

**Supplemental Figure S1** Expression of LPA receptors (LPAR) in primary human hepatic stellate cells from 3 different donors. Data represent relative mRNA levels normalised versus levels of RNA-polymerase II (RNAPol2). Dots represent three individual donors.

**Supplemental Figure S2** IC<sub>50</sub> curves of Ex<sub>31</sub> from the (A) biochemical and (B) rat whole blood assay. The potency of Ex<sub>31</sub> was determined as described recently (Bretschneider *et al.*, 2017). Briefly, the reaction buffer consisted of 50 mM Tris (pH 8.0), 3 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM Mg Cl<sub>2</sub>, 0.14 mM NaCl and 0.1% bovine serum albumin, which was supplemented with 5 nM recombinant rat ATX. Ex<sub>31</sub> was added to the assay solution in a concentration range from 0.1 nM to 10 μM. To 2.5 μL of this solution, 2.5 μL of a 10 μM 18:1 LPC substrate solution was added to initiate the biochemical reaction. After 2 h incubation at room temperature the samples were analysed by LC-MS. The whole blood potency was determined by incubation of 45 μL heparinized rat whole blood with a serial dilution of Ex<sub>31</sub> (0.12 to 100 μM). After 1 h at 37 °C the reaction was stopped by addition of 100 μL 40 mM disodium hydrogen phosphate buffer containing 30 mM citric acid (pH 4) and 1 μM 17:0 LPA (internal standard). Subsequently, LPA was extracted by liquid-liquid extraction using 500 μL butanol. The samples were analysed by LC-MS. IC<sub>50</sub> values were calculated by applying a sigmoidal Hill equation.

**Supplemental Table S3** Eurofin Cerep profile of Ex<sub>31</sub>. Values represent relative activities in Ex<sub>31</sub> treated samples (10 μM) compared to untreated controls.

**Supplemental Table S4** In the CCl<sub>4</sub> study, male Sprague Dawley rats were administered CCl<sub>4</sub> (0.25 mL kg<sup>-1</sup> in olive oil, twice weekly) for a total duration of 10 weeks. After 6 weeks, animals were stratified based on plasma collagen IV (Col. IV) and 18:1 LPA levels. Each row represents one individual animal, which was assigned to the Vehicle (columns 1 and 2) or Ex<sub>31</sub> (columns 3 and 4) group.

**Supplemental Table S5** Male Wistar rats were fed a CDAA diet for a total duration of 14 weeks. After 9 weeks, animals were stratified based on histology assessment of liver biopsies. Values represent quantification of fibrotic area based on collagen I-stained liver sections. Each row represents one individual animal, which was assigned to the Vehicle (column 1) or Ex<sub>31</sub> (column 2) group.

**Supplemental Figure S6** Normalised plasma lysophosphatidylcholine (LPC) levels and lysophosphatidic acid to LPC ratios in choline-deficient amino acid-defined diet-induced liver injury model. Data represent mean  $\pm$  SEM, n=12, \*\*p<0.01.

**Supplemental Table S7** Permeability of LPA species in  $\text{cm} \cdot \text{sec}^{-1}$ .

**Supplemental Table S8** List of TaqMan assays and primers/probes for RT-PCR.

**Supplemental Figure S9** Synthesis scheme for ATX inhibitor Ex\_31 (Example 31(24)).

**Supplemental Figure S10** Analytical Data for Ex\_31:  $R_f = 0.4$  (10% MeOH:Dichloromethane); m.p.: 176-180 °C; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 12.01 (s, 1 H), 8.20 (dd, J = 4.7, 1.3 Hz, 1 H), 7.93-7.90 (m, 1 H), 7.50-7.46 (m, 1 H), 7.20-7.17 (m, 1 H), 7.11 (dd, J = 7.8, 4.8 Hz, 1 H), 6.90-6.83 (m, 1 H), 5.53-5.48 (m, 2 H), 4.66 (s, 2 H), 3.82-3.75 (m, 2 H), 2.79-2.66 (m, 2 H), 2.34-2.06 (m, 3 H), 1.90-1.65 (m, 5 H), 1.34-1.22 (m, 2 H), 1.05-0.86 (m, 2 H); m/z = 484.2 [M+H]<sup>+</sup>.

**Supplemental Figure S11** *Ex-vivo* measurement of ATX activity in plasma and homogenates from un-perfused and perfused livers. Heparin plasma and un-perfused and perfused livers rat livers were collected and immediately frozen at -20 °C until analysis. 100 mg liver were homogenised in 500  $\mu\text{L}$  PBS buffer using a dry iced cooled Precyllis Evolution (Bertin Technologies). The cell debris were pelleted by centrifugation at 10,000 rpm, 10 min and 4 °C. 400  $\mu\text{L}$  of the supernatants or 35  $\mu\text{L}$  plasma were supplemented with 10  $\mu\text{M}$  18:2 LPC and samples were incubated at 37 °C. After 1 h the reaction was stopped by the addition of 40 mM disodiumhydrogenphosphate buffer containing 30 mM citric acid (pH 4) and 1  $\mu\text{M}$  17:0 LPA (internal standard). Subsequently, LPA was extracted by liquid-liquid extraction using 500  $\mu\text{L}$  butanol as described above. Data represent individual values and mean  $\pm$  SD, n=3.

**Supplemental Figure S12A** Individual relative plasma LPA species in CCl<sub>4</sub> model. LPA levels were determined as described in the materials and methods section. Data represent mean  $\pm$  SEM, n=10 (control) and n=13 (CCl<sub>4</sub>-treated), \*p<0.05, n.s. = not significant. Levels were below detection limit in several samples of the vehicle and CCl<sub>4</sub>/Ex\_31 groups for 16:0 LPA and in the CCl<sub>4</sub>/Ex\_31 group for 18:0 LPA and 18:1 LPA. Thus statistical power calculations are not applicable for these groups (NA).

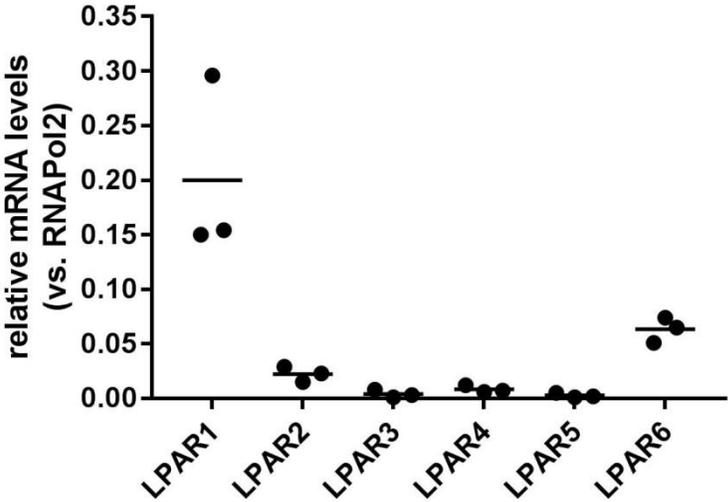
**Supplemental Figure S12B** Individual relative liver LPA species in CCl<sub>4</sub> model. LPA levels were determined as described in the materials and methods section. Data represent mean  $\pm$  SEM, n=10 (control) and n=13 (CCl<sub>4</sub>-treated), \*p<0.05, n.s. = not significant.

**Supplemental Figure S12C** Individual relative plasma LPA species in CDAA model. LPA levels were determined as described in the materials and methods section. Data represent mean  $\pm$  SEM, n=12, \*p<0.05, n.s. = not significant. Levels were below detection limit in several

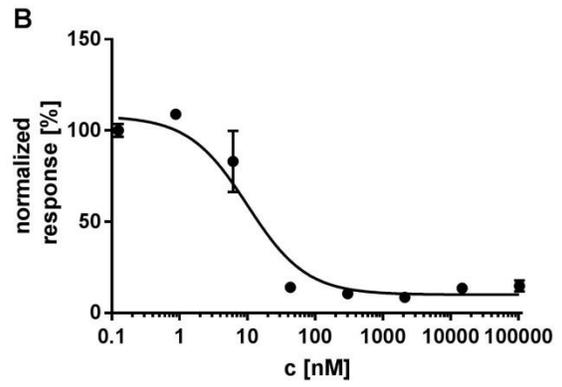
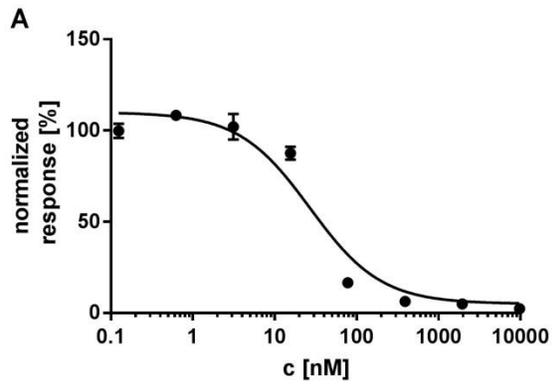
samples of the CDAA/Ex\_31 groups for 16:0 LPA, 18:0 LPA and 18:1 LPA. Thus statistical power calculations are not applicable for these groups (NA).

**Supplemental Figure S12D** Individual relative liver LPA species in CDAA model. LPA levels were determined as described in the materials and methods section. Data represent mean  $\pm$  SEM, n=12, \*p<0.05, n.s. = not significant.

Supplemental Figure S1



Supplemental Figure S2



## Supplemental Table S3

Method	Activity (%)
5-HT transporter (antagonist radioligand)	-6
5-HT2A (h) (agonist radioligand)	3
5-HT2B (agonist site) / [125I](±)DOI / human	3
Acetylcholinesterase ACE (human, AMTCh 400 uM)	-12
Adrenergic alpha2A / [3H]RX 821002 / human	-3
Adrenergic beta1 / [3H](-)CGP 12177 / Human recombinant	9
Adrenergic beta2 / [3H](-)CGP 12177 / Human recombinant	-2
alpha 1A (h) (antagonist radioligand)	18
Benzodiazepine BZD central / [3H]flunitrazepam / Rat brain	-7
Ca <sup>2+</sup> channel L(dihydropyridine site) / [3H](+)PN200-110 / Rat brain	7
COX-1 / arachidonic acid / human	6
COX-2 / arachidonic acid / human	5
D2S (h) (agonist radioligand)	16
Dopamine D1 / [3H]SCH 23390 / Human recombinant	-22
Dopamine transporter / [3H]GBR12935 / Human recombinant	-9
Endothelin ETA / [125I]endothelin-1 / Human recombinant	5
Glutamate NMDA / [3H]CGP 39653 / Rat brain	5
Histamine H1 / [3H]pyrilamine / Human recombinant	5
K <sup>+</sup> channel volt-dependent / [125I]a-dendrotoxin / Rat brain	-1
kappa (KPO) / [3H]U 69593 / Rat	-3
Lck kinase (h)	-4
MAO-A (antagonist radioligand)	11
MCH1 (h) (agonist radioligand)	-4
Muscarinic M2 / [3H]AF-DX 384 / Human recombinant	-8
Muscarinic M3 / [3H]4-DAMP / Human recombinant	15
N neuronal alpha4beta2 (agonist radioligand)	0
Na <sup>+</sup> channel site 2 / [3H]batrachotoxinin / Rat brain	10
Norepinephrine transporter / [3H]nisoxetine / Human recombinant	-1
Opiate delta2 (DOP) / [3H]DADLE / Human recombinant	19
Phosphodiesterase PDE3A (human)	6
Phosphodiesterase PDE4D2 (human)	75
Potassium Channel hERG (human), [3H] Dofetilide	1
RB. Cannabinoid central CB1/[3H]WIN55212-2/Human recombinant	27
RB. Cannabinoid peripheral CB2/[3H]WIN55212-2/Human recombinant	10
RB. Cholecystokinin CCKA/[3H]devazepide/Human recombinant	3
RB.Adenosine A2a/ CGS 21680/ Human recombinant	-11
RB.Histamine H2/[125]APT/Human recombinant	-8
RB.Muscarinic M1/[3H]pirenzepine/Human recombinant (expressed in mammalian cells)	-14
RB.Opiate mu/[3H]DAMGO/Human recombinant (expressed in mammalian cells)	10
Serotonin 5-HT1A / [3H]8-OH-DPAT / Human recombinant	-1
Serotonin 5-HT1B / [125I]CYP / Rat brain	4
Serotonin 5-HT3/ [H3]BRL 43694 / human recombinant (HEK 293 cells)	1
Steroid androgen (AR) / [3H]methyltrienolone / Human	-5
Steroid glucocorticoid / [3H]triamcinolone acetonide / Human lymphoblastoid cells	7
Vasopressin V1a / [3H]AVP / Human recombinant	13

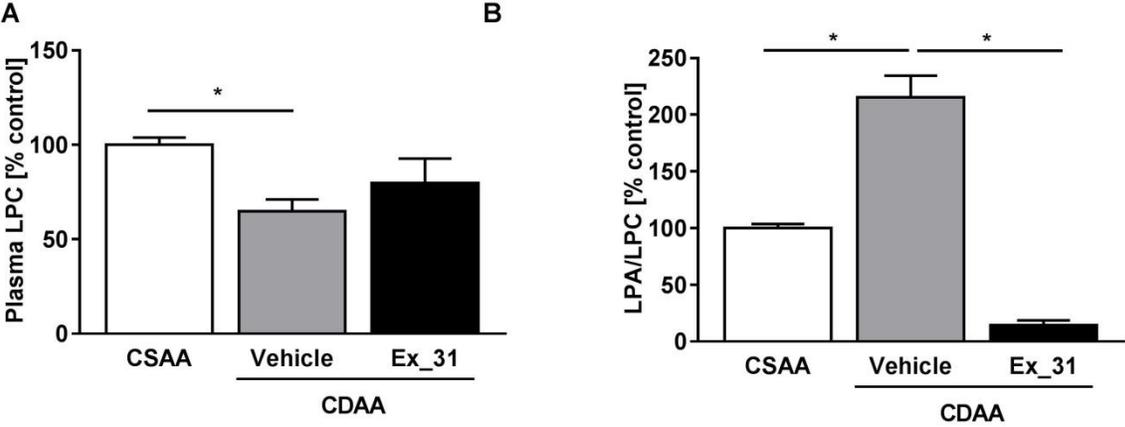
Supplemental Table S4

CCl <sub>4</sub> / Vehicle		CCl <sub>4</sub> / Ex_31	
Col. IV [μg/mL]	LPA (18:1) [nM]	Col. IV [μg/mL]	LPA (18:1) [nM]
4.1	44.7	3.4	34.3
5.7	45.4	3.9	56.1
2.6	44.1	4.6	38.4
12.3	50.9	6.2	39.8
5.1	37.5	7.2	62.6
6.0	30.7	7.0	52.7
3.9	35.2	5.4	36.2
7.2	33.2	5.7	30.9
5.7	41.2	10.8	31.6
3.4	34.3	6.8	37.9
6.2	31.1	2.6	22.4
3.3	42.2	3.1	31.7
4.6	36.1	6.2	47.1
7.1	42.8	4.0	29.9

Supplemental Table S5

<b>CDA Vehicle</b>	<b>CDA Ex_31</b>
<b>fibrotic area [%]</b>	<b>fibrotic area [%]</b>
0.75	0.88
4.00	0.55
0.32	0.47
1.05	2.36
1.95	0.82
2.61	2.15
0.65	1.24
1.46	3.03
2.54	1.77
0.99	1.88
1.59	2.71
2.11	1.28

Supplemental Figure S6



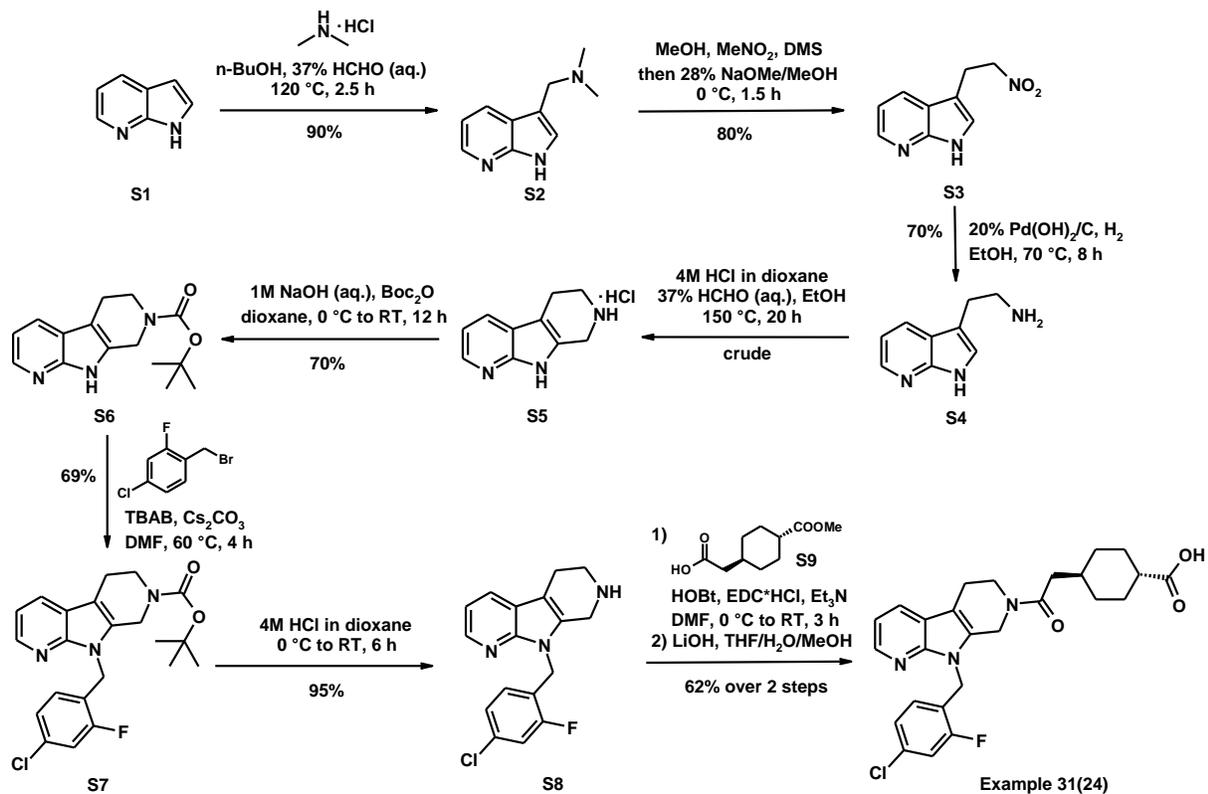
Supplemental Table S7

<b>LPA species</b>	<b>pH 5</b>	<b>pH 6.5</b>	<b>pH 7.4</b>
16:0	$2.6 \cdot 10^{-7}$	$<7.2 \cdot 10^{-8}$	$4.6 \cdot 10^{-7}$
18:1	$<1.8 \cdot 10^{-7}$	$<1.6 \cdot 10^{-7}$	$<1.6 \cdot 10^{-7}$
18:2	$4.1 \cdot 10^{-7}$	$3.6 \cdot 10^{-7}$	$2.9 \cdot 10^{-7}$
20:4	$6.3 \cdot 10^{-7}$	$4.1 \cdot 10^{-7}$	$6.6 \cdot 10^{-7}$
18:0	$<1.5 \cdot 10^{-7}$	$<6.8 \cdot 10^{-8}$	$<9.8 \cdot 10^{-8}$

Supplemental Table S8

<b>Species</b>	<b>Gene</b>	<b>Assay # (or sequence)</b>
rat	RNAPol2 fwd	GCA GGC GAG AGC GTT GAG
rat	RNAPol2 rev	CAT TGG TAT AAT CAA AAC GGA ACT TC
rat	RNAPol2 probe	CTG GCT ACA CTT AAG CCT TCT AAT AAA GC
rat	Lpar1	Rn00588435_m1
rat	Itgam	Rn00709342_m1
rat	Emr1	Rn01527631_m1
rat	Tnfa	Rn01525859_g1
rat	Tgfb	Rn00572010_m1
rat	Ccl2	Rn00580555_m1
rat	Cxcl1	Rn00578225_m1
human	ACTA2	Hs00426835_g1
human	CTGF	Hs01026927_g1
human	CCL2	Hs00234140_m1
human	CXCL1	Hs00236937_m1
human	LPAR1	Hs00173500_m1
human	LPAR2	Hs01109356_m1
human	LPAR3	Hs00173857_m1
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human	RNAPol 2	Hs00172187_m1

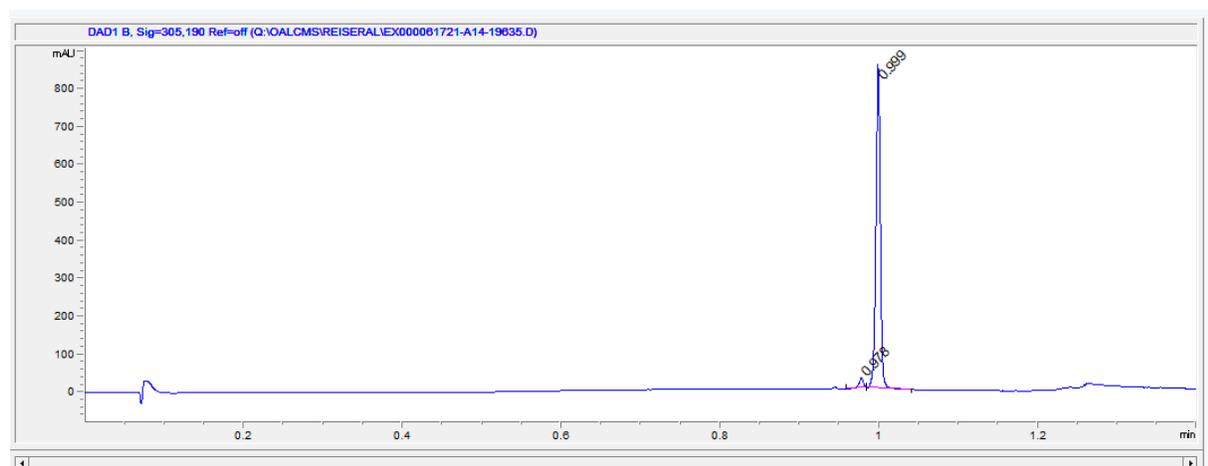
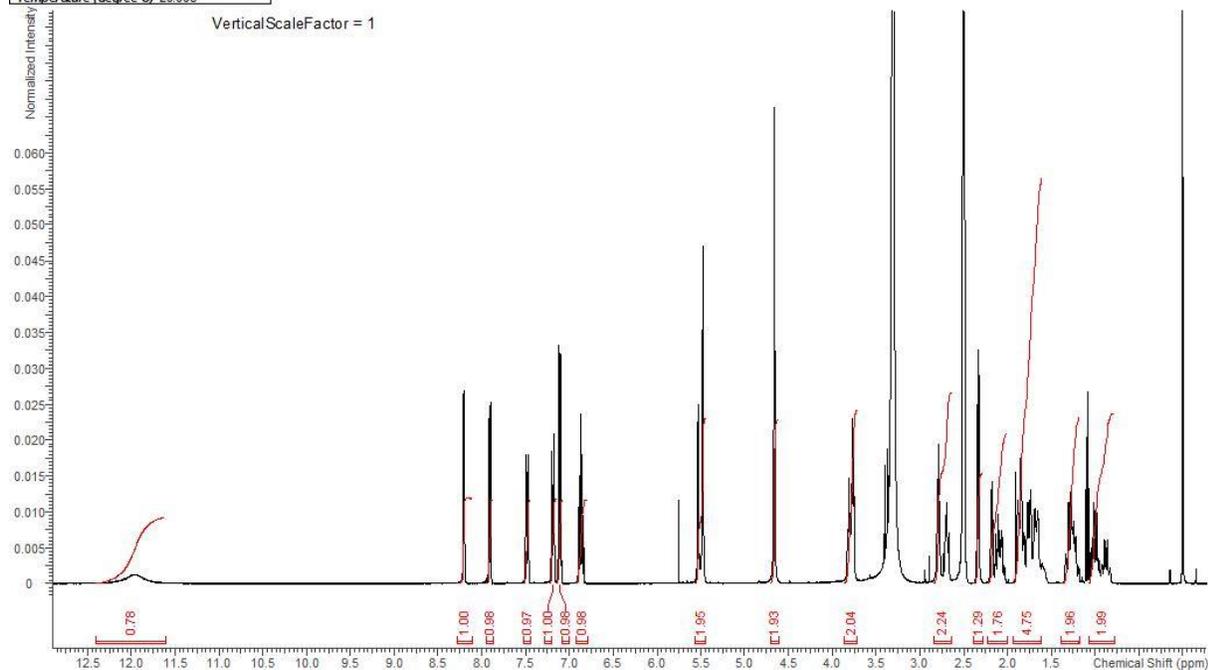
## Supplemental Figure S9



# Supplemental Figure S10

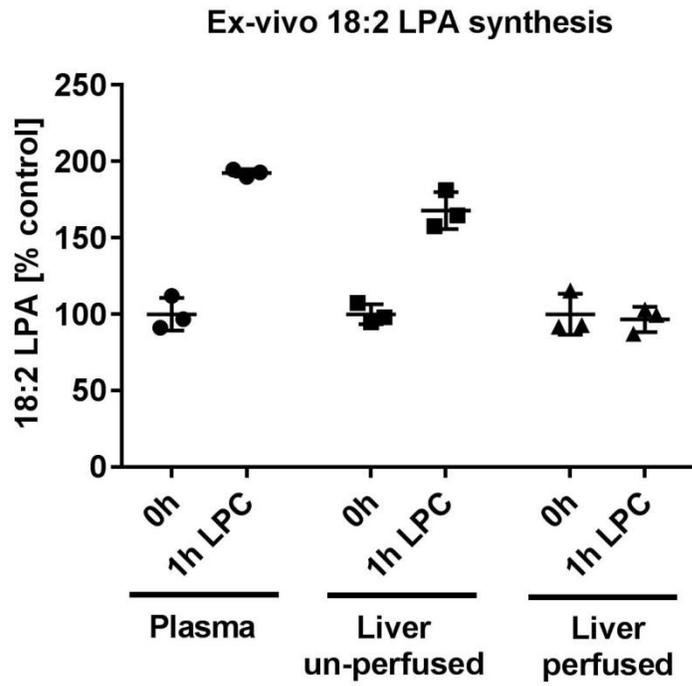
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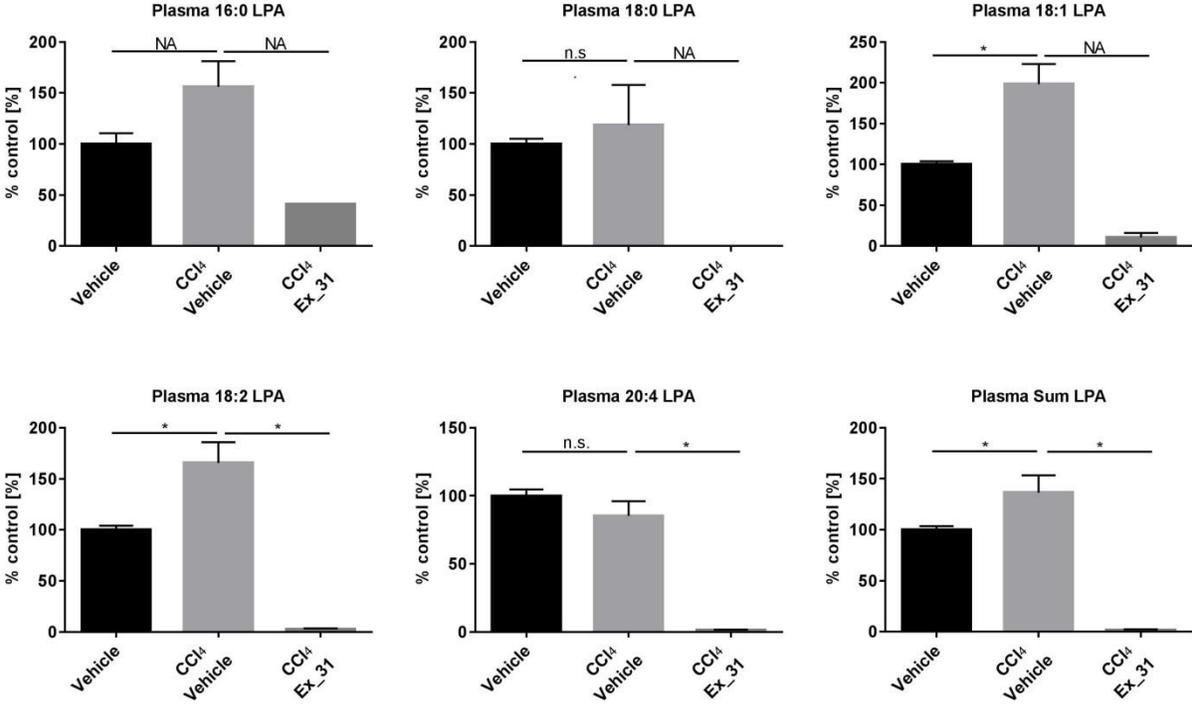


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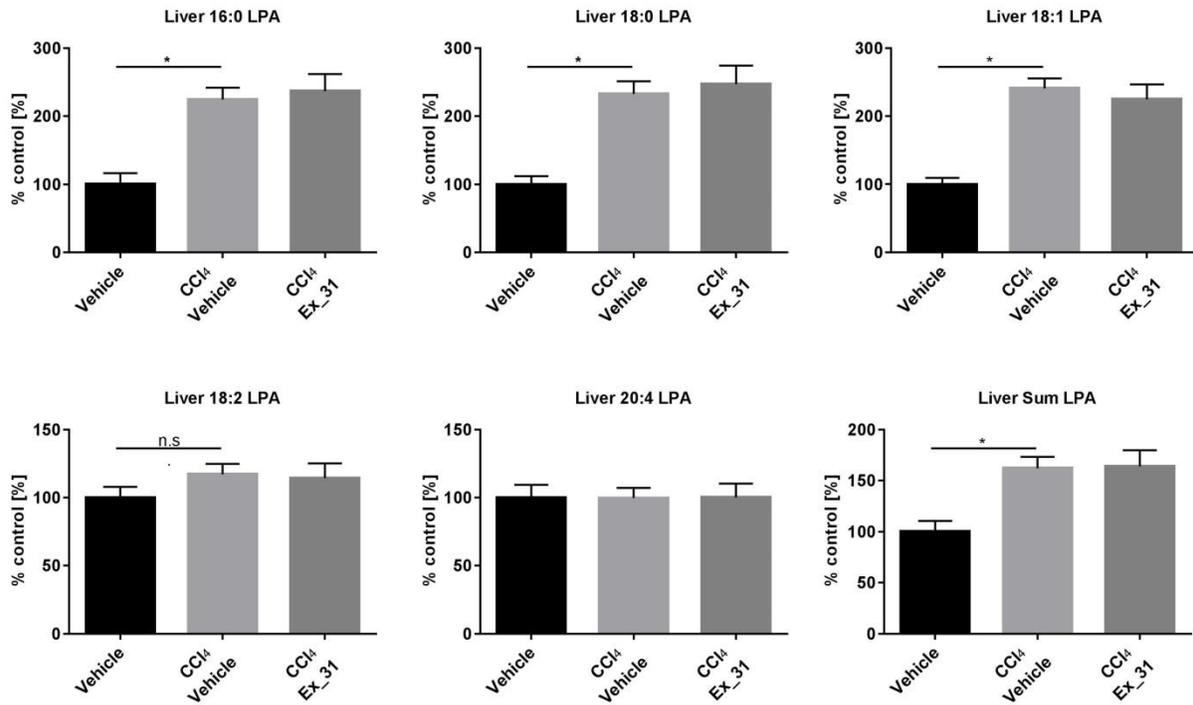
Supplemental Figure S11



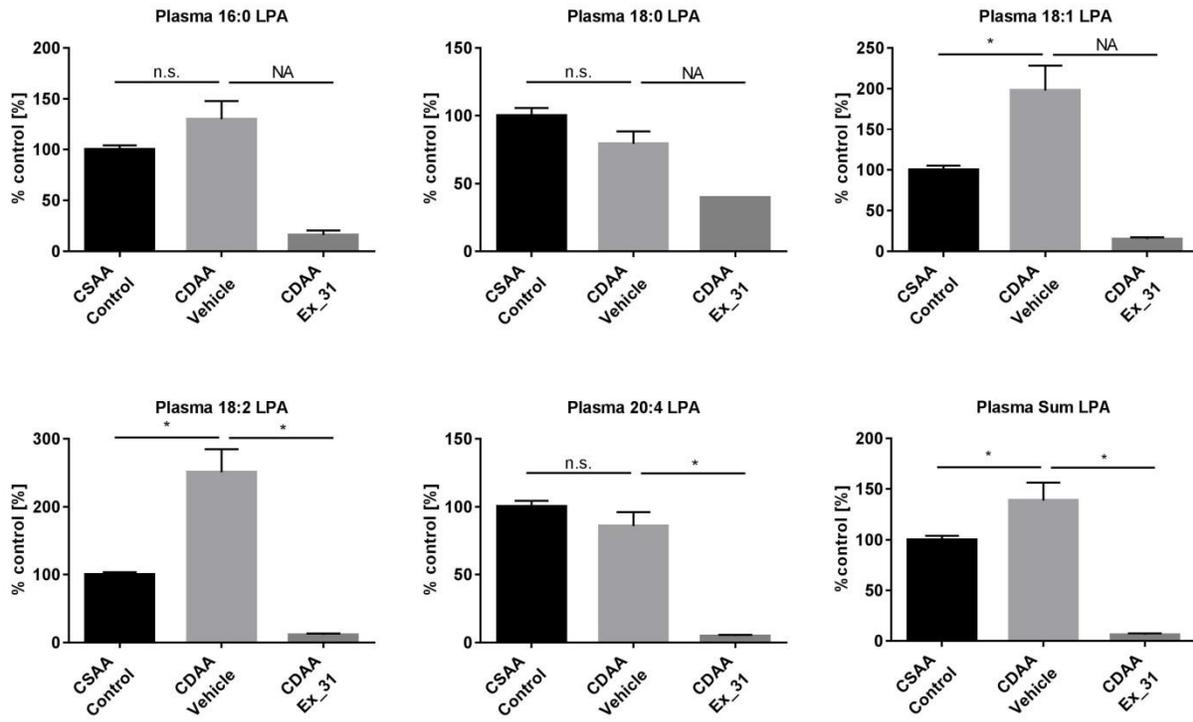
Supplemental Figure S12A



Supplemental Figure S12B



Supplemental Figure 12C



Supplemental Figure 12D

