SUPPLEMENTARY DATA

Absence of TXNIP in human gives lactic acidosis and low serum methionine linked to deficient respiration on pyruvate

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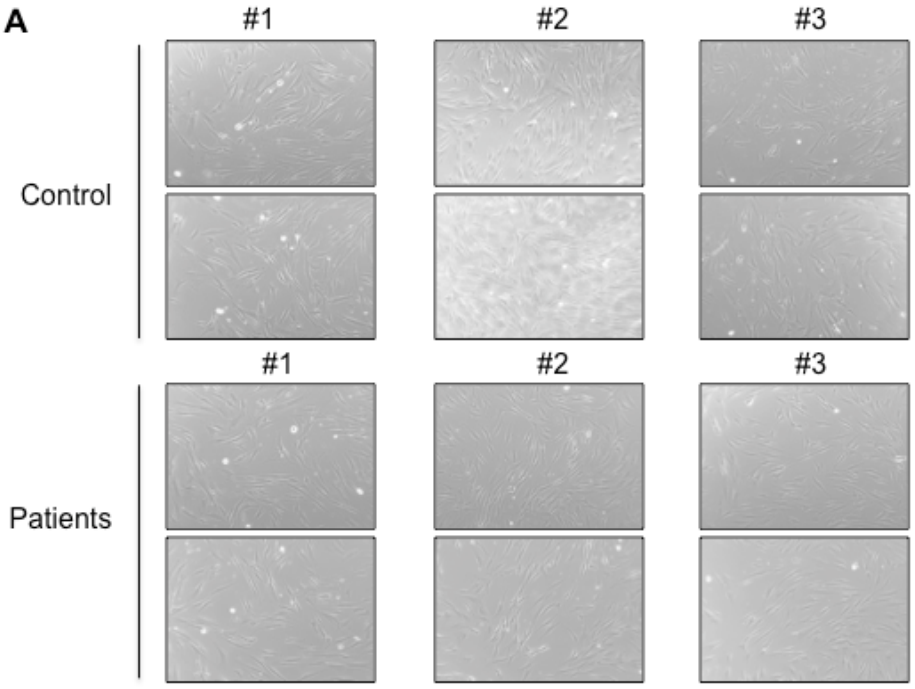
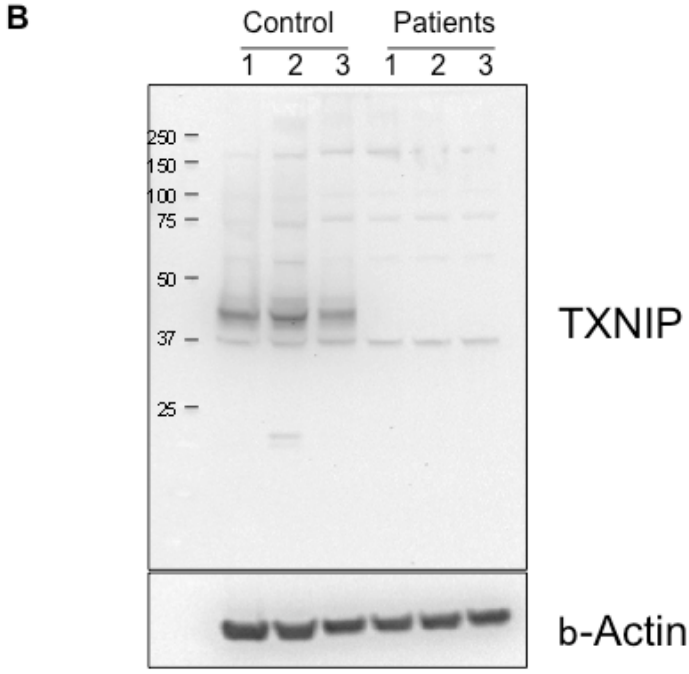
Supplementary Figure S1. Characterization of primary fibroblasts from patients bearing a homozygous point nonsense mutation in *TXNIP*.

A. Bright field images of three sets of primary fibroblasts from patients and three sets of healthy donors. B. Western-blot of TXNIP in primary fibroblasts isolated from healthy subjects compared with primary cells from patients with a point nonsense mutation in the *TXNIP* gene.

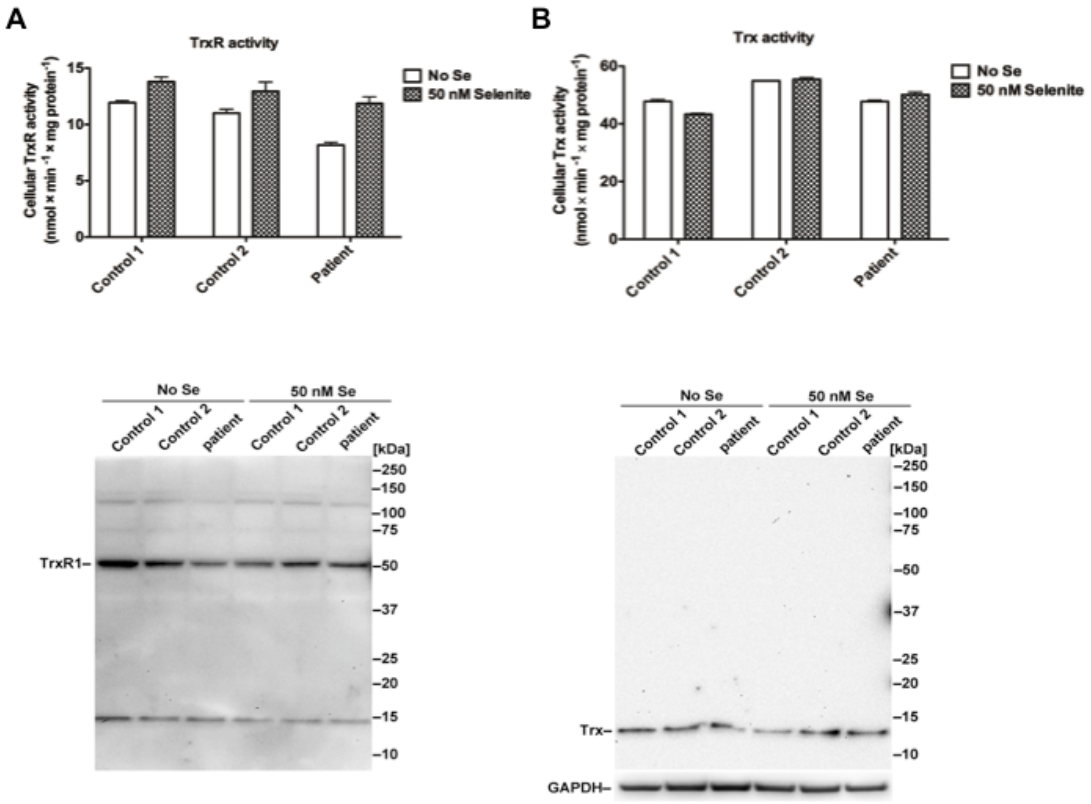




SUPPLEMENTARY DATA

Supplementary Figure S2. TXN and TXNRD activities and levels in primary fibroblasts from patients with a nonsense mutation in TXNIP.

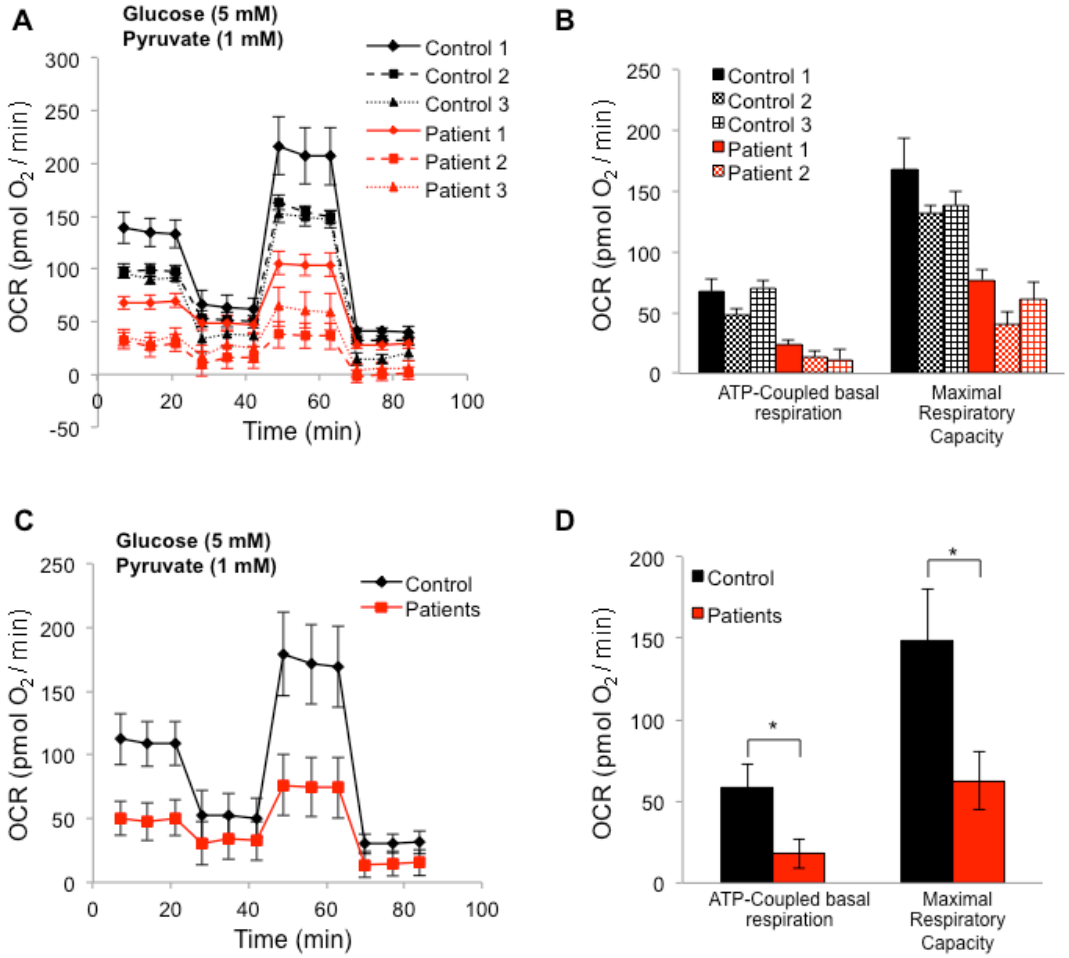
TXN (A) and TXNRD (B) activities were determined in whole cell lysates of fibroblasts from control subjects or a patient, as indicated. The activities were determined in cells grown in regular culture medium or supplemented with 50 nM sodium selenate (grey) to ensure synthesis of selenoproteins involved in redox homeostasis control, including TXNRD isoforms. Below the activity graphs are western blot analyses of TXNRD1 and TXN expression using the same lysates.



SUPPLEMENTARY DATA

Supplementary Figure S3. Mitochondrial respiration in the presence of glucose and pyruvate in patient-derived *TXNIP*-null fibroblasts

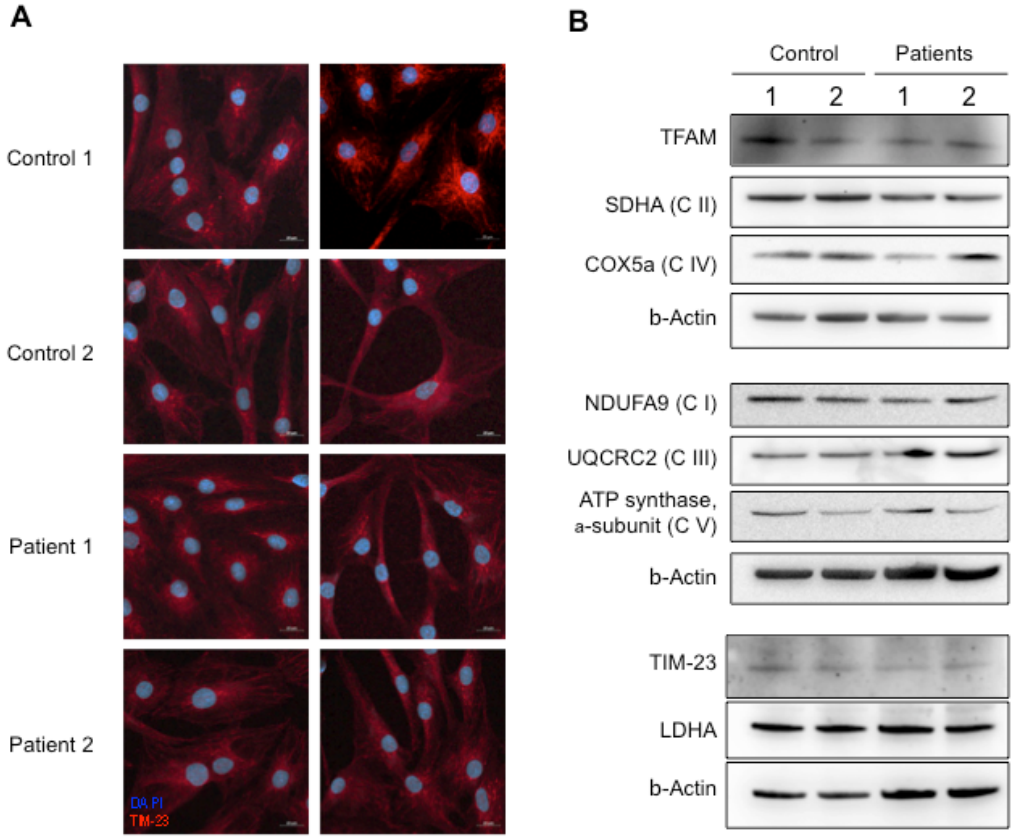
A. Monitoring of oxygen consumption rate (OCR) in real time in the presence of 5 mM glucose + 1 mM pyruvate using extracellular flux analysis in primary fibroblasts from healthy donors (black) and from patients (red). The sequential addition of oligomycin (O), FCCP (F) and antimycin (A) was used to determine ATP-coupled basal respiration and maximal respiratory capacity, respectively. B. Assessment of ATP-coupled basal respiration and maximal respiratory capacity in primary fibroblasts from control subjects (black) and patients (red), based on the results obtained in (A). C. & D. Average results of all controls and all patients as depicted in A and B. Data are represented as mean ± S.E.M. (n=3 independent experiments, 4-8 wells / set of cells / experiment).



SUPPLEMENTARY DATA

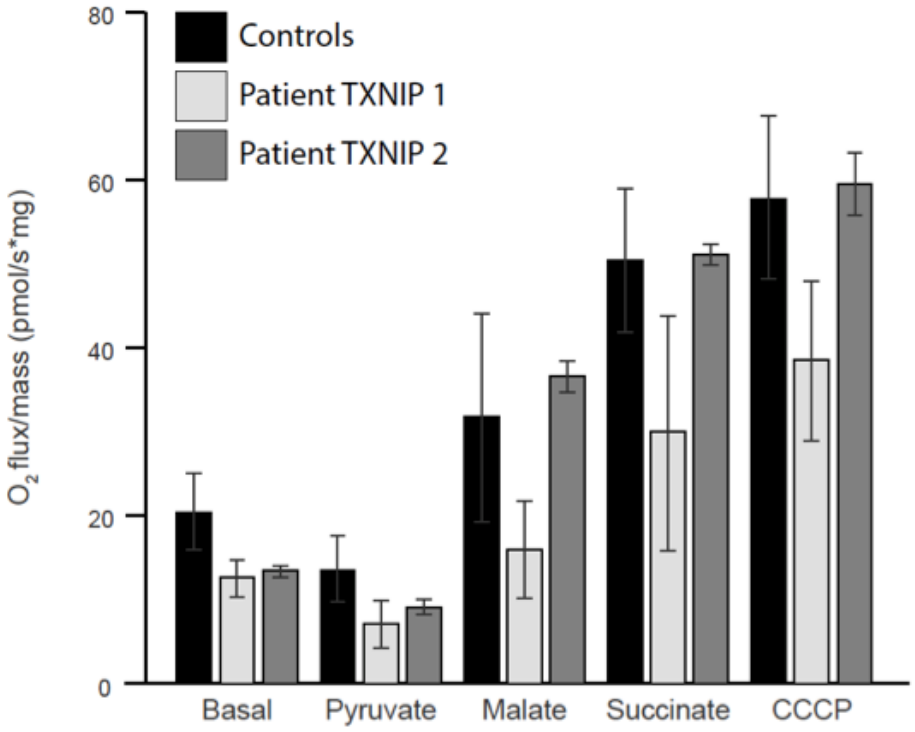
Supplementary Figure S4. Mitochondrial content in absence of TXNIP

A. Confocal images of myoblasts from control subjects and patients immunolabeled with an antibody agains the mitochondrial protein TIM23 (red) and counterstained in the nuclei with DAPI (blue) to visualize the mitochondrial network. Scale bar: 20 μm. B. Western-blot of a panel of several mitochondrial proteins in whole-cell extracts from myoblasts from control subjects and patients. β-Actin was used as loading control. Please note that the β-Actin loading control in the top set of western blots is the same as depicted in Figure 1A, since antibodies against TXNIP, TFAM, SDHA and COX5a were probed in the same membrane. There are uncropped version of the full probed membranes shown in Fig. S6.



SUPPLEMENTARY DATA

Supplementary Figure S5. Mitochondrial function in permeabilised myoblasts.
Mitochondrial respiration in response to pyruvate, malate or succinate was determined in permeabilised cells, as indicated in the figure (see also materials and methods). Both patient cell lines present with reduced basal and state 3 respiration in the presence of pyruvate, which is fully rescued in Patient 2, and partially rescued in Patient 1 in the presence of malate. Relative increase in complex II-driven respiration (succinate) and maximal respiration (CCCP) does not differ between patient and control lines.



SUPPLEMENTARY DATA

Supplementary Figure S6. Reconstituition of cells with TXNIP expression does not alter their respiration on malate.

Top graph: Monitoring of oxygen consumption rate (OCR) in real time in the presence of 5 mM malate using extracellular flux analysis in fibroblasts from healthy donors (black) or patients (red) transduced with an empty control vector (pBaBe) or with a vector overexpressing TXNIP (pBaBe-TXNIP). The sequential addition of oligomycin (O), FCCP (F) and antimycin (A) was used to determine ATP-coupled basal respiration and maximal respiratory capacity, respectively. Bottom bar graphs: Assessment of ATP-coupled basal respiration and maximal respiratory capacity in the cells from control subjects (black) or patients (red), based on the results obtained in the top graph.