S1 Table. Sequence analysis of randomly chosen clones from HB library.

Clone	Identity(blastN)	Nt sequence	Homology	ORF
HB1	Homo sapiens neurofilament 3 (150kDa medium) (NEF3), mRNA	2315-2676	99%	IF
HB2	Homo sapiens ribosomal protein SA (RPSA), transcript variant 1, mRNA	107-418	99%	IF
HB3	Homo sapiens chromosome 5 genomic contig	42251786-42251655	100%	IF
HB4	Homo sapiens cold shock domain containing C2, RNA binding (CSDC2), mRNA	10-62	98%	OF
HB5	Homo sapiens chromosome 2 genomic contig	75938720-75938686	100%	IF
HB6	Human DNA sequence clone RP11-378J18 on chromosome 1	4894-4807	98%	IF
HB7	Homo sapiens brain expressed, X-linked 1 (BEX1), mRNA	232-489	100%	IF
HB8	Homo sapiens polymerase (RNA) I polypeptide D, 16kDa (POLR1D), transcript variant 2, mRNA	101-370	100%	IF
HB9	Homo sapiens enolase 2 (gamma, neuronal) (ENO2), mRNA	2010-1859	99%	IF
HB10	Homo sapiens microtubule-associated protein 1B (MAP1B), transcript variant 2, mRNA	1808-2034	100%	IF
HB11	Fas apoptotic inhibitory molecule 2 (FAIM2)	3615-3545	98%	IF
HB12	Vesicle-associated membrane protein 1 (VAMP1)	2038-1806	100%	IF
HB13	Transient receptor potential cation channel, subfamily V, member 6	1487-1604	100%	IF
HB14	GNAS complex locus (GNAS)	173-414	100%	OF
HB15	Chromatin assembly factor 1 (p150) (CHAF1A)	1275_1428	100%	IF

BlastN analysis of 15 randomly picked clones from the ORF-selected phage display cDNA library from human brain. The portion of each identified nucleotide sequence and the percentage of homology are indicated. The sequences of clones that were also coding for the corresponding peptide (“in frame” clones, IF) are indicated; the “out-of-frame” clones (OF) were non coding sequences.

S2 Table. Antigens selected with the anti-human IgG.

Identity (blastN)
HS GRB10 interacting GYF protein 2 (GIGYF2)
HS DExH-box helicase 9 (DHX9)
WD repeat domain

List of antigens identified by the selection of phage display cDNA library from human brain with anti-human IgG.

S3 Table. Nucleotide sequence alignments of clones selected with CSF pool.

Clone	Freq.	Identity - blastN	GeneBank No.	% identity	start	end
HB_CSFD3	3/24	HS DEAD-box helicase 24 (DDX24)	NM_020414.3	100	392	744
HB_CSFD12	1/24	HS DEAD-box helicase 24 (DDX24)	NM_020414.3	100	357	744
HB_CSFD8	2/24	HS erythrocyte membrane protein band 4.1 (EPB41)	NM_001166005.1	100	273	436
HB_CSFD6	1/24	HS pleckstrin homology domain containing B2 (PLEKHB2)	NM_017958.2	98	535	751
HB_CSFE12	1/24	HS ST13, Hsp70 interacting protein (ST13)	NM_003932.4	100	554	749
HB_CSFB2	3/24	HS adenylate kinase 5 (AK5)	NM_174858.2	100	1897	1719
HB_CSFE9	3/24	HS CAP-Gly domain containing linker protein 1 (CLIP1)	NM_002956.2	100	3341	3148
HB_CSFA3	1/24	n.a.	n.a			
HB_CSFD4[a]	2/24	n.a.	n.a			
HB_CSFA2[b]	7/24	HS GRB10 interacting GYF protein 2 (GIGYF2)	NM_001103147.1	99	2628	2976

BlastN analysis of 24 positive clones identified by the selection of phage display cDNA library from human brain with pooled and purified IgG from CSF of MS patients. For each clone are indicated: the code of the clone (“Clone”); the clone frequency (“Freq.”) corresponding to the number of clones, over the total clones sequenced, that map to the same antigen; the nucleotide sequence identified by blastN analysis (“Identity-blastN”); the NCBI accession number (“GeneBank No.”) of the identified sequence; the percentage of homology (“% identity”) with the identified sequence; the first and last nucleotide of the identified sequence.

[a]out of frame clones

[b]background clones shared with the anti-human IgG selection

S4 Table. Protein sequence alignments of clones selected with CSF pool.

Clone	Freq.	Identity - blastP	ProtBank No.	% identity	start	end	classification
HB_CSFD3	3/24	DEAD-box helicase 24	NP_065147.1	100	99	215	ORF
HB_CSFD12	1/24	DEAD-box helicase 24	NP_065147.1	100	87	215	ORF
HB_CSFD8	2/24	Erythrocyte membrane protein band 4.1	NP_001159477.1	100	50	103	ORF
HB_CSFD6	1/24	Pleckstrin homology domain containing B2	NP_001296379.1	97	1	29	ORF
HB_CSFE12	1/24	Hsp70 interacting protein	NP_003923.2	97	30	95	ORF
HB_CSFB2	3/24	Flotillin 2	NP_004466.2	55	28	38	mimotope
HB_CSFE9	3/24	n.a					unidentified
HB_CSFA3	1/24	n.a					unidentified
HB_CSFD4	2/24	n.a					out-of-frame
HB_CSFA2	7/24	GRB10-interacting GYF protein 2	NP_001096618.1	97	761	876	background

BlastP analysis of 24 positive clones identified by the selection of phage display cDNA library from human brain with pooled and purified IgG from CSF of MS patients. For each clone are indicated: the code of the clone (“Clone”); the clone frequency (“Freq.”) corresponding to the number of clones, over the total clones sequenced, that map to the same antigen; the aminoacid sequence identified by blastP analysis using the translation of the clone nucleotide sequence (“Identity-blastN”); the NCBI accession number (“ProtBank No.”) of the identified sequence; the percentage of homology (“% identity”) with the identified sequence; the first and last nucleotide of the identified sequence. The last column reports as the clone can be classified: “ORF” if the clone was in frame and an aminoacid sequence was identified; “mimotope” if the nucleotide and aminoacid sequences identified belong to different gene; “unidentified” if an aminoacid sequence was not identified; “out-of-frame” if the clone was apparently not coding; “background” if the clone was also identified in the selection against only human IgG.

S5 Table. Nucleotide sequence alignments of clones selected with sera pool.

Clone	Freq.	Identity - blastN	GeneBank No.	% identity	start	end
HB_RRG7	1/42	HS amyloid beta precursor protein (APP)	NM_201413.2	95	1037	1298
HB_RRF1	1/42	HS ATP synthase, H+ transporting, mitochondrial Fo complex subunit E (ATP5I)	NM_007100.3	0	87	240
HB_RRB7	1/42	HS brain expressed X-linked 2 (BEX2)	NM_001168399.1	100	444	700
HB_RRH5	1/42	HS DEAD-box helicase 24 (DDX24)	NM_020414.3	100	357	744
HB_RRC8	1/42	HS DEAD-box helicase 24 (DDX24)	NM_020414.3	99	392	658
HB_RRH11	1/42	HS heterogeneous nuclear ribonucleoprotein U like 2 (HNRNPUL2)	NM_001079559.2	100	2214	2379
HB_RRH2	1/42	HS microtubule associated protein 1B (MAP1B)	NM_005909.4	100	2189	2523
HB_RRB8	1/42	HS adenylate kinase 5 (AK5)	NM_174858.2	100	1897	1719
HB_RRE2	1/42	HS RNA, 28S ribosomal (LOC109910382), ribosomal RNA	NR_146154.1	97	1861	2009
HB_RRC3	1/42	HS cystathionine-beta-synthase (CBS)	NM_000071.2	100	1622	1854
HB_RRA12	1/42	HS hypoxia up-regulated 1 (HYOU1), transcript variant 2	NM_001130991.2	100	3998	3580
HB_RRA11	1/42	HS RANBP2-like and GRIP domain containing 5 (RGPD5)	NM_005054.2	100	3487	3470
HB_RRC6[a]	1/42	HS protein phosphatase 4 regulatory subunit 4 (PPP4R4)	NM_058237.1	100	3113	2891
HB_RRH9[a]	6/42	n.a	n.a			
HB_RRB10[b]	20/42	HS GRB10 interacting GYF protein 2 (GIGYF2)	NM_001103147.1	99	2628	2976
HB_RRD12[b]	3/42	HS DExH-box helicase 9 (DHX9)	NM_001357.4	100	3720	3975

BlastN analysis of 42 positive clones identified by the selection of phage display cDNA library from human brain with pooled and purified IgG from sera of MS patients. For the meaning of column heading see the legend of S3 Table.

[a]out of frame clones

[b]background clones shared with the anti-human IgG selection

S6 Table. Protein sequence alignments of clones selected with sera pool.

Clone	Freq.	Identity - blastP	ProtBank No.	% identity	start	end	classification
HB_RRG7	1/42	Amyloid beta precursor protein	NP_958816.1	99	299	366	ORF
HB_RRF1	1/42	ATP synthase membrane subunit e	NP_009031.1	97	1	35	ORF
HB_RRB7	1/42	Brain expressed X-linked 2	NP_001161871.1	99	29	114	ORF
HB_RRH5	1/42	DEAD-box helicase 24	NP_065147.1	100	87	215	ORF
HB_RRC8	1/42	DEAD-box helicase 24	NP_065147.1	99	99	186	ORF
HB_RRH11	1/42	Heterogeneous nuclear ribonucleoprotein U like 2	NP_001073027.1	98	663	717	ORF
HB_RRH2	1/42	microtubule-associated protein 1B	NP_005900.2	100	643	753	ORF
HB_RRB8	1/42	Flotillin 2	NP_004466.2	55	28	38	mimotope
HB_RRE2	1/42	Uridine-cytidine kinase 1 like 1	NP_060329.2	50	69	94	mimotope
HB_RRC3	1/42	n.a					unidentified
HB_RRA12	1/42	n.a					unidentified
HB_RRA11	1/42	n.a					unidentified
HB_RRC6	1/42	n.a					out-of-frame
HB_RRH9	6/42	n.a					out-of-frame
HB_RRB10	20/42	GRB10-interacting GYF protein 2	NP_001096618.1	97	761	876	background
HB_RRD12	3/42	ATP-dependent RNA helicase A DHX9	NP_001348.2	100	1183	1267	background

BlastP analysis of 42 positive clones identified by the selection of phage display cDNA library from human brain with pooled and purified IgG from sera of MS patients. For the meaning of column heading see the legend of S4 Table.

S7 Table. Nucleotide sequence alignments of clones selected with the scFV library.

Clone	Freq.	Identity - blastN	GeneBank No.	% identity	start	end
HB_scFv-CSFA1	3/15	HS DEAD-box helicase 24 (DDX24)	NM_020414.3	100	357	744
HB_scFv-CSFB5	2/15	HS DEAD-box helicase 24 (DDX24)	NM_020414.3	100	515	786
HB_scFv-CSFA5	1/15	HS DEAD-box helicase 24 (DDX24)	NM_020414.3	100	392	744
HB_scFv-CSFB7	1/15	HS transcription elongation factor A like 4 (TCEAL4)	NM_024863.5	100	591	743
HB_scFv-CSFE5	1/15	HS transcription elongation regulator 1 (TCERG1)	NM_006706.3	100	2085	2418
HB_scFv-CSFB6	2/15	HS adenylate kinase 5 (AK5)	NM_174858.2	100	1897	1719
HB_scFv-CSFA8	2/15	HS Fas apoptotic inhibitory molecule 2 (FAIM2)	NM_012306.3	99	3655	3585
HB_scFv-CSFG6	1/15	HS cathepsin C (CTSC)	NM_148170.4	93	4132	4078
HB_scFv-CSFG7	1/15	HS vesicle associated membrane protein 1 (VAMP1)	NM_014231.4	99	2402	2128
HB_scFv-CSFF4[a]	1/15	HS RNA, 28S ribosomal (LOC109910382), ribosomal RNA	NR_146154.1	100	3933	3734

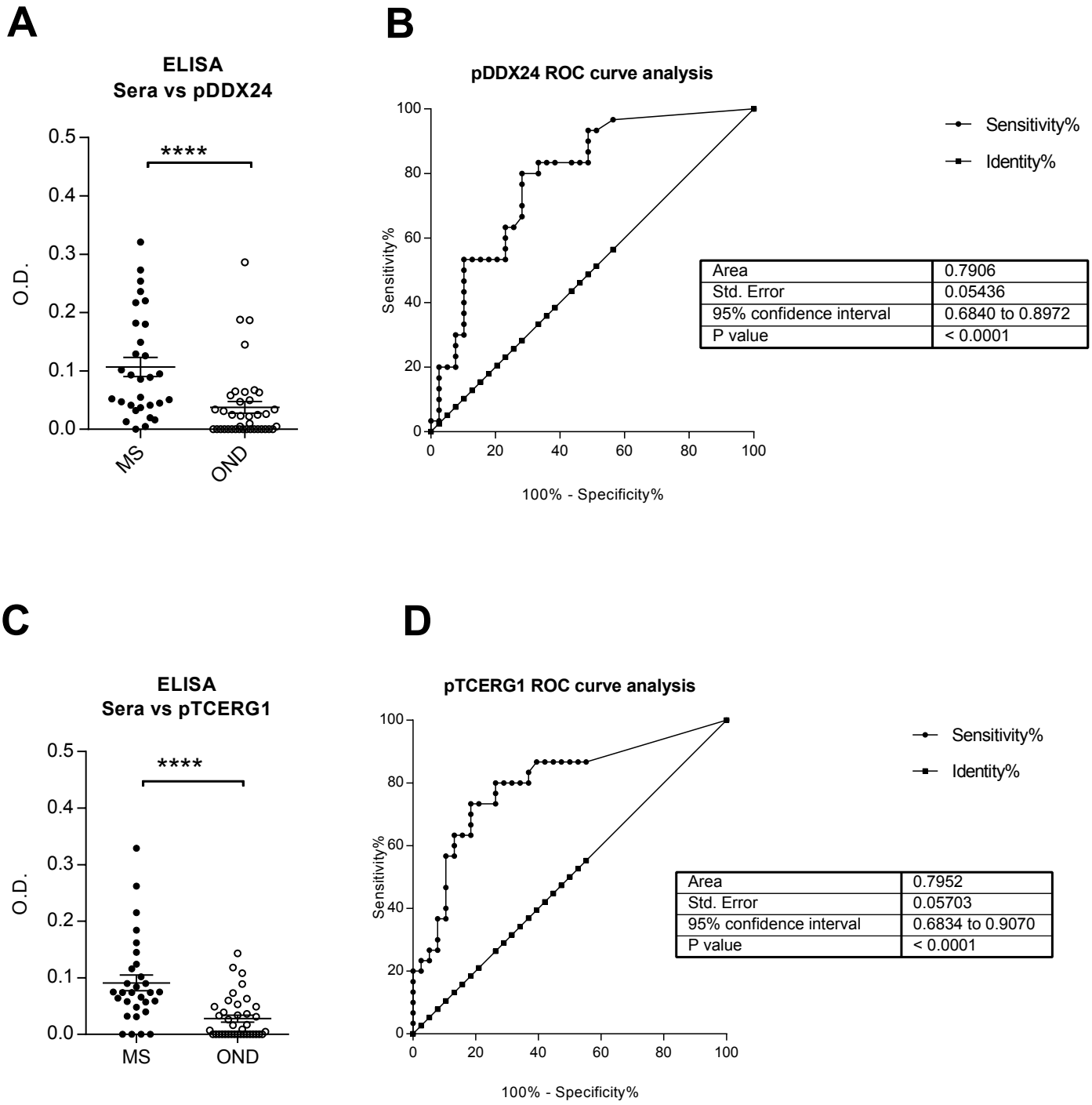
BlastN analysis of 15 positive clones identified by the selection of phage display cDNA library from human brain with the scFv phage display library from CSF of two RR-MS patients. For the meaning of column heading see the legend of S3 Table.

[a]out of frame clones

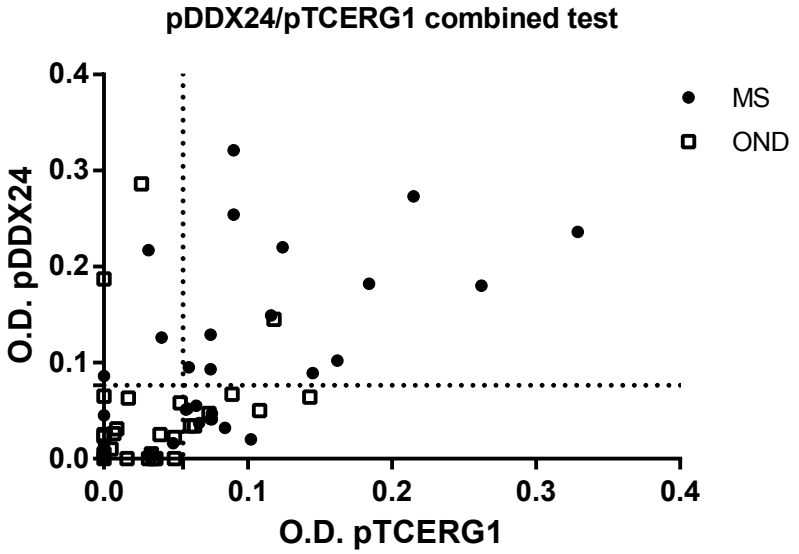
S8 Table. Protein sequence alignments of clones selected with the scFv library.

Clone	Freq.	Identity - blastP	ProtBank No.	% identity	start	end	classification
HB_scFv-CSFA1	3/15	DEAD-box helicase 24	NP_065147.1	100	87	215	ORF
HB_scFv-CSFB5	2/15	DEAD-box helicase 24	NP_065147.1	100	140	229	ORF
HB_scFv-CSFA5	1/15	DEAD-box helicase 24	NP_065147.1	100	99	215	ORF
HB_scFv-CSFB7	1/15	Transcription elongation factor A like 4	NP_001006936.1	100	98	148	ORF
HB_scFv-CSFE5	1/15	Transcription elongation regulator 1	XP_004042807.1	99	683	793	ORF
HB_scFv-CSFB6	2/15	Flotillin 2	NP_004466.2	55	28	38	mimotope
HB_scFv-CSFA8	2/15	Phosphatidyl inositol-4-phosphate 5-kinase type 1 alpha	XP_006711630.1	50	450	465	mimotope
HB_scFv-CSFG6	1/15	n.a					unidentified
HB_scFv-CSFG7	1/15	n.a					unidentified
HB_scFv-CSFF4	1/15	n.a					out-of-frame

BlastP analysis of 15 positive clones identified by the selection of phage display cDNA library from human brain with the scFv phage display library from CSF of two RR-MS patients. For the meaning of column heading see the legend of S4 Table.



S1 Figure. Evaluation of the diagnostic value of pDDX24 and pTCERG1 in the prediction of MS.
The diagnostic value of DDX24 and TCERG1 was further investigated testing the reactivity of some sera samples (30 MS from RR-MS samples utilized in the selections and 38 OND with a mean age of 62 and a ratio of female/male of 14/24) against synthetic peptides pTCERG1 (A) and pDDX24 (C) by an ELISA assay. The synthetic peptides named pDDX24 (aa SQSTAAKVPKKAKTWIPEVHD) and pTCERG1 (aa AAKHAKDSRFKAIEKMKDRE) are included in the aminoacidic portion of antigens recognized in the selections. Unpaired t-test has been used in A and C (**** p< 0.0001). A significantly higher reactivity of MS patients against pDDX24 and pTCERG1 compared to the control group was observed (Fig S1 A, C).
The data of the Receiver operating characteristic (ROC) curve analysis for the pDDX24 and pTCERG1 ELISA are showed near the graph (Fig S1 B, D). For pDDX4 at O.D. cut off of 0.0765 the sensitivity for discriminating patients with and without MS is of 53.33% (95% confidence interval 34.33-71.66) and specificity of 89.74% (95% confidence interval 75.78-97.13) with a prevalence weighted likelihood positive ratio (LR+) of 5.2 for the diagnosis of MS. For pTCERG1 at O.D. cut-off of 0.055 the test showed a sensitivity of 73.33% (95% confidence interval 54.11-87.72) and a specificity of 81.58% (95% confidence interval 65.67-92.26) with a LR+ of 3.98.



S2 Fig. Evaluation of the diagnostic value of pDDX24/pTCERG1 combined test in the prediction of MS.

Serum response against synthetic peptides pTCERG1 and pDDX24 in MS (n=30) and OND (n=38) patients measured by ELISA (S1 Fig). Individual biological replicates are shown. Dotted lines represent the cut-off with the highest Youden's index calculated for each antigen.

Considering positive only the double positive samples the test showed a sensitivity of 43.33% and a specificity of 97.37%; the PPV and FPR were respectively 92.86% and 2.63% with a LR+ of 16.47.