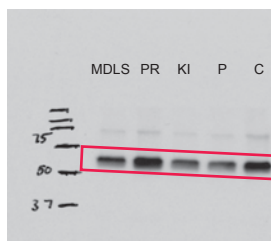
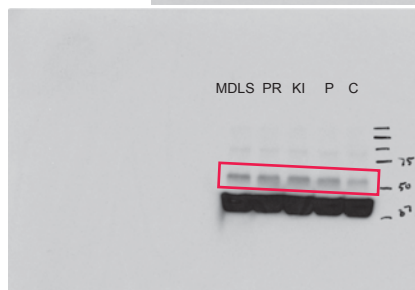

Supplementary information

Dysregulation of mTOR signalling is a converging mechanism in lissencephaly

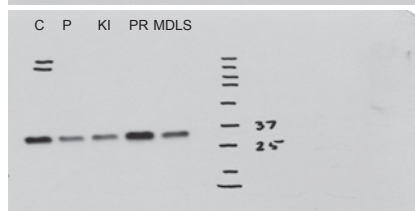
In the format provided by the
authors and unedited

a

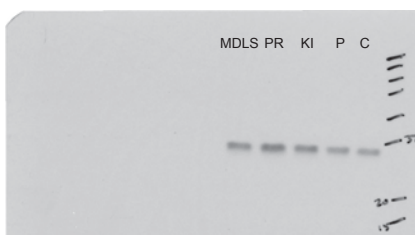
p-AKT (rb)



AKT(rb)
+ beta-actin (ms)



p-S6 (rb)



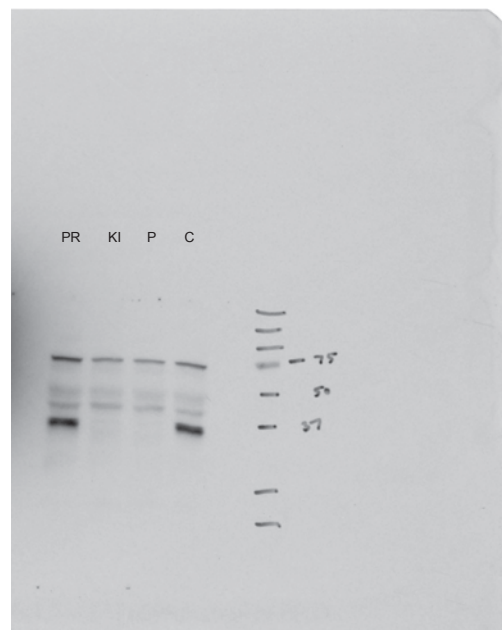
S6 (rb)



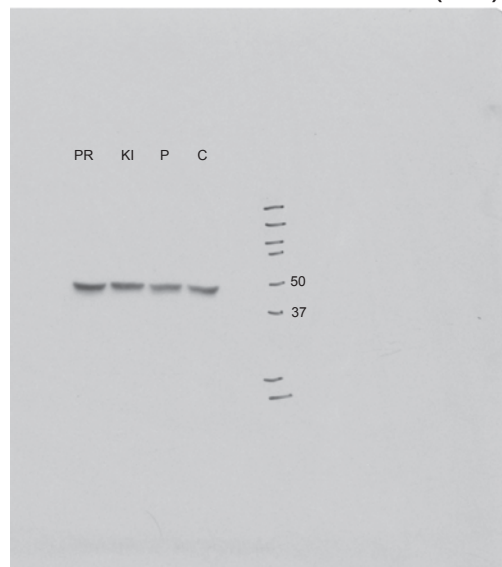
beta-actin (ms)

b

PIDD1 (ms)

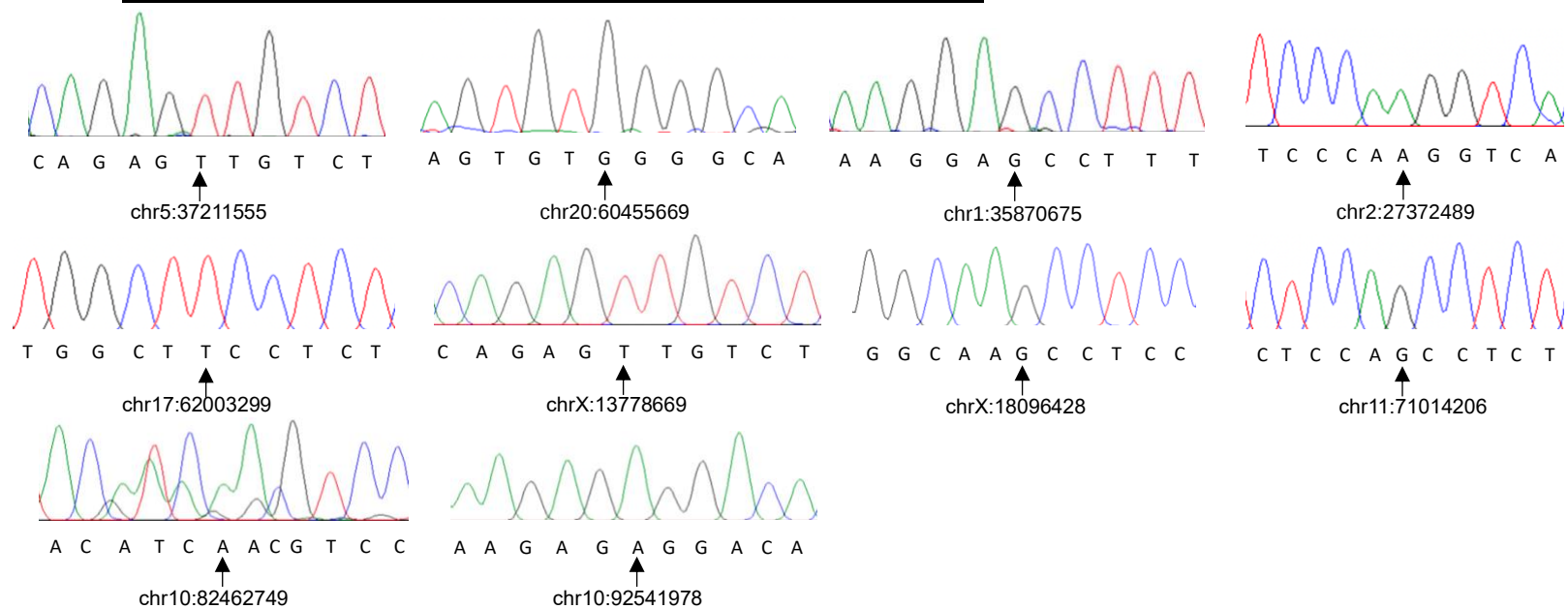


Beta-actin (ms)

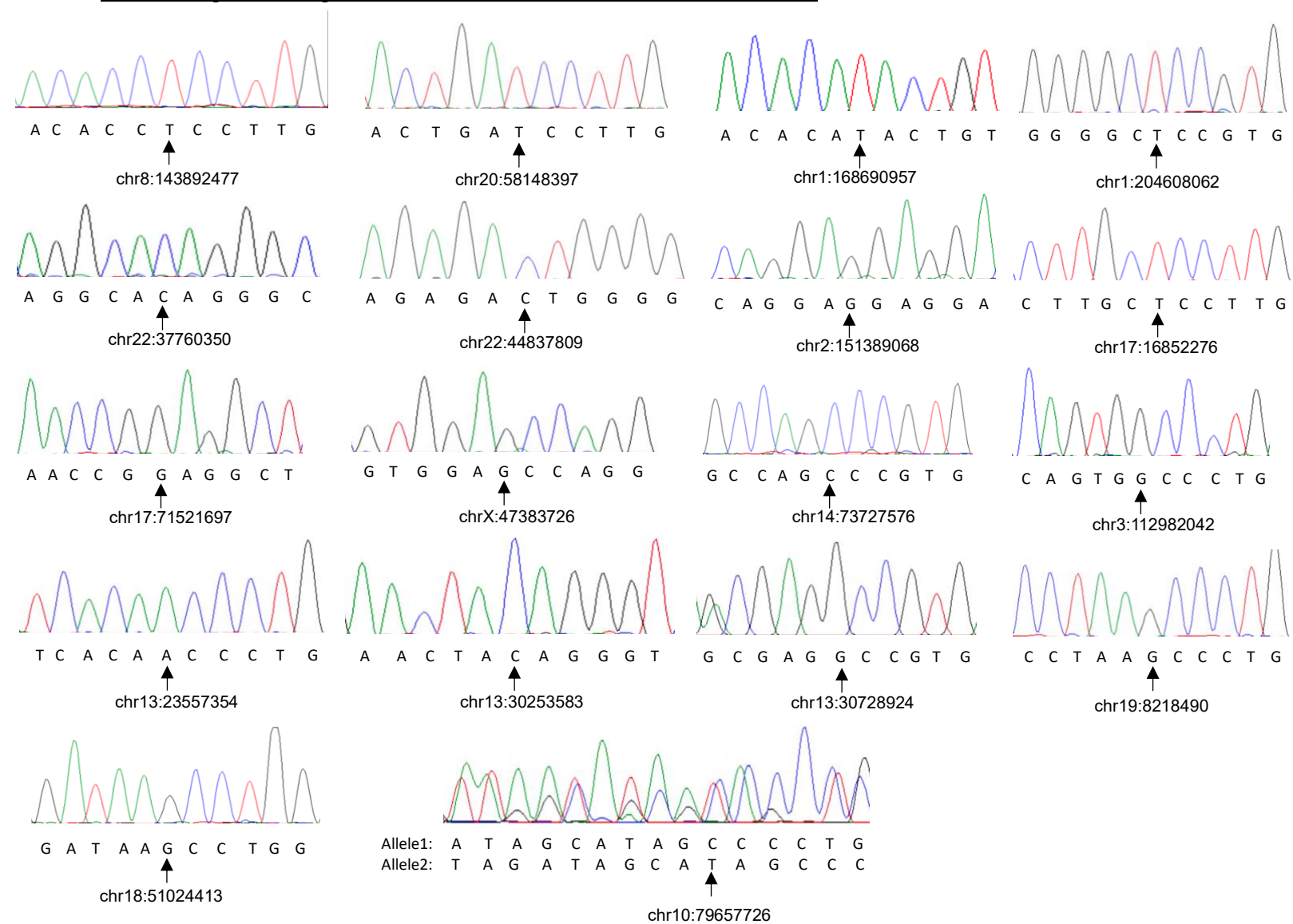


Supplementary Figure 1: Raw data (uncropped and unprocessed images of western blots). **a**, Scans for p-AKT, AKT, p-S6, S6, beta-actin in Figure 5c. Primary antibodies including loading control were all applied to the same membrane. Drawn red rectangle indicates cropped area for p-AKT and AKT. **b**, Scans for PIDD1, beta-actin in Extended Data Fig. 3i. Primary antibodies including loading control were all applied to the same membrane.

a. KI off-target sites, guide RNA: GGTCAGTCTGAGCAGCCTCGAG



b. PR off-target sites, guide RNA: AGGGTCACTGAGCAGCCTCA



Supplementary Figure 2

Supplementary Figure 2: Off-target site validation in iPSCs by Sanger sequencing.

a,b Sanger sequencing chromatograms for off-target sites predicted by CAS-OFFinder for Knock-In (**a**) and Patient Rescue (**b**) iPSC lines.

SAMPLE ID	Chr	Position	Gene	Ref	Variant	HGVSc	HGVSp	zygoti ty	Function GVS	dbSNP	omim	Allele freqs	Deleteriousne ss
NG375-1	chr1 6	69335066	COG8	G	A	NM_032382.5:c.868C>T, NM_001374871.1:c.868C> T, NM_001379261.1:c.868C> T, NM_001379262.1:c.868C> T, NM_001379263.1:c.868C> T, NM_001379264.1:c.868C> T, NM_001379265.1:c.868C> T, NM_001379266.1:c.868C> T	NP_115758.3:p.Arg290Cys, NP_001361800.1:p.Arg290Cys, NP_001366190.1:p.Arg290Cys, NP_001366191.1:p.Arg290Cys, NP_001366192.1:p.Arg290Cys, NP_001366193.1:p.Arg290Cys, NP_001366194.1:p.Arg290Cys, NP_001366195.1:p.Arg290Cys	HOM	missense_variant	rs37725906 5	Congenital disorder of glycosylation, type IIh, 611182 (3)	gnomAD V2: 0.00001 Turkish_AF: 0.0002	CADD: 30 ClinPred: D BayesDel: D FATHMM: T MetaSVM: T
NG375-1	chr6	146543968	RAB32	G	C	NM_006834.5:c.97G>C	NP_006825.1:p.Glu33Gln	HOM	missense_variant			Turkish_AF: 0.0002	CADD: 27.5 ClinPred: D BayesDel: D FATHMM: T MetaSVM: D
NG375-1	chr9	77358421	VPS13 A	C	T	NM_033305.3:c.8018C>T, NM_001018037.2:c.7901 C>T, NM_001018038.3:c.8018C >T, NM_015186.4:c.8018C> T	NP_150648.2:p.Ser2673Leu, NP_001018047.1:p.Ser2634Leu , NP_001018048.1:p.Ser2673Leu , NP_056001.1:p.Ser2673Leu	HOM	missense_variant	rs37519380 1	Choreoacanthocytosis, 200150 (3), Autosomal recessive	gnomAD V2: 0.00006 gnomAD V3: 0.00006 Turkish_AF: 0.0003	CADD: 28.4 ClinPred: D BayesDel: T FATHMM: D MetaSVM: D
NG375-1	chr1 1	3666492	CHRNA10	T	C	NM_001303034.2:c.350A> G, NM_001303035.2:c.350A> G, NM_020402.4:c.968A>G	NP_001289963.1:p.His117Arg, NP_001289964.1:p.His117Arg, NP_065135.2:p.His323Arg	HOM	missense_variant			gnomAD V3: 0.000006 Turkish_AF: 0.0005	CADD: 26 ClinPred: D BayesDel: D FATHMM: T MetaSVM: D
NG375-1	chr1 1	802380	PIDD1	G	A	NM_145886.4:c.991C>T, NM_145887.4:c.991C>T	NP_665893.2:p.Gln331Ter, NP_665894.2:p.Gln331Ter	HOM	stop_gained	rs77064071 7	Intellectual developmental disorder, autosomal recessive 75, with neuropsychiatric features and variant lissencephaly, 619827 (3), Autosomal recessive		CADD: 37 BayesDel: D
NG375-1	chr7	64928211	ZNF27 3	T	C	NM_021148.3:c.883T>C, NM_001385644.1:c.688T> C, NM_001385645.1:c.688T> C, NM_001385646.1:c.592T> C, NM_001385649.1:c.688T> C, NM_001385652.1:c.688T> C, NM_001388021.1:c.688T> C	NP_066971.2:p.Cys295Arg, NP_001372572.1:p.Cys230Arg, NP_001372573.1:p.Cys230Arg, NP_001372574.1:p.Cys230Arg, NP_001372575.1:p.Cys198Arg, NP_001372578.1:p.Cys230Arg, NP_001372581.1:p.Cys230Ar g, NP_001374950.1:p.Cys230Arg	HOM	missense_variant	rs75823901 7		gnomAD V2: 0.00003 gnomAD V3: 0.000006 Turkish_AF: 0.0007	CADD: 22.4 ClinPred: D BayesDel: T FATHMM: D MetaSVM: D
NG375-1	chr1 1	5453791	OR510	G	T	NM_001004754.3:c.305,3 0del	NP_001004754.1:p.Phe102Serf sTer19	HOM	frameshift_variant	rs77382824 7		gnomAD V2: 0.0003 gnomAD V3: 0.0001 Turkish_AF: 0.0002	
NG1801-1	chr1 1	800453	PIDD1	T	C	NM_145886.4:c.2042- 2A>G		HOM	splice_acceptor_variant	rs19975224 8	Intellectual developmental disorder, autosomal recessive 75, with neuropsychiatric features and variant lissencephaly, 619827 (3), Autosomal recessive	gnomAD V2: 0.0001 gnomAD V3: 0.00009 Turkish_AF: 0.0002	CADD: 33 BayesDel: T FATHMM: D CADD: 29.6 ClinPred: D BayesDel: T FATHMM: T MetaSVM: T
NG1801-1	chr1 1	800449	PIDD1	G	A	NM_145886.4:c.2044C>T, NM_145887.4:c.2044C>T	NP_665893.2:p.Arg682Cys, NP_665894.2:p.Arg682Cys	HOM	missense_variant&splice_region_ variant	rs20029064 0	Intellectual developmental disorder, autosomal recessive 75, with neuropsychiatric features and variant lissencephaly, 619827 (3), Autosomal recessive	gnomAD V2: 0.00007 gnomAD V3: 0.00008 Turkish_AF: 0.0002	CADD: 25.7 ClinPred: D BayesDel: T FATHMM: T MetaSVM: T
NG1801-1	chr X	124737149	TENM1	G	A	NM_001163278.2:c.584C> T, NM_001163279.1:c.584C> T, NM_014253.3:c.584C>T	NP_001156750.1:p.Pro195Leu, NP_001156751.1:p.Pro195Leu, NP_055068.2:p.Pro195Leu	HOM	missense_variant			gnomAD V3: 0.00001 Turkish_AF: 0.0002	CADD: 25.7 ClinPred: D BayesDel: T FATHMM: T MetaSVM: T
NG1801-1	chr1 0	60200206	ANK3	T	C	NM_020987.5:c.1414A>G, NM_001204403.2:c.1396A >G, NM_001204404.2:c.1363A >G, NM_001320874.2:c.1414A >G	NP_066267.2:p.Met472Val, NP_001191332.1:p.Met466Val, NP_001191333.1:p.Met455Val, NP_001307803.1:p.Met472Val	HOM	missense_variant		Intellectual developmental disorder, autosomal recessive 37, 615493 (3), Autosomal recessive	Turkish_AF: 0.0003	CADD: 25.4 ClinPred: D BayesDel: D FATHMM: T MetaSVM: T
NG8 Shared	chr11	800912	PIDD1	C	T	NM_145886.4:c.1767G> A, NM_145887.4:c.1767G> A	NP_665893.2:p.Tyr589Ter, NP_665894.2:p.Tyr589Ter	HOM	stop_gained&splice_region_varian t		Intellectual developmental disorder, autosomal recessive 75, with neuropsychiatric features and variant lissencephaly, 619827 (3), Autosomal recessive	Turkish_AF: 0.0009	CADD: 48 BayesDel: D
NG8 Shared	chr11	280782	NLRP6	T	A	NM_001276700.2:c.1048T >A, NM_138329.2:c.1048T>A	NP_001263629.1:p.Ser350Thr , NP_612202.2:p.Ser350Thr	HOM	missense_variant	rs374161810		gnomAD V2: 0.00001 gnomAD V3: 0.00007 Turkish_AF: 0.001	CADD: 23.4 ClinPred: D BayesDel: T FATHMM: D MetaSVM: T
NG8 Shared	chr11	18083701	SAAL1	C	T	NM_138421.3:c.1073G>A	NP_612430.2:p.Arg358Gln	HOM	missense_variant	rs755925079		gnomAD V2: 0.00002 gnomAD V3: 0.00021 Turkish_AF: 0.001	CADD: 23.7 ClinPred: T BayesDel: T FATHMM: T MetaSVM: T
NG8 Shared	chr11	5453589	OR5102	T	C	NM_001004754.3:c.101T> C	NP_001004754.1:p.Met34Thr	HOM	missense_variant	rs779753439		gnomAD V2: 0.000008 Turkish_AF: 0.0009	CADD: 20.6 ClinPred: D BayesDel: T FATHMM: T MetaSVM: T
NG8 Shared	chr11	6259989	CKBR	C	CT	NM_176875.4:c.62dup, NM_001318029.2:c.62dup, NM_001363552.2:c.62dup	NP_795344.1:p.Cys22ValfsTer3 , NP_001304958.1:p.Cys22Valfs Ter147, NP_001350481.1:p.Cys22Valfs Ter36	HOM	frameshift_variant			Turkish_AF: 0.0007	

NG8 shared are variants co-segregated in NG8-3/8-4/8-5

Supplementary Table 1

Supplementary Table 1: Candidate variants identified by whole exome sequencing.

List of candidate variants for NG375-1, NG1801-1 and those shared by NG8-3, NG-8-4, and NG8-5. Chr: Chromosome; Position: genomic coordinate; Ref: reference allele; Variant (mutated allele); HGVS_c: HGVS coding sequence name organized by RefSeq accession; HGVS_p: HGVS protein sequence name organized by RefSeq accession; zygosity: homozygous (HOM); Function GVS: functional impact of genetic variants; dbSNP: Single Nucleotide Polymorphism Database (dbSNP), reference single nucleotide polymorphism (rs) ID; omim: genetic phenotypes listed in Online Mendelian Inheritance in Man (OMIM) database; Allele freqs: minor allele frequencies organized by genomic databases; Deleteriousness: variant deleteriousness scores organized by in silico prediction tools.

Family		NG-8	NG-375	NG-1801
Patients	1	<u>NG8-3</u> Head circumference: 56 cm Disease onset 2-3 years old Crawled at 18 months old Walked at 30 months old Convulsions Aggression Toilet trained Forgetfulness Normal vision and hearing Unsteady gait Worsening speech Dependent on mother for personal hygiene care No help needed with eating Poor sleep Normal development	<u>NG375-1</u> Started walking at 14 months, speaking at 2.5-3 years old Convulsions Problems with understanding speech but can talk, IQ 42 Abnormal development Aggression Antipsychotic drug use Head circumference at 17 years: 61cm No help needed with eating, toilet use Trouble with performing fine movements (not cerebellum related) Normal cranial nerve function and reflexes Normal hearing and sight	<u>NG1801-1</u> Due to congenital dislocation of the hip, walked at 18 months old History of one febrile convulsion Toilet-trained Strabismus No speech Mentally stable Misalignment in one eye History of one convulsion
	2	<u>NG8-4</u> Head circumference: 54 cm No convulsions Loss of balance Worsening of speech Phobias	<u>NG375-2</u> Mild intellectual disability Normal gait and speech	
	3	<u>NG8-5</u> Head circumference: 56 cm Convulsions (stopped at 4-5 years old) Crawling at 5 years old Most aggressive among siblings		

		Worsening of speech Normal development		
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Supplementary Table 2

Supplementary Table 2: Clinical histories. Clinical histories of patients NG8-3, NG8-4, NG-1801, and NG375-1, organized by family.

	NG8-3	NG8-4	NG8-5	NG375-1	NG1801-1
Read Length (bp)	100	100	100	74	76
Num reads (M)	54,1	111,7	53,8	77,7	80,1
Num bases (Gb)	5,5	11,3	5,4	5,7	6,1
Mean coverage (X)	53,5	114	59,1	43,3	84,4
Median coverage (X)	48	104	53	42	73
PCR duplicates	17,49%	16,40%	11,31%	43,84%	2,93%
Multiply mapped	6,99%	6,98%	7,79%	3,72%	6,15%
Unmapped	0,09%	0,11%	0,19%	1,21%	0,29%
Reads on-target	50,84%	52,54%	56,32%	36,37%	65,03%
Bases on-target	37,98%	39,33%	42,28%	27,25%	49,08%
Mean error rate	0,20%	0,21%	0,27%	0,36%	0,48%
1x target base coverage	99,00%	99,00%	99,00%	96,6%	98,3%
10x target base coverage	97,50%	98,50%	97,90%	90,2%	93,5%
15x target base coverage	94,90%	98,00%	96,10%	85,9%	90,8%
20x target base coverage	90,50%	97,30%	92,70%	80,8%	87,9%
50x target base coverage	48,20%	85,60%	54,60%	38,8%	66,7%

Supplementary Table 3

Supplementary Table 3: Whole exome sequencing metrics. Whole exome sequencing metrics for patients NG8-3, NG8-4, NG8-5, NG375-1, and NG1801-1 (refer to Extended Figure 2a-c for pedigrees and MRI scans).

Figure 1B	Significance P-Value	Figure 1D	Significance P-Value	Figure 1E	Significance P-Value
Control vs. Patient	** 0.001	Control vs. Patient	** 0.001	Control vs. Patient	ns 0.425
Control vs. Control KI	** 0.002	Control vs. Control KI	** 0.004	Control vs. Knock-in	ns 0.882
Control vs. Patient Rescue	ns 0.2128	Control vs. Patient Rescue	ns 0.9996	Control vs. Patient Rescue	ns 0.3631
Patient vs. Patient Rescue	0.0214	Patient vs. Patient Rescue	** 0.0002	Patient vs. Patient Rescue	ns 0.9702

Figure 2B	Significance P-Value	Figure 2D	Significance P-Value	Figure 2E	Significance P-Value
Control vs. Patient CP	*** <0.0001	Bin 1 Control vs. Patient	* 0.0105	Control vs. Patient	*** <0.0001
Control vs. Knock-in CP	*** <0.0001	Bin 1 Control vs. Knock-in	*** 0.0002	Control vs. Knock-in	*** <0.0001
Control vs. Patient Rescue CP	ns >0.9999	Bin 1 Control vs. Patient Rescue	ns 0.7329	Control vs. Patient Rescue	ns 0.9787
Patient vs. Patient Rescue CP	*** <0.0001	Bin 1 Patient vs. Patient Rescue	* 0.0217	Patient vs. Patient Rescue	*** <0.0001
Control vs. Patient SVZ	*** <0.0001	Bin 2 Control vs. Patient	** 0.0085		
Control vs. Knock-in SVZ	** 0.0022	Bin 2 Control vs. Knock-in	** 0.0023		
Control vs. Patient Rescue SVZ	ns 0.9985	Bin 2 Control vs. Patient Rescue	ns 0.6369		
Patient vs. Patient Rescue SVZ	*** 0.0003	Bin 2 Patient vs. Patient Rescue	* 0.0205		
Control vs. Patient VZ	ns 0.2144	Bin 3 Control vs. Patient	** 0.0113		
Control vs. Knock-in VZ	** 0.0158	Bin 3 Control vs. Knock-in	*** <0.0001		
Control vs. Patient Rescue VZ	ns >0.9999	Bin 3 Control vs. Patient Rescue	ns 0.1331		
Patient vs. Patient Rescue VZ	ns 0.0786	Bin 3 Patient vs. Patient Rescue	* 0.0113		
		Bin 4 Control vs. Patient	ns 0.1417		
		Bin 4 Control vs. Knock-in	* 0.0488		
		Bin 4 Control vs. Patient Rescue	ns 0.5543		
		Bin 4 Patient vs. Patient Rescue	ns 0.055		
		Bin 5 Control vs. Patient	ns 0.1701		
		Bin 5 Control vs. Knock-in	ns 0.1065		
		Bin 5 Control vs. Patient Rescue	ns 0.5276		
		Bin 5 Patient vs. Patient Rescue	ns 0.0866		

Figure 4E	Significance P-Value
Control vs. Patient	** 0.0003
Control vs. Knock-in	*** <0.0001
Control vs. MDLS	* 0.0021
Patient vs. Knock-in	ns 0.5084
Patient vs. MDLS	ns 0.6055
Knock-in vs. MDLS	ns 0.4139

Figure 5B	Significance P-Value	Figure 5D	Significance P-Value	Figure 5E	Significance P-Value
Control vs. Patient	*** <0.0001	Control vs. Patient	** 0.0074	Control vs. Patient	*** <0.0001
Control vs. Knock-in	*** 0.0001	Control vs. Knock-in	** 0.0058	Control vs. Knock-in	*** <0.0001
Control vs. Patient Rescue	ns 0.9909	Control vs. Patient Rescue	ns 0.9977	Control vs. Patient Rescue	ns 0.9955
Control vs. MDLS	** 0.0005	Control vs. MDLS	** 0.0067	Control vs. MDLS	*** 0.0001
Patient vs. Patient Rescue	*** <0.0001	Patient vs. Patient Rescue	* 0.0136	Patient vs. Patient Rescue	** 0.0019

Figure 6C	Significance P-Value	Figure 6D	Significance P-Value	Figure 6F	Significance P-Value	Figure 6G	Significance P-Value	Figure 6H	Significance P-Value
Ctrl No Treatment vs. Ctrl Treatment	** 0.0005	Control vs. knock-in	*** <0.0001	C vs. P	*** <0.0001	Control No Treatment vs. Control Treatment	*** 0.0003	Bin 1 Control vs. Row 1 Knock-in	*** <0.0001
Ki No Treatment vs. Ki Treatment	*** <0.0001	control vs. knock-in+HV	ns 0.9448	C vs. P	*** <0.0001	Patient No Treatment vs. Patient Treatment	*** <0.0001	Bin 1 Control vs. Row 1 Knock-in+HV	ns >0.9999
PR No Treatment vs. PR Treatment	*** <0.0001	control vs. MDLS	*** <0.0001	C vs. PR	ns >0.9999	Knock-in No Treatment vs. Knock-in Treatment	*** <0.0001	Bin 1 Control vs. Row 1 Patient Rescue +HV	ns >0.9999
MDLS No Treatment vs. MDLS Treatment	** 0.0002	control vs. MDLS+HV	ns >0.9999	C vs. MDLS	*** <0.0001	Control No Treatment vs. Patient Treatment	** 0.2104	Bin 1 Patient vs. Row 1 Patient Rescue	*** <0.0001
Ctrl No Treatment vs. Ki No Treatment	** 0.0068	patient vs. patient rescue	*** <0.0001	P vs. PR	*** <0.0001	Control No Treatment vs. Knock-in No Treatment	** 0.009	Bin 2 Control vs. Row 2 Knock-in	* 0.0302
P No Treatment vs. PR No Treatment	** 0.0013		ns 0.0807			Control No Treatment vs. Knock-in Treatment	ns 0.6132	Bin 2 Control vs. Row 2 Patient Rescue +HV	ns >0.9999
Ctrl No Treatment vs. MDLS No Treatment	*** <0.0001							Bin 2 Patient vs. Row 2 Patient Rescue	*** <0.0001
								Bin 3 Control vs. Row 3 Knock-in	ns >0.9999

Ext Figure 3H	Significance P-Value
C vs C+CHK	** 0.0056
P vs P+CHK	* 0.0266
C vs P	0.0329
P vs PR	0.0287
C+CHK vs P+CHK	ns 0.3489
C vs PR	ns 0.3071
PR vs PR+CHK	ns 0.0611

Ext Figure 4B	Significance P-Value	Ext Figure 4D	Significance P-Value	Ext Figure 4F	Significance P-Value	Ext Figure 4H	Significance P-Value	Ext Figure 4I	Significance P-Value
Control vs. Patient	** 0.0017	Control vs. Patient	*** <0.0001	Control vs. Patient	ns 0.4425	Control vs. Knock-in	** 0.0027	Control vs. Knock-in	ns 0.9735
Control vs. Control KI	** 0.003	Control vs. Control KI	*** <0.0001	Control vs. Control KI	ns 0.5549	Control vs. Patient	** 0.0027	Control vs. Patient	ns 0.8
Control vs. Patient Rescue	ns 0.8455	Control vs. Patient Rescue	ns 0.9489	Control vs. Patient Rescue	ns 0.9711	Control vs. Patient Rescue	ns 0.6472	Control vs. Patient Rescue	ns 0.9838
Patient vs. Patient Rescue	** 0.0178	Patient vs. Patient Rescue	*** <0.0001	Patient vs. Patient Rescue	ns 0.224	Patient vs. Patient Rescue	* 0.0361	Patient vs. Patient Rescue	ns 0.9929

Ext Figure 5B	Significance P-Value	Ext Figure 5D	Significance P-Value	Ext Figure 5F	Significance P-Value	Ext Figure 5H	Significance P-Value
Control vs. Patient	ns 0.7503	Control vs. Patient	ns 0.349	Control 0-30° (Horizontal) v	*** <0.0001	Control vs. Patient MAP2	** 0.0002
Control vs. Knock-in	ns 0.9689	Control vs. Knock-in	ns 0.9984	Control 0-30° (Horizontal) v	*** <0.0001	Control vs. Knock-in MAP2	*** <0.0001
Control vs. Patient Rescue	ns >0.9999	Control vs. Patient Rescue	ns 0.6982	Control 30-60° (Horizontal) v	ns 0.996	Control vs. Patient Rescue MAP2	ns 0.9996
Patient vs. Patient Rescue	ns 0.7055	Patient vs. Patient Rescue	ns 0.9353	Control 30-60° (Oblique) v	ns 0.2729	Patient vs. Patient Rescue MAP2	*** <0.0001
				Control 30-60° (Oblique) v	ns 0.8986	Control vs. Patient SOX2	*** <0.0001
				Control 30-60° (Oblique) vs	ns >0.9999	Control vs. Knock-in SOX2	*** <0.0001
				Control 60-90° (Vertical) v	*** <0.0001	Control vs. Patient Rescue SOX2	ns >0.9999
				Control 60-90° (Vertical) vs	*** <0.0001	Patient vs. Patient Rescue SOX2	*** 0.0002
				Control 60-90° (Vertical) v	ns >0.9999		
				Patient 0-30° (Horizontal) v	*** <0.0001		
				Patient 30-60° (Oblique) v	ns 0.5061		
				Patient 60-90° (Vertical) v	*** <0.0001		

Ext Figure 6B	Significance P-Value	Ext Figure 6F	Significance P-Value
Control vs. Patient CP	*** <0.0001	Control vs. Patient SATB2+CTIP2+	*** <0.0001
Control vs. Knock-in CP	*** 0.0004	Control vs. Knock-in SATB2+CTIP2+	*** <0.0001
Control vs. Patient Rescue CP	ns >0.9999	Control vs. Patient Rescue SATB2+CTIP2+	ns 0.9996
Patient vs. Patient Rescue CP	*** <0.0001	Patient vs. Patient Rescue SATB2+CTIP2+	*** <0.0001
Control vs. Patient VZ	*** <0.0001	Control vs. Patient SATB2+CTIP2	** 0.0007
Control vs. Knock-in VZ	*** <0.0001	Control vs. Knock-in SATB2+CTIP2	** 0.0002
Control vs. Patient Rescue VZ	ns 0.9837	Control vs. Patient Rescue SATB2+CTIP2	ns >0.9999
Patient vs. Patient Rescue VZ	*** <0.0001	Patient vs. Patient Rescue SATB2+CTIP2	** 0.0002
Control vs. Patient SVZ	ns 0.1386	Control vs. Patient SATB2+CTIP2+	*** <0.0001
Control vs. Knock-in SVZ	ns >0.9999	Control vs. Knock-in SATB2+CTIP2+	*** <0.0001
Control vs. Patient Rescue SVZ	ns 0.9992	Control vs. Patient Rescue SATB2+CTIP2	ns 0.9917
Patient vs. Patient Rescue SVZ	** 0.0097	Patient vs. Patient Rescue SATB2+CTIP2	** 0.0012

Ext Figure 9C	Significance P-Value	Ext Figure 9E	Significance P-Value	Ext Figure 9F	Significance P-Value
p-S6+ HOPK+ vs P-S6+ TBK2+	*** <0.0001	Control vs. Patient	** 0.0017	Control vs. Patient	*** <0.0001
		Control vs. Knock-in	* 0.0028	Control vs. Knock-in	*** <0.0001
		Control vs. Patient Rescue	ns 0.9431	Control vs. Patient Rescue	ns 0.9808
		Patient vs. Patient Rescue	** 0.0072	Patient vs. Patient Rescue	*** <0.0001

Ext Figure 10C	Significance P-Value	Ext Figure 10D	Significance P-Value
Control No Treatment vs. Control Treatment	** 0.0099	Control vs. Control+HV	*** <0.0001
Control No Treatment vs. Patient No Treatment	* 0.0012	Patient vs. Patient +HV	*** <0.0001
Control No Treatment vs. Patient Treatment	ns 0.9967	MDLS vs. MDLS+HV	*** <0.0001
Control No Treatment vs. MDLS No Treatment	* 0.0045	Control vs. Patient	** 0.0074
Control No Treatment vs. MDLS Treatment	ns 0.9613	Control vs. Patient+HV	** 0.0004
Patient No Treatment vs. Patient Treatment	*** 0.0004	Control vs. MDLS	* 0.043
MDLS No Treatment vs. MDLS Treatment	*** 0.0005	Control vs. MDLS+HV	** 0.0022

Supplementary Table 5

Supplementary Table 5: List of P-values for quantifications presented in figures. P-values for all statistical comparisons organized by figure/panels.

Mass Spectrometry deposited file names (Accession PXD056364 in PRIDE)	Associated Sample names in Figures
YB7-1	Control1ExtFig8
YB7-2	Control2ExtFig8
YB7-3	Control3ExtFig8
C91	Patient1ExtFig8
C92	Patient2ExtFig8
C93	Patient3ExtFig8
YB7-4	Control1Fig4
YB7-5	Control2Fig4
YB7-6	Control3Fig4
C94	Patient1Fig4
C95	Patient2Fig4
C96	Patient3Fig4
KI1	Knockin1Fig4
KI2	Knockin2Fig4
KI3	Knockin3Fig4
MDS1	MDLS1Fig4
MDS2	MDLS2Fig4
MDS3	MDLS3Fig4

scRNA-seq deposited file names (Accession PRJNA1168541 in SRA)	Associated Sample names in Figures
RevCOrg 1	Control1ExtFig10
RevCOrg 2	Control2ExtFig10
P1	Patient1ExtFig10
P2	Patient2ExtFig10
MDS1	MDLS1ExtFig10
MDS2	MDLS2ExtFig10
CT1	Control+NV1Fig10
CT2	Control+NV2Fig10
PT1	Patient+NV1Fig10
PT2	Patient+NV2Fig10
MDSD1	MDLS+NV1Fig10
MDSD2	MDLS+NV2Fig10
YB7D70Org1	Control1Fig3
YB7D70Org2	Control2Fig3
YB7D70Org3	Control3Fig3
YB712522	Control4Fig3
C9Org12522	Patient1Fig3
3751D70Org1	Patient2Fig3
3751D70Org2	Patient3Fig3
3751D70Org3	Patient4Fig3
YB7Homo12522	KI1Fig3
YB7HomoOrg2	KI2Fig3
C9HomoOrg1	PR1Fig3
C9HomoOrg2	PR2Fig3

Supplementary Table 6

Supplementary Table 6: Deposited file names associated with figures for scRNA-seq and mass spectrometry. List of deposited file names in PRIDE (mass spectrometry public database) and SRA (sc-RNA seq public database) and their matching samples and figures used in the study.