Supplementary methods

Development of algorithms for quantification procedures

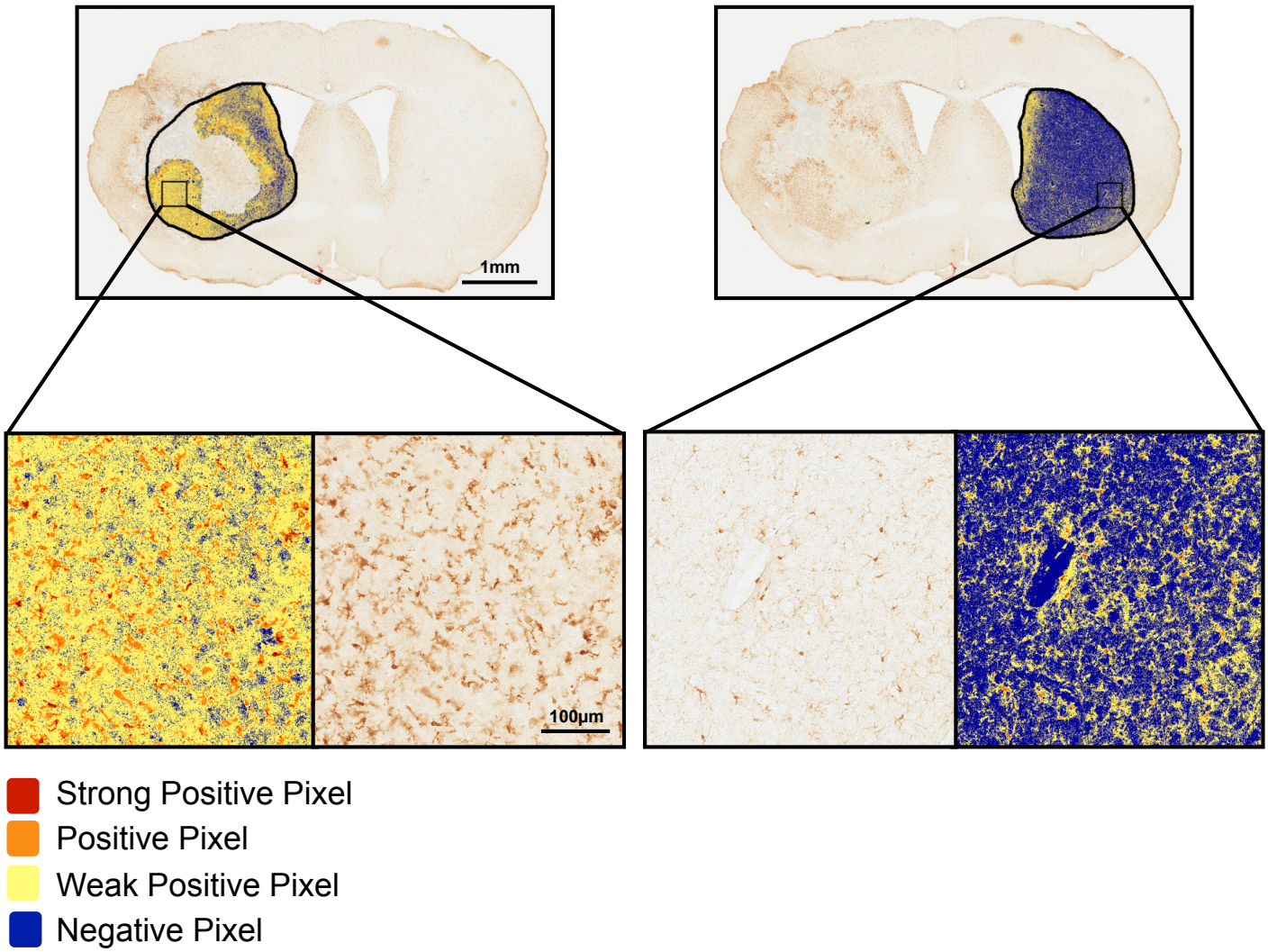
Blood accumulation, ferric iron content, and immunohistochemical stains were quantified using the ImageScope 'positive pixel count' algorithm. This Algorithm was tuned for each of the individual stains such that the appropriate signal (e.g. blue pixels for ferric iron content vs. brown pixels for Iba1) and strength of signal was evaluated. SI Table 1 provides the relevant algorithm parameters used for each histological stain. For all stains, thresholds were set intermediate between the signal seen for WT and $EP3^{-/-}$ mice on a representative slide in each group such that the algorithm allowed for optimal detection in either direction (i.e. more intense vs. less intense).

SI Table 1. ImageScope positive pixel count algorithm parameters

Histological Stain	Hue Value	Hue Width	Color Saturation Threshold	Weak Positive Pixel (Upper Limit)	Positive Pixel (Upper Limit)	Positive Pixel (Lower Limit)	Strong Positive Pixel (Lower Limit)	Negative Pixel
Blood Accumulation	0.06	0.21	0.00	214	214	204	0	-1
Ferric Iron Content	0.66	0.28	0.04	220	175	100	0	-1
Iba1	0.10	0.50	0.04	255	175	100	0	-1
GFAP	0.10	0.50	0.04	255	175	100	0	-1
MPO	0.10	0.10	0.05	255	175	60	0	-1
IgG	0.02	0.25	0.12	255	159	137	90	-1

Regarding the strength of signal used in the quantification, strong positive pixels were used for blood accumulation and GFAP, total positive pixels (weak positive, positive, and strong positive) for IgG, and positive and strong positive pixels were used for the remaining histological stains. SI Figure 1 shows an example of the Iba1 algorithm and demonstrates the rationale for including positive and strong positive pixels for this particular staining intensity-algorithm combination, but excluding weak positive pixels since these, in this case, represent background and non-specific signal. Identical procedures were used for other stain-algorithm combinations. After running a given algorithm, all slides were checked for specificity and accuracy and to ensure minimal interference from artifact.

It should be noted that these specific algorithms and choice of pixel intensity to include in the quantification (e.g. only strong positive vs. positive and strong positive) are applicable for other quantification procedures only if the same staining intensity is present as shown in Figures 1 and 3-7.



SI Figure 1. Example of an ImageScope positive pixel count algorithm used for quantification procedures. Images demonstrate the Iba1 algorithm (parameters are provided in SI Table 1) used for quantification of ipsilateral (left panels) and contralateral (right panels) striatal microgliosis and cortical microgliosis (example not shown). In this case, positive and strong positive pixels were used to generate the corresponding experimental results shown in Figures 4B and 4D.