

Compound heterozygous TYK2 mutations underlie primary immunodeficiency with T-cell lymphopenia

Michiko Nemoto^{1,2}, Hiroyoshi Hattori^{1,3*}, Naoko Maeda³, Nobuhiro Akita³, Hideki Muramatsu⁴, Suzuko Moritani⁵, Tomonori Kawasaki⁵, Masami Maejima¹, Hirotaka Ode¹, Atsuko Hachiya¹, Wataru Sugiura¹, Yoshiyuki Yokomaku¹, Keizo Horibe^{1,3} and Yasumasa Iwatani^{1, 6*}

¹Clinical Research Center, National Hospital Organization Nagoya Medical Center, Aichi 460-0001, Japan;

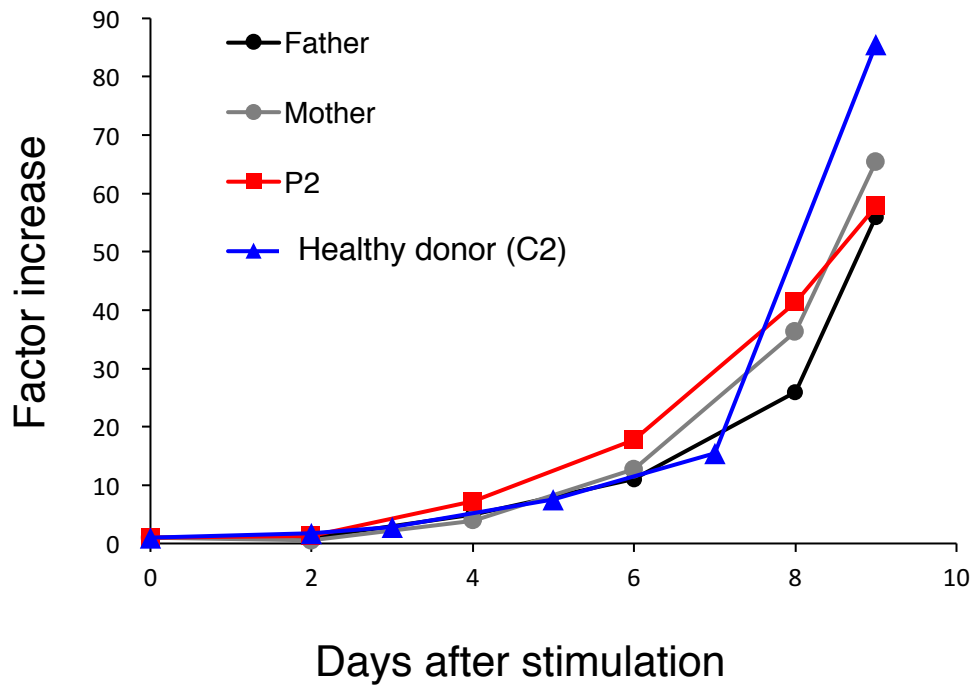
²Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan;

³Department of Pediatrics, National Hospital Organization Nagoya Medical Center, Aichi 460-0001, Japan;

⁴Department of Pediatrics, Nagoya University Graduate School of Medicine, Aichi 466-8550, Japan;

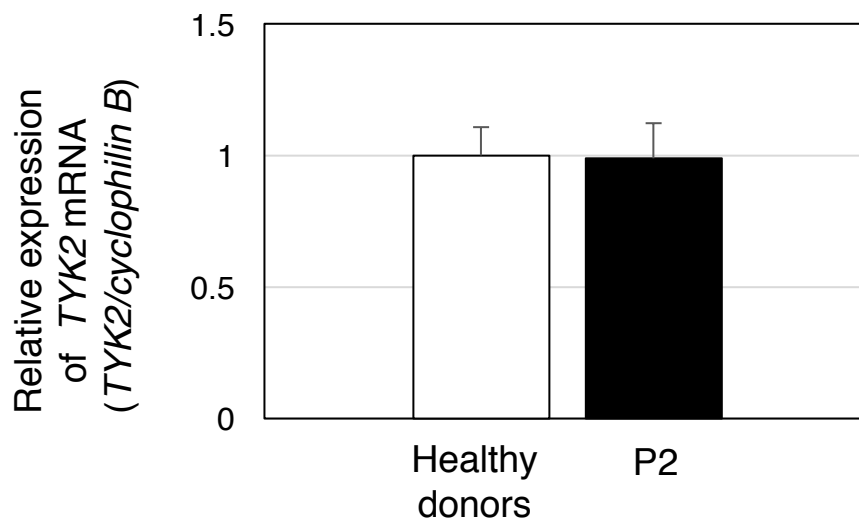
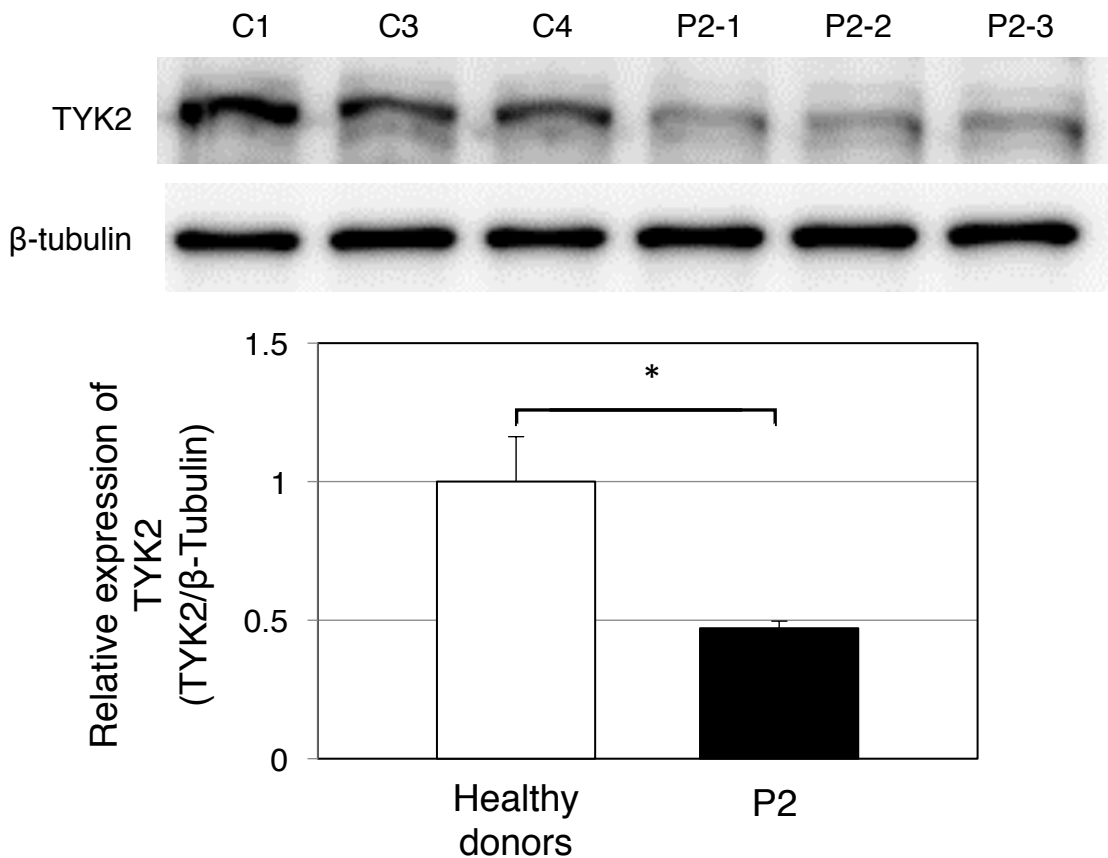
⁵Department of Pathology, National Hospital Organization Nagoya Medical Center, Aichi 460-0001, Japan;

⁶Division of Basic Medicine, Nagoya University Graduate School of Medicine, Aichi 466-8550, Japan,



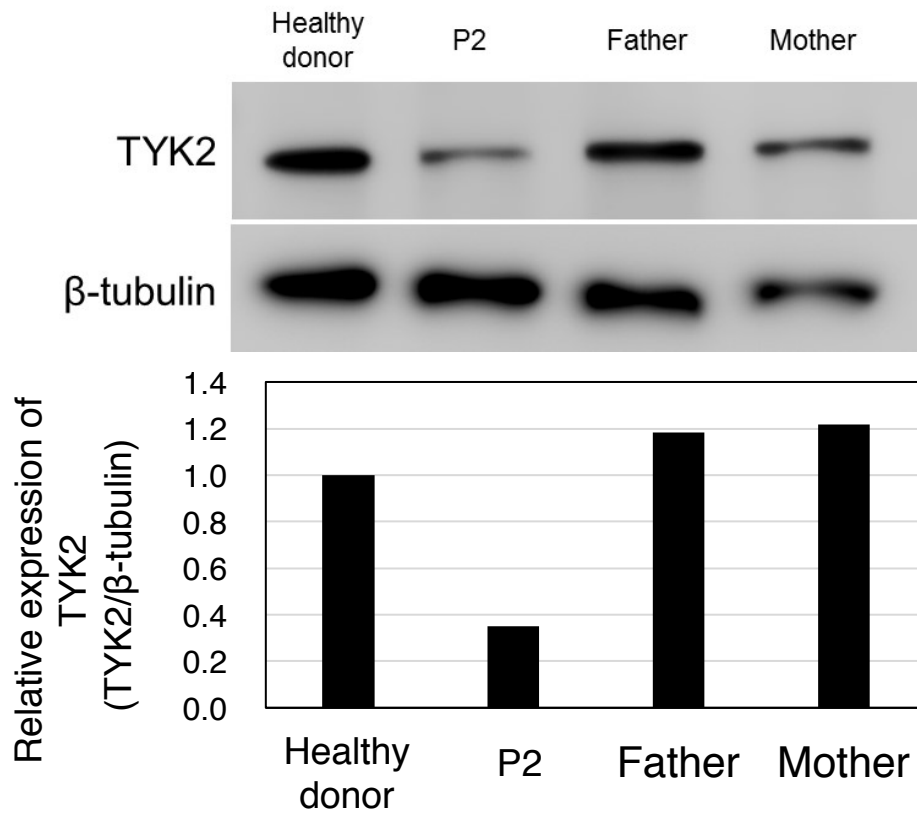
Supplementary Figure S1

Growth curves of T cells stimulated with anti-CD3 plus anti-CD28 coated beads and expanded in culture with IL-2.

a**b**

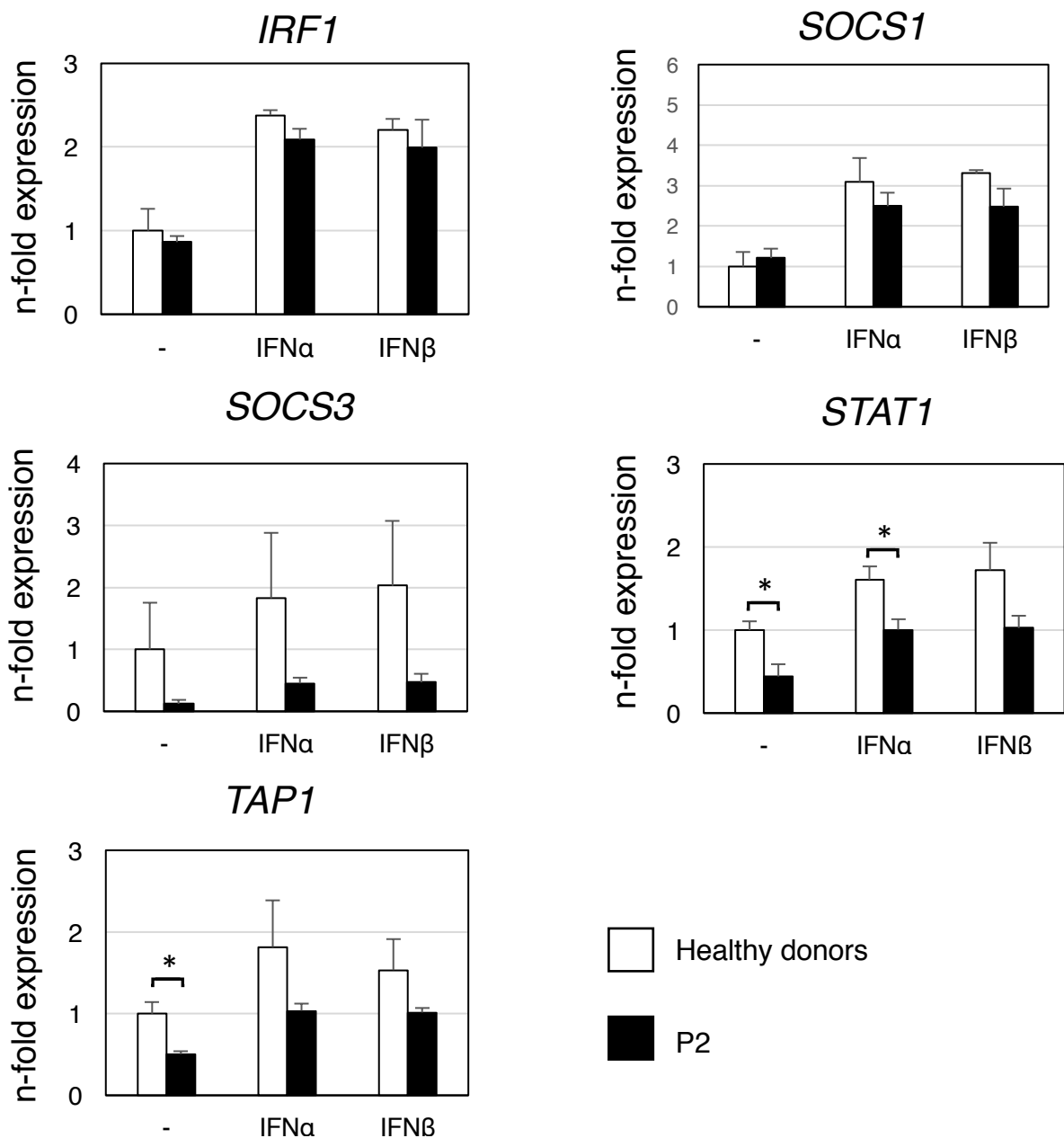
Supplementary Figure S2

Expression Levels of *TYK2* mRNA and protein in EBV-BCLs. (a) *TYK2* mRNA expression in EBV-BCLs from healthy donors (C1, C3, C4) and a *TYK2*-mutated patient (P2). (b) Immunoblots for *TYK2* expression in EBV-BCLs from healthy donors (C1, C3, C4) and a *TYK2*-mutated patient (P2). P2-1, P2-2, and P2-3 are different batches of EBV-BCLs from the same patient. β -tubulin serves as a loading control.



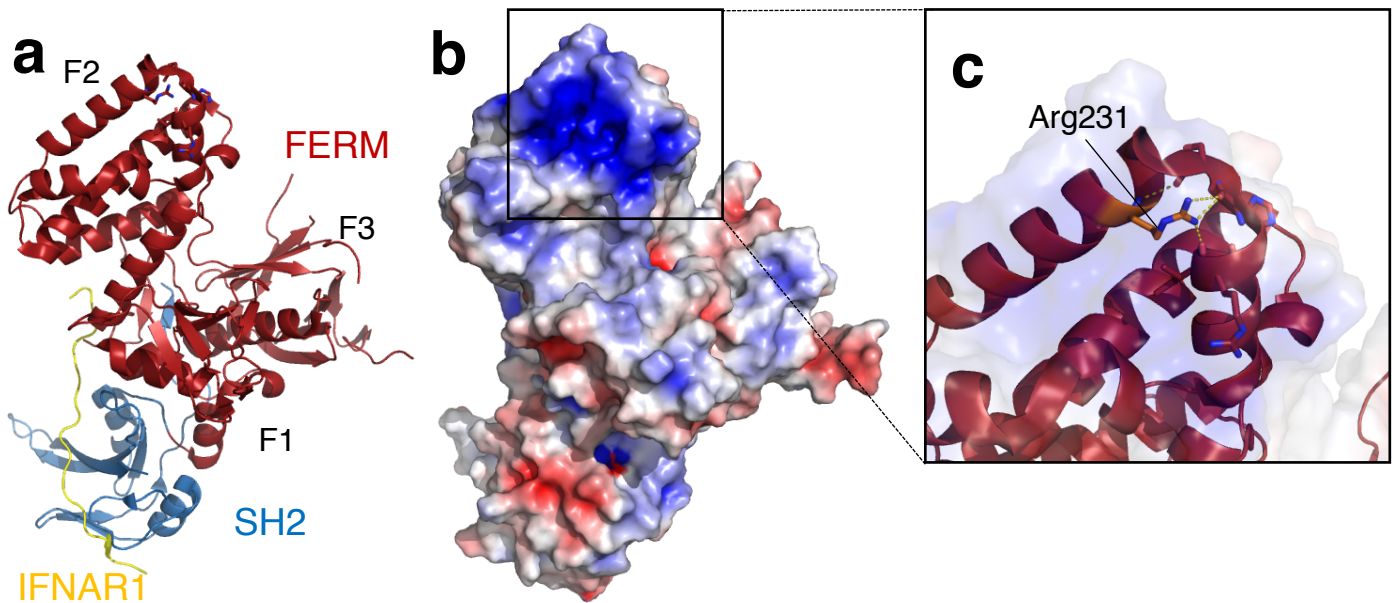
Supplementary Figure S3

Expression Levels of TYK2 protein in CD4⁺ T cells . Immunoblots for TYK2 expression in CD4⁺ T cells from a healthy donor (C1), a TYK2-mutated patient and her parents. β -tubulin serves as a loading control.



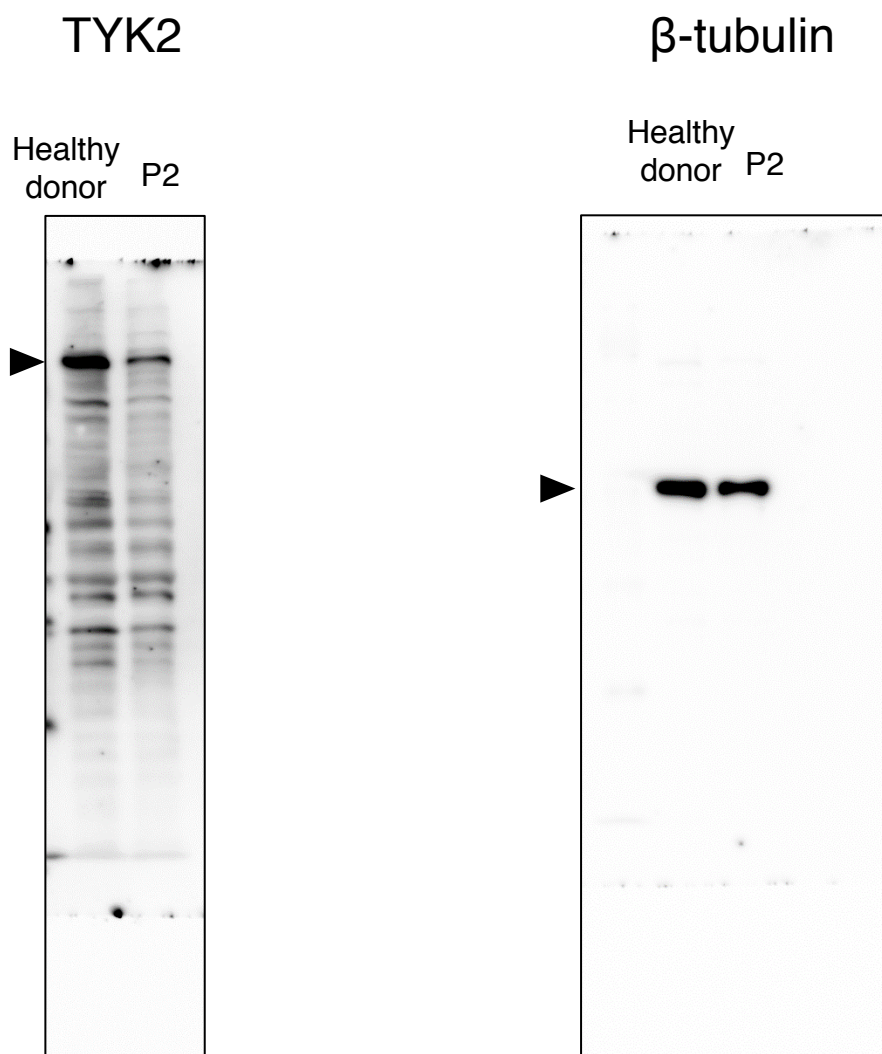
Supplementary Figure S4

Analysis of type I IFN-inducible gene induction in EBV-BCLs from healthy donors (C1, C3, C4) and a TYK2-mutated patient. Cells were stimulated with IFN α (1000 U/mL), IFN β (500 U/mL) or without any IFN for 2 h. The cDNAs generated from the total RNA were quantified with qPCR assays. The expression levels of *IRF1*, *SOCS1*, *SOCS3*, *STAT1* and *TAP1* mRNAs were determined by normalizing each with *cyclophilin B* levels. The induction level is presented as n-fold expression that in the untreated healthy donor cell control, which was set as 1. The data are derived from three independent experiments using 3 EBV-BCLs per genotype. Data represent mean $\pm$ SEM. *P < 0.05. P values were derived from 2-tailed Student's t-test.



Supplementary Figure S5

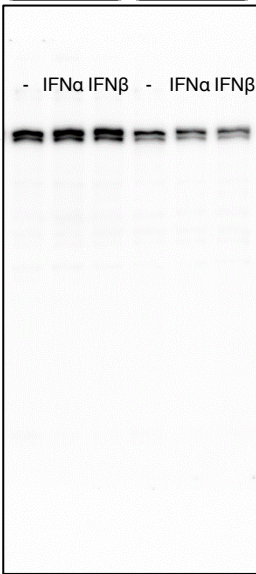
Mapping of the Arg231 in the crystal structure of human TYK2 structure (PDB 4PO6). (a) Ribbon representation of the structure of the FERM and SH2 domains is in red and blue, respectively. IFN- α/β receptor subunit 1 (IFNAR1) interacts with the FERM F2 and SH2. (b) Electrostatic surface potential, contoured at -5 (red) to +5 (blue) kT/e , is shown for the complex. The FERM F2 contains a highly basic patch, which likely adheres to the plasma membrane. (c) The detailed view around Arg231 (orange) is shown. The Arg231 constitutes the basic patch and likely stabilizes the F2 conformation by forming intramolecular hydrogen bonds. The Arg231Trp mutation may lead to disruption of the positively charged-F2 conformation, which shows defects in function.



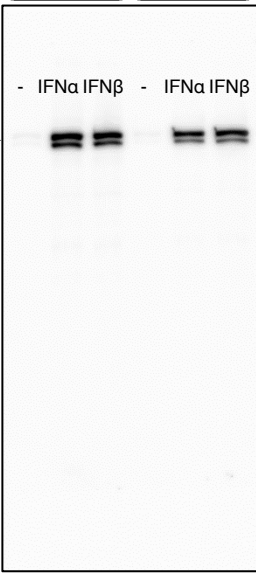
Supplementary Figure S6
Full-length immunoblot images of Figure 1d.
Each arrow head highlights bands for TYK2 or β-tubulin.

STAT1**pSTAT1****STAT2****pSTAT2**

Healthy donor P2



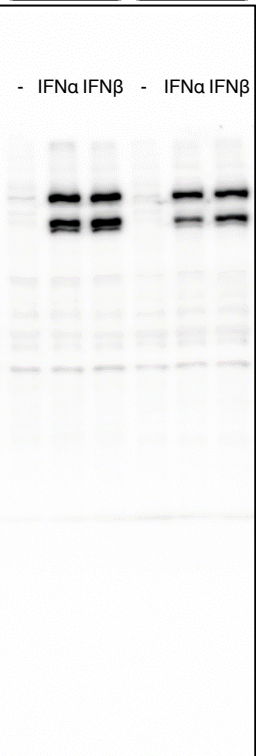
Healthy donor P2



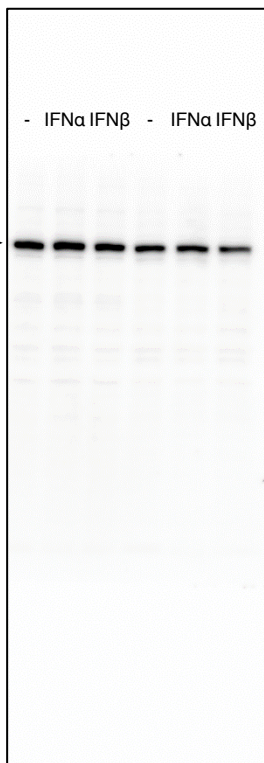
Healthy donor P2



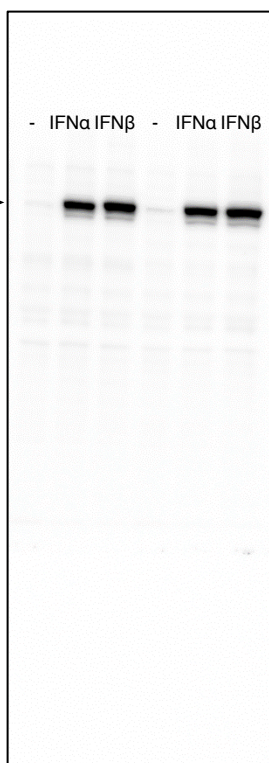
Healthy donor P2

**STAT3****pSTAT3** **β -tubulin**

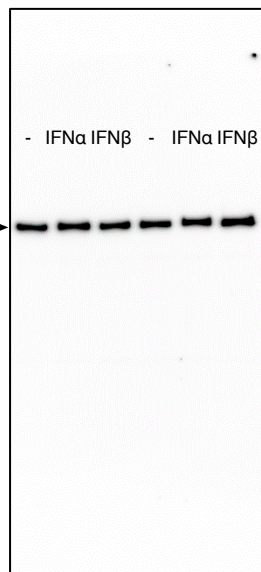
Healthy donor P2



Healthy donor P2

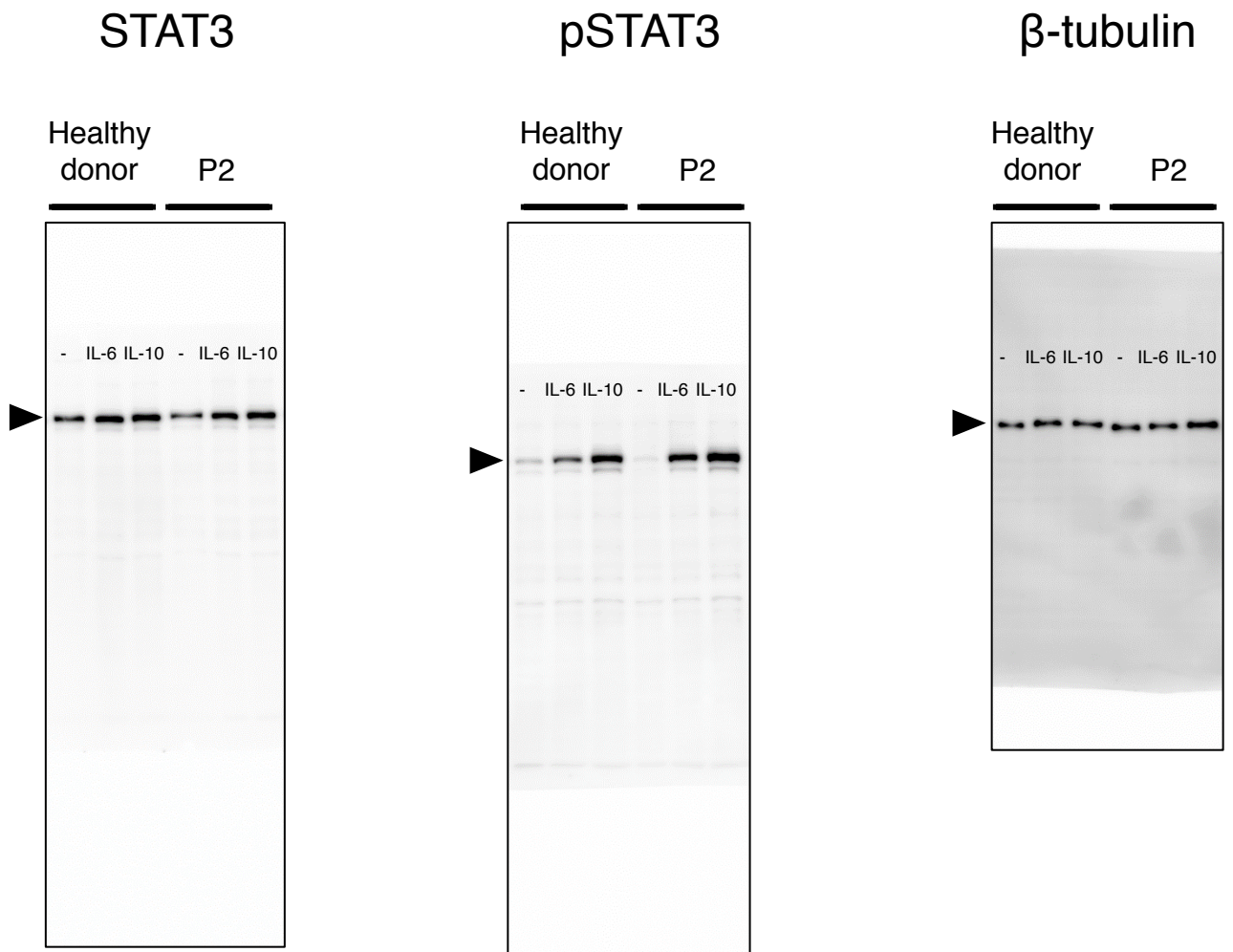


Healthy donor P2



Supplementary Figure S7

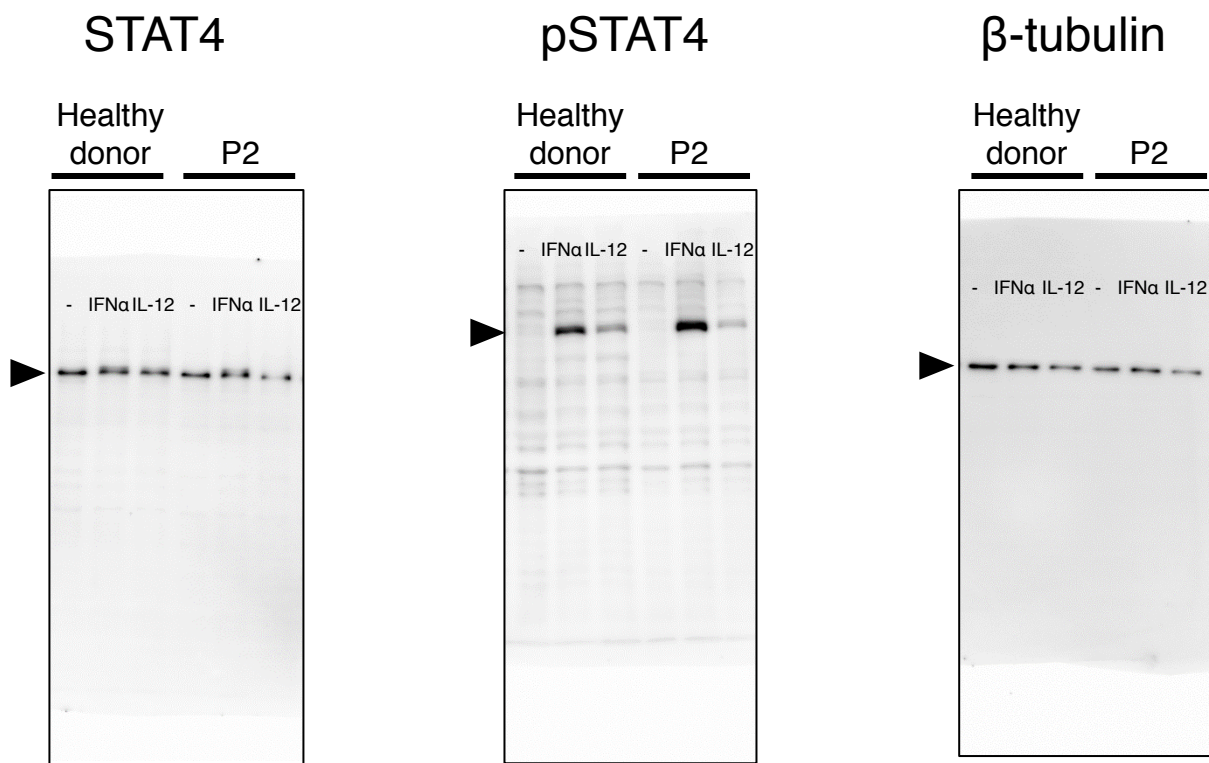
Full-length immunoblot images of Figure3a. Each arrow head highlights bands for STAT1, pSTAT1, STAT2, pSTAT2, STAT3, pSTAT3, or β -tubulin.



Supplementary Figure S8

Full-length immunoblot images of Figure 5a.

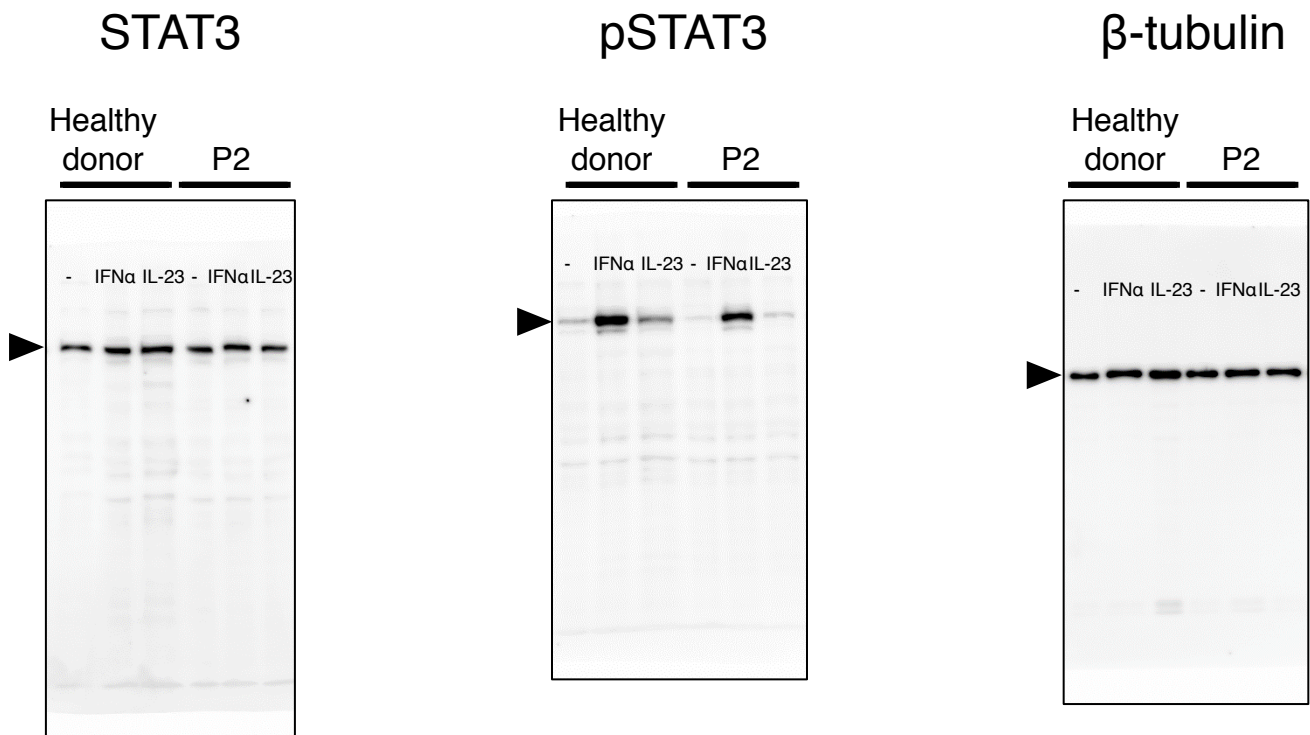
Each arrow head highlights bands for STAT3, pSTAT3, or β -tubulin.



Supplementary Figure S9

Full-length immunoblot images of Figure 5b.

Each arrow head highlights bands for STAT4, pSTAT4, or β-tubulin.



Supplementary Figure S10

Full-length immunoblot images of Figure 5c.

Each arrow head highlights bands for STAT3, pSTAT3, or β-tubulin.