

Figure S1. Sequence homologies between dehydrogenases implicated in NADH-shuttling in *P. pastoris* and *S. cerevisiae*. (A) Malate dehydrogenases for *S. cerevisiae* (NP_012838.1, NP_014515.2, and NP_010205.1) and *P. pastoris* (XP_002491128.1 and XP_002494265.1). (B) Glycerol 3-phosphate dehydrogenases for *S. cerevisiae* (NP_010262.1 and NP_014582.1) and *P. pastoris* (XP_002492095.1). Protein sequences were aligned using the Clustal Omega Multiple Clustal Alignment. * indicates identity and : denotes similarity. Mitochondrial presequences are highlighted in red, and peroxisomal targeting signals are depicted in green.

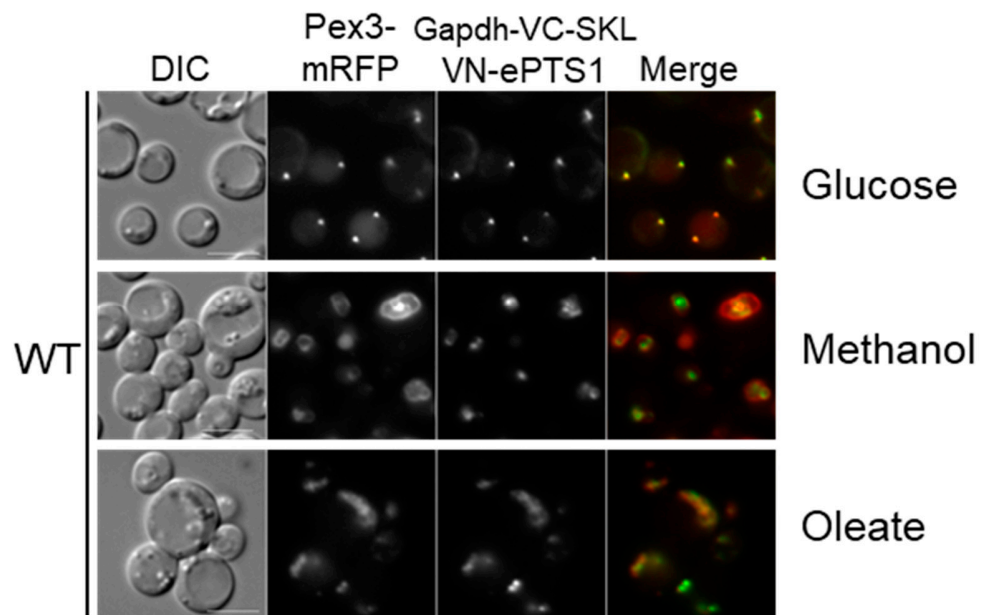


Figure S2. Gapdh-VC with a C-terminal SKL is capable of interaction with VN-ePTS1 at the peroxisome. Strains were grown in glucose, methanol, and oleate overnight. Peroxisomes were visualized using Pex3-mRFP. Bars: 5 μ m.

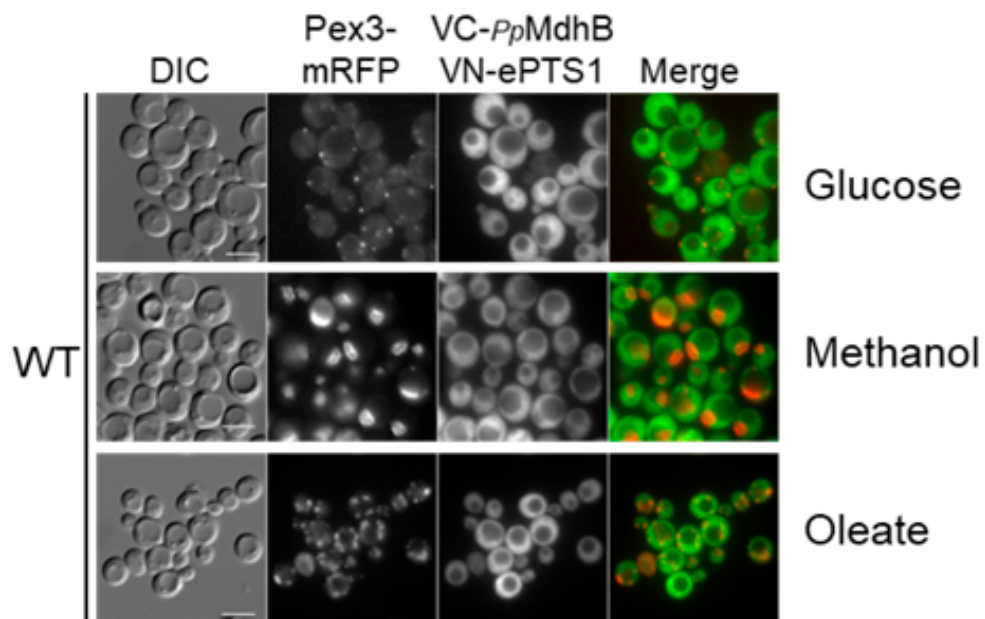


Figure S3. *Pp*MdhB fused with VC at its N-terminus shows only cytosolic localization using the divergent BiFC assay. Strains were grown overnight in glucose, methanol, and oleate. Peroxisomes were visualized using Pex3-mRFP. Bars: 5 μ m.

Table S1. Yeast strains.

<i>Yeast Strains</i>	<i>Description</i>	<i>Source</i>
<i>GS115</i>	<i>his4</i>	Lab stock
<i>PPY12h</i>	<i>his4 arg4</i>	Lab stock
<i>Δpex7</i>	<i>Δpex7::ARG4 his4</i>	Lab stock
<i>JC404</i>	<i>Δpex14::ARG4 his4</i>	Lab stock
<i>sPL66</i>	GS115 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL21 (P _{HTX1} - <i>Gapdh-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL67</i>	GS115 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL14 (P _{HTX1} - <i>Pot1-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL68</i>	GS115 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL18 (P _{HTX1} - <i>MdhA-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL69</i>	GS115 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL19 (P _{HTX1} - <i>MdhB-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL70</i>	GS115 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL20 (P _{HTX1} - <i>GpdA-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL75</i>	Δpex7 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL14 (P _{HTX1} - <i>Pot1-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL77</i>	Δpex7 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL19 (P _{HTX1} - <i>MdhB-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL79</i>	JC404 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL14 (P _{HTX1} - <i>Pot1-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL81</i>	JC404 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL19 (P _{HTX1} - <i>MdhB-VC</i> + VN-ePTS1)::HIS4	This study
<i>sPL93</i>	GS115 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pPL23 (P _{HTX1} - <i>VC-MdhB</i> + VN-ePTS1)::HIS4	This study
<i>sJCF2169</i>	PPY12h + pJCF523(P _{TOM20} - <i>Tom20-2xmCherry</i>)::ARG4(Hygro ^R) pJCF402 (P _{GAPDH} - <i>BFP-SKL</i>)::ARG4 <i>his4</i>	This study
<i>sJCF2771</i>	sJCF2169 + pPL26(P _{AOX1} - <i>MdhB-GFP</i>)::HIS4	This study
<i>sJCF2683</i>	sJCF2169 + pJCF855(P _{GPD} - <i>GpdA-GFP</i>)::HIS4	This study
<i>sJCF2682</i>	sJCF2169 + pJCF854(P _{MDHA} - <i>MdhA-GFP</i>)::HIS4	This study
<i>sJCF2770</i>	sJCF2169 + pJCF853(P _{MDHB} - <i>MdhB-GFP</i>)::HIS4	This study
<i>sJCF2772</i>	GS115 + pJCF235-Zeo (P _{PEX3} - <i>Pex3-mRFP</i>)::HIS4(Zeocin ^R) + pJCF856 (P _{HTX1} - <i>Gapdh-VC</i> + VN-ePTS1)::HIS4	This study

Table S2. Plasmids.

<i>Plasmid</i>	<i>Description</i>	<i>Source</i>
<i>pPL14</i>	P _{HTX1} - <i>Pot1-VC</i> + VN-ePTS1, HIS4, AMP ^R	This study
<i>pPL18</i>	P _{HTX1} - <i>MdhA-VC</i> + VN-ePTS1, HIS4, AMP ^R	This study
<i>pPL19</i>	P _{HTX1} - <i>MdhB-VC</i> + VN-ePTS1, HIS4, AMP ^R	This study
<i>pPL20</i>	P _{HTX1} - <i>GpdA-VC</i> + VN-ePTS1, HIS4, AMP ^R	This study
<i>pPL21</i>	P _{HTX1} - <i>Gapdh-VC</i> + VN-ePTS1, HIS4, AMP ^R	This study
<i>pPL23</i>	P _{HTX1} - <i>VC-MdhB</i> + VN-ePTS1, HIS4, AMP ^R	This study
<i>pPL26</i>	P _{AOX1} - <i>MdhB-GFP</i> , HIS4, AMP ^R	This study
<i>pJCF235-ZEO</i>	P _{PEX3} - <i>Pex3-mRFP</i> , HIS4/Zeocin ^R , AMP ^R	Lab stock
<i>pJCF402</i>	P _{GAPDH} - <i>BFP-SKL</i> , ARG4, AMP ^R	Lab stock
<i>pJCF523</i>	P _{TOM20} - <i>Tom20-2xmCherry</i> , ARG4/Hygro ^R , AMP ^R	Lab stock
<i>pJCF853</i>	P _{MDHA} - <i>MdhA-GFP</i> , HIS4, AMP ^R	This study
<i>pJCF854</i>	P _{MDHA} - <i>MdhA-GFP</i> , HIS4, AMP ^R	This study
<i>pJCF855</i>	P _{GPD} - <i>GpdA-GFP</i> , HIS4, AMP ^R	This study
<i>pJCF856</i>	P _{HTX1} - <i>Gapdh-VC-SKL</i> + VN-ePTS1, HIS4, AMP ^R	This study