

The microglial translocator protein (TSPO) in Alzheimer's disease reflects a phagocytic phenotype.

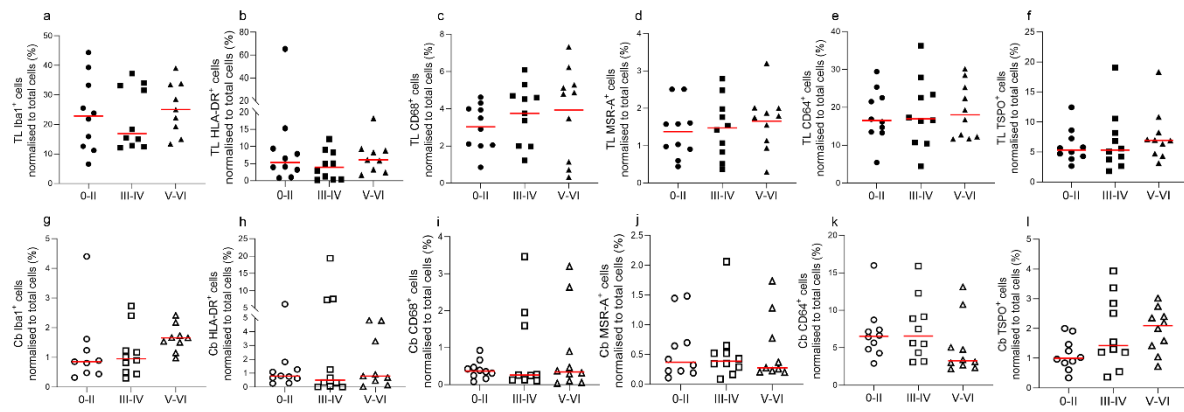
Emma F. Garland¹, Henrike Antony¹, Laura Kulagowska¹, Thomas Scott¹, Charlotte Rogien¹, Michel Bottlaender^{2,3}, James A.R. Nicoll^{1,4}, Delphine Boche¹.

¹Clinical Neurosciences, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

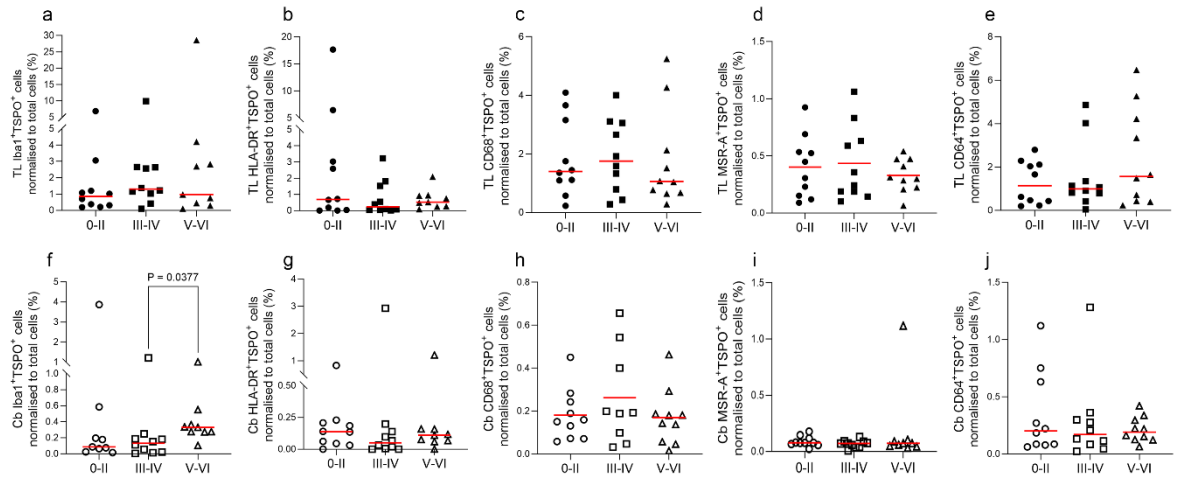
²Paris-Saclay University, CEA, CNRS, Inserm, BioMaps, Service Hospitalier Frederic Joliot, Orsay, France

³UNIACT Neurospin, CEA, Gif-sur-Yvette, France

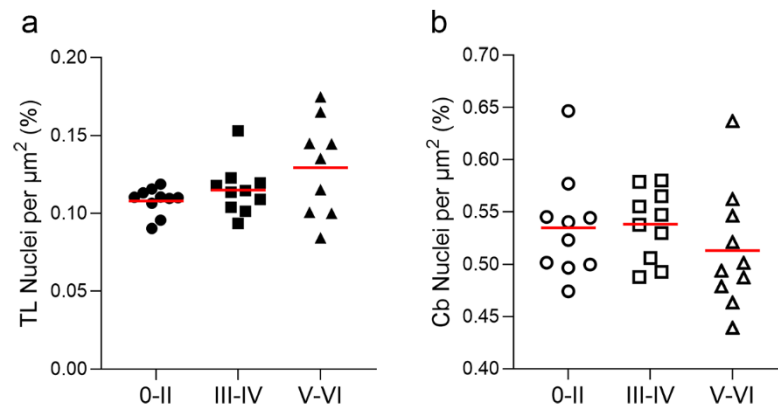
⁴Department of Cellular Pathology, University Hospital Southampton NHS Trust, Southampton, UK



Supplementary Figure 1. Quantification of positive cell counts for Iba1 (a, g), HLA-DR (b, h), CD68 (c, i), MSR-A (d, j), CD64 (e, k) and TSPO (f, l) normalised to total cells (%) in the temporal lobe (a-f) and cerebellum (g-l), separated by Braak group (0-VI).



Supplementary Figure 2. Quantification of positive cell counts for Iba1 (**a, f**), HLA-DR (**b, g**), CD68 (**c, h**), MSR-A (**d, i**) and CD64 (**e, j**) with TSPO, normalised to total cells (%) in the temporal lobe (**a-e**) and cerebellum (**f-j**), separated by Braak group (0-VI).



Supplementary Figure 3. Quantification of nuclei counted (DAPI) per μm^2 (%) in the temporal lobe (a) and cerebellum (b), separated by Braak group (0-VI).

Supplementary Table 1. QuPath thresholds for positive cell counts of Iba1 (FITC) and TSPO (CY3)

Temporal	DAPI+FITC	DAPI+CY3	FITC+CY3	Cerebellum	DAPI+FITC	DAPI+CY3	FITC+CY3
Background radius	3µm	3µm	3µm	Background radius	3µm	3µm	3µm
Min area	10µm ²	10µm ²	10µm ²	Min area	10µm ²	10µm ²	10µm ²
Max area	100µm ²	100µm ²	100µm ²	Max area	100µm ²	100µm ²	32µm ²
DAPI threshold	80	80	250	DAPI threshold	80	80	200
Cell expansion	1µm	2µm	3µm	Cell expansion	1µm	2µm	3µm
Threshold +1	250	300	300	Threshold +1	200	1000	1000

Supplementary Table 2. QuPath thresholds for positive cell counts of HLA-DR (FITC) and TSPO (CY3)

Temporal	DAPI+FITC	DAPI+CY3	FITC+CY3	Cerebellum	DAPI+FITC	DAPI+CY3	FITC+CY3
Background radius	3µm	3µm	5µm	Background radius	3µm	3µm	5µm?
Min area	10µm ²	10µm ²	10µm ²	Min area	10µm ²	10µm ²	10µm ²
Max area	100µm ²	100µm ²	100µm ²	Max area	100µm ²	100µm ²	100µm ²
DAPI threshold	120	120	250/400	DAPI threshold	100	100	400/300
Cell expansion	1µm	2µm	3µm	Cell expansion	1µm	2µm	3µm
Threshold +1	500/400	550/350	480/350	Threshold +1	600/400	550/350	480/350

Supplementary Table 3. QuPath thresholds for positive cell counts of CD68 (FITC) and TSPO (CY3)

Temporal	DAPI+FITC	DAPI+CY3	FITC+CY3	Cerebellum	DAPI+FITC	DAPI+CY3	FITC+CY3
Background radius	3µm	3µm	3µm	Background radius	3µm	3µm	3µm
Min area	10µm ²	10µm ²	10µm ²	Min area	10µm ²	10µm ²	10µm ²
Max area	100µm ²	100µm ²	100µm ²	Max area	100µm ²	100µm ²	100µm ²
DAPI threshold	120	120	400	DAPI threshold	120	120	400
Cell expansion	2µm	2µm	3µm	Cell expansion	2µm	2µm	3µm
Threshold +1	400	650	650	Threshold +1	400	650	650

Supplementary Table 4. QuPath thresholds for positive cell counts of MSR-A (FITC) and TSPO (CY3)

Temporal	DAPI+FITC	DAPI+CY3	FITC+CY3	Cerebellum	DAPI+FITC	DAPI+CY3	FITC+CY3
Background radius	3µm	3µm	3µm	Background radius	3µm	3µm	3µm
Min area	10µm ²	10µm ²	10µm ²	Min area	10µm ²	10µm ²	10µm ²
Max area	100µm ²	100µm ²	100µm ²	Max area	100µm ²	100µm ²	100µm ²
DAPI threshold	80	80	600	DAPI threshold	80	80	600
Cell expansion	1µm	2µm	3µm	Cell expansion	1µm	2µm	3µm
Threshold +1	600	450	450	Threshold +1	600	900	900

Supplementary Table 5. QuPath thresholds for positive cell counts of CD64 (FITC) and TSPO (CY3)

Temporal	DAPI+FITC	DAPI+CY3	FITC+CY3	Cerebellum	DAPI+FITC	DAPI+CY3	FITC+CY3
Background radius	3µm	3µm	3µm	Background radius	3µm	3µm	3µm
Min area	10µm ²	10µm ²	10µm ²	Min area	10µm ²	10µm ²	10µm ²
Max area	100µm ²	100µm ²	100µm ²	Max area	100µm ²	100µm ²	100µm ²
DAPI threshold	80	80	450	DAPI threshold	80	80	450
Cell expansion	1µm	2µm	3µm	Cell expansion	1µm	2µm	3µm
Threshold +1	450	500	500	Threshold +1	450	550	550