Supplemental information

Tetracycline-induced mitochondrial dysfunction is a confounder for research and poses an environmental hazard

Norman Moullan[1,4], Laurent Mouchiroud[1,4], Xu Wang[1], Dongryeol Ryu[1], Evan G. Williams[1], Adrienne Mottis[1], Virginija Jovaisaite[1], Michael V. Frochaux[2], Pedro M. Quiros[1], Bart Deplancke[2], Riekelt H. Houtkooper[3]*, Johan Auwerx[1]*

Supplementary data

Table S1. Mouse primers list. Related to Figure 7.

Gene name	*Sequence*
36B4-fw	AGATTCGGGATATGCTGTTGG
36B4-rv	AAAGCCTGGAAGAAGGAGGTC
B2m-fw	TTCTGGTGCTTGTCTCACTG
B2m-rv	TATGTTCGGCTTCCCATTCT
Hsp60-fw	GCTGTAGCTGTTACAATGGGG
Hsp60-rv	TGACTTTGCAACAGTGACCC
Tfam-fw	AAGTGTTTTTCCAGCATGGG
Tfam-rv	GGCTGCAATTTTCCTAACCA
Opa1-fw	TGACAAGCATTACAGGAAGGTGTCAGA
Opa1-rv	TCATCTCGCCGGACCCTCTCG
Mfn2-fw	ACGTCAAAGGGTACCTGTCCA
Mfn2-rv	CAATCCCAGATGGCAGAACTT
Fis1-fw	ATTTGAATATGCCTGGTGCC
Fis1-rv	GCTGTTCCTCTTTGCTCCCT
Mrpl28-fw	CACTACCCTCCAGAGTCCCA
Mrpl28-rv	CACCTTCTTCACCCTCTTGG

Table S2. Plant primers list. Related to Figure 5.

Gene name	*Sequence*
AOX1A-fw	GCCTACCGATTTGTTCTTCCAG
AOX1A -rv	CAGTGTAGTAACATTCCTCCAACCA
CPN10X-fw	CGGGAGAGTTATAGCAGTTGGTC
CPN10X -rv	CGCCAAATTCAGGCAAAAGA
HSC70-5-fw	CAGCTCTCCTCCGCTCTATTC
HSC70-5-rv	CGTTTCCAGCAGGCTTCG
BCS1-fw	GTTCCCACCTCCCACCAAT
BCS1-rv	TAGACGGGATTCGGCGGA
UBQ10-fw	CACACTCCACTTGGTCTTGCGT
UBQ10-rv	TGGTCTTTCCGGTGAGAGTCTTCA

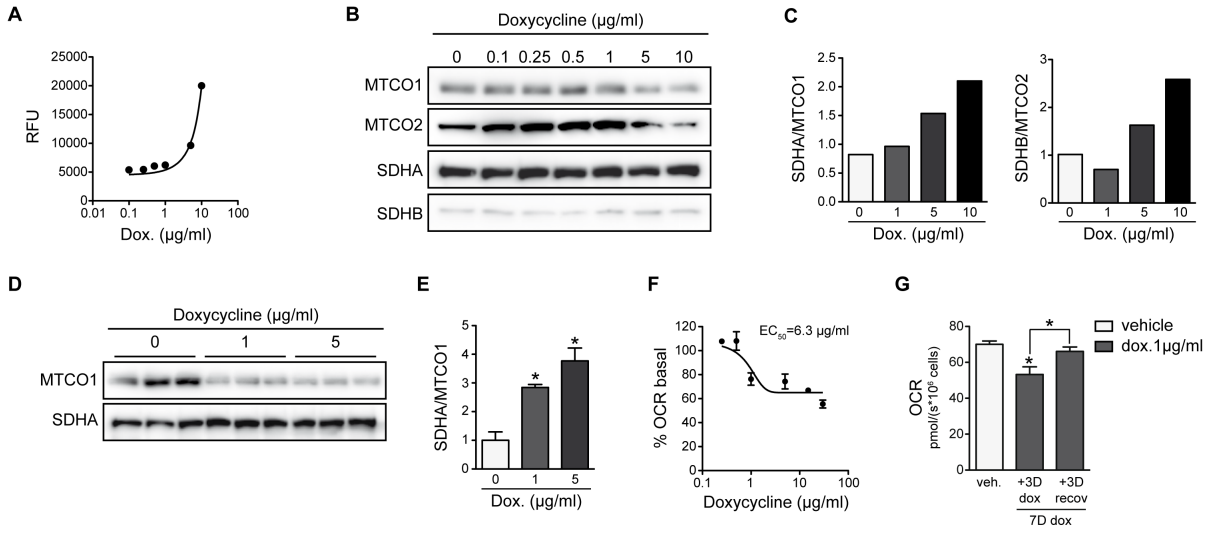

Figure S1. Doxycycline effects in cell lines. Related to Figure 1. (A) Doxycycline induces Tet-controlled luciferase expression in the A549 cell line at the concentration of 5-10 µg/ml. (B) Doxycycline dose-dependently causes mitonuclear protein imbalance as evidenced by the altered ratio between mtDNA-encoded (MTCO1 and MTCO2) and nDNA-encoded (SDHA and SDHB) OXPHOS subunits. (C) Quantification of the mitonuclear protein imbalance observed in panel B. (D) Doxycycline treatment in A549 "Tet-on" cells at 1 and 5 µg/ml for 48 hours causes mitonuclear protein imbalance as evidenced by the altered ratio between mtDNA-encoded (MTCO1) and nDNA-encoded (SDHA) OXPHOS subunits. (E) Quantification of the mitonuclear protein imbalance observed in panel D. (F) EC_{50} determination of doxycycline on mitochondrial respiration in HeLa cells. Oxygen consumption rate (OCR) without treatment is used as 100%. (G) Ten days doxycycline treatment reduces OCR in Hela cells. Conversely, when cells were allowed to recover for the last 3 days in regular medium (+3D recov), this leads to restoration of oxygen consumption.

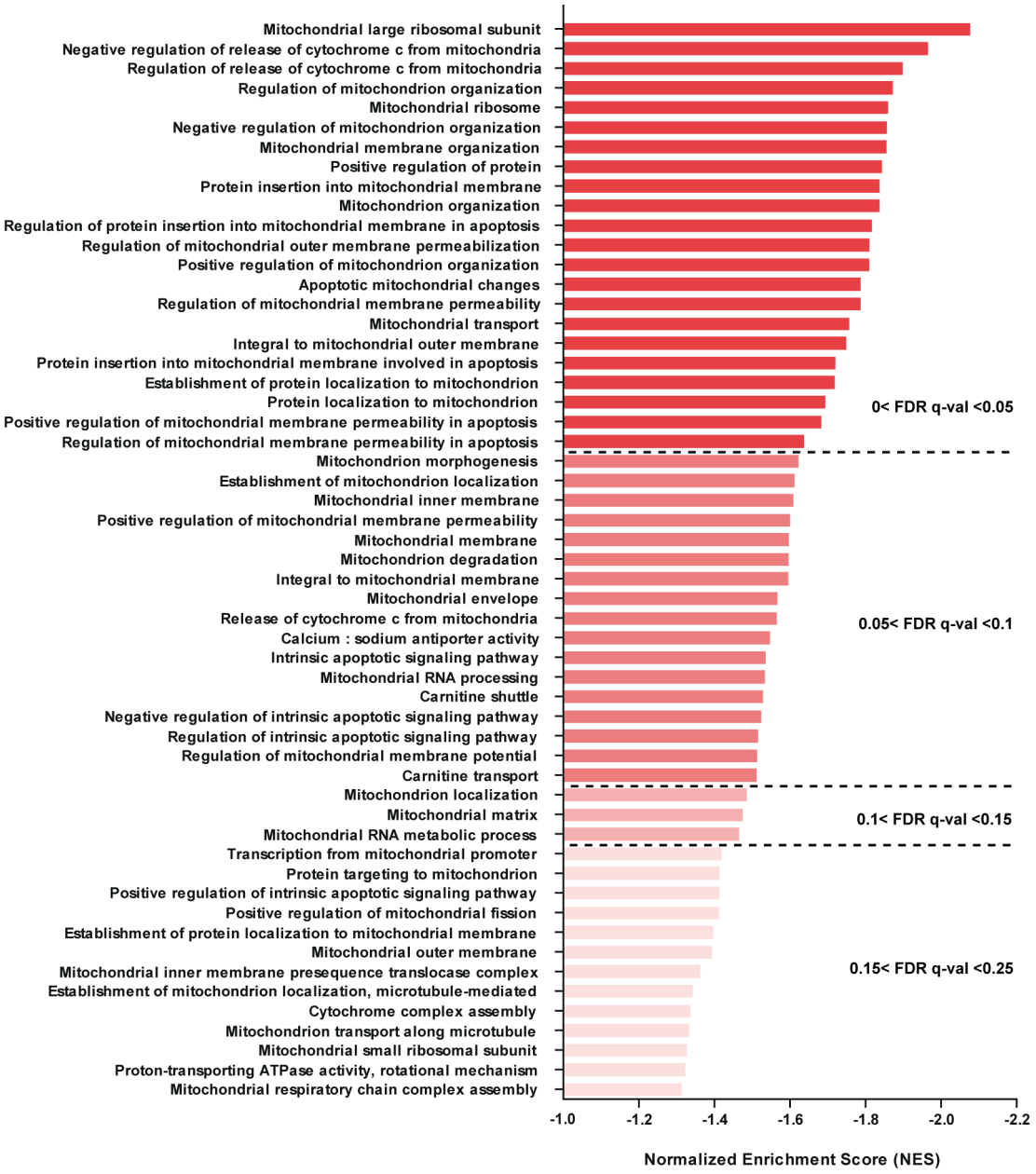

Figure S2. Geneset enrichment of doxycycline-treated RT112 cells. Related to Figure 3. Mitochondrial genesets modulated by doxycycline treatment in human bladder cancer cell line RT112. The normalized enrichment scores (NES) were used to compare analysis results across gene sets. Significant enriched gene sets after doxycycline treatment were indicated by a false discovery rate (FDR) of less than 25%, which is adjusted for gene set size and multiple hypotheses testing.