**Supplementary materials**

**Supplementary Figures legends**

**Supplementary Figure S1. Chemical structure of mitochondria-targeted doxorubicin.**

**Supplementary Figure S2. Expression of Pgp and MRP1 in doxorubicin-sensitive and doxorubicin-resistant osteosarcoma cells**

Human Dox-sensitive U-2OS cells and Dox-resistant variants (U-2OS/DX30, U-2OS/DX100, U-2OS/DX580), human Dox-sensitive Saos-2 cells and Dox-resistant variants (Saos-2/DX30, Saos-2/DX100, Saos-2/DX580) were analyzed for the surface expression of ABCB1/Pgp and ABCC1/MRP1 by flow cytometry in duplicate. The figure is representative of 1 out of 3 experiments.

**Supplementary Figure S3. Intracellular accumulation and cytotoxicity of mitochondria-targeted doxorubicin in drug-sensitive Saos-2 cells and in their resistant variants,** **and in K7M2 cells**

**A.** Dox-sensitive Saos-2 cells and Dox-resistant variants (Saos-2/DX30, Saos-2/DX100, Saos-2/DX580) were incubated with 5 μmol/L Dox or mtDox for 24 h. The amount of Dox in whole cell lysates was measured spectrofluorimetrically in duplicate. Data are presented as means + SD (n= 3). Versus Saos-2 cells: \* p < 0.01; mtDox versus Dox: ° p < 0.05. **B.** Cells were grown for 72 h in fresh medium (Ctrl), in medium containing 5 μmol/L Dox or mtDox, then stained with neutral red solution in quadruplicate. The results were expressed as a percentage of viable cells versus untreated cells. Data are presented as means + SD (n= 3). Versus respective Ctrl: \* p < 0.005; mtDox versus Dox: ° p < 0.005. C**.** Murine osteosarcoma K7M2 cells were lysed and subjected to the Western blot analysis of ABCB1/Pgp. The resistant U-2OS/DX580 and Saos-2/DX580 variants were included as control of Pgp-overexpressing cells. The β-tubulin expression was used as a control of equal protein loading. The figure is representative of 1 out of 2 experiments. **D.** K7M2 cells were incubated for 24 h with 5 μmol/L Dox or mtDox. The amount of Dox was measured spectrofluorimetrically in duplicate. Data are presented as means + SD (n= 3). mtDox versus Dox: \* p < 0.01. **E**. Cells were grown for 72 h in fresh medium (Ctrl), in medium containing 5 μmol/L Dox or mtDox, then stained with neutral red solution in quadruplicate. The results were expressed as a percentage of viable cells versus untreated cells. Data are presented as means + SD (n= 3). Versus Ctrl: \* p < 0.002; mtDox versus Dox: ° p < 0.002.

**Supplementary Figure S4. Intracellular accumulation and cytotoxicity of mitochondria-targeted doxorubicin in non-transformed osteoblasts and cardiomyocytes**

Human non-transformed osteoblasts and rat neonatal H9c2 cardiomyocytes were cultured for 24 h (panels **A, B, D, E**) or 72 h (panels **C**, **F**) in fresh medium (Ctrl), in medium containing 5 μmol/L Dox or mtDox. **A** and **D.** The amount of Dox in whole cell lysates was measured spectrofluorimetrically in duplicate. Data are presented as means + SD (n= 3). mtDox versus Dox: \* p < 0.02. **B** and **E.** The release of LDH in the extracellular medium was measured spectrophotometrically in duplicate. Data are presented as means + SD (n= 3). Versus respective Ctrl: \* p < 0.005; mtDox versus Dox: ° p < 0.01. **C** and **F.** Cells were stained with neutral red solution in quadruplicate. The results were expressed as a percentage of viable cells versus untreated cells. Data are presented as means + SD (n= 4). Versus respective Ctrl: \* p < 0.01; mtDox versus Dox: ° p < 0.02.

**Supplementary Figure S5. Immunohistochemical analysis of drug-resistant osteosarcoma treated with mitochondria-targeted doxorubicin**

Six week-old female NOD SCID BALB/c mice were inoculated s.c. with 1 x 107 U-2OS cells. When the tumor reached the volume of 50 mm3 (day 7), the animals were randomized and treated on days 7, 14, 21, 28 as follows: 1) Ctrl group, treated with 0.1 mL saline solution i.v.; 2) Dox group, treated with 5 mg/kg Dox i.v.; 3) mtDox group, treated with 5 mg/kg mitochondria-targeted Dox i.v. Animals were sacrificed on day 35. **A.**Sections of tumors from each group of animals were stained with hematoxylin and eosin (HE) or immunostained for the proliferation marker Ki67, the apoptotic marker cleaved caspase 3, the immunogenic death marker calreticulin (CRT), the DC marker CD11c. Nuclei were counterstained with hematoxylin. Bar = 10 µm. The photographs are representative of sections from 5 tumors/group. **B. Q**uantification of immunohistochemical images, performed on sections from 5 animals of each group (105-83 nuclei/field). The percentage of proliferating cells was determined by the ratio Ki67-positive nuclei/total number (hematoxylin-positive) of nuclei using ImageJ software (<http://imagej.nih.gov/ij/>). The ctrl group percentage was considered 100%. The percentage of caspase 3-positive and CRT-positive cells was determined by Photoshop program. The number of CD11c-positive cells/field was calculated by ImageJ software. Data are presented as means + SD. Versus Ctrl group: \* p *<* 0.02; mtDox group versus Dox group: ° p *<* 0.01.

**Supplementary Figure S6. Dose-response effects of mitochondria-targeted doxorubicin on drug-resistant osteosarcoma *in vivo***

Six week-old female BALB/c mice were inoculated s.c. with 1 x 106 K7M2 cells. When the tumor reached the volume of 50 mm3 (day 7), the animals (10 mice/group) were randomized and treated on days 7, 14, 21, 28 with 0.1 mL saline solution i.v. (Ctrl group) or with 0.5, 1, 2.5, 5 mg/kg mtDox. Tumor growth monitored by caliper measurements.Arrows represent saline or mtDox injections.Data are presented as means ± SD. 1, 2.5, 5 mg/kg mtDox group versus Ctrl group: \* p < 0.01.

**Supplementary Figure S7. Effects of mitochondria-targeted doxorubicin on drug-sensitive osteosarcoma *in vivo***

Six week-old female NOD SCID BALB/c mice were inoculated s.c. with 1 x 107 U-2OS cells. When the tumor reached the volume of 50 mm3 (day 7), the animals were randomized and treated on days 7, 14, 21, 28 as it follows: 1) Ctrl group, treated with 0.1 mL saline solution i.v.; 2) Dox group, treated with 5 mg/kg Dox i.v.; 3) mtDox group, treated with 5 mg/kg mitochondria-targeted Dox i.v. Arrows represent saline or drug injections.Data are presented as means ± SD. Dox/mtDox group versus Ctrl group: \* p < 0.001.

**Supplementary Tables**

**Supplementary Table S1. IC50 (μmol/L) of doxorubicin and mitochondria-targeted doxorubicin**

|  |  |  |
| --- | --- | --- |
| **Cell line** | **Dox** | **mtDox** |
| U-2OS | 3.91 + 0.43 | 1.09 + 0.12 ° |
| U-2OS/DX30 | 25.62 + 1.16 \* | 2.35 + 0.28 ° |
| U-2OS/DX100 | 79.67 + 6.17 \* | 4.68 + 0.99 ° |
| U-2OS/DX580 | 124.18 + 11.07 \* | 10.71 + 0.87 ° |
| Saos-2 | 4.55 + 0.71 | 2.31 + 0.23 ° |
| Saos-2/DX30 | 18.78 + 2.37 \* | 3.81 + 0.42 ° |
| Saos-2/DX100 | 53.29 + 8.93 \* | 6.17 + 0.52 ° |
| Saos-2/DX580 | 109.82 + 21.09 \* | 9.71 + 0.87 ° |
| K7M2 | 75.71 + 4.77 | 8.54 + 0.41 ° |
| Primary osteoblasts | 4.93 + 0.29 | 27.52 + 4.11 ° |
| H9c2 | 0.72 + 0.08 | 12.39 + 5.27 ° |

Cells were incubated for 72 h with increasing concentrations (1 nmol/L-1 mmol/L) of Dox or mtDox, then stained in quadruplicate with neutral red. Data are presented as means + SD (n = 3). Dox-resistant variants versus their parental cells: \* p < 0.001; mtDox versus Dox: °p < 0.001.

**Supplementary Table S2. Expression of mitochondria-related genes in U-2OS cells and resistant variants**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Fold change**  **DX30**  **versus**  **U-2OS** | **p value** | **Fold change**  **DX100**  **versus**  **U-2OS** | **p value** | **Fold change**  **DX580**  **versus**  **U-2OS** | **p value** | **Biological function** |
| *AIFM2* | 1.66 | 0.02 | 1.42 | 0.005 | 1.04 | Ns | Apoptosis induction |
| *ATP12A* | **0.34** | 0.005 | **0.45** | 0.02 | 1.70 | Ns | H+/ATP exchange |
| *ATP4A* | **0.33** | 0.005 | **0.45** | 0.02 | 1.70 | Ns | H+/ATP exchange |
| *ATP4B* | **0.34** | 0.005 | **0.45** | 0.02 | 1.67 | Ns | H+/ATP exchange |
| *ATP50* | 1.29 | Ns | **2.40** | 0.001 | **3.71** | 0.001 | ATP synthase subunit |
| *ATP5A1* | **2.24** | 0.002 | **5.82** | 0.001 | **6.38** | 0.001 | ATP synthase subunit |
| *ATP5B* | **2.09** | 0.005 | **2.96** | 0.001 | **3.93** | 0.001 | ATP synthase subunit |
| *ATP5C1* | 1.38 | 0.05 | **2.91** | 0.001 | **2.26** | 0.001 | ATP synthase subunit |
| *ATP5F1* | 1.70 | 0.01 | **4.62** | 0.001 | **4.91** | 0.001 | ATP synthase subunit |
| *ATP5G1* | **3.40** | 0.001 | **2.53** | 0.001 | **2.42** | 0.001 | ATP synthase subunit |
| *ATP5G2* | 1.72 | 0.01 | **2.24** | 0.001 | **2.42** | 0.001 | ATP synthase subunit |
| *ATP5G3* | 1.80 | 0.01 | **2.29** | 0.001 | **5.41** | 0.001 | ATP synthase subunit |
| *ATP5I* | 1.82 | 0.005 | **3.4** | 0.001 | **3.93** | 0.001 | ATP synthase subunit |
| *ATP5J* | 1.59 | 0.001 | **5.92** | 0.001 | **6.38** | 0.001 | ATP synthase subunit |
| *ATP5J2* | **3.17** | 0.001 | **2.06** | 0.001 | **3.93** | 0.001 | ATP synthase subunit |
| *ATP5L* | **4.41** | 0.001 | **4.49** | 0.001 | **10.38** | 0.001 | ATP synthase subunit |
| *BAK1* | 1.55 | 0.02 | 1.10 | Ns | 1.42 | Ns | Apoptosis induction |
| *BBC3* | 1.44 | Ns | 1.52 | Ns | 0.88 | Ns | Apoptosis induction |
| *BCL2* | 1.35 | 0.05 | 0.88 | Ns | 1.13 | Ns | Apoptosis inhibition |
| *BCL2L1* | 1.78 | 0.001 | 0.73 | Ns | 1.17 | Ns | Apoptosis induction |
| *BCS1L* | 1.82 | 0.005 | 1.82 | 0.001 | 1.39 | 0.01 | Ubiquinol-cytochrome c reductase assembly |
| *BID* | 1.55 | 0.02 | 1.19 | Ns | 1.59 | Ns | Apoptosis induction |
| *BNIP3* | 1.18 | Ns | 1.99 | Ns | 1.90 | Ns | Apoptosis induction |
| *COX10* | 1.66 | 0.01 | 1.41 | Ns | **4.02** | 0.05 | Cytochrome c oxidase assembly |
| *COX18* | 1.78 | 0.02 | 1.40 | Ns | **2.05** | 0.02 | Cytochrome c oxidase assembly |
| *COX412* | 1.70 | Ns | **0.45** | 0.02 | **0.34** | 0.01 | Cytochrome c oxidase subunit |
| *COX4I1* | 0.79 | Ns | **2.40** | 0.001 | **2.39** | 0.01 | Cytochrome c oxidase assembly |
| *COX5A* | 1.95 | 0.005 | **4.16** | 0.001 | **2.59** | 0.001 | Cytochrome c oxidase subunit |
| *COX5B* | 1.95 | 0.005 | **4.16** | 0.001 | **5.81** | 0.001 | Cytochrome c oxidase subunit |
| *COX6A1* | 1.70 | 0.01 | 0.81 | 0.001 | 0.71 | 0.02 | Cytochrome c oxidase subunit |
| *COX6A2* | **3.32** | 0.01 | **0.41** | 0.001 | **0.34** | 0.001 | Cytochrome c oxidase subunit |
| *COX6B1* | **2.41** | 0.005 | 1.16 | 0.001 | 1.71 | 0.001 | Cytochrome c oxidase assembly/regulation |
| *COX6C* | 1.38 | 0.05 | 1.49 | 0.05 | **2.26** | 0.001 | Cytochrome c oxidase assembly/regulation |
| *COX7A2* | **2.24** | 0.005 | 1.86 | 0.001 | 1.71 | 0.001 | Cytochrome c oxidase assembly/regulation |
| *COX7A2L* | 1.29 | Ns | **3.81** | 0.001 | **4.21** | 0.001 | Cytochrome c oxidase assembly/regulation |
| *COX7B* | 0.85 | 0.002 | 1.96 | 0.001 | **2.11** | 0.001 | Cytochrome c oxidase subunit |
| *COX8A* | **2.09** | 0.001 | **4.53** | 0.001 | **4.51** | 0.001 | Cytochrome c oxidase regulation |
| *CPT1* | 1.66 | 0.01 | **2.05** | 0.05 | **3.30** | 0.05 | Long chain fatty acylcoA import/β-oxidation |
| *CPT2* | 1.55 | 0.02 | **2.04** | 0.05 | **3.42** | 0.01 | Long chain fatty acylcoA import/β-oxidation |
| *CYC1* | 1.95 | 0.005 | **3.24** | 0.001 | **3.98** | 0.001 | Electron transport |
| *DMM1L* | 1.91 | 0.01 | **2.16** | 0.02 | **3.18** | 0.05 | Control of mitochondria morphology |
| *FIS1* | 1.35 | 0.05 | 1.52 | Ns | **2.71** | 0.05 | Control of mitochondria fission |
| *FXC1* | 1.45 | 0.05 | 0.78 | Ns | 1.05 | Ns | Mitochondrial proteins import |
| *HSP90A1* | 1.26 | Ns | **2.26** | 0.05 | **4.40** | 0.05 | Proteins chaperon |
| *HSPD1* | 1.26 | Ns | **2.16** | 0.05 | **3.33** | 0.05 | Mitochondrial proteins chaperon |
| *IMMP1L* | 1.45 | 0.05 | 1.24 | 0.05 | **2.01** | 0.05 | Mitochondrial proteins processing/import |
| *IMMP2L* | 0.59 | 0.001 | 0.80 | Ns | 1.56 | Ns | Mitochondrial proteins processing/import |
| *LRPPRC* | 1.10 | Ns | 1.42 | Ns | 1.59 | Ns | Mitochondrial transcription factor |
| *MFN1* | 0.63 | 0.02 | 0.86 | Ns | 1.15 | Ns | Control of mitochondria fusion |
| *MFN2* | **2.19** | 0.002 | **2.43** | 0.05 | **3.49** | 0.05 | Control of mitochondria fusion |
| *MIPEP* | 1.78 | 0.01 | 1.39 | Ns | **2.47** | 0.05 | Mitochondrial proteins processing |
| *MPV17* | 1.66 | 0.01 | 1.18 | Ns | 1.45 | 0.005 | Metabolism of mitochondrial ROS |
| *MTX2* | 0.72 | 0.05 | 1.18 | Ns | **3.11** | 0.001 | Mitochondrial proteins import |
| *NDUFA1* | 1.82 | 0.005 | 1.40 | 0.001 | 1.71 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA10* | 1.70 | 0.01 | 1.38 | 0.001 | 1.70 | 0.002 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA11* | **2.58** | 0.001 | **5.29** | 0.001 | **6.84** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA2* | 1.70 | 0.01 | **5.16** | 0.001 | **6.41** | 0.001 | NADH:ubiquinone oxidoreductase assembly |
| *NDUFA3* | 1.95 | 0.005 | **0.29** | 0.001 | **0.40** | 0.05 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA4* | 1.82 | 0.005 | 1.91 | 0.001 | **3.96** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA5* | 1.70 | 0.01 | **2.19** | 0.001 | **2.26** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA6* | **2.09** | 0.005 | 1.91 | 0.001 | **2.11** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA7* | 1.48 | 0.02 | **2.24** | 0.001 | **4.21** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA8* | 1.70 | 0.01 | **2.78** | 0.001 | **8.38** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFAB1* | **2.76** | 0.001 | **4.12** | 0.001 | **6.35** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFAB10* | **2.76** | 0.001 | **3.91** | 0.001 | **3.93** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB2* | 1.95 | 0.005 | **5.92** | 0.001 | **6.38** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB3* | **2.09** | 0.005 | **3.81** | 0.001 | **3.97** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB4* | 1.82 | 0.005 | **2.59** | 0.001 | **5.16** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB5* | 0.79 | Ns | 1.71 | 0.001 | **4.19** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB6* | 1.59 | 0.01 | **3.67** | 0.001 | **3.91** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB7* | **3.65** | 0.005 | **2.24** | 0.001 | **7.86** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB8* | 1.82 | 0.005 | **2.42** | 0.001 | **8.38** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB9* | **2.96** | 0.001 | 1.83 | 0.001 | **6.35** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFC1* | **2.24** | 0.002 | 1.26 | 0.001 | **4.19** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFC2* | 1.38 | 0.05 | **2.26** | 0.001 | **3.40** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS1* | 0.85 | Ns | **4.81** | 0.001 | **3.67** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS2* | 1.29 | Ns | **3.65** | 0.001 | **3.19** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS3* | **2.41** | 0.002 | **3.19** | 0.001 | **5.53** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS4* | 1.59 | 0.01 | **2.59** | 0.001 | **4.49** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS5* | **2.24** | 0.002 | **2.78** | 0.001 | **5.16** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS6* | **2.24** | 0.005 | **4.81** | 0.001 | **4.84** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS7* | 1.82 | 0.001 | **3.93** | 0.001 | **4.49** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS8* | **2.76** | 0.001 | **14.49** | 0.001 | **16.76** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV1* | **4.19** | 0.001 | **2.98** | 0.001 | **3.65** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV2* | 1.59 | 0.01 | 1.29 | 0.001 | 1.05 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV3* | 1.20 | Ns | 1.49 | 0.002 | **2.24** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *OPA1* | 1.45 | 0.05 | 1.79 | 0.05 | **2.01** | 0.05 | Control of mitochondria network |
| *OXA1L* | 1.59 | 0.005 | **2.11** | 0.001 | **3.65** | 0.001 | Cytochrome c oxidase assembly |
| *RHOT1* | 1.55 | 0.02 | 1.39 | Ns | **2.71** | 0.05 | Control of mitochondria fission and fusion |
| *RHOT2* | 1.91 | 0.005 | 0.62 | Ns | 1.15 | Ns | Control of mitochondria fission and fusion |
| *SDHA* | 1.17 | Ns | 1.48 | 0.005 | 1.83 | 0.001 | Succinate dehydrogenase subunit |
| *SDHB* | 1.59 | 0.01 | **2.29** | 0.001 | **4.21** | 0.001 | Succinate dehydrogenase subunit |
| *SDHC* | 1.38 | 0.05 | 1.58 | 0.001 | 1.83 | 0.001 | Succinate dehydrogenase subunit |
| *SDHD* | 1.29 | Ns | 1.35 | 0.001 | **2.98** | 0.001 | Succinate dehydrogenase subunit |
| *SH3SGLB1* | 1.45 | 0.05 | 1.84 | Ns | 1.53 | Ns | Apoptosis induction |
| *SLC25A1* | **2.52** | 0.002 | **2.74** | 0.02 | **5.29** | 0.05 | Tricarboxylic acids import |
| *SLC25A10* | **2.35** | 0.005 | **2.66** | 0.02 | **6.22** | 0.05 | Mitochondrial proteins import |
| *SLC25A12* | 1.78 | 0.01 | 1.39 | 0.02 | 1.96 | 0.02 | Dicarboxylic acids import |
| *SLC25A13* | 1.02 | Ns | 1.50 | Ns | 1.87 | Ns | Aspartic acid/glutamic acid exchange |
| *SLC25A14* | 1.91 | 0.01 | 1.42 | Ns | **2.30** | 0.02 | Aspartic acid/glutamic acid exchange |
| *SLC25A15* | 1.55 | 0.02 | **2.01** | 0.05 | **3.57** | 0.05 | Ornithine import |
| *SLC25A19* | **3.10** | 0.002 | **4.01** | 0.05 | **6.93** | 0.05 | Thiamine pyrophosphate import |
| *SLC25A2* | **4.09** | 0.001 | **4.53** | 0.05 | **4.70** | 0.05 | Mitochondrial proteins import |
| *SLC25A20* | **2.46** | 0.002 | **3.10** | 0.05 | **3.74** | 0.001 | Carnitine/acylcarnitine translocation |
| *SLC25A21* | **2.76** | 0.01 | **5.07** | 0.05 | **5.77** | 0.05 | Oxodicarboxylic acids import |
| *SLC25A22* | 1.35 | 0.05 | **0.14** | 0.05 | 1.29 | 0.05 | Glutamate import |
| *SLC25A23* | 0.72 | 0.05 | 1.39 | Ns | **2.01** | 0.05 | Phosphate import |
| *SLC25A24* | 1.66 | 0.01 | 1.71 | 0.01 | **2.77** | 0.05 | Phosphate import |
| *SLC25A25* | 1.35 | 0.05 | 0.96 | 0.01 | 1.07 | Ns | Phosphate import |
| *SLC25A27* | 0.59 | 0.01 | **0.16** | 0.01 | **0.13** | 0.001 | OXPHOS/ATP synthesis uncoupling |
| *SLC25A3* | 1.78 | 0.01 | 0.82 | Ns | **4.30** | 0.05 | Phosphate/hydroxyl ions exchange |
| *SLC25A31* | **2.44** | 0.01 | **2.52** | 0.05 | **2.41** | 0.005 | Adenine nucleotide translocation |
| *SLC25A37* | 1.55 | 0.02 | 0.92 | Ns | 0.74 | Ns | Iron import |
| *SLC25A4* | 1.10 | Ns | **2.98** | 0.05 | **3.18** | 0.05 | Adenine nucleotide translocation |
| *SLC25A5* | 1.26 | Ns | **2.72** | 0.05 | **2.65** | 0.05 | Adenine nucleotide translocation |
| *SOD1* | 1.26 | Ns | 1.09 | Ns | **4.01** | 0.05 | ROS protection (cytosol) |
| *SOD2* | 1.48 | 0.005 | **3.39** | 0.001 | **4.30** | 0.001 | ROS protection (mitochondria) |
| *STARD3* | 1.02 | Ns | 0.94 | Ns | 1.07 | Ns | Cholesterol import |
| *TIMM10* | **2.05** | 0.005 | **3.34** | 0.05 | **4.30** | 0.05 | Protein insertion in the inner membrane |
| *TIMM17A* | **2.35** | 0.005 | **2.78** | 0.02 | **4.72** | 0.05 | Protein insertion in the inner membrane |
| *TIMM17B* | 1.10 | Ns | 1.45 | Ns | **2.01** | 0.05 | Protein insertion in the inner membrane |
| *TIMM8A* | 1.91 | 0.01 | 1.88 | Ns | **3.66** | 0.05 | Protein insertion in the inner membrane |
| *TIMM8B* | 1.66 | 0.01 | **3.58** | 0.02 | **5.54** | 0.05 | Protein insertion in the inner membrane |
| *TIMM9* | 1.26 | Ns | **2.84** | 0.05 | **2.30** | 0.05 | Protein insertion in the inner membrane |
| *TOMM20* | 1.35 | 0.05 | 1.46 | Ns | 1.35 | 0.05 | Mitochondrial proteins import |
| *TOMM22* | **3.35** | 0.01 | **3.13** | 0.05 | **3.01** | 0.05 | Mitochondrial proteins import |
| *TOMM34* | 1.45 | 0.05 | 0.90 | Ns | **3.66** | 0.05 | Mitochondrial proteins import |
| *TOMM40* | **2.19** | 0.005 | **2.48** | 0.02 | **2.65** | 0.01 | Mitochondrial proteins import |
| *TOMM70A* | 1.78 | 0.01 | 1.75 | 0.01 | **3.74** | 0.002 | Mitochondrial proteins import |
| *TSPO* | 1.18 | Ns | 1.42 | Ns | **2.25** | 0.05 | Cholesterol import |
| *UCP1* | **2.44** | 0.05 | 1.34 | Ns | **0.42** | 0.05 | OXPHOS/ATP synthesis uncoupling |
| *UCP2* | **3.34** | 0.005 | **2.12** | 0.05 | **2.10** | 0.05 | OXPHOS/ATP synthesis uncoupling |
| *UCP3* | **9.19** | 0.001 | **9.01** | 0.05 | **3.12** | 0.05 | OXPHOS/ATP synthesis uncoupling |
| *UQCR11* | **2.58** | 0.001 | **3.42** | 0.001 | **4.81** | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRC1* | 1.29 | Ns | **2.22** | 0.001 | **2.96** | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRC2* | **2.41** | 0.002 | **3.67** | 0.001 | **5.53** | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQRCFS1* | **2.09** | 0.002 | **8.42** | 0.001 | **15.63** | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRH* | **2.41** | 0.002 | **5.56** | 0.001 | **10.31** | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRQ* | 1.95 | 0.005 | 1.71 | 0.001 | **3.40** | 0.001 | Ubiquinol-cytochrome c reductase subunit |

OXPHOS: oxidative phosphorylation.

Fold-Change (2^(- Delta Delta Ct)) is the normalized gene expression (2^(- Delta Ct)) in U-2OS/DX30, U-2OS/DX100 or U-2OS/DX580 cells, divided the normalized gene expression (2^(- Delta Ct)) in U-2OS cells (n= 4), where Ct is the threshold cycle in qRT-PCR; when the fold-change is less than 1, the value is the negative inverse of the fold-change. Ns: not significant. Bold characters: up- or down-regulation more than two-fold.

**Supplementary Table S3. Expression of mitochondria-related genes in U-2OS cells untreated and treated with doxorubicin or mitochondria-targeted doxorubicin**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Fold change**  **Dox**  **versus**  **Ctrl** | **p value** | **Fold change**  **mtDox**  **versus**  **Ctrl** | **p value** | **Biological function** |
| *AIFM2* | 1.83 | 0.05 | 0.71 | 0.02 | Apoptosis induction |
| *ATP12A* | 0.80 | 0.001 | **0.21** | 0.001 | H+/ATP exchange |
| *ATP4A* | 0.71 | 0.001 | **0.22** | 0.001 | H+/ATP exchange |
| *ATP4B* | 0.80 | 0.001 | **0.21** | 0.001 | H+/ATP exchange |
| *ATP50* | **0.35** | 0.001 | **0.06** | 0.001 | ATP synthase subunit |
| *ATP5A1* | 1.29 | 0.005 | 1.99 | 0.005 | ATP synthase subunit |
| *ATP5B* | 1.21 | 0.02 | 1.39 | 0.002 | ATP synthase subunit |
| *ATP5C1* | 0.86 | 0.05 | 1.13 | Ns | ATP synthase subunit |
| *ATP5F1* | 1.39 | 0.005 | 1.26 | 0.01 | ATP synthase subunit |
| *ATP5G1* | 1.30 | 0.01 | 1.06 | Ns | ATP synthase subunit |
| *ATP5G2* | 0.65 | 0.001 | 1.39 | 0.02 | ATP synthase subunit |
| *ATP5G3* | 0.99 | 0.05 | 1.21 | 0.05 | ATP synthase subunit |
| *ATP5I* | 0.70 | 0.002 | 0.68 | 0.01 | ATP synthase subunit |
| *ATP5J* | 1.49 | 0.002 | 1.11 | 0.01 | ATP synthase subunit |
| *ATP5J2* | 1.06 | Ns | 1.97 | 0.001 | ATP synthase subunit |
| *ATP5L* | 1.84 | 0.001 | 1.21 | 0.02 | ATP synthase subunit |
| *BAK1* | **3.42** | 0.05 | **3.72** | 0.001 | Apoptosis induction |
| *BBC3* | **3.46** | 0.05 | **3.17** | 0.001 | Apoptosis induction |
| *BCL2* | **0.20** | 0.05 | **0.10** | 0.001 | Apoptosis inhibition |
| *BCL2L1* | **0.48** | 0.05 | **0.31** | 0.005 | Apoptosis inhibition |
| *BCS1L* | **0.28** | 0.001 | **0.21** | 0.001 | Ubiquinol-cytochrome c reductase assembly |
| *BID* | 1.65 | 0.05 | **2.76** | 0.05 | Apoptosis induction |
| *BNIP3* | **2.48** | 0.05 | **2.06** | 0.05 | Apoptosis induction |
| *COX10* | 1.01 | Ns | 1 | Ns | Cytochrome c oxidase assembly |
| *COX18* | 0.54 | 0.05 | 0.66 | 0.005 | Cytochrome c oxidase assembly |
| *COX412* | **3.20** | 0.001 | **0.21** | 0.001 | Cytochrome c oxidase subunit |
| *COX4I1* | 0.92 | Ns | 1.97 | 0.001 | Cytochrome c oxidase assembly |
| *COX5A* | 1.30 | 0.01 | 0.99 | 0.005 | Cytochrome c oxidase subunit |
| *COX5B* | 1.06 | Ns | 1.60 | 0.001 | Cytochrome c oxidase subunit |
| *COX6A1* | 0.70 | 0.002 | 0.57 | 0.001 | Cytochrome c oxidase subunit |
| *COX6A2* | 1.13 | 0.005 | **0.10** | 0.001 | Cytochrome c oxidase subunit |
| *COX6B1* | **3.20** | 0.001 | 0.92 | Ns | Cytochrome c oxidase assembly/regulation |
| *COX6C* | 1.21 | 0.02 | **0.21** | 0.001 | Cytochrome c oxidase assembly/regulation |
| *COX7A2* | 0.75 | 0.005 | 1.21 | 0.02 | Cytochrome c oxidase assembly/regulation |
| *COX7A2L* | 1.81 | 0.001 | **2.26** | 0.002 | Cytochrome c oxidase assembly/regulation |
| *COX7B* | 0.80 | 0.01 | 1.30 | 0.01 | Cytochrome c oxidase subunit |
| *COX8A* | 1.71 | 0.001 | 1.30 | 0.01 | Cytochrome c oxidase regulation |
| *CPT1* | **0.18** | 0.001 | **0.18** | 0.001 | Long chain fatty acylcoA import/β-oxidation |
| *CPT2* | **2.06** | 0.05 | 0.54 | 0.005 | Long chain fatty acylcoA import/β-oxidation |
| *CYC1* | 0.98 | Ns | 1.06 | Ns | Electron transport |
| *DMM1L* | 0.96 | Ns | 1.06 | Ns | Control of mitochondria morphology |
| *FIS1* | 1.42 | 0.05 | 1.24 | Ns | Control of mitochondria fission |
| *FXC1* | 0.56 | 0.05 | 0.60 | 0.05 | Mitochondrial proteins import |
| *HSP90A1* | 0.92 | Ns | 1.20 | Ns | Proteins chaperon |
| *HSPD1* | 0.58 | Ns | 1.09 | Ns | Mitochondrial proteins chaperon |
| *IMMP1L* | 0.66 | 0.05 | **0.41** | 0.001 | Mitochondrial proteins processing/import |
| *IMMP2L* | **0.16** | 0.001 | **0.24** | 0.001 | Mitochondrial proteins processing/import |
| *LRPPRC* | 0.58 | Ns | 0.95 | Ns | Mitochondrial transcription factor |
| *MFN1* | 0.55 | 0.05 | 1.09 | Ns | Control of mitochondria fusion |
| *MFN2* | 1.39 | 0.05 | **0.36** | 0.001 | Control of mitochondria fusion |
| *MIPEP* | 1.13 | Ns | 0.79 | 0.01 | Mitochondrial proteins processing |
| *MPV17* | 1.33 | Ns | 0.83 | 0.05 | Metabolism of mitochondrial ROS |
| *MTX2* | 1.63 | 0.005 | 1.15 | Ns | Mitochondrial proteins import |
| *NDUFA1* | 0.75 | 0.005 | 1.30 | 0.01 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA10* | **0.46** | 0.001 | **0.35** | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA11* | **0.43** | 0.001 | **0.27** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA2* | 1.71 | 0.001 | 1.91 | 0.005 | NADH:ubiquinone oxidoreductase assembly |
| *NDUFA3* | **0.24** | 0.001 | **0.10** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA4* | 0.70 | 0.002 | 1.13 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA5* | 0.75 | 0.005 | 1.06 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA6* | 1.06 | Ns | 1.30 | 0.01 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA7* | 0.86 | 0.05 | 1.30 | 0.01 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA8* | 1.26 | 0.002 | 1.42 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFAB1* | 1.39 | 0.005 | 1.71 | 0,001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFAB10* | 0.86 | 0.05 | 1.13 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB2* | 0.79 | 0.001 | 1.46 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB3* | **0.42** | 0.001 | **0.36** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB4* | 0.98 | Ns | 1.84 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB5* | 1.06 | Ns | 1.26 | 0.01 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB6* | **0.36** | Ns | **0.37** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB7* | 3.20 | 0.001 | 1.97 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB8* | 1.13 | Ns | 1.30 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB9* | 1.20 | 0.05 | 0.75 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFC1* | 1.06 | Ns | 1.49 | 0.002 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFC2* | 1.30 | 0.01 | 1.60 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS1* | 0.86 | 0.05 | 1.11 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS2* | 0.92 | Ns | 1.60 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS3* | **2.11** | 0.001 | **2.11** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS4* | 0.70 | 0.002 | 0.97 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS5* | 1.30 | 0.01 | 1.39 | 0.02 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS6* | 1.84 | 0.005 | 1.84 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS7* | 1.60 | 0.001 | 1.84 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS8* | **4.22** | 0.001 | **3.43** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV1* | 0.98 | Ns | 0.70 | 0.02 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV2* | **0.30** | 0.001 | **0.25** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV3* | 0.92 | Ns | 1.49 | 0.002 | NADH:ubiquinone oxidoreductase subunit |
| *OPA1* | 0.84 | Ns | 0.60 | 0.005 | Control of mitochondria network |
| *OXA1L* | 0.80 | 0.001 | **0.21** | 0.001 | Cytochrome c oxidase assembly |
| *RHOT1* | 0.56 | 0.05 | 1 | Ns | Control of mitochondria fission and fusion |
| *RHOT2* | 1.03 | Ns | **0.22** | 0.02 | Control of mitochondria fission and fusion |
| *SDHA* | 0.65 | 0.001 | 0.94 | Ns | Succinate dehydrogenase subunit |
| *SDHB* | 1.49 | 0.002 | 1.65 | 0.01 | Succinate dehydrogenase subunit |
| *SDHC* | 0.91 | Ns | 1.71 | 0.02 | Succinate dehydrogenase subunit |
| *SDHD* | 1.71 | 0.001 | 1.42 | 0.01 | Succinate dehydrogenase subunit |
| *SH3SGLB1* | 1.27 | 0.005 | 1.51 | Ns | Apoptosis induction |
| *SLC25A1* | **2.42** | 0.001 | 0.76 | 0.005 | Tricarboxylic acids import |
| *SLC25A10* | **2.98** | 0.001 | 0.69 | 0.005 | Mitochondrial proteins import |
| *SLC25A12* | 0.59 | 0.05 | 0.62 | 0.005 | Dicarboxylic acids import |
| *SLC25A13* | **0.41** | 0.01 | 0.52 | 0.02 | Aspartic acid/glutamic acid exchange |
| *SLC25A14* | 1.15 | Ns | 0.66 | 0.05 | Aspartic acid/glutamic acid exchange |
| *SLC25A15* | 1.48 | 0.05 | 1.06 | Ns | Ornithine import |
| *SLC25A19* | 1.88 | 0.05 | **0.29** | 0.01 | Thiamine pyrophosphate import |
| *SLC25A2* | 1.88 | 0.02 | **0.19** | 0.001 | Mitochondrial proteins import |
| *SLC25A20* | 0.98 | Ns | 0.55 | 0.01 | Carnitine/acylcarnitine translocation |
| *SLC25A21* | 0.91 | Ns | 0.76 | 0.005 | Oxodicarboxylic acids import |
| *SLC25A22* | 0.82 | Ns | **0.40** | 0.001 | Glutamate import |
| *SLC25A23* | 0.96 | Ns | 1.26 | Ns | Phosphate import |
| *SLC25A24* | 1.10 | Ns | 0.91 | Ns | Phosphate import |
| *SLC25A25* | 0.56 | 0.05 | **0.41** | 0.02 | Phosphate import |
| *SLC25A27* | **2.10** | 0.001 | **2.55** | 0.05 | OXPHOS/ATP synthesis uncoupling |
| *SLC25A3* | 1.49 | 0.05 | 1.51 | Ns | Phosphate/hydroxyl ions exchange |
| *SLC25A31* | 1.01 | Ns | 1.09 | Ns | Adenine nucleotide translocation |
| *SLC25A37* | **0.41** | 0.01 | **0.19** | 0.001 | Iron import |
| *SLC25A4* | 1.60 | 0.05 | 1.58 | Ns | Adenine nucleotide translocation |
| *SLC25A5* | 1.05 | Ns | 1.34 | Ns | Adenine nucleotide translocation |
| *SOD1* | **0.20** | 0.001 | **0.38** | 0.02 | ROS protection (cytosol) |
| *SOD2* | **0.42** | 0.001 | **0.40** | 0.05 | ROS protection (mitochondria) |
| *STARD3* | 0.86 | 0.02 | **0.45** | 0.02 | Cholesterol import |
| *TIMM10* | 1.56 | 0.01 | 1.20 | Ns | Protein insertion in the inner membrane |
| *TIMM17A* | 1.71 | 0.001 | 0.95 | Ns | Protein insertion in the inner membrane |
| *TIMM17B* | 1.63 | 0.02 | 0.79 | 0.01 | Protein insertion in the inner membrane |
| *TIMM8A* | 1.67 | 0.02 | 1.00 | Ns | Protein insertion in the inner membrane |
| *TIMM8B* | **3.63** | 0.001 | 1.74 | 0.05 | Protein insertion in the inner membrane |
| *TIMM9* | 0.60 | Ns | 1.20 | Ns | Protein insertion in the inner membrane |
| *TOMM20* | **0.41** | 0.01 | **0.20** | 0.001 | Mitochondrial proteins import |
| *TOMM22* | 1.67 | 0.01 | 1.20 | Ns | Mitochondrial proteins import |
| *TOMM34* | 1.21 | Ns | 1.51 | Ns | Mitochondrial proteins import |
| *TOMM40* | 1.67 | 0.02 | **0.40** | 0.02 | Mitochondrial proteins import |
| *TOMM70A* | 1.21 | Ns | 1.09 | Ns | Mitochondrial proteins import |
| *TSPO* | 1.13 | Ns | 1.15 | Ns | Cholesterol import |
| *UCP1* | **3.46** | 0.001 | **3.17** | 0.002 | OXPHOS/ATP synthesis uncoupling |
| *UCP2* | **4.12** | 0.001 | **3.44** | 0.001 | OXPHOS/ATP synthesis uncoupling |
| *UCP3* | **4.12** | 0.001 | **3.18** | 0.005 | OXPHOS/ATP synthesis uncoupling |
| *UQCR11* | 1.39 | 0.005 | 1.60 | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRC1* | 1.49 | 0.002 | 1.30 | 0.01 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRC2* | 1.06 | Ns | 1.60 | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQRCFS1* | **0.42** | 0.001 | 0.68 | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRH* | 1.60 | 0.001 | 1.11 | 0.05 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRQ* | 1.13 | Ns | 0.92 | Ns | Ubiquinol-cytochrome c reductase subunit |

Ctrl: untreated cells; Dox: cells treated with 5 μmol/L Dox for 24 h; mtDox: cells treated with 5 μmol/L mtDox for 24 h; OXPHOS: oxidative phosphorylation.

Fold-Change (2^(- Delta Delta Ct)) is the normalized gene expression (2^(- Delta Ct)) in Dox- or mtDox-treated U-2OS cells, divided the normalized gene expression (2^(- Delta Ct)) in untreated cells (n= 4), where Ct is the threshold cycle in qRT-PCR; when the fold-change is less than 1, the value was the negative inverse of the fold-change. Ns: not significant. Bold characters: up- or down-regulation more than two-fold.

**Supplementary Table S4. Expression of mitochondria-related genes in U-2OS/DX580 cells untreated and treated with doxorubicin or mitochondria-targeted doxorubicin**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Fold change**  **Dox**  **versus**  **Ctrl** | **p value** | **Fold change**  **mtDox**  **versus**  **Ctrl** | **p value** | **Biological function** |
| *AIFM2* | 1.58 | Ns | 0.71 | 0.002 | Apoptosis induction |
| *ATP12A* | 1.22 | Ns | **0.23** | 0.001 | H+/ATP exchange |
| *ATP4A* | 1.26 | Ns | **0.21** | 0.001 | H+/ATP exchange |
| *ATP4B* | 1.28 | Ns | **0.21** | 0.001 | H+/ATP exchange |
| *ATP50* | 1.66 | 0.05 | 1.62 | 0.001 | ATP synthase subunit |
| *ATP5A1* | 1.67 | 0.01 | 1.46 | 0.001 | ATP synthase subunit |
| *ATP5B* | 1.34 | 0.02 | 1.31 | 0.01 | ATP synthase subunit |
| *ATP5C1* | 1.22 | Ns | 1.44 | 0.001 | ATP synthase subunit |
| *ATP5F1* | **2.01** | 0.02 | 1.76 | 0.002 | ATP synthase subunit |
| *ATP5G1* | 1.26 | Ns | 0.76 | 0.005 | ATP synthase subunit |
| *ATP5G2* | 1.37 | Ns | **0.41** | 0.001 | ATP synthase subunit |
| *ATP5G3* | 0.89 | Ns | 0.51 | 0.001 | ATP synthase subunit |
| *ATP5I* | 1.11 | Ns | 1.23 | Ns | ATP synthase subunit |
| *ATP5J* | 1.26 | 0.05 | 1.62 | 0.005 | ATP synthase subunit |
| *ATP5J2* | **2.07** | 0.01 | **0.22** | 0.001 | ATP synthase subunit |
| *ATP5L* | 1.21 | 0.02 | **0.33** | 0.001 | ATP synthase subunit |
| *BAK1* | 1.00 | Ns | **2.72** | 0.001 | Apoptosis induction |
| *BBC3* | 1.23 | Ns | **2.19** | 0.001 | Apoptosis induction |
| *BCL2* | 1.03 | 0.05 | **0.43** | 0.001 | Apoptosis inhibition |
| *BCL2L1* | 0.93 | Ns | **0.29** | 0.005 | Apoptosis inhibition |
| *BCS1L* | 0.66 | 0.05 | **0.41** | 0.001 | Ubiquinol-cytochrome c reductase assembly |
| *BID* | 1.89 | 0.05 | **2.76** | 0.01 | Apoptosis induction |
| *BNIP3* | 1.26 | Ns | **2.09** | 0.05 | Apoptosis induction |
| *COX10* | 1.44 | Ns | 1 | Ns | Cytochrome c oxidase assembly |
| *COX18* | 0.72 | 0.02 | 0.66 | 0.05 | Cytochrome c oxidase assembly |
| *COX412* | 1.21 | Ns | **0.20** | 0.001 | Cytochrome c oxidase subunit |
| *COX4I1* | **2.11** | 0.01 | **2.14** | 0.001 | Cytochrome c oxidase assembly |
| *COX5A* | 1.78 | 0005 | 1.86 | 0.001 | Cytochrome c oxidase subunit |
| *COX5B* | 1.09 | Ns | 1.11 | Ns | Cytochrome c oxidase subunit |
| *COX6A1* | 0.98 | Ns | **0.41** | 0.001 | Cytochrome c oxidase subunit |
| *COX6A2* | 1.00 | Ns | **0.29** | 0.001 | Cytochrome c oxidase subunit |
| *COX6B1* | 0.92 | Ns | 1.20 | 0.05 | Cytochrome c oxidase assembly/regulation |
| *COX6C* | 1.21 | Ns | **0.43** | 0.001 | Cytochrome c oxidase assembly/regulation |
| *COX7A2* | 1.23 | Ns | 1.23 | Ns | Cytochrome c oxidase assembly/regulation |
| *COX7A2L* | 1.26 | Ns | 1.28 | 0.02 | Cytochrome c oxidase assembly/regulation |
| *COX7B* | 1.39 | Ns | 1.41 | 0.02 | Cytochrome c oxidase subunit |
| *COX8A* | 1.30 | Ns | 1.51 | 0.02 | Cytochrome c oxidase regulation |
| *CPT1* | 1.04 | 0.05 | **0.24** | 0.05 | Long chain fatty acylcoA import/β-oxidation |
| *CPT2* | 0.81 | Ns | **0.37** | 0.02 | Long chain fatty acylcoA import/β-oxidation |
| *CYC1* | 1.51 | 0.002 | 1.23 | Ns | Electron transport |
| *DMM1L* | 1.04 | Ns | 1.24 | Ns | Control of mitochondria morphology |
| *FIS1* | 1.44 | Ns | 1.06 | Ns | Control of mitochondria fission |
| *FXC1* | 1.55 | 0.02 | **0.40** | 0.05 | Mitochondrial proteins import |
| *HSP90A1* | 1.44 | Ns | 1.20 | Ns | Proteins chaperon |
| *HSPD1* | 1.15 | Ns | 1.09 | Ns | Mitochondrial proteins chaperon |
| *IMMP1L* | 1.79 | 0.01 | **0.41** | 0.005 | Mitochondrial proteins processing/import |
| *IMMP2L* | 1.81 | Ns | 1.82 | Ns | Mitochondrial proteins processing/import |
| *LRPPRC* | 0.91 | Ns | 0.95 | Ns | Mitochondrial transcription factor |
| *MFN1* | 0.95 | Ns | **0.36** | 0.05 | Control of mitochondria fusion |
| *MFN2* | 0.83 | 0.05 | **0.39** | 0.02 | Control of mitochondria fusion |
| *MIPEP* | 1.05 | Ns | 0.79 | Ns | Mitochondrial proteins processing |
| *MPV17* | 0.79 | 0.01 | 1.15 | Ns | Metabolism of mitochondrial ROS |
| *MTX2* | **0.45** | 0.02 | **0.31** | 0.02 | Mitochondrial proteins import |
| *NDUFA1* | 0.77 | 0.01 | 1.23 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA10* | 0.97 | Ns | 0.76 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA11* | 1.56 | 0.005 | 1.62 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA2* | 1.34 | 0.01 | **0.29** | 0.001 | NADH:ubiquinone oxidoreductase assembly |
| *NDUFA3* | 1.51 | 0.01 | **0.08** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA4* | 1.13 | Ns | 1.29 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA5* | 1.78 | 0.02 | 1.63 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA6* | 1.30 | 0.05 | 1.51 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA7* | 1.24 | Ns | 1.17 | 0.05 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFA8* | 1.61 | 0.001 | 1.02 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFAB1* | 1.23 | Ns | 1.41 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFAB10* | 1.45 | 0.05 | 1.15 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB2* | 1.42 | 0.05 | 1.71 | 0.05 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB3* | 1.26 | 0.05 | **0.50** | 0.02 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB4* | 1.84 | 0.02 | 0.63 | 0.05 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB5* | 1.23 | Ns | **0.40** | 0.01 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB6* | 1.56 | Ns | 1.14 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB7* | 1.56 | 0.05 | 1.41 | 0.01 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB8* | 1.29 | 0.05 | 1.99 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFB9* | 0.78 | 0.05 | 0.81 | 0.02 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFC1* | 1.42 | 0.01 | 1.41 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFC2* | 1.60 | 0.01 | 1.23 | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS1* | 1.72 | 0.002 | 1.46 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS2* | 1.11 | Ns | 1.74 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS3* | 0.78 | Ns | **0.24** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS4* | 1.62 | 0.001 | 1.13 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS5* | 1.28 | 0.05 | **0.38** | 0.005 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS6* | 1.36 | Ns | 1.46 | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS7* | 1.17 | Ns | 1.51 | 0.002 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFS8* | **2.11** | 0.05 | **3.46** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV1* | 1.01 | Ns | **0.37** | 0.001 | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV2* | 1.32 | Ns | 1.12 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *NDUFV3* | 0.71 | Ns | 0.81 | Ns | NADH:ubiquinone oxidoreductase subunit |
| *OPA1* | 1.02 | 0.002 | **0.16** | 0.001 | Control of mitochondria network |
| *OXA1L* | 1.56 | 0.02 | **0.46** | 0.005 | Cytochrome c oxidase assembly |
| *RHOT1* | 0.63 | 0.01 | 0.52 | 0.05 | Control of mitochondria fission and fusion |
| *RHOT2* | 1.16 | 0.001 | **0.22** | 0.05 | Control of mitochondria fission and fusion |
| *SDHA* | 1.32 | Ns | 0.61 | 0.001 | Succinate dehydrogenase subunit |
| *SDHB* | 1.89 | 0.001 | 1.34 | 0.001 | Succinate dehydrogenase subunit |
| *SDHC* | 1.42 | 0.002 | 0.87 | 0.05 | Succinate dehydrogenase subunit |
| *SDHD* | 1.70 | 0.002 | 1.99 | 0.001 | Succinate dehydrogenase subunit |
| *SH3SGLB1* | 1.32 | Ns | 1.51 | 0.05 | Apoptosis induction |
| *SLC25A1* | 0.79 | 0.005 | 0.56 | 0.01 | Tricarboxylic acids import |
| *SLC25A10* | 1 | Ns | **0.46** | 0.005 | Mitochondrial proteins import |
| *SLC25A12* | 0.83 | 0.05 | **0.49** | 0.005 | Dicarboxylic acids import |
| *SLC25A13* | 0.76 | 0.01 | **0.42** | 0.01 | Aspartic acid/glutamic acid exchange |
| *SLC25A14* | 1.91 | 0.005 | 0.56 | 0.005 | Aspartic acid/glutamic acid exchange |
| *SLC25A15* | 1.71 | Ns | **0.29** | 0.001 | Ornithine import |
| *SLC25A19* | 1.32 | Ns | 1.09 | Ns | Thiamine pyrophosphate import |
| *SLC25A2* | **0.10** | 0.05 | **0.19** | 0.05 | Mitochondrial proteins import |
| *SLC25A20* | 1.07 | Ns | **0.45** | 0.05 | Carnitine/acylcarnitine translocation |
| *SLC25A21* | 0.72 | 0.01 | 0.76 | 0.05 | Oxodicarboxylic acids import |
| *SLC25A22* | 1.69 | 0.01 | **0.40** | 0.05 | Glutamate import |
| *SLC25A23* | 1.32 | Ns | 1.26 | Ns | Phosphate import |
| *SLC25A24* | 1.15 | Ns | 0.70 | Ns | Phosphate import |
| *SLC25A25* | 1.45 | 0.002 | **0.41** | 0.05 | Phosphate import |
| *SLC25A27* | 1.18 | Ns | **2.45** | 0.02 | OXPHOS/ATP synthesis uncoupling |
| *SLC25A3* | 1.38 | Ns | 1.51 | Ns | Phosphate/hydroxyl ions exchange |
| *SLC25A31* | 0.72 | 0.005 | 1.09 | Ns | Adenine nucleotide translocation |
| *SLC25A37* | 0.81 | 0.05 | **0.21** | 0.002 | Iron import |
| *SLC25A4* | 1.74 | 0.05 | 1.32 | Ns | Adenine nucleotide translocation |
| *SLC25A5* | 1.26 | Ns | 1.58 | Ns | Adenine nucleotide translocation |
| *SOD1* | 1.38 | Ns | 0.38 | Ns | ROS protection (cytosol) |
| *SOD2* | 1.09 | Ns | **0.40** | 0.05 | ROS protection (mitochondria) |
| *STARD3* | 1.00 | 0.02 | **0.45** | 0.02 | Cholesterol import |
| *TIMM10* | 1.26 | Ns | 1.20 | Ns | Protein insertion in the inner membrane |
| *TIMM17A* | 1.04 | Ns | 0.75 | Ns | Protein insertion in the inner membrane |
| *TIMM17B* | 1.26 | Ns | **0.49** | 0.01 | Protein insertion in the inner membrane |
| *TIMM8A* | 1.15 | Ns | **0.41** | 0.005 | Protein insertion in the inner membrane |
| *TIMM8B* | 1.58 | Ns | 1.74 | 0.05 | Protein insertion in the inner membrane |
| *TIMM9* | 1.32 | Ns | 1.20 | Ns | Protein insertion in the inner membrane |
| *TOMM20* | 0.87 | Ns | 1.20 | Ns | Mitochondrial proteins import |
| *TOMM22* | **0.40** | 0.02 | **0.21** | 0.001 | Mitochondrial proteins import |
| *TOMM34* | 1.51 | Ns | 1.51 | Ns | Mitochondrial proteins import |
| *TOMM40* | 1.06 | 0.005 | **0.33** | 0.05 | Mitochondrial proteins import |
| *TOMM70A* | 1.20 | Ns | **0.47** | 0.02 | Mitochondrial proteins import |
| *TSPO* | 1.09 | Ns | **0.17** | 0.005 | Cholesterol import |
| *UCP1* | 1.21 | Ns | **2.19** | 0.05 | OXPHOS/ATP synthesis uncoupling |
| *UCP2* | 1.66 | 0.05 | 1.44 | Ns | OXPHOS/ATP synthesis uncoupling |
| *UCP3* | 1.21 | Ns | **2.18** | 0.05 | OXPHOS/ATP synthesis uncoupling |
| *UQCR11* | 1.34 | Ns | 0.93 | Ns | Ubiquinol-cytochrome c reductase subunit |
| *UQCRC1* | 1.65 | 0.005 | 1.62 | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRC2* | 1.32 | 0.05 | **0.32** | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQRCFS1* | 1.72 | 0.05 | **0.30** | 0.001 | Ubiquinol-cytochrome c reductase subunit |
| *UQCRH* | 1.19 | Ns | 1.15 | Ns | Ubiquinol-cytochrome c reductase subunit |
| *UQCRQ* | 0.89 | Ns | **0.46** | 0.01 | Ubiquinol-cytochrome c reductase subunit |

Ctrl: untreated cells; Dox: cells treated with 5 μmol/L Dox for 24 h; mtDox: cells treated with 5 μmol/L mtDox for 24 h; OXPHOS: oxidative phosphorylation.

Fold-Change (2^(- Delta Delta Ct)) is the normalized gene expression (2^(- Delta Ct)) in Dox- or mtDox-treated U-2OS/DX580 cells, divided the normalized gene expression (2^(- Delta Ct)) in untreated cells (n= 4), where Ct is the threshold cycle in qRT-PCR; when the fold-change is less than 1, the value is the negative inverse of the fold-change. Ns: not significant. Bold characters: up- or down-regulation more than two-fold.

**Supplementary Tables S5. qRT-PCR validation of PCR-arrays results in U-2OS, U-2OS/DX30, U-2OS/DX100, U-2OS/DX580 cells**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene name** | **Relative expression**  **U-2OS/DX30 vs U-2OS** | **Relative expression**  **U-2OS/DX30 vs U-2OS** | **Relative expression**  **U-2OS/DX30 vs U-2OS** | **Biological function** |
| *TIMM8B* | 1.92 + 0.32 \* | 2.89 + 0.34 \* | 5.01 + 0.47 \* | Protein insertion in the inner membrane |
| *TOMM70A* | 1.72 + 0.21 \* | 1.81 + 0.29 \* | 3.34 + 0.27 \* | Mitochondrial proteins import |
| *MFN2* | 2.92 + 0.37 \* | 2.82 + 0.71 \* | 3.72 + 0.52 \* | Control of mitochondria fusion |
| *OPA1* | 1.26 + 0.12 | 1.54 + 0.12 \* | 2.91 + 0.43 \* | Control of mitochondria network |
| *SLC25A10* | 2.12 + 0.61 \* | 2.73 + 0.37 \* | 5.88 + 0.34 \* | Dicarboxylic acids import |
| *CPT1* | 1.52 + 0.26 | 2.44 + 0.23 \* | 3.17 + 0.28 \* | Long chain fatty acylcoA import/β-oxidation |
| *NDUFA2* | 1.43 + 0.21 | 5.03 + 0.47 \* | 5.99 + 0.71 \* | NADH:ubiquinone oxidoreductase assembly |
| *NDUFS5* | 2.35 + 0.36 \* | 2.67 + 0.11 \* | 5.56 + 0.43 \* | NADH:ubiquinone oxidoreductase subunit |
| *UQRCQ* | 1.76 + 0.33 \* | 1.82 + 0.32 \* | 2.47 + 0.55 \* | Ubiquinol-cytochrome c reductase subunit |
| *UQRCFS1* | 2.21 + 0.65 \* | 7.13 + 1.29 \* | 13.09 + 2.24 \* | Ubiquinol-cytochrome c reductase subunit |
| *OXA1L* | 1.26 + 0.21 | 2.53 + 0.12 \* | 4.82 + 0.33 \* | Cytochrome c oxidase assembly |
| *ATP5L* | 3.67 + 0.56 \* | 4.45 + 0.87 \* | 8.33 + 1.21 \* | ATP synthase subunit |
| *UCP1* | 2.02 + 0.35 \* | 1.18 + 0.21 | 0.19 + 0.05 \* | OXPHOS/ATP synthesis uncoupling |
| *SLC25A27* | 0.62 + 0.12 \* | 0.26 + 0.11 \* | 0.13 + 0.06 \* | OXPHOS/ATP synthesis uncoupling |
| *SOD2* | 1.54 + 0.31 | 2.28 + 0.54 \* | 3.66 + 0.29 \* | ROS protection (mitochondria) |
| *BAK1* | 1.23 + 0.22 | 1.09 + 0.03 | 0.98 + 0.15 | Apoptosis induction |
| *BCL2* | 0.93 + 0.21 | 1.22 + 0.11 | 1.32 + 0.09 | Apoptosis inhibition |

The expression levels of specific mitochondria-related genes, representative of the main biological categories screened by PCR arrays (Supplementary Table S2), were validated by qRT-PCR (n = 3). The expression level of each gene in U-2OS cells was considered as 1. The relative expression of the other genes was calculated with PrimePCR™ Analysis Software. *S14* gene was used as the housekeeping gene. Versus U-2OS cells: \* p < 0.05.

**Supplementary Tables S6. qRT-PCR validation of PCR-arrays results in U-2OS cells treated with doxorubicin or mitochondria-targeted doxorubicin**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene name** | **Relative expression**  **Dox vs Ctrl** | **Relative expression**  **mtDox vs Ctrl** | **Biological function** |
| *TIMM8B* | 3.56 + 0.34 \* | 1.65 + 0.41 | Protein insertion in the inner membrane |
| *TOMM70A* | 1.12 + 0.11 | 1.34 + 0.26 | Mitochondrial proteins import |
| *MFN2* | 1.11 + 0.19 | 0.27 + 0.11 \* | Control of mitochondria fusion |
| *OPA1* | 0.79 + 0.27 | 0.51 + 0.09 \* | Control of mitochondria network |
| *SLC25A10* | 3.09 + 0.45 \* | 0.62 + 0.20 \* | Dicarboxylic acids import |
| *CPT1* | 0.32 + 0.11 \* | 0.19 + 0.08 \* | Long chain fatty acylcoA import/β-oxidation |
| *NDUFA2* | 1.83 + 0.14 \* | 1.89 + 0.25 \* | NADH:ubiquinone oxidoreductase assembly |
| *NDUFS5* | 1.27 + 0.06 | 1.35 + 0.09 | NADH:ubiquinone oxidoreductase subunit |
| *UQRCQ* | 1.01 + 0.28 | 1.14 + 0.28 | Ubiquinol-cytochrome c reductase subunit |
| *UQRCFS1* | 0.59 + 0.18 \* | 0.63 + 0.12 \* | Ubiquinol-cytochrome c reductase subunit |
| *OXA1L* | 0.92 + 0.31 | 0.42 + 0.11 \* | Cytochrome c oxidase assembly |
| *ATP5L* | 1.57 + 0.33 | 1.26 + 0.05 | ATP synthase subunit |
| *UCP1* | 3.01 + 0.72 \* | 3.35 + 0.61 \* | OXPHOS/ATP synthesis uncoupling |
| *SLC25A27* | 2.13 + 0.27 \* | 2.61 + 0.64 \* | OXPHOS/ATP synthesis uncoupling |
| *SOD2* | 0.61 + 0.18 \* | 0.42 + 0.19 \* | ROS protection (mitochondria) |
| *BAK1* | 2.93 + 0.34 \* | 3.37 + 0.71 \* | Apoptosis induction |
| *BCL2* | 0.39 + 0.12 \* | 0.18 + 0.08 \* | Apoptosis inhibition |

The expression levels of specific mitochondria-related genes, representative of the main biological categories screened by PCR arrays (Supplementary Table S3), were validated by qRT-PCR (n = 3). The expression level of each gene in untreated U-2OS cells was considered as 1. The relative expression of the other genes was calculated with PrimePCR™ Analysis Software. *S14* gene was used as the housekeeping gene. Versus untreated U-2OS cells: \* p < 0.05.

**Supplementary Tables S7. qRT-PCR validation of PCR-arrays results in U-2OS/DX580 cells treated with doxorubicin or mitochondria-targeted doxorubicin**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene name** | **Relative expression**  **Dox vs Ctrl** | **Relative expression**  **mtDox vs Ctrl** | **Biological function** |
| *TIMM8B* | 1.28 + 0.21 | 1.54 + 0.33 | Protein insertion in the inner membrane |
| *TOMM70A* | 1.07 + 0.21 | 0.52 + 0.13 \* | Mitochondrial proteins import |
| *MFN2* | 0.95 + 0.13 | 0.43 + 0.18 \* | Control of mitochondria fusion |
| *OPA1* | 0.91 + 0.08 | 0.21 + 0.10 \* | Control of mitochondria network |
| *SLC25A10* | 1.14 + 0.21 | 0.33 + 0.06 \* | Dicarboxylic acids import |
| *CPT1* | 0.95 + 0.18 | 0.26 + 0.05 \* | Long chain fatty acylcoA import/β-oxidation |
| *NDUFA2* | 1.04 + 0.12 | 0.21 + 0.03 \* | NADH:ubiquinone oxidoreductase assembly |
| *NDUFS5* | 1.04 + 0.19 | 0.41 + 0.23 \* | NADH:ubiquinone oxidoreductase subunit |
| *UQRCQ* | 1.08 + 0.11 | 0.61 + 0.15 \* | Ubiquinol-cytochrome c reductase subunit |
| *UQRCFS1* | 1.52 + 0.13 | 0.33 + 0.07 \* | Ubiquinol-cytochrome c reductase subunit |
| *OXA1L* | 1.24 + 0.11 | 0.61 + 0.17 \* | Cytochrome c oxidase assembly |
| *ATP5L* | 1.06 + 0.14 | 0.41 + 0.19 \* | ATP synthase subunit |
| *UCP1* | 1.17 + 0.25 | 2.49 + 0.36 \* | OXPHOS/ATP synthesis uncoupling |
| *SLC25A27* | 0.97 + 0.21 | 2.27 + 0.31 \* | OXPHOS/ATP synthesis uncoupling |
| *SOD2* | 0.87 + 0.11 | 0.36 + 0.14 \* | ROS protection (mitochondria) |
| *BAK1* | 1.32 + 0.22 | 3.11 + 0.39 \* | Apoptosis induction |
| *BCL2* | 1.059 + 0.19 | 0.37 + 0.13 \* | Apoptosis inhibition |

The expression levels of specific mitochondria-related genes, representative of the main biological categories screened by PCR arrays (Supplementary Table S4), were validated in qRT-PCR (n = 3). The expression level of each gene in untreated U-2OS/DX580 cells was considered as 1. The relative expression of the other genes was calculated with PrimePCR™ Analysis Software. *S14* gene was used as the housekeeping gene. Versus untreated U-2OS/DX580 cells: \* p < 0.05.