# Supplement: CXCR4-Targeted PET Imaging of Glioblastoma Using [$^{68}$Ga]Ga-TD-01: From Pharmacokinetics and Dosimetry to Theranostic Potential

## Table of Contents

# 1. Radiochemistry

## 1.1 Radio labeling and quality control of [$^{68}$Ga]Ga-TD-01

The BFC containing DOTA for $^{68}$Ga complexation was successfully synthesized. Radiolabeling with $^{68}$Ga was performed via a 10-minute reaction at 60 °C in sodium acetate solution (pH 4.0). The crude product was purified by solid phase extraction (HLB SPE Cartridge) to remove unreacted $^{68}$Ga. Consequently, this condition (Fig. 1) was selected for labeling TD-01 and conducting quality control of the [$^{68}$Ga]Ga-TD-01 complex. The radio-TLC and HPLC chromatograms confirmed the successful radioactive labeling of $^{68}$Ga with TD-01 (Fig. 2 and 3). After removing excess Ga-68 using an HLB SPE cartridge, [$^{68}$Ga]Ga-TD-01 exhibited radiochemical purity (RCP) exceeding 97% (n = 4). The calculated molar activity of the produced [$^{68}$Ga]Ga-TD-01 was 51.14±4.74 GBq/μmol. The quality control analysis results of [$^{68}$Ga]Ga-TD-01 for CXCR4 imaging are reported in Table 1.

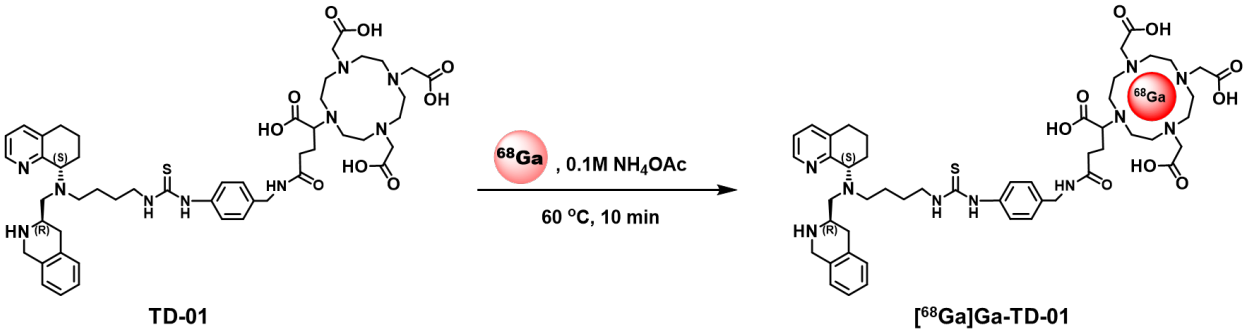



Figure 1 Radiosynthesis of [$^{68}$Ga]Ga-TD-01.

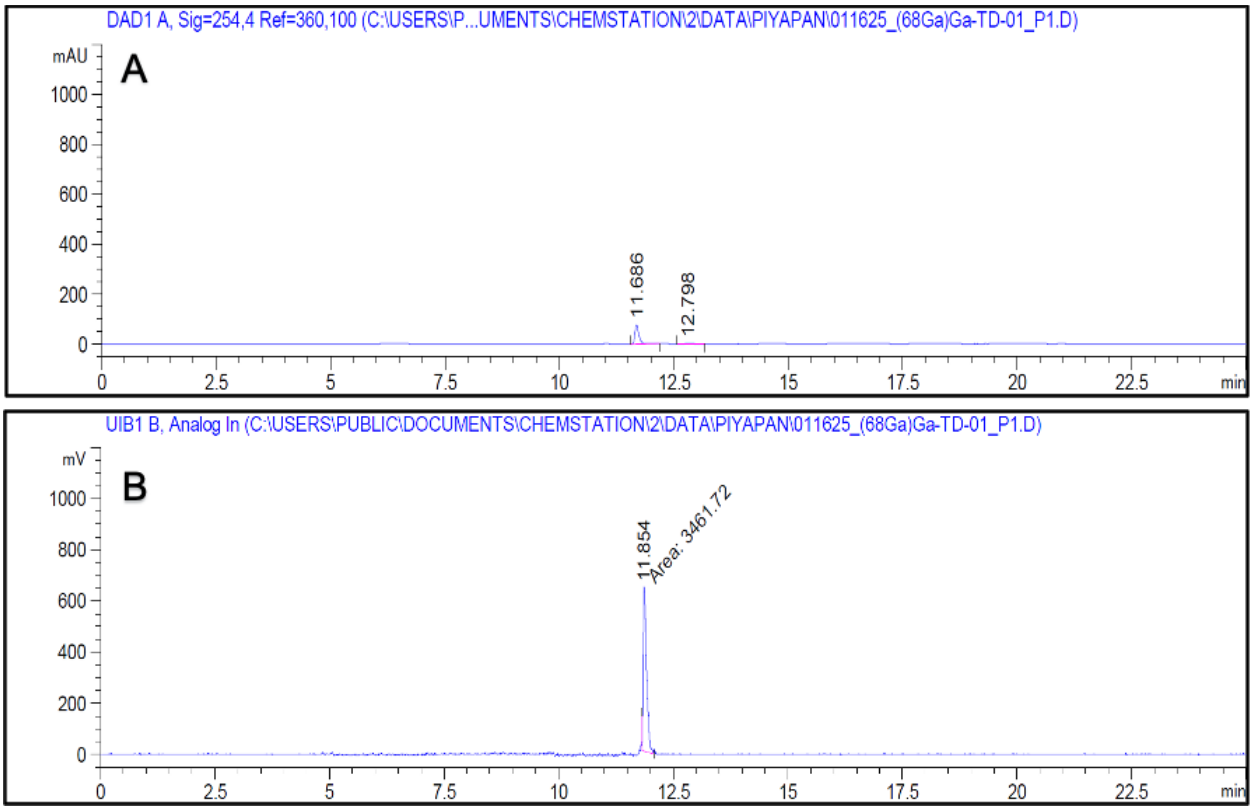



*Figure 2: HPLC chromatogram of [$^{68}$Ga]Ga-TD-01. (A) UV-detector and (B) Radio-detector*

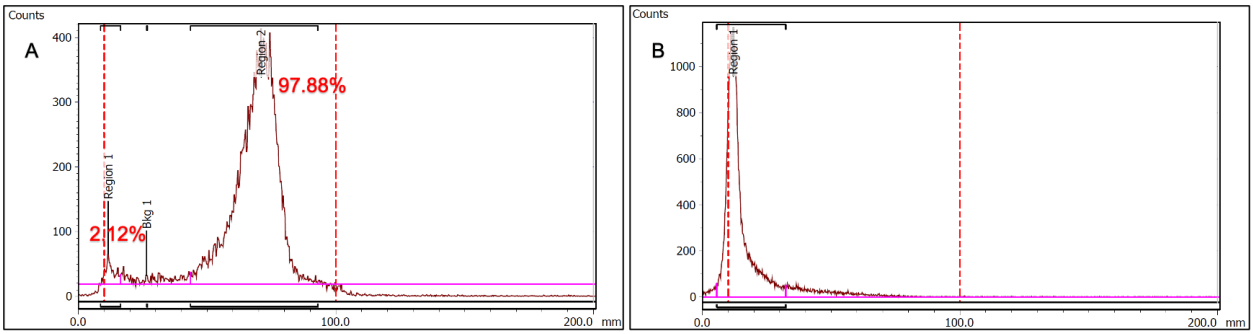
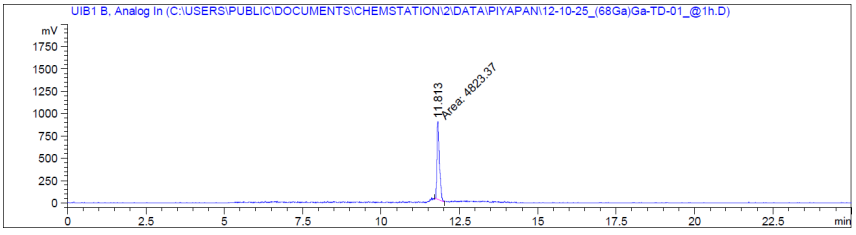
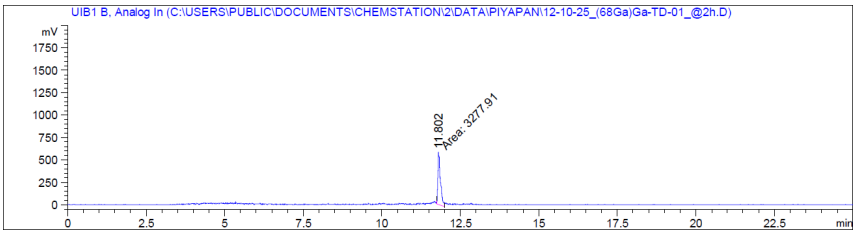
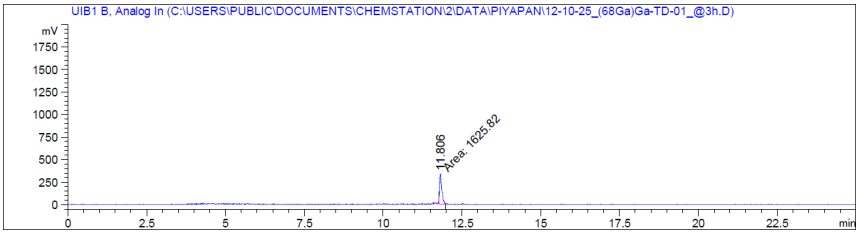
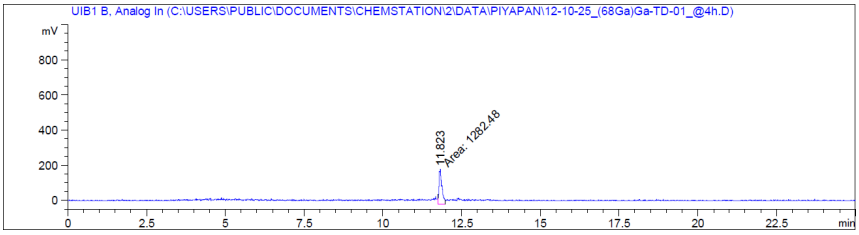
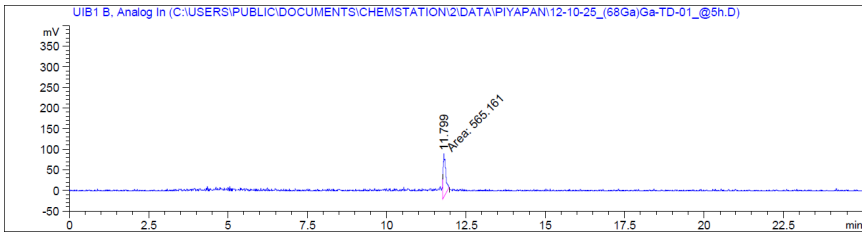
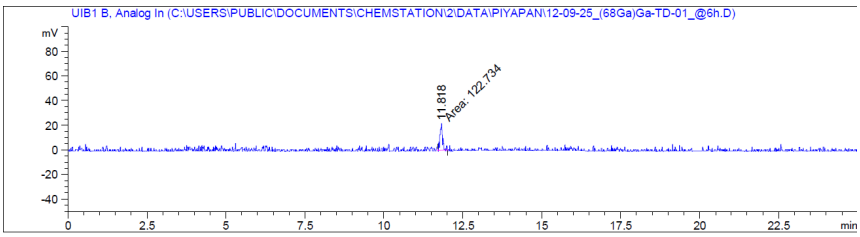



*Figure 3: iTLC profile of [$^{68}$Ga]Ga-TD-01. (A) 0.1 M $NH_4OAc$ : MeOH = 4:6 and (B) 100% of sodium citrate.*

*Table1:* ***Shelf life of [$^{68}$Ga]Ga-TD-01 in normal saline at 1, 2, 3, 4, 5 and 6 hours (n = 3)***

| Time (h) | HPLC chromatogram | %RCP |
|---|---|---|
| 1 |  | > 99 |

| | | |
|---|---|---|
| **2** |  | **> 99** |
| **3** |  | **> 99** |
| **4** |  | **> 99** |
| **5** |  | **> 99** |
| **6** |  | **> 99** |

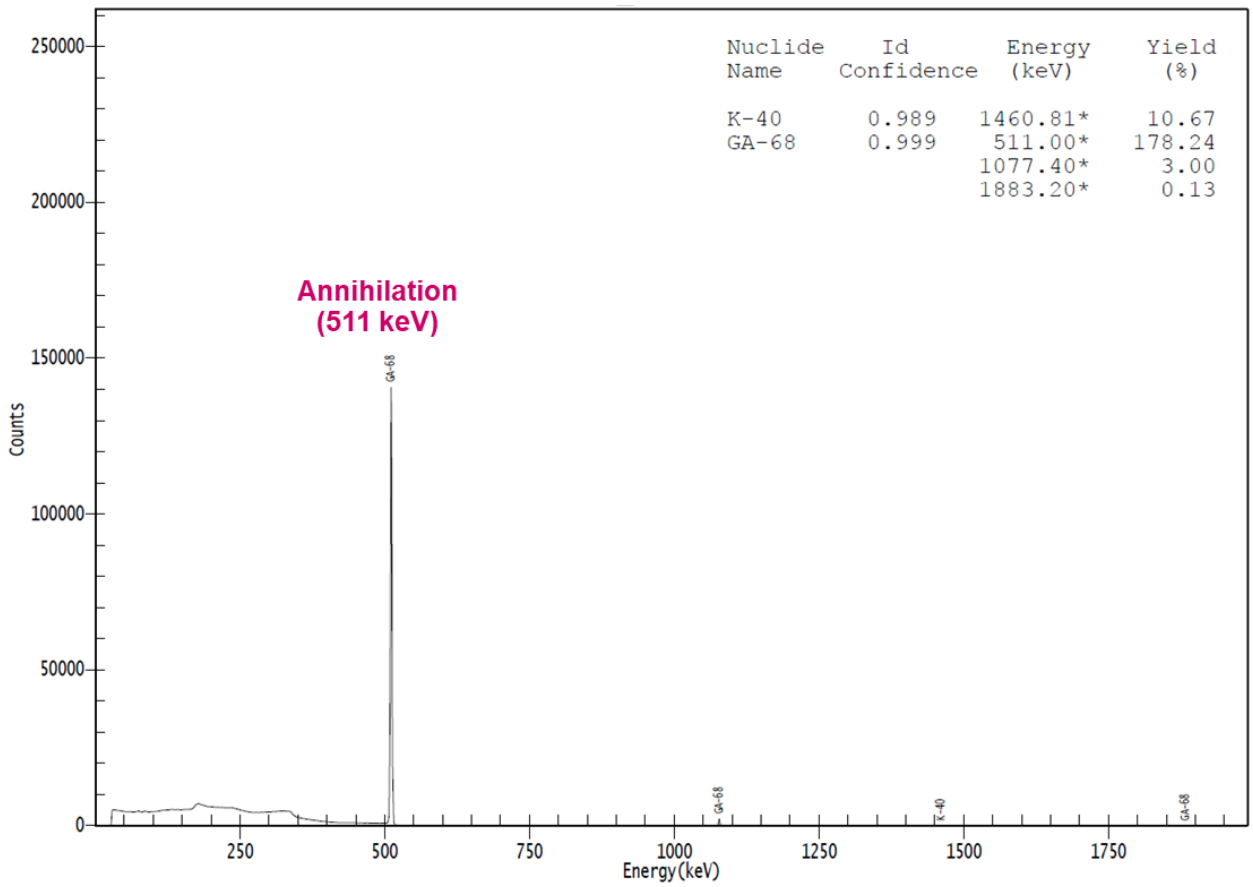



Figure 4: Gamma spectrum of Ga-68 sample. The gamma spectrum displays the energy distribution of counts obtained from a Ga-68 sample. The measurement was conducted over a live time of 3496.241 seconds and a real time of 3600.000 seconds. The prominent peak at approximately 511 keV corresponds to Ga-68. Additional smaller peaks are also identified at various energies. The spectrum indicates a high radionuclidic purity for Ga-68, as evidenced by the dominant peak at the expected energy for Ga-68 emissions.

## 2. PET/MR image segmentation

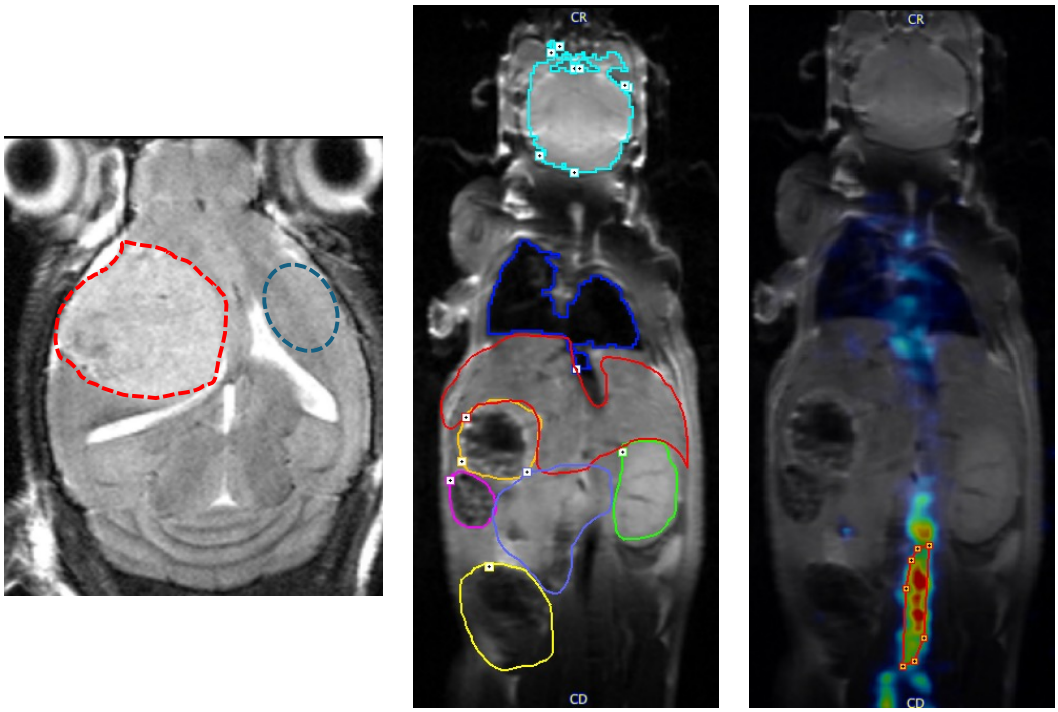



Figure 5: Examples of MRI based organ/VOI segmentation for left:T2-weighted FSE MRI, the tumor region (red) and contra lateral healthy brain region (blue) or middle: T1-weighted FSE MRI and whole body organ segmentation to extract time-activity data of dose relevant organs. Here brain (light blue), lungs (dark blue), liver (red), stomach (orange), spleen (magenta), right kidney (green) and small intestines (yellow) is visible. Right: Segmentation of the inferior vena cava (red) 5 s after radiotracer injection to extract the imaged derived input function for subsequent pharmacokinetic modeling.

## 3. Power analysis

**A priori: baseline vs. blocking tumor study**

t tests - Means: Difference between two independent means (two groups)

| | | | | | |
|---|---|---|---|---|---|
| Analysis: | A priori: Compute required sample size | | | | |
| Input: | Tail(s) | = | Two | | |
| | Effect size d | = | 2,7 | | |
| | α err prob | = | 0,05 | | |
| | Power (1-β err prob) | | = | 0,95 | |
| | Allocation ratio N2/N1 | | = | 1 | |
| Output: | Noncentrality parameter δ | | | = | 4,2690748 |
| | Critical t | = | 2,3060041 | | |
| | Df | = | 8 | | |
| | Sample size group 1 | | = | 5 | |
| | Sample size group 2 | | = | 5 | |
| | Total sample size | | = | 10 | |
| | Actual power | | = | **0,9606290** | |

**Post hoc: baseline vs. blocking tumor study**

t tests - Means: Difference between two independent means (two groups)

| | | | | | |
|---|---|---|---|---|---|
| Analysis: | Post hoc: Compute achieved power | | | | |
| Input: | Tail(s) | = | Two | | |
| | Effect size d | = | 2,85 | | |
| | α err prob | = | 0,05 | | |
| | Sample size group 1 | | = | 6 | |
| | Sample size group 2 | | = | 6 | |
| Output: | Noncentrality parameter δ | | | = | 4,9363448 |
| | Critical t | = | 2,2281389 | | |
| | Df | = | 10 | | |
| | Power (1-β err prob) | | = | **0,9931020** | |

A post hoc power analysis was performed in G*Power 3.1 for the primary pharmacokinetic endpoint (tumor Vt, 1TCM). Based on the observed means and standard deviations (0.541 ± 0.065 vs. 0.325 ± 0.085), the standardized effect size was d = 2.85. With α = 0.05 and n = 6 animals per group, the achieved power was >0.95.

## 4. Whole-body organ dosimetry

*Table 2: OD, ED contributions and the ED based on mouse biokinetic data extrapolated to human entity and estimated with OLINDA 1.1 for the adult male model.*

| | OD (µSv/MBq) | | ED contr (µSv/MBq) | |
|---|---|---|---|---|
| **Adult male (73.7 kg)** | mean | stdev | mean | stdev |
| **Adrenals** | 9.82E-03 | 1.28E-03 | 7.58E-05 | 3.17E-05 |
| **Brain** | 5.29E-03 | 3.00E-03 | 4.53E-05 | 3.25E-05 |
| **Breasts** | 7.49E-03 | 1.79E-03 | 8.41E-04 | 3.61E-04 |
| **Gallbladder Wall** | 1.31E-02 | 1.52E-03 | 9.08E-05 | 5.38E-05 |
| **LLI Wall** | 1.31E-02 | 4.90E-03 | 1.07E-03 | 8.61E-04 |
| **Small Intestine** | 2.52E-02 | 1.59E-02 | 1.57E-04 | 1.91E-05 |
| **Stomach Wall** | 1.18E-02 | 1.07E-03 | 1.40E-03 | 1.10E-04 |
| **ULI Wall** | 2.08E-02 | 1.20E-02 | 7.92E-04 | 3.90E-04 |
| **Heart Wall** | 2.60E-02 | 2.54E-02 | 1.30E-04 | 3.40E-05 |
| **Kidneys** | 5.01E-02 | 2.32E-02 | 6.58E-04 | 7.58E-04 |
| **Liver** | 1.82E-02 | 1.17E-02 | 5.90E-04 | 3.67E-04 |
| **Lungs** | 2.45E-02 | 3.14E-02 | 1.35E-03 | 5.56E-04 |
| **Muscle** | 9.51E-03 | 1.87E-03 | 6.83E-05 | 2.40E-05 |
| **Ovaries** | 1.18E-02 | 3.77E-03 | 1.42E-03 | 1.25E-03 |
| **Pancreas** | 9.68E-03 | 1.73E-03 | 7.63E-05 | 3.37E-05 |
| **Red Marrow** | 7.84E-03 | 1.08E-03 | 9.59E-04 | 1.41E-04 |
| **Osteogenic Cells** | 1.38E-02 | 3.37E-03 | 1.41E-04 | 3.38E-05 |
| **Skin** | 8.53E-03 | 2.16E-03 | 8.72E-05 | 2.22E-05 |
| **Spleen** | 1.17E-02 | 4.02E-03 | 8.63E-05 | 4.80E-05 |

| | | | | |
|---|---|---|---|---|
| **Testes** | 1.00E-02 | 3.05E-03 | 0.00E+00 | 0.00E+00 |
| **Thymus** | 8.44E-03 | 1.85E-03 | 6.58E-05 | 3.12E-05 |
| **Thyroid** | 9.72E-03 | 2.49E-03 | 4.27E-04 | 1.56E-04 |
| **Urinary Bladder Wall** | 5.15E-02 | 2.52E-02 | 2.08E-03 | 9.50E-04 |
| **Uterus** | 1.24E-02 | 3.45E-03 | 8.78E-05 | 2.74E-05 |
| **Total Body** | 8.95E-03 | 2.47E-03 | 0.00E+00 | 0.00E+00 |
| **ED** | - | - | **1.25E-02** | **8.16E-04** |

## 5. PET pharmacokinetic modeling

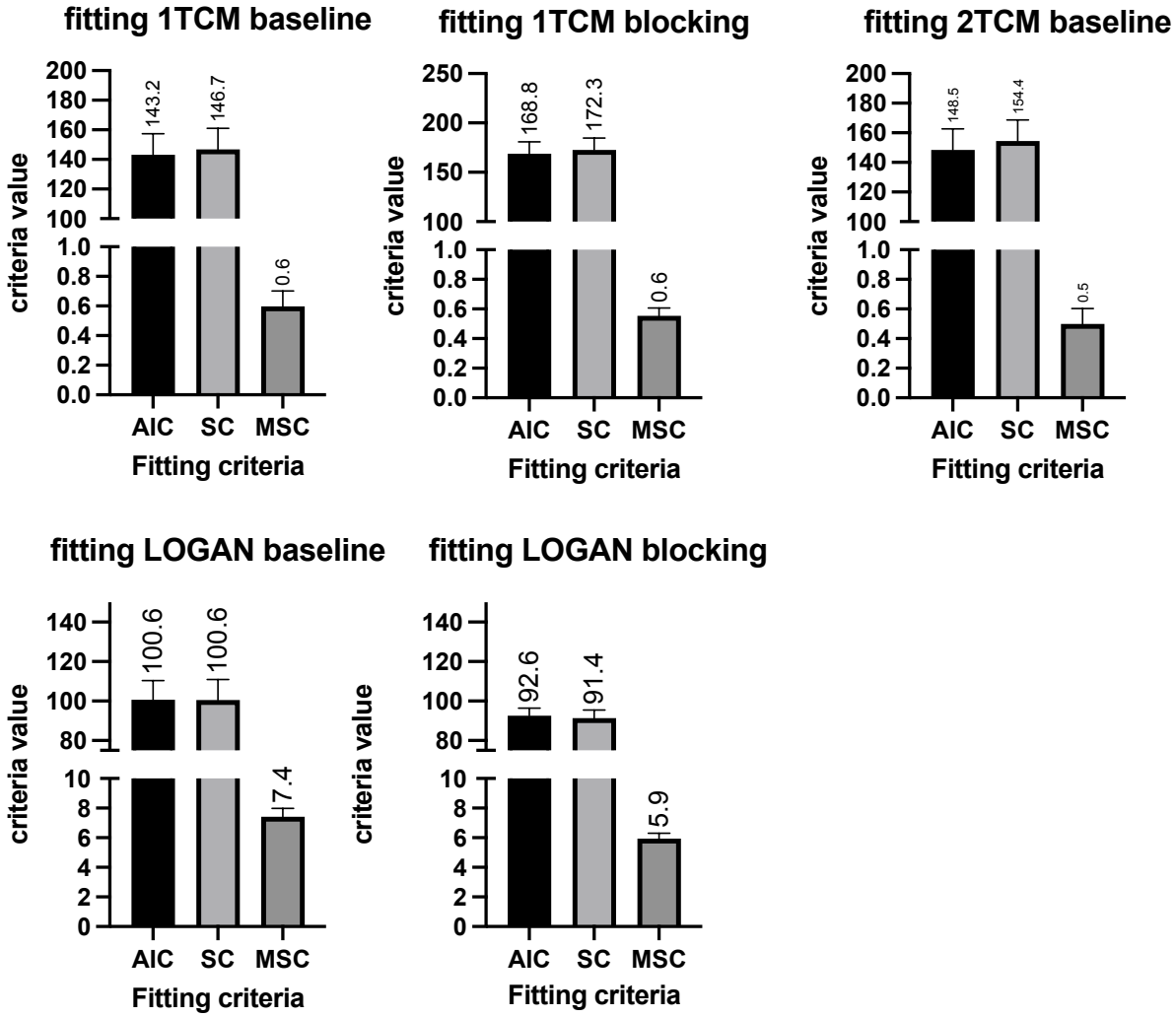



*Figure 6: Evaluation of model fitting criteria using Akaike Information Criterion (AIC), Schwartz Criterion (SC) and Model Selection Criterion (MSC).*

## 6. Ex vivo stability

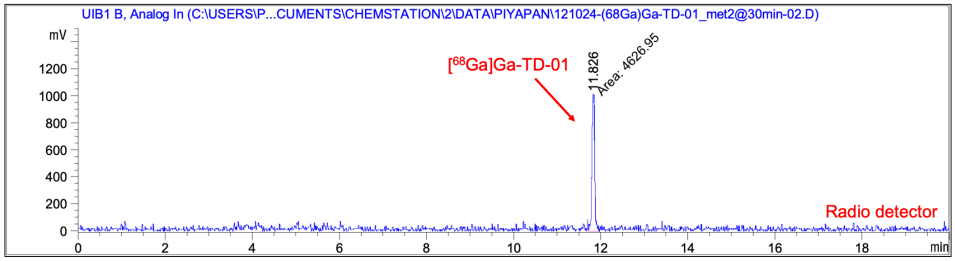
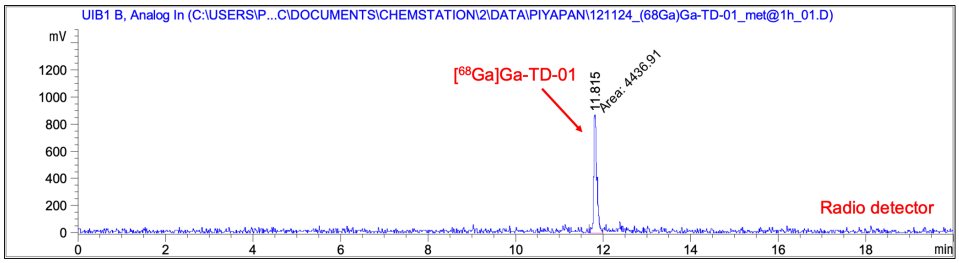
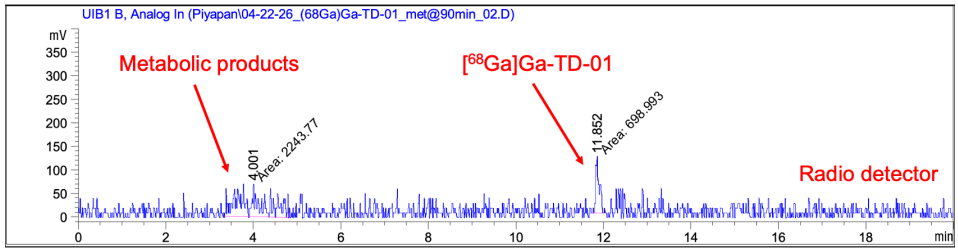
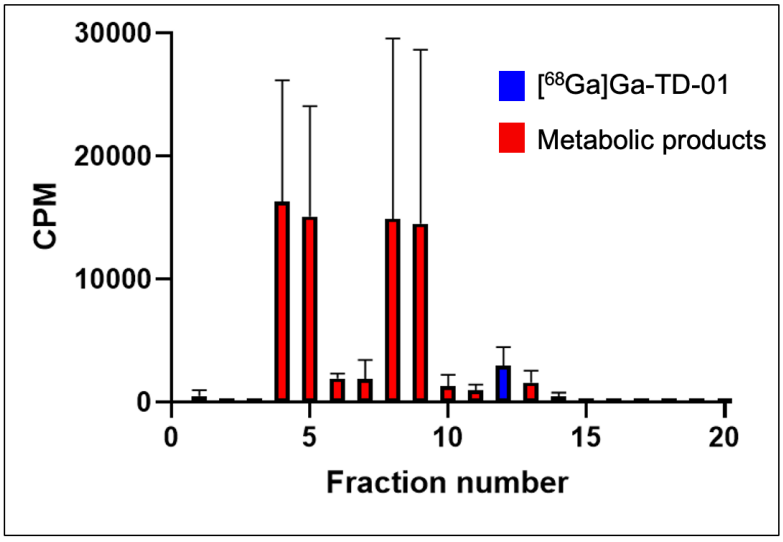
| Time (min) | HPLC chromatogram / gamma counter signal | Intact tracer (%) |
|---|---|---|
| 30 |  | > 99 |
| 60 |  | > 99 |
| 90 |  | 45 ± 13 |
| 120 |  | 14 ± 11 |

Figure 7: Radio-HPLC chromatogram and gamma counter fractions of [$^{68}$Ga]Ga-TD-01 in the extracted supernatant at 0.5, 1, 1.5 and 2-hours post-injection.

## 7. RNA scope group comparison

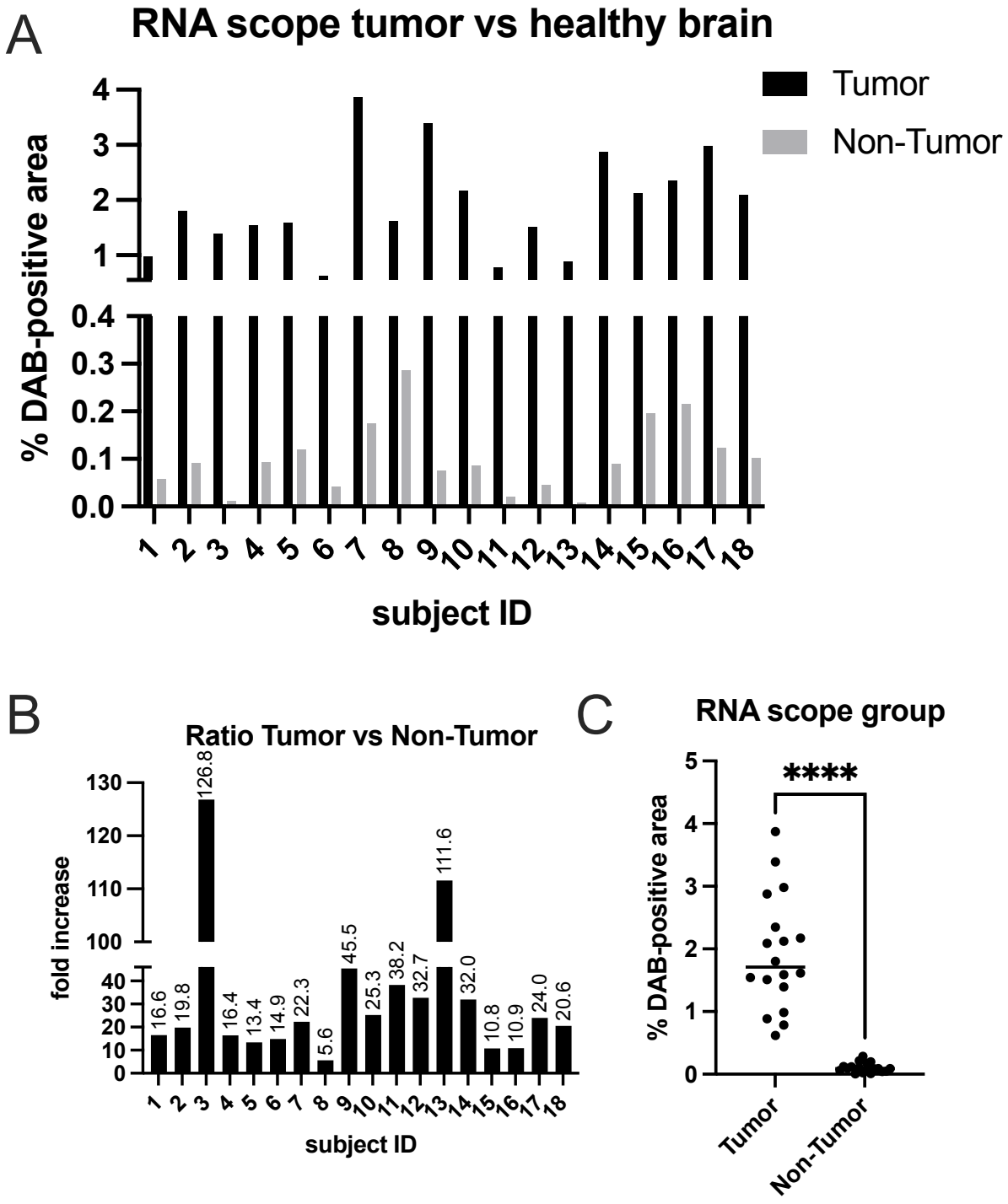



Figure 8: Quantitative analysis of CXCR4 RNAscope signal in orthotopic GL261 glioblastoma tissue and contralateral healthy brain. (A) Quantification of RNAscope-derived DAB-positive area in tumor and non-tumor brain regions for individual animals (n = 18). Tumor tissue demonstrated consistently higher CXCR4-associated signal compared to contralateral healthy brain tissue. (B) Tumor-to-brain fold increase in RNAscope signal intensity for each subject, illustrating elevated CXCR4 expression within the tumor microenvironment relative to normal brain tissue. (C) Grouped

analysis of RNAscope DAB-positive area (two-sided, unpaired t test) demonstrating significant increased CXCR4 expression in tumor tissue compared to non-tumor brain regions. Data are presented as mean ± SD.