Thomas Cullup, Ay L. Kho, Carlo Dionisi-Vici, Birgit Brandmeier, Frances Smith, Zoe Urry, Michael A. Simpson, Shu Yau, Enrico Bertini, Verity McClelland, Mohammed Al-Owain, Stefan Koelker, Christian Koerner, Georg F. Hoffmann, Frits A. Wijburg, Amber E. ten Hoedt, Curtis Rogers, David Manchester, Rie Miyata, Masaharu Hayashi, Elizabeth Said, Doriette Soler, Peter M. Kroisel, Christian Windpassinger, Francis M. Filloux, Salwa Al-Kaabi, Jozef Hertecant, Miguel Del Campo, Stefan Buk, Istvan Bodi, Hans-Hilmar Goebel, Caroline A. Sewry, Stephen Abbs, Shehla Mohammed, Dragana Josifova, Mathias Gautel, Heinz Jungbluth

Recessive mutations in *EPG5* cause Vici syndrome, a multisystem disorder with defective autophagy

SUPPLEMENTARY FILES

F	Pat	ACC	CNS[1]	Seizures	Cataracts[2]	Sensorineural Deafness	Cardiac involvement[3]	Hypopigmentation	Immunodeficiency[4]	Myopathy	Other features	Outcome[5]
1	**1.1**	ND	ND	+	+	ND	+	+	+	ND	Microcephaly, nystagmus, cleft lip, hypospadias, FTT, thymus aplasia	Died 2y
	1.2	+	+	+	+	ND	+	+	+	ND	Microcephaly, Cleft lip and palate, thymus hypoplasia	Died 3y
2	**2.1**	+	+	-	+	+	+	+	+	+	Microcephaly, Nystagmus, obstructive sleep apnoea	Alive 3y
3	**3.1**	+	+	-	+	+	+	+	+	+	Nystagmus	Died 3m
4	**4.1**	+	+	+	+	ND	-	+	+	+	PFO, hepatomegaly, liver dysfunction	Alive 3m
5	**5.1**	+	-	+	+	ND	+	+	+	ND	-	Alive 4y
	5.2	+	-	-	+	-	+	+	+	ND	-	Alive 3y
6	**6.1**	+	-	-	+	ND	+	+	+	+	Renal impairment, Chronic anaemia	Died 9m
7	**7.1**	+	-	+	+	+	+	+	+	+	-	Alive 3y
8	**8.1**	+	+	+	+	ND	+	+	+	ND	Thymus hypoplasia, thyroid agenesis, Hypothyroidism	Died 8y
	8.2	+	+	+	+	ND	+	+	+	ND	2^{nd}-3^{rd} toe syndactyly, scoliosis	Died 8y
9	**9.1**	+	+	+	+	ND	+	+	+	+	Pulmonary hypoplasia	Died 9m
10	**10.1**	+	+	+	-	ND	+	+	+	ND	Microcephaly, Nystagmus, renal tubular acidosis, anaemia	Died 1y
11	**11.1**	+	-	-	+	-	+	+	+	+	Liver dysfunction	Died 2y
12	**12.1**	+	+	+	+	ND	+	+	+	+	-	Alive 2y
13	**13.1**	+	+		+	ND	+	+	+	ND	High arched palate, 2^{nd}-3^{rd} toe syndactyly	Alive 2y
14	**14.1**	+	-	-	+	ND	-	+	+	ND	Microcephaly, PFO, ASD, hydronephrosis	Alive 10y
15	**15.1**	+	-	-	+	ND	-	+	+	ND	Cleft palate	Alive 4y

Supplementary Table 1

SUPPLEMENTARY TABLE 1

Clinical features in patients with Vici syndrome. Patients were included in the study if 4 of the 5 major diagnostic criteria (callosal agenesis, cataracts, cardiomyopathy, hypopigmentation and immunodeficiency) were fulfilled (for review,[5]). 1) Additional CNS abnormalities included (in order of frequency) cerebellar and pontine hypoplasia, paucity of white matter and ventricular dilatation, heterotopias, abnormalities of the septum pellucidum, and schizencephaly. 2) Cataracts were reported as central lens opafications in patient 13.1, but no specific information regarding the nature and location of cataracts was available in other patients. 3) 15/18 patients had evidence of a cardiomyopathy, based on the presence of marked cardiomegaly on chest X-ray, and/or specific findings on cardiac US, and/or findings post mortem examination. Some patients were historical cases who presented at a time where cardiac US was not routinely performed, or had died before a more detailed cardiac assessment had been performed. Specific findings suggested a dilated cardiomyopathy with left ventricular emphasis in patients 1.2, 2.1, 8.2, 10.1, whereas findings in patients 3.1, 9.1, 11.1 and 13.1 were suggestive of a hypertrophic cardiomyopathy, again with left ventricular emphasis. Interestingly, cardiac ultrasound in patient 8.1 suggested a hypertrophic cardiomyopathy, whereas findings on post mortem in the same patients were suggestive of a dilated cardiomyopathy. In some patients, cardiac assessment was normal when done very early in life but abnormal findings evolved over time. Patient 12.1 had evidence of a transient cardiomyopathy during an intercurrent illness that appeared to have resolved on subsequent cardiology follow-up. 4) In most patients the suspicion of an associated immunodeficiency was based on clinical findings of an increased number and/or unusual types of infection. Findings were suggestive of a combined immunodeficiency in those patients (1.1, 1.2, 2.1)[1,8] who had the most detailed immunological assessments. 5) In our cohort, Vici syndrome presented as a severe condition with only half of all patients still alive at the time of last

follow-up. The most common causes of death were progressive cardiac failure and recurrent infections. The oldest survivor, a girl without confirmed *EPG5* mutation and no cardiac involvement (Patient 14.1), was 10 years, whereas the oldest genetically confirmed case (Patient 5.1) was 4 years at the last follow-up. None of our patients had achieved independent ambulation. ACC = agenesis of the corpus callosum; PFO = persistent foramen ovale; ASD = atrial-septal defect; FTT = failure to thrive; + = feature present, - = feature absent, ND = not determined.

Patient	2	4	5.1	5.2
Total mapped reads	61375961	65964183	61538769	61771882
Reads mapped to target	35130476	41436491	34807833	34788484
Percentage	57.24	62.82	56.56	56.32
Reads mapped to target +/-150bp	40445422	48862241	40235007	41517629
Percentage	65.9	74.07	65.38	67.21
Mean Coverage	64.24	73.62	63.34	62.37
Target bases	27812282	27812282	27812282	27812282
Target bases at 1x coverage	27226331	27161438	27159756	27140738
- Percentage	97.89	97.66	97.65	97.58
Target bases at 5x coverage	26367664	26238303	26249885	25986174
- Percentage	94.81	94.34	94.38	93.43
Target bases at 10x coverage	25295211	25103044	25073500	24522650
- Percentage	90.95	90.26	90.15	88.17
Target bases at 20x coverage	22766150	22750565	22539027	21693999
- Percentage	81.86	81.8	81.04	78

SUPPLEMENTARY TABLE 2

Data output from the KCL/Linux pipeline.

	NextGene		Linux	
	P5.1	P5.2	P5.1	P5.2
Total variants called	19103	18459	19963	19375
Novel variants	2545	2558	684	664
- Novel non-synonymous variants	1920	1939	485	469
Total shared non-synonymous variants	990		-	
Total genes with 2x novel variants	122		25	
- Compound heterozygous	113		14	
- Homozygous	9		11	
Total shared homozygous genes		5		

SUPPLEMENTARY TABLE 3

Numbers of variants detected by NextGene/Linux pipelines for Patients 5.1 and Patient 5.2

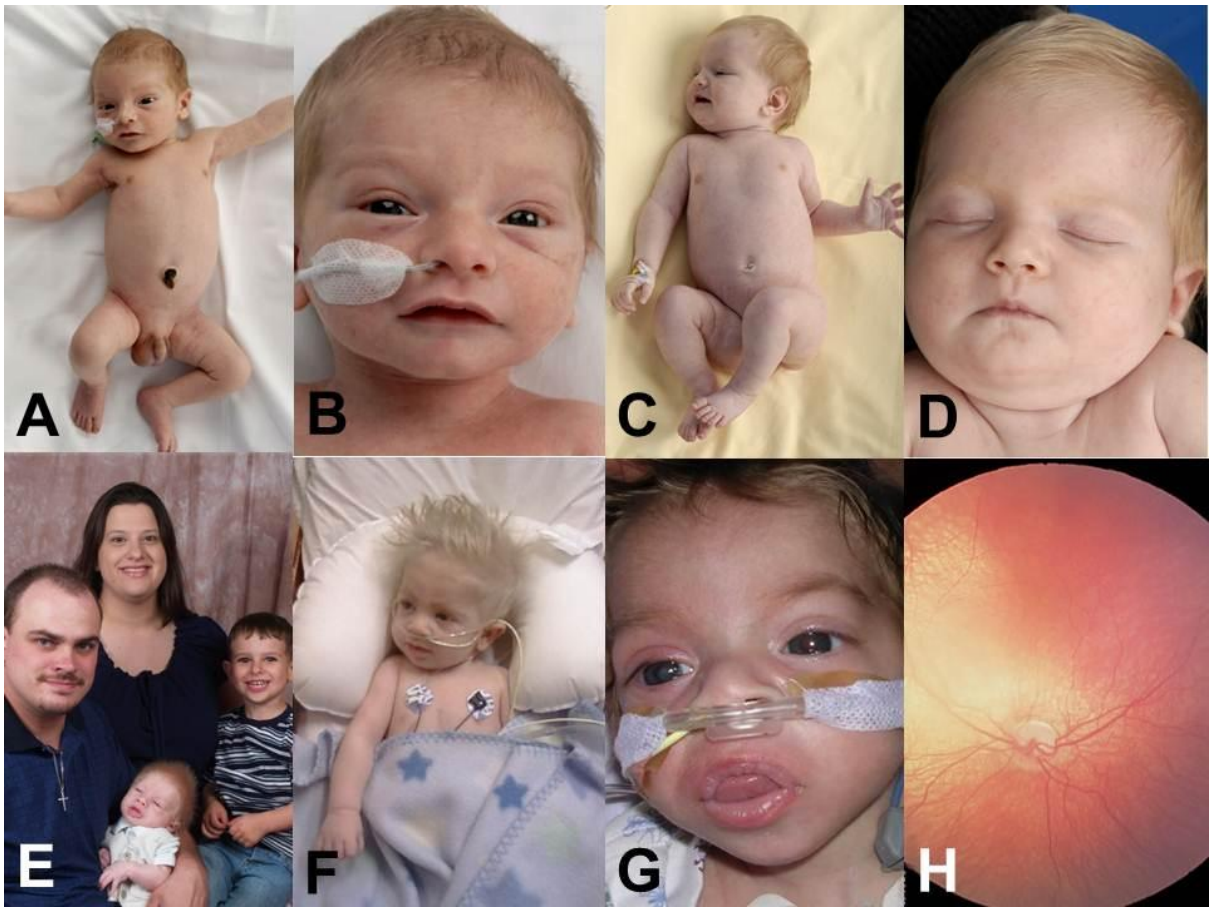
KIAA1632 / EPG5 Sequencing primers	
KIAA1632_Ex01_F_T1_v1	GGAGTGGTTTGTCCTACGTT
KIAA1632_Ex01_R_T1_v1	ATCAAATATTGGAAGGTGGAG
KIAA1632_Ex02_1_F_T1_v1	CACATTTCTGTTTCATATTTGTTC
KIAA1632_Ex02_1_R_T1_v1	ACCGACATATTTTCCTCTACCT
KIAA1632_Ex02_2_F_T1_v1	ATAAGCAATGAAGAGTCCCTGA
KIAA1632_Ex02_2_R_T1_v1	GGTACTAGTTCCAGTTGAGACG
KIAA1632_Ex02_3_F_T1_v1	TGGTTTGTTCTTCAGAGGTG
KIAA1632_Ex02_3_R_T1_v1	TGATCCCTGCACTCTATTACTC
KIAA1632_Ex03_F_T1_v1	TCCTCACCATGATTATAAGCTC
KIAA1632_Ex03_R_T1_v1	AAATGGTACCTTGTAAGACCAA
KIAA1632_Ex04_F_T1_v1	AACTTTTCTCCTTTTATCTGCAC
KIAA1632_Ex04_R_T1_v1	TCATCAAATAAATTAGCTGAAAAA
KIAA1632_Ex05_F_T1_v1	TAGATTTGTAGGCCTCGTAGAA
KIAA1632_Ex05_R_T1_v1	TAGCCCAACAAGTTTCAGTCTA
KIAA1632_Ex06_F_T1_v2	GGCTGTGCTTTACTCTTGTGT
KIAA1632_Ex06_R_T1_v2	CAGTTGAAGGGTCACTTTGA
KIAA1632_Ex07_F_T1_v2	GCAGTTTTATTAGCCTTGGTTT
KIAA1632_Ex07_R_T1_v1	AAGTCTTTACCTCATGGAGTTG
KIAA1632_Ex08_F_T1_v2	CCATTGAGACCTGTGATAATTT
KIAA1632_Ex08_R_T1_v2	GCCACTACCACATTTTACACTT
KIAA1632_Ex09_F_T1_v1	GAGACTGTCAGTTCTTCTCCAC
KIAA1632_Ex09_R_T1_v1	CCTGAGGTTTACAACTAAAACG
KIAA1632_Ex10_F_T1_v1	TCAGAACCTGGACAATAGTAGG
KIAA1632_Ex10_R_T1_v1	GAGAGAGAGACCATTTCAGTCA
KIAA1632_Ex11_F_T1_v1	GGTGGTTTGTATTTGCTGTTAT
KIAA1632_Ex11_R_T1_v1	ATCAAAACTGTGAAGCAGTCTC
KIAA1632_Ex12_F_T1_v1	ATTTTGGAACAAAGAAAGACCT
KIAA1632_Ex12_R_T1_v1	TCCCTGTAGCAAAATAATGTTC
KIAA1632_Ex13_F_T1_v1	TGTAAGATTCTTTCTGCTTTGC
KIAA1632_Ex13_R_T1_v1	GCTGGCACAGTAAATGTTAGTT
KIAA1632_Ex14_F_T1_v1	AATTAAACAAAAACTCCAGTTCC
KIAA1632_Ex14_R_T1_v1	GCAAATGGTACTCAGAAATGTT
KIAA1632_Ex15_F_T1_v1	GGAACATTTAGCTTCTACATCG
KIAA1632_Ex15_R_T1_v1	TCTTTATCAAACCATAGAAGTCAA
KIAA1632_Ex16_F_T1_v1	GGTCACAAAAGGAGATGAGTGT
KIAA1632_Ex16_R_T1_v1	CTCCCCTTGATAAGCTGTTAAT
KIAA1632_Ex17_F_T1_v1	CTGAGATAACCCAAGGTATTGA
KIAA1632_Ex17_R_T1_v1	ATACGCTGTTGAAATAGCATTC
KIAA1632_Ex18_F_T1_v1	CTTAAATAGTGCTTTGGTCGAA
KIAA1632_Ex18_R_T1_v1	CAAAAGCACACTAAGCCAAG
KIAA1632_Ex19_F_T1_v1	GGGATTTGTAGTGGAGGTAGAT
KIAA1632_Ex19_R_T1_v1	TCTACGCTAGTGGACTACGATT
KIAA1632_Ex20_F_T1_v1	GCTTGTTACATTTGGCTGTAAT
KIAA1632_Ex20_R_T1_v1	ACCAAAGGGCTACTGTAAAGAT
KIAA1632_Ex21_F_T1_v2	GGTGTTAATTTTAGAAACTGCAA
KIAA1632_Ex21_R_T1_v2	AAGTATTGGTTTGGTTGTTGAG
KIAA1632_Ex22_F_T1_v1	AGAATAAAAAGCTGCCCTACAT
KIAA1632_Ex22_R_T1_v1	AGAATGATTCCAACTCACACTC
KIAA1632_Ex23_F_T1_v1	TGGTGTATATCCACAATCACAG
KIAA1632_Ex23_R_T1_v1	CCACCTTCCATATTCAATCTAA
KIAA1632_Ex24_F_T1_v1	TTGGCCACATTTTCTGTATTAT
KIAA1632_Ex24_R_T1_v1	CATCTGAGTGACTACCCATTTT
KIAA1632_Ex25_F_T1_v1	GAGGACTTGGAGTAGACCACTT
KIAA1632_Ex25_R_T1_v1	GAAATCTCTTTTTAAAGCACCA
KIAA1632_Ex26_F_T1_v1	GGCCTGTCTAGGGTAATAATTT

KIAA1632_Ex26_R_T1_v1	TTGGTCAAACTATCCTCATTGT
KIAA1632_Ex27_F_T1_v1	TTATGTGTCTTTTGCTGCACTA
KIAA1632_Ex27_R_T1_v1	GGACTCATCTGGTACCTACAAA
KIAA1632_Ex28_F_T1_v1	TCCTACAAAAGGCTTTGAACTA
KIAA1632_Ex28_R_T1_v1	TAAAAATAAGTAGCAGGCCAGA
KIAA1632_Ex29_F_T1_v1	ACATTTTCATGGGTGCTCTG
KIAA1632_Ex29_R_T1_v1	TGGGTAAGACTCTGAACCTGTA
KIAA1632_Ex30_F_T1_v1	TTGGAAGCAATAGGCTTATCTA
KIAA1632_Ex30_R_T1_v1	GCTGTACCTGGAAACAAAACT
KIAA1632_Ex31_F_T1_v1	ATAAAATGCTGGGTAATTTTTG
KIAA1632_Ex31_R_T1_v1	CAAGCCCTTTAGATGACAATTA
KIAA1632_Ex32_F_T1_v1	GTTTTGGATTTTGGCTTTATTT
KIAA1632_Ex32_R_T1_v1	TAACACAAAGAAAAACACATGG
KIAA1632_Ex33_F_T1_v1	CCTACTATATACACCCCCATCA
KIAA1632_Ex33_R_T1_v1	CTTTCTCTCCTTGACACTCAAT
KIAA1632_Ex34_F_T1_v1	TGTAGCCAATGAAAACGTAAAC
KIAA1632_Ex34_R_T1_v1	ATGTCTTAAGTTTTGAAAAGAAGC
KIAA1632_Ex35_F_T1_v1	GGTGTTACAAGCTACAATTCG
KIAA1632_Ex35_R_T1_v1	TTCAGTCACAAATAACTGCCTA
KIAA1632_Ex36_F_T1_v1	GGTCAATCATGAGAACCTTAGA
KIAA1632_Ex36_R_T1_v1	ACACTGCCACTATGCCTAAC
KIAA1632_Ex37_F_T1_v1	AAGTGATATAATCTGGGGAAAAA
KIAA1632_Ex37_R_T1_v1	CTGGAAAAACAAATGAAAAACT
KIAA1632_Ex38_F_T1_v1	ACTGTTCTGTGCTTACCATAGG
KIAA1632_Ex38_R_T1_v1	TGATAATACACATCCTCCGACT
KIAA1632_Ex39_F_T1_v1	CCTGTTCTGTCCCCTCTACT
KIAA1632_Ex39_R_T1_v1	CTCTCTGACCATTGCTTCTCT
KIAA1632_Ex40_F_T1_v1	AGAACAGCTAAGATTGAAGCAC
KIAA1632_Ex40_R_T1_v1	ATTCAGAAACATCCACAACATT
KIAA1632_Ex41_F_T1_v1	TCTCATAGGTTGCTATGTCAAA
KIAA1632_Ex41_R_T1_v1	ATCTTTCCAAAACAACTGTCAC
KIAA1632_Ex42_F_T1_v1	CTTCAAACTCTGATGTGGACTT
KIAA1632_Ex42_R_T1_v1	GTCCAAACACAACAGGAGATAC
KIAA1632_Ex43_F_T1_v1	TGAACAACACAGTAGATCTGGA
KIAA1632_Ex43_R_T1_v1	GAAACCATTTTCCCGTAAGTAG
KIAA1632_Ex44_F_T1_v2	CCTTCTGGTTGTGGTTAAGTT
KIAA1632_Ex44_R_T1_v2	CTTAAAGAGTCCCCAGAAGGT
KIAA1632 / EPG5 cDNA sequencing primers	
KIAA1632_RNA_Primary_F	TATGTGAATCGTGAAGAACAGA
KIAA1632_RNA_Primary_R	AGTGAAGAAAGGAGACAGCAG
KIAA1632_RNA_Secondary_F	CAGTTGTCACGGTTCAGTTT
KIAA1632_RNA_Secondary_R	GGTTTCAAGTACTTTTCTGCAC
KIAA1632 / EPG5 qPCR primers and probe	
KIAA1632_qPCR_F	GCTGAAGTGGCTTTAATGGTTC
KIAA1632_qPCR_R	TTGGTTAAATGAGGTCTCGGG
KIAA1632_qPCR_Probe	CCAGCATATGGCTTCTGTGCAAGGTA
ACTB qPCR primers and probe	
ACTB_qPCR_F	ACCTTCTACAATGAGCTGCG
ACTB_qPCR_R	CCTGGATAGCAACGTACATGG
ACTB_qPCR_Probe	CAACCGCGAGAAGATGACCCAGAT
TMEM49/VMP1 (hEPG3)	
TMEM49_Ex02_F_T1_v1	AGTCACAGCTACACAGCAGAA

TMEM49_Ex02_R_T1_v1	TGACCCATAATTTAATTTACTAAGAAG
TMEM49_Ex03_F_T1_v1	ACTAGTTGCCATTTTTCACAAG
TMEM49_Ex03_R_T1_v1	AGAGGGCAGAAAGTAAGAGATT
TMEM49_Ex04_F_T1_v1	AGGTAACCTAACTGTTTCAGCA
TMEM49_Ex04_R_T1_v1	ACCACATATAAATCCTCAGCAG
TMEM49_Ex05_F_T1_v1	AAAGAAATTAGGCCTCTTCAAT
TMEM49_Ex05_R_T1_v1	TCAGAATGCTTACAAAACACAA
TMEM49_Ex06_F_T1_v1	GAAAGCTAAGATTTTTCTTACACAG
TMEM49_Ex06_R_T1_v1	AGGTAGCTGGTTACTGACTGAA
TMEM49_Ex07_F_T1_v1	ATATTGCATCTCAAAATGCTTC
TMEM49_Ex07_R_T1_v1	GGTTTCTGACAAGGTGTTTTAG
TMEM49_Ex08_F_T1_v1	TCATCAGATTGGGAGATCTTTA
TMEM49_Ex08_R_T1_v1	AGCACTTTTGTTAATTCAGGAG
TMEM49_Ex09_F_T1_v1	GCAGAATACCACATATCAATGG
TMEM49_Ex09_R_T1_v1	AAATAATTCACTTAGGAATGAGCA
TMEM49_Ex10_F_T1_v1	CCCCAGCTAATTTTTGTATTTT
TMEM49_Ex10_R_T1_v1	CAAGTTATTTTTGCTCCTCAAA
TMEM49_Ex11_F_T1_v1	TCCAGGTGGTAAGTACATTTTC
TMEM49_Ex11_R_T1_v1	GGCAAACCAACTTCACTTATT
TMEM49_Ex12_F_T1_v1	AGTAGTTGGGGTTGCTTACTTT
TMEM49_Ex12_R_T1_v1	TACAGGTTGGAAAAGGGAAT
EI24 (hEPG4) sequencing primers	
EI24_Ex02_F_T1_v1	CTCCATTATGTTCCATCTGTTT
EI24_Ex02_R_T1_v1	AAAAGTTCCAAAAACCCTAAAG
EI24_Ex03a_F_T1_v1	GATCATTTGAACCCAGGAGT
EI24_Ex03a_R_T1_v1	AAATCACTTGCAACATTTTTCT
EI24_Ex03b_F_T1_v1	CCCCACACTTTCTCTTAATCTT
EI24_Ex03b_R_T1_v1	ACGGCTGTAATCCTAACACTTT
EI24_Ex04_F_T1_v1	ACATTAGAACATTGGGAGAACA
EI24_Ex04_R_T1_v1	TCAGTGTTTCAAGAAAACAGTTC
EI24_Ex05_F_T1_v1	AAGTCACTCCCATCTATAAAATCA
EI24_Ex05_R_T1_v1	AAATAAACAGTGAAAGCTCCTG
EI24_Ex06_F_T1_v1	ACAGAGAATAGTCACGAGATGG
EI24_Ex06_R_T1_v1	GAAACATTAAGGCTGAAAAACA
EI24_Ex07_F_T1_v1	CTTTTCTTAACTGGCAGCTCTA
EI24_Ex07_R_T1_v1	TAAAAATCATTACCTCCCACCA
EI24_Ex08_F_T1_v1	CAGCTAGGAAGCTATCTCAGAC
EI24_Ex08_R_T1_v1	TTGAGAGTTAAACCCACTAAGC
EI24_Ex09_F_T1_v1	AGCAAGACTCCATCTCAAATAA
EI24_Ex09_R_T1_v1	CTGATGGACAAATGATGATGTA
EI24_Ex10_11_F_T1_v1	GTTGAGATCTTGCCACTGTACT
EI24_Ex10_11_R_T1_v1	TTTTAAAAAGCCTCACTGACAT
EI24_Ex12_F_T1_v1	TATTCTTTAGTTGCCTGTTCCA
EI24_Ex12_R_T1_v1	ATTCCTCAGAGAGAAGGGAATA

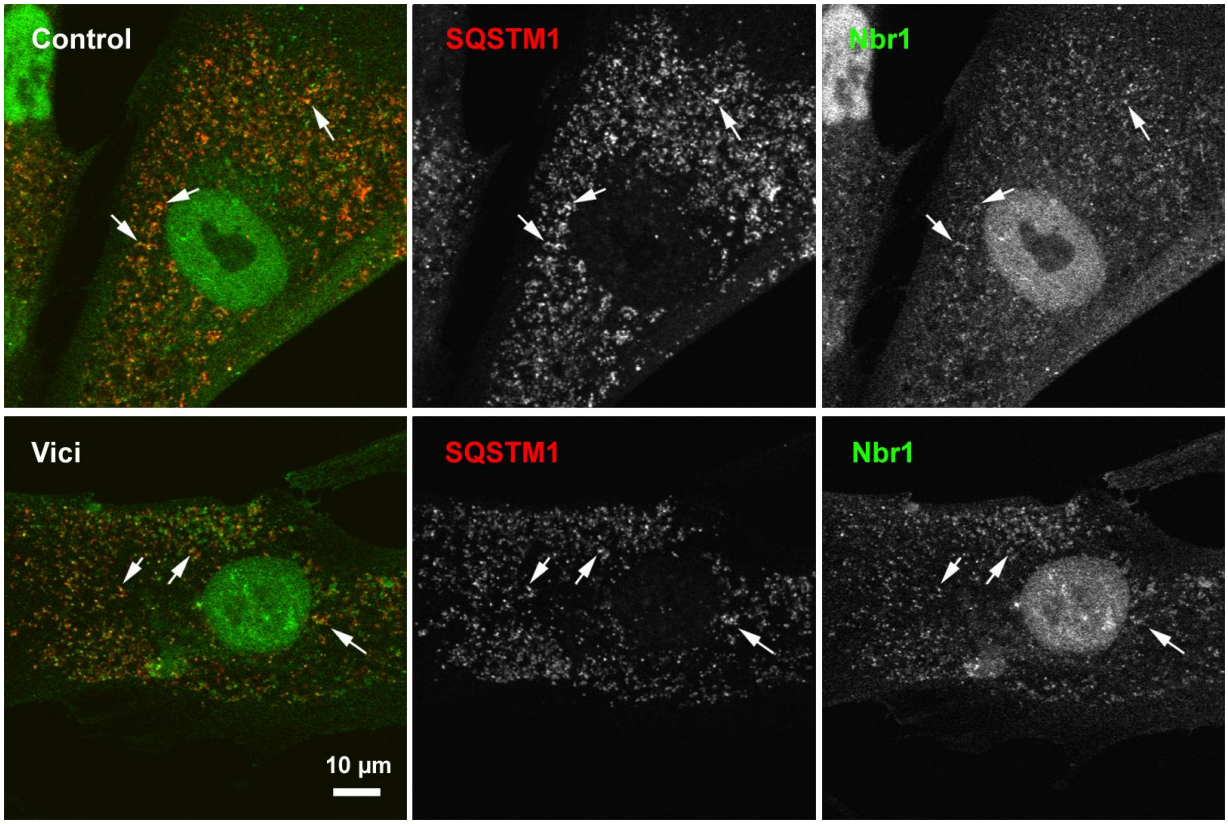
SUPPLEMENTARY TABLE 4

List of primer sequences used in this study.



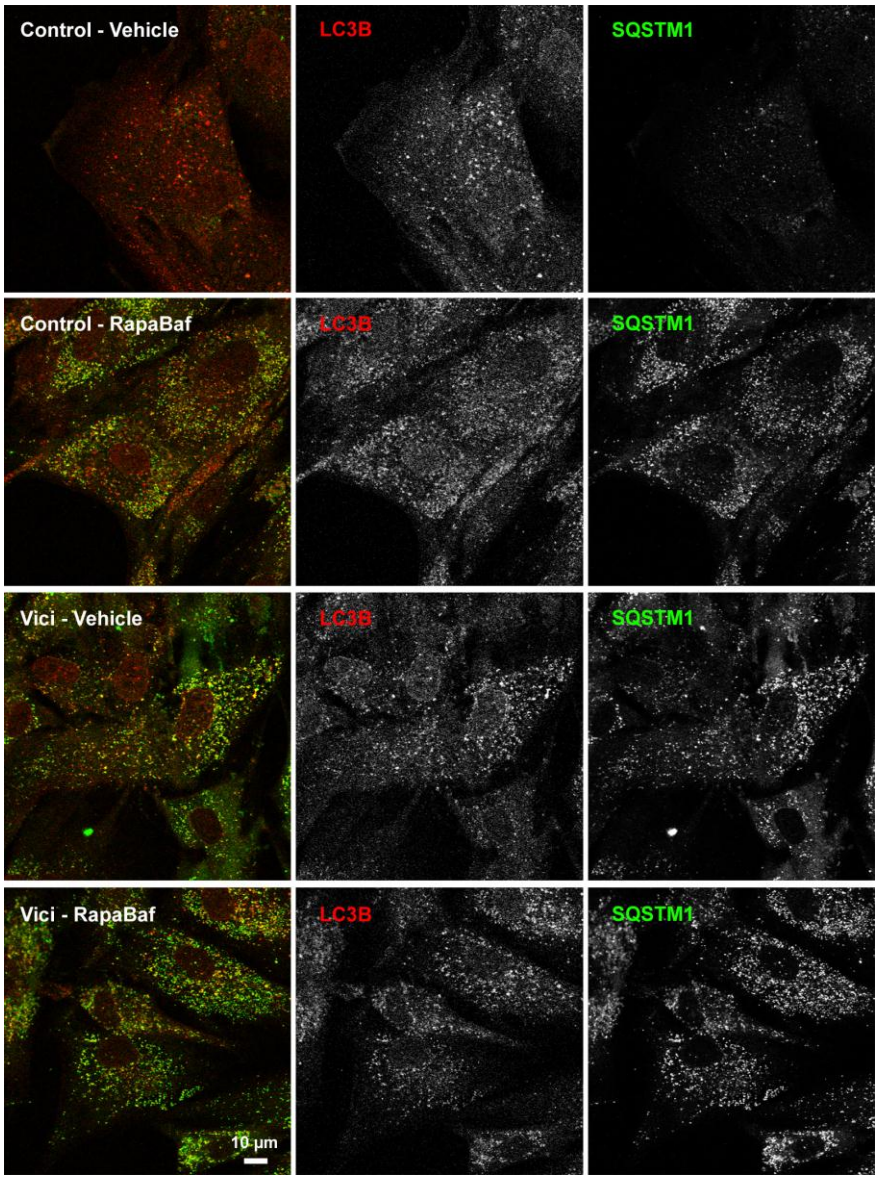
SUPPLEMENTARY FIGURE 1

Clinical features in patients with Vici syndrome. Clinical photographs from Patients 5.1 (A,B) and 5.2 (C,D), Patient 6.1 (E,F), Patient 3.1 (G) and Patient 12.1 (H). There is marked generalized hypopigmentation relative to the ethnic background (A-D) (Patients 5.1 and 5.2 are siblings of Turkish origin) and other family members (E,F) (Patient 6.1). Coarsening of facial features with full lips and macroglossia is noted in some older children (G). There is marked retinal hypopigmentation on fundoscopy (H). Microcephaly was either present at birth or developed over time. Failure to thrive was a common finding. Informed consent was obtained from all individuals or the legal guardian of minors. Supplemental Figure 1G adapted from reference 5.



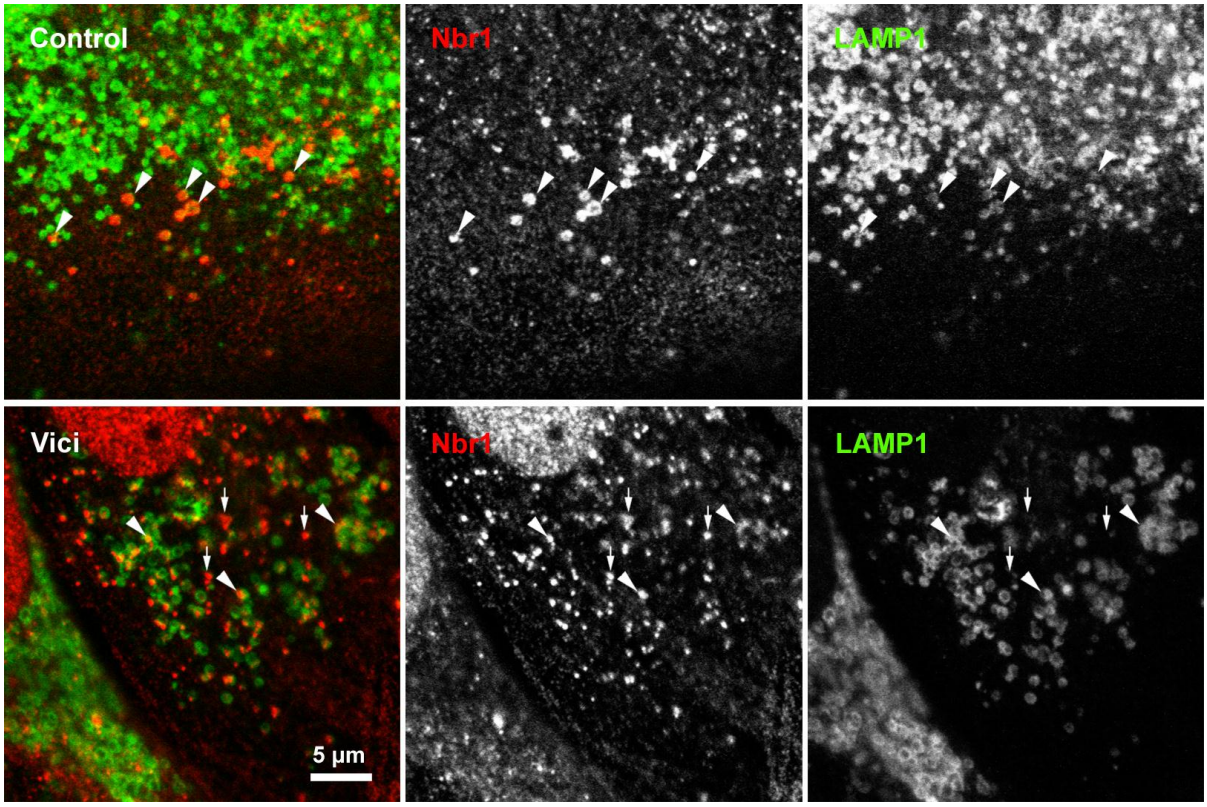
SUPPLEMENTARY FIGURE 2

Colocalisation of Nbr1- and p62/SQSTM1 positive puncta in Vici patient fibroblasts. The colocalisation of p62/SQSTM1 with Nbr1 in puncta was assessed in control and Vici-patient fibroblasts in rapamycin/bafilomycin treated cells. The induction of autophagy and block of autolysosomal degradation with this treatment causes accumulation of Nbr1 and p62/SQSTM1 in both control and Vici-patient fibroblasts. Colocalising puncta (arrows) occur in both cells with a frequency of around 30%. Scale bar: 10 µm.



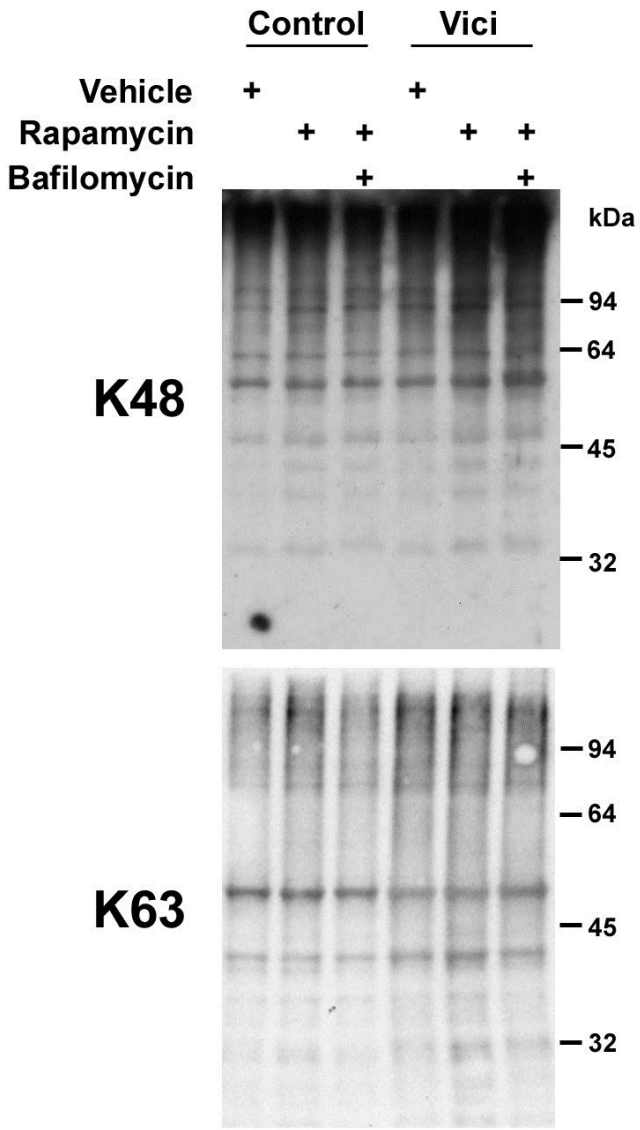
SUPPLEMENTARY FIGURE 3

Recruitment of p62/SQSTM1 to LC3-positive puncta is normal in Vici syndrome. The colocalisation of p62/SQSTM1 with LC3-positive puncta was assessed in control and Vici-patient fibroblasts under control conditions and in rapamycin/bafilomycin treated cells (RapaBaf). While induction of autophagy and block of autolysosomal degradation (RapaBaf) causes accumulation of LC3 and p62/SQSTM1 in control cells, Vici-patient fibroblasts show massive accumulation of autophagosome-like puncta under baseline conditions that does not change appreciably under RapaBaf. Scale bar: 10 µm.



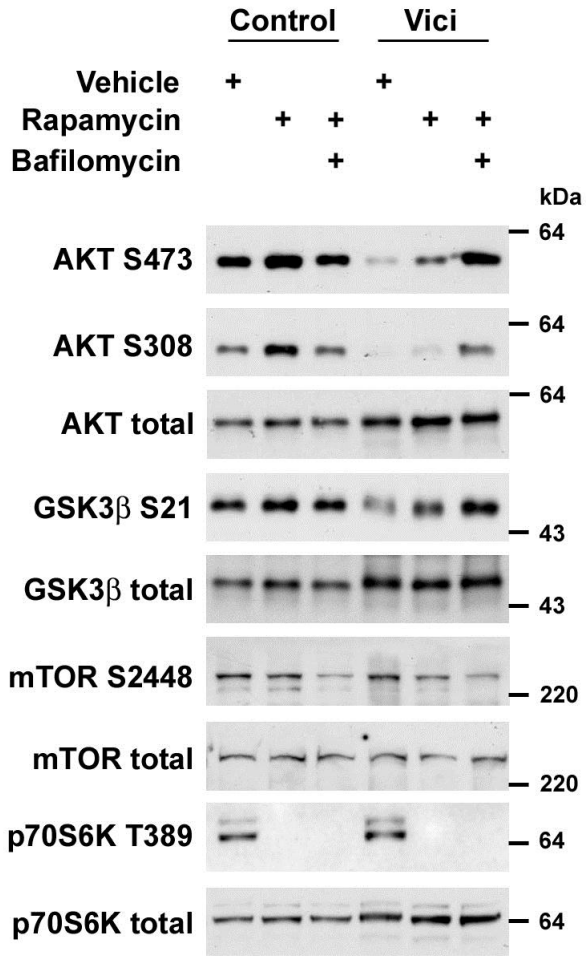
SUPPLEMENTARY FIGURE 4

Abnormal fusion of Nbr1-positive puncta with lysosomes in Vici syndrome. The autophagy adaptor Nbr1 and the lysosome membrane component LAMP1 colocalise in ring-shaped vesicular structures in control fibroblasts after 6 h treatment with bafilomycin (arrowheads). In Vici-patient fibroblasts (Patient 4.1), occasional fusion occurs in small puncta (arrowheads), but many non-fused Nbr1-positive puncta are observed (arrows). Scale bar: 5 µm



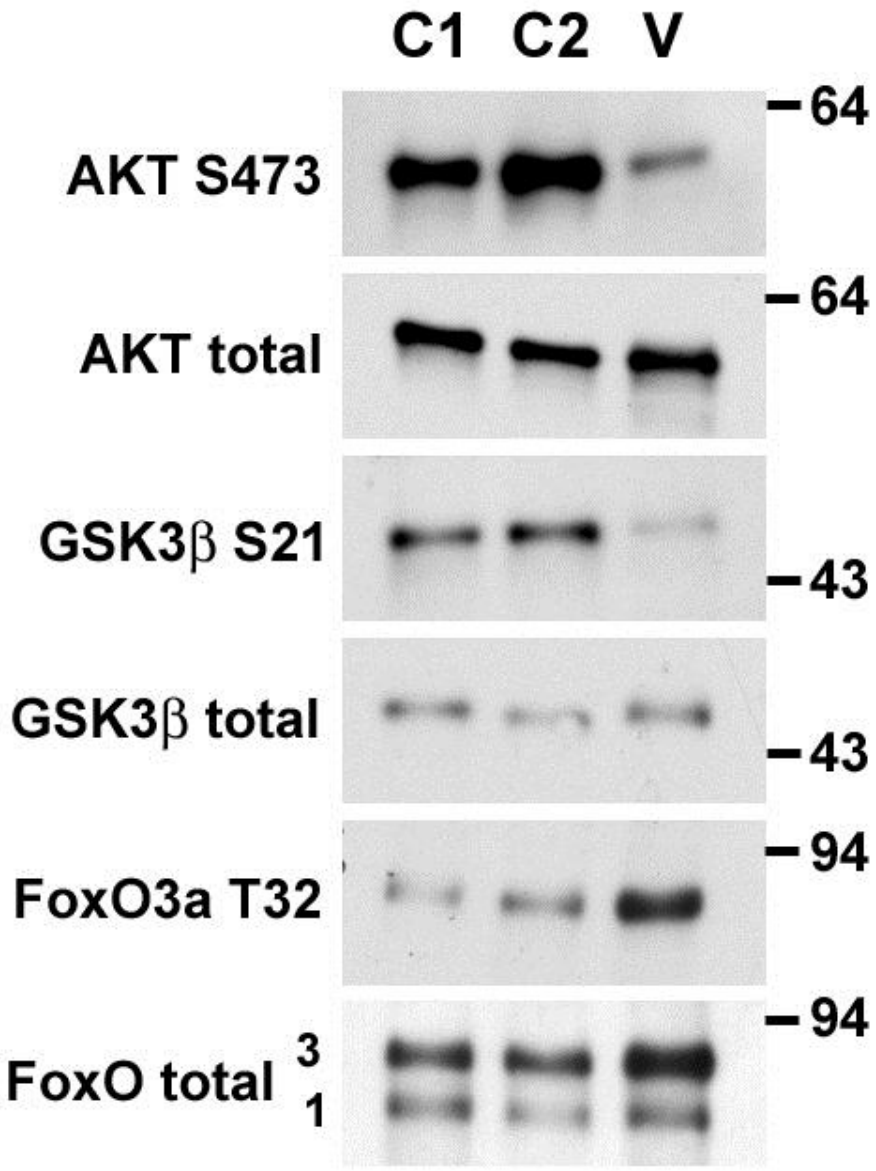
SUPPLEMENTARY FIGURE 5

Defects in autophagy in Vici syndrome lead to elevated K63-poly-ubiquitylated proteins. Western blots of control and Vici-patient fibroblasts under baseline conditions and after 12h treatment with rapamycin, or dual treatment with rapamycin and bafilomycin with monoclonal antibodies against Lysine-48 (K48) and Lysine-63 (K63) polyubiquitin chains. K48-linked chains increase under rapamycin and rapamycin/bafilomycin treatment in both cells. K63-linked chains are elevated under baseline conditions in Vici cells, suggesting a defect of specific clearance of this type of poly-ubiquitinylated products.



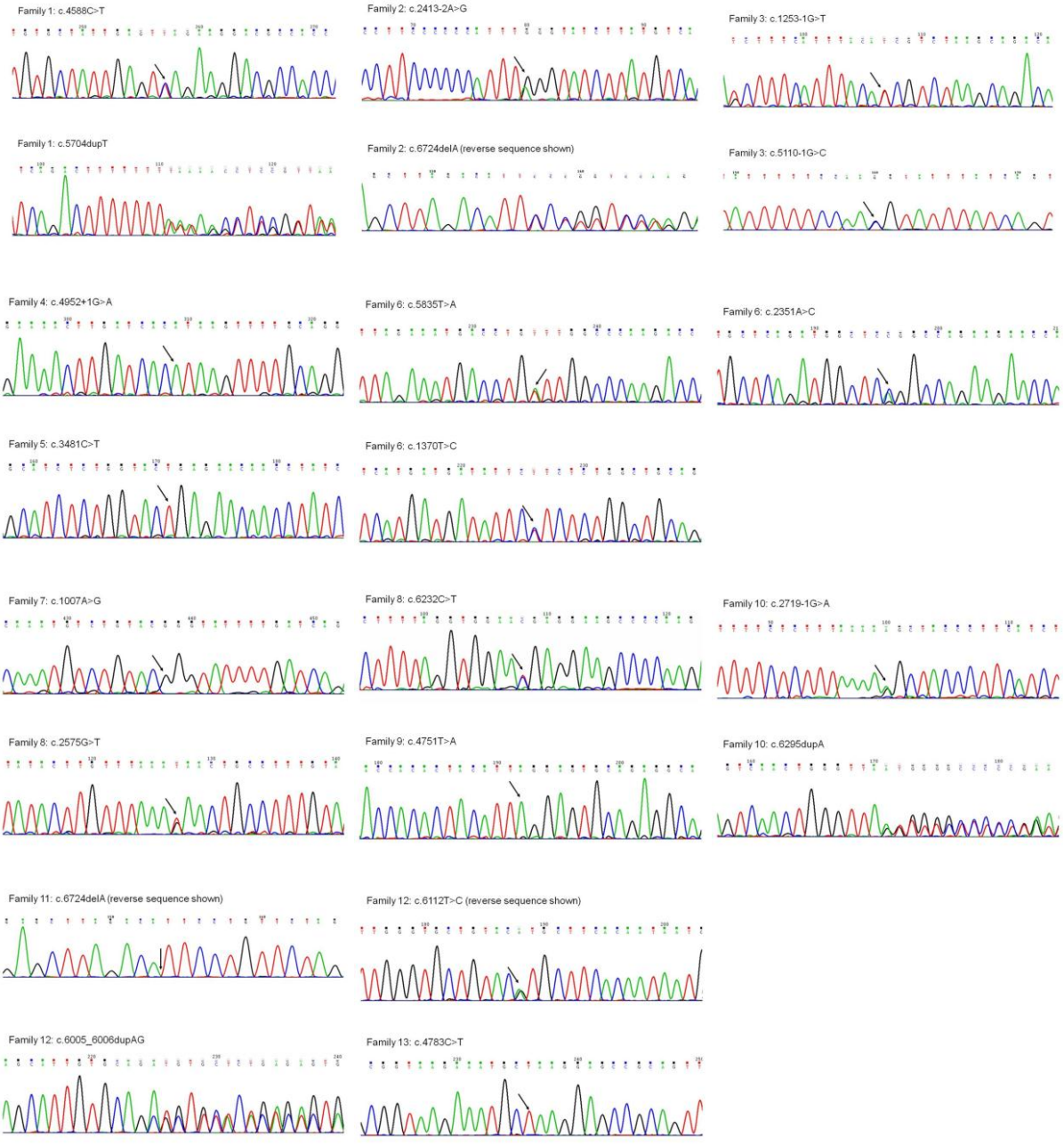
SUPPLEMENTARY FIGURE 6

Abnormal AKT-mTOR signalling in Vici syndrome. The response of the AKT-mTOR pathway was probed in control and Vici-patient fibroblasts after 12h treatment with rapamycin, or dual treatment with rapamycin and bafilomycin. In control cells, rapamycin inhibits p70S6-kinase (p70S6K) phosphorylation as expected and induces slight increase in AKT (serines 473 and 308) and downstream glycogen-synthase kinase 3-beta (GSK3beta) phosphorylation due to inhibition of the negative feedback loop via p70S6kinase. Additional treatment with bafilomycin results in decrease of AKT, GSK3beta and mTOR phosphorylation in control cells. In Vici patient fibroblasts, strong baseline reduction of AKT and GSK3beta phosphorylation is observed that is paradoxically increased in rapamycin/bafilomycin treated cells.



SUPPLEMENTARY FIGURE 7

Abnormal AKT-FoxO signalling in Vici fibroblasts. The AKT-FoxO pathway was probed in control and Vici-patient fibroblasts. In control cells (C1 and C2), phosphorylation of AKT (serines 473 and 308) and the downstream substrates glycogen-synthase kinase 3-beta (GSK3beta) and Foxo3a is high. In Vici patient fibroblasts (V), baseline reduction of AKT and GSK3beta phosphorylation is observed. As the levels of FoxoO are strongly upregulated, the ratio of phospho-FoxO3a (T32) to total FoxO3a are about 50% of control levels.



SUPPLEMENTARY FIGURE 8

ABI traces for mutant alleles identified in Families 1-13.