Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Schiff M, Veauville-Merllié A, Acquaviva-Bourdain C, et al. *SLC25A32* mutations and riboflavin-responsive exercise intolerance. N Engl J Med 2016;374:795-7. DOI: 10.1056/NEJMc1513610

SUPPLEMENTAL APPENDIX

Mitochondrial FAD transporter mutations and riboflavin-responsive exercise intolerance

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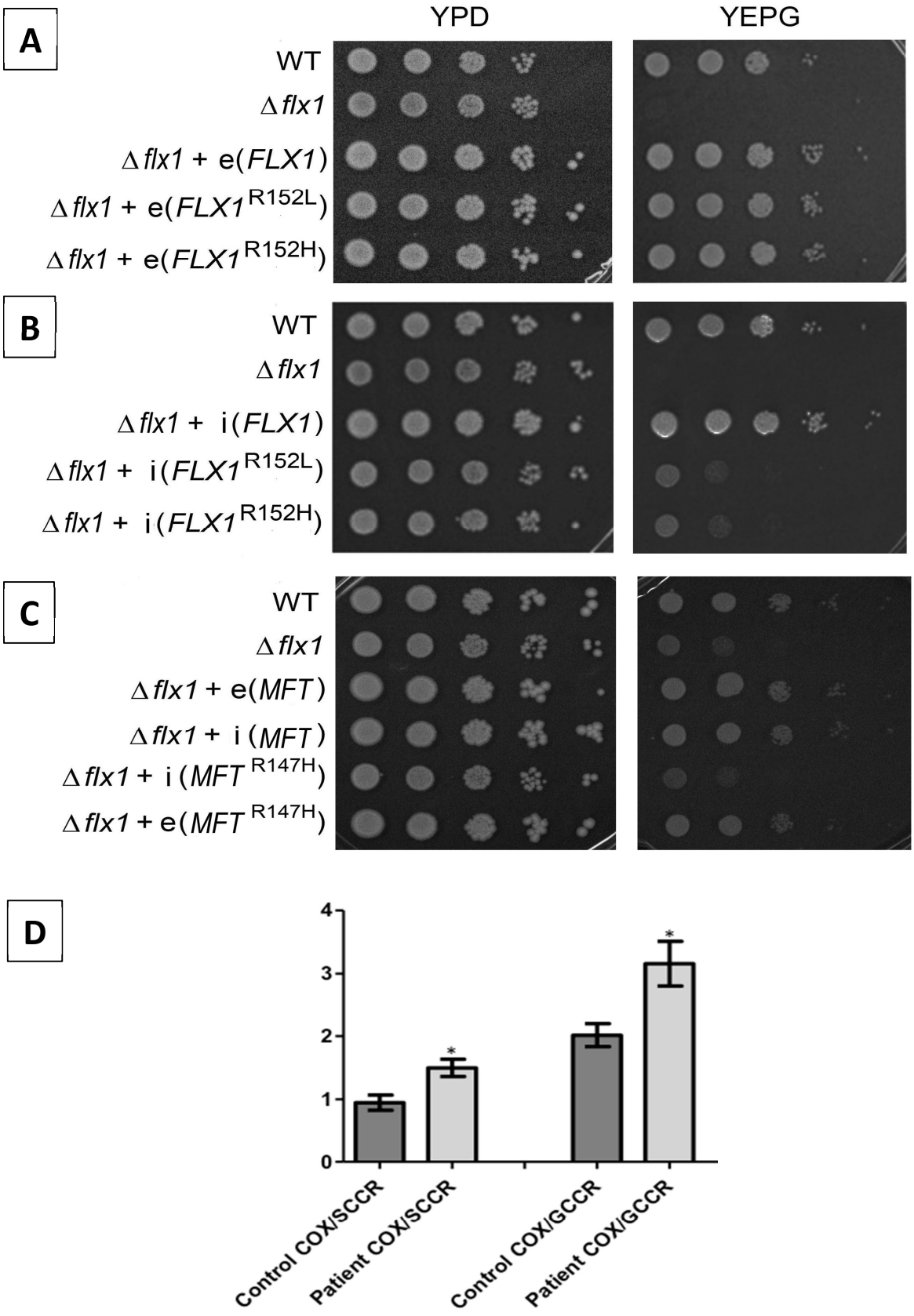

FIGURE S1: Evidence for the deleterious impact of the *SLC25A32* mutations on yeast growth and mitochondrial flavoproteins

LEGEND

A to C: Yeast studies. The wild type strain (WT), the flx1 null mutant (*Δflx1*) and the flx1 mutant transformed with either the wild type gene (Δflx1 + FLX1), the R152H or the R152L mutation (this other substitution, R152L was introduced to assess the effect of a non-charged hydrophobic residue) on a high copy plasmid (prefix e in B) or integrated into chromosomal DNA (prefix i in A) were grown in rich glucose to early stationary phase. The cultures were serially diluted and equal volumes spotted on rich glucose (YPD) and rich glycerol/ethanol (YEPG) plates. The flx1 mutant was also transformed with the *SLC25A32* cDNAs for the wild type and the R147H human FAD transporter (MFT), either in multicopy or integrative plasmids (panel C).

D: Activities of FAD-dependent mitochondrial enzymes succinate dehydrogenase (SDH) and glycerol-3-phosphate dehydrogenase (G3PDH) in *SLC25A32*-deficient fibroblasts. Compared to COX (cytochrome *c* oxidase) activity, the two FAD-dependent activities, SCCR (succinate cytochrome *c* reductase, representative of the SDH activity) and GCCR (G3P cytochrome *c* reductase, representative of G3PDH activity) activities appeared significantly decreased, resulting in a 50% higher ratio of COX/SCCR and COX/GCCR. Values are means ± SEM (n=4 for each cell type); *: $P < 0.05$.