Import of a major mitochondrial enzyme depends on synergy between two distinct helices of its presequence

Ester Kalef-Ezra, Dimitra Kotzamani, Ioannis Zaganas, Nitsa Katrakili, Andreas Plaitakis, Kostas Tokatlidis
Biochemical Journal

Supplementary Table 1
Clonings and mutagenesis design

| Constructs                  | Cloning Methodology | Restriction sites |
|-----------------------------|---------------------|-------------------|
| pSP64-GLUD1                 | Subcloning          | BamHI/EcoRI      |
| pSP65-GLUD2                 | Subcloning          | EcoRI/EcoRI      |
| pSP64-Δ53GLUD1              | PCR                 | BamHI/EcoRI      |
| pSP64-Δ53GLUD2              | PCR                 | BamHI/EcoRI      |
| pSP64-Δα1GLUD2              | PCR                 | BamHI/EcoRI      |
| pSP65-α1-DHFR*              | PCR                 | EcoRI/BamHI      |
| pSP65-α2-DHFR*              | Synthetic oligonucleotides | EcoRI/BamHI |
| pSP65-α1α2-DHFR*            | Synthetic oligonucleotides | EcoRI/BamHI |
| pSP64-α1-Δ53GLUD2*          | Synthetic oligonucleotides | PstI/BamHI |
| pSP64-α1α2-Δ53GLUD2*        | Synthetic oligonucleotides | PstI/BamHI |
| pEGFPN3-α1-EGFP**           | Synthetic oligonucleotides | EcoRI/BamHI |
| pEGFPN3-α2-EGFP**           | Synthetic oligonucleotides | EcoRI/BamHI |
| pEGFPN3-α1α2-EGFP**         | Synthetic oligonucleotides | EcoRI/BamHI |
| pEGFPN3-α2α1-EGFP**         | Synthetic oligonucleotides | EcoRI/BamHI |
| pEGFPN3-α1-Δ53GLUD2-EGFP*   | Synthetic oligonucleotides | XhoI/EcoRI |
| pSP65-GLUD2(R3A)            | Site-directed Mutagenesis |
| pSP65-GLUD2(K7A)            | Site-directed Mutagenesis |
| pSP65-GLUD2(R3A-K7A)        | Site-directed Mutagenesis |
| pSP65-GLUD2(R3A-K7P-R13A)   | Site-directed Mutagenesis |
| pSP65-GLUD2(R3A-L5A)        | Site-directed Mutagenesis |
| pSP65-GLUD2(L5P)            | Site-directed Mutagenesis |
| pSP65-GLUD2(K7P)            | Site-directed Mutagenesis |
| pSP65-GLUD2(K7P-P13A)       | Site-directed Mutagenesis |
| pSP65-GLUD2(G35R)           | Site-directed Mutagenesis |
| pSP64-GLUD1(R51D)           | Site-directed Mutagenesis |
| pSP64-GLUD1(R50G-R51D)      | Site-directed Mutagenesis |
| pSP64-GLUD1(Y53D)           | Site-directed Mutagenesis |
| pSP64-GLUD1(Y53D-S54D)      | Site-directed Mutagenesis |
| pSP65-GLUD2(Y53D)           | Site-directed Mutagenesis |
| pSP65-GLUD2(Y53D-S54D)      | Site-directed Mutagenesis |
| pSP65-α3(R3A)-DHFR         | Site-directed Mutagenesis |
| pSP65-α3(R3A-L5A)-DHFR     | Site-directed Mutagenesis |

* to ensure that only one translation product was obtained we mutagenised the first methionine to glycine of DHFR or Δ53GLUD2 respectively

** to ensure that only one translation product was obtained we deleted the first methionine of EGFP.