

# Supplementary information -

## Evidence of traumatic brain injury in headbutting bovids

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**Table S1: Exhaustive counts of different pSer202 tau-immunoreactive structures in the neocortical layers of the muskox brain.**

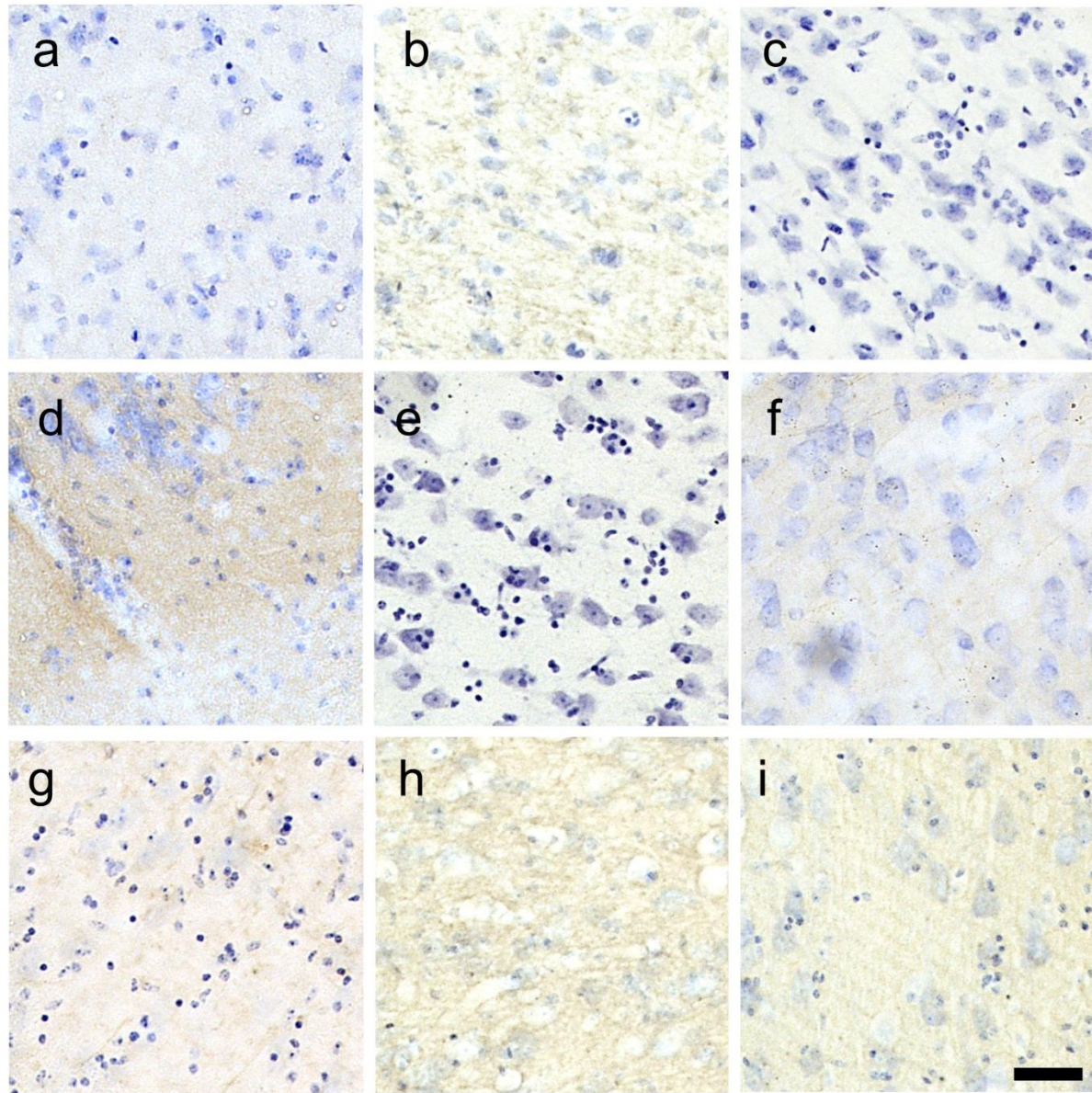
| Individual         | Block    | Region | Neuropil | NTC | Neuron |
|--------------------|----------|--------|----------|-----|--------|
| Male               | PFC      | I      | 679      | 130 | 1      |
|                    |          | II     | 347      | 67  | 5      |
|                    |          | III    | 585      | 92  | 4      |
|                    |          | IV-VI  | 879      | 48  | 11     |
|                    |          | WM     | 131      | 7   | 1      |
|                    | parietal | I      | 299      | 1   | 0      |
|                    |          | II     | 167      | 5   | 0      |
|                    |          | III    | 293      | 15  | 0      |
|                    |          | IV-VI  | 284      | 10  | 2      |
|                    |          | WM     | 57       | 0   | 0      |
| Middle-aged female | PFC      | I      | 5557     | 680 | 2      |
|                    |          | II     | 2515     | 319 | 21     |
|                    |          | III    | 6205     | 743 | 25     |
|                    |          | IV-VI  | 7089     | 838 | 38     |
|                    |          | WM     | 4071     | 377 | 0      |
|                    | parietal | I      | 417      | 5   | 0      |
|                    |          | II     | 175      | 4   | 1      |
|                    |          | III    | 321      | 9   | 0      |
|                    |          | IV-VI  | 317      | 5   | 0      |
|                    |          | WM     | 88       | 1   | 0      |
| Old female         | PFC      | I      | 1658     | 44  | 0      |
|                    |          | II     | 528      | 50  | 0      |
|                    |          | III    | 2047     | 112 | 2      |
|                    |          | IV-VI  | 776      | 36  | 3      |
|                    |          | WM     | 314      | 20  | 0      |
|                    | parietal | I      | 1316     | 49  | 0      |
|                    |          | II     | 1494     | 78  | 3      |
|                    |          | III    | 1420     | 68  | 1      |
|                    |          | IV-VI  | 573      | 42  | 0      |
|                    |          | WM     | 112      | 2   | 1      |

WM = white matter, PFC = prefrontal cortex

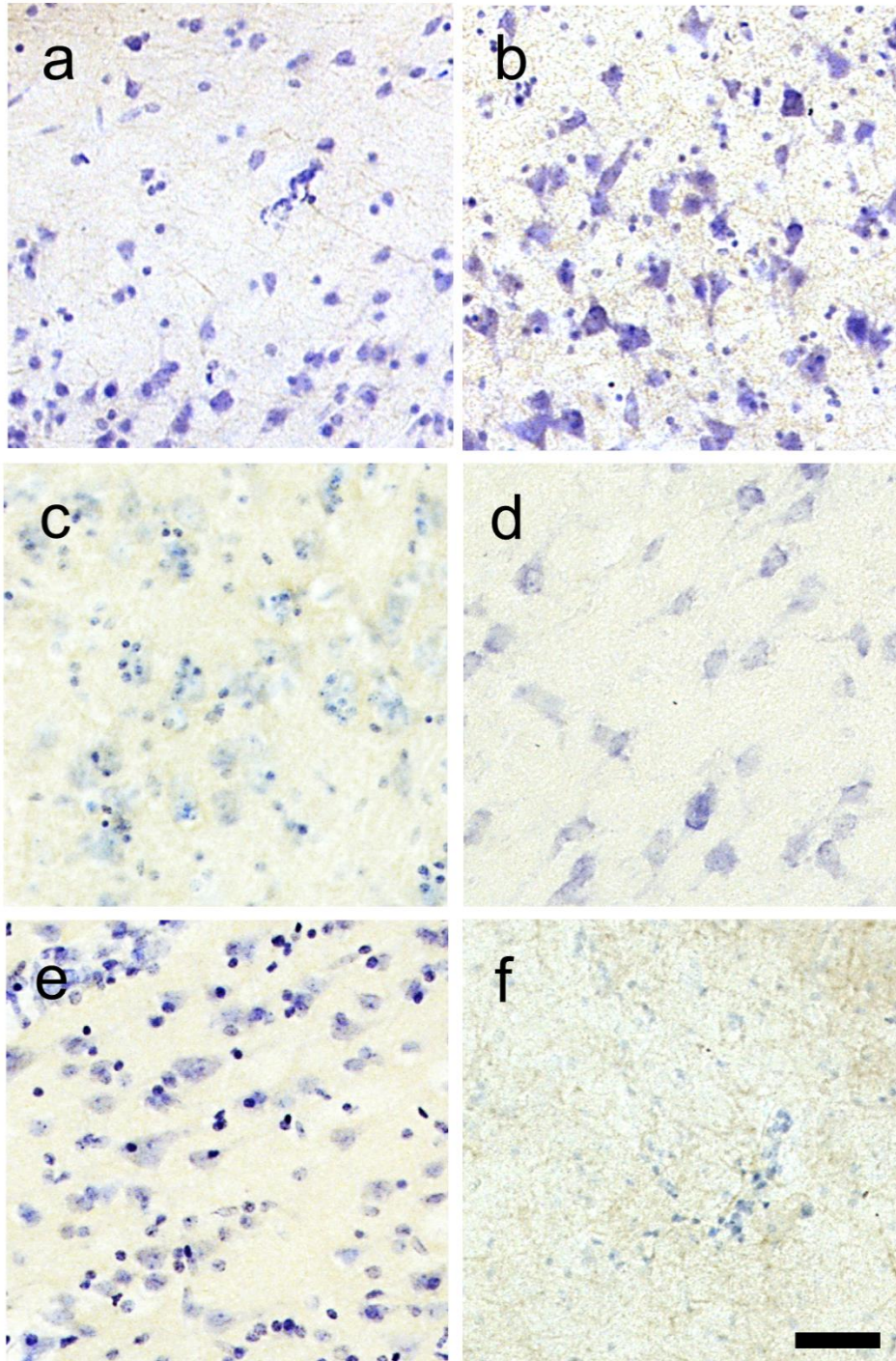
**Table S2: Coefficient of error for estimated counts of pSer202 tau-immunoreactive structures in neocortical layers of the muskox brain.**

| Individual         | Block    | Region | Neuropil | NTC  | Neuron |
|--------------------|----------|--------|----------|------|--------|
| Male               | PFC      | I      | 0.04     | 0.09 | 1.00   |
|                    |          | II     | 0.05     | 0.12 | 0.45   |
|                    |          | III    | 0.04     | 0.10 | 0.50   |
|                    |          | IV-VI  | 0.03     | 0.15 | 0.30   |
|                    |          | WM     | 0.09     | 0.38 | 1.00   |
|                    | parietal | I      | 0.07     | 1.00 | NA     |
|                    |          | II     | 0.08     | 0.45 | NA     |
|                    |          | III    | 0.07     | 0.26 | NA     |
|                    |          | IV-VI  | 0.07     | 0.32 | 0.71   |
|                    |          | WM     | 0.14     | NA   | NA     |
| Middle-aged female | PFC      | I      | 0.03     | 0.04 | 0.71   |
|                    |          | II     | 0.03     | 0.06 | 0.22   |
|                    |          | III    | 0.02     | 0.04 | 0.20   |
|                    |          | IV-VI  | 0.02     | 0.04 | 0.16   |
|                    |          | WM     | 0.03     | 0.06 | NA     |
|                    | parietal | I      | 0.05     | 0.45 | NA     |
|                    |          | II     | 0.08     | 0.50 | 1.00   |
|                    |          | III    | 0.06     | 0.33 | NA     |
|                    |          | IV-VI  | 0.06     | 0.45 | NA     |
|                    |          | WM     | 0.11     | 1.00 | NA     |
| Old female         | PFC      | I      | 0.03     | 0.15 | NA     |
|                    |          | II     | 0.05     | 0.14 | NA     |
|                    |          | III    | 0.03     | 0.09 | 0.71   |
|                    |          | IV-VI  | 0.04     | 0.17 | 0.58   |
|                    |          | WM     | 0.06     | 0.22 | NA     |
|                    | parietal | I      | 0.04     | 0.14 | NA     |
|                    |          | II     | 0.03     | 0.12 | 0.58   |
|                    |          | III    | 0.03     | 0.12 | 1.00   |
|                    |          | IV-VI  | 0.05     | 0.16 | NA     |
|                    |          | WM     | 0.10     | 0.71 | 1.00   |

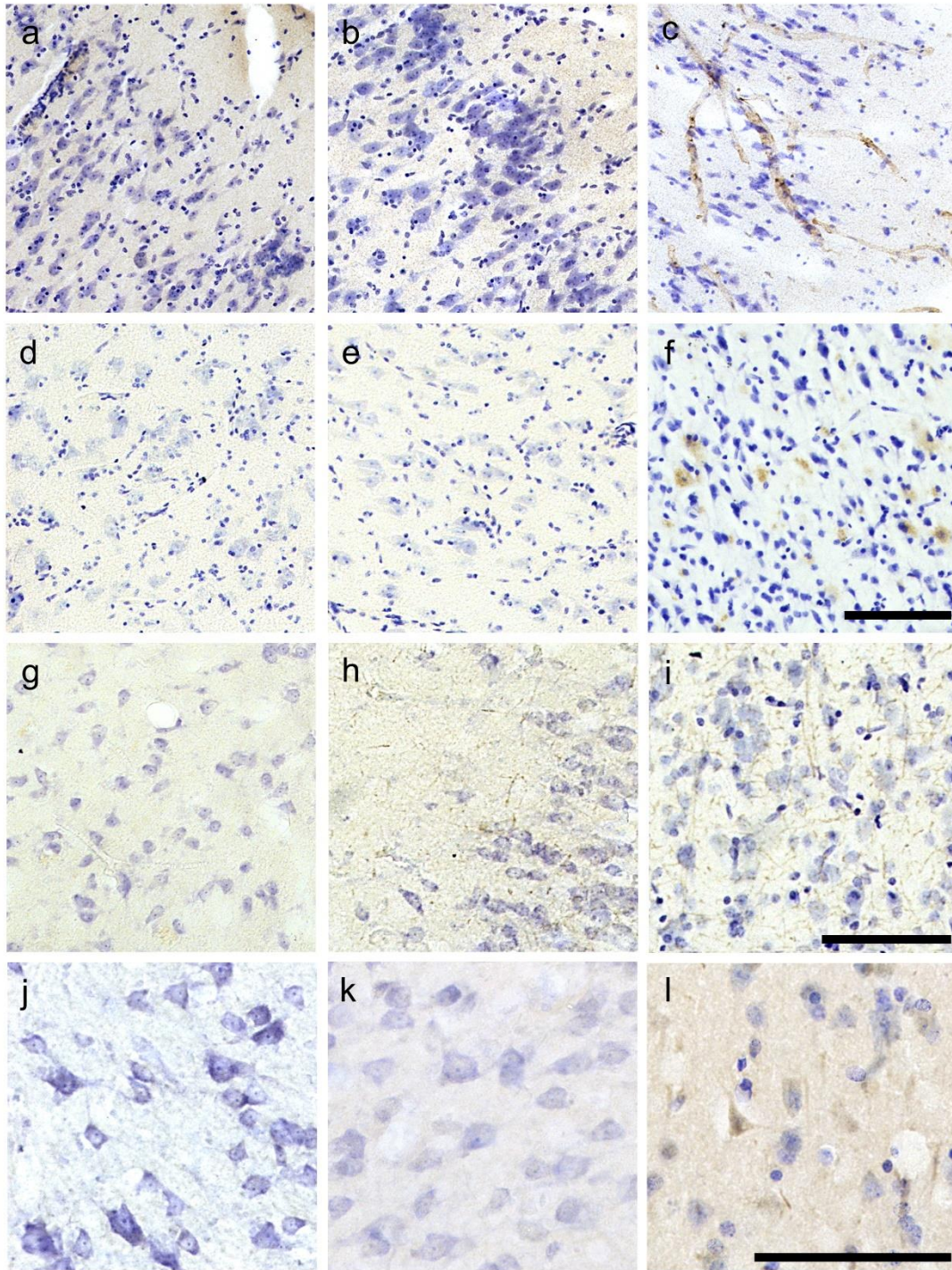
NTC= neuritic thread cluster, WM= white matter, NA= no neurons counted,  
PFC = prefrontal cortex



**Figure S1. Photomicrographs of controls for abnormally phosphorylated tau immunostained for different antibodies in brains of human (a-c), old male muskox (d-f), and a male bighorn sheep (g-i).** Samples were taken from the prefrontal cortex and subjected to immunohistochemistry in the absence of anti-pSer396/Ser404 tau (a, d, g), anti-pSer202/Thr205 tau (b, e, h), and pSer202 tau (c, f, i). The same control was used for both the Alzheimer's disease and CTE human specimens. Immunolabeled images can be found in Fig. 2. Scale bar = 100  $\mu$ m.

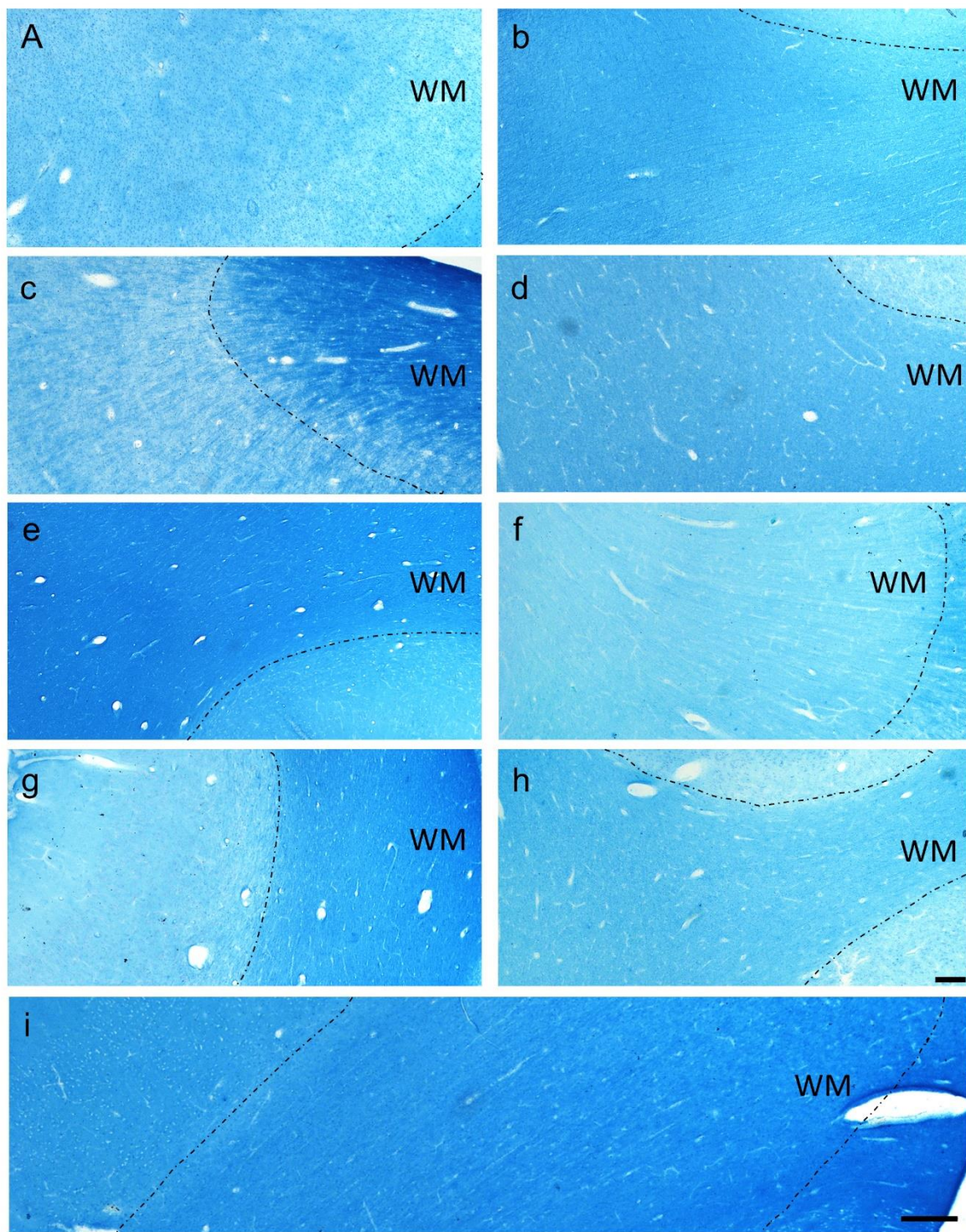


**Figure S2. Photomicrographs of controls for anti-Iba1 (a-e) and anti-GFAP (b-f) immunohistochemical stain in brains of human with late stage CTE (a, b), an old male muskox (c, d), and a male bighorn sheep (e, f).** Samples were taken from the prefrontal cortex and subjected to immunohistochemistry in the absence of Iba1 or GFAP. The same control was used for both the Alzheimer's disease and CTE human specimens. Immunolabeled images can be found in Fig. 7. Scale bar = 100  $\mu$ m.

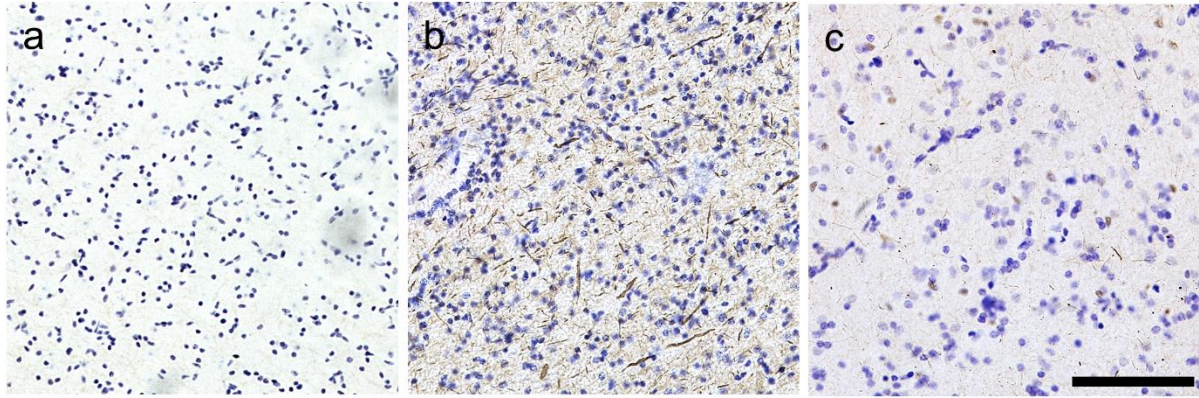


**Figure S3. Immunohistochemical staining on muskox as compared to human brain tissue.**

(a, d, g, j) Negative controls, (b, e, h, k) and light or no immunostaining in muskox prefrontal cortex, (c, f, i, l) immunostaining in the human CTE sample. (a-c) Anti-collagen IV antibody to highlight blood vessel morphology. Note immunoreactive blood vessels in (c) human tissue versus lack of labeling in (b) muskox. (d-f) Antibody A $\beta$  for presence of A $\beta$ . Note presences of plaques in (f) human tissue as opposed to (e) muskox tissue. (g-i) Antibody for degenerating myelin basic protein (dMBP). Note light immunostaining in both (h) muskox and (i) human tissue. (j-l) anti-pSer409/410 showed immunostaining in human tissue but not in muskox tissue. Scale bar = 100  $\mu$ m.



**Fig S4. Photomicrographs of Luxol Fast Blue staining in human (a, b), muskox (c-h), and bighorn sheep (i) brain tissue to characterize myelination.** (a) Human prefrontal cortex with advanced Alzheimer's disease with cloudy appearance of staining within the white matter. (b) Human superior frontal region with CTE, showing non-uniform staining in white matter. (c) Prefrontal cortex and (d) parietal regions of the old male muskox. (e) Prefrontal cortex and (f) parietal region of the middle-aged female muskox. (g) Prefrontal cortex and (h) parietal region of the old female muskox. (i) Prefrontal cortex of the male bighorn sheep. Dashed lines indicates white matter border, WM = white matter. Scale bar = 200  $\mu$ m



**Figure S5. Photomicrographs of pNFP immunoreactivity using pNFP in muskox (a, b) and human (c).** (a) Primary antibody control. (b) pNFP immunoreactivity in the old male muskox without any axonal anomalies. (c) pNFP immunoreactivity in a human sample without any axonal anomalies. Scale = 100  $\mu$ m