Supplementary Table 1 Review of literature on saturation TSPO binding levels in autopsied brain of healthy human subjects

Reference	Ligand	Sample (n)	B_{max}* (fmol/mg protein)	K_d (nM)	Method	Age (range) (years)	Comments
Doble et al. 1987[38]	[^{3}H]PK11195	Ctx (3)	357	4.3	Homogenate, 4ºC, 120 min; Autoradiography 25ºC, 90 min	40-46	~30% non-specific binding in GM; no specific binding in WM; regional autoradiography (single 2 nM) showed heterogeneous binding of 100-2000 fmol/mg protein across regions.
		Fctx (1)	481	1.4			
Broaddus et al. 1990[39]	[^{3}H]PK11195	Fctx (3)	4,000	28	Homogenate, RT, 60 min	Unknown	Human renal cortex had a total Bmax (two sites) of ~58,000 fmol/mg protein.
Lavoie et al. 1990[40]	[^{3}H]PK11195	Fctx (9)	669	2.4	Homogenate, 4ºC, 120 min	58 (49-67)	<20% in non-specific binding.
		CN (9)	469	2.1			
Diorio et al. 1991[41]	[^{3}H]PK11195	Tctx (6)	413	2.1	Homogenate, 4ºC, 120 min	70 (60-75)	30-40% in non-specific binding.
		Fctx (6)	568	2.8			
Awad & Gavish 1991[42]	[^{3}H]PK11195	Ctx (5)	128[a]	2.1	Crude membrane, 4ºC, 60 min	45±8 (SD)	90% non-specific binding for [^{3}H]Ro5-4864
	[^{3}H]Ro5-4864		ND (Ro5-4864)	ND			
Rao et al. 1997[37]	[^{3}H]PK11195	Octx (6)	462[a]	2.7	Crude membrane, 4ºC, 120 min	59 (52-65)	Extensively washed crude membrane fraction was employed; <10% (PK11195) and <30% (Ro5-4864) in non-specific binding; highest binding was observed in occipital cortex as compared to thalamus, hippocampus and substantia nigra
		Tctx (6)	356[a]	2.9			
		Cereb (6)	173[a]	3.0			
	[^{3}H]Ro5-4864	Octx (6)	172[a]	13.2			
		Tctx (6)	145[a]	16.7			
		Cereb (6)	68[a]	15.5			
Kurumaji et al. 1997[43]	[^{3}H]PK11195	Octx (7)	431[a]	14.9	Crude membrane, 4ºC, 90 min	67 (52-74)	No obvious regional distribution was observed among cerebral cortices, thalamic subregions, caudate, putamen, globus pallidus, substantia nigra and red nucleus.
		Pctx (9)	384[a]	10.2			
		Put (4)	224[a]	5.9			
Messmer & Reynolds 1998[44]	[^{3}H]PK11195	Put (10)	932[b]	5.7	Crude membrane, 4ºC, 120 min	58±15 (SD)	
		Fctx (10)	790[b]	2.1			
		Tctx (10)	938[b]	3.8			

Reference	Ligand	Sample (n)	B_{max}* (fmol/mg protein)	K_d (nM)	Method	Age (range) (years)	Comments
Sauvageau et al. 2002[45]	[^{3}H]PK11195	Hippo (5)	141[a]	0.94	Crude membrane, 4ºC, 60 min	Unknown	Increased TSPO immunoreactivity in epileptic hippocampal biopsy was confined to astrocytes.
Venneti et al. 2008a,b,c, 2009[47-50]	[^{3}H](*R*)-PK11195	Fctx (3-6)	290-354[a]	12-24	Crude membrane, 4ºC, 120 min	Variable, on average 61-66	<20% in non-specific binding; higher K_d reported for [^{3}H](*R*)-PK11195 as compared to earlier reports using the racemic [^{3}H]PK11195 under a similar condition of incubation (4ºC).
		Cereb (6)	445[a]	19			
		BG (3)	325[a]	24			
	[^{3}H]DAA1106	Fctx (3-6)	273[a]	0.7			
		BG (3)	268[a]	3.4			
Owen et al. 2010[46]	[^{3}H]PK11195	Brain region not clear (15)	2163[a] (HAB/MAB)	29	Crude membrane, 37ºC, 60 min	55 (38-88)	HAB (n=6), MAB (n=4), LAB (n=5); K_d was lower in autoradiography at room temperature as compared to saturation binding using washed membrane fraction at 37 ºC for [^{3}H]PK11195 (1-2 vs 30 nM) but not for [^{3}H]PBR28.
			2532[a] (LAB)	31			
	[^{3}H]PBR28		965[a] (HAB)	2.2			
			931[a] (MAB)	1.3/135			
			497[a] (LAB)	52			

[a]B_{max} in fmol/mg tissue protein was converted from values reported in fmol/mg membrane protein by assuming a membrane to homogenate protein ratio of 0.5;

[b]B_{max} in fmol/mg tissue protein was converted from values reported in fmol/mg wet tissue by assuming a protein/tissue ratio of 0.05;

*Note the range of B_{max} varying between <100 to 4,000 fmol/mg tissue protein and apparently higher levels of B_{max} (and also K_d values) when binding assays of [^{3}H]PK11195 were performed at room temperature (RT)[39] or 37ºC[46] as compared to those at 4ºC.

Ctx = cerebral cortex; Fctx = frontal cortex; Tctx = temporal cortex; Pctx = parietal cortex; Octx = occipital cortex; Cereb = cerebellum; Hippo = hippocampus; CN = caudate; Put = putamen; BG = basal ganglia. HAB = high affinity binder; MAB = mix affinity binder; LAB = low affinity binder; ND = not detectable.

Supplementary Results.

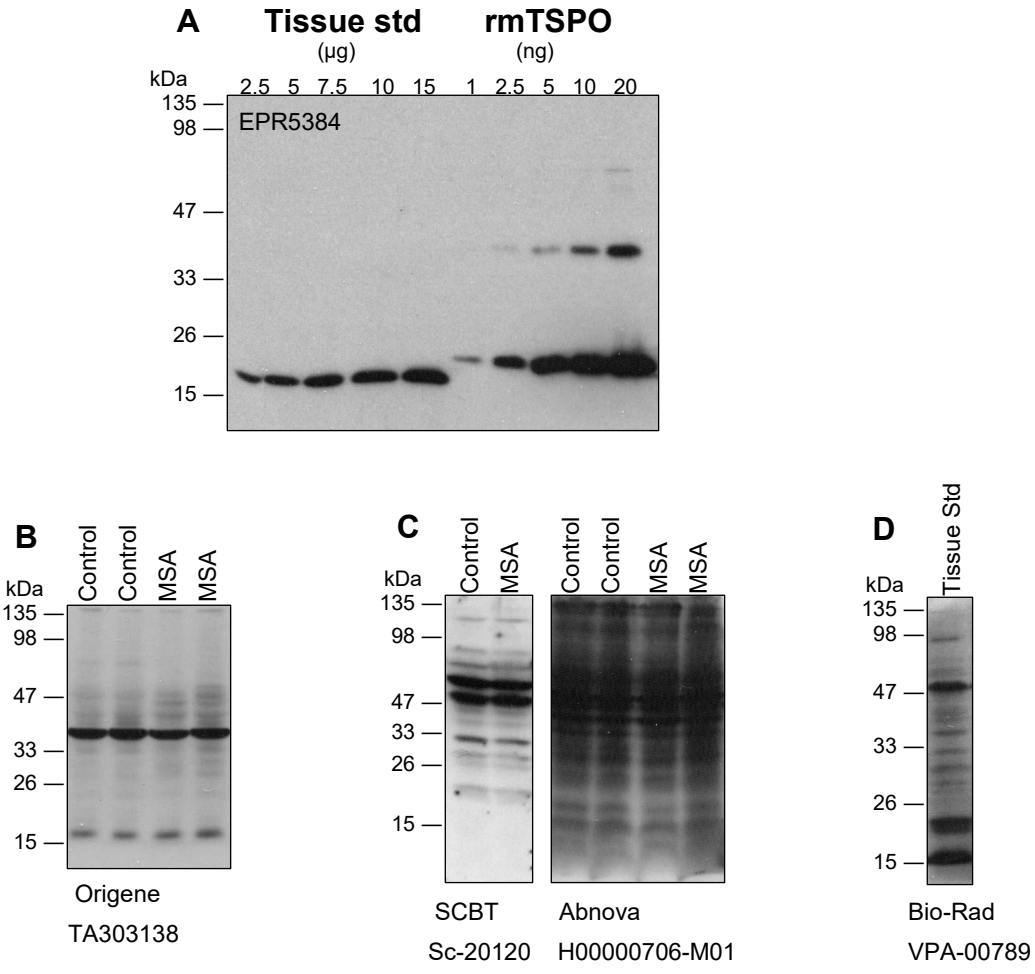
Characterization of TSPO antibodies for Western blotting in human brain

The rabbit monoclonal TSPO antibody EPR5384 detected in normal human brain homogenate one single protein band at the expected 18 kDa position (Fig. 1A). The same protein band was also detected in human adrenal samples with higher levels, in agreement with more abundant presence of TSPO reported in peripheral organs than in brain. This protein band was found in the membrane but not in the soluble fraction of human brain (data not shown), consistent with known subcellular localization of TSPO in the mitochondria.[38] The specificity of EPR5384 was also confirmed by using three recombinant human TSPO proteins from different sources. The N-tagged recombinant proteins including N-His tagged TSPO-3462H (MW = 21 kDa) and N-His/GST tagged TSPO-1696H (MW = 48.8 kDa) from Creative BioMart were all detected with high sensitivity (as low as 0.1 ng for the partially purified TSPO-3462H under our WB conditions, see Fig. 1A), whereas the C-Myc/DDK tagged human TSPO from Origene (MW = 23 kDa) was detected with very low sensitivity (>40 ng needed), which is consistent with the epitope of EPR5384 being at the C-terminal of TSPO (Table 1) and the C-Myc/DDK tag influencing antibody binding. Indeed, the sequence differences at the C-terminal among human, mouse and rat TSPO also influenced the immunoreactivity of EPR5384. The N-His tagged recombinant mouse TSPO (from Dr. J. J. Lacapere)[74] was detected by EPR5384 but with lower sensitivity (>1 ng, Supplement Fig. 1A) whereas the C-Myc/DDK tagged mouse and rat TSPO could not be detected. Several other TSPO antibodies were also tested. The rabbit monoclonal (MA5-24844, 4H2) and polyclonal (PA5-75544) antibodies from ThermoFisher detected the same major 18 kDa protein band in the human brain and the 21 kDa N-His tagged recombinant TSPO-3462H (Fig. 1A) though with lower sensitivity, whereas several other commercial antibodies were found not suitable for human brain immunoblotting for TSPO, mainly because of much non-specific reactivity (Table 1 and Supplement Fig. 1B-D).

We found that the recombinant TSPO-3462H, as well as the recombinant mouse TSPO, had a small amount of aggregates, likely dimers, trimers and tetramers of

TSPO,[75] which was also detected by the antibodies (Fig. 1A and Supplement Fig 1A). However, no TSPO polymer was detectable in the human brain SDS-PAGE sample preparation. The few high MW protein bands detected by PA5-75544 in human brain and adrenal were probably non-specific. Indeed, PA5-75544 was unable to detect native TSPO in human adrenals but rather detected a smaller MW band that is possibly a truncated fragment of TSPO in the adrenal gland (Fig. 1A).

Supplementary Figure 1. (A) Western blot with EPR5384 on the tissue standard and N-His-tagged recombinant mouse TSPO (rmTSPO, a kind gift from Dr. Jean J. Lacapere). Note a small amount of polymers of the recombinant protein. (B-D) Western blot in human brain homogenates, both control subjects and patients with multiple system atrophy (MSA), with TSPO antibodies from different sources. Note much non-specific reactivity with these antibodies as compared to EPR5384 and no obvious difference in immunoreactivity between controls and MSA putamen by these antibodies as compared to that by EPR5384 (see Fig. 5).



Supplementary Figure 2. Relationship between protein levels of TSPO and GFAP in the frontal cortex and the time of death in 24 adult subjects with the information.