

Supplemental Information

Table S1, Related to Figure 1. Summary characteristics of the Leigh syndrome patient derived fibroblast cell lines including the patient phenotype at diagnosis, the mutations identified, and published literature using the cell line.

Table S2, Related to Figure 4. Day 10 LS derived neutralized embryoid bodies relative metabolite abundance for metabolites used in pathway analysis. LC-MS measured metabolite peak areas were normalized to the total ion count (TIC) by sample and fold change was determined by dividing each LS TIC normalized peak area by the control TIC normalized peak area for each metabolite. Metabolites were identified and filtered for pathway analysis using a threshold fold change (FC >5 and <0.4). Fold change values shown are averages for 2 independent runs with ~150 neutralized EBs per run.

Table S3, Related to Figure 4. Summary of the metabolic pathways analysis for metabolites enriched in day 10 LS derived neutralized embryoid bodies. Statistical p values from enrichment analysis are adjusted for multiple hypothesis testing. Total: total number of compounds in the pathway. Hits: matched number from the uploaded data. Raw p: original p value calculated from the enrichment analysis. Holm p: p value adjusted by Holm-Bonferroni method. FDR p: adjusted p value using False Discovery Rate. Impact: pathway impact value calculated from pathway topology analysis.

Figure S1, Related to Figure 2. Characterization of Leigh syndrome iPSCs. A. Schematic representation of the fibroblast reprogramming protocol. B-C. Pluripotency characterization of the LS iPSCs. Samples were analyzed against samples in a reference data set (The International Stem Cell Initiative, 2018) (B). The distribution of the samples compared with a non-iPSC control shows clustering of the samples in the high pluripotency and low novelty quadrant (C). D. qPCR

for the pluripotency genes *POU5F1* and *NANOG*. E. Karyotype characterization results for LS and control cell lines.

Figure S2, Related to Figure 3. Leigh syndrome derived NPCs are multipotent and do not present increased sensitivity to pharmacological stressors. A. Quantification of protein expression of neural markers Pax6, Nestin and Sox2. B. Leigh syndrome NPCs do not show enhanced sensitivity towards different stressors. LS NPCs have similar cell viability compared to control when exposed to the DNA damaging agents etoposide and neocarzinostatin, the mitochondrial toxicant CCCP, and the microtubule destabilizer nocodazole.

Figure S3, Related to Figure 5. MT-ATP6/PDH brain organoids display defective differentiation at day 10. A. Brightfield images (4X) of day 10 brain organoids. MT-ATP6/PDH shows disorganized cellular growth that do not resemble neuroepithelial buds. B. Quantification of the defective organoids at day 10 by cell line.

Figure S4, Related to Figure 7. Heat map of metabolomic analysis of day 40 organoids. A total of four batches of 40-day organoids per line were analyzed as described in methods. Each column represents a replicate and each row represents a metabolite. Relative abundance is independently scaled by row with higher z scores represented by red color and lower z scores represented by blue color.

Cell line	Phenotype	Mutation	References
GM03672	1 y/o female, Caucasian. Developmental regression; microcephalic; elevated blood lactic acid and pyruvate; only affected family member	PDH (E1) LOF Pyruvate dehydrogenase (79delC, Arg27fs)	Hinman et al. 1989; Huh et al. 1990; Johnson et al. 2018
GM01503	3y/o female, Caucasian. Lactic acidosis, psychomotor delay; sister also affected. Subnormal activation of pyruvate dehydrogenase complex in disrupted fibroblasts	DLD (E3) Dihydrolipoamide dehydrogenase (A100G, Thr34Ala)	Cooper et al, 1969; Murphy JV, 1973; Sorbi& Blass, 1982; Hinman et al. 1989; Huh et al. 1990; Vo et al. 2007; Johnson et al. 2018
GM13411	8 month old male, Chinese. Lactic acidosis; developmental delay. Hypertrophic cardiomyopathy. Symmetric neural necrosis. Fibroblast: 90% heteroplasmy	MT-ATP6 (T8993G, Leu156Arg) PDH (E1) LOF Pyruvate dehydrogenase (79delC, Arg27fs)	Pastores et al. 1994; Iyer et al.2012; Ma et al. 2014; Galera-Monge et al. 2016; Zheng et al. 2016; Johnson et al. 2018

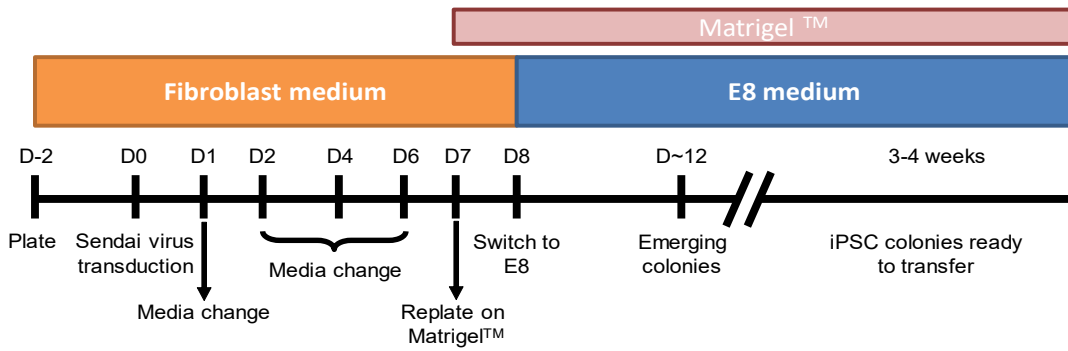
Query	HMDB	PubChem	KEGG	PDH/Control	DLD/Control	MT-ATP6-PDH/ Control
2-Hydroxybutyric acid	HMDB0000008	11266	C05984	0.41	0.67	0.36
D-2-Hydroxyglutaric acid	HMDB0000606	439391	C01087	6.79	1.18	1.08
3-Methylguanine/ 7-Methylguanine	HMDB0001566/ HMDB0000897	76292/ 11361	C02230/ C02242	32.83	7.63	6.08
3-Phosphoglyceric acid	HMDB0000807	724	C00597	8.94	4.58	4.16
Phosphoserine	HMDB0000272	68841	C01005	6.83	4.76	2.88
p-Hydroxyphenylacetic acid	HMDB0000020	127	C00642	0.60	0.23	0.39
5-Methylcytosine	HMDB0002894	65040	C02376	11.61	6.71	1.88
5'-Methylthioadenosine	HMDB0001173	439176	C00170	18.81	8.50	8.87
6-Phosphogluconic acid	HMDB0001316	91493	C00345	26.19	9.48	4.96
6-Phosphonoglucono-D-lactone	HMDB0001127	439452	C01236	0.00	0.00	0.00
Acetoacetic acid	HMDB0000060	96	C00164	0.64	0.80	0.13
Acetylcholine	HMDB0000895	6060	C01996	6.07	3.72	4.50
cis-Aconitic acid	HMDB0000072	643757	C00417	6.75	8.62	7.20
Adenylsuccinic acid	HMDB0000536	440122	C03794	13.71	11.06	5.76
ADP	HMDB0001341	6022	C00008	7.03	4.00	3.45
Agmatine	HMDB0001432	199	C00179	8.02	0.00	16.86
Oxoglutaric acid (a-KG)	HMDB0000208	51	C00026	8.20	15.06	19.39
L-Alanine	HMDB0000161	5950	C00041/ C00133	12.70	5.25	4.19
Alpha-ketoisovaleric acid	HMDB0000019	49	C00141	13.96	7.30	10.47
Anandamide	HMDB0004080	5281969	C11695	29.79	1.99	2.60
L-Arginine	HMDB0000517	6322	C02385	7.06	3.27	3.33
Argininosuccinic acid	HMDB0000052	16950	C03406	10.76	3.52	3.25
L-Asparagine	HMDB0000168	6267	C16438	9.09	4.52	3.93
L-Aspartic acid	HMDB0000191	5960	C16433	5.81	3.83	2.03
Adenosine triphosphate (ATP)	HMDB0000538	5957	C00002	11.05	6.03	3.88
Beta-Alanine	HMDB0000056	239	C00099	12.15	6.85	0.50
Betaine	HMDB0000043	247	C00719	6.74	5.60	3.57
Carbamoyl phosphate	HMDB0001096	278	C00169	0.00	5.91	2.29
L-Carnitine	HMDB0000062	2724480	C00487	1.36	8.78	3.02
Citric acid	HMDB0000094	311	C00158	126.65	49.58	34.21
Cytidine monophosphate (CMP)	HMDB0000095	8117	C00055	5.96	4.46	4.52
Creatine	HMDB0000064	586	C00300	24.56	15.50	19.10
Cytidine triphosphate	HMDB0000082	6176	C00063	9.58	5.77	4.27
L-Cystathionine	HMDB0000099	439258	C02291	11.04	5.92	4.11
L-Cysteine	HMDB0000574	5862	C00097	16.61	3.61	8.56
L-Cystine	HMDB0000192	67678	C00491	0.01	0.17	0.61
Cytidine	HMDB0000089	6253	C00475	13.76	4.12	4.44
Cytosine	HMDB0000630	597	C00380	28.76	7.89	5.83
D-Arabinono-1,4-lactone	NA	NA	C00652	12.31	4.88	5.36
dCMP	HMDB0001202	13945	C00239	0.00	0.00	0.00
dCTP	HMDB0000998	65091	C00458	25.73	10.28	0.00
Deoxyguanosine	HMDB0000085	187790	C00330	29.29	9.02	5.09
Deoxyinosine	HMDB0000071	65058	C05512	49.11	14.94	6.69
Deoxyuridine	HMDB0000012	13712	C00526	264.71	78.97	23.73
Fructose 1,6-bisphosphate/ D-Fructose	HMDB0001058/ 2,6-bisphosphate	445557/ 105021	C00354 / C00665	8.22	6.10	5.02
Fructose 1-phosphate	HMDB0001076	10400369	C01094	12.62	7.55	8.20
Galactonic acid	HMDB0000565	128869	C00880	10.39	6.53	4.31
dGDP	HMDB0000960	439220	C00361	19.75	8.44	4.62
Glucose 6-phosphate	HMDB0001401	5958	C00092	6.68	3.81	4.19
D-Glyceraldehyde 3-phosphate/	HMDB0001112/	729/	C00118 /	7.65	4.01	3.60
Dihydroxyacetone phosphate	HMDB0001473	668	C00111			
4,5-Dihydroorotic acid	HMDB0000528	648	C00337	6.70	4.34	3.98
DL-Dopa	HMDB0000609	836	C00355	0.16	1.19	0.46
3,4-Dihydroxyphenylglycol (DOPEG)	HMDB0000318	91528	C05576	0.11	0.64	0.52
Hydroxytyrosol (DOPET)	HMDB0005784	82755	NA	0.46	0.72	0.43
D-Sedoheptulose 7-phosphate	HMDB0001068	22833559	C00281	14.26	6.50	4.82
Fructose 6-phosphate	HMDB0000124	69507	C00085	9.73	4.43	3.96
Fumaric acid	HMDB0000134	444972	C00122	5.39	4.16	3.13
GDP-L-fucose	HMDB0001095	439211	C00325	14.13	5.67	6.02
Glucosamine 6-phosphate	HMDB0001254	439217	C00352	13.26	6.49	7.38
L-Glutamine	HMDB0000641	5961	C00303	10.30	4.88	4.37
L-Glutamic acid	HMDB0000148	33032	C00302	6.65	3.18	2.72
Glutathione	HMDB0000125	124886	C02471	59.41	23.87	28.72
Oxidized glutathione	HMDB0003337	975	C00127	5.16	3.35	2.59
Glycerol 3-phosphate	HMDB0000126	439162	C00093	9.59	3.84	3.86
Glycine	HMDB0000123	750	C00037	5.05	4.19	3.23
Guanosine monophosphate (GMP)	HMDB0001397	6804	C00144	6.51	2.92	2.63
Guanosine triphosphate (GTP)	HMDB0001273	6830	C00044	12.01	5.85	4.38
Guanine	HMDB0000132	764	C00242	15.87	5.37	3.29
Guanosine	HMDB0000133	6802	C00387	8.15	3.86	2.18
L-Gulonolactone	HMDB0003466	439373	C01040	1.29	0.34	1.09
L-Homoserine	HMDB0000719	12647	C00263	4.85	2.84	2.33
Iminodiacetic acid	HMDB0011753	8897	C19911	14.77	4.33	3.91
Imidazoleacetic acid	HMDB0002024	96215	C02835	7.21	2.50	2.65
Inosinic acid (IMP)	HMDB0000175	8582	C00130	8.83	4.42	4.41
Itaconic acid	HMDB0002092	811	C00490	9.96	3.81	4.44

L-Lysine	HMDB0000182	5962	C00047/ C00739	7.95	2.62	2.84
Maleic acid	HMDB0000176	444972	C01384	18.82	5.87	14.75
Malic acid	HMDB0000744	525	C00711	5.16	3.09	2.09
L-Methionine	HMDB0000696	6137	C00073	7.27	3.68	4.27
N1-Acetylspermidine	HMDB0001276	496	C00612/ C01029	1.04	1.35	7.34
N-Acetyl-L-aspartic acid	HMDB0000812	65065	C01042	8.84	4.17	5.22
N-Acetylaspartylglutamic acid	HMDB0001067	5255	C12270	12.24	5.66	7.83
N-acetyl-b-alanine	NA	NA	C01073	3.99	5.66	0.91
N-Acetyl-D-Glucosamine 6-Phosphate	HMDB0001062	440996	C04501/ C00357	10.29	5.01	3.75
NAD+	HMDB0000902	5893	C00003	7.43	3.60	3.18
NADP+	HMDB0000217	5886	C00006	7.75	4.09	3.32
Ureidosuccinic acid	HMDB0000828	93072	C00438	7.53	5.11	3.97
Niacinamide	HMDB0001406	936	C00153	8.25	3.05	3.74
Norepinephrine	HMDB0000216	439260	C00547	0.54	0.74	1.33
Ornithine	HMDB0000214	6262	C00077	11.26	3.69	3.88
Phosphocreatine	HMDB0001511	587	C02305	14.01	9.17	5.58
Phosphoenolpyruvic acid	HMDB0000263	1005	C00074	28.03	3.97	3.85
Phosphorylcholine	HMDB0001565	8691	C00588	16.04	6.46	7.14
Pseudouridine	HMDB0000767	15047	C02067	7.12	3.12	2.07
Putrescine	HMDB0001414	1045	C02896	0.95	0.53	0.20
Pyruvic acid	HMDB0000243	1060	C00022	12.46	2.20	5.47
D-Ribulose 5-phosphate	HMDB0000618	439184	C00199	8.64	4.76	4.06
S-Adenosylmethionine	HMDB0001185	16757548	C00019	5.75	4.54	2.92
L-Serine	HMDB0000187	5951	C00716	12.90	4.78	3.57
Thymidine	HMDB0000273	5789	C00214	39.07	10.42	7.04
Uridine 5'-diphosphate (UDP)	HMDB0000295	6031	C00015	10.94	5.82	4.69
Uridine diphosphate glucose	HMDB0000286	53477679	C00029	9.60	4.82	3.14
Uridine diphosphate-N-acetylgalactosamine/ Uridine diphosphate-N-acetylglucosamine	HMDB0000304/ HMDB0000290	1167/ 9547196	C00203/ C00043	8.73	4.87	3.37
Uridine	HMDB0000296	6029	C00299	5.11	2.47	1.44
Uridine triphosphate (UTP)	HMDB0000285	6133	C00075	14.49	7.88	5.13

Supplemental Table 3

Pathway	Hits	Total	Expected	Raw p	-LOG(p)	Holm adjust	FDR	Impact
Alanine, aspartate and glutamate metabolism	16	28	1.8968	9.88E-13	27.643	8.30E-11	8.30E-11	0.76282
Synthesis and degradation of ketone bodies	1	5	0.33871	0.29616	1.2169	1	0.85783	0.6
Arginine biosynthesis	9	14	0.94839	3.24E-08	17.244	2.69E-06	1.36E-06	0.5736
Glycine, serine and threonine metabolism	8	33	2.2355	0.001137	6.7797	0.088659	0.01364	0.55868
D-Glutamine and D-glutamate metabolism	3	6	0.40645	0.005201	5.259	0.38485	0.036405	0.5
Cysteine and methionine metabolism	9	33	2.2355	0.000209	8.4735	0.016923	0.003721	0.47436
Nicotinate and nicotinamide metabolism	4	15	1.0161	0.015176	4.188	1	0.091058	0.42895
beta-Alanine metabolism	2	21	1.4226	0.42186	0.86309	1	1	0.39925
Pyrimidine metabolism	14	39	2.6419	6.64E-08	16.527	5.45E-06	1.86E-06	0.32533
Arginine and proline metabolism	8	38	2.5742	0.00301	5.806	0.22873	0.028089	0.28068
Citrate cycle (TCA cycle)	6	20	1.3548	0.001488	6.5105	0.11456	0.015622	0.27512
Pentose phosphate pathway	3	22	1.4903	0.18299	1.6984	1	0.61483	0.27226
Purine metabolism	12	65	4.4032	0.000959	6.9499	0.075743	0.013423	0.27171
Tyrosine metabolism	7	42	2.8452	0.02008	3.908	1	0.11245	0.2369
Pyruvate metabolism	3	22	1.4903	0.18299	1.6984	1	0.61483	0.20684
Glycolysis / Gluconeogenesis	3	26	1.7613	0.25592	1.3629	1	0.7962	0.20594
Glyoxylate and dicarboxylate metabolism	7	32	2.1677	0.004395	5.4272	0.32966	0.036405	0.20371
Amino sugar and nucleotide sugar metabolism	6	37	2.5065	0.034767	3.3591	1	0.17272	0.19203
Aminoacyl-tRNA biosynthesis	11	48	3.2516	0.000221	8.4152	0.017718	0.003721	0.16667
Starch and sucrose metabolism	3	18	1.2194	0.11742	2.142	1	0.44831	0.16651
Glutathione metabolism	6	28	1.8968	0.00926	4.6821	0.66671	0.059833	0.1406
Butanoate metabolism	3	15	1.0161	0.075729	2.5806	1	0.3348	0.11111
Glycerophospholipid metabolism	4	36	2.4387	0.22346	1.4985	1	0.72196	0.10926
Fructose and mannose metabolism	4	20	1.3548	0.041479	3.1826	1	0.19357	0.09559
Folate biosynthesis	1	27	1.829	0.85201	0.16016	1	1	0.09111
Pentose and glucuronate interconversions	2	18	1.2194	0.34738	1.0573	1	0.97267	0.07812
Glycerolipid metabolism	2	16	1.0839	0.29605	1.2172	1	0.85783	0.04361
Pantothenate and CoA biosynthesis	4	19	1.2871	0.034955	3.3537	1	0.17272	0.02143
Valine, leucine and isoleucine degradation	2	40	2.7097	0.76775	0.26429	1	1	0.01084
Primary bile acid biosynthesis	1	46	3.1161	0.96223	0.038502	1	1	0.00758
Galactose metabolism	1	27	1.829	0.85201	0.16016	1	1	0.00228
Nitrogen metabolism	3	6	0.40645	0.005201	5.259	0.38485	0.036405	0
Histidine metabolism	3	16	1.0839	0.088774	2.4217	1	0.37285	0
Ascorbate and aldarate metabolism	2	8	0.54194	0.097365	2.3293	1	0.38946	0
Neomycin, kanamycin and gentamicin biosynthesis	1	2	0.13548	0.13094	2.033	1	0.4782	0
Thiamine metabolism	1	7	0.47419	0.3886	0.9452	1	1	0
Valine, leucine and isoleucine biosynthesis	1	8	0.54194	0.43021	0.84349	1	1	0
Taurine and hypotaurine metabolism	1	8	0.54194	0.43021	0.84349	1	1	0
Propanoate metabolism	2	23	1.5581	0.46908	0.75698	1	1	0
Biotin metabolism	1	10	0.67742	0.50519	0.68283	1	1	0
Inositol phosphate metabolism	2	30	2.0323	0.61489	0.48631	1	1	0
Porphyrin and chlorophyll metabolism	2	30	2.0323	0.61489	0.48631	1	1	0
Mannose type O-glycan biosynthesis	1	17	1.1516	0.69847	0.35886	1	1	0
Selenocompound metabolism	1	20	1.3548	0.75632	0.27929	1	1	0
Sphingolipid metabolism	1	21	1.4226	0.77304	0.25742	1	1	0
Lysine degradation	1	25	1.6935	0.8293	0.18717	1	1	0

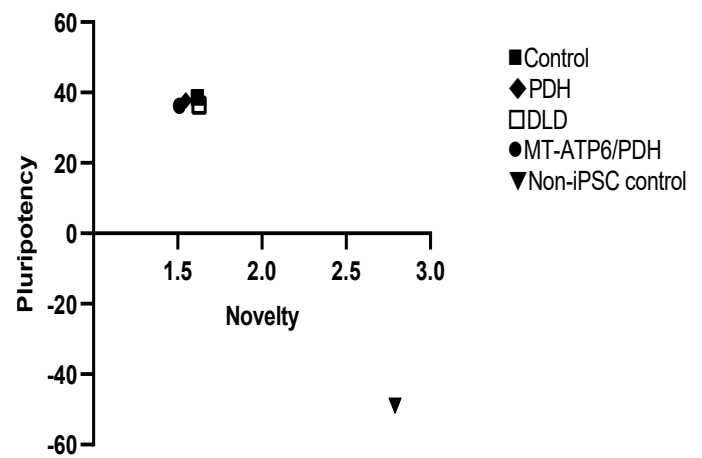
A



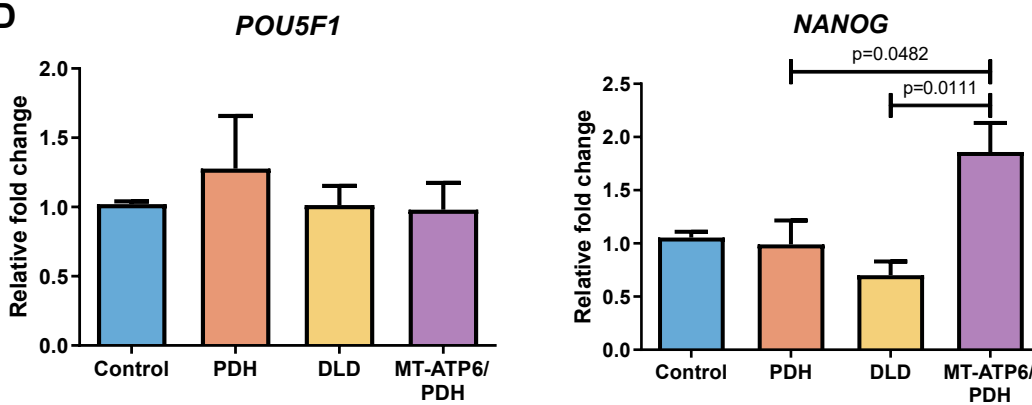
B

Sample	Pluritest Result	Pluricor	NovelCor
Control	Pass	38.62112	1.61562
PDH	Pass	37.78063	1.547368
DLD	Pass	36.43402	1.626429
MT-ATP6/ PDH	Pass	36.21865	1.509551
Non iPSC control	Fail	-48.9629	2.790166

C



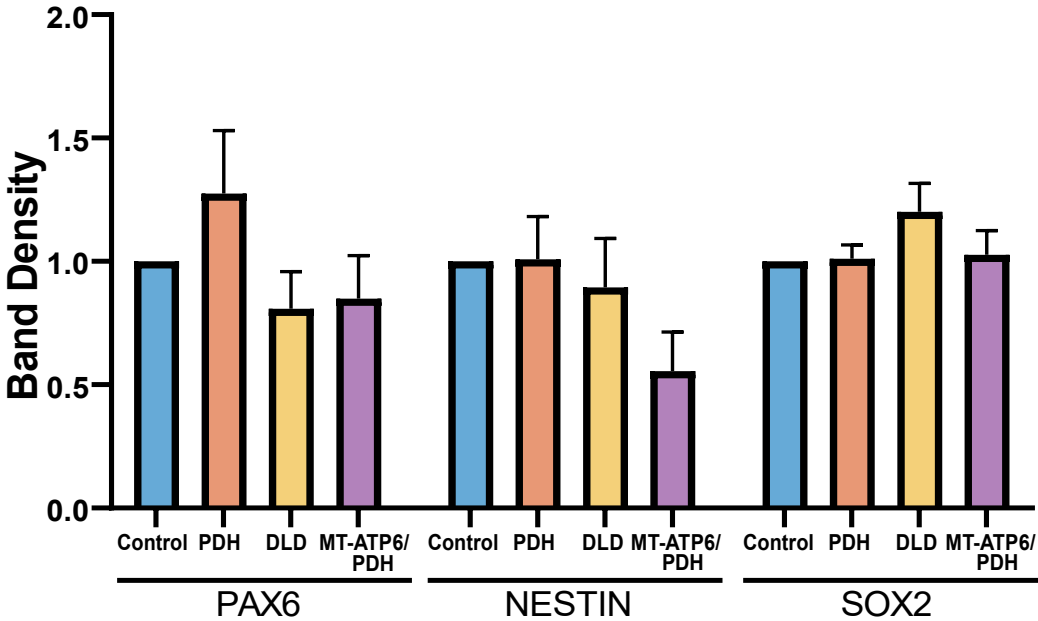
D



E

Sample	Karyotype
Control	Normal
MT-ATP6/PDH	Normal
PDH	Normal
DLD	Normal

A



B

