Supporting Information

Hatakeyama et al. 10.1073/pnas.1105057108

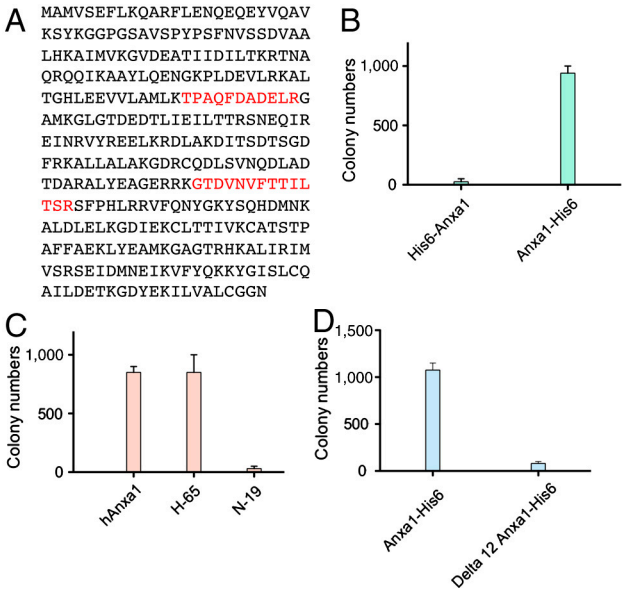

Fig. S1. Identification of annexin 1 as IF7 peptide binding peptide. (*A*) The peptide sequences of the full-length mouse annexin 1 protein and the tryptic peptides identified by proteomics. I-peptide receptor (IPR) proteins, which are responsible for carbohydrate-dependent cancer cell colonization to the lung were purified by an affinity chromatography using carbohydrate-mimicry IELLQAR-peptide (I-peptide) conjugated agarose beads from the rat lung membrane fraction as described previously. The major component was identified as pre-mRNA splicing factor (1). Peptide sequences of the tryptic 15 kDa fragments identified by proteomics analysis are shown by red. (*B*) Binding of IF7 phage to Anxa1. Recombinant Anxa1 proteins expressed in bacteria were coated on plastic wells, IF7 peptide displaying phage was added, and phage binding to Anxa1 proteins was determined by colony forming assay. Binding of IF7 phage to His_6-Anxa1 and Anxa1-His_6. Effect of Anti-Anxa1 antibodies on IF7 phage binding to Anxa1-His_6. Binding of IF7 phage to wild type Anxa1-His_6 and delta 12 Anxa1-His_6.

1 Hatakeyama S, et al. (2009) Identification of mRNA splicing factors as the endothelial receptor for carbohydrate-dependent lung colonization of cancer cells. *Proc Natl Acad Sci USA* 106:3095–3100.

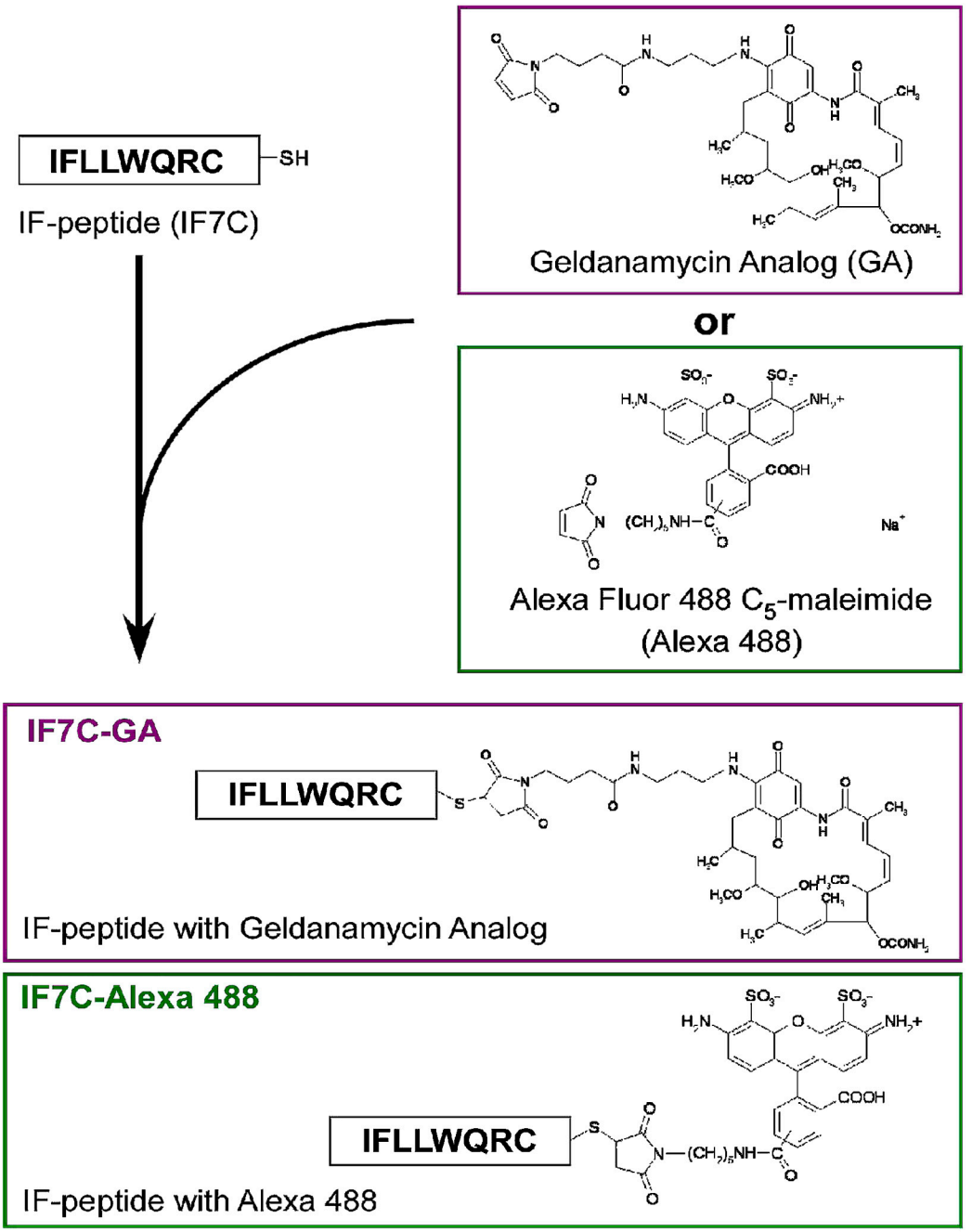

Fig. S2. Conjugation of IF7C peptide with GA and with Alexa 488. A geldanamycin analogue, 17-GMB-APA-GA (GA), was purchased from Invivogen (San Diego, CA). GA was also synthesized from geldanamycin (LC labs, Woburn, MA) as described by Mandler, et al. (1). Briefly, GA was dissolved in chloroform and mixed with 1,3-diaminopropane (Sigma-Aldrich) under argon gas at room temperature for 20 h. Diaminopropane cross-linked GA was precipitated with hexane. The precipitate was dissolved in chloroform, and was reacted with N-[g-maleimidobutyryloxy] succinimide ester (Pierce, Rockford, IL) at room temperature for 2 h. The product or 17-GMB-APA-GA was purified by thin layer chromatography using preparative TLC plate (1.5 mm silica gel, Analtech, Newark, DE) in solvent system, dichloromethane:methanol (92:8, vol/vol). The structure of 17- GMB-APA-GA was verified by ESI mass spectrometry (Micromass ZQ) with MASSLYNX ver3.5 (Waters Corp., Milford, MA). To conjugate IF7C peptide with 17-GMB-APA-GA, they were dissolved in methanol at 1:1 molar ratio. Equal volume of purified water was added for the mixture, and was left at room temperature for 2 h. The product, IF7C-GA, was purified by C18 reverse-phase HPLC column (10 × 150 mm) by gradient elution from 40% to 50% acetonitrile in water containing 0.1% (vol/vol) trifluoroacetic acid at a flow rate of 2.5 mL/min. The purity and structure of IF7C-GA was assessed by ESI mass spectrometry. IF7C was also conjugated with Alexa fluor 488 C5-maleimide (Invitrogen, Carlsbad, CA) and purified by HPLC in a similar manner as described above.

1 Mandler R, et al. (2000) Immunoconjugates of geldanamycin and anti-HER2 monoclonal antibodies: antiproliferative activity on human breast carcinoma cell lines. *J Natl Cancer Inst* 92:1573–1581.

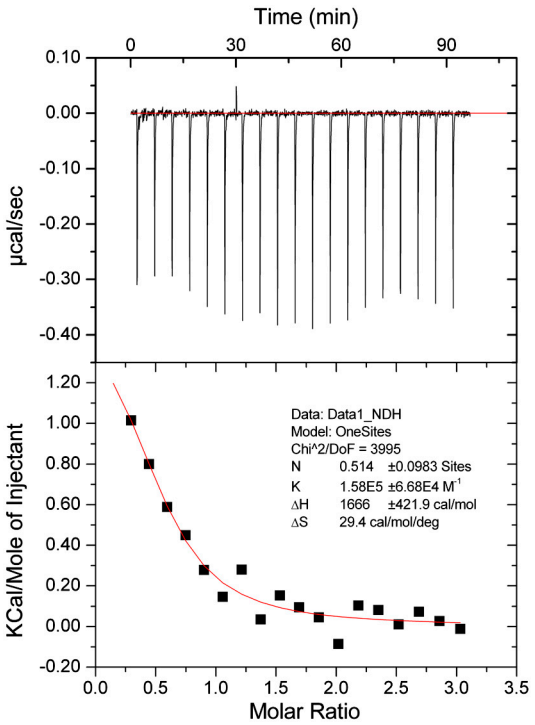

Fig. S3. Isothermal colorimetry analysis of Anxa1-His_6 and IFLLWQR-C-RR peptide. Recombinant Anxa1-His_6 protein was produced in bacteria as described in the main body text under *Materials and Methods*. Two additional arginine residues were added after cysteine residue to increase solubility of IF7 peptide in water. Note that the binding sites/Anxa1-His_6 is 0.514, suggesting that dimer of Anxa1-His_6 interacts with IF7 peptide.

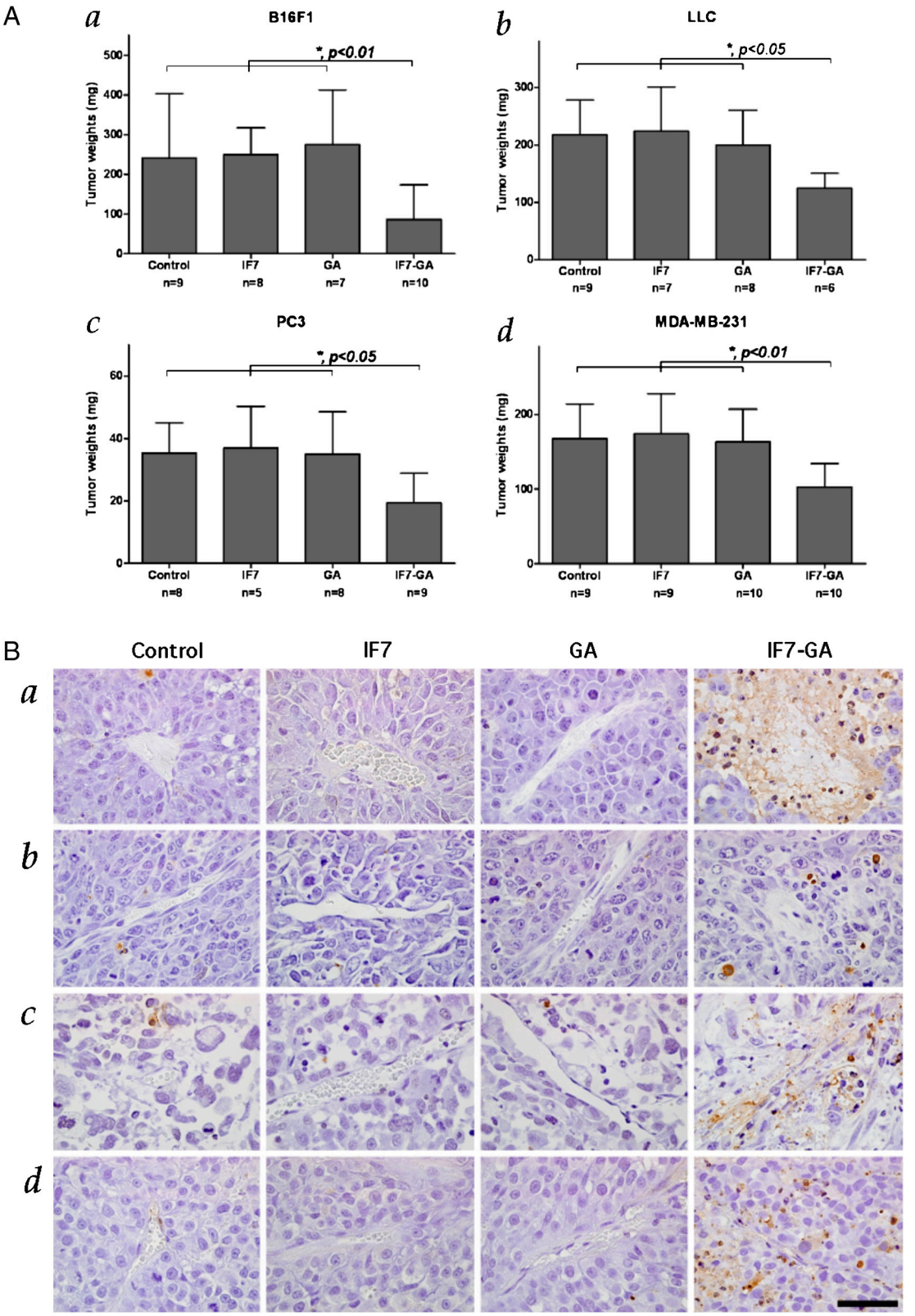

Fig. S4. Effect of IF7-GA on melanoma, lung carcinoma, prostate cancer, and breast cancer models in the mouse. (*A*) Size of tumors measured by caliper. (*a*) Mouse melanoma B16F1 tumors were grown subcutaneously in C57BL6 mice. On day 10, each mouse was injected intravenously with either 100 μL of 5% glucose or that containing 0.13 μmoles of each IF7, GA, or IF7-GA. Injections were administered every other day, for a total of three injections, until day 14. Mice were euthanized on day 15 to measure tumor weight. (*b*) Mouse Lewis lung carcinoma (LLC) lung carcinoma tumors were grown subcutaneously in C57BL6 mice. On day seven, each mouse was injected intravenously with the compounds as in *A*–*a*, and injections administered every other day, for a total of three injections, until day 11. Mice were euthanised on day 13 and tumors weighed. (*c*) Human prostate cancer PC3 tumors were grown orthotopically in the prostate of SCID mice. On day seven, each mouse was injected intravenously with the compounds as in *A*–*a*, and injections administered every 4 d, for a total of four injections, until day 22. Mice were euthanized on day 28 and tumors weighed. (*d*) Human breast cancer MDA-MB-231 tumors were grown orthotopically in fat

pads of SCID mice. On day seven, each mouse was injected intravenously with the compounds as in *A–a*, and injections performed every 4 days, for a total of four injections, until day 22. Mice were euthanized on day 28 and tumors weighed. Asterisks show statistical significance (Mann-Whitney's U test). (*B*) Histochemistry of tumors from the mice intravenously injected with the compounds shown in (*A*). Apoptotic tumor cells along blood vessels in transplanted tumors were detected by TUNEL assay. B16 melanoma (*a*), LLC lung carcinoma (*b*), PC3 prostate tumor (*c*) and MDA-MB-231 breast tumor (*d*) from mice treated with each compound. Note that perivascular cancer cells rarely show apoptosis in control IF7, and GA groups, while perivascular tumor cells in the IF7-GA groups show greater numbers of apoptotic cells. (Scale bar, 50 μm).

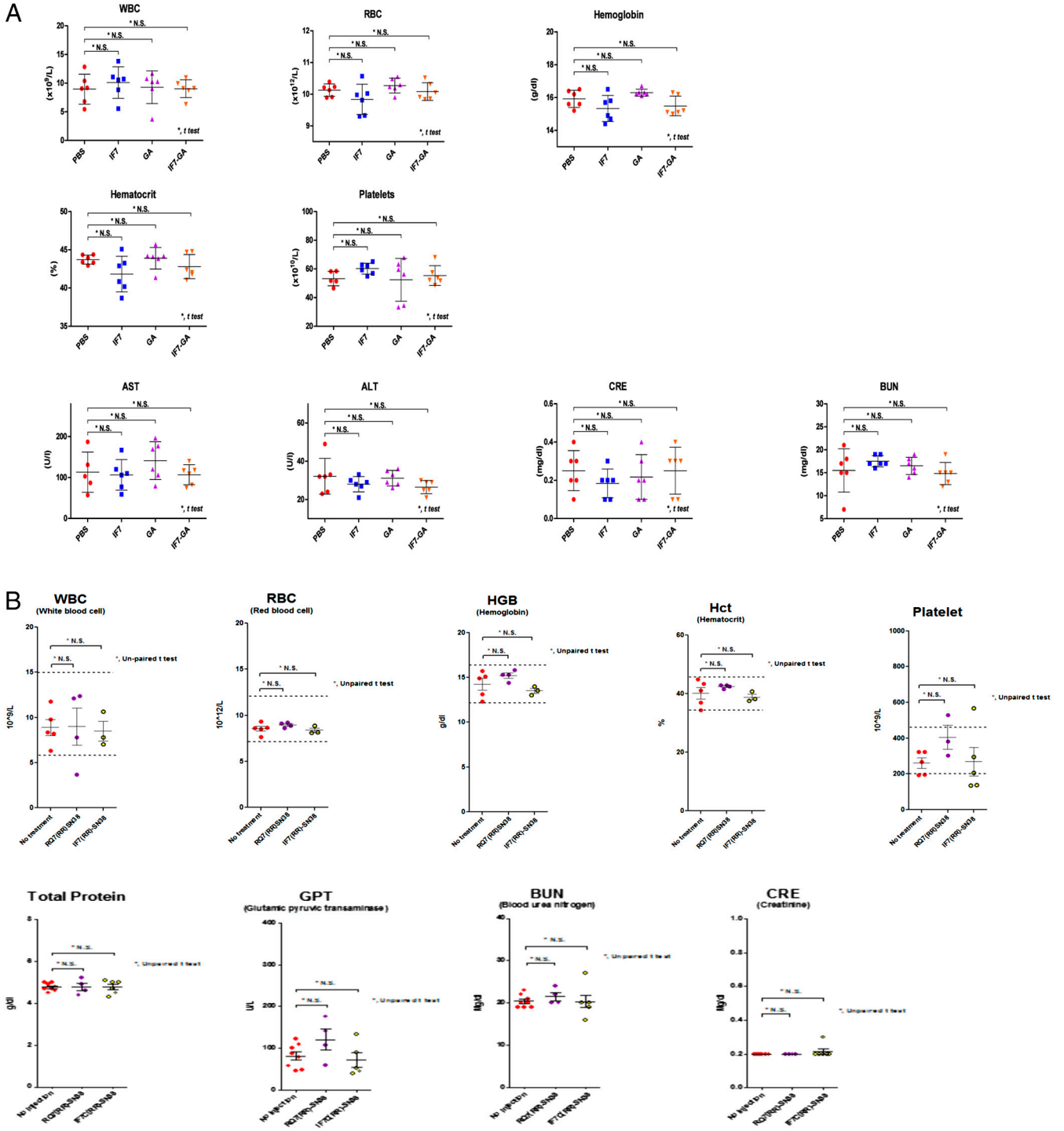

Fig. S5. Blood test of mice subjected to intravenous injection of IF7-GA and IF7C(RR)-SN38. (*A*) Blood test of mice injected with IF7-GA. (*B*) Blood test of mice injected with IF7C(RR)-SN38. WBC, white blood cells; RBC, red blood cells; AST, aspartate aminotransferase to evaluate liver function; ALT, alanine transaminase to evaluate liver function; CRE, creatine to evaluate kidney function; BUN blood urea to evaluate kidney function. Statistical analysis unpaired *t*-test was applied. *N.S.: statistically not significant.

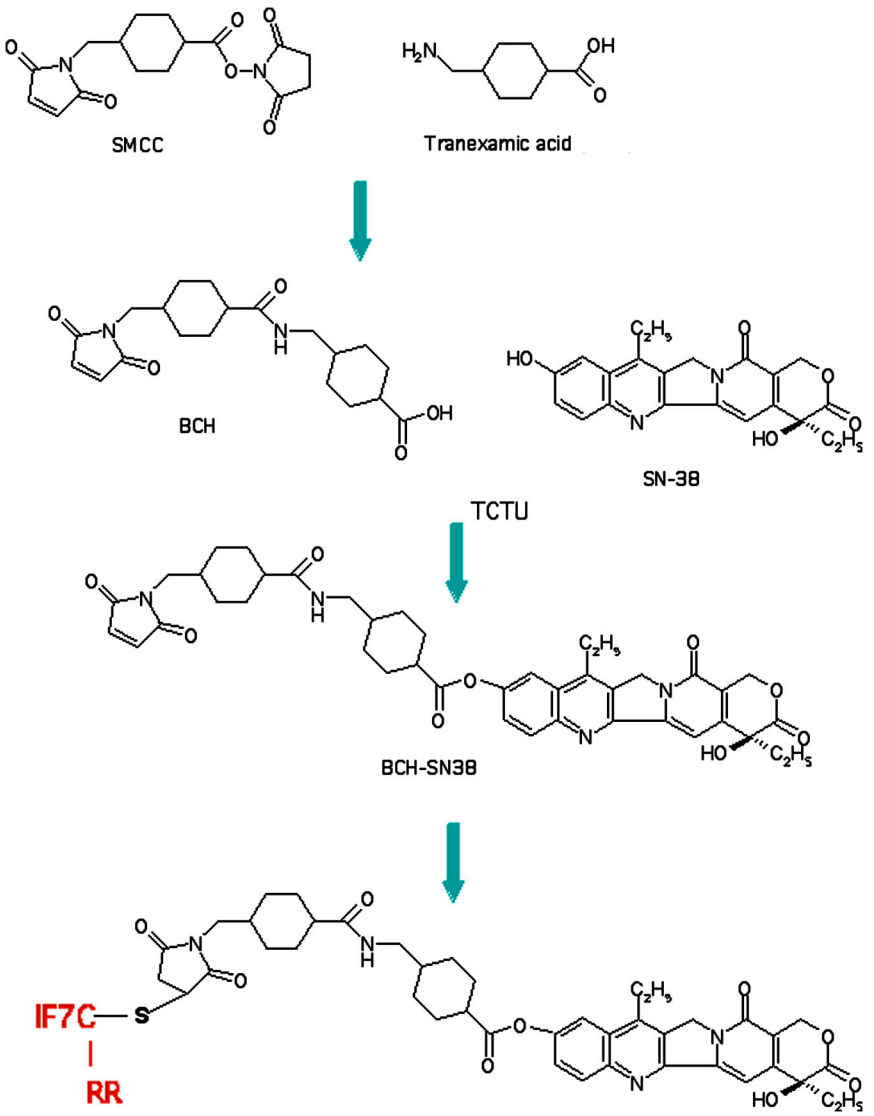

Fig. S6. Conjugation of IF7C(RR) with SN-38. Conjugation of IF7C(RR) with SN-38 followed the method described by Meyer-Losic F, et al (1) with modifications. Details are described in main body text under *Materials and Methods*.

1 Meyer-Losic F, et al. (2008) DTS-108, a novel peptidic prodrug of SN38: in vivo efficacy and toxicokinetic studies. *Clin Cancer Res* 14:2145–2153.

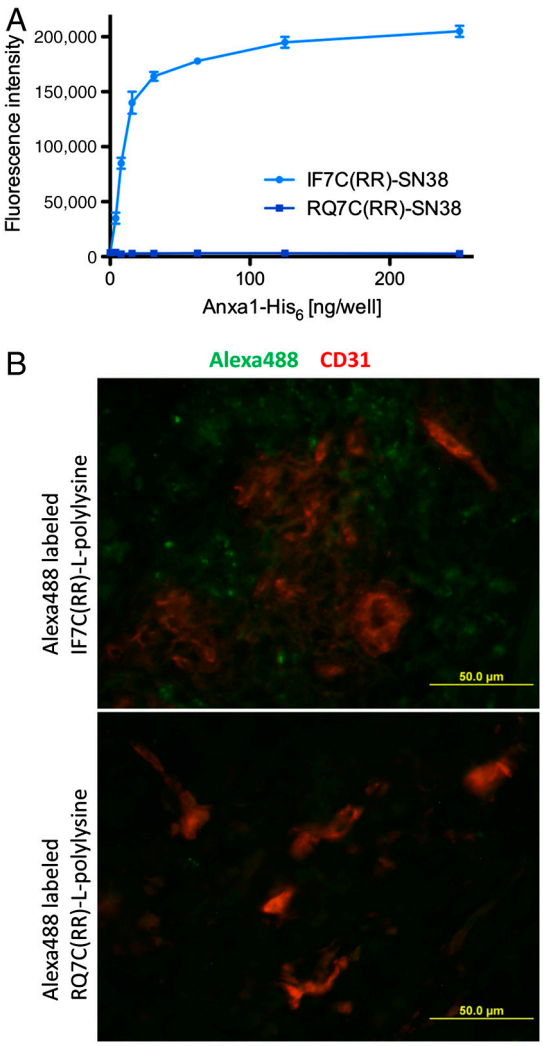

Fig. S7. Binding of IF7C(RR) to Anxa1 in vitro and in vivo. (*A*) Binding of IF7C(RR)-SN38 to Anxa1-His_6 protein. Binding assay was performed in the same manner as for IF7-A488 shown in Fig. 2*A*. Binding was monitored by fluorescence by SN-38. (*B*) Fluorescence micrographs of HCT116-Luc tumors from mice intravenously injected with Alexa 488-labeled IF7C(RR)-conjugated poly-L-lysine or with Alexa488-labeled RQ7C(RR)-conjugated poly-L-lysine. Tumors were isolated 40 min after the injection of each probe. Note that IF7C(RR)-conjugated probe penetrated the tumor tissue. Tissue section was stained by anti-CD31 antibody followed by rhodamine-conjugated anti-rat IgG antibody.

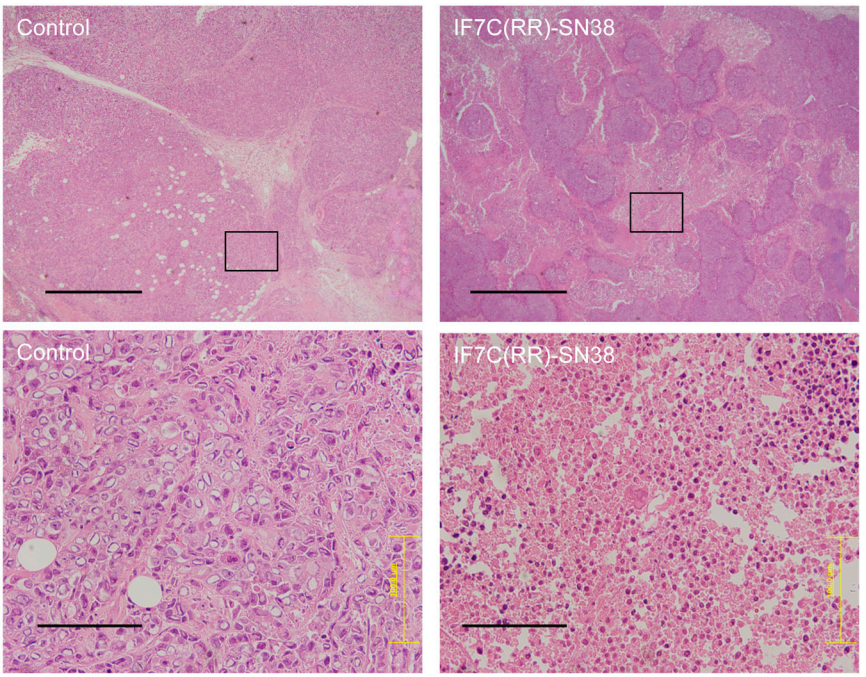

Fig. S8. Histologies of HCT116-Luc tumors from mice injected with IF7C(RR)-SN38. Tumor tissue sections from mice intravenously injected daily with or without low dose (1.63 μmoles/kg) IF7C(RR)-SN38 for 10 d were stained by hematoxylin and eosin. Representative images for low magnification [upper box, (scale bar, 1,000 μm)] and high magnification [lower box, (scale bar, 100 μm)] are shown. Lower box images are enlargement of each insert shown in upper box.

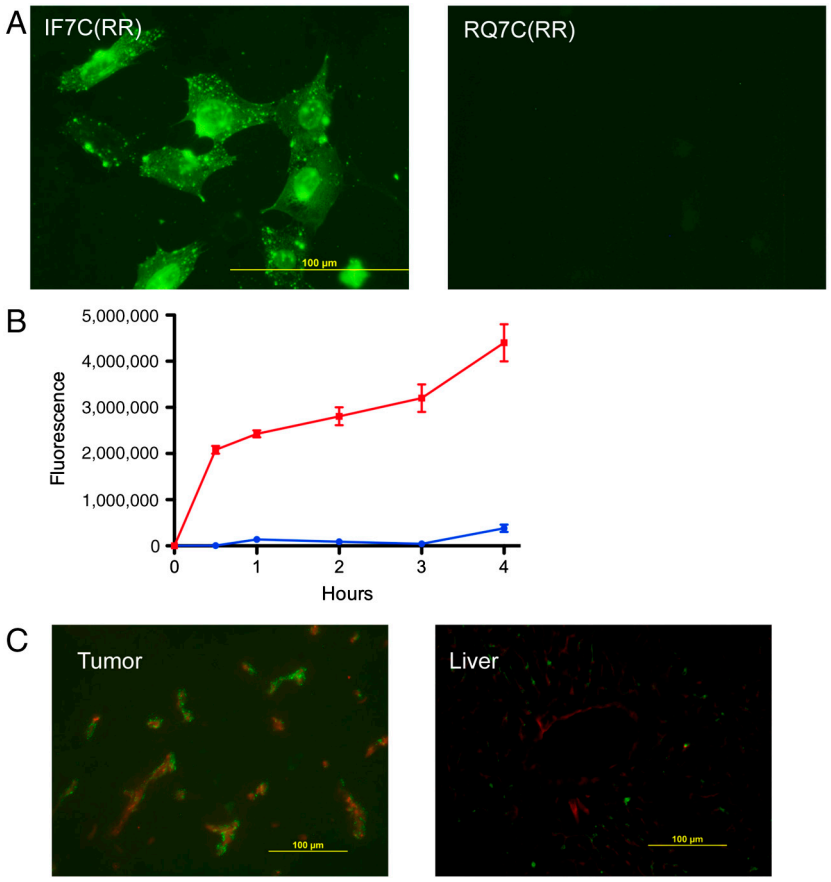

Fig. S9. Binding and penetration of FITC-labeled and IF7C(RR)-conjugated poly-L-lysine to mouse endothelial F-2 cells expressing Anxa1 on cell surface. (*A*) Fluorescence microscopy of mouse endothelial F-2 cells added with FITC-labeled and IF7C(RR)-conjugated poly-L-lysine (left) or FITC-labeled RQ7C(RR)-conjugated poly-L-lysine (right). Fluorescent probe was added to live F-2 cell, washed with PBS, and images were observed under a fluorescence microscope. (*B*) FITC-labeled and IF7C(RR)-conjugated poly-L-lysine were bound to F-2 cells cultured on a filter of transwell insert. After washing cells, fluorescence in the lower chamber at 37 °C (red) and at 4 °C (blue) was measured. (*C*) Tumor tissue sections of FITC-labeled and IF7C(RR)-conjugated poly-L-lysine intrevenously injected to B16 tumor bearing mice. Frozen tissue sections were made at 20 min after injection and immunostainined by monoclonal rat anti-CD31 antibody followed by rhodamine-anti-rat IgG antibody.

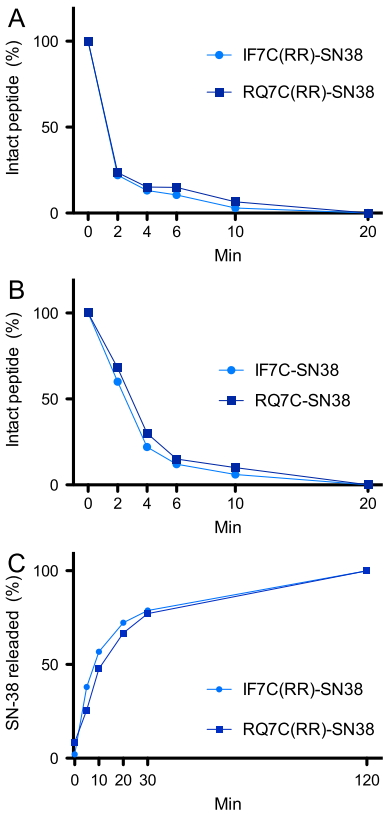

Fig. S10. Stability of IF7C(RR)-SN38 and RQ7C(RR)-SN38 in the mouse plasma. Fresh mouse plasma was prepared by collecting mouse blood in serum separation tube (BD Microtainer) and immediately centrifuged at 12,000 rpm for 10 min. Each 20 μg IF7C(RR)-SN38 and IF7C(RR)-SN38 was dissolved in 1 μl DMSO, added to 100 μL plasma, and incubated at 37 °C. Aliquot (10 μL) was withdrawn at the time indicated, added to 200 μL acetonitrile: water (25:75) containing 0.1% TFA and 25 ng CPT-11 (an internal marker for quantitative analysis as reported by Guo et al. (1). Insoluble material was removed by centrifugation and supernatant was injected to Shimadzu SCL-10A HPLC equipped with ZORBAX ODS reverse-phase column (4.6 × 250 mm), which was eluted by gradient from acetonitrile 20% to 70% over 20 min at flow rate 1 mL/ min. Elution was monitored by Shimaddzu RF-535 fluorescence HPLC monitor at excitation wave length of 355 nm and emission wave length of 515 nm. (*A*) Relative amount of IF7C(RR)-SN38 ($IC_{50} = 1.28$ min) and RQ7C(RR)-SN38 ($IC_{50} = 1.30$ min) remained in mouse plasma incubated at 37 °C. (*B*) Relative amount of IF7C-SN38 ($IC_{50} = 2.50$ min) and RQ7C-SN38 ($IC_{50} = 3.12$ min) in mouse plasma incubated at 37 °C. (*C*) Relative amount of SN-38 released from IF7C(RR)-SN38 and RQ7C(RR)-SN38 in mouse plasma incubated at 37 °C.

1 Guo W, et al. (2003) Determination by liquid chromatography with fluorescence detection of total 7-ethyl-10-hydroxy-camptothecin (SN-38) in beagle dog plasma after intravenous administration of liposome-based SN-38 (LE-SN38). *J Chromatogr B Analyt Technol Biomed Life Sci* 791:85–92.