**Spatial proteomic multiplex immunofluorescence (IF) staining**

Sequential IF was performed using Lunaphore's COMET™ system with automated staining, imaging, and elution cycles. The multiplex immunofluorescence panel included X antibodies (**Supplementary table 1**). All antibodies were validated using conventional immunohistochemistry and/or immunofluorescence (IF) staining, in conjunction with the corresponding fluorophore and the spectral 4',6-diamidino-2-pheynlindole (DAPI; ThermoFisher Scientific) counterstain. For optimal concentration and the best signal/noise ratio, all antibodies were tested at three different dilutions, starting with the manufacturer-recommended dilution (MRD), then MRD/2, and MRD/4. Secondary Alexa Fluor 555 (ThermoFisher Scientific) and Alexa Fluor 647 (ThermoFisher Scientific) were used at 1/200 and 1/400 dilutions, respectively. The optimizations and full runs of the panel were executed using the sequential IF (seqIF™) methodology integrated into the COMET™ control software (*Rivest, F., et al., Fully Automated Sequential Immunofluorescence (seqIF) for Hyperplex Spatial Proteomics. bioRxiv, 2023*). The staining is performed following automated cycles of 2 antibodies' staining at a time, followed by imaging and elution, where no human intervention is required. All reagents were diluted in Multistaining Buffer (BU06, Lunaphore Technologies). The elution step lasted 2min for each cycle and was performed with Elution Buffer (BU07-L, Lunaphore Technologies) at 37°C. Quenching lasted for 30sec and was performed with Quenching Buffer (BU08-L, Lunaphore Technologies). Imaging was performed with Imaging Buffer (BU09, Lunaphore Technologies). The incubation times were set at 4min for all primary antibodies and secondary antibodies at 2min. Imaging was performed with an integrated epifluorescent microscope at 20x magnification. Image registration was performed immediately after concluding the staining and imaging procedures by COMET™ control software. Each protocol resulted in a multi-stack OME-TIFF file where the imaging outputs from each cycle were stitched and aligned. The OME-TIFF files contain a DAPI image, intrinsic tissue autofluorescence in TRITC and Cy5 channels, and a single fluorescent layer per marker. Subsequent analysis was performed using the HORIZON Viewer™ software from Lunaphore. The background autofluorescence cycle was subtracted from each subsequent cycle using the subtraction feature integrated within the Viewer™. Each marker was then pseudo-colored and its signal thresholded for visualization and generation of snapshot Tiff files.

**Supplementary Table 1**: Primary and secondary antibodies used for multiplex IF staining.

| MARKER | MANUFACTURER | CATALOGUE NUMBER | CLONE | DILUTION |
|---|---|---|---|---|
| **DAPI** | Thermofisher | 62248 | N/A | 1/1500 |
| **AF 555** | Thermofisher | A32727 | N/A | 1/200 |
| **AF 647** | Thermofisher | A32733 | N/A | 1/400 |
| **CD31** | Abcam | Ab225883 | EPR17259 | 1/1500 |
| **ACTA2** | Abcam | Ab7517 | 1A4 | 1/1000 |
| **GFAP** | Sigma | MAB360 | GA5 | 1/3000 |
| **MAP2** | Abcam | Ab183830 | EPR19691 | 1/100,000 |
| **Tau** | Thermofisher | MN1020 | AT8 | 1/1500 |
| **β-Amyloid** | Biolegend | 800701 | 4G8 | 1/1000 |
| **P2RY12** | Atlas Antibodies | HPA014518 | Polyclonal | 1/1000 |
| **CD11c** | Abcam | Ab52632 | EP1347Y | 1/300 |
| **CD68** | Dako Agilent | GA613 | PG-M1 | No dilution required |
| **LAMP1** | Cell Signaling | 9091S | D2D11 | 1/400 |
| **RAB5** | Cell Signaling | 3547S | C8B1 | 1/200 |
| **Fibronectin** | Abcam | Ab2413 | Polyclonal | 1/1000 |
| **Fibrinogen** | Abcam | Ab34269 | Polyclonal | 1/500 |
| **MBP** | Santa Cruz | sc-271524 | F-6 | 1/1000 |

N/A: not applicable.

**Data availability:** Underlying, deidentified data may be obtained from the corresponding author upon request.