Supplementary Material

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Ligase-4 deficiency causes distinctive immune abnormalities in asymptomatic individuals

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Supplementary Methods

Genetic sequencing

Primer sequences for mutations in LIG4:

c.1345A>C: 558bp

F:TTCCAGGTAGAATAGAAATAGTGC

R:CAATTCGTGGAAAACGC

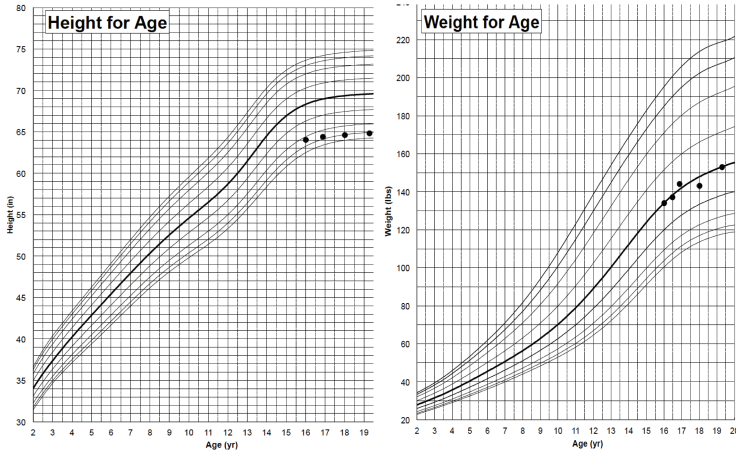
c. 2440C>T 462bp

F: GCCCGTGAATATGATTGC

R: CTGGTTTTCTTCTTGTAATTCACAC

Supplementary Figures

P1



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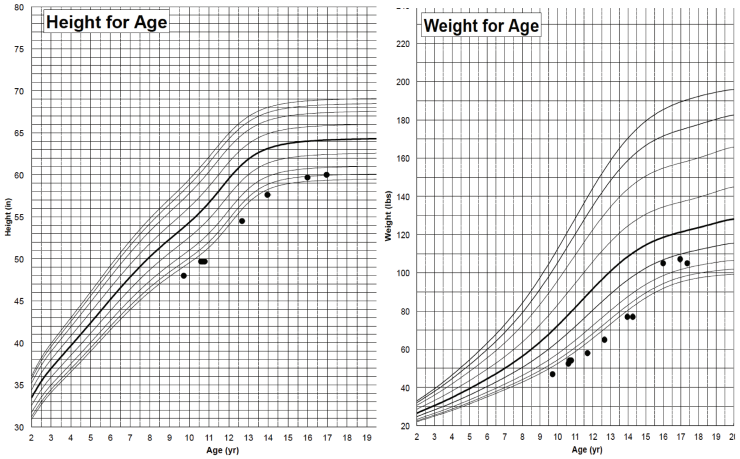

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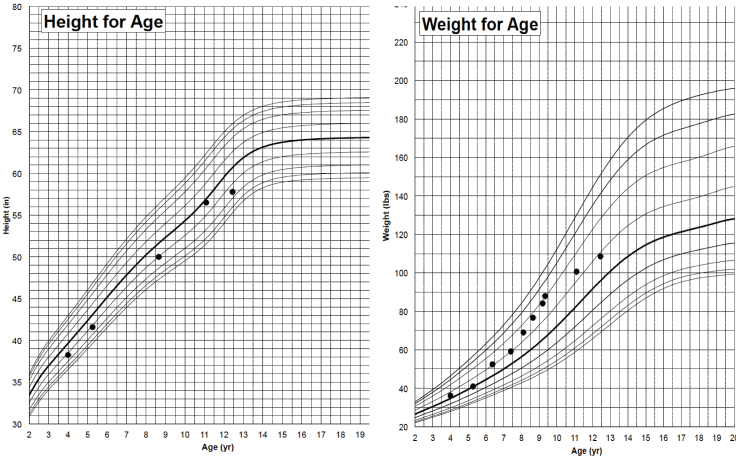

Fig. S1 Growth charts of P1-P3

The growth charts for height and weight (3^{rd},5^{th},10^{th},25^{th},50^{th},75^{th},90^{th},95^{th},97^{th} percentiles) were graphed for P1-P3 using the webtool developed by Ernest M. Post, MD and Zimi Medical Technologies, LLC from CDC data on http://www.cdc.gov/growthcharts.

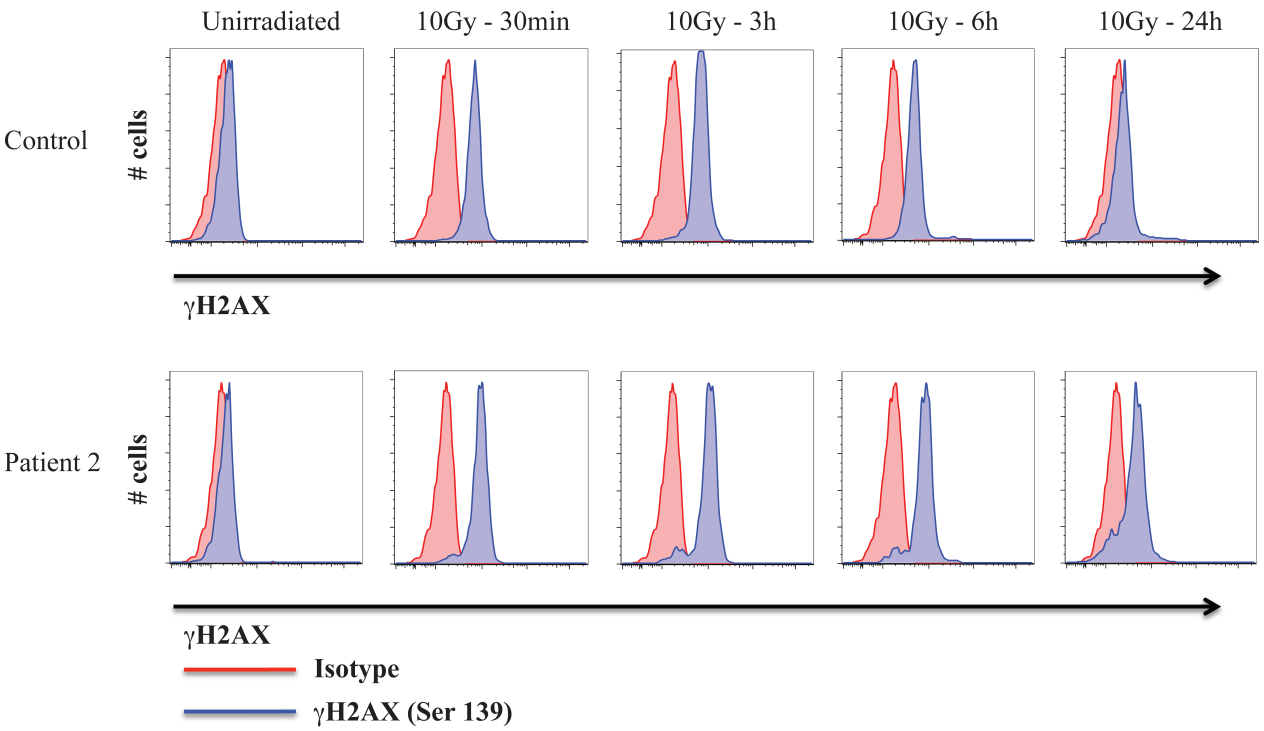

Fig. S2 Radiosensitivity assessed in PBMCs by γH2AX staining and flow-cytometry

PBMCs of P2 and a healthy control were irradiated with 10Gy and fixed at given time points. Induction and resolution of phosphorylation of the histone protein H2AX (γH2AX) at sites of DNA DSB was analyzed by flow-cytometry. Mean fluorescence intensities (MFI) are shown as histograms (blue) compared to isotype (red).

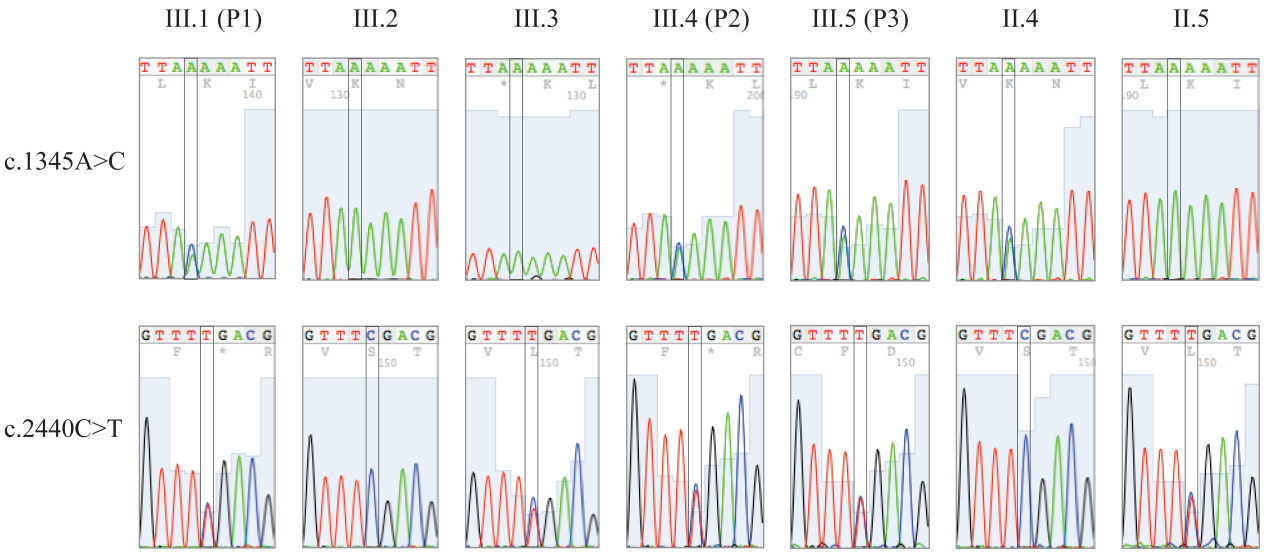

Fig. S3 Genetic analysis of parents and siblings

Both parents (II.4 and II.5) and their five siblings (III.1-III.5) were analyzed for the two mutations found in the index case III.4 (P2). Sanger sequencing was performed on DNA isolated from peripheral blood cells (PBMCs) and mutated base pairs are indicated in boxes. An older brother, III.1 (P1), and a younger sister, III.5 (P3), were found to be compound heterozygous for both mutations. III.2 has two wild type alleles, and III.3 is a carrier of c.2440C<T.

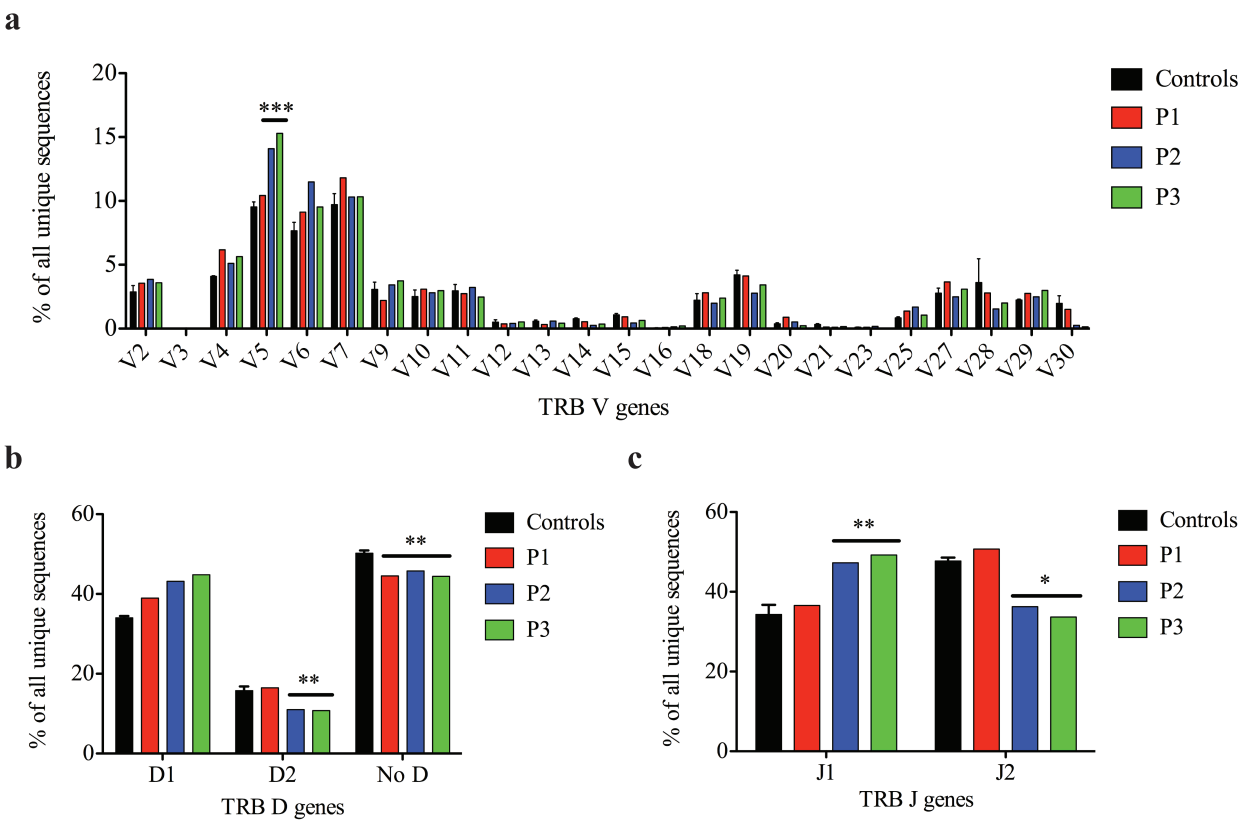

Fig. S4 V, D and J gene families used in unique *TRB* sequences

Percentages of all used V, D, and J gene families were evaluated in *TRB* repertoires (A-C) of three healthy controls and patients P1-P3 (mean values and standard deviation are shown) (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

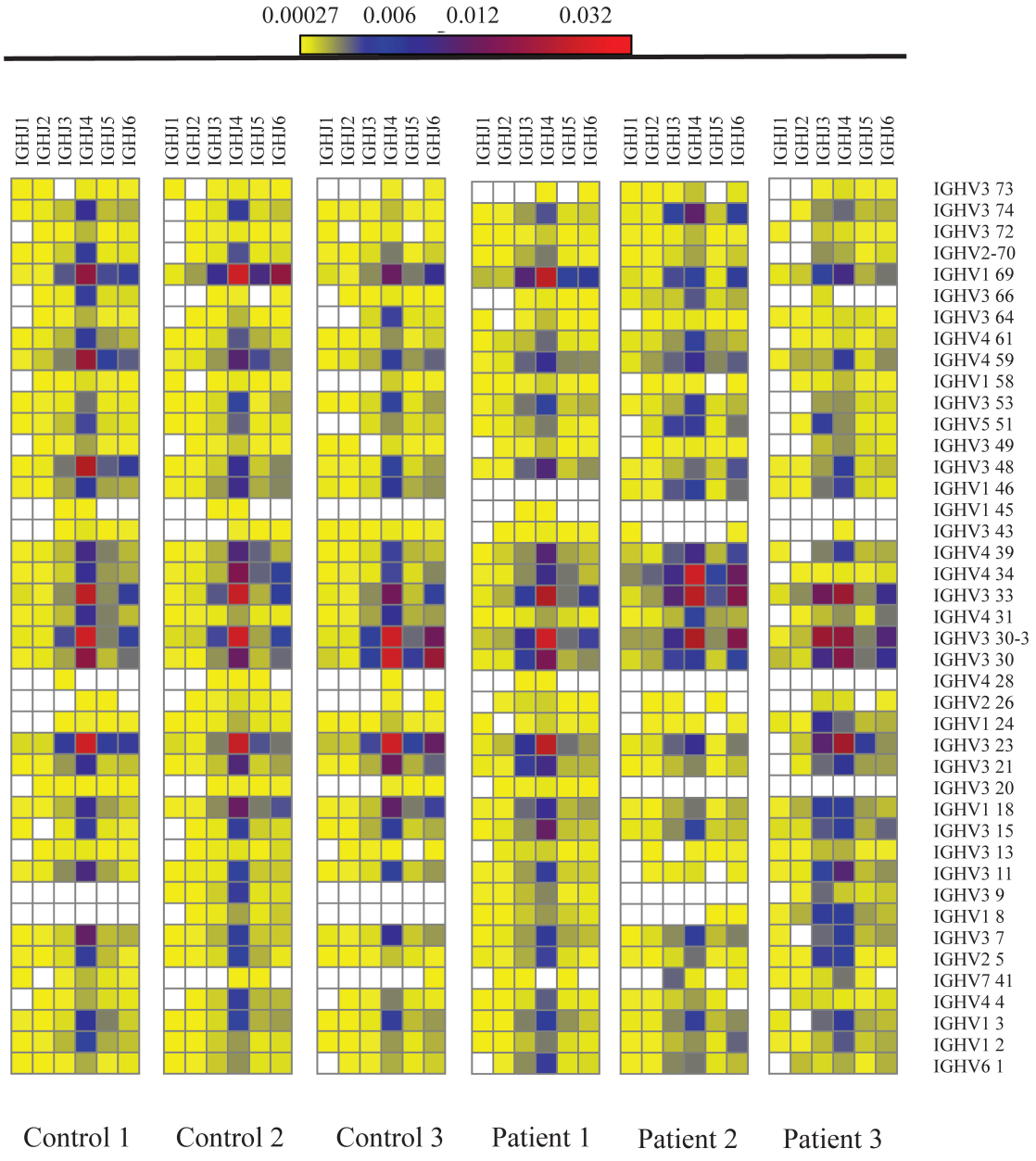

Fig. S5 Modest skewing in the *IGH* repertoire of LIG4-deficient patients P1-P3

Global usage of unique *IGH* sequences is demonstrated for 3 healthy controls and P1-P3 on a heat map showing *IGHV* genes joint with *IGHJ* genes. The frequency of gene usage is color coded, using yellow as very rarely and red as most frequently used; blank spots indicate no usage at all.

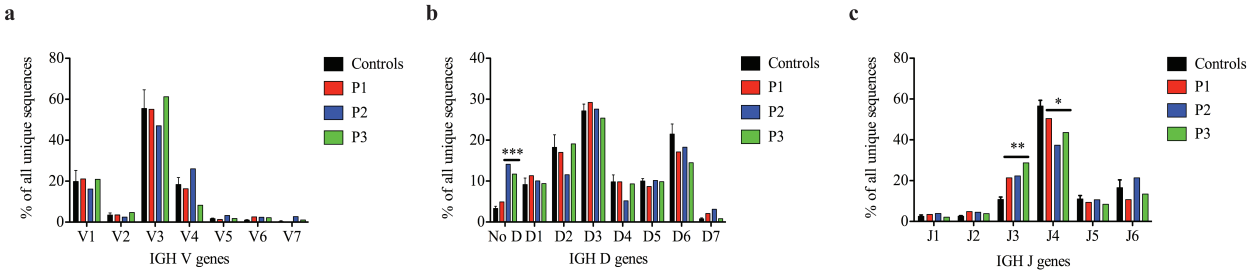

Fig. S6 V, D and J gene families used in unique *IGH* sequences

Percentages of all used V, D, and J gene families were evaluated in the BCR heavy chain (*IGH*) locus (A-C) of three healthy controls and patients P1-P3 (mean values and standard deviation are shown) (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

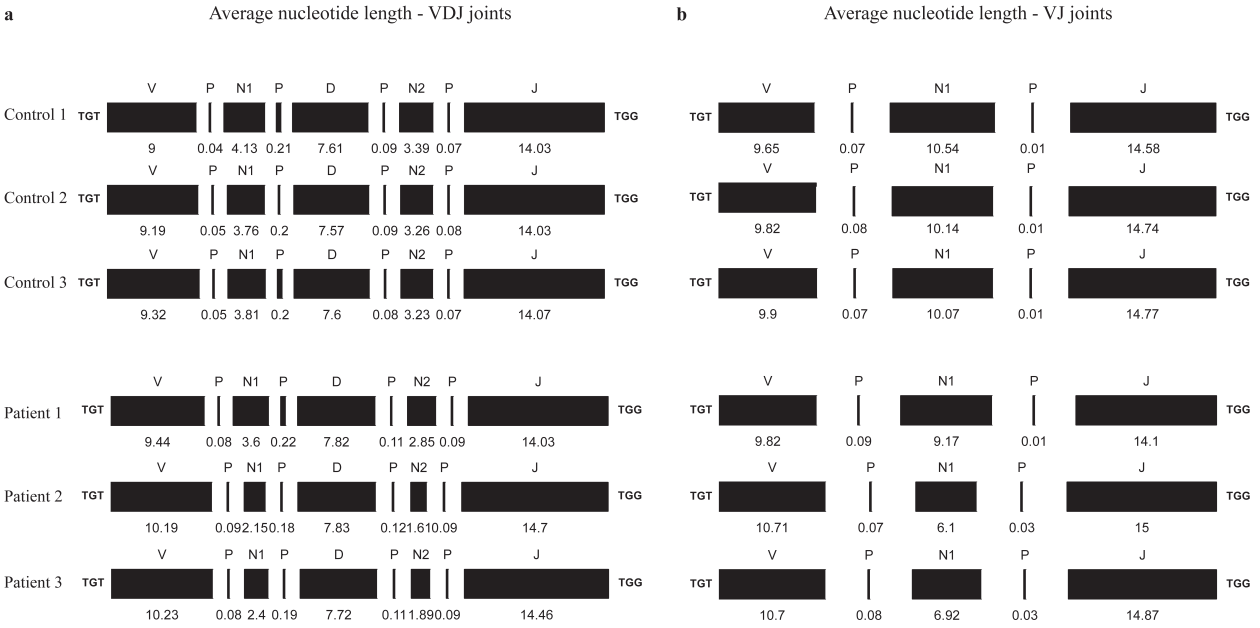

Fig. S7 Average length of V, D and J elements with P and N nucleotide insertions in *TRB* sequences

Average lengths of V, D and J (A), and V and J gene elements (B) with average length of interspaced P and N nucleotides are shown for *TRB* sequences. N1 nucleotides are added between V and D or V and J gene elements, N2 nucleotides between D and J elements. P nucleotides are located before and after N nucleotides, or directly between joint elements, in case no N nucleotides are added.

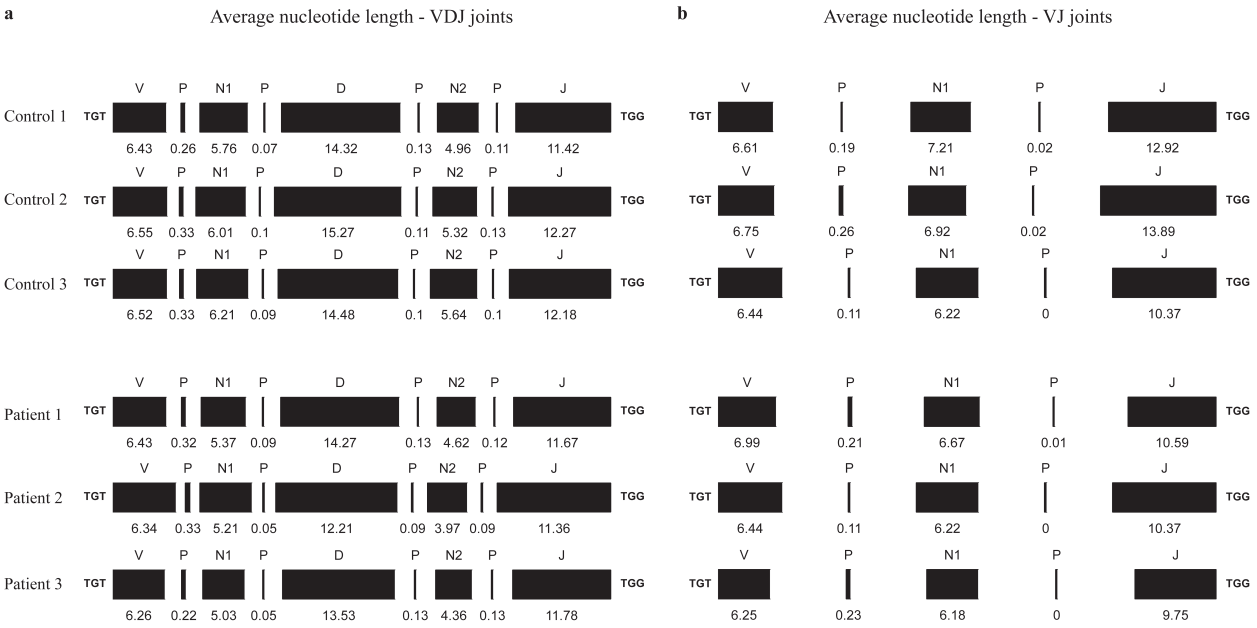

Fig. S8 Average length of V, D and J elements with P and N nucleotide insertions in *IGH* sequences

Average lengths of V, D and J (A), and V and J gene elements (B) with average length of interspaced P and N nucleotides are shown for *IGH* sequences.

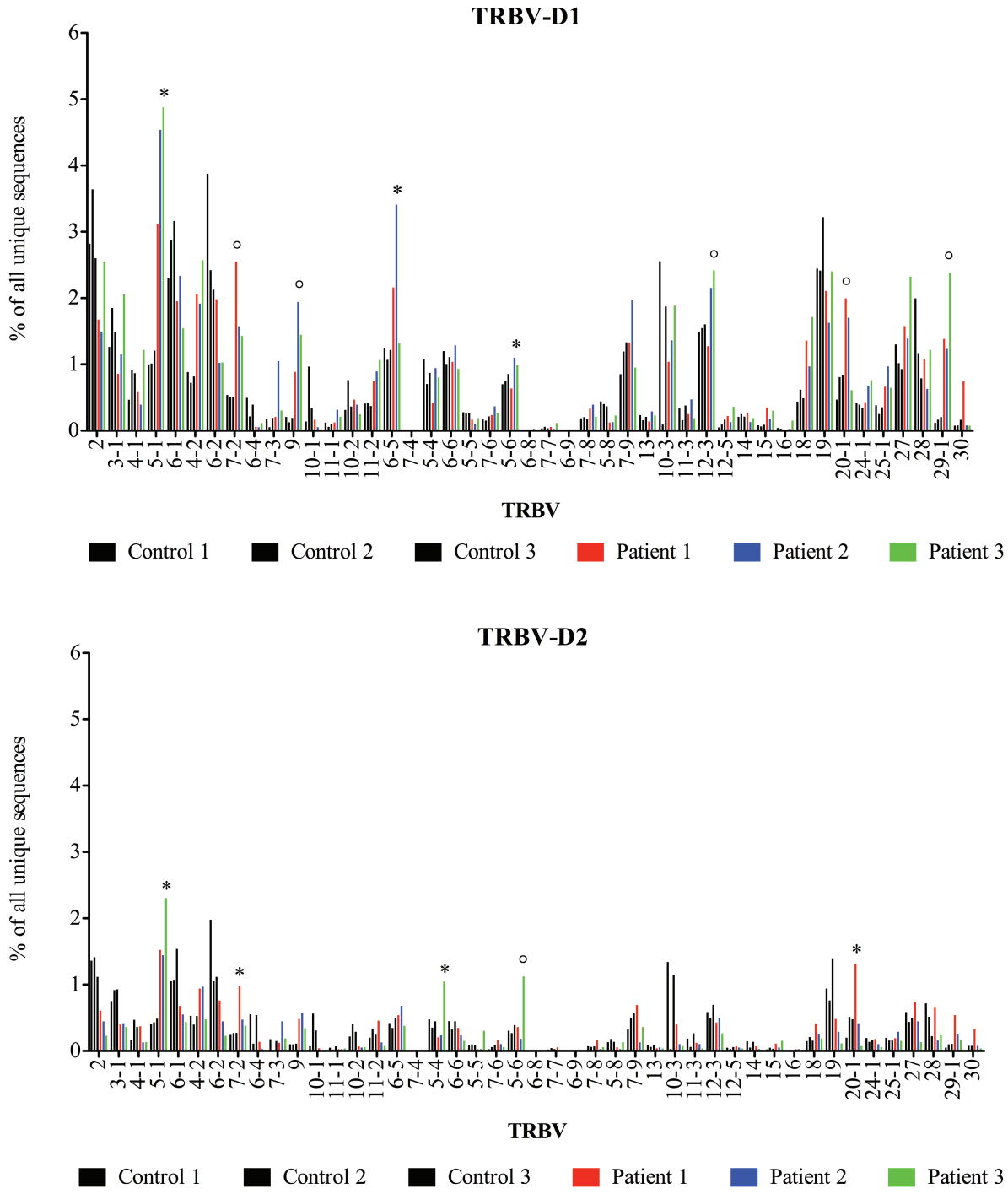

Fig. S9 Frequency of joins between *TRBV* and *TRBD* genes

The frequencies of all possible joins between *TRBV* and *TRBD1* or *TRBD2*, respectively, are shown as percentage of all unique sequences for 3 healthy controls and patients P1-P3. Joins in which MHMEJ has been found (*) or in which MHMEJ has been excluded (°) are indicated accordingly.