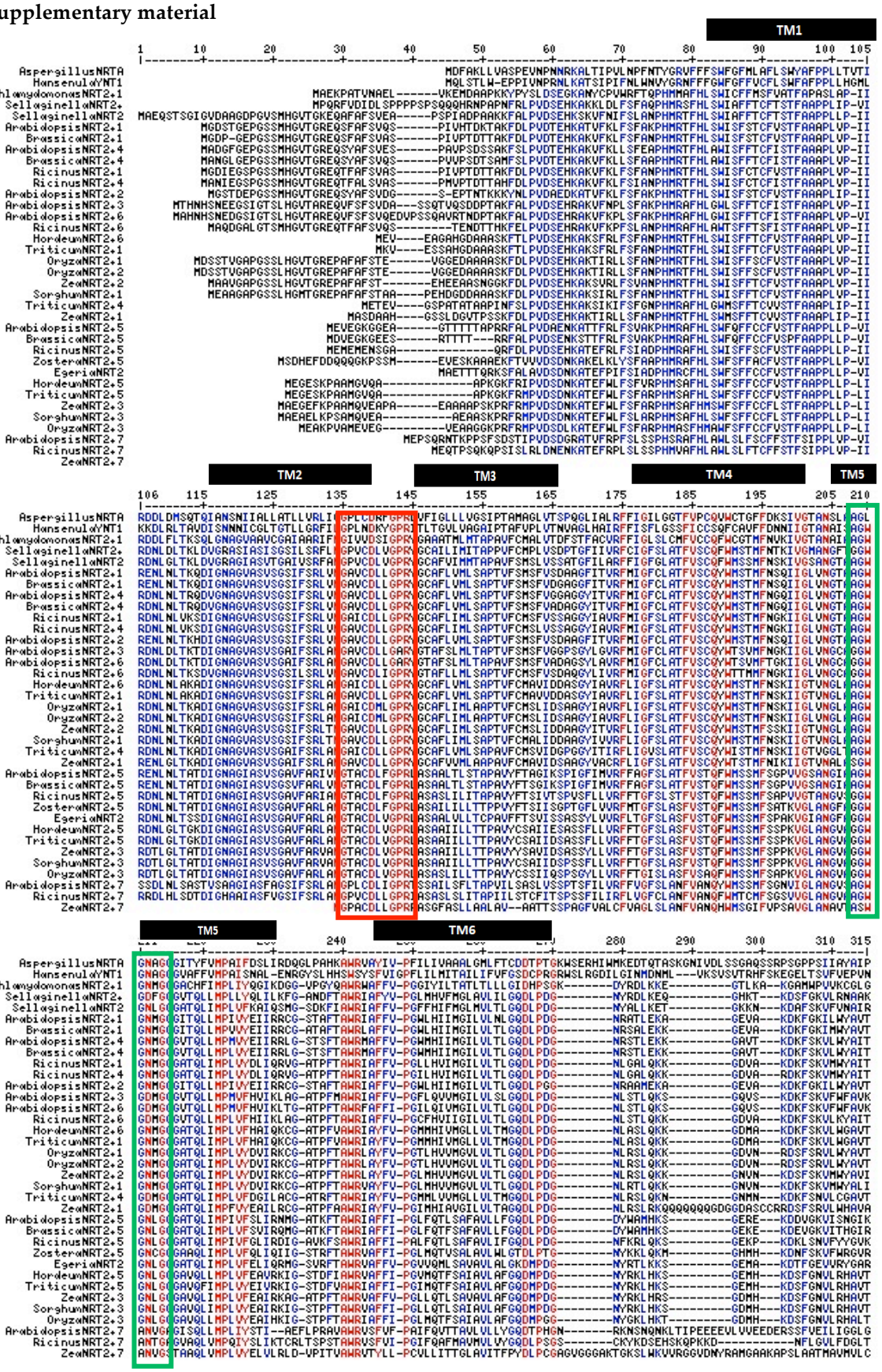
1 **Supplementary material**

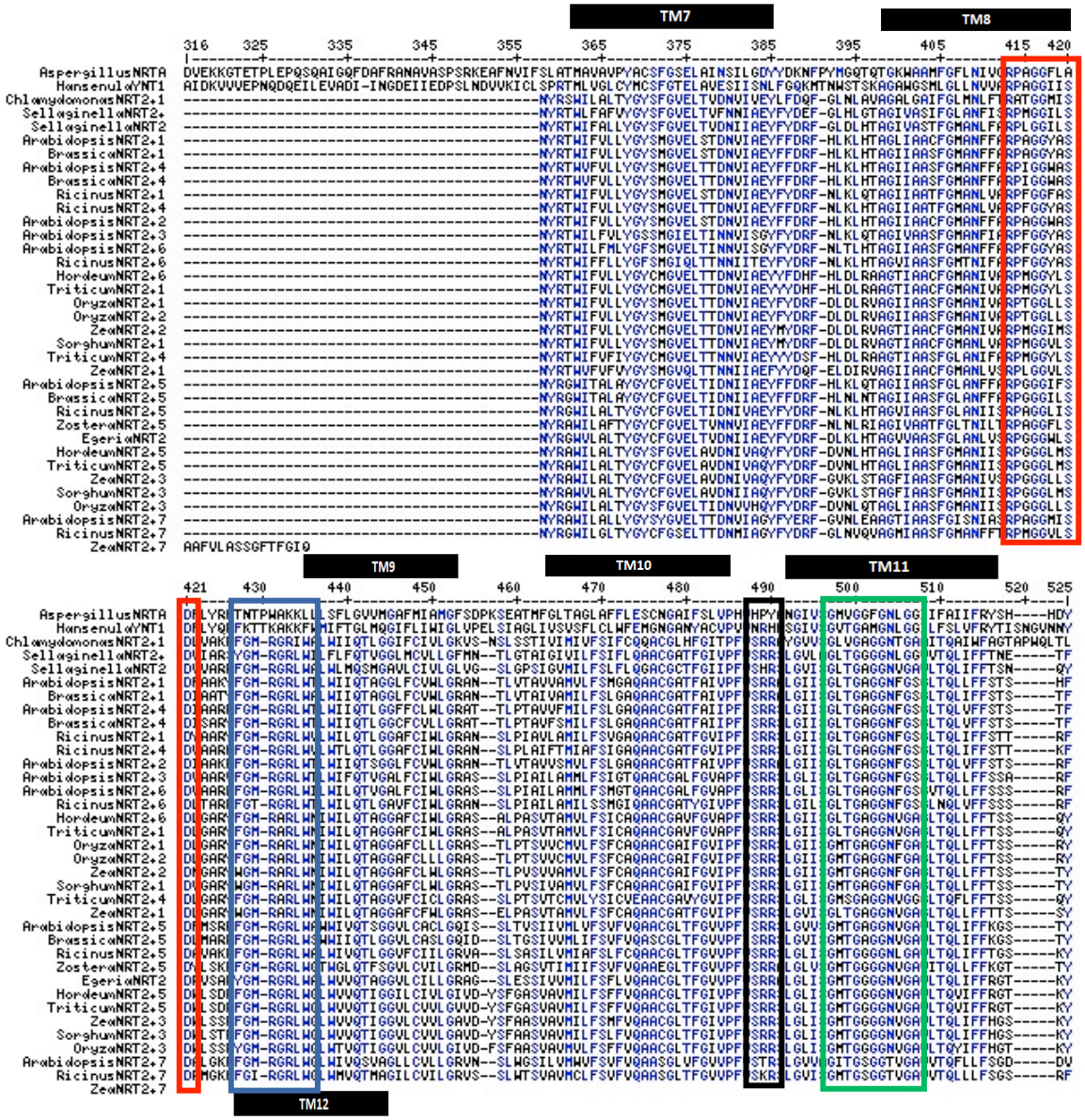


2

3

4

1


TM7
TM8
TM9
TM10
TM11
TM12
AspergillusNRTA
HansenulaYNT1
ChlamydomonasNRT2.1
SelaginellaNRT2.
SelaginellaNRT2
ArabidopsisNRT2.1
BrassicaNRT2.1
ArabidopsisNRT2.4
BrassicaNRT2.4
RicinusNRT2.1
RicinusNRT2.4
ArabidopsisNRT2.2
ArabidopsisNRT2.3
ArabidopsisNRT2.6
RicinusNRT2.6
HordeumNRT2.6
TriticumNRT2.1
OryzaNRT2.1
OryzaNRT2.2
ZeaNRT2.2
SorghumNRT2.1
TriticumNRT2.4
ZeaNRT2.1
ArabidopsisNRT2.5
BrassicaNRT2.5
RicinusNRT2.5
ZosteraNRT2.5
EgeriaNRT2
HordeumNRT2.5
TriticumNRT2.5
ZeaNRT2.3
SorghumNRT2.3
OryzaNRT2.3
ArabidopsisNRT2.7
RicinusNRT2.7
ZeaNRT2.7

2

3

Figure S1. Multiple alignment of NNP family transporters. Sequence alignment was obtained using the MultAlin alignment tool [44]. Red and blue amino acids indicate highly conserved residues, higher than 90% and 50%, respectively. Transmembrane domains of ZosmaNRT2 are overlined and numbered above the sequences. The conserved amino acids for Mayor Facilitator Superfamily motifs (MFS I and II) are framing in red, green for Nitrate Nitrite Porter motifs (NNP I and II), blue for the Photosynthetic NRT2 motif and blak for phosphorylation sites of S/T-X-R/K motifs. The accession numbers of each protein in the NCBI used for the analysis were: *Zostera marina* (NRT2.1, KMZ59016); *Arabidopsis thaliana* (NRT2.1, O82811; NRT2.2, Q9LMZ9; NRT2.3, AED97376; NRT2.4, Q9FJH8; NRT2.5, Q9LPV5; NRT2.6, Q9LXH0; NRT2.7, Q9LYK2); *Brassica napus* (NRT2.1, XP_013729508; NRT2.4, XP_013665864; NRT2.5, XP_013657329) *Ricinus communis* (NRT2.1, XP_002523687; NRT2.4, XP_002523688; NRT2.5, XP_002527899; NRT2.6, XP_002523689; NRT2.7, XP_002524664); *Oryza sativa* (NRT2.2, XP_015623596; NRT2.3, XP_015628524); *Sorghum bicolor* (NRT2.1, XP_002453159; NRT2.3, XP_002456219); *Triticum urartu* (NRT2.1, EMS65311; NRT2.4, EMS46096, NRT2.5, EMS50263); *Zea mays* (NRT2.1, XP_008645163; NRT2.2, NP_001105195; NRT2.3, XP_008656795; NRT2.7, AQK44570); *Hordeum vulgare* (NRT2.5, ABG20828; (NRT2.6, ABG20829); *Egeria densa* (NRT2, BAK51923); *Selaginella moellendorffii* (NRT2.1, XP_002993278; NRT2.4, XP_002966266); *Chlamydomonas reinhardtii* (NRT2.1, XP_001696789); *Hansenula polymorpha* (YNT1; CAA93631); *Aspergillus nidulans* (CRNA, XP_658612).

Table S1. List and description of primers used for cloning into pGEM®-T Easy and pDONR vectors**.** The restriction targets added to the gene-specific sequence are highlighted in gray. Oligonucleotide primer sequences containing attB1/attB2 Gateway® recombination sites are highlighted in bold (attB short sites) and underlined (attB adapter sites).

Gene		Primer Sequence 5′ → 3′	Aplication
NRT2	Fw attB1 short	**AAAAAGCAGGCT**ATGTCTGATCATGAGTTTGATG	Addition of attB short sites PCR diagnostic
	Rv attB2 short	**AGAAAGCTGGGT**CTAAACAATGATGTTTGGGGAT	
	Fw attB1 short	GGGGACAAGTTTGTACA**AAAAAGCAGGCT**	Addition of attB adapter sites
	Rv attB2 short	GGGGACCACTTTGTACA**AGAAAGCTGGGT**	
NAR2	Fw attB1 short	**AAAAAAGCAGGCT**ATGTATTCTTCCTTCCTCTCC	Addition of attB short sites PCR diagnostic
	Rv attB2 short	**AGAAAGCTGGGT**CTTTGTTCTTCCTTCTCCGGTC	
	Fw attB1 adapter	GGGGACAAGTTTGTACA**AAAAAGCAGGCT**	Addition of attB adapter sites
	Rv attB2 adapter	GGGGACCACTTTGTACA**AGAAAGCTGGGT**	
NRT2	Fw BamHI	GGATCCATGTCTGATCATGAGTTTGATGATC	cDNA amplification and restriction sites addition
	Rv XbaI	TCTAGACTAAACAATGATGTTTGGGGATTGG	
NAR2	Fw BamHI	GGATCCATGTATTCTTCCTTCCTCTCCG	cDNA amplification and restriction sites addition
	Rv XbaI	TCTAGATTATTTGTTCTTCCTTCTCCGGT	