A human immunodeficiency caused by mutations in the *PIK3R1* gene.

Supplementary Methods

Whole-exome sequencing. Whole-exome sequencing libraries were prepared from 3 µg of genomic DNA extracted from total blood after shearing with a S2 Ultrasonicator (Covaris). Exome capture was performed as recommended by the manufacturer, using the 51 Mb SureSelect Human All Exon kit V5 (Agilent Technologies). The barcoded exome library was sequenced on a HiSeq2500 machine (Illumina) in HighOutput mode (48 libraries per FlowCell), which generated 76+76 paired-end reads. After demultiplexing, paired-end sequences were mapped onto the human genome reference (NCBI build37/hg19 version) using the Burrows-Wheeler Alignment software package (http://bio-bwa.sourceforge.net/). The mean depth of coverage obtained for each sample was >90X, and at least 95% of the exome was covered at least 15X. Single nucleotide polymorphism and indel calling was performed with GATK software tools. An in-house software package (PolyWeb) was used to annotate and filter the variants. Sanger sequencing of genomic DNA extracted from total blood was used to confirm the splice-site mutation in *PI3KR1*. DNA was amplified using the primers 5' –AGAAAACATTTGAAGCAGATGAA– 3' and 5' –CAGTGACTGCTTCAGTTATCACG– 3' and the PCR product was subsequently sequenced with one of the two primers.

Cell culture. Peripheral blood mononuclear cells were isolated by Ficoll-Paque density-gradient centrifugation and washed twice with RPMI 1640 GlutaMax medium (Invitrogen). T cell blasts were obtained by stimulating $1x10^6$ cells per ml in RPMI 1640 GlutaMax supplemented with 10% human AB serum, penicillin and streptomycin (Invitrogen), PMA (20 ng/mL; Sigma-Aldrich) and ionomycin (1 µmol/L). After 2 to 3 days of activation, viable cells were separated by Ficoll-Paque density-gradient

centrifugation, washed twice with RPMI 1640 GlutaMax and then cultured in RPMI 1640 GlutaMax supplemented with 10% human AB serum and 100 U/mL IL-2.

Activation-induced cell death. T cell blasts ($3x10^5$ cells) were incubated with 3μM of PI3Kδ inhibitor (IC87114, Calbiochem) for 15 minutes before and during stimulation with plate-bound anti-CD3 (2.5 μg/ml: OKT3, ebioscience) for 16h. The viability of CD4 and CD8 T cell blasts was determined using 7-amino-actinomycin D (BD Via Probe, Bioscience) and annexin-V (BD Pharmingen) staining, together with CD4 (RPA-T4; Biolegend) and CD8 (BW135/80, Miltenyi Biotec) staining in accordance with the manufacturer's protocol (BD Pharmingen). Cells were measured with flow cytometry (BD FACSCalibur) and the data were analyzed with FlowJo software (Tree Star Inc.).

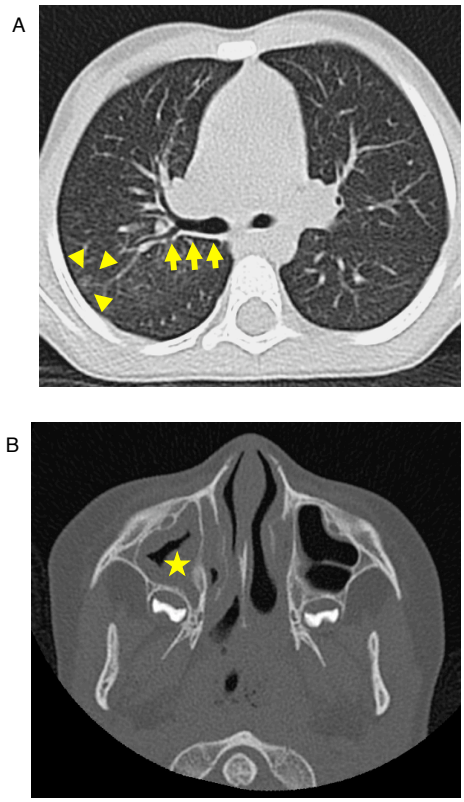
Plasmids and transfection. The entire coding sequence of human wildtype p85α and mutated $p85\alpha^{\Delta 434\_475}$ was amplified using reverse transcription PCR from P1's T cell blast mRNA incooperating flanking 5' BamHI site. The PCR products were subcloned into Topo-TA vector (Invitrogen). After sequencing the inserts, p85α and $p85\alpha^{\Delta 434\_475}$ containing inserts were excised with BamHI and were inserted into BglII digested p3XFLAG-MYC-CMV-26 expression vector (Sigma) to encode FLAG-p85α and FLAG-$p85\alpha^{\Delta 434\_475}$. Transient transfection of FLAG-p85 mutant or wildtype expressing plasmids into NIH3T3 cells were carried out using lipofectamine 2000 (Invitrogen) according to the manufacturer's directions. Cells were lysed 48h after transfection immunoblot analysis.

Immunoblot analysis. T cell blasts (5×10^6 cells/ml) were stimulated with anti-CD3 (0.1 μg/mL OKT3, ebioscience) cross-linked with rabbit-anti-mouse IgG (2 μg/mL) for 10

minutes. The cells were then lysed in TNE (50 mmol/L Tris at pH 8.0, 1% Nonidet P-40, and 2 mmol/L EDTA) buffer supplemented with protease and phosphatase inhibitors. Immunoblots were performed according to standard protocols. Antibodies against p110δ (H-219; Santa Cruz), phospho-Akt (Ser473; #9271; Cell Signaling and Thr308; #9275S; Cell Signaling), Akt (#9272; Cell Signaling) PTEN (#9188S; Cell Signaling), Ku-70 (H-308; Santa Cruz) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 6C5; Santa Cruz) were used for immunoblotting.

Intracellular phospho-Akt staining. After a 10-minute stimulation with anti-CD3 (0.1 μg/mL; OKT3) cross-linked with rabbit-anti-mouse IgG (2 μg/mL), cells were fixed in 4% formaldehyde prior to cell membrane permeabilization with methanol. Staining was performed according to the manufacturer's instructions (Cell Signaling), with incubation with anti-phospho-AKT Ser 473 Alexa 647 (#4075, Cell Signaling) for 1 hour in the dark at room temperature. Cells were measured by flow cytometry (BD FACSCalibur) and the data were analyzed with FlowJo software (Tree Star Inc.). For the analysis of phosphorylated S6 with anti-phospho-S6 Ribosomal Protein (Ser240/244) Alexa488 (#5018, Cell Signaling) cells were fixed in the medium they were cultured with.

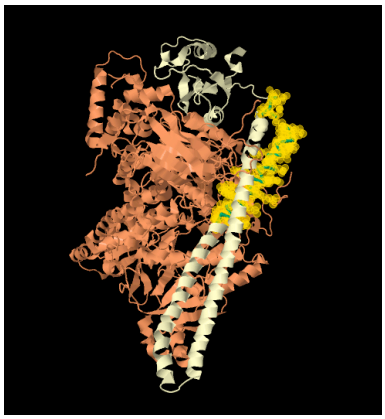
Statistical analysis. Analyses were performed with PRISM software (version 4 for Macintosh, GraphPad Inc.). Statistical hypotheses were tested using 2-tailed *t* test. A *P* value less than 0.05 was considered significant.



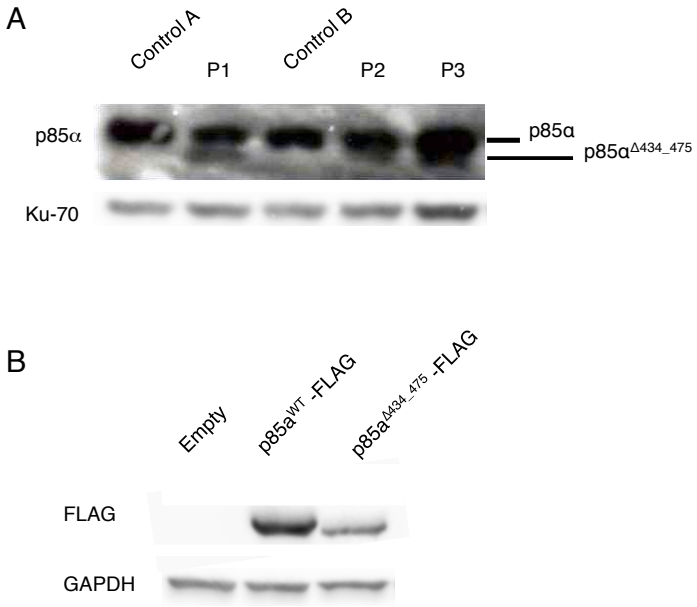
Supplementary Figure 1. Imaging infection of upper and lower respiratory tract.

(A) Thoracic computed tomography (CT) scan showing bronchitis with wall thickening of bronchial tract (arrows) and distal pneumonitis with multifocal ground glass opacities (arrow heads).

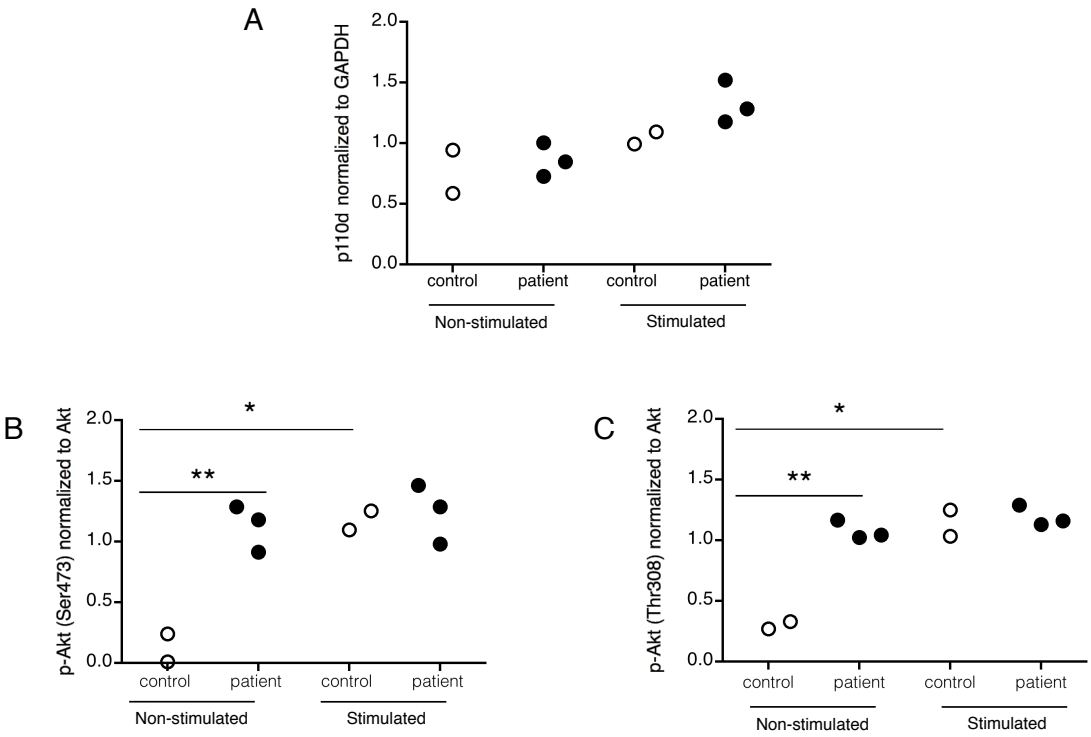
(B) Facial CT scan showing right maxillary sinusitis with inflammatory thickening of the mucosal lining (star). Both CT scans were performed in P1.



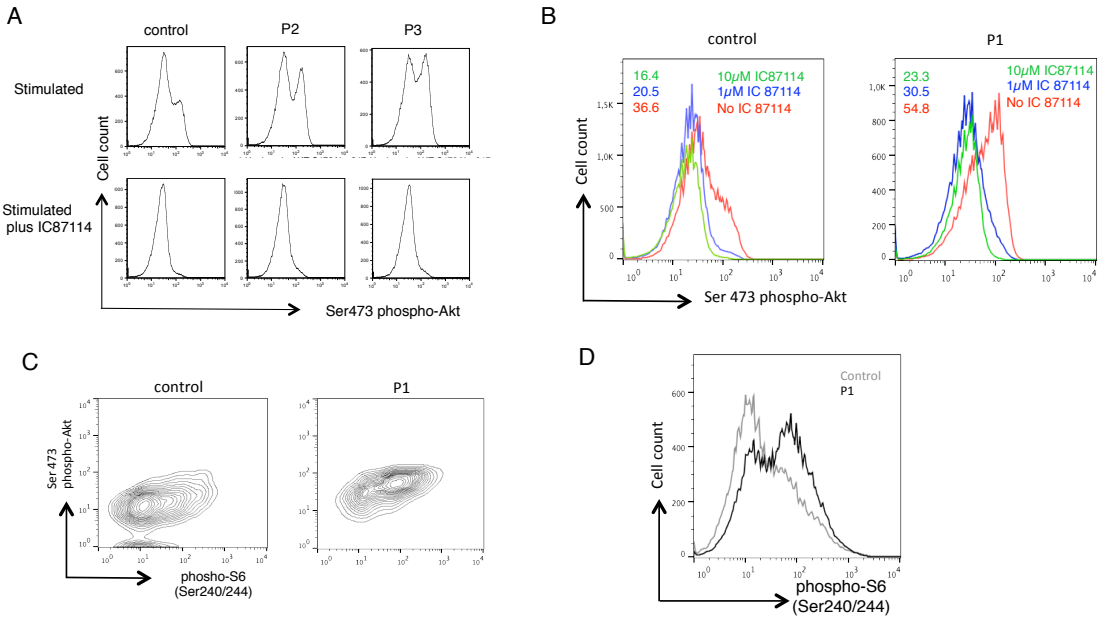
Supplementary Figure 2. Location of the deleted amino acid residues (434-475) in p85α. The figure shows the structure of human p110α (in orange) bound to the p85α regulatory component (in white) of the PI3K complex (Protein Data Bank accession code: 3HHM); the amino acid residues 434-475 (encoded by exon 10 of the *PIK3R1* gene) are highlighted in yellow and their backbone in green.



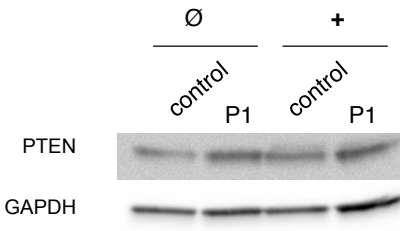
Supplementary Figure 3. Western blot for p85α. A) Expression of p85α and Ku-70 (as loading control) by Western blotting of cell lysates derived from T cell blasts from P1, P2 and P3 and healthy controls. B) A representative experiment showing the expression of flag-tagged p85α wildtype (WT), flag-tagged $p85\alpha^{\Delta 434\_475}$ and GAPDH by Western blotting of cell lysates derived from 293T cells 2 days after transfection with the indicated expression vector.



Supplementary Figure 4. Increased phosphorylation of Akt in patient T cell blasts detected by immunoblotting. A) Quantification of band intensity of p110δ normalized to band intensity of GAPDH from immunoblots presented in Figure 2a. B) Quantification of band intensity of phosphorylated Akt at Ser473 normalized to total Akt from immunoblots presented in Figure 2a. C) Quantification of band intensity of phosphorylated Akt at Thr308 normalized to total Akt from immunoblots presented in Figure 2a. * P=0.05, ** P=0.01 (unpaired t-test).



Supplementary Figure 5. Hyperphosphorylation of Akt and S6 detected by flow cytometry. A) Phosphorylation of Akt at Ser473 in T cell blasts from patients and a control following activation with anti-CD3 and anti-CD28 antibodies for 30 minutes in the presence or absence of 10μM IC87114 inhibitor for the last 20 minutes of activation, and as measured by intracellular staining and flow cytometry. T cell blasts from patients and controls were cultured for the same period of time. B) Intracellular staining of phosphorylated Akt at Ser473 in T cell blasts from patients and a control following activation with 1μg of anti-CD3 antibody in the absence (red) or presence of either 1μM (blue) or 10μM (green) IC87114 inhibitor. Numbers indicate the geometric mean fluorescence intensity. C, D) Intracellular staining of phosphorylated Akt (Ser473) and phosphorylated S6 (Ser240/244) of T cell blasts fixed immediately in medium. C) Contour plot showing phosphorylated Akt (Ser473) and phosphorylated S6 (Ser240/244) staining of non-stimulated T cell blasts from patient and control D) Histogram showing the overlay of phosphorylated S6 (Ser240/244) from control (grey) and patient (black).



Supplementary Figure 6. Abundance of PTEN protein in T cell blasts.

Expression of PTEN and GAPDH detected by Western blotting of cell lysates derived from T cell blasts from P1 and healthy control before and after stimulation with anti-CD3 antibody.