Supplementary materials

Supplementary methods:

Lesion segmentation

White matter lesions were first manually marked by putting a cursor into the lesion and were then semi-automatically segmented using intensity thresholding with Amira 3.1.1 (Mercury Computer System Inc.). Manual adjustments were performed when necessary. The lesions were marked on proton density weighted images, while the according slices of T2-weighted (T2w) images were displayed in parallel to confirm the lesion site and extent. All raters undergo a training period with consecutive reliability testing before working on any study. Reliability is retested in all raters at fixed intervals (once a year) to ensure a consistently high quality of lesion marking and segmentation. After lesion marking and segmentation, a final quality control step included the verification of all segmentations by a radiologist.[1]

Image processing - Registering to the Montreal Neurological Institute (MNI) space

First, the lesion masks were transformed to T1-weighted (T1w) coordinates, applying the transformation parameters resulting from linearly registering the T2w image to the T1w image.[2, 3] These registered lesion masks were used to perform lesion filling on T1w images[4] to reduce the effect on the next step.[5] Then, a two-stage linear and nonlinear registration[6] was carried out to align the T1w images to the MNI152 standard brain

template (with a resolution of 2x2x2 mm^3). The obtained transformation matrices were applied to the lesion masks previously registered on the T1w image. Quality assessment of the results was performed by two independent MRI experts and, in case of disagreement, a consensus was reached by involving a third expert. Patients were excluded if failures of the registration process were identified.

Supplementary Table e-1. Percentage of patients with lesions at Baseline and with new and enlarging lesions at M24 by location.

Brain regions		**Baseline** (%)	**M24** (%)
BR	Superior corona radiata	83.4	52.3
	Anterior corona radiata	77.5	42.3
	Superior longitudinal fasciculus	79.7	45.9
	Body of CC	75.5	43.9
	Splenium of CC	74.1	39.0
	External capsule	42.4	19.4
	Posterior limb of internal capsule	38.9	19.6
	Genu of CC	61.2	29.1
	Posterior thalamic radiation	82.8	47.5
	Posterior corona radiata	85.5	51.0
	Anterior limb of internal capsule	36.8	19.1
	Retrolenticular part of internal capsule	59.4	31.5
	Sagittal stratum	56.5	26.4
	Cingulum (cingulate gyrus)	15.9	10.1

	Fornix (cres) / Stria terminalis	10.6	5.9
	Cingulum (hippocampus)	5.7	1.6
	Superior fronto-occipital fasciculus	39.1	20.5
	Uncinate fasciculus	3.1	1.4
	Tapetum	67.9	32.7
CR	Middle cerebellar peduncle	20.1	13.5
	Inferior cerebellar peduncle	4.3	2.0
	Superior cerebellar peduncle	2.2	1.4
BS	Cerebral peduncle	12.1	4.7
	Corticospinal tract	9.5	5.2
	Pontine crossing tract	4.6	4.1
	Medial lemniscus	5.7	3.8

BR, supratentorial brain; BS, brainstem; CC, corpus callosum; CR, cerebellum; M, month

Supplementary Table e-2. Definition of disability worsening.

Disability scores	**Worsening**
PASAT	$\begin{cases} 1 \; if \; \dfrac{(Value_{Month\ 24} - Value_{Baseline}) - (Mean_{Month\ 24} - Mean_{Baseline})}{SD_{Month\ 24-Baseline}} \leq -1 \\ 0 \; otherwise \end{cases}$
T25FWT	$\begin{cases} 1 \; if \; (Value_{Month\ 24} - Value_{Baseline} \geq 20\% Value_{Baseline} \\ 0 \; otherwise \end{cases}$
NHPT	$\begin{cases} 1 \; if \; (Value_{Month\ 24} - Value_{Baseline} \geq 20\% Value_{Baseline} \\ 0 \; otherwise \end{cases}$
EDSS	6-month confirmed disability progression [7]
EDSS subscores	$\begin{cases} 1 \; if \; (EDSS_{Month\ 24} - EDSS_{Baseline}) \geq 0.5 \; AND \; (Value_{Month\ 24} - Value_{Baseline}) \geq 1 \\ 0 \; otherwise \end{cases}$

EDSS, Expanded Disability Status Scale; NHPT, 9-Hole Peg Test; PASAT, Paced Auditory Serial Addition Test; SD, standard deviation; T25FWT, Timed 25-Foot Walk Test

Supplementary Table e-3. Bootstrap analysis results: Percentage of bootstrap samples with significant and stronger association between pre-existing baseline lesions the specific brain region and the disability score than between the 'average whole brain lesion' and the disability score that were re-confirmed: values are between 0-100%, higher numbers indicate better reproducibility of the association.

		PASAT	T25FWT	NHPT	EDSS	EDSS-BB	EDSS-BS	EDSS-CB	EDSS-CE	EDSS-PY	EDSS-SE	EDSS-VI
BR	Frontal	4	9	1	0	0	0	0	0	4	0	29
	Sublobar	88	93	96	96	75	78	91	79	2	22	46
	Temporal	0	3	0	0	0	0	0	0	1	1	0
	Parietal	37	0	1	1	2	14	4	41	0	13	0
	Limbic	12	6	6	56	15	1	76	8	81	56	0
	Occipital	4	3	38	40	4	61	3	9	15	1	63
CR	Posterior	40	2	10	29	12	12	10	17	11	2	2
	Anterior	0	1	0	1	3	1	2	3	0	2	0
BS	Pons	55	0	2	9	46	12	31	5	10	2	6
	Midbrain	7	8	45	31	1	20	4	11	24	2	7
	Medulla	2	39	0	3	5	80	1	21	11	2	32

BR, supratentorial brain; BS, brainstem; CR, cerebellum; EDSS, Expanded Disability Status Scale; EDSS-BB, EDSS bowel and bladder; EDSS-BS, EDSS brainstem; EDSS-

CB, EDSS cerebellar; EDSS-CE, EDSS cerebral; EDSS-PY, EDSS pyramidal; EDSS-SE, EDSS sensory; EDSS-VI, EDSS visual; NHPT, 9-Hole Peg Test; PASAT, Paced Auditory Serial Addition Test; T25FWT, Timed 25-Foot Walk Test.

Supplementary Table e-4. Bootstrap analysis results: Percentage of bootstrap samples with significant and stronger association between NE lesions in the specific brain region and the disability score than between the 'average whole brain lesion' and the disability score that were re-confirmed: values are between 0-100%, higher numbers indicate better reproducibility of the association.

		PASAT	T25FWT	NHPT	EDSS	EDSS-BB	EDSS-BS	EDSS-CB	EDSS-CE	EDSS-PY	EDSS-SE	EDSS-VI
BR	Frontal	11	3	0	1	46	0	9	3	18	7	1
	Sublobar	5	4	0	75	4	9	47	5	77	11	4
	Temporal	22	54	4	2	13	35	0	16	2	1	7
	Parietal	21	10	39	10	1	0	4	10	0	0	0
	Limbic	5	1	3	0	3	70	2	20	0	12	1
	Occipital	0	1	4	0	0	0	4	0	3	31	0
CR	Posterior	0	1	1	0	37	0	13	1	0	1	9
	Anterior	1	5	18	0	0	0	0	9	1	1	0
BS	Pons	65	1	33	0	6	17	1	15	0	3	30
	Midbrain	3	2	16	8	16	38	25	0	0	46	0
	Medulla	1	28	6	38	27	0	46	13	42	2	0

BR, supratentorial brain; BS, brainstem; CR, cerebellum; EDSS, Expanded Disability Status Scale; EDSS-BB, EDSS bowel and bladder; EDSS-BS, EDSS brainstem; EDSS-

CB, EDSS cerebellar; EDSS-CE, EDSS cerebral; EDSS-PY, EDSS pyramidal; EDSS-SE, EDSS sensory; EDSS-VI, EDSS visual; NHPT, 9-Hole Peg Test; PASAT, Paced Auditory Serial Addition Test; T25FWT, Timed 25-Foot Walk Test.

Supplementary Table e-5. Bootstrap analysis results: Percentage of bootstrap samples with significant and stronger association between pre-existing baseline lesions in the specific white matter tract and the disability score than between the 'average whole brain lesion' and the disability score that were re-confirmed: values are between 0-100%, higher numbers indicate better reproducibility of the association.

	PASAT	T25FWT	NHPT	EDSS	EDSS-BB	EDSS-BS	EDSS-CB	EDSS-CE	EDSS-PY	EDSS-SE	EDSS-VI
Superior corona radiata	30	4	1	8	1	19	6	0	18	2	7
Anterior corona radiata	4	2	20	1	2	1	0	1	0	9	2
Superior longitudinal fasciculus	2	2	1	0	0	1	0	0	1	0	19
Body of CC	0	2	16	0	0	0	27	1	5	1	0
Splenium of CC	7	1	54	92	37	38	58	55	44	33	3
External capsule	4	5	10	2	4	0	18	22	0	6	0
Posterior limb of internal capsule	30	11	59	2	23	16	11	6	0	6	2
Genu of CC	6	0	3	15	3	17	25	23	20	12	29
Posterior thalamic radiation	21	23	3	2	1	7	4	0	18	1	27

Posterior corona radiata	18	41	52	48	10	17	16	64	3	15	0
Anterior limb of internal capsule	29	19	13	3	14	36	2	0	3	0	18
Retrolenticular part of internal capsule	2	7	26	14	0	11	1	24	2	2	8
Sagittal stratum	1	6	2	4	9	9	4	1	28	0	3
Cingulum (cingulate gyrus)	14	3	4	6	4	44	17	0	19	3	3
Fornix (cres) / Stria terminalis	11	6	5	10	0	2	26	0	5	9	13
Cingulum (hippocampus)	1	0	0	0	0	0	0	1	0	2	1
Superior fronto-occipital fasciculus	14	3	0	1	30	0	0	91	0	37	1
Uncinate fasciculus	6	13	0	0	12	44	0	21	0	1	0
Tapetum	1	5	0	37	38	11	40	1	0	13	21
Middle cerebella	17	0	0	1	38	3	6	7	3	0	0

r peduncle											
Inferior cerebellar peduncle	0	3	3	4	4	8	3	16	0	9	1
Superior cerebellar peduncle	0	4	0	0	0	2	7	0	3	1	4
Cerebral peduncle	8	1	48	32	17	11	3	47	23	1	9
Corticospinal tract	18	8	1	1	5	3	1	2	22	13	4
Pontine crossing tract	9	0	6	5	0	9	5	16	0	1	3
Medial lemniscus	3	16	2	15	58	49	3	2	5	11	35

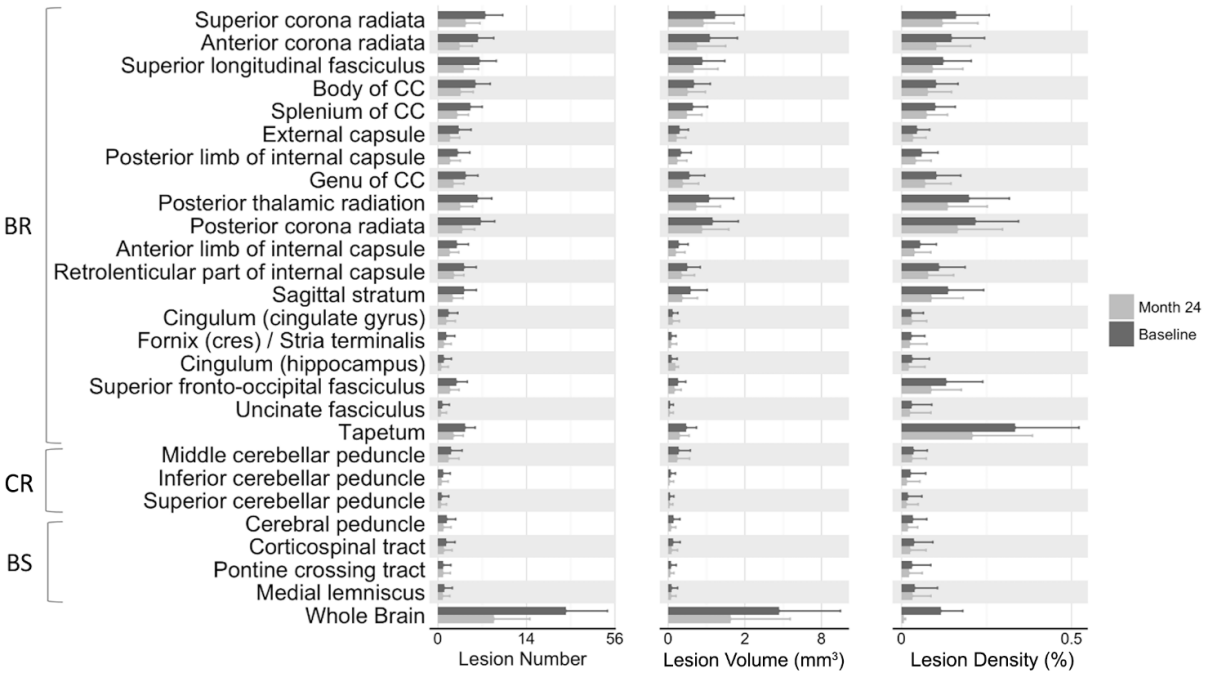
CC, corpus callosum; EDSS, Expanded Disability Status Scale; EDSS-BB, EDSS bowel and bladder; EDSS-BS, EDSS brainstem; EDSS-CB, EDSS cerebellar; EDSS-CE, EDSS cerebral; EDSS-PY, EDSS pyramidal; EDSS-SE, EDSS sensory; EDSS-VI, EDSS visual; NHPT, 9-Hole Peg Test; PASAT, Paced Auditory Serial Addition Test; T25FWT, Timed 25-Foot Walk Test

Supplementary Table e-6. Bootstrap analysis results: Percentage of bootstrap samples with significant and stronger association between NE lesions in the specific white matter tract and the disability score than between the 'average whole brain lesion' and the disability score that were re-confirmed: values are between 0-100%, higher numbers indicate better reproducibility of the association.

	PASAT	T25FWT	NHPT	EDSS	EDSS-BB	EDSS-BS	EDSS-CB	EDSS-CE	EDSS-PY	EDSS-SE	EDSS-VI
Superior corona radiata	6	12	22	15	15	38	39	17	42	30	7
Anterior corona radiata	38	3	21	5	17	35	6	7	44	3	33
Superior longitudinal fasciculus	22	7	1	0	0	15	1	13	0	0	1
Body of CC	13	1	1	0	1	1	1	3	0	4	7
Splenium of CC	86	24	3	33	41	59	26	24	8	2	6
External capsule	0	11	0	23	4	2	0	0	13	6	19
Posterior limb of internal capsule	11	2	7	5	6	53	5	29	3	0	3
Genu of CC	0	1	1	2	2	0	3	0	0	8	1
Posterior thalamic radiation	0	60	10	4	4	0	2	2	2	27	3
Posterior corona radiata	0	0	27	14	9	0	12	1	33	1	0
Anterior limb of internal capsule	4	2	11	1	2	0	3	0	0	8	0
Retrolenticular part of internal capsule	0	10	1	2	1	7	24	5	0	16	0
Sagittal stratum	27	21	19	11	25	77	12	2	54	1	1
Cingulum (cingulate gyrus)	0	40	56	34	1	1	34	12	48	30	0
Fornix (cres) / Stria terminalis	3	1	6	55	16	20	32	37	38	2	0

Superior fronto-occipital fasciculus	2	6	1	32	28	10	45	47	15	0	24
Tapetum	44	4	2	15	0	26	16	0	4	31	1
Middle cerebellar peduncle	34	3	76	0	4	0	13	4	7	8	4
Inferior cerebellar peduncle	0	12	65	20	12	19	33	13	13	34	2
Cerebral peduncle	8	2	5	1	14	29	22	0	0	44	0
Corticospinal tract	2	33	5	60	44	2	11	13	9	4	19
Pontine crossing tract	57	1	2	0	0	44	7	2	13	23	2
Medial lemniscus	0	14	0	1	14	1	10	2	4	4	1

CC, corpus callosum; EDSS, Expanded Disability Status Scale; EDSS-BB, EDSS bowel and bladder; EDSS-BS, EDSS brainstem; EDSS-CB, EDSS cerebellar; EDSS-CE, EDSS cerebral; EDSS-PY, EDSS pyramidal; EDSS-SE, EDSS sensory; EDSS-VI, EDSS visual; NHPT, 9-Hole Peg Test; NE, new and enlarging; PASAT, Paced Auditory Serial Addition Test; T25FWT, Timed 25-Foot Walk Test



Supplementary Figure e-1. The distribution of all T2 lesions at baseline (Baseline), and of new or enlarging lesions between baseline and M24 (Month 24).

The mean and standard deviation of the lesion number, volume and density in each brain region and in the whole brain at Baseline (i.e. pre-existing lesions at study entry) and at M24 (i.e. new or enlarging lesions between baseline and Month 24) are presented. The majority of the lesions at both time points were concentrated in the WM tracts in the supratentorial brain, rather than in the CR and BS.

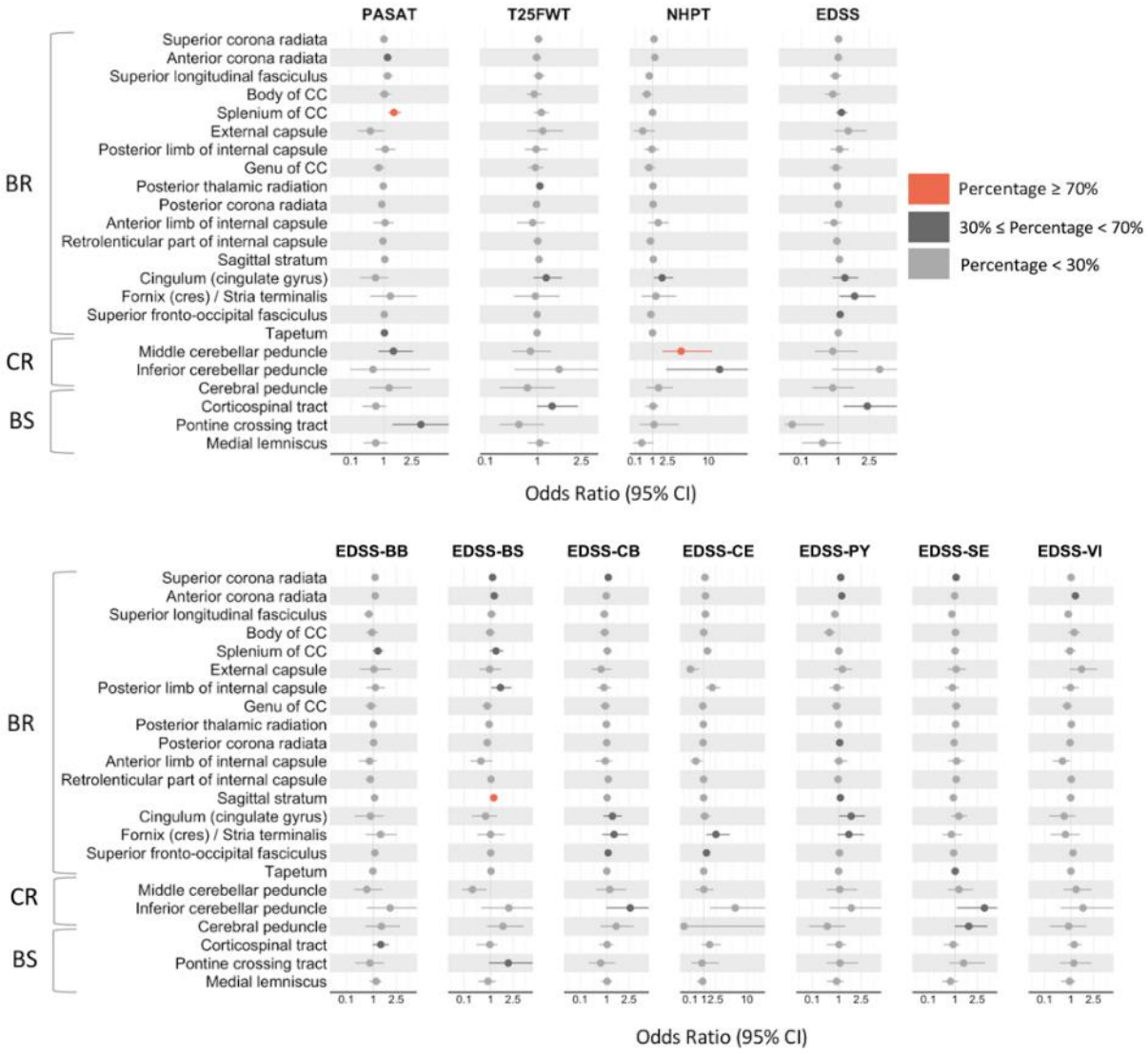
BR, supratentorial brain; BS, brainstem; CC, corpus callosum; CR, cerebellum; WM, white matter

Supplementary Figure e-2. Association between lesions at baseline in each region and the different disability scores.

The estimates/odds ratios and the CIs plotted here were derived from the model defined as (2) using the whole dataset. The colour associated with each estimate and CI was

derived from the bootstrap analysis and indicates the percentage of times the condition in model (3) was satisfied: values are between 0-100%, higher values indicate better reproducibility. A high lesion density in the splenium of the CC showed a stronger association with an increase in disability measured by EDSS compared to the association obtained using the lesion density defined in the whole brain. A stronger relationship was also found between lesion density in the superior fronto-occipital fasciculus and EDSS-CE.

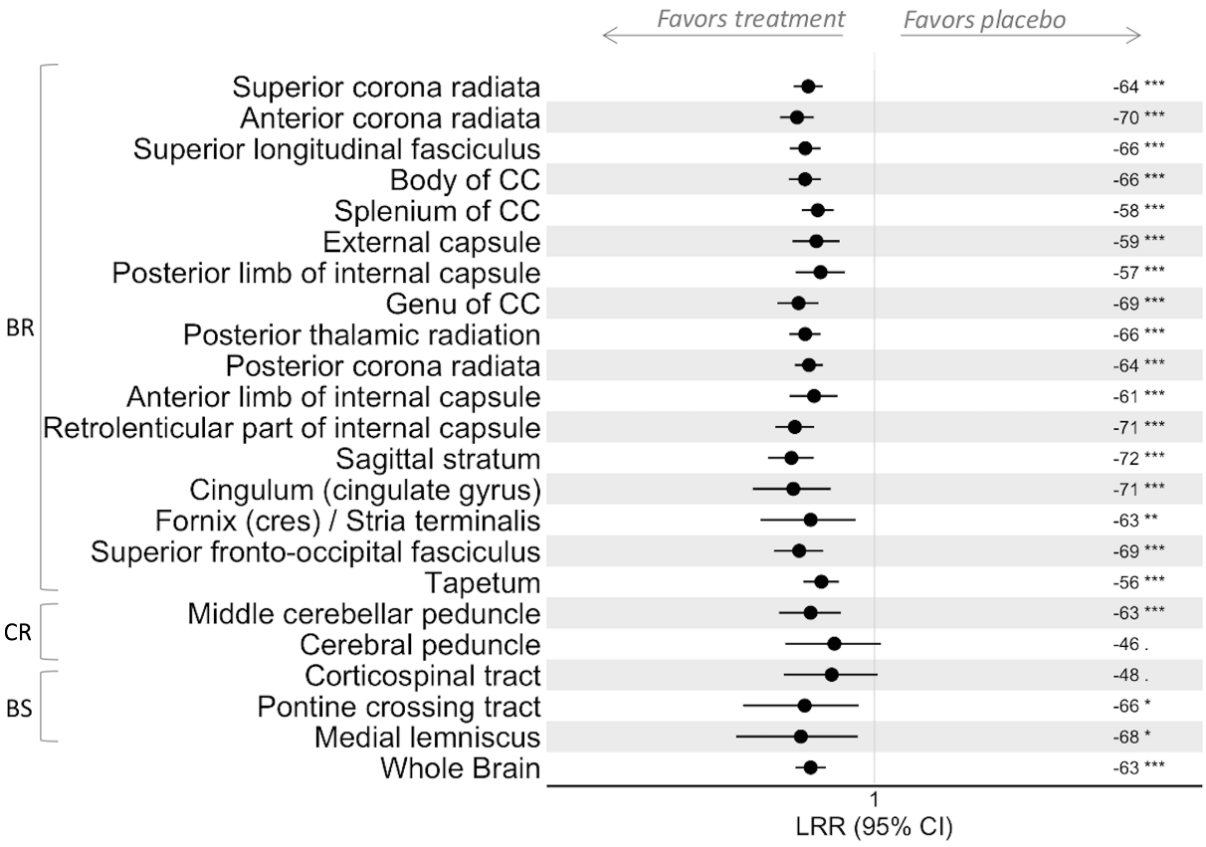
BR, supratentorial brain; BS, brainstem; CC, corpus callosum; CI, confidence interval; CR, cerebellum; EDSS, Expanded Disability Status Scale; EDSS-BB, EDSS bowel and bladder; EDSS-BS, EDSS brainstem; EDSS-CB, EDSS cerebellar; EDSS-CE, EDSS cerebral; EDSS-PY, EDSS pyramidal; EDSS-SE, EDSS sensory; EDSS-VI, EDSS visual; NHPT, 9-Hole Peg Test; PASAT, Paced Auditory Serial Addition Test; T25FWT, Timed 25-Foot Walk Test



Supplementary figure e-3. Association between new and enlarging lesions in each region and the different disability worsening.

The odds ratios and confidence intervals plotted here were derived from the model defined as (5) using the whole dataset. The color associated with each odds ratio and confidence interval was derived from the bootstrap analysis and indicated the percentage of times the condition (6) was satisfied. A high lesion density in the splenium of the corpus callosum showed an association with PASAT worsening, which was stronger than the association obtained using the lesion density defined in the whole brain. A stronger relationship was also found between lesion density in the middle cerebellar peduncle and NHPT, and between the sagittal stratum and EDSS-BS.

BR, supratentorial brain; BS, brainstem; CC, corpus callosum; CI, confidence interval; CB, cerebellum; EDSS, Expanded Disability Status Scale; EDSS-BB, EDSS bowel and bladder; EDSS-BS, EDSS brainstem; EDSS-CB, EDSS cerebellar; EDSS-CE, EDSS cerebral; EDSS-PY, EDSS pyramidal; EDSS-SE, EDSS sensory; EDSS-VI, EDSS visual; NHPT, 9-Hole Peg Test; PASAT, Paced Auditory Serial Addition Test; T25FWT, Timed 25-Foot Walk Test.



Supplementary figure e-4. Treatment effect of fingolimod 0.5 mg on the occurrence of new/enlarging lesions by location.

In each brain region and in the whole brain, the effect of fingolimod was investigated using a negative binomial in which the new/enlarging lesion number was the dependent variable and treatment, clinical trial and baseline lesion number were the independent variables. The LRR between the fingolimod-treated and placebo patients was calculated,

as were the confidence intervals. The percentage reduction in lesion number was calculated as $(LRR - 1) * 100$ and reported in the right side of the plot, together with the p value level (i.e. ' ': p>0.1; '.': 0.1≤p<0.05; '*': 0.05≤p<0.01; '**': 0.01≤p<0.001; '***': p≤0.001). Fingolimod 0.5 mg significantly and consistently reduced new/enlarging lesions compared with placebo in almost every tract. Fingolimod 1.25 mg tended to have a slightly stronger effect than fingolimod 0.5 mg. However, the treatment effect in each region was homogenous and broadly consistent with the overall effect for the whole brain.

BR, supratentorial brain; BS, brainstem; CC, corpus callosum; CI, confidence interval; CB, cerebellum; LRR, lesion rate ratio.

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