

ImmunoPET imaging of Trop2 in patients with solid tumours

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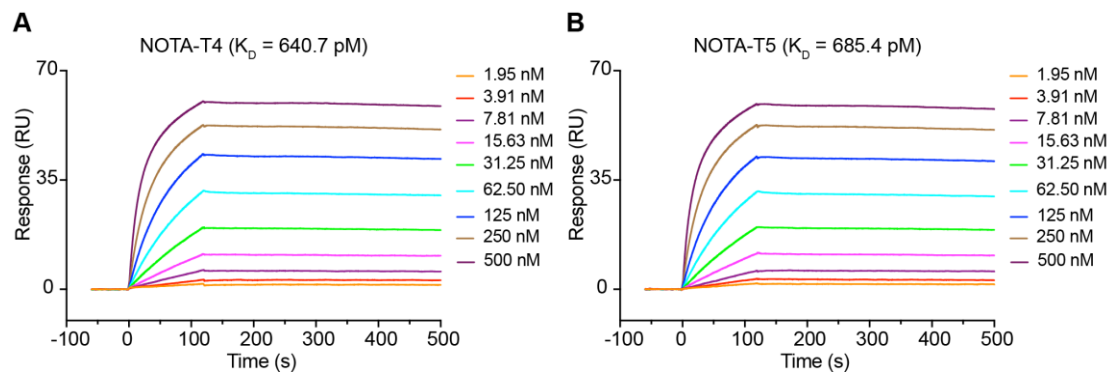
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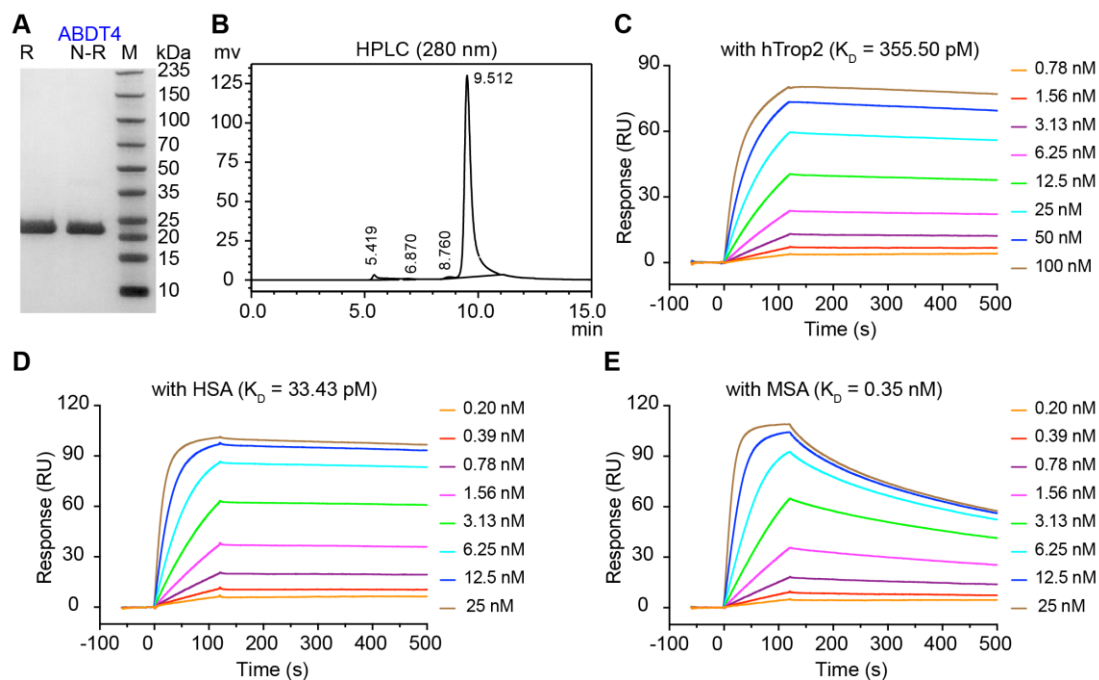
Appendix Figure and Figure legends

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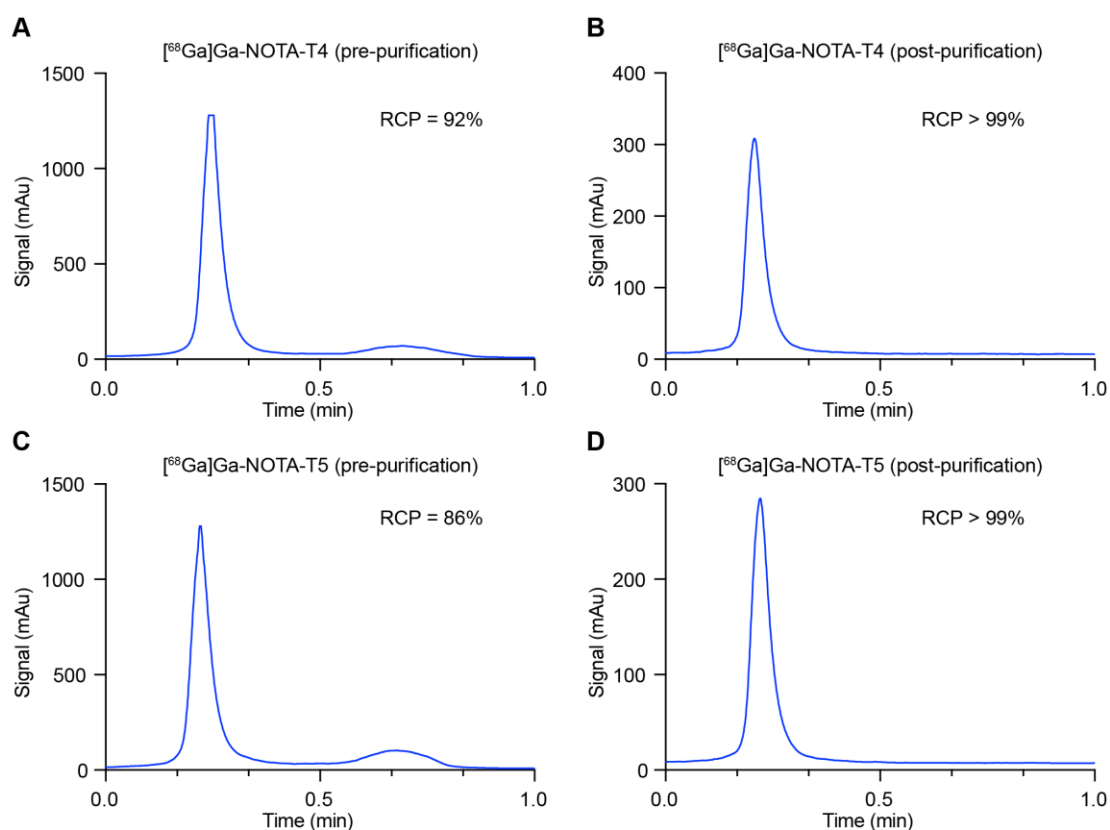
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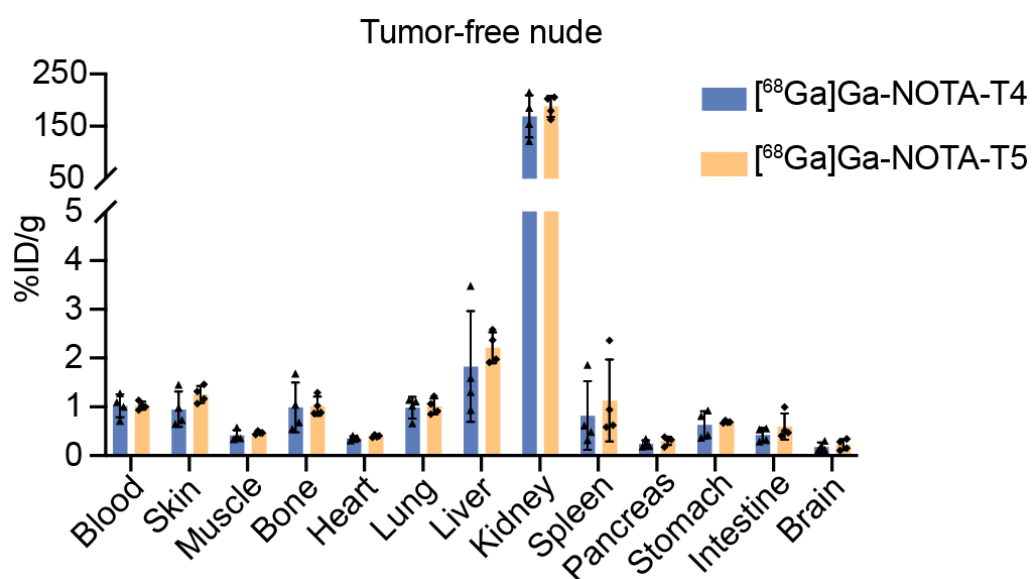
Appendix Figure S1. Surface plasmon resonance (SPR) analysis revealed the binding affinity of NOTA-T4 (A) and NOTA-T5 (B) interacting with recombinant human Trop2 protein.



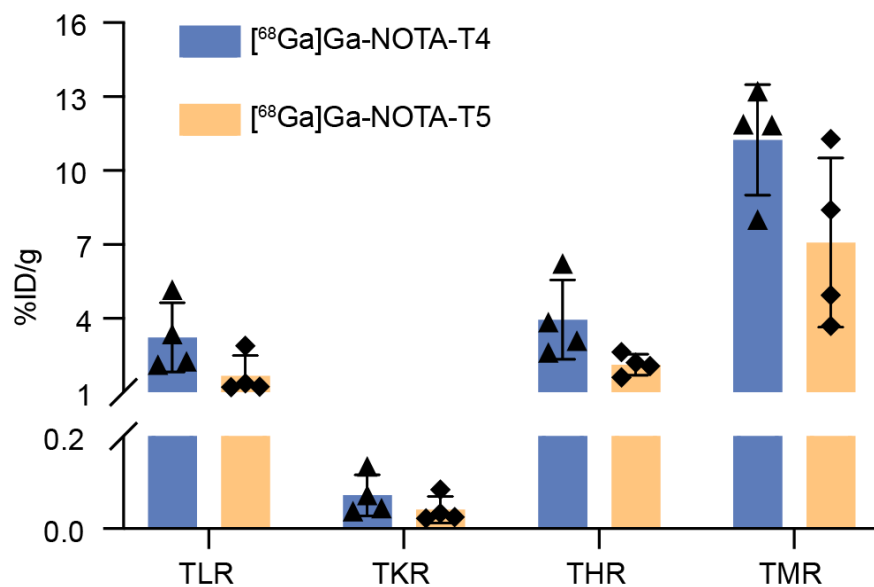
Appendix Figure S2. Expression and Characterization of ABDT4. (A) SDS-PAGE tested the purity of ABDT4. R: reducing conditions; N-R: non-reducing conditions. (B) Characterisation of ABDT4 by HPLC. (C–E) The binding affinities of ABDT4 to immobilised recombinant human Trop2 protein (C), human serum albumin (D), and murine serum albumin (E) were assayed by SPR, respectively. Source data are available online for Figure S2A.



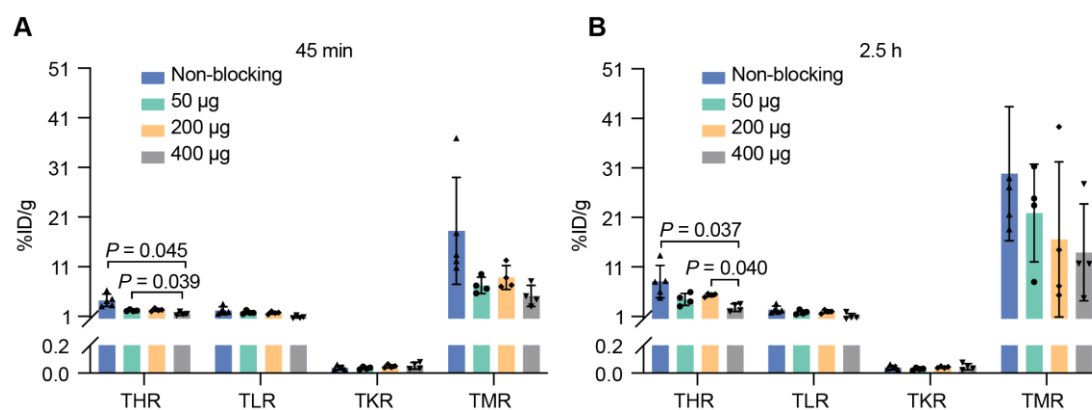
Appendix Figure S3. Assessment of radiochemical purity (RCP) of the tracers. The pre-purification (A) and post-purification (B) RCP of $[^{68}\text{Ga}]\text{Ga-NOTA-T4}$ were 92% and >99%, respectively. The pre-purification (C) and post-purification (D) RCP of $[^{68}\text{Ga}]\text{Ga-NOTA-T5}$ were 86% and >99%, respectively.



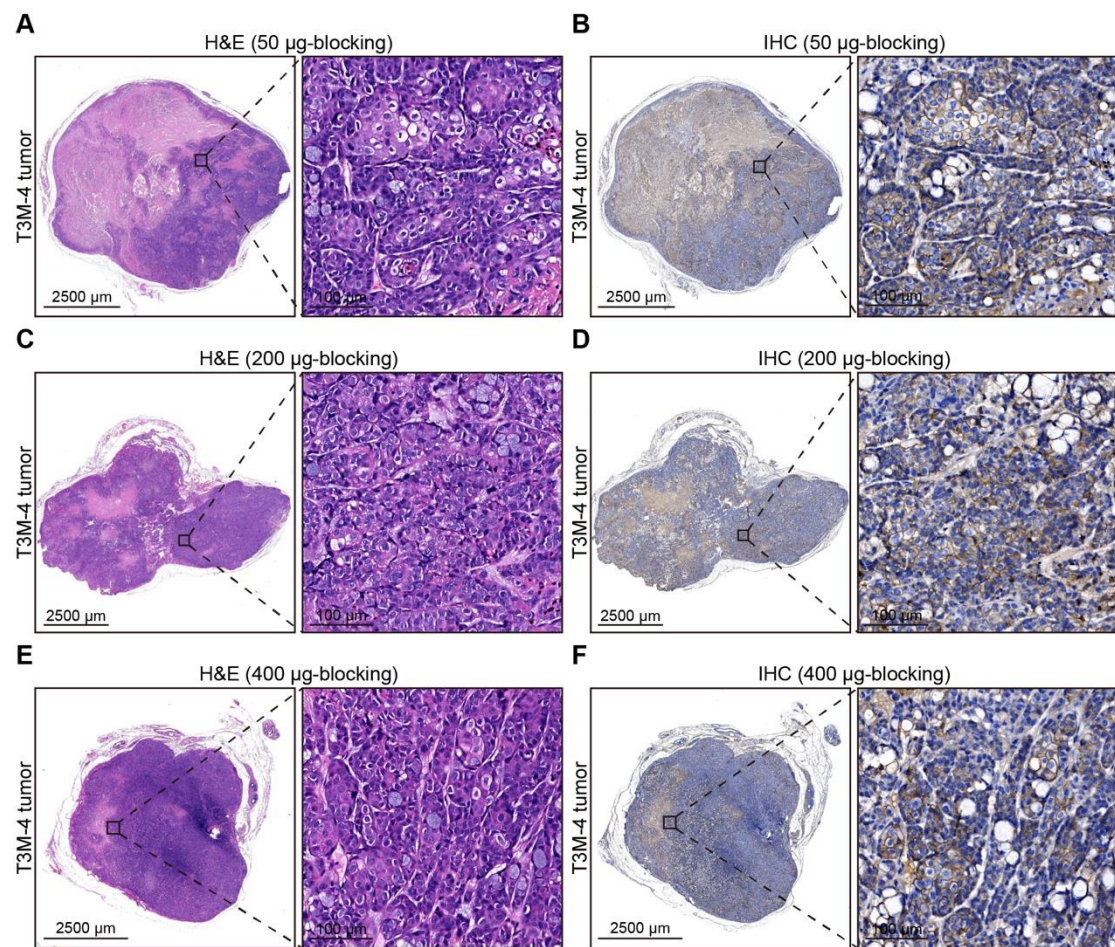
Appendix Figure S4. Distribution of radioactivity in each organ/tissue of tumour-free mice models after [⁶⁸Ga]Ga-NOTA-T4 ($n = 4$) and [⁶⁸Ga]Ga-NOTA-T5 ($n = 4$) immunoPET imaging.



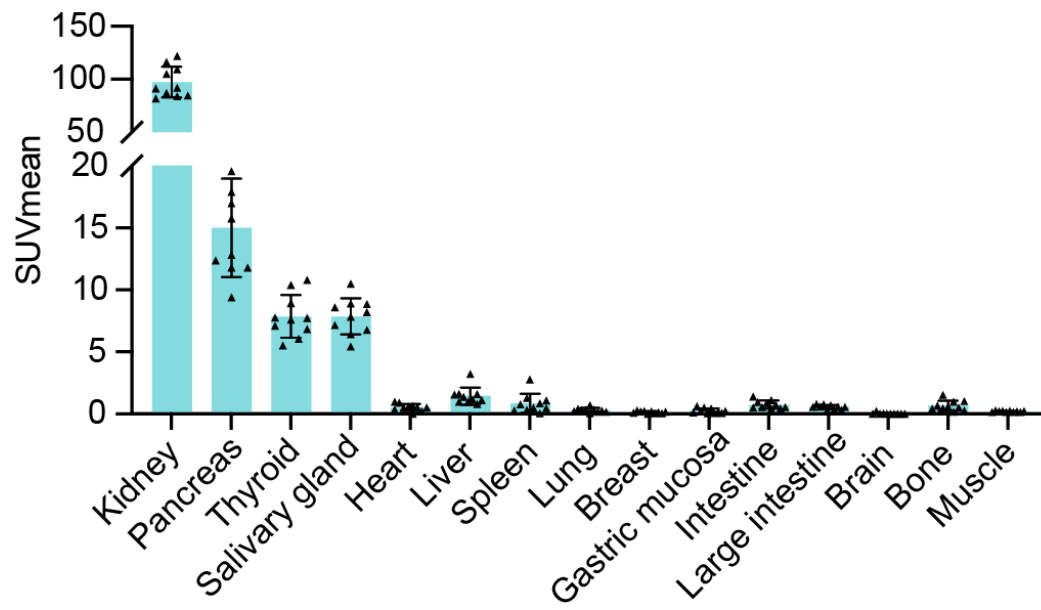
Appendix Figure S5. Comparison of tumor-to-organ ratios at [⁶⁸Ga]Ga-NOTA-T4 and [⁶⁸Ga]Ga-NOTA-T5. TLR: tumor-to-liver; TKR: tumor-to-kidney; THR: tumor-to-heart; TMR: tumor-to-muscle. *T*-test (Mean ratio $\pm$ SD, $n = 4$ for each group).



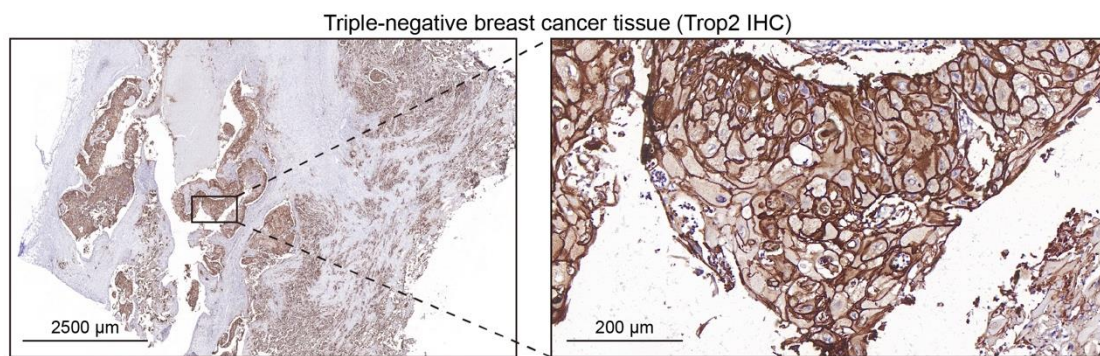
Appendix Figure S6. Comparison of tumour-to-organ ratios at two time points (45 min, A; 2.5 h, B) between the unblocking and the three blocking groups. THR: tumor-to-heart; TLR: tumor-to-liver; TKR: tumor-to-kidney; TMR: tumor-to-muscle. Two-way ANOVA.



Appendix Figure S7. Hematoxylin-eosin (H&E) and immunohistochemistry (IHC) staining results of tumour tissues from different blocked groups. (A, C, and E) H&E staining of tumours from the blocking dose of 50 µg (A), 200 µg (C), and 400 µg (E). Scale bar: 2500 µm and 100 µm. (B, D, and F) IHC staining of Trop2 expression in the 50 µg (B), 200 µg (D), and 400 µg (F) blocking groups. Scale bar: 2500 µm and 100 µm.



Appendix Figure S8. Radioactivity uptake values in normal tissues from 10 patients with malignant tumours regarding SUVmean.



Appendix Figure S9. Trop2 IHC staining of triple-negative breast cancer tissue. IHC staining result showed Trop2 high expression. Scale bar: 2500 µm and 200 µm.