Supplementary Information

The impact of resveratrol and hydrogen peroxide on muscle cell plasticity shows a dose-dependent interaction

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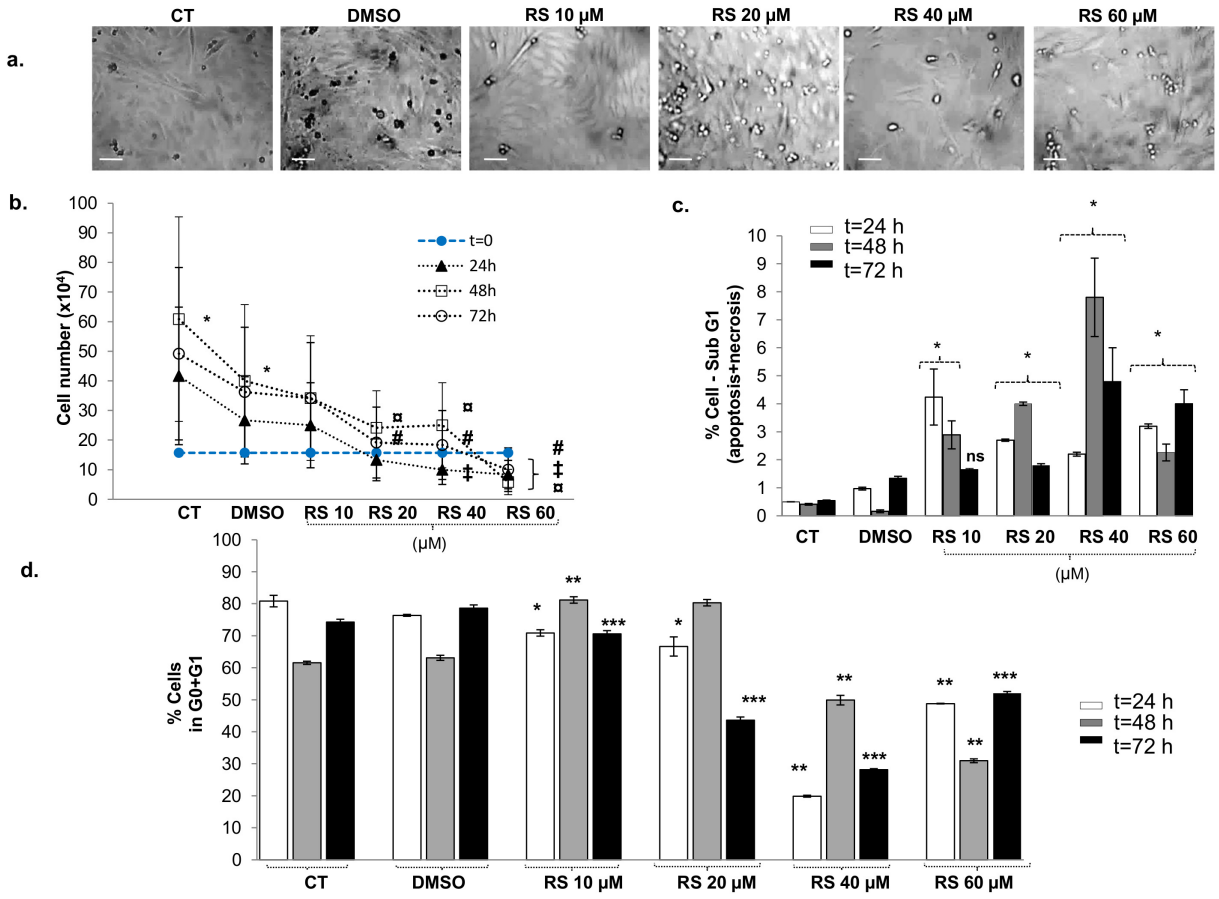
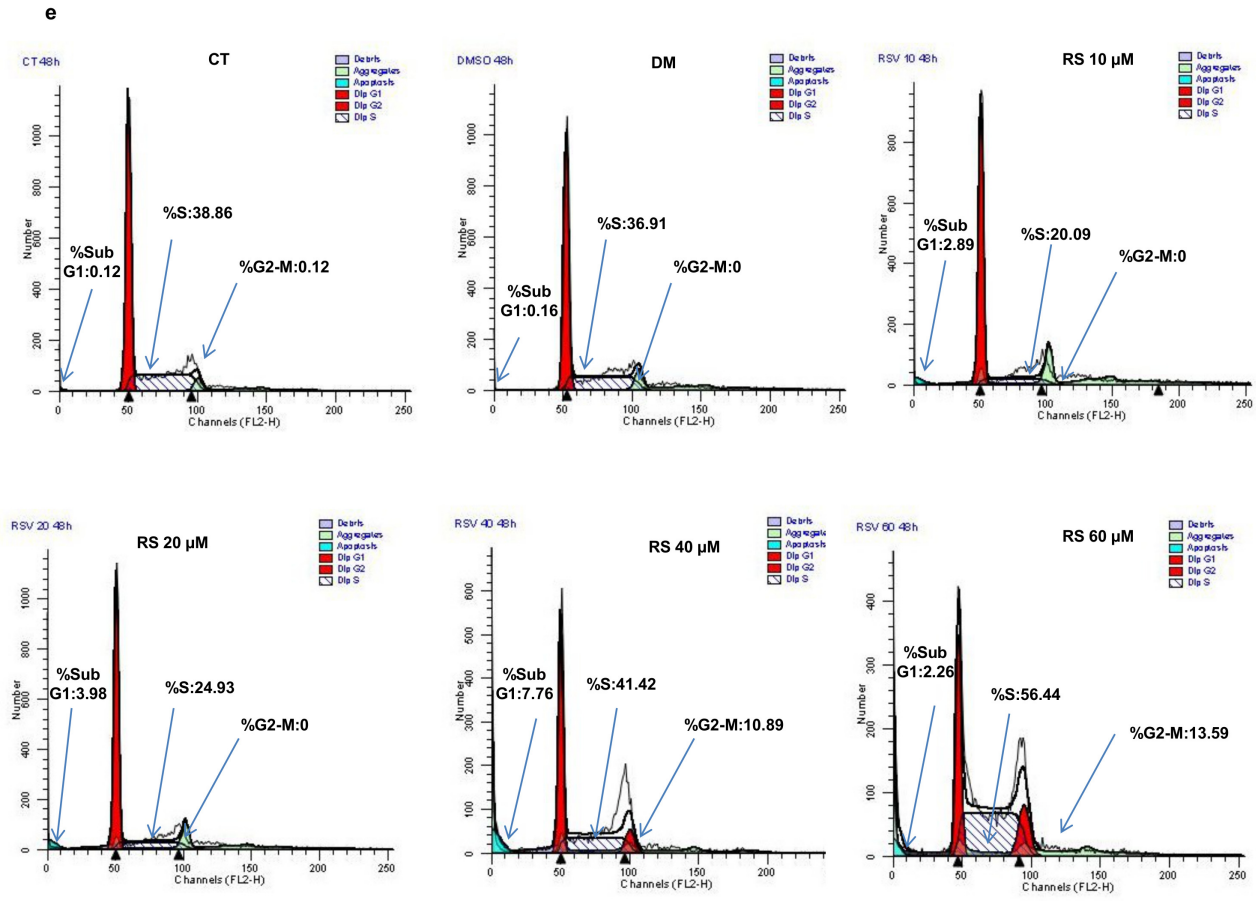
Supplementary Figure 1



(Supplementary Figure 1) Panel e



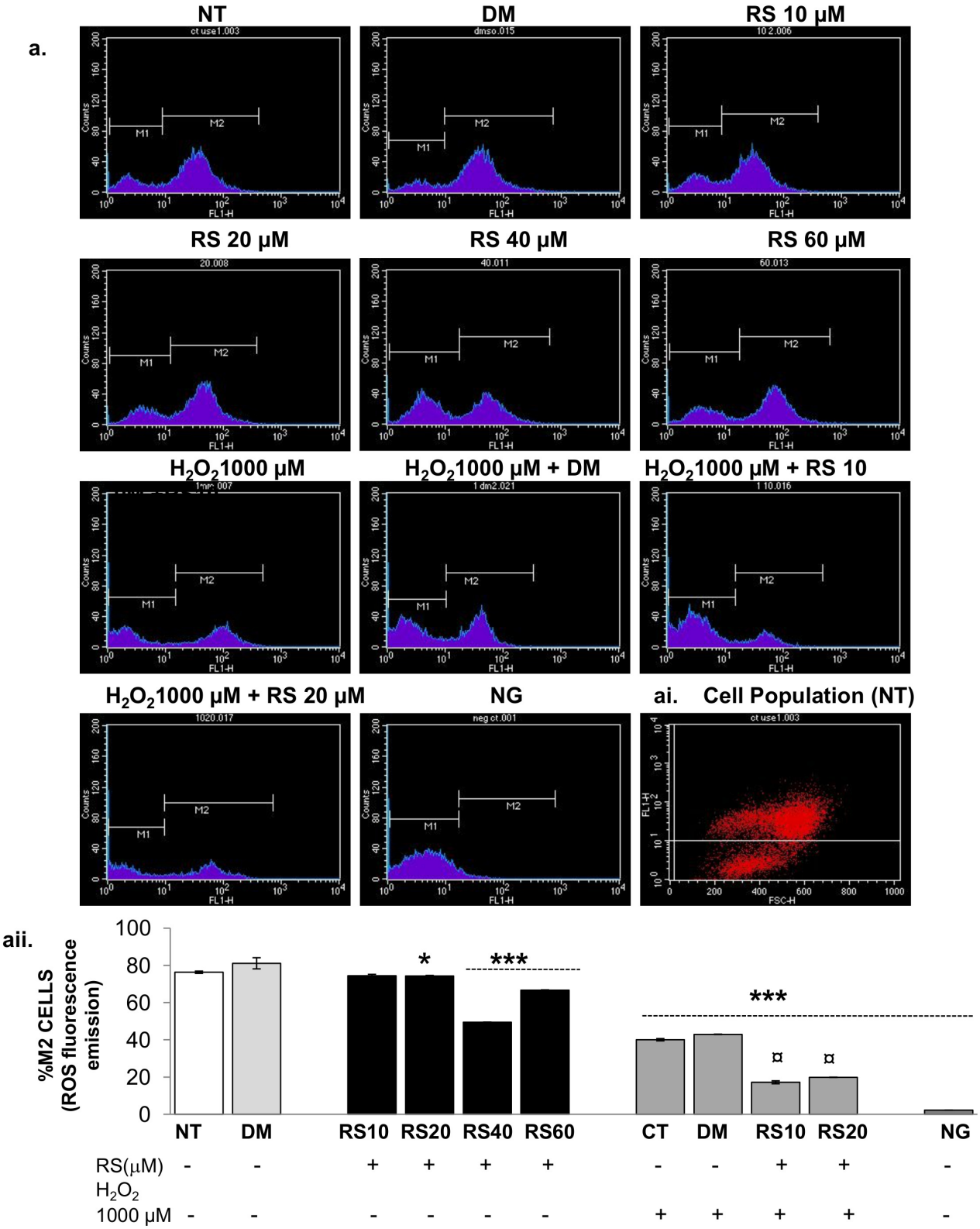
Supplementary Figure 1. Resveratrol retarded C2C12 cell growth and exerted a cytotoxic effect. (**a**) Phase contrast phase images showing the effect of 10, 20, 40 and 60 µM resveratrol (RS) on C2C12 cell morphology, captured at 72 h after treatment.

(**b**) Effect of RS on cell proliferation as reflected by cell number at 24, 48 and 72 h after treatment. (**c**) Effect of RS on cell viability expressed as % apoptotic and necrotic cells (Sub G1 cell population), determined by propidium idodide inclusion and FACS analysis. (**d**) Graph showing proportion of cells in G0+G1 phase. (**b**) Cell number was reduced with increasing dose of RS. (**c**) All doses of RS induced an increased % sub-G1 cell population. (**d**) Compared to controls, RS 10 µM (at 48 h) induced an increased proportion of cells in

G0+G1 phases and reduced those in S+G2 phases, suggesting cell cycle arrest. Higher RS concentrations (40-60 µM) induced a decrease in the proportion of cells in the G0+G1 phases, starting as early as after 24 h treatment.

Data are expressed as mean ±s.e.m. of biological triplicates. *P*-value calculated using a two-tailed Student's *t*-test. In **b**, *: $P<0.01$ vs. CT basal (t=0); ‡: $P<0.05$ vs CT at 24, ¤: $P<0.05$ vs CT at 48h; and #: $P<0.05$ vs CT 72h. In **c,** *: $P<0.05$ vs CT. In **d**, *: $P<0.05$ vs CT; **: $P<0.01$ vs CT; ***: $P<0.005$ vs CT. DMSO does not differ significantly from CT. Bars 20 μm.

Supplementary Figure 2

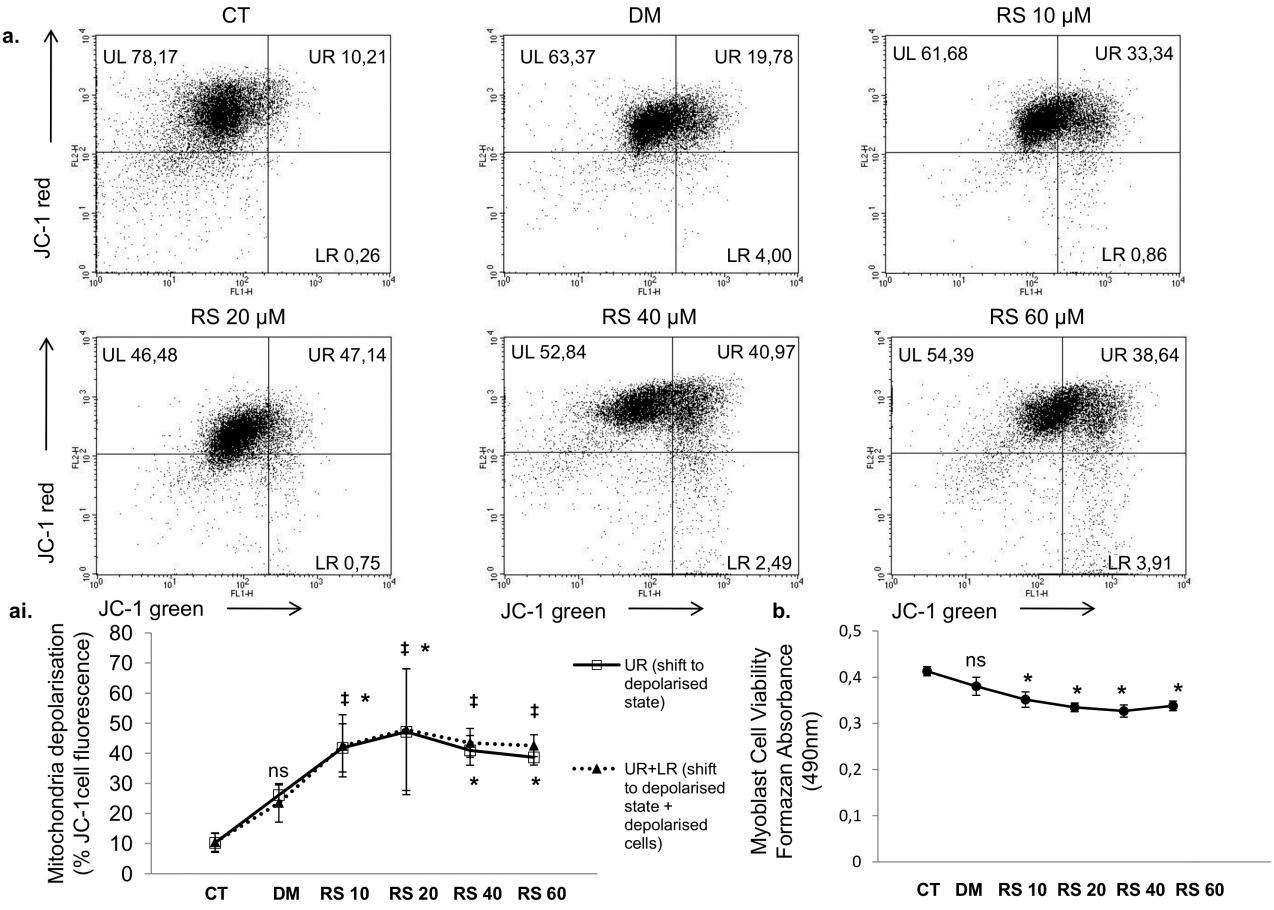


Supplementary Figure 2. The antioxidant effect of resveratrol is dose dependent

(**a**) Measure of intracellular ROS production following 24 h of treatment with 10, 20, 40 and 60 µM RS, or 1000 µM H_2O_2 compared to baseline ROS levels in controls (NT). FACS results were analyzed by "M2 and M1 percentage" of fluorescence variation. Cells without DCFH-DA treatment served as negative control (NG) to set M1 (non-stressed cells) and M2 (stressed) boundary.

(**ai**) Representative images of the M1 and M2 cell populations in control cells (NT) depicted by fluorescence of DCF on FACS-FL-1 channel (525 nm). (**aii**) Graph showing % M2, representing fluorescence emission for ROS species. RS-treated cells (20, 40 and 60 µM) showed a reduction of intracellular ROS, which was not significant at the lowest doses (10 µM). After 24 h treatment with 1000 µM H_2O_2, the surviving cells showed a marked reduction of intracellular ROS compared to CT only. RS pre-conditioning further reduced intracellular ROS level as a consequence of 1000 µM H_2O_2 even at the lowest doses (10 µM).Data are expressed as mean ± s.e.m. of biological triplicates. *P*-value calculated using a two-tailed Student's *t*-test.*: $P=0.03$ vs untreated cells (NT); ***: $P<0.0001$ vs NT; ¤: $P<0.01$ vs 1000 µM H_2O_2. DMSO (DM) did not differ significantly from NT.

Supplementary Figure 3



Supplementary Figure 3. Resveratrol induced mitochondrial membrane depolarisation in C2C12 myoblasts

Analysis of mitochondrial membrane depolarisation, indicated by a fluorescent emission shift from green (~525 nm) to red (~590 nm). **a**) Representative images of each condition and the cell percentages in each gate (*Upper Left*-polarised mitochondria, UL; *Upper Right*-mixed cell population i.e. polarised and depolarised mitochondria, UR; *Lower Right*-depolarised mitochondria, LR). The total % JC-1 green fluorescence cell population, including shift in depolarisation (gate UR+LR) was calculated. Percentages of depolarised cells under different conditions are reported in the graph (**ai**). (**b**) Graph showing cell

viability of myoblasts after 24 h RS treatment. 24 h RS induced mitochondrial membrane depolarisation, which was higher at higher RS doses. This was associated with significant reduction of cell viability. Data are expressed as mean ±s.e.m. of biological quadruplicate. *P*-values calculated using a two-tailed Student's *t*-test. In **ai**; gate UR+LR (shift to depolarised state+depolarised cells): ‡: $P<0.05$ vs. CT; gate UR (shift to depolarised state): *: $P<0.04$ vs CT. In **b**, *: $P<0.05$ vs CT; ns: no statistical differences vs. CT.