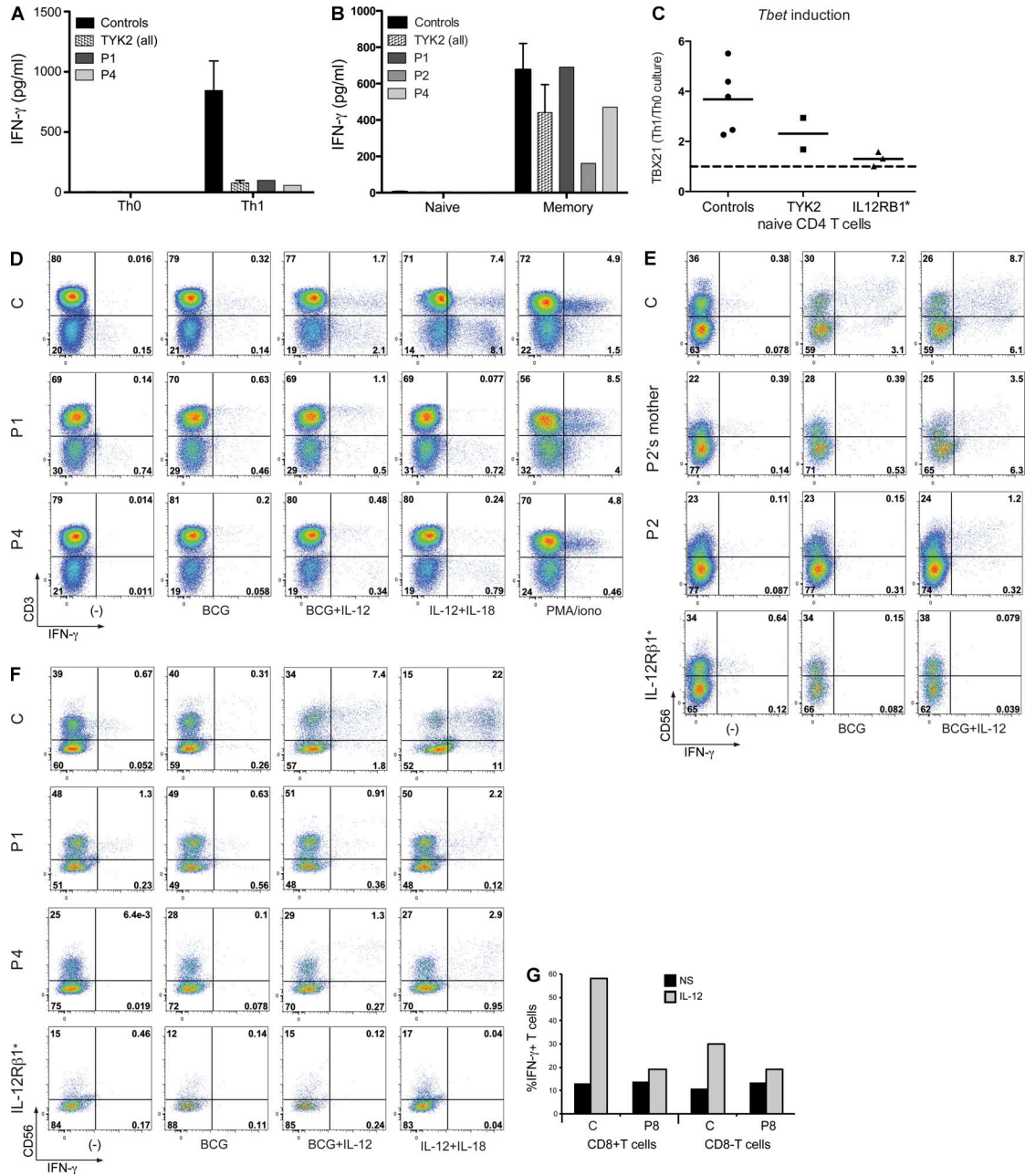


SUPPLEMENTAL MATERIAL

Kreins et al., <http://www.jem.org/cgi/content/full/jem.20140280/DC1>



**Figure S1. Impaired IL-12 responses in TYK2-deficient patients.** (A) Cytokine production by in vitro-differentiated CD4<sup>+</sup> T cells from control donors and TYK2-deficient patients. Naive (CD45RA<sup>+</sup>CCR7<sup>+</sup>) CD4<sup>+</sup> T cells from controls ( $n = 4$ ) or TYK2-deficient patients P1 and P4 were cultured for 5 d with TAE beads (anti-CD2/CD3/CD28), either alone (Th0) or together with polarizing stimuli to induce the differentiation of Th1 (IL-12) type cells. Culture supernatants were then assessed for IFN- $\gamma$  secretion in cytometric bead assays. (B) Naive and memory (defined as CD45RA<sup>-</sup>) CD4<sup>+</sup> T cells from WT controls ( $n = 5$ ) and TYK2-deficient patients ( $n = 3$ ; P1, P2, and P4) were sorted and purified (>98% purity) and cultured with TAE beads for 5 d. Culture supernatants were then assessed for IFN- $\gamma$  secretion in cytometric bead assays. All error bars indicate SEM. (C) *Tbet* mRNA induction by naive CD4 T cells from five controls, two TYK2-deficient patients, and three IL-12R $\beta$ 1-deficient patients stimulated with TAE beads (anti-CD2/CD3/CD28) and IL-12 (Th1) compared with cells stimulated with TAE beads only (Th0). Mean values for each set of conditions are indicated by solid lines. (D-F) Flow cytometry analysis showing intracellular IFN- $\gamma$  production in PBMCs after stimulation with BCG (MOI = 20), BCG + 100 ng/ml IL-12, or 25 ng/ml IL-12 + 50 ng/ml IL-18 for 48 h or after stimulation with 40 ng/ml PMA + 10<sup>-5</sup> M ionomycin for 24 h, in PBMCs from a healthy control (C), TYK2-deficient patients P1, P2, and P4, the mother of patient P2, and an IL-12R $\beta$ 1-deficient patient (IL-12R $\beta$ 1\*). (D) An anti-CD3 Ab was used to identify CD3<sup>+</sup> T cells. (E and F) An anti-CD56 Ab was used to identify CD3<sup>-</sup>CD56<sup>+</sup> NK cells. (G) Flow cytometry assay assessing the proportion of IFN- $\gamma$ <sup>+</sup> T cells among CD8<sup>+</sup> and CD8<sup>-</sup> T cells from a healthy control (C) and P8, with and without stimulation with IL-12.

**Table S1.** Summary of the functional experiments performed in TYK2-deficient cells

Parameter	Method	Cells	Patient							
			P1	P2	P3	P4	P5	P6	P7	P8
Response to IL-12	ELISA (IFN- $\gamma$ )	Whole blood/CD4 T cells	impaired	impaired	impaired	impaired	null	null	impaired	
	Flow cytometry (IFN- $\gamma$ <sup>+</sup> cells)	PBMCs	impaired	impaired		impaired				impaired
	WB (pSTAT4)	HVS-T cells		abolished						
	EMSA (GAS)	HVS-T cells		abolished						
	ELISA (IFN- $\gamma$ )	HVS-T cells		abolished						
Response to IL-23	Microarray	HVS-T cells		impaired						
	WB (pSTAT3)	EBV-B cells	abolished	abolished	abolished		impaired		abolished	impaired
	WB (pSTAT3)	HVS-T cells		abolished						
	ELISA (IFN- $\gamma$ )	HVS-T cells		abolished						
Response to IFN- $\alpha/\beta$	Microarray	HVS-T cells		impaired						
	WB (pSTAT1)	EBV-B cells	impaired	impaired	impaired		impaired		impaired	impaired
	WB (pSTAT3)	EBV-B cells	abolished	abolished	abolished		abolished		abolished	abolished
	EMSA (GAS and ISRE)	EBV-B cells	impaired	impaired	impaired					
	Viral assay (VSV)	EBV-B cells	no protection	no protection	no protection					
	Q-PCR (ISG15, Mx1, SOCS3)	EBV-B cells	impaired	impaired						
	WB (pSTAT1)	SV40-fibroblasts		impaired						
	EMSA (GAS and ISRE)	SV40-fibroblasts		abolished						
	Viral assay (VSV)	SV40-fibroblasts		impaired						
	WB (pSTAT1)	HVS-T cells		abolished						
Response to IL-10	Viral assay (VSV)	HVS-T cells		no protection						
	WB (pSTAT3)	EBV-B cells	impaired	impaired	impaired		impaired		impaired	impaired
	EMSA	EBV-B cells	impaired	impaired						
	Q-PCR (SOCS3)	EBV-B cells	impaired	impaired						
	LPS and TNF	PBMCs		impaired						
Response to IL-6	WB (pSTAT3)	SV40-fibroblasts		impaired						
	WB (pSTAT3)	EBV-B cells	impaired	normal	normal		normal		normal	normal
	EMSA (GAS) and EMSA ELISA	EBV-B cells	impaired	normal						
	WB (pSTAT3)	Fibroblasts	impaired	normal						
IL-17 cells	Q-PCR (SOCS3)	Fibroblasts	impaired	normal						
	Flow cytometry (IL17A <sup>+</sup> cells after PMA iono)	PBMC		normal			normal			normal
	Flow cytometry (IL17A <sup>+</sup> cells after TAE beads)	Naive and memory CD4 T cells	normal	normal			normal			
	ELISA (IL-17A and F)	Naive T cells	impaired	impaired			impaired			
Response to IL-29/IL-28	Q-PCR (IFIT1)	EBV-B cells	impaired	impaired						
Response to IL-21	Flow cytometry (pSTAT3)	EBV-B cells	normal	normal	normal					
	WB (pSTAT3)	EBV-B cells	normal	normal						

**Table S1.** Summary of the functional experiments performed in TYK2-deficient cells (*Continued*)

Parameter	Method	Cells	Patient							
			P1	P2	P3	P4	P5	P6	P7	P8
Response to IL-27	WB (pSTAT1 and STAT3)	EBV-B cells	normal	normal						
	EMSA (GAS)	EBV-B cells	normal	normal	normal					
	Q-PCR (IRF1)	EBV-B cells	normal	normal						
Response to IFN- $\gamma$	WB (pSTAT1)	EBV-B cells								
	EMSA (GAS)	EBV-B cells	normal	normal	normal					
	Q-PCR (IRF1)	EBV-B cells	normal	normal						
	ELISA (IL-12p40)	Whole blood		normal	normal	normal	normal	normal	normal	normal
Expression of IFN- $\alpha$ 1	Flow cytometry	EBV-B cells	impaired	impaired	impaired		impaired		impaired	impaired
Expression of IL-10R2	Flow cytometry	EBV-B cells	impaired	impaired	impaired		impaired		impaired	impaired
Expression of IL-12R $\beta$ 1	Flow cytometry	EBV-B cells	impaired	impaired	impaired		impaired		impaired	impaired
Expression of IFN- $\alpha$ 2, IFN- $\gamma$ R1, IFN- $\gamma$ R2	Flow cytometry	EBV-B cells	normal	normal	normal		normal		normal	normal
Response to LIF	WB (pSTAT3)	SV40 and primary fibroblasts		normal						

Q-PCR, quantitative PCR; WB, Western blotting.