Electronic Supplementary Material

Pre-clinical Evaluation of a Cyanine Based SPECT Probe for Multimodal Tumor Necrosis Imaging

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Supplementary Figure 1: Structural characteristics and *in vitro* necrosis avid properties of HQ4 vs HQ5.

a) Chemical and structural characteristics of the carboxylated cyanine dyes HQ4 and HQ5.

b) *In vitro* necrosis targeting properties of HQ4 and HQ5 utilizing the dry ice assay. Fluorescent signal intensity was obtained from the area of dead cells in the centre of a culture well after incubation with different concentrations of HQ4 or HQ5 (1-100 nM) and is subtracted by the background signal from the area of the living cells.
Reversed-phase chromatography showed a clear peak indicating the high grade of purity (98%) of this conjugate. Mass spectrometric analyses of HQ4-DTPA further showed the expected molecular weight (calc.: 1331.59 for $C_{66}H_{90}N_{8}O_{17}S_{2}$ and MALDI-TOF found 1332.4 [M+1]+ 1354.6 [M+Na]), indicating the high grade of purity of this conjugate.
Confluent cultures of 4T1 cells were incubated for 24 hr with various concentrations HQ4, HQ4-DTPA, HQ5 or the natural anti-cancer compound Gambogic Acid (GA). Relative cell viability (%) was expressed as a percentage relative to the untreated control. HQ4, HQ4-DTPA and HQ5 did not affect cell viability, whereas, GA induced cell death with an IC50 of around 6µM.
Supplementary Figure 4: Biodistribution of $^{111}$InDTPA.

a) Biodistribution of the free chelate $^{111}$InDTPA in 4T1 tumor bearing mice. 24h after probe injection (10 µg, 30-35 MBq), mice (n=4) were sacrificed and the organs, body fluids and tumors were dissected, weighted and measured for radioactivity in a gamma counter. At each time point, the amount of radioactivity in each organ and tumor is expressed as percentage of the injected dose divided by the weight (%ID/w).

b) Total amount of remaining $^{111}$InDTPA-HQ4 and $^{111}$InDTPA in the whole mouse body (% of ID) at the indicated time points 6 to 72h after probe injection.