Supplemental methods:

Clinical description of the SJIA patient undergoing bone marrow examination

14 m/o Caucasian female who was initially admitted with 4 month history of intermittent fevers, rash and joint pain. Her initial exam was remarkable for mild urticarial rash, mild splenomegaly, swelling and tenderness in both elbows, left wrist, both knees and ankle as well as decreased range of motion in the cervical spine. On the day preceding the BM biopsy her hemoglobin was 7.1 g/dL, MCV 68.8 fL, platelet count 368 K/mcL, white blood cell count 8.0 K/mcL with absolute neutrophil count of 2.4, CRP 12.7 mg/dL and ESR 39 (down from 105 3 days earlier). Her serum ferritin was 312 ng/mL (up from 121 three days earlier). After bone marrow examination she initially responded to the combination of corticosteroids and anakinra but the symptoms returned with corticosteroid taper. She failed canakinumab but responded well to biweekly tocilizumab infusions at the dose of 12 mg/kg started approximately at month 5 since the initial diagnosis. Her disease has subsequently been well controlled with continuous tocilizumab monotherapy. Since her course of the disease has been typical of SJIA and she has not had recurrences of MAS, genetic testing has not been performed.

Transcriptional signatures of *in vitro* polarized control monocytes were determined by using Ampliseq Transcriptome. Briefly, healthy control monocytes were isolated and incubated for 24h as follows: **M(LPS+IFNγ)** (20 ng/ml IFNγ (R&D Systems, Minneapolis, MN) and 10 ng/ml LPS (Sigma Aldrich)), **M(IL-4)** (20 ng/ml IL-4 (R&D Systems)), **M(LPS+IC)** (100 ng/ml LPS in IgG-coated tissue culture wells), **M(IL-10)** (50 ng/ml IL-10 (R&D Systems)). The Ion AmpliSeq Library Kit Plus (Life Technologies) and the Ion AmpliSeq Transcriptome Human Gene Expression Core Panel was used to amplify target genes per manufacturer's directions, and each sample was barcoded with the Ion Express Barcode Adapters. Each library was then purified, amplified, and size selected, followed by amplification through emulsion PCR. Libraries were then sequenced on the Ion 540 Chip using the Ion S5 sequencer, and the amplicon regions were mapped with hg19_AmpliSeq_Transcriptome_ERCC_V1 reference from Ion Community. Transcriptional profiles in each of these cell populations were compared to untreated monocytes to generate M(LPS+IFNγ), M(IL-4), M(LPS+IC) and M(IL-10) signatures, respectively.

Bulk monocyte isolation and gene expression analysis: Monocytes were purified as described using positive selection [1] to maximize cell purity [2], and were found to be 95-98% pure by flow cytometry without notable neutrophil contamination (absence of CD15+CD16+ cells). CD119 surface expression was determined Total RNA was extracted immediately following cell separation using the mirVana miRNA isolation kit (Life technologies, Carlsbad, CA). RNA quantity was assessed on the Qubit Fluorometric quantitation system (Life Technologies) and on a Bioanalyzer (Agilent, Wilmington,DE). Genomic libraries were created using TruSeq Stranded mRNA Sample Prep Kits (Illumina, San Diego, CA, USA) and sequenced using an Illumina HiSeq2500 to generate an average of over 40 million raw, single-end reads per sample. Data was analyzed using the *limma* Bioconductor package and accounting for surrogate variables discovered with *sva*[3]. SJIA patient samples were processed in separate batches from healthy control samples. RNA-seq reads were quantified by pseudoalignment to the[4] reference genome (hg38) using *kallisto*[5]. Statistical analysis to identify genes differentially expressed between all SJIA patients and age-matched controls was performed using the *limma* Bioconductor package and accounting for surrogate variables discovered with *sva*[3]. Bonferroni-corrected $P < 0.05$ was used as evidence of statistical significance. For exploratory plots, counts were normalized and transformed using the regularized logarithm from *DESeq2*[6] and surrogate variables were regressed out. using the cleaningY function from the R package jaffelab version 0.99.29. Analyses of over-represented gene ontologies (limma[4]) was used to identify pathways showing differences between all SJIA patients and controls. Gene set overlap analysis with derived polarization signatures was performed using the R packages GeneOverlap version 1.26.0.

CD119 surface expression: Frozen patient and control PBMC was thawed, washed and stained with Live/ Dead cell stain (Invitrogen), APC-conjugated anti-CD14 (clone M5E2) and FITC-conjudated anti-CD16 (clone NKP15), and PE-conjugated CD119 (clone GIR-208) as described previously [7] and analyzed using LSR Fortessa. Cells were gated for live cells, CD14, and CD16 positive cells. All antibodies were from BD Biosciences.

Single cell RNA-seq analysis: Leftover BM aspirates were obtained from deidentified patients evaluated for fever and/or cytopenias but where no hematologic or oncologic abnormalities were found (control BM) or from a patient with SJIA and early/subclinical MAS. After red cell lysis, BM cells were stored at -80C in freezing

media until sequencing. After thawing, macrophage single cell suspensions were obtained using cell sorting for live (7AAD-) populations expressing the monocyte and macrophage surface markers CD14 and CD163 while excluding cells expressing the granulocyte/monocyte marker CD15. This suspension was then loaded onto the Fluidigm C1 Single-Cell Auto Prep System, which captures up to 96 single cells per chip. These cells were subsequently lysed, and extracted RNA converted into cDNA and sequenced as a pooled library using the Illumina platform, providing approximately 3.5-4 million reads per single cell. All single-cell libraries were pseudo-aligned to the human Ensembl version 72 transcriptome using the Kallisto through AltAnalyze version 2.1.3. as described in our previous publication[8]. To ensure quality control, libraries with less than 300,000 paired-end reads or estimated read percentage alignment <20% were excluded. Unsupervised population discovery using control BMM samples was performed using the software ICGS version 2[8] and PCA-loading gene analysis and HOPACH clustering in AltAnalyze. PCA-loading genes were selected as the top-100 correlated and top-100 anti-correlated genes associated with the first 4 principal components from the initial variance filtered expression file (filteredExp ICGS output file), corresponding to the software defaults (Figure 4B). The number of principal components to retain (n=4) was verified using the findElbowPoint function supplied in the PCAtools package (version 2.2.0), and we note that 27% of the total variance could be attributed to the top-4 PCs. In order to identify further cellular subpopulations, the initial identified predominant gene clusters from this PCA-loading analysis were excluded prior to ICGS2 [9], by identifying genes correlated to the centroid of the gene clusters with a Pearson rho > 0.1 (21,719 genes remaining after exclusion) (Figure S2B). This threshold was selected after iteratively testing Pearson rho thresholds of 0.1 to 0.9 in 0.1 increments, followed by ICGS2 analysis to observe the presence of these predominant gene signatures. ICGS2 was run using the default program parameters. To expand the TRIM8-overexpressing cell-cluster identified from the analysis of SJIA with donor BMM, an aggregate score (sum log2 TPM of the *TRIM8* cluster maker genes), was used to identify additional SJIA patient cells (n=12, with an aggregate score greater than the average) enriched in *TRIM8*-associated genes prior to differential expression analyses. For the identification of *TRIM8*-cluster specific genes, we applied empirical Bayes moderated (eBayes) t-test comparing *TRIM8*-cluster SJIA cells (excluding all other SJIA cells) to all donor macrophage cells (AltAnalyze). Only genes with a minimum average TPM >1 in the two compared populations, eBayes t-test p<0.05 (following Benjamini-Hochberg correction) and fold > 1.5 were considered differentially expressed. To confirm the validity

of this expanded *TRIM8* SJIA cluster, we performed a supervised analysis of the expanded *TRIM8* cluster cells versus all other dataset cell profiles, using marker genes (MarkerFinder) as the reference, using the software cellHarmony [10]. To define genes up and down-regulated in the expanded *TRIM8* SJIA cluster, SJIA cells in this cluster were compared to all healthy control cells.

Supplemental references:

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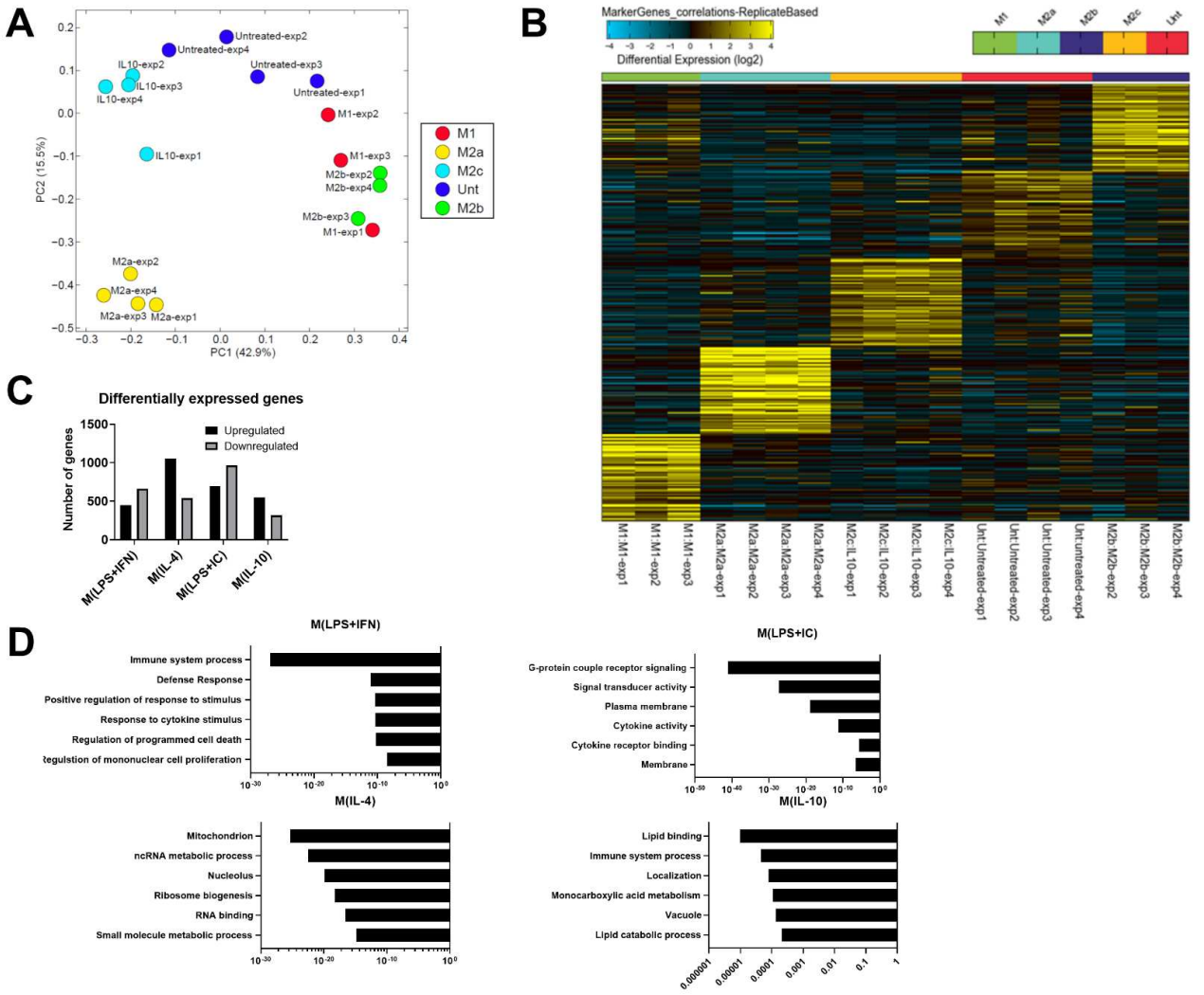
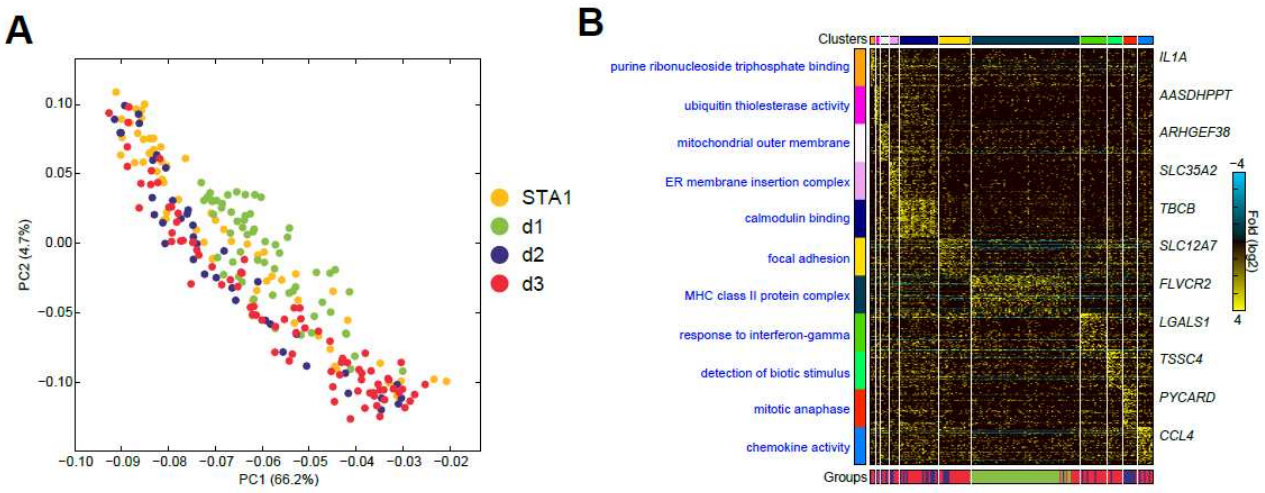


Supplemental Figure 1: Empirically derived polarization signatures of in vitro treated peripheral blood monocytes. A, principle component analysis of gene expression profiles obtained using Ampliseq Transcriptome from peripheral blood monocytes from heatlhy donors (n=4) left untreated or polarized towards M(LPS+IFN)/M1, M(IL-4)/M2a, M(LPS+IC)/M2b, or M(IL-10)/M2c. B, hierarchical clustering of gene expression profiles using MarkerFinder genes as determined by AltAnalyze. C, upregulated and downregulated differentially expressed genes in polarization conditions compared to untreated monocytes ($p<0.05$). D, significantly enriched Gene Ontology pathways of upregulated genes in each condition compared to control monocytes.



Supplemental Figure 2: A, principle component analysis of single cell RNA-seq profiles from BM macrophages obtained from three normal donors (d1, d2, d3) and a patient with SJIA and subclinical MAS (STA1). B, Unsupervised clustering of scRNA-seq (ICGS version 2) from three normal control samples (d1=green, d2=blue, d3=red). Along left-side of the plot are enriched functional pathways within the gene cluster (PathwayCommons), and representative genes listed along right edge.