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Supporting Information

A MIF-Derived Cyclopeptide that Inhibits MIF Binding and Atherogenic Signaling via the Chemokine Receptor CXCR2

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SUPPLEMENTARY METHODS

Label-free dynamic mass redistribution: Dynamic mass redistribution (DMR) analysis[19] was performed according to Perkin Elmer's instructions for label-free DMR measurements. Briefly, cells were seeded at a density of 40,000 cells per well of an EnSpire label-free 96-well fibronectin-coated cell assay microplate (Perkin Elmer, Rodgau, Germany). Post-seeding, the cells were equilibrated at room temperature (RT) for 30 min and cultivated overnight in serum-containing DMEM/F-12 media. The next day, the cells were washed four times with assay buffer containing 20 mM Hepes and 1% DMSO in HBSS (pH 7.4), using an aspiration wand leaving a residual volume of 35 µL. The plate was equilibrated at RT for 2 h. MIF-derived peptides were reconstituted at a concentration of 5 mM in PBS and further diluted to a working concentration of 2.5 mM in assay buffer. Before adding the peptides, baseline measurements were performed for 10 min (30 sec intervals) using the EnSpire Label-free Multimode Plate Reader (Perkin Elmer). Then, 20 µL of the respective peptides were added to the cells at a final concentration of 500 µM followed by real-time measurements over a time period of 40 min (30 s intervals). The signal output represents a cumulative response overlay of all cells.

SUPPLEMENTARY FIGURES

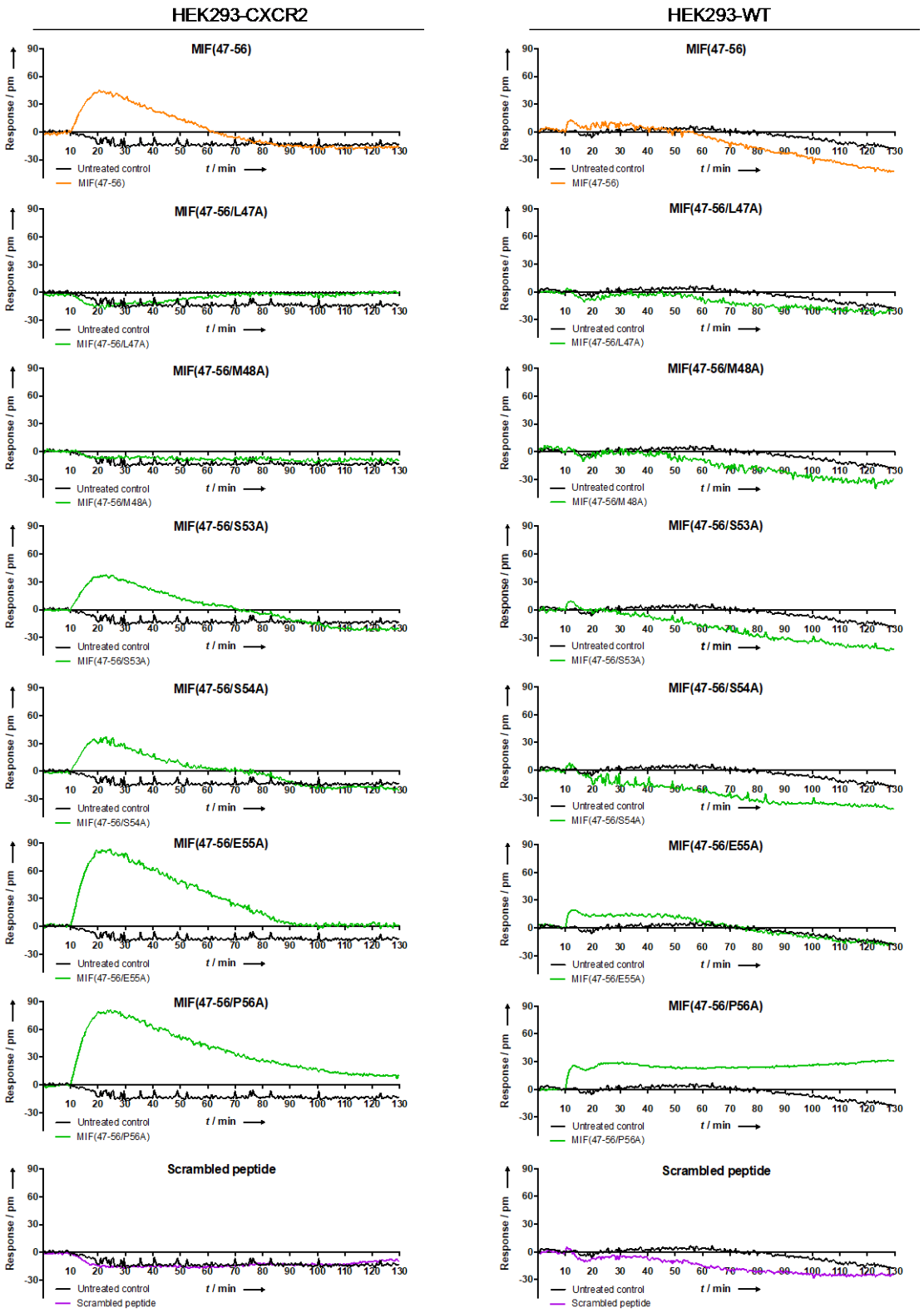

Figure S1. Screening of MIF(47-56) Ala variants for their binding capacity to CXCR2 by DMR technology using HEK-CXCR2 transfectants (left) compared to non-transfected control cells (WT, right). A scrambled sequence of MIF(47-56) was used to determine unspecific binding (bottom). All peptides were applied at a concentration of 500 µM).

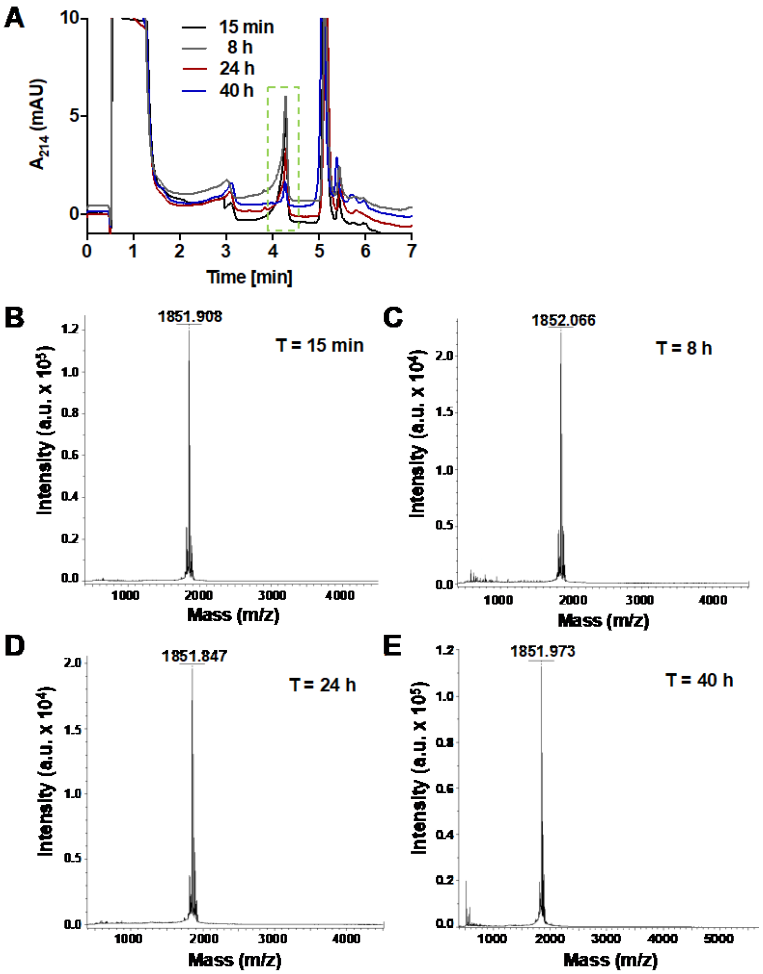

Figure S2. MIF-derived cyclic peptide MIF(cyclo10) exhibits high proteolytic stability in human blood plasma. The HPLC chromatogram (A) and the mass spectra of recovered peptide MIF(cyclo10) at the various time points (B-E) are shown. MIF(cyclo10) was incubated (225 µg/mL) in human plasma at 37°C for the indicated time intervals, recovered peptides quantitated by C18 HPLC and their molecular weights verified by MALDI-MS (see also Figure 4). (A) HPLC chromatograms with the chromatograms of the four incubation times indicated by color code. The peaks at 4.2 min that are marked by a dotted green box are the peptide fractions that were isolated and subjected to MALDI-MS. The peaks at 5.1 min represent remaining (non-precipitated) bulk plasma proteins. The chromatograms shown are representative of three independent incubations. (B-E) Mass spectra of the isolated peptide peaks at 4.2 min according to (A), representing the different incubation intervals (B, 15 min; C, 8 h; D, 24 h; E, 40 h).