Supplemental Information

Experimental procedure for ChIP-sequencing experiments

Cells were crosslinked with 2% formaldehyde (Sigma-Aldrich) and after 10 min crosslinking was stopped by adding 0.15 M glycine. Nuclei were isolated in in 50 mM Tris (pH 7.5), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, and 1% Triton X-100 and lysed in 20 mM Tris (pH 7.5), 150 mM NaCl, 2 mM EDTA, 1% NP-40 and 0.3% SDS. Lysates were sheared using Covaris microTUBE (duty factor 20%, peak incident power 105, 200 cycles per burst, 480 sec cycle time) and diluted in 20 mM Tris (pH 8.0), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100. Sheared DNA was incubated overnight with anti-histone H3 acetyl K27 antibody (ab4729; Abcam) pre-coupled to protein A/G magnetic beads (Pierce). Samples were washed and crosslinking was reversed by adding 1% SDS, 100 mM NaHCO3, 200 mM NaCl, and 300 ug/ml proteinase K (Invitrogen). DNA was purified using ChIP DNA Clean & Concentrator kit (Zymo Research), endrepair, a-tailing, and ligation of sequence adaptors was done using Truseq nano DNA sample preparation kit (Illumina). Samples were PCR amplified and barcoded libraries were sequenced 75 bp single-end on Illumina NextSeq500. After quality control, 9 million reads or more were obtained for each sample. Peaks were called using Cisgenome 2.0 (–e 150 -maxgap 200 –minlen 200) [1]. Peak coordinates were stretched to at least 2000 base pairs and collapsed into a single list. Overlapping peaks were merged based on their outmost coordinates. Only peaks identified by at least 2 independent datasets were further analyzed. Peaks with differential H3K27ac occupancy were identified using DESeq (padj<0.1) [2]. Associated genes were defined as genes with a transcription start site within 20 kB from the H3K27ac peak. To assess enrichment of STAT1 and H3K27ac ChIP-seq data of IFNγ-treated monocytes (GSE43036) in JIA SF H3K27ac peaks, peak coordinates of GSM1057010 and GSM1057016 were overlapped with significantly upregulated or downregulated genes in JIA SF vs. JIA Blood.

Experimental procedure for RNA-sequencing experiments

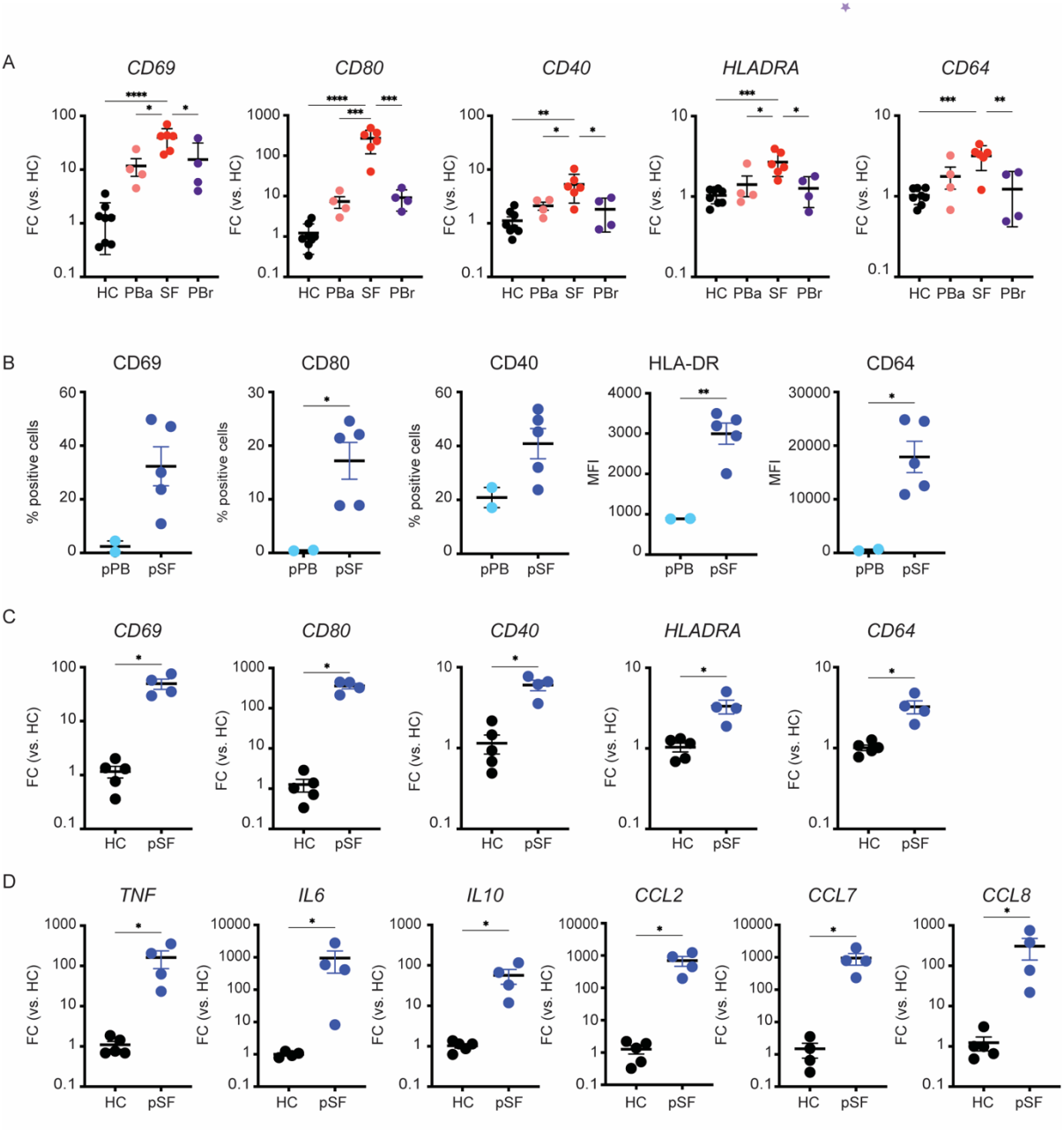
For analysis of JQ1 sensitive genes, RNA-sequencing was performed as described before[3]. For analysis of *ex vivo* gene expression, total RNA was extracted using Trizoll, mRNA was isolated using Poly(A)Purist MAG kit (Life Technologies) and additionally purified with a mRNA-ONLY Eukaryotic mRNA Isolation Kit (Epicentre). Transcriptome libraries were then constructed using SOLiD total RNA-seq kit (Applied Biosystems) and sequenced using 5500 W Series Genetic Analyzer (Applied

Biosystems) to produce 40-bp-long reads. After quality control, 9 million reads or more were obtained for each sample and the total coverage was 8X. Reads were aligned to the human reference genome GRCh37 using STAR version 2.4.2a. Picard's AddOrReplaceReadGroups (v1.98) was used to add read groups to the BAM files, which were sorted with Sambamba v0.4.5 and transcript abundances were quantified with HTSeq-count version 0.6.1p1 using the union mode. Subsequently, reads per kilobase million reads sequenced (RPKMs) were calculated with edgeR's RPKM function. Differentially expressed genes were identified using the DESeq2 package with standard settings (adjusted p-value was calculated using the Benjamini and Hochberg method to account for multiple testing correction). Genes with padj<0.05, with a base mean ≥ 10 and a log2 FC ≥1 or ≤ -1 were considered as differentially expressed and used for further analysis. For analysis of Ruxolitinib-affected gene expression, total RNA was purified using the PicoPure RNA Isolation kit (Thermo Fisher Scientific). mRNA was isolated using NEXTflex®Poly(A) Beads (Bio Scientific), libraries were prepared using the NEXTflex®Rapid Directional RNA-Seq Kit (Bio Scientific) and samples were sequenced 75 base pair single-end on Illumina NextSeq500 (Illumina, Utrecht DNA Sequencing Facility) and processed as described above. Genes with absolute padj<0.1 were considered as differentially expressed and used for further analysis.

Gene ontology (GO) enrichment analysis was performed using the ToppFunn tool of ToppGene Suite[4]. GO terms with an adjusted (Benjamini-Hochberg procedure) p-value <0.05 were considered being enriched. Gene set enrichment analysis was performed using GSEA pre-ranked[5]. Significance of the enrichment was calculated based on 1000 cycles of permutations and the normalized enrichment score (NES) and the p-value (FDR) are annotated.

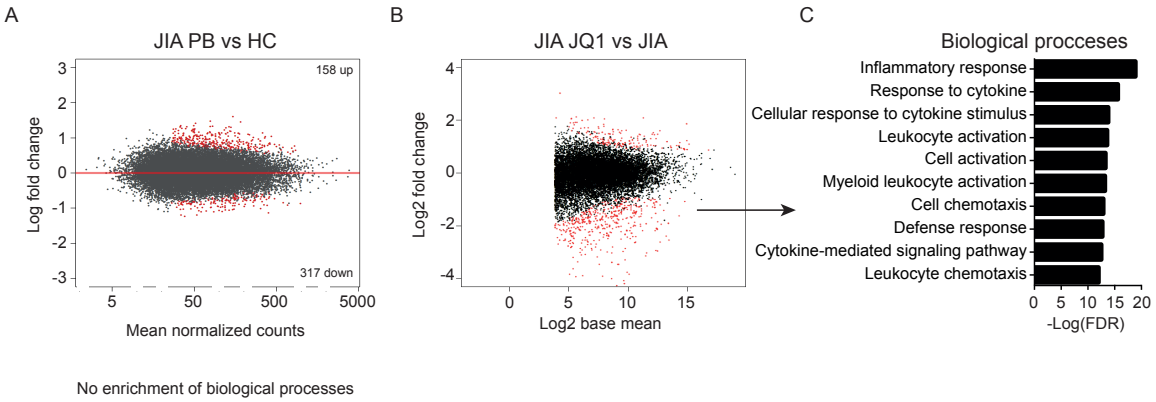
References

1. Jiang H, Wang F, Dyer NP, Wong WH. CisGenome Browser: a flexible tool for genomic data visualization. Bioinformatics 2010;26:1781–2.
2. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.
3. Peeters JGC, Vervoort SJ, Tan SC, Mijnheer G, de Roock S, Vastert SJ, et al. Inhibition of Super-Enhancer Activity in Autoinflammatory Site-Derived T Cells Reduces Disease-Associated Gene Expression. Cell Rep. 2015;12.
4. Chen J, Bardes EE, Aronow BJ, Jegga AG. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res. 2009;37:W305–11.
5. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U. S. A. 2005;102:15545–50.



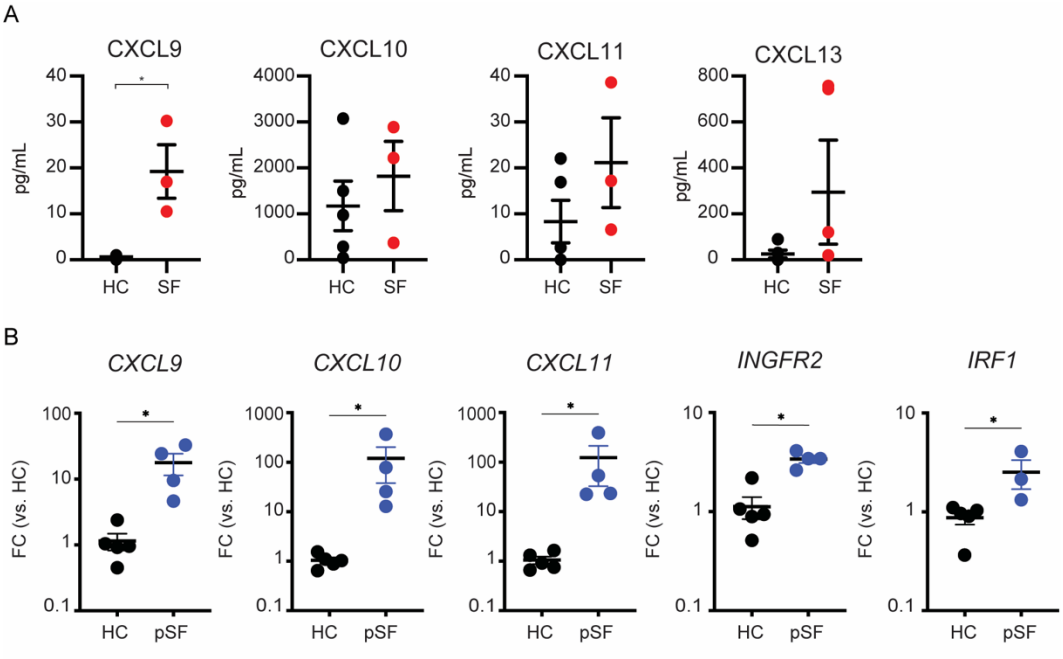
Supplemental Figure 1.

JIA SF monocytes are transcriptionally different. (**A**) Gene expression of selected surface markers in monocytes derived from either the PB of HC, PB of JIA patients undergoing active disease (PBa), SF of JIA patients, and PB of JIA patients in remission (PBr). (**B**) Protein levels of selected surface markers in monocytes derived from either the PB or SF of polyarticular (p) JIA patients. (**C**) Gene expression of selected cytokines and chemokines in monocytes derived from either the PB of HC or SF of polyarticular JIA patients. P values for A were calculated using an ordinary one-way ANOVA, for B using a student's t-test, and for C using a Mann-Whitney test. * = $p<0.05$; ** = $p<0.01$, *** = $p<0.001$.



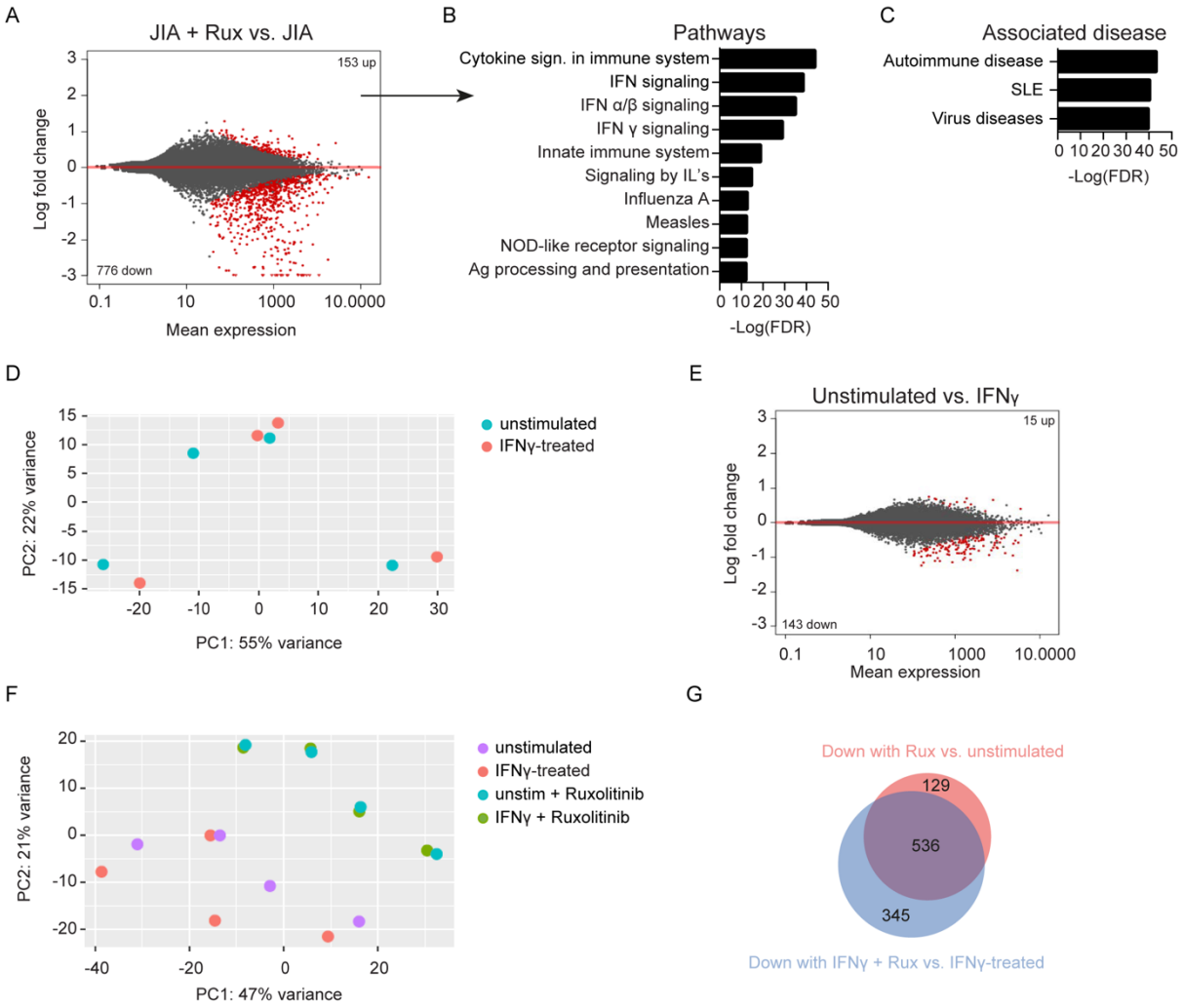
Supplemental Figure 2.

JIA SF monocytes are epigenetically different. (**A**) MA plot of genes differentially expressed between JIA PB and HC PB monocytes. Red dots indicate genes that are significantly different. (**B**) MA plot for genes differentially expressed in JIA SF monocytes after JQ1 treatment. Red dots indicate genes that are significantly different. (**C**) Top 10 biological processes associated with genes that are significantly decreased by JQ1 treatment.



Supplemental Figure 3.

Expression levels of IFNγ-induced chemokines. (**A**) Protein levels of selected chemokines in JIA SF monocytes and HC PB monocytes. (**B**) Gene expression of selected IFNγ signaling-associated genes in monocytes derived from either the PB of HC or SF of polyarticular (p) JIA patients. P values were calculated using a Mann-Whitney test. * = $p<0.05$; ** = $p<0.01$, *** = $p<0.001$.



Supplemental Figure 4.

Ruxolitinib decreases the activated phenotype of JIA SF monocytes. (**A**) MA plot of genes differentially expressed within JIA SF monocytes after Ruxolitinib treatment. Red dot indicate genes with a FDR<0.1. (**B**) Top 10 pathways associated with genes downregulated by Ruxolitinib in JIA SF monocytes. (**C**) Top 3 diseases associated with genes downregulated by Ruxolitinib in JIA SF monocytes. (**D**) PCA of gene expression in JIA SF monocytes cultured *in vitro* with or without IFNγ for 4 hours. (**E**) MA plot of genes differentially expressed between JIA SF monocytes cultured *in vitro* with or without IFNγ for 4 hours. (**F**) PCA of gene expression in JIA SF monocytes cultured *in vitro* with or without IFNγ for 4 hours in the absence or presence of Ruxolitinib. (**G**) Overlap of genes significantly different between untreated and Ruxolitnib treated JIA SF monocytes vs. genes significantly different between IFNγ-treated and Ruxolinib + IFNγ-treated JIA SF monocytes.