Supplementary Methods

Cell lines and cell culture. The cell lines SKBR3, BT474, MCF7, SKOV3, NCI-N87, and Calu3 were all obtained from American Type Culture Collection. The human breast cancer cell line MDA MB435S was Her2/neu transfected cell line, from Prof. Dihua Yu (UT MDACC, Houston, TX). The mouse breast cancer cell line 4T1 was kindly provided by Prof. Toshiyuki Yoneda (UT Health Science Center, San Antonio, TX). Cells were maintained in DMEM or RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, plus 2mM L-glutamine and 1mM antibiotics. The transfected cell line MDA MB435S was maintained in the DMEM complete medium containing 200 μg/mL geneticin (Invitrogen).

TUNEL assay. To assess apoptosis using terminal deoxyribonucleotidyltransferase-mediated dUTP nick end-labeling assay, SKOV3 cells were plated into chamber slides and then treated with doses of 25nM immunotoxins for 72h. The slides were fixed in 3.7% paraformaldehyde followed by a brief rinse with PBS, and then permeabilized in PBS containing 0.2% Triton X-100. After washing, fixed cells were then stained with an in situ cell death detection kit (Roche).

Lactate dehydrogenase assay. Lactate dehydrogenase (LDH) release was measured as a physiological indicator of necrosis. SKOV3 cells were incubated with 200nM immunotoxins for up to 48h. LDH release from lysed cells was detected using a LDH cytotoxicity detection kit (Roche). Maximum LDH release was detected by adding 1% Triton X-100 to the cells.