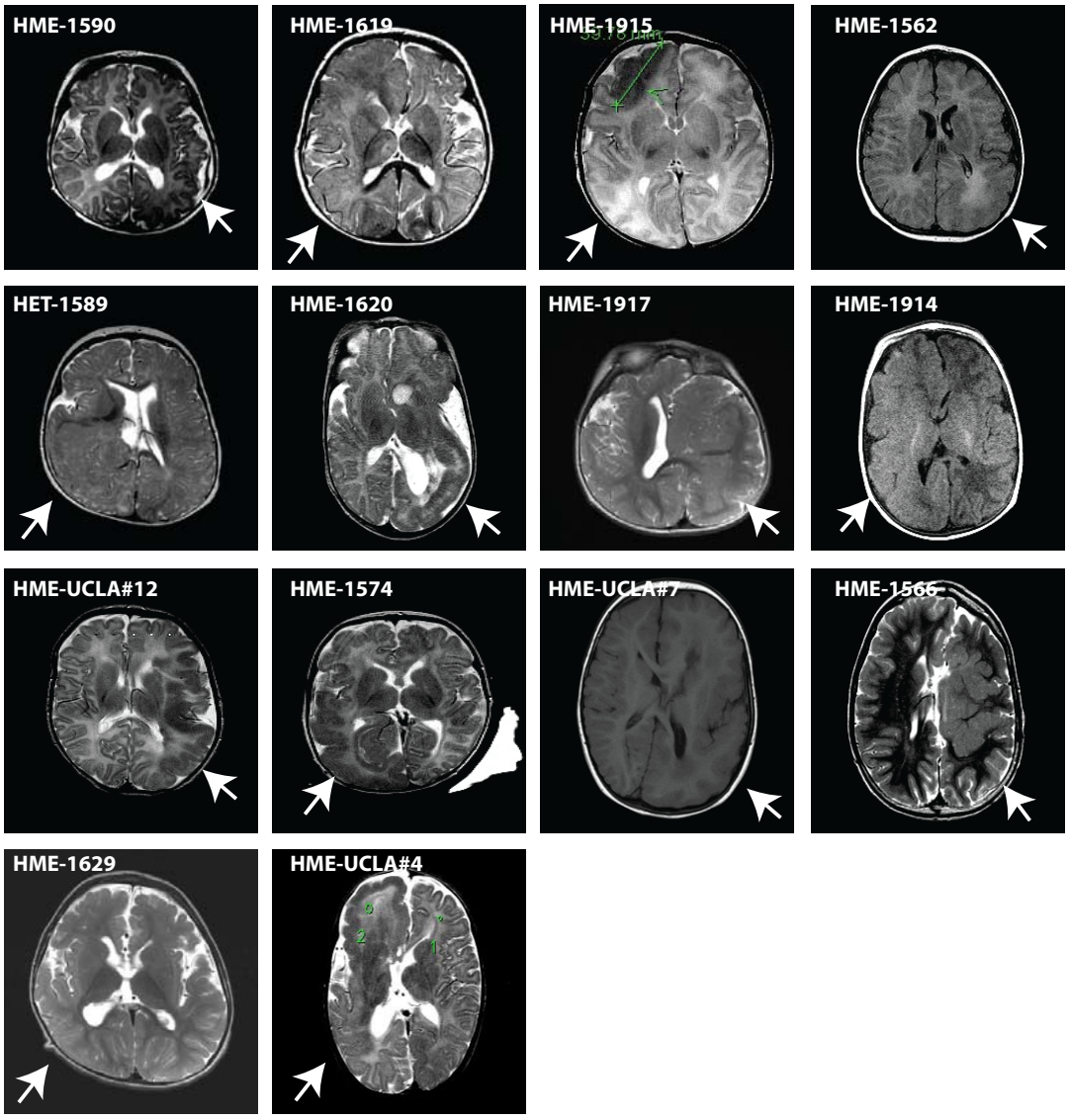
Supplementary Data

Supplementary Table 1. Clinical and molecular data from all HME patients in the cohort

Patient ID	Somatic Mutation	CN	BC	PMG	Heterotopia	Age Sz onset (yr)	Sz Status Post	Gender	Age at Surgery(yr)	Other
HME-1565	AKT3 p.Glu17Lys	Yes	No	Yes	Yes	0.48	Not Sz Free	Female	0.5	
HME-1563	MTOR p.Cys1483Tyr	Yes	No	No	Yes	4.4	Sz Free	Male	4.3	Hypomelanosis of Ito
HME-1573	PIK3CA p.Glu545Lys	Yes	No	Yes	Yes	0.32	Sz Free	Male	0.33	
HME-1916	PIK3CA p.Glu545Lys	Yes	No	Yes	Yes	0.34	Sz Free	Male	0.33	
HME-1564	PIK3CA p.Glu545Lys	No	No	Yes	Yes	0.48	Sz Free	Male	0.5	
HME-1855	PIK3CA p.Glu545Lys	Yes	No	Yes	Yes	3.72	Sz Free	Male	3.7	Hemihypertrophy of right hand/foot
UCLA-HME#4	Unknown	Yes	Yes	No	No	0.19	Sz Free	Male	0.2	
HME-1915	Unknown	Yes	No	Yes	No	0.23	Sz Free	Male	0.2	
HME-1574	Unknown	Yes	Yes	Yes	No	0.3	Not Sz Free	Female	0.25	CD in opposite mesial frontal lobe
HME-1914	Unknown	Yes	No	No	Yes	0.33	Sz Free	Female	0.33	
HME-1620	Unknown	Yes	Yes	No	Yes	0.44	Sz Free	Male	0.45	
HME-1619	Unknown	Yes	Yes	Yes	Yes	0.63	Sz Free	Female	0.67	
HME-1590	Unknown	Yes	No	No	No	0.66	Sz Free	Female	0.75	
UCLA-HME#7	Unknown	No	No	Yes	No	1.5	Sz Free	Female	1.4	
HME-1908	Unknown	No	No	No	Yes	0.01	Sz Free	Male	1.5	
HET-1589	Unknown	No	No	No	Yes	1.5	Sz Free	Male	1.5	
HME-1917	Unknown	No	No	No	Yes	1.7	Sz Free	Male	1.7	
HME-1629	Unknown	No	No	Yes	Yes	2.41	Not Sz Free	Male	2.4	
HME-1566	Unknown	No	No	Yes	Yes	4.1	Sz Free	Male	4	
HME-1562	Unknown	No	No	Yes	Yes	0.83	Sz Free	Male	9.5	

CN=cytomegalic neuron, BC=Balloon cells, PMG=polymicrogyria, Sz=Seizure, CD=cortical dysplasia
+Ordered by gene name and age at surgery

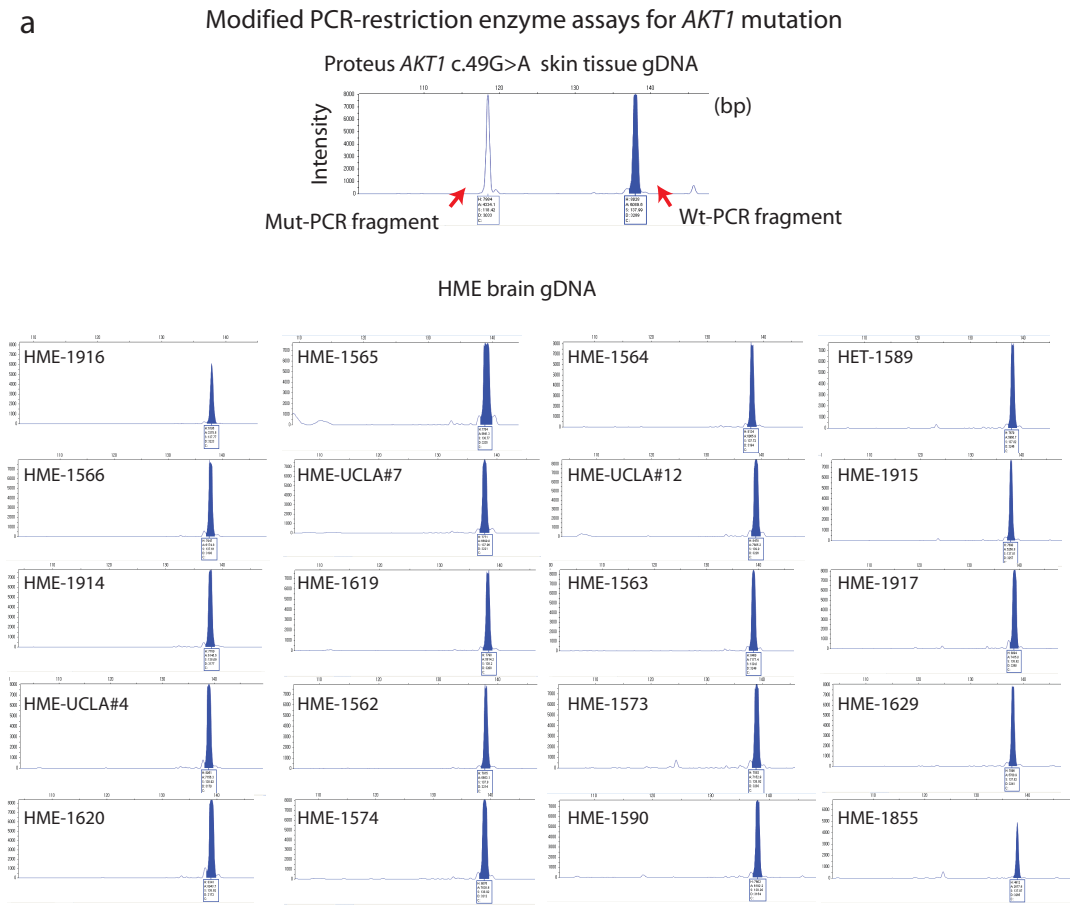
Nature Genetics: doi:10.1038/ng.2329



Supplementary Figure 1. Brain MRIs from mutation-negative patients involved in this study. Arrows highlights the affected hemispheres.

Nature Genetics: doi:10.1038/ng.2329

a



b

Exclusion of significant CNVs in HME brain using SNP genotyping arrays

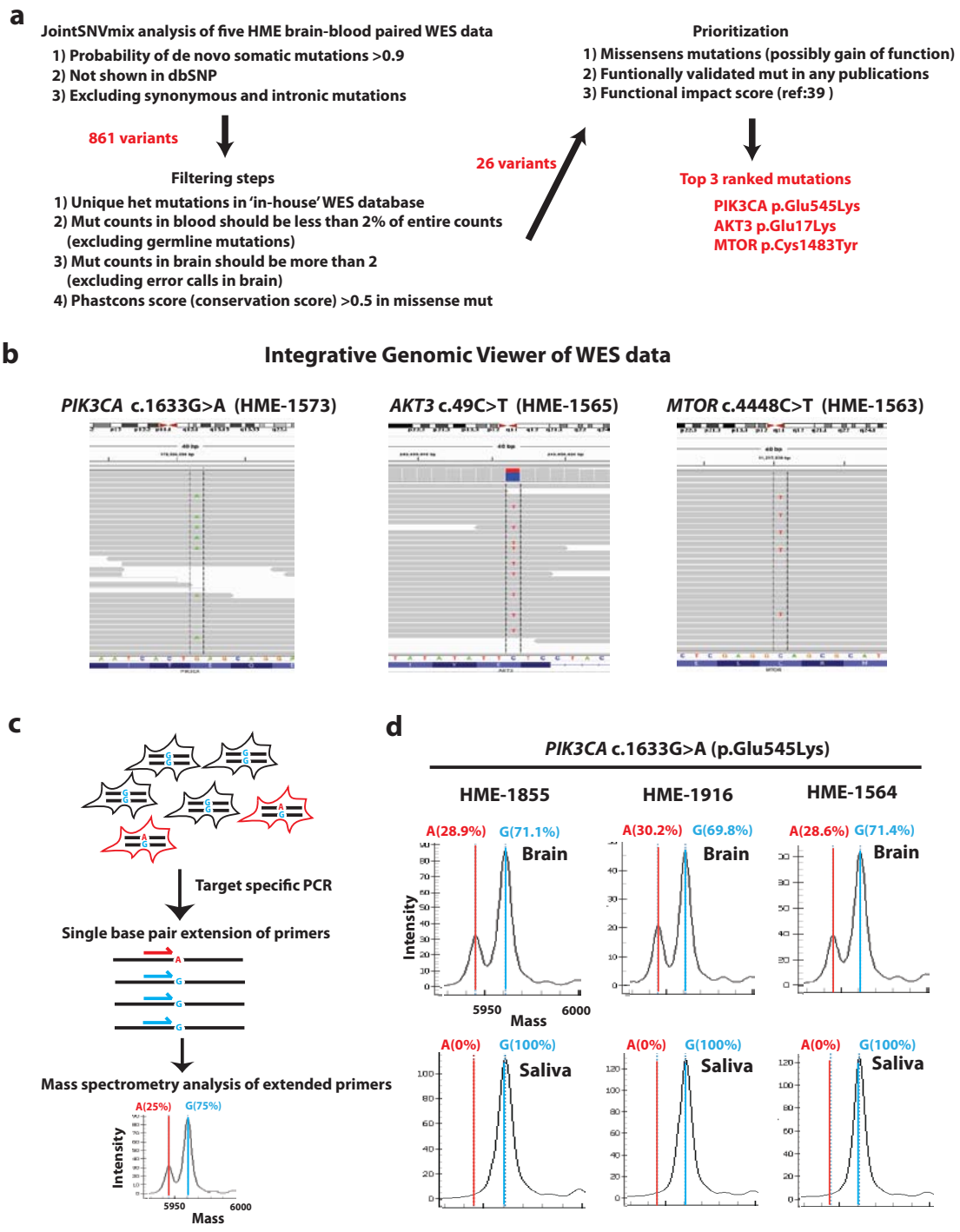
Patients	Tissues	Positions(hg19)	Number of SNP	Length(bp)	Copy number	Start/End SNP
HME-1563	Brain	chr6:168356152-168599333	37	243,182	3	startsnp=rs9346633 endsnp=rs2306286
		chr12:85171056-85550132	8	379,077	4	startsnp=rs1452243 endsnp=rs2174754
	Blood	chr6:168356152-168576278	32	220,127	3	startsnp=rs9346633 endsnp=rs6941655
		chr12:83695187-85603531	9	432,476	4	startsnp=rs1452243 endsnp=rs1354839
HME-1565	Brain	chr10:47596804-47701570	9	104,767	3	startsnp=rs1870519 endsnp=rs11596854
	Blood	chr10:47596804-47701570	9	104,767	3	startsnp=rs1870519 endsnp=rs11596854
HME-1573	Brain	chr1:149039120-149211496	7	172,377	3	startsnp=rs11579261 endsnp=rs12409037
HME-1574	Brain	chr12:31359210-31398147	4	38,938	3	startsnp=rs2164498 endsnp=rs7957449
	Blood	chr12:31385094-31398147	3	13,054	3	startsnp=rs4931471 endsnp=rs7957449
HME-1620	Brain	chr14:43876895-44236516	16	359,622	3	startsnp=rs974953 endsnp=rs11157364
	Blood	chr14:43876895-44246488	17	369,594	3	startsnp=rs974953 endsnp=rs11157365

Supplementary Figure 2. Lack of *AKT1* c.49G>A mutation or significant *de novo* copy number variations in HME brains. (**a**) Top: Proteus syndrome sample testing positive for the *AKT1* c.49G>A mutation, with approximately 41% of PCRed alleles showing the mutation using the modified PCR-restriction enzyme assays. Filled peak: wildtype allele, Unfilled peak: Mutant allele. (**b**) Exclusion of significant *de novo* CNVs in HME brain using SNP genotyping array. Most detected CNVs in this

Nature Genetics: doi:10.1038/ng.2329

cohort were identified in both brain and blood samples. Only HME-1573 showed a *de novo* CNV in brain, but this CNV is reported in healthy controls.

Nature Genetics: doi:10.1038/ng.2329



Supplementary Figure 3. The work-flow of whole exome sequencing and mass-spectrometry analysis **(a)** The general work-flow of analysis and prioritization of JointSNVMix data. JointSNVMix utilized aligned sequence data in base space stored in the BAM format to generate JointSNVMix output with 7036 variant calls. To stringently detect HME brain specific mutations, we selected JointSNVMix output

Nature Genetics: doi:10.1038/ng.2329

with a high threshold probability of somatic mutation (P[somatic]>0.9, meaning less than 1:10 of the disagree rate) and excluded variants found in dbSNP (normal variants database), as well as synonymous or intronic variants. After this process, we found 861 variants specific to HME brains. We subsequently filtered any germline mutations in HME blood, erroneous calls in HME brain, and variants in evolutionarily non-conserved sequence to 26 novel heterozygous variants. We prioritized variants for those likely to be gain-of-function by considering previous reports and functional impact scores reflecting the biological importance of evolutionarily conserved sequence [42]. **(b)** Visualization of identified somatic mutation calls in WES. Variants were visually inspected in Integrative Genomic Viewer. **(c)** Schematic figure of mutation burden analysis. A single base pair extension on target sequence was followed by mass spectrometry analysis, allowing detection of the mass difference due to a single nucleotide change. **(d)** Mutation burden analysis in HME cohort identified a recurrent *PIK3CA* c.1633G>A in three more patients (HME-1855, -1916, and -1564) with ~30% mutation frequencies. Saliva samples from all these patients were negative for the *PIK3CA* c.1633G>A mutation.

Nature Genetics: doi:10.1038/ng.2329