

## Appendix E1

### Calculation of rPMC Average MTR

MTR analyses were performed using the MINC Tool Kit (<https://www.mcgill.ca/bic/software/minc/minc toolkit>, McConnell Brain Imaging Centre, Montreal, Quebec, Canada) (30). To calculate rPMC average MTR, the intensity nonuniformity corrected (1) proton-density MR imaging without magnetization transfer prepulse (MT<sub>OFF</sub>) and T1w were linearly coregistered (2). The inverse of the resultant transformation matrix was used to transform the Freesurfer segmentation rPMC mask to the MT<sub>OFF</sub>, which was subsequently eroded using a 3-dimensional 8-connectivity kernel. rPMC average MTR was calculated within the eroded segmentation mask after eliminating all MTR values below the 2.5th percentile and above the 97.5th percentile.

### Immunostaining rPMC for Neurons

Histopathology quantification was performed using a stereological-based approach. rPMC was sampled at  $\sim 4$  cm intervals along the superior-inferior axis of the rPMC starting from the superior aspect; sections were sliced 30  $\mu$ m thick using a freezing-sliding microtome. Free-floating sections were immunostained with HuR mouse monoclonal antibody (1:1000, Santa Cruz Biotechnology, Dallas, TX) (31).

### Digitization of Immunostained Slides

Uniformity in the quality of immunostaining was ensured by using rapid-autopsy tissues that were short-fixed. De-identified immunostained slides were digitized with a Zeiss Mirax SCAN digital whole slide scanning system (Carl Zeiss MicroImaging, Jena, Germany) using a Zeiss Plan-Apochromat 20 $\times$  Objective/0.8 NA (Carl Zeiss Optronics GmbH, Oberkochen, Germany) yielding a scan resolution of 0.23  $\mu$ m/pixel in the X and Y directions. ROIs within L5 PMC were selected (the majority of neurons were required to be in the plane of the section, exhibiting cell body and apical dendrite) for neuron density quantification from the 20 $\times$  view of the digitized immunostained slide using Panoramic Viewer (3DHISTECH Ltd., Budapest, Hungary).

### Statistical Analysis

In initial statistical analyses, we performed a repeated measures analysis of variance to determine if rPMC mean MN density was significantly different when comparing the PMC subregions. To determine if rPMC mean MN density may be associated with any underlying clinical features: we calculated the Welch Two Sample T-test to compare males versus females, fALS versus sALS, bulbar versus limb onset, and presence versus absence of either C9orf72 or SOD1; and we calculated Pearson linear correlations with age at onset, age at death and disease duration. For fALS and sALS separately, we calculated Pearson linear correlations between rPMC mean MN density and all MR imaging metrics.

To determine if MR imaging metrics associated with the rPMC could estimate rPMC mean MN density, an iterative unbiased procedure using the *lm* function was performed

considering three MR imaging metrics:  $rPMC_{\text{volume}}$ ,  $rPMC_{\text{thickness}}$  and  $rPMC$  average MTR. All metrics were centered and scaled prior to linear modeling. Initially,  $rPMC$  mean MN density was fitted with straight line with a single MR imaging metric as slope and a constant. The best fit was decided by the highest adjusted  $R^2$ . Iteratively, linear models with additional MR imaging metrics (and related interaction terms) were tested (if additional MR imaging metrics were not correlated). Models were compared using the F-statistic from the “*anova*” function to determine if the additional complexity yielded a significantly improved estimation.

1. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* 1998;17(1):87–97.
2. Collins DL, Holmes CJ, Peters TM, Evans AC. Automatic 3-D Model-Based Neuroanatomical Segmentation. *Hum Brain Mapp* 1995;3(3):190–208.

**Table E1: MR Imaging Sequence Parameters**

Parameter	T1-weighted magnetization-prepared rapid acquisition gradient echo	Proton density weighted gradient recalled echo MR imaging with/without magnetization transfer prepulse
No. of partitions	176	160
Resolution (mm isotropic)	0.94	1
Field of view (mm <sup>2</sup> )	240 × 240	256 × 256
Matrix	256 × 256	256 × 256
Repetition time (msec)	1860	24
Echo time (msec)	2.8	3.8
Inversion time (msec)	1100	Not applicable
Flip angle (°)	10	10
GRAPPA acceleration factor	2	2
Magnetization transfer contrast offset frequency (Hz)	Not applicable	2000