

Expanding the clinical and immunological phenotypes and natural history of MALT1 deficiency

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Abstract

Purpose: MALT1 deficiency is a combined immune deficiency characterized by recurrent infections, eczema, chronic diarrhea, and failure to thrive. Clinical and immunological characterizations of the disease have not been previously reported in large cohorts. We sought to determine the clinical, immunological, genetic features, and the natural history of MALT-1 deficiency.

Methods: The clinical findings and treatment outcomes were evaluated in nine new MALT1-deficient patients. Peripheral lymphocyte subset analyses, cytokine secretion, and proliferation assays were performed. We also analyzed ten previously reported patients to comprehensively evaluate genotype/phenotype correlation.

Results: The mean age of patients and disease onset were 33 ± 17 and 1.6 ± 0.7 months, respectively. The main clinical findings of the disease were recurrent infections (100%), skin involvement (100%), failure to thrive (100%), oral lesions (67%), chronic diarrhea (56%), and autoimmunity (44%). Eosinophilia and high IgE were observed in six (67%) and two (22%) patients, respectively. The majority of patients had normal T and NK cells, while eight (89%) exhibited reduced B cells. Immunoglobulin replacement and antibiotics prophylaxis were mostly ineffective in reducing the frequency of infections and other complications. One patient received hematopoietic stem cell transplantation (HSCT) and five patients died as a complication of life-threatening infections. Analyzing this cohort with reported patients revealed overall survival in 58% (11/19), which was higher in patients who underwent HSCT ($P=0.03$).

Conclusion: This cohort provides the largest analysis for clinical and immunological features of MALT1 deficiency. HSCT should be offered as a curative therapeutic option for all patients at the early stage of life.

Keywords: Inborn errors of immunity, primary immunodeficiency, MALT1, combined immune deficiency, immune dysregulation, recurrent infections, skin involvement, failure to thrive, hematopoietic stem cell transplantation.

Introduction

The mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a caspase-like cysteine protease, expressed broadly and plays an essential role in nuclear factor- κ B (NF- κ B) activation (1). During cell activation, scaffold MALT1 protein is recruited to B-cell lymphoma 10 (BCL-10), then each assembled to the caspase recruitment domain (CARD)-containing coiled-coil proteins to generate CARD-BCL-10-MALT1 (CBM) signalosome, which plays as the main driver for the activation of NF- κ B in immune and nonimmune cells (2, 3). Homologous tissue-specific CARD proteins (like CARD9, CARD10, CARD11, and CARD14) coupled with ubiquitously expressed BCL-10 and MALT1 conduct broad effector functions. This signalosome controls the activation and differentiation of lymphocytes, recruitment of immune cells, and releasing of proinflammatory cytokines from hematopoietic and non-hematopoietic cells (4, 5). Apart from scaffold protein function, MALT1 also acts as protease thereby controlling NF- κ B activity by cleaving various substrates, resulting in activation or termination of the signaling (3, 6).

Inborn errors of immunity (IEI) are a group of diseases consisting of approximately 450 genetic disorders causing developmental or functional defects in the immune system (7, 8). The group of IEI presenting with combined cellular defects has been strictly expanded in the last two decades. This phenomenon helps us to understand the molecular basis of the immune system in more detail, thus providing early diagnosis and better disease management. In 2013, Jabara et al. described a biallelic loss-of-function (LOF) *MALT1* variant (OMIM #615468) in two siblings who presented as a combined immune deficiency (CID), resulting in a severe disease course, which led to the patients' demise (9). Later, additional patients with MALT1 deficiency have been described with a combination of recurrent bacterial, viral, and fungal infections. Furthermore, those patients demonstrated various symptoms characterized by oral lesions, mainly aphthous ulcers, chronic diarrhea, dermatitis, and failure to thrive, mimicking immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)-like disorder (9-16).

The immunological features of MALT1 deficiency are variable and characterized by normal lymphocyte counts, normal or reduced immunoglobulin (Ig) levels, low antigen-specific antibody titers, and abnormal T cell proliferation response to mitogenic stimuli. Lymphocyte subgroup analysis generally showed normal CD3⁺ T and CD16⁺/CD56⁺ NK cells, while high T-cell numbers and impaired B-cell maturation were also described in some patients (3, 17). Due to the defective signaling characterized by impaired NF-κB activation and IL-2 secretion, MALT1-deficient patients exhibited reduced regulatory T cells (Tregs) and T helper 17 (T_H17) cells (9-16). Heretofore, the majority of patients were reported under investigational research studies without detailed history. Thus, further studies are needed to better characterize the features of MALT1 deficiency.

Herein, we report clinical and immunological findings of nine new patients with biallelic LOF *MALT1* variants. Our well-defined expansive cohort exhibits a broad clinical and immunological phenotype of the disease and provides detailed analysis by combining new and ten previously reported patients. This comprehensive evaluation would facilitate early diagnosis of the disease and potentially improves patients' outcomes.

Materials and Methods

This multicenter study included patients with MALT1 deficiency. The genetic diagnosis was made by whole-exome sequencing, confirmed by Sanger sequencing, as described previously (18-24). Details are provided in the **Supplementary file**. Clinical and demographic features of the patients were documented. The local ethics committee from Marmara University and Tehran University of Medical Sciences approved the study protocol and written informed consent was obtained from all patients and/or their parents.

Clinical evaluation

A questionnaire, including demographic and clinical data (age at onset of symptoms, age at diagnosis, follow-up period, family history, past infections, systemic involvements, treatments) was filled for every patient. Clinical and molecular diagnoses of IEI were performed based on the European Society for Immunodeficiencies (ESID) and International Union of Immunological Societies (IUIS) criteria, respectively (8, 25, 26).

Immunological assessments

Peripheral lymphocyte subset analyses, intracellular protein and proliferation assays were performed by flow cytometry as described previously (27-29). Cytokine secretion was detected by the enzyme-linked immunosorbent assay. The plausible effects of variants on 3D protein structure were investigated based on the predicted unfolding free energy change (30, 31). The details are provided in the **Supplementary file**.

Statistical analysis

The data were presented as mean \pm standard deviation and median with interquartile range. Group comparisons were performed by unpaired Student's t-test. Within-group, comparisons were carried out with paired t-test and 2-way analysis of variance (ANOVA) with Holm-Sidak's post hoc analysis, as indicated. Fisher's Exact Test was used for the comparison of categorical values. Analysis of overall survival was done using the Kaplan-Meier method (log-rank test). A statistical significance was considered at a p -value <0.05 . Statistical analyses were done using GraphPad Prism 8 (GraphPad Software Inc, San Diego, Calif).

Results

Genetic Diagnosis and Variant Analysis

We combined ten previously published cases (P1-P10) (9-15) and our nine patients (P11-P19) to provide an up-to-date overview of MALT1 deficiency. We presented detailed information about a previously reported patient (P14) (16). All of our patients had novel homozygous variants (**Fig.1A** and **Fig.S1A**). Most of the newly identified variants were missense (P11, P14, P15, P16, P17), while the others were frameshift (P13 and P19), inframe (P12), and start-loss (P18). The Combined Annotation Dependent Depletion, SIFT, Polyphen, MutationTaster, and PROVEAN scores of all identified variants are provided in **Table S1**, predicting a deleterious impact of novel mutations. The other rare and homozygous variants, which were detected by the WES are presented in **Table S2**, indicating no other known IEI genes mutated in those patients. The variant types were similar in nature to the previously reported variants, where the majority of them demonstrated missense changes (P1, P2, P3, P5, P6, P7, P8, P10), except for the P4 (11) and P9 (13), who had compound heterozygous variants (**Fig.1A**).

From the variants, which are represented in **Fig.1B**, Leu386Pro (between a beta-strand and alpha-helix) and Asp535Asn (on alpha-helix) are located on the caspase-like domain, while Trp580Arg is located at the Ig-like domain on alpha-helix of MALT1. These residues are defined as buried in the protein core, based on the solvent-accessible surface area of the mentioned locations (30). The potential effects of these variants on protein structure were investigated based on the predicted unfolding free energy change (31). As shown in **Table S3**, all of the newly detected missense variants have a destabilizing effect on the MALT1, supporting the potential pathogenicity of the variants (32, 33). Furthermore, intramolecular interactions for Trp580Arg and Leu386Pro cause an apparent reduction in the hydrophobic interactions and disappearance of a hydrogen bond (**Fig.1B**). Moreover, variation from Asp to Asn at 535 position results in the disappearance of two ionic bonds compared to the wild type. Multiple sequence alignment demonstrated that all identified variants in our cohort are highly conserved (**Fig.S1B**).

While MALT1 mutations leading to a frame shifts (P13, P19) or loss of start site (P18) are destructive mutants, we wanted to analyze the effect of missense mutants P11, P14, P15, P16, P17 on MALT1 protein expression. Since we lack sufficient amounts of primary cells from the patients for expression analyses, we cloned the mutations in context of MALT1A into mammalian expression vector and transfected HEK293 cells. MALT1 expression was analyzed by Western blotting using two different MALT1 antibodies and the Strep antibody recognizing the Strep-Tactin (ST) epitope tag (**Fig. 1C**). In line with previous results for mutation W580S (P3), also the MALT1 W580R (P17) mutation led to decreased protein expression compared to MALT1 WT. Further, variants L386P (P15, P16), L401LI (P12) and N485S (P14) provoke reduced MALT1 protein expression, while MALT1 D535N (P11) was expressed at equivalent levels compared to MALT1 WT.

The current and previously reported variants were located mostly in the caspase-like domain and Ig-like domains, followed by the death domain. The clinical and immunological comparisons between all reported variant types did not reveal any differences and were not associated with survival (**Table S4, Fig.S1C**). Therefore, we concluded that there was no strong genotype-phenotype relationship that governed the manifestations of MALT1 deficiency.

Clinical and Immunological Overview of MALT1 Deficiency

Demographic, clinical, and immunological characteristics of our newly identified (n=9) and previously known (n=10) patients are summarized in **Table 1**. Detailed clinical and laboratory information of our patients are provided in the **Supplementary file**. The mean age of the patients was 33±17 months, and the mean age of disease onset was 1.6±0.7 months. There was no significant gender predilection in our cohort (Male/Female: 4/5). Of total 19 patients, 52% (n=10) were female, 48% (n=9) were male. All of our patients had parental consanguinity.

The clinical features observed in MALT1 deficiency (n=9, **Table 1, Fig.2A and B**) were consistent with CID and all the patients showed onset of disease in the first six months of life. The cardinal clinical findings of the disease were recurrent infections (100%), skin involvement (100%), failure to thrive (FTT) (100%), oral lesions (67%), chronic diarrhea (56%), and autoimmunity (44%).

Spectrum of Infections

Recurrent infections were among the most common findings of patients (100%), including bacterial, viral, and fungal infections. All of our patients and previously published patients had recurrent pneumonia, requiring hospitalization. P17 and P19 also had upper respiratory tract infections characterized by severe otitis media and sinusitis, respectively. P17 had bronchiectasis and when evaluated together with previously described patients, 37% of the patients had bronchiectasis, as a complication of infections (**Table 1**).

Severe life-threatening infections were documented in seven (78%) of our patients. P11 suffered from *Acinetobacter baumannii* meningitis and sepsis, leading to disseminating intravascular coagulopathy and extremities necrosis. P12 and P14 had sepsis due to staphylococcal infections. P13 experienced *Candida parapsilosis* septic arthritis. P15 and P17 had a severe necrotizing skin infection, requiring prolonged hospitalization. P18 suffered severe meningitis, due to varicella-zoster virus (VZV), causing death. When all previously reported and our patients were evaluated together, severe life-threatening infections occurred in 68% of MALT1 deficiency. Notably, sepsis (53%) and meningitis (21%) were the most common severe infections.

Gram-positive strains (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were the most isolated microorganisms in the site of infections, followed by gram-negative bacterial agents (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*). In the review of reported and current patients, bacterial infections were noticed in 100% of MALT1-deficient patients (**Table 1**).

In our cohort, fungal infections were described mainly by candidal infections (n=7, 78%). There were two patients with *Aspergillus* infection in the skin (P14) and lung (P17). Viral infections were observed in four (44%) patients. P11, P14, P18 suffered from cytomegalovirus (CMV) infection and Epstein-Barr virus (EBV) was detected in P14 and P18. P12 showed dermatitis complicated with the Herpes simplex virus (HSV) and *Staphylococcus aureus*. P18 had severe VZV meningitis. In a total of 19 patients, viral infections were reported in 74%, and CMV was the most common viral agent (47%), followed by HSV (26%) (**Table 1**).

Opportunistic infections were not observed in our cohort, while *Pneumocystis jirovecii* pneumonia was reported previously in one patient (P5) (15).

Dermatological Manifestations

Eczema was observed in seven cases (78%) of our cohort, started in the early period of life, and was considered severe atopic dermatitis in most patients before diagnosis. Six (67%) patients had diffuse erythroderma (**Fig.2B**). P12 and P14 also showed seborrheic dermatitis. Skin prick tests for food allergy were negative, and eczema did not respond to diet modification and local treatments. Only P18 has a history of multiple food allergies. P11 showed extensive skin necrosis leading to extremities amputation (**Fig.2B**). Other skin involvements were alopecia (n=3, 33%) and vitiligo (n=1, 11%). P13 had widespread progressive vitiligo in the genital and perianal area, and fingertips (**Fig.2B**).

Other Prominent Clinical Findings

Failure to thrive was a common feature among patients and detected in all of our patients, likewise to the reported patients. Overall, 17 (94%) of MALT1-deficient patients had FTT, except P6 (15) and not specified in P10 (14). Treatment-resistant, recurrent oral lesions such as aphthous ulcers, cheilitis, gingivitis, and thrush were other common findings observed in six (67%) of our patients, emerging in the early stages of the disease. Accompanied dysmorphic features were observed in one of our patients, while described in five cases, previously (**Table 1**).

Since CBM signalosome is required for the appropriate inflammatory responses and Treg development (5), IPEX-like chronic and/or intermittent diarrhea was a common finding of disorder. Five of our patients (56%) and seven (78%) of previously reported patients suffered from mild intermittent or severe diarrhea, causing growth retardation, FTT, malabsorption, and sometimes requiring hospitalization. P12 and P17 also displayed hypoalbuminemia because of loss from the gastrointestinal tract. Pathological dysregulation findings such as villus atrophy, mucosal lymphocyte accumulation, and inflammation were observed in P12 and P15.

Laboratory and Immunological Assessments of MALT1 Deficiency

Laboratory findings of all previously reported and current MALT1-deficient patients are presented in **Table 2**. In addition, detailed immunological evaluations of our cohort are presented in **Table 3**.

Although all patients presented as CID, lymphopenia was not detected in our cohort. Whereas due to severe infections, we usually detected mild leukocytosis in the peripheral blood (n=6, 78%). Eosinophilia was prominent in six (67%) patients. Decreased serum Ig levels before Ig replacement therapy (IgRT) were detected in our cohort; consistent with previously reported patients (**Table 2**). IgE level was high in two (22%) of our patients. Post-vaccination-specific antibody responses were available in six patients (**Table 3**). Pneumococcal antibody responses were non-protective in two patients (P17, P19), while was protective in P16; similarly, the tetanus toxoid response was non-protective in three patients (P15, P17, P19), but was protective in P16 and P18. Overall, nine of previously reported and present patients (64% of tested cases) demonstrated impaired vaccine responses.

Flow cytometric analyses are presented in **Table 2** and **Table 3**. Interestingly, the majority of patients had normal total T and NK cell frequencies. Eight (89%) exhibited mild to moderately reduced B cells, and P13 had normal B cells but showed decreased memory B cells. Compared to healthy controls, we observed slightly decreased naive CD4⁺ T and increased memory CD4⁺ T cells in P11, P12, and P18. These alterations were higher than previously reported patients (only P5 had low naïve CD4⁺ T cells) (15). Effector T-cell subtypes and recent thymic emigrants were detected in the normal range (P13, P18), consistent with previous reports showing normal thymic activity (12).

MALT1 dependent signaling (34, 35) and NF- κ B activation (36, 37) are important for the thymic Treg development, lymphocyte maturation, and proliferation (9, 35). Therefore, we evaluated Treg cells, T cell proliferation responses to mitogenic stimulus, and cytokine responses in our patients. Our P11, P12, P13 patients and other reported patients (P4, P5, P6, P7, P8) had low CD4⁺ CD25⁺ FOXP3⁺ Treg cells (11, 12, 15) (**Fig.3A**). Anti-CD3/CD28 or Phytohemagglutinin (PHA)-stimulated T cells revealed blunted expansion (P13, P14, P15, P17, P18, P19), and P13 showed reduced cytokine responses (IL-2, IL-4, IFN- γ , IL-6, IL-10, IL-17) (**Fig.3B**, **Fig.S2**, **Fig.S3** and **Table 3**). Overall, impaired T-cell proliferation was observed in most of the

reported and present patients (P1, P2, P3, P4, P5, P6, P7, P8, P13, P14, P15, P17, P18, P19) (9-12, 15). While P10's T cells were able to proliferate *in-vitro* in response to PHA, which was described as hypomorphic variant in nature compared to others with LOF variants, leading to absent MALT1 protein expression (14).

The CBM complex phosphorylates the NF- κ B inhibitor I κ B α , which results in its proteasomal degradation, hence ensuring the translocation of the NF- κ B subunits p50 and p65 into the nucleus, leading to T cell activation and proliferation (3). As a result of defective MALT1 function, we demonstrated poor CD25 and inducible T-cell costimulator (ICOS) upregulations in T cells of P13 (**Fig.4A** and **Fig.S4**). Furthermore, phorbol myristate acetate/ionomycin-mediated short stimulation clearly showed impaired NF- κ B activation in P13, as measured by phospho-p65 level compared to the healthy control (**Fig.4B** and **Fig.S5**).

Treatments and Outcome

Because of recurrent infections and dysgammaglobulinemia with inadequate antibody responses, seven (78%) and nine (100%) of patients were commenced on antibiotic prophylaxis, and IgRT, respectively. The detail of the regimens is presented in **Table 2** and **Table 3**.

The combined therapies, including IgRT and antibiotics prophylaxis, were mostly ineffective in reducing the frequency of infections and other complications. In our cohort, only four (44%) patients survived, one of them (P12) received hematopoietic stem cell transplantation (HSCT) and is presently more than one year after transplantation with the complete disease control (**Fig.1C** and **Supplementary File**). P13 is doing well under medications. P17 and P19 are suffering from infections, chronic diarrhea, and FTT, both are waiting for transplantation. P16 deceased at age 38 months due to respiratory failure. Four patients died as a complication of sepsis or meningitis (P11, P14, P15, P18), approaching statistical significance of better OS probability in patients without meningitis ($P=0.06$) (**Fig.S6A** and **B**).

When previously reported patients were evaluated, the HSCT was performed in four (44.4%) by using reduced-intensity conditioning regimens, all of them are alive (10,

11, 15, 38). In contrast, three (30%) patients without transplantation were demise due to infections and associated complications.

The pooled overall survival (OS) for present and previous patients was 58% (11/19) and it was higher in patients with HSCT compared to non-transplanted cases ($P=0.03$) (**Fig.5A** and **B**). The OS probability was not changed in terms of chronic diarrhea and bronchiectasis (**Fig.5C** and **D**).

Discussion

This report evaluated the clinical and laboratory features, and outcomes of nine patients with novel *MALT1* variants. In addition, we aimed to create comprehensive diagnostic and therapeutic approaches to MALT1 deficiency by combining all defined patients (this report and previously reported). The most prominent symptoms were recurrent infections, most commonly affect the lower respiratory tract, caused frequently by bacterial microorganisms, followed by fungal and viral infections. Patients also had dermatologic lesions (eczema, seborrheic dermatitis, erythroderma), chronic diarrhea, and FTT. Life-threatening infections, mostly sepsis and meningitis can determine patients' outcomes. All patients presented as CID, albeit had normal lymphocyte numbers with variable Ig levels, and had reduced B, Treg, and T_H17 cells. The definitive treatment of the disease is HSCT. Until HSCT, patients can benefit from antibiotic prophylaxis and IgRT but are still susceptible to infections, which determine the outcome.

This cohort demonstrated that MALT1-deficient patients have a cluster of symptoms, characterized by recurrent/severe infections, skin involvement, and chronic enteropathy with FTT. The symptoms of the disease begin at an early life period and require early therapeutic interventions (9-15). The most frequently isolated pathogens are *Staphylococcus aureus*, *Candida albicans*, and CMV. Lymphocyte responses in T and B cells were impaired in MALT1 deficiency, as showed in patients and *Malt1* knockout (KO) mice (39), leading to low production of cytokines, defective proliferation, and reduced antibody responses, contributing to the patients' phenotype. The reduced T cells proliferation can be rescued by IL-2 supplementation (12). Pronounced reduction in T_H17 cells is thought to be the main reason for the tendency of staphylococcal and *Candida* infections. Expression of CARD14 in keratinocytes with defective MALT1 can lead to impaired production of T_H17, explaining the skin involvement with *Staphylococcus aureus* and *Candida albicans* pathogens. MALT1 is necessary for mouse marginal zone B and peritoneal B1 cells development (35). This can explain the mild to moderate reduction of B cells in our cohort. Furthermore, KO mice NK cells show reduced cytokine production, but normal cytotoxicity (40). These factors can contribute to the mechanisms that pave a way for the increased viral infection rates in the patients. Additionally, specific functions of the CARD protein

component in different cell types can impart an accumulation of some unique features in MALT1 deficiency, resembling hyper-IgE syndrome and IEI with chronic mucocutaneous candidiasis. However, the elevated IgE and eosinophilia are not observed to be as high in MALT1 deficiency as a hyper-IgE syndrome.

In our cohort, we observed very early onset eczema and diffuse erythroderma, mimicking Omenn syndrome in the majority of patients. Interestingly, some patients also demonstrated chronic diarrhea, lymphoproliferation, and autoimmune manifestations, which are mainly localized to the skin (vitiligo and alopecia). It is well known that the CBM complex and NF- κ B activation are required for the development of thymus-derived Treg cells (41, 42) and *Malt1* KO mice exhibit very low Foxp3⁺ Treg cells (35, 43). Consistent with the mouse model, Treg development is also impaired in human MALT1-deficiency, explaining the IPEX-like phenotype observed in those patients. It would be also worth mentioning that mice lacking protease activity (*Malt1*^{PD/PD}) show a roughly 60% decrease in CD4⁺ Foxp3⁺ T cells, but exhibit exaggerated inflammatory responses characterized by T_H1 and T_H2 skewing, reflecting by enhanced IgG1 and IgE levels (35, 39). Interestingly, KO and *Malt1*^{PD/PD} mice demonstrate different lymphocyte compositions in lymphoid organs, which are dissecting by lacking germinal center and T follicular helper cells (T_{FH}) in the KO phenotype. Diminished T_{FH} cells can prevent the production of autoantibodies due to inappropriate B-cell co-stimulation (44). This phenotype can explain the less spectrum of autoimmune manifestations in MALT1 deficiency, which are usually ample in IPEX patients (45). Furthermore, MALT1 paracaspase activity regulates alanine-serine-cysteine transporter 2 requiring for glutamine influx, thereby controlling the mechanistic target of rapamycin complex 1 (mTOR1) that is important for appropriate Treg functioning (46). Dominant-negative mutations in *CARD11*, similarly, lead to defective mTOR1 activity by enhanced T_H2 responses (47). This mechanism potentially contributes to dysregulated T_H2 skewing in MALT1 deficiency.

Reported MALT1-deficient patients experienced fungal infections as onychomycosis and moniliasis (9-12, 15). Invasive fungal infections like candida-related pneumonia, esophagitis, and duodenitis have been also described in four patients (9-11). One of our newly defined patients had *Candida parapsilosis* septic arthritis, and the other two had Aspergillosis, while the rest suffered from moniliasis and onychomycosis.

Heretofore, *Aspergillus* infection has been only reported in one MALT1-deficient patient (16). This type of invasive fungal infection mirrors the important role of MALT1 in neutrophils, where acts together with CARD9, which is known to be important for fungal immunity (48).

Functionally, destructive frame shift (P13, P19) or start site (P18) mutations will lead to a loss of MALT1 function. Further, with MALT1 W580R (P17), L386P (P15, P16), L401LI (P12) and N485S (P14), four of five newly identified MALT1 missense mutations in the Caspase-like and Ig3 domains cause a decrease in MALT1 expression, which has also been found for other MALT1 variants (**Lu et al. *Frontiers in Immunology*, 2018; DOI:10.3389/fimmu.2018.02078**). For patient P3, it is well documented that decreased stability of the homozygous MALT1 W580S variant leads to CID, which resembles the phenotypic changes seen with the other destabilizing MALT1 missense mutations leading identified here. Interestingly, MALT1 expression was not affected by the D535N exchange (P11) in the Caspase-like domain, suggesting that this mutation and potentially also some of the other missense variants, may directly impact on MALT1 function, for instance MALT1-catalyzed substrate cleavage. Thus, the level of MALT1 destabilization and functional impairment can cause partial to complete loss of MALT1 function, which will differentially affect the severity of the clinical manifestations in the individual patients.

In our cohort only four (44%) patients survived, delineating the poor prognosis and high fatality of the disease. HSCT has been conducted on one of our patients and four previously reported patients (44.4%), all of them are alive with good condition, highlighting the decisive role of the HSCT in successful and sustained disease control. Matched and mismatched donor sources were used during transplantation and the majority of patients showed a high percentage of chimerism without major complication after transplantation (10, 11, 15, 38). Interestingly, one patient demonstrated mixed chimerism with 11.2% of peripheral blood nucleated donor cells but showed normal immune reconstitution for T and B cells (15, 38). This result explores the minimal required level of MALT1 in the immune system to prevent disease manifestations and achieves desirable control. Importantly, prophylactic antimicrobial agents and IgRT should be started in all of the patients until HSCT.

The present study has a limitation in terms of verifying the pathogenicity of the genetic variants. While protein expression of most MALT1 missense mutations was decreased in a heterologous setting, lack of appropriate primary patient material often precludes an in depth functional analyses of the deleteriousness of the mutations in vivo. Nevertheless, our patients had clinical pictures of CID, and showing the onset of disease in the first six months of life. All of the patients had family consanguinity, prioritizing autosomal recessive disorders. Whole exome sequencing was evaluated with the clinical and laboratory findings, and only alterations in the *MALT1* gene can explain the phenotype of these patients.

In conclusion, human MALT1 deficiency causes a CID or IPEX-like disorder characterized by defective NF- κ B signaling with lacking paracaspase activity. The defective Treg and T_H17 development accompanied by impaired T- and B-cell responses determine the clinical phenotype of the patients. Our study extends the clinical spectrum of the disease by combining and analyzing all reported MALT1-deficient cases. Current experience suggests that MALT1 deficiency should be envisaged in patients with severe infections, skin involvement, and chronic diarrhea who have normal lymphocyte numbers. HSCT should be provided for all patients at an early stage of life before end-organ damage to enhance the survival rates.

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Code availability: Not applicable.

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Consent to participate: Informed consent for participation was obtained from all individuals.

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Figure and Table Legends

Figure 1. Variants in MALT1 deficiency are distributed throughout the gene. (A) Schematic diagram of MALT1 protein domains, with locations of previously known homozygous variants (Black), compound heterozygous variants (Blue-P4 and Green-P9) and variants observed in our cohort (Red). Asterisk (*) indicates a premature stop codon. The depicted domains are the death domain, three Immunoglobulin (Ig)-like domains, and Caspase-like domain. **(B)** Reduced intra-molecular interactions as a result of the identified missense variants (Trp580Arg, Asp535Asn, and Leu386Pro). Intra-molecular interactions for hydrogen bonds (purple), ionic bonds (brown), van der Waals (VDW) bonds (green), hydrophobic interaction (blue), aromatic interactions (fuchsia), carbonyl interaction (orange), polar interactions (cyan), and clashes (pink) are depicted. **(C)** HEK293 cells were transfected with MALT1A WT and respective missense mutants and protein expression was analyzed by Western blotting using two different anti-MALT1 antibodies and the anti-Strep antibody against the Strep-Tactin epitope tag. Representative blots from three independent experiments are shown.

Figure 2. Clinical phenotypes of our MALT1-deficient patients (n=9). (A) The bars are depicted as percentages. Disease symptom clusters are indicated with different colors. Red bars indicate infections, black bars show skin manifestations, blue bar demonstrates failure to thrive, brown bar exhibits oral lesions, pink bars indicate autoimmune manifestations, grey bar shows lymphoproliferation, dark green bar depicts dysmorphic findings. *RTI*, respiratory tract infection. **(B)** Representative pictures of patients' phenotypes: Alopecia, generalized eczema and extensive extremity necrosis of P11; erythroderma, facial eczema complicated with superficial *Staphylococcus aureus* of P12 before hematopoietic stem cell transplantation and resolution after transplantation; general appearance and vitiligo on fingertips and perineal area of P13; facial eczema of P14; colonoscopic examination of P15 revealing hyperemia and beading pattern in ileal and cecal mucosa; necrotizing skin infection involving right plantar area and phalanges of P17.

Figure 3. Reduced Treg and impaired proliferation of T cells in MALT1-deficient patients. (A) Flow cytometry analysis of CD4⁺ CD25⁺ FOXP3⁺ Treg cells in P12 and healthy control. Corresponding percentages are indicated in the upper right quadrants, and the summary of CD4⁺ CD25⁺ FOXP3⁺ staining data from P11, P12, and P13 along with age-matched healthy control (n=7), is shown on the right. Asterisks indicate significance levels (**p<0.01, unpaired Student's t-test). (B) T cell proliferation assay with anti-CD3/28 of P13 and three healthy controls in unstimulated and stimulated (anti-CD3 and anti-CD28) conditions. Asterisks indicate significance levels (**p<0.01, paired t-test).

Figure 4. Defective T cells stimulation in MALT1 deficiency. (A) Flow cytometric analysis and summary of percentages of CD25 and inducible T-cell costimulator (ICOS) expression in CD4⁺ T cells in P13 (two technical replicates) and healthy controls (n=6) in unstimulated and stimulated (anti-CD3 and anti-CD28). Asterisks indicate significance levels (***p<0.001 and ****p<0.0001, paired t-test). (B) Flow cytometric analysis and summary of mean fluorescence intensity (MFI) of Phospho-p65 expression in CD4⁺ T cells (P13, three technical replicates) and healthy controls (n=3) in unstimulated and stimulated conditions at indicated time courses with phorbol myristate acetate and ionomycin (P-I). Asterisks indicate significance levels (*p<0.05, 2-way ANOVA with Holm-Sidak's posttest analysis). *US*, Unstimulated; *S*, Stimulated; *ns*, not significant.

Figure 5. Kaplan-Meier survival curves of all new and defined MALT1-deficient patients (n=19). Survival curves demonstrating overall survival (A), survival analysis comparing patients with/without HSCT (B), with/without chronic diarrhea (C), and with/without bronchiectasis (D). *HSCT*, hematopoietic stem cell transplantation.

Figure 6. Clinical red flags and diagnostic algorithm for recognizing patients with MALT1 mutations. *CBM*, CARD-BCL10-MALT1, *HIES*, Hyper-IgE syndrome, *IPEX*, Immune dysregulation, polyendocrinopathy, enteropathy, X-linked, *WAS*, Wiskott–Aldrich syndrome.

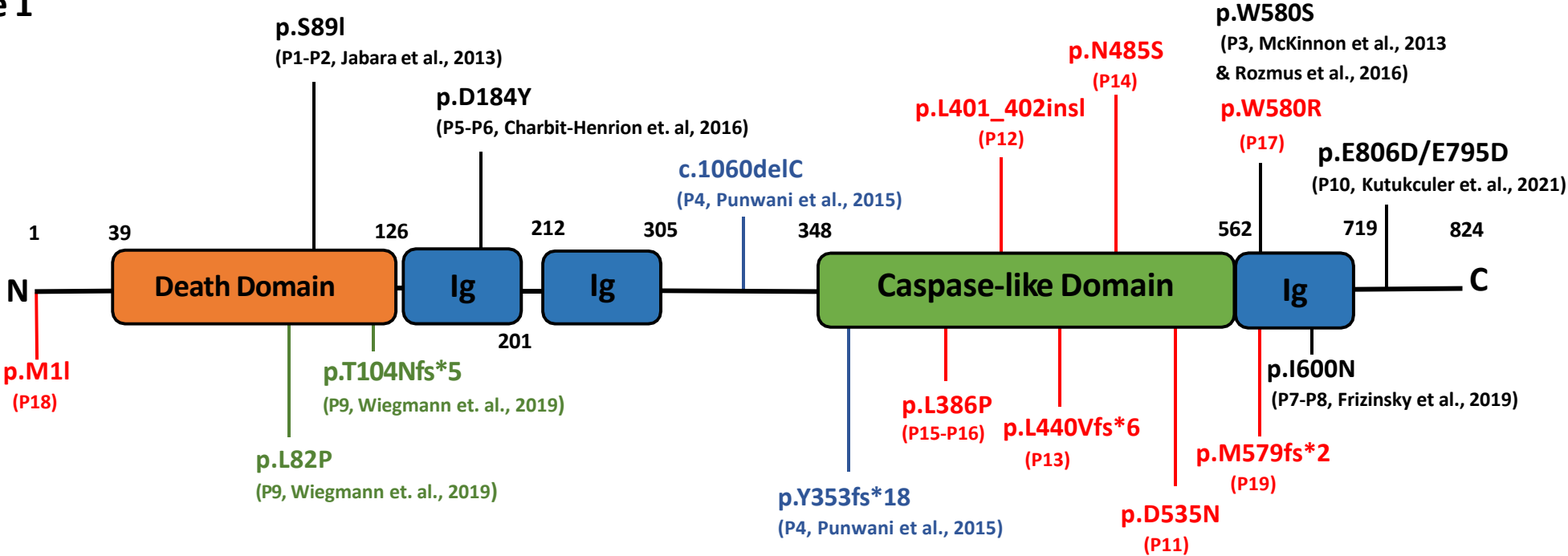
Table 1. The Demographic and clinical features of all described MALT1-deficient patients.

Table 2. The laboratory findings and outcome of all described MALT1-deficient patients.

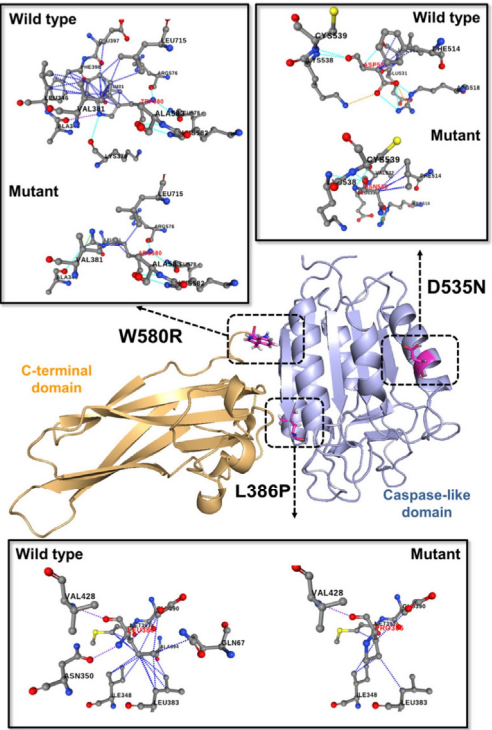
Table 3. Immunological evaluation and treatment options of the newly defined MALT1-deficient patients.

Figure 1

A



B



C

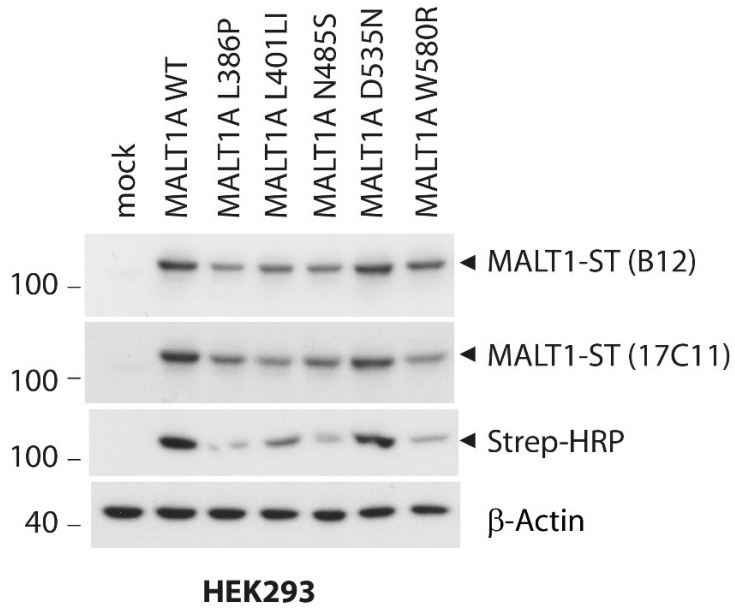
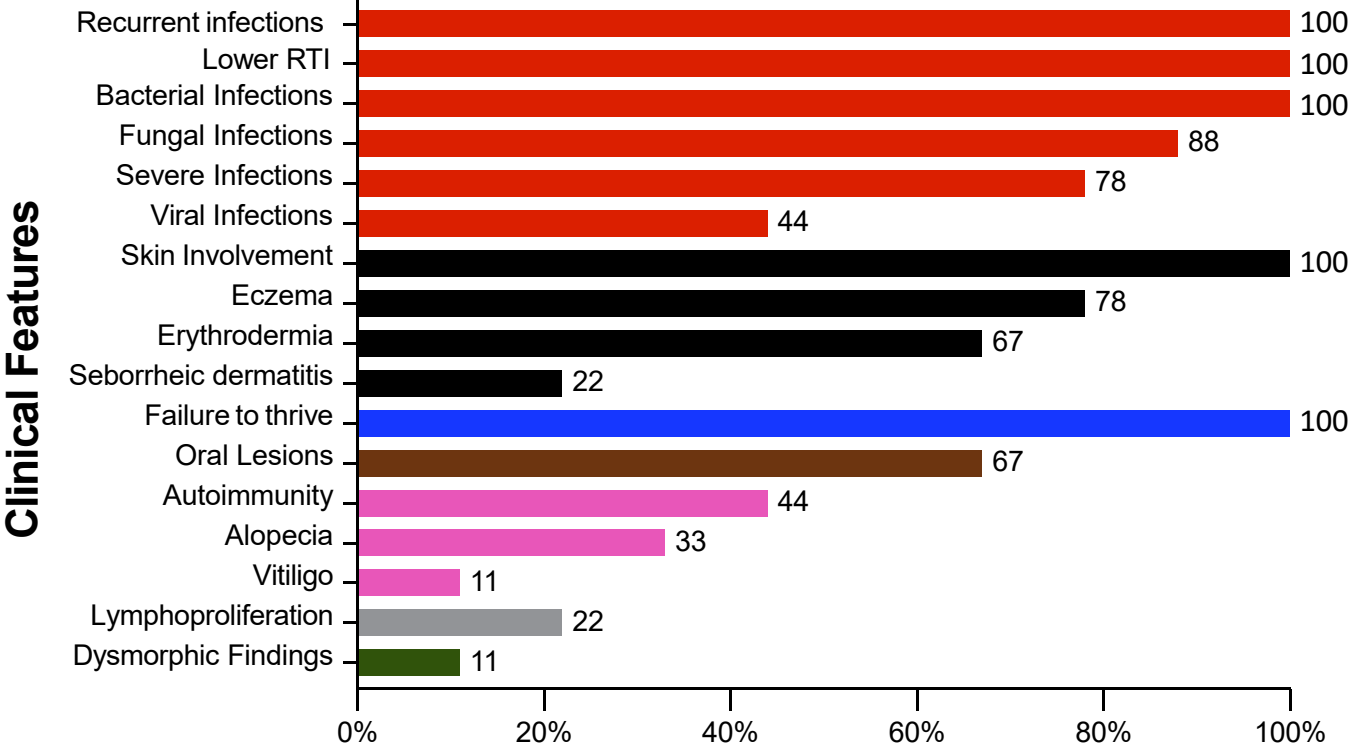


Figure 2

A



B

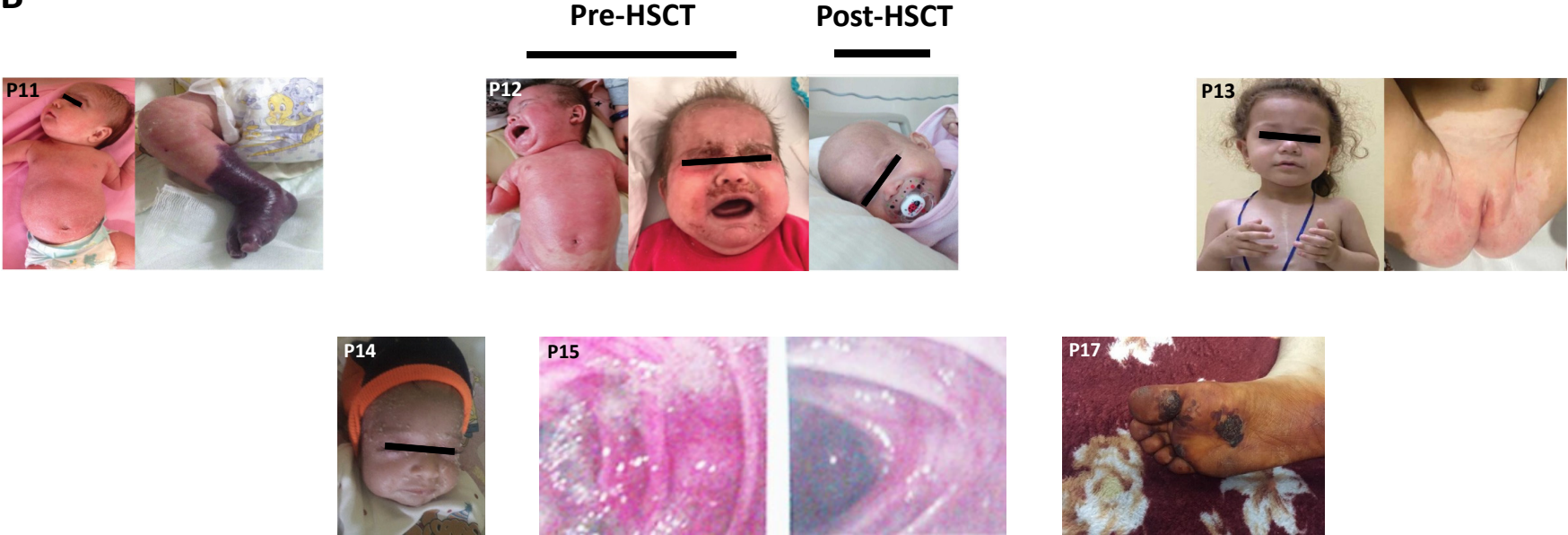
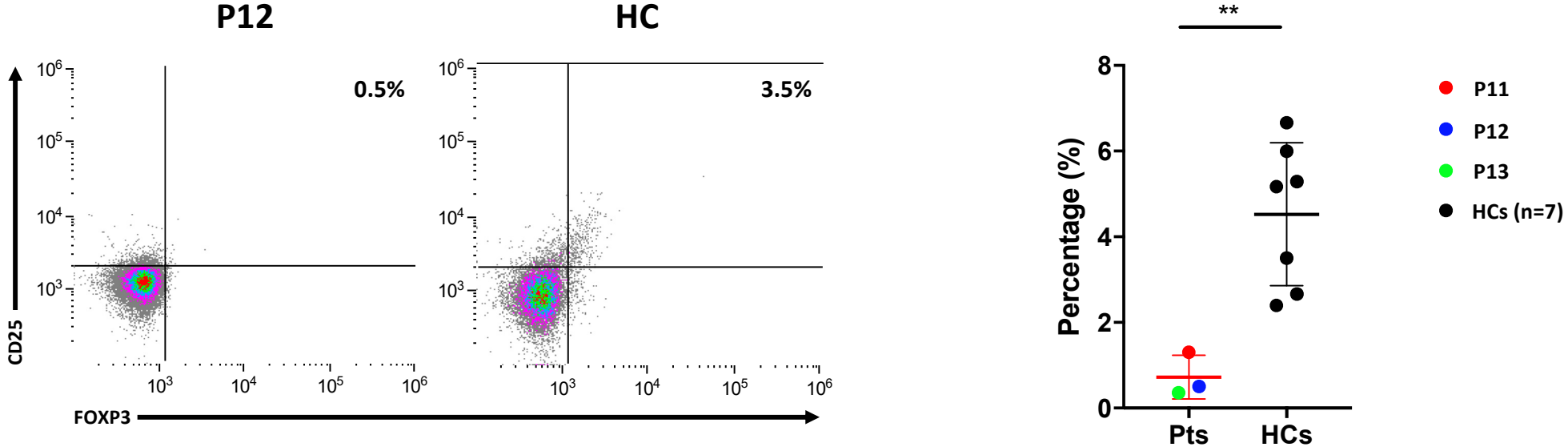


Figure 3

A



B

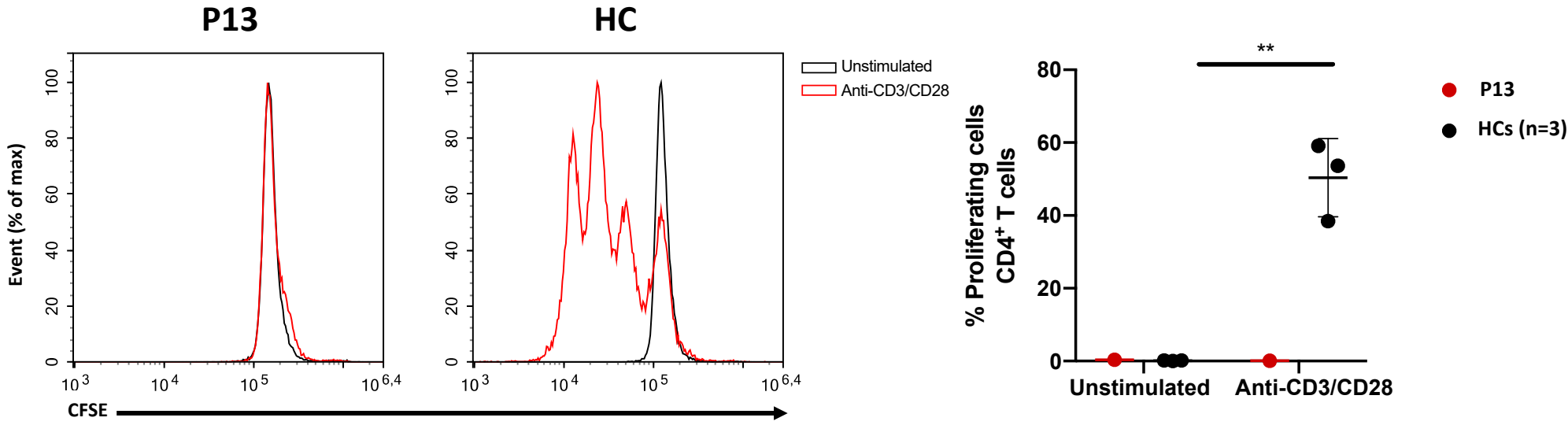
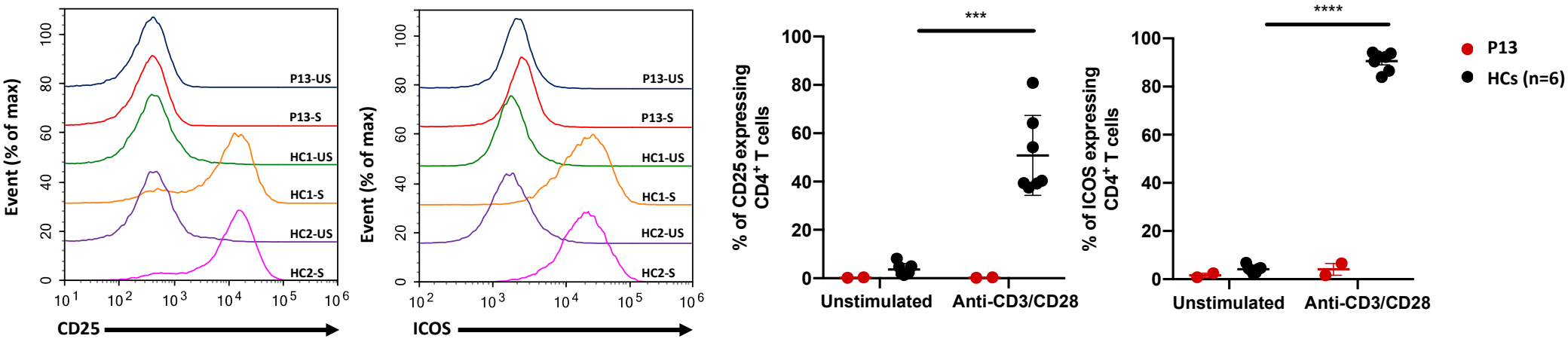


Figure 4

A



B

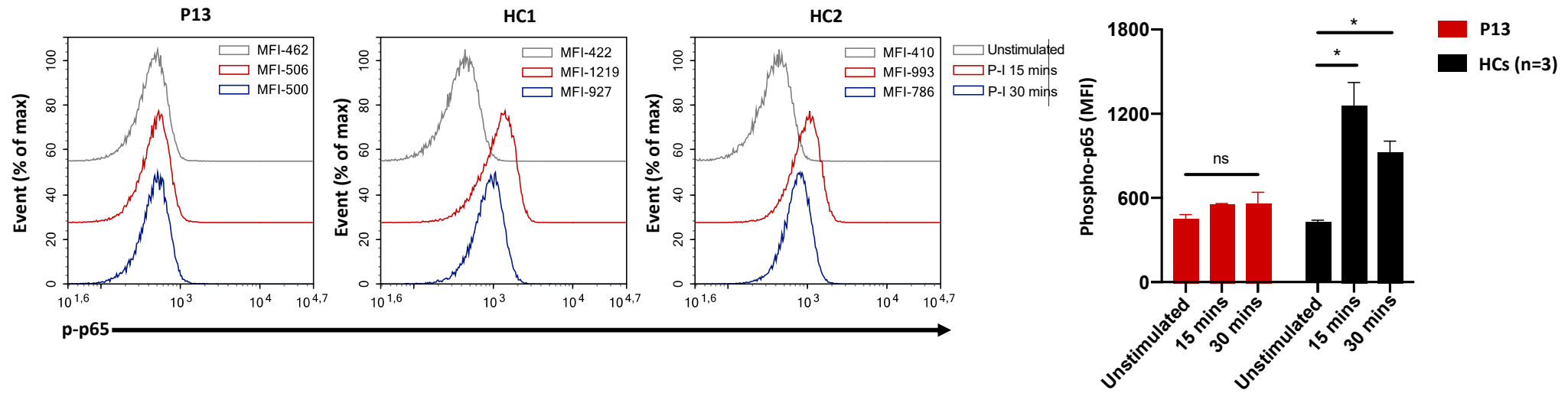


Figure 5

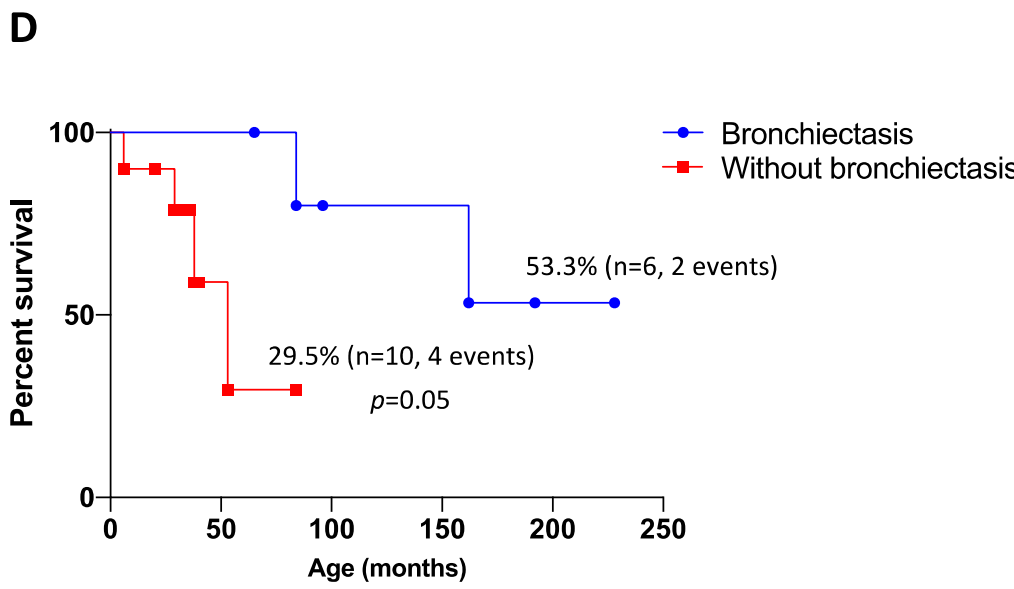
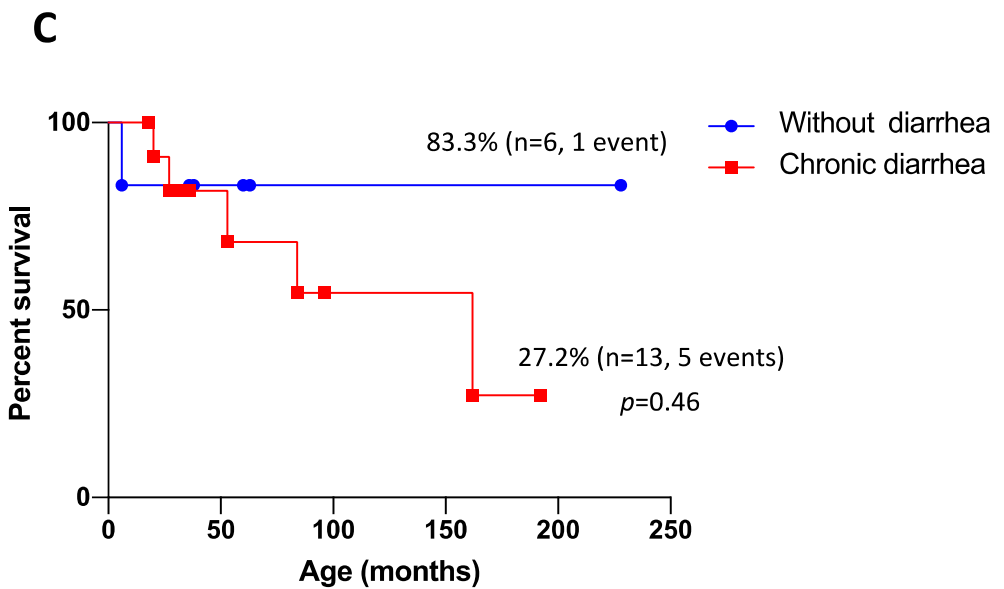
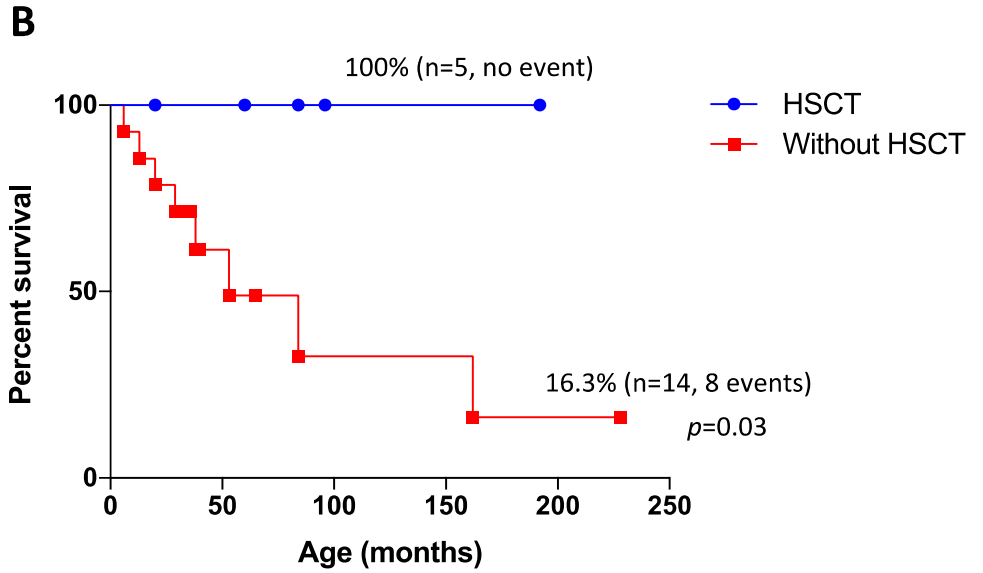
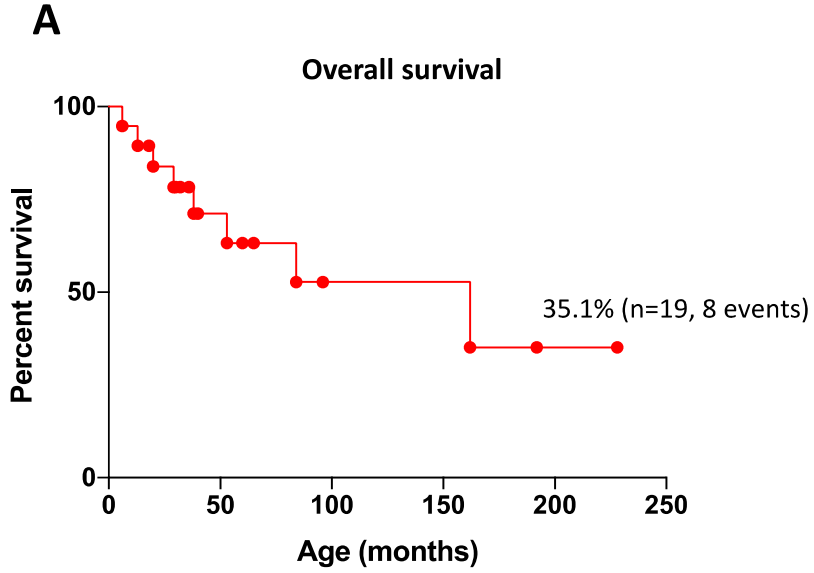


Figure 6

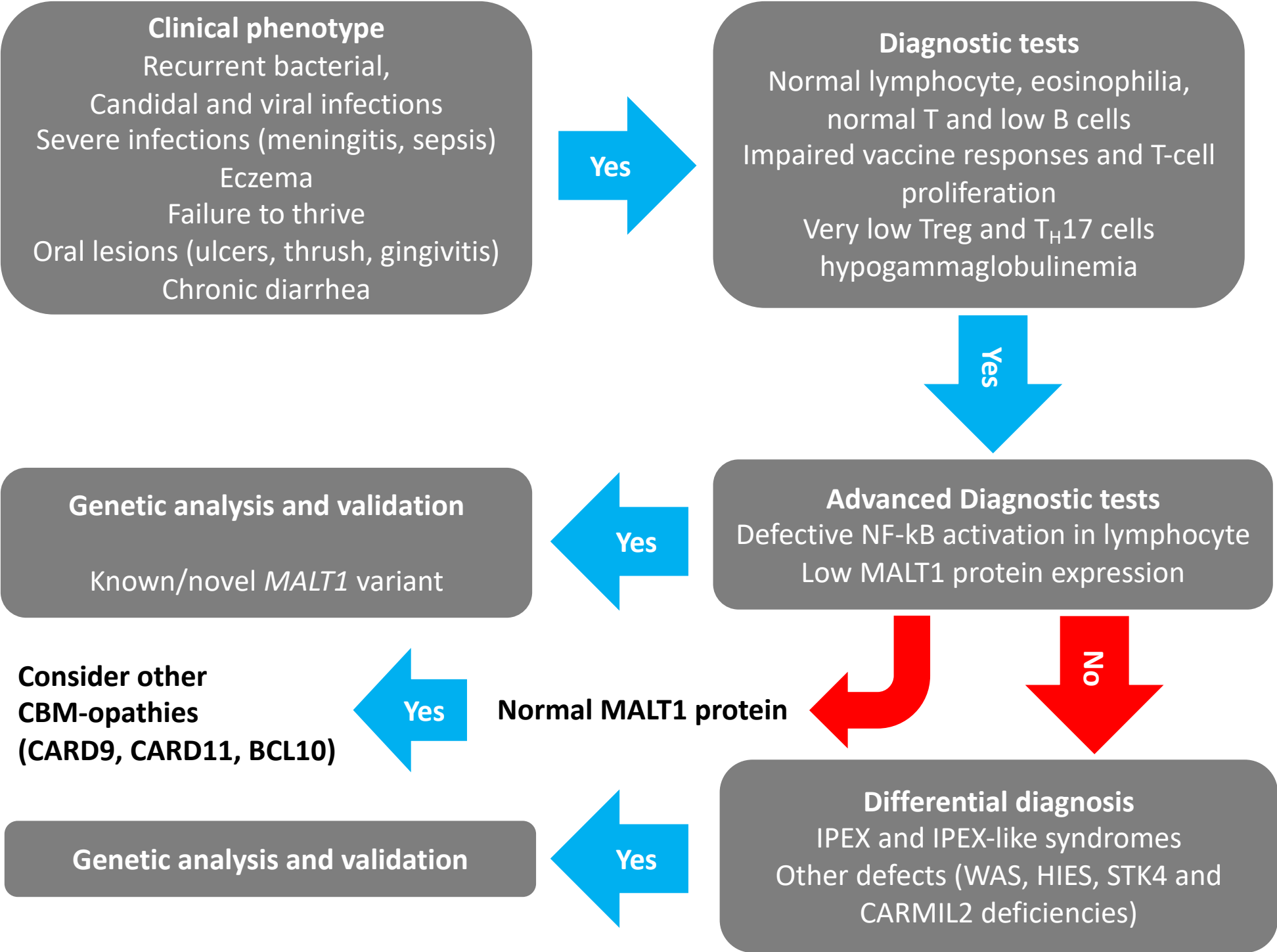
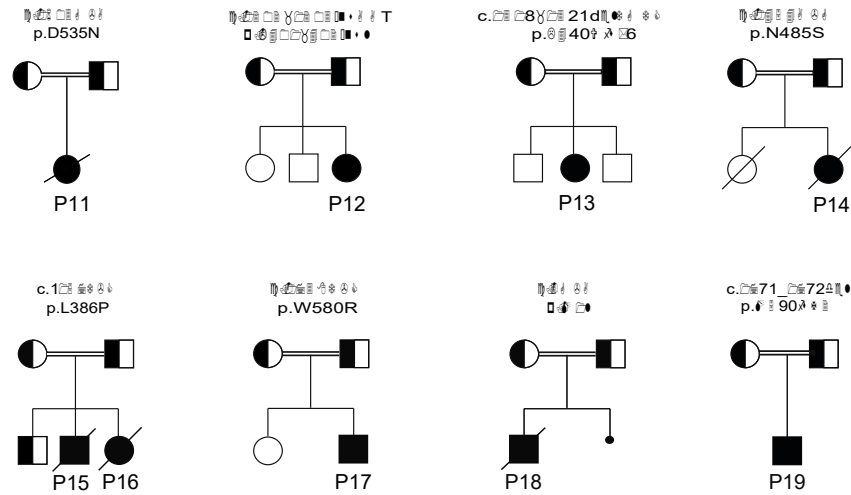
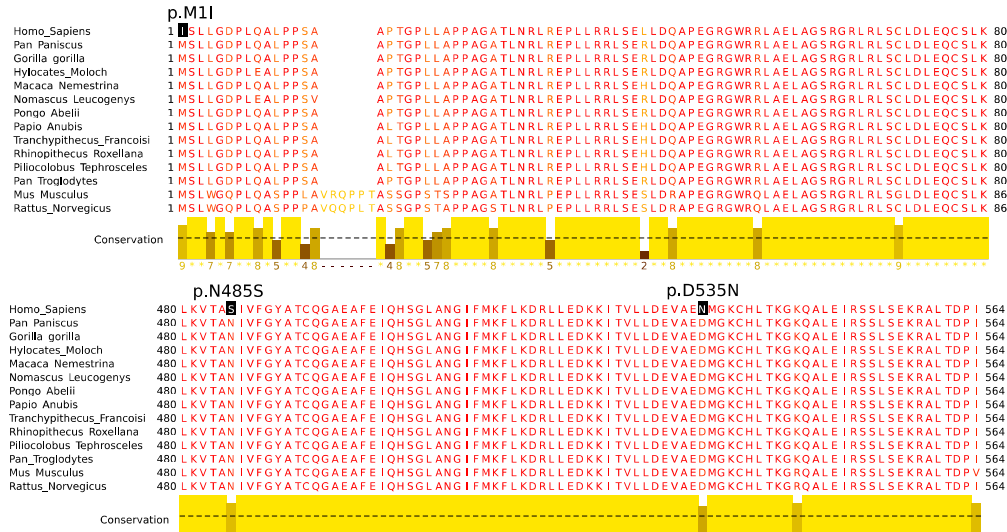


Figure S1

A



B



C

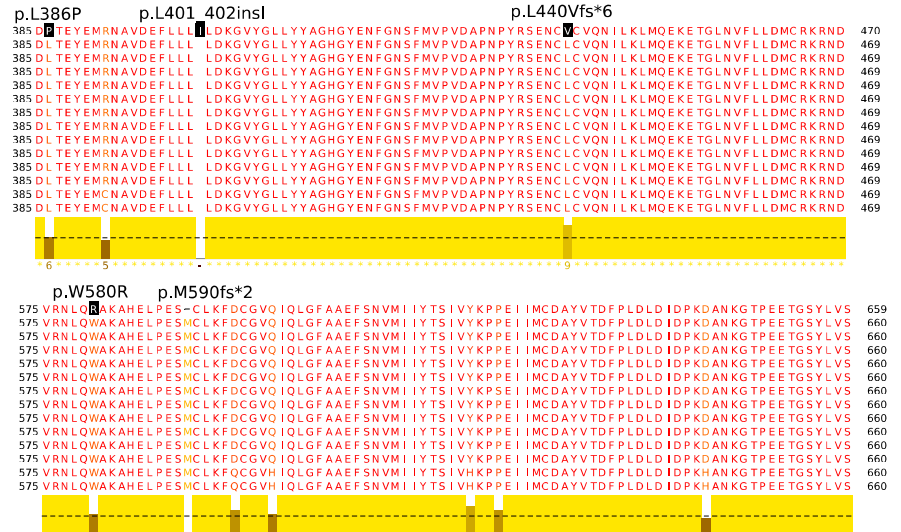
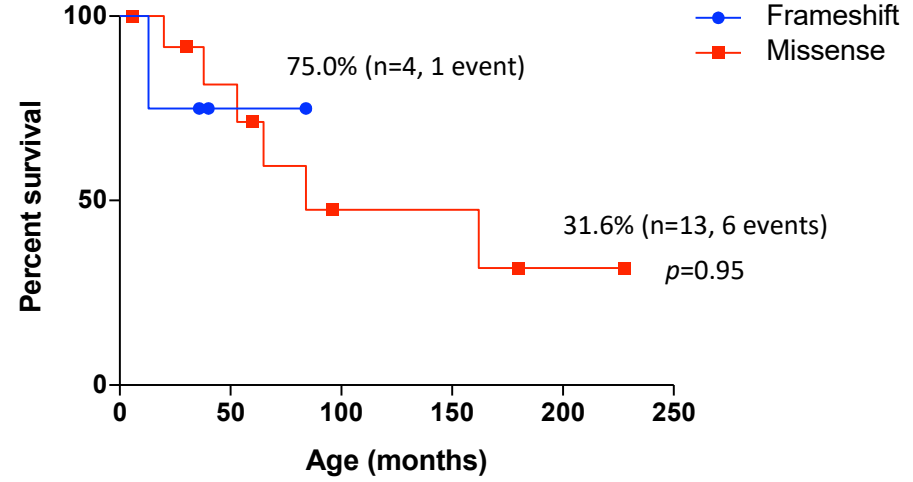


Figure S2

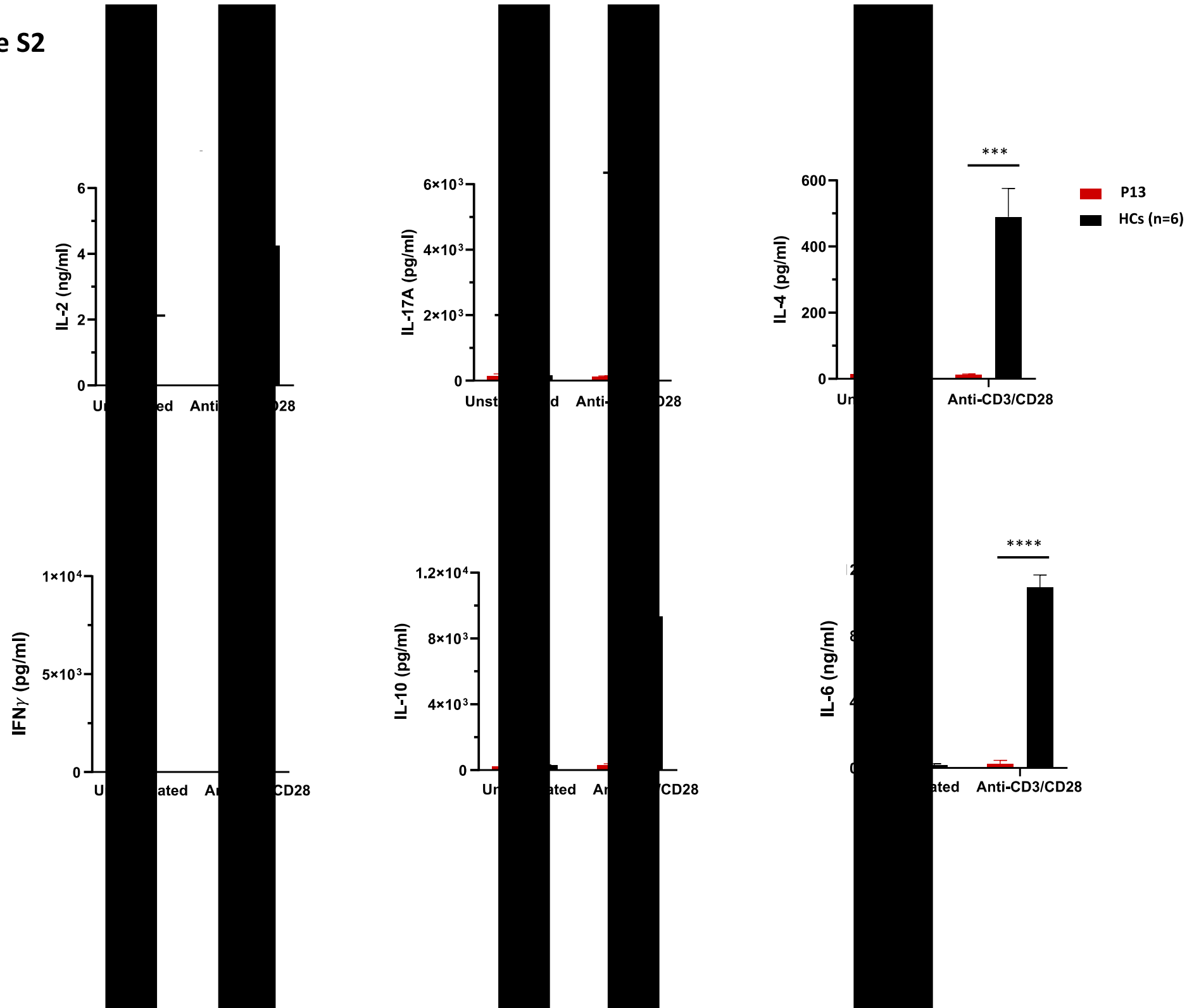


Figure S3

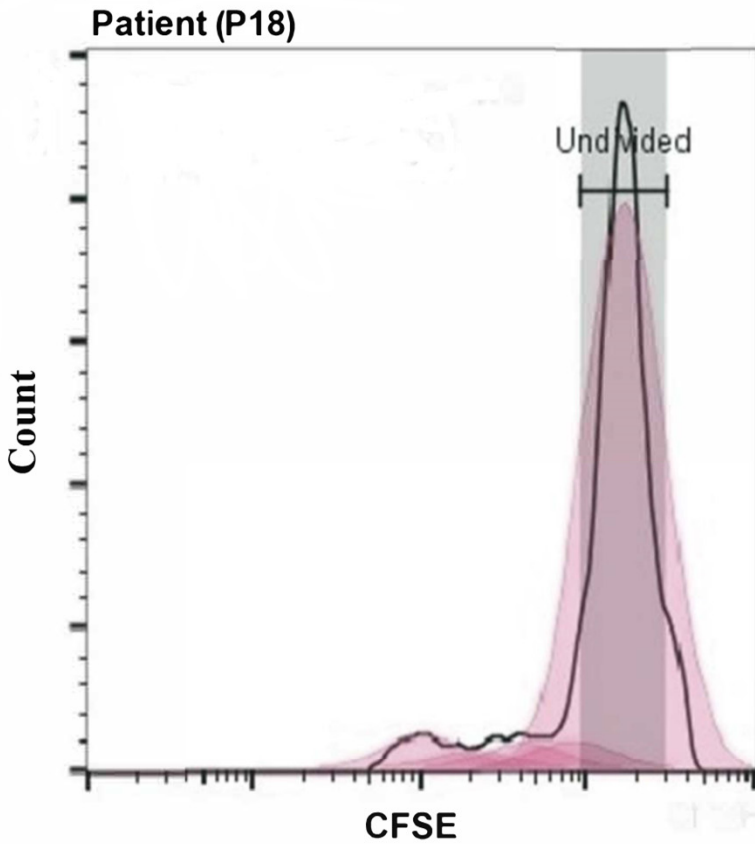
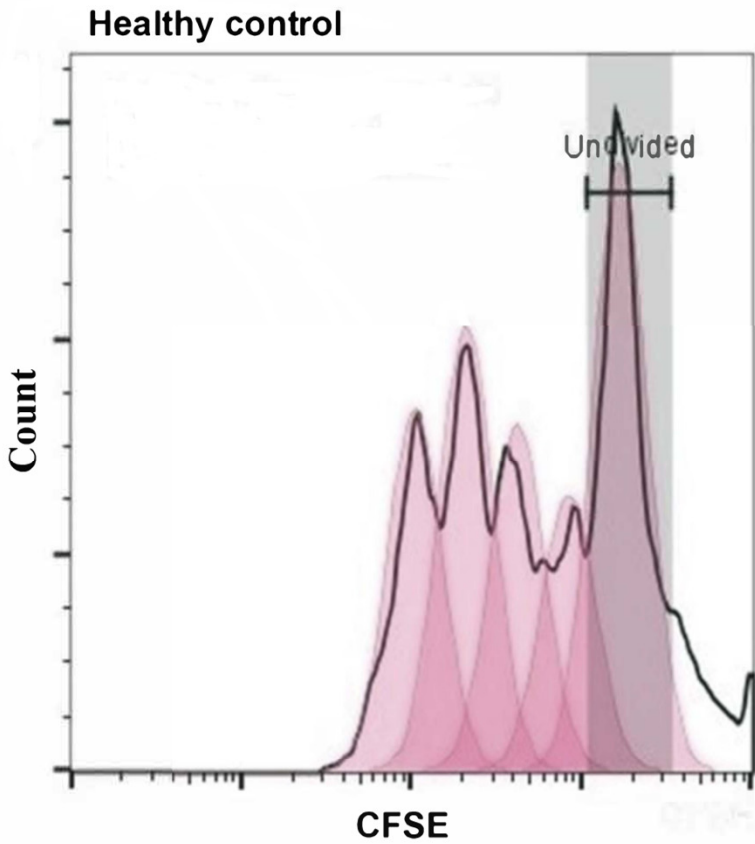
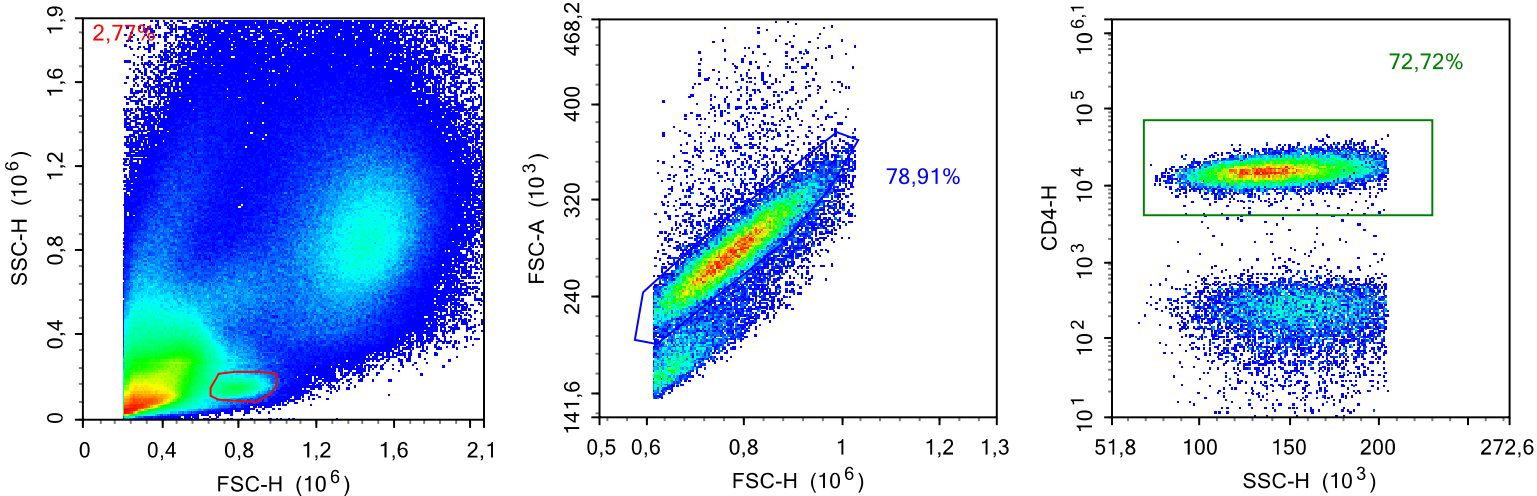
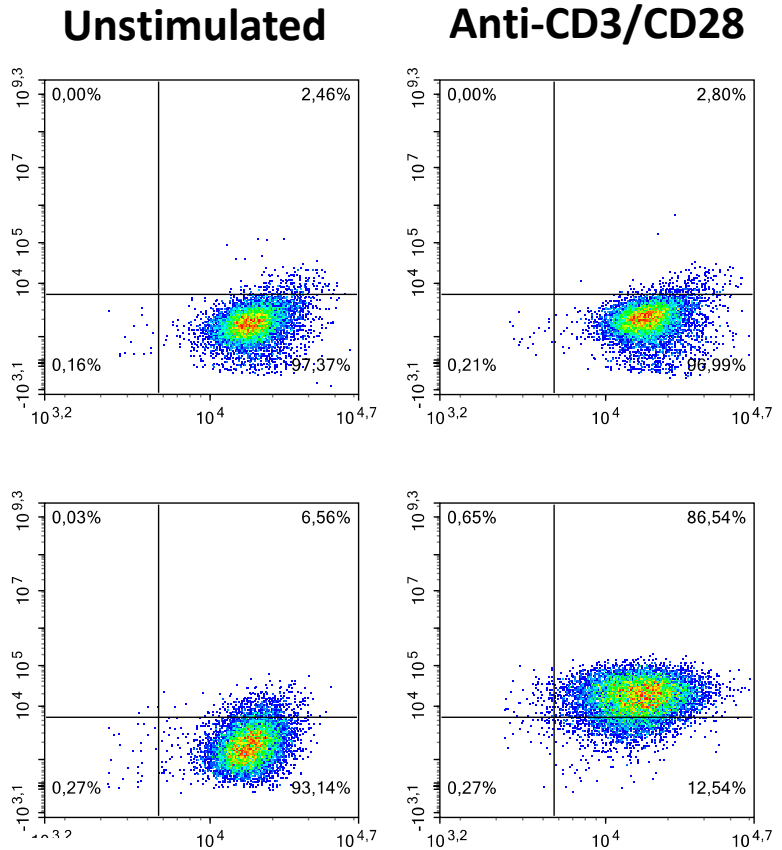


Figure S4

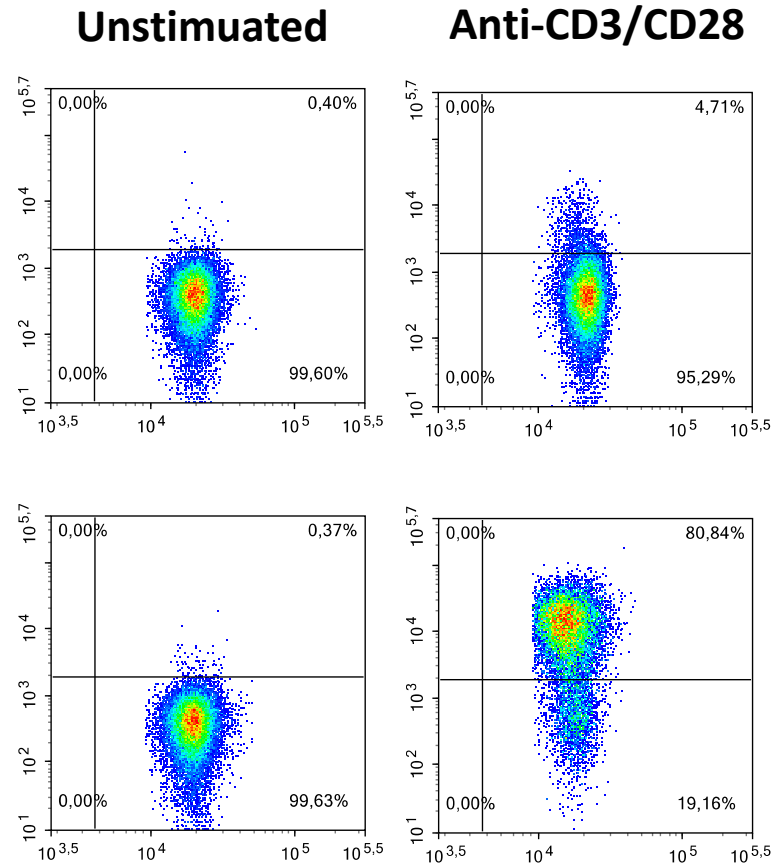


ICOS

Healthy control



CD25



CD4



Figure S5

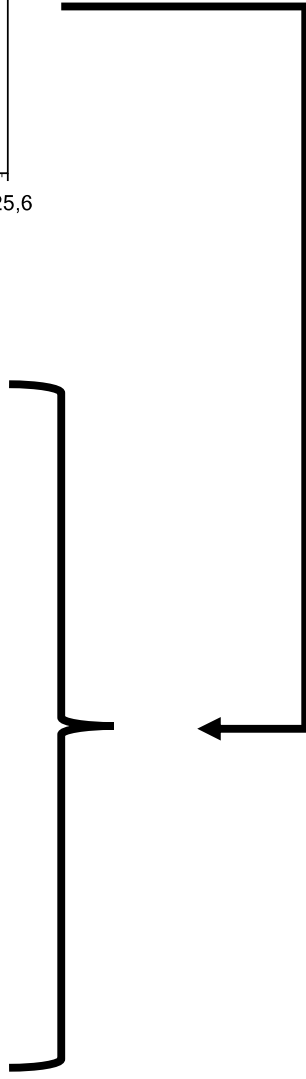
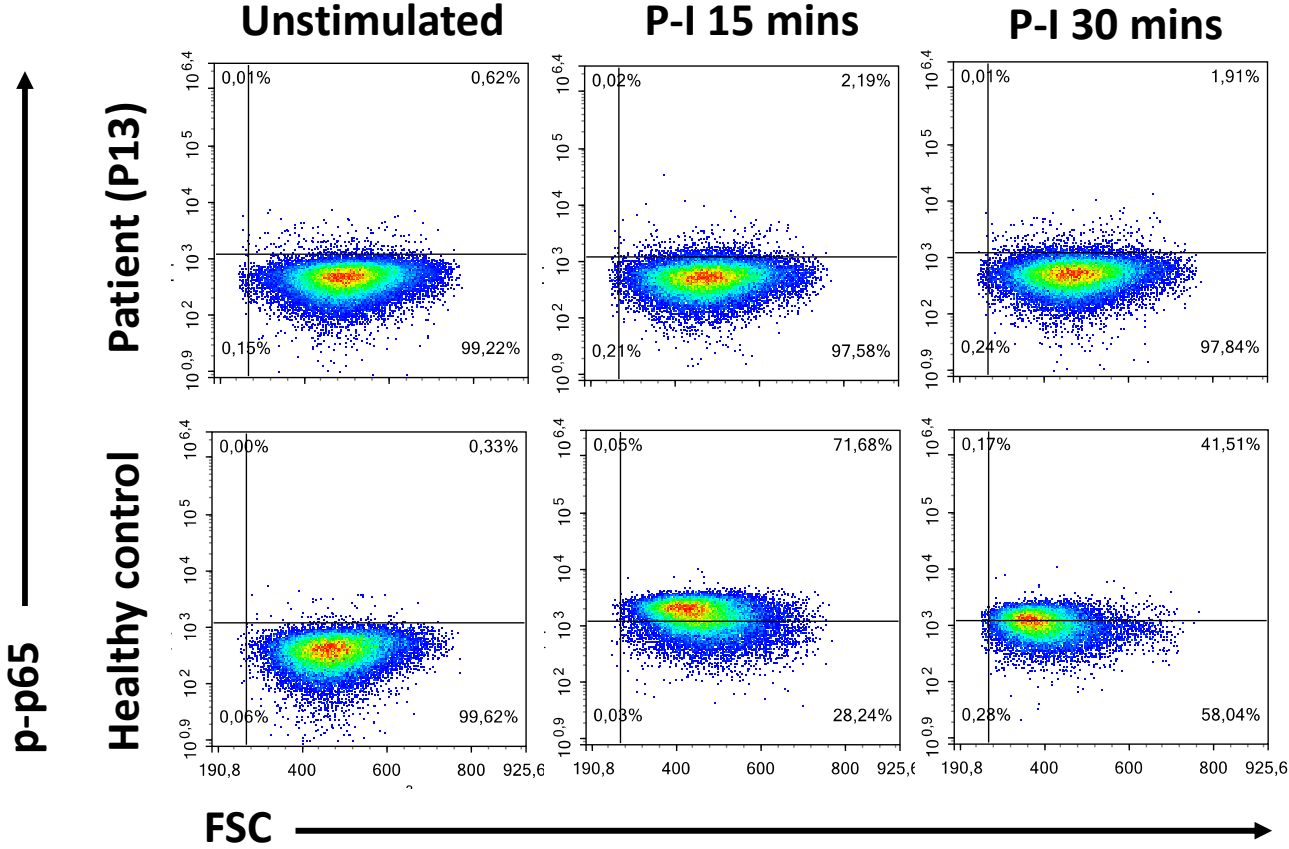
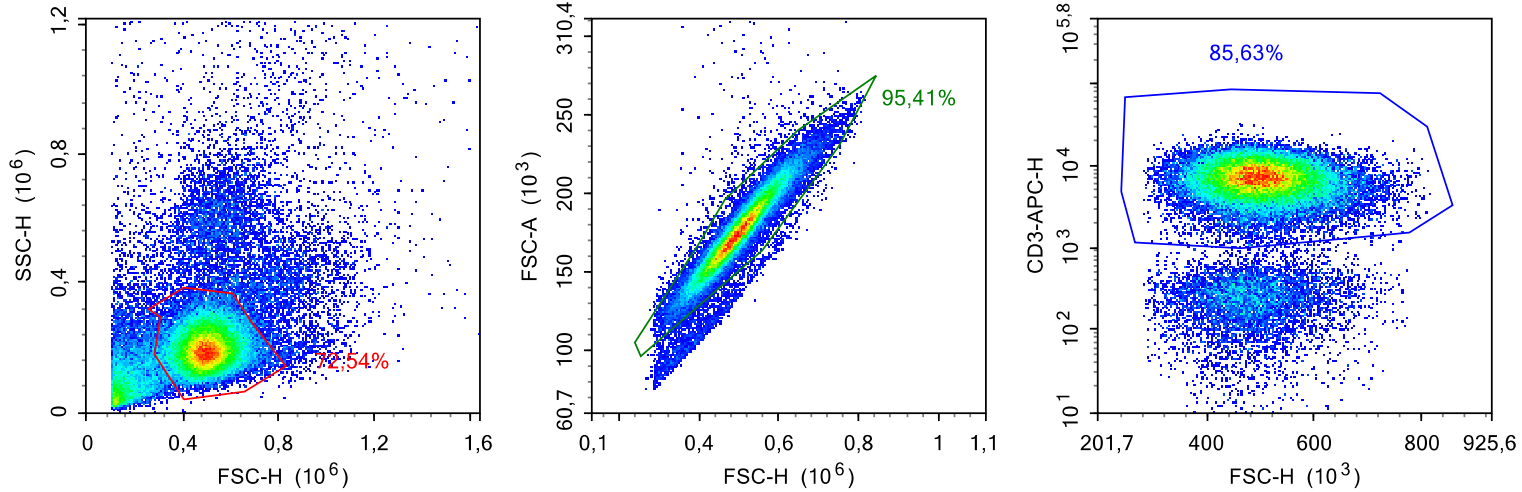
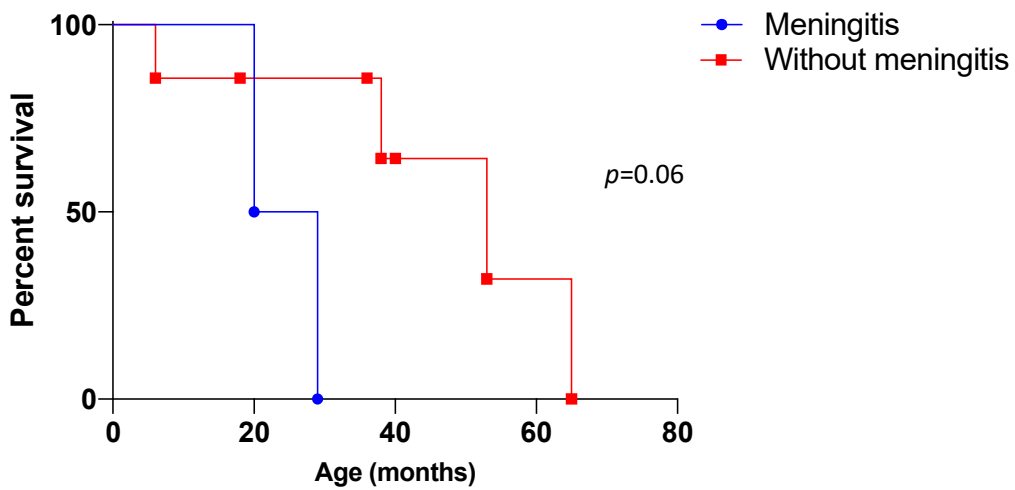


Figure S6

A



B

