Supplementary Materials for

First-in-Human DR5 PET Reveals Insufficient DR5 Expression in GI Cancer Patients

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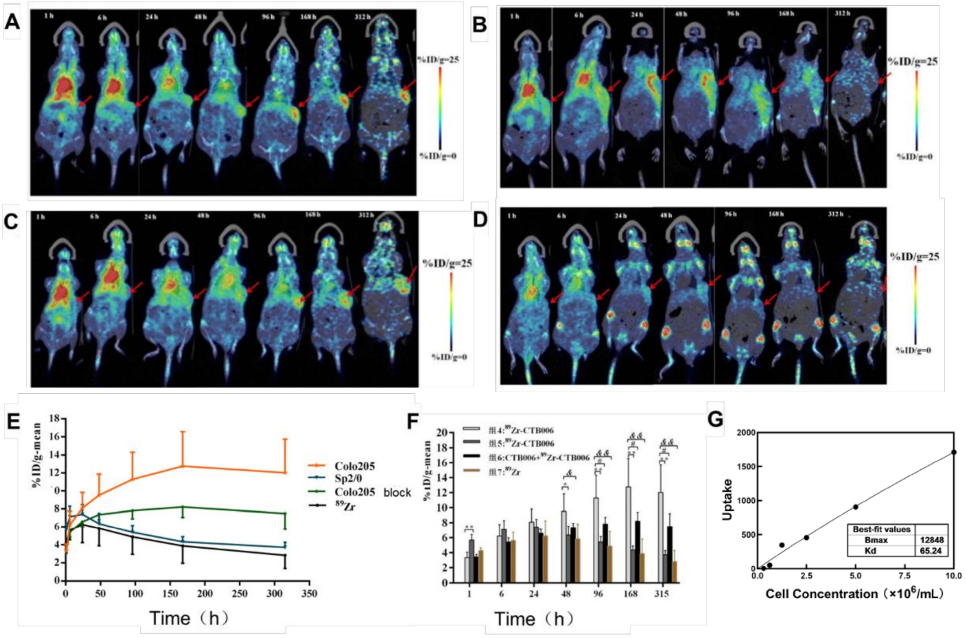

Figure S1. The Colo205 tumor in the experiment group showed obvious uptake of ^{89}Zr-CTB006 and obtained the highest uptake at 168h after administration (%ID/g: 12.75±3.81). The experiment group showed significant higher uptake in tumors than that in the blocking group (%ID/g: 12.75±3.81 vs 8.21±1.16, $P < 0.05$), the control group (%ID/g: 12.75±3.81 vs 4.34±0.55, $P < 0.01$) and the free ^{89}Zr group (%ID/g: 12.75±3.81 vs 3.88±1.93, $P < 0.01$). **A.** The PET/CT images of the Colo205 mouse in the experiment group. **B.** The PET/CT images of the Sp2/0 mouse in the control group. **C.** The PET/CT images of the Colo205 mouse in the blocking group. **D.** The PET/CT images of the Colo205 mouse in the free ^{89}Zr group. **E-F.** The tumor %ID/g in the four groups. **G.** The affinity of ^{89}Zr-CTB006 in Colo205 cells.

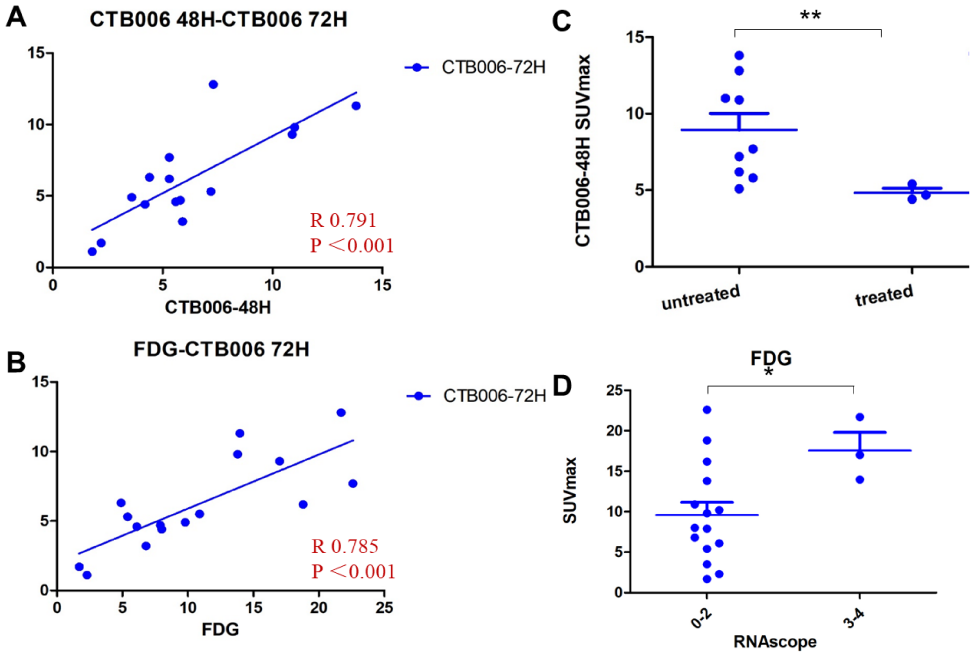

Figure S2. A. There was a significant positive correlation between the SUV_{max} of tumors on ^{89}Zr-CTB006 PET/CT at 48h and that at 72h (P < 0.001). **B.** There was a significant positive correlation between the SUV_{max} of tumors on ^{89}Zr-CTB006 PET/CT at 72h and that on ^{18}F-FDG PET/CT (P < 0.001). **C.** SUV_{max} in colorectal cancer that had received radiotherapy or chemotherapy was significantly lower than that without treatment on 48h ^{89}Zr-CTB006 PET/CT (4.83 ± 0.51 vs 8.94 ± 3.28, P = 0.005). **D.** The tumor uptake of ^{18}F-FDG was also significantly higher in in patients with RNAscope score 3-4 than that with score 0-2 (SUV_{max}: 17.55±3.90 vs 9.60± 6.05, P =0.046).

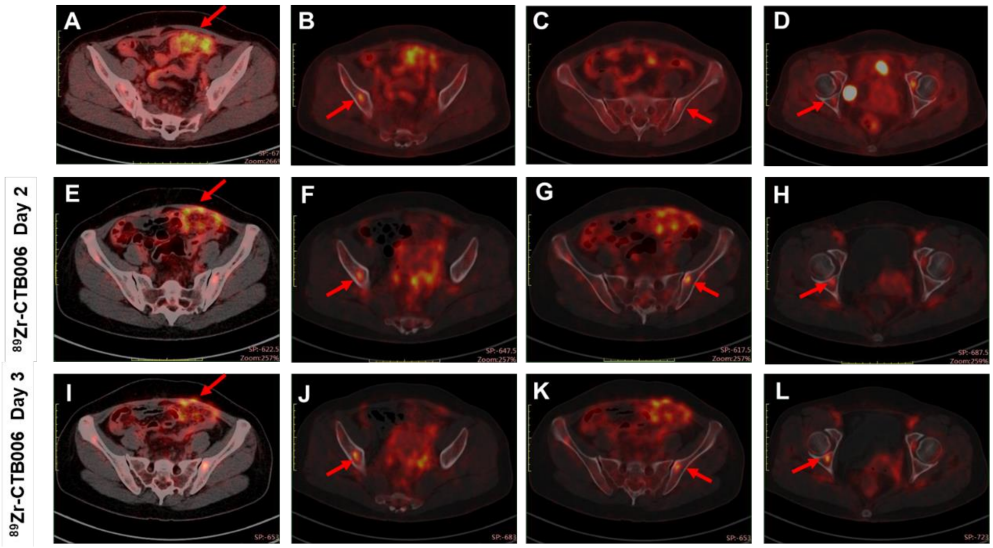

Figure S3. ^{18}F-FDG PET/CT showed high uptake of the thickened peritoneum (**A**, arrow, SUVmax: 4.9) and the metastasis on the right iliac bone (**B**, arrow, SUVmax: 3.9), slight uptake of the metastasis on the left iliac bone (**C**, arrow, SUVmax: 2.1) and the right acetabulum (**D**, arrow, SUVmax: 2.1). ^{89}Zr-CTB006 PET/CT showed inhomogeneous high uptake of the thicked peritoneum (**E**, arrow, SUVmax: 6.3 at 48h; **I**, arrow, SUVmax: 4.4 at 72h) and the metastases on the right iliac bone (**F**, arrow, SUVmax:4.3 at 48h; **J**, arrow, SUVmax:4.4 at 72h), the left iliac bone (**G**, arrow, SUVmax: 5.3 at 48h; **K**, arrow, SUVmax: 3.7 at 72h), the right acetabulum (**H**, arrow, SUVmax: 3.7 at 48h; **L**, arrow, SUVmax: 4.4 at 72h).

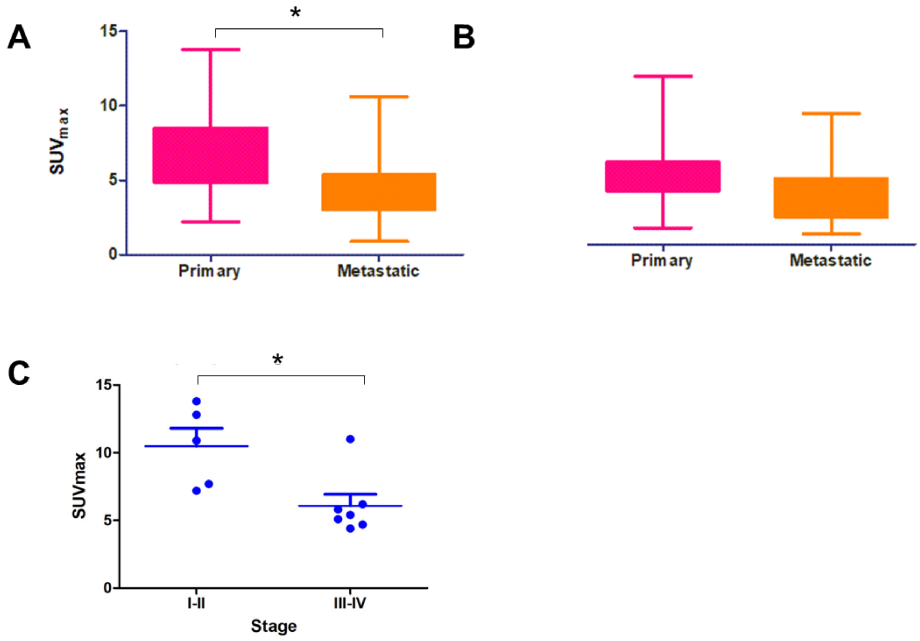

Figure S4. A. The uptake of ^{89}Zr-CTB006 at 48h in the metastatic lesions was significantly lower than that in the primary lesions (6.97 ±3.14 vs 4.22±2.45, P = 0.0135). **B.** The uptake of ^{89}Zr-CTB006 at 72h in the metastatic lesions was not significantly different from that in the primary lesions (5.18 ±2.63 vs 3.52±2.19, P = 0.0704). **C.** The uptake in colorectal cancer patients with III-IV stage was significantly lower than I-II stage (6.09±2.25 vs 10.48±2.96, P = 0.015).

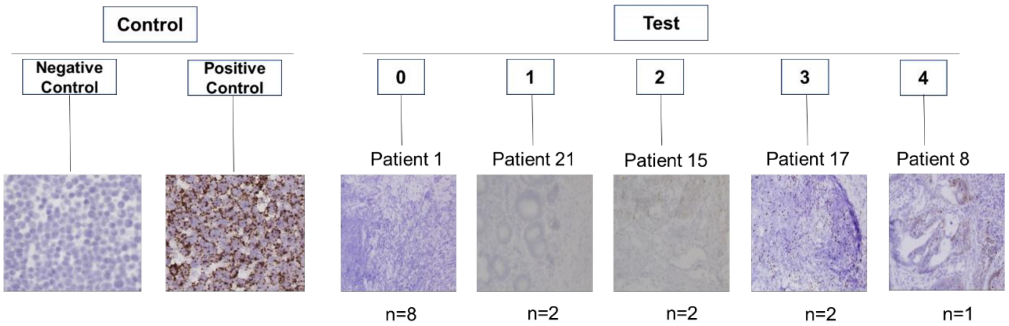

Figure S5. The negative and positive control images and the images of the representative sample with different RNAscope scores.

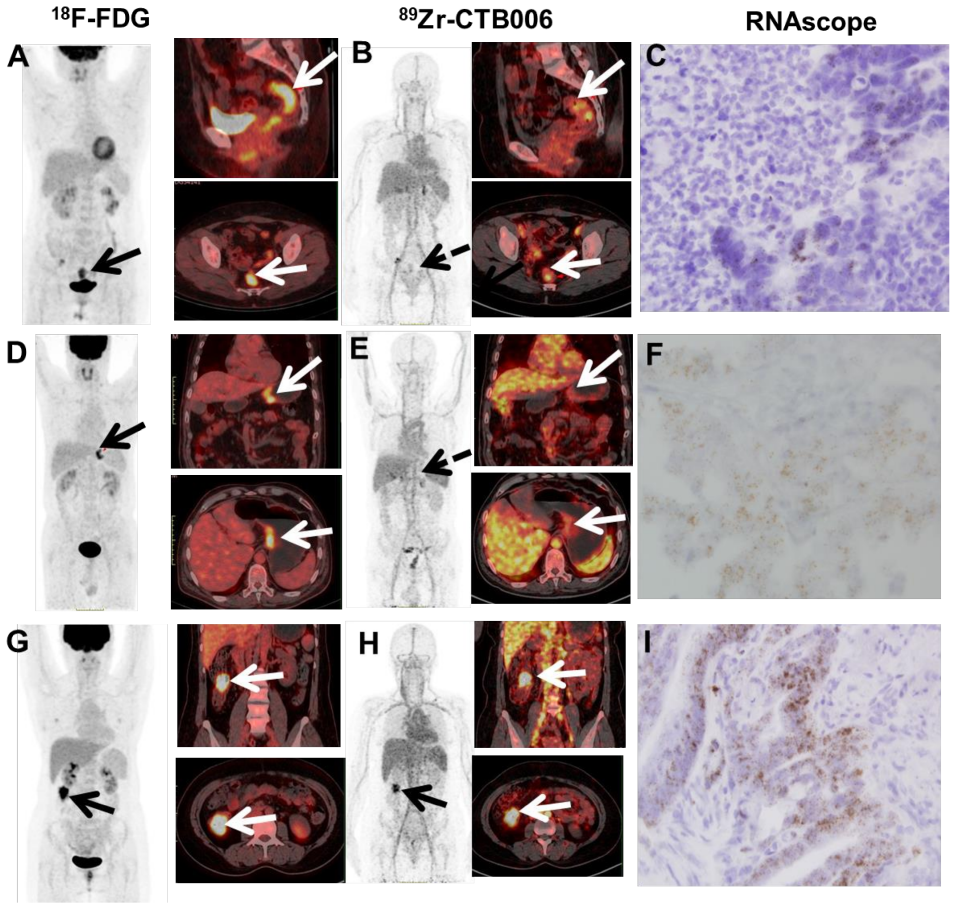

Figure S6. PET/CT images and RNAscope staining of patient 11, 15 and 8**. A.** MIP image, the axial and coronal PET-CT fused images of ^{18}F-FDG PET/CT of patient 11 showed high uptake of ^{18}F-FDG in tumor (arrow). **B.** MIP image, the axial and coronal PET-CT fused images of ^{89}Zr-CTB006 PET/CT of patient 11 showed low uptake of ^{89}Zr-CTB006 in tumor (arrow). **C.** RNAscope score of patient 11 was 1 (40X). **D.** MIP image, the axial and coronal PET-CT fused images of ^{18}F-FDG PET/CT of patient 15 showed high uptake of ^{18}F-FDG in tumor (arrow). **E.** MIP image, the axial and coronal PET-CT fused images of ^{89}Zr-CTB006 PET/CT of patient 15 showed low uptake of ^{89}Zr-CTB006 in tumor (arrow; the dash arrow indicates the unclearly tumor lesion). **F.** RNAscope score of patient 15 was 2 (40X).

G. MIP image, the axial and coronal PET-CT fused images of ^{18}F-FDG PET/CT of patient 8 showed high uptake of ^{18}F-FDG in tumor (arrow). **H.** MIP image, the axial and coronal PET-CT fused images of ^{89}Zr-CTB006 PET/CT of patient 8 showed low uptake of ^{89}Zr-CTB006 in tumor (arrow; the dash arrow indicates the unclearly tumor lesion). **I.** RNAscope score of patient 8 was 2 (40X).

Table S1. RNAscope Score

RNAscope score	Objective scoring by microscope
0	0 or less than 1 signal per 10 cells
1	1-3 signals per cell
2	4-10 signals per cell, very few cluster points
3	more than 10 signals per cell, positive cells with clusters are less than 10%
4	more than 10 signals per cell, positive cells with clusters are more than 10%

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Table S2. Quality Control of ^{89}Zr-CTB006 for Clinical Application

Parameter	QC specification	QC result
Appearance	Clear, colorless	Pass
Volume	1.5-2.5ml	2.5ml
Injection dose	18.5-74MBq	37MBq
pH	6.5-8.0	7.0
Radio-thin-layer Chromatography	＞95%	＞99%
Radio-high-performance Liquid chromatography	＞95%	＞99%
Ethanol	＜5%	0
Endotoxins	＜15EU/ml	Pass
Sterility	Sterile	Pass

Table S3. ^{89}Zr-CTB006 Gender Averaged Organ Doses [mSv/MBq]

Target Organ	Effective Dose
Adrenals	8.77E-03
Brain	5.34E-04
Left colon	2.18E-02
Small Intestine	1.21E-03
Stomach Wall	7.63E-02
Heart Wall	8.66E-03
Kidneys	8.21E-03
Liver	5.68E-02
Lungs	6.61E-02
Ovaries	2.69E-03
Pancreas	5.60E-03
Prostate	2.72E-03
Salivary Glands	2.89E-04
Red Marrow	1.86E-02
Osteogenic Cells	8.86E-04
Spleen	1.08E-02
Urinary Bladder Wall	1.86E-03
Uterus	1.63E-03
Total Body	0.00E+00
Effective Dose	3.49E-01