SUPPLEMENTARY MATERIAL

Analysis of the stimulated $O_2^{\cdot-}$ production by Cb_5R using DHE

Data analysis was performed based on the Cyt *c* stimulated $O_2^{\cdot-}$ production by C*b*$_5$R (sensitive to SOD) that reacts with DHE [9, 10] and Cyt *c* [11] following the two mainly proposed reactions:

1) $$DHE + O_2^{\cdot-} \rightarrow 2OH\text{-}E^+$$

2) $$\text{oxidized Cyt } c + O_2^{\cdot-} \rightarrow \text{reduced Cyt } c + O_2$$

Therefore, a K_m and k_{cat} can be determined using a two-substrate Michaelis-Menten kinetic model and data were analyzed by using the following equation:

$$v = \frac{[E]_0}{\left(\frac{1}{k_{\mathrm{cat}}}\right) + \left(\frac{K_{\mathrm{mA}}}{k_{\mathrm{cat}}[A]}\right) + \left(\frac{K_{\mathrm{mB}}}{k_{\mathrm{cat}}[B]}\right)}$$

Where: *v* is the initial rate concentration; $[E]_0$ is C*b*$_5$R concentration; [A] and [B] are Cyt *c* and DHE concentrations, respectively, and K_{mA} and K_{mB} are their corresponding K_m values.

Details for human soluble and membrane Cb_5R cloning

Commercially available construct for soluble CYB5R3 (GenScript; CloneID:OHu12696) with the inserted sequence of *Homo sapiens* NADH-cytochrome b5 reductase 3 isoform 2 and membrane CYB5R3 (GenScript; CloneID:OHu22339) with the inserted sequence of *Homo sapiens* cytochrome b5 reductase 3 (CYB5R3), transcript variant 1, were used as templates for cloning, as indicated in [5]. Primers (0.1µM)(FW- 5'-CAATGCCATGGCTATGAAGCTGTTCCAGCGC-3'and RW-5'-CCCAAGCTTGCCCCGTCCGAAGACGAAGCAGCGCTC-3') for the soluble isoform and the commercial plasmid (GenScript; CloneID:OHu12696) (20ng) were added to the buffer (MgCl2 1.5 mM, dNTPs (0.25 mM) and enzyme (NZY proof DNA polymerase kit cat#MB14601, NZYtech), and PCR was used to prepare the insert. The procedure for preparing the insert of the membrane isoform was the same with Primers (0.1µM) (FW- 5'- CAATG CCATGGGGGCCCAGCTC-3' and RV-5' CCC AAGCTTGCCCCGTCC GAAGACGAAGCAGCGCTC -3') using 20ng of plasmid (GenScript; CloneID:OHu22339). The adjusted PCR parameters were: 30 s at 95 ºC, 30 s at 60ºC and 60 s at 72ºC ºC for the soluble isoform, and 30 s at 95ºC, 30 s at 58ºC and 60 s at 72ºC for the membrane isoform. The insert was ligated into to the cut and dephosphorylated pet22d plasmid previously dephosphorylated.

Ligation was accomplished using the Rapid DNA Ligation kit (cat#11635379001, Roche). Transformation was performed and positive colonies were picked to grow in LB Ampicillin. Purification of plasmids was done to obtain stock solutions that were frozen at -80ºC.