eTable 1 Demographics of subjects and positivity rates of anti-DRG satellite glial cell antibodies by tissue-based IFAs

Subjects	Patients with CIDP n = 113	Controls[#] n = 127	*p*
Male/female, n (ratio)	76/37 (0.5:1)	45/82 (1:0.5)	<0.001
Age at onset (age ± SD range) (years)	46.1 ± 17.5 (5.0-86.0)	Not available	
Age at examination (age ± SD range) (years)	52.4 ± 16.9 (18.0-91.0)	48.6 ± 17.8 (13.0-87.0)	NS
Positive rate of IFA using mice DRGs, n (%)	4/113 (3.5%)	0/127 (0%)	0.048

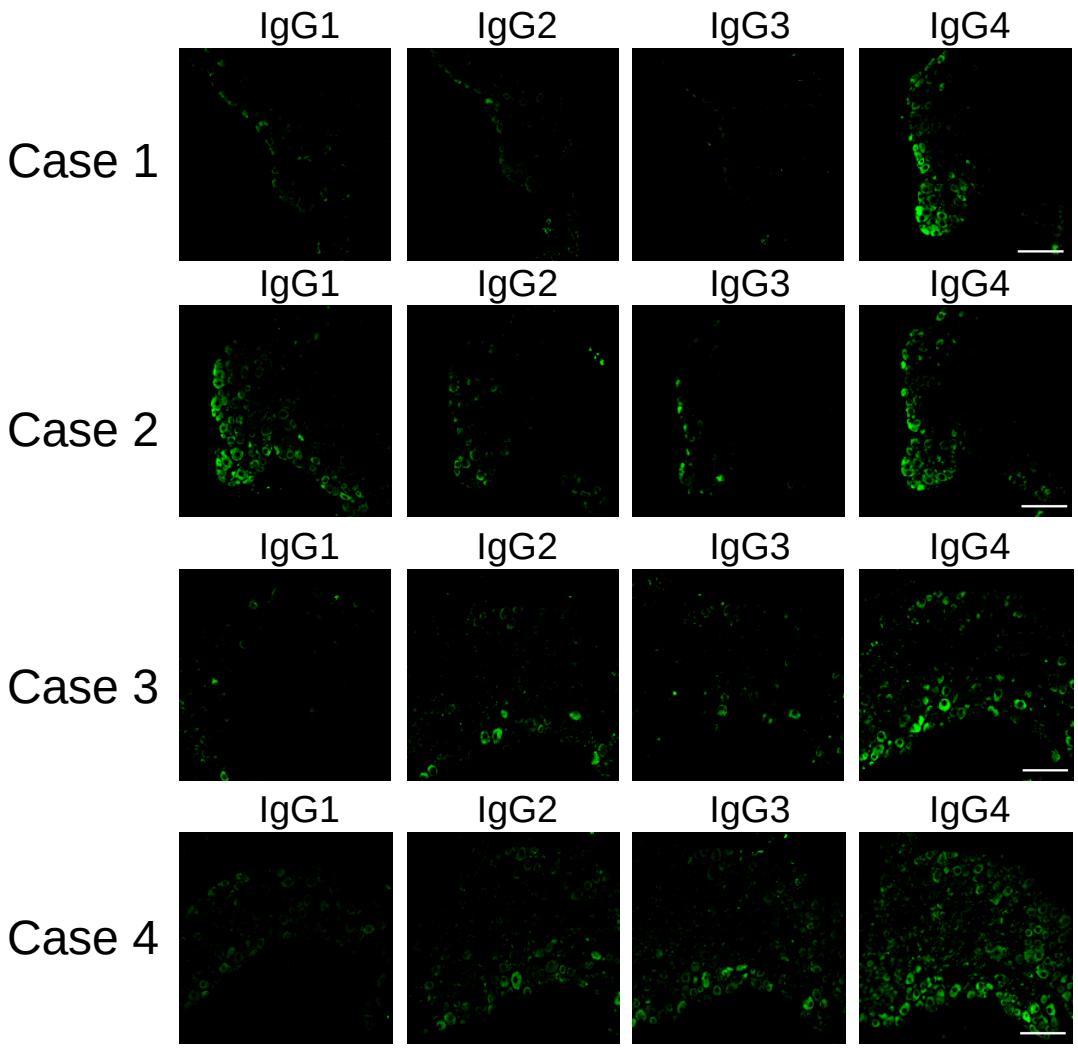
[#]Thirty five healthy controls and 92 patients with other neurological diseases were enrolled. Other neurological diseases include 19 with multiple sclerosis, 11 with Parkinsonism, 7 with amyotrophic lateral sclerosis, 8 with myelitis, 4 each with Guillain–Barré syndrome and autoimmune encephalitis, 3 each with neuromyelitis optica spectrum disorders, hereditary spinocerebellar degeneration, normal pressure hydrocephalus, myelopathy, and small fiber neuropathy, 2 each with dementia with Lewy bodies, spastic paraparesis, myasthenia gravis, and neuralgic pain, and 1 each with Alzheimer's disease, herpes simplex encephalitis, encephalopathy, epilepsy, tremor, optic neuritis, rheumatoid myeloradiculitis, Charcot–Marie–Tooth disease, mononeuritis multiplex, Bell's palsy, perineurioma, myokymia, piriformis syndrome, Isaacs syndrome, paresthesia, and reflex sympathetic dystrophy. Abbreviations: CIDP = chronic inflammatory demyelinating polyneuropathy; DRG = dorsal root ganglia; HC = healthy control; IFA = immunofluorescence assay; LGI4 = leucine-rich repeat LGI family member 4; NS = not significant.

eTable 2 Primer sequences

mRNAs		Sequence
Human *LGI4*	Forward	5′ -CACACGCTACATTGGGGACTC-3′
	Reverse	5′ -AGCCCCTTGTCAGGCTCAAG-3′
Rat *Lgi4*	Forward	5′ -TCGTGGAGTATGCGTCTCTG-3′
	Reverse	5′ -TGGAAAAAGGTACCCAGTGC-3′
Human *GAPDH*	Forward	5′ -ACCCACTCCTCCACCTTTGAC-3′
	Reverse	5′ -TGTTGCTGTAGCCAAATTCGTT-3′
Rat *Gapdh*[5]	Forward	5′ -AGGGCTGCCTTCTCTTGTGAC-3′
	Reverse	5′ -TGGGTAGAATCATACTGGAACATGTAG-3′
Rat *Krox20*	Forward	5′ -GGTGTGTGTACCATGTCCCA-3′
	Reverse	5′ -CCAGAGAGGAGGTGGAAGTG-3′
Rat *Periaxin*	Forward	5′ -AATGTGCCGAGCCCTACAAG-3′
	Reverse	5′ -AGGGGACAGACTCTGGATGT-3′

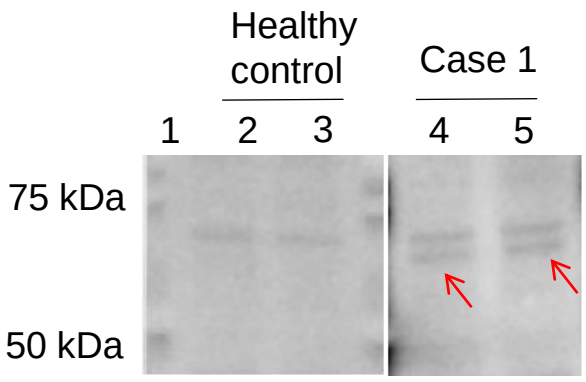
Abbreviations: *GAPDH = glyceraldehyde-3-phosphate dehydrogenase*; *Lgi4 = leucine rich repeat LGI family member 4*.

eFigure 1

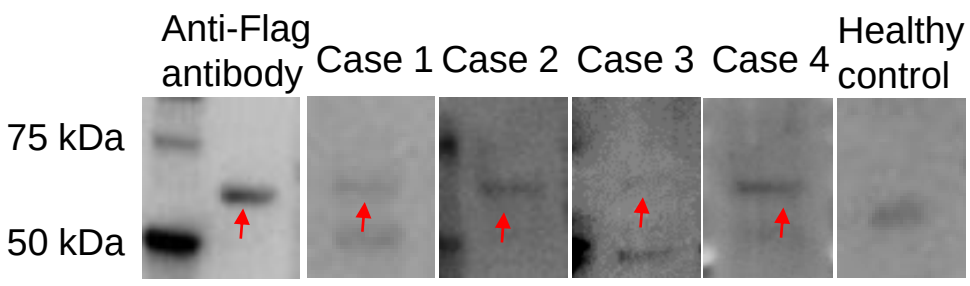

IgG1
IgG2
IgG3
IgG4
Case 1
IgG1
IgG2
IgG3
IgG4
Case 2
IgG1
IgG2
IgG3
IgG4
Case 3
IgG1
IgG2
IgG3
IgG4
Case 4

eFigure 2

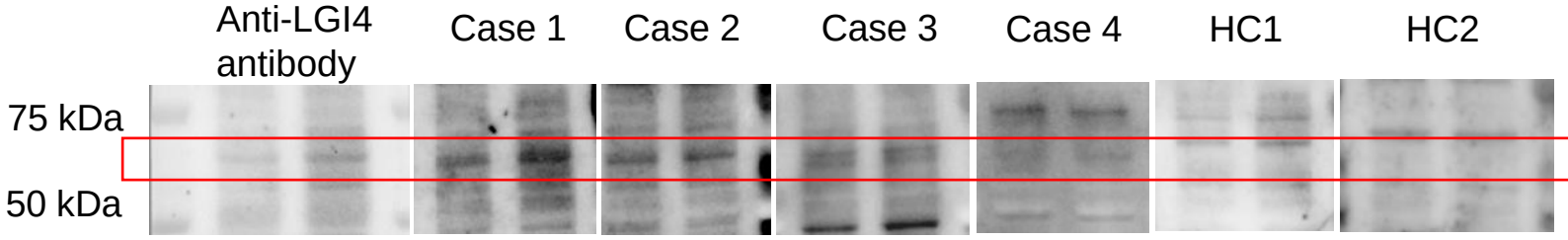
A



B



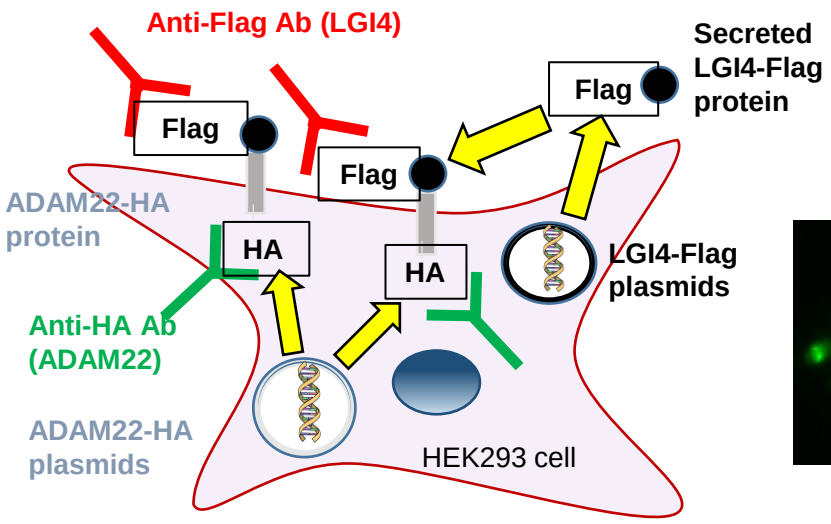
C

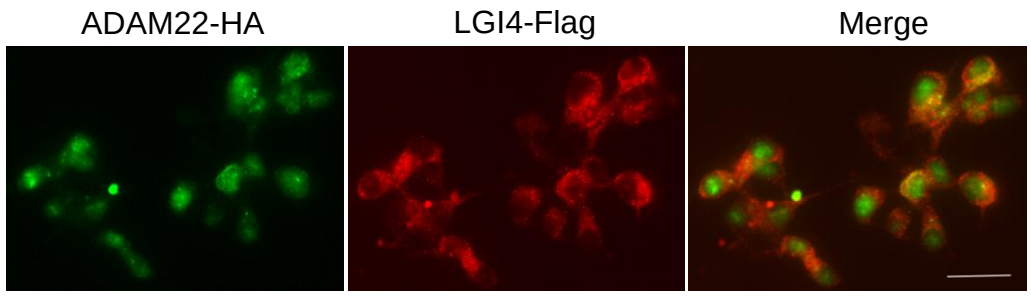


eFigure 3

A

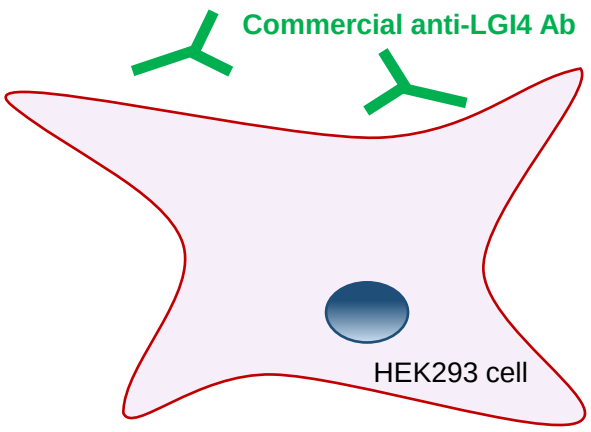
LGI4-Flag- and ADAM22-HA-cotransfected HEK293T cells stained by anti-HA and anti-Flag antibodies

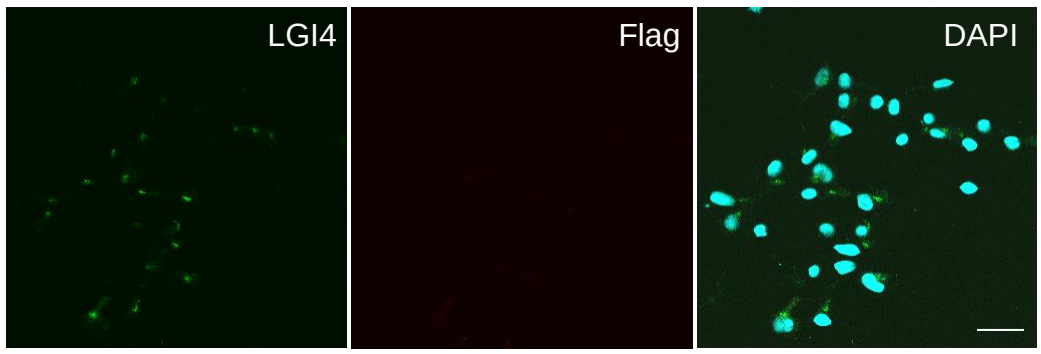




B

Naïve HEK293T cells stained by anti-Flag and commercial anti-LGI4 antibodies

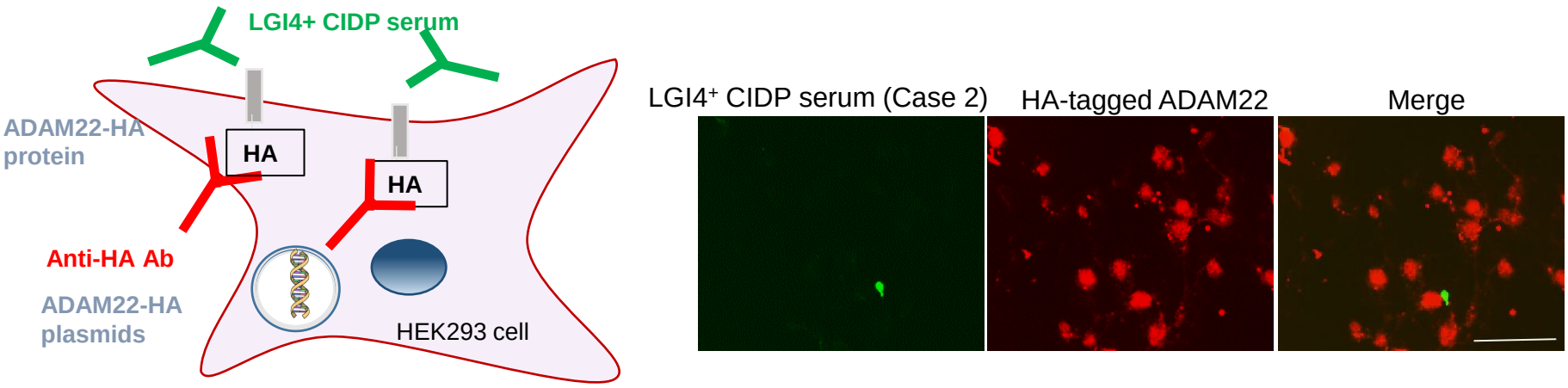




eFigure 4

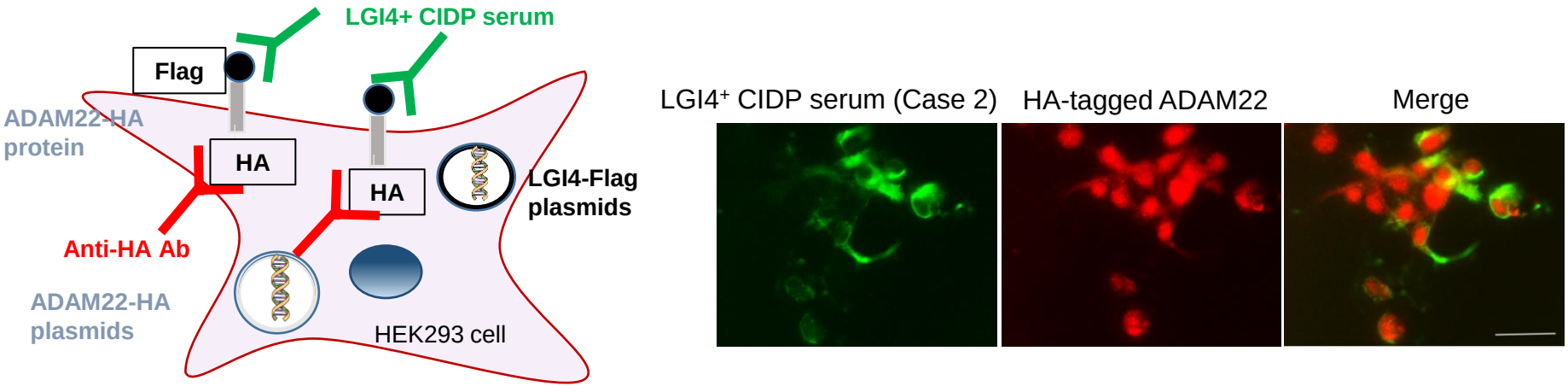
A

ADAM22-HA-transfected HEK293T cells stained by anti-HA antibodies and the seropositive patient's IgG



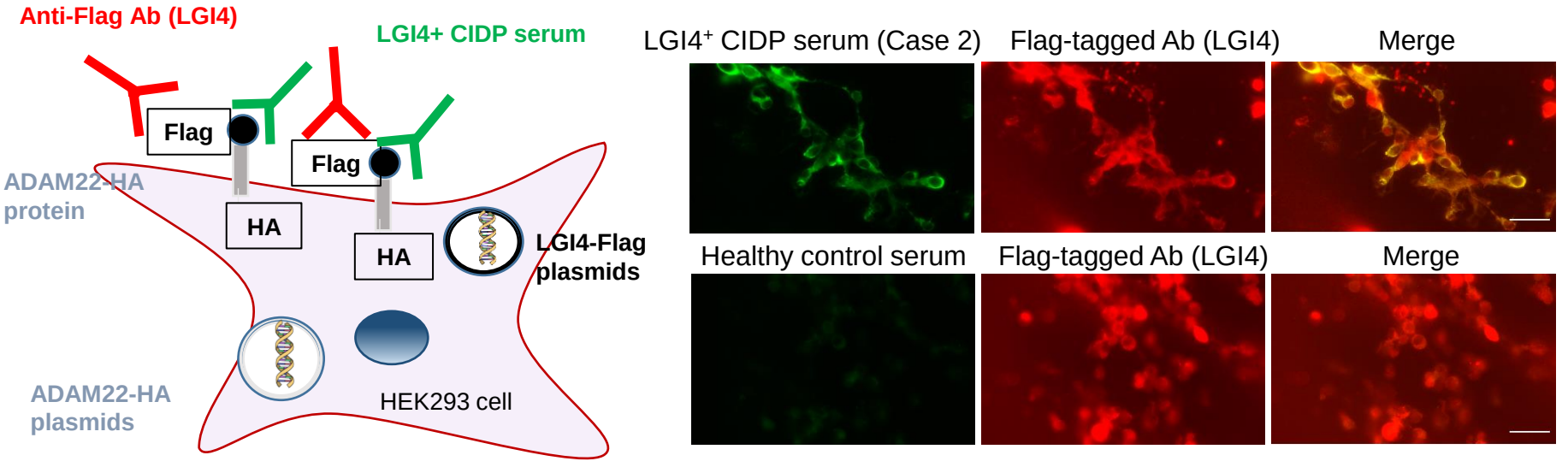
B

LGI4-Flag- and ADAM22-HA-cotransfected HEK293T cells stained by anti-HA antibodies and the seropositive patient's IgG

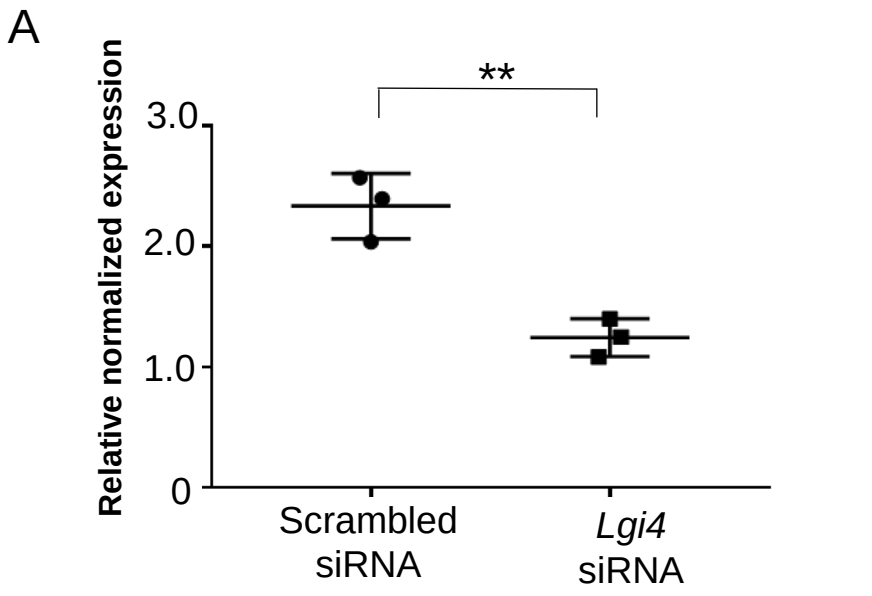


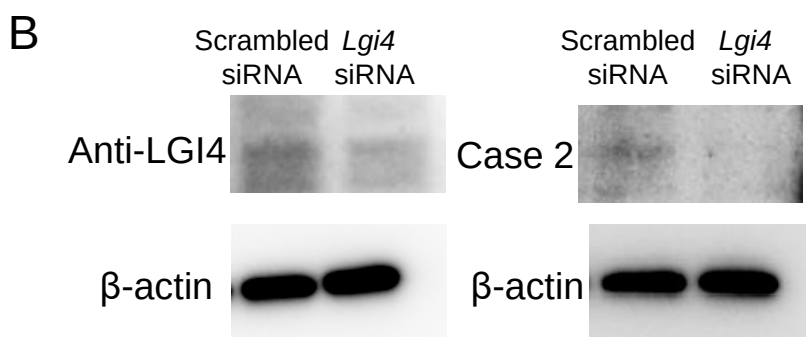
C

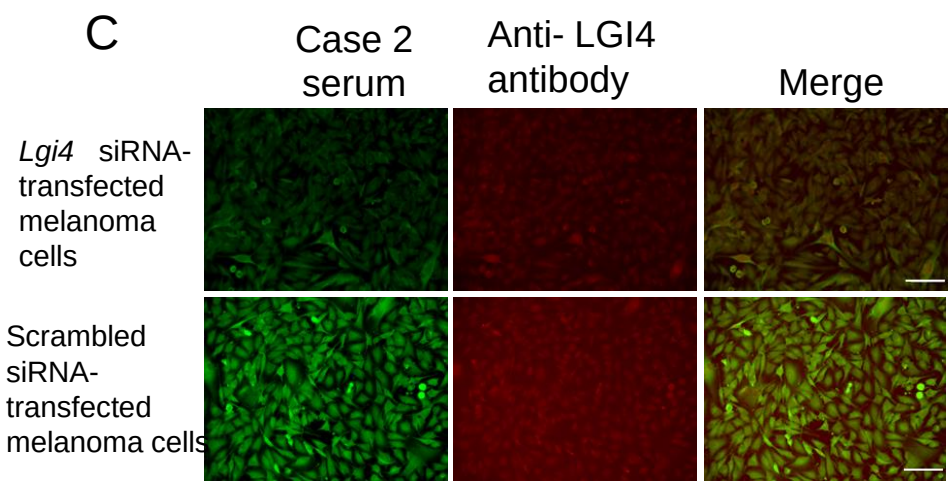
LGI4-Flag- and ADAM22-HA-cotransfected HEK293T cells stained by anti-Flag antibodies and the seropositive patient's IgG

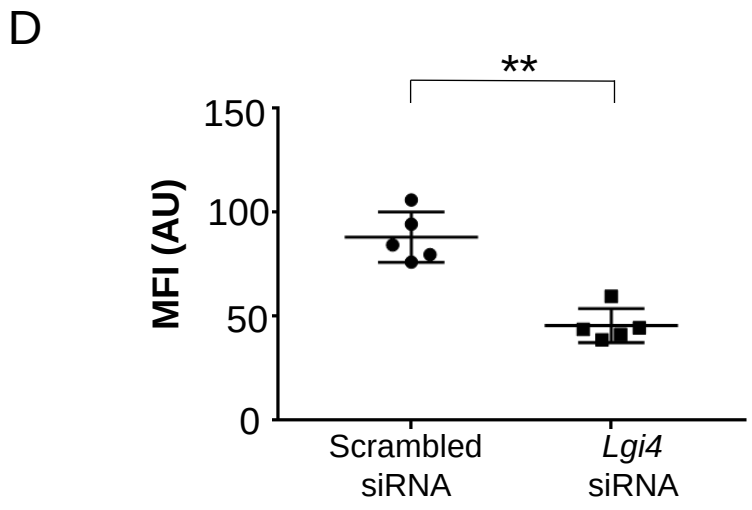


eFigure 5


A
Relative normalized expression
3.0
2.0
1.0
0
**
Scrambled siRNA
Lgi4 siRNA


B
Scrambled siRNA
Lgi4 siRNA
Anti-LGI4
β-actin
Scrambled siRNA
Lgi4 siRNA
Case 2
β-actin

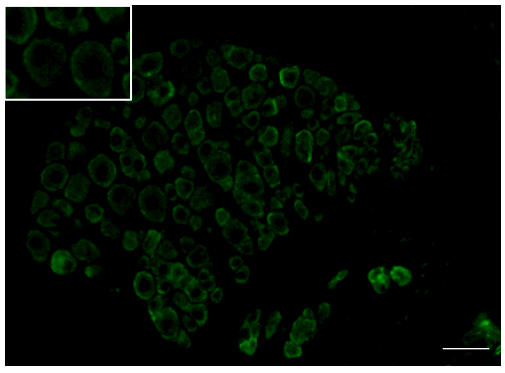

C
Case 2 serum
Anti- LGI4 antibody
Merge
Lgi4 siRNA-transfected melanoma cells
Scrambled siRNA-transfected melanoma cells


D
**
150
100
50
0
MFI (AU)
Scrambled siRNA
Lgi4 siRNA

eFigure 6

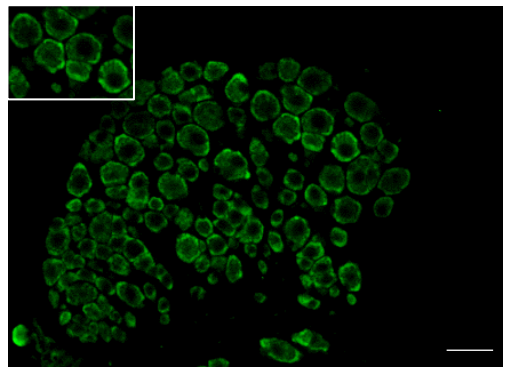
A

Pre-incubation with LGI4- and ADAM22 - co-transfected HEK293T cells

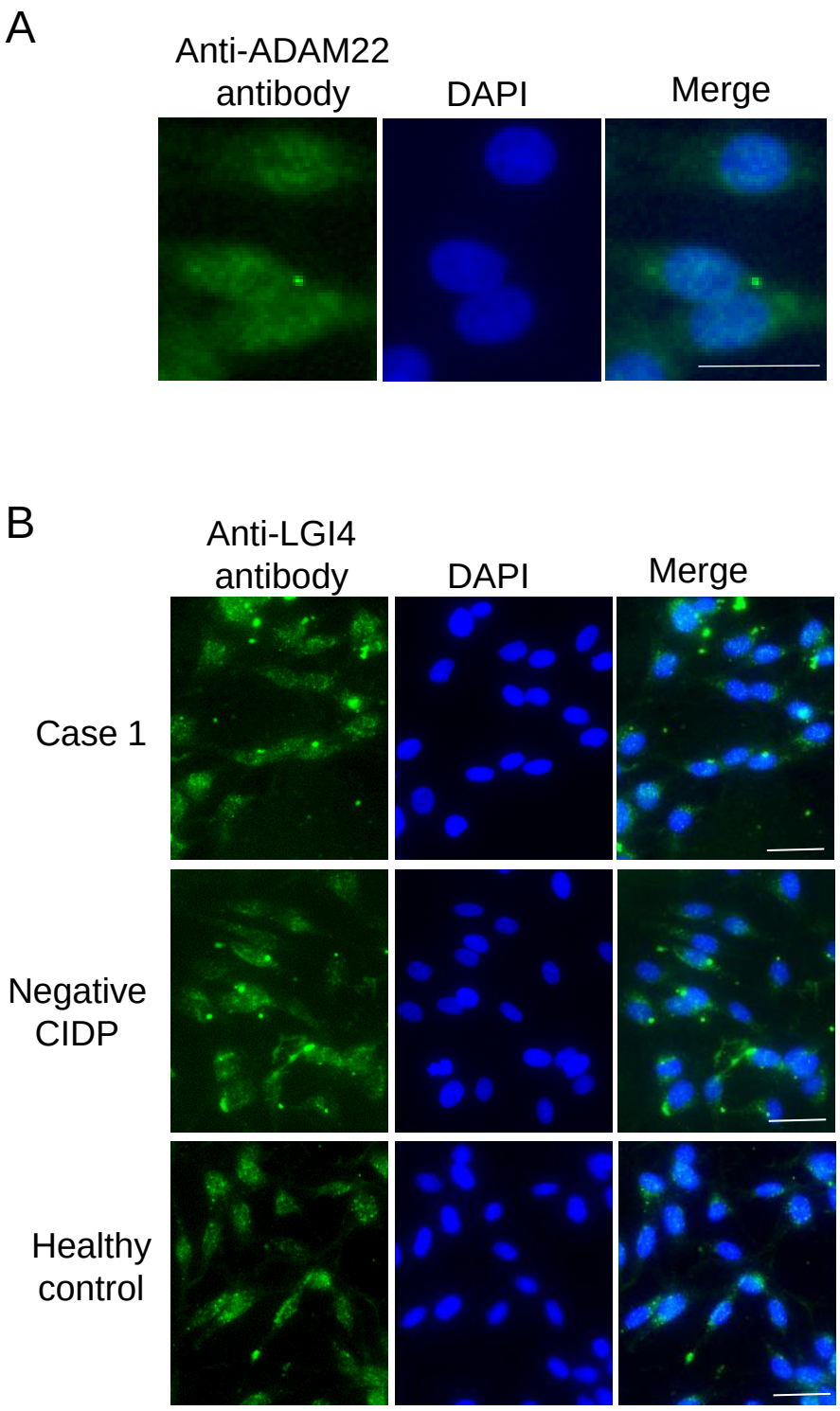


B

Pre-incubation with non-transfected HEK293T cells



eFigure 7


A
Anti-ADAM22 antibody
DAPI
Merge
B
Anti-LGI4 antibody
DAPI
Merge
Case 1
Negative CIDP
Healthy control

Supplementary Figure Legends

eFigure 1: IgG subclass analysis by DRG-based IFAs.

Scale bar: 30 μm. DRG = dorsal root ganglia; IFA = immunofluorescence assay.

eFigure 2: Identification of anti-LGI4 antibodies in sera from tissue-based IFA-positive CIDP patients by WB analysis.

(A) WB analysis of sera from a healthy control (lanes 2 and 3) and a representative tissue-based IFA-positive CIDP patient (Case 1 in Table 1, lanes 4 and 5) using mouse DRG tissue. Lane 1, molecular weight marker; lanes 2, 3, 4, and 5, mouse DRG lysate; a protein band of approximately 60 kDa is visible in DRG lysates in Case 1 but not in the healthy control. **(B)** WB analysis of anti-Flag antibody and sera from four tissue-based IFA-positive CIDP patients and a healthy control using Flag-tagged LGI4-overexpression cell lysates from HEK293T cells. IgG from all four patients bound to a lysate band of approximately 60 kDa, which is similar to the binding of the anti-Flag antibody. The healthy control showed no 60 kDa band. **(C)** WB analysis of the commercial anti-LGI4 antibody and IgG from four seropositive CIDP patients and two healthy controls using rat Schwann cell lysates. A 60-kDa band is visible with sera from all four CIDP patients and the commercial anti-LGI4 antibody but not with the healthy control sera. CIDP = chronic inflammatory demyelinating polyneuropathy; CASPR1 = contactin-associated protein 1; DRG = dorsal root ganglia; HEK = human embryonic kidney; IFA = immunofluorescence assay; LGI4 = leucine-rich repeat LGI family member 4; WB = western blotting.

eFigure 3: Immunostaining of LGI4-Flag- and ADAM22-HA-cotransfected HEK293T cells and naïve HEK293T cells by a cell-based IFA.

(**A**) The secreted Flag-tagged LGI4 was immunostained (red) by anti-Flag antibodies on the cell surface near ADAM22 immunostained (green) by anti-HA antibodies. **(B)** Naïve HEK293T cells stained with anti -Flag and commercial anti-LGI4 antibodies. Scale bar: 50 μm; ADAM22 = a disintegrin and metalloprotease domain-containing protein 22, CIDP = chronic inflammatory demyelinating polyneuropathy; HEK = human embryonic kidney; IFA = immunofluorescence assay; LGI4 = leucine-rich repeat LGI family member 4.

eFigure 4: Verification of patients' autoantibody specificity for LGI4 using a cell-based IFA.

(**A**) ADAM22-HA-transfected HEK293T cells double immunostained with anti-HA antibodies and IgG from a seropositive patient (Case 2) showed no binding of the patient's IgG. **(B)** LGI4-Flag- and ADAM22-HA-cotransfected HEK293T cells double immunostained with anti-HA antibodies and serum from a seropositive patient (Case 2) showed binding of the patient's IgG to the cotransfected cell surface. **(C)** LGI4-Flag- and ADAM22-HA-cotransfected HEK293T cells double immunostained with anti-Flag antibodies and serum from a seropositive patient (Case 2) or healthy control showed binding of the patient's IgG, but not that of the healthy control, to the cotransfected cell surface. Scale bar: **A**–**C**, 50 μm. ADAM22 = a disintegrin and metalloprotease domain-containing protein 22; HEK = human embryonic kidney; IFA = immunofluorescence assay; LGI4 = leucine-rich repeat LGI family member 4.

eFigure 5: Confirmation of the autoantibody specificity for LGI4 using a genetic strategy with a human melanoma cell line.

(A) Assessment of the effect of *Lgi4* siRNA transfection on *Lgi4* mRNA levels in human melanoma WM115 cells by quantitative real-time PCR. *Lgi4* mRNA levels were decreased after *Lgi4* siRNA treatment compared with those after scrambled siRNA treatment in human melanoma cells (**$p = 0.0039$, n = 3 cultures/group). The results are expressed as the mean ± SEM. The expression of the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* was determined. **(B)** WB analysis of LGI4 protein using human melanoma cells after *Lgi4* siRNA or scrambled siRNA treatment. IgG from one representative seropositive CIDP patient (Case 2) and a commercial anti-LGI4 antibody showed decreased signals after *Lgi4* siRNA treatment compared with those after scrambled siRNA treatment in human melanoma cells. **(C)** Cell-based IFA using *Lgi4* siRNA or scrambled siRNA-treated human melanoma cells. Signals in human melanoma cells from the serum of Case 2 and the anti-LGI4 antibody were significantly decreased after *Lgi4* siRNA treatment compared with those after scrambled siRNA treatment. **(D)** Comparison of the MFI from a CIDP patient's IgG between *Lgi4* siRNA and scrambled siRNA-treated human melanoma cells. *Lgi4* siRNA treatment decreased the MFI of IgG binding signals in the representative CIDP patient (Case 2) by 52% compared with scrambled siRNA treatment in human melanoma cells (**$p = 0.0046$, n = 5 cultures/group), indicating binding of the patient's IgG to LGI4. Nuclei are counterstained with DAPI (blue). The results are expressed as the mean ± SEM. Scale bars: **C**, 30 μm. AU = arbitrary units; CIDP = chronic inflammatory demyelinating polyneuropathy; DAPI = 4',6-diamidino-2-phenylindole; IFA =

immunofluorescence assay; LGI4 = leucine-rich repeat LGI family member 4; MFI = mean fluorescence intensity; WB = western blotting.

eFigure 6: Confirmation of the autoantibody specificity for LGI4 using an immunoadsorption assay.

Immunoadsorption assay including DRG immunostaining (green) with serum from a representative antibody-positive CIDP patient (Case 1 in Table 1) pre-incubated with LGI4-Flag- and ADAM22-HA-cotransfected HEK293T cells **(A)** or non-transfected HEK293T cells **(B)**. Scale bar: 50 µm. ADAM22 = a disintegrin and metalloprotease domain-containing protein 22; CIDP = chronic inflammatory demyelinating polyneuropathy; DRG = dorsal root ganglia; HEK = human embryonic kidney; IFA = immunofluorescence assay; LGI4 = leucine-rich repeat LGI family member 4.

eFigure 7: The effects of sera from anti-LGI4 antibody-seropositive CIDP patients and controls on LGI4 protein expression in Schwann cells.

(A) A cell-based IFA using a commercial anti-ADAM22 antibody shows that ADAM22, an LGI4 receptor, is expressed by rat Schwann cells. Nuclei are counterstained with DAPI (blue). **(B)** Treatment of rat Schwann cells with serum IgG from either $LGI4^+$ CIDP patients, seronegative CIDP patients, or healthy controls did not apparently alter LGI4 expression in cultured Schwann cells upon immunostaining. Scale bars: **A**, 20 µm; **B**, 50 µm. ADAM22 = a disintegrin and metalloprotease domain-containing protein 22; CIDP = chronic inflammatory demyelinating polyneuropathy; DAPI = 4′,6-diamidino-2-phenylindole; IFA = immunofluorescence assay; LGI4 = leucine-rich repeat LGI family member 4.