



**Figure 1S.** Quantitation of radiotracer uptake during dynamic imaging. ROIs were drawn for the whole-body (WB), tumor (T), heart (H), kidneys (K), and urinary bladder (UB) for each 2-min image (**A**, representative ROIs). The counts per pixel (cpp) for each tissue ROI were then expressed as a percentage of the WB cpp, which represented 100% of the injected activity, and plotted over 60 min (**B-D**). The remainder (R) was derived by subtracting the sum of the tissue ROI cpp from the WB cpp.

Quantitation of radioactivity in each of the 30, 2-min, images indicated that by 6 min post-injection, there was more signal in the tumor than in the heart of the pretargeted animal (**B**). There was a steady accumulation of signal in the tumor, with maximal uptake at ~10% of the WB radioactivity, and a rapid decline of signal from the heart and kidneys, with a concomitant decrease in the normal tissue ROI and accumulation in the bladder. In contrast, the tumor signal remained fixed at ~2% of WB radioactivity in the mouse that received  $^{99m}\text{Tc}$ -anti-CEA Fab' (**C**). At the last time-point, ~60% of the total radioactivity was in the remainder of the body of this mouse, with all other organs having substantially more radioactivity than the tumor, indicating no specific tumor localization. The radiotracer used alone, without pretargeting, also showed a constant level in the tumor that was higher than the  $^{99m}\text{Tc}$ -Fab', but about 3-fold lower than that of the pretargeted mouse. The amount of radiotracer alone in the tumor was higher than in the heart, but ~2.5-fold lower than in the kidneys (**D**).