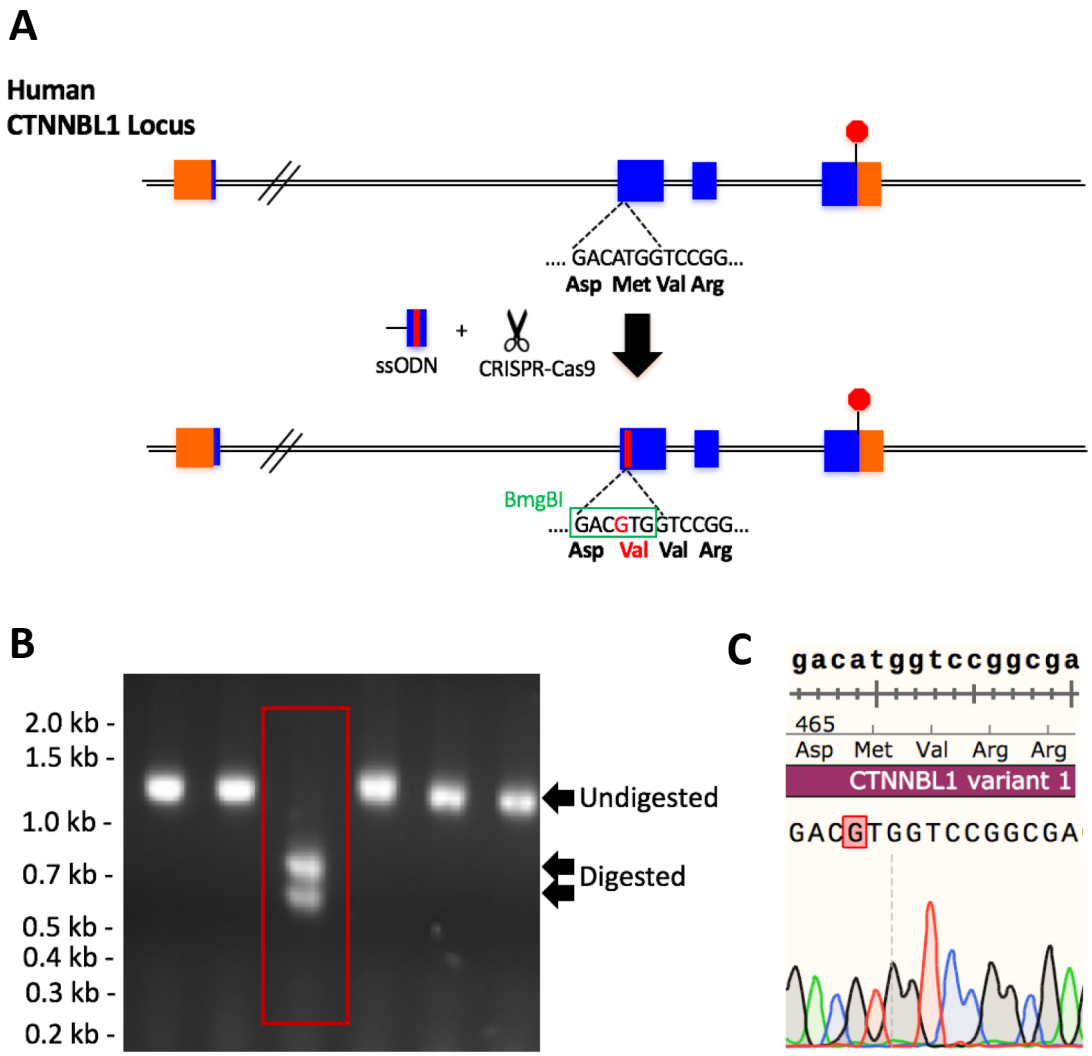
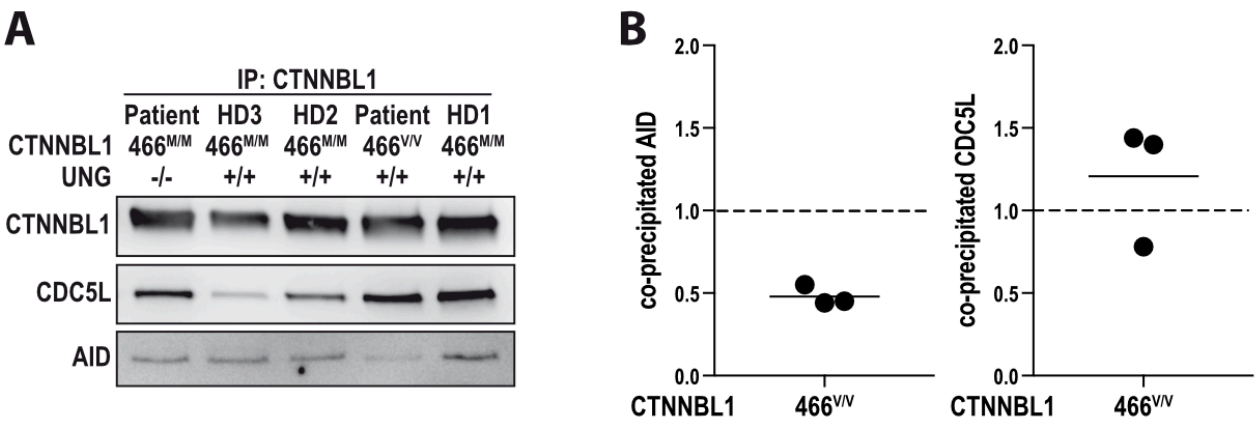
Supplemental Table 1.
Clinical features of CTNNBL1 $466^{V/V}$ patient.

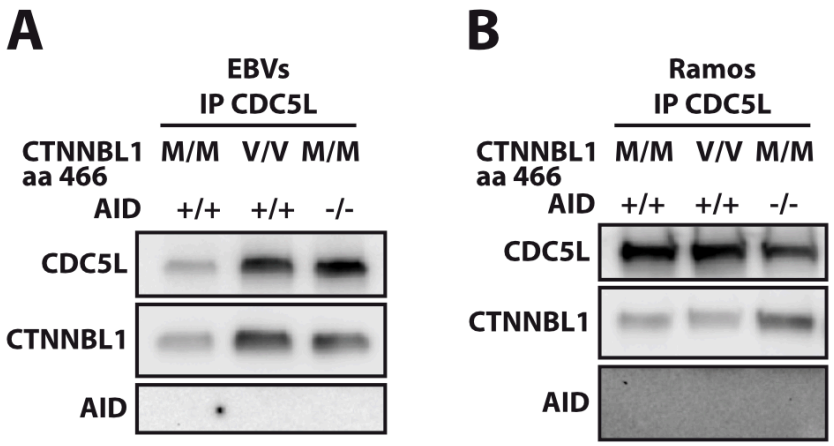
Patient:	
CTNNBL1	Chr20:36488304_A>G: c.A1396G:p.M466V (hg19, NM_030877)
Age presentation	2 years
Sex	F
Infections:	Sinopulmonary HSV
Skin	Herpes Simplex V2 facial lesions Vitiligo
Hematology	Thrombocytopenia
Endocrine	Hypothyroidism Growth delay – now resolved



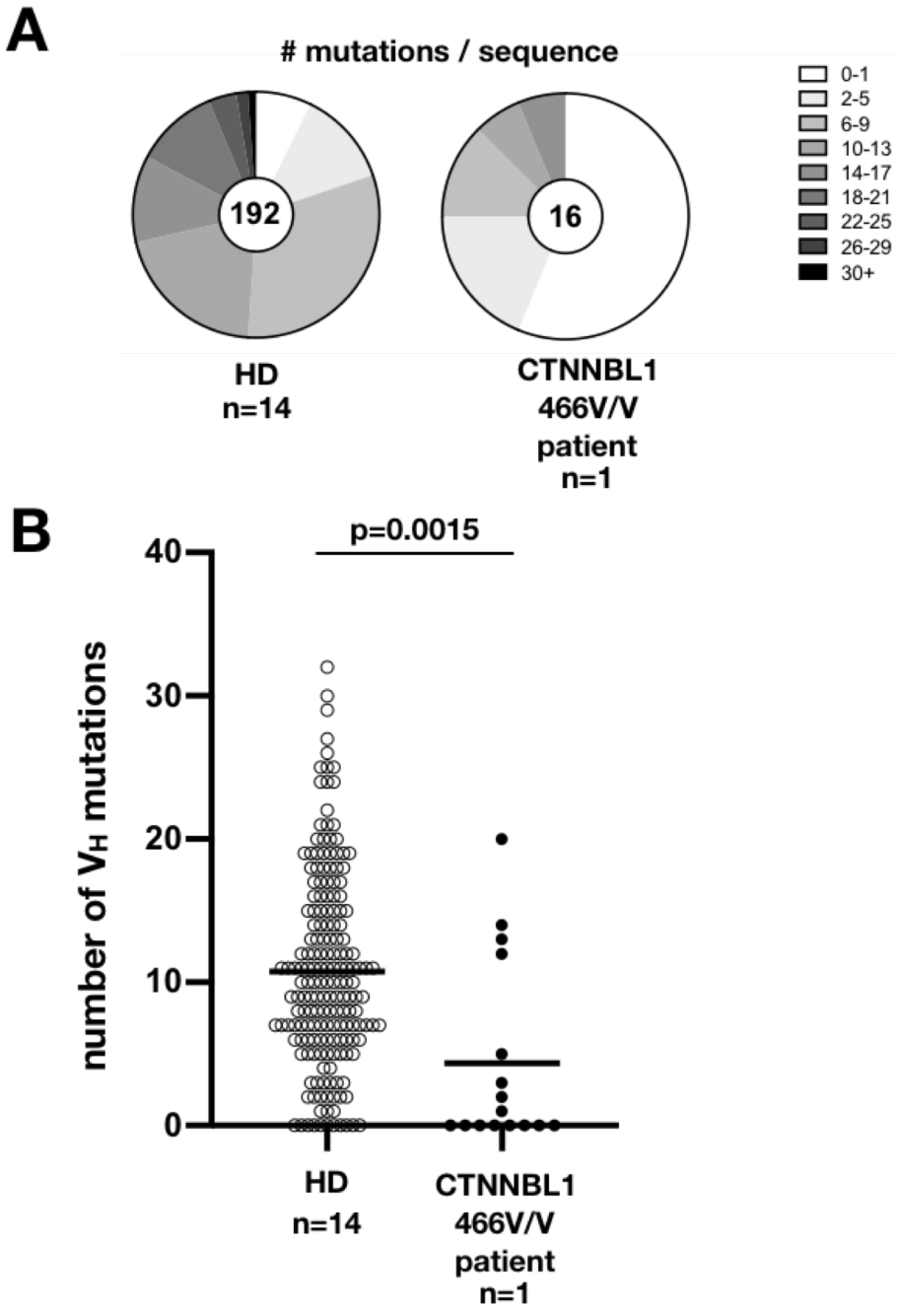
Supplemental Figure 1. Generation of CTNNBL1 466$^{V/V}$ Ramos B cells. (A) Diagram of knock-in approach to introduce the CTNNBL1 466V mutation into Ramos B cells. ssODN - single-stranded donor oligonucleotide. Orange boxes indicate untranslated regions (UTRs), blue boxes indicate coding regions (CDS), and red box indicates the mutated base pair/amino acid. Green outline indicates the introduction of a novel BmgBI restriction enzyme site upon successful genome editing. **(B)** Agarose gel of a BmgBI digest of PCR products amplifying the region surrounding exon 14 from genomic DNA of potential CTNNBL1 466$^{V/V}$ Ramos subclones. Red box highlights clone that had undergone successful genome editing. **(C)** Representative Sanger sequence of CTNNBL1 466$^{V/V}$ clone aligned with the sequence of the CTNNBL1 transcript (variant 1).



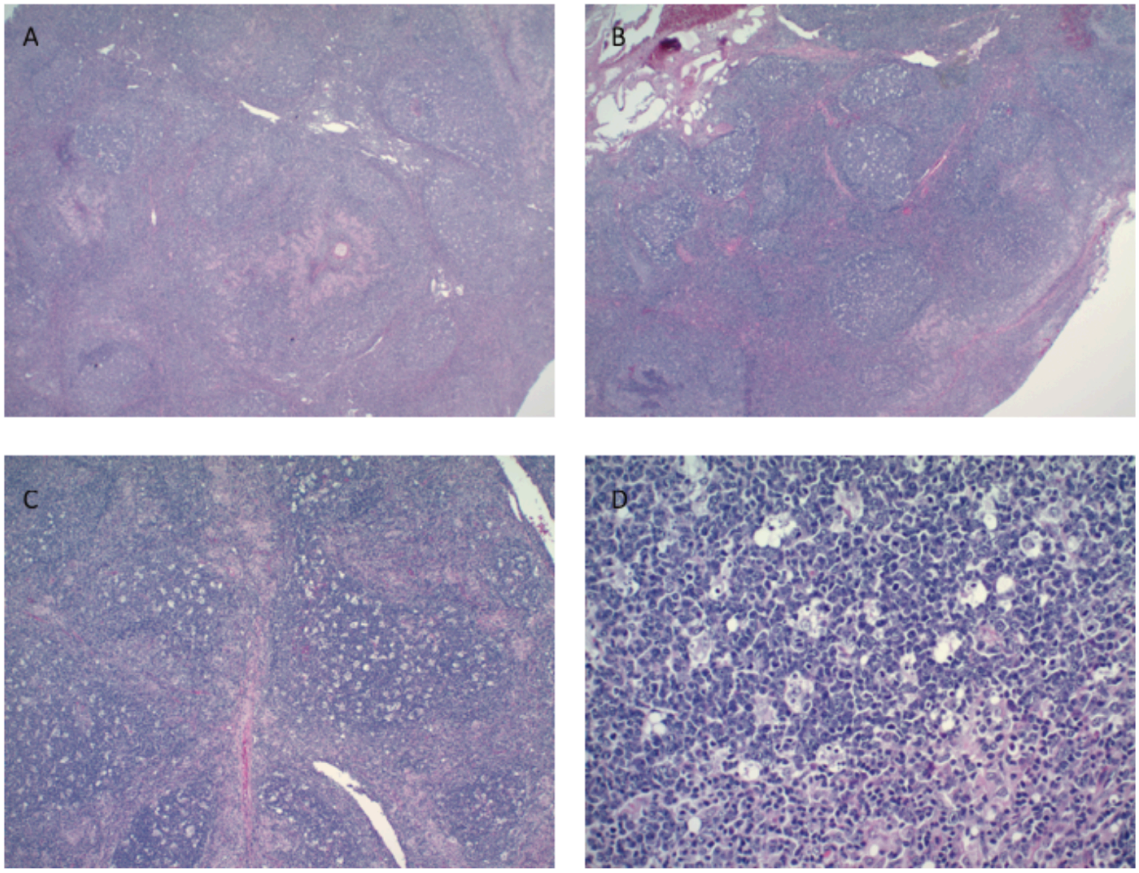
Supplemental Figure 2. UNG-deficient B cells do not display reduced interaction of CTNNBL1 and AID. (**A**) Lysates from EBV BLCLs from healthy donors (HD), patient carrying the 466V mutation, and UNG-deficient patient were subjected to immune precipitation with anti-CTNNBL1 antibody; precipitates were analyzed by immune blotting with indicated antibodies. (**B**) Densitometric quantification. Represented are values relative to UNG deficient cells (dashed lines); bar represents mean.

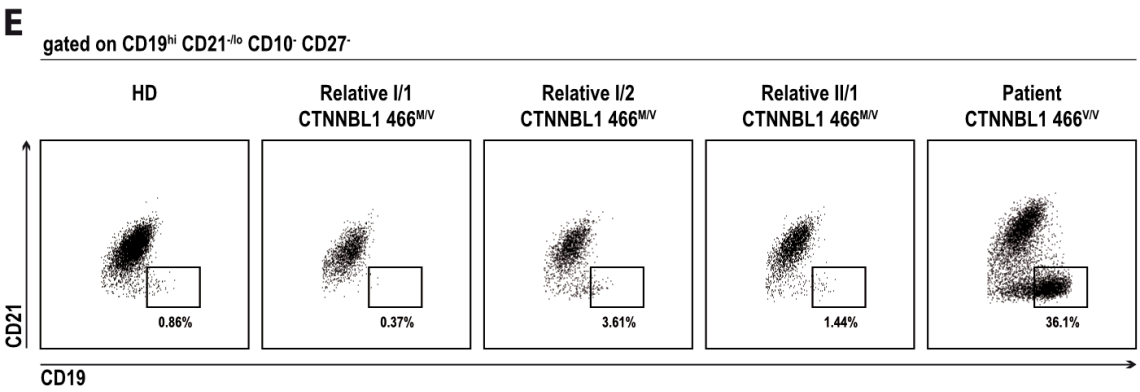


Supplemental Figure 3. AID and CDC5L form distinct complexes with CTNNBL1. Lysates of (**A**) EBV BLCLs from healthy donor (HD), CTNNBL1 $466^{V/V}$ patient, or AID-deficient patient or (**B**) parental WT, CTNNBL1 $466^{V/V}$, and $AID^{-/-}$ Ramos B cells were subjected to immune precipitation with anti-CDC5L antibody; precipitates and total cell lysates were analyzed by immune blotting with indicated antibodies.

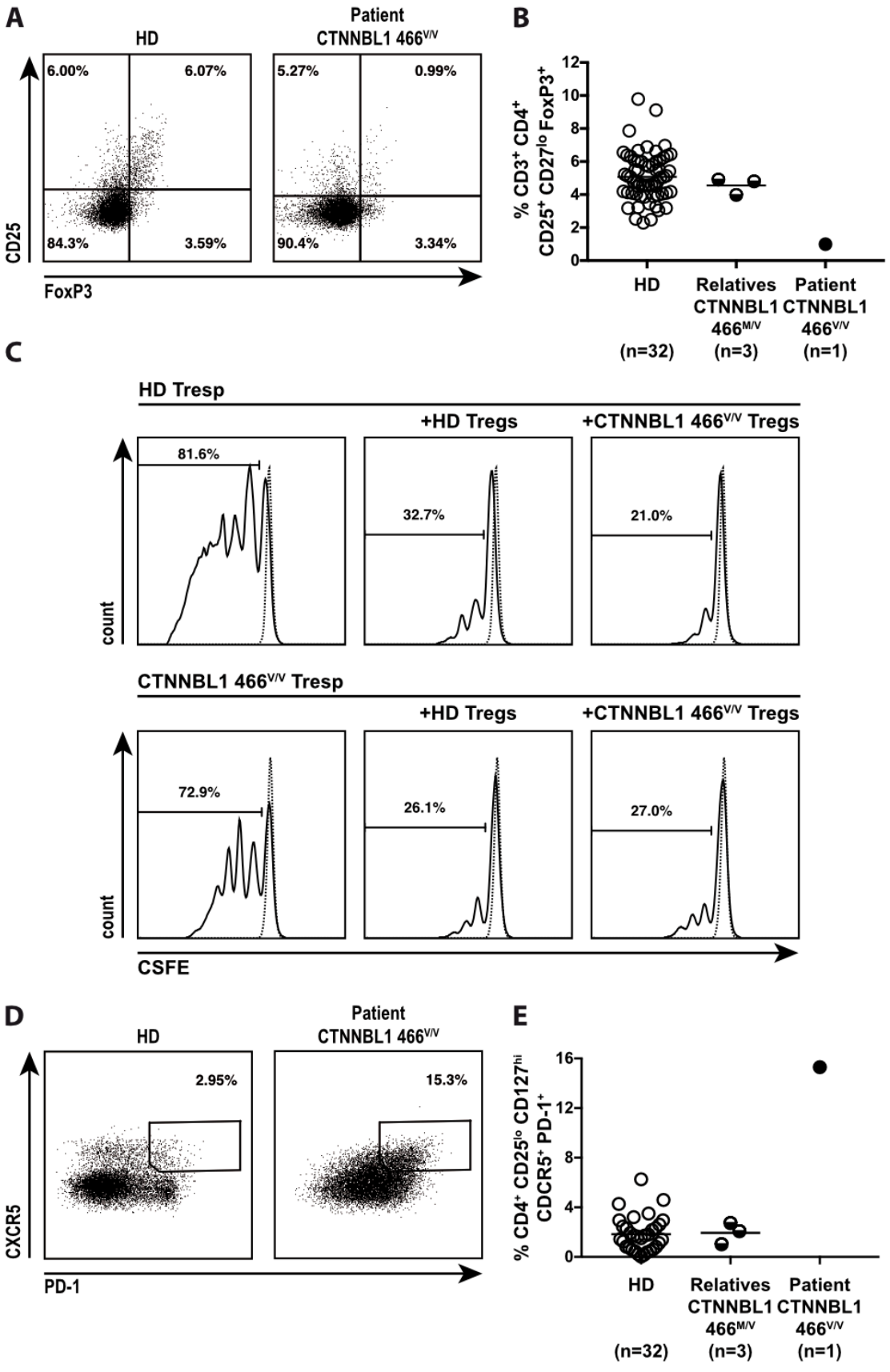


Supplemental Figure 4. Decreased SHM in CD27$^+$IgM$^+$ B cells from the CTNNBL1 466$^{V/V}$ patient. (**A**) Distribution of number of mutations per *VH* sequence and (**B**) number of mutations evaluated in V_H sequences derived from single CD27$^+$IgM$^+$ B cells from healthy donors (HD, n=14) and the CTNNBL1 466$^{V/V}$ patient is shown. Bar represents mean. P values were obtained with unpaired two-tailed Student's t-test.





Supplemental Figure 5. Lymphadenopathy and expanded CD19^{hi}CD21$^{-/lo}$CD10^{-}CD27^{-} B cells in the CTNNBL1 466$^{V/V}$ patient. (**A**) Lymph node architecture shows expanded and distorted follicles with surrounding epithelioid histiocytes in loose clusters 20x; (**B**) some areas demonstrate follicular hyperplasia with back-to-back follicles 20x; (**C**) there is attenuation of the mantle zones and some coalescent follicles 40x; (**D**) germinal centers show prominent tingible body macrophages with some peripheral epithelioid histiocytes 200x. (**E**) Dot plots of PBMCs from a representative healthy donor (HD), asymptomatic CTNNBL1 466$^{M/V}$ relatives, and CTNNBL1 466$^{V/V}$ patient, gated on CD19^{hi}CD21$^{-/lo}$CD10^{-}CD27^{-}.


A
HD
Patient
CTNNBL1 466V/V
6.00%
6.07%
84.3%
3.59%
5.27%
0.99%
90.4%
3.34%
CD25
FoxP3
B
% CD3+ CD4+
CD25+ CD27lo FoxP3+
HD
(n=32)
Relatives
CTNNBL1
466M/V
(n=3)
Patient
CTNNBL1
466V/V
(n=1)
C
HD Tresp
+HD Tregs
+CTNNBL1 466V/V Tregs
81.6%
32.7%
21.0%
count
CTNNBL1 466V/V Tresp
72.9%
26.1%
27.0%
CSFE
D
HD
Patient
CTNNBL1 466V/V
2.95%
15.3%
CXCR5
PD-1
E
% CD4+ CD25lo CD127hi
CDCR5+ PD-1+
HD
(n=32)
Relatives
CTNNBL1
466M/V
(n=3)
Patient
CTNNBL1
466V/V
(n=1)

Supplemental Figure 6. Altered frequencies of $CD3^+CD4^+CD25^{hi}CD127^{-/lo}FOXP3^+$ Treg and circulating $CD3^+CD4^+CXCR5^+PD\text{-}1^{hi}$ Tfh-like in the CTNNBL1 $466^{V/V}$ patient. (**A**) Dot plots of CD25 and FOXP3 expression in $CD3^+CD4^+CD127^{-/lo}$ T cells from a representative healthy donor (HD) and the CTNNBL1 $466^{V/V}$ patient. (B) Frequency of $CD3^+CD4^+CD25^{hi}CD127^{-/lo}FOXP3^+$ regulatory T cells (Treg) in 32 HDs, 3 patient's relatives (CTNNBL1 $466^{M/V}$), and the CTNNBL1 $466^{V/V}$ patient. Bar indicates mean. (**C**) Histograms of heterologous CFSE-labeled HD T responder cell (Tresp) proliferation on day 4 co-cultured with $CD3^+CD4^+CD25^{hi}CD127^{-/lo}$ Tregs from a HD or the CTNNBL1 $466^{V/V}$ patient. Dashed lines show unstimulated Tresp. (**D**) Dot plots of CXCR5 and PD-1 expression on $CD3^+CD4^+$ T cells from a representative HD and the CTNNBL1 $466^{V/V}$ patient. (**E**) Frequency of circulating $CD3^+CD4^+CXCR5^+PD\text{-}1^{hi}$ T follicular helper (Tfh)-like in 32 HDs, 3 unaffected relatives (CTNNBL1 $466^{M/V}$), and the CTNNBL1 $466^{V/V}$ patient.