

Supplemental material:

Table 1: FACS antibodies

Receptors and intracellular proteins were grouped in 5 panels as shown. Activation and functional panels were performed with and without stimulation as described in Materials and Methods. All panels included CD56, CD3 and CD45 to identify NK cells as CD45⁺CD56⁺CD3⁻.

Supplemental Table 2. Heterozygous and homozygous variants detected by whole exome sequencing of the proband and his affected siblings that are present at low frequency in the ExAC database.

Supplemental Figure 1. Genetic confirmation of PI3K110δ mutation. A) Pedigree of our proband showing affected (filled) and unaffected (open) family members accompanied by the Sanger confirmation of the E525K (Chr1:9780851_G>A:NM_005026:exon13:c.1573G>A) mutation. B) Schematic of PI3K110δ protein showing the positions of E525K and E1021K mutations. ABD: adaptor binding domain; RBD: Ras-binding domain.

Supplemental Figure 2. Intracellular cytokine staining. PBMC from 5 healthy donors (black circles) and PI3K110δ patients with E525K (red) or E1021K (blue) mutations were analyzed by flow cytometry following 4 hours of stimulation with phorbol myristate acetate and ionomycin in the presence of Brefeldin A. A) Intracellular cytokine expression in CD56^{bright} NK cells. **p*<0.05 by Student's two-tailed T test.

	Fluorophore	Marker	Clone; Source
Anchor (all panels)	QD605	CD56	HCD56, Biolegend
	QD711	CD3	SK7, Biolegend
	PE Cy7 or APC Cy7	CD45	J.33, Beckman Coulter
Maturation	APC	CD94	DX22, Biolegend
	APC Cy7	CD34	581, Biolegend
	Pacific Blue	CD57	NC1, Beckman Coulter
	QD655	CD62L	DREG56, Biolegend
	PE Cy7	CD117	104D2, Biolegend
	CF 594	CD16	3G8, Biolegend
	FITC	IL15Ra	151303, R&D Systems
	PE	CD122	TU27, Biolegend
Adhesion	PE Cy5	CD27	O323, Affymetrix eBioscience
	Pacific Blue	CD2	39C1.5, Beckman Coulter
	PE	CD28	L293, BD Biosciences
	PE Cy7	CD11a	HI111, BD Biosciences
	CF 594	CD11b	ICRF44, BD Biosciences
	APC	CD18	6.7, BD Pharmingen
Inhibitory	APC Cy7	KLRG1	REA261, Miltenyi Biotec
	Bv421	CD158e	DX9, Biolegend
	PE Cy7	CD158a	HP-MA4, eBioscience
	FITC	CD158b	DX27, Biolegend
	AlexaFluor 700	NKG2A	131411, R&D Systems
	PE	NKG2C	134591, R&D Systems
	PE-Cy7	NKG2D	1D11, Biolegend
Function	AlexaFluor 700	IFN γ	4S.B3, Biolegend
	FITC	Perforin	δ G9, BD Pharmingen
	Pacific Blue	GM-CSF	BVD2-21C11, Biolegend
	PE Cy7	IL-10	JES3-9D7, Biolegend
	CF 594	Granzyme B	GB11, Invitrogen
	APC	IL-13	JES10-5A2
	QD655	TNF α	Mab11, Biolegend
Activating	Pacific Blue	NKp46	9E2, Biolegend
	Bv785	CD8a	RPA-T8, Biolegend
	CF 594	CD69	FN50, BD Biosciences
	APC	NKp30	P30-15, Biolegend
	AlexaFluor 700	CD25	B1.49.9, Beckman Coulter
	FITC	CD244	2-69, BD Biosciences

Patient 1: homozygous					
Gene	Coordinates	vR	tR	Type	Frequency (EXaC)
APEX2	X:55032975_C>T	265	266	NS	0.0006
C6ORF120	6:170102970_G>A	5	6	NS	0.0006
CFHR1	1:196799651_C>T	587	587	NS	1.75E-05
CFHR3	1:196744044_A>G	280	280	NS	1.75E-05
DEFB106B	8:7686465_T>C	22	27	NS	0.0003
DMD	X:32398793_G>A	12	12	NS	
FAM83E	19:49116502_G>A	17	20	NS	0.0012
FAM123B	X:63412689_G>A	51	51	NS	0.0002
GPR119	X:129519219_C>T	400	400	NS	3.42E-05
KIR2DL1	19:55286731_G>C	5	6	NS	9.27E-06
MTMR8	X:63563592_A>T	34	34	NS	0.0006
PCDHA8	5:140222138_G>C	15	16	NS	0.0002
PCDHA8	5:140222140_G>A	8	9	NS	5.94E-05
SPANXC	X:153200861_C>T	13	13	SP	0.0078
RIN3	14:93118230_G>A	18	22	NS	0.0014
SHROOM2	X:9863139_G>T	58	59	NS	
SHROOM4	X:50377101_T>C	111	111	NS	0.0013
SPANXC	X:140335820_T>G	47	47	NS	0.0072
STARD8	X:67943959_C>T	83	83	NS	0.0018
TAF1	X:70614091_A>G	213	213	S	0.0007
ZNF91	19:23544656_C>A	126	148	NS	0.002
Patient 1 : Heterozygous					
CLPX	15:65477449_G>A	62	112	NS	0
KIAA0182	16:85691198_A>G	10	33	NS	0
CSMD2	1:34049296_T>C	160	315	NS	8.24E-06
PFDN2	1:161070592_T>C	70	150	NS	8.24E-06
MKL2	16:14354915_G>A	113	223	NS	8.24E-06
PRG2	11:57156551_C>G	142	263	NS	8.24E-06
OSBP	11:59368096_T>A	66	125	NS	8.24E-06
PPP2R1B	11:111624231_ATAG>A	36	83	NS	8.25E-06
PSG6	19:43414917_T>C	125	274	NS	8.25E-06
MFSD9	2:103340182_A>G	53	101	NS	8.25E-06
CD59	11:33743924_C>T	250	504	SP	8.26E-06
SMC2	9:106892056_A>G	50	106	NS	8.26E-06
PLCXD2	3:111394102_G>T	123	241	NS	8.26E-06
CUL7	6:43019122_C>T	80	170	NS	8.26E-06

LRRN1	3:3887010_G>A	14	30	NS	8.27E-06
CDH3	16:68716386_G>A	234	472	NS	8.27E-06
SEMA4C	2:97533568_A>G	113	242	NS	8.27E-06
ZNF860	3:32030804_C>G	128	291	NS	8.28E-06
MTHFD2	2:74434861_C>A	55	106	NS	8.29E-06
GRHPR	9:37428554_G>A	41	71	NS	8.31E-06
MLKL	16:74719433_G>T	57	127	NS	8.35E-06
TMEM184B	22:38643835_C>A	77	160	NS	8.71E-06
SCYL1	11:65303419_C>T	108	217	SP	8.98E-06
ZNF786	7:148768980_G>C	3	6	NS	9.08E-06
DEDD2	19:42713982_TG>T	4	50	SL	9.66E-06
OSR2	8:99963921_G>A	41	88	NS	1.04E-05
AGRN	1:983186_G>A	10	14	NS	1.31E-05
GRIK3	1:37285433_C>T	163	324	NS	1.61E-05
GTF2E2	8:30510979_C>G	40	85	NS	1.65E-05
CPS1	2:211512716_A>G	392	770	NS	1.65E-05
ZFYVE26	14:68250051_G>A	222	461	NS	1.65E-05
FAM13C	10:61022330_G>T	57	106	NS	1.65E-05
KIF14	1:200572990_G>T	36	115	NS	1.65E-05
PUS7L	12:44148765_G>C	66	121	NS	1.65E-05
SULF1	8:70536224_G>A	102	214	NS	1.65E-05
ABCA12	2:215819927_G>A	122	243	NS	1.65E-05
ANKMY1	2:241420464_C>T	100	213	NS	1.65E-05
MAP1LC3A	20:33137836_C>G	78	123	SP	1.65E-05
SHANK2	11:70331487_C>G	104	172	NS	1.65E-05
ZNF407	18:72343804_C>T	123	238	NS	1.66E-05
CCL20	2:228680208_C>T	20	44	NS	1.66E-05
ADAM29	4:175898929_T>A	58	282	NS	1.66E-05
ABI3BP	3:100585752_A>G	49	89	NS	1.66E-05
IRAK4	12:44167807_G>A	25	65	NS	1.67E-05
OR10H4	19:16060734_A>G	20	37	NS	1.68E-05
ZDHHC24	11:66311391_C>T	76	129	NS	1.69E-05
FAM83C	20:33875024_C>A	26	52	NS	1.72E-05
H2AFV	7:44874160_A>G	5	42	NS	1.72E-05
TGFB1I1	16:31485899_C>T	77	164	NS	1.73E-05
OR1L6	9:125512745_G>A	46	94	NS	1.73E-05

Patient 2: homozygous					
Gene	Coordinates	vR	tR	Type	Frequency (EXaC)
AHNAK2	14:105416756_C>T	45	50	NS	4.20E-05
ANKRD36B	2:98148841_T>G	134	135	NS	0.0063
DUSP1	5:172197687_C>T	5	6	NS	
FAM157B	9:141124247_A>G	5	6	NS	0.0009
GNL1	6:30520367_C>T	87	87	NS	0.0074
GPR119	X:129519219_C>T	66	67	NS	3.42E-05
KAL1	X:8504901_G>T	107	109	NS	0.0018
KRTDAP	19:35979745_G>T	30	30	SP	0.0003
MAGEC1	X:140994018_C>G	14	15	NS	7.94E-05
MUC1	1:155161505_G>C	5	6	NS	
MUC4	3:195506369_C>A	77	88	NS	6.06E-05
MUC4	3:195506630_T>C	51	54	NS	5.91E-05
PITRM1	10:3182940_C>T	35	35	NS	0.0001
RENBP	X:153200861_C>T	33	33	SP	0.0078
SPANXC	X:140335820_T>G	30	32	NS	0.0072
STK19	6:31948509_C>T	14	14	NS	0.0005
TG	8:133906136_G>A	111	111	NS	0.0062
Patient 2 : Heterozygous					
ATOH8	2:85981731_C>G	5	11	NS	0
CEP170B	14:105350603_G>A	22	51	NS	0
CLPX	15:65477449_G>A	70	145	NS	0
FAM20A	17:66596762_CCAG>C	3	48	NFS	0
GSE1	16:85691198_A>G	16	42	NS	0
PCSK4	19:1481916_G>A	25	51	NS	0
RGPD5	2:113147238_A>G	3	7	NS	0
CSMD2	1:34049296_T>C	42	99	NS	8.24E-06
PFDN2	1:161070592_T>C	100	214	NS	8.24E-06
PRG2	11:57156551_C>G	37	87	NS	8.24E-06
SREBF2	22:42280858_C>G	55	114	NS	8.24E-06
OSBP	11:59368096_T>C	138	242	NS	8.24E-06
PPP2R1B	11:111624231_ATAG>A	24	49	NS	8.25E-06
LAMP3	3:182872138_G>A	112	224	NS	8.25E-06
CD59	11:33743924_C>T	27	66	SP	8.26E-06
SMC2	9:106892056_A>G	55	103	NS	8.26E-06
PCDHGA4	5:140736922_TG>T	32	63	FS	8.26E-06

CUL7	6:43019122_C>T	12	36	NS	8.26E-06
LRRN1	3:3887010_G>A	21	42	NS	8.27E-06
CDC42BPG	11:64606626_T>G	23	63	NS	8.27E-06
CDH3	16:68716386_G>A	20	40	NS	8.27E-06
C3ORF67	3:58899563_C>T	32	63	NS	8.27E-06
CTU2	16:88776631_G>C	135	279	NS	8.28E-06
ADAM29	4:175897197_G>A	20	51	NS	8.29E-06
GRHPR	9:37428554_G>A	17	31	NS	8.31E-06
BSN	3:49698340_G>A	22	40	NS	8.34E-06
MLKL	16:74719433_G>T	63	141	NS	8.35E-06
ZNF513	2:27601855_C>T	32	71	NS	8.84E-06
SCYL1	11:65303419_C>T	28	72	SP	8.98E-06
C4ORF21	4:113482122_C>G	149	320	NS	1.02E-05
AGRN	1:983186_G>A	36	77	NS	1.31E-05
GRIK3	1:37285433_C>T	32	58	NS	1.61E-05
EYA1	8:72184042_C>T	98	158	NS	1.65E-05
IFT122	3:129231240_C>T	52	102	NS	1.65E-05
MUC4	3:195487823_C>T	106	187	NS	1.65E-05
FIBP	11:65653878_G>A	22	46	NS	1.65E-05
GTF2E2	8:30510979_C>G	71	153	NS	1.65E-05
FAM13C	10:61022330_G>T	67	148	NS	1.65E-05
PRSS37	7:141536197_AC>A	62	138	FS	1.65E-05
RTCB	22:32788315_C>T	41	92	NS	1.65E-05
ABCA12	2:215819927_G>A	75	157	NS	1.65E-05
GIPC3	19:3589438_C>T	22	38	SP	1.66E-05
ZNF407	18:72343804_C>T	86	165	NS	1.66E-05
ADAM29	4:175898929_T>A	20	64	NS	1.66E-05
ABI3BP	3:100585752_A>G	69	147	NS	1.66E-05
NUP98	11:3765754_G>A	40	99	NS	1.67E-05
OR10H4	19:16060734_A>G	25	55	NS	1.68E-05
ZDHHC24	11:66311391_C>T	35	86	NS	1.69E-05
PRDM1	6:106553812_G>C	21	40	SP	1.74E-05
MAMDC4	9:139751207_G>A	31	56	NS	1.75E-05

Patient 3: homozygous					
Gene	Coordinates	vR	tR	Type	Frequency (EXaC)
AHNAK2	14:105416757_G>C	5	6	NS	0.0012
DMD	X:32398793_G>A	16	16	NS	
DNMT1	19:10260337_G>A	9	11	SP	0.0077
GNL1	6:30520367_C>T	63	63	NS	0.0074
GPR119	X:129519219_C>T	45	45	NS	3.42E-05
IGFN1	1:201181672_C>T	6	7	NS	0.0002
MAGEC1	X:140994018_C>G	16	17	NS	7.94E-05
MUC4	3:195506369_C>A	44	49	NS	6.06E-05
MUC4	3:195506630_T>C	69	73	NS	5.91E-05
PITRM1	10:3182940_C>T	26	26	NS	0.0001
POTEI	2:131221663_G>A	13	13	NS	0.0003
PRRT1	6:32118278_T>G	10	12	NS	0.0011
RIMBP3	22:20456768_C>T	6	7	NS	
SHROOM2	X:9863139_G>T	32	32	NS	
SPANXC	X:140335820_T>G	22	23	NS	0.0072
STK19	6:31948509_C>T	10	10	NS	0.0005

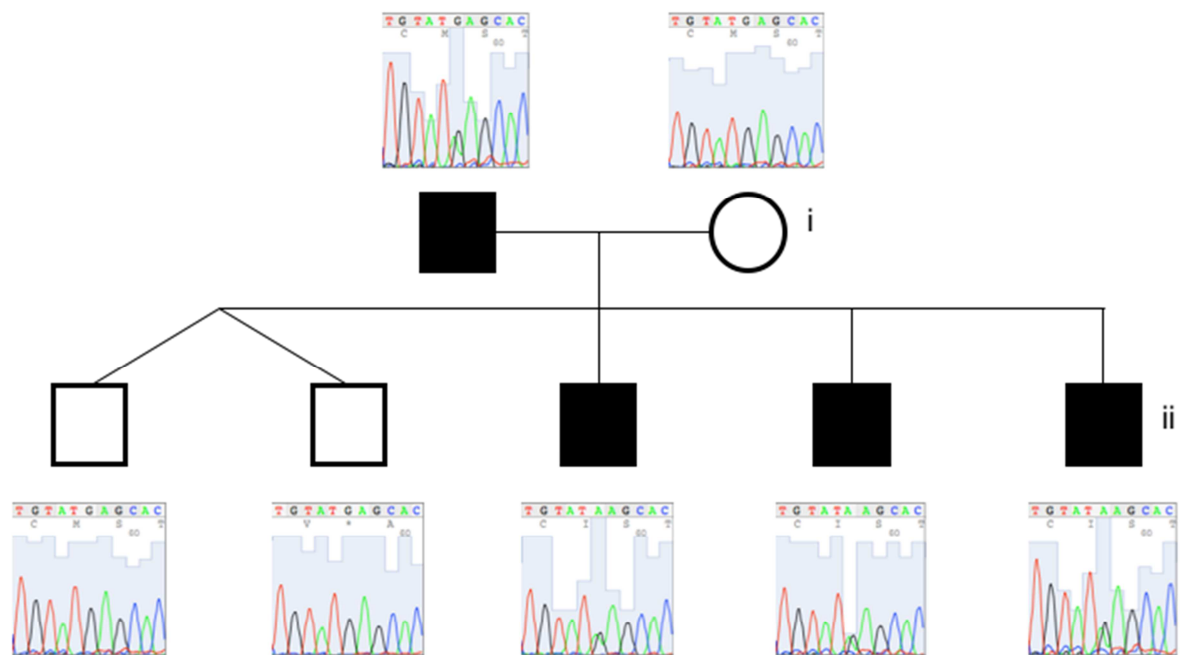
Patient 3 : Heterozygous					
CEP170B	14:105350603_G>A	18	42	NS	0
CLPX	15:65477449_G>A	44	97	NS	0
GSE1	16:85691198_A>G	15	36	NS	0
MSN	X:64956727_AAGG>A	10	81	NS	0
PCSK4	19:1481916_G>A	12	32	NS	0
THBD	20:23029435_G>C	8	12	NS	0
WT1	11:32456484_AGCC>A	3	23	NS	0
CSMD2	1:34049296_T>C	41	78	NS	8.24E-06
C2CD3	11:73843946_G>C	67	124	NS	8.24E-06
MKL2	16:14354915_G>A	49	87	NS	8.24E-06
PPP2R1B	11:111624231_ATAG>A	20	46	NS	8.25E-06
LAMP3	3:182872138_G>A	76	149	NS	8.25E-06
PLCXD2	3:111394102_G>T	12	36	NS	8.26E-06
CUL7	6:43019122_C>T	16	31	NS	8.26E-06

CDC42BPG	11:64606626_T>G	29	50	NS	8.27E-06
CDH3	16:68716386_G>A	13	28	NS	8.27E-06
C3ORF67	3:58899563_C>T	32	71	NS	8.27E-06
USP54	10:75277060_T>C	31	76	NS	8.27E-06
CTU2	16:88776631_G>C	101	175	NS	8.28E-06
MTHFD2	2:74434861_C>A	48	96	NS	8.29E-06
ADAM29	4:175897197_G>A	14	41	NS	8.29E-06
CDHR2	5:176005574_CAGG>C	2	17	NFS	8.30E-06
GRHPR	9:37428554_G>A	10	24	NS	8.31E-06
COG4	16:70557397_A>G	33	59	NS	8.32E-06
BSN	3:49698340_G>A	27	47	NS	8.34E-06
OAS3	12:113403657_G>A	27	50	NS	8.55E-06
TMEM184B	22:38643835_C>A	8	27	NS	8.71E-06
DNMT1	19:10270741_TG>T	2	20	SP	8.76E-06
ZNF786	7:148768980_G>C	19	31	NS	9.08E-06
CBWD1	9:178947_G>A	22	188	NS	1.16E-05
AGRN	1:983186_G>A	40	67	NS	1.31E-05
EYA1	8:72184042_C>T	59	126	NS	1.65E-05
MUC4	3:195487823_C>T	59	122	NS	1.65E-05
CTNNA3	10:67680222_C>T	71	179	NS	1.65E-05
FIBP	11:65653878_G>A	22	41	NS	1.65E-05
GTF2E2	8:30510979_C>G	52	123	NS	1.65E-05
RASGRF2	5:80366311_A>G	15	27	NS	1.65E-05
CPS1	2:211512716_A>G	91	157	NS	1.65E-05
KIF14	1:200572990_G>T	40	91	NS	1.65E-05
PUS7L	12:44148765_G>C	99	188	NS	1.65E-05
ABCA12	2:215819927_G>A	47	120	NS	1.65E-05
ANKMY1	2:241420464_C>T	16	25	NS	1.65E-05
MAP1LC3A	20:33137836_C>G	12	27	SP	1.65E-05
GIPC3	19:3589438_C>T	15	27	SP	1.66E-05
XIRP1	3:39228954_GTGTC>G	2	33	FS	1.66E-05
NAV2	11:20067171_A>G	11	30	NS	1.66E-05
ADAM29	4:175898929_T>A	11	44	NS	1.66E-05
IRAK4	12:44167807_G>A	20	33	NS	1.67E-05
EME2	16:1824297_TTGC>T	3	44	NFS	1.67E-05
OR10H4	19:16060734_A>G	20	50	NS	1.68E-05

vR, variant reads; tR, total reads; NS, nonsynonymous; SP, splice; FS, frameshift; NFS, non-frameshift

Supplemental Figure 1

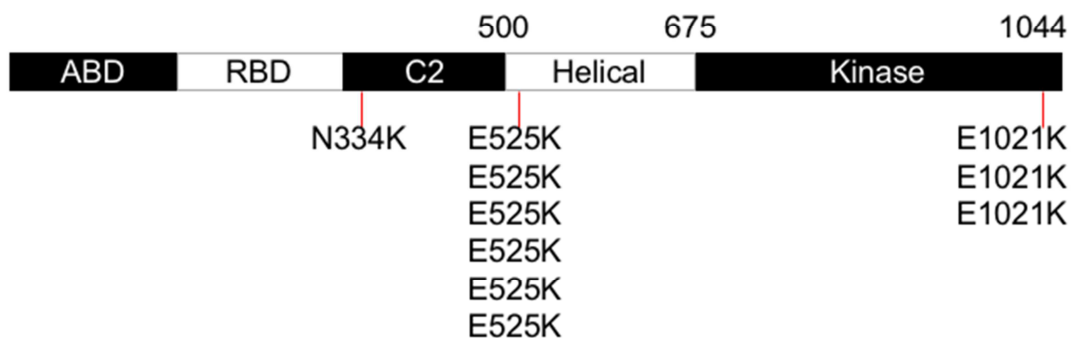
A



B

Family Relationship	# Mapped Reads	Variant Read Breakdown (%)			
		G (ref)	A	T	C
Father	289,095	51%	49%	1%	0.1%
Proband (Patient 1)	319,229	51%	49%	1%	0.1%
Affected Sibling (Patient 2)	238,666	51%	49%	1%	0.04%
Affected Sibling (Patient 3)	282,084	50%	49%	1%	0.1%
Unaffected Sibling	308,459	99%	0.4%	0.4%	0.01%
Unaffected Sibling	330,711	99%	0.4%	0.4%	0.01%

C



Supplemental Figure 2

