Supplemental Material

Amino acid identity (similarity)

	GpAMT1	GpAMT2	GpAMT3	GiAMT1
GpAMT2	60% (74%)			
GpAMT3	59% (76%)	63% (78%)		
GiAMT1	57% (74%)	61% (77%)	65% (81%)	
GiAMT2	61% (74%)	58% (74%)	65% (81%)	63% (79%)

Fig. S1 Comparison of glomeromycotan AMT amino acid sequences. GpAMT1, GpAMT2, GpAMT3, GiAMT1 and GiAMT2 amino acid sequences were analyzed pairwise with the BLAST algorithm and Blosum62 (1), and percentages of identical (similar) amino acids are listed. The comparison of *Geosiphon pyriformis* ammonium transporters is marked in dark grey.

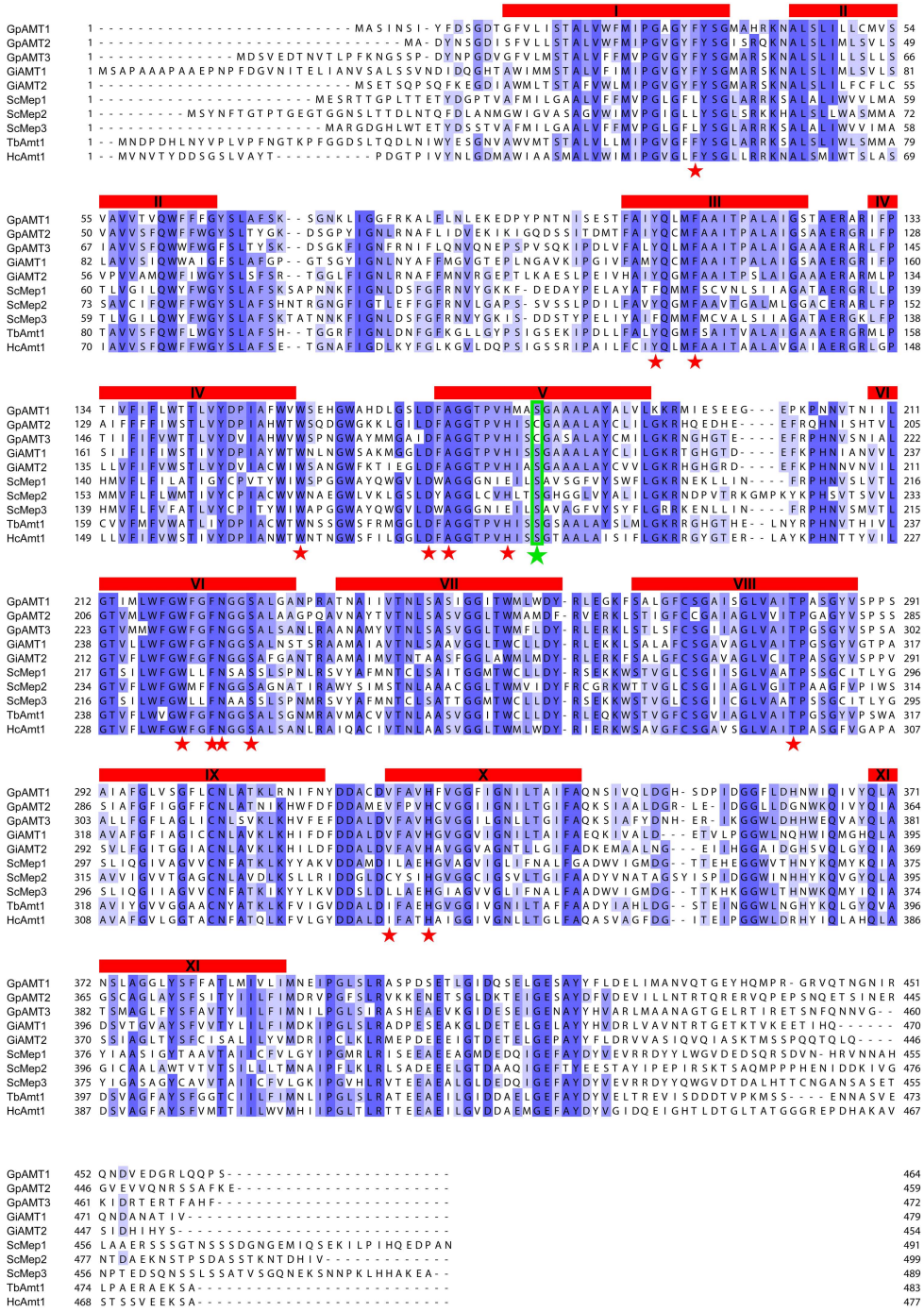

Fig. S2 Alignment of 10 fungal AMT amino acid sequences. The 14 conserved residues reported to be of functional significance for conducting ammonium through the pore region (2, 3) are marked with red stars. Sequences were obtained from the GenBank database with the following accession numbers: *Geosiphon pyriformis* (GpAMT1, JX535577; GpAMT2, JX535578; GpAMT3, JX535579), *Rhizophagus irregularis* (GiAMT1, CAI54276; GiAMT2, CAX32490), *Saccharomyces cerevisiae* (ScMep1, P40260; ScMep2, P41948; ScMep3, P53390), *Tuber borchii* (TbAmt1, AAL11032), *Hebeloma cylindrosporum* (HcAmt1, AAM21926). Residues shaded in light, medium, and dark blue indicate at least 50%, 70%, and 90% conservation of the 10 sequences, respectively. Red thick lines indicate positions of the 11 transmembrane domains predicted for GpAMT1. Green box and star mark the unusual cysteine residue in GpAMT2 and GpAMT3 located in close proximity to the histidine dyad (see Fig. S5) that plays an important role in ammonium transduction (4).

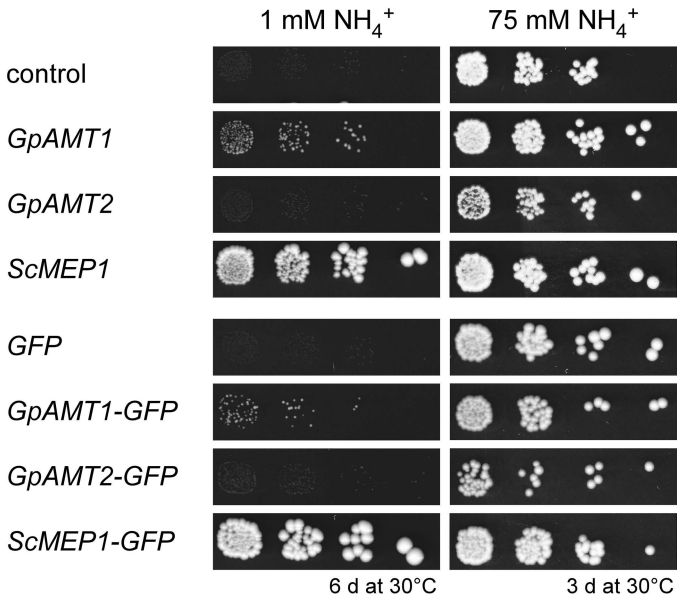

Fig. S3 Complementation assay of yeast strain MLY131a/α (*mep1-3*Δ). The strain was transformed with the empty vector or *GFP* as negative controls, *ScMEP1* or *ScMEP1-GFP* as positive controls, and the annotated AMT genes or genes encoding AMT-GFP fusion proteins. Strains were cultivated on minimal medium containing 1 mM NH_4^+ as sole nitrogen source or on HC-U medium containing 75 mM NH_4^+. Note that transformation with GFP-tagged AMTs resulted in the same functional complementation as transformation with untagged versions.

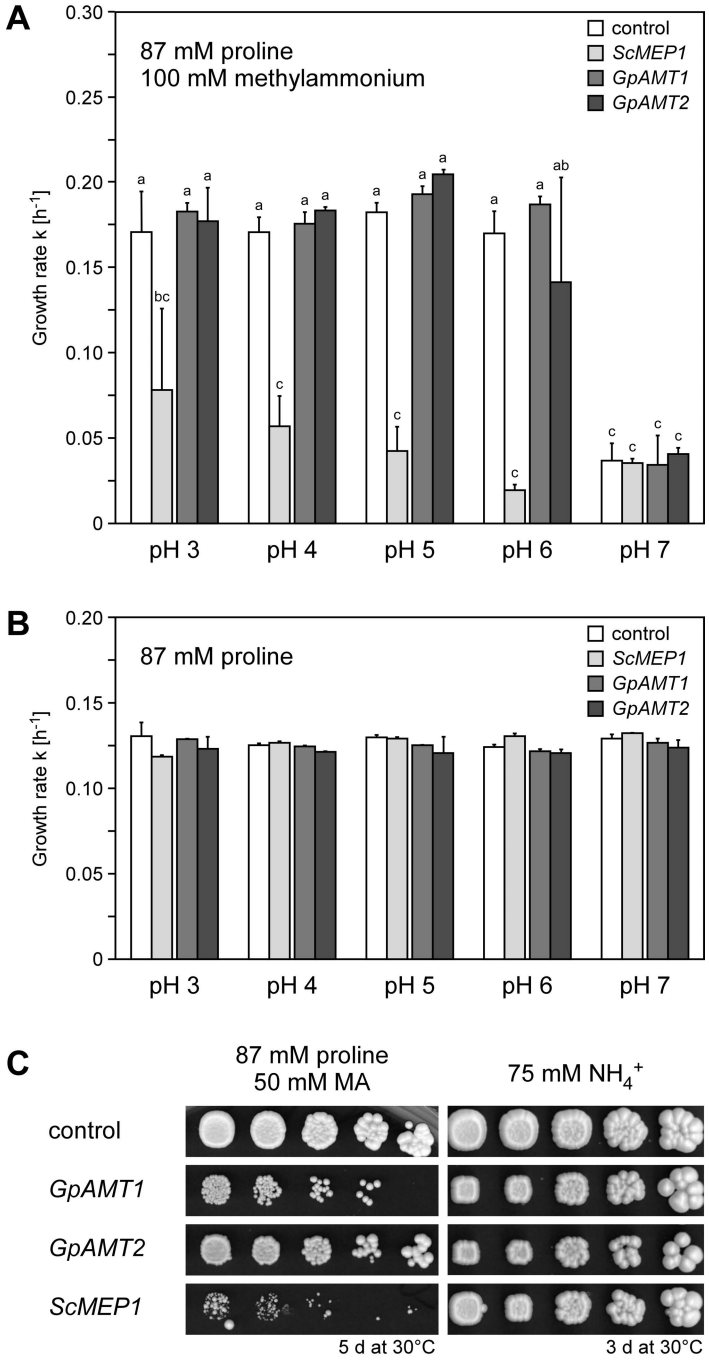

Fig. S4 Growth assays in the presence of cytotoxic methylammonium (MA). The yeast strain MLY131a/α (*mep1-3*Δ) was transformed with the empty vector (negative control), *ScMEP1* (positive control), *GpAMT1*, or *GpAMT2*. (A) pH dependence of MA uptake and cytotoxicity. Yeast cells were cultivated in buffered liquid medium containing 0.1% proline as nitrogen source and 100 mM MA. At pH values below 7, clear differences between *ScMEP1* expressing yeast and the other strains were observed. At pH 7 passive influx of MA seems to limit growth of all strains. Error bars, ±SD; n = 4 biological replicates. Different letters above bars indicate highly significant differences ($P \leq 0.001$, ANOVA test) between pairwise comparisons. (B) Growth in buffered liquid medium with 0.1% proline as sole nitrogen source, at pH values of 3-7. Yeast growth was not affected by medium pH. Error bars, ±SD; n = 4 biological replicates. (C) Growth on solid mimimal medium containing 0.1% proline as nitrogen source and 50 mM MA (left), or on HC-U medium containing 75 mM NH_4^+ (right).

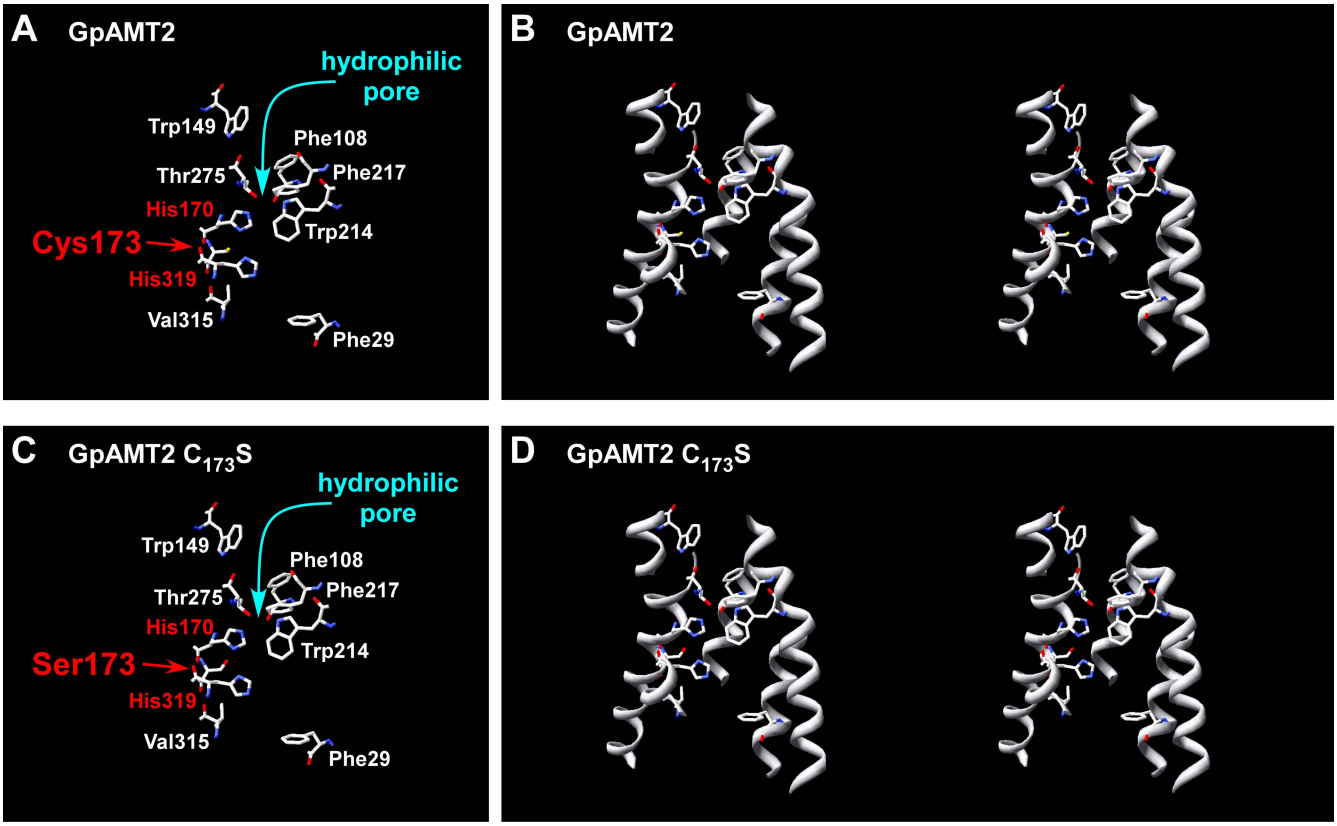

Fig. S5 Structural model of the hydrophilic pore in GpAMT2. (A,C) Representation of the conserved and functionally important residues inside the hydrophilic pore for ammonium transduction and of cysteine/serine 173. (B,D) Stereo images of the 3D structure of the pore region. Cysteine/serine 173 is located in close proximity to the histidine dyad. Structures of GpAMT2 and GpAMT2 $C_{173}S$ were generated using SWISS-MODEL and the PDB template 2b2h of *Archaeoglobus fulgidus* amt-1.

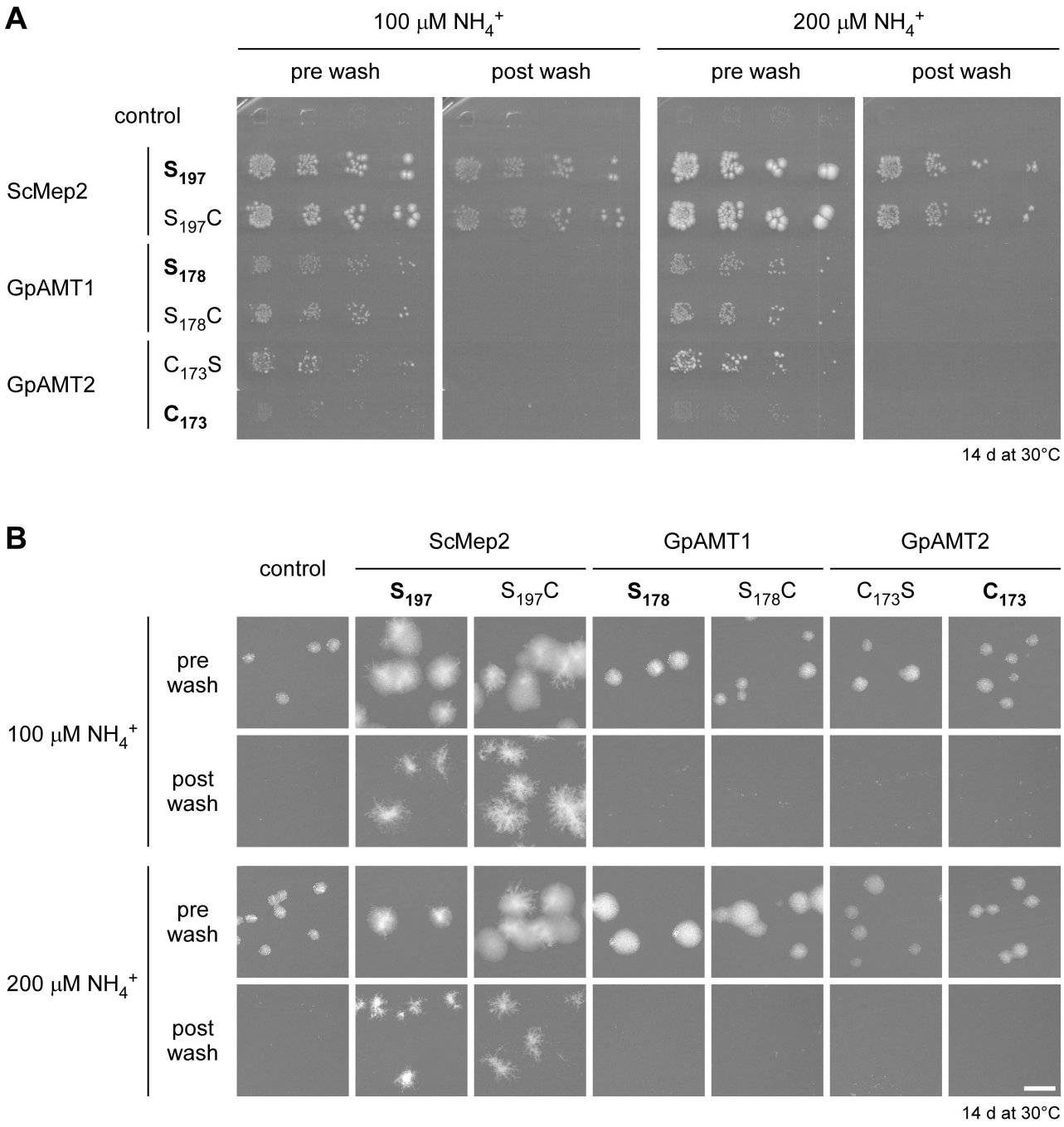

Fig. S6 Test for invasive pseudohyphal growth on solid medium. The yeast strain MLY131a/α (*mep1-3*Δ) was transformed with the empty vector (negative control), and with vectors expressing wild type and mutant yeast and *Geosiphon pyriformis* AMTs. The respectice wild type constructs are indicated in bold. Yeast strains were grown on solid minimal medium with 100 μM or 200 μM NH_4^+ as sole nitrogen source. After 14 days at 30°C colonies were documented and subsequently washed away with water, leaving only invasively growing cells. (A) Photographs of fivefold serial dilutions of the indicated strains. Representative examples from 4 biological replicates are shown. (B) Photographs of colony morphologies were taken directly from Petri plates with a Leica MZ 16 FA stereomicroscope with a 63x primary objective and a 0.8x camera adaptor (bar, 200 μm). Representative examples from 4 biological replicates are shown.

Note that only ScMep2 constructs complement for pseudohyphal growth and that the $S_{197}C$ mutation does not interfere with pseudohyphal growth.

Table S1 Plasmids used in this study.

Plasmid	Backbone	Insert	Reference
pDR196sfi	pDR196	SfiI restriction sites	(5)
pRU4		*EGFP*	(6)
pBL101	pDR196sfi	*GpAMT1* with UTRs	This study
pBL102	pDR196sfi	*GpAMT2* with UTRs	This study
pBL103	pDR196sfi	*ScMEP1*	This study
pBL104	pDR196sfi	*ScMEP2*	This study
pBL106	pDR196sfi	*GFP*	This study
pBL107	pDR196sfi	*GpAMT1-GFP*	This study
pBL108	pDR196sfi	*GpAMT2-GFP*	This study
pBL109	pDR196sfi	*ScMEP1-GFP*	This study
pBL110	pDR196sfi	*GpAMT1* ORF	This study
pBL111	pDR196sfi	*GpAMT2* ORF	This study
pBL130	pDR196sfi	*GpAMT1_S_{178}C*	This study
pBL131	pDR196sfi	*GpAMT2_C_{173}S*	This study
pBL134	pDR196sfi	*ScMEP2_S_{197}C*	This study
pBL151	pDR196sfi	*GiAMT1*	This study
pBL154	pDR196sfi	*GiAMT1-GFP*	This study
pBL179	pDR196sfi	*GpAMT3* with UTRs	This study
pBL180	pDR196sfi	*cGpAMT3*	This study
pBL181	pDR196sfi	*cGpAMT3_C_{189}S*	This study
pBL183	pDR196sfi	*gGpAMT3*	This study
pBL185	pDR196sfi	*gGpAMT3_C_{189}S*	This study
pBL186	pDR196sfi	*gGpAMT3-GFP*	This study

Table S2 Primers used in this study.

Primer name	Sequence (5'-3')[a]	Application
Primers in vector backbone		
ADHclose	AATACGACTCACTATAGG	pDR196sfi backbone, 3' of insertion site
ADH109-REV	CGACTCACTATAGGGCG	Nested to ADHclose
PMA5	CTCTCTTTTATACACACATTC	pDR196sfi backbone, 5' of insertion site
PMA16	CGGGCTGCAGGAATTCGG	Nested to PMA5
Cloning of GpyrAMT genes		
MEP1-50-FWD	GGTATGTATGATGGCTATTTGTGTTGTTATNYTNCARTGGT	Degenerate PCR
MEP1-53-FWD	ATGGCTATTTGTGTTGTTATTTTTCARTGGTWYTT	Degenerate PCR
MEP1-178-REV	AATTTCAACAGGACCACCACCNGCVHARTC	Degenerate PCR
GpAMT2-FWD1	CATATATTGGCAATTTAAGGAATG	Cloning of *GpAMT2* 3' end
GpAMT2-FWD2	GGCAATTTAAGGAATGCTTTCC	Nested to GpAMT2-FWD1
GpAMT2-REV3	CCAAATGCAATAGAAGATGAGG	Cloning of *GpAMT2* 5' end
GpAMT2-REV5	GGTCTTCTTGATGTCGCTTTCC	Nested to GpAMT2-REV3
GpAMT3-clone-FWD	CGGTTGGTTTGGATTTAACG	Cloning of *GpAMT3*
GpAMT3-clone-REV	CCCAGCAAGGAAACCAAATA	Cloning of *GpAMT3*
Cloning of yeast expression constructs		
GpAMT1-sfi-FWD	CAAGGCCATTACGGCCAACATGGCTTCGATAAATTCGATATATTTCG	Cloning of *GpAMT1* CDS
GpAMT1-sfi-REV	GATGGCCGAGGCGGCCTTAAAGAGACGGTTGCTGAAGTCT	Cloning of *GpAMT1* CDS
GpAMT2-sfi-FWD	CCAGGCCATTACGGCCTACAAAATGGGCCGATTATAATTC	Cloning of *GpAMT2* CDS
GpAMT2-sfi-REV	GATGGCCGAGGCGGCCTTAATTTTCCTTGAAAGCACTCGA	Cloning of *GpAMT2* CDS
GpAMT3-sfi-FWD	CAAGGCCATTACGGCCAACATGGATTCTGTGGAAGATACGAATG	Cloning of *GpAMT3* CDS
GpAMT3-sfi-REV	GATGGCCGAGGCGGCCTTAAAAATGAGCAAATGTTCTTTCGG	Cloning of *GpAMT3* CDS
GpAMT3-gen-FWD	CACAATGGATTCTGTGGAAGATACG	Constructing gDNA variant of *GpAMT3*
GpAMT3-gen-REV	CAGAGCACAAAAATATTTGCATTATTGCC	Constructing gDNA variant of *GpAMT3*
GiAMT1-sfi-FWD	CCAGGCCATTACGGCCAACATGTCTGCTCCCGCTGCTGC	Cloning of *GiAMT1*
GiAMT1-sfi-REV	CCTAGGCCGAGGCGGCCTTATACAATTGTTGCATTTGCGTCATTTTG	Cloning of *GiAMT1*
ScMEP1-sfi-FWD	CAAGGCCATTACGGCCTACAAAATGGAGAGTCGAACTAC	Cloning of *ScMEP1* CDS
ScMEP1-sfi-REV	CCAGGCCGAGGCGGCCTTACCTATTGGCAGGATCTTCTTGATG	Cloning of *ScMEP1* CDS
ScMEP2-sfi-FWD	CCAGGCCATTACGGCCAACATGTCTTACAATTTTACAGGTACG	Cloning of *ScMEP2* cDNA
ScMEP2-sfi-REV	CCAGGCCGAGGCGGCCTTATACTATATGGTCAGTGTTCTTA	Cloning of *ScMEP2* cDNA
Cloning of GFP-tags		
X-S-EGFP-FWD	GGACTCGAGTAACACGGCCGCCTCGGCCATGGTGAGCAAGGGCGAGG	Cloning of *GFP* into pDR196sfi
EGFP-A-REV	CATGGGCCCTTACTTGTACAGCTCGTCCATGCC	Cloning of *GFP* into pDR196sfi
ORF-FWD	CGGCCTACAAAATGTCGGCCGCCT	Cloning of pBL106 (soluble GFP)
ORF-REV	CGGCCGACATTTTGTAGGCCGTAA	Cloning of pBL106 (soluble GFP)
GpAMT1-tag-sfi-REV	GATGGCCGAGGCGGCCGAAAGAGACGGTTGCTGAAGTCT	Reverse primer for tagging *GpAMT1*
GpAMT2-tag-sfi-REV	GATGGCCAGGCGGCCGAATTTTCCTTGAAAGCACTCGA	Reverse primer for tagging *GpAMT2*
GpAMT3-tag-sfi-REV	CCTAGGCCGAGGCGGCCAAAAAATGAGCAAATGTTCTTTCGG	Reverse primer for tagging *GpAMT3*
ScMEP1-tag-sfi-REV	GATGGCCGAGGCGGCCGACCTATTGGCAGGATCTTCTTG	Reverse primer for tagging *ScMEP1*
Cloning of CtoS and StoC variants		
GpAMT1-StoC-FWD	GTTCATATGGCATGTGGAGCTGCTGCACTCG	Mutation $S_{178}C$ in GpyrAMT1
GpAMT1-StoC-REV	CGAGTGCAGCAGCTCCACATGCCATATGAAC	Mutation $S_{178}C$ in GpyrAMT1
GpAMT2-CtoS-FWD	GTACACATTTCTTCCGGAGCTGCTGTTTAG	Mutation $C_{173}S$ in GpyrAMT2
GpAMT2-CtoS-REV	CTAAGCAGCAGCTCCGGAAGAAATGTGTAC	Mutation $C_{173}S$ in GpyrAMT2
GpAMT3-CtoS-FWD	CTGTTCATATTTCTTCCGGAGCTTCTGCAC	Mutation $C_{189}S$ in cGpyrAMT3
GpAMT3-CtoS-REV	GTGCAGAAGCTCCGGAAGAAATATGAACAG	Mutation $C_{189}S$ in cGpyrAMT3
gGpAMT3-CtoS-FWD	CTGTTCATATTTCTTCCGGAGCTGCTGCAC	Mutation $C_{189}S$ in gGpyrAMT3
gGpAMT3-CtoS-REV	GTGCAGCAGCTCCGGAAGAAATATGAACAG	Mutation $C_{189}S$ in gGpyrAMT3
ScMEP2-StoC-FWD	GTGTCCATCTCACGTGTGGACATGGTGGTC	Mutation $S_{197}C$ in ScMep2
ScMEP2-StoC-REV	GACCACCATGTCCACACGTGAGATGGACAC	Mutation $S_{197}C$ in ScMep2

[a] Overhangs are underlined

Supplemental References

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