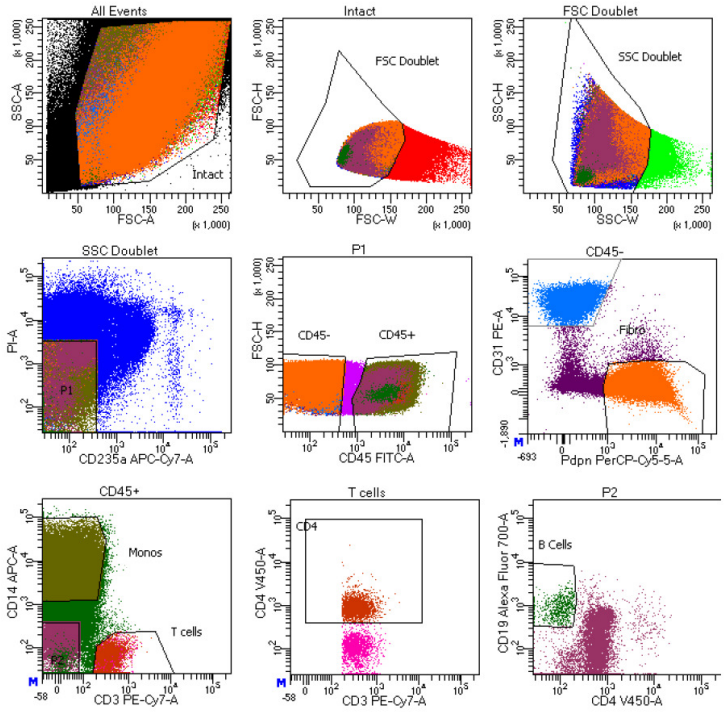
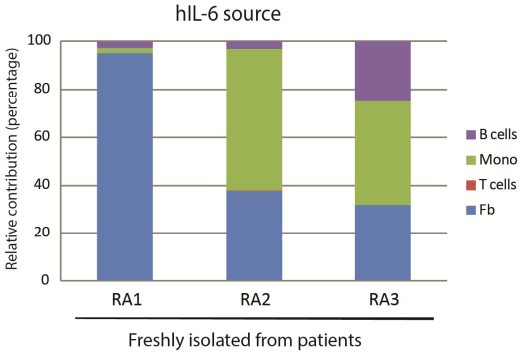
SUPPLEMENTAL DATA

A



B



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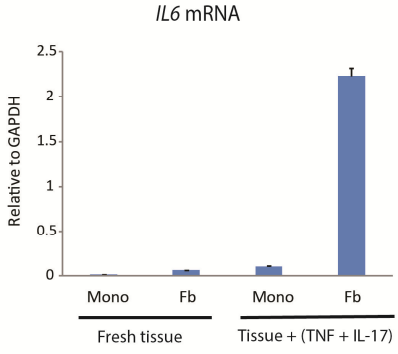


D

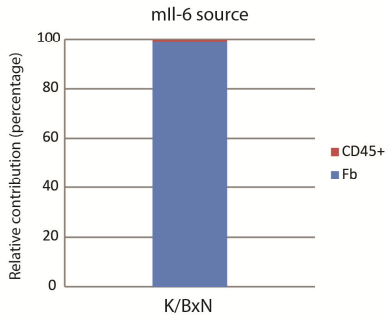


Figure S1, related to Figure 1. FACS sorting scheme and IL-6 source in human and K/BxN mice. (**A**) FACS sorting. Freshly isolated synovial fibroblasts, T cells, B cells and monocytes were prepared by digesting pieces of tissues with 4 mg/ml collagenase type 4 (Worthington), 0.8 mg/ml DispaseII (Roche), and 0.2 mg/ml DNase I (Roche) in DMEM at 37^{o}C for 1 hour, followed by sorting cells enriched for CD45-, CD31-, CD235a-, Pdpn+ surface proteins as fibroblasts (Fibro); for CD45+, CD14+ as monocytes (Monos); for CD45+, CD3+, CD4+ as T cells; for CD45+, CD4-, CD19+ as B cells using the FACSAria Fusion (BD). (**B**) *IL6* expression in various synovial cell types. Fibroblasts (Fb), B cells, CD4+ T cells (T cells) and monocytes (Mono) were freshly isolated from human rheumatoid arthritis (RA) arthroplasty synovium by FACS sorting and each number denotes a different donor. Relative contribution of *IL6* for each cell type was calculated based on the amount of *IL6* mRNA produced per cell using GAPDH as the normalization control and the relative abundance of each cell population. Of note, the relative contribution by fibroblasts is probably an underestimate because it is harder to release all fibroblasts compared to leukocytes by enzymatic digestion of synovium. (**C**) *IL6* mRNA in tissue fibroblasts and monocytes under inflammatory conditions. Fibroblasts (Fb) and monocytes (Mono) were isolated from human rheumatoid arthritis (RA) arthroplasty synovium under fresh conditions or after freshly isolated tissues have been placed in media containing TNF (1ng/mL) + IL-17 (1ng/mL) for 48 hours. The amount of *IL6* mRNA was measured by qPCR and normalized to GAPDH. Error bars represent s.d. of triplicate technical replicates. Data are representative of 2 independent experiments. (**D**) Mouse *Il6* source. Mouse fibroblasts (Fb) and CD45+ cells (CD45+) were freshly isolated by pooling the ankles from 15 K/BxN arthritis mice (age 8-9 weeks) and digesting them with 4 mg/ml collagenase type 4 (Worthington), 0.8 mg/ml DispaseII (Roche), and 0.2 mg/ml DNase I (Roche) in DMEM at 37^{o}C for 1 hour. Fibroblasts were sorted using the markers as in (A). Relative contribution of *Il6* for each cell type was calculated based on the amount of *Il6* mRNA produced per cell using beta-actin as the normalization control and the relative abundance of each cell type. Data are representative of 2 independent experiments.

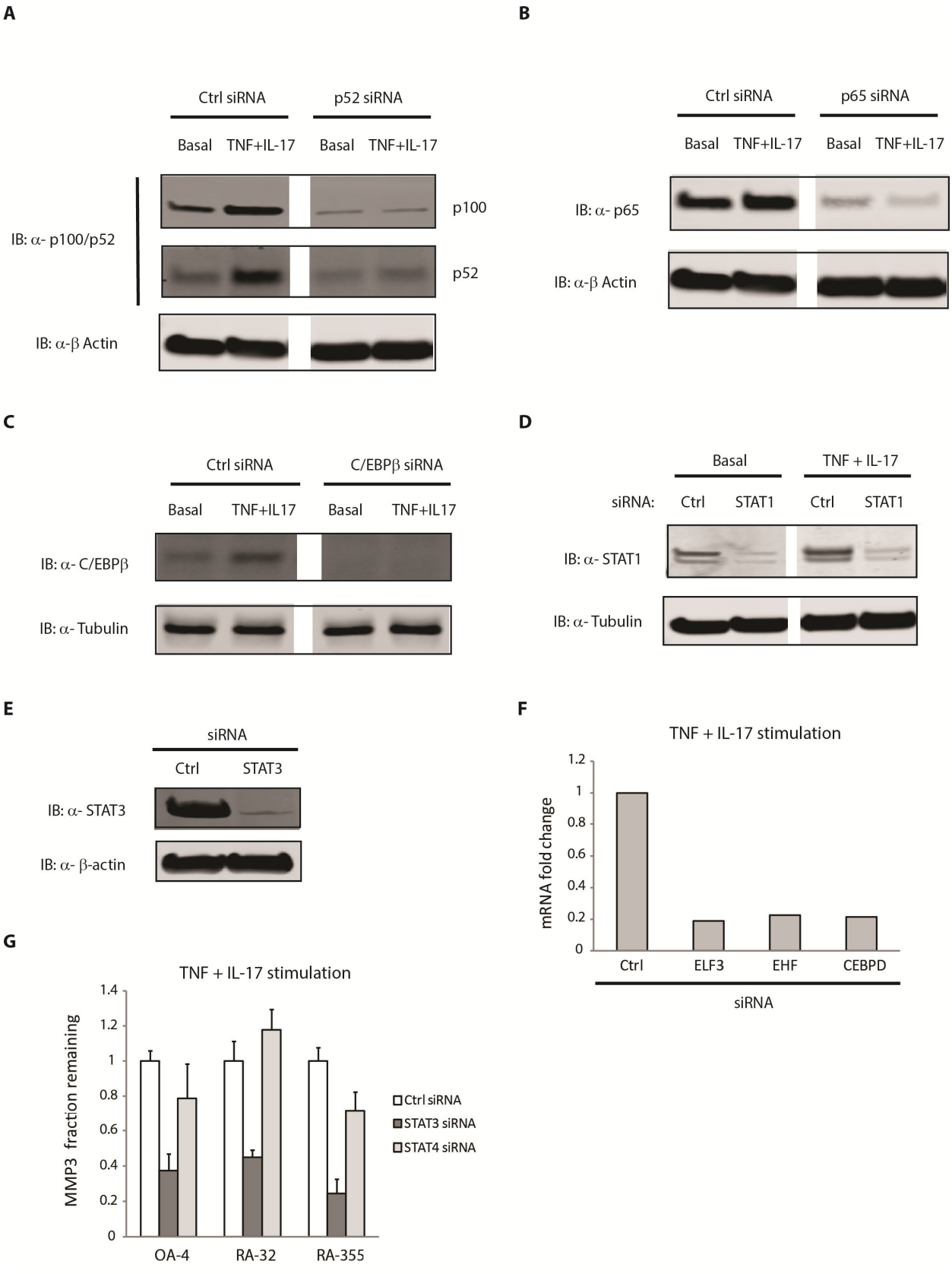

A
Ctrl siRNA
p52 siRNA
Basal
TNF+IL-17
Basal
TNF+IL-17
IB: α- p100/p52
p100
p52
IB: α-β Actin
B
Ctrl siRNA
p65 siRNA
Basal
TNF+IL-17
Basal
TNF+IL-17
IB: α- p65
IB: α-β Actin
C
Ctrl siRNA
C/EBPβ siRNA
Basal
TNF+IL17
Basal
TNF+IL17
IB: α- C/EBPβ
IB: α- Tubulin
D
Basal
TNF + IL-17
siRNA:
Ctrl
STAT1
Ctrl
STAT1
IB: α- STAT1
IB: α- Tubulin
E
siRNA
Ctrl
STAT3
IB: α- STAT3
IB: α- β-actin
F
TNF + IL-17 stimulation
mRNA fold change
1.2
1
0.8
0.6
0.4
0.2
0
Ctrl
ELF3
EHF
CEBPD
siRNA
G
TNF + IL-17 stimulation
MMP3 fraction remaining
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OA-4
RA-32
RA-355
Ctrl siRNA
STAT3 siRNA
STAT4 siRNA

Figure S2, related to Figure 2. Validation of transcription factor silencing. Fibroblasts expressing an siRNA against a transcription factor as indicated were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for approximately 20 hours before samples were collected either for Western blotting (A, B, C, D, E) or qPCR (F). mRNA fold change was calculated as the ratio of the normalized expression value (against GAPDH mRNA) in cells expressing a specific siRNA relative to cells expressing a control (Ctrl) siRNA. (G) Fibroblasts expressing siRNA against a transcription factor as indicated were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for approximately 20 hours, supernatants were collected and the amounts of MMP3 produced were measured by ELISA. Fold reduction was calculated by dividing the amount of MMP3 release from cells expressing an siRNA specific for a transcription factor with that from cells expressing a control (Ctrl) siRNA. Error bars represent s.d. of triplicate technical replicates. Data are representative of two independent experiments. Basal represents protein levels under unstimulated conditions.

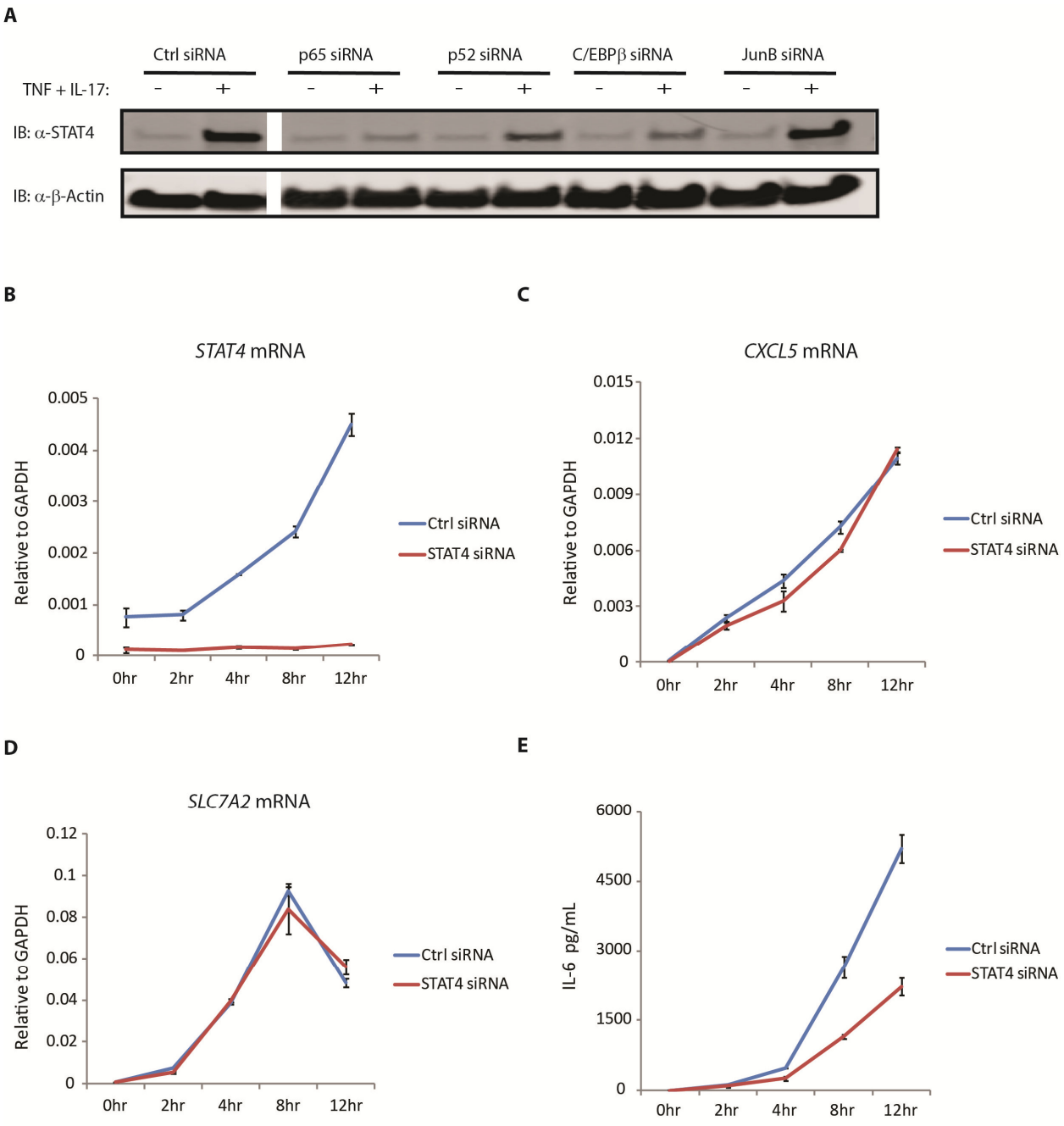


Figure S3, related to Figure 2. Transcription factors involved in STAT4 regulation (A) and effect of *STAT4* siRNA on IL-6 protein (E) and on STAT4's non-target genes (C, D). (**A**) Transcription factors involved in upregulation of STAT4. RA-1357 fibroblasts expressing siRNA against a transcription factor were either unstimulated (-) or stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) (+) for approximately 20 hours

before whole cell lysates were collected. Western blotting was performed using an anti-STAT4 antibody with anti-β-actin as loading controls. (**B, C, D, E**) RA-1357 fibroblasts expressing control (Ctrl) and *STAT4* siRNA were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for different amounts of time, RNA (B, C, D) and supernatant (E) samples were collected. The level of *STAT4*, *CXCL5* and *SLC7A2* mRNA was measured by qPCR and normalized to GAPDH (B, C, D). The amount of IL-6 release was measured by ELISA and the amount of IL-6 at each time point was adjusted by subtracting it with that at 0hr (E). Error bars represent s.e.m. of biological duplicates. hr, hours.

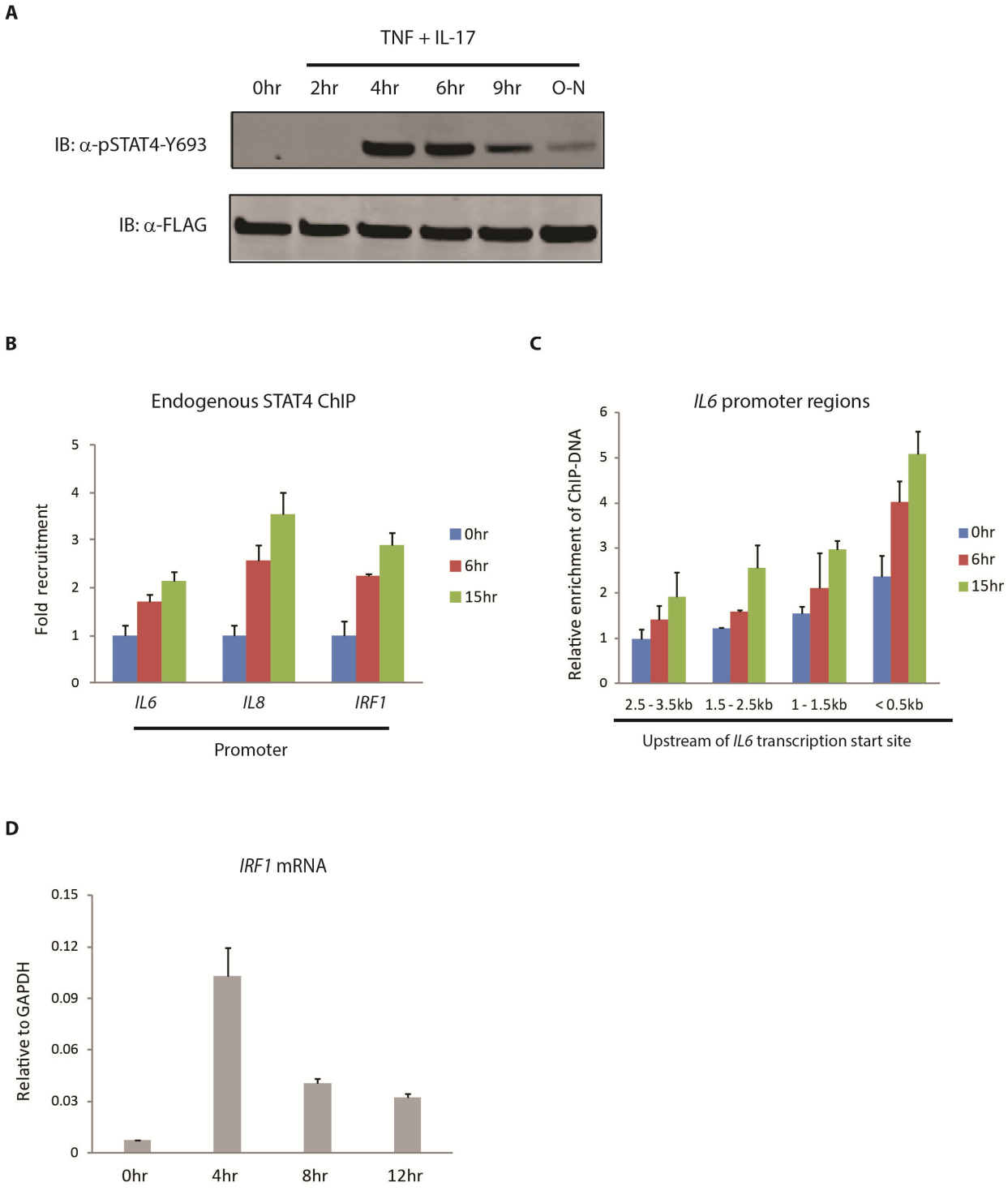
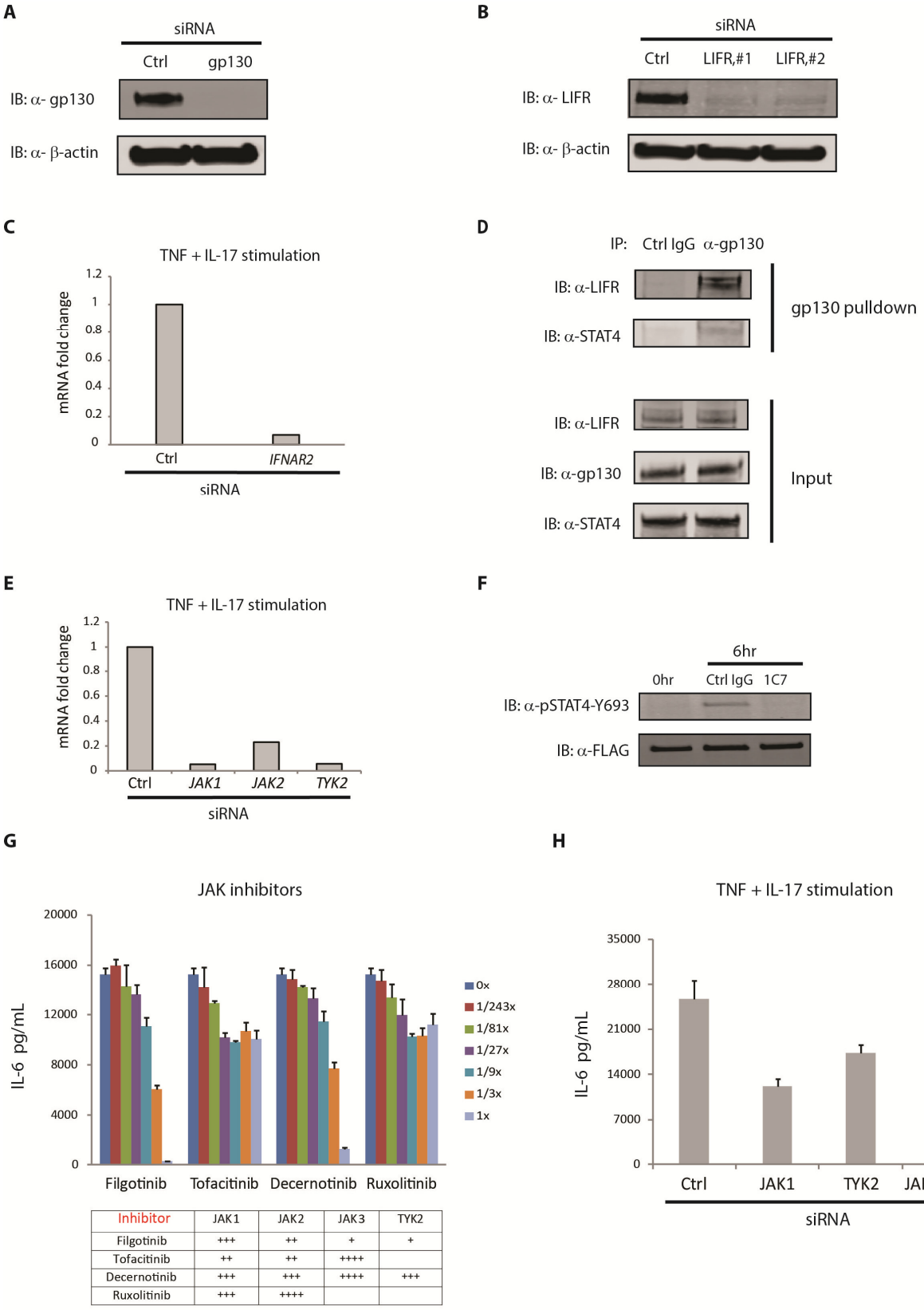


Figure S4, related to Figure 3. Pattern of STAT4 phosphorylation, endogenous STAT4 recruitment and *IRF1* expression. (**A**) Pattern of STAT4 phosphorylation following stimulation. Lung fibroblasts

overexpressing wild type STAT4 were stimulated with TNF (1 ng/mL) + IL-17 (1 ng/mL) for various amounts of time before whole cell lysates were collected. Western blots were performed using anti-pSTAT4-Y693 for phosphorylated STAT4 and anti-FLAG for total STAT4. O-N, overnight. (**B, C**) Pattern of endogenous STAT4 recruitment. Lung fibroblasts without STAT4 overexpression were stimulated with TNF (1 ng/mL) + IL-17 (1 ng/mL) for 0, 6 and 15 hours (hr) followed by formaldehyde fixation. Chromatin immunoprecipitation (ChIP) was carried out using an anti-STAT4 antibody. The amount of DNA precipitated was quantified using qPCR. Fold recruitment was calculated by dividing the amount of ChIP DNA at 6 and 15 hr (normalized with input DNA) with that at 0hr (normalized with input DNA) (B). Relative enrichment of ChIP-DNA was calculated by first normalizing the ChIP-DNA in each region with the input DNA in that region and then selecting the smallest of the normalized DNA across all regions for the second normalization (C). Error bars represent s.e.m. of duplicate biological replicates. (**D**) Pattern of *IRF1* expression. RA-1357 fibroblasts were stimulated with TNF (1 ng/mL) + IL-17 (1 ng/mL) for various durations (x-axis) before RNA samples were collected. The amount of *IRF1* mRNA was measured by qPCR and normalized to GAPDH. Error bars represent s.e.m. of duplicate biological replicates.



Inhibitor	JAK1	JAK2	JAK3	TYK2
Filgotinib	+++	++	+	+
Tofacitinib	++	++	++++	
Decernotinib	+++	+++	++++	+++
Ruxolitinib	+++	++++		

Figure S5, related to Figure 4. Validation of siRNA silencing, interaction of LIFR, STAT4 and gp130, effect of LIFR blockade on STAT4 phosphorylation and effect of JAK inhibitors on IL-6 production. (**A, B, C, E**) Validation of siRNA silencing. Fibroblasts expressing siRNA against a receptor or a kinase were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for approximately 20 hours before samples were collected either for Western blotting (A, B) or qPCR (C, E). mRNA fold change was calculated as the ratio of the normalized expression value (against GAPDH mRNA) in cells expressing a specific siRNA relative to cells expressing a control (Ctrl) siRNA. (**D**) Interaction of gp130 with LIFR and STAT4. Cell lysates were collected from lung fibroblasts overexpressing wild type STAT4 under basal conditions. Immunoprecipitation was performed using an anti-gp130 or control antibody (Ctrl IgG). Antibodies against LIFR, STAT4 and gp130 were used to detect the presence of these proteins in the gp130 pulldown fraction. (**F**) Effect of LIFR blockade on STAT4 phosphorylation. RA-1357 fibroblasts were stimulated with TNF (1 ng/mL) + IL-17 (1 ng/mL) for 6 hours in the presence of mouse monoclonal antibodies, mAb 1C7 or IgG control, before whole cell lysates were collected. Western blots were performed using anti-pSTAT4-Y693 for phosphorylated STAT4 and anti-FLAG for total STAT4. Data are representative of two independent experiments. (**G**) Effect of JAK inhibitors on IL-6 production. JAK inhibitors were added approximately 1 hour before cells were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for about 20 hours and IL-6 release in the supernatants was measured by ELISA. Error bars represent s.d. of triplicate technical replicates. Data are representative of two independent experiments. Filgotinib 1x = 10μM; tofacitinib 1x = 10μM; decernotinib 1x = 10μM; and ruxolitinib 1x = 5μM. Number of '+' indicates the degree of inhibitor specificity. (**H**) Effect of *JAK1* and *TYK2* silencing on IL-6 production. Fibroblasts expressing a control (Ctrl) siRNA or siRNA against indicated kinase(s) were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for approximately 20 hours, supernatants were collected and IL-6 was measured by ELISA. Error bars represent s.d. of triplicate technical replicates. Data are representative of two independent experiments.

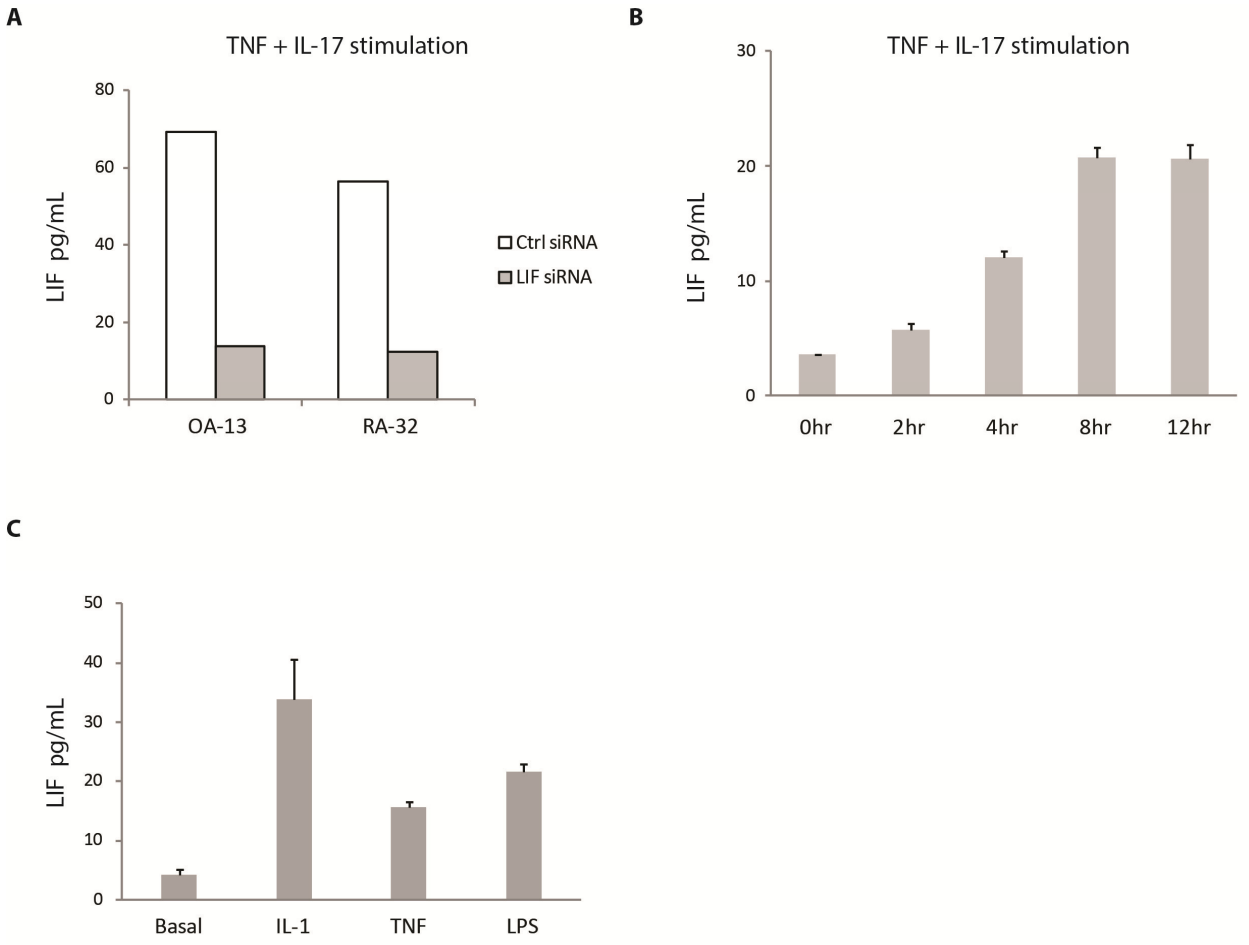


Figure S6, related to Figure 5 and 6. Validation of *LIF* siRNA and LIF production under different conditions. (**A**) Validation of *LIF* siRNA. Fibroblasts expressing siRNA against *LIF* and a control (Ctrl) siRNA were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for approximately 20 hours before supernatants were collected and the amount of LIF measured by ELISA. (**B**) LIF release following stimulation. RA-1357 fibroblasts were stimulated with TNF (0.1 ng/mL) + IL-17 (1 ng/mL) for various durations, supernatants were collected and the amount of LIF measured by ELISA. Error bars represent s.d. of triplicate technical replicates. Data are representative of two independent experiments. (**C**) LIF production in human fibroblasts following stimulation by IL-1, TNF and LPS. RA-1357 fibroblasts were stimulated with IL-1β (5 pg/mL), TNF (0.1 ng/mL), LPS (2.5 μg/mL) for approximately 20 hours, supernatants were collected and

the amount of LIF measured by ELISA. Basal represents the amount of LIF produced under unstimulated conditions. Error bars represent s.d. of triplicate technical replicates. Data are representative of two independent experiments.

a) qPCR primers

Primer	Sequence
IL-6	Forward: CCACTCACCTCT TCAGAACG Reverse: CATCTTTGGAAGGTTCAGGTTG
GAPDH	Forward: ACATCGCTCAGACACCATG Reverse: TGTAGTTGAGGTCAATGAAGGG
IFNAR1	Forward: CTTTTGAGCCAGAATGCCTTC Reverse: GGACCGGAAATTTCGCAATG
JAK1	Forward: AACCTCTTTGCCCTGTATGAC Reverse: CTGCTCATTGTCGTTGGTTC
JAK2	Forward: AGAATGTCTTGGGATGGCAG Reverse: TGATAGTCTTGGATCTTTGCTCG
Tyk2	Forward: ACAAATGTCCCTGTGAGGTC Reverse: ACACGCTGAACACTGAAGG
LIF	Forward: ATACGCCACCCATGTCAC Reverse: CCACATAGCTTGTCCAGGTTG
CEBPD	Forward: AGAACGAGAAGCTGCACCAG Reverse: CGCTCCTATGTCCCAAGAAACTG
ELF3	Forward: AAGCTGAGCAAAGAGTACTGG Reverse: TTCATGCCGATTCTCCCAC
EHF	Forward: GCCTTCCATCATGAACACCT Reverse: GGGTTCTTGTCTGGGTTCAA
CXCL5	Forward: TCTGCAAGTGTTCGCCATAG Reverse: CAGTTTTCCTTGTTTCCACCG
SLC7A2	Forward: TCTCGTCCTTCTGTTTGTGATG Reverse: GCCCCATAGATACTTGTTCCG
IRF1	Forward: AGTGATCTGTACAACTTCCAGG Reverse: CCTTCCTCATCCTCATCTGTTG
LIFR	Forward: GTTGCTCTGGACAAGTTAAATCC Reverse: AAGTATCAGGCCCCTTTGAAG
Mouse IL-6	Forward: TACCACTTCACAAGTCGGAGGC Reverse: CTGCAAGTGCATCATCGTTGTTC
Mouse beta-actin	Forward: GCTCTGGCTCCTAGCACCAT Reverse: GCCACCGATCCACACAGAGT

b) ChIP primers

Primer	Sequence
IL-6, <0.5 kb	Forward: AGGACTGGAGATGTCTGAGGCTCATTCT Reverse: GTTCCAGGGCTAAGGATTTCCTGCACTT
IL-6, 1 – 1.5 kb	Forward: GATCCTCCTGCAAGAGACACCATCCTG Reverse: GGAGGACCTTGTGGCATCTTGCAGAG
IL-6, 1.5 – 2.5 kb	Forward: AGCTCTCCAAGGCAGAGACTCTGAGC Reverse: GATCCTGGCTGTTACATCAAGGACCTG
IL-6, 2.5 – 3.5 kb	Forward: GGAGCAAGGTAGAGCTCATCTCTCCCAC Reverse: TCAAACTCCTGATAGTGGCCCTCGAGG
IRF1	Forward: CTTCGCCGCTAGCTCTACAACAG Reverse: GCTCCGGGTGGCCTCGGTTCG
IL-8	Forward: CATCCATGATCTTGTTCTAACACCTGCC Reverse: CTCAGGGCAAACCTGAGTCATCACA

Table S1, related to Experimental Procedures. List of primer sequences used in qPCR and ChIP assays. (**a**) qPCR and (**b**) ChIP primers were used at the concentration of 0.25μM per primer in a real time PCR reaction.

EXTENDED EXPERIMENTAL PROCEDURES

Cell Stimulation and Antibody Blocking Assays. Fibroblasts were plated on day 1 at 10,000 cells per well in 96-well plates in 10% FBS containing media. Cells were serum-starved on day 2 by changing to 1% FBS-containing media. Cells were stimulated as indicated on day 3 or blocking antibodies were added 1 hour prior to cytokine stimulation on day 3.

siRNA Silencing. Fibroblasts were transfected with an siRNA by reverse transfection at 30 nM using the RNAi Max reagent (Life Technologies) in 10% FBS containing media. Cells were then switched to serum-starving media containing 1% FBS on day 2. Cells were stimulated as indicated on day 3. Efficiency of siRNA silencing was assessed by Western blotting and qPCR (Supplemental Figure S2, S3, S5, S6).

Plasmid Constructs. A STAT4 overexpression construct was generated by PCR amplifying STAT4 from cDNA made from RA fibroblast cells. The PCR product containing the NotI and BamHI restriction sites was cloned into the pQCXIX vector (Clontech). A FLAG tag sequence was added for immunoprecipitation and Western blot detection. Point mutations were created using the site-directed mutagenesis kit (Agilent). pQCXIX, pCL-ampho vectors were gifts from Dr. Joshua Sims, Department of Systems Biology, Harvard Medical School, Boston, USA.

Generation of Cells Stably Expressing STAT4 Protein. HEK 293T cells were transfected with a pQCXIX-STAT4 and pCL-ampho vector using the TurboFect transfection reagent (Fisher Scientific). Media was changed after 4 hours and viruses were collected after 48 hours post transfection. Lung fibroblasts were infected with viruses for 24 hours in the presence of polybrene. Cells stably expressing a STAT4 protein were selected using 2-4 μg/mL puromycin (Sigma).

Immunoprecipitation. Total cell lysates were collected by washing cells once with cold PBS followed by addition of lysate buffer (50mM HEPES pH 7.5, 5% glycerol, 100 mM NaCl, 0.25% TritonX-100, supplemented with a protease inhibitor cocktail (Roche) and phosphatase inhibitors (sodium orthovanadate, sodium fluoride, and beta-glycerol phosphate). Cells were lysed for 30 minutes on ice followed by centrifugation at 15000rpm for 15 minutes at 4^{o}C. Immunoprecipitation was done overnight at 4^{o}C using either an anti-FLAG resin or a Protein A resin together with a rabbit antibody against the relevant target or an isotype control antibody.

Quantitative Real-Time PCR. mRNA samples were extracted from cells using an RNeasy Micro Kit (Qiagen). cDNA synthesis was carried out using the QuantiTect Reverse Transcription Kit (Qiagen). qPCR reactions were performed in duplicate using the Brilliant III Ultra-Fast SYBR reagent (Agilent). Relative transcription level was calculated by using the ΔΔCt method with GAPDH (for human) and beta-actin (for mouse) as the normalization control. Fold induction was calculated by dividing the normalized mRNA at

a certain time point with that at time 0hr. Fold change is the ratio of the normalized mRNA from cells expressing a specific siRNA vs. Ctrl siRNA.

Chromatin Immunoprecipitation Assays. Fibroblasts were stimulated with TNF (1 ng/mL) + IL-17 (1 ng/mL) for different amounts of time as indicated followed by fixation with formaldehyde (1% final concentration) for 10 minutes. Cells were lysed in swelling buffer for 15 minutes (25mM HEPES pH 7.8, 1.5 mM $MgCl_2$, 10mM KCl, 0.1% NP-40, 1mM DTT, protease inhibitor cocktail) then in sonication buffer (50mM HEPES pH 7.8, 140mM NaCl, 1mM EDTA, 0.1% SDS, 1% TritonX-100 supplemented with protease inhibitor cocktail). Samples were sonicated for 2 minutes with 20-second pulse intervals and 1 minute off at each interval at 4^{o}C using a Qsonica sonicator set at 28% output. Chromatin was immunoprecipitated using anti-FLAG resins or anti-STAT4 antibody at 4^{o}C overnight. DNA was purified using the phenol/chloroform precipitation method. The amount of DNA precipitated was quantified using qPCR. Fold recruitment was calculated by dividing the amount of ChIP-ed DNA at indicated time point (normalized with input DNA) with that at 0hr (normalized with input DNA).

Western Blotting. Total cell lysates were collected by washing cells once with cold PBS followed by addition of lysate buffer (50mM HEPES pH 7.5, 10% glycerol, 100 mM NaCl, 0.1% SDS, 1% NP-40, supplemented with protease inhibitors and phosphatase inhibitors sodium orthovanadate, sodium fluoride, and beta-glycerol phosphate). Cells were lysed for 30 minutes on ice followed by centrifugation at 15000rpm for 15 minutes at 4^{o}C. Protein concentration was measured by the microBCA kit (Pierce). Equal amounts of total protein (~20 μg per lane) were separated on an 8% SDS-PAGE gel. Proteins were transferred onto a PVDF membrane and blocked with 5% BSA in PBS and probed with primary antibodies overnight at 4^{o}C, followed by secondary antibodies conjugated with IRDye 680 or 800 (Rockland). Membranes were scanned with a Li-COR Odyssey scanner.