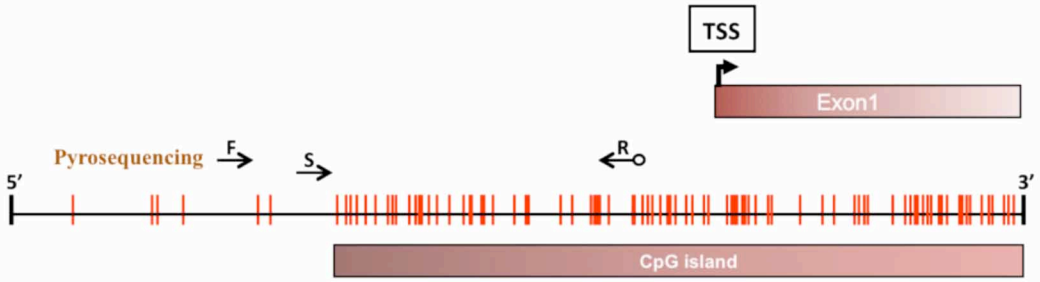
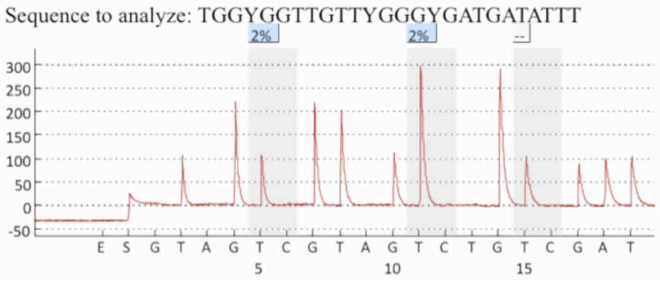
A



B

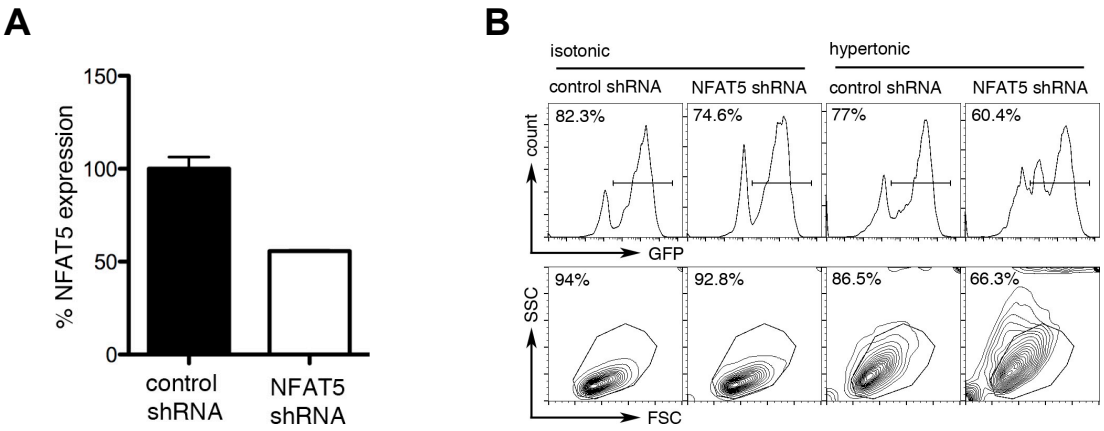


C

Methylation levels of NFAT5 in cases and controls

	N	% NFAT5 Methylation (Mean ± SD)	*p-value*
Cases	4	0.37 ± 0.75	0.28
Controls	7	0.89 ± 0.69	

Supplemental Figure 1. Methylation analysis of NFAT5 promoter lesion. (**A**) Schematic illustration of NFAT5 genomic loci. Orange box: CpG island; vertical tick marks: CpG sites; black arrows: pyrosequencing primer location; TSS: Transcription start site; F, Forward primer; R, Reverse primer, and S, Sequencing primer. (**B**) Results of bisulfite pyrosequencing of NFAT5. Grey horizontal bars represent the mean methylation levels; grey vertical boxes in the pyrograms illustrate individual CpG sites analyzed. (**C**) Summarizes methylation levels of NFAT5 in cases and controls. P-value was calculated with a one-way ANOVA.



Supplemental Figure 2. Assessment of survival in hyperosmotic condition by flow cytometry. (**A**) Jurkat cells were retrovirally transduced with control shRNA or NFAT5 shRNA constructs. RNA was extracted from transduced, sorted GFP$^+$ Jurkat cells, and cDNA was synthesized using reverse transcription. NFAT5 mRNA expression level was measured using qPCR using β-actin as a reference gene. (**B)** Jurkat cells were retrovirally transduced with control or NFAT5 shRNA constructs and cultured in isotonic (280 mOsm/kg) or hypertonic (420 mOsm/kg) media. Cell survival was assessed by flow cytometry after 5 days. Cells expressing these constructs were detected and gated on the basis of GFP positivity. The percentage of live lymphocytes was determined by forward scatter (FSC) and side scatter (SSC) properties. Viability in hyperosmotic condition was expressed as % cell death and calculated with the following equation: (% live lymphocytes in isotonic conditions – % live lymphocytes in hypertonic conditions) / % live lymphocytes in isotonic conditions. The same methodology was applied to assess survival of human PBMCs subjected to hypertonic conditions.

Supplemental Table I. Laboratory Evaluation

Variable	Reference Range, Adults	Patient
Serum Osmolality (Calculated)[1]	278-305	284
Immunoglobulin analysis[1]		
Immunoglobulin G (mg/dL)	522 – 1703	1078
Immunoglobulin A (mg/dL)	70 – 400	82
Immunoglobulin M (mg/dL)	28 – 179	106

Immunoglobulin G Sub-class analysis[1]	**Normal range**	**Month 0**	**Month 18**
IgG Sub-Class 1 (mg/dL)	382 – 929	594	736
IgG Sub-Class 2 (mg/dL)	241 – 700	134	145
IgG Sub-Class 3 (mg/dL)	22 – 178	150	197
IgG Sub-Class 4 (mg/dL)	4 – 26	< 1.0	< 1.0
Neutrophil Function[2]			
Oxidative Burst			
(% Oxidation positive Neutrophils)	≥ 90		96
Regulatory T cell analysis			
Foxp3[2]			
% Positive of $CD4^+CD25^+CD127^-$	87 – 100		92
Absolute Foxp3 (cells/mcL)	23 – 232		36
% Natural Tregs (% CD4 cells)[3]	0.6 – 4.3		2.7
Absolute Natural Treg (cells/mcL)	5 – 43		16
% Naïve Tregs (% CD4 cells)	0.1 – 2.3		0.7

Absolute Naïve Treg (cells/mcL)	0.4 – 22.0	4.2
Evaluation for CVID[4]		
CVID Confirmation Flow Panel (% of Total $CD19^+$ B cells)		
% $CD19^+TACI^+$ (% Total B cells)	> 3.4	34.4
% $CD19^+$BAFF $Receptor^+$ (% Total B cells)	> 90.2	93.0
BAFF level (pg/mL)	241 – 1,748	711
Autoimmune Serologies[1]		
Adrenal Antibody	Negative	Negative
Thyroid Peroxidase Antibody (IU/mL)	< 10	< 35
Islet Cell Autoantibody 512 (U/mL)	< 0.8	< 0.8
Anti-Tissue Transglutaminase IGA Antibody	0 – 19	Negative
T cell Phenotyping[3]		
% Naïve $CD4^+CD45RA^+$ T cells (% of CD4 cells)	3 – 59	18
Absolute (cells/mcL)	27 – 833	120
% Naïve $CD4^+CD62L^+CD27^+$ T cells (% of CD4 cells)	2 – 58	17
Absolute (cells/mcL)	11 – 824	113
% Naïve $CD8^+CD45RA^+$ T cells (% of CD8 cells)	6 – 84	83
Absolute (cells/mcL)	19 – 508	149
% Naïve $CD8^+CD62L^+CD27^+$ T cells (% of CD8 cells)	2 – 78	77
Absolute (cells/mcL)	5 – 475	138
% $CD4^+CD45RO^+$ Memory T cells (% of CD4 cells)	15 – 69	78
Absolute (cells/mcL)	167 – 670	520
% $CD4^+CD62L^+CD27^+CD45RO^+$cells (% of CD4 cells)	6 – 47	69
Absolute (cells/mcL)	58 – 413	460

% $CD4^+CD62L^+CD27^-CD45RO^+$ (% of CD4 cells)	0.7 – 12.0	0.7
Absolute (cells/mcL)	7 – 99	5
% $CD8^+CD45RO^+$ Memory T cells (% of CD8 cells)	4 – 49	10
Absolute (cells/mcL)	15 – 275	18
% $CD8^+CD62L^+CD27^+CD45RO^+$ (% of CD8 cells)	1 – 18	7
Absolute (cells/mcL)	6 – 135	13
% $CD8^+CD62L^+CD27^-CD45RO^+$ (% of CD8 cells)	0 – 6	0
Absolute (cells/mcL)	0 – 36	0
Evaluation of Thymic Function[3]		
CD4 Recent Thymic Emigrants (% of CD4)	6.4 – 51.0	15.4
CD8 Recent Thymic Emigrants (% of CD8)	0.0 – 0.6	0.5
Antibody Response to Vaccination[1]		
Diphtheria Antitoxoid Ab (IU/mL)[1]	≥ 0.01	0.15
Haemophilus Influenza Type B Ab (mcg/mcL)	>1.0	1.15
Hepatitis B surface Ab	Non-Reactive	Reactive
Measles IgG Ab	≥ 1.10	4.70
Mumps Virus IgG Ab	≥ 1.10	2.02
Bordetella Pertussis IgG Ab		
Pertussis Toxin IgG (IU/mL)	< 45	5
Filamentous Hemagglutinin IgG (IU/mL)	<90	40
Poliovirus Ab Neutralization		
Polio 1 titer	≥ 1:8	>1:128
Polio 2 titer	≥ 1:8	>1:128

Polio 3 titer	≥ 1:8	>1:128
Rubella IgG Ab	≥ 1.10	4.28
Tetanus Antitoxoid Ab (IU/mL)	> 0.15	0.56
Varicella Zoster Virus IgG Ab	≥ 1.10	1.48

[1] denotes reference range from Quest Diagnostics; [2] denotes reference range from Focus Diagnostics; [3] denotes reference range from Mayo Medical Laboratories; and [4] denotes reference range from Cincinnati Children's Hospital Medical Center. Abbreviations: mg: milligram, dL: deciliter, Foxp3: forkhead box P3, mcL: microliter; Treg: Regulatory T cell, BAFF: B cell activating factor, CVID: common variable immune deficiency, TACI: tumor necrosis factor receptor superfamily member 13B, pg: picogram, mL: milliliter, IgA: immunoglobulin A, IU: international units, U: units, Ab: antibody, mcg: microgram, IgG: immunoglobulin, NA: not applicable.

Supplemental Table II. Long-Range PCR Primers for Sequencing

Gene	Primer Sequence	
NFAT5 -1 sequencing	Forward	CTCTGCTACCCTGTATACTGACTAATG
	Reverse	GACGCTATGCTAGGCAATTTAATACAC
NFAT5 -2 sequencing	Forward	TGTCATAATCATAGAGATGATCAGGGAGG
	Reverse	AATAGGGATACTGGTACTACATCGCAGG
NFAT5 -3 sequencing	Forward	CGATGTAGTACCAGTATCCCTATTTCGT
	Reverse	AAGTCATCTGTAGTCTAGTCTGGCTTATC
NFAT5 -4 sequencing	Forward	CATAATTGTGTGGTATGTGGAAAGGAG
	Reverse	AAGTCTCTTAATCATCTGGGACGTTTG
NFAT5 -5 sequencing	Forward	CTGGAATTGGAACTTTTCTGCAGTCC
	Reverse	CATTATTTCAGCAAATCTCACCCAACC
NFAT5 -6 sequencing	Forward	TATGGCAGCCCTCTAAATCTTCTTCA
	Reverse	CAGTTACTCAGAACACGGAAGTTACAAG
NFAT5 -7 sequencing	Forward	CAGGTACAATGTTGTTTCTTCACCACT
	Reverse	GGCTACTTCATGTTATGCCACAGTATC
NFAT5 -8 sequencing	Forward	GGACAGATTCTTGATCTTCTGAACTTGC
	Reverse	TCTAAGGCTTAACTATCTTTCCACTGGG
NFAT5 -9 sequencing	Forward	AAGTGTGTACAGTGGAAATCTTGGTTG
	Reverse	CCAGTAACGATGGTGAACTTGAATTGT
NFAT5 -10 sequencing	Forward	CCACTATTTGTTCACTTAGCACTCTTG
	Reverse	CATTTGGTCTAACTAGTGTGCAGTTCC
NFAT5 -11 sequencing	Forward	CATAGGTCACCAGAAGGATTATGCGG
	Reverse	GTATCCCAAGTATTCACCTCTAGTTTGG
NFAT5 -12 sequencing	Forward	GCGTAGGGATATTGAAATTGAGGAATGC
	Reverse	TACGAATTCCTAAGTCAGGTCTTGATCC
NFAT5 -13 sequencing	Forward	CTTTCACTTGGACTTGGCACATTACTTC
	Reverse	GATGCAGAAGCTCATCAGGAAACAAATC
NFAT5 -14 sequencing	Forward	GAACTCTGCCTTTCTCTTATAAGGATGC
	Reverse	AAGTGAGACTAGGTAGAGAAAGTTGAGC
NFAT5 -15 sequencing	Forward	CCACTTGGAGATTGGAGAATTTAGAGG
	Reverse	CGATGTTCTGGTTGGTGTTTATAGAGGA
NFAT5 -16 sequencing	Forward	GTTCAGAATAGTGGTACCCAACAACAAG
	Reverse	TAAGAAGGGAGGAATGTAACTAGAGCAG