Supplemental materials and methods

Induction of the Fra-1 deletion by the inducible promoter Mx1

The deletion of Fra-1 by Cre under the control of the Mx1 promoter was induced by 250 µg poly(I:C) (Invivogen, San Diego, USA) injected intraperitoneally (i.p.) three times in a 2-day interval (1). Control littermates were also treated with poly(I:C). The mice were used 7 to 10 days later for the generation of thioglycollate-elicited macrophages, bone marrow transfer or serum-induced arthritis. Detailed information about the time-line of the experiments performed with the Mx-Cre strain is illustrated in supplementary fig. 12A-C. Additionally, the effect of the poly(I:C) treatment of the mice during the serum induced was excluded (Supplemental Fig. 12D).

In vivo modulation of Arginase activity

To inhibit arginase activity in vivo, mice with K/BxN induced arthritis were injected daily i.p. with 100 mg/kg bodyweight of N^{ω}-hydroxy-nor-L-arginine (NOHA) in a volume of 0.2 mL PBS from day 0 of the experiment until day 10 (2). The control groups were injected with 0.2 mL PBS. To increase arginase activity in vivo, the drinking water of the mice was supplemented with 40 g/L L-arginine (Sigma-Aldrich, Munich, Germany). Previous measurements found that daily water intake of a male mouse weighting 28 g was 2.7±0.2 ml, which results in an expected daily intake of approximately 110 mg surplus arginine (3).

Bone marrow transfer (BMT) reconstitution

Before BMT reconstitution, Fra-$1^{\Delta Mx}$ and control (graft and host) mice were injected with poly(I:C), as described above, to induce the Mx1 promoter for proper deletion of Fra-1 in bone marrow cells. Two weeks later, 6 week-old C57BL/6 wild type recipient male mice were lethally irradiated with 6 Gy for 230 s and with 5 Gy for 190 s 5 hours after the first irradiation.

The mice were then subsequently reconstituted retro-orbitally with 2 x 10^6 bone marrow cells derived from Fra-$1^{\Delta Mx}$ mice or littermate control mice (4). The mice were used for further experiments 6 weeks post injection, when the K/BxN model was applied.

Generation of apoptotic cells (AC)

A single cell suspension of splenocytes was incubated in RPMI-1640 (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts) supplemented with 10 % (v/v) FCS (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts), 1 % (v/v) penicillin/streptomycin (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts), in presence of 1 µM dexamethasone (Sigma-Aldrich, Munich, Germany). Following a 12-h incubation, the AC were washed several times with PBS, centrifuged through a FCS cushion and resuspended in RPMI-1640 with supplements added as described above. The viability of the cells was checked by annexin V/PI staining and subsequent flow cytometry analysis. Samples containing at least 90 % apoptotic cells (annexin V^+/PI^-) were used for further experiments.

Histological analyses

Histological analysis of mice paws was carried out as previously described (5). Briefly, hind paws were dissected, fixed overnight in 4% formalin and then decalcified in 14% EDTA until bones were pliable. Following embedding, serial paraffin sections (2 µm) were obtained and stained with haematoxylin and eosin, and tartrate-resistant acid phosphatase (TRAP) using the Acid Phosphatase Kit (Sigma-Aldrich, Munich, Germany). The sections were evaluated using a light microscope (Nikon, Tokio, Japan) and a histomorphometry image analysis system (Osteomeasure, OsteoMetrics, Decatur, USA).

Staining for CD68 (CD68 antibody [clone: FA-11], GeneTex, Irvine, California, US), Fra-1 (Fra-1 [clone: D-3), Santa Cruz Biotechnology, Dallas, Texas, US) and Arg1 (Arginase-1 (D4E3M™) XP® Rabbit mAb, Cell Signaling Technology, Danvers, Massachusetts, US) in

the synovium of RA patients was performed as described previously (5). Briefly, antigen retrieval was performed using citrate buffer, the sections were blocked with 1% horse serum followed by antibody staining with mouse anti-mouse/human/rat Fra-1 (D-3, Santa Cruz, Dallas, Texas, US), rabbit anti-human/mouse Arg1 (Cell Signaling Technology, Danvers, Massachusetts, US), rat anti-human/mouse CD68-Biotin (GeneTex, Irvine, California, US), horse anti-mouse DyLight488 (Vector Laboratories, Burlingame, California, US), horse anti-rabbit DyLight549 (Vector Laboratories, Burlingame, California, US) and Cy5 Streptavidin (BioLegend, San Diego, California, US). The slides were covered with fluorescence mounting medium and acquired with the BZ-X 710 All-in-One Fluorescence Microscopes (Keyence, Osaka, Osaka Prefecture, Japan).

RNA Isolation, reverse transcription and real-time polymerase chain reaction (RT-PCR)

To isolate RNA from paws, the skin was removed and the total paw tissues were homogenized in peqGOLD TriFast™ (Peqlab, Life Science, VWR, Radnor, Pennsylvania, USA) using a Precellys® Ceramic Kit on a Precelllys®24 tissue grinder (Peqlab, Life Science, VWR, Radnor, Pennsylvania, USA) with the conditions 2 x 30 s at 6,500 rpm, followed by the RNA isolation according to the manufacturer's instruction. RNA was isolated from cells with TriFast™ (Peqlab, Life Science, VWR, Radnor, Pennsylvania, USA) according to the manufacturer's instruction. 1 µg of total RNA was digested with 1 Unit DNase I (Invitrogen, Life Technologies GmbH, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and reverse transcribed to cDNA using the *High Capacity cDNA Reverse Transcription Kit* (Applied Biosystems, Life Technologies GmbH, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The RT-PCR reactions were performed using *SYBR® Select Master Mix* (Applied Biosystems, Life Technologies GmbH, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The data analysis was performed according to the $2^{-\Delta\Delta ct}$ method with *β-actin* used as a housekeeping gene for normalization.

Chromatin Immunoprecipitation (ChIP)

ChIP was performed as described previously (6). *Nos2* and *Arg1* promoter regions were amplified with specific primers for the corresponding promoter sites by real-time PCR. The percent binding of input was determined as $2^{(Ct_{input}-Ct_{sample})}*100$.

Luciferase reporter assay

The putative AP-1 binding regions in the promoter of Arg1 and Nos2 were amplified by PCR from genomic DNA extracted from splenocytes in C57BL/6 WT mice and cloned using XhoI and SacI into the pGL4.23 firefly reporter vector (Promega). To delete 6 bp containing the AP-1 binding sites, the Q5® Site-Directed Mutagenesis Kit (New England Biolabs, Ipswich, Massachusetts, United States) was used. All the constructs were verified by sequencing. Then 293T cells were co-transfected with the luciferase reporter construct, Renilla plasmid and the Fra-1 expression vector using calcium phosphate. Transfected cells were then lysed and luciferase activity was quantified and normalized to the activity of the co-transfected Renilla reporter gene.

Flow cytometry

For flow cytometry analysis, a single-cell suspension was prepared from paws by mincing the tissue and subsequent collagenase A (Biochrom, Merck Millipore, Berlin, Germany) digestion for 60 min at 37 °C. For cell surface staining, the cell suspension was blocked with a final concentration of 0.5 µg/ml anti-mouse CD16/32 (clone: 93) for 10 min at 4 °C and subsequently stained with the following directly conjugated antibodies: anti-mouse F4/80-APC (clone: BM8), anti-mouse Ly-6G-FITC (clone: 1A8), anti-mouse CD11b-PerCP (clone: ICRF44), anti-mouse Siglec F-BrilliantViolet421 (clone: S17007L), anti-mouse CD11b-APCCy7 (clone: ICRF44), anti-mouse Ly6G-PE (clone: 1A8), anti-mouse Ly6C-PECy7

(clone: HK1.4) and anti-mouse Ly-6C-BrilliantViolet421 (clone: HK1.4) (all BioLegend, San Diego, California, US). The gating strategy is illustrated in supplementary fig. 8A. Briefly, the events were gated for $CD11b^+/Ly6G^+$ indicating neutrophils, the $CD11b^+/Ly6G^-$ population was further gated for $Ly6C^+$ cells indicating monocytes and the $CD11b^+/Ly6C^-$ population was then gated for $F4/80^+$ cells indicating macrophages. Intracellular staining was carried out using the intracellular fixation and permeabilization buffer set (eBioscience, Thermo Fisher Scientific, Waltham, Massachusetts, US) according to the manufacturer's instructions and the staining of Arg1 was carried out with a directly labelled anti-mouse arginase I-PE (clone: E-2, Santa Cruz Biotechnologies, Dallas, Texas, US). Samples were analysed using the *CytoFLEX S Flow Cytometer* (Beckman Coulter, Krefeld, Germany), for sorting the *MoFlo Astrios* (Beckman Coulter, Brea, California, US) was used instead.

Western Blot analysis

Cells were lysed in Frackelton buffer (10 mM Tris, 50 mM NaCl, 30 mM NaPPi, 50mM NaF, 1 % (v/v) Triton X-100) supplemented with protease inhibitor cocktail (cOmplete™ ULTRA, Roche, Basel, Switzerland) followed by three freeze/thaw cycles performed by flash-freezing the sample in liquid nitrogen followed by thawing on ice. Lysates were boiled at 95 °C for 5 minutes in Laemmli buffer. Cell lysates from 0.25 x 10^6 macrophages were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in a 10 % (w/v) polyacrylamide gel. Semidry immunoblotting was carried out on PVDF membranes, blocked with 10 % (w/v) milk powder in TBS/ 0.05% (v/v) tween 20 and further incubated overnight at 4 °C with rabbit anti-mouse Fra-1 1:1000 (R-60, Santa Cruz, Dallas, Texas, US) and mouse anti-mouse β-actin antibody 1:5000 (Sigma-Aldrich, Munich, Germany) and the respective secondary antibody, goat anti-rabbit IgG or goat anti-mouse IgG conjugated with horseradish peroxidase (both used at a dilution of 1:20,000; Promega, Madison, US). Chemiluminescence was carried out using Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific, Waltham, Massachusetts,

US) and monitored with *Celvin* chemiluminescence-imager (Biostep, Burkhardtsdorf, Germany).

Arginase activity assay and Griess assay

The arginase assays were performed as described previously (7). 100 µl human serum samples from patients were depleted of urea through centrifugal filter units with 10 kDa cut off (Amicon, Merck, Kenilworth, New Jersey, US). For the preparation of the paw lysates, the skin was removed and the total paw tissues were homogenized using a Precellys® Ceramic Kit on a Precelllys®24 tissue grinder (bertin instruments, Frankfurt am Main, Germany) with the conditions 2 x 30 s at 6,500 rpm, subsequently samples were sonificated on ice (settings: cycle5, power 50%, 40 s) and centrifuged at 23,000 x g for 10 min. Arginase assays of *in vitro* cell culture were performed with lysates from 1 x 10^6 cells.

To measure the accumulation of nitrite, which is a stable end product of the synthesized NO, the Griess Reagent System from Promega was used according to the manufacturer's instructions (Promega, Madison, US).

Network reconstruction

With the lists of targeted gene loci in the ChIP-Seq, sets of unique gene loci per treatment group were prepared via standard set operations (union and difference). A network was then reconstructed by querying interactions from the databases TRANSFAC (release 2015/3) (8), miRTarBase (release 6.1) (9), and RegPhos (release 2.0) (10) for each set and the connected components (genes with interactors) extracted and used for enrichment analyses. See Wentker *et al.* 2017 for a more involved description of the network reconstruction procedure (11).

Supplemental references

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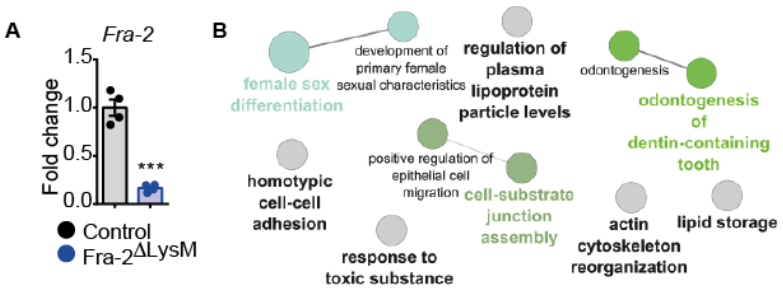
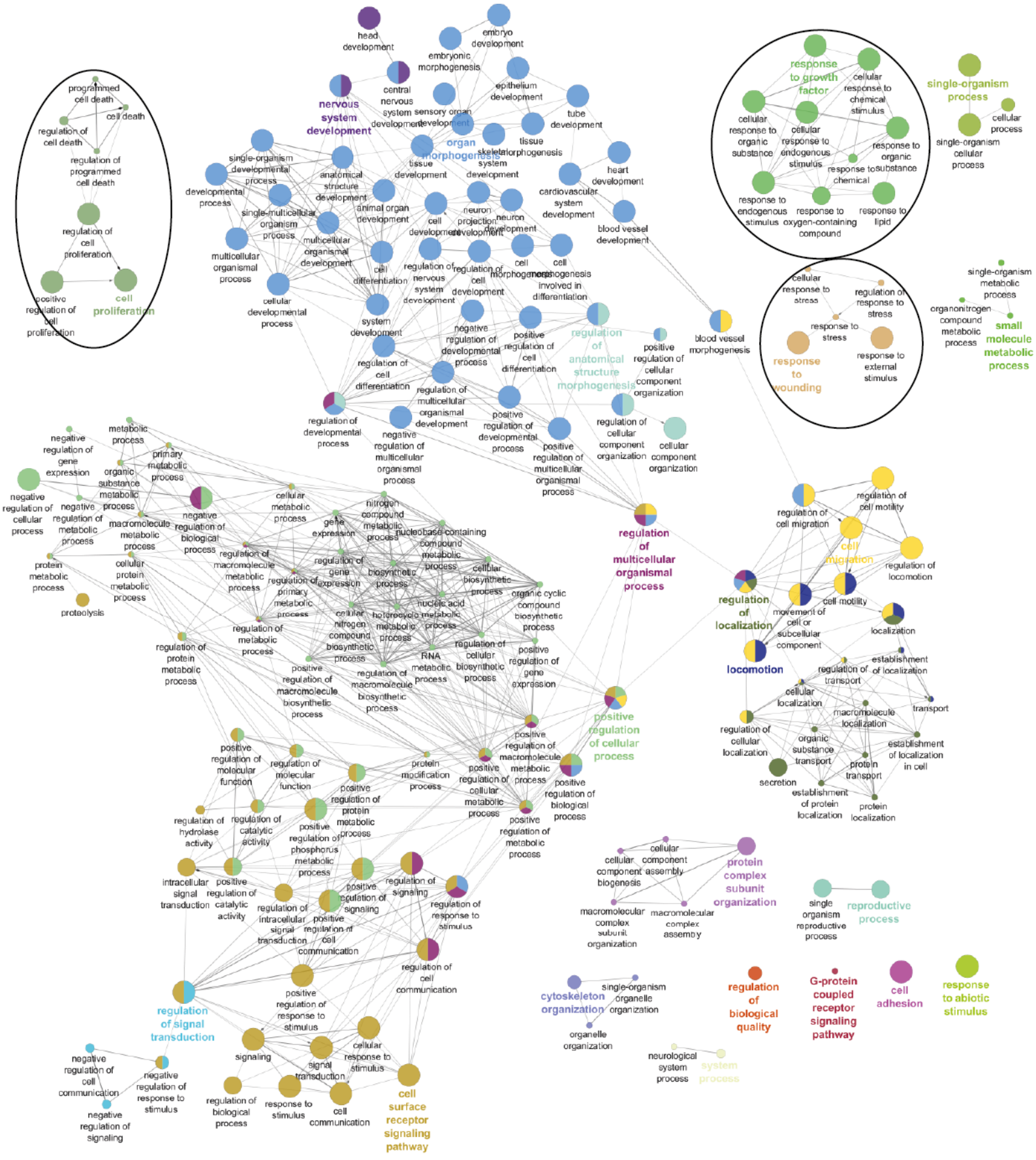
Supplemental figures



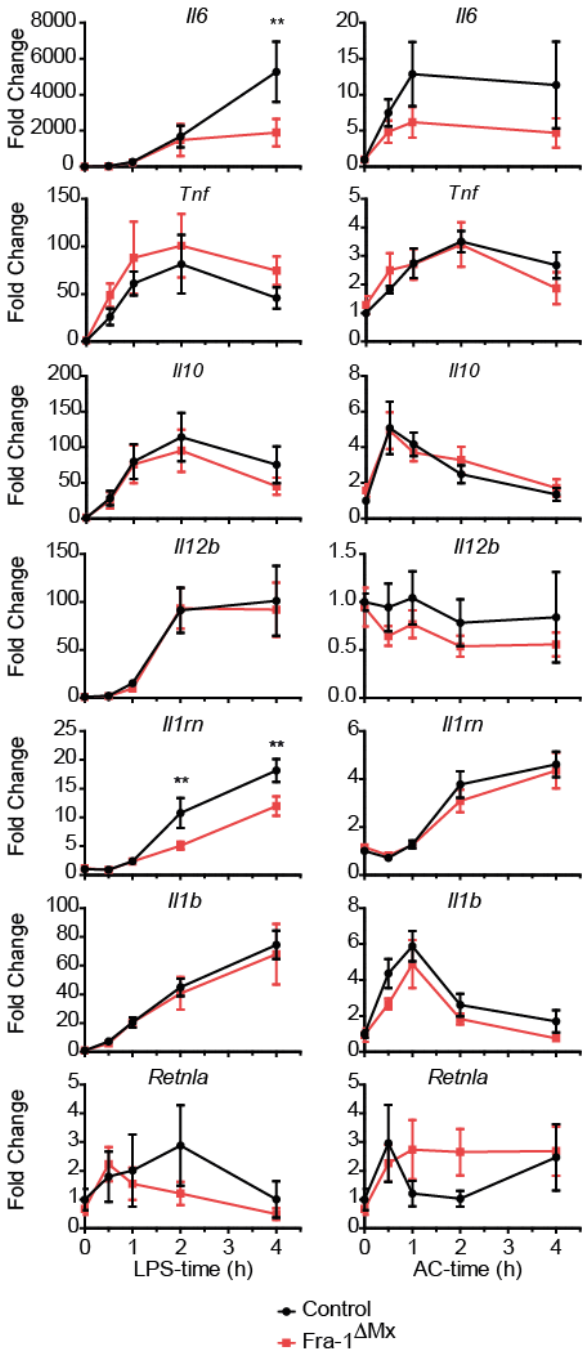
Supplemental Figure 1: GO cluster analyses links Fra-2 *expression in macrophages to developmental processes.*

Thioglycollate-elicited macrophages were isolated from Fra-$2^{\Delta LysM}$ (n=2) and control mice (n=2). (A) The deletion efficiency of *Fra-2* was quantified by real-time PCR. Data are shown as mean of 2 samples with duplicates and the error bars represent S.E.M. ANOVA ***P<0.01 was considered as significant.

(B) *Agilent* microarrays were performed comparing Fra-$2^{\Delta LysM}$ macrophages to their respective control macrophages and the differentially expressed genes were enriched into GO groups.

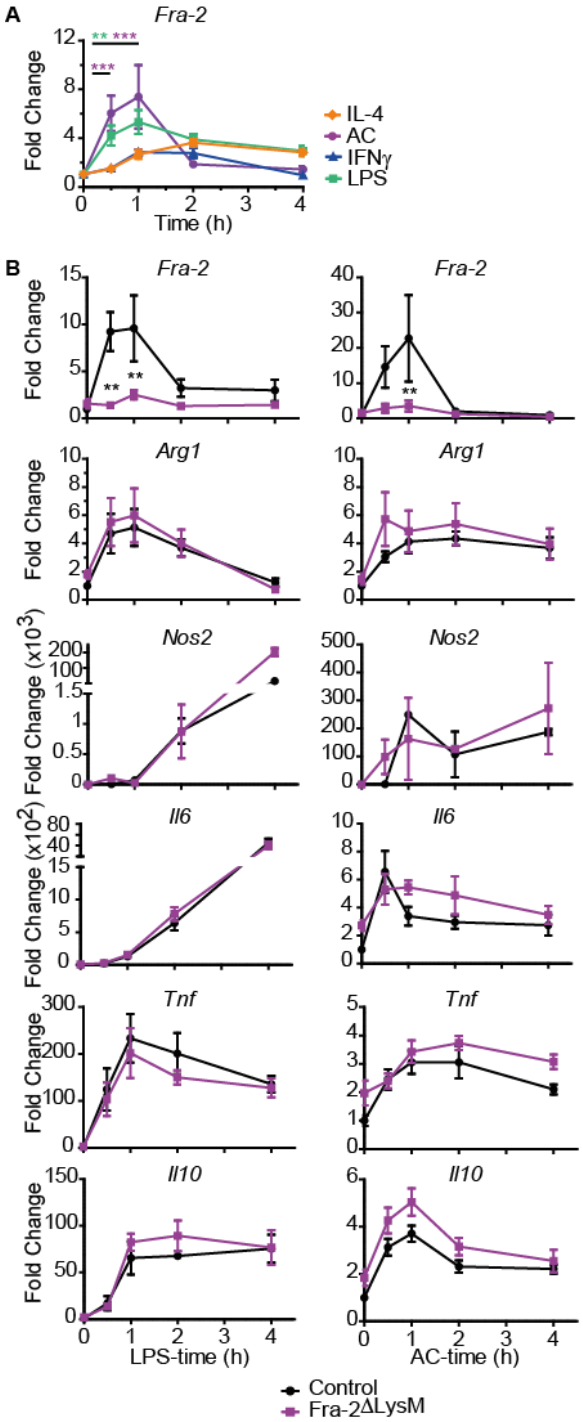

programmed cell death
cell death
regulation of cell death
regulation of programmed cell death
regulation of cell proliferation
positive regulation of cell proliferation
cell proliferation
head development
embryo development
embryonic morphogenesis
central nervous system development
nervous system development
sensory organ development
epithelium development
tube development
organ morphogenesis
tissue development
skeletal system development
tissue morphogenesis
heart development
cardiovascular system development
blood vessel development
single-organism developmental process
developmental process
anatomical structure development
animal organ development
single-multicellular organism process
multicellular organismal development
multicellular organismal process
cell development
neuron projection development
neuron development
cell differentiation
regulation of nervous system development
regulation of cell development
cell morphogenesis
cell morphogenesis involved in differentiation
cellular developmental process
system development
negative regulation of developmental process
positive regulation of cell differentiation
regulation of anatomical structure morphogenesis
positive regulation of cellular component organization
blood vessel morphogenesis
regulation of cell differentiation
regulation of multicellular organismal development
regulation of developmental process
negative regulation of multicellular organismal process
positive regulation of developmental process
positive regulation of multicellular organismal process
regulation of cellular component organization
cellular component organization
response to growth factor
cellular response to chemical stimulus
cellular response to organic substance
cellular response to endogenous stimulus
response to organic substance
response to endogenous stimulus
response to oxygen-containing compound
response to lipid
response to chemical
single-organism process
cellular process
single-organism cellular process
cellular response to stress
regulation of response to stress
response to stress
response to wounding
response to external stimulus
single-organism metabolic process
organonitrogen compound metabolic process
small molecule metabolic process
metabolic process
negative regulation of gene expression
primary metabolic process
organic substance metabolic process
negative regulation of cellular process
negative regulation of metabolic process
macromolecule metabolic process
negative regulation of biological process
cellular metabolic process
protein metabolic process
cellular protein metabolic process
proteolysis
nitrogen compound metabolic process
gene expression
nucleobase-containing compound metabolic process
regulation of macromolecule metabolic process
regulation of gene expression
cellular biosynthetic process
regulation of primary metabolic process
organic cyclic compound biosynthetic process
nucleic acid metabolic process
cellular nitrogen compound biosynthetic process
heterocycle metabolic process
regulation of metabolic process
RNA metabolic process
regulation of cellular biosynthetic process
positive regulation of gene expression
regulation of protein metabolic process
positive regulation of macromolecule biosynthetic process
regulation of macromolecule biosynthetic process
regulation of multicellular organismal process
regulation of cell migration
regulation of cell motility
cell migration
regulation of locomotion
regulation of localization
movement of cell or subcellular component
cell motility
localization
locomotion
establishment of localization
regulation of transport
cellular localization
macromolecule localization
transport
regulation of cellular localization
organic substance transport
establishment of localization in cell
protein transport
secretion
establishment of protein localization
protein localization
positive regulation of cellular process
positive regulation of molecular function
regulation of molecular function
protein modification process
positive regulation of macromolecule metabolic process
positive regulation of cellular metabolic process
positive regulation of biological process
positive regulation of protein metabolic process
regulation of hydrolase activity
regulation of catalytic activity
positive regulation of phosphorus metabolic process
positive regulation of metabolic process
intracellular signal transduction
positive regulation of catalytic activity
regulation of intracellular signal transduction
positive regulation of signaling
regulation of signaling
regulation of response to stimulus
positive regulation of cell communication
regulation of cell communication
cellular component biogenesis
cellular component assembly
protein complex subunit organization
macromolecular complex subunit organization
macromolecular complex assembly
single organism reproductive process
reproductive process
cytoskeleton organization
single-organism organelle organization
organelle organization
regulation of biological quality
G-protein coupled receptor signaling pathway
cell adhesion
response to abiotic stimulus
neurological system process
system process
regulation of signal transduction
signaling
signal transduction
cellular response to stimulus
response to stimulus
regulation of biological process
cell communication
cell surface receptor signaling pathway
negative regulation of cell communication
negative regulation of response to stimulus
negative regulation of signaling

Supplemental Figure 2: GO cluster analyses links Fra-1 *expression in macrophages to cellular responses, such as wound response, proliferation and the response to diverse stimuli.*
Thioglycollate-elicited macrophages were isolated from Fra-1$^{\Delta Mx}$ (n=2) and control mice (n=2). *Agilent* microarrays were performed comparing Fra-1$^{\Delta Mx}$ macrophages to control macrophages and the differentially expressed genes were enriched into GO groups.

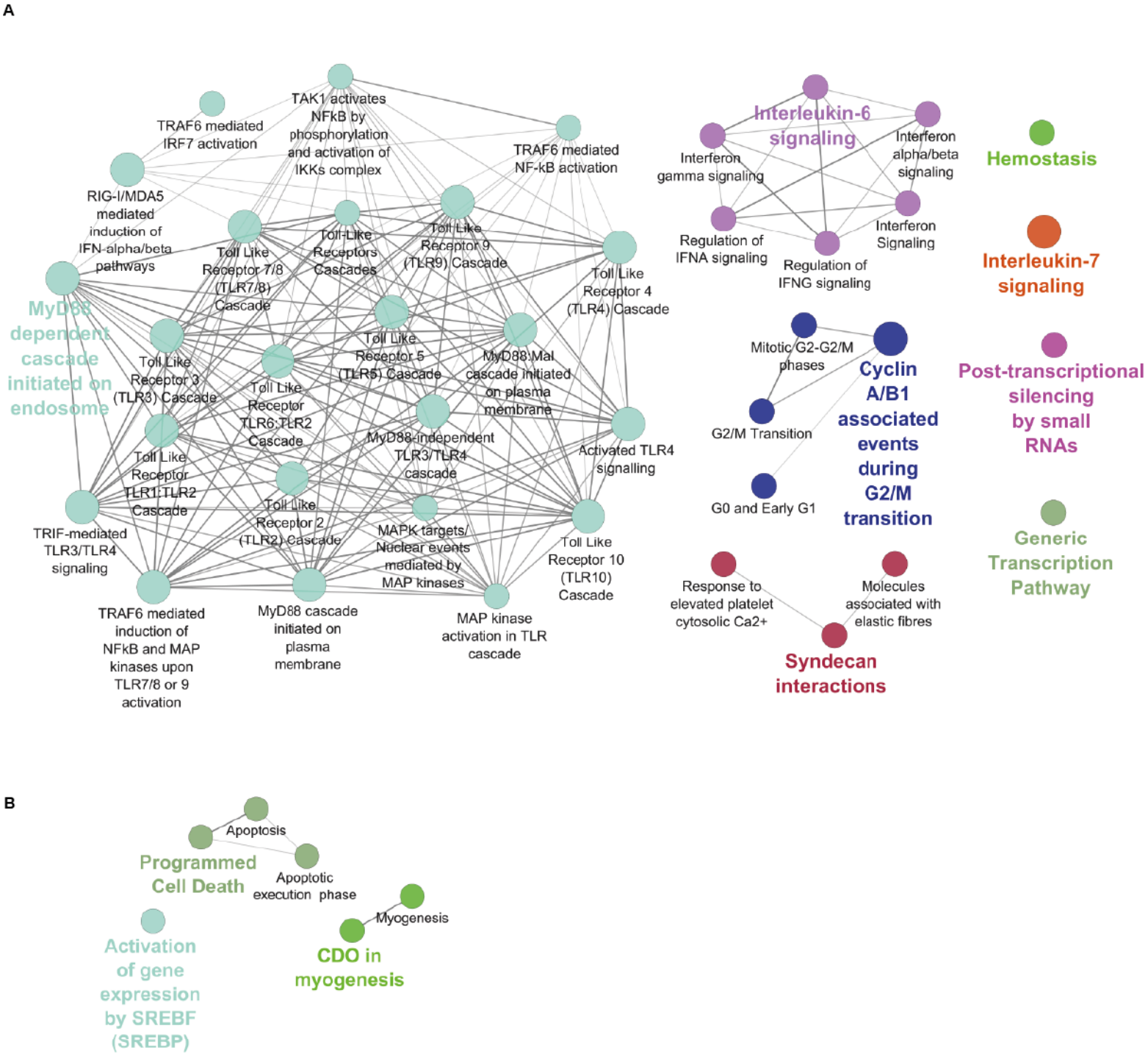


Supplemental Figure 3: Decreased Il6 expression in thioglycollate-elicited macrophages following AC and LPS stimulations.

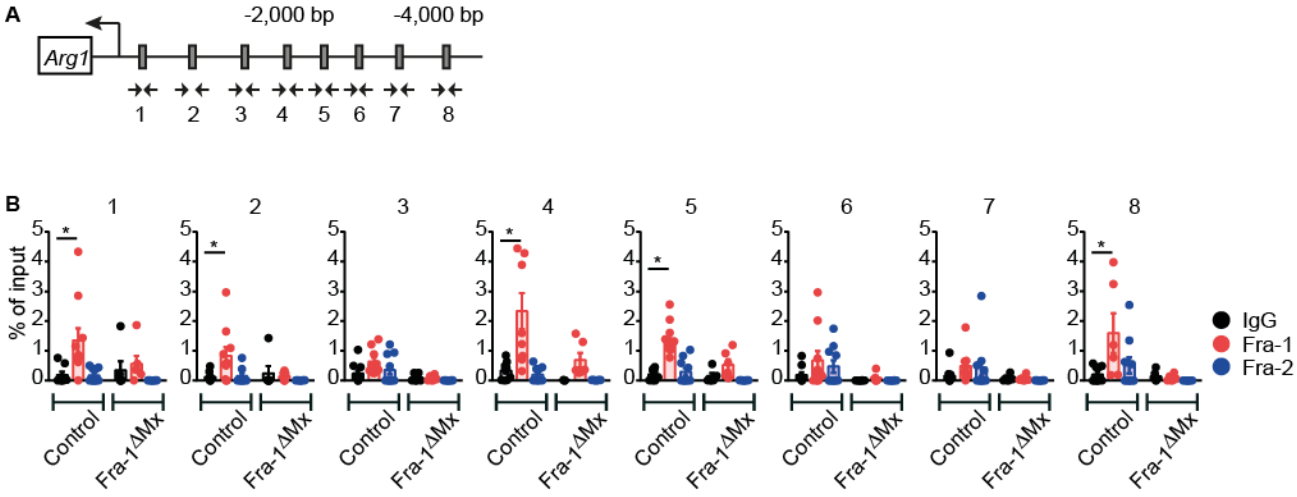
$1x10^6$ thioglycollate-elicited macrophages isolated from Fra-$1^{\Delta Mx}$ or control littermate mice were stimulated with 1 µg/ml LPS or $5x10^6$ AC. The mRNA levels of *Il6*, *Tnf, Il10*, *Il12b*, *Il1rn*, *Il1b* and *Retnla* were determined by quantitative PCR at the indicated time points post stimulation. Data are shown as mean of 3 independent experiments and the error bars represent S.E.M. Student's *t*-test with $^{**}P<0.01$ was considered as significant.



Supplemental Figure 4: No regulation of Arg1/Nos2 *expression by Fra-2 in macrophages.*
(A) 1x10^6 thioglycollate-elicited macrophages isolated from wildtype mice were stimulated with 50 ng/ml IFNγ, 1 µg/ml LPS, 100 ng/ml IL-4 or 5x10^6 AC. *Fra-2* mRNA levels were determined by quantitative PCR at the indicated time points post stimulation.
(B) 1x10^6 thioglycollate-elicited macrophages isolated from Fra-2$^{\Delta LysM}$ or control littermate mice were stimulated with 1 µg/ml LPS or 5x10^6 AC. *Fra-2*, *Arg1*, *Nos2*, *Il6*, *Tnf* and *Il10* mRNA levels were determined by quantitative PCR following LPS (left panel) or AC (right panel) stimulation. Data are shown as mean of 3 independent experiments and the error bars represent S.E.M. ANOVA $^{**}P<0.01$ and $^{***}P<0.001$ were considered as significant.



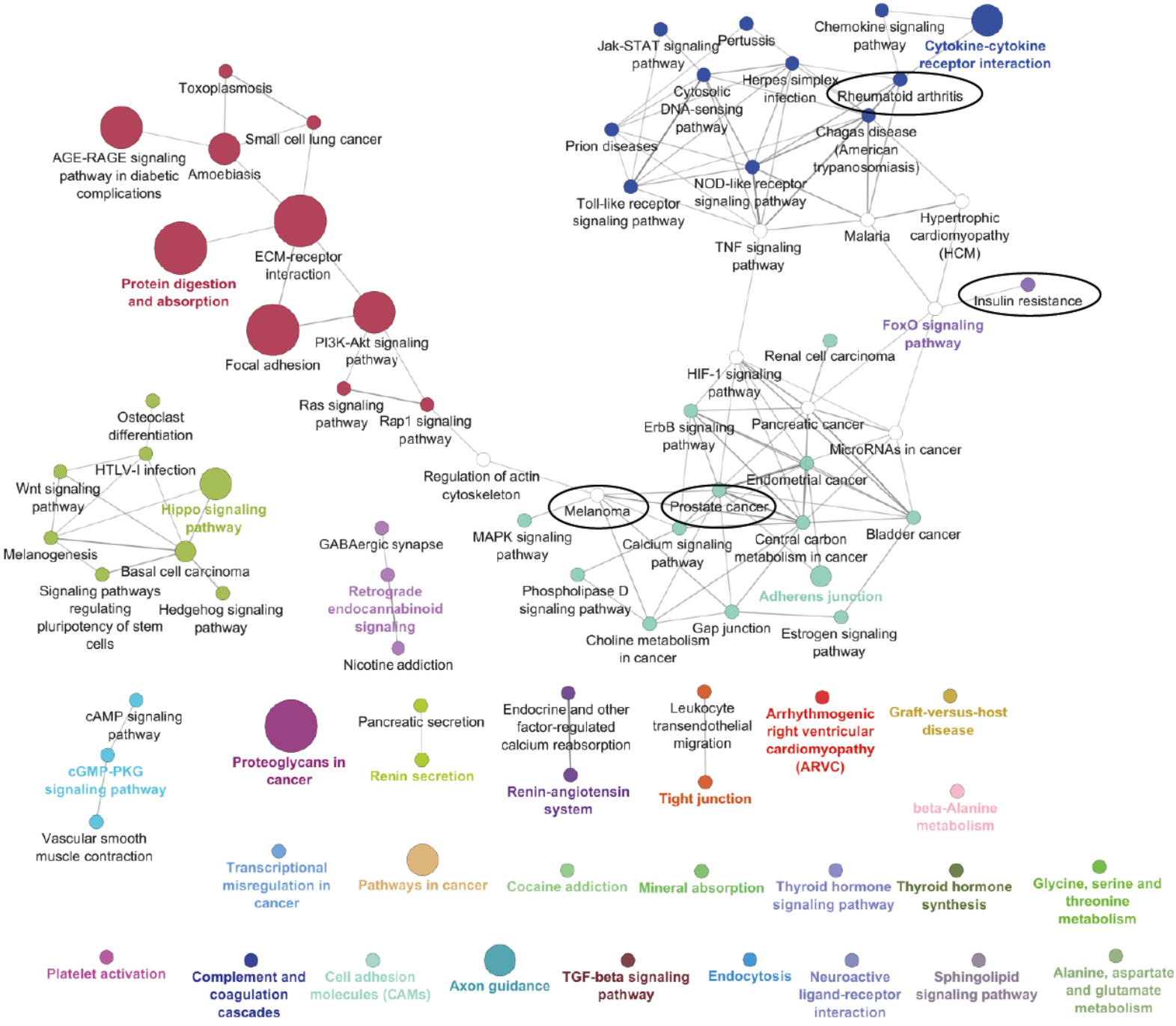
Supplemental Figure 5: ChIP-Sequencing analyses highlighted Fra-1 *expression in macrophages involved in immune responses.*
Thioglycollate-elicited macrophages from wildtype mice unstimulated or stimulated for 1 h with (A) 1 µg/ml LPS or (B) $5x10^6$ AC were used for ChIP-Sequencing analysis. Chromatin was precipitated using an anti-mouse Fra-1 antibody or IgG isotype control (n=3 for each group), the obtained eluate was sequenced and the predicted genes bound by Fra-1 were clustered into their GO groups.



Supplemental Figure 6: No binding of Fra-2 on Arg1 *promoter, neither in control macrophages nor in Fra-1$^{\Delta Mx}$ macrophages.*

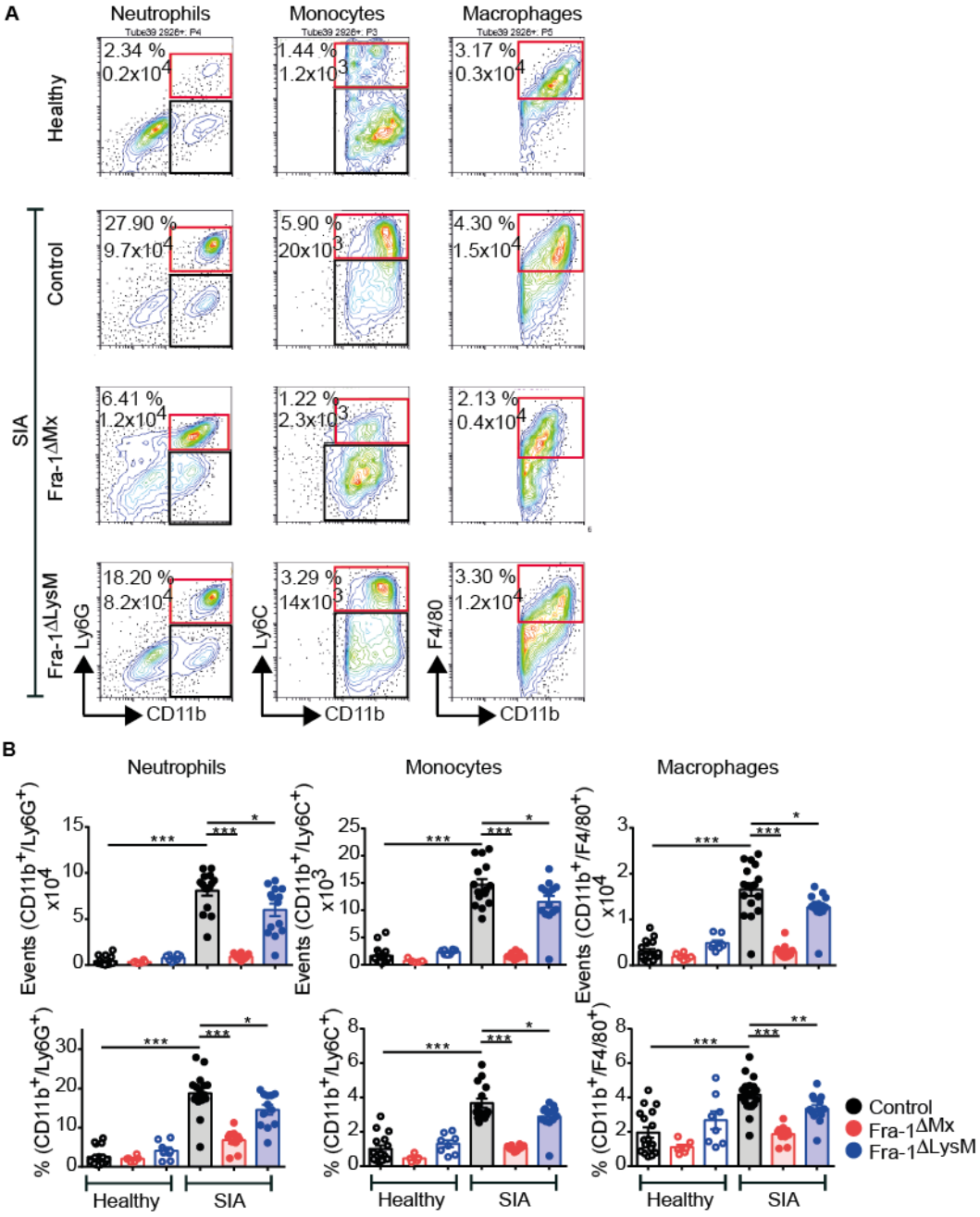
(A) Scheme of AP-1 consensus sequences on the *Arg1* promoter determined by the online tool TF search and indicated by grey boxes. Arrows below indicate the primer binding sites for ChIP analysis.

(B) Chromatin from thioglycollate-elicited macrophages isolated from Fra-1$^{\Delta Mx}$ or control littermate mice were precipitated using anti-mouse Fra-1, anti-mouse Fra-2 or IgG isotype. Eluates were analysed by RT-PCR using primer specific for the respective binding sites of *Arg1* promoter. The Ct values are normalized to input.



Supplemental Figure 7: KEGG cluster analyses linked Fra-1 expression in macrophages to diseases.

KEGG cluster analysis based on microarray data differentially expressed genes from Fra-1$^{\Delta Mx}$ (n=2) and control (n=2) thioglycollate-elicited macrophages.

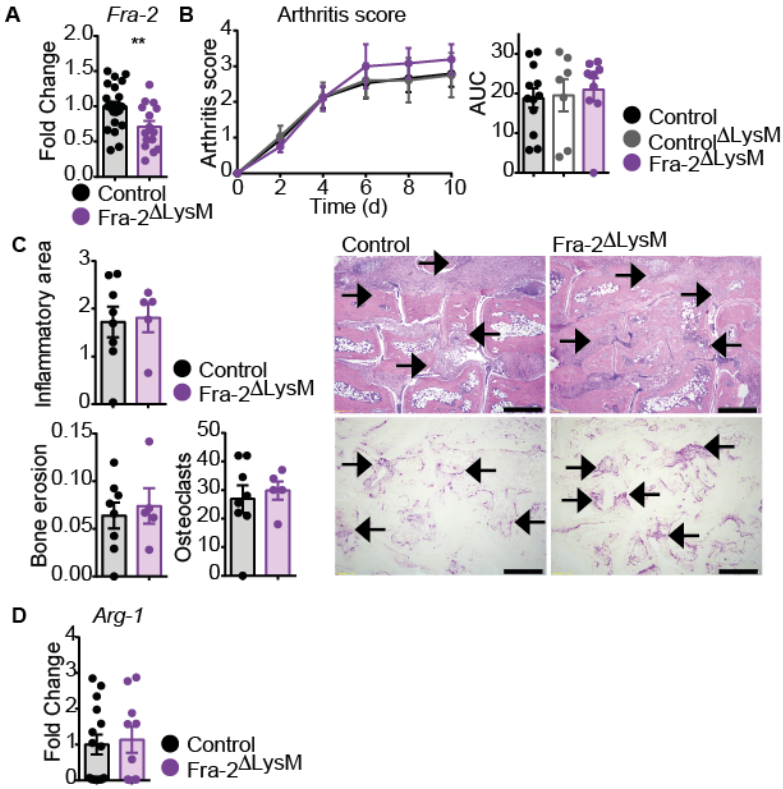


Supplemental Figure 8: Decreased immune cell infiltration in joints of arthritic Fra-1$^{\Delta Mx}$ and Fra-1$^{\Delta LysM}$ mice.

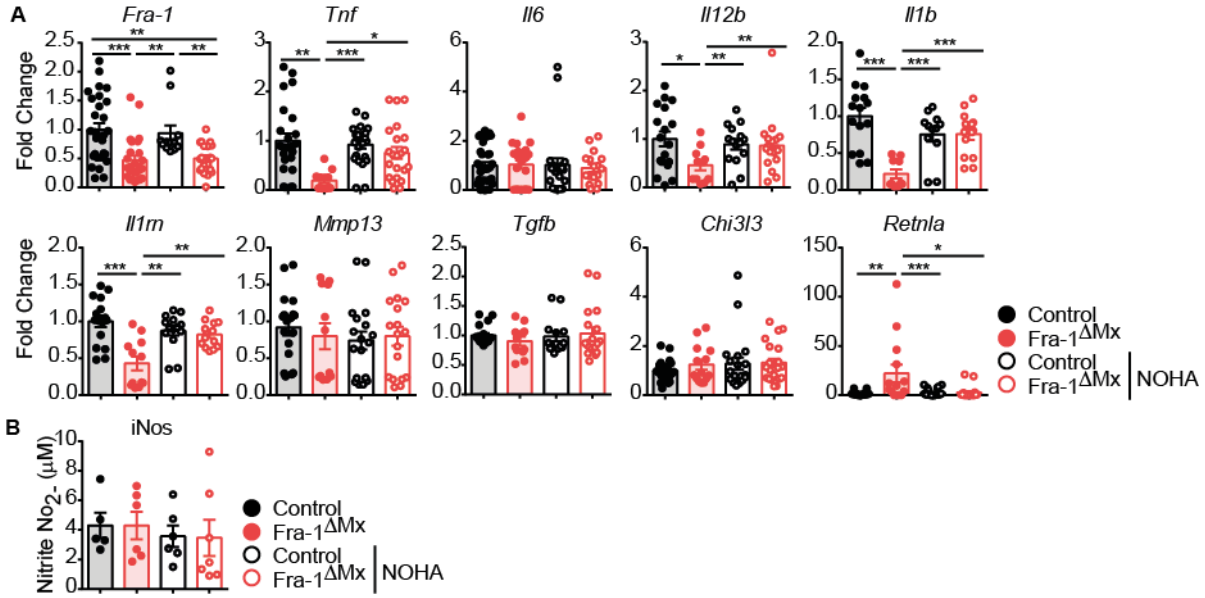
Fra-1$^{\Delta Mx}$, Fra-1$^{\Delta LysM}$ or control littermate mice were analysed in steady state or after arthritis induced by K/BxN serum transfer.

(A) 10 days after serum transfer a single cell suspension of the joints were analysed for infiltrated immune cell populations and consecutive gates are represented by the black arrows. Representative contour plots including absolute cell numbers and the percentage are shown for healthy and arthritic control, Fra-1$^{\Delta Mx}$ and Fra-1$^{\Delta LysM}$ joints.

(B) Quantification of the flow cytometric analysis for infiltrated neutrophils, monocytes and macrophages (n>5). Data are shown as mean values and the error bars represent S.E.M. ANOVA with *P<0.05, **P<0.01 and ***P<0.001 were considered as significant.



Supplemental Figure 9: No altered course of rheumatoid arthritis in Fra-2$^{\Delta LysM}$ mice.
K/BxN arthritis was applied to Fra-2$^{\Delta LysM}$ (n=9), control$^{\Delta LysM}$ (n=7) and control (n=12) littermate mice. The mice were analysed 10 days post serum transfer.
(A) *Fra-2* mRNA levels in whole paws.
(B) The arthritis score and ist quantification of area under the curve (AUC).
(C) Quantification of the inflammatory area, erosion area and number of osteoclast and representative images ascertained from H&E (top) and TRAP (bottom) staining of arthritic paws. Scale bars indicate 500 µm.
(D) *Arg1* mRNA level in total paw lysates. Data are shown as mean values and the error bars represent S.E.M. Student's *t*-test with $^{**}P<0.01$ were considered as significant.

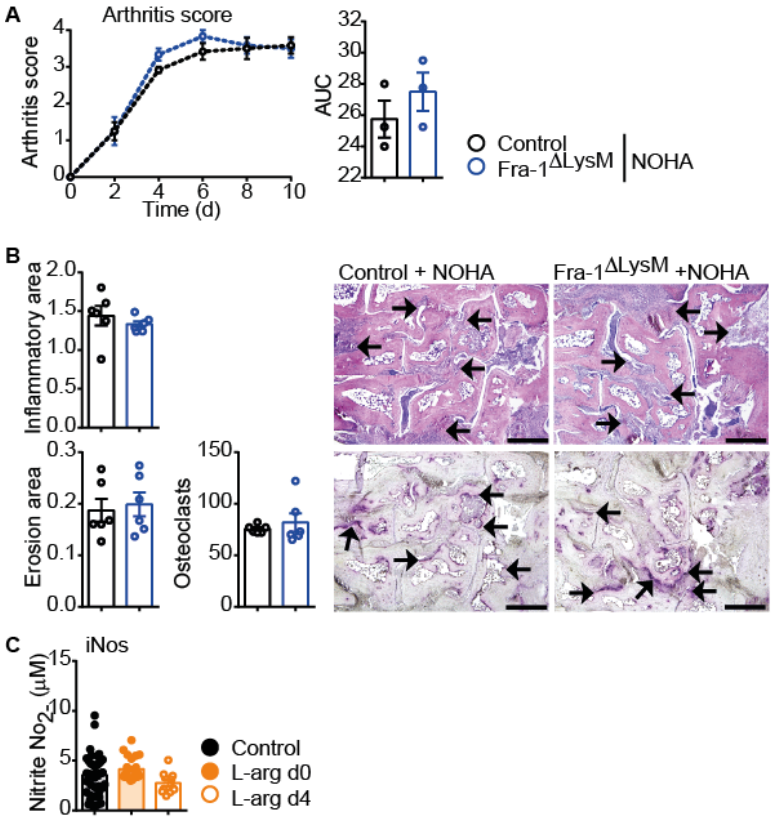


Supplemental Figure 10: Arginase inhibition by NOHA rescues the pro- and anti-inflammatory gene expression in arthritic Fra-1$^{\Delta Mx}$ mice.

Arthritis was induced by K/BxN serum-transfer in Fra-1$^{\Delta Mx}$ or control mice and the mice were injected daily *intra peritoneally* with NOHA (100 mg/kg body weight) or PBS.

(A) *Fra-1*, *Tnf*, *Il6*, *Il12b*, *Il1b*, *Il1rn*, *Mmp13*, *Tgfb, Chi3l3* and *Retnla* mRNA levels day 10 post-serum transfer in total paw lysates.

(B) iNos activity was determined in paw lysates. Data are shown as mean values and the error bars represent S.E.M. Student's *t*-test with **P*<0.05, ***P*<0.01 and ****P*<0.001 were considered as significant.



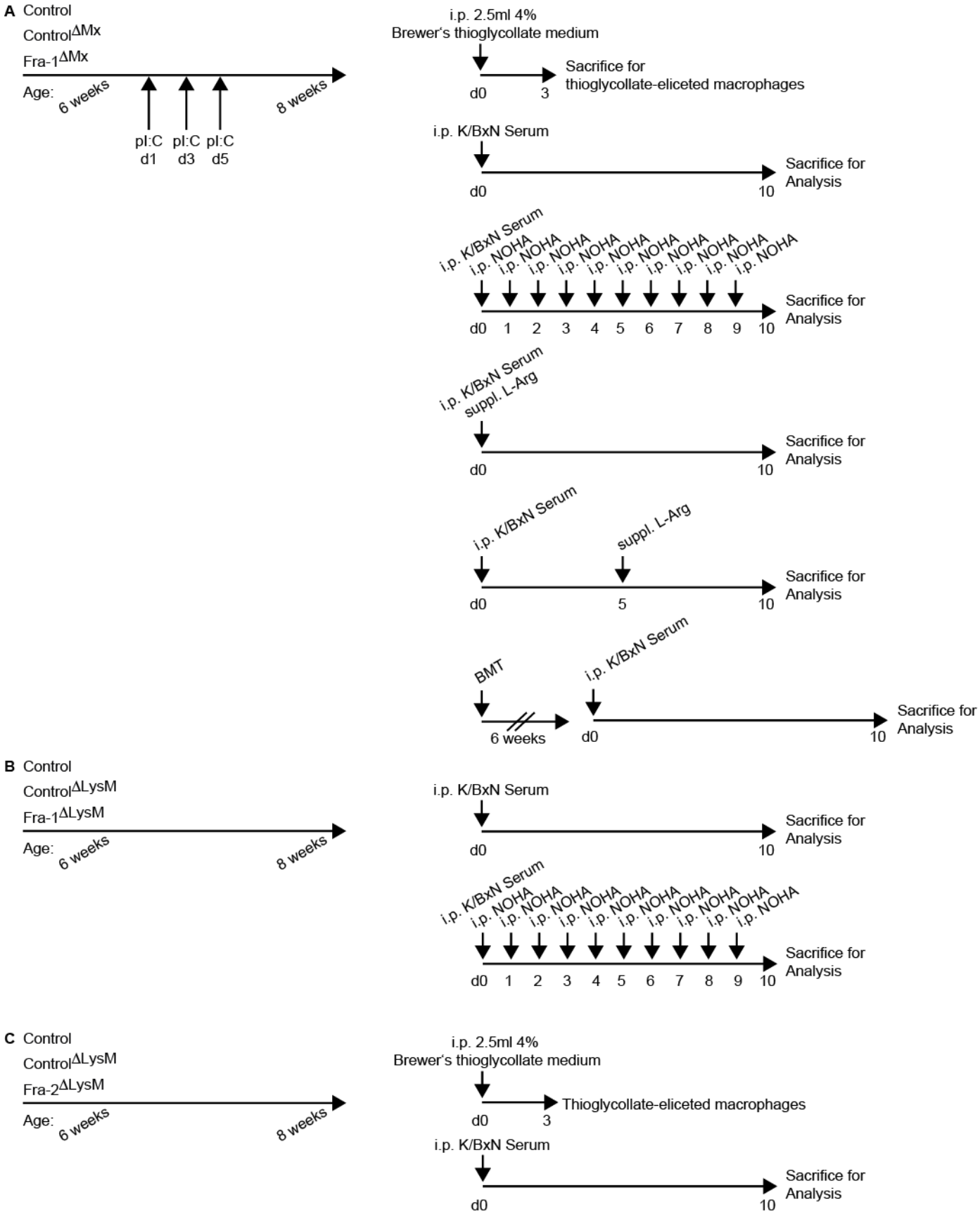
Supplemental Figure 11: Arginase inhibition by NOHA rescues the pro- and anti-inflammatory gene expression in arthritic Fra-1$^{\Delta LysM}$ mice.

(A-B) Arthritis was induced by K/BxN serum-transfer in Fra-1$^{\Delta Mx}$ or control mice, the mice were intraperitoneally injected with NOHA (100 mg/kg body weight, Fra-1$^{\Delta Mx}$ [n=3] or control [n=3]) daily from the day of arthritis induction and the mice were analysed 10 days post-serum transfer.

(A) Arthritis score and its quantification of AUC.

(B) Quantification of the inflammatory area, erosion area and number of osteoclasts from the histological analysis of H&E (top) and TRAP (bottom) staining and its representative images. Scale bars indicate 500 μm.

(C) Arthritis was induced by K/BxN serum-transfer to wild type mice, supplemented with 40 g/L L-arginine in the drinking water, either simultaneously with the K/BxN serum transfer (d0; n=11) or at d4 post serum transfer (n=6). iNOS enzyme activity in whole-paw lysates were quantified 10 days post serum transfer. Data are shown as mean values and the error bars represent S.E.M.


A
Control
Control ΔMx
Fra-1 ΔMx
Age:
6 weeks
8 weeks
pI:C d1
pI:C d3
pI:C d5
i.p. 2.5ml 4% Brewer's thioglycollate medium
d0
3
Sacrifice for thioglycollate-eliceted macrophages
i.p. K/BxN Serum
d0
10
Sacrifice for Analysis
i.p. K/BxN Serum
i.p. NOHA
d0 1 2 3 4 5 6 7 8 9 10
Sacrifice for Analysis
i.p. K/BxN Serum
suppl. L-Arg
Sacrifice for Analysis
i.p. K/BxN Serum
suppl. L-Arg
5
Sacrifice for Analysis
BMT
6 weeks
i.p. K/BxN Serum
Sacrifice for Analysis
B
Control
Control ΔLysM
Fra-1 ΔLysM
Age:
6 weeks
8 weeks
i.p. K/BxN Serum
Sacrifice for Analysis
i.p. K/BxN Serum
i.p. NOHA
Sacrifice for Analysis
C
Control
Control ΔLysM
Fra-2 ΔLysM
Age:
6 weeks
8 weeks
i.p. 2.5ml 4% Brewer's thioglycollate medium
Thioglycollate-eliceted macrophages
i.p. K/BxN Serum
Sacrifice for Analysis
D
Arthritis score
Arthritis Score
time (d)
Control
poly(I:C)

Supplemental Figure 12: Schematic time-line of in vivo experiments performed with the different mice strains.

(A) The deletion of Fra-1 by Cre under the control of the Mx1 promoter was induced with the age of 6 weeks by 250 µg poly(I:C) injected intraperitoneally (i.p.) three times in a 2-day interval. Control littermates without Cre were also treated with poly(I:C).

(B) Control mice with and without LysMCre or Fra-$1^{\Delta LysM}$ mice were used at the age of 8 weeks for the indicated experiments.

(C) Control mice with and without LysMCre or Fra-$2^{\Delta LysM}$ mice were used at the age of 8 weeks for the indicated experiments.

(D) Wildtype control mice with the age of 8 weeks were left untreated or treated with poly(I:C) i.p. three times in a 2-day interval and arthritis was induced 10 d later. The arthritis score was determined to the indicated times points.