

## SUPPLEMENTARY INFORMATION

### Supplementary Table S1. Primers used in this study.

#### **Primers used to construct protein expression vectors**

AmtriPGDH1-F	<u>AAAGCTTTGACTAGTGCGAAGCCACGGTTTTGGTGGCGGAGAAG</u>
AmtriPGDH1-R	<u>CGGGCTTATGCGGCCCTACAAGTTGAGGAAAACAACTCTTCAAC</u>
AhPGDH-F	<u>CAAAGCTTTGACTAGTGCAAAAGTTTTAGTTTCAGACTCTGTGGATC</u>
AhPGDH-R	<u>CGGGCTTATGCGGCCCTAGAGTTTAATGGTGTAAAGCATCACGAATCCC</u>
OsPGDH1-F	<u>AAGCTCTTCAAAGCTTTGACTAGTGCCGTACCGGGGAAGCCGAC</u>
OsPGDH1-R	<u>CGGGCTTATGCGGCCCTAGAGCTTGAGGAAAAC</u>
OsPGDH2-F	<u>AAGCTCTTCAAAGCTTTGACTAGTGCGCTGTGGCCGAAGCCG</u>
OsPGDH2-R	<u>CGGGCTTATGCGGCCCTCATAGCTCGAGGAAGAC</u>
OsPGDH3-F	<u>AAAGCTTTGACTAGTGGAAGGCCGACGGTGCTTGTGACGGAGAAG</u>
OsPGDH3-R	<u>CGGGCTTATGCGGCCCTAAAGCTTAATAAAGACAACTCCTCAAC</u>
PpPGDH2-F	<u>AAAGCTTTGACTAGTAACCCCTGATCTTGCTACCGTCTTGG</u>
PpPGDH2-R	<u>CGGGCTATGCGGCCCTCAAAGCCGAAGAAACACCA</u>
PpPGDH3-F	<u>AAAGCTTTGACTAGTTCTGTAGCGAAGCCCACCGTGTTGG</u>
PpPGDH3-R	<u>CGGGCTTATGCGGCCCTACAGTTTGAGGAATACAACTCC</u>
PpPGDH4-F	<u>AAAGCTTTGACTAGTAACCCCGATCTTGCCACTGTTCTCG</u>
PpPGDH4-R	<u>CGGGCTTATGCGGCCCTCAAACTTCAAAAATACC</u>

#### **Primers used to construct complementation vectors**

AmtriPGDH1-F	<u>AGGAAACAGACCATGGCGAAGCCACGGTTTTGGTGGCGGAGAAG</u>
AmtriPGDH1-R	<u>CTAGAGGATCCCCGGCTACAAGTTGAGGAAAACAACTCTTCAAC</u>
OsPGDH1-F	<u>AGGAAACAGACCATGGCCGTACCGGGGAAGCCGACGGTGCTCGTGG</u>
OsPGDH1-R	<u>CTAGAGGATCCCCGGCTAGAGCTTGAGGAAAACGAATTCTTCAATCG</u>
OsPGDH2-F	<u>AGGAAACAGACCATGGCGCTGTGGCCGAAGCCGGCGGTGCTGGTGG</u>
OsPGDH2-R	<u>CTAGAGGATCCCCGGTCATAGCTCGAGGAAGACGAACTCCTCGATCG</u>
OsPGDH3-F	<u>AGGAAACAGACCATGGGAAGGCCGACGGTGCTTGTGACGGAGAAG</u>
OsPGDH3-R	<u>CTAGAGGATCCCCGGCTAAAGCTTAATAAAGACAACTCCTCAAC</u>
PpPGDH2-F	<u>AGGAAACAGACCATGAACCCTGATCTTGCTACCGTCTTGGTGTCTG</u>
PpPGDH2-R	<u>CTAGAGGATCCCCGGTCAAAGCCGAAGAAACACCAGCTCTTCAATCG</u>
PpPGDH3-F	<u>AGGAAACAGACCATGTCTGTAGCGAAGCCCACCGTGTTGGTAGCTG</u>
PpPGDH3-R	<u>CTAGAGGATCCCCGGTTACAGTTTGAGGAATACAACTCCTCCACGGC</u>
PpPGDH4-F	<u>AGGAAACAGACCATGAACCCCGATCTTGCCACTGTTCTCGTGGC</u>
PpPGDH4-R	<u>CTAGAGGATCCCCGGTCACAACTTCAAAAATACCAATTCACCAATGG</u>

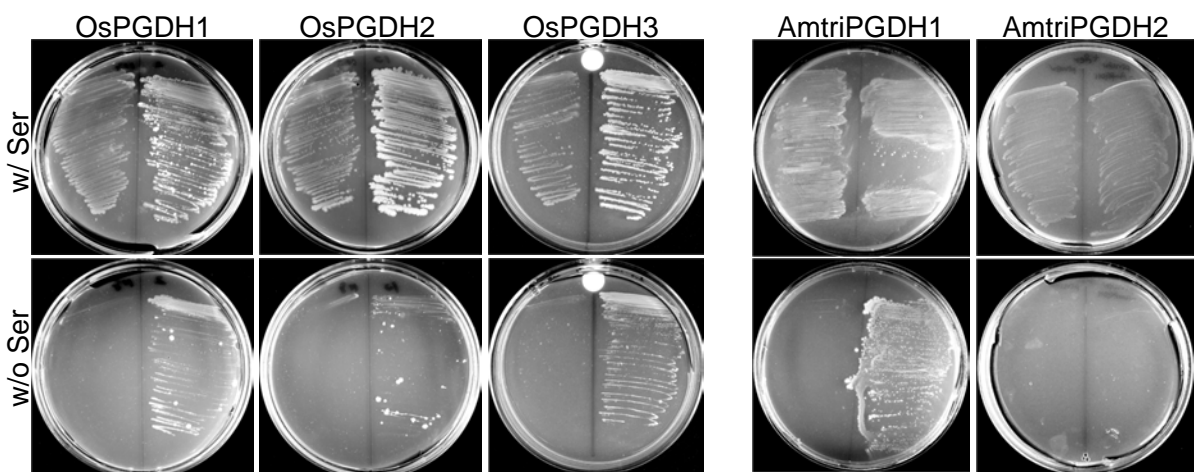
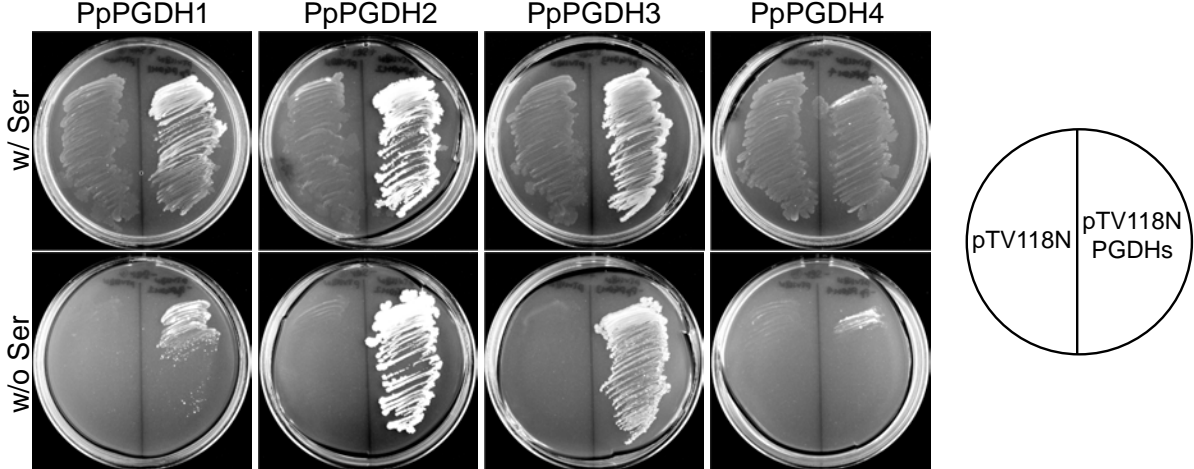
**Primers used for site-directed mutagenesis experiments**

AtPGDH1-N556A-F GGAGAGTCTAATGTCGCTGTTAACTTCATGAGC  
AtPGDH1-N556A-R GTCATGAAGTTAACAGCGACATTAGACTCTCC

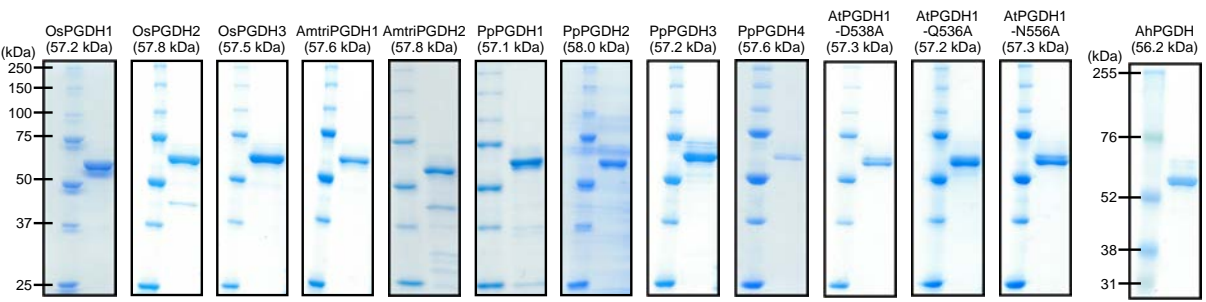
AtPGDH1-D538A-F CTGTGCAGGCAGGTGGCTCAACCTGGTATGATC  
AtPGDH1-D538A-R GATCATACCAGGTTGAGCCACCTGCCTGCACAG

AtPGDH1-Q536A-F ATCATACTGTGCAGGGCTGTGGATCAACCTGGT  
AtPGDH1-Q536A-R ACCAGGTTGATCCACAGCCCTGCACAGTATGAT

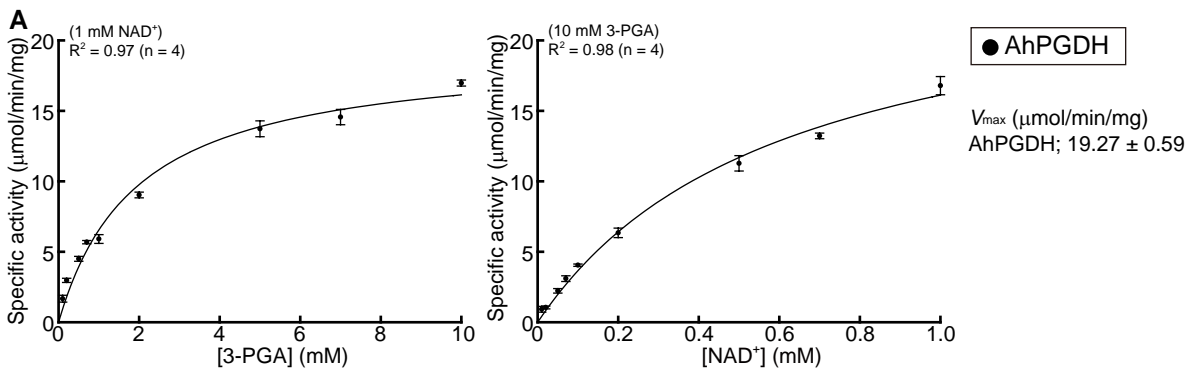
Primer sequences are written in the 5' to 3' direction. Underlined, double-underlined, and bold sequences denote pPAL7 vector sequence, pTV118N vector sequence, and mutated codon sequences of AtPGDH1 for N556, D538, and Q536, respectively.



**Figure S1.** Complementation of a PGDH-defective *E. coli* mutant with *PGDH* cDNAs. *E. coli* strain JW2880 ( $TG1\Delta serA::KmFRT$ ) was transformed with the expression vector pTV118N carrying *PGDH* cDNAs (*PpPGDH1*, *PpPGDH2*, *PpPGDH3*, and *PpPGDH4* from *P. patens*; *OsPGDH1*, *OsPGDH2*, and *OsPGDH3* from *O. sativa*; *AmtriPGDH1* and *AmtriPGDH2* from *A. trichopoda*) or with the empty pTV118N as negative control. PGDHs without the transit peptide were used. The transformed *E. coli* were cultured on M9 minimal medium agar plates with or without L-serine.



**Figure S2.** SDS-PAGE analysis of the recombinant PGDHs. The purified recombinant PGDH proteins were subjected to SDS-PAGE analysis (right lane) along with a molecular weight marker (left lane) and were stained with Coomassie Brilliant Blue. The theoretical molecular weight of PGDHs without the transit peptide are shown in parentheses.



**B**

	$k_{\text{cat}}$ ( $\text{s}^{-1}$ )	$K_m^{\text{app}}$ (mM)		$k_{\text{cat}} / K_m$ ( $\text{M}^{-1} \cdot \text{s}^{-1}$ )	
		3-PGA	$\text{NAD}^+$	3-PGA ( $\times 10^4$ )	$\text{NAD}^+$ ( $\times 10^5$ )
AhPGDH	$72.5 \pm 2.25$	$1.94 \pm 0.17$	$0.608 \pm 0.069$	$3.73 \pm 0.34$	$1.19 \pm 0.14$

**Figure S3.** Michaelis-Menten plot and kinetic parameters of AhPGDH. (A) Specific activities at various concentrations of 3-PGA (left) and  $\text{NAD}^+$  (right) are shown. Data are presented as the means and standard error from two technical replicates, using enzymes purified from two independent batches of cells ( $n = 4$ ). (B) Kinetic parameters of AhPGDH. The apparent Michaelis constants ( $K_m^{\text{app}}$ ) were calculated from Fig. S3A. Standard errors (SE) are shown.

1 10 20 30 40 50  
AtPGDH1 . . . . . MSATAAASSSIAVATNSLRNVLTSSRSLPSAISVAFPPSGRNTLQRRLLVLS  
AtPGDH2 . . . . . MAFSSSCSSSVKAVNSRWTSPSPSPSSRFVAVLPAPLHRRYATSVKLTATSAALKTVEQTT  
AtPGDH3 . . . . . . . . . . MATSLSLSSIPSSSSRLVTPSPSVFFPIRQ. . . . . RRILVLS  
OsPGDH1 . . . . . . . . . . MAAPSQTTATTHHRVLLPSHHHHRAVPPSLRLPLRAARRGRLSAAAV  
OsPGDH2 . . . . . . . . . . MATAAAAARAAALSRPHAAAAAARASPAAGAVTLPRRRSRVSAVIVSSSASAAV  
OsPGDH3 . . . . . . . . . . MALAPPLCHLLAAPPPSTSAAADAAHDHAGALPHRRASPLRRRGRLLFLACRV  
PpPGDH1 METASMAATAGSATLVGAKLAVNYGVSRREPQLSSRVSLGRNSVFLSKAGVYSGRGNVSGRVRGASKRVVAVNSAAAS  
PpPGDH2 . . . . . MATIAGLTMAVNVKEMQLQSVTLGRQQAQCNRRQLASRVSLHKNSETLRR. . . . . QPVCSSRRHSVVMNVTTHS  
PpPGDH3 . . . . . MATTARSSTCIGTMAVRSVGAQKQECHLARSVSGRNSVFLASRAGVSGRVVNVGVRWVAVGVMAVN. . . . .  
PpPGDH4 . . . . . MAMVMGKSMTANAKHTLQFAPSTQKSGQKHRLLSSVAFQTSSSLIGAKGTTTICTLTPKRSLSAVKAVVNT. . . . .  
AmtriPGDH1 . . . . . . . . . . MAASSSKLLSSPSFRGIGRERIAASASNLRPSPLLVGRGRERSVLSGQLGIGS  
AmtriPGDH2 . . . . . . . . . . MASSNAKPLLTPIISRRLPSLQSLYLSLQKQSNRPRSLASASVFLGQPLK  
MpPGDH . . . . . . . . . . MAATSAAVAVVAGALGAVPQRTDLAAAAAVSRACSSVSLASQSLASCKTARSARAVSKVGGRO. . . . .  
AhPGDH  
MtPGDH

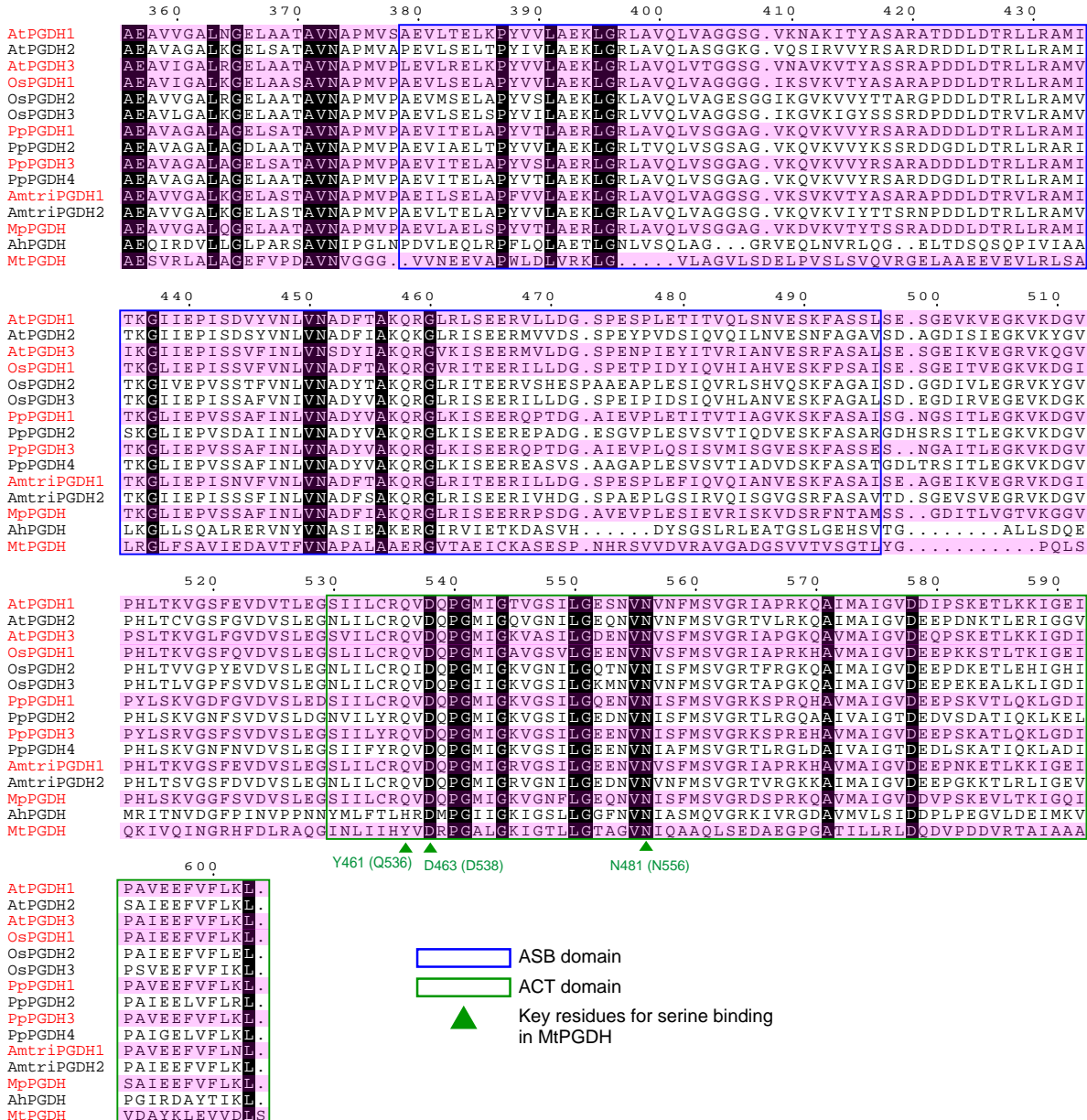
60 70 80 90 100 110  
AtPGDH1 . . . . . CSTGDG. . . . . SKPTTIVAEKLGDA GIKLLEDVANVDCSYNMTPELNIKISLCLDALTVRSGLKVGREVF  
AtPGDH2 . . . . . LTEDNRFSTVGSDSDEYNPLTLPKPRILEVTEKLGEGAGVNLIRFEGDVDCSYDLSPELKKKVAESDALTVRSGLKVTREVF  
AtPGDH3 . . . . . SSSGGG. . . . . GKPTILEVTEKLGQAGIDLLKKYANVDCSYDLSPELCTKISLCLDALTVRSGLKVGDRDFV  
OsPGDH1 . . . . . AAAAPAAATAPSEPAAGAVPGKPTIVAEKLGGAAGLELIRGFADVDCSYGLSPELRRAKIPLCDALTVRSGLKVGDRDFV  
OsPGDH2 . . . . . AAEPAAAGRTLGAAGTDLGALWPKPAVVAEKLSEAGLVRGFADVECAVYGMSPALAKVAQFDALTVRSGLKVTREVL  
OsPGDH3 . . . . . PAAAPASARGVAAEAASG. . . . . RPTVILEVTEKLGDA GLELRRFVANVDCAYELTAEELRAKVSLDALVVRSGAFVTRVF  
PpPGDH1 . . . . . SSSSSSSAVAVTSSDGQKSVAKPTIVAEKLGGAAGIELLEKMAVNDCSYNLTSQEDLCAKVAECDALTVRSGLKVTREVF  
PpPGDH2 . . . . . SSPAVTQTLSEQSNQSRNPLATVILESEKLDGGGLDIRKNSVNDCIYNLTNEELCAKISDYDALTVRSGLKVTREVF  
PpPGDH3 . . . . . SASTAASPTATKNEKSVAKPTIVAEKLGGAAGIELLEKVAVNDCSYNLTSQEDLCAKISDYDALTVRSGLKVTREVF  
PpPGDH4 . . . . . SSATVSKPTTEEAVKTRNPLATVILEAEKLEGGGLDIRKNSVNDALYNLTNEELCEKISNCDALTVRSGLKVTREVF  
AmtriPGDH1 . . . . . SILRHGNPRTLASSQVRASLEAKPTIVAEKLEGAAGLELRSFVANVDCSYNLTQEBLCTKISLCLDALTVRSGLKVTREVF  
AmtriPGDH2 . . . . . SSI SRVVSLSKRRDLTVKASDKPTVILEKLEGAAGLELRSFVANVDCSYNLTPEELCKSISLCLDALTVRSGLKVTREVF  
MpPGDH . . . . . IVCVSAAPSTSPRSASTEIVGKPTIVAEKLEGAAGLELKKIANVDCSYNLSQEBLCAKISLCLDALTVRSGLKVTREVF  
AhPGDH . . . . . . . . . . MAKVIVSDSVDQAGIDLISQVAQVDVKTKLSPBELVATIEPYDALVVRSGKVTREVI  
MtPGDH . . . . . . . . . . MVSLPVVILADKLPASTVAALGDQVEVRWVDGPPDRDKLLAAVPEADALTVRSATTVVDABVL

120 130 140 150 160 170 180 190  
AtPGDH1 . . . . . ESSHGRLLKVVGRAGVGINVLDLAATEFGCLLVNAPANTAVAAEHGIALMAAMARNVAQADASV KAGEWKRNKYVGVSL  
AtPGDH2 . . . . . EAAKGRLLKVVGRAGVGINVLDLAATEHGCCLLVNAPANTAVAAEHGIALLAMARNVAQADASIKAGWKERNKYVGVSL  
AtPGDH3 . . . . . ESSRGRLLKVVGRAGVGINVLDLAATEYGCCLLVNAPANTAVAAEHGIALLTAMARNVAQADASIKAGWKERNKYVGVSL  
OsPGDH1 . . . . . EASGGRLLKVVGRAGVGINVLDLAATEHGCCLLVNAPANTAVAAEHGIALLTAMARNVAQADASIKAGWKERNKYVGVSL  
OsPGDH2 . . . . . EAGRGRLLKVVGRAGVGINVLDLAATEAGCCLLVNAPANTAVAAEHGIALLAMARNVAQADASIKAGWKERNKYVGVSL  
OsPGDH3 . . . . . EAARGRLLKVVGRAGVGINVLDLAATEAGCCLLVNAPANTAVAAEHGIALLAMARNVAQADASIKAGWKERNKYVGVSL  
PpPGDH1 . . . . . EASKGRLLKVVGRAGVGINVLDLAATEFGCCLLVNAPANTAVAAEHGIALLTAMARNVAQASAMKAGWKERNKYVGVSL  
PpPGDH2 . . . . . EASKGRLLKVVGRAGVGINVLDLAATEELGCCLLVNAPANTAVAAEHGIALLTAMARNVAPSSSMKSGWKERNKYVGVSL  
PpPGDH3 . . . . . EASKGRLLKVVGRAGVGINVLDLAATEELGCCLLVNAPANTAVAAEHGIALLTAMARNVAQASAMKAGWKERNKYVGVSL  
PpPGDH4 . . . . . QASNGRLLKVVGRAGVGINVLDLAATEELGCCLLVNAPANTAVAAEHGIALLTAMARNVAPSSSMKSGWKERNKYVGVSL  
AmtriPGDH1 . . . . . ESSRGRLLKVVGRAGVGINVLDLAATEHGCCLLVNAPANTAVAAEHGIALVAAMARNVAQADASIKAGWKERNKYVGVSL  
AmtriPGDH2 . . . . . EASKGRLLKVVGRAGVGINVLDLAATEHGCCLLVNAPANTAVAAEHGIALMVAMARNVAQDASAMKSGWKERNKYVGVSL  
MpPGDH . . . . . EASNGRLLKVVGRAGVGINVLDLAATEELGCCLLVNAPANTAVAAEHGIALTLALARNVAQASAMKAGWKERNKYVGVSL  
AhPGDH . . . . . EAGNQLKIIIRAGVGINVLDLVAATRRGIMVNSPEGNTIAAAEHALAMMLLSRHIPLEANQSVKKEKWRKNFVIGSEV  
MtPGDH . . . . . AAAP.KLKIIVARAGVGINVLDVAATARGVLLVNAPTSNIHSAEHALALLLAASRQIPAADASLRHTWKRSSFSGTEI

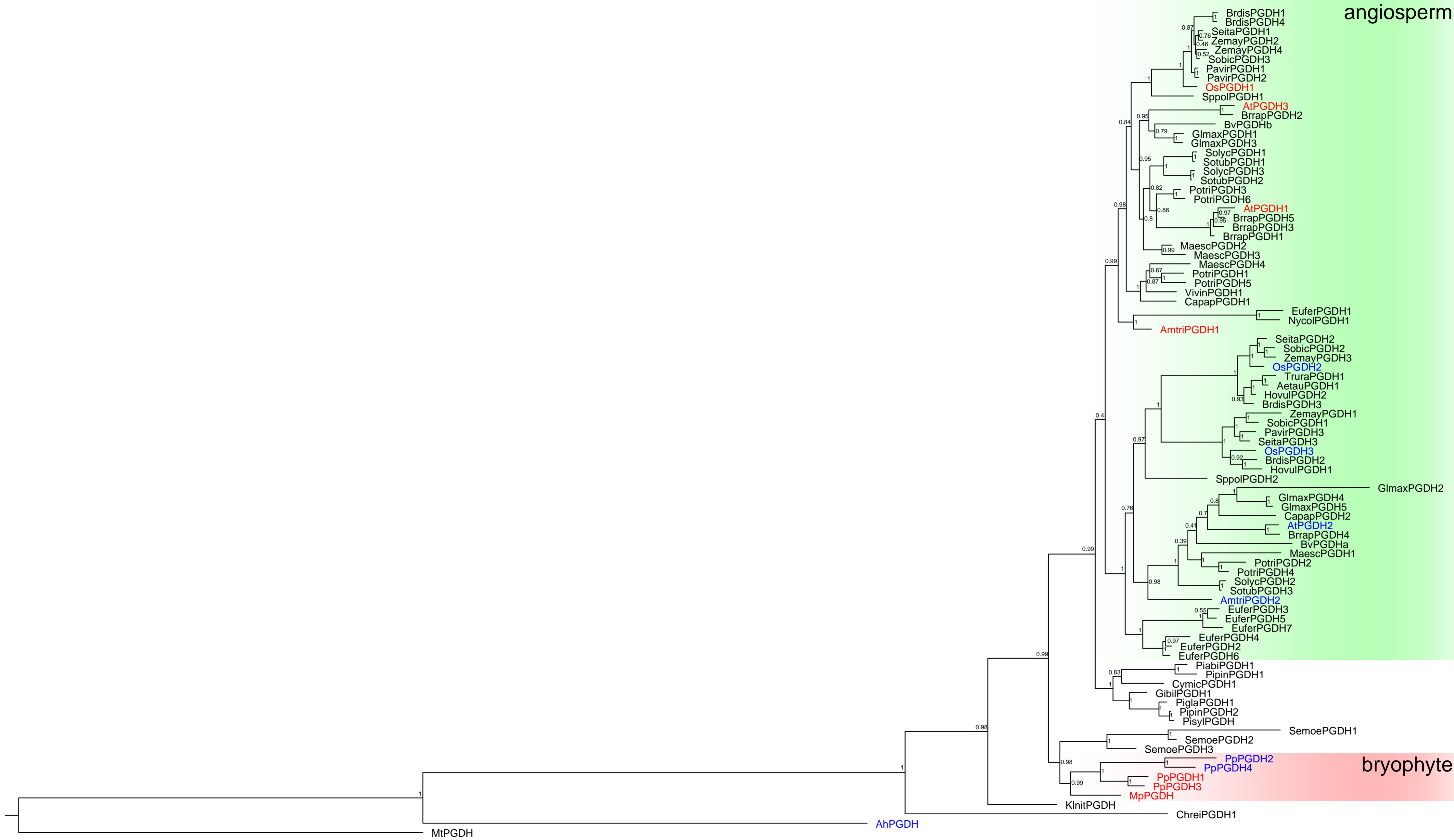
200 210 220 230 240 250 260 270  
AtPGDH1 . . . . . VGKTLAVLGRGKVGTSVARRAKGLGMRVIAHDPYAPADRARAIGVGLVSPDEALATADFISLHMLPTPTTKSKLLNDETFA  
AtPGDH2 . . . . . VGKTLAVMGRGKVGTSVARRAKGLGMVVIHSDPYAPADRARAIGVGLVSPDEAISTADFISLHMLPTPTTKKVFNDSTFS  
AtPGDH3 . . . . . VGKTLAVLGRGKVGTSVARRAKGLGMVVIHSDPYAPADRARAIGVGLVSPDEAISTADFISLHMLPTPTTKSKMNDVTFA  
OsPGDH1 . . . . . VGKTLAILGRGKVGTSVARRAKGLGMVIAHDPYASADRARAIGVGLVSPMEDALTADFISLHMLPTPTANKMLNDETFA  
OsPGDH2 . . . . . VGKTLAVMGRGKVGTSVARRAKGLGMVIAHDPYAPADRARAIGVGLVSPDEAIGRADFISLHMLPTPTASKVFNDESFS  
OsPGDH3 . . . . . VGKTLAIMGRGKVGTSVARRAKGLGMDIISHDPYAPADRARAIGVGLVSPDEAISTADFISLHMLPTPTSTAKLFDDETFA  
PpPGDH1 . . . . . VDKTLAVMGRGKVGTSVARRAKGLGMVVVHSDPYASADRARAIGVGLVSPDEALAAADFISLHMLPTPTDKIFNDESFG  
PpPGDH2 . . . . . VDKTLAVMGRGKVGTSVARRAKGLGMVVIHSDPYASADRARAIGVGLVSPDDALARADFISLHMLPTPTTKKVFNDSTFS  
PpPGDH3 . . . . . VDKTLAVMGRGKVGTSVARRAKGLGMVVVHSDPYASADRARAIGVGLVSPDEALAAADFISLHMLPTPTDKIFNDESFG  
PpPGDH4 . . . . . VDKTLAVMGRGKVGTSVARRAKGLGMVVIHSDPYASADRARAIGVGLVSPDEALAAADFISLHMLPTPTDKIFNDDTFA  
AmtriPGDH1 . . . . . VGKTLAVLGRGKVGTSVARRAKGLGMVIAHDPYAPADRARAIGVGLVSPDEAISTADFISLHMLPTPTTKSKLLNDETFA  
AmtriPGDH2 . . . . . VGKTLAVMGRGKVGTSVARRAKGLGMVVIHSDPYAPADRARAIVDMVSPDEAISTADFISLHMLPTPTTKKVFNDSTFS  
MpPGDH . . . . . VDKTLAVMGRGKVGTSVARRAKGLGMVIAHDPYAPADRARAIGVGLVSPDEALQRAFDFISLHMLPTPTDKCFNDESFA  
AhPGDH . . . . . YKKTGLVVGCKIGISHVAVARAMGMKLLAYDFEISABRAEQLGCRLVLDLDFITFADYITLHPIRTPETENLINAEBALA  
MtPGDH . . . . . FGKTVGVVGRGIGQLVAQRIAAFGA VYVADYVSPARAAQLGIELLSLDDLARADFISVHLKPTPTAGLIDKEALA

280 290 300 310 320 330 340 350  
AtPGDH1 . . . . . KMKKGVRIINVARGGVIDEALVLRALDAGIVAQAALDVFTEPPAKDS. . . . . KLVQHERVTVTPHLGASTMEAQEGVAIEI  
AtPGDH2 . . . . . KMKKGVRIINVARGGVIDEALVLRALDAGIVAQAALDVFTEPPSKDS. . . . . KLIQHENVTVTPHLGASTMEAQEGVAIEI  
AtPGDH3 . . . . . MMKKGVRINVARGGVIDEALLRALDAGIVAQAALDVFTEPPVKDN. . . . . KLVLHESVTA TPHLGASTMEAQEGVSIEI  
OsPGDH1 . . . . . KMKKGVRIINVARGGVIDEALLRALDAGIVAQAALDVFTEPPAPDS. . . . . KVLVHENVTVTPHLGASTMEAQEGVAIEI  
OsPGDH2 . . . . . RMKNGVRINVARGGVIDEALLRALDAGKVAQAALDVFTEPPAKDS. . . . . KVLVHENVTVTPHLGASTMEAQEGVAIEI  
OsPGDH3 . . . . . NMKKGVRINVARGGVIDEALLRALDGNVTSGVAQAALDVFTEPPKDS. . . . . KVLVHENVTVTPHLGASTMEAQEGVAIEI  
PpPGDH1 . . . . . KCKKGVRIINVARGGVIDEALVLRALDAGIVAQAALDVFTEPPKGGD. . . . . ALVNHENVIVTPHLGASTMEAQEGVAIEI  
PpPGDH2 . . . . . KCKKGVRIINVARGGVIDEALLRALDAGIVAQAALDVFTEPPKGGDFALVQHKNVIVTPHLGASTMEAQEGVAIEI  
PpPGDH3 . . . . . KCKKGVRIINVARGGVIDEALVLRALDAGIVAQAALDVFTEPPKGGD. . . . . ALVNHENVIVTPHLGASTMEAQEGVAIEI  
PpPGDH4 . . . . . KCKKGVRIINVARGGVIDEALLRALDAGIVAQAALDVFTEPPKGGSSWALVQHKNVIVTPHLGASTMEAQEGVAIEI  
AmtriPGDH1 . . . . . KMKKGVRIINVARGGVIDEALLRALDAGIVAQAALDVFTEPPKDS. . . . . KLVQHENVTVTPHLGASTMEAQEGVAIEI  
AmtriPGDH2 . . . . . KMKKGVRIINVARGGVIDEALLRALDAGIVAQAALDVFTEPPKEN. . . . . KLIQHERVTVTPHLGASTMEAQEGVAIEI  
MpPGDH . . . . . KMKKGVRIINVARGGVIDEALLRALDAGIVAQAALDVFTEPPAKDD. . . . . KLIQHENVTVTPHLGASTMEAQEGVAIEI  
AhPGDH . . . . . KMKPTTRINCSRGGVIDEALATAVENCTIGGAALDVFTEPPESK. . . . . LRFEGSNIVLTPHLGASTMEAQPNVAVDV  
MtPGDH . . . . . KTKPGVIVINVARGGVIDEALADAVTGGHVRAGLDVFTEPPCTDSP. . . . . LVELAQVVVTPHLGASTMEAQDRAGTDV

↑ R51 (R108)  
↑ W130 (W188)  
↑ R132 (R190)  
↑ G149, 151, 154 (G207, 209, 212)  
↑ D172 (D230)  
↑ R233 (R291)  
↑ R280 (H339)



**Figure S4.** Multiple sequence alignments of full-length amino acid sequences of PGDH isoforms. The full-length amino acid sequences of PGDHs from *A. thaliana* (AtPGDHs), *O. sativa* (OsPGDHs), *A. trichopoda* (AmtriPGDHs), *P. patens* (PpPGDHs), *M. polymorpha* (MpPGDH), *A. halophytica* (AhPGDH), and *M. tuberculosis* (MtPGDH) are shown. Isozymes regulated by all six effector amino acids are highlighted by red font and pink shading. The predicted transit peptides are underlined. The predicted ASB domains and ACT domains are surrounded by blue and green rectangles, respectively. Green triangles, red triangles and red pentagon with number represent key residues for serine binding, substrate binding and active site histidine in MtPGDH, respectively. Corresponding residues on AtPGDH1 are also shown in parentheses.





**Figure S5.** Phylogenetic tree of PGDHs across multiple plant lineages. Amino acid sequences of PGDHs from eudicots, monocots, basal angiosperms, gymnosperms, lycophyte, bryophytes, charophyte, chlorophyte, cyanobacterium, and actinobacterium were obtained from databases (Supplementary Table S2). Red and blue letters indicate the isozymes sensitive to six effector amino acids and those insensitive or sensitive to some effectors, respectively. Posterior probabilities are indicated at the nodes. Bar indicates substitutions per site.