Supplementary Material for

Humanized mouse model of Rasmussen's encephalitis supports the immune-mediated hypothesis

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Other Supplementary Material for this manuscript includes the following:

Supplementary Video 1. Video EEG recording of an RE-NSG mouse
Please see attached .mp4 file

	Control					RE Patient						
	1	2	3	4	5	1	2	3	4	5	6	7
Diagnosis	TLE	Healthy	Healthy	Healthy	Healthy	RE	RE	RE	RE	RE	RE	RE
Gender	M	F	F	M	F	M	F	M	F	M	F	M
Age at symptom onset	7	—	—	—	—	11	51	10	8	8	19	8
Age at transfer	8	8	26	11	12	11	54	11	10	10	20	19
Focal seizure	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Seizure secondarily generalized	No	No	No	No	No	No	Yes	No	Yes	Yes	No	Yes
EPC	No	No	No	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes
Paresis	No	No	No	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes
Staging of RE												
Stage 1	NR	NR	NR	NR	NR	3 wk	11 wk	5 wk	18 wk	1 y	12 wk	1 y
Stage 2	NR	NR	NR	NR	NR	Ong.	20 mo	Ong.	10 mo	Ong.	Ong.	Ong.
Stage 3	NR	NR	NR	NR	NR	NR	Ong.	NR	Ong.	NR	NR	NR
MRI hyperintensity	No	NA	NA	NA	NA	Left	Left	Right	Left	No	Right	Left
Atrophy	HPC	NA	NA	NA	NA	No	Yes	Yes	Yes	Yes	Yes	Yes
Spikes	LT	NA	NA	NA	NA	LCT	LCT	RFT	LCT	LTP	RFT	LCT
EEG findings												
Focal slow	No	NA	NA	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ictal	Yes	NA	NA	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes	Yes
AutoAbs (NMDA, VGKC, others)	NR	NR	NR	NR	NR	No	No	No	No	No	No	No
Response to tx												
AEDs	Partial	NR	NR	NR	NR	No	No	No	No	No	No	No
Steroids	NA	NR	NR	NR	NR	No	No	Partial	No	No	No	No
IVIG	NA	NR	NR	NR	NR	Yes	Partial	Partial	No	No	NA	No
Surgery	NA	NR	NR	NR	NR	NA	Yes	Yes	Yes	Yes	Yes	Yes
Other	—	—	—	—	—	NA	NA	NA	NA	NA	RTX	RTX

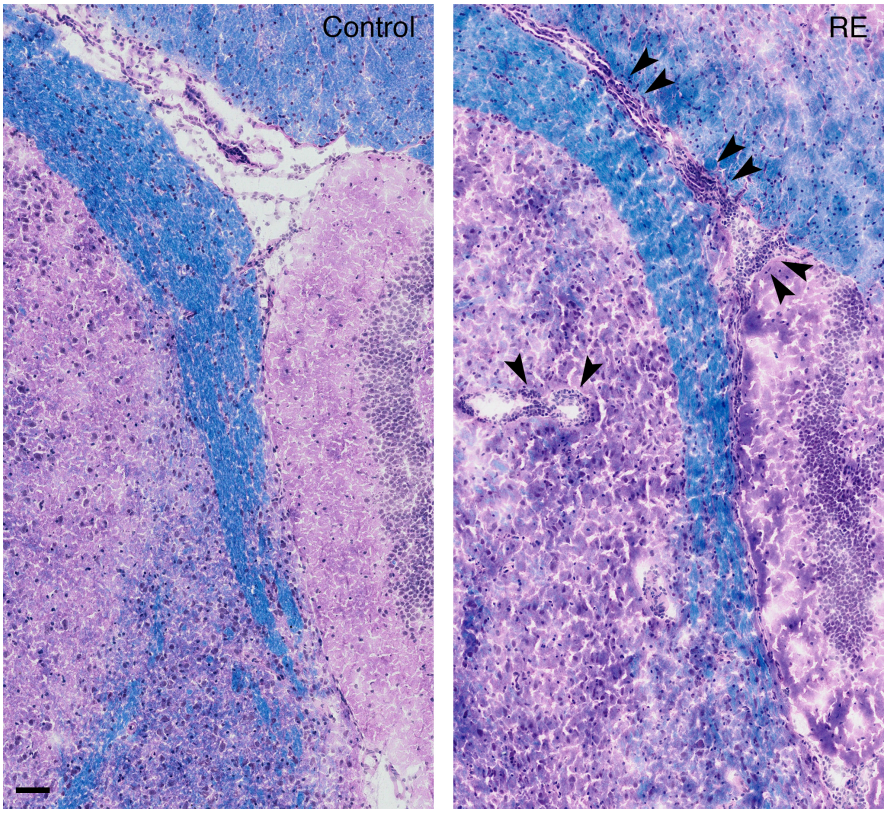
Abbreviations: Ab, antibody; AED, anti-epileptic drugs; EPC, Epilepsia Partialis Continua; HPC, hippocampus; IVIG, intravenous immunoglobulin; LCT, left centro-temporal; LT, left temporal, LTP, left temporo-parietal; MRI, magnetic resonance imaging; NA, not attempted; NMDA, N-methyl-d-aspartate; NR, not relevant; Ong.,Ongoing; RFT, right fronto-temporal; RTX, Rituximab; TLE, temporal lobe epilepsy; Tx, treatment; VGKC, voltage gated potassium channel.

Supplementary Table 1. Characteristics of control subjects and RE patients

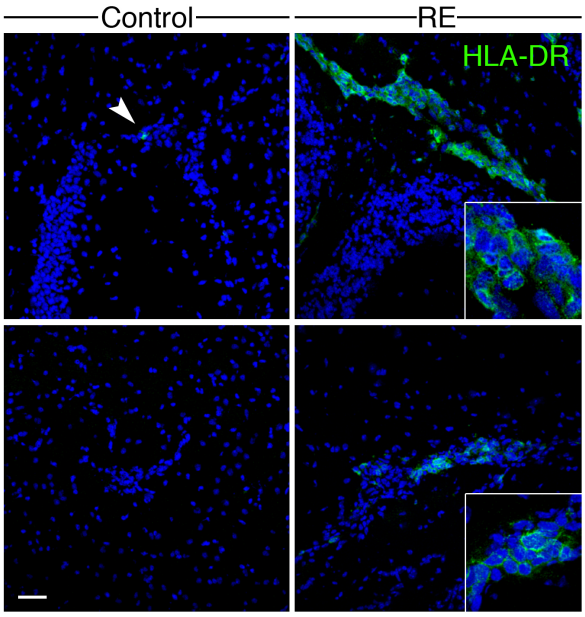
Input human PBMCs	Injected PBMCs	Nbr of injected mice	Nbr of mice used for Tx or pathology[†]	Nbr of video & EEG-recorded mice (no Tx)	Nbr of video & EEG-recorded mice with seizures (in %)[‡]	
Control 1	Fresh	4	2	2	0/2	(0%)
Control 2	Fresh	4	2	2	0/2	(0%)
Control 3	Fresh	5	0	5	0/5	(0%)
Control 4	Fresh	4	0	4	0/4	(0%)
Control 5	Fresh	4	0	4	0/4	(0%)
Total		**21**	**4**	**17**	**0/17**	**(0%)**
Patient 1	Fresh/ Frozen	4	0	4	3[§]/4	(75%)
Patient 2	Fresh/ Frozen	8	5	3	3/3	(100%)
Patient 3	Frozen	19[*]	12	2	1/2	(50%)
Patient 4	Fresh	10	6	4	4/4	(100%)
Patient 5	Frozen	20	18	2	2/2	(100%)
Patient 6	Frozen	9	7	2	2/2	(100%)
Patient 7	Frozen	8	6	2	1/2	(50%)
Total		**78**	**54**	**19**	**16/19**	**(84%)**

[*]5 of the 19 mice injected with frozen PBMCs from RE patient #3 died.
[†]This group includes mice used for pathology assessment, for kinetic study and mice treated with either IVIG, PBS, isotype control or anti-a4 antibody. EEG-recorded mice are excluded from this group.
[‡]Seizures are defined by convulsing rhythmic activity lasting more than 10 sec at EEG with compatible behaviour recorded on video
[§]One mouse had video-recorded seizures but no convulsing rhythmic activity at EEG.

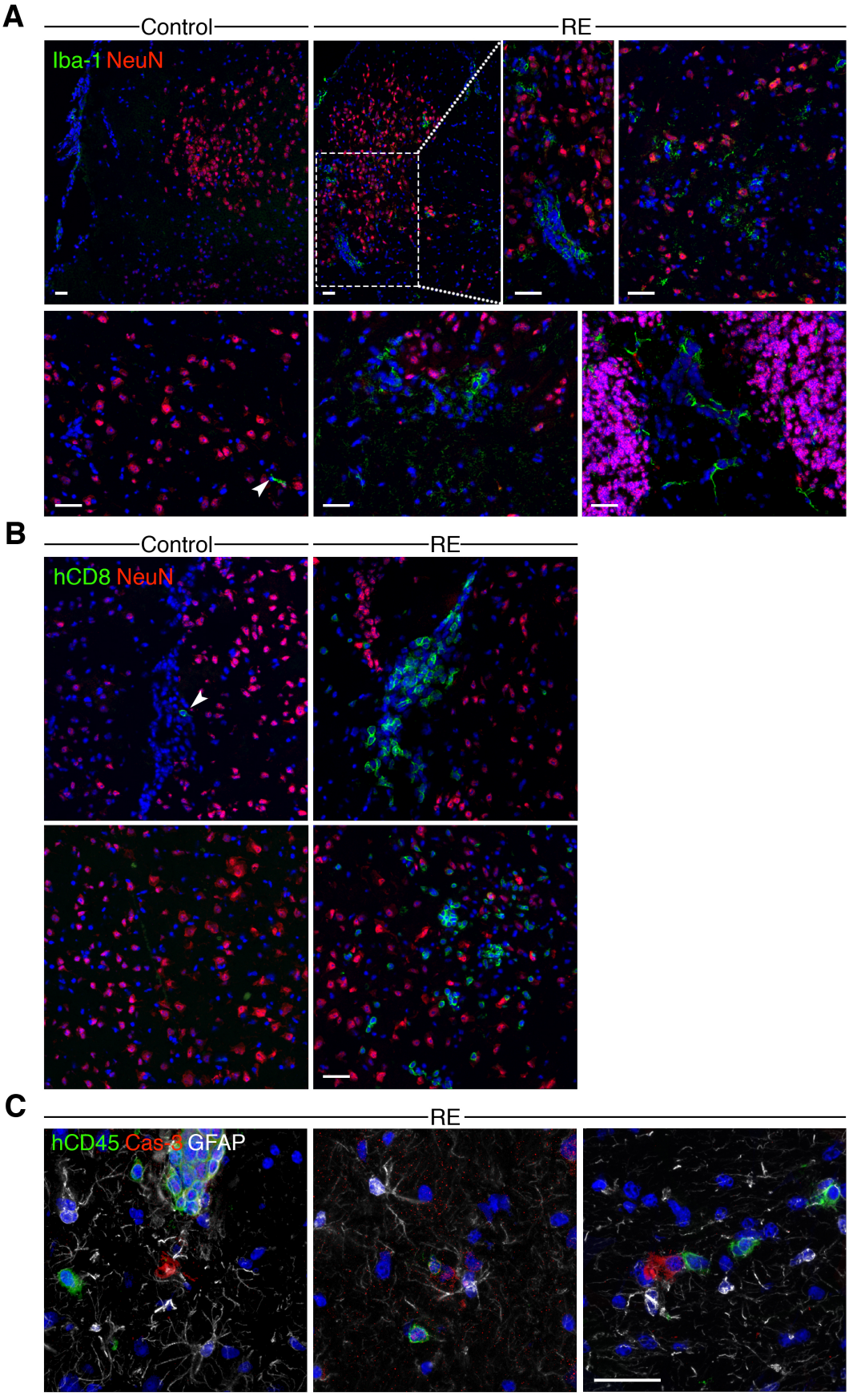
Supplementary Table 2. Mice engrafted with human PBMCs from control subjects and RE patients



Supplementary Figure 1. Luxol Fast Blue and haematoxylin-eosin (LHE) stained brain sections from control-NSG and RE-NSG mice. Brain sections were taken 5 weeks post PBMC transfer. Arrowheads indicate mononuclear perivascular cuffs. Scale bar = 50 μm.

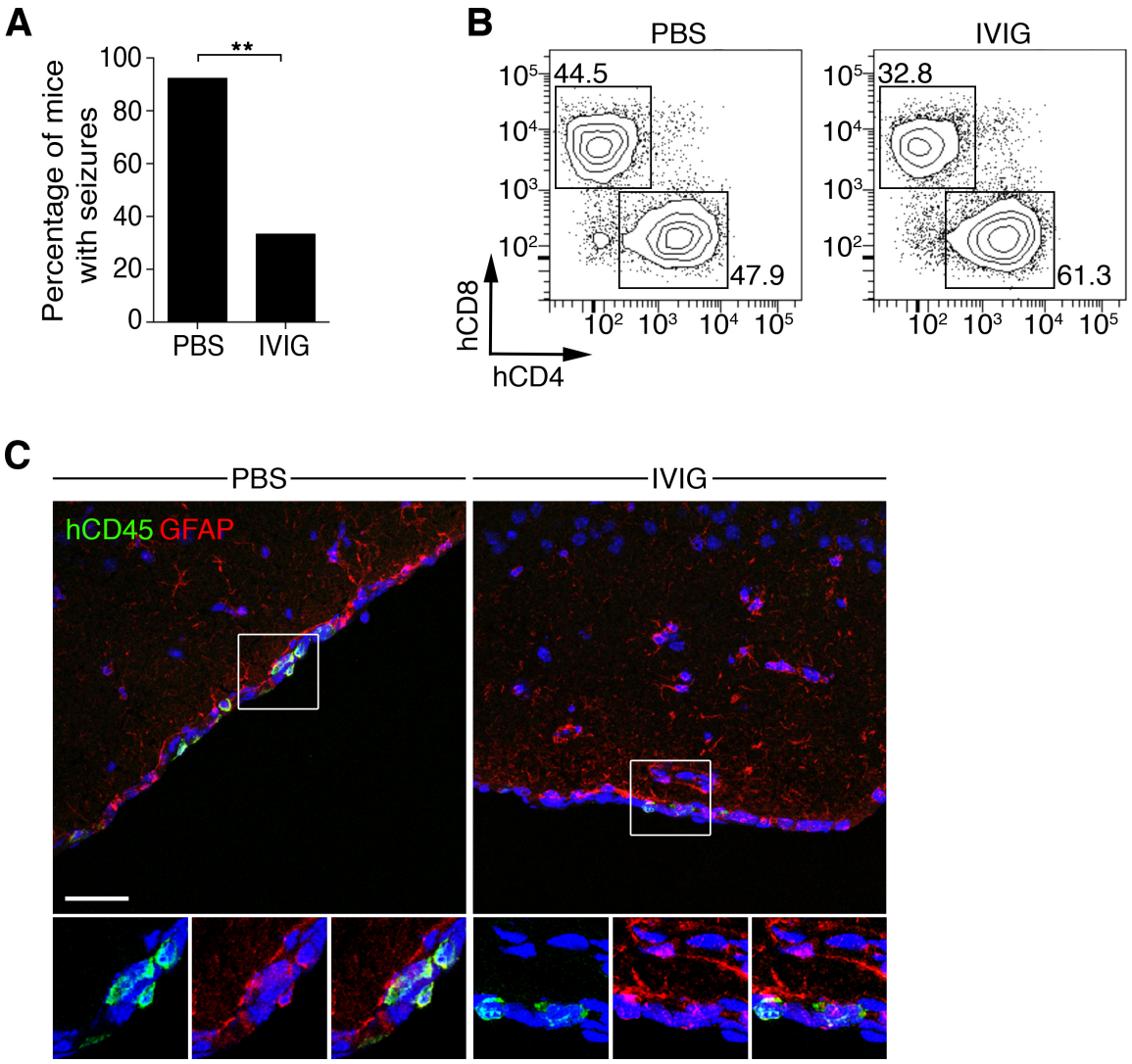


Supplementary Figure 2. HLA-DR expression in the brain of control-NSG and RE-NSG mice. Images shown are representative of 6–8 fields from 6 sections obtained from 3 animals per group. Scale bar = 30 μm.



Supplementary Figure 3. Neuropathology in the brain of control-NSG and RE-NSG mice. (**A**) Double immunohistofluorescent staining for Iba-1 (green) and NeuN (red) in the brain of

control-NSG or RE-NSG mice. Images shown are representative of 12 fields from 6 sections obtained from 3 animals per group. Scale bars = 30 μm. (**B**) Double immunohistofluorescent staining for hCD8 (green) and NeuN (red) in the brain of control-NSG or RE-NSG mice. Brain sections are from NSG mice sacrificed 5 weeks post PBMC transfer. Images shown are representative of 8 fields from 4 sections obtained from 2 animals per group. Scale bar = 30 μm. (**C**) Immunofluorescent staining for hCD45 (green), cleaved caspase-3 (Cas-3, red), and GFAP (white) in the brain of RE-NSG mice. DAPI (blue) was used as nuclear staining. Images shown are representative of 7 fields from 3 sections obtained from 3 RE-NSG mice. Scale bar = 20 μm.



Supplementary Figure 4. Effect of intravenous immunoglobulin on the clinical and immunological parameters of RE in NSG mice. (**A**) Percentage of RE-NSG mice that developed clinical and electrographic seizures upon treatment with either PBS or IVIG. $^{**}P < 0.01$, by chi-square test. (**B**) FACS analyses showing the presence of hCD4 and hCD8 in the CNS of RE-NSG mice treated with PBS or IVIG. Data shown are representative of $n = 2–4$ animals. (**C**) Confocal microscopy photomicrographs for GFAP (red) and hCD45 (green) in the CNS of RE-NSG mice treated with PBS or IVIG, 4 weeks post transfer. Images shown are representative of 6 fields from 4 sections obtained from 2 animals per group. Scale bar = 30 μm.