

Table S1

	AIPAT02	AIPAT03	AIPAT04	AIPAT05	AIPAT06	AIPAT07	AIPAT08	AIPAT09	AIPAT10	AIPAT11	AIPAT12	AIPAT13	AIPAT14	AIPAT15	AIPAT16	AIPAT17	AIPAT18	AIPAT19	AIPAT20	AIPAT21	AIPAT22	AIPAT23	AIPAT24
AIPAT01	73.0	57.4	53.3	44.6	47.5	47.7	45.6	44.8	24.7	18.1	21.6	21.4	19.6	22.4	24.8	26.9	17.8	17.3	18.2	17.7	15.4	18.7	19.9
AIPAT02		57.1	51.0	41.7	45.8	44.7	42.1	41.3	25.5	17.7	20.1	19.9	19.7	23.7	23.2	26.0	16.1	16.3	15.4	16.9	15.8	18.6	19.4
AIPAT03			53.4	45.5	46.1	45.0	43.3	44.0	25.5	16.7	19.4	20.4	21.0	22.3	23.5	23.9	19.7	16.5	17.1	19.7	17.5	18.0	19.4
AIPAT04				40.6	44.6	42.0	41.9	42.5	27.7	14.8	20.2	21.8	20.1	22.0	25.1	25.5	16.5	15.0	16.4	18.3	17.4	19.9	20.7
AIPAT05					54.7	55.3	46.9	44.8	26.0	13.9	22.2	23.4	19.5	23.4	21.7	22.3	16.6	13.4	14.3	14.6	16.5	20.0	19.7
AIPAT06						80.3	50.7	48.9	25.8	15.8	22.1	23.2	20.2	23.0	22.5	26.6	17.5	15.9	17.9	18.0	17.1	21.1	20.6
AIPAT07							53.3	49.9	25.7	16.1	23.3	23.3	20.5	22.5	21.5	26.1	17.3	14.2	15.4	17.9	17.2	20.6	20.7
AIPAT08								74.0	28.9	19.6	22.4	21.3	22.7	25.4	24.9	27.8	18.5	16.2	17.4	17.9	19.6	23.9	22.1
AIPAT09									26.0	17.3	19.6	22.0	21.6	24.6	25.0	27.9	18.4	14.7	15.4	15.6	18.0	22.8	22.7
AIPAT10										16.9	19.9	18.7	19.8	25.3	23.1	19.9	17.0	17.4	18.1	21.8	19.4	22.7	21.4
AIPAT11											20.8	17.0	17.8	23.8	24.2	16.0	12.2	12.9	13.5	13.5	13.7	18.3	16.3
AIPAT12												53.0	57.0	31.6	31.7	27.2	16.7	16.9	16.6	19.1	16.7	19.7	22.2
AIPAT13													66.4	33.1	30.2	25.3	17.8	15.0	15.0	19.5	16.8	23.0	19.4
AIPAT14														33.2	31.8	24.3	16.5	17.2	16.3	21.6	17.3	21.6	21.2
AIPAT15															50.8	26.8	20.2	19.0	21.0	23.0	17.3	25.0	24.2
AIPAT16																25.7	20.4	20.0	20.1	21.5	19.1	27.0	24.2
AIPAT17																	17.5	15.5	16.2	14.7	16.9	18.8	20.2
AIPAT18																		28.9	29.0	31.6	31.1	21.1	20.3
AIPAT19																			74.4	50.8	36.0	17.8	18.4
AIPAT20																				48.6	34.2	17.8	18.6
AIPAT21																					37.3	20.0	17.7
AIPAT22																						15.8	16.0
AIPAT23																							49.9

10-20 %

>20-30%

>30-40%

>40-60%

>60%

Table S1: Protein identity matrix of Arabidopsis PATs

Identity scores of the amino acid sequences are given in percent. The background colours represent score ranges. Low values are given in yellow (10-20% light yellow, >20-30% dark yellow), middle values in green (>30-40% yellowish green, >40-60% green) and high values (in AtPAT1-AtPAT2, AtPAT6-AtPAT7, AtPAT8-AtPAT9, AtPAT19-AtPAT20) by red colour (>60%) and additionally by bold letters. The lowest score (AtPAT11-AtPAT18) is indicated by the black frame and italic letters.

Figure S1

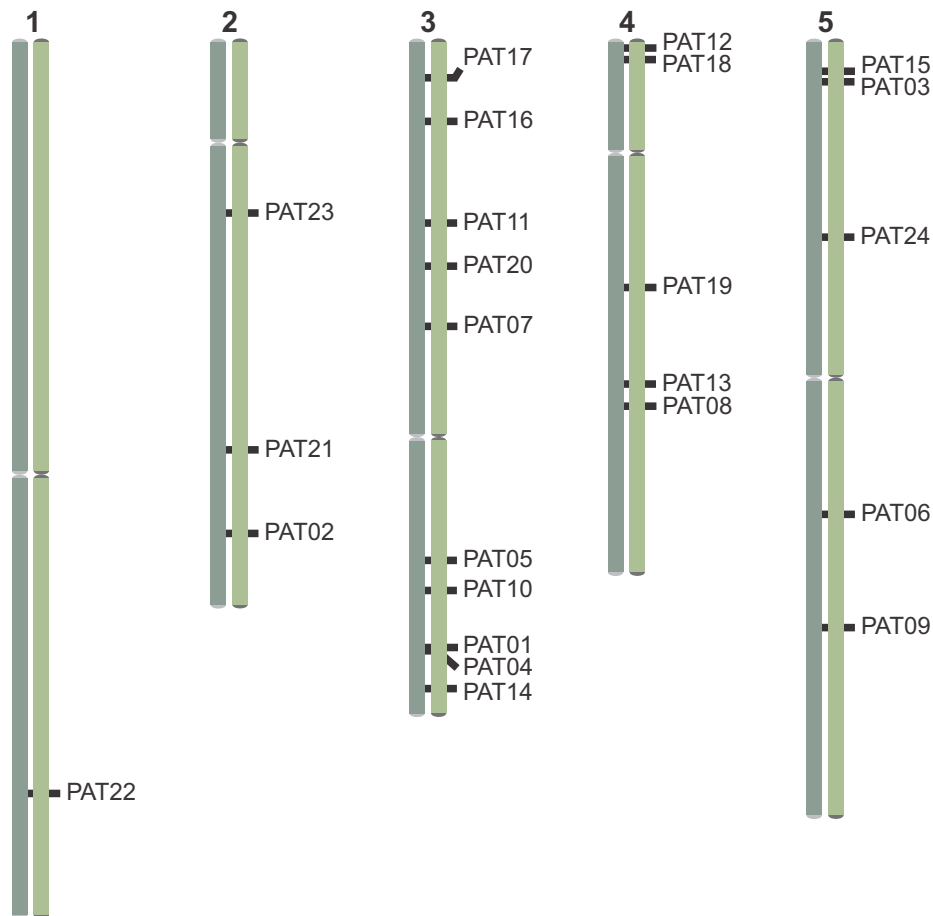


Figure S1: Distribution of Arabidopsis PAT gene loci

Distribution of *Arabidopsis thaliana* PAT gene loci within the genome. Most gene loci are distributed on chromosome 3, while a single PAT locus is located on chromosome 1. Each 5 loci are located on the chromosomes 4 and 5, respectively. The residual 3 loci are located on chromosome 2. AtPAT1 and AtPAT4 are directly organized in tandem.

Figure S2

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AtPAT09-1 -----MAGRVFEAWKGSNKFLFGGRLIFGPDAWSI
AtPAT09-2 MNDSWSAGPDRILQFVRECSEECILRSLILMAGRVFEAWKGSNKFLFGGRLIFGPDAWSI
AtPAT09-3 -----MAGRVFEAWKGSNKFLFGGRLIFGPDAWSI
*****

AtPAT09-1 PFTFLLIITPVCFVSVFVATHLRRELLPNNAGHVFLVAGVLFVFLVLLFLTSARDPGI
AtPAT09-2 PFTFLLIITPVCFVSVFVATHLRRELLPNNAGHVFLVAGVLFVFLVLLFLTSARDPGI
AtPAT09-3 PFTFLLIITPVCFVSVFVATHLRRELLPNNAGHVFLVAGVLFVFLVLLFLTSARDPGI
*****

AtPAT09-1 VPRNSHPPEEELCYDTTVSSDGRQTPTVQIPRTKEVMVYGVSVRVKYCDTCMLYRPPRCs
AtPAT09-2 VPRNSHPPEEELCYDTTVSSDGRQTPTVQIPRTKEVMVYGVSVRVKYCDTCMLYRPPRCs
AtPAT09-3 VPRNSHPPEEELCYDTTVSSDGRQTPTVQIPRTKEVMVYGVSVRVKYCDTCMLYRPPRCs
*****

AtPAT09-1 HCSICNNCVERFDHHCpw-----RNYRYFFMFVSSATILCIYIFSMSALYIKVLMDNH
AtPAT09-2 HCSICNNCVERFDHHCpwVGOcIGVRNYRYFFMFVSSATILCIYIFSMSALYIKVLMDNH
AtPAT09-3 HCSICNNCVERFDHHCpwVGOcIGVRNYRYFFMFVSSATILCIYIFSMSALYIKVLMDNH
*****

AtPAT09-1 QGTVWRAMRESPWAVMLMIYCFISLWVFGGLTGFHLYLISTNQTTYENFRYRSDNRINVY
AtPAT09-2 QGTVWRAMRESPWAVMLMIYCFISLWVFGGLTGFHLYLISTNQTTYENFRYRSDNRINVY
AtPAT09-3 QGTVWRAMRESPWAVMLMIYCFISLWVFGGLTGFHLYLISTNQTTYENFRYRSDNRINVY
*****

AtPAT09-1 NRGCSNNFFETFCSKVKPSRNDFRAFIKEEPPRNITLATTWERPEEAEENREERRQKVE
AtPAT09-2 NRGCSNNFFETFCSKVKPSRNDFRAFIKEEPPRNITLATTWERPEEAEENREERRQKVE
AtPAT09-3 NRGCSNNFFETFCSKVKPSRNDFRAFIKEEPPRNITLATTWERPEEAEENREERRQKVE
*****

AtPAT09-1 DDLIDIDEDVMKLQQRNLNDEEGSDTAHKKIDIDQMRRIGSNERAPTIRSEARHGNGWARSNA
AtPAT09-2 DDLIDIDEDVMKLQQRNLNDEEGSDTAHKKIDIDQMRRIGSNERAPTIRSEARHGNGWARSNA
AtPAT09-3 DDLIDIDEDVMKLQQRNLNDEEGSDTAHKKIDIDQMRRIGSNERAPTIRSEARHGNGWARSNA
*****

AtPAT09-1 QEEDVIAGSSVRESRSYAAAEEGR
AtPAT09-2 QEEDVIAGSSVRESRSYAAAEEGR
AtPAT09-3 QEEDVIAGSSVRESRSYAAAEEGR
*****

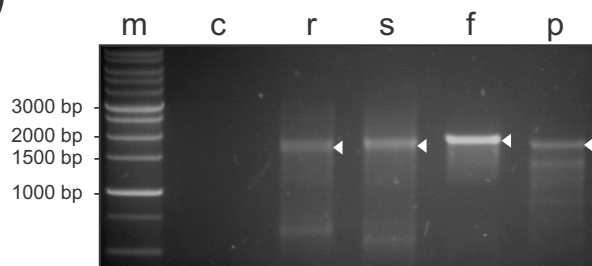
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Figure S2: Protein sequence alignment of AtPAT9 isoforms

Protein sequence alignment of the TAIR annotated AtPAT9-1 and 9-2 isoforms and of the isoform (AtPAT 9-3) which was obtained and used in this study. The DHHC motif is given in bold. Underlined sequence (and yellow background) is missing in AtPAT9-1. AtPAT9-2 contains a N-terminal extension which is missing in AtPAT9-1 and 9-3.

Figure S3

A)



B)

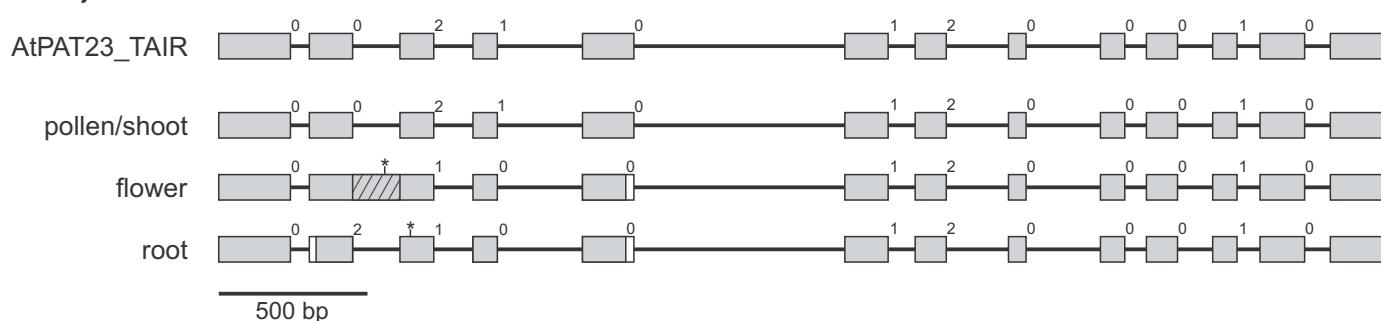


Figure S3: PCR on cDNAs and transcript structure of AtPAT23

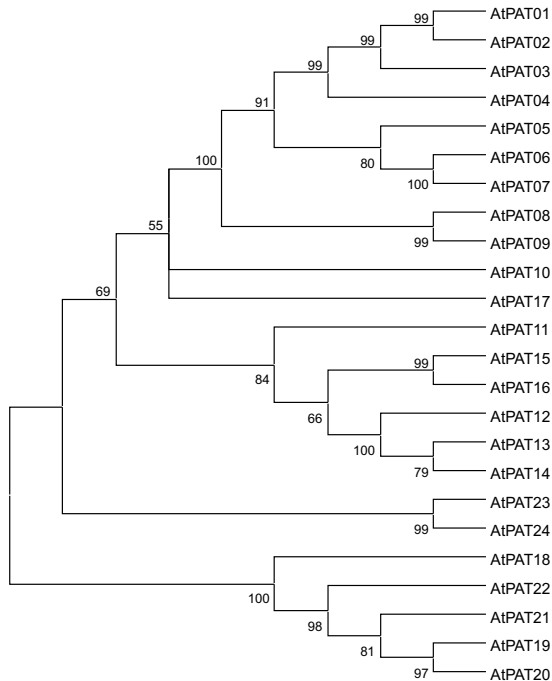
A) PCRs, using AtPAT23 specific primers, were performed on cDNAs from different tissues (r= root, s= shoot, f= flower, p= pollen specific). m= marker, c= water control. The PCR products which were further analyzed (see B) are indicated by the arrowheads.

B) Transcript structure of AtPAT23 as annotated by TAIR, and of transcripts which were obtained from the RT-PCR (see A; pollen/shoot, flower, root). Grey boxes represent exons, the line between the boxes represent introns. The phase of the introns is indicated by the numbers. Missing parts of exons are indicated by the empty boxes. The unspliced intron in the flower transcript is indicated by the grey box with diagonal lines. The positions of the first premature STOP codons, which are in frame of the initiator ATG, are indicated by the asterisks. The scale is indicated (bar = 500 basepairs).

Figure S4

A)

NJ tree



B)

ML tree

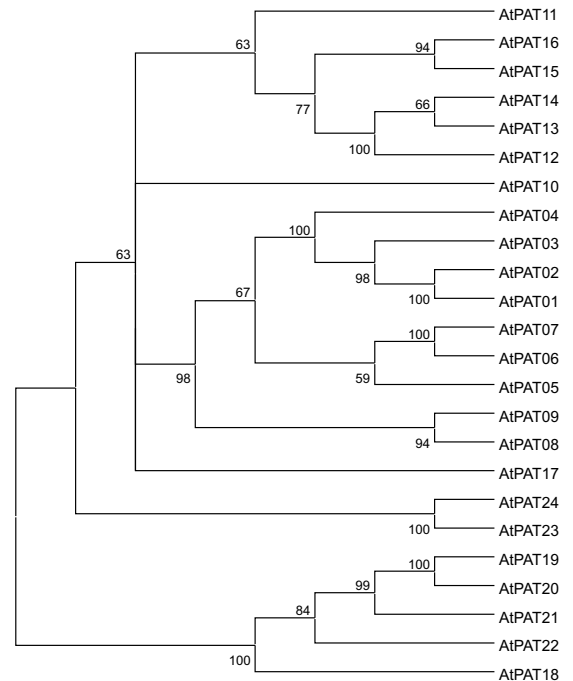


Figure S4: Comparison of the Arabidopsis PAT phylogeny by a neighbour joining and maximum likelihood approach.

A) Phylogenetic relationship of Arabidopsis PATs obtained by a neighbour joining (NJ) method. Nodes with less than 50% bootstrap confidence were collapsed. Bootstrap factors are given at the respective nodes.

B) Phylogenetic relationship of Arabidopsis PATs obtained by a maximum likelihood (ML) approach using the PALM pipeline. Nodes with less than 50% bootstrap confidence were collapsed. Bootstrap factors are given at the respective nodes.

Figure S5

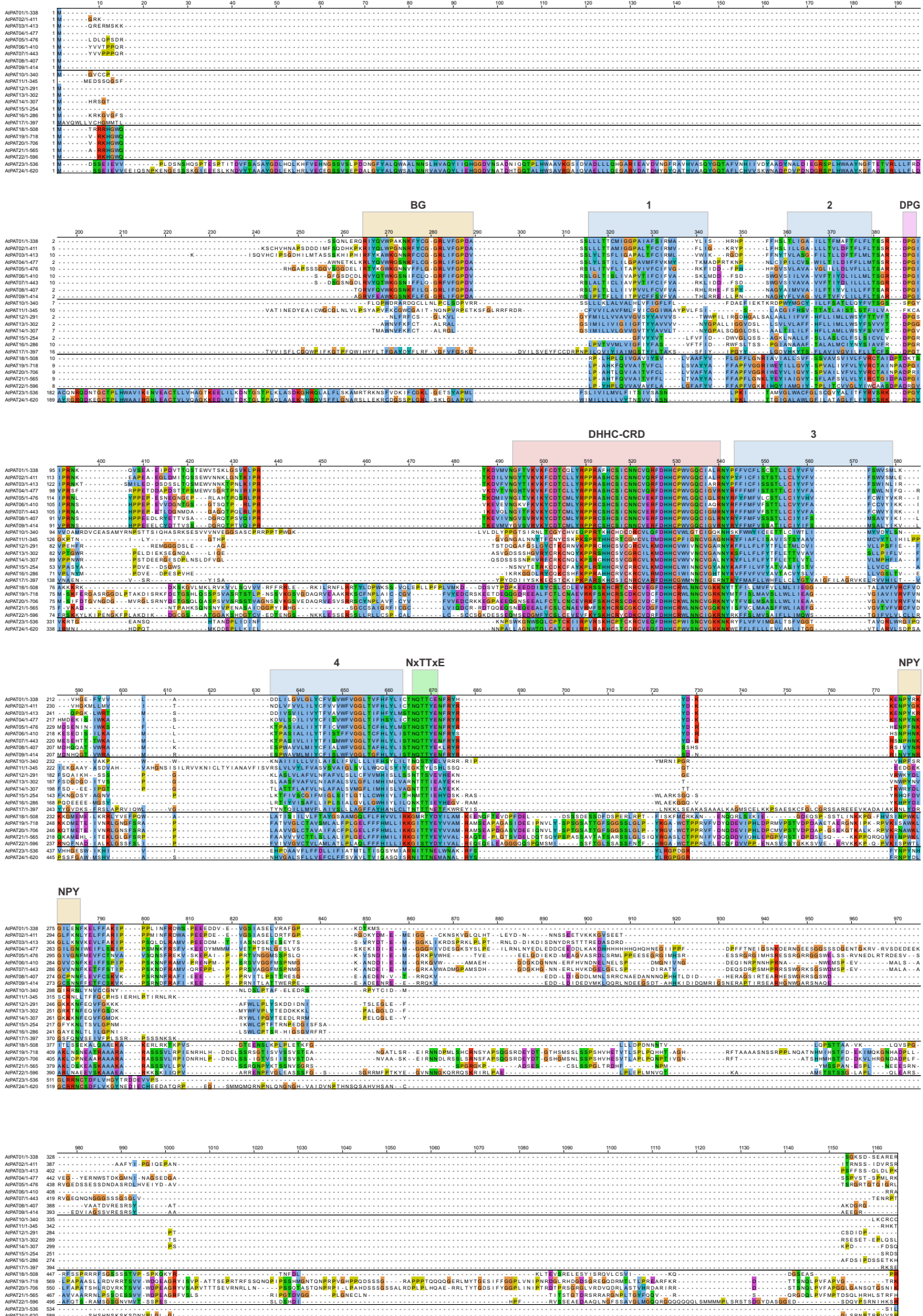


Figure S5 (continued)

Figure S5: Alignment of Arabidopsis PAT proteins

Full alignment of the Arabidopsis PAT proteins. Conserved amino acids were highlighted using the ClustalX colour code and colouring was performed groupwise (AtPATs 1-9, 10-17, 18-22, 23-24), to identify conserved blocks within sub-groups. Conserved in all proteins is the central DHHC-CRD. Upstream and downstream hydrophobic stretches are indicated with blue boxes (1 - 4), as well as the position of short, conserved peptide motifs (DPG in purple , and NxTTxE in green) which follow the second and fourth hydrophobic stretch.

AtPATs 1-9 contain a conserved N-terminal region which harbours several basic and glycine residues (BG). A less conserved region was discovered in the C-terminal region, which in most proteins consists of the amino acid sequence NPYxxGxxxN (NPY).

Figure S6

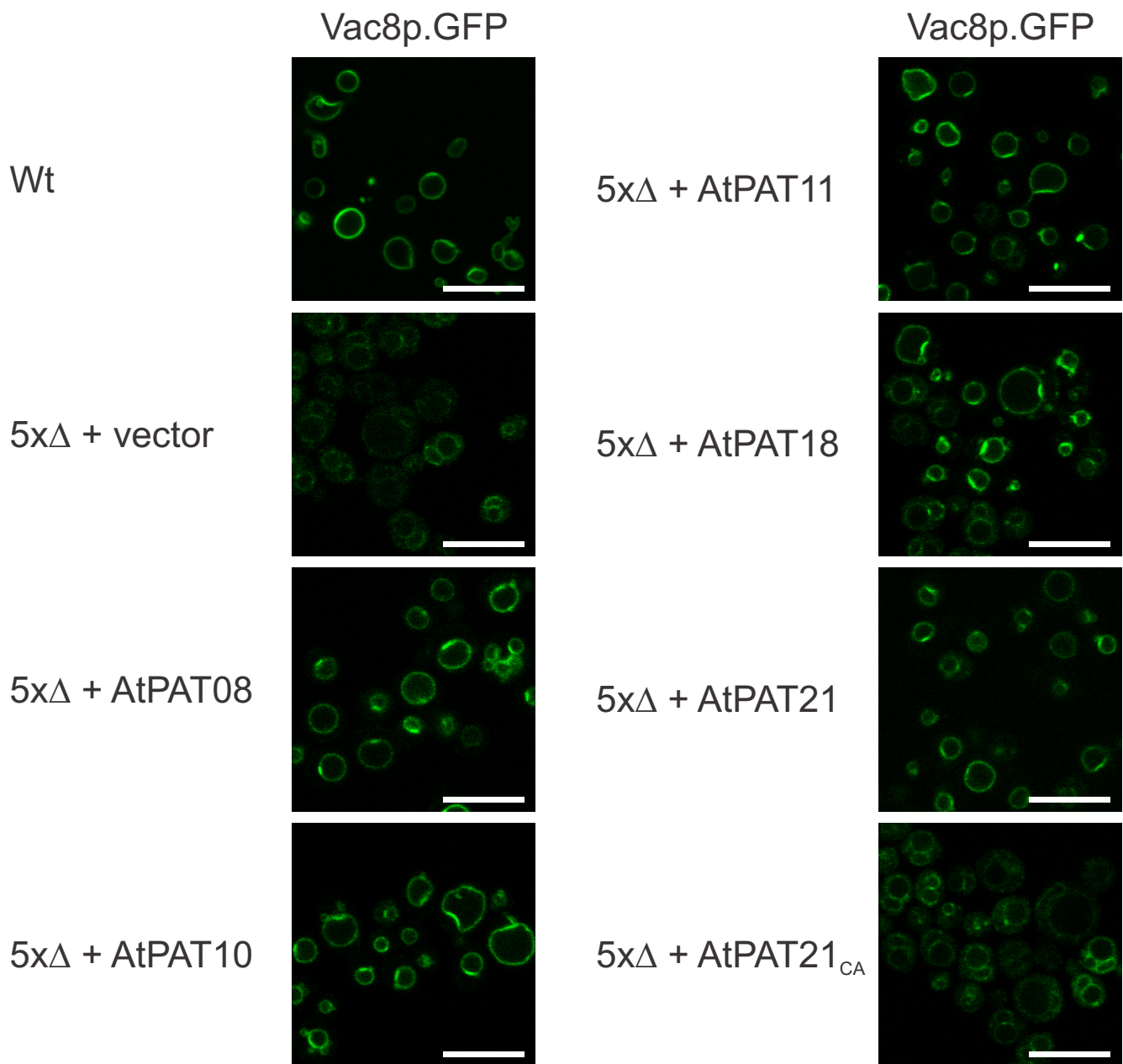


Figure S6: Targeting of Vac8p in yeast mutant cells by AtPATs

GFP fluorescence of Vac8p in wild type cells (Wt) and in the yeast mutant cells 5xΔ harbouring an empty vector (vector) or expressing an AtPAT. Bar represent 10 μm.

Figure S7

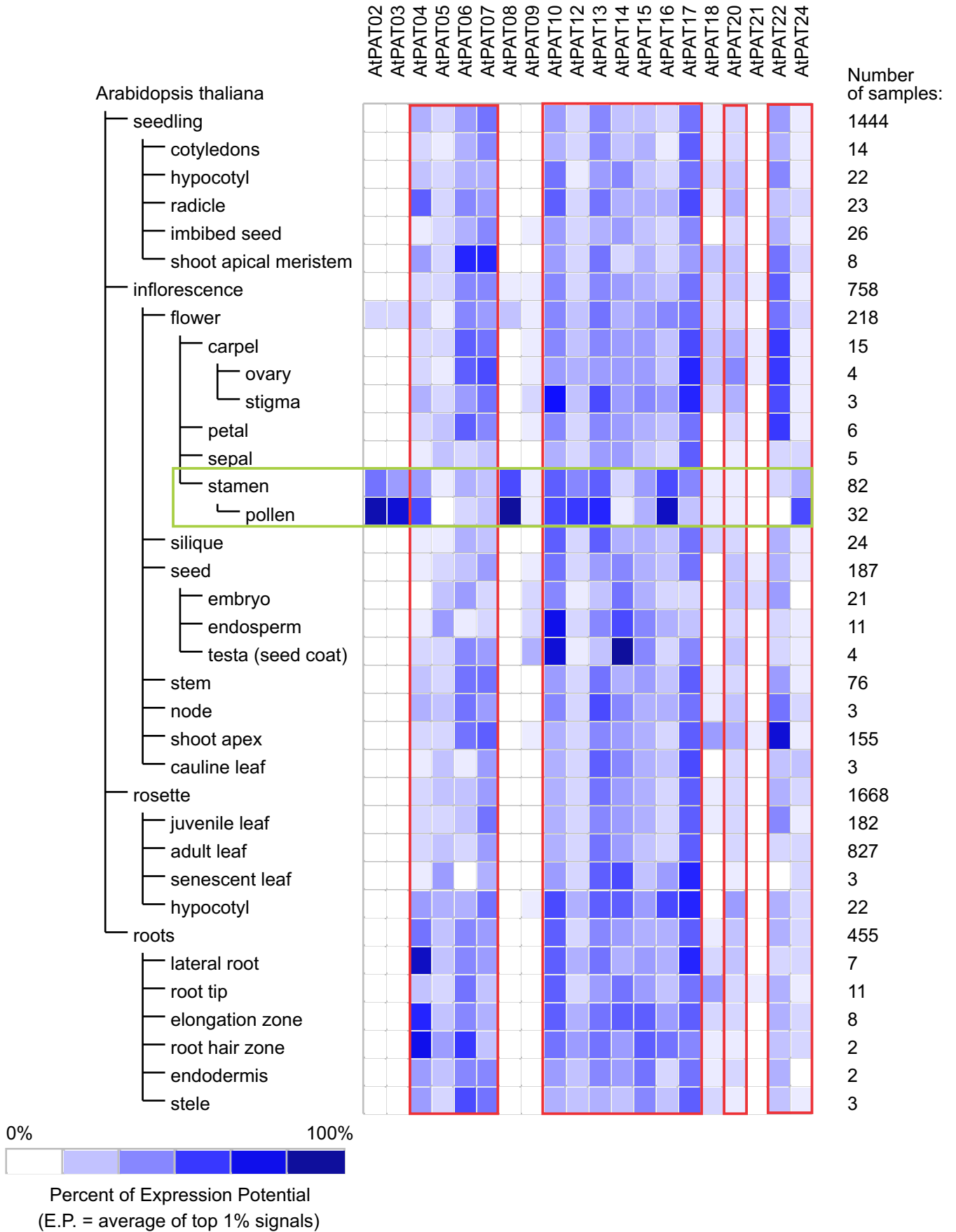
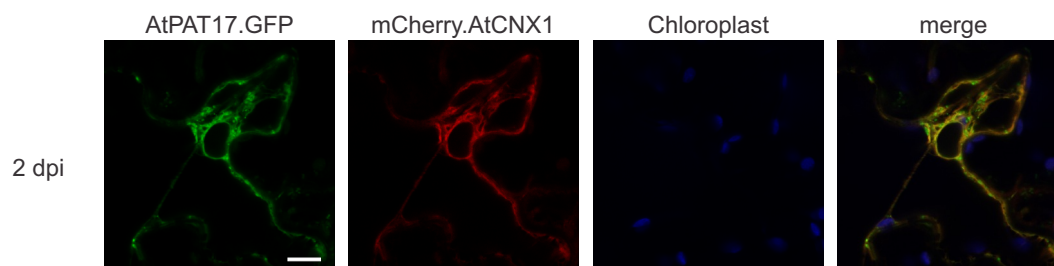


Figure S7: Expression heat map of Arabidopsis PATs

Heat map of the PAT gene expression in Arabidopsis. AtPATs 1, 11, 19, 23 are missing on the ATH1 microarray chips. The relative expression of the genes is indicated by the colour intensity (lower panel). Red frames highlight PAT genes which are expressed in nearly all samples. Green frame highlights expression in stamen and pollen.

Figure S8

A)



B)

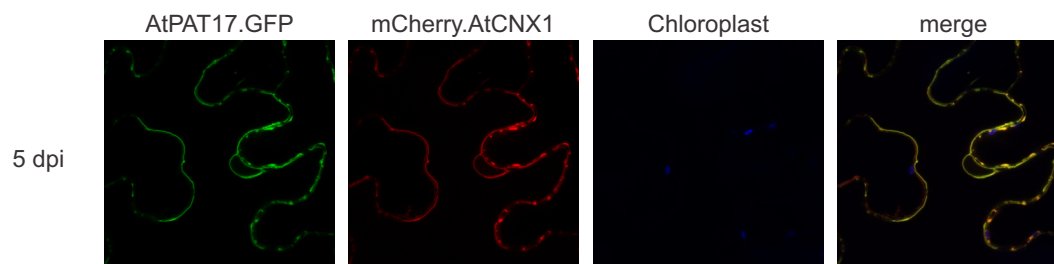
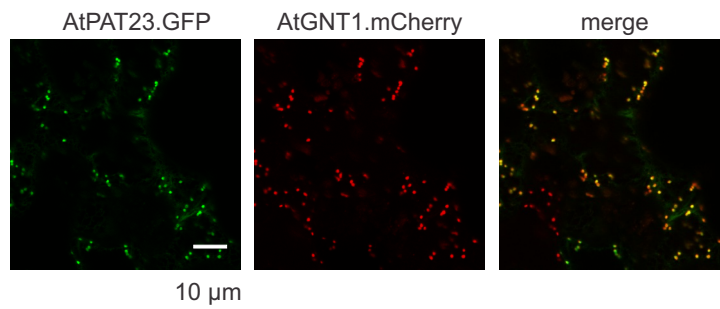
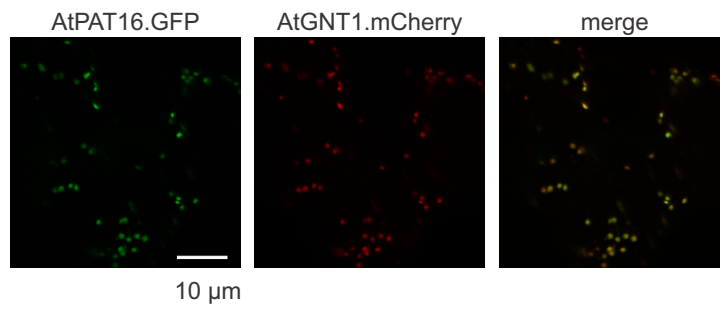


Figure S8: Co-localization studies of Arabidopsis PAT17

AtPAT17.GFP was co-expressed with mCherry.AtCNX1. Both proteins co-localized at the ER. AtPAT17.GFP was additionally targeted to vesicles. Localization was analyzed after 2 (A) and 5 days (B) (dpi = days post infiltration). Bar represents 10 μ m.

Figure S9

A)



B)

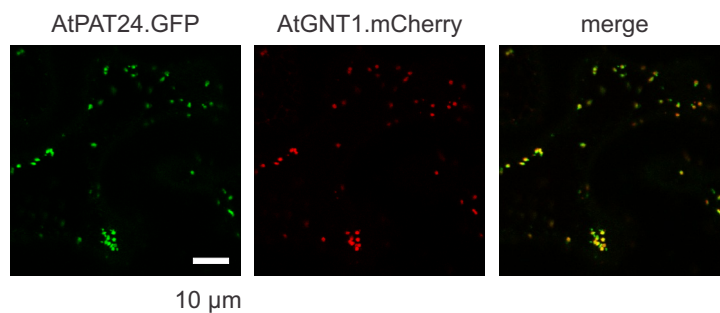
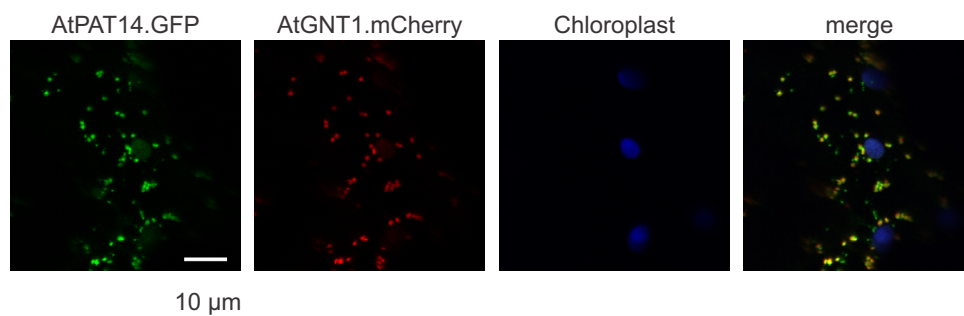


Figure S9: Co-localization studies of selected PATs.

A) AtPAT16.GFP and AtPAT23.GFP co-localize with the Golgi marker protein AtGNT1.mCherry. No additional vesicles were observed for AtPAT16 and AtPAT23. Bar represents 10 μm .

B) AtPAT14.GFP and AtPAT24 co-localize with Golgi marker protein AtGNT1.mCherry. In addition, smaller vesicles were observed which are separated from the Golgi. Chloroplast autofluorescence (in AtPAT14) is shown in blue. Bar represents 10 μm .

Figure S10

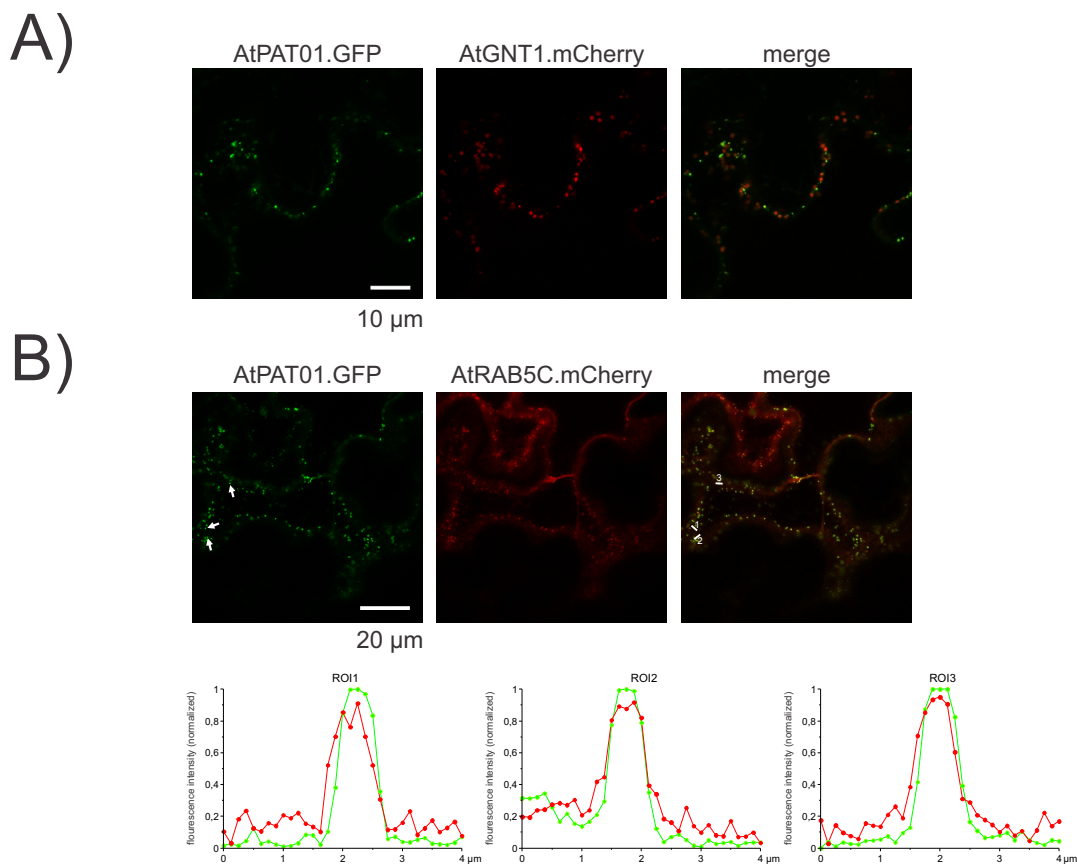


Figure S10: Co-localization studies of selected PATs.

A) AtPAT1.GFP was co-expressed with AtGNT1.mCherry. PAT1 was found mostly in vesicles which do not co-localize with the Golgi marker protein. Only residual fluorescence co-localized with the Golgi. Bar represents 10 µm.

B) AtPAT1.GFP was co-expressed with AtRAB5C.RFP. Co-localization was observed at vesicles. Intensity scans (shown below) are shown for three vesicles which are indicated in the merged picture in the right panel by the roi and by arrows in the left panel. Bar represents 20 µm.

Figure S11

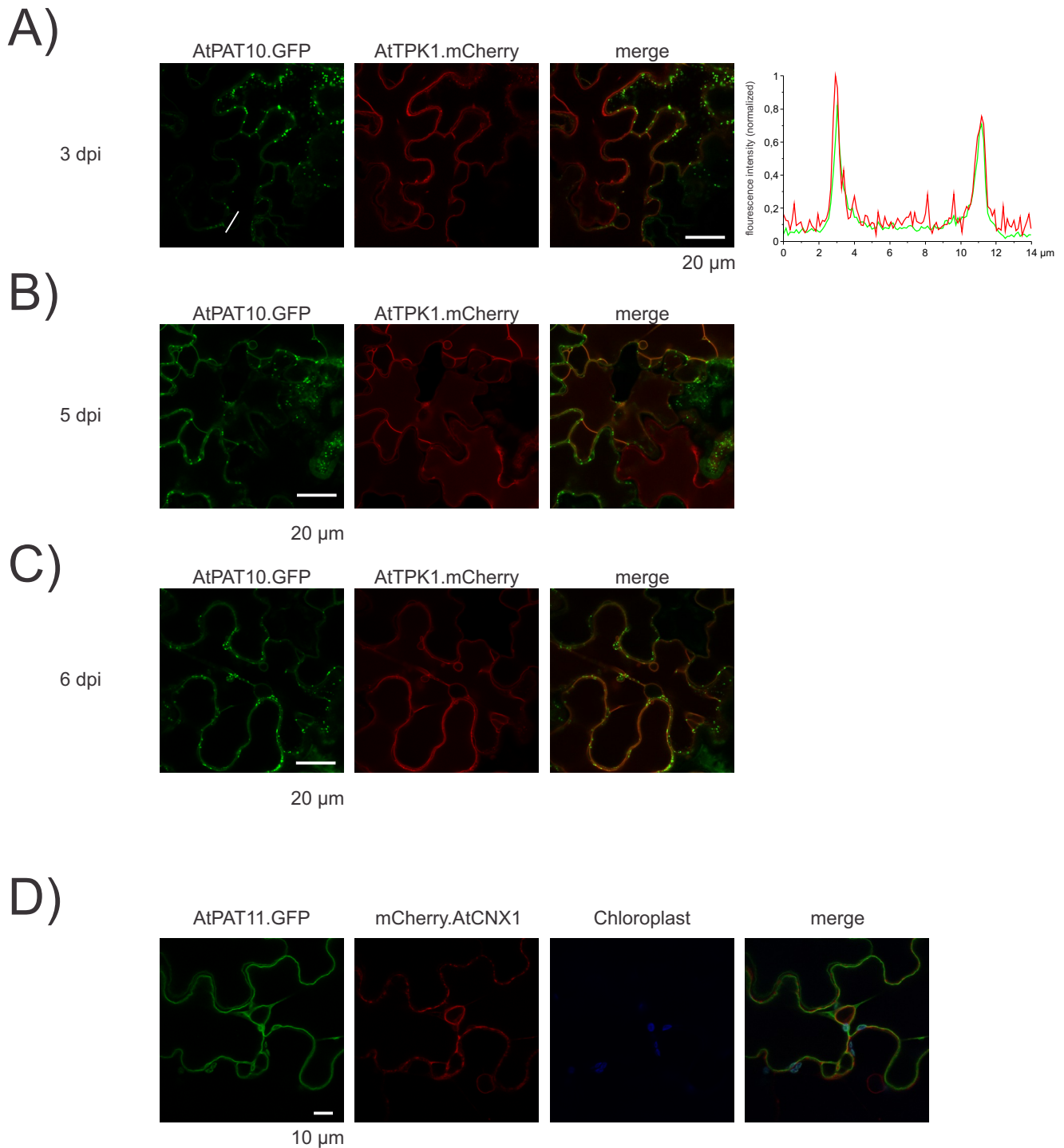


Figure S11: Co-localization studies of selected PATs.

A) AtPAT10.GFP was co-expressed with AtTPK1.mCherry for three days. Co-localization was analyzed at the respective region of interest (line in the left panel showing fluorescence of GFP). The intensity of both fluorescences (green and red channel) is shown in the right graph implicating co-localization of AtPAT10 and AtTPK1. Bar in merged figure represents 20 μm .

B-C) AtPAT10.GFP was co-expressed with AtTPK1.mCherry for further five (B) to six (C) days (dpi = days post infiltration). Bar in merged figure represents 20 μm .

D) PAT11.GFP co-expressed with the ER marker mCherry.AtCNX1. Both proteins stain different intracellular membranes, showing that AtPAT11 is not targeted to the ER. Chloroplast autofluorescence is shown in blue. Bar represents 10 μm .

Figure S12

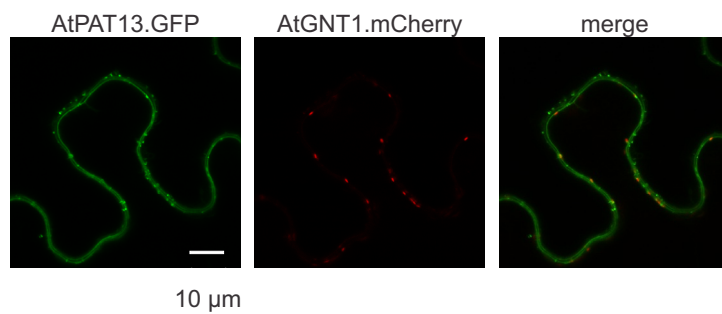


Figure S12: Co-localization studies of AtPAT13 and AtGNT1

AtPAT13.GFP is localized to the plasma membrane and to vesicles. These vesicles do not co-localize with Golgi vesicles as observed for the AtGNT1.mCherry. Bar represents 10 μm.

