Supporting Information Appendix

Definition of the contribution of an Osteopontin-producing CD11c^{+} microglial subset to Alzheimer's disease

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Figures S1 – S8

Tables S1 + S2

Other Supplementary Materials for this manuscript include the following:

Dataset S1

Movies S1 and S2

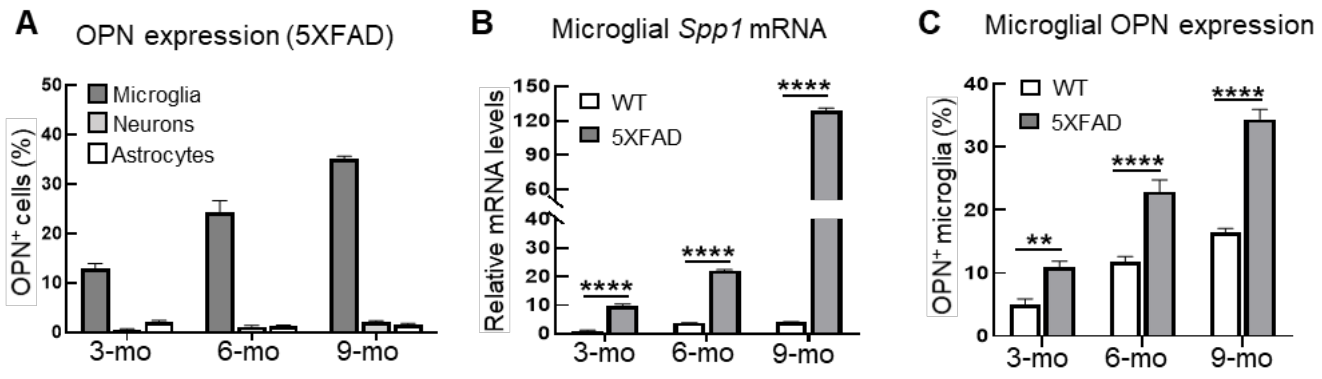

Figure S1. Microglial expression of OPN in 5XFAD mice.

(A) Flow cytometric analysis of OPN expression in microglia ($CD11b^+$), astrocytes ($GFAP^+$) and neurons ($MAP2^+$) obtained from 5XFAD mice (n=3) at different stages of disease development.

(B, C) Microglial OPN expression at the mRNA level (RT-qPCR) (n=4) and protein level (n=6) was analyzed by flow cytometry in 5XFAD mice compared with age-matched WT mice at the indicated ages during disease progression. ****$p < 0.0001$, **$p < 0.01$ by two-way ANOVA with Bonferroni's multiple comparisons test. Data are presented as mean ± s.e.m.

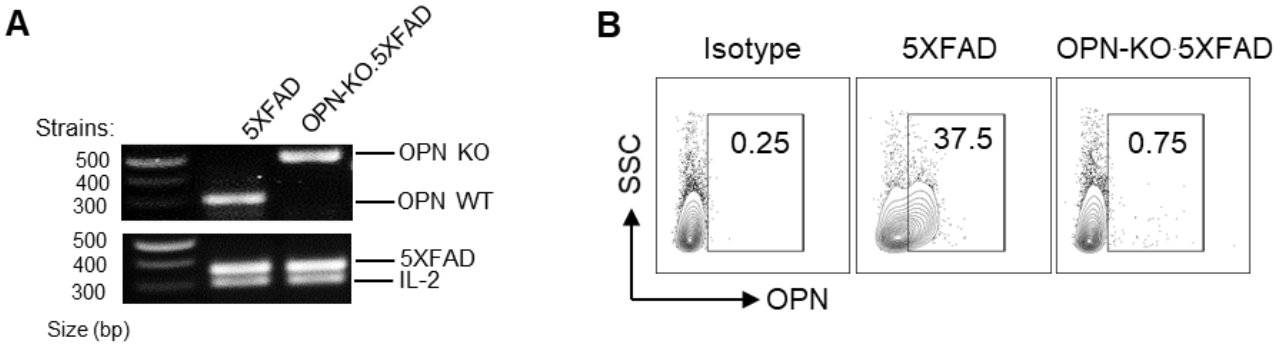

Figure S2. Confirmation of OPN KO in OPN-KO.5XFAD mice.

(A) PCR genotyping results of the indicated mouse strains: OPN-KO.5XFAD: 500 bp; OPN^{WT}: 300 bp; 5XFAD transgene: 377 bp; IL-2 (internal positive control): 324 bp.

(B) Validation of OPN KO at the protein level in microglia of 9-mo old OPN-KO.5XFAD mice. Isotype control was used as negative control. Microglia from age-matched 5XFAD mice were used as positive controls. The contour plots were representative results from 3 independent experiments.

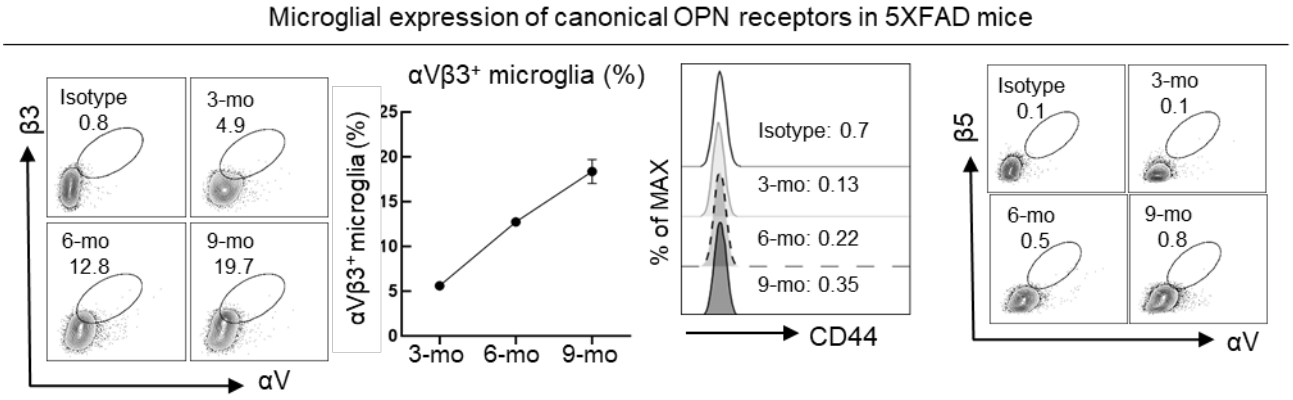

Figure S3. Microglial expression of canonical OPN receptors in 5XFAD mice at different stages of disease.

Flow cytometric analysis of microglial expression of αVβ3, CD44 and αVβ5 in 5XFAD mice at the indicated ages during disease progression. Histogram of CD44 and contour plots of αVβ3 and αVβ5 are the representative results from 3 independent experiments.

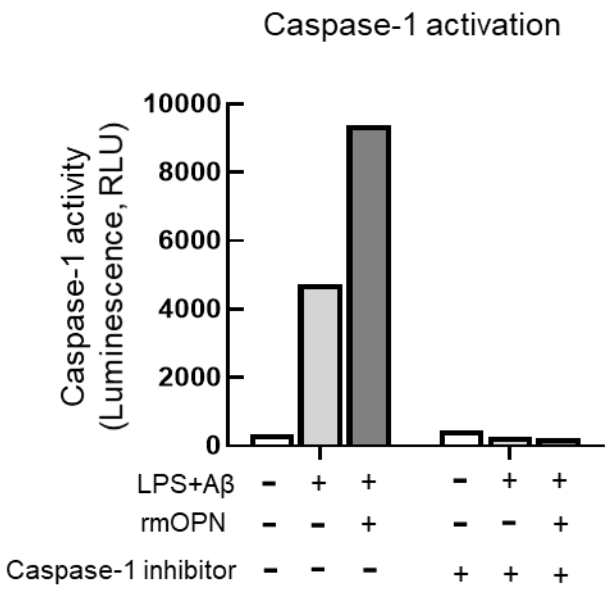

Figure S4. Validation of the specificity of Caspase-1 activation.

Intracellular Caspase-1 activity was measured by bioluminescent assay of microglia from 9-mo 5XFAD mice. Detection of the specificity of Caspase-1 activity was confirmed by a selective Caspase-1 inhibitor (Ac-YVAD-CHO, 1 μM). Bar plots are representative results from 3 independent experiments.

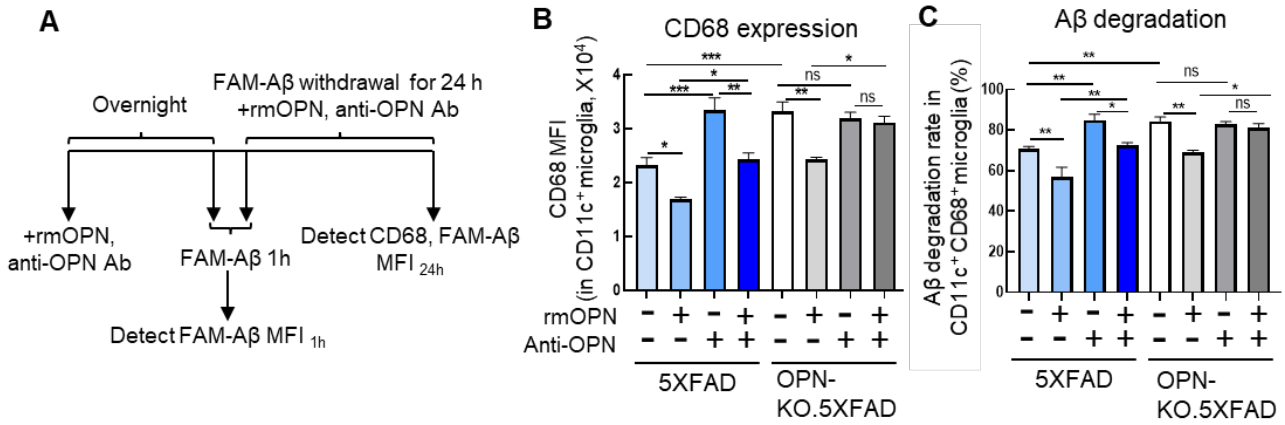

Figure S5. In vitro analysis of OPN-dependent inhibition of lysosomal Aβ degradation.

(A) Protocol of in vitro analysis of OPN-dependent inhibition of lysosomal Aβ degradation in $CD11c^+$ microglia from 9-mo old 5XFAD and OPN-KO.5XFAD mice.

(B) CD68 expression (MFI) in $CD11c^+$ microglia from 5XFAD and OPN-KO.5XFAD mice in the presence or absence of rmOPN and anti-OPN Ab (n=3). ***p < 0.001, **p < 0.01, **p < 0.05 by one-way ANOVA with Bonferroni's multiple comparisons test.

(C) Mean fluorescence intensity (MFI) of FAM-$A\beta_{1-42}$ after 1 h incubation determined by flow cytometry in lysosomes of $CD11c^+$ microglia ($CD11c^+CD68^+$) were defined as Aβ MFI_{1h}. MFI of retained FAM-Aβ in lysosomes of $CD11c^+$ microglia ($CD11c^+CD68^+$) 24 h after FAM-$A\beta_{1-42}$ withdrawal was determined and defined as Aβ MFI_{24h}. $CD11c^+$ microglial Aβ degradation rate (n=3) was calculated as (Aβ MFI_{1h} - Aβ MFI_{24h}) / Aβ MFI_{1h}. **p < 0.01, *p < 0.05 by one-way ANOVA with Bonferroni's multiple comparisons test. All data are presented as mean ± s.e.m.

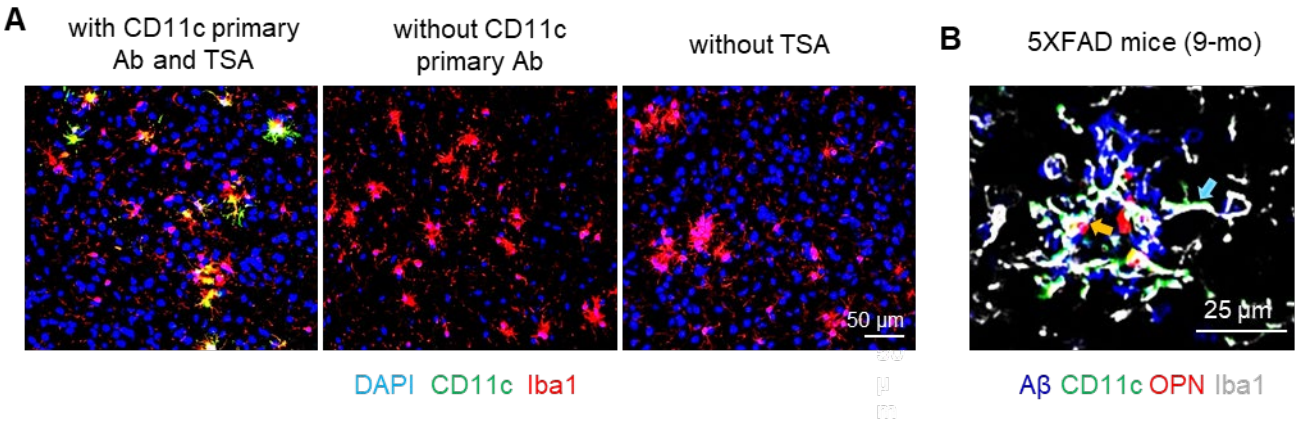

Figure S6. Immunofluorescent staining of CD11c microglial subsets in brain cryosections of 5XFAD mice.

(A) Immunofluorescent signal of microglial CD11c expression was validated in 9-mo old 5XFAD mice. Brain cryosections incubated without anti-CD11c primary Ab or Tyramide Signal Amplification (TSA) reagent were used as negative controls. Scale bar = 50 μm.

(B) Representative immunofluorescent staining of $CD11c^{+}OPN^{+}$ microglia ($CD11c^{+}OPN^{+}Iba\text{-}1^{+}$, yellow arrow) and $CD11c^{+}OPN^{-}$ microglia ($CD11c^{+}Iba\text{-}1^{+}$, cyan arrow) in brain sections of 9-mo old 5XFAD mice. Scale bar = 25 μm.

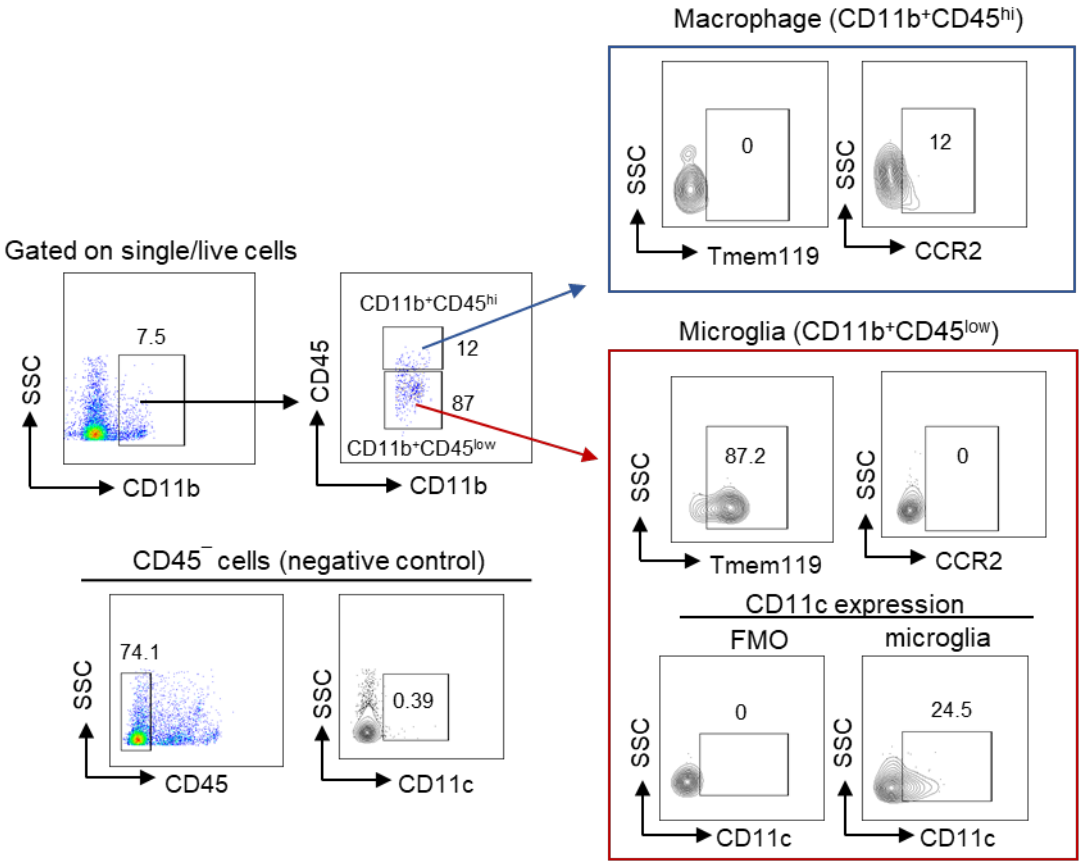

Figure S7. Validation of microglial CD11c expression in 5XFAD mice by flow cytometry.

Flow cytometric analysis of microglial CD11c expression in 9-mo old 5XFAD mice. $CD11b^+$ cells were gated on single/live cells. Microglia are identified as $CD11b^+$ $CD45^{low}$ $Tmem119^+CCR2^-$ cells and macrophage are identified as $CD11b^+CD45^{hi}$ $Tmem119^-CCR2^+$ cells. The specificity of CD11c staining was confirmed using FMO negative control. Brain $CD45^-$ cells containing primarily non-immune cells that do not express CD11c were included as negative controls.

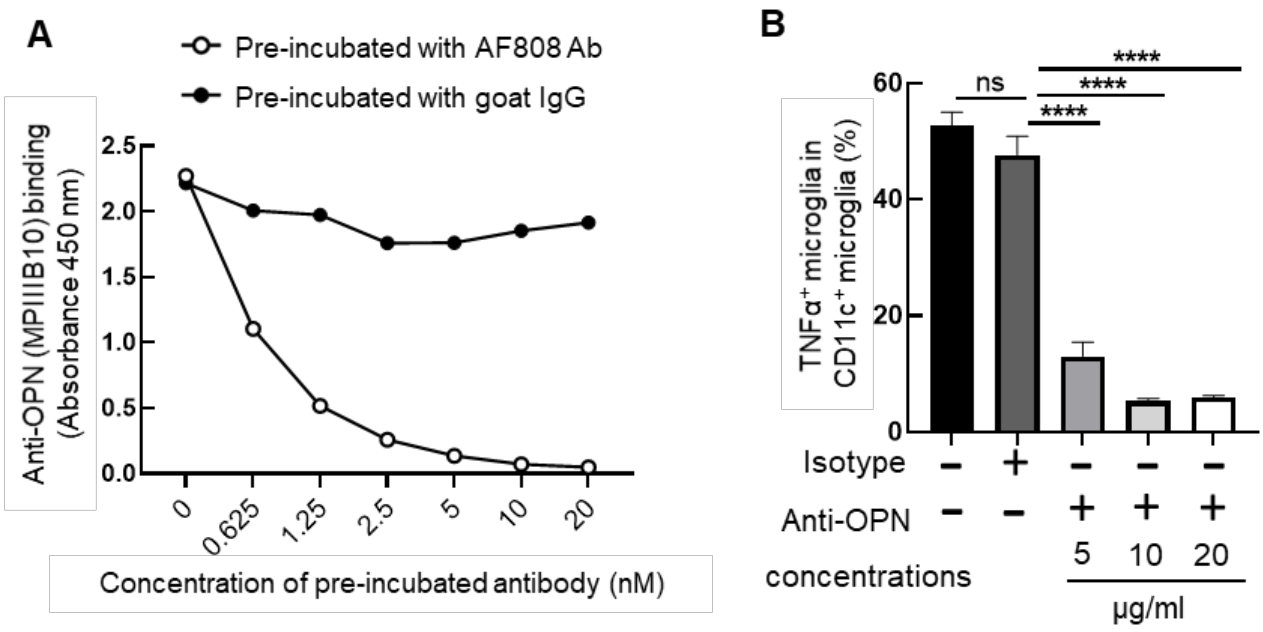

Figure S8. Binding specificity and in vitro function of anti-OPN mAb (MPIIIB10).

(A) The binding specificity of anti-OPN mAb (clone: MPIIIB10, isotype: mouse IgG1) to rmOPN was determined by competitive ELISA immunoassay. Plates coated with 2 µg/ml rmOPN (R&D) were pre-incubated with another anti-OPN Ab (clone: AF808, goat IgG) or its isotype goat IgG at graded concentrations followed by incubation with 200 nM anti-OPN mAb (MPIIIB10).

(B) Microglia isolated from 9-mo old 5XFAD mice were incubated at increasing concentrations (5, 10, 20 µg/ml) of anti-OPN mAb (MPIIIB10) or an isotype-matched (mouse IgG1) control for 24 hours followed by flow cytometric analysis of TNF-α production by $CD11c^+$ microglia (n=3). ****$p < 0.0001$, ns: not significant by one-way ANOVA with Bonferroni's multiple comparisons test. Data are presented as mean ± s.e.m.

See Excel file

Dataset S1. Differentially expressed genes (DEGs) of CD11c$^+$ microglia from 9-mo old OPN-KO.5XFAD and 5XFAD mice.

A full list of differentially expressed genes (DEGs) in CD11c$^+$ microglia from 9-mo old OPN-KO.5XFAD mice compared with 5XFAD mice identifies 2,985 DEGs. Gene expression was considered upregulated if $\log_2$FC >1 or downregulated if $\log_2$FC <−1. DEGs were considered significant with an FDR-adjusted p value < 0.05.

Table S1. Summary of human samples included in the study.

	N for ELISA	N for IF	Age (years ± SD)	Sex (F/M)	PMI (min ± SD)
Normal (CDR=0)	11	5	81.1 ± 9.6	6/5	787 ± 425
MCI (CDR=0.5)	10	9	82.6 ± 9.4	6/5	730 ± 425
AD (CDR>1)	11	8	82.7 ± 9.5	7/5	533 ± 237

PMI, Post-mortem Interval (min); CDR, Clinical Dementia Rating; IF, Immunofluorescence staining

Samples matched for age and sex were analyzed for OPN levels as measured by ELISA of frozen samples followed by immunofluorescence staining for Iba-1/CD11c/OPN (fixed, paraffin embedded sections).

Table S2. Characterization of frozen and fixed human samples.

	Subject number	Age	Sex	PMI (min)	CDR	Used for ELISA	Used for IF	Plaque rating[1]	Tangle rating[1]
Normal	23983	79	Male	964	0	+		1	0
	36472	89	Female	353	0	+		1	0
	57242	85	Female	320	0	+	+	1	0
	61911	95	Female	456	0	+		0	0
	80875	83	Male	834	0	+		1	0
	201539	85	Male	488	0	+		3	1
	395695	91	Female	290	0	+	+	0	0
	503571	81	Female	1364	0	+		0	0
	576228	72	Male	986	0	+	+	0	0
	808460	66	Male	1390	0	+	+	0	0
	921781	66	Female	1211	0	+	+	0	0
MCI	13090	71	Male	1285	0.5	+	+	1	0
	24420	83	Male	740	0.5	+		0	0
	27409	68	Female	1155	0.5	+	+	0	0
	38519	92	Female	225	0.5	+	+	3	3
	46426	68	Male	225	0.5	+		0	0
	83284	89	Female	495	0.5	+	+	0	0
	271140	89	Male	776	0.5	+	+	1	0
	582757	87	Male	925	0.5	+	+	0	0
	604571	93	Female	248	0.5	+	+	1	0
	754833	82	Female	553	0.5	+	+	5	3
	852223	87	Female	1398	0.5		+	3	1
AD	5323	91	Female	420	2	+		5	5
	29188	91	Female	285	2	+	+	3	1
	1100	84	Female	480	3	+		3	1
	64891	68	Male	1075	3	+	+	5	5
	74931	79	Female	390	3	+	+	5	5
	354049	85	Male	392	3	+	+	5	1
	639732	69	Female	500	3	+	+	5	5
	762124	72	Male	375	3		+	3	5
	782872	99	Female	360	3	+		1	0
	31742	87	Female	885	4	+	+	5	5
	39413	87	Male	540	5	+	+	5	5
	524179	80	Male	688	5	+		5	5

[1]Determined postmortem by a neuropathologist before storage of samples in the Mount Sinai brain bank.
PMI: Post-mortem Interval (min); CDR: Clinical Dementia Rating; IF: Immunofluorescence staining

Table S3. Characterization of frozen and fixed human samples.
Detailed characterization of all human samples included in the study.

See MP4 file

Movie S1. Confocal 3D images of human brain section stained for CD11c^{+}OPN^{+}Iba-1^{+} microglia from a normal control.

Microglia shown in the images were stained for Iba1 (red), CD11c (green) and OPN (cyan). 3D images consist of 34 Z stacks, each slice 0.29 µm thick accumulating to a total of 9.85 µm in depth for the entire image. The image was acquired at a magnification of ×63 with a zoom in effect.

See MP4 file

Movie S2. Confocal 3D images of human brain section stained for $CD11c^{+}OPN^{+}Iba\text{-}1^{+}$ microglia from an AD patient.

Microglia represented in the images were stained for Iba1 (red), CD11c (green) and OPN (cyan). The 3D image consists of 29 Z stacks, each slice 0.29 μm thick accumulating to a total of 8.36 μm in depth for the entire image. The image was acquired at a magnification of ×63 with a zoom in effect.