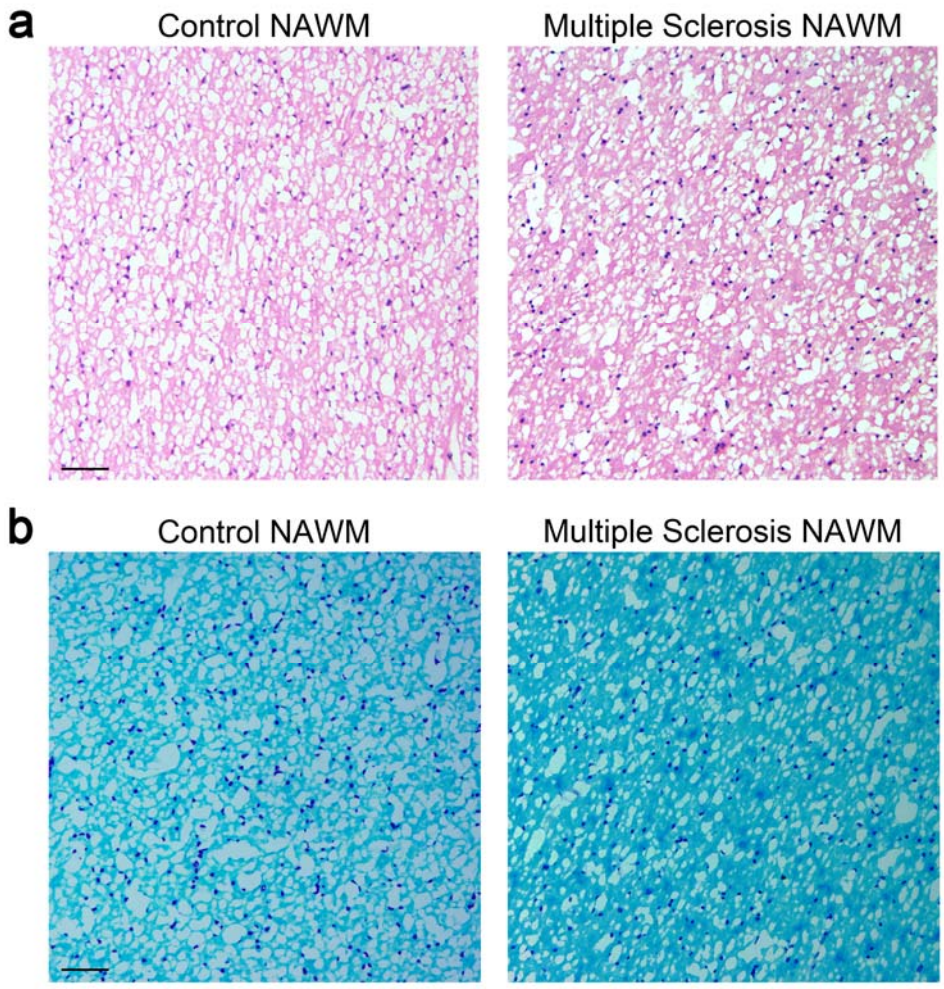
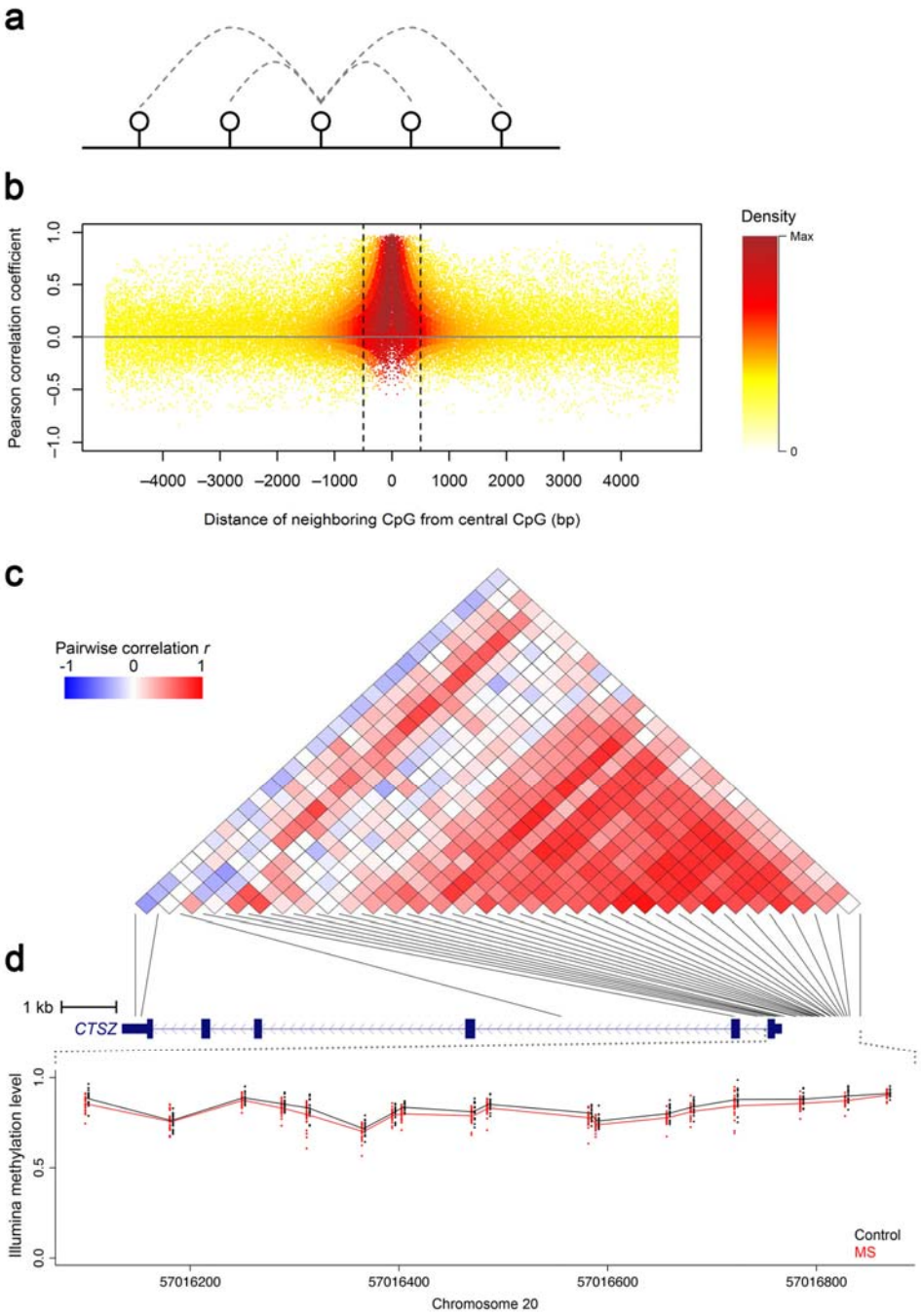
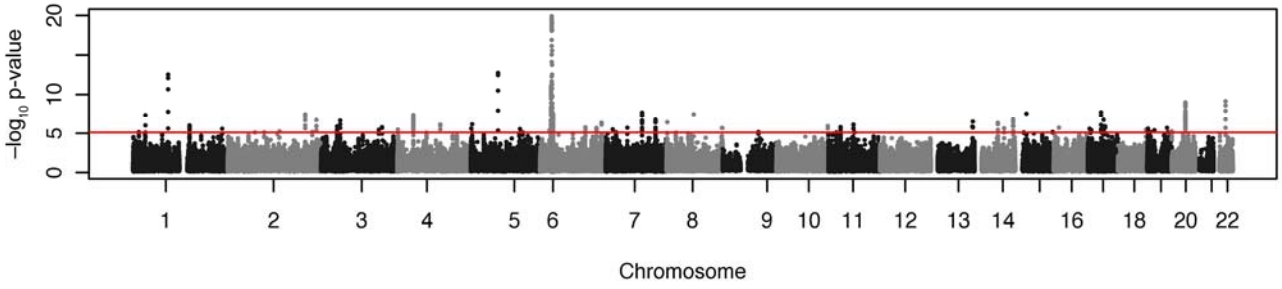


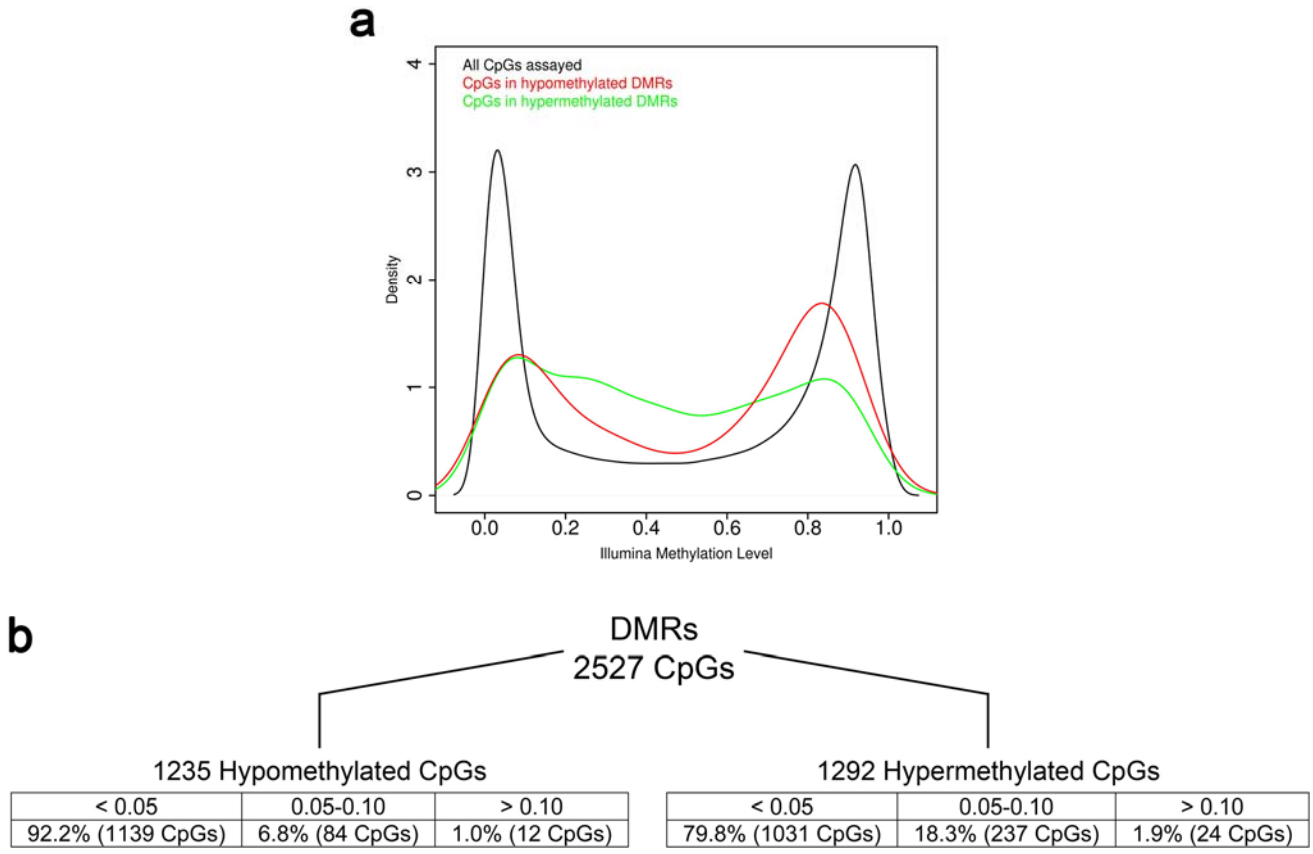
Supplementary Figure 1. Hematoxylin and eosin and Luxol fast blue staining shows absence of overt inflammation and demyelinating lesions. (a) Representative hematoxylin and eosin staining for control (n=5 brain samples) and multiple sclerosis (n=15 brain samples) sections shows an absence of inflammatory infiltrates. (b) Representative Luxol fast blue staining for control (n=5 brain samples) and multiple sclerosis (n=15 brain samples) sections shows an absence of demyelinating lesions. Scale bar = 100 μm. NAWM, normal-appearing white matter.



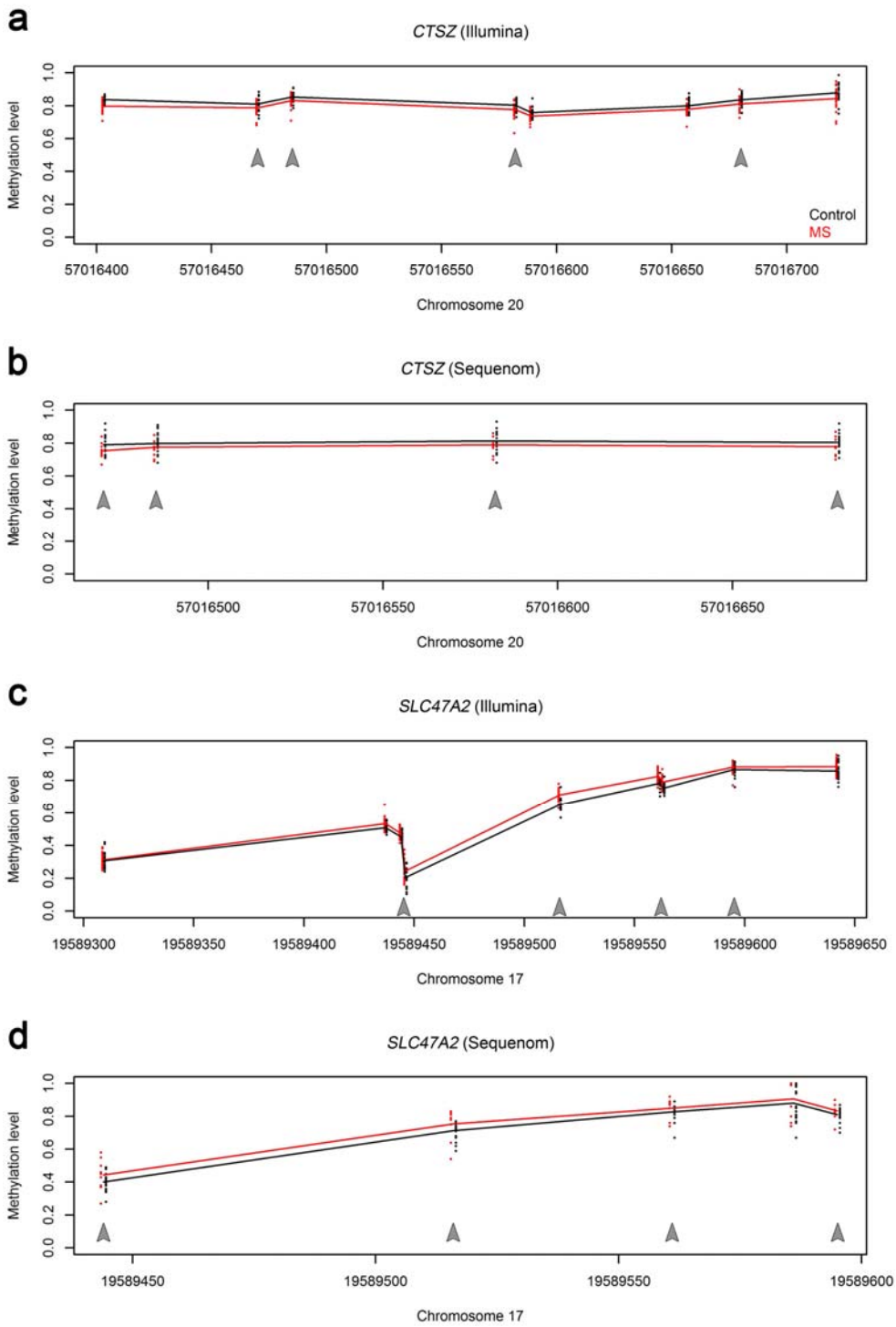
Supplementary Figure 2. Pairwise comparison of neighboring CpGs shows high correlation within a 1-kb window. (a) Schematic of pairwise comparison between the central CpG within DMRs and neighboring CpGs. (b) A ±5 kb window was extended around each CpG within the DMRs. Pearson correlation was calculated for each CpG (central CpG) and the neighboring CpGs within each respective window. Vertical dashed lines demarcate 1-kb window (±500 bp) around the central CpG, which shows the strongest level of correlation. Heatmap shows density of correlations. (c) DNA methylation block shows pairwise Pearson correlation between CpGs in the promoter and gene body of *CTSZ*. Lines connect with the gene structure below at the location of the corresponding CpG. (d) Plot of DNA methylation levels for individual CpGs within a hypomethylated DMR in the promoter of *CTSZ* as measured by Illumina array. Above the plot is the complete gene structure, with dashed lines indicating the zoomed region containing the DMR shown in the plot. Methylation levels for individual MS cases and controls are plotted in red and black, respectively, with red and black lines connecting the mean methylation level for each consecutive CpG assayed. MS, multiple sclerosis.



Supplementary Figure 3. Minimal association of disease duration with methylation levels in normal-appearing white matter. The x-axis shows autosomal chromosomes and y-axis the $-\log_{10}$(p-value). Each dot represents nominal Fisher's combined p-value for CpGs within a 1-kb sliding window, where individual p-values were generated using the correlation between disease duration and methylation across the genome. The red line denotes 5% FDR.



Supplementary Figure 4. Distribution of methylation difference between multiple sclerosis and controls reveal most changes to be subtle. (a) Density plots based on β-values obtained from 461,272 autosomal CpGs show a bimodal distribution of DNA methylation levels in the genome (black). Density plots of CpGs within hypomethylated (red) and hypermethylated (green) DMRs show a significantly different distribution ($p<10^{-10}$, Kolmogorov-Smirnov test), with an excess of intermediate methylation levels. (b) Difference taken as the β-value average of MS minus the β-value average of controls. Distribution of methylation level difference for each CpG identified in differentially methylated regions are divided into three categories: <0.05, 0.05–0.10, or >0.10.



Supplementary Figure 5. Validation of changes in DNA methylation between multiple sclerosis and controls in an independent cohort of samples. (a) Plot of DNA methylation levels for individual CpGs within a window of the *CTSZ* DMR as measured by Illumina array of the discovery cohort. Methylation levels for individual MS cases and controls are plotted in red and black, respectively, with red and black lines connecting the mean methylation level for each consecutive CpG assayed. Arrowheads indicate CpGs that were assayed in a second cohort (b). (b) Methylation levels for individual CpGs assessed using MassARRAY EpiTYPER of a second cohort. Plots are as shown in (a) for the CpGs marked by arrowheads. Arrowheads indicate CpGs with corresponding Illumina probes in (a). (c–d) Same as described in (a,b) for a window of the *SLC47A2* DMR. MS, multiple sclerosis.

Supplementary Table 1. Description of MS and non-neurological control samples assessed with Illumina Infinium HumanMethylation450 BeadChip.

Patient ID	Gender	Age	MS type	MS duration (yrs)	Post-mortem interval (hrs)	Additional Diagnosis
MS4218	F	63	CP	18	15	Cerebral atherosclerosis, chronic UTI
MS3931	F	74	PP	11	9.5	Pneumonia
MS2771	F	64	SP	33	17.3	
MS377	F	50	SP	NA	22	Pneumonia
MS094	F	42	PP	6	11	Bronchopneumonia
MS088	F	54	SP	NA	22	Bronchopneumonia
MS342	F	35	SP	5	9	
MS076	F	49	SP	18	31	Chronic renal failure, heart disease
MS058	F	51	SP	21	15	
MS4201	F	75	CP	29	13.8	Chronic UTI, HPN, hypothyroidism, migraine
MS3010	F	50	SP	24	15	
MS062	F	49	SP	19	10	Respiratory infection
MS2765	F	51	CP	32	9	
MS109	F	60	SP	25	22	Cerebral atherosclerosis, MI
MS3840	F	61	PP	17	22.8	Chronic UTI, Pneumonia
MS093	F	57	SP	26	25	Pneumonia
MS231	F	59	PP	27	12	Bronchopneumonia
MS273	M	61	PP	31	24	Septicemia, UTI
MS097	M	55	SP	22	31	Bronchopneumonia
MS286	M	45	SP	16	7	
MS2946	M	59	PP	12	15	Dementia
MS3502	M	78	SP	52	15.5	Bell's Palsy, CVA, seizure disorder
MS100	M	46	SP	8	7	Acute polyarthritis, peptic ulceration, pneumonia
MS141	M	NA	NA	NA	NA	
MS083	M	54	PP	16	13	Bronchopneumonia
MS060	M	55	SP	43	16	
MS122	M	44	SP	10+	16	Bronchopneumonia
MS104	M	53	SP	11	12	UTI
N3175	F	54	-	-	21.5	Hypothyroidism, pancytopenia, T1DM
N3348	F	76	-	-	9	CHD, HPN, T1DM
PDC08	F	71	-	-	NA	Cerebral atherosclerosis
N3406	F	72	-	-	20.1	Breast cancer, CHF, TSC
N3482	F	79	-	-	14	CHD, HPN
N3558	F	59	-	-	19.5	Non-Hodgkin's lymphoma
N3543	F	73	-	-	12	COPD
CO14	M	64	-	-	18	MI, TIA
PDC05	M	58	-	-	NA	Cervical ependymoma
CO51	M	68	-	-	24	IHD
N3276	M	54	-	-	19	CHD, CKD, PVD

N3602	M	66	-	-	13.1	Metastatic larynx cancer, T1DM
CO36	M	68	-	-	30	CAD, cor pulmonale, IPF
N3529	M	58	-	-	9	Colon cancer
N3611	M	64	-	-	17.5	CAD, lymphoma
N3535	M	81	-	-	14	Non-Hodgkin's lymphoma
N3606	M	71	-	-	11.5	COPD
N3589	M	53	-	-	15	Melanoma
PDC022	M	71	-	-	NA	

CP=chronic progressive. PP=primary progressive. SP=secondary progressive. NA=not available. UTI=urinary tract infection. HPN=hypertension. MI=myocardial infarction. CVA=cerebral vascular accident. T1DM=type 1 diabetes mellitus. CHD=coronary heart disease. CHF=congestive heart failure. TSC=tuberous sclerosis complex. COPD=chronic obstructive pulmonary disease. TIA=transient ischemic attack. IHD=ischemic heart disease. CKD=chronic kidney disease. PVD=peripheral vascular disease. CAD=coronary artery disease. IPF=idiopathic pulmonary fibrosis.

Supplementary Table 4. Description of MS and non-neurological control samples used for independent validation.

Patient ID	Gender	Age	MS type	MS duration (yrs)	Post-mortem interval (hrs)	Additional Diagnosis
M1	F	70	SP	45	4.3	
M2	F	51	SP	25	5.5	
M4	F	46	SP	14	5.6	
M5	F	56	SP	27	4.5	
M7	F	66	SP	35	13.3	
M11	F	65	SP	10	9.2	
M12	F	73	SP	46	7	
M3	M	52	SP	25	4.8	
M8	M	61	SP	6	9.3	
M10	M	53	SP	13	17.7	
N19	F	48	-	-	28.8	
N21	F	43	-	-	28	
N25	F	66	-	-	22.6	
N27	F	68	-	-	24	
N28	F	73	-	-	28.3	
N41	F	73	-	-	3	
N42	F	67	-	-	16.5	
N49	F	61	-	-	25.1	
N6	F	64	-	-	19.1	
N69	F	60	-	-	14.9	
N7	F	73	-	-	3.4	
N71	F	45	-	-	32	
N77	F	52	-	-	24.3	
N83	F	66	-	-	16	
N53	M	53	-	-	20.2	
N55	M	51	-	-	13.8	
N58	M	53	-	-	15.2	
N64	M	53	-	-	17.5	
N79	M	61	-	-	24.1	
N8	M	61	-	-	18.8	

SP=secondary progressive.

Supplementary Table 5. Description of MS and non-neurological control samples used for independent validation.

Patient ID	Gender	Age	MS type	MS duration (yrs)	Post-mortem interval (hrs)	Additional Diagnosis
MS1	F	61	SP	NA	10	
MS2	M	46	SP	NA	3	
MS3	M	49	RR	NA	18.8	
MS4	F	57	PP	NA	5.5	
MS5	M	56	SP	NA	3.1	
C1	F	47	-	-	15	
C2	M	53	-	-	12	
C3	M	77	-	-	12	

SP=secondary progressive. RR=relapsing remitting. PP=primary progressive.