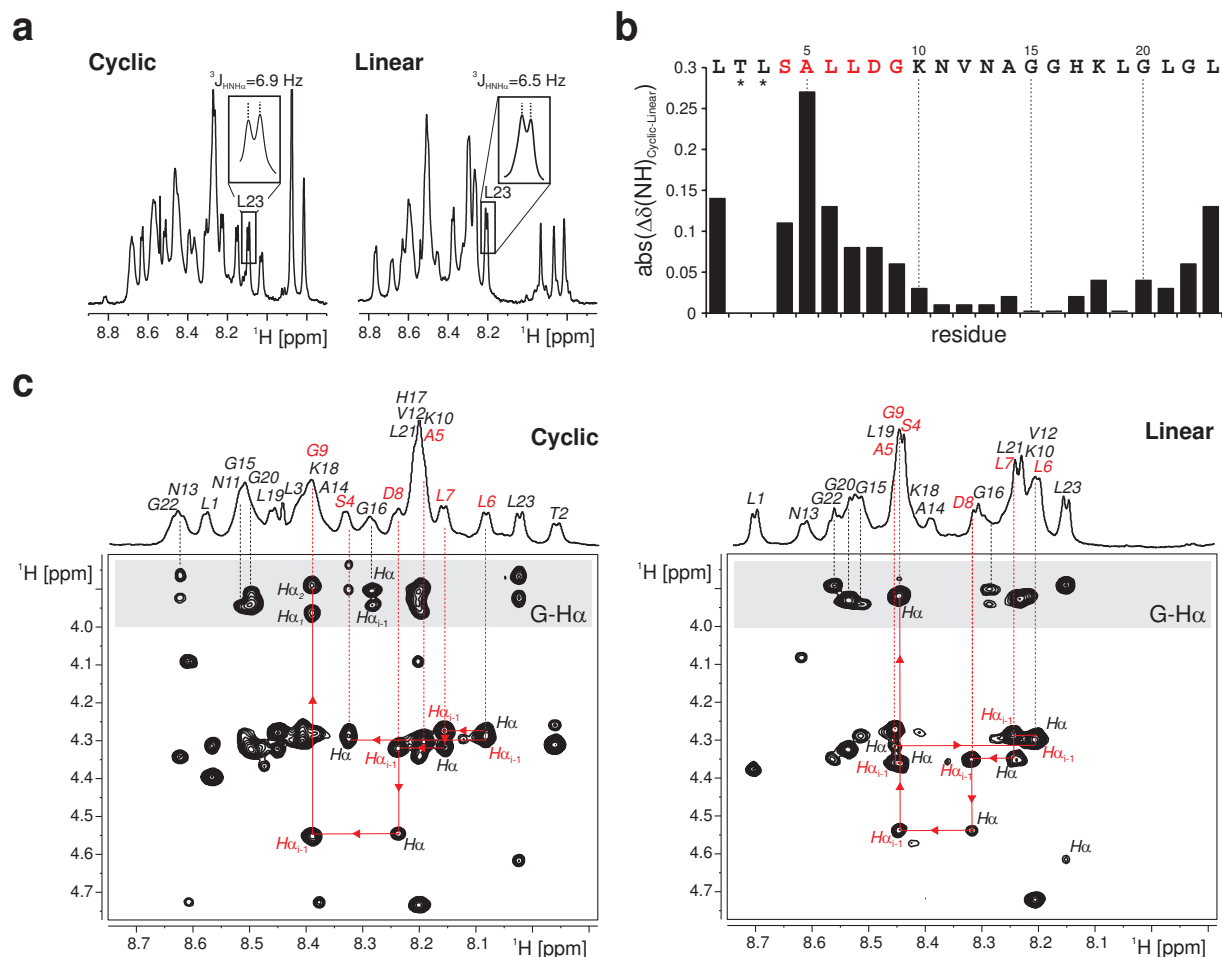
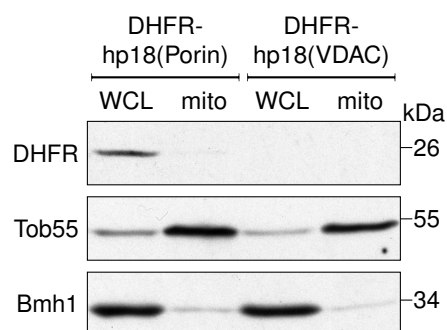
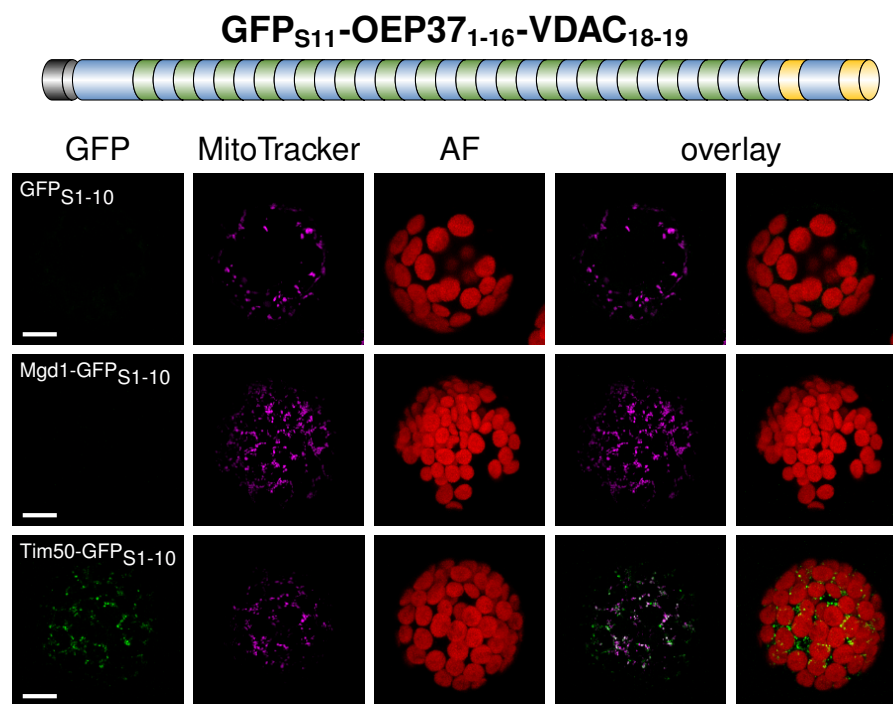




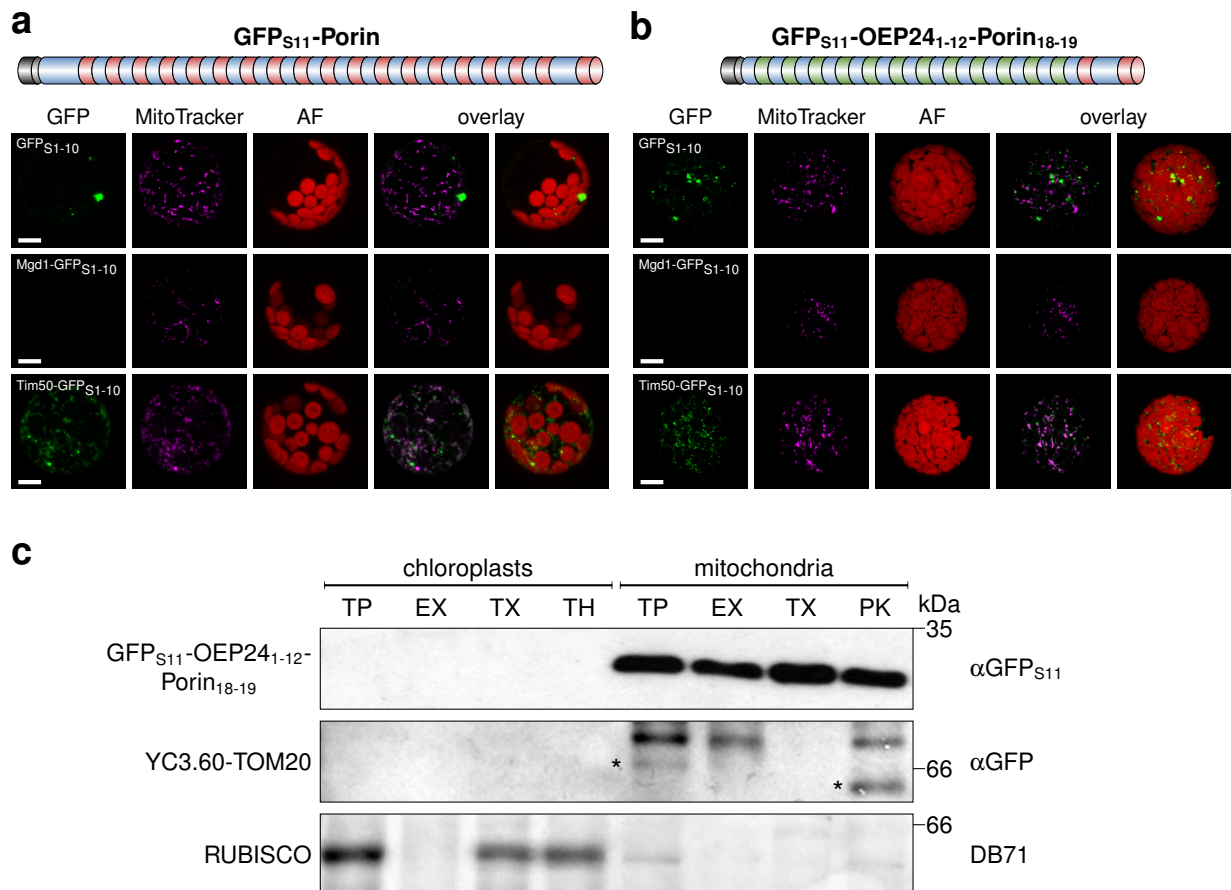
Supplementary Figure 1 | Structural analysis by NMR of the two peptides used for import competition. (a) Amide region of 1D ^1H spectra of the cyclic (left) and the linear (right) peptide at 10 $^\circ\text{C}$. The inset shows the amide resonance of L23. The $^3J(\text{HN},\text{H}\alpha)$ couplings splitting these resonances are indicated. (b) Amide proton chemical shift differences between the cyclic and the linear peptide at 5 $^\circ\text{C}$. The sequence stretch, for which the sequential assignment is shown in (c), is highlighted in red. Residues, for which the difference could not be determined due to a lack in NH assignment, are labeled with an asterisk. (c) HN-H α region of 2D $^1\text{H},^1\text{H}$ NOESY spectra of the cyclic (left) and the linear (right) peptides at 5 $^\circ\text{C}$. The assigned 1D ^1H spectrum of the amide region is shown on top. Intraresidual (HN-H α_i , labeled in black) and sequential (HN-H α_{i-1} , labeled in red) connections are indicated for the sequence stretch S4 to G9. The spectral region typical of glycine HN-H α cross peaks residues is labeled in grey.



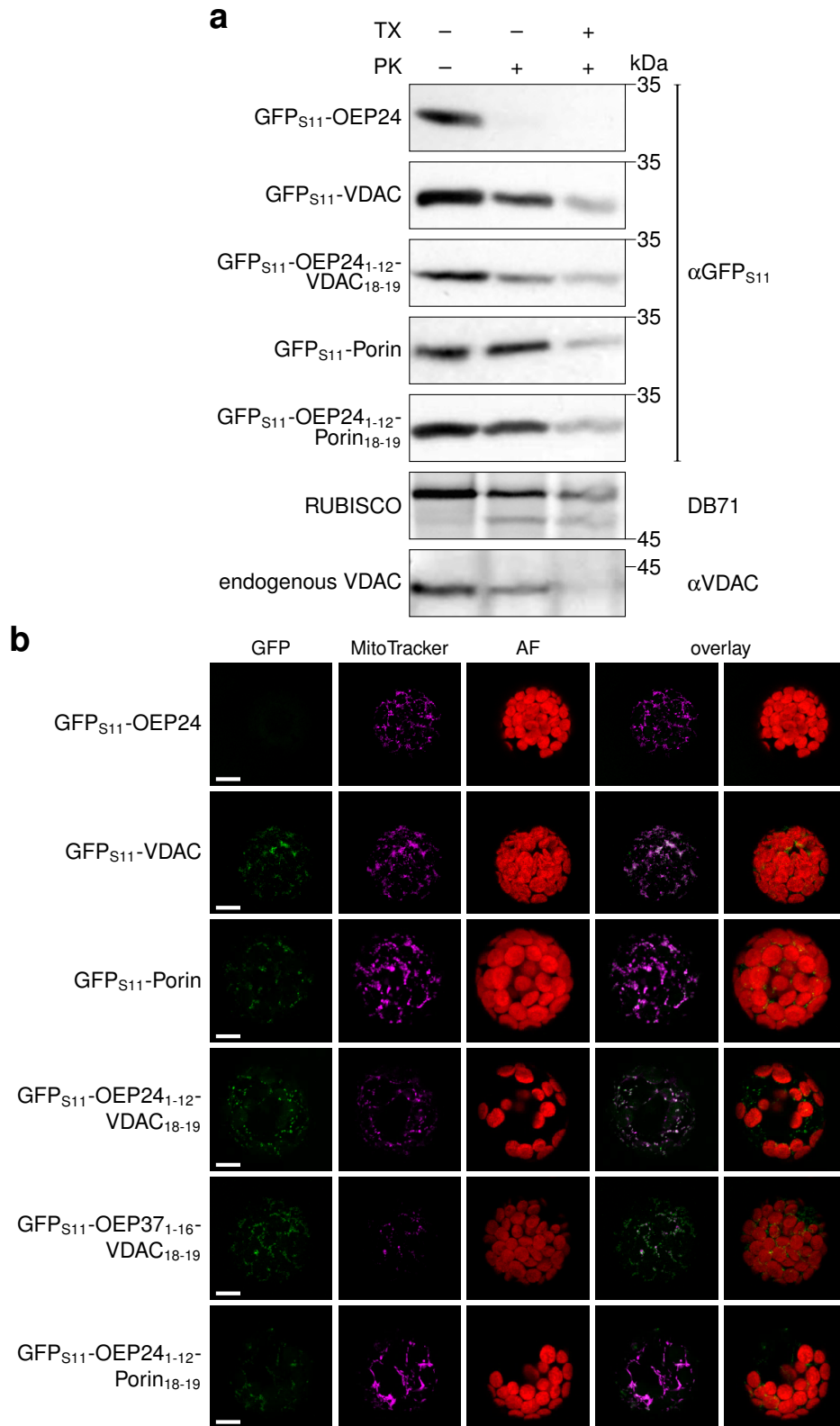
Supplementary Figure 2 | Fusion proteins with a β -hairpin fused C-terminally to DHFR are not targeted to mitochondria. Crude mitochondria were isolated from yeast cells expressing DHFR alone or the indicated fusion proteins. Samples from the whole cell lysate (WCL) and the crude mitochondria (mito) were analyzed by SDS-PAGE and immunodecoration with antibodies against the indicated proteins. Tob55, mitochondrial β -barrel protein; Bmh1, cytosolic protein.



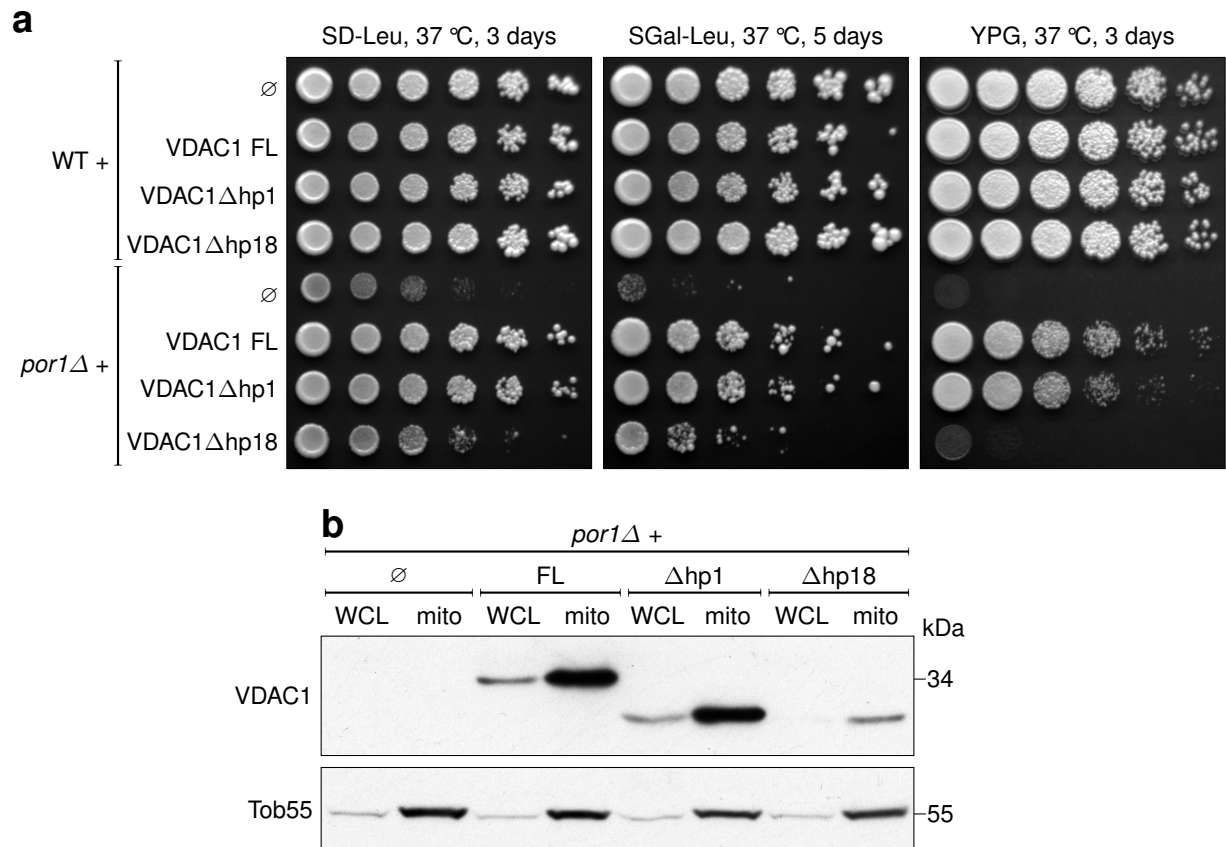
Supplementary Figure 3 | The last β -hairpin of atVDAC1 directs psOEP37 to mitochondria. GFP_{S11}-OEP37₁₋₁₆-VDAC₁₈₋₁₉ and the indicated reporter constructs were co-transformed into *A. thaliana* protoplasts. Signals and schemes are as in Fig. 3a. Scale bar: 10 μ m.



Supplementary Figure 4 | The last β -hairpin of scPorin targets psOEP24 to mitochondria. (a and b) GFP_{S11}-Porin (a) or GFP_{S11}-OEP24₁₋₁₂Porin₁₈₋₁₉ (b) and the indicated reporter constructs were co-transformed into *A. thaliana* protoplasts. Images were taken as described in the legend to Fig. 3a. Schemes of scPorin and the construct generated are shown. Red/green sections indicate transmembrane β -sheets of scPorin and psOEP24, respectively. The black section represents the GFP_{S11}-tag. Scale bar: 10 μ m. (c) Organelles were carbonate extracted (EX), solubilized by addition of Triton X-100 (TX), or treated with either thermolysin (TH) or proteinase K (PK). Further treatment and analysis were as described in the legend to Figure 3b. Bands resulting from cross-reactivity of the GFP antibody are marked with an asterisk.

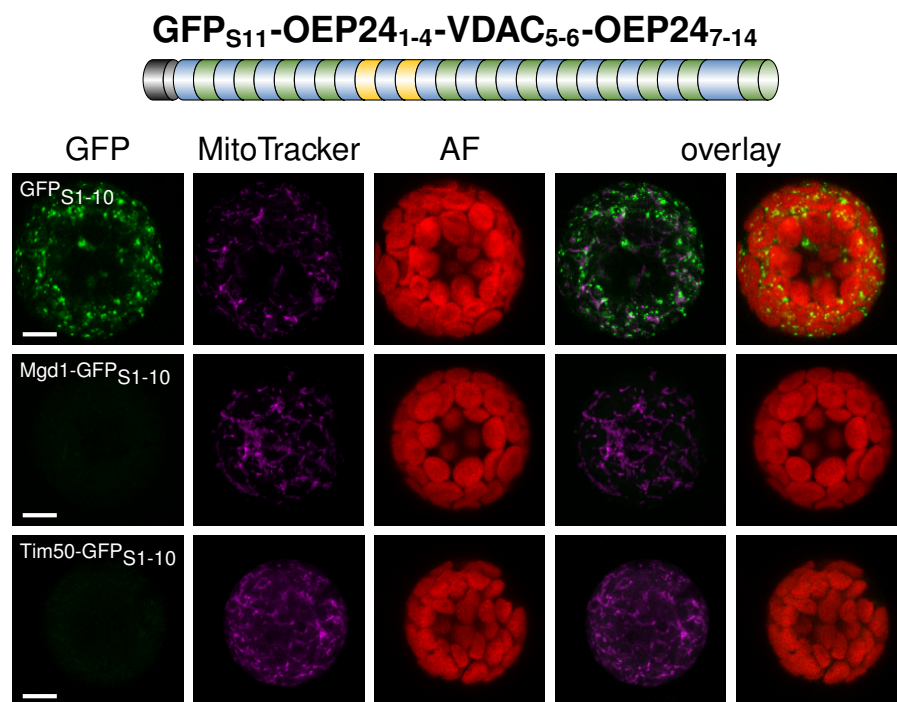


Supplementary Figure 5 | The last β -hairpin of atVDAC1 or scPorin targets chloroplast β -barrel proteins to the mitochondrial intermembrane space. (a) Plasmids coding for the indicated GFP_{S11}-tagged proteins were transformed individually into *A. thaliana* protoplasts. Protoplasts were lysed and incubated with proteinase K (PK, 5 μ g/mL final concentration) and Triton X-100 (TX, 1 % final) where indicated. After incubation, the cell lysate was subjected to SDS-PAGE followed by Western blotting with antibodies against GFP_{S11}. DB71 staining was used to visualize RUBISCO and immunodecoration with antibodies against the endogenous mitochondrial protein atVDAC1 was used to confirm the experimental procedure. (b) The indicated proteins and the reporter construct Tim21(N)-GFP_{S1-10} were co-transformed into *A. thaliana* protoplasts. Images were taken as described in the legend to Fig. 3a. Scale bar: 10 μ m.

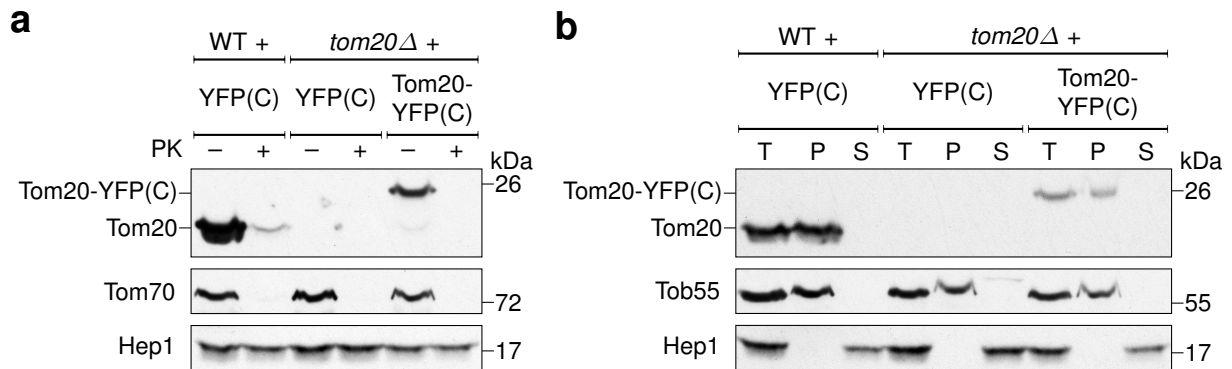


Supplementary Figure 6 | The last β -hairpin of atVDAC1 is important for mitochondrial targeting.

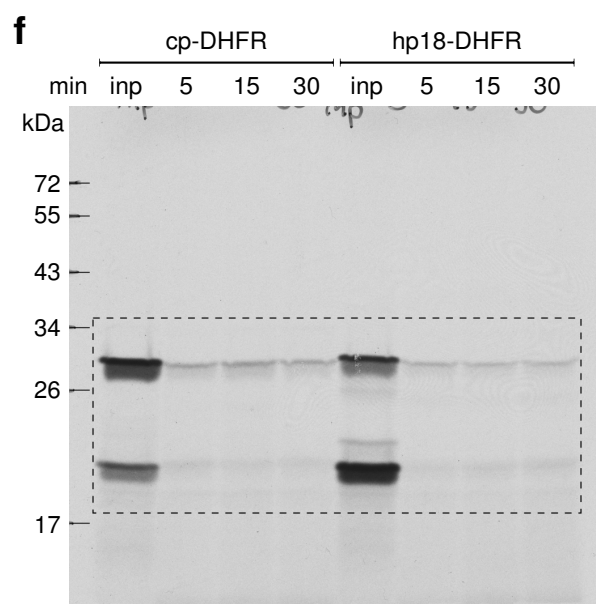
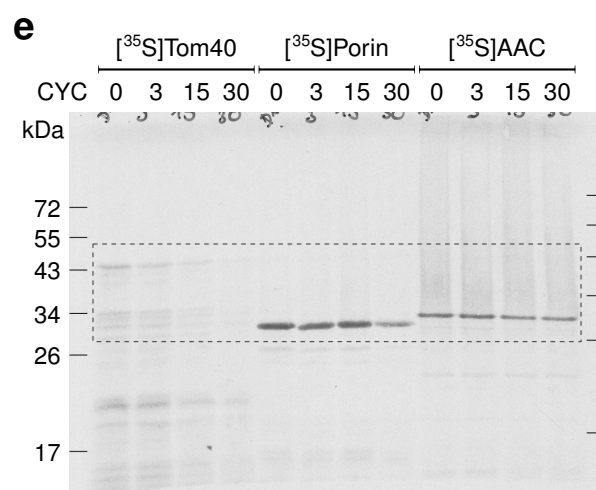
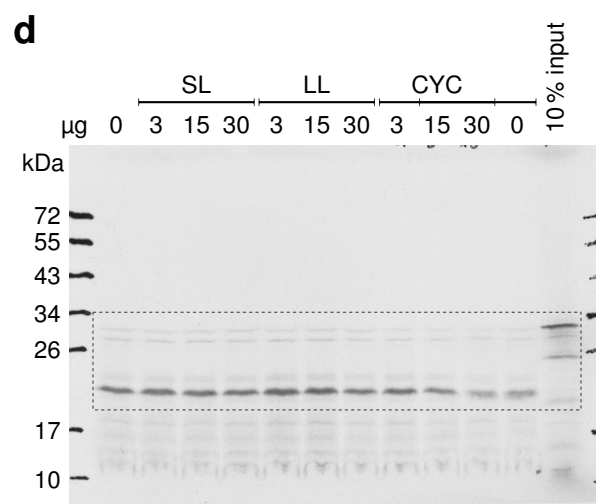
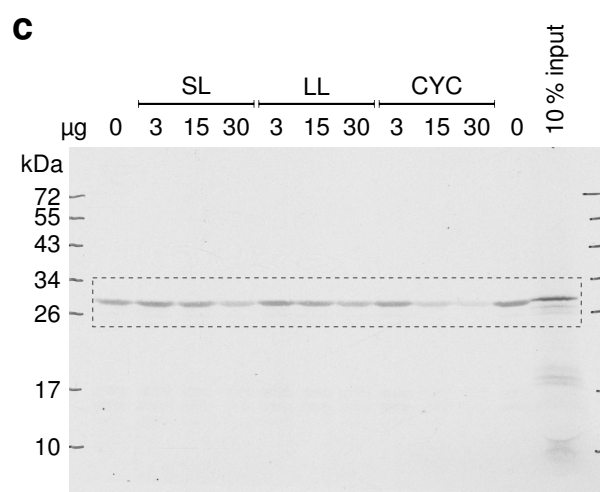
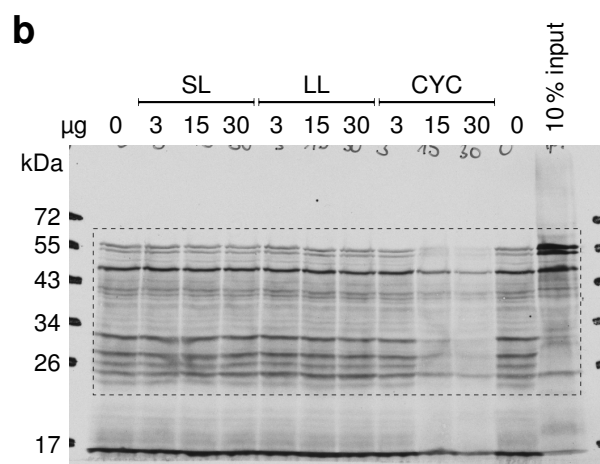
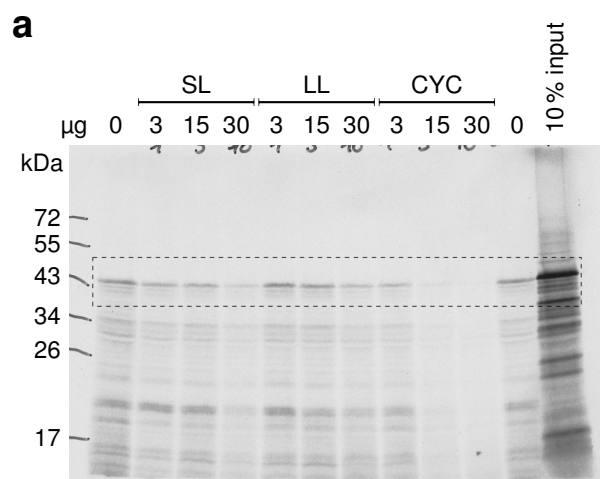
(a) WT and *por1Δ* cells were transformed with an empty plasmid (∅) or with a plasmid encoding either full-length VDAC1 (VDAC1 FL) or a truncated VDAC1 variant where the first (VDAC1Δhp1) or last (VDAC1Δhp18) β -hairpin was deleted. The growth of the cells at elevated temperature (37 °C) was analyzed by drop-dilution assay on the indicated media. (b) Crude mitochondria from the *por1Δ* cells described in (a) were isolated. The whole cell lysate (WCL) and the mitochondrial fraction (mito) were subjected to SDS-PAGE and immunoblotting with antibodies against VDAC1. Tob55 was used as a loading control.

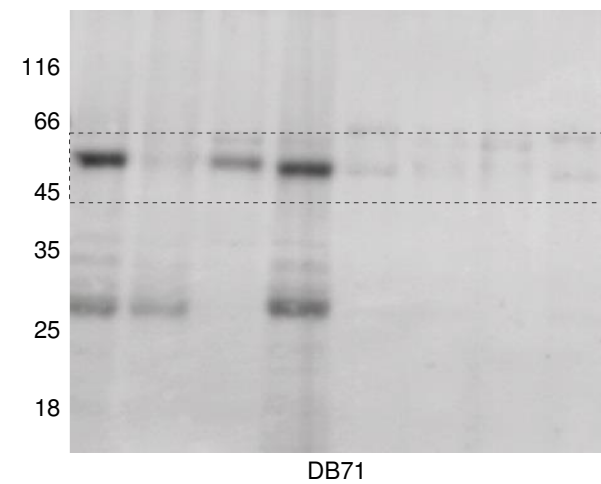
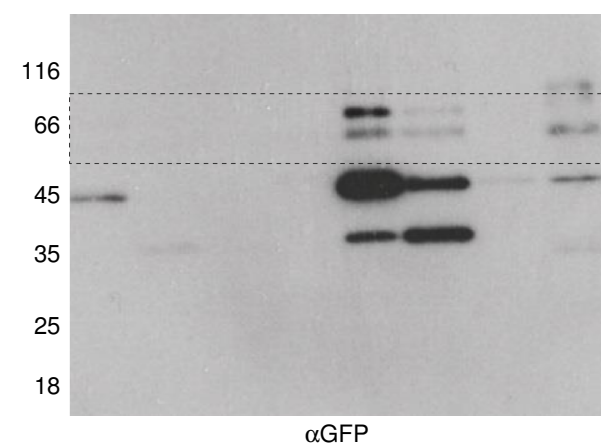
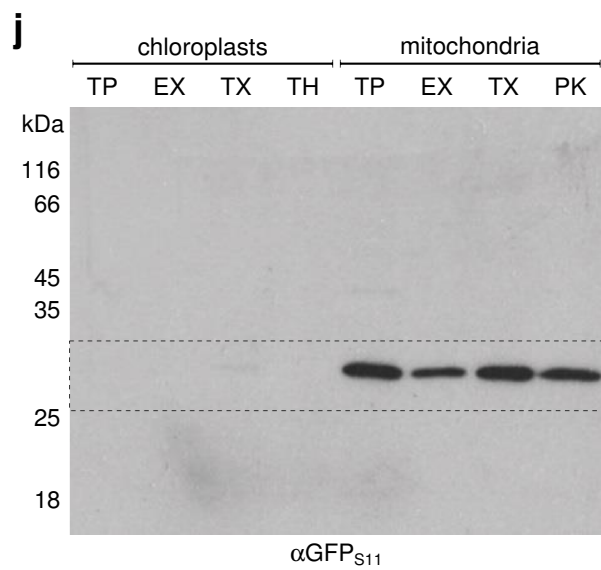
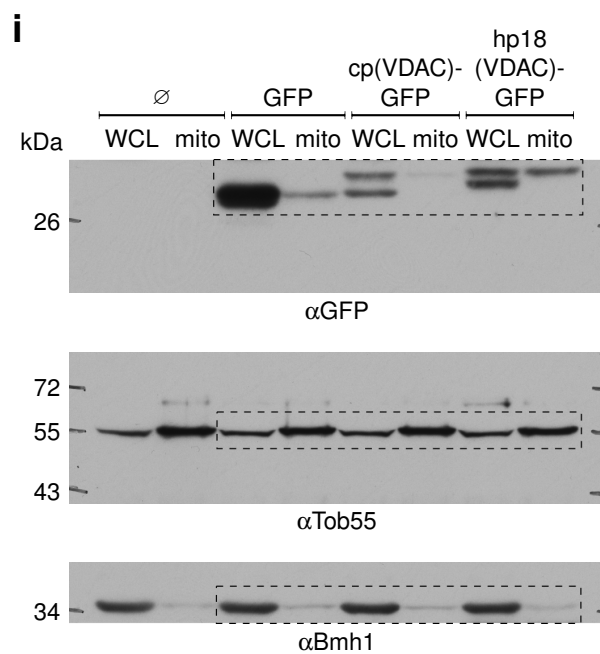
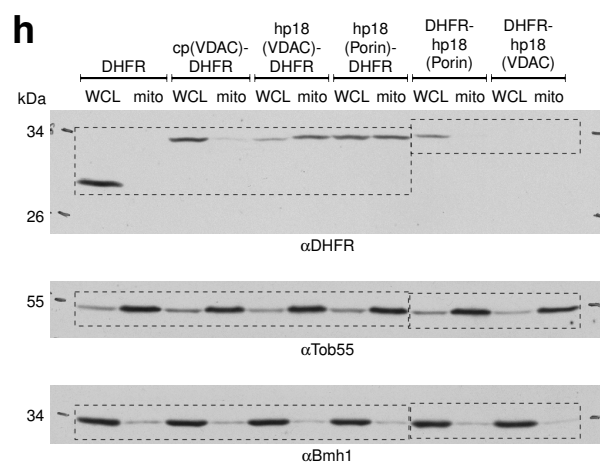
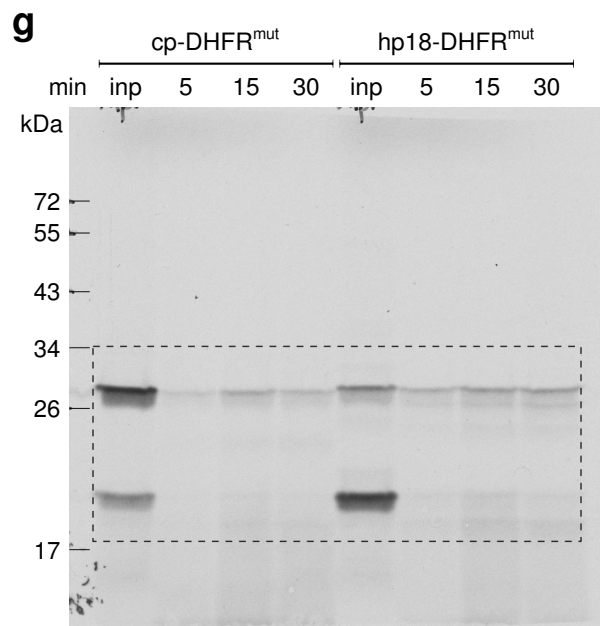


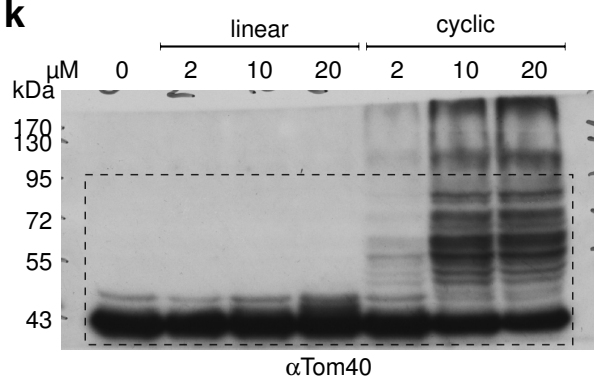
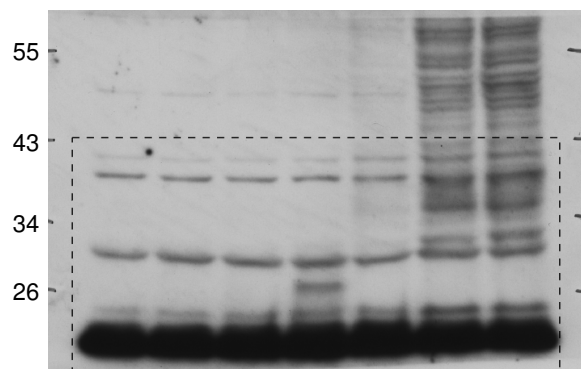
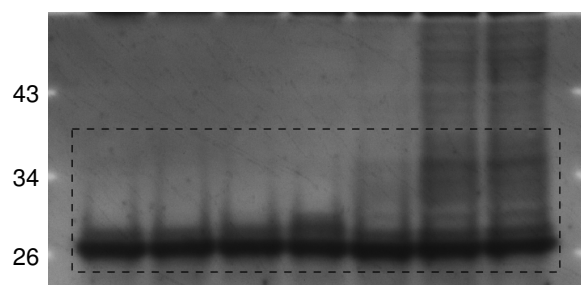
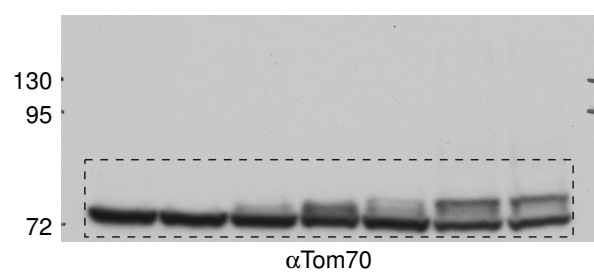
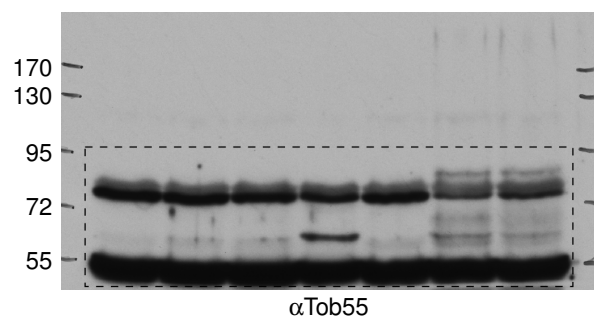
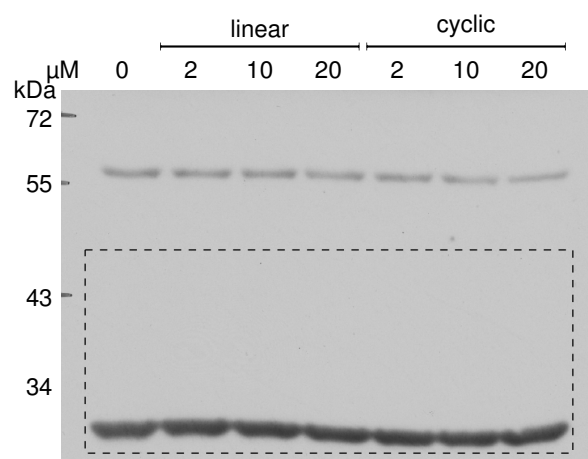
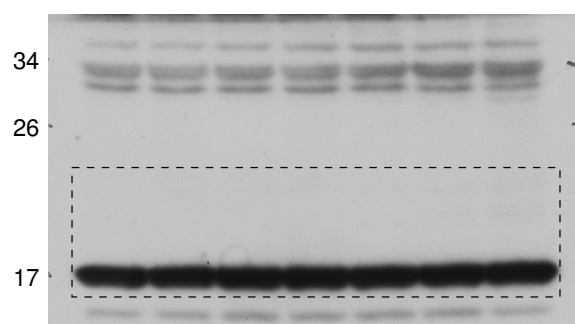
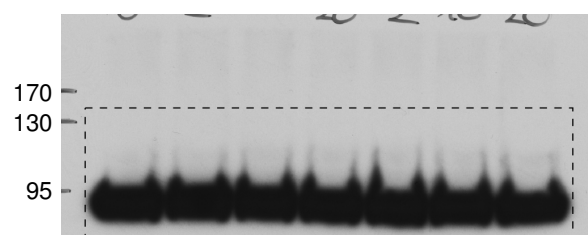
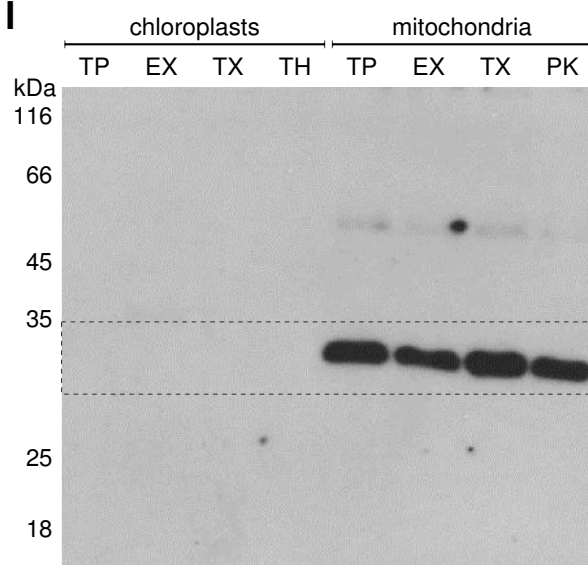
Supplementary Figure 7 | The fifth and sixth β -strands of atVDAC1 do not direct psOEP24 to mitochondria. The indicated fusion protein and reporter constructs were co-transformed into *A. thaliana* protoplasts. Signals and schemes are as in Fig. 3a. Scale bar: 10 μ m.

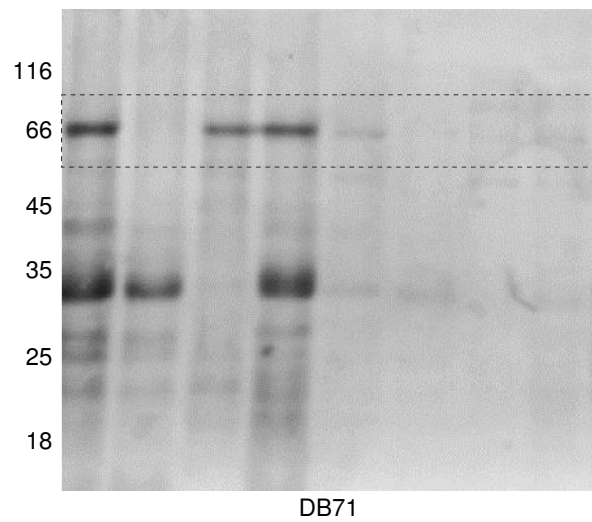
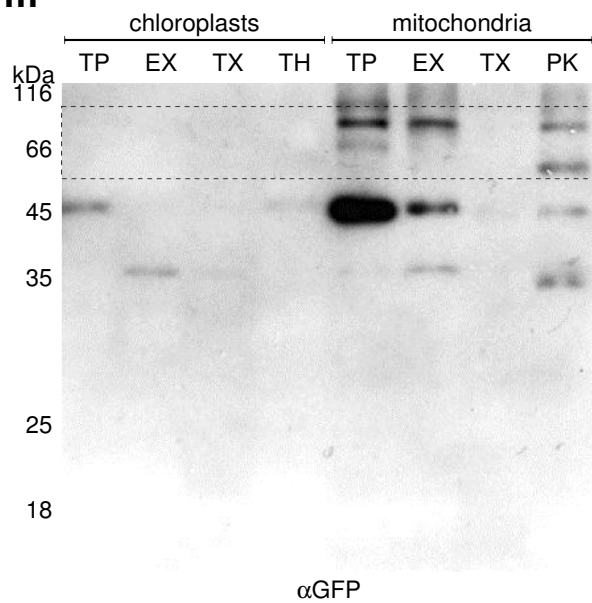
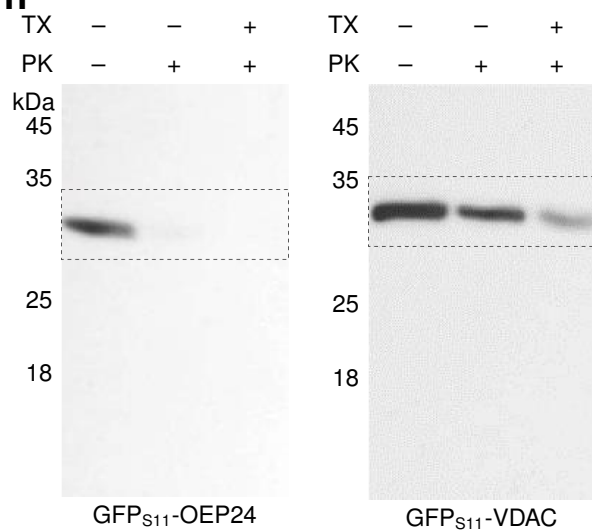
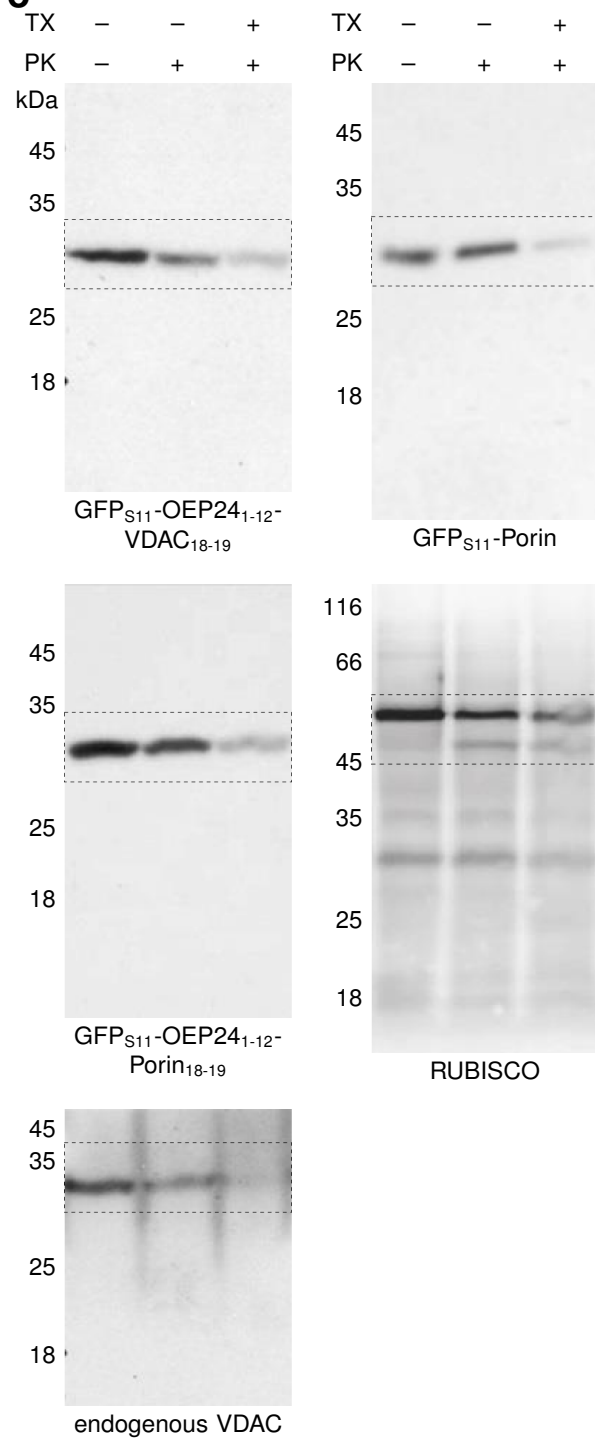


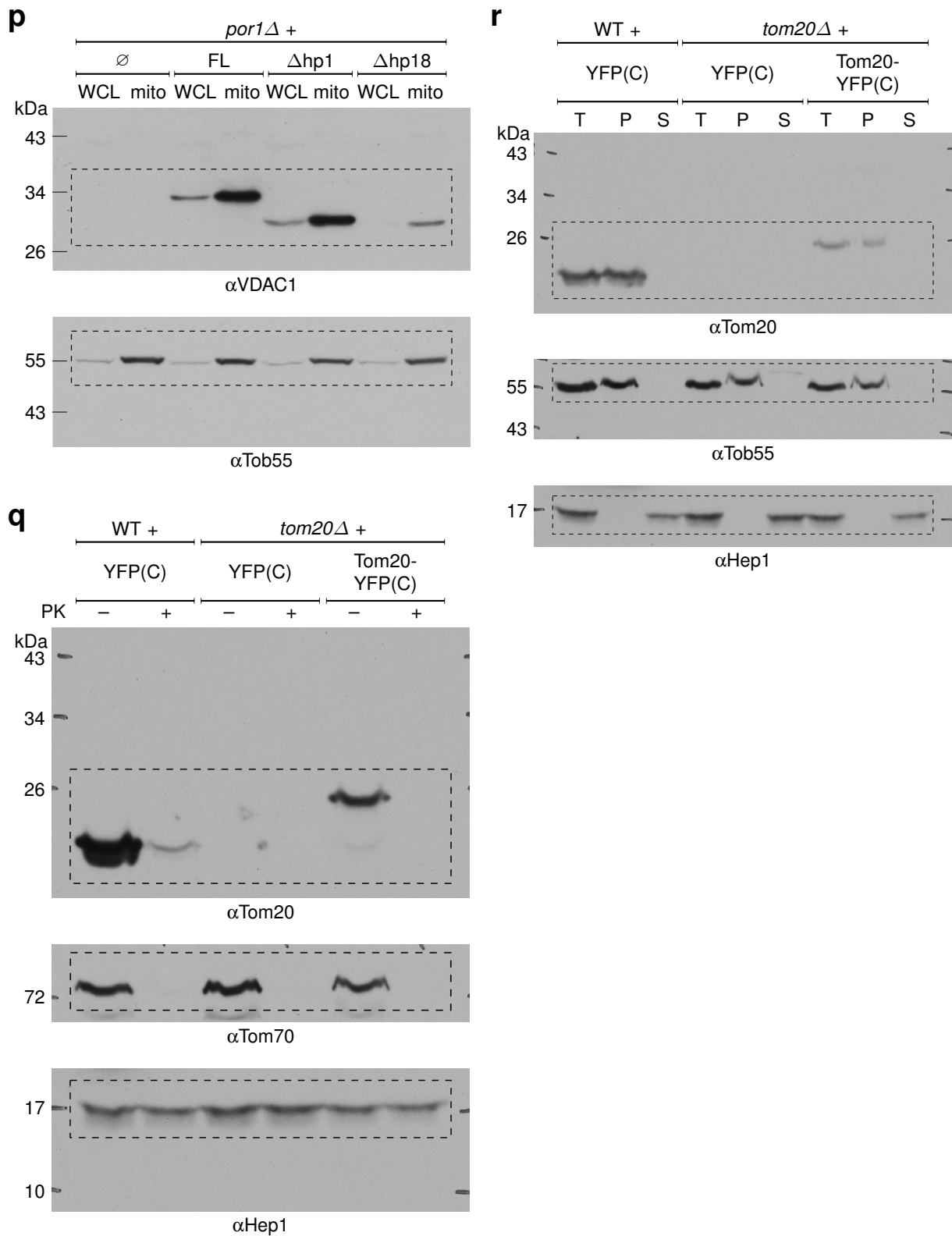
Supplementary Figure 8 | Tom20-YFP(C) is imported into the outer membrane of mitochondria in a native-like conformation. (a) Isolated mitochondria from either WT or *tom20Δ* strains expressing the C-terminal part of YFP (YFP(C)) or Tom20 fused N-terminally to YFP(C) were treated with 50 μg/mL proteinase K (PK) or left untreated. The samples were analyzed by SDS-PAGE and immunodecoration. Tom70, outer membrane protein exposed to the cytosol; Hep1, matrix protein. (b) Carbonate extraction of mitochondria isolated from the strains described in (a) was performed to separate membrane-embedded proteins in the pellet fraction (P) from soluble protein in the supernatant (S). T, total; Tob55, membrane-embedded β-barrel protein; Hep1, soluble matrix protein.





k α Tom40 α Tom20 α Tom22 α Tom70 α Tob55 α Porin α Fis1 α Aconitase**l** α GFP_{S11}

m**n****o**



Supplementary Figure 9 | Full immunoblots and autoradiography films. (a-r) Original images of the blots shown in Figs. 1b (a), 1c (b), 1d (c), 1e (d), 1f (e), 2a (f and g), 2c (h), 2f (i), 3e (j), 7 (k) and in Supplementary Figs. 2 (h), 4c (l and m), 5a (n and o), 6b (p) 8a (q), and 8b (r). Cropped areas are marked by dashed lines.

Supplementary Table 1 | Primers used in this study.

Construct	Primer	Sequence (5' → 3')
GFP _{S11} -VDAC	atVDAC-1_FW	AATGGTACCATGGTGAAAGGTCCCGGTCTCTACACC
	atVDAC-1_RV	AATACTAGTAGGCTTGAGTGCGAGAGCCAATCC
GFP _{S11} -OEP24 ₁₋₁₀ -VDAC ₁₆₋₁₉	OEP+4VD_FW	CTACCTATGATGTTGCTCCCTTGACCTCTGTGAAGG C
	OEP+4VD_RV	CACAGAGGTCAAGGGAGCAACATCATAGGTAGGTT C
GFP _{S11} -OEP24 ₁₋₁₂ -VDAC ₁₈₋₁₉	OEP+2VD FW	GACAAGGAATTCCAAACCCAAGTCATTCTTCACAAT C
	OEP+2VD_RV	GAAGAATGACTTGGGTTTGAATTCCTTGTCCTC
GFP _{S11} -OEP24 ₁₋₁₃ -VDAC ₁₉	O-L1VD_RV	GTACACTAGTAGGCTTGAGTGCGAGAGCCAATCCAA CTTTAGCACTCTTGTCATTGACTTTGTGTTAACGGA CGCCACAAC
	O-1VD_RV	CTAGTAGGCTTGAGTGCGAGAGCCAATCCAACTTTT TTGGGAATTTTC
GFP _{S11} -OEP24 ₁₋₄ -VDAC ₅₋₆ -OEP24 ₇₋₁₄	O(1-4)-V(5-6)- O(7-14)_ FW	TCTACTTTTCTGATCACCGCTACCGTTGATGAGGCT GCACCCGACTGAGGTCAATCTTCAGCTTCAAGGTT CCTGAGAAACCGTTGAATTTGGC
	O(1-4)-V(5-6)- O(7-14)_ RV	AGGAACCTTGAAGCTGAAGATTGACCTCAGTCCGG GTGCAGCCTCATCAACGGTAGCGGTGATCAGAAAA GTAGAGGGTTTCTCTACGGCGAGGACTAGGCC
GFP _{S11} -Porin	scPOR1Acc_FW	AATGGTACCATGTCTCCTCCAGTTTACAGCG
	scPOR1Bcu_RV	AATACTAGTAGCGTCGAAGGACAAAGACCAACC
GFP _{S11} -OEP24 ₁₋₁₂ -Porin ₁₈₋₁₉	O_2Por1_FW	CCTGGCGTCACTCTGGGTGTGGTTCTCTTTTCGAT GCTTTGAAGTTGTCTGAACCTGTTCAAGCTAGGT TGGTCTTTGTCTTCGACGCTACTAGTTAATCTAGA GTCCGCAAAAAT
	O_2Por1_RV	TAACTAGTAGCGTCGAAGGACAAAGACCAACCTAGC TTGTGAACAGGTTTCAGACAACCTCAAAGCATCGAAA GAGGAACCGACACCCAGAGTGACGCCAGGTTGTTT GGAATTCCTTG
GFP _{S11} -VDAC ₁₋₁₇ -OEP24 ₁₃₋₁₄	VDAC+2O_FW	CAACACGAGTGGAACAAACTGGTTGCTTCAAGGTT G
	VDAC+2O_RV	GAAGCAACCAGTTTGTTCCTCGTGTGTAATGAG
GFP _{S11} -VDAC ₁₋₁₇ -OEP24 ₁₃ VDAC ₁₉	AK3-INT_FW	TCATTCTTCACAATCTCTGGAGAAGTCGACACAAAG TCAAAAATTCCCAAACCTCAGTGTTGAGTCC
	AK3-INT_RV	GTCGACTTCTCCAGAGATTGTGAAGAATGACTTGGG TTTGAATTCCTTGTCCTCACTCCAATCC
GFP _{S11} -OEP37 ₁₋₁₆ -VDAC ₁₈₋₁₉	OEP37_ 2VDAC1_FW	GGAGATGAAGGTGGGAAAGCATGGAAACCCAAGTC ATTCTTCACAATCTCTGG
	OEP37_ 2VDAC1_RV	GATTGTGAAGAATGACTTGGGTTTCCATGCTTTCCC ACCTTCATCTCCAACCCAAAGG
Tim21(N)-GFP _{S1-10}	Tim21-L1_KpnI_ FW	AATGGTACCATGGTGAAACCATCGGATTTCAAAGC

Construct	Primer	Sequence (5' → 3')
	Tim21-L1_ TPTMD_SpeI_ RV	ATTACTAGTTGTAAACCTTGAGCGGAGGTATAATCC
DHFR	TJ009	CCCGGTACCATGGTTCGACCATTGAACTGC
	TJ011	CCCGGATCCTTAGTCTTTCTTCTCGTAGAC
DHFR ^{mut}	TJ081	CCCGGTACCATGGTTCGACCATTGAAC
	TJ011	CCCGGATCCTTAGTCTTTCTTCTCGTAGAC
cp(VDAC)	TJ022	CCCGAATTCATGGCTGTGCCACCCACG
	TJ023	CCCGGTACCACCAGCACCAGCACCCAAATCAAGCT TTATTAAGCCAAATC
hp18(VDAC)	TJ014	CCCGAATTCATGAAGCCAGGTATTAAACTGAC
	TJ015	CCCGGTACCACCAGCACCAGCACCTGCTTGAAATTC CAGTCCTAGACC
hp18(Porin)	TJ012	CCCGAATTCATGAAGCAATTGTTAAGACCTGG
	TJ013	CCCGGTACCACCAGCACCAGCACCGTCGAAGG ACAAAGACC
hp2(Porin)	TJ060	CCCGAATTCATGAATGGCATTAAAGTTCTCATTGAAG
	TJ061	CCCGGTACCACCAGCACCAGCACCGTTTGCTTGT CATTCAACTTTG
hp3(Porin)	TJ037	CCCGAATTCATGAAAGACGGTCCACTGTC
	TJ038	CCCGGTACCACCAGCACCAGCACCGTTTGTGTTAG ACCAGCC
hp17(VDAC)	TJ058	CCCGAATTCATGAACTCCAGCCTGATAGGTTTAG
	TJ059	CCCGGTACCACCAGCACCAGCACCGTTCTTGCCAT CCAGAAGAG
hp3(VDAC)	TJ039	CCCGAATTCATGGAGACCACCAAAGTGAC
	TJ040	CCCGGTACCACCAGCACCAGCACCATTTGTCGGTATT CCATTTCTC
GFP	TJ032	CCCGGTACCATGAGTAAGGGTGAAGAACTTTTCAC
	TJ034	CCCGGATCCTTATTTGTATAGTTCATCCATGCC
VDAC1 FL	TJ092	CCCGGATCCAATGGCTGTGCCACCCACG
	TJ093	CCCGTCTGACTTATGCTTGAAATTCCAGTCCTAGAC
VDAC1Δhp1	TJ107	GGCTATGGATTTGGCACTGAGACCACCAAAGTGAC G
	TJ108	CTTTGGTGGTCTCAGTGCCAAATCCATAGCCCTTGG
VDAC1Δhp18	TJ092	CCCGGATCCAATGGCTGTGCCACCCACG
	TJ094	CCCGTCTGACTTATAGAGTCTGAGTGTATCCTAAACC
hp18(F281A)-DHFR	TJ014	CCCGAATTCATGAAGCCAGGTATTAAACTGAC
	TJ066	CCCGGTACCACCAGCACCAGCACCTGCTTGTGCTT CCAGTCCTAGACC
hp18(F281Q)-DHFR	TJ014	CCCGAATTCATGAAGCCAGGTATTAAACTGAC

Construct	Primer	Sequence (5' → 3')
hp18(L259Q, F281Q)-DHFR	TJ046	CCCGGTACCACCAGCACCAGCACCTGCTTGTTGTTCCAGTCCTAGACC
	TJ077	GTATTAAACTGACACAATCAGCTCTTCTG
	TJ078	CAGAAGAGCTGATTGTGTCAGTTTAATAC
hp18(L259Q, L277Q, F281Q)-DHFR	TJ079	ACAAGCTTGGTCAAGGACTGGAACAA
	TJ080	TTGTTCCAGTCCTTGACCAAGCTTGT
hp17(Q249L)-DHFR	TJ095	GTTTAGGATACACTTTGACTCTAAAGCC
	TJ096	GGCTTTAGAGTCAAAGTGTATCCTAAAC
	TJ097	GATAGGTTTAGGATTCACTTTGACTCTAAAGCC
hp17(Y247F, Q249L)-DHFR	TJ098	GGCTTTAGAGTCAAAGTGAATCCTAAACCTATC
	TJ052	CCCGGATCCATGTCCCAGTCGAACCCTATC
	TJ053	CCCAAGCTTGTTCATCGATATCGTTAGCTTCAG
Mcr1(R4E, R7E)-YFP(C)	TJ067	CCCGAATTCATGTTTTCCGAATTATCCGAATCTCACTCAAAAGC
	TJ068	CCCAAGCTTAAATTTGAAAAGTTGGTCCTTGGAG
YFP(N)	TJ054	CCCGGTACCATGGTGAGCAAGGGCGAG
	TJ055	CCCGGATCCTCACTCGATGTTGTGGCG

Supplementary Table 2 | Plasmids used in this study.

Construct	Accession Number(s)	Reference
GFP _{S11} -OEP24	CAA04468	1
GFP _{S11} -VDAC1	AT3G01280	current study
GFP _{S1-10}		2
Mgd1-GFP _{S1-10}	AT4G31780	2
Tim50-GFP _{S1-10}	AT1G55900	3
Tim21(N)-GFP _{S1-10}	AT2G40800	current study
YC3.60-Tom20	AT3G27070	1
GFP _{S11} -OEP24 ₁₋₁₀ -VDAC1 ₁₆₋₁₉	CAA04468 / AT3G01280	current study
GFP _{S11} -OEP24 ₁₋₁₂ -VDAC1 ₁₈₋₁₉	CAA04468 / AT3G01280	current study
GFP _{S11} -OEP24 ₁₋₁₃ -VDAC1 ^{**} ₁₉	CAA04468 / AT3G01280	current study
GFP _{S11} -OEP24 ₁₋₁₃ -VDAC1 ₁₉	CAA04468 / AT3G01280	current study
GFP _{S11} -OEP24 ₁₋₄ -VDAC1 ₅₋₆ -OEP24 ₇₋₁₄	CAA04468 / AT3G01280	current study
GFP _{S11} -Porin	EDN62755	current study
GFP _{S11} -OEP24 ₁₋₁₂ -Porin ₁₈₋₁₉	CAA04468 / EDN62755	current study
GFP _{S11} -VDAC1 ₁₋₁₇ -OEP24 ₁₃₋₁₄	CAA04468 / AT3G01280	current study
GFP _{S11} -VDAC1 ₁₋₁₇ -OEP24 ₁₃ -VDAC1 ₁₉	CAA04468 / AT3G01280	current study
GFP _{S11} -OEP37 ₁₋₁₆ -VDAC1 ₁₈₋₁₉	CAB50915 / AT3G01280	current study
pGEM4-hVDAC1	P21796	4
pGEM4-Tom40	P23644	5
pGEM4-Tob55	P53969	5
pGEM4-Porin	EDN62755	6
pGEM4-pSu9-DHFR	P00842 / P00375	7
pGEM4-pSu9-DHFR ^{mut}	P00842	8
pGEM4-cp(VDAC)-DHFR ^{mut}	P21796	current study
pGEM4-hp18(VDAC)-DHFR ^{mut}	P21796	current study
pGEM4-AAC	P04710	9
pYX142-DHFR	P00375	current study
pYX142-cp(VDAC)-DHFR	P21796 / P00375	current study
pYX142-hp18(VDAC)-DHFR	P21796 / P00375	current study
pYX142-hp18(F281A)-DHFR	P21796 / P00375	current study
pYX142-hp18(F281Q)-DHFR	P21796 / P00375	current study
pYX142-hp18(L259Q, F281Q)-DHFR	P21796 / P00375	current study

Construct	Accession Number(s)	Reference
pYX142-hp18(L259Q, L277Q, F281Q)-DHFR	P21796 / P00375	current study
pYX142-hp18(Porin)-DHFR	EDN62755 / P00375	current study
pYX142-hp2(Porin)-DHFR	EDN62755 / P00375	current study
pYX142-hp3(Porin)-DHFR	EDN62755 / P00375	current study
pYX142-hp17(VDAC)-DHFR	P21796 / P00375	current study
pYX142-hp17(Q249L)-DHFR	P21796 / P00375	current study
pYX142-hp17(Y247F, Q249L)-DHFR	P21796 / P00375	current study
pYX142-hp3(VDAC)-DHFR	P21796 / P00375	current study
pYX142-mtGFP		10
pYX142-eGFP		current study
pYX142-cp(VDAC)-eGFP	P21796	current study
pYX142-hp18(VDAC)-eGFP	P21796	current study
pYX142-VDAC1	P21796	current study
pYX142-VDAC1 Δ hp1	P21796	current study
pYX142-VDAC1 Δ hp18	P21796	current study
pRS426-TPI-Tom20	P35180	11
pGEM4-Mcr1	P36060	12
C-YC426ADH		13
p426ADH-Tom20-YFP(C)	P35180	current study
p426ADH-Mcr1(R4E, R7E)-YFP(C)	P36060	current study
C-YN425ADH		13
pYX142-YFP(N)		current study
pYX142-cp(VDAC)-YFP(N)	P21796	current study
pYX142-hp18(VDAC)-YFP(N)	P21796	current study

Supplementary Table 3 | Antibodies used in this study.

Antibody directed against	Dilution	Number
<i>S. cerevisiae</i> (S.c.) Aconitase (purified full-length protein)	1:5000	sc-Aco
S.c. Bmh1 (QQPPAAAEAGEAPK)	1:1000	sc-Bmh1
mouse DHFR	1:1000	610697 (BD Biosciences)
S.c. Fis1 (purified protein comprising AA 1-98 fused to MBP)	1:1000	309
GFP	1:5000	11814460001 (Roche)
GFP (β -strand #11, RDHMLVHEYVNAAGIT)	1:2000	(PSL GmbH)
N.c. Porin (SDIAKSANDLLNKDC, antiserum detects S.c. Porin)	1:3000	PorN5
S.c. Tob55 (purified his-tagged full-length protein)	1:2000	321
S.c. Tom20 (CKAESDAVAEANDIDD)	1:1000	sc-Tom20
S.c. Tom22 (CMVELTEIKDDVV)	1:1000	YTom22-N11
S.c. Tom40 (CADGNPLQALPQL)	1:4000	45300
S.c. Tom70 (purified protein comprising AA 33-616)	1:2000	312
<i>A. thaliana</i> VDAC1	1:5000	AS07 212 (Agrisera)
<i>H. sapiens</i> VDAC1	1 μ g/mL	ab15895 (Abcam)

Supplementary References

- 1 Machettira, A. B. *et al.* The localization of Tic20 proteins in *Arabidopsis thaliana* is not restricted to the inner envelope membrane of chloroplasts. *Plant Mol. Biol.* **77**, 381-390 (2011).
- 2 Sommer, M. S. *et al.* Chloroplast Omp85 proteins change orientation during evolution. *Proc. Natl. Acad. Sci. U S A* **108**, 13841-13846 (2011).
- 3 Ulrich, T., Gross, L. E., Sommer, M. S., Schleiff, E. & Rapaport, D. Chloroplast β -barrel proteins are assembled into the mitochondrial outer membrane in a process that depends on the TOM and TOB complexes. *J. Biol. Chem.* **287**, 27467-27479 (2012).
- 4 Engl, G., Florian, S., Tranebjærg, L. & Rapaport, D. Alterations in expression levels of deafness dystonia protein 1 affect mitochondrial morphology. *Hum. Mol. Gen.* **21**, 287-299 (2012).
- 5 Paschen, S. A. *et al.* Evolutionary conservation of biogenesis of β -barrel membrane proteins. *Nature* **426**, 862-866 (2003).
- 6 Mayer, A., Lill, R. & Neupert, W. Translocation and insertion of precursor proteins into isolated outer membranes of mitochondria. *J. Cell Biol.* **121**, 1233-1243 (1993).
- 7 Pfanner, N., Muller, H. K., Harmey, M. A. & Neupert, W. Mitochondrial protein import: involvement of the mature part of a cleavable precursor protein in the binding to receptor sites. *EMBO J.* **6**, 3449-3454 (1987).
- 8 Vestweber, D. & Schatz, G. Point mutations destabilizing a precursor protein enhance its post-translational import into mitochondria. *EMBO J.* **7**, 1147-1151 (1988).
- 9 Pfanner, N. & Neupert, W. Distinct steps in the import of ADP/ATP carrier into mitochondria. *J. Biol. Chem.* **262**, 7528-7536 (1987).
- 10 Westermann, B. & Neupert, W. Mitochondria-targeted green fluorescent proteins: convenient tools for the study of organelle biogenesis in *Saccharomyces cerevisiae*. *Yeast* **16**, 1421-1427 (2000).
- 11 Waizenegger, T., Stan, T., Neupert, W. & Rapaport, D. Signal-anchor domains of proteins of the outer membrane of mitochondria: structural and functional characteristics. *J. Biol. Chem.* **278**, 42064-42071 (2003).
- 12 Meineke, B. *et al.* The outer membrane form of the mitochondrial protein Mcr1 follows a TOM-independent membrane insertion pathway. *FEBS Lett.* **582**, 855-860 (2008).
- 13 Skarp, K. P., Zhao, X., Weber, M. & Jantti, J. Use of bimolecular fluorescence complementation in yeast *Saccharomyces cerevisiae*. *Methods Mol. Biol.* **457**, 165-175 (2008).