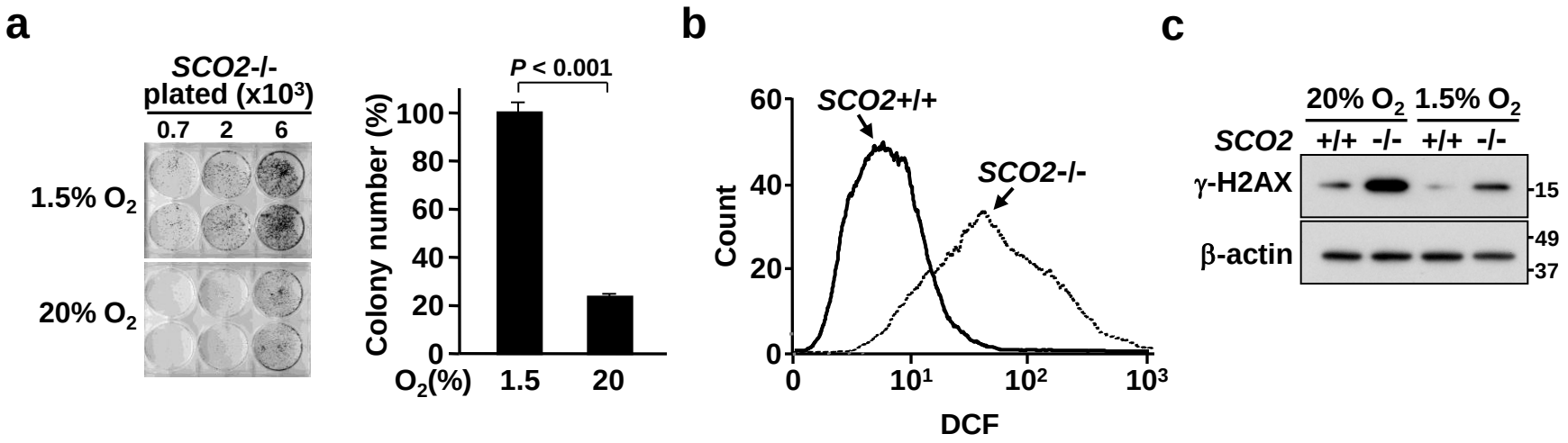
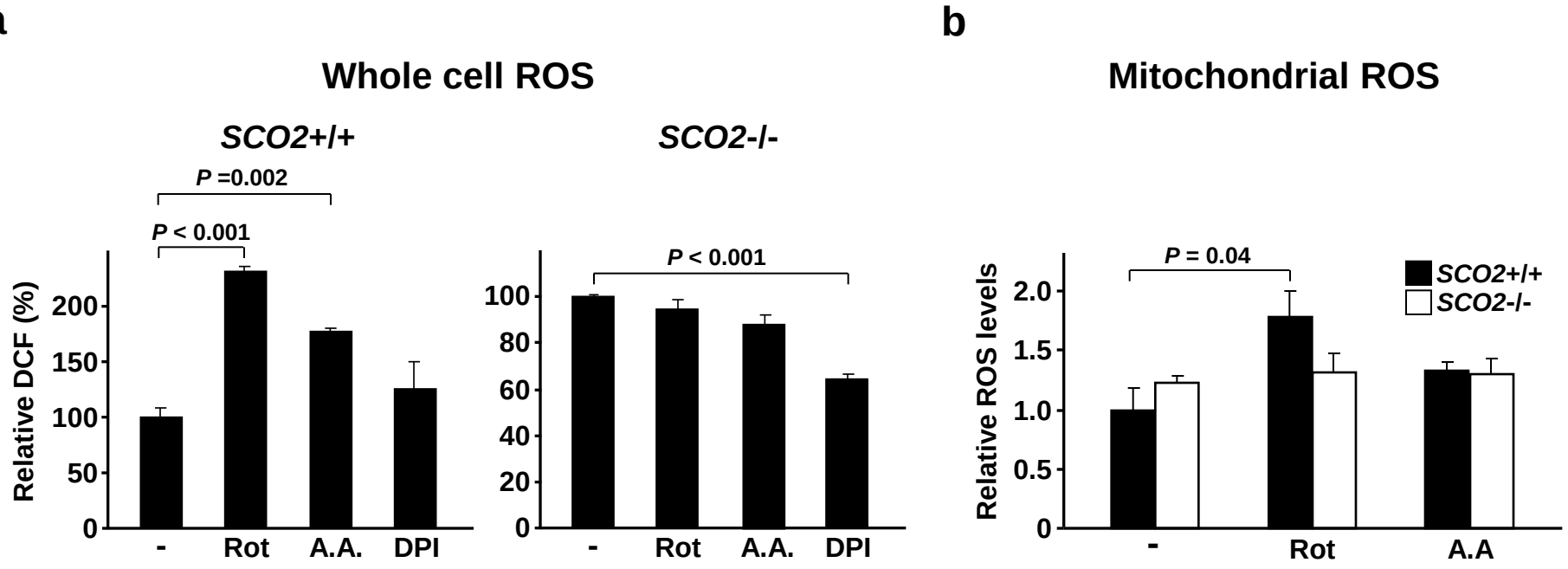
Supplementary Figure 1



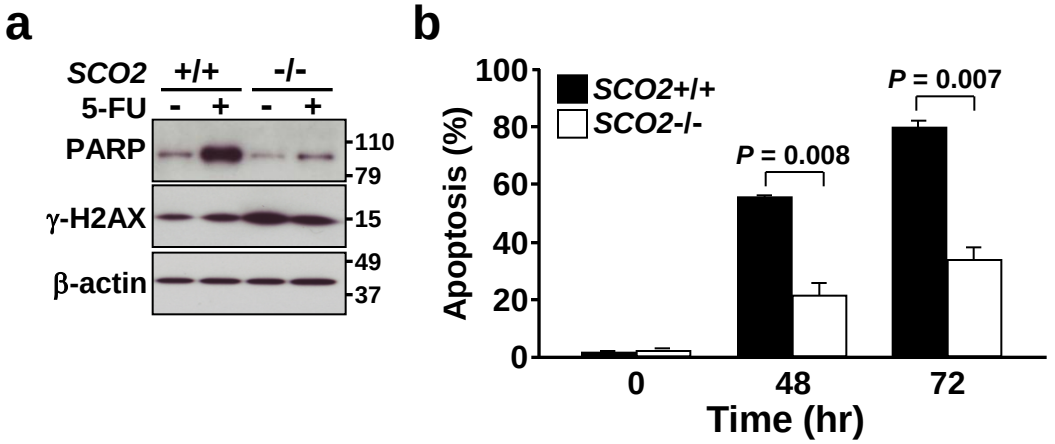
Supplementary Figure 1. An independently derived *SCO2-/-* clone also displays increased ROS production, increased DNA damage response and growth inhibition by oxygen. An independent *SCO2*-/- clone was generated by AAV-mediated homologous recombination and selected under 1.5% oxygen. **a**, Cells were plated at the indicated densities in 6-well plates, cultured under 1.5% or 20% oxygen for 10-12 d, colony formation visualized and quantified by crystal violet staining (n = 4). **b**, ROS levels were detected by DCF staining and quantified by FACS analysis. **c**, Cells were exposed for 48 h at the indicated oxygen concentration and γ-H2AX DNA damage response detected by western blotting. Values shown as mean and s.e.m.; *P*-values determined by Student's *t*-test.

Supplementary Figure 2



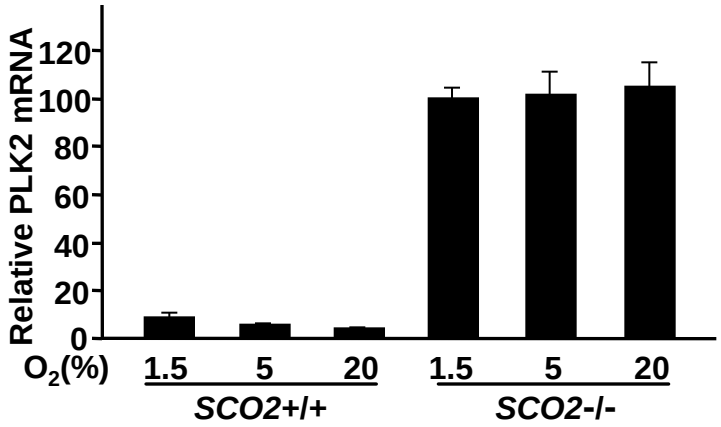
Supplementary Figure 2. ROS production in *SCO2-/-* cells is sensitive to the cytoplasmic NADPH oxidoreductase inhibitor but not to mitochondrial respiratory inhibitors. **a**, Relative whole cell ROS production measured by DCF FACS analysis after treatment with mitochondrial respiratory inhibitors rotenone (Rot) or antimycin A (A.A.), or cytoplasmic NADPH oxidoreductase inhibitor DPI, (n = 3). Cells were attached for 24 h before being treated with 0.1 μM rotenone, 10 μM antimycin A or 1 μM DPI for 24 h. To measure mitochondrial ROS production, mitochondria were purified from cells by standard differential centrifugation and 10 μg of mitochondrial protein was used for ROS detection by the Amplex Red kit (Invitrogen) per manufacturer's instruction [1]. Fluorescence signals were collected using the VICTOR 3TM fluorescence reader (PerkinElmer). **b**, ROS production in response to rotenone or antimycin A was determined in purified mitochondria using Amplex Red (n = 3). Values shown as mean and s.e.m.; *P*-values determined by Student's *t*-test.

Supplementary Figure 3



Supplementary Figure 3. DNA damage response and apoptosis are dissociated. **a**, Western blot of cleaved PARP and γ-H2AX proteins after 24 h of 5-FU treatment under 20% oxygen condition. *SCO2*+/+ cells undergo apoptosis as expected but do not show increased DNA damage response. *SCO2*-/- cells show high basal DNA damage response but do not significantly increase their level of apoptosis or DNA damage response after 5-FU treatment. **b**, Confirmation of the apoptotic response by Hoechst staining using an Axioskop2 Plus fluorescence microscope with Axiovision software (version 4.6) [2] after 5-FU treatment at the indicated times in *SCO2*+/+ and *SCO2*-/- cells (n = 4). Values shown as mean and s.e.m.; *P*-values determined by Student's t-test.

Supplementary Figure 4



Supplementary Figure 4. PLK2 expression in *SCO2-/-* cells is not affected by oxygen. After exposing cells to the indicated oxygen concentrations for 48 h, PLK2 mRNA expression levels were quantified and normalized using an ABI HT7900 real-time PCR thermal cycler (Applied Biosystems) as previously described [3]. Values shown as mean and s.e.m. relative to 1.5% oxygen exposed *SCO2-/-* cells (n=3).

REFERENCES

1. Frezza, C., Cipolat, S. & Scorrano, L. Organelle isolation: functional mitochondria from mouse liver, muscle and cultured fibroblasts. *Nat Protoc* **2**, 287-295 (2007).
2. Waldman, T., Lengauer, C., Kinzler, K. W. & Vogelstein, B. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. *Nature* **381**, 713-716 (1996).
3. Matsumoto, T. et al. Polo-like kinases mediate cell survival in mitochondrial dysfunction. *Proc Natl Acad Sci U S A* **106**, 14542-14546 (2009).