

## Supplementary Information

### **Context-dependent activities of mitrephorone link lipid redirection, anti-inflammatory action, and ferroptosis control to hepatocyte protection**

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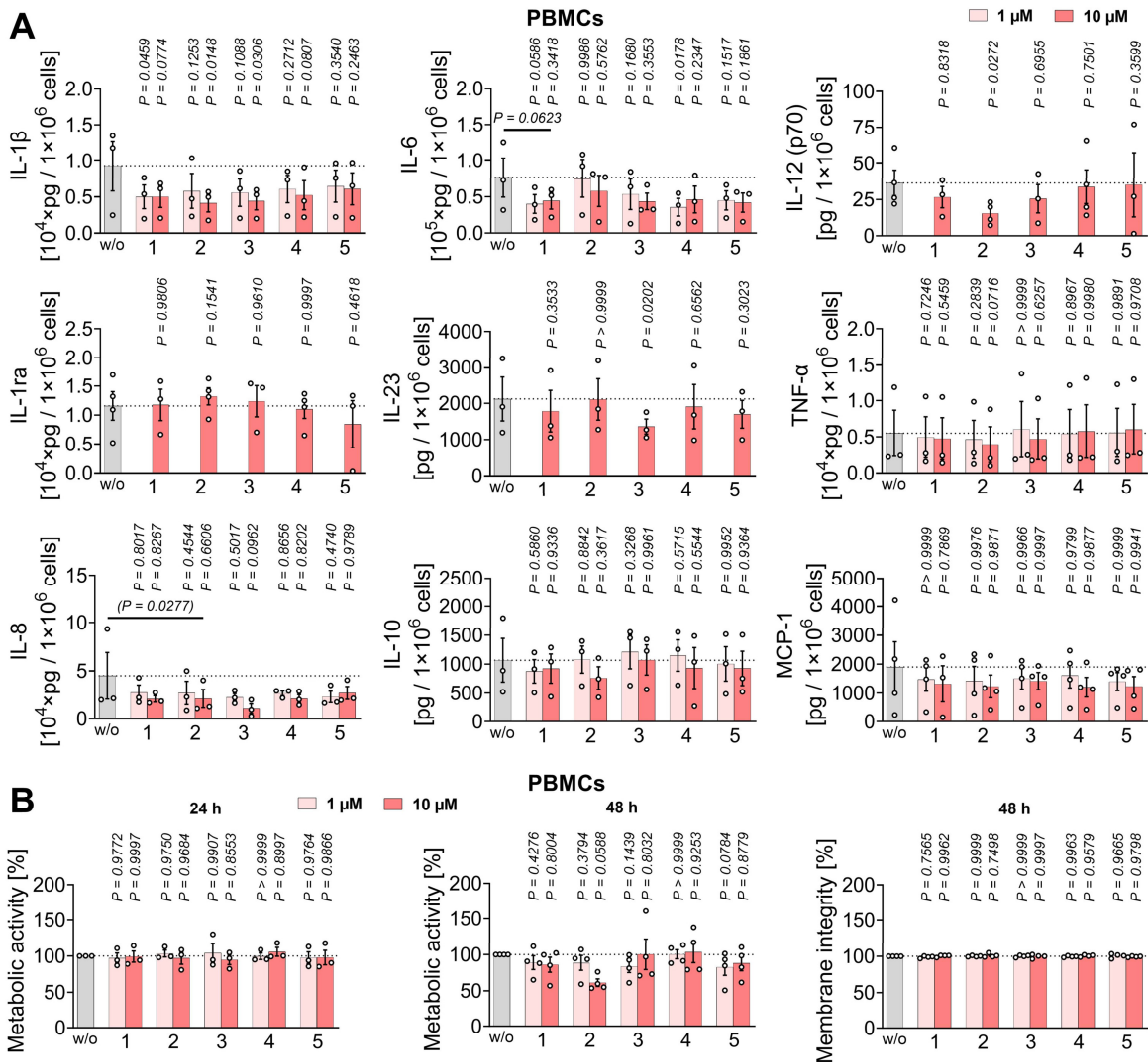
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## Supplementary tables

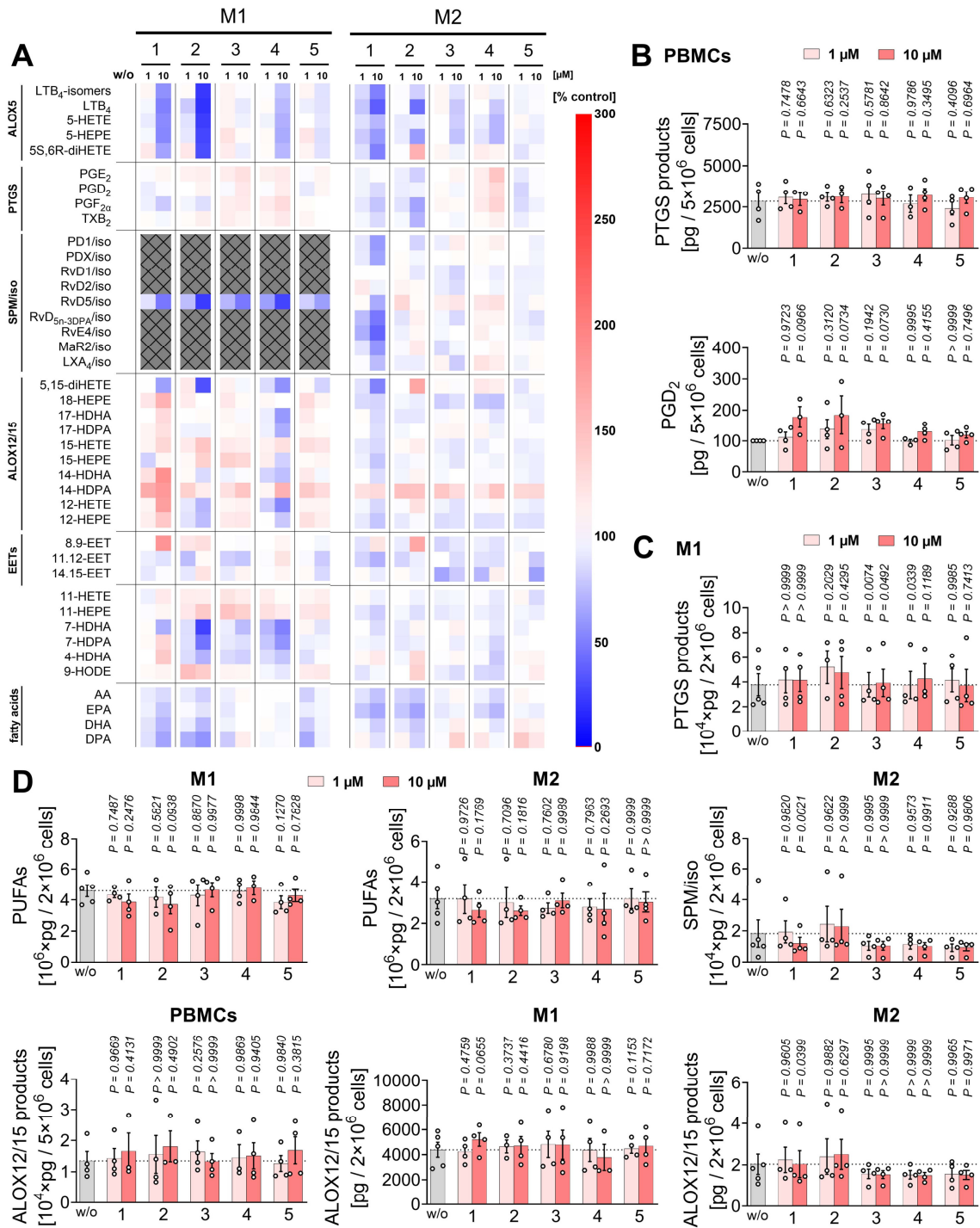
**Table S1. Human-specific primers for qPCR**

Gene	RefSeq-ID	Sequence (5' → 3')
FTH1	NM_002032.3	fwd: TGAAGCTGCAGAACCAACGAGG rev: GCACACTCCATTGCATTCAGCC
FTL	NM_000146.4	fwd: TACGAGCGTCTCCTGAAGATGC rev: GGTTCAGCTTTTTCTCCAGGGC
SLC40A1 (FPN1)	NM_014585.6	fwd: CTA CTTGGGGAGATCGGATGT rev: CTGGGCCACTTTAAGTCTAGC
SLC11A2 (DMT1)	NM_000617.3	fwd: AGCTCCACCATGACAGGAACCT rev: TGGCAATAGAGCGAGTCAGAACC
STEAP3	NM_018234.3	fwd: TGCAAACCTCGCTCAACTGGAGG rev: AGGCAGGTAGAACTTGTAGCGG
SLC25A37 (MFRN1)	NM_016612.4	fwd: CCACATGACAGCAGGAGCGATG rev: CTTGTGTACTGGGCTTTGGGATC
SLC25A28 (MFRN2)	NM_031212.4	fwd: AGCCACTGTCACCACGCACATG rev: GTCAGGCTGTAGACTCTGCATC
FECH	NM_000140.5	fwd: TCTTCTTGACCGAGACCTCATG rev: TCCAATCCTGCGGTACTGCTCT
GPX4	NM_002085.3	fwd: AGGCAAGACCGAAGTAAACTACAC rev: TACTACTCTTTCGTTACTCCCTGGCT
SLC7A11	NM_014331.3	fwd: TGCTCTTCTCTGGAGACCTCGAC rev: ACAGTGGCACCTTGAAAGGACG
PTGS2	NM_000963.4	fwd: GTTCCACCCGCAGTACAGAA rev: AGGGCTTCAGCATAAAGCGT
PRDX1	NM_002574.2	fwd: TCGCGAGATCCCTACTGGC rev: ACCAGTCCCAACACAAGTCG
PRDX6	NM_004905.3	fwd: CAGCTACCACTGGCAGGAAGT rev: GGAAGGACCATCACACTATCCC
CAT	NM_001752.4	fwd: GTGCGGAGATTCAACACTGCCA rev: CGGCAATGTTCTCACACAGACG
GCH1	NM_000161.3	fwd: GCCATGCAGTTCTTACCAAGG rev: ATGGAACCAAGTGATGCTCACAC
SQOR	NM_021199.4	fwd: GCTGACCTGATGCCTTTCCTGT rev: CCAGAAGCCATCCAAGATGAGC
POR	NM_001395413	fwd: ACTCTGCTCTCGTCAACCAGCT rev: TGGGTGCTTCTTGTGGACTCC
FSP1/AIFM1	NM_004208.4	fwd: GACTCCTTCCACCACAATGTGG rev: CAGCACCATCTGGTTCTTCAGG
ACSL4	NM_022977.3	fwd: GCTATCTCCTCAGACACACCGA rev: AGGTGCTCCAACTCTGCCAGTA
LPCAT3	NM_005768.6	fwd: CAGGATACCTGGTCTGCTTCCA rev: TGAAGAGCCAGTGGATGGTCTG
GAPDH	NM_002085.3	fwd: AGGCAAGACCGAAGTAAACTACAC rev: TACTACTCTTTCGTTACTCCCTGGCT

## Supplementary figures

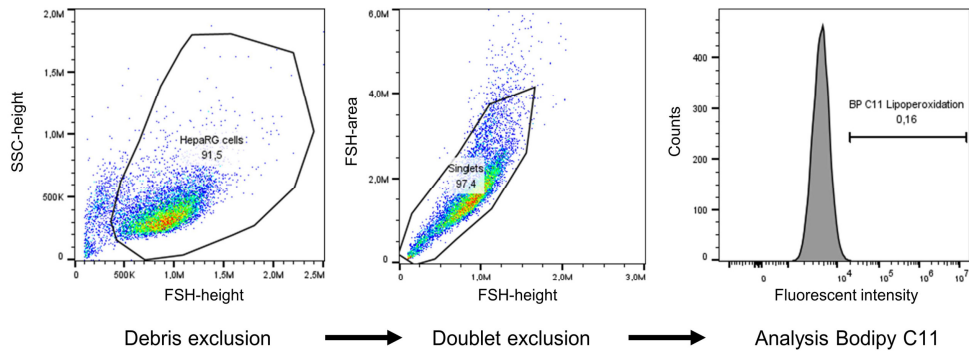


**Supplementary Figure S1. Effect of mitrephorones 1–5 on cytokine expression, metabolic activity, and membrane integrity of PBMCs.** (A) Cytokine and chemokine levels in PBMCs ( $1.4 \times 10^6$ ) preincubated with vehicle (DMSO, 0.1%) or mitrephorones for 30 min and stimulated with LPS for 4 h (TNF- $\alpha$ , IL-8) or 18 h (IL-1 $\beta$ , IL-6, IL-10, MCP-1, IL-12 (p70), IL-23, IL-1ra). (B) PBMCs ( $2 \times 10^5$  for metabolic activity;  $4 \times 10^6$  for membrane integrity) were preincubated with vehicle (DMSO, 0.5% for metabolic activity; 0.1% for membrane integrity) or mitrephorones for 24 or 48 h. Metabolic activity (MTT assay) and membrane integrity were determined. Mean  $\pm$  SEM and single data from  $n = 3-4$  independent experiments.  $P$  values given vs. vehicle control; repeated measures one-way ANOVA of log data (A, B) or mixed-effects model (REML) ANOVA of log data (A) + Dunnett's *post hoc* tests, or two-tailed paired Student's *t* test of log data for pairwise comparisons as indicated by bars (A). The vehicle controls shown in A and B are identical to those published previously [1].

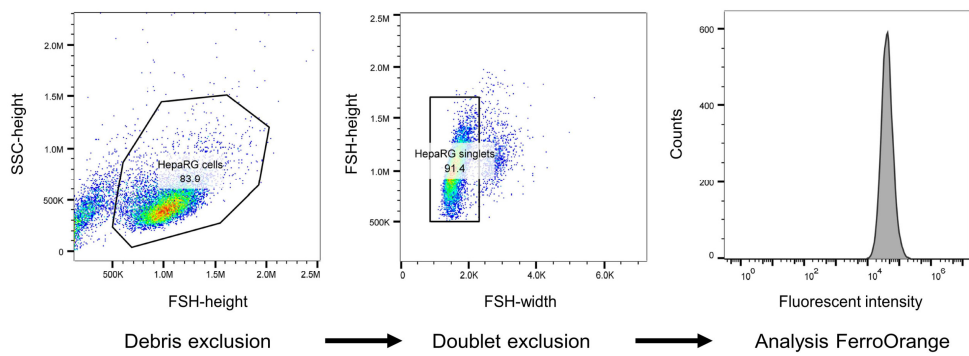


**Supplementary Figure S2. Effect of mitrephorones 1–5 on lipid mediator profiles of activated human PBMCs and macrophages.** (A, C, D) M1-like or M2-like macrophages ( $2 \times 10^6$ ) were preincubated with vehicle (DMSO, 0.1%) or mitrephorones for 15 min and stimulated with SACM (1%) for 180 min. (A) Heatmap of mean percentage changes in oxylipin and fatty acid levels relative to vehicle. Lipid mediators are grouped as ALOX5 products, PTGS products, SPM/iso, monohydroxylated ALOX12/15 products, EETs, and free PUFAs. Crossed boxes indicate lipid mediators that were not detected. (B, D) PBMCs ( $5 \times 10^6$ ) were preincubated with vehicle (DMSO, 0.1%) or mitrephorones for 10 min and treated with A23187 for 10 min. (B) Levels of PTGS products and PGD<sub>2</sub> in PBMCs. (C) PTGS product formation in M1-like macrophages. (D) Levels of PUFAs, ALOX12/15 products, and SPM/iso. Mean (A) or mean  $\pm$  SEM and single data (B–D) from  $n = 3$ –4 independent experiments.  $P$  values given vs. vehicle control; mixed-effects model (REML) ANOVA of log data + Dunnett's *post hoc* tests. The vehicle controls shown in A–D are identical to those published previously [1].

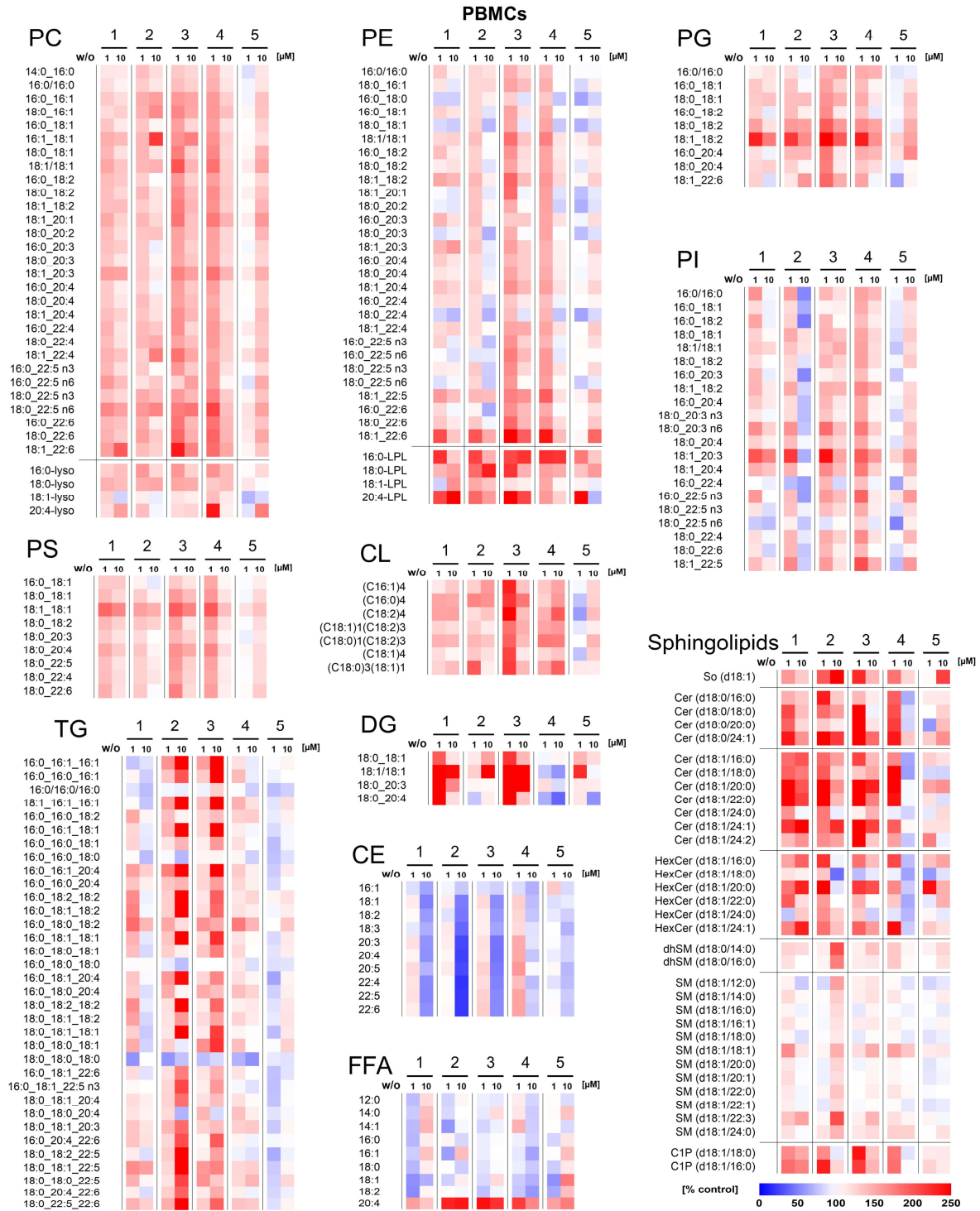
**A** Gating strategy for BODIPY-C11 staining



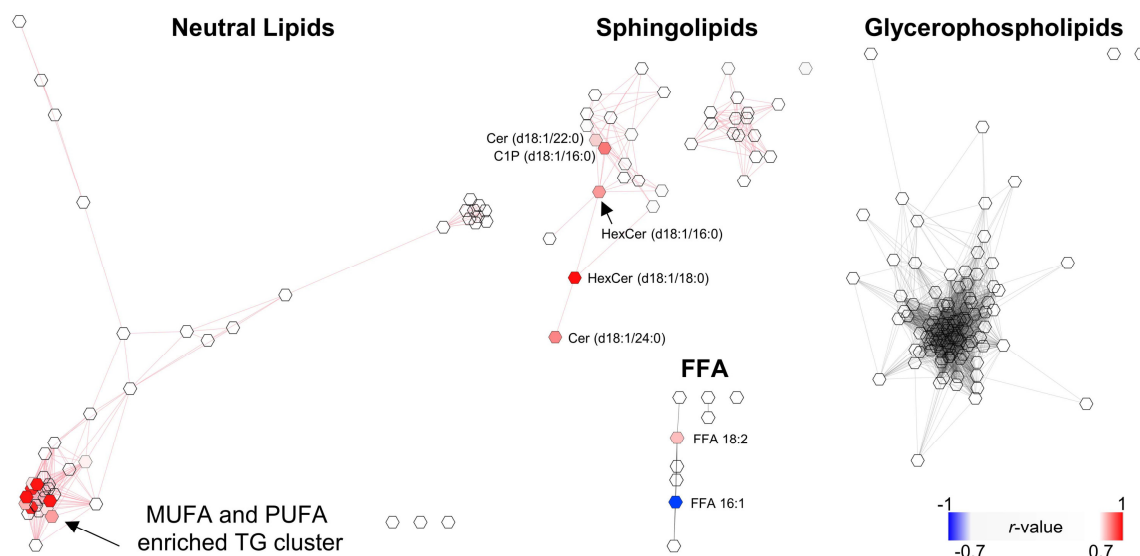
**B** Gating strategy for FerroOrange staining



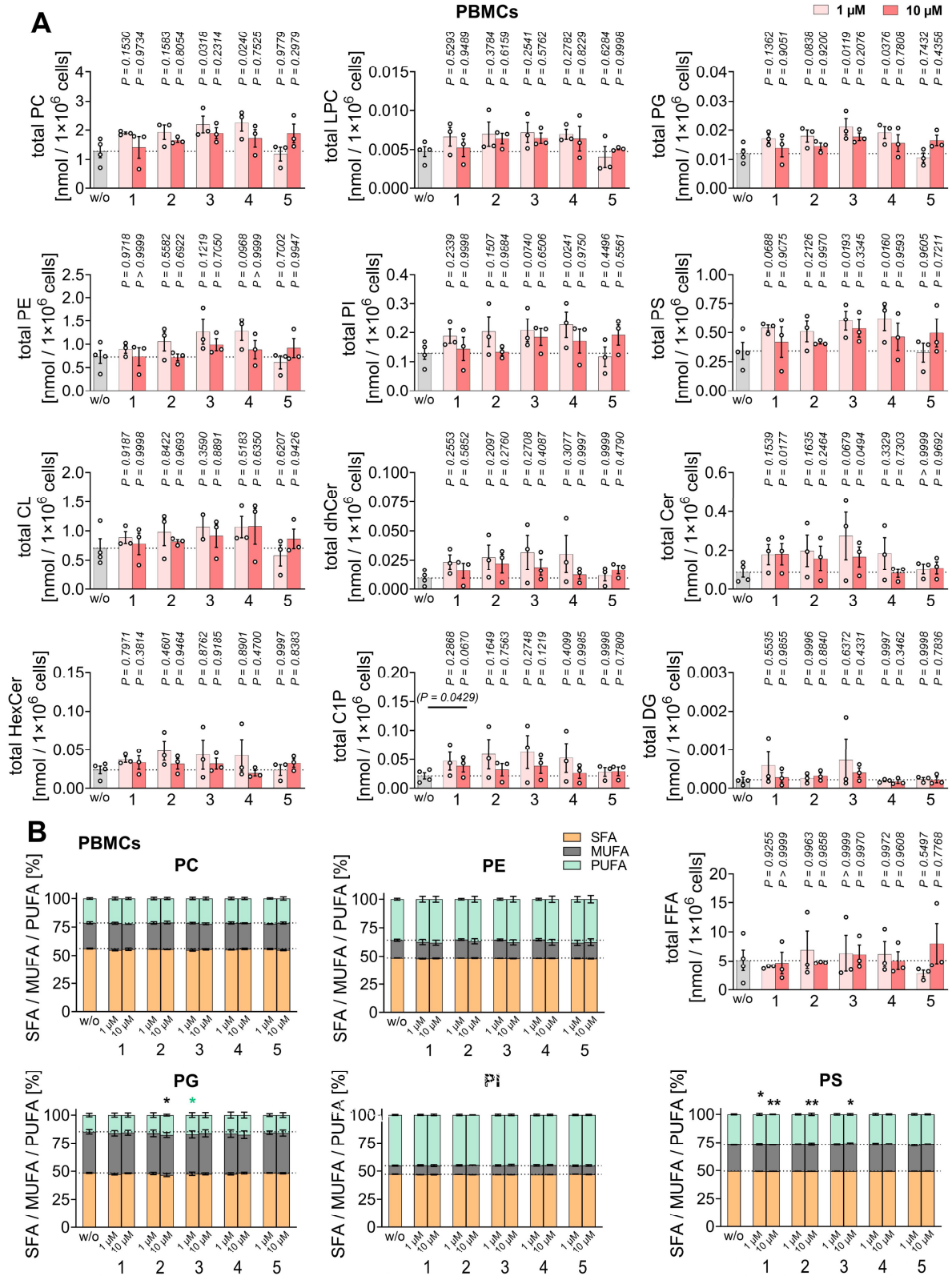
**Supplementary Figure S3. Gating strategies. (A) Figure 3A, B. (B) Figure 4B.**



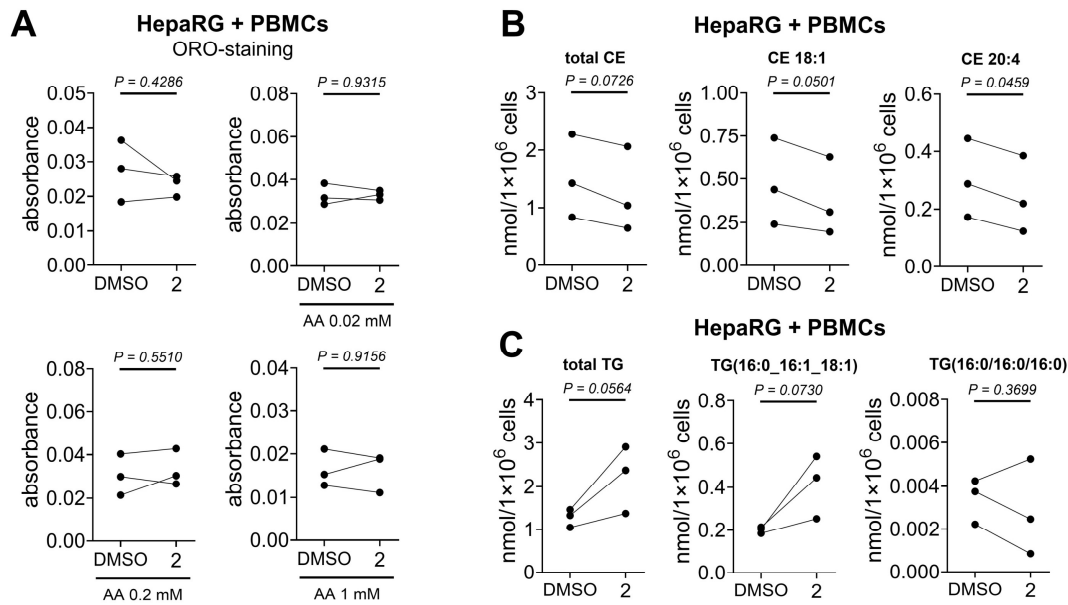
**Supplementary Figure S4. Effect of mitrephorones 1–5 on the lipid composition of human PBMCs.** Cells ( $4 \times 10^6$ ) were treated with vehicle (DMSO, 0.1%) or mitrephorones for 48 h. Heatmaps depict mean percentage changes in the amount of lipid species relative to vehicle. Mean from  $n = 2$  (CL 3 – 1  $\mu\text{M}$ ) or  $n = 3$  independent experiments. The vehicle controls shown are identical to those published previously [1].



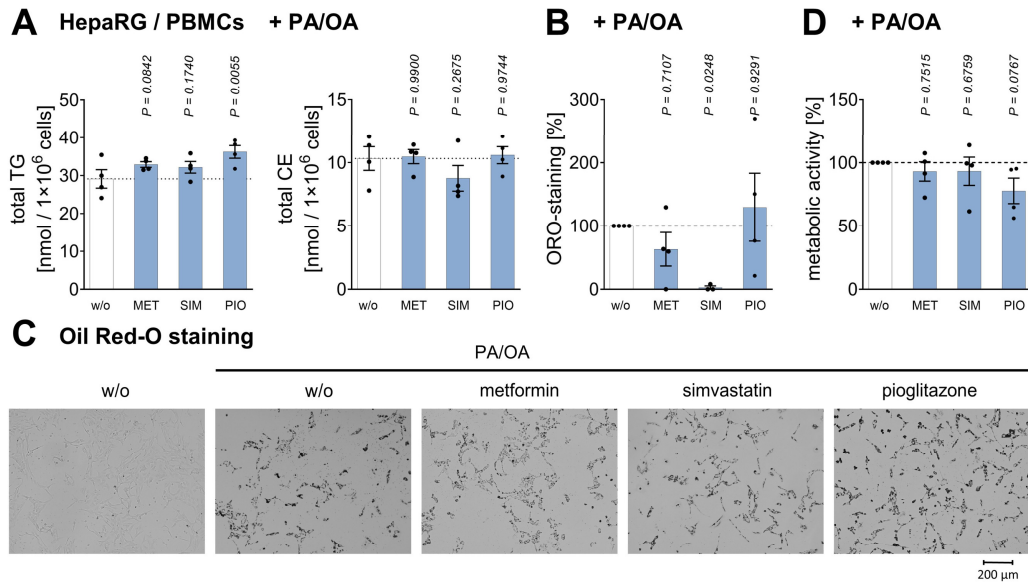
**Supplementary Figure S5. Correlation between 1-5-induced changes in PBMC relative lipid proportions and ferroptosis protection in hepatocytes.** Correlation-based lipid network showing positive lipid–lipid correlations ( $r \geq 0.7$ ) among neutral lipids, sphingolipids, glycerophospholipids, or free fatty acids (FFAs). Lipid species are represented as nodes (see Figure S4 for detailed information). The network combines data from PBMCs treated with compounds **1–5** (1 and 10  $\mu\text{M}$  each) for 48 h and was calculated from the mean percentage changes in relative lipid proportions relative to vehicle control from  $n = 2$  (CL **3** – 1  $\mu\text{M}$ ) or  $n = 3$  independent experiments. Node color indicates the strength and direction of Pearson correlations between mitrephorone **1–5** (1  $\mu\text{M}$ )-induced changes in the proportion of PBMC lipid species and the mean percentage changes in the metabolic activity of RSL3-treated HepaRG cells (Figure 2A) within 48 h relative to vehicle control: positive correlations ( $r \geq 0.7$ ) are shown in red, negative correlations ( $r \leq -0.7$ ) in blue;  $n = 2$  (CL **3** – 1  $\mu\text{M}$ ) or  $n = 3$  independent experiments.



**Supplementary Figure S6. Effect of mitrephorones 1-5 on the amount of lipid subclasses and the ratio of phospholipid-bound SFAs, MUFAs, and PUFAs in PBMCs.** Cells ( $4 \times 10^6$ ) were treated with vehicle (DMSO, 0.1%) or mitrephorones for 48 h. (A) Amounts of lipid subclasses in pg per  $1 \times 10^6$  cells. (B) Ratios of SFAs, MUFAs, and PUFAs in glycerophospholipids. Mean  $\pm$  SEM (B) and single data (A) from  $n = 2$  (A CL 3 – 1  $\mu\text{M}$ ) or  $n = 3$  (A, B) independent experiments.  $P$  values given vs. vehicle control (A) or  $*P < 0.05$  vs. vehicle control; mixed-effects model (REML) ANOVA (B) of log data (A) + Dunnett's *post hoc* tests. The vehicle controls shown are identical to those published previously [1].



**Supplementary Figure S7. Effect of mitrephorone 2 on lipid droplet staining and neutral lipid content in unchallenged and AA/20:4-challenged PBMCs.** HepaRG cells and PBMCs (1:1) were co-cultured and preincubated with or without AA/20:4 for 24 h. Cells were then treated with vehicle (DMSO, 0.1%) or **2** (10  $\mu$ M) for an additional 24 h. (A) Absorbance of organic extracts from Oil-Red-O (ORO)-stained cells. (B) Content of CE and selected CE species. (C) Content of TG and selected TG species. Interconnected single data from  $n = 3$  independent experiments.  $P$  values given vs. vehicle control; two-tailed paired Student's  $t$  tests of log data.



**Supplementary Figure S8. Effect of metformin, simvastatin, and pioglitazone on lipid overload in cocultures of hepatocytes and PBMCs.** HepaRG cells and PBMCs were co-cultured (1:1) and preincubated with PA/16:0 and OA/18:1 (1 mM) for 24 h. Cells were then treated with vehicle (DMSO, 0.1%) or metformin (1 mM), simvastatin (1 μM), or pioglitazone (100 μM) and incubated for another 24 h. (A) Total TG and CE content. (B) ORO staining – absorbance of organic extracts. (C) ORO staining – microscopic images. (D) Metabolic activity (MTT assay). Mean ± SEM and single data from  $n = 3-4$  independent experiments.  $P$  values given vs. vehicle control; repeated measures one-way ANOVA of log data + Dunnett's *post hoc* tests.

## References

1. Waltl L, Speck K, Wildermuth R, Haut F, Permann P, D'Avino D, et al. Reorganization of innate immune cell lipid profiles by bioinspired meroterpenoids to limit inflammation. bioRxiv. 2024: doi: <https://doi.org/10.1101/2024.05.24.595516>.