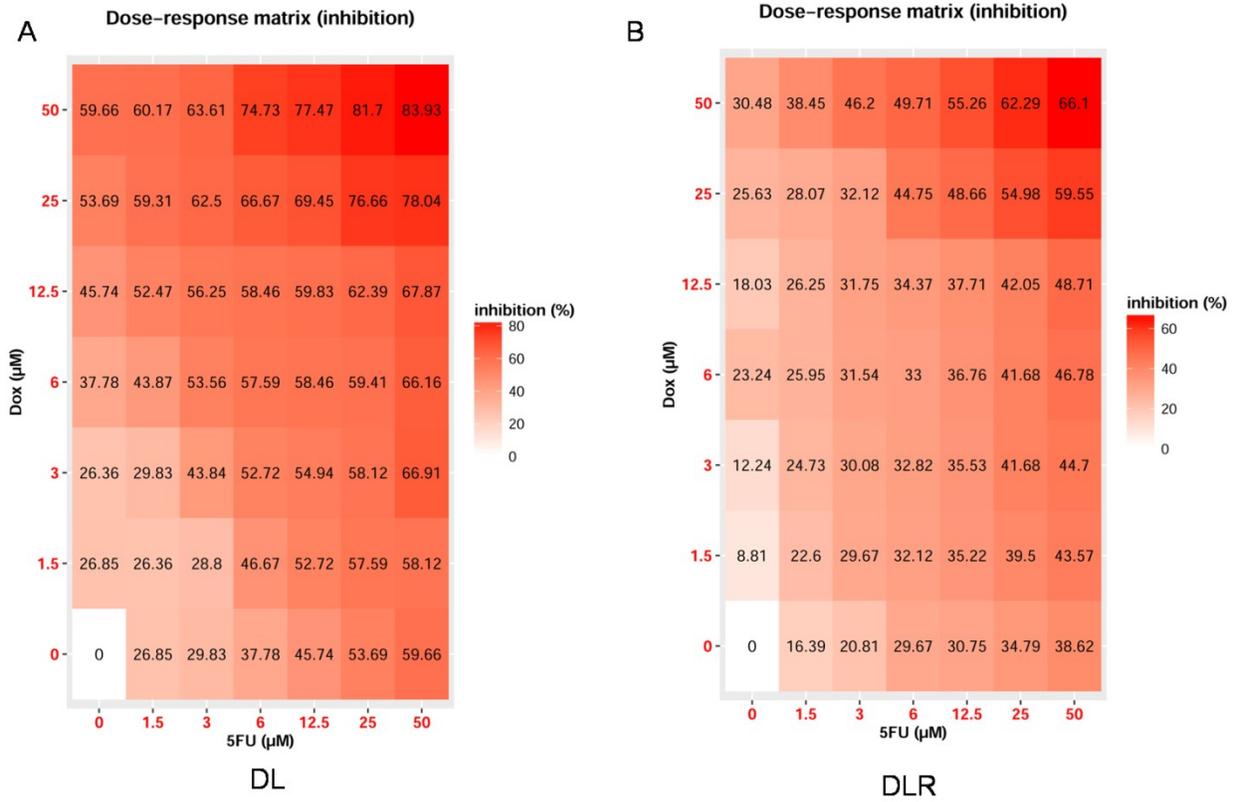
**Figure S10.** Two dimensional (A & C) and three dimensional (B & D) maps of Synergistic and antagonistic interaction landscapes between anti-cancer activity of DOX and 5FU using delta scores ( $\delta$ ) calculated with the zero interaction potency models in DL and DLR cells.

## Supporting information



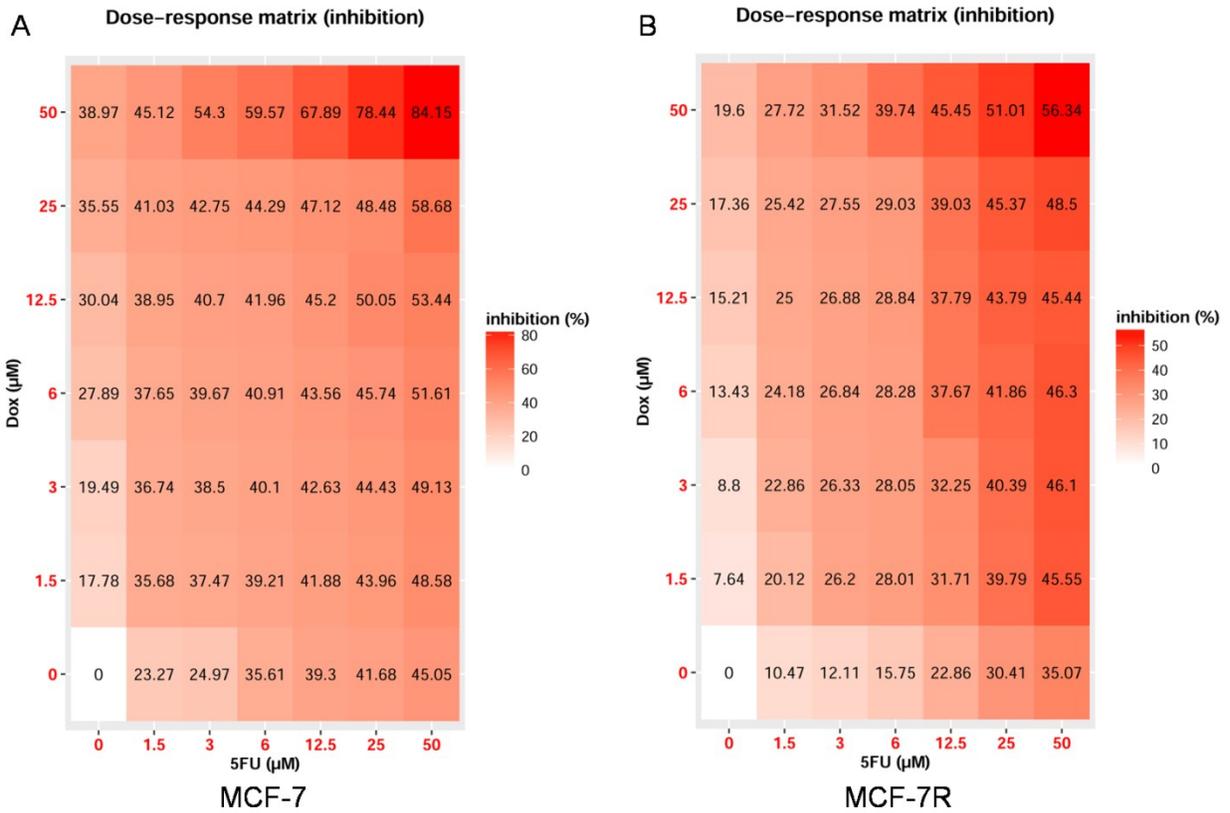
**Figure S11.** Two dimensional (A & C) and three dimensional (B & D) maps of Synergistic and antagonistic interaction landscapes between anti-cancer activity of DOX and 5FU using delta scores ( $\delta$ ) calculated with the zero interaction potency models in MCF-7 and MCF-7R cells.

## Supporting information



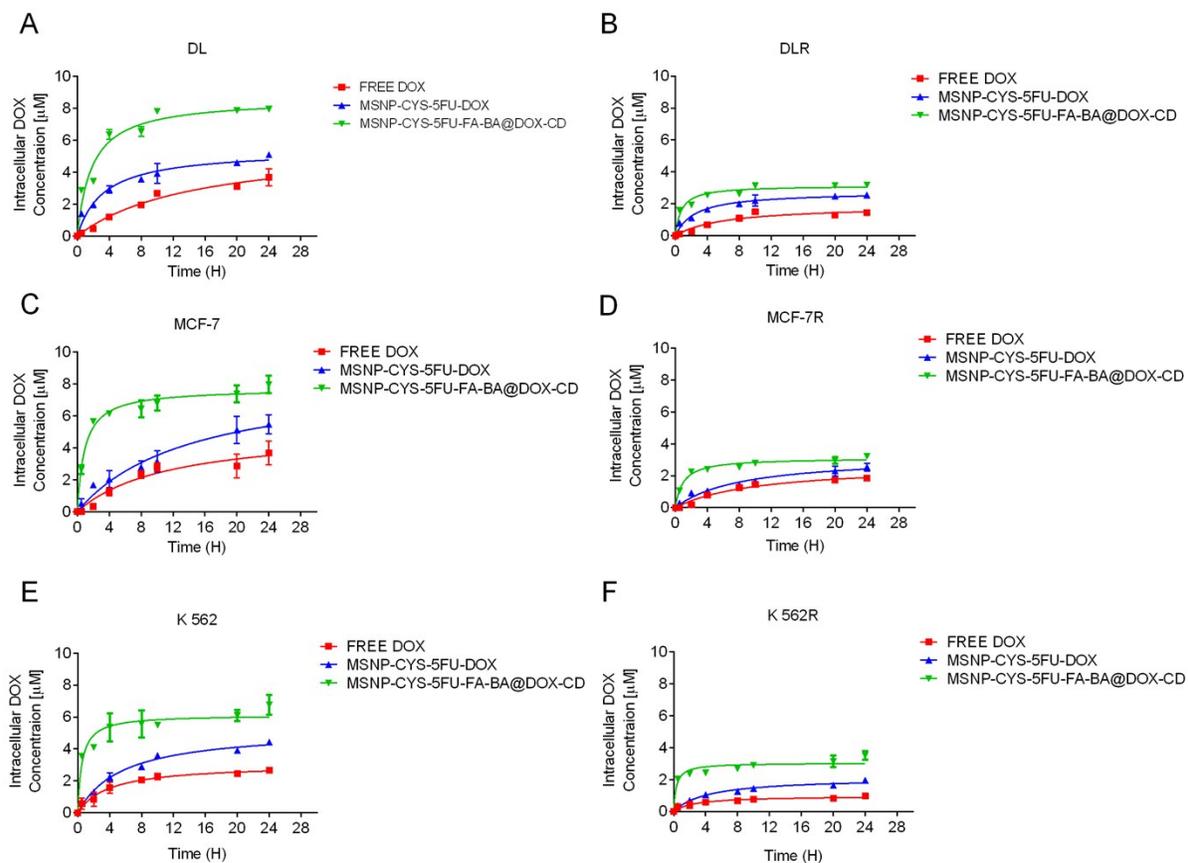
**Figure S12.** Demonstration of dose response matrix for identification of the combination effects of doxorubicin and 5FU across multiple dosages and response levels in DL (A) and DLR (B) cells.

## Supporting information



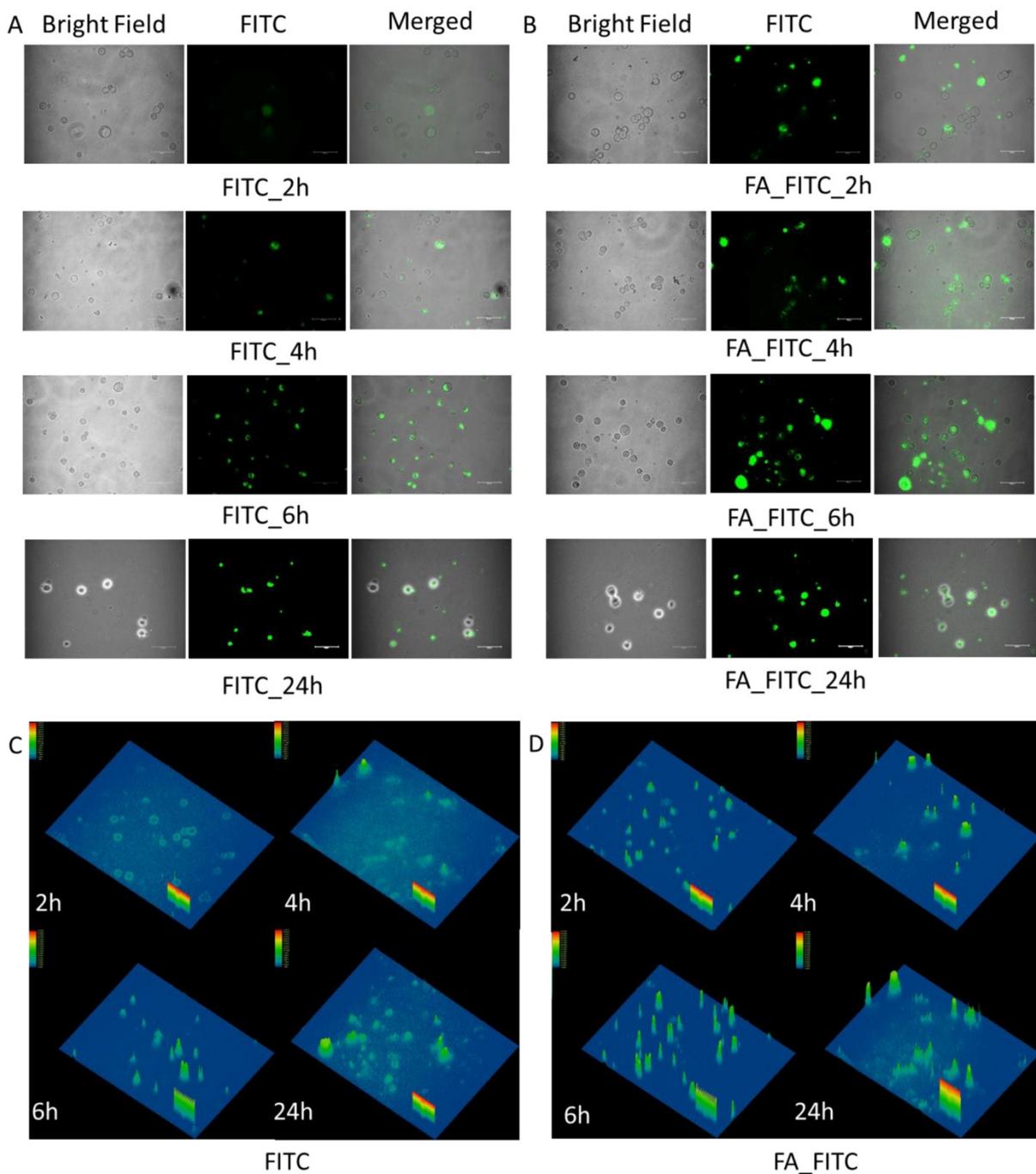
**Figure S13.** Demonstration of dose response matrix for identification of the combination effects of doxorubicin and 5FU across multiple dosages and response levels in MCF-7 (A) and MCF-7R (B) cells.

# Supporting information



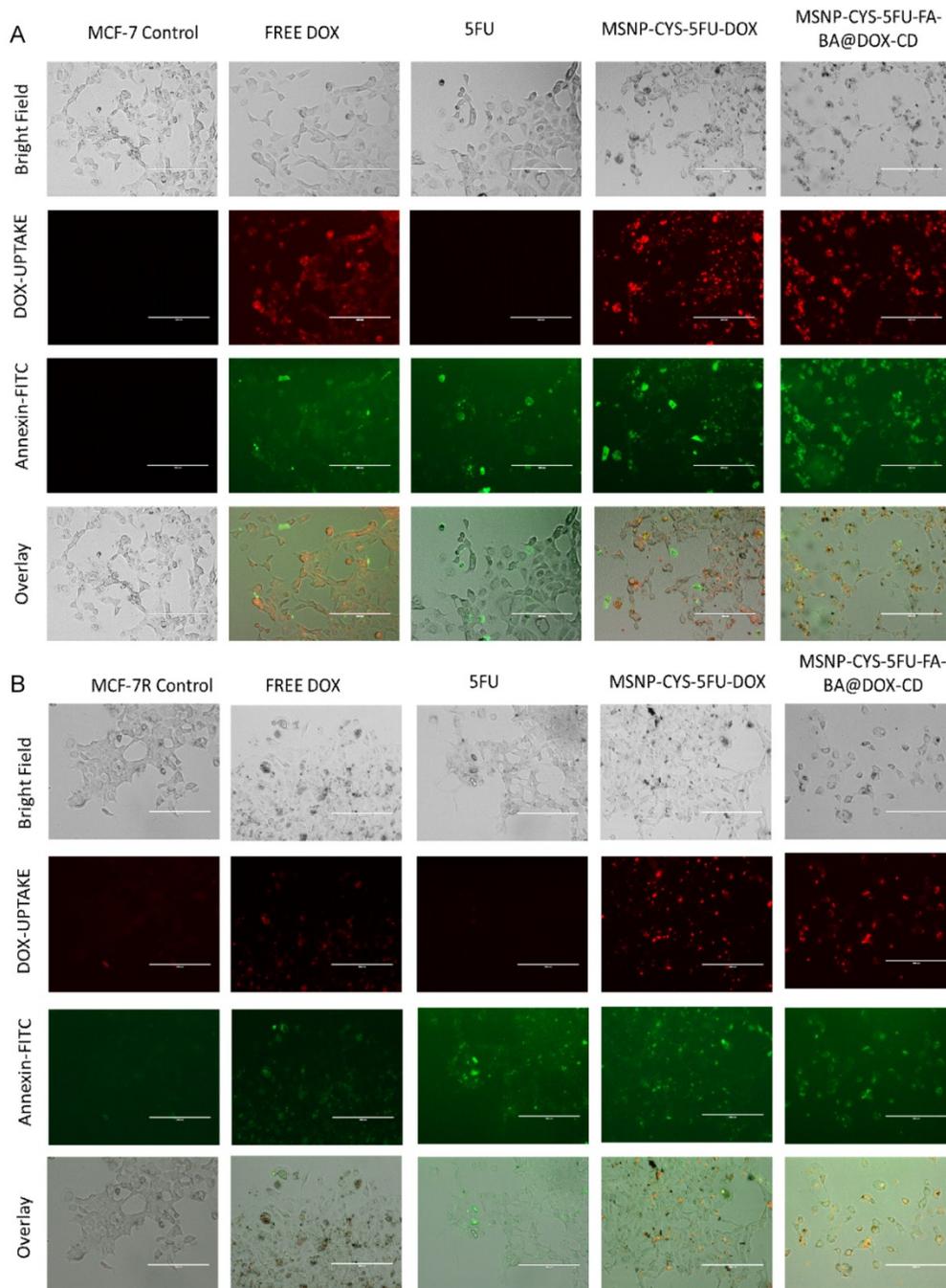
**Figure S14.** Quantitative estimation of doxorubicin uptake in DL and DLR (A & B), MCF-7 and MCF-7R (C & D) and K562 and K562R (E & F) cells

## Supporting information



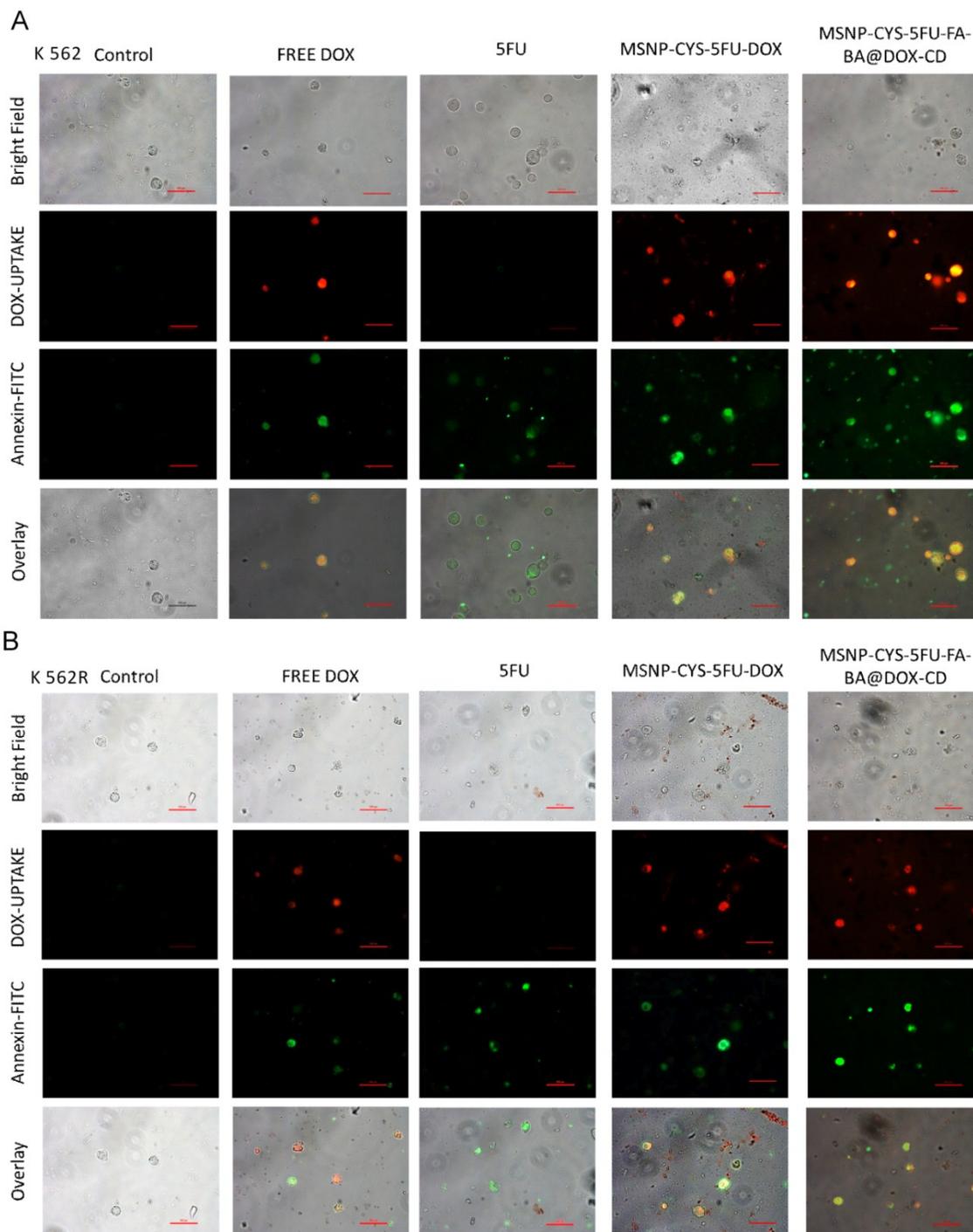
**Figure S15.** Time dependent uptake of FITC conjugated bare MSNP (A) or MSNP tagged with FA (B) in the DL cells. Quantitative analysis of uptake for the above treatment in the DL cells (C & D).

## Supporting information



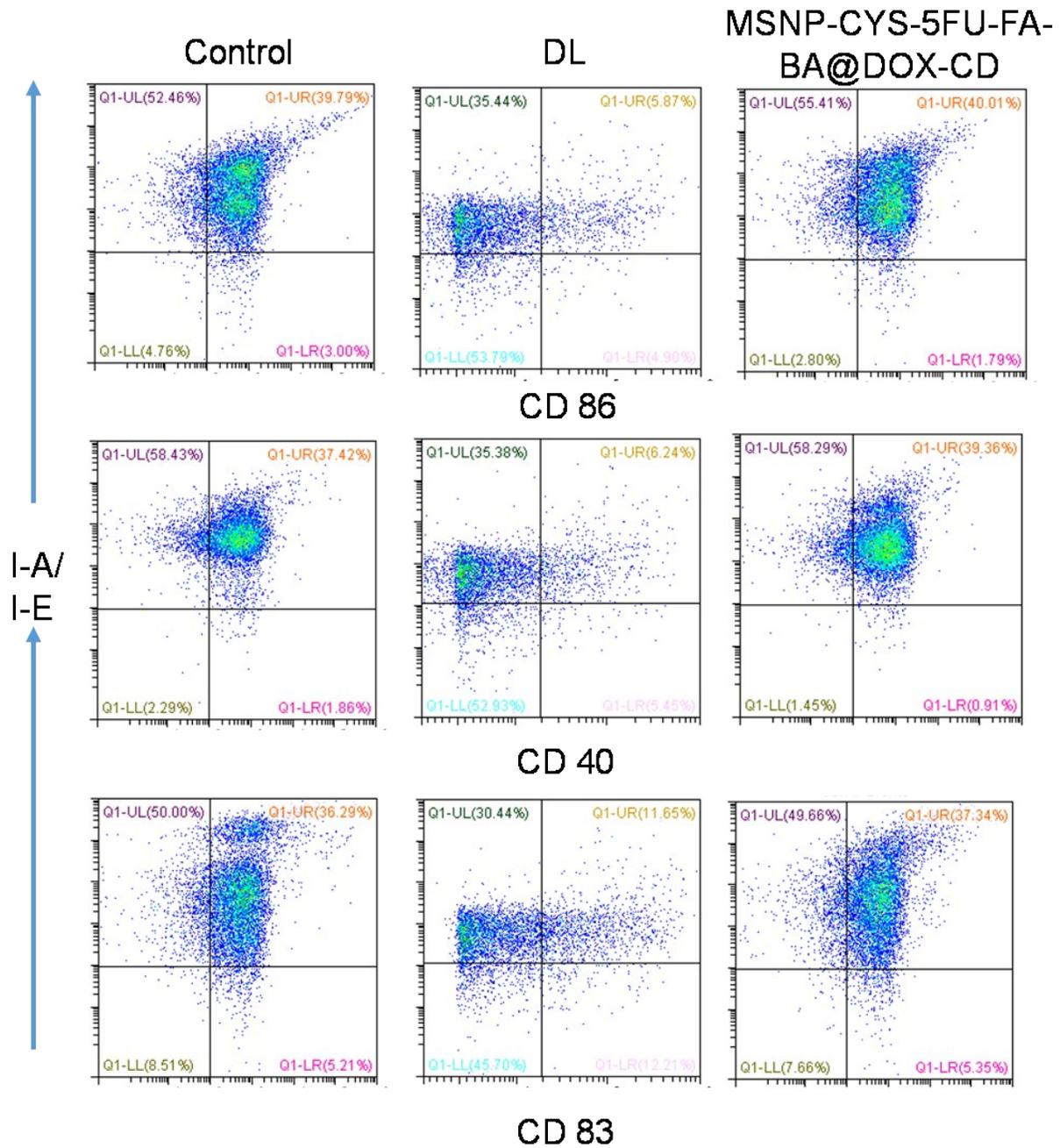
**Figure S16.** Apoptosis of MCF-7 and MCF-7R cells in the presence of MSNP-CYS-5FU-FA-BA@DOX-CD and free doxorubicin or 5FU

## Supporting information



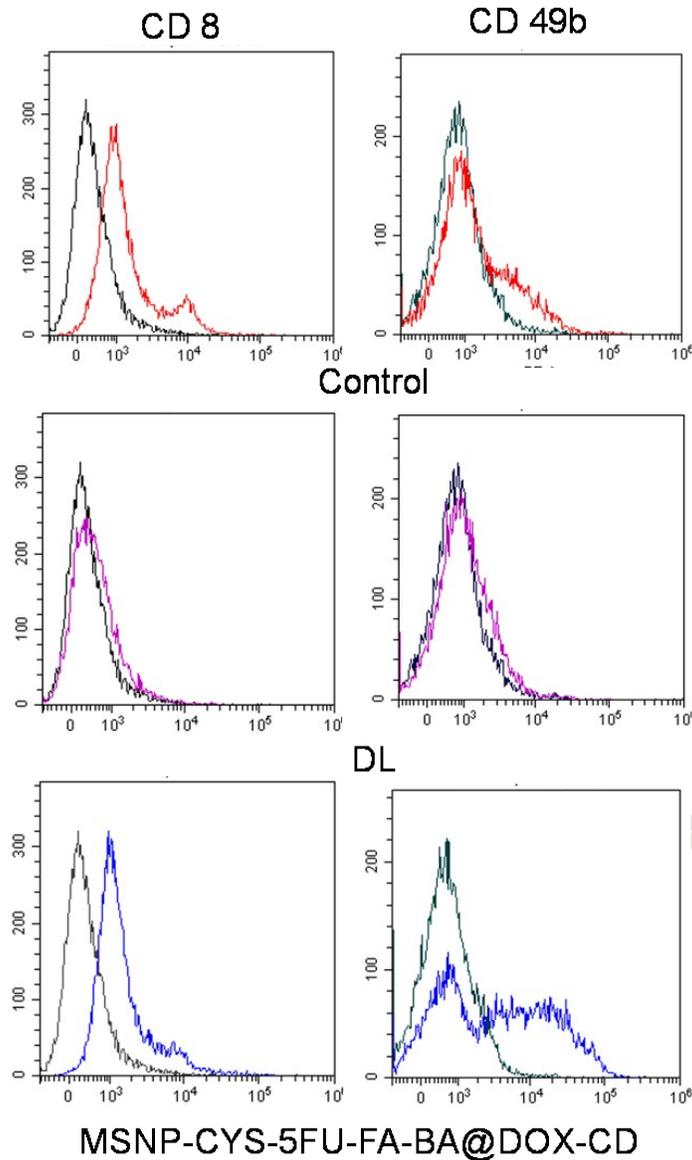
**Figure S17.** Apoptosis of K562 and K562R cells in the presence of MSNP-CYS-5FU-FA-BA@DOX-CD, free doxorubicin or 5FU

## Supporting information



**Figure S18.** FACS analysis for the characterization of purified DC from control, DL tumor bearing and tumor bearing mice treated with MSNP-CYS-5FU-FA-BA@DOX-CD. Dot plot analysis was performed in purified DC using antibodies specific to MHC class II (I-A/I-E) vs CD86, CD40 or CD83.

## Supporting information



**Figure S19.** Histogram analysis of partially purified CD8<sup>+</sup>T cells and natural killer (NK) cells (CD49b) in monocytes, CD19<sup>+</sup> B cells and CD4<sup>+</sup> T cell depleted splenocytes from control, DL tumor bearing and MSNP-CYS-5FU-FA-BA@DOX-CD treated animals. The black histogram represents the isotype control and the overlaid color histograms are the specific staining of the indicated markers