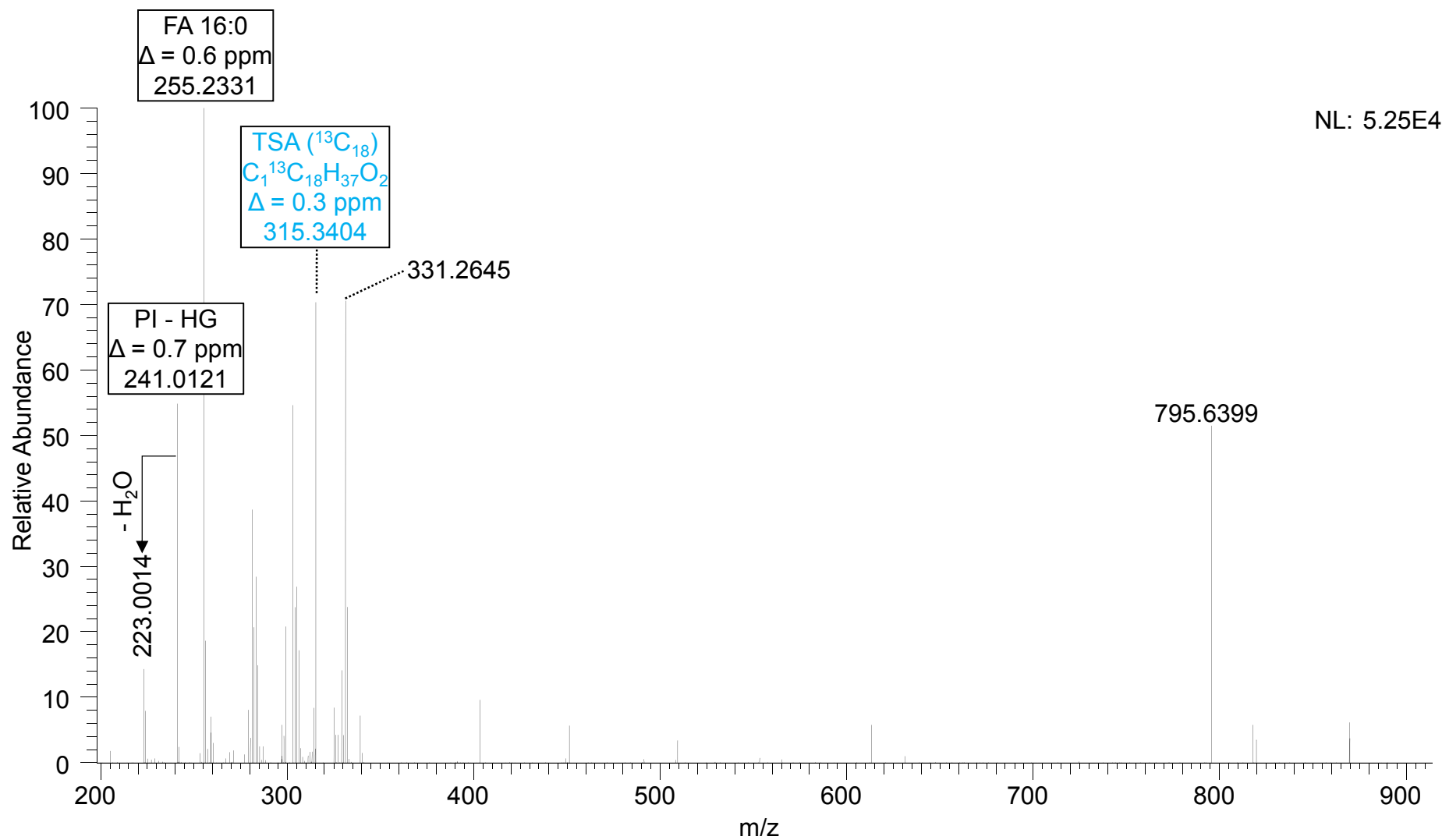
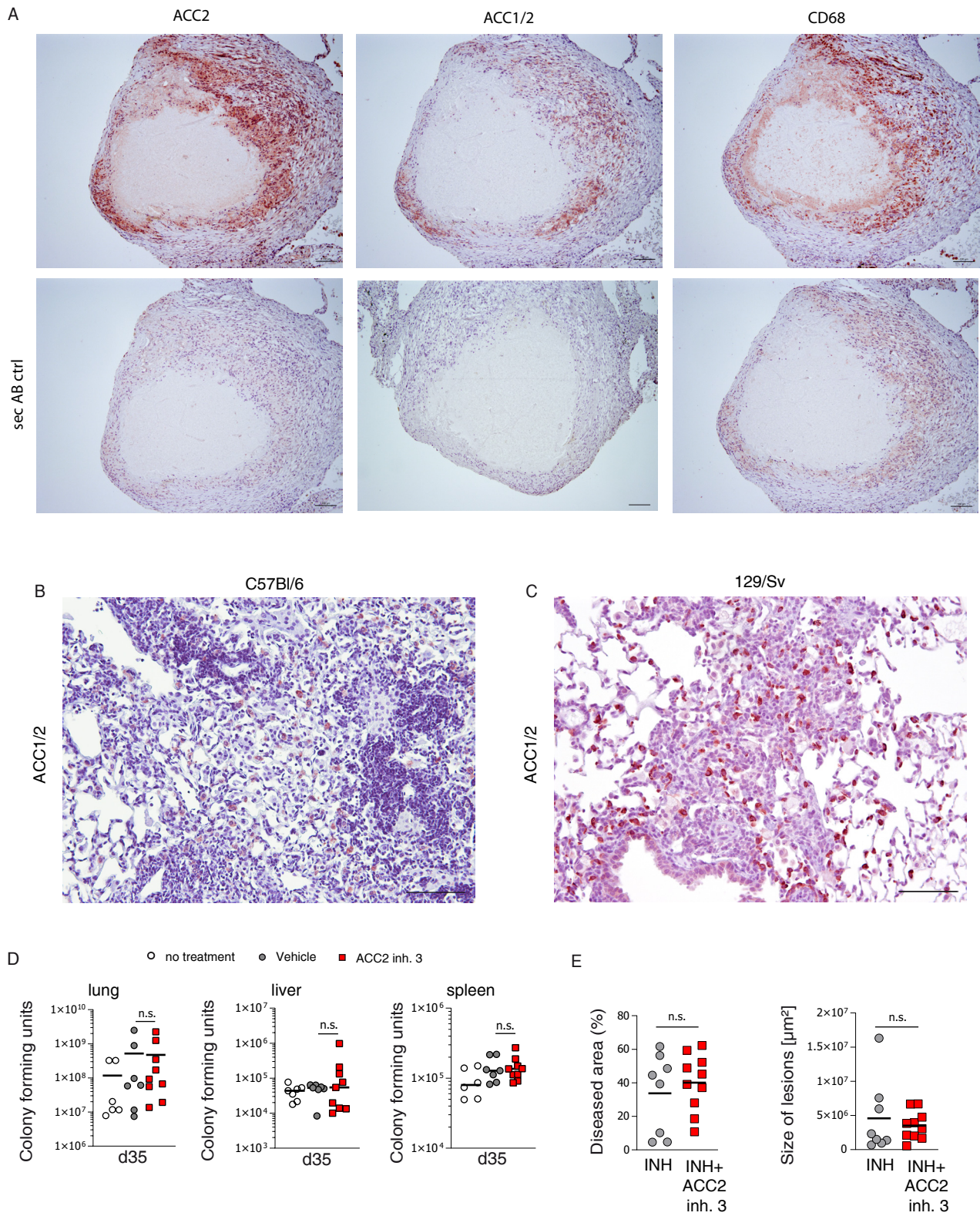
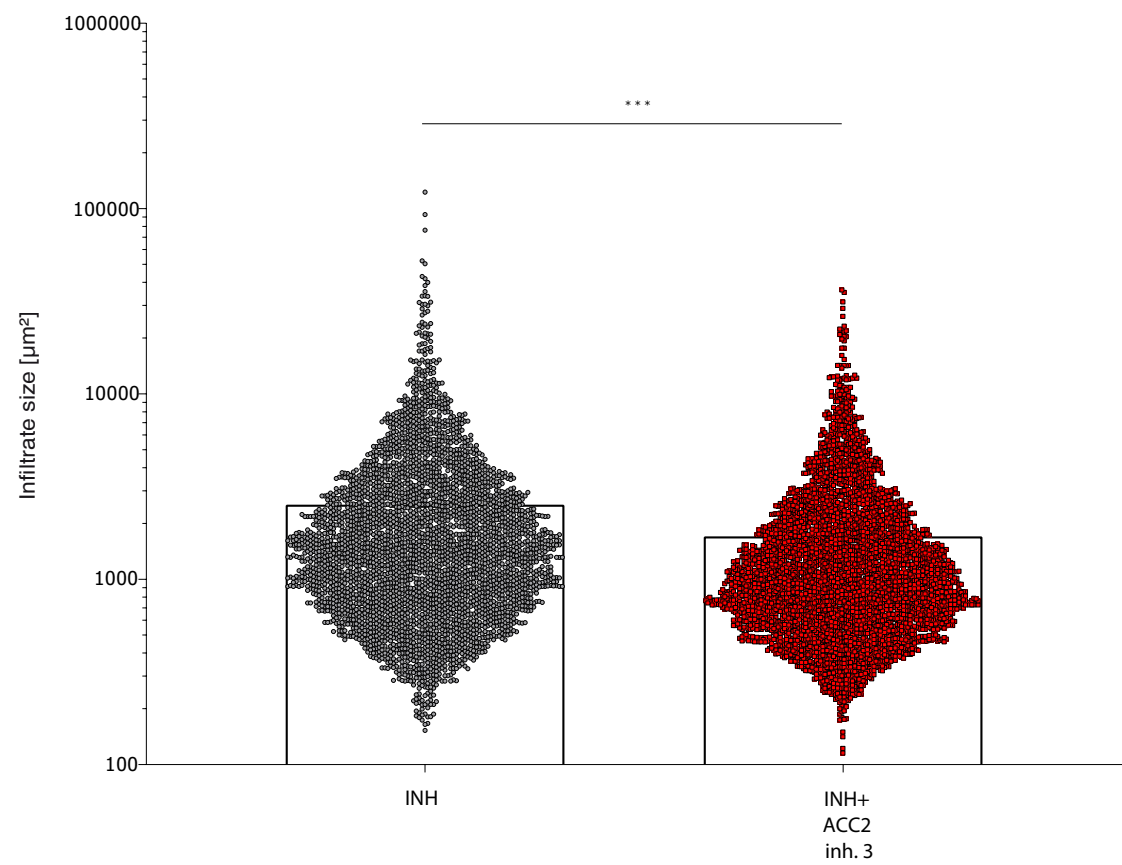


MS² at m/z 869.6 – PI 16:0_19:0 (TSA-¹³C₁₈)





Supplementary figure legends:

Figure S1: WNT6 expression in lungs of TB patients.

Immunohistochemical analyses of formalin-fixed and paraffin-embedded lung tissue from three tuberculosis patients. Sections (1 μm) were incubated with antibodies specific for WNT6 (**A, D**), the macrophage/monocyte marker CD68 (**B**), the lipid droplet scaffolding protein Perilipin 2 (ADFP). (**E**) or without the respective primary antibody as a control (**C, F ctrl**). Antigens were visualized with a horseradish-peroxidase (HRP)-based detection system using AEC as chromogen. Scale bar: 50 μm and 100 μm (ctrl of patient 3)).

Figure S2: **WNT6 and neutral lipids in Mtb-infected *IL-13* overexpressing mice.**
Frozen lung tissue sections (5 µm) derived from Mtb-infected *IL-13* overexpressing mice were stained for neutral lipids with Oil-Red O (**A, B**; d104 p.i.) or BODIPY 493/503 (**C**; d63 p.i.). The section in (C) was stained for neutral lipids in the absence of primary antibody for WNT6 (sec. AB ctrl.), serving as a control to images presented in Figure 1D. Nuclei were stained with DAPI (blue); N, Necrosis; Scale bar, 20µm (C); (A,B), total magnification of 200x and 400x, respectively.

Figure S3: **WNT6 reduces CE levels, while not affecting PC.**

Mass spectrometry-based quantification of total PC **(A)** and CE **(B)** species in WNT6-overexpressing and control (ctrl, LacZ) NIH3T3 cells. Data are from the same set of experiments as depicted in Figure 2C; each n=3. **(C)** Mass spectrometry-based quantification of PC (36:2) levels in *Wnt6*^{+/+} or *Wnt6*^{-/-} BMDMs incubated for 24h in the absence (BSA, ctrl) or presence of fatty acids (oleate-BSA, 200 μ M). Data are from the same set of experiments as depicted in Figure 2E; n=2. Statistical analyses were carried out using One-Way ANOVA with a Holm-Sidak's multiple comparisons test; **p \leq 0.01. n.s.; not significant. All data are depicted as mean \pm SEM.

Figure S4: Effect of *Wnt6*-deficiency on Mtb-induced gene expression, Mtb uptake and growth, nitrite formation and acidification of Mtb-containing compartments in murine BMDM.

(A) Microarray-based expression analyses of Mtb-infected (MOI 3:1) *Wnt6*^{+/+} and *Wnt6*^{-/-} BMDMs. Depicted are the top 10 enriched gene sets derived from a gene set enrichment analysis (Reactome database) of all differentially regulated genes. Further details are found in the *Material and Methods* section; n=3. **(B)** qRT-PCR based *Plin3* mRNA expression analyses of *Wnt6*^{+/+} and *Wnt6*^{-/-} BMDMs infected for 24h with various doses (MOIs) of Mtb; n=3. **(C)** CFU analysis of Mtb-infected (MOI 0.5:1) *Wnt6*^{+/+} and *Wnt6*^{-/-} BMDMs at different time points. Bacterial growth was related to the number of macrophages (normalized CFU); n=3-4. **(D)** Quantification of nitrite (NO₂⁻) in culture supernatants of Mtb-infected *Wnt6*^{+/+} and *Wnt6*^{-/-} BMDMs by Griess reaction (samples are derived from the set of experiments shown in Figure S4C and 3G); n=2. **(E)** Fluorescence microscopy-based visualization (left panel) and quantification (right panel) of acidified (LysoTracker, red, 400 nM) Mtb-containing compartments from *Wnt6*^{+/+} and *Wnt6*^{-/-} BMDMs 4h post infection with GFP-Mtb, which was heat-inactivated (85°C, 5 minutes) or remained viable (untreated); DAPI (blue, 1 µg/ml). Evaluation of LysoTracker positive phagosomes was conducted in a blinded fashion of over 200 compartments per condition in a total of 3 independent experiments. Scale bar, 10µm). **(F)** CFU analysis of Mtb-infected (MOI as indicated) hMDMs at day 0 (4h) and day 7 post infection; n=4 (MOI 0.5); n=6 (MOI 1:1). Cells were incubated with 0.1% DMSO and are from the same set of experiments shown in Figures 4B,C,D and 4F. Statistical analyses were carried out using One-Way ANOVA with a suitable post-hoc test for multiple comparison; **p≤0.01, ***p≤0.001. All data are depicted as mean +/- SEM.

Figure S5: **ACC2 inhibitor treatment does not affect human macrophage viability, cytokine formation, lactate dehydrogenase and TNF α release of human macrophages, neither does it reduce Mtb growth in liquid culture.**

(A,B) Real-time impedance based estimation of human macrophage viability (expressed as cell index) in the presence of various ACC2 inhibitors, isoniazid and staurosporine (positive control). Depicted is representative data from 2 independent experiments with similar results. **(C)** Mtb growth in liquid culture in the absence (solvent, DMSO) and presence of various ACC2 inhibitors or rifampicin as determined by measuring fluorescence of GFP-expressing Mtb in a microplate reader at day 7 p.i.; n=2 (left panel), n=3 (right panel). **(D)** TNF α release of hMDMs infected for 24h with Mtb and simultaneously incubated with solvent (DMSO, ctrl) or the indicated concentrations of ACC2 inhibitor 1; n=3. **(E)** CFU analysis of Mtb-infected (MOI 0.5:1) hMDMs at day 7 p.i. after treatment with various doses of fatty acids (oleate-BSA or palmitate-BSA). Cells were incubated in the presence of 0.1% DMSO. Data are derived from the same set of experiments depicted in Figure 4F; n=4. **(F)** Flow cytometry-based quantification of relative mitochondrial activity in Mtb-infected (MOI 0.1:1) wild-type (WT) and ACC2 KO human macrophage-like cells (BLaER1 macrophages) as assessed by Rhodamine 123 signals relative to MitoTracker Deep Red signals ((both aMFI) x100). Data is expressed in % of signals from uninfected WT cells (=100%). **(G)** Quantification of Lactate Dehydrogenase (LDH) release from hMDMs treated with various doses of ACC2 inhibitor 1 for 7 days; Data was normalized to the maximum release when cells were incubated with 1% Triton X-100 for 10 minutes (=100%); n=3. **(H)** Pre-labelled (200 μ M 13 C-oleate-BSA) hMDMs were infected with Mtb (MOI 1:1) and subsequently incubated in the absence (“-“, solvent) and presence of ACC2 inhibitor 3 (“+”, 400 nM) for 7 days. % isotope labeling (12 C vs 13 C) in tuberculostearic acid (TSA, C19:0) from the mycobacterial membrane lipid PI 16:0_19:0 as determined by mass-spectrometry. Data is derived from the same set of experiments shown in Figure 4I (left panel); n=4. Statistical analyses were carried out using One-Way ANOVA with a suitable post-hoc test for multiple comparison (d); * p \leq 0.05, ***p \leq 0.001. All data are depicted as mean +/- SEM.

Figure S6: Incorporation of $^{13}\text{C}_{18}$ -labelled oleic acid into PC, TAG and CE of human macrophages.

Representative spectra of samples from Mtb-infected (MOI 1:1) hMDMs (d7 p.i.), demonstrating the incorporation of $^{13}\text{C}_{18}$ oleic acid into TAGs, PCs and CEs. The mass shifts and $^{13}\text{C}_{18}$ -labelled lipid species are highlighted in blue. **(A)** Positive ion mode MS^1 spectrum with assignments of selected major abundant lipids. **(B)** Zoom into the MS^1 spectrum showing signals for CE 18:1 and CE 18:1 ($^{13}\text{C}_{18}$) (upper panels) and the respective cholesterol-derived MS^2 fragment ion at m/z 369.35 (lower panels). The indicated transitions are utilized for quantitation of CEs in this study. Data from donor 2 is shown (see also Figure 4H and 4I). * - known signals of chemical background.

Figure S7: Metabolization of oleic acid into tuberculostearic acid (TSA) containing PI 16:0_19:0 (TSA) by Mtb.

Representative MS² spectrum of samples from Mtb-infected (MOI 1:1) hMDMs at day 7 p.i.. MS² spectrum of the precursor with the *m/z* 869.6 shows expected specific fragment ions for identification (PI - HG: fragments ion of the phosphatidylinositol head group, FA 16:0: palmitic acid, and TSA (¹³C₁₈): labelled tuberculostearic acid with incorporation of one ¹²C methyl group as shown previously (<https://doi.org/10.1101/2021.02.04.429149>)). ¹³C₁₈ labelled PI 16:0_19:0 (TSA) in negative ion mode. ¹³C₁₈-labelled lipid species are highlighted in blue. Data from donor 2 is shown (see also Figure 4H and 4I).

Figure S8: **The role of ACC2 in vivo.**

(A) Immunohistochemical detection of ACC2 (left panel), ACC1/2 (middle panel) and the macrophage/monocyte marker CD68 (right panel) in lung tissue sections derived from a tuberculosis patient (Patient 1, consecutive sections). The upper panel shows sections (1 μ m) incubated with primary and secondary antibody. The lower panel shows the respective consecutive section, which was incubated without primary antibody (sec. AB ctrl). Stainings shown for ACC2 and CD68 are the same that are shown in Figure 5A and 5B. Scale bar, 100 μ m. **(B,C)** Immunohistochemical detection of ACC1/2 in lung tissue sections derived from Mtb-infected (~1000 CFU) C57BL/6 (B) or (~100 CFU) 129/Sv (C) mice (both day 42 p.i.). A representative staining of two independent observations is shown. Scale bar, 100 μ m. **(D)** Effect of a low-dose ACC2 inhibitor treatment on mycobacterial loads in Mtb-infected mice. After 28 days of infection with Mtb (~200 CFU), 129/Sv mice were either left untreated (white symbols), were treated with vehicle solution (grey) or with ACC2 inhibitor 3 (ND-646, 25 mg/kg BW, red) for 7 days. At day 35 p.i., Mtb bacterial burden was determined in lung, liver and spleen (n=6-10 animals per group). **(E)** Effect of adjunct ACC2 inhibitor treatment on lung histopathology of Mtb-infected 129/Sv mice. After 28 days of infection with Mtb (~200 CFU), 129/Sv mice were treated for 14 days with isoniazid (INH) alone (10 mg/kg bodyweight (BW), n=8, grey) or with ACC2 inhibitor 3 (ND-646, 25 mg/kg BW) in combination with INH (n=10, red). Quantification of the diseased area (% per lung section) and the average size of the lesions (per section) are shown. Statistical analyses were carried out using an one-tailed, unpaired Student's t-test; n.s.= not significant. Data are depicted as scatter dot plot with line at mean.

Figure S9: Adjunct ACC2 inhibitor treatment reduces the average size of mononuclear infiltrates in the livers of Mtb-infected mice.

After 28 days of infection with Mtb (~200 CFU), 129/Sv mice were either treated for 14 days with isoniazid (INH) alone (10 mg/kg bodyweight (BW), n=8, grey) or with ACC2 inhibitor 3 (ND-646, 25 mg/kg BW) plus INH (n=10, red). Shown is the size of all identified mononuclear infiltrates from each mouse of the respective treatment groups. Analyses was performed on two liver lobes per mouse. The figure derives from the same dataset as Figure 5K. Statistical analysis was carried out using a Mann-Whitney U-Test; *** $p \leq 0.001$.

Supplementary table I:

MASS	NAME	time point status inhibitor	4h inf	7d inf	7d inf ND646	4h inf	7d inf	7d inf ND646	4h inf	7d inf	7d inf ND646	4h inf	7d inf	7d inf ND646
	donor		1	1	1	2	2	2	3	3	3	4	4	4
	TAG [52:2]	%label	89,3	44,8	36,4	90,4	53,4	44,6	57,7	17,0	13,0	78,6	50,6	60,9
	TAG [52:3]	%label	72,0	37,8	28,3	78,1	48,0	49,6	33,3	10,8	9,7	60,0	38,3	53,8
	TAG [54:2]	%label	92,6	53,5	42,5	93,2	61,9	66,2	69,1	21,0	21,6	84,5	59,0	70,6
	TAG [54:3]	%label	98,2	68,7	63,9	98,6	77,9	74,0	80,9	27,4	21,1	94,6	74,5	85,0
	TAG [54:4]	%label	87,4	35,0	29,7	85,9	40,1	50,2	46,7	9,5	5,7	72,4	37,9	54,4
	TAG [56:6]	%label	71,8	27,9	27,5	75,2	33,0	38,2	29,5	7,9	10,1	54,2	26,5	44,1
	TAG [52:2]	pmol label	2,81E+03	7,57E+01	5,70E+00	2,06E+03	4,42E+02	1,03E+01	4,15E+02	2,08E+02	7,78E+00	3,18E+03	2,64E+03	3,49E+02
	TAG [52:3]	pmol label	9,32E+02	4,64E+01	2,82E+00	8,12E+02	3,60E+02	2,95E+01	1,02E+02	6,35E+01	4,96E+00	1,12E+03	8,56E+02	2,59E+02
	TAG [54:2]	pmol label	1,03E+03	5,13E+01	4,19E+00	5,89E+02	2,57E+02	8,54E+00	2,98E+02	1,16E+02	4,67E+00	1,06E+03	1,52E+03	2,34E+02
	TAG [54:3]	pmol label	1,27E+04	3,87E+02	2,41E+01	7,95E+03	3,01E+03	9,50E+01	1,19E+03	4,51E+02	2,24E+01	9,63E+03	8,04E+03	1,72E+03
	TAG [54:4]	pmol label	3,13E+03	4,45E+01	3,22E+00	1,51E+03	1,97E+02	1,46E+01	2,79E+02	6,47E+01	2,42E+00	2,08E+03	8,75E+02	3,08E+02
	TAG [56:6]	pmol label	8,96E+02	7,24E+01	8,99E+00	7,84E+02	1,76E+02	2,10E+01	1,57E+02	8,34E+01	1,46E+01	7,72E+02	6,26E+02	3,61E+02
	912,9225 TAG [52:2]	H106 C19 C136 O6 N1	1,86E+03	3,17E+01	2,33E+00	1,39E+03	1,92E+02	4,36E+00	1,99E+02	4,81E+01		1,81E+03	1,11E+03	1,68E+02
	894,8621 TAG [52:2]	H106 C37 C18 O6 N1	1,09E+03	7,89E+01	6,68E+00	7,65E+02	4,06E+02	1,06E+01	3,40E+02	3,88E+02	1,80E+01	1,75E+03	2,55E+03	2,75E+02
	876,8018 TAG [52:2]	H106 C55 O6 N1	1,93E+02	5,83E+01	6,64E+00	1,26E+02	2,29E+02	8,00E+00	1,80E+02	7,87E+02	3,97E+01	4,89E+02	1,54E+03	1,31E+02
	910,9065 TAG [52:3]	H104 C19 C136 O6 N1	4,19E+02	1,67E+01	1,17E+00	4,22E+02	1,45E+02	1,26E+01	2,88E+01	6,97E+00		4,57E+02	3,19E+02	1,13E+02
	892,8462 TAG [52:3]	H104 C37 C18 O6 N1	6,79E+02	5,67E+01	4,55E+00	4,93E+02	3,67E+02	2,86E+01	1,43E+02	1,45E+02	1,24E+01	9,83E+02	1,02E+03	2,37E+02
	874,7861 TAG [52:3]	H104 C55 O6 N1	1,96E+02	4,94E+01	4,78E+00	1,25E+02	2,37E+02	1,82E+01	1,35E+02	4,38E+02	4,06E+01	4,22E+02	8,86E+02	1,34E+02
	940,9539 TAG [54:2]	H110 C21 C136 O6 N1	7,21E+02	2,37E+01	1,55E+00	4,17E+02	1,25E+02	3,69E+00	1,54E+02	3,02E+01		6,46E+02	7,39E+02	1,19E+02
	922,8937 TAG [54:2]	H110 C39 C18 O6 N1	3,48E+02	4,53E+01	4,75E+00	1,90E+02	1,98E+02	6,79E+00	2,00E+02	1,99E+02	9,79E+00	4,96E+02	1,22E+03	1,59E+02
	904,8336 TAG [54:2]	H110 C57 O6 N1	4,74E+01	2,69E+01	3,61E+00	2,50E+01	9,20E+01	2,41E+00	7,59E+01	3,26E+02	8,27E+00	1,09E+02	6,25E+02	5,60E+01
	938,9381 TAG [54:3]	H108 C21 C136 O6 N1	5,39E+03	1,80E+02	1,03E+01	3,36E+03	1,45E+03	4,56E+01	5,58E+02	1,62E+02	6,40E+00	4,63E+03	3,84E+03	8,35E+02
	956,9984 TAG [54:3]	H108 C3 C154 O6 N1	5,67E+03	4,28E+01	4,39E+00	3,65E+03	3,75E+02	1,27E+01	1,98E+02	8,75E+00		2,87E+03	1,05E+03	2,93E+02
	920,8778 TAG [54:3]	H108 C39 C18 O6 N1	1,69E+03	2,32E+02	1,40E+01	9,71E+02	1,52E+03	4,92E+01	5,34E+02	6,19E+02	3,72E+01	2,30E+03	4,19E+03	7,02E+02
	902,8176 TAG [54:3]	H108 C57 O6 N1	1,81E+02	1,09E+02	8,99E+00	9,04E+01	5,23E+02	2,09E+01	1,76E+02	8,53E+02	6,28E+01	3,85E+02	1,71E+03	1,92E+02
	936,9221 TAG [54:4]	H106 C21 C136 O6 N1	2,02E+03	1,32E+01	1,72E+00	9,77E+02	6,29E+01	6,40E+00	1,26E+02	5,70E+00		1,13E+03	3,11E+02	1,49E+02
	918,8620 TAG [54:4]	H106 C39 C18 O6 N1	1,30E+03	6,03E+01	3,55E+00	6,40E+02	2,44E+02	1,39E+01	2,73E+02	1,53E+02	6,50E+00	1,29E+03	1,07E+03	2,61E+02
	900,8020 TAG [54:4]	H106 C57 O6 N1	2,57E+02	5,37E+01	5,58E+00	1,42E+02	1,84E+02	8,86E+00	2,01E+02	5,20E+02	3,58E+01	4,53E+02	9,24E+02	1,56E+02
	960,9217 TAG [56:6]	H106 C23 C136 O6 N1	4,78E+02	2,37E+01	3,97E+00	4,38E+02	5,64E+01	8,27E+00	5,10E+01	3,81E+00		3,43E+02	1,77E+02	1,49E+02
	942,8620 TAG [56:6]	H106 C41 C18 O6 N1	5,69E+02	1,05E+02	1,16E+01	4,57E+02	2,39E+02	2,46E+01	2,23E+02	2,09E+02	3,65E+01	6,94E+02	9,67E+02	3,82E+02
	924,8022 TAG [56:6]	H106 C59 O6 N1	2,00E+02	1,31E+02	1,72E+01	1,46E+02	2,38E+02	2,22E+01	2,59E+02	8,41E+02	1,08E+02	3,90E+02	1,22E+03	2,89E+02
	sum TAG OA bound (pmol)	OA bound selected	2,33E+04	1,34E+03	1,17E+02	1,48E+04	6,88E+03	3,08E+02	4,05E+03	5,74E+03	4,17E+02	2,16E+04	2,55E+04	4,80E+03
	sum all TAG (pmol)	all	2,79E+04	5,05E+03	2,50E+02	2,10E+04	1,30E+04	6,68E+02	8,90E+03	1,43E+04	1,21E+03	3,33E+04	5,08E+04	1,02E+04
	PC [32:1]	%label	21,82	6,05	1,65	18,22	10,73	5,22	6,50	1,96	0,66	13,55	6,61	3,08
	PC [34:1]	%label	62,30	26,24	17,52	63,47	32,47	28,59	38,09	11,23	10,07	50,48	28,01	32,14
	PC [34:2]	%label	24,01	17,50	12,01	29,99	22,52	25,30	7,98	5,47	8,19	19,73	17,67	22,74
	PC [36:1]	%label	62,33	31,07	22,11	63,34	35,72	32,89	43,43	13,32	15,35	50,44	34,00	37,69
	PC [36:2]	%label	86,26	42,34	26,88	88,01	52,58	45,59	53,47	15,92	14,13	76,12	45,64	49,46
	PC [36:3]	%label	54,88	19,37	13,44	54,63	22,45	23,23	29,46	6,99	7,62	44,45	21,32	26,12
	PC [38:2]	%label	80,57	41,29	39,97	82,80	50,62	55,43	52,65	21,58	36,36	63,52	44,04	54,24
	PC [38:4]	%label	18,23	7,67	1,93	11,18	8,88	4,73	7,82	4,11	3,02	14,27	8,57	5,08
	PC [38:5]	%label	37,03	18,56	10,59	35,20	20,44	17,90	21,17	8,54	6,63	35,56	23,88	20,83
	PC-O [34:1]	%label	81,27	52,96	43,67	83,49	59,38	57,27	63,02	24,62	21,34	72,44	63,21	64,50
	PC [32:1]	pmol label	4,91E+01	1,63E+01	1,36E+00	2,76E+01	3,20E+01	7,68E+00	1,25E+01	1,69E+01	5,41E+00	3,59E+01	4,00E+01	1,36E+01
	PC [34:1]	pmol label	2,91E+03	6,02E+02	9,08E+01	1,41E+03	7,33E+02	1,21E+02	1,57E+03	6,57E+02	1,95E+02	1,99E+03	1,53E+03	9,55E+02
	PC [34:2]	pmol label	2,04E+02	9,42E+01	1,71E+01	1,15E+02	1,27E+02	7,89E+01	5,97E+01	6,57E+01	1,25E+02	1,51E+02	1,63E+02	2,08E+02
	PC [36:1]	pmol label	4,49E+02	1,57E+02	3,87E+01	1,85E+02	1,54E+02	3,09E+01	4,12E+02	1,22E+02	4,38E+01	2,79E+02	4,81E+02	3,02E+02
	PC [36:2]	pmol label	8,92E+03	1,64E+03	1,87E+02	3,66E+03	2,46E+03	4,73E+02	2,56E+03	7,97E+02	3,35E+02	4,55E+03	2,85E+03	2,22E+03
	PC [36:3]	pmol label	9,90E+02	9,28E+01	1,38E+01	2,93E+02	1,02E+02	3,14E+01	3,57E+02	7,79E+01	5,24E+01	4,85E+02	1,93E+02	1,95E+02
	PC [38:2]	pmol label	1,54E+02	9,69E+01	1,36E+01	7,22E+01	1,61E+02	3,21E+01	2,37E+02	7,30E+01	6,65E+01	1,23E+02	2,09E+02	1,58E+02
	PC [38:4]	pmol label	7,09E+01	3,51E+01	6,87E+00	2,53E+01	3,24E+01	6,85E+00	4,81E+01	2,88E+01	2,21E+01	4,64E+01	7,40E+01	5,96E+01

PC [38:5]	pmol label	2,30E+02	1,44E+02	5,77E+01	1,29E+02	1,29E+02	7,74E+01	1,43E+02	8,09E+01	1,23E+02	1,73E+02	2,63E+02	4,48E+02
PC-O [34:1]	pmol label	3,52E+02	5,20E+01	1,01E+01	9,10E+01	5,98E+01	9,47E+00	1,86E+02	6,56E+01	5,16E+00	1,87E+02	1,65E+02	4,35E+01
750,6141 PC [32:1]	C22 H79 C18 O8 N1 P1	8,05E+01	3,07E+01	2,68E+00	4,67E+01	5,78E+01	1,46E+01	2,36E+01	3,31E+01	1,07E+01	6,32E+01	7,51E+01	2,63E+01
732,5539 PC [32:1]	H79 C40 N1 O8 P1	1,44E+02	2,39E+02	7,98E+01	1,06E+02	2,40E+02	1,32E+02	1,69E+02	8,25E+02	7,88E+02	2,02E+02	5,31E+02	4,14E+02
778,6454 PC [34:1]	C24 H83 C18 O8 N1 P1	3,58E+03	9,53E+02	1,55E+02	1,73E+03	1,11E+03	1,88E+02	2,27E+03	1,18E+03	3,54E+02	2,64E+03	2,39E+03	1,44E+03
760,5850 PC [34:1]	H83 C42 N1 O8 P1	1,08E+03	1,34E+03	3,64E+02	4,97E+02	1,15E+03	2,34E+02	1,85E+03	4,67E+03	1,58E+03	1,29E+03	3,07E+03	1,52E+03
776,6298 PC [34:2]	C24 H81 C18 O8 N1 P1	3,29E+02	1,60E+02	3,06E+01	1,77E+02	2,08E+02	1,26E+02	1,11E+02	1,25E+02	2,31E+02	2,52E+02	2,77E+02	3,39E+02
758,5695 PC [34:2]	H81 C42 N1 O8 P1	5,18E+02	3,78E+02	1,12E+02	2,06E+02	3,57E+02	1,86E+02	6,37E+02	1,08E+03	1,29E+03	5,12E+02	6,47E+02	5,76E+02
806,6768 PC [36:1]	C26 H87 C18 O8 N1 P1	5,54E+02	2,40E+02	6,34E+01	2,26E+02	2,27E+02	4,65E+01	5,74E+02	2,16E+02	7,60E+01	3,71E+02	7,18E+02	4,38E+02
788,6164 PC [36:1]	H87 C44 N1 O8 P1	1,68E+02	2,66E+02	1,12E+02	6,55E+01	2,05E+02	4,74E+01	3,74E+02	7,03E+02	2,10E+02	1,82E+02	6,97E+02	3,62E+02
804,6611 PC [36:2]	C26 H85 C18 O8 N1 P1	3,82E+03	1,87E+03	2,82E+02	1,50E+03	2,39E+03	5,24E+02	2,22E+03	1,52E+03	6,65E+02	2,68E+03	3,00E+03	2,23E+03
822,7215 PC [36:2]	C8 H85 C136 O8 N1 P1	5,72E+03	6,03E+02	5,68E+01	2,37E+03	9,75E+02	1,69E+02	1,22E+03	1,78E+02	7,07E+01	2,49E+03	1,15E+03	8,88E+02
786,6007 PC [36:2]	H85 C44 N1 O8 P1	8,10E+02	1,40E+03	3,59E+02	2,84E+02	1,31E+03	3,43E+02	1,35E+03	3,31E+03	1,63E+03	8,01E+02	2,10E+03	1,37E+03
802,6455 PC [36:3]	C26 H83 C18 O8 N1 P1	1,28E+03	1,55E+02	2,43E+01	3,78E+02	1,66E+02	5,10E+01	5,51E+02	1,46E+02	9,73E+01	6,72E+02	3,17E+02	3,09E+02
784,5851 PC [36:3]	H83 C44 N1 O8 P1	5,28E+02	3,24E+02	7,81E+01	1,57E+02	2,87E+02	8,43E+01	6,60E+02	9,69E+02	5,91E+02	4,21E+02	5,87E+02	4,37E+02
832,6925 PC [38:2]	C28 H89 C18 O8 N1 P1	1,70E+02	1,37E+02	1,94E+01	7,90E+01	2,13E+02	4,12E+01	3,10E+02	1,20E+02	9,76E+01	1,50E+02	2,90E+02	2,05E+02
814,6322 PC [38:2]	H89 C46 N1 O8 P1	2,05E+01	9,75E+01	1,46E+01	8,20E+00	1,04E+02	1,66E+01	1,39E+02	2,18E+02	8,62E+01	4,32E+01	1,82E+02	8,66E+01
828,6609 PC [38:4]	C28 H85 C18 O8 N1 P1	1,20E+02	6,52E+01	1,35E+01	4,54E+01	5,95E+01	1,31E+01	8,93E+01	5,53E+01	4,29E+01	8,13E+01	1,36E+02	1,13E+02
810,6007 PC [38:4]	H85 C46 N1 O8 P1	2,70E+02	3,92E+02	3,43E+02	1,80E+02	3,05E+02	1,32E+02	5,26E+02	6,44E+02	6,80E+02	2,44E+02	7,19E+02	1,06E+03
826,6449 PC [38:5]	C28 H83 C18 O8 N1 P1	3,35E+02	2,43E+02	1,04E+02	1,91E+02	2,14E+02	1,31E+02	2,37E+02	1,49E+02	2,30E+02	2,54E+02	4,25E+02	7,42E+02
808,5850 PC [38:5]	H83 C46 N1 O8 P1	2,85E+02	5,33E+02	4,43E+02	1,76E+02	4,16E+02	3,01E+02	4,41E+02	7,99E+02	1,63E+03	2,30E+02	6,78E+02	1,41E+03
746,6062 PC-O [34:1]	H85 C42 N1 O7 P1	8,09E+01	4,65E+01	1,30E+01	1,80E+01	4,09E+01	7,07E+00	1,09E+02	2,01E+02	1,90E+01	7,11E+01	9,60E+01	2,37E+01
764,6663 PC-O [34:1]	C24 H85 C18 O7 N1 P1	3,52E+02	5,20E+01	1,01E+01	9,10E+01	5,98E+01	9,47E+00	1,86E+02	6,56E+01	5,16E+00	1,87E+02	1,65E+02	4,35E+01
sum PC OA bound (pmol)	OA bound selected	2,02E+04	9,52E+03	2,68E+03	8,54E+03	1,01E+04	2,80E+03	1,40E+04	1,72E+04	1,04E+04	1,38E+04	1,82E+04	1,40E+04
sum all PC (pmol)	all	2,41E+04	1,39E+04	4,73E+03	1,05E+04	1,39E+04	4,30E+03	2,04E+04	2,66E+04	1,91E+04	1,76E+04	2,69E+04	2,30E+04
668,6343 Chol [18:1]	H82 C45 O2 N1	1,40E+02	9,79E+01	3,14E+01	2,24E+01	1,18E+02	4,32E+00	2,46E+01	2,30E+02	7,61E+01	8,67E+01	3,65E+02	1,45E+02
686,6944 Chol [18:1]	H82 C27 C18 O2 N1	3,43E+02	5,01E+01	6,04E+00	3,05E+01	8,06E+01	2,62E+00	1,54E+01	5,81E+01	1,98E+01	9,89E+01	2,72E+02	8,76E+01
TAG / PC Ratio (OA bound)	OA bound selected	1,15	0,14	0,04	1,74	0,68	0,11	0,29	0,33	0,04	1,56	1,40	0,34
TAG (all) / PC Ratio (all)	all	1,16	0,36	0,05	2,00	0,94	0,16	0,44	0,54	0,06	1,90	1,89	0,44
Chol 18:1 / PC Ratio (all)	OA bound selected	0,024	0,016	0,014	0,006	0,020	0,002	0,003	0,017	0,009	0,013	0,035	0,017
% Label TAG	OA bound selected	92,2	50,6	42,0	92,5	64,6	58,1	60,1	17,2	13,6	82,4	57,2	67,3
% Label PC	OA bound selected	70,8	30,8	16,3	70,4	39,5	31,0	39,8	11,5	9,4	57,9	32,7	32,8
% Label Chol [18:1]	OA bound selected	71,0	33,7	16,1	57,4	40,6	37,0	38,2	20,2	20,5	54,1	42,9	38,4
869,6272 PI [i-19:0_16:0]	H84 C26 C18 O13 P1	0,00E+00	8,70E+00	1,15E+01	0,00E+00	3,03E+01	8,02E+00	0,00E+00	6,08E+00	6,42E+00	0,00E+00	6,79E+00	1,50E+01
851,5672 PI [19:0_16:0]	H84 C44 O13 P1	8,19E+00	4,98E+00	1,19E+01	3,85E+00	1,27E+01	4,38E+00	6,04E+00	7,39E+00	9,01E+00	4,83E+00	4,31E+00	9,31E+00
% Label PI [19:0_16:0]		0,0	63,5	49,3	0,0	70,4	64,7	0,0	45,1	41,6	0,0	61,2	61,7

values

Data referring to Fig. 4H,I and Fig. S5H

values

pmol lipid species

values

pmol of ¹³C₁₈ labelled OA in lipid species

values

percentage of lipid labelled with ¹³C₁₈

Supplementary material and methods

Macrophages

To generate bone-marrow derived macrophages (BMDM), mice were sacrificed, and bone-marrow cells were flushed out from femora and tibiae with ice-cold DMEM as described previously (1). To yield high purity and remove contaminating fibroblasts (2), bone-marrow cells were first cultivated in Nunclon Delta cell culture dishes (Thermo Fisher, Waltham, USA) for 24 hours. Only non-adherent cells were collected, and incubated for 7 days in cell culture dishes (Sarstedt, Nümbrecht, Germany) in DMEM containing 10 mM HEPES, 1 mM sodium pyruvate, 4 mM glutamine (Biochrome, Berlin, Germany), 10% of heat-inactivated fetal calf serum (FCS; Pan-Biotek, Aidenbach, Germany), supplemented with 50 ng/ml macrophage colony stimulating factor (M-CSF; Bio-Techne, Minneapolis, USA) (3). To obtain quiescent tissue macrophages (4), peritoneal exudate cells (PEC) were isolated from the resting peritoneal cavity of mice as described previously (1).

Real-time quantitative PCR

Gene-specific primer pairs and TaqMan probes (Universal Probe Library (UPL), Roche Applied Science, Mannheim, Germany) were designed with the UPL assay design center (ProbeFinder Version 2.45 and earlier versions); sequences and probes are given in supplement table II. Crossing point values of target and reference gene (hypoxanthine-guanine phosphoribosyltransferase, HPRT) were determined by the second derivative maximum method. Relative gene expression was calculated with the E-Method (5) considering the individual efficiency of each PCR setup determined by a standard curve or, if this was not possible, by the $2^{-\Delta\Delta CT}$ method.

Neutral lipid quantification by fluorescence microscopy

Isolated peritoneal macrophages were seeded on Chamber Slides (Nunc™ Lab-Tek™ II, Thermo Fisher Scientific, Waltham, US), infected with mCherry-expressing *M. tuberculosis* (MOI 1:1, 24h) and fixed (1% PFA, 24h, 4°C). Subsequently, cells were stained with 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY 493/503, 5 µg/ml; Thermo Fisher) and DAPI (1 µg/ml; Roche Applied Science) and slides mounted with ProLong™ Antifade Reagents (Thermo Fisher). Images were acquired with an Axio Observer microscope, equipped with an ApoTome,

and the AxioVision Software 4.8 or earlier (Carl Zeiss, Oberkochen, Germany). To quantify neutral lipids, images were analyzed with ImageJ software (Version 1.51n) using a macro-script. This analysis included following steps. First, the nuclear area of cells was identified by DAPI staining. This was followed by measuring the area of the BODIPY signals, which was normalized to the nuclear area of the cells. Finally, in order to determine the threshold of BODIPY positive area signals within the images, BODIPY background signals within the nuclear area, which are essentially devoid of neutral lipids, were determined. A BODIPY signal above the average background multiplied by two times the standard deviation was considered positive. At least 200 cells per condition in each individual experiment were analyzed.

Sample preparation and lipid extraction

Total lipids were extracted according to a customized methyl-tert-butyl ether (MTBE) method (6). Briefly, samples were dried in a SpeedVac and solved in 50 μ l of water. Completely dried lung homogenate samples were solved in 20 μ l of 50 mM ammonium acetate instead. Then 270 μ l methanol, containing 3% acetic acid, were added. After vortexing the mixture, internal standard solution (either, standard mixture according to supplement table III) or SPLASH® Lipidomix® Mass Spec Standard according to supplement table IV (330707, Avanti Polar Lipids, Alabaster, US) were added. Afterwards, 1 ml of MTBE was added and the solution was incubated for 1 hour at room temperature with continuous shaking at 600 rpm (Eppendorf, MixMate). Next, 250 μ l of water was added and subsequently incubated for 10 mins at room temperature with continuous shaking at 600 rpm. For phase separation, were centrifuged for 10 min at 15.000 \times g and then the upper phase was collected in a separate tube. The lower phase was re-extracted with 400 μ l theoretical upper phase, vortexed and incubated for 20 min at room temperature with continuous shaking (1300 rpm). The solution was once more centrifuged as described above. The resulting upper phases were combined and subsequently dried in a SpeedVac (Thermo Fisher Scientific). The dried extracts were dissolved in a mixture of chloroform, methanol and water (60/30/4.5; v/v/v) and stored at -80 °C.

Tracing experiments with ¹³C-oleic acid

Lipids were extracted and quantified using shotgun lipidomics as described earlier. Briefly, ¹³C-labeled oleic acid incorporation in macrophages was traced by high

resolution MS1 using the Q Exactive Plus. Incorporation rates for hMDMs were determined for the lipid classes PC, PC-O, TAG, CE and SM using the positive ion mode (Supplement Table I). Quantitation was performed in reference to SPLASH® Lipidomix® (Table III). Metabolization of ^{13}C -labeled OA in Mtb was traced using the major abundant phospholipid PI 16:0_19:0 (TSA) with a semi-targeted lipid analysis in the negative ion mode. The isotopic labelled $^{12}\text{C}_1^{13}\text{C}_{18}$ TSA fragment in MS2 (m/z 315.34) and the $^{12}\text{C}_{19}$ signal (m/z 297.28) were utilized to determine incorporation rates (7).

Supplement table II: Primers used for qRT-PCR

Species	Target gene	Input sequence	Forward primer	Reverse primer	Probe UPL#
<i>H. sapiens</i>	<i>ACACB</i>	NM_001093	tgtcccaggtgctggact	ctgggccacacagctcat	7
	<i>TNF</i>	X01394.1	cagcctcttctcctcctgat	gccagagggctgattagaga	29
	<i>HPRT</i>	NM_000194.1	tgacctgattattttgcatacc	cgagcaagacgttcagtcct	73
	<i>DGAT2</i>	NM_001253891; NM_032564	tactccaagcccatcaccac	ggtgtggtacaggtcgatgtc	78
<i>M. musculus</i>					
	<i>Acacb</i>	NM_133904.2	gcgaaaaccagatgagg	gttcttggtgctgcggaag	17
	<i>Plin3</i>	NM_025836.3	ggaggaacctgtgtgcag	accatcccatacgtggaact	34
	<i>Plin2</i>	NM_007408.3	cctcagctctcctgttaggc	cactactgctgctgccattt	79
	<i>Hprt</i>	NM_013556.2	tcctcctcagaccgctttt	cctggttcacatcgctaadc	95
	<i>Cpt1b</i>	NM_009948.2	gagtgactggtgggaagaatatg	gctgcttgacatttgtgtt	92
	<i>Dgat2</i>	NM_026384.3	ggcgctacttccgagactac	tggtcagcaggtgtgtgtc	42

Supplement table III: Internal standards used for lipid quantification of *in vitro* cultivated cells.

Abbr.	Substance	Supplier/ID	Amount added Apex Qe (pmol)*	Amount added Q Exactive (pmol)#
SM-IS	17:0 SM (d18:1/17:0)	Avanti/ 860585	143.4	14.3
LPC-IS	17:0 Lyso PC	Avanti/ 855676	303.7	30.4
TAG-IS	Glycerol triheptadecanoate	Sigma/ T2151	111.7	11.2
PE-IS	4ME 16:0 Diether PE	Avanti/ 999985	306.7	30.7
PC-IS	4ME 16:0 Diether PC	Avanti/ 999984	181.2	18.1
CE-IS	17:0 Cholesteryl Ester	Avanti/ 110864	335.3	33.5

* IS amounts used for the analysis with the Apex Qe instrument for Figure 2E.

IS amounts used for the analysis with Q Exactive Plus instrument for Figure 2C.

Supplement table IV, SPLASH® Lipidomix® Mass Spec Standard).

Abbr.	Substance	Supplier/ ID	Amount added Q Exactive (pmol)*	Amount added Q Exactive (pmol) [#]
PC-IS	15:0-18:1(d7) PC	Avanti/ 791637	83.65	1175.85
PE-IS	15:0-18:1(d7) PE	Avanti/ 791638	3.14	43.8
PS-IS	15:0-18:1(d7) PS	Avanti/ 791639	2.12	29.55
PG-IS	15:0-18:1(d7) PG	Avanti/ 791640	14.94	205.5
PI-IS	15:0-18:1(d7) PI	Avanti/ 791641	4.19	58.95
PA-IS	15:0-18:1(d7) PA	Avanti/ 791642	4.19	58.8
LPC-IS	18:1(d7) LPC	Avanti/ 791643	18.89	264.75
LPE-IS	18:1(d7) LPE	Avanti/ 791644	4.27	59.25
CE-IS	18:1(d7) Chol Ester	Avanti/ 700185	212.11	2940.15
MAG-IS	18:1(d7) MAG	Avanti/ 791646	2.16	29.1
DAG-IS	15:0-18:1(d7) DAG	Avanti/ 791647	6.27	88.05
TAG-IS	15:0-18:1(d7)-18:1 TAG	Avanti/ 791648	27.64	382.2
SM-IS	18:1(d9) SM	Avanti/ 791649	16.43	235.8
Chol-IS	Cholesterold(d7)	Avanti/ 700041	97.96	1469.55
Cer-IS	Ceramide C17	Avanti/ 860681	37.68	565.35

* IS amounts used for the analysis of ¹³C labeled OA macrophages with Q Exactive Plus instrument for Figure 4H,I [#] IS amounts used for the analysis of lung homogenates with Q Exactive Plus instrument for Figure 5G

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