Expanded View Figures

Figure EV1. Ectopic overexpression (OE) of MALSU1 does not protect ND1 mutant cybrid cells from nutrient deprivation. ND1 cells were transduced with lentiviral vector containing MALSU1 under the constitutive control of the CMV-promotor.

A ND1 control (MOCK) and MALSU1-OE cells exposed to varying extent of glucose deprivation (96 h) follow similar cell death responses (error bars represent the average ± s.e.m. of $n = 3$ biological replicates per concentration).

B MALSU1-OE ND1 cells do not synergize with doxycycline (1 μM) in promoting survival (96 h, error bars represent the average ± s.e.m. of $n = 9$ biological replicates across $N = 3$ independent experiments). Statistical significance ($P < 0.05$) between doxycycline treated and untreated cells across MOCK and MALSU1-OE cells was calculated using two-way ANOVA, multiple comparisons.

C Stable MALSU1-flag tag expression in ND1 cells.

D Quantitative PCR of ND1 cells (galactose, 48 h) treated with varying concentrations of doxycycline or inactive tetracycline analog CMT3 indicate mRNA expression levels of MALSU1 are unchanged (error bars represent the average ± s.e.m. of $n = 4$ biological replicates). Statistical significance ($P < 0.05$) between doxycycline or CMT3 treated and untreated cells was calculated using one-way ANOVA, multiple comparisons.

E, F Time-course experiments indicate that doxycycline does not alter (E) MALSU1 or (F) MTIF3 transcript levels ($n = 2$ technical replicates).

G ND1 cells exposed to glucose deprivation for indicated time periods with and without doxycycline indicate stability of MALSU1 by doxycycline at the 24 h time point. Note MTIF3 expression levels are unchanged. Note doxycycline suppresses the phosphorylation of p38 MAPK at later time points (12–24 h) as a control.

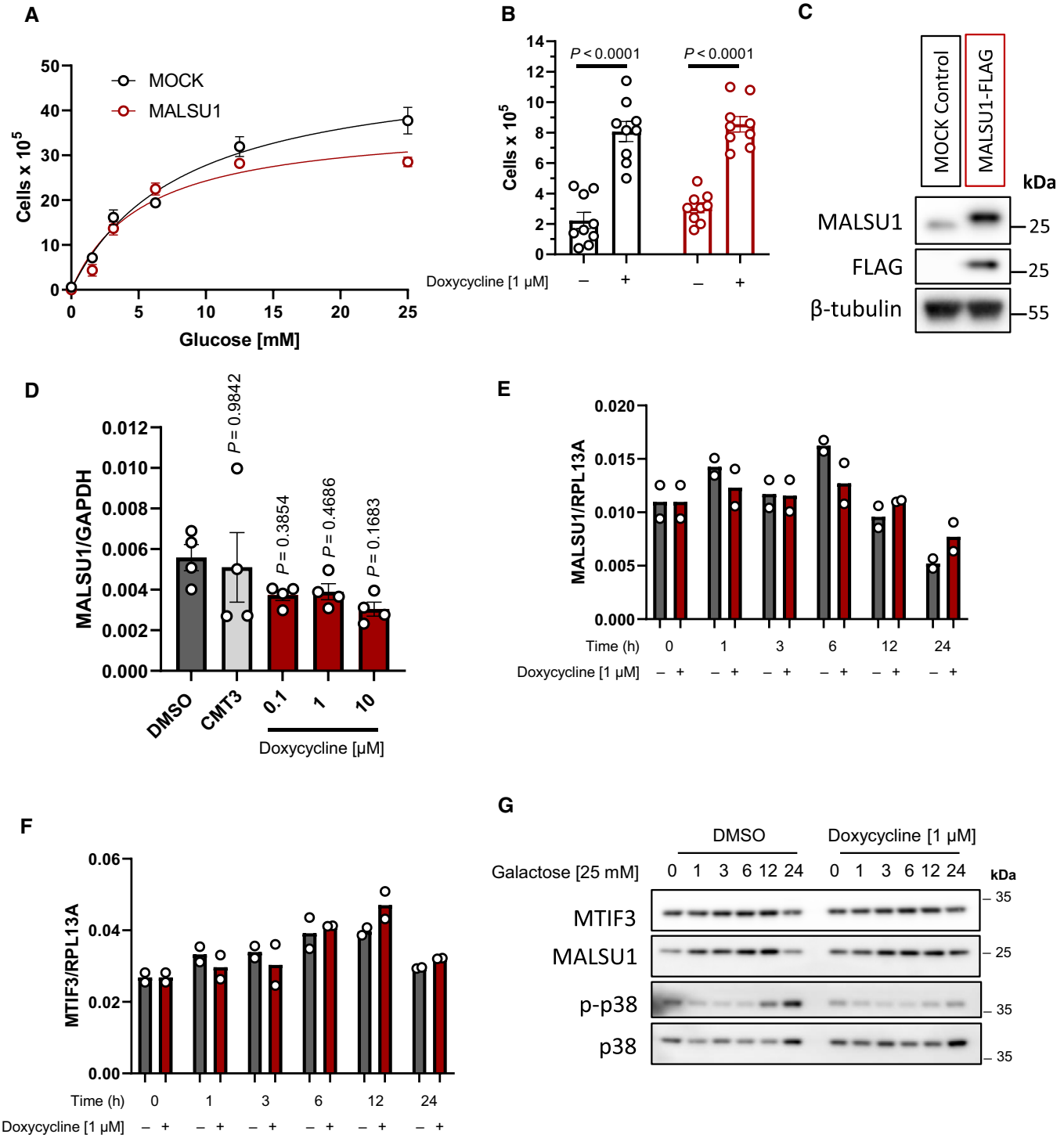

Figure EV1.

Figure EV2. Inhibition of mitochondrial translation with tetracyclines inhibits IRE1α and MAPK signaling, dependent on MALSU1.

A Whole cell IRE1α oligomerization states under galactose conditions assessed by blue-native PAGE. BN-PAGE of IRE1α with whole cell extracts does not capture doxycycline regulation of high-order oligomers when compared to ER concentrated at the mitochondria (Mito/ER extracts, Fig 4B). Glimepiride (25 μM) and IRE1α inhibitor 4μ8C (20 μM), compounds previously identified to rescue complex I mutants from nutrient deprivation, were used as controls. Note compensatory increase in IRE1α expression and oligomerization in 4μ8C treated cells.

B The activation of p38 MAPK is downstream of IRE1α and can be modulated by ERAD and mitochondrial translation. WT and ND1 cells were cultured in galactose media for 48 h and analyzed for the activation of MAPK and UPR signaling as evidenced by phosphorylation of p38 (P-p38) and expression of XBP1s, respectively. Pharmacological inhibition of IRE1α with 4μ8C (20 μM) and p97 inhibitor ESI (20 μM) indicate P-p38 and XBP1s are downstream of IRE1α and can be controlled by ERAD. CMT3 (1 μM) does not affect P-p38 MAPK or XBP1s levels when compared to doxycycline (1 μM) indicating inhibition of mitochondrial translation, not off target effects of tetracyclines, modulates UPR and MAPK signaling.

C UPR and MAPK signaling are activated after 48 h of galactose stress in ND1 cybrids, where doxycycline (1 μM) and chemically distinct 7002 (1 μM), but not CMT3 (1 μM) attenuate these responses.

D Doxycycline suppresses p38 MAPK phosphorylation depending on MALSU1. Data represents the average ± s.e.m. p38 phosphorylation level normalized to total p38 using western blot densitometry (error bars represent the average ± s.e.m. of $n = 3$ biological replicates). Statistics across glucose, galactose, and doxycycline treated cultures in WT, ND1, and sgMALSU1 cells was calculated using two-way ANOVA, multiple comparisons.

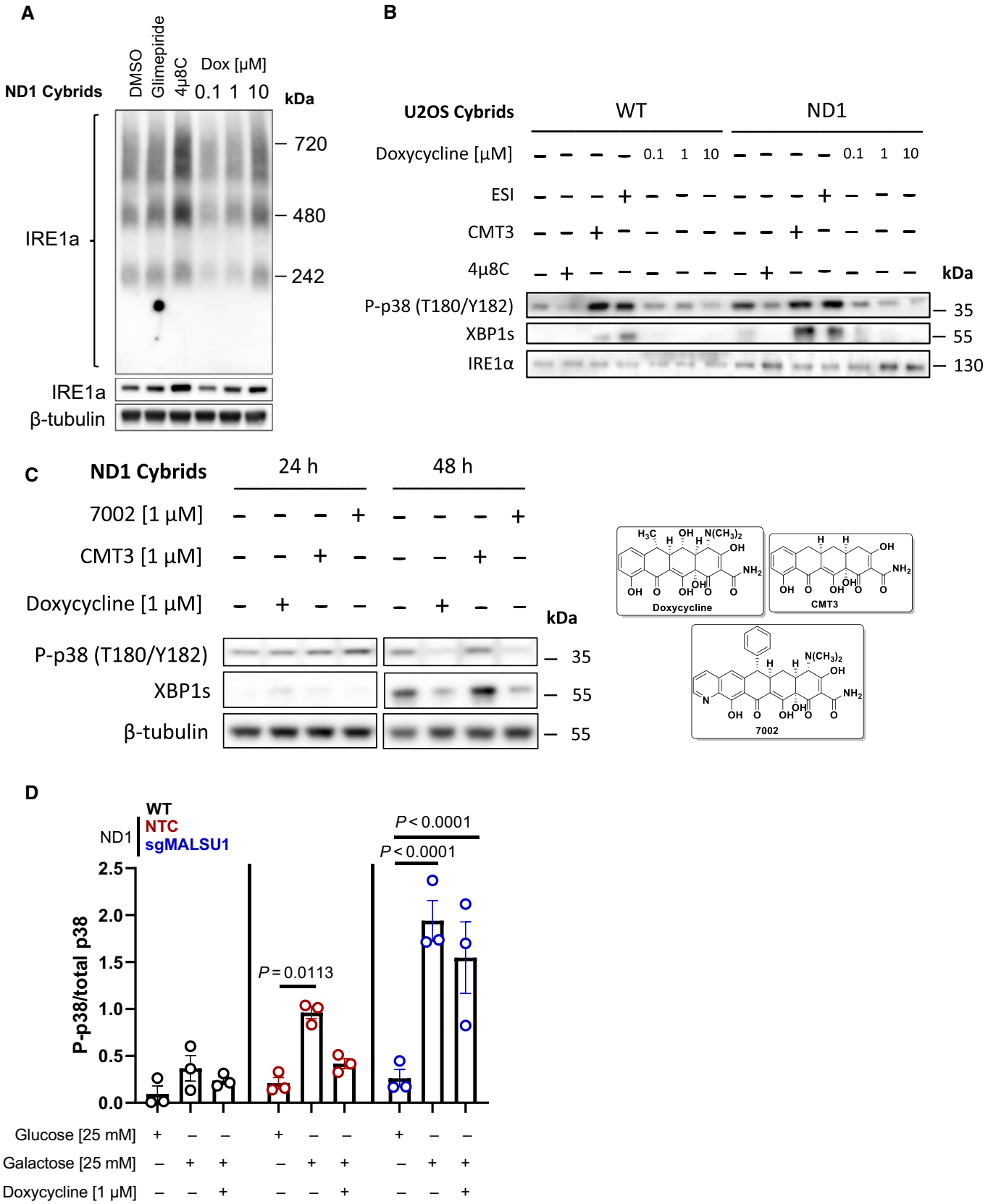

Figure EV2.

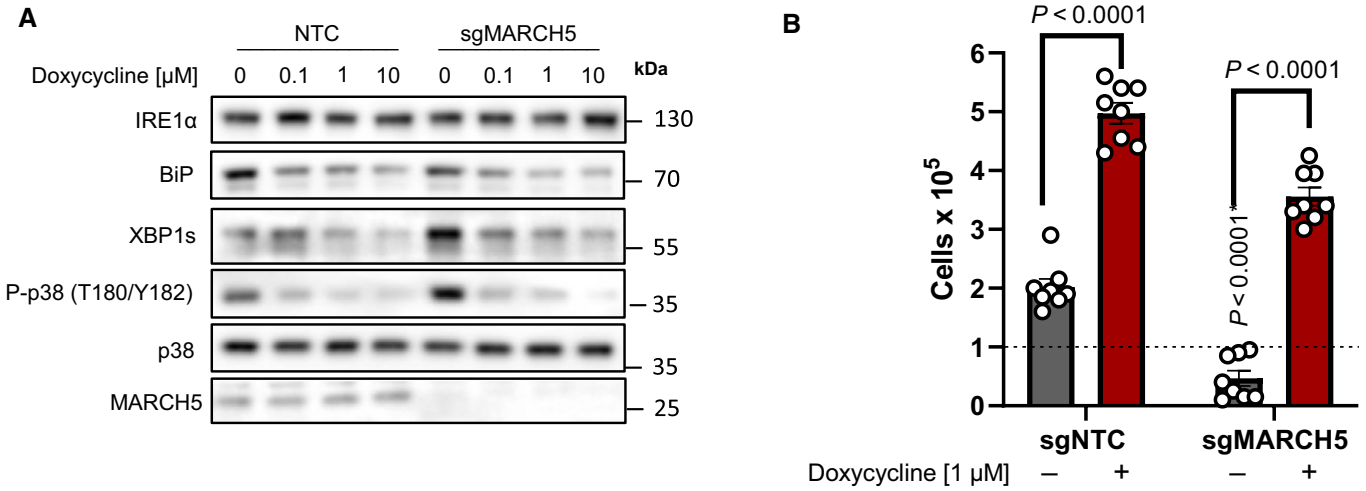

Figure EV3. Outer-mitochondrial membrane E3-ubiquitin ligase MARCH5 is not required for tetracyclines pro-survival mechanism in mitochondrial mutant cells.

A Doxycycline dose-dependently inhibits UPR and MAPK signaling in ND1 non-target control (NTC) cells and cells depleted of MARCH5 under galactose conditions. Note basal levels of XBP1s and P-p38 are increased in sgMARCH5 cells, indicating enhanced sensitivity to ER stress under these conditions.

B ND1 cybrids cultured in galactose conditions for 4 days can be rescued by doxycycline independent of MARCH5 expression status (error bars represent the average ± s.e.m. of $n = 8$ independent biological replicates across $N = 3$ independent experiments). Statistics of doxycycline treated and untreated cultures across NTC and sgMARCH5 cells were calculated using two-way ANOVA, multiple comparisons.