Supplementary Information

Assessment of TSPO in a Rat Experimental Autoimmune Myocarditis Model: A Comparison Study between $[^{18}F]$Fluoromethyl-PBR28 and $[^{18}F]$CB251

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I. Representative HPLC chromatogram for $[^{18}F]$Fluoromethyl-PBR28

II. Representative HPLC chromatogram for $[^{18}F]$CB251

III. *In vitro* binding assay

IV. PET images based time-activity curves analysis

V. Reference
I. Representative HPLC chromatogram for [$^{18}$F]Fluoromethyl-PBR28

![Representative HPLC chromatogram of [$^{18}$F]Fluoromethyl-PBR28 ([$^{18}$F]1). Column: Xterra RP-18, 10 $\mu$m, 10 $\times$ 250 mm with a guard cartridge (Phenomenex, 10 $\times$ 10 mm); Eluent: 45% CH$_3$CN/H$_2$O; Flow rate: 3 mL/min; Blue line: UV-254 nm; Red line: gamma-ray.](image1)

![Analytical HPLC chromatogram of pure [$^{18}$F]Fluoromethyl-PBR28 ([$^{18}$F]1). Column: Xterra RP-18, 5 $\mu$m, 4.6 $\times$ 250 mm; Eluent: 65% CH$_3$CN/H$_2$O; Flow rate: 1 mL/min; Upper: gamma-ray; Bottom: UV-254 nm.](image2)
II. Representative HPLC chromatogram for \([^{18}\text{F}]\text{CB251}\)

**Fig. S3** Preparative-HPLC chromatogram of \([^{18}\text{F}]\text{CB251}\) (\([^{18}\text{F}]\text{F}\)_2). Column: Xterra RP-18, 10 μm, 10 × 250 mm with a guard cartridge (Phenomenex, 10 × 10 mm); Eluent: 55% CH₃CN/H₂O; Flow rate: 4 mL/min; Bottom: UV-254 nm; Upper: gamma-ray.

**Fig. S4** Analytical HPLC chromatogram of pure \([^{18}\text{F}]\text{CB251}\) (\([^{18}\text{F}]\text{F}\)_2). Column: Xterra RP-18, 5 μm, 4.6 × 250 mm; Eluent: 60% CH₃CN/H₂O; Flow rate: 1 mL/min; Upper: gamma-ray; Bottom: UV-254 nm.
III. *In vitro* TSPO binding assay

**Method**

Binding affinity to the TSPO was assessed using *in vitro* receptor binding assay. This analysis was conducted as previously described by Denora et al [1].

**Result**

The binding affinities of fluoromethyl-PBR28 and CB251 for TSPO and CBR, expressed as inhibition constants (Kᵢ), were compared with those of unlabeled PK11195. Fluoromethyl-PBR28 was characterized by a binding affinity for TSPO (Kᵢ = 1.85 nM) while CB251 showed a subnanomolar binding affinity for TSPO (Kᵢ = 0.27 nM).
IV. PET images based time-activity curves analysis

Fig. S5 MicroPET time-activity curves for [\(^{18}\text{F}\)]fluoromethyl-PBR28 ([\(^{18}\text{F}\)]1) and [\(^{18}\text{F}\)]CB251 ([\(^{18}\text{F}\)]2) in control (n = 5) and EAM model (n = 5) during 60 min after injection. Time-activity curves with [\(^{18}\text{F}\)]1 (A), [\(^{18}\text{F}\)]2 (B), and PK11195 (10 mg/kg, n = 3) treatment before injection of [\(^{18}\text{F}\)]2 (C). The time–activity curves for the heart and lung are plotted 1 min after injection of [\(^{18}\text{F}\)]1 or [\(^{18}\text{F}\)]2. The uptake values in ROI were normalized for the peak uptake value of lung.
V. Reference