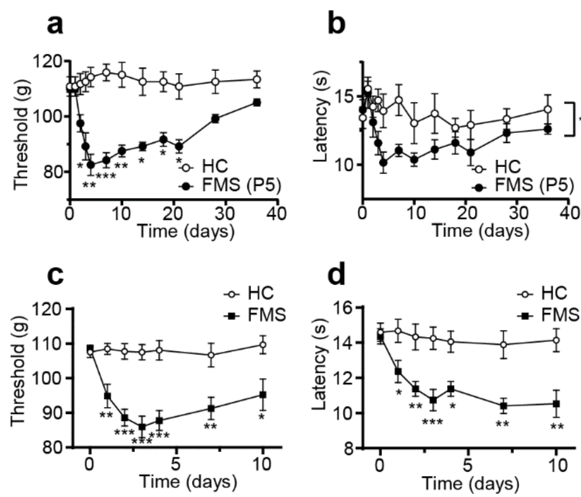
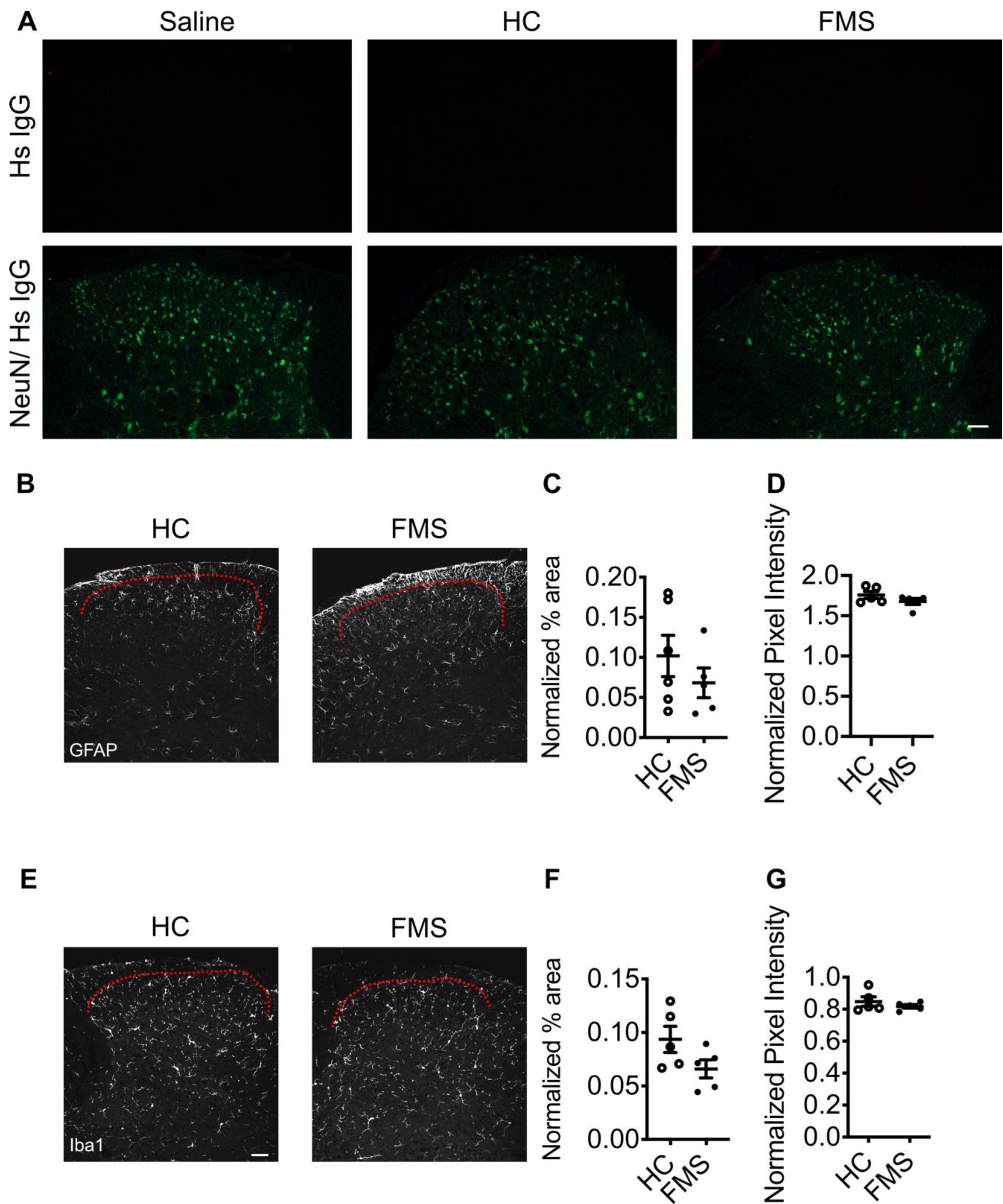
Supplementary Materials:

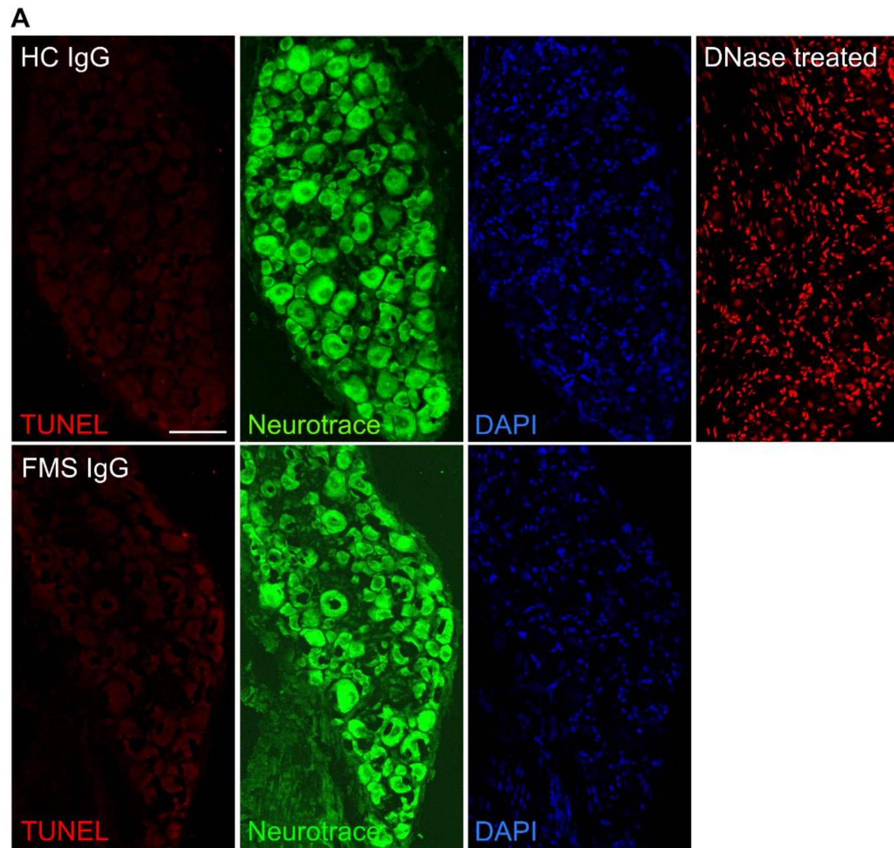


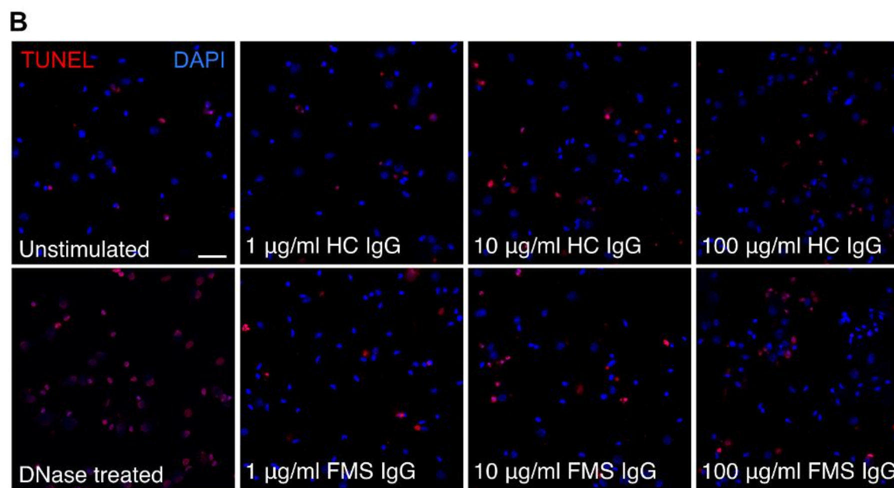
Supplemental figure 1. Time course of hypersensitivities. Examples of the full time course of mechanical (a) and cold (b) hypersensitivity produced by IgG from an FMS patient compared to a healthy control subject. Data in (**a**, **b**) are mean ± SEM of n=6 mice. Mean sensitivity in the paw-pressure test (**c**) and cold plate test (**d**) following administration of IgG from 8 patients and 6 control subjects (summarizing results from fig. 1). *P<0.05, **P<0.01, ***P<0.001, FMS IgG compared to HC IgG, two-way ANOVA followed by Sidak's correction.

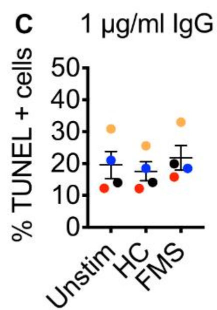


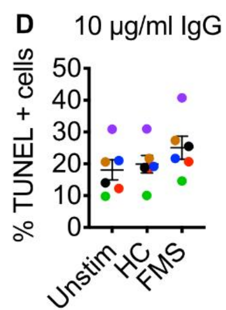
Supplemental Figure 2. FMS IgG does not accumulate in the spinal cord or activate dorsal horn glia. Neither HC IgG nor FMS IgG accumulates in the dorsal horn of the spinal cord (**A**). Sections were probed with an anti-Hs (human) IgG antibody (top row) and anti-NeuN antibody (bottom row, merged with anti-Hs IgG, **A**). FMS IgG did not change the percent area of

GFAP immunoreactivity of GFAP intensity in the dorsal horn compared to HC IgG (**B-D**), suggesting that FMS IgG does not alter astrocyte activity. Iba1 percent area of immunoreactivity and intensity were also unchanged by FMS IgG compared to HC IgG (**E-G**), suggesting that FMS IgG does not alter microglia activity. Iba1 and GFAP signal intensities were normalized to DAPI. Red dotted lines indicate dorsal edge of lamina I of the dorsal horn. Line and whiskers indicate mean ± SEM, n=5-6, data were analyzed by unpaired t-tests. Scale bars are 50 µm.


A
HC IgG
TUNEL
Neurotrace
DAPI
DNase treated
FMS IgG
TUNEL
Neurotrace
DAPI


B
TUNEL
DAPI
Unstimulated
1 µg/ml HC IgG
10 µg/ml HC IgG
100 µg/ml HC IgG
DNase treated
1 µg/ml FMS IgG
10 µg/ml FMS IgG
100 µg/ml FMS IgG


C
1 µg/ml IgG
% TUNEL + cells
50
40
30
20
10
0
Unstim
HC
FMS


D
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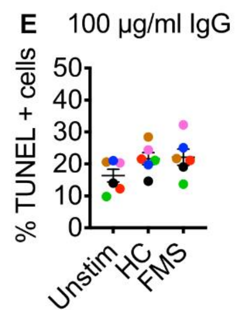
% TUNEL + cells
50
40
30
20
10
0
Unstim
HC
FMS


E
100 µg/ml IgG
% TUNEL + cells
50
40
30
20
10
0
Unstim
HC
FMS

Supplemental figure 3. FMS IgG does not induce apoptosis in vivo or in vitro. Very few, if any, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive cells were found in the DRGs of mice injected with HC IgG (top row) or FMS IgG (bottom row) (pooled, 8mg per day for four consecutive days, tissue collected after last injection) indicating that IgG injection does not induce apoptosis (n=6/group and the scale bar indicates 100 μm) (**A**). The right most panel in the top row is an example of a DNase treated slide that results in DNA ligation that is similar to apoptosis and thus reacts with TUNEL and serves as a positive control (**A**). Increasing concentrations of pooled HC or FMS IgG did not increase the number of TUNEL positive cells compared to unstimulated cells in dissociated DRG cultures (**B-E**), indicating that neither HC nor FMS IgG induce apoptosis in vitro. The colour of data points in c-e indicate that the data are from the same experiment. Data points are mean ± SEM, n=4-6, data were analyzed by one-way ANOVA with Bonferroni's *post hoc* test and the scale bar indicates 20 μm.

Table S1. *Patient characteristics*. Age - mean (range), duration of FMS pain – median (interquartile range); pain intensity - average pain intensity over the past week ± SD (range); UK sample – numeric rating scale pain score on an 11-point scale (0-10), Swedish sample – visual analogue scale pain score (0-100), with 10, 100 = 'worst pain imaginable'; tender point count – average (range) number of defined points which are tender to pressure out of a maximal 18[1].

	Age; %female	Duration (years)	Pain intensity	Tender point count
UK sample, n=8	46 (19-58); 88	2.5 (1.3-18)	8.1±0.8 (6.5-9)	15 (12-18)
Swedish sample, n=36	48 (34-59); 100	10 (1-26)	69±18 (35-100)	17 (11-18)

Table S2. *Characteristics of blood donors for IgG pools*. Fibromyalgia (FMS) characteristics are in **A)** and healthy control characteristics are in **B**). Age - mean (range), duration of FMS pain – median (interquartile range); pain intensity - average pain intensity over the past week ± SD (range) assessed by visual analogue scale pain score (0-100), with 10, 100 = 'worst pain imaginable'; tender point count – average (range) number of defined points which are tender to pressure out of a maximal 18.[1] Pressure pain threshold (PPT), mean±SD (range); Fibromyalgia Impact Questionnaire (FIQ), mean±SD (range).

A)

FMS	Age (all female)	Duration (years)	Pain intensity	Tender point count	PPT	FIQ
Pool 1, n=8	46 (39-56)	9.5 (2.25-13.75)	81±17 (47-100)	17 (15-18)	141±55 (96-228)	90±3.3 (85-95)
Pool 2, n=14	49 (35-58)	11.5 (3.75-26)	74±16 (35-98)	16 (11-18)	134±61 (39-243)	75±10 (62-90
Pool 3, n=14	48 (34-59)	10 (6-34.5)	58±15 (39-86)	17 (12-18)	152±51 (86-238)	60±12 (46-88)

B)

Healthy controls	Age All female	Pain intensity	PPT
Pool 1, n=12	50 (40-59)	1.5±2.6 (0-7)	343±130 (138-633)

Pool 2, n=10	50 (38-59)	3.9±6.7 (0-29)	264±103 (77-446)
Pool 3, n=11	46.65 (36-55)	7.5±7.2 (0-26)	327±106 (200-562)

Table S3 *Serum IgG subclass concentrations.* Serum concentrations of IgG subclasses were measured using ELISA in the samples collected in the UK (**a**) and Sweden (**b**). Data are mean (95% C.I.) and were assessed by Mann-Whitney.

A)

UK samples	HC (5)	FMS (8)	p-value
IgG1 (µg/ml)	2.93 (1.7-4.1)	5.30 (2.2-8.4)	0.17
IgG2 (µg/ml)	1.25 (0.5-2.0)	1.06 (0.65-1.5)	0.62
IgG3 (µg/ml)	0.36 (0.1-0.6)	0.49 (0.3-0.7)	0.28
IgG4 (µg/ml)	0.18 (0-0.4)	0.19 (0-0.4)	0.62

B)

Swedish samples	HC (33)	FMS (32)	p-value
IgG1 (mg/ml)	6.426 (5.47-7.39)	5.201 (4.162-6.24)	0.021
IgG2 (mg/ml)	1.656 (1.17-2.14)	1.655 (1.38-1.93)	0.252
IgG3 (mg/ml)	1.190 (1.04-1.34)	1.074 (0.923-1.22)	0.248
IgG4 (mg/ml)	0.664 (0.48-0.85)	0.905 (0.67-1.14)	0.184

Table S4. *Antibodies used*. Primary antibody details are in **A**) and secondary antibody details are in **B**). GS (glutamate synthase), GFAP (Glial fibrillary acidic protein), NeuN (Neuronal N), MBP (Myelin Basic Protein), PGP (protein gene product), TrkA (Tropomyosin receptor kinase A), AF (Alexa Fluor).

A)

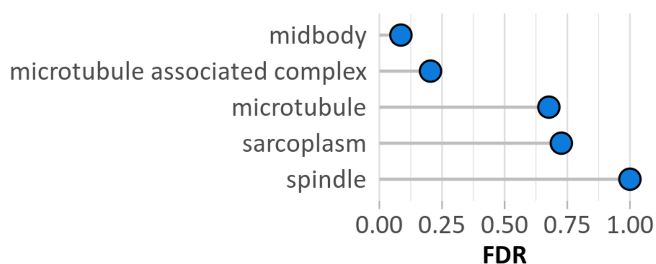
Primary Antibodies				
Target	**Host**	**Manufacturer**	**Catalogue #**	**Dilution**
GS	Rabbit	Abcam	ab73593	1:500
GFAP	Mouse	Millipore	Mab360	1:500 (DRG) 1:1000 (SC)
GFAP	Rabbit	Dako	Z0334	1:500
Iba1	Rabbit	Wako	019-19741	1:500 (DRG) 1:1000 (SC)
NeuN conj. AF488	Mouse	Millipore	MAB377X	1:100
NF200	Chicken	Neuromics	CH22104	1:500
MBP	Rat	Abcam	550274	1:500
β-III tubulin	Mouse	Promega	G7121	1:2000
TrkA	Goat	R&D Biosystems	AF5479	1:100
PGP 9.5	Rabbit	Cedarlane	CL7756AP	1:2000
CD31	Rat	BD Pharmigen	Ab739	1:500

Secondary Antibodies					
Target	**Fluorophore**	**Host**	**Manufacturer**	**Catalogue #**	**Dilution**

Rabbit IgG	AF488	Goat	ThermoFisher	A11008	1:250
Human IgG	AF488	Goat	ThermoFisher	A11013	1:300
Goat IgG	Cy2	Donkey	Jackson Immunoresearch	706-225-148	1:300
Rabbit IgG	Cy5	Donkey	Jackson Immunoresearch	711-175-152	1:600
Rabbit IgG	Cy5	Donkey	Jackson Immunoresearch	11-165-152	1:600
Human IgG	Cy3	Donkey	Jackson Immunoresearch	709-165-149	1:600
Human IgG	AF594	Goat	ThermoFisher	A11014	1:200
Mouse IgG	AF488	Goat	ThermoFisher	A11029	1:250
Rat IgG	AF488	Goat	ThermoFisher	A11006	1:250

Table S6. Gene set enrichment analysis of proteins with fragments recognised by at least one sample in the peptide array (see separate table S5 for details). Gene Ontology (GO) terms for cellular components were queried in webgestalt.org.

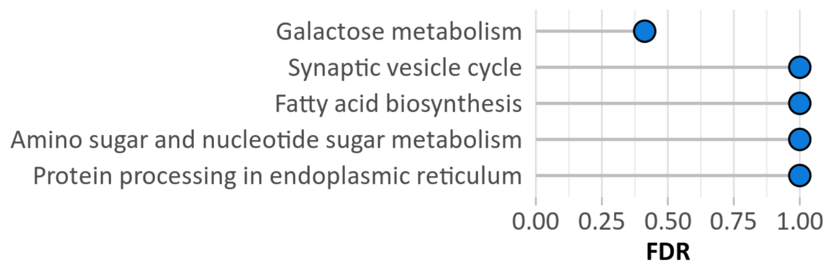
Gene set	description	size	overlap	expected	enrichment Ratio	pValue	FDR
GO:0030496	midbody	171	11	3.33	3.31	5.01E-04	0.086
GO:0005875	microtubule associated complex	148	9	2.88	3.13	2.37E-03	0.204
GO:0005874	microtubule	402	15	7.82	1.92	0.012	0.676
GO:0016528	sarcoplasm	77	5	1.50	3.34	0.017	0.726
GO:0005819	spindle	328	11	6.38	1.72	0.055	1
GO:0042629	mast cell granule	22	2	0.43	4.67	0.067	1
GO:0005788	endoplasmic reticulum lumen	306	10	5.95	1.68	0.075	1
GO:0031252	cell leading edge	393	12	7.64	1.57	0.082	1
GO:0099568	cytoplasmic region	479	14	9.32	1.50	0.084	1
GO:0042581	specific granule	160	6	3.11	1.93	0.092	1



Supplemental figure S4. Lollipop plot of the 5 most strongly enriched GO terms for cellular components (from table S6).

Table S7. Pathway analysis of proteins with fragments recognised by at least one sample in the peptide array (see separate table S5 for details). Gene Ontology (GO) terms for cellular components were queried in webgestalt.org.

Gene set	description	size	overlap	expected	enrichment ratio	pValue	FDR
hsa00052	Galactose metabolism	31	4	0.49	8.23	0.0013	0.412
hsa04721	Synaptic vesicle cycle	63	4	0.99	4.05	0.0168	1
hsa00061	Fatty acid biosynthesis	13	2	0.20	9.81	0.0170	1
hsa00520	Amino sugar and nucleotide sugar metabolism	48	3	0.75	3.99	0.0390	1
hsa04141	Protein processing in endoplasmic reticulum	165	6	2.59	2.32	0.0447	1
hsa04974	Protein digestion and absorption	90	4	1.41	2.83	0.0524	1
hsa00310	Lysine degradation	59	3	0.93	3.24	0.0648	1
hsa04020	Calcium signaling pathway	183	6	2.87	2.09	0.0673	1
hsa04392	Hippo signaling pathway	29	2	0.45	4.40	0.0752	1
hsa05016	Huntington disease	193	6	3.03	1.98	0.0822	1



Supplemental figure S5. Lollipop plot of the 5 most strongly enriched KEGG pathways (from table S7).

Supplementary methods

[Ca^{2+}]$_i$-measurements

DRG neurons were dissociated as previously described (1) and seeded on poly-d-lysine coated coverslips. Neurons were maintained at 37° in 95% O_2 and 5% CO_2 in MEM AQ (Sigma-Aldrich) supplemented with NGF (50ng/ml) and cytosine arabinoside (10µM) and used within 24h. Neurons were incubated with Fura-2 AM (2.5µM) and probenecid (1mM) for ~1h in a physiological salt solution (PSS) containing (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 glucose buffered to pH 7.4 (with NaOH). IgG was diluted to 200µg/ml in PSS and applied locally to the neurons, followed by a challenge with 50mM KCl to identify viable neurons. Fluorescence emission images (>520nm) were collected after excitation at 340 and 380nm using a microscope-based imaging system (Photon Technology International) and analyzed using ImageMaster 5.

Immunocytochemistry

In order to label cells in a non-permeabilized condition, cells were washed and 100 µg/ml of HC IgG (pooled) or FMS IgG (pooled) in media with 10% FBS and 1x Penicillin-Streptomycin was added for 3 hours and cells were kept in an incubator at 37°C. Cells were then washed with PBS, fixed with 4% PFA for 10 min, washed and a blocking solution of PBS with 5% normal goat serum was applied. SGC and neurons were visualized by adding primary antibodies (anti-GS or anti-β-III tubulin, respectively) diluted in the blocking solution to the cells. Following primary antibody incubation cells were washed and then incubated with fluorescently conjugated antibodies against human IgG and appropriate secondary antibodies.

Following washing, the slides were incubated with Hoechst (1:5000), washed again, and cover slipped with Prolong Gold mounting media. Cultures were imaged using a confocal microscope (Zeiss LSM800) operated by LSM ZEN2012 (Zeiss) software. Five random areas per condition per experiment were imaged. The binding of antibodies to cells and intensity of binding (assessed by integrated density) was analyzed in ImageJ after applying pixel intensity thresholds. A size threshold based on pixel area of a cell above the threshold was also used to determine if the cells were immunoreactive with a particular antibody.

Western blot analysis

Mice were deeply anesthetized with isoflurane and euthanized by decapitation. Brain, spinal cord and lumbar DRG were collected and flash frozen for later use. Protein was extracted from the tissue by sonication in an extraction buffer (0.5% Triton X-100, 50mM Tris, 150 mM NaCl, 1mM EDTA and 1% SDS in water, pH 7.4). Protein concentration was measured using the BCA Assay (Thermofisher Scientific). Samples were diluted in an LDS loading buffer (Thermofisher Scientific) and denatured with DTT at 95°C. An equal amount of protein per sample was loaded into 2-12% gradient NuPage Bis-Tris gels (Thermofisher Scientific) and protein was separated by electrophoresis. Protein was transferred to a nitrocellulose membrane using a dry transfer system (Thermofisher Scientific). The membrane was blocked with 5% non-fat milk powder (Biorad) in TBS.T (tris-buffered saline with Tween 20) for 1 hour at room temperature. The membrane was then probed with an anti-human HRP conjugated antibody (Santa Cruz) diluted in TBS.T with 5% non-fat milk powder. SuperSignal West Pico Chemiluminescent Substrate (Thermofisher Scientific) was incubated with the membrane and protein was visualized using a Biorad ChemiDoc Image System.

Autoimmunity profiling assay

Whole proteome protein fragment arrays were generated as previously described (47). Each array consisted of 58k spots representing 42k unique protein fragments (2 glass slides x 21k array), covering approximately 94% of the human protein coding genes. The antigens were produced within the Human Protein Atlas project (46) and consisted of protein fragments of approximately 50-150 aa selected based on sequence uniqueness within the human proteome. The antigens were produced in *E.coli* with a common purification and immune stimulating tag consisting of 6 histidines and an albumin binding protein (His6ABP). Samples (sera pools) were diluted 1:100 in assay buffer (0.1% PBS-Tween20, 3% BSA, 5% milk, supplemented with 160µg/ml His6ABP). The samples were incubated in assay buffer for 15 minutes, and then transferred to the slides containing the antigen arrays. The samples were incubated on the arrays for 1 hour at RT on the bench. After washing in 0.01% PBS-Tween20, the slides were then incubated with hen anti-His6ABP with dilution 1:40,000 (Hen anti-His6ABP, concentration 25mg/ml) for 1 hour on shaker. The secondary anti-human IgG (H+L) conjugated with Alexa 647 (Life Technology Cat. #A21445, 2mg/ml) with dilution 1:15,000 and Goat anti-chicken IgY Alexa 555 (Invitrogen Cat. #A-21437) with dilution 1:15,000 were used with 1 hour incubation at RT. After washing in 0.01% PBS-Tween20, the slides were then scanned using Capital Bio LuxScan HT24 scanner at a resolution of 10 µm. The images from the scanner were analysed using GenePix Pro 5.1 image analysis program. R was used for the statistical analysis. The result from the image analysis was background subtracted using the local background and the data was filtered for features that were negative after background subtraction, features that had been flagged as bad or not found during image analysis, and features that were less than 30

pixels in size. The data from the two arrays were then combined by calculating the mean of replicates if the replicates had a coefficient of variation less than 20% and discarding all replicates except those that had the highest protein content, as measured by an anti-tag antibody. Results were considered positive if the level exceeded the mean fluorescence intensity (MFI) plus 4 times the SD of the MFI.

TUNEL staining

The number of apoptotic cells in DRGs was assessed using Click-iT™ Plus TUNEL Assay for In Situ Apoptosis Detection, Alexa Fluor™ 594 dye Kit (Thermofisher Scientific) according to the manufacturer's protocol, except for increased number of washes (3x5 min each). Positive control slides were incubated with 1U DNase (1mg/ml, Sigma-Aldrich) for 30 minutes. Following washing with PBS + 0.1% Triton X-100 for 10 min, and 2x5 min with PBS, slides were incubated with NeuroTrace™ 640/660 Deep-Red Fluorescent Nissl Stain (Thermofisher Scientific) for 20 minutes at a dilution 1:200 in PBS. Slides were then washed as previously described, incubated with DAPI (1:20000) and cover slipped with Prolong Gold mounting media (Thermofisher Scientific). At least 4 DRG sections per animal, separated by at least 50 µm were stained. DRGs were imaged as described above.

To assess IgG-induced apoptosis in vitro DRG cultures were incubated with 1, 10 and 100 µg/ml of HC or FMS IgG for 24 hours. Following fixation with 4% PFA, the cultures were stained using the modified TUNEL procedure described above and imaged.

qPCR

Mice were deeply anesthetized with isoflurane, euthanized by decapitation and the L3-L5 DRGs were removed, flash frozen, and kept at -80°C until use. RNA was extracted using the TRIzol (Thermofisher Scientific)-chloroform method according to manufacturer's instructions. RNA was reverse transcribed into cDNA using MultiscribeTM Reverse Transcriptase (Applied Biosystems). Quantitative real-time PCR analysis was performed using a CFX384 instrument (Bio-Rad) with TaqMan™ Fast Advanced Master Mix (Thermofisher Scientific) and validated TaqMan Gene Expression Assays for *Hprt1* (Mm03024075_m1), *Gfap* (Mm01253033_m1), *s100b* (Mm00485897), *Itgam* (Mm00434455_m1) and *Aif1* (Mm00479862_g1). Data was analysed using the 2-ΔΔCt method and is normalized to *Hprt1* and then to HC IgG injected mice. Data is presented as the mean fold-difference ± SEM.

Serum cytokine and chemokine analysis

Mice were euthanized on day four following two injections of 8mg IgG and serum was collected and stored at -80°C until analysis. Serum concentrations of cytokines and chemokines were measured using the V-Plex Proinflammatory Panel 1 (mouse) kit (Mesoscale Discovery, catalogue # K15048D-1, lot # K0081524) according the manufacturer's instructions. All serum samples were diluted 1:2 and measured as technical duplicates. The assay was read using a MESO QuickPlex SQ120 (Mesoscale Discovery) and data was analyzed using the Discovery Workbench 4.0 software (Mesoscale Discovery). The lower limit of detection for each analyte was defined as the mean signal of the blank plus 2.5 standard deviations and the lower limit of quantification was obtained from the manufacturer.

Serum IgG subclass quantification

IgG1, IgG2, IgG3 and IgG4 ELISAs (Thermofisher Scientific, catalogue numbers BMS2092, BMS2093, BMS2094, BMS2095), or an IgG subclass ELISA (Thermofisher Scientific,

catalogue number 991000) were used to quantify serum concentrations of each IgG isotype. ELISAs were performed according to the manufacturer's instructions, read with a Spectramax iD3, or a Flexstation 3 plate reader (Molecular Devices) and analyzed with Softmax Pro software (Molecular Devices).

1. Quallo T, et al. TRPM8 is a neuronal osmosensor that regulates eye blinking in mice. *Nat Commun*. 2015;6:7150.

Figure 7a, human IgG blot

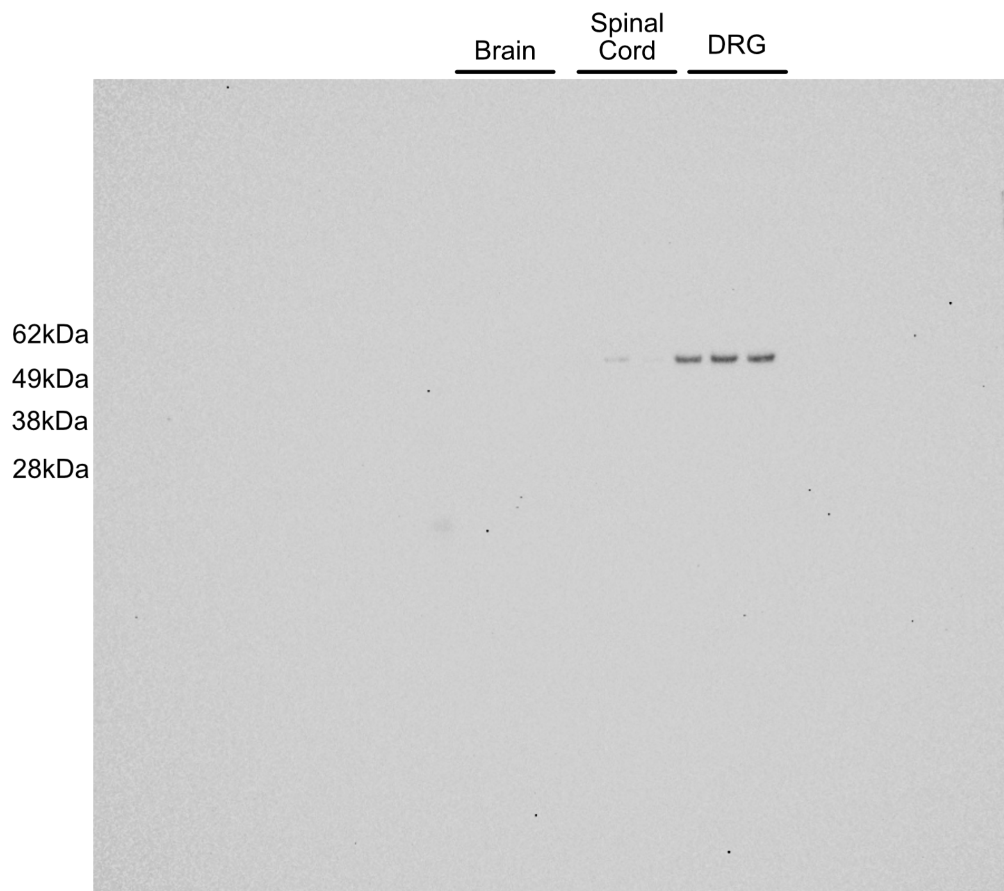


Figure 7a, GAPDH blot