eMETHODS

1. Patient cohorts and sample acquisition
2. Electrodiagnostics
3. Rat dorsal root ganglia neurons and Schwann cells culture
4. DRG neuron and Schwann cell immunocytochemistry
5. Myelinating cell co-cultures
6. Myelinating cell co-culture immunofluorescence labelling
7. Peripheral nerve immunohistochemistry
8. Chemical and enzymatic characterisation of target antigens
9. Assessment of serum-induced demyelination
10. Glycoarray
11. Immunoprecipitation (IP) and mass spectrometry (MS)
12. Protein electrophoresis and western blotting and target protein identification
13. ELISA
14. Transfected-cell based assays
15. Statistical analysis

eFIGURES AND LEGENDS

eFigure 1. Simplified heat map showing all screening performed in ZIKV-GBS, ZIKV-OND, ZIKV-CON, DENV-CON, ALS, Dysferlin (DYS) and healthy controls (CON) patients.

eFigure 2. Glycolipid microarray results.

eFigure 3. Reactivity of ZIKV patients' serum IgG and IgM antibodies against primary cultured rat DRG neurons and Schwann cells.

eFigure 4. Patterns of ZIKV patients' serum IgM antibody reactivity against myelinating cell co-cultures.

eFigure 5. Patterns of ZIKV patients' serum IgG antibody reactivity against myelinating cell co-cultures.

eFigure 6. Confirmation of complement-dependent demyelinating activity in ZIKV-CON patient B serum.

eTABLES

eTable 1. Summary of ZIKV patient cohorts and controls.

eREFERENCES

eMETHODS

1. Patient cohorts and sample acquisition

Serum samples were collected from informed and consenting: a) patients with ZIKV infections with and without neurological complications, b) patients with other infectious (e.g. DENV: dengue virus) and non-infectious diseases, as well as c) healthy and convalescent ZIKV controls from three separate patient cohorts from different regions of Colombia: Cali (n=89), Cucuta (n=118) and Barranquilla (n=11). ZIKV patients with other inflammatory neurological diseases (ZIKV-OND) included myeloradiculopathy (n=1), encephalitis (n=5), meningoencephalitis (n=1), peripheral facial palsy (n=3) or transverse myelitis (n=7). The Spanish subjects (n=38) included 28 healthy controls and 10 patients with non-immune neuromuscular disorders amyotrophic lateral sclerosis (ALS) (n=5) and dysferlin muscular dystrophy (Dysferlin) (n=5). Sample collection was performed in compliance with Act 008430/1993 of the Ministry of Health of the Republic of Colombia, and the institutional review board of the Universidad del Rosario. Additional samples from healthy subjects with non-immune mediated neurological disorders, and nodal/paranodal antibody positive patients, were obtained for use from local collections in Oxford, UK (South Central - Oxford A Research Ethics Committee approval number 14/SC/0280) or Barcelona, Spain (The Sant Pau Biomedical Research Institute ethical approval number IIBSP-AUT-2016-69). All of these samples were coded and anonymized. Group sizes were based on sample availability and were not decided *a priori*. Whole blood was collected by venepuncture in SST II BD Vacutainer Tubes (BD Bioscience) and after clot retraction they were centrifuged at >2000 x g for 20-30 min. The separated serum was then aliquoted and stored at -80°C, with transportation on dry ice where required.

2. Electrodiagnostics

Electrodiagnostics was employed to classify GBS patients as either acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN) or acute motor and sensory axonal neuropathy (AMSAN). Nerve conduction studies were evaluated according to Uncini's criteria [e1]. Motor conduction studies, were evaluated in the median, ulnar, peroneal and tibial motor nerves whereas sensory nerve action potential and conduction velocity were measured in median, sural and ulnar nerves. F wave and H reflex were also registered. Where electrodiagnostics were unavailable patients were categorised as 'unclassified'.

3. Rat dorsal root ganglia neurons and Schwann cells culture

Animal procedures were performed according to Schedule 1 of the UK Home Office Animals (Scientific Procedures) Act 1986, and a protocol approved by the Animal Ethics' Committee of Hospital de la Santa Creu i Sant Pau. Dorsal root ganglia (DRG) were dissected from E16 rat embryos, dissociated and plated on glass coverslips coated with laminin (2.5 µg/ml) (Invitrogen, CA, USA) and poly-D-lysine (PDL) (25 µg/ml) (Sigma, MO, USA). These cells were then grown in neurobasal medium supplemented with B-27 (Cat 12587-010) and Glutamax (Cat. 35050-038) (all Gibco, Life Technologies) and mouse nerve growth factor (mNGF) (50 ng/ml) (Invitrogen). After 24 hours, cytosine arabinoside (Ara-C) (1 µM) (C1768, Sigma), floxuridine (40 µM) (PHR2589, Sigma) and uridine (40µM) (U3003, Sigma) were added to the medium to remove fibroblasts. This culture medium was replaced every other day until complete growth and differentiation of the DRG neurons were attained.

Schwann cells (SCs) were harvested from the sciatic nerves of 3 days-old rat pups, and cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose (Thermo Fisher scientific, MA, USA), 10% fetal bovine serum (FBS) (Gibco), mNGF and penicillin-streptomycin (Lonza, Basel, Switzerland).

After 24h, Ara-C (Sigma) was added to the medium to remove fibroblasts. Three days later the medium was changed to a commercial Schwann cell medium (ScienCell, CA, USA) supplemented with 10% FBS, penicillin-streptomycin, forskolin (F3917,Sigma) and neuregulin 1-β1 (NRG1-β1) (R&D systems). This medium was replaced every 2 days until the cells had sufficiently proliferated for harvest. These cells were dissociated by trypsin (0.25%, 5 min) diluted in DMEM and then plated on PDL coated coverslips until 70-80% confluency was reached. The cells were then fixed with 4% paraformaldehyde (PFA) in PBS, blocked for 1 hour with 5% goat serum in PBS and frozen at -80°C until immunocytochemistry experiments were performed.

4. DRG neuron and Schwann cell immunocytochemistry

Live DRG neurons were incubated for 1 hour with patients' sera diluted 1:100 (for IgG antibody experiments) or 1:40 (for IgM antibody experiments) in culture medium at 37°C. These cells were then fixed for 10 min with 4% PFA, followed by incubation with Alexa Fluor 488-conjugated goat anti-human IgG or IgM (1:1000 dilution) secondary antibodies (Thermo Fisher Scientific). Immunocytochemistry experiments on Schwann cells were performed on previously fixed and blocked frozen cells using patients' sera diluted 1:100

(for IgG antibody labelling) or 1:40 (for IgM antibody labeling) and appropriate secondary antibodies (1:1000 dilution). Coverslips were mounted with Vectashield with DAPI (Vector Laboratories, CA, USA) and the fluorescence signal intensities were scored by two independent researchers blinded to the diagnostic category within three weeks of immunostaining. Immunocytochemistry results were grouped according to three separate categories: moderate to strong positives (including scores 2 and 3), all positives (including scores 1, 2 and 3), and negatives (score 0). Images were obtained with an Olympus BX51 Fluorescence Microscope (Olympus Corporation, Tokyo, Japan).

5. Myelinating cell co-cultures

Myelinating cell co-cultures were prepared using human induced pluripotent stem cell (hiPSC)-derived sensory neurons and primary rat Schwann cells as previously described [e2]. hiPSCs from control subjects were obtained via the University of Oxford StemBANCC consortium. In brief, hiPSCs were differentiated into sensory neurons using a combination of small-molecule mediated dual SMAD (mothers against decapentaplegic family transcription factor) inhibition and Wnt pathway activation. On day 11 of differentiation, the sensory neuron precursors were seeded onto 13 mm diameter glass coverslips (approximately 20,000 cells per coverslip) or tissue culture-treated 10 cm petri dishes (approximately 200,000 per dish). Coverslips were previously cleaned by acid-washing, and coated with PDL (10 µg/ml) and reduced growth-factor Matrigel (Corning). Neurons were matured for 2-4 weeks in neurobasal media supplemented with 1x N2 (Cat. 17502-048), B-27 (Cat 12587-010), Glutamax (Cat. 35050-038) and 1x antibiotic-antimycotic (penicillin, streptomycin and amphotericin) mixture (Cat. 15240-062) (all Gibco, Life Technologies) ('complete' neurobasal medium) (see above) plus recombinant human β-NGF (rhNGF) (Cat. 450-01, Peprotech), NT3 (Cat. 450-03, Peprotech), GDNF (Cat. 450-10, Peprotech), and BDNF (Cat. PHC7074, Life Technologies). All growth factors were used at 25 ng/ml. Primary Schwann cells were isolated from rat pups (P2-3) according to the protocol above, added to the neuronal cultures (25,000 cells per coverslip; 200,000 cell per 10 cm dish) and allowed to proliferate and align with the axons for 1 week in basal media containing insulin (5 µg/ml) (I9278,Sigma), holo-transferrin (100 µg/ml) (T0665,Sigma), rhNGF (25 ng/ml) (Peprotech) (Sigma), selenium (25 ng/ml) (S5261,Sigma), thyroxine (25 ng/ml) (T1775,Sigma), progesterone (30 ng/ml) (P6149,Sigma), triiodothyronine (25 ng/ml) (T6397,Sigma) and putrescine 8 µg/ml (P6024,Sigma) in DMEM/F12 media (Gibco, Life Technologies). From this point on, these cell cultures were maintained in 'myelination medium' containing 5% CS-FBS, ascorbic acid (25 µg/ml), phenol-free Matrigel (1:300

dilution) (Corning) and hrNGF (25 ng/ml) in 'complete' neurobasal medium. Myelinating cultures were matured for at least 4 weeks before use in subsequent experiments.

6. Myelinating cell co-culture immunofluorescence labelling

To assess the immuno-reactivity against myelinating co-cultures, sera from ZIKV-exposed subjects was diluted 1:100 in 'complete' neurobasal media supplemented with rhNGF (25 ng/ml) 1% bovine serum albumin (BSA), and added to these live co-cultures for 1h at 37°C. These cultures were then washed in three changes of DMEM containing 20mM HEPES (DMEM/HEPES) and fixed in 1-2% paraformaldehyde (PFA) for 30 minutes at room temperature (RT). Alexa Fluor-488 conjugated goat anti-human IgG (H+L) (A11013, Life Technologies), F(ab')2 goat anti-human IgG Fc (H10120, Life Technologies) or goat anti-human IgM (μ chain specific) (A21215, Life Technologies) secondary antibodies were then added at a 1:750 dilution for 1h RT in DMEM/HEPES with 1% BSA, before washing in DMEM/HEPES. For the IgG antibody subclass determinations, mouse anti-human IgG1 to 4 (I2513, I5635, I7260, I7385, Sigma) were added at a 1:50 dilution in DMEM/HEPES plus 1% BSA for 1h at RT, followed by Alexa 488-conjugated goat anti-mouse IgG secondary antibodies (1:1000) (A-11029, Life Technologies).

For the assessment of complement deposition, rabbit anti-human complement C3c-specific antibody (A006202-2, Dako) at a dilution of 1:200 was added and after reaction and washing the bound anti-C3c IgG was detected by Alexa 488-conjugated goat anti-rabbit IgG (A-11008, Life Technologies) (1:1000 dilution). These cells were then permeabilised with ice-cold 100% methanol for 25 minutes, rinsed in PBS, blocked in 5% NGS for 1h at RT and incubated with chicken anti-neurofilament heavy chain (NF200) (ab4680, Abcam) (1:10,000 dilution) and rat anti-myelin basic protein (MBP) (ab7349, Abcam) (1:500 dilution) primary antibodies overnight at 4°C to label neuronal processes and myelin internodes, respectively. After washing in PBS, these cells were incubated with biotinylated goat anti-chicken IgY (BA-9010, Vector) (1:500 dilution) and Alexa 546-labeled goat anti-rat IgG (A11081, Life Technologies) (1:1000 dilution) secondary antibodies for 1h at RT, followed by streptavidin-conjugated Pacific Blue (S11222, Life Technologies) (1:500 dilution) for 45-60 min at RT. The coverslips were then mounted onto microscope slides (Superfrost Plus, Thermo Scientific) using Vectashield mounting medium (H1000, Vector Laboratories) and stored at -20°C until imaging. The images were then acquired using a confocal laser scanning microscopy (LSM 700, Zeiss) with a 63x oil-immersion lens (with a 10x eye-piece = 630x magnification) and exported as

maximum intensity projection of 4-5 z-sections at 1 µm intervals. These images were then assessed for IgG or IgM antibody reactivity to distinct topographical domains by an operator blinded to the diagnostic category of the samples. These serum reactions were categorised as either positive or negative, and the topographical binding patterns were recorded for the following categories: myelin/abaxonal (including outpouchings), and/or Schwann cell and nodal/axonal binding.

7. Peripheral nerve immunohistochemistry

Macaque peripheral nerve tissue slides (Inova Diagnostics, Inc., San Diego, CA) were blocked with 5% normal goat serum in PBS; followed by incubation with patients' sera at 1:10 (for IgM) or 1:20 (for IgG). Monkey-adsorbed Alexa Fluor-488 conjugated goat anti-human IgG (Southern Biotech, Alabama, USA) or goat anti-human IgM (Molecular Probes) were used as secondary antibodies at 1/500 concentration. Finally, slides were mounted with Fluoromount medium (Sigma) and examined by two independent observers. Immunostaining patterns were analysed scoring fluorescence signal intensity of each nerve structure in a 0-3 scale. The nerve structures analysed were: nodes or paranodes, myelin from small myelinated fibers, myelin from large myelinated fibers, Schwann cells from unmyelinated fibers, large fiber axons, and small fiber axons. Reactivity against other non-nerve structures (fibroblasts, connective tissue, vessels) was not considered in the analysis. To further study the staining patterns, peripheral nerve tissue slides were coated with mouse anti-human CD56 antibody (Becton Dickinson, New Jersey, USA) at 1:50 to stain non-myelinating Schwann cells (Remak bundles). Alexa Fluor-594 conjugated goat anti-mouse IgG (Life Technologies) was used as secondary antibody (1:500 dilution). Images were acquired using Leica TSC SP5 confocal microscope.

8. Chemical and enzymatic characterisation of target antigens

For lipid-extraction, serum-incubated cell cultures were washed with PBS and fixed with 4% PFA for 30 min at RT before treatment with or without a solvent mixture of chloroform, methanol and water at a ratio of 4:8:3 [e3] for 1h on ice. For the enzymatic digestion of sialylated antigens, live myelinated cell co-cultures were pre-treated with 1U/ml neuraminidase (from *Clostridium perfringens,* Sigma, N2876) in PBS containing Ca^{2+} and Mg^{2+} (D-PBS) for 14 h at 37°C. After washing 3 times in D-PBS, these cultures were subsequently incubated with patients' serum for 1h at 37°C before washing in PBS and fixing with 2% PFA for 20 min at RT. Subsequently, the coverslips were immunolabelled for human IgG antibodies, MBP and NF200 following the protocol above.

9. Assessment of serum-induced demyelination

Myelinating cell cultures were incubated for 1h in 'complete' neurobasal media supplemented with fluoromyelin red (F34652, Fisher Scientific) (1:300 dilution), washed 3 times in PBS, and placed back into myelination medium. Baseline myelin coverage was imaged on an inverted microscope (Leica DM IL) fitted with LED fluorescence excitation (pE-300, CoolLED) and the results were obtained using a digital camera. Serum-free myelination medium was then supplemented with or without ZIKV patients' sera at a 1:100 dilution containing 20% normal human serum (NHS) as a source of serum complement proteins and added to these co-cultures. After 24h incubation at 37°C, the culture medium was replaced, re-stained with fluoromyelin and re-imaged. To illustrate the change in myelination after the 24h incubation period with serum compared to baseline, the layers images were overlaid using the transparency function in Photoshop (Adobe). For quantification, the myelin internodes on the original, unprocessed baseline and 24h images were counted and measured using the line measurement function on ImageJ (NIH). Time-lapse images of demyelination during ZIKV patients' serum-treatment (1:50 dilution) at 37°C were acquired every 15 min at 10 times magnification using phase contrast and red channel (565–605 nm excitation) epi-fluorescence on an IncuCyte S3 live-cell imager (Sartorius). Fluoromyelin staining was used to confirm myelin internodes at the beginning (0h) and end (16h) of the experiment. The complement-fixing human monoclonal antiganglioside IgM antibody HA1 used a positive control was cloned after EBV transformation of peripheral blood mononuclear cells taken from a patient with CANOMAD, as previously described [e4].

For assessment of demyelination in post-fixed cultures, duplicate co-cultures on 13 mm diameter, coverslips were treated with one of four ZIKV patient sera (1:50 dilution) in N2 'complete' media supplemented with 1% BSA and huNGF (25 ng/ml) for 1hr at 37°C (250 µl per well). Normal human serum (NHS) (from F, 36-40y donor) was added to one of duplicate wells (62.5 µl) for final concentration of 20% NHS and incubated 24h at 37°C. N2 'complete' media only was added to control wells. Wells were washed 4x PBS and fixed 4% PFA, 30 min at RT. Cells were immunostained for human IgG, as well as MBP and NF200, as described. For quantification of demyelination, coverslips were mounted on glass slides for confocal imaging. A pre-set of 5x5 grid of positions (or 7x7 for sparsely myelinated coverslips) at x20 magnification (0.5 digital zoom) was used for automated acquisition across each coverslip. NF200 (405 nm, blue) and MBP (555 nm, red) were set to multitrack for simultaneous acquisition. 5-7x 3 µm z-sections were collected to ensure cell culture features were imaged at all points along each coverslip. Images at

each position were exported as maximum projections and used for counting intact and fragmented internodes in each field of view. For quantification of myelin feature size, the MBP signal was thresholded in ImageJ. The Analyse Particle function was used to count and measure the size of myelin features (5-infinity μm^2) per region of interest (25-49 ROI per coverslip). Average myelin features size was calculated as: total myelin area/number of features. Smaller values indicate greater fragmentation of myelin.

10. Glycoarray

Serum samples were screened on a glycolipid microarray as previously described [e5]. In brief, all sera were screened against a panel of 16 single glycolipids (GM1, GM2, phosphatidylserine, GM4, GA1, GD1a, GD1b, GT1a, GT1b, GQ1b, GD3, SGPG, LM1, cholesterol, GalC and sulphatide) and 120 heteromeric 1:1 (v:v) complexes, printed in duplicate. Array slides were blocked with 2% BSA in PBS for 1 hour at room temperature, prior to addition of the patients' serum samples.

FAST incubation chambers and frames (GVS, USA) were used to separate the individual arrays on each slide, to which 100µl of each human serum sample, diluted at 1:50 in 1% BSA/PBS was added for 1h at 4°C. Following two 15 min washes in 1% BSA/PBS, anti-glycolipid antibody reactivities were detected using fluorescently conjugated, isotype specific, anti-human IgG and IgM antibodies (see above). Arrays were scanned with a Genepix 4300A (Molecular Devices, USA) and the average of duplicate median fluorescent intensity for each glycolipid target was calculated. Anti-glycolipid antibody binding intensities were displayed on a heat map (MeV software) using Pearson's correlation hierarchical clustering.

11. Immunoprecipitation and mass spectrometry

Human sera that showed moderate or strong (scores 2 or 3) reactivity against rat DRG neurons were used for the immunoprecipitation (IP) experiments using the same cell types as substrates as previously reported [e6]. Briefly, protein A and G agarose beads (Invitrogen) or anti-human IgM-agarose antibody (Sigma) were used to isolate human serum IgG or IgM which was allowed to bind overnight to the rat DRG neuron cell culture extract. The precipitated proteins were detached from the agarose beads using Laemmli sample buffer (BioRad, CA, USA) containing with 5% 2-mercaptoethanol and separated by electrophoresis. The protein bands which appeared using the patients' serum IPs but not in the controls, were analysed by mass spectrometry. Proteins were selected as candidate antigens using Anaxomics (Anaxomics Biotech SL, Spain). Before filtering, the

software deleted non-specific and low-signal antigens from the uploaded data, and only entries that met the following criteria were analysed: a) protein scores >100, b) peptide sequence coverage >5% or c) two or more peptides identified with the absence of the same criteria in the control sample. After this treatment, the software applied a set of sequential filters of inclusion, including: nerve or brain expressed proteins, membrane proteins and surface proteins.

Correspondingly, myelinating cell co-cultures or sensory neuron monocultures were scaled up into 10 cm diameter petri dishes to provide a substrate for immunoprecipitation (IP) of the positive human sera identified in the co-culture system. After >8 weeks of maturation in myelination medium, the sera from selected ZIKV-exposed and control subjects were diluted 1:100 in neurobasal media supplemented with huNGF (25/ng/ml) and 1% BSA and added to live co-cultures for 1h at 37°C. The cells were then washed 3 times with ice-cold PBS and lysed for 15 minutes on ice with 500 μl of RIPA buffer supplemented with a 1X protease inhibitor cocktail (Halt, ThermoFisher Scientific). These cells were then gently scraped from the plate into a 1.5 ml tube and incubated for 30 min at 4ºC with gentle mixing / inversion. Homogenisation was ensured through repeated pipetting before the samples were then centrifuged in a benchtop centrifuge at >10,000 x g for 5 min at 4°C. These supernatants were then collected and incubated with 50 μl of protein G dynabeads (Invitrogen) for 2hr at 4ºC on a rotating mixer and then washed 3 times with 200 μl PBS using a magnetic rack. Elution was performed using 200 μl of 0.1M glycine/HCl (pH 2.6) added for 2 min at RT and repeated a further 2 times (total 600 μl).

For mass spectrometry, IP eluates from myelinating cell co-cultures were prepared by chloroform:methanol precipitation and in-solution trypsin digestion. These peptides were then subjected to C18 reversed-phase HPLC column chromatography and analysed by data-dependant MS/MS on a ThermoFisher Fusion Lumos mass spectrometer. Antigen-bound IgG from the ZIKV patients' sera was compared with serum containing IgG antibodies reactive with known nerve cell antigens to enable calculations of their relative protein enrichments. For comparison of the ZIKV-CON patient B's serum and the anti-neurofascin-155 (NF155) antibody positive sera, IPs were generated from lysates from myelinated cultures of sensory neurons derived from four donor hiPSC lines.

For statistical analysis of biological replicates, sample peptide data were aligned with concatenated human, rat and ZIKV reference proteomes (UniProt.org) and split using Progenesis QI (Nonlinear Dynamics) to generate raw abundance values. Protein

enrichment was quantified by transforming their normalised abundance values and presented in a volcano plot using Perseus (MaxQuant) [e7]. For single sample comparison of ZIKV-GBS patient A's serum, label free quantification was performed using Peaks software v7 (Bioinformatics Solutions Inc.). For in-gel digests, raw files from each LC-MS/MS injection were converted to a MASCOT Generic Format (MGF) and searched on the MASCOT server (version 2.5.1) against the UPR *Homo sapiens* and *Rattus norvegicus* databases (UniProt.org). Unique peptides were exported and compared for presence or absence in either co-culture or neuronal culture lysates. Full data sets are available upon request.. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [e8] partner repository with the dataset identifier PXD028476 and 10.6019/PXD028476

12. Protein electrophoresis and western blotting for target protein identification

Lysates from mature myelinating cell co-cultures, hiPSC-derived sensory neurons monocultures, and primary rat Schwann cells were prepared with 500μl RIPA buffer supplemented with a 1X protease inhibitor cocktail (Halt, ThermoFisher Scientific). Total protein content was quantified using a bicinchoninic acid assay kit (Pierce BCA Protein Assay Kit, ThermoFisher Scientific) according to manufacturer's instructions. 10, 20 and 40μg of each lysate was prepared in lithium dodecyl sulfate (LDS) sample buffer, heated to 37°C for 10 minutes, loaded into separate wells of 4-12% continuous acrylamide Bis-Tris mini-gels and separated by electrophoresis in MOPS running buffer (ThermoFisher Scientific) on a Mini Gel tank electrophoresis system (ThermoFisher Scientific).

The separated proteins were then either wet-transferred to 0.45 μm pore-sized nitrocellulose membranes using a mini-blot module with Bolt transfer buffer (ThermoFisher Scientific) for western blotting, or in-gel stained with Pierce Imperial Protein Stain (ThermoFisher Scientific) according to manufacturer's instructions.

Following the protein transfer, the nitrocellulose membranes were blocked in 5% milk powder (Sigma) dissolved in Tris-buffered saline containing 0.1% v/v Tween-20 (TBS-T) for 1h at room temperature.

ZIKV-CON patient B's serum diluted 1:2500 in blocking solution was then allowed to react with the membrane overnight at 4°C. These blots were then washed using 7 changes of TBS-T buffer. A horse-radish peroxidase (HRP)-conjugated Fc-specific anti-human-IgG secondary antibody (A0170, Sigma) diluted 1:3000 in blocking solution was

then reacted with the membrane for 1h at RT. Detection of the bound IgG autoantibodies was then performed with ECL Prime substrate (GE Healthcare) and the results were developed using radiographic film.

For the subsequent mass spectrometry-based target antigen identification, the bands detected by western blotting were aligned with those in parallel-developed gel stained with the Pierce Imperial Protein Stain (ThermoFisher Scientific). To identify the target protein bound by patient B's IgG autoantibodies gel, the bands identified in the co-culture lysate, along with corresponding positions in the neuron-only and Schwann cell lysates were excised from the chemically stained gel and subjected to in-gel trypsin digestion.

The peptides were then subjected to C18 reverse-phase column chromatography and analysed by data-dependant MS/MS on a ThermoFisher Fusion Lumos mass spectrometer as described above.

13. ELISA

Recombinant human nidogen-1 protein (2570-ND, R&D Systems), laminins (111, 121, 211, 221, 411, 421, 511 and 521) (BioLamina), prosaposin (sulphated glycoprotein-1) (Enzo Life Sciences) and vinculin (VINC, Origene) were prepared according to the manufacturer's instructions and were added at 1 μg/ml (100 μl per well) to Nunc Maxisorp ELISA plates overnight at 4°C. Sulfatides (24323-1 mg-CAY, Cambridge) and Ganglioside GM3 from bovine brain (Insight Biotechnology) were applied at 5 μg/ml and 2 μg/ml, respectively, to Immulon 2HB plates overnight at 4°C. The following day, residual solutions were removed and the wells were blocked with 5% skimmed milk powder (Sigma) in PBS for 1h at room temperature. Human serum samples, diluted 1:100 in 5% milk powder/PBS were then incubated in the wells for 1h at room temperature. Following five washes with PBS, HRP-conjugated Fc-specific anti-human-IgG secondary antibody (A0170, Sigma, 1:3000) diluted in 5% milk powder/PBS at 1:3000 was added to the wells and incubated for 1h at RT.

Following further PBS washing cycles, the bound antibodies were detected by reaction with SigmaFast OPD substrate (Sigma) and terminated after 20 min incubation in the dark at RT with 4M H_2SO_4. Optical density measurements were determined at 492 nm and were corrected by subtracting OD values obtained using uncoated wells. The anti-ganglioside GM3 ELISA was performed as previously described [e9].

For the detection of patients' anti-ZIKV antibodies, Maxisorp (NUNC Immulon 4HB) ELISA plates were coated with the anti-flavivirus monoclonal antibody 4G2 at 2 µg/ml (GTX57154, Genetex) in 0.05 M carbonate-bicarbonate buffer (pH 9.6) overnight at 4°C. ZIKV (PF13 strain) or mock infected C6/36 cells were cultured in Leibovitz L-15 (Sigma) containing 2% FBS, 2 mM L-glutamine, 100 U/ml of penicillin and 100 µg/ml of streptomycin at 28°C. After 4 days the media supernatants were collected and stored in screw top vials at -80°C [e10]. Duplicate wells were treated with either ZIKV-infected or mock-infected cell culture supernatants for 1 h at 37ºC to capture the Zika virions. After washing the wells with PBS containing 0.1% v/v Tween-20 and blocking them with 3% BSA in PBS, the patients' and healthy control persons' sera diluted at 1:100 in 10% FBS in RPMI were added to the wells and incubated for 1 h at 37ºC. The bound human IgG was then detected with sequential reaction steps with an HRP-conjugated goat anti-human IgG Fc-specific secondary antibody (A0170, Sigma, 1:3000) and OPD (Sigma Fast) substrate followed by stopping the reaction with 4M H_2SO_4 (see above). The optical density (Absorbance) values were then determined at 492 nm and the resultant anti-ZIKV OD values were determined by subtraction with the OD values obtained from mock-ZIKV treated wells, with the cut-off threshold value of 0.2 for positive reaction determinations.

For measurement of total human serum IgG levels, Nunc Maxisorp plates (Invitrogen, 44-2404-21), were coated with an anti-human IgG capture antibody overnight in PBS according to the manufacturer's instructions (Invitrogen, Cat. 88-50550-86). These plates were then blocked with 2X assay buffer (0.1% Tween-20, 1% BSA in PBS) for 2h at room temperature before incubation with patient serum diluted 1:500,000 for 2h at room temperature on orbital shaker (400 rpm) followed by washing four times in PBS including 0.05% Tween-20 (PBS-T). The plates were then incubated with detection antibody for 1h at room temperature on orbital shaker (400 rpm) followed by washing four times in PBS-T. The plates were incubated in dark with peroxidase substrate Tetramethylbenzidine (TMB) solution (15 min at room temperature), the reaction was stopped with an equal volume of 1M H_2SO_4, and the absorbance values were read at 450 nm and 570 nm (background). All serum samples were run in duplicate and standard curves (again in duplicate) were run on every plate (1-100 ng/ml IgG in two-fold dilution series). Absorbance (optical density) values at 450 nm were background subtracted with those obtained at 570 nm and IgG concentrations interpolated from a four-parameter fit of the standard curve using Prism 9 (Graphpad).

14. Transfected-cell based assays

HEK293 cells were seeded on 13 mm diameter coverslips coated with PDL (10-25 µg/ml) in medium containing 10% FBS in DMEM with penicillin-streptomycin, L-glutamine and sodium pyruvate. After 24h, these cells were transfected overnight with mammalian expression vectors (0.5-1 µg plasmid cDNA per well) encoding human AHNAK2 (His-tagged) (HG15220-CH, Sino Biological), ANXA2 (GFP tagged) (P#107196, Addgene), CD44S (#19127, Addgene), CNTN1 (EX-A1153-M02, Genecopoeia), CNTNAP1 (Caspr1) (pReceiver-M02 backbone; Genecopoeia), GFRA1 (OHu12279, Genscript), ITGA6 (#53352, Addgene), ITGA7 (HG13425-CM, Stratech), MAG (mCherry tagged) (EX-D0078-M56, GeneCopoeia), NEP (MME) (#12338, Addgene), NFASC (transcript variant 1) (RC228652, Origene), NFASC (transcript variant 2) (Querol lab), NKCC1 (SLC12A2) (YFP-tagged) (#49085, Addgene) L-PRX (OHu25883, Genscript), S-PRX (OHu25782, Genscript) and TGBR3 (HA-tagged) (#83095, Addgene) using JetPEI (Polyplus). A minimum of 16-24 hours after transfection, human sera were diluted 1:100 in 1% BSA in DMEM containing 20 mM HEPES and added to the live transfected cells for 1h at RT. Serum-treated cells were then washed in three changes of DMEM/HEPES and fixed in 4% PFA, before IgG binding was assessed using an Alexa Fluor-488 conjugated goat anti-human IgG (Fc-specific) secondary antibody (H10120, Life Technologies, 1:750) and fluorescent microscopic analyses. Additionally, human ALCAM (RC219251, Origene), AXL (RC206431, Origene), DPYSL2 (EX-M0208-M02-10, Genecopoeia), GAS6 (RC207916, Origene), L1CAM (RC211601, Origene), NCAM1 (EX-X0019-M77 Genecopoeia) and NrCAM (EX-H0649-M02, Genecopoeia) gene expression plasmids were transfected at 37°C using Lipofectamine 2000 (Invitrogen).

These cells were subsequently fixed with 4 % PFA, blocked for 1h with 5% NGS and frozen at -80°C until immunocytochemistry experiments were performed. Coverslips containing the transfected HEK293 cells were thawed with 5% NGS. Human sera diluted at 1:100 were added and after incubation the binding of their IgG and IgM autoantibodies were assessed using the method described above for their detection in primary cells. Successful transfection and transgene expression was confirmed by labelling these cells with a commercial antibody, or visualisation of fluorescent tag, and stained coverslips were mounted with Vectashield with DAPI. All plasmid cDNA was sequenced by Sanger Sequencing by a commercial provider (Source Bioscience) and compared to the known open reading frame sequence to prior to transfection assays. Successful heterologous expression was confirmed with antigen-specific commercial antibodies (Sheep anti-CD44, AF6127, R&D Systems, 1:1000; Goat anti-CNTN1, AF904, R&D Systems,

1:2000; Goat anti-NEP, AF1182, R&D Systems, 1:1000; Chicken anti-pan neurofascin, AF3235, R&D Systems, 1:1000; Sheep anti-PRX, gift from P. Brophy, 1:2000) or detection of the corresponding fluorescent or motif tag (Rabbit anti-His, 2365T, Cell Signalling, 1:2000; Rabbit anti-HA (C29F4), #3724, Cell Signalling, 1:1000; Rabbit-anti-FLAG, F7425, Sigma, 1:500; Rabbit anti-Myc, A9106, Abcam, 1:1000).

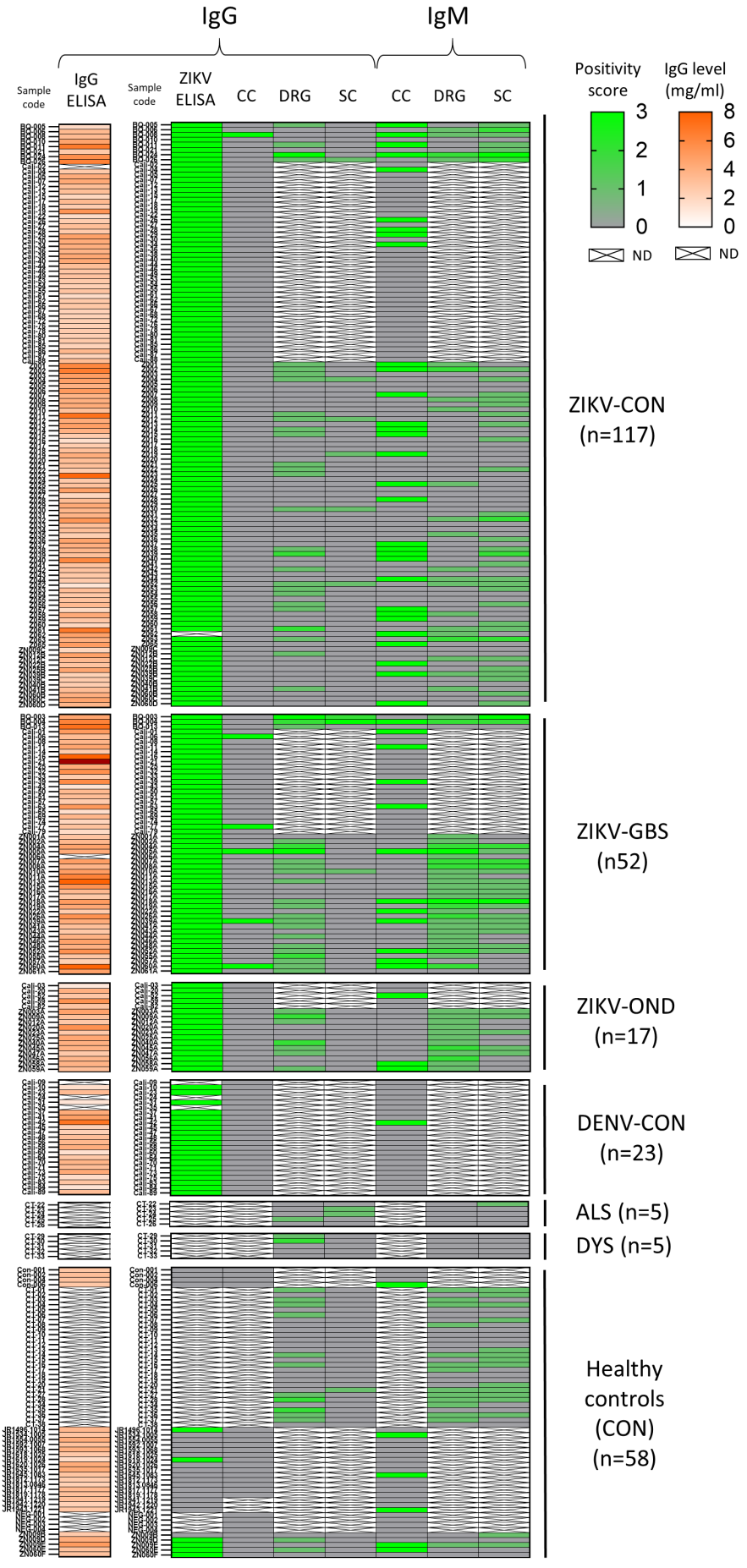
15. Statistical analysis

The results were analysed in Prism v9.1.0 (GraphPad Software). Statistical comparison of the proportions of seropositive patients among the ZIKV groups were performed using contingency analysis with the application of a two-tailed Fisher's exact test for individual group-group comparisons, and Chi square testing for comparisons between all groups. For the fluorescence intensity profiles of the human IgG antibody, human IgM antibody and myelin basic protein (MBP) immunolabelling, confocal images were acquired using identical settings and analysed using the plot profile function on ImageJ. For the analysis of IgM antibody deposition on myelin outpouchings, two images were acquired per serum sample and analysed by an investigator blinded to the sample grouping. IgM antibody fluorescence values for the plot profile were integrated according to the signal overlap with MBP. The outpouching intensity score was background subtracted and normalised to the signal at the adjacent internode. For the analysis of the internodes of solvent or neuraminidase-treated cultures, the images of two internodes per field of view, 6-14 fields of view per treatment from two independent experiments were acquired. Immunofluorescence images were adjusted for brightness and contrast for presentation only. For the comparison of the peptide enrichment in the IP eluates from the human anti-ZIKV serum and positive control serum-treated cultures, respectively, was performed on normalised abundance values by t-test using a false discovery rate of 1% and significance threshold of 0.1 (Perseus) [e7]. Data were presented as mean ± SEM unless otherwise stated. An alpha-level of <0.05 was accepted for statistical significance.

eFIGURES AND LEGENDS

eFigure 1. Simplified heat map showing all screening performed in ZIKV-GBS, ZIKV-OND, ZIKV-CON, DENV-CON, ALS, Dysferlin (DYS) and healthy control (CON) patients. *Left*) Total serum IgG levels (mg/ml) as measured by ELISA. *Right*) The reaction scores for each patients' IgG and IgM antibodies from each group (ordered alphanumerically) against ZIKV (ELISA), rat dorsal-root ganglia neurons (DRG) and Schwann cells (SC) are shown by their colour intensity of each square (0=negative, 1=mildly positive, 2=moderately positive, 3=strongly positive), while their IgG and IgM antibody reactions with the myelinating cell co-cultures (CC) were scored as either negative (0) or positive (3). The serum samples that were not tested on a particular assay (due to lack of sample or availability) appear blank (ND, not determined).

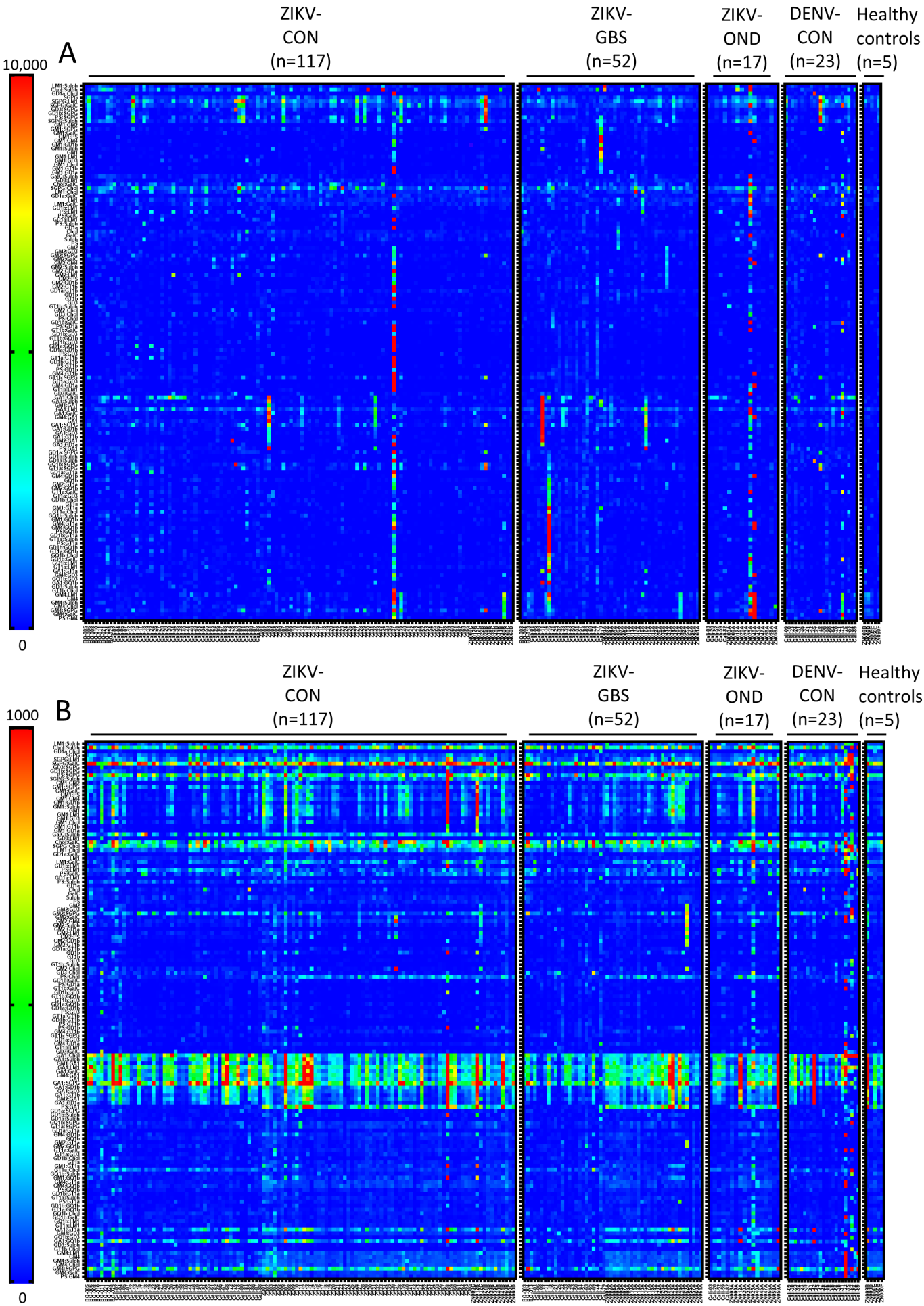
eFigure 1.


IgG
IgM
Sample code
IgG ELISA
Sample code
ZIKV ELISA
CC
DRG
SC
CC
DRG
SC
Positivity score
3
2
1
0
ND
IgG level (mg/ml)
8
6
4
2
0
ND
ZIKV-CON (n=117)
ZIKV-GBS (n52)
ZIKV-OND (n=17)
DENV-CON (n=23)
ALS (n=5)
DYS (n=5)
Healthy controls (CON) (n=58)

eFigure 2. Glycolipid microarray results.

Graphical display of the IgG (**A**) and IgM (**B**) antibody reaction intensities presented as heat maps from blue (negative) to red (strong) for each patients' serum sample from each clinically classified group (ZIKV-GBS, ZIKV-OND, ZIKV-CON, CON) when reacted against 136 different glycolipid targets shown in the 136 rows. No significant differences were observed between the IgG or IgM antibody reactions against glycolipids and glycolipid complexes of the ZIKV-GBS patients compared with those of the control group (CON).

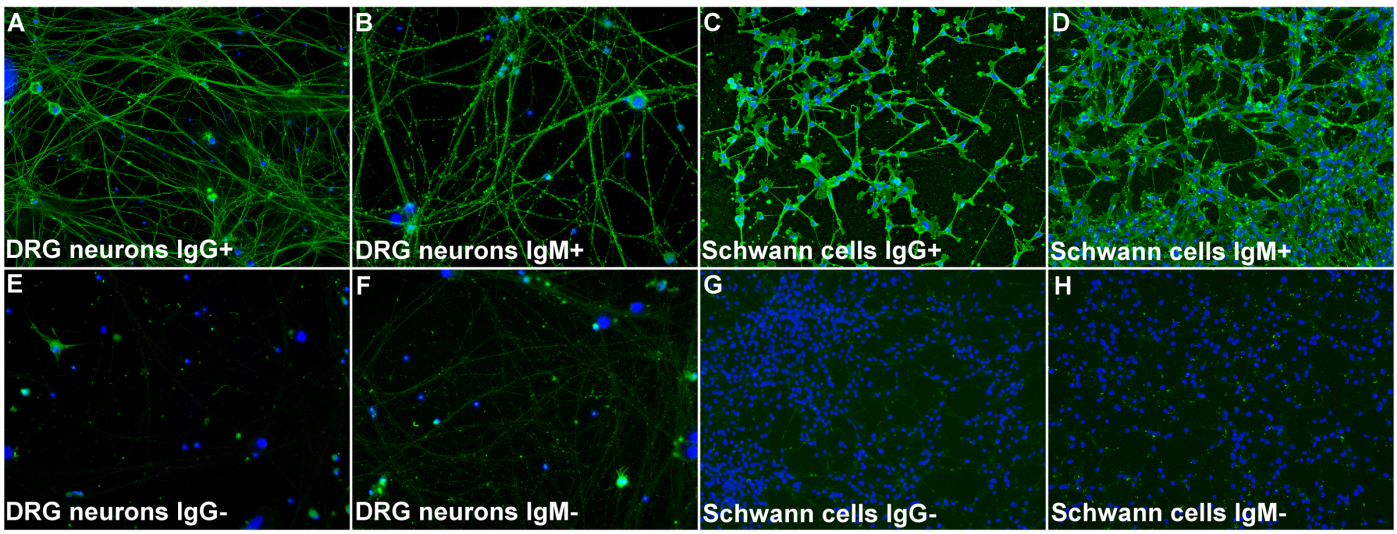
eFigure 2.

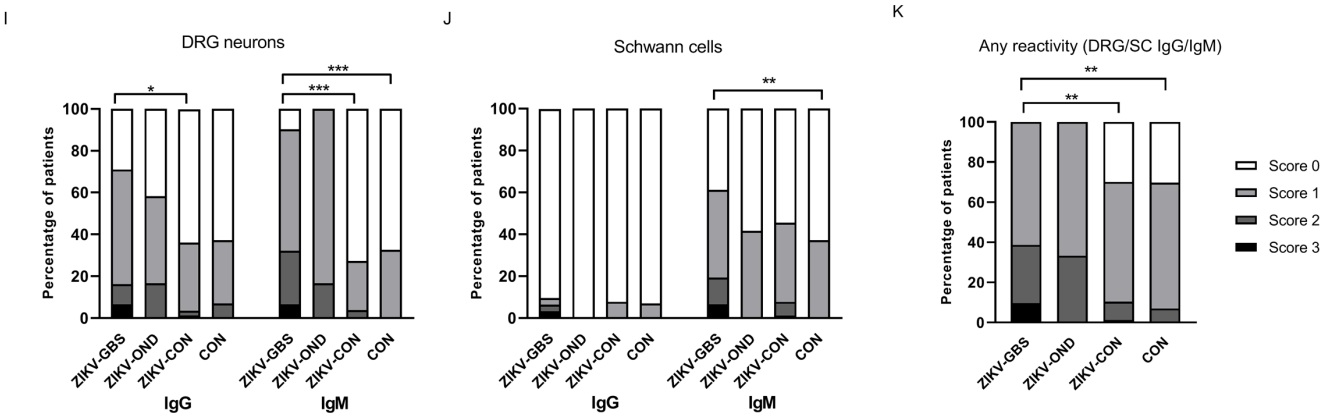

A
10,000
0
ZIKV-CON (n=117)
ZIKV-GBS (n=52)
ZIKV-OND (n=17)
DENV-CON (n=23)
Healthy controls (n=5)
B
1000
0
ZIKV-CON (n=117)
ZIKV-GBS (n=52)
ZIKV-OND (n=17)
DENV-CON (n=23)
Healthy controls (n=5)

eFigure 3. Reactivity of ZIKV patients' serum IgG and IgM antibodies against primary cultured rat DRG neurons and Schwann cells.

Immunofluorescent assay photomicrographs using ZIKV-GBS patients' serum samples showing strong IgG (**A**) or IgM (**B**) antibody reactivities against DRG neurons, strong IgG (**C**) and IgM (**D**) antibody reactivities against Schwann cells compared to the negative control (CON) human sera which showed negligible/weak non-specific IgG (**E**) or IgM (**F**) antibody reactivity against DRG neurons or IgG (**G**) or IgM (**H**) antibody reactivities against Schwann cells. The percentage of IgG and IgM reactive antibodies of different intensities: 0 = negative (blank bars), 1 = weak (light-grey bars),: 2 = moderate (medium-grey bars) or 3 = strong (dark-grey bars) for the different human patient groups (ZIKV-GBS, ZIKV-OND, ZIKV-CON and healthy controls (CON)) against DRG neurons, Schwann cells (SCs) or either DRG or SCs are shown in **I**, **J** and **K** respectively and the p-values of 0.05, < 0.01 or < 0.001 are shown as *, **, and *** respectively.

eFigure 3.

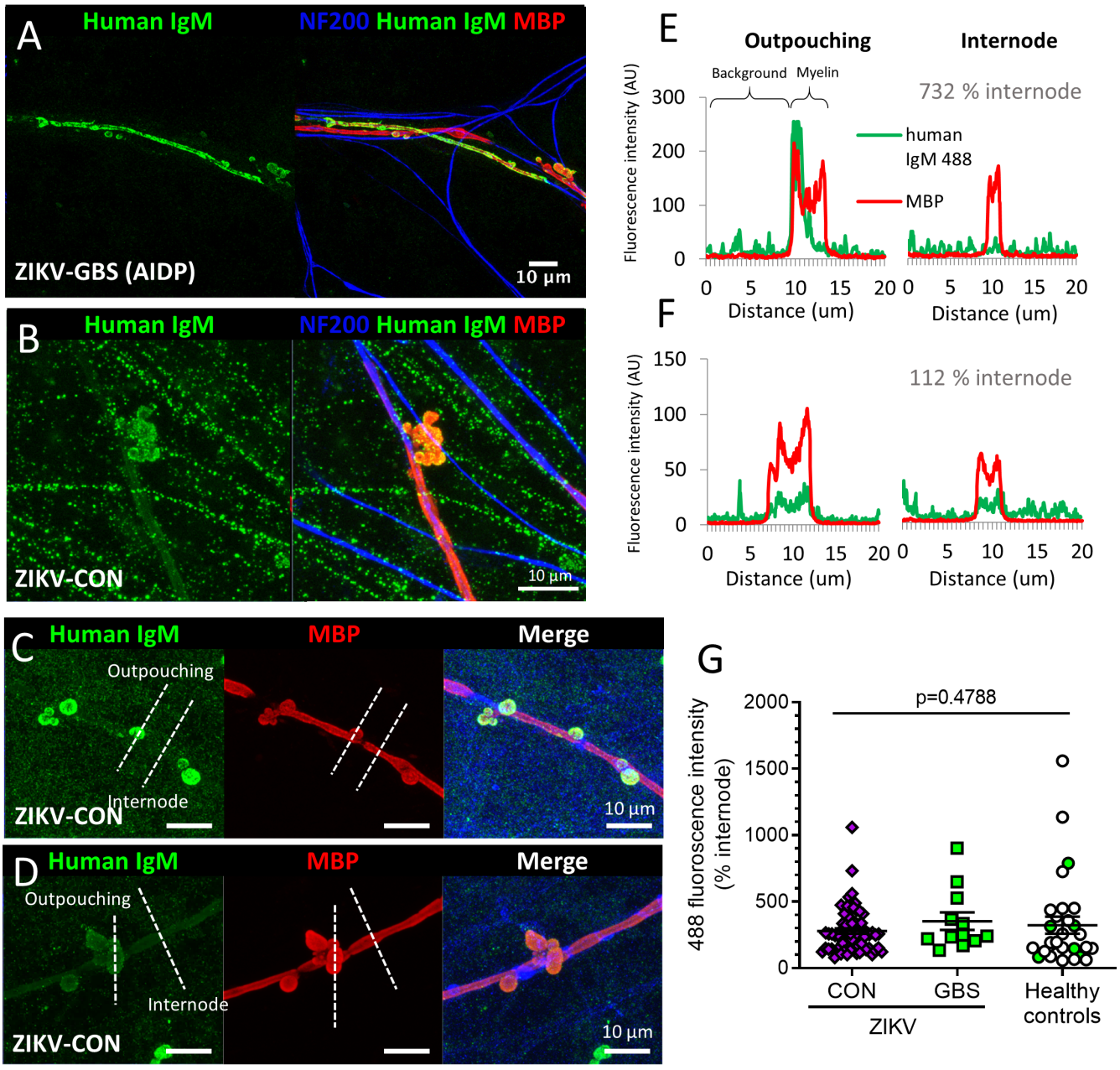

A
DRG neurons IgG+
B
DRG neurons IgM+
C
Schwann cells IgG+
D
Schwann cells IgM+
E
DRG neurons IgG-
F
DRG neurons IgM-
G
Schwann cells IgG-
H
Schwann cells IgM-


I
DRG neurons
Percentatge of patients
100
80
60
40
20
0
*
***
***
ZIKV-GBS
ZIKV-OND
ZIKV-CON
CON
IgG
IgM
J
Schwann cells
**
K
Any reactivity (DRG/SC IgG/IgM)
Score 0
Score 1
Score 2
Score 3

eFigure 4. Patterns of ZIKV patients' serum IgM antibody reactivity against myelinating cell co-cultures.

Immunofluorescent photomicrographs of IgM autoantibodies (**A**) from a ZIKV-GBS (AIDP) patient's sera (patient D, *ZN005A*) with deposition on individual myelin internodes, (**B**) from a male ZIKV-CON patient's serum (patient X, *ZN039B*, 41-45 years old) with deposition on non-myelinating Schwann cell processes, (**C**) from a female ZIKV-CON patient's serum (patient Y, *Z040*, 51-55 years old) showing strong reactivity against myelin outpouchings and **D**) from another female ZIKV-CON patient's serum (patient Z, *Z010*, 81-85 years old) showing weak reactivity against the myelin outpouchings. Counterstaining for NF200 (blue) and MBP (red) are also shown. Scale bars, 10 µm. The dashed lines illustrate the transection of myelin outpouchings and internodes for profile analysis of IgM signal intensity. (**E** and **F**) Fluorescent intensity profile plots of patients' IgM autoantibodies (green) and myelin (red) across myelin outpouchings and internodes for the (**E**) strong and (**F**) weak labelling antisera. (**G**) The IgM (Alexa 488) autoantibody fluorescence intensities of the ZIKV-GBS and ZIKV-CON patients' sera compared to the healthy controls against myelin outpouchings normalised to internode labelling. One way ANOVA (all groups): $F(2,106)=0.7417$, $p=0.4788$). The healthy control serum samples which had detectable anti-ZIKV antibodies are represented by solid green circles.

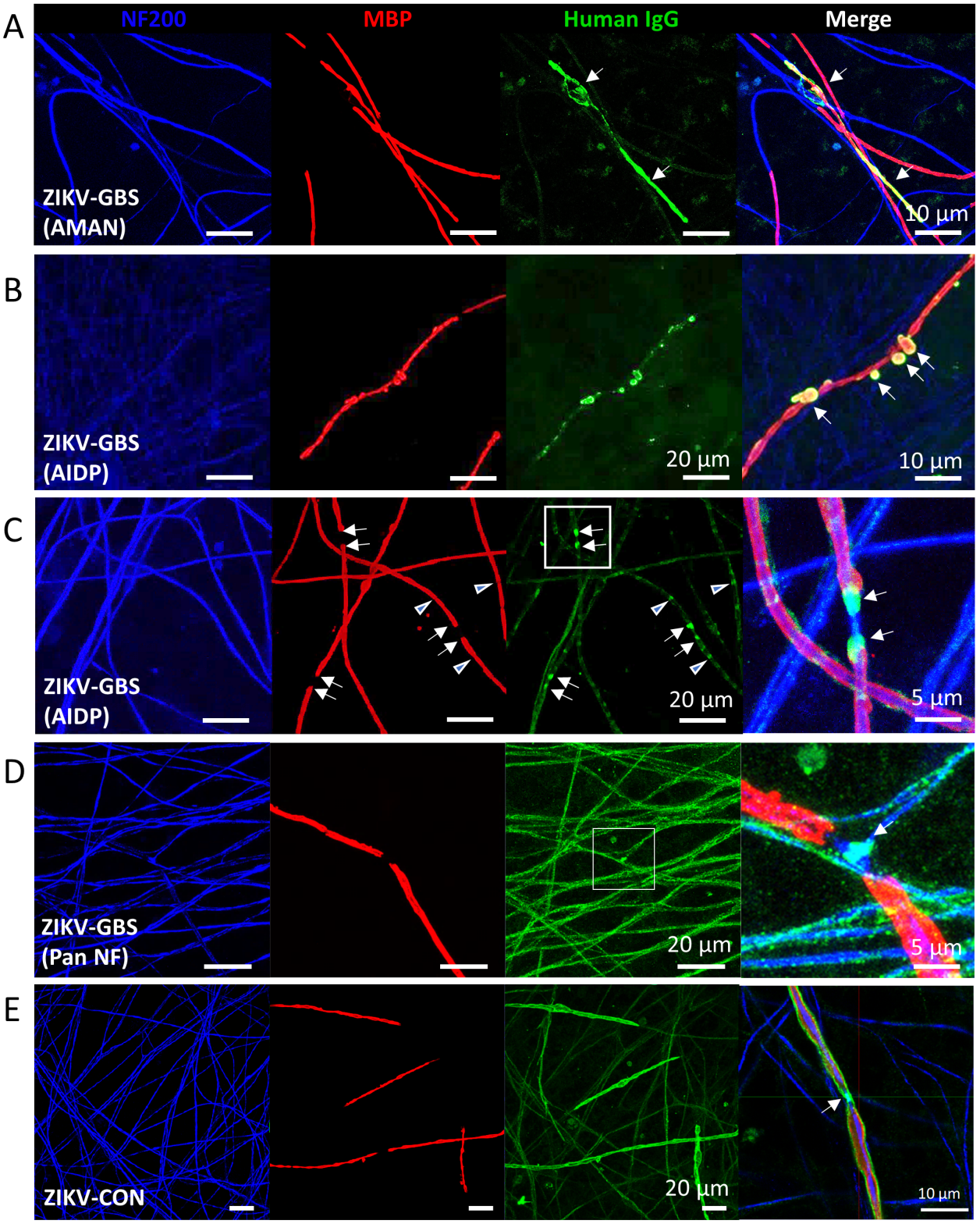
eFigure 4.


A
Human IgM
NF200 Human IgM MBP
ZIKV-GBS (AIDP)
10 µm
B
Human IgM
NF200 Human IgM MBP
ZIKV-CON
10 µm
C
Human IgM
MBP
Merge
Outpouching
Internode
ZIKV-CON
10 µm
D
Human IgM
MBP
Merge
Outpouching
Internode
ZIKV-CON
10 µm
E
Outpouching
Internode
Background
Myelin
732 % internode
human IgM 488
MBP
Fluorescence intensity (AU)
300
200
100
0
0 5 10 15 20
Distance (um)
F
112 % internode
Fluorescence intensity (AU)
150
100
50
0
0 5 10 15 20
Distance (um)
G
p=0.4788
488 fluoroscence intensity (% internode)
2000
1500
1000
500
0
CON
GBS
ZIKV
Healthy controls

eFigure 5. Patterns of ZIKV patients' serum IgG antibody reactivity against myelinating cell co-cultures.

(**A**) The selective labelling of individual internodes by IgG antibodies from one female ZIKV-GBS patient (*ZN060A*, 31-35 years old) with the AMAN subtype. Note a similar IgG antibody binding pattern was also seen using the sera from a male ZIKV-GBS patient (*Cali006*, 41-45 years old). (**B**) Myelin outpouchings or 'blebs' labelled by IgG autoantibodies in the serum of a female ZIKV-GBS patient (patient D, *ZN005A*, 46-50 years old) with the AIDP subtype. (**C**) Myelin paranodes (arrows) and Schmidt-Lanterman incisors (arrow heads) labelled by IgG autoantibody reactions from the serum of a male ZIKV-GBS patient (*ZN039A*, 16-20 years old) with the AIDP subtype. (**D**) Axonal and labelling with enrichment at the node shown by IgG autoantibody reactions from the serum of a male ZIKV-GBS patient (patient A, *Cali077*, 41-45 years old) fatal case. Nodal regions corresponding to insets in (C) and (D) are shown on the far right. (**E**) The labelling of the abaxonal membranes of all myelinating Schwann cells by the IgG autoantibodies in the serum of a female ZIKV-CON patient (patient B, *BQ008*, 41-45 years old) with previous uncomplicated ZIKV infection. Note that the staining is particularly intense at the Schwann cell microvilli overlying the node of Ranvier (right). Co-cultures were counterstained with NF200 (blue) and myelin basic protein (MBP) (red). Scale bars 5, 10 or 20 μm as indicated.

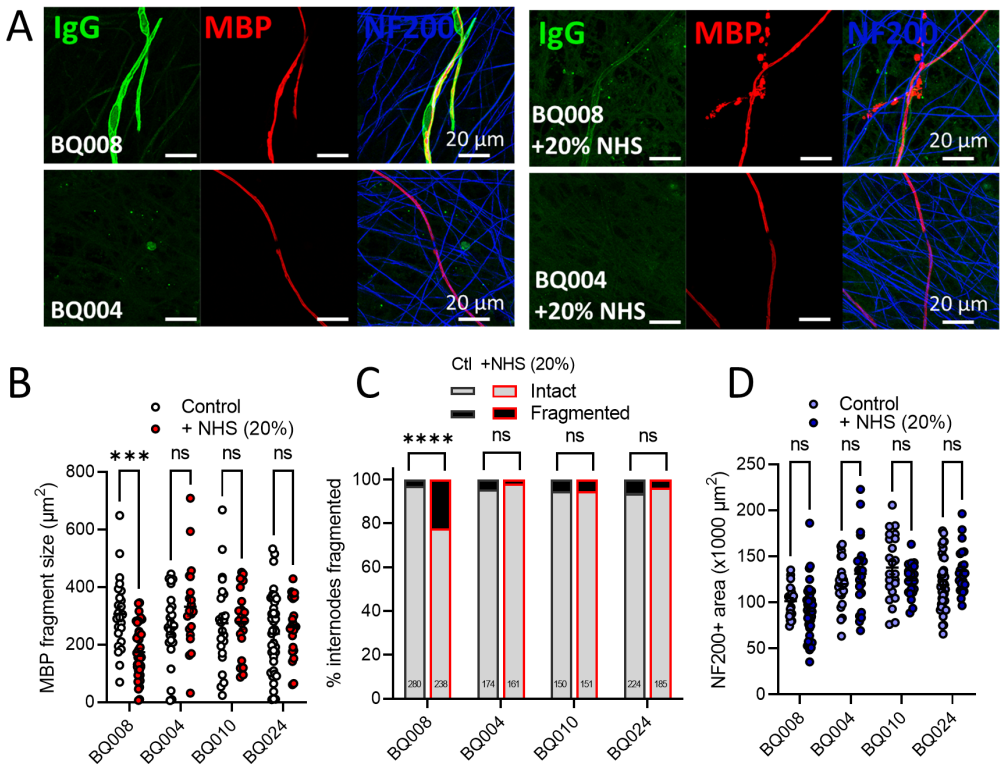
eFigure 5.


A
NF200
MBP
Human IgG
Merge
ZIKV-GBS
(AMAN)
10 µm
B
ZIKV-GBS
(AIDP)
20 µm
10 µm
C
ZIKV-GBS
(AIDP)
20 µm
5 µm
D
ZIKV-GBS
(Pan NF)
20 µm
5 µm
E
ZIKV-CON
20 µm
10 µm

eFigure 6. Confirmation of complement-dependent demyelinating activity in ZIKV-CON patient B serum.

(**A**) Representative confocal images of IgG binding of each serum (1:50) 24 h after the addition of media only (control) or a source of complement (fresh normal human serum, 20%). Note fragmentation of myelin basic protein (MBP, red), but not axons (NF200, blue) in BQ008 treated cultures supplemented with 20% NHS. (**B**) Quantification of MBP fragmentation. Two-way ANOVA. Significant interaction between serum sample and complement: $F(3,211)=7.003$, $p=0.0002$. Sidak's multiple comparison test. ***$p=0.0002$; ns, $p>0.05$. (**C**) Proportion of intact versus fragmented internodes. Fisher's Exact test (two sided). ***$p<0.0001$; ns, $p>0.05$. (**D**) Quantification of NF200 area. Two-way ANOVA. Interaction between serum sample and complement: $F(3, 211) = 3.651$, Sidak's multiple comparison test. ns, $p>0.05$.

eFigure 6.


A
IgG
MBP
NF200
BQ008
20 µm
BQ004
BQ008
+20% NHS
BQ004
+20% NHS
B
Control
+ NHS (20%)
MBP fragment size (µm²)
***
ns
BQ008
BQ004
BQ010
BQ024
C
Ctl
+NHS (20%)
Intact
Fragmented
% internodes fragmented
****
280
238
174
161
150
151
224
185
D
NF200+ area (x1000 µm²)

eTABLES

eTable 1. Summary of ZIKV patient cohorts and controls.

Group	Number of samples	Age: median; range [# samples]	% Female	ZIKV symptoms to sampling (days) median; range [# samples]	Neurological onset to sampling (days) median; range [# samples]
ZIKV-GBS (overall)	**52**	**44; 10-71 [47/52]**	**46.2% [52/52]**	**113; 65-579 [31/52]**	**97; 51-534 [31/52]**
• AIDP	16	41; 10-69 [16/16]	56.3% [16/16]	102; 67-164 [16/16]	93; 51-131 [16/16]
• AMAN/AMSAN	10	44; 27-70 [10/10]	50.0% [10/10]	112; 65-271 [10/10]	99; 58-242 [10/10]
• Unclassified	26	45; 20-71 [21/26]	38.5% [26/26]	564; 99-579 [5/26]	482; 94-534 [5/26]
Controls (overall)	**225**	**40; 2-88 [221/225]**	**56.9% [225/225]**	**N/A**	**N/A**
• ZIKV-OND*	17	26; 8-41 [13/17]	52.9% [17/17]	125; 82-211 [12/17]	103; 28-168 [12/17]
• ZIKV-CON	117	43; 2-83 [117/117]	64.1% [117/117]	105; 1-615 [116/117]	N/A
• DENV-CON	23	36; 21-71 [23/23]	34.8% [23/23]	N/A	N/A
• Local healthy*	9	32; 4-41 [9/9]	55.6% [9/9]	N/A	N/A
• ALS	5	68; 35-81 [5/5]	60.0% [5/5]	N/A	N/A
• Dysferlin	5	51; 17-88 [5/5]	40.0% [5/5]	N/A	N/A
• Healthy controls	49	42; 21-80 [49/49]	55.1% [49/49]	N/A	N/A

When the data was available, the ZIKV infected GBS (ZIKV-GBS) patients were subdivided into those diagnosed as acute inflammatory demyelinating polyneuropathy (AIDP), predominantly axonal (AMAN/AMSAN: acute motor axonal neuropathy or acute motor sensory axonal neuropathy) or 'unclassified' where the data were unavailable. Control samples obtained locally included ZIKV patients who presented with other neurological disease (OND*), including myeloradiculopathy (n=1), encephalitis (n=5), meningoencephalitis (n=1), peripheral facial palsy (n=3), transverse myelitis (n=7), uncomplicated ZIKV infections (ZIKV-CON), post-dengue virus infections (DENV-CON) (n=23) and otherwise healthy local controls* (n=9). 38 serum samples from Spanish subjects included patients with the non-immune neuromuscular disorders amyotrophic lateral sclerosis (ALS) (n=5) and dysferlinopathy (Dysferlin) (n=5). 28 healthy Spanish controls were grouped with 21 healthy controls from Oxford. Age and time to sampling data are presented as median, range and the proportion of patients for whom this data was available.

eREFERENCES

e1 Uncini A, Gonzalez-Bravo DC, Acosta-Ampudia YY et al., Clinical and nerve conduction features in Guillain-Barre syndrome associated with Zika virus infection in Cucuta, Colombia. *Eur J Neurol* 2018;25(4):644-650. DOI: 10.1111/ene.13552.

e2 Clark AJ, Kaller MS, Galino J, Willison HJ, Rinaldi S, and Bennett DLH, Co-cultures with stem cell-derived human sensory neurons reveal regulators of peripheral myelination. *Brain* 2017;140(4):898-913. DOI: 10.1093/brain/awx012.

e3 Svennerholm L and Fredman P, A procedure for the quantitative isolation of brain gangliosides. *Biochim Biophys Acta* 1980;617(1):97-109. DOI: 10.1016/0005-2760(80)90227-1.

e4 Willison HJ, O'Hanlon GM, Paterson G et al., A somatically mutated human antiganglioside IgM antibody that induces experimental neuropathy in mice is encoded by the variable region heavy chain gene, V1-18. *J Clin Invest* 1996;97(5):1155-64. DOI: 10.1172/JCI118529.

e5 Halstead SK, Kalna G, Islam MB et al., Microarray screening of Guillain-Barre syndrome sera for antibodies to glycolipid complexes. *Neurol Neuroimmunol Neuroinflamm* 2016;3(6):e284. DOI: 10.1212/NXI.0000000000000284.

e6 Siles AM, Martinez-Hernandez E, Araque J et al., Antibodies against cell adhesion molecules and neural structures in paraneoplastic neuropathies. *Ann Clin Transl Neurol* 2018;5(5):559-569. DOI: 10.1002/acn3.554.

e7 Tyanova S, Temu T, Sinitcyn P et al., The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature Methods* 2016;13(9):731-740. DOI: 10.1038/nmeth.3901.

e8 Perez-Riverol Y, Csordas A, Bai J et al., The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res* 2019;47(D1):D442-D450. DOI: 10.1093/nar/gky1106.

e9 Willison HJ, Veitch J, Swan AV et al., Inter-laboratory validation of an ELISA for the determination of serum anti-ganglioside antibodies. *Eur J Neurol* 1999;6(1):71-7. DOI: 10.1046/j.1468-1331.1999.610071.x.

e10 Dejnirattisai W, Supasa P, Wongwiwat W et al., Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nat Immunol* 2016;17(9):1102-8. DOI: 10.1038/ni.3515.