Supplementary

Materials and Methods:

Neuropathological semiquantification of cell loss/ gliosis. Biopsy hippocampal brain tissue of pharmacoresistant TLE patients who underwent epilepsy surgery for seizure relief with *I²GO* and HS pathology (n=12 each) was processed as described elsewhere in detail before.[1] In brief, tissue was fixed in PFA overnight and embedded in paraffin. Deparaffinized 4 µm sections were stained with hematoxylin and eosin (HE) and commercial antibodies specific for neuronal nuclei (NeuN) and glial fibrillary acidic protein (GFAP). Staining was visualized using the avidin-biotin-peroxidase method. The immunostained sections were used in all cases to analyze semiquantitatively the extent of neuronal cell loss (estimated in percent of regular densities), and the fibrillary as well as cellular astrogliosis (absent, weak, intermediate, strong) by a blinded neuropathology expert (AJB) adapting a semiquantitative scoring procedure described before for quantification of epilepsy surgery pathology.[2,3]

Neuroradiology: For this analysis, we have evaluated presurgical MR images (N=40) from the matched pair cohort. All MRI data were acquired at a 3 T Philips Achieva or Philips Intera scanner (2007-2012) and for all patients digital 3D isotropic 1 mm T1-weighted (T1-w) data (gradient echo) and high-resolution (0.2–0.8 mm in-plane) coronal T2-weighted (T2-w) and coronal FLAIR images were available. We used blinded visual rating, automatic volumetric analysis and manually segmentation of regions of interest for MRI analysis of aspect, volumes, and signal intensity of the hippocampus and/or amygdala. These procedures were described elsewhere in detail.[4]

Normalized volumes of the hippocampus:

Only patients with 3D T1-weighted data sets were analyzed (n=46, 30 patients with hippocampus sclerosis (HS) and 16 patients with 'gliosis only'). Brain volumes without ventricular structures [mm^3] and hippocampus volumes [mm^3] were extracted using Freesurfer for each subject to calculate normalized hippocampus volumes [%]. Kruskal-Wallis test was used to compare normalized volumes of the affected hippocampus and the normalized volumes of the contralateral hippocampus. Significance level was set at $P < .05$

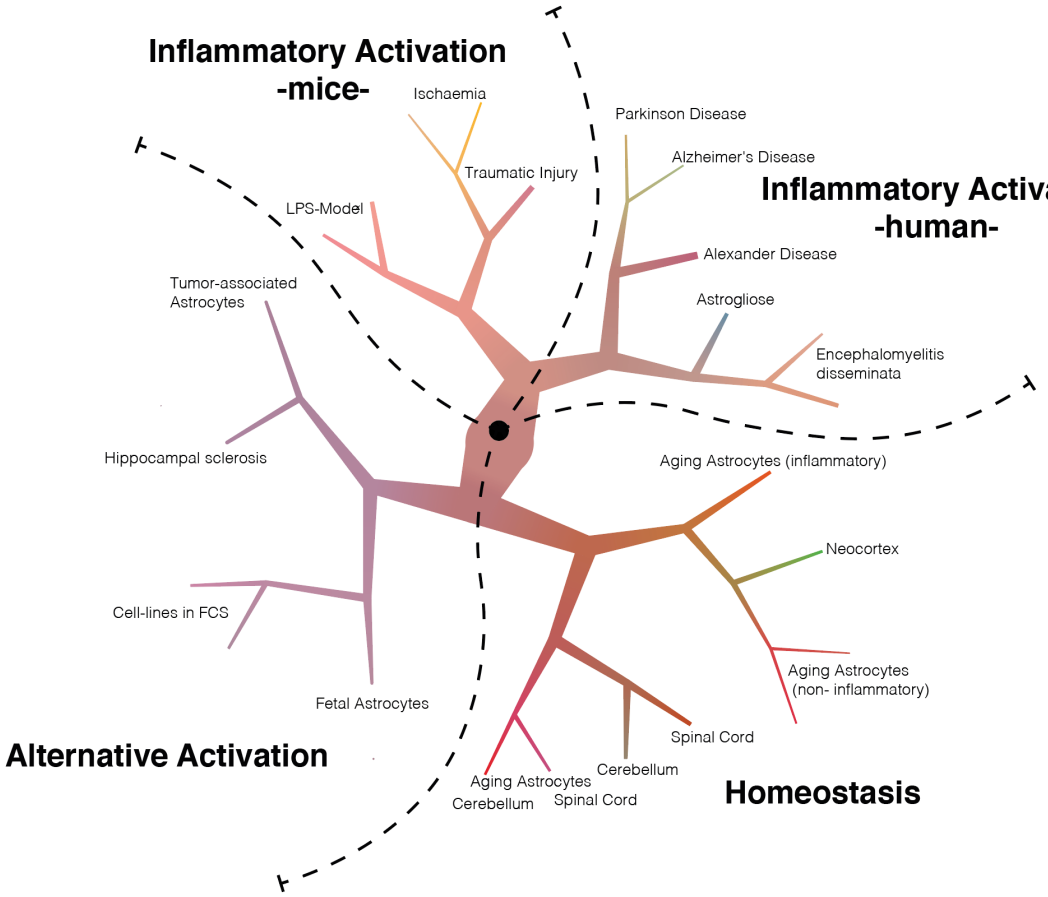
FLAIR signal intensity:

Only patients with FLAIR data sets without severe motion artefacts and with appropriate delineation of the hippocampi were analyzed. Since 3D FLAIR was not included in the standard "epilepsy protocol", only 2D data sets were available for most of the patients.

Calculation of normalized FLAIR maps was performed following the MRI post-processing procedure described by Focke et al. and Huppertz et al.[5,6]. For this, we performed brain extraction and tissue segmentation using the FSL tools BET and FAST, before the WM (white matter) and GM (gray matter) were thresholded at 90% to create a binary mask, excluding the diseased hippocampus. Finally, the mean value from each segmented FLAIR image was calculated to perform image normalization.

Results:

Supplementary Figure 1: Transcriptional signature of astrocytes



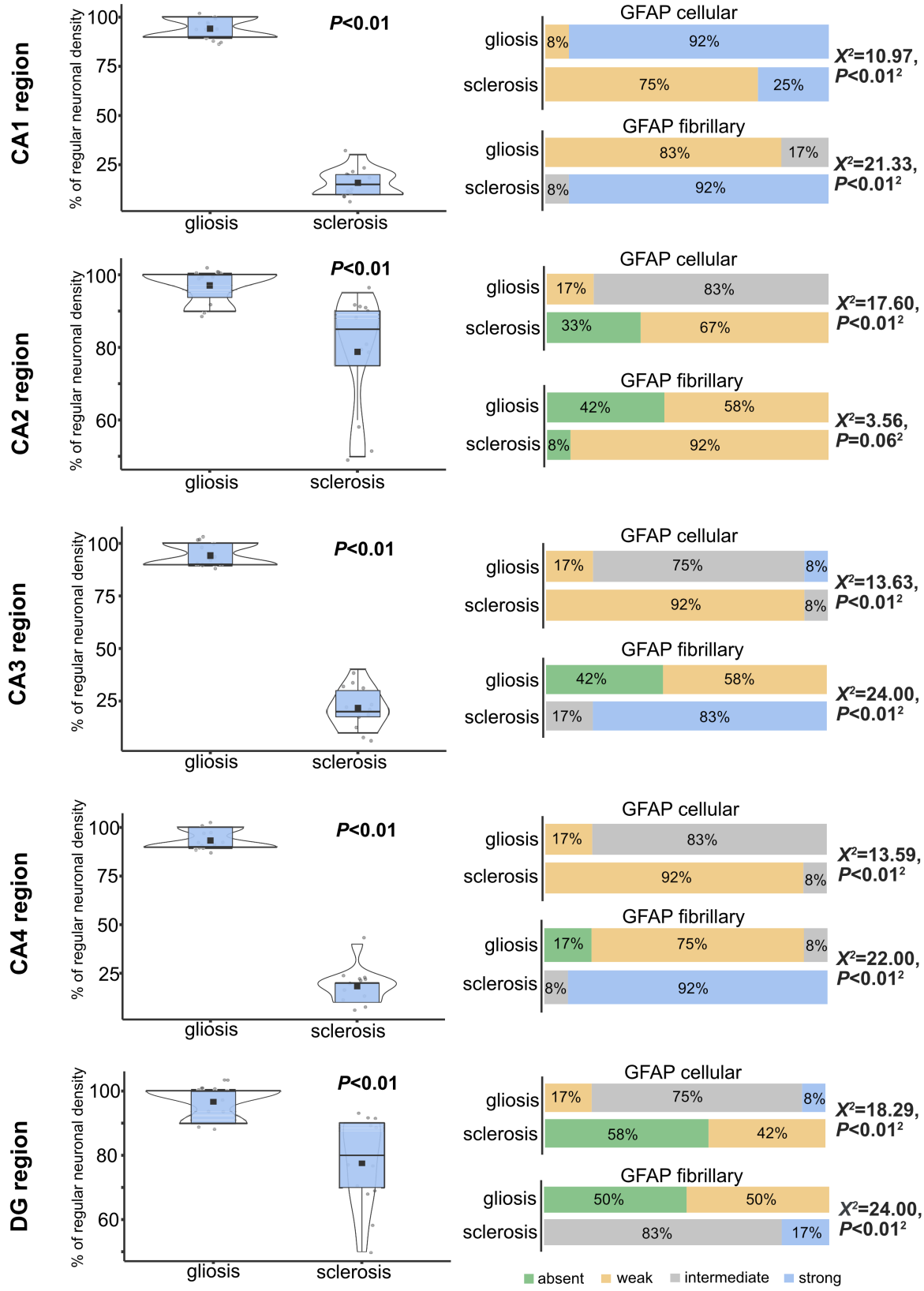
Supplementary Figure 1: Visualization of various transcriptional signature of astrocytes. For the analysis, multiple public available datasets were merged and adopted. Hierarchical clustering was performed to separate distinct profiles of astrocytic differentiation and reactive transformation.

Supplementary Table 1: Additional binominal logistic regression model

	Electronically available cohort (total N=302; sclerosis N=232)				Matched pairs (total N=140, sclerosis N=70)			
			95% CI				95% CI	
Predictor	***P***	**Odds Ratio**	**Lower**	**Upper**	***P***	**Odds Ratio**	**Lower**	**Upper**
Side of surgery Right – Left	0.447	0.824	0.501	1.356	0.946	1.026	0.489	2.15
Duration of epilepsy	0.508	0.994	0.976	1.012	0.334	0.984	0.953	1.02
Type of surgery sAHE – ATL	0.957	1.015	0.571	1.804	0.231	0.625	0.290	1.35
Invasive EEG Yes – No	**0.029**	1.764	1.059	2.938	0.323	1.491	0.675	3.29
Histology I^2GO – Sclerosis	**<0.001**	1.605	1.187	2.169	**0.023**	1.545	1.061	2.25
Meningitis / Encephalitis Yes – No	0.610	1.115	0.661	2.020	0.240	1.629	0.721	3.68
Early childhood seizures Yes – No	0.536	0.854	0.496	1.440	0.058	0.436	0.185	1.03
Current ASM	0.054	1.412	0.994	2.007	0.739	0.899	0.480	1.68
FBTCS Yes – No	0.861	1.04	0.624	1.356	0.571	1.258	0.568	2.78

Supplementary Table 1: An additional binominal logistic regression analysis for a subset of patients´ (N=302) and the matched pair cohort (N=70 pairs =140 patients) with ILAE1 seizure outcome as the dependent variable was performed. In consistency to the whole cohort (table 2, main text) invasive eeg and the histology finding of 'gliosis only' were independent prognostic factors for worse seizure outcome. Within the matched pair group the analysis confirms the histopathological finding of 'gliosis only' as the only independent prognostic factor for poor seizure outcome, while invasive eeg lost its influence. The additional included parameters: meningitis, early childhood convulsion, current ASM and FBTCS did not influence the seizure outcome. Estimates represent the log odds of "outcome = seizures" vs. "outcome = seizure free". Significant values are highlighted in bold. Abbreviations: sAHE = selective Amygdalohippocamectomy, ATL = anterior temporal lobe resection, ASM = anti-seizure medication, FBTCS = focal to bilateral tonic clonic seizure.

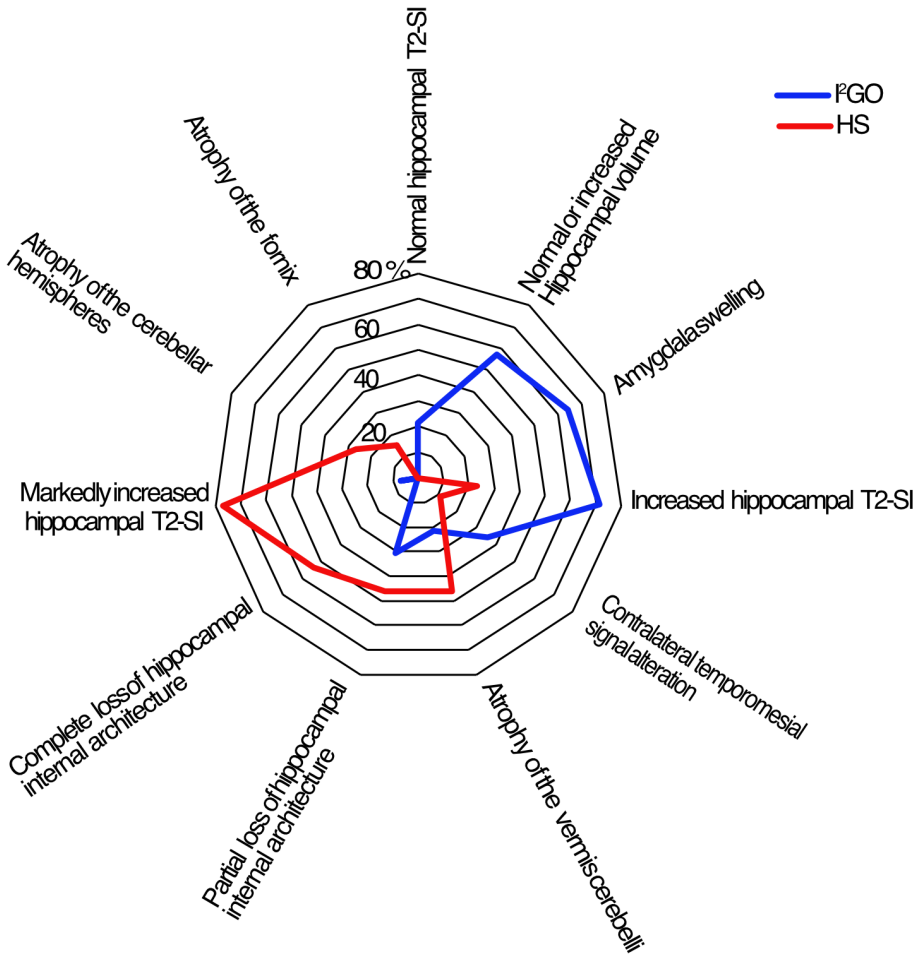
Supplementary Figure 2: Neuronal cell loss / gliosis patterns clearly separate 'gliosis only' from hippocampus sclerosis according to hippocampal subfields.[x]



[x]The combinatorial pattern of neuronal conservation (left panel) in concert with the extent of cellular and fibrillary astrogliosis (right panel) significantly differentiates 'gliosis only' from

HS in all hippocampal subfields under study. Neuronal degeneration is substantial in HS compared to *I²GO* most strikingly in CA1, CA3/4 (each $P<0.01$); but also, in the CA2 and DG subfields, where it is less robust but still detectable. Of note, prominent differences are present in all subfields regarding cellular and fibrillary astrogliosis (all $P<0.01$; exception GFAP fibrillary in the CA2 region: p=0.06; see right panel of Suppl. Fig. 2). Whereas varying degrees of cellular astrogliosis are predominant in 'gliosis only' hippocampi, there is preponderant fibrillary astrogliosis in HS – not rarely accompanied by only rather sparse cellular reactive astroglial infiltrates. The fibrillary, 'scar-like' astrogliosis pattern is particularly pronounced in HS in the hippocampal subfields with extensive neuronal degeneration (CA1, CA3, CA4). Astrogliotic changes are slighter in CA2 and DG with less severe neuronal damage. In the *I²GO* hippocampi, cellular reactive gliosis rather homogeneously affects all hippocampal subfields despite the presence of some variation.

Supplementary Figure 3: Distinct MRI feature profiles between I²GO and HS.[y]

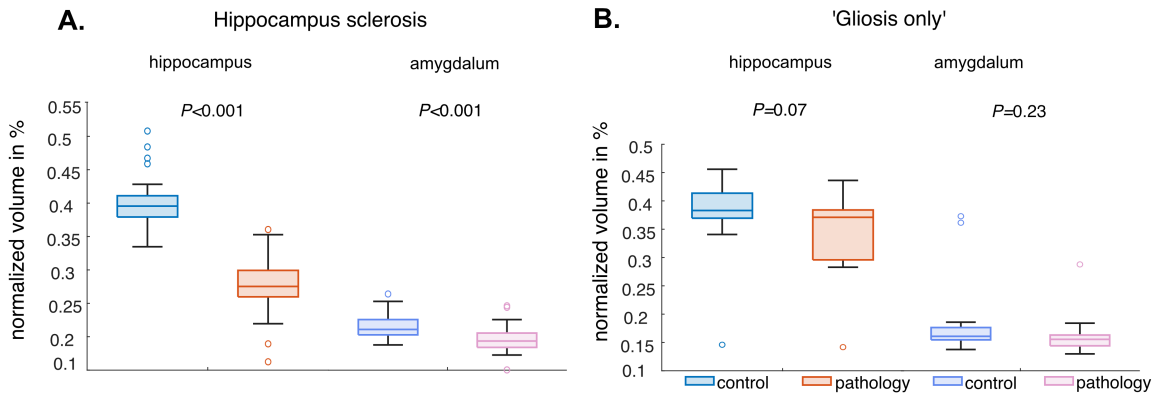


[y]A radar chart demonstration of the different MRI characteristics in patients with I²GO and HS. The percentages reflect the results of a visual evaluation done by a blinded rater with >15 years of neuroradiological experience.

Normalized volumetric analysis of the hippocampus

The normalized volumes of the affected hippocampus (0.3±0.06) and amygdala (0.16±0.03) were significantly smaller compared to the contralateral hippocampus (0.4±0.05) and amygdala (0.18±0.05) (P<0.001) after analyzing all subjects. This was in particularly true for patients with HS showing normalized hippocampus volumes of 0.28±0.04 (affected side) vs. 0.4±0.04 (contralateral side) (P<0.001) and normalized amygdala volumes of 0.17±0.04 (affected side) vs. 0.20±0.08 (contralateral side), (P<0.001); (suppl. Fig. 4A). In contrast, no significant difference could be found between the normalized volumes of the affected vs. the contralateral hippocampus and amygdala in patients with 'gliosis only' (0.34±0.07 vs. 0.38±0.08, P=0.07) and (0.15±0.02 vs. 0.17±0.02, P=0.23) (suppl. Fig. 4B).

Supplementary Figure 4: Analysis of the normalized hippocampus and amygdalum volumes for patients with hippocampal sclerosis (HS) and 'gliosis only'



Supplementary Figure 4: Wisker plots with median, first and third quartile (box), highest and lowest non-outlier value (Wisker) and outliers (circles) of normalized hippocampus and amygdala volumes for (A) patients with hippocampus sclerosis (HS) (n=30) and with (B) 'gliosis only' (n=16)

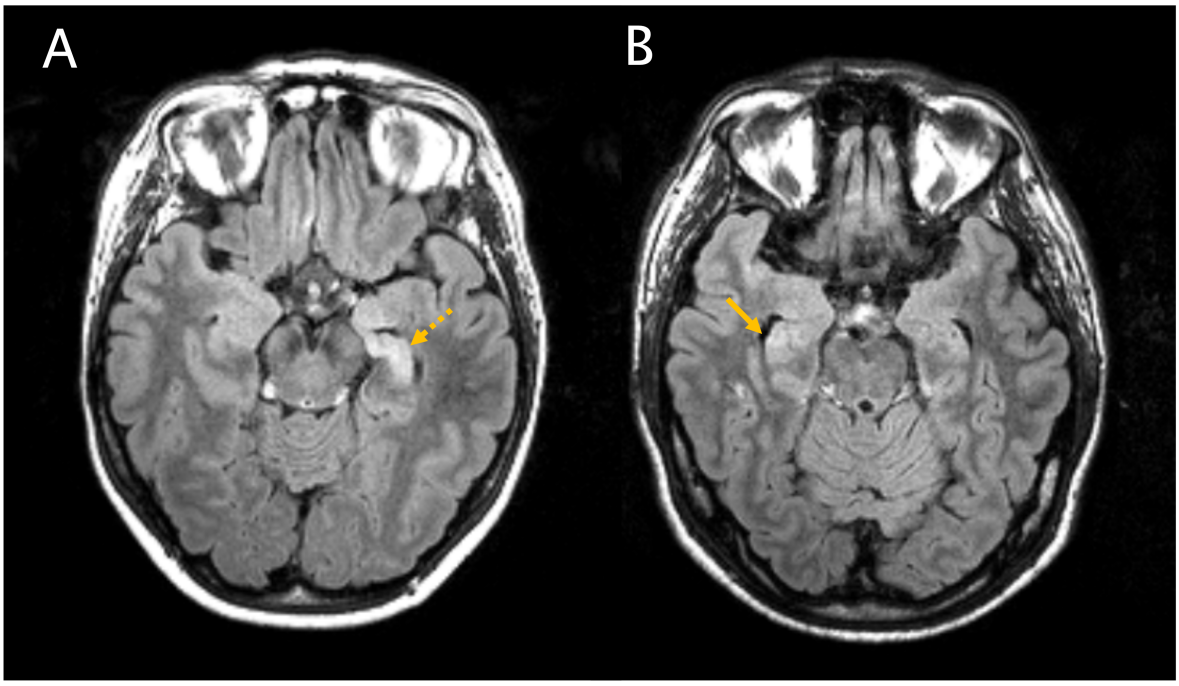
Flair signal intensity

Since hippocampus could not automatically be segmented on 2D data, standard ROIs were manually drawn by a neuroradiologist with more than 20 years' experience. Results revealed that FLAIR signal intensities of the affected hippocampus were increased compared to the contralateral side in HS patients. In contrast, no obvious difference was found between the affected and contralateral hippocampus in patients with 'gliosis only' (suppl. Table 2 and suppl. Fig. 5).

Supplementary Table 2: FLAIR analysis

	Affected amygdala		Contralateral amygdala		Affected hippocampus		Contralateral hippocampus	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
'Gliosis only'	1.44	0.10	1.38	0,07	**1.37**	0.07	1.36	0.07
Hippocampal sclerosis	1.43	0.06	1.44	0.07	**1.59**	0.11	1.34	0.12

Supplementary Table 2: Relative signal intensities measured on normalized FLAIR maps, within standard ROIs in the amygdala and anterior hippocampus of eight patients with 'gliosis only' and eight age-matched patients with hippocampal sclerosis.



Supplementary Figure 5: Normalized FLAIR maps for HS and 'gliosis only'. Example of normalized FLAIR maps for a patient with left-sided HS (A) and a patient with right-sided "gliosis only" (B)

References:

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