

## **SUPPLEMENTARY INFORMATION**

### **Automated image analysis of neuroinflammation and neurodegeneration enables quantitative assessment of virus neurovirulence in primates**

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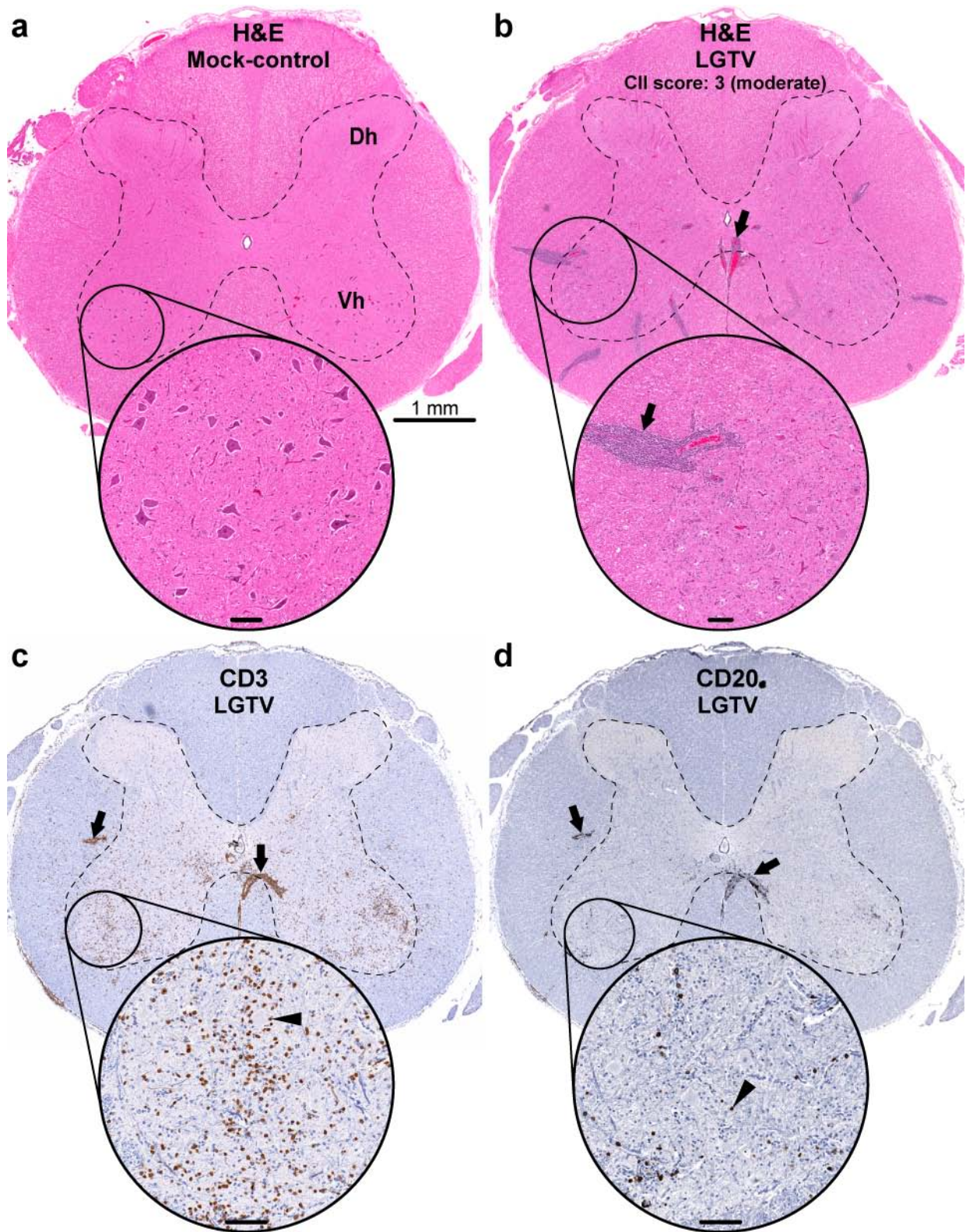
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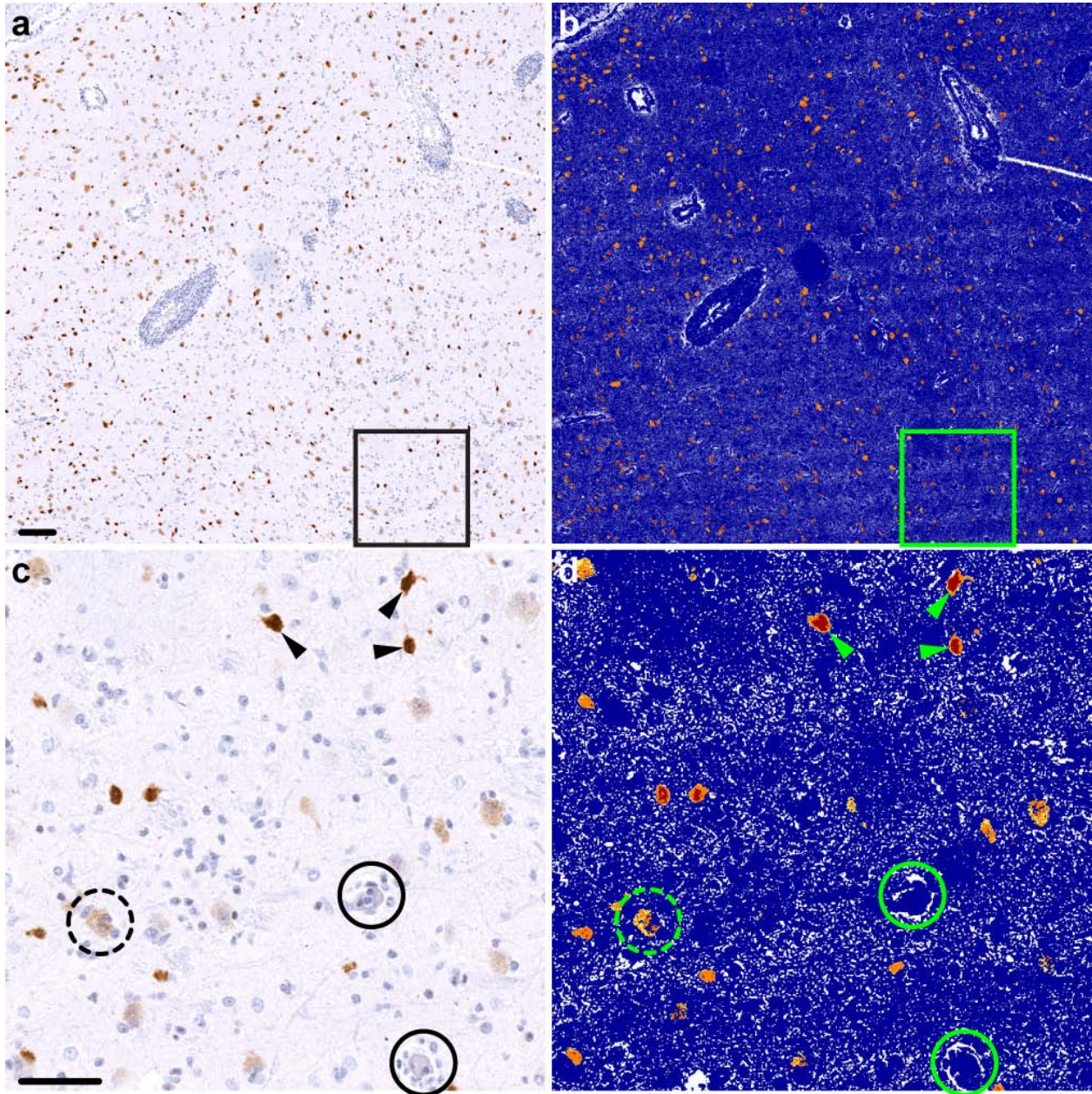
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**Supplementary Figure 1. Assessment of cellular lymphocytic infiltration in the spinal cord.** Representative transverse sections of the lumbar spinal cord of a mock-inoculated monkey (**a**) or LGTV-infected monkey (**b – d**; adjacent sections) showing H&E staining (**a** and **b**), CD3-immunostaining (**c**), or CD20-immunostaining (**d**). The dashed lines show approximate boundaries of the grey matter. **Arrows** in **b – d**: large perivascular inflammatory infiltrates. The circled areas in **a – d** are shown at higher magnification in corresponding insets. Inset in **c** shows T-cell infiltration within the parenchyma of ventrolateral column containing motor neurons. Note: some T cells were seen in close contact with motor neurons (**arrowhead**). Inset in **d** shows intraparenchymal B-cell infiltration (**arrowhead**). Inset bars: 100  $\mu$ m.



**Supplementary Figure 2. Image analysis of NeuN-immunoreactivity for assessment of neuronal degeneration in the thalamus.** (a) Original image showing the medial nucleus of pulvinar of a TBEV/DEN4 $\Delta$ 30-infected monkey. (b) Corresponding markup image showing the results of applied NeuN-IR algorithm (strong positive pixels - red; moderate positive pixels - orange; weak positive pixels - yellow; negative pixels - blue; neutral pixels - white). (c and d) Corresponding original and markup images of the boxed areas in a and b are shown at higher magnification. **Arrowheads (black in c or green in d):** strong NeuN-immunoreactivity in apparently normal neurons. **Note:** degenerating neurons show either significantly decreased NeuN-immunoreactivity (**dashed circles**) or completely devoid NeuN-immunoreactivity (**closed circles**). Bars: 50  $\mu$ m.

**Supplementary Table 1.** Grading scale used for evaluation of the cellular inflammatory infiltration and microglial activation/neuronal degeneration in the CNS

<b>CNS region</b>	<b>Score</b>	<b>Description</b>
<b>CII</b>		
Basal ganglia Thalamus	0	No inflammatory lesions
Spinal cord	1 (minimal)	1 to 3 perivascular cellular inflammatory infiltrates (perivascular cuffs) with or without focal inflammatory infiltration in leptomeninges
	2 (mild)	4 to 10 perivascular cuffs and mild diffuse inflammatory infiltration of the parenchyma, with or without focal infiltration in leptomeninges
	3 (moderate)	11 to 30 perivascular cuffs and diffuse inflammatory infiltration of the parenchyma, with or without focal infiltration in leptomeninges
	4 (severe)	More than 30 perivascular cuffs and widespread diffuse infiltration of the parenchyma, usually with focal infiltration in leptomeninges
<b>MGA/ND</b>		
Basal ganglia Thalamus	0	No lesions other than injection-related lesions
	1 (minimal)	Up to 3 small microglial nodules (MGNs), no evidence of neuronal degeneration
	2 (mild)	4 to 20 MGNs with degenerative neuronal changes within or on the periphery of MGN
	3 (moderate)	21 to 30 MGNs accompanied by diffuse microglial activation, neuronal degeneration, neuronophagia, and neuronal loss
	4 (severe)	More than 30 MGNs, diffuse microglial activation, neuronal degeneration, neuronophagia, and neuronal loss
Spinal cord	0	No lesions
	1 (minimal)	Minimal diffuse microglial activation and mild neuronal degeneration
	2 (mild)	1 or 2 MGNs accompanied by diffuse microglial activation and neuronal degeneration
	3 (moderate)	3 to 6 MGNs accompanied by diffuse microglial activation, neuronal degeneration, neuronophagia, and neuronal loss
	4 (severe)	Multiple coalescent MGNs, neuronophagia, and severe neuronal loss

**Abbreviations:** CII - cellular inflammatory infiltration; MGA/ND - microglial activation/neuronal degeneration.