



**Supplementary Data Figure 6: Non-biased quantitation of endothelial HIF-1 $\alpha$  expression using image analysis.** Representative images illustrate the annotation and quantification workflow for HIF-1 $\alpha$  expression in the endothelium of mock-infected control brain (AGM5). Raw images of basal ganglia (**a**), brainstem (**e**), and cerebellum (**i**) show blood vessels with HIF-1 $\alpha$  immunoreactivity. Endothelial regions were manually annotated to delineate vessel walls (**b**, **f**, **j**), and pixel-level optical density was quantified within these boundaries (**c**, **g**, **k**). Solid green lines indicate the specific areas included in the analysis. Panels **d**, **h**, and **l** show the markup alone, confirming that only endothelial regions were assessed. Scale bars = 50  $\mu$ m.