Supporting Information for:

SerpinB1 controls encephalitogenic T helper cells in neuroinflammation

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

Five videos (Video 1.mp4 through Video 5.mp4) related to Fig. 5 *C-E* show the behavior of littermate *wt* mice (5 cages) that developed MOG-induced EAE and were then treated (therapeutic protocol) with anti-CXCR6 mAb or isotype control.

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SI - MATERIALS AND METHODS

Human samples

Whole human blood of healthy donors and patients with MS were obtained with informed written consent and approval of the Institutional Review Board of Boston Children's Hospital under IRB protocols P003270 and 09-02-0043. Discarded synovial fluid specimens were obtained from patients with inflammatory arthritis undergoing diagnostic and/or therapeutic arthrocentesis for active joint inflammation under approved IRB protocols 2007P002441 (Brigham and Women's Hospital) and S09-10-0557 (Boston Children's Hospital). Associated clinical information, when available, was obtained from medical record review within 2 wk of sample collection, before associated identifying linkers were destroyed. Other synovial fluid samples and peripheral blood samples and associated clinical information were collected from patients who provided informed consent under IRB protocol 2014P002558 from Partners HealthCare. Patients were categorized as 'inflammatory arthritis' or 'control'; the latter were recruited from orthopedics and rheumatology clinics and were being seen for musculoskeletal complaints and were screened to exclude patients with a diagnosis of inflammatory conditions including rheumatoid arthritis, lupus, psoriasis, multiple sclerosis, type 1 diabetes, and inflammatory bowel disease or prior or current use of disease modifying anti-rheumatic drugs including methotrexate, sulfasalazine, azathioprine, hydroxychloroquine, mycophenolate mofetil, and biologic immunosuppressants. All patient specimens were de-identified prior to use in our study. Information on patients is provided in Table S2.

Synovial fluid specimens that were processed fresh were diluted in RPMI-1640 medium containing 10% FCS, pelleted and enriched by Ficoll-Hypaque density centrifugation at room temperature. For synovial fluid and blood samples that were analyzed as cryopreserved samples, the density purified mononuclear cells were washed and cryopreserved in FBS/10%DMSO. Cryopreserved samples were thawed rapidly at 37 ℃, washed, and analyzed. Single cell suspensions were prepared for surface staining or were stimulated with PMA and ionomycin (P+I) in the presence of Brefeldin A for 4 h and stained for cytokines and/or SB1 (clone ELA-5, in house).

Mice

SerpinB1 deficient mice (*serpinb1a*$^{-/-}$, hereafter *Sb1*$^{-/-}$) were generated in 129S6/SvEv/Tac (129S6) background (1) and were backcrossed to C57BL/6J (B6) (CD45.2^{+}) background for more than 10 generations. Congenic B6.SJL-CD45.1 (CD45.1, *wt*), OT-II, CD4-Cre, and *Rag1*$^{-/-}$ mice were from the Jackson Laboratory. CD45.1 *sb1*$^{-/-}$ and OT-II *sb1*$^{-/-}$ strains were generated by mating *Sb1*$^{-/-}$ B6 mice with CD45.1 or OT-II mice and intercrossing the resulting heterozygotes. *Il23r*$^{fl/fl}$ mice were originally described in Aden et al (2). *Il23r*$^{\Delta CD4}$ mice were generated by mating *Il23r*$^{fl/fl}$ mice with *Cd4*^{cre} mice and intercrossing the resulting heterozygotes. Mice were maintained in the animal facility of Boston Children's Hospital or the animal facility of Institute of Experimental Immunology, University of Zurich. To generate mixed *wt:Sb1*$^{-/-}$ bone marrow chimeras, *Rag1*$^{-/-}$ mice were lethally irradiated with two doses of 550 rads separated by a 4 h interval. T cell-depleted wild type and mutant bone marrow cells with traceable congenic CD45 markers were mixed at 1:1 ratio, and injected i.v. To generate mixed *wt*:*Il23r*$^{\Delta CD4}$ bone marrow chimeras, a total of 5x10^{6} bone marrow cells from *wt* (CD45.1) and *Il23r*$^{\Delta CD4}$ (CD45.2) mice

were injected in the tail veil of *wt* CD45.1xCD45.2 mice irradiated 2 x 550 rad with a 24 h interval. To prevent bacterial infection, the mice were provided with autoclaved drinking water containing Sulfatrim 1 wk before until 4 wk after irradiation (Boston site) or 0.2% (vol/vol) Borgal was added to the drinking water for 2 wk (Zurich site).

T helper cell differentiation

Single cell suspensions were prepared from spleens of 4-6 wk old B6 mice. For in vitro polarization studies, naïve CD4 T cells ($CD4^+CD25^{neg}CD44^{neg}CD62L^+$) were FAC-sorted and were cultured for 3 days in 24-well plates (Costar) pre-coated with anti-CD3 and anti-CD28 in the presence of previously described cytokine combinations in the case of T_H0, T_H1, Treg and T_H17 cells (3). For the T_H17 cells, these cytokines were IL-6 and TGFβ. For the studies of Fig. S1*A*, T_H17 cells were also generated with mIL-1β (10 ng/ml, Peprotec), mIL-6 (20 ng/ml, Peprotec), mIL-23 (40 ng/ml, Peprotec), anti-mIFN-γ (XMG1.2, 5 μg/ml, BioXcell) and anti-mIL-4 (11B11, 5 μg/ml, BioXcell). To generate T_H2 cells, the differentiating cytokines were mIL-4 (10 ng ml^{-1}, Biolegend) and anti-mIFN-γ (XMG1.2, 5 μg/ml, BioXcell). For two-stage cultures (4), freshly differentiated T_H17 cells were rested for 2 days in the presence of mIL-2 (2 ng/ml) and then were collected, washed and re-stimulated with anti-CD3 and anti-CD28 (both 1 μg/ml, plate coated) in the absence or presence of mIL-2 (20 ng/ml), mIL-12 (20 ng/ml) or mIL-23 (50 ng/ml) for additional 24 h.

Induction of EAE

Mice were injected with myelin oligodendrocyte glycoprotein (MOG) amino acid 35-55 (ProSpec, 150 μg per mouse) emulsified with complete Freund's adjuvant containing heat-killed *Mycobacterium tuberculosis* strain H37Ra (4 mg/ml) (Difco) at three sites on the back and were injected i.p with 200 ng pertussis toxin (List Biological Labs) on days 0 and 2 (hereafter called 'MOG immunization'). Both male and female mice were used, and in each experiment, the animals being compared were matched for age and gender. Disease was scored as (0) asymptomatic, (1) limp tail, (2) hindlimb weakness, (3) hindlimb paralysis, (4) hindlimb paralysis and partial or complete forelimb paralysis. Mice were euthanized when they reached stage 4 or stage 3 accompanied with 25% bodyweight loss per institutional regulations.

Adoptive transfer EAE

MOG-immunized *wt* or *sb1*$^{-/-}$ mice were sacrificed late during the "induction phase" prior to development of clinical symptoms (*i.e.,* days 7-10). Lymph nodes and spleen were harvested and cultured with MOG peptide plus IL-23. The expanded CD4 T cells were enriched by negative magnetic chromatography (Miltenyi Biotec) and injected i.v (5×10^6 cells per mouse) through tail vein into naïve *wt* or *sb1*$^{-/-}$ mice. Mice were injected i.p with 200 ng pertussis toxin on days 0 and 2.

Naïve CD4 cell transfer model of EAE

Naïve CD4 T cells were isolated from spleens of naive wt or *sb1*$^{-/-}$ mice by negative magnetic selection (Miltenyi Biotec) and 5×10^6 cells per mouse were injected in the tail vein of naive *Rag1*$^{-/-}$ recipient mice. One day later, the mice were immunized with MOG_{35-55}/CFA followed by pertussis toxin injection as above to induce EAE.

OT-II cell tracking study

Congenic WT CD45.1 mice were i.v. transferred with 2×10^5 naïve CD45.2$^+$OT-II cells or naïve CD45.2$^+$ *sb1*$^{-/-}$OT-II cells and s.c. immunized with $OVA_{323-339}$/CFA. Draining lymph nodes were harvested on days 4 and 12 post immunization, and OT-II cells were quantified and phenotyped by flow cytometry.

DTH reaction

Wt recipients of OT-II cells or *sb1*$^{-/-}$OT-II cells were immunized with $OVA_{323-339}$/CFA and were challenged in one hind footpad with 50 µg $OVA_{323-339}$ in saline as described (5). For the MOG-DTH reaction, *wt* or *sb1*$^{-/-}$ mice were immunized with MOG according to the EAE-induction protocol and, in the pre-disease phase, were challenged in one hind footpad with 50 µg MOG_{35-55} in saline. In both cases, the contralateral footpad was injected with saline. Foot thickness was measured with calipers; swelling is calculated by subtracting the foot thickness prior to challenge.

Isolation of spinal cord-infiltrating cells

EAE mice were sacrificed, and spinal cords were removed. Tissues were mechanically dissociated and digested for 30 min at 37 ℃ by 1 mg/ml collagenase D (Sigma-Aldrich) and 50 unit/ml DNAse I (Roche) in complete RPMI 1640 medium containing 5% FCS. Leukocytes were further enriched by 30% versus 80% percoll gradient.

Spinal cord histology

Spinal cords were fixed by immersion in Bouin's solution (Sigma-Aldrich) and were embedded in paraffin wax. Sections were cut from various locations and stained with H & E. Sections were evaluated by a pathologist and scored for severity of inflammation and degeneration as (0) asymptomatic, (1) mild, (2) moderately severe, (3) severe. Scoring was done blindly.

Intracellular staining and flow cytometry

Cells were stimulated for 4h with PMA (50 ng/ml) and ionomycin (750 ng/ml) (Sigma-Aldrich) in the presence of Brefeldin A (P+I stimulation). Cells were stained with fluorochrome-conjugated antibodies to surface markers. After washing, cells were fixed for flow cytometry analysis, or were permeabilized and stained intracellularly with fluorochrome-conjugated antibodies using fixation/permeabilization reagents and protocols from BD Bioscience. In case of LAMP1 staining, anti-LAMP1 antibody was added into the culture at the beginning of P+I stimulation. Fluorochrome-conjugated antibodies or cell death related dyes are: from Biolegend: FITC- or PE-Cy7-anti-mCD3 (145-2C11), Pacific blue- or APC-anti-mCD45.1 (A20), Pacific blue- or PE-anti-mCD45 (30-F11), Pacific blue-anti-mCD45.2 (104), Pacific blue- or PE-Cy7- or APC- anti-mCD4 (GK1.5), Alexa fluor488-anti-Brdu (3D4), PE-anti-mGranzymeC (SFC1D8), FITC-anti-h/mGranzymeB (GB1), PE-anti-mIL1R1 (JAMA-147), FITC-anti-mCD44 (IM7), APC-anti-mCXCR6 (SA051D1), APC-anti-mCCR2 (SA203G11), APC- or PE-Cy7-anti-mCCR6 (29-2L17), PE-Cy7-anti-mCD11b (M1/70), Alexa Fluor488-anti-mGr1 (RB6-8C5), FITC-anti-mIntegrinβ2 (M18/2), PE-anti-mIntegrinαL (M17/4), PE-anti-mCD25 (3C7), PE-anti-mIL7Rα (A7R34), FITC-anti-h/m/rat ICOS (C398.4A), PE-Cy7-anti-mPD1 (29F.1A12), PE-anti-mCD62L (MEL-14), Pacific blue- or APC-anti-mIL-17 (TC11-18H10.1), Pacific blue- or PE-anti-mIFN-γ (XMG1.2), FITC- or PE-anti-mGM-CSF (MP1-22E9), Alexa Fluor488-anti-FoxP3 (FJK-16s). From eBioscience: APC-anti-perforin (eBioOMAK-D), PE-anti-LAMP1 (1D4B), PE-Cy7-anti-Ki67 (S01A15), FITC-anti-mIntegrin β1 (eBioHMb1-1), PE-anti-mIntegrinα4 (R1-2), FITC-anti-mIntegrinβ3 (2C9.G3), PE-anti-mIntegrinαV (RMV-7). From R&D system: PE-anti-mIL-23R (753317). From BD Biosciences: PE-anti-mCD69 (H1.2F3),

FITC-rabbit-anti-active caspase3 (C92-605), FITC-Annexin V. Data were acquired on a Canto II cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star).

BrdU labeling and detection

Mice were immunized to induce EAE. At day 10 post EAE induction, BrdU (1 mg/mouse) was i.v. injected or i.p. injected. Lymph node cells were harvested and stained for surface expression of various markers, and detection of BrdU was carried out following the manufacturer's protocol (BD PharMingen). To monitor the BrdU incorporation in cytokine producing cells, mice were i.p injected with Brdu (1 mg/mouse) for 6 h. Then, lymph node cells were stimulated for 2.5 h with PMA (50 ng/ml) and ionomycin (750 ng/ml) (Sigma-Aldrich) in the presence of Brefeldin A, followed by surface marker staining, and intracellular co-staining of cytokine and BrdU.

Mitochondrial membrane potential

Freshly harvested lymph node leukocytes were incubated with 3,3′-dihexyloxacarbocyanine iodide ($DIOC_6$) (6) (10 nM, Sigma-Aldrich) for 15 min at 37 ℃, washed, and stained with fluorescein-labeled mAbs**.** The cells were evaluated by flow cytometry without fixation. Alternatively, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolocarbo-cyanine iodide (JC-1) (7) (2 μg/ml) (Thermo Fisher) was used as mitochondrial probe in the same protocol.

Western blot

Samples were resolved on 12% Tris-glycine gels and transferred onto PVDF. Membranes were blocked with 5% or 20% milk solids and stained with rabbit antiserum generated to human SerpinB1 or IgG fraction (arC70688) of rabbit 428A antiserum to granzyme-C (8) followed by HRP-conjugated secondary antibodies (Cell Signaling or BioRad). Bands were visualized by enhanced chemiluminescence (ECL Plus, Amersham Biosciences or West Pico, Pierce). SerpinB1 blots were stripped and restained with rabbit mAb to GAPDH (Cell Signaling).

Serpin-protease complex formation

Recombinant E193G-granzymeC (9) (20 ng/ml) was co-incubated with 1, 2 or 4 molar equivalents of recombinant human SerpinB1(10). The reactions were prepared for Western blot (as above) by heating with SDS and 2-mercaptoethanol. PVDF transfers of parallel reactions were stained for protein using Aurodye (colloidal gold, Amersham).

Reverse transcription and qPCR analysis.

RNA was isolated using RNeasy Plus kits (74134, Qiagen) according to the manufacturer's protocol and reverse-transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad). The qPCR assays were performed on the CFX96™ Real-Time System (Bio-Rad) with the iTaq™ Universal SYBR Green Supermix (Bio-Rad) using 30 sec denaturation at 95 ℃ and 40 cycles of 5 sec at 95 ℃ and 30 sec at 61 ℃ using the primers (Table S1). Relative expression level for each gene was calculated by using the $^{\Delta\Delta}$Ct method and normalizing to *Actb*.

RNA sequencing

Sb1 related RNAseq: CD4 effector cells ($CD44^{+}CD4$) were sort-purified from pooled lymph node cells of MOG-immunized *wt* and *sb1*$^{-/-}$ mice at disease onset. The cells were stimulated for 4 h ± SEM with P+I, and RNA was purified using QIAGEN RNeasy Plus Mini kits and quantified by optical density at 260/280/230nm RNA (1 μg per genotype) was shipped to Macrogen Corp (Seoul, Korea) and TruSeq RNA V2 kits were used to construct transcript-

specific libraries that were sequenced on Illumina HiSeq2500. The resulting 4.5 Gb/genotype of raw data was trimmed, and 20 million reads were mapped. Of the ≥ 24,000 genes evaluated in the resulting 2-way data sets, the 9,600 genes that had expression levels (FPKM) ≥1.0 were analyzed for differential expression.
IL-23r related RNAseq: Chimeric mice (*wt*:*Il23r*$^{\Delta CD4}$) mice were immunized with MOG. Nine days later, effector CD4 cells (CD44^{hi}CD62L^{lo} CD4^{+} T cells) were sorted from lymph nodes (axillary, brachial and inguinal) using the following antibodies: CD45.1 (clone A20), CD11b (M1/70), CD8a (53-6.7) from BD Pharmingen; CD45.2 (104), CD4 (RM4-5), CD62L (MEL-14) from BioLegend; CD3 (17A2) and CD44 (IM7) from eBioscience. Doublets exclusion was performed by FSC-A/FSC-H gating, and cell death exclusion with Zombie Aqua Fixable Viability Kit (BioLegend). Cell sorting was performed on the FACS Aria III (BD Biosciences). Total RNA was isolated with QIAGEN RNeasy Plus Micro Kit according to manufacturer's instructions. For RNA preamplification and library preparation, the Smart-seq2 protocol was used in combination with Illumina's Nextera XT DNA Library Preparation Kit (Illumina). Library preparation and NGS were performed by the Genomics Facility Basel (ETH Zurich and University of Basel, Switzerland) using the HiSeq 2500 v4 System (Illumina). Quality control included the fastqc analysis.

Anti-CXCR6 antibody treatment
Isotype rat IgG2b antibody (RTK4530) and rat-anti-mouse CXCR6 (SA051D1) were from Biolegend. The antibodies (ULEAF purity) were sterile-filtered (0.2 μm filter), contained no preservative, no azide, and endotoxin < 0.01 EU/μg protein. Isotype or anti-CXCR6 antibodies were i.p. injected. Disease prevention protocol: *wt* mice were immunized with MOG and treated with anti-mouse CXCR6 mAb or isotype control (300 μg) on days 5 (pre-disease), 7, 9 and 12; Therapeutic protocol: *Wt* mice were MOG-immunized, and when disease was first detected (clinical score 1-3), the mice were randomly assigned to receive either anti-CXCR6 antibody (n=8) or isotype control (n=11) (400 μg) on that day and 2 and 4 days later.

Statistical analysis
Statistical analyses were performed using Prism 4 (Graphpad Software). Student's *t*-test, unpaired and paired, and one-way ANOVA were used according to the type of experiment. p-values ≤ 0.05 were considered significant.

SI – REFERENCES

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SI – FIGURES and LEGENDS

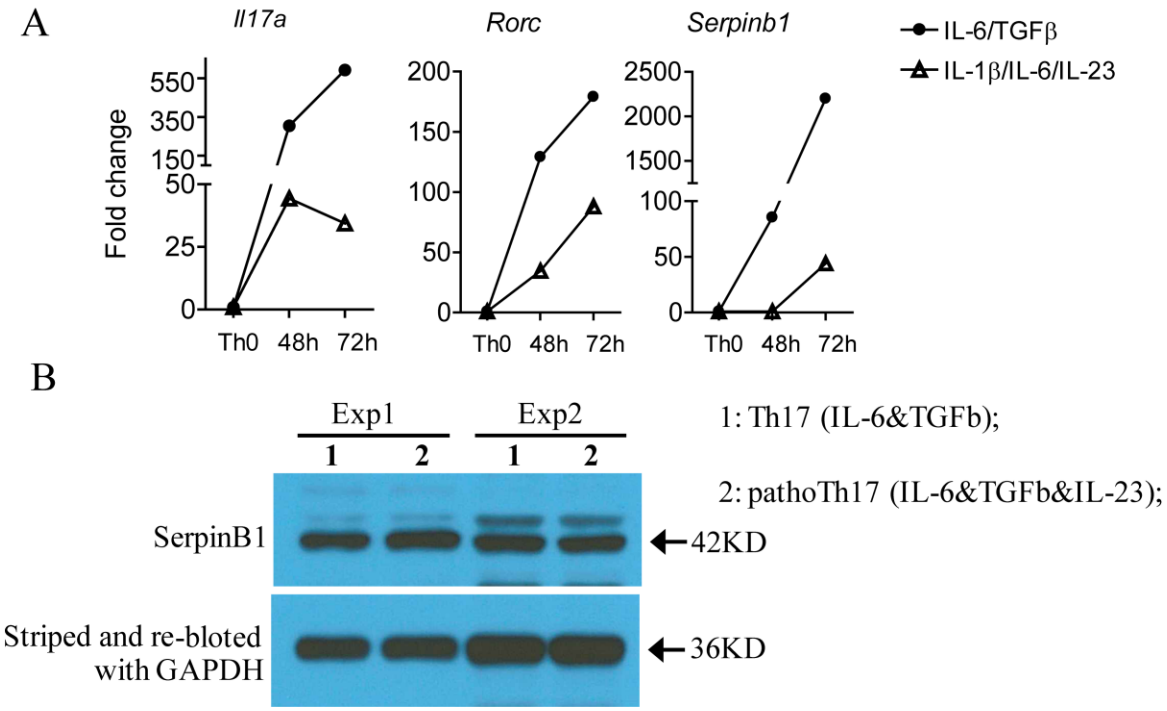

Fig. S1. *In vitro* differentiation of Th17 cells. (*A*) Differentiation of T_H17 cells with different cytokine cocktails. Naïve CD4 cells were FACS sorted and differentiated by the addition of IL-6 + TGFβ or IL-1β + IL6 + IL-23. Cells collected at the indicated times were subjected to qRT-PCR analysis. (*B*) Effect of IL-23 on IL-6+TGFβ induced differentiation of T_H17 cells. Naïve CD4 cells were differentiated into Th17 cells for 3 days by IL-6 + TGFβ with or without IL-23. Cells were collected and subjected to western blot analysis for Sb1 and GAPDH. Shown are findings from two independent experiments.

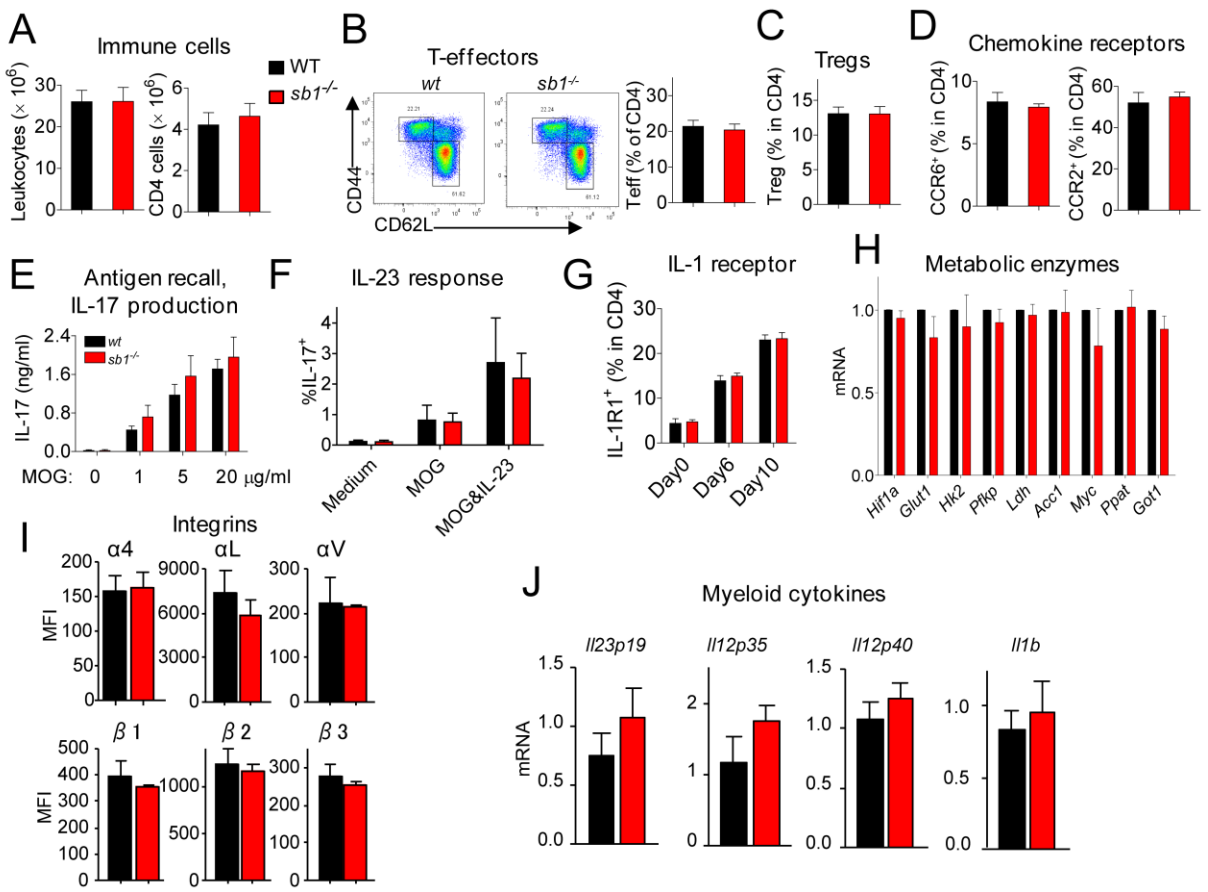

Fig. S2. Serpinb1-deficient CD4 cells are not defective in the priming phase of EAE.
(*A-E*< *G-I*) *Wt* and *sb1*$^{-/-}$ mice were immunized with MOG, and draining lymph node (LN) cells were studied at onset of EAE. (A) Immune cell counts. (*B*) T effector cell frequency. (*C*) Tregs (CD4^{+}CD25^{hi}FoxP3^{+}) frequency. (*D*) CCR2^{+} and CCR6^{+} CD4 cell frequency. Data were determined by flow cytometry and are means for 9 mice each genotype. (*E*) IL-17 production in antigen-recall reaction. (*F*) Responsiveness to IL-23 in antigen recall. Splenocytes (*E*) or LN cells (*F*) harvested at disease onset were cultured with or without MOG in the presence or absence of IL-23 for 48 h and (*E*) IL-17 in supernatants was quantified by ELISA or (*F*) BFA was added during the last 8 h, and IL-17^{+} cells were quantified by intracellular flow cytometry. Depicted data are means for (*E*) 8 and (*F*) 5 mice per genotype representative of 2-3 experiments. (*G*) IL-1 receptor upregulation. Frequency of IL-1R^{+} CD4 cells in LN on days 0, 6 (pre-disease) and day 10 (onset of EAE) post-immunization of *Rag1*$^{-/-}$ mice transferred with naïve *wt* and *sb1*$^{-/-}$ CD4 cells. Depicted data are means for 3-4 mice per genotype per time point. (*H*) Relative expression of metabolic enzymes determined by qRT-PCR of effector (CD44^{+}) CD4 cells. (*I*) Cell surface expression (mean fluorescence intensity; MFI) of integrin subunits of LN effector CD4 cells. Data are mean for 3-5 mice each genotype representative of three experiments. (*J*) Relative expression of myeloid cell cytokines determined by qRT-PCR analysis of total LN immune cells. (*H,J*) Depicted data are mean for pooled cells of 3-5 mice per genotype in three experiments. *P<0.05 by Student's t-test. Error bars represent SEM.

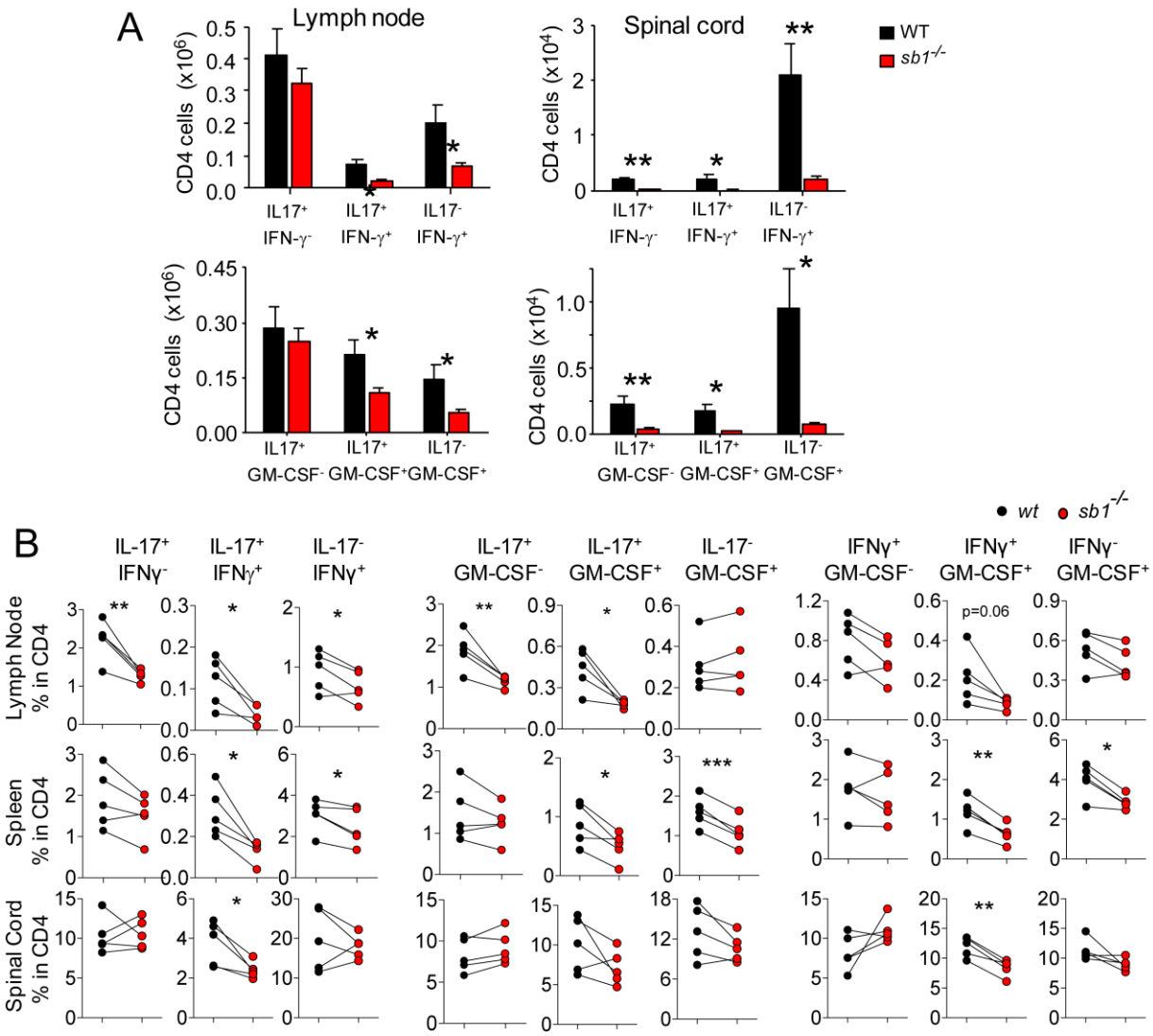

Fig. S3. Decrease of cytokine-expressing *sb1*$^{-/-}$ CD4 cells in EAE (related to Fig. 3)
(*A*) Decreased number of IFNγ^{+} and GM-CSF^{+} CD4 cells at disease onset in (left) LN and (right) spinal cord. Shown are absolute cell numbers for the experiments of Fig. 3*B*,*C*.
(*B*) Frequency of cytokine-producing *wt* and *sb1*$^{-/-}$ CD4 cells in mixed chimeric mice at peak disease determined by flow cytometry after ex vivo stimulation with P+I. Lines connect *wt* (black circles) and *sb1*$^{-/-}$ (red circles) cells from the same chimera. Experiments were repeated twice with the same pattern. **P* <0.05, ***P* <0.01, ****P*<0.001 by Student's paired t-test.

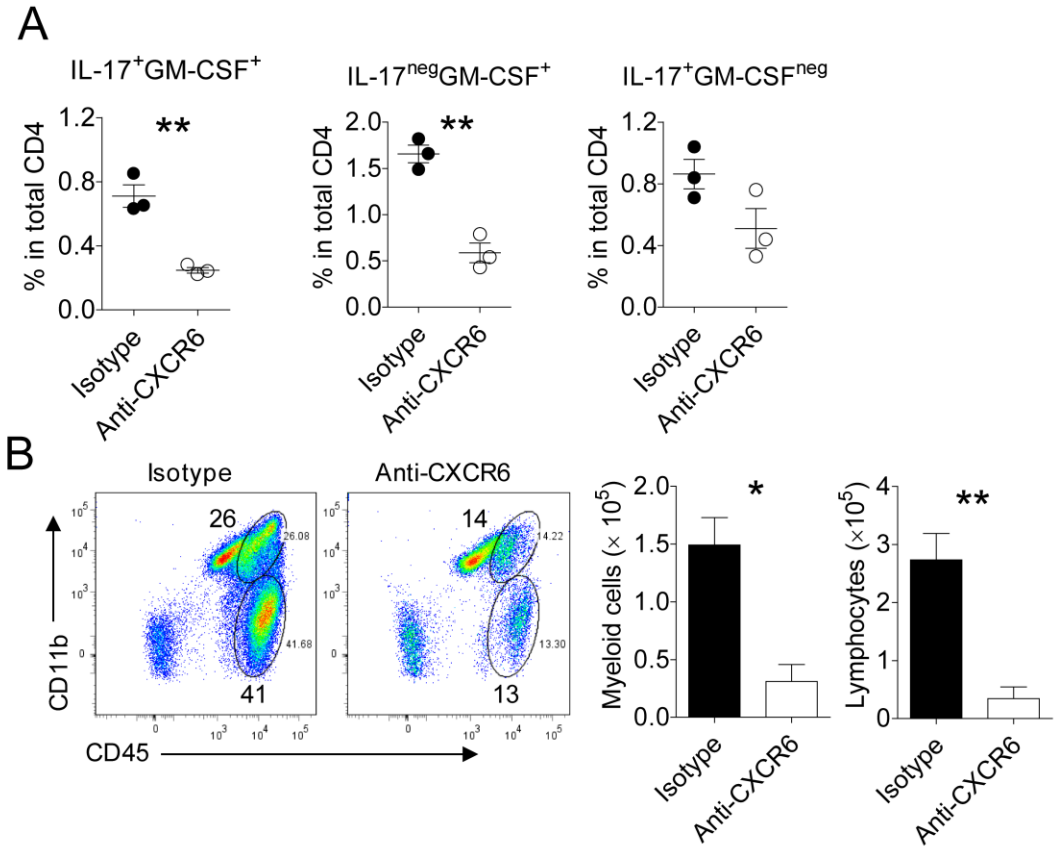

Fig. S4. Treatment with CXCR6 antibody decreases splenic IL-17/GM-CSF DP and GM-CSF SP CD4 cells and prevents accumulation of lymphocytes and myeloid cells in the spinal cord (related to Fig. 5)

(*A*) Feasibility study: MOG-immunized *wt* mice received a single dose (400 μg i.p.) of anti-CXCR6 mAb or isotype control at disease onset and were sacrificed 24 h. Cytokines were analyzed by intracellular staining post P+I for 4 h. Symbols represent individual mice. Results are representative of two experiments. (*B*) Prevention protocol: Myeloid cells and lymphocytes harvested from spinal cord of isotype-treated and anti-CXCR6 treated *wt* mice at day 30 (termination) of the Fig. 5*A*,*B* study. (Left) Representative dot plots. (Right) Mean cell counts **P*<0.05, ***P*<0.01 by Student's t-test. Error bars represent ± SEM.

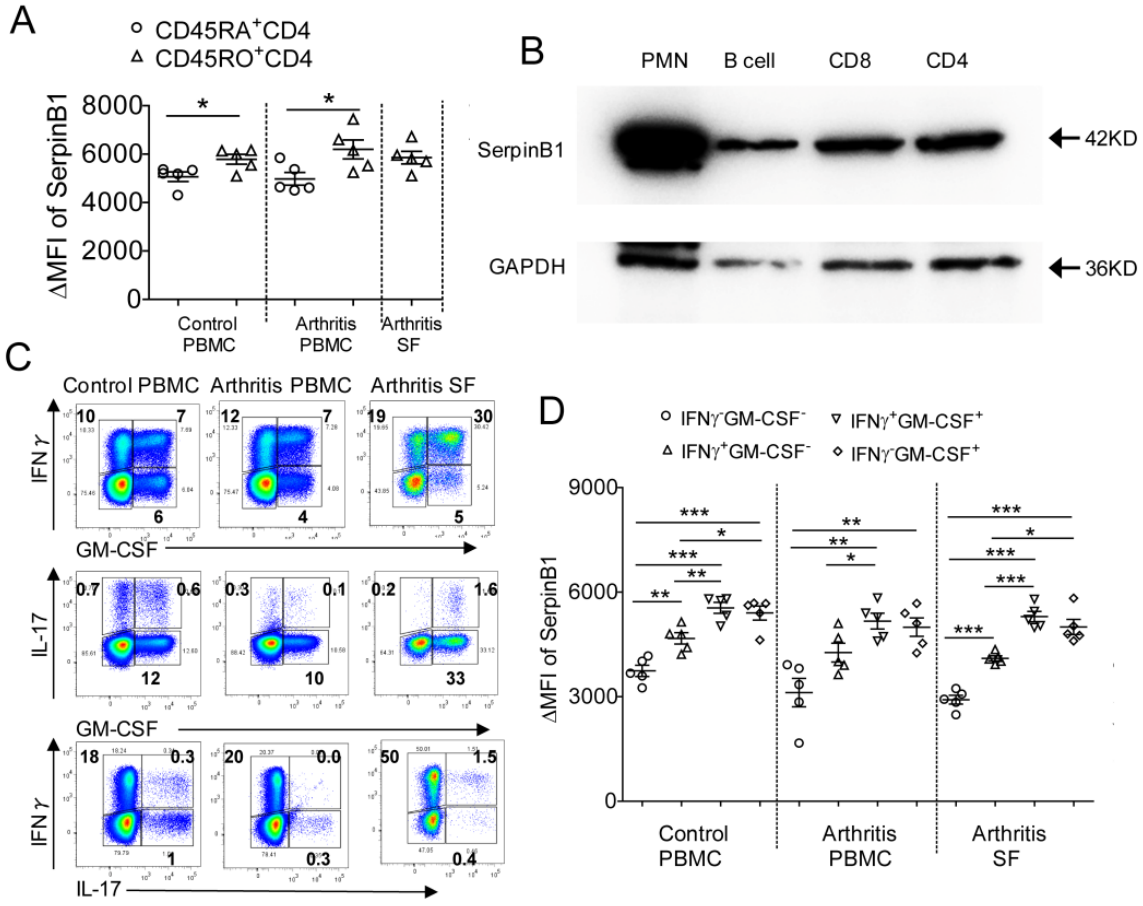

Fig. S5. *SerpinB1* (*SB1*) expression in human leukocytes. (*A-B*) *SB1* expression in peripheral blood and synovial fluid CD4 cells of control and inflammatory arthritis patients. (*A*) Mononuclear cells from the indicated sources were surface stained with marker antibodies, stimulated with P+I for 4 h, stained intracellularly for SB1 (clone ELA-5) and analyzed by FACS. Shown are MFI. (*B*) SB1 expression in neutrophils, CD4 cells and CD8 cells of normal healthy donors examined by Western blot. Neutrophils isolated by conventional techniques (≥ 95% pure) and CD4 and CD8 cells that were FACS-sorted (>98% pure) were subjected to Western blot using HI-443 rabbit anti-SerpinB1 and rabbit anti-GAPDH. (*C,D*) *SB1* expression in the indicated cytokine-producing CD4 cells. (*C*). Representative FACS plots showing cytokine staining; (*D*) Mononuclear cells were stained for cytokines and SB1 after 4 h stimulation with P+I and were analyzed by FACS. (*A, D*) Symbols indicate individual patients. **P*<0.05, ***P*<0.01, ***P*<0.001 by Student's t-test.

SI – VIDEO LEGENDS

Videos 1.mp4, 2.mp4, 3.mp4, 4.mp4 and 5.mp4. Treatment with anti-CXCR6 reverses established EAE Behavior of anti-CXCR6-treated and isotype-treated mice. Therapeutic protocol. Nineteen *wt* mice, 5 cages of 4-5 littermates/cage, were immunized with MOG, and when disease was first detected (clinical score 1-3), the mice were randomly assigned to receive 400 μg i.p. of either anti-CXCR6 antibody or isotype control (n=11) on that day ('Day 0') and 2 and 4 days later. Clinical scores were recorded daily beginning on Day 0 (Fig. 5*C*). Videos were prepared during a single scoring session corresponding to treatment days 3 to 6, at which time three of the isotype-treated mice had reached score 4 and been sacrificed per protocol. All mice had been identified at weaning by an ear punch system, which was supplemented for videotaping by marker pen labeling of the tail. Marker pen labeling system: #1:one horizontal line; #2: two horizontal lines; #3: Three horizontal lines; #4: Four horizontal lines; #5: one vertical line. Mice in each cage were littermates and remained together throughout the study. In videos of each cage the mice that moved continuously or frequently were mAb treated mice and those that remained in place, in most cases lying prone, or that moved only slowly and infrequently were isotype-treated mice. Detailed information on anti-CXCR6 mAb treated and isotype-treated mice were provided in the following.

Video 1 (cage 1197288). Three mice:
Anti-CXCR6 mAb, mouse #3, score 2 on Day 0, videotaped on Day 5 (3 treatments)
Anti-CXCR6 mAb, mouse #5, score 2 on Day 0, videotaped on Day 4 (2 treatments)
Isotype-treated, mouse #1, score 1 on Day 0, videotaped on Day 4 (2 treatments)

Video 2 (cage 1197287). Three mice:
Anti-CXCR6 mAb, mouse #4, score 2 on Day 0, videotaped on Day 4 (2 treatments)
Isotype-treated, mouse #2, score 3 on Day 0, videotaped on Day 5 (3 treatments)
Isotype-treated, mouse #3, score 1 on Day 0, videotaped on Day 3 (2 treatments)

Video 3 (cage 1197271). Three mice:
Anti-CXCR6 mAb, mouse #2, score 3 on Day 0, videotaped on Day 6 (3 treatments)
Isotype-treated, mouse #1, score 1 on Day 0, videotaped on Day 2 (1 treatment)
Isotype-treated, mouse #3, score 2 on Day 0, videotaped on Day 4 (2 treatments)

Video 4 (cage 1197304). Four mice:
Anti-CXCR6 mAb, mouse #3, score 2 on Day 0, videotaped on Day 6 (3 treatments)
Anti-CXCR6 mAb, mouse #5, score 1 on Day 0, videotaped on Day 4 (2 treatments)
Isotype-treated, mouse #1, score 1 on Day 0, videotaped on Day 4 (2 treatments)
Isotype-treated, mouse #4, score 1 on Day 0, videotaped on Day 5 (3 treatments)

Video 5 (cage 1197298). Three mice:
Anti-CXCR6 mAb, mouse #1, score 2 on Day 0, videotaped on Day 3 (2 treatments)
Anti-CXCR6 mAb, mouse #3, score 1 on Day 0, videotaped on Day 6 (3 treatments)
Isotype-treated, mouse #2, score 2 on Day 0, videotaped on Day 4 (2 treatments)

Table S1. Primer sequences.

	Forward (5'-3')	Reverse (5'-3')
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Hif1α	AGCTTCTGTTATGAGGCTCACC	TGACTTGATGTTCATCGTCCTC
Glut1	CAGTTCGGCTATAACACTGGTG	GCCCCCGACAGAGAAGATG
Hk2	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
Pfkp	CGCCTATCCGAAGTACCTGGA	CCCCGTGTAGATTCCCATGC
Ldha	CATTGTCAAGTACAGTCCACACT	TTCCAATTACTCGGTTTTTGGGA
Acc1	ATGGGCGGAATGGTCTCTTTC	TGGGGACCTTGTCTTCATCAT
Myc	ATGCCCCTCAACGTGAACTTC	CGCAACATAGGATGGAGAGCA
Ppat	GCGAGGAATGTGGTGTGTTTG	TTTAGGCACTGCACTCCCATC
Got1	GCGCCTCCATCAGTCTTTG	ATTCATCTGTGCGGTACGCTC
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ccl3	TTCTCTGTACCATGACACTCTGC	CGTGGAATCTTCCGGCTGTAG
Ccl4	TTCCTGCTGTTTCTCTTACACCT	CTGTCTGCCTCTTTTGGTCAG
Ccl5	GCTGCTTTGCCTACCTCTCC	TCGAGTGACAAACACGACTGC
Ccl20	GCCTCTCGTACATACAGACGC	CCAGTTCTGCTTTGGATCAGC
Il2	TGAGCAGGATGGAGAATTACAGG	GTCCAAGTTCATCTTCTAGGCAC
Il23r	TTCAGATGGGCATGAATGTTTCT	CCAAATCCGAGCTGTTGTTCTAT
Ccr6	CCTGGGCAACATTATGGTGGT	CAGAACGGTAGGGTGAGGACA
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Il17a	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
Il21	TCATCATTGACCTCGTGGCCC	ATCGTACTTCTCCACTTGCAATCC
Il22	GTGGGATCCCTGATGGCTGTCCTGCAG	AGCGAATTCTCGCTCAGACTGCAAGCAT
Il10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Csf2	GGCCTTGGAAGCATGTAGAGG	GGAGAACTCGTTAGAGACGACTT
Ifng	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
Il7ra	GCGGACGATCACTCCTTCTG	AGCCCCACATATTTGAAATTCCA
Tbx21	CCACTGGATGCGCCAGGAAGTT	TTCACCTCCACGATGTGCAGCC
Rorc	CGCGGAGCAGACACACTTA	CCCTGGACCTCTGTTTTGGC
Gzmc	GCAGAGGAGATAATCGGAGGC	GCACGAATTTGTCTCGAACCA
Prf1	AGCACAAGTTCGTGCCAGG	GCGTCTCTCATTAGGGAGTTTTT
Gzma	TGCTGCCCACTGTAACGTG	GGTAGGTGAAGGATAGCCACAT
Gzmb	CCACTCTCGACCCTACATGG	GGCCCCCAAAGTGACATTTATT
Serpinb9	TCAGGTGGCTCCGTCGATT	GGCATGTCCATTGTGTACTCTT
Serpinb1	ACATCCATTCACGCTTCCAAA	GGCCAAGTCAGCACCATACAT
Cxcr6	GAGTCAGCTCTGTACGATGGG	TCCTTGAACTTTAGGAAGCGTTT

Table S2. Human subject information.

	Sample	Age	Gender	Source	Disease	Treatment	Processing
Control PBMCs	1	74	F	PBMC	Control	NSAIDs	frozen
	2	57	F	PBMC	Control	none	frozen
	3	22	F	PBMC	Control	none	frozen
	4	50	F	PBMC	Control	none	frozen
	5	22	F	PBMC	Control	none	frozen
Arthritis PBMCs	1	54	F	PBMC	RA	sulfasalazine, NSAIDs	frozen
	2	57	F	PBMC	RA	prednisone, NSAIDs	frozen
	3	20	M	PBMC	JIA	etanercept, methotrexate	frozen
	4	22	M	PBMC	JIA	hydroxychloroquine	frozen
	5	22	F	PBMC	spondyloarthritis	adalimumab	frozen
Arthritis synovial fluid MNCs	1	15	F	knee	JIA	NSAIDs	fresh
	2	19	F	knee	JIA	none	fresh
	3	11	M	knee	JIA	NSAIDs	fresh
	4	59	M	knee	spondyloarthritis	NSAIDs	fresh
	5	54	F	knee	RA	prednisone, methotrexate	fresh
	6	18	F	knee	JIA	NSAIDs	fresh
	7	47	M	knee	RA	NSAIDs	fresh
	8	83	M	knee	pseudogout	none	fresh
	9	56	F	wrist	RA	NSAIDs	fresh
	10	n/a	n/a	knee	RA	n/a	frozen
	11	n/a	n/a	knee	RA	n/a	frozen
	12	n/a	n/a	knee	JIA	n/a	frozen

	13	n/a	n/a	knee	JIA	n/a	frozen
	14	n/a	n/a	knee	spondyloarthritis	n/a	frozen
Multiple sclerosis PBMC	1	18	F	PBMC	MS	Gilenya	fresh
	2	16	F	PBMC	MS	Steroids	fresh
	3	18	F	PBMC	MS	Tecfidera	fresh