Supporting Information

Selective Delivery of Dicarboxylates to Mitochondria by Conjugation to a Lipophilic Cation via a Cleavable Linker

Hiran A. Prag,[†,#] Duvaraka Kula-Alwar,[‡,#] Laura Pala,[§] Stuart T. Caldwell,[§] Timothy E. Beach,[||] Andrew M. James,[†] Kourosh Saeb-Parsy,[||] Thomas Krieg,[‡] Richard C. Hartley,[§,] and Michael P. Murphy[†,‡,]**

[†]MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge Biomedical Campus, CB2 0XY, UK

[‡]Department of Medicine, University of Cambridge, Cambridge, CB2 0QQ, UK

[§]School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, UK

[||]Department of Surgery and Cambridge NIHR Biomedical Research Centre, University of Cambridge, Cambridge, CB2 0QQ, UK

[#]Equal contribution

*Corresponding authors: M.P. Murphy (mpm@mrc-mbu.cam.ac.uk)

R.C. Hartley (Richard.Hartley@glasgow.ac.uk)

Chemical Synthesis, Figures S1-S6, Supplementary Methods

Contents

1. Chemical Syntheses.

1.1. Overview

The TPP-malonates were prepared by first converting the appropriate haloalkanols **1**-**3** into the corresponding (hydroxyalkyl)triphenylphosphonium salts **4**-**6**, and then monoesterifying malonic acid using *N*-*N'*-dicyclohexylcarbodiimide (DCC) as the coupling agent (Figure S1).

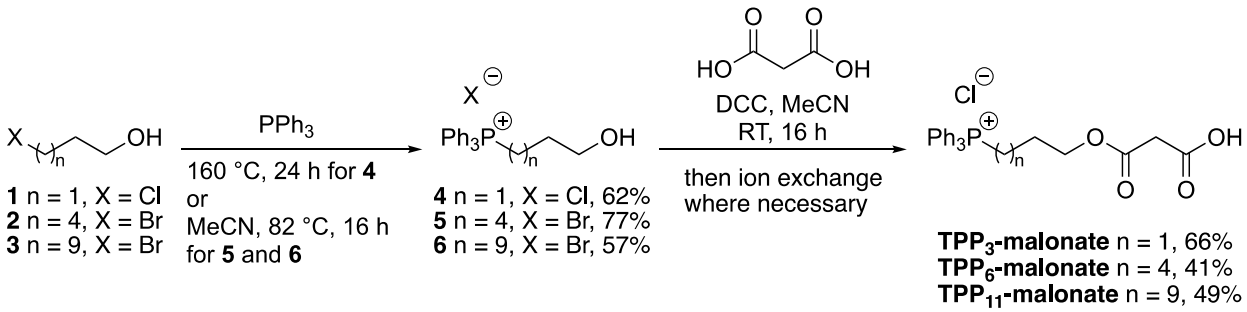

Figure S1. Synthesis of TPP-malonate monoesters

1.2. General

All reactions under an inert atmosphere were carried out using flame-dried glassware and solvents were added *via* syringe. Reagents were obtained from commercial suppliers and used without further purification. Dry solvents were collected from a Puresolv solvent purification system or obtained from commercial suppliers. ^{1}H NMR spectra were obtained using Bruker AVIII 400 and AVIII 500 spectrometers, ^{13}C NMR spectra at 101 and 126 MHz, ^{31}P NMR spectra at 162 and 202 MHz, ^{19}F spectra at 377 and 471 MHz. Signal splitting patterns were described as: singlet (s), doublet (d), triplet (t), quartet (q), quintet (qn), sextuplet (sx), septuplet (sept), multiplet (m), broad singlet (broad s), or any combination of the above. All coupling constants were recorded in Hz. In ^{1}H NMR spectra tentative assignment was done on the base of the chemical shift, definitive on the base on COSY (when made). DEPT was used to assign the signals in ^{13}C NMR spectra as C, CH,

CH_2 and CH_3. 2D techniques including COSY and HSQC were used to help assignment. Deuterated solvents contained trimethylsilane (TMS) as a reference compound. All spectra were assigned using following reference solvent peaks: $CDCl_3$ (7.26 ppm for ^{1}H NMR; 77.16 ppm for ^{13}C NMR), CD_3CN (1.94 ppm for ^{1}H NMR; 118.26 ppm for ^{13}C NMR), DMSO-*d*$_6$ (2.50 ppm for ^{1}H NMR, 39.52 ppm for ^{13}C NMR). LRMS (ESI^+) and HRMS (ESI^+) spectra were collected on a Bruker MicroTOF-Q, EI spectra were collected on a Jeol JMS700 (MStation) spectrometer. IR spectra were obtained using Shimadzu FTIR-8400S. Reactions were monitored by thin layer chromatography (TLC) performed on aluminium sheets pre-coated with silica gel (Merck or Fluorochem Silica Gel 60 F254) and visualization was performed using UV light (λ_{max} = 254 or 365 nm) or by staining with a potassium permanganate solution dip. Purification of products was carried out by re-crystallization, distillation under vacuum or Biotage® Isolera™ One Flash Chromatography system using Biotage® SNAP Ultra silica gel cartridges.

1.3. Specific compounds

1.3.1. Synthesis of (3-hydroxypropyl)triphenylphosphonium chloride 4.

Following the procedure of Dolle *et al,*[1] triphenylphosphine (2.00 g, 7.62 mmol, 1 eq.) and 3-hydroxypropyl chloride **1** (640 μL, 7.62 mmol, 1 eq.) were heated at 160 °C for 16 h while stirring under an atmosphere of argon. The mixture was allowed to cool to RT. The solvent was removed under reduced pressure and the resulting white solid was dissolved in dichloromethane (10 mL), precipitated from diethyl ether (200 mL), collected by filtration and recrystallized from acetonitrile to yield 3-OH propyl-TPP **4** as a white amorphous solid (1.67 g, 4.96 mmol, 62%). δ_H (400 MHz, $CDCl_3$): 7.83-7.64 (15H, m, 15 × CH, Ar), 5.81 (1H, t, *J* = 7.0 Hz, OH), 3.88-3.76 (4H, m, CH_2P + CH_2OH), 1.88-1.76 (2H, m, CH_2CH_2O). δ_C (126 MHz, $CDCl_3$): 135.09 (d, *J* = 2.7 Hz, CH), 133.47 (d, *J* = 10.0 Hz, CH), 130.57 (d, *J* = 12.3 Hz, CH), 118.42 (d, *J* = 86.2 Hz, C), 60.44 (d, *J*

= 16.6 Hz, CH_2), 25.99 (d, J = 4.2 Hz, CH_2), 19.94 (d, J = 52.7 Hz, CH_2). δ_P (202 MHz, $CDCl_3$): 24.21 (PPh_3). LRMS (ESI^+): 321 (M^+, 100%). HRMS (ESI^+): 321.1392. $C_{21}H_{22}OP^+$ requires M^+, 321.1403. ^1H and ^{13}C NMR data agree with literature data for the bromide salt.[2]

1.3.2. Synthesis of (6-hydroxyhex-1-yl)triphenylphosphonium bromide 5.

Triphenylphosphine (2.00 g, 7.63 mmol, 1.2 eq.) and 6-hydroxyhex-1-yl bromide **2** (831 µL, 6.35 mmol, 1.0 eq.) were dissolved in anhydrous acetonitrile (20 mL) under an atmosphere of argon. The reaction was heated under reflux for 16 h. The formed slurry was dissolved in the minimum amount of dichloromethane (5 mL) and precipitated with diethyl ether (30 mL). The precipitate was collected *via* filtration under vacuum to afford 6-OH hexyl-TPP **5** as a white amorphous solid (2.18 g, 4.91 mmol, 77%). ν_{max} (ATR): 3550 (OH), 3053 (OH), 2934 (CH), 2862 (CH), 1587 (CH) 1485 (Ph), 1439 (PC) cm^{-1}. δ_H (400 MHz, $CDCl_3$): 7.90-7.73 (9H, m, 9 × CH, Ar), 7.73-7.64 (6H, m, 6 × CH, Ar), 3.79-3.65 (2H, m, CH_2P), 3.60 (2H, broad t, CH_2O), 2.96-2.57 (1H, bs, OH), 1.73-1.56 (4H, m, 2 × CH_2), 1.55-1.42 (4H, m, 2 × CH_2). δ_C (101 MHz, $CDCl_3$): 135.13 (d, J = 3.0 Hz, CH), 133.80 (d, J = 10.0 Hz, CH), 130.63 (d, J = 12.5 Hz, CH), 118.50 (d, J = 85.8 Hz, C), 61.59 (CH_2), 32.05 (CH_2), 29.58 (d, J = 16.0 Hz, CH_2), 24.90 (CH_2), 22.60 (d, J = 50.0 Hz, CH_2), 22.48 (d, J = 4.4 Hz, CH_2). δ_P (162 MHz, $CDCl_3$): 24.49 (PPh_3). LRMS (ESI^+): 363 (M^+, 100%). HRMS (ESI^+): 363.1862. $C_{24}H_{28}OP^+$ requires M^+, 363.1872. ^1H and ^{13}C NMR data agree with literature.[3]

1.3.3. Synthesis of (11-hydroxyundec-1-yl)triphenylphosphonium bromide 6.

Triphenylphosphine (5.27 g, 20.10 mmol, 1.01 eq) was added to a solution of 11-bromoundecan-1-ol **3** (5.00 g, 19.90 mmol, 1.0 eq) in MeCN (30 ml). The solution was heated under reflux overnight under an atmosphere of argon. After cooling to RT the solution was slowly added to rapidly stirred diethyl ether (~200 ml) and the resulting precipitate filtered and washed with diethyl

ether to give the alcohol **6** as a white solid (5.85 g, 57%). ν_{max}(ATR): 3273 (OH), 2928 (CH), 2852 (CH), 1437 (P-C), 1112 (C-OH) cm^{-1}. δ_H (400 MHz: $CDCl_3$): 7.87-7.76 (9H, m, Ar), 7.72-7.67 (6H, m, Ar), 3.81-3.74 (2H, m, PCH_2), 3.60 (2H, t, *J* = 6.6 Hz, C*H*$_2$OH), 1.78 (1H, broad s, OH), 1.63-1.57 (4H, m, 2×CH_2), 1.56-1.52 (2H, m, CH_2), 1.34-1.19 (12H, m, 6×CH_2). δ_C (101 MHz: $CDCl_3$): 135.01 (d, *J* = 3.0 Hz, CH), 133.47 (d, *J* = 9.9 Hz, CH), 130.46 (d, *J* = 12.6 Hz, CH), 118.10 (d, *J* = 85.8 Hz, C), 62.41 (CH_2), 32.58 (CH_2), 30.24 (d, *J* = 15.6 Hz), 29.23 (CH_2), 29.16 (CH_2), 29.11 (CH_2), 28.91 (CH_2), 28.88 (CH_2), 25.60 (CH_2), 22.68 (d, *J* = 42.6 Hz, CH_2), 22.41 (d, *J* = 2.9 Hz, CH_2). δ_P (162 MHz: $CDCl_3$): 24.04 (s). m/z (ESI): 433.2655. $C_{29}H_{28}OP$ requires M^+, 433.2646.

1.3.4. Synthesis of mono(3-triphenylphosphonioprop-1-yl) malonate, chloride salt (TPP$_3$-malonate).

Malonic acid (70 mg, 0.67 mmol, 1.2 eq.), 3-OH propyl-TPP **4** (200 mg, 0.561 mmol, 1.0 eq.) and *N-N'*-dicyclohexylcarbodiimide (139 mg, 0.673 mmol, 1.2 eq.) were dissolved in anhydrous acetonitrile (2 mL) under an atmosphere of argon while stirring. The reaction mixture was stirred at RT for 16 h, the solid dicyclohexylurea was filtered out and the product was purified by column chromatography [SiO_2, dichloromethane-methanol from (93:7) to (85:15)], to afford TPP$_3$-malonate as a white sticky foam (163 mg, 0.367 mmol, 66%). ν_{max} (ATR): 2879 (CH), 2808 (OH), 1731 (C=O), 1716 (C=O), 1587, (Ph), 1439 (PC) cm^{-1}. R_f [SiO_2, dichloromethane-methanol (9:1)] = 0.21. δ_H (400 MHz, $CDCl_3$): 7.89-7.63 (15H, m, 15 × CH, Ar), 4.38-4.19 (2H, m, CH_2O), 3.81-3.55 (2H, m, CH_2P), 3.65 (2H, s, CH_2CO), 2.05-1.88 (2H, m, CH_2CH_2P). δ_C (101 MHz, $CDCl_3$): 168.26 (C), 167.20 (C), 135.35 (d, *J* = 2.8, CH), 133.76 (d, *J* = 10.0 Hz, CH), 130.80 (d, *J* = 12.6 Hz, CH), 117.87 (d, *J* = 86.4 Hz, C), 63.65 (d, *J* = 17.2 Hz, CH_2), 43.05 (CH_2), 22.43 (d, *J* = 3.1

Hz, CH_2), 19.68 (d, J = 53.3 Hz, CH_2). δ_P (162 MHz, $CDCl_3$): 24.36 (PPh_3). LRMS (ESI^+): 407 (M^+, 100%). HRMS (ESI^+): 407.1395. $C_{24}H_{24}O_4P^+$ requires M^+, 407.1407.

1.3.5. Synthesis of mono(6-triphenylphosphoniohex-1-yl) malonate, chloride salt (TPP_6-malonate).

(6-Hydroxyhex-1-yl)triphenylphosphonium bromide **5** (400 mg, 0.902 mmol, 1.0 eq.), malonic acid (113 mg, 1.08 mmol, 1.2 eq.) and *N,N'*-dicyclohexylcarbodiimide (224 mg, 1.08 mmol, 1.2 eq.) were dissolved in anhydrous acetonitrile while stirring under an atmosphere of argon. The reaction mixture was stirred at RT for 16 h, washed with brine (50 mL), extracted with dichloromethane (3 × 30 mL), dried over magnesium sulfate, filtered and the concentrated under reduced pressure. The crude was purified by column chromatography [SiO_2, dichloromethane-methanol from (93:7) to (83:17)] to yield TPP_6-malonate as a white hygroscopic sticky foam (178 mg, 0.366 mmol, 41%). R_f [SiO_2, dichloromethane-methanol (85:15)] = 0.30. ν_{max} (ATR): 2951 (OH), 2920 (CH), 2853 (CH), 1734 (C=O), 1716 (C=O), 1439 (PC) cm^{-1}. δ_H (500 MHz, $CDCl_3$): 7.91-7.75 (9H, m, 9 × CH, Ar), 7.75-7.62 (6H, m, 6 × CH, Ar), 4.18 (2H, t, J = 4.8 Hz, CH_2O), 3.77-3.64 (2H, m, CH_2P), 3.58 (2H, s, CH_2CO), 1.73-1.46 (8H, m, 4 × CH_2). δ_C (126 MHz, $CDCl_3$): 167.62 (C), 166.71 (C), 135.08 (d, J = 2.6 Hz, CH), 133.86 (d, J = 9.6 Hz, CH), 130.63 (d, J = 12.4 Hz, CH), 118.65 (d, J = 85.7 Hz, C), 64.79 (CH_2), 43.30 (CH_2), 29.48 (d, J = 11.4 Hz, CH_2), 27.90 (CH_2), 25.41 (CH_2), 22.54 (CH_2), 21.94 (d, J = 21.94 Hz, CH_2). δ_P (202 MHz, $CDCl_3$): 24.64 (PPh_3). LRMS (ESI^+): 449 (M^+, 100%). HRMS (ESI^+): 449.1862. $C_{27}H_{30}O_4P^+$ requires M^+, 449.1876.

1.3.6. Synthesis of mono(11'-triphenylphosphinoundec-1-yl) malonate, chloride salt (TPP_{11}-malonate). DCC (47 mg, 0.23 mmol, 1.2 eq) was added to a solution of 11-hydroxyundec-1-

yl)triphenylphosphonium bromide **6** (100 mg, 0.19 mmol, 1.0 eq), and malonic acid (24 mg, 0.23 mmol, 1.2 eq) in MeCN (2 ml). The solution was stirred overnight at R.T. and concentrated under vacuum. The residue was extracted into DCM and washed with water (2 × 20 ml). The organic layer dried over magnesium sulfate and concentrated under vacuum. The residue was purified by column chromatography [SiO_2, dichloromethane-methanol from (100:0) to (85:15)]. The product containing fractions were containing fractions were concentrated to near dryness then precipitated from ether to give the acid as colourless viscous hygroscopic oil (52 mg, 49%). δ_H (400 MHz: $CDCl_3$): 8.48 (1H, broad s, OH), 7.78-7.64 (15H, m, Ar), 3.99 (2H, t, *J* = 6.5 Hz, OCH_2), 3.56-3.48 (2H, m, PCH_2), 3.37 (2H, s, CH_2), 1.62-1.48 (6H, m, 3×CH_2), 1.29-1.15 (12H, m, 6×CH_2). δ_C (101 MHz: $CDCl_3$): 169.10 (C), 168.18 (C), 135.13 (d, *J* = 3.0 Hz, CH), 133.59 (d, *J* = 9.9 Hz, CH), 130.58 (d, *J* = 12.4 Hz, CH), 118.35 (d, *J* = 85.8 Hz, C), 64.92 (CH_2), 43.02 (CH_2), 30.23 (d, *J* = 15.6 Hz, CH_2), 28.97 (CH_2), 29.31 (CH_2), 28.91 (CH_2), 28.80 (CH_2), 28.78 (CH_2), 28.73 (CH_2), 25.51 (CH_2), 22.56 (d, *J* = 4.5 Hz, CH_2), 22.26 (d, *J* = 50.4 Hz, CH_2). δ_P (162 MHz: $CDCl_3$): 24.0 (s).

1.3.7. Synthesis of (3-hydroxypropyl)tri(pentadeuterophenyl)phosphonium bromide (d_{15}-3-OH propyl-TPP). Triphenylphosphine-d15 (100 mg, 0.36 mmol, 1.0 eq) was added to a solution of 3-bromopropan-1-ol (75 µl, 0.54 mmol, 1.5 eq) in MeCN (2ml). The solution was added to 80 °C under an atmosphere of argon overnight. After cooling to R.T. the solution was slowly added to Et_2O (~20 ml), the resulting precipitate filtered and washed with ether (10 ml) to give the salt as off white hygroscopic solid (77 mg, 51%). δ_H (400 MHz: $CDCl_3$): 1.79-2.00 (2H, m, CH_2), 3.78-3.87 (4H, m, CH_2). δ_P (162 MHz: $CDCl_3$): 24.39 (s).

2. Supporting Figures

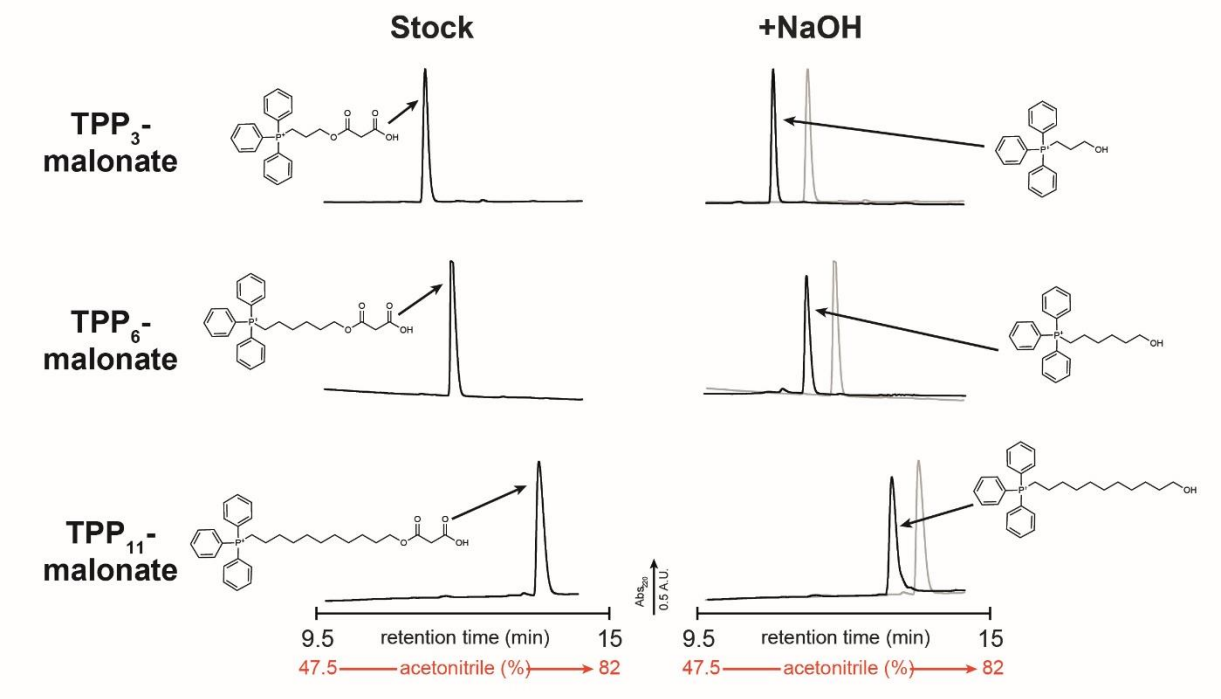

Figure S2. Base hydrolysis of TPP-malonate monoesters. TPP-malonate monoesters (1 nmol) were treated with excess NaOH (50 nmol) and analyzed by UV-HPLC. Representative traces of 3 independent experiments.

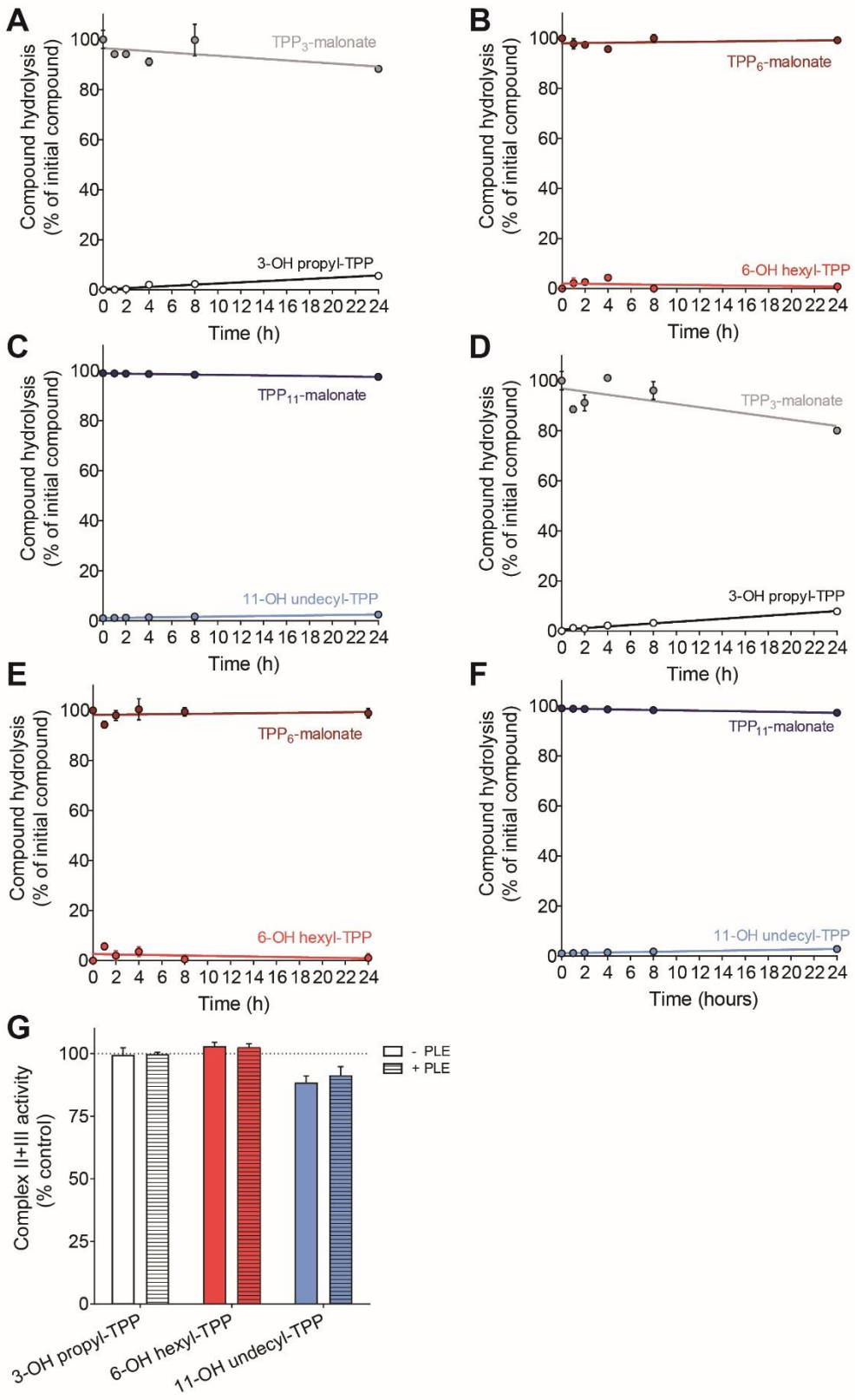
A
Compound hydrolysis (% of initial compound)
TPP3-malonate
3-OH propyl-TPP
Time (h)
B
TPP6-malonate
6-OH hexyl-TPP
C
TPP11-malonate
11-OH undecyl-TPP
D
E
F
Time (hours)
G
Complex II+III activity (% control)
- PLE
+ PLE
3-OH propyl-TPP
6-OH hexyl-TPP
11-OH undecyl-TPP

Figure S3. Non-enzymatic hydrolysis of TPP-malonate monoesters. (A-F) TPP_3-malonate (A and D), TPP_6-malonate (B and E) or TPP_{11}-malonate (C and F) (all at 200 μM) together with internal standard (propyl-TPP or isoamyl-TPP to avoid retention time overlap; 200 μM) were incubated in KCl buffer (37 °C) at pH 7.2 (A-C) or pH 8 (D-F) and sampled at 0, 1, 2, 4, 8 or 24 hours. Samples were analyzed by RP-HPLC-UV at 220 nm. Peak areas from each sample were normalized to the peak area of the internal control and presented as a percentage of the peak area of the initial parent compound (mean ± S.E.M, n=3). (G) Complex II + III activity assay with TPP-alcohols. The assay was carried out as in Figure 2E but with TPP-alcohols (10 μM) ± PLE (mean ± S.E.M. % of EtOH control activity, n=3).

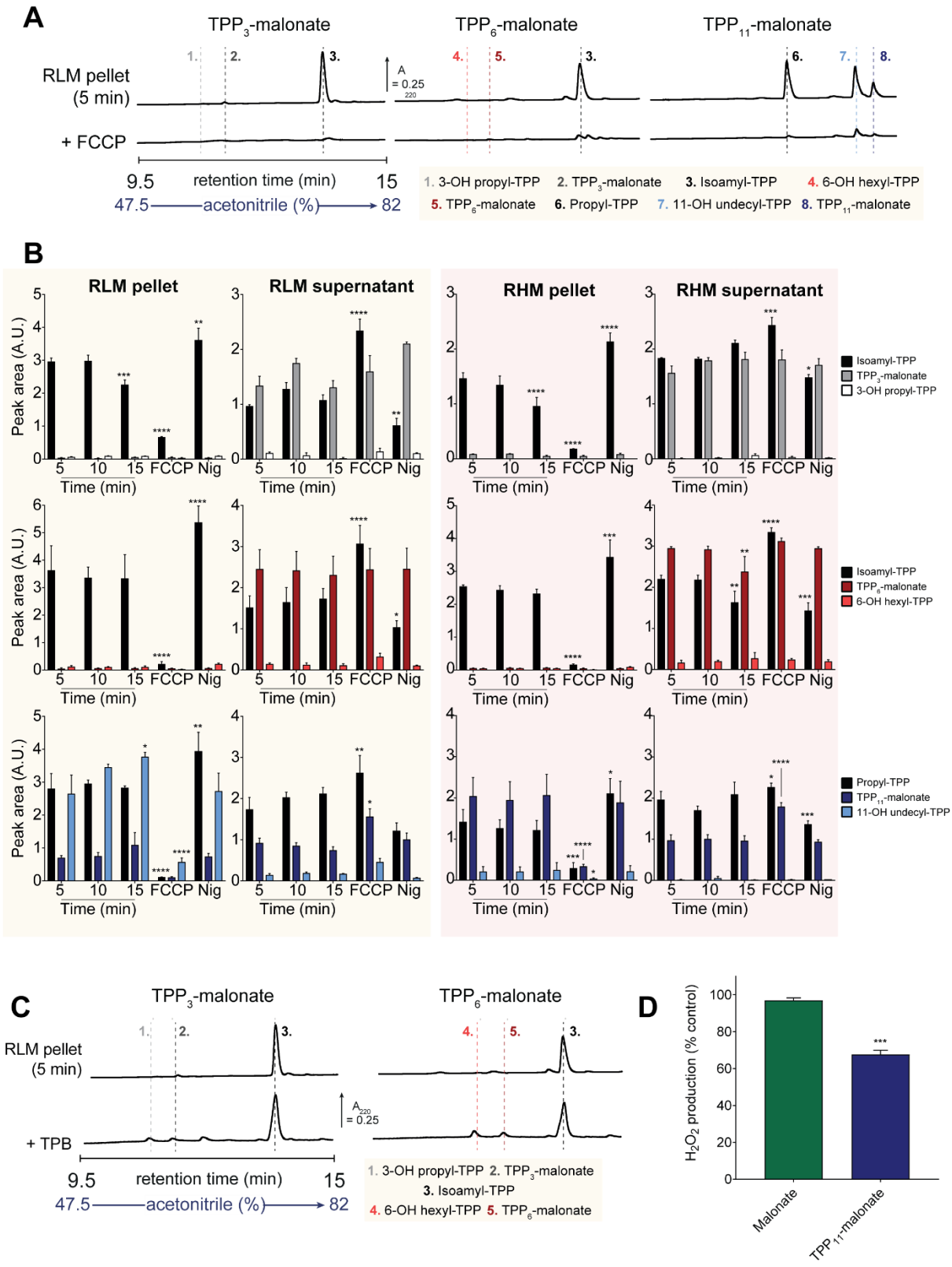
A
TPP3-malonate
TPP6-malonate
TPP11-malonate
RLM pellet (5 min)
+ FCCP
A220 = 0.25
retention time (min)
9.5
15
47.5
acetonitrile (%)
82
1. 3-OH propyl-TPP
2. TPP3-malonate
3. Isoamyl-TPP
4. 6-OH hexyl-TPP
5. TPP6-malonate
6. Propyl-TPP
7. 11-OH undecyl-TPP
8. TPP11-malonate
B
RLM pellet
RLM supernatant
RHM pellet
RHM supernatant
Peak area (A.U.)
Time (min)
5 10 15 FCCP Nig
Isoamyl-TPP
TPP3-malonate
3-OH propyl-TPP
TPP6-malonate
6-OH hexyl-TPP
Propyl-TPP
TPP11-malonate
11-OH undecyl-TPP
C
TPP3-malonate
TPP6-malonate
RLM pellet (5 min)
+ TPB
A220 = 0.25
retention time (min)
9.5
15
47.5
acetonitrile (%)
82
1. 3-OH propyl-TPP 2. TPP3-malonate
3. Isoamyl-TPP
4. 6-OH hexyl-TPP 5. TPP6-malonate
D
H2O2 production (% control)
Malonate
TPP11-malonate

Figure. S4. Uptake and hydrolysis of mitochondria-targeted malonate esters in rat liver and heart mitochondria. RLM or RHM (0.5mg protein/ml), respiring with 10 mM succinate/4 μg/ml rotenone, were incubated with TPP-malonate monoesters (5 μM) and internal control (5 μM) and incubated at 37 °C for 5, 10 or 15 min. Where FCCP (1 μM) or nigericin (100 nM) were added, the incubation time was 5 min. Mitochondria were pelleted and both mitochondrial pellet and supernatant extracted before analysis by RP-HPLC-UV at 220 nm. (A) Representative trace of mitochondrial pellet from RLM and (B) quantification of mitochondrial pellets and supernatants from treated RLM and RHM (mean ± S.E.M. of quantified peak areas, n=3). Statistical significance was assessed (comparing against the relevant 5 min value) by two-way ANOVA with Dunnett's correction for multiple comparisons, where *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (C) RLM were treated as in (A) with TPP_3-malonate and TPP_6-malonate but in the presence of TPB (5 μM) and incubated for 5 min (representative trace of 3 biological replicates). (D) H_2O_2 inhibition by TPP_{11}-malonate. H_2O_2 production was measured as in Figure 3F in the presence of either 10 μM malonate or 10 μM TPP_{11}-malonate and presented as % of control of values (KCl buffer for malonate or 10 μM 11-OH undecyl-TPP for TPP_{11}-malonate). Mean ± S.E.M., n=3, statistical significance assessed by unpaired, two-tailed Student's t-test where ****p*<0.001.

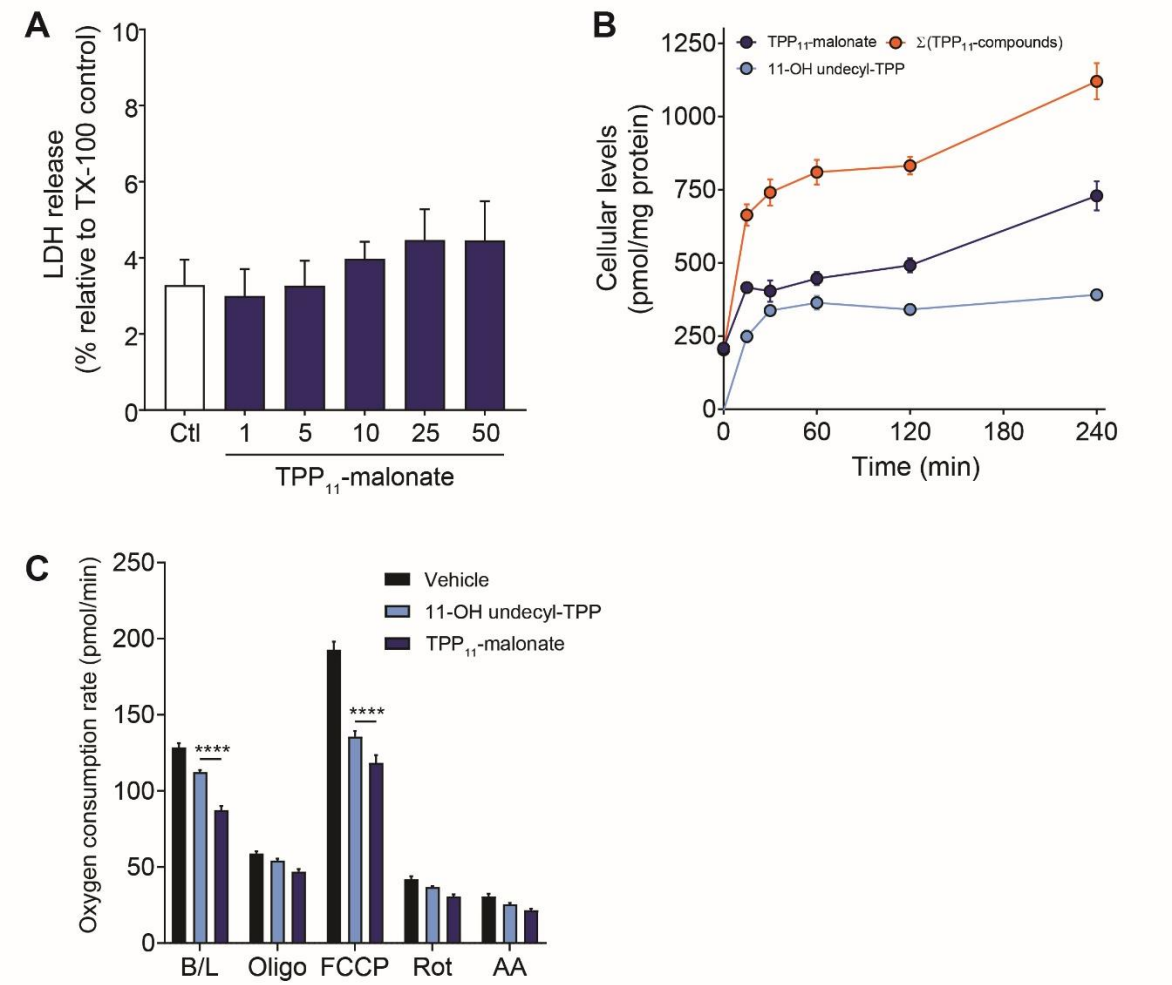

Figure S5. TPP_{11}-malonate incubation with cells. (A) TPP_{11}-malonate is not toxic to cells. C2C12 mouse myoblasts were incubated with 0.1% EtOH (Ctl) or TPP_{11}-malonate for 24 hours before measuring LDH release (mean ± S.E.M. of the percentage of cell death compared to Triton-X100 treated cells; n=3). (B) Uptake of TPP_{11}-malonate in HeLa cells. HeLa cells were treated with TPP_{11}-malonate like Figure 4C and the uptake measured by LC-MS/MS (mean ± S.E.M., n=3). (C) Cellular oxygen consumption with TPP_{11}-malonate or 11-OH undecyl-TPP. Oxygen consumption was measured in the presence of 0.1% EtOH (vehicle), TPP_{11}-malonate or 11-OH undecyl-TPP (both 5 µM) ± oligomycin (1.5 µM), FCCP (0.5 µM), rotenone (4 µg/ml) and antimycin A (10 µM) (mean ± S.E.M., n=3 biological replicates with 10 technical replicates per condition).

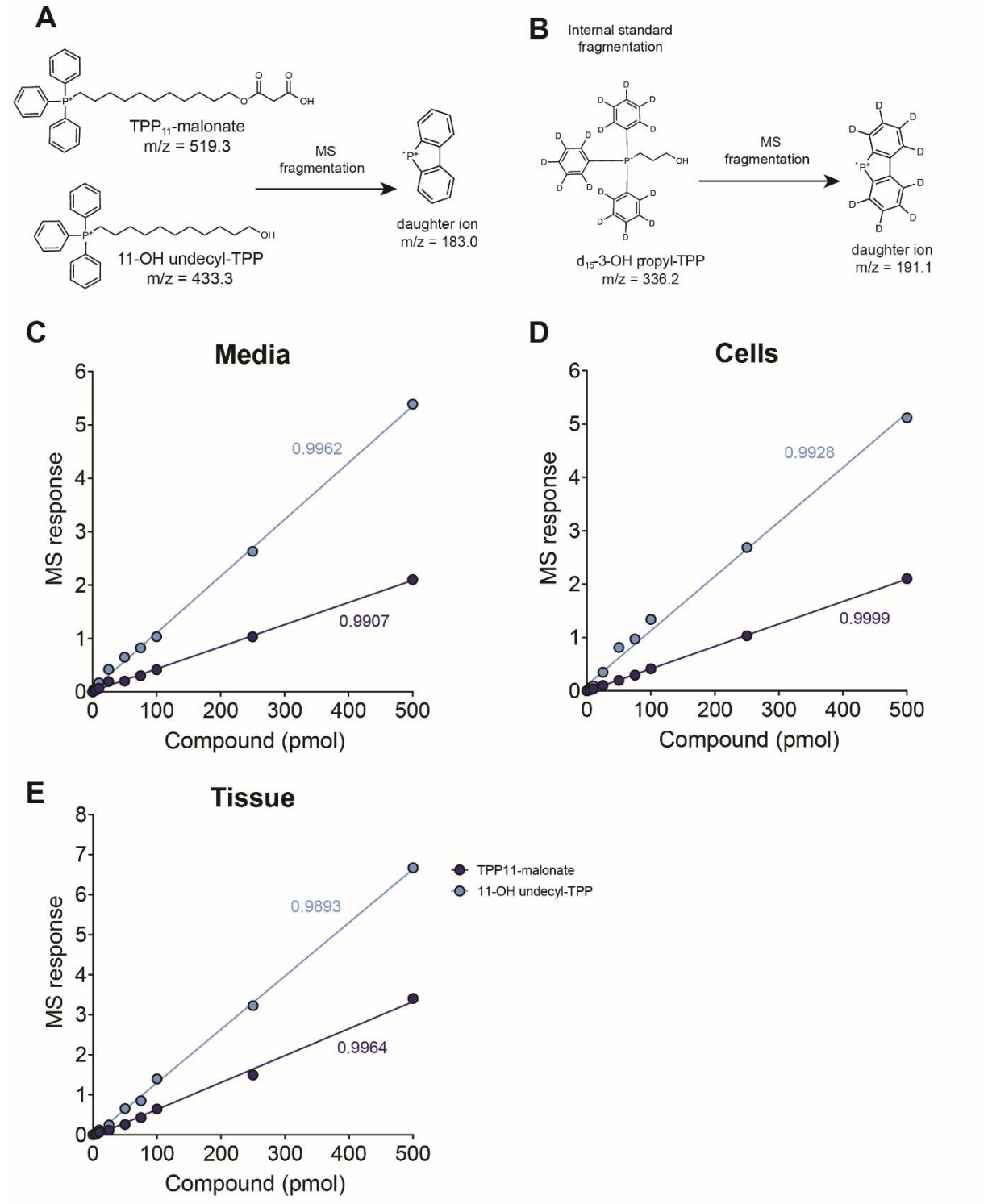

Figure S6. Detection of TPP_{11}-malonate and 11-OH undecyl-TPP by LC-MS/MS. (A) TPP_{11}-malonate and 11-OH undecyl-TPP with daughter ion used for analysis. (B) Structure of d_{15}-3-OH propyl-TPP (MS internal standard) and daughter ion used for analysis. (C-E) Standard curves of

compound peak area relative to MS internal standard peak area (MS response) in the presence of cell culture medium (C), cells (D) or tissue (E), with r^2 values shown (mean ± range of 2 independent replicates).

3. Supplementary Methods

3.1. LDH assay for cellular toxicity. C2C12 mouse myoblasts were plated in 96-well plates (10,000 cells/well) and adhered overnight in a humidified incubator at 37 °C and 5% CO_2. The following day, media was replaced with 200 µl phenol red-free DMEM media supplemented with 10% FBS and the indicated treatments or vehicle (0.1% EtOH) and incubated for 24 h. Substance controls without cells and 2% Triton X-100 were used as low and high controls. After 24 h, the plate was centrifuged (250 x g, 10 min) and 100 µl supernatant removed and assessed for LDH release using a cytotoxicity detection kit (Roche, UK) according to the manufacturer's instructions.

3.2. Measurement of cellular oxygen consumption rate (OCR). C2C12 mouse myoblasts were plated in Seahorse 96-well plates (10,000 cells/well; Agilent) and adhered overnight in a humidified incubator at 37 °C and 5% CO_2. The following day, the media was replaced with Seahorse assay buffer (Agilent) containing 0.1% EtOH (vehicle control), 11-OH undecyl-TPP (5 µM) or TPP_{11}-malonate (5 µM) and incubated for 30 min before measuring oxygen consumption rate by a Seahorse XFe96 analyzer (Agilent). Sequential port additions of oligomycin, FCCP, rotenone and antimycin A were used at final concentrations of 1.5 µM, 0.5 µM, 4 µg/ml and 10 µM respectively.

4. Supplementary References

(1) Dolle, R. E.; Li, C. S.; Novelli, R.; Kruse, L. I.; Eggleston, D. Enantiospecific Synthesis of (-)-Tabtoxinine. Beta.-Lactam. *J. Org. Chem.* **1992**, *57* (1), 128–132.

(2) Couturier, M.; Dory, Y. L.; Rouillard, F.; Deslongchamps, P. Studies Directed towards the Total Synthesis of Aldosterone and Naturally Occurring Analogues. A Unified Approach Using the Transannular Diels-Alder Reaction. *Tetrahedron* **1998**, *54* (8), 1529–1562.

(3) Culcasi, M.; Casano, G.; Lucchesi, C.; Mercier, A.; Clément, J.-L.; Pique, V.; Michelet, L.; Krieger-Liszkay, A.; Robin, M.; Pietri, S. Synthesis and Biological Characterization of New Aminophosphonates for Mitochondrial PH Determination by 31 P NMR Spectroscopy. *J. Med. Chem.* **2013**, *56* (6), 2487–2499.