SUPPLEMENTARY DATA

Mutations in the colony stimulating factor 1 receptor (*CSF1R*) cause hereditary diffuse leukoencephalopathy with spheroids

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Legend Supplementary Figure 1. Genome-wide linkage analysis in family VA using Illumina Infinium HumanLinkage-12 microarrays. (a) Multipoint LOD scores are shown for all chromosomes from non-parametric linkage analyses calculated with Merlin 1.0. Five loci on five different chromosomes generated LOD scores greater than 1.0; chromosome 5 (LOD=2.67), chromosome 10 (LOD=1.86), chromosome 11 (LOD=1.07), chromosome 20 (LOD=1.22) and chromosome 21 (LOD=1.35). **(b)** Parametric linkage analyses for the five loci with suggestive evidence for linkage identified in (a). Multipoint LOD scores were generated assuming an autosomal dominant, affecteds only disease model with a disease allele frequency of 0.0001. Only the locus on chromosome 5 reached significance, with a maximum LOD score of 3.71 at 165.3cM (rs13178296).

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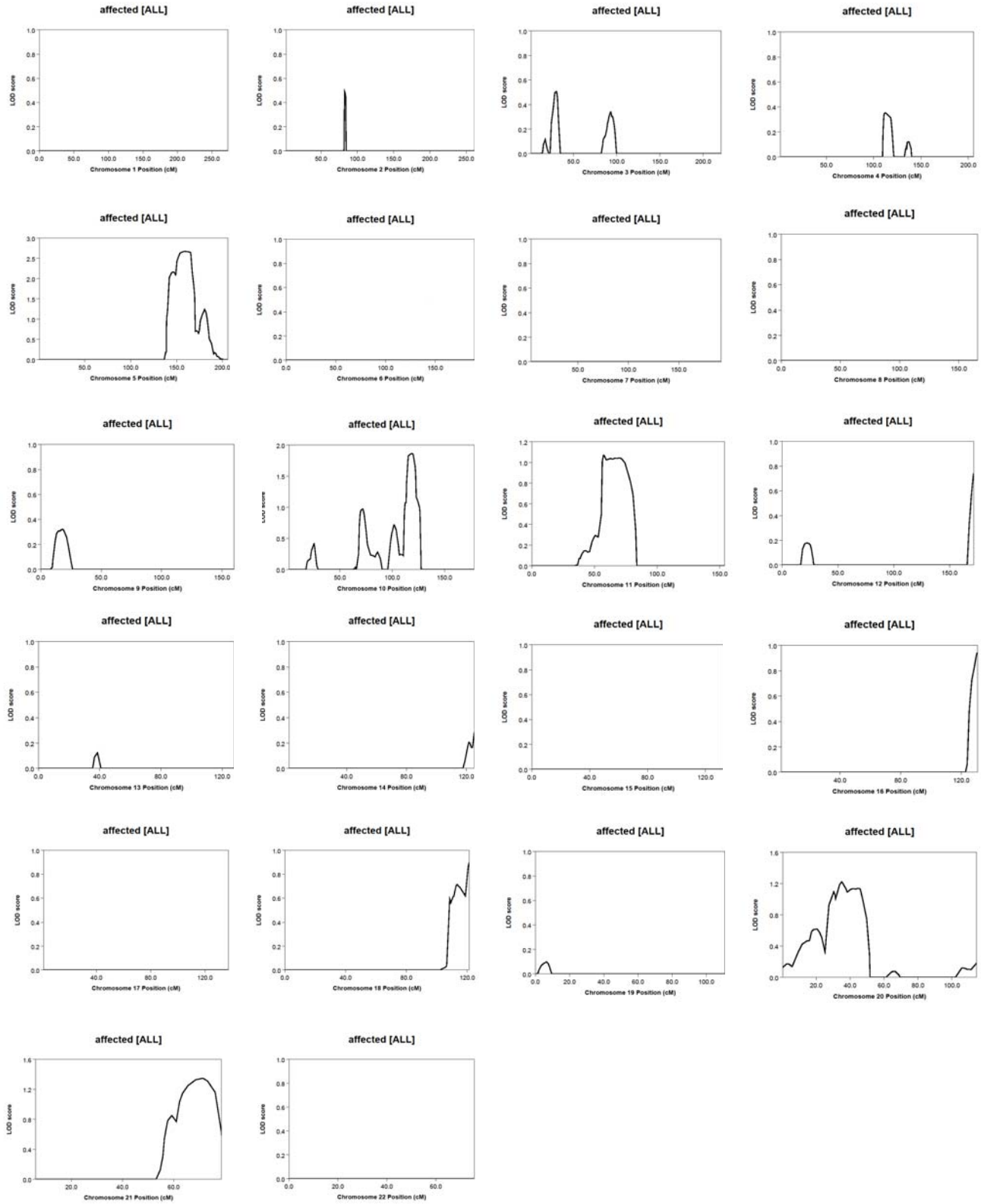
Supplementary Figure 1a.



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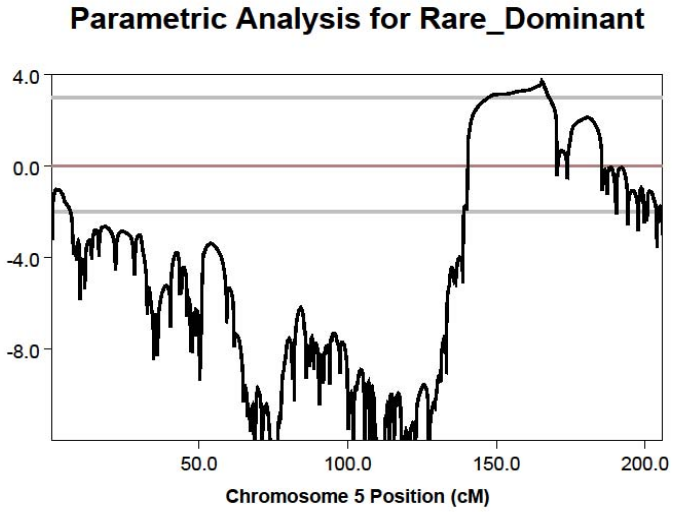
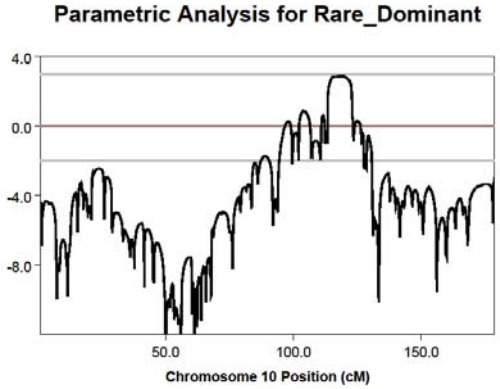
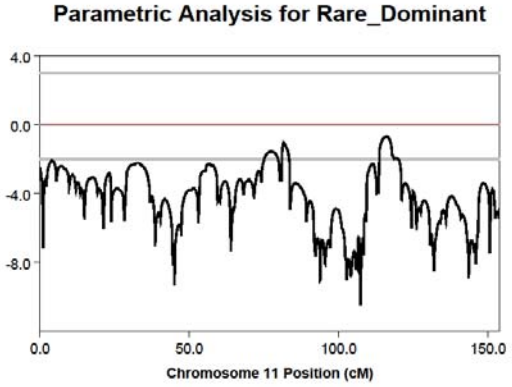
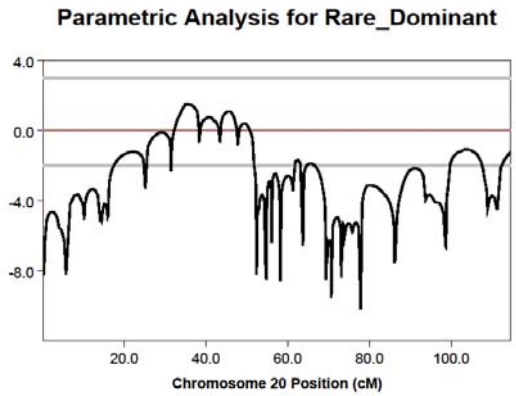
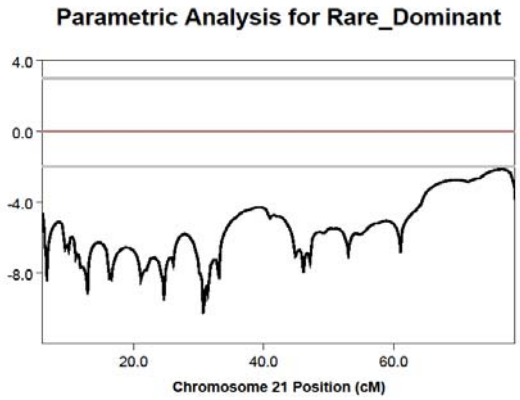
Supplementary Figure 1b.











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Legend Supplementary Figure 2. Segregation analyses of chromosome 5q34 disease haplotype in affected members of HDLS family VA. Black symbols represent patients affected with HDLS. White symbols represent unaffected individuals or at-risk individuals with unknown phenotype. The Roman numeral to the left of the pedigree denotes the generation; Arabic numbers above the individuals denote unique patient numbers (UPN) corresponding to **Table 1**. Extended pedigree is shown in **Figure 2.** Haplotypes are based on 18 single nucleotide polymorphisms (SNPs) on chromosome 5q, selected from the Infinium HumanLinkage-12 microarrays. Haplotypes for individual IV-29 are inferred from genotype data of siblings and offspring. Black bars represent the disease haplotype; white bars represent non-disease haplotypes. Red arrows indicate three obligate recombination events together defining a maximal candidate region of 30.3cM between rs801399 and rs1445716.

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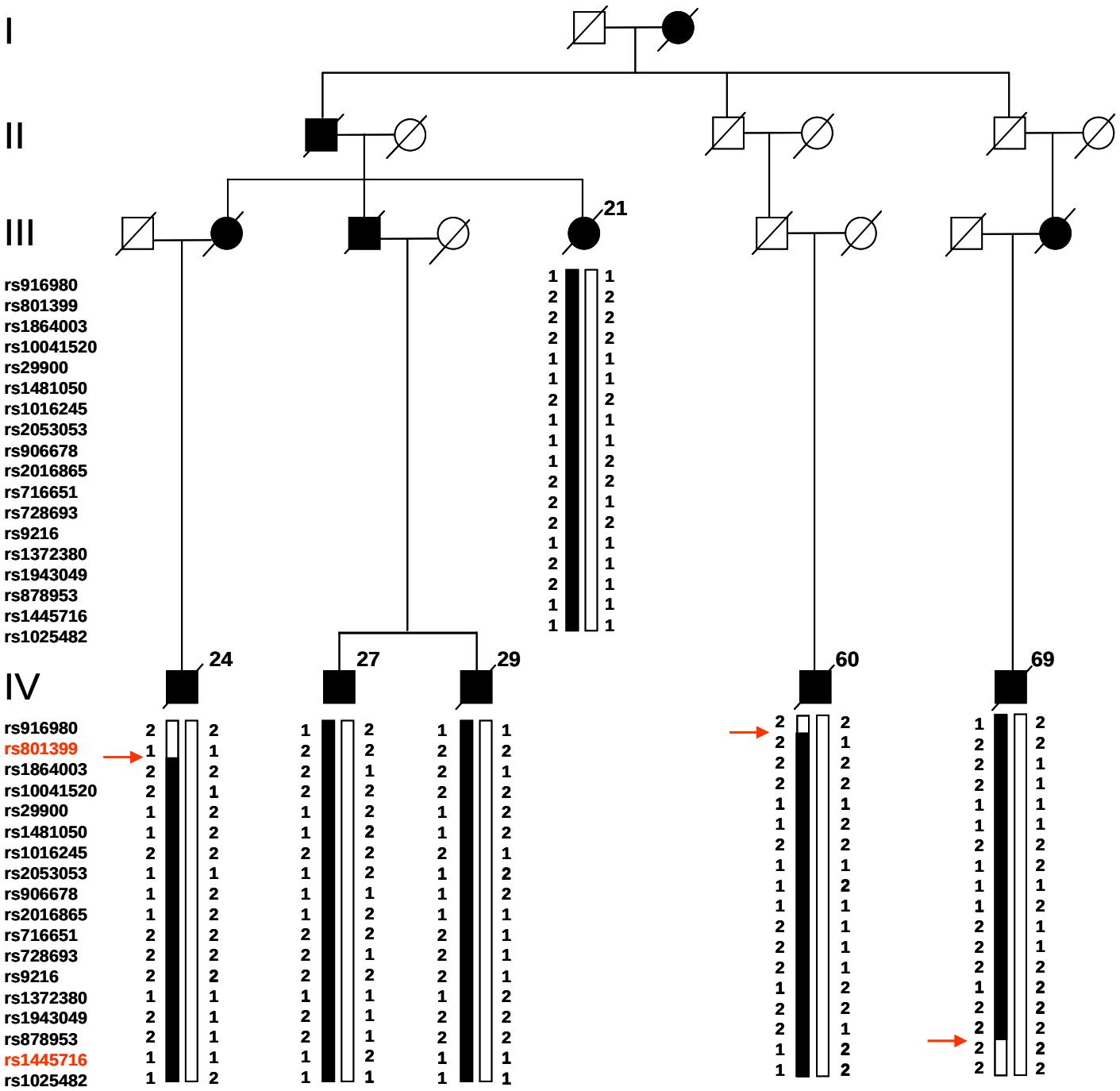
Supplementary Figure 2.



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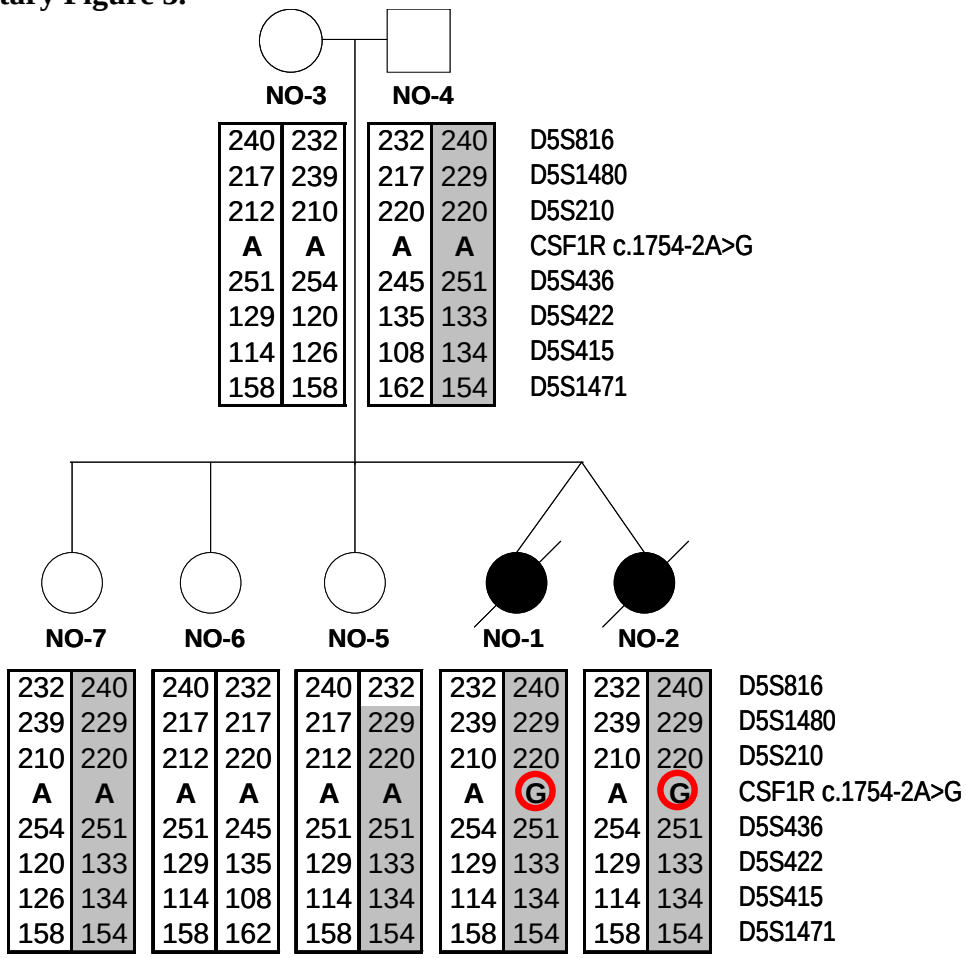
Legend Supplementary Figure 3. *De novo CSF1R* mutation in HDLS family from Norway. Pedigree of family NO showing that the *CSF1R* c.1754-2A>G mutation observed in the monozygotic twin-pair (NO-1 and NO-2) is not present in gDNA samples collected from their parents (NO-3 and NO-4). Segregation analysis using 7 short tandem repeat markers flanking *CSF1R* revealed that the mutation occurred *de novo* in this family. The mutant G-allele of c.1754-2A>G (circled in red) was arbitrarily assigned to the haplotype inherited from the father (NO-4). The *de novo* occurrence of the *CSF1R* mutation in this family combined with the fact that we identified 14 different *CSF1R* mutations in 14 HDLS families, suggests a high *de novo* mutation rate for *CSF1R*.

Supplementary Figure 3.



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Legend Supplementary Figure 4. cDNA transcript analyses of the *CSF1R* splice-site mutations identified in families NO, FL2 and CA2. **(a)** The A>G transition identified in the acceptor site of intron 12 of the NO kindred was predicted to lead to exon 13 skipping. RT-PCR in patient NO-1 with cDNA primers CSF1R c11F/c19R (**Supplementary Table 4**) resulted in an aberrant PCR product in addition to the wild-type product (data not shown). Gel-excision followed by sequence analyses confirmed the loss of exon 13 in the mutated allele. The mutation results in the in-frame deletion of 34 consecutive amino acids combined with an insertion of one novel Alanine residue (p.G585_K619delinsA). **(b)** The A>G substitution in the acceptor site of intron 17 observed in the FL2 family was predicted to result in the skipping of exon 18. PCR of cerebellum derived cDNA from patient FL2-1 using primers CSF1R c17F/c20R (**Supplementary Table 4**), gel-excision and sequencing confirmed that exon 18 is absent, leading to the in-frame deletion of 40 consecutive amino acids (p.C774_N814del). **(c)** The mutation identified in the CA2 family destroys the donor site in intron 18 which leads to the use of alternative acceptor and donor sites in intron 17. This was evidenced by PCR of blood lymphocyte derived cDNA from patient CA2-1 using primers CSF1R c17F/c20R (**Supplementary Table 4**), gel-excision and subsequent sequencing analyses. Sequencing in the reverse direction (bottom panel) shows skipping of exon 18 combined with the splicing in of a 57bp fragment of intron 17 (marked IVS). The in-frame addition of this intronic sequence creates 19 novel amino acids. Interestingly, forward sequencing analyses (top panel) showed evidence of two alternative splice isoforms, one including 54bp and one including 57bp of intron 17. This is likely the result of alternate acceptor sites being used upstream of the IVS insert; the sequence of this region reads TCACTCCAGCAG, where either 'AG' may be adopted as a splice-site. The longer (57bp) IVS insert was used for nomenclature of the mutation (p.C774_N814delinsQGLQSHVGPSLPSSSPQAQ).

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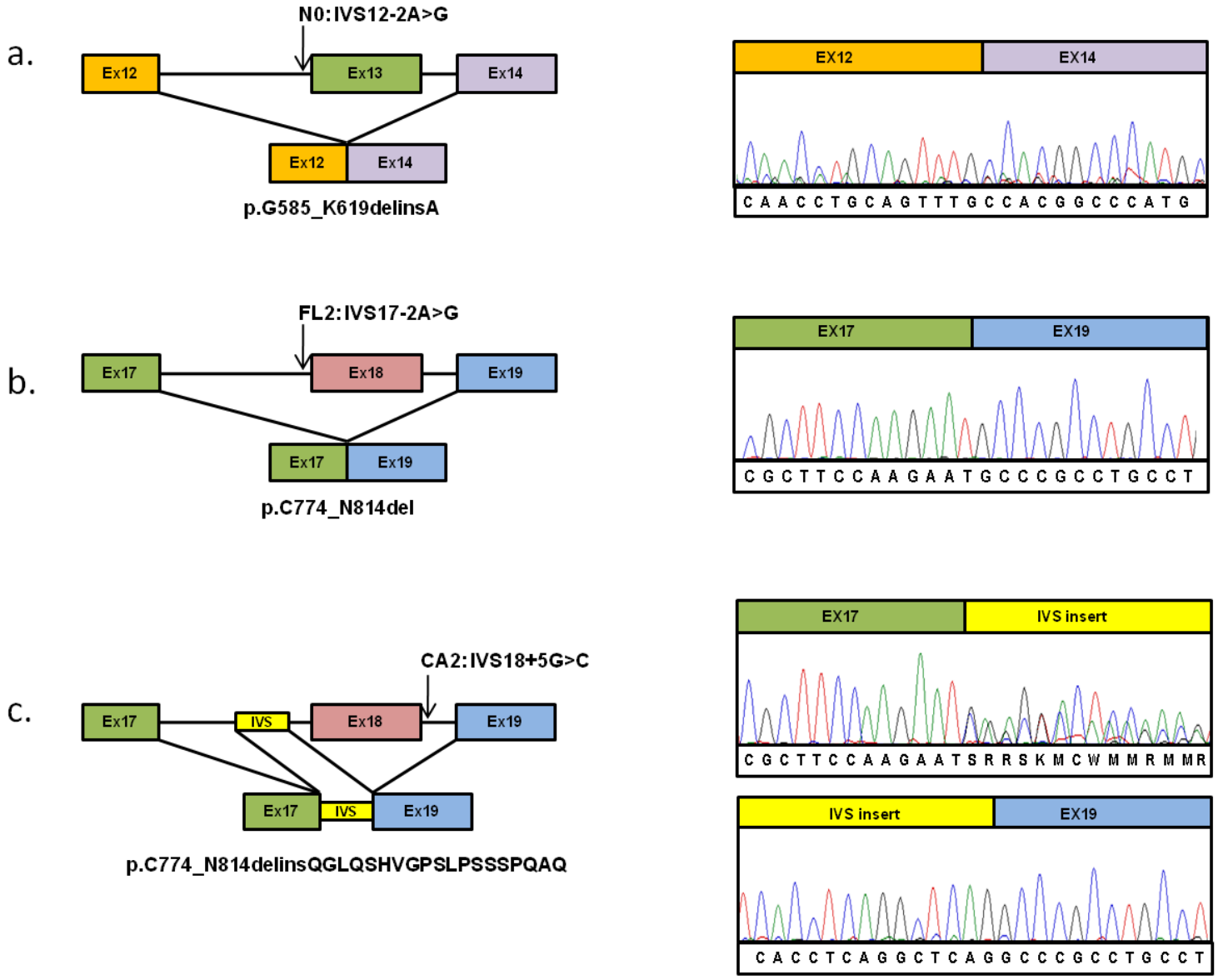
Supplementary Figure 4.



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Legend Supplementary Figure 5. *In-vitro* functional analyses of $CSF1R_{E633K}$ and $CSF1R_{M766T}$. (a) Western blot of lysates from $CSF1R_{WT}$ or mutant $CSF1R_{E633K}$ transfected HeLa cells treated with CSF-1 for 5, 15, or 30 minutes. Lysates from untreated CSF1R-transfected cells are included as a control and GAPDH immunoreactivity is shown to ensure equal protein loading. Total CSF1R immunodetection for the wild-type and mutant DNA construct was robust. Further, we observed strong phosphorylation of wild-type CSF1R after 5 minutes of CSF-1 treatment, which decreased over the course of 15 and 30 minutes, as determined by immunoblotting using CSF1R phospho-specific tyrosine (p-Y) antibodies. In contrast, no CSF1R autophosphorylation at any of the selected tyrosine residues was detected after CSF-1 treatment in $CSF1R_{E633K}$ transfected cells. Experiments were repeated three times with similar outcome. (**b**) Western blot of lysates from $CSF1R_{WT}$ or mutant $CSF1R_{M766T}$ transfected HeLa cells treated with CSF-1 for 5, 15, or 30 minutes showing a comparable defect in $CSF1R_{M766T}$ autophosphorylation.

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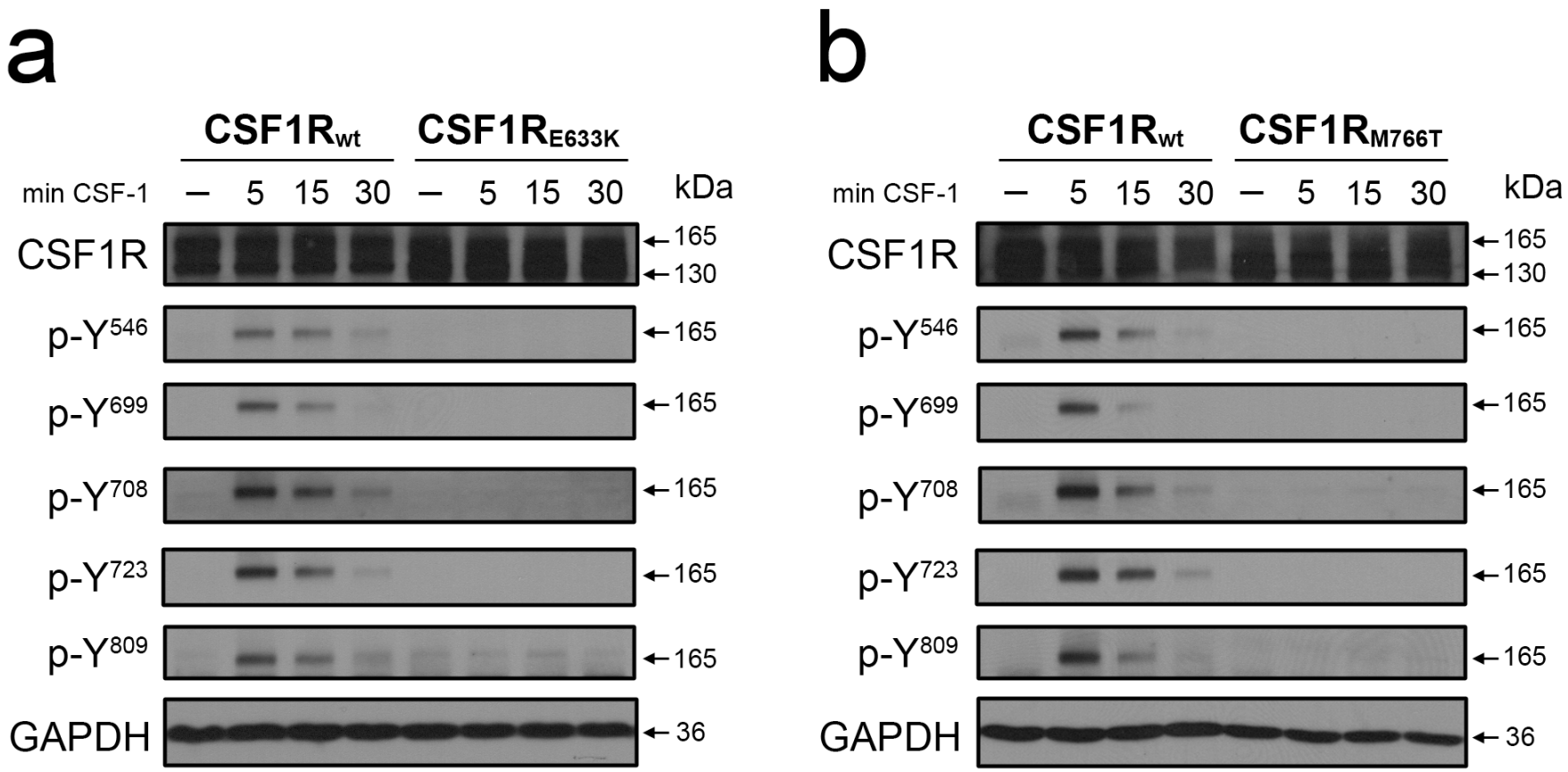
Supplementary Figure 5.



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Legend Supplemental Figure 6. CSF1R phosphorylation is unchanged in blood and brain of affected individuals. CSF1R autophosphorylation was assessed in blood obtained from a healthy control and HDLS patient CA1-1 **(a)**, as well as in frontal cortex brain tissue from a selection of healthy controls, HDLS, AD, and ALS patients **(b)**. Tissue samples from AD and ALS patients were included as disease controls to exclude the possibility that potential CSF1R phosphorylation changes in HDLS would be the result of global cell death. Each gel was loaded by equal protein and blots were reacted with CSF1R phospho-specific tyrosine (p-Y) antibodies. GAPDH was used to ensure equal protein loading for brain lysates. In blood, total and phosphorylated CSF1R levels appeared unchanged in the HDLS patient compared to the controls (**a**). Additionally, in brain, HDLS frontal cortex total and phosphorylated CSF1R levels were not significantly different from normal and disease controls (one-way ANOVA; $p > 0.05$) (**b**). Please refer to the main text of this manuscript for details on these results and a discussion on how they relate to the results obtained *in vitro*.

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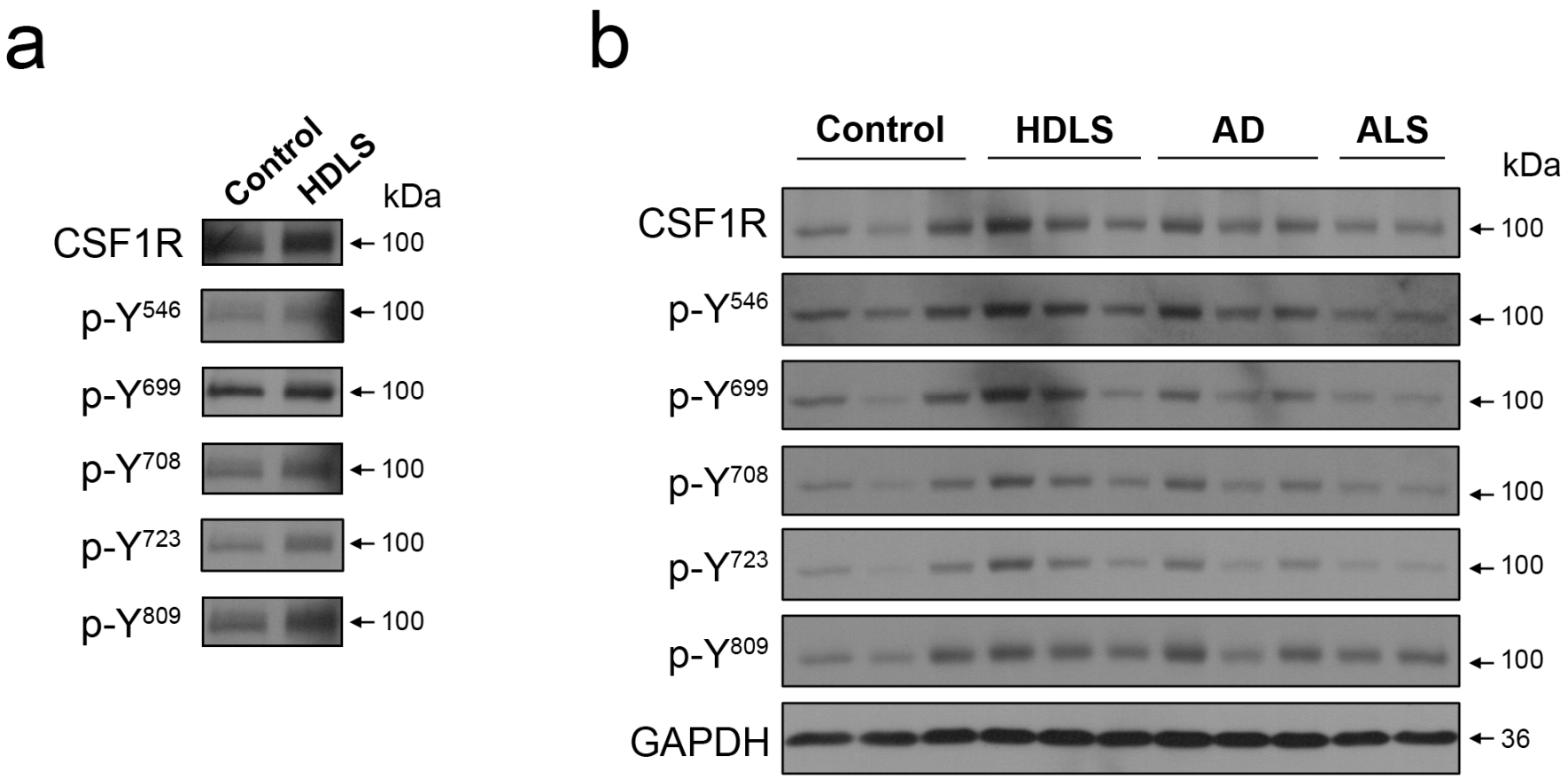
Supplementary Figure 6.



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Supplementary Table 1. List of mutations identified in *CSF1R*.

Family	cDNA Mutation[a]	Gene Site	Protein Mutation[b]	Genomic position[c]	Controls assayed[d]
NO	c.1754-2A>G	IVS12	p.G585_K619delinsA	chr5:149441160	1447
MD	c.1766G>A	EX13	p.G589E	chr5:149441146	1463
FL1	c.1897G>A	EX14	p.E633K	chr5:149440498	1471
CA1	c.2297T>C	EX17	p.M766T	chr5:149436873	1449
DE	c.2308G>C	EX17	p.A770P	chr5:149436861	1464
FL2	c.2320-2A>G	IVS17	p.C774_N814del	chr5:149435906	1444
MI	c.2324T>A	EX18	p.I775N	chr5:149435900	1467
SC	c.2381T>C	EX18	p.I794T	chr5:149435843	1458
CA2	c.2442+5G>C	IVS18	p.C774_N814delinsQG-LQSHVGPSLPSSSPQAQ	chr5:149435777	1444
CBS186[e]	c.2509G>T	EX19	p.D837Y	ch5:149435634	1436
SCT	c.2546T>C	EX19	p.F849S	chr5:149435597	1454
IN	c.2546_2548delTCT	EX19	p.F849del	chr5:149435597-149435599	1466
MO3	c.2603T>C	EX20	p.L868P	chr5:149434851	1477
VA	c.2624T>C	EX20	p.M875T	chr5:149434830	1452
MO2	c.2632C>A	EX20	p.P878T	chr5:149434822	1456

[a] Numbering relative to NM_005211.3 starting at the translation initiation codon.

[b] Numbering relative to NP_005202.2.

[c] Numbered accordingly to assembly Feb 2009 (GRC37/hg19) available at http://genome.ucsc.edu/.

[d] Caucasian controls assayed by TaqMan genotyping.

[e]Clinical CBS patient identified in the follow-up sequencing analysis (see **Supplementary Table 2**).

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Supplementary Table 2. Demographic information of Mayo Clinic patient cohorts included in *CSF1R* mutation screening.

Study cohorts	N	M:F	Mean age of onset ± SD (years)	Family history Yes:No
Frontotemporal dementia	93	53:40	59 ± 7	78:15
Corticobasal syndrome[a]	93	39:54	63 ± 10	32:61
Alzheimer's disease	93	36:57	55 ± 5	47:46
Parkinson's disease	93	59:34	52 ± 10	51:43
Ischemic Stroke	93	64:29	62 ± 14	33:60
Multiple sclerosis	61	6:55	32 ± 9	15:46

[a] In one CSB patient (CBS186) a novel *CSF1R* missense mutation (c.2509G>T; p.D837Y) was identified, which was absent from 1436 controls. This 43-year-old Caucasian female experienced gradual decline in expressing herself, finding her words and performing motor tasks with her right hand, soon spreading to her right lower limb. During the evaluation at the Mayo Clinic, approximately 24 months after symptom onset, she demonstrated executive dysfunctions, slight bradykinesia in all extremities (more on the right side), exaggerated deep tendon reflexes with extensor plantar response on the right, slight right limb apraxia, right upper extremity rigidity, bilateral lower extremity spasticity (more on the right) and circumduction of the right leg while walking. Over the next months, there was rapid deterioration of cognitive and motor functions with spasticity and multiple falls. She was followed by her local physician and died at the age of 50 years old.. A head MRI performed during her fist year of symptom onset showed localized white matter T2 hyperintense foci in the bifrontal and biparietal white matter (more on the left), with associated cortical atrophy (films are not longer available). Her clinical diagnosis at the Mayo Clinic was CBS. Her father apparently was diagnosed with Alzheimer disease and died 79 years old of malignancy. Her mother and two siblings are alive and healthy.

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Supplementary Table 3. Non-synonymous polymorphisms in *CSF1R* identified in the clinical patient screening.

cDNA Mutation[a]	Gene Site	Protein Mutation[b]	dbSNP ID
c.2129G>A	EX15	p.R710H	n/a[a]
c.2239G>A	EX17	p.G747R	rs41355444
c.2760G>C	EX21	p.E920D	rs34030164

[a] n/a, not available. Mutation p.R710H is a novel mutation which was identified in one ischemic stroke patient. Genotyping using a Taqman assay also identified this variant in 1/1453 controls. The arginine at position 710 is part of the kinase insert and is evolutionary not well conserved. Based on these findings we concluded that p.R710H is most likely a benign polymorphism.

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Supplementary Table 4. Demographic information of Mayo Clinic brain bank samples included in the CSF1R immunoblot experiment.

Study cohorts	N	M:F	Mean age at death ± SD (years)	Brain weight range (g)	Braak Stage range
Normal controls	3	1:2	67 ± 9	1180-1600	0-2
HDLS	3	2:1	57 ± 13	1120-1440	0-2
Alzheimer's disease	3	3:0	65 ± 7	1020-1180	6
Amyotrophic lateral sclerosis	2	2:0	64 ± 8	1220-1400	1.5-2.5

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Supplementary Table 5. *CSF1R* gDNA and cDNA sequencing primers.

CSF1R gDNA PCR Primer Sequences (M13 labeled)

CSF1R x1 F	CACGACGTTGTAAAACGACGCCTCCACCATGTGCTTTG
CSF1R x1 R	GGATAACAATTTCACACAGGGCAGGAGAGCCTATGACTCATG
CSF1R x2 F	CACGACGTTGTAAAACGACACATGTCTCTTCTCCCAA
CSF1R x2 R	GGATAACAATTTCACACAGGACTACCTCTCCAAAGCAG
CSF1R x3 F	CACGACGTTGTAAAACGACGCTGAGCAAATGACCAGG
CSF1R x3 R	GGATAACAATTTCACACAGGGGTCCTTGCTCATAGCCA
CSF1R x4 F	CACGACGTTGTAAAACGACTGGTGTGACCTCAGATGAATG
CSF1R x4 R	GGATAACAATTTCACACAGGGAAAGAGGACATCCCACTGC
CSF1R x5 F	CACGACGTTGTAAAACGACCAAGGTCTCTTTAGGACAG
CSF1R x5 R	GGATAACAATTTCACACAGGAGCTACTGACTGGGAGTG
CSF1R x6 F	CACGACGTTGTAAAACGACTTTACAAGTGAAGCAACAG
CSF1R x6 R	GGATAACAATTTCACACAGGAAACTCCAGCAGAGATATC
CSF1R x7 F	CACGACGTTGTAAAACGACGGGACTATGTACCTGTGAC
CSF1R x7 R	GGATAACAATTTCACACAGGGACTTGCTCAAGGTCATAC
CSF1R x8 F	CACGACGTTGTAAAACGACGTCTGAGTATCTGGCTTACCC
CSF1R x8 R	GGATAACAATTTCACACAGGCCCAAGTCACACTTTCCC
CSF1R x9 F	CACGACGTTGTAAAACGACCCTTCGATGTGAGTGCTGG
CSF1R x9 R	GGATAACAATTTCACACAGGCACTTGGGCCTCATCACAC
CSF1R x10 F	CACGACGTTGTAAAACGACGGCCACACTGATAGGTAAG
CSF1R x10 R	GGATAACAATTTCACACAGGTGGACCTTGGTACTGCTAG
CSF1R x11 F	CACGACGTTGTAAAACGACCCAGTGGAGGATGCTTCATTC
CSF1R x11 R	GGATAACAATTTCACACAGGCCCTTGCTTTAATGGCTATTTG
CSF1R x12 F	CACGACGTTGTAAAACGACTATGTTCATGAGACGACCTC
CSF1R x12 R	GGATAACAATTTCACACAGGCACACAGGTCCCTTAAGTC
CSF1R x13 F	CACGACGTTGTAAAACGACCAGTTTGGTGAGATGGCAG
CSF1R x13 R	GGATAACAATTTCACACAGGAAACCCAAGTCCTCACCC
CSF1R x14 F	CACGACGTTGTAAAACGACTTTGGCTTCTGGTCACTC
CSF1R x14 R	GGATAACAATTTCACACAGGATGCTCCCTATCAGTCTCTC
CSF1R x15 F	CACGACGTTGTAAAACGACCACAGAGCCGAGGTTAGAAC
CSF1R x15 R	GGATAACAATTTCACACAGGGGTGGCTACTTCCCATGAC
CSF1R x16F	CACGACGTTGTAAAACGACGACCAATGCTCACTCAGATG
CSF1R X16R	GGATAACAATTTCACACAGGTGGAGAAGTGAAGCAGGTC
CSF1R x17F	CACGACGTTGTAAAACGACACTTCTTCAAATGACTCCTTC
CSF1R x17R	GGATAACAATTTCACACAGGACTCTCTCATACTCTGTCTCCTC
CSF1R x18&19 F	CACGACGTTGTAAAACGACAACTGTTGTTCACTCCAGC
CSF1R x18&19 R	GGATAACAATTTCACACAGGCCATTCCTGCACTCTCAC
CSF1R x20 F	CACGACGTTGTAAAACGACGGTAAGAGTAGCTGCTGTTG
CSF1R x20 R	GGATAACAATTTCACACAGGGTTGTGTCCACTATGGGAG
CSF1R x21 F	CACGACGTTGTAAAACGACGGTAAAGCATGCCAAGGAC
CSF1R x21 R	GGATAACAATTTCACACAGGGTATAGTCCTGAGGGTGGGAG
CSF1R x22 F	CACGACGTTGTAAAACGACTTCAGGAGCAGGCCCAAGAG
CSF1R x22 R	GGATAACAATTTCACACAGGCGAGGCCAACACCATGAGAAC

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CSF1R cDNA PCR Primer Sequences (M13 labeled)

CSF1R c17F	CACGACGTTGTAAAACGACGGGACCTGCTTCACTTCTC
CSF1R c20R	GGATAACAATTTCACACAGGGCCATTTGGTATCCATCCTTC
CSF1R c11F	CACGACGTTGTAAAACGACTATAAGCAGAAGCCCAAGTAC
CSF1R c19R	GGATAACAATTTCACACAGGGGATGCCATAGGACCAGAC

HTR4 gDNA PCR Primer Sequences (M13 labeled)

HTR4 1F	CACGACGTTGTAAAACGACGACACTCGGCTGTACCATAC
HTR4 1R	GGATAACAATTTCACACAGGATAGGAAGCTGGAAATGTTTAC
HTR4 2F	CACGACGTTGTAAAACGACTTGGTGTACATTAAACGCATG
HTR4 2R	GGATAACAATTTCACACAGGCACTGAAATGTTTACACTGGTTG
HTR4 3F	CACGACGTTGTAAAACGACGGGAAATTGAATCTCACGG
HTR4 3R	GGATAACAATTTCACACAGGCAAAGGCTGGAGAATGACTG
HTR4 4F	CACGACGTTGTAAAACGACCTGTTCTCTATCCCTTGCTCC
HTR4 4R	GGATAACAATTTCACACAGGCACCCAGACCACTATCAGATTC
HTR4 5-1F	CACGACGTTGTAAAACGACTTTATCTTCCAATCCTCCAC
HTR4 5-1R	GGATAACAATTTCACACAGGAGGGACAGTGTAGTCTATGAAAG
HTR4 5-2F	CACGACGTTGTAAAACGACCCGCATCTATGTCACAGCTAAG
HTR4 5-2R	GGATAACAATTTCACACAGGGTCTGGCCCAGAATGGAAG
HTR4 5-3F	CACGACGTTGTAAAACGACTGTGGATCCTTTCATAGACTAC
HTR4 5-3R	GGATAACAATTTCACACAGGGTTCTTAACCATTGCTGTATTC
HTR4 5aF	CACGACGTTGTAAAACGACAGCCTGGAGATAGTAAATATTGC
HTR4 5aR	GGATAACAATTTCACACAGGGCACCCACTCAAACTAGACC
HTR4 5bF	CACGACGTTGTAAAACGACGCCTGTCAATGGAAATTCC
HTR4 5bR	GGATAACAATTTCACACAGGCTGGGAAGAGGGAGTGTTG
HTR4 5cF	CACGACGTTGTAAAACGACTTTCAAATACCACCTCCTACCTG
HTR4 5cR	GGATAACAATTTCACACAGGGCAGAGCAGCACTGAGATTTG
HTR4 5dF	CACGACGTTGTAAAACGACGCACTTTAAGAATATTGGCAAG
HTR4 5dR	GGATAACAATTTCACACAGGATATGGGCTCTGCAGTTCTG
HTR4 6F	CACGACGTTGTAAAACGACTGGGTGCAGTTCATTTGG
HTR4 6R	GGATAACAATTTCACACAGGGGAGGCTGAATCTGACAATG

Sequencing Primers

M13Forward	CACGACGTTGTAAAACGAC
M13Reverse	GGATAACAATTTCACACAGG

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Supplementary Table 6. *CSF1R* Mutagenesis Primers.

CSF1R mut E633K F	GGCCCTCATGTCCAAGCTGAAGATCATG
CSF1R mut E633K R	CATGATCTTCAGCTTGGACATGAGGGCC
CSF1R mut M766T F	CAAGTAGCCCAGGGCACGGCCTTCCTCGCTTC
CSF1R mut M766T R	GAAGCGAGGAAGGCCGTGCCCTGGGCTACTTG
CSF1R mut M875T F	GAAGGATGGATACCAAACGGCCCAGCCTGCATTTG
CSF1R mut M875T R	CAAATGCAGGCTGGGCCGTTTGGTATCCATCCTTC
CSF1R c66F	GATAGAGCCCAGTGTCCCTGA
CSF1R c96R	CACCGTTGCTCCTGGCTTC
CSF1R c464R	ATGCCAGGGCGAGAAGG
CSF1R c501F	CAAGTTCATTCAGAGCCAGG
CSF1R c981R	CCTTGCAGGCCTGGGTAG
CSF1R c1011F	CTACCTGGGACCCTTTTCTG
CSF1R c1491R	CCTGCAGAGATGGGTATGAAG
CSF1R c1581F	CTTGCTGCTGCTGCTGCT
CSF1R c2081R	CTTATAGTCGACGCCTCCCT
CSF1R c2111F	TCGAGAAGAAATATGTCCGC
CSF1R c2561R	AGGATGCCAGGGTAGGGAT
CSF1R c2651F	ATATATACAGCATCATGCAG

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Supplementary Note. Description of human subjects included in this study.

This study included 14 families with a proven diagnosis of HDLS as determined by autopsy or brain biopsy in at least one family member: 11 US families of which 4 were previously described (FL1[4], CA2[9], SC[12] and VA[10]), one Norwegian family (NO), one German family (DE) and one Scottish family (SCT). All autopsy material was reviewed, and living patients and family members were prospectively enrolled using protocols approved by the Mayo Clinic IRB. The study was also approved by local ethical committees from each participating institution. Patient study populations further included 93 patients with Alzheimer's disease, 93 patients with frontotemporal dementia, 93 patients with Parkinson's disease, 93 patients with corticobasal syndrome, 61 patients with multiple sclerosis and 93 patients with ischemic stroke, all ascertained at Mayo Clinic Jacksonville, Florida. Demographic information on these patients is summarized in **Supplementary Table 2**. A cohort of 1487 unrelated controls free of neurodegenerative diseases ascertained at Mayo Clinic Jacksonville, Florida, were also available. These controls were recruited either through the Movement Disorder Clinic (n=752) and had a single clinical and neurological assessment or through the Dementia Clinic (n=735) and have repeated evaluations annually between 2 and 15 years. Mean age at examination of this cohort was 74 ± 11 years (range 22-99 years). For sequencing analyses of the complete *CSF1R* gene, 24 normal brains (mean age at death 79±12 years; range 53-93 years) were selected from the Mayo Clinic Jacksonville Brain Bank. The mean brain weight in this cohort was 1300g and none showed a Braak stage greater than 3. For the analyses of CSF1R phosphorylation in human tissue, frontal cortex brain samples from three normal brains, three HDLS patients, three AD patients and two patients with amyotrophic lateral sclerosis were selected from the Mayo Clinic

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Florida brain bank. Demographic information on these patients is summarized in **Supplementary Table 4**.

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