Supporting Information

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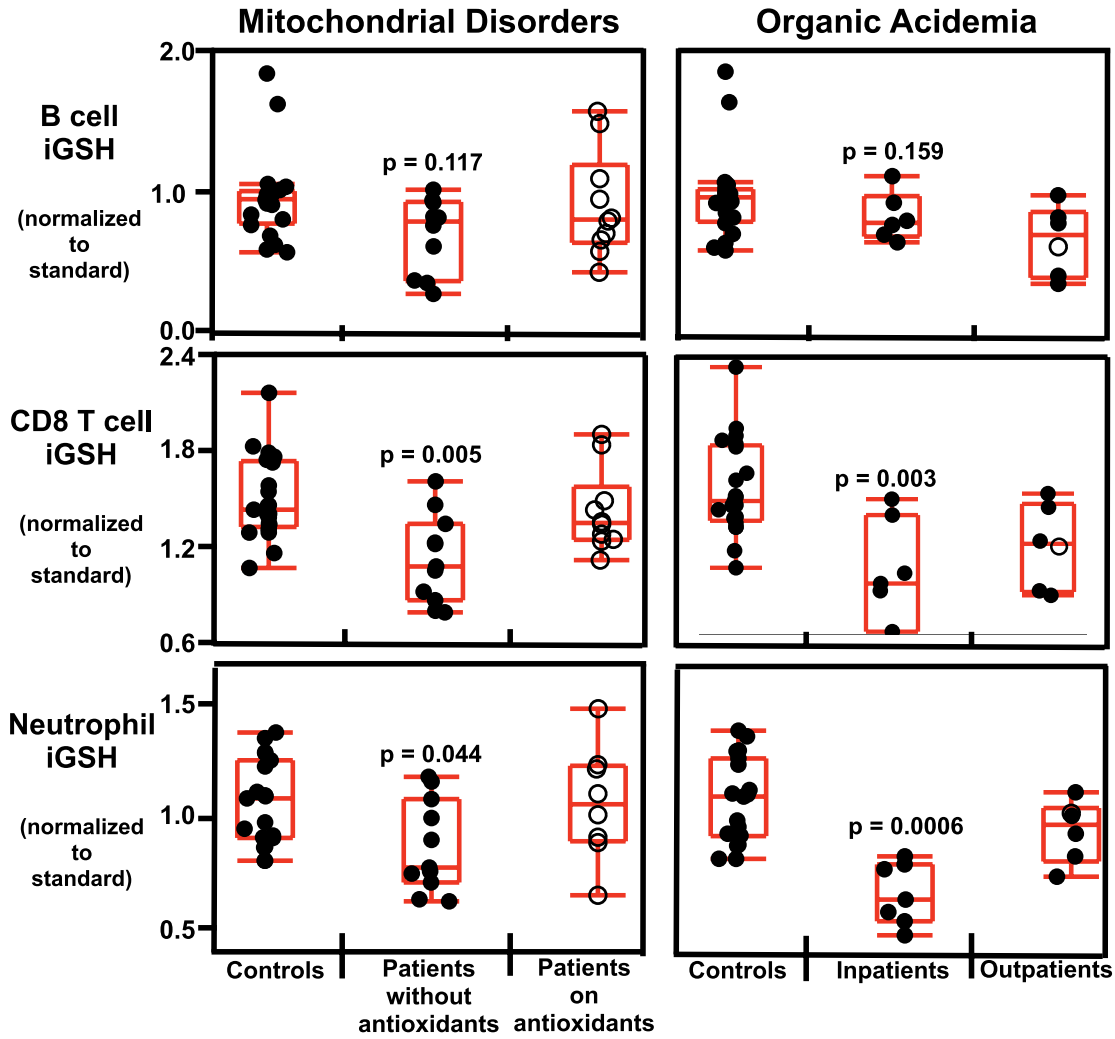

Fig. S1. iGSH levels are lower in patients with mitochondrial disorders and organic acidemias. Peripheral blood was obtained and immediately processed as described in *Material and Methods*. iGSH levels were measured by the MCB assay on whole blood and analyzed by Hi-D FACS within 3 h of staining. iGSH levels are normalized to iGSH levels of a standard PBMC preparation stained and analyzed at the same time as patient samples. *Top*: iGSH levels in CD8 T cells in mitochondrial disorders (*Left*) and organic acidemias (*Right*); *Middle*: iGSH levels in neutrophils in mitochondrial disorders (*Left*) and organic acidemias (*Right*); *Lower*: iGSH levels in B cells in mitochondrial disorders (*Left*) and organic acidemias (*Right*). Statistics was determined by Wilcoxon/Kruskal Wallis non-parametric test for ranked sums using JMP software. Each point represents a single sample. All controls are adults and are not age matched. Solid circles represent subjects not on antioxidants and open circles represent subjects on antioxidants. Control $n = 21$. For mitochondrial disorders, subjects not an antioxidant therapy ($n = 10$); and subjects on antioxidant therapy ($n = 11$). For organic acidemias, inpatient subjects ($n = 7$); and outpatient subjects ($n = 6$).

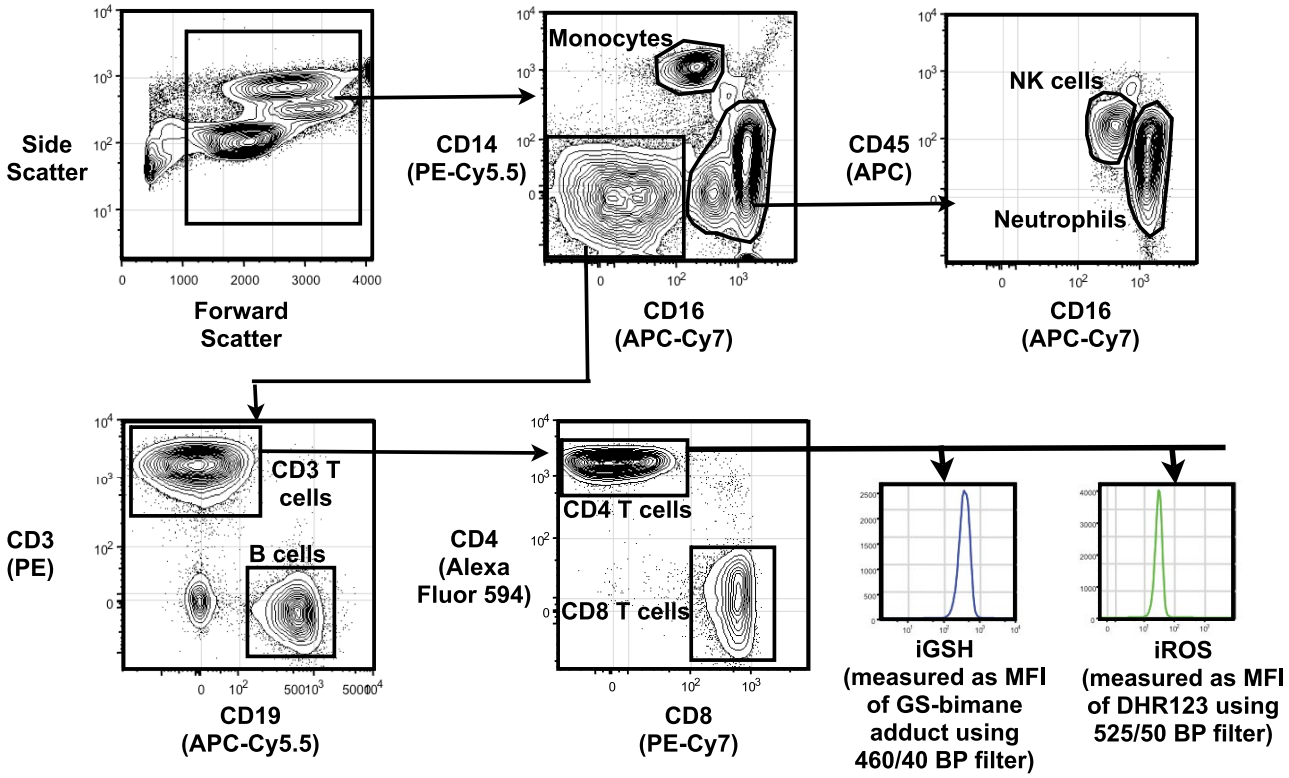

Fig. S2. Hi-D FACS Gating Scheme. Processed peripheral blood was stained with MCB and DHR123, washed and stained with a mixture of antibodies against pan-leukocyte subset markers as described in *Materials and Methods*. Pan markers used to identify leukocyte subsets are as follows: neutrophils (CD16), monocytes (CD14), B cells (CD19), CD4 T cells ($CD3^+CD4^+$), CD8 T cells ($CD3^+CD4^+$), and erythrocytes (CD235a, Glycophorin *A*). Contaminating erythrocytes were eliminated during data acquisition by gating on GS-bimane$^-$CD235a$^+$ cells. ''Fluorescence-minus-1'' controls (29) were included to determine the level of nonspecific staining and autofluorescence associated with subsets of cells in each fluorescence channel. BD CompBeads (anti-mouse Ig, κ beads) were used for single stain controls for fluorescence compensation. Eleven-color data were collected on either on a modified FACStar Plus (Becton Dickinson), with Moflow electronics (Cytomation) or BD FACSAria (BD Biosciences). Data were analyzed using Flowjo Software (Treestar) and plotted on a logicle scale. All data acquisition was done at $<$1,000 events/s to minimize acquisition of doublets of erythrocytes with other leukocytes.

Table S1. Details of subjects with mitochondrial disease or organic acidemias

Subject ID	Age at sample collection	Sex	Genotype (if known), Diagnosis, and Clinical Syndrome	Classification	Supplement Usage
S02A	3 y 8 m	F	PA	NA	None
S02B	5 y 8 m	F	PA	NA	None
S03A	16 y	F	$tRNA^{Leu}$3243A > G, MELAS	Definite	VBC, VB3, VC
S04A	19 y	F	TK2 deficiency	Definite	None
S04B	19 y 11 m	F	TK2 deficiency	Definite	VB2, VC, Q, Nic
S05A	7 y 8 m	F	mtDNA deletion	Definite	MV
S06A	4 y 7 m	F	CIII deficiency	Probable	VC, VK, Q
S07A	4 y 6 m	M	CII/III deficiency	Definite	Q
S08A	3 y 8 m	F	CI deficiency	Probable	None
S13A	18 y	F	$tRNA^{Leu}$3243A > G	Definite	None
S14A	36 y	F	$tRNA^{Leu}$3243A > G	Definite	None
S15A	20 y	M	$tRNA^{Leu}$3243A > G, MELAS	Definite	VBC, Q
S21A	7 y	F	CI deficiency	Definite	VB2, VC, S
S22A	12 y	M	CIV deficiency	Definite	None
S23A	2 y 5 m	M	CI deficiency	Probable	VB1, VB2, VC, VE, Q, LA
S25A	8 y 10 m	F	$tRNA^{Leu}$3243A > T, CI/IV deficiency, MCAD deficiency	Definite	Bi, Cr, Z
S26A	26 y	F	↑ fibroblast L/P ratio, ↓ CI/IV staining	Probable	VBC, VB1, VB2, VC, VE, NAC
S27A	1 m	M	MMA	NA	None
S29A	1 w	F	MMA	NA	VB12
S29B	2 w	F	MMA	NA	VB12
S30A	1 1/2w	M	MMA	NA	VB12
S30B	2 w	M	MMA	NA	VB12
S30C	10 m	M	MMA	NA	VB12, VC
S31A	9 y	F	CI/CIII deficiency	Probable	VA, VB1, VE, Q, F
S32A	9 y 8 m	M	CI/III deficiency	Probable	None
S33A	8 y 3 m	F	CIV deficiency	Definite	None
S37A	2 w	M	IVA	NA	None
S38A	2 w	M	MMA	NA	VB12
S39A	14 y	F	Hearing loss, cataracts, basal ganglia calcification	Probable	MV, Q
S42A	10 y 9 m	M	Leigh Syndrome	Probable	VBC, LP
S43A	1 w	M	MMA	NA	VB12
S44A	6 y 9 m	M	CI deficiency	Probable	None
S51A	1 y 1 m	F	IVA	NA	None
S52A	4 y 8 m	M	MMA	NA	VB12

Mitochondrial disease patients were classified according to the system of Bernier *et al.* into *definite*, *probable*, or *possible* disease. Major and minor diagnostic criteria encompassing clinical, histological, functional, and molecular data were used as described in Bernier FP, *et al.* [(2002) Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 59:1406–1411]. Four subjects with possible mitochondrial disease were excluded from the analyses (data not shown). The mean age ± standard deviation for the mitochondrial disease patients overall was 12.6 ± 8.3 years; mitochondrial disease patients taking antioxidants: 11.5 ± 8.0 years; and mitochondrial disease patients without antioxidants 13.4 ± 8.8 years. The mean age ± standard deviation for organic acidemia subjects overall was 1.3 ± 2.1 years. A second blood sample was available for 1 PA patient, 2 MMA patients, and 1 mitochondrial disease patient with *TK2* deficiency (noted as ″B″ samples in the *Identification* column). A third blood sample was available from a single MMA patient (noted as the ″C″ sample in the *Identification* column). Subject S25A, in addition to a mtDNA point mutation in the mitochondrial $tRNA^{Leu}$ gene (3243A>T), had MCAD deficiency confirmed by molecular analysis. Subject S42A has Leigh syndrome, but respiratory chain activities in muscle and skin fibroblasts were normal, as was mtDNA analysis for common mutations and deletions. Fibroblast enzymology for pyruvate dehydrogenase complex, pyruvate carboxylase, and phospholenolpyruvate carboxykinase was also normal in subject S42A. Because of the clinical variability associated with the 3243A>G mtDNA mutation, further details of these patients are also provided. Subjects S03A and S15A had a classic MELAS presentation with seizures and stroke-like episodes. Subject S13A is a maternal half-sister of subject S03A. She has a history of hydrocephalus requiring ventriculoperitoneal shunt placement, short stature, mild cognitive impairment, and cerebral palsy. Subject S14A is the mother of subjects S03A and S13A. She has sensorineural hearing loss and nephropathy requiring dialysis, but is cognitively normal. Only patients taking pharmacological doses of typical redox cyclers such as Vitamin C, Vitamin E, coenzyme Q_{10}, Lipoic acid, or N-acetylcysteine were included in the cohort of patients with antioxidant supplements. *Abbreviations:* C − complex; Cr–creatine; F–folate; IVA–isovaleric acidemia; LP–lipoic acid; m–month; MMA–methylmalonic acidemia; NA–not applicable; NAC–N-acetylcysteine; Nic–nicotinamide; PA–propionic acidemia; S–succinate; Q–coenzyme Q_{10}; VA–vitamin A; VBC–vitamin B complex; VB1–thiamine; VB2–riboflavin; VB3–niacin; VB12–vitamin B_{12}; VC–vitamin C; VE–vitamin E; VK–vitamin K; y–year; Z–zinc.