**Animals**

Adult CD-1 and C57BL/6 mice (Charles River, Sulzfeld, Germany) transferred into the animal facility of the Institute of Clinical Neurobiology or Department of Neurology of the University Hospital Würzburg (Würzburg, Germany) were used for primary neuronal cultures. Experiments were approved by the local veterinary authority (Veterinäramt der Stadt Würzburg) and the Ethics Committee of Animal Experiments, i.e. Regierung von Unterfranken, Würzburg (FBVVL 568/200-324/13). Mice were housed in cages with filter top (EU Direction 2010/63/EU) with access to water and food *ad libitum* at a 12 hours light/dark rhythm with lights on at 6.30 a.m. C57BL/6 mice were used for preparation of embryonal and adult DRG neuron culture and CGN culture. CD-1 mice were used occasionally for preparation of adult DRG neuron culture as there were no differences in binding of patients’ anti-CNTN1 autoantibodies between neurons from C57BL/6 mice or CD-1 mice.

**Adult DRG neurons**

Adult male/female wild-type C57Bl/6J mice or male/female wild-type CD-1 mice were sacrificed by overdose exposure to CO2. DRGs were dissected as previously described ([Sleigh et al., 2016](#_ENREF_32)). Briefly, spinal columns were excised and cut dorsally into two halves. After removing the spinal cord, DRGs were pulled out of the intravertebral foramen and anterior and posterior nerve roots were cut off. DRG neuron cultures were established by digestion with LiberaseTM TH for 30 min at 37 °C and 900 rpm followed by incubation with LiberaseTM TM (Roche, Mannheim, Germany) for 10 min at 37 °C and 900 rpm. Both enzymes contain two isoforms of collagenase with high and medium concentrations of thermolysin, respectively. Cells were plated on poly-D-lysine (0.1 mg/ml) coated coverslips in DRG medium (DMEM/F-12 supplemented with GlutaMAX™ (Life Technologies, Darmstadt, Germany), 10% FCS, penicillin (10,000 U/ml)/streptomycin (10,000 µg/ml) and nerve growth factor (NGF, 100 ng/ml) and incubated at 37 °C and 5% CO2. Adult DRG neurons were used for binding assays, immunocytochemistry of β III tubulin, electrophysiological analysis of sodium channels and super-resolution microscopy.

**Embryonal DRG neurons**

Embryonal DRGs were dissected from age Day 14 wild-type C57Bl/6J mice according to [Hall (2006)](#_ENREF_14). Cultivation of embryonal DRG neurons was established by enzymatic digestion of DRGs with 0.05% trypsin-EDTA (Thermo Fisher Scientific, Waltham, United States) in Neurobasal medium (Life Technologies, Darmstadt, Germany) for 30 min at 37 °C. After removing the trypsin-EDTA, cells were triturated and plated on poly-D-lysine/laminin coated coverslips (Corning Inc., Corning, NY, USA). DRG neurons were grown in Neurobasal medium (Life Technologies, Darmstadt, Germany) supplemented with 1% B-27 (Life Technologies, Darmstadt, Germany), 1 mM GlutaMAX™ and penicillin (10,000 U/ml)/ streptomycin (10,000 µg/ml) at 37 °C and 5% CO2. Embryonal DRG neurons were used for binding assays and incubation experiments with anti-CNTN1 autoantibody positive patients’ sera.

**CGN**

Primary culture of CGN was established by dissection of P5 mouse (male and female) cerebellum. Dissociation of cells was performed with a papain dissociation system (Worthington Biochemical Corporation, Lakewood, NJ, USA) as described by the manufacturer. Cells were plated on poly-D-lysine/laminin coated coverslips (Corning Inc., Corning, NY, USA) and grown in Neurobasal medium (Life Technologies, Darmstadt, Germany) supplemented with 1% B-27 (Life Technologies, Darmstadt, Germany), 1 mM GlutaMAX™ and penicillin (10,000 U/ml)/ streptomycin (10,000 µg/ml) at 37 °C and 5% CO2.

**Binding assays**

For HEK293 cells and adult DRG neurons, unspecific binding sites were blocked for 20 min in 10% BSA/PBS at 37 °C and 5% CO2 following incubation with serum or PE material of anti-CNTN1 positive patients or serum of HCs or anti-CNTN1 antibody (AF904, RD Systems, Minneapolis, MN, USA) at a dilution of 1:500 for HEK293 cells or 1:250 for DRG neurons in 2% BSA/PBS for 1 h at 37 °C and 5% CO2. After fixation with 4% paraformaldehyde, cells were incubated with appropriate secondary fluorescent antibodies (goat anti-human Cy3, 1:500, 109-165-003; donkey anti-goat Dylight488, 1:500, 705-485-147; donkey anti-goat Cy3, 1:500, 705-165-147; goat anti-human AF488, 1:500, 109-545-003; Dianova, Hamburg, Germany) in 2% BSA/PBS for 1 h at room temperature (21 °C) and embedded in VECTASHIELD® Antifade Mounting Medium with DAPI. CGNs and embryonal DRG neurons were first fixed in 4% paraformaldehyde following blocking of unspecific binding sites for 20 min in 10% BSA/PBS. Primary antibodies (anti-CNTN1, 1:500, AF904, RD Systems, Minneapolis, MN, USA) or sera of anti-CNTN1 positive patients (1:500) were then incubated overnight at 4 °C in PBS. The next day, CGNs or embryonal DRG neurons were incubated with appropriate secondary antibodies (goat anti-human Cy3, 1:300, 109-165-003; donkey anti-goat Cy3, 1:300, 705-165-147; Dianova, Hamburg, Germany) in 2% BSA/PBS for 1 h at room temperature and embedded in VECTASHIELD® Antifade Mounting Medium with DAPI.

**Stainings of pan-neurofascin, β III tubulin and pan sodium channel**

24 h after plating adult DRG neurons or CGNs, cells were incubated with patients’ material or serum of a HC for specific periods of time at a dilution of 1:250 for DRG neurons or 1:100 for CGNs in medium at 37 °C and 5% CO2. Afterwards neurons were fixed in 4% paraformaldehyde for 20 min and unspecific binding sites were blocked with 10% BSA/PBS. For co-stainings of CNTN1 and pan sodium channels, adult DRG neurons were incubated with anti-CNTN1 antibody (1:250, AF904, RD Systems, Minneapolis, MN, USA) for 1 h at 37 °C and 5% CO2 prior to fixation. Then, DRGs were permeabilized in 0.1% Triton X-100 (X100-100ML, Sigma-Aldrich, Saint-Louis, MO, USA) for 20 min. Primary antibodies (anti-pan neurofascin, 1:2,000 or 1:1,000, AF3235, RD Systems, Minneapolis, MN, USA; anti-beta III tubulin, 1:250 or 1:500, ab41489, abcam, Cambridge, USA; anti-pan sodium channel, 1:100, S8809, Sigma-Aldrich, Saint-Louis, MO, USA) were applied to DRG neurons in 2% BSA/PBS or CGNs in PBS overnight at 4 °C. Cells were incubated with appropriate secondary antibodies (donkey anti-goat Cy3, 1:500, 705-165-147; goat anti-human AF488, 1:500 or 1:300, 109-545-003; goat anti-chicken Cy3, 1:250 or 1:300, 103-165-155; Dianova, Hamburg, Germany) for 1 h at room temperature in 2% BSA/PBS before embedding in VECTASHIELD® Antifade Mounting Medium with DAPI. Additionally, for SIM measurements goat anti-mouse IgG was custom labelled using an ATTO-643 NHS-ester (AD643-31, ATTO-TEC, Siegen, Germany) to stain sodium channels in adult DRG neurons. DRG neurons used for SIM were embedded in ProLong™ Glass Antifade Mountant (P36982, Thermo Fisher Scientific, Waltham, United States).

**Validation of IgG Fab and F(ab’)2 purification**

Both anti-contactin-1 IgG Fab and F(ab’)2 digestion and purification were complete in pat1 and pat3, as purified Fab and F(ab’)2 showed low ODs (OD450nm < 0.5) in anti-contactin-1 ELISA using IgG Fc-specific secondary antibody, and remnants using IgG Fab specific secondary antibody. IgG, IgG Fab and F(ab’)2 of the seronegative control resulted in low ODs (OD450nm < 0.5) in all assays and dilutions.