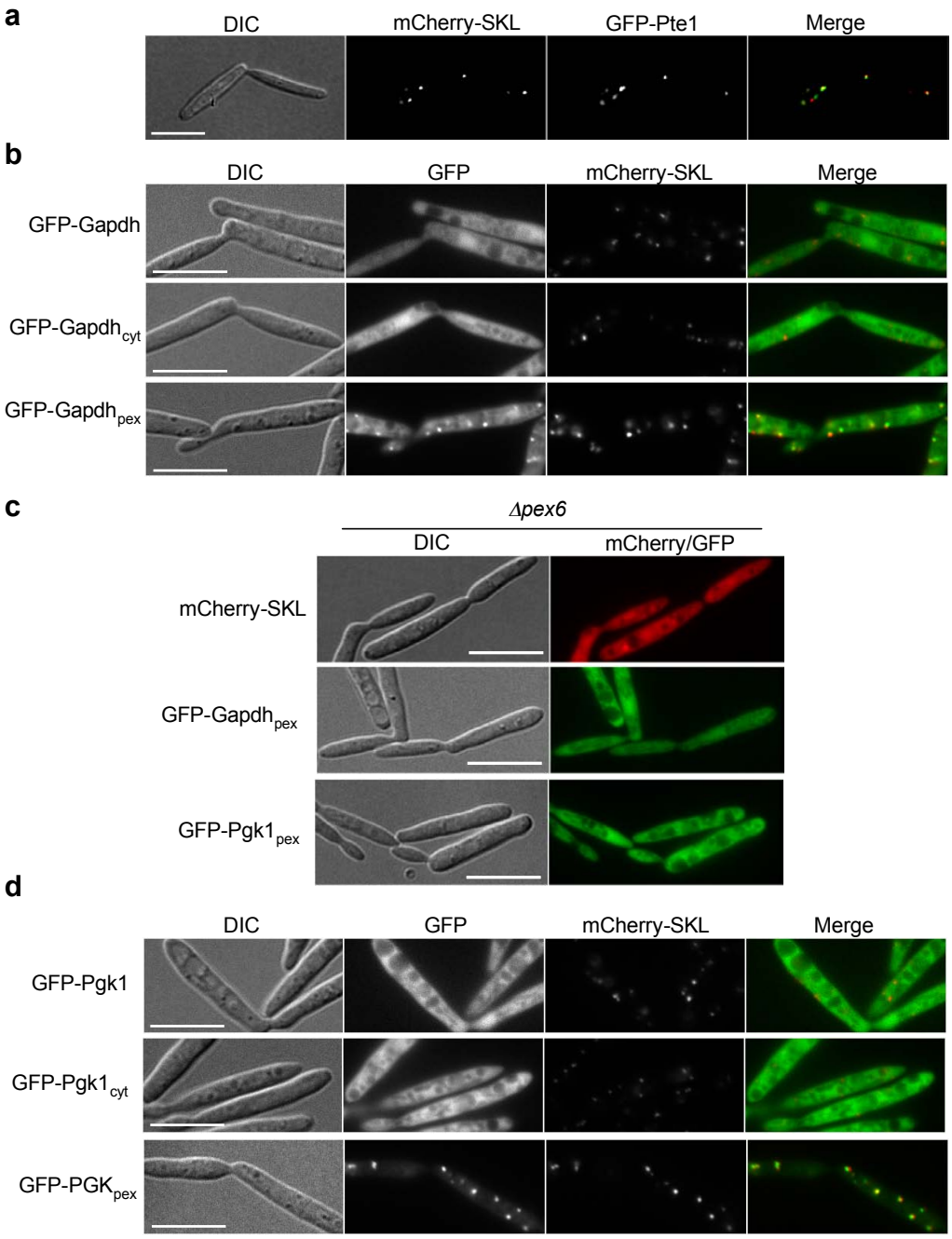
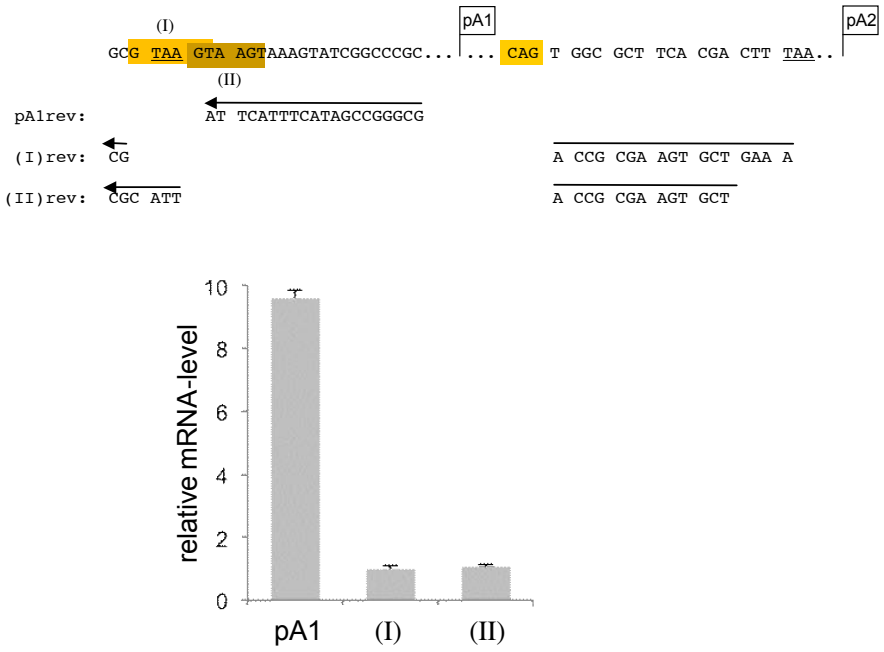
doi:10.1038/nature11051

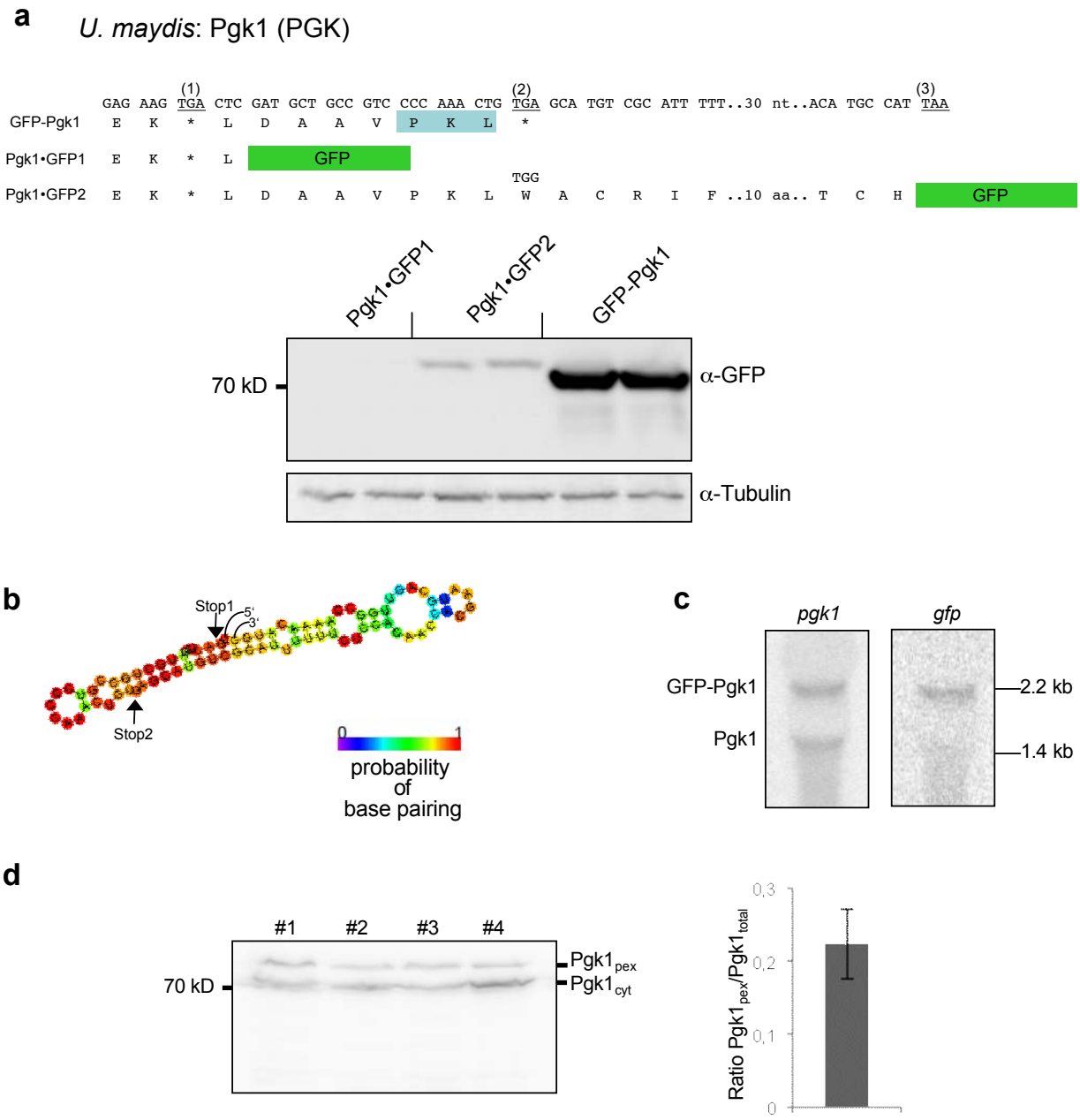


Supplementary Figure 1. Peroxisomal localisation of GAPDH and PGK in *U. maydis*.
a, Colocalisation of mCherry-SKL and GFP-Pte1 in *U. maydis* peroxisomes. Scale bar represents 10 μm.
b, GFP-fusion proteins of different full-length Gapdh derivatives were co-expressed with mCherry-SKL. GFP-Gapdh carries the genomic sequence and allows alternative polyadenylation and splicing. GFP-Gapdh$_{cyt}$ is deleted for the sequence encoding the PTS1. GFP-Gapdh$_{pex}$ mimics splice variant (I), which terminates with a PTS1. Scale bars represent 10 μm.
c, mCherry-SKL, GFP-Gapdh$_{pex}$ and GFP-Pgk1$_{pex}$ were expressed in Δ*pex6* mutants. In GFP-Pgk1$_{pex}$ the stop codon was exchanged by a serine codon thus mimicking translational read-through. Scale bars represent 10 μm.
d, GFP-fusion proteins of different full-length PGK derivatives were co-expressed with mCherry-SKL. GFP-Pgk1 carries the genomic sequence, while GFP-Pgk1$_{cyt}$ is deleted for the sequence encoding the PTS1. Scale bars represent 10 μm.

U. maydis: GAPDH

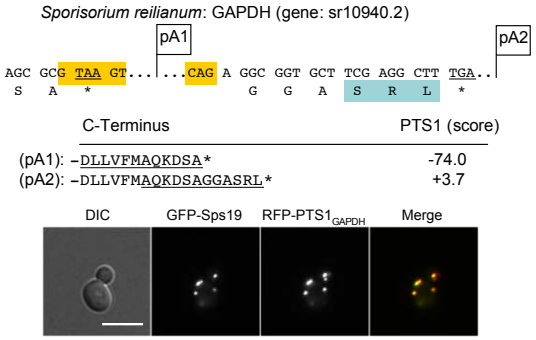


Supplementary Figure 2. Quantification of alternative *gapdh* mRNA transcripts of *U. maydis*. Schematic drawing of the *gapdh* gene of *U. maydis*. The position and sequences of reverse primers designed to distinguish between alternatively processed transcripts by rtPCR are shown. Quantification of different *gapdh* mRNA isoforms is given as relative mRNA levels. Data are presented as mean +/- SD.

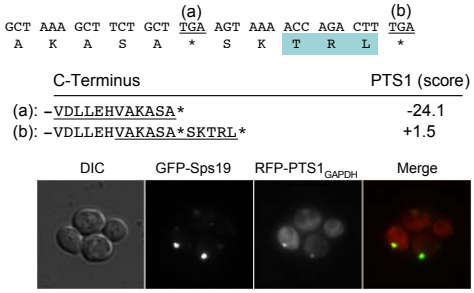


Supplementary Figure 3. Translational read-through of *pgk1* transcripts in *U. maydis*.
a, Translational read-through of the *pgk1* termination codon requires additional sequences downstream of the bypassed stop codon. C-terminal GFP-reporter constructs were expressed under control of the constitutive *otef*-promoter. Translational read-through was observed for Pgk1•GFP2 but not for Pgk1•GFP1. N-terminally fused GFP-Pgk1 served as control. Please note that the second stop codon (TGA) has been changed to tryptophan-coding TGG.
b, The RNA sequence downstream of the bypassed stop codon could form a stable stem-loop structure. The secondary structure was predicted using RNAfold (Gruber et al., 2008)
c, GFP was fused to the N-terminus of PGK and expressed under control of the endogenous *pgk1*-promoter. Northern blotting demonstrates comparable amounts of endogenous transcripts and *gfp*-tagged transcripts.
d, Western blotting allowed quantification of the amount of GFP-PGK$_{pex}$ resulting from translational read-through. To facilitate detection of the readt-hrough product the second stop codon (TGA) was mutated to TGG as depicted in (a) to generate an extended protein. Data are presented as mean +/- SD.

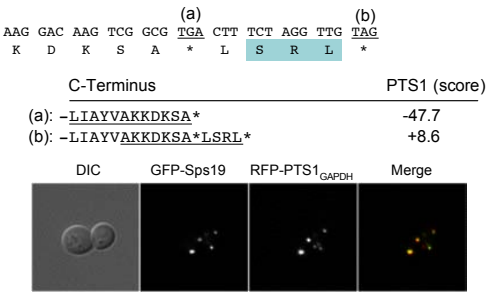
Sporisorium reilianum: GAPDH (gene: sr10940.2)



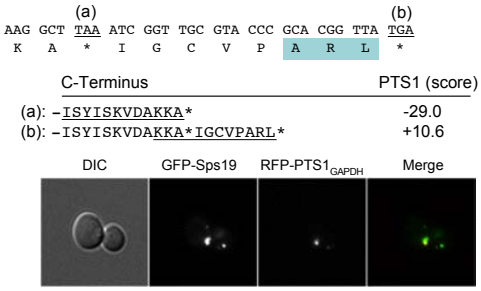
Candida albicans: GAPDH (gene: 3638640)



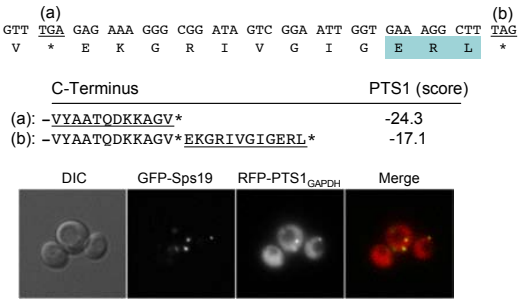
Cryptococcus curvatus: GAPDH (accession: AF126158.1)



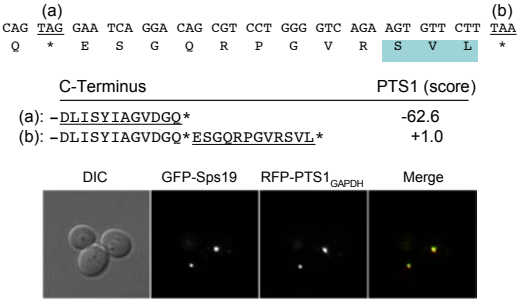
Neurospora crassa: GAPDH (gene: 3873099)



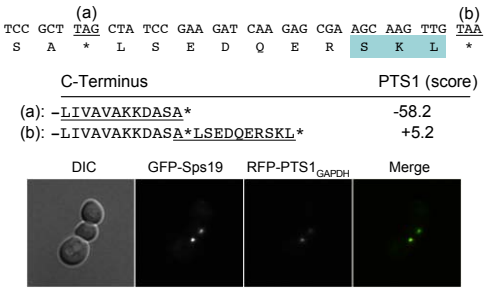
Laccaria bicolor: GAPDH (gene: 6082971)



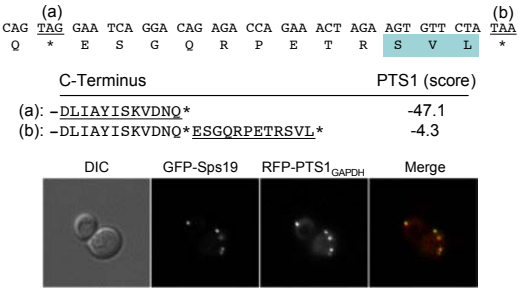
Penicillium chrysogenum: GAPDH (gene: 8308441)



Yarrowia lipolytica: GAPDH (gene: 2909309)

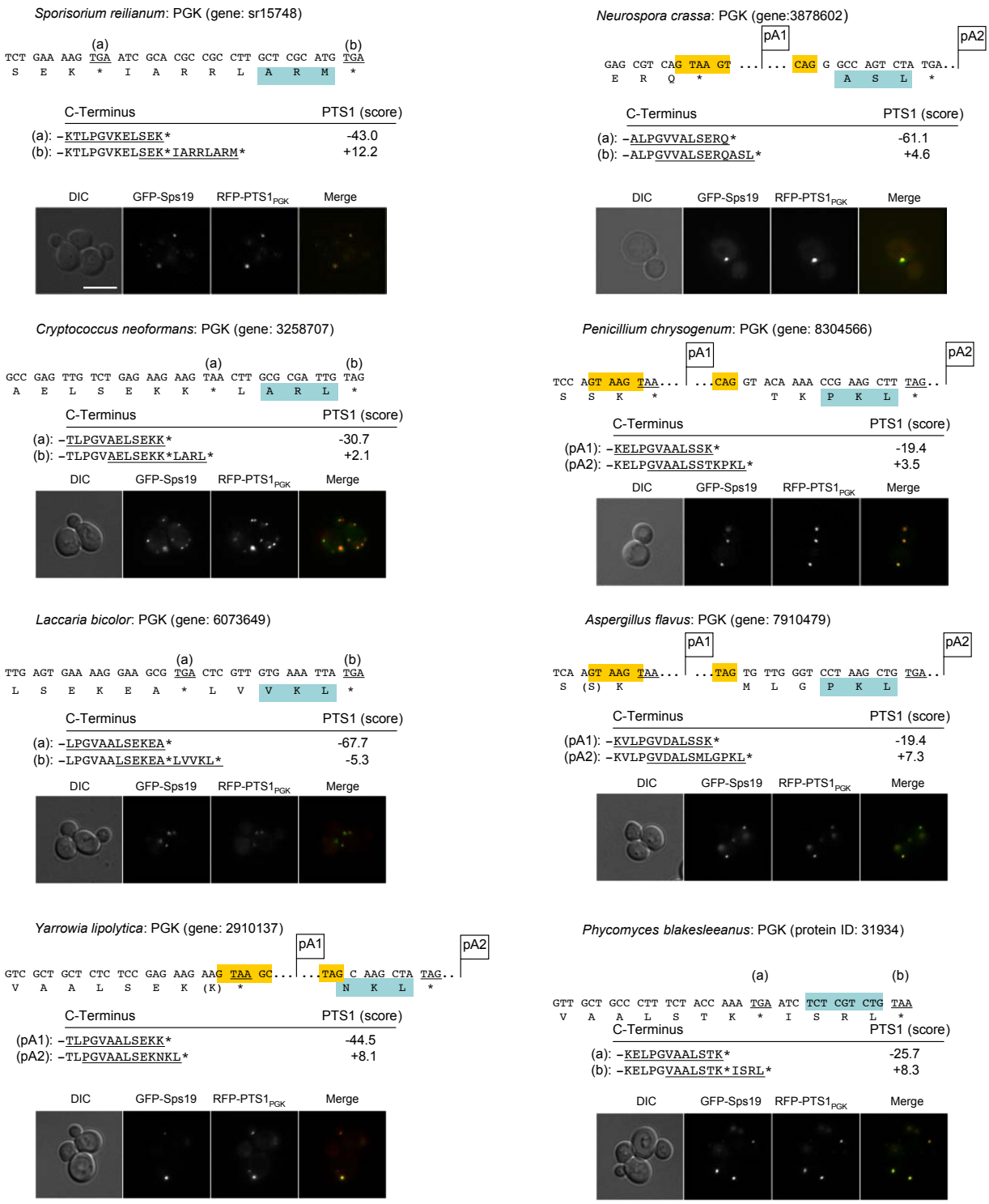


Aspergillus flavus: GAPDH (gene: 7909200)



Supplementary Figure 4, Cryptic peroxisomal targeting signals of GAPDH proteins from different fungal species.

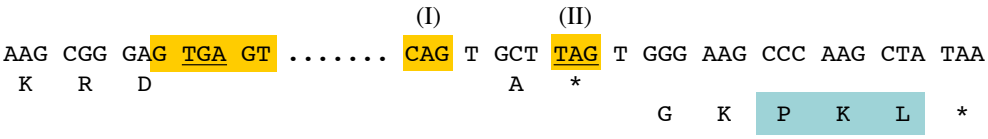
The schemes illustrate the predicted cryptic PTS1-motifs in different fungal GAPDH genes. DNA and derived protein sequences are shown. The underlined dodecamers were used for PTS1 prediction using an online prediction tool (Neuberger et al., 2003). The putative PTS1-motifs were fused to RFP and expressed in *S. cerevisiae*. Verification of peroxisomal targeting was done by colocalisation with the peroxisomal protein GFP-SPS19. Gene IDs or GeneBank accessions numbers are indicated. The *Sporisorium reilianum* sequence can be accessed via MSRDB (http://mips.helmholtz-muenchen.de/genre/proj/sporisorium/).



Supplementary Figure 5. Cryptic peroxisomal targeting signals of PGK proteins from different fungal species

The schemes illustrate the predicted cryptic PTS1-motifs in different fungal PGK-encoding genes. DNA and derived protein sequences are shown. The underlined dodecamers were used for PTS1 prediction using an online prediction tool (Neuberger et al., 2003). The putative PTS1-motifs were fused to RFP and expressed in *S. cerevisiae*. Verification of peroxisomal targeting was done by colocalisation with the peroxisomal protein GFP-SPS19. Gene IDs or GeneBank accessions numbers are indicated. The genome of *Phycomyces blakesleeanus* is available on JGI (http://genome.jgi-psf.org/Phybl2/Phybl2.home.). The *Sporisorium reilianum* sequence can be accessed via MSRDB (http://mips.helmholtz-muenchen.de/genre/proj/sporisorium/).

Puccinia graminis-tritici: GAPDH (PGTG_04956.2)

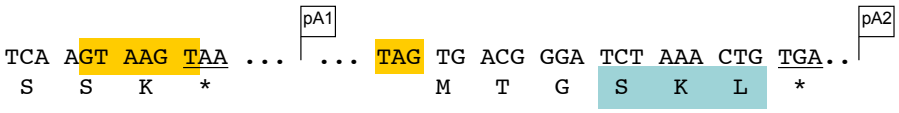


	ESTs (#)	C-Terminus	PTS1 (score)
(I):	5	–VDLVCEMAKRDA*	-55.6
(II):	1	–VDLVCEMAKRDGKPKL*	+4.3

Uromyces appendiculatus: GAPDH (EST_NCBI)

	ESTs (#)	C-Terminus	PTS1 (score)
(I):	18	–VDLVCEMAKRDA*	-55.6
(II):	3	–VDLVCEMAKRDGRAKL*	+5.1

Aspergillus terreus: PGK (gene: 4354973)



	ESTs (#)	C-Terminus	PTS1 (score)
(pA1):	33	–KELPGVTALSSK*	-17.9
(pA2):	2	–KELPGVTALSMTGSKL*	+5.8

Verticillium dahliae: PGK (VDAG_07805)

	ESTs	C-Terminus	PTS1 (score)
(pA1):	✓	–KELPGVVALSSK*	-22.9
(pA2):	✓	–KELPGVVALSSAPRL*	+2.7

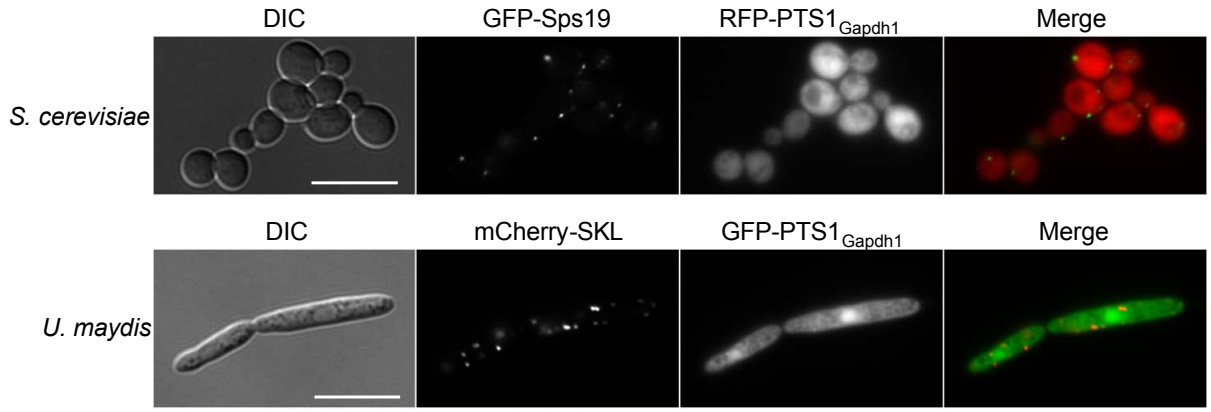
Supplementary Figure 6. ESTs deposited in databases confirm peroxisomal isoforms of GAPDH and PGK in various fungal species

In the basidiomycetous rust fungi *Puccinia graminis-tritici* and *Uromyces appendiculatus* peroxisomal variants of GAPDH are generated by differential splicing using alternative 3'splice sites. PTS1 scores were obtained using an online prediction tool (Neuberger et al., 2003). EST sequences were extracted from NCBI or Broad Institute.

In the ascomycetes *Aspergillus terreus* and *Verticillium dahliae* peroxisomal variants of PGK are generated by alternative splicing and polyadenylation. PTS1 scores were obtained using the online PTS1 predictor (Neuberger et al., 2003). ESTs were extracted from NCBI. The Gene IDs are from NCBI and JGI.

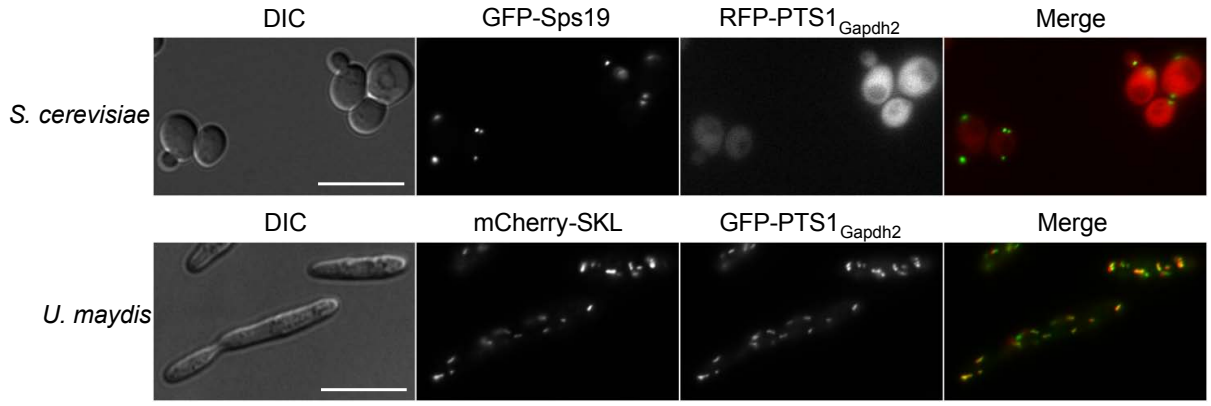
Phycomyces blakesleeanus: Gapdh1 (protein ID: 40333), PTS1 score: -42.1

TTG ATC GTC CAC GCC GCC AAG GTC GAT GGT GCC CTC TAA
L I V H A A K V D G A L *



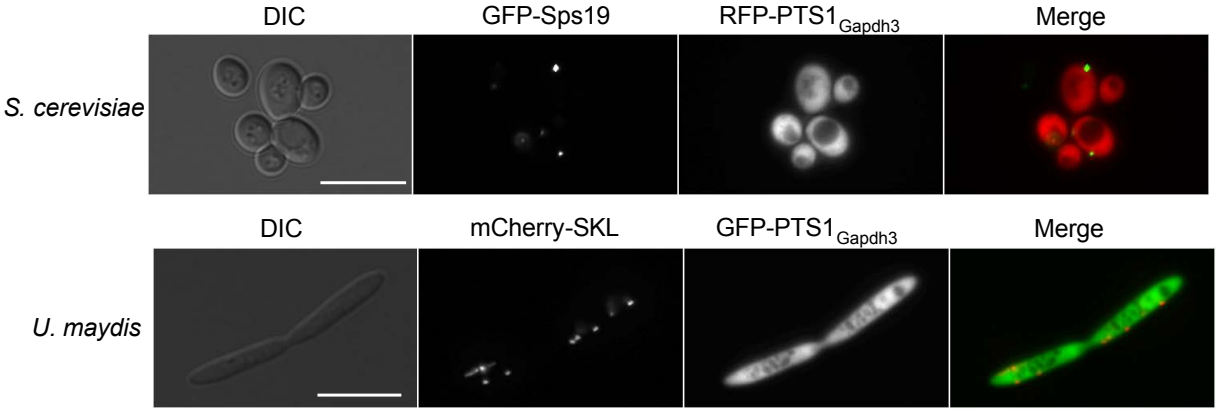
Phycomyces blakesleeanus: Gapdh2 (protein ID: 76956), PTS1 score: -35.3

CTT TTG GTT CAT GTT GCA AAG GTT GAT GGC AAT CTG TAA
L L V H V A K V D G N L *



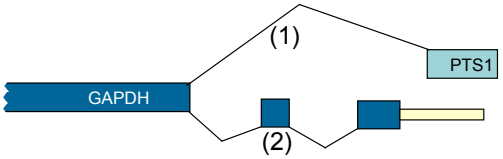
Phycomyces blakesleeanus: Gapdh3 (protein ID: 57879), PTS1 score: -59.5

TTG CTT CTT CAC GTC GCA AAG GTT GAT GGC AAC GCC TAA
L L L H V A K V D G N A *

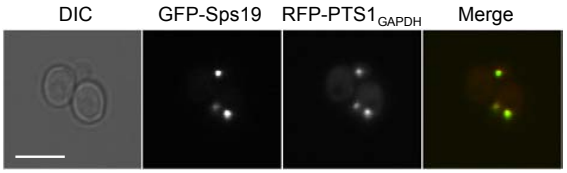


Supplementary Figure 7. *Phycomyces blakesleeanus* contains three paralogous GAPDH genes. The C-terminal dodecamers of all three paralogous *P. blakesleeanus* GAPDH proteins were tested for peroxisomal targeting in *S. cerevisiae* and *U. maydis*. Although the calculated PTS1 scores (Neuberger et al., 2003) for the C-terminal dodecamers did not predict peroxisomal localisation of either GAPDH isoform, the dodecamer from Gapdh2 was able to target GFP to peroxisomes in *U. maydis*. Notably, peroxisomal localisation of RFP-PTS1$_{Gapdh2}$ was not observed in *S. cerevisiae*. Scale bars represent 10 μm. The protein IDs are based on JGI.

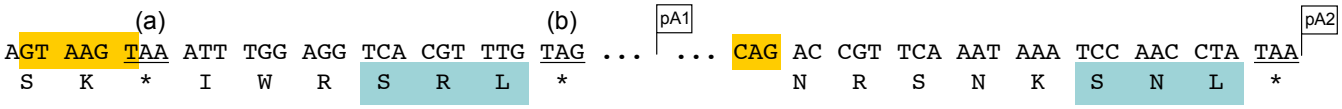
Botrytis cinerea: GAPDH (gene ID:154302816)



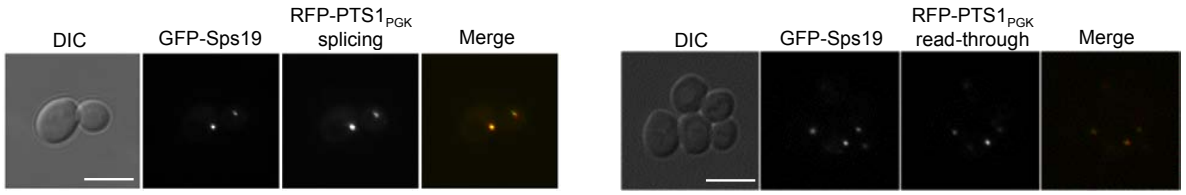
	C-Terminus	PTS1 (score)
splicing1	-VELKPNAHPQKL*	+0.6
splicing2	-LSGDSAIATQNS*	-28.8



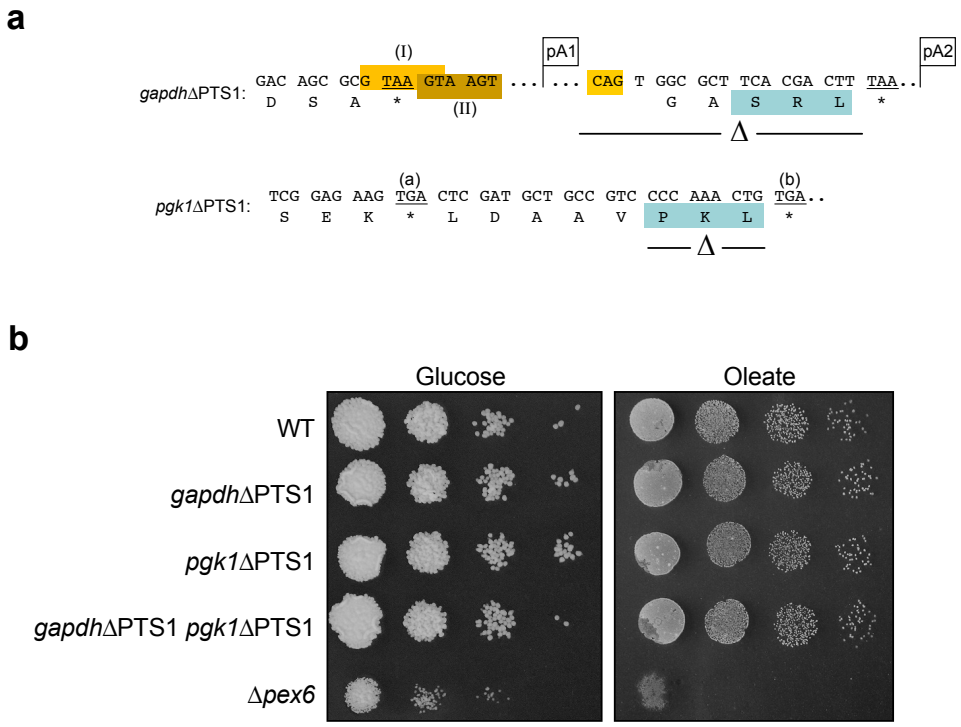
Botrytis cinerea: PGK (gene ID: 5428785)



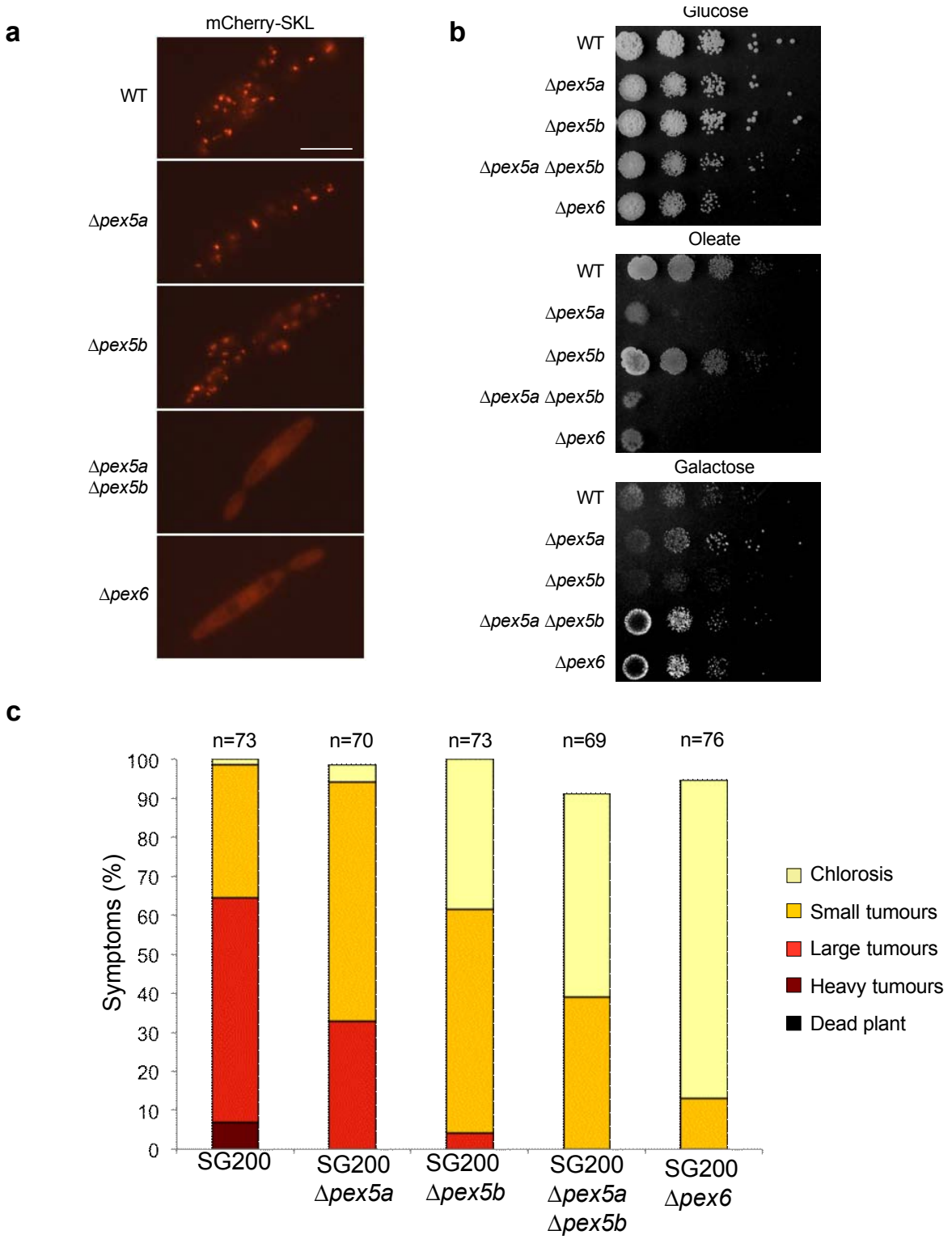
	C-Terminus	PTS1 (score)
splicing	-KDLPGVSALSYRSNKSNL*	+5.3
termination at (a)	-KDLPGVSALSSK*	-17.9
termination at (b)	-KDLPGVSALSSK*IWRSRL*	+13.3



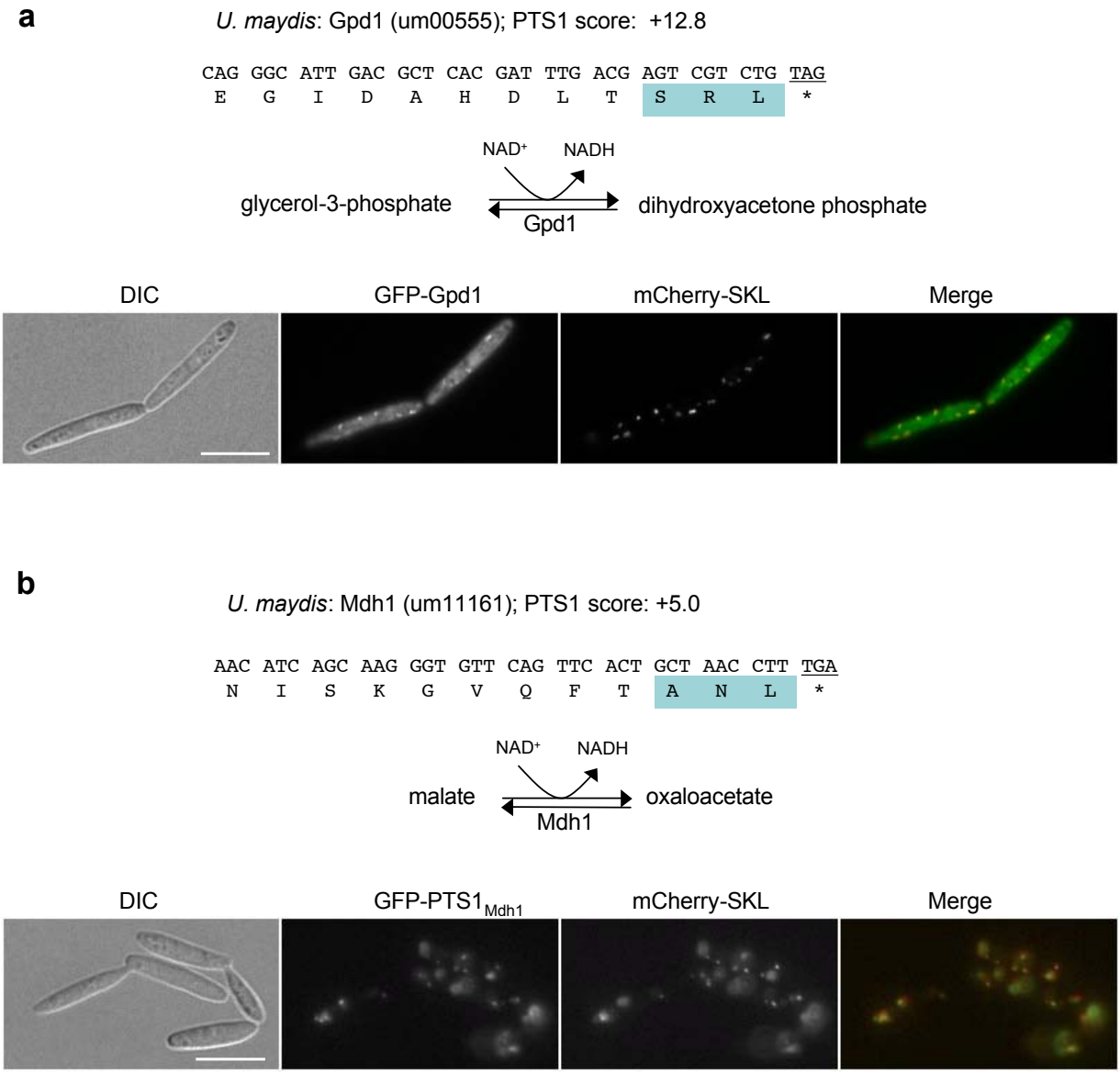
Supplementary Figure 8. *Botrytis cinerea* uses unusual mechanisms to generate PTS1 motifs in GAPDH and PGK.
Differential use of alternative 3' splice sites coupled to exon skipping could generate a PTS1 in the GAPDH of *B. cinerea*. For PGK, two mechanisms, alternative splicing and translational read-through, are able to generate a peroxisomal variant. Both predicted C-terminal sequences display a positive PTS1 score (Neuberger et al., 2003). To demonstrate peroxisomal targeting, respective C-termini were fused to RFP and compared to GFP-SPS19 in *S. cerevisiae*. Scale bars represent 10 μm.



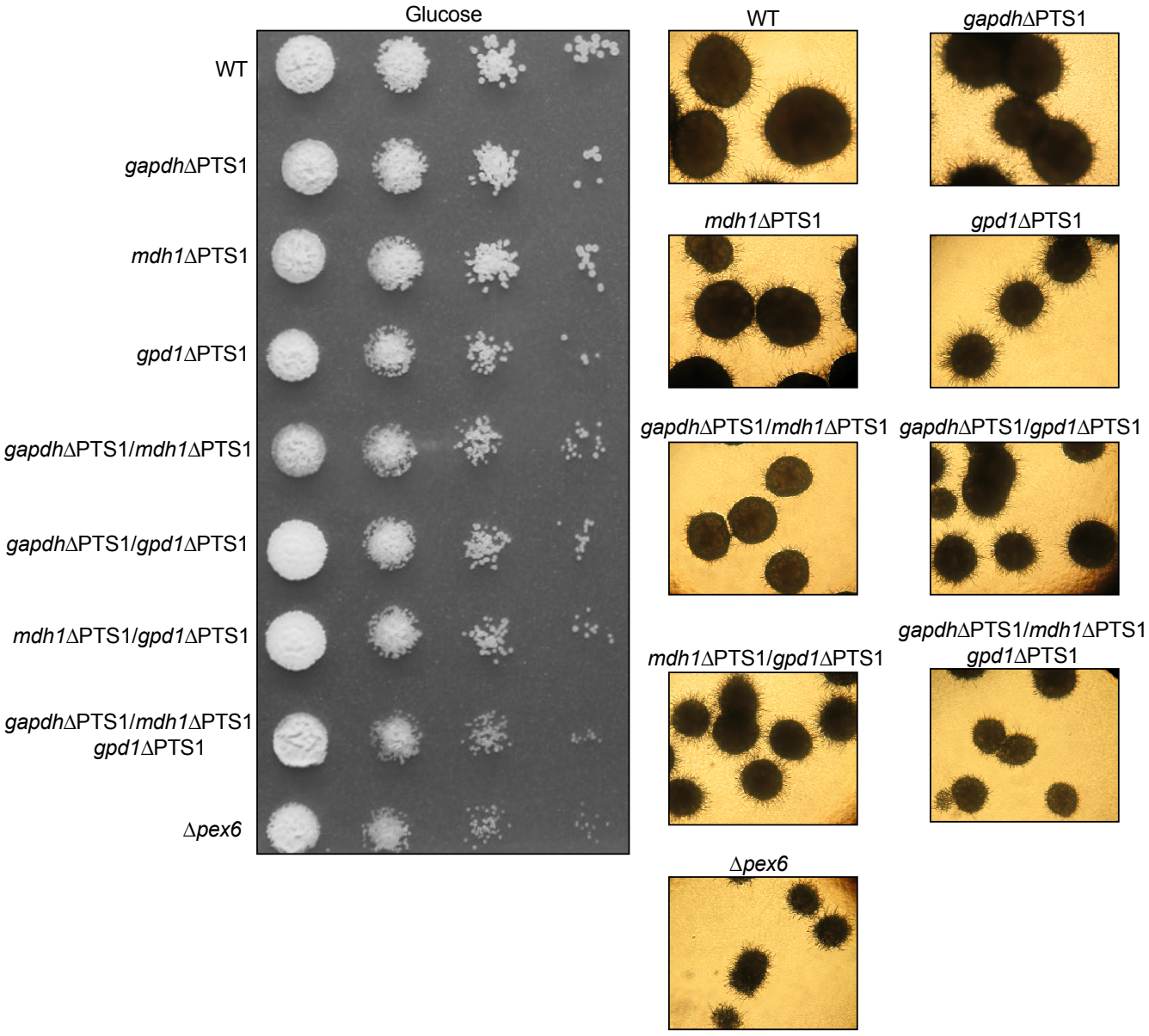
Supplementary Figure 9, *U. maydis* mutants expressing PTS1-less *gapdh* and *pgk1* genes.
a, Sequences encoding the cryptic PTS1 motifs were deleted in *gapdh* and *pgk1* in the genomic context by homologous recombination.
b, PTS1-less mutants of *gapdh* and *pgk1* were grown on glucose and oleate containing media in serial dilutions. Growth of single and double mutants was compared to corresponding wild type and Δ*pex6* strains.



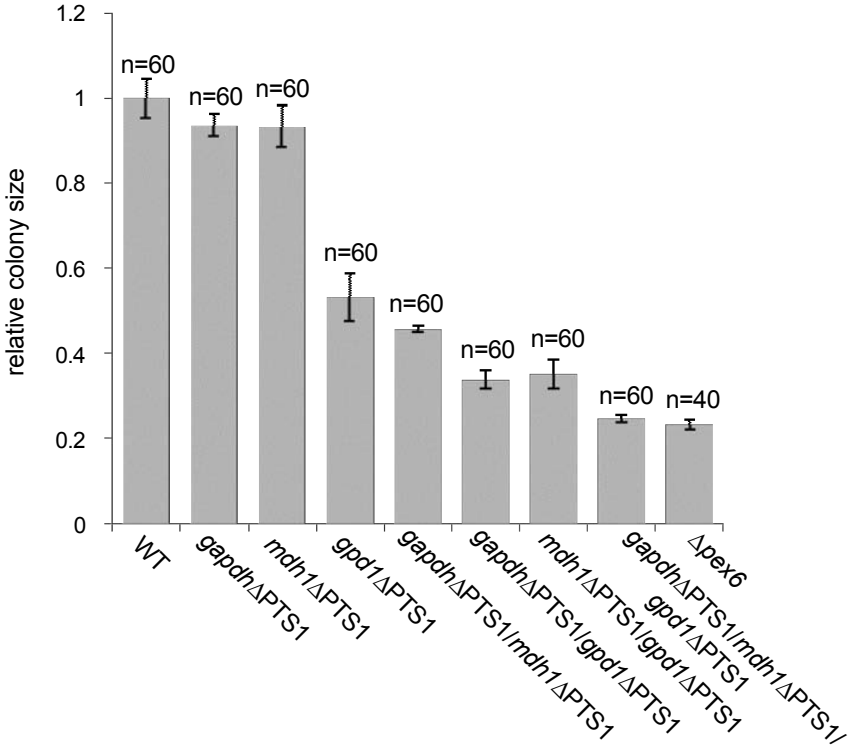
Supplementary Figure 10. Mutational analysis of *U. maydis pex5a* and *pex5b* genes.
a, Peroxisomal targeting of mCherry-SKL was tested in indicated strains.
b, Indicated strains were tested for growth on different carbon sources. Serial dilutions were spotted on YNB solid medium containing either 2% of indicated sugar or 0.2% oleate.
c, SG200, SG200Δ*pex5a*, SG200Δ*pex5b*, the double mutant SG200Δ*pex5a*Δ*pex5b* and SG200Δ*pex6* were used to infect seven day old maize seedlings. Disease symptoms were scored 10 days post infection. Symptoms were grouped into categories depicted on the right. Average values of three independent experiments are given as percentage of the total number (n) of infected plants.



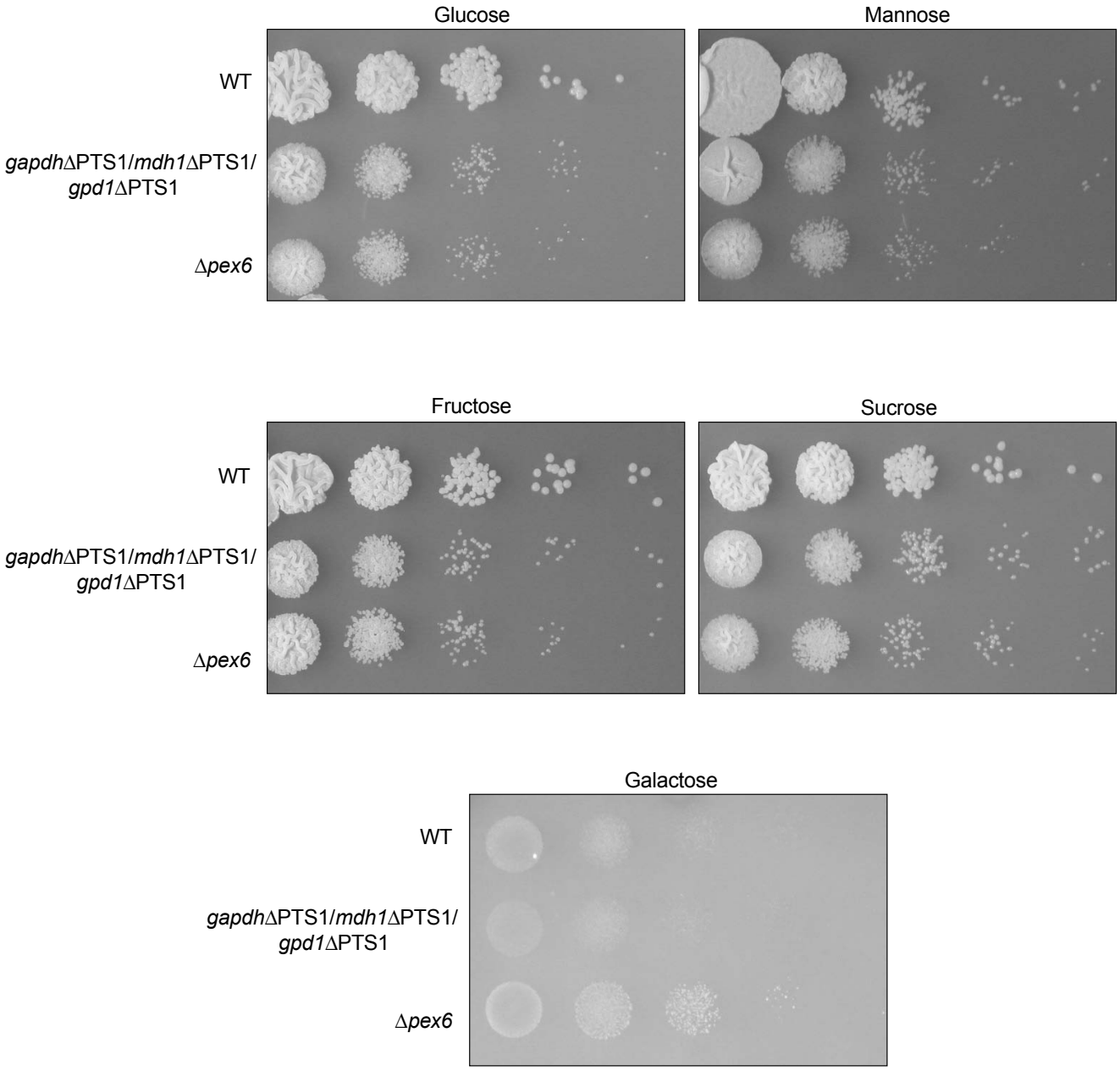
Supplementary Figure 11. Peroxisomal localisation of glycerol-3-phosphate dehydrogenase (Gpd1) and malate dehydrogenase (Mdh1) in *U. maydis*.
a, The C-terminal sequence and the redox reaction catalyzed by Gpd1 are shown. Gpd1 exhibits a positive PTS1 score (Neuberger et al., 2003). Partial peroxisomal localisation of full-length GFP-Gpd1 was observed by colocalisation with mCherry-SKL. Scale bar represents 10 μm.
b, The C-terminal sequence and the redox reaction catalyzed by Mdh1 are shown. Mdh1 exhibits a positive PTS1 score (Neuberger et al., 2003). The C-terminal dodecamer of Mdh1 was fused to GFP and co-expressed with mCherry-SKL. Scale bar represents 10 μm.



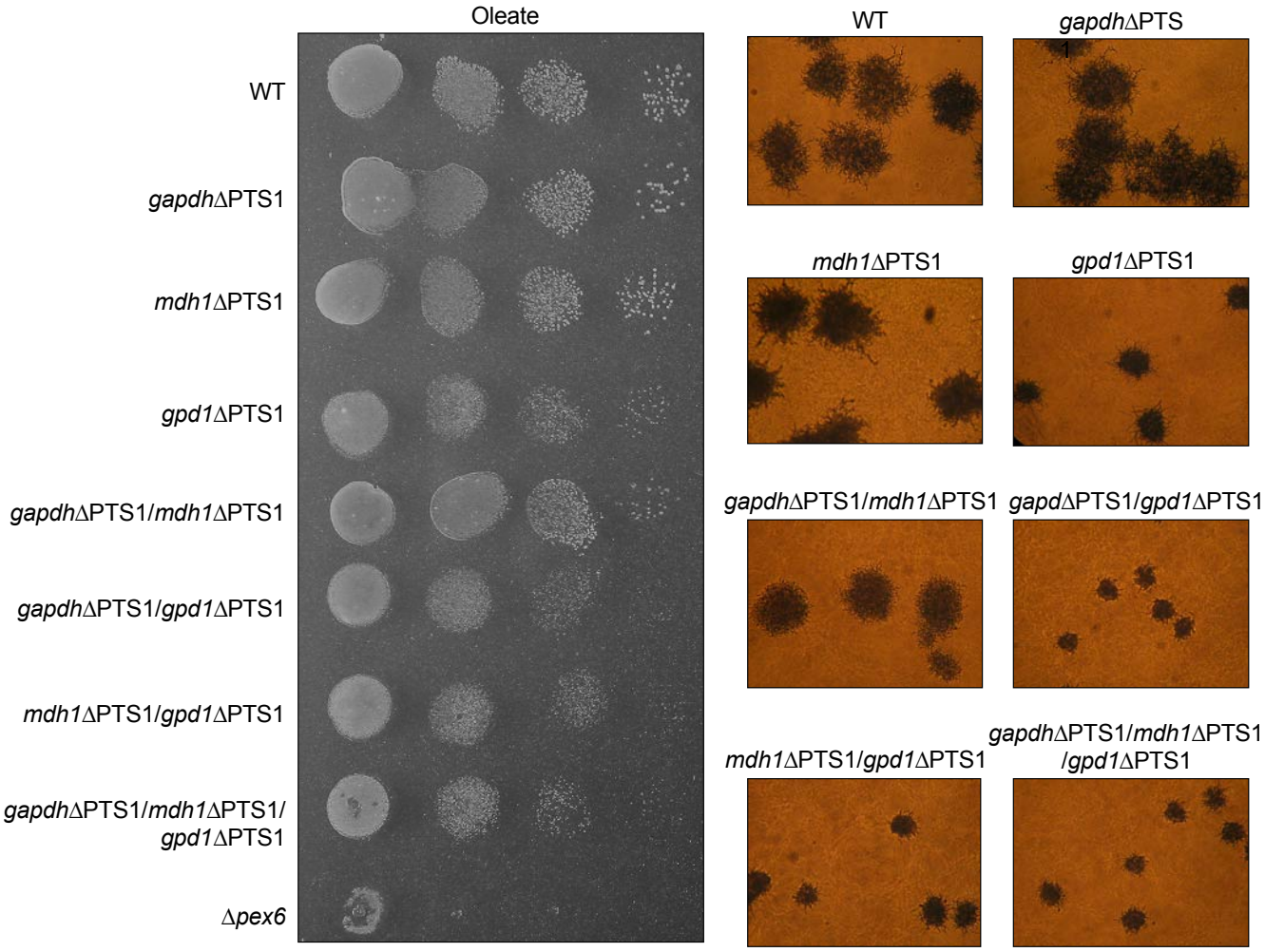
Supplementary Figure 12. Growth phenotype of mutants expressing PTS1-less mutants of peroxisomal dehydrogenases on glucose containing medium.
Serial dilutions of indicated mutants were spotted on minimal medium containing glucose and compared to the corresponding wild type and Δ*pex6* strains. The plate was photographed after 48 h at 28 °C, while single colonies were photographed after 36 h at 28 °C.



Supplementary Figure 13. Quantification of colony size on glucose containing minimal medium. The area of single colonies was calculated using ImageJ (http://rsb.info.nih.gov/ij/). At least 40 colonies from three biological replicates were used for quantification. Data are presented as mean +/- SD.



Supplementary Figure 14. Peroxisomes affect growth on different sugars.
Serial dilutions of indicated strains were spotted on YNB solid medium containing 2% of indicated sugars. Note, that on galactose containing medium peroxisome deficient mutants grow better than wild type strains.



Supplementary Figure 15. Growth phenotype of mutants expressing PTS1-less dehydrogenases on oleate medium.
Serial dilutions of indicated mutants were spotted on minimal medium containing oleate and compared to the corresponding wild type and Δ*pex6* strains. The plate and single colonies were photographed after 48 h at 28 °C.

a

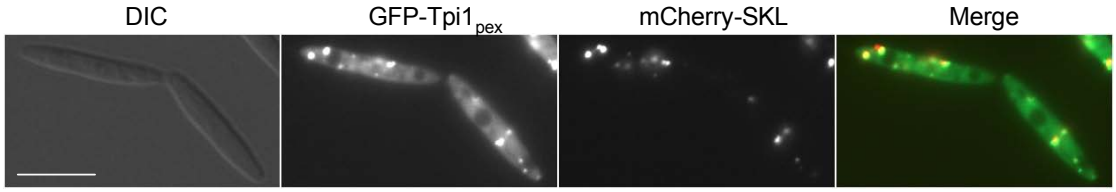
U. maydis: triose-phosphate isomerase (*tpi1*: um03299)

(a) (b)

AAC GCT AAC GCT TGA CTA GCT GGC CAG TCG GCT AGG ATC TGA

N A N A * L A G Q S A R I *

	C-Terminus	PTS1(score)
(a):	–KPEFVDIVNANA*	-21.5
(b):	–KPEFVDIVNANA* LAGQARI*	+0.7

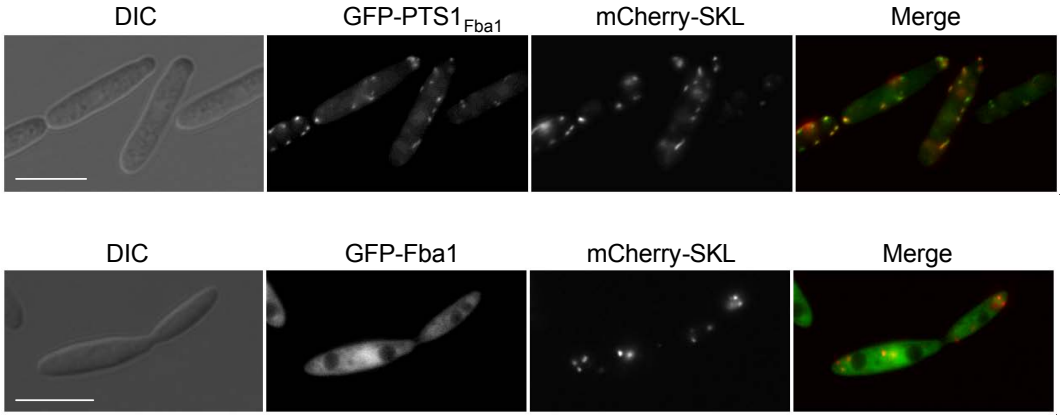


b

U. maydis: fructose-bisphosphate aldolase (*fba1*: um00674): PTS1 score: -0.35

GAG GCC TTT ACT GAC CTT GGC TCC ACT GGC AAG CTC TAA

E A F T D L G S T G K L *

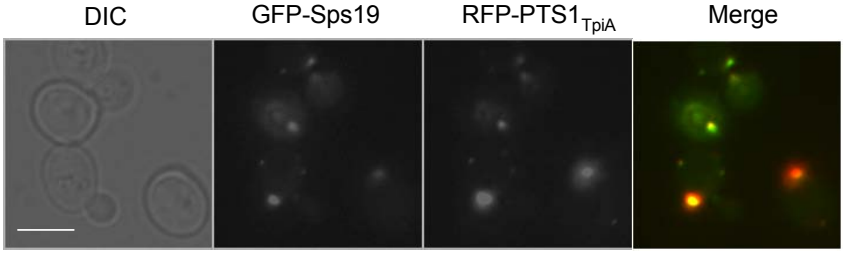


Supplementary Figure 16. *U. maydis* triose-phosphate isomerase (Tpi1) and fructose-bisphosphate aldolase (Fba1) contain peroxisomal targeting signals.
a, Protein sequences of *U. maydis* Tpi1 resulting from normal translational termination and translational read-through are indicated. The PTS1 scores of C-terminal dodecamers (underlined) were obtained using the PTS1 predictor tool (Neuberger et al., 2003). The C-terminal dodecamer of the read-through product (b) was expressed as GFP-fusion and colocalized with mCherry-SKL. Scale bar represents 10 μm.
b, *U. maydis* Fba1 contains a putative PTS1 motif. The C-terminal dodecamer was expressed as GFP-fusion and colocalized with mCherrySKL. Scale bar represents 10 μm.

a

A. nidulans: triose-phosphate isomerase (*tpiA*: gene ID: 2870611): PTS1 score: -10.5

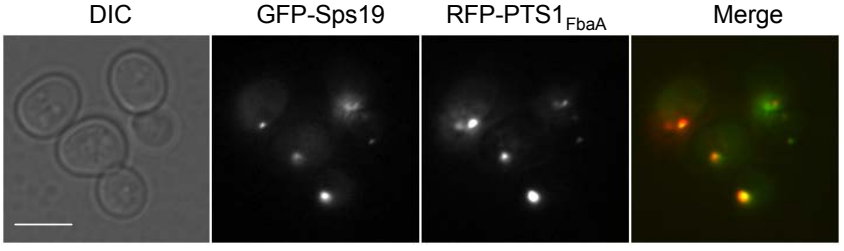
```
AAG CCT GCC T GTACGT ..... TAG TC GTC GAT ATT GTC AAT GCC CGC CTG TAA
 K   P   A                    F   V   D   I   V   N   A   R   L   *
```



b

A. nidulans: fructose-bisphosphate aldolase (*fbaA*: gene ID: 67524834): PTS1 score: -3.6

```
GTT GCT CTT GAG GAC TTC AAC ACT GCT GGC AAG CTC TAA
 V   A   L   E   D   F   N   T   A   G   K   L   *
```

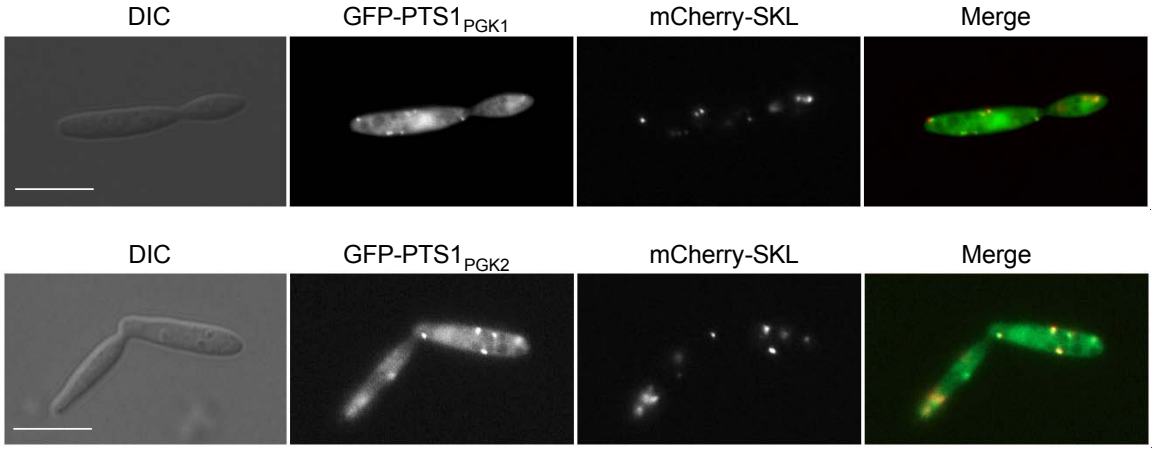


Supplementary Figure 17. *A. nidulans* triose-phosphate isomerase and fructose-bisphosphate aldolase contain peroxisomal targeting signals.
a, *A. nidulans* TpiA contains a putative PTS1. The C-terminal dodecamer was expressed as RFP-fusion in *S. cerevisiae* and colocalized with GFP-Sps19. Scale bar represents 5 μm.
b, *A. nidulans* FbaA contains a putative PTS1. The C-terminal dodecamer was expressed as RFP-fusion in *S. cerevisiae* and colocalized with GFP-Sps19. Scale bar represents 5 μm.

a

Homo sapiens

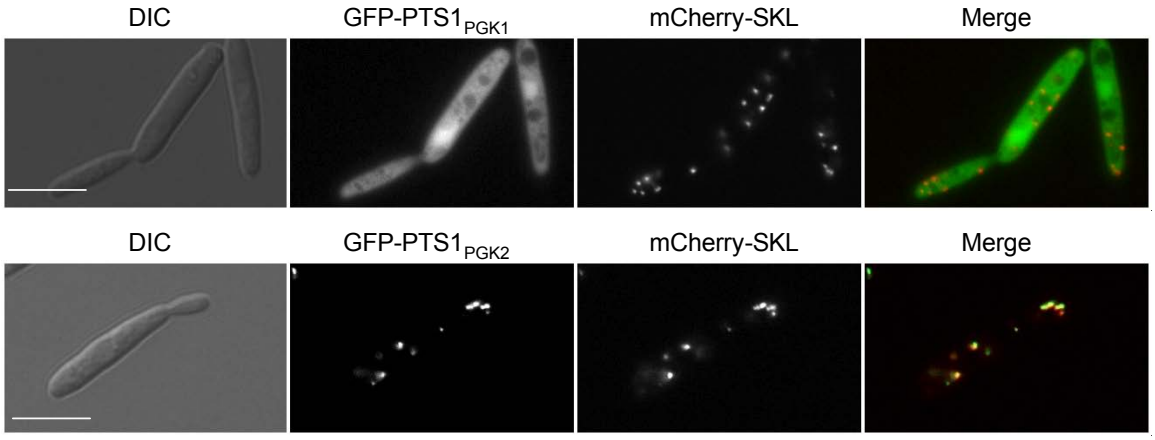
	C-Terminus	PTS1 (score)
PGK1 (GeneID: 48145549):	–KVLPGVDALSNI*	-5.7
PGK2 (GeneID: 31543397):	–KILGGVEALSNM*	+1.6



b

Bos taurus

	C-Terminus	PTS1 (score)
PGK1 (GeneID: 296470852):	–KVLPGVDALSSV*	-10.3
PGK2 (GeneID: 296474436):	–KVLPGVNALSNL*	+6.0



Supplementary Figure 18. Mammalian PGK1 and PGK2 proteins contain functional PTS1 motifs.
a, GFP fusion proteins of the C-terminal dodecamers of human PGK1 and PGK2 colocalized with mCherry-SKL in *U. maydis*. Scale bars represent 10 μm.
b, GFP fused to the C-terminal dodecamer of bovine PGK2 colocalized with mCherry-SKL in *U. maydis*, while the bovine PGK1 dodecamer was unable to target GFP to peroxisomes. Scale bars represent 10 μm.

Supplementary Table1: Growth assays in liquid medium

strain	doubling time (min) 2% glucose	doubling time (min) 0.4% oleate
wild type	119.72 +/- 0.73	555.23 +/- 29.2
*gapd*ΔPTS1	127.87 +/- 0.94	660.26 +/- 53
*mdh1*ΔPTS1	123.94 +/- 2.1	633.22 +/- 85.64
*gpd1*ΔPTS1	120.64 +/- 1.6	1066.58 +/- 149.45
*gapd*ΔPTS1 *mdh1*ΔPTS1	140.77 +/- 2.6	644.18 +/- 120.82
*gapd*ΔPTS1 *gpd1*ΔPTS1	147.01 +/- 1.05	1769.24 +/- 78.66
*mdh1*ΔPTS1 *gpd1*ΔPTS1	153.10 +/- 8.63	1372.31 +/- 190.82
*gapd*ΔPTS1 *mdh1*ΔPTS1 *gpd1*ΔPTS1	149.32 +/- 4.47	1663.49 +/- 172.61
Δ*pex6*	151.74 +/- 1.29	no growth

Supplementary table 2: *U. maydis* strains used in this study

Strain	Genotype	Resistance	Reference
Bub8	*a2 b4*	_	Weinzierl et al. (2002)
SG200	*a1::mfa2 bW2 bE1*	Phleo^R	Kämper et al. (2006)
Bub8 mCherry-SKL	*a2 b4 P_{otef}:mcherry-SKL*	Hyg^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{GAPDH}$	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-PTS1$_{GAPDH}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{PGK}$	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-PTS1$_{PGK}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-Pte1	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-pte1]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{PGK}$	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-pte1]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-GAPDH	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-gapdh]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-GAPDH$_{pex}$	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-gapdh$_{pex}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-GAPDH$_{cyt}$	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-gapdh$_{cyt}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PGK	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-pgk1]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PGK$_{pex}$	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-pgk1$_{pex}$]ip^S*	Hyg^R, Cbx^R	This study
SG200 gapdhΔPTS1	*a1::mfa2 bW2 bE1 gapdh*ΔPTS1	Hyg^R, Phleo^R	This study
SG200 Δpex6 mCherry-SKL	*a1::mfa2 bW2 bE1 Δpex6 P_{otef}:mcherry-SKL*	Hyg^R, Phleo^R, Nat^R	This study
Bub8 mCherry-SKL GFP-PGK$_{cyt}$	*a2 b4 P_{otef}:mcherry-SKL ip^R[P_{otef}:egfp-pgk1$_{cyt}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 Δpex6	*a2 b4 Δpex6*	Hyg^R	This study
Bub8 Δpex6 mCherry-SKL	*a2 b4 Δpex6 P_{otef}:mcherry-SKL*	Hyg^R, Cbx^R	This study
Bub8 Δpex6 GFP-GAPDH$_{pex}$	*a2 b4 Δpex6 ip^R[P_{otef}:egfp-gapdh$_{pex}$]ip^S*	Hyg^R, Cbx^R	This study

Supplementary table 2: continued

Strain	Genotype	Resistance	Reference
Bub8 Δpex6 GFP-PGK$_{pex}$	*a2 b4 Δpex6 ip^{R}[P$_{otef}$:egfp-pgk1$_{pex}$]ip^{S}*	Hyg^{R}, Cbx^{R}	This study
Bub8 PGK•GFP1	*a2 b4 ip^{R}[P$_{otef}$:pgk1-stop+3-egfp]ip^{S}*	Cbx^{R}	This study
Bub8 PGK•GFP2	*a2 b4 ip^{R}[P$_{otef}$:pgk1-stop+81-egfp]ip^{S}*	Cbx^{R}	This study
Bub8 gapdΔPTS1	*a2 b4 gapd*ΔPTS1	Hyg^{R}	This study
Bub8 pgk1ΔPTS1	*a2 b4 pgk1*ΔPTS1	Nat^{R}	This study
Bub8 gapdΔPTS1 pgk1ΔPTS1	*a2 b4 pgk1*ΔPTS1	Hyg^{R}, Nat^{R}	This study
Bub8 mCherry-SKL GFP-Gpd1	*a2 b4 P$_{otef}$:mcherry-SKL ip^{R}[P$_{otef}$:egfp-gpd1]ip^{S}*	Hyg^{R}, Cbx^{R}	This study
Bub8 mCherry-SKL GFP-PTS1$_{Mdh1}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^{R}[P$_{otef}$:egfp-PTS1$_{Mdh1}$]ip^{S}*	Hyg^{R}, Cbx^{R}	This study
Bub8 mdh1ΔPTS1	*a2 b4 mdh1*ΔPTS1	Nat^{R}	This study
Bub8 gapdΔPTS1 mdh1ΔPTS1	*a2 b4 gapd*ΔPTS1 *mdh1*ΔPTS1	Hyg^{R}, Nat^{R}	This study
Bub8 gpd1ΔPTS1	*a2 b4 gpd1*ΔPTS1	Hyg^{R}	This study
Bub8 gapdΔPTS1 gpd1ΔPTS1	*a2 b4 gapd*ΔPTS1 *gpd1*ΔPTS1	Hyg^{R}, Nat^{R}	This study
Bub8 gpd1ΔPTS1 mdh1ΔPTS1	*a2 b4 gpd1*ΔPTS1 *mdh1*ΔPTS1	Hyg^{R}, Nat^{R}	This study
Bub8 gapdΔPTS1 mdh1ΔPTS1 gpd1ΔPTS1	*a2 b4 gapd*ΔPTS1 *mdh1*ΔPTS1 *gpd1*ΔPTS1	Hyg^{R}, Nat^{R}, Phleo^{R}	This study
Bub8 mCherry-SKL GFP-PTS1$_{Pb\text{-}GAPDH1}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^{R}[P$_{otef}$:egfpPTS1$_{Pb\text{-}GAPDH1}$]ip^{S}*	Hyg^{R}, Cbx^{R}	This study
Bub8 mCherry-SKL GFP-PTS1$_{Pb\text{-}GAPDH2}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^{R}[P$_{otef}$:egfpPTS1$_{Pb\text{-}GAPDH2}$]ip^{S}*	Hyg^{R}, Cbx^{R}	This study
Bub8 mCherry-SKL GFP-PTS1$_{Pb\text{-}GAPDH3}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^{R}[P$_{otef}$:egfpPTS1$_{Pb\text{-}GAPDH3}$]ip^{S}*	Hyg^{R}, Cbx^{R}	This study

Supplementary table 2: continued

Strain	Genotype	Resistance	Reference
Bub8 PGK•GFP#1	*ip^R[P$_{pgk1}$:pgk1-stop+81-egfp]ip^S*	Cbx^R	This study
Bub8 PGK•GFP#2	*ip^R[P$_{pgk1}$:pgk1-stop+81-egfp]ip^S*	Cbx^R	This study
Bub8 PGK•GFP#3	*ip^R[P$_{pgk1}$:pgk1-stop+81-egfp]ip^S*	Cbx^R	This study
Bub8 PGK•GFP#4	*ip^R[P$_{pgk1}$:pgk1-stop+81-egfp]ip^S*	Cbx^R	This study
SG200 pgk1ΔPTS1	*a1::mfa2 bW2 bE1 pgk1*ΔPTS1	Nat^R, Phleo^R	This study
SG200 gapdhΔPTS1 pgk1ΔPTS1	*a1::mfa2 bW2 bE1 gapdh*ΔPTS1 *pgk1*ΔPTS1	Hyg^R, Nat^R , Phleo^R	This study
Bub8 mCherry-SKL Δpex5a	*a2 b4 P$_{otef}$:mcherry-SKL Δpex5a*	Hyg^R, Nat^R	This study
Bub8 mCherry-SKL Δpex5b	*a2 b4 P$_{otef}$:mcherry-SKL Δpex5b*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL Δpex5b Δpex5b	*a2 b4 P$_{otef}$:mcherry-SKL Δpex5a Δpex5b*	Hyg^R, Cbx^R , Nat^R,	This study
SG200 mCherry-SKL Δpex5a	*a1::mfa2 bW2 bE1 P$_{otef}$:mcherry-SKL Δpex5a*	Hyg^R, Nat^R, Phleo^R	This study
SG200 mCherry-SKL Δpex5b	*a1::mfa2 bW2 bE1 P$_{otef}$:mcherry-SKL Δpex5b*	Hyg^R, Cbx^R, Phleo^R,	This study
SG200 mCherry-SKL Δpex5a Δpex5b	*a1::mfa2 bW2 bE1 P$_{otef}$:mcherry-SKL Δpex5a Δpex5b*	Hyg^R, Cbx^R , Phleo^R Nat^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{FBA}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^R[P$_{otef}$:egfpPTS1$_{FBA}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-TPI$_{pex}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^R[P$_{otef}$:egfp-tpi1$_{pex}$]ipS*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{Hs\text{-}PGK1}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^R[P$_{otef}$:egfpPTS1$_{Hs\text{-}pgk1}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{Hs\text{-}PGK2}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^R[P$_{otef}$:egfpPTS1$_{Hs\text{-}pgk2}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{Bt\text{-}PGK1}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^R[P$_{otef}$:egfpPTS1$_{Bt\text{-}pgk1}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-PTS1$_{Bt\text{-}PGK2}$	*a2 b4 P$_{otef}$:mcherry-SKL ip^R[P$_{otef}$:egfpPTS1$_{Bt\text{-}pgk2}$]ip^S*	Hyg^R, Cbx^R	This study
Bub8 mCherry-SKL GFP-FBA	*a2 b4 P$_{otef}$:mcherry-SKL ip^R[P$_{otef}$:egfp-fba1]ip^S*	Hyg^R, Cbx^R	This study