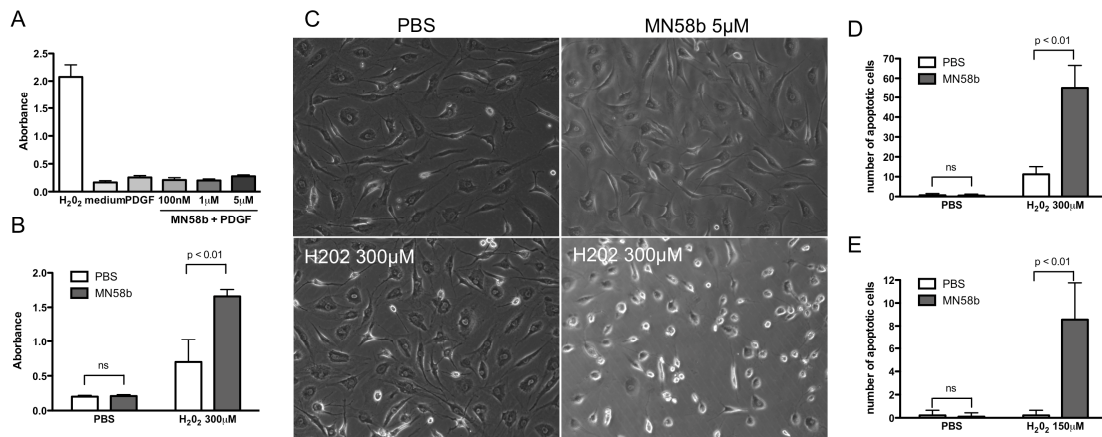


SUPPLEMENTARY INFORMATION

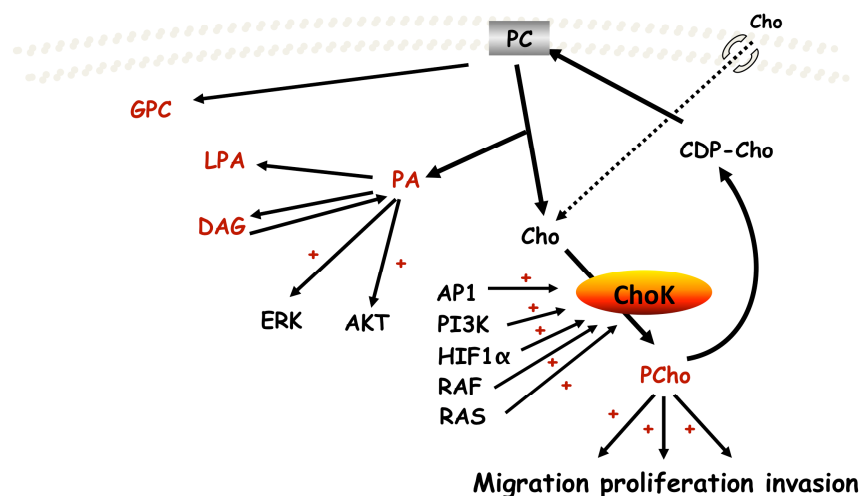
Supplementary Figure 1. Choka inhibition regulates cell death. (A) On day 3, apoptosis was determined by histone associated DNA fragmentation. There was no effect on apoptosis under these conditions. (B) FLS were exposed to 0.3mM H₂O₂ for 6 hours with or without pretreatment with MN58b (5μM). Apoptosis was determined by histone associated DNA fragmentation. (C) FLS were exposed to 0.3mM H₂O₂ for 6 hours with or without pretreatment with MN58b (5μM). Representative images after 6 hours of treatment. Quantification (as the average of number of dead cells in three different fields from 3 different RA FLS cell lines) of apoptotic cells after 0.3 mM (D) or 0.15 mM (E) H₂O₂ with or without pretreatment with MN58b (5uM). Representative of 3 different experiments.

Supplementary Fig 1



Supplementary Figure 2 Scheme of choline metabolism, its regulatory pathways and downstream effectors. PCho: Phosphatidylcholine; DAG: Diacylglycerol; Pcho: phosphocholine; PA: Phosphatidic Acid; LPA: Lysophosphatidic acid; CDP-Cho: cytidine 5'-diphosphocholine; GPC: Glycerophosphocholine. Cho: Choline.

Supplementary Fig 2



Supplementary methods

Immunohistochemistry. Paraffin sections prepared from RA and OA synovial tissue were incubated overnight at 4°C with mouse anti-human ChoK α antibody [58] at a 1:50 dilution. Citrate buffer (S1700; Dako) was used at 96°C for 40 minutes for antigen retrieval. The signal was developed using diaminobenzidine and sections were counterstained with hematoxylin.

ELISA. IL-1 β , IL-6 and MMP3 has evaluated by DuoSet enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN), following the manufacturer's protocol.

MTT assay. For the MTT assay, 3×10^3 FLS/well were plated into 96-well plates in 10% FBS/DMEM. After 24 hours, the medium was replaced with low-serum medium (0.1%

FBS/DMEM) for 24 hours for synchronization. On day 0, medium was replaced with 1% FBS and cells were treated with ChoK α inhibitor at the indicated concentration or with PBS for 1 hour. PDGF or medium alone was added to the appropriate wells. The experiment was performed in triplicate wells. Cell viability was estimated on day 3 after incubation with MTT for 4 hours and was read at 550 nm with a spectrophotometer.

BrdU assay. For the BrdU assay, 3×10^3 FLS/well were plated into 96-well plates in 10% FBS/DMEM. After 24 hours, the medium was replaced with low-serum medium (0.1% FBS/DMEM) for 24 hours for synchronization. On day 0, medium was replaced with 1% FBS and cells were treated with ChoK α inhibitor at the indicated concentration or with PBS for 1 hour. PDGF or medium alone was added to the appropriate wells. The experiment was performed in triplicate wells. Cell growth was estimated on day 6 after incubation with BrdU for 24 hours and was read at 550 nm with a spectrophotometer (Cell Proliferation ELISA kit by Roche Applied Science, Mannheim, Germany).

Cell Survival and Apoptosis Assays. For cell survival and apoptosis assays, 1×10^6 FLS/well were plated onto 6-well plate in 10% FBS/DMEM, then after 24 hours was replaced with starving medium (0.1% FBS/DMEM) for 24 hours for synchronization. Cells were incubated with MN58b inhibitors for 1 hour, then cells were treated with 300 μ M H₂O₂ for 6 hours and viability was determined in H₂O₂ treated cells by phase contrast light microscopy. Apoptosis was determined using a Cell Death Detection ELISAPLUS kit (Roche Applied Science, Mannheim, Germany).

Migration scratch assay. FLS were seeded onto 24-well plates and allowed to come to confluence. Cells were incubated with MN58b for 1 hour. A single scratch wound was induced through the middle of each well with a sterile pipette tip. Cells were

subsequently exposed to PDGF (10ng/ml). SFC migration across the wound margins from 36h was assessed, photographed and measured by ImageJ.

Real-time quantitative PCR (qPCR). FLS, synovium and joints were collected. For FLS, cells were collected after FLS stimulation. Total RNA was extracted with Trizol (Invitrogen) and reverse-transcribed with random hexamers and Superscript II Kit (Invitrogen). qPCR was performed with SYBR Green PCR Master Mix Kit (Applied Biosystems, Foster City, CA). The relative amounts of transcripts were compared to those of HRPT and normalized to untreated samples by the $\Delta\Delta C_t$ method.

Western blot analysis. FLS, synovium or joints were disrupted in lysis buffer (PhosphoSafe™, Novagen, Gibbstown, NJ) containing a protease inhibitor cocktail. Proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane. Blots were probed with antibodies against ChoK α (Proteintech), phospho-ERK1/2, phospho-AKT, ERK1/2, phosphoRb and Rb (Cell Signaling Technology, Danvers, MA), and cyclin D1 and total AKT (Santa Cruz) Biotechnology Inc, Santa Cruz, CA). Horseradish peroxidase-conjugated anti-IgG (Cell Signaling Technology, Danvers, MA) was used as secondary antibody. Membranes were developed using a chemiluminescence system (ECL detection reagent: Amersham Life Science, Aylesbury, UK). Densitometry analysis was done by using Quantity One 1-D analysis software (Bio-Rad).

Serum transfer and arthritis scoring. Sera from arthritic adult K/BxN mice were pooled and recipient mice were injected intraperitoneally (i.p.) with 150 μ l of K/BxN serum on day 0. Clinical arthritis scores were evaluated as described [59]. MN58b (3mg/kg) was injected daily i.p. beginning on day 0 (for 5 days) or day 4 after serum administration.

Histology analysis. Joints were fixed in 10% formalin, decalcified in 10% EDTA for 2-3 weeks and paraffin embedded. Sections were prepared from the tissue blocks and stained with hematoxylin and eosin (H&E) and O-safranin. A blinded semiquantitative scoring system was used to assess synovial inflammation, extra-articular inflammation, bone erosion and cartilage damage (0-5 scale), as previously described [59].

Reference:

59. Guma, M., Ronacher, L., Liu-Bryan, R. et al. Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. *Arthritis Rheum* 2009;60:3642-50.