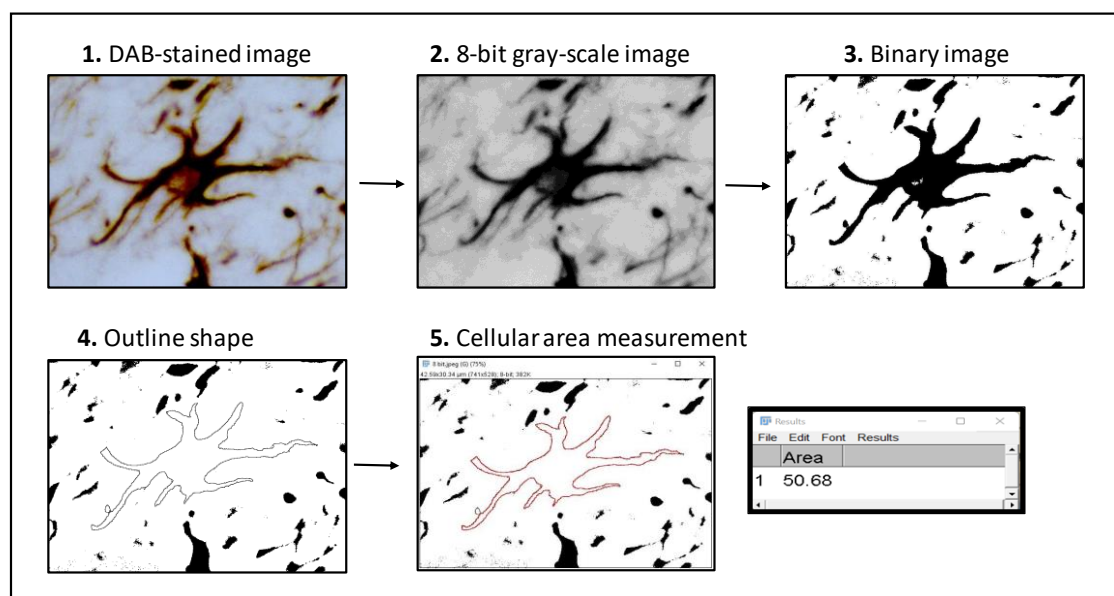
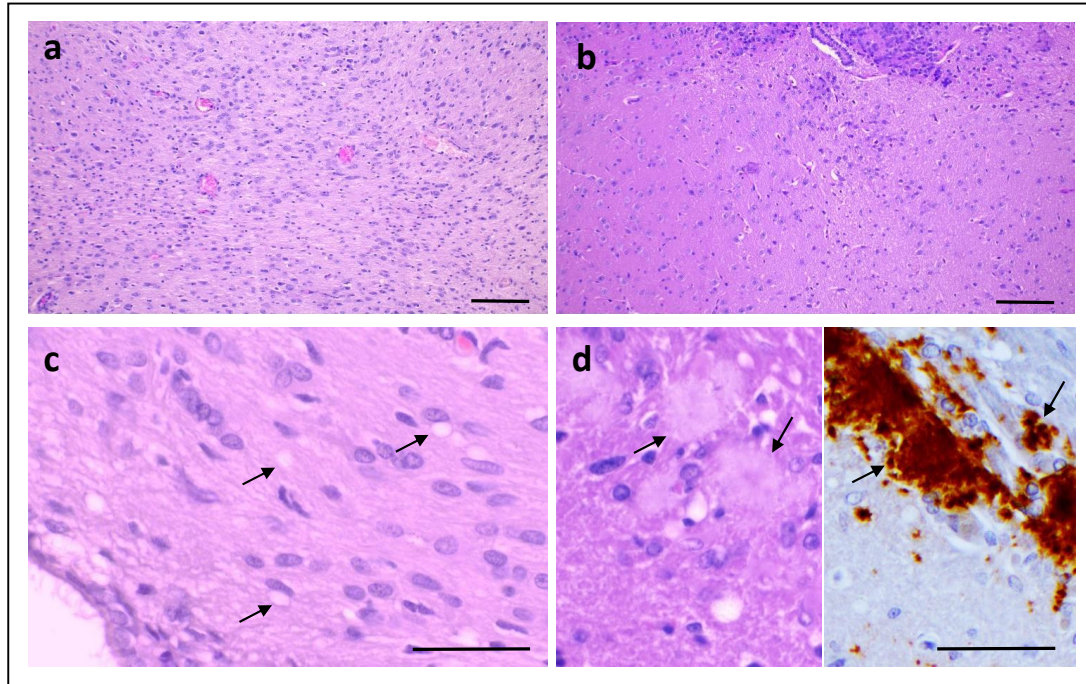


Supplementary materials of:

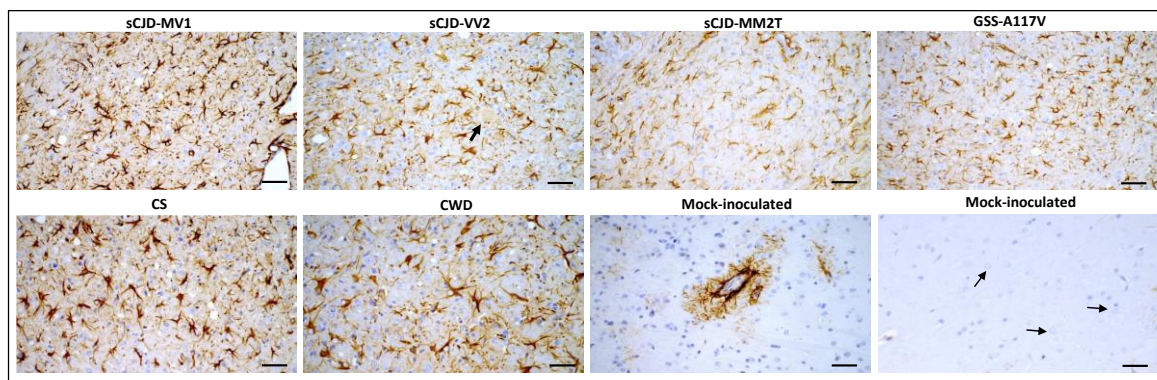
**Strain-Dependent Morphology of Reactive Astrocytes in Human- and Animal-Vole-Adapted Prions**



**Figure S1: Progressive representation of Fiji protocol developed to measure the cellular area starting from a DAB-stained image.**



**Figure S2 - Specific neuropathological features of sCJD-MM2T, GSS-A117V and sCJD-VV2.** Haematoxylin and eosin staining highlighted thalamic atrophy in sCJD-MM2T (a) in comparison to the thalamus of mock-inoculated vole (b) (scale bars, 50  $\mu$ m); (c) small vacuoles in corpus callosum of GSS-A117V (scale bar, 20  $\mu$ m); (d) (d) panel with haematoxylin and eosin (left) and immunohistochemistry (right) of plaques accumulated in the alveus of vole infected with sCJD-VV2 (scale bar, 20  $\mu$ m).



**Figure S3 - Immunohistochemical detection of GFAP in MDTN of selected vole-adapted prion strains.** Prominent astrogliosis affected all prion-infected vole brains. In sCJD-VV2, amyloid plaque encircled by few astrocytes is detected in the neuropil (arrow). In the thalamus of mock-inoculated voles, few astrocytic processes surrounding blood vessels are visible, while any GFAP immunolabelling is associated with astrocyte cellular bodies scattered within thalamic parenchyma. Scale bars, 20  $\mu$ m.

Strain	Vole ID	Number of astrocytes	Mean of cellular area ( $\mu\text{m}^2$ )	Standard Deviation	Prob>f (Anova)
sCJD-MM2T	#1	12	23.85	3.89	0.0016
	#2	4	18.81	3.14	
	#3	9	17.46	3.33	
GSS-A117V	#1	15	23.55	4.20	0.3001
	#2	5	20.70	3.20	
	#3	6	23.67	3.44	
sCJD-MV1	#1	10	32.92	4.28	0.0529
	#2	11	34.62	3.79	
	#3	6	38.38	4.20	
	#4	8	32.95	3.53	
sCJD-VV2	#1	4	31.34	1.30	0.0124
	#2	5	39.33	8.36	
	#3	7	40.22	6.81	
	#4	4	27.43	3.33	
CS	#1	21	38.85	5.29	0.1889
	#2	5	35.09	5.78	
	#3	4	33.29	3.17	
	#4	12	35.98	6.06	
	#5	7	39.58	5.92	
CWD	#1	10	45.26	4.70	0.1183
	#2	6	49.54	8.89	
	#3	3	57.57	11.53	
	#4	3	50.31	7.75	

**Table S1 – Dataset of cellular areas of astrocytes in prion strains investigated.** Table includes: i) number of animals per strain; ii) astrocytes per vole analyzed for each strain; iii) the mean values  $\pm$  standard deviation of the cellular area obtained from every single animal; iv) and results of one-way parametric ANOVA. Medium-high consistency was observed among voles inoculated with the same strain, except for sCJD-VV2 and sCJD-MM2T, for which between-subject variability was found.