Supplementary materials and methods

Protein extraction and immunoblotting

FLS were lysed in 1x Laemmli's buffer or FLS nuclear fractions were extracted using a Nuclear Extraction Kit (Active Motif, Carlsbad, CA) and protein content quantitated with a BCA Protein Assay Kit (Pierce, Rockford, IL). Equivalent amounts of lysate or nuclear extract were resolved by electrophoresis and analyzed by immunoblotting using primary antibodies recognizing acetylated lysine, acetylated histone 3 (H3), acetylated histone 4 (H4), H3, phospho (p)-IκBα, p-p38, p-ERK, p-JNK, p38, ERK (all from Cell Signaling Technology, Beverly, MA), H4 (Upstate, Temecula, CA), NF-κB p65 and p50 (both from Santa Cruz Biotechnology, Santa Cruz, CA), and tubulin (Sigma-Aldrich), followed by development with IRDye-680-labelled anti-rabbit or IRDye-800-labelled anti-mouse immunoglobulin secondary antibodies (LI-COR Biosciences, Bad Homburg, Germany) and visualization using an Odyssey infrared imaging system (LI-COR Biosciences).

Quantitative measurement of mRNA expression

FLS were left unstimulated or were stimulated for 1-8 h with IL-1β in the presence or absence of TSA. Alternatively, cells were treated with IL-1β for 4 h with or without TSA and cycloheximide (CHX) (Sigma-Aldrich). Total RNA was extracted using a GenElute RNA isolation kit (Sigma-Aldrich) and reverse transcribed using a First-Strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania). Quantitative (q)PCR was performed on a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA) using Power SybrGreen PCR Master Mix (Applied Biosystems) and the following primers: IL-6 forward, GACAGCCACTCACCTCTTCA; IL-6 reverse, CCTCTTTGCTGCTTTCACAC; c-Jun forward, GGAAGCTGGAGAGAATCGCC; c-Jun reverse,

TTCTGTTTAAGCTGTGCCACCTG; 18S forward, CGGCTACCACATCCAAGGAA; 18S reverse, GCTGGAATTACCGCGGCT (Invitrogen). For measurement of mRNA stability, FLS or macrophages were incubated in medium alone or IL-1β or LPS, respectively, for 4 h in the presence or absence of TSA. Culture medium was replaced with medium containing 10 μg/ml actinomycin D (ActD, Sigma-Aldrich), and RNA isolated at 0, 1, 2, 4 and 20 h following ActD exposure. qPCR reactions were performed in duplicate. Specific PCR product amplification was confirmed by dissociation curve analysis for each primer pair. Relative gene expression was calculated using StepOne Software v2.1 (Applied Biosystems) and expressed as the ratio between the gene of interest and 18S.

Measurement of NF-κB transcriptional activity in RA FLS

Adenoviral 4xNF-κB-MLP-luciferase (Luc) reporter vector was generated using previously described methodologies.[47] A 4xNF-κB enhancer was excised from a 4xNF-κB-TK-SEAP plasmid (Clontech/BD Biosciences) and cloned into pGL3-MLP-Luc in front of a major late adenoviral minimal promoter (MLP). Next, a cassette containing an artificial polyA track, 4xNF-κB enhancer, MLP promoter, Luc ORF and a polyA signal was re-cloned into pShuttle vector and recombined with an Easy-1 adenoviral backbone in BJ1583 bacteria. The linearized cosmid was transfected into HEK-293 cells and amplified as an adenovirus. Adenoviruses encoding 4xNF-κB-MLP-Luc and control CMV-β-galactosidase (β-Gal) were amplified and titrated in transcomplemental HEK-293 cells and purified by cesium chloride gradient ultracentrifugation.[48] FLS were co-transduced with 4xNF-κB-MLP-Luc and CMV-β-Gal at a multiplicity of infection of 300 and 30, respectively. 48 hours after transduction, cells were serum starved for 24 hours and then left unstimulated or stimulated with IL-1β in the presence or absence of TSA for 2-8 hours. Cells were lysed in a 1x Passive

Lysis Buffer (Promega, Madison, WI). Luc activity was measured with a Luciferase Assay reagent (Promega) using a Victor3 Multilabel Reader (Perkin Elmer, Norwalk, CT). β-Gal activity, measured at OD_{405} in 100 mM Na_2HPO_4/NaH_2PO_4, 1 mM $MgCl_2$, 100 mM β-mercaptoethanol, and 0.67 mg/ml *O*-nitrophenyl-β-D-galactopyranoside, was used to correct for transduction efficiency.