



**Assessment of ROS production with the fluorescent probes dihydroethidium (DHE) and dihydrorhodamine 123 (Rho123), and effect of *Endog* silencing/deletion and ROS inhibition with N-Acetyl-L-Cysteine (NAC, 200  $\mu$ M), Glutathione (GSH, 2 mM) or MitoTEMPO™ (MTP, 25  $\mu$ M).** **A)** Rat-1 cells were left untreated (NT) or treated 30 min with Antimycin-A 50  $\mu$ M (antibiotic interrupting the electron flux through the electron transport chain and inducing  $O_2^{\cdot-}$  within the mitochondria), or with 0.1  $\mu$ M Phorbol myristate acetate (PMA, an agonist of PKC, which in turn activates NADPH oxidase generating  $O_2^{\cdot-}$  in the cytosol), and fluorescence was measured after Rho123 staining (N=3). **B)** *Endog*<sup>+/+</sup> and *Endog*<sup>-/-</sup> skin fibroblasts were treated with NAC, GSH or MTP, stained with Rho123 and fluorescence was detected as described in the Materials and Methods section (N=3; 3 *Endog*<sup>+/+</sup> and 3 *Endog*<sup>-/-</sup> plates). **C)** *Endog*<sup>+/+</sup> and *Endog*<sup>-/-</sup> skin fibroblasts were treated and cultured as in B, counted at time 0 and 48 h later and proliferation was calculated as cell cycles in 48h. **D)** Rat-1 fibroblasts were transduced with lentiviral particles carrying scrambled (Scr) or *Endog*-specific shRNA silencing (shRNA) constructs and cultured as described in the Materials and Methods section. Cells were left untreated or treated with NAC during 48 hours and fluorescence was measured after DHE staining (N=5). **E)** Rat-1 cells were transduced with Scr or *Endog*-specific shRNA and treated with NAC, GSH or MTP and fluorescence (ROS) was quantified after Rho123 staining (N=4). **F)** Following the same treatments as in (E), cells were counted at time 0 and 48 h later and proliferation was calculated (N=10). One-way ANOVA or 2W ANOVA, followed by Tukey's test were performed to analyze the global effects of treatments on the measured parameter, interaction between treatment and *Endog* expression, and against untreated (NT) cells (A, B, C, E, F). Ns: not significant, \*, P<0.05; \*\*, P<0.01, \*\*\*, P<0.001, \*\*\*\*, P<0.0001. Plots depict each experimental measurements plus means  $\pm$  SD.