SUPPORTING INFORMATION

"The BCKDK inhibitor BT2 is a chemical uncoupler that lowers mitochondrial ROS production and *de novo* lipogenesis"

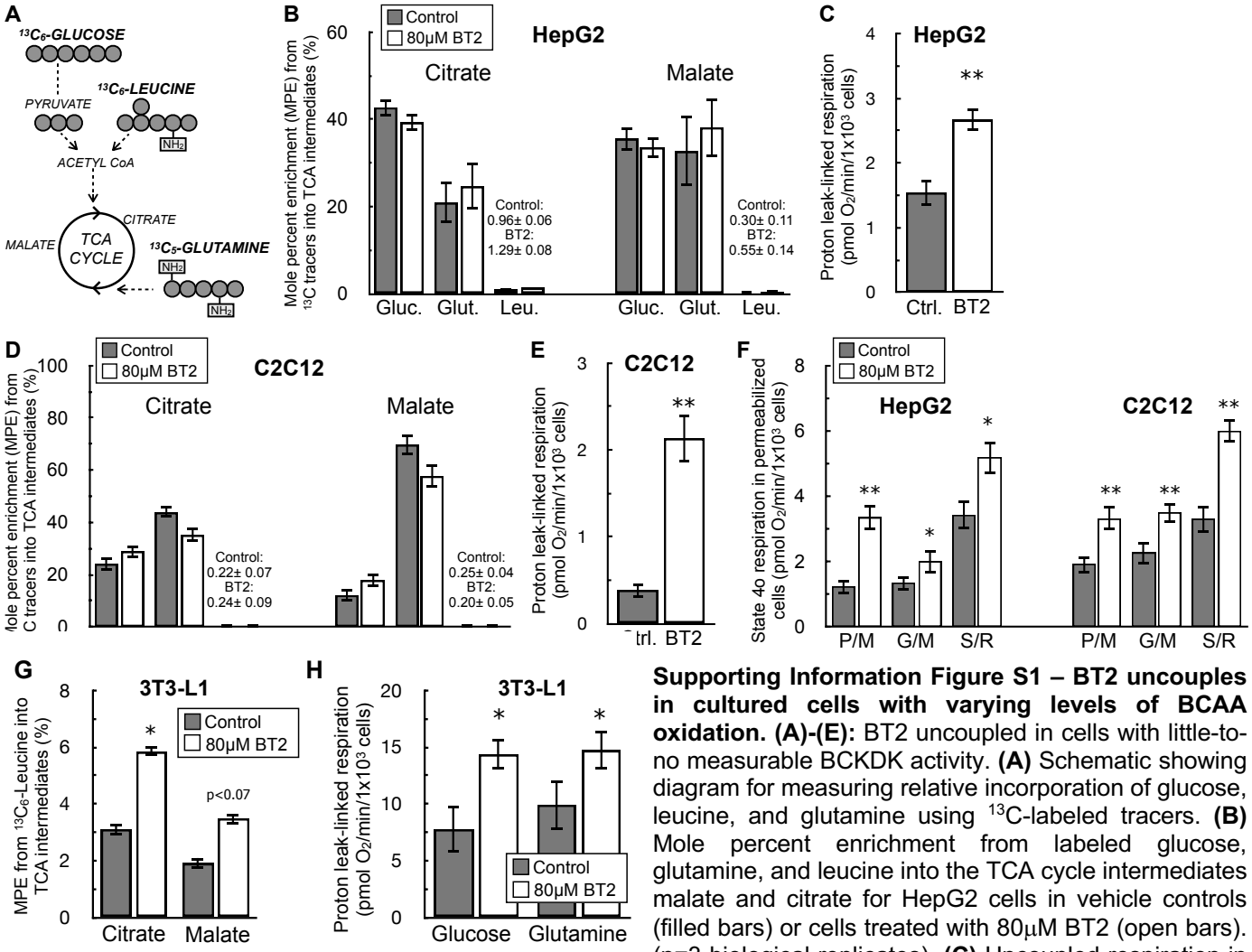
Includes:

Supporting Information Figure S1 – BT2 uncouples in cultured cells with varying levels of BCAA oxidation.

Supporting Information Figure S2 – 200μM BT2 results in proton conductance measurements that cannot be accurately quantified

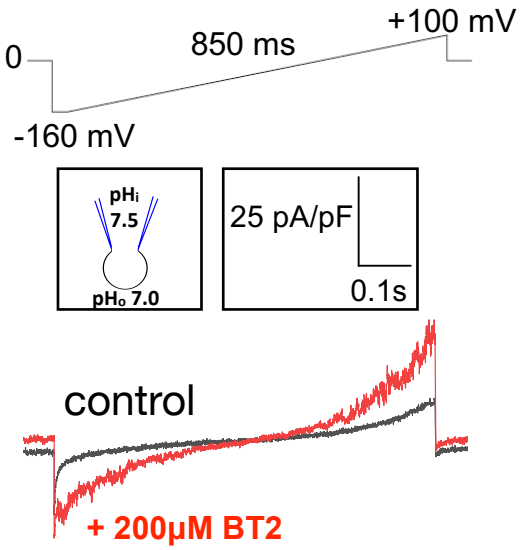
Supporting Table 1 – ISA modeled values and 95% confidence intervals for individual technical replicates

Supporting Information Figure S1 – BT2 uncouples in cultured cells with varying levels of BCAA oxidation.



Supporting Information Figure S1 – BT2 uncouples in cultured cells with varying levels of BCAA oxidation. (A)-(E): BT2 uncoupled in cells with little-to-no measurable BCKDK activity. **(A)** Schematic showing diagram for measuring relative incorporation of glucose, leucine, and glutamine using ^{13}C-labeled tracers. **(B)** Mole percent enrichment from labeled glucose, glutamine, and leucine into the TCA cycle intermediates malate and citrate for HepG2 cells in vehicle controls (filled bars) or cells treated with 80μM BT2 (open bars). (n=3 biological replicates). **(C)** Uncoupled respiration in HepG2 cells offered glucose, glutamine, and pyruvate in DMEM assay medium. (n=4 biological replicates). **(D)** Mole percent enrichment from labeled glucose, glutamine, and leucine into the TCA cycle intermediates malate and citrate for C2C12 myoblasts in vehicle controls (filled bars) or treated with 80μM BT2 (open bars). (n=4 biological replicates). **(E)** Uncoupled respiration in C2C12 cells offered glucose, glutamine, and pyruvate in DMEM assay medium. (n=3 biological replicates). **(F)** BT2 uncouples respiration in permeabilized cells with substrates that bypass BCKDK: State 4$_o$ respiration in permeabilized HepG2 (left) and C2C12 (right) cells in vehicle controls (filled bars) or cells treated with 80μM BT2 (open bars). Permeabilized cells were offered pyruvate/malate (P/M), glutamate/malate (G/M), or succinate/rotenone (S/R). [n=4 (C2C12) or n=5 (HepG2) biological replicates]. **(G)-(H):** As a 'positive control' for measurable leucine oxidation, 3T3-L1 adipocytes show BT2 behaves as expected, but uncouples irrespective of the respiratory substrate provided. **(G)** Mole percent enrichment from uniformly labeled $^{13}C_6$-leucine in differentiated 3T3-L1 adipocytes in vehicle controls (filled bars) or cells treated with 80μM BT2 (open bars). (n=4 biological replicates) **(H)** Uncoupled respiration in differentiated 3T3-L1 adipocytes in the presence or absence of BT2 as before. Cells were assayed in a simple-salts, Krebs-Henseleit buffer supplemented with either glucose or glutamine. (n=3 biological replicates). All data are presented as mean ± S.E.M.

Supporting Information Figure S2 – 200μM BT2 results in proton conductance measurements that cannot be accurately quantified



Supporting Information Figure S2: Patch-clamp electrophysioloigy with cardiac mitoplasts demonstrates that 200μM BT2 yields a bigger current amplitude than 100μM, but disrupts the integrity of the inner membrane. As such, a proton conductance cannot be reliably quantified.

Supporting Information Table S1: ISA modeled values and 95% confidence intervals for individual technical replicates.

Sample	Measurement	Value	Lower range	Upper range	Sample	Measurement	Value	Lower range	Upper range
N1-NT1	D(M2-AcCoA)	0.309	0.264	0.353	N3-NT1	D(M2-AcCoA)	0.558	0.509	0.605
	D(M1-AcCoA)	0.027	0.006	0.052		D(M1-AcCoA)	0.034	0.010	0.066
	1-D(AcCoA)	0.665	0.621	0.708		1-D(AcCoA)	0.408	0.360	0.457
	g(t) palmitate	0.419	0.375	0.459		g(t) palmitate	0.456	0.408	0.498
	TOTAL AcCoA contribution	0.335	0.270	0.405		TOTAL AcCoA contribution	0.592	0.519	0.671
N1-NT2	D(M2-AcCoA)	0.288	0.240	0.335	N3-NT2	D(M2-AcCoA)	0.292	0.236	0.348
	D(M1-AcCoA)	0.027	0.006	0.055		D(M1-AcCoA)	0.030	0.005	0.065
	1-D(AcCoA)	0.685	0.638	0.731		1-D(AcCoA)	0.677	0.624	0.730
	g(t) palmitate	0.394	0.349	0.435		g(t) palmitate	0.354	0.307	0.396
	TOTAL AcCoA contribution	0.315	0.246	0.390		TOTAL AcCoA contribution	0.323	0.241	0.412
N1-NT3	D(M2-AcCoA)	0.244	0.194	0.291	N3-NT3	D(M2-AcCoA)	0.326	0.248	0.398
	D(M1-AcCoA)	0.032	0.009	0.064		D(M1-AcCoA)	0.030	0.000	0.078
	1-D(AcCoA)	0.724	0.678	0.769		1-D(AcCoA)	0.644	0.574	0.715
	g(t) palmitate	0.395	0.348	0.437		g(t) palmitate	0.262	0.211	0.306
	TOTAL AcCoA contribution	0.276	0.204	0.355		TOTAL AcCoA contribution	0.356	0.248	0.476
N1-BT2-1	D(M2-AcCoA)	0.322	0.255	0.385	N3-BT2-1	D(M2-AcCoA)	0.327	0.282	0.372
	D(M1-AcCoA)	0.029	0.000	0.070		D(M1-AcCoA)	0.023	0.002	0.048
	1-D(AcCoA)	0.650	0.588	0.712		1-D(AcCoA)	0.650	0.605	0.694
	g(t) palmitate	0.285	0.236	0.329		g(t) palmitate	0.366	0.325	0.403
	TOTAL AcCoA contribution	0.350	0.255	0.455		TOTAL AcCoA contribution	0.350	0.284	0.420
N1-BT2-2	D(M2-AcCoA)	0.299	0.221	0.370	N3-BT2-2	D(M2-AcCoA)	0.295	0.223	0.362
	D(M1-AcCoA)	0.030	0.000	0.079		D(M1-AcCoA)	0.030	0.000	0.075
	1-D(AcCoA)	0.671	0.603	0.739		1-D(AcCoA)	0.675	0.611	0.739
	g(t) palmitate	0.258	0.209	0.303		g(t) palmitate	0.235	0.191	0.274
	TOTAL AcCoA contribution	0.329	0.221	0.449		TOTAL AcCoA contribution	0.325	0.223	0.437
N1-BT2-3	D(M2-AcCoA)	0.231	0.076	0.320	N3-BT2-3	D(M2-AcCoA)	0.316	0.245	0.383
	D(M1-AcCoA)	0.046	0.004	0.169		D(M1-AcCoA)	0.028	0.000	0.072
	1-D(AcCoA)	0.723	0.646	0.826		1-D(AcCoA)	0.655	0.591	0.720
	g(t) palmitate	0.275	0.218	0.325		g(t) palmitate	0.248	0.202	0.289
	TOTAL AcCoA contribution	0.277	0.079	0.489		TOTAL AcCoA contribution	0.345	0.245	0.455
N1-DNP-1	D(M2-AcCoA)	0.355	0.236	0.455	N3-DNP-1	D(M2-AcCoA)	0.551	0.483	0.615
	D(M1-AcCoA)	0.024	0.000	0.095		D(M1-AcCoA)	0.023	0.000	0.062
	1-D(AcCoA)	0.621	0.524	0.721		1-D(AcCoA)	0.426	0.360	0.495
	g(t) palmitate	0.197	0.141	0.247		g(t) palmitate	0.111	0.088	0.134
	TOTAL AcCoA contribution	0.379	0.236	0.550		TOTAL AcCoA contribution	0.574	0.483	0.677
N1-DNP-2	D(M2-AcCoA)	0.387	0.243	0.505	N3-DNP-2	D(M2-AcCoA)	0.338	0.000	0.483
	D(M1-AcCoA)	0.022	0.000	0.105		D(M1-AcCoA)	0.031	0.000	0.287
	1-D(AcCoA)	0.591	0.476	0.713		1-D(AcCoA)	0.631	0.492	0.993
	g(t) palmitate	0.171	0.114	0.221		g(t) palmitate	0.175	0.110	0.231
	TOTAL AcCoA contribution	0.409	0.243	0.610		TOTAL AcCoA contribution	0.369	0.000	0.770
N1-DNP-3	D(M2-AcCoA)	0.346	0.201	0.456	N3-DNP-3	D(M2-AcCoA)	0.295	0.178	0.386
	D(M1-AcCoA)	0.025	0.000	0.109		D(M1-AcCoA)	0.029	0.000	0.100
	1-D(AcCoA)	0.629	0.524	0.743		1-D(AcCoA)	0.677	0.592	0.766
	g(t) palmitate	0.178	0.123	0.228		g(t) palmitate	0.172	0.127	0.212
	TOTAL AcCoA contribution	0.371	0.201	0.565		TOTAL AcCoA contribution	0.323	0.178	0.486
N2-NT1	D(M2-AcCoA)	0.224	0.187	0.260	N4-NT1	D(M2-AcCoA)	0.346	0.306	0.386
	D(M1-AcCoA)	0.031	0.012	0.054		D(M1-AcCoA)	0.023	0.004	0.045
	1-D(AcCoA)	0.746	0.711	0.780		1-D(AcCoA)	0.631	0.591	0.672
	g(t) palmitate	0.395	0.358	0.430		g(t) palmitate	0.378	0.340	0.412
	TOTAL AcCoA contribution	0.254	0.198	0.314		TOTAL AcCoA contribution	0.369	0.309	0.431
N2-NT2	D(M2-AcCoA)	0.387	0.337	0.436	N4-NT2	D(M2-AcCoA)	0.523	0.475	0.571
	D(M1-AcCoA)	0.024	0.002	0.052		D(M1-AcCoA)	0.029	0.007	0.059
	1-D(AcCoA)	0.589	0.540	0.638		1-D(AcCoA)	0.447	0.399	0.496
	g(t) palmitate	0.420	0.373	0.462		g(t) palmitate	0.429	0.383	0.470
	TOTAL AcCoA contribution	0.411	0.339	0.488		TOTAL AcCoA contribution	0.553	0.481	0.630
N2-NT3	D(M2-AcCoA)	0.249	0.203	0.293	N4-NT3	D(M2-AcCoA)	0.435	0.381	0.489
	D(M1-AcCoA)	0.030	0.008	0.059		D(M1-AcCoA)	0.023	0.000	0.052
	1-D(AcCoA)	0.721	0.679	0.763		1-D(AcCoA)	0.541	0.487	0.595
	g(t) palmitate	0.393	0.350	0.432		g(t) palmitate	0.412	0.363	0.455
	TOTAL AcCoA contribution	0.279	0.212	0.352		TOTAL AcCoA contribution	0.459	0.381	0.541
N2-BT2-1	D(M2-AcCoA)	0.398	0.295	0.492	N4-BT2-1	D(M2-AcCoA)	0.300	0.246	0.352
	D(M1-AcCoA)	0.029	0.000	0.094		D(M1-AcCoA)	0.026	0.001	0.058
	1-D(AcCoA)	0.573	0.480	0.669		1-D(AcCoA)	0.674	0.624	0.724
	g(t) palmitate	0.231	0.172	0.281		g(t) palmitate	0.266	0.226	0.302
	TOTAL AcCoA contribution	0.427	0.295	0.586		TOTAL AcCoA contribution	0.326	0.247	0.411
N2-BT2-2	D(M2-AcCoA)	0.282	0.203	0.354	N4-BT2-2	D(M2-AcCoA)	0.317	0.266	0.367
	D(M1-AcCoA)	0.032	0.000	0.084		D(M1-AcCoA)	0.023	0.000	0.053
	1-D(AcCoA)	0.685	0.619	0.753		1-D(AcCoA)	0.660	0.611	0.709
	g(t) palmitate	0.279	0.227	0.325		g(t) palmitate	0.232	0.198	0.263
	TOTAL AcCoA contribution	0.315	0.203	0.437		TOTAL AcCoA contribution	0.340	0.266	0.420
N2-BT2-3	D(M2-AcCoA)	0.369	0.271	0.459	N4-DNP-1	D(M2-AcCoA)	0.365	0.247	0.468
	D(M1-AcCoA)	0.030	0.000	0.091		D(M1-AcCoA)	0.026	0.000	0.093
	1-D(AcCoA)	0.601	0.514	0.690		1-D(AcCoA)	0.609	0.508	0.714
	g(t) palmitate	0.252	0.193	0.303		g(t) palmitate	0.183	0.133	0.229
	TOTAL AcCoA contribution	0.399	0.271	0.549		TOTAL AcCoA contribution	0.391	0.247	0.561
N2-DNP-1	D(M2-AcCoA)	0.464	0.319	0.590	N4-DNP-2	D(M2-AcCoA)	0.340	0.271	0.408
	D(M1-AcCoA)	0.021	0.000	0.117		D(M1-AcCoA)	0.019	0.000	0.056
	1-D(AcCoA)	0.515	0.390	0.650		1-D(AcCoA)	0.641	0.576	0.706
	g(t) palmitate	0.150	0.094	0.201		g(t) palmitate	0.163	0.129	0.195
	TOTAL AcCoA contribution	0.485	0.319	0.707		TOTAL AcCoA contribution	0.359	0.271	0.464
					N4-DNP-3	D(M2-AcCoA)	0.316	0.239	0.388
						D(M1-AcCoA)	0.021	0.000	0.064
						1-D(AcCoA)	0.663	0.594	0.732
						g(t) palmitate	0.162	0.126	0.194
						TOTAL AcCoA contribution	0.337	0.239	0.452