

SUPPLEMENTAL MATERIAL

Ma et al., <http://www.jem.org/cgi/content/full/jem.20151467/DC1>

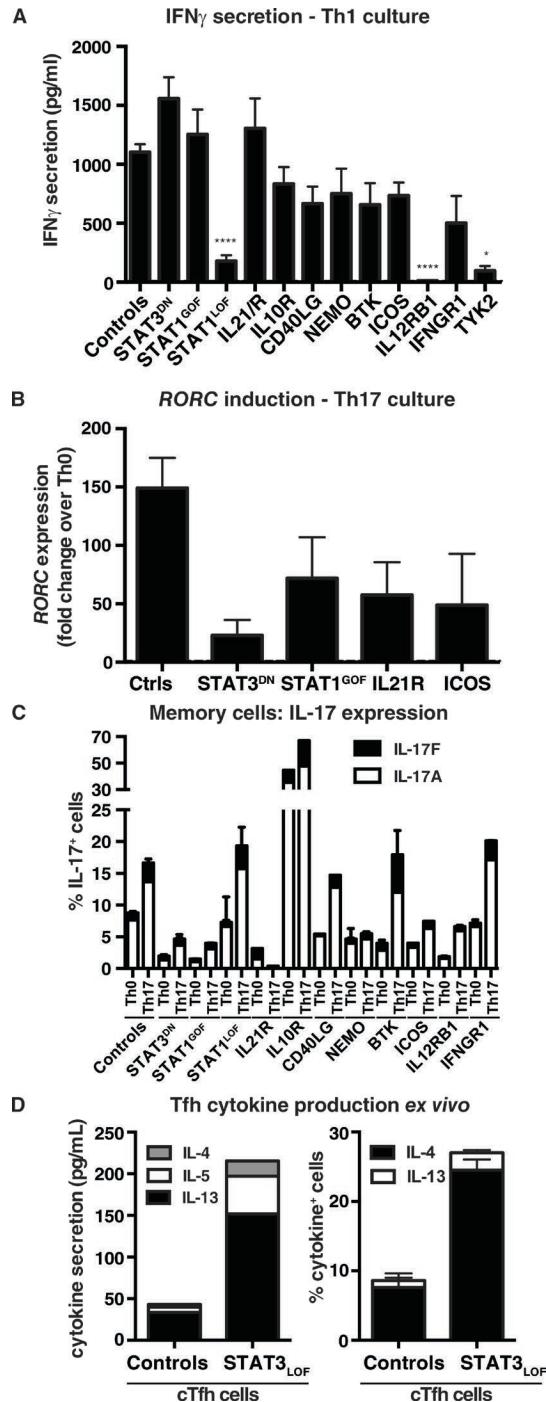


Figure S1. Effect of monogenic mutations on Th1, Th17, and Th2 cells. (A and B) Naive CD4⁺ T cells were isolated from healthy donors or patients with mutations in *STAT3*, *STAT1*, *IL21R*, *IL10R*, *CD40LG*, *NEMO*, *BTK*, *ICOS*, *IL12RB1*, *IFNGR1*, or *TYK2*, and then cultured with TAE beads alone (Th0) or under Th1 (A) or Th17 (B) conditions for 5 d. After this time, secretion of IFN- γ (CBA; A) and expression of *RORC* (qPCR; B) were determined. Data for IFN- γ secretion by TYK2-deficient naive CD4⁺ T cells was also presented in Kreins et al. (2015), but is shown here for comparison. *, P < 0.01; **, P < 0.001, compared with controls (ANOVA). (C) Memory CD4⁺ T cells were isolated from peripheral blood of healthy donors ($n = 9$) or patients with mutations in *STAT3* ($n = 2$), *STAT1^{GOF}* ($n = 2$), *STAT1^{LoF}* ($n = 2$), *IL21R* ($n = 1$), *IL10R* ($n = 1$), *CD40LG* ($n = 2$), *NEMO* ($n = 2$), *BTK* ($n = 2$), *ICOS* ($n = 1$), *IL12RB1* ($n = 2$), or *IFNGR1* ($n = 2$), and then cultured under Th0 or Th17 conditions for 5 d. Cells were then harvested and analyzed for expression of IL-17A and IL-17F. (D) cTfh (CD4⁺CD45RA⁻CXCR5⁺) cells were isolated from peripheral blood of healthy donors or *STAT3*-deficient patients and cultured for 5 d with TAE beads. Secretion of IL-4, IL-5, or IL-13 (left) or the proportion of cells expressing IL-4 or IL-13 (right) was then determined. The values represent the mean of four experiments using cells from different donors and patients.

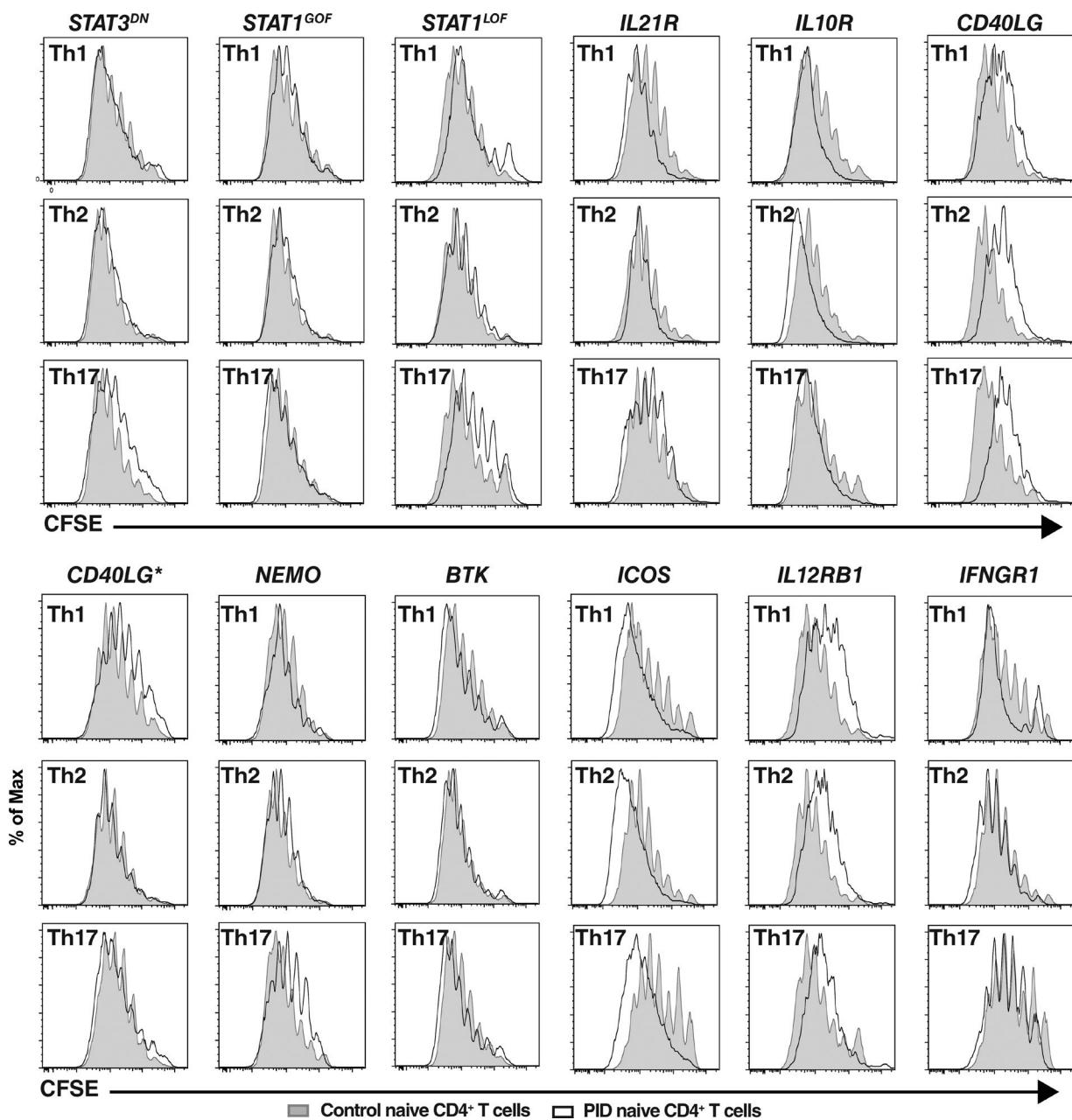


Figure S2. Effect of disease-causing mutations on naive CD4⁺ T cell proliferation induced by Th1, Th2, and Th17 culture conditions. Naive CD4⁺ T cells from healthy donors (solid gray histograms) and patients (overlay black histograms) with the indicated gene mutations were labeled with CFSE and then cultured under Th1, Th2, or Th17 conditions. After 5 d, the cells were harvested and analyzed for proliferation by assessing CFSE dilution. Histograms are representative of experiments performed using naive CD4⁺ T cells from nine different healthy controls, two patients with mutations in *STAT3*, *STAT1*, *CD40LG*, *NEMO*, *BTK*, *IL12RB1*, or *IFNGR1*, or individual patients with mutations in *IL21R*, *IL10R*, or *ICOS*.

Table S1. Primary immunodeficient patients used in this study

Gene	Allele	Disease	Patient	Mutation	References
STAT3 (<i>n</i> = 10)	•Heterozygous, autosomal dominant •DN	•Hyper-IgE syndrome •CMC, Staph infections •Impaired functional antibodies	#1 #2 #3 #4 #5 #6 #7 #8 #9 #10	N647D/WT R382W/WT R382Q/WT R382W/WT F384S/WT V463del/WT V637M/WT V637M/WT V637M/WT L706M/WT	Holland et al., 2007; de Beaucaire et al., 2008; Ma et al., 2008; 2015; Deenick et al., 2013
STAT1 (<i>n</i> = 7)	•Heterozygous, autosomal dominant •LOF (DN)	•MSMD	#1 #2 #3 #4 #5 #6 #7	V701C/WT V701C/WT G250E/WT G250E/WT Y701C/WT M654K/WT p.F378_R379del/WT	Deenick et al., 2013; Hirata et al., 2013; Ma et al., 2015
STAT1 (<i>n</i> = 13)	•Heterozygous, autosomal dominant •GOF	•CMC •Autoimmunity •Reduced humoral immunity in some patients	#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13	L351F/WT N355D/WT R321S/WT R274Q/WT A267V/WT P329L/WT P329L/WT P329L/WT T385M/WT R274Q/WT R321S/WT N355D/WT G384D/WT	Liu et al., 2011; Sampaio et al., 2013; Mizoguchi et al., 2014
IL21 (<i>n</i> = 1)	•Homozygous/biallelic autosomal recessive •LOF	•IBD	#1	L49P/L49P	Salzer et al., 2014
IL21R (<i>n</i> = 5)	•Homozygous/biallelic autosomal recessive •LOF	•CVID (reduced serum/Ag specific Ig) •IBD •Cryptococcal, pneumocystis infection	#1 #2 #3 #4 #5	C81_H82del/C81_H82del C81_H82del/C81_H82del W138S/W138S R201Q/R201Q R201Q/R201Q	Deenick et al., 2013; Kotlarz et al., 2014; Stepensky et al., 2015
IL10R (<i>n</i> = 2)	•Homozygous/biallelic, autosomal recessive	•Severe early onset IBD	#1 #2	Q159X/Q159X (IL10RB) I169T/E431X	Lee et al., 2014
CD40LG (<i>n</i> = 6)	•X-linked null •LOF	•X-linked HIGM (reduced serum/Ag specific Ig)	#1 #2 #3 #4 #5 #6	W140C c.526T->G, p.Y169D c.526T->G, p.Y169D 13.3 kB del 736_740 del 5 (Q246fsXext22) p.Y145N	Fan et al., 2012
CD40LG* (<i>n</i> = 3)	•X-linked hypomorphic •LOF	•X-linked HIGM (reduced serum/Ag specific Ig) •Milder clinical phenotype	#1 #2 #3	G>A, +1 intron 4 c.289-15T>A c.289-15T>A	Lovell et al., 2016
NEMO (<i>n</i> = 8)	•X-linked •LOF	•X-HIGM/ectodermal dysplasia •Poor Ab responses •MSMD	#1 #2 #3 #4 #5 #6 #7 #8	R254Q C417R R254Q F312L E315A E57K R254G c.0.1-16G>C	Keller et al., 2011; Huppmann et al., 2015

Table S1. Primary immunodeficient patients used in this study (Continued)

Gene	Allele	Disease	Patient	Mutation	References
BTK (<i>n</i> = 10)	•X-linked •LOF	•XLA •Agammaglobulinemia •B cell deficient	#1	L295P	
			#2	Q151X	
			#3	M228V/FX15	
			#4	C165Y	
			#5	G909C splice mutation (intron8)	
			#6	C165Y	
			#7	C155Y	
			#8	c.1102+2dup T. D326T (frameshift)	
			#9	c. 136dupC; p.R>Pfs*7	
			#10	L512P	
ICOS (<i>n</i> = 6)	•Homozygous, autosomal recessive •LOF	•CVID (reduced serum/Ag specific Ig)	#1	Del exons 2, 3 (443 bp del)	Grimbacher et al., 2003;
			#2		Warnatz et al., 2006;
			#3		Robertson et al., 2015
			#4		
			#5		
			#6	c.321_330 del	
IL12RB1 (<i>n</i> = 7)	•Bi-allelic, autosomal recessive •LOF	•MSMD •Some candidiasis	#1	c.1623_1624delinsTT	de Beaucoudrey et al., 2010
			#2	c.557_563delins8	
			#3	c.1791+2T>G	
			#4	c.1425delC	
			#5	R486X/1791+2GT	
			#6	c.1791+2T>G	
			#7	[E67X]+[1623_1624delinsTT]	
IFNGR1 (<i>n</i> = 4)	•Heterozygous, partial deficiency	•MSMD	#1	774del4/WT	Dorman et al., 2004; Okada et al., 2007
			#2	818del4/WT	
			#3	818del4/WT	
			#4	NA	
IFNGR1 (<i>n</i> = 3)	•Biallelic (homozygous or compound heterozygous); complete deficiency	•MSMD	#1	22delC	Dorman et al., 2004; Okada et al., 2007
			#2	373(+1)g>t and 202(-1)g>c	
			#3	561del4 and 182 T>G; p: Val61Gln	
TYK2 (<i>n</i> = 3)	•Homozygous, autosomal recessive •LOF	•MSMD •(1 pt with mild CMC)	#1	C70HfsX21/C70HfsX21	Minegishi et al., 2006; Kreins et al., 2015
			#2	T110HfsX4/T110HfsX4	
			#3	767X/767X	

Abbreviations: CMC, chronic mucocutaneous candidiasis; CVID, common variable immunodeficiency; IBD, inflammatory bowel disease; MSMD, Mendelian susceptibility to mycobacterial disease; HIGM, hyper-IgM syndrome; NA, not available.

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