
Supplementary information

**Small-molecule inhibitors of human
mitochondrial DNA transcription**

In the format provided by the
authors and unedited

Supplementary Information

Supplementary Figures

Supplementary Figure 1: Uncropped gels of Figures 1, 3-4 and Extended Data Figures 1-4, 6-8.

Figure 1h

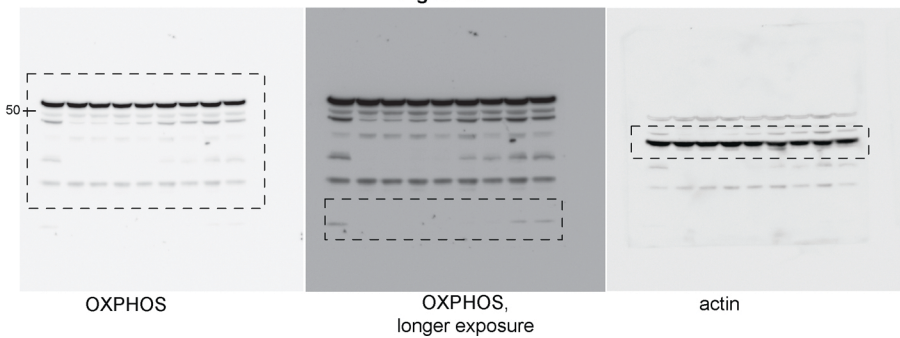
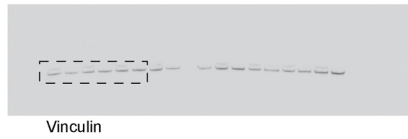
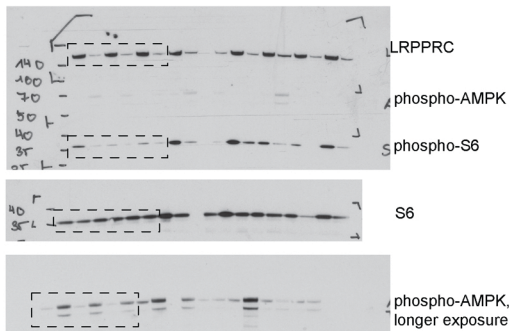
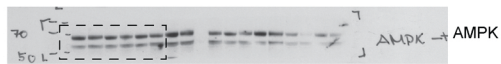


Figure 3d



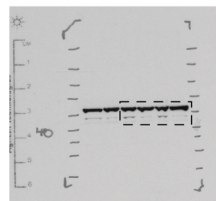
Gel 1



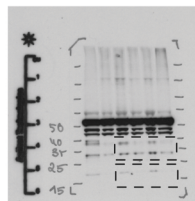
Gel 2

Figure 4c

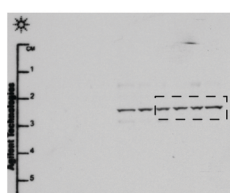
Tumour



OXPHOS



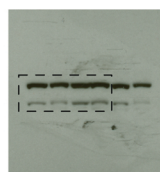
OXPHOS
longer exposure
actin



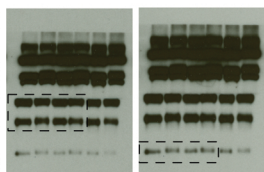
actin
short exposure

Gel 1

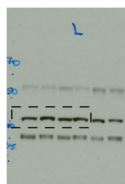
Liver



OXPHOS



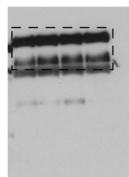
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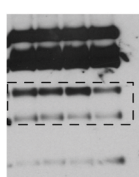
actin

Gel 2

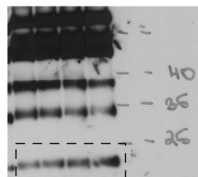
Heart



OXPHOS



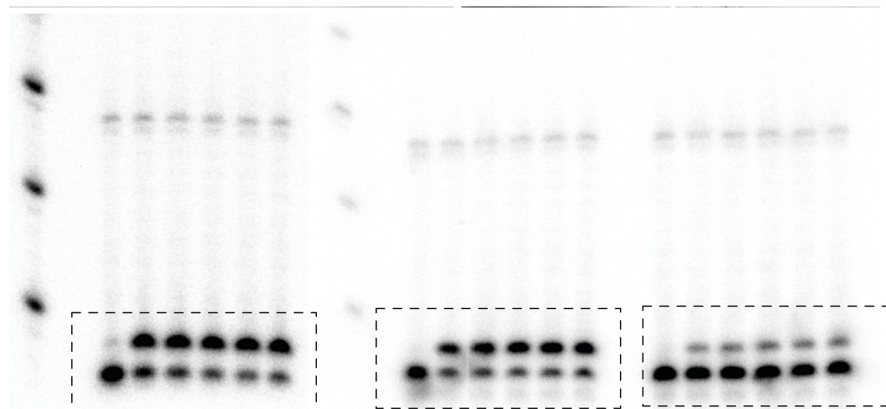
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longer exposure



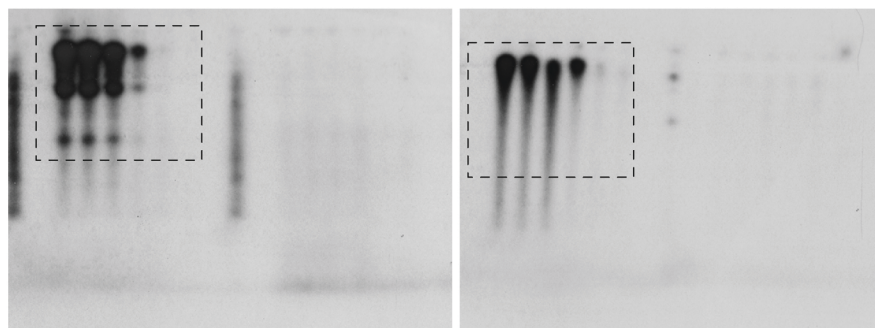
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Gel 3

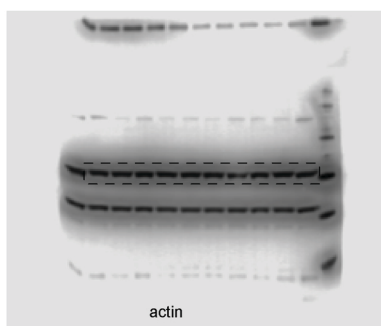
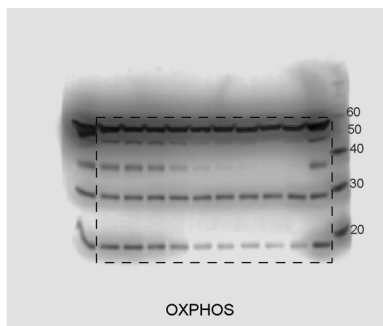
Extended Data Figure 1e



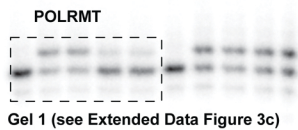
Extended Data Figure 1h



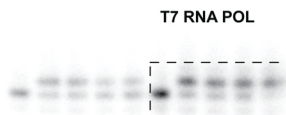
Extended Data Figure 2c



Extended Data Figure 3a



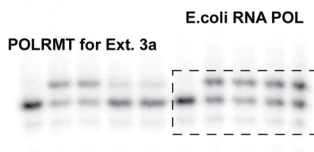
Extended Data Figure 3b



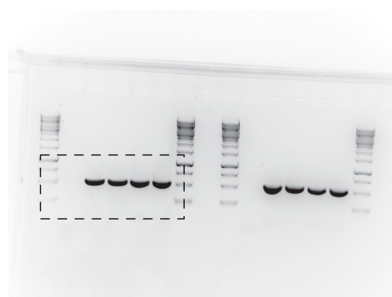
RPO41

Gel 2

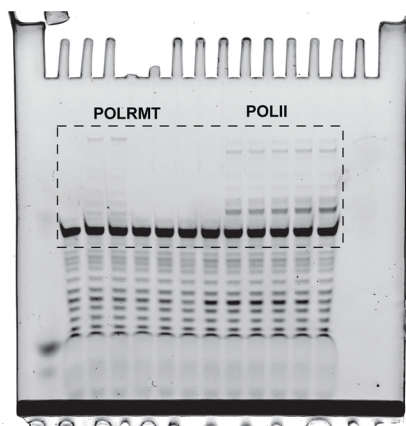
Extended Data Figure 3c



Extended Data Figure 3d



Extended Data Figure 3e



Extended Data Figure 3g

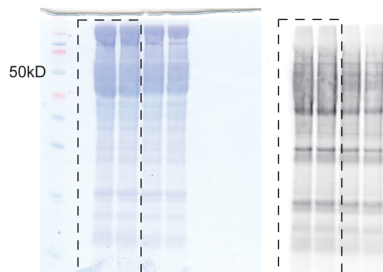
POLG



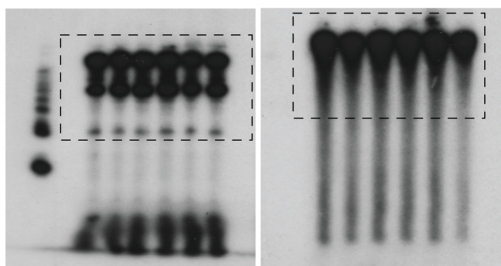
Extended Data Figure 3h

Coomassie

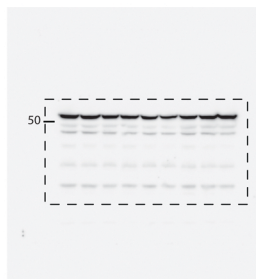
S35-Methionine



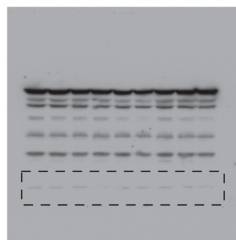
Extended Data Figure 3k



Extended Data Figure 3o



OXPHOS

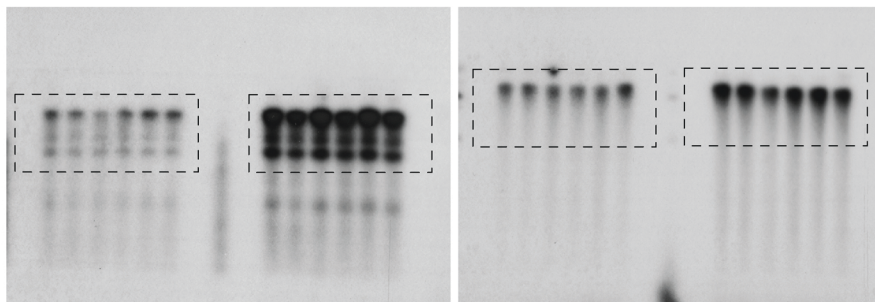


**OXPHOS
longer exposure**

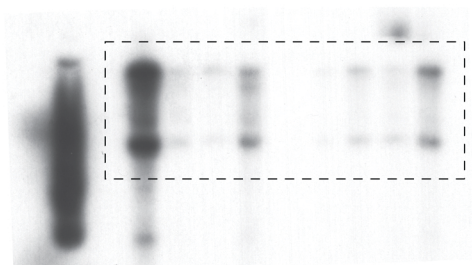


actin

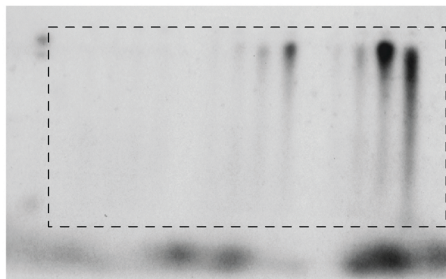
Extended Data Figure 4 b



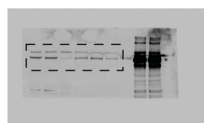
Extended Data Figure 4 d



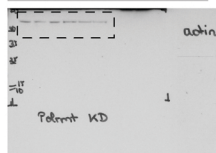
Extended Data Figure 4 e



Extended Data Figure 6b



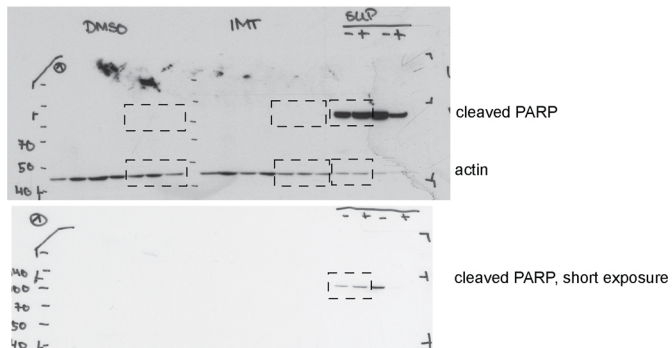
POLRMT
*recombinant protein



actin

POLRMT KD

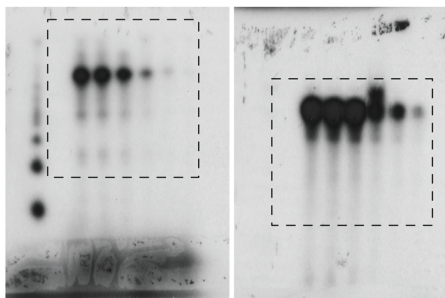
Extended Data Figure 7b



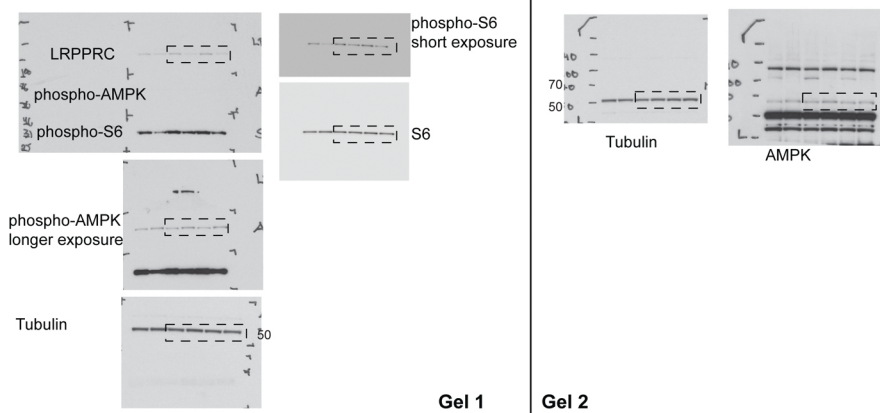
Extended Data Figure 7c



Extended Data Figure 8 d



Extended Data Figure 8 k



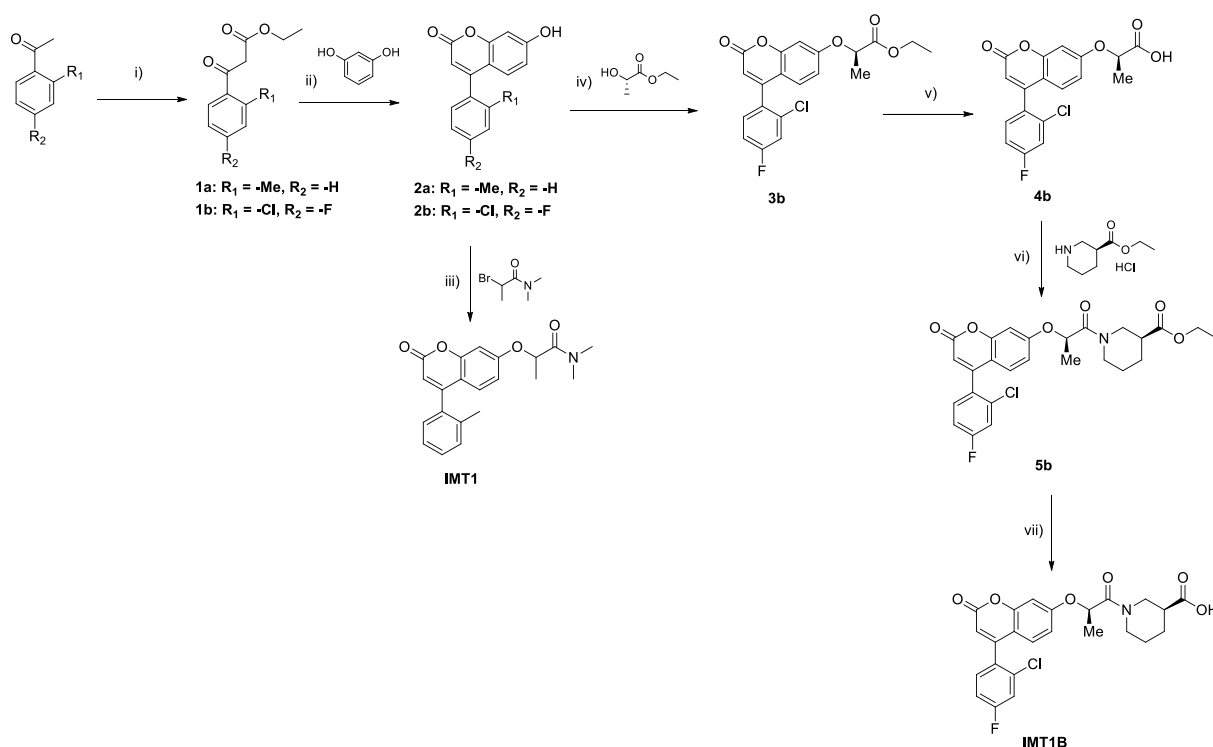
Supplementary Methods

Synthesis of IMTs

General Chemistry

Anhydrous solvents and reagents were purchased from various fine chemical suppliers and were used without further purification. ^1H NMR spectra were taken on a Oxford Varian 400/54 (400 MHz) spectrometer or a Bruker Avance II (300 MHz) with residual protonated solvent (CHCl_3 δ 7.26; DMSO δ 2.49) as reference. Data was reported as follows: chemical shift, multiplicity (bs, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant, and integration. ^{13}C NMR spectra were taken on a Bruker Biospin with power gated H1 decoupling (150 MHz) spectrometer and showed an unusually complicated splitting pattern, due to the presence of diastereomers, Carbon-Fluorine Coupling and rotational effects. For this reason, the peaks assigned to the same carbon are shown between brackets. Final compounds were judged to be $\geq 95\%$ pure by HPLC analysis using a Waters Acquity UPLC (Sample Manager, Binary Solvent Manager, Column Heater/Cooler, PDA e λ Detector; SQ Detector), Column Acquity UPLC BEH C18 1.7 μm , 2.1 x 50 mm, eluted with H_2O with 0.05% formic acid (Solvent A) and MeCN with 0.05% formic acid (Solvent B) at 0.5 mL/min. Gradient: elution from 5% to 100% B over 3.5 min with an initial hold of 0.5 min and a final hold at 100% B of 0.5 min. Total run time: 5 min or a HPLC/MS Waters (2767 Sample Manager, 515 HPLC Pump, 2525 Binary Gradient Module, 2996 Photodiode array Detector, Micromass ZQ Detector), Column Xterra[®] MS C18 5 μm 100 x 4.6 mm, eluted with H_2O with 0.1% formic acid (Solvent A) and MeCN (Solvent B) at 2 mL/min. Gradient: elution from 5% to 100% B over 7 min and a final hold at 100% B of 1.5 min. Total run time: 8.5 min. Reported yields are not optimized, with emphasis on purity of products rather than quantity.

Scheme 1: synthesis of compounds IMT1 and IMT1B



i) (EtCO)₂O, 60% NaH in mineral oil, toluene, 50°C, 18h; ii) MsOH, 45°C, 2 h; iii) Cs₂CO₃, DMF, r.t., 1.5 h; iv) PPh₃, DIAD, THF, 0°C to r.t., 4 h; v) 2 M NaOH, THF, 0°C to r.t., 1 - 3 h, b) 2 M HCl, 30 min; vi) EDC.HCl, HOBT.xH₂O, Et₃N, DMF, r.t., 1.5 h; vii) a) 2 M NaOH, THF, MeOH, 0°C to r.t., 1 - 3 h, b) 2 M HCl, 30min.

Experimental procedures

Condensation reaction to obtain intermediate 1b

Ethyl 3-(2-chloro-4-fluorophenyl)-3-oxopropanoate (1b)

60% NaH in mineral oil (4.86 g, 121.7 mmol) was washed with pentane and dried under a flow of nitrogen. Dry toluene (350 mL) was added and the suspension was cooled to 0°C. Diethyl carbonate (27.4 g, 231.8 mmol) was added dropwise over a period of 25 min, followed by 2-chloro-4-fluoroacetophenone (10 g, 57.9 mmol) over a period of 20 min. The cooling bath was removed, and the reaction heated to 50°C and stirred for 18 h. The reaction mixture was allowed to cool to r.t. and was poured into ice cold water (500 mL). The aqueous layer was acidified to pH 2 with 10% aq. HCl and extracted with Et₂O. The combined organic phases were dried over MgSO₄, filtered and evaporated *in vacuo* to yield the title compound **1b** (6.0 g, 42%) as a mixture of tautomers, which was used in the following step without further purification.

Tautomer 1, ethyl 3-(2-chloro-4-fluorophenyl)-3-oxopropanoate: ¹H NMR (300 MHz, Chloroform-*d*) δ 7.63 (dd, *J* = 8.8, 6.0 Hz, 1H), 7.14-6.93 (m, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.96 (s, 2H), 1.18 (t, *J* = 7.1 Hz, 3H); LCMS *t*_R = 5.18 min, MS (ESI+) *m/z* 245.19, 247.18 (M+H)⁺, 43%.

Tautomer 2, (Z)-ethyl 3-(2-chloro-4-fluorophenyl)-3-hydroxyacrylate: ¹H NMR (300 MHz, Chloroform-*d*) δ 12.43 (s, 1H), 7.52 (dd, *J* = 8.7, 6.1 Hz, 1H), 7.14-6.93 (m, 2H), 5.48 (s, 1H),

4.21 (q, $J = 7.1$ Hz, 2H), 1.27 (t, $J = 7.1$ Hz, 3H); LCMS $t_R = 6.38$ min, MS (ESI+) m/z 245.19, 247.18 (M+H)⁺, 34%.

General Procedure 1: Pechmann reaction to obtain intermediates 2

Intermediate **1** (1.05 eq) was dissolved in methansulfonic acid (30 mL/g) and resorcinol (1 eq) was added. The mixture was stirred at 45°C for 2 h, upon which the reaction was allowed to cool to r.t. EtOH was added dropwise, followed by water. The suspension was extracted with EtOAc and washed with brine. The combined organic layers were dried over MgSO₄ filtered, and evaporated *in vacuo*. The crude product was purified by column chromatography using a gradient of EtOAc in cHex to yield the title compound **2**.

7-Hydroxy-4-(*o*-tolyl)-2H-chromen-2-one (2a)

Ethyl (2-methylbenzoyl)acetate **1a** (11.3 g, 54.8 mmol) was reacted with resorcinol (5.7 g, 51.8 mmol) according to **General Procedure 1** to yield the title compound **2a** (6.6 g, 51%) as a white solid. δ ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.65 (s, 1H), 7.48 – 7.28 (m, 3H), 7.23 (dd, $J = 7.5, 1.3$ Hz, 1H), 6.80 (dd, $J = 5.5, 3.2$ Hz, 2H), 6.72 (dd, $J = 8.7, 2.2$ Hz, 1H), 6.09 (s, 1H), 2.11 (s, 3H); LCMS $t_R = 5.33$ min, MS (ESI+) m/z 253.13 (M+H)⁺, 99%.

4-(2-Chloro-4-fluorophenyl)-7-hydroxy-2H-chromen-2-one (2b)

Intermediate **1b** (28.2 g, 115 mmol) was reacted with resorcinol (12 g, 109 mmol) according to **General Procedure 1** to yield the title compound **2b** (25.4 g, 80%) as a pink solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.70 (bs, 1H), 7.69 (dd, $J = 8.9, 2.5$ Hz, 1H), 7.55 (dd, $J = 8.6, 6.1$ Hz, 1H), 7.42 (td, $J = 8.5, 2.6$ Hz, 1H), 6.91 – 6.77 (m, 2H), 6.74 (dd, $J = 8.7, 2.3$ Hz, 1H), 6.21 (s, 1H); LCMS $t_R = 5.28$ min, MS (ESI+) m/z : 291.10, 293.16 (M+H)⁺, 95%.

Alkylation reaction to obtain compound IMT1

N,N-dimethyl-2-((2-oxo-4-(*o*-tolyl)-2H-chromen-7-yl)oxy)propanamide (IMT1)

Intermediate **2a** (70 mg, 0.277 mmol) was dissolved in DMF (2 mL) and Cs₂CO₃ (121 mg, 0.555 mmol), followed by 2-bromo-*N,N*-dimethylpropanamide (58.1 g, 0.416 mmol) were added. The reaction was stirred at r.t. for 1.5 h. The mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water and brine, dried over MgSO₄, filtered, and evaporated *in vacuo*. The crude product was purified by flash chromatography on silica gel using a gradient of EtOAc in cHex to yield the title compound **IMT1** (79.8 mg, 82%) as an off-white solid. $[\alpha]_D$ (deg cm³ g⁻¹ dm⁻¹) = -0,004 ($c = 1$ mg cm⁻³, CHCl₃, 20 °C); m.p.: 86.2°C; ¹H NMR (400 MHz, Chloroform-*d*) δ 7.31 (td, $J = 7.5, 1.5$ Hz, 1H), 7.27 – 7.20 (m, 2H), 7.10 – 7.06 (m, 1H), 6.89 (d, $J = 8.5$ Hz, 1H), 6.72 – 6.67 (m, 2H), 6.09 (s, 1H), 4.97 (qd, $J = 6.7, 1.7$ Hz, 1H), 3.07 (d, $J = 3.1$ Hz, 3H), 2.95 – 2.86 (m, 3H), 2.08 (d, $J = 2.3$ Hz, 3H), 1.58 – 1.55 (m, 3H); ¹³C

NMR (150 MHz, DMSO-*d*₆) δ 169.64, 160.99, 160.49, 155.81, 155.35, 135.32, 135.11, 130.83, 129.58, 128.81, 128.19, 126.54, [113.63, 113.45], [113.07, 113.04], 112.52, 102.28, 71.26, 36.68, 35.80, 19.77, 17.58; LCMS t_R = 2.68 min, MS (ESI+) m/z : 351.72 (M+H)⁺, 99%; HRMS (FAB, *m*-NBA) m/z calc. for C₂₁H₂₂NO₄ 352.15433, found 352.15399 [M+H]⁺.

Mitsunobu reaction to obtain intermediate **3b**

(R)-Ethyl 2-((4-(2-chloro-4-fluorophenyl)-2-oxo-2H-chromen-7-yl)oxy)propanoate (3b)

Intermediate **2b** (12 g, 41.28 mmol) and PPh₃ (11.91 mg, 45.41 mmol) were dissolved in THF (7 mL), and (-)-Ethyl (*S*)-2-hydroxypropionate (7.07 ml, 61.92 mmol) was added. The reaction was cooled to 0°C and DIAD (8.94 ml, 45.41 mmol) was added dropwise. The reaction was then stirred at r.t. for 4 h. The mixture was diluted with EtOAc and washed with a sat. NaHCO₃ solution, a sat. NH₄Cl solution, and water. The organic layer was dried over MgSO₄, filtered, and evaporated *in vacuo*. The crude product was purified by flash chromatography on silica gel using a gradient of EtOAc in cHex to yield the title compound **3b** (13.53 g, 83%) as a colorless glue. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.70 (ddd, *J* = 8.9, 2.5, 1.5 Hz, 1H), 7.57 (ddd, *J* = 8.6, 6.1, 1.5 Hz, 1H), 7.43 (tdd, *J* = 8.5, 2.6, 1.3 Hz, 1H), 7.02 (dd, *J* = 5.9, 2.4 Hz, 1H), 6.95 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.88 (ddd, *J* = 8.9, 3.6, 2.5 Hz, 1H), 6.33 (s, 1H), 5.18 (dd, *J* = 6.8, 1.1 Hz, 1H), 4.16 (qd, *J* = 7.1, 1.4 Hz, 2H), 1.54 (dd, *J* = 6.8, 0.5 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H); LCMS t_R = 3.23min, MS (ESI+) m/z : 391.29, 393.32 (M+H)⁺, 100%.

General Procedure 3: Hydrolysis reaction to obtain intermediate **4b** or final compound **IMT1B**

Intermediate **3b** or **5b** (1 eq) was dissolved in THF and 2 M NaOH aq. solution was added at 0°C. A few drops of MeOH were added until the mixture was homogeneous. The reaction was allowed to warm up to r.t. stirring for 1 - 3 h, neutralized with 2 M HCl, and stirred for a further 30 min. The mixture was extracted with EtOAc and the combined organic phases were washed with water, dried over MgSO₄, filtered, and evaporated *in vacuo* to yield the desired product **4b**. Alternatively, the crude product was purified by flash chromatography on silica gel using a gradient of MeOH in DCM to yield the desired products **IMT1B**.

(R)-2-((4-(2-chloro-4-fluorophenyl)-2-oxo-2H-chromen-7-yl)oxy)propanoic acid (4b)

Intermediate **3b** (13.53 g, 34.62 mmol) was hydrolyzed with 2 M NaOH aq. solution according to **General Procedure 3** to yield the title compound **4b** (12.55 g, 100%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.21 (s, 1H), 7.68 (ddd, *J* = 8.9, 2.5, 1.9 Hz, 1H), 7.54 (dd, *J* = 8.6, 6.1 Hz, 1H), 7.46 – 7.37 (m, 1H), 7.00 – 6.89 (m, 2H), 6.85 (ddd, *J* = 8.9, 2.5, 1.5 Hz, 1H), 5.04 (qd, *J* = 6.8, 2.0 Hz, 1H), 1.51 (d, *J* = 6.7 Hz, 3H); LCMS t_R = 2.70min, MS (ESI+) m/z : 363.30, 365.25 (M+H)⁺, 100%.

Amide coupling to obtain intermediate 5b

(S)-ethyl 1-((R)-2-((4-(2-chloro-4-fluorophenyl)-2-oxo-2H-chromen-7-yl)oxy)propanoyl)piperidine-3-carboxylate (5b)

Intermediate **4b** (13.51 g, 37.24 mmol) EDC.HCl (10.71 g, 55.87 mmol), and HOBT.xH₂O (8.56 g, 55.87 mmol) were dissolved in DMF (370 mL) and (S)-piperidine-3-carboxylic acid ethyl ester (2R,3R)-2,3-dihydroxybutanedioate (7.03 g, 44.69 mmol) was added slowly. Et₃N (7.8 mL, 55.87 mmol) was added, and the mixture was stirred at r.t. for 1.5 h. The reaction was diluted with EtOAc and washed with water, a sat. NaHCO₃ solution, a sat. NH₄Cl solution, and again with water. The organic phase was dried over MgSO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on silica gel using a gradient of EtOAc in cHex to yield the title compound **5b** (18.13 g, 97%) as a colorless glue.

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.70 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.60-7.52 (m, 1H), 7.43 (t, *J* = 8.6 Hz, 1H), 7.01 - 6.88 (m, 2H), 6.88 - 6.76 (m, 1H), 6.31 (s, 1H), 5.54 - 5.42 (m, 1H), 4.27 - 3.97 (m, 3H), 3.89 - 3.73 (m, 1H), 3.70 - 3.52 (m, 1H), 3.27 - 2.95 (m, 1H), 2.64 - 2.37 (m, 1H), 2.01 - 1.86 (m, 1H), 1.85 - 1.50 (m, 3H), 1.50 - 1.40 (m, 3H), 1.22 - 1.13 (m, 3H); LCMS *t*_R = 3.08 min, MS (ESI+) *m/z*: 502.25, 504.27 (M+H)⁺, 100%.

(S)-1-((R)-2-((4-(2-chloro-4-fluorophenyl)-2-oxo-2H-chromen-7-yl)oxy)propanoyl)piperidine-3-carboxylic acid (IMT1B)

Intermediate **5b** (18.13 g, 36.12 mmol) was hydrolyzed with 2 M NaOH aq. solution according to **General Procedure 3** to yield the title compound **IMT1B** (14.37 g, 84%) as a white solid. [α]_D (deg cm³ g⁻¹ dm⁻¹) = +0.022 (*c* = 1 mg cm⁻³, CHCl₃, 20 °C); m.p.: 149.8 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.46 (bs, 1H), 7.68 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.58 - 7.50 (m, 1H), 7.41 (tdd, *J* = 8.5, 2.5, 0.9 Hz, 1H), 6.99 - 6.86 (m, 2H), 6.86 - 6.74 (m, 1H), 6.33 - 6.23 (m, 1H), 5.54 - 5.41 (m, 1H), 4.33 - 3.16 (m, 1H), 3.90 - 3.72 (m, 1H), 3.68 - 3.47 (m, 1H), 3.15 - 2.77 (m, 1H), 2.55 - 2.21 (m, 1H), 2.02 - 1.83 (m, 1H), 1.80 - 1.65 (m, 1H), 1.65 - 1.30 (m, 5H); ¹³C NMR (150 MHz, DMSO-*d*₆) δ [174.90, 174.56], 168.27, [163.66, 162.00], [161.30, 161.07], [160.31, 160.25], 155.26, 152.65, [132.98, 132.90], [132.66, 132.63, 132.60, 132.57], 130.81, [128.29, 128.21, 128.00], [117.77, 117.60], [115.64, 115.49], [113.68, 113.59], [113.51, 113.43, 113.27, 113.17], [112.60, 112.53, 112.47, 112.43], [102.65, 102.55, 102.39], [71.47, 71.39, 71.29, 71.24], [46.50, 45.28], [44.00, 42.17], [41.25, 41.00, 40.93], [27.17, 27.02], [25.29, 23.89, 23.84], [18.18, 18.15, 17.86, 17.81]; LCMS *t*_R = 2.64 min, MS (ESI+) *m/z*: 474.27, 476.29 (M+H)⁺, 100%; HRMS (FAB, *m*-NBA) *m/z* calc. for C₂₄H₂₂NO₆ClF 474.11142, C₂₄H₂₂NO₆³⁷ClF 476.10847, found 474.10996 / 476.10717 [M+H]⁺.

Supplementary Tables

Supplementary Table 1: Cryo-EM data collection, refinement and validation statistics.

	POLRMT-IMT (EMDB-11679) (PDB 7A8P)
Data collection and processing	
Magnification	105,000
Voltage (kV)	300
Electron exposure (e ⁻ /Å ²)	36
Defocus range (μm)	0.5 – 3.0
Pixel size (Å)	0.834
Symmetry imposed	C1
Initial particle images (no.)	1,446,981
Final particle images (no.)	193,651
Map resolution (Å)	3.5
FSC threshold	0.143
Map resolution range (Å)	3.4 – 4.5
Refinement	
Initial model used (PDB code)	4BOC
Model resolution (Å)	3.5
FSC threshold	0.5
Model resolution range (Å)	3.4 – 4.5
Map sharpening <i>B</i> factor (Å ²)	-130
Model composition	
Non-hydrogen atoms	7305
Protein residues	911
Ligands	1
<i>B</i> factors (Å ²)	
Protein	54.32
Ligand	27.65
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.878
Validation	
MolProbity score	1.12
Clashscore	2.23
Poor rotamers (%)	0.63
Ramachandran plot	
Favored (%)	97.77

Allowed (%)	2.23
Disallowed (%)	0.00

Supplementary Table 2: Cell lines used in this study.

Tissue origin	Cell line	Reference
bladder	5637	ATCC® HTB-9
	CLS-439	CLS; 300150
	J82	ATCC® HTB-1
	T24	ATCC® HTB-4
	UMUC3	ATCC® CRL-1749
bone	MG63	ATCC® CRL-1427
	MHH-ES1	CLS; 300136
	RD-ES	ATCC® HTB-166
	SAOS2	ATCC® HTB-85
	U2OS	ATCC® HTB-96
brain	SF-295	NCI-DTP, SF-295
	SK-N-AS	ATCC® CRL-2137
	SK-N-SH	ATCC® HTB-11
	SNB75	NCI-DTP, SN-75
	U87MG	ATCC® HTB-14
breast	BT-20	ATCC® HTB-19
	Hs578T	ATCC® HTB-126
	JIMT1	DSMZ, ACC 589
	MCF7	ATCC® HTB-22
	MDA-MB-231	ATCC® HTB-26
	MDA-MB-436	ATCC® HTB-130
	MDA-MB-468	ATCC® HTB-132
	SKBR3	ATCC® HTB-30
cervix	C33A	ATCC® HTB-31
	Ca Ski	ATCC® CRM-CRL-1550
	HeLa	ATCC® CCL-2
colon	Caco-2	ATCC® HTB-37
	COLO 205	ATCC® CCL-222
	COLO-678	DSMZ, ACC 194

	DLD-1	ECACC, 90102540
	HCT-116	ATCC® CCL-247
	HCT-15	ATCC® CCL-225
	HT-29	ATCC® HTB-38
	LoVo	ATCC® CCL-229
	SW620	ATCC® CCL-227
connective tissue	HT-1080	ATCC® CCL-121
hematological	GRANTA-519	DSMZ, ACC 342
	HL-60	DSMZ, ACC 3
	K-562	DSMZ, ACC 10
	Kasumi-1	DSMZ, ACC 220
	L-363	DZMZ, ACC 49
	Mino	DSMZ, ACC 687
	MV-4-11	DSMZ, ACC 102
	PBMC	donor specific
	Ramos	DSMZ, ACC602
	SU-DHL-10	DSMZ, ACC 576
	SU-DHL-6	DSMZ, ACC 572
	THP-1	DSMZ, ACC 16
	WSU-NHL	DSMZ, ACC 58
kidney	786-O	ATCC® CRL-1932
	ACHN	ATCC® CRL-1611
	Caki-1	ATCC® HTB-46
	Hek293	ATCC® CRL-1573
	UO-31	NCI-DTP, UO-31
liver	HepG2	ATCC® HB-8065
	PLC/PRF/5	ATCC® CRL-8024
	SK-HEP-1	ATCC® HTB-52
lung	A549	ATCC® CCL-185
	Calu-6	ATCC® HTB-56
	IMR-90	ATCC® CCL-186
	NCI-H292	ATCC® CRL-1848
	NCI-H358M	NCI-DTP, NCI-H358M
	NCI-H460	ATCC® HTB-177

	NCI-H82	ATCC® HTB-175
muscle	A-204	ATCC® HTB-82
	A-673	ATCC® CRL-1598
	Hs729	ATCC® HTB-153
	RD	ATCC® CCL-136
ovary	A2780	ECACC 93112519
	EFO-21	DSMZ, ACC 235
	IGROV1	NCI-DTP, IGR-OV1
	OVCAR3	ATCC® HTB-161
	OVCAR4	NCI-DTP, OVCAR-4
	SK-OV-3	ATCC® HTB-77
pancreas	AsPC-1	ATCC® CRL-1682
	BxPC-3	ATCC® CRL-1687
	MIA PaCa-2	ATCC® CRM-CRL-1420
	PANC-1	ATCC® CRL-1469
	Panc 10.05	ATCC® CRL-2547
placenta	JAR	ATCC® HTB-144
	JEG-3	ATCC® HTB-36
prostate	22Rv1	ATCC® CRL-2505
	DU 145	ATCC® HTB-81
	PC-3	ATCC® CRL-1435
skin	A-375	ATCC® CRL-1619
	A-431	ATCC® CRL-1555
	SK-MEL-28	ATCC® HTB-72
	SK-MEL-5	ATCC® HTB-70
vulva	SK-LMS-1	ATCC® HTB-88

ATCC, American Type Culture Collection (Manassas, VA, USA); CLS, Cell Line Service GmbH (Eppelheim, Germany); NCI-DTP, National Institutes of Cancer – Developmental Therapeutics Program (Bethesda, MD, USA); DSMZ, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany); ECACC, European Collection of Authenticated Cell Cultures (Salisbury, UK).

Supplementary Table 3: Mission esiRNA pools used in this study.

ID	esiRNA against EGFP
Catalogue-#	EHUEGFP (Sigma-Aldrich)
esiRNA cDNA target sequence:	
GTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGGCCATCCTGGTCGAGCTGGACGGCGACG TAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGAC CTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGC CATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTT CAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACAAGCCACAACGTCTAT ATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGG ACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTG CTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGA TCACATGGTCTCTGCTGGAGTTCGTGACCGCCGCGGGGATCACTCTCGGCATGGACGAGCTGTA	
ID	esiRNA against human POLRMT
Catalogue-#	EHU012581 (Sigma-Aldrich)
esiRNA cDNA target sequence:	
CAGGTGCTGGAAGGTTTCATCACCCGCAAGGTGGTGAAGCAGACGGTGATGACGGTGGTGTACG GGGTCACGCGCTATGGCGGGCGCCTGCAGATTGAGAAGCGCCTCCGGGAGCTGAGCGACTTTCC CCAGGAGTTCGTGTGGGAGGCCTCTCACTATCTCGTACGCCAGGTCTTCAAGAGTCTACAGGAGA TGTTCTCGGGGACCCGGGCCATCCAGCACTGGCTGACCGAGAGTGCCCGCCTCATCTCCACATG GGCTCTGTGGTGGAGTGGGTACACCCCTGGGCGTCCCCGTCATCCAGCCCTATCGCCTGGACTCC AAGGTCAAGCAAATAGGAGGTGGAATTCAGAGCATCACCTACACCCACAACGGAGACATCAGCC GAAAGCCCAACACACGTAAGCAGAAGAACGGCTTCCCGCCCAACTTCATCCACTCGCTGGACTCCT CCCACATGATGCTCACCGCCCTGCACTGCTACAGGAAGGGCCTGACCTTCGTCTCTGTGCAC	

Supplementary Table 4: Taqman probes used in this study.

Reagent	Source	Identifier
Taqman Assay: human CytB	Life Technologies	Hs02596867_s1
Taqman Assay: human ND1	Life Technologies	Hs02596873_s1
Taqman Assay: human COX1	Life Technologies	Hs02596864_g1
Taqman Assay: human RNA, 45S pre-ribosomal 5	Life Technologies	Hs05627131_gH
Taqman Assay: human RNA, 5S ribosomal 1	Life Technologies	Hs03682751_gH
Taqman Assay: human POLRMT	Life Technologies	Hs04187596_g1
Taqman Assay: human 18S	Life Technologies	Hs99999901_s1
Taqman Assay: mouse ND1	Life Technologies	Mm04225274_s1

Taqman Assay: mouse CytB	Life Technologies	Mm04225271_g1
Taqman Assay: mouse COX1	Life Technologies	Mm04225243_g1
Taqman Assay: mouse ACTB	Life Technologies	Mm01205647_g1
Taqman Assay: mouse beta-2-microglobulin	Life Technologies	Mm00437762_m1

Supplementary Table 5: MRM transitions.

Compound class	Compound	Precursor	Fragment	dwell time	cone V	collision	Function	RT	Ionization mode
deoxynucleotide triphosphate	dATP	490,02	158,89	0,066	30	26	33	quantifier	[M-H ⁺]-
deoxynucleotide triphosphate	dATP	490,02	391,97	0,066	30	24	33	qualifier	[M-H ⁺]-
deoxynucleotide triphosphate	dCTP	465,98	158,81	0,078	28	30	29	quantifier	[M-H ⁺]-
deoxynucleotide triphosphate	dCTP	465,98	368,01	0,078	28	20	29	qualifier	[M-H ⁺]-
deoxynucleotide triphosphate	dGTP	505,98	158,88	0,138	30	34	34	quantifier	[M-H ⁺]-
deoxynucleotide triphosphate	dGTP	505,98	408	0,138	30	20	34	qualifier	[M-H ⁺]-
deoxynucleotide triphosphate	dTTP	480,83	158,88	0,066	28	46	30	quantifier	[M-H ⁺]-
deoxynucleotide triphosphate	dTTP	480,83	383	0,066	28	20	30	qualifier	[M-H ⁺]-
glycolysis	3-Phosphoglyceric acid	184,97	96,95	0,057	20	12	9	quantifier	[M-H ⁺]-
glycolysis	3-Phosphoglyceric acid	184,97	78,91	0,057	20	10	9	qualifier	[M-H ⁺]-
glycolysis	Fructose 1,6-P	339,06	96,95	0,066	26	22	20	quantifier	[M-H ⁺]-
glycolysis	Fructose 1,6-P	339,06	241,06	0,066	26	14	20	qualifier	[M-H ⁺]-
glycolysis	Phosphoenolpyruvic acid	166,97	78,95	0,057	14	10	7	quantifier	[M-H ⁺]-
glycolysis	Phosphoenolpyruvic acid	166,97	138,93	0,057	14	8	7	qualifier	[M-H ⁺]-
glycolysis	Pyruvic acid	86,84	42,95	0,245	18	16	1	quantifier	[M-H ⁺]-
glycolysis	Pyruvic acid	86,84	58,97	0,245	18	8	1	qualifier	[M-H ⁺]-
IS	ATP_C13	516,16	158,98	0,066	38	36	36	quantifier	[M-H ⁺]-
IS	ATP_C13	516,16	418,16	0,066	38	20	36	qualifier	[M-H ⁺]-
IS	Citric acid d4	195,1	113,11	0,057	16	12	12	quantifier	[M-H ⁺]-
IS	Citric acid d4	195,1	88,77	0,057	16	18	12	qualifier	[M-H ⁺]-
nucleotide diphosphate	ADP	425,98	158,85	0,078	32	28	27	quantifier	[M-H ⁺]-
nucleotide diphosphate	ADP	425,98	272,88	0,078	32	28	27	qualifier	[M-H ⁺]-
nucleotide diphosphate	CDP	401,96	158,86	0,057	32	26	25	quantifier	[M-H ⁺]-
nucleotide diphosphate	CDP	401,96	303,98	0,057	32	18	25	qualifier	[M-H ⁺]-
nucleotide diphosphate	GDP	441,98	150	0,138	30	30	28	quantifier	[M-H ⁺]-
nucleotide diphosphate	GDP	441,98	344,06	0,138	30	16	28	qualifier	[M-H ⁺]-
nucleotide diphosphate	UDP	402,94	158,86	0,066	34	26	26	quantifier	[M-H ⁺]-
nucleotide diphosphate	UDP	402,94	110,94	0,066	34	20	26	qualifier	[M-H ⁺]-
nucleotide monophosphate	AMP	346	133,97	0,061	30	30	22	quantifier	[M-H ⁺]-

nucleotide monophosphate	AMP	346	96,89	0,061	30	24	22	qualifier	[M-H+]-
nucleotide monophosphate	CMP	322,03	96,92	0,066	28	22	17	quantifier	[M-H+]-
nucleotide monophosphate	CMP	322,03	210,98	0,066	28	16	17	qualifier	[M-H+]-
nucleotide monophosphate	GMP	362,12	78,9	0,066	30	24	24	quantifier	[M-H+]-
nucleotide monophosphate	GMP	362,12	210,96	0,066	30	14	24	qualifier	[M-H+]-
nucleotide monophosphate	IMP	346,98	78,86	0,066	28	18	23	quantifier	[M-H+]-
nucleotide monophosphate	IMP	346,98	96,9	0,066	28	20	23	qualifier	[M-H+]-
nucleotide monophosphate	UMP	322,96	210,96	0,057	28	12	18	quantifier	[M-H+]-
nucleotide monophosphate	UMP	322,96	96,9	0,057	28	20	18	qualifier	[M-H+]-
nucleotide triphosphate	ATP	506,1	158,94	0,066	36	32	35	quantifier	[M-H+]-
nucleotide triphosphate	ATP	506,1	408,04	0,066	36	16	35	qualifier	[M-H+]-
nucleotide triphosphate	CTP	481,93	384,02	0,066	26	20	31	quantifier	[M-H+]-
nucleotide triphosphate	CTP	481,93	158,9	0,066	26	22	31	qualifier	[M-H+]-
nucleotide triphosphate	GTP	521,96	158,86	0,138	36	32	37	quantifier	[M-H+]-
nucleotide triphosphate	GTP	521,96	424,02	0,138	36	20	37	qualifier	[M-H+]-
nucleotide triphosphate	UTP	482,92	158,85	0,066	30	20	32	quantifier	[M-H+]-
nucleotide triphosphate	UTP	482,92	385	0,066	30	20	32	qualifier	[M-H+]-
TCA	alfa_Ketoglutaric acid	144,82	56,94	0,061	16	10	6	quantifier	[M-H+]-
TCA	alfa_Ketoglutaric acid	144,82	72,94	0,061	16	14	6	qualifier	[M-H+]-
TCA	Cis-Aconitic acid	172,86	84,9	0,057	16	14	8	quantifier	[M-H+]-
TCA	Cis-Aconitic acid	172,86	110,93	0,057	16	8	8	qualifier	[M-H+]-
TCA	Citric acid	190,9	110,92	0,057	20	12	11	quantifier	[M-H+]-
TCA	Citric acid	190,9	86,92	0,057	20	18	11	qualifier	[M-H+]-
TCA	Fumaric acid	114,97	70,96	0,061	18	6	3	quantifier	[M-H+]-
TCA	Fumaric acid	114,97	44,96	0,061	22	20	3	qualifier	[M-H+]-
TCA	Isocitric acid	190,9	72,93	0,057	20	20	10	quantifier	[M-H+]-
TCA	Isocitric acid	190,9	154,94	0,057	20	12	10	qualifier	[M-H+]-
TCA	Malic acid	132,87	42,94	0,066	18	12	5	quantifier	[M-H+]-
TCA	Malic acid	132,87	70,28	0,066	20	18	5	qualifier	[M-H+]-
TCA	Succinic acid	116,97	72,97	0,066	20	10	4	quantifier	[M-H+]-
TCA	Succinic acid	116,97	98,99	0,066	20	12	4	qualifier	[M-H+]-

Supplementary Table 6: Definition of boxplots shown in Extended Data Figure 2d (IMT1) and Extended Data Figure 3p (Con IMT).

category	comparison	compound	lower whisker	lower box hinge	median	upper box hinge	upper whisker
Complex I	0h	IMT1	-0,297	-0,056	0,049	0,108	0,199
Complex II	0h	IMT1	-0,017	-0,017	0,086	0,188	0,188
Complex III	0h	IMT1	-0,086	-0,08	-0,038	0,104	0,177
Complex IV	0h	IMT1	-0,206	-0,125	-0,113	0,012	0,184
Complex V	0h	IMT1	-0,099	-0,076	-0,026	0,008	0,128
cytosolic ribosome	0h	IMT1	-0,35	-0,144	-0,078	-0,006	0,156
mitochondrial ribosome	0h	IMT1	-0,217	-0,089	-0,026	0,027	0,167
Complex I	24h	IMT1	-1,409	-0,819	-0,601	-0,273	0,461
Complex II	24h	IMT1	-0,088	-0,088	-0,041	0,007	0,007
Complex III	24h	IMT1	-1,019	-0,584	-0,211	0,076	0,484
Complex IV	24h	IMT1	-0,54	-0,502	-0,324	-0,17	0,035
Complex V	24h	IMT1	-0,205	-0,13	-0,071	0,09	0,181
cytosolic ribosome	24h	IMT1	-0,358	-0,147	-0,06	0,024	0,277
mitochondrial ribosome	24h	IMT1	-2,337	-1,376	-1,052	-0,725	0,067
Complex I	48h	IMT1	-1,539	-1,193	-0,837	-0,387	0,217
Complex II	48h	IMT1	0,018	0,018	0,084	0,15	0,15
Complex III	48h	IMT1	-1,086	-0,941	-0,668	0,089	0,238
Complex IV	48h	IMT1	-1,45	-1,006	-0,997	-0,701	-0,701
Complex V	48h	IMT1	-0,04	-0,018	0,006	0,055	0,159
cytosolic ribosome	48h	IMT1	-0,114	-0,007	0,026	0,069	0,153
mitochondrial ribosome	48h	IMT1	-2,171	-1,6	-1,352	-1,109	-0,406
Complex I	6h	IMT1	-0,216	-0,124	-0,053	0,034	0,201
Complex II	6h	IMT1	-0,024	-0,024	0,014	0,052	0,052
Complex III	6h	IMT1	-0,31	-0,205	-0,01	0,096	0,329
Complex IV	6h	IMT1	-0,324	-0,153	0,01	0,041	0,061
Complex V	6h	IMT1	-0,285	-0,163	-0,087	-0,016	0,108
cytosolic ribosome	6h	IMT1	-0,363	-0,169	-0,095	-0,031	0,118
mitochondrial ribosome	6h	IMT1	-0,616	-0,301	-0,204	-0,068	0,221
Complex I	96h	IMT1	-2,381	-1,918	-1,532	-1,053	0,168
Complex II	96h	IMT1	0,049	0,049	0,067	0,085	0,085
Complex III	96h	IMT1	-1,747	-1,562	-1,449	-0,062	0,304
Complex IV	96h	IMT1	-2,026	-1,718	-1,496	-0,755	-0,138
Complex V	96h	IMT1	-0,065	0,003	0,034	0,075	0,132
cytosolic ribosome	96h	IMT1	-0,084	0,006	0,033	0,069	0,14
mitochondrial ribosome	96h	IMT1	-2,595	-2,019	-1,815	-1,477	-0,72
Complex I	0h	Con IMT	-0,098	-0,018	0,027	0,063	0,157
Complex II	0h	Con IMT	0,077	0,077	0,081	0,086	0,086
Complex III	0h	Con IMT	-0,1	0,018	0,035	0,159	0,209
Complex IV	0h	Con IMT	-0,004	0,001	0,011	0,041	0,079
Complex V	0h	Con IMT	-0,044	-0,015	0,012	0,039	0,049
cytosolic ribosome	0h	Con IMT	-0,143	-0,051	-0,014	0,03	0,114

mitochondrial ribosome	0h	Con IMT	-0,041	-0,002	0,016	0,048	0,107
Complex I	48h	Con IMT	-0,167	-0,097	-0,049	-0,005	0,104
Complex II	48h	Con IMT	0,018	0,018	0,045	0,072	0,072
Complex III	48h	Con IMT	-0,046	-0,046	-0,037	-0,016	0,01
Complex IV	48h	Con IMT	-0,336	-0,255	-0,133	-0,091	0,087
Complex V	48h	Con IMT	-0,13	-0,06	-0,035	-0,006	0,052
cytosolic ribosome	48h	Con IMT	-0,178	-0,089	-0,056	-0,009	0,09
mitochondrial ribosome	48h	Con IMT	-0,159	-0,087	-0,048	-0,016	0,071
Complex I	120h	Con IMT	-0,228	-0,129	-0,08	-0,053	0,041
Complex II	120h	Con IMT	-0,003	-0,003	0,007	0,017	0,017
Complex III	120h	Con IMT	-0,177	-0,069	0,004	0,058	0,115
Complex IV	120h	Con IMT	-0,432	-0,323	-0,271	-0,16	-0,046
Complex V	120h	Con IMT	-0,111	-0,031	0,022	0,033	0,083
cytosolic ribosome	120h	Con IMT	-0,108	-0,018	0,024	0,054	0,134
mitochondrial ribosome	120h	Con IMT	-0,107	-0,038	-0,007	0,019	0,068