Supporting Information

Hyaluronan Like Polysaccharide Based Nanodrugs with Enhanced CD44 Avidity for Image-guided Drug Delivery to Breast Cancer

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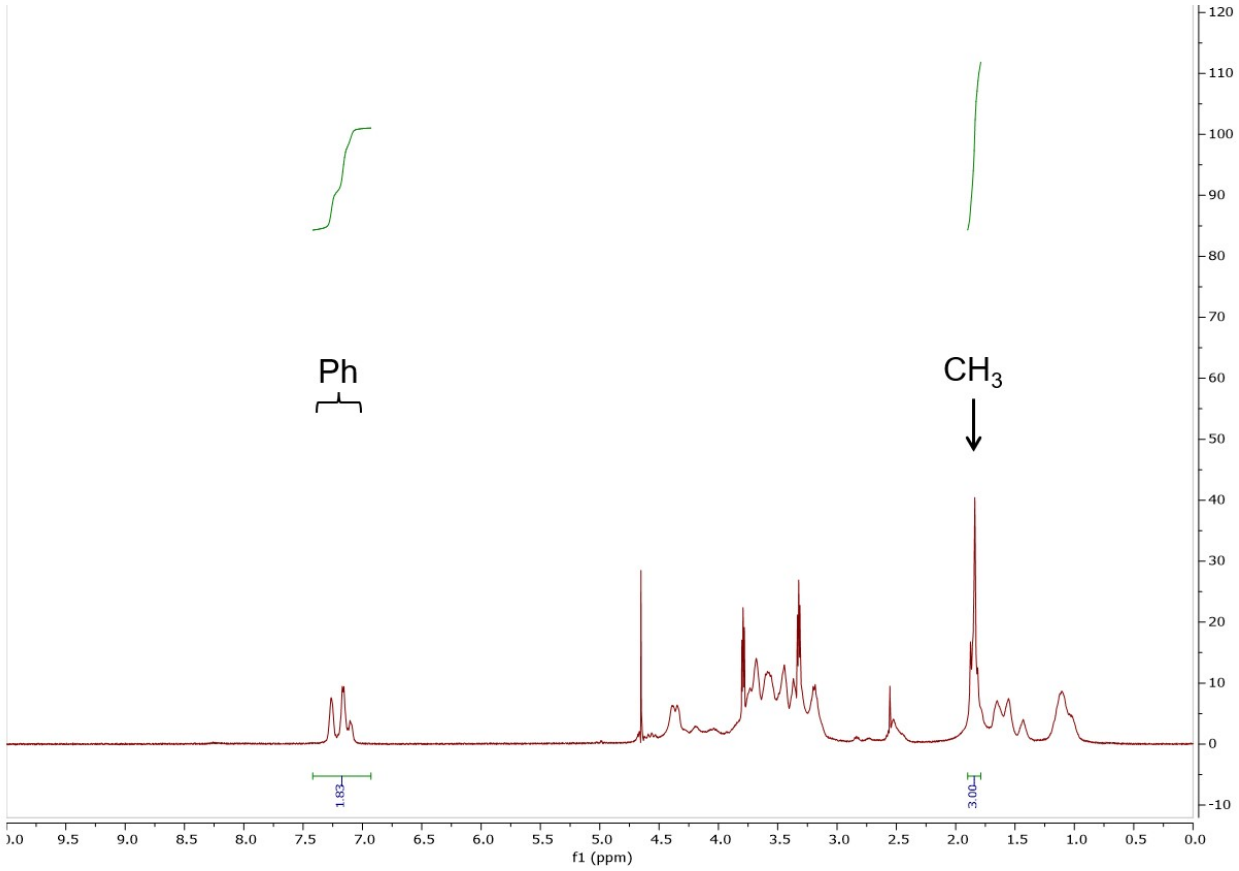
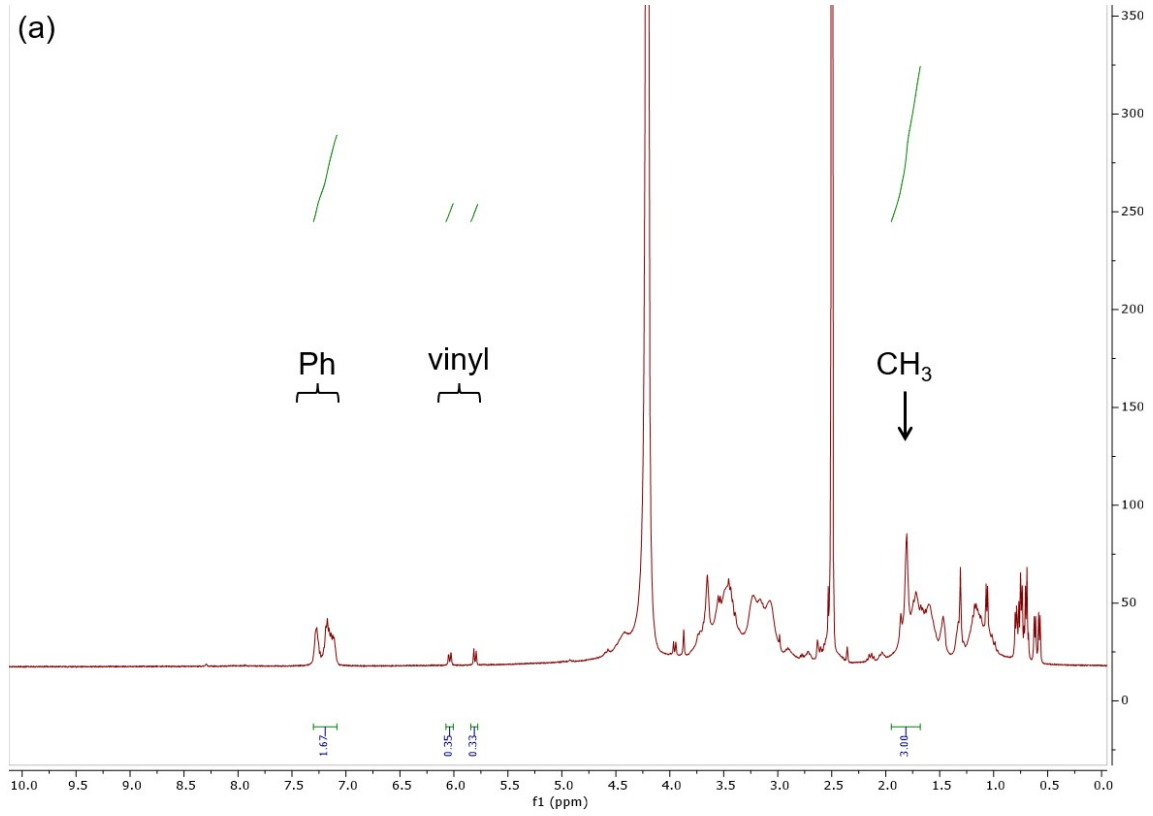
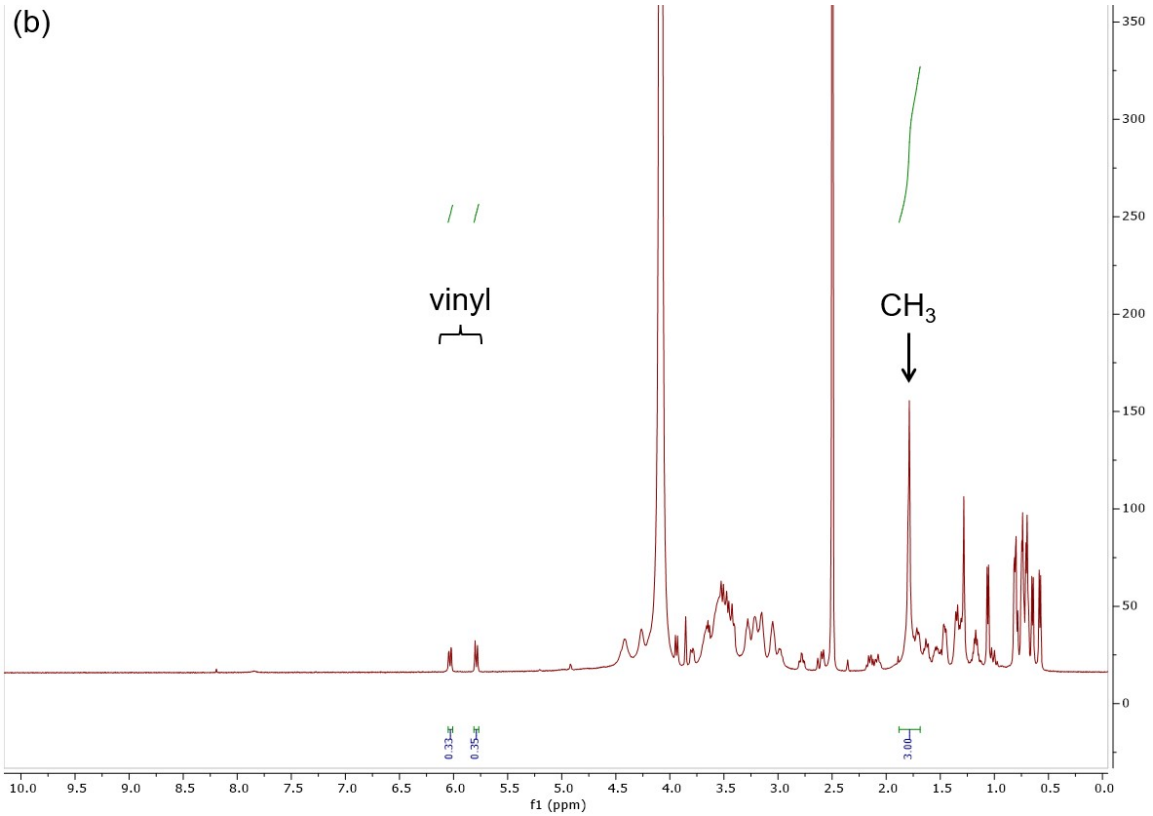


Figure S1. ^{1}H-NMR spectrum of G2 in D_2O. Peak at 1.8 ppm is from the methyl group of acetamide of HA. Peaks between 7.1 ppm and 7.3 ppm are from the phenyl rings. The level of modification was determined to be 36.6 % according to the ratio of the integration value of phenyl ring between 7.1 ppm and 7.3 ppm and the methyl group peak at 1.8 ppm.


(a)
Ph
vinyl
CH3
1.67
0.35
0.33
3.00
f1 (ppm)


(b)
vinyl
CH3
0.33
0.35
3.00
f1 (ppm)

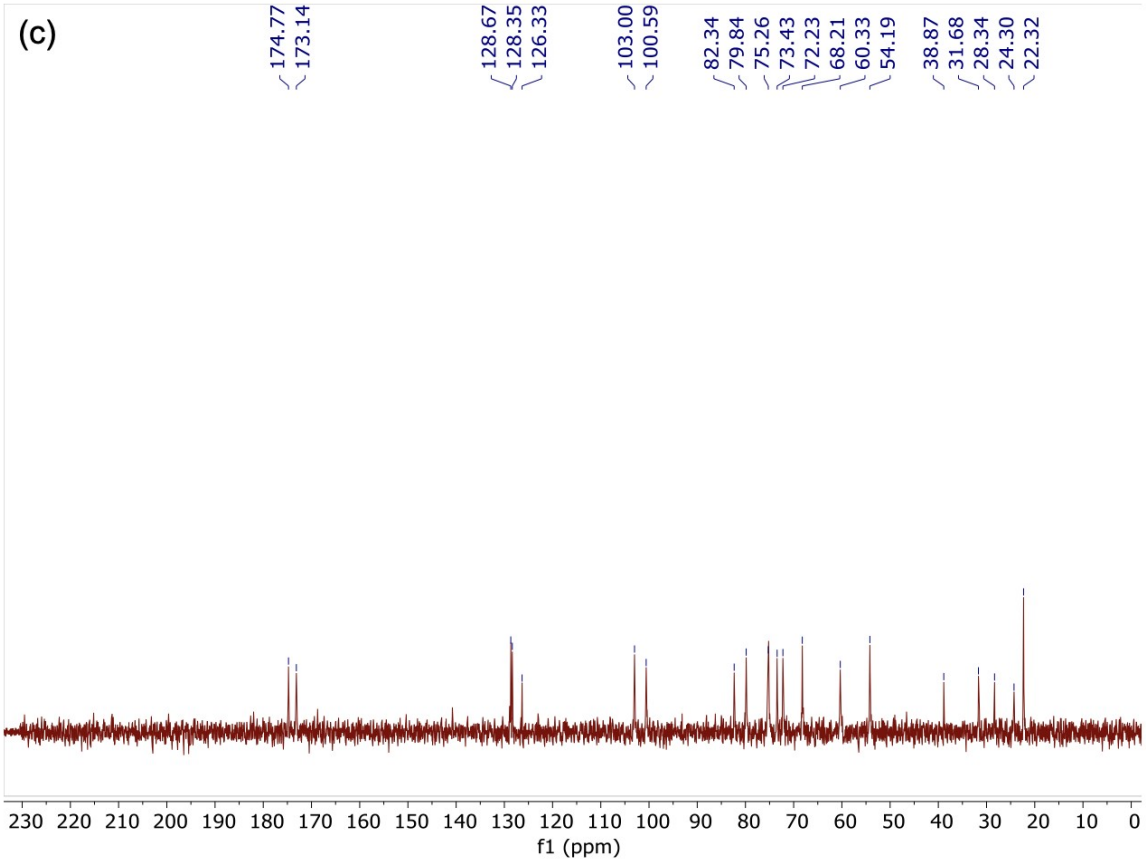


Figure S2. ^{1}H-NMR spectrum of (a) G2-Sal and (b) HA-Sal in d-DMSO/D_2O mixture (2:1). The peak at 1.8 ppm is from the methyl group of acetamides in HA. Peaks at chemical shift 5.8 and 6.0 ppm are from the vinyl group in salinomycin. The level of modification was estimated as 34 % according to the ratio of integration value of vinyl group peaks at 5.8 ppm and 6.0 ppm and the methyl group peak at 1.8 ppm. (c) ^{13}C-NMR spectrum of G2-Sal in d-DMSO/D_2O mixture (2:1).

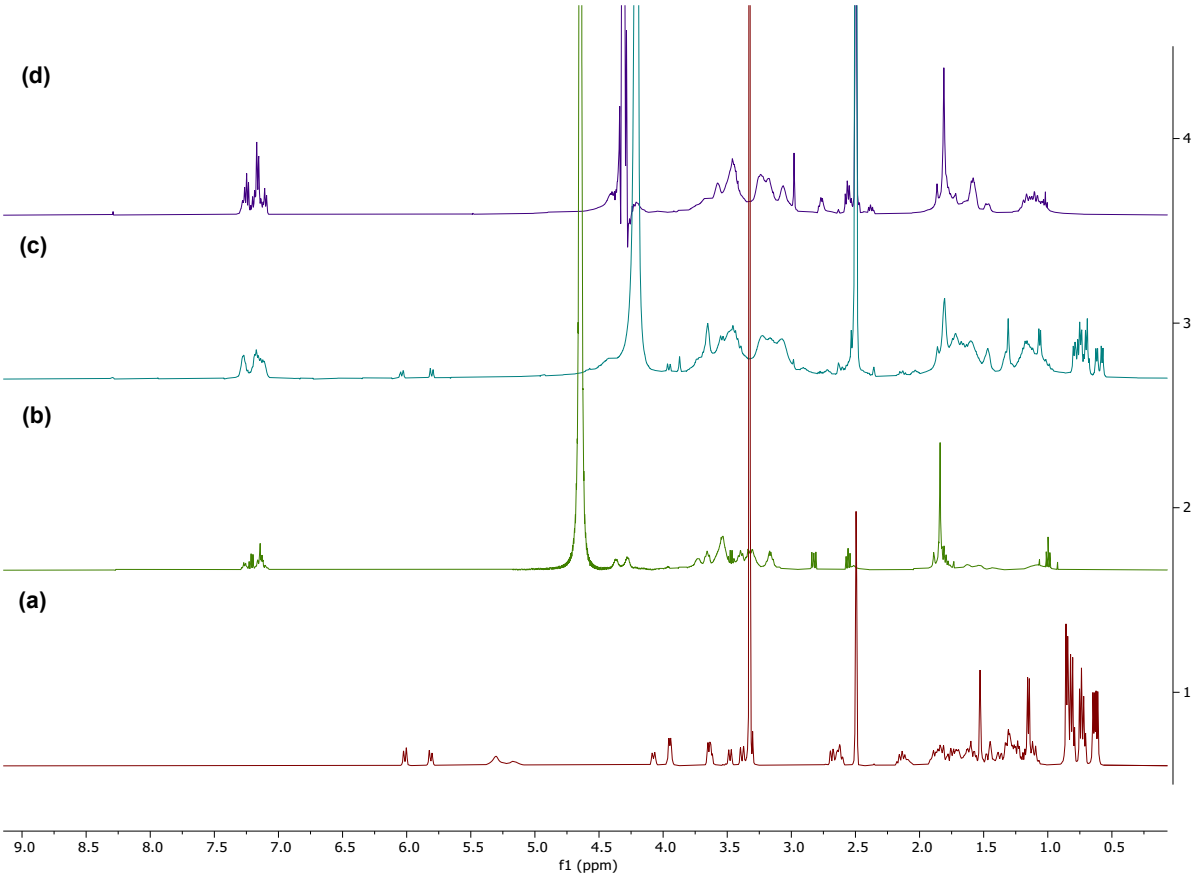


Figure S3. Stacked ^{1}H-NMR spectra of (a) salinomycin, (b) G2, (c) G2-Sal formed with DCC, and (d) mixture of G2 and Sal without DCC after dialysis. Salinomycin was dissolved in d-DMSO. G2 was dissolved in D_2O. G2-Sal was dissolved in d-DMSO/D_2O mixture (2:1). Salinomycin characteristic peaks are at chemical shift 5.8 and 6.0 ppm. The lack of signals from Sal in sample d, mixture of G2 and Sal without DCC after dialysis suggested that there were no covalent bond forming between G2 and Sal in the absence of DCC.

Concentration (mg/mL)	Z-average Size (nm)
0.001	No detectable particles
0.007	128 ± 12
0.009	163 ± 12
0.01	274 ± 11
0.1	288 ± 8

Table S1. Determination of Critical Association Concentration (CAC) of G2-Sal-ICG Nanodrug via DLS Measurement

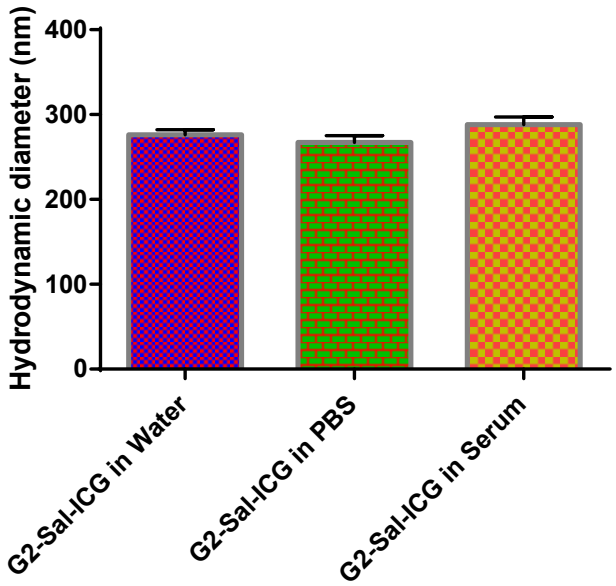


Figure S4. Stability test for G2-Sal-ICG nanodrugs in different media, including water, PBS, and serum. The G2-Sal-ICG nanodrugs were incubated with different media on a rotator for 24 h at room temperature. The hydrodynamic size was measured by DLS.

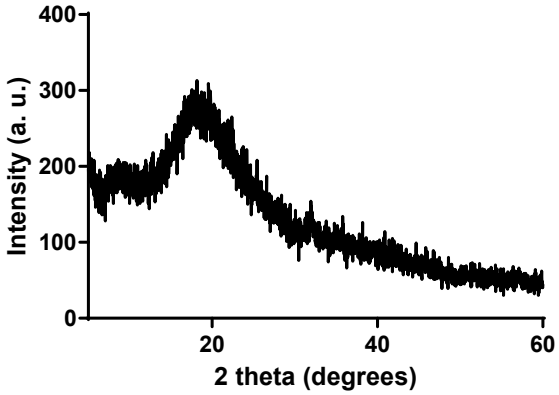


Figure S5. XRD analysis of G2-Sal-ICG nanodrugs.

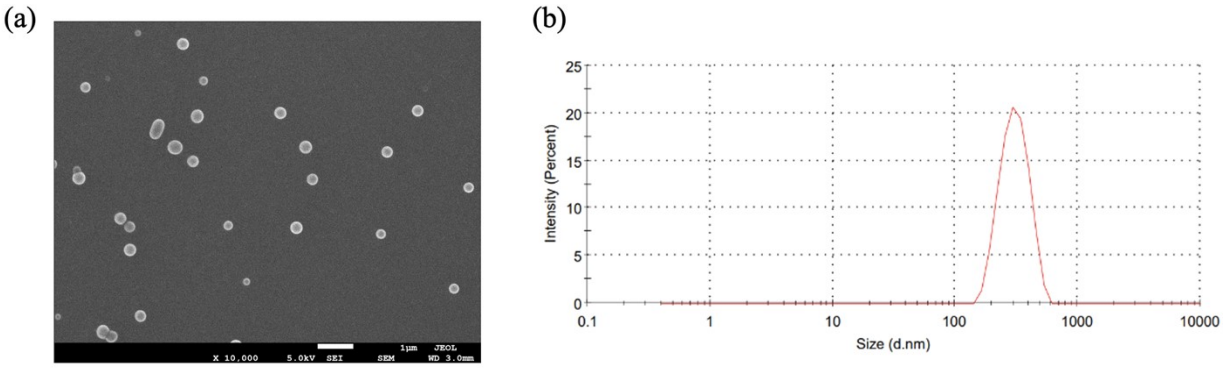


Figure S6. Characterizations of HA-Sal-ICG nanodrug (a) SEM image and (b) hydrodynamic diameter. The scale bar is 1 μm. The zeta potential of HA-Sal-ICG was -19.4 ± 0.8 mV in PBS. HA-Sal-ICG was diluted to 0.5 mg/mL in PBS for above measurements.

	Z-average Size (nm)	Zeta potential (mV)
G2-Sal-ICG	273	-16.7 ± 0.6
HA-Sal-ICG	312	-19.4 ± 0.8

Table S2. The hydrodynamic size and zeta potential of G2-Sal-ICG and HA-Sal-ICG nanodrugs

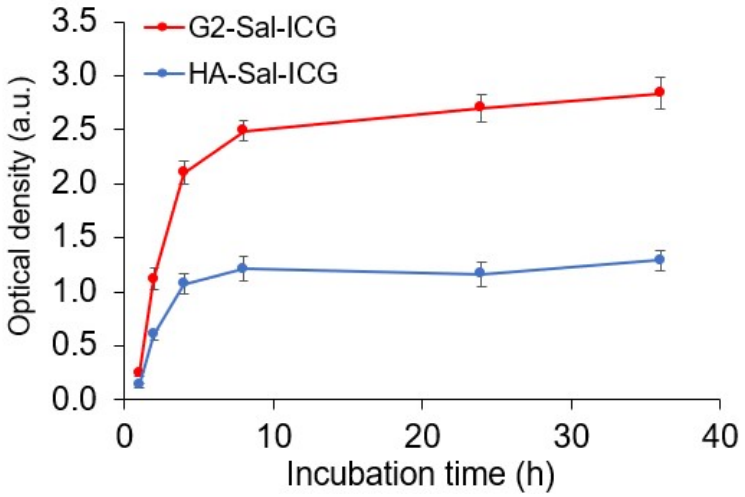


Figure S7. 4T1 cells were incubated with G2-Sal-ICG or HA-Sal-ICG nanodrug for 1, 2, 4, 8, 24, 36 h at 37°C then washed with PBS for three times then measured the fluorescence with excitation: 700 nm and emission: 820 nm.

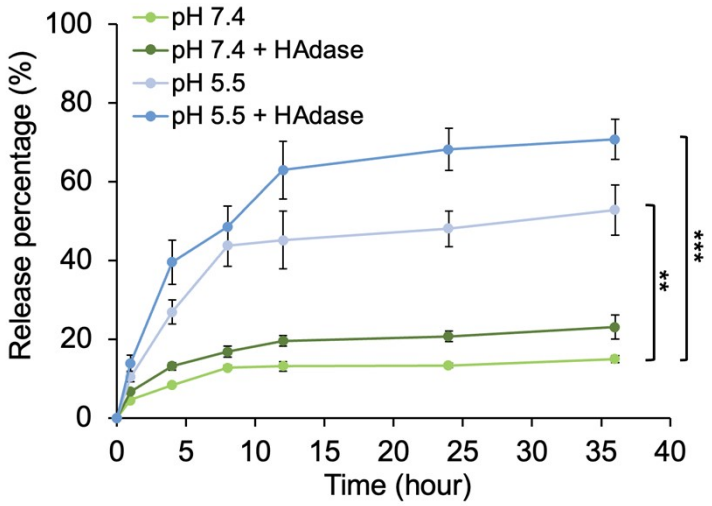


Figure S8. *In vitro* release rate (%) of salinomycin from G2-Sal under different conditions. Statistical analysis was performed through one-way ANOVA analysis. **$p < 0.05$, ***$p < 0.001$.

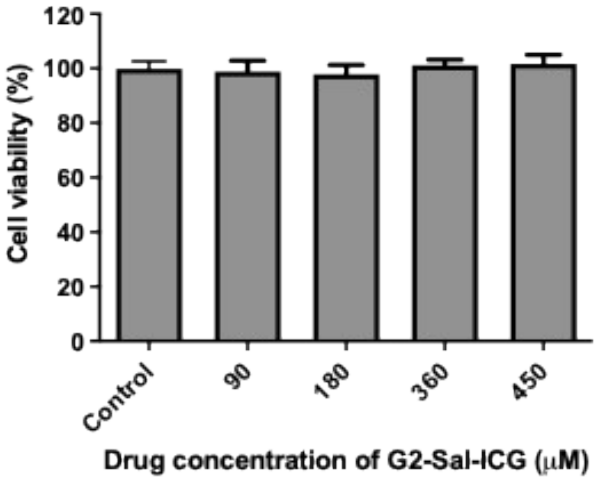


Figure S9. Cell viability (MTS) assays performed using HC11 cells with different concentrations of G2-Sal-ICG nanodrugs.

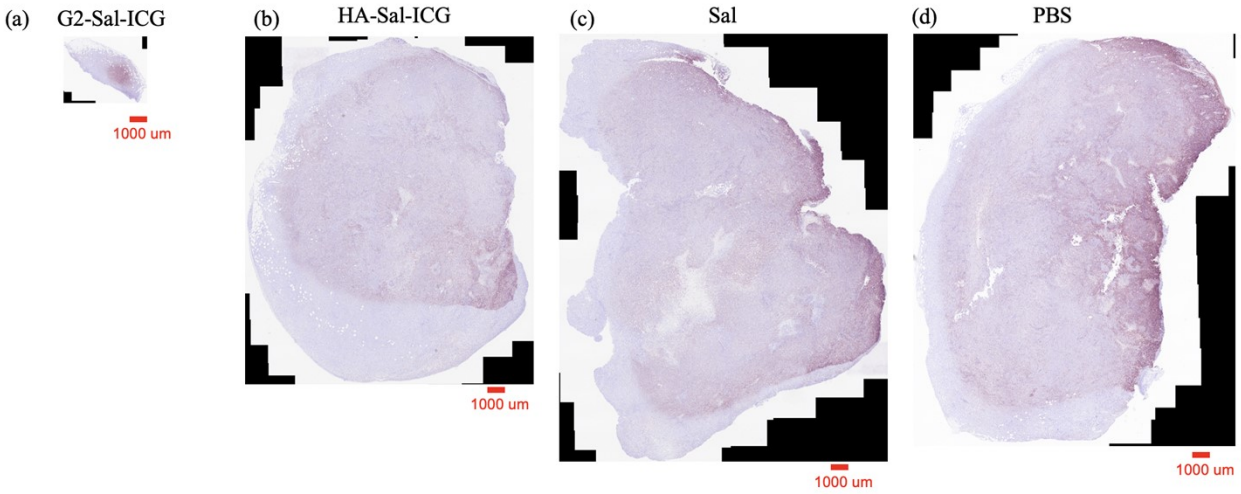


Figure S10. Excised breast tumor from different treatment groups: (a) G2-Sal-ICG (b) HA-Sal-ICG (c) salinomycin (d) PBS. Tumor slides were performed with CD44 IHC staining (brown color). The scale bar is 1000 μm.