# SUPPLEMENTARY MATERIAL

## Methotrexate promotes the release of granulocyte-macrophage colony-stimulating factor from rheumatoid arthritis fibroblast-like synoviocytes via autocrine interleukin-1 signaling

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## Supplementary tables

**Supplementary Table 1.** Demographics and clinical data of a cohort of early RA patients.

| | Early RA patients (*n* = 24) | |
|---|---|---|
| **Variable** | **Baseline** | **2-year follow-up** |
| **Age**, median, years (range) | 56.5 (22-74) | |
| **Female sex**, n (%) | 21 (88%) | |
| **Symptom duration at diagnosis**, median, months (range) | 6 (1-31) | |
| **Erosive disease**, n (%) | 5 (21%) | |
| **SJC (28)**, mean (± SD) | 7.4 (± 4.0) | 0.8 (± 1.2) |
| **TJC (28)**, mean (± SD) | 6.5 (± 4.2) | 1.9 (± 3.4) |
| **CRP**, mean (± SD) | 18.3 (± 24.0) | 4.1 (± 5.0) |
| **ESR**, mean (± SD) | 27.6 (± 24.8) | 16.7 (± 13.2) |
| **RF positive**, n (%) | 20 (83%) | |
| **ACPA positive**, n (%) | 19 (79%) | |
| **RF and ACPA positive**, n (%) | 16 (67%) | |
| **DAS28**, mean (± SD) | 4.8 (± 1.1) | 2.7 (± 1.0) |
| **DAS28-CRP**, mean (± SD) | 4.6 (± 1.0) | 2.4 (± 0.9) |
| **CDAI**, mean (± SD) | 22.6 (± 9.0) | 5.9 (± 6.2) |
| **Therapy at 2 years** | | |
| MTX monotherapy, n (%) | | 7 (29%) |
| MTX combination therapy[a], n (%) | | 11 (46%) |
| No MTX[b], n (%) | | 6 (25%) |

[a] MTX combined with other bDMARD, csDMARD or prednisone

[b] Other DMARDs or prednisone

**Supplementary Table 2.** TaqMan Gene Expression Assays used for qPCR.

| Gene | | Assay ID |
|---|---|---|
| *CDKN1A* | Cyclin Dependent Kinase Inhibitor 1A | Hs00355782_m1 |
| *CSF2* | Granulocyte-Macrophage Colony-Stimulating | Hs00929873_m1 |
| *GAPDH* | Glyceraldehyde-3-Phosphate Dehydrogenase | Hs99999905_m1 |
| *IL1A* | Interleukin 1 Alpha | Hs00174092_m1 |
| *IL1B* | Interleukin 1 Beta | Hs01555410_m1 |
| *IL6* | Interleukin 6 | Hs00174131_m1 |
| *TGFA* | Transforming Growth Factor Alpha | Hs00608187_m1 |

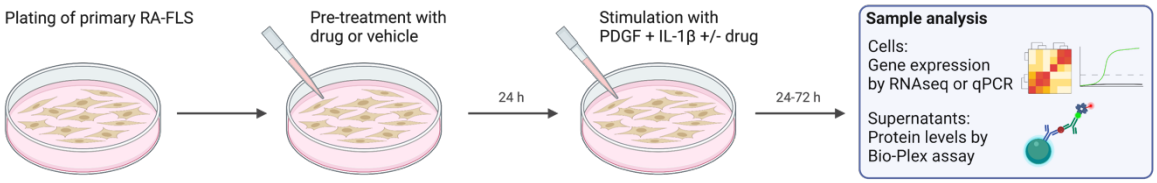
**Supplementary Table 3.** RA risk genes differentially expressed (adj. $p \leq 0.05$) in methotrexate-treated PDGF + IL-1β-activated RA-FLS compared to untreated control.

| Gene symbol | Description |
|---|---|
| *AFF3* | ALF Transcription Elongation Factor 3 |
| *ARID5B* | AT-Rich Interaction Domain 5B |
| *B3GNT2* | UDP-GlcNAc:BetaGal Beta-1,3-N-Acetylglucosaminyltransferase |
| *BACH2* | BTB Domain And CNC Homolog 2 |
| *C1QBP* | Complement C1q Binding Protein |
| *CD83* | CD83 Molecule |
| *CDK2* | Cyclin Dependent Kinase 2 |
| *CDK6* | Cyclin Dependent Kinase 6 |
| *CEP57* | Centrosomal Protein 57 |
| *COG6* | Component Of Oligomeric Golgi Complex 6 |
| *CSF2* | Granulocyte-Macrophage Colony-Stimulating Factor |
| *EOMES* | Eomesodermin |
| *ETS1* | ETS Proto-Oncogene 1, Transcription Factor |
| *ETV7* | ETS Variant Transcription Factor 7 |
| *IFNGR2* | Interferon Gamma Receptor 2 |
| *IL20RB* | Interleukin 20 Receptor Subunit Beta |
| *ILF3* | Interleukin Enhancer Binding Factor 3 |
| *IRF4* | Interferon Regulatory Factor 4 |
| *JAZF1* | JAZF Zinc Finger 1 |
| *LBH* | LBH Regulator Of WNT Signaling Pathway |
| *NFKBIE* | NFKB Inhibitor Epsilon |
| *PLCL2* | Phospholipase C Like 2 |
| *POU3F1* | POU Class 3 Homeobox 1 |
| *PPIL4* | Peptidylprolyl Isomerase Like 4 |
| *PRDM1* | PR/SET Domain 1 |
| *PRKCH* | Protein Kinase C Eta |
| *PTPN22* | Protein Tyrosine Phosphatase Non-Receptor Type 22 |
| *PVT1* | Pvt1 Oncogene |
| *RAD51B* | RAD51 Paralog B |
| *RCAN1* | Regulator Of Calcineurin 1 |
| *REL* | REL Proto-Oncogene, NF-KB Subunit |
| *SPRED2* | Sprouty Related EVH1 Domain Containing 2 |
| *STAT4* | Signal Transducer And Activator Of Transcription 4 |
| *TNFAIP3* | TNF Alpha Induced Protein 3 |
| *TNFRSF9* | TNF Receptor Superfamily Member 9 |
| *TRAF1* | TNF Receptor Associated Factor 1 |
| *WDFY4* | WDFY Family Member 4 |

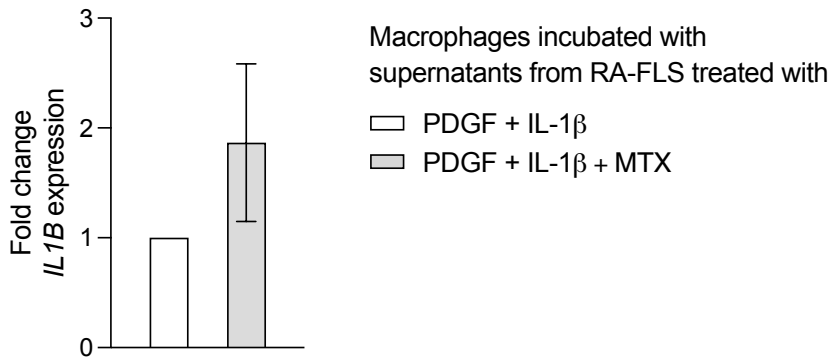
**Supplementary Table 4.** RA risk genes differentially expressed (adj. $p \leq 0.05$) in tofacitinib-treated PDGF + IL-1β-activated RA-FLS compared to untreated control.

| Gene symbol | Description |
|---|---|
| *ARID5B* | AT-Rich Interaction Domain 5B |
| *CDK6* | Cyclin Dependent Kinase 6 |
| *ETV7* | ETS Variant Transcription Factor 7 |
| *HLA-A* | Major Histocompatibility Complex, Class I, A |
| *HLA-B* | Major Histocompatibility Complex, Class I, B |
| *PRDM1* | PR/SET Domain 1 |
| *RCAN1* | Regulator Of Calcineurin 1 |
| *RUNX1* | RUNX Family Transcription Factor 1 |
| *TLE3* | TLE Family Member 3, Transcriptional Corepressor |
| *TNFAIP3* | TNF Alpha Induced Protein 3 |
| *TNFRSF9* | TNF Receptor Superfamily Member 9 |

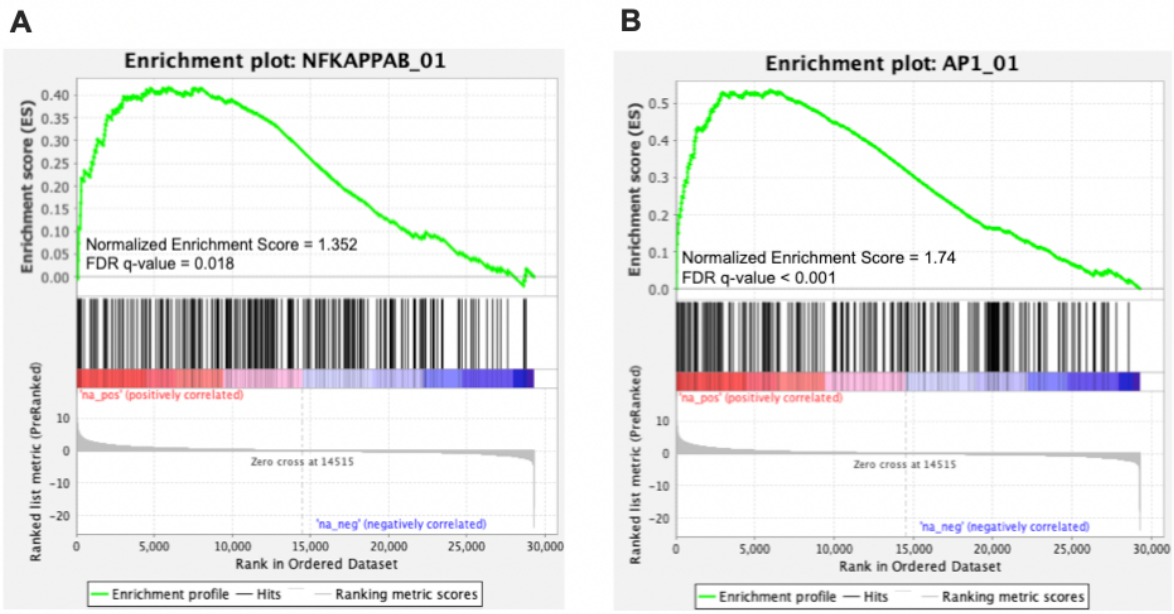
## Supplementary figures



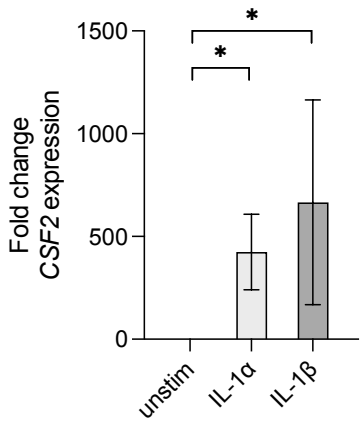

**Supplementary Fig. 1** Overview of the experimental setup. Primary RA-FLS were plated, pre-treated with methotrexate or tofacitinib or vehicle for 24 h, followed by stimulation with PDGF-BB and IL-1β in the presence or absence of the drug for 24-72 h prior to analysis. Illustration created with BioRender.com.



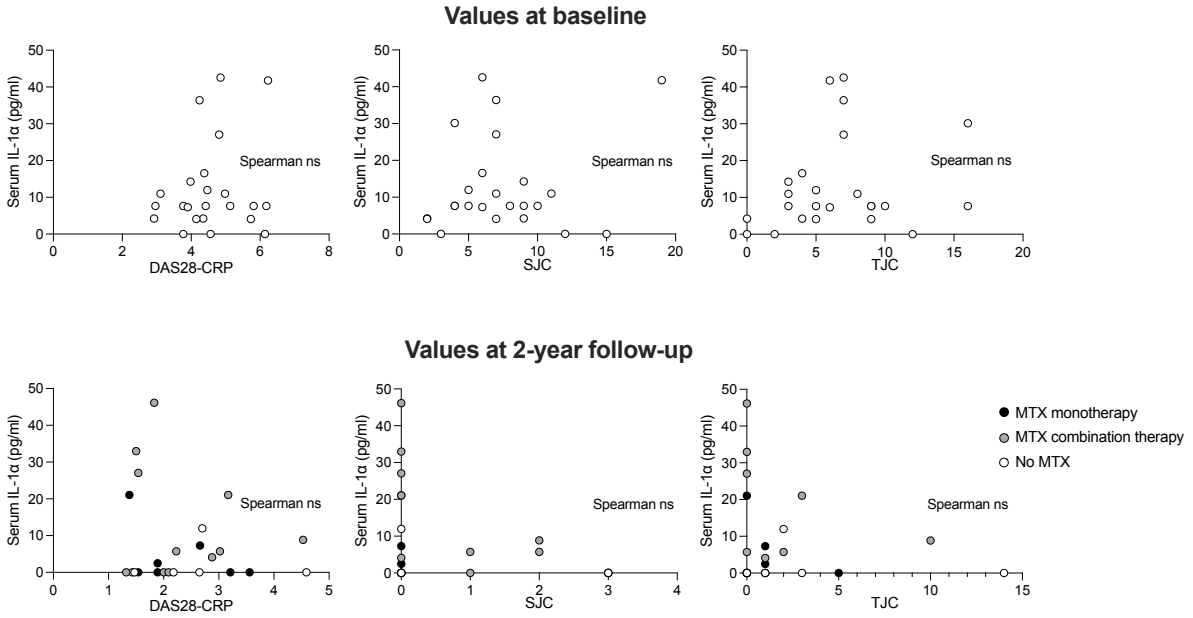

**Supplementary Fig. 2** Expression of *IL1B* in macrophages incubated with supernatants from MTX-treated activated RA-FLS. Monocytes were isolated from buffy coats from healthy blood donors, plated and differentiated into macrophages. In parallel, RA-FLS were pre-treated with MTX or vehicle for 24 h followed by stimulation with PDGF + IL-1β in the presence or absence of MTX for 6 h. Media were then removed and the RA-FLS were incubated with fresh 1% FBS media for 72 h. Thereafter, the supernatants were collected and transferred to the macrophages. After 24 h incubation, macrophages were subjected to RNA isolation and analysis of *IL1B* expression by qPCR. Bar graph shows mean ± SEM ($n$ = 3 different RA-FLS lines).




**Supplementary Fig. 3** Gene Set Enrichment Analysis (GSEA) for **A** NF-κB transcription factor target genes/'NFKAPPAB_01' gene set (MSigDB) or **B** AP-1 transcription factor targes genes/'AP1_01' gene set (MSigDB), ran against a $\log_2$ fold change-ranked list of genes from the RNA-seq expression dataset of MTX-treated versus untreated activated RA-FLS.




**Supplementary Fig. 4** Effects of IL-1α and IL-1β on *CSF2* expression in RA-FLS. Primary RA-FLS were serum-starved overnight (in 1% FBS-DMEM), then stimulated with IL-1α or IL-β (both at 2 ng/mL) for 24 h prior to analysis of *CSF2* expression by qPCR. Bar graphs show mean ± SEM ($n$ = 3 different RA-FLS lines). *$p < 0.05$ by one-way repeated measures ANOVA with multiple comparisons.




**Supplementary Fig. 5** Serum levels of IL-1α in relation to disease activity marker DAS28-CRP, swollen joint count (SJC), or tender joint count (TJC) in a cohort of early RA patients at baseline and after two years of treatment as indicated.