# Identification and functional analysis of two mitoferrins, CsMIT1 and CsMIT2, participating in iron homeostasis in cucumber

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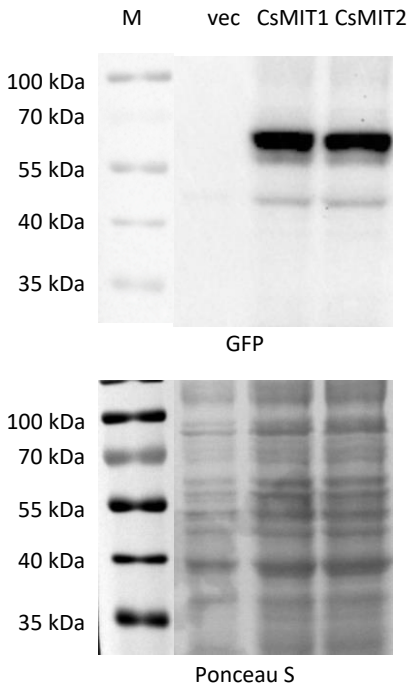


Figure S1- Immunoblotting of the mitochondria isolated from *Δmrs3Δmrs4* strain transformed with either pUG23-GFP (vec), pUG23-CsMIT1-GFP (CsMIT1) or pUG23-CsMIT2-GFP (CsMIT2) vector, using the antibodies against GFP.

Table S1. The sequences of primers used in this work. The restriction sites are underlined.

| Primers | Sequences (5'-3') |
|---|---|
| ***Primers used for amplification of full coding sequence of CsMIT1 and CsMIT2*** | |
| **for ligation of CsMIT1 into pUG23 vector** | forward: AAATCTAGAATGGCTACCGAGGCGA |
| | reverse: TTTGAATTCGGTACCGTTGTGGCTGC |
| **for ligation of CsMIT2 into pUG23 or pUG35 vector** | forward: AAAGAATTCATGGCCACAAGCGTATC |
| | reverse: TTTGTCGACATTGTTGTGATTGTGGAGATGT |
| **for ligation of CsMIT1 into pA7-GFP vector** | forward: AAAACTAGTATGGCTACCGAGGCGA |
| | reverse: TTTACTAGTAGGTACCGTTGTGGCTGC |
| **for ligation of CsMIT2 into pA7-GFP vector** | forward: AAAGTCGACATGGCCACAAGCGTATC |
| | reverse: TTTACTAGTATTGTTGTGATTGTGGAGATGT |
| ***Primers used for real-time PCR*** | |
| **CsMIT1** | forward: CAATTAAATCTGTTGGAGTTCGAC |
| | reverse: ACTTCTTACAATTCTCGTAAACTG |
| **CsMIT2** | forward: CTTAGGTGGTGGATCGTCA |
| | reverse: CCTCTTTCGCAAACTCGTA |
| **CsCACS [51]** | forward: TGGGAAGATTCTTATGAAGTGC |
| | reverse: CTCGTCAAATTTACACATTGGT |

Table S2. Strains and plasmids used in this work.

| Strain | Genotype | Source or reference |
|---|---|---|
| **DY150** | *MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-52* | [54] |
| **DY150 *mrs3mrs4*** | *mrs3::KanMX mrs4::KanMX* | [55] |
| **BY4742** | *MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0* | [56] |
| **BY4742 *zrc1*** | *zrc1::KanMX4* | Euroscarf, Germany |
| **BY4742 *cot1*** | *cot1::kanMX4* | Euroscarf, Germany |
| **BY4742 *ycf1*** | *ycf1*:: *kanMX4* | Euroscarf, Germany |
| **BY4742 *ace1*** | *ace1::KanMX4* | Euroscarf, Germany |
| **Plasmid** | **Description** | **Source or reference** |
| **pUG23** | *Escherichia coli*-yeast shuttle vector with the multiple cloning site (MCS) between the constitutive MET25 promoter and CYC1 termination sequence with C-terminal GFP reporter gene. HIS selectable marker and $Amp^r$ | [52] |
| **pUG35** | *Escherichia coli*-yeast shuttle vector with the multiple cloning site (MCS) between the constitutive MET25 promoter and CYC1 termination sequence with C-terminal GFP reporter gene. URA selectable marker and $Amp^r$ | [52] |
| **pA7-GFP** | pUC18-based vector for the transient expression of C-terminal GFP-fusion proteins in plant cells under the CaMV 35S promoter. $Amp^r$ | [53] |
| **pRS426** | *Escherichia coli*-yeast shuttle vector carrying the *gentisate 1,2-dioxygenase* (*GDO*) gene from *Pseudaminobacter salicylatoxidans* tagged with FLAG epitope. URA selectable marker and $Amp^r$ | kindly provided by Prof. Jerry Kaplan from University of Utah (USA) |