Supplemental Methods

Microscopy Photomicrographs were systematically acquired from each area using NEUN as anatomical reference. For each PFC slide, a total of 4 photomicrographs were acquired at Layer III along the ventral bank of the prefrontal cortex (PFC), and 4 additional photomicrographs were acquired from the underlying white matter (for a total of 12 PFC gray matter [PFCgm] images and 12 PFC white matter [PFCwm] micrographs). For each hippocampus slide, a total of 4 photomicrographs centered on the pyramidal layer were acquired from the CA1 field, two from the CA3 field, and two from the CA4/hilus field (for a total of 24 hippocampal formation photomicrographs per animal). The experimenter was unable to visualize IBA1 and HLADR channels during area selection to prevent biases.

Photomicrographs were acquired using an upright AxioImager Z2 microscope coupled to an LSM800 confocal head equipped with 2 GaAsP photomultiplier tubes, a 32-channel Airyscan detector, and a laser module capable of generating 405nm, 488nm, 561nm, and 633nm laser lines. All photomicrographs were acquired using a 20X objective (0.8NA), 1024p resolution, 16-bit depth, 2X averaging, 0.76 us pixel time, and the following individual channel settings: DAPI) 0.98AU | 3.5% 405nm laser power | 410-470nm detection wavelength | 500V detector gain | -1,500 detector offset | 0.3 detector digital gain; NEUN) 0.98AU | 5.0% 640nm laser power | 656-700nm detection wavelength | 500V detector gain | 0 detector offset | 1.0 detector digital gain; IBA1) 0.5 AU | 2.0% 488nm laser power | 410-545 detection wavelength | 500V detector gain | -2,560 detector offset | 1.0 detector digital gain; HLADR) 0.51 AU | 5% 561nm laser power | 545-620nm detection wavelength | 750V detector gain | 0 detector offset | 1.5 detector digital gain. All images were saved, unmodified and uncompressed, using Zeiss ZEN 3.8 software in czi format.

Image Segmentation. All image processing and quantitative mask generation were performed using a custom Python 3.11 pipeline built around the *scikit-image* (0.22), *numpy* (1.26), *tifffile* (2023.x), and *scipy* (1.11) libraries, following OME-TIFF conversion and voxel size adjustment. The analysis pipeline was executed independently for each scene, producing per-channel 3D binary masks and a per-scene quantitative summary table. Each OME-TIFF file contained four fluorescence channels corresponding to HLA-DR (channel 0), IBA1 (channel 1), DAPI (channel 2), and NEUN (channel 3). All stacks were converted to a canonical C×Z×Y×X format for uniform processing.

To limit computation to the region of optimal tissue focus, each scene was cropped to a *DAPI-based Z-band* determined through a robust coverage analysis. For each Z slice of the DAPI channel, the fraction of above-threshold foreground pixels was computed using an Otsu threshold(66). The per-slice coverage profile was then smoothed with a Gaussian kernel, and a central “plateau” of consistently high coverage was selected. The coverage threshold for inclusion was defined as the median of the central 10% of slices, and the selected region was symmetrically extended by one slice above and below to ensure inclusion of the nuclear boundaries. The resulting cropped interval was applied identically across all channels for downstream segmentation.

The DAPI channel was processed to define nuclear volumes and exclude non-nuclear debris. Each 2D slice was thresholded using the Triangle method(67), which provided robust performance in both sparse and densely stained regions. The resulting binary mask was optionally subjected to a 2D morphological closing (disk radius = 1 pixel) to merge fragmented nuclei. Connected components were then labeled in 3D, and small objects (< 20 μm^3) were removed. The final DAPI mask thus represented the union of all nuclei within the selected Z-band and was used for downstream normalization and quality control.

IBA1 segmentation was performed using a two-step approach designed to preserve fine microglial processes while maintaining clean somatic boundaries. *Vesselness-enhanced process map(68):*

A 3D Frangi filter (scale range = 1–2 voxels) was applied to enhance tubular structures. Each slice was then thresholded at 20% of its maximum vesselness response. The resulting binary map was lightly dilated (1-pixel radius) to ensure connectivity along fine arbors. *Soma/body mask:* The raw IBA1 intensity was thresholded using Otsu's method and morphologically refined by one iteration of 2D erosion followed by a 2D closing (disk radius = 1 pixel). Small holes (< 15 μm^2) were filled using a 2D binary fill-holes operation. The two masks were merged by pixelwise maximum operation to generate a single 3D IBA1 mask, which was then pruned of isolated noise components smaller than 20 μm^3. This fusion approach was empirically optimized to capture both fine processes and cell bodies with minimal background inclusion.

The HLA-DR channel exhibited variable background and uneven illumination, so a multistage adaptive normalization and thresholding strategy was used: *1) Normalization***:** Intensities were normalized per slice using percentile stretching between the 3rd and 99.5th percentiles to mitigate inter-slice brightness fluctuations; *2) Background subtraction:* A morphological *rolling ball* filter (69) (radius = 50 pixels) was applied to suppress slow-varying background and emphasize punctate or membranous signal. *3) Adaptive hysteresis thresholding:* High thresholds were computed using the *Yen* method on each slice. The low threshold was set to 90 % of the high threshold. Pixels exceeding the high threshold were immediately classified as foreground, whereas pixels between the two thresholds were included only if connected to a high-threshold region. 4) *Morphological cleanup*: Binary opening (disk radius = 1 pixel) and closing (radius = 1 pixel) were applied in 2D to remove salt-and-pepper noise and bridge small gaps. Connected components smaller than 20 μm^3 were removed to exclude noise and tissue edge artifacts, respectively. This method was selected over simpler global thresholding to achieve consistent labeling of microglial and vascular HLA-DR across variable immunostaining intensities.

Microglia Morphological Reconstruction. Three-dimensional microglial arbors were reconstructed from binary IBA1 masks using a custom Python 3.11 workflow designed to associate each 3D arbor skeleton with its corresponding soma and quantify morphometric and spatial features on a per-cell basis. Soma masks were first refined by removing very small objects and closing small gaps to ensure continuous volumes. Adjacent somata separated by fewer than 2 voxels were merged to correct for over-segmentation. Each connected soma was then uniquely labeled and its centroid and volume computed in physical units (μm^3).

To isolate microglial processes, a process mask was dilated around each soma and cleared within a thin exclusion shell to prevent double counting at soma boundaries, followed by a light binary closing to connect fragmented branches. A 3D skeletonization (70) pass was then applied, producing a one-voxel-thick representation of each arbor. Any skeleton voxels lying within somata were removed to confine process tracing to the parenchymal compartment. Each skeleton was then assigned to its nearest soma. Skeleton voxels within a small search radius of each soma served as seed points, and a breadth-first propagation (71) was performed through contiguous skeleton pixels to label all connected segments. This ensured that each microglial arbor was traced from its soma outward, preserving topological continuity. Voxels not connected to any soma were ignored. For each soma, the assigned skeleton was used to compute total arbor length (in μm), as well as the number of branch points and endpoints, by convolving the skeleton with a 26-connectivity kernel to estimate local degree.

For quality control, full 3D overlays were also produced as OME-TIFF volumes with RGB channels corresponding to soma and skeleton colorization. Nearest-neighbor distances were used to calculate cell density (cells/mm^2), the Clark–Evans R index and Z-score for regularity, the Hopkins H statistic for clustering tendency, and Ripley's $L(r) - r$ function across radii from 5–150 μm.

Statistical Analysis of Microglial Assessments. All quantitative measurements were summarized at the level of the biological unit (animal). For each animal, values from individual imaging scenes within a given region were averaged to yield a single representative value per metric. Each brain region was treated as an independent variable, with the experimental groups (SIV-, α4, IgG) as the variables of interest. Prior to hypothesis testing, the distribution of each metric was examined for normality and variance homogeneity. Data were evaluated using the Brown–Forsythe test for equality of variances and by assessing skewness ($|Sk| \leq 1$) and kurtosis ($|K| \leq 3$). Metrics satisfying all three criteria were analyzed parametrically; those that failed were further examined after transformation. When appropriate, square-root or $\log(1 + x)$ transformations were applied. If the transformed data met the parametric assumptions, the transformed values were used; otherwise, non-parametric procedures were retained.

Parametric data were analyzed using one-way ANOVA, followed by Tukey's honestly significant difference (HSD) test for pairwise group comparisons. Non-parametric data were analyzed using the Kruskal–Wallis test, followed by Mann–Whitney U tests for pairwise contrasts with Holm's step-down correction for multiple comparisons. Statistical significance was set at $\alpha = 0.05$ for all analyses. Data visualization was performed using a combination of box and violin plots, which display the full distribution of measurements together with the group median and interquartile range. All statistical analyses and visualizations were conducted using custom Python scripts designed for this project.