SI Appendix

Inhibition of Δ24-dehydrocholesterol-reductase activates pro-resolving lipid mediator biosynthesis and inflammation resolution

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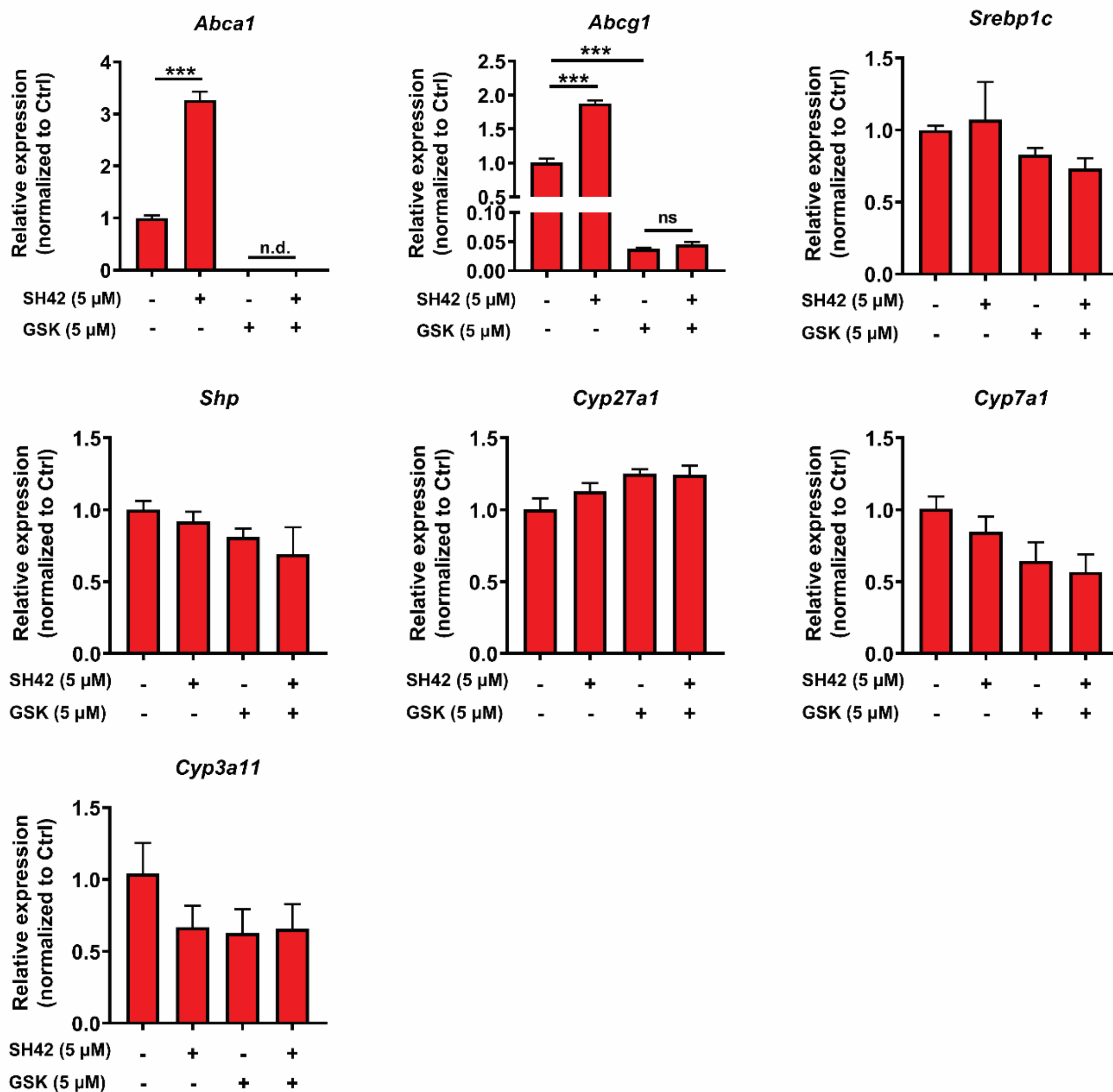
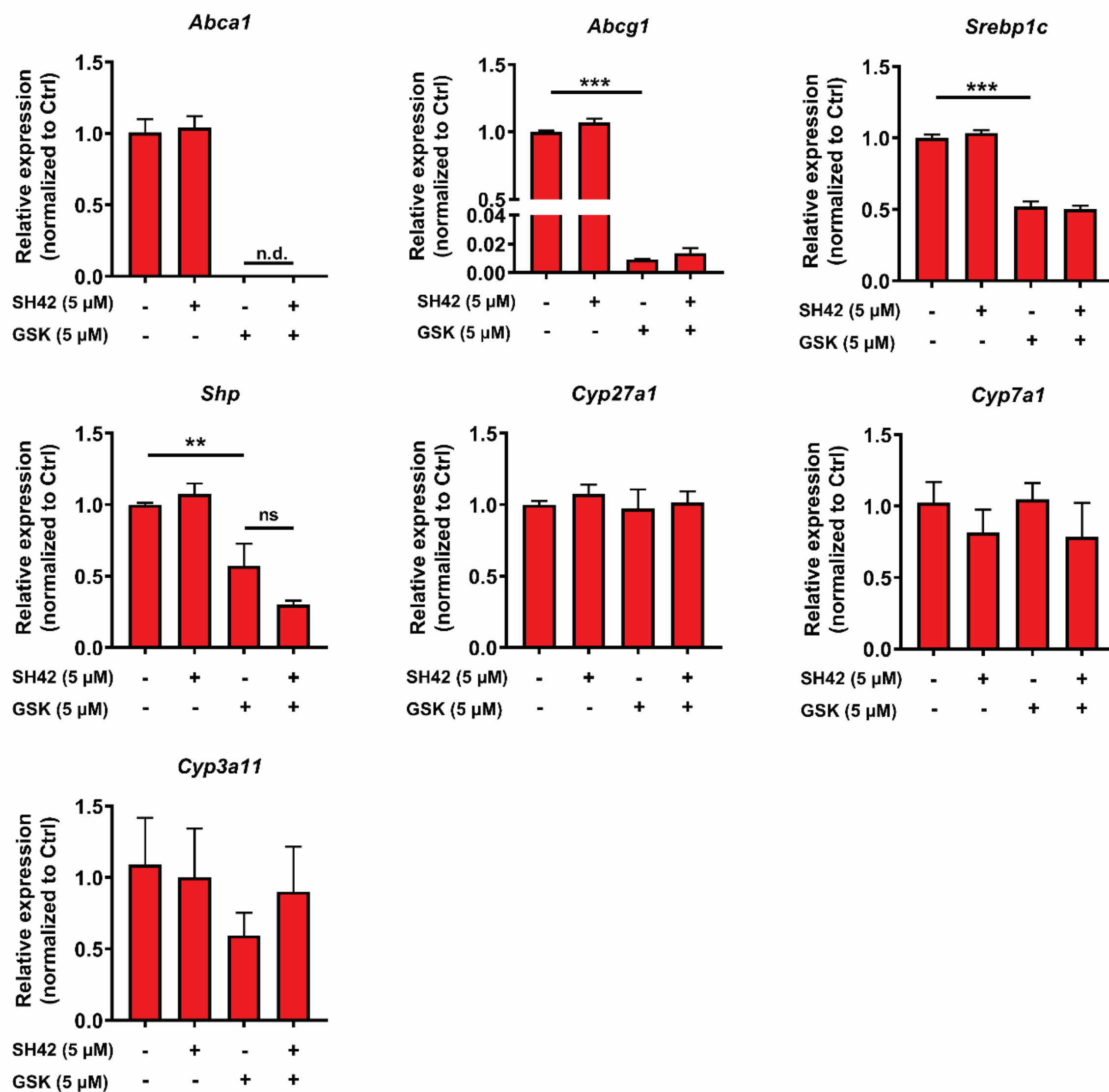
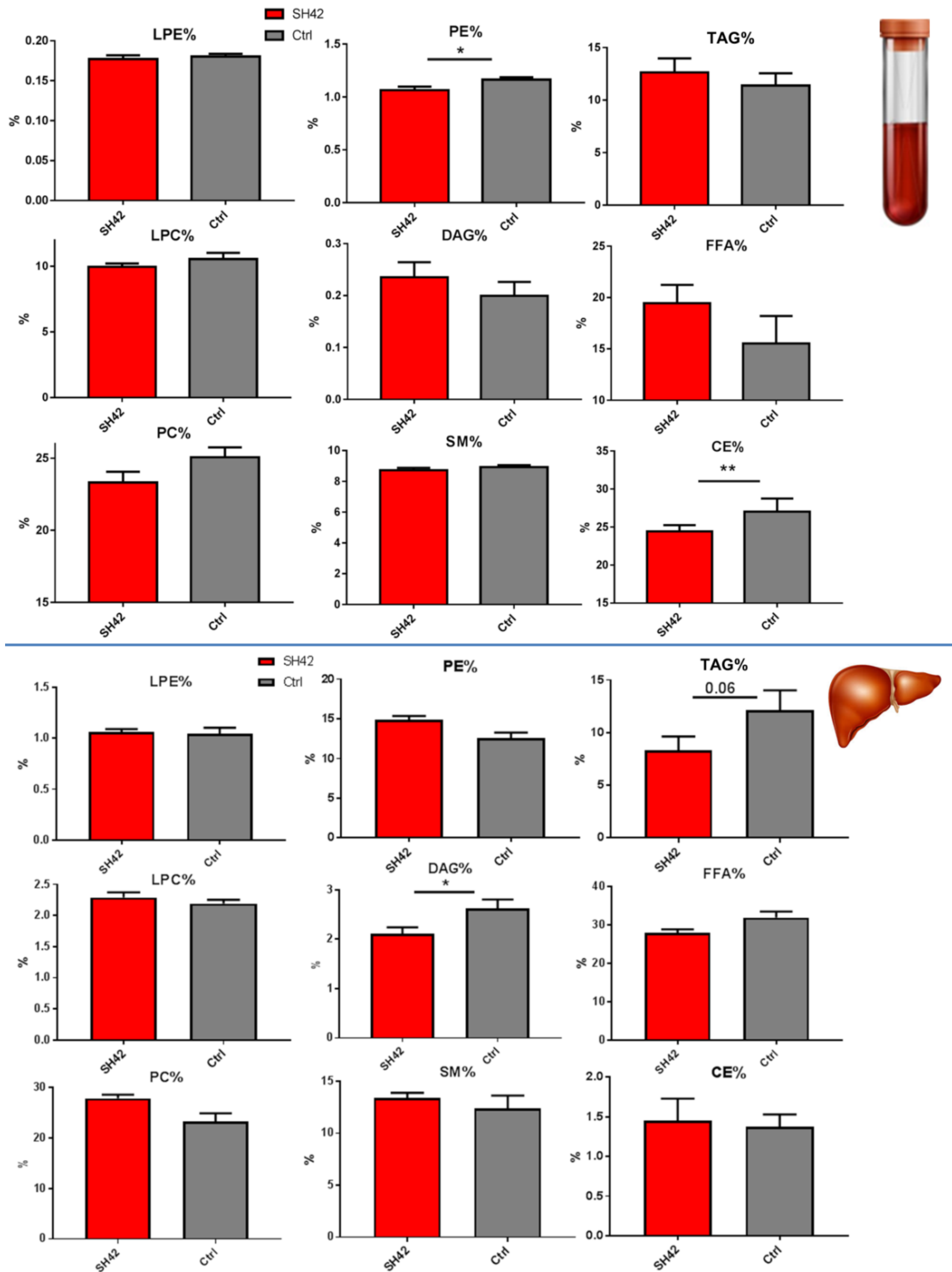
S1: **qPCR analysis of SH42/GSK2033 co-treatment and evaluation of additional transcription factor target genes without FCS being present (=active distal cholesterol biosynthesis).** n.d. not detected. Mean±SEM, non-parametric students t-test (Mann-Whitney, one per conditions against control). Inhibitor experiments with GSK2033 were carried out as follows: In the GSK 2033 treated groups, cells were pretreated with GSK 2033 (5 µM) for 2 hours before SH42 treatment. Cells were ultimately incubated with SH42 (5 µM). RNA was isolated using Tripure RNA Isolation reagent (Roche) according to the manufacturer's protocol. Total RNA (1 µg) was reverse transcribed using Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Promega) for RT-qPCR according to the manufacturer's instructions to produce cDNA. mRNA expressions were normalized to β-actin and Cyclophilin A mRNA expressions and expressed as fold change compared to vehicle-treated groups using the ΔΔCT method. Primer sequences can be found in **SI Appendix S11**. For further details please refer to the materials and methods section **LXR selectivity assessment**.



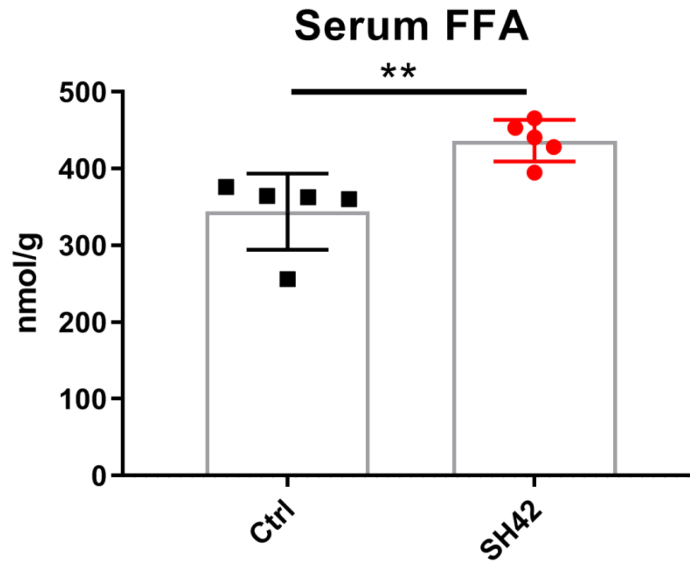
S2: **qPCR analysis of SH42/GSK2033 co-treatment and evaluation of additional transcription factor target genes with FCS being present (=inactive distal cholesterol biosynthesis), n.d. not detected.** Inhibitor experiments with GSK2033 were carried out as follows: In the GSK 2033 treated groups, cells were pretreated with GSK 2033 (5 µM) for 2 hours before SH42 treatment. Cells were ultimately incubated with SH42 (5 µM). RNA was isolated using Tripure RNA Isolation reagent (Roche) according to the manufacturer's protocol. Total RNA (1 µg) was reverse transcribed using Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Promega) for RT-qPCR according to the manufacturer's instructions to produce cDNA. mRNA expressions were normalized to β-actin and Cyclophilin A mRNA expressions and expressed as fold change compared to vehicle-treated groups using the ΔΔCT method. Primer sequences can be found in **SI Appendix S11**. For further details please refer to the materials and methods section **LXR selectivity assessment**.



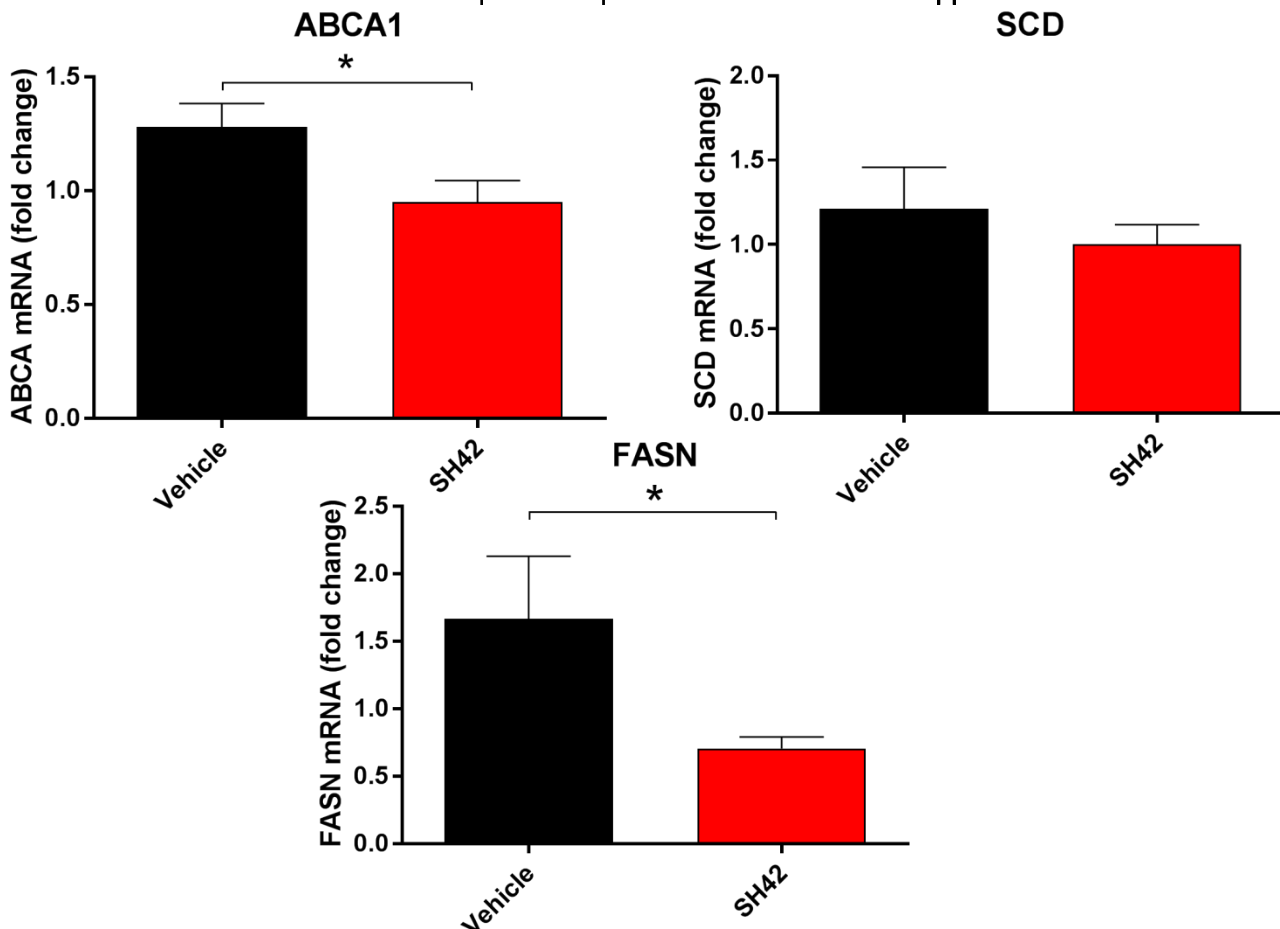
S3: Serum (above) and liver (below) lipid class composition obtained after three day treatment with 0.5 mg/d SH42 versus vehicle control (Ctrl). All experiments n=5, mean±SEM, non-parametric students t-test (Mann-Whitney), * p<0.05, **p<0.01. LPE lysophosphatidylethanolamine, LPC lysophosphatidylcholine, PC phospatidylcholine, PE phospatidylethanolamine, DAG diacylglyceride, SM sphingomyeloid, TAG triacylglyceride, FFA free fatty acid, CE cholesteryl ester. Lipid compositional analysis for the aforementioned lipid classes is shown. Lipidomics analysis was carried out as described in **SI Appendix S15.**



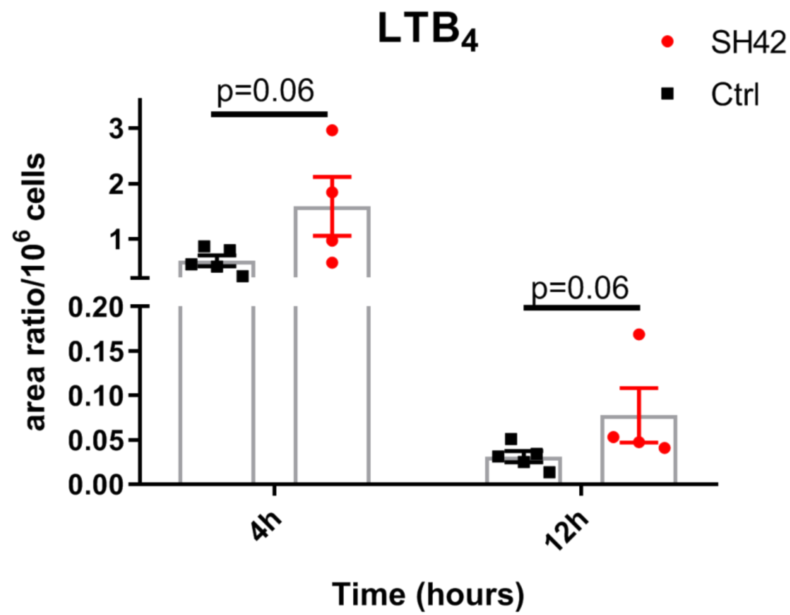
S4: Serum free fatty acid (FFA) concentration, ** p<0.01, mean±SEM, non-parametric students t-test (Mann-Whitney) n=5, FFA = free fatty acids. The serum FFA concentration as obtained by quantitative lipidomics analysis (**SI Appendix S15**) is shown. The absolute concentration of the FFA lipid class is shown as nmol/g serum.



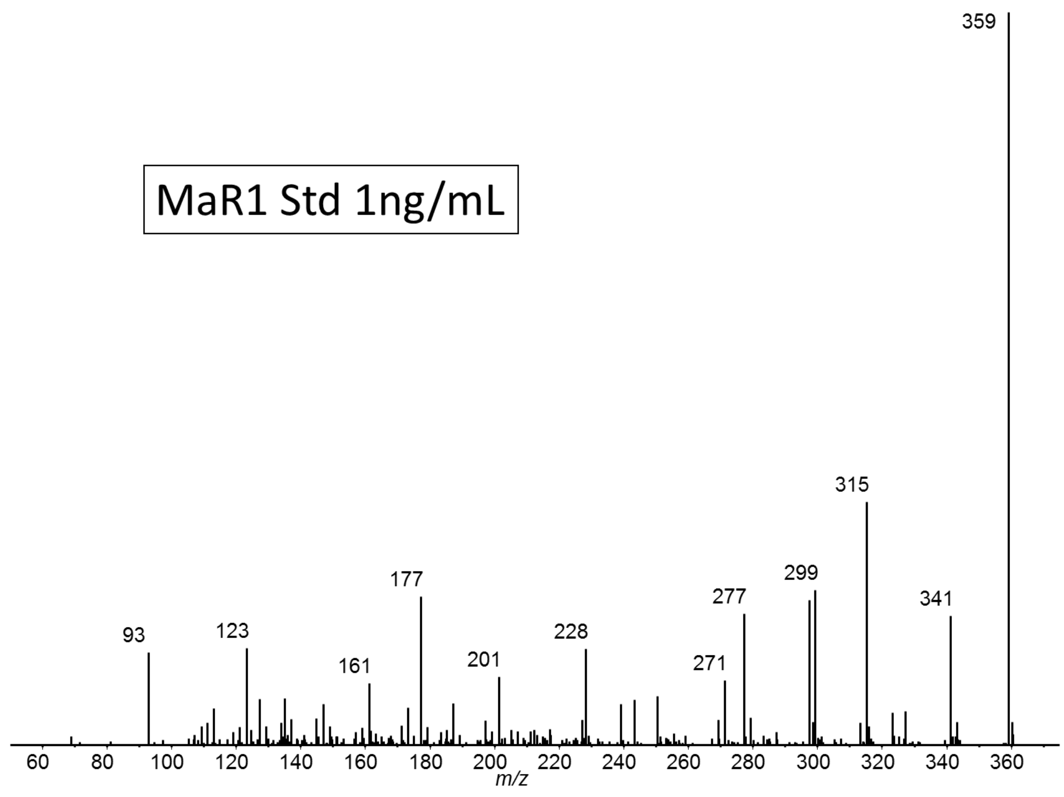
S5: qPCR analysis of liver genes comparing vehicle and 3 day SH42 treatment. All experiments n=5. non-parametric students t-test (Mann-Whitney), mean±SEM, * p<0.05. Murine liver samples were first carefully disrupted using a tissue homogenizer (Peqlab Precellys 24). Then, RNA was isolated using peqGOLD TriFast (VWR) and subsequently cDNA synthesized via reverse transcription using iScript™ cDNA Synthesis Kit (Bio-Rad). All procedures were performed according to the manufacturer's instructions. The primer sequences can be found in **SI Appendix S11.**

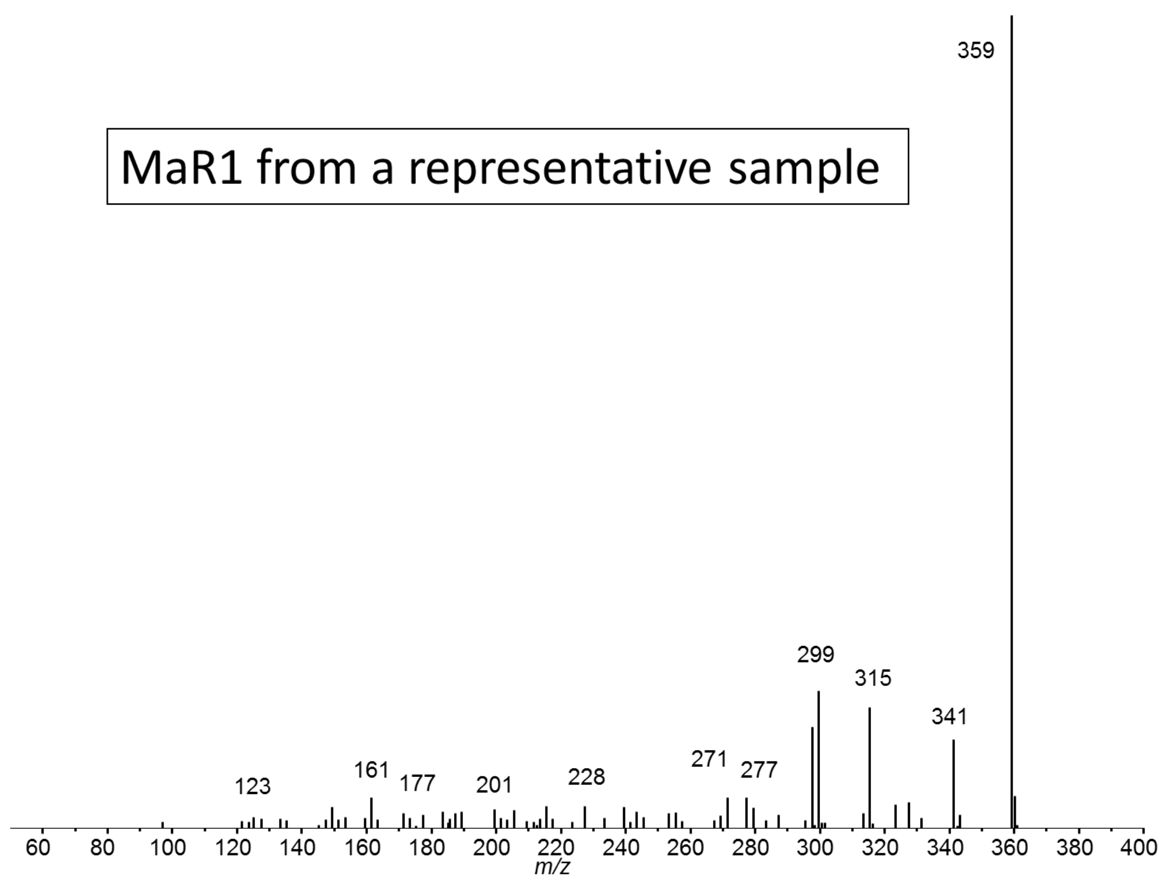


S6: LTB_4 analysis, non-parametric students t-test (Mann-Whitney), mean±SEM, n=4-5. LTB_4 was assessed using LC-MS analysis as described in **SI Appendix S16**. Mice were sacrificed 4 and 12 hours after zyA injection with and without SH42 treatment (0.5mg for 3 days). Area ratios corrected for the internal standard d4-LTB_4 and cell numbers are given.

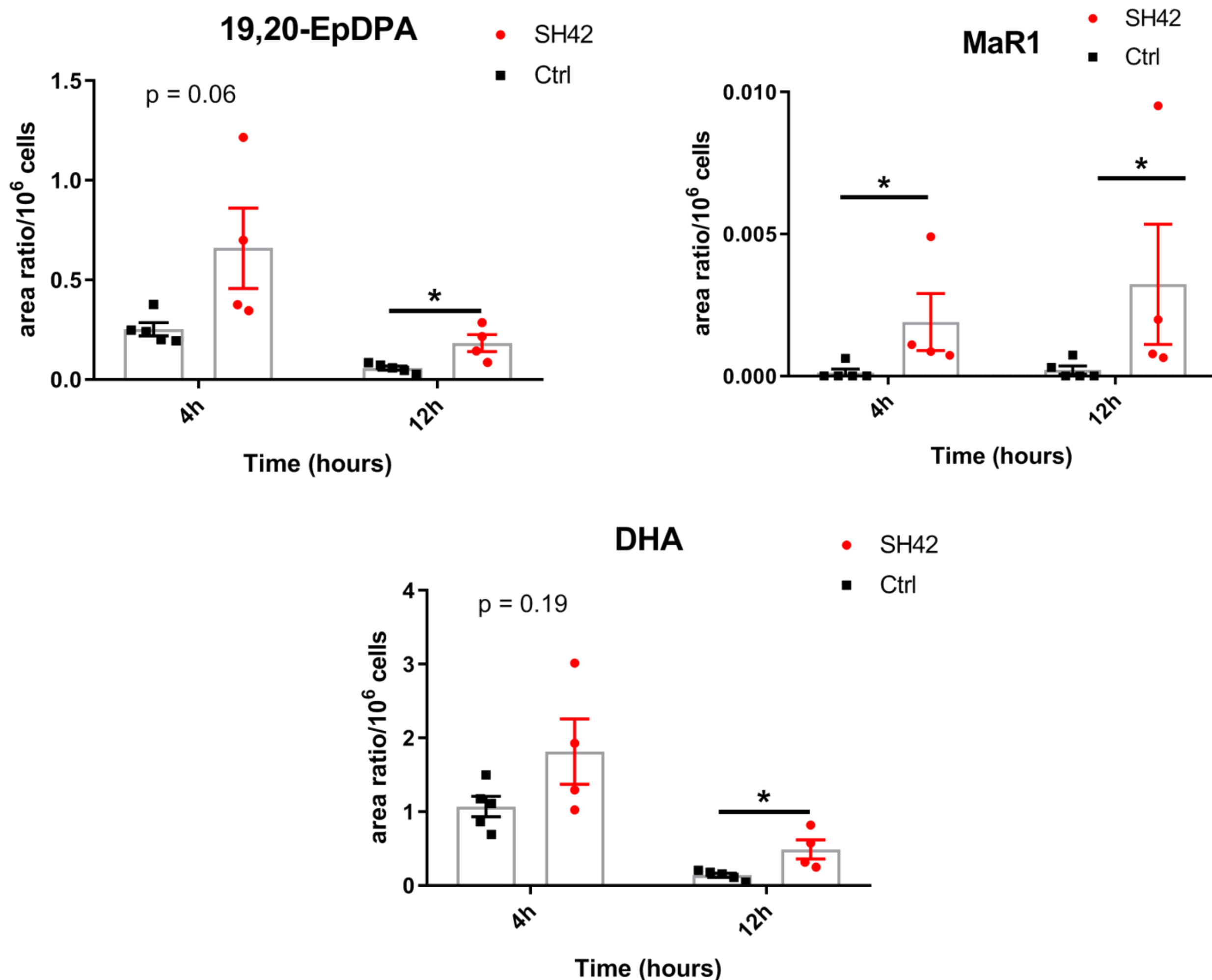


S7: Tandem MS spectra for MaR1 in comparison to authentic standard material.





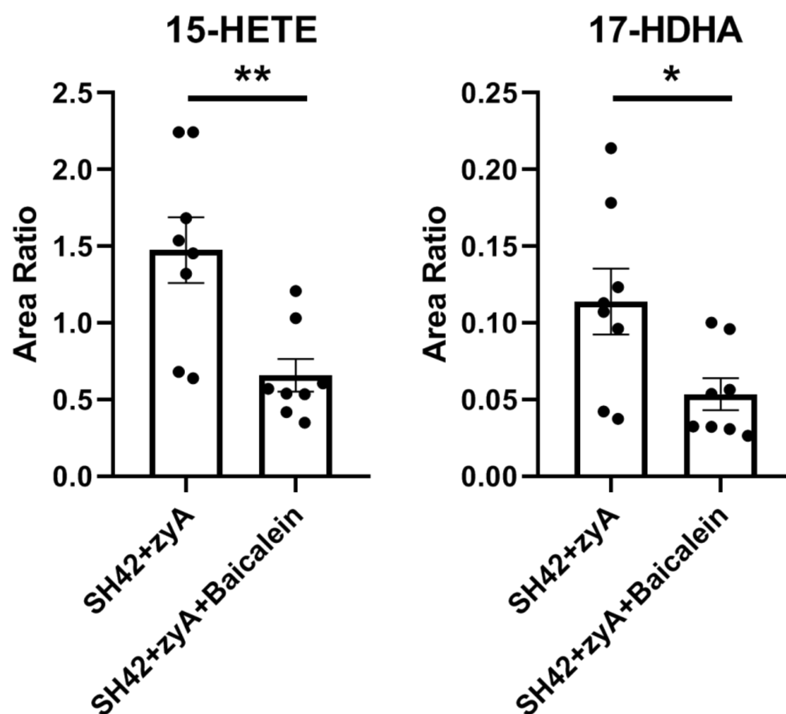
S8: Validation experiment for DHA, MaR1 and 19,20-EpDPA, non-parametric students t-test (Mann-Whitney), one per time point, mean±SEM, n=4-5, * p<0.05. Lipids were assessed using LC-MS analysis as described in **SI Appendix S16**. Mice were sacrificed 4 and 12 hours after zyA injection with and without SH42 treatment. Area ratios corrected for the internal standards d4-LTB_4 (19,29-EpDPA and MaR1) or d5-DHA (DHA) and cell numbers are given.



S9 Procedures used for cell shape analysis. Cell shape analysis has become an accepted parameter differentiating M1 and M2 MΦs (1). Image analysis was carried out as described elsewhere (2) and lined out below. Human peripheral blood monocytes (PBMCs) were isolated from human leukapheresis collars from the Blood Bank of Eberhard Karls University of Tübingen. Cells were then cultured in RPMI 1640 medium supplemented with either 10 ng/ml human recombinant GM-CSF (Macs Milteny) for M1 differentiation, 100 ng/ml M-CSF (Macs Milteny) for M2 differentiation, 0.01 µg/ml Desmosterol (Sigma Aldrich) or 250 µg/ml SH42 at 37°C for 7 d. The cell morphology was then analyzed by phase contrast images using a Leica inverted microscope equipped with a AxioCam MRc color camera (Zeiss) and measurements of cell shape, cell length and perimeter in each high-power

field (magnification ×200) performed with AxioVision software (Zeiss). Representative pictures are shown in Figure 8. The obtained data from n=80-90 cells for each condition is plotted in Figure 8.

S10 Baicalein blocks the SH42 induced accumulation of the 15-LOX pathway markers 15-HETE and 17-HDHA. M1 macrophages (5×10^6 cells) were treated with zyA and SH42 with and without 1 µg/mL Baicalein for 24 hours. N=8, mean±SEM, (4 biological (donors) and 2 technical repeats). Non parametric students t-test (Mann-Whitney), * p<0.05, ** p<0.01. 15-HETE and 17-HDHA were assessed using LC-MS analysis as described in **SI Appendix S16**. Area ratios corrected for the internal standard d8-15-HETE are shown.



S11 Primer sequences

Liver gene expression (Figure S5)

Gene	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
Abca1	GTGGGCTCCTCCCTGTTTTT	TCTGAGAAACACTGTCCTCCTTTT
SCD1	AGGATTTTCTACTACATGACCAGCG	CACGTCATTCTGGAACGCCA
FASN	GACTCGGCTACTGACACGAC	CGAGTTGAGCTGGGTTAGGG

Abca1, ATP-binding cassette sub-family A member 1; *SCD-1, Stearoyl-CoA desaturase-1*, *FASN* fatty acid synthase

LXR and LXR selectivity assessment

Transcription factor	Gene	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
LXR	*Abca1*	CCCAGAGCAAAAAGCGACTC	GGTCATCATCACTTTGGTCCTTG
LXR	Abcg1	AGGTCTCAGCCTTCTAAAGTTCCTC	TCTCTCGAAGTGAATGAAATTTATCG
	β-actin	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA
	Cyclophilin A	ACTGAATGGCTGGATGGCAA	TGTCCACAGTCGGAAATGGT
PXR	*Cyp3a11,*	CACAAACCGGAGGCCTTTTG	ATCCATGCTGTAGGCCCCAA
PXR; FXR	*Cyp7a1*	CAGGGAGATGCTCTGTGTTCA	AGGCATACATCCCTTCCGTGA
PXR; RXR	*Cyp27a1*	TCTGGCTACCTGCACTTCCT	CTGGATCTCTGGGCTCTTTG
FXR	*Shg*	TCTGCAGGTCGTCCGACTATT	TGTCTTGGCTAGGACATCCA
LXR	*Srebp1c*	AGCCGTGGTGAGAAGCGCAC	ACACCAGGTCCTTCAGTGATTTGCT

Abca1, ATP-binding cassette sub-family A member 1; *Abcg1*, ATP-binding cassette sub-family G member 1; *Srebp1c*, sterol regulatory element-binding protein 1 isoform c, *Cyp, cytochrome P*.

Peritoneal cell analysis

Gene	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
SCD-1	TCGGGATTTTCTACTACATGACCA	ACGTCATTCTGGAACGCCA
Elovl1	GCCTCGAATCATGGCTAATC	CCTTGGAAAGCATGAAGAGC
Elovl5	ATGGACACCTTTTTCTTCATCCTT	ATGGTAGCGTGGTGGTAGACATG
Elovl6	TCGAACTGGTGCTTACATGC	GAGCACAGTGATGTGGTGGT
Fads6	CAGCACATCGGACTGCCTAT	ACACCAAGGGCTTCACCTTC

SCD-1, Stearoyl-CoA desaturase-1*; Elovl1, 5, 6* Elongation of very long chain fatty acids protein 1, 5, 6; *Fads6,* fatty acid desaturase 6

Macrophage phenotype determination (human)

Gene	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
ALX/FPR2	TGT TCT GCG GAT CCT CCC ATT	CTC CCA TGG CCA TGG AGA CA
Arg1	TGG ACA GAC TAG GAA TTG GCA	CCA GTC CGT CAA CAT CAA AAC T
BAI1	AGC CAC TGA CAT CAG CTT CC	ATG CTT CAT CGG CCT CTG TC
CD36	CTTGGCTTAATGAGACTGGGAC	GCA ACA AAC ATC ACC ACA CCA
CD80	AGC CTC ACC TCT CCT GGT TG	TGG GGC AAA GCA GTA GGT CA
CD163	ACA ACA GGT CGC TCA TCC C	GTG TGG CTC AGA ATG GCC T
CD206	CCC TCA GAA AGT GAT GTG CCT	TCT CCA CGA AGC CAT TTG GT
ChemR23	AGG GAC TGA TTG GCT GAG GA	ATC CTC CAT TCT CAT TCA CCG T
CX3CR1	AAT GCC TGG CTG TCC TGT GT	GCC TGC TCC TTT GTG ATT CAG
GPR18	ACA GAA GTG GAA GTG CTG AAA A	CAG AAA GCA GCA CCT CCT GT
GPR32	GGG CCT GCA AAC TCT ACA	GGA GGC AGT TAC TGG CAA

IL-6	CAC CAG GCA AGT CTC CTC AT	GAC AGC CAC TCA CCT CTT CA
IL-10	AAT CGA TGA CAG CGC CGT AG	GGT TGC CAA GCC TTG TCT GA
STAT-1	ATC AGG CTC AGT CGG GGA ATA	TGG TCT CGT GTT CTC TGT TCT
TIM4	ACA GGA CAG ATG GAT GGA ATA CCC	AGC CTT GTG TGT TTC TGC G

FPR2, N-formyl peptide receptor 2; *Arg1*, Arginase 1; *STAT-1*, Signal transducer and activator of transcription 1; *TIM4,* T-cell immunoglobulin mucin protein 4.

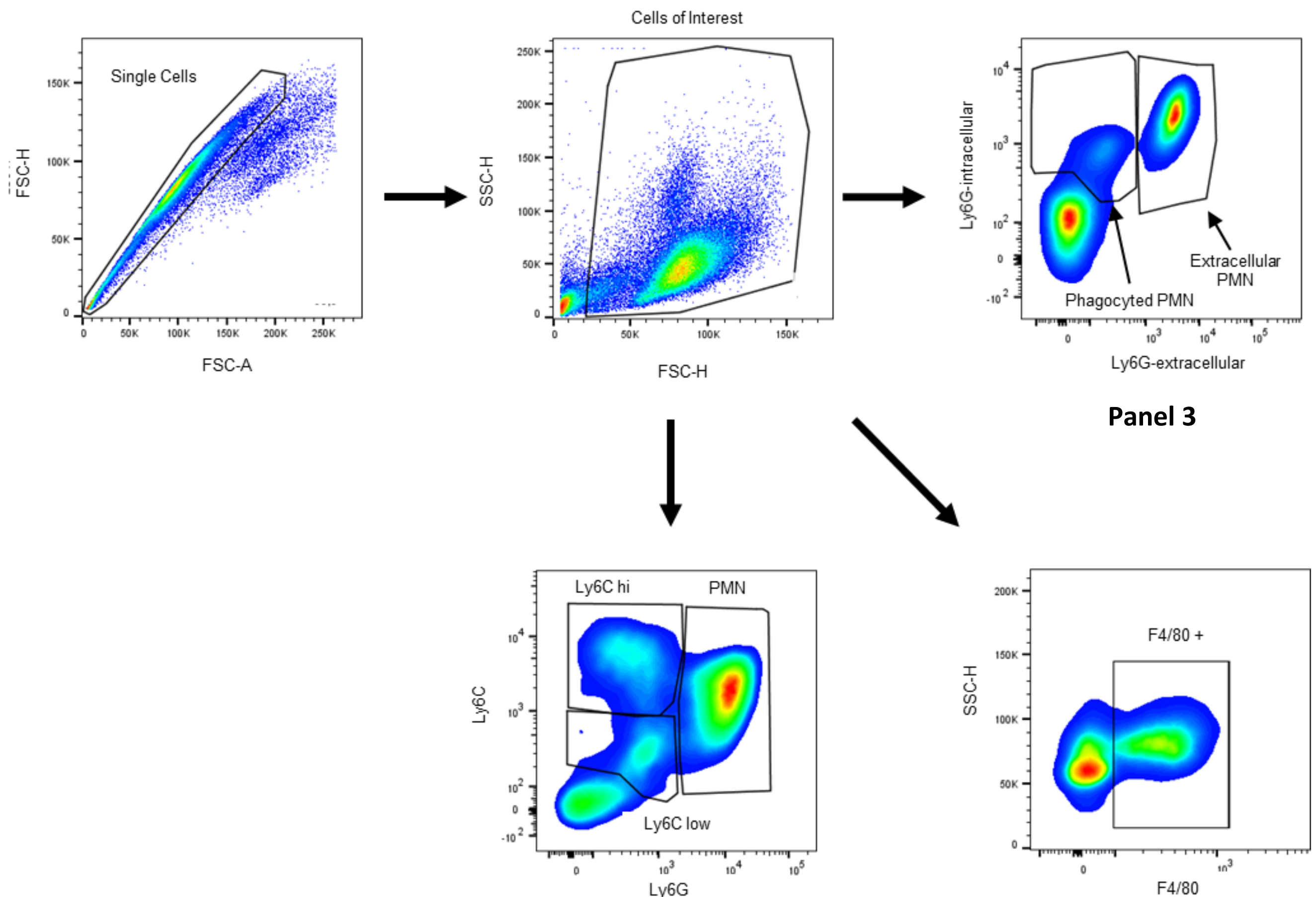
S12 GC-MS analysis.

We used a Bruker Scion GC/MS system, operated in scan mode from *m/z* 100-550. Helium 99.9990% was used as carrier gas at a constant flow rate of 1.4 mL/min. Injector and transfer line were operated at 280 °C. The oven program started at 90 °C held for 0.5 minute, then ramped to 270 °C at 30 °C/minute, ramped to 310 °C at 10 °C/minute. The following masses were used to quantify desmosterol levels against an external calibration line (0 – 10 ppm) *m/z* 445 + 355 for cholestanol (IS), *m/*z 357 and 271 for cholestan (IS) and *m/z* 343 + 253 for desmosterol.

Serum desmosterol levels were determined as described elsewhere (3). Liver desmosterol levels, were monitored as follows: livers samples were homogenized in a bullet blender after the addition of 500 µL LC-MS grade water for 2 min. To 100 µL of the homogenate was added 100 µL 10M NaOH and 10 µL of a cholestanol solution (100 µg/mL, serving as internal standard). The sample was hydrolysed for 30 minutes at 60°C. After the addition of 100 µL LC-MS grade water, lipids were extracted with 500 µL methyl-*tert*-butyl ether (MTBE). The organic extract was dried under a gentle stream of nitrogen and 29 µL MTBE, 20 µL MSTFA and 1 µL pyridine were added. After storing the sample for 30 minutes at room temperature, 1 µL was injected splitless. In the case of peritoneal cell desmosterol levels, cells were pelleted and washed once with PBS. The cell pellet was resuspended in 250 µL 2-propanol and washed into a glass vial. The tube was subsequently washed with another portion of 250 µL 2-propanol and the organic solvent was combined. After drying under a gentle stream of nitrogen 100 µL water and 50 µL 10 M NaOH and 10 µL cholestan solution (100 µg/mL, serving as internal standard) (cholestanol in the case of macrophages) were added to the lavage cells and hydrolysis was accomplished for 30 min at 60°C, lipids were extracted twice with 500 µL methyl-tert.-butyl ether (MTBE). The organic extract was dried under a gentle stream of nitrogen and 79 µL MTBE, 20 µL MSTFA and 1 µL pyridine were added. After storing the sample for 30 minutes at room temperature, 1 µL was injected splitless. For whole blood analysis after 1 or 2 days of SH42 treatment, 50 µL whole blood was spiked with 10 µL cholestan solution and 25 µL 10M NaOH and 100 µL water for hydrolyisis were added. Extraction and derivatization was carried out as described

for the lavage cells. For all experiments a freshly prepared calibration line with authentic desmosterol (0-10 ppm) was prepared on the day of analysis.

S13 Leukocyte differentiation and flow cytometry analysis (gating strategy shown below). Peritoneal lavages (4 mL in PBS(-/-) were taken at dedicated time points after zyA injection and prepared to determine the cellular distributions. First, a 2mL lavage aliquot was spun down (300xg, 5 min, 4°C) and the pellet resuspended in 300 µL of Flow Cytometry Staining Buffer (1x PBS(-/-), 1% BSA, 2 mM NaN3)(FCSB) and 100 µL sample volume was then transferred to a 96 well plate (round bottom). Antibodies were diluted with FCSB before use (see materials and methods section **Zymosan A induced peritonitis and FACS analysis**). For differential cell count cells were blocked with mouse anti-CD16/CD32 antibodies (Biolegend) for 10 min at room temperature. Then, cells were stained with anti-mouse APC-Ly6G, e450-F4/80 (eBioscience) and FITC-Ly6C (BioLegend) antibodies for 30 min at 4°C. For defining MΦ efferocytosis of apoptotic PMNs, the differentiation of intra- and extracellular PMN was assessed by using differential Ly6G-staining with a permeabilization step. Cells were permeabilized using a fixation and permeabiliziation kit (BD) according to the manufacturer's instructions. After this step, the second Ly6G staining (Ly6G intracellular) was performed to also determine phagocytized apoptotic PMN. Phagocytized PMN were Ly6G intracellular positive (+) and Ly6G extracellular negative (-) (Panel 3). The cells were analyzed by flow cytometry on a FACSCanto II (BD) using FlowJo (Tree Star Inc.). For further details please also refer to the materials and methods section **Zymosan A induced peritonitis and FACS analysis**.



S14 Protein array analysis

Average signal intensity of 6 replicates was normalized to the median signal of all antibodies on the array. The presented fold change represents the ratio of the normalized signal from SH42 treated mice compared with vehicle treated controls. GAPDH and beta actin were used as housekeeping proteins. Data analysis was performed with IPA software (Qiagen). Pathways were substantiated and updated with recent literature.

S15 Quantitative lipidomics analysis

Briefly: for liver samples, one liver lob was homogenized in an Eppendorf tube with 600 µL LCMS grade water and pre-washed stainless steel beads. An aliquot corresponding to approximately 2 mg liver tissue was worked up. For serum samples, 100 µL was worked up. To the samples in 2 mL Eppendorf tubes was added 100 µL internal standard mix (Lipidyzer kit) in MTBE. Subsequently 500 µL MTBE and 160 µL methanol were added, the mixture was shaken for 30 minutes at room temperature, before 200 µL of water was added. The samples were spun at 16,100 ×g for 3 minutes

and the upper organic layer transferred to a glass vial. The remaining sample was extracted again by the addition of 300 µL MTBE, 100 µL methanol and 100 µL for 30 minutes. After centrifugation the organic extracts were combined and dried under a gentle stream of nitrogen. The dry extract was dissolved in 250 µL Lipidyzer running buffer and analysed according to the manufacturers protocol (4, 5). For data analysis the Excel spread sheets (**SI datasets S1, S2 and S3**) were transformed into comma separated files (.csv). FA composition analysis calculates the total of a given FA within a class divided by the total of all the FA within that class. In this way a FA fractional analysis for each class of analysed lipids has been carried out. For further analysis volcano plots were built in order to visualize the different FA compositions of liver, serum and peritoneal cells. For this, missing values in each dataset were imputed with half of the minimum concentration value and all determined fractional values were centred around the mean value of each analyte. For the volcano plot we set the fold change threshold to 1.5 and the p-value threshold to 5%, and the control group was considered as the reference in all comparisons.

S16 Targeted lipid mediator analysis

Peritoneal lavage samples were spiked with 4 µL of an internal standard solution (containing d4-PGE4, d4-LTB4 d8-15-HETE and d5-DHA at a concentration of 50 ng/ml (500ng/mL for d5-DHA) in methanol). The samples were transferred to a 12-ml glass vial, and 1.75 ml of methanol was added. The samples were centrifuged at 4,000 rpm for 5 min at 6 °C, and the supernatant was transferred to a fresh 12-ml glass vial. The pellet was re-extracted with 500 µl of methanol and centrifuged as described above, and the organic extracts were combined. The methanol was partially removed under a gentle stream of nitrogen at 40 °C for 30 min. The remaining methanolic extract (approximately 1.5 ml) was diluted with 8 mL of water, and 10 µL of 6 M HCl was added. The prepared samples were cleaned via solid phase extraction (SPE) (SepPak C18 200 mg, Waters, MA, USA). The samples were loaded onto preconditioned SPE cartridges (2 ml methanol, followed by 2 ml water), the cartridges were washed with 3 ml of water followed by 3 ml of n-hexane, and then the samples were eluted with 3 ml of methylformate. The eluate was dried under a gentle stream of nitrogen, reconstituted in 200 µL of 40% methanol, and injected.

LC-MS/MS analysis was performed as described below. Briefly, a QTrap 6500 mass spectrometer operating in negative ESI mode (Sciex, Nieuwerkerk aan den Ijssel, The Netherlands) was coupled to an LC system employing two LC-30AD pumps, a SIL-30AC autosampler, and a CTO-20AC column oven

(Shimadzu,’s-Hertogenbosch, The Netherlands). A 1.7 µm Kinetex C18 50 × 2.1 mm column protected with a C8 precolumn (Phenomenex, Utrecht, The Netherlands) was used, and the column was maintained at 50 °C. A binary gradient of water (A) and MeOH (B) containing 0.01% acetic acid was generated as follows: 0 min 30% B, held for 1 min, then ramped to 45% B at 1.1 min, 53.5% B at 2 min, 55.5% B at 4 min, 90% B at 7 min, and 100% B at 7.1 min, and held for 1.9 min. The injection volume was 40 µl, and the flow rate was 400 µL/min. For analyte identification, the mass transition used for each analyte was combined with its relative retention time (RRT). The calibration lines constructed with standard material for each analyte were used for quantification, and only peaks with a signal to noise (S/N) ratio > 10 were quantified

In the case of treated M1 MΦ, quenched cells in 1.5 mL methanol were spun for 5 min at 4000 ×g and the supernatant transferred to a 1.5 mL glass vial. After spiking samples to a final concentration of 1 ng/mL (d4-PGE2, d4-LTB4 and d8-15HETE) and 10 ng/mL (d5-DHA) with internal standards, samples were dried under a gentle stream of nitrogen, resuspended in 150 µL 40% methanol, spun again for 3 min at 18.000 ×g and transferred to micro vial inserts for LC-MS/MS analysis.

Please refer to **Table 1** below for further details.

Table S1 LC-MS/MS settings

Group	Compound	Absolute Quantitation	Lipid Maps ID	Retention time [min]	m/z in Q1	m/z in Q3	Declustering potential [V]	Collision energy [V]	Collision cell exit potential [V]
Epoxydocosapentaenoic acids	19(20)-EpDPA		LMFA04000038	8.1	343.1	281.1	-70	-16	-11
Epoxyeicosatrienoic acids (EET)	11(12)-EET		LMFA03080004	8.2	318.9	166.9	-90	-18	-19
Epoxyeicosatrienoic acids (EET)	14(15)-EET		LMFA03080005	8.1	319	218.9	-5	-16	-55
Epoxyeicosatrienoic acids (EET)	8(9)-EET		LMFA03080003	8.2	319	154.9	-60	-18	-13
Hydroxydocosahexaenoic acids (HDHA)	10-HDHA	Yes	LMFA04000027	7.9	343.1	153	-25	-20	-15
Hydroxydocosahexaenoic acids (HDHA)	14(S)-HDHA		LMFA04000058	8.0	343.1	204.9	-60	-18	-27
Hydroxydocosahexaenoic acids (HDHA)	17-HDHA	Yes	LMFA04000072	7.9	343.1	245	-65	-16	-15
Hydroxydocosahexaenoic acids (HDHA)	4-HDHA		LMFA04000058	8.2	343.1	101	-50	-18	-9
Hydroxydocosahexaenoic acids (HDHA)	7-HDHA	Yes	LMFA04000025	8.0	343.1	141.1	-85	-18	-23
Hydroxydocosapentaenoic acids (HDPA)	19,20-DiHDPA	Yes	LMFA04000043	7.4	361.1	273	-55	-22	-15
Hydroxydocosapentaenoic acids (HDPA)	7,17-DiHDPA	Yes	N/A	7.0	361.1	198.9	-45	-26	-23
Hydroxyeicosapentaenoic acids (HEPE)	12-HEPE		LMFA03070031	7.6	317	179	-60	-18	-17
Hydroxyeicosapentaenoic acids (HEPE)	15-HEPE	Yes	LMFA03070009	7.5	317.1	219	-65	-18	-19
Hydroxyeicosapentaenoic acids (HEPE)	18-HEPE	Yes	LMFA03070038	7.4	317.1	259	-5	-16	-7
Hydroxyeicosapentaenoic acids (HEPE)	5-HEPE		LMFA03070027	7.7	317	114.9	-55	-18	-11
Hydroxyeicosatetraenoic acids (HETE)	11-HETE	Yes	LMFA03060003	7.9	319.1	167	-70	-22	-15
Hydroxyeicosatetraenoic	12-HETE	Yes	LMFA03060007	7.9	319.1	179	-65	-20	-23

acids (HETE)									
Hydroxyeicosatetraenoic acids (HETE)	14,15-diHETE	Yes	LMFA03060077	7.0	335.1	207	-65	-24	-21
Hydroxyeicosatetraenoic acids (HETE)	15-HETE	Yes	LMFA03060001	7.8	319.1	219.1	-55	-18	-9
Hydroxydocosatetraenoic acids	17-OH-DH-HETE		N/A	8.2	347.1	247	-110	-22	-27
Hydroxyeicosatetraenoic acids (HETE)	20-HETE		LMFA03060009	7.7	319	289.1	-70	-24	-15
Hydroxyeicosatetraenoic acids (HETE)	5,15-diHETE	Yes	LMFA03060010	6.8	335	173.1	-55	-20	-11
Hydroxyeicosatetraenoic acids (HETE)	5-HETE	Yes	LMFA03060002	8.0	319.1	115	-65	-18	-11
Hydroxyeicosatetraenoic acids (HETE)	8(S),15(S)-diHETE	Yes	LMFA03060050	6.7	335.1	207.9	-55	-22	-17
Hydroxyeicosatetraenoic acids (HETE)	8-HETE	Yes	LMFA03060006	7.9	319.1	154.9	-70	-20	-19
Hydroxyoctadecadienoic acids (HoDE)	13-HoDE		LMFA02000228	7.7	295	194.9	-110	-24	-21
Hydroxyoctadecadienoic acids (HoDE)	9-HoDE		LMFA02000188	7.7	295	171	-130	-22	-7
Hydroxyoctadecatrienoic acids (HoTrE)	13-HoTrE		LMFA02000051	7.4	293	195	-45	-24	-19
Hydroxyoctadecatrienoic acids (HoTrE)	9-HoTrE		LMFA02000024	7.4	293	170.9	-75	-20	-15
Internal standards	15-HETE-d8		LMFA03060080	7.8	327.2	226	-85	-18	-11
Internal standards	DHA-d5		LMFA01030762	8.8	332	288.1	-75	-16	-13
Internal standards	LTB4-d4		LMFA03020030	6.9	339.1	196.9	-70	-22	-19
Internal standards	PGE2-d4		LMFA03010008	4.9	355.1	193	-50	-26	-17
Isoprostanes (IsoP) and neuroprostanes (NeuroP)	17-F2t-dihomo-IsoP		LMFA03110167	6.1	381.1	318.9	-115	-32	-41
Isoprostanes (IsoP) and neuroprostanes (NeuroP)	4-F4t-NeuroP		N/A	5.2	377.1	270.9	-15	-26	-13
Isoprostanes (IsoP) and neuroprostanes (NeuroP)	5-F3t-IsoP		N/A	3.8	351	114.8	-95	-26	-13
Keto-eicosatetraenoic acids (KETE/OxoETE)	12-KETE		LMFA03060019	7.9	317	153	-60	-22	-9
Keto-eicosatetraenoic acids (KETE/OxoETE)	15-KETE		LMFA03060051	7.8	317	113	-10	-22	-5
Keto-eicosatetraenoic acids (KETE/OxoETE)	5-KETE		LMFA03060011	8.1	317	203.1	-70	-24	-11
Leukotrienes (LT)	20-OH-LTB4	Yes	LMFA03020018	4.0	351.1	195	-60	-24	-17
Leukotrienes (LT)	6-trans-12-epi-LTB4	Yes	LMFA03020014	6.8	335.1	194.9	-80	-22	-25
Leukotrienes (LT)	6-trans-LTB4	Yes	LMFA03020013	6.7	335.1	194.9	-105	-22	-11
Leukotrienes (LT)	LTB4	Yes	LMFA03020001	6.9	335.1	195	-65	-22	-21
Leukotrienes (LT)	LTD4	Yes	LMFA03020006	6.7	495.1	177	-70	-28	-19
Leukotrienes (LT)	LTE4	Yes	LMFA03020002	7.0	438.1	333.1	-55	-26	-15
Lipoxins (LX)	AT-LXA4	Yes	LMFA03040003	5.6	351.1	114.9	-20	-22	-11
Lipoxins (LX)	LXA4	Yes	LMFA03040001	5.5	351.1	114.8	-40	-20	-11
Lipoxins (LX)	LXB4	Yes	LMFA03040002	5.1	351.1	220.9	-60	-22	-13
Maresins (MaR)	Mar1	Yes	LMFA04050001	7.0	359.2	250.2	-65	-20	-13
Maresins (MaR)	7(S)-MaR1		N/A	6.6	359.1	249.9	-20	-20	-19
Polyunsaturated fatty acids	AA	Yes	LMFA01030001	8.8	303	205.1	-155	-20	-11
Polyunsaturated fatty acids	AdA	Yes	LMFA01030178	9.1	331.1	233	-130	-22	-11
Polyunsaturated fatty acids	ALA/GLA	Yes	LMFA01030152 LMFA01030141	8.6	277	233	-90	-22	-29
Polyunsaturated fatty acids	ALA*	Yes	LMFA01030152	8.6	277	182.1	-55	-24	-7
Polyunsaturated fatty acids	DGLA		LMFA01030158	9.0	305.1	261.2	-85	-22	-13
Polyunsaturated fatty acids	DHA	Yes	LMFA01030185	8.8	327.1	229.2	-115	-18	-11
Polyunsaturated fatty acids	DPAn-3	Yes	LMFA04000044	8.9	329.1	231.1	-50	-20	-17
Polyunsaturated fatty acids	DPAn-6		LMFA01030182						
Polyunsaturated fatty acids	EPA	Yes	LMFA01030759	8.6	301	202.9	-125	-18	-21
Polyunsaturated fatty acids	LA	Yes	LMFA01030120	8.8	279	261	-115	-28	-13
Prostaglandins (PG)	13,14-dihydro-15-keto-PGF2alpha	Yes	LMFA03010027	5.4	353.1	195	-110	-32	-11
Prostaglandins (PG)	15-deoxy-PGJ2		LMFA03010021	7.3	315	203	-50	-28	-19

Prostaglandins (PG)	15-keto-PGE2	Yes	LMFA03010030	4.5	349	234.9	-65	-20	-13
Prostaglandins (PG)	8-iso-PGE2	Yes	LMFA03110003	4.6	351.1	271	-5	-24	-19
Prostaglandins (PG)	8-iso-PGF2alpha	Yes	LMFA03110001	4.5	353.1	193	-135	-34	-11
Prostaglandins (PG)	PGD2	Yes	LMFA03010004	5.0	351.1	233	-30	-16	-13
Prostaglandins (PG)	PGE2	Yes	LMFA03010003	4.9	351.2	271.1	-50	-22	-21
Prostaglandins (PG)	PGF2alpha	Yes	LMFA03010002	5.2	353.1	193	-80	-34	-11
Prostaglandins (PG)	PGJ2	Yes	LMFA03010019	6.1	333	271	-30	-22	-17
Protectins (PD)	PD1		LMFA04040001	6.9	359.1	153	-70	-22	-9
Protectins (PD)	PDX	Yes	LMFA04040003	6.8	359.1	153	-70	-22	-9
Resolvins (Rv)	18R-RvE3		LMFA03140006	7.1	333.1	245	-55	-18	-23
Resolvins (Rv)	18S-RvE3		LMFA03140007	6.7	333.1	245.2	-25	-16	-17
Resolvins (Rv)	AT-RvD1		LMFA04030005	5.7	375	215	-50	-26	-11
Resolvins (Rv)	RvD1	Yes	LMFA04030011	5.6	375.1	215	-50	-26	-11
Resolvins (Rv)	RvD2	Yes	LMFA04030001	5.3	375.1	277.1	-60	-18	-15
Resolvins (Rv)	RvE1		LMFA03140003	3.8	349.1	195	-95	-22	-13
Resolvins (Rv)	RvE2		LMFA03140011	6.1	333.1	114.9	-35	-18	-15
Thromboxanes (Tx)	TXB2	Yes	LMFA03030002	4.6	369.1	169	-55	-24	-15

*due to technical limitations the signal obtained for the here applied QqQ settings for ALA might result in an approximate 10% overlap with the signal obtained from GLA

References SI Appendix

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