



Supplemental Figure 7. Non-targeted 4-1BB agonist accumulates in the liver in contrast with FAP-targeted 4-1BBL. (A) Schematic representation of radiolabeling assay in which TS/A+CAF tumor bearing mice received radiotherapy (RT, blue arrows) of the primary tumor (PT) but not contralateral tumor (CT). For combination treatment mice received additionally muFAP-4-1BBL (green arrow) or DP47-4-1BBL (brown arrow) labeled with [^{67}Ga] as indicated. *In vivo* biodistribution analysis measuring radioactivity performed by SPECT/CT images obtained in vivo (day 17, 21 and 23) and ex vivo by harvesting at day 23 (orange arrows). (B) SPECT/CT images showing the mean of standardized uptake values (SUV) of coronal sections measured at day 17, 21 and 23 of mice treated with RT+muFAP-4-1BBL or RT+muDP47-4-1BBL. (C) Quantification of [^{67}Ga]-DP47-1BBL and [^{67}Ga]-FAP-1BBL meanSUV as mean \pm SEM in the liver (n=3 mice/group) (Two-way ANOVA) * $p < .05$, *** $p < .001$. (D) Ex vivo biodistribution analysis at day 23 performed by gamma counter and displayed as percent of injected dose per gram of liver (%ID/g) of indicated mice (n=2 mice/group, mean \pm SEM), (t-test one-tailed); * $p < .05$. (E) Transaminase levels (ALT and AST) measured in serum 72h after first dose of therapy of indicated groups in 4T1-mCherry engrafted tumors (n=6-8 mice/group). Each symbol represents one mouse, shown is the mean \pm SEM.