Table E1. Clinical features of DOCK8 immunodeficient patient cohorts followed at the NIH

	Patient	Age (yr)	HPV	MCV	HSV	VZV	EBV	URTI	LRTI	Staph skin infxn	Pneumocystis	Fungal infxn	Severe infxn	Lymphopenia	Specific Abs	Eczema	Asthma	Food allergy	Eosinophilic GI ds	Poor growth	IgE	Eosinophilia	Liver ds	HPV malignancy	Lymphoma	Vascular abnl	AIHA/ITP	Death	Age adjustment	Total score	Viral burden	Atopic burden	Infectious burden	Malignancy
Repaired	1	29	3	1	0	0	2	1	2	0	0	1	2	2	2	3	0	2	0	0	1	0	2	0	0	0	0		24	6	6	12	0
	2	21	3	3	3	0	2	1	1	1	0	0	0	2	2	3	0	2	0	2	1	2	2	0	0	2	0		32	11	8	14	0
	3	23	3	3	0	1	0	2	1	0	0	0	0	2	3	2	2	1	0	0	0	1	1	0	0	0	0	0		22	7	5	12	0
	4	19	3	3	2	0	0	2	1	0	0	1	2	2	2	2	1	0	0	0	0	1	1	0	0	0	0	0		23	8	5	14	0
	5	19	1	3	2	0	0	2	2	1	1	2	0	3	2	2	1	2	3	0	1	1	0	0	3	0	0		32	6	10	14	3
	6	10	1	0	3	1	2	0	0	1	0	2	0	1	1	3	0	2	0	2	1	0	0	0	0	0	0		20	7	6	10	0
	7	5	0	0	3	0	0	1	1	1	0	0	2	3	1	3	1	2	3	2	1	2	0	0	0	0	0	5	31	3	12	8	0
	8	4	1	0	3	0		1	1	1	1	2	2	2	1	3	1	1	0	0	1	2		0	0	0	0	5	28	4	8	12	0
	9	28	1	0	0	2		1	2	1	0	0	0	3	2	3	1	0	0	0	1	1	0	0	3	2	0	2	25	3	6	7	3
	10	27	3	0	3	0	2	1	2	0	0	0	0	3	2	0	0	2	0	0	0	1	1	0	2	0	0	0	22	8	4	11	2
	11	25	3	0	0	0	2	2	3	0	0	1	0	3	2	0	0	0	0	2	0	0	0	1	0	0	0		19	5	0	11	1
	12	6	0	0	3	0	2	2	2	1	1	1	2	1	1	2	1	0	0	0	0	2	0	0	0	0	0		21	5	5	14	0
	13	18	3	0	0	0	0	2	2	1	0	2	2	3	2	2	0	2	0	0	1	1	0	0	0	0	0		23	3	6	12	0
	14	18	3	3	0	0	2	0	1	1	0	0	0	1	1	2	0	0	0	0	0	1	1	0	0	0	0	0	16	8	4	10	0
	15	16	1	1	0	0	2	3	2	0	0	0	0	1	2	2	0	0	0	0	0	1	1	0	0	3	0	0	19	4	4	9	3
	16	17	3	0	0	0	0	2	1	0	1	0	2	3	2	0	0	0	0	0	0	0	0	0	0	3	0	0	17	3	0	9	3
	17	11	3	0	2	0	0	2	1	0	0	1	0	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	14	5	2	9	0
Unrepaired	18	21	3	0	0	1		2	2	1	0	0	2	3	2	2	1	2	2	0	1	2	0	3	0	0	0	2	31	4	10	11	3
	19	18	1	0	2	2	2	2	2	1	0	2	2	2	2	2	0	1	0	2	1	2	0	0	0	0	0		28	7	6	16	0
	20	10	1	1	2	0	0	3	1	1	0	1	2	3	2	3	1	1	0	0	1	2	0	0	0	0	0	3	28	4	8	12	0
	21	8	0	0	2	0	0	2	2	1	1	1	2	3	2	2	0	1	0	2	1	2	2	0	0	0	2		28	2	6	11	0
	22	8	1	0	2	0	2	1	2	1	0	0	0	3	2	3	1	1	0	2	1	2	0	0	0	0	0		24	5	8	9	0
	23	6	0	0	2	0	2	1	1	1	0	0	0	2	2	3	1	1	0	2	1	2	0	0	0	0	0		21	4	8	7	0
	24	2	0	0	2	0	0	0	0	1	0	1	0	1		2	0	1	0	2	1	2	0	0	0	0	0	5	18	2	6	4	0
	25	14	3	0	0	0	0	2	1	1	0	1	2	2	2	3	1	1	0	0	1	2	0	0	0	0	0		22	3	8	10	0
	26	6	0	1	0	1	0	0	1	1	1	2	2	3	2	3	1	2	0	2	1	1	2	0	0	0	0		26	2	8	9	0
	27	13	0	3	0	2	2	1	2	1	0	0	2	3	2	2	1	0	3	2	1	2	0	0	0	2	0		31	7	9	13	0
	28	9	0	0	2	0	0	2	2	0	0	2	2	1	0	2	0	2	0	2	1	2	2	0	0	0	0		22	2	7	10	0
	29	8	0	3	0	0	0	2	2	1	1	0	0	3	2	3	0	2	0	2	1	2	0	0	0	0	0		24	3	8	9	0

The patients' mutational analyses are shown in Table 1 for those with somatic repair, and in Table 2 for those incapable of somatic repair.
Five additional patients in the NIH cohort were not included in this analysis because of insufficient material to determine whether they showed any somatic repair.
Key to scores:
Age: Age at last evaluation, time of transplant or death.
HPV/MCV: 0= none; 1= localized; 2=disseminated
HSV: 0=none; 1=severe primary disease exluding keratitis; 2=recurrent HSV>3x/year or on chronic prophylaxis; 3=keratitis or systemic infection
VZV: 0=none; 1=severe primary or zoster once; 2=recurrent zoster
EBV: 0=no viremia; 2=persistent EBV viremia (>2 episodes)
URTI: 1=URI>4 episodes/year; 2=myringotomy tubes or sinus surgery; 3=mastoiditis or invasive disease complicating sinusitis
LRTI: 1=pneumonia/bronchitis>1 episode; 2=bronchiectasis; 3=baseline oxygen requirement
SSTI: 1=cellulitis or skin abscess
Pneumocystis: 1=infection
Fungal infxn: 0=none; 1=recurrent oral/vaginal; 2=fingernail; 3=disseminated histoplasmosis/cryptococcus/coccidiodes
Severe infection: 0=none; 2= present
Lymphopenia: 0=none; 1=CD4 lymphopenia; 2=CD8 lymphopenia; 3=NK lymphopenia
Specific abs: 0=normal responses; 1=abnormal polysacchaide pneumococcal response; 2=abnormal protein conjugate response
Eczema: at worse stage,0=absent; 1=mild; 2=moderate; 3=severe
Food allergy: 0=no food allergy; 1=food allergy; 2=food allergy with anaphylaxis
Eosinophilic GI disease: 0=none; 2=esophagitis; 3=GI tract beyond esophagus
Poor growth: 2=<5th percentile for weight and/or height
IgE: 0=normal; 2=elevated serum IgE
Eosinophilia: 0=<600/uL; 1=600-5000/ul; 2=>5000/ul
Liver disease: 2=persistent AST/ALT>2x upper limits of normal; 3=sclerosing cholangitis
HPV malignancy: 0=absent; 1=dysplasia; 2=localized squamous cell carcinoma; 3=metastatic squamous cell carcinoma
Lymphoma: 3=present; 4= relapse
Vascular abnormality: 2=arterial dilation, calcification or vasculitis
AIHA/ITP: 2= hemolytic anemia and/or ITP
Death: 2= by age 30; 3= by age 20; 4=by age 10
Age adjustment: add 5 points for age 5 or less

Table E2. Primer sets used in this study (5' to 3')

Amplicon	Forward Primer Sequence	Reverse Primer Sequence
DOCK8 cDNA sequencing		
DOCK8cDNA4-836	TTTGTCTCCTGTAACAATTTACGC	TTTGAACTGGTCAGAGTTCAGG
DOCK8cDNA736-1503	AAATTGAGCCCCTGTTTGC	TTTCAGGAAAGGGTTTCACG
DOCK8cDNA1400-2162	CAAGTCAATTCCAGGCTTGC	GAAGGTCACCTGGCTCTCC
DOCK8cDNA2090-2869	TGTACACACCCAGGACAACC	ACCAGAAGCTCAAAGAAGAACC
DOCK8cDNA2772-3533	CATTTCCATGAGGAGCTTGC	GGCGATTTTGACCTTCACC
DOCK8cDNA3467-4197	CAGCCTGCTAAGTTCTCACG	TGCTTCTGTAGCCAGATTGC
DOCK8cDNA4113-4851	CGGCAAGCTAATGAGAAGC	GTTTCTCTGCCATGTTCTGG
DOCK8cDNA4729-5503	TGAAAATGAGGGAATTTCAGG	TCCACAAAAGTGATCTGTATGTAGG
DOCK8cDNA5463-6183	TTTTGGTGCAGAATTTGTGG	ACAACTGGGTTTCACATTTCC
DOCK8cDNA6114-7014	TTCAGAGTTGAGAGTCAAAAGAGG	AGAAACTTGGTCCCAGAGAGG
mRNA splicing analyses in Patients 16 and 17		
Exon 2-Exon 13	AGGACTTTGCAGCCCTCTTTGCC	TTTCAGGAAAGGGTTTCACG
Exon 23-Exon 24	GTGAGAGAAACAGTCTTCAAG	TGGTTTTTACTAAAAGGGCTG
cDNA cloning for SNP analyses in Patient 12		
Exon 32-Exon 34	CCGATTTCCAGGCCTAAATG	TGCAGTGGTGCAGGACTTGG

Table E3. *DOCK8* mutational analyses in the NIH patients incapable of somatic repair

Patient	Germline mutations	Nomenclature
18	Homozygous large deletion (exons 5 to 24)	Chr9:g.301,734_388,139del, c.426_3017, p.109A_972Kdel
19	Homozygous large deletion (exons 28 to 35)	Chr9:g.414,668_430,067 del
20	Homozygous large deletion (exons 5 to 9)	Chr9:g.(300,972_391,582)_(323,232_323,291)del, c.325_921del, p.A109_K307del
21	Homozygous large deletion (exons 10 to 23)	Chr9:g.333,830_394,034del, c.921_2765del, p.308S_922Ldel
22	Homozygous small indel (exon 13)	c.1325_1326delTG, p.L442RfsX16
23		
24		
25	Large deletion (exons 1 to 45) + small indel with frameshift mutation (exon 17)	Chr9:g.271,361_452,291del, plus Chr9:g.372,260_272,348del89
26		
27	Large deletion (exons 3 to 25) + large deletion (exons 22 to 32), with overlap from exons 22 to 25	Chr9:g.(287,561_287,591)_(399,673_399,716)del, plus Chr9:g.(381,489_390,463)_(421,985_434,742)del
28	Large deletion (exons 39 to 47) + small indel with frameshift mutation (exon 45)	Chr9:g.439,006_465,450del, plus c.5815_5816insT, p.Y1939LfsX12
29	Large deletion (exons 1 to 47) + splicing mutation (exon 6)	Chr9:g.214,340_465,450del, plus c.623+1G>A, p.E180VfsX4

Germline mutational analyses were performed on genomic DNA isolated from neutrophils, and in some cases also *Herpesvirus saimiri*-transformed T cells or Epstein-Barr Virus-transformed B cells. Parenthetical information indicates where the mutation is located. Genomic sequencing was also performed on both parents to establish that the patients' mutations were inherited on different alleles, except for Patients 20, 27, 28, and 29, whose fathers' DNA was unavailable.

Table E4. Somatic repair in PBMC subsets from Patients 10 and 11

	Cells	# clones wildtype (wt)	# clones with indel mutation	wt/indel	% of cells with repaired genotype
Patient 10	$CD4^+$ T cells	69	24	2.9	48
	$CD4^+$ $CD45RA^+$ T cells	48	40	1.2	9
	$CD4^+$ $CD45RO^+$ T cells	78	12	6.5	73
	$CD8^+$ T cells	68	11	6.2	72
	$CD8^+$ $CD45RA^+$ T cells	79	9	8.8	80
	$CD8^+$ $CD45RO^+$ T cells	81	8	10.1	82
	$CD19^+$ B cells	53	41	1.3	13
	$CD56^+$ $CD3^-$ NK cells	49	46	1.1	3
	$CD14^+$ Monocytes	40	53	0.8	-14
	Fibroblasts	47	45	1.0	2
Patient 11	$CD4^+$ T cells	55	23	2.4	41
	$CD4^+$ $CD45RA^+$ T cells	57	36	1.6	23
	$CD4^+$ $CD45RO^+$ T cells	70	20	3.5	57
	$CD8^+$ T cells	78	10	7.8	77
	$CD8^+$ $CD45RA^+$ T cells	65	24	2.7	46
	$CD8^+$ $CD45RO^+$ T cells	80	9	8.9	80
	$CD19^+$ B cells	30	26	1.2	7
	$CD56^+$ $CD3^-$ NK cells	55	38	1.4	18
	$CD14^+$ Monocytes	44	29	1.5	20
	Fibroblasts	39	38	1.0	1

Leukocyte subsets from Patients 10 and 11 were purified from PBMC by fluorescence-activated cell sorting, and their genomic DNAs isolated. As a control, primary fibroblast cell lines, which do not normally express DOCK8 protein, [E1] were also established from skin biopsies for similar analyses. A short sequence encompassing the small indel mutation in exon 12 (c.1266delC) was PCR amplified for 25 cycles and cloned. Plasmid DNA containing cloned products were isolated from transformed bacteria. At least 56 transformant colonies were analyzed for the presence or

absence of the indel by sequencing. Colony counts were used to calculate estimated proportions of cells having a repaired genotype, as follows:

unrepaired cells having indel/wt genotype = # clones indel

repaired cells having wt/wt genotype = [(# clones wt) – (# clones indel)]/2

% repaired cells = (# repaired cells)/[(# unrepaired cells) + (# repaired cells)] *100

When compared to flow cytometric detection of DOCK8-expressing cells, this methodology underestimated the proportions of repaired T cells and overestimated the proportions of repaired B and NK cells.

REFERENCE

E1. Su HC, Jing H, Zhang Q. DOCK8 deficiency. Ann N Y Acad Sci 2011; 1246:26-33.

Figure E1

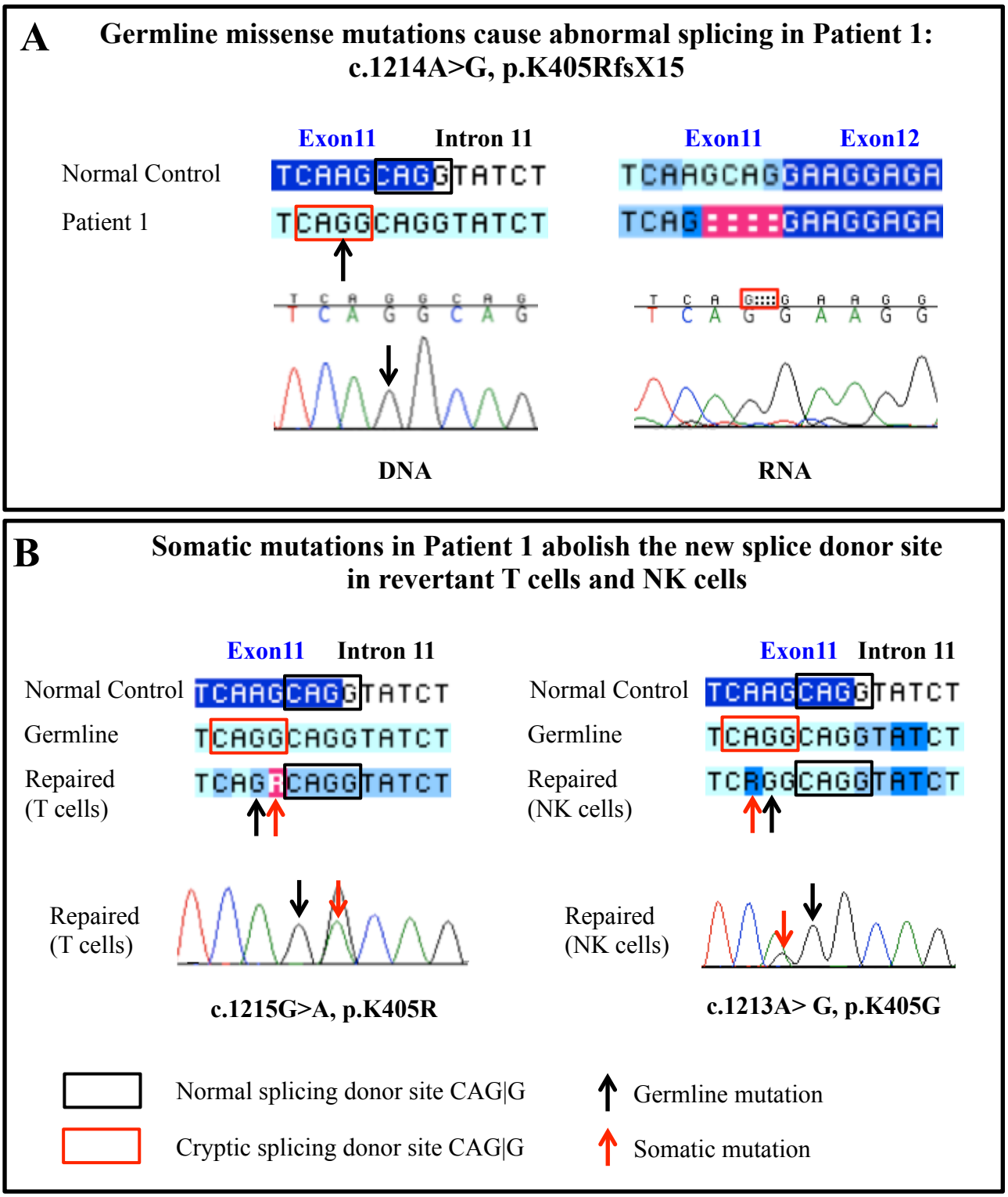

A
Germline missense mutations cause abnormal splicing in Patient 1:
c.1214A>G, p.K405RfsX15
Exon11
Intron 11
Exon11
Exon12
Normal Control
Patient 1
DNA
RNA
B
Somatic mutations in Patient 1 abolish the new splice donor site
in revertant T cells and NK cells
Exon11
Intron 11
Exon11
Intron 11
Normal Control
Germline
Repaired
(T cells)
Normal Control
Germline
Repaired
(NK cells)
Repaired
(T cells)
Repaired
(NK cells)
c.1215G>A, p.K405R
c.1213A> G, p.K405G
Normal splicing donor site CAG|G
Cryptic splicing donor site CAG|G
Germline mutation
Somatic mutation

Figure E2

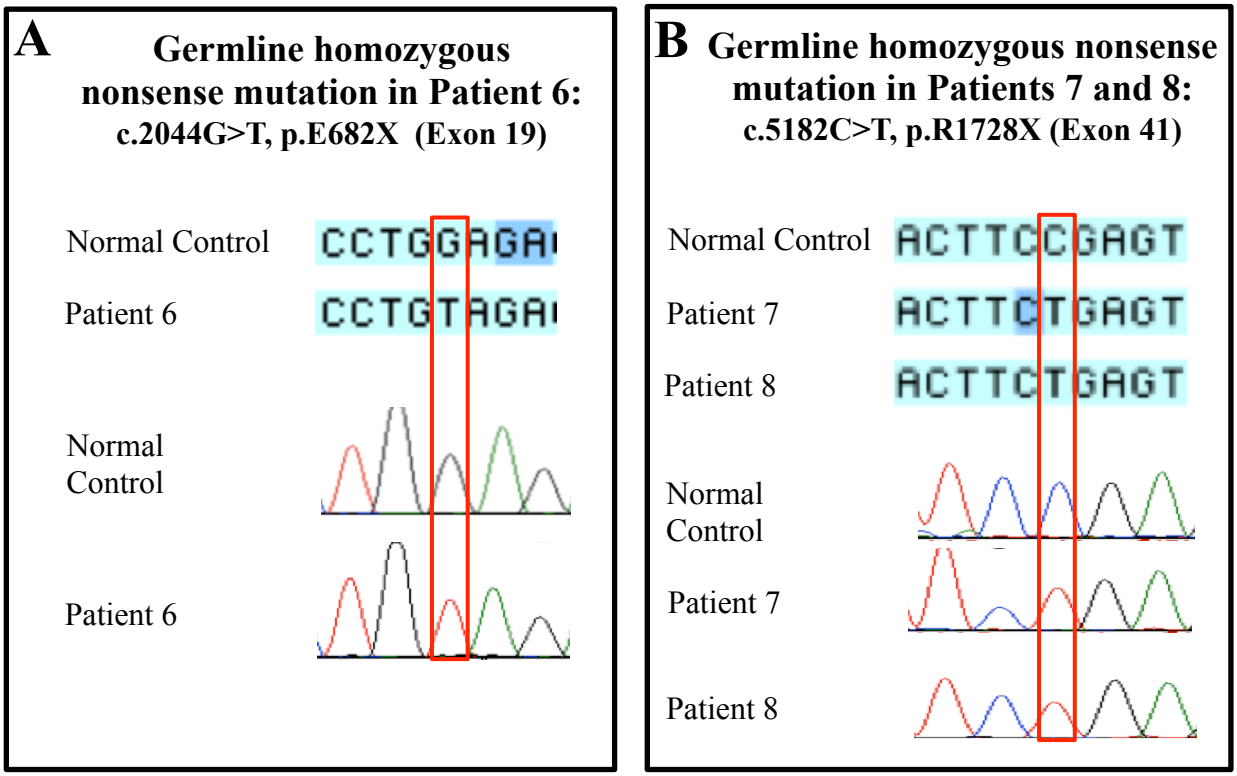

A
Germline homozygous nonsense mutation in Patient 6:
c.2044G>T, p.E682X (Exon 19)
Normal Control
CCTGGAGA
Patient 6
CCTGTAGA
Normal Control
Patient 6
B
Germline homozygous nonsense mutation in Patients 7 and 8:
c.5182C>T, p.R1728X (Exon 41)
Normal Control
ACTTCCGAGT
Patient 7
ACTTCTGAGT
Patient 8
ACTTCTGAGT
Normal Control
Patient 7
Patient 8

Figure E3

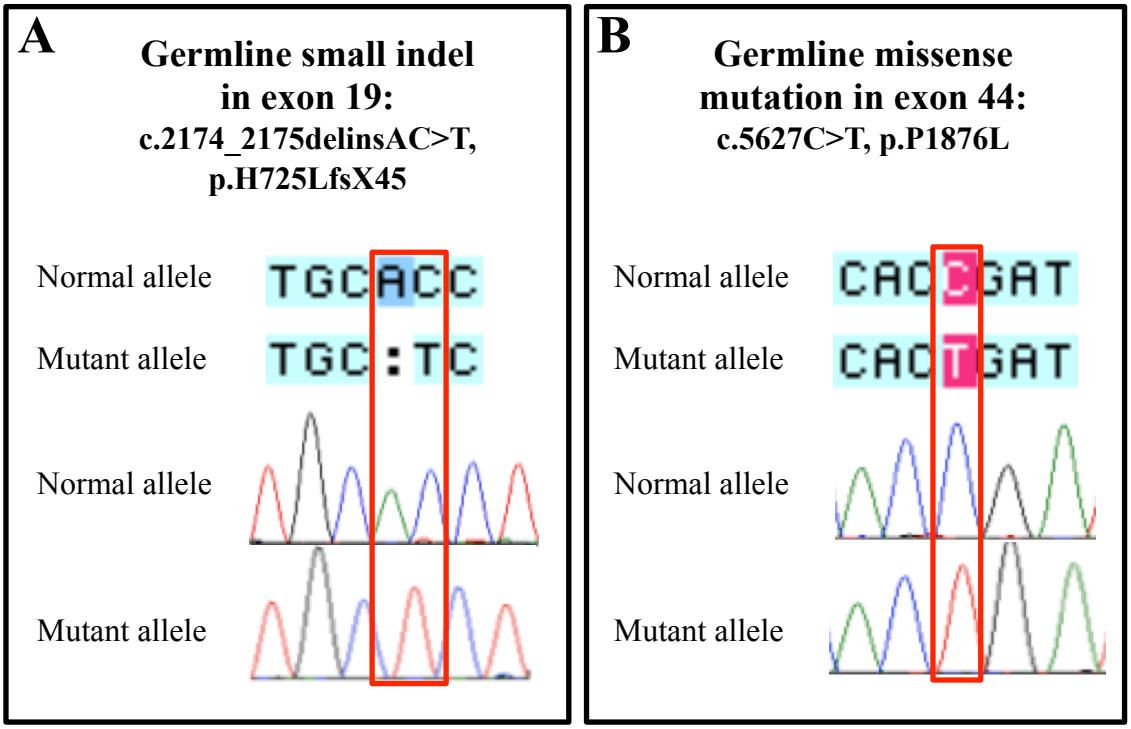

A
Germline small indel in exon 19:
c.2174_2175delinsAC>T, p.H725LfsX45
Normal allele
TGCACC
Mutant allele
TGC:TC
Normal allele
Mutant allele
B
Germline missense mutation in exon 44:
c.5627C>T, p.P1876L
Normal allele
CAC
GAT
Mutant allele
CAC
T
GAT
Normal allele
Mutant allele

Figure E4

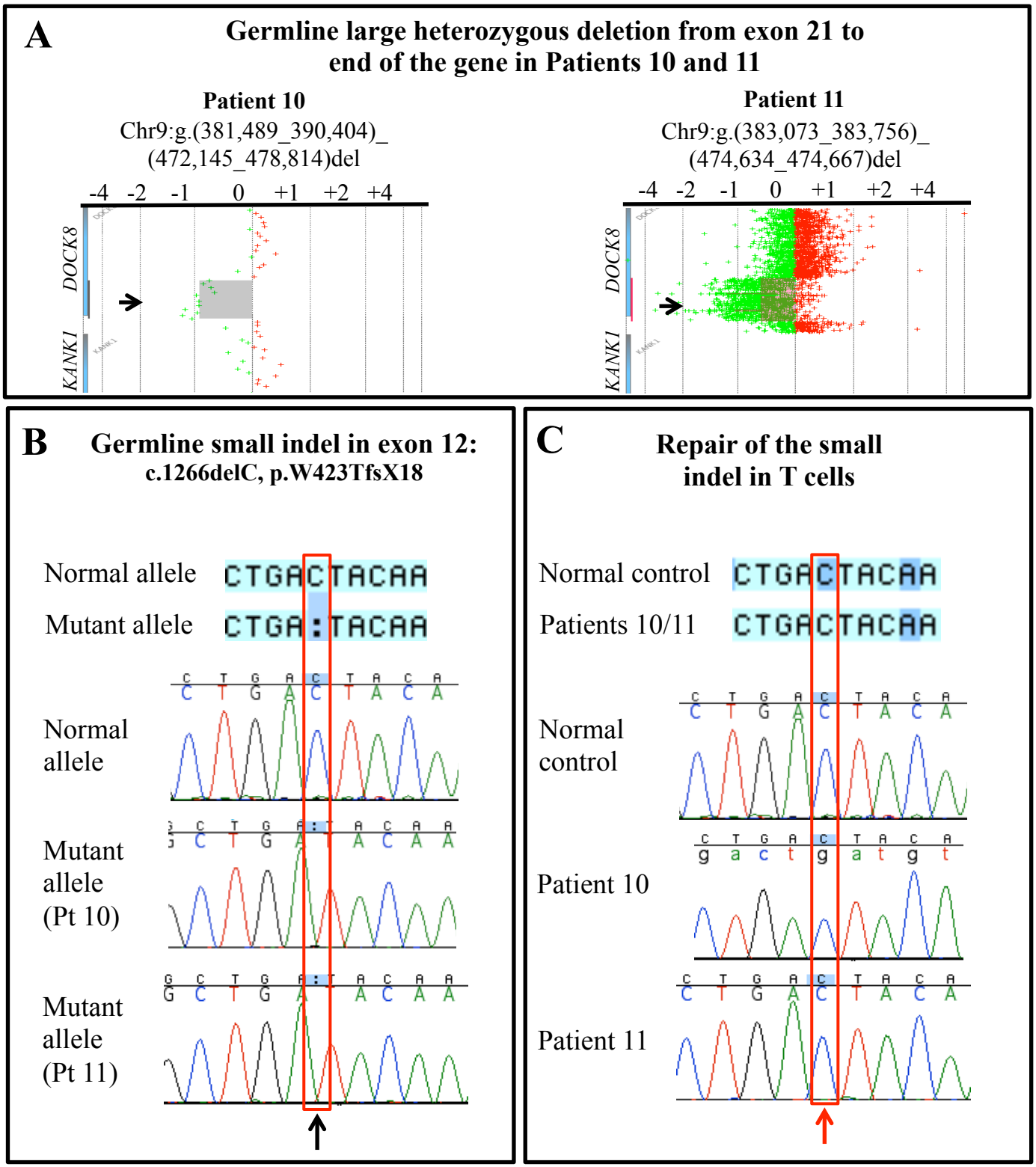
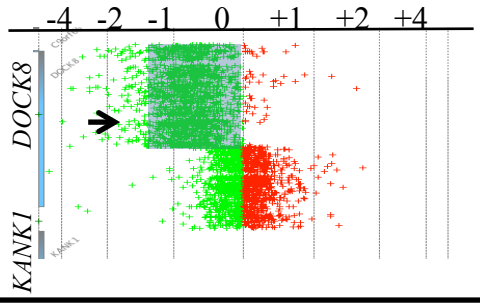

A
Germline large heterozygous deletion from exon 21 to end of the gene in Patients 10 and 11
Patient 10
Chr9:g.(381,489_390,404)_(472,145_478,814)del
Patient 11
Chr9:g.(383,073_383,756)_(474,634_474,667)del
-4 -2 -1 0 +1 +2 +4
DOCK8
KANK1
B
Germline small indel in exon 12:
c.1266delC, p.W423TfsX18
Normal allele
CTGACTACAA
Mutant allele
CTGA:TACAA
Normal allele
Mutant allele (Pt 10)
Mutant allele (Pt 11)
C
Repair of the small indel in T cells
Normal control
CTGACTACAA
Patients 10/11
CTGACTACAA
Normal control
Patient 10
Patient 11

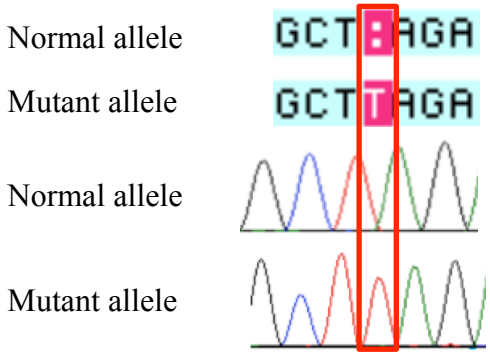
Figure E5

A **Germline large heterozygous deletion from promoter to exon 13:**

Chr9:g.(1_163,131)_(368,288_368,361)del

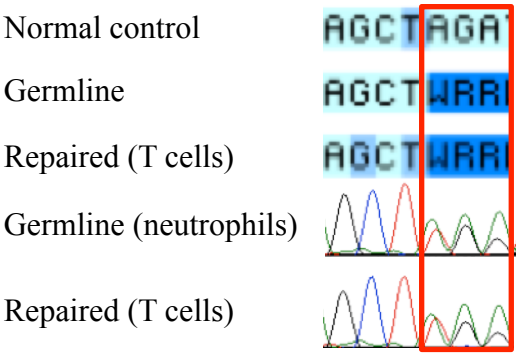


B **Germline small indel in exon 32:**
c.4031_4032insT, p.D1344RFsX2

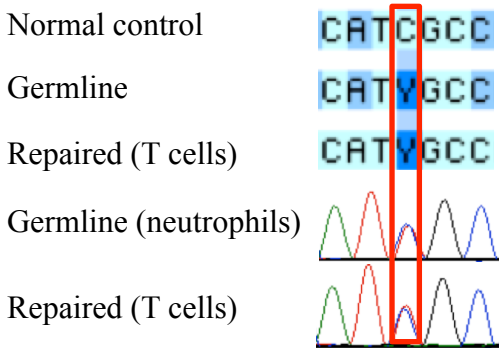


C **No change at genomic DNA level in T cells, suggesting intragenic crossover**

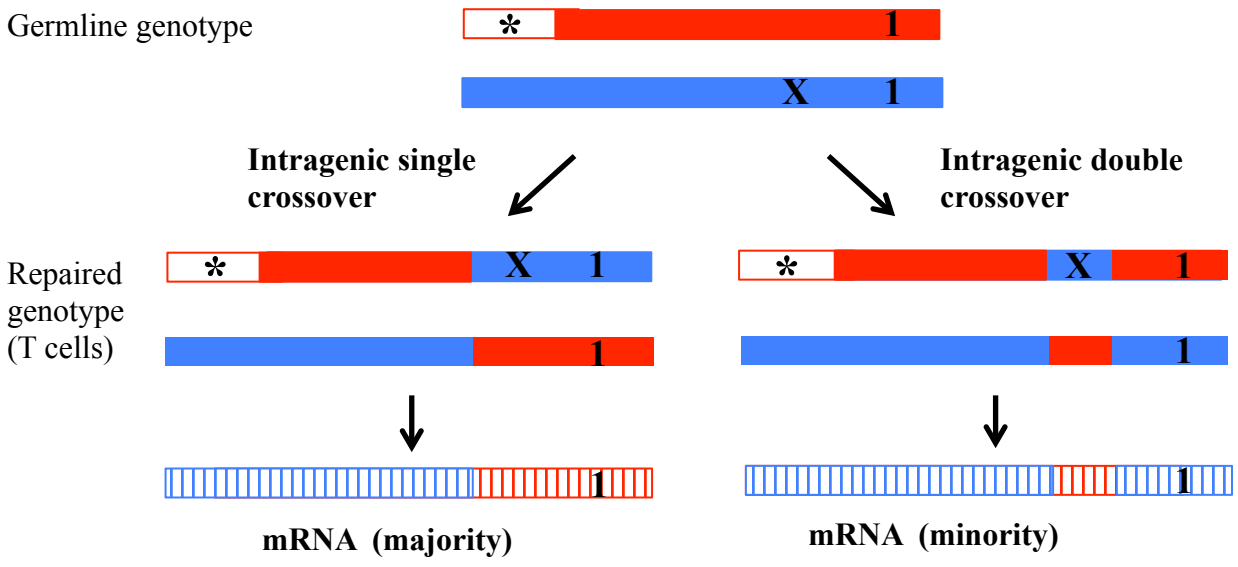
Indel: c.4031_4032insT, p.D1344RFsX2



SNP: c.4263C>T, p. I1421I



D **cDNA sequencing indicated that the small indel was repaired by intragenic crossover**



*: large deletion

X: c.4031_4032insT, p.D1344RfsX2

1: c.4263C>T, p. (=)
Maternal allele: C
Paternal Allele: T

Figure E6

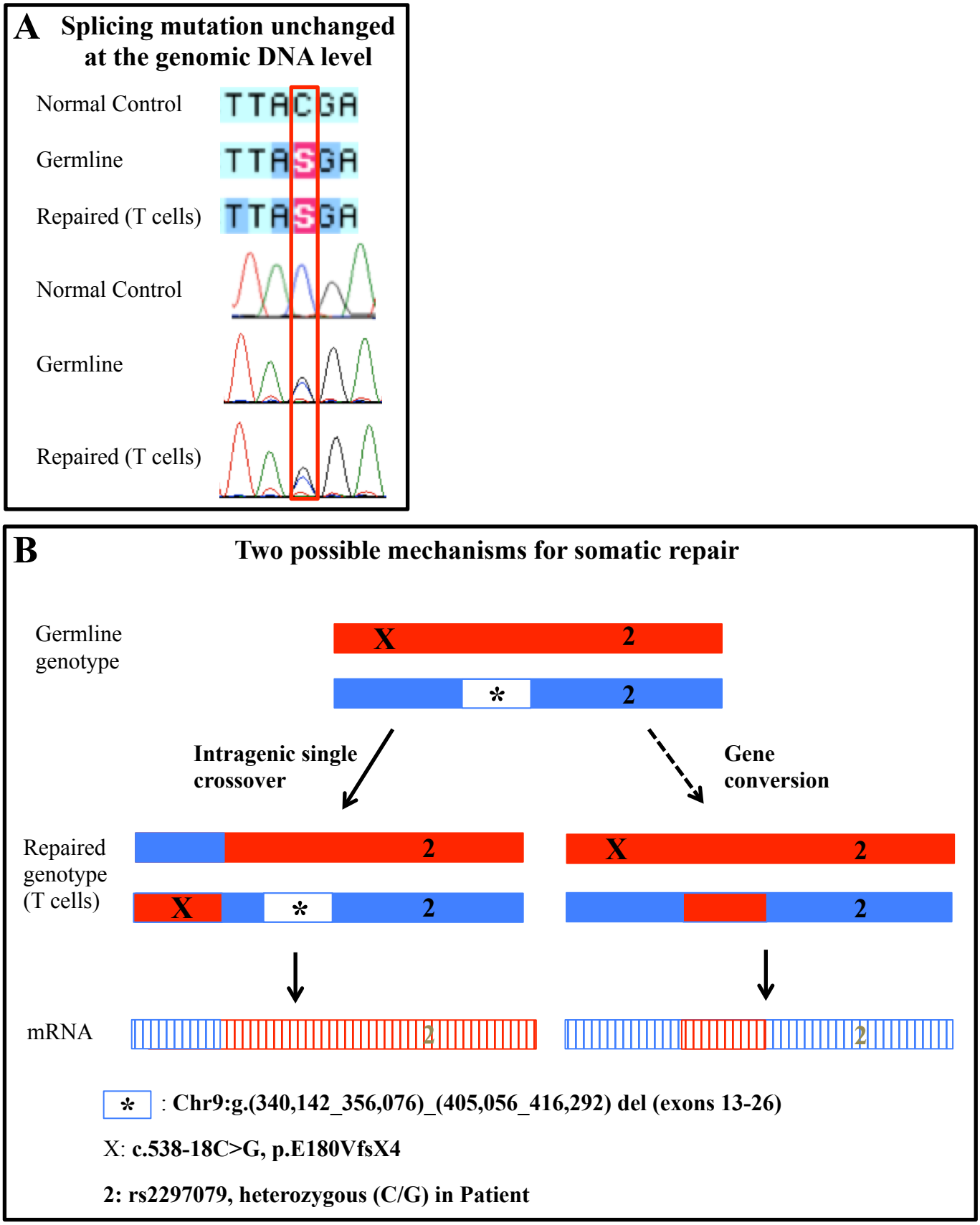
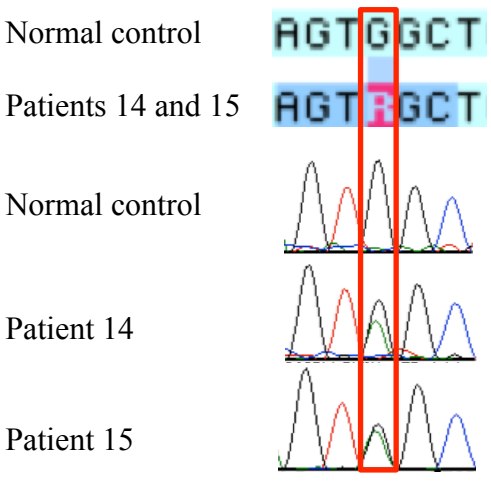
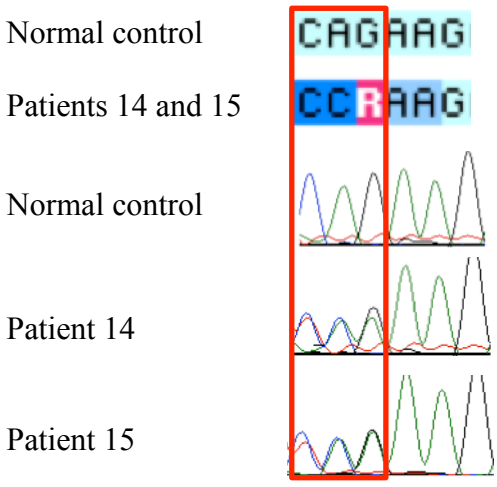

A
Splicing mutation unchanged at the genomic DNA level
Normal Control
TTACGA
Germline
TTASGA
Repaired (T cells)
TTASGA
Normal Control
Germline
Repaired (T cells)
B
Two possible mechanisms for somatic repair
Germline genotype
X
2
*
2
Intragenic single crossover
Gene conversion
Repaired genotype (T cells)
2
X
*
2
X
2
2
mRNA
2
2
* : Chr9:g.(340,142_356,076)_(405,056_416,292) del (exons 13-26)
X: c.538-18C>G, p.E180VfsX4
2: rs2297079, heterozygous (C/G) in Patient

Figure E7

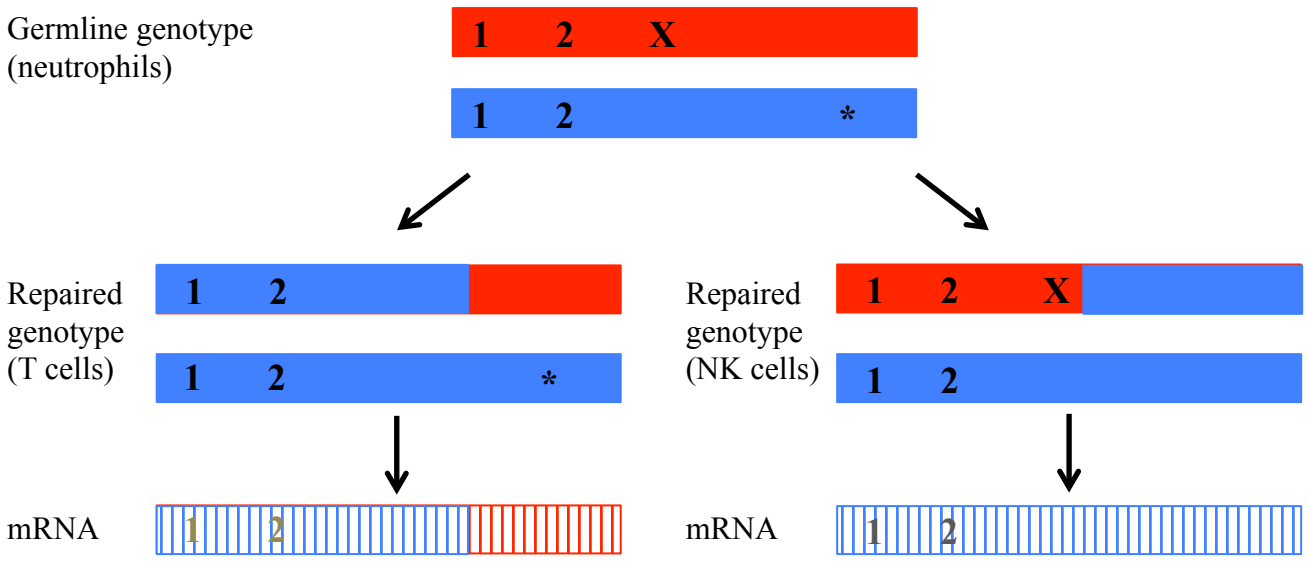
A **Germline heterozygous nonsense mutation in Patients 14 and 15: c.1805G>A, p.W602X (exon 17)**



B **Germline heterozygous indel in Patients 14 and 15: c.4540delG, p.E1514KfsX8 (exon 36)**



C **Genomic DNA sequencing in Patient 14 indicate that different gene conversion events repaired the *DOCK8* mutant alleles in T cells and NK cells**



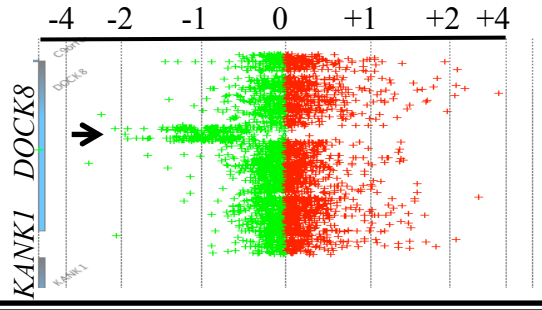
Marker	Position	Maternal	Paternal
1	rs529208	C	G
2	rs10970979	G	A

X: c.1805G>A, p.W602X

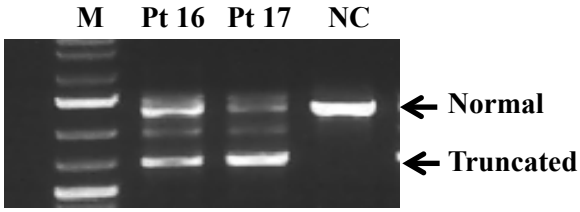
*: c.4540delG, p.E1514KfsX8

Figure E8

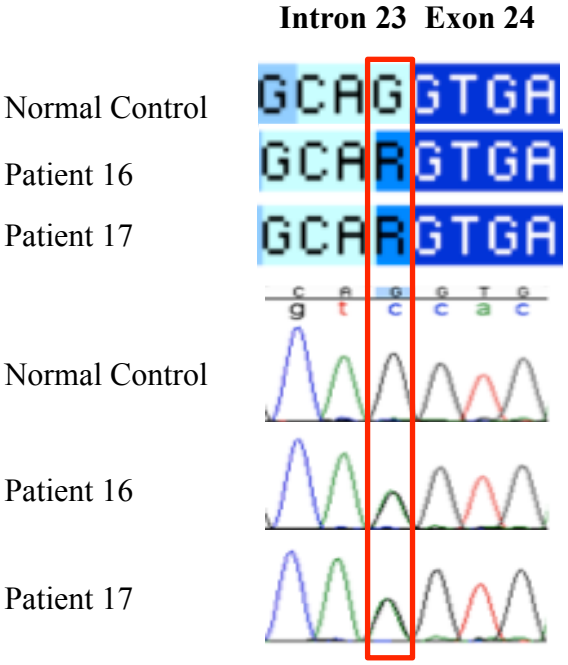
A **Germline heterozygous deletion from exons 5 to 9 in Patient 16:** Chr9:g.(300,972_301,582) _ (323,232_323,291)del



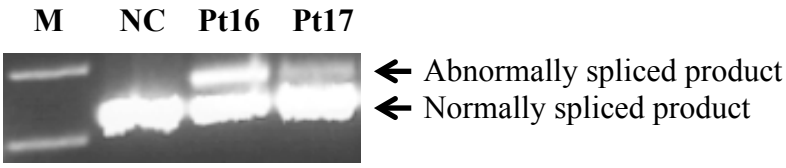
B **Large deletion resulted in a truncated mRNA: c.325_921del, p.A109_K307del**



C **Germline heterozygous splicing mutation: c.2767-1G>A, p.K924TfsX15 (intron 23)**



D **cDNA sequencing of abnormal splice products**



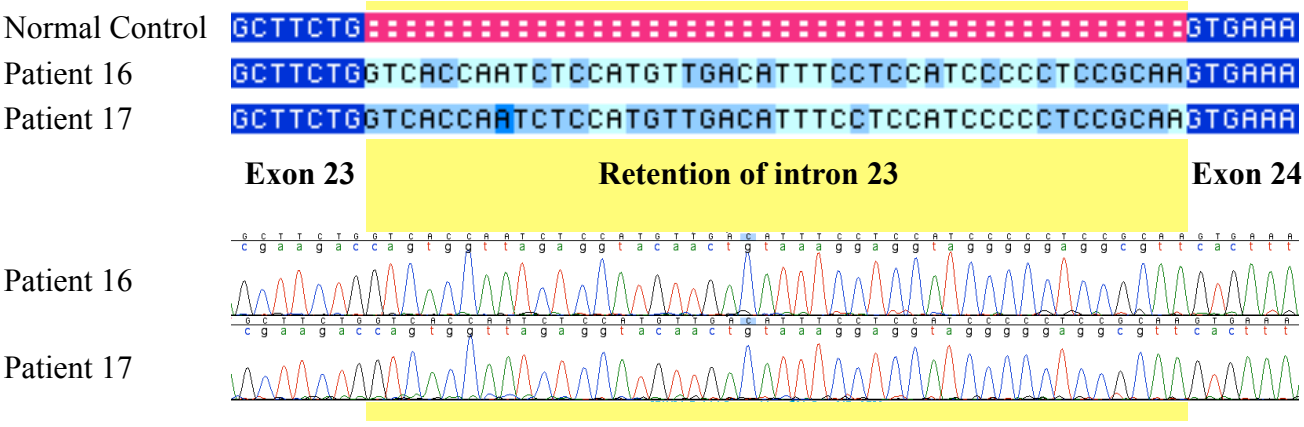


Figure E8

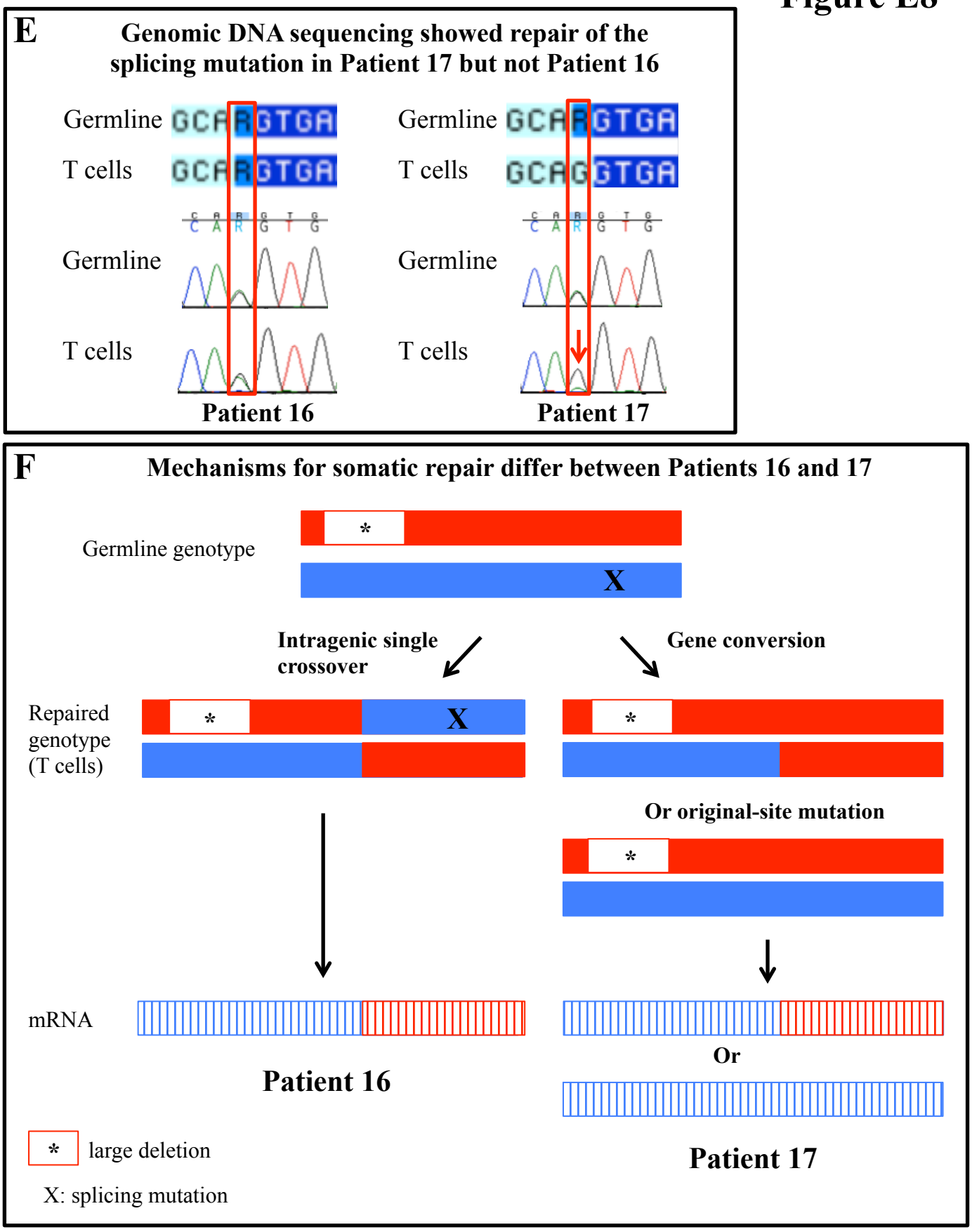
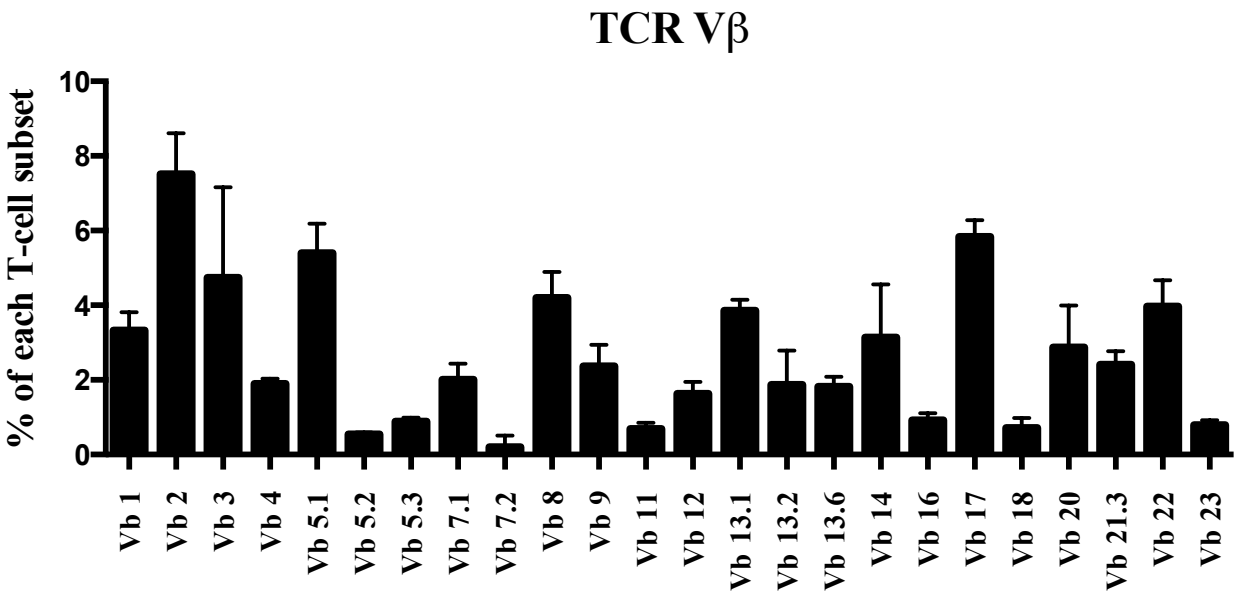

E
Genomic DNA sequencing showed repair of the splicing mutation in Patient 17 but not Patient 16
Germline
T cells
Germline
T cells
Patient 16
Germline
T cells
Germline
T cells
Patient 17
F
Mechanisms for somatic repair differ between Patients 16 and 17
Germline genotype
*
X
Intragenic single crossover
Gene conversion
Repaired genotype (T cells)
*
X
*
Or original-site mutation
*
mRNA
Or
Patient 16
Patient 17
* large deletion
X: splicing mutation

Figure E9


TCR Vβ
% of each T-cell subset
10
8
6
4
2
0
Vb 1
Vb 2
Vb 3
Vb 4
Vb 5.1
Vb 5.2
Vb 5.3
Vb 7.1
Vb 7.2
Vb 8
Vb 9
Vb 11
Vb 12
Vb 13.1
Vb 13.2
Vb 13.6
Vb 14
Vb 16
Vb 17
Vb 18
Vb 20
Vb 21.3
Vb 22
Vb 23

Online Repository Materials

METHODS

Information regarding study subjects can be found in the Methods section in this article's main text.

Cells

Peripheral blood mononuclear cells (PBMC) and cell purifications were prepared as described.[E1] In some cases, lymphocyte subsets were separated by fluorescence-activated cell sorting using a BD FACSAria Fusion cell sorter, after staining with fluorescence-conjugated anti-human CD3, CD4, CD8, CD19, CD45RA, CD56 (all from BD Biosciences), and CCR7 (R & D Systems) antibodies. Isolated populations had purities ≥ 99%, with viabilities of >99%. Fibroblast cultures were established from skin punch biopsies. Tissues were incubated overnight with Dispase (BD Biosciences) to separate the dermis from epidermis. Minced pieces of dermis were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) for 1 to 2 weeks, when fibroblasts emerged.

Array comparative genomic hybridization

Comparative genomic hybridization (CGH) analyses were performed on standard human 1 x 244K microarrays (Agilent) as described.[E1] In some cases, we used customized human 8 x 15K

SurePrint HD microarrays, which were designed using Agilent's eArray application. For each customized microarray, 3,666 unique 60-mer probes covered the *DOCK8* gene from chromosome 9 positions 0 to 500,000, with an average density of 12 probes per kb.

Immunoblotting

Preparation of cell lysates, separation and transfer of proteins, and immunoblotting for DOCK8 proteins were performed as described,[E1, E2] except that cells were lysed in 2% SDS buffer and 30 µg of protein lysate were separated per lane. Blots were probed using polyclonal rabbit anti-DOCK8 and mouse anti-β-actin antibodies (Sigma-Aldrich).

Flow cytometry

Flow cytometry was performed as described.[E1] Fluorescence-conjugated anti-human CD3, CD4, CD8, CD19, CD45RA, CD56, TCRγδ (all from BD Biosciences), and CCR7 (R & D Systems) antibodies were also used for cell surface staining. T-cell receptor Vβ repertoire analysis was performed using the IOTest beta Mark TCR V kit, per manufacturer's recommendations (Beckman Coulter). For intracellular flow cytometric detection of DOCK8 protein, PBMC were stained for surface molecules before fixation and permeabilization using the Cytofix/Cytoperm kit (BD Biosciences). Mouse anti-DOCK8 monoclonal antibody clone G2 (Santa Cruz Biotechnology) and purified mouse IgG1 isotype control (Biolegend) were non-covalently labeled using the Zenon® Alexa Fluor® 647 mouse IgG1 kit (Invitrogen). For labeling, each µg of antibody was incubated for 10 min with addition of 10 µL of labeling component A and then

10 µL of blocking component B. Fixed cells were resuspended in perm/wash buffer and incubated with the freshly labeled anti-DOCK8 antibodies for 1 h at 4 ^{o}C in the dark. Cells were washed with perm/wash buffer and PBS, resuspended in PBS, and acquired on the flow cytometer. Analysis on gated populations was performed using FlowJo software (TreeStar).

Sequencing

Isolation of genomic DNA or RNA, preparation of cDNA, PCR amplification, purification of amplified products, and sequencing reactions were performed as described.[E1] To estimate the proportions of somatically repaired cells, genomic DNA was PCR amplified for 25 cycles, and the purified products cloned using TOPO TA Cloning Kit for Sequencing (Invitrogen). Plasmid DNA was isolated from transformed TOP10 *E. coli* colonies using the PureLink 96 Plasmid Purification System (Invitrogen). Sequencing of at least 56 clones per reaction was analyzed using M13 Forward and Reverse primers. Clones showing germline or repaired sequences were counted, and counts were used to calculate the percentages of diploid cells having germline vs. repaired genotypes. Sequencing was performed by the Genomics Unit of the Rocky Mountain Laboratories Research Technologies Section of the National Institute of Allergy and Infectious Diseases. In some cases, RT-PCR amplified products were visualized by ethidium bromide staining after gel electrophoresis through 1.2% agarose in 1x TAE buffer. Primers used for amplification and sequencing, if not previously published, are listed in Table E2. All genomic variants are described according to Human Genome Variation Society recommendations (http://www.hgvs.org/mutnomen/recs.html), using GenBank Reference Sequences NC_000009.10 (DNA), NM_001193536.1 (mRNA; previously NM_203447.1), and

NP_001180465.1 (protein; previously NP_982272.1) based upon NCBI Build 37.3. Coding DNA variations are described with the A of the ATG translation initiation codon designated as nucleotide +1.

Statistical analyses

The Prism 5 software package (GraphPad) was used to calculate *p*-values using Mann-Whitney, Fisher's exact, and Chi square tests, as indicated.

SUPPLEMENTAL FIGURE LEGENDS

FIG E1. *DOCK8* genetic analyses for Patient 1. **A**, Left: Sequencing of genomic DNA from neutrophils or an HVS-transformed T-cell line showed a germline homozygous splicing mutation in exon 11: c.1214A>G, p.K405RfsX15. The same mutation was previously reported in the affected brother who is Patient 2. Right: cDNA sequencing showed that this mutation uses a cryptic splice donor site to cause abnormal splicing. **B**, Sequencing of genomic DNA revealed second-site mutations in exon 11 that abolished the cryptic splice donor site. Left: c.1215G>A, p.K405R in primary T cells, expanded in IL-2 after activation with anti-CD3 plus anti-CD28 antibodies. Right: c.1213A>G, p.K405G in purified NK cells. Black and red arrows designate germline and somatic mutations, respectively. Black and red boxes designate normal and cryptic splice donor consensus site sequences, respectively.

FIG E2. DOCK8 genetic analyses for Patients 6, 7, and 8. Genomic DNA from neutrophils showed homozygous nonsense mutations. **A**, For Patient 6, in exon 19: c.2044G>T, p.E682X. **B**, For Patients 7 and 8, in exon 41: c.5182C>T, p.R1728X. Because of very low frequencies of DOCK8-expressing cells, genetic analyses for somatic mutations were not undertaken.

FIG E3. *DOCK8* genetic analyses for Patient 9. Genomic DNA isolated from neutrophils or HVS-transformed T cells showed germline compound heterozygous mutations. Representative chromatograms are shown of sequenced cloned PCR-amplified products. **A**, Small indel in exon 19: c.2174_2175delinsAC>T, p.H725LfsX45. **B**, Missense mutation in exon 44 encoding part of the conserved DHR2 domain: c.5267C>T, p.P1876L. Additional genetic analyses showed that the indel was inherited from mother (data not shown), whereas the missense mutation was presumed inherited from the father (sample unavailable for analysis). See Fig 2, *C*, which shows that intragenic single crossover was responsible for somatic repair in primary T cells. cDNA sequencing established the parental origin of six exonic SNPs and two mutations, from which a wild-type RNA derived from both maternal (red bars) and paternal (blue bars) alleles could be deduced. For simplicity, only three of the genotyped SNPs are shown in Fig 2, *C*. The genetic material change by intragenic single crossover was reciprocal, with no change observed by genomic DNA sequencing (data not shown). Markers on cDNA (grey font) are inferred from the markers identified by genomic DNA sequencing (black font).

FIG E4. *DOCK8* genetic analyses for Patients 10 and 11. **A**, Genomic DNA isolated from neutrophils showed a germline heterozygous large deletion from exon 21 to the end of the gene by array CGH: Chr9:g.(381,489_390,404)_(472,145_478.814)del in Patient 10 (left), or Chr9:g.(383,073_383,756)_(474,634_474,667)del in Patient 11 using a higher density chip (right). Each plus sign represents a unique probe whose location on the X-axis represents the

log_2(ratio of fluorescence intensities of patient vs. reference DNA) and whose location on the Y-axis represents the chromosomal location. The shaded area indicates the region of the heterozygous deletion in the *DOCK8* gene. **B**, Representative chromatograms are shown of sequenced cloned PCR-amplified products, which demonstrated a germline small indel in exon 12: c.1266delC, p.W423TfsX18. Additional genetic analyses showed that the large deletion was inherited from the father, whereas the small indel was inherited from the mother (data not shown). **C**, Sequencing of genomic DNA revealed repair of the small indel in primary T cells. See Fig 2, *B*, for genomic DNA sequencing showing gene conversion, in which the maternally-inherited frameshift mutation was repaired by using the intact portion of the truncated paternally-inherited *DOCK8* allele in T cells. This repair generated normal RNA but resulted in loss of heterozygosity in the DNA sequence. Markers on cDNA (grey font) are inferred from the markers identified by genomic DNA sequencing (black font).

FIG E5. *DOCK8* genetic analyses for Patient 12. Genomic DNA isolated from neutrophils showed germline compound heterozygous mutations. **A**, Heterozygous large deletion from the promoter to exon 13 by array CGH: Chr9:g.(1_163,131)_(368,288_368,361)del. **B**, Representative chromatogram of sequenced cloned PCR-amplified products, which demonstrated a small indel in exon 32: c.4031_4032insT, p.D1344RfsX2. Additional genetic analyses showed that the large deletion was inherited from the mother, and the small indel was inherited from the father (data not shown). **C**, Sequencing of genomic DNA from primary T cells showed no changes in the relative proportions of the wildtype and mutant nucleotides (left), or an SNP 3' to this mutation (right). **D**, cDNA sequencing of cloned RT-PCR amplified products from primary T cells, to identify parental origin of a distal exonic SNP relative to the indel. The maternally-derived SNP was disproportionately present in the transcripts (67% of clones). These

findings suggested that somatic repair primarily resulted from intragenic single crossover (left panel). The presence of a minor proportion of transcripts containing paternally-derived SNPs (33% of clones) also suggested intragenic double crossover (right panel), although original-site reversion could not be excluded. Red and blue bars designate maternally- and paternally-derived alleles, respectively, as inferred by the listed SNP and mutations. Hatched bars indicate mRNA.

FIG E6. *DOCK8* genetic analyses for Patient 13. Patient 13 has germline compound heterozygous mutations with a large deletion from exons 13 to 26, plus a splicing mutation in exon 5: Chr9:g.(340,142_356,076)_(405,056_416,292)del, plus c.538-18C>G, p.E180VfsX4.[E1] **A**, Sequencing of genomic DNA revealed no repair of the splicing mutation in primary T cells. **B**, Schematic diagram showing that either intragenic crossover or gene conversion could account for these results. SNPs between the splicing mutation in exon 5 and the beginning of the deletion in exon 13 are lacking, so it was not possible to directly demonstrate either mechanism by sequencing, and array CGH was not performed to distinguish between the two possibilities. Red and blue bars designate maternally- and paternally-derived alleles, and hatched bars indicate mRNA. Markers on cDNA (grey font) are inferred from the markers identified by genomic DNA sequencing (black font).

FIG E7. *DOCK8* genetic analyses for Patients 14 and 15. Genomic DNA isolated from neutrophils showed germline compound heterozygous mutations. **A**, Nonsense mutation in exon 17: c.1805G>A, p.W602X. **B**, Small indel in exon 36: c.4540delG, p.E1514KfsX8. Additional genetic analyses showed that the nonsense mutation was inherited from the mother, and the small indel was inherited from the father (data not shown). **C**, Sequencing of genomic DNA was performed in sorted primary T cells and NK cells to determine the parental origin of two exonic SNPs and the two mutations. One gene conversion event repaired the maternally-inherited

nonsense mutation using the intact portion of the paternally-inherited *DOCK8* allele in T cells. Another gene conversion event repaired the paternally-inherited indel using the intact portion of the maternally-inherited *DOCK8* allele in NK cells. Red and blue bars designate maternally- and paternally-derived alleles, respectively, as inferred by the listed SNPs and mutations. Hatched bars indicate mRNA. Markers on cDNA (grey font) are inferred from the markers identified by genomic DNA sequencing (black font).

FIG E8. *DOCK8* genetic analyses for Patients 16 and 17. **A**, Genomic DNA isolated from neutrophils showed a germline heterozygous large deletion from exons 5 to 9 by array CGH and cDNA sequencing in Patient 16: c.325_921del, p.A109_K307del. **B**, Agarose gel electrophoresis after RT-PCR amplification of a region encompassing exons 2 to 13 showed a truncated product in primary T cells from both patients, suggesting that Patient 17 carries the same large deletion. **C**, Genomic DNA sequencing also showed a germline heterozygous splicing point mutation in intron 23 in both patients: c.2767-1G>A, p.K924TfsX15. **D**, Agarose gel electrophoresis after RT-PCR amplification of a region encompassing exons 23 and 24 showed an abnormally long spliced product (upper panel). cDNA sequencing of this abnormal product showed partial retention of intron 23 (bottom panel, shaded region). Additional genetic analyses showed that the large deletion was inherited from the mother, whereas the splicing point mutation was inherited from the father (data not shown). **E**, Genomic DNA isolated from primary T cells showed somatic repair of the splicing point mutation in intron 23 in Patient 17 but not Patient 16. **F**, Schematic diagram showing the repair mechanisms used. Because of a high level of DOCK8-expressing T cells that still had the deletion and splicing mutations, Patient 16 was inferred as having repaired the splicing mutation by intragenic single crossover. By contrast, Patient 17 was inferred as having repaired the splicing mutation through either gene conversion or original-site

reversion. SNPs between the end of the deletion in exon 9 and the splicing mutation in intron 23 were lacking, so it was not possible to directly demonstrate these mechanisms.

FIG E9. Normal TCR Vβ repertoire**.** TCR Vβ repertoire analyses were performed on purified T cells from four normal controls. Shown are means ± SD.

REFERENCES

E1. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, et al. Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med 2009; 361:2046-55.

E2. Sanal O, Jing H, Ozgur T, Ayvaz D, Strauss-Albee DM, Ersoy-Evans S, et al. Additional diverse findings expand the clinical presentation of DOCK8 deficiency. J Clin Immunol 2012; 32:698-708.