Supporting Information

Positron Emission Tomography Probes Targeting Bromodomain and Extra-Terminal (BET) Domains to Enable *In Vivo* Neuroepigenetic Imaging

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Materials and Methods

All reagents and solvents were of ACS-grade purity or higher and used without further purification. Analytical separation was conducted on an Agilent 1100 series HPLC fitted with a diode-array detector, quaternary pump, vacuum degasser, and autosampler. ^{1}H NMR spectra were recorded at room temperature in $CDCl_3$ solution using a Varian INOVA 500 NMR spectrometer. Chemical shifts are reported in parts per millions (ppm) relative to tetramethylsilane (TMS). Mass spectrometry data were recorded on an Agilent 6310 ion trap mass spectrometer (ESI source) connected to an Agilent 1200 series HPLC with quaternary pump, vacuum degasser, diode-array detector, and autosampler. [^{11}C]CO_2 (1.2 Ci) was obtained via the ^{14}N (p, α) ^{11}C reaction on nitrogen with 2.5% oxygen, with 11 MeV protons (Siemens Eclipse cyclotron), and trapped on molecular sieves in a TRACERlab FX-MeI synthesizer (General Electric). [^{11}C]CH_4 was obtained by the reduction of [^{11}C]CO_2 in the presence of Ni/hydrogen at 350 °C and recirculated through an oven containing I_2 to produce $^{11}CH_3I$ via a radical reaction.

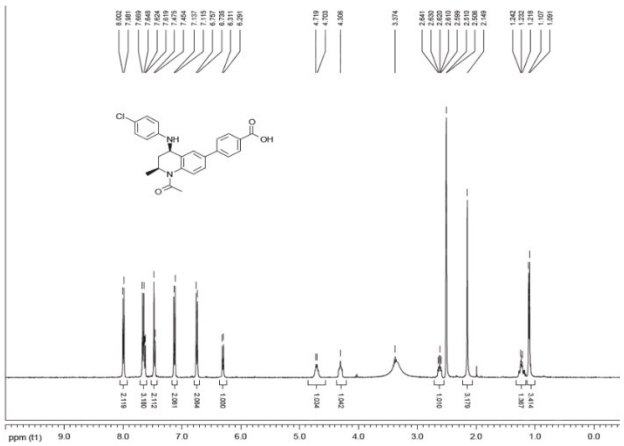
All animal studies were carried out at Massachusetts General Hospital (PHS Assurance of Compliance No. A3596-01). The Subcommittee on Research Animal Care (SRAC) serves as the Institutional Animal Care and Use Committee (IACUC) for the Massachusetts General Hospital (MGH). SRAC reviewed and approved all procedures detailed in this paper.

PET-CT imaging was performed in anesthetized (isoflurane) male Balb/c mice to minimize discomfort. Highly trained animal technicians monitored animal safety throughout all procedures, and veterinary staff were responsible for daily care. All rats were socially housed in cages appropriate for the physical and behavioral health

of the individual animal and were given unlimited access to food and water, with additional nutritional supplements provided as prescribed by the attending veterinary staff.

PET-MR imaging was performed in anesthetized (ketamine, isoflurane) macaque to minimize discomfort. Highly trained animal technicians monitored animal safety throughout all procedures, and veterinary staff were responsible for daily care. All animals were socially housed in cages appropriate for the physical and behavioral health of the individual animal and were fed thrice perdiem, with additional nutritional supplements provided as prescribed by the attending veterinarian. Audio, video, and tactile enrichment was provided on a daily basis to promote psychological well-being. No nonhuman primates were euthanized to accomplish the research presented.

Synthesis of compound CW22. To a mixture of I-BET726 (150 mg, 0.35 mmol) and anhydrous K_2CO_3 (72.6 mg, 0.53 mmol) in DMSO were added the iodomethane (0.02 mL). The reaction mixture was stirred for 2 h at room temperature. After completed reaction, the reaction solvent was evaporated under reduced pressure and extracted with dichloromethane and water. The aqueous phase and the organic phase were separated and the organic phase was washed with saturated aqueous sodium chloride (30 mL) and dried over sodium sulfate. After filtered, the solvent was evaporated to dryness under reduced pressure. The crude product was purified on silica gel chromatography (CH_2Cl_2/CH_3OH = 30: 1) to afford light brown solid compound (60 mg, 38.7%). LC-MS calculated for $C_{26}H_{25}ClN_2O_3$ expected [M]: 447.16.; Found $[M-H]^+$: 448.94. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 8.10 − 8.06 (m, 2H), 7.59 − 7.55 (m, 3H), 7.19 − 7.15 (m, 2H), 6.64 − 6.59 (m, 2H) , 3.94 (s, 3H), 2.74 – 2.63 (m, 2H), 2.41 (m, 1H), 2.26 (s, 3H), 1.36 (m, 2H), 1.27 (m, 2H), 1.23 – 1.20 (s, J = 6.2 Hz, 3H).



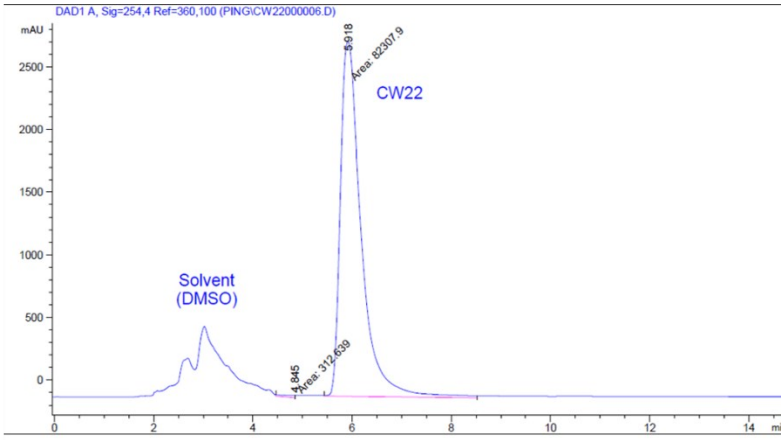


Fig. S1. The ^{1}H NMR data of precursor I-BET726 (left) and the HPLC profile of CW22 (HPLC conditions: 35% H_2O + 0.1% TFA/65% CH_3CN + 0.1% TFA, at the flow rate of 5.0 mL/min.)

Radiosynthesis of [^{11}C]CW22. [^{11}C]CO_2 was produced by the cyclotron, the [^{11}C]CH_3I was obtained from [^{11}C]CH_4 which is obtained by the reduction of [^{11}C]CO_2 and with a radical reaction with iodine. [^{11}C]CH_3I was trapped in a TRACERlab FX-M synthesizer reactor (General Electric) preloaded with a solution of I-BET726 (0.5 mg) in 300 µL dry DMSO. The mixture was heated at 100°C for 3 min followed by water (1.2 mL) adding. The reaction mixture was cooled to room temperature and injected onto reverse phase semi-preparative HPLC (Phenomenex Luna 5u C18(2), eluting with a mobile phase of 30% H_2O + 0.1% TFA/60% CH_3CN, at the flow rate of 5.0 mL/min. The radioactivity product was collected and reformulated by loading onto a solid-phase exchange (SPE) C-18 SepPak cartridge. Then the SepPak was rinsed with 5 mL water and followed with 1 mL ethanol. The ethanol was collected and diluted by saline solution (0.9%, 9 mL). The radiosynthesis process took an average of 30-40 minutes to complete. The average radiochemical yield was 20−28% (nondecay corrected to trapped [^{11}C]CH_3I) with the purity over 95%. Specific activity of [^{11}C]CW22 (at time of injection): 1.1 − 1.8 mCi/nmol.

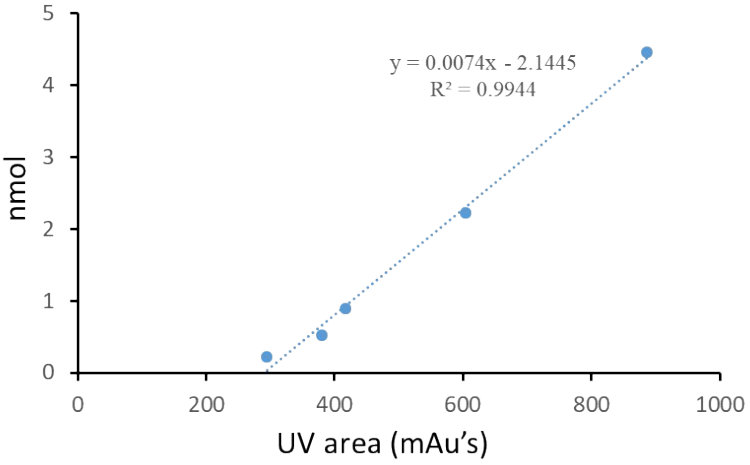


Fig. S2. Mass/UV calibration curve from five concentrations (0.5, 1.0, 2.0, 5.0, 10.0 µM) of standard, 100 µL CW22 of each concentration were injected in triplicate into analytical HPLC column (UV: 254 Nm, Agilent; Gemini C18, 150 × 4.6 mm)

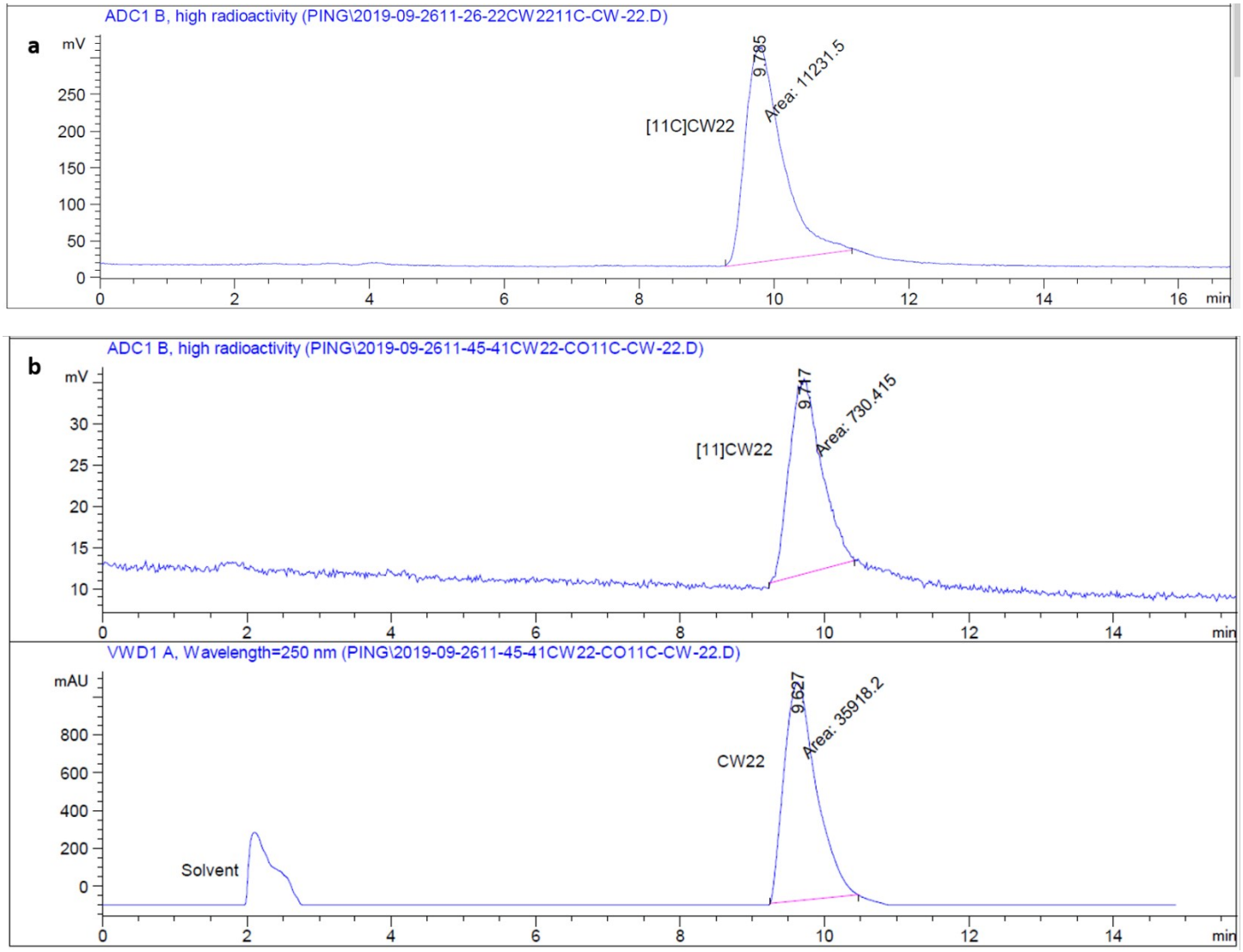


Fig. S3. HPLC profile of formulated [^{11}C]CW22 (a) and co-injection of [^{11}C]CW22 and CW22 (b). HPLC condition: Agilent Eclipse XDB–C18, 5 μm, 9.4×250 mm, flow rate = 5.0 mL/min, mobile phase = 0.1% TFA in water / 0.1% TFA in acetonitrile, 40/60, v/v.

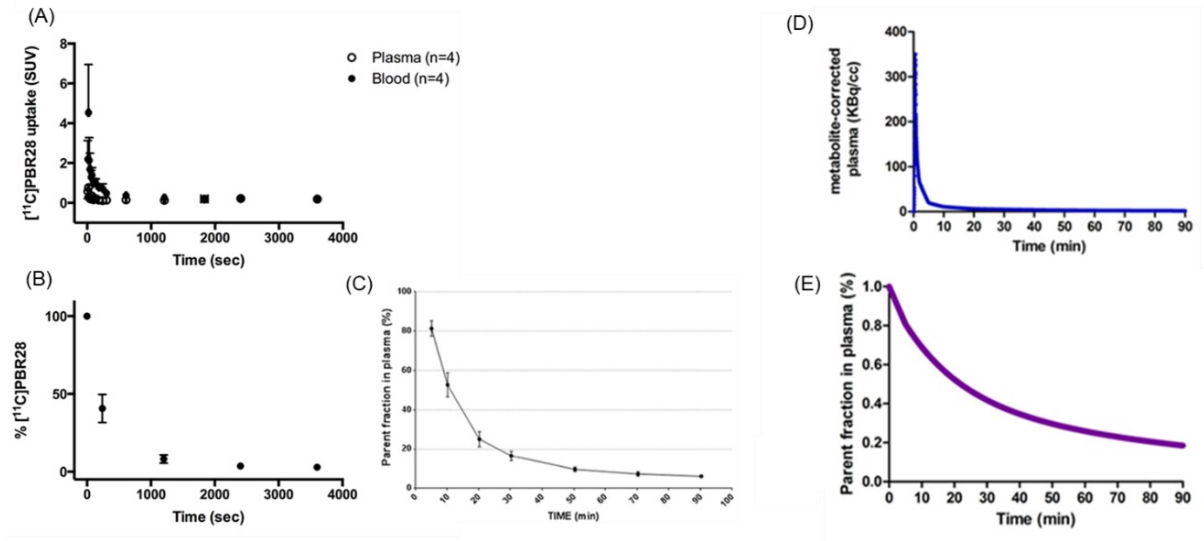


Fig. S4. Plasma/blood activity comparison. (A), (B) are Figures from a [^{11}C]PBR28 rodent study paper (PLOS ONE, DOI:10.1371/journal.pone.0125917), (C) is from a [^{11}C]PBR28 human study paper (European Journal of Nuclear Medicine and Molecular Imaging, 2018, 45, 1432–1441); (D) and (E) are from our [^{11}C]CW22 study.

Log D Determination. The formulated [^{11}C]CW22 was added to a mixture of octanol (2.5 mL) and phosphate buffer solution (2.5 mL, pH 7.4).The mixture was then centrifuged for 2 min to fully separate the aqueous and organic phase. Took radioactivity measurement for the octanol layer (0.1 mL) and the aqueous layer (1.0 mL). Took another octanol layer (2.0 mL) to a new test tube containing 0.5 mL of octanol and 2.5 mL of phosphate buffer solution (pH 7.4). Six samples were prepared in parallel. The radioactivity of each sample was measured in a well counter (PerkinElmer, Waltham, MA). Then the log *D* was calculated by the formula: log *D* = log (10/decay-corrected radioactivity in phosphate buffer sample × decay-corrected radioactivity in octanol sample).

BRD4 Binding Assays. 20 μm thick rat brain cryo-sections were used. Sections were pre-incubated in 2% bovine serum albumin (BSA) in phosphate buffer saline (PBS) solution (pH = 7.4) for 10 minutes. Then the sections were sinked in a solution that contained [^{11}C]CW22 (100 μCi) and either (i) 5% DMSO as control; (ii) JQ1 (1mM) or AZD5153 (1mM) in 2% BSA in PBS and incubated for 10 min in room temperature. The sections were washed with two times with 2% BSA in PBS for 2 min, then two times with PBS for 2 min and dried. Radioactivity distribution was detected using digital autoradiography, where imaging plate was exposure with tissue sections for 1 h and then analyzed using a Cyclone Plus phosphorimager (PerkinElmer). Individual images of sections were cropped using ImageJ with no additional adjustment to color levels/thresholds.

Plasma Protein Binding Assay. The formulated [^{11}C]CW22 in saline (10 μL) was added to macaque or human plasma (1.2 mL). The mixture was incubated for 10 min at room temperature. Took a sample of the incubated mixture (20 μL) to measure the radioactivity in the plasma sample (A_T; $A_T = A_{bound} + A_{unbound}$). Then the plasma sample (200 μL) was placed in the upper compartment of a Centrifree tube (Amicon, Inc., Beverly, MA) and centrifuged for 10 min. Discarded the upper part of the Centrifree tube, determine the amount of plasma radioactivity in bottom part that passed through the membrane ($A_{unbound}$). The plasma protein binding can be measured by the following equation: $\%_{unbound} = A_{unbound} \times 100/A_T$.

In Vitro Autoradiography. The rat brain sections were cut to 20 μm thick slides, fixed in 4% paraformaldehyde with 2% ethanol for 60 min at 4 °C. After that, the sections were washed with cold Tris-HCl (10 mM, pH = 7.4) for 10 minutes. Then the sections were incubated in a 50 mL bath containing either at room temperature (i) 5% DMSO as control; (ii) JQ1 (1mM) or AZD5153 (1mM) for 10 min. [^{11}C]CW22 (100 μCi) was added to each bath and incubated for 10 minutes at room temperature. The sections were washed with Tris-HCl (10 mM, pH 7.4) and dried. After 1 h exposures, sections were developed using a Cyclone Plus phosphorimager (PerkinElmer). Individual images of sections were cropped using ImageJ with no additional adjustment to color levels/thresholds.

Rodent PET-CT Acquisition and Post Processing. Mice were utilized in this study. Animals were anesthetized with inhalational isoflurane (Forane) at 3% in a carrier of 1.5-2 L/min medical oxygen and maintained at 2% isoflurane during the imaging scan. The mice were then arranged in a Triumph Trimodality PET/CT/SPECT scanner (Gamma Medica, Northridge, CA). [^{11}C]CW22 (100-200 μCi per animal) were administrated after JQ1

(1 mg/kg) or CW22 (10 mg/kg) were injected via a lateral tail vein catheterization 5-min prior to the start of PET acquisition. Each dynamic PET scan performed for 60 min and followed by computed tomography (CT).

Rodent PET-CT Image Analysis. After scanning, dynamic PET data were collected and the corresponding images were reconstructed by 3D-MLEM method. The spheres in brain regions were defined as the volumes of interest (VOIs) according to the high-resolution CT structural images and summed PET data, with a radius of no less than 1 mm to minimize partial volume effects. Reconstructed images are exported from the scanner in DICOM format along with an anatomic CT for rodent studies. These files are imported to PMOD (PMOD Technologies, Ltd.).

Macaque PET-MR Acquisition. Macaques was used in this PET-MR scan. The macaque was immobilized with intramuscular xylazine (0.5-2.0 mg/kg) and ketamine (10 mg/kg). V-line and A-line were inserted into the limb for radiotracer and blocking drug injection. A femoral arterial line was placed for blood sampling. Dynamic PET-MR scan was performed and the images of the brain regions were acquired in a Biograph mMR scanner (Siemens, Munich, Germany). Dynamic PET image acquisition was initiated followed by administration of 2 mCi [^{11}C]CW22. A T1-werghting MEMPRAGE sequence begin at the same time of the PET scan for anatomic co-registration. Imaging data were collected for 120 min and corrected for decay, scatter and attenuation.

Macaques PET-MR Image Analysis. The PET data was motion-corrected and registered to the INIA19 Template for brain imaging analysis in macaques. Kinetic modeling was carried out in PMOD (PMOD3.3, PMOD Technologies Ltd.). VOIs were selected according to the brain atlas and TACs were exported from the whole brain, cerebellum, primary motor cortex, putamen, thalamus, primary visual cortex, caudate, and white matter VOIs for analysis. The regional volume of distribution (V_T) was measured by a Logan plot model with a metabolite-corrected plasma TAC. Voxel-wise V_T maps will be calculated using Logan plot from the dynamic PET data. The time until linearity of the plot achieved will be determined for each scan.

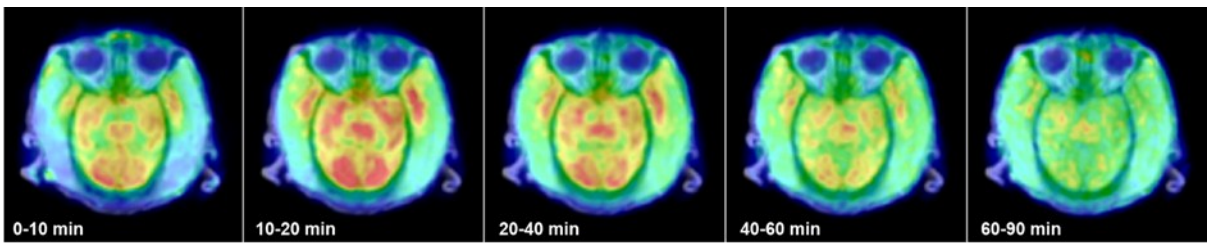


Fig. S4. The radioactivity in the brain and surrounding tissue of macaque during scan time points.

***Plasma and Metabolite Analysis*.** Arterial blood samples drawn from the macaques were collected during the image scan for plasma and metabolite analyses. The blood was centrifuged to obtain plasma and placed in an automated gamma counter. The analysis of radiolabeled metabolites was conducted on a custom automated robot, fitted with Phenomenex SPE Strata-X 500 mg solid phase extraction cartridges that were primed with ethanol (2 mL) and deionized water (20 mL). To achieve protein precipitation, addition of an aliquot of plasma (300 μL) was added to acetonitrile (300 μL) and centrifuged for 1 min to obtain protein-free plasma (PFP). Next, 300 μL of PFP/acetonitrile solution was diluted into deionized water (3 mL), loaded onto the C18 cartridge, and removed of polar metabolites with 100% water. Next, a series of extractions were performed using water and acetonitrile. Each sample was counted in a WIZARD2 Automatic Gamma Counter to determine the presence of radiolabeled metabolites.