SUPPORTING INFORMATION

Matching the decay half-life with the biological half-life: ImmunoPET imaging with ^{44}Sc-labeled Cetuximab Fab fragment

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Experimental Section

Optimization of radiolabeling condition

The pH of the reaction medium plays a crucial role in affecting the radiolabeling yield and specific activity of the radiolabeled agent. In order to determine the optimum pH, the radiolabeling of CHX-A″-DTPA with ^{44}Sc was carried out under different pH conditions. For this purpose, ^{44}Sc (74 MBq) was diluted in 500 μL of 0.5 M sodium acetate buffer and added to 20 μg of CHX-A″-DTPA. The pH of the reaction mixture was carefully adjusted to the desired values (3.0, 4.5, 6.5, and 8.0) and incubated for 30 min at room temperature (25 °C) with constant shaking. The radiolabeling yield was determined by thin layer chromatography (TLC), adopting the reported procedure.[1] The chromatogram was developed using 50% aqueous acetonitrile as the eluent and it was observed that ^{44}Sc-CHX-A″-DTPA migrated towards the solvent front (R_f = 0.8-0.9), while under identical conditions unlabeled $^{44}Sc^{3+}$ remained at the point of application (R_f = 0). The radiolabeling yield of ^{44}Sc-CHX-A″-DTPA was found to be maximum (> 80%) when the reaction was carried out at pH ~4.5 (***Figure S2***). Therefore, all subsequent ^{44}Sc-labeling reactions using CHX-A″-DTPA-Cetuximab-Fab was carried out at this pH to maximize the radiolabeling yield and specific activity of the radiolabeled agent.

Histology

To further validate that tumor uptake of ^{44}Sc-CHX-A″-DTPA-Cetuximab-Fab was indeed EGFR specific, U87MG (high EGFR expression) and Caco-2 (low EGFR expression) tumor-bearing mice were each injected with a larger dose of Alexafluor350-Cetuximab-Fab (5 mg/kg of mouse body weight) and euthanized at 4 h p.i. The tissues (tumor, liver, kidney and muscle) were dissected and frozen in the Tissue-TEK embedding medium to prevent the tissues from degradation. The frozen tissue samples were sectioned to obtain slices of 7 μm thickness. The

frozen tissue slices were then fixed with cold acetone for 10 min and dried in the air for 30 min. After rinsing with PBS and blocking with 10 % donkey serum for 30 min at room temperature, the slices were incubated with Cetuximab (0.5 μg/mL) for 1 h at 4 °C and visualized using FITC-labeled donkey anti-rat IgG. After washing with PBS, all images were acquired with a Nikon Eclipse Ti microscope.

In tissues from U87MG tumor bearing mice, accumulation of Alexafluor350-Cetuximab-Fab (blue fluorescence) co-localized with the expression of EGFR in tumor tissues (green fluorescence) (***Figure S5***). The excellent overlay of blue and green signals in U87MG tumors suggested that Alexafluor350-Cetuximab-Fab binds specifically to EGFR. Very faint signals were observed in muscles which serve as the control. The absence of blue signal from Caco-2 tumors further confirms the EGFR specificity of Cetuximab-Fab.

Supplementary Table

Table S1: Determination of ^{44}Sc labeling yields when different BFCs were conjugated with Cetuximab-Fab. All reactions were carried out at room temperature (25 °C) for 30 min.

BFC used for conjugation with Cetuximab-Fab	**Radiolabeling yield (%)**	**Theoretical stability constant (log K_{ML})$^{@}$**	**Reference**
DOTA	21±3	30.8	(*2*)
NOTA	14±1	16.5	(*3*, *4*)
DTPA	68±2	27.4	(*2*)
CHX-A″-DTPA	66±5	#	#

$^{@}K_{ML}$=[ML]/[M][L]; #not reported.

Supplementary Figures

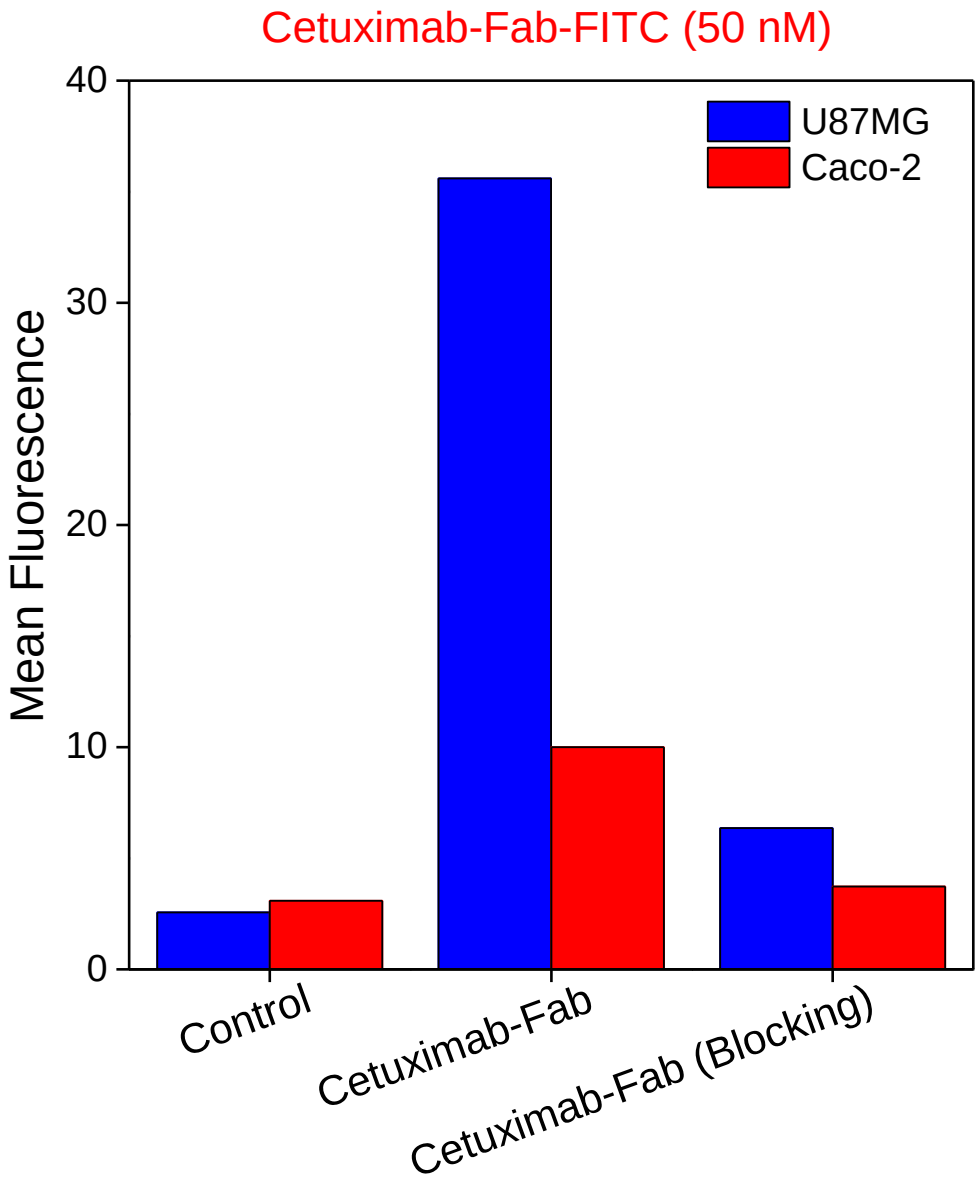

Figure S1: Mean fluorescence intensities of U87MG and Caco-2 cells for targeted and blocking groups in flow cytometry studies.

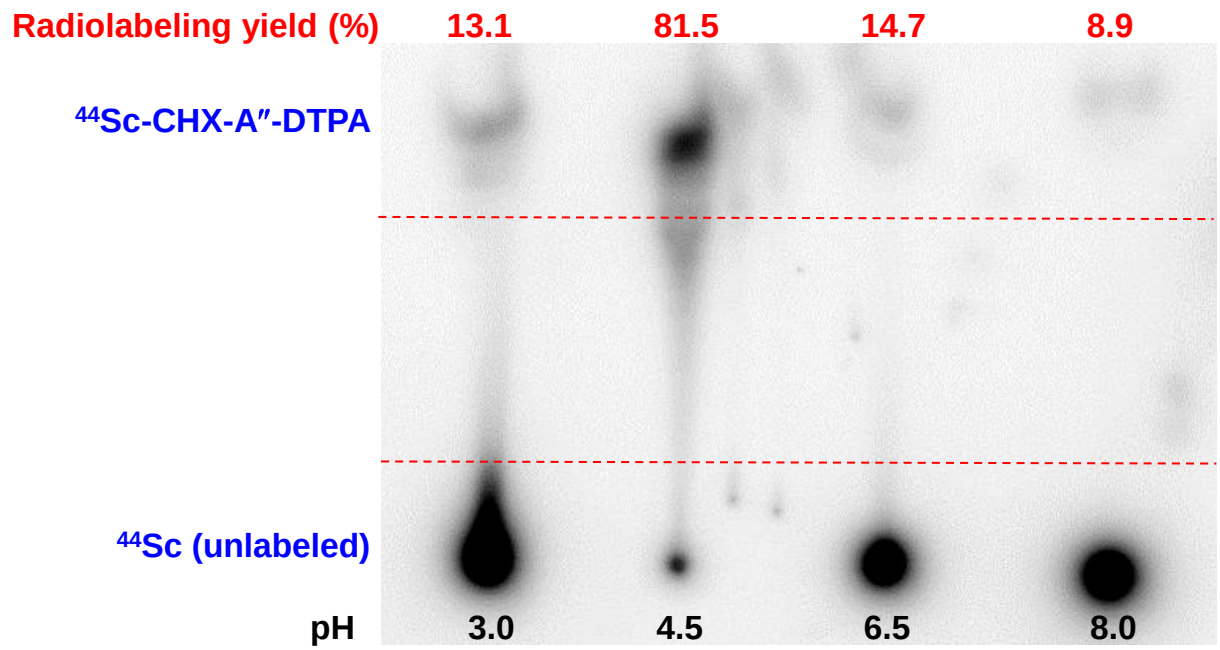

Figure S2: Determination of radiolabeling yields of ^{44}Sc-CHX-A″-DTPA when reaction was carried out under different pH conditions. The unlabeled ^{44}Sc remains at the point of application while ^{44}Sc- CHX-A″-DTPA migrates to the solvent front.

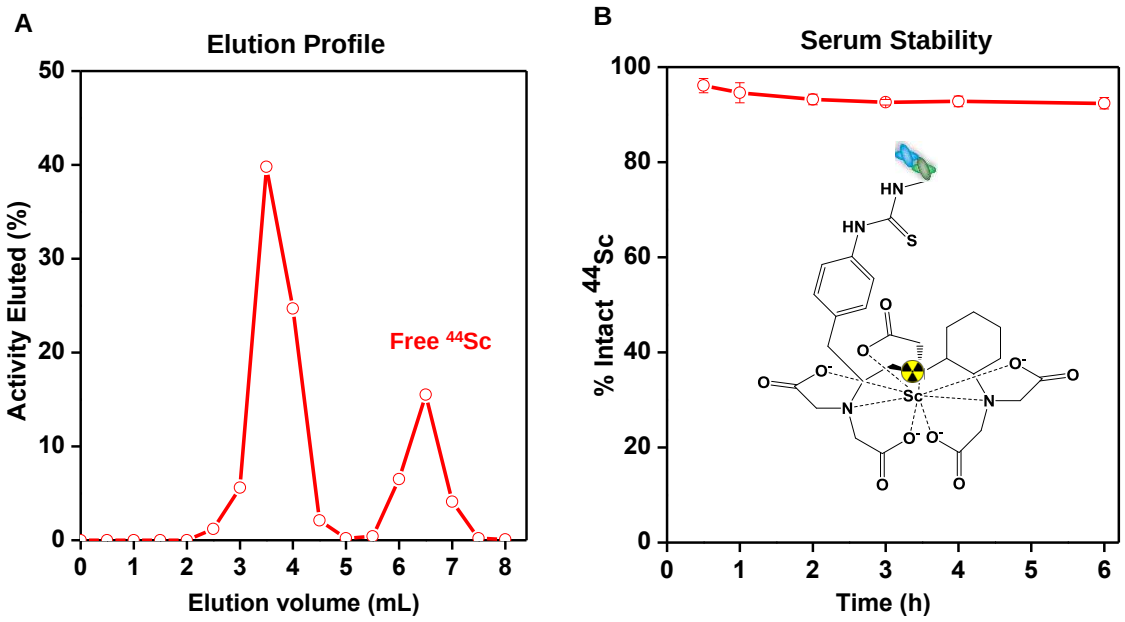

Figure S3: (A) Elution profile of ^{44}Sc-CHX-A″-DTPA-Cetuximab-Fab from PD-10 column. **(B)** Serum stability of ^{44}Sc-CHX-A″-DTPA-Cetuximab-Fab.

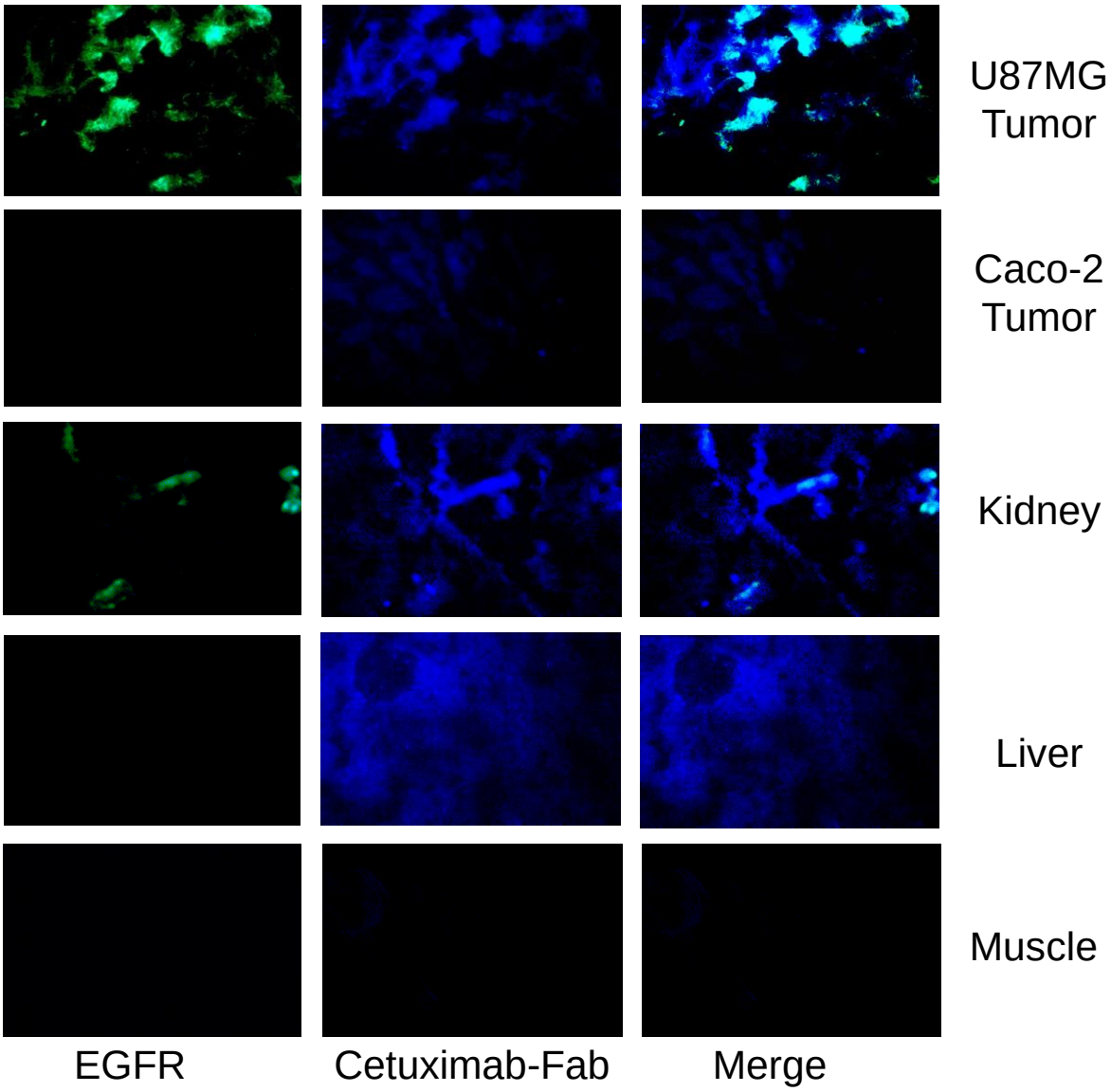

Figure S4: Immunofluorescence staining of U87MG tumor, Caco-2 tumor, kidney, liver, and muscle tissue sections.

References

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