Supplementary material

Immunoblot of soluble LGI1 and determination of concentration of LGI1

Media from the indicated LGI1-expressing HEK293 cells, and media from non-transfected cells, were normalized in protein concentration, and 25 μg of protein diluted 1:4 with Rotiload (#K929.1, Roth) boiled for 5 minutes, and separated in a 4-12% BisTris electrophoresis gel (#NP03222 NuPage, Invitrogene). After transferring the proteins to a nitrocellulose membrane (#1704158, Biorad), they were probed with a polyclonal rabbit antibody against LGI1 (1:200, #ab30868, Abcam) overnight at 4°C, followed by 1 hour incubation with a peroxidase-labeled secondary anti-rabbit antibody (1:1000, NIF824, GE HealthCare) and the signal developed with ECL western blotting

detection reagent (#RPN2108, GE Healthcare) visualized with LAS4000 imager (GE Healthcare).

To determine the concentration of secreted LGI1 in the media of HEK LGI1-expressing cells, 25 μg of total protein from media of HEK LGI1-expressing cells, and non-transfected HEK cells (control), were run in a gel side by side with known concentrations (0.4 μg; 0.2 μg) of a commercially available recombinant protein (H00009211-P01, Abnova). After probing with the indicated polyclonal rabbit antibody against LGI1 and the corresponding secondary antibody, the LGI1 signal was developed with the ECL detection reagent, as above, and quantified with ImageQuant TL software (GE Healthcare).

Immunoabsorption of patients' LGI1 IgG with HEK293 cells expressing LGI1

HEK293 cells expressing LGI1 or not expressing LGI1 (control) were seeded in multiple wells of a 24-well cell culture plate at a density of 150,000 cells/well. LGI1 IgG pooled from patients (4 μg/μl) was added to the first well of HEK293 expressing or non-expressing LGI1 (total volume of media 300μl/well; final dilution of the antibody, 1:150) for 1 hour, and subsequently the media was serially transferred to 9 additional wells (1 hour each) of the corresponding HEK293 cultures. The media was then removed by aspiration, filtered, and used for immunohistochemistry of rat brain tissue, HEK293 cells expressing LGI1, and cultured neurons.

Mice, surgery, placement of ventricular catheters and osmotic pumps

Male C57BL6/J mice (Charles River), 8-10 weeks old (25-30 grams) were housed in cages of five until one week before surgery when they were housed individually. The room was maintained at a controlled temperature (21±1ºC) and humidity (55±10%) with illumination at 12-hour cycles; food and water were available *ad libitum*. All

experiments were performed during the light phase, and animals were habituated to the experimental room for 1 week before starting the tests.

Cerebroventricular infusion of patients' LGI1 IgG or control IgG was performed using osmotic pumps (model 1002, Alzet, Cupertino, CA) with the following characteristics: volume 100 µl, flow rate 0.25 µl/hour, and duration 14 days, as reported (Planaguma *et al.*, 2015). In brief, 24 hours before surgery, two osmotic pumps per animal were each loaded with 100 µl of patients' LGI1 IgG or control IgG (1µg/µl). Mice were then placed in a stereotaxic frame, and a bilateral cannula (model 3280PD-2.0/SP, PlasticsOne) was inserted into the ventricles (coordinates: 0.2 mm posterior and ±1.00 mm lateral from bregma, depth 2.2 mm), as reported (Planaguma *et al.*, 2015). Each arm of the cannula was connected to one osmotic pump, which was subcutaneously implanted on the back of the mice (two pumps per animal). Appropriate ventricular placement of the catheters was assessed in randomly selected mice injecting methylene blue through the catheters, as reported (Planaguma *et al.*, 2015).

Behavioral tasks

Multiple behavioral tasks were applied at different time points related to the day of pump implantation and initiation of the ventricular infusion of patients' LGI1 IgG or control IgG. They included the novel object recognition (NOR) index and spontaneous locomotor activity (LOC), which were tested 1 day before surgery and on days 3, 10, 18, 25 and 47 after surgery, black and white test (BW, day 7), elevated plus maze (EPM, day 17), and resident-intruder test (RI, days 11 and 26). The distribution of behavioral tests in relation to the infusion period is shown in Supplementary Fig. 4. Detailed information on these tests has been previously reported (Planaguma *et al.*, 2015).

Golgi-Cox staining

Golgi silver impregnation was conducted with the FD Rapid GolgiStainTM Kit (#PK401, FD NeuroTechnologies, Inc., Columbia, USA) according to manufacturer's instructions. Briefly, the tissue was rinsed in aCSF1 to remove blood residues, and was then transferred into equal amounts of solution A+B of the FD Rapid GolgiStainTM Kit. After renewal of solutions on the second day the tissue was subsequently left in the dark for two weeks at room temperature. Thereafter, brain hemispheres were transferred into solution C of the FD Rapid GolgiStainTM Kit, which was changed after one day, and were left in solution at 4°C for 5 days. Hemispheres were then stored at -80°C until further use. Thereafter, hemispheres were cut into 150µm thick coronal slices in the area of the hippocampus using a Leica CM3050S cryostat and mounted on object slides. For the last step of staining procedure, slices were washed in purified water (2 x 4 minutes), incubated in a mix of 1/4 solution D, 1/4 solution E and 1/2 purified water for 10 minutes, washed again, and then dehydrated in an ascending alcohol series (50%, 75%, 95% for 4 minutes each, and 2 x 100 % for 8 minutes). Finally, slices were immersed in xylene (2 x 5 minutes), and then quickly coverslipped with Entellan® (Merck KGaG, Darmstadt, Germany) and dehumidified overnight.

Sholl analysis

To evaluate dendritic morphology, a Zeiss Axioskop 2 mot plus (Zeiss, Oberkochen, Germany) and a computer-based system (Neurolucida; MicroBrightField) was used to generate three-dimensional neuron tracings that were subsequently analyzed using the NeuroExplorer Software (MicroBrightField). Golgi-impregnated cells were selected for reconstruction if they fulfilled the following criteria: (1) the neuron was located in the dentate gyrus with soma in outer granule layer of the superior or inferior blade, (2) the neuron was distinguishable from neighboring cells to allow for identification of

dendrites, (3) the dendrites were not truncated or broken, and (4) the cell exhibited dark and well filled impregnation throughout whole dendrites including spines. Selected cells were traced manually using the Neurolucida system at 40x magnification. For each reconstructed neuron an estimate of dendritic complexity was obtained using the Sholl ring method (Zhou *et al.*, 2009). Additionally, the total dendritic length, the number of branch ends and the number of dendrites per branch order were calculated.

For analysis of dendritic spines, positively stained neurons in the CA region were randomly selected and used for analysis as described previously (Orlowski and Bjarkam, 2012). Neuronal apical dendrites were followed up to tertiary branches without visible disruptions and without further split up using a Zeiss Axioskop 2 mot plus with a 100x oil immersion objective (PLAN-Neofluar). For correct analysis of dendrite length, tertiary branches were chosen to be in an even z-layer. Number and type of spines (thin, mushroom, stubby, filopodia or branched) were counted manually by the same blinded investigator according to previous reports (McKinney, 2010). Total spine density was calculated as number of spines per 10 µm of dendritic length. Morphological subclassification was outlined in proportion of all spines regardless of spine properties.

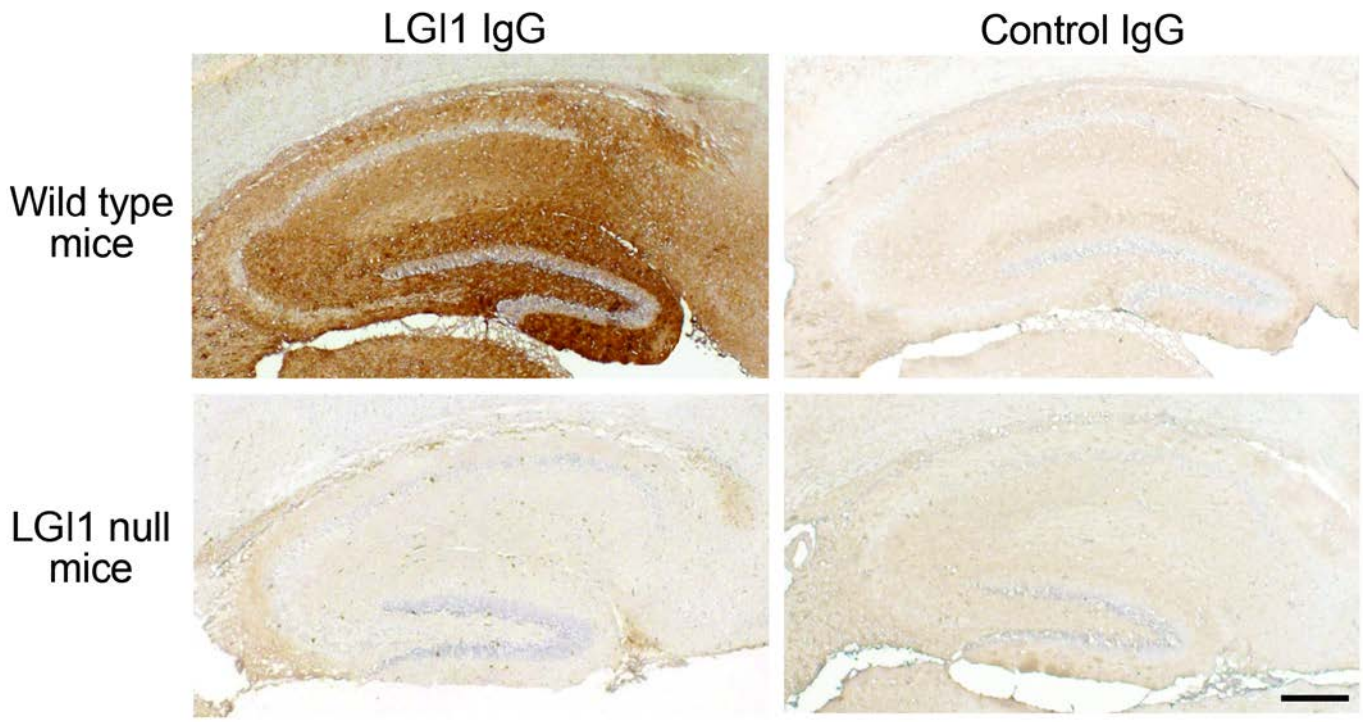
Supplementary Table 1: Clinical and MRI features of patients with anti-LGI1 encephalitis*

Patient	Age	Gender	Facio-brachial dystonic seizures	Other seizure types	MRI T2/ FLAIR increased signal
1**	62	F	No	Generalized	No
2**	54	F	Yes	No	Unilateral mesiotemporal
3**	73	M	Yes	No	No
4**	65	M	No	Generalized	Unilateral mesiotemporal
5**	65	M	No	Focal	Bilateral mesiotemporal
6**	63	F	No	Focal and generalized	Bilateral mesiotemporal
7**	52	F	No	Generalized	Bilateral mesiotemporal
8**	59	M	Yes	Focal and generalized	Basal ganglia, frontal, insula
9	71	F	Yes	Focal and generalized	No
10	57	M	No	Focal and generalized	Bilateral mesiotemporal
11	63	M	Yes	Focal and generalized	Bilateral mesiotemporal
12	72	M	Yes	No	Unilateral mesiotemporal sclerosis
13	80	M	No	Focal	No
14	71	F	No	Generalized	Bilateral mesiotemporal
15	61	M	No	Focal and generalized	Bilateral mesiotemporal and basal ganglia
16	74	M	Yes	Focal	Bilateral mesiotemporal
17	59	M	Unknown	Focal and generalized	No
18	56	M	No	Focal	Unilateral mesiotemporal
19	54	F	Unknown	NA	Bilateral mesiotemporal
20	58	M	No	No	Bilateral mesiotemporal
21	65	M	Yes	Focal and generalized	Unilateral insula
22	41	M	Yes	Focal	No
23	56	M	Unknown	Focal	Unilateral mesiotemporal
24	53	F	Yes	Focal	No
25	58	F	Yes	Focal	Bilateral mesiotemporal

*In all patients the main syndrome was limbic encephalitis

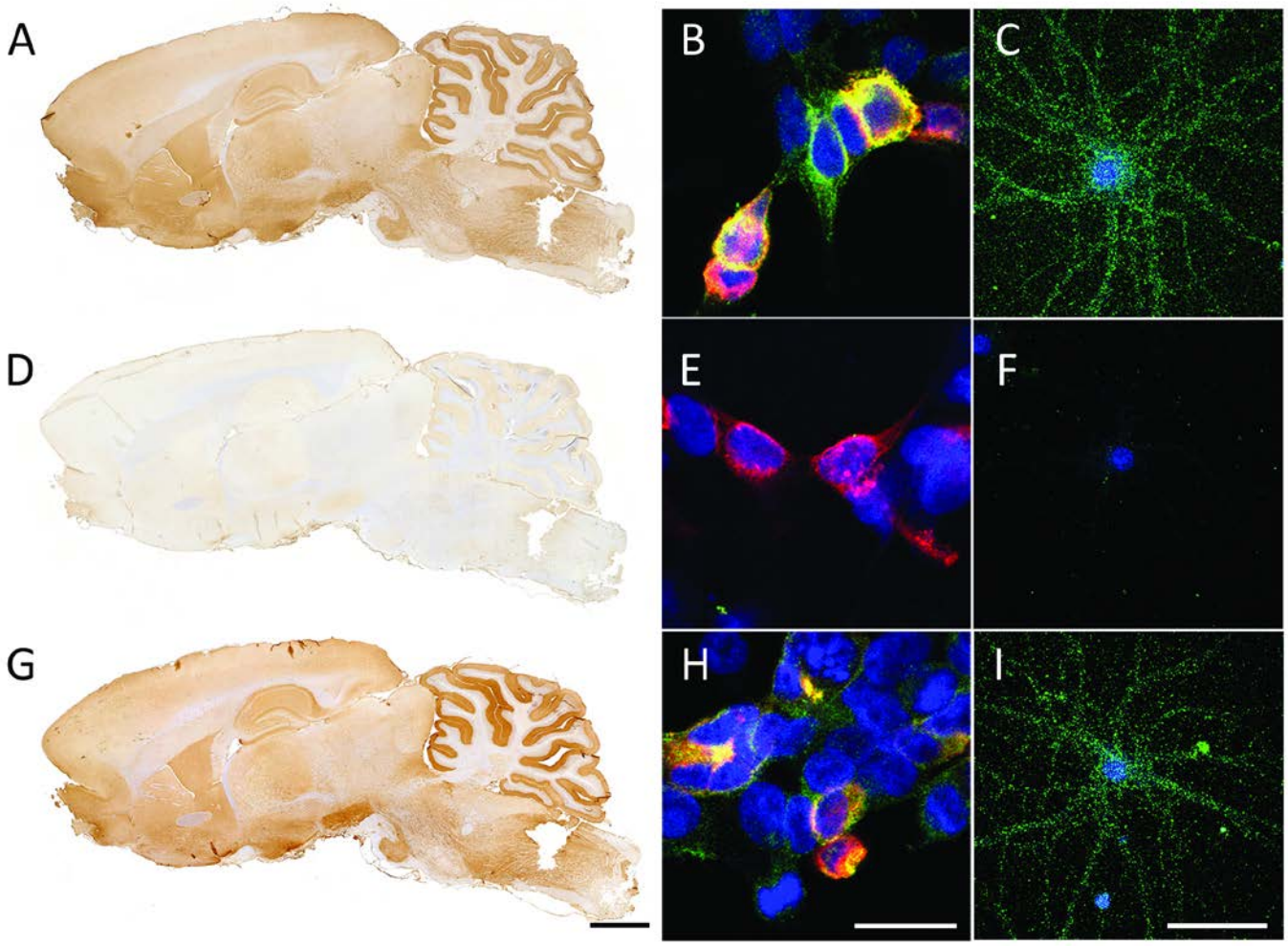
**Pooled IgG from these samples were used for animal experiments

Supplementary figures



Supplementary Figure 1: Patients' LGI1 IgG does not react with brain of LGI1 null mice

LGI1 IgG from patients intensively reacts with hippocampus of wild type mice but it does not react with hippocampus of LGI1 null mice. Control IgG from healthy participants does not show reactivity with any of the brains. Scale bar = 200 μm.

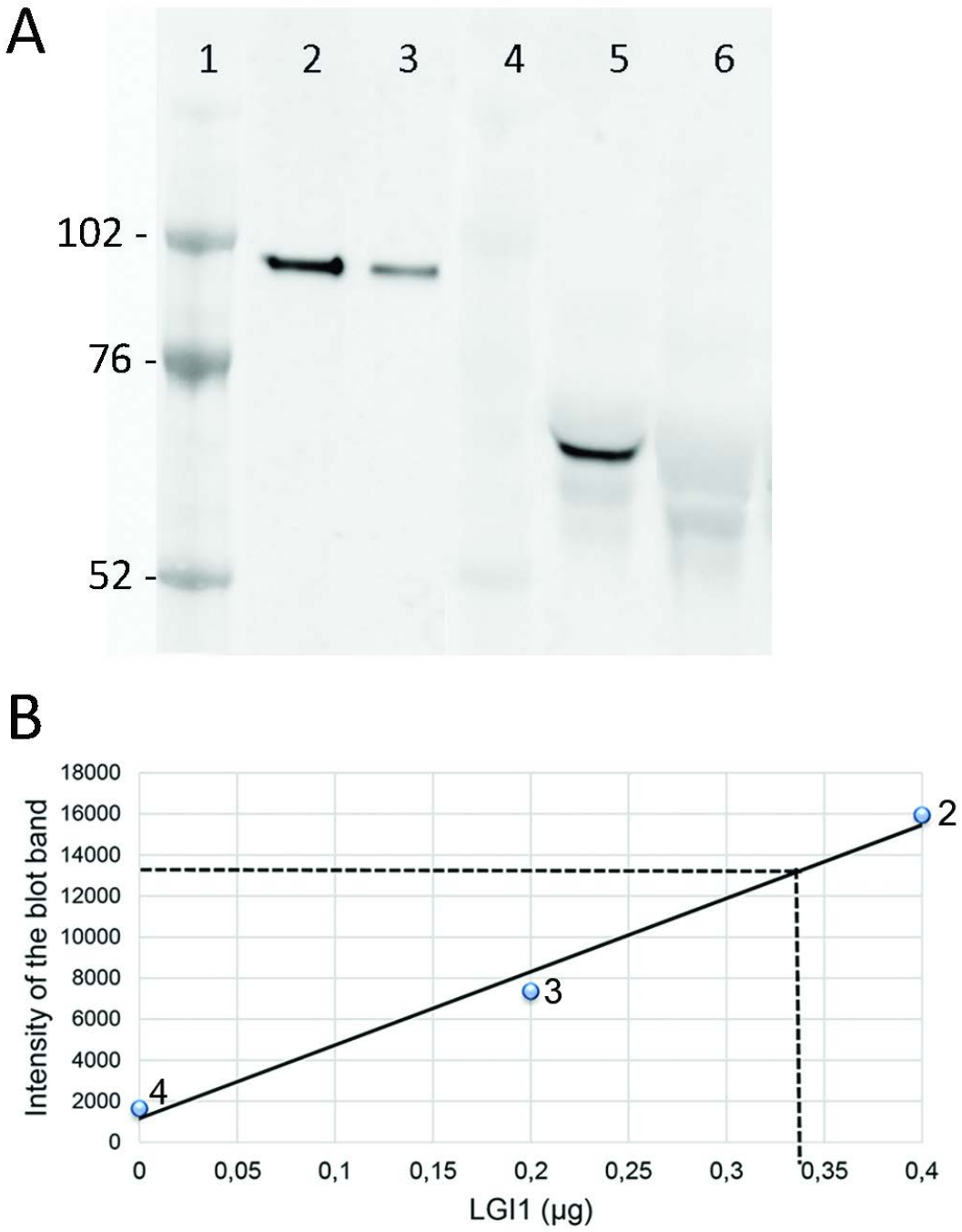


Supplementary Figure 2: Immunoabsorption of patients' purified IgG with LGI1 expressed in HEK293 cells

A-C) Reactivity of patients' LGI1 IgG with rat brain, HEK293 cells that express LGI1, and live cultures of neurons, respectively.

D-F) Pre-absorption of patients' LGI1 IgG with LGI1 expressed in HEK293 cells abolishes the reactivity of patients' IgG with brain, HEK293 cells that express LGI1, and cultured neurons, respectively.

G-I) In contrast to D-E, pre-absorption of patients' LGI1 IgG with HEK293 cells that do not express LGI1 does not alter the reactivity with brain, HEK cells that express LGI1, or cultured neurons. Scale bar in G = 2 mm; Scale bar in H = 20 μm; Scale bar in I = 50 μm.

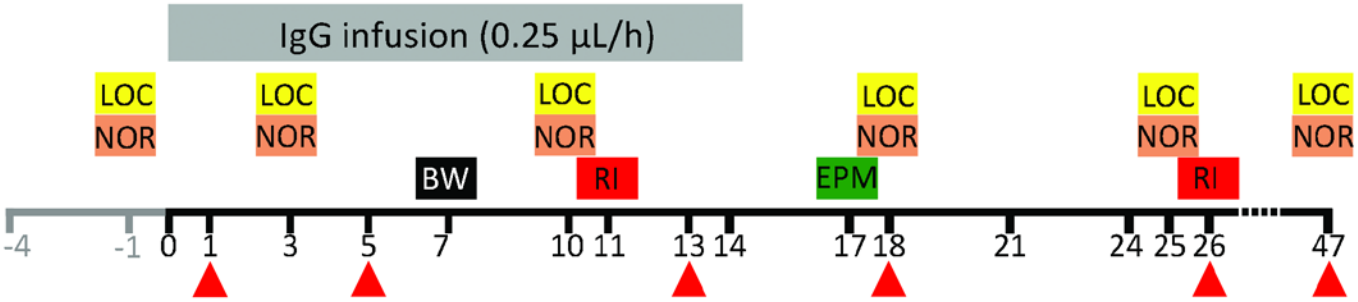


Supplementary Figure 3: Immunoblot quantification of soluble LGI1 contained in the media of HEK LGI1-expressing cells

A) Lanes 2 and 3 show the LGI1 band that resulted from immunoblot of serial dilutions of a commercially available (Abnova, H00009211-P01) human recombinant LGI1 protein (lane 2, 0.4 µg; lane 3, 0.2 µg). Lane 4 does not contain protein, Lane 5 shows the LGI1 band that resulted from the load of 25 µg of total protein from media of HEK293 cells that express LGI1, and Lane 6 shows the absence of LGI1 band that resulted from the load of 25 µg of total protein from media of HEK cells that do not express LGI1. Lane 1 is the

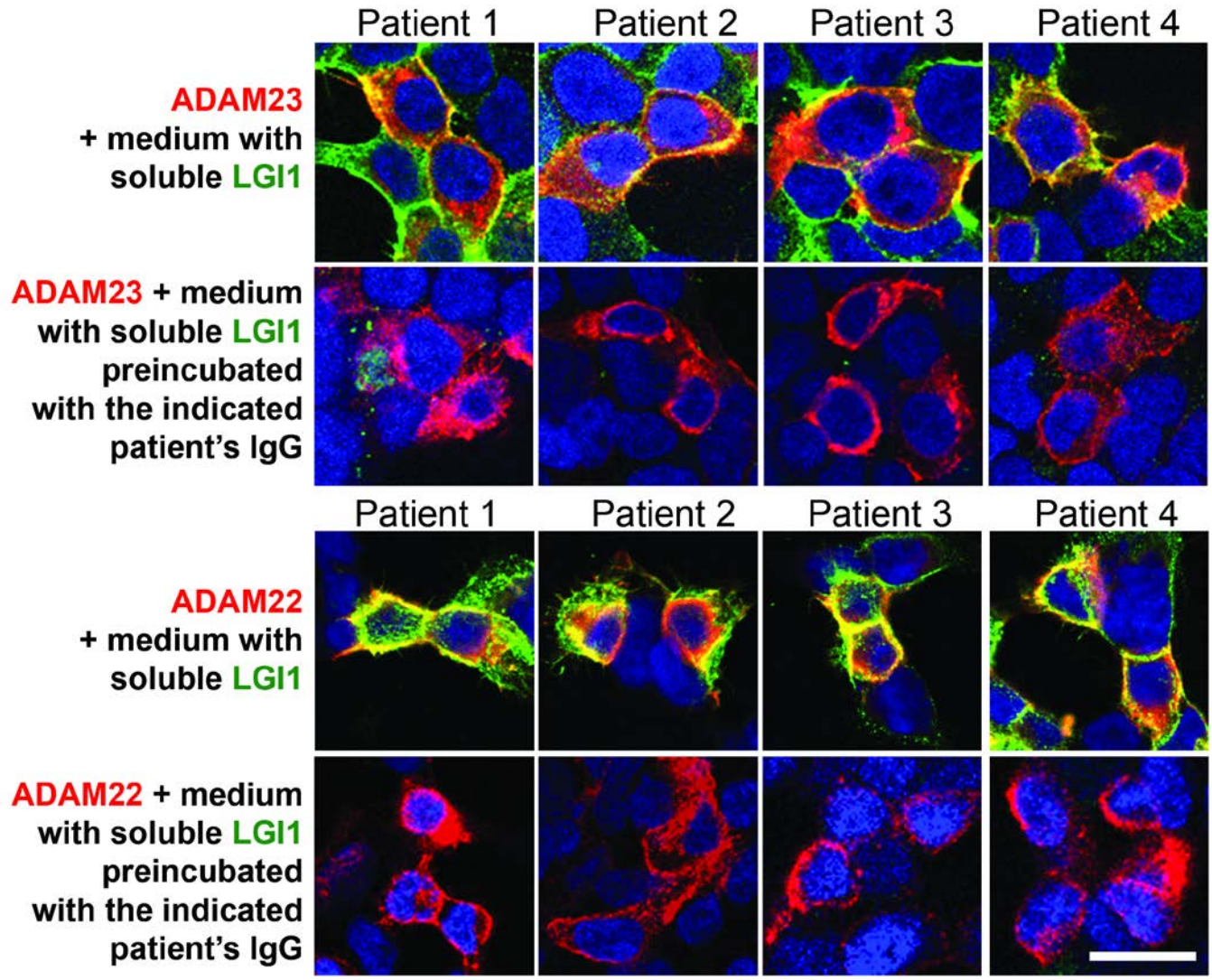
molecular weight marker. The higher molecular weight of the commercial recombinant LGI1 protein (lanes 2 and 3) compared with the LGI1 protein contained in the media from HEK LGI1-expressing cells is due to the presence of a GST-tag at the N-terminal of the recombinant protein.

B) Graph showing the linear distribution of the LGI1 band signal obtained with the recombinant protein at the indicated protein concentrations (circles indicate the signal intensity derived from lanes 2, 3 and 4 in panel A). The concentration of LGI1 in the media of HEK cells (Lane 5) was obtained by comparing its signal with that of the recombinant protein (dotted line in B). Lane 5 was loaded with 8.15 μl of LGI1 enriched media, demonstrating a concentration of LGI1 of 4.2 μg/100μl.



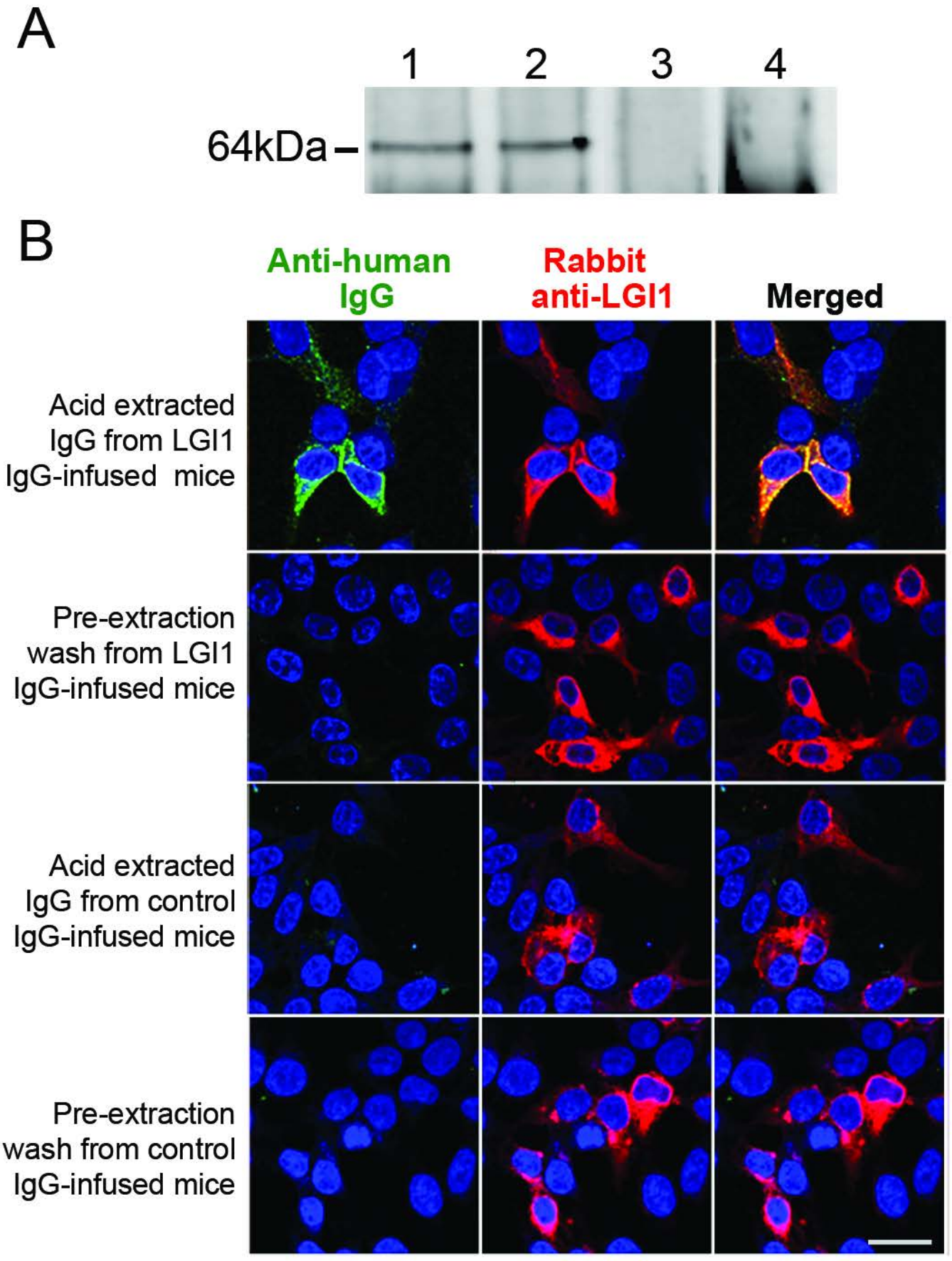
Supplementary Figure 4: Distribution of behavioral tests, period of infusion, and brain tissue studies

At day 0, catheters and osmotic pumps were placed and bilateral ventricular infusion of patients' LGI1 IgG or control IgG started. Infusion lasted for 14 days. Memory (novel object recognition [NOR]), locomotor activity (LOC), anxiety (black and white test [BW] and elevated plus maze test [EPM]), and aggressiveness (resident intruder test [RI]) were assessed blinded to treatment at the indicated days. Animals were habituated for 1 to 4 days before surgery (baseline) to NOR and LOC. Red arrowheads indicate the days of sacrifice for studies of effects of antibodies in brain.



Supplementary Figure 5: Patients' LGI1 IgG blocks the binding of LGI1 to ADAM23 and ADAM22

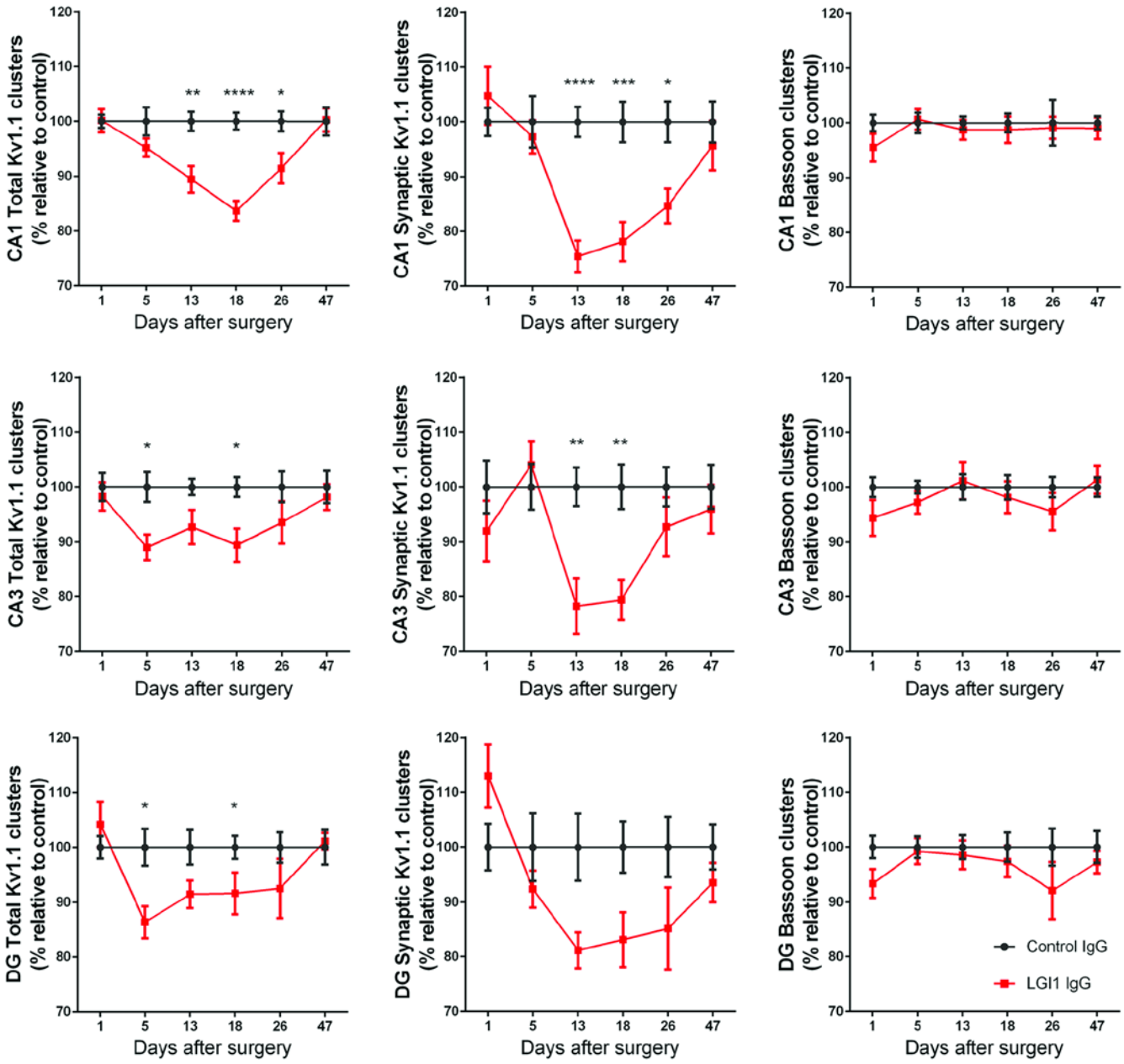
Sera from four additional representative patients show that all block the binding of LGI1 to ADAM23 and ADAM22. Scale bar = 20 μm.



Supplementary Figure 6: Presence of human LgI1 IgG in brain of infused mice

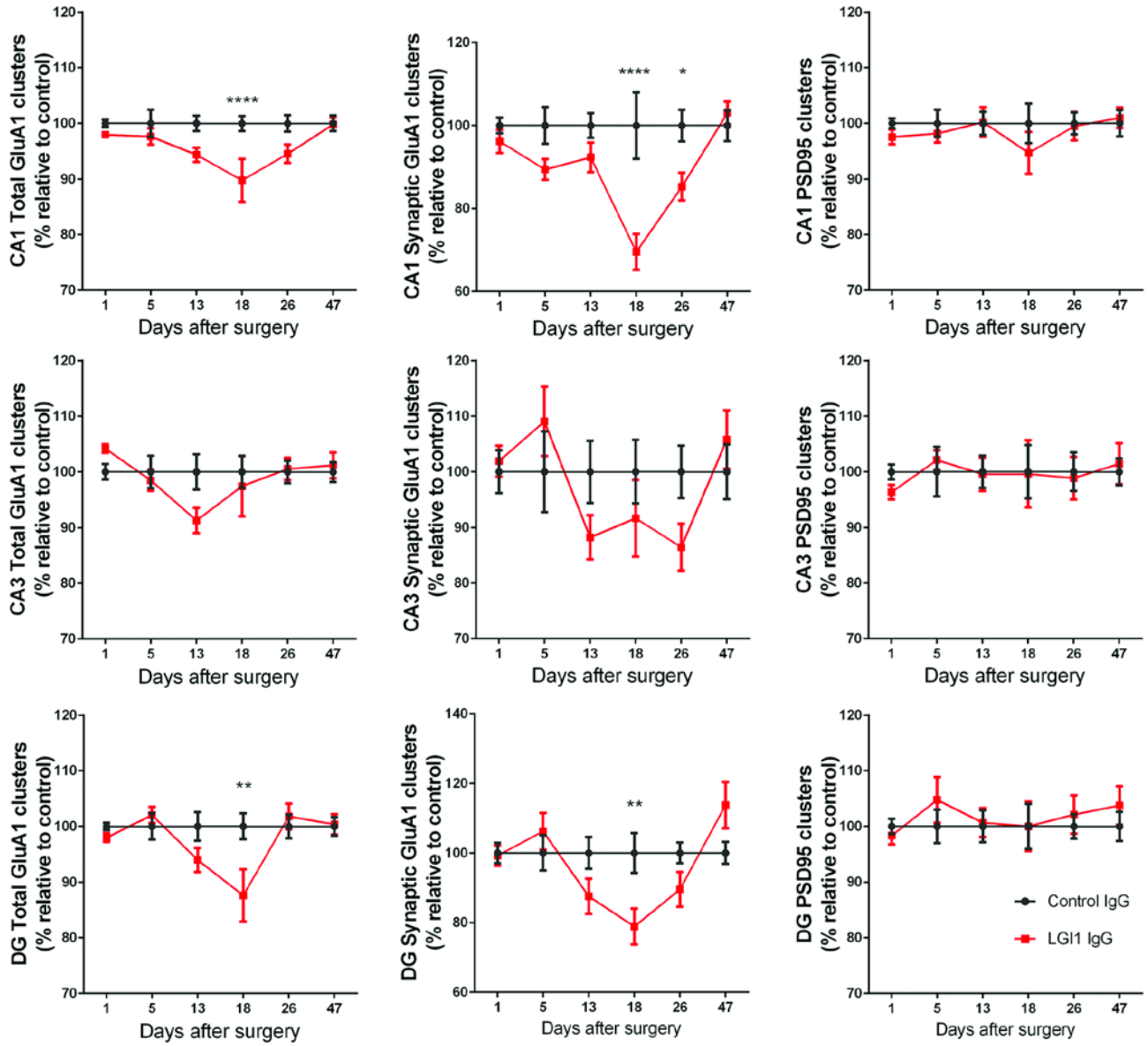
A) In mice infused with patients' LGI1 IgG (lanes 1 and 2), but not in mice infused with control IgG (lanes 3 and 4), the extraction and precipitation of human IgG from brain associated with the precipitation of LGI1 (~64 kDa protein band in lanes 1 and 2).

B) In another experiment, acid-extracted human IgG from hippocampus of mice infused with patients' LGI1 IgG shows the presence of LGI1 antibodies, which are demonstrated in a cell-based assay with HEK293 cells expressing LGI1 (first row, green, which colocalizes with the reactivity of a commercially available polyclonal rabbit antibody against LGI1, red). In contrast no LGI1 antibodies are detected in the tissue-wash before the acid extraction (second row) indicating that the acid-extracted IgG contained antibodies specifically bound to LGI1 in tissue. Similar experiments with samples obtained from mice infused with control IgG show absence of LGI1 antibodies (third and fourth rows). For all rows the expression of LGI1 in HEK293 cells was confirmed with the indicated polyclonal rabbit antibody against LGI1 (1:1000, #ab30868, Abcam) showing the merged reactivities of the human and polyclonal antibodies. Scale bar = 20 μm.



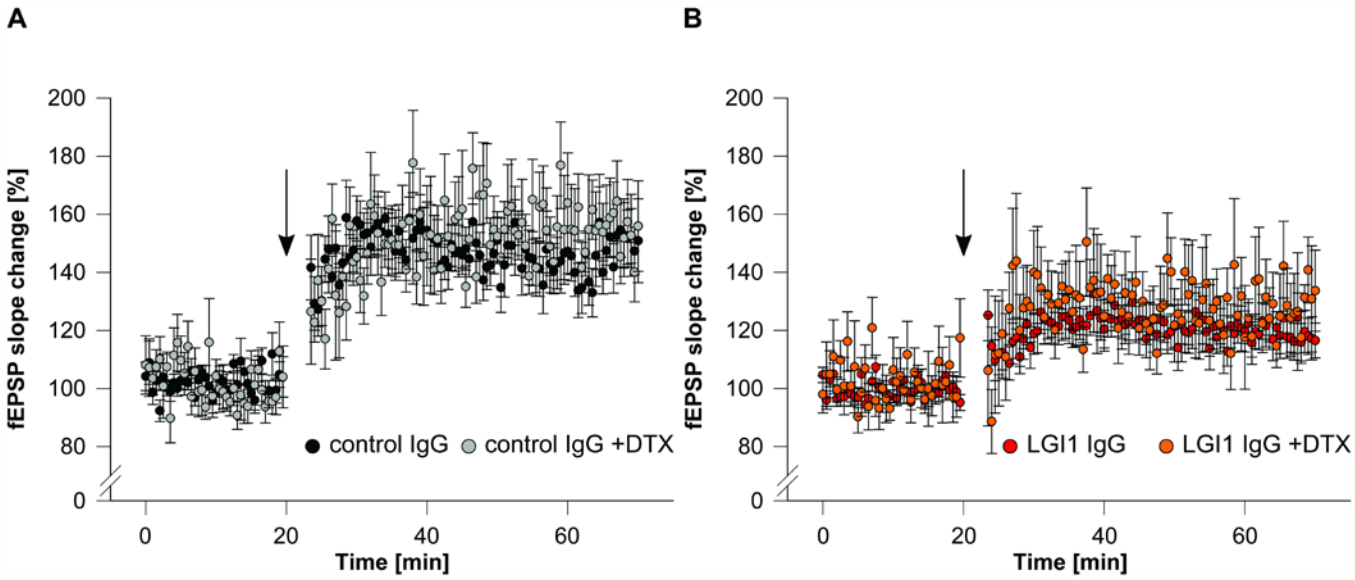
Supplementary Figure 7: Analysis of the effect of patients' LGI1 IgG on Kv1.1 clusters in subregions of the hippocampus

Quantification of the density of total and synaptic Kv1.1 clusters, and Bassoon clusters in the indicated subregions of the hippocampus in mice treated with patients LGI1 IgG (red) or control IgG (black) on the indicated days. Mean density of clusters in control IgG treated animals was defined as 100%. Data are presented as mean ± SEM. For each time point five animals infused with patients LGI1 IgG and five with control IgG were examined. Significance of treatment effect was assessed by two-way ANOVA with an alpha-error of 0.05 (asterisks) and *post-hoc* testing with Sidak-Holm adjustment. ***P*< 0.01; ****P*<0.001; *****P*<0.0001.



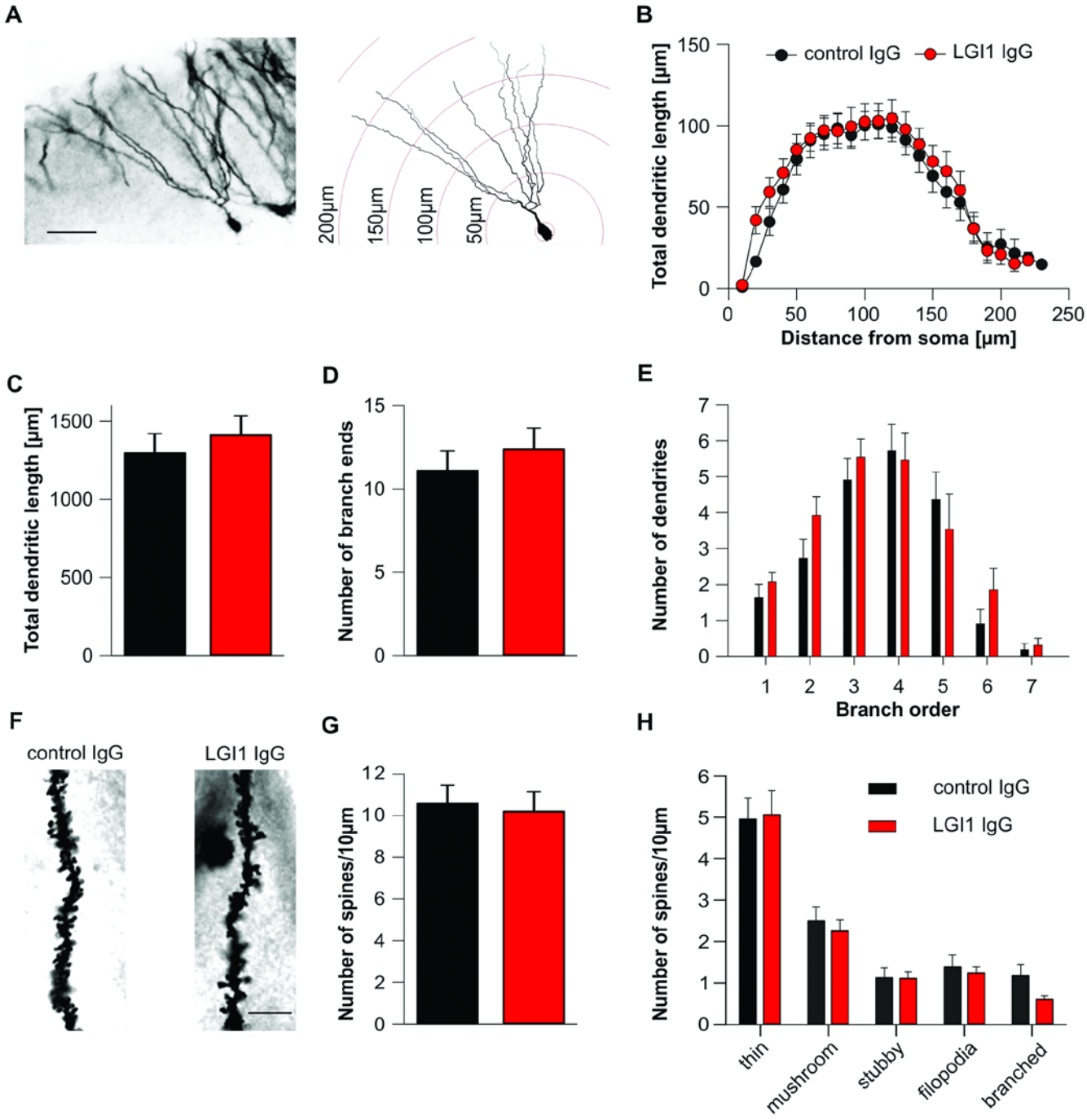
Supplementary Figure 8: Analysis of the effect of patients' LGI1 IgG on AMPA receptor clusters in subregions of the hippocampus

Quantification of the density of total and synaptic GluA1 AMPA receptor clusters, and PSD95 clusters in the indicated subregions of the hippocampus in mice treated with patients' LGI1 IgG (red) or control IgG (black) on the indicated days. Mean density of clusters in control IgG treated animals was defined as 100%. Data are presented as mean ± SEM. For each time point five animals infused with patients' LGI1 IgG and five with control IgG were examined. Significance of treatment effect was assessed by two-way ANOVA with an alpha-error of 0.05 (asterisks) and *post-hoc* testing with Sidak-Holm adjustment. ***P*< 0.01.



Supplementary Figure 9: Effects of DTX on hippocampal long term potentiation

A) DTX does not affect the time course of long-term potentiation (LTP) in acute hippocampal slices of mice infused with control IgG ($n_{control\ IgG}$ = 14; $n_{control\ IgG\ +\ DTX}$ = 9); B) DTX does not further influence the reduced LTP in acute hippocampal slices of mice infused with patients' LGI1 IgG (B; $n_{LGI1\ IgG}$ = 18; $n_{LGI1\ IgG\ +\ DTX}$ = 8).



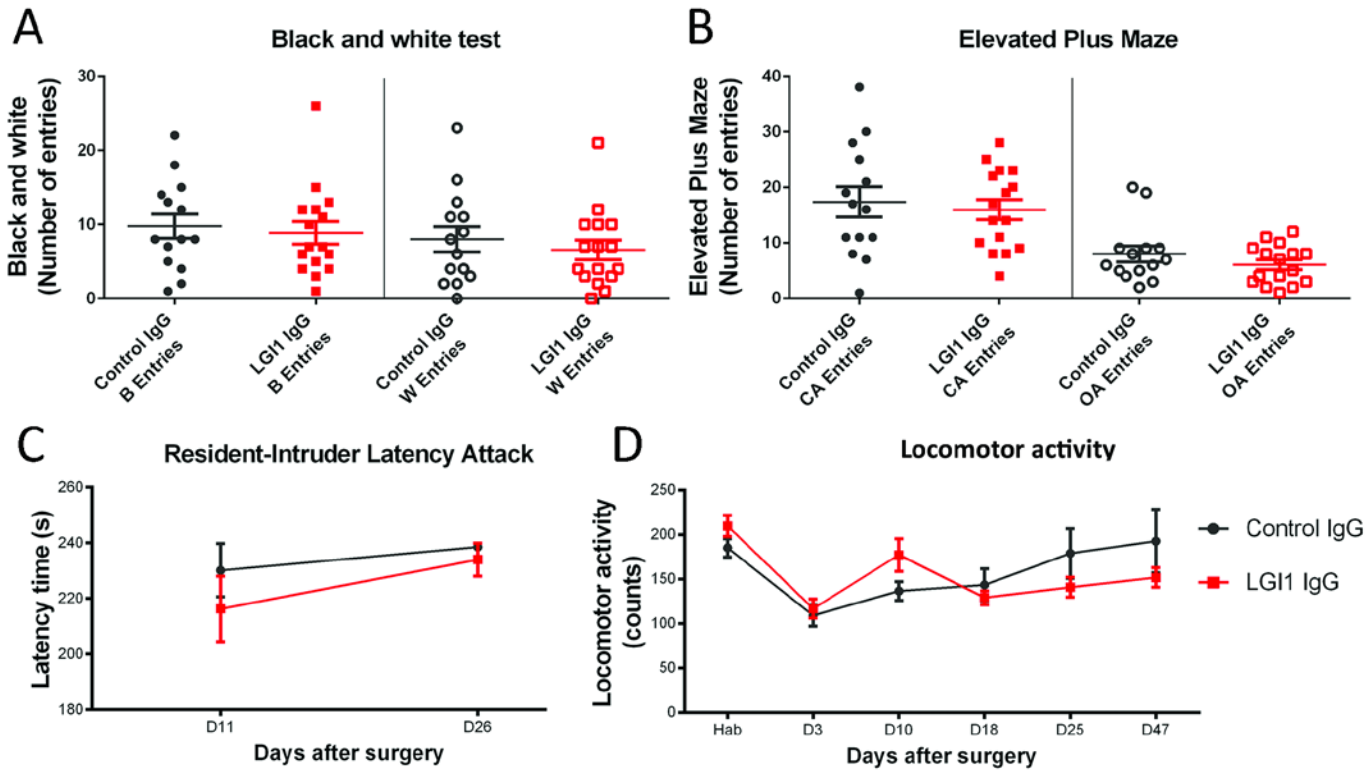
Supplementary Figure 10: Dendritic pruning is unaffected by patients' LGI1 IgG

A) Dendritic arborization of a dentate gyrus granule cell; example neuron is shown after Golgi silver impregnation (left) and in Neurolucida reconstruction for Sholl analysis (right) (scale bar = 50 µm).

B to E) Sholl analysis revealed unchanged dendritic arborization in control IgG and patients' LGI1 IgG infused mice as shown by the length of hippocampal granule cell dendrites (B and C) and by number and intersection of dendritic branches (D and E).

F) Synaptic spines of tertiary dendrites of CA1 pyramidal neurons (F, scale bar = 10µm).

G and H) Quantification of spine density (G) and of spine morphology (H) is unchanged in control IgG and patients' LGI1 IgG infused mice (all data are presented as mean ± SEM).



Supplementary Figure 11: Tests of anxiety, aggression, and locomotor activity

A) Tests of anxiety (black and white test), and (B) elevated plus maze, (C),aggression (resident intruder), and (D) locomotor activity did not show significant differences between mice infused with patients' LGI1 IgG and control (CT) IgG. "B entries" = black entries; "W entries" = white entries; "CA entries" = closed arm entries; "OA entries" = open arm entries. In panel D, "D" = day of the experiment related to first day of IgG infusion; Hab = habituation.

Supplementary References

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