The American Journal of Human Genetics, Volume *98*

Supplemental Data

Mutations in *GANAB*, Encoding the Glucosidase IIα Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease

Binu Porath, Vladimir G. Gainullin, Emilie Cornec-Le Gall, Elizabeth K. Dillinger, Christina M. Heyer, Katharina Hopp, Marie E. Edwards, Charles D. Madsen, Sarah R. Mauritz, Carly J. Banks, Saurabh Baheti, Bharathi Reddy, José Ignacio Herrero, Jesús M. Bañales, Marie C. Hogan, Velibor Tasic, Terry J. Watnick, Arlene B. Chapman, Cécile Vigneau, Frédéric Lavainne, Marie-Pierre Audrézet, Claude Ferec, Yannick Le Meur, Vicente E. Torres, Genkyst Study Group, HALT Progression of Polycystic Kidney Disease Group, Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease, and Peter C. Harris

SUPPLEMENTAL FIGURES

Figure S1

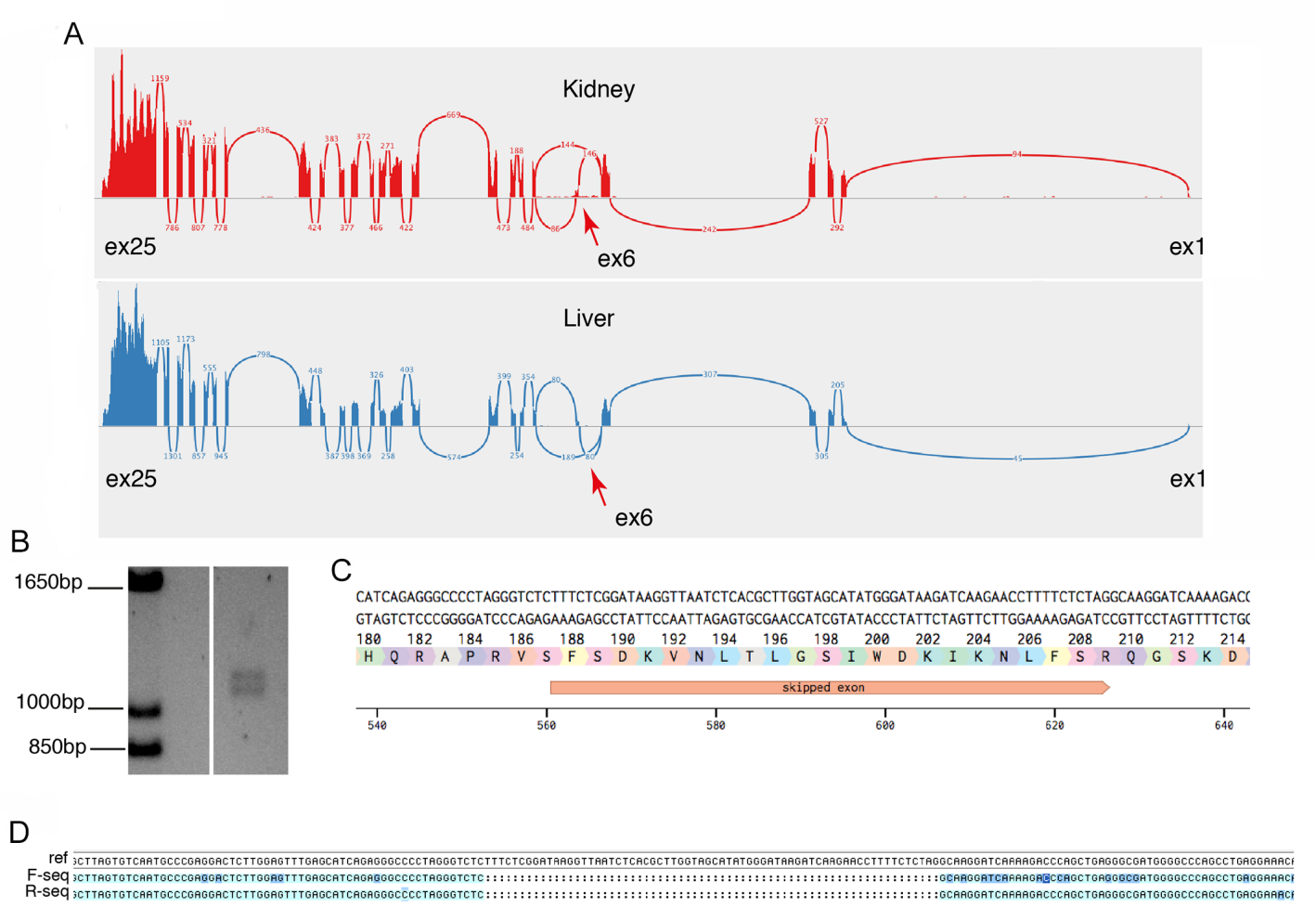

Figure S1: Analysis of *GANAB* isoforms. (**A**) Sashimi plots summarizing the results of the Illumina BodyMap 2.0 RNAseq data set (ENA archive: ERP000546) for *GANAB* mRNA expression in human liver and kidney tissue visualized using Integrated Genome Viewer (IGV, Broad Institute; 45 read threshold). Note in both liver and kidney the splice forms with (Isoform 3; NM_198335) and without exon 6 (Isoform 2; NM_198334) are approximately equally represented. (**B**) RT-PCR of RCTE cells showing two distinct products with primers flanking exon 6, which are confirmed by sequencing to be isoforms 3 and 2 (**D**). (**C**) Diagram showing the inframe exon 6 region (66bp, 22aa) skipped in isoform 2.

Figure S2

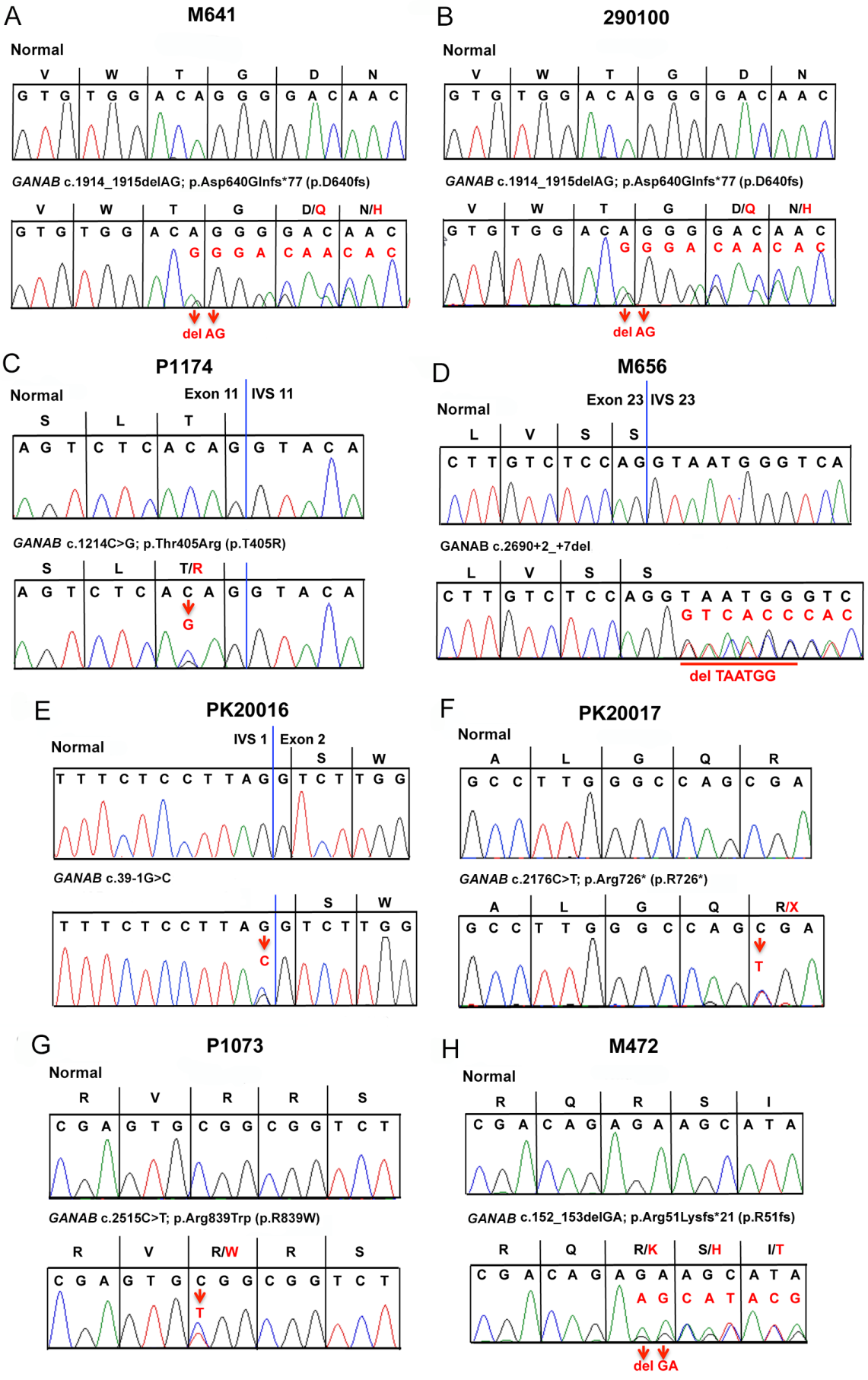
A
M641
Normal
V W T G D N
G T G T G G A C A G G G G A C A A C
GANAB c.1914_1915delAG; p.Asp640GInfs*77 (p.D640fs)
V W T G D/Q N/H
G T G T G G A C A G G G G A C A A C
G G G A C A A C
del AG
B
290100
Normal
V W T G D N
G T G T G G A C A G G G G A C A A C
GANAB c.1914_1915delAG; p.Asp640GInfs*77 (p.D640fs)
V W T G D/Q N/H
G T G T G G A C A G G G G A C A A C
G G G A C A A C
del AG
C
P1174
Normal
Exon 11 IVS 11
S L T
A G T C T C A C A G G T A C A
GANAB c.1214C>G; p.Thr405Arg (p.T405R)
S L T/R
A G T C T C A C A G G T A C A
G
D
M656
Exon 23 IVS 23
Normal
L V S S
C T T G T C T C C A G G T A A T G G G T C A
GANAB c.2690+2_+7del
L V S S
C T T G T C T C C A G G T A A T G G G T C
G T C A C C C A C
del TAATGG
E
PK20016
Normal
IVS 1 Exon 2
S W
T T T C T C C T T A G G T C T T G G
GANAB c.39-1G>C
S W
T T T C T C C T T A G G T C T T G G
C
F
PK20017
Normal
A L G Q R
G C C T T G G G C C A G C G A
GANAB c.2176C>T; p.Arg726* (p.R726*)
A L G Q R/X
G C C T T G G G C C A G C G A
T
G
P1073
Normal
R V R R S
C G A G T G C G G C G G T C T
GANAB c.2515C>T; p.Arg839Trp (p.R839W)
R V R/W R S
C G A G T G C G G C G G T C T
T
H
M472
Normal
R Q R S I
C G A C A G A G A A G C A T A
GANAB c.152_153delGA; p.Arg51Lysfs*21 (p.R51fs)
R Q R/K S/H I/T
C G A C A G A G A A G C A T A
A G C A T A C G
del GA

Figure S2: Sequence of *GANAB* mutations detected by Sanger sequencing in eight families. (**A**) M641, c.1914_1915delAG; p.Asp640Glnfs*77 (p.D640fs), (**B**) 290100, c.1914_1915delAG; p.Asp640Glnfs*77 (p.D640fs), (**C**) P1174, c.1214C>G; pThr405Arg (p.T405R), (**D**) M656, c2690+2_+7del, (**E**) PK20016, c.39-1G>C, (**F**) PK20017, c.2176C>T; p.Arg726* (p.R726*), (**G**) P1073, c.2515C>T; p.Arg839Trp (p.R839W), (**H**) M472, c.152_153delGA; p.Arg51Lysfs*21 (p.R51fs). Each component figure shows the sequence of the specific *GANAB* variant indicated in red (bottom panel), compared to the normal sequence (top panel). Note that the deletion in M656 disrupts the canonical donor site. The substitution in P1174 is close to end of exon 11 but is not predicted to alter splicing.

Figure S3

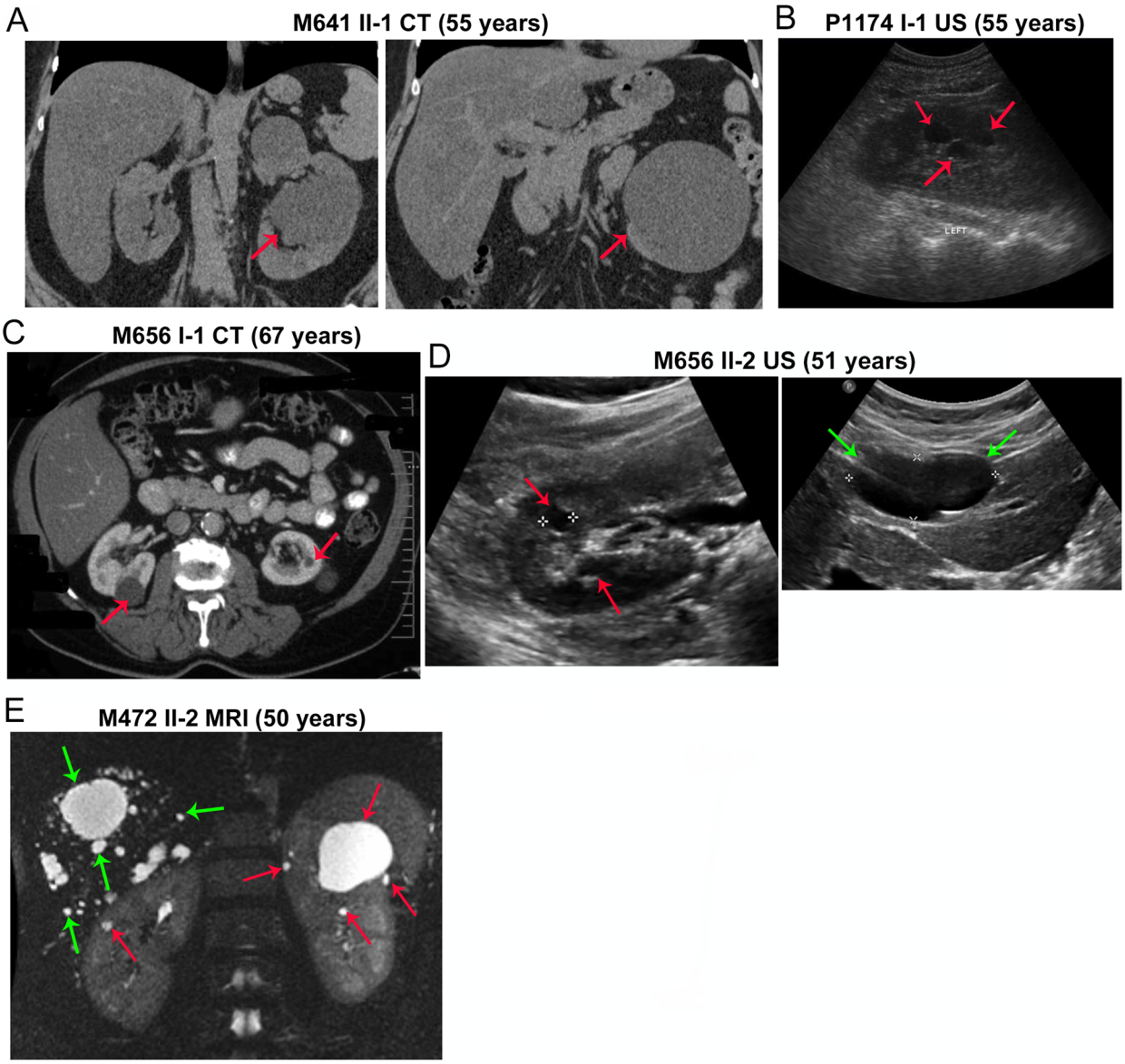

Figure S3: Kidney and liver images from affected members of families with *GANAB* mutations. Kidney and liver cysts are indicated with red or green arrows, respectively. Just representative cysts are highlighted where multiple cysts are present. (**A**) CT scan without contrast of kidneys and liver from M641, II-1 at 55y showing several kidney cysts, including a large one causing organ enlargement. Liver cysts are not evident in this image without contrast and organ enlargement may be due to fatty infiltrate (Table 1). (**B**) US image of kidneys from P1174, I-1 at 55y showing three moderately sized cysts. (**C**) CT scan image with contrast from M656, I-1 at 67y showing a few kidney cysts, but no apparent liver cysts. (**D**) Ultrasound of M656, II-2 at 51y showing a few renal cysts (left) and two large liver cysts (right). (**E**) T2-weighted MRI images from M472, II-2 at 50y showing a few renal cysts and multiple scattered liver cysts.

Figure S4

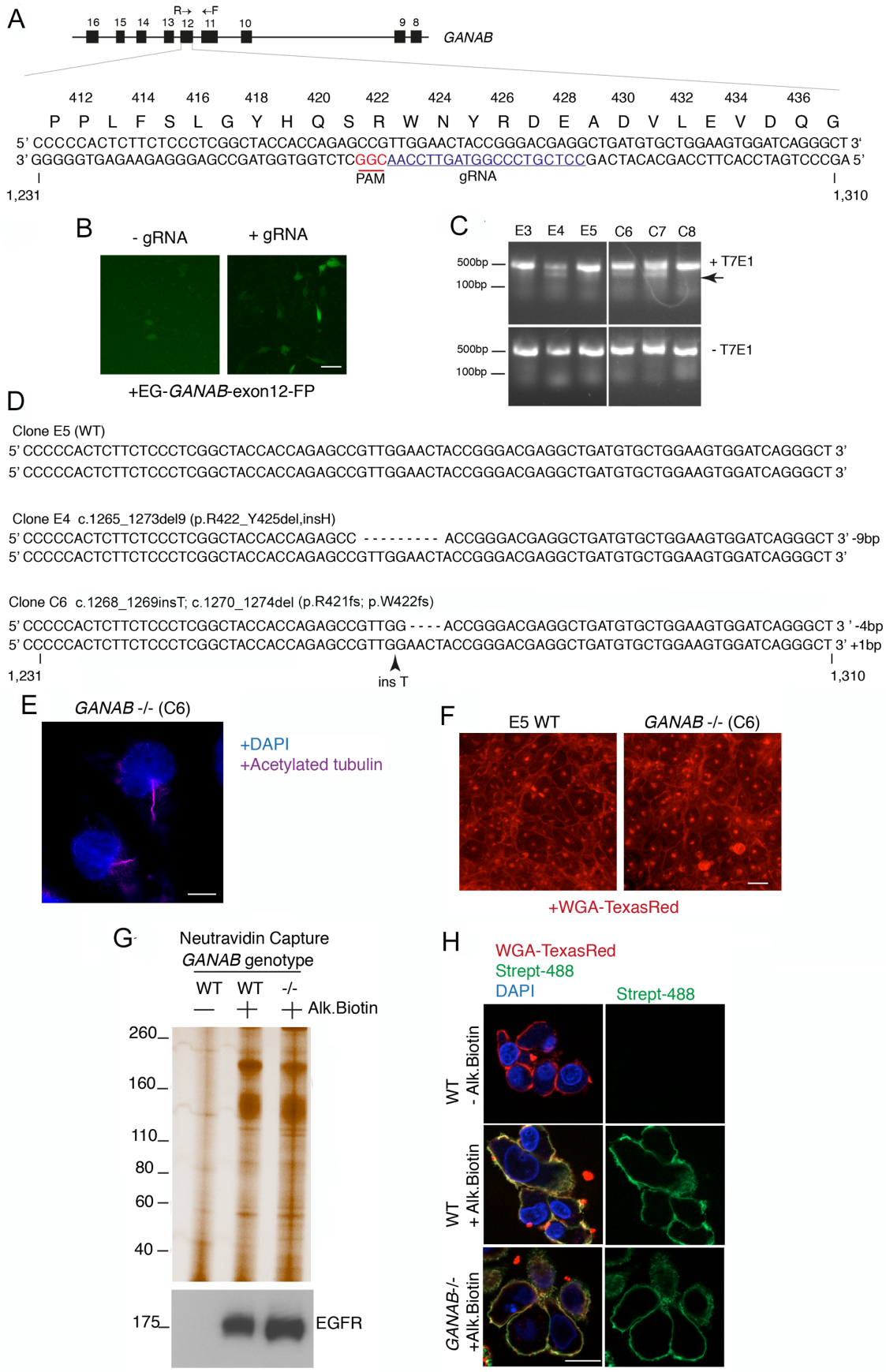
A
R→ ←F
16 15 14 13 12 11 10 9 8
GANAB
412 414 416 418 420 422 424 426 428 430 432 434 436
P P L F S L G Y H Q S R W N Y R D E A D V L E V D Q G
5' CCCCCACTCTTCTCCCTCGGCTACCACCAGAGCCGTTGGAACTACCGGGACGAGGCTGATGTGCTGGAAGTGGATCAGGGCT 3'
3' GGGGGTGAGAAGAGGGAGCCGATGGTGGTCTCGGCAACCTTGATGGCCCTGCTCCGACTACACGACCTTCACCTAGTCCCGA 5'
PAM
gRNA
1,231
1,310
B
- gRNA
+ gRNA
+EG-GANAB-exon12-FP
C
E3 E4 E5 C6 C7 C8
500bp
100bp
+ T7E1
- T7E1
D
Clone E5 (WT)
5' CCCCCACTCTTCTCCCTCGGCTACCACCAGAGCCGTTGGAACTACCGGGACGAGGCTGATGTGCTGGAAGTGGATCAGGGCT 3'
5' CCCCCACTCTTCTCCCTCGGCTACCACCAGAGCCGTTGGAACTACCGGGACGAGGCTGATGTGCTGGAAGTGGATCAGGGCT 3'
Clone E4 c.1265_1273del9 (p.R422_Y425del,insH)
5' CCCCCACTCTTCTCCCTCGGCTACCACCAGAGCC --------- ACCGGGACGAGGCTGATGTGCTGGAAGTGGATCAGGGCT 3' -9bp
5' CCCCCACTCTTCTCCCTCGGCTACCACCAGAGCCGTTGGAACTACCGGGACGAGGCTGATGTGCTGGAAGTGGATCAGGGCT 3'
Clone C6 c.1268_1269insT; c.1270_1274del (p.R421fs; p.W422fs)
5' CCCCCACTCTTCTCCCTCGGCTACCACCAGAGCCGTTGG - - - - ACCGGGACGAGGCTGATGTGCTGGAAGTGGATCAGGGCT 3 ' -4bp
5' CCCCCACTCTTCTCCCTCGGCTACCACCAGAGCCGTTGGAACTACCGGGACGAGGCTGATGTGCTGGAAGTGGATCAGGGCT 3' +1bp
ins T
1,231
1,310
E
GANAB -/- (C6)
+DAPI
+Acetylated tubulin
F
E5 WT
GANAB -/- (C6)
+WGA-TexasRed
G
Neutravidin Capture
GANAB genotype
WT WT -/-
Alk.Biotin
260
160
110
80
60
40
175
EGFR
H
WGA-TexasRed
Strept-488
DAPI
Strept-488
WT - Alk.Biotin
WT + Alk.Biotin
GANAB-/- +Alk.Biotin

Figure S4: Generation and characterization of RCTE cells with targeted *GANAB* deletion using the CRISPR/Cas9 system. (**A**) Diagram of *GANAB* showing the targeted region and sequence plus the position of the employed gRNA and protospacer adjacent motif (PAM). (**B**) Testing of the *GANAB* exon 12 gRNA using targeted sequence flanked by two halves of EGFP (EGxxFP)[1], EG-*GANAB*_exon12-FP. Cas9/gRNA co-transfected with EG-GANAB_exon12-FP in RCTE cells promotes recombination and EGFP fluorescence in transfected cells (Bar = 100μm). (**C**) PCR of a 550bp genomic region including *GANAB* exon 12 showing digestion due to mismatched sequence in clones E4, C6 and C7 (arrow) following T7E1 treatment. (**D**) DNA sequencing showing the inframe deletion in clone E4 and biallelic, frameshifting mutations in clone C6. (**E**) Confluent *GANAB*$^{-/-}$ RCTE cells detected with acetylated α-tubulin and stained with DAPI showing that they form normal cilia. Bar = 10μm. (**F**) Wheat Germ Agglutinin (WGA), which reacts with mature surface sugars, staining of WT and *GANAB*$^{-/-}$ RCTE clones shows similar staining, indicating no gross terminal glycosylation defects. (**G**) Biotinylation of terminally processed sialylated sugars with alkoxyamine biotin (Alk. Biotin) and neutravidin capture followed by silver staining showing similar surface glycoprotein detection in WT and *GANAB*$^{-/-}$ RCTE cells. (**H**) Cells employed for neutravidin capture (**G**), detected with WGA and streptavidin-488 to confirm surface glycoprotein labeling specificity. Scale bar = 20μm.

Figure S5

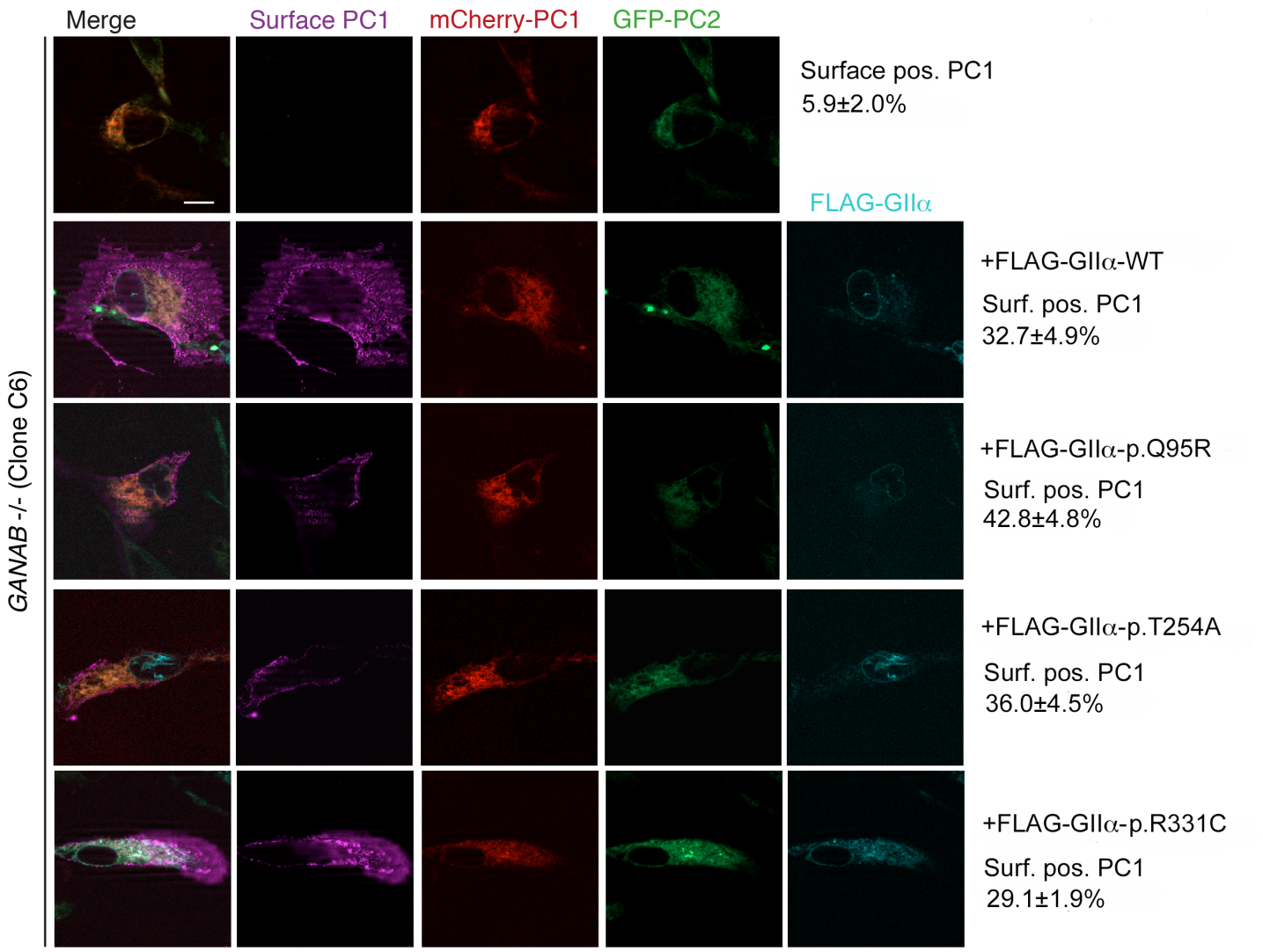

Figure S5: Surface localization of PC1 tested in *GANAB*$^{-/-}$ cells transfected with GIIα variants. Three *GANAB* missense variants either poorly predicted to be pathogenic from bioinformatics analysis (p.Gln95Arg [p.Q95R] and p.Thr254Ala [p.T254A]) or that are commonly found in the ExAC server (p.Arg331Cys [p.R331C]) were assayed for surface localization of PC1. *GANAB*$^{-/-}$ RCTE cells were co-transfected with mCherry-PC1 and GFP-PC2 as well as FLAG tagged GIIα plasmids that are WT (+FLAG-GIIα-WT) or contain the variants and examined for live-cell surface mCherry-PC1. In triple transfected *GANAB*$^{-/-}$ cells, p.Gln95Arg (p.Q95R; P=0.1), p.Thr254Ala (p.T254A; P=0.6), and p.Arg331Cys (p.R331C; P=0.5) were not significantly different from WT in their able to rescue PC1 surface localization, indicating that they are likely neutral variants. Students T-test was performed to determine significance in at least 100 triple-transfected cells analyzed between three independent experiments. Scale bar=20μm.

Table S1: Identified possible pathogenic variants detected by whole exome sequencing

Family[1]	Chr.	Position	Gene	Transcript	Exon	HGVS Coding	HGVS Protein	#Passed Filters[2]
P75	5	66438330	MAST4	NM_001164664	21	c.2699A>C	p.Q900P	5
	5	131940620	RAD50	NM_005732	16	c.2647C>T	p.R883C	5
	8	25261105	DOCK5	NM_024940	48	c.4958G>A	p.R1653H	5
	11	66328939	ACTN3	NM_001104	17	c.2006G>A	p.R672Q	5
	16	10867236	TVP23A	NM_001079512	5	c.387A>G	p.I129M	5
	19	49364918	PLEKHA4	NM_020904	4	c.224G>A	p.R75H	5
M199	4	190876287	FRG1	NM_004477	5	c.413G>A	p.W138*	6
	12	50598436	LIMA1	NM_001113546	6	c.763C>T	p.R255*	6
	12	51773085	GALNT6	NM_007210	3	c.481C>T	p.R161*	6
	14	64676186	SYNE2	NM_182914	102	c.18430G>A	p.D6144N	6
	16	2376215	ABCA3	NM_001089	5	c.115C>T	p.L39F	6
	1	22448067	WNT4	NM_030761	3	c.316A>G	p.T106A	5
	4	190876272	FRG1	NM_004477	5	c.398G>A	p.G133E	5
	6	12161747	HIVEP1	NM_002114	8	c.6563A>G	p.N2188S	5
	12	14927737	H2AFJ	NM_177925	1	c.333C>G	p.N111K	5
M263	1	26887275	RPS6KA1	NM_001006665	14	c.1301G>A	p.G434D	6
	2	215798885	ABCA12	NM_173076	52	c.7597G>A	p.A2533T	6
	3	120449574	RABL3	NM_173825	2	c.107C>A	p.S36*	6
	3	128694748	KIAA1257	NM_020741	7	c.897-2A>G	p.W300fs	6
	4	190876287	FRG1	NM_004477	5	c.413G>A	p.W138*	6
	9	35102108	STOML2	NM_013442	3	c.267G>T	p.Q89H	6
	11[3]	**62398260**	**GANAB**	**NM_198335**	**12**	**c.1265G>T**	**p.R422L**	**6**
	11	67377897	NDUFV1	NM_007103	5	c.556G>C	p.A186P	6
	12	106820980	POLR3B	NM_018082	13	c.1107A>T	p.L369F	6
	13	25671369	PABPC3	NM_030979	1	c.1033G>T	p.E345*	6
	15	90934098	IQGAP1	NM_003870	2	c.148G>A	p.A50T	6
	17	21319792	KCNJ12	NM_021012	3	c.1138G>A	p.E380K	6
	19	8322803	CERS4	NM_024552	10	c.782G>T	p.C261F	6
	22	18613671	TUBA8	NM_018943	5	c.1118G>A	p.R373Q	6
	2	152293802	RIF1	NM_018151	13	c.1420C>A	p.H474N	5
	2	233655546	GIGYF2	NM_001103147	12	c.917G>C	p.S306T	5
	3	49761081	GMPPB	NM_013334	1	c.79G>C	p.D27H	5
	4	190876272	FRG1	NM_004477	5	c.398G>A	p.G133E	5
	5	149782705	CD74	NM_001025159	7	c.796C>T	p.R266C	5
	8	144688809	PYCRL	NM_023078	4	c.413A>G	p.N138S	5
	9	101980978	ALG2	NM_033087	2	c.489G>C	p.E163D	5
	10	26446251	MYO3A	NM_017433	26	c.2806G>A	p.D936N	5
	11	1017041	MUC6	NM_005961	31	c.5760C>A	p.Y1920*	5
	11	1477645	BRSK2	NM_001256627	17	c.1736G>T	p.G579V	5
	12	53647451	MFSD5	NM_001170790	2	c.1153G>A	p.G385R	5
	14	23311809	MMP14	NM_004995	4	c.571C>A	p.P191T	5
	14	105613735	JAG2	NM_002226	20	c.2407A>T	p.I803F	5
	17	65944369	BPTF	NM_182641	23	c.7873G>A	p.E2625K	5
M560	1	1572296	CDK11B	NM_033487	14	c.995T>A	p.L332Q	6
	3	1337405	CNTN6	NM_014461	6	c.575G>A	p.G192D	6
	16[4]	2140803	PKD1	NM_001009944	44	c.12010C>T	p.Q4004*	6
	1	236228234	NID1	NM_002508	1	c.146G>A	p.G49E	5
	2	170042319	LRP2	NM_004525	50	c.9539C>T	p.P3180L	5

	2	179431879	TTN	NM_001267550	326	c.78980G>A	p.R26327Q	5
	5	78977820	PAPD4	NM_001114394	14	c.1316C>T	p.T439I	5
	13	52532497	ATP7B	NM_000053	8	c.2305A>G	p.M769V	5
	18	11889468	MPPE1	NM_023075	5	c.412C>T	p.R138W	5
	21	33711066	URB1	NM_014825	26	c.4460C>T	p.P1487L	5
	21	34889894	GART	NM_000819	15	c.1724C>T	p.P575L	5
P907	1	21903087	ALPL	NM_000478	11	c.1262A>G	p.Y421C	6
	1	24706274	STPG1	NM_001199013	5	c.331G>A	p.A111T	6
	2	170072887	LRP2	NM_004525	35	c.5702C>T	p.A1901V	6
	7	7495725	COL28A1	NM_001037763	16	c.1321G>A	p.G441R	6
	7	141759383	MGAM	NM_004668	32	c.3931C>T	p.R1311W	6
	11	18048098	TPH1	NM_004179	6	c.742C>T	p.R248*	6
	14	102749873	MOK	NM_014226	2	c.64C>T	p.Q22*	6
	15	78882221	CHRNA5	NM_000745	5	c.488C>T	p.P163L	6
	1	43032040	CCDC30	NM_001080850	6	c.749G>T	p.R250L	5
	1	145273300	NOTCH2NL	NM_203458	3	c.154C>T	p.R52*	5
	2	172931008	METAP1D	NM_199227	5	c.514G>A	p.G172R	5
	5	33596160	ADAMTS12	NM_030955	17	c.2533C>T	p.R845C	5
	5	140166534	PCDHA1	NM_018900	1	c.659C>A	p.P220Q	5
	5	140222006	PCDHA8	NM_018911	1	c.1100C>T	p.T367I	5
	11	65631296	MUS81	NM_025128	10	c.983T>C	p.L328P	5
	15	34649630	NUTM1	NM_001284292	8	c.3421C>T	p.R1141C	5
	16	23208666	SCNN1G	NM_001039	6	c.995A>G	p.H332R	5
	17	74005483	EVPL	NM_001988	22	c.3803G>A	p.R1268H	5
	22	50886828	SBF1	NM_002972	38	c.5197C>T	p.R1733C	5
P1272	1	93998617	FNBP1L	NM_001164473	8	c.778G>A	p.E260K	5
	1	216166437	USH2A	NM_206933	35	c.6730G>A	p.V2244M	5
	2	190922360	MSTN	NM_005259	3	c.752C>T	p.P251L	5
	2	197706020	PGAP1	NM_024989	27	c.2707T>C	p.Y903H	5
	3	160783259	PPM1L	NM_139245	3	c.643G>A	p.G215R	5
	5	13752253	DNAH5	NM_001369	64	c.11018C>T	p.S3673F	5
	12	104144483	STAB2	NM_017564	60	c.6565G>A	p.D2189N	5
	20	54824351	MC3R	NM_019888	1	c.452G>A	p.R151H	5

1: Details of subjects screened per family
P75 5 members screened: 3 affected siblings, 1 unaffected sibling, and unaffected mother
M199 3 members screened: 3 affected, maternal uncle and 2 nieces
M263 2 members screened: 2 affected, father and daughter
M560 3 members screened: 3 affected, mother and 2 daughters
P907 3 members screened: 2 affected daughters and an unaffected mother
P1272 2 members screened: 2 affected, maternal grandfather and grandchild
2: Only variants listed are those considered likely neutral by ≤ 1/6 programs, with the number of programs predicting pathogenicity listed (see Online Methods for details)
3: *GANAB* mutation is bolded
4: *PKD1* mutation missed by Sanger sequencing detected in this family

Table S2: Details of CRISPR/Cas9 guide RNA sequences

Name	DNA Sequence
gRNA1	TGCCTCATTCTTCTGCTCTT
gRNA2	CGGCAATATGCTAGTCTCAC
gRNA3	CCAGAGCCGTTGGAACTACC
gRNA4	CCTCGTCCCGGTAGTTCCAA
gRNA5	TGTGATGTCATCTGGCTAGA

Table S3: Details of primer sequences for generating site directed mutations

Mutation	Forward sequence	Reverse sequence
c.284A>G; p.Gly95Arg	CAGGGGCTTCgAAAGAACATG	AAGCTCTAGCACCAGCAAC
c.760A>G; p.Thr254Ala	GACATTCAAAgCTCACTCTGACAGCAAGC	TCCTCCCAGGCTCCTGGC
c.991C>T; p.Arg331Cys	CAACCCTCATtGCGACTTGGG	TGTGCCAGGAGCACAGGC
c.1214 C>G; p.Thr405Arg	GCTAGTCTCAgAGGAACCCAG	ATATTGCCGGAAAACATCAG
c.1265 G>T; p.Arg422Leu	CACCAGAGCCtTTGGAACTAC	GTAGCCGAGGGAGAAGAG
c. 2515 C>T; p.Arg839Trp	TGCGAGTGtGGCGGTCTTCAG	TCCATCGAGGCACGATTGT

REFERENCE

1. Mashiko, D. *et al.* Generation of mutant mice by pronuclear injection of circular plasmid expressing Cas9 and single guided RNA. *Sci Rep* **3**, 3355 (2013).