

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 Cb_5R (sensitive to SOD) that reacts with DHE [9, 10] and Cyt c [11] following the two mainly proposed reactions:

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

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

Where: v is the initial rate concentration; $[E]_0$ is Cb_5R 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 (0.1 μ M)(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 (0.1 μ M) (FW- 5'- CAATGCCATGGGGGCCAGCTC-3' and RV-5' CCC AAGCTTGCCCCGTCCGAAGACGAAGCAGCGCTC -3') using 20ng of plasmid (GenScript; CloneID:OHu22339). The adjusted PCR parameters were: 30 s at 95 °C, 30 s at 60°C and 60 s at 72°C °C for the soluble isoform, and 30 s at 95°C, 30 s at 58°C and 60 s at 72°C 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 -80°C.