

Mitochondrial hormesis links nutrient restriction to improved metabolism in fat cells

Supplementary Material

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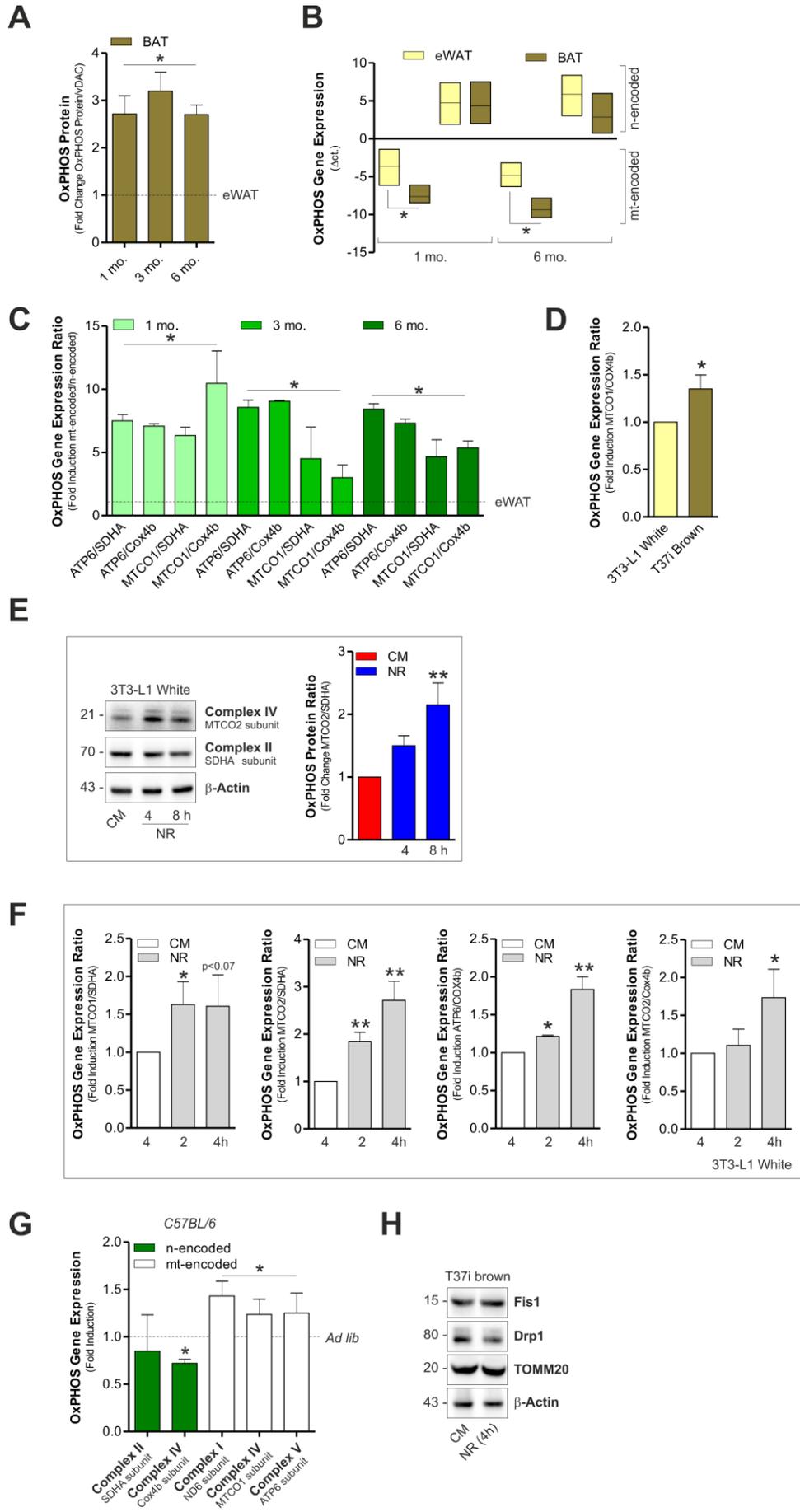


Figure S1. Mitonuclear OxPHOS proteins and genes expression

(A) Densitometric analysis of OxPHOS protein levels in adipose depots (see Figure 1A) (n=2 mice per group).

(B) OxPHOS gene expression, reported as Δ ct, analysed by RT-qPCR (see Figure 1C) (n=2 mice per group).

(C) OxPHOS gene expression, reported as fold induction *vs* eWAT, analysed by RT-qPCR (see Figure 1C) (n=2 mice per group).

(D) OxPHOS gene expression ratio evaluated by calculating the ratio between ^{mt}DNA-encoded and ⁿDNA-encoded OxPHOS genes after RT-qPCR.

(E) OxPHOS protein ratio (*right panel*) evaluated by calculating the ratio between ^{mt}DNA-encoded (MTCO2) and ⁿDNA-encoded (SDHA) proteins after Western blot (see Figure 1D).

(F) OxPHOS gene expression ratio evaluated in 3T3-L1 cells by calculating the ratio between ^{mt}DNA-encoded (MTCO1, ATP6 and MTCO2) and ⁿDNA-encoded (SDHA and Cox4b) OxPHOS genes after RT-qPCR (see Figure 1E).

(G) ^{mt}DNA-encoded and ⁿDNA-encoded OxPHOS mRNAs measured by RT-qPCR (n = 4 mice per group).

(H) Fis1, Drp1 and TOMM20 proteins evaluated by Western blot analysis.

Actin staining served as loading control. Bar graphs are expressed as mean \pm S.D. (n=4; *p<0.05; **p<0.01 *vs* CM).

NR: nutrient restriction; CM: complete medium.

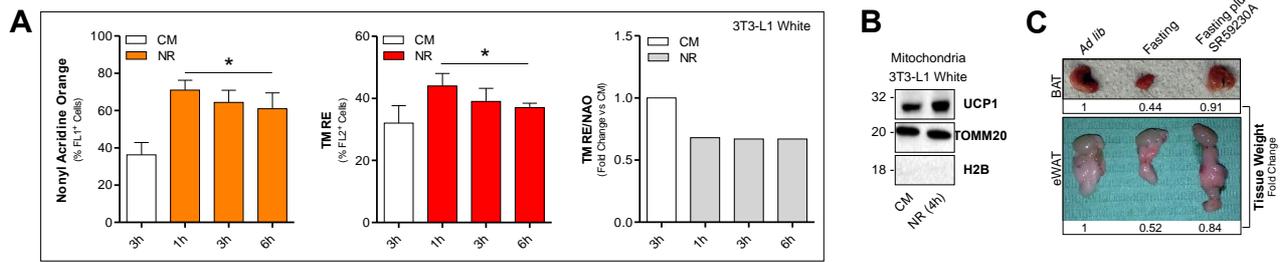


Figure S2. Brown fat-like change and adipose tissue remodelling upon NR

(A) Cytofluorimetric detection of mitochondrial amount and membrane potential after Nonyl Acridine Orange (NAO) and Tetramethyl Rhodamine Ester (TMRE) staining, respectively. Polarized mitochondria determined by calculating TMRE-to-NAO ratio (See Figure 2F, G and H).

(B) UCP1 protein level evaluated in mitochondrial extracts by Western blot analysis (see Figure 3B).

(C) eWAT and BAT explanted from *ad libitum* fed or 20 h fasted C57BL/6 mice treated or not with the selective β_3 -receptor antagonist SR59230A (n=4 mice per group).

TOMM20 and H2B staining served as loading controls or for assessing the purity of protein fraction.

Bar graphs are expressed as mean \pm S.D. (n=3; *p<0.05 vs CM).

NR: nutrient restriction; CM: complete medium.

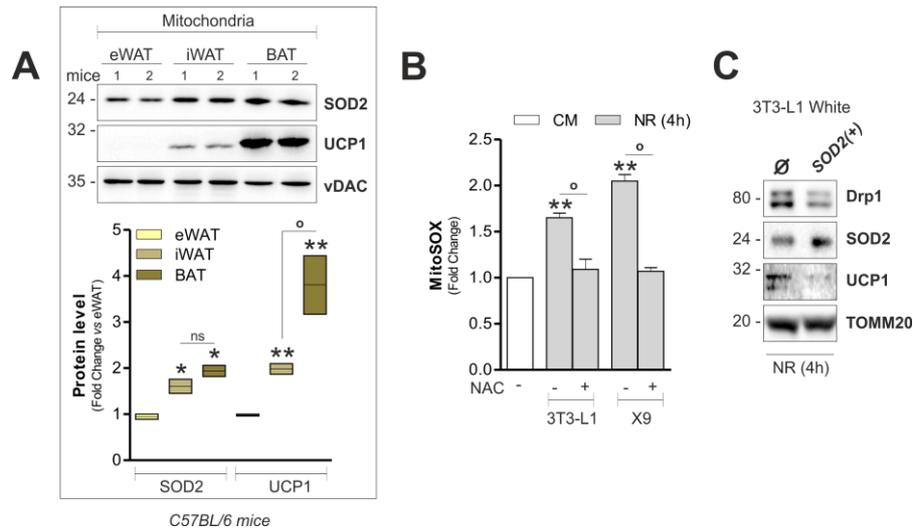


Figure S3. Mitochondrial antioxidant proteins and redox state of fat cells.

(A) Western blot (upper panel) and relative densitometric analysis (bottom panel) of SOD2 and UCP1 in crude mitochondria (n = 4 mice per group).

(B) Cytofluorimetric detection of mitochondrial ROS after staining with MitoSOX in cells treated or not with NAC.

(C) Western blot analysis of Drp1, SOD2 and UCP1 in cells transfected with a SOD2 cDNA [SOD2(+)] or with an empty vector (∅).

TOMM20 and vDAC staining served as loading controls. Bar graphs are expressed as mean \pm S.D. (n=4; *p<0.05; **p<0.01 vs CM; °p<0.05).

NR: nutrient restriction; CM: complete medium.

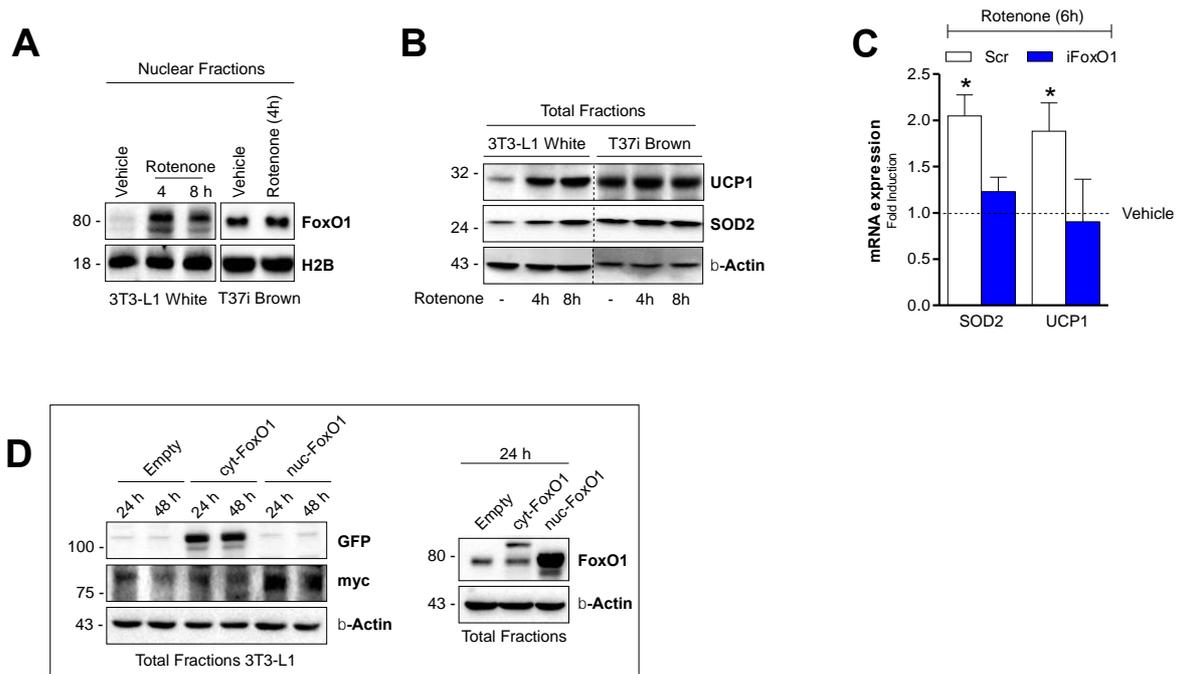


Figure S4. NR-like responses upon mitochondrial complex I inhibition

(A) Western blot analysis of FoxO1 in nuclear fractions of white and brown adipocytes treated with rotenone.

(B) Western blot analysis of UCP1 and SOD2 in total fractions of white and brown adipocytes treated with rotenone.

(C) SOD2 and UCP1 mRNA expression analysed by RT-qPCR in 3T3-L1 white adipocytes treated with rotenone.

(D) Western blot analysis of GFP-tagged, myc-tagged FoxO1 (*left panel*) and untagged FoxO1 (*right panel*) in 3T3-L1 cells transfected with nuc-FoxO1, cyt-FoxO1 cDNAs or empty vector.

Actin and H2B served as loading controls. Bar graphs are expressed as mean \pm S.D. (n=4; *p<0.05 vs vehicle).