

Supplementary Material

Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')
TNF- α	TCTGTCTACTGAACTTCGGGGTGATCG	GTATGAGATAGCAAATCGGCTGACGGTG
IL-6	CTTCCATCCAGTTGCCTTCTTG	AATTAAGCCTCCGACTTGTGAAG
TGF- β	GGAGAGCCCTGGATACCAAC	CAACCCAGGTCCTTCCTAAA
JAK1	GAACCACCTCAAGAAGCAGAT	GGCTTTCTTAGTGGCTACGAG
STAT1	TTGACAAAGACCACGCCTT	GACTTCAGACACAGAAATCAACTC
STAT6	GCCACCATCAGACAAATACTTC	AGTTCTTCCTGCTTCCGATG
NFKBIA	TGCCTGGCCAGTGTAGCAGTCTT	CAAAGTCACCAAGTGCTCCACGAT
KEAP1	GGAGTATATCTACATGCACTTCGG	GCAGCGTACGTTTCAGATCA
NFE2L2	TGATGGACTTGGAGTTGCC	TCAAACACTTCTCGACTTACTCC
ACTB (β -actin)	GATTACTGCTCTGGCTCCTAG	GACTCATCGTACTCCTGCTTG

Table S1. Primer sequences used for RT-qPCR analysis

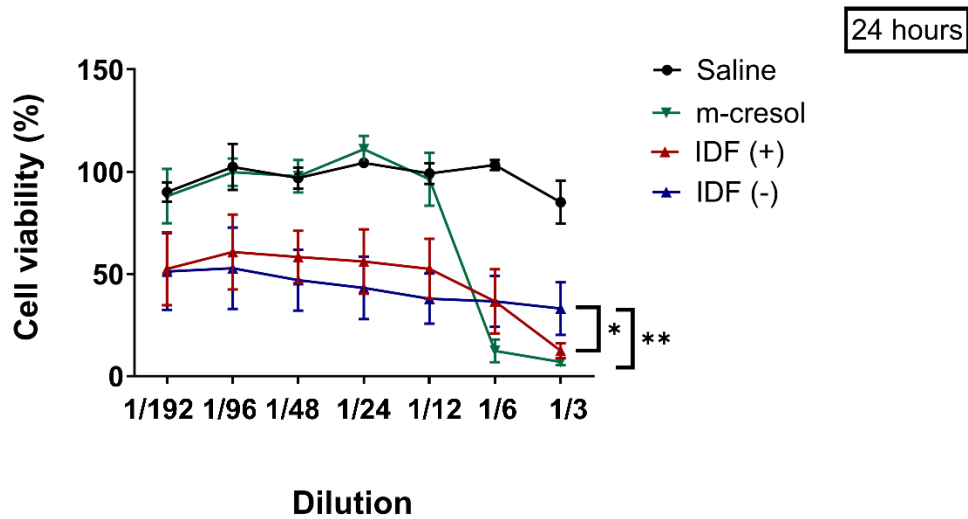


Figure S1. Cytotoxic effects of insulin-derived fibrils (IDFs) and m-cresol in BMDMs as a function of exposure time and concentration, with IDF(+) exhibiting increased cytotoxicity relative to IDF(-) at higher dilution. Bone Marrow-Derived Macrophages (BMDMs) were exposed to IDF (+), IDF (-), or m-cresol solution (3.15 mg/mL) at serial dilutions for 24h. Cell viability was measured using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). Results are depicted as a percentage of untreated controls. Data represent the mean $\pm$ SD of at least two independent experiments performed in triplicate. **p < 0.01, ***p < 0.001, ****p < 0.0001, as determined by two-way ANOVA with Tukey's post hoc test for multiple comparisons.

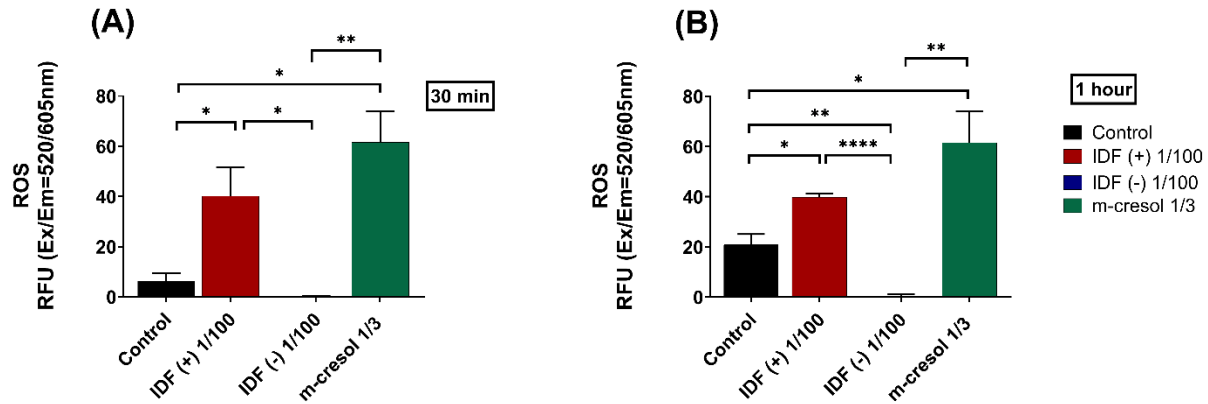


Figure S2. Intracellular reactive oxygen species (ROS) production in Bone Marrow-Derived Macrophages (BMDMs) following stimulation with insulin-derived fibrils (IDFs) and m-cresol. IDF (+) at dilution 1/100 induced significantly higher ROS levels than IDF (-) and produced ROS levels comparable to m-cresol at 1/3 dilution. BMDMs were treated with IDF (+), IDF (-), or m-cresol for **(A)** 30 minutes and **(B)** 1 hour. Intracellular ROS levels were measured using a fluorescence-based assay. Data represent mean $\pm$ SEM of three independent experiments performed in triplicate. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ as determined by unpaired t -test.

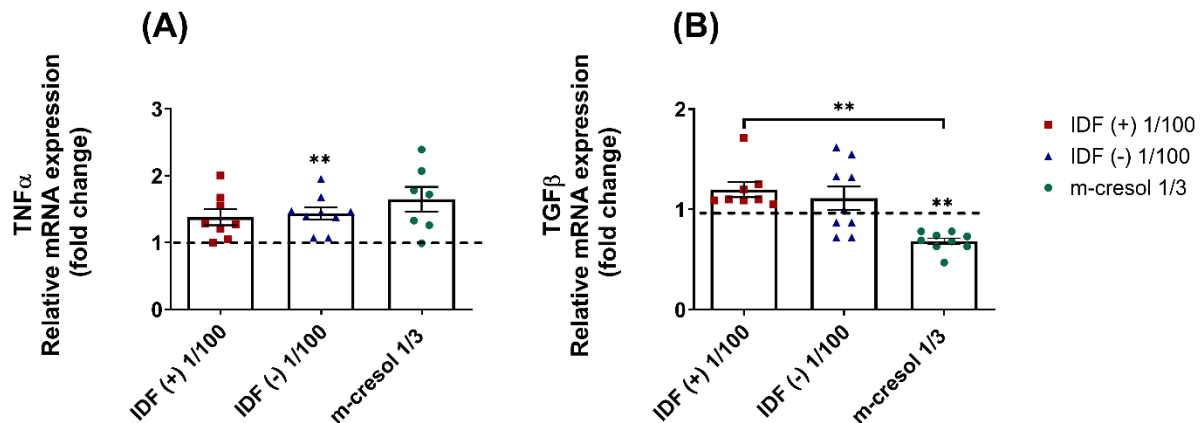


Figure S3. Cytokine gene expression profiles in bone marrow-derived macrophages (BMDMs) following stimulation with insulin-derived fibrils (IDFs) and m-cresol. IDF(-) significantly induces TNF- α whereas m-cresol significantly reduced TGF- β compared to control and IDF(+) 1/100. BMDMs were treated with IDF (+), IDF (-), or m-cresol for 1 hour. Relative mRNA expression levels of **(A)** TNF α , and **(B)** TGF β were quantified by RT-qPCR. Data are presented as mean $\pm$ SEM of fold change relative to the untreated control (control = 1, shown as a black dashed line) from at least four independent experiments performed in duplicate. Statistical analysis was performed using Δ Ct values, and the data was plotted as fold change. ** $p < 0.01$, as determined by Mixed-effect analysis or One-way ANOVA with Tukey's post hoc test for multiple comparisons.