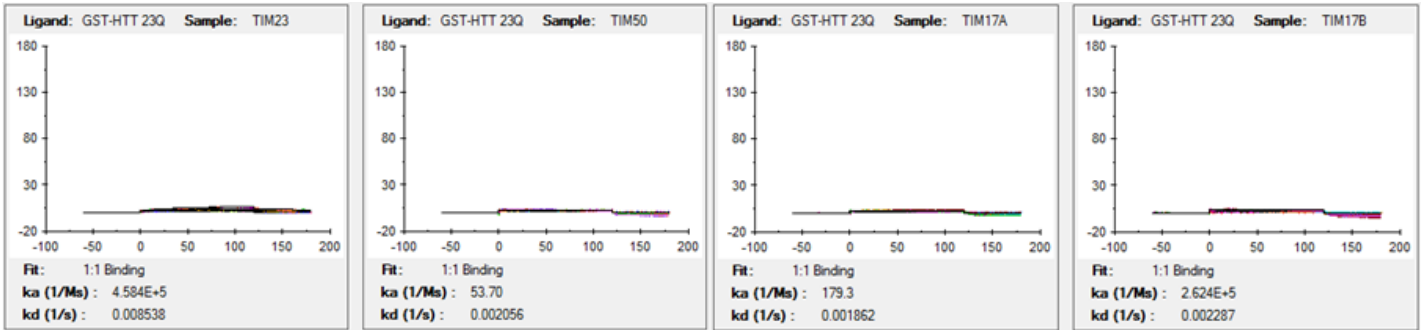


SI Appendix

A



B

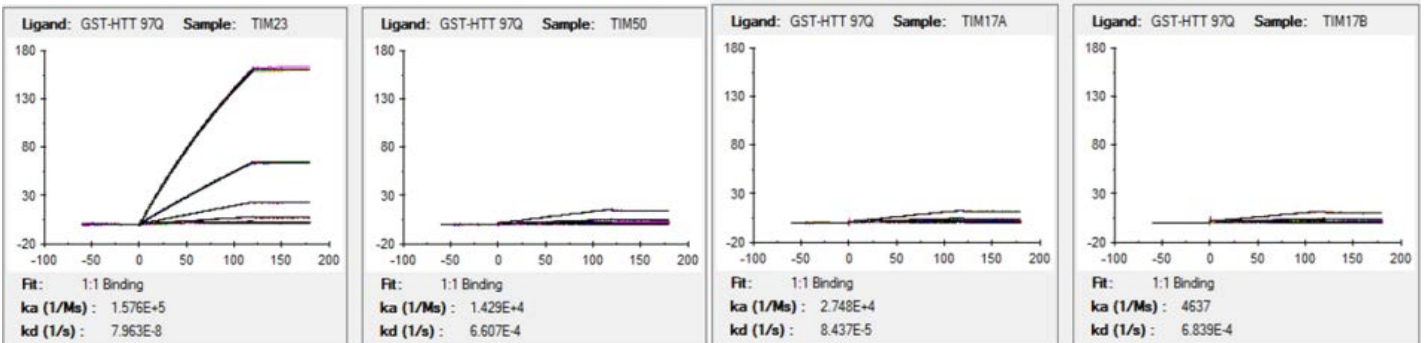


Fig. S1. Representative SPR sensorgrams displaying binding of TIM23, TIM50, TIM17A and TIM17B proteins (sample) to immobilized ligand wild type HTT (GST-HTT-23Q) (A) and mutant HTT (GST-HTT-2Q) (B).

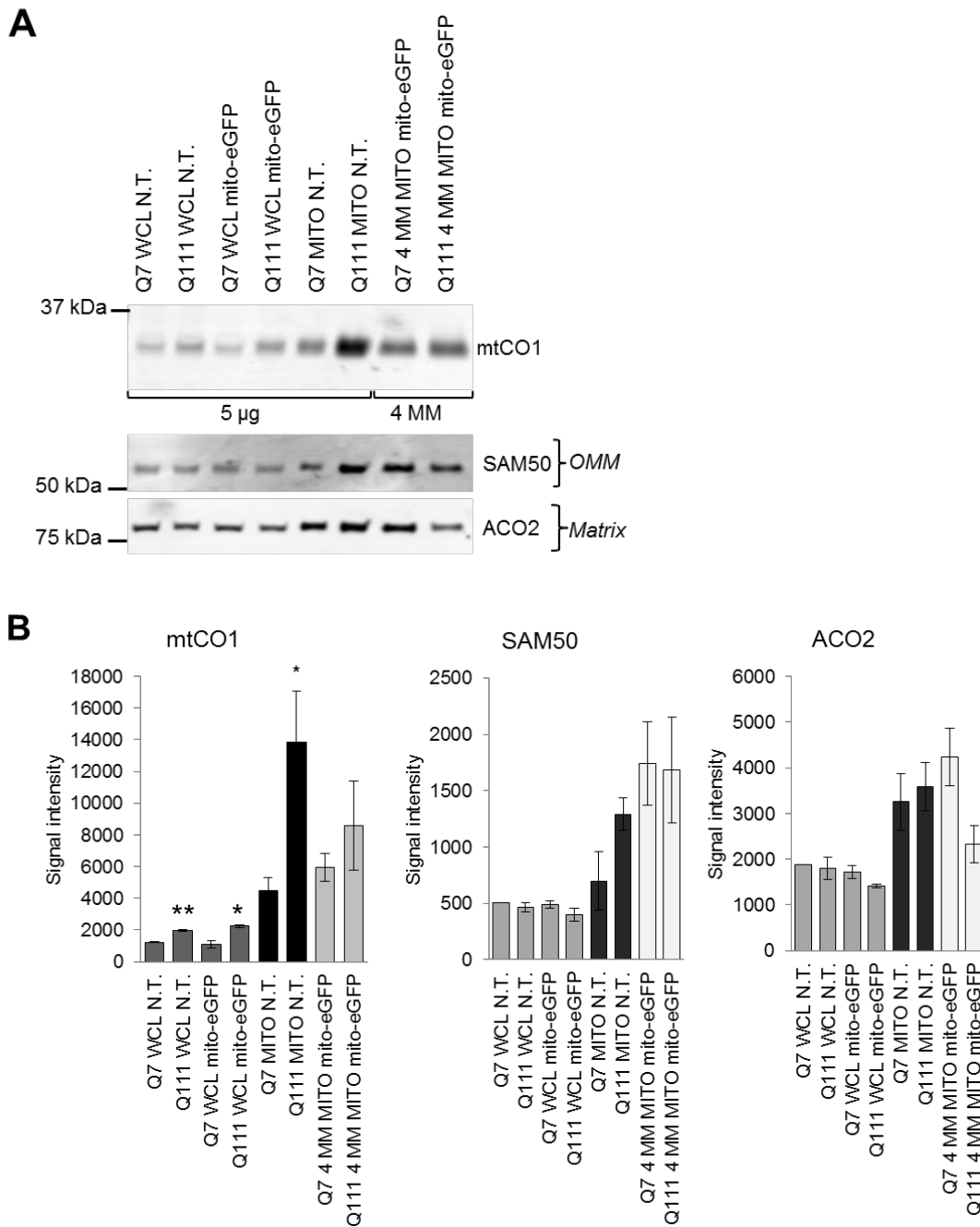


Fig. S2. The level of mtCO1 protein is equal in equivalent number of mitochondria from ST-Hdh-Q7/Q7 and -Q111/Q111 cells. Representative immunoblot (A) and quantification (B) of mtCO1 level in FACS sorted mitochondria isolated from mito-eGFP transfected cells. Four million (4 MM) eGFP labeled mitochondria and 5 µg protein samples were immunoblotted for mtCO1, and nuclearly-encoded matrix aconitase 2 (ACO2) and outer membrane (SAM50) proteins. WCL – whole cell lysate, MITO – mitochondria, N.T. – not transfected, OMM – outer mitochondrial membrane. (n=3, data shown as mean+s.e.m. ** $P < 0.001$, * $P < 0.05$, t-Test).

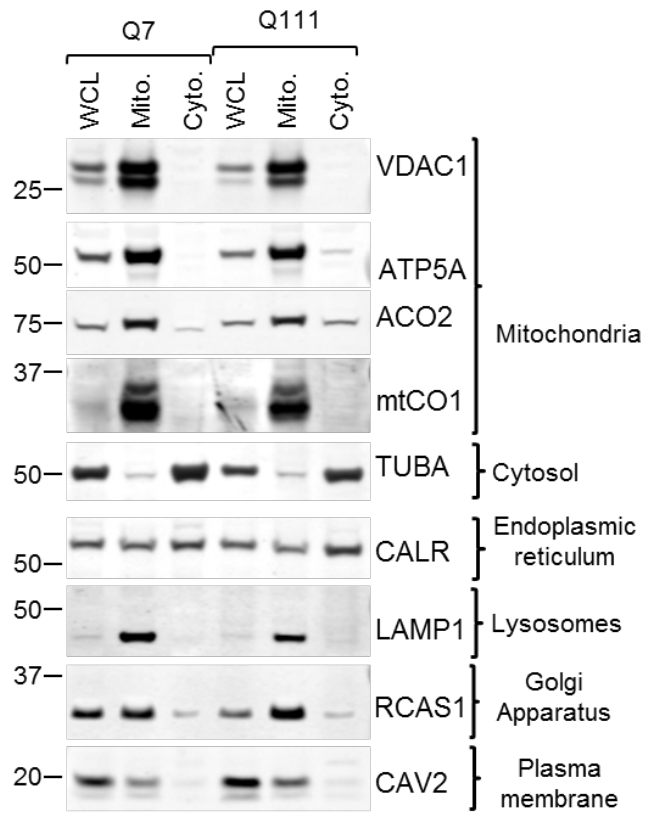
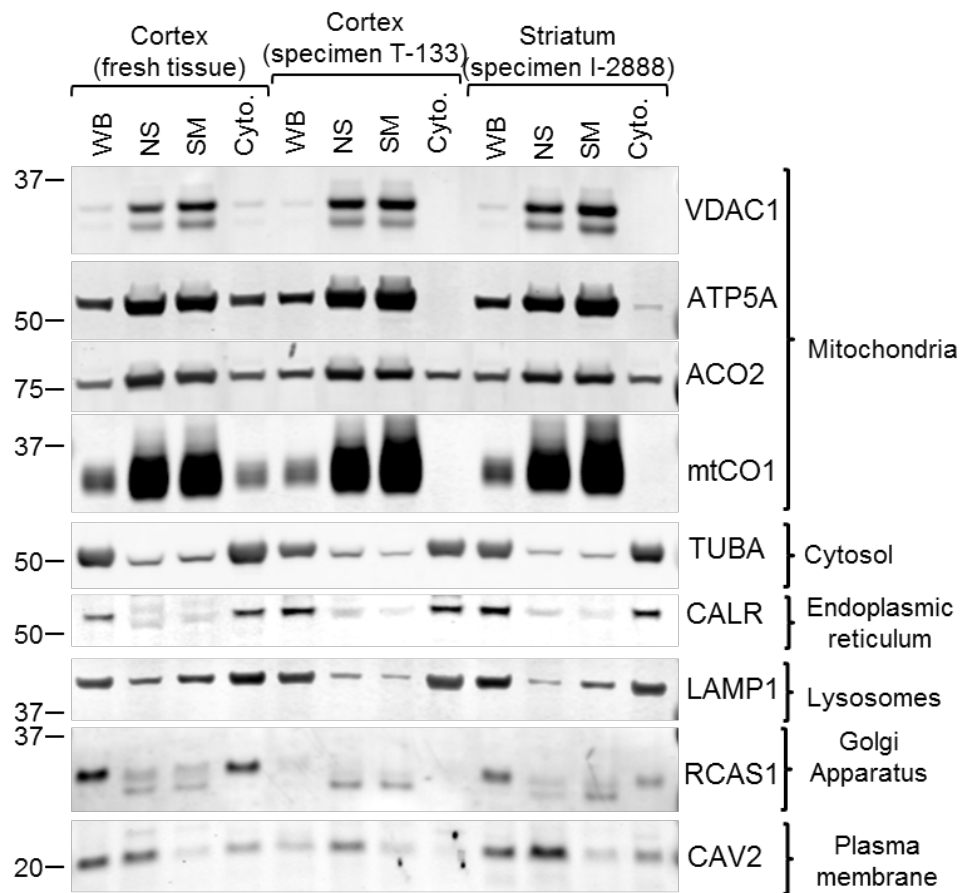
A**B**

Fig. S3. Purity of mitochondria enriched fractions. (A) Representative immunoblot of Q7 and Q111 whole cell lysates (WCL), mitochondrial (Mito.) and cytosolic fractions (Cyto.) probed for mitochondrial markers (VDAC1, ATP5A, ACO2 and mtCO1) and typical mitochondrial fraction contaminants and contaminating organelles: cytosol (TUBA), endoplasmic reticulum (CALR), lysosomes (LAMP1), Golgi apparatus (RCAS1) and plasma membrane (CAV2). (B) Nonsynaptosomal (NS) and synaptosomal (SM) mitochondrial fractions isolated from fresh post-surgical cortex and frozen specimens of cortex and striatum (tissue blocks listed in Table 1) were tested for enrichment with mitochondrial markers and contamination with markers listed in (A) compared to whole brain homogenate (WB) and cytosolic fraction.

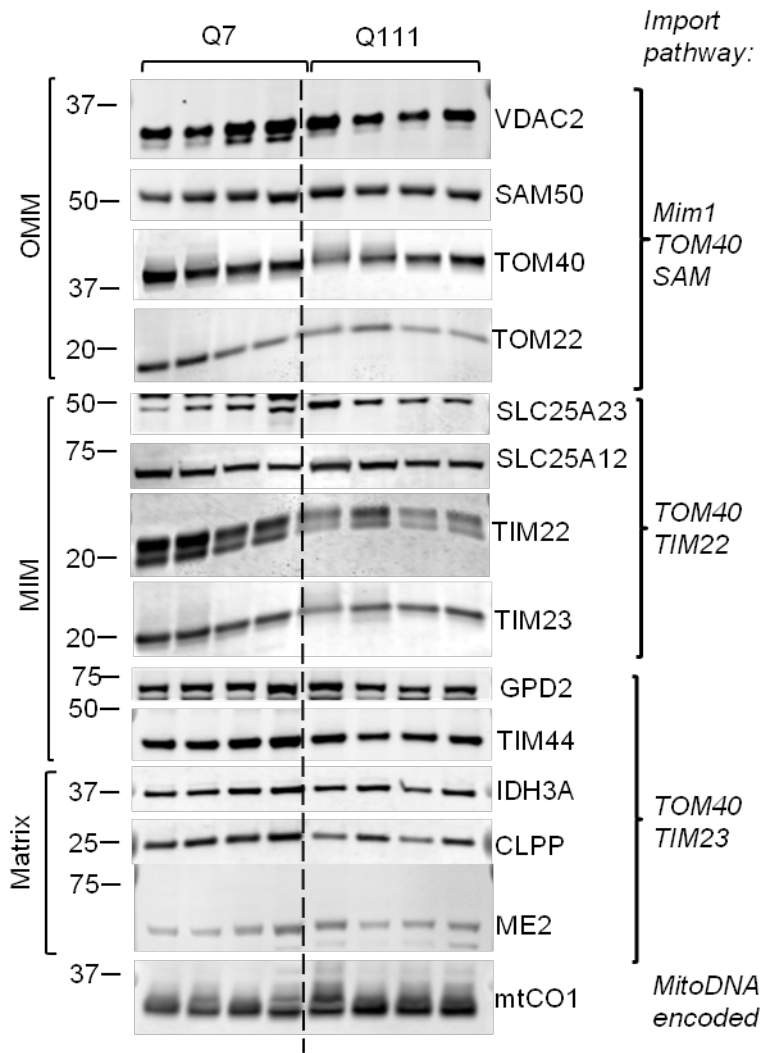
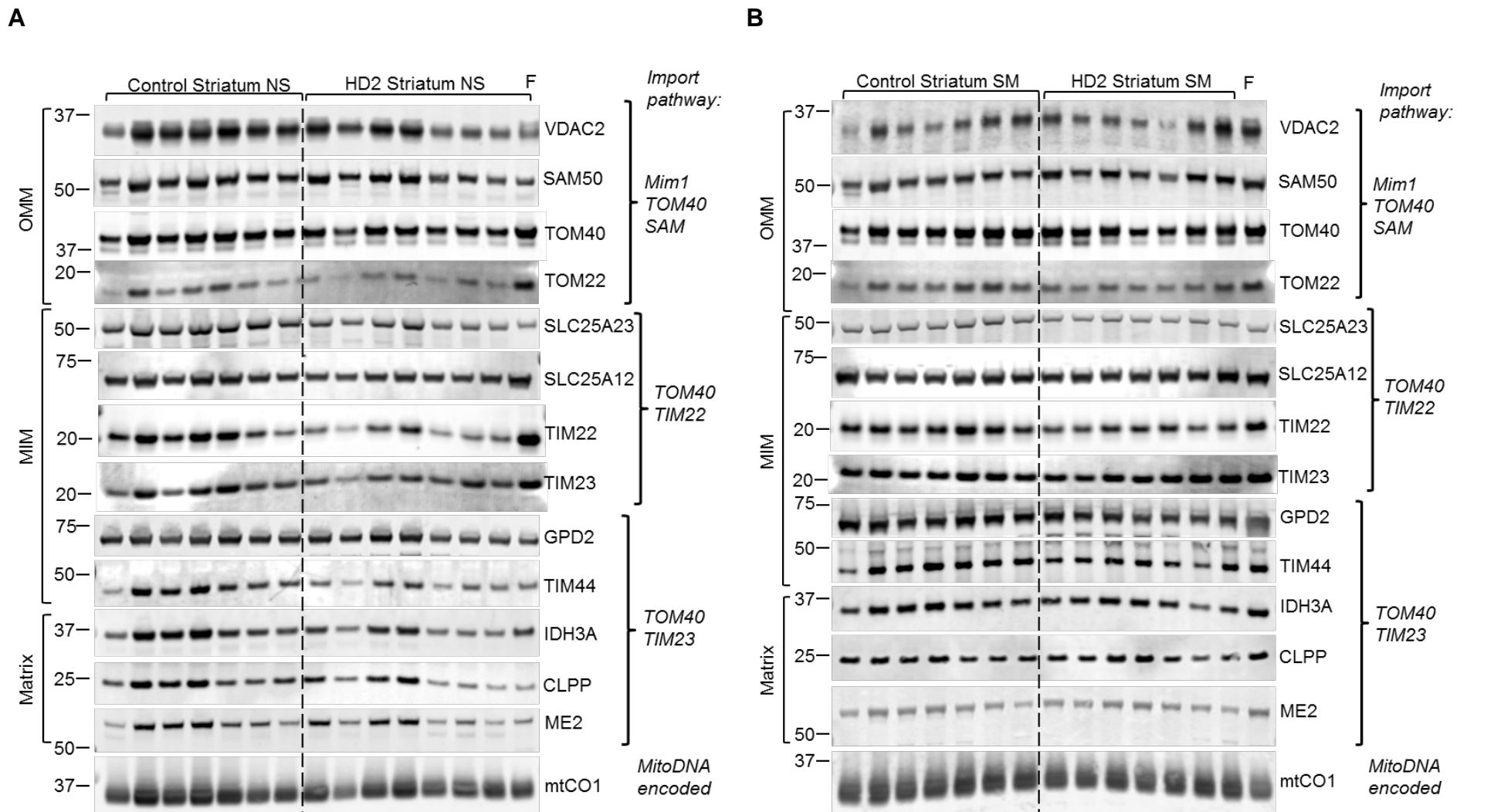


Fig. S4. Representative immunoblots of mitochondrial proteins from Q7 and Q111 cells (shown here to supplement Fig. 3E). Nuclearly encoded mitochondrial protein levels are reduced in the Q111 striatal cell line. OMM indicates outer mitochondrial membrane protein, MIM indicates mitochondrial inner membrane proteins, (n=4).



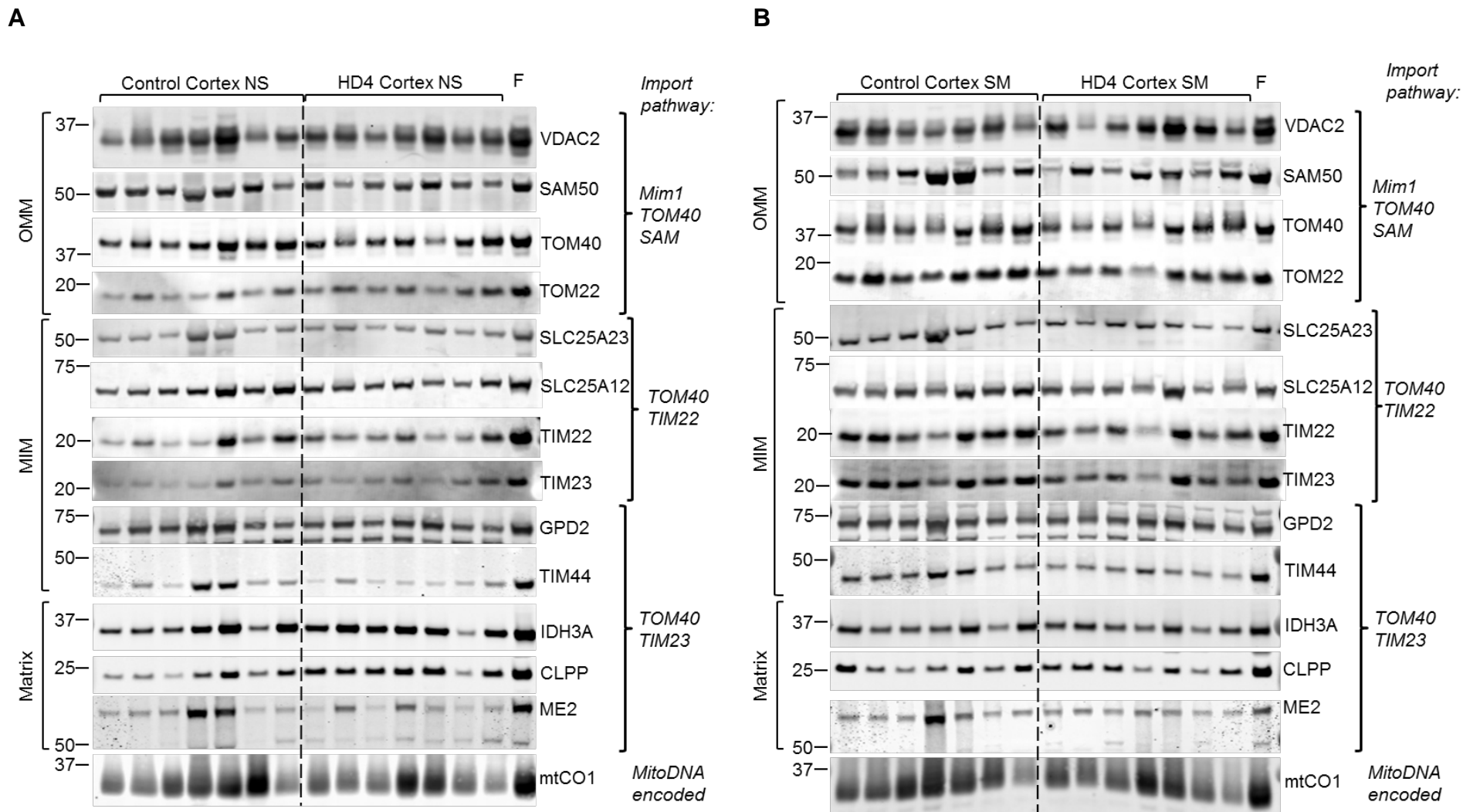


Fig. S6. Protein dysregulation in cortical mitochondria of HD grade 4 patients. Immunoblots of mitochondrial proteins in (A) nonsynaptosomal (NS) and (B) synaptosomal (SM) mitochondria (shown here to supplement Fig. 5A and 5C). OMM indicates outer mitochondrial membrane protein. MIM indicates mitochondrial inner membrane proteins. Samples prepared from cortex of non-HD patients were used as a control, and mitochondrial lysates from fresh (F) surgically resected cortex tissue of non-HD patients were included. Each lane represents the mitochondrial fraction from independent tissue block (n=7).

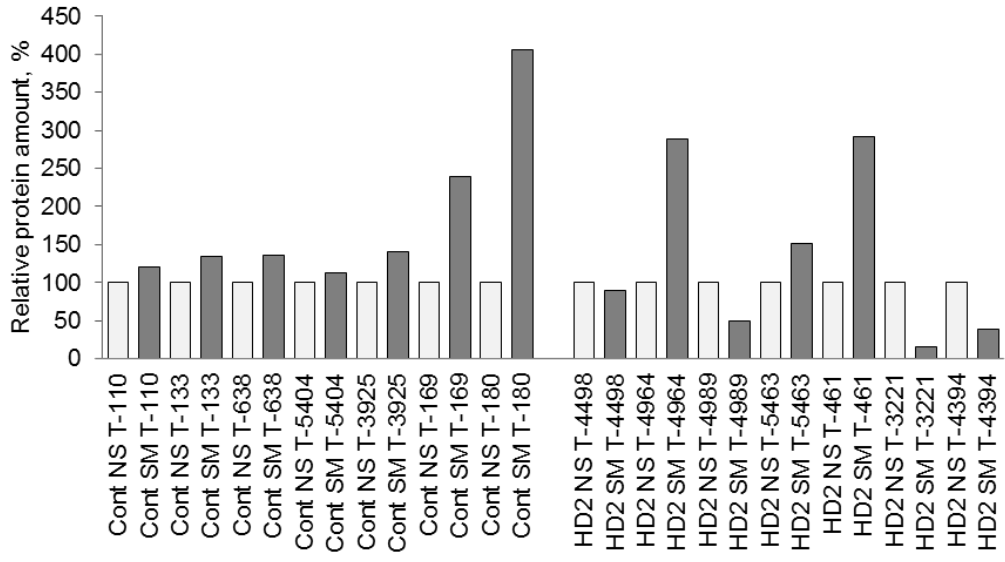
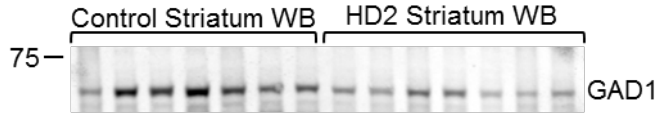
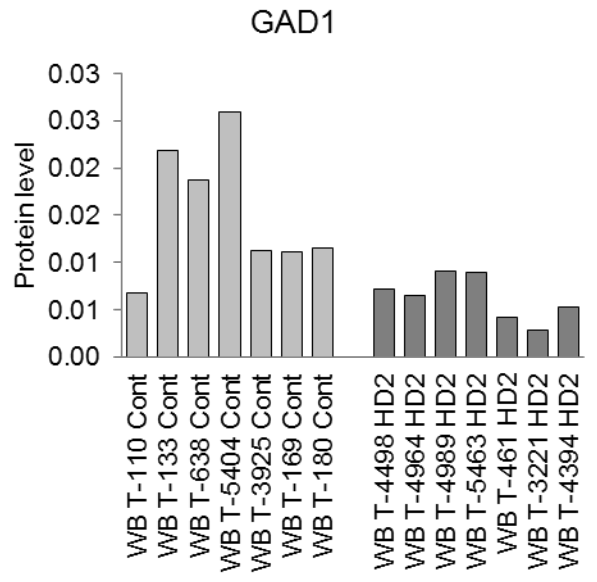
A**B****C**

Fig. S7. Synaptic mitochondria loss in striatum of HD grade 2 (HD2) patients and controls. (A) Quantification of relative protein amount in mitochondrial fraction isolated from striatal tissues. The total protein amount (μg) in nonsynaptosomal samples was taken as 100% and used to recalculate relative protein amount in synaptosomal samples originating from the same tissue block (listed in

Table 1). (B) Immunoblot and (C) quantification of protein level of striatal neuronal marker GAD1 in whole brain (WB) lysates.

Table S1. Binding affinity constants of TIM23 complex subunits with HTT exon1 fragments.

Ligand	Sample	Curve	Temp (°C)	Fit	k_a	k_d	KD
GST-HTTex1-23Q	TIM23	Fc=2-1	25	1:1 Binding	1.22E+04	4.18E-05	3.43E-09
GST-HTTex1-23Q	TIM50	Fc=2-1	25	1:1 Binding	3.87E+05	0.2054	5.31E-07
GST-HTTex1-23Q	TIM17A	Fc=2-1	25	1:1 Binding	2540	0.03182	1.25E-05
GST-HTTex1-23Q	TIM17B	Fc=2-1	25	1:1 Binding	5.38E+05	0.0539	1.00E-07
GST-HTTex1-97Q	TIM23	Fc=4-3	25	1:1 Binding	1.58E+05	7.96E-08	5.05E-13
GST-HTTex1-97Q	TIM50	Fc=4-3	25	1:1 Binding	1.43E+04	6.61E-04	4.62E-08
GST-HTTex1-97Q	TIM17A	Fc=4-3	25	1:1 Binding	2.75E+04	8.44E-05	3.07E-09
GST-HTTex1-97Q	TIM17B	Fc=4-3	25	1:1 Binding	4637	6.84E-04	1.48E-07

note:

k_a – association constant

k_d - dissociation constant

KD - equilibrium dissociation constant

Table S2. Human brain tissue information.

Type	Grade	Specimen	Age	Gender	CAG repeats	Standard brain block (SBB) ¹	Postmortem interval before frozen
Cortex							
Control	n.a.	T-145	57	F	N.E.	SBB4.1	9:14
Control	n.a.	T-159	54	F	N.E.	SBB2.1	15:40
Control	n.a.	T-169	67	M	N.E.	SBB4.2	49:20
Control	n.a.	T-171	89	M	N.E.	SBB1	6:05
Control	n.a.	T-172	89	M	N.E.	SBB4.1	8:45
Control	n.a.	T-206	49	M	N.E.	SBB2.2	15:05
Control	n.a.	T-551	77	F	N.E.	SBB3.1	13:34
Control	n.a.	T-4931	66	M	N.E.	SBB3.2	26:55
HD4	4	T-4522	42	M	52/17	SBB1.3	10:58
HD4	4	T-4584	41	M	49/17	SBB4.2	38:20
HD4	4	T-4638	39	F	52/18	SBB3.1	28:46
HD4	4	T-4929	50	M	49/16	SBB1.4	43:08
HD4	4	T-5017	66	F	44/17	SBB3.3	5:30
HD4	4	T-5301	64	F	46/19	SBB3.1	18:52
HD4	4	T-5321	50	M	N.E.	SBB3.1	31:38
Striatum							
Control	n.a.	T-110	62	M	N.E.	SBB7.1	N.E.
Control	n.a.	T-133	33	F	N.E.	SBB7.1	11:25
Control	n.a.	T-169	69	M	N.E.	SBB6.2	49:20
Control	n.a.	T-180	52	F	N.E.	SBB7.2	6:07
Control	n.a.	T-638	78	M	N.E.	SBB7.1	8:00
Control	n.a.	T-3925	79	F	N.E.	SBB6.1	18:50
Control	n.a.	T-5404	54	F	N.E.	SBB7.1	16:36
HD2	2	T-461	77	M	41/15	SBB7.2	19:28
HD2	2	T-3221	58	M	42/15	SBB7.2	85:11
HD2	2	T-4394	75	F	41/17	SBB7.2	22:50
HD2	2	T-4498	59	M	43/17	SBB6.2	32:45
HD2	2	T-4964	89	M	40/17	SBB6.0	10:35
HD2	2	T-4989	75	F	42/15	SBB6.1	37:35
HD2	2	T-5263	55	F	54/30	SBB6.2	30:50

note:

Grade - diagnosed HD grade, Specimen - frozen tissue samples, Age - years at death, F - female, M - male, N.E. - not estimated, n.a. - not applicable, CAG repeats - number of CAG repeats of both alleles.

1 Vonsattel, J. P., Del Amaya, M. P. & Keller, C. E. Twenty-first century brain banking. Processing brains for research: the Columbia University methods. *Acta Neuropathol* **115**, 509-532, doi:10.1007/s00401-007-0311-9 (2008).

Table S3. DIGE map spot identifier.

Spot #	Identified protein		Protein name	Localization
	UniProt ID	Gene		
1	Q64521	GPD2	Glycerol-3-phosphate dehydrogenase, mitochondrial	Inner mitochondrial membrane
	P48678	LMNA		NM
2	Q8BH04	PCK2	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	Mitochondrial matrix
	Q8K2B3	SDHA	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	Inner mitochondrial membrane
3	Q8BH04	PCK2	See spot 2	
	Q8K2B3	SDHA	See spot 2	
4	P80318	CCT3		NM
5	Q99KE1	ME2	NAD-dependent malic enzyme, mitochondrial	Mitochondrial matrix
	O08749	DLD (LAD)	Dihydrolipoyl dehydrogenase, mitochondrial	Mitochondrial matrix
6	Q61753	PHGDH		NM
7	P80314	CCT2		NM
8	P47738	ALDH2	Aldehyde dehydrogenase, mitochondrial	Mitochondrial matrix
9	P50544	ACADVL	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	Inner mitochondrial membrane
10	Q5SX75	P4HA2		NM
	P63038	HSP60	60 kDa heat shock protein, mitochondrial	Mitochondrial matrix *
11	Q5SX75	P4HA2		NM
	P27773	PDIA3		NM
	Q60715	P4HA1		NM
12	P27773	PDIA3		NM
13	P11983	TCP1		NM
14	Q99JY	ACTR3		NM
	P56480	ATP5B	ATP synthase subunit beta, mitochondrial	Inner mitochondrial membrane
15	P29758	OAT	Ornithine aminotransferase, mitochondrial	Mitochondrial matrix
16	P29758	OAT	See spot 15	
17	P17182	ENO1		NM
	Q9D8N0	EEF1g		NM
18	Q8BFR5	TUFM	Elongation factor Tu, mitochondrial	Mitochondrial matrix
19	Q8BMD8	SLC25A24	Calcium-binding mitochondrial carrier protein SCaMC-1	Inner mitochondrial membrane
	P35486	PDHA1	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	Mitochondrial matrix

	Q9QYR9	ACOT2	Acyl-coenzyme A thioesterase 2, mitochondrial	Mitochondrial matrix
20	Q9JHI5	IVD	Isovaleryl-CoA dehydrogenase, mitochondrial	Mitochondrial matrix
	Q8K248	HPDL		NM
	O88986	GCAT	2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial	Mitochondrial matrix *
	Q8BMD8	SLC25A24	See spot 19	
21	P10107	ANXA1		NM
22	O88544	COPS4		NM
23	Q9D6R2	IDH3A	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	Mitochondrial matrix
24	P47754	CAPZA2		NM
28	P67778	PHB	Prohibitin	Inner mitochondrial membrane
29	O88696	CLPP	ATP-dependent Clp protease proteolytic subunit, mitochondrial	Mitochondrial matrix
32	P20108	PRDX3	Thioredoxin-dependent peroxide reductase, mitochondrial	Mitochondrial matrix *

note:

NM – non mitochondrial, * - multiple intracellular localization.

Spots 25, 26, 27, 30, 31 were not confirmed due to low level of protein.

Table S4. List of all identified mitochondrial proteins.

Identified protein	UniProt ID	MW
A2AJP9_MOUSE MCG54027, isoform CRA_a OS=Mus musculus GN=Pdp1 PE=3 SV=2	A2AJP9 (+3)	64 kDa
A2AQR0_MOUSE Glycerol-3-phosphate dehydrogenase, mitochondrial OS=Mus musculus GN=Gpd2 PE=2 SV=1	A2AQR0 (+1)	83 kDa
XPP3_MOUSE Probable Xaa-Pro aminopeptidase 3 OS=Mus musculus GN=Xpnpep3 PE=2 SV=1	B7ZMP1	57 kDa
D3YXX5_MOUSE NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial (Fragment) OS=Mus musculus GN=Ndufv1 PE=2 SV=1	D3YXX5 (+2)	13 kDa
D3Z0L4_MOUSE Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial (Fragment) OS=Mus musculus GN=Chchd3 PE=2 SV=1	D3Z0L4 (+3)	22 kDa
GLSK_MOUSE Glutaminase kidney isoform, mitochondrial OS=Mus musculus GN=Gls PE=1 SV=1	D3Z7P3	74 kDa
GLSK_MOUSE Isoform 2 of Glutaminase kidney isoform, mitochondrial OS=Mus musculus GN=Gls	D3Z7P3-2	66 kDa
E9PUB7_MOUSE Protein misato homolog 1 OS=Mus musculus GN=Msto1 PE=2 SV=1	E9PUB7 (+2)	61 kDa
F2Z471_MOUSE Voltage-dependent anion-selective channel protein 1 OS=Mus musculus GN=Vdac1 PE=2 SV=1	F2Z471 (+2)	28 kDa
F8WIT2_MOUSE Annexin OS=Mus musculus GN=Anxa6 PE=2 SV=1	F8WIT2 (+1)	75 kDa
G3UX26_MOUSE Voltage-dependent anion-selective channel protein 2 (Fragment) OS=Mus musculus GN=Vdac2 PE=4 SV=1	G3UX26	30 kDa
DLDH_MOUSE Dihydrolipoyl dehydrogenase, mitochondrial OS=Mus musculus GN=Dld PE=1 SV=2	O08749	54 kDa
PRDX4_MOUSE Peroxiredoxin-4 OS=Mus musculus GN=Prdx4 PE=1 SV=1	O08807	31 kDa
PHB2_MOUSE Prohibitin-2 OS=Mus musculus GN=Phb2 PE=1 SV=1	O35129	33 kDa
TIM44_MOUSE Mitochondrial import inner membrane translocase subunit TIM44 OS=Mus musculus GN=Timm44 PE=2 SV=2	O35857	51 kDa
PTRF_MOUSE Polymerase I and transcript release factor OS=Mus musculus GN=Ptrf PE=1 SV=1	O54724	44 kDa
1433S_MOUSE 14-3-3 protein sigma OS=Mus musculus GN=Sfn PE=1 SV=2	O70456	28 kDa
CLPP_MOUSE Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial OS=Mus musculus GN=Clpp PE=1 SV=1	O88696	30 kDa
KBL_MOUSE 2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial OS=Mus musculus GN=Gcat PE=1 SV=2	O88986	45 kDa
AATM_MOUSE Aspartate aminotransferase, mitochondrial OS=Mus musculus GN=Got2 PE=1 SV=1	P05202	47 kDa
ANXA1_MOUSE Annexin A1 OS=Mus musculus GN=Anxa1 PE=1 SV=2	P10107	39 kDa
PRDX3_MOUSE Thioredoxin-dependent peroxide reductase, mitochondrial OS=Mus musculus GN=Prdx3 PE=1 SV=1	P20108	28 kDa
VIME_MOUSE Vimentin OS=Mus musculus GN=Vim PE=1 SV=3	P20152	54 kDa
CATA_MOUSE Catalase OS=Mus musculus GN=Cat PE=1 SV=4	P24270	60 kDa
DHE3_MOUSE Glutamate dehydrogenase 1, mitochondrial OS=Mus	P26443	61 kDa

musculus GN=Glud1 PE=1 SV=1		
OAT_MOUSE Ornithine aminotransferase, mitochondrial OS=Mus musculus GN=Oat PE=1 SV=1	P29758	48 kDa
MP2K1_MOUSE Dual specificity mitogen-activated protein kinase kinase 1 OS=Mus musculus GN=Map2k1 PE=1 SV=2	P31938	43 kDa
NLTP_MOUSE Non-specific lipid-transfer protein OS=Mus musculus GN=Scp2 PE=1 SV=3	P32020	59 kDa
ODPA_MOUSE Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial OS=Mus musculus GN=Pdha1 PE=1 SV=1	P35486	43 kDa
GRP75_MOUSE Stress-70 protein, mitochondrial OS=Mus musculus GN=Hspa9 PE=1 SV=3	P38647	73 kDa
ACADM_MOUSE Medium-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Mus musculus GN=Acadm PE=1 SV=1	P45952	46 kDa
ALDH2_MOUSE Aldehyde dehydrogenase, mitochondrial OS=Mus musculus GN=Aldh2 PE=1 SV=1	P47738	57 kDa
GSHR_MOUSE Glutathione reductase, mitochondrial OS=Mus musculus GN=Gsr PE=1 SV=3	P47791 (+1)	54 kDa
LMNA_MOUSE Prelamin-A/C OS=Mus musculus GN=Lmna PE=1 SV=2	P48678	74 kDa
LMNA_MOUSE Prelamin-A/C OS=Mus musculus GN=Lmna PE=1 SV=2	P48678 (+1)	74 kDa
LMNA_MOUSE Isoform C of Prelamin-A/C OS=Mus musculus GN=Lmna	P48678-2	65 kDa
ODBA_MOUSE 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial OS=Mus musculus GN=Bckdha PE=1 SV=1	P50136 (+1)	50 kDa
VATA_MOUSE V-type proton ATPase catalytic subunit A OS=Mus musculus GN=Atp6v1a PE=1 SV=2	P50516	68 kDa
ACADV_MOUSE Very long-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Mus musculus GN=Acadvl PE=1 SV=3	P50544	71 kDa
ACADL_MOUSE Long-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Mus musculus GN=Acadl PE=1 SV=2	P51174	48 kDa
KPYM_MOUSE Pyruvate kinase PKM OS=Mus musculus GN=Pkm PE=1 SV=4	P52480	58 kDa
IDHP_MOUSE Isocitrate dehydrogenase [NADP], mitochondrial OS=Mus musculus GN=ldh2 PE=1 SV=3	P54071	51 kDa
ATPB_MOUSE ATP synthase subunit beta, mitochondrial OS=Mus musculus GN=Atp5b PE=1 SV=2	P56480	56 kDa
RUVB1_MOUSE RuvB-like 1 OS=Mus musculus GN=Ruvb1 PE=1 SV=1	P60122	50 kDa
1433G_MOUSE 14-3-3 protein gamma OS=Mus musculus GN=Ywhag PE=1 SV=2	P61982	28 kDa
CH60_MOUSE 60 kDa heat shock protein, mitochondrial OS=Mus musculus GN=Hspd1 PE=1 SV=1	P63038	61 kDa
PP1G_MOUSE Serine/threonine-protein phosphatase PP1-gamma catalytic subunit OS=Mus musculus GN=Ppp1cc PE=1 SV=1	P63087 (+1)	37 kDa
PHB_MOUSE Prohibitin OS=Mus musculus GN=Phb PE=1 SV=1	P67778	30 kDa
TCPH_MOUSE T-complex protein 1 subunit eta OS=Mus musculus GN=Cct7 PE=1 SV=1	P80313	60 kDa

FUMH_MOUSE Fumarate hydratase, mitochondrial OS=Mus musculus GN=Fh PE=1 SV=3	P97807 (+1)	54 kDa
ATPA_MOUSE ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	Q03265	60 kDa
ACADS_MOUSE Short-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Mus musculus GN=Acads PE=1 SV=2	Q07417	45 kDa
P4K2A_MOUSE Phosphatidylinositol 4-kinase type 2-alpha OS=Mus musculus GN=Pi4k2a PE=1 SV=1	Q2TBE6	54 kDa
HSDL2_MOUSE Hydroxysteroid dehydrogenase-like protein 2 OS=Mus musculus GN=Hsd12 PE=1 SV=1	Q2TPA8	54 kDa
FXRD1_MOUSE FAD-dependent oxidoreductase domain-containing protein 1 OS=Mus musculus GN=Foxred1 PE=2 SV=1	Q3TQB2 (+1)	54 kDa
Q3U3J1_MOUSE 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial OS=Mus musculus GN=Bckdha PE=2 SV=1	Q3U3J1	51 kDa
C1TM_MOUSE Monofunctional C1-tetrahydrofolate synthase, mitochondrial OS=Mus musculus GN=Mthfd1l PE=1 SV=2	Q3V3R1	106 kDa
P4HA1_MOUSE Prolyl 4-hydroxylase subunit alpha-1 OS=Mus musculus GN=P4ha1 PE=2 SV=2	Q60715	61 kDa
VDAC2_MOUSE Voltage-dependent anion-selective channel protein 2 OS=Mus musculus GN=Vdac2 PE=1 SV=2	Q60930	32 kDa
MP2K2_MOUSE Dual specificity mitogen-activated protein kinase kinase 2 OS=Mus musculus GN=Map2k2 PE=1 SV=2	Q63932 (+1)	44 kDa
GPDM_MOUSE Glycerol-3-phosphate dehydrogenase, mitochondrial OS=Mus musculus GN=Gpd2 PE=1 SV=2	Q64521	81 kDa
Q6P8N8_MOUSE ATP-dependent Clp protease ATP-binding subunit clpX-like, mitochondrial OS=Mus musculus GN=Clpx PE=2 SV=1	Q6P8N8 (+1)	67 kDa
LPPRC_MOUSE Leucine-rich PPR motif-containing protein, mitochondrial OS=Mus musculus GN=Lrpprc PE=1 SV=2	Q6PB66	157 kDa
Q6PF96_MOUSE Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial OS=Mus musculus GN=Etfdh PE=2 SV=1	Q6PF96 (+1)	61 kDa
EFTU_MOUSE Elongation factor Tu, mitochondrial OS=Mus musculus GN=Tufm PE=1 SV=1	Q8BFR5	50 kDa
SAM50_MOUSE Sorting and assembly machinery component 50 homolog OS=Mus musculus GN=Samm50 PE=1 SV=1	Q8BGH2	52 kDa
ALAT2_MOUSE Alanine aminotransferase 2 OS=Mus musculus GN=Gpt2 PE=1 SV=1	Q8BGT5	58 kDa
PCKGM_MOUSE Phosphoenolpyruvate carboxykinase [GTP], mitochondrial OS=Mus musculus GN=Pck2 PE=2 SV=1	Q8BH04	71 kDa
ODPX_MOUSE Pyruvate dehydrogenase protein X component, mitochondrial OS=Mus musculus GN=Pdhx PE=2 SV=1	Q8BKZ9	54 kDa
SCMC1_MOUSE Calcium-binding mitochondrial carrier protein SCaMC-1 OS=Mus musculus GN=Slc25a24 PE=2 SV=1	Q8BMD8	53 kDa
ODP2_MOUSE Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial OS=Mus musculus GN=Dlat PE=1 SV=2	Q8BMF4	68 kDa
CPSM_MOUSE Carbamoyl-phosphate synthase [ammonia], mitochondrial OS=Mus musculus GN=Cps1 PE=1 SV=2	Q8C196	165 kDa
NAKD2_MOUSE NAD kinase 2, mitochondrial OS=Mus musculus	Q8C5H8	51 kDa

GN=Nadk2 PE=1 SV=2		
NAKD2_MOUSE Isoform 2 of NAD kinase 2, mitochondrial OS=Mus musculus GN=Nadk2	Q8C5H8-2 (+1)	43 kDa
IMMT_MOUSE Isoform 2 of Mitochondrial inner membrane protein OS=Mus musculus GN=Immt	Q8CAQ8-2	83 kDa
LONM_MOUSE Lon protease homolog, mitochondrial OS=Mus musculus GN=Lonp1 PE=1 SV=2	Q8CGK3	106 kDa
DHSA_MOUSE Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial OS=Mus musculus GN=Sdha PE=1 SV=1	Q8K2B3	73 kDa
PREP_MOUSE Presequence protease, mitochondrial OS=Mus musculus GN=Ptrm1 PE=1 SV=1	Q8K411 (+1)	117 kDa
THIL_MOUSE Acetyl-CoA acetyltransferase, mitochondrial OS=Mus musculus GN=Acat1 PE=1 SV=1	Q8QZT1	45 kDa
SUOX_MOUSE Sulfite oxidase, mitochondrial OS=Mus musculus GN=Suox PE=2 SV=2	Q8R086	61 kDa
COQ6_MOUSE Ubiquinone biosynthesis monooxygenase COQ6 OS=Mus musculus GN=Coq6 PE=2 SV=3	Q8R1S0	51 kDa
AT2A1_MOUSE Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 OS=Mus musculus GN=Atp2a1 PE=2 SV=1	Q8R429	109 kDa
ACSF2_MOUSE Acyl-CoA synthetase family member 2, mitochondrial OS=Mus musculus GN=Acsf2 PE=1 SV=1	Q8VCW8	68 kDa
NDUS2_MOUSE NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial OS=Mus musculus GN=Ndufs2 PE=1 SV=1	Q91WD5	53 kDa
ETFD_MOUSE Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial OS=Mus musculus GN=Etfdh PE=1 SV=1	Q921G7	68 kDa
SELR1_MOUSE Sel1 repeat-containing protein 1 OS=Mus musculus GN=Selrc1 PE=2 SV=1	Q921H9	26 kDa
STML2_MOUSE Stomatin-like protein 2, mitochondrial OS=Mus musculus GN=Stoml2 PE=1 SV=1	Q99JB2	38 kDa
MAOM_MOUSE NAD-dependent malic enzyme, mitochondrial OS=Mus musculus GN=Me2 PE=2 SV=1	Q99KE1	66 kDa
ACON_MOUSE Aconitate hydratase, mitochondrial OS=Mus musculus GN=Aco2 PE=1 SV=1	Q99K10	85 kDa
NDUAA_MOUSE NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial OS=Mus musculus GN=Ndufa10 PE=1 SV=1	Q99LC3	41 kDa
GRPE1_MOUSE GrpE protein homolog 1, mitochondrial OS=Mus musculus GN=Grpel1 PE=1 SV=1	Q99LP6	24 kDa
TRAP1_MOUSE Heat shock protein 75 kDa, mitochondrial OS=Mus musculus GN=Trap1 PE=1 SV=1	Q9CQN1	80 kDa
PUR9_MOUSE Bifunctional purine biosynthesis protein PURH OS=Mus musculus GN=Atic PE=1 SV=2	Q9CWJ9	64 kDa
COQ5_MOUSE 2-methoxy-6-polyprenyl-1,4-benzoquinol methylase, mitochondrial OS=Mus musculus GN=Coq5 PE=2 SV=2	Q9CXI0	37 kDa
MPPB_MOUSE Mitochondrial-processing peptidase subunit beta OS=Mus musculus GN=Pmpcb PE=2 SV=1	Q9CXT8	55 kDa
QCR1_MOUSE Cytochrome b-c1 complex subunit 1, mitochondrial	Q9CZ13	53 kDa

OS=Mus musculus GN=Uqcrc1 PE=1 SV=2		
Q9CZN7_MOUSE Serine hydroxymethyltransferase OS=Mus musculus GN=Shmt2 PE=2 SV=1	Q9CZN7	56 kDa
GATA_MOUSE Glutamyl-tRNA(Gln) amidotransferase subunit A, mitochondrial OS=Mus musculus GN=Qrs11 PE=2 SV=1	Q9CZN8	57 kDa
EFTS_MOUSE Elongation factor Ts, mitochondrial OS=Mus musculus GN=Tsfm PE=2 SV=1	Q9CZR8	35 kDa
CISY_MOUSE Citrate synthase, mitochondrial OS=Mus musculus GN=Cs PE=1 SV=1	Q9CZU6	52 kDa
TOM70_MOUSE Mitochondrial import receptor subunit TOM70 OS=Mus musculus GN=Tom70a PE=1 SV=2	Q9CZW5	68 kDa
ODPB_MOUSE Pyruvate dehydrogenase E1 component subunit beta, mitochondrial OS=Mus musculus GN=Pdhb PE=1 SV=1	Q9D051	39 kDa
TRM5_MOUSE tRNA (guanine(37)-N1)-methyltransferase OS=Mus musculus GN=Trmt5 PE=2 SV=1	Q9D0C4 (+1)	57 kDa
SCOT1_MOUSE Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial OS=Mus musculus GN=Oxct1 PE=1 SV=1	Q9D0K2	56 kDa
ODO2_MOUSE Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial OS=Mus musculus GN=Dlst PE=1 SV=1	Q9D2G2	49 kDa
IDH3A_MOUSE Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial OS=Mus musculus GN=Idh3a PE=1 SV=1	Q9D6R2	40 kDa
TIM50_MOUSE Mitochondrial import inner membrane translocase subunit TIM50 OS=Mus musculus GN=Timm50 PE=1 SV=1	Q9D880	40 kDa
QCR2_MOUSE Cytochrome b-c1 complex subunit 2, mitochondrial OS=Mus musculus GN=Uqcrc2 PE=1 SV=1	Q9DB77	48 kDa
AL7A1_MOUSE Alpha-aminoacidic semialdehyde dehydrogenase OS=Mus musculus GN=Aldh7a1 PE=1 SV=4	Q9DBF1 (+1)	59 kDa
MPPA_MOUSE Mitochondrial-processing peptidase subunit alpha OS=Mus musculus GN=Pmpca PE=1 SV=1	Q9DC61	58 kDa
NDUS3_MOUSE NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial OS=Mus musculus GN=Ndufs3 PE=1 SV=2	Q9DCT2	30 kDa
ETFB_MOUSE Electron transfer flavoprotein subunit beta OS=Mus musculus GN=Etfb PE=1 SV=3	Q9DCW4	28 kDa
MMSA_MOUSE Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial OS=Mus musculus GN=Aldh6a1 PE=1 SV=1	Q9EQ20	58 kDa
RM46_MOUSE 39S ribosomal protein L46, mitochondrial OS=Mus musculus GN=Mrpl46 PE=2 SV=1	Q9EQI8	32 kDa
GSDMA_MOUSE Gasdermin-A OS=Mus musculus GN=Gsdma PE=2 SV=1	Q9EST1	50 kDa
IVD_MOUSE Isovaleryl-CoA dehydrogenase, mitochondrial OS=Mus musculus GN=Ivd PE=1 SV=1	Q9JHI5	46 kDa
SYSM_MOUSE Serine--tRNA ligase, mitochondrial OS=Mus musculus GN=Sars2 PE=1 SV=2	Q9JL8	58 kDa
AL9A1_MOUSE 4-trimethylaminobutyraldehyde dehydrogenase OS=Mus musculus GN=Aldh9a1 PE=1 SV=1	Q9JLJ2	54 kDa
ACOT2_MOUSE Acyl-coenzyme A thioesterase 2, mitochondrial OS=Mus musculus GN=Acot2 PE=1 SV=2	Q9QYR9	50 kDa

CAH5B_MOUSE Carbonic anhydrase 5B, mitochondrial OS=Mus musculus GN=Ca5b PE=2 SV=2	Q9QZA0	37 kDa
AIFM1_MOUSE Apoptosis-inducing factor 1, mitochondrial OS=Mus musculus GN=Aifm1 PE=1 SV=1	Q9Z0X1	67 kDa
NFS1_MOUSE Cysteine desulfurase, mitochondrial OS=Mus musculus GN=Nfs1 PE=2 SV=3	Q9Z1J3	51 kDa
SUCB2_MOUSE Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial OS=Mus musculus GN=Suclg2 PE=1 SV=3	Q9Z2I8	47 kDa
SUCB2_MOUSE Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial OS=Mus musculus GN=Suclg2 PE=1 SV=3	Q9Z2I8 (+1)	47 kDa
SUCB1_MOUSE Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial OS=Mus musculus GN=Sucla2 PE=1 SV=2	Q9Z2I9	50 kDa

Table S5. Source Extractor protein counts of the Q7 and Q111 proteome changes in Figure 2.

Guide star spots					
	Cy3-Flux	Cy5-Flux	Cy3/Cy5	C3/Cy5 Normalized	
*1	4663.9	11935.8	0.39	0.977	
*2	1491.9	3621.6	0.41	1.030	
*3	13976.7	36562.2	0.38	0.956	
*4	240047.0	518705.1	0.46	1.157	
*5	168505.5	438975.3	0.38	0.960	
*6	36092.9	92785.3	0.39	0.972	
*7	27635.7	67814.5	0.41	1.019	
*8	41361.2	110093.2	0.38	0.939	
*9	8832.0	21893.6	0.40	1.009	
		Average	0.40	1.002	
		% Standard deviation	2.63%	6.57%	

Difference protein spots					
	Cy3-Flux	Cy5-Flux	Cy3/Cy5	C3/Cy5 Normalized	Fold change
1†	27529.5	34460.7	0.80	1.997	2.00
2	1906.4	72904.4	0.03	0.065	-15.30
3	41395.1	38849.4	1.07	2.664	2.66
4	100417.3	156390.9	0.64	1.605	1.61
5	59180.5	211718.0	0.28	0.699	-1.43
6	110601.5	50169.4	2.20	5.511	5.51
7	139736.9	118210.5	1.18	2.955	2.96
8	64777.6	345285.2	0.19	0.469	-2.13
9	15608.6	2283.7	6.83	17.087	17.09

note:

† This spot was not detected by Source Extractor. These values were obtained manually using ImageJ.

Table S6. Mitochondrial proteins grouped by import pathway based on primary structure as indicated in UniProt database or stated in references.

Pathway	Gene	Protein structure and mitochondrial localization
Mim1, TOM40+SAM50	TOM70A	Outer membrane single-pass protein. Contains: no MTS, 1 helical transmembrane stretch, and domains protruding into mitochondrial intermembrane space and cytoplasmic side.
	SAM50	Outer membrane multi-pass protein. Contains: no MTS, C-terminal 16 transmembrane β -barrel structures.
	TOM40	Outer membrane multi-pass protein. Contains: no MTS, 19 membrane-spanning β -barrel strands.
	TOM22	Outer membrane single-pass protein. Contains: no MTS, 1 helical transmembrane stretch, and domains protruding into mitochondrial intermembrane space and cytoplasmic side.
	VDAC2	Outer membrane multi-pass protein. Contains: no MTS, 19 membrane-spanning β -barrel strands.
	VDAC1	Outer membrane multi-pass protein. Contains: no MTS, 19 membrane-spanning β -barrel strands.
TOM40+TIM22	SLC25A24	Inner membrane multi-pass protein. Contains: no MTS, 6 transmembrane helical strands.
	SLC25A23	Inner membrane multi-pass protein. Contains: no MTS, 6 transmembrane helical strands.
	SLC25A12	Inner membrane multi-pass protein. Contains: no MTS, 6 transmembrane helical strands.
	TIM22	Inner membrane multi-pass protein. Contains: no MTS, 3 transmembrane helical strands.
	TIM23	Inner membrane multi-pass protein. Contains: no MTS, 3 transmembrane helical strands.
TOM40+TIM23 Membrane bound	TIM44	Peripheral inner membrane protein associated with the matrix side of the membrane ^{1,2} .
	TIM50	Single-pass inner membrane protein. Contains: MTS, 1 helical transmembrane stretch, and domains protruding into mitochondrial intermembrane space and matrix.
	GPD2	Integral protein of inner membrane. Contains: MTS, 3 transmembrane stretches, big intermembrane space domain and N-terminus facing matrix ³ .
	ACADVL	Inner membrane anchored protein. Contains: MTS and C-terminal membrane-anchored region.
	PHB	Inner membrane anchored protein. Contains: N-terminal unconventional targeting sequence, and hydrophobic stretches. Uses TIM23 for insertion into inner membrane ⁴ .
TOM40+TIM23 Matrix soluble	OTC	Matrix protein. Contains MTS.
	TUFM	Matrix protein. Contains MTS.
	IDH3A	Matrix protein. Contains MTS.
	CLPP	Matrix protein. Contains MTS.
	OAT	Matrix protein. Contains MTS.
	ME2	Matrix protein. Contains MTS.

note:

MTS – N-terminal mitochondrial targeting sequence.

- 1 Berthold, J. *et al.* The MIM complex mediates preprotein translocation across the mitochondrial inner membrane and couples it to the mt-Hsp70/ATP driving system. *Cell* **81**, 1085-1093, doi:S0092-8674(05)80013-3 [pii] (1995).
- 2 Rassow, J. *et al.* Mitochondrial protein import: biochemical and genetic evidence for interaction of matrix hsp70 and the inner membrane protein MIM44. *J Cell Biol* **127**, 1547-1556 (1994).
- 3 MacDonald, M. J. & Brown, L. J. Calcium activation of mitochondrial glycerol phosphate dehydrogenase restudied. *Arch Biochem Biophys* **326**, 79-84, doi:S0003-9861(96)90049-1 [pii] 10.1006/abbi.1996.0049 (1996).
- 4 Tatsuta, T., Model, K. & Langer, T. Formation of membrane-bound ring complexes by prohibitins in mitochondria. *Mol Biol Cell* **16**, 248-259, doi:E04-09-0807 [pii] 10.1091/mbc.E04-09-0807 (2005).

Table S7. Comparison of mitochondrial protein levels between Q7 and Q111 mitochondria.

	Pathway	Protein	Q111 mitochondria		Average per group, %	MANOVA	
			% of control	<i>P</i> value, t-test		Dependent variables, proteins	Q111 vs Q7, <i>P</i> value
Non-TIM23 imported	Mim1, TOM40+ SAM50	VDAC1	51.0	0.0024	58	VDAC1 TOM40 TIM22 SLC25A23 SLC25A24	0.025
		VDAC2	38.0	0.0146			
		SAM50	63.6	0.0040			
		TOM70A	61.5	0.0435			
		TOM40	52.6	0.0627			
		TOM22	40.7	0.1116			
	TOM40+ TIM22	SLC25A24	67.2	0.1028			
		SLC25A23	101.9	0.9668			
		SLC25A12	74.4	0.4100			
		TIM22	33.3	0.0491			
	TIM23	55.5	0.0788				
TIM23 imported	TOM40+ TIM23 Membrane bound	PHB	72.5	0.0023	49	CLPP ACADVL OTC ME2	0.008
		TIM50	57.2	0.0131			
		TIM44	37.9	0.0080			
		GPD2	49.1	0.0004			
		ACADVL	81.3	0.5157			
	TOM40+ TIM23 Matrix soluble	OTC	22.0	0.0165			
		TUFM	38.4	0.0004			
		IDH3A	46.1	0.0001			
		CLPP	32.8	0.00001			
		OAT	49.3	0.0021			
		ME2	53.8	0.1107			

note:

MANOVA *P*-value was for Q7 vs. Q111 and when considering the listed proteins together. One of the assumptions of MANOVA analysis is that there should be no collinearity among the dependent variables; thus, highly correlated dependent variables (correlation coefficient > 0.8) were removed from the comparison. Average per group – average value for group of TIM23-imported and non-TIM23 imported proteins.

Table S8. Comparison of mitochondrial protein levels between Control and HD grade 2 striatum.

	Pathway	Protein	HD2 Striatum NS		AVRG per group, %	MANOVA		HD2 Striatum SM		AVRG per group, %	MANOVA	
			% of control	P value, t-test		Dependent variables, proteins	NS HD2 vs Control, P value	% of control	P value, t-test		Dependent variables, proteins	SM HD2 vs Control, P value
Non-TIM23 imported	Mim1, TOM40+ SAM50	VDAC1	95.9	0.6328	92	VDAC2	0.019	104.3	0.7960	97	VDAC2	0.005
		VDAC2	80.7	0.1295		SAM50		120.2	0.4474		SAM50	
		SAM50	118.6	0.1317		TOM70A		122.5	0.0198		TOM70A	
		TOM70A	47.3	0.0180		TOM40		55.7	0.0353		TOM40	
		TOM40	117.8	0.1102		SLC25A23		100.6	0.9591		SLC25A23	
		TOM22	82.4	0.3616		SLC25A12		76.5	0.1052		SLC25A24	
											SLC25A12	
	TOM40+ TIM22	SLC25A24	123.1	0.1616		TIM22		121.4	0.0997		TIM23	
		SLC25A23	87.9	0.1171		TIM23		86.3	0.0014			
		SLC25A12	95.6	0.5754				103.4	0.7437			
TIM23		73.9	0.5945		75.8	0.5774						
	TIM22	94.2	0.0218		95.0	0.0196						
TIM23 imported	TOM40+ TIM23 Membrane bound	PHB	86.5	0.0632	82	PHB	0.003	106.1	0.2888	93	PHB	0.035
		TIM50	55.8	0.0474		TIM44		61.1	0.0110		TIM50	
		TIM44	62.6	0.0278		GPD2		77.5	0.1529		TIM44	
		GPD2	97.6	0.7661		ACADVL		83.4	0.0177		GPD2	
		ACADVL	137.8	0.1339		CLPP		155.5	0.0540		OTC	
	TOM40+ TIM23 Matrix soluble	OTC	66.1	0.0080		OAT		81.6	0.1736			
		TUFM	70.5	0.0082		ME2		80.1	0.2142			
		IDH3A	62.5	0.0235				91.8	0.6529			
		CLPP	56.8	0.1188				97.7	0.9063			
		OAT	80.2	0.5163				76.2	0.3597			
	ME2	122.4	0.3387		107.0	0.7542						

note:

MANOVA P-value was for control vs. HD when considering the listed proteins together. NS – nonsynaptosomal mitochondria, SM – synaptosomal mitochondria additionally purified with MACS kit. One of the assumptions of MANOVA analysis is that there should be no collinearity among the dependent variables; thus, highly correlated dependent variables (correlation coefficient > 0.8) were removed from the comparison. Average (AVRG) per group – average value for group of TIM23-imported and non-TIM23 imported proteins.

Table S9. Comparison of mitochondrial protein levels between Control and HD grade 4 cortex.

	Pathway	Protein	HD4 Cortex NS		AVRG per group, %	MANOVA		HD4 Cortex SM		AVRG per group, %	MANOVA	
			% of control	P value, t-test		Dependent variables, proteins	NS HD4 vs Control, P value	% of control	P value, t-test		Dependent variables, proteins	SM HD4 vs Control, P value
Non-TIM23 imported	Mim1, TOM40+ SAM50	VDAC1 VDAC2 SAM50 TOM70A TOM40 TOM22	98.2 95.9 76.2 73.6 123.6 183.0	0.9278 0.6962 0.0113 0.1768 0.0769 0.0003	118	VDAC1 VDAC2 SAM50 TOM70A TOM22 SLC25A23	0.002	89.8 85.8 57.6 53.2 113.9 91.1	0.5986 0.4531 0.0077 0.0597 0.5481 0.6780	88	VDAC2 SAM50 TOM70A TOM40 SLC25A23 SLC25A24	0.326
	TOM40+ TIM22	SLC25A24 SLC25A23 SLC25A12 TIM23 TIM22	143.2 77.4 119.5 161.7 147.8	0.1057 0.3464 0.2396 0.0360 0.0052		SLC25A24 SLC25A12		100.7 71.7 110.3 105.1 90.4	0.9765 0.0854 0.4861 0.4433 0.7160		SLC25A12 TIM23	
TIM23 imported	TOM40+ TIM23 Membrane bound	PHB TIM50 TIM44 GPD2 ACADVL	97.1 76.3 41.9 90.8 73.1	0.8751 0.4604 0.1271 0.4114 0.1678	95	TIM50 TIM44 GPD2 ACADVL OTC	0.309	104.2 48.7 66.9 101.2 73.6	0.6806 0.0516 0.0435 0.9299 0.3016	76	PHB TIM44 ACADVL OTC CLPP	0.003
	TOM40+ TIM23 Matrix soluble	OTC TUFM IDH3A CLPP OAT ME2	96.2 93.1 130.4 219.2 65.1 60.9	0.8486 0.5670 0.1821 0.0070 0.3642 0.1013		TUFM CLPP ME2		35.8 89.0 97.0 84.7 64.2 65.8	0.0068 0.5790 0.8878 0.5665 0.1102 0.1423		ME2	

note:

MANOVA P-value was for control vs. HD when considering the listed proteins together. NS – nonsynaptosomal mitochondria, SM – synaptosomal mitochondria additionally purified with MACS kit. One of the assumptions of MANOVA analysis is that there should be no collinearity among the dependent variables; thus, highly correlated dependent variables (correlation coefficient > 0.8) were removed from the comparison. Average (AVRG) per group – average value for group of TIM23-imported and non-TIM23 imported proteins.

Table S10. List of antibodies.

Antibody	Vendor	Catalog #	Description	Dilution	Notes
Primary:					
Anti-polyglutamine	Millipore	MAB1574	Mouse monoclonal	1:5000	
Anti-ACADVL	AbCam	ab54698	Mouse monoclonal	1:1000	Hm samples
Anti-ACADVL	AbCam	ab155138	Rabbit polyclonal	1:500	Ms samples
Anti-ACO2	AbCam	Ab110321	Mouse monoclonal	1:2000	
Anti-ATP5A	AbCam	ab14748	Mouse monoclonal	1:5000	
Anti-CALR	Millipore	MABT145	Rabbit monoclonal	1:10.000	
Anti-CAV2	AbCam	ab133484	Rabbit monoclonal	1:5000	
Anti-CLPP	AbCam	ab124822	Rabbit monoclonal	1:5000	
Anti-DIABLO	AbCam	ab32023	Rabbit monoclonal	1:1000	
Anti-FLAG	Sigma	F1804	Mouse monoclonal	1:1000	
Anti-GAD1	Sigma	SAB4501074	Rabbit polyclonal	1:1000	
Anti-GPD2	AbCam	ab188585	Rabbit monoclonal	1:5000	
Anti-HTT	AbCam	ab109115	Rabbit monoclonal	1:5000	
Anti-HTT	Millipore	MAB2166	Mouse monoclonal	1:1000	
Anti-IHD3A	Proteintech	15909-1-AP	Rabbit polyclonal	1:1000	
Anti-LAMP1	AbCam	ab13523	Mouse monoclonal	1:1000	Ms samples
Anti-LAMP1	AbCam	ab108597	Rabbit monoclonal	1:1000	Hm samples
Anti-ME2	AbCam	ab139686	Rabbit monoclonal	1:5000	
Anti-MIA40	Proteintech	21090-1-AP	Rabbit polyclonal	1:1000	
Anti-mtCO1	AbCam	ab14705	Mouse monoclonal	1:2000	
Anti-OAT	AbCam	ab100845	Mouse monoclonal	1:500	
Anti-OTC	AbCam	ab228646	Rabbit polyclonal	1:1000	Hm samples
Anti-OTC	Sigma	HPA000243	Rabbit polyclonal	1:500	Ms samples
Anti-polyQ	Millipore	MAB1574	Mouse monoclonal	1:1000	
Anti-RCAS1	Cell Signaling	#12290	Rabbit monoclonal	1:1000	
Anti-SAMM50	AbCam	ab133709	Rabbit monoclonal	1:1000	
Anti-SLC25A23	Santa Cruz Biotechnology	sc-377109	Mouse monoclonal	1:200	
Anti-SLC25A24	AbCam	ab68796	Mouse polyclonal	1:1000	Hm samples
Anti-SLC25A12	AbCam	Ab200201	Rabbit monoclonal	1:1000	
Anti-SLC25A10	Millipore	MABN457	Mouse monoclonal	1:1000	
Anti-TIMM22	AbCam	Ab167423	Rabbit monoclonal	1:1000	
Anti-TIMM23	BD Bioscience	611222	Mouse monoclonal	1:2000	
Anti-TIMM44	AbCam	ab194829	Rabbit monoclonal	1:1000	Hm samples
Anti-TIMM44	Proteintech	66149-1-Ig	Mouse monoclonal	1:1000	Ms samples
Anti-TIMM50	AbCam	ab109436	Rabbit monoclonal	1:2000	
Anti-TOMM20	Santa Cruz Biotechnology	sc-11415	Rabbit polyclonal	1:2000	
Anti-TOMM22	Proteintech	11278-1-AP	Rabbit polyclonal	1:1000	
Anti-TOMM40	Santa Cruz Biotechnology	D-2	Mouse monoclonal	1:1000	Hm samples
Anti-TOMM40	Proteintech	18409-1-AP	Rabbit polyclonal	1:1000	Ms samples
Anti-TOMM70A	AbCam	ab83841	Rabbit polyclonal	1:1000	

Anti-TUBA	Sigma	T5168	Mouse monoclonal	1:10.000	
Anti-VDAC1	Millipore	AB10527	Rabbit polyclonal	1:2500	
Anti-VDAC2	AbCam	ab155803	Rabbit polyclonal	1:1000	
Secondary:					
IRDye 800CW Goat anti-Rabbit	Li-Cor	926-32211	Goat	1:15.000	
IRDye 800CW Goat anti-Mouse	Li-Cor	926-32210	Goat	1:15.000	
IRDye 680LT Goat anti-Rabbit	Li-Cor	926-68021	Goat	1:15.000	
IRDye 680LT Goat anti-Mouse	Li-Cor	926-68020	Goat	1:15.000	

Materials and methods

Plasmids and recombinant proteins preparation. Plasmid constructs pGEX-4T3-HTT_{exon1-23Q} and -HTT_{exon1-97Q} for bacterial expression of GST-fused HTT fragments were generated as described (1). Recombinant mouse subunits of TIM23 complex (TIM23, TIM50, TIM17A, TIM17B) were generated by cDNA subcloning into pGEX-4T3 vector (GE Healthcare). GST-fused HTT exon 1 proteins (GST-HTT_{exon1-23Q}, -97Q) and subunits of TIM23 complex (GST-TIM23, -TIM50, -TIM17A, -TIM17B), and GST control were purified from transformed One Shot™ BL21 Star™ (DE3) cells (Life Technologies) as described (1). GST-TIM23, GST-TIM50, GST-TIM17A, GST-TIM17B proteins bound to Glutathione Sepharose 4B beads (GE Healthcare) were digested with Thrombin protease (GE Healthcare) overnight at 25°C and eluted with PBS to remove GST proteins and concentrated using Amicon®Ultra-10K (Millipore) filter.

The wtHTT (HTT_{171-Q17}) and mHTT (HTT_{171-Q68}) fragments were overexpressed in HEK293t cells using pcDNA3.1+ vectors for eukaryotic expression encoding 171 amino acids long N-terminal sequence of human HTT labeled with flag-tag (DYKDDDDK) at the C-terminus.

Mitochondria fractionation from cell lines. Immediately prior to each experiment, cells were collected by trypsinization, washed twice in ice-cold low-salt buffer (138 mM NaCl, 2.7 mM KCl, 10 mM HEPES, pH 8 adjusted with NaOH) and subjected to mitochondria fractionation using Mitochondria Isolation MACS Kit (Miltenyi Biotec) according to the manufacturer's protocol. Briefly, cells were ruptured with 27G-needled syringe in ice-cold cell-lysis buffer provided with the kit (1 mL cell-lysis buffer per 10x10⁷ cells) and incubated with iron nano-beads conjugated with anti-TOM22 antibody in provided buffer for 1 h at 4°C. The suspension was applied to a column placed in magnetic field of the MACS Separator, which retains magnetically labeled mitochondria. Mitochondria then were eluted with isolation buffer (225 mM Sucrose, 75 mM Mannitol, 10 mM HEPES, pH 8 adjusted with NaOH) upon column removal from the magnetic field and pelleted at 13,000 g for 4 min.

Immunoblotting. Mitochondrial lysates were used to prepare samples with Laemmli buffer (BioRad) augmented with 10% β-Mercaptoethanol (BioRad). Before loading into Bolt™ 4-12% Bis-Tris Plus gels (Invitrogen), samples were heated at 55°C for 10 min. Proteins were separated at 155 V in MES SDS Running buffer (Life Technologies) and transferred to Immobilon®-FL PVDF membrane (EMD Millipore) overnight at 35 V in 1X TG buffer (BioRad) supplemented with 10% methanol at 45 V and 4°C. PVDF membranes were blocked in SEA Blocking buffer (Thermo Scientific) for 1 h at ambient temperature. Primary antibodies were reconstituted in SEA Blocking buffer and incubated at 4°C overnight. The full list of primary antibodies and dilutions used in this study is indicated in *SI Appendix* Table S10. IRDye infrared fluorescent dye-labeled secondary antibodies (LI-COR Biosciences)

detectable at 700 or 800 nm were reconstituted (1:15.000) in SEA Blocking buffer and applied for 1 h at ambient temperature. All secondary antibodies are listed in *SI Appendix* Table S10. Both primary and secondary antibodies were washed 3 times with 1X PBST for 15 min per wash. Final PVFD-FL membranes were scanned on Odyssey CLx near-infrared fluorescence imager (LI-COR Biosciences) to visualize protein bands. The signal intensity of protein bands was analyzed in Image Studio Version 2.1 software. Images were converted to monochrome for presentation.

1. Yano H, *et al.* (2014) Inhibition of mitochondrial protein import by mutant huntingtin. *Nature neuroscience* 17(6):822-831.