Supplementary material for:

A potential protective role of the nuclear receptor-related factor 1 (Nurr1) in multiple sclerosis motor cortex: a neuropathological study

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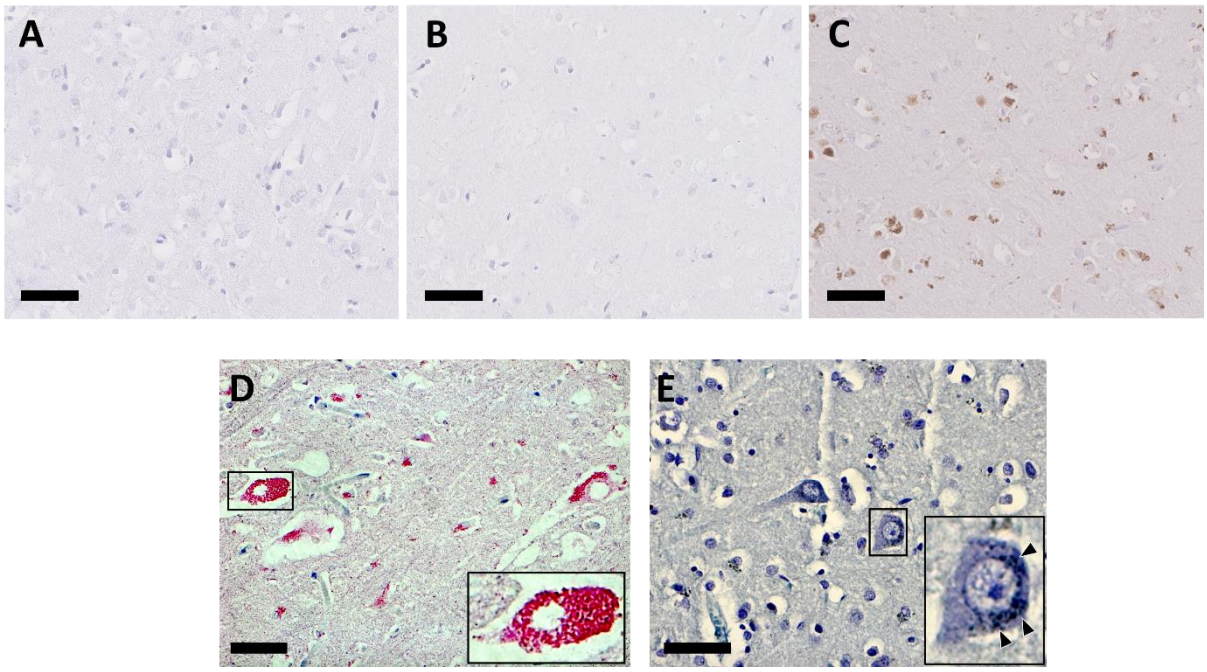
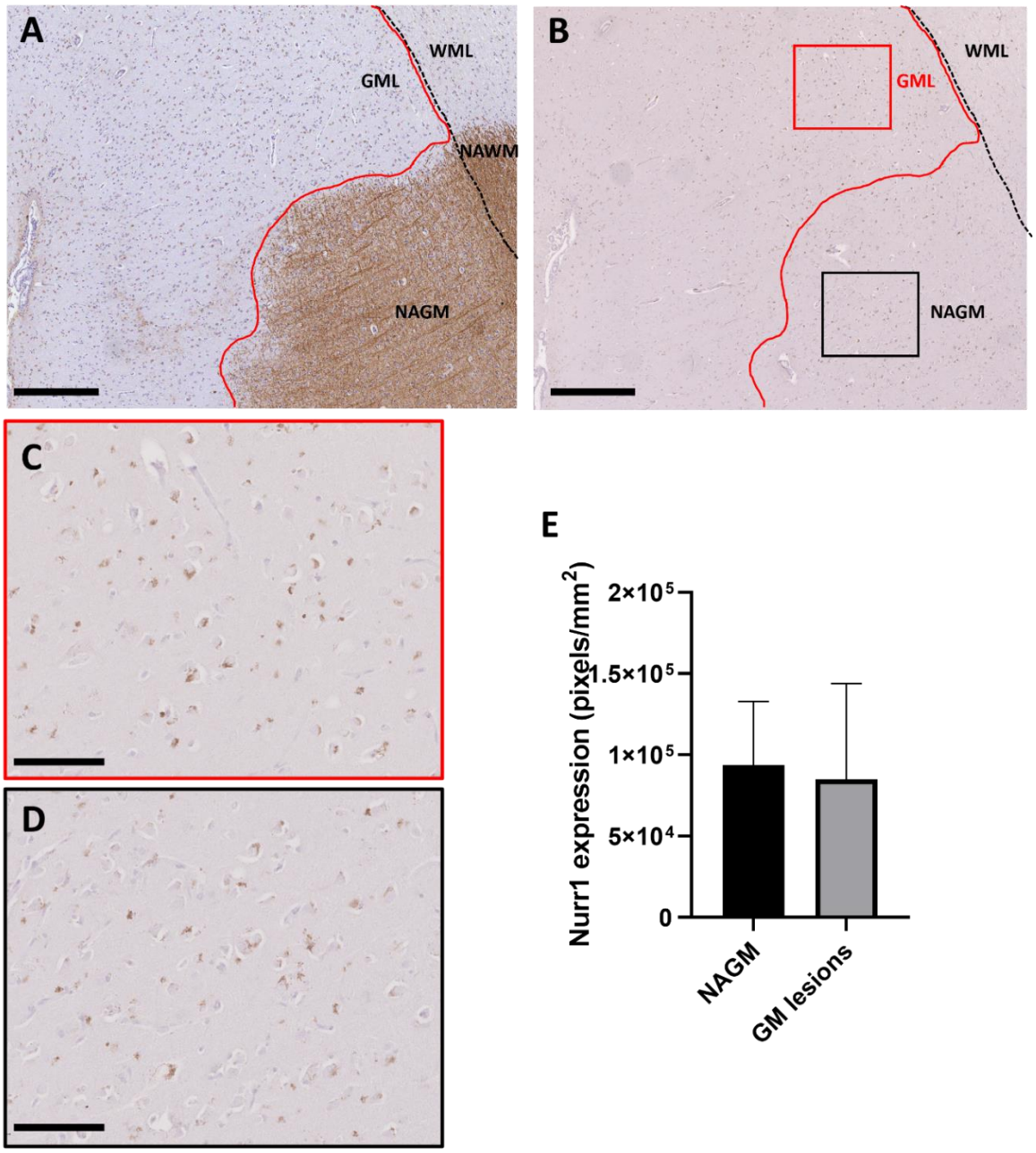
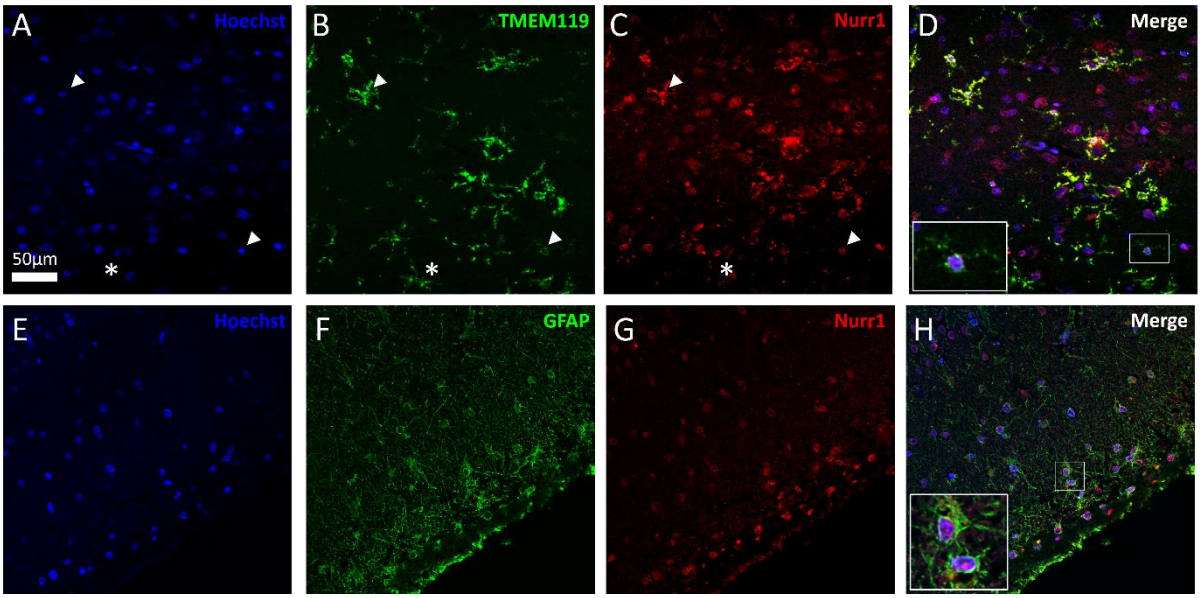
Supplementary Figures



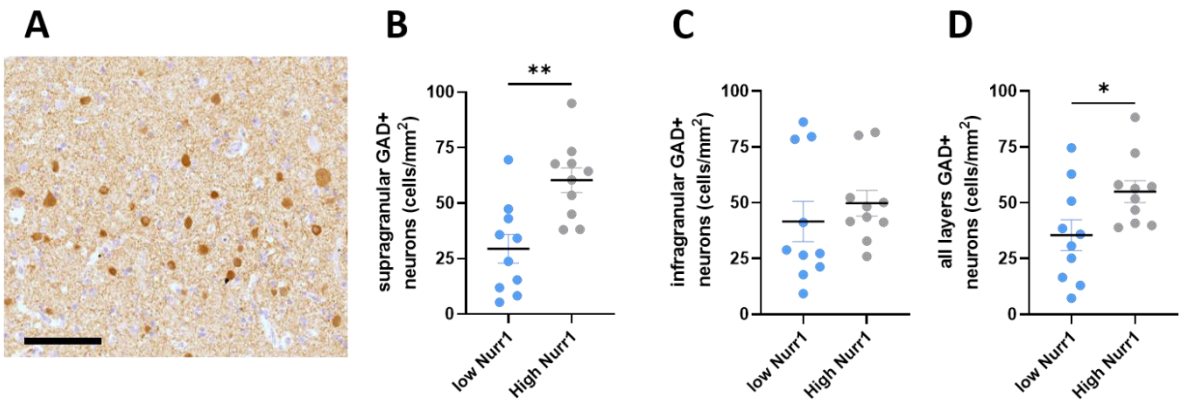
Supplementary Fig.1 Nurr1 cytoplasmic expression in neurons of cortical layer 5 from MS case. (A-C) Representative immunolabelling of Nurr1 revealed by DAB with omission of **(A)** primary or **(B)** secondary antibodies, showing no staining. **(C)** Representative immunolabelling of Nurr1 revealed by DAB staining (brown). **(D)** Representative immunolabelling of Nurr1 revealed by Vector red staining (purple) shows widely distributed, irregular and coarse granules within cytoplasm of neurons compared with **(E)** lipofuscin staining revealed by Sudan Black B staining (black, arrowheads in the insert). Scale bars 50 µm.



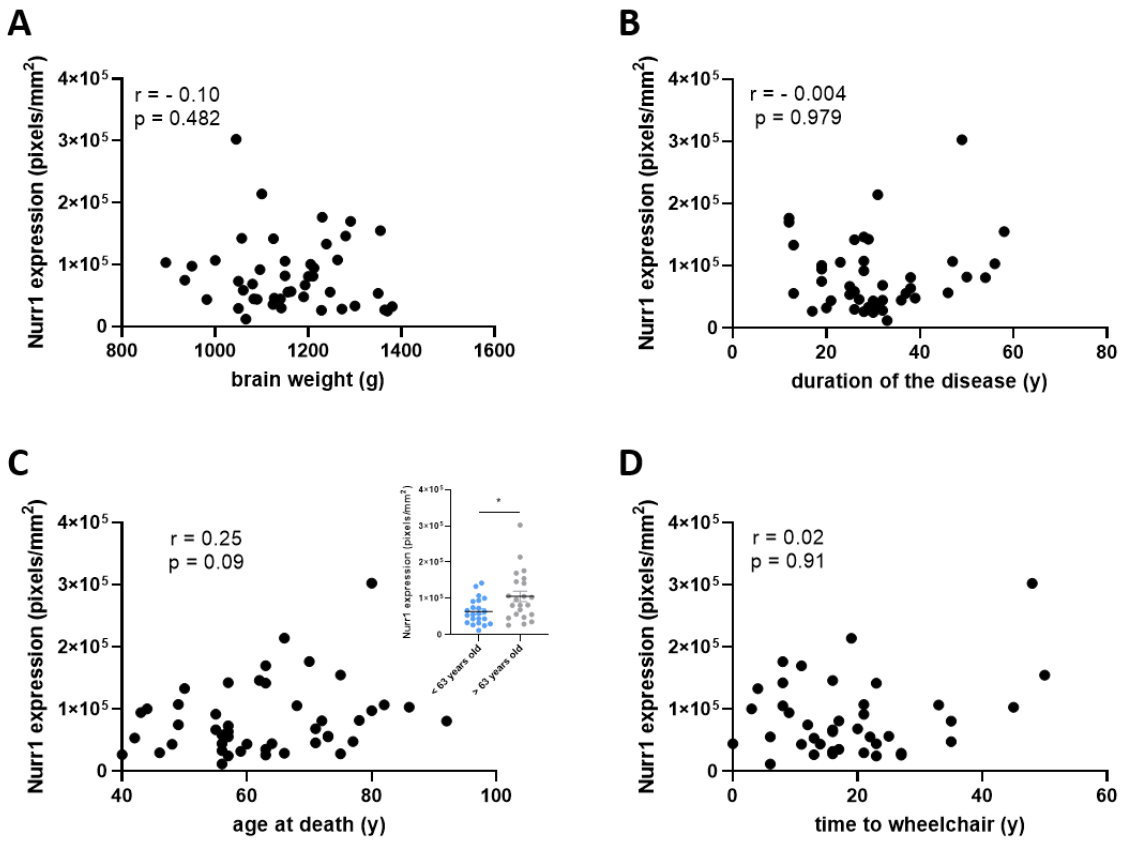
Supplementary Fig.2 No difference of Nurr1 expression comparing NAGM with GM lesions (A-D) Representative immunolabelling of **(A)** PLP and **(B)** Nurr1 revealed by DAB illustrating the demyelinated areas (red line) and corresponding Nurr1 expression, respectively (Scale bar 400µm). **(C,D)** Higher magnification of Nurr1 expression in **(C)** GM lesions and **(D)** NAGM, illustrating the absence of difference in Nurr1 expression in NAGM and GM lesions (Scale bar 100µm). **(E)** No differences in Nurr1 expression comparing NAGM and GM lesions was found in MS cases showing large demyelinated areas (Wilcoxon test, n=18, p = 0.09). (NAWM = normal-appearing white matter ; NAGM = normal-appearing grey matter ; WML = white matter lesion ; GML = grey matter lesion ; border between WM and GM is delineated by dotted line).



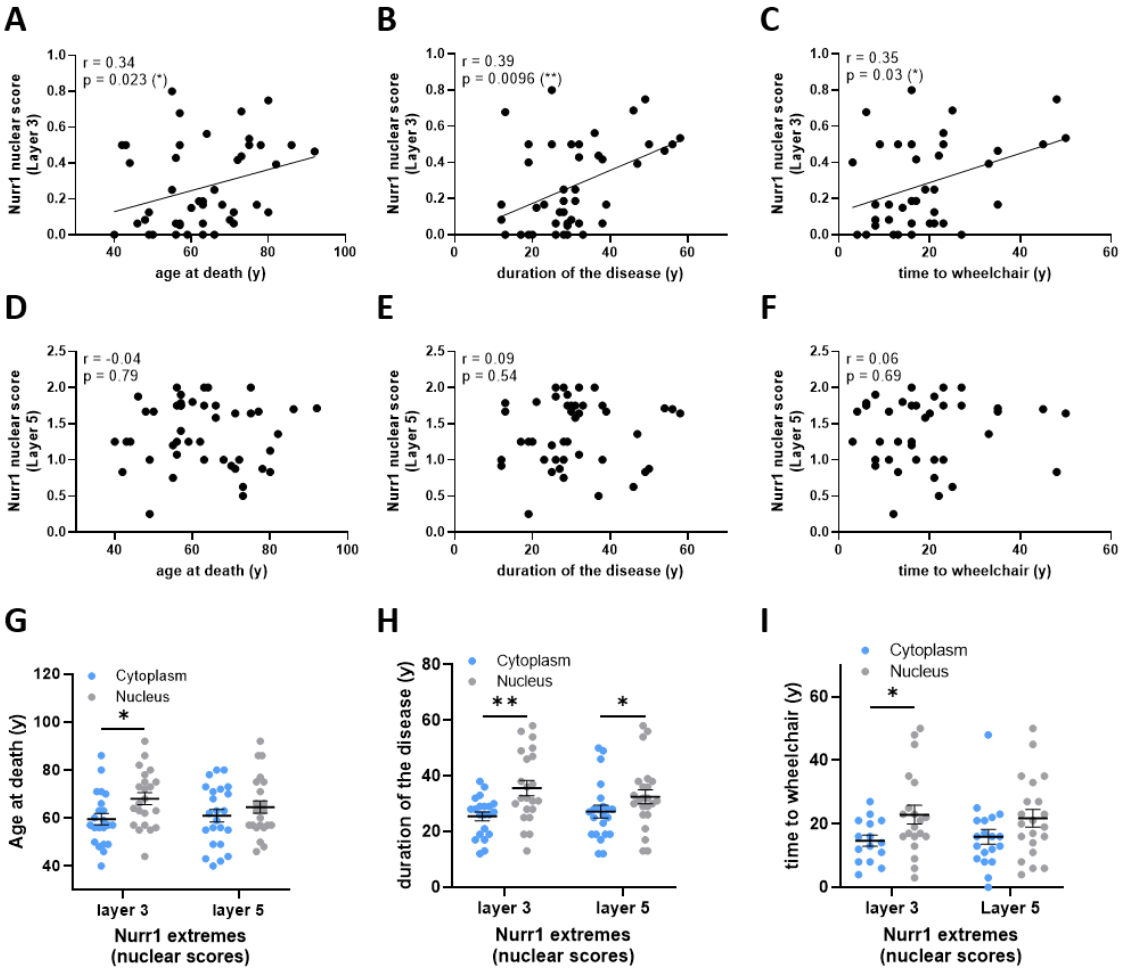
Supplementary Fig. 3. Co-localisation of Nurr1 with glial cells. (A-D) Confocal images of layer 3 microglia in MS case are labelled for nucleus (Hoechst, blue, **A**), microglia (TMEM119, green, **B**), and Nurr1 (Nurr1, red, **C**) at 400x magnification, and representative merge image **(D).** Arrowheads indicates co-labelling of Nurr1 with TMEM119+ microglia, while asterisk illustrate TMEM119+ microglia without Nurr1 co-localisation. Intra-cellular Nurr1 is illustrated in the insert. **(E-F)** Confocal images of layer 1-2 astrocytes in MS case are labelled for nucleus (Hoechst, blue, **E**), astrocytes (GFAP, green, **F**), and Nurr1 (Nurr1, red, **G**) at 400x magnification, and representative merge image **(H).** Nuclear and cytoplasmic Nurr1 is illustrated in the insert.



Supplementary Fig. 4. Relationships between Nurr1 expression and GAD+ neuronal density in MS cases lying at the lowest extremes of Nurr1 expression. (A) Representative labelling of GAD+ neurons using DAB immunostaining in supragranular layer 3 in MS. **(B-D)** Further assessment shows increased density of GAD+ neurons in the MS subgroup with high levels of Nurr1, a finding restricted to supragranular layers. (Mann-Whitney test, data presented as mean ± SEM.* $p < 0.05$, **$p < 0.01$; Scale bar 100 µm).



Supplementary Fig. 5. Relationship of Nurr1 expression with clinical features in MS. No correlation was found between Nurr1 expression and **(A)** brain weight, **(B)** duration of the disease, **(C)** age at death and **(D)** time to wheelchair. However, Nurr1 expression in NAGM is greater in cases older than median age (insert in **C,** Mann-Whitney test, p = 0.019). (Spearman rank-correlation coefficient was used in A-D ; * $p < 0.05$)



Supplementary Fig. 6. Relationship of Nurr1 neuronal localisation with clinical features in MS. (A-C) Correlation was found between Nurr1 nuclear score in layer 3 and age at death, duration of the disease and time to wheelchair (Spearman rank-correlation coefficients), while **(D-F)** no correlation was found between Nurr1 nuclear score in layer 5 and these features (Spearman rank-correlation coefficients). **(G)** Only Nurr1 preferential nuclear location in layer 3 show increased age at death (Mann-Whitney test ; layer 3 : $p = 0.019$; layer 5 : $p = 0.36$), while **(H)** both preferential Nurr1 nuclear location in layer 3 and layer 5 show increased duration of the disease (Mann-Whitney test ; layer 3: $p = 0.006$; layer 5 : $p = 0.03$), and **(I)** only Nurr1 nuclear location in layer 3 show increased time to wheelchair (Mann-Whitney test ; layer 3: $p = 0.044$; layer 5 : $p = 0.12$). (* $p < 0.05$, ** $p < 0.01$)