

SUPPLEMENTARY MATERIAL

Supplementary Table 1: ddPCR probe specifications. Commercially available probes were purchased from Biorad and used following manufacturer's instructions for amplification cycles, with annealing temperatures as shown (in °C)

Probeset description	Assay ID	Annealing temperature
ddPCR™ Mutation Assay: AKT3 p.E17K, Human	dHsaMDS121759946	55
ddPCR™ Mutation Assay: PIK3CA p.E542K, Human	dHsaMDV2010073	57
ddPCR™ Mutation Assay: PIK3CA p.E545K, Human	dHsaMDV2010075	57
ddPCR™ Mutation Assay: PIK3CA p.H1047R, Human	dHsaMDV2010077	55
ddPCR™ Mutation Assay: MTOR p.S2215F, Human	dHsaMDS365202467	55
ddPCR™ Mutation Assay: MTOR p.S2215Y, Human	dHsaMDS2514998	55

Supplementary Table 2: Complete list of samples and ddPCR results for the 17 mutation positive patients and comparison with orthogonal methods. Patients' de-identified code (Patient-ID), sex, diagnosis based on neuroimaging (Dx-MRI), Diagnosis based on neuropathology (Dx-NPath), genetic variant, sample type and results from the different variant identification methods (ddPCR, Oncoplex, smMIPs, Exome sequencing, and Amplicon sequencing) are listed for each of the 17 mutation-positive patients. For ddPCR results, Variant allele fraction (VAF%), Delta confidence interval (DCI), and number of wild-type and mutant droplets (average of quadruplicate) are reported. Samples were considered positive for a given mutation via ddPCR when $DCI > 0.045$ and $VAF > 0.05\%$ (green text). Negative samples are indicated in red text. False positive results are indicated in green bolded text and *, while false negative results are indicated in red italic text and **. Empty cells represent lack of testing due to exhaustion of sample.

Patient ID	Sex	Dx - MRI	Dx - NPATH	Genetic variant	Sample type	ddPCR			Oncoplex		smMIPs		Exomes		Amplicon Seq	
						Mutant/total reads	VAF%	DCI	Mutant/total reads	VAF %	Mutant/total reads	VAF %	Mutant/total reads	VAF %	Mutant/total reads	VAF %
LR16-470	M	HMEG	NA	PIK3CA (p.E542K)	Saliva	704568	1.47	1.056	7836	0.837						
LR13-197	F	DMEG	FCD 1b	PIK3CA (p.E545K)	Blood	49280	0.04	-0.077								
					Saliva	7923776	20.68	19.398								
					Brain, FFPE	2461297	18.86	16.691	23185	12.432						
LR15-251	M	HMEG	NA	PIK3CA (p.E545K)	Blood	231069	0.02	-0.049	1/1241	0.081	0/205	0.00				
					Buccal	86473	0.12	-0.015			0/164	0.00				
LR16-242	F	HMEG	NA	PIK3CA (p.E545K)	Brain	17577865	21.76	20.780	1691015	16.650	47165	28.485				
					Brain	18278430	21.01	19.996			95522	18.179				
LR16-413	M	HMEG	FCD	PIK3CA (p.E545K)	Peripheral blood						0636	0.000				
					Skin fibroblasts						0582	0.000				
					Saliva	13775937	22.67	21.459	2271086	20.902						
					Brain, anterior dysplasia	39796	0.03	-0.072								
					Brain, superior temporal	1014226	0.06	-0.050								
					Brain, temporal tip	1711562	0.13	0.000								
					Brain, inferior temporal	4414122	0.28	0.120	0/004**	0						
					Brain, anterior inferior	2112662	0.15	0.010								
					Brain, middle temporal	109044	0.10	-0.040								
					Brain, amygdala	2115168	0.12	0.000								
					Brain, hippocampus	76259	0.11	-0.050								
					Brain, cusa	24268	0.05	-0.072								
					Brain, FFPE, superior temporal	39620	0.03	-0.26								
					Brain, FFPE, inferior temporal 1	45482	0.07	0.00								
					Brain, FFPE, inferior temporal 2	96090	0.14	0.05								
					Brain, FFPE, inferior temporal 3	38068	0.03	0								
LR12-251	F	FCD	FCD 2a	PIK3CA (p.H1047R)	Saliva	06774	0.00	-0.06	0/650	0						
					Brain	57411451	4.58	4.186								
					Brain, frontal lobe	7215114	13.76	12.771			1041063	9.784				
					Brain, operculum	10677604	13.51	12.703			92932	9.871				
					Brain, superior temporal	14047549	17.93	16.987			2111172	18.003				
					Brain, anterior temporal 1	169711371	14.06	13.352			1921168	7.877				
					Brain, anterior temporal 2	10626515	15.78	14.820								
					Brain, dura	888502	0.95	0.668								
					Brain, parietal	5877862	7.08	6.437								
					Brain, other	8396792	11.80	10.963								
					Brain, hippocampus	10396864	14.54	13.64			1641090	15.046				
					Epidermis	8411348	0.67	0.44	10789	1.267						
					Cultured fibroblasts	47448	0.05	-0.09								
LR12-317	M	HMEG	NA	AKT3 (p.E17K)	Brain, FFPE	99762	12.93	10.461								
					Saliva	142347	0.58	0.208	3/1143	0.262						
LR13-351	F	FCD	FCD 2a	AKT3 (p.E17K)	Brain	537955	0.62	0.428								
					Blood	38093	0.03	-0.023								
					Brain, temporal lobe	573263	1.66	1.231								
					Brain, hippocampus	1611254	1.26	0.639								
					Brain, insula-1	2453262	7.26	6.384								
					Brain, insula-2	4537056	5.93	5.395								
					Brain, temporal tip	803618	2.13	1.670								
					Brain, FFPE-1	1342048	6.44	5.315								
					Brain, FFPE-2	1892621	7.08	6.033								
					Brain, FFPE-3	1692018	8.25	6.990	38/497	7.646						
					Brain, FFPE-4	391576	2.44	1.608								
					Skin	03688	0.00	0.000								
LR12-246	M	HMEG	FCD 2a	MTOR (p.S2215F)	Brain	1910964	0.16	0.047	5/2879	0.002	0/48	0.000				
					Brain, lateral frontal lobe	210948	0.02	-0.051			0/7	0.000				
					Brain, medial frontal lobe	6/12085	0.04	-0.043								
					Brain, orbital frontal lobe	7511983	0.57	0.388	11/1640**	0.0067	0/15**	0.000				
					Brain, FFPE, frontal lobe	8/1292	0.06	-0.034								
					Brain, FFPE, parietal lobe	5/10161	0.05	-0.047								
					Brain, FFPE, posterior temporal lobe	3011250	0.24	0.104								
					Brain, FFPE, occipital lobe	4/11120	0.03	-0.051								
					Brain, FFPE, white matter	68714	0.06	-0.040								
					Brain, FFPE, superior frontal lobe	9/10528	0.08	-0.025								
					Brain, superior temporal	557789	0.65	0.390			0/351*	0.000	0/435*	0		
					Brain, inferior temporal	2130292	0.05	-0.030			0/178	0.000	0/2798	0		
					Brain, middle temporal	1830290	0.04	-0.030			0/266	0.000	0/2416	0		
					Brain, basal temporal	6/15644	0.03	-0.050			0/238	0.000	0/2941	0		
					Brain, amygdala	29287	0.02	-0.047			0/302	0.000	0/2941	0		
					Brain, hippocampus	48328	0.04	-0.047			0/202	0.000	0/2941	0		
					Brain, parietal lobe	2439276	2.44	2.090			3/346*	1.220	9/7583*	0.11886654		
					Brain, posterior temporal-1	120016993	6.22	5.824			0/281**	0.000	0/569**	0		
					Brain, posterior temporal-2	29410678	2.54	2.202			15/158	9.494	761828	4.15754923		
					Brain, frontal operculum	169395	0.16	0.032			7/220	3.182	474016	1.17031873		
					Brain, mid-frontal	48203	0.05	-0.047			6/204	2.941	542361	2.28716645		
					Brain, posterior frontal	4219411	4.16	3.727								
					Brain, posterior temporal	1438092	1.66	1.346								
					Brain, inferior frontal	98096	0.10	-0.012								
					Brain, orbital frontal	97531	0.11	-0.010								
					Saliva	2/167	0.03	-0.047								
LR14-046	M	FCD	FCD	MTOR (p.S2215F)	Brain, deep temporal	3237638	3.91	3.379	75/2513	0.030						
					Brain, posterior frontal	3810187	0.34	0.121	6/1419	0.423						
LR14-155	F	FCD	FCD 2b	MTOR (p.S2215F)	Brain, anterior frontal	8/11312	0.06	-0.091	0/1417	0.000						
					Saliva							0/91	0.00			
					Brain, lateral temporal lobe	6017966	7.18	6.626			2/21	9.524				
					Brain, inferior temporal lobe	897954	1.05	0.836			1/69	1.449				
					Brain, temporal tip	2485042	4.72	4.143			3/23	13.043				
					Brain, hippocampus	26077	0.03	0.000			0/5	0.000				
					Brain, anterior frontal lobe	16009	0.02	0.000			0/102	0.000				
					Brain, middle frontal lobe	8776238	13.59	12.756			0/0	0.000				
					Brain, parietal lobe	7907623	9.88	9.224			5/75	6.667				
					Brain, insula and operculum	7988803	8.54	7.973			8/58	13.793	16/111	14.41		
					Brain, ventricle wall	1478551	1.60	1.341			3/53	5.660				
					Brain, frontal basal cortex	2336872	3.20	2.798			4/29	13.793				
					Brain, gyrus rectus	08727	0.00	0.000								
					Saliva	928998	0.96	0.761					2/78	2.56		

Supplementary Table 3: Summary of clinical and neuroimaging features for the 17 mutation positive patients. Abbreviations: d, day; DMEG, dysplastic megalencephaly; FCD focal cortical dysplasia; HMEG, hemimegalencephaly; y, year; m, month; PQD, posterior quadrant dysplasia; N/A, not available. Data from two patients have been previously published, namely: LR13-389 (PMID 27159400); LR11-443 (PMID 28969385, 25722288, 27159400).

Mutation	Patient ID	Sex	Ethnicity	Age last assessed	Diagnosis	Head size (cm) at birth	Head size (cm) at last assessed	Seizure - age at onset	Seizure - type	Seizure - medical Rx	Brain MRI - ages	Brain MRI - findings	Epilepsy surgery - type	Neuropathology	Post surgery - epilepsy recurrence	Somatic findings (vascular malformations, overgrowth)
P/KC/A P/E52/K	LR16-470	M	N/A	N/A	HMEG	N/A	N/A	N/A	Focal epileptic spasms	N/A	N/A	N/A	N/A	N/A	N/A	Abnormal swelling skin pigmentation on several areas of the body
	LR13-197	F	N/A	-2.5y	DMEG	N/A	38cm	-29d	Starting episodes, back arching, focal	Medically intractable	37d	HMEG	N/A	N/A	N/A	Right lower extremity hemihypertrophy with some left-sided facial asymmetry
	LR15-251	M	Caucasian	16y	HMEG	N/A	N/A (WNL by report)	1d	Status epilepticus: tonic-clonic with breath arrest	Currently well-controlled, last seizure 4 years ago	3y	HMEG	Temporo-parietal-occipital functional disconnection (2005), inferotemporal (May 2008), temporo-parietal-occipital functional disconnection more anterior than 1st procedure (Sept 2009)	N/A	N/A	Fat foot, trunkless skin, sebaceous nevus on left cheek
	LR16-242	F	Caucasian	3 y	HMEG	37.2 cm	51 cm at 3y7m, 95% for age	1 d	Flexor spasms, complex partial seizures	Medically intractable, much improved on ketogenic diet	2d	R HMEG, with L hemispherectomy	No	N/A	N/A (died at age 4y 1m from respiratory arrest after status epilepticus)	Enlarged R cheek and eye, swelled brown nodule on R cheek extending to neck
P/KC/A P/H1047R	LR16-413	M	Caucasian	N/A	HMEG	46cm	N/A	2d	Abnormal eye movements, TC, EEG low Ohabara syndrome	Medically intractable	N/A	L HMEG and R parieto-occipital FCD (likely FCD type 2)	N/A	N/A	N/A	Hypertrophic left lip/cheek
	LR16-024	F	N/A	17y	FCD	N/A	N/A	6y	Abnormal eye movements, complex partial	Medically intractable	5y, 15y, 17y	Non-specific small focal areas of T2/FLAIR prolongation in the L superior frontal periventricular white matter, hypomyelination in R anterior temporal region	Misrodinectomy	No post-surgical recurrence	-	-
	LR12-251	F	Asian/Hawaiian/ Pacific Islander	12y	FCD	N/A, referred after 1st seizure	50cm	3y	Absent, partial seizures	Medically intractable	5y	FCD with mass effect	R temporo-parietal craniotomy, R occipital lobectomy	FCD2a	No post-surgical recurrence	-
	LR11-443	F	Caucasian	8y	HMEG (+ comititant focus)	36cm	48-4cm	3d	Generalized, focal	Medically intractable, ketogenic diet	4y	HMEG with involvement of comititant mesial occipital lobe	Craniotomy hemispherectomy total, craniotomy	FCD2a	Post-surgical recurrence 2 for days postop #1, and after 4-6m postop#2	Cuts mammae, epigomomygaly, 1: retroorbital vascular malformation
P/KC/A P/E17K	LR12-317	M	Caucasian	14m	HMEG	N/A	44.3	7d	[stiffness, flexion of trunk, rhythmic lip smacking/chewing, rigid extremities which moved from flexion into extension 3 times]	Medically intractable	13d	HMEG	Hemispherectomy	[Sections show disorganized cortical layering; neurons disorganized with focal clustering and vary immensely in size, likely representing a form of HMEG/MGAP, Ohabara syndrome]	No post-surgical recurrence	Caucasian vascular lesions on extremities and trunk
	LR13-351	F	N/A	N/A	FCD	N/A	N/A	N/A	CPS	N/A	N/A	Right occipital lobectomy	FCD2a	Best seizure	-	
	LR16-313	F	Caucasian	3y	FCD	36.8 (1m)	45.9 (2y)	-4d	ISS	Medically intractable	1m	HMEG	Right craniotomy hemispherectomy	FCD2a	No post-surgical recurrence	-
	LR12-246	M	Caucasian	13y	HMEG (POD)	38.5cm at 1m	43.8cm	3d	Multiple types	Medically intractable	6m	FCD	Hemispherectomy (S2)	FCD2a	No post-surgical recurrence	-
	LR13-129	M	Caucasian	5y	FCD	N/A	49cm	11m	drop attacks, gaze seizures, intractable	Medically intractable	5y	FCD	Craniotomy NSR (3), craniotomy hemispherectomy	FCD2b (x3, not seen in last resection)	Possible 1 seizure recurrence	-
	LR13-389	F	Caucasian	6y	FCD	37.1cm at 6w	48.2cm at 3y	5w	CPS	Medically intractable	7m	HMEG	Craniotomy NSR (2/2/13), craniotomy hemispherectomy (2/2/14)	FCD2a	Only one seizure of a different semiology post 2nd procedure	-
P/KC/A P/S2215F	LR16-046	M	Caucasian	6y	FCD	N/A	53.4cm	7m	Starting spells with occasional left upper extremity twitching, tonic posturing	Medically intractable	5y	FCD	Craniotomy placement distal, craniotomy removal grid w/ resection	FCD2b	No post-surgical recurrence	-
	LR14-155	F	Caucasian	17y	FCD	N/A	53.5cm (12y)	5y	Localization-related (focal) epilepsy, CPS	Medically intractable	12y, 15y	FCD	Craniotomy NSR	FCD2b	No post-surgical recurrence	-
P/KC/A P/S2215Y	LR16-004	M	Caucasian	3y	HMEG	N/A	45.9 (3y)	3w	ISS	Medically intractable	6m	HMEG	Craniotomy, left hemispherectomy total (1/7/16)	FCD2a	Movements continued to be jerky after first craniotomy/epilepsy recurrence	-

Supplementary Table 4: Data summary of patients and samples who tested negative for the 6 PI3K-AKT-MTOR hotspot mutations. Patients' de-identified code (Patient-ID), diagnosis based on neuroimaging (Dx-MRI), Diagnosis based on neuropathology (Dx-NPath), and sample type are listed for the mutation-negative patients (n=41). All samples were tested via ddPCR for all 6 hotspots in quadruplicates and in multiple independent runs. All samples had a DCI<0.045, thus they were considered negative (cells shaded in red). The average of each quadruplicate is reported here. Number of WT droplets is reported as measure of quality of the ddPCR run; samples with over 3000 were considered robust and subsequently analyzed (green shaded cells). If samples had less than 3000 WT droplets (yellow shaded cells) and VAF<0.1%, they were deemed negative. When multiple runs were performed for a given sample, results from the run yielding the highest number of wild-type droplets (WT droplets) is reported here. Patient LR12-245 was previously published in PMID: 27159400.

Di (neuropathology)	Patient ID	Sex	DS (Neuropathology)	Stimulus - age of onset (mo)	# of surgeries	Sample type	FPGC/P-R54SK			FPGC/P-R418R			MTOF p-R221F8			MTOF p-R221F9			AFTJ p-R17K							
							V.A.P.%	DCI	WT Drops	V.A.P.%	DCI	WT Drops	V.A.P.%	DCI	WT Drops	V.A.P.%	DCI	WT Drops	V.A.P.%	DCI	WT Drops	V.A.P.%	DCI	WT Drops		
ECD	LR12-244	F	FCD 1b	7:00	2	Brain - Saliva	0.03	-0.01	3469	1	0.04	0.00	2310	1	0.01	0.00	3562	0	0.00	0.00	3379	0	0.00	0.00	3573	2
	LR12-245	F	FCD 2a	6:00	3	Brain - medial parietal	0.02	-0.04	8014	4	0.03	-0.02	9286	3	0.01	0.00	6763	1	0.03	0.00	6784	3	0.02	-0.026	10784	2
	LR12-246	F	FCD 2b	4:00	3	Brain	0.02	-0.00	4435	2	0.03	-0.02	5933	1	0.02	0.00	5933	0	0.02	0.00	5526	0	0.02	-0.021	7112	5
	LR12-247	F	FCD 2b	4:00	3	Brain	0.02	-0.00	4435	2	0.03	-0.02	5933	1	0.02	0.00	5933	0	0.02	0.00	5526	0	0.02	-0.021	7112	5
	LR12-248	M	FCD 2b	48:67	3	Brain	0.03	-0.00	10974	4	0.03	-0.02	9819	3	0.02	0.00	8485	1	0.02	0.00	10213	2	0.02	-0.026	12121	3
	LR12-249	M	FCD 1a	12:17	2	Brain	0.03	-0.00	10609	4	0.02	-0.02	10186	2	0.04	-0.02	10186	0	0.00	0.00	10765	0	0.02	-0.008	11776	8
	LR12-250	M	FCD 1a	10:00	2	Brain	0.02	-0.00	10609	4	0.02	-0.02	10186	2	0.04	-0.02	10186	0	0.00	0.00	10765	0	0.02	-0.008	11776	8
	LR12-251	F	FCD 2b	10:00	2	Brain	0.02	-0.00	10609	4	0.02	-0.02	10186	2	0.04	-0.02	10186	0	0.00	0.00	10765	0	0.02	-0.008	11776	8
	LR12-252	F	FCD 2a	71:00	2	Saliva	0.03	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9
	LR12-253	F	FCD 2a	11:467	2	Brain	0.02	-0.00	11467	2	0.04	-0.02	9124	2	0.01	0.00	8960	1	0.02	0.00	11832	2	0.02	-0.079	9880	2
	LR12-254	F	FCD 2b	13:57	2	Brain	0.02	-0.00	11467	2	0.04	-0.02	9124	2	0.01	0.00	8960	1	0.02	0.00	11832	2	0.02	-0.079	9880	2
	LR12-255	M	FCD 2b	4:07	2	Brain	0.02	-0.00	11467	2	0.04	-0.02	9124	2	0.01	0.00	8960	1	0.02	0.00	11832	2	0.02	-0.079	9880	2
	LR12-256	F	FCD 2b	18:50	3	Brain	0.02	-0.00	11467	2	0.04	-0.02	9124	2	0.01	0.00	8960	1	0.02	0.00	11832	2	0.02	-0.079	9880	2
	LR12-257	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9
	LR12-258	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9
	LR12-259	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9
	LR12-260	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9
	LR12-261	M	FCD 2b	18:50	3	Brain	0.02	-0.00	11467	2	0.04	-0.02	9124	2	0.01	0.00	8960	1	0.02	0.00	11832	2	0.02	-0.079	9880	2
	LR12-262	F	FCD 2b	18:50	3	Brain	0.02	-0.00	11467	2	0.04	-0.02	9124	2	0.01	0.00	8960	1	0.02	0.00	11832	2	0.02	-0.079	9880	2
	LR12-263	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9
LR12-264	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-265	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-266	F	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-267	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-268	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-269	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-270	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-271	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-272	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-273	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-274	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-275	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-276	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-277	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-278	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-279	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-280	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-281	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-282	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-283	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-284	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-285	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-286	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-287	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-288	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-289	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-290	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-291	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	7429	1	0.07	-0.002	11102	9	
LR12-292	M	FCD 1b	N/A	1	Brain	0.02	-0.00	8211	3	0.04	-0.02	9741	3	0.00	0.00	6591	1	0.01	0.00	742						

Supplementary Table 5: Neuropathological classification for cohort. The neuropathological diagnosis for the 58 patients reported here, sub-classified by their primary clinical diagnosis, and the number of cases within each category. Abbreviations: FCD, focal cortical dysplasia; HIE, hypoxic-ischemic encephalopathy; MTS, mesial temporal sclerosis; Multifocal CD, multifocal cortical dysplasia; PMG, polymicrogyria

Clinical Diagnosis	Neuropathology	Cases
FCD/HMEG/DMEG	FCD	5
	FCD 1	3
	FCD 1a	3
	FCD 1b	3
	FCD 1c	1
	FCD 2	1
	FCD 2a	19
	FCD 2b	4
	FCD 3d	1
MCD	PMG	2
	Multifocal CD/ FCD 1c	1
	Multifocal CD/ FCD 2b	2
Other	Gliosis	3
	MTS	1
	Meningoangiomas	1
	Stroke	1
	HIE	1
	N/A	6
TOTAL		58

Supplementary Table 6: Genotype-neuropathology correlation for patient LR13-129; LR16-313 and LR18-024. Results from ddPCR analysis and neuropathology evaluation for these three representative patients are listed below. For neuropathologic evaluation, hematoxylin and eosin staining (H&E) was performed and cortical layering, as well as presence/absence of balloon/abnormal neurons was evaluated. The H&E results refer to the percentage of abnormal area as evaluated by the pathologist. When possible, pS6 staining was performed and presence of positive neurons (dark or light staining, percentage relative to the area of the section) was performed. For ddPCR results, cells shaded in gray represent brain regions that are positive for the relative hotspot mutation, while cells shaded in gray represent brain regions that do not present the mutation. For LR18-204, FFPE and fresh frozen samples are listed. Two representative brain regions (inferior temporal, positive; and superior temporal, negative) from patient LR18-024 were selected for a comparison of VAF% detected via ddPCR in fresh frozen vs FFPE samples. See Figure 5 for a schematic representation of the two LR18-024 specimens and relative neuropathology findings, and Supplementary Fig. 3 for neuropathology analysis for samples relative to LR13-129 and LR16-313. Blocks highlighted in Fig.5 and Supplementary Fig. 3 are highlighted here in yellow (positive blocks) and gray (negative blocks).

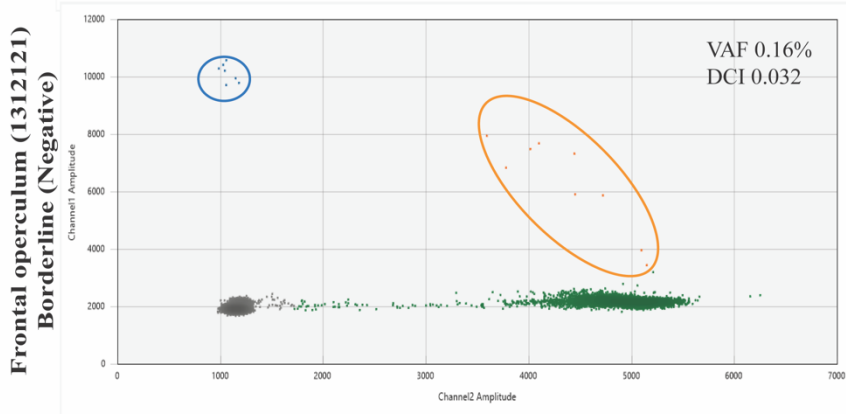
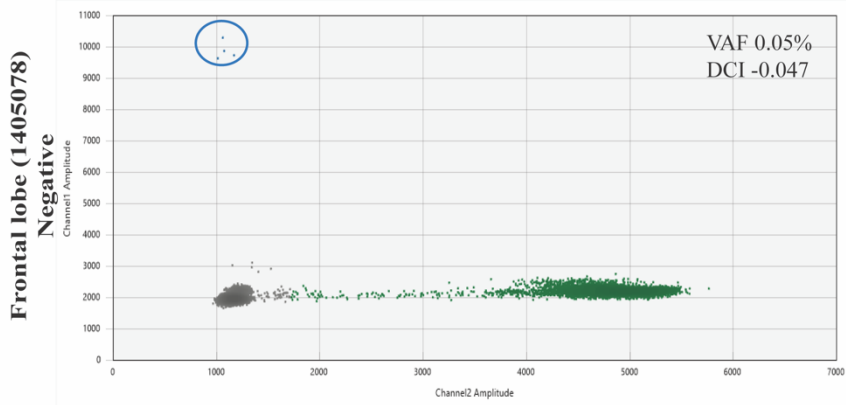
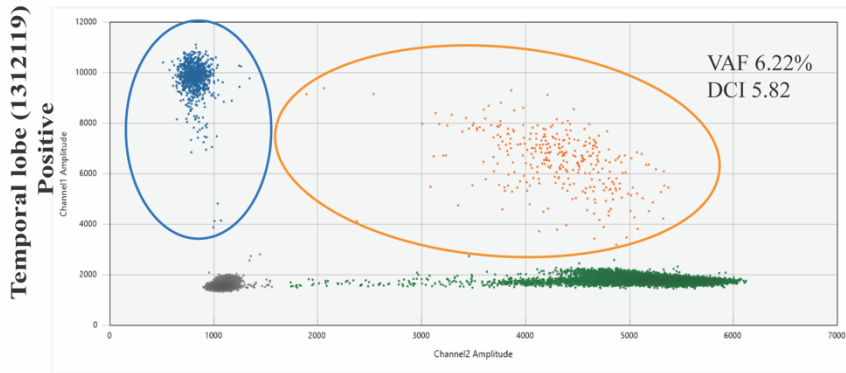
Sample description	ddPCR										Neuropathology									
	VAF%	DCI	WT droplets	Mutant droplets	H&E		Not able to evaluate	Dark		Light		None								
					Dysplastic	Non-Dysplastic		Comment	%	Comment	%									
LR13-129	Left	0.02	-0.05	10946	2	50	0	Dysplastic area	15%	Dysplastic area	80%									
	Medial Frontal	0.04	-0.04	12079	6	0	100	Dysplastic area	15%	Dysplastic area	80%									
	Inferior Frontal	0.57	0.39	11908	75	100	0		80%		0%									
	Frontal	0.06	-0.03	12784	8	5%	80%		20%		0%									
	Parietal	0.05	-0.05	10155	5	20%	75%	Mostly dysplastic area	20%		55%									
	Posterior temporal	0.24	0.01	11220	30	0%	100%	Diffusely distributed mostly in layer V	80%		10%									
	Occipital	0.03	-0.05	11116	4	20%	60%		20%		55%									
	White matter	0.06	-0.04	8708	6	30%	30%	Concentrated in dysplastic area	20%	Concentrated in dysplastic area	60%									
	Superior Frontal	0.08	-0.02	10519	9	20%	50%		15%		70%									
	Temporal lobe (A)	1.66	1.23	3206	57	20	60	Most prevalent in dysplastic foci and layer III	75%	Layers III and V	10%									
	Temporal Lobe (B)	1.26	0.64	1238	16	25	10	Most prevalent in dysplastic foci and layer III	40%	Layers III and V	5%									
	Hippocampus	7.26	6.38	3017	245	70	20	Most prevalent in dysplastic foci	50%	Layers III and V	5%									
	Insula (D1)	5.93	5.39	6603	453	50	40	Most prevalent in dysplastic foci and layer III	45%		5%									
	Insula (D2)	6.44	5.32	3538	80	50	10	Most prevalent in dysplastic foci and layer III	75%		5%									
	Insula (D3)	7.08	6.03	1914	134	50	40	Most prevalent in dysplastic foci and layer III	75%		5%									
Insula (D4)	8.25	6.99	2432	189	75	15	Most prevalent in dysplastic foci and layer III	75%		5%										
Insula (D5)	2.44	1.61	1849	169	10	90	Mostly scattered in layer V	10%		65%										
temporal lip	2.13	1.67	1537	39	10	90		0%	N/A											
FFPE	Inferior temporal, FFPE, IT1	0.070	0.000	5478	4	10%	30%		0%		100%									
	Inferior temporal, FFPE, IT2	0.140	0.046	6081	9	0%	50%		3%		95%									
	Inferior temporal, FFPE, IT3	0.030	0.000	8065	3	0%	60%		0%		95%									
	Superior temporal, FFPE, ST1, dysplastic	0.020	-0.260	5811	1															
	Superior temporal, FFPE, ST1, non-dysplastic	0.050	-0.260	7012	4	15	65	perfect correlation with dysplasia	85		0									
	Superior temporal, FFPE, ST1, full scroll	0.030	-0.260	9617	3															
	Anterior Dysplasia	0.03	-0.072	9793	3	50%	50%													
	Superior Temporal	0.06	-0.050	14216	10	10%	30%													
	Temporal Lip	0.13	0.000	11545	17	0	70													
	Inferior Temporal	0.28	0.170	14078	44	0%	75%													
Fresh Frozen	Anterior Inferior	0.15	0.010	13641	21	10%	60%													
	Middle Temporal	0.10	-0.040	9024	10	0	20													
	Antygalla	0.12	0.000	15447	21	0	20													
	Hippocampus	0.11	-0.050	6252	7	0	90													
	Cyst Specimen	0.03	-0.072	4266	2			too poorly oriented to evaluate for dysplasia												

SUPPLEMENTARY FIGURES

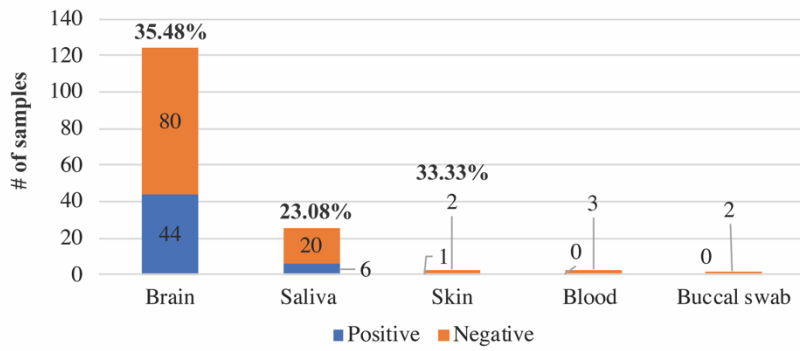
Supplementary Figure 1. ddPCR representative results. (A) Examples of 2D Quantasoft plots for brain tissues from patient LR13-389, carrying the *MTOR* p.S2215F mutation. Variant allele fractions (VAF%) and Delta confidence intervals (DCI) are shown for each sample. The temporal lobe (sample 1312119) shows a clear positive result, with mutant droplets (in the blue oval) clustering in the upper left quadrant and in higher number than the double positive droplets (in the orange oval), with wild-type droplets in green clustering in the lower right quadrant. A frontal lobe sample from the second surgical procedure (sample 1405078) was negative, with 4 mutant droplets (in the blue oval) detected which were also present in the wild-type control in the same run, as demonstrated by the negative DCI. A sample from the frontal operculum region (1312121) had a “borderline” result. Samples with VAF 0.16% and DCI=0.032 would be considered positive by the Biorad analysis protocol. However, as the plot shows, the number of mutant droplets (in the blue oval, n=7) are fewer than the double positive droplets (in the orange oval, n=9). The high number of double positive droplets leads to a false positive result, thus samples with DCI<0.045 are considered negative in this study. **(B)** The solve rate per type of tissue based on number of positive specimens vs. total. Hotspot mutations were not detectable in blood and buccal swabs.

A

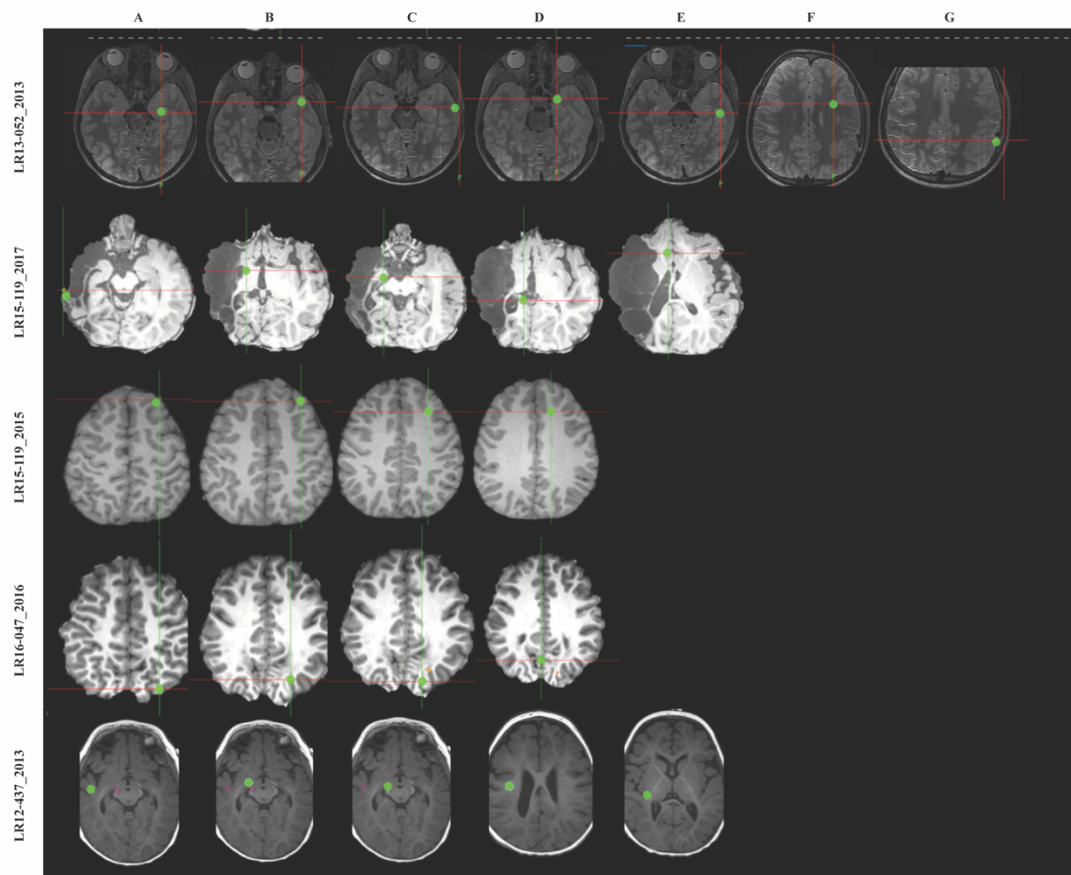
LR13-389 *MTOR* (p.S2215F)



B



Supplementary Figure 2: Brain MRI of four patients with FCD/HMEG/DMEG who tested negative for the 6 PI3K-AKT-MTOR hotspot mutations. Summary MRI images showing biopsy locations. For each image, cross-hairs reflect the sample site, with letters reflecting the multiple samples taken. Green dots represent the exact location of resection. The year in which the surgery was performed is indicated after the underscore, as patient LR15-119 underwent multiple brain surgeries. These samples were tested via ddPCR for the 6 hotspot mutations and were all negative.

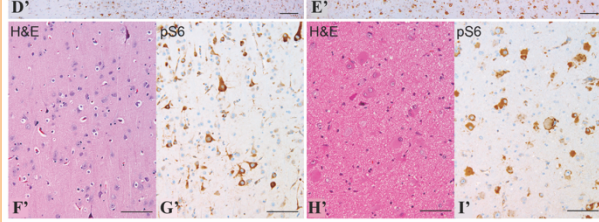
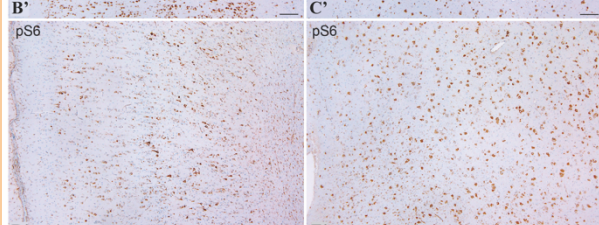
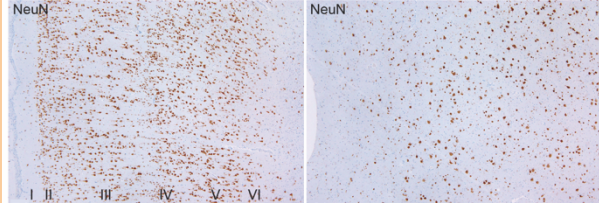


Supplementary Figure 3: Histopathological findings and correlations with genotype in tissue samples from individuals LR13-129 and LR16-313. (A') Schematic representation of the resection locations for patient LR13-129. Immunohistochemical and ddPCR findings in the histologically non-dysplastic parietal (B',D',F',G') and dysplastic (FCD type IIB) inferior frontal (C', E', H', I') resection specimens from patient LR13-129. (B') NeuN labeling of neurons in a low magnification field from the parietal resection specimen shows intact six-layered cortical gray matter as indicated by Roman numerals. The neurons appear cytologically normal (F'), but a significant subset show strong perikaryal pS6 immunoreactivity (D',G'). (C') In contrast, NeuN immunostaining shows marked disorganization of the cortical lamina in the inferior temporal region with balloon cells (H') and diffuse positive pS6 immunoreactivity in nearly the entire neuronal population (I'). Scale bars: B'-E', 200 μ m; F'-I', 100 μ m. (A'') Schematic representation of the resection location for patient LR16-313. Immunohistochemical and ddPCR findings in the histologically non-dysplastic (B'', D'', F'', G'') and dysplastic area (C'', E'', H'', I'') from the temporal lobe resection (block B) LR16-313. (B'') NeuN labeling of neurons in a low magnification field from a portion of the temporal lobe resection specimen shows intact six-layered cortical gray matter as indicated by Roman numerals. The neurons appear cytologically normal (F''), despite having a detectable mosaic burden in this region (VAF 1.26%). In contrast, NeuN immunostaining shows disorganization of the cortical lamina in a portion of the same region (C'') with atypical large neurons (H'') and intense positive pS6 immunoreactivity in a subset of the neuronal population (I''). Scale bars: B-E, 200 μ m; F-I, 100 μ m. Overview of ddPCR results for all samples belonging to these three individuals with matching neuropathology evaluation is presented in Supplementary Table 6, with the negative blocks (red text) and positive blocks (green text) matching the ones represented in this figure.

A' **LR13-129**
MTOR p.S2215F VAF% 0-0.57%



Parietal (Non-dysplastic, VAF 0.05%) Inferior Frontal (Dysplastic, VAF 0.57%)



A'' **LR16-313**
AKT3 p.E17K VAF% 1.26-8.25%



Temporal B (Non-dysplastic, VAF 1.26%) Temporal B (Dysplastic, VAF 1.26%)

