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Plasma cell deficiency in humans with heterozygous mutations in *SEC61A1*

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Keywords: SEC61A1; translocon; protein translocation; antibody deficiency; plasma cell; multiple myeloma; calcium homeostasis; ER stress.

Abstract

Background: Primary antibody deficiencies (PAD) are the most frequent primary immunodeficiencies in humans. The genetic causes for PADs are largely unknown. Sec61 translocon alpha 1 subunit (SEC61A1) is the major subunit of the Sec61 complex, which is the main polypeptide-conducting channel in the endoplasmic reticulum (ER) membrane. *SEC61A1* is a target gene of XBP1s and strongly induced during plasma cell differentiation.

Objective: Characterization of a novel genetic defect and its pathological mechanism in eleven patients from two unrelated families with PAD.

Methods: Whole exome sequencing (WES) and targeted sequencing were conducted to identify novel genetic mutations. Functional studies were carried out *ex vivo* in primary cells of patients and *in vitro* in different cell lines to assess the effect of *SEC61A1* mutations on B cell differentiation and survival.

Results: We investigated two families with patients suffering from hypogammaglobulinemia, severe recurrent respiratory tract infections and normal peripheral B- and T cell subpopulations. Upon *in vitro* stimulation, B cells showed an intrinsic deficiency to develop into plasma cells (PCs). Genetic analysis and targeted sequencing identified novel heterozygous missense (c.254T>A, p.V85D) and nonsense (c.1325G>T, p.E381*) mutations in *SEC61A1*, segregating with the disease phenotype. SEC61A1-V85D was deficient in co-translational protein translocation and it disturbed the cellular calcium homeostasis in HeLa cells. Moreover, SEC61A1-V85D triggered the terminal unfolded protein response (UPR) in multiple myeloma (MM) cell lines.

74 **Conclusion:** We describe a monogenic defect leading to a specific plasma cell deficiency in
75 humans, expanding our knowledge about the pathogenesis of antibody deficiencies.

Capsule Summary

Heterozygous mutations in *SEC61A1* are associated with plasma cell deficiency in patients with early-onset hypogammaglobulinemia and severe, recurrent respiratory tract infections but with normal B cell subpopulations in the peripheral blood.

Key Messages

SEC61A1, a target gene of XBP1s during plasma cell differentiation, was mutated in patients diagnosed with primary antibody deficiency.

The disease phenotype was limited to severe, recurrent infections, mainly of the respiratory tract and patients responded well to immunoglobulin replacement therapy.

All patients had normal subpopulations of B and T cells in the peripheral blood but *in vitro* plasma cell homeostasis was impaired due to unresolvable ER-stress.

Introduction

Primary antibody deficiencies (PADs) are the most frequent primary immunodeficiencies (PIDs) in humans¹. PADs are a group of diseases generally characterized by impaired B cell differentiation and function, which results in low immunoglobulin (Ig) production. They have a spectrum of underlying causes and phenotypical expressivity².

Differentiation of B lymphocytes into plasma cells (PCs) requires extensive morphological and homeostatic changes³ such as the expansion of the endoplasmic reticulum (ER), which allows PCs to become secretory factories releasing thousands of antibodies per second⁴. This massive Ig production is accompanied by the accumulation of misfolded proteins in the ER, which activates the unfolded protein response (UPR). The UPR must be constitutively maintained in plasma cells to cope with perpetual ER stress to ensure longevity⁵. Of the existing signaling pathways regulating the UPR in mammals (IRE1A/XBP1s, PERK/ATF4 and ATF6/ATF6f), only the IRE1A/XBP1s pathway is required for the differentiation of B cells into PCs^{6, 7}. After sensing ER stress, inositol-requiring enzyme 1 alpha (IRE1A) is activated by phosphorylation. Active IRE1A triggers the splicing of the “unspliced” transcriptional activator x-box binding protein 1 (*XBP1u*) by the excision of a 23-base pair intron from its mRNA, resulting in the active “spliced” form of the *XBP1* mRNA (*XBP1s*)^{8, 9}. XBP1s increases the synthetic capacities of cells by inducing the transcription of genes encoding mediators of protein synthesis, transport, folding and degradation¹⁰. However, which of the many target genes of XBP1s are critical for plasma cell differentiation is poorly understood. Interestingly, the PERK/ATF4 pathway of the UPR is specifically suppressed during the differentiation of B cells into PCs⁶. PKR-like ER kinase (PERK) activation leads to a global downregulation of protein synthesis and concomitantly increased levels of the activating transcription factor 4 (ATF4), which induces the transcription of C/EBP homologous protein (*CHOP*). CHOP is a

mediator of the terminal UPR that induces apoptosis in situations of unresolvable ER stress¹¹. Therefore, activation of the PERK/ATF4 pathway would be incompatible with the development of a long-lived humoral immune response.

The genes encoding the Sec61 complex are targets of the UPR¹⁰. This protein complex co- or post-translationally transports nascent polypeptides from the cytosol into the ER lumen or inserts them into the ER membrane¹². The heterotrimeric Sec61 complex is composed of the large SEC61A1 subunit and the two much smaller SEC61B and SEC61G subunits¹³. In its open state, the Sec61 channel forms a large aqueous pore in the ER membrane that is penetrable for small molecules and ions¹⁴. Indeed, the Sec61 complex acts as a passive calcium (Ca^{2+}) leakage channel¹⁵. Therefore, SEC61A1 contains a highly conserved domain called the “pore ring” that tightly surrounds the nascent polypeptide to limit Ca^{2+} leakage through the opened channel¹³.

Here, we describe two families suffering from early-onset, severe, recurrent bacterial infections of the respiratory tract with antibody isotype deficiencies involving IgM, IgG and IgA. By whole exome sequencing (WES) in the first family and targeted sequencing in the second family, we identified a heterozygous missense mutation and a heterozygous nonsense mutation in *SEC61A1*. The missense mutation is located within the pore ring of SEC61A1. Affected individuals had normal numbers and subsets of peripheral B cells, but these failed to differentiate into PCs *in vitro*. We show that *SEC61A1-V85D* overexpression boosts ER stress due to an increased Ca^{2+} leakage and impaired protein translocation in HeLa cells, and activates the terminal UPR in multiple myeloma (MM) cell lines.

Materials and methods

Ethics approval

All individuals donated samples following informed written consent under local ethics board–approved protocols: 295/13_140782 from 19th August 2014 “Klinik und molekulargenetischer Defekt des variablen Immundefekts (CVID)” (ethics committee of the Albert-Ludwigs-University Freiburg).

B cell stimulation assay

B cells were isolated from PBMCs with the B cell isolation kit II (Miltenyi Biotec). 30,000 CD19⁺ B cells (purity >90%, mostly >99%) were seeded in triplicates for each time point and stimulated in 200 µl medium (IMDM supplemented with 10% FBS, 1% penicillin/streptomycin, 2.5 µg/ml Transferrin, 1 µg/ml Glutathion, 1 µg/ml Insulin, 2 mM L-Glutamine, 1x non-essential amino acids and 0.1% fatty acid supplement) containing 2 µg/ml anti-IgM antibody (SouthernBiotech #2020-10), 0.5 µM CpG (Apara Bioscience #153100) and 0.1 µg/ml Baff-3mer or CD40L and IL21 (Baff-3mer, CD40L and IL21 were produced, titrated and kindly provided by the laboratory of Professor Dr. Eibel) in a 96-well round bottom culture plate for nine days. The medium was changed (100 µl medium were replaced by fresh, twofold concentrated stimulation medium) every three days.

SEC61A1 silencing and expression

To rescue the phenotype of *SEC61A1* silencing and functionally characterize the SEC61A1-V85D mutant, the respective cDNAs of wild type (wt) or mutant were inserted into the multi-cloning sites of the pCMV6-AC-IRES-GFP-vector (Origene). For gene silencing, 6 x 10⁵ HeLa cells were seeded per 6 cm culture plate. The cells were transfected with *SEC61A1*-UTR

(CACUGAAAUGUCUACGUUtt) siRNA (Qiagen) or control siRNA (AllStars Negative Control siRNA, Qiagen) using HiPerFect Reagent (Qiagen) for 96 hours as described previously¹⁶ (final concentration of siRNAs: 20 nM). SiRNA transfection was repeated after 24 hours. Six hours after the first siRNA transfection, the cells were transfected with pCMV6 expression plasmids using Fugene HD (Promega).

Pre-prolactin processing and Cytochrome B5 glycosylation

Two established model precursor polypeptides were used for quantitative analysis of SEC61-dependent (bovine preprolactin (ppl)) as well as SEC61-independent (tail-anchored hybrid protein Cytb5-ops28) protein transport into the ER of semi-permeabilized HeLa cells *in vitro*¹⁷⁻¹⁹. Both precursor polypeptides were synthesized in reticulocyte lysate (nuclease treated; Promega) in the presence of [³⁵S]methionine (Perkin Elmer) plus buffer or semi-permeabilized HeLa cells (final concentration: 40,000 cell equivalents/ μ l) for 60 minutes at 30°C. Semi-permeabilized-cells were prepared from identical cell numbers by digitonin treatment. Transport of [³⁵S]methionine-labeled ppl *via* the Sec61 complex was assessed by SDS-PAGE and phosphorimaging (Typhoon-Trio imaging system, Image Quant TL software 7.0).

Live cell calcium imaging

HeLa cells were transfected using FuGene HD (Promega) 8h after seeding with *SEC61A1* expression plasmids in combination with a *SEC61B-IRES-SEC61G* encoding pCDNA3-IRES-GFP expression plasmid. Live cell calcium imaging for cytosolic Ca²⁺ was carried out as described previously²⁰.

Viral transduction of cell lines

176 The respective cDNAs of wt or mutant *SEC61A1* were inserted into the multi-cloning sites of
177 the pMXS-IRES-GFP retroviral expression vector. HEK293T cells were transiently transfected
178 using X-tremeGene HP DNA Transfection Reagent (Roche) with 5 µg expression plasmid and
179 5 µg pCL-ampho retrovirus packaging vector (Imgenex). The medium was changed after 24
180 hours and virus was harvested 48 and 72 hours after transfection. Cell lines were treated on
181 two consecutive days with virus-containing medium and fresh medium in a 1:1 ratio. Spin
182 infection was carried out for 2-3 hours at 870 g. Infection efficiencies were analyzed by flow
183 cytometry. Fluorescence activated cell sorting (FACS) was carried out with a MoFlo Astrios
184 cell sorter (Beckman Coulter). Multiple myeloma cells were sorted one day after the second
185 viral transduction. Transduced Hek293T cells were expanded in culture for one week before
186 sorting.

Results

Clinical description of the families

We investigated 10 individuals in Family I (Figure 1A), who had antibody isotype deficiencies involving IgM, IgG and IgA (Table S1) and who suffered from severe recurrent bacterial infections such as tonsillitis, otitis, sinusitis, pneumonias and gastrointestinal infections. The disease onset was mostly in the first year of life. Affected individuals did not respond to polysaccharide vaccination and responded variably to *tetanus* toxin vaccination (Table S1). They have successfully maintained a marked decrease in the number and severity of infections since initiating immunoglobulin replacement therapy with intravenous immunoglobulins (IVIG) (detailed case reports in Supplemental Information). The index patient of Family II (Figure 1A) was an eight year old boy who had hypogammaglobulinemia since birth but owing to IVIG treatment has not experienced any severe or recurrent infections (II.P3, detailed case report in Supplemental Information). Attempts to withdraw IVIG treatment repeatedly failed due to persistently low Ig serum levels (Table S1). His mother (who is affected by a secondary immunodeficiency and therefore not included in this study) and grandmother (II.P1) are also mutation carriers; the latter reported an increased frequency of upper respiratory infections during childhood with recurrent bilateral bacterial otitis media. Now, at age 66, she has normal immunoglobulin serum levels (Table S1).

Identification of heterozygous mutations in *SEC61A1*

We performed linkage analysis using microsatellite markers to identify the genetic region harboring the disease-causing mutation in Family I. A genome-wide scan of all autosomes revealed only one region, on chromosome 3, with LOD scores greater than 2. The high scores were achieved at consecutive markers. Fine mapping confirmed genetic linkage to

chromosome 3. The peak single-marker and multiple-marker LOD score was 3.31. The genetic linkage analysis established that the mutated gene should be between markers D3S3697 and D3S3684 (Table S2).

Next, we performed WES on six members of Family I (I.P7, P8, P9, P10, H1 and H2). Evaluation of all variants shared among the four affected individuals and absent in the two unaffected family members revealed three novel single-nucleotide variants (SNV) in the genes *SEC61A1*, *GOLGA8K* and *SLC47A2*, with only *SEC61A1* located in the linkage region on chromosome 3 (Table S3). By Sanger sequencing we confirmed that the heterozygous missense mutation (c.254T>A, p.V85D) in *SEC61A1* perfectly segregated with the disease within Family I (Figure 1B). c.254T>A in *SEC61A1* translates into a p.Val85Asp change (p.V85D), predicted as “damaging” by programs SIFT²¹ (score 0.000), Proven²² (score -6.26) and MutationTaster²³ (score 4.15). The V85 residue of SEC61A1 (Figure 1C, in magenta) is one of six bulky and hydrophobic amino acids that form the pore ring in the middle of the Sec61 channel (blue and magenta side chains in Figure 1C). Furthermore, V85 is highly conserved (Figure 1D) and a valine to aspartic acid change substitutes a hydrophobic and neutral amino acid for a hydrophilic and negatively charged residue, which is predicted to alter the structure and/or functional properties of the Sec61 channel.

Seeking additional individuals with *SEC61A1* mutations, we performed targeted next generation sequencing in >200 additional patients with primary antibody deficiency. We identified a heterozygous nonsense mutation (c.1325G>T, p.E381*) in individual II.P3 from Family II (SIFT score: 0.003). By Sanger resequencing, we confirmed this mutation and identified two additional mutation carriers in the family (Figure 1B). In the genome aggregation database (gnomAD) which includes data from 123,136 exomes (ExAc data) and additional 15,496 genomes, there are no mutations reported in healthy individuals leading

to a premature stop codon in *SEC61A1*. Moreover, *SEC61A1* was a promising candidate since it is a target gene of XBP1s during plasma cell differentiation²⁴⁻²⁶. As determined by mass spectrometry-based proteomics²⁷, the relative abundance of SEC61A protein is two-fold higher in plasmablasts from human peripheral blood as compared to naïve and memory B cells (Figure 1E).

The p.E381* nonsense mutation in Family II leads to SEC61A1 haploinsufficiency

Nonsense mutations lead to a premature stop of translation resulting in either the synthesis of a shortened protein or in nonsense-mediated mRNA decay. We analyzed SEC61A1 protein levels in sorted naïve B cells (CD19⁺CD27⁻), and CD3⁺CD4⁻ T cells of II.P1 and II.P3. We observed reduced expression levels of SEC61A1 in naïve B cells of II.P1 and II.P3 whereas expression levels in CD8⁺ T cells were comparable to the control (Figure 1F). Using a second antibody recognizing the N-terminal domain of SEC61A1 in lysates from an EBV-transformed B cell line of II.P3, we excluded the presence of a shortened protein product from the mutated allele in Family II (Figure S1), supporting the hypothesis that the nonsense mutation p.E381* likely results in nonsense-mediated mRNA decay and leads to SEC61A1 haploinsufficiency in Family II.

Affected individuals in both families have normal peripheral B and T cell subpopulations.

The circulating B and T cell populations of patients from both families were analyzed by flow cytometry. B-, T- and NK cells were present at normal ratios (Figure 2A and Table S4) and the patients did not show any abnormalities in the detailed T cell phenotyping looking at naïve, memory, and effector T cell stages in CD8⁺ and CD4⁺ T cells. They also had normal numbers of regulatory T cells and of recent thymic emigrants (Table S4). Even more surprising, the peripheral subpopulations of B cells including naïve, unswitched memory, switched memory,

IgA⁺, IgG⁺, transitional and CD21^{low} B cells were also normal (Figure 2B, all lymphocyte values are displayed in Table S4). Only the very small population of peripheral CD27⁺CD38^{high} plasmablasts appeared to be reduced in patients of Family I (Figure 2B). However, since this is a very small cell population, which can be lower than 1% of B cells in healthy individuals but increase dramatically during infection, this value is rather error-prone.

B cells of mutation carriers have an intrinsic deficiency to develop into plasma cells.

The initial observations on B cells prompted us to study plasma cell development. To assess whether the B cells of patients have an intrinsic differentiation defect, we stimulated purified B cells for nine days mimicking T cell dependent (CD40L and IL21) and T cell independent (anti-IgM, BAFF, CpG) activation. The differentiation of resting peripheral B cells into CD27⁺CD38^{high} plasmablasts and CD38^{high}CD138⁺ plasma cells was significantly decreased in mutation carriers from both families upon T cell dependent (Figure 3A, B) and T cell independent (Figure S2A, B) stimulation. Furthermore, upon CD40L/IL21 stimulation, B cells from affected individuals had decreased IgG and IgA production, but no significantly different IgM production (Figure 3C). Similarly, IgG and IgA levels were significantly lower for cells from affected individuals upon polyclonal stimulation with CpG, BAFF and anti-IgM (Figure S2C), whereas IgM was not secreted by the cells following this stimulation.

EBV-transformed B cell lines carrying *SEC61A1* mutations secrete less immunoglobulin.

To substantiate the results of the primary B cell stimulation assay, we studied EBV-transformed B cell lines of several individuals of Family I and II. First, we analyzed protein and mRNA levels in four healthy donor (HD) cells lines, three cell lines generated from Family I and one cell line from II.P3. We observed significantly reduced protein and mRNA levels in all cell lines from patients (Figures 4A and B), with the cell line of II.P3 having the lowest

mRNA expression levels (triangle in Figure 4B). If the reduced protein levels were a product of decreased stability of the p.V85D mutant protein, we would have expected similar or even increased mRNA levels in Family I compared to the controls. Since mRNA and protein levels were both reduced and EBV cell lines are known to have a high phenotypic variability, we stained five control and four patient EBV cell lines for B cell surface markers. Intriguingly, we observed that all HD cell lines contained a subpopulation of CD38^{high}CD138⁺ plasma cells that was absent in all patient-derived cell lines (Figure 4C). This result prompted us to analyze immunoglobulin secretion levels of the cells by ELISPOT. The mean spot size, representing the secretion capacity per cell, was significantly reduced in cells from the patients compared to controls for IgG, IgA and IgM (Figure 4D). Taken together, these data provide a link between low *SEC61A1* expression levels and reduced immunoglobulin secretion by B lymphoblasts.

Protein translocation by the SEC61A1-V85D channel is impaired.

The main function of SEC61A1, as the central subunit of the Sec61 channel, is the transport of nascent polypeptides into the ER. To test whether co-translational protein transport was still functional in mutated translocons, we silenced the endogenous *SEC61A1* mRNA in HeLa cells followed by complementation with either the wt or the mutant SEC61A1 protein using their respective expression plasmids (Figure 5A). The transport of preprolactin (ppl) *via* the Sec61 complex was assessed in semipermeabilized cells. Ppl is an established model precursor polypeptide for studying general effects of *SEC61A1* missense mutations on Sec61-dependent cotranslational protein translocation. Upon transport into the ER, the signal peptide of the ppl is cleaved by a signal peptidase leaving the mature prolactin (pl) protein. We observed that co-translational transport of ppl through the SEC61A1-V85D channel was significantly decreased compared to the transport through the wt channel (Figure 5B). In

contrast, SEC61A1-independent membrane insertion of the tail-anchored protein Cytochrome B5 and concomitant N-glycosylation was not impaired (Figure 5C), reflecting the integrity of the ER membrane in SEC61A1-V85D-producing cells.

Dissipation of the ER/cytosol calcium gradient in SEC61A1-V85D-producing HeLa cells.

The localization of the p.V85D mutation in the pore ring of SEC61A1 suggested that the ion permeability of the ER membrane, especially concerning the maintenance of the calcium gradient between ER lumen and cytosol, may play a role in the disease pathogenesis. To investigate the influence of the p.V85D mutation on the leakiness of the SEC61A1 channel for Ca^{2+} ions, we transiently overproduced SEC61A1-V85D together with its interaction partners SEC61B and SEC61G in HeLa cells (Figure 6A). The cells were then loaded with the calcium indicator Fura2 and treated with thapsigargin, an inhibitor of the SERCA-ATPase, to visualize leakage of calcium from the ER into the cytosol by live cell imaging. Overproduction of the wt protein resulted in an increased leakage (Figure 6B), in accordance with its role as a calcium leak channel. Unexpectedly, we observed a significant decrease in calcium leakage in cells overproducing SEC61A1-V85D compared to cells with endogenous SEC61A1 protein levels or cells overproducing SEC61A1-wt (Figure 6B). This could be explained either by a decreased leakiness of the SEC61A1-V85D channel or, on the contrary, by a severe increase of its leakiness resulting in a depletion of the ER/cytosol Ca^{2+} gradient.

Indeed, treating the cells with ionomycin to assess the total amount of calcium ions in the ER lumen revealed that the calcium load of the ER was significantly decreased in cells overproducing the mutant channel in comparison to cells overproducing the wt form (Figure 6C), thus supporting the latter hypothesis. Since these experiments were carried out in cells

overexpressing *SEC61A1-V85D* without prior silencing of endogenous *SEC61A1*, we conclude that the effect on the ER/cytosol Ca^{2+} gradient is a dominant effect of *SEC61A1-V85D*.

***SEC61A1-V85D* production selectively affects plasma cells.**

To study the effect of stable expression of *SEC61A1-V85D* on plasma cells, we utilized three different multiple myeloma (MM) cell lines: JK6L, U266 and L-363. While JK6L cells still secrete high amounts of IgG, U266 and L-363 cells do not secrete antibodies (Figure S3); nevertheless, they still have the morphology of plasma cells²⁸. Stable transduction with a vector encoding *SEC61A1-V85D-IRES-GFP* resulted in the disappearance of GFP^+ cells of all MM cell lines after several days in culture, whereas the expression of wt *SEC61A1-IRES-GFP* was stable (Figure 7A and S4). Interestingly, the IgG-secreting JK6L cells appeared to be more sensitive to *SEC61A1-V85D* protein than the non-Ig-secreting U266 and L-363 cells. Stable overexpression of wt and mutant *SEC61A1* in other lymphocytic (EBV-transformed B cells, Jurkat T cells) and non-lymphocytic (THP-1 monocytes, Hek293T, HeLa, HT29) cell lines did not affect the proliferation capacity or viability of those cells (Figure 7A and S4). Taken together, these observations indicate that *SEC61A1-V85D* production selectively impairs the survival of plasma cell lines. In line with the hypothesis, patients did not present additional clinical symptoms to infections and lab values of proteins secreted by the liver and the pancreas into the serum were all within the normal ranges (Table S5).

***SEC61A1-V85D* production and *SEC61A1* knock down induces the terminal UPR in plasma cell lines.**

Based on the experiments in HeLa cells (Figures 5, 6), we hypothesized that disturbed cellular calcium homeostasis and/or dysfunctional protein translocation caused by *SEC61A1-V85D* might induce unresolvable ER stress and the terminal UPR in MM cells. To study this

hypothesis, we analyzed MM cells overproducing SEC61A1-V85D by western blotting (WB) and RT-qPCR. We observed significantly increased protein levels of the ER stress sensors IRE1A and PERK and their downstream transcription factors XBP1s and ATF4 in U266 cells (Figure 7B). Comparable results were obtained from the JK6L (Figure S5A) and the L-363 (Figure S5B) MM cell lines. Notably, the PERK/ATF4 pathway, which acts upstream of the apoptosis-inducing transcription factor CHOP, seemed to be activated. By RT-qPCR analysis, we verified the activation of this pathway. In addition to *XBP1s*, mRNA levels of *ATF4* and *CHOP* were upregulated upon *SEC61A1-V85D* overexpression in L-363 cells (Figure 7C), pointing to the activation of the terminal UPR in this plasma cell line. To study the effect of *SEC61A1* haploinsufficiency on plasma cell homeostasis, we attempted to partially knock down *SEC61A1* in JK6L multiple myeloma cell lines using lentiviral vectors encoding four different shRNAs against *SEC61A1*. However, after puromycin selection, none of the four stably transduced cell lines had reduced SEC61A1 protein levels (data not shown), indicating that we had selected for clones that evaded shRNA knock down while maintaining the puromycin resistance. Next, we directly transfected the cells with siRNA against *SEC61A1*. In independent repeats optimizing the ratio of siRNA to transfection reagent and checking at different time points in JK6L, U266 and L363 cell lines, we found only once a partial knock down of *SEC61A1* at 24 hours post-transfection in JK6L cells. Intriguingly, those cells with a partial knock down had increased expression levels of IRE1A and PERK, showing an increase of ER stress and induction of the UPR (Figure 7E). In summary, these results indicate that multiple myeloma cells do not easily tolerate a knock down of *SEC61A1* and that a knock down might induce the UPR in JK6L cells.

Dominant effect of mutant SEC61A1-V85D in sensitized HEK293T cells.

In accordance with the insensitivity of non-MM cell lines to the overexpression of *SEC61A1-V85D* (Figure S4), we did not observe an induction of the terminal UPR in Hek293T nor in HT29 cells (data not shown). This suggested that they were likely able to adapt to the effects of the mutant protein. To sensitize them towards the overproduction of the mutant *SEC61A1*, we treated HEK293T cells stably overexpressing *SEC61A1-V85D* for 24h with thapsigargin to induce additional ER stress by mimicking a disturbance of the Ca^{2+} homeostasis. As expected, we observed a significantly higher induction of *XBP1s* in cells overproducing *SEC61A1-V85D* compared to cells overproducing the wt protein (Figure 7D). Interestingly, it made no difference whether the mutant was overexpressed alone or co-overexpressed with *SEC61A1-wt* (Figure 7D), thus showing a dominant effect of *SEC61A1-V85D*.

Discussion

Currently known genetic causes for antibody deficiencies, some of which are diagnosed as CVID, impair early B-cell development, B cell receptor signaling or the functionality of B- or T-cells in the germinal center reaction²⁹. Even though CVID patients suffering only from infections have been reported to present a considerable subset in CVID cohorts (e.g. Resnick et al.: 32% of 473 subjects³⁰), hardly any of the known monogenic disorders present with this clinical phenotype^{1, 29}. To our knowledge, this is the first monogenic defect that specifically impairs plasma cell homeostasis without interfering with B cell development, activation, and memory formation.

We found two heterozygous *SEC61A1* mutations in patients of two unrelated families with an early-onset, infection-only antibody deficiency and normal (sub-)populations of peripheral B cells. First, a missense mutation in the pore ring affected cotranslational protein transport and increased Ca^{2+} leakage from the ER, the latter representing a potential mechanism for a toxic, dominant-negative effect of the mutant over the wt protein. Second, a nonsense mutation abrogated protein production from one of the two *SEC61A1* alleles. It is known that SEC61A1 proteins with severe mutations compete poorly with wt proteins for binding of complex partners and are therefore degraded in a heterozygous situation³¹⁻³³. In plasma cells, concomitant increase of SEC61B and SEC61G levels might stabilize protein levels of previously degraded p.V85D mutants, resulting in the dissipation of the ER/cytosol calcium gradient and increased ER-stress. In addition, plasma cells, in contrast to most other cells, might require two functional *SEC61A1* alleles to sufficiently induce SEC61A1 protein levels for excessive immunoglobulin production. We suggest that *SEC61A1* haploinsufficiency is causing (possibly transient) hypogammaglobulinemia with variable expressivity and incomplete penetrance in Family II. In contrast, a dominant negative mutation in *SEC61A1*

resulting in a functional haploinsufficiency in combination with a dominant negative disturbance of the calcium homeostasis may be responsible for the fully penetrant PAD in Family I ranging from transient hypogammaglobulinemia and specific antibody deficiency to CVID.

In humans, Sec61-channelopathies have been linked for example to polycystic liver disease, diabetes, and congenital disorder of glycosylation due to mutations in the translocon-associated proteins SEC63, ERJ6 and SSR4, respectively³⁴⁻³⁶. Moreover, in a mouse model, a homozygous point mutation in *Sec61a1* impaired binding of Sec61a1 to its interaction partner BiP and caused type 1 diabetes³⁷. Evidence that variations in the *SEC61* genes may be relevant to plasma cell deficiency comes from a murine study, in which the conditional B cell specific inactivation of *Xbp1* resulted in reduced production of IgM and IgG and in reduced expression levels of the *Xbp1*s target gene *Sec61a1*²⁵. A recent study reported two heterozygous missense mutations in *SEC61A1* in patients with autosomal dominant tubulointerstitial and glomerulocystic kidney disease (ADTKD) with anemia³⁸. Laboratory values that were found altered in ADTKD patients were normal in our patients (Table S6). The effects of *SEC61A1* missense mutations in ADTKD on protein-protein interactions, intrinsic functionality, or protein stability were not investigated. However, heterozygous mutations that reduce the hydrophobicity of the signal peptide of renin cause ADTKD with a clinical phenotype similar to the phenotype associated with *SEC61A1* mutations. These mutations interfere with SEC61 complex-dependent translocation of renin across the ER membrane and signal peptide cleavage, hence the Sec61 complex is directly involved in the disease pathogenesis³⁹⁻⁴¹. It therefore seems possible that the *SEC61A1* mutations in ADTKD specifically impair renin translocation and processing and therefore mirror the clinical phenotype of renin mutations.

431 In summary, we provide evidence that the disturbance of the ER homeostasis interferes with
432 plasma cell functionality in humans and can cause primary antibody deficiency as previously
433 proposed by others⁴². We hypothesize that sensors, signaling components and target genes
434 of the UPR that play an important role in protein synthesis, modification and secretion, and
435 in the maintenance of the ER homeostasis in PCs are likely to be mutated or dysregulated in
436 other antibody-deficient patients with normal numbers of peripheral B cells.

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Author contributions

Conceptualization, D.S., B.G., R.Z.; Methodology, D.S., M.F., M.R., H.E.; Software, S.W., S.R., A.B.; Formal Analysis, S.P., A.B.; Investigation, D.S., M.C.K., S.H., L.Y., M.P., Ja.K., Jo.K, S.W., A.A.S., H.C., F.Y., Y.L., J.M.P., A.M., B.P.P.; Resources, H.E., R.K., A.L.M., R.W.H., J.M.P.; Writing – Original Draft, D.S.; Writing – Review & Editing, M.C.K., An.C., A.A.S., J.M.P, R.Z., B.G.; Supervision, D.S., A.A.S., Ad.C., R.Z., B.G.; Funding Acquisition, D.S., B.G., R.Z.

Figure legends

Figure 1: Mutations in *SEC61A1* in patients with hypogammaglobulinemia. **A:** Pedigrees of two families with germline *SEC61A1* mutations. Squares: males; circles: females; black filled symbols: mutation carriers; asterisk: genetically tested. **B:** Confirmation of mutations by Sanger sequencing showing the heterozygous changes in the coding sequence (c) and the protein sequence (p). Wt: wild type; mut: mutant. **C:** Atomic model of the heterotrimeric Sec61 complex in the laterally closed conformation seen from the cytoplasm, composed of SEC61A1 (blue), SEC61B (orange) and SEC61G (yellow). Pore ring residues in the center of the channel are shown in blue with the mutated V85 in magenta. The plug domain (transparent) reaches into the pore from the back. **D:** Protein sequence alignment of SEC61A1 from various species including vertebrates and fungi. The mutated V85 is highlighted in magenta. **E:** Log2 of label free quantitation (LFQ) values of SEC61A1 as determined by mass spectrometry-based proteomics in human cells sorted from peripheral blood. Data were taken from Rieckmann et al.²⁷. nB: naïve B cells; mB: memory B cells; PB: plasmablasts **F:** Western blot analysis of SEC61A1 levels in sorted primary CD19⁺CD27⁺ naïve B cells and CD3⁺CD4⁺ T cells. Bar graphs show the quantification of the blots with SEC61A1 normalized to GAPDH levels.

Figure 2: Flow cytometric analysis of PBMCs. Relative amount of indicated cells among peripheral blood lymphocytes (**A**) or among CD19⁺ B cells (**B**) of patients from Family I (squares), Family II (triangles) and travel controls (circles) divided in three age groups. Grey background indicates the normal range for each age group.

Figure 3: *In vitro* differentiation of primary B cells into plasma cells upon stimulation with CD40L and IL21. Scatter plots summarize results of independent experiments for travel controls (circles) and patients of Family I (squares) and Family II (triangles). **A:** Representative flow cytometry plots showing CD38^{high}CD27⁺ plasmablasts among CD19⁺ primary B cells. **B:** Representative flow cytometry plots showing CD38^{high}CD138⁺ and CD27⁺CD138⁺ plasma cells among CD19⁺ primary B cells. **C:** Ig in the cell culture supernatant measured by ELISA upon CD40L/IL21 stimulation at Day 9. Data are represented as mean $\pm$ SD; **p<0.005, *p<0.05, ns: not significant (unpaired t test).

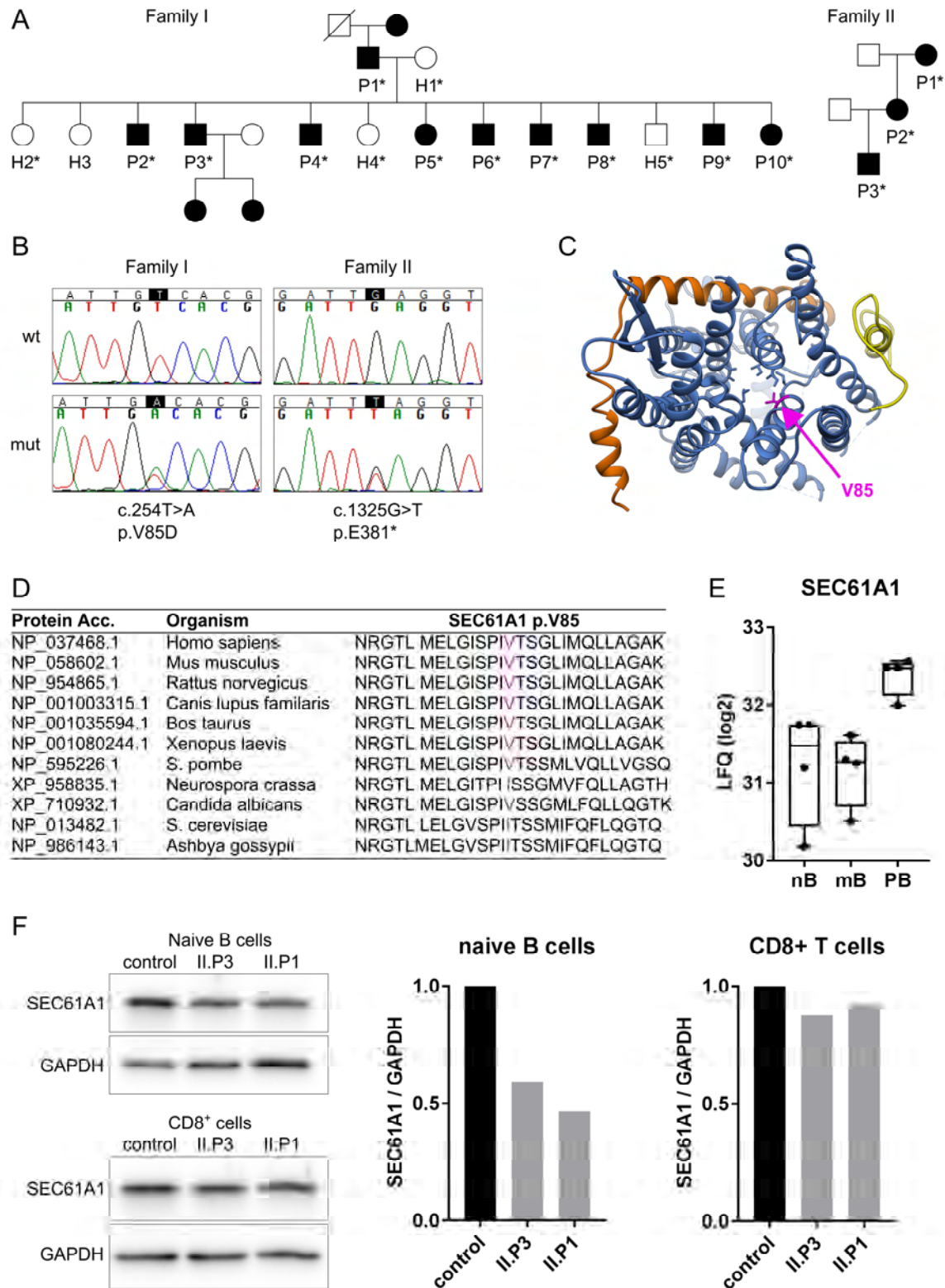
Figure 4: Analysis of the phenotype of EBV-transformed B cell lines *SEC61A1* mutation carriers. Data are shown for subjects of Family I (squares), Family II (triangles) and unrelated healthy donors (HD) (circles). **A:** Quantification of Western blot analysis of SEC61A1 relative to tubulin levels in four HD EBV cell lines and four cell lines from different patients. (n=2) **B:** *SEC61A1* mRNA levels relative to the reference genes *HPRT*, *TBP*, *RPLP0* in EBV cell lines from (A). (n=1) **C:** Flow cytometric analysis of surface marker expression in five HD and four patient EBV cell lines. The bar graph indicates the frequency of CD38^{high}CD138⁺ cells per cell line. **D:** ELISPOT analysis of the mean spot size of secreted IgG, IgA and IgM. Data in bar graphs are represented as mean $\pm$ SD, ns: not significant, *p<0.05, **p<0.005, ***p<0.001 (unpaired t test).

Figure 5: Effect of p.V85D on Sec61 complex-dependent co-translational protein translocation into the ER. **A:** Representative Western blot and respective quantification of SEC61A1 levels in HeLa cells treated with the indicated siRNA and transiently transfected with the indicated plasmids. **B:** Representative phosphorimaging blot and respective quantification of the efficiency of co-translational transport of pre-prolactin (ppl) into the ER

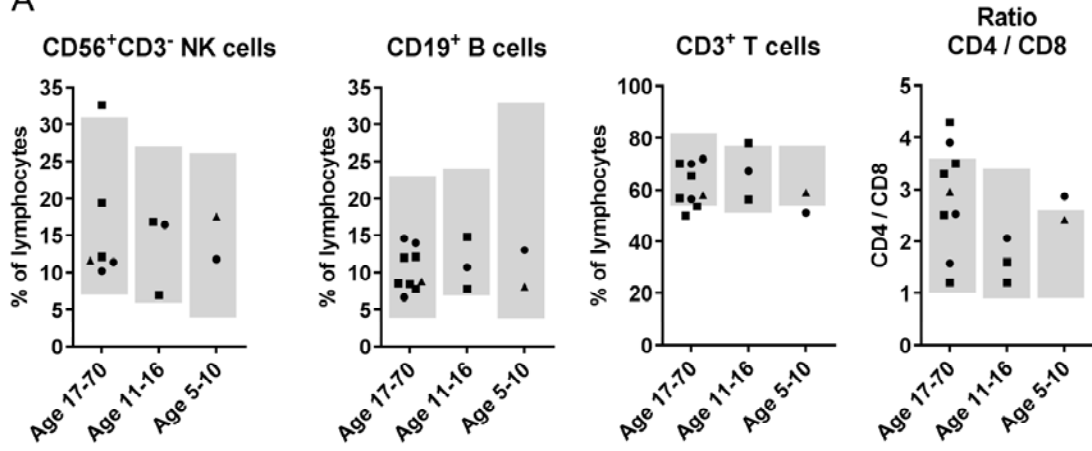
by HeLa cells given as relative percentage of mature, processed protein prolactin (pl). **C:** Representative phosphorimaging blot (n=3) of the efficiency of Sec61 complex-independent post-translational transport of pre-Cytochrome B5 (pre) into the ER (in HeLa cells) given as the relative percentage of mature, glycosylated protein (glyc). Data are represented as mean $\pm$ SD; ns: not significant, * p <0.05, ** p <0.005 (unpaired t test).

Figure 6: Effect of SEC61A1-V85D on Ca²⁺ leakage from the ER into the cytosol. A: Protein levels of SEC61A1 and SEC61B two days after transient transfection of HeLa cells relative to endogenous levels. Data (n=5) represented as mean $\pm$ SEM, ns: not significant (unpaired t-test). **B:** Thapsigargin-treatment and live cell imaging of calcium-indicator (Fura-2) loaded in HeLa cells overproducing SEC61A1 (n=5). **C:** Ionomycin-treatment and live cell imaging of calcium-indicator (Fura-2) loaded in HeLa cells overproducing SEC61A1 (n=4). **B and C:** Data in bar graphs represented as mean $\pm$ SEM with the total number of analyzed cells shown at the bottom of the bars. *** p <0.001 (unpaired t test).

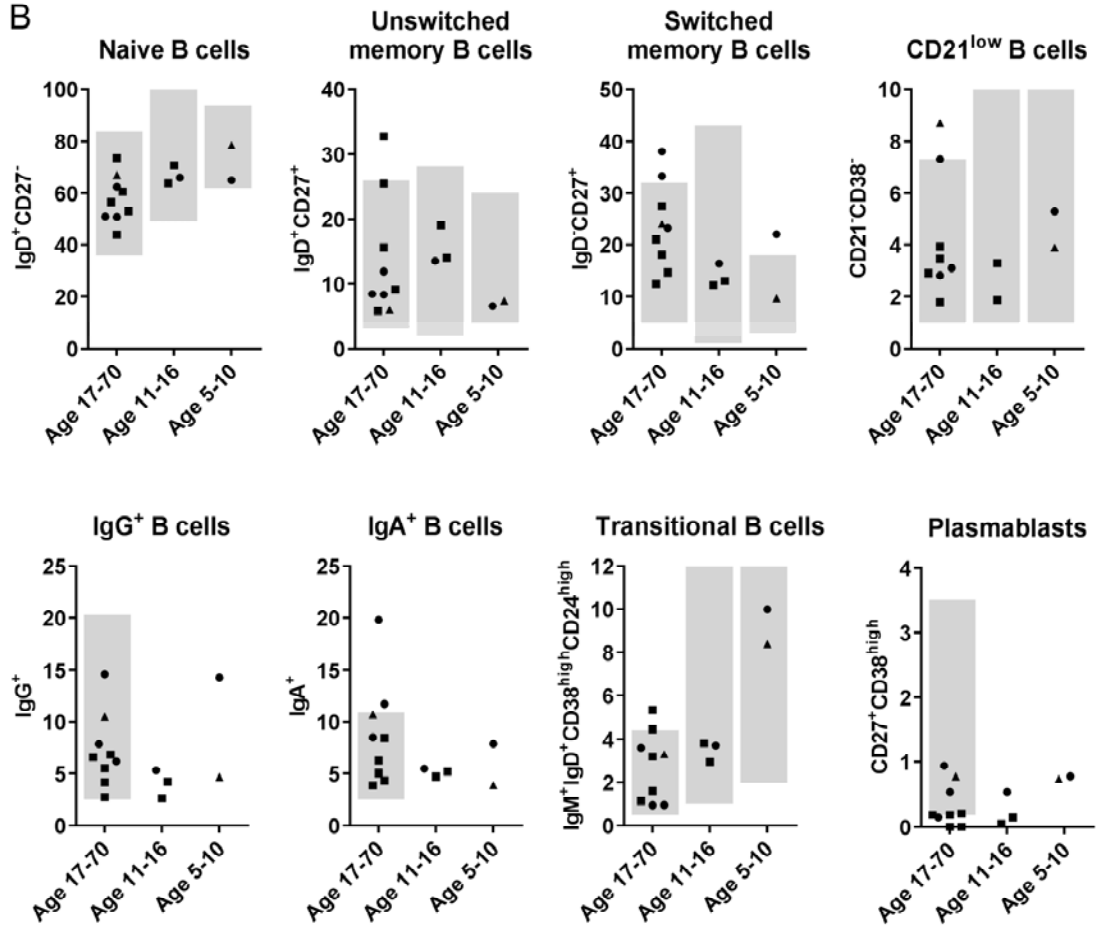
Figure 7: Effect of UPR induction on plasma cells and sensitized Hek293T cells after SEC61A1-V85D overproduction. A: Flow cytometric analysis of the indicated cell lines after stable transduction with an expression vector encoding SEC61A1-wt or -V85D and GFP separated by an IRES site. **B:** Representative Western blots and respective statistical analysis showing levels of the indicated proteins in U266 cells stably overexpressing SEC61A1-wt and -V85D normalized to tubulin levels and relative to cells transduced with the empty vector. **C:** Relative mRNA expression of the indicated transcription factors in L363 cells stably overexpressing SEC61A1-wt or -V85D. **D:** Representative Western blots showing levels of the indicated proteins in Thapsigargin-treated Hek293T cells stably overexpressing SEC61A1-wt and/or -V85D (n=4). The bar graph shows the respective statistical analysis of XBP1s levels normalized to tubulin levels and relative to cells transduced with the empty vector. **E:** Western blot (n=1) showing levels of indicated proteins in JK6L cells treated for 24 hours with siRNA against SEC61A1. Data in bar graphs is represented as mean $\pm$ SD, ns: not significant, * p <0.05, ** p <0.005, *** p <0.001 (unpaired t test).

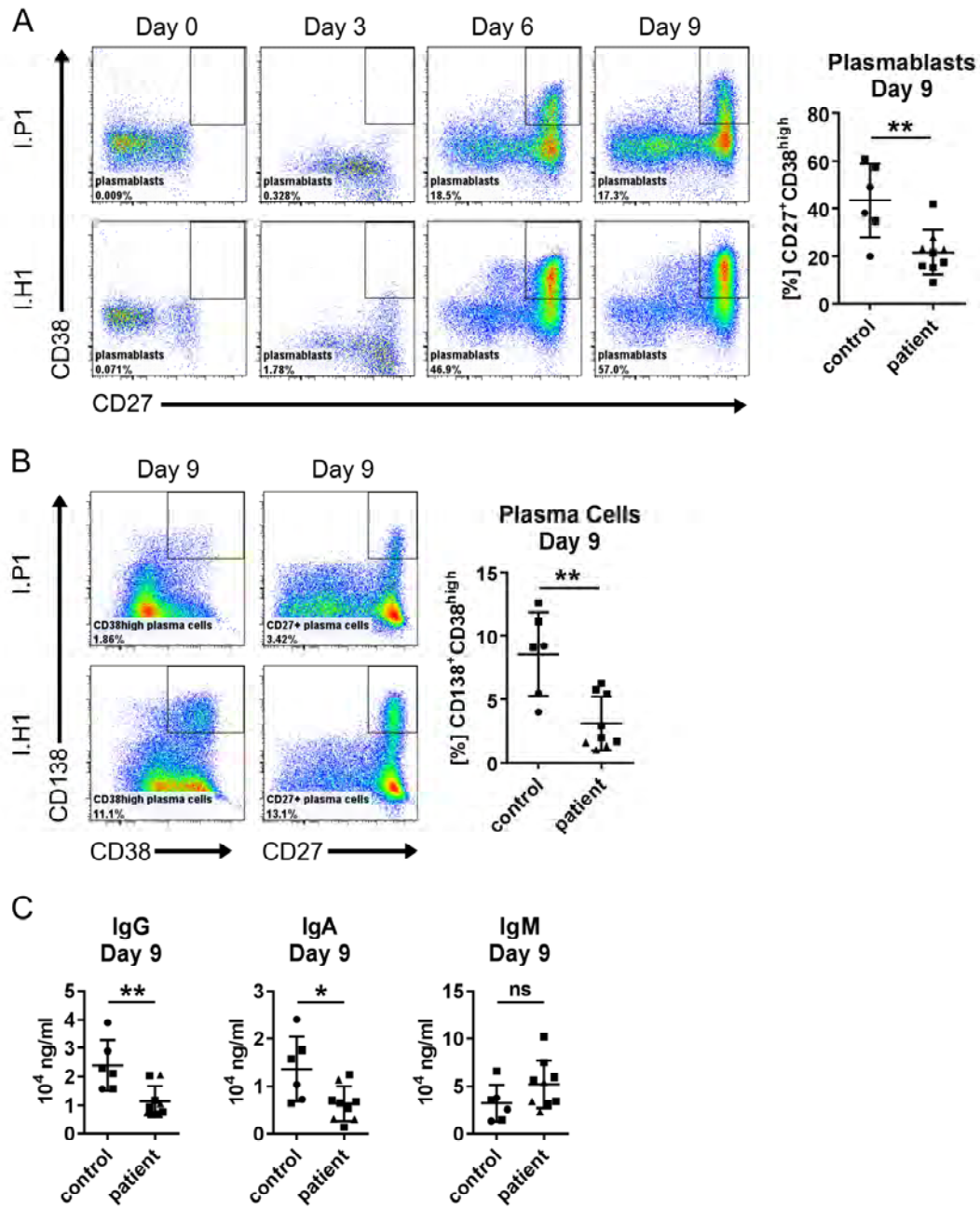


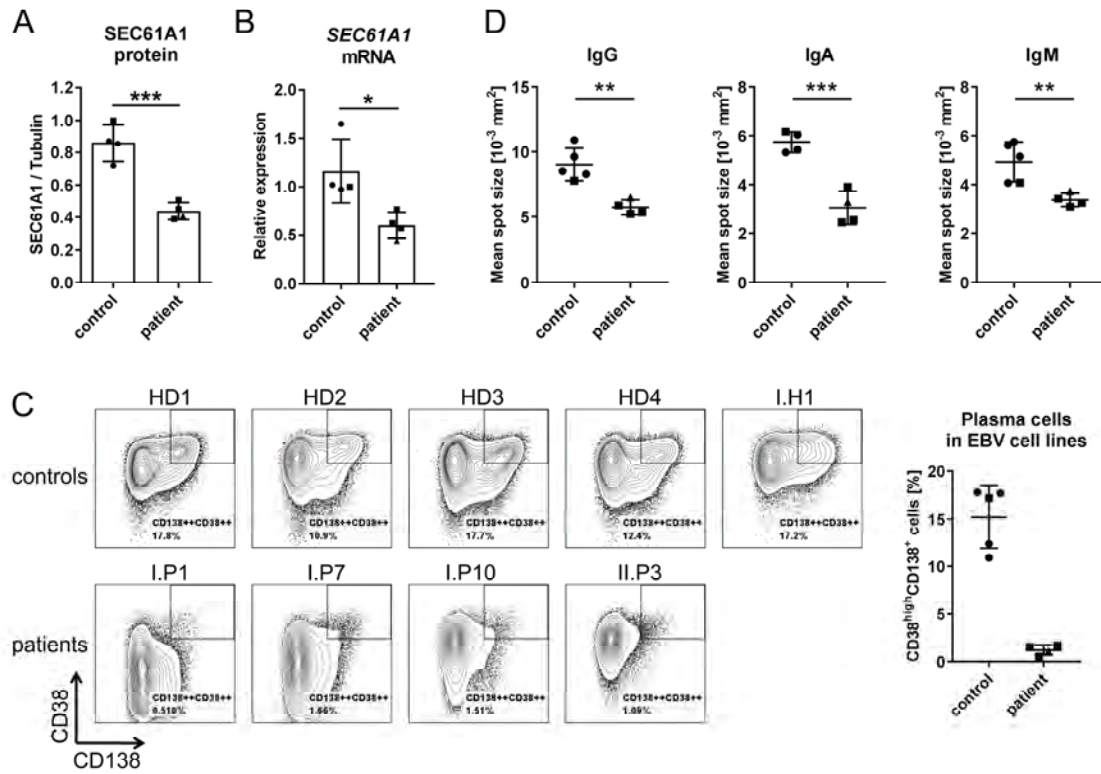
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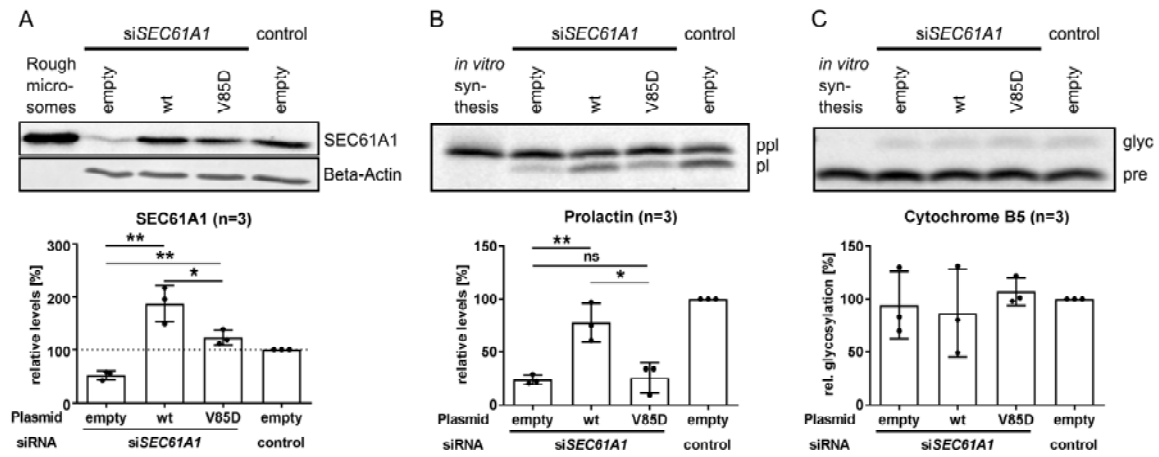


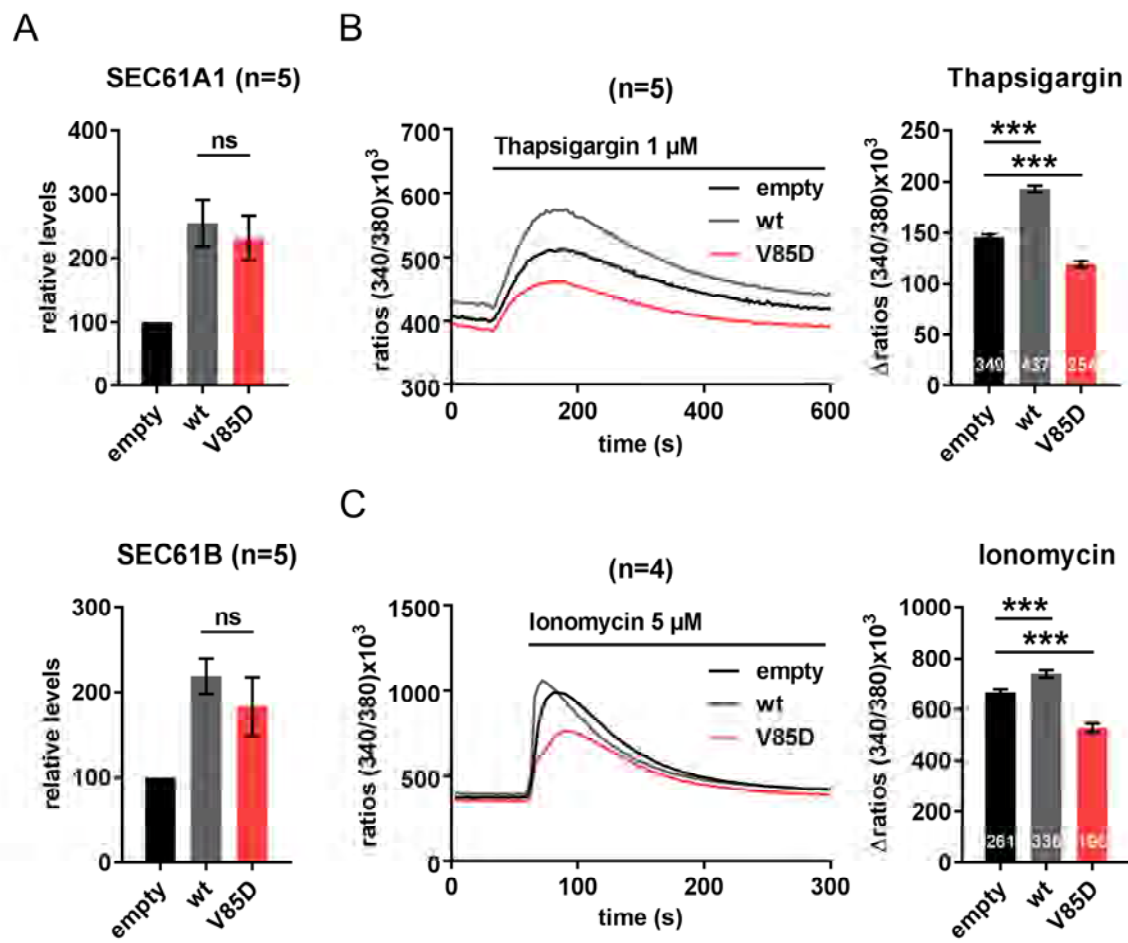
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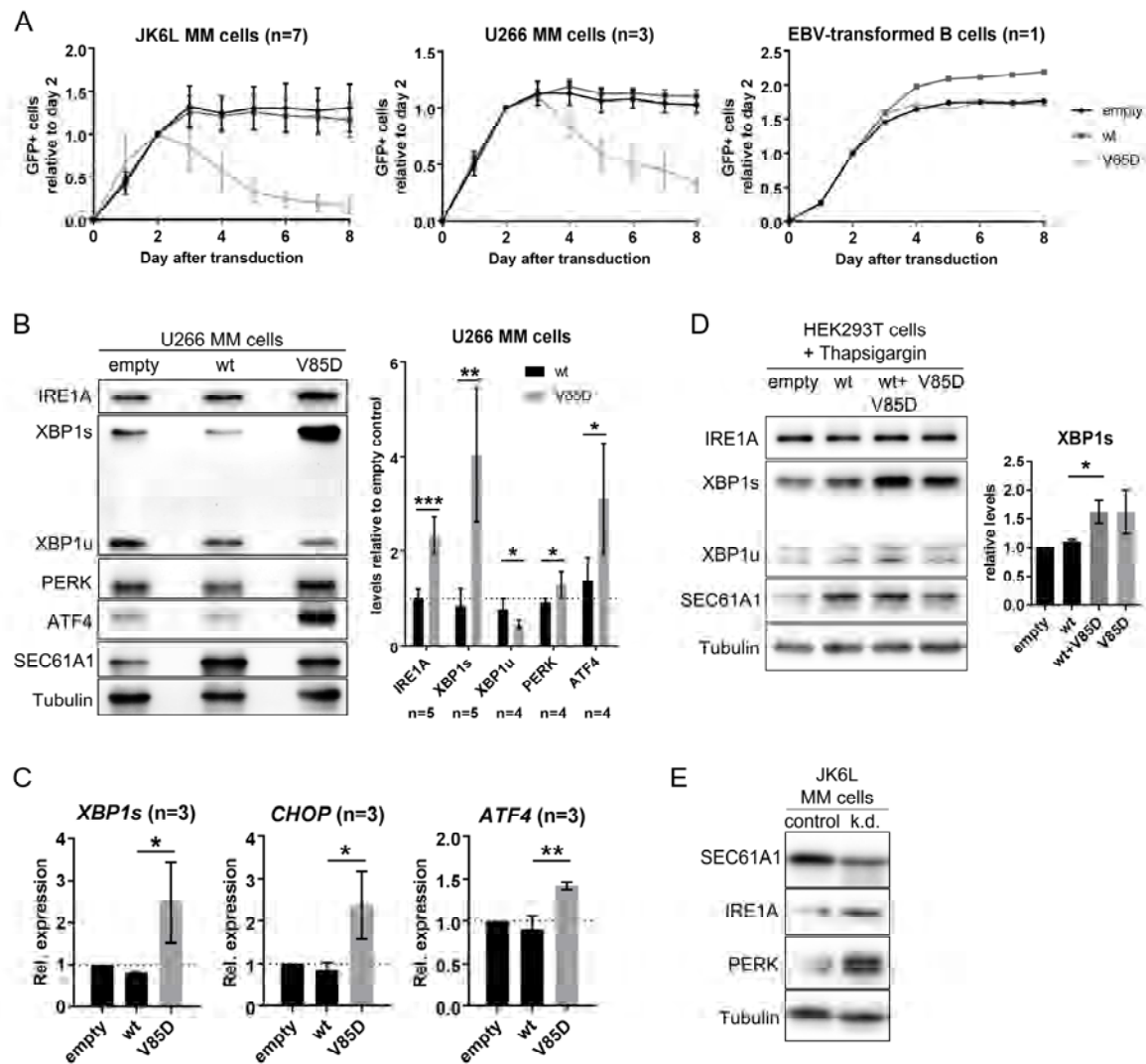












1 **Supplements**

2 **1. Supplemental figures and tables**

3 **2. Supplemental case reports**

4 **3. Supplemental material and methods**

5

6 **Figure S1. SEC61A1 haploinsufficiency caused by a p.E381* heterozygous**
7 **mutation.** Representative western blot (n=3) showing SEC61A1 protein levels in EBV-
8 transformed B cell lines of two unrelated healthy donors (HD) and II.P3 using an
9 antibody which recognizes the N-terminus of SEC61A1. Two exposure times of the
10 same membrane are shown to highlight differences in expression levels (upper image)
11 and absence of a shortened protein in II.P3 (lower image). β -actin was used as loading
12 control.

13
 14 **Figure S2: *In vitro* differentiation of primary B cells into plasma cells upon**
 15 **stimulation with anti-IgM, BAFF and CpG.** Scatter plots summarize results of all
 16 independent experiments for travel controls (circles) and patients of Family I (squares)
 17 and Family II (triangles). **A:** Representative flow cytometry plots showing CD38^{high}CD27⁺
 18 plasmablasts among CD19⁺ primary B cells. **B:** Representative flow cytometry plots
 19 showing CD38^{high}CD138⁺ and CD27⁺CD138⁺ plasma cells among CD19⁺ primary B
 20 cells. **C:** Ig secretion levels in supernatants from patients and healthy controls measured
 21 by ELISA at Day 9. Data are represented as mean $\pm$ SD; **p<0.005; ns: not significant
 22 (unpaired t test).

23
 24 **Figure S3: Ig secreting capacities of EBV and MM cell lines.** **A:** Representative
 25 images of an ELISPOT assay of 3 different EBV and 3 different MM cell lines where
 26 single Ig secreting cells can be spotted. Box plot displays size distribution (sq.mm) of
 27 ICS for JK6L MM line (red) in comparison to the three EBV-lines (blue). **B:** Frequency of
 28 IgG, A or M secreting cells for different EBV or MM cell lines, obtained from an ELISPOT
 29 assay. Data represented in box-plots (2 repetitions). **C:** Amount of IgG, A or M secreted
 30 at day 3 by the different EBV or MM cell lines measured by ELISA.

31

32 **Figure S4: Overexpression of *SEC61A1-V85D* affects multiple myeloma cells but**
33 **not non-plasma cell types.** Flow cytometric analysis of the indicated lymphocytic and
34 non-lymphocytic cell lines after stable transduction with an expression vector encoding
35 *SEC61A1-wt* or *-V85D* and *GFP* separated by an IRES site. **A:** Frequency of GFP⁺ cells
36 relative to frequency on day 2, the first time point after two consecutive transductions. **B:**
37 Results of all independent experiments in MM cell lines in separate tables with graphs
38 displaying the frequency of GFP⁺ cells as a measure for transduction efficiency and
39 growth/survival of transduced cells.

40

41 **Figure S5: SEC61A1-V85D overexpression strongly induces UPR in plasma cells.**
42 **A:** Representative Western blots and respective statistical analysis showing production
43 levels of the indicated proteins in JK6L cells stably overexpressing *SEC61A1-wt* or -
44 *V85D* relative to cells transduced with the empty vector. **B:** Representative Western
45 blots and respective statistical analysis showing production levels of the indicated
46 proteins in L-363 cells stably overexpressing *SEC61A1-wt* or -*V85D* relative to cells
47 transduced with the empty vector. Data in bar graphs are represented as mean $\pm$ SD,
48 ns: not significant, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$ (unpaired t test).

Table S1. Immunoglobulin levels and vaccination responses. Serum levels of Ig isotypes and vaccination responses for members of Family I and II. The interval between vaccination and titer measurements in all cases was between one and four month.

ID	Age at exam. (years)	Age in case report	Het. carrier	Status	Total IgG (mg/dl)	Total IgA (mg/dl)	Total IgM (mg/dl)	IgG1 (mg/dl)	IgG2 (mg/dl)	IgG3 (mg/dl)	IgG4 (mg/dl)	IgE (IU/ml)	Pneumococcal response	Tetanus antitoxoid IgG (IU/ml)
I.P1	41	54	Yes	Affected	703 (700-1600)	32↓ (70-400)	6↓ (40-230)	n.a.	n.a.	n.a.	n.a.	n.a.	(age 45)	(age 45)
	45				504↓ (700-1600)	39↓ (70-400)	33↓ (40-230)	308↓ (422-1292)	154 (117-747)	23↓ (41-129)	19 (1-291)	n.a.	No response	1.34 Protective
I.P3	14	26	Yes	Affected	430↓ (759-1549)	23↓ (58-358)	9↓ (35-239)	315↓ (347-993)	130↓ (140-440)	30 (23-117)	9 (1-83)	5 (0-158)	(age 15 and 20)	(age 15)
	15				576↓ (580-1584)	26↓ (75-425)	33↓ (57-330)	n.a.	n.a.	n.a.	n.a.	7 (<100)	No response	2.18 Protective
	17				780 (549-1584)	61 (61-348)	31 (23-259)	534 (422-1292)	185 (117-747)	21↓ (41-129)	30 (1-291)	n.a.		
I.P4	16	24	Yes	Affected	693 (549-1584)	58↓ (61-348)	14↓ (23-259)	425 (422-1292)	167 (117-747)	35↓ (41-129)	30 (1-291)	n.a.	No response	n.a.
I.P5	13	21	Yes	Affected	468↓ (759-1549)	27↓ (58-358)	26↓ (35-239)	262↓ (347-993)	152 (140-440)	70 (23-117)	27 (1-83)	n.a.	No response	0.29 Intermediate
I.P6	11	20	Yes	Affected	634↓ (698-1560)	22↓ (53-204)	22↓ (31-179)	477 (456-938)	129↓ (147-493)	28 (12-179)	8 (1-168)	n.a.	No response	n.a.
I.P7	9	17	Yes	Affected	517↓ (572-1474)	37 (34-305)	27↓ (31-208)	381↓ (456-938)	98↓ (163-513)	33 (26-113)	3 (1-121)	n.a.	(age 16)	n.a.
	16				615↓ (670-1760)	43↓ (89-580)	23↓ (39-330)	403 (315-855)	114 (60-495)	29 (23-200)	8,9↓ (10-157)	4 (<88)	No response	
I.P8	6	14	Yes	Affected	421↓ (504-1464)	31 (27-195)	12↓ (24-210)	285↓ (292-816)	125 (83-513)	22 (8-111)	31 (1-121)	n.a.	(age 13)	(age13)
	13				482↓ (670-1760)	26↓ (89-580)	16↓ (39-330)	281↓ (342-1150)	180 (100-455)	52 (28-125)	62 (4-136)	5 (<88)	No response	0.16 Intermediate
I.P9	2	11	Yes	Affected	345↓ (453-916)	9↓ (20-100)	16↓ (19-146)	234↓ (286-680)	80 (30-327)	28 (13-82)	1 (1-65)	n.a.	(age 9)	(age 9)
	9				436 (670-1760)	15 (33-236)	17 (48-242)	254 (432-1020)	125 (70-430)	30 (13-85)	10 (2-95)	<1 (<88).	No response	0.34 Intermediate
I.P10	9 month	8	Yes	Affected	159↓ (572-1474)	<7↓ (34-305)	<10↓ (31-208)	128↓ (456-938)	38↓ (163-513)	11↓ (26-113)	<5 (1-121)	n.a.	(age 7)	(age 7)
	7				580↓ (610-1570)	44 (33-236)	17↓ (48-242)	351 (288-920)	163 (44-375)	27 (16-85)	100 (0.4-98)	5 (<88)	No response	<0.1 Undetected
I.H1	44	n.d.	No	Unaffected	1360 (700-1600)	259 (70-400)	103 (40-230)	781 (422-1292)	486 (117-747)	18↓ (41-129)	38 (1-291)	n.a.	n.a.	n.a.
I.H2	22	n.d.	No	Unaffected	1330 (700-1600)	146 (70-400)	145 (40-230)	692 (422-1292)	634 (117-747)	61 (41-129)	63 (1-291)	50.9 (1-214)	n.a.	n.a.

ID	Age at exam. (years)	Age in case report	Het. carrier	Status	Total IgG (mg/dl)	Total IgA (mg/dl)	Total IgM (mg/dl)	IgG1 (mg/dl)	IgG2 (mg/dl)	IgG3 (mg/dl)	IgG4 (mg/dl)	IgE (IU/ml)	Pneumococcal response	Tetanus antitoxoid IgG (IU/ml)
I.H4	14	n.d.	No	Unaffected	1060 (716-1711)	110 (47-249)	70 (15-188)	740 (347-993)	255 (140-440)	22↓ (23-117)	70 (1-83)	n.a.	n.a.	n.a.
I.H5	5	n.d.	No	Unaffected	832 (504-1464)	94 (27-195)	57 (24-210)	568 (292-816)	207 (83-513)	19 (8-111)	<5 (1-121)	n.a.	n.a.	n.a.
II.P3 (when not on IVIG)	5 month	8	Yes	Affected	143↓ (231-1411)	<5 (0-83)	<25 (0-145)	n.a.	n.a.	n.a.	n.a.	<15 (<90)	See Table S7	See Table S7
	9 month				126↓ (231-1411)	<5 (0-83)	<25 (0-145)	n.a.	n.a.	n.a.	n.a.	<15 (<90)		
	2.5				296↓ (453-916)	22.2 (20-100)	26 (19-146)	n.a.	n.a.	n.a.	n.a.	<4 (<90)		
	6.5				449↓ (504-1464)	34 (27-195)	37 (24-210)	n.a.	n.a.	n.a.	n.a.	20 (<90)		
	7				445↓ (572-1474)	36.5 (34-305)	42 (31-208)	260↓ (422-802)	155 (113-480)	33 (15-133)	37 (1-84)	13.5 (1-403)		
	8				493↓ (572-1474)	45 (34-305)	61 (31-208)	n.a.	n.a.	n.a.	n.a.	42 (<90)		
	8.3				530↓ (572-1474)	44 (34-305)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.		
II.P1	~65	n.d.	Yes	Affected	861 (700-1600)	234 (70-400)	64 (40-230)	650 (422-1292)	280 (117-747)	22↓ (41-129)	111 (1-291)	208	See Table S7	See Table S7

Het.: heterozygous; n.a.: not available; n.d.: not described; () Ig reference values for Family II and unaffected members of Family I from Medscape.

Table S2. Linkage analysis in Family I. Single-marker LOD scores for pertinent markers on chromosome 3 for various values of the recombination fraction (θ) between the putative location of the disease gene and the marker. Marker positions in Mbp are from human genome build 19.

Marker	Position (Mbp)	$\theta=0.00$	$\theta=0.01$	$\theta=0.03$	$\theta=0.05$	$\theta=0.10$	$\theta=0.15$
D3S3697	66.4	$-\infty$	0.67	1.07	1.21	1.27	1.30
D3S1566	70.3	2.71	2.67	2.58	2.49	2.25	2.00
D3S2406	73.3	3.31	3.26	3.15	3.04	2.76	2.46
D3S4529	85.9	3.31	3.26	3.15	3.04	2.76	2.46
D3S3045	107.0	3.31	3.26	3.15	3.04	2.76	2.46
D3S2460	117.4	3.31	3.26	3.15	3.04	2.76	2.46
D3S3558	123.7	2.71	2.67	2.58	2.49	2.25	2.00
D3S3606	127.2	3.31	3.26	3.15	3.04	2.76	2.46
D3S3684	133.9	$-\infty$	0.97	1.36	1.49	1.55	1.48
D3S1764	139.2	$-\infty$	0.37	0.78	0.93	1.04	1.02

θ = recombination fraction

Table S3. Genes with novel SNVs identified by WES. Genes containing novel heterozygous SNVs shared by four affected individuals (I.P7, P8, P9, P10) and not mutated in two unaffected individuals (I.H1 and H2) from Family I.

Gene	Chromosome	Position	Ref. allele	Alt. allele	aa. change	Transcript ID	Effect	Function
SEC61A1	3	127775585	T	A	V85D	NM_013336.3	NON_SYNONYMOUS_CODING	Protein translocation in ER
GOLGA8K	15	32686425	T	TCTC	E429GE	NM_001282493.1	CODON_INSERTION	Golgin subfamily A member 8K
SLC47A2	17	19611067	G	A	T240M	NM_001099646.1	NON_SYNONYMOUS_CODING	Excretion of toxic electrolytes

Ref.: reference, Alt.: altered, aa.: amino acid

Table S4. Detailed B and T cell phenotyping. Frequency of PBMC, lymphocyte, B cell and T cell subpopulations of mutation carriers from Family I and II and age-matched travel controls.

	Healthy			Affected							Healthy		Affected	
Family member/ travel control	I.H1	I.H2	I.H5	I.P1	I.P4	I.P6	I.P7	I.P8	I.P9	I.P10	ctrl1	ctrl2	II.P1	II.P3
Age at analysis [years]	55	30	16	57	24	20	20	17	14	12	adult	8	~65	7
	PBMCs [%]													
CD14+ Monocytes	8	n.a.	8	17.2	n.a.	n.a.	13.1	8.22	21.2	8.3	3	2.6	2.1	1.64
	Lymphocytes [%]													
CD3-CD56+ NK cells	11.4	n.a.	16.4	32.6	n.a.	n.a.	19.4	12.1	16.8	6.9	10.2	11.8	11.6	17.5
CD3+ T cells	71.8	56.5	67.2	49.8	53.55	56.8	65.3	70.1	56.3	77.9	70	51	58	59
CD19+ B cells	6.6	14.6	10.7	12	7.7	12.1	8.4	8.5	14.8	7.7	14	13	8.75	8.03
	CD3+ T cells [%]													
CD4+	55.4	66.9	58.8	71.3	75.15	71.9	67.4	50.2	48.5	56.1	78	66	71	65
CD8+	33.7	26.6	29.3	23	17.3	20.5	26.7	39.4	39.8	35.6	20	23	24	27
CD4/CD8 ratio	1.58	2.52	2.06	3.30	4.34	3.5	2.54	1.20	1.23	1.60	3.9	2.87	2.96	2.41
CD4-CD8- DNT	9.4	6.06	12.5	4.8	6.76	6.53	5.2	9.5	11.5	7.5	2.2	10.5	2.8	8.3
gdTCR	0.9	n.a.	1.3	0.3	n.a.	n.a.	0.6	1.1	4.2	2.2	2.04	8.69	2.2	7.4
CD4+HLA-DR+	0.46	n.a.	0.29	0.55	n.a.	n.a.	0.20	0.17	0.28	0.12	0.3	0.37	1.64	0.29
CD8+HLA-DR+	0.71	n.a.	0.55	1.70	n.a.	n.a.	0.44	0.48	0.93	0.55	0.68	0.91	4.65	0.58
CD4+CD25 ^{high} CD127- Treg	4.5	4.61	5.0	6.2	5.85	5.39	4.8	5.3	7.0	4.9	3.94	4.99	6.25	4.74
CD4+CD45RA+ naive	63.7	79.7	74.9	44.1	71.55	79.5	78.6	77.1	75.2	83.5	57	79	31	86
CD4+CD45RO+ memory	23.9	16.1	18.2	38.6	24.6	16.5	10.8	14.7	17.3	11.4	33	10.4	60	7
CD4+CD45RA+CD31+ recent thymic emigrants	37.7	n.a.	50.3	22.3	n.a.	n.a.	47.9	51.9	55.8	63.7	38	64	15	78
CD4+CD45RA+CCR7+ naive	65.3	n.a.	66.8	53.1	n.a.	n.a.	81.7	75.7	69.9	79.3	64	85	34	90
CD4+CD45RA-CCR7+ central memory	9.70	n.a.	9.16	22.2	n.a.	n.a.	7.41	7.24	8.27	6.60	20	6.3	39	5.3
CD4+CD45RA-CCR7- effector memory	16.7	n.a.	13.9	16.5	n.a.	n.a.	5.91	10.0	9.82	7.84	14	6.3	26	3.4
CD4+CD45RA+CCR7- TEMRA	8.3	n.a.	10.1	8.31	n.a.	n.a.	4.99	7.05	12.1	6.3	1.6	2.7	1.3	1.3
CD4+CD45RO-CD62L+ recirculating naive	75.4*	73.7	n.a.	49.2*	73	77.6	78.7*	85.2*	80.2	n.a.	n.a.	n.a.	n.a.	n.a.
CD4+CD45RO+CD62L+ central memory	12.5*	7.43	n.a.	25.9*	13.55	9.24	7.6*	6.37*	7.24*	n.a.	n.a.	n.a.	n.a.	n.a.
CD4+CD45RO+CD62L- effector memory	9.1*	8.72	n.a.	20.6*	10.95	7.3	6.54*	4.15*	6.18*	n.a.	n.a.	n.a.	n.a.	n.a.

	Healthy			Affected							Healthy		Affected	
Family member/ travel control	I.H1	I.H2	I.H5	I.P1	I.P4	I.P6	I.P7	I.P8	I.P9	I.P10	ctrl1	ctrl2	II.P1	II.P3
Age at analysis [years]	55	30	16	57	24	20	20	17	14	12	adult	8	~65	7
	CD3+ T cells [%]													
CD4+CD45RO-CD62L- TEMRA	3.01*	10.2	n.a.	4.27*	2.46	5.87	7.18*	4.25*	6.34*	n.a.	n.a.	n.a.	n.a.	n.a.
CD8+CD45RA+CCR7+ naive	28.3	n.a.	72.2	43.8	n.a.	n.a.	69.2	69.3	74.3	83.2	42	23	13	43
CD8+CD45RA-CCR7+ Central Memory	1.74	n.a.	1.58	2.13	n.a.	n.a.	2.09	1.17	0.86	0.77	3.1	0.34	12	0.53
CD8+CD45RA-CCR7- Effector Memory	26.0	n.a.	13.5	24.9	n.a.	n.a.	17.2	12.5	9.25	7.04	18.2	15.6	42	11
CD8+CD45RA+CCR7- Effector Memory RA	43.9	n.a.	12.8	29.1	n.a.	n.a.	11.5	16.9	15.5	8.98	37	61	33	45
CD8+CD27+CD28+ effector memory 1	17.4	n.a.	12.1	11.4	n.a.	n.a.	10.3	8.16	7.91	5.40	51	28	46	41
CD8+CD27+CD28- effector memory 2	43.2	n.a.	82.4	60.7	n.a.	n.a.	88.5	87.6	91.3	93.8	39	67	42	57
CD8+CD27-CD28- effector memory 3	37.6	n.a.	4.79	27.3	n.a.	n.a.	1.05	3.81	0.53	0.52	7.9	4.2	9.9	1.9
CD8+CD27-CD28+ effector memory 4	1.81	n.a.	0.68	0.51	n.a.	n.a.	0.17	0.47	0.21	0.24	1.5	0.59	2.2	0.07
CD8+CD57+ replicative senescence	36.6	n.a.	10.1	29.3	n.a.	n.a.	3.70	12.6	4.88	3.46	11.4	10.3	13.6	5.2
	CD19+ B cells [%]													
IgD+CD27- naive	62.4	50.8	66.0	56.6	73.4	53.1	43.8	60.5	63.8	70.6	51	65	67	78.5
IgD+CD27+	11.9	8.4	13.5	25.5	5.8	9.07	32.8	15.6	19.0	14.0	8.3	6.5	5.95	7.35
IgD-CD27+	23.2	33.3	16.4	14.7	12.5	27.5	21.0	18.1	13.1	12.3	38	22	24	9.7
IgM+CD27- naive	57.3	51.6	68.4	57.7	70.55	53.7	42.4	56.7	63.7	67.1	45	68	63.33	81
IgM+CD27+ MZ-like memory	16.4	32.5	21.1	30.0	11.35	25.5	36.7	19.7	22	17.5	13.6	13.4	18.67	12
IgM-CD27+ class switched memory	16.7	9.19	9.29	6.11	6.92	11.1	14.7	10.6	6.15	7.49	33	14.8	18.67	4.97
IgD+IgM+ naive	66.3	55	79.3	82.9	71.4	56.8	73.3	70.1	80.0	80.2	52	70	65.5	85
IgD-IgM+ IgM-only memory	7.38	29.1	10.2	4.81	10.45	22.4	5.77	6.33	5.71	4.40	6.7	10.7	20.15	8.5
IgD-IgM- class switched memory	17.3	10.3	9.99	7.49	9.35	13.9	14.9	11.9	7.92	9.61	34	17.9	19.35	65.5
IgM+IgD+CD38highCD24high transitional (or **CD38highCD10+ transitional)	0.94	0.95**	3.71	4.45	5.35**	3.19**	1.14	1.6	2.93	3.82	3.6	10	3.3	8.4
CD38-CD21low	2.83*	3.1	n.a.	2.92*	1.78	3.93	3.47*	1.87*	3.29*	na	7.3	5.3	8.7	3.9
CD27+CD38high plasmablasts	0.54	0.15	0.54	0.21	0.19	0.19	0.00	0.00	0.15	0.05	0.94	0.78	0.78	0.74
IgA+	11.7	8.53	5.46	5.03	4.35	3.98	8.46	6.25	5.20	4.72	19.8	7.9	10.7	3.9
IgG+	7.87	6.18	5.29	2.73	4.17	6.83	6.59	5.51	2.63	4.23	14.6	14.3	10.5	4.7

*values from 3 years before; **CD38highCD10+ transitional; n.a. not available

Table S5: Serum levels of proteins secreted by the liver and the pancreas.

	II.P3 Age 7	I.P1 Age 46	I.P4 Age 15	I.P5 Age 14
Total protein	70.6 g/l (60-80 g/l)	6.7 g/dl (6.4-8.2)	7.6 g/dl (6.4-8.2)	6.6 g/dl (6.4-8.2)
Albumin	43 g/l (35-50 g/l)	3.8 g/dl (3.4-5 g/dl)	4.9 g/dl (3.4-5 g/dl)	4.3 g/dl (3.4-5 g/dl)
Cholinesterase	8.12 kU/l (5-13 kU/l)	n.a.	n.a.	n.a.
Transferrin	2.4 g/l (2-3.6 g/l)	n.a.	n.a.	n.a.
Insulin	9.4 mU/l (4-19 mU/l)	n.a.	n.a.	n.a.
Prothrombin time	110.6% (80-130%)	n.a.	n.a.	n.a.

n.a.: not available

Table S6. Physical and hematological observations. Values of individuals from Families I and II detected as altered by Bolar *et al.* (2016) in *SEC61A1* mutation carriers with ADTKD and anemia.

Individual	Age at examination	Height (cm)	Weight (Kg)	BMI (Kg/m ²) (percentile)	Hemoglobin (g/dl)	Hematocrit (%)	Creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)
I.P7	19y 5m	161	55.47	21.4 (31 st)	15.5 (13.6-17.5)	42.6 (41-53)	1.13 (0.71-1.22)	13 (6-22)
I.P8	16y 3m	168	52.21	18.5 (17 th)	14.3 (12.6-17)	40.4 (38-49)	0.75 (0.3-0.9)	12 (6-22)
I.P9	12y 11m	159	50.56	20 (71 st)	14.4 (12.3-16)	42 (37-49)	0.43 (0.3-0.9)	10 (6-22)
I.P10	10y 8m	132,5	30.72	17.5 (53 rd)	13 (11.6-15.5)	37.9 (35-45)	0.34 (0.3-0.9)	5 (6-22)*
II.P3	7y 3m	122,7	21.6	14.3 (16 th)	12.5 (11.1-14.7)	35.7 (32-41)	0.36 (0.2-0.7)	11 (7-17)

y: years, m: months, BMI: Body Mass Index. Reference values for healthy individuals given in parenthesis. *Below normal range.

Table S7: Specific antibody titers of patients from Family II. The interval between vaccination and titer measurements in II.P3 was between one and two month.

Antigen	II.P1	II.P3 (post-vaccination titers)	Reference values
Pneumococci IgG	40.7 mg/l	Post-Prevenar: negativ 1 month post-Pneumovax: 230.6 mg/l 4 month post-Pneumovax: 190.5 mg/l	15-270 mg/l
Measles IgG	2900 IU/ml	negativ	> 150 IU/ml
Mumps	n.a.	negativ	
Rubella	n.a.	negativ	
Diphtheria IgG	negativ	> 2 IU/ml	> 0.05 IU/ml
Tetanus IgG	0.189 IU/ml	> 5 IU/ml	> 0.2 IU/ml
Pertussis IgG	negativ; 21 IU/ml	Negative; 45 IU/ml	> 50 IU/ml

n.a.: not available

2. Supplemental case reports

2.1 Family I case reports

In this family, the mother and affected father had eight children (seven males and one female) with various forms of hypogammaglobulinemia described below in birth order. Five additional full siblings (one male and four females) are healthy and are not described further.

The father is of northern European heritage. As a child he suffered from recurrent tonsillitis and eventually received a tonsillectomy and adenoidectomy. In his adolescence he developed an inflamed appendix which ruptured causing septicemia. As an adult, he was hospitalized for five days for dysentery caused by *Shigella*; later he experienced inflammation of the dorsum of the left hand and was hospitalized with an elevated white count and treated with vancomycin and ceftriaxone. The inflammation eventually spread to the other hand, ankles and ribs. This was later diagnosed as a viral arthropathy. He had decreased IgG and IgA levels and absent responses to vaccination with polysaccharide antigens, but his antibody response to tetanus was normal (Table S1).

His 26-year-old son developed a periorbital cellulitis at 10 days of age and was hospitalized for several weeks. During his first year of life he was ill with otitis media and bronchitis every month. At four years of age he was hospitalized for RSV pneumonia. Tonsillectomy, adenoidectomy and two sets of tympanostomy tubes were required prior to 10 years of age. His tympanic membranes ruptured twice producing hearing loss in his right ear. Serum immunoglobulin evaluation at age 14 showed decreased IgG, IgA and IgM, and he failed to make responses to polysaccharide antigens. In addition he had developed two pneumonias per year until

14 years of age when IVIG replacement was started. He received only three infusions and then stopped. His infections have decreased to one infection every other month. Despite normal Ig levels at age 17, he repeatedly failed to mount a specific antibody response to pneumococcal antigens at age 20.

The 24-year-old son developed recurrent otitis media which required tympanostomy tube placement in early childhood. At age fifteen he developed recurrent episodes of *Giardia lamblia* despite multiple courses of standard treatment regimens. At age 16 he presented with shingles. Serum immunoglobulin levels showed decreased IgG, IgA, and IgM with absent antibody response to polysaccharide vaccination (Table S1).

The 21-year-old daughter developed RSV as a child without complication other than occasional wheezing with upper respiratory tract infection. She was diagnosed at 13 years of age with Reynaud's syndrome. Serum immunoglobulin levels showed decreased IgG, IgA, IgM and diminished response to polysaccharide vaccination.

The 20-year-old son had dietary intolerance and failure to thrive as an infant but recovered with appropriate formula. He experienced recurrent sino-pulmonary infections and received a tonsillectomy and adenoidectomy at six years of age. Serum immunoglobulin evaluation showed decreased IgG, IgA, IgM and poor antibody responses following polysaccharide vaccination.

The 17-year-old male suffered from Raynaud's syndrome. Recurrent pneumonia and croup occurred in his youth. Evaluation of his immune systems showed low IgG, IgA and IgM as well as absent antibody responses to polysaccharide vaccinations.

The 14-year-old male suffered from recurrent pneumonias and chronic otitis media. Evaluation of his immune systems showed low IgG, IgA and IgM as well as absent antibody responses to polysaccharide vaccinations.

The 11-year-old son developed his first ear infection at 8 months of age and experienced chronic otitis despite tympanostomy tubes were placed a few months later. A life-threatening episode of croup/asthma occurred at 15 months of age requiring hospitalization. At 4 years of age he was diagnosed with pneumonia and treated as an outpatient. Evaluation of his immune systems showed low serum IgG, IgA and IgM as well as absent antibody responses to polysaccharide vaccinations. He was pulled out of school which decreased the number of infections for 5 months, but after an episode of sepsis, he was started on IVIG replacement.

The 8-year-old daughter presented at 12 months of age with recurrent sino-pulmonary infections. Her serum IgG was low. She was started on IVIG replacement.

2.2 Family II case reports

During the perinatal period, the patient II.P3 was exposed to HIV-1, as his asymptomatic 29-year old HIV-1-positive mother (CDC stage A0), who also carries the *SEC61A1* mutation, had been diagnosed to be HIV-1 positive by routine screening in early pregnancy. Maternal antiretroviral therapy (lopinavir/ritonavir and ziduvudine/lamivudine) had been initiated but because the HIV-1 viral load at time of delivery was still >50 copies (76 copies/ml), the boy was born by caesarean section (gestational age 37+3 weeks, weight 2930 g (21. percentile), length 50 cm, APGAR 10/10/10). Being exposed to maternal HIV-1 infection, the boy was not breastfed and he received post-exposure prophylaxis with zidovudine (4 x 2 mg/kg body weight for 4 weeks) as recommended by national guidelines. During regular follow-up visits for

the exclusion of vertical HIV-1 infection, transient hypogammaglobulinemia of infancy was diagnosed with normal B-lymphocyte count and functional antibody response after tetanus and diphtheria vaccination. He did not respond to repeated measles, mumps and rubella vaccination. Vaccination against pneumococci antigens with Prevenar was not successful. However, after Pneumovax vaccination at age 7, II.P3 developed protective antibody titers against pneumococci (Table S7). Although no severe infections occurred until the age of 9 months, he was given IVIG because of persisting and further decreasing very low levels of serum IgG (Table S1). At the ages of 24 and 48 months, respectively, after exclusion of HIV-1 infection, attempts to discontinue IgG substitution failed because autonomously produced IgG did not reach protective levels. At the age of 6.5 years, IgG infusions were stopped. Over one year, he was free from recurrent infections, maintaining low but acceptable IgG levels. Shortly before his 8th birthday, he suffered from a clinically apparent varicella infection despite regular vaccination (there were also no anti-measles antibodies detectable). At age 8, he started suffering from frequent infections of the gastrointestinal and upper respiratory tract, followed by otitis media and two episodes of scarlet fever. Furthermore, the parents reported him to be constantly tired and suffering from concentration weakness. Therefore, restarting immunoglobulin replacement therapy after live-attenuated MMR vaccination is being considered.

The grandmother (a *SEC61A1* mutation carrier) reported an increased frequency of upper respiratory infections during childhood with recurrent bilateral bacterial otitis media, sudden hearing loss at 24 years of age and an acute intraabdominal abscess at age 48 years. At age 66, with the last vaccinations more than 10 years ago, she did not have specific antibodies against Diphtheria and Pertussis. She had low

95 antibody titers against tetanus and pneumococci and normal antibody titers against
96 measles (Table S7).

3. Supplemental material and methods

3.1 Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated through a Ficoll step gradient (Lymphoprep™ from Axis-Shield) and stained on ice in PBS containing 3% FBS for cell surface markers with fluorochrome-conjugated antibodies to the proteins listed in Table S4. Samples were acquired on a FACS-Canto II flow cytometer (BD) and analyzed using FlowJo version 7.6.5 analysis software (Treestar, Ashland, OR).

3.2 Genotyping and genetic linkage analysis

The identification of the genomic region likely to contain the gene mutated in Family I was done in 2006 using microsatellite markers and genetic linkage analysis, techniques that are now considered old-fashioned. Genotyping for both a genome scan and fine mapping was done using the same methods as described in¹. A genome scan with microsatellites was done using the two parents and 11 children. The 12th child was genotyped only during the fine mapping stage. Some microsatellites were added on chromosome 3, which contained the only promising region in the analysis of the genome scan.

For genetic linkage analysis, LOD scores were computed using FASTLINK²⁻⁴. Both single-marker and multi-marker LOD scores were computed; for this particular analysis several single markers are fully informative, so combining them does not strengthen the evidence for linkage. Most of the genetic linkage analysis of Family I was done using a model of autosomal dominant inheritance with 95% penetrance, since that seemed to fit the inheritance pattern in Family I. No genetic linkage analysis was done in Family II, for which the penetrance may indeed, be lower. To protect against the effects of known genetic heterogeneity in familial CVID, we used a

1% phenocopy rate that exceeds the CVID rate of incidence. The disease allele frequency was set to 0.001. The marker allele frequencies were set all equal; due to the family structure and the parents being genotyped, the LOD scores are insensitive to changes in the allele frequencies.

3.3 Whole exome sequencing and Sanger sequencing confirmation in Family I

Genomic DNA was isolated from blood samples of the four affected family members with the lowest IgG levels using QIAamp Kits (QIAGEN). Whole Exome Sequencing (WES) of 6µg of purified gDNA was performed on an Illumina HiSeq 2000 platform (BGI–Shenzhen) and raw image files were processed using the Illumina Base-calling Software 1.7 with default parameters.

An in-house WES analysis pipeline was previously created⁵. The raw sequenced reads were mapped against the human reference genome build UCSC hg19 using Bowtie 2 v2.2.3⁶, reordered, sorted and converted to BAM format, followed by the removal of PCR duplicates with Picard v1.115 (<http://picard.sourceforge.net>). Local realignment around InDels and base quality score recalibration as well as variant calling and variant quality score recalibration were performed with GATK v3.1 according to their best practice recommendations⁷. Genetic variation data stored in VCF format files was handled using VCFtools program package⁸. For the annotation of discovered variants with the IDs from the short genetic variations database dbSNP v147 (<http://www.ncbi.nlm.nih.gov/SNP/>) the program SnpSift was used⁹. The annotation of variants and their effects was studied with the prediction tool SnpEff v3.6.

A 500bp genomic fragment of *SEC61A1* comprising exon 5 was amplified by PCR with the primers forward (CAGGCTCCTTTTCCAGACTC) and reverse

(TTTGGGCTCCGTTGAAGA). PCR primers were used for Sanger sequencing confirmation according to standard techniques.

3.4 Targeted next generation sequencing and Sanger sequencing confirmation in Family II

Targeted NGS of a panel of 99 candidate CVID genes was performed on patient II.P3 using Agilent's HaloPlex Target Enrichment System for Illumina Sequencing and following the manufacturer's instructions. Sample enrichment was validated on a 2100 Bioanalyzer and subjected to multiplexed sequencing on an Illumina Miseq system following the manufacturer's instructions. Analysis of variants was performed with Agilent's SureCall software. Sanger re-sequencing of a 435 bp fragment in exon 10 of *SEC61A1* was done on members of Family II with primers forward (ACTGTGGGCACCGAGTAAAA) and reverse (AACTGCCCCCTAAAGGACGA).

3.5 Visualization of the V85 position in the Sec61 heterotrimer

An atomic model of the detergent-solubilized mammalian Sec61 protein-conducting channel (PDB 3j7q) bound to the idle ribosome determined by single particle cryo-electron microscopy¹⁰ was used for visualization of the V85 position in UCSF Chimera¹¹. Sec61 subunits in the atomic model were colored separately and V85 was highlighted in magenta.

3.6 Cell culture

All cells were cultured at 37°C in a humidified environment with 5% CO₂. HeLa cells (ATCC no. CCL-2) were cultivated in Dulbecco's modified Eagle's medium (DMEM) (Gibco) with 10% fetal bovine serum (FBS) (Biochrom) and 1% penicillin/streptomycin (GE Healthcare). For live cell calcium imaging, cells were grown on 25-mm glass coverslips. Cell growth was monitored using the Countess®

Automated Cell Counter (Invitrogen) according to the manufacturer's instructions. Hek293T cells were cultivated in DMEM (Gibco) with 10% FBS (Gibco) and 1% penicillin/streptomycin (Sigma-Aldrich). JK6L cells¹² (kindly provided by Prof. Voll), L-363 and U266 cells were cultured in RPMI (Gibco) supplemented with 20% FBS (Gibco), 1% Sodium pyruvate (Gibco) and 1% penicillin/streptomycin (Sigma-Aldrich). EBV-transformed B cell lines, Jurkat cells and THP-1 cells were cultured in RPMI (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Sigma-Aldrich).

3.7 Western blot

Sorted multiple myeloma cell lines stably transduced with empty, *SEC61A1* or *SEC61A1-V85D* encoding pMXs-IRES-GFP-vector were lysed in RIPA buffer (50 mM Tris pH 8.0, 1% Igepal (NP40), 0.5% Sodium Deoxycholate, 150 mM Sodium chloride, 1 mM EDTA, 0.1% SDS in water) containing protease inhibitor (Sigma Aldrich P8340) and phosphatase inhibitors (Sigma Aldrich P5726, P0044) and sonicated (3 x 5 seconds, 20% amplitude) to fragment the gDNA. The protein concentration was measured with a BCA Protein Assay Kit (Pierce), Lämmli buffer was added and samples were incubated for 15 minutes at 56°C for protein denaturation. After SDS-PAGE and Western blot, the membranes were incubated with the following primary antibodies: IRE1A (#3294s), PERK (#3192), XBP1s (#12782), ATF4 (#11815) from Cell Signaling; SEC61A1 and SEC61B provided by Prof. Dr. Richard Zimmermann; α -Tubulin (ab108629, Abcam). The primary antibodies were visualized using Horse Reddish Peroxidase conjugated secondary antibody donkey-anti-rabbit-HRP (sc-2077, Santa Cruz) and SignalFire ECL™ Reagent or SignalFire Plus ECL™ Reagent (Cell Signaling) and the Fusion FX7

Spectra (peqlab) luminescence imaging system in combination with the Fusion-Capt software (peqlab).

Silencing and expression efficiencies in HeLa cells used for protein transport assay were evaluated by western blot analysis using mouse anti- β -actin antibody (Sigma) and rabbit antibodies raised against the carboxy-terminal peptide (14-mer) of human SEC61A and amino-terminal peptide (9-mer) of human SEC61B, respectively, as previously described¹³. Primary antibodies were visualized using goat-anti-rabbit IgG-Peroxidase conjugate (Sigma) and Enhanced Chemiluminescence (ECLTM) or ECLTM Plex goat-anti-mouse IgG-Cy3 conjugate (GE Healthcare) and the Fusion SL (peqlab) luminescence imaging system in combination with the Image Quant TL software 7.0 (GE Healthcare).

3.8 Quantitative reverse transcriptase PCR

Total RNAs from virally transduced, sorted L363 cells were isolated with the RNeasy Kit according to the manufacturer's instructions (QIAGEN). RNA concentration was measured with a NanoDrop 2000c Spectrophotometer (Thermo Scientific) and equal RNA amounts were reverse transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative PCR reactions were performed using the QuantiTect SYBR Green PCR kit (Qiagen) with 30-50 ng cDNA per reaction in 20 μ l reaction volume with a primer concentration of 250 nM. Specific primers for human *ATF4*, *XBP1s*, *CHOP* and *GUSB1* (reference gen) mRNA were designed using the open access NCBI Primer-Blast software. Primer sequences were as follows: *ATF4*: fw_TGTGGATGGGTTGGTCAGTC; rev_ACAGGGCATCCAAGTCGAAC; *XBP1s*: fw_GCGGAAGCCAAGGGGAATGA; rev_CTGCACCTGCTGCGGACTCAG; *CHOP*: fw_TAAAGATGAGCGGGTGGCAG rev_GCTTTCAGGTGTGGTGATGT; *GUSB*: fw_GAGCAAGACAGTGGGCTGGT; rev_CCAGTCCCATTGCGCCACGAC.

3.9 ELISA

Human immunoglobulins were detected using human IgG/IgA/IgM ELISA quantitation sets (E80-104/E80-108/E80-102, Bethyl Laboratories, US). According to the manufacturer's instructions with some modifications, ELISA plates were coated with 25µl of 10µg/ml goat anti-human IgG/A/M antibodies for one hour at room temperature. Plates were blocked with 100µl of [PBS + 1% BSA] for one hour, and washed thoroughly with [PBS + 0.05% Tween-20]. Afterwards, 25µl of sample was added and incubated for one hour. IgG/A/M antibodies were detected with horseradish peroxidase conjugated antibodies using the recommended dilutions. ELISA plates were developed using TMB substrate by measuring absorbance at 450nm.

3.10 ELISPOT

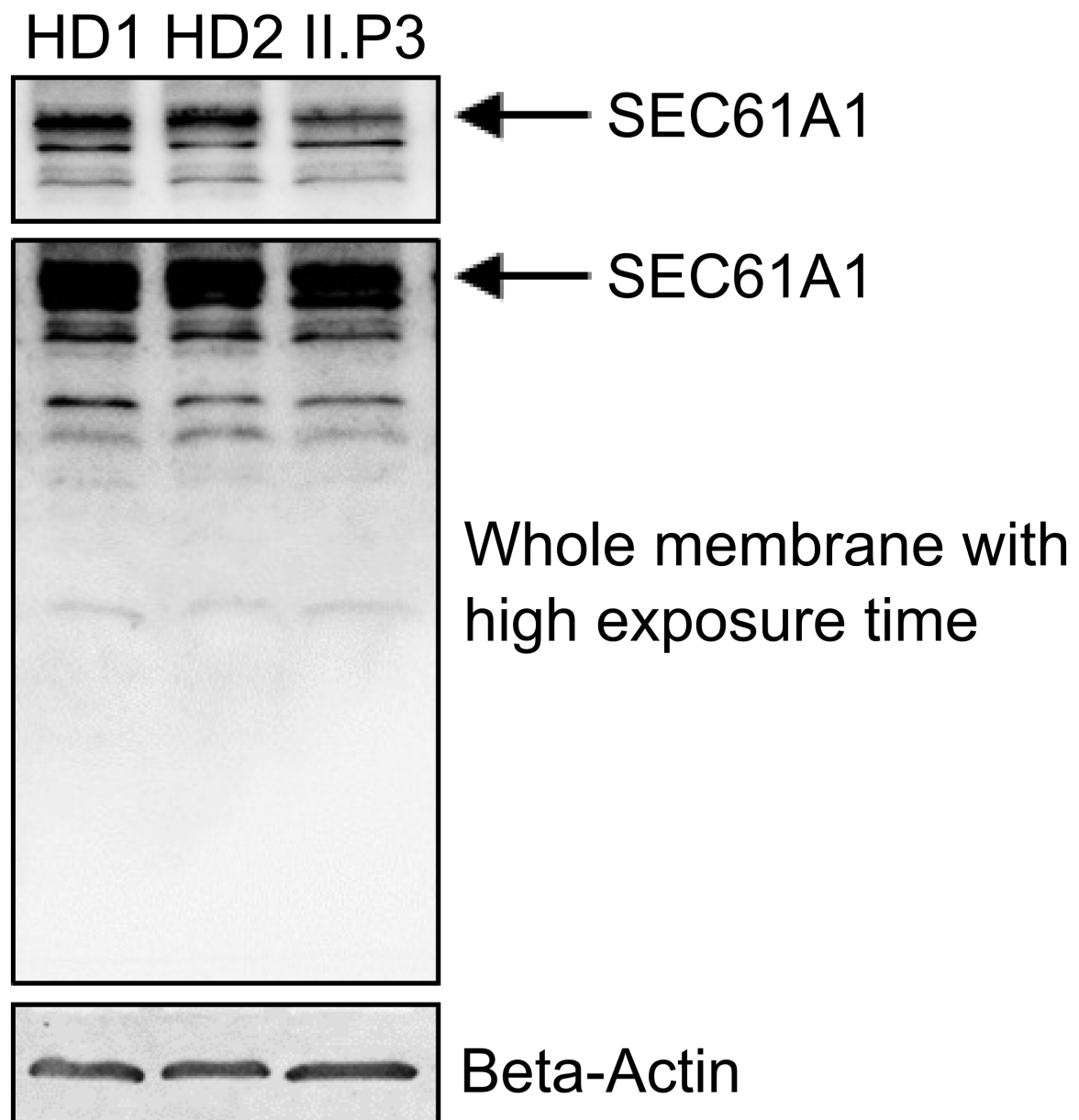
EBV-transformed B cell lines and plasma cells secreting IgA, IgG or IgM were detected using ELISPOT assay. Briefly, 96-well plates (Millipore) were coated with 10 µg/ml purified polyclonal goat anti-human IgA, IgG or IgM (Southern Biotechnologies) overnight at 4°C. After subsequent washings (3x PBS) and blocking with 1% BSA in PBS for 30 minutes at RT, serial dilutions (1000, 500, 250) of cultured cells were seeded and incubated at 37°C and 5% CO₂ for 24h. Plates were then washed (4x [PBS + 0,25% Tween]; 3x PBS) and incubated with isotype-specific, biotin-conjugated goat anti-human IgA, IgG or IgM antibodies (Southern Biotechnologies) for 2h at RT; washed again (4x [PBS + 0,25% Tween]; 3x PBS) and incubated with streptavidin-HRP (Sigma-Aldrich) for 1h at RT. After final washing (4x [PBS + 0,25% Tween]; 3x PBS) plates were developed with 3-Amino-9-ethylcarbazole (AEC) dye (Sigma-Aldrich). Plates were scanned and immunospots counted with a CTL ELISPOT-reader.

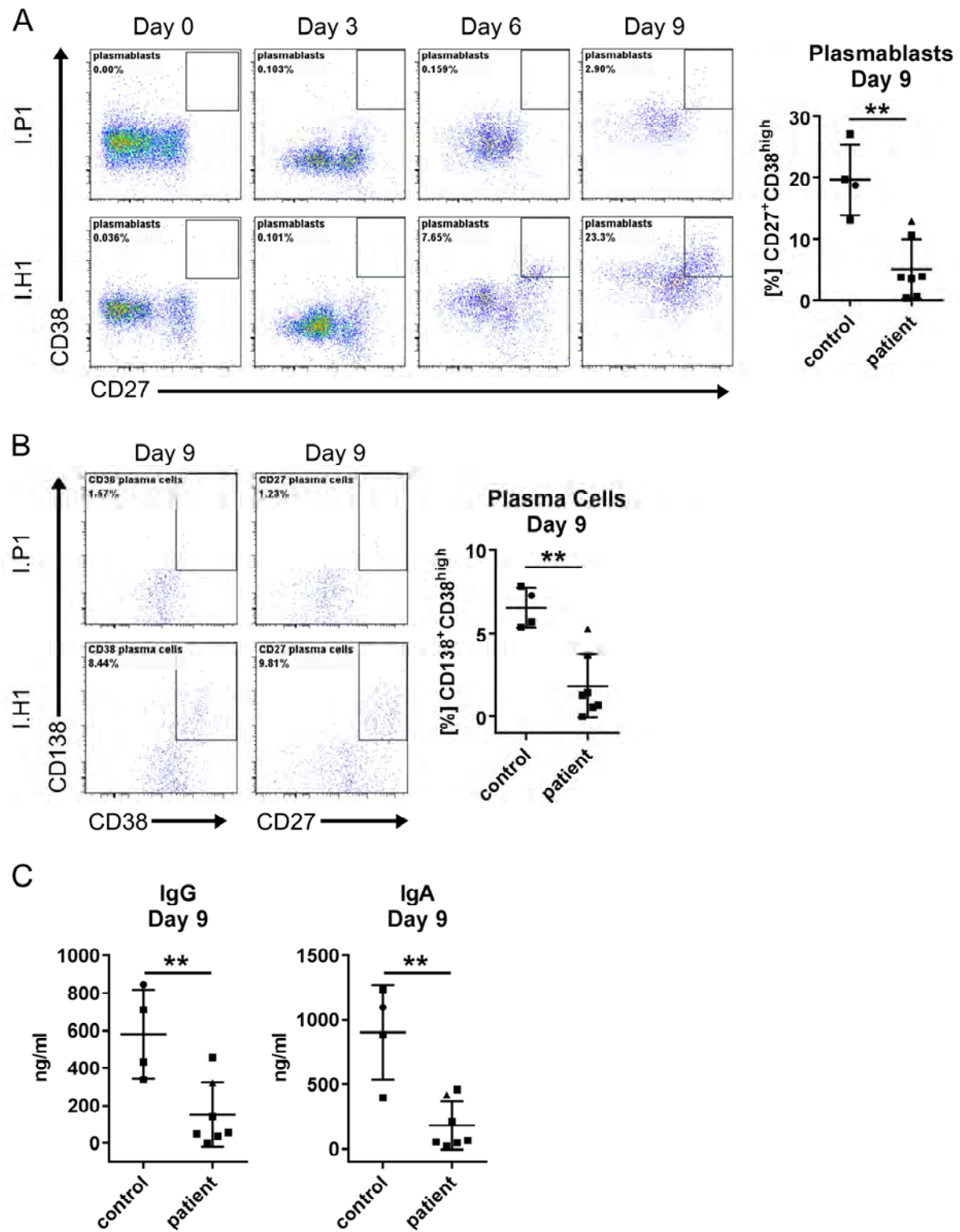
3.11 Statistical analyses

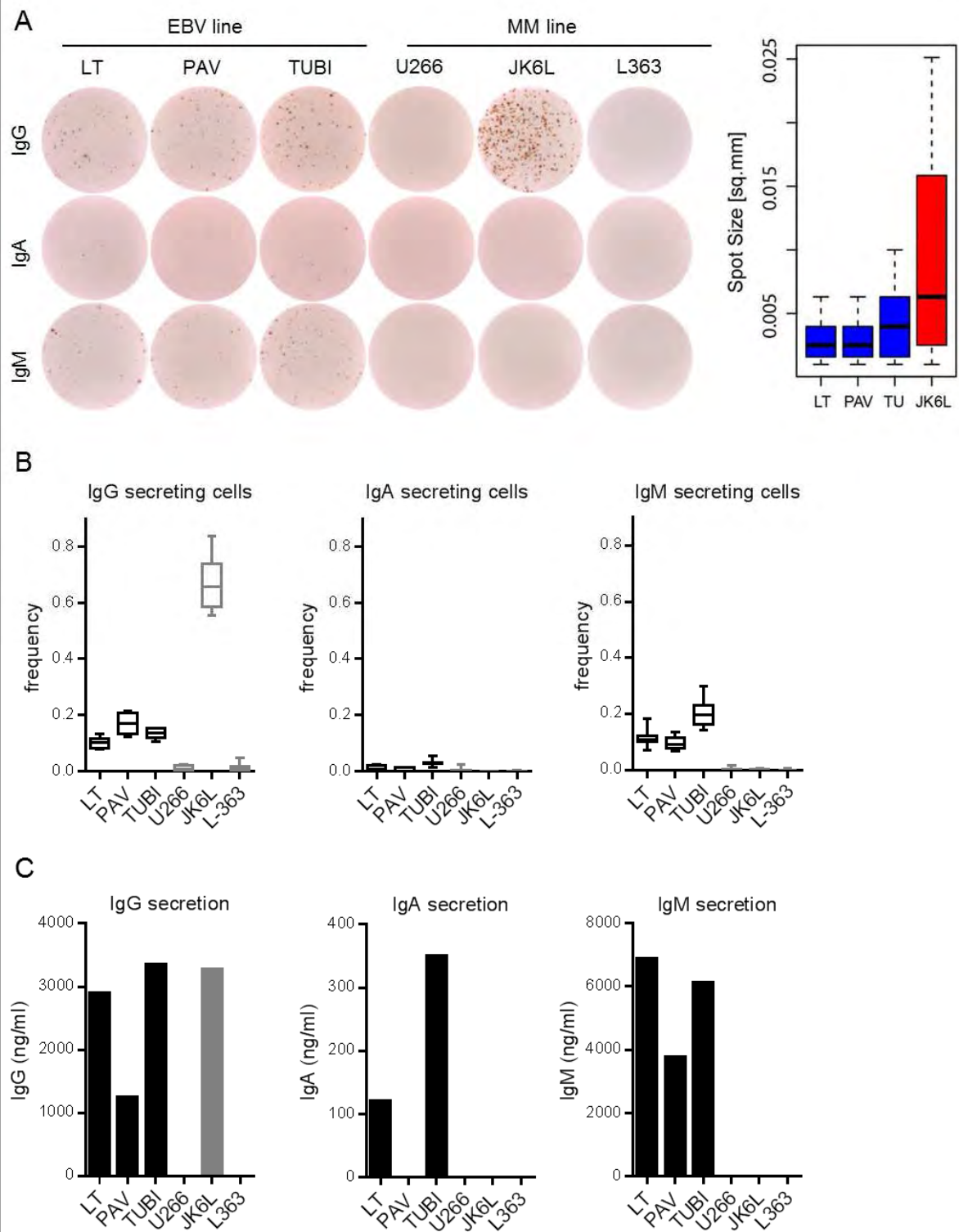
Unless otherwise indicated, statistical analysis was performed using GraphPad Prism version 6, and p values were calculated by two-tailed unpaired Student's t-test for the means with a 95% confidence interval.

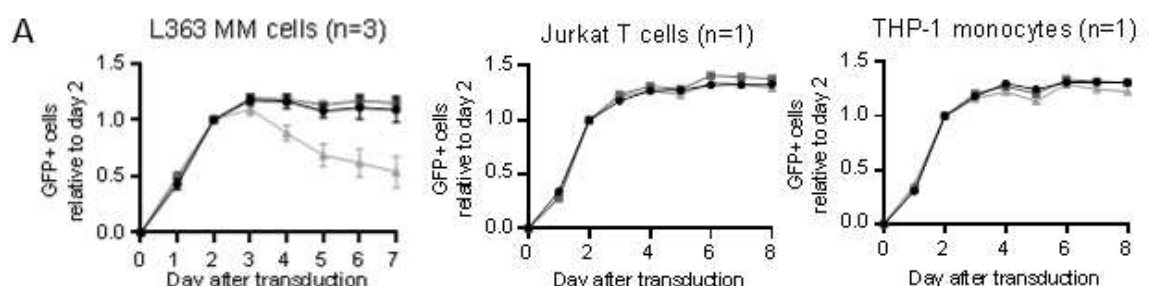
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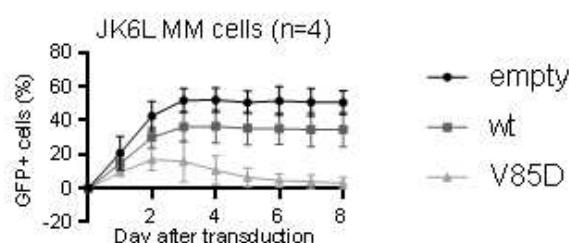




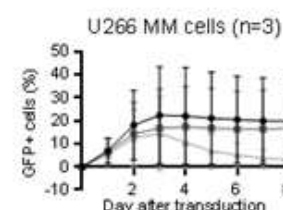
B

● empty ● V85D ● wt

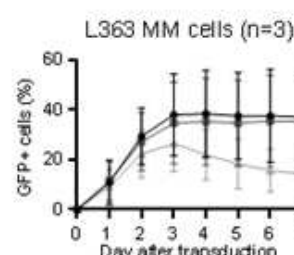
Day	JK6L empty										JK6L wt										JK6L V85D									
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	4.63*	33.7	15.1*	22.8	13.9	12.7	3.62*	7.47*	5.82*	20.8	9.24*	13.1	14	10.7	2.99*	17.51*	3.99*	9.45	10.3*	9.97	10.1	7.52	1.23*	5.11*						
2	11.2*	55.1		38.8	40	38	8.01*	30.4*	19*	35.9		26.3	34.2	23.6	5.48*	15.4*	11.2*	13.1		14.1	26.5	13.7	1.88*	3.76*						
3		56.8		48.7	58.2	43.6		50.6*		36.1		29.2	48.6	30.8		22.2*		8.5						3.03*						
4		55.5	25.9*	50.3	59.2	42.7				35.9	14*	29.7	50	29.6				4.27	5.19*	8.79	23.3	6.27								
5	14.5*	51.7	25.4*	49.1	58.4	42.6		51.2*	26.1*	32.8	14.6*	30	49.4	28.7		22.1*	3.59*	2.48	5.98*	5.36	14.5	3.43		1.05*						
6	12.9*	52.7		49.7	61.9	41	13.9*		22.1*	33.6		28.9	48.7	29.1	7.76*		2.06*	1.84		3.86	10.1	2.33	0.524*							
7	12.6*	52.9		51.2	59.2	39.8		50.8*	22.6*	32		28.8	49.4	28.1		21.1*	1.77*	1.12		2.94	9.66	1.84		0.868*						
8		52	24*	51.8	57.4	40.9	13.4*			32.2	13.1*	29.1	49.2	27.6	6.73*			0.981	2.5*	2.64	7.86	1.05	0.318*							



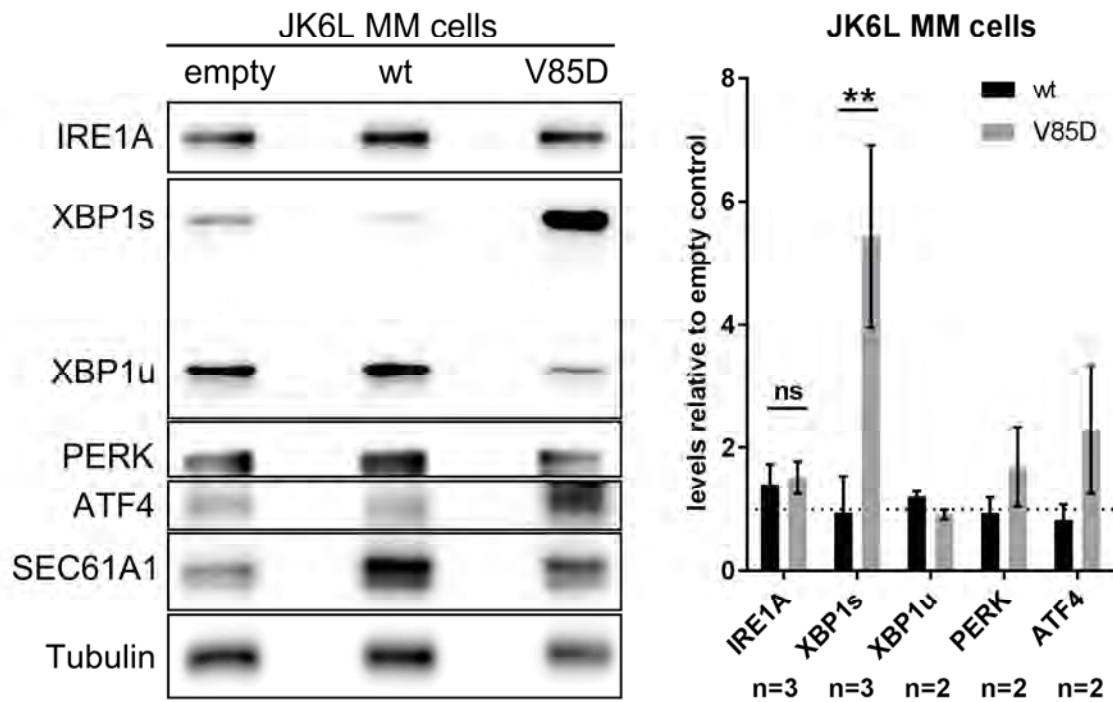
Day	U266 empty					U266 wt					U266 V85D				
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1	6.66	12.5	1.48	1.75*	5.27	12.8	1.28	2.68*	5.63	9.74	0.602	1.35*			
2	15.5	34.2	4.51	6.82*	10.8	29.4	2.58	4.81*	10.2	25.8	1.43	2.17*			
3	16.8	45.5	4.5	7.71*	11.8	35.6	2.73	5.31*	8.92	32.5	1.8	1.93*			
4	15.7	45.4	4.66		11.6	37	3.28		6.65	23.5	1.29				
5	15.2	43.3	4.44	6.77*	11.1	36.1	3.06	4.29*	3.78	15.2	1.33	1.22*			
6	14.4	41.6	5.28		11.1	34.8	3.15		3.23	10.7	1.2				
7	14.2	40.7	4.67	6.19*	10.9	35.4	2.92	4.49*	2.43	7.94	1.01	0.885*			
8	14	40.4	4.7		10.9	35.7	2.89		2.23	6.7	0.584				



Day	L363 empty				L363 wt				L363 V85D			
0	0	0	0	0	0	0	0	0	0	0	0	0
1	6.83	21.2	4.41	6.15	20.5	7.89	5.8	17.1	4.88			
2	17.7	40.4	30	14	35.7	31.7	13.8	32.5	20.5			
3	19.6	50.9	43.3	16.1	41.2	46.8	13.8	35.5	30.3			
4	18.4	49.9	46.9	15.3	42.4	47.9	10.5	28.8	26.6			
5	17.5	47.9	47.3	15.1	42.2	46.9	7.25	21.3	26.6			
6	16.2	46.6	49.7	14.8	42	49.6	6.22	17.7	22.8			
7	15.7	44.4	51	14.4	41.7	49.5	4.99	14.2	23.7			
8*	15.6*	43.7*		14*	42.3*		4.76*	12.3*				



A



B

