Supplemental Tables

Table S1. Anti-mPEG affinities of TsAb and BsAbs

Antibodies	EC50 (nM) [a)]
anti-HER2/anti-mPEG BsAb	3.505
anti-FAP/anti-mPEG BsAb	3.581
anti-HER2/anti-FAP/anti-mPEG TsAb	3.687

[a)] EC50 was determined by the value from anti-mPEG ELISA. Methoxyl-PEG5000-NH_2-coating ELISA plate was stained with serially diluted TsAb and BsAbs. The bound TsAb and BsAbs were further detected with HRP-conjugated goat anti-human IgG F(ab')2 and ABTS substrate.

Table S2. IC50 (μg/mL) of TsAb- or BsAb-Lipo-Dox against co-culture of MCF-7/HER2 and WS-1/FAP.

Nanomedicines [a)]	Ratios of MCF-7/HER2 to WS-1/FAP [b)]		
	1:0.2	1:1	1:5
Lipo-Dox	>100 [c)]	>100 [c)]	48.806
anti-HER2-Lipo-Dox	39.231	29.928	70.991
anti-FAP-Lipo-Dox	>100 [c)]	37.912	20.352
anti-HER2/anti-FAP-Lipo-Dox	39.688	16.845	24.021

[a)] Nanomedicines were prepared by mixing Lipo-Dox with buffer (PBS + 0.05 BSA), anti-HER2/anti-mPEG BsAb, anti-FAP/anti-mPEG BsAb, anti-HER2/anti-FAP/anti-mPEG TsAb.

[b)] MCF-7/HER2 cells and WS-1/FAP cells were mixed with at the indicated cell ratios (1:0.2, 1:1, and 1:5), seeded into 96-well plates (4000 cells per well), and then treated with the serially diluted nanomedicines.

[c)] Values of > 100 μg/mL indicate the IC50 value is greater than the maximum concentration.

Table S3. Tumor size of SCID mice bearing with TAF-rich breast tumor at week 6.

Nanomedicines [a)]	Tumor size (mm^3) [b)]	*P* value [c)]
Vehicle	879.2 ± 155.0	
Lipo-Dox	456.1 ± 174.1	
anti-HER2-Lipo-Dox	353.0 ± 54.6	0.3092
anti-FAP-Lipo-Dox	362.1 ± 81.3	0.3777
anti-HER2/anti-FAP-Lipo-Dox	211.6 ± 63.4	0.0054

[a)] Nanomedicines were prepared by mixing Lipo-Dox with buffer (PBS + 0.05% BSA), anti-HER2/anti-mPEG BsAb, anti-FAP/anti-mPEG BsAb, anti-HER2/anti-FAP/anti-mPEG TsAb, and then were weekly i.v. injected for 5 times.

[b)] mean ± SD, n = 5.

[c)] *P* value when compared with the Lipo-Dox treatment group.

Table S4. Tumor size of SCID mice bearing with TAF-free breast tumor at week 6.

Nanomedicines [a)]	Tumor size (mm^3) [b)]	*P* value [c)]
Vehicle	924.3±124.5	
Lipo-Dox	485.7±111.2	
anti-HER2-Lipo-Dox	296.4±26.7	0.2581
anti-FAP-Lipo-Dox	520.8±143.7	0.6277
anti-HER2/anti-FAP-Lipo-Dox	293.2±75.7	0.7125

[a)] Nanomedicines were prepared by mixing Lipo-Dox with buffer (PBS + 0.05% BSA), anti-HER2/anti-mPEG BsAb, anti-FAP/anti-mPEG BsAb, anti-HER2/anti-FAP/anti-mPEG TsAb, and then were weekly i.v. injected for 5 times.

[b)] mean ± SD, n = 5.

[c)] *P* value when compared with the Lipo-Dox treatment group.

Supplemental Figures

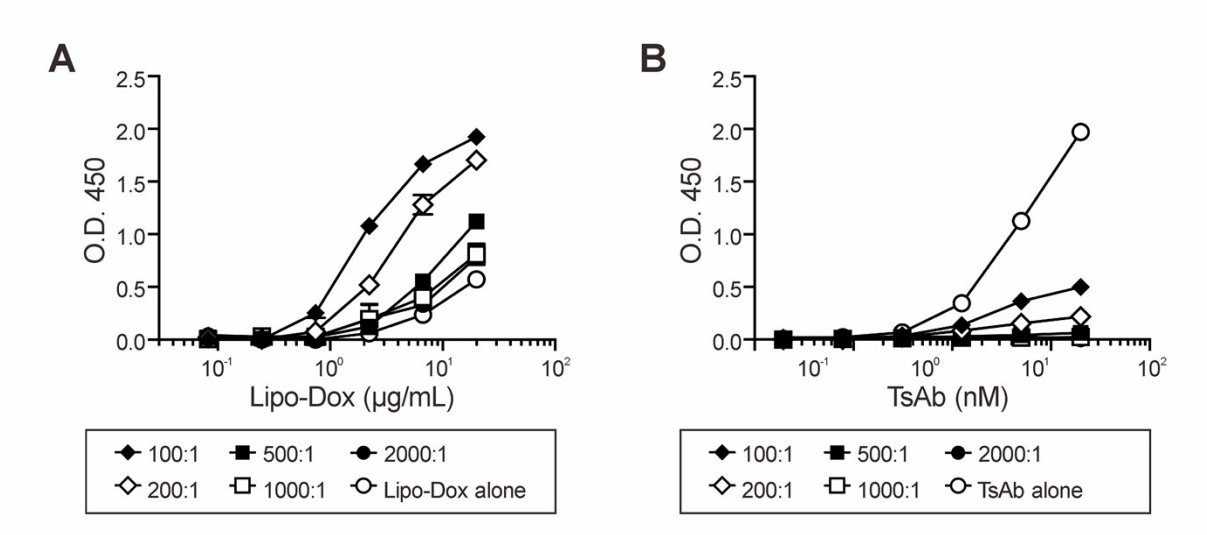

Figure S1. Determination of optimal TsAb modification ratio. (A) The cellular binding of TsAb-Lipo-Dox prepared with various mPEG:TsAb ratios was assessed by cell-based ELISA with MCF-7/HER2 cells. (n = 3. Bar, SD.) **(B)** The amount of free TsAb in TsAb-Lipo-Dox solution was assessed by ELISA with mPEG-coated plates. All TsAb-Lipo-Dox solution was diluted to the same molar concentration of TsAb. (n = 3. Bar, SD.)

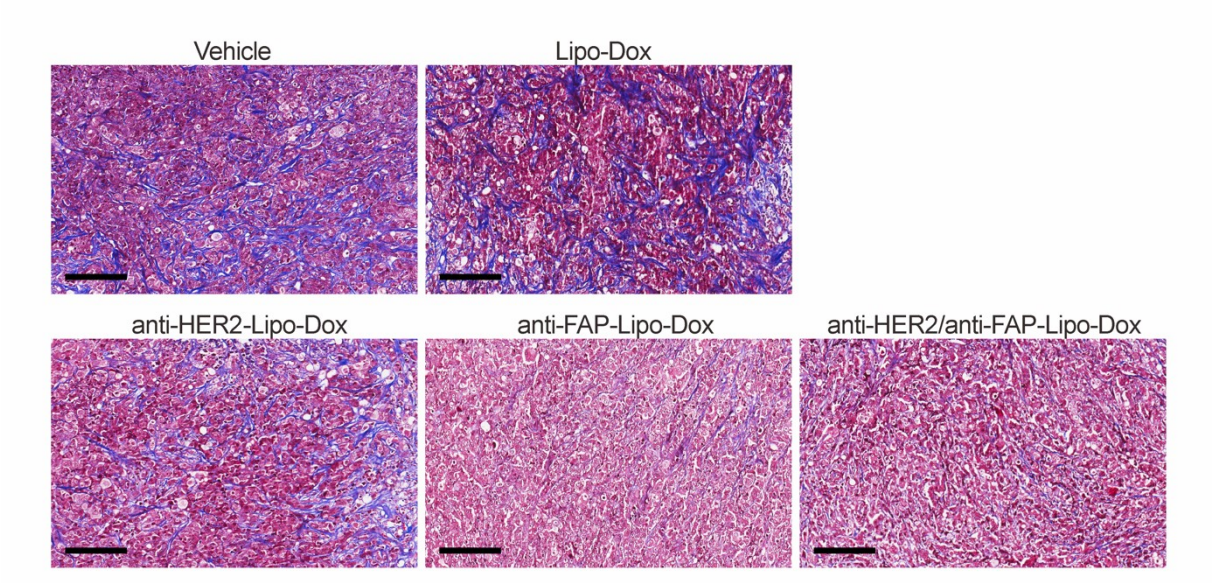

Figure S2. Masson's trichrome staining of treated TAF-rich tumors. Tumors were excised 2 weeks after the last dose of vehicle (PBS+0.05% BSA) or nanomedicines, and then were fixed in 10% formalin and embedded in paraffin. The tissue sections were cut at 4 μm, and stained with Masson's trichrome. Blue, collagen; dark purple, nuclei; and light pink, cytoplasm. Scale bar, 200 μm.

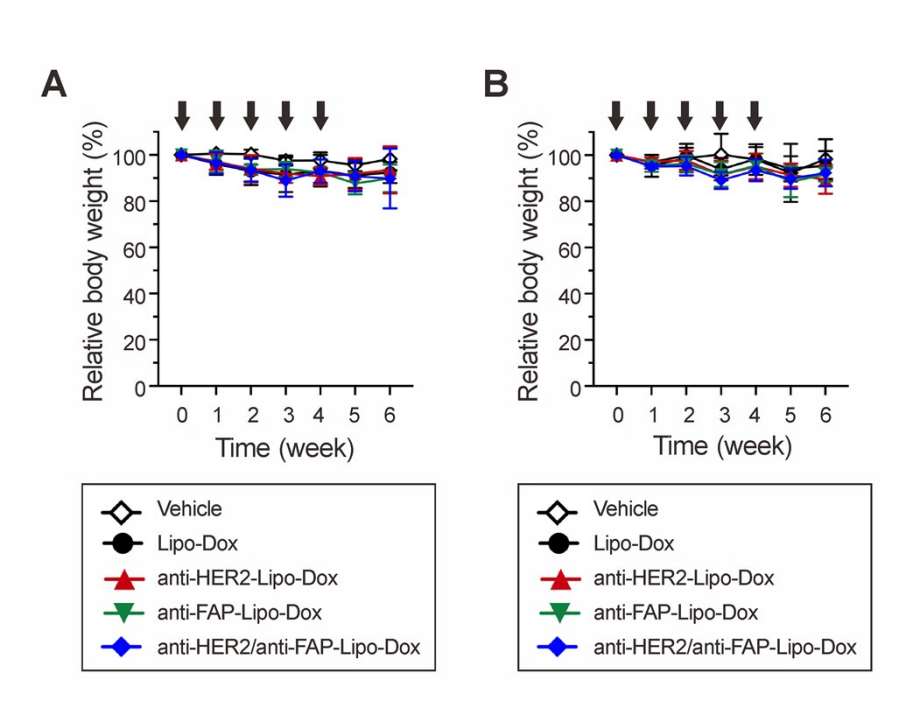

Figure S3. Relative body weights of SCID mice receiving treatment with the nanomedicines. Body weights of mice bearing **(A)** s.c. tumors consisting of a mixture of MCF-7/HER2 cells and WS-1/FAP cells or **(B)** s.c. tumors consisting of MCF-7/HER2 cells alone were measured weekly. Arrows indicate the time points of the administration. (n = 5. Bar, SD.)

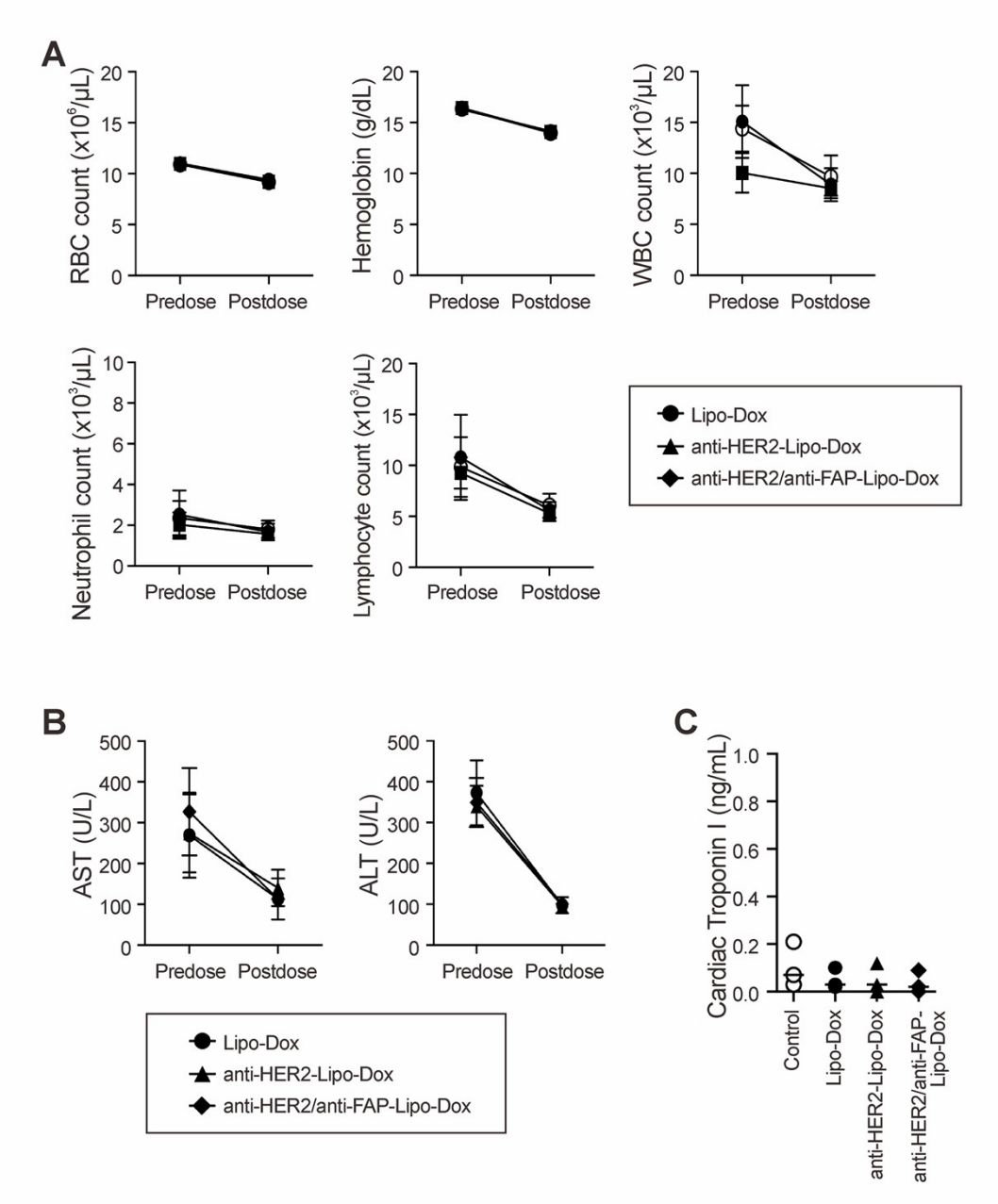

Figure S4. Side effect evaluations of TsAb- and BsAb-Lipo-Dox. BALB/c mice received three doses of the nanomedicines, and then blood was collected from their sublingual veins to measure **(A)** RBC, hemoglobin, WBC, neutrophil, and lymphocyte levels for myelotoxicity (n = 5. Bar, SD.), **(B)** AST and ALT levels for liver toxicity (n = 5. Bar, SD.), and **(C)** troponin I levels for cardiac toxicity (n = 3. Bar, SD.).