Identification of an Arg-Leu-Arg tripeptide that contributes to the binding interface between the cytokine MIF and the chemokine receptor CXCR4

Michael Lacy\textsuperscript{1,§}, Christos Kontos\textsuperscript{2,§}, Markus Brandhofer\textsuperscript{1,§}, Kathleen Hille\textsuperscript{2}, Sabine Gröning\textsuperscript{3}, Dzmitry Sinitski\textsuperscript{1}, Priscila Bourilhon\textsuperscript{1}, Eric Rosenberg\textsuperscript{4}, Christine Krammer\textsuperscript{1}, Tharshika Thavayogarajah\textsuperscript{1}, Georgios Pantouris\textsuperscript{4}, Maria Bakou\textsuperscript{2}, Christian Weber\textsuperscript{5,6,7}, Elias Lolis\textsuperscript{4}, Jürgen Bernhagen\textsuperscript{1,6,8,*}, Aphrodite Kapurniotu\textsuperscript{2,*}

\textsuperscript{1}Department of Vascular Biology, Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig-Maximilians-University of Munich, Feodor-Lynen-Str. 17, D-81377 Munich, Germany; \textsuperscript{2}Division of Peptide Biochemistry, Technische Universität München, D-85354 Freising-Weihenstephan, Germany; \textsuperscript{3}Department of Anaesthesiology, RWTH Aachen University Hospital, D-52074 Aachen, Germany; \textsuperscript{4}Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT, USA; \textsuperscript{5}Institute for Cardiovascular Prevention, Klinikum der Universität München, Ludwig-Maximilians-University of Munich, Pettenkofer Str. 8, D-80336 Munich, Germany; \textsuperscript{6}Munich Heart Alliance, D-80802 Munich, Germany; \textsuperscript{7}Cardiovascular Research Institute Maastricht, Maastricht University, 6229 Maastricht, The Netherlands; \textsuperscript{8}Munich Cluster for Systems Neurology, D-81377 Munich, Germany.

\textsuperscript{§}Equally contributing first authors

*Corresponding authors:

Professor Dr. Aphrodite Kapurniotu, Tel.: +49 8161 713542; Fax: +49 8161 713298; \textit{E-mail address}: akapurniotu@wzw.tum.de

Professor Dr. Jürgen Bernhagen, Tel.: +49 89 4400 46151; Fax: +49 89 4400 46148; \textit{E-mail address}: juergen.bernhagen@med.uni-muenchen.de
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Supplementary Figure 1: Randomization of RLR-containing MIF sequence 75-90 leads to reduction in binding to CXCR4(1-27). Binding of peptide sequences to biotin-CXCR4(1-27) was analyzed by the micro slide-based peptide array technology (Intavis). Sequence 75-90 was randomized by the 'shuffle protein' method (www.bioinformatics.org) to generate the randomized peptides scrambled 1 (scr1), scr2, scr3, scr4, and scr5. Bars represent mean signal values of duplicate slides (mean signal intensity) relative to the binding signal of the wildtype peptide MIF(75-90).
Supplementary Figure 2: Comparison of the hydrophobicity of R87A-L88A-R89A-MIF and wild-type MIF (WT-MIF) using GRAVY. The grand average of hydropathy (GRAVY) index is based on the Kyte & Doolittle algorithm and involved plotting the relative hydrophobicity (y-axis) versus the MIF or RLR mutant sequence (x-axis). The sequence region around RLR(87-89) is framed in red.
Supplementary Figure 3: Purification of R87A-L88A-R89A-MIF by size exclusion chromatography. Representative chromatogram of a Superdex 75 gel filtration run of the RLR mutant. Fractions F1 through F6 were collected and analyzed for RLR mutant content (see Fig. 2a-b for SDS-PAGE and Western blot analysis). Fractions F5 contained most of the enriched mutant protein. F3 and 4 contained minor portions of RLR mutant, which tended to form high-molecular weight oligomers. The chromatogram shown is plotted as UV absorption at 280 nm (in [mAU]; blue line) over the elution volume in [ml]. The column was run at 0.75 ml/min. The green line indicates that elution was done with one buffer (100% B; no gradient); orange line indicates conductivity, which was read for control. Small font numbers next to the fraction captions F1-F6 indicate the exact elution volumes of the peaks.
Supplementary Figure 4: The RLR triple alanine mutant does not bind to CXCR4(1-27). (a) Fluorescence spectroscopic titration of Alexa-488-R87A-L88A-R89A-MIF with increasing ratios of CXCR4(1-27). The solution mixture of CXCR4(1–27) peptide with Alexa-488-R87A-L88A-R89A-MIF does not evoke a conformational change in the mutant protein as indicated by a lack of change in fluorescence. Peptide was added at molar ratios of 1:0.76, 1:1.52, 1:7.63, 1:76.3, 1:763 and spectra were recorded between wavelengths 500 and 600 nm (arbitrary units, a.u.). (b) Concentration dependence of the CXCR4 peptide plotted against the fluorescence change at 522 nm. The horizontal dashed line is a fit through the data points and indicates no conformational change in the mutant protein.
**Supplementary Figure 5:** JVM-3 B lymphocytes express marked levels of CXCR4. Flow cytometry analysis of JVM-3 cells. (a) FACS histogram showing cell counts against staining for surface CXCR4 in JVM-3 by allophycocyanin (APC)-A label (black curve). Grey curves indicates the non-specific signal using isotype IgG control instead of anti-CXCR4-antibody. Inset, dot-plot of forward scatter (FSC-A) versus APC-A signal. (b) Comparison of CXCR4 expression (plotted as mean fluorescence intensity (MFI)) in JVM-3 B cells and primary human B cells isolated via MACS selection from peripheral blood mononuclear cells (PBMCs) of healthy volunteers. Specific signals using anti-CXCR4 antibody versus controls applying isotype immunoglobulin (IgG2a). Triplicate measurements ± SD.
Supplementary Figure 6: Structure models of the human MIF monomer and trimer, highlighting the interaction motifs implicated in CXCR4 binding. (a) Surface structure model. The MIF motifs implicated in CXCR4 binding, i.e. N-like loop (pink), RLR motif (red), and Pro-2- residue (green) are highlighted. (b) Ribbon structures. Same color code and captions as in (a). Protein structures were produced/visualized with PyMOL.
**Supplementary Table 1:** Purification and enrichment of R87A-L88A-R89A-MIF compared to wildtype MIF. Overview of the purification scheme.

<table>
<thead>
<tr>
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<th>Total protein in bacterial lysate [mg] / % purity</th>
<th>Purification by anion exchange column [mg] / % purity</th>
<th>Secondary purification step (C8 or SEC*) [mg] / % purity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT-MIF</strong></td>
<td>96.9 / 10-20%</td>
<td>20.5 / 80-85%</td>
<td>3.2 / 95-98%</td>
</tr>
<tr>
<td><strong>R87A-L88A-R89A-MIF</strong></td>
<td>87.7 / 10-20%</td>
<td>4.2 / 50-60%</td>
<td>1.4 / &gt;90%</td>
</tr>
</tbody>
</table>

For each step, total protein in [mg] (left number) and enrichment factor in % purity of total protein (right number) is given. C8, last step of the WT-MIF purification scheme via C8 reverse phase column that has been established previously to result in 95-98% pure MIF protein in endotoxin-free form (see ref. 29); SEC, size exclusion chromatography, last step of the R87A-L88A-R89A-MIF purification procedure (see Methods).
**Supplementary Table 2**: Circular dichroism (CD) spectropolarimetry shows that the folding and secondary structure profile of R87A-L88A-R89A-MIF is similar to that of WT-MIF. Quantification of the secondary structure contents of the CD spectra shown in Fig. 2b.

![Table](attachment:table.png)

Conformations in the CD spectra were measured as mean residue ellipticity versus the wavelength in the far-UV range. Quantification of the secondary structure fractions of WT-MIF and R87A-L88A-R89A-MIF as analyzed by the Dichroweb online software webtool. Secondary structure contents (%) were calculated by deconvolutions of CD spectra which was performed using ContinLL at DichroWeb and the reference spectra set 7. NRMSD (normalized root mean square deviation) of fits.
The original full gel underlying Figure 2 is shown above. To produce Figure 2a, the following 3 lanes on the left hand side of the gel were not shown: i) molecular weight markers (lane 1); ii) non-induced bacteria (not concentration-adjusted); iii) induced bacteria (not concentration-adjusted). Lane 4 equals the first lane in Figure 2a, i.e. the total lysate lane. The right-hand side of the gel also was cropped (empty lanes).
Full-length blot of Figure 2b

To produce Figure 2b, the blot image was cropped on the left and right side (empty space).