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Authentication of Key Biological and/or Chemical Resources

Recombinant proteins – Aim 1 will use recombinant human Cytoglobin (Cygb) as a key biologic, which we have generated and engineered. Recombinant human Cygb is produced in our laboratory as follows. The Cygb gene was transferred from the Cygb cDNA clone SC321813 (accession number NM_134268; from Origene, Rockville, MD) into the pET-28a plasmid (Novagen) using the NcoI/HindIII restriction sites. The plasmid pET28-HsaCygb was transformed into SoluBL21 cells (Genlantis) and protein expression and purification is carried out as previously reported for human neuroglobin with minor changes [1-4]. Site directed mutagenesis has been (or will be) performed using the QuikChange II site-directed mutagenesis kit (Stratagene, Palo Alto, CA) with suitable oligonucleotides. The nucleotide sequences were confirmed by DNA sequencing at the University of Pittsburgh, Health Sciences Core Research Facilities, Genomics Research Core.

Lentivirus – Aim 2 uses lentiviral plasmids to overexpress Cygb in SH-SY5Y and HEK293 cells. Lentiviral plasmids for the overexpression of wild-type and mutant Cygbs are (or will be) generated using the pLVX-AcGFP-N1 plasmid (Clontech). The wild-type Cygb pLVX-AcGFP-Cygb plasmid has been generated by cloning the Cygb gene Cygb cDNA clone SC321813 (accession number NM_134268; from Origene, Rockville, MD) into the pLVX-AcGFP-N1 plasmid (Clontech) using the EcoRI/BamHI restriction sites. Site directed mutagenesis of the resulting pLVX-AcGFP-Cygb plasmid has been (or will be) performed using the QuikChange II site-directed mutagenesis kit (Stratagene, Palo Alto, CA) with suitable oligonucleotides. The nucleotide sequences were confirmed by DNA sequencing at the University of Pittsburgh, Health Sciences Core Research Facilities, Genomics Research Core.

Antibodies – Our antibody of choice against Cygb (sc-66855, Santa Cruz) is validated by immunoblot against the recombinant protein produced as described above. The specificity of other antibodies – GAPDH (ab-37168, Abcam), AIF (sc-13116 antibody, Santa Cruz), is validated by immunoblotting.

Cell lines – Human neuroblastoma SH-SY5Y cells and human HEK293 cells are purchased from American Type Culture Collection, Manassas, VA (ATCC). Initial cell stocks from ATCC are expanded during passages 1 to 2 (HEK293) or 3 to 4 (SH-SY5Y), aliquoted and kept in liquid nitrogen. Samples of this secondary stock are kept to establish an authentication reference for later passages of the same cell line. Cells from the secondary stock are used for 20 passages or less before they are discarded, then new cells from the stock are used.

Lipids – Lipids (free fatty acids and phospholipids) are obtained from Sigma-Aldrich (St. Louis, MO) or Avanti Polar Lipids, Inc (Alabaster, AL)

Other reagents and chemicals – Reagents and chemicals not described above are obtained from commercial sources.

[1] Corti, P., M. Ieraci, and J. Tejero, *Characterization of zebrafish neuroglobin and cytoglobins 1 and 2: Zebrafish cytoglobins provide insights into the transition from six-coordinate to five-coordinate globins.* Nitric Oxide, 2016. **53**: p. 22-34.

[2] Tiso, M., J. Tejero, S. Basu, I. Azarov, X. Wang, V. Simplaceanu, S. Frizzell, T. Jayaraman, L. Geary, C. Shapiro, C. Ho, S. Shiva, D.B. Kim-Shapiro, and M.T. Gladwin, *Human neuroglobin functions as a redox-regulated nitrite reductase.* J Biol Chem, 2011. **286**(20): p. 18277-89. PMCID: 3093900.

[3] Tejero, J., C.E. Sparacino-Watkins, V. Ragireddy, S. Frizzell, and M.T. Gladwin, *Exploring the mechanisms of the reductase activity of neuroglobin by site-directed mutagenesis of the heme distal pocket.* Biochemistry, 2015. **54**(3): p. 722-33. PMCID:PMC4410703

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