Figure legends for supplementary figures Erickson, et al.,

Supplementary Figure S1. Effects of chloroquine on the mitotic arrest induced by huC242-SMCC-DM1. FACS histograms (FL2-A) display DNA contents of samples of asynchronous, exponentially growing COLO 205 cells either untreated or treated for 20 h with 10^{-8} M DM1-SMe, 3 x 10^{-9} M huC242-SMCC-DM1, in the presence or absence of 30 μM chloroquine. In the presence of chloroquine, G_2/M arrest induced by huC242-SMCC-DM1 was almost completely abrogated (14% in G_2), whereas chloroquine had only a modest effect on G_2/M arrest caused by DM1-SMe (51% in G_2).

Supplementary Figure S2. Treatment of metabolites in the medium from COLO 205 cells treated with huC242-SPDB-[^{3}H]DM4 with DTT and selenol. Metabolites were extracted with acetone from the spent media following a 26-h exposure of cells to conjugate. The extract was divided into two equal portions. One portion was subjected directly to chromatography (a) and the other portion (b) was treated with DTT and selenol before chromatography to reduce all disulfide bonds. The effluent from the column was monitored for tritium using an in-line flow scintillation analyzer with an output in mV^2; thus the chromatograms show retention time on the abscissa and mV^2 as a measure of [^{3}H] on the ordinate.

Supplementary Figure S3. Generation of maytansinoid metabolites from cell surface-bound huC242-SPDB-[^{3}H]DM4. Proliferating COLO 205 cells were placed on ice and incubated with a saturating amount of huC242-SPDB-[^{3}H]DM4 (10^{-7} M) to allow the conjugate to bind to the surface receptors without being internalized. The cells were

then washed, and the amount of bound radioactivity was quantified by subjecting small fractions of the samples to scintillation counting. The total amount of radioactivity associated with the cell sample after conjugate binding at 0 ^{o}C is shown on the ordinate at T = 0 (open triangle, 78 pmol) and after 22 hours of incubation (open triangle, 57 pmol). The curves show the accumulation of various metabolites in the cells (closed symbols) and in the medium (open symbols) for samples incubated for 5, 9, 22, and 30 hours. Maytansinoid metabolites are *S*-methyl-DM4 (squares), lysine-N^{ε}-SPDB-DM4 (circles), and *S*-cysteinyl-DM4 (diamonds).

Supplementary Figure S4. Antigen dependence of cellular metabolism. Maytansinoid metabolites were studied with conjugates that don't bind to COLO 2005 cells. Panel A (a,b) shows chromatograms of acetone extracts of cells (a) and medium (b) after treatment of COLO 205 cells for 26 hours with Tras-SMCC-[^{3}H]DM1. Correspondingly, Panel A (c) (cells) and d (medium) are for the conjugate huB4-SPDB-[^{3}H]DM4. Panel B shows chromatograms associated with acetone extracts of Tras-SMCC-[^{3}H]DM1 (a) and huB4-SPDB-[^{3}H]DM4 (b).

Supplementary Figure S5. **Maytansinoid metabolites formed upon treatment of COLO 205 cells with huC242-SPDB-D-[^{3}H]DM4.** COLO 205 cells were treated with huC242-SPDB-D-[^{3}H]DM4 and cells and media were harvested at 5, 9, 22, and 30 h and analyzed for metabolites as described in Fig. 1. (A) The Left Panels show the chromatograms of the cell extracts at the four consecutive time points, and the Right Panels represent the chromatograms of the corresponding medium samples. (B) Total

amounts of maytansinoid metabolites generated from huC242-SPDB-D-[^{3}H]DM4 in the cells (closed circles) and from medium (open squares) were quantified as described in Fig. 2.