

Figure S1 Identification of *zmpt7* mutants. (a) Diagram of *ZmPT7* showing two target sites (C1 and C2) for CRISPR/Cas9 technology. PAM motifs are indicated with bold letters. The coding region and untranslated regions of *ZmPT7* are indicated by black and gray boxes, respectively. (b) The *zmpt7* mutants generated by CRISPR/Cas9 technology. The mutations of *ZmPT7* in the *zmpt7* mutants were evaluated by sequencing, and indicated by red arrows and letters.

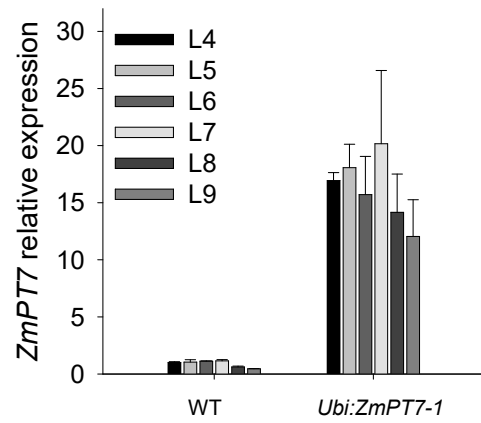


Figure S2 Analysis of transcript abundance of *ZmPT7* in different leaves of 40-d-old *Ubi:ZmPT7-1* line by qRT-PCR. Data are means \pm SE of three plants.

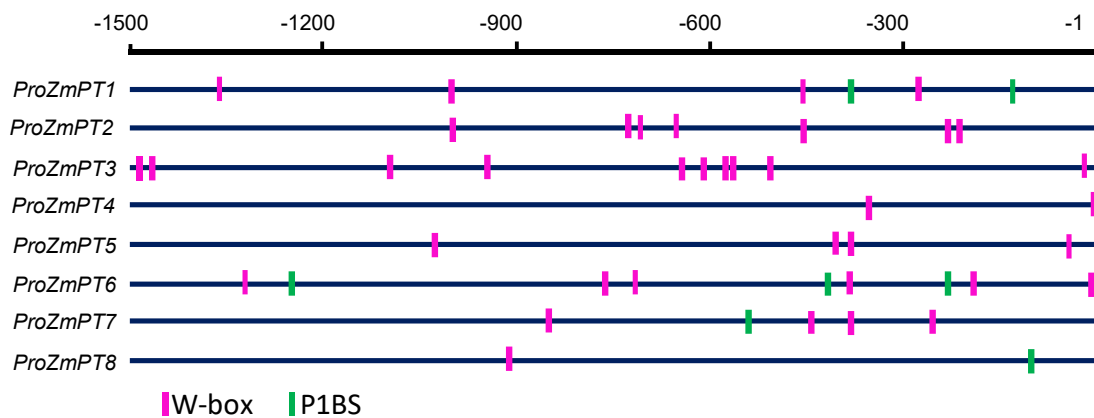


Figure S3 In silico analysis of *ZmPT* promoter sequences. The 1.5-kb promoter regions of *ZmPT* genes were retrieved from the MaizeGDB database (www.maizegdb.org) and predicted the putative binding sites of WRKY or PHR transcription factor. The +1 indicates the transcriptional start site, pink line shows the W-box site, and green line shows the P1BS site.

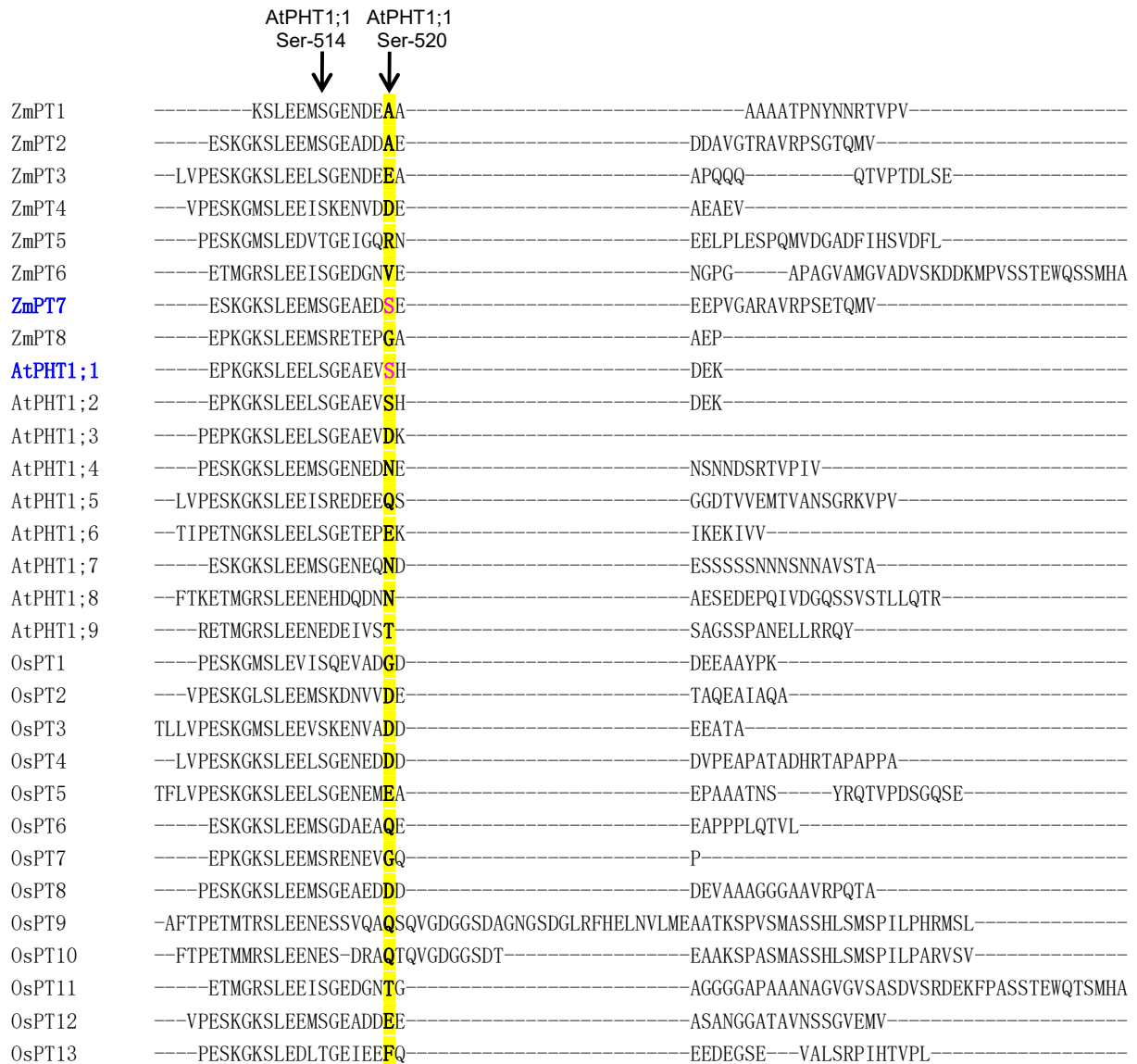


Figure S4 Sequence alignment of the hydrophilic C-termini (CT) of the PHT1 transporters in maize, *Arabidopsis*, and rice. The CT sequences were retrieved from TMHMM (<http://www.cbs.dtu.dk/services/TMHMM>) and analyzed in Clustalw (<https://www.genome.jp/tools-bin/clustalw>). The ZmPT7 Ser-521 and AtPHT1;1 Ser-520 are indicated with pink bold words, and the conserved residues in PHT1 transporters are bright highlighted.

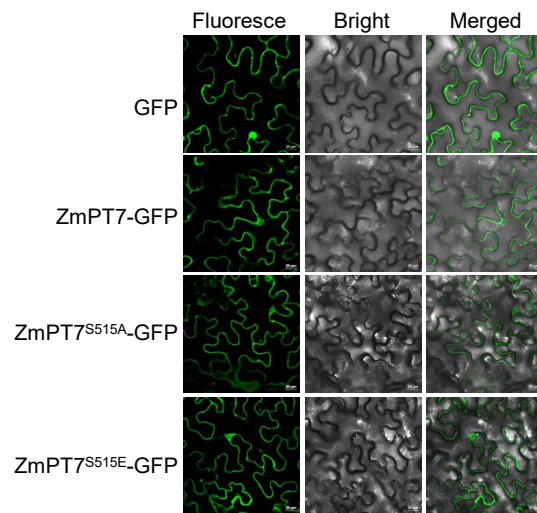


Figure S5 Location of ZmPT7-GFP and Ser515 variant of ZmPT7 in tobacco leaves. Bars = 20 μ m.

Table S1 PHT1 transporters in maize, rice, soybean, and *Arabidopsis*.

Gene name	Gene ID	GenBank accession number	Chromosome	Length of CDS sequence (bp)	Length of amino acids sequence (AA)	TM domains	PHT1 signature GGDYPLSATIxSE
ZmPT1	GRMZM2G112377_T01	NM_001112347	Chr1	1632	543	12	GGDYPLSATIMSE
ZmPT2	GRMZM2G154090_T01	NM_001112346	Chr1	1626	541	12	GGDYPLSATIMSE
ZmPT3	GRMZM2G045473_T01	MG787470	Chr2	1608	535	12	GGDYPLSATIMSE
ZmPT4	GRMZM2G070087_T01	NM_001196972	Chr1	1575	524	12	GGDYPLSATIMSE
ZmPT5	GRMZM2G159075_T01	MG787471	Chr10	1794	597	12	GGDYPLSATIMSE
ZmPT6	GRMZM5G881088_T01	MG787472	Chr8	1665	554	12	GGDYPLSATIMSE
ZmPT7	GRMZM2G326707_T01	NM_001111799	Chr5	1620	539	12	GGDYPLSATIMSE
ZmPT8	GRMZM2G075870_T01	BT086132	Chr1	1764	587	12	GGDYPLSATIMSE
OsPT1	Os03g05620	XM_015775809	Chr3	1584	527	12	GGDYPLSATIMSE
OsPT2	Os03g05640	AF536962	Chr3	1587	528	12	GGDYPLSATIMSE
OsPT3	Os10g30770	AF536963	Chr10	1581	526	12	GGDYPLSATIMSE
OsPT4	Os04g10750	AF536964	Chr4	1617	538	12	GGDYPLSATIMSE
OsPT5	Os04g10690	XM_015780498	Chr4	1647	548	12	GGDYPLSATIMSE
OsPT6	Os08g45000	AF536966	Chr8	1605	534	12	GGDYPLSATIMSE
OsPT7	Os03g04360	AF536967	Chr3	1581	526	12	GGDYPLSATIMSE
OsPT8	Os10g30790	AF536968	Chr10	1626	541	12	GGDYPLSATIMSE
OsPT9	Os06g21920	AF536969	Chr6	1749	582	12	GGDYPLSATIMSE
OsPT10	Os06g21950	AF536970	Chr6	1659	552	10	GGDYPLSATIMSE
OsPT11	Os01g46860	AF536971	Chr1	1668	555	12	GGDYPLSATIMSE
OsPT12	Os03g05610	AF536972	Chr3	1626	541	12	GGDYPLSATIMSE
OsPT13	Os04g10800	XM_015778982	Chr4	1527	508	10	GGDYPLSATIMSE
AtPHT1;1	At5g43350	NM_123701	Chr5	1575	524	12	GGDYPLSATIMSE
AtPHT1;2	At5g43370	NM_123703	Chr5	1575	524	12	GGDYPLSATIMSE
AtPHT1;3	At5g43360	NM_123702	Chr5	1566	521	12	GGDYPLSATIMSE
AtPHT1;4	At2g38940	NM_129452	Chr2	1605	534	12	GGDYPLSATIMSE
AtPHT1;5	At2g32830	NM_128843	Chr2	1629	542	12	GGDYPLSATIMSE
AtPHT1;6	At5g43340	NM_123700	Chr5	1551	516	12	GGDYPLSATIMSE
AtPHT1;7	At3g54700	NM_001339687	Chr3	1608	535	12	GGDYPLSATIMSE
AtPHT1;8	At1g20860	NM_101939	Chr1	1605	534	12	GGDYPLSATIMSE
AtPHT1;9	At1g76430	NM_001332472	Chr1	1599	532	12	GGDYPLSATIMSE

Table S1 (continued) PHT1 transporters in maize, rice, soybean, and *Arabidopsis*.

Gene name	Gene ID	GenBank accession number	Chromosome	Length of CDS sequence (bp)	Length of amino acids sequence (AA)	TM domains	PHT1 signature GGDYPLSATIxSE
GmPT1	Glyma02G00840	FJ814697	Chr2	1602	533	12	GGDYPLSATIMSE
GmPT2	Glyma03G31950	FJ814696	Chr3	1620	539	12	GGDYPLSATIMSE
GmPT3	Glyma07G34870	FJ814701	Chr7	1551	516	12	GGDYPLSATIMSE
GmPT4	Glyma10G00950	JQ518269	Chr10	1602	533	12	GGDYPLSATIMSE
GmPT5	Glyma10G04230	FJ814694	Chr10	1566	521	12	GGDYPLSATIMSE
GmPT6	Glyma10G33020	FJ814693	Chr10	1509	502	12	GGDYPLSATIMSE
GmPT7	Glyma10G33030	FJ814695	Chr10	1611	536	12	GGDYPLSATIMSE
GmPT8	Glyma13G08720	FJ814700	Chr13	1560	519	12	GGDYPLSATIMSE
GmPT9	Glyma14G28780	FJ814698	Chr14	1578	525	12	GGDYPLSATIMSE
GmPT10	Glyma14G36650	FJ814699	Chr14	1590	529	12	GGDYPLSAVIMSE
GmPT11	Glyma19G34710	JQ518270	Chr19	1620	539	12	GGDYPLSATIMSE
GmPT12	Glyma20G02660	FJ814692	Chr20	1521	506	12	GGDYPLSSTIMSE
GmPT13	Glyma20G34610	FJ789662	Chr20	1611	536	12	GGDYPLSATIMSE
GmPT14	Glyma20G34620	JQ518271	Chr20	1584	527	12	GGDYPLSATIMSE

Table S2 Percentage of amino acid identity among maize PHT1 proteins, AtPHT1;1, and OsPT8.

% ident.	ZmPT1	ZmPT2	ZmPT3	ZmPT4	ZmPT5	ZmPT6	ZmPT7	AtPHT1;1	OsPT8
ZmPT1								73.1	77.1
ZmPT2	77.1							72.6	86.6
ZmPT3	73.8	74.2						70.2	74.8
ZmPT4	72.1	72.7	71.1					72.9	73.6
ZmPT5	48.5	48.7	50.0	48.2				47.8	49.4
ZmPT6	55.7	56.9	54.3	55.3	41.4			56.9	57.6
ZmPT7	76.0	93.9	73.7	73.6	49.5	56.9		72.4	84.8
ZmPT8	69.4	65.8	64.5	63.3	47.9	48.3	65.3	64.1	66.4

Table S3 Primer sequences used in this study.

Primer name	Forward primer (5'→3')	Reverse primer (5'→3')
For gene cloning		
ZmPT1	ATGGCCGCCGGGGACCTT	TTACACGGGCACTGTGCG
ZmPT2	ATGGCGCGCGGGGG	CTACACCATCTGGGTCCCC
ZmPT3	ATGGCACACGATCACAAAGGT	TTATTCACTCAAATCTGTCGGCAC
ZmPT4	ATGGCGGGAGGCCAGCT	CTACACTTCGGCCTCAGCTTCAT
ZmPT5	ATGGGCCTGTGTAGTGCCGT	TTATAAAAAATCAACACTGTGGATGAAG
ZmPT6	ATGGCGGCGCGGGGCGG	TCACGCATGCATGGAGCTC
ZmPT7	ATGGCGCGCGGGGAGA	CTACACCATCTGGGTCTCCGAC
ZmPT8	ATGGACGACTCGGCGCC	TTACGGCTCGGCGGCG
For qRT-PCR assay		
ZmPT7-qPCR	GAACCAGGACAGGAGCAAGA	CCTCCTCTGAGTCTTCAGCC
ZmPT2-qPCR	CATCGGGGCCTTTGGGTTC	AGGATAAAGCCAAGCAAGTTGGA
ZmUBQ	CTGGTGCCCTCTCCATATGG	CAACACTGACACGACTCATGACA
For RT-PCR assay		
ZmPT7	ATGGCGCGCGGGGAGA	CTACACCATCTGGGTCTCCGAC
EF1 α	ATGCCCCAGGACATCGTGATTTCA	TTGGCGGCACCCTTACGTGGATCA
For yeast complementation assay		
ZmPT7	TTCCTGCAGCCCGGGGATCCATGGCGCGGG GGGAGA	CTCCACCGCGGTGGCGGCCGCTACACCATCTGGGTCTCCG
AtPHT1;1	TTCCTGCAGCCCGGGGATCCATGGCCGAACA CAACTAGGAGT	CTCCACCGCGGTGGCGGCCGCTTATTCTCGTCATGGCTAAC
For <i>pht1;1A4A/ZmPT7</i> and <i>Ubi:ZmPT7</i> transgenic lines		
ZmPT7	tataagcttATGGCGCGCGGGGAGA	gactagtCTACACCATCTGGGTCTCCGAC
For ProZmPT7:GUS transgenic line		
ProZmPT7	tgcactgcagtgcaacACACGCATGCTAAGGTCA	ggggtaccccGGCGGATGTTAGCTACCTAG
For protein expression in <i>E. coli</i>.		
ZmPT7-4T-1	gaattcGAGTCCAAGGGCAAGTCG	ataagaatgcgccgctaactatCTACACCATCTGGGTCTCC
For subcellular localization		
ZmPT7	tataagcttATGGCGCGCGGGGAGA	gactagtCTACACCATCTGGGTCTCCGAC
For in situ hybridization		
ZmPT7-Probe	GATTTAGGTGACACTATAGAATGCTATCGACGCG TGTTCCCTTC	TGTAATACGACTCACTATAGGGCGCGGTAACCGATTATATATG