

Figure W1. Gel image of Affibody molecules labeled with IR-Dye800CW or DY-682. Labeled Affibody molecules were purified by passing reaction mixture through a Zeba Spin Desalting Column. Purified samples (0.2 pmol each) were separated on a Bis-Tris gel.

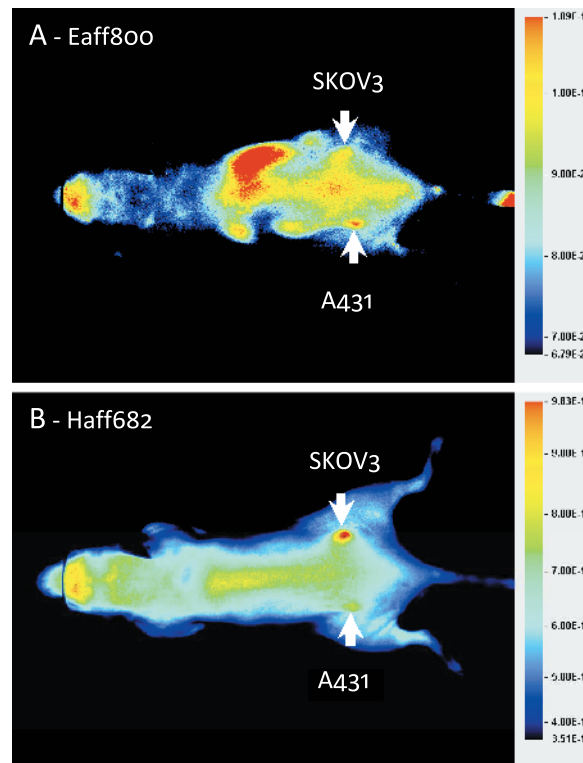


Figure W2. Pseudocolored images of mouse with Eaff800 signal (A) or Haff682 signal (B). Note that S2A and S2B were pseudocolored images of Figure 6, A-a and A-b, respectively. See Figure 6 for details of experiment.

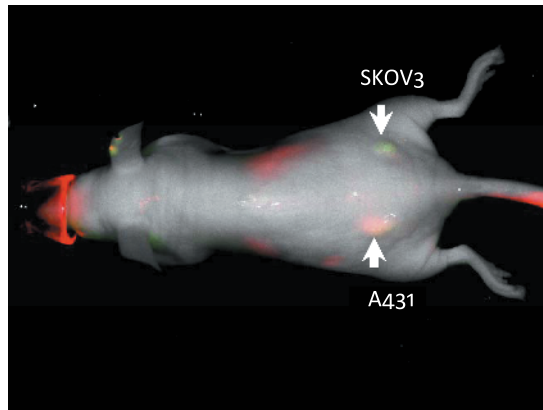


Figure W3. Two-color *in vivo* optical imaging with Eaff682 and Haff800. Nude mice bearing A431 and SKOV3 tumors on the left and right sides, respectively, were injected with 100 μ l of PBS containing 0.5 nmol of Eaff682 and 0.5 nmol of Haff800. Whole body images (dorsal view) were acquired 1 day after agent injection. Green and red represent IRDye800CW and DY-682 fluorescence signals, respectively. The tumors are indicated with arrows.

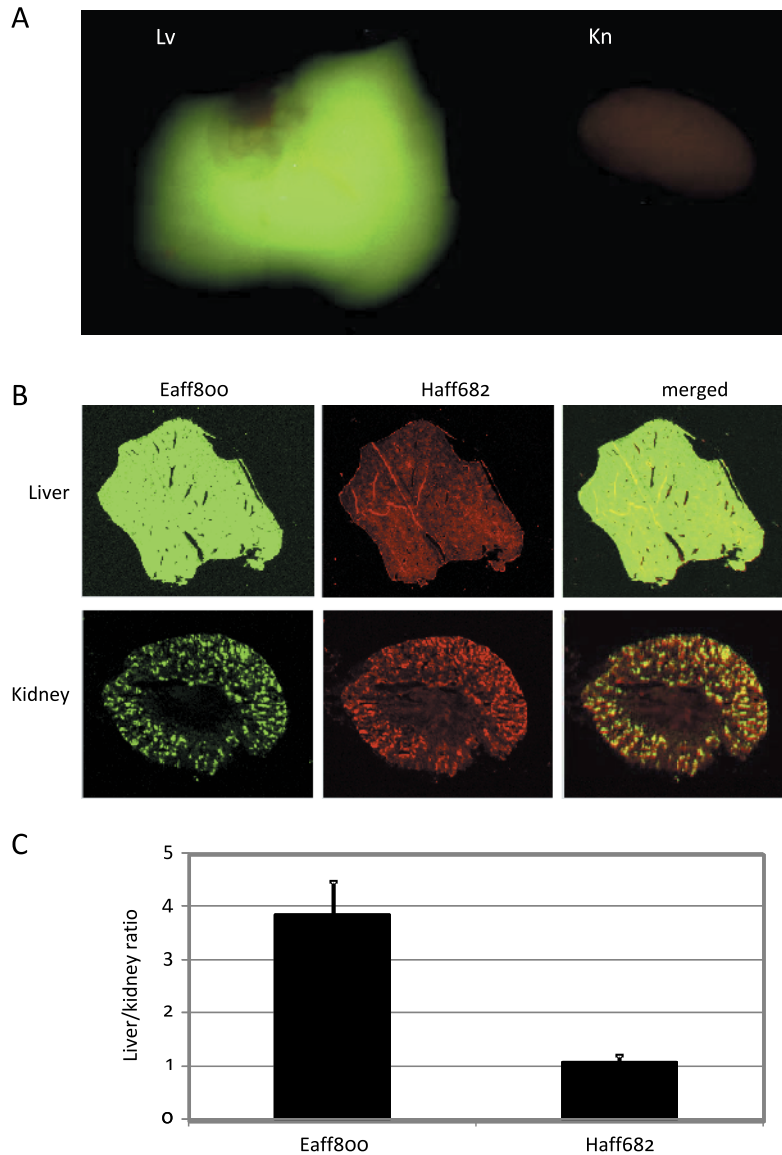


Figure W4. Accumulation of Eaff800 and Haff682 in mouse liver and kidney. (A) Images of the liver and kidney. Mice were killed 1 day after imaging agent injection. The organs were collected and rinsed in PBS before imaging. Green and red represent IRDye800CW and DY-682 fluorescence signals, respectively. *Kn* indicates kidney; *Lv*, liver. (B) Sections of mouse liver and kidney. The organs were snap-frozen in OCT compound and sectioned at 8- μ m thickness. (C) Liver-to-kidney ratio of Eaff800 and Haff682 signal intensities. Average signal intensities were calculated using ROIs with the same sizes from different tissue sections. Liver-to-kidney ratio was calculated by dividing liver signal intensity by kidney signal intensity.

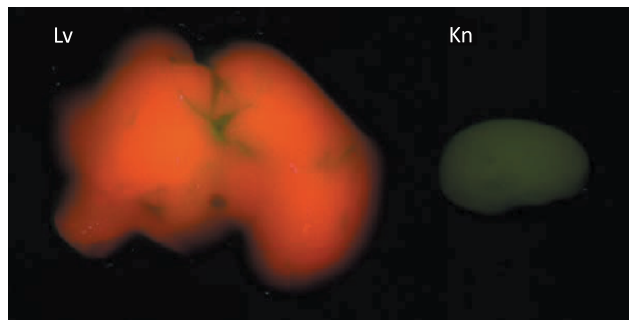


Figure W5. Accumulation of Eaff682 and Haff800 in mouse liver and kidney. Mice were killed 1 day after imaging agent injection. The liver and kidney were collected and rinsed in PBS before imaging. Green and red represent IRDye800CW and DY-682 fluorescence signals, respectively. Note the predominant Eaff682 signal in the liver. *Kn* indicates kidney; *Lv*, liver.