Bier et al. JACI-D-18-01161. ***Activating mutations in PIK3CD disrupt the differentiation and function of human and murine CD4$^+$ T cells***

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Supplementary Figure Legends

Supplementary Figure 1: Aberrant cytokine profiles of *PIK3CD* GOF memory CD4$^+$ T and cTfh cells

(A) Naïve and total memory CD4$^+$ T cells or (B) cTfh cells were sort-purified from the peripheral blood of healthy donors and patients with *PIK3CD* GOF mutations and then cultured with beads coated with anti-CD2/CD3/CD28 mAbs. After 5 days, secretion of the indicated cytokines was determined by cytometric bead arrays. (C) CFSE-labelled naïve CD4$^+$ T cells from healthy donors or patients with *PIK3CD* GOF mutations were cultured under Th0 or Th1 polarizing conditions. After 4 days, the proportion of activated naïve CD4$^+$ T cells in each division (left panel), or expressing IL-21 (middle panel) or IFNγ (right panel) was determined. Significant differences were determined by unpaired Students t-tests, **p<0.01, ***p<0.001.

Supplementary Figure 2: Thymic T cell development in *Pik3cd* GOF mice

Thymi from WT and *Pik3cd* GOF intact mice aged 8-12 or 30-40 weeks were stained to identify different stages of T cell development. Percentages of double negative (DN), double positive (DP), single positive CD4$^+$ or CD8$^+$ T cell are shown in the graphs (mean ± SEM, n=8-10).

Supplementary Figure 3: Extrinsic factors contribute to activated phenotype of CD4$^+$ T cells activation in aged *Pik3cd* GOF mice

Spleens from WT:WT and WT:*Pik3cd* GOF mixed bone marrow chimeras were stained to identify different CD4$^+$ T cell populations 36 weeks after reconstitution. (A) Total CD4$^+$ T cells within either CD45.1 or CD45.2 populations. Percentages of (B) naïve (CD44^{lo}CD62L^{hi}), (C) central memory (CD44^{hi}CD62L^{hi}), (D) effector memory (CD44^{hi}CD62L^{lo}), (E) Tfh and (F) Treg cells amongst the CD45.1$^+$CD4$^+$ or CD45.2$^+$CD4$^+$ T cells (mean ± SEM, n=9-10). Significant differences were determined by 2-way ANOVA, **p<0.01; ****p<0.0001.

Supplementary Figure 4: *Pik3cd* GOF OT-II cells show altered activation following immunization

WT or *Pik3cd* GOF OT-II cells were transferred to WT recipients, which were then immunized with OVA/Alum. Spleens were harvested 7 days later. (A-E) Flow cytometric analysis was performed to assess expression of surface molecules. Graphs depict mean fluorescent intensity relative to expression by recipient CD4$^+$ T cells (dotted line) of (A) CXCR5, (B) PD-1, (C) CD62L, (D) CCR7, and (E) CD127. (F-H) Cells were stimulated for 6 hours in the presence of PMA/ionomycin and cytokine production assessed by flow cytometry. Graph shows percentage of OT-II cells expressing (F) IL-2, (G) TNFα and (H) IL17A. All graphs show mean ± SEM, n=15-17. Significant differences were determined by unpaired Students t-tests, *p<0.05; ****p<0.0001.

Table 1: Demographics of individuals diagnosed with *PIK3CD* GOF mutations

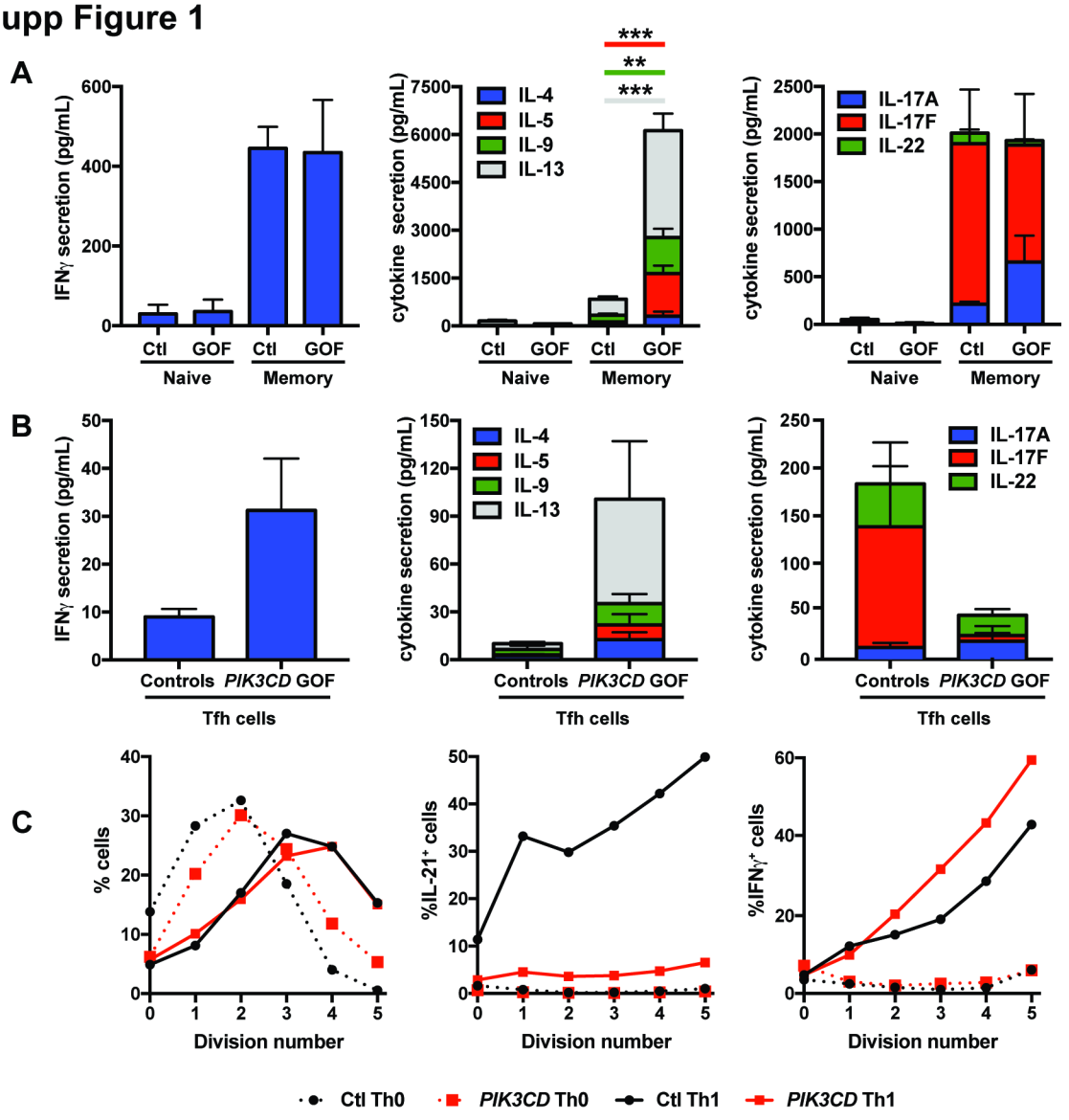
PIK3CD Patient	Mutation	Age	Gender	Ethnicity	EBV (serology or PCR)	CD4+ T cell count ($x10^9$/L)[a]	Ig levels	Th2-type disease	Ab responses	Respiratory infections/ complications
1	c.3061G>A p.E1021K	15	F	Caucasian	IgM, IgG+	0.14 (L)	↑IgG, Normal IgM, IgA;	Asthma	↓Pneumo, Hib response	Bronchiecstasis
2	c.3061G>A p.E1021K	47	M	Caucasian	-		On IVIg	-	ND (on IVIg)	Bronchiecstasis
3	c.3061G>A p.E1021K	17	F	Caucasian	-		↑IgM, normal IgG, IgA	Asthma	↓Pneumo, Hib response	Bronchiecstasis
4	c.3061G>A p.E1021K	10	F	Caucasian	-		Normal	Asthma	↓Pneumo; normal Hib and tetanus response	Pneumonia
5	c.3061G>A p.E1021K	12	F	Caucasian	+	0.5	Normal (↑IgM during EBV infection)	Eosinophilic esophagitis, eczema	↓Pneumo response	Yes
6	c.3061G>A p.E1021K	20	F	Caucasian	-	0.51	↑IgM, ↓IgG, ↓IgA; IgE[b]: 2 U/ml	Asthma	↓Pneumo response	Recurrent pneumonia, bronchiecstasis
7	c.3061G>A p.E1021K	9	F	Polynesian	++	0.42	↑IgM, ↑IgG, normal IgA; IgE: 83U/mL	-	↓Pneumo response	Recurrent pneumonia, bronchiecstasis
8	c.3061G>A p.E1021K	12	M	Lebanese Caucasian	-	0.54	↑IgM, ↓IgG; IgE: normal	-	ND	Bronchiecstasis
9	c.3061G>A p.E1021K	7						-		
10	c.3061G>A p.E1021K	10	M	Caucasian	-	0.26 (L)	IgM, G, A, E: Normal	-	Intact	
11	c.3061G>A p.E1021K	15	F	European	-	0.23-0.38 (L)	↑IgM, hypogamma; IgE: normal	-	↓Pneumovax response	Recurrent pneumonia
12	c.3061G>A p.E1021K	11	M	Moroccan	++	0.57	↑IgM, hypogamma; IgE: normal	Urticarial, allergic rhinoconjunctivitis, mild eczema	Weak/absent	Recurrent pneumonia
13	c.3061G>A p.E1021K	6	M	Caucasian	-	0.34 (L)	↑IgM ↓IgG, normal IgA; IgE: 12U/mL	-	ND (on IVIg)	Otitis media, Recurrent pneumonia, bronchiecstasis
14	c.3061G>A p.E1021K	10	F	Caucasian	++	0.19-0.39 (L)	↑IgM, normal IgG and IgA;	-	↓Pneumo response; normal	Bronchiecstasis

							IgE: 3 U/mL		tetanus	
15	c.3061G>A p.E1021K	19	F	Caucasian	-	0.41	↑IgM, ↓IgG, □□□IgA	-	↓Pneumo response	Pneumonia, ear infections
16	c.3061G>A p.E1021K	46	F	Caucasian	-	0.62	Normal	-	↓Pneumo response	Recurrent conjunctivitis, sinusitis, ear infections
17	c.3061G>A p.E1021K	12	M	Caucasian	-	0.5	↑IgM, ↓IgG, ↓IgA	-	↓Pneumo, Hib response	Pneumonia, conjunctivitis, ear infections
18	c.3061G>A p.E1021K	8	F	Cauc, Persian Sth Amer descent	+	0.34 (L)	↑IgM, hypogamma; IgE: 2U/mL	-	↓□□□□□□□□ response, normal diphtheria response	Yes
19	c.3061G>A p.E1021K	10	M	Cauc, Persian Sth Amer descent	+	0.72	Normal; IgE: 8U/mL	-	↓□□□□□□□□ response, normal diphtheria response	Yes
20	c.3061G>A p.E1021K	12	M	Caucasian, American descent	-	0.24-0.38 (L)	↑IgM, normal IgG; IgE: <1U/mL	Atopic dermatitis, eczema	ND (on IVIg)	
21	c.3061G>A p.E1021K	19	F	Hispanic	+	0.2 (L)	↑IgM, hypogamma; IgE: 6U/mL	-	ND (on IVIg)	
22	c.1573G>A p.E525K	60	M	Caucasian, American descent	+	0.71	Normal; IgE: 160 U/mL (H)	Eosinophilic esophagitis, eczema, food allergy	↓□□□□□□□□ response, normal diphtheria response	
23	c.1573G>A p.E525K	28	M	Caucasian, American descent	+	0.74	Normal; IgE: 41U/mL	Eosinophilic esophagitis, eczema	↓□□□□□□□□ response, normal diphtheria response	
24	c.1573G>A p.E525K	22	F	Caucasian, American descent	++	1.36	↑IgM, normal IgG; IgE: 26U/mL	Eosinophilic esophagitis, eczema, hives	↓□□□□□□□□ response, normal diphtheria response	
25	c.3061G>A p.E1021K	15	M	Caucasian, American descent	-	0.19 (L)	↑IgM, hypogamma; IgE: <1U/mL	-	ND (on IVIg)	
26	c.3061G>A p.E1021K	22	M	Caucasian, American descent	+	0.27 (L)	□□rm□□□□gM, hypogamma; IgE: 24U/mL	Asthma	↓□□□□□□□□ response, normal diphtheria response	

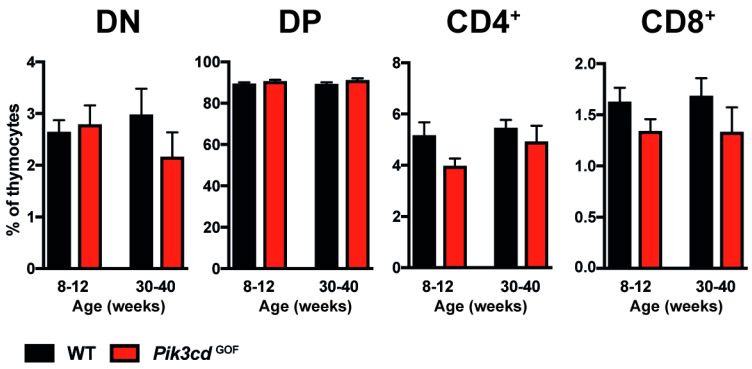
27	c.3061G>A p.E1021K	16	M	Caucasian, American descent	-	0.32 (L)	↑IgM, hypogamma; IgE: <1U/mL	-	↓□□□□□□□□ response, normal diphtheria response	
28	c.1573G>A p.E525K	12	F	Asian	++	0.35 (L)	□□rm□□□□gM, hypogamma; IgE: 450 U/mL (H)	Asthma	↓□□□□□□□□ response, normal diphtheria response	
29	c.371G>A p.G124D	40	F		+					
30	c.3061G>A p.E1021K	29	F							
31	c.3061G>A p.E1021K	16	M							
32	c.3061G>A p.E1021K	23	F					Eosinophilic esophagitis		
33	c.1573G>A p.E525K	65	F		+					
34	c.1573G>A p.E525K	15	M					Eosinophilic esophagitis		
35	c.3061G>A p.E1021K	10	M							
36	c.1002C>A, p.N334K	12	F							
37	c.1573G>A p.E525K	13	F							

[a]Normal range: 0.4-2 x 10^9/L
[b]IgE normal range: 0-90 U/mL
ND – Not done; IVIG – intravenous immunoglobulin

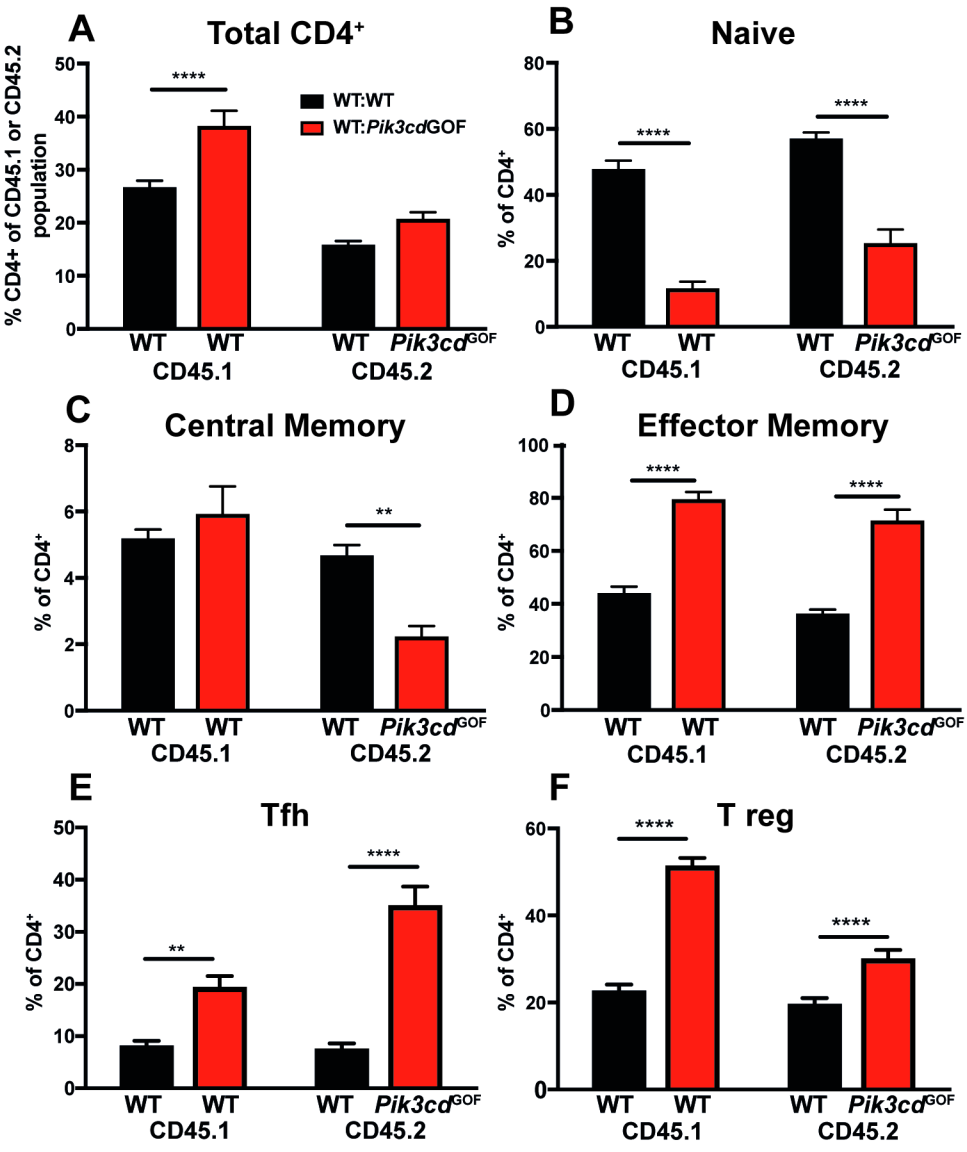
Supp Figure 1



Supp Figure 2



Supp Figure 3



Supp Figure 4