

SUPPLEMENTARY INFORMATION

A new class of natural anthelmintics targeting lipid metabolism

Hala Zahreddine Fahs^{1,2}, Fathima S. Refai^{1,†}, Suma Gopinadhan^{1,†}, Yasmine Moussa¹, Hin Hark Gan², Yamanappa Hunashal¹, Gennaro Battaglia^{1,3}, Patricia G. Cipriani^{1,2}, Claire Ciancia⁴, Nabil Rahiman¹, Stephan Kremb¹, Xin Xie¹, Yanthe E. Pearson¹, Glenn L. Butterfoss¹, Rick M. Maizels⁴, Gennaro Esposito^{1,5}, Antony P. Page⁶, Kristin C. Gunsalus^{*1,2}, Fabio Piano^{*1,2}

¹ Center for Genomics and Systems Biology, New York University Abu Dhabi, Saadiyat Island, Abu Dhabi, United Arab Emirates

² Center for Genomics and Systems Biology, Department of Biology, New York University, New York, NY, USA

³ Dipartimento di Scienze Chimiche, Università di Napoli “Federico II”, 80138 Naples, Italy

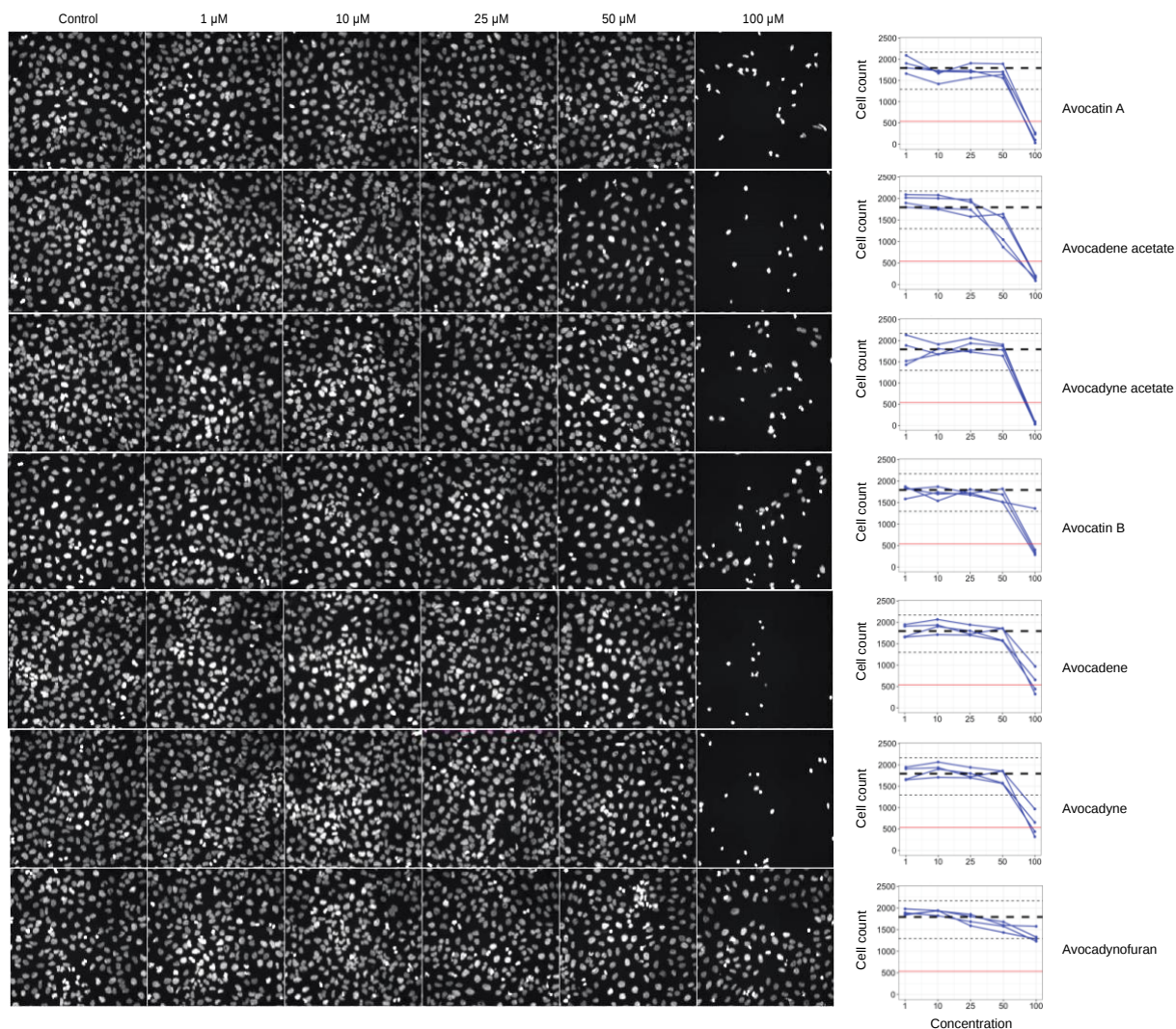
⁴ School of Infection and Immunity, University of Glasgow, Scotland, UK

⁵ Istituto Nazionale Biostrutture e Biosistemi, 00136 Rome, Italy

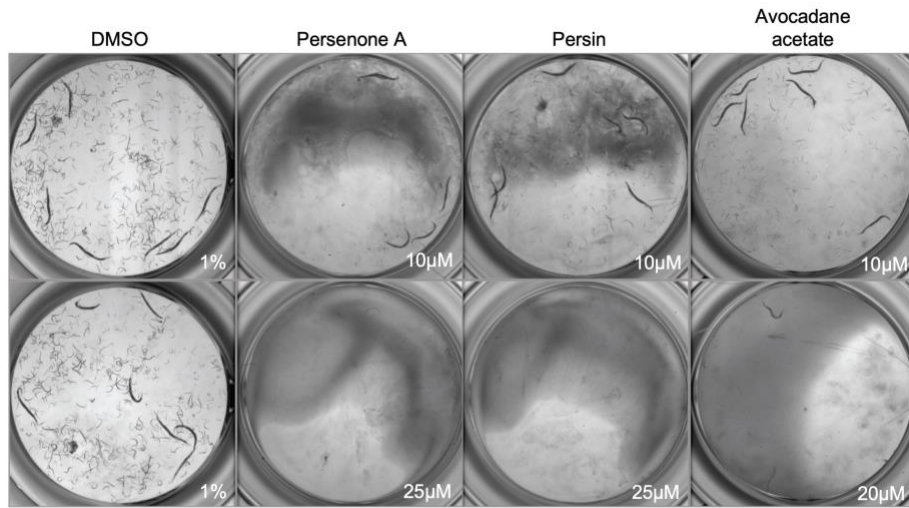
⁶ School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Scotland, UK

† These authors contributed equally

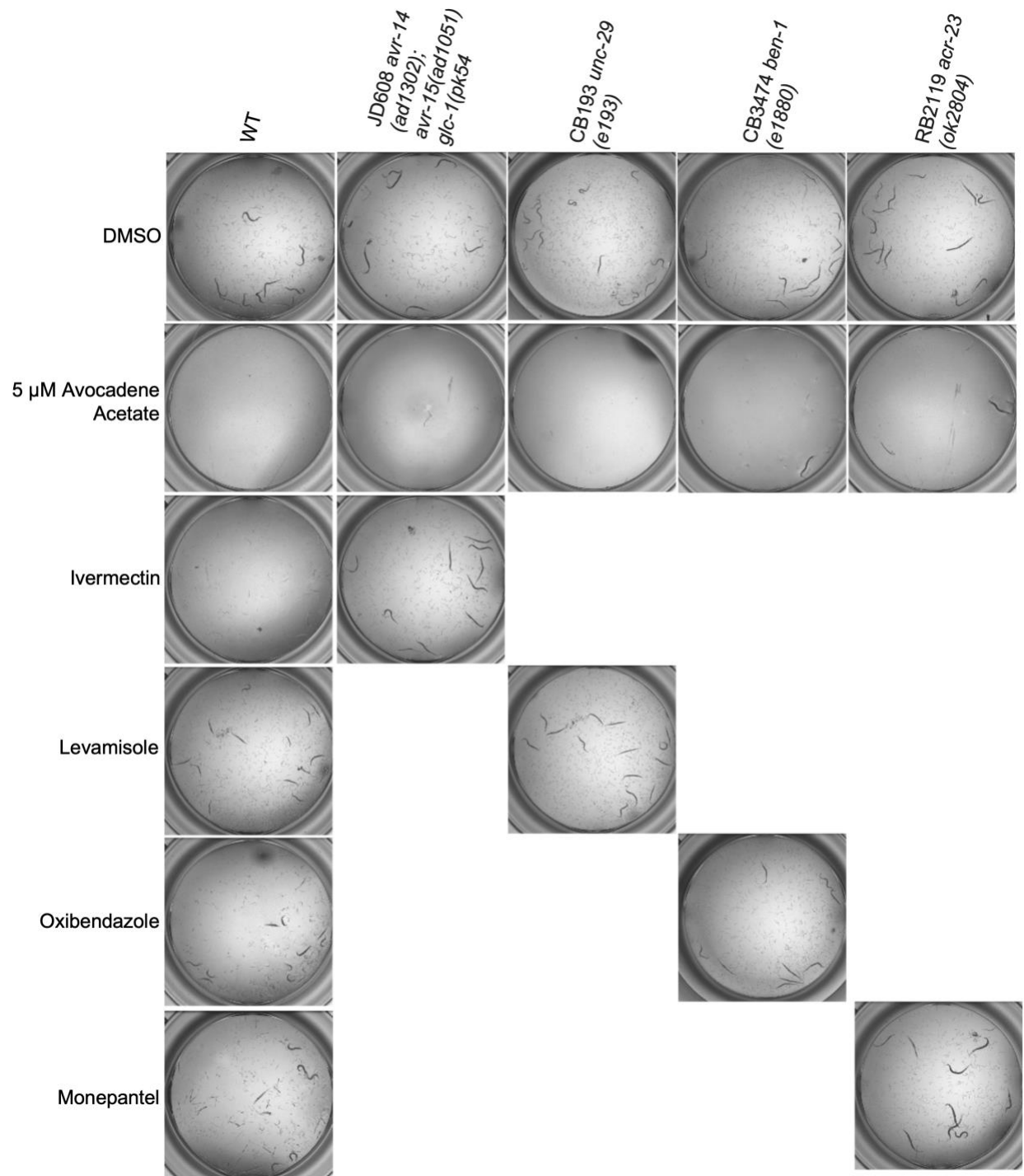
* Corresponding authors



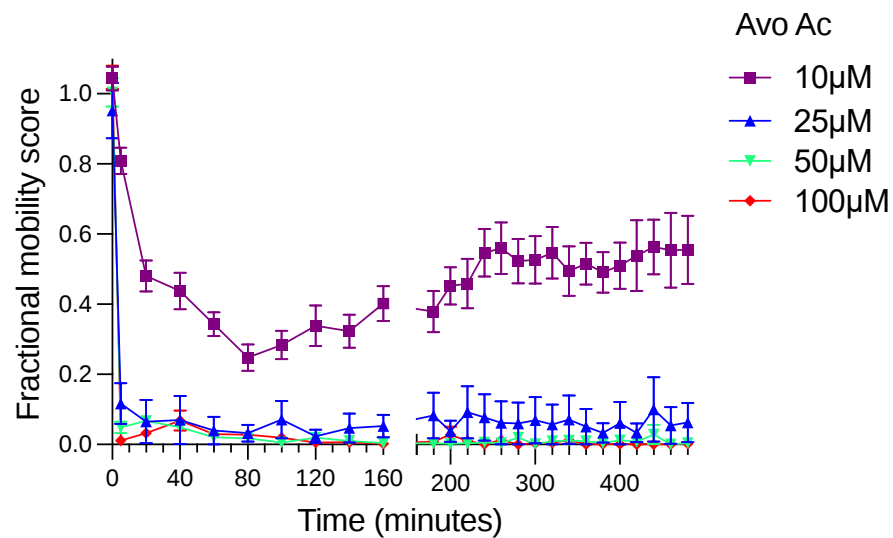
Supplementary Fig. 1. Dose-dependent responses to AFA compounds in HCS panels. Left: Representative images of U2OS cells stained with Hoechst to reveal nuclei. Cells were imaged and quantified after 24-hour treatment with control (1% DMSO) or AFA compounds. Right: Dose response curves for replicate assays (blue), showing per-well cell counts (y-axis) vs. compound concentration in μM (x-axis). Median (dashed line) and range (dotted lines, minimum and maximum) of cell counts in DMSO controls and 30% threshold for cytotoxicity (red line) are shown for comparison. Source data are provided as a Source Data file.



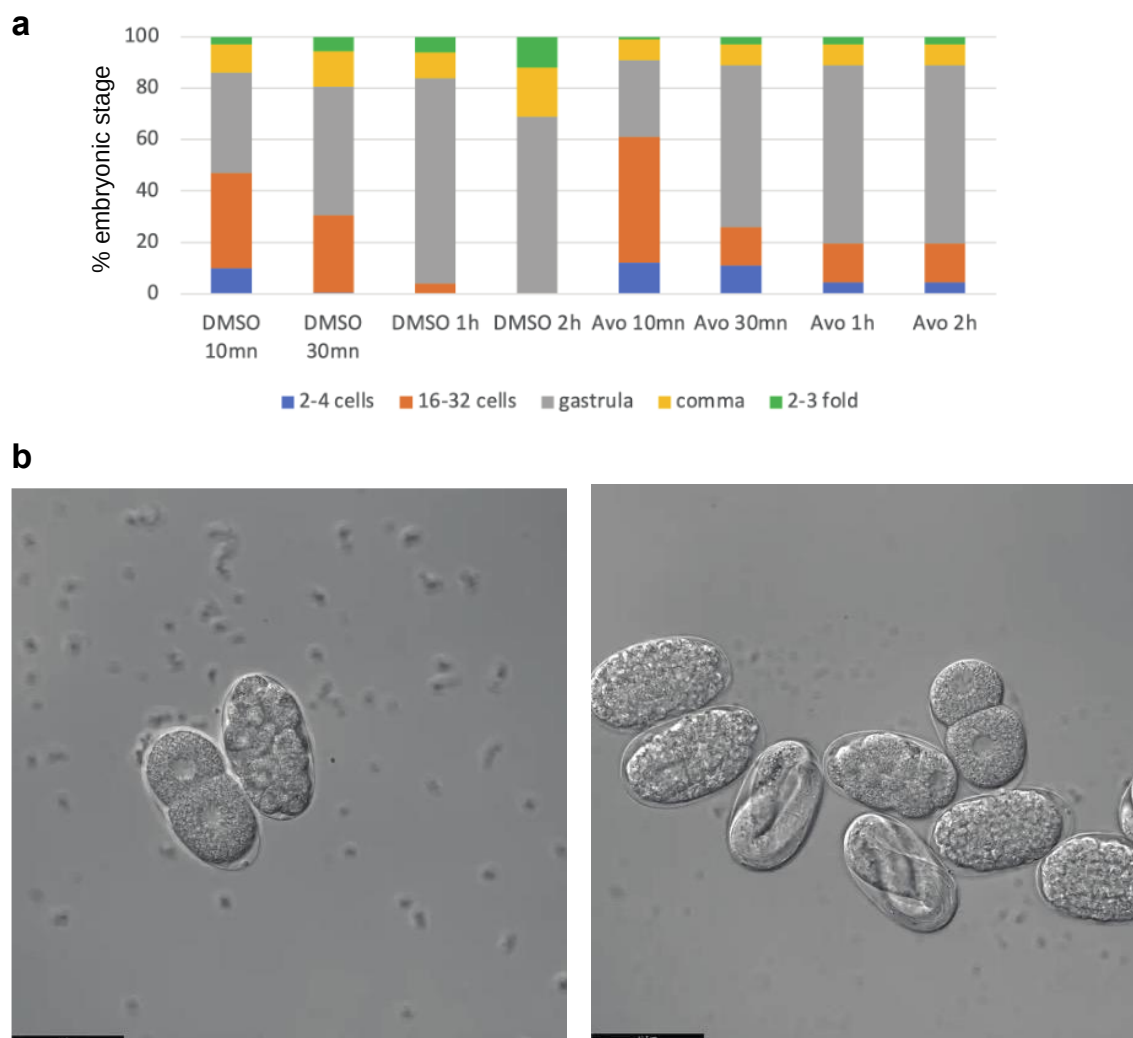
Supplementary Fig. 2. Persenone A, persin and avocadane acetate impair L1 development of *C. elegans*. Representative images show the inhibition of development in N2 (WT) L1 stage worms upon treatment with persenone A, persin and avocadane acetate at different concentrations.



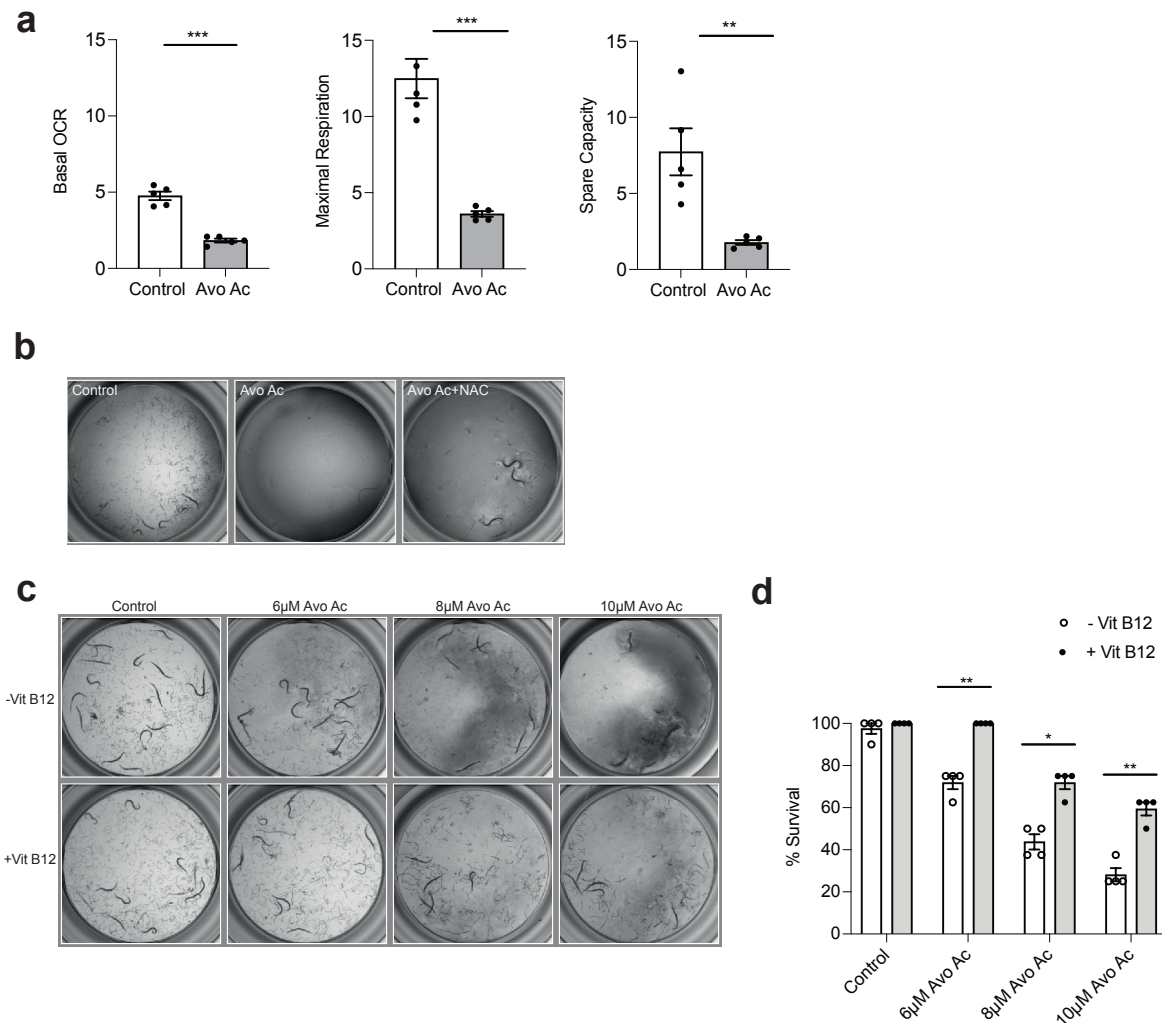
Supplementary Fig. 3. Anthelmintic-resistant *C. elegans* mutants are sensitive to AFA compounds. Avocadoene acetate is effective against ivermectin-resistant mutant strain JD608 *avr-14(ad1302); avr-15(ad1051) glc-1(pk54)*, levamisole-resistant mutant strain CB193 *unc-29(e193)*, monepantel resistant mutant strain RB2119 *acr-23(ok2804)* and benzimidazoles resistant mutant strain CB3474 *ben-1(e1880)*.



Supplementary Fig. 4. Kinetic response of young adult *C. elegans* worms treated with varying concentrations of avocadene acetate over a time course of 8 hours. All data points were normalized by dividing by the fractional mobility score of DMSO control wells for each time point. Data represents mean $\pm$ S.E.M.; $n = 3$ BRs; >50 individual worms per concentration. Source data are provided as a Source Data file.

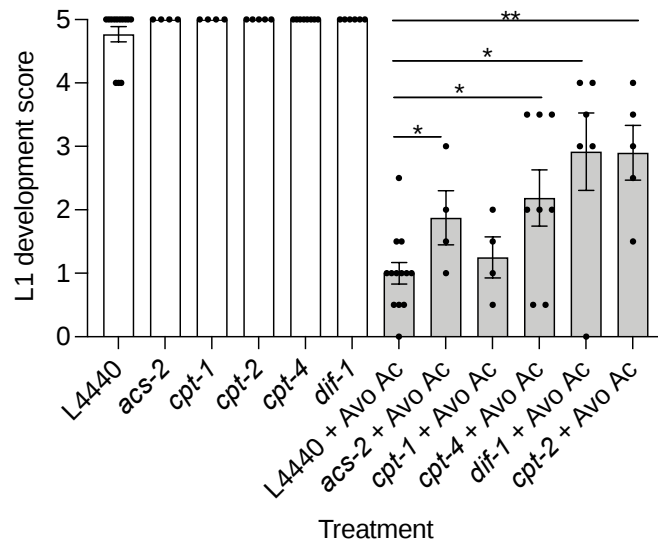


Supplementary Fig. 5. AFA affects the development of all embryonic stages in *C. elegans*. (a) Stacked bar chart showing relative proportion of different embryonic stages after direct treatment of embryos with DMSO (control) or 20 μ M avocadene acetate for 10 min, 30 min, 1 hr or 2hr. (b) Timelapse videos showing embryo development after treatment with DMSO control (left) or 20 μ M avocadene acetate (right). AFA-treated embryos showed developmental arrest within around 20 minutes, $n = 10$, (scale bar = 41.7 μ m). Source data are provided as a Source Data file.

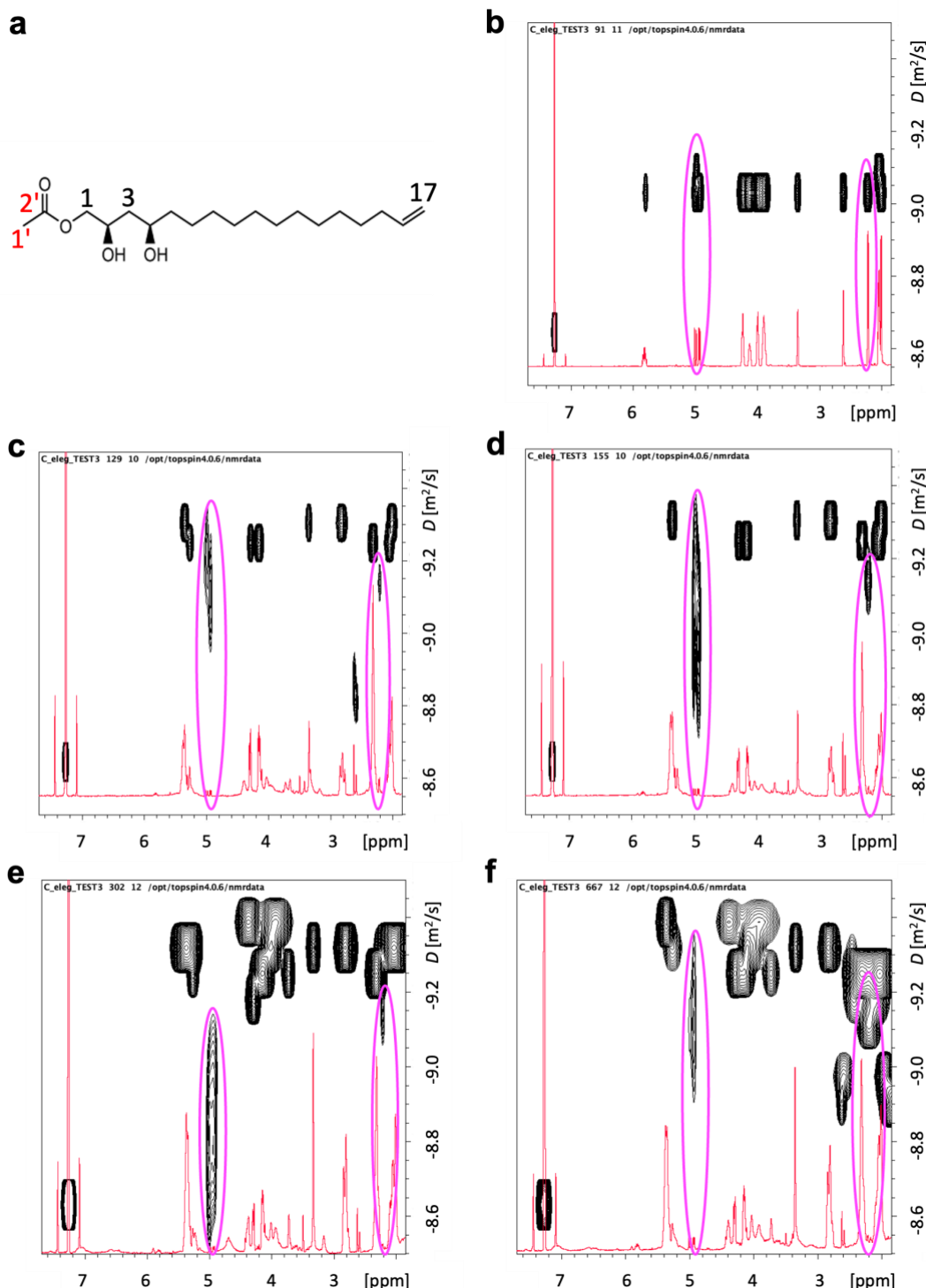


Supplementary Fig. 6. Reduced mitochondrial respiration and lethality induced by avocadyne acetate treatment can be rescued by pre-treatment with NAC and Vitamin B12.

(a) Avocadyne acetate treatment impairs mitochondrial respiration. Basal oxygen consumption rate (OCR, left), maximal respiration (middle) and spare capacity (maximal OCR – basal OCR, right) per animal in DMSO controls compared with 5 μ M concentration avocadyne acetate treatment of L1 stage animals for 44 hours. $n = 3$ (b) Representative images showing that pre-treatment with 10mM N-acetyl cysteine partially rescued avocadyne acetate-induced lethality. (c) Representative images and (d) bar charts illustrating rescue of avocadyne acetate-induced lethality by pre-treatment with vitamin B12. N2 (WT) L1 stage worms were incubated without or with 64nM vitamin B12 until stage L4, followed by treatment with 1% DMSO (control) or varying concentrations of avocadyne acetate for 5 days. Percent development/survival is shown as mean $\pm$ S.E.M. ($n = 4$ BRs; >50 individual worms per assay). Statistical significance (unpaired two-tailed t-test): * p -value < 0.05, ** p -value < 0.001 and *** p -value < 0.0001. Source data are provided as a Source Data file.

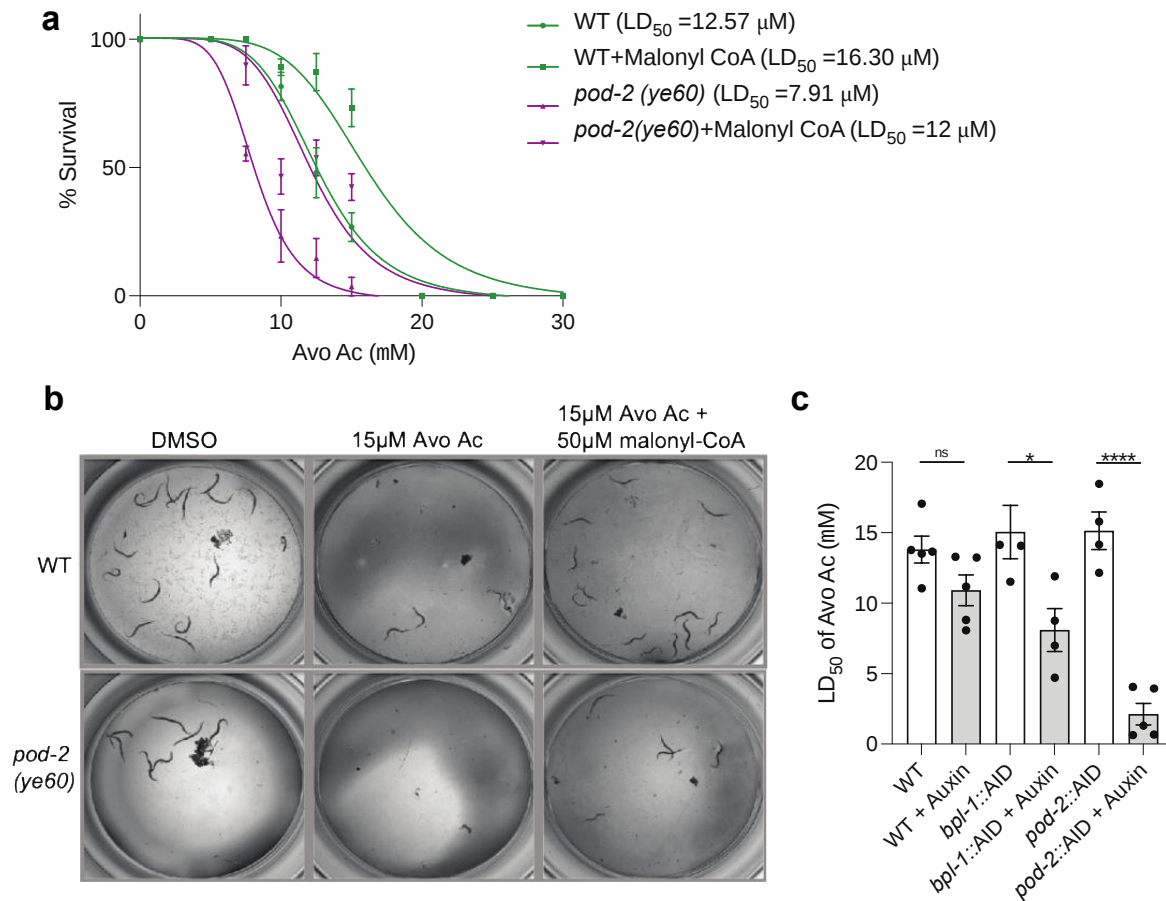


Supplementary Fig. 7. Knockdown of carnitine shuttle rescues avocadyne acetate phenotype in *C. elegans*. The bar chart illustrates the effect on development of N2 L1 stage worms grown in the presence of 1% DMSO (empty bars) or 5μM avocadyne acetate (filled bars) and subjected to RNAi by feeding with empty vector (L4440 control) or RNAi of *acs-2*, *cpt-1*, *cpt-4*, *dif-1* or *cpt-2*. Development scores represent the estimated proportion of worms that developed beyond the L1 stage or were alive after 5 days of treatment, with 0 being 100% arrested/lethal and 5 being 100% developed/alive. Shown are mean ± S.E.M.; $n > 3$ BRs; >50 individual worms. Statistical significance (Mann-Whitney U test): * p -value < 0.05 , ** p -value < 0.001 and *** p -value < 0.0001 . Source data are provided as a Source Data file.

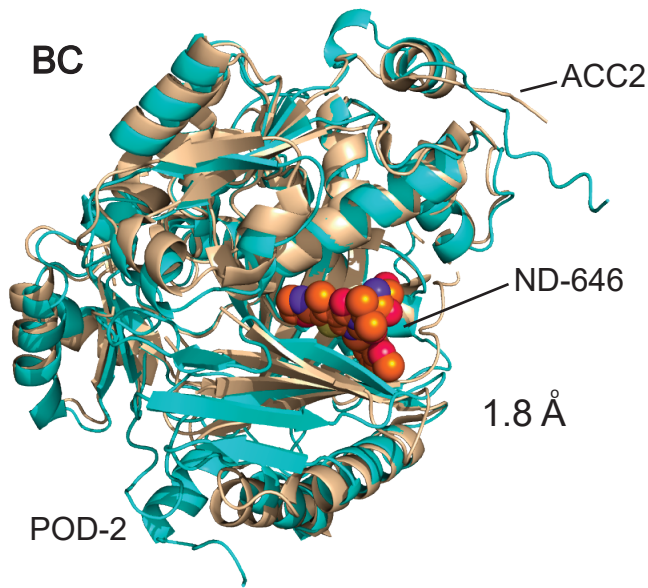


Supplementary Fig. 8. NMR assessment of molecular integrity of avocadoene acetate in whole animal extracts. (a) Molecular structure of avocadoene acetate highlighting carbon atoms of the acetyl moiety (1', 2') and the terminal CH₂ (17). The molecular species employed for the experiments was synthesized with ¹³C labeling at the acetyl moiety (red labels). (b-f) Overlay of ¹H DOSY map (black contours) and corresponding ¹H NMR region (red traces) of various samples in deuterium-labeled chloroform (CDCl₃) to highlight the uniformity of the translational diffusion coefficient (*D*) of the molecule and rule out hydrolysis giving free avocadoene and acetate. Signals

from the acetyl methyl (1', ~2.2 ppm) and the terminal CH₂ (17, ~5.0 ppm) are circled (purple). **(b)** DOSY map and spectrum overlay for free avocadene acetate in solution. The acetyl methyl (1') and the terminal CH₂ (17) exhibit the same *D* value, i.e. $7.66 \pm 0.04 \times 10^{-10}$ m²/s and $7.56 \pm 0.13 \times 10^{-10}$ m²/s, respectively. **(c-f)** Overlaid DOSY maps and spectra for chloroform extracts of *C.elegans* embryos **(c,d)** or L4 larvae **(e,f)**, following **(c,e)** 30 or **(d,f)** 120 minutes treatment with 20 μM or 10 μM avocadene acetate. The measured *D* values for the acetyl methyl (1') and the terminal CH₂ (17) of avocadene acetate in the extracts were: **(c)** $6.15 \pm 0.50 \times 10^{-10}$ m²/s and $5.34 \pm 1.92 \times 10^{-10}$ m²/s, **(d)** $6.26 \pm 0.58 \times 10^{-10}$ m²/s and $6.43 \pm 2.71 \times 10^{-10}$ m²/s, **(e)** $6.23 \pm 1.79 \times 10^{-10}$ m²/s and $8.56 \pm 2.22 \times 10^{-10}$ m²/s and **(f)** $5.78 \pm 0.41 \times 10^{-10}$ m²/s and $5.98 \pm 2.81 \times 10^{-10}$ m²/s, respectively. Despite the errors due to the low intensity of the avocadene acetate signals, the reliability of the determinations is ensured by the observed *D* value of the CHCl₃ isotopic impurity of the solvent at ~ 7.2 ppm (invariably $1.90 \pm 0.02 \times 10^{-9}$ m²/s, vs. 2.01×10^{-9} m²/s expected).



Supplementary Fig. 9. Malonyl-CoA supplementation alleviates the embryonic lethality phenotype of *pod-2*(*ye60*) mutant and partially rescues the avoacene acetate lethality phenotype in both N2 and *pod-2* worms. (a) Dose response and LD₅₀ shift curves showing that the *pod-2*(*ye60*) mutant (purple) is more sensitive to avoacene acetate than WT animals (green) at L4 stage, and that 50 μM malonyl-CoA supplementation partially rescues the lethality. The mean $\pm$ S.E.M. of $n = 3$ BRs with 3 technical replicates per condition are shown. **(b)** Representative images illustrate the partial rescue of the avoacene acetate lethality phenotype by malonyl CoA after 48 hours of treatment, in both WT and *pod-2* mutants. **(c)** Average LD₅₀ of wild type, *bpl-1::AID* and *pod-2::AID* strains pre-treated with 1 mM auxin at L4 stage followed by treatment with avoacene acetate at concentrations ranging from 7.5 to 22.5 μM at young adult stage for 48 hours. Each bar in the plot represents mean $\pm$ S.E.M. ($n > 3$ BRs; > 50 individual worms per assay). Statistical significance (unpaired two-tailed t-test): ns - not significant; * p -value < 0.05 ; **** p -value < 0.0001 . Source data are provided as a Source Data file.



cePOD-2	210	220	230	240	250
hsACC1	LDQGLAL	-----HIRSS	MSGLHLVKQG	RDRKKIDSQR	DFTVASPAEF
hsACC2	LEAYLTGEEA	ETRVPTMRPS	MSGLHLVKRG	REHKKIDLHR	DFTVASPAEF
Consistency	521835000	0000001325	7**45*6636	5713747536	6*54754*76

cePOD-2	260	270	280	290	300
hsACC1	YTRFGGDKVI	EKVLIANNGI	AAVKCMRSIR	RWYEMFRNE	RAIRFVVMVT
hsACC2	YTRFGGDRVI	EKVLIANNGI	AAVKCMRSIR	RWYEMFRNE	RAIRFVVMVT
Consistency	551333174*	777*9*6**	*77*85757	761655**7	65*5*5*6*

cePOD-2	310	320	330	340	350
hsACC1	PEDIKANAEY	IKMAD-HYVP	VFGGPPNNNNY	ANVELIDIA	KRIPVQAVNA
hsACC2	PEDIKANAEY	IKMAD-HYVP	VFGGPPNNNNY	ANVELIDIA	KRIPVQAVNA
Consistency	57787774*	87*564565	5*6*5*6*7	**74*754*	755*6*6**

cePOD-2	360	370	380	390	400
hsACC1	GWGHASNDP	LRRLLNDHNI	AFRGPPASAM	FSLGDKIAST	IAQATVGVPT
hsACC2	GWGHASNDP	LRRLLNDHNI	AFRGPPASAM	FSLGDKIAST	IAQATVGVPT
Consistency	5*****5	*64*15669	*46**75**	67*****7	99**549**

cePOD-2	410	420	430	440	450
hsACC1	VWSSGSGITM	ESITRS--HG	DFVY-VBEDL	LEQAQVANYK	EGLEALKTHN
hsACC2	VWSSGSGITM	ESITRS--HG	DFVY-VBEDL	LEQAQVANYK	EGLEALKTHN
Consistency	75*****87	732*36106	54921*777	577645565	74*7*0654

cePOD-2	460	470	480	490	500
hsACC1	GGFFLMIKAS	EGGGGKGIRK	CFWVDFDKSM	FEEVAQEVQG	SPHFLMKQVD
hsACC2	GGFFLMIKAS	EGGGGKGIRK	CFWVDFDKSM	FEEVAQEVQG	SPHFLMKQVD
Consistency	9*747*****	*****	33467*4538	67*54*95*	**7*7*564

cePOD-2	510	520	530	540	550
hsACC1	QSRHLEVQIL	ADQYGNALIS	FDRDCSIQRR	HQKIVEEAPA	TIAPLAFVFFH
hsACC2	QSRHLEVQIL	ADQYGNALIS	FDRDCSIQRR	HQKIVEEAPA	TIAPLAFVFFH
Consistency	7*8*8*8*8*	*7*4*6*9*7	74*****9**	3*****	5*4249472

cePOD-2	560	570	580	590	600
hsACC1	MEQDAVRLAK	YVGYESAGTV	EYLYLPASEG	QEHHYFPLEL	NPRLQVEHPT
hsACC2	MEQDAVRLAK	YVGYESAGTV	EYLYLPASEG	QEHHYFPLEL	NPRLQVEHPT
Consistency	773*97***	2**4*****	*****000006	74576*6**	*****5

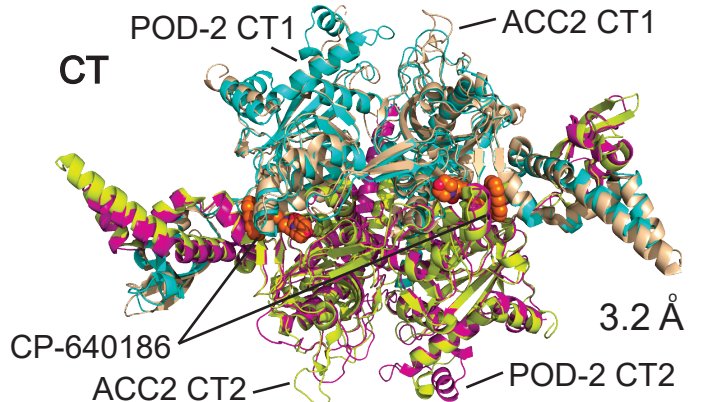
cePOD-2	610	620	630	640	650
hsACC1	TEMVSNESIP	ATQLQIAMGL	PLHKIKDIRK	LYDLPIDGGS	ELPDVRLVDT
hsACC2	TEMVSNESIP	ATQLQIAMGL	PLHKIKDIRK	LYDLPIDGGS	ELPDVRLVDT
Consistency	**76878*	*5*****7	*678*****4	9*53543*7	583422443

cePOD-2	660	670	680	690	700
hsACC1	KI--HAIAA	RITCENPDGS	FRPSTGKVEE	INFTSSQDAW	AYFSGVGRSS
hsACC2	KI--HAIAA	RITCENPDGS	FRPSTGKVEE	INFTSSQDAW	AYFSGVGRSS
Consistency	551336**	***5*****76	47*7*5*5*	8*4*7766*	6**663366

cePOD-2	710	720	730	740	750
hsACC1	VHQFADSDQFG	HIFTATSTRT	EAHNTTCSTL	KHMTIRSSFF	TQVNLVDLM
hsACC2	VHQFADSDQFG	HIFTATSTRT	EAHNTTCSTL	KHMTIRSSFF	TQVNLVDLM
Consistency	747*****8*	*5*79*875*	*776*556*	4587*666*	5*6*9*948

cePOD-2	760	770	780	790	800
hsACC1	HDADFNNAPF	NTQWLDKRIA	MKKK--KQSL	SMSVLAISA	AVIGSSRVTG
hsACC2	HDADFNNAPF	NTQWLDKRIA	MKKK--KQSL	SMSVLAISA	AVIGSSRVTG
Consistency	5556*44*26	6*4*****34*	4*37377364	586*90056*	5296643453

Unconserved 112345678910 Conserved



cePOD-2	1710	1720	1730	1740	1750
hsACC1	ETGVTTTEV	---ITTFSSA	GLGV	---VIN	---IE
hsACC2	ETGVTTTEV	---ITTFSSA	GLGV	---VIN	---IE
Consistency	7*5457933	3339*74546	4845422313	33*03337**	333333387

cePOD-2	1760	1770	1780	1790	1800
hsACC1	KRRLAARVY	SCYIYDFPII	FMAAANNPK	SASSAMHLN	EMAAALKEQR
hsACC2	KRRLAARVY	SCYIYDFPII	FMAAANNPK	SASSAMHLN	EMAAALKEQR
Consistency	4*65*7476	77*4*6*47	*675466*3	4311103*5	1235000000

cePOD-2	1810	1820	1830	1840	1850
hsACC1	REFFQRLREL	VLEN-GVTE	ISDAEILKKR	SANALNCCG	VAWMTLYTF
hsACC2	REFFQRLREL	VLEN-GVTE	ISDAEILKKR	SANALNCCG	VAWMTLYTF
Consistency	8476555**	*753*4*65	770000000*	55660*64**	*64*56475

cePOD-2	1860	1870	1880	1890	1900
hsACC1	REFFQRLREL	VLEN-GVTE	ISDAEILKKR	SANALNCCG	VAWMTLYTF
hsACC2	REFFQRLREL	VLEN-GVTE	ISDAEILKKR	SANALNCCG	VAWMTLYTF
Consistency	8476555**	*753*4*65	770000000*	55660*64**	*64*56475

cePOD-2	1910	1920	1930	1940	1950
hsACC1	SGARIGLSRK	ISKLVKLOLE	NEDKBEQGF	YIYIDREHA	QEEB-EVVY-
hsACC2	SGARIGLSRK	ISKLVKLOLE	NEDKBEQGF	YIYIDREHA	QEEB-EVVY-
Consistency	*****8757	*458557544	65753*7*75	*8*85*7543	7865365*63

cePOD-2	1960	1970	1980	1990	2000
hsACC1	SGARIGLSRK	ISKLVKLOLE	NEDKBEQGF	YIYIDREHA	QEEB-EVVY-
hsACC2	SGARIGLSRK	ISKLVKLOLE	NEDKBEQGF	YIYIDREHA	QEEB-EVVY-
Consistency	132*71466*	55*549**8*	5748*4*6*7	***8***77	4*4*3*9355

cePOD-2	2010	2020	2030	2040	2050
hsACC1	VYTCRSVGIC	AYTARIAARI	VQHKQSHLIL	TCYEAANTLL	GKKVYTSNNQ
hsACC2	VYTCRSVGIC	AYTARIAARI	VQHKQSHLIL	TCYEAANTLL	GKKVYTSNNQ
Consistency	5*43*87***	*56*6*65*	9*476***8*	*43*57*	477*****

cePOD-2	2060	2070	2080	2090	2100
hsACC1	LGGEVDFVRN	GVTAAVVDND	LEGIAKVRNR	MSFLPTPTTE	LPFFSKHED-
hsACC2	LGGEVDFVRN	GVTAAVVDND	LEGIAKVRNR	MSFLPTPTTE	LPFFSKHED-
Consistency	***379*52*	*7*26*16*	6**935984*	8*78*3336	5*555320*

cePOD-2	2110	2120	2130	2140	2150
hsACC1	DCSARDVVIP	SDSEQNTYV	ADLIDSK---	NLSNQ--TG	IPFFSKHED-
hsACC2	DCSARDVVIP	SDSEQNTYV	ADLIDSK---	NLSNQ--TG	IPFFSKHED-
Consistency	55624457*	7000335*4	388467333	646653076	438*4*4*5

cePOD-2	2160	2170	2180	2190	2200
hsACC1	QWAKSKIVAG	RARLCQPIPG	VVSSEFRNPS	TIAPADPAID	GQVQNTQRA
hsACC2	QWAKSKIVAG	RARLCQPIPG	VVSSEFRNPS	TIAPADPAID	GQVQNTQRA
Consistency	35*779*4*	***3***9*	*975*4*656	449*****43	5*76745*7*

cePOD-2	2210	2220	2230	2240	2250
hsACC1	GQVVPDPSAF	KTAEAINDLN	KENLPLMIIA	SLRGFSGGGR	DMYDMVLKFG
hsACC2	GQVVPDPSAF	KTAEAINDLN	KENLPLMIIA	SLRGFSGGGR	DMYDMVLKFG
Consistency	***779*4*	*47*966*6*	7*3*****36*	74*****6*	***6*****

cePOD-2	2260	2270	2280	2290	2300
hsACC1	ACIYDAVAVY	NRPVIVYIPE	AGELRGGAWA	VLDSEIRPEF	IHLVADEKSR
hsACC2	ACIYDAVAVY	NRPVIVYIPE	AGELRGGAWA	VLDSEIRPEF	IHLVADEKSR
Consistency	5*5*6*534	27*989***5	26*****7*6	8*74*6*21	7585**57**

cePOD-2	2310	2320	2330	2340	2350
hsACC1	GVLEPEGTV	EIKFRKKDLI	KYMRIDPAY	KKLHEQLGEP	DLSPDKRKDL
hsACC2	GVLEPEGTV	EIKFRKKDLI	KYMRIDPAY	KKLHEQLGEP	DLSPDKRKDL
Consistency	6*9**666*	4***75486	74*7*4*4*	25*464535*	753112*75*

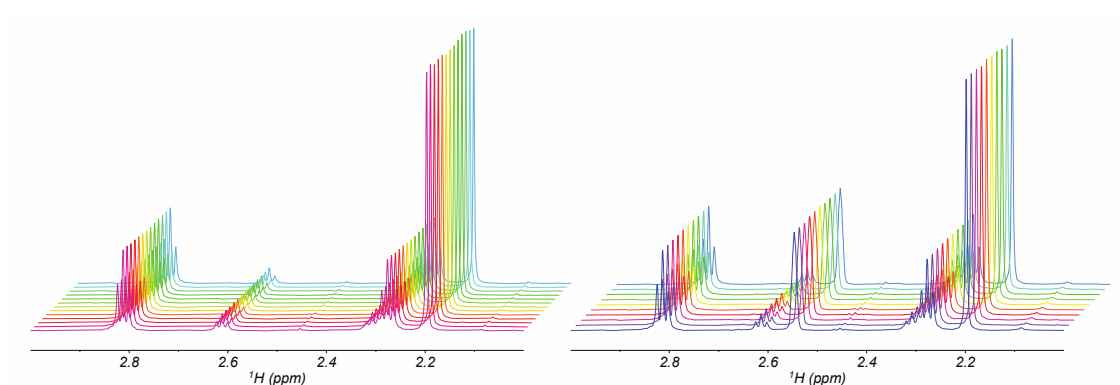
cePOD-2	2360	2370	2380	2390	2400
hsACC1	E---ERYEE	LSKTYRNASV	EFADADRQPI	RMSVGVGVHE	VTSLTNRRLL
hsACC2	E---ERYEE	LSKTYRNASV	EFADADRQPI	RMSVGVGVHE	VTSLTNRRLL
Consistency	1*2335*472	*455*5667*	7*4**3*534	*3*64**965	9554567*56

cePOD-2	2410	2420	2430	2440	2450
hsACC1	FELLRLRLLL	EDLVKKKKIHN	ANPELTDGQI	QAMLRRLGTF	VEGTAKYAVW
hsACC2	FELLRLRLLL	EDLVKKKKIHN	ANPELTDGQI	QAMLRRLGTF	VEGTAKYAVW
Consistency	6434*64*5	7334547*34	7445542118	555835*3**	6631777*74

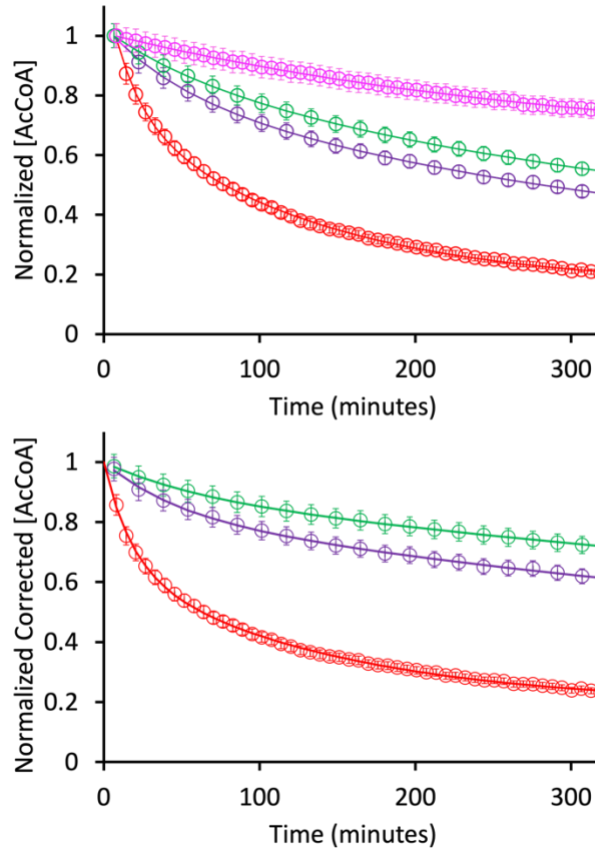
cePOD-2	2460	2470	2480	2490	2500
hsACC1	PNSB--M	DEQYTO---	---EQ---	YETQFVDDLI	IAINEARRRY
hsACC2	PNSB--M	DEQYTO---	---EQ---	YETQFVDDLI	IAINEARRRY
Consistency	5*74021238	7553540330	131*673314	44363*3539	4584746565

cePOD-2	2510	2520	2530	2540	2550
hsACC1	EQQLNTFMNK	--ISKRR--	---	---	---
hsACC2	EQQLNTFMNK	--ISKRR--	---	---	---
Consistency	5462354857	3**535*323	2123333333	033	

Supplementary Fig. 10. Comparison of biotin carboxylase (BC) and carboxyltransferase (CT) domains of *C. elegans* POD-2 and human ACC proteins. Top: Structural alignments of predicted models for POD-2 BC domain (left) and CT domain (right), superimposed with solved structures of corresponding human ACC2 domains bound to inhibitor compounds. Root mean square deviations (RMSD) between worm and human structures are shown (1.8 Å for BC domain; 3.2 Å for CT domain). The ACC2 BC structure (PDB: 5kkn) contains compound ND-646 at the active site; the ACC2 CT structure (PDB: 3ff6) contains compound CP-640186 at the active site and is shown as a homodimer, with two active sites. Predicted structural models for POD-2 were generated using AlphaFold. Color codes: POD-2, cyan (BC, CT1) and magenta (CT2); ACC2, beige (BC, CT1) and chartreuse (CT2). Bottom: Multiple sequence alignments of corresponding domains from POD-2 with human ACC1 and ACC2.



Supplementary Fig. 11. Stacked plot highlighting the constancy of the acetyl methyl peak of acetyl CoA (AcCoA) at 2.20 ppm in the ¹H NMR spectra of 50 μM AcCoA dissolved in D₂O with 50 μM ATP and 30 mM (NH₄)HCO₃, without (left) and with (right) 50 μM avocadene acetate. The time intervals spanned by the displayed plots are 2.5 hours (left) and 1 hour (right).



Supplementary Fig. 12. Time course of the acetyl-CoA normalized concentrations [AcCoA] assessed from NMR measurements after addition of purified POD-2. The data represent the experimental trends of the standard reaction mixture referred to as reference – 50 μM AcCoA in D_2O + 50 μM ATP + 30 mM $(\text{NH}_4)\text{HCO}_3$ – (red), the reference solution with 20 μM (purple) or 50 μM (green) avocadene acetate, and the reference solution devoid of ATP (magenta). In the top plot, data are normalized with respect to starting values. In the lower plot, the three remaining datasets are shown scaled with respect to the data collected without ATP at each timepoint. The difference between any of the reported curves with respect to the reference ones is significant ($p < 0.0001$, Wilcoxon matched-pairs signed rank test). The AcCoA reduction in the absence of ATP is due to mitochondrial AcCoA-acetyltransferase and AcCoA-hydrolase, according to the proteomic assessment of the NMR samples (not shown). The error bars represent the signal-area maximal uncertainty.

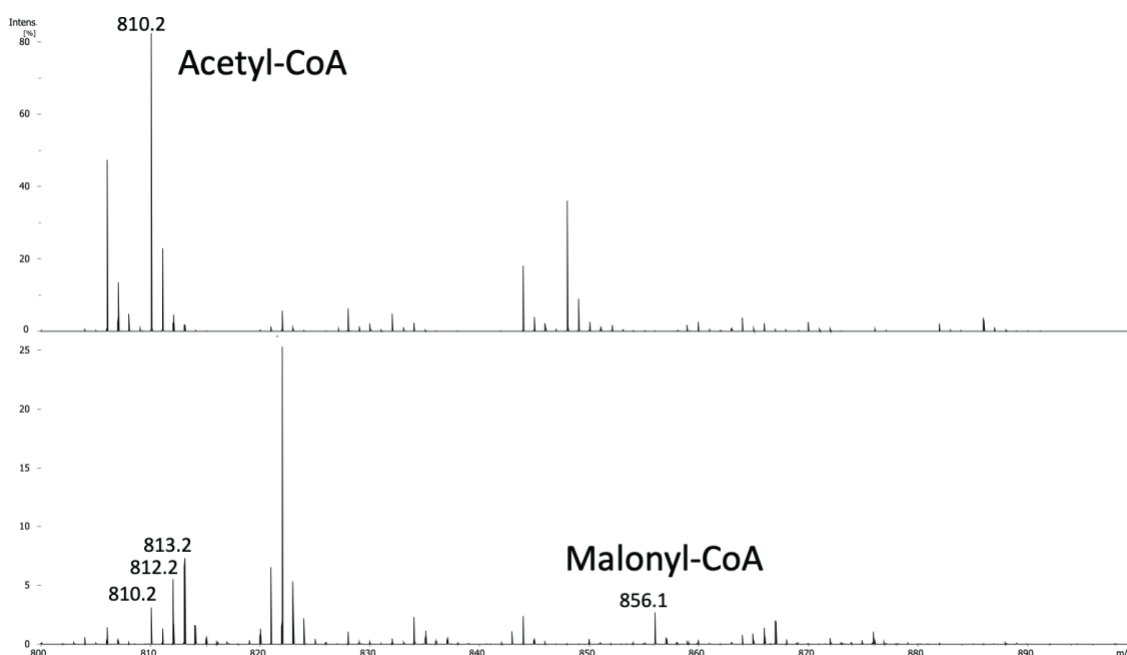
The double-exponential fitting curves follow the equation:

$$y = A_0 + A_1 e^{-k_1 t} + A_2 e^{-k_2 t}$$

or, for the solution without ATP, the equation:

$$y = A_0 + A_1 e^{-k_1 t}$$

where t is time, A_0 , A_1 and A_2 are pre-exponential constants, k_1 and k_2 are slow and fast kinetic constants featuring the minimal empirical model fitting the experimental data (see Table S3).



Supplementary Fig. 13. MALDI-FTICR mass spectra of reference solution after completion of NMR acquisitions without (upper trace) and with (lower trace) a *C. elegans* protein fraction enriched for POD-2. The reference solution contains the necessary substrates for conversion of acetyl-CoA (AcCoA) to malonyl-CoA (MalCoA) and consists of 50 μ M AcCoA, 50 μ M ATP, and 30 mM $(\text{NH}_4)\text{HCO}_3$ in D_2O . The upper trace shows the AcCoA $[\text{M}+\text{H}]^+$ molecular ion at 810.2 Th (Th = thompson, i.e. mass-over-charge ratio unit), as expected without the conversion catalyzed by POD-2. The lower trace contains the weak $[\text{M}+\text{H}]^+$ molecular peak of MalCoA, at 856.1 Th, as expected from POD-2 activity, along with a peak of unconverted AcCoA at 810.2 Th. Note that the MalCoA molecular ion occurs with an increment of 2 Th because of the incorporation of 2 deuterium atoms from the keto-enol equilibrium in D_2O . The low intensity of MalCoA $[\text{M}+\text{H}]^+$ molecular ion is related to inherently weak ionization and fragmentation, with a step involving CO_2 neutral loss leading to AcCoA. The fragmentation-derived AcCoA occurs at 812.2 and 813.2 Th, with the incorporation of 2 or 3 deuterium atoms, which is the signature of the malonyl precursor in D_2O .

<i>Haemonchus contortus</i>		Total eggs	% emb lethality	% larval lethality
DMSO	1%	857	40	10
Avocatin A	10uM	215	77	100
	100uM	196	97	100
Avocadene acetate	10uM	237	78	100
	100uM	210	96	100
Avocadyne acetate	10uM	207	76	100
	100uM	256	81	100
Avocatin B	10uM	212	58	78
	100uM	238	61	100
Avocadene	10uM	148	52	75
	100uM	204	56	100
Avocadyne	10uM	187	39	98
	100uM	196	51	100

Supplementary Table 1. AFA compounds impair survival of the parasitic nematode *Haemonchus contortus* UGA strain. Embryonic and larval lethality counts after treatment with different AFA compounds in comparison with 1% DMSO control.

Genes	RNAi + AFA	Mutant + AFA	Mutant genotype
Mitochondrial β -oxidation			
<i>acs-2</i>	rescue*		
<i>cpt-1</i>	no effect		
<i>cpt-4</i>	rescue*		
<i>dif-1</i>	rescue*		
<i>cpt-2</i>	rescue**	rescue**	<i>him-9(e1487) ii; unc-24(e138) let(t...)/nt1 [let(m435)] iv; cpt-2(t1879); dpy-11(e224)/nt1 [let(m435)] v</i>
<i>acdh-2, acdh-3, acdh-4, acdh-6, acdh-7, acdh-8, acdh-9, acdh-10, acdh-11</i>	no effect		
<i>acdh-12</i>	no effect	no effect	<i>acdh-12(gk5631)</i>
<i>acdh-13</i>	no effect		
<i>ech-1.1</i>	no effect	no effect	<i>ech-1.1(gk5134[loxp + myo-2p::gfp::unc-54 3' utr + rps-27p::neor::unc-54 3' utr + loxp]) v.</i>
<i>ech-1.2</i>	no effect		
<i>ech-2</i>	no effect		
<i>ech-4</i>	no effect		
<i>ech-5</i>	no effect		
<i>ech-7</i>	no effect		
Propionate/shunt pathway			
<i>pcca-1</i>	variable		
<i>pccb-1</i>	variable		
<i>mce-1</i>	variable		
<i>mmcm-1</i>	variable	enhancement*	<i>mmcm-1(ok1637) iii.</i>
<i>acdh-1</i>	variable	not significant enhancement	<i>acdh-1(ok1489) i.</i>
<i>ech-6</i>	no effect		
<i>hach-1</i>	variable		
<i>alh-1</i>	variable		
Peroxisomal β -oxidation			
<i>acox-1.1</i>	no effect		
<i>ech-3</i>	no effect		
<i>ech-8</i>	no effect		
<i>ech-9</i>	no effect		
Uncoupling protein			
<i>ucp-4</i>	variable	variable	<i>ucp-4(ok195) V.</i>
Lipid synthesis and storage			
<i>pod-2</i>		enhancement**	<i>pod-2(ye60) ii.</i>
<i>mlcd-1</i>	rescue*		
<i>bpl-1</i>		enhancement*	<i>bpl-1(db1372[bpl-1::degron(aid)]) iesi57[eft-3p::tir1::mruby::unc-54 3'utr+cbr unc-119(+)] ii</i>
<i>fasn-1</i>	enhancement*		
<i>emb-8</i>		enhancement*	<i>emb-8(hc69) iii</i>
<i>c32e8.9 (echdc1)</i>	no effect		
<i>fat-5</i>	no effect		
<i>fat-6</i>	no effect		
Other biotin binding carboxylases			
<i>mccc-1</i>	no effect		

Supplementary Table 2. Chemical genetic interactions screen reveals a modified effect of RNAi knockdowns in the presence of AFA. *mild effect. **strong effect.

Sample	A_0	A_1	A_2	k_1	k_2	P -value
Reference	0.223±0.015	0.551±0.041	0.394±0.041	7.21±0.80×10 ⁻³	5.66±1.05×10 ⁻²	2×10 ⁻⁵
Reference + 20µM Avocadene Acetate	0.297±0.126	0.550±0.042	0.167±0.042	1.64±0.58×10 ⁻³	2.21±0.57×10 ⁻²	3×10 ⁻³
Reference + 50µM Avocadene Acetate	0.166±0.026	0.731±0.067	0.107±0.067	8.57±3.98×10 ⁻⁴	1.73±0.35×10 ⁻²	5×10 ⁻³
Reference – ATP	0.604±0.009	0.396±0.144	–	3.27±0.14×10 ⁻³	–	1×10 ⁻³

Supplementary Table 3. Fitting parameters for the experimental data reported in Supplementary Fig. 12. The experimental data fitting was obtained using Prism 10 (GraphPad Software LLC). All parameters are reported with the respective 95% confidence interval (profile likelihood). The reference solution was 50 µM AcCoA dissolved in D₂O + 50 µM ATP + 30 mM (NH₄)HCO₃. The reported parameter values refer to the fitting for normalized AcCoA concentration data, further adjusted by scaling with respect to the corresponding data collected without ATP at each timepoint. This further correction was not applied for the normalized AcCoA concentration data collected in the absence of ATP. The p values represent the probability of the experimental data to fit the exponential models by chance and were computed by comparing the experimental data with their corresponding fitting curves using two-sample two-tailed t-tests.