

Figure S1

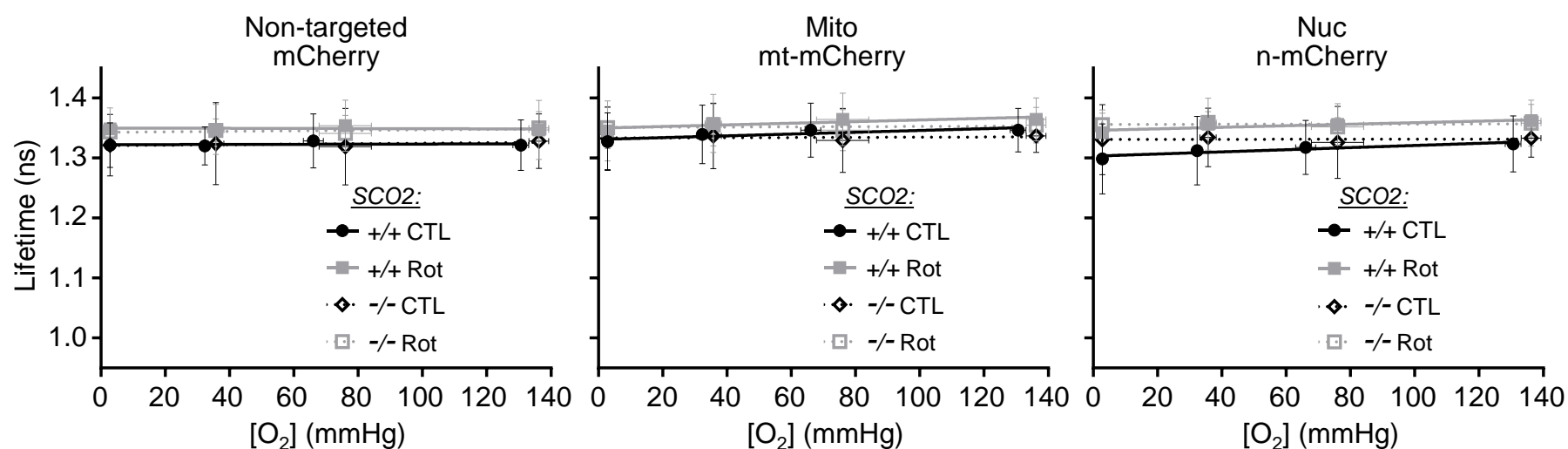


Figure S1. The effects of refractive index on the lifetime values of mCherry in the cytosolic, mitochondrial, and nuclear compartment. *SCO2*^{+/+} and *SCO2*^{-/-} cells were transfected with mCherry (without MB) that was non-targeted (“cytosol”) or targeted to the mitochondria (Mito, TFAM MTS fused mt-mCherry) or to the nucleus (Nuc, SV40 NLS fused n-mCherry) to serve as corrections for the refractive index. The cells were treated with diluent DMSO as control (CTL) or rotenone (Rot, 1 μ M), and FLIM analysis performed. The imposed [O₂] (in mmHg) in the medium was measured at O₂ levels of 0.5, 5, 10, and 18.6% (corresponding to a range of ~2.8 to 130 mmHg) using an OxyLite Pro 1 probe. mCherry has a mean lifetime value of ~1.32 ns in non-respiring *SCO2*^{-/-} cells and this number increased by a maximum of 3% only in rotenone treated respiring *SCO2*^{+/+} cells, possibly due to the effects of the refractive index or pH changes. The effects of the refractive index on the lifetime data was calculated from the lifetime values measured for *SCO2*^{+/+} CTL and *SCO2*^{+/+} Rot. Values are mean \pm SD.

Figure S2

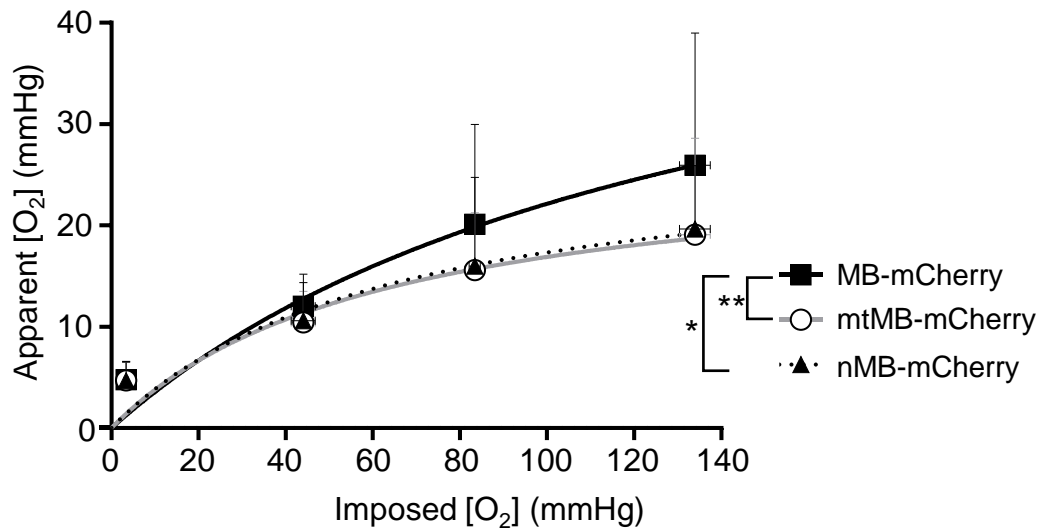
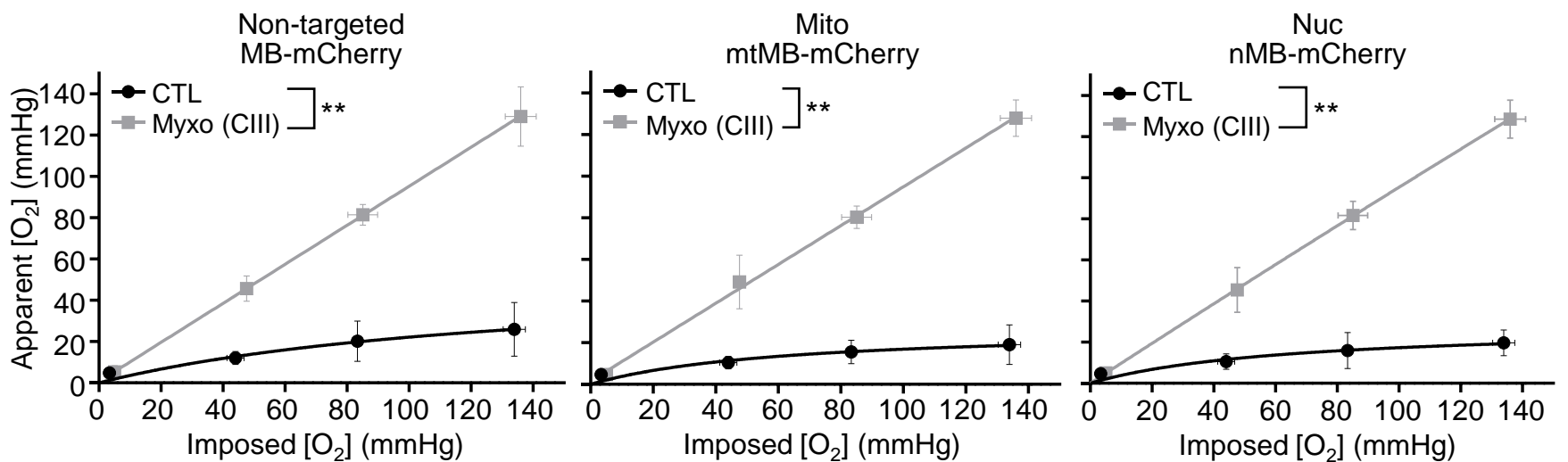
A**B**

Figure S2. Subcellular measurement of [O₂] in HEK293T cells and the effect of mitochondrial respiration. HEK293T cells were transfected with non-targeted MB-mCherry, mitochondrial mtMB-mCherry (Mito), and nuclear nMB-mCherry (Nuc). A, FLIM measurements were made under different imposed O₂ levels ranging from 0.5% (~2.8 mmHg) to 18.6% (~130 mmHg) and used to estimate apparent compartmental [O₂]. B, Apparent [O₂] measurements of untreated control (CTL) cells or those treated with inhibitor of mitochondrial respiratory complex III (CIII) myxothiazol (Myxo). Except at the lowest imposed [O₂] (~2.8 mmHg), there was a statistically significant difference between the apparent [O₂] values of the indicated probes or treatment conditions. Statistical difference by 2-way ANOVA with Tukey's post-test. Values are mean ± SD. **P* < 0.05; ***P* < 0.01.