Supplemental Data

Determining pathogenicity of variants of uncertain significance and identification of a founder variant in the epilepsy-associated gene, *SZT2*.

Jeffrey D Calhoun, Miriam C Aziz, Hannah C Happ, Jonathan Gunti, Colleen Gleason, Najma Mohamed, Kristy Zeng, Meredith Hiller, Emily Bryant, Divakar Mithal, Irena Bellinski, Lisa Kinsley, Mona Grimmel, Eva MC Schwaibold, Constance Smith-Hicks, Anna Chassevent, Marcello Scala, Andrea Accogli, Annalaura Torella, Pasquale Striano, Valeria Capra, Lynne M. Bird, Issam Ben-Sahra, Nina Ekhilevich, Tova Hershkovitz, Karin Weiss, John Millichap, Elizabeth E Gerard, Gemma L Carvill

SUPPLEMENTAL DATA

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Supplemental materials and methods

Exon skipping PCR

RNA was extracted from HEK 293T cells with Trizol (Invitrogen 15596026) according to manufacturer's recommendations. cDNA was synthesized using iScript (Biorad) and 1000 ng of input RNA according to manufacturer's recommendations. Primers amplifying from *SZT2* exon 9 to exon 11 were used to generate amplicons (5' aatgagcacctggtctctgc 3' and 5' ggcactgaggagaaggactg 3'). Amplicons were separated on 2% agarose followed by gel purification and Sanger sequencing (Figure S3).

Supplementary Figures

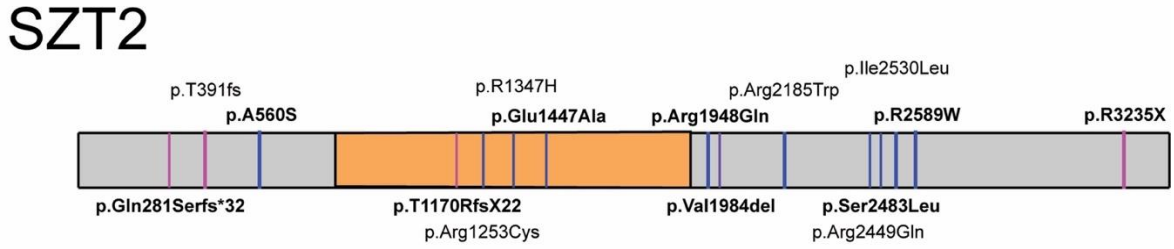

Figure S1: Distribution of variants throughout *SZT2*. Location of truncation variants indicated by purple line, while missense or in-frame deletions are represented by blue lines. The multi-exon deletion is displayed in orange.

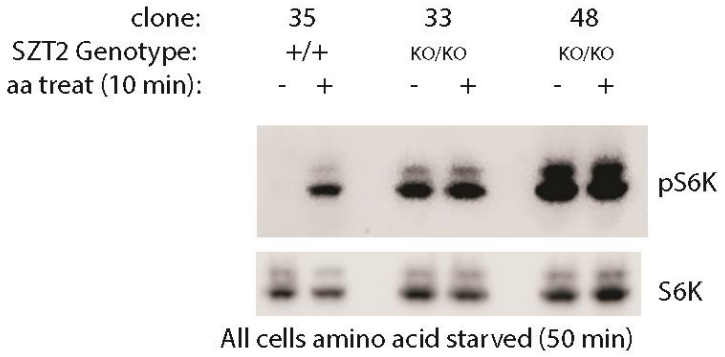

Figure S2: Amino acid treatment of HEK *SZT2*$^{KO/KO}$ cells. *SZT2*$^{KO/KO}$ cells were generated using a gRNA (GTGGCAGCCAGATGAACCAG) targeting exon 3 as previously described followed by puromycin treatment and limited dilution cloning to establish clonal lines [1]. We observed excess mTORC1 activity in amino acid starved *SZT2*$^{KO/KO}$ cells relative to control *SZT2*$^{+/+}$ cells. AA: - denotes cells starved of amino acids for 60 min; AA: + denotes cells starved of amino acids for 50 min followed by subsequent treatment with amino acids for 10 min. pS6K = phosphorylated S6K. For genotype, + = wildtype or reference allele.

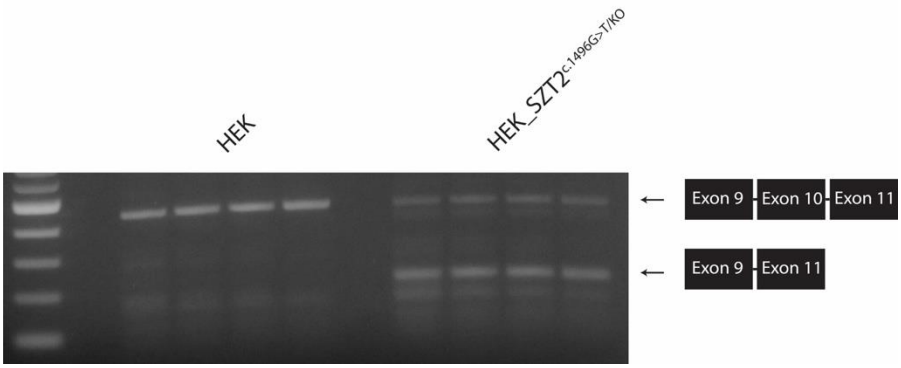

Figure S3: Exon skipping in *SZT2* transcript caused by c.1496G>T. Sanger sequencing of smaller PCR products in cells heterozygous for *SZT2* c.1496G>T confirmed exon skipping due to disruption of splice donor site. The four lanes of control HEK (HEK_SZT2$^{WT/WT}$) and four lanes of compound heterozygous HEK_SZT2$^{c.1496G>T/KO}$ each contain two biological replicates and two technical replicates.

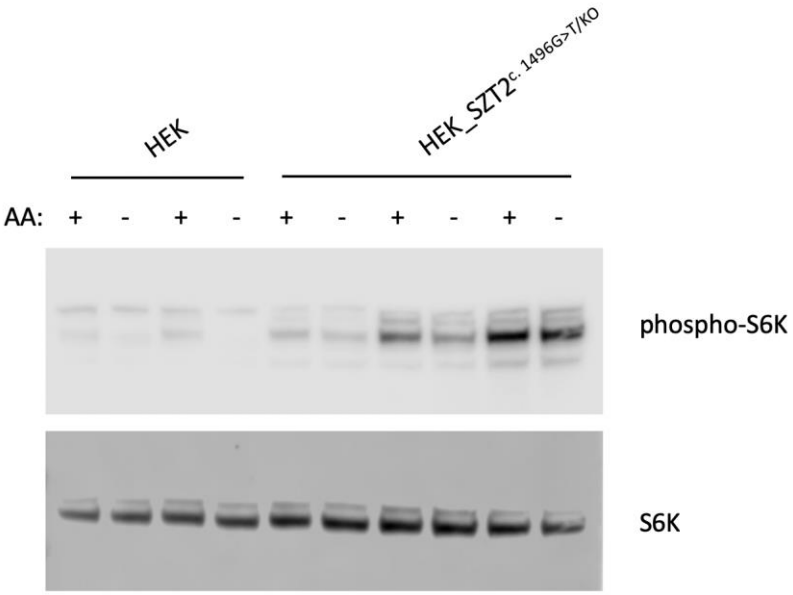

Figure S4: Amino acid treatment of HEK *SZT2*$^{c.1496G>T/KO}$ cells. *SZT2*$^{c.1496G>T/KO}$ cells exhibit excessive mTORC1 activity under amino acid starvation, in support of *SZT2* c.1496G>T as a loss-of-function allele. Two biological replicates shown for control HEK (HEK_SZT2$^{WT/WT}$). Three biological replicates are shown for compound heterozygous HEK_SZT2$^{c.1496G>T/KO}$. AA: - denotes cells starved of amino acids for 60 min; AA: + denotes cells starved of amino acids for 50 min followed by subsequent treatment with amino acids for 10 min.

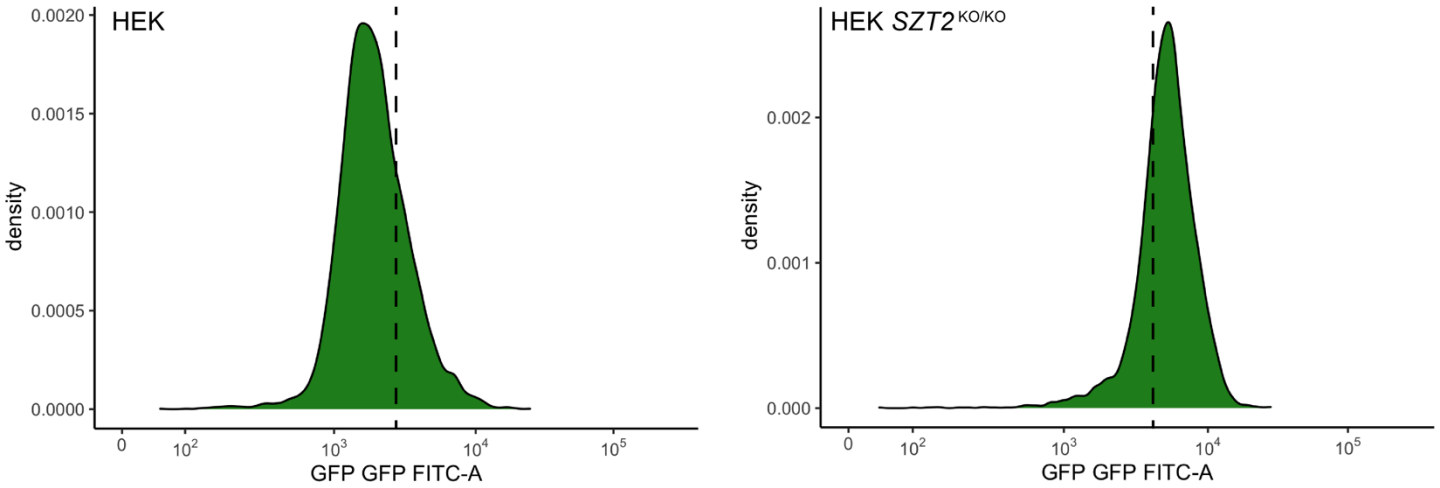

Figure S5: Flow cytometry assay for mTORC1 activity in amino acid starved cells. P-S6 levels are higher in amino acid starved *SZT2*$^{KO/KO}$ cells relative to control HEK cells due to constitutive mTORC1 activity. Dashed line denotes the gating cutoff used to separate P-S6^{LOW} from P-S6^{HIGH} cell populations. GFP = signal from Alexa488 conjugated anti P-S6.

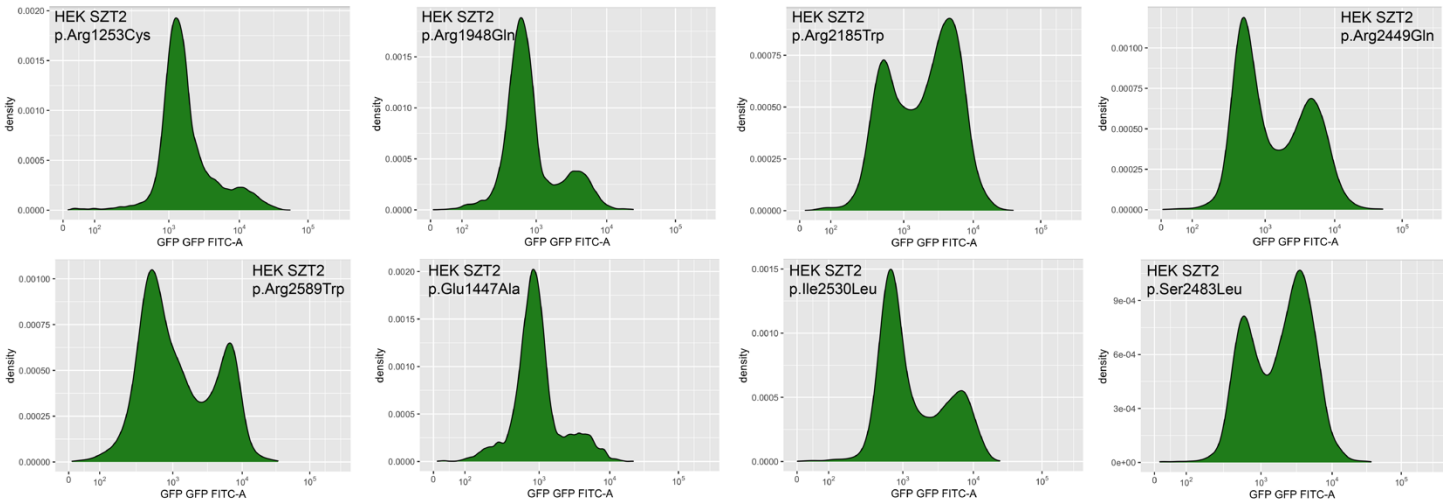

Figure S6: Flow cytometry assay for mTORC1 activity in amino acid starved HEK cells with gene editing for multiple *SZT2* variants. Representative flow cytometry plots from *SZT2* missense variants tested using the pooled method. GFP = signal from Alexa488 conjugated anti P-S6.

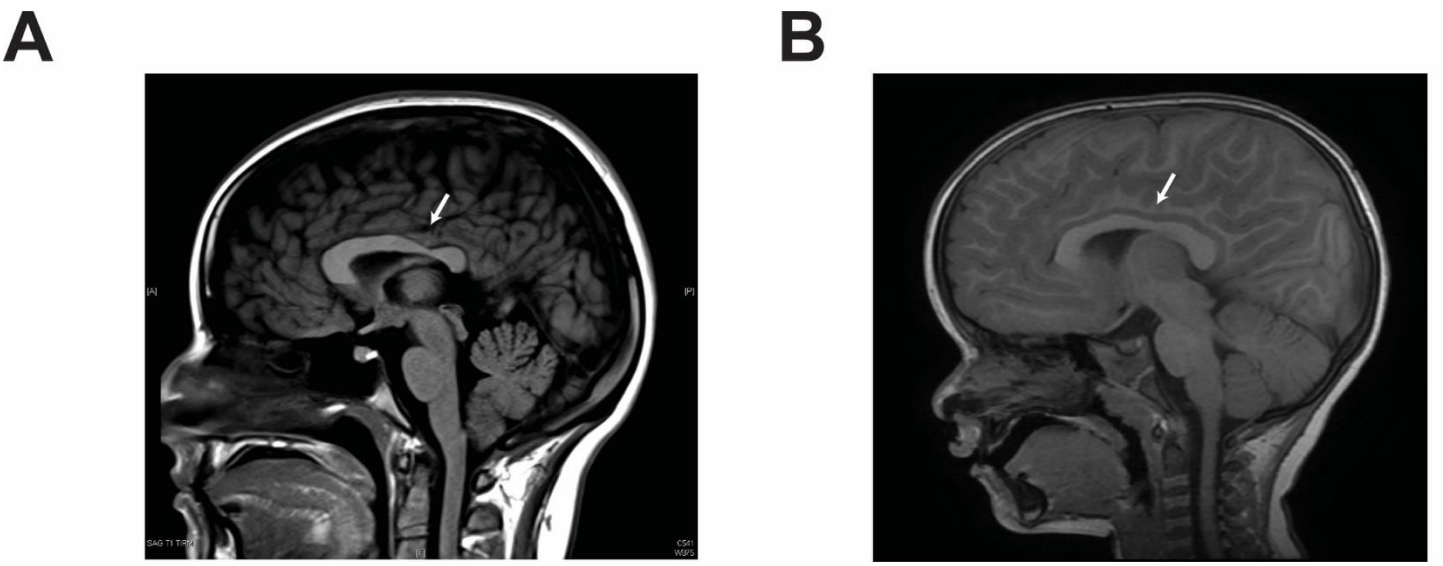

Figure S7: MRI images from individuals 10 and 11. Arrows indicating dysgenesis of corpus callosum in individual 11 (A) and possible corpus callosum abnormality (upper limit of normal) in individual 10 (B).

Table S1: *SZT2* gRNAs and primers.

Primer seq (5' to 3')	**Name**	**Purpose**	**HDR/patient allele**
CACCGGTGGCAGCCAGATGAACCAG	SZT2_ex3s	gRNA pair	$SZT2^{KO/KO}$ (exon 3)
AAACCTGGTTCATCTGGCTGCCACC	SZT2_ex3as		
CACCGTTTCTGGAACACGCTGCAG	SZT2_cG1496Ts	gRNA pair	*SZT2* c.1496G>T
AAACCTGCAGCGTGTTCCAGAAAC	SZT2_cG1496Tas		
CTCCCCAGCTCCCGCCTTTGCAAATCTGTCCCTC CATACAACAAATGGGGCAGCACTCATTGAGTGAT GACTTCACTGACATCTGCAGCGTGTTCCAGAAAC GCCGGATAACATGGGTACGATACAATGAACGAA TGGGCTGCCTTAGTGCACAGGACACA	SZT2_cG1496TssODN	Repair oligo	
TTGGAGGTAAAGCTGGTGCT	SZT2_cG1496TsurvF	Primer pair for T7eI assay / Sanger sequencing	
GTCAGGAAGCGTGAAATGCT	SZT2_cG1496TsurvR		
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGAGGTAAGGGTGGTGAGT	SZT2_cG1496TampSeq F	Primer pair for amplicon sequencing	
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA GCCTGTGGGTGTGTCCTCTGT	SZT2_cG1496TampSeq R		
CACCGTTCTCCTGTGACGTTGTGTG	SZT2_1984DELs	gRNA pair	*SZT2* p.Val1984del
AAACCACACAACGTCACAGGAGAAC	SZT2_1984DELas		
ATGCCAATCACATACCCCGAGAGACCCCCATGC TGGGCCCCATTTTGAGGCGTGAATGGACTCGGA TCACAGTTCCCCACACGTCACAGGAGAAATGGC CAGGGACAAACTGCATTGTGGCTGCCAGGTACC CACGGGGCGCACAGCTCTCATCAGCAGC	SZT2_1984DELssODN	Repair oligo	
TTGCCTGCCTTGATACCTCT	SZT2_1984DELsurvF		

TTTCCAGATCCTTCCAATGC	SZT2_1984DELsurvR	Primer pair for T7eI assay / Sanger sequencing	
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGGTGGTGTGTCCCATTT	SZT2_1984DELampSeqF	Primer pair for amplicon sequencing	
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA GATCCTTCCAATGCTCACCTG	SZT2_1984DELampSeqR		
CACCGAGACACATCTGCCTGCTGTG	SZT2_1447s	gRNA pair	*SZT2* p.Glu1447Ala
AAACCACAGCAGGCAGATGTGTCTC	SZT2_1447as		
GCGAGGCAGAGTCTAGCCCAGCAGGCCCCAGG TCTGATTCACGGCTCTCCCGGTATTCTACCTCTA GCTCTGGGTCACTCGCAGTGACGACACAGCAGG CAGATGTGTCTCCTAGTAGTGCATGTCACATGGT CAATGAGAGAGGCTGGCAGTGGAACCCT	SZT2_1447ssODN	Repair oligo	
CCTGTTGAGGGGTGACTGAC	SZT2_1447survF	Primer pair for T7eI assay / Sanger sequencing	
CACTTAGGCAGGCTGTTCCA	SZT2_1447survR		
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCGAGATTCAAGGGTTCCA	SZT2_1447ampSeqF	Primer pair for amplicon sequencing	
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA GGTCTACGTCTGACAGCGAGG	SZT2_1447ampSeqR		
CACCGTCCCTTCCACTCCCGTCAGC	SZT2_1948s	gRNA pair	*SZT2* p.Arg1948Gln
AAACGCTGACGGGAGTGGAAGGGAC	SZT2_1948as		
TCGGTAAAAGAAAGGGCGGCAGCACTGCCTTGT GAGGGAGGCTCCTGGGTGGGATCTCACCATCAC TGGGCAGTGGTGCCTGCTGACGGCTGTGGAAG GGAGTCTCACTGCGCCACAGATCTTCTTCACTCT CGGCCACCAGAAGAGAGTTACACACGTGG	SZT2_1948ssODN	Repair oligo	
GACCACCACCACCCACTTAG	SZT2_1948survF	Primer pair for T7eI assay / Sanger sequencing	
CCTTGCCCCCATCTTCCTTT	SZT2_1948survR		
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGACCTTCATGACAGCCACG	SZT2_1948ampSeqF	Primer pair for amplicon sequencing	
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA GACTCTGCCCTCAGCTTTCAC	SZT2_1948ampSeqR		
CACCGGCGAAATGCTCCCCGGCAG	SZT2_2589s	gRNA pair	*SZT2* p.Arg2589Trp
AAACCTGCCGGGGAGCATTTCGCC	SZT2_2589as		
ACGGAACCCCTCTTCCCACCCAGTCCCTGGCCC CATATTTACCTTCTTGTCCACAACCTCTAGTAGCA AGAGTCTCTGCCAGGGAGCATTTCGCCCTGAGC TCCCATCACCTCCTGGCTCGAAGCGCTGCATGG CTTTGGCAGCTACAGGGTGGGGGAGGG	SZT2_2589ssODN	Repair oligo	
AGATCTTCGGCCCTTGTTCC	SZT2_2589survF	Primer pair for T7eI assay / Sanger sequencing	
CCTTAGGGCCTCGTCAAAGG	SZT2_2589survR		
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATCTGTTCCCAGGCCCCTAT	SZT2_2589ampSeqF	Primer pair for amplicon sequencing	
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA GTGAGATCACGGAACCCCTCT	SZT2_2589ampSeqR		
CACCGAGTATGTGGCTATGGCACCC	SZT2_446s	gRNA pair	*SZT2* p.Pro446Ser
AAACGGGTGCCATAGCCACATACTC	SZT2_446as		
ACATCATGCAAAATGTCGTAGCCGCCTTCCATCG TCACTTCCACCCGTGTTACTCGAGGGCCCTCAG GCTCCAGGGGCCAGGAAGCCATAGCCACATACT	SZT2_446ssODN	Repair oligo	

CAATGCGCATGTTGTGTTTCCACAGCAGCACCA GCTTTACCTCCAATTGGGACCCTCCTT			
TTGGAGGTAAAGCTGGTGCT	SZT2_446survF	Primer pair for T7eI assay / Sanger sequencing	
GTCAGGAAGCGTGAAATGCT	SZT2_446survR		
AGGTAGGTGGGGGTTTCAGA	SZT2_446ampSeqF	Primer pair for amplicon sequencing	
ATGCAAAATGTCGTAGCCGC	SZT2_446ampSeqR		
CACCGGACAGCACTGGCTTCCAGT	SZT2_560s	gRNA pair	*SZT2* p.Ala560Ser
AAACACTGGAAGCCAGTGCTGTCC	SZT2_560as		
CAGGATTAGCACCAGGCGATGCATGTGCAGCCA TCGCTGCCAGGAATTTGCATCCATGGACAGCAC TGGCTTCCAGTAGCTAGCAAACTGGGCATGGGA TGAGTCAGAACCACTGGGCTGGAGGGAGAGCAC CTGAGGACAGGAAGGACAGCACTATAGGA	SZT2_560ssODN	Repair oligo	
TTCCTGTCCTCAGGTGCTCT	SZT2_560ampSeqF	Primer pair for amplicon sequencing	
CCCTTCCCATCATTCCCTAT	SZT2_560ampSeqR		
CACCGCAGCCCTGGCATGGTACCTA	SZT2_2185s	gRNA pair	*SZT2* p.Arg2185Trp
AAACTAGGTACCATGCCAGGGCTGC	SZT2_2185as		
GTGCCATCTCACTTGGAAGTGGTTCCGGCTGTT GCTATCTGTGTACTTGGGAGAGTGCAGGAAGAT GAGCAAGTTCTGCCATAAGTACCATGCCAGGGC AGGAAGCCAGGGCCTGCAGGAGGTGGGTAGGG CCAGATGCAGCACCTCTGAGGGGAGACTTC	SZT2_2185ssODN	Repair oligo	
CATTCAGCTCTGCCCTCTTC	SZT2_2185ampSeqF	Primer pair for amplicon sequencing	
TGAGTGCCATCTCACTTGGA	SZT2_2185ampSeqR		
CACCGTTTCAGAGCACCCGTGTCCC	SZT2_1347s	gRNA pair	*SZT2* p.Arg1347His
AAACGGGACACGGGTGCTCTGAAAC	SZT2_1347as		
GGGCACCATGGGCCTCTCCTCTGAGCTGGCAGA CATCTGTCAGAGAGATCTCTGGCCCAGGGTCTG GAATGCCAGGGACATGGGTGGACTGAAACTCAG GCTCGCTTAGGTCCTCGGTCTCTATGCTGAATG GTTGGGGTAAGGATGTTGAAGTGTTAAGA	SZT2_1347ssODN	Repair oligo	
CCAGTTGAGAGGCAGGCTAC	SZT2_1347ampSeqF	Primer pair for amplicon sequencing	
CACCTGGATGACGCTATGAA	SZT2_1347ampSeqR		
CACCGGTTCAGAGCCAGAGATCTT	SZT2_2530s	gRNA pair	*SZT2* p.Ile2530Leu
AAACAAGATCTCTGGCTCTGAACC	SZT2_2530as		
CCTTCCCAGAAGCAGCAGATGCCGCTCAGCTGC AGGGCGGGGAGAGGGGCCCAGTTGCCCAGGGG AACAAGGGCCGAAGAGCTCTGGCTCACTACCCC TATTGAAAGGGAGGACAGACAGAAAGGCCATCC ATGACACCACTCCCTGGCCTAGCCTTCACC	SZT2_2530ssODN	Repair oligo	
CCAAACCTGAGGAAGCTGAG	SZT2_2530ampSeqF	Primer pair for amplicon sequencing	
CCTCCACTGCAAGAAGTTCC	SZT2_2530ampSeqR		
CACCGCGGACAACACAGCTAGAAGA	SZT2_2449s	gRNA pair	*SZT2* p.Arg2449Gln
AAACTCTTCTAGCTGTGTTGTCCGC	SZT2_2449as		
TAAACTCCATCCAGCGCTGAGCAACACGGGCAA ACACAGGATGAAGGGTCCCCACCTCACCCTCTT CTAACTGAGTTGTCTGGCGCCTGAAGACAGTGG GAGTAAGAGCCTCATGTCCATACATGACACTTGT ATGTTTATACAGACCAAGGCCCATCTGT	SZT2_2449ssODN	Repair oligo	
TGGAGCCCAGAGACAAAAGT	SZT2_2449ampSeqF	Primer pair for amplicon sequencing	
GGGGTCTCACCAATCTGAAC	SZT2_2449ampSeqR		

CACCGGCCCCTTCCACTTCCATCA	SZT2_1253s	gRNA pair	*SZT2* p.Arg1253Cys
AAACTGATGGAAGTGGAAGGGGCC	SZT2_1253as		
TTCTTGTAGTAGCTCCTCACAGGCATCTACCGCT GTCAGCAAATCCTGGGAGGTCACACTCTGTGCT TGCTGCAAGCTGCAGAATAGTCCTGATGGAAGT GGAAGGGGCCATGAGGCTTGAGCTCTGGAATGG CCTCTCCAGTCCCCACCCTGTAATCCCT	SZT2_1253ssODN	Repair oligo	
ATTCCAGAGCTCAAGCCTCA	SZT2_1253ampSeqF	Primer pair for amplicon sequencing	
GCTGAGAGGACCAGGACTTG	SZT2_1253ampSeqR		
CACCGGCAGAGCTTCTGGACACTG	SZT2_2483s	gRNA pair	*SZT2* p.Ser2483Leu
AAACCAGTGTCCAGAAGCTCTGCC	SZT2_2483as		
AGGTGACCAGTGCGGTGAACTCAGACAGGATGG ATGGAAGGAGGAACCGGGACACCATGTGGGCA GAGCTTCTGGACACTAATGCACAACCTAGGAGG CAGAGGAGTCACTGTGGAGCTGCAGGATGGAGA CCAGGCTGGGGTTGAGGGATGGGAGGGGAG	SZT2_2483ssODN	Repair oligo	
CGCTGGATGGAGTTTATGGT	SZT2_2483ampSeqF	Primer pair for amplicon sequencing	
CAAATGCTGCTCAAAGATGC	SZT2_2483ampSeqR		

Abbreviations: s = sense, as = antisense, ssODN = single strand oligo donor, surv = surveyor, ampSeq = amplicon sequencing, F = forward, R = reverse. Amplicon sequencing primers were synthesized with standard Illumina adaptors.

Table S2: Antibodies.

Primary antibodies		**Secondary antibodies**	
Vendor information	**Binding condition**	**Vendor information**	**Binding condition**
Cell Signaling Technologies (CST) rabbit anti-phospho-S6K (108D2)	1:1000 overnight at 4 deg C (Western blot)	Abcam HRP-conjugated goat anti-rabbit (ab205718)	1:50,000 room temperature for 1 hr
CST rabbit anti-S6K (49D7)	1:1000 overnight at 4 deg C (Western blot)	Abcam HRP-conjugated goat anti-rabbit (ab205718)	1:50,000 room temperature for 1 hr
CST Alexa488 conjugated rabbit anti-phospho-S6 (#5018; D68F8)	1:50 30 min at 4 deg C (labeling for FACS)	N/A	N/A

Table S3: Percentage of alleles and constitutive mTOC1 activity score (CMAS) determined by amplicon sequencing in unsorted and P-S6 sorted cells.

Alleles (Replicate 1):	HDR (1)				LoF (1)			
Cell pool:	Unsorted	P-S6^{LOW}	P-S6^{HIGH}	CMAS	Unsorted	P-S6^{LOW}	P-S6^{HIGH}	CMAS
***SZT2* p.Glu1447Ala**	20.65	26.99	9.03	0.44	36.5	25.39	50.94	1.40
***SZT2* p.Arg1948Gln**	20.64	28.91	3.14	0.15	5.98	4.22	9.93	1.66
***SZT2* p.Arg2589Trp**	19.03	26.29	7.67	0.40	5.92	4.35	9.3	1.57
***SZT2* p.Val1984del**	25.91	29.17	25.56	0.99	40.17	26.34	43.77	1.09
***SZT2* p.Pro446Ser**	29.08	43.78	5.95	0.20	21.72	14.18	30.84	1.42
***SZT2* p.Ala560Ser**	22.05	ND	22.95	1.04	14.68	ND	15.92	1.08
***SZT2* p.Arg1253Cys**	25.7	ND	23.41	0.91	18.94	ND	18.71	0.99
***SZT2* p.Arg1347His**	16.68	ND	10.74	0.64	8.08	ND	15.11	1.87
***SZT2* p.Arg2185Trp**	15.69	ND	6.7	0.43	11.03	ND	13.75	1.25
***SZT2* p.Arg2449Gln**	11.4	ND	6.38	0.56	11.75	ND	33.68	2.87
***SZT2* p.Ile2530Leu**	26.24	ND	15.95	0.61	17.31	ND	29.03	1.68
***SZT2* p.Ser2483Leu**	14.12	ND	6.65	0.47	59.71	ND	68.15	1.14
Alleles (Replicate 2):	**HDR (2)**				**LoF (2)**			
Cell pool:	**Unsorted**	**P-S6^{LOW}**	**P-S6^{HIGH}**	**CMAS**	**Unsorted**	**P-S6^{LOW}**	**P-S6^{HIGH}**	**CMAS**
***SZT2* p.Glu1447Ala**	22.5	26.27	9.44	0.42	31.26	24.87	50.37	1.61
***SZT2* p.Arg1948Gln**	17.61	27.34	3.42	0.19	7.45	6.31	9.76	1.31
***SZT2* p.Arg2589Trp**	14.75	24.69	9.76	0.66	5.76	3.78	8.57	1.49
***SZT2* p.Val1984del**	23.76	34.29	20.67	0.87	39.15	28.93	42.88	1.10
***SZT2* p.Pro446Ser**	29.51	43.68	9.18	0.31	20.59	14.46	30.17	1.47
***SZT2* p.Ala560Ser**	13.9	ND	13.52	0.97	16.44	ND	17.98	1.09
***SZT2* p.Arg1253Cys**	17.99	ND	14.31	0.80	25.48	ND	21.51	0.84
***SZT2* p.Arg1347His**	16.29	ND	4.83	0.30	14.62	ND	20.74	1.42
***SZT2* p.Arg2185Trp**	10.55	ND	3.89	0.37	15.04	ND	16.98	1.13
***SZT2* p.Arg2449Gln**	8.45	ND	2.43	0.29	30.94	ND	41.9	1.35
***SZT2* p.Ile2530Leu**	24.6	ND	9.18	0.37	24.58	ND	38.98	1.59
***SZT2* p.Ser2483Leu**	9.88	ND	3.16	0.32	68.78	ND	75.95	1.10
Alleles (Replicate 3):	**HDR (3)**				**LoF (3)**			
Cell pool:	**Unsorted**	**P-S6^{LOW}**	**P-S6^{HIGH}**	**CMAS**	**Unsorted**	**P-S6^{LOW}**	**P-S6^{HIGH}**	**CMAS**
***SZT2* p.Glu1447Ala**	25.11	30.1	7.09	0.28	35.31	25.85	58.62	1.66
***SZT2* p.Arg1948Gln**	ND	ND	ND	ND	ND	ND	ND	ND
***SZT2* p.Arg2589Trp**	22.09	31.4	8.54	0.39	6.36	3.87	9.23	1.45
***SZT2* p.Val1984del**	25.51	35.66	22.21	0.87	45.71	33.24	49.58	1.08
***SZT2* p.Pro446Ser**	ND	ND	ND	ND	ND	ND	ND	ND
***SZT2* p.Ala560Ser**	12.84	ND	12.47	0.97	14.85	ND	16.99	1.14
***SZT2* p.Arg1253Cys**	18.99	ND	17.95	0.95	21.89	ND	21.8	1.00
***SZT2* p.Arg1347His**	16.93	ND	10.78	0.64	12.85	ND	15.7	1.22
***SZT2* p.Arg2185Trp**	10.05	ND	3.23	0.32	14.73	ND	16.13	1.10
***SZT2* p.Arg2449Gln**	8.4	ND	3.61	0.43	27.25	ND	34.56	1.27
***SZT2* p.Ile2530Leu**	23.63	ND	11.86	0.50	25.14	ND	34.79	1.38
***SZT2* p.Ser2483Leu**	6.84	ND	2.88	0.42	69.12	ND	72.58	1.05

Abbreviations: Ref= Reference. For LoF variants, percentage of only the most abundant variant is reported. ND=not determined.

CMAS = % alleles in P-S6^{HIGH} / % alleles in unsorted cells. For example, for *SZT2* p.Ala560Ser replicates 1-3: (1) 22.95/22.05 = 1.04, (2) 13.52/13.90 = 0.97, and (3) 12.47/12.84 = 0.97.

Table S4: Seizure characteristics and epilepsy diagnosis in individuals with biallelic *SZT2* variants

Affected individual	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Diagnosis	DEE	DEE	DEE	Suspected neonatal seizure	DEE	Infantile epilepsy	DEE	Infantile epilepsy	Infantile epilepsy	Suspected neonatal seizure	Focal epilepsy	DEE
Seizure onset	2 y	2 m	4 y	3 y	2 DOL	2 y	No seizures*	9 m	3 y	3 DOL	20 y	6 y
Current Age, Sex	5y, M	8y, M	6y, F	10y; M	7y, M	5y9m; F	5y10m; M	10y; F	5y, M	6y; M	23y, F	9y; M
Febrile Seizures	Yes	No	No	Unk	No	No	No	Yes	Yes	Unk	N	No
Seizure type(s)	Focal Impaired Awareness Focal to bilateral tonic-clonic	Focal Impaired Awareness Focal to bilateral tonic-clonic	Focal Impaired Awareness Focal to bilateral tonic-clonic	GTC	Focal Impaired Awareness Focal to bilateral tonic-clonic	GTC (rare)	NA	Atonic Absence Focal motor GTC	Absence GTC	GTC	Focal Impaired Awareness Focal to bilateral tonic-clonic	Myoclonic Absence GTC
Current seizure control	Intractable	Intractable	Well controlled	Seizures resolved without medication	Intractable	Well controlled	N/A	Well controlled	Well controlled	Seizures resolved without medication	Partial control	Intractable
History of status epilepticus	Yes	Yes	Yes	N/A	Yes	No	N/A	No	No	N/A	No	No
Effective Therapies	CLB, OXC	CBD, ESL, LEV, ZNS, VNS (partially effective)	CLB, VPA	N/A	BRV, LCS, VPA	OXC	N/A	CLB, LEV	LEV	N/A	LEV	CLB, VPA
Ineffective Therapies	VPA	ACZ, BRV, CLB, CLZ, RFM, LCS, LTG, OXC, PER, PB	LEV	N/A	ACZ, CBD, ESM, FBM, RFM, LCS, LEV, OXC, TPM, PHT, CLB, VPA	N/A	N/A	CLZ, LTG, TPM	OXC	N/A	N/A	N/A
Other ineffective therapies	N/A	KD	N/A	N/A	KD, MPN, VNS	N/A	N/A	N/A	N/A	N/A	Epidermoid cyst resection	N/A

ACZ, acetazolamide; BRV- brivaracetam; CBD, cannabadiol; CLB, clobazam; CLZ, clonazepam; DOL – day of life; ETX, ethosuximide; ESL, eslicarbamazepine; FBM, felbamate; GTC, generalized tonic clonic seizure (or presumed generalized); KD, ketogenic diet; LEV, levetiracetam; LCS, lacosamide; LTG, lamotrigine; m, month;, MPN, methylprednisolone; N/A, not applicable; OXC, oxcabarbazepine; PER, perampanel; PB, phenobarbital; PHT, phenytoin; RFM, rufinamide; Unk, uknown; VNS, Vagal Nerve Stimulator; VPA, valproate; TPM, topiramate; y, years; ZNS, zonisamide. Colors mark individuals within reclassification groups; red = Pathogenic (P) or likely pathogenic (LP) / P or LP, yellow = P or LP / benign or likely benign (BLB), green = BLB/BLB.

* This patient did not show evident motor seizures but his EEG was characterized by severe abnormalities consistent with encephalopathy (slow abnormalities and centro-parietal irritative elements)

Table S5 Developmental History and Other Features in individuals with biallelic *SZT2* variants

Affected individual	1	2	3	4	5	6	7	8	9	10	11	12
Developmental delay /regression	DD: Walked at 21 m (requires AFOs), Non-verbal (uses sign-language)	DD: Walked at 3 y (requires walker), Non-verbal	DD: Walked at 15 m independently, Minimally verbal (uses communication device)	DD: Motor and speech regression reported during first year. Does not sit or talk, no eye contact	DD with regression at 7 y Walked at 2y but unable to walk after 7y, Non-verbal (uses sign language)	DD: Motor & speech regression at 2y4m. No crawling, no walking, only sitting & holding upright with assistance Non-verbal	DD: Non-verbal, Unable to walk, feeding issues	DD: Walked at 2y, in main-stream school with some assistance	DD: Independent steps at 20m, fine motor delay, Non-verbal at 20m	DD: Not walking at 2.5 y, global DD with cognitive impairment	No: Normal development	Developmental regression (speech& motor). Normal walking, Speech & cognitive challenges noted at 12m
Other neurological features	ASD	Hypotonia, ASD	ASD	Mixed muscle tone Central hypotonia, hypertonia of mainly the left upper limb No social contact	Hypotonia, hypertonia / spasticity, Unilateral conductive hearing loss, Self-injurious behavior	Stereotyped behavior	Hypotonia, Stereotyped movements	ADHD suspected	Hypotonia, Astigmatism, and amblyopia, Poor social interaction	Hypotonia	Epidermoid cyst (seizures persisted after resection), Depression	Intellectual disability (IQ 63)
Head circumference	Macrocephaly (>98th %ile)	Macrocephaly (97.3 %ile)	Macrocephaly (100%ile)	Microcephaly (-3 SD)	Macrocephaly (>98th %ile)	Macrocephaly, (>99th %ile)	Microcephaly (OFC 47 cm at 4 y, -2.11 SDS)	Normal	Normal - 52cm at 3y 11m	Macrocephaly At 30 m 56 cm +4.5SD	Macrocephaly as child; Normal as adult (94 %ile)	Normal 52 cm at 7y10m (41 %ile)
Dysmorphic features	None noted	Frontal bossing, Downslanted palpebral fissures, Hypertelorism (mild)	Wide nasal bridge	Down slanting palpebral fissures	Frontal bossing (mild), Flattened nasal bridge, Low set ears	Frontal bossing	Narrow forehead, Plagiocephaly, Hypotelorism, Single palmar crease	None noted	Frontal bossing, High arched palate	None noted	Large forehead, Arched eyebrows	Protruding ears, Upslanting palpebral fissures, Thick eyebrows, Diastema
Dysgenesis of the corpus callosum	No	No	No	No	No	Yes - Slight narrowing of trunci corpori callosi	No	No	No*	Yes - Thick corpus callosum (See Fig S7A)	Yes – Dysgenesis (See Fig S7A)	No
Other MRI findings	Non-specific diffusion restriction in the bilateral cerebellar hemispheres	Right PVNH & abnormal perisylvian gyral configuration; Pineal cyst (13mm); Small pars intermedia cyst	None	Ischemic changes (s/p premature delivery and hemorrhage)	bilateral & multifocal areas of MCD involving both hemispheres with the appearance of PMG and/or associated deep sulci. particular	No	Narrowing of the middle third of the Sylvian aqueduct (mild), Myelination delay (mild)	Normal	None	None	Bilateral PVNH Epidermoid cyst	None

Affected individual	1	2	3	4	5	6	7	8	9	10	11	12
					frontal involvement							

Abbreviations: AFO, Ankle foot orthotics; ADHD, attention deficit hyperactivity disorder; ASD, Autism spectrum disorder; DD, developmental delay; m, months; mm, milimieters; MCD, malformations of cortical development; MRI, Magnetic Resonance Imaging; PMG, polymicrogyria; PVNH, periventricular nodular heterotopia; y, years. Colors mark individuals within reclassification groups; red = Pathogenic (P) or likely pathogenic (LP) / P or LP, yellow = P or LP / benign or likely benign (BLB), green = BLB/BLB.
* Noted as upper limit of normal'

REFERENCES

1. Wolfson RL, Chantranupong L, Wyant GA, et al. KICSTOR recruits GATOR1 to the lysosome and is necessary for nutrients to regulate mTORC1. Nature. 2017;543(7645):438-442.