Supplemental information

Inherited GINS1 deficiency underlies growth retardation along with neutropenia and NK cell deficiency

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Patient	P1 (died at 18 mo)	P2 (17y)	P3 (7y)	P4 (29y)	P5 (18y)
Intrauterine growth retardation (birth)	Weight – 3,5 SD Height – 4 SD	Weight – 3,5 SD Height – 3,5 SD	Weight – 6,5 SD Height – 6,5 SD	Weight – 2,5 SD Height – 2 SD	Weight – 3,5 SD Height – 3,5 SD
Extrauterine growth retardation	Weight – 3,5 SD Height – 4 SD	Weight – 2,5 SD Height – 2 SD	Weight – 6 SD Height – 6 SD	No	Weight – 3 SD Height – 4 SD
Facial dysmorphy	Yes	Yes	Yes (with some preaging features)	Yes, mild	Yes, mild
Chest infection	Yes (*)	Yes (*)	Adenovirus RSV	Yes (*)	*Aspergillus nidulans* *Streptococcus agalactiae*
Digestive infection	*Enterobacter cloacae* Other (*)	Rotavirus *Clostridium* spp.	Rotavirus	Yes (*)	*Enterococcus faecalis* *Escherichia coli* *Candida glabrata* *Candida albicans* *Acinetobacter lowfii* *Pseudomonas aeruginosa*
Other viral infections	CMV	No	CMV VZV	VZV HSV	VZV Flu
Other bacterial infections	*Enterobacter cloacae*	*Streptococcus* spp. *Clostridium* spp.	ND	*Staphylococcus* spp.	*Enterobacter cloacae* *Escherichia coli* *Pseudomonas* *Staphylococcus hominis*
Lympho-adenopathies	Yes	Yes	Yes	Yes	No
Others clinical manifestations	Eczema	Eczema GH treatment (4y – 12y) Osteosarcoma + Chemotherapy (12y)	Protein loosing enteropathy Hypothyroidism	Dry skin - Ichtyosis Psoriatic scalp lesion (14 y)	Eczema GH treatment (3 - 4y) Glaucoma (5y-18y) G-CSF treatment (13y – 18y) AIHA (IgG+C3D) (9y, 10y, 14y, 17y)
Prophylactic treatment	No	Cotrimoxazole (3mo to 6y)	Cotrimoxazole (0 to 6mo) Azithromycin Immunoglobulins	Cotrimoxazole (15y to 17y, 18y to 24y)	Cotrimoxazole (3y – 18y) Immunoglobulins (7y – 18y)

Supplemental Table 1. Summary of the clinical phenotype of each patient in terms of developmental phenotype, infections, and treatments. (*) undetermined infectious etiology.

Patient / Stimulation	P1	P2			P3	P4			P5		
Age	18m	1y	15y	16y	4y	9y	15y	17y	9y	11y	13y
PHA (normal range >50) cpm 10^3	85	135	28	48	ND	87,7	106,6	46,5	26	68,5	21,5
OKT3 (50ng/ml) (normal range >30) cpm 10^3	ND	ND	13	ND	ND	ND	ND	ND	50,5	ND	ND
Beads anti-CD3/CD28 + IL2	ND	ND	ND	ND	0	ND	ND	ND	ND	ND	ND
Candidin (normal range >10) cpm 10^3	1	10	0,67	0,4	ND	6	4,7	0,2	5,3	7	13
Tuberculin (normal range >10) cpm 10^3	ND	ND	ND	0,8	ND	2	ND	ND	6,8	ND	ND
Tetanus (normal range >10) cpm 10^3	2	15	5	9	ND	53	16	7,1	6,3	1,4	2,45

Supplemental Table 2. T lymphocytes proliferation of each patient.

Patient		P2				P3		P4				P5
Experiment		1	2	3	4	1	2	1	2	3	4	1
Lymphocytes	Cells per µl	1253	1297	1558	1077	1053	688	1105	1018	1070	879	360
Total NK cells	% among lymphocytes	0.1	0.2	0.2	0.2	0.7	0.3	0.1	0.2	0.1	0.1	0.1
	Cells per µl	1	3	3	2	7	2	1	2	1	1	0.4
$CD56^{bright}$ NK cells	% among NK cells	0	3	10	NA	29	13	27	25	30	NA	20
$CD56^{dim}$ NK cells	% among NK cells	100	97	90	NA	71	87	73	75	70	NA	80

Supplemental Table 3. Count and percentage of total NK, $CD56^{bright}$, $CD56^{dim}$ cells of each patient.

	P1	P2	P3	P4	P5
Single/Paired-end	Paired-end	Paired-end	Paired-end	Paired-end	Paired-end
Bait-set	50Mb	50Mb	71Mb	71Mb	71Mb
Total reads	30893815	34310743	101158606	67204180	76147288
% Mapped	0,9169	0,9343	0,9884	0,9898	0,9912
Mean coverage	21,3426	21,4846	89,21	63,08	69,3
Target bases covered by > 2X	91,732	94,146	99,9	99,7	99,8
Target bases covered by > 5X	79,52	82,502	99,4	99	99,2
Target bases covered by > 10X	65,036	68,16	98	96,8	97,2
Target bases covered by > 20X	42,032	43,594	92,8	88,6	89,8
Target bases covered by > 30X	25,56	25,702	85,2	77,5	79,7

Supplemental Table 4. Exome sequencing data. Whole Exome sequencing quality for each independent patient (P1, P2, P3, P4, P5).

Whole Exome Sequencing		# of variants
Nonsense (stop-gained)	Total	0
	Homozygous	0
	Heterozygous	0
Readthrough (stop-lost)	Total	0
	Homozygous	0
	Heterozygous	0
Missense	Total	6
	Homozygous	1
	Heterozygous	6
Silent	Total	1
	Homozygous	0
	Heterozygous	1
Frameshift	Total	23
	Homozygous	7
	Heterozygous	16
UTR	Total	112
	Homozygous	3
	Heterozygous	109
Splice	Total	3
	Homozygous	0
	Heterozygous	3

Supplemental Table 5. Summary of reported GINS1 variants found in our in-house database of 3,000 exomes from patients without GINS1 deficiency.

P1 & P2	P3	P4	P5
PINX1	**AK2,**	SCNN1D	THAP3
CLDN9	SPATA6	**SLC27A3**	**CROCC**
	PSRC1,	**IGSF9**	RUNX3
	XPR1	POU2F1	PAFAH2
	AGT,	**REN**	PSRC1
	SIX2	MDM4	SMG7
	CCNT2,	**OBSCN**	GTDC1
	TTN	MTR	**TTN**
	CTDSPL,	EHD3	PECR
	DAG1	**HOXD1**	**MROH2A**
	RAD54L2	**TTN**	FANCD2
	EXOC1	**ANKAR**	**SCN5A**
	F13A1	SGPP2	WDR6
	CAPN11	SNED1	**HTRA3**
	HS3ST5	SLC6A11	**OCIAD2**
	CCR6	**CDC20B**	**KIAA1211**
	CREB5	**SPINK14**	**CNOT6L**
	CUX1	FAM26E	DCHS2
	NAPEPLD	**GLI3**	**EXOC3**
	TMEM168	**ZAN**	SNX18
	SHH	ERMP1	ARRDC3
	ST3GAL1	**FAM208B**	CDYL
	FRMD4A	SFMBT2	**GSTA5**
	LDB3	ITPRIP	HEBP2
	ANKRD1	**CNTN5**	**TULP4**
	ART5	POU6F1	TNRC18
	CDC42BPG	NUPL1	ZC3HAV1
	CIT	**ELMSAN1**	**CHRNA2**
	CDH24	CHP1	**ADAMTS13**
	AHNAK2	ALPK3	PLXDC2
	CHTF18	IGF1R	**MICALCL**
	CACNA1H	**MFSD6L**	USH1C
	AMFR	**KRTAP4-8**	TNKS1BP1
	DHX38	ALDH3A1	**HOXC8**
	DPEP1	**SLC38A10**	STK24
	SHPK	**USHBP1**	**KCNK10**
	MYO15A	**ZNF772**	YY1
	RHOT1	PCK1	TMCO5A
	ZNF207	**SLC19A1**	**ZACN**
	TAC4	**TNRC6B**	**EPG5**
	RYR1	PNPLA3	**SIPA1L3**
	BCR	PHEX	**ZNF805**
	XIAP		DZANK1
	TMEM185A		SPO11
	BGN		APOBEC3F
	CR1		TSPAN7
	KLHL23		**TM4SF2**
			GPR112
			SMG7
			EIF2AK2
			CRAMP1L
			IGF1R

Supplemental Table 6. List of genes with rare, homozygous or compound heterozygous variants (MAF<1%), predicted to be damaging (CADD>MSC). The genes with at least one variant in the coding region are in bold.

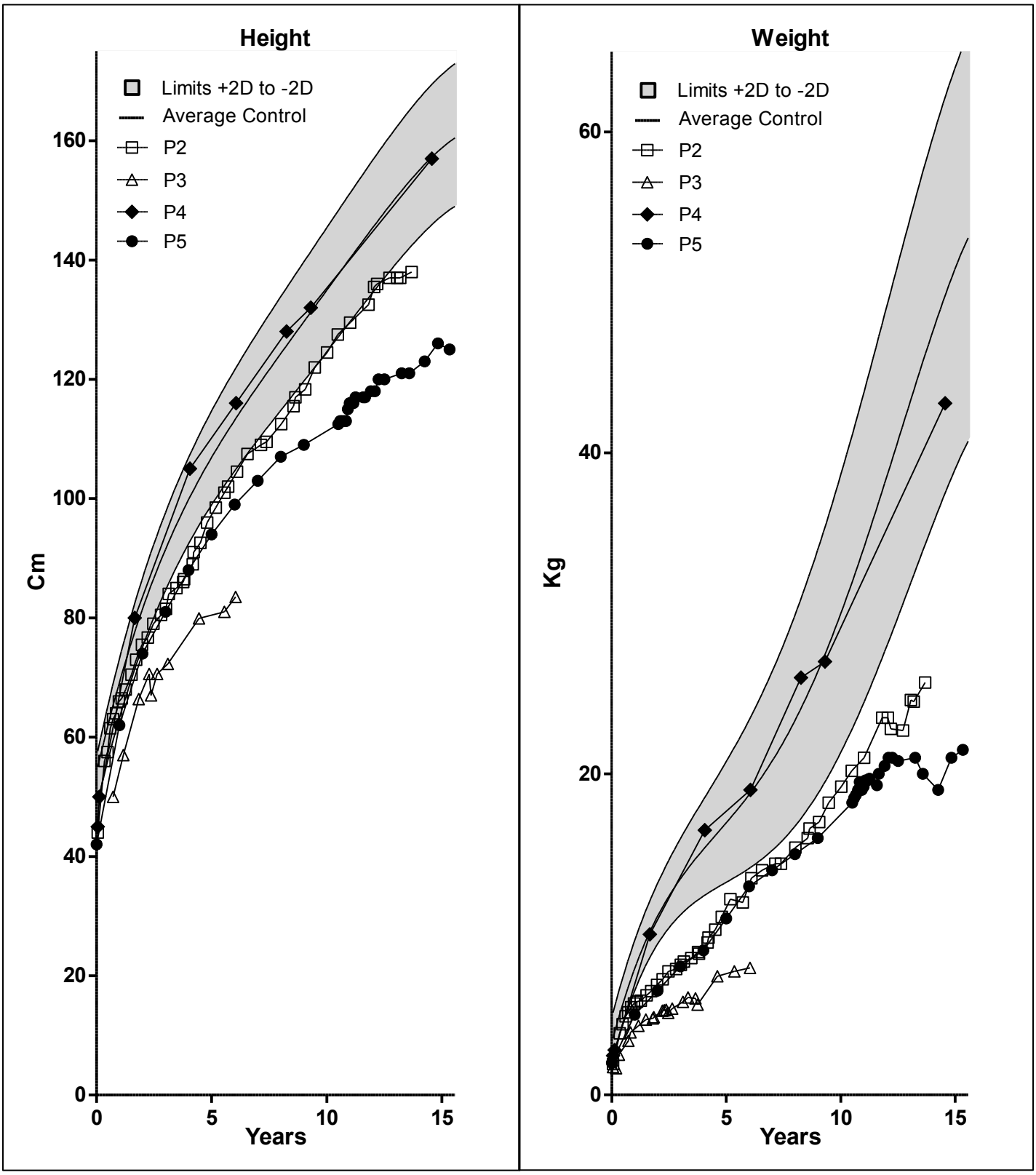
Protein	CTL	P2	P3	P4	P5
GINS1	100%	43%	36%	53%	29%
GINS2	100%	129%	116%	127%	106%
GINS3	100%	19%	10%	27%	13%
GINS4	100%	69%	44%	88%	59%
MCM4	100%	128%	59%	124%	120%

Supplemental Table 7. Quantification of GINS complex components expression. Signals' intensities obtained on western blot analysis (Figure 3, C and D) were assessed by ImageJ software and normalized to GAPDH expression and to control expression (one experiment).

	NT					0.5 mM HU					2 mM HU				
Protein	**CTL**	**P2**	**P3**	**P4**	**P5**	**CTL**	**P2**	**P3**	**P4**	**P5**	**CTL**	**P2**	**P3**	**P4**	**P5**
P-CHK1 (%)	100	122	195	100	66	100	35	16	31	62	100	37	29	45	70
P-RPA (%)	100	20	73	37	39	100	28	35	38	91	100	64	71	88	80

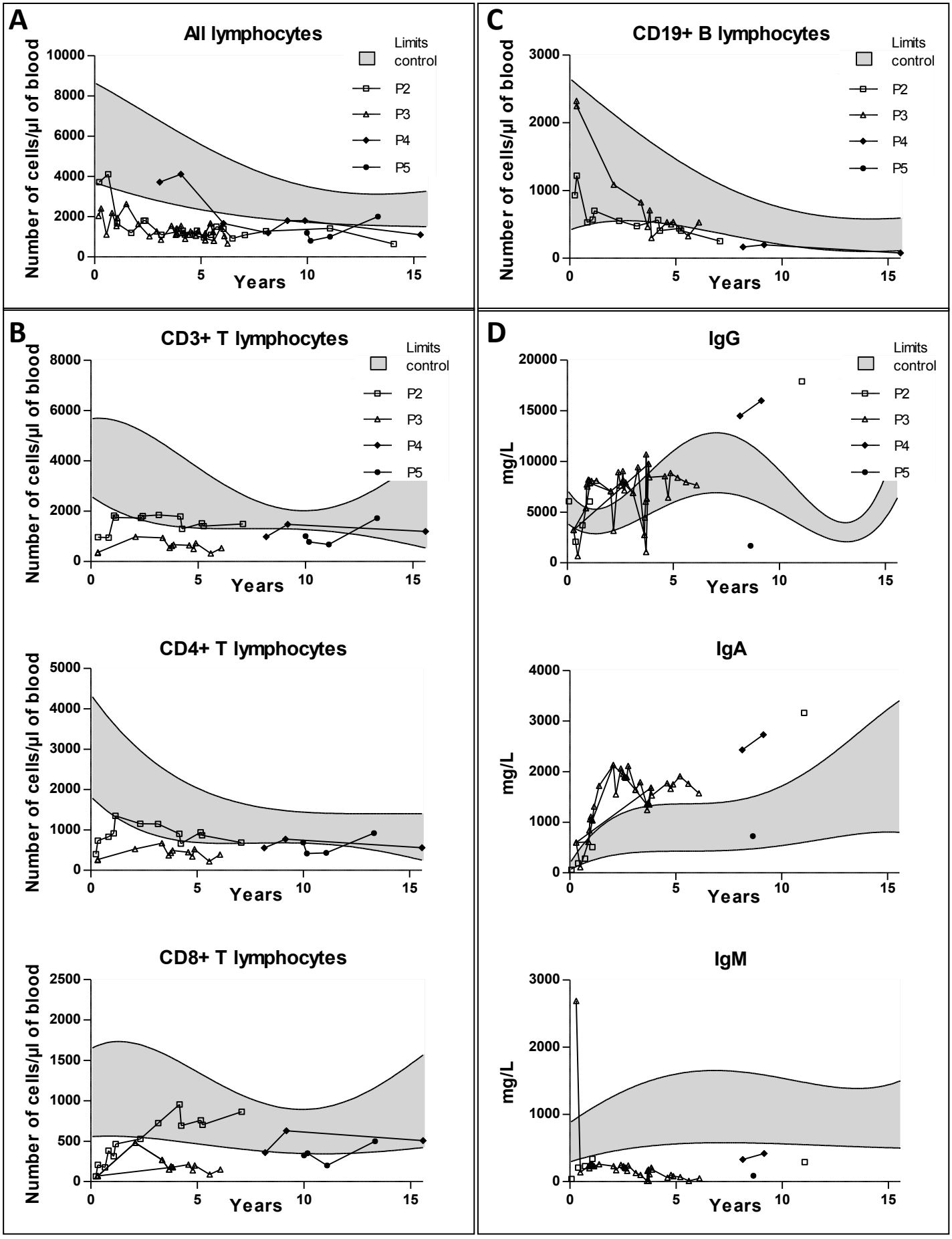
Supplemental Table 8. Quantification of CHK1 and RPA phosphorylation. Signals' intensities obtained on western blot analysis (Figure 5C) were assessed by ImageJ software and normalized to GAPDH and expression control (one experiment).

Supplemental Figure 1



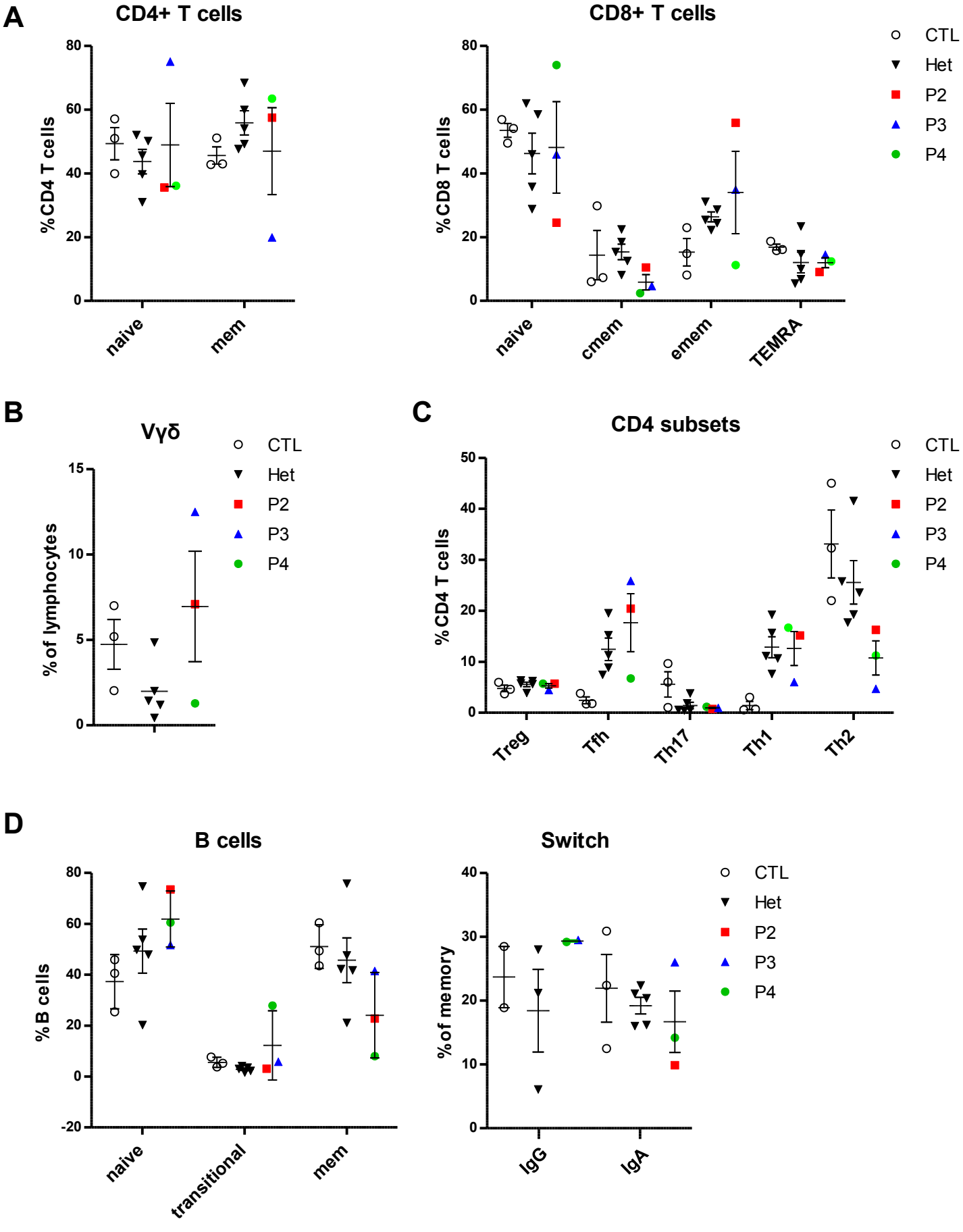
Supplemental Figure 1. **Developmental growth**. Height and weight curves of all GINS1-deficient patients. The black line mean growth and the gray area indicates two standard deviations on either side of the mean.

Supplemental Figure 2

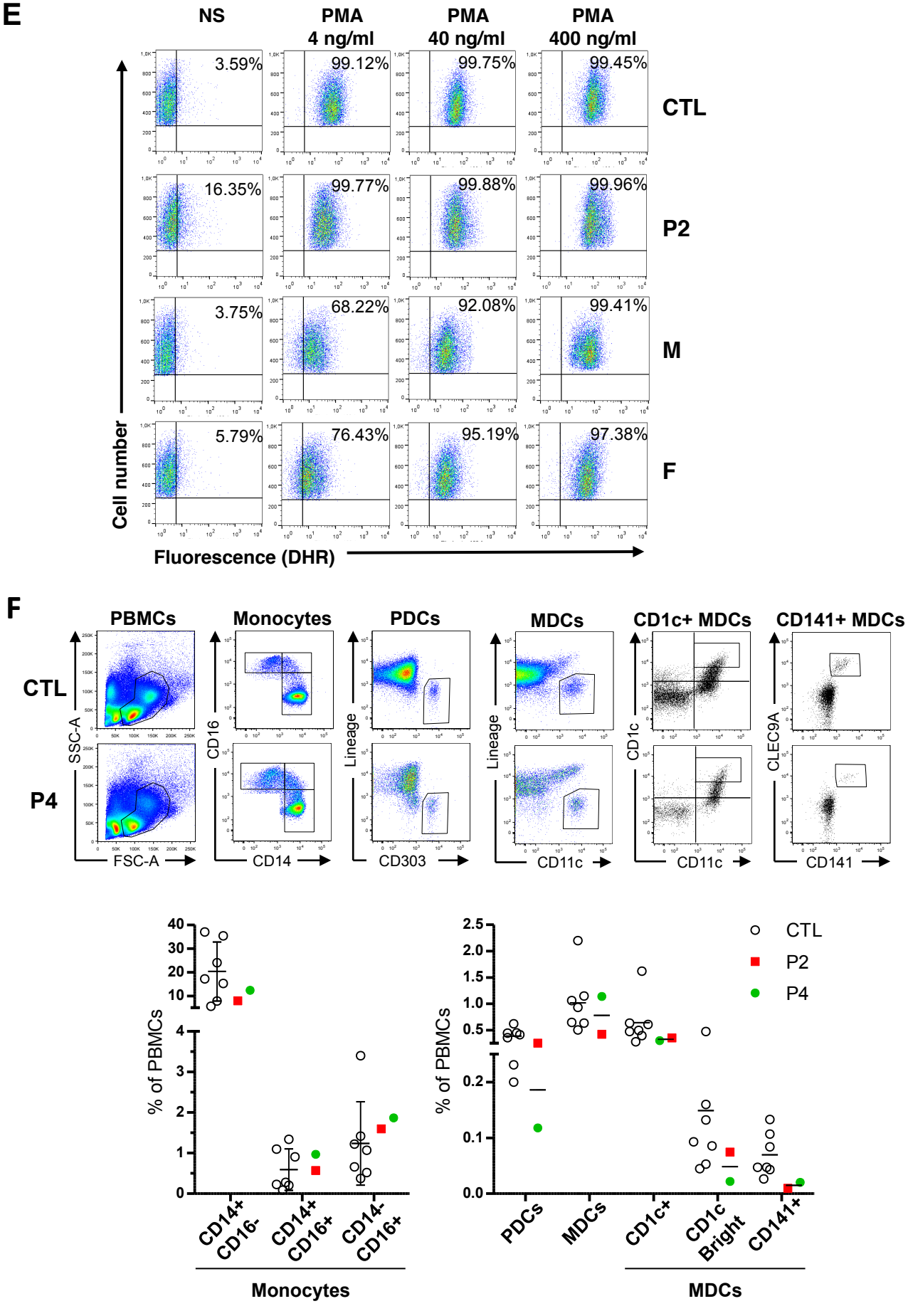


Supplemental Figure 2. Classical immunophenotype of patients. (**A**) Total lymphocytes. (**B**) $CD3^+$, $CD4^+$, $CD8^+$ T lymphocytes. (**C**) $CD19^+$ B lymphocytes. (**D**). Immunoglobulins. The gray area corresponds to the range observed in controls.

Supplemental Figure 3

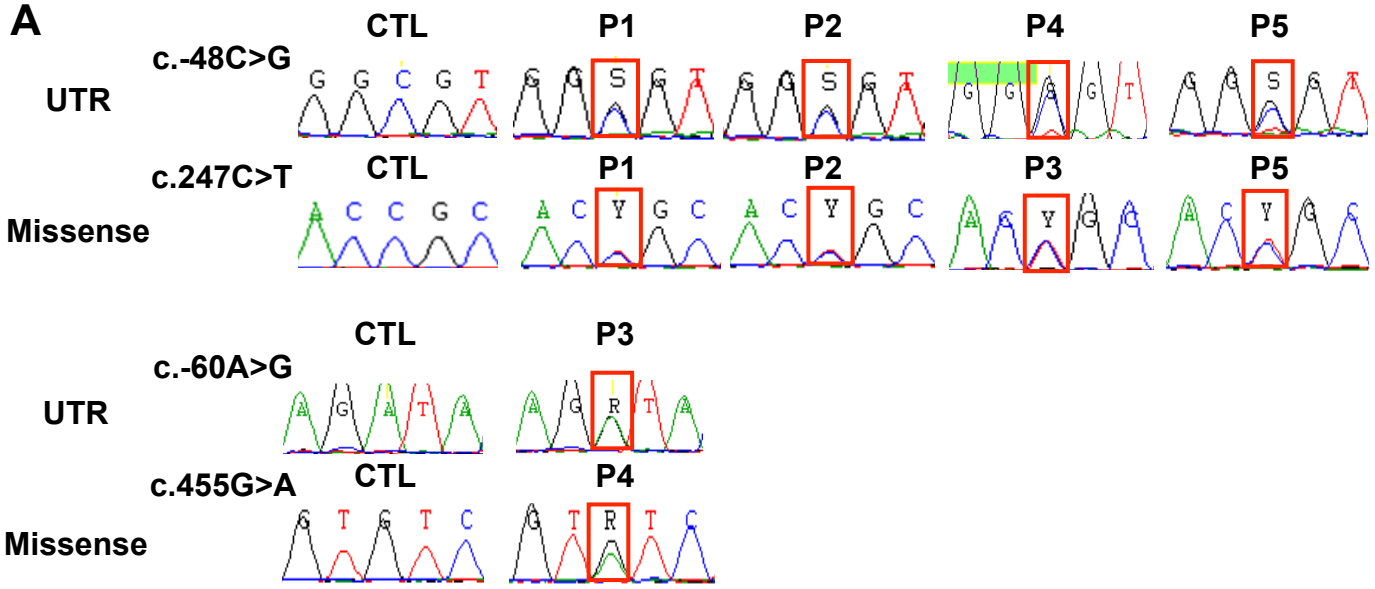


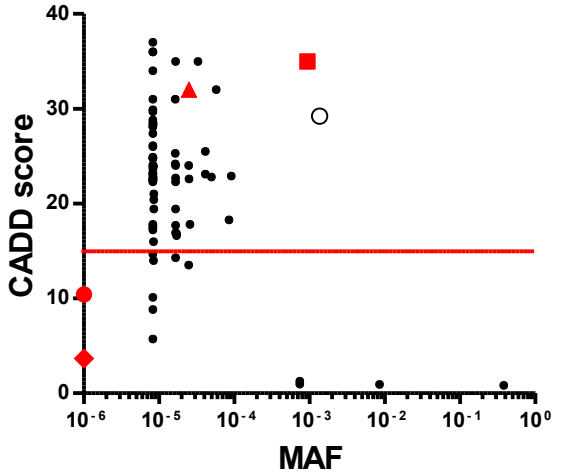
Supplemental Figure 3

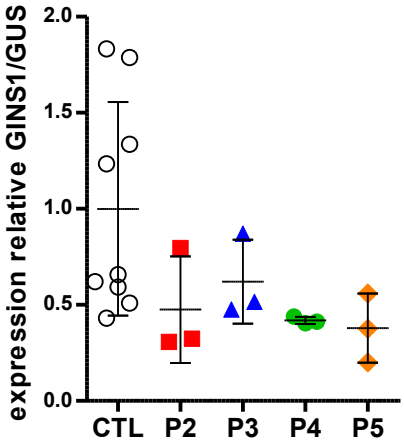


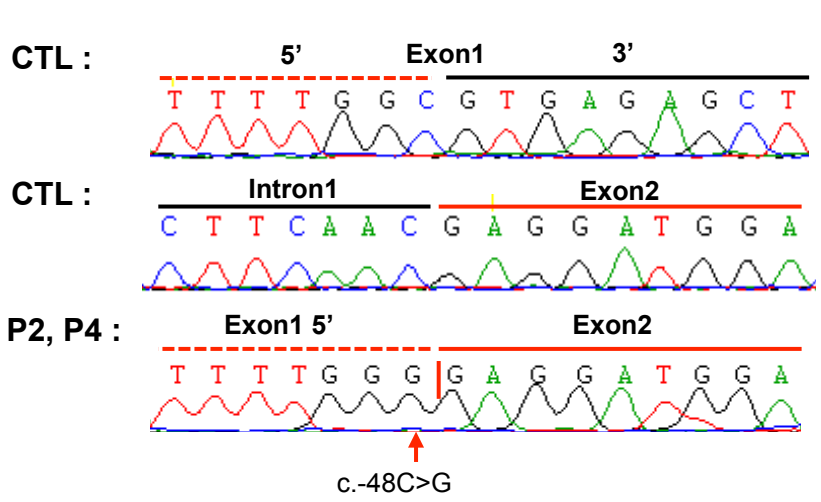
Supplemental Figure 3. **Lymphoid and myeloid cells subsets phenotype**. Frozen PBMCs from healthy control subjects or patients. (**A**) Proportion of naïve ($CD45RA^{+}CCR7^{+}$) and memory ($CD45RA^{-}CCR7\pm$) cells among $CD4^{+}$ lymphocytes (left panel) and of naïve ($CD45RA^{+}CCR7^{+}$), central memory (cmem) ($CD45RA^{-}CCR7^{+}$), effector memory (emem) ($CD45RA^{-}CCR7^{-}$ and TEMRA ($CD45RA^{+}CCR7^{-}$) cells among $CD8^{+}$ lymphocytes (right panel). (**B**) Percentage of Vγδ cells in total lymphocytes. (**C**) Quantification of $CD4^{+}$ T-cell subsets, Treg cells ($CD25^{+}CD127^{lo}$), T follicular helper (Tfh) cells ($CD45RA^{-}CXCR5^{+}$), Th1 cells ($CD45RA^{-}CXCR3^{+}CCR6^{-}$), Th17 cells ($CD45RA^{-}CCR6^{+}CXCR3^{-}$), Th2/ Th9 cells ($CD45RA^{-}CXCR3^{-}CCR6^{-}$). (**D**) Percentage of transitional ($CD10^{+}$), naïve ($CD27^{-}$) , memory ($CD27^{+}$) B cells. (**E**) Flow cytomety analysis of intracellular H_2O_2 production, using the fluorescent DHR123 probe in neutrophils from healthy controls (CTL), P2 and P2's parents (F: father, M: mother) stimulated by incubation with different concentrations of PMA for 10 minutes. The results shown are representative of two independent experiments. (**F**) Percentage of different myeloid cells, Left : $CD14^{+}$ $CD16^{-}$ cells, $CD14^{+}$ $CD16^{+}$ cells, $CD14^{-}$ $CD16^{+}$ cells, Right: PDCs (Lin^{-}, $CD303^{+}$), MDCs (Lin^{-}, $CD11c^{+}$), $CD1c^{+}$ MDCs ($CD1c^{+}$, $CD11c^{+}$), $CD141^{+}$ MDCs ($CLEC9A^{+}$, $CD141^{+}$) in controls (n=7) and patients (n=2).

Supplemental Figure 4



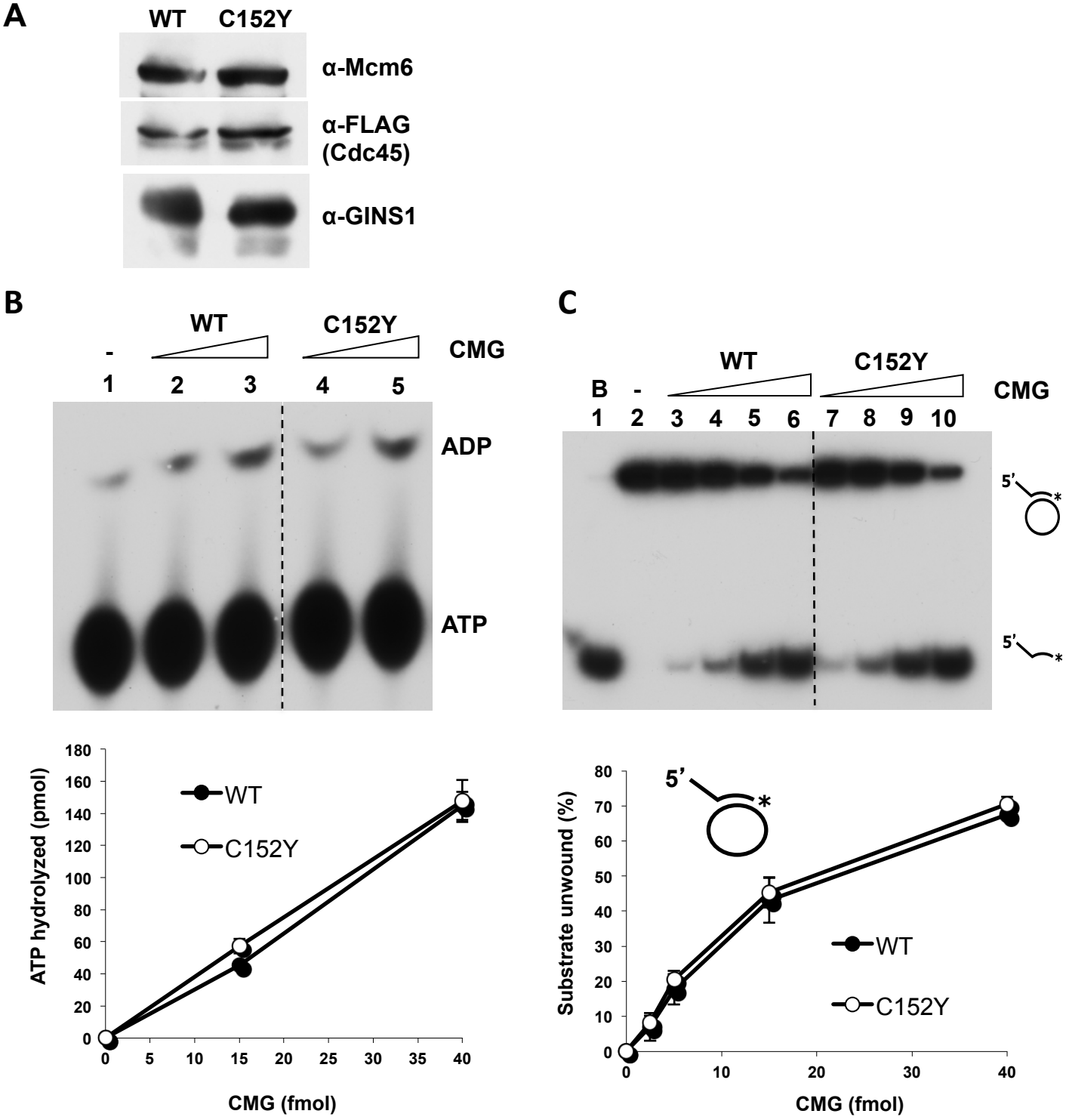






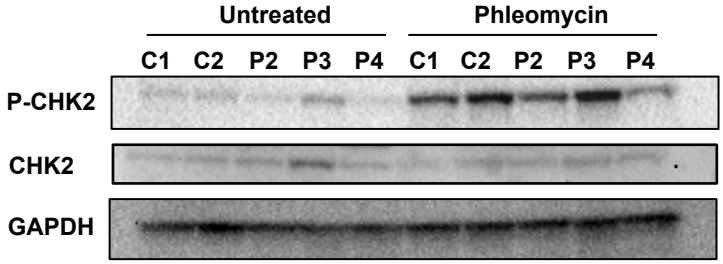
Supplemental Figure 4. gDNA sequence and mRNA levels. (**A**) Sanger sequence of the gDNA, for all patients. The heterozygous profile of each mutation is shown for P1 to P5. (**B**) Representation of the frequency of missense variants in the ExAC database as a function of their impact, as predicted by CADD score. (**C**) GINS1 mRNA levels in E6/E7-fibroblasts from GINS1-deficient patients, as compared with control cells by q-PCR. (**D**) Sanger sequence of a *GINS1* cDNA with a deletion of part of the exon1 from P2 and P4, aligned with control sequences.

Supplemental Figure 5



Supplemental Figure 5. **Biochemical activities of WT and mutant CMG**. (**A**) Soluble extracts (2 µl) prepared from Sf9 cells infected with CMG-expressing baculoviruses (including either WT or C152Y GINS1) were separated on 10% polyacrylamide gel electrophoresis. Expression of Mcm6, Cdc45, and GINS1 were detected by western blotting. (**B**) ATP hydrolysis activities were measured with increasing levels (15 and 40 fmol) of WT (lanes 2 and 3) and mutant (lanes 4 and 5) CMG complexes as described in materials and methods (top). The amount of hydrolyzed ATP was calculated and plotted against the level of CMG added (bottom). (**C**) DNA unwinding activities were measured with increasing levels (2.5, 5, 15, and 40 fmol) of WT (lanes 3-6) and mutant (lanes 7-10) CMG complexes as described in materials and methods (top). The structure of the DNA substrate and the unwound oligomer are illustrated on the right side of the gel. The amount of unwound substrate was calculated and plotted against the level of CMG added (bottom). B: boiled substrate.

Supplemental Figure 6



Supplemental Figure 6. **Phosphorylation of CHK2**, assessed by western blot of total protein extracts from untreated or treated (incubation for 2 h with 80 µg/ml of phleomycin) E6/E7-fibroblasts from controls and patients, with antibodies against P-CHK2 and CHK2. GAPDH was used as a loading control. (*n*=3)