ELISA, CBA and IFA supplemental Methods.

ELISA

ELISA were performed as previously described [1]. Briefly, MaxiSorp microtiter plates (ThermoFisher; 44-2404-21) were coated with 50 ng in PBS of human Nfasc155, Nfasc186, CASPR1, or 20 ng of CNTN1 overnight at 4°C. Wells were then blocked with 0.5% casein + 0.05% Tween-20 in PBS for 1 hour at 37°C and then incubated overnight at 4°C with sera diluted 1:500 in blocking solution. The day after, the membranes were incubated with HRP-conjugated secondary antibodies and the reactivity was revealed with SIGMAFAST OPD (Merck-Millipore), and optical density (OD) were measured at 450 nm. Sera were considered positive when the OD was higher than 3 standard deviations above the average of eight HC signal at 1:500 dilution.

CBA

HEK293T cells were transiently transfected using JetPEI (Polyplus-transfection, Illkirch-Graffenstaden, France) with plasmids encoding full length Human myc tagged Nfasc155, myc tagged Nfasc186, CNTN1 or CNTN1 together with CASPR1, as previously described [2]. Living cells were incubated for 20 min at 37°C with sera diluted 1:50 in OptiMEM (ThermoFisher), quickly washed in PBS, and fixed with 2% paraformaldehyde in PBS for 20 min at RT. After several washes, cells were blocked for 20 min at RT with PBS + 5% fish skin gelatin + 0.1 % Triton-X100, incubated for 60 min at RT with primary antibodies: a mouse monoclonal antibody against Myc (1:500; 11667149001; Roche, Bale, Switzerland) or goat antibodies against CNTN1 (1:2000; AF904; R&D systems). Cells were then revealed for 30 min at RT with secondary antibodies, counterstained with DAPI, and mounted. Cells were

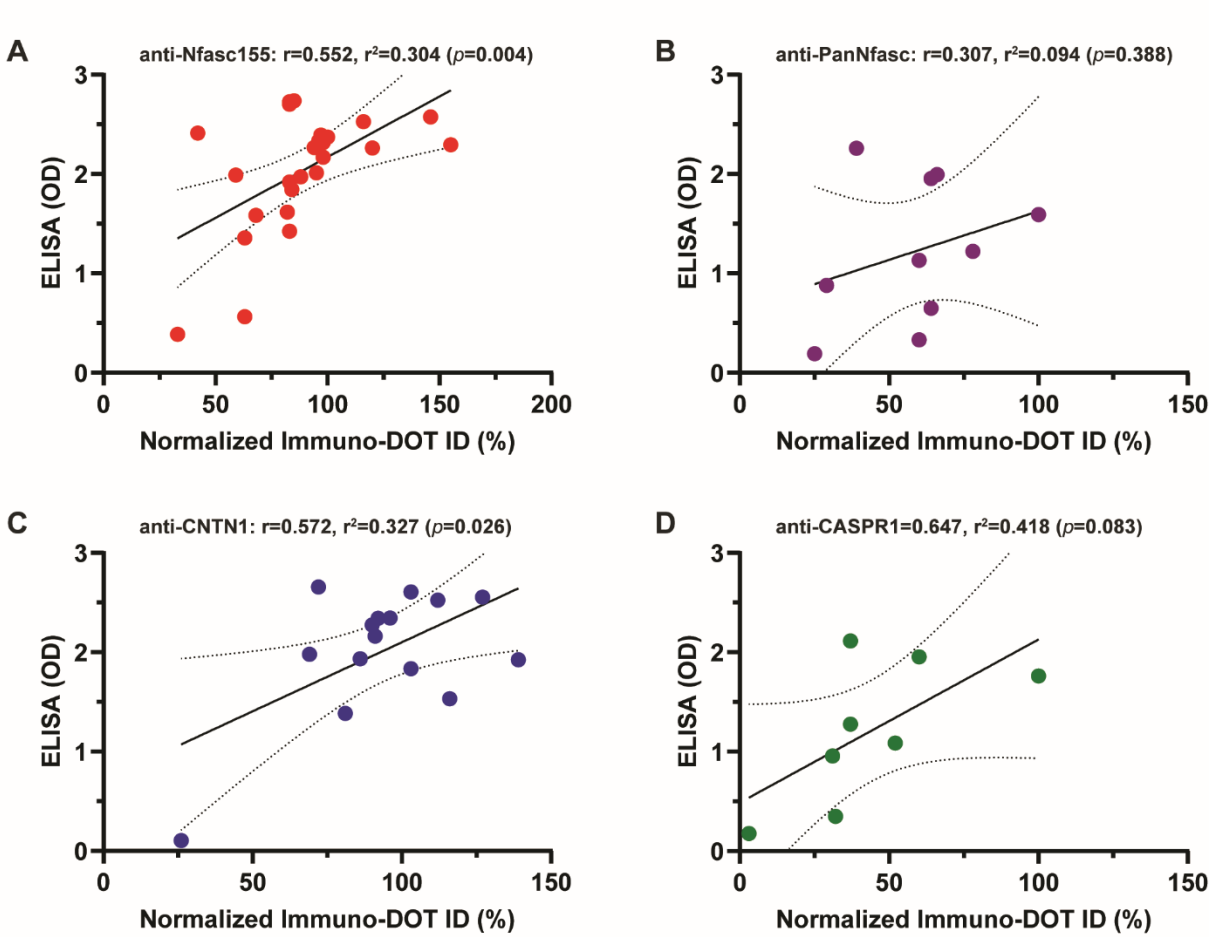
observed using an ApoTome fluorescence microscope (Carl Zeiss MicroImaging GmbH, Oberkochen, Deutschland).

IFA

Immunolabeling was performed as previously described [3]. Briefly, sciatic nerves from C57/Bl6 mice were dissected out and fixed in 2% paraformaldehyde in PBS for 1 hour at 4°C, then rinsed in PBS. Axons were gently teased and stored at –20°C. Teased fibers were permeabilized by immersion in -20°C acetone for 10 minutes, washed in PBS, then blocked with PBS + 5% fish skin gelatin + 0.1 % Triton-X100 for 60 min at RT. Tissues were then incubated overnight with sera diluted 1:200 in blocking solution with a goat antibody against CNTN1 (1/2000). The day after, slides were washed and incubated 60 min at RT with the following secondary antibodies: Alexa 488 conjugated donkey anti-goat IgG (1:500; 705-545-147; Jackson Immunoresearch) and Alexa 594 conjugated donkey anti-human IgG (1:500; 709-585-149; Jackson Immunoresearch). Slides were mounted and examined using an ApoTome fluorescence microscope.

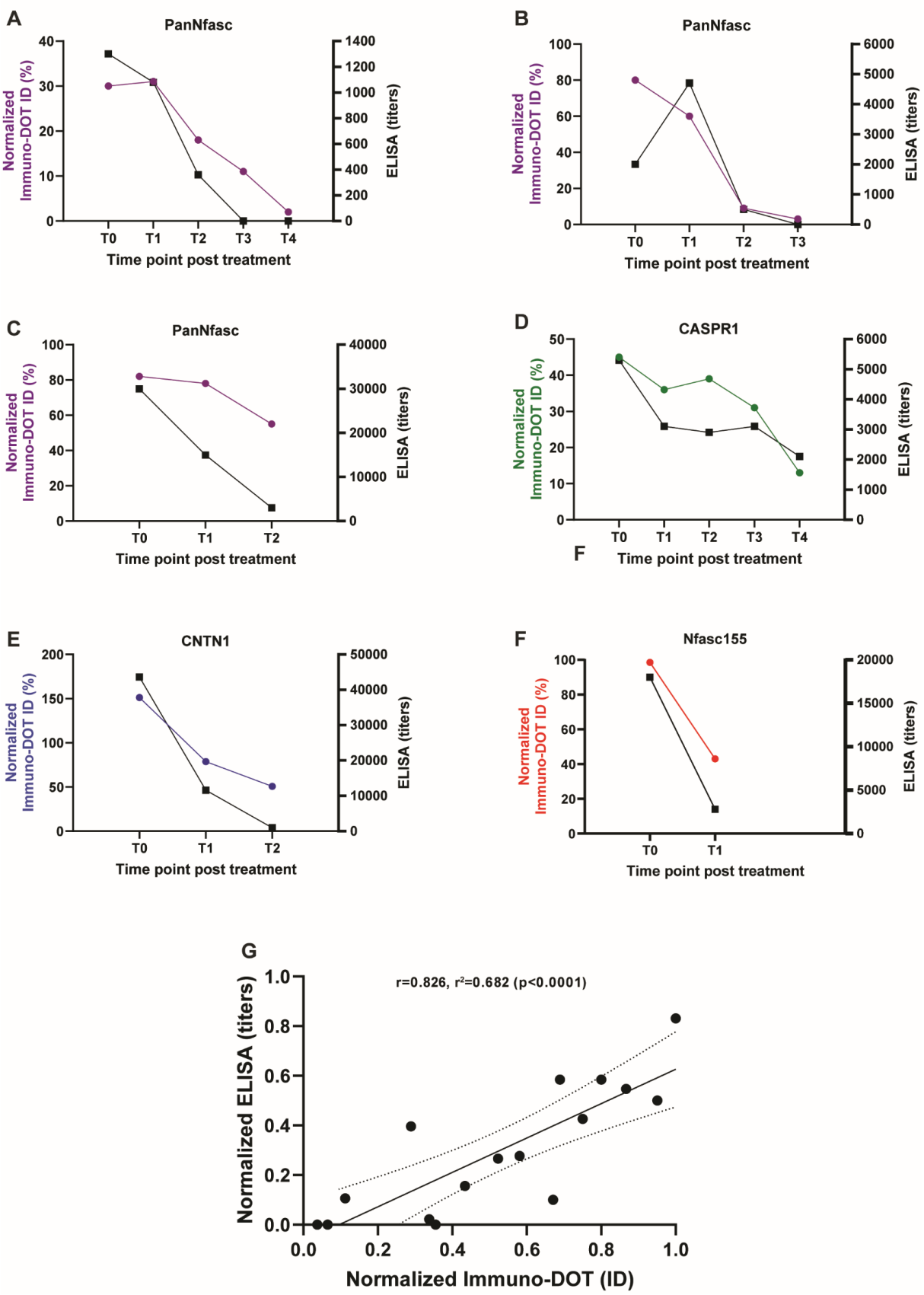
References of supplemental Methods

1. Jentzer A, Attal A, Roué C, Raymond J, Lleixà C, Illa I, et al. IgG4 Valency Modulates the Pathogenicity of Anti-Neurofascin-155 IgG4 in Autoimmune Nodopathy. Neurol Neuroimmunol Neuroinflamm 2022;9:e200014.
2. Delmont E, Manso C, Querol L, Cortese A, Berardinelli A, Lozza A, et al. Autoantibodies to nodal isoforms of neurofascin in chronic inflammatory demyelinating polyneuropathy. Brain 2017;140:1851–1858.
3. Manso C, Querol L, Lleixà C, Poncelet M, Mekaouche M, Vallat J-M, et al. Anti-Neurofascin-155 IgG4 antibodies prevent paranodal complex formation in vivo. J Clin Invest 2019;129:2222–2236.



Supplemental Figure 1: Comparison of the normalized ID of the Immuno-DOT and the OD of the ELISA

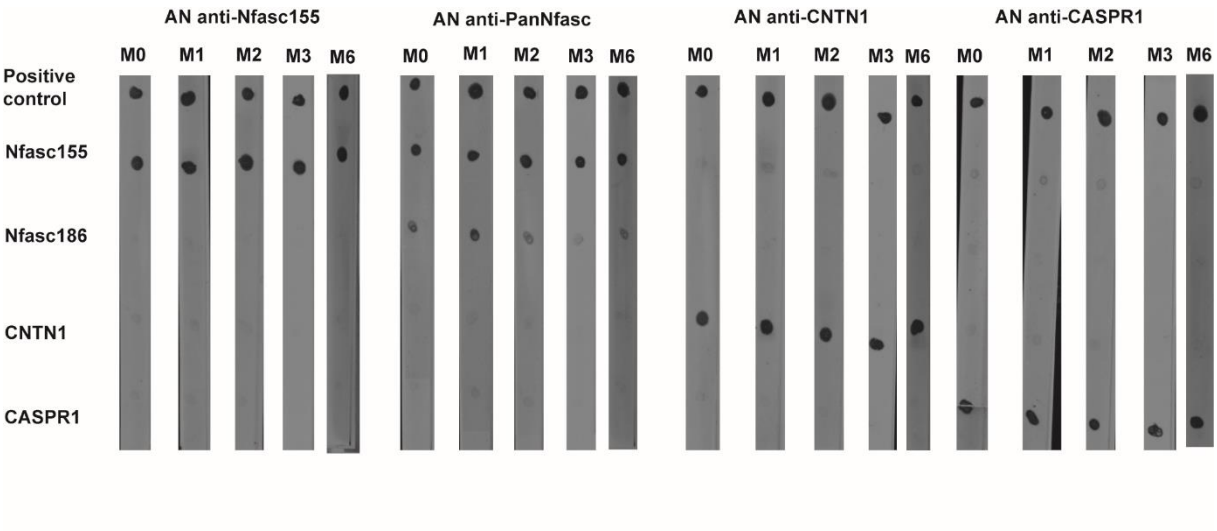
Pearson correlation coefficient (r), r square (r2), and 95% confidence band (dotted lines) are indicated on the graphs that showed the normalized integrated density (ID) and the optic density (OD) for anti-Nfasc155 (A), anti-PanNfasc (B), anti-CNTN1 (C), anti-CASPR1 (D) reactive patients.



Supplemental Figure 2: Immuno-monitoring following immunosuppressive therapies

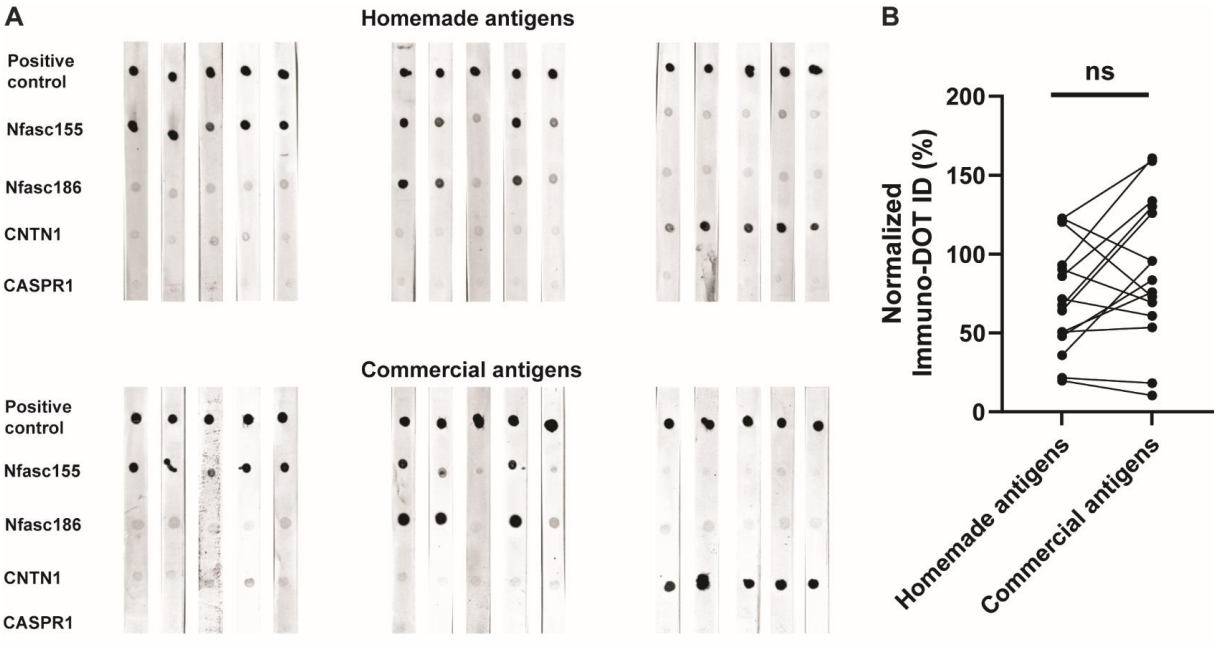
(A-F) The reactivity of six AN patients who benefited from treatments with rituximab or plasma exchange was examined by immuno-DOT and ELISA: 3 PanNfasc+ (5, 4 and 3 samples,

respectively) (A,B,C), 1 CASPR1+ (5 samples) (D), 1 CNTN1+ (3 samples) (E), and 1 Nfasc155+ (2 samples) (F). The evolution of antibody titers measured by ELISA (black) and the normalized ID values of immuno-DOT (in colors) are represented in the graphs. (G) Antibody titers (ELISA) or ID values (immuno-DOT) were normalized to the maximal values in each series. Pearson correlation coefficient (r), r square (r2), and 95% confidence band (dotted lines) are indicated on the graph between the optic density (OD) for ELISA and the integrated density (ID) for immuno-DOT.



Supplemental Figure 3: Stability assay of immuno-DOT

Images of immuno-DOTs tested at 0, 1, 2, 3 and 6 months after freezing with the sera of one AN anti-Nfasc155+, one AN anti-PanNfasc+, one AN anti-CNTN1+ or one AN anti-CASPR1+, as indicated.



Supplemental Figure 4: Homemade antigens *vs* commercial antigens

(A) Images of immuno-DOTs prepared with homemade (upper panel) or commercial (lower panel) antigens and tested with 5 anti-Nfasc155+ AN (left), 5 anti-PanNfasc AN (middle) or 5 anti-CNTN1+ AN (right). (B) Scatter plots of the normalized ID obtained with homemade and commercial antigens. No significant difference was found (n.s.; paired two tailed Student's t tests).