**Sterol derivatives specifically increase anti-inflammatory oxylipin formation in M2-like macrophages by LXR mediated induction of 15-LOX**

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# Supplementary Materials

**Contents**

**Selection of peptides**

For analysis of proteins using a quantitative targeted proteomics method, unique proteotypic peptides (PTPs) [85] are selected to ensure that the target protein is unambiguously identified and can be detected.

The following human proteins were selected to characterize macrophage differentiation and to quantify LXR:

- Oxysterols receptor LXR-alpha (LXRα; Uniprot accession no.: Q13133)
- Oxysterols receptor LXR-beta (LXRβ; Uniprot accession no.: P55055)
- Peroxisome proliferator-activated receptor gamma (PPARγ; Uniprot accession no.: P37231)
- Interleukin-1 receptor type 2 (IL-1RII; Uniprot accession no.: P27930)
- Toll-like receptor 2 (TLR2; Uniprot accession no.: O60603)
- Toll-like receptor 4 (TLR4; Uniprot accession no.: O00206)
- Inducible Nitric oxide synthase (iNOS; Uniprot accession no.: P35228)

The detailed procedure is described here exemplarily iNOS (gene: NOS2). The petides for the other proteins were selected accordingly.

An *in silico* tryptic digestion using ExPASy peptide mass and peptide cutter [86] was carried out, whereby 128 peptides with lengths between 2 and 43 amino acids (aa) were initially obtained. These peptides were analyzed for their suitability as PTPs based on certain criteria. Peptides shorter than 7 (low probability of unique sequence) and longer than 22 aa (poor MS ionization or out of measuring range) were discarded [83] and 58 peptides remained (Table S1).

These peptides were checked for uniqueness in the human proteome using the NextProt peptide uniqueness checker [87] and NCBI Blast [88] databases. Two of the checked peptides were not unique, as they are also found in the sequence of other proteins. The theoretical cleavage probability for the C-terminal cleavage site of the peptides was calculated using ExPASy peptide cutter [86] and additionally the overall cleavage probability was predicted using the decision tree CP-DP [89]. Peptides with a cleavage probability <90% or a total cleavage probability <70% were excluded, leaving 48 peptides.

The remaining peptides were screened for posttranslational modifications (PTMs) (Uniprot [90], Phosphosite Plus [91]), such as phosphorylation or glycosylation, or

naturally occurring variants resulting from single nucleotide polymorphisms (SNPs) [90]. Alternative splice variants of the corresponding protein were also considered [90] and 33 peptides remained. Furthermore, the presence of certain aa (cysteine (C), asparagine (N) and glutamine (Q, especially N-terminal), methionine (M), tryptophan (W)) may be probe to chemical modifications [92-94], such that a maximum of two of these aa are tolerated in a peptide. Finally, 18 peptides were left.

In order to also ensure that the selected peptides elute within an optimal detection window of the chromatographic method, an estimated retention time was calculated using Sequence Specific Retention Calculator (SSRCalc [95]; gradient delay time: 1.5 min, acetonitrile gradient: 1.05 %B/min, "100 Å C18 column, 0.1% Formic Acid 2015"). After these calculations, 4 peptides remained (Table S2) and they were ordered as standards as well as corresponding heavy labeled peptides were obtained from JPT Peptides (Berlin, Germany).

For the selected peptides, product ion spectra were recorded and 3-5 of the most intensive fragments per peptide were selected to achieve the highest possible sensitivity. The SRMAtlas database [96] was used to assist the characterization of the fragments. The collision energy was optimized for all transitions. Constant values were used for all other parameters, e.g. 40 V for the declustering potential, which were optimized for the detection of peptides [83]. The same fragments and potentials were selected for both unlabeled and heavy labeled peptides with the terminal arginine and lysine being uniformly labeled by $^{13}$C and $^{15}$N isotopes.

After optimization of the transitions, the peptides were spiked into primary M0 macrophage lysates at a concentration of 50 nM. Two of the selected peptides showed poor sensitivity and matrix interference. Finally, for the two remaining peptides, two transitions (one quantifier and one qualifier) each, which are particularly sensitive and have only few matrix interferences, were selected (Table S3). In addition, the ratio between the two transitions is monitored to ensure peak identity and thus reliable quantification of the peptides in the samples.

**Table S1: Selection of iNOS peptides from *in silico* tryptic digest.** Selection of peptides was based on peptide length (7-22 aa), uniqueness, cleavage probability and prediction, known posttranslational modifications (PTMs), single nucleotide polymorphism (SNPs), number of unfavored aa and predicted retention time (tR). Shown are only peptides with lengths between 7-22 aa. Peptides selected for the method are shown in bold.

| peptide sequence | position | [M+H]+ | peptide length [aa] | uniqueness [1] | C-terminal cleavage probability [%] [2] | overall cleavage probability [%] [3] | SNPs [4] | PTM [5] | unfavoured aa [6] | predicted tR [min] [7] |
|---|---|---|---|---|---|---|---|---|---|---|
| DPELFEIPPDLVLEVAMEHPK | 315-335 | 2418.2 | 21 | unique | 100 | 84 | | | 1 x M | - |
| APCATSSPVTQDDLQYHNLSK | 31-51 | 2275.1 | 21 | unique | 100 | 88 | | S37: p; S50: p | 1 x N; 2 x Q; 1 x C | - |
| EIETTGTYQLTGDELIFATK | 172-191 | 2230.1 | 20 | unique | 100 | 86 | 177:G → V | | 1 x Q | - |
| LLLVVTSTFGNGDCPGNGEK | 585-604 | 2021.0 | 20 | unique | 95 | 89 | | | 2 x N; 1 x C | - |
| SEALAWDLGALFSCAFNPK | 550-568 | 2040.0 | 19 | unique | 100 | 87 | 552:A → G | | 1 x N; 1 x W; 1 x C | - |
| DHTPTEIHLTVAVVTYHTR | 915-933 | 2190.1 | 19 | unique | 100 | 94 | 933:R → G | | | - |
| GDPANVEFTQLCIDLGWKPK | 279-298 | 2231.1 | 18 | unique | 100 | 12 | | | 1 x N; 1 x Q; 1 x W; 1 x C | - |
| FTNSPTFLEVLEEFPSLR | 873-890 | 2126.1 | 18 | unique | 100 | 92 | | | | 30.0 |
| QNVTIMDHHSAAESFMK | 429-445 | 1945.9 | 17 | unique | 100 | 93 | 439:A → T | | 2 x M; 1 x N; 1 x Q | - |
| DQAVVEINIAVLHSFQK | 412-428 | 1911.0 | 17 | unique | 100 | 90 | 423:L → I | | 1 x N; 2 x Q | - |
| YHEDIFGAVFPYEAK | 1123-1137 | 1785.8 | 15 | unique | 91 | 81 | | | | 18.8 |
| NWGSGMTFQDTLHHK | 89-103 | 1758.8 | 15 | unique | 100 | 87 | | | 1 x M; 1 x N; 1 x Q; 1 x W | - |
| LEALDESGSYWVSDK | 803-817 | 1698.8 | 15 | unique | 74 | 77 | 805:A → D | | 1 x W | - |
| LNEEQVEDYFFQLK | 1105-1118 | 1801.9 | 14 | unique | 100 | 79 | | | 1 x N; 2 x Q | 11.1 |
| LYTSNVTWDPHHYR | 697-710 | 1788.8 | 14 | unique | 100 | 90 | | | 1 x N; 1 x W | - |
| VSAGFLLSQLPILKPR | 891-906 | 1739.1 | 14 | unique | 50 | 17 | | S892: p | 1 x Q | - |
| QQNESPQPLVETGK | 52-65 | 1554.8 | 14 | unique | 93 | 84 | | | 1 x N; 3 x Q | - |
| YAVFGLGSSMYPR | 621-633 | 1447.7 | 13 | unique | 100 | 94 | | | 1 x M | 17.6 |
| FDVVPLVLQANGR | 302-314 | 1427.8 | 13 | unique | 100 | 86 | | | 1 x N; 1 x Q | 19.5 |
| VAVQPSSLEMSAL | 1141-1153 | 1331.7 | 13 | unique | - | - | | | 1 x M; 1 x Q | 19.8 |
| IQWSNLQVFDAR | 204-215 | 1476.8 | 12 | unique | 100 | 94 | | | 1 x N; 2 x Q; 1 x W | - |
| LEALCQPSEYSK | 859-870 | 1367.7 | 12 | unique | 100 | 77 | | | 1 x Q; 1 x C | - |
| YAGYQMPDGSIR | 267-278 | 1357.6 | 12 | unique | 100 | 93 | | | 1 x M; 1 x Q | 10.9 |
| EPGHLYVCGDVR | 1075-1086 | 1344.6 | 12 | unique | 100 | 75 | | | 1 x C | - |
| LVQDSQPLDLSK | 711-722 | 1342.7 | 12 | unique | 100 | 90 | | | 2 x Q | 12.3 |
| GVLHAVHTAYSR | 1036-1047 | 1310.7 | 12 | unique | 100 | 87 | | | | 7.7 |
| VVDGPTPHQTVR | 791-802 | 1305.7 | 12 | unique | 100 | 93 | 800:T → A | | 1 x Q | 5.2 |
| FCAFAHDIDQK | 634-644 | 1294.6 | 11 | unique | 100 | 91 | | | 1 x Q; 1 x C | - |
| LAQVATEEPER | 846-856 | 1242.6 | 11 | unique | 100 | 92 | | | 1 x Q | 7.5 |
| **VTILFATETGK** | **539-549** | **1179.7** | **11** | **unique** | **91** | **87** | | | | **15.3** |
| FHQYAMNGEK | 13-22 | 1224.5 | 10 | unique | 83 | 78 | | | 1 x M; 1 x N; 1 x Q | - |
| AVLFACMLMR | 521-530 | 1154.6 | 10 | unique | 100 | 84 | | | 2 x M; 1 x C | - |
| QNLQSPTSSR | 741-750 | 1117.6 | 10 | unique | 92 | 86 | 747:T → A | S745: p | 1 x N; 2 x Q | - |
| LDATPLSSPR | 74-83 | 1056.6 | 10 | unique | 100 | 85 | | T77: p; S81: p | | - |
| SCLGSIMTPK | 114-123 | 1036.5 | 10 | unique | 100 | 76 | | | 1 x M; 1 x C | - |
| QQLASEVLR | 1062-1070 | 1043.6 | 9 | unique | 100 | 95 | | | 2 x Q | - |
| **YNILEEVGR** | **389-397** | **1092.6** | **9** | **unique** | **96** | **93** | | | **1 x N** | **15.1** |
| YSTNNGNIR | 233-241 | 1038.5 | 9 | unique | 100 | 94 | | S234: p | 3 x N | - |
| SAITVFPQR | 242-250 | 1018.6 | 9 | P29474; P35228 | 100 | 88 | | | 1 x Q | - |
| AACETFDVR | 679-687 | 1011.5 | 9 | unique | 100 | 94 | 679:A → S | | 1 x C | - |
| THVWQDEK | 498-505 | 1042.5 | 8 | unique | 92 | 80 | | | 1 x Q; 1 x W | 5.5 |
| EMFEHICR | 222-229 | 1064.5 | 8 | unique | 100 | 87 | | | 1 x M; 1 x C | - |
| ALSSMHAK | 723-730 | 844.4 | 8 | unique | 88 | 89 | | | 1 x M | 3.3 |
| VYVQDILR | 1054-1061 | 1005.6 | 8 | unique | 100 | 85 | | Y1055: p | 1 x Q | - |
| VWNAQLIR | 259-266 | 999.6 | 8 | unique | 100 | 94 | 266:R → H | | 1 x N; 1 x Q; 1 x W | - |
| LSCLEEER | 577-584 | 978.5 | 8 | unique | 100 | 87 | | S578: p | 1 x C | - |
| SWAVQTFK | 671-678 | 966.5 | 8 | unique | 86 | 97 | 676:T → I | | 1 x Q; 1 x W | - |
| FYSISSSR | 907-914 | 946.5 | 8 | unique | 84 | 90 | 913:S → P | Y1055: p | x C | - |
| DINNNVEK | 23-30 | 945.5 | 8 | unique | 94 | 81 | 23:D → G | | 3 x N | - |
| MTLVFGCR | 1010-1017 | 926.5 | 8 | P29474; P35228 | 91 | 87 | | | 1 x M; 1 x C | - |
| DFCDVQR | 382-388 | 882.4 | 7 | unique | 100 | 95 | | | 1 x Q; 1 x C | - |
| IEEHLAR | 159-165 | 867.5 | 7 | unique | 100 | 89 | | | | 4.3 |
| LHDSQHK | 997-1003 | 864.4 | 7 | unique | 100 | 85 | | | 1 x Q | 3.4 |
| QHIQIPK | 690-696 | 863.5 | 7 | unique | 100 | 86 | | | 2 x Q | - |
| YMQNEYR | 446-452 | 1003.4 | 7 | unique | 100 | 92 | | | 1 x M; 1 x N; 1 x Q | - |
| MGLETHK | 399-405 | 815.4 | 7 | unique | 100 | 86 | | | 1 x M | 3.3 |
| DVAHTLK | 1090-1096 | 783.4 | 7 | unique | 100 | 86 | | | | 3.9 |
| SPESLVK | 67-73 | 759.4 | 7 | unique | 100 | 73 | | | | 6.4 |

[1] from BLAST [88] and NeXtprot [87]; [2] calculated from peptide cutter [86] (≥ 90%); [3] calculated from CP-DT [89] (≥ 70%); [4] SNPs from Uniprot [90]; [5] PTMs from Uniprot [90] and Phosphosite Plus [91] (p – phosphorylation); [6] unfavored aa (C, M, N, Q, W; max. 2); [7] predicted retention time (tR) from SSRCalc [95] (3 – 30 min); -: not evaluated

**Table S2: Selected proteotypic peptides (PTPs) for LXRα, LXRβ, PPARγ, IL-1RII, TLR2, TLR4, iNOS.** The selection is based on peptide length (7-22 aa), uniqueness, cleavage probability or prediction, occurrence of single nucleotide polymorphisms (SNPs), variation in splice variants or posttranslational modifications (PTMs), unfavored amino acids and predicted retention time (Table S1).

| peptides | position | [M+H]+ | length [aa] | uniqueness [1] | C-terminal cleavage probability [2] [%] | overall cleavage probability [3] [%] | SNPs [4] | variation in splice variants [5] | PTMs [6] | unfavored aa [7] | pred. tR [8] [min] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Oxysterols receptor LXR-alpha (LXRα; Q13133; gene: NR1H3)** | | | | | | | | | | | |
| EECVLSEEQIR * | 165-175 | 1334.6 | 11 | unique | 100 | 86 | - | - | - | 1 x Q | 12.0 |
| MLGNELCSVCGDK * | 92-104 | 1368.6 | 13 | unique | 93 | 83 | - | - | - | 1 x M; 1 x N | 12.6 |
| TSAIEVMLLETSR | 292-304 | 1449.8 | 13 | unique | 86 | 95 | - | - | - | 1 x M | 20.1 |
| **Oxysterols receptor LXR-alpha (LXRα; Q13133; gene: NR1H3) / Oxysterols receptor LXR-beta (LXRβ; P55055; gene: NR1H2)** | | | | | | | | | | | |
| TLSSVHSEQVFALR | 416-429 | 1573.8 | 14 | no | 100 | 88 | - | - | - | 1 x Q | 13.8 |
| **Oxysterols receptor LXR-beta (LXRβ; P55055; gene: NR1H2)** | | | | | | | | | | | |
| VEALQQPYVEALLSYTR | 391-407 | 1980.0 | 17 | unique | 100 | 77 | - | - | - | 2 x Q | 21.6 |
| VTPWPLGADPQSR | 248-260 | 1423.7 | 13 | unique | 84 | 87 | - | - | - | 1 x W; 1 x Q | 13.7 |
| YNHETECITFLK | 319-330 | 1497.7 | 12 | unique | 86 | 92 | - | - | - | 1 x N; 1 x H | 10.4 |
| **Peroxisome proliferator-activated receptor gamma (PPARγ; P37231, gene: PPARG)** | | | | | | | | | | | |
| LNHPESSQLFAK | 451-462 | 1370.7 | 12 | unique | 95 | 93 | - | missing in isoform 3 | - | 1 x N; 1 x Q; 1 x H | 9.4 |
| HLYDSYIK | 245-252 | 1038.5 | 8 | unique | 100 | 92 | - | missing in isoform 3 | - | 1 x H | 9.1 |
| TDPVVADYK | 86-94 | 1007.5 | 9 | unique | 100 | 90 | - | - | - | - | 9.2 |
| **Interleukin-1 receptor type 2 (IL-1RII; P27930; gene: IL1R2)** | | | | | | | | | | | |
| EDLHMDFK | 318-325 | 1034.5 | 8 | unique | 100 | 90 | - | missing in isoform 2 | - | 1 x M; 1 x H | 9.8 |
| TVPGEEETR | 77-85 | 1017.5 | 9 | unique | 100 | 91 | - | - | - | - | 4.2 |
| TISASLGSR | 245-253 | 891.5 | 9 | unique | 100 | 86 | - | - | - | - | 7.9 |
| **Toll-like receptor 2 (TLR2; O60603; gene: TLR2)** | | | | | | | | | | | |
| TVFVLSENFVK | 699-709 | 1282.7 | 11 | unique | 100 | 87 | - | - | - | 1 x N | 19.3 |
| EQLDSFHTLK | 517-526 | 1217.6 | 10 | unique | 100 | 92 | - | - | - | 1 x Q; 1 x H | 11.4 |
| VGNMDTFTK | 156-164 | 1012.5 | 9 | unique | 100 | 90 | - | - | - | 1 x N; 1 x M | 9.1 |
| **Toll-like receptor 4 (TLR4; O00206; gene: TLR4)** | | | | | | | | | | | |
| DFIPGVAIAANIIHEGFHK | 711-729 | 2049.1 | 19 | unique | 100 | 77 | - | - | - | 1 x N; 2 x H | 21.6 |
| NLDLSFNPLR | 58-67 | 1188.6 | 10 | unique | 100 | 92 | - | - | - | 2 x N | 17.4 |
| LVLGEFR | 258-264 | 833.5 | 7 | unique | 100 | 90 | - | - | - | - | 13.4 |
| **Nitric oxide synthase, inducible (iNOS; P35228; gene: NOS2)** | | | | | | | | | | | |
| VTILFATETGK | 539-549 | 1179.7 | 11 | unique | 91 | 87 | - | - | - | - | 15.3 |
| YNILEEVGR | 389-397 | 1092.6 | 9 | unique | 86 | 92 | - | - | - | 1 x N | 15.1 |

[1]from BLAST [88] and NeXtprot [87]; [2]calculated from peptide cutter [86] (≥ 90%); [3]calculated from CP-DT [89] (≥ 70%); [4]SNPs from Uniprot [90]; [5] splice variants from Uniprot; [6] PTMs from Uniprot [90] and Phosphosite Plus [91] (ac – acetylation, gl – glycosylation, n – nitrosylation, p – phosphorylation); [7]unfavored aa; [8]predicted retention time (tR) from SSRCalc [95] (3 – 30 min)

- not evaluated

* carbamidomethylated C

**Table S3: Selected transitions for (A) unlabeled and (B) heavy labeled (lys: U-13C6; U-15N2; arg: U-13C6; U-15N4) peptide data for LXRα, LXRβ, PPARγ, IL-1RII, TLR2, TLR4, iNOS.** Shown are Q1 and Q3 *m/z*, optimized collision energies (CE), area ratios of qualifier to quantifier transitions and retention time (tR, mean ± range, within one analytical batch, n = 20)). (**A**) Linear calibration range, limit of detection (LOD) and limit of quantification (LLOQ), as well as the transitions of the corresponding internal standards (IS) are shown for quantifier transitions (**bold**). Accuracy of calibrators was within a range of ± 15% (20% for LLOQ). (**B**) The heavy labeled peptides are spiked at a concentration of 25 nM for all peptides (concentration in vial).

## (A)

| gene / protein | peptide | transitions | Q1 *m/z* | Q3 *m/z* | tR [min] | rel. ratio to quantifier [%] | CE (V) | IS Transitions | calibration range [nM] | LOD [pM] | LLOQ [pM] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **NR1H3 / Oxysterols receptor LXR-alpha** | | | | | | | | | | | |
| | EECVLSEEQIR | $\mathbf{M^{2+} \to y_7^+}$ | 696.33 | 874.46 | 12.04 ± 0.08 | | 35 | $M^{2+} \to y_7^+$ | 0.08 - 1060 | 50 | 80 |
| | | $M^{2+} \to y_6^+$ | 696.33 | 761.38 | | 82 | 35 | | | | |
| | MLGNELCSVCGDK | $\mathbf{M^{2+} \to y_6^+}$ | 741.82 | 665.29 | 13.38 ± 0.12 | | 40 | $M^{2+} \to y_6^+$ | 0.45 - 897 | 230 | 450 |
| | | $M^{2+} \to y_7^+$ | 741.82 | 825.32 | | 47 | 40 | | | | |
| | TSAIEVMLLETSR | $\mathbf{M^{2+} \to y_7^+}$ | 725.39 | 849.45 | 21.41 ± 0.15 | | 35 | $M^{2+} \to y_7^+$ | 0.87 - 1167 | 530 | 870 |
| | | $M^{2+} \to y_5^+$ | 725.39 | 605.20 | | 47 | 30 | | | | |
| **NR1H3 / Oxysterols receptor LXR-alpha ; NR1H2 / Oxysterols receptor LXR-beta** | | | | | | | | | | | |
| | TLSSVHSEQVFALR | $\mathbf{M^{3+} \to b_2^+}$ | 525.28 | 215.14 | 14.15 ± 0.07 | | 20 | $M^{3+} \to b_2^+$ | 0.45 - 1270 | 300 | 450 |
| | | $M^{3+} \to y_{12}^+$ | 525.28 | 680.36 | | 20 | 25 | | | | |
| **NR1H2 / Oxysterols receptor LXR-beta** | | | | | | | | | | | |
| | VEALQQPYVEALLSYTR | $\mathbf{M^{3+} \to y_5^+}$ | 660.69 | 639.35 | 23.63 ± 0.08 | | 30 | $M^{3+} \to y_5^+$ | 0.61 - 339 | 250 | 610 |
| | | $M^3 \to y_4^+$ | 660.69 | 526.26 | | 63 | 25 | | | | |
| | VTPWPLGADPQSR | $\mathbf{M^{2+} \to y_4^+}$ | 712.37 | 487.26 | 16.71 ± 0.10 | | 45 | $M^{2+} \to y_4^+$ | 0.47 - 1050 | 280 | 470 |
| | | $M^{2+} \to y_9^{++}$ | 712.37 | 940.49 | | 61 | 40 | | | | |
| | YNHETECITFLK | $\mathbf{M^{3+} \to y_5^+}$ | 518.91 | 621.40 | 13.75 ± 0.06 | | 25 | $M^{3+} \to y_5^+$ | 3.0 - 1220 | 1800 | 3000 |
| | | $M^3 \to y_6^+$ | 518.91 | 781.43 | | 97 | 25 | | | | |
| **PPARG / Peroxisome proliferator-activated receptor gamma** | | | | | | | | | | | |
| | LNHPESSQLFAK | $\mathbf{M^{3+} \to y_2^+}$ | 457.57 | 218.15 | 10.35 ± 0.11 | | 30 | $M^{3+} \to y_2^+$ | 2.7 - 1002 | 1500 | 2700 |
| | | $M^{3+} \to y_{11}^{++}$ | 457.57 | 629.32 | | 43 | 27 | | | | |
| | HLYDSYIK | $\mathbf{M^{3+} \to y_2^+}$ | 346.85 | 260.20 | 12.18 ± 0.08 | | 17 | $M^{3+} \to y_2^+$ | 5.7 - 1230 | 3000 | 5700 |
| | | $M^{3+} \to b_2^+$ | 346.85 | 251.15 | | 58 | 23 | | | | |
| | TDPVVADYK | $\mathbf{M^{2+} \to y_7^+}$ | 504.26 | 791.43 | 9.07 ± 0.05 | | 21 | $M^{2+} \to y_7^+$ | 0.03 - 1035 | 10 | 28 |
| | | $M^{2+} \to b_2^+$ | 504.26 | 217.08 | | 49 | 21 | | | | |
| **IL1R2 / Interleukin-1 receptor type 2** | | | | | | | | | | | |
| | TVPGEEETR | $\mathbf{M^{2+} \to y_7^{++}}$ | 509.25 | 409.19 | 5.42 ± 0.07 | | 21 | $M^{2+} \to y_7^{++}$ | 0.05 - 559 | 24 | 54 |
| | | $M^{2+} \to b_2^+$ | 509.25 | 201.12 | | 10 | 21 | | | | |
| | TISASLGSR | $\mathbf{M^{2+} \to y_7^+}$ | 446.25 | 677.36 | 8.54 ± 0.12 | | 22 | $M^{2+} \to y_7^+$ | 0.01 - 1110 | 4 | 11 |
| | | $M^{2+} \to y_5^+$ | 446.25 | 519.29 | | 18 | 25 | | | | |
| **TLR2 / Toll-like receptor 2** | | | | | | | | | | | |
| | TVFVLSENFVK | $\mathbf{M^{2+} \to y_7^+}$ | 641.86 | 836.45 | 19.35 ± 0.11 | | 29 | $M^{2+} \to y_7^+$ | 0.5 - 1054 | 280 | 500 |
| | | $M^{2+} \to b_2^+$ | 641.86 | 201.12 | | 77 | 27 | | | | |
| | EQLDSFHTLK | $\mathbf{M^{3+} \to y_8^{++}}$ | 406.54 | 480.76 | 11.49 ± 0.07 | | 18 | $M^{3+} \to y_8^{++}$ | 0.12 - 1200 | 75 | 120 |
| | | $M^{3+} \to y_6^{++}$ | 406.54 | 366.71 | | 20 | 19 | | | | |
| | VGNMDTFTK | $\mathbf{M^{2+} \to y_2^+}$ | 506.74 | 248.16 | 10.15 ± 0.08 | | 34 | $M^{2+} \to y_2^+$ | 0.25 - 1050 | 100 | 250 |
| | | $M^{2+} \to b_3^+$ | 506.74 | 271.14 | | 88 | 26 | | | | |
| **TLR4 / Toll-like receptor 4** | | | | | | | | | | | |
| | NLDLSFNPLR | $\mathbf{M^{2+} \to y_8^+}$ | 594.82 | 961.51 | 20.19 ± 0.12 | | 26 | $M^{2+} \to y_8^+$ | 0.24 - 1140 | 109 | 243 |
| | | $M^{2+} \to y_6^+$ | 594.82 | 733.40 | | 62 | 29 | | | | |
| | LVLGEFR | $\mathbf{M^{2+} \to b_2^+}$ | 417.25 | 213.16 | 15.48 ± 0.10 | | 18 | $M^{2+} \to b_2^+$ | 0.1 - 1080 | 75 | 98 |
| | | $M^{2+} \to y_2^+$ | 417.25 | 322.19 | | 58 | 29 | | | | |
| **NOS2 / Nitric oxide synthase, inducible** | | | | | | | | | | | |
| | VTILFATETGK | $\mathbf{M^{2+} \to y_8^+}$ | 590.34 | 866.46 | 16.82 ± 0.08 | | 27 | $M^{2+} \to y_8^+$ | 0.028 - 1006 | 15 | 28 |
| | | $M^{2+} \to y_9^+$ | 590.34 | 979.55 | | 68 | 23 | | | | |
| | YNILEEVGR | $\mathbf{M^{2+} \to y_6^+}$ | 546.79 | 702.38 | 16.57 ± 0.07 | | 27 | $M^{2+} \to y_6^+$ | 0.069 - 964 | 43 | 69 |
| | | $M^{2+} \to y_5^+$ | 546.79 | 589.29 | | 73 | 28 | | | | |

**(B)**

| Gene / Protein | Peptide | Transitions | Q1 $m/z$ | Q3 $m/z$ | tR [min] | Rel. Ratio to quantifier [%] |
|---|---|---|---|---|---|---|
| **NR1H3 / Oxysterols receptor LXR-alpha** | | | | | | |
| | EECVLSEEQIR | $M^{2+} \rightarrow y_7^+$ | 701.33 | 884.46 | 12.04 ± 0.08 | |
| | | $M^{2+} \rightarrow y_6^+$ | 701.33 | 771.38 | | 82 |
| | MLGNELCSVCGDK | $M^{2+} \rightarrow y_6^+$ | 745.83 | 673.30 | 13.38 ± 0.12 | |
| | | $M^{2+} \rightarrow y_7^+$ | 745.83 | 833.32 | | 46 |
| | TSAIEVMLLETSR | $M^{2+} \rightarrow y_7^+$ | 730.39 | 859.45 | 21.41 ± 0.15 | |
| | | $M^{2+} \rightarrow y_5^+$ | 730.39 | 615.20 | | 44 |
| **NR1H3 / Oxysterols receptor LXR-alpha ; NR1H2 / Oxysterols receptor LXR-beta** | | | | | | |
| | TLSSVHSEQVFALR | $M^{3+} \rightarrow b_2^+$ | 528.62 | 215.14 | 14.15 ± 0.07 | |
| | | $M^{3+} \rightarrow y_{12}^+$ | 528.62 | 685.36 | | 20 |
| **NR1H2 / Oxysterols receptor LXR-beta** | | | | | | |
| | VEALQQPYVEALLSYTR | $M^{3+} \rightarrow y_5^+$ | 664.02 | 649.35 | 23.63 ± 0.08 | |
| | | $M^3 \rightarrow y_4^+$ | 664.02 | 536.27 | | 71 |
| | VTPWPLGADPQSR | $M^{2+} \rightarrow y_4^+$ | 717.37 | 497.27 | 16.71 ± 0.10 | |
| | | $M^{2+} \rightarrow y_9^{++}$ | 717.37 | 950.49 | | 63 |
| | YNHETECITFLK | $M^{3+} \rightarrow y_5^+$ | 521.59 | 629.40 | 13.75 ± 0.06 | |
| | | $M^3 \rightarrow y_6^+$ | 521.59 | 789.43 | | 97 |
| **PPARG / Peroxisome proliferator-activated receptor gamma** | | | | | | |
| | LNHPESSQLFAK | $M^{3+} \rightarrow y_2^+$ | 460.24 | 226.15 | 10.35 ± 0.11 | |
| | | $M^{3+} \rightarrow y_{11}^{++}$ | 460.24 | 633.31 | | 57 |
| | HLYDSYIK | $M^{3+} \rightarrow y_2^+$ | 349.51 | 268.20 | 12.18 ± 0.08 | |
| | | $M^{3+} \rightarrow b_2^+$ | 349.51 | 251.15 | | 58 |
| | TDPVVADYK | $M^{2+} \rightarrow b_2^+$ | 508.26 | 217.08 | 9.07 ± 0.05 | |
| | | $M^{2+} \rightarrow y_7^+$ | 508.26 | 799.43 | | 50 |
| **IL1R2 / Interleukin-1 receptor type 2** | | | | | | |
| | TVPGEEETR | $M^{2+} \rightarrow b_2^+$ | 514.25 | 201.12 | 5.42 ± 0.07 | |
| | | $M^{2+} \rightarrow y_7^{++}$ | 514.25 | 414.19 | | 11 |
| | TISASLGSR | $M^{2+} \rightarrow y_7^+$ | 451.25 | 687.36 | 8.54 ± 0.12 | |
| | | $M^{2+} \rightarrow y_5^+$ | 451.25 | 529.29 | | 10 |
| **TLR2 / Toll-like receptor 2** | | | | | | |
| | TVFVLSENFVK | $M^{2+} \rightarrow y_7^+$ | 645.86 | 844.45 | 19.35 ± 0.11 | |
| | | $M^{2+} \rightarrow b_2^+$ | 645.86 | 201.12 | | 69 |
| | EQLDSFHTLK | $M^{3+} \rightarrow y_8^{++}$ | 409.21 | 484.76 | 11.49 ± 0.07 | |
| | | $M^{3+} \rightarrow y_6^{++}$ | 409.21 | 370.71 | | 21 |
| | VGNMDTFTK | $M^{2+} \rightarrow y_2^+$ | 510.74 | 256.16 | 10.15 ± 0.08 | |
| | | $M^{2+} \rightarrow b_3^+$ | 510.74 | 271.14 | | 86 |
| **TLR4 / Toll-like receptor 4** | | | | | | |
| | NLDLSFNPLR | $M^{2+} \rightarrow y_8^+$ | 599.82 | 971.51 | 20.19 ± 0.12 | |
| | | $M^{2+} \rightarrow y_6^+$ | 599.82 | 743.40 | | 62 |
| | LVLGEFR | $M^{2+} \rightarrow b_2^+$ | 422.25 | 213.16 | 15.48 ± 0.10 | |
| | | $M^{2+} \rightarrow y_2^+$ | 422.25 | 332.19 | | 35 |
| **NOS2 / Nitric oxide synthase, inducible** | | | | | | |
| | VTILFATETGK | $M^{2+} \rightarrow y_8^+$ | 594.34 | 874.46 | 16.82 ± 0.08 | |
| | | $M^{2+} \rightarrow y_9^+$ | 594.34 | 987.55 | | 67 |
| | YNILEEVGR | $M^{2+} \rightarrow y_6^+$ | 551.79 | 712.38 | 16.57 ± 0.07 | |
| | | $M^{2+} \rightarrow y_5^+$ | 551.79 | 599.29 | | 72 |

**Table S4: Oxylipin concentrations and 15-LOX levels in M2-like macrophages induced by LXR, FXR and RXR agonists and cholesterol precursors and metabolites.** Primary blood monocytic cells were differentiated to M2-like macrophages with 10 ng/mL M-CSF for 8 days and incubated with IL-4 for the final 48 h. For 24 h, M2-like macrophages were incubated with known LXR antagonist (GSK2033), FXR agonists (fexaramine and HDCA) and RXR agonists (bexarotene and 9-RA). In order to find an endogenous ligand inducing 15-LOX activity the cells were also treated with different sterols. The results are shown as mean ± SEM (n=3-5).

| | Oxylipin concentrations | | | | | | | | | 15-LOX level |
|---|---|---|---|---|---|---|---|---|---|---|
| | [pmol/mg protein] | | | | | | | | | |
| | 12-HETE | 15-HETE | 5,15-diHETE | 7,17-diHDHA | 12-HEPE | 15-HEPE | 14-HDHA | 7-HDHA | 17-HDHA | 15-LOX |
| Ctrl | 5.96 ± 2.10 | 85.2 ± 35.8 | 3.88 ± 1.87 | 6.10 ± 3.75 | 0.54 ± 0.12 | 7.06 ± 2.00 | 8.04 ± 3.47 | 1.95 ± 0.75 | 27.1 ± 11.73 | 0.92 ± 0.13 |
| GSK2033 (1 µM) | 13.6 ± 3.77 | 163 ± 49.3 | 23.4 ± 9.12 | 24.5 ± 18.2 | 1.06 ± 0.03 | 12.5 ± 0.56 | 14.4 ± 1.32 | 2.33 ± 0.03 | 38.4 ± 9.99 | 1.73 ± 0.82 |
| Fexaramine (1 µM) | 15.7 ± 8.60 | 206 ± 113 | 25.9 ± 1.19 | 44.9 ± 8.60 | 1.18 ± 0.03 | 14.8 ± 0.07 | 23.6 ± 0.43 | 3.54 ± 0.02 | 60.0 ± 3.05 | 1.77 ± 0.56 |
| HDCA (10 µM) | 4.29 ± 4.16 | 63.8 ± 63.2 | 5.49 ± 5.49 | 24.9 ± 24.9 | 0.54 ± 0.46 | 7.05 ± 6.82 | 6.30 ± 6.13 | 1.36 ± 1.33 | 22.7 ± 22.4 | 1.09 ± 0.79 |
| Bexarotene (1 µM) | 27.7 ± 8.06 | 309 ± 88.2 | 42.1 ± 18.4 | 49.5 ± 20.8 | 1.89 ± 0.01 | 19.1 ± 0.13 | 25.8 ± 0.61 | 4.31 ± 0.05 | 70.7 ± 1.67 | 2.25 ± 0.46 |
| 9-RA (10 µM) | 4.15 ± 1.52 | 41.8 ± 21.4 | 0.60 ± 0.29 | 1.44 ± 0.81 | 0.54 ± 0.20 | 5.12 ± 2.22 | 4.71 ± 2.47 | 1.47 ± 0.88 | 13.7 ± 8.16 | 0.82 ± 0.47 |
| 22(R)-OH Chol (10 µM) | 14.6 ±5.74 | 172 ± 62.6 | 26.2 ± 13.5 | 30.7 ± 14.6 | 1.11 ± 0.41 | 13.7 ± 5.15 | 16.9 ± 6.12 | 2.26 ± 0.42 | 43.3 ± 13.5 | 1.94 ± 0.20 |
| 24(S)-OH Chol (5 µM) | 3.81 ± 2.94 | 47.8 ± 44.3 | 2.06 ± 1.99 | 7.76 ± 7.32 | 0.52 ± 0.37 | 6.00 ± 5.31 | 4.81 ± 4.43 | 1.18 ± 1.09 | 14.9 ± 14.0 | 0.57 ± 0.31 |
| 25-OH Chol (10 µM) | 3.92 ± 1.78 | 50.3 ± 24.8 | 4.95 ± 3.35 | 6.28 ± 3.79 | 0.54 ± 0.22 | 6.49 ± 2.76 | 4.42 ± 1.95 | 1.07 ± 0.54 | 13.4 ± 6.06 | 1.10 ± 0.39 |
| 24(S),25-Ep Chol (1 µM) | 7.26 ± 2.98 | 101 ± 36.6 | 8.77 ± 5.13 | 10.0 ± 5.04 | 0.95 ± 0.34 | 11.3 ± 3.81 | 7.81 ± 2.65 | 1.43 ± 0.41 | 22.8 ± 7.30 | 1.91 ±0.30 |
| 24(S),25-Ep Chol (10 µM) | 30.8 ± 20.0 | 238 ± 96.9 | 21.9 ± 8.94 | 27.9 ± 10.9 | 1.85 ± 0.92 | 16.9 ± 5.27 | 20.7 ± 7.81 | 3.01 ± 1.00 | 55.7 ± 21.6 | 2.13 ± 0.40 |
| 7-keto Chol (10 µM) | 4.27 ± 3.80 | 49.4 ± 47.0 | 4.41 ± 4.38 | 12.0 ± 11.7 | 0.50 ± 0.38 | 5.41 ± 4.83 | 5.36 ± 5.12 | 1.38 ± 1.32 | 14.7 ± 14.2 | 1.01 ± 0.70 |
| Desmosterol (1 µM) | 10.5 ± 10.4 | 151 ± 133 | 18.7 ± 24.9 | 136 ± 181 | 0.78 ± 0.69 | 9.47 ± 9.13 | 11.5 ± 11.4 | 1.72 ± 1.70 | 30.8 ± 30.5 | 2.68 ± 0.52 |
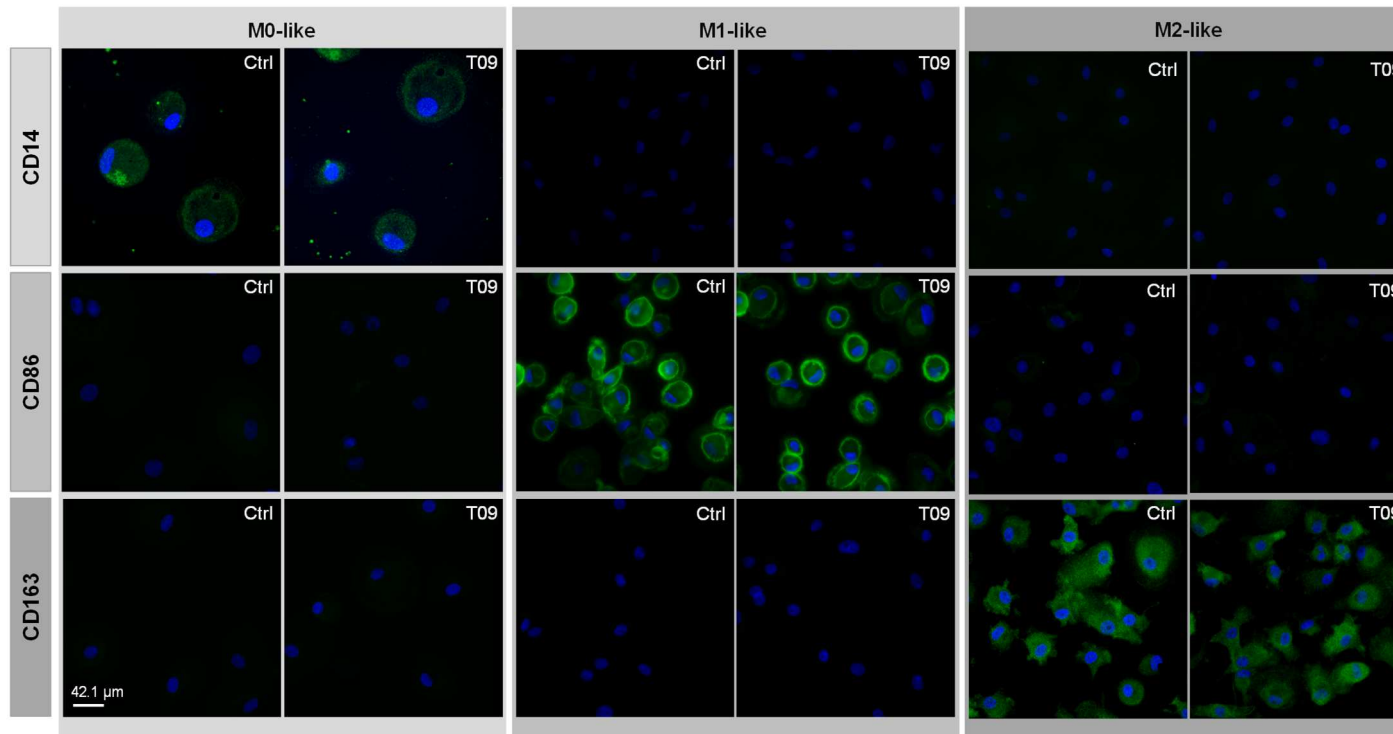
**Figure S1: Immunostaining of macrophage surface markers.** Primary human blood monocytic cells were treated with 10 ng/mL GM-CSF (M1 type) and M-CSF (M2 type) for 8 days as well as 10 ng/mL IFNγ (M1 type) or IL-4 (M2 type) for the final 2 days. Additionally, M1 cells were challenged with 100 ng/mL LPS for the final 6 h. For M0 type, the adhered monocytes were left untreated for 8 days. The three different macrophage phenotypes were incubated w/ or w/o the synthetic LXR agonist T09 (1 µM) for the final three hours. The three macrophage phenotypes were immunostained for specific macrophage marker CD14, CD86, or CD163 (all mouse anti-Human; Bio-Rad Laboratories, Feldkirchen, Germany) using goat anti-Mouse IgG Alexa Fluor secondary antibodies from Thermo Scientific, Langenselbold, Germany (**green**). Nuclei were counterstained with Hoechst (**blue**). Immunostaining shows that CD14 is only expressed in M0-like, CD86 in M1-like and CD 163 in M2-like macrophages. T09 has no influence on the expression of the surface markers.
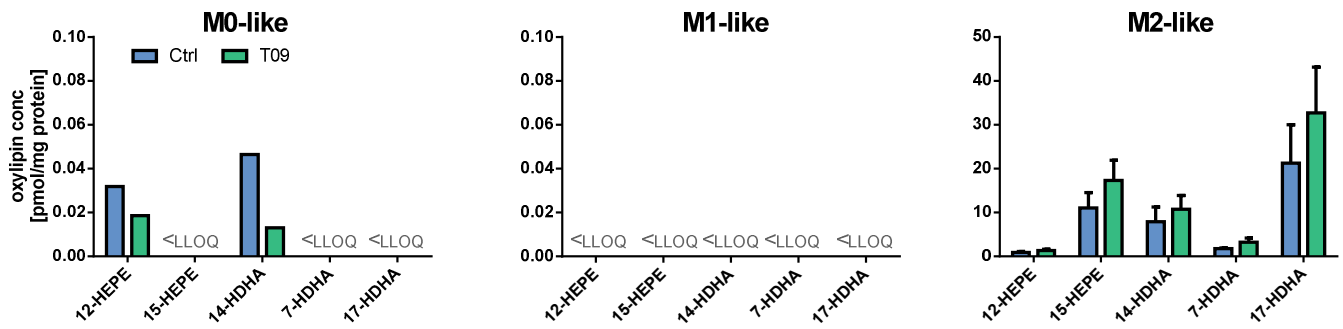
**Figure S2: Effect of the LXR agonist T09 on the monohydroxylated oxylipin formation.** Primary human blood monocytic cells were treated with 10 ng/mL GM-CSF (M1 type) for 8 days or with M-CSF (M2 type) for 8 days as well as 10 ng/mL IFNγ (M1 type) or IL-4 (M2 type) for the final 2 days. Additionally, M1 cells were challenged with 100 ng/mL LPS for 6 h. For M0 type, the adhered monocytes were left untreated for 8 days. The three different macrophage phenotypes were incubated w/ (**blue**) or w/o the synthetic LXR agonist T09 (1 µM; **green**) for the final three hours. Shown are monolydroxylated oxylipin concentrations of 12-HEPE and 15-HEPE (EPA-derived) and 14-HDHA, 7-HDHA and 17-HDHA (DHA-derived) (mean ± SEM; cells from 3-5 donors).

**(A) time dependence (1 µM T09)**
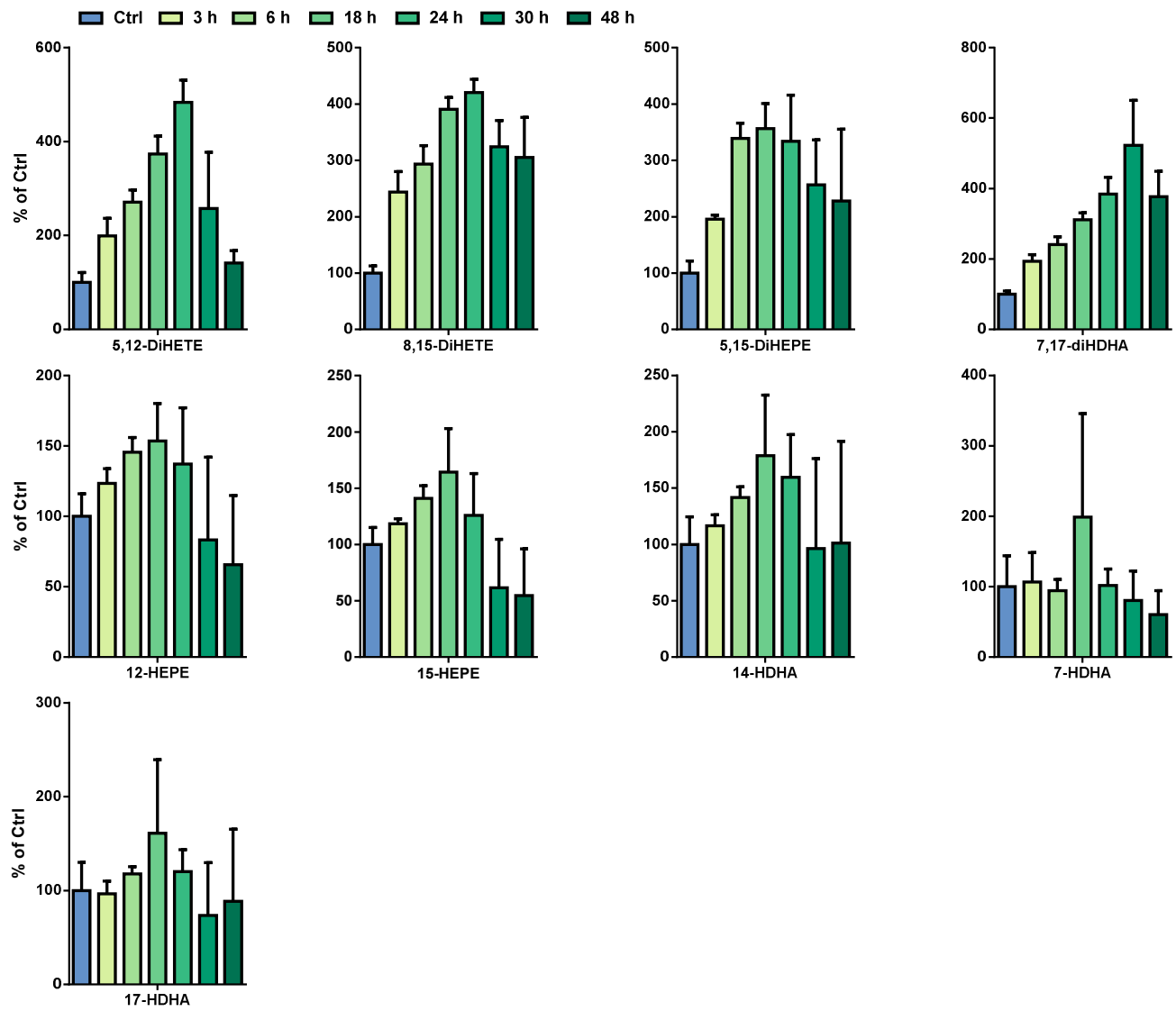
**(B) dose dependence (24 h)**
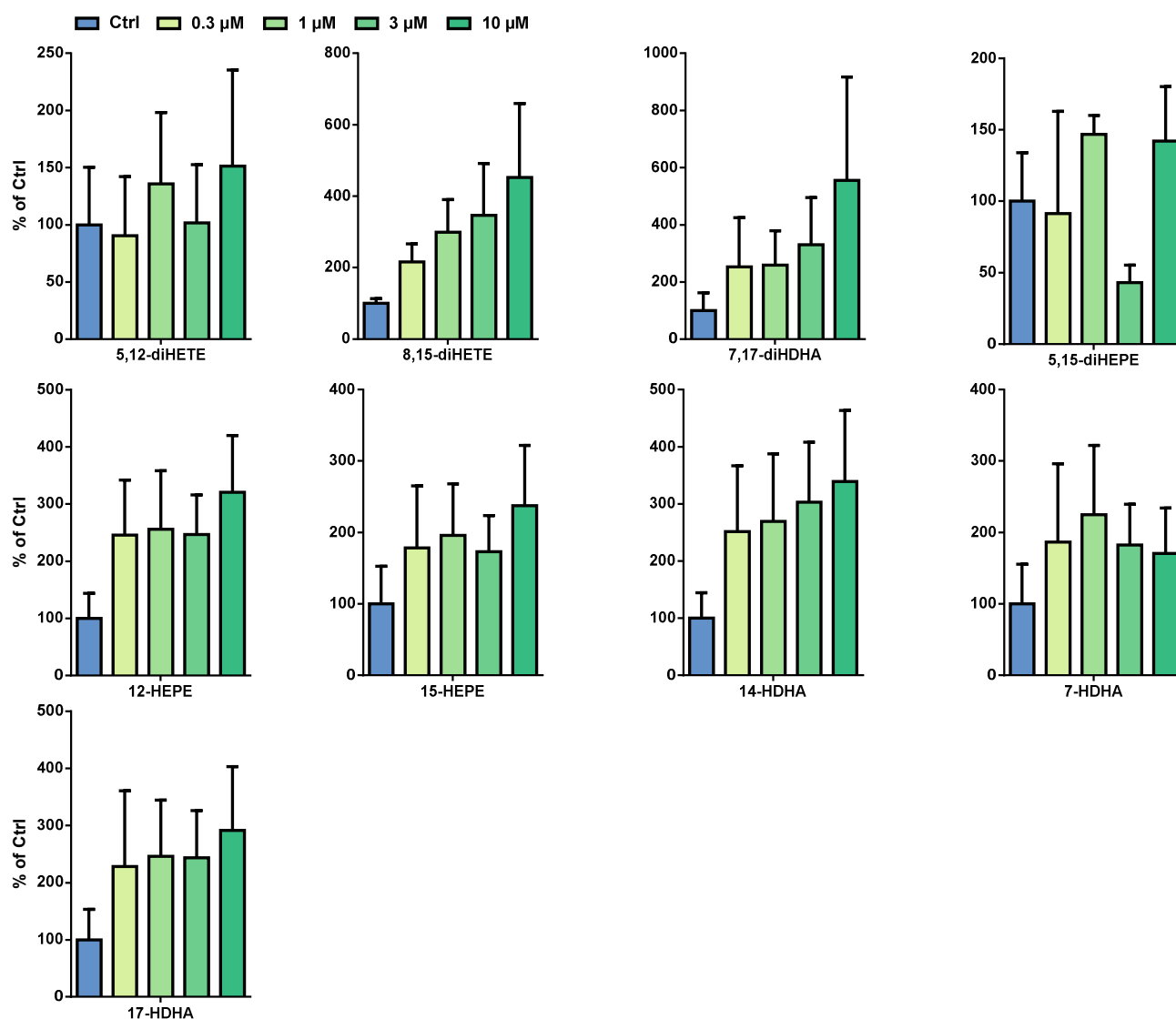


**Figure S3: Time- and dose-dependent 15-LOX activity.** (**A**) M2-like macrophages were incubated with 1 µM T09 for different periods of time to investigate the time-dependent of 15-LOX activity. (**B**) For the evaluation of the dose-dependence, M2-like macrophages were treated with different T09 concentrations for 24 h. Shown are relative amounts of multiple hydroxylated oxylipins 5,12-diHETE, 8,15-diHETE (ARA-derived), 5,15-diHEPE (EPA-derived) and 7,17-diHDHA (DHA-derived) and monohydroxylated oxylipins 12-HEPE and 15-HEPE (EPA-derived) and 14-HDHA, 7-HDHA and 17-HDHA (DHA-derived) (mean ± SEM, n=3-6). All incubations were repeated on two different days. Results are shown as % of Ctrl (0.1% DMSO). The concentrations determined in the cells can be found in the Table S4.
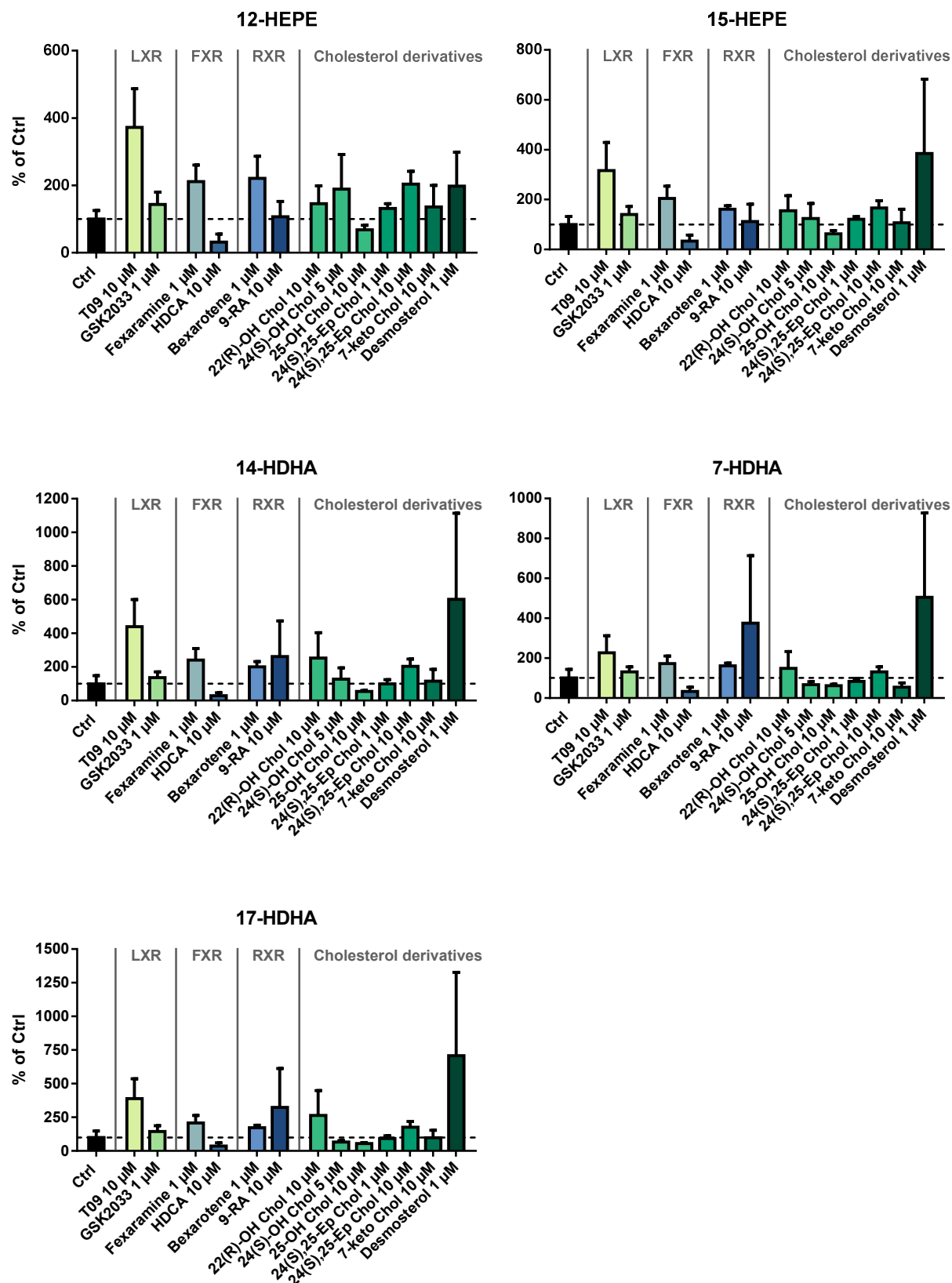
**Figure S4: 15-LOX activity induced by LXR, FXR and RXR agonists and identification of cholesterol precursors and metabolites.** M2-like macrophages were incubated with test compounds for 24 h. Shown is the increase of 15-LOX derived mono-hydroxylated metabolites 12-HEPE and 15-HEPE (**top**: EPA-derived) and 14-HDHA, 7-HDHA and 17-HDHA (**middle** and **bottom**: DHA-derived). Results are shown as % of Ctrl (mean ± SEM, cells from 3-5 donors).
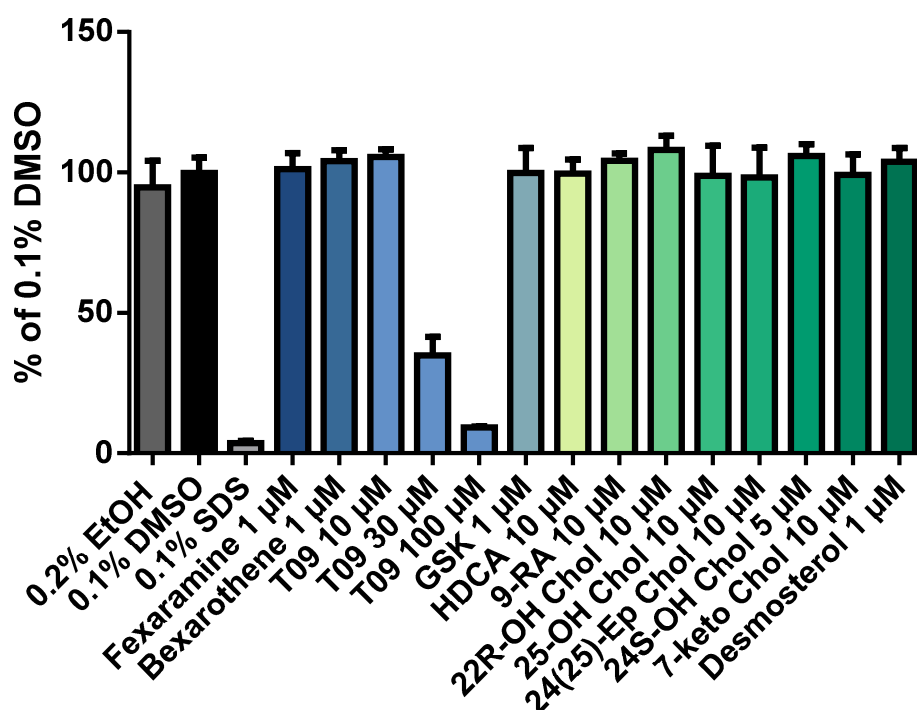
**Figure S5: Cell viability assay of the test compounds in M2-like macrophages.** Cell viability was determined by resazurin assay [79]. The cells were incubated with the different test compounds at the indicated concentrations for 24 h. DMSO and ethanol (EtOH) served as vehicle control and sodium dodecyl sulfate (SDS) as positive control. Dehydrogenase activity was measured as resorufin formation from resazurin (5 µg/mL) by fluorometric readout at 590 nm after excitation at 560 nm [79]. Shown are mean ± SD for n = 6-12 technical replicates from a pool of 4 donors.