

TNF receptor superfamily member 13b (*TNFRSF13B*) hemizygosity reveals transmembrane activator and CAML interactor haploinsufficiency at later stages of B-cell development

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Background: Heterozygous C104R or A181E TNF receptor superfamily member 13b (*TNFRSF13B*) mutations impair removal of autoreactive B cells, weaken B-cell activation, and convey to patients with common variable immune deficiency (CVID) an increased risk for autoimmunity. How mutant transmembrane activator and CAML interactor (TACI) influences wild-type TACI function is unclear; different models suggest either a dominant negative effect or haploinsufficiency. **Objective:** We investigated potential TACI haploinsufficiency by analyzing patients with antibody-deficient Smith-Magenis syndrome (SMS) who possess only 1 *TNFRSF13B* allele and antibody-deficient patients carrying one c.204insA *TNFRSF13B* null mutation. **Methods:** We tested the reactivity of antibodies isolated from single B cells from patients with SMS and patients with a c.204insA *TNFRSF13B* mutation and compared them with counterparts from patients with CVID with heterozygous C104R

or A181E *TNFRSF13B* missense mutations. We also assessed whether loss of a *TNFRSF13B* allele induced haploinsufficiency in naive and memory B cells and recapitulated abnormal immunologic features typical of patients with CVID with heterozygous *TNFRSF13B* missense mutations.

Results: We found that loss of a *TNFRSF13B* allele does not affect TACI expression, activation responses, or establishment of central B-cell tolerance in naive B cells. Additionally, patients with SMS and those with a c.204insA *TNFRSF13B* mutation display normal regulatory T-cell function and peripheral B-cell tolerance. The lack of a *TNFRSF13B* allele did result in decreased TACI expression on memory B cells, resulting in impaired activation and antibody secretion.

Conclusion: *TNFRSF13B* hemizygosity does not recapitulate autoimmune features of CVID-associated C104R and A181E *TNFRSF13B* mutations, which likely encode dominant negative products, but instead reveals selective TACI haploinsufficiency at later stages of B-cell development. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *TNFRSF13B*, transmembrane activator and CAML interactor, B-cell tolerance, common variable immune deficiency, Smith-Magenis syndrome

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Common variable immune deficiency (CVID) is a heterogeneous group of primary antibody deficiency disorders.¹ Approximately 20% of all cases of CVID are further complicated by autoimmune manifestations, a rate that is further increased (36% to 55%) in patients carrying a mutation in the CVID risk gene TNF receptor superfamily member 13b (*TNFRSF13B*).²⁻⁴ *TNFRSF13B* encodes transmembrane activator and CAML interactor (TACI), a trimeric transmembrane receptor that plays an essential role during counterselection of early B cells expressing self-reactive B-cell receptors (BCRs) in bone marrow.⁵ At later stages of B-cell development, TACI can support class-switch recombination, plasma cell differentiation, and antibody secretion.⁶⁻⁹ The extracellular domain of TACI binds 2 ligands: a proliferation-inducing ligand (APRIL) and B-cell activation factor (BAFF).¹⁰ Intracellular TACI domains interact with several signaling molecules, including myeloid differentiation primary response gene-88, as well as activated endosomal Toll-like receptor (TLR) 7 and TLR9.^{5,11} Ninety percent of all CVID-associated *TNFRSF13B* mutations consist of either a C104R mutation, which alters ligand binding, or the A181E mutation, which affects transmembrane function.¹²⁻¹⁵ The mechanism by which C104R

Abbreviations used

ANA:	Anti-nuclear antibody
APC:	Allophycocyanin
APRIL:	A proliferation-inducing ligand
BAFF:	B-cell activation factor
BCR:	B-cell receptor
CFSE:	Carboxyfluorescein succinimidyl ester
CVID:	Common variable immune deficiency
Foxp3:	Forkhead box protein 3
PE:	Phycoerythrin
PerCP:	Peridinin-chlorophyll-protein complex
SMS:	Smith-Magenis syndrome
TACI:	Transmembrane activator and CAML interactor
T _{FH} :	T follicular helper
TLR:	Toll-like receptor
TNFRSF13B:	TNF receptor superfamily member 13b
Treg:	Regulatory T
Tresp:	Responder T

or A181E mutated TACI molecules exert their influence over wild-type TACI is unclear. Evidence generated from one transgenic mouse model suggests a role for haploinsufficiency,¹² whereas another mouse model and experiments with transfected cell lines indicate that mutant proteins can function as dominant negative products.^{13,14}

We investigated TACI haploinsufficiency in human subjects by analyzing several conditions that reflect hemizygoty (ie, lack of an allele at the *TNFRSF13B* locus). Patients with CVID with one 204insA frameshift *TNFRSF13B* mutation have been reported; this functionally null allele yields a severely truncated gene product that lacks ligand binding and transmembrane and intracellular signaling domains.^{2,16}

Smith-Magenis syndrome (SMS) is a complex neurodevelopmental disorder that results from a heterozygous 3.5-Mb deletion of chromosome 17p11.2, a region encompassing the entire *TNFRSF13B* locus.¹⁷ Although the most overt neurological aspects of this syndrome stem from heterozygous loss of a nonimmunologic gene or genes, patients with SMS routinely experience chronic otitis and vaccine failure, suggesting an underlying humoral immune deficiency.^{17,18}

Herein we report that *TNFRSF13B* hemizygoty in patients with SMS and patients with a 204insA frameshift *TNFRSF13B* mutation does not result in defective naive B-cell activation or antibody repertoire selection, which are associated with the C104R and A181E *TNFRSF13B* mutations. This suggests that these mutated *TNFRSF13B* alleles do not encode functionally inert products but rather dominant negative molecules favoring the development of autoimmunity.^{2,5} The loss of one *TNFRSF13B* allele reveals TACI haploinsufficiency in later stages of B-cell development, when its expression should normally be upregulated; the failure to enhance TACI expression in memory B cells of patients with SMS and patients with a 204insA frameshift *TNFRSF13B* mutation correlates with activation defects and clinical antibody deficiency.

METHODS**Patients**

Patients with SMS with a documented 17p11.2 deletion were recruited for the study (Table I). Healthy donors with and without *TNFRSF13B* mutations,

patients with CVID with a C104R or A181E *TNFRSF13B* mutation, and antibody-deficient patients with a c.204insA *TNFRSF13B* mutation were previously described.^{5,16} All participants provided informed consent before participation in this study. All aspects of the study were approved by the Yale University School of Medicine Human Investigation Committee (New Haven, Conn).

Cell staining and sorting, cDNA, RT-PCR, antibody production, ELISAs, and indirect fluorescent assays

Single CD19⁺CD21^{lo}CD10⁺IgM^{hi}CD27[−] new emigrant/transitional and CD19⁺CD21⁺CD10[−]IgM⁺CD27[−] mature naive B cells from patients and healthy donors were sorted on a FACSaria flow cytometer (Becton Dickinson, Mountain View, Calif) into 96-well PCR plates, and antibody reactivity was tested, as previously described.¹⁹ For indirect immunofluorescence assays, HEP-2 cell-coated slides (Bion Enterprises, Des Plaines, Ill) were incubated in a moist chamber at room temperature with recombinant IgG antibodies at a standardized concentration of 100 µg/mL or patient plasma samples at 1:80 and 1:320 dilutions in PBS. Fluorescein isothiocyanate-conjugated goat anti-human IgG was used as detection reagent for fluorescent microscopy. Serum BAFF concentrations were determined by means of ELISA, according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn).

B-cell activation

B cells were enriched from the blood of research subjects either by means of positive selection with CD20 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) or negative selection with the Naïve B Cell Isolation Kit II (Miltenyi Biotec) and plated at 150,000 to 200,000 cells per well in a 96 well-plate in RPMI 10% FBS and either 2.5 µg/mL polyclonal F(ab)₂ rabbit anti-human IgM or BCR (Jackson ImmunoResearch, West Grove, Pa), 1.0 µg/mL Gardiquimod (TLR7 agonist; InvivoGen, San Diego, Calif), or 0.5 µg/mL CpG (TLR9 agonist; Invitrogen, San Diego, Calif) for 48 hours. Induction of surface CD86 and CD69 was detected on gated CD19⁺CD21⁺CD27[−] naive or CD19⁺CD21⁺CD27⁺ memory B-cell populations with an LSRII flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and analyzed with FlowJo software (TreeStar, Ashland, Ore).

B-cell proliferation and antibody secretion

B cells were enriched from peripheral blood of research subjects by means of negative selection with the EasySep Human B cell Enrichment Kit (STEMCELL Technologies, Vancouver, British Columbia, Canada) and then further separated into CD27⁺ memory and CD27[−] naive B-cell fractions by using an allophycocyanin (APC)-conjugated anti-CD27 IgG followed by anti-APC-conjugated magnetic beads (Miltenyi Biotec). Each fraction was labeled with CellTrace CFSE (Life Technologies, Grand Island, NY) at 0.05 µmol/L and cultured at 100,000 cells per well in a 384-well plate in RPMI 10% FBS and 500 ng/mL APRIL (R&D Systems) with and without 2.5 µg/mL polyclonal F(ab)₂ rabbit anti-human BCR (Jackson ImmunoResearch) and 0.5 µg/mL CpG (Invitrogen). After 6 days in culture, cells were stained with the LIVE/DEAD kit (Life Technologies) to measure viability. B-cell proliferation was analyzed by using carboxyfluorescein succinimidyl ester (CFSE) dilution with flow cytometry. IgM concentrations in culture supernatants were measured by means of ELISA.

In vitro regulatory T-cell suppression assay

CD4⁺ T cells were enriched with the EasySep Human CD4⁺ T Cell Enrichment Kit (STEMCELL Technologies). CD4⁺CD25^{hi}CD127^{lo/−} regulatory T (Treg) cells were sorted by means of flow cytometry, whereas CD3⁺CD4⁺CD25[−] responder T (Tresp) cells were obtained after depletion of CD25⁺ cells with anti-human CD25 microbeads (Miltenyi Biotec) and then labeled with CFSE at 5 µmol/L. Treg and Tresp cells were cocultured at a 1:1 ratio in the presence of beads loaded with anti-CD2, anti-CD3, and anti-CD28 (Treg Suppression Inspector, human; Miltenyi Biotec). At 3.5 to

TABLE I. Clinical characteristics of research subjects

Subject ID	Age (y)	Sex	<i>TNFRSF13B</i> mutation	Diagnosis	Laboratory abnormality	Recurrent infections	IVIG/SQIG	Autoimmune diseases
SMSY1	7	F	Del	SMS	Hypogammaglobulinemia	PNA, OM, S	Yes	—
SMSY2	6	F	Del	SMS	Vaccine failure	PNA, OM	No	—
SMSY3	12	F	Del	SMS	IgA def, vaccine failure	PNA, OM	No	—
SMSY4	26	F	Del	SMS	IgG ₂ def, vaccine failure	Warts	No	—
SMSY5	20	F	Del	SMS	Vaccine failure	OM, GI	No	—
SMSY6	9	M	Del	SMS	Hypogammaglobulinemia	OM	No	—
SMSY7	20	M	Del	SMS	IgA def and IgM def	OM, PNA	No	—
C12B*	17	F	204insA	CVID	Hypogammaglobulinemia	AOM, S	Yes	—
C12C*	13	M	204insA	CVID	Hypogammaglobulinemia	OM, PNA, S	Yes	—
HD G.II†	38	F	A181E	Healthy	ND	—	No	—
HD E.V†	80	F	C104R	Healthy	ND	—	No	—
HD D.II†	82	M	C104R	Healthy	ND	—	No	—
HD E.II†	79	M	C104R	Healthy	ND	—	No	—
HD D.III†	14	M	C104R	Healthy	ND	—	No	—
M.I.1†	78	M	C104R	Healthy	ND	—	No	—
M.I.2†	78	F	C104R	Healthy	ND	—	No	—
CVID321†	27	F	A181E	CVID	Hypogammaglobulinemia	GI, PNA	Yes	ITP, arthritis, uveitis
CVIDY15†	40	M	C104R	CVID	Hypogammaglobulinemia	PNA, S	Yes	—
CVIDY17†	48	F	C104R	CVID	Hypogammaglobulinemia	PNA, S	Yes	AH
CVID B.I†	19	F	A181E	CVID	Hypogammaglobulinemia	S	Yes	—
CVID292†	45	F	C104R	CVID	Hypogammaglobulinemia	—	Yes	ITP
CVID G.I†	11	F	A181E	CVID	Hypogammaglobulinemia	—	Yes	ITP, AIHA
CVID376†	45	M	C104R	CVID	Hypogammaglobulinemia	S	Yes	ITP
C12A*	12	M	C104R	CVID	Hypogammaglobulinemia	OM, S	Yes	—

AH, Autoimmune hepatitis; AIHA, autoimmune hemolytic anemia; AOM, acute otitis media; Def, deficient; F, female; GI, gastrointestinal infection; ITP, idiopathic thrombocytopenia; M, male; ND, not done; OM, otitis media; PNA, pneumonia; S, sinusitis.

*As described in Castigli et al.¹⁶

†As described in Romberg et al.⁵

4.5 days, cocultures were stained for viability with the LIVE/DEAD kit, and proliferation of the viable Tresp cells was analyzed by means of CFSE dilution.

Flow cytometry

The following antibodies were used for flow cytometric staining: anti-TACI phycoerythrin (PE; clone 1A1), anti-CD19 APC-Cy7, anti-CD27 peridinin-chlorophyll-protein complex (PerCP)—Cy5.5 or APC, anti-CD10 PE-Cy7, anti-IgM fluorescein isothiocyanate, anti-CD21 APC, anti-CD69 PE-Cy7, anti-CD86 PE, anti-CD4 APC-Cy7, anti-CD25 PE-Cy7, anti-CD127 PerCP-Cy5.5, anti-CD45RO Pacific Blue, anti-CXCR5 PerCP-Cy5.5, anti-PD-1 PE-Cy7, anti-CD25 PE, anti-CD25 PE-Cy7 (all from BioLegend, San Diego, Calif), anti-CD3 eFluor 605NC, anti-CD21 BD Horizon V450 (Becton Dickinson), and goat polyclonal anti-TACI biotin (R&D Systems). Intracellular staining with anti-forkhead box protein 3 (Foxp3) Alexa Fluor 488 and anti-B-cell lymphoma protein 6 PE (eBioscience, San Diego, Calif) was performed with the Foxp3/Transcription Factor Staining Buffer Set (eBioscience) in accordance with the manufacturer's instructions.

Statistics

Differences between patients and healthy donors were analyzed for statistical significance with unpaired Student *t* tests or χ^2 tests by using GraphPad Prism software (GraphPad Software, La Jolla, Calif). *P* values of less than .05 were considered statistically significant.

RESULTS

A single *TNFRSF13B* allele is sufficient to establish central B-cell tolerance

We previously reported that *TNFRSF13B* mutations interfere with establishment of central B-cell tolerance, a checkpoint in

the bone marrow normally responsible for removal of most polyreactive and anti-nuclear clones before they enter the new emigrant/transitional B-cell compartment.^{5,19} To determine whether *TNFRSF13B* hemizyosity can affect central B-cell tolerance, we tested the reactivity of recombinant antibodies cloned from single CD19⁺CD10⁺CD21^{lo}IgM^{hi}CD27[−] new emigrant/transitional B cells from 3 patients with SMS and 2 patients with a heterozygous 204insA *TNFRSF13B* mutations, both of whom met the clinical criteria for diagnosis of CVID (Table I and see Tables E1-E10 in this article's Online Repository at www.jacionline.org).²⁰ For comparison, we represented previously reported reactivity data for recombinant antibodies cloned from new emigrant/transitional B cells from 11 healthy donors without *TNFRSF13B* mutations and 8 subjects heterozygous for the C104R or A181E *TNFRSF13B* missense mutations; 4 were patients with CVID, and 4 were immunocompetent carriers of a mutated *TNFRSF13B* allele.⁵

We found that new emigrant/transitional B cells from patients with SMS and patients with a 204insA *TNFRSF13B* mutation expressed low frequencies of polyreactive antibodies, which represented 6.6% to 9.1% and 7.4% to 9.5% of the clones, respectively, and were similar to those of healthy donors without *TNFRSF13B* mutations (5% to 11.5%, Fig 1). New emigrant/transitional B cells from patients with SMS and patients with a 204insA *TNFRSF13B* mutation were also normally purged from anti-nuclear clones because none were detected in B cells from these patients (see Fig E1 in this article's Online Repository at www.jacionline.org). In contrast, subjects harboring one C104R or a A181E *TNFRSF13B* mutation displayed a defective central B-cell tolerance checkpoint characterized by increased frequencies of new emigrant/transitional B cells expressing

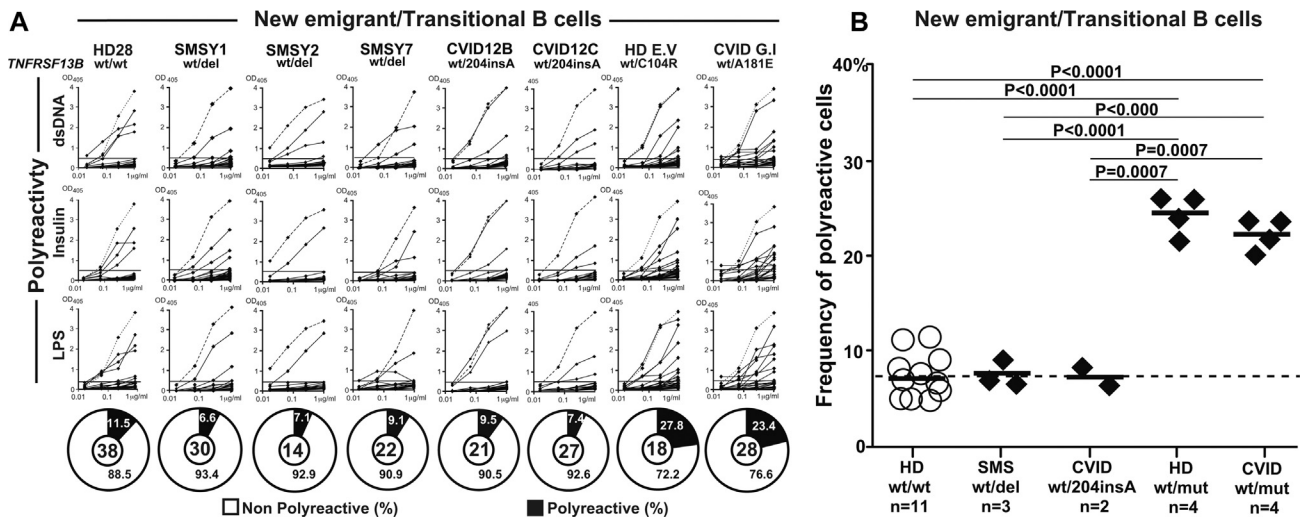


FIG 1. Central B-cell tolerance is functional in patients with SMS and patients with a 204insA *TNFRSF13B* mutation. **A**, Recombinant antibodies cloned from new emigrant/transitional B cells were tested by means of ELISA for reactivity against double-stranded DNA (dsDNA), insulin, and LPS.¹⁹ Antibodies were considered polyreactive if reacting against all 3 antigens. Dashed lines show the ED38 antibody-positive control, and solid lines show binding for each cloned recombinant antibody. Horizontal solid lines define the cutoff OD₄₀₅ for positive reactivity. For each patient, the frequency of polyreactive and nonpolyreactive clones is summarized in pie charts, with the total number of antibodies tested indicated in the center. **B**, The frequency (black bars) of polyreactive new emigrant/transitional B cells is increased in all patients carrying a C104R or A181E *TNFRSF13B* mutation (*TNFRSF13B* wt/mut) but not in patients with SMS or patients with a 204insA *TNFRSF13B* mutation. Statistical significance determined by using the unpaired Student *t* test is indicated. HD, Healthy donors.

polyreactive and anti-nuclear antibodies (ANAs; Fig 1 and see Fig E1). Hence *TNFRSF13B* hemizyosity does not affect the establishment of central B-cell tolerance in human subjects.

TNFRSF13B hemizyosity decreases TACI expression and activation responses in memory but not naive B cells

Impaired central counterselection of polyreactive and anti-nuclear B cells has been associated with defects in BCR- and TLR-mediated B-cell activation.^{5,21-23} In agreement with this hypothesis, we previously reported that C104R or A181E *TNFRSF13B* mutations altered induction of the B-cell activation markers CD86 and CD69 on naive B cells after stimulation with F(ab')₂ anti-IgM, TLR7, or TLR9 agonists.⁵ In contrast, we found that naive B cells from patients with SMS or patients carrying a 204insA *TNFRSF13B* mutation were able to normally upregulate CD86 after stimulation through the BCR, TLR7 (Gardiquimod stimulation), or TLR9 (CpG stimulation; Fig 2, A). Other activation markers, including CD69, were poorly induced on naive B cells from patients with CVID after stimulation with BCR, or TLR agonists were also normally upregulated in B cells with only one productive *TNFRSF13B* allele (Fig 2, B). We also measured induction of CD86 and CD69 on activated CD27⁺ memory B cells. In contrast to their naive counterparts, memory B cells from patients with SMS and patients carrying a 204insA *TNFRSF13B* mutation showed impaired CD86 induction after BCR, TLR7, and TLR9 stimulation and decreased CD69 expression after BCR stimulation (Fig 2, A and B).

Correlating with memory B-cell activation defects in these patients, TACI cell-surface expression levels on freshly isolated memory B cells from patients with SMS and patients with a

204insA *TNFRSF13B* mutation were decreased by 39% to 57% compared with those of memory B cells from healthy donors, revealing TACI haploinsufficiency at this late stage of B-cell development ($P < .0001$; Fig 2, C). In contrast, TACI expression on freshly isolated naive B cells from patients with SMS and patients with a 204insA *TNFRSF13B* mutation was similar to that observed on healthy donor naive B cells (Fig 2, C). Hence TACI expression can be sustained by a single functional *TNFRSF13B* allele on naive but not memory B cells. Of note, TACI expression was not quantitatively diminished on naive or memory B cells harboring one missense C104R *TNFRSF13B* mutation; the C104R mutant TACI protein, which is not detected by the 1A1 anti-TACI mAb, represented about half of all TACI cell-surface molecules (see Fig E2 in this article's Online Repository at www.jacionline.org).^{2,24} Induction of TACI by the TLR9 agonist CpG, which typically increases its expression on naive and memory B cells from healthy donors, was significantly diminished (2.0- to 4.2-fold induction, $P < .0001$) on memory B cells from patients with SMS (Fig 2, D). Induction of TACI expression by the TLR7 agonist Gardiquimod was similarly decreased in memory B cells from patients with SMS compared with their healthy donor B-cell counterparts (see Fig E3 in this article's Online Repository at www.jacionline.org). In contrast, TACI induction was not significantly affected in naive B cells from patients with SMS and correlated with normal activation of these B cells after BCR and TLR stimulations (Fig 2, D).

We further assessed the effect of the lower expression and impaired induction of TACI in memory B cells from patients with SMS by testing their proliferation and antibody secretion ability *in vitro* after CpG and BCR stimulation for 6 days in the presence or absence of the TACI ligand APRIL. We found that memory

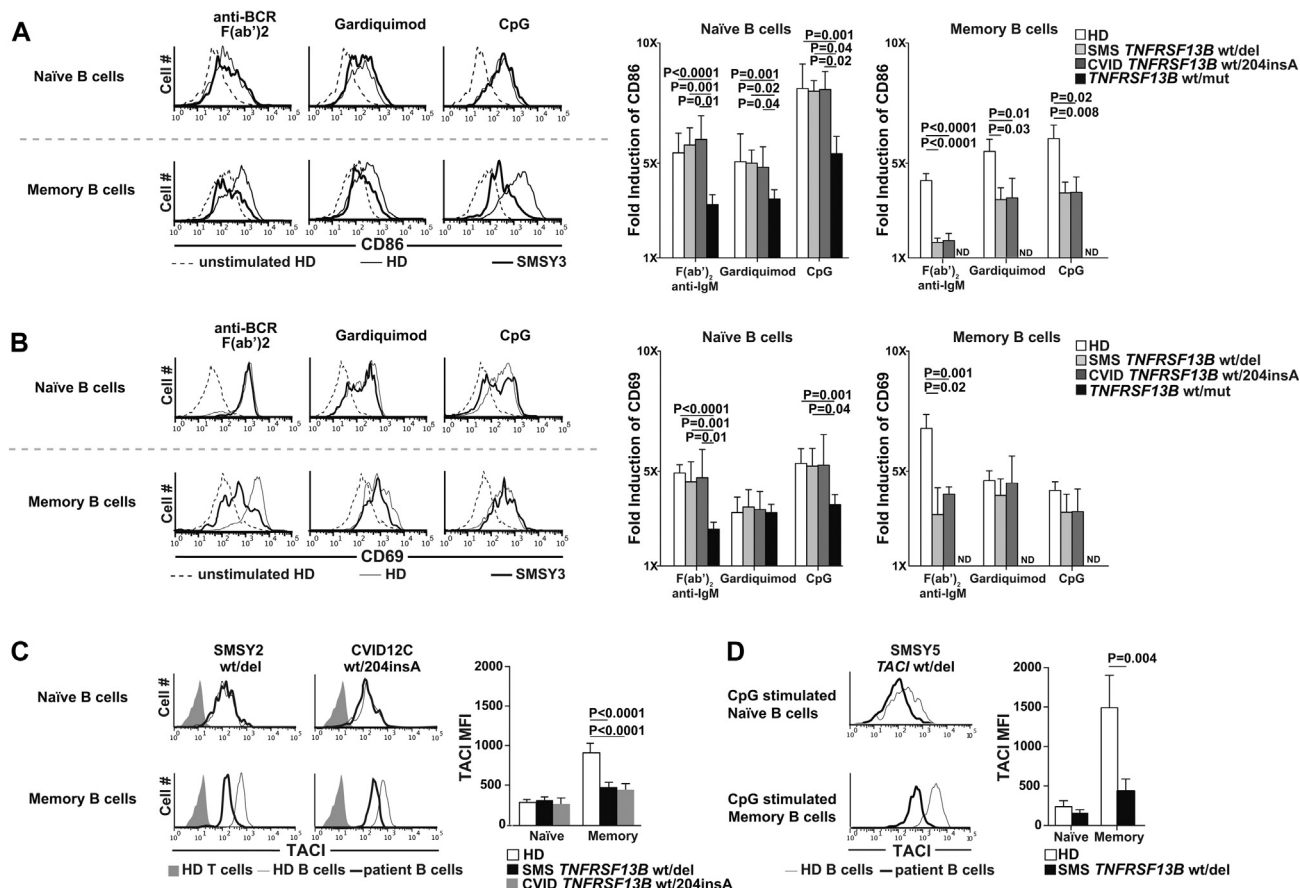


FIG 2. *TNFRSF13B* hemizygosity disturbs memory but not naive B-cell activation by selectively altering TACI expression on memory B cells. **A** and **B**, Histograms display CD86 (Fig 2, A) and CD69 (Fig 2, B) induction after stimulation for 48 hours with either F(ab')₂ anti-BCR (2.5 μg/mL), the TLR7 agonist Gardiquimod (1 μg/mL), or the TLR9 agonist CpG (0.5 μg/mL), which is normal in CD19⁺CD21⁺CD27⁺ naive B cells (upper panels) but defective in CD19⁺CD21⁺CD27⁺ memory B cells (lower panels) from a representative patient with SMS. Bar graphs on the right represent CD86 and CD69 induction on naive and memory B cells from patients with SMS with a heterozygous deletion of 17p11.2 (n = 6) and patients with CVID with 204insA (n = 2) or a missense (C104R or A181E) *TNFRSF13B* mutation (n = 15) relative to same-day healthy donor control subjects (n = 8–17). **C**, The mean fluorescent intensity of cell-surface TACI is significantly lower on memory B cells from patients with SMS (n = 3) and patients with a 204insA *TNFRSF13B* mutation (n = 2) than on counterparts from healthy donor control subjects (n = 8), whereas TACI expression on naive B cells is not altered by the lack of a *TNFRSF13B* allele. **D**, CpG-mediated (0.5 μg/mL) TACI induction is decreased on memory B cells from patients with SMS (n = 3) compared with that on healthy donor memory B cells (n = 6) at 48 hours, whereas TACI induction on naive B cells from patients with SMS appears unaffected. Error bars represent the means ± SEMs. Statistical significance determined by using the unpaired Student *t* test is indicated. HD, Healthy donors; MFI, mean fluorescent intensity; ND, experiments not performed.

B cells from patients with SMS did not become activated properly, as evidenced by a profound decrease in blasting cell numbers after 6 days of stimulation. Naive B cells from the same patients blasted normally after BCR and TLR9 triggering (Fig 3, A). The decrease in blasting memory B cells in patients with SMS was associated with reduced cell viability, regardless of the stimulations, which included CpG and BCR with or without APRIL (Fig 3, B). In addition, memory B-cell activation impairments in patients with SMS were further evidenced by the decreased proportion of memory B cells from patients with SMS undergoing 2 or more divisions, a fraction reduced by 30% to 52% compared with healthy donor B cells, and also by their low IgM secretion (Fig 3, C and D, see Fig E4 in this article's Online Repository at www.jacionline.org). In contrast, naive B

cells from patients with SMS displayed normal proliferation and antibody secretion responses (Fig 3, B–D). We conclude that *TNFRSF13B* hemizygosity induces abnormal memory B-cell responses and that a single *TNFRSF13B* allele cannot therefore compensate for the loss of the other allele in memory B cells, where TACI expression is normally enhanced.

TNFRSF13B hemizygosity affects isotype-switched memory B cells *in vivo*

To determine whether *TNFRSF13B* hemizygosity can affect memory B cells *in vivo*, we analyzed the peripheral B-cell compartments of patients lacking a productive *TNFRSF13B* allele. We found that the frequency of

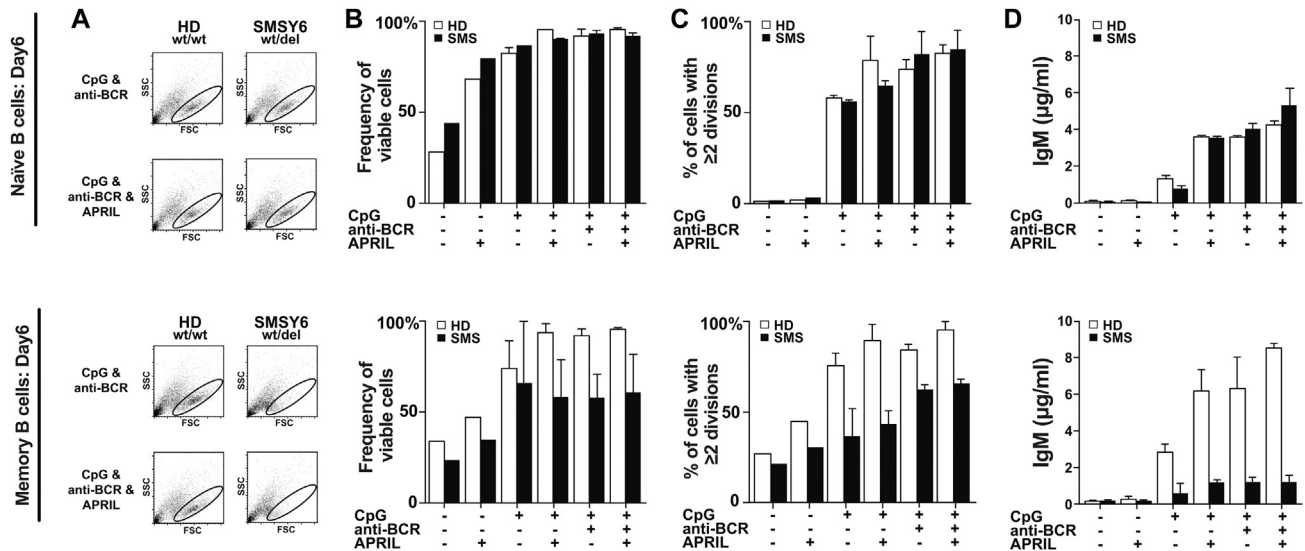


FIG 3. *TNFRSF13B* hemizygosity results in impaired memory B-cell responses *in vitro*. **A**, Representative dot plots display forward scatter (FCS) and side scatter (SSC) of naïve and memory B cells from a representative healthy donor and patient with SMS after CpG and BCR stimulation with or without APRIL for 6 days. The absence of blasting B cells among memory but not naïve B cells from patients with SMS reveals defective memory responses in these patients. **B–D**, Bar graphs display the proportion of viable cells (Fig 3, **B**), cells undergoing multiple (≥ 2) divisions (Fig 3, **C**), and production of IgM secreted into culture supernatants (Fig 3, **D**) in patients with SMS ($n = 2$) and healthy donors ($n = 2$) on day 6. Various culture conditions are indicated. Error bars represent means $\pm$ SEMs. Whereas responses of naïve B cells from patients with SMS and healthy donors were similar, the lack of a *TNFRSF13B* allele resulted in abnormal memory B-cell proliferation, survival, and antibody production. HD, Healthy donors.

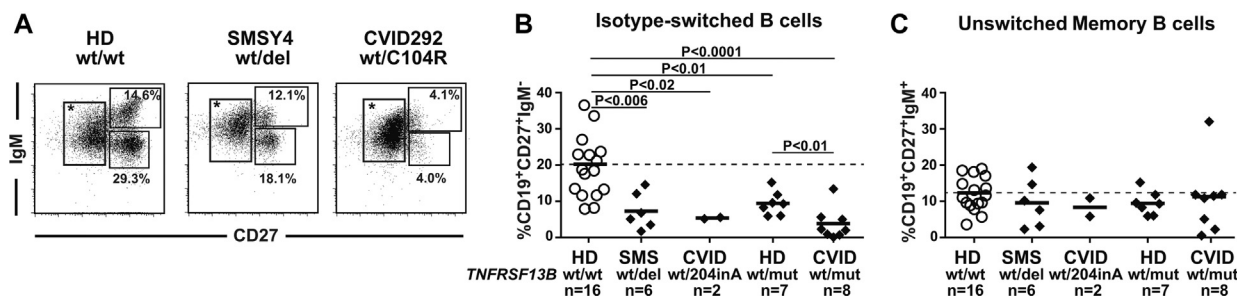


FIG 4. *TNFRSF13B* hemizygosity correlates with decreased class-switched memory B-cell frequencies. Dot plots for CD27 and IgM expression on CD19-gated B cells in representative subjects (**A**) and frequencies of CD27⁺IgM[−] isotype-switched (**B**) and unswitched CD27⁺IgM⁺ (**C**) memory B cells in enrolled patients reveal decreased numbers of isotype-switched memory B cells in patients with SMS (mean age, 14.3 years) and in patients with one 204insA *TNFRSF13B* mutation (mean age, 15 years) compared with those in age-matched healthy donor control subjects (mean age, 19.9 years). Error bars represent means $\pm$ SEMs. Statistical significance determined by using the unpaired Student *t* test is indicated. HD, Healthy donors.

IgM[−]CD27⁺ isotype-switched memory B cells was significantly decreased in both patients with SMS and patients carrying a 204insA *TNFRSF13B* mutation in whom they represented only 1.7% to 14.6% and 5.1% to 5.5% of peripheral B cells, respectively, compared with 7.9% to 37% in age-matched healthy control subjects (Fig 4, **A** and **B**). These low frequencies of isotype-switched memory B cells were similar to those of patients with CVID with missense C104R/A181E *TNFRSF13B* mutations (Fig 4, **A** and **B**). In contrast, null *TNFRSF13B* mutations did not affect frequencies of IgM⁺CD27⁺ memory B cells (Fig 4, **C**) or those of new emigrant/transitional and CD21^{lo} B cells, numbers of which are increased in patients with CVID with missense *TNFRSF13B* mutations (see Fig E5 in this article's Online Repository at

www.jacionline.org).²⁵ Hence TACI plays an important role in the production or maintenance of isotype-switched memory B cells in patients with SMS and patients carrying a 204insA *TNFRSF13B* mutation.

Normal peripheral B-cell tolerance checkpoint and normal Treg cell function in subjects with one productive *TNFRSF13B* allele

In healthy human subjects autoreactive new emigrant/transitional B cells capable of recognizing peripheral self-antigens are further eliminated at a second, more peripheral B-cell tolerance checkpoint before they enter the mature naïve B-cell compartment.¹⁹ We previously reported that a general feature of CVID is a defective peripheral B-cell tolerance

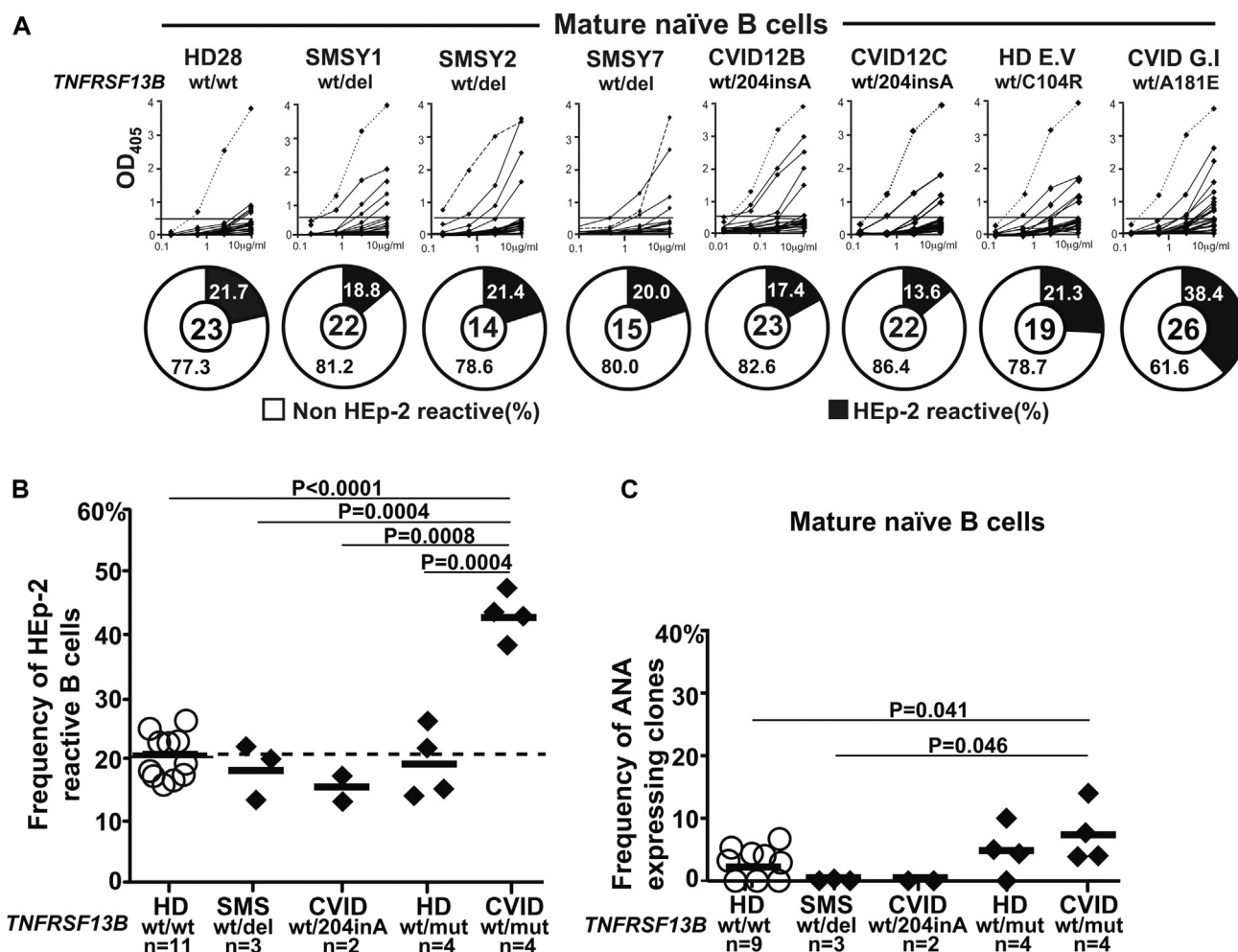


FIG 5. *TNFRSF13B* hemizygosity is compatible with a normal peripheral B-cell tolerance checkpoint. **A**, Recombinant antibodies cloned from mature naïve B cells were tested by using ELISA for anti-HEp-2 cell reactivity. Dashed lines show an ED38⁺ control.¹⁹ Horizontal lines define cutoff OD₄₀₅ values for positive reactivity. For each patient, the frequencies of HEp-2-reactive and non-HEp-2-reactive clones are summarized in pie charts, with the number of antibodies tested indicated in the center. **B**, The mean frequency of HEp-2-reactive mature naïve B cells (black bars) is normal in patients with a single productive *TNFRSF13B* allele, whereas it is increased in patients with CVID carrying a heterozygous C104R or A181E *TNFRSF13B* mutation (CVID wt/mut). **C**, Mature naïve B cells from patients with SMS and patients with a 204insA *TNFRSF13B* mutation are virtually devoid of anti-nuclear clones. Statistical significance determined by using the unpaired Student *t* test is indicated. HD, Healthy donors.

checkpoint that occurs independently of *TNFRSF13B* mutations.⁵ Notably, peripheral B-cell tolerance is also defective in patients with other primary immune deficiency diseases affecting antibody production, including CD40 ligand, MHC class II, activated cytidine deaminase, and dedicator of cytokinesis 8 deficiencies.^{26–28} To determine whether the peripheral B-cell tolerance checkpoint was dysfunctional in patients with SMS and patients with a 204insA *TNFRSF13B* mutation who display various antibody production defects (Table I), we tested the reactivity of recombinant antibodies from their CD10⁺CD21⁺IgM⁺CD27⁺CD20⁺ mature naïve B cells against HEp-2 cell lysates using ELISA (see Tables E6–E10). As previously reported, 38.5% to 47.6% of mature naïve B cells from patients with CVID with heterozygous C104R or A181E *TNFRSF13B* mutations expressed HEp-2-reactive antibodies, and 30.8% to 34.6% expressed polyreactive antibodies.⁵ In contrast, we found a much lower frequency of mature naïve

B cells expressing HEp-2-reactive antibodies in patients with SMS (18.8% to 21.4%, *P* < .0004) and patients with a 204insA *TNFRSF13B* mutation (13.6% to 17.4%, *P* < .0008) that were comparable with frequencies reported in healthy donors with and without *TNFRSF13B* mutations (16% to 26.3%; Fig 5, A and B). The frequencies of polyreactive mature naïve B cells in patients with loss of a *TNFRSF13B* allele and healthy donors with 2 unmutated alleles were also similarly low (3.5% to 13.6%, see Fig E6 in this article's Online Repository at www.jacionline.org). In addition, anti-nuclear clones, which were found to be increased (3.8% to 14%) in mature naïve B cells from patients with CVID with heterozygous C104R or A181E *TNFRSF13B* mutations, were not identified in patients with only 1 productive *TNFRSF13B* allele (Fig 5, C). Thus patients with SMS and patients with a 204insA *TNFRSF13B* mutation display an intact peripheral B-cell tolerance checkpoint.

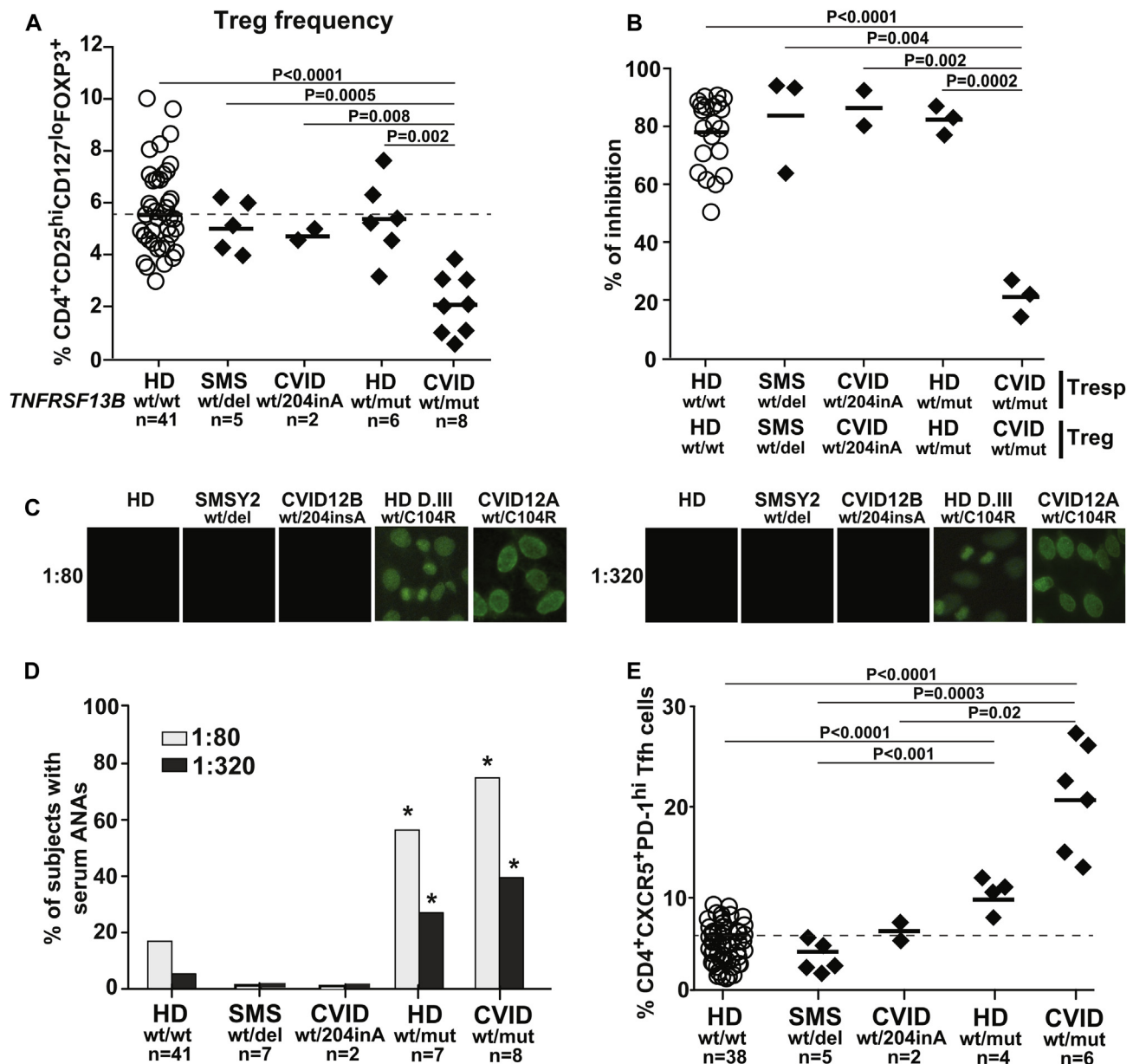


FIG 6. *TNFRSF13B* hemizygosity does not alter Treg cell function, autoantibody production, or circulating T_{FH} cell frequency. **A** and **B**, Normal Treg cell frequency (Fig 6, A) and Treg cell suppressive function in patients with SMS and patients with a 204insA *TNFRSF13B* mutation, whereas both are altered in patients with CVID carrying one C104R or one A181E *TNFRSF13B* mutation (CVID wt/mut). Each symbol represents a patient, and horizontal bars show the average. **C**, Representative ANA staining patterns of 1:80 (left) and 1:320 (right) dilutions of plasma from patients with SMS, patients with a 204insA *TNFRSF13B* mutation, and patients with a C104R or A181E *TNFRSF13B* mutation. **D**, ANAs are absent in the plasma of patients with SMS and patients with a 204insA *TNFRSF13B* mutation, whereas they are commonly detected in patients carrying a C104R or a A181E *TNFRSF13B* mutation (wt/mut). **E**, Normal CD4⁺PD-1^{hi}CXCR5⁺ T_{FH} cell frequencies in patients with SMS and patients with a 204insA *TNFRSF13B* mutation, whereas numbers are increased in patients with CVID with a C104R or A181E *TNFRSF13B* mutation (wt/mut). Each symbol represents a patient, and horizontal bars show the average. Statistical significance is indicated.

Defects in peripheral B-cell tolerance have been correlated with altered T regulatory (Treg) cell frequency and function, as well as increased plasma BAFF concentrations: both features are commonly associated with CVID.^{5,29-31} To further investigate the effect of a loss of a *TNFRSF13B* allele, we analyzed the frequency and function of Treg cells in patients with SMS and patients with a 204insA *TNFRSF13B* mutation and compared them with those in

patients with CVID. Previously, we reported low frequencies of CD4⁺CD25^{hi}CD127^{lo}FOXP3⁺ Treg cells in patients with CVID with heterozygous C104R or A181E *TNFRSF13B* mutations (0.7% to 4% of CD4⁺ T cells) and defective suppressive function.⁵ In contrast, we found that Treg cell frequencies in patients with SMS and patients with a 204insA *TNFRSF13B* mutation were similar to those of healthy donors

with or without *TNFRSF13B* mutations (Fig 6, A, and see Fig E7 in this article's Online Repository at www.jacionline.org). Direct assessments of Treg cell suppressive function *in vitro* demonstrated that unlike the Treg cells from most patients with CVID, Treg cells from patients with SMS and patients with 204insA frameshift *TNFRSF13B* mutations were capable of inhibiting the proliferation of CFSE-labeled CD3⁺CD4⁺CD25[−] autologous and heterologous responder T cells (Fig 6, B, and see Fig E8 in this article's Online Repository at www.jacionline.org).⁵ Measurement of plasma BAFF concentrations in patients with SMS and patients with a 204insA *TNFRSF13B* mutation revealed normal BAFF concentrations that were similar to those in healthy donors and significantly lower than those in patients with CVID (see Fig E9 in this article's Online Repository at www.jacionline.org).^{5,30} We conclude that *TNFRSF13B* hemizyosity does not affect Treg cell frequency or function and does not significantly increase plasma BAFF concentrations.

Absence of plasma autoantibody production in patients with one productive *TNFRSF13B* allele

Despite an impaired production of immune protective antibodies, IgG ANAs were detected in the plasma of a large majority of patients with CVID and healthy donors with heterozygous C104R or A181E *TNFRSF13B* mutations, revealing that TACI plays an important role in preventing autoantibody secretion.⁵ To assess whether *TNFRSF13B* hemizyosity also leads to autoantibody production, we measured plasma ANA titers in 7 patients with SMS and 2 patients with a 204insA *TNFRSF13B* mutation. We found that plasma of patients with SMS and patients with 204insA frameshift *TNFRSF13B* mutations were devoid of IgG ANAs (Fig 6, C and D). High-titer plasma ANAs in patients with CVID correlated with increased frequencies of peripherally circulating CXCR5⁺PD-1^{hi}CD4⁺ T follicular helper (T_{FH})-like cells, which favor late B-cell development and antibody secretion.^{5,32} Frequencies of circulating T_{FH}-like cells in the blood of patients with SMS and patients with 204insA frameshift *TNFRSF13B* mutation were low and similar to those in healthy donors (Fig 6, E, and see Fig E10 in this article's Online Repository at www.jacionline.org). In addition, T_{FH}-like cells from patients with SMS and patients with a 204insA *TNFRSF13B* mutation did not express high amounts of the B-cell coactivation molecule inducible T-cell costimulator or the intracellular transcription factor B-cell lymphoma protein 6, whereas both of these molecules were expressed in classical T_{FH} cells from germinal centers³³ and circulating T_{FH} cells from patients with CVID (see Fig E10).⁵ Hence in contrast to common C104R and A181E heterozygous *TNFRSF13B* mutations, *TNFRSF13B* hemizyosity does not lead to a breach in peripheral B-cell tolerance or autoantibody production.

DISCUSSION

We report that *TNFRSF13B* hemizyosity does not recapitulate the naive B-cell selection and activation defects caused by the presence of one C104R or A181E mutant *TNFRSF13B* allele. TACI is essential for the establishment of central B-cell tolerance because all subjects carrying missense *TNFRSF13B* mutations had an inability to remove developing autoreactive B cells in the bone marrow.⁵ Here, using 2 different human genetic models

of *TNFRSF13B* hemizyosity, we demonstrate that a single unmutated *TNFRSF13B* allele is sufficient for normal surface expression of TACI on naive B cells and to maintain TACI-dependent central B-cell tolerance. Thus the impairment of central B-cell tolerance observed when a normal *TNFRSF13B* allele is paired with a C104R and A181E mutated product reveals that these 2 most common *TNFRSF13B* mutations do not exert their effects through haploinsufficiency but more likely through negative interference. In agreement with this hypothesis is the normal activation of naive B cells from patients with SMS and patients with a 204insA *TNFRSF13B* mutation who display a single productive *TNFRSF13B* allele, whereas naive B cells carrying one C104R or A181E *TNFRSF13B* allele do not properly respond to BCR, TLR7, and TLR9.⁵ Therefore mutant TACI molecules might limit the ability of wild-type TACI to enhance BCR- and TLR-mediated B-cell activation. Because we previously reported that TACI binds TLR7 and TLR9,⁵ mutated TACI variants might associate with these TLRs and therefore decrease the nonmutated TACI-TLR complexes required to mediate TLR7 and TLR9 responses. Other B-cell intrinsic defects disrupting TLR signaling and function, such as IL-1 receptor-associated kinase 4 and myeloid differentiation primary response gene-88 deficiencies, also interfere with the establishment of central B-cell tolerance, suggesting an important role for TLRs in the removal of developing autoreactive B cells, especially cells expressing ANAs, which might recognize nucleic acids bound by TLR7 and TLR9.^{5,22}

The peripheral B-cell tolerance checkpoint operates independently of the central tolerance checkpoint and appears to rely on B-cell extrinsic factors.³⁴ Indeed, FOXP3-deficient patients who lack functional Treg cells displayed a defective peripheral B-cell tolerance checkpoint, whereas central B-cell tolerance was established normally in these patients.²⁶ Interestingly, healthy donors carrying a C104R or an A181E *TNFRSF13B* mutation who showed normal Treg cell frequencies and function were able to prevent the accumulation of autoreactive clones in their mature naive B-cell compartment despite their impaired central B-cell tolerance checkpoint, demonstrating a dominant role for Treg cells in ensuring tolerance.^{5,35} The numerically and functionally replete Treg cell pool with corresponding intact peripheral B-cell tolerance checkpoints we observed in patients with SMS and patients with a 204insA *TNFRSF13B* mutation further supports this hypothesis.

The C104R and A181E *TNFRSF13B* mutations are also associated with a more distal breach in B-cell tolerance characterized by production of autoreactive antibodies, increased numbers of circulating T_{FH} cells, and often the development of autoimmune manifestations.^{2,5} All these features are unrelated to the loss of a *TNFRSF13B* allele because there is no evidence of autoimmunity in patients with SMS or the 2 patients with a 204insA *TNFRSF13B* mutation reported here. In addition, we could not detect ANAs in the plasma of these patients, who also displayed normal low frequencies of T_{FH}-like cells in their blood. Hence C104R and A181E *TNFRSF13B* mutations likely generate dominant negative products that also interfere with TACI's tolerogenic functions in the periphery. However, similar to patients with CVID who harbor a single C104R or A181E *TNFRSF13B* mutation and mice with only a single *TNFRSF13B* allele,¹² *TNFRSF13B* hemizyosity in human subjects decreases circulating isotype-switched memory B-cell numbers, serum immunoglobulin production, and vaccine responses.

Both patients with a 204insA *TNFRSF13B* mutation¹⁶ and at least 2 of the 7 patients with SMS we studied fulfill the accepted diagnostic criteria for CVID (Table I). The remaining 5 patients with SMS experienced recurrent sinopulmonary infections or did not properly respond to vaccines, illustrating abnormal antibody responses when one *TNFRSF13B* allele is lost (Table I). The decrease in TACI expression, which is restricted to memory B cells from patients with SMS and patients with a 204insA *TNFRSF13B* mutation, reveals that the loss of a *TNFRSF13B* allele results in haploinsufficiency at this stage of B-cell development, which has also been observed in EBV-transformed B cells from these patients.¹⁸ In light of B cells from patients carrying 2 nonfunctional *TNFRSF13B* alleles, which are virtually irresponsive to many stimulations,⁵ decreased TACI expression on memory B cells is likely responsible for their diminished responses to BCR, TLR7, and TLR9 stimulation and alters antibody responses by affecting B-cell proliferation, as well as plasma cell differentiation and survival.^{11,36,37} Further exploration will be necessary to determine the mechanisms through which steps of terminal B-cell differentiation require 2 functional *TNFRSF13B* alleles.

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Key messages

- Unlike heterozygous C104R and A181E *TNFRSF13B* mutations, complete loss of one *TNFRSF13B* allele does not affect B-cell functions at early stages of B-cell development.
- Two functional *TNFRSF13B* alleles are required at later stages of B-cell development when TACI expression is upregulated.

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Table E1: Repertoire and reactivity of antibodies from new emigrant/transitional B cells of SMS patient Y1

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vk	Jk	CDR3 (aa)	Length	Poly	HEp-2	Staining
neSMSY1 02	3-33	/	/	6	EPGDEGYYYYYGMDV	15	3-11	4	QQRSNWPPGLT	11	-	+	-
neSMSY1 03	3-15	2-2	2	6	ADCSTSCPLYYYYGMDV	18	1-13	2	QQYYSTPYS	9	-	-	-
neSMSY1 04	4-31	3-16	1	4	GILGGFDY	8	1-5	2	QQYNSYSYS	9	-	+	-
neSMSY1 05	3-15	/	/	4	DRGGYFDY	8	1-39	1	QQSYSTPWT	9	-	+	-
neSMSY1 06	3-21	5-24	2	3	GSRDGYTIVSPVGCAFDI	18	4-1	1	QQYYSTPPS	9	-	-	-
neSMSY1 08	3-13	6-6	1	3	VSRLGQLVPGAFDI	14	1-39	4	QQSYSTPPGVT	11	-	-	-
neSMSY1 09	3-15	2-21	3	4	VVVVATKEIRYYFDY	16	3-15	1	QYNNWPPWT	9	-	-	-
neSMSY1 11	1-3	/	/	3	PYRRDAFDI	9	1-5	2	QQYNSYPYS	9	-	-	-
neSMSY1 13	3-21	2-15	2	6	VPCSGGSCYHLSPIYYYGMDV	21	1-39	3	QQSYSTPFT	9	-	+	-
neSMSY1 14	4-59	3-22	2	3	HVEYYYDSSGYSPHAFDI	18	2-28	4	MQALQTPIT	9	-	-	-
neSMSY1 15	3-23	2-2	2	4	DRQRTTSCHDY	11	1-39	2	QQSYSTPYT	9	-	+	-
neSMSY1 17	4-59	3-22	2	4	SPFYDSSGRYYFDY	16	3-20	4	QQYGSSPIL	9	-	+	-
neSMSY1 23	4-59	1-26	1	4	RQWELGVFDY	10	4-1	3	QQYYSTPFT	9	-	-	-
neSMSY1 24	3-7	6-13	3	5	AKRVAAALSPWWFDP	15	1-5	1	QQYNSYSLT	9	-	+	-
neSMSY1 25	4-39	6-13	2	5	HGPYSSSLWFDP	12	3-15	4	QQYNNWPPLT	10	-	+	-
neSMSY1 26	4-34	2-8	2	5	GAMLCTNGVCYTFWFDP	17	4-1	3	QQYYSTPPT	9	-	-	-
neSMSY1 31	3-48	1-7	3	6	LFGTTGPYYYYGMDV	15	1-5	1	QQYNSYWT	8	-	-	-
neSMSY1 33	4-39	3-10	1	4	HWRFGELFDY	10	3-20	4	QQYGSSPRALT	11	-	-	-
neSMSY1 34	3-23	3-22	2	4	ADSSGYYVY	9	2-24	3	MQATQFPHT	9	-	-	-
neSMSY1 36#	4-4	2-2	2	6	HTHCSSTSCYSVAGYYYYYMDV	22	2-14	2	SSYTSSSTVV	10			
neSMSY1 43#	4-39	3-22	2	6	ATTLGRYYYDSSGYSPGR	18	3-20	1	QQYGSSPPWT	10			
neSMSY1 46	1-3	3-3	1	4	SLRFLEWPPLSGFDY	15	3-11	3	QQRSNWPPGFT	11	+	-	-
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
neSMSY1 01	1-2	1-26 5-12	3 3	2	GGREGIVGATSAGDRRHYWYFDL	23	2-23	3	CSYAGSSTRV	10	-	+	-
neSMSY1 07	4-39	2-21	2	3	QRGGDYCGGDCPGMRDAFDI	21	1-40	3	QSYDSSLGFKV	12	-	-	-
neSMSY1 10	3-21	5-5	2	4	GPGGYSYGPDY	11	1-44	3	AAWDDSLNGWV	11	-	+	-
neSMSY1 12#	3-49	3-3	2	5	PFSGSPSTYWFDP	13	2-14	2	SSYTSSSTVV	10	-	+	-
neSMSY1 16	4-59	6-13	3	6	DRRAAAGSSPPTVYYYYGMDV	21	2-14	3	SSYTSSSTRV	10			
neSMSY1 20	4-39	6-19	3	4	RVGIAVAGSMPYYFDY	16	2-14	3	SSYTSSSTSLV	11	-	-	-
neSMSY1 21	3-48	1-26	1	4	WSWDNEIDY	9	1-47	3	AAWDDSLSGRV	11	-	-	-
neSMSY1 23					see kappa	/	2-14	2	SSYTSSSTVV	10	-	-	-
neSMSY1 25					see kappa	/	2-14	2	SSYTSSSTVV	10	+	+	c
neSMSY1 27	3-23	/	/	6	YPPVRSPLYYYMDV	15	1-51	3	GTWDDSSLGAGV	11	-	-	-
neSMSY1 36	4-4	2-2	2	6	HTHCSSTSCYSVAGYYYYYMDV	22	1-40	3	QSYDSSLGSEV	12	-	-	-

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E2: Repertoire and reactivity of antibodies from new emigrant/transitional B cells of SMS patient Y2

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vk	Jk	CDR3 (aa)	Length	Poly	HEp-2	Staining
neSMSY2 02	4-30	3-10	1	5	ARARFGEFDP	10	1-5	3	QQYNSYFT	8	-	ND	-
neSMSY2 03#	3-7	2-21	2	4	ARVDPYSKRDDY	12	3-11	5	KQRKNCPLPPS	11			
neSMSY2 04	4-39	3-3	2	3	ARQANYDFWSDNWFDP	16	2-28	4	MQALQTPHT	9	-	ND	-
neSMSY2 12	1-3	3-10	2	6	ARDPPQYYYGSGPAKIYYYYGMDV	24	1-33	2	QQYDNLQPVT	10	+	ND	c
neSMSY2 13	3-33	5-5	2	4	AREEEAGYSYGVL DY	15	3-15	1	QQYNNWPRT	9	-	ND	-
neSMSY2 17#	3-21	2-21	3	6	ARAVIRRFGPALLHQYGM DV	20	3-20	1	QQYGSSSWT	9			
neSMSY2 18	3-30	5-12	2	4	ARAPGYSGYDYWDY	14	1-39	4	QQSYSTPLS	9	-	ND	-
neSMSY2 19	4-30	3-10	2	4	ARMAGSGSGSYLFDY	15	1-8	4	QQYYSYPLT	9	-	ND	c
neSMSY2 20	3-23	5-5	2	6	AKSRARGRGYSYGSR IYYGMDV	22	3-20	1	KQYGSSPRT	9	-	ND	-
neSMSY2 21	4-59	4-17	2	6	ARCLHPYGDYVEHYYYYY GMDV	22	1-39	1	QQSYSTPWT	9	-	ND	c
neSMSY2 23#	3-11	2-2	2	5	ARAYCSSTSCPPNWFDP	17	4-1	1	QQYYSTPIT	9			
neSMSY2 24#	1-69	2-2	3	6	ARASLDIVVPAAPNPPHP SHVKETYYYYY GMDV	34	1-39	3	QQSYSTPFT	9			
neSMSY2 25#	3-48	2-2	3	4	ARGPRSDVVVPAAMPPT IIDYFDY	24	1-5	2	QQYNSYPVT	9			
neSMSY2 29	3-21	6-13	3	5	ARAPRRVAAAGSWFDP	12	1-5	2	QQNSRYT	7	-	ND	-
neSMSY2 30	4-59	3-3	2	6	ARGRRSDRSGSEGGM DV	17	1-5	1	QQYNSFWT	8	-	ND	-
neSMSY2 10							3-20	1	QQYGSSPSIT	10			
neSMSY2 35							2-28	2	MQALQTPPYT	10			
	VH	D	RF		CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
neSMSY2 08	3-33	3-10	2	6	ARDTRYYYGSERYYYGMDV	20	3-21	2	QVWDSSSDHV V	11	-	ND	-
neSMSY2 14	3-74	3-22	2	3	ARATYYDSSPGGAFDI	17	2-23	2	CSYAGSSTPF	10			
neSMSY2 15	3-33	1-26	3	3	ARDRRIVGADDAFDI	15	3-9	1	QVWDSSSTLYV	10	-	ND	-
neSMSY2 22	3-23	2-15	2	4	AKVPVSPLGYCSGGSCSY YFDY	23	3-21	2	QVWDSSSDQP VV	12	-	ND	-
neSMSY2 27	1-8	I	I	6	ARYPPPGDY YGMDV	14	1-51	2	GTWDSSSLAVV	11	-	ND	-
neSMSY2 31#	3-73	2-15	2	4	TSGQYCSGGSCYDY	14	2-11	1	CSYAGSYKV	9			
neSMSY2 34#	7-4	1-26	1	4	ARGVRAGRWELHWAY YFDY	19	1-40	2	CSYAGSSTPVV	11			
	VH	D	RF		CDR3 (aa)	Length							
neSMSY2 09	1-3	3-3	1	6	ARVDVLRFN GMDV	13							
neSMSY2 16	3-7	2-2	1	6	ARGPYQLRSHYYY GMDV	17							
neSMSY2 28#	5-a	5-12	2	6	ARHEPLSIGYGNYYYYY GMDV	21							
neSMSY2 33#	3-30	3-16	2	6	ARDFLSYDYV	10							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E3: Repertoire and reactivity of antibodies from new emigrant/Transitional B cells of patient SMSY7

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3	Length	Vk	Jk	CDR3 (aa)	Length	Poly	HEp-2	Stainin
neSMSY7 01	3-30	4-17	2	4	GDKEDYGDYVWYFDY	15	1-21	4	QQANSFPLT	9	-	ND	-
neSMSY7 02	4-30	6-13	3	4	GAAAGTWFD	10	1-5	4	QQYNSYLT	8	-	ND	-
neSMSY7 03	4-59	3-10	1	6	VRFGGYYYYGMDV	13	1-5	1	QQYNSYPWT	9	+	ND	-
neSMSY7 05	4-31	/	/	6	YVPVITLDV	9	1-6	4	LQDYNYSLT	9	-	ND	-
neSMSY7 10	3-33	3-22	2	6	DPLYYYDSSGYSYYYYGMDV	20	1-6	2	LQDYNYPRT	9	-	ND	-
neSMSY7 12	3-23	/	/	4	DLHGVFDY	8	3-15	2	QQYNNWPLYT	10	-	ND	-
neSMSY7 17	3-23	4-17	2	6	DKAGDYATIPYYYYYYGMDV	20	3-11	1	QQRSNWPPAWT	11	+	ND	-
neSMSY7 18	3-30	3-10	2	4	LYYYGRGDY	9	1D-13	2	QQFNYPYT	9	-	ND	-
neSMSY7 21#	3-33	6-19	3	4	SIAAVRYFD	11	3-11	4	QQRSNWPPLT	10			
neSMSY7 24#	3-33	3-10	1	3	DPLWFGELLGAFDI	14	1-5	2	QQYNSYSNT	9			
neSMSY7 33	4-34	2-15	2	5	GVLEKYPCSGGSCYSTKNLSNWFD	25	3-15	1	QQYNNWPRT	9	-	ND	-
neSMSY7 34	4-59	6-19	2	6	AGYSSGWDDTTYGMDV	16	1-39	2	QQSYSTPYT	9	-	ND	-
neSMSY7 37	4-31	6-13	2	4	GTPMYSSTPDY	11	1-39	4	QQSYSTPLT	9	-	ND	-
neSMSY7 38	4-4	6-19	3	4	DGEIAVAGTDYFDY	14	3-20	1	QQYGSSPRT	9	-	ND	-
neSMSY7 42	4-34	1-26	2	4	GGGSYPPFDY	11	1-12	2	QQANSFPYT	9	-	ND	-
neSMSY7 46#	4-4	/	/	4	RREGAVDY	8	1-9	2	QQLNSYPWT	9			
neSMSY7 07							3-11	5	QQRSNWPPGIT	11			
	VH	D	RF	JH	CDR3	Length	Vλ	Jλ	CDR3 (aa)		Poly	HEp-2	Stainin
neSMSY7 09	3-53	/	/	6	YTYYGMDV	8	3-21	2	QVWDSSSDHPVV	12	-	ND	-
neSMSY7 13	4-39	6-19	3	4	SGLGIAVAGPRWALSQTDFY	20	1-51	2	GTWDSSLSAGV	11	-	ND	-
neSMSY7 14	3-74	3-22	2	5	EFSGYYYDSSGYP	15	1-47	1	AAWDDSLSVSYV	12	-	ND	-
neSMSY7 16	4-39	/	/	5	PLNPPHWF	10	2-14	1	SSYTSSSTLG	10	-	ND	-
neSMSY7 19	3-33	2-2	2	6	DRGYCSSTSCSPHYYYGMDV	20	1-44	3	AAWDDSLNGWV	11	-	ND	-
neSMSY7 31#	4-61	4-17	3	4	ENTVTSFDY	9	3-21	3	QVWDSSSDHRV	11			
neSMSY7 36	3-48	3-3	2	4	DWWNDSSGGYSSGPFY	18	3-21	1	QVWDSSSDHYV	12	-	ND	-
neSMSY7 39	1-18	/	/	4	GGFRTRAPFY	11	1-40	1	QSYDSSLSGV	10	-	ND	-
neSMSY7 44	4-59	6-19	3	4	DQMEVAGTGFGY	12	3-1	1	QAWDSSTYYV	10	-	ND	-
neSMSY7 48	3-9	3-10	1	4	ARFGLSPD	10	2-23	2	CSYAGSSAVV	10	-	ND	-
	VH	D	RF	JH	CDR3	Length							
neSMSY7 06	1-18	3-10	2	4	DGRSNYYGSGIDY	13							
neSMSY7 26	3-9	2-2	3	5	DGVPAAPALQTTAPTGGFDP	20							
neSMSY7 30	3-23	3-10	2	4	DQSFQGTPHYGSGSYYSY	18							
neSMSY7 35	3-30	3-3	1	6	TSLRFLEHYGMDV	14							
neSMSY7 40	5-51	6-19	3	4	GVAGTLYY	8							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E4: Repertoire and reactivity of antibodies from new emigrant/transitional B cells of patient C12B.

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	(-)	Len	Vk	Jk	CDR3 (aa)	Length	Poly	HEP-2	Staining
neC12B 05	3-43	5-5	2	6	DLSGYSYGSVNYYYGMDV	1	1-39	3	QQSYSTPFT	9	-	ND	-
neC12B 15	4-39	4-17	2	4	SLQVG DYEDY	1	3-11	3	QQRSNWPFT	9	-	ND	-
neC12B 16	3-33	3-3	2	3	ARGGDFWSGYTQDEAFDI	1	1-5	1	QQYNSYSPTWT	11	-	ND	-
neC12B 22#	5-51	3-3	2	5	HGGWFDP	7	3D-15	4	QQYNNWPPLT	10			
neC12B 23	3-33	3-10	2	6	SGGSGSYNNYYYGMDV	1	2-28	1	MQALQTPPT	9	-	ND	-
neC12B 25#	3-64D	2-2	2	4	ADAYCSSTSCYIDY	14	3-20	2	QQYGSFLYT	9			
neC12B 32	3-48	3-3	2	4	DVWSGYRFDY	11	1-5	1	QQYNSYSRT	9			
neC12B 34	6-1	6-19	2	6	GATLGYSYGWYKGGYYYYYYMDV	2	1-12	1	QQANSFP	7	-	ND	-
neC12B 35#	3-23	3-16	3	6	AKVIGMITFGGVIASPRSGSYYYYYGMDV	2	2-28	1	MQALQTPST	9			
neC12B 48#	4-31	2-2	2	5	AREGYCSSTSCYKGAWFDP	1	1-33	4	QQYDNLPLT	9			
neC12B 40							1-39	1	QQTYSAPRT	9			
neC12B 45							3-20	1	QQYGSSPWT	9			
	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	(-)	Len	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEP-2	Staining
neC12B 03	4-39	3-22	2	3	PQPYYYDSSGYYPAAFDI	1	3-21	2	QVWDSSSDRGVV	12	-	ND	-
neC12B 06	4-4	3-22	2	6	ESLYYYDSSGYYSKYYYGMDV	2	2-8	2	SSYAGSNKYVV	11	-	ND	-
neC12B 07	4-39	3-3	2	5	SSNGYYLSYWYDLT	1	3-21	2	QVWDSSSDHVV	11	-	ND	-
neC12B 08	3-30	2-2	1	6	EMDAQLLYYYGMDV	1	1-40	2	QSYDSSLGSGV	11	-	ND	-
neC12B 09	3-33	2-2	2	6	PHRAFSSSTLHYYYYMDV	1	2-14	2	SSYTSSSTLMV	11	-	ND	-
neC12B 10	3-30	1-26	3	4	GATFYRYSEKHFY	1	2-11	1	CSYAGSYTYV	10	+	ND	-
neC12B 11	1-18	2-2	1	3	VLGEYQLLAGPAAFDI	16	2-14	2	SSYTSSSTLVV	11	-	ND	-
neC12B 14	4-31	3-10	3	4	DRGRITWDY	9	2-11	3	SSYTSSSTPRV	11	-	ND	-
neC12B 18	3-33	3-3	1	6	DQGLRFLEWFLPYYGMDV	1	1-51	2	GTWDSSSLSAVV	11	+	ND	-
neC12B 26	3-33	6-13	2	6	ADYSSSWYEVEEPSYGMDV	1	1-51	2	GTWDSSLTEVV	11	-	ND	-
neC12B 29	4-31	5-5	3	3	GVSSAMVG	9	3-1	2	QAWDSSNVV	9	-	ND	-
neC12B 30	4-31	3-3	2	3	SLSDFWSLYGGFADF	1	1-51	2	GTWDSSSLSAVV	11	-	ND	-
neC12B 31	3-33	3-22	2	6	DLKGVNYDYYYGMDV	1	3-1	2	QAWDSSFVV	9	-	ND	-
neC12B 33	4-34	2-2	2	5	GVDCSSTSCSHRTRKNWFDP	2	3-21	2	QVWDSSSDHVV	11	-	ND	-
neC12B 36	3-30	4-23	3	6	ARSTVVPPVYGMDV	1	1-36	2	AAWDDSLNGVV	11	-	ND	-
neC12B 37	1-18	3-22	2	4	ARGYYDSSGYYYFDY	1	3-21	2	QVWDSSSDHPV	11	-	ND	-
neC12B 01#	1-18	6-13	3	5	ARPSIAAAGENWFDP	1	3-21	1	QVWDSSSDHYV	11			
neC12B 38#	1-2	6-19	1	4	ARGAQWLVRDLY	1	2-23	2	CSYAARDVV	9			
neC12B 43#	3-48	6-13	3	2	ARDFYKAAVSYGMDV	1	3-21	1	QVWDSSSDHYV	11			
neC12B 46#	4-39	6-6	2	6	ARLVRLYSSSHSIDYMDV	1	2-14	2	SSYTSSSTRV	10			

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E5: Repertoire and reactivity of antibodies from new emigrant/transitional B cells of CVID patient C12C

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vk	Jk	CDR3 (aa)	Length	Poly	HEp-2	Staining
neC12C 01	4-4	1-20	2	6	DKKPNWNDVAVDNERYYYYMDV	20	3-11	4	QQRSNWPPPT	9	-	ND	-
neC12C 03	4-31	2-8	2	4	GMVKIPTNFDY	11	1-39	4	QQSYSTPLT	9	+	ND	-
neC12C 05#	4-39	6-19	2	4	HELVNYTVGW	10	2-24	2	MQDTQFPHYT	10			
neC12C 07	3-33	3-22	2	4	DQDSSGYNMGVGVSDY	16	1-5	1	QQYNSYPWT	9	-	ND	-
neC12C 08#	1-69	2-2	3	6	EAGGARVGQGAGLVVPAAYYYYYGMDV	27	1-39	5	QQSYSTPIT	9			
neC12C 09	3-33	1-26	2	4	DLPYSGSYSLEGG	13	1-5	2	QQYNSYPYT	9	+	ND	A, F
neC12C 13	3-15	4-11	3	6	GVTTVTQQDYYYYYMDV	16	1-5	1	QQYNSPQCT	9	-	ND	-
neC12C 14	4-4	3-10	2	6	YKPSGVYYYYYMDV	14	1-5	1	QQYNSYSLT	9	-	ND	-
neC12C 17	1-69	5-24	2	4	DGVSGPFDY	9	4-1	4	QQYYSTPPT	9	-	ND	-
neC12C 19	3-7	1-26	3	4	DLALWLGAADY	11	1-13	4	QQFNSYPRT	9	-	ND	-
neC12C 22	4-4	3-10	2	6	VKSGSGSYLPLDGMDV	16	4-1	3	QQYYSTPLT	9	-	ND	-
neC12C 27#	3-53	4-17	2	6	DIGELTPYGDYEDYYYGMDV	20	3-20	1	QQYGSSPPWT	10			
neC12C 30#	5-A	3-10	2	6	RHYGSGSYSDYYYYGMDV	20	3-20	3	QQYGSSPPET	10			
neC12C 34	4-39	2-2	2	4	QWGYCSSTSCYPNFDY	17	3-20	4	QQYGSSSLT	9	-	ND	-
neC12C 40	4-34	2-2	2	6	VWVCSSTSCNYGMDV	15	3-20	1	QQYGSSPKT	9	-	ND	-
neC12C 42	4-34	6-6	2	4	GDSSSSGPADY	11	3-20	3	QQYGSSPKVT	10	-	ND	-
neC12C 44	3-23	3-16	3	4	GGVRYYFDY	9	3-15	1	QQYNNWPQT	9	-	ND	-
neC12C 46	4-31	3-10	2	4	DNPGTPYGSGLGVY	15	1-27	3	QKYNAPPFT	10	-	ND	-
neC12C 47#	4-31	2-2	3	3	DRRSALIPWAFDI	13	2-28	2	MQALQTPLYT	10			
	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
neC12C 04	3-30	3-3	2	4	DGADFWSGYYPGYFDY	17	1-47	2	AAWDDSLSGVV	11	-	ND	-
neC12C 11	3-9	6-13	3	4	DIAAAGRGYFDY	12	2-14	2	SSYTSSSTPVV	11	-	ND	-
neC12C 12	3-15	6-6	3	6	DKAEIAARPRLYYYYYYMDV	20	1-40	2	QSYDSSLGVV	11	-	ND	-
neC12C 14					see kappa		2-14	2	SSYTSSSTGEV	11	-	ND	-
neC12C 15	3-11	3-22	2	4	VRPHYDSSGYYYFDY	16	2-14	1	SSYTSSSTLPG	11	-	ND	-
neC12C 18	3-33	3-3	1	6	ARDQGLRFLEWFLPYGMDV	20	1-40	2	QSYDSSLGVV	10	-	ND	-
neC12C 23	4-59	5-5	3	4	ITDTAMGYEGGFDY	14	3-1	2	QAWDSSSVV	9	-	ND	-
neC12C 25	3-7	/	/	3	EGMGAFDI	8	2-23	2	CSYAGSSTLV	10	-	ND	-
neC12C 28	1-3	1-7	3	6	TGITGTAKYYYYGMDV	16	3-25	2	QSADSSGTVV	10	-	ND	-
neC12C 29	3-33	3-3	3	4	DFLRDFGVVILGY	13	2-11	3	CSYSDTSTRWV	11	-	ND	-
neC12C 33	4-39	3-22	2	5	AVLVYYDSSGQSNWFDV	17	1-51	2	GTWDSSLSAVV	11	-	ND	-
neC12C 43	4-34	2-2	2	6	FPYCSSTSCYYYYYMDV	18	2-14	2	SSYTSSSTPVV	11	-	ND	-
neC12C 47	4-31	2-2	3	3	DRRSALIPWAFDI	13	2-23	1	CSYAGSSFYV	10	-	ND	-

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E6: Repertoire and reactivity of antibodies from mature naive B cells of SMS patient Y1.

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vk	J	CDR3 (aa)	Length	Poly	Hep-2	Staining
mnSMSY1 49	4-34	1-26	2	6	GQSGSPLTDYYYYMDV	15	1-39	3	QQSYSTPFT	9	-	-	-
mnSMSY1 50	3-7	6-19	3	4	HTLIAVAGPEQVTGFDY	17	2-28	2	MQALQTPLYT	10	-	-	-
mnSMSY1 56#	4-4	3-3	2	6	VPGDFWSGSMFSYYGMDV	18	3-20	1	QQYGSSPWT	9			
mnSMSY1 57	3-23	3-22	2	4	AGDGGSGYSPLRSPFDY	17	1-8	1	QQYYSYPQT	9	-	+	-
mnSMSY1 58	1-2	6-13	2	3	HPTSKGGSWSDAFDI	15	3-15	1	QQYNNWPRT	9	-	-	-
mnSMSY1 59	3-48	1-26	3	5	DRVVGATNPMAP	12	4-1	4	QQYYSTPLT	9	-	-	-
mnSMSY1 60	3-23	3-22	3	4	GPITMIVVVEYYFDY	15	1-33	4	QQYDNLPSLT	10	-	-	-
mnSMSY1 62	3-23	2-2	3	4	DRPAALDY	8	1-5	1	QQYNSYWT	8	-	-	-
mnSMSY1 63	4-4	6-19	2	4	GPRSSGWYGPLYFDY	15	1-39	2	QQSYSTHT	8	-	-	-
mnSMSY1 66	3-30	3-16	3	4	DLGAFGGVIVIGYPDY	16	1-5	2	QQYNSYSYT	9	-	-	-
mnSMSY1 67	3-33	5-24	3	6	DAAMAPTLSDV	11	3-15	1	QQYNNWPPWT	10	-	-	-
mnSMSY1 68	1-3	5-12	3	5	DKQVATWWNWFDP	13	1-13	3	QQYYSTPD	8	-	-	-
mnSMSY1 74	3-21	1-20	2	3	AYNWNLDLDAFDI	12	1-5	1	QQYNSYWRT	9	-	+	-
mnSMSY1 77#	3-21	1-26	3	3	DRSGATYGVDI	11	1-39	5	QQSYSTPA	8			
mnSMSY1 80	3-7	3-22	2	4	GGPWWYYDSSGGYYLSY	16	3-20	4	QQYGSSPELT	10	-	-	-
mnSMSY1 84	1-69	3-16	3	6	DELMITFGGVIVRPPYYGMDV	22	2-28	4	MQALQTPQLT	10	-	-	-
mnSMSY1 88	3-11	3-3	2	6	ERGRRDYDFWSGYPPFYYYYYMDV	25	3-20	1	QQYGSSLWT	9	+	+	-
mnSMSY1 90#	3-15	3-3	2	4	TTRFWSGYTYIYFDY	16	3-11	4	QQRSN	5			
mnSMSY1 91	3-15	/	/	4	DLIGAKHHP	9	3-11	4	QQRSNWPPLT	10	-	-	-
mnSMSY1 96#	1-18	6-13	3	4	DLAVAAPSIAAGLGDY	16	3-15	3	QQYNNWPLFT	10			
mnSMSY1 51							3-15	4	QQYNNWPLT	10			
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	Hep-2	Staining
mnSMSY1 52	3-23	3-22	2	4	DYYYDSSGYYYESYFDY	17	3-9	1	QVWDSSTGV	9	-	-	-
mnSMSY1 55	1-2	3-3	3	6	DDLSPRFTIFGVVTRGGV	18	1-36	3	AAWDDSLNGPV	11	-	-	-
mnSMSY1 60					SEE KAPPA		3-25	2	QSADSSGTYVV	11	-	-	-
mnSMSY1 61	4-59	2-21	2	3	RKNYCGGDCYEGQDAFDI	18	1-51	3	GTWDSSLSAGV	11	-	-	-
mnSMSY1 71	4-39	4-11	1	4	LYVKLQECFDY	11	1-40	2	QSYDSSLSGPE	11	+	-	-
mnSMSY1 76#	5-a	6-19	2	2	LTRLVSDLRSGWYKRGNWYFDL	22	2-14	2	SSYTSSSTVV	10			
mnSMSY1 78#	5-a	3-10	2	4	FHGSGSYVVDY	11	3-1	2	QAWDSSTTVV	10			
mnSMSY1 79	3-30	3-22	2	4	GYYYDSSEFDY	11	6-57	3	QSYDQFWV	8	-	-	-
mnSMSY1 75							2-14	2	SSYTSSSTVV	10			
	VH	D	RF	JH	CDR3 (aa)	Length							
mnSMSY1 75	1-18	4-17	3	4	DGAHIVAVTATWVAYFDY	18							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E7: Repertoire and reactivity of antibodies from mature naive B cells of SMS patient Y2.

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vk	Jk	CDR3 (aa)	Length	Poly	HEp-2	Staining
mnSMSY2 49	1-46	4-17	2	3	EGGDYARDAFDI	12	3-15	3	QQYNNWPPIFT	11	-	-	-
mnSMSY2 52	3-11	3-10	3	6	DPSREGMVRGVNSPNSYYYYYGMDV	25	3-11	1	QQRSNWPPIT	10	-	+	-
mnSMSY2 53#	1-46	5-24	3	4	EMATKPDPFDY	10	3-11	4	QQRSNWLT	8	-	-	-
mnSMSY2 56#	3-33	6-6	3	4	DRIAARSMEFDY	12	1-5	1	QQYNSYSWT	9			
mnSMSY2 58#	1-2	3-10	2	3	GRDYYGSGSYRSPYAFDI	19	1-13	3	QQFNSYPQKALT	12			
mnSMSY2 60	3-53	3-22	2	4	EVDYYDSSGGYYDY	13	1-5	2	QQYNSYSPYT	10			
mnSMSY2 64	4-59	3-22	2	4	TNYYDSSGGYYLDRDY	15	1-5	2	QQYNSPYT	8	-	-	-
mnSMSY2 67	3-30	6-19	3	4	DLGIAVAGTGY	11	1-5	2	QQYNSYSPYT	10	+	-	-
mnSMSY2 69	4-31	3-10	2	4	DGGRNYYGSDY	11	1-39	3	QQSYSTPPFT	10	-	-	-
mnSMSY2 70#	3-64D	4-17	3	4	ELNTVTYEGDY	11	1-33	3	QQYDNLFT	8			
		4-23											
mnSMSY2 71	3-30	5-5	2	4	DRGYSLDY	8	3-15	4	QQYNNWLLT	9	-	-	-
mnSMSY2 74	4-59	5-24	3	4	LSVEMATTLFDY	12	1-39	3	QQSYSTPPFT	10	-	-	-
mnSMSY2 75	3-23	3-22	2	3	DPSTYYYDSSGGGAFDI	17			BAD SEQ		-	-	-
mnSMSY2 78	3-7	2-15	3	6	GDIWVVVAATLTLPNYYYYYGMDV	24			BAD SEQ		-	+	-
mnSMSY2 79	1-3	2-2	3	5	LMDNLPAAIPRSWFDP	16	1-5	2	QQYNSYSYT	9	-	-	-
mnSMSY2 80	3-23	3-22	2	4	DRLLGDSGY	9	1-5	2	QQYNSYSYT	9	-	-	-
mnSMSY2 82#	5-51	3-9	2	5	LQYYDILTGYNNVRGSWFDP	20	2-28	2	MQALQTLTY	9			
mnSMSY2 83	3-30	6-19	1	6	EQGRLVNNYEGVDV	14	2-28	2	MQALQTPRT	9	-	-	-
		4-11	2										
	VH	D	RF		CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mnSMSY2 54	3-73	4-23	2	6	RGYGGNASGLGMDV	14	3-21	3	QVWDSSSDHWV	11	-	+	-
mnSMSY2 57#	1-69	3-16	3	6	GVWGDYTPYY	10	2-28	2	SSYAGSNNGV	10			
mnSMSY2 62#	3-23	3-10	2	4	TLYGSGSGGVDY	12	3-1	1	QAWDSSTASYV	11			
mnSMSY2 65#	1-69	1-26	2	4	DQGSYYRY	8	2-14	2	SSYTSSSTLGV	11			
mnSMSY2 66#	4-59	3-10	3	5	DGAPITMVRGVIFGSGGNWFDP	22	7-43	3	LLYYGGAQGV	10			
mnSMSY2 76#	1-3	3-16	1	4	GRLGELSWFDY	11	1-44	2	AAWDDSLNGP	10			
mnSMSY2 85#	1-8	3-22	2	5	LVDSSGYYYIGNWFDP	16	1-40	1	QSYDSSLGPPYV	13			
mnSMSY2 63							2-14	2	SSYTSSSTLEGV	12			
	VH	D	RF		CDR3 (aa)	Length							
mnSMSY2 81#	5-51	5-24	1	5	HGRWLQFSWFDP	12							
mnSMSY2 84#	3-53	3-10	2	6	DKGSGSYNFDYYYYYGMDV	19							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E8: Repertoire and reactivity of antibodies from mature naive B cells of SMS patient Y7

Ig	HEAVY						LIGHT				REACTIVITY					
	VH	D	RF	JH	CDR3 (aa)	Length	V	J	CDR3 (aa)	Length	Poly	HEp-2	Staining			
mnSMSY7 51#	4-34	3-10	1	1	GRTPVLHTSFQH	12	3-15	1	QQYNNWPPGRT	11						
mnSMSY7 59#	4-61	3-9	2	4	GLTYDILTGYSEFDY	15	3-20	3	QQYGSSPTT	9						
mnSMSY7 62	4-30	6-19	3	4	ESGIAVAGTIIFDY	14	1-6	1	LQDYNYPRT	9						
mnSMSY7 64#	1-3	2-15	2	6	IYCSGGSCSQAYYGMDV	17	1-5	1	QQYNSYWT	8						
mnSMSY7 65	4-31	6-13	2	3	RGSSSSTNAFDI	12	1-33	4	QQYDNLPLT	9						
mnSMSY7 73	3-30	6-13	2	6	DLAGSSSLGMDV	12	1-5	2	QQYNSYSRYT	10						
mnSMSY7 78	4-34	5-24	2	4	DGDREVARGFRPFDY	15	3-15	4	QQYNNWPPLT	10	-	-	-			
mnSMSY7 80	3-30	5-5	3	3	YRVDTAMVDAFDI	13	1-5	2	QQYNSYPLA	9	-	-	-			
mnSMSY7 87#	1-8	3-3	1	6	QLRFLEWLDYYGMDV	15	3-11	2	QQRSNWPYT	9						
mnSMSY7 88	3-30	6-19	2	4	GSPSSGWYFDY	11	3-20	4	QQYGSSPSLT	10						
mnSMSY7 58							1-39	2	QQSYSTPRT	9						
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	Hep2	Staining			
mnSMSY7 53	4-31	4-4	3	4	FLSPTTVTIFDY	12	1-51	2	GTWDSSLSARV	11	-	+	-			
mnSMSY7 54	3-9	1-7	1	6	VELRFAYYYYGMDV	14	1-47	3	AAWDDSLSGGV	11	-	+	c			
mnSMSY7 61	1-8	6-19	2	6	GRGVGSGWYGEQDYYYYGMDV	21	1-40	2	QSYDSSLSGWV	11	-	-	-			
mnSMSY7 63	3-30	5-5	2	4	DGYSIDY	7	1-51	2	GTWDSSLSAVV	11	-	-	c			
mnSMSY7 68	3-21	/	/	6	DHTGMDV	7	1-40	1	QSYDSSLSGYV	11	-	-	-			
mnSMSY7 77	3-30	5-12	2	4	GVTDSGYDCDFDY	13	2-14	3	SSYTSSSTVV	10	-	-	-			
mnSMSY7 90	3-30	/	/	4	DQVLFFDY	8	1-51	3	GTWDSSLSAVV	11	-	-	c			
mnSMSY7 95	4-30	3-10	2	4	EMWGSGPLGFY	11	2-14	3	SSYTSSSTLV	10	-	-	-			
mnSMSY7 82	4-61	3-22	2	4	GPYYDSSGYELGY	14.0	2-14	3	SSYTSSSTHV	11	-	-	-			
mnSMSY7 72							7-46	2	LLSYSGARPV	10						
	VH	D	RF	JH	CDR3 (aa)	Length										
mnSMSY7 85	3-30	2-21	2	6	ERCGGDCYFYYYYGMDV	18										
mnSMSY7 94	4-39	1-7	2	6	HFLNNWNYWSYYYYGTDV	18										

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E9: Repertoire and reactivity of antibodies from mature naive B cells of CVID patient C12B.

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vk	Jk	CDR3 (aa)	Length	Poly	HEp-2	Staining
mnC12B 52	3-48	3-3	2	4	EYDFWSGYDY	10	1-16	1	QQYNSYPRT	9	-	-	-
mnC12B 59	4-39	4-17	3	3	EEATVTT	7	2-30	4	MQGTHWPLT	9	-	-	-
mnC12B 61	3-23	6-19	3	4	AKLNIALDY	9	3-11	1	QQRSNWWT	8	-	-	-
mnC12B 64#	4-34	3-3	3	6	GLITIFGVVLGMDV	14	3-11	3	QQRSNWPPGGT	11			
mnC12B 65	4-34	3-3	3	1	RSGITIFGVVIPTGYFQH	18	1-8	3	QQYYSYPFT	9	+	+	c
mnC12B 70	4-39	2-2	2	6	HRDDICSSTSCYYYYYMDV	20	4-1	4	QQYYSTLALT	10	-	-	-
mnC12B 74	1-69	2-21	2	6	PCVGGDCEYYYYGMDV	16	1-39	1	QQSYSTPPT	9	-		-
mnC12B 77	3-30	3-3	2	6	ELNDFWSGYPEGFYGMDV	19	1-16	2	QQYNSYPYT	9	-	-	-
mnC12B 79#	1-69	2-2	3	6	DCGFCDVVPAAATHYHYYYMDV	22	2-28	1	MQALQTPRT	9			
mnC12B 80	1-69	6-19	2	5	ARSSGWYNWFDP	12	1-33	4	QQYDNLPLT	9	-	-	-
mnC12B 83	3-23	1-7	2	4	DGENWRDLPLYFDY	14	4-1	3	QQYYSTPFT	9	-	-	-
mnC12B 86	3-33	6-19	2	6	GLQGEDSSGWYDYYYYYGMDV	21	1-9	2	QQLNSYPYT	9	-	+	-
mnC12B 87	4-34	2-2	3	5	GWKIVVVPAAIFWVLGACGPFDP	23	1-39	1	QQSYSTPGT	9	-	+	-
mnC12B 95	4-34	4-11	2	6	ARGGNYAWLAYMDV	14	1-39	1	QQSYSTPRT	9	-	-	-
mnC12B 96	1-69	3-3	2	6	ARGPPFDYDFWSGYDAYYGMDV	23	1-5	5	QQYNSYSGT	9	-	-	-
mnC12B 53							1-39	2	QQSYSTPYT	9			
mnC12B 84							4-1	1	QQYYSTPT	8			
	VH	D	RF		CDR3 (aa)	Length	VI	Jl	CDR3 (aa)	Length	Poly	HEp-2	Staining
mnC12B 49	3-43	3-22	2	6	DIGYYDSSGYYYPPYYGMDV	20	1-47	2	AAWDDSLSGVV	11	-	-	-
mnC12B 55	4-34	2-2	3	4	GLGVVVPAAAITLGGFDY	18	1-40	1	QSYDSSLGYV	11	-	-	-
mnC12B 56	4-61	2-21	2	4	GPEGESSYSGFDY	13	3-1	2	QAWDSSTVV	9	-	-	-
mnC12B 66	4-34	3-22	2	5	EYDSTIGRLDP	11	2-14	2	SSSKV	5	-	-	-
mnC12B 68	3-30	2-2	2	6	GPKRDAYCSSTSCYTQYYYYYYGMDV	27	3-1	2	QAWDSSTSGVV	11	-	-	-
mnC12B 72	4-B	6-13	2	6	VFSSSWYPPYYYGMDV	17	8-61	3	VLYMGSSWV	9	+	+	c
mnC12B 76	3-33	6-19	3	4	NPGIAVAGWYFYDY	14	3-21	2	QVWDSSSDHVV	11	-	-	-
mnC12B 82	3-15	6-6	3	4	GPSTISIAY	9	1-47	2	AAWDDSLSGPL	11	-	-	c
mnC12B 89	3-53	3-3	2	3	TPGPYDFWSGYGVTPRDAFDI	22	1-47	3	AAWDDSLGLVWV	13	-	-	-
	VH	D	RF		CDR3 (aa)	Length							
mnC12B 60	4-39	1-26	2	4	DLEGSSPFDY	10							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

Table E10: Repertoire and reactivity of antibodies from mature naïve B cells of CVID patient C12C

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vk	Jk	CDR3 (aa)	Length	Poly	HEp-2	Staining
mnC12C 04	3-73	3-16	2	4	PGADGGDY	8	3-15	3	QQYNNWPPFT	10	-	-	-
mnC12C 05	4-31	4-17	2	6	DFGGYGDYGNPYYYYGMDV	19	3-11	2	QQRSNWPPRYT	11	-	-	-
mnC12C 06	1-46	4-11	3	6	SGTTVSYYYYYGMDV	15	1-12	3	QQANSFPF	8	-	-	-
mnC12C 11#	4-31	4-17	2	3	EYGDHDAFDI	10	1-33	3	QQNDNLLVT	9			
mnC12C 17	4-39	3-3	3	6	EWRTVVIIYYYGMDV	15	1-12	5	QQANSFPIT	9	-	-	A, F
mnC12C 20	4-59	6-19	2	6	MGGQNSRSGWYYEKDHYYGMDV	22	3-20	1	QQYGSSPWT	9	+	+	-
mnC12C 21	3-23	2-2	3	6	EAKYQLTDIVVPAATNHRDYYYYYMDV	28	4-1	4	QQYYSTLLT	9	-	-	-
mnC12C 23	3-49	2-2	3	4	YPPWGEVVPAAIEYFDY	18	1D-17	5	LQHNSYPFT	9	-	-	-
mnC12C 28	1-69	2-21	2	6	LSRPYCGGDCTTDYYYYYMDV	21	3-15	4	QQYNNWPLT	9	-	-	-
mnC12C 29	4-B	2-2	3	6	DDIVVVPAAIRGAYYYYYGMDV	22	3-11	3	QQRSNWSFT	9	-	-	-
mnC12C 33	3-23	7-27	2	4	GVGRSGEEIY	10	1-33	5	QQYDNLPIIT	9	-	-	-
mnC12C 38#	1-2	3-10	3	4	GYTMVRGVIIKIRGGDHFDY	20	3-20	2	QQYGSSPYT	9			
mnC12C 41	4-34	3-10	3	5	GRTAGVRHGWFDY	13	3-15	2	QQYNNWPPYT	10	-	-	-
mnC12C 44	3-49	6-6	2	4	VSSSSAPGGY	10	1D-33	4	QQYDNLPLT	9	-	-	-
mnC12C 45#	1-69	3-16	3	3	RGPGPFGGVIVRPLGGDDDAFDI	23	3-15	1	QQYNNWPRT	9			
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mnC12C 10	4-59	5-12	2	4	DIGYGQHDY	9	2-23	3	CSYAGSSTFR	10	-	-	-
mnC12C 11					see kappa		2-23	1	CSYAGSSTFSYV	12	-	-	-
mnC12C 12	1-46	4-23	1	4	ELRWYIDY	8	2-14	1	SSYTSSSTHV	10	-	+	-
mnC12C 13	4-59	3-3	2	4	ADYDFWSGYYYFDY	14	2-14	2	SSYTSSSTSVV	11	-	-	-
mnC12C 19	1-69	2-2	3	6	DRVVVVPAARVTTGIGYYYGMDV	23	2-14	2	SSYTSSSTLVV	11	-	-	-
mnC12C 20					see kappa		2-14	2	SSYTSGSTVV	10	-	-	-
mnC12C 22	3-33	3-3	2	4	RMGYIDDFWSPIDY	15	3-21	3	QVWDSSSDHHVV	12	-	-	-
mnC12C 30	4-34	2-2	3	6	VGTPYIVVPAAIGNHYGMDV	21	2-23	1	CSYAGSSTYYV	11	-	-	-
mnC12C 36	3-48	3-3	2	6	DRSTYYDFWSGYYTNDYYYYYMDV	24	2-14	1	SSYTSSSTPYV	11	+	+	-
mnC12C 39	4-39	2-2	3	4	GPIVVVPAAILGET	14	2-23	1	CSYAGSSTYV	10	-	-	-
mnC12C 47#	1-69	1-26	1	3	ELEGINPHAIDI	12	2-14	2	SSYTSSSTLAVV	12			
mnC12C 48#	3-21	3-3	2	6	DNYDFWSALRTYYYYYYYMDV	21	2-14	2	SSYTSSSTLV	10			
mnC12C 25							2-11	2	CSYAGSYTSVV	11			

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

c, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

ND, not determined

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Figure E1. New emigrant/transitional B cells from Smith Magenis Syndrome (SMS) patients and patients with a 204insA *TNFRSF13B* mutation do not express antinuclear B-cell receptors. Recombinant antibodies derived from single new emigrant/transitional B cells of healthy donors, SMS patients and patients with one 204insA *TNFRSF13B* mutation do not stain nuclear structures on slide-mounted HEp-2 cells as frequently as recombinant antibodies derived from single new emigrant/transitional B cells of healthy carriers or CVID patients with a C104R or A181E *TNFRSF13B* mutation. The frequencies of ANA expressing clones is displayed for individual subjects and as a mean (black bar) for each subject group. Statistical significance by unpaired Student t-testing is indicated.

Figure E2. Similar TACI expression in B cells carrying the wildtype or the C104R mutated *TNFRSF13B* allele. Using the gating strategy shown in (A) to analyze freshly isolated (B) naïve or (C) memory CD19⁺ purified B cells for TACI expression using a polyclonal anti-TACI antibody which does recognize C104R mutated TACI or monoclonal anti-TACI antibody which does not. Histograms (left) display expression from representative patients, bar graphs (right) represent the mean TACI MFI from all subjects analyzed. Error bars represent the mean $\pm$ SEM. Statistical significance by unpaired student t-test is indicated.

Figure E3. *TNFRSF13B* hemizyosity results in decreased TACI expression on memory B cells activated with the TLR7 agonist gardiquimod. Histograms display TACI expression on CD19⁺CD21⁺CD27⁻ naïve (upper panel) and CD19⁺CD21⁺CD27⁺ memory B cells (lower panel) from a representative healthy donor and Smith Magenis Syndrome patient after 48hrs of gardiquimod (1 μ g/ml) stimulation *in vitro*. The bar graph (right) represents TACI induction on indicated B cell populations from healthy donors (n=6) and SMS patients (n=3). Error bars represent the mean $\pm$ SEM. Statistical significance by unpaired student t-test is indicated.

Figure E4: Altered proliferative responses in Smith Magenis Syndrome (SMS) memory B cells. Histograms display proliferation by CFSE dilution of naïve (A) and memory B cells (B) from healthy donors (n=2) and SMS patients (n=2) after 6 days in the indicated culture conditions. Gating denotes the frequency of cells undergoing 2 or more divisions.

Figure E5. Normal CD27⁻ B cell subsets in Smith Magenis Syndrome (SMS) patients and

patients with a 204insA *TNFRSF13B* mutation. Dot plots (right) of (A) CD27⁺CD21^{lo}CD10⁺IgM^{hi} new emigrant B cells and (B) CD27⁺CD19^{hi}CD21^{lo} B cells from a healthy donor (female, 30 years), an SMS patient (female, 29 years) and a CVID patient with a heterozygous C104R *TNFRSF13B* mutation (female, 45 years), graphs (left) display cell frequencies for each individual subject and as a mean (black bar) for all enrolled subjects. Statistical significance by unpaired student t-test is indicated.

Figure E6. Polyreactive clones do not emerge in the mature naïve B-cell pool of Smith Magenis Syndrome (SMS) patients or patients with a 204insA *TNFRSF13B* mutation. **(A)** Recombinant antibodies derived from mature naïve B cells of healthy donors, SMS patients, patients with a 204insA *TNFRSF13B* mutation, healthy carriers with a C104R or A181E *TNFRSF13B* mutation and CVID patients with a C104R or A181E *TNFRSF13B* mutation were tested for polyreactivity via ELISA. **(B)** The frequency of polyreactive mature naïve B cells clones is displayed for each individual subject and as a mean (black bar) for each subject group. Statistical significance by unpaired student t-test is indicated.

Figure E7. Normal Treg frequency in patients with *TNFRSF13B* hemizygosity. Dot plots represent CD4⁺ gated CD25^{hi}FOXP3⁺ T cells of a healthy donor control, a Smith Magenis Syndrome patient, a patient with a 204insA mutation and his brother a CVID patient with one C104R *TNFRSF13B* mutation.

Figure E8. Tregs from Smith Magenis Syndrome (SMS) patients or patients with a 204insA *TNFRSF13B* mutation display normal suppressive capacity. **(A)** Representative histograms of Treg-mediated suppression of autologous and heterologous CFSE labeled Tresp cells on day 4.5 from a healthy donor (top row), her daughter, a SMS patient (second row), a male patient with a 204insA *TNFRSF13B* mutation (third row) and his younger brother a CVID patient with a C104R *TNFRSF13B* mutation (bottom row). **(B and C)** Heterologous Treg suppression using T effector cells and Tregs from healthy donors (n=21) SMS patients (n=3), patients with a 204insA *TNFRSF13B* mutation (n=2), healthy carriers of one C104R or A181E *TNFRSF13B* mutation (n=3) and CVID patients with one C104R or A181E *TNFRSF13B* mutation (n=3). Inhibition in each assay is displayed as a percentage of T effector cell proliferation in the absence of Tregs. The mean percent inhibition is represented by a black bar. Statistical significance by unpaired student t-testing is indicated.

Figure E9. Circulating B-cell Activating Factor (BAFF) concentrations are not elevated in Smith Magenis Syndrome (SMS) patients or patients with a 204insA *TNFRSF13B* mutation. Log plasma BAFF concentrations (pg/ml) as determined by ELISA are displayed for individual

healthy donors, SMS patients, patients with a 204insA *TNFRSF13B* mutation, healthy carriers with a C104R or A181E *TNFRSF13B* mutation and CVID patients with a C104R or A181E *TNFRSF13B* mutation. The mean BAFF concentration in each subject group is represented by a black bar. Statistical significance by unpaired Student t-testing is indicated.

Figure E10. T follicular helper cells are not expanded in the peripheral blood of Smith Magenis Syndrome patients or patients with one 204insA *TNFRSF13B* mutation.

Representative dot plots and histograms of peripheral CD4⁺ T cells reveal PD-1^{hi}CXCR5⁺BCL6⁺ICOS^{hi} T follicular helper cells are expanded in the peripheral blood of CVID patients with one C104R or one A181E *TNFRSF13B* mutation.

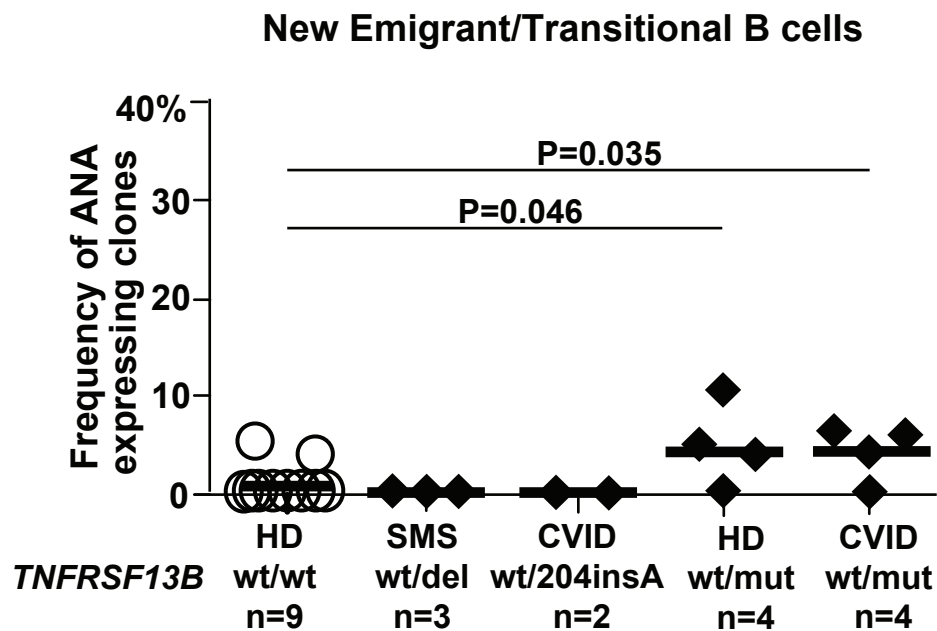


Figure E1. New emigrant/transitional B cells from Smith Magenis Syndrome (SMS) patients and patients with a 204insA *TNFRSF13B* mutation do not express antinuclear B-cell receptors. Recombinant antibodies derived from single new emigrant/transitional B cells of healthy donors, SMS patients and patients with one 204insA *TNFRSF13B* mutation do not stain nuclear structures on slide-mounted HEp-2 cells as frequently as recombinant antibodies derived from single new emigrant/transitional B cells of healthy carriers or CVID patients with a C104R or A181E *TNFRSF13B* mutation. The frequencies of ANA expressing clones is displayed for individual subjects and as a mean (black bar) for each subject group. Statistical significance by unpaired Student t-testing is indicated.

Figure E2

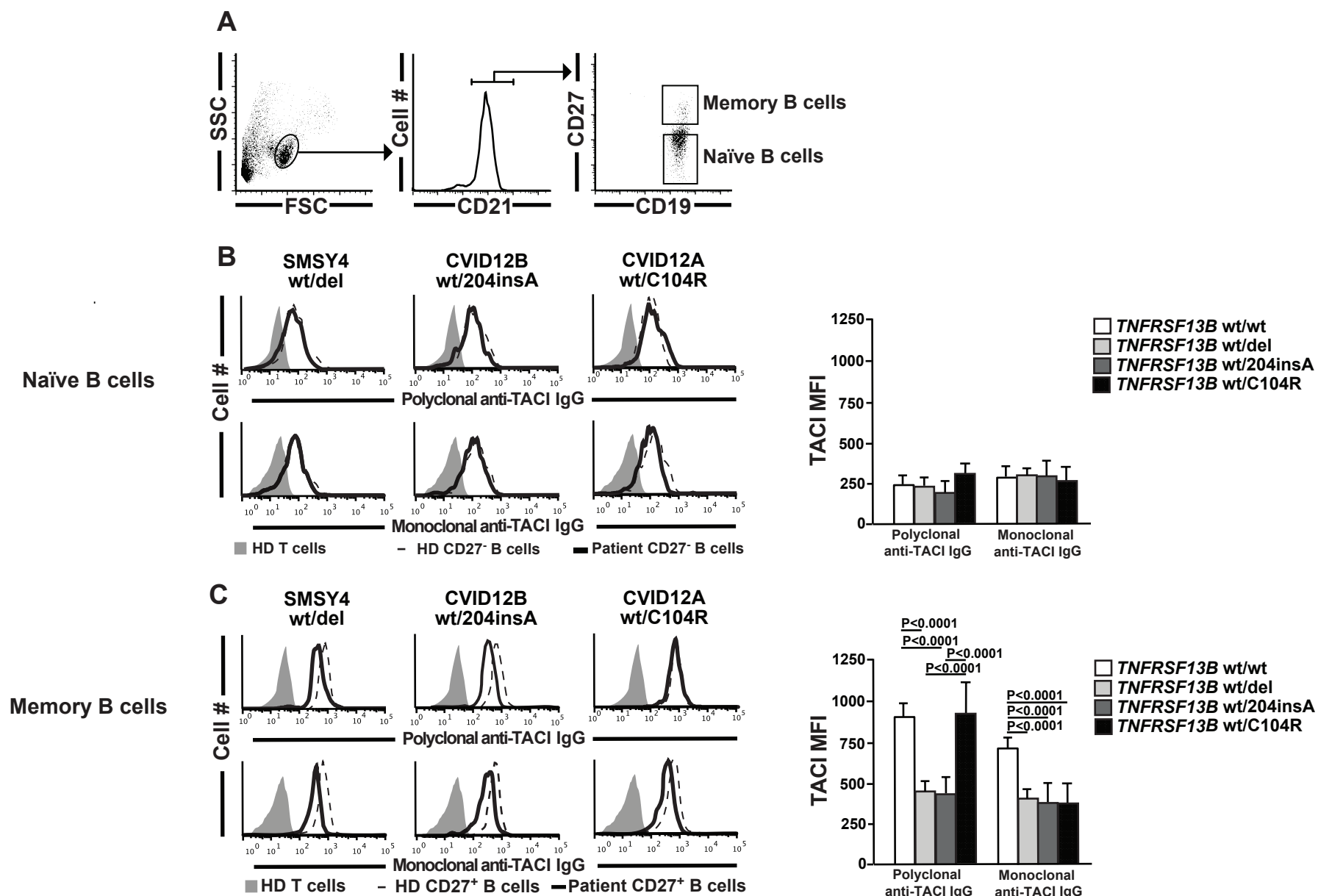


Figure E2. Similar TACI expression in B cells carrying the wildtype or the C104R mutated *TNFRSF13B* allele. Using the gating strategy shown in (A) to analyze freshly isolated (B) naïve or (C) memory CD19⁺ purified B cells for TACI expression using a polyclonal anti-TACI antibody which does recognize C104R mutated TACI or monoclonal anti-TACI antibody which does not. Histograms (left) display expression from representative patients, bar graphs (right) represent the mean TACI MFI from all subjects analyzed. Error bars represent the mean $\pm$ SEM. Statistical significance by unpaired student t-test is indicated.

Figure E3

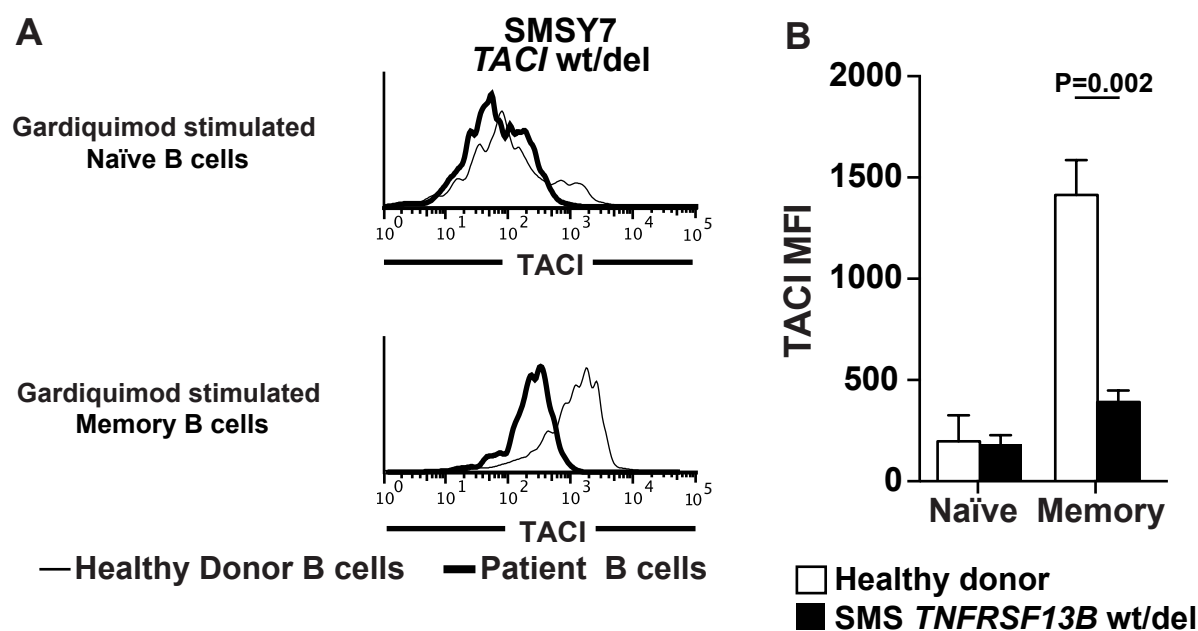


Figure E3. *TNFRSF13B* hemizyosity results in decreased TACI expression on memory B cells activated with the TLR7 agonist gardiquimod. Histograms display TACI expression on CD19+CD21+CD27- naïve (upper panel) and CD19+CD21+CD27+ memory B cells (lower panel) from a representative healthy donor and Smith Magenis Syndrome patient after 48hrs of gardiquimod (1µg/ml) stimulation *in vitro*. The bar graph (right) represents TACI induction on indicated B cell populations from healthy donors (n=6) and SMS patients (n=3). Error bars represent the mean ± SEM. Statistical significance by unpaired student t-test is indicated.

Figure E4

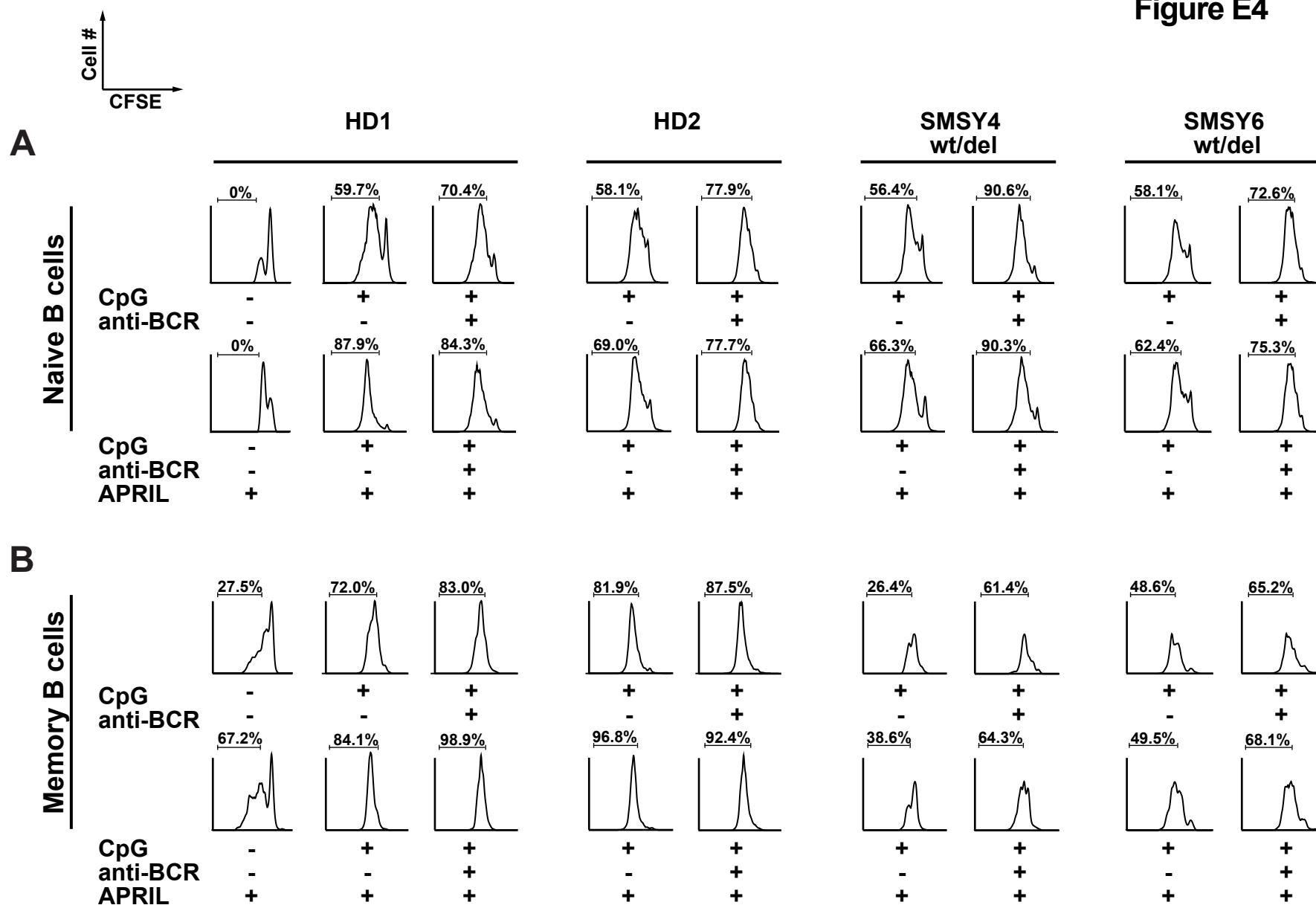


Figure E4: Altered proliferative responses in Smith Magenis Syndrome (SMS) memory B cells. Histograms display proliferation by CFSE dilution of naïve (A) and memory B cells (B) from healthy donors (n=2) and SMS patients (n=2) after 6 days in the indicated culture conditions. Gating denotes the frequency of cells undergoing 2 or more divisions.

FIGURE E5

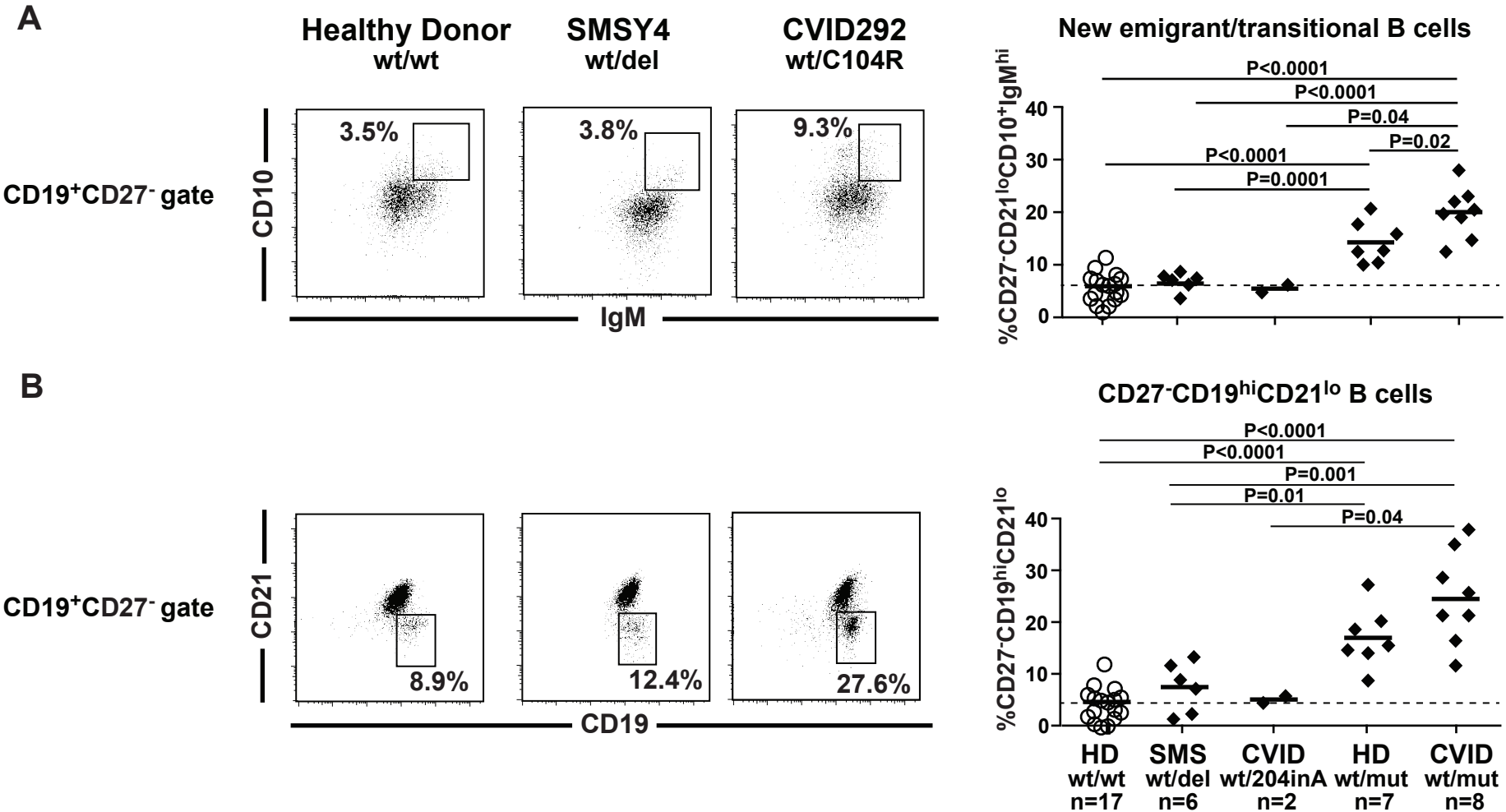


Figure E5. Normal CD27⁻ B cell subsets in Smith Magenis Syndrome (SMS) patients and patients with a 204insA *TNFRSF13B* mutation. Dot plots (right) of (A) CD27⁻CD21^{lo}CD10⁺IgM^{hi} new emigrant B cells and (B) CD27⁻CD19^{hi}CD21^{lo} B cells from a healthy donor (female, 30 years), an SMS patient (female, 29 years) and a CVID patient with a heterozygous C104R *TNFR13B* mutation (female, 45 years), graphs (left) display cell frequencies for each individual subject and as a mean (black bar) for all enrolled subjects. Statistical significance by unpaired student t-test is indicated.

Figure E6

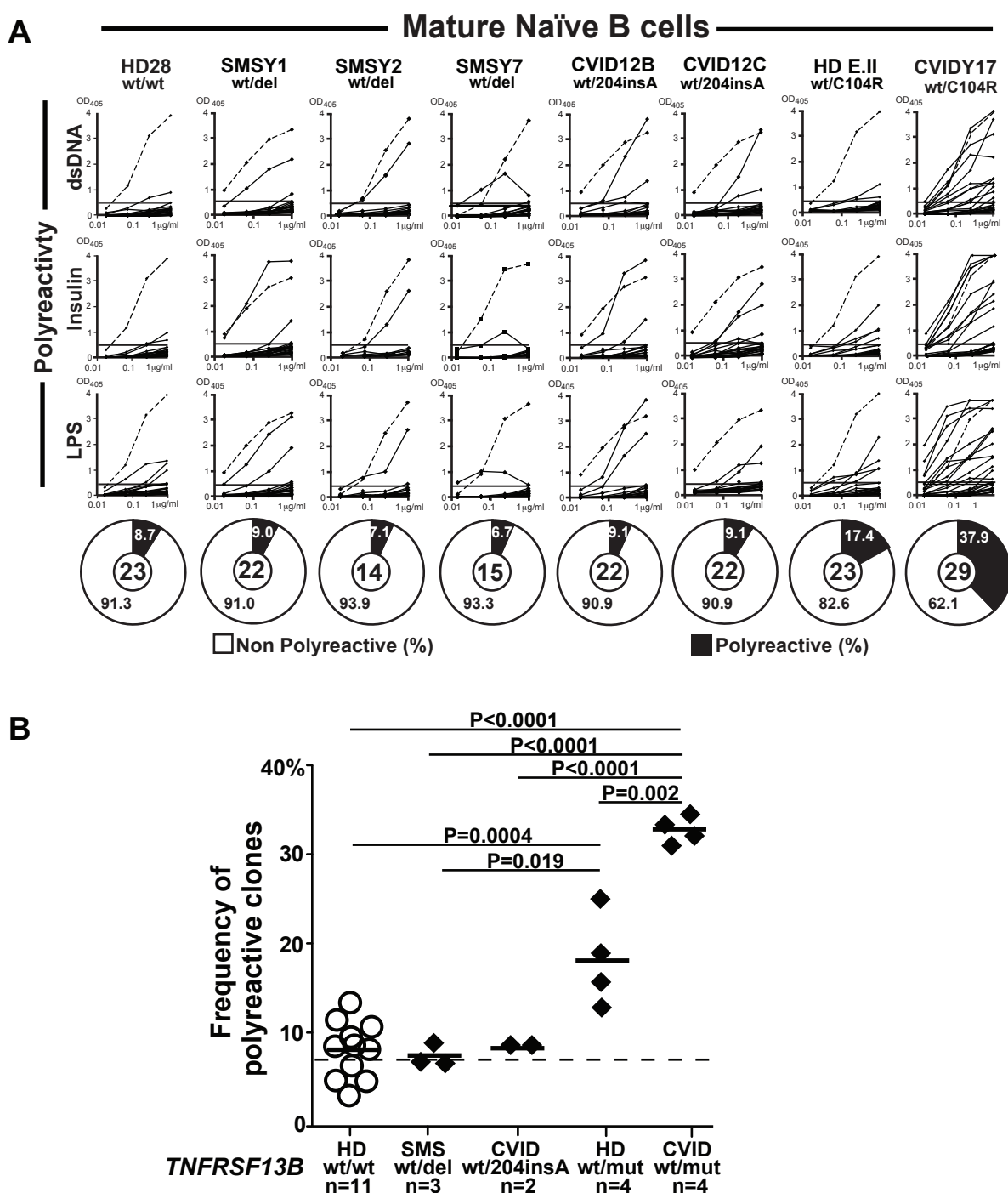


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Figure E7

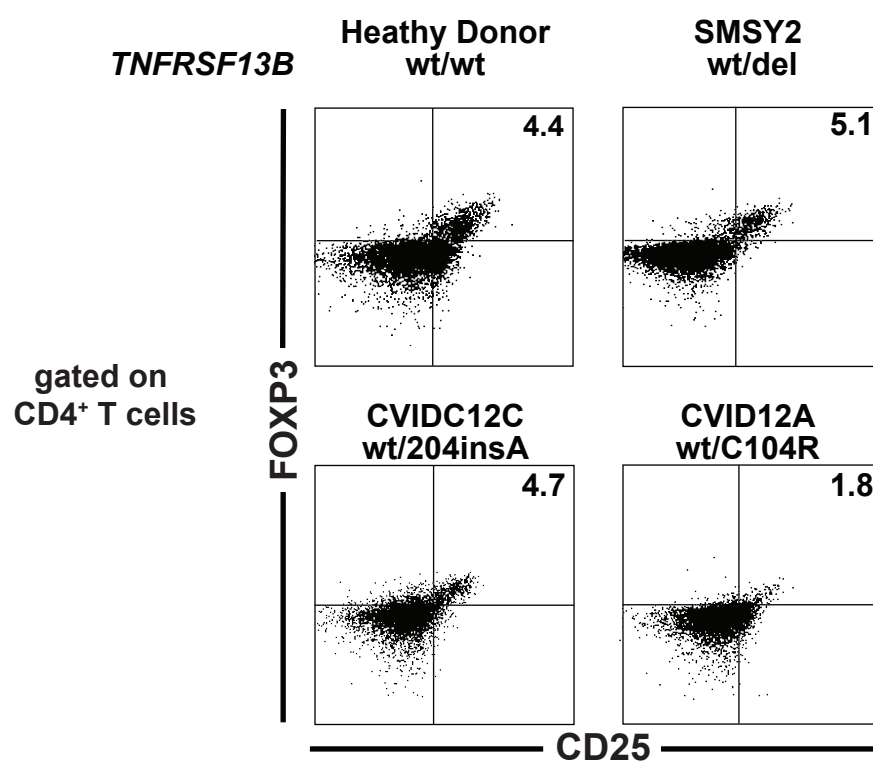


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Figure E8

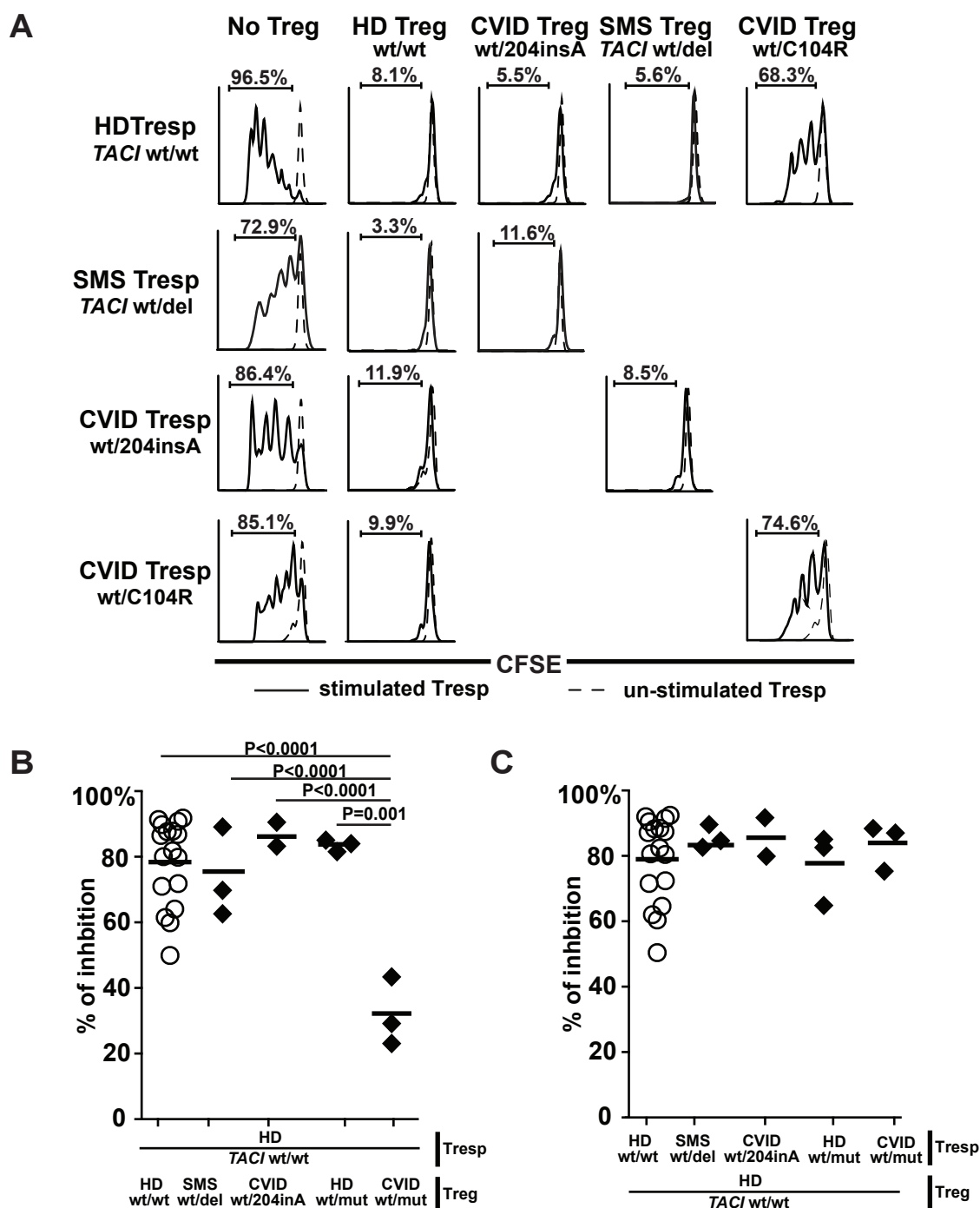


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Figure E9

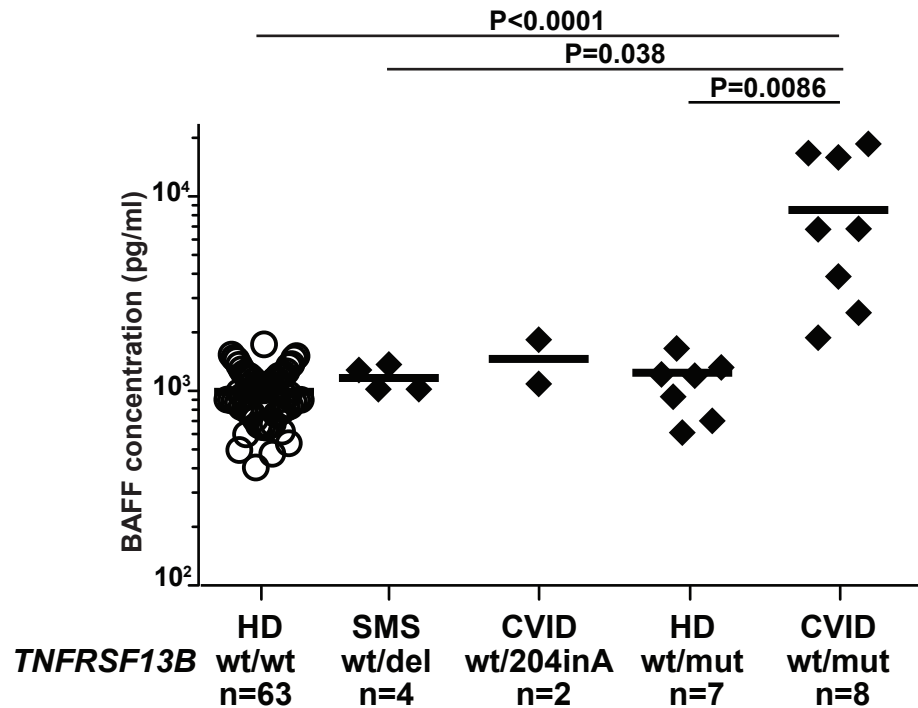


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Figure E10

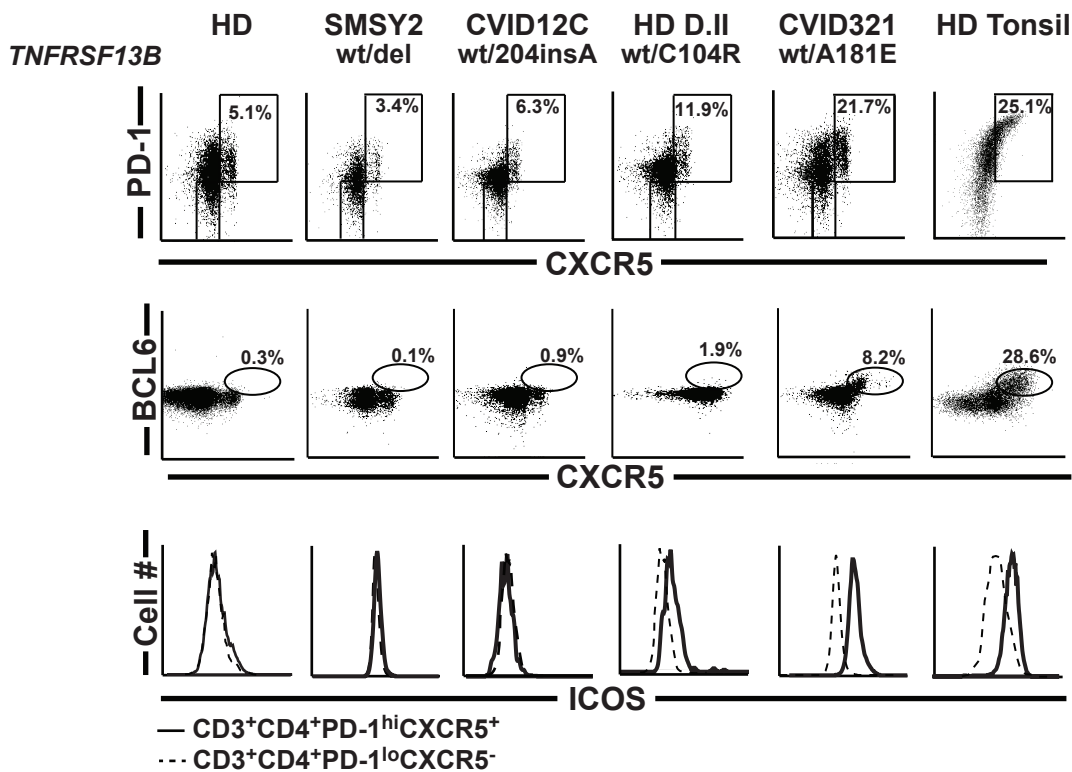


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