SUPPORTING INFORMATION

A new spin on antibody-drug conjugates: trastuzumab-fulvestrant colloidal drug aggregates target HER2-positive cells

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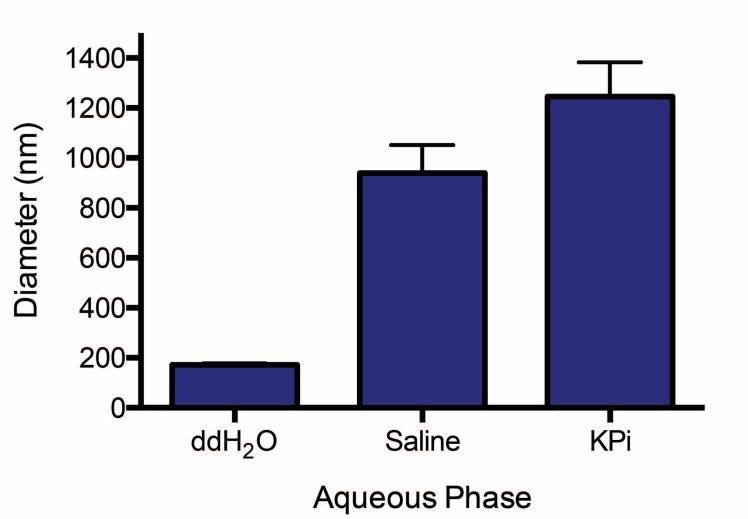

Figure S1 Formulation of 50 μM fulvestrant colloids in water, saline and 100 mM phosphate buffer (KPi). (n=3, mean+SD)

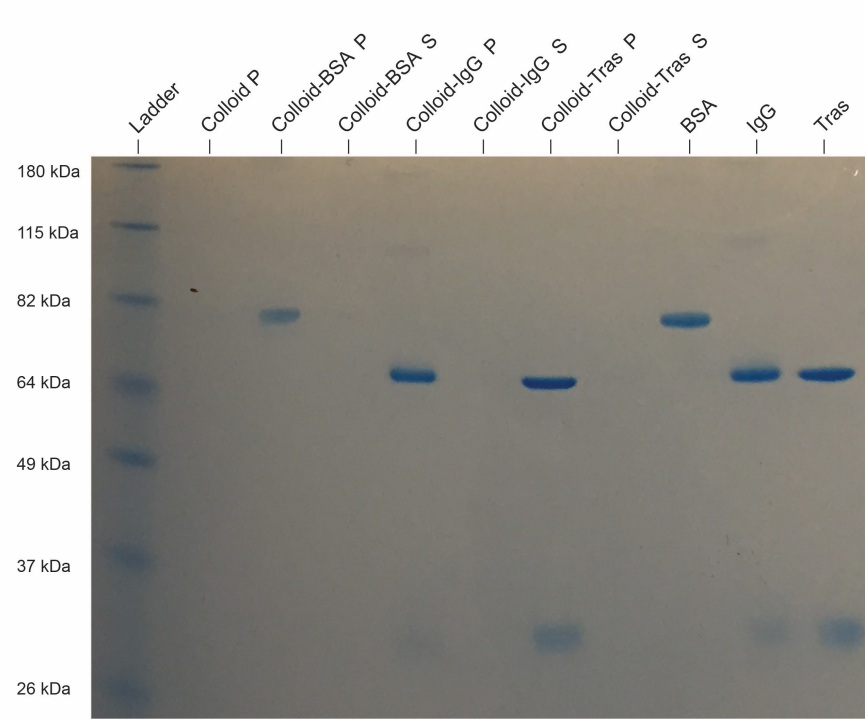

Figure S2 SDS-PAGE of protein corona based colloidal formulations (50 nM protein). Pellet (P) and supernatant (S) fractions of formulations were isolated by centrifugation at 16000x *g* for 1 h at 4 ^{o}C. Proteins were only found associated with the pellet fraction indicating that they were bound to the colloid surface forming a protein corona. Protein-only controls indicate where protein was loaded directly onto gel (lanes 9-11): BSA is bovine serum albumin; IgG is immunoglobulin G; Tras is trastuzumab. Representative image of 3 repeats.

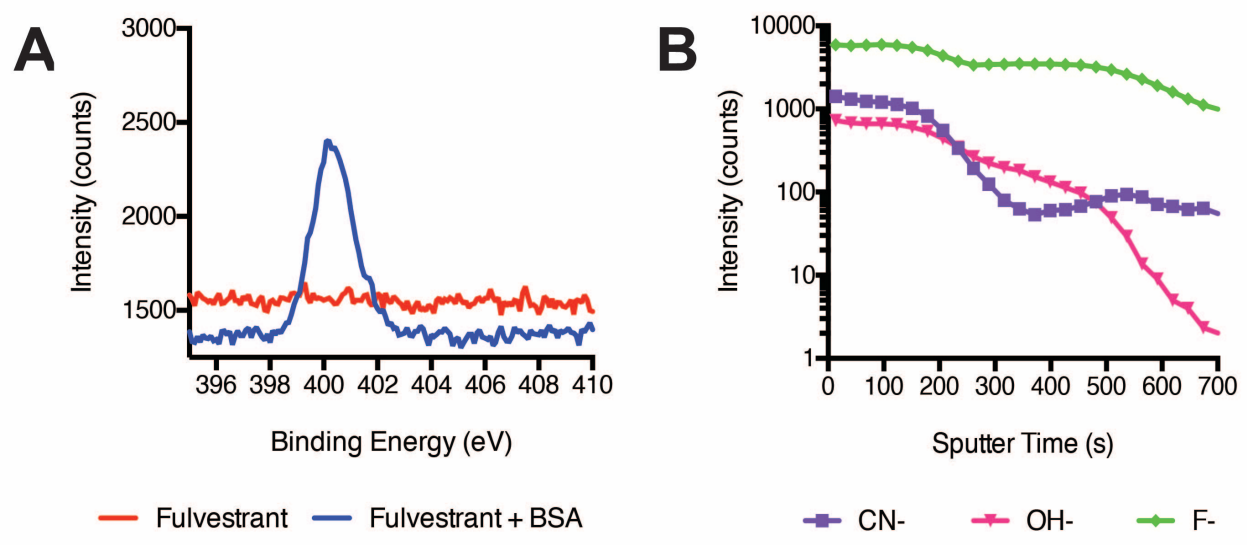

Figure S3 (A) Only fulvestrant colloids formulated with BSA, but not fulvestrant colloids alone, have a nitrogen peak by XPS, confirming the presence of surface-bound protein. (B) Depth profile of fulvestrant-BSA colloids by TOF-SIMS shows protein-specific secondary CN- ion signal decreasing in intensity through depth of sample, confirming surface-bound protein.

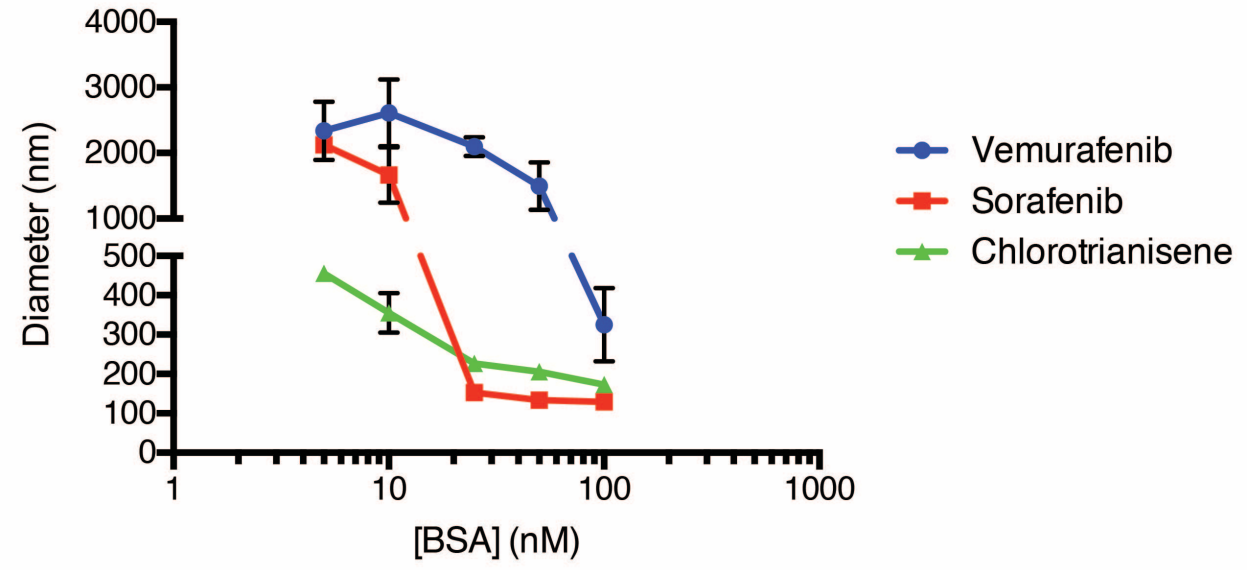

Figure S4 Formulations of 50 µM each of vemurafenib, sorafenib and chlorotrianisene form colloids in water that are stabilized with protein coronas of bovine serum albumin (BSA). The protein corona controls the size of all three colloid-forming compounds in a concentration-dependent manner. (n=3, mean±SD)

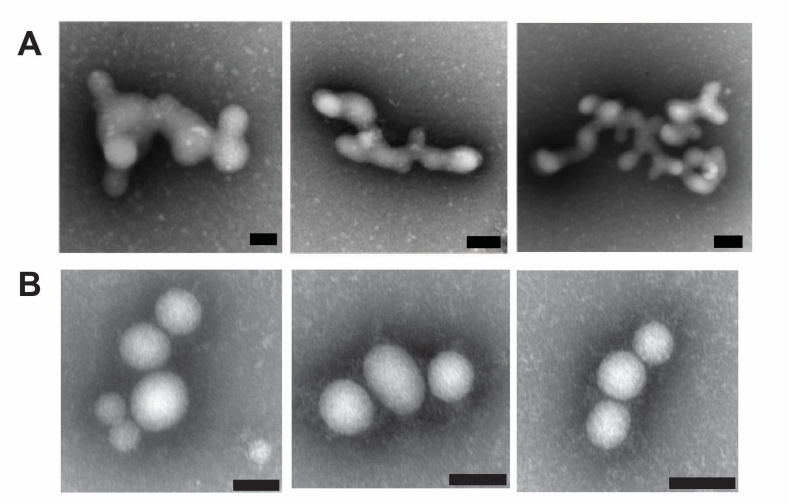

Figure S5 Additional TEM fields of view of (A) non-stabilized and (B) trastuzumab-stabilized fulvestrant colloids. 50 µM fulvestrant were formulated with 1% DMSO and 3.5 µM trastuzumab and incubated in 5% serum for 4 h prior to imaging. Scale bar represents 100 nm.

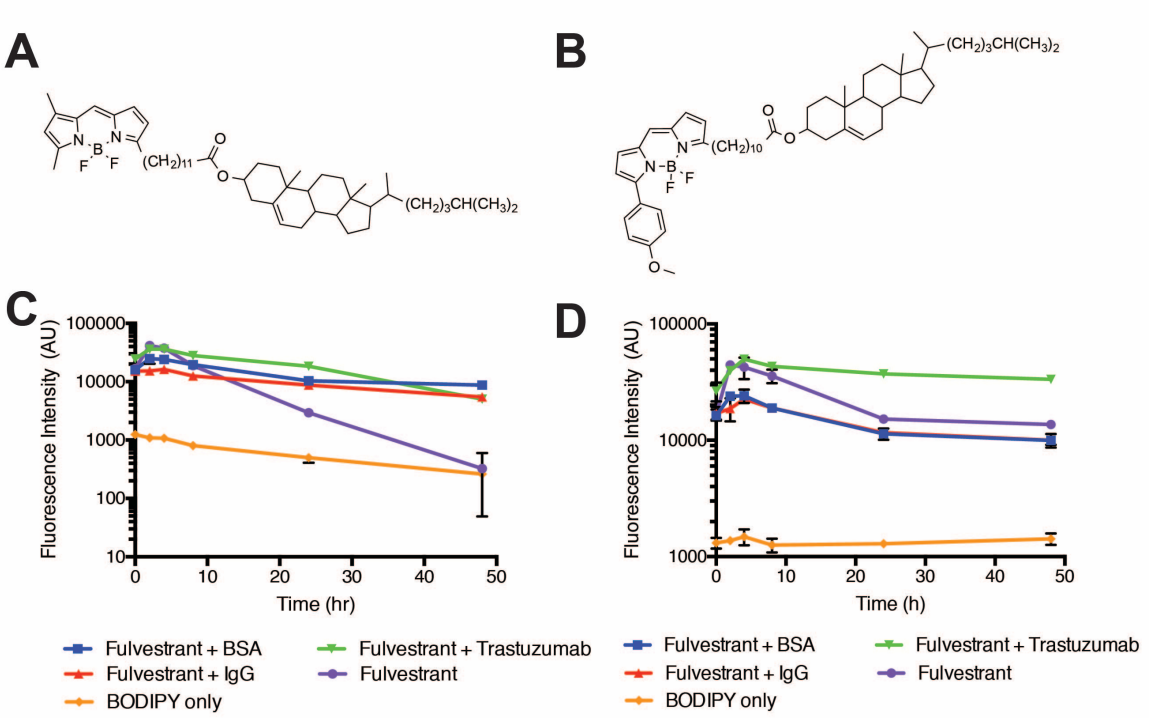

Figure S6 FRET donor (A) cholesteryl BODIPY FL C12 (1.75 mol%) and (B) acceptor cholesteryl BODIPY 542/563 C11 (0.25 mol%) dyes were co-formulated with fulvestrant colloids to measure of stability. (C) Fulvestrant colloids with or without stabilizing protein coronas were incubated in PBS over 48 hours at 37 ^{o}C. The decrease in fluorescence for fulvestrant-only colloids corresponds to the decrease in colloidal species observed in solution in Figure 1B. (D) In media containing 5% serum, all formulations remain relatively stable. (n=3, mean±SD)

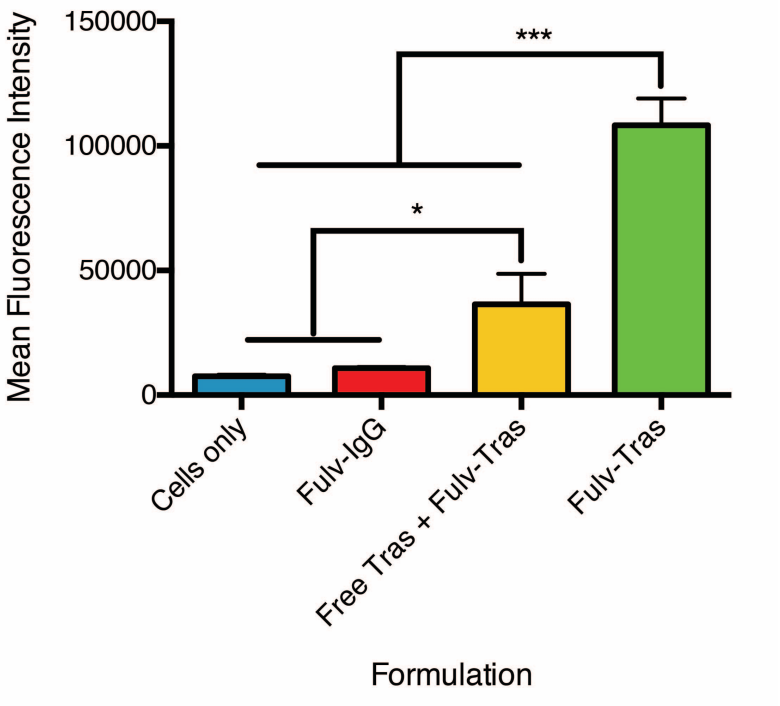

Figure S7 Quantification of colloid uptake by HER2 overexpressing BT-474 cells using flow cytometry. Trastuzumab-stabilized fulvestrant colloids (green) have significantly increased uptake by BT-474 cells compared to IgG-stabilized colloids (red) after 3-h incubation. Pre-incubation of cells with free trastuzumab (yellow) significantly reduced uptake of trastuzumab-stabilized colloids by cells. (n=3 biological replicates, mean+SD, *$p<0.05$, ***$p<0.001$)

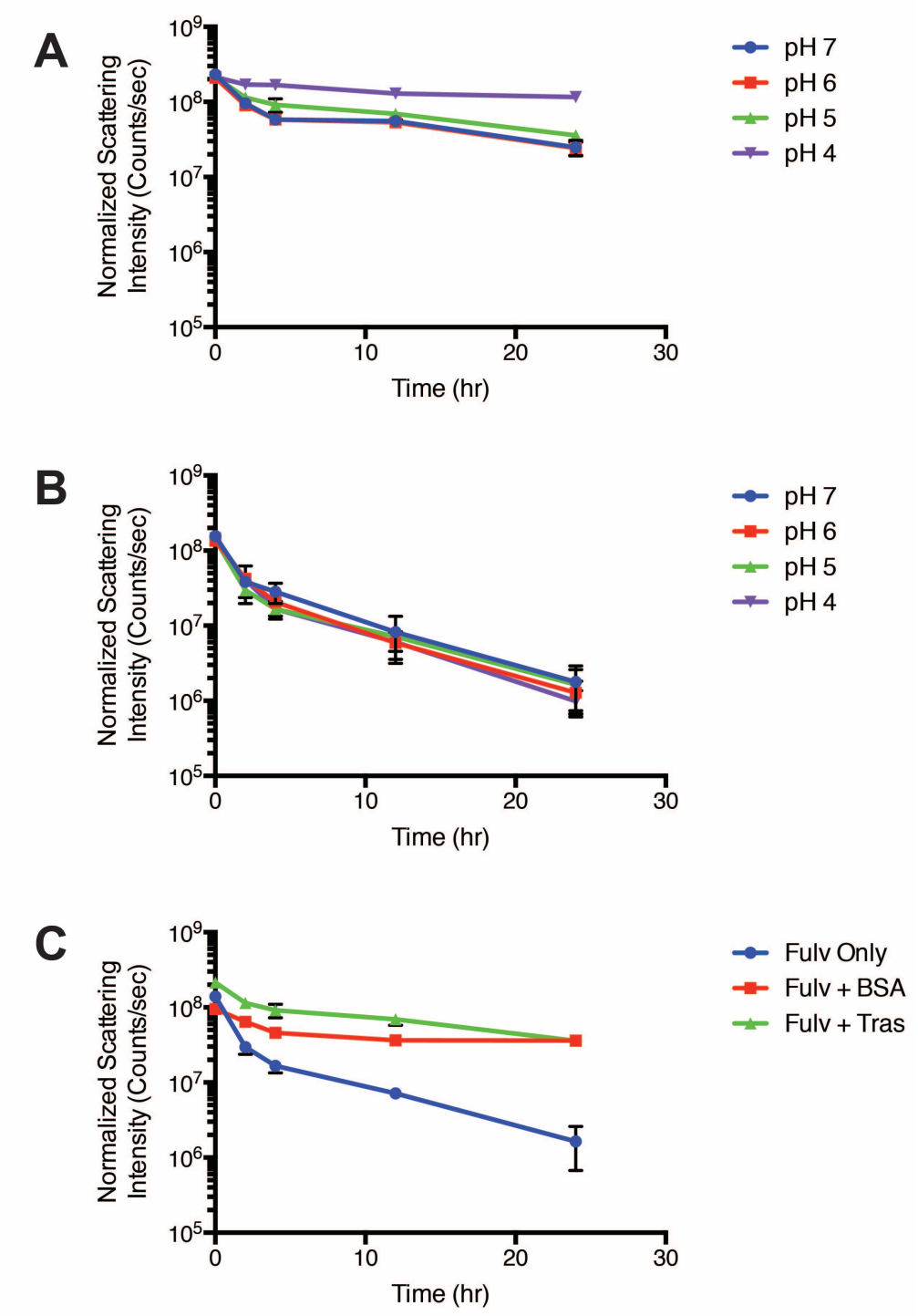

Figure S8 Proteins improve the stability of colloids in acidic environments such as those of the endo-lysosomal pathway. (A) Trastuzumab-stabilized fulvestrant colloids maintain scattering intensity over a 24-hour incubation in buffers of different pH while (B) non-stabilized colloids decrease in scattering indicating a decrease in colloidal species. (C) At pH 5, protein coronas stabilize colloids in a citric acid-phosphate buffer system. 50 µM fulvestrant, 1% DMSO, 100 nM BSA or 3.5 µM trastuzumab. (n=3, mean±SD)

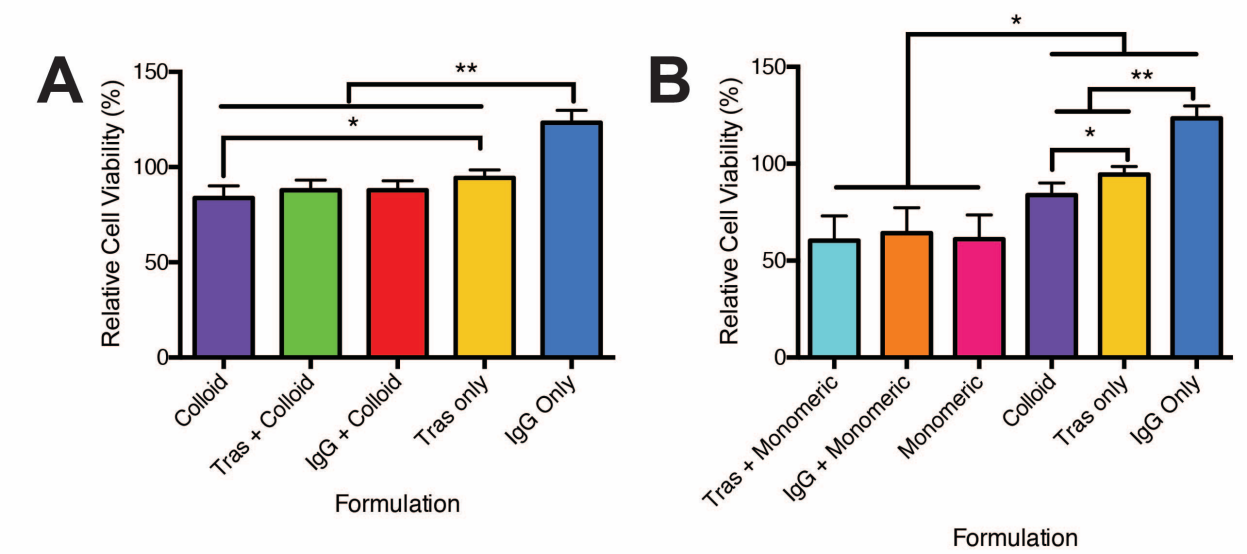

Figure S9 (A) Fulvestrant colloids formulated with trastuzumab do not selectively target low HER2-expressing MCF-7 cells as shown with similar cell viability as non-targeted fulvestrant colloids formulated with IgG. (B) All monomeric fulvestrant formulations reduce cell viability to the same extent irrespective of the presence of either trastuzumab or IgG. Cells were incubated with 50 µM fulvestrant, 3.5 µM antibody, 1% DMSO and 0.015% UP80 to maintain monomeric formulation for 24 hours. Cell viability was assessed by the PrestoBlue assay after a 72-hour total incubation. * $p<0.05$, ** $p<0.01$. (n=4 biological replicates, mean+SD)

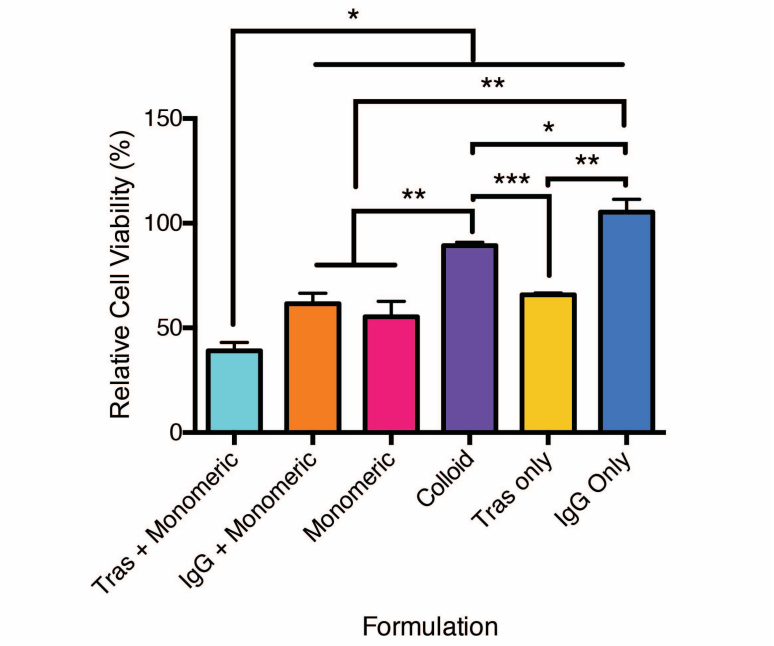

Figure S10 All monomeric formulations reduce cell viability of BT-474 cells. Cells were incubated with 50 µM fulvestrant, 3.5 µM antibody, 1% DMSO and 0.015% UP80 to maintain monomeric formulation for 24 hours. Cell viability was assessed by the PrestoBlue assay after a 72-hour total incubation and represented as percentage of vehicle control. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. (n=4 biological replicates, mean+SD)