**Appendix e-1: Supplementary methods**

**Cell purification**

Primary human fibroblasts, expressing TLR3 were obtained from skin biopsies of

patient and healthy controls, and were cultured in DMEM medium (ThermoFisher, USA, ref. 41965-047) supplemented with 10% fetal calf serum (FCS) (Linus Cultek, Spain, ref. 501805).

Freshly isolated human PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation (Lymphoprep, Serumwerk, Germany, ref. 04-03-9391/01) from whole blood samples obtained from patient and healthy volunteers.

For the differentiation and culture of monocyte-derived dendritic cells (MDDCs), PBMCs freshly isolated by Ficoll-Hypaque density gradient centrifugation or previously frozen PBMCs from healthy controls and patient were incubated in RPMI 1640 (ThermoFisher, USA, ref. 21875-034) supplemented with 10% FCS in 6-well culture plates for 1 hour, at 37°C, under an atmosphere containing 5% CO2. The adherent cells (monocytes) were resuspended in RPMI 1640 supplemented with 10% FCS in the presence of GM-CSF (Miltenyi Biotec, Germany, 50 ng/mL) and IL-4 (Miltenyi Biotec, Germany, 10 ng/ml) and cultured in 96-well culture plates (0.5x105 cells/well) for 8 days. The differentiation and purity of dendritic cells was assessed by microscopic evaluation and/or staining with anti-CD1a and anti-CD14 antibodies (Beckton Dickinson).

**TLR –pathway evaluation**

The MDDCs and the fibroblasts were stimulated using two different TLR3 agonists: polyinosinic-polycytidylic acid (Poly (I:C) 25µg/mL and low molecular weight Poly(I:C) (Poly(I:C) LMW) 25µg/mL (Invivogen, San Diego, California; ref: tlr-kit1hw)1; and its own culture media as negative control.

**Cytokine determination**

The supernatant obtained after stimulation was tested, following kit manufacturer´s instruction, for IFN-b on stimulated fibroblasts and IL-6 on stimulated MDDCs. IFN-b was determined by ELISA (VeriKine Human IFN beta, PBL Assay Science, New Jersey, ref. 41410). IL-6 was determined by LUMINEX (Milliplex Map, Merk, Germany).

**Neuronal surface antibodies determination**

Serum and CSF samples of the patient were examined using rat brain tissue immunohistochemistry and cultured live neurons. In brief, adult Wistar rats were sacrificed and the brains removed, split sagitally, fixed with 4% paraformaldehyde for 1 hour at 4ºC, cryopreserved with 40% sucrose for 48 hours, embedded in freezing media, and frozen in methylbutane chilled with liquid nitrogen. Seven-micron thick cyrostat sections were sequentially incubated with 0.3% H2O2 for 15 minutes, blocked with 5% goat serum for 1 hour at room temperature, and exposed to patient’s CSF (diluted 1:5) overnight at 4ºC. Sections were then incubated with biotinylated goat anti-human IgG (1:2000; Vector Laboratories, ref BA-3000) and the reactivity was developed with avidin-Biotin peroxidase (Elite kit; Vector Laboratories, ref PK-6100) and diaminobenzidine (Vector Laboratories, ref SK-4105). The tissue was counterstained with haematoxylin. Rat hippocampal neuronal cultures were prepared as reported.2,3 Fourteen days live neurons grown on coverslips were treated for 1 hour at 37º C with patients’ or control serum (final dilution 1:200) or CSF (1:5). After removing the media and extensive washing with PBS, neurons were fixed with 4% PFA, and incubated with anti-human IgG (diluted 1:1000) Alexa Fluor secondary antibody (Molecular Probes, OR). Results were photographed under a fluorescence microscope using Zeiss Axiovision software (Zeiss, Thornwood, NY).2,3

In addition serum and CSF samples were analyzed by in house cell-based assays for the following antibodies: NMDAR, AMPAR, GABAA R, GABABR, Dopamine 2 R, Glycine R, mGluR5, neurexin3-alfa, LGI1, Caspr2, DPPX, MOG, AQP4 and IgLON5, using previously reported techniques.2,3

**Supplementary References**

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2. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 2008;7:1091-1098.

3. Lai M, Hughes EG, Peng X, et al. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. AnnNeurol 2009;65:424-434.