**Supplemental Methods**

**Cell Culture, STAT3 Phosphorylation, and Activation Assays**

EBV-B cell lines were cultured in RPMI 1640 medium (Invitrogen Life Technologies, France) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Invitrogen). EBV-B cells from patients and controls were plated in 24-well plates (2.5 × 106 cells/well) and starved of serum for 12 hours in 1 mL of RPMI supplemented with 2% FCS. The cells were stimulated with 50 ng/mL recombinant human IL-6 (R&D Systems 206-IL/CF), 50 ng/mL recombinant human IL-10 (R&D Systems 217-IL/CF), 30 ng/mL recombinant human IL-21 (GIBCO PHC0214), or 1000 U/mL IFN-α (Boehringer Ingelheim). STAT3 tyrosine 705 (Y705) phosphorylation was assessed by flow cytometry in EBV-B cells from patients and controls. Briefly, cells were stimulated with IL-6, IL-10, IL-21, and IFN-α for 15 min at 37-C (5% Co2). Cells were then fixed, washed, and stained with Alexa-Fluor 647-conjugated mouse antihuman phosphorylated tyrosine 705 STAT3 (pY705-STAT3) mAb (BD Biosciences) or with isotopic antibody, and analyzed by flow cytometry (BD FACSCanto II) with FACSDiva 6.0 software (BD). Nuclear translocation and DNA binding of STAT3 were assessed in EBV-B cells from patients and controls, with the Nuclear Extract Kit (Active Motif, Carlsbad, CA). EBV-B cells were starved of serum for 12 h and treated with IL-6, IL-10, IL-21, and IFN-α for 30 min. Nuclear STAT3 and STAT1 (used as a control) DNA binding activities were assessed with the TransAM STAT Family Kit (Active Motif), according to the manufacturer’s instructions.

**Quantification of mRNA Levels by RT-PCR**

Total RNAwas isolated from EBV-B cells with the RNeasy kit (Qiagen, Crawley, UK), according to the manufacturer’s instructions, and was left unstimulated or stimulated with IL-6(50 ng/mL), IL-10 (50 ng/mL), IL-21 (30 ng/mL), or IFN-α (1000 U/mL) for 1 h. RNA was treated with RNase-free DNase (Qiagen). Reverse transcription was carried out with oligo dT and random primers, according to the High Capacity RNA-to-cDNA Kit protocol (Applied Biosystems). STAT3, SOCS3, and IRF1 Taqman assays were carried out with Taqman Universal Fast Master Mix (Applied Biosystems) and analyzed with the ABI Fast 7500 system. STAT3, SOCS3, and IRF1 mRNA levels were analyzed by GUS staining with an endogenous control (Applied Biosystems). Relative expression levels were calculated by the delta-delta Ct method.53a

**Statistical Methods**

Experimental datawere summarized as means with standard errors. Differences between controls and STAT3 patients were tested for significance in the Kruskal-Wallis algorithm test: 1) mean ratio (stimulated/nonstimulated) of number of phospho-STAT3-positive EBV-B cells upon cytokine stimulation, 2) mean ratios (stimulated/nonstimulated) of STAT3 nuclear translocation, 3) mean ratios (stimulated/nonstimulated) for expression of the SOCS3 gene upon cytokine stimulation in each group of subjects, represented on scatter plots with the median line indicated.

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**Supplemental Figure 1*.*** STAT3 phosphorylation in EBV-B cells from patients and controls, as assessed by intracellular flow cytometry. Induction of phospho-STAT3 in EBV-B cell lines from healthy controls (squares); 8 STAT3-deficient patients with DNA-binding domain (DBD) mutations (R382W [Patient 18], R382Q [Patient 22], T412S [Patient 36], Vdel463 [Patients 14, 15, 16, 44], N472D [Patient 46]) (triangles); 2 STAT3-deficient patients with SH2 domain mutations (S614G [Patient 27], V637M [Patient 1]) (diamonds); and 3 STAT3-deficient patients with TA domain mutations (T708N [Patient 5], K709E [Patient 50], V713M [Patient 26]) (filled circles), in response to IL-6 (a), IL-10 (b), IL-21 (c), and IFN-α (d). Scatter plots (with median line) showing the mean percentages of phospho-STAT3-positive cells obtained following cytokine stimulation, for each subject for whom at least 2 independent experiments were carried out. The gray circle designates the patient (Patient 50) with heterozygous K709E STAT3 mutation.

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**Supplemental Figure 2*.*** SSTAT3 nuclear translocation in EBV-B cells from patients and controls, as assessed by EMSA ELISA. The values shown represent ratios of absorbance at 450 nm, for nuclear samples extracted from cytokine-stimulated or nonstimulated cells. STAT3 nuclear translocation from control EBV-B cell lines (squares); 8 STAT3-deficient patients with DNA-binding domain (DBD) mutations (R382W EPatient 18^, R382Q [Patient 22], T412S [Patient 36], Vdel463 [Patients 14, 15, 16, 44], N472D [Patient 46]) (triangles); 3 STAT3-deficient patients with SH2 domain mutations (S614G [Patient 27], V637M [Patients 1, 34]) (diamonds); and 5 STAT3-deficient patients with TA domain mutations (T708N [Patient 5], K709E [Patients 31, 50], V713M [Patient 26], T714I [Patient 13]) (filled circles), in response to IL-6 (a), IL-10 (b), IL-21 (c), and IFN-α (d). Each dot represents a mean value obtained for a single subject for whom at least 3 independent experiments were performed. The gray circle designates the patient (Patient 50) with heterozygous K709E STAT3 mutation.

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**Supplemental Figure 3**. Defective expression of the STAT3 target gene SOCS3. SOCS3 gene induction in EBV-B cell lines from healthy controls (squares); 8 STAT3-deficient patients with heterozygous DNA-binding domain (DBD) mutations (R382W [Patient 18], R382Q [Patient 22], T412S [Patient 36], Vdel463 [Patients 14, 15, 16, 44], N472D [Patient 46]) (triangles); 4 STAT3-deficient patients with heterozygous SH2 domain mutations (S614G [Patient 27], V637M [Patients 1, 34], S668Y [Patient 21]) (diamonds); and 5 STAT3-deficient patients with heterozygous TA domain mutations (T708N [Patient 5], K709E [Patients 31, 50], V713M [Patient 26], T714I [Patient 13]) (filled circles), in response to IL-6 (a), IL-10 (b), IL-21 (c), and IFN-α (d), as evaluated by quantitative RT-PCR. The gray circle designates the patient (Patient 50) with heterozygous K709E STAT3 mutation.