**Supplementary figure legends**

Figure S1.

CX-072, PbCtrl and CX-075 binding properties. (A) Binding of CX-075 parental antibody to human and murine PD-L1 determined by ELISA. Bound CX-075 is presented as optical density (OD) measured at 450 nm. Data is shown as mean ± standard deviation (SD). (B) CX-072 and CX-075 binding affinity for human and murine PD-L1. Affinity for PD-L1 is expressed as the apparent binding constant (Kapp). (C) Binding of CX-072, PbCtrl and CX-075, after conjugation with TFP-N-SucDf, to PD-L1 determined by ELISA. Bound CX-075 or conjugated antibody (mAb-N-sucDf) is presented as OD measured at 450 nm. Data is shown as mean ± SD. (D) CX-072 and CX-075 immunoreactivity to PD-L1 before and after conjugation with TFP-N-sucDf. Immunoreactivity to PD-L1 is presented as the effective concentration needed for 50% receptor occupation (EC50).

**Figure S2.**

***Ex vivo* biodistribution with escalating protein dose of 89Zr-CX-072, 89Zr-PbCtrl and 89Zr-CX-075.** Biodistribution of **(A)** 89Zr-CX-072, **(B)** 89Zr-PbCtrl and **(C)** 89Zr-CX-075 at increasing total protein dose of 10, 50 and 250 µg, respectively, in MDA-MB-231 tumor-bearing mice at 6 days pi. Tracer uptake per organ is presented as percentage of injected dose per gram tissue (%ID/g). Data is shown as mean ± SD.

**Figure S3.**

***In vivo* quantification of 89Zr-CX-072, 89Zr-PbCtrl and 89Zr-CX-075 PET imaging.** Quantification of **(A)** 89Zr-CX-072, **(A)** 89Zr-PbCtrl and **(C)** 89Zr-CX-075 uptake 6 days pi in MDA-MB-231 tumor and blood pool at increasing total protein dose. Tracer uptake is presented as mean standardized uptake value (SUVmean). Data is shown as mean ± SD.

**Figure S4.**

**PET imaging and biodistribution in syngeneic MC38 tumor-bearing mice. (A)** *In vivo* quantification of 89Zr-CX-072, 89Zr-PbCtrl and 89Zr-CX-075 uptake in MC38 tumor and blood pool at 6 days pi. Tracer uptake is presented as SUVmean. **(B)** *Ex vivo* biodistribution of 89Zr-CX-072, 89Zr-PbCtrl and 89Zr-CX-075 in MC38 tumor-bearing mice at 6 days pi. Tracer uptake per organ is presented as %ID/g. Data is shown as mean ± SD.

**Figure S5.**

***In vitro* PD-L1 expression versus *ex vivo* uptake in MC38 and MDA-MB-231 tumors. (A)** PD-L1 expression detected with flow cytometry in MDA-MB-231 and MC38 cell lines. CX-075 was used for detection of PD-L1 positive cells, and IgG4 antibody was used as isotype control. Data from a representative experiment is shown. **(B)** PD-L1 expression in MDA-MB-231 and MC38 cell lines presented as mean fluorescent intensity (MFI). Data is shown as mean ± SD. **(C)** Internalization of 89Zr-CX-075-PD-L1 complexes in MDA-MB-231 and MC38 cells. Internalization was determined after 1 and 2 hours incubation at 37 °C, while control samples were kept at 4 °C. Internalization is presented as percentage of total PD-L1-bound 89Zr-CX-075. Data is shown as mean ± SD. **(D)** Uptake of 89Zr-CX-072, 89Zr-PbCtrl and 89Zr-CX-075 in MDA-MB-231 and MC38 tumors at 6 days pi. Tracer uptake per organ is presented as %ID/g. Data is shown as mean ± SD. \*: *p* < 0.05; \*\*: *p* < 0.01; ns: not significant.

**Figure S6.**

***In vivo* kinetics of 89Zr-CX-072, 89Zr-PbCtrl and 89Zr-CX-072.** Residual 89Zr activity inBALB/c nude and C57BL/6J tumor-bearing mice measured at 1, 3 and 6 days pi of 89Zr-CX-072, 89Zr-CX-PbCtrl and 89Zr-CX-075. Results are presented as radioactive decay corrected percentage of injected dose (%ID). Data is shown as mean ± SD.