**Optoacoustic detection of early therapy-induced tumor cell death using a targeted imaging agent**

**Supplementary data**

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**Figure S1. Blocking binding of C2Am-750 to Colo205 cells using unlabeled C2Am.** Cells were untreated or treated with 1 pM MEDI3039 for 24 h. Blocked cell samples (right column) were incubated with 30 μM unlabeled C2 for 30 min. All samples were incubated subsequently with a cell necrosis marker, Sytox Green (upper panels), C2Am-750 (middle panels) or iC2Am-680 (lower panels). Low levels of NADH autofluorescence (x axis) and staining with Sytox Green were used to identify dead cells. The numbers in the scatter plots represent the percentage of cells in the respective quadrants.



**Figure S2. Measurements of fluorescence intensities in MEDI3039-treated HT-29 tumors *in vivo* and ex vivo.** Nude mice (n = 3) bearing subcutaneous HT-29 tumors were treated with a single dose of 0.4 mg/kg MED3039. After 16 h, the mice were injected with a mixture of 0.1 µmole/kg C2Am-750 and 0.1 µmole/kg iC2Am-680 followed by whole body FLI measurements using an IVIS 200 camera at 0 and 3 hours post probe injection. Untreated mice (n = 3) were injected with drug vehicle. (**A**) Quantitation of tumor fluorescence intensities *in vivo* at 3 h post injection of C2Am-750 and iC2Am-680. P value for T-test between treated and untreated groups was 0.85 for C2Am-750 and 0.26 for iC2Am-680. (**B**) Quantitation of fluorescence intensities in excised tumors from animals injected with C2Am-750 and iC2Am-680. The intensities have been normalized to tumor weight. P value for T-test between treated and untreated groups was 0.76 for C2Am-750 and 0.14 for iC2Am-680.



**Figure S3. Spectral validation of the C2Am-750 signal *in vivo*.** (**A**) Optical absorption spectrum of C2Am-750 measured at a concentration of 1 uM in PBS. (**B**) MSOT spectrum measured in the center of a tumor from a region with high signal intensity before and 3 h after C2Am-750 injection. Similar size regions of interest were drawn in the tumor before (**C**) and after (**D**) probe injection (shown by purple lines). The tumor margin is indicated by a white line.



**Figure S4. MSOT of 5-fluorouracil-induced tumor cell death in mice injected with C2Am-750.** (**A**) Representative axial, coronal and sagittal views of a tumor before and 24 h after a single dose (250 mg/kg) of 5-FU. (**B**) Volumetric quantification comparing the total MPI of the 3D reconstructed VOI in the tumor area, expressed as mean voxel intensity, before and after 5-FU treatment. (**C**) C2Am-750 biodistribution in 5-FU treated tumors and major organs 3 h after injection. The fluorescence intensities have been normalized to tumor or organ weight. \* P<0.05. Scale bar = 3 mm.

**Supplementary videos. Representative reconstructed 3D rendering of a Colo205 tumor before and 3 h after injection of 0.2 µmole/kg of C2Am-750.** Signals from C2Am-750, oxygenated hemoglobin (HbO2), deoxygenated hemoglobin (Hb) are shown in green, red and blue respectively.