

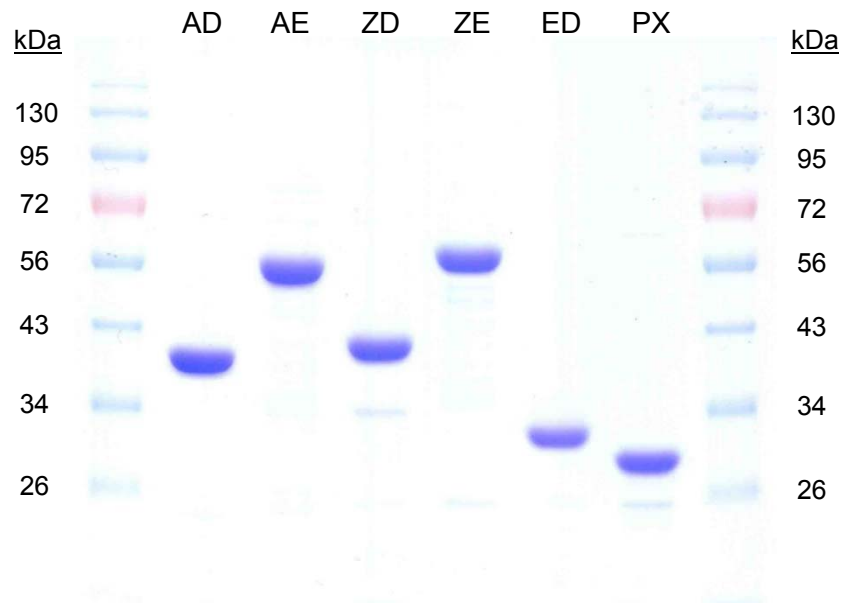
A

Arabidopsis			MLVKPSI	ISGLVRLTSH	SPSSSSSVLR	RQEFVLRITLC	GSPIIR---A	MSSTSEADAE	SVLRTVTPSL	DLKRHKGQAG	KIAVIGGCRE	
Maize	MSRPTGAWR	LGMATCTIPA	APRAHVGRLP	GIRQAARPAL	LLPSFSSSTL	LCHRLPPILP	LVLQLLRVRA	NHAMAA---S	AGTVYEADAE	AVVRRITPAL	DRARHKGQAG	KIAVIGGCRE
Onion			MNSLA	IFSSCTESRL	LLSSSILRRQ	L--FLIKTLG	AYCCRAHMDT	NQTEAS---G	ITLALAEADAE	TVIRDITPAL	DPKRHKGQAG	KIAVIGGCRE
Switchgrass	MSRPTGAWR	HQPQHRGRMW	AASPFRRQL	VLLRSLLPQS	PPPSVAGCRF	AHLSSPSGTS	GSRAARAMA	AATVYEADAE	AVVRRITPPL	DRARHKGQAG	KIAVIGGCRE	
Wheat			MW	AASPAFRRQL	FLLRSLAPT-	-----CAD	GGRASSSSLR	PHLMYA---A	AGPVYEADAE	AVVRRITPPL	DRARHKGQAG	KIAIIGGCRE
Orange			MLIRYGVST	GFASLGSVKN	CMLASSAVFR	RQQFLIRSLG	GYSDHIEPRR	MQDIRS---MS	GTTF-EADAE	NVMREITPVL	DPSKHKGQAG	KIAVIGGCRE
Oak			MFVKHGMNS	QFTLLGSAKH	CMLASSAVLR	RQQFLIRTLG	GYTDQNHLLR	MQQTKA---MS	GTAF-EADSE	NILRAITPPL	DPSKHKGQAG	KIAVIGGCRE
Beech			MFVRHGMNC	QFTLLGSAKH	CMLDSSAVFR	RQQFLIRTLG	GYTDQTHQKR	MQQTKA---LS	GTAF-EADAE	NILRAITPPL	DPSKHKGQAG	KIAVIGGCRE
Iris	MLSDLLFFGR	RLVFHDDMSS	WLTFSGCLGN	RLLSSSAIYR	RQLFLIRSLG	--CCKAHMEE	GRGVPG---VG	VSTS-QTDGE	TVIQRITPVL	DPSRHKGHAG	KIAVIGGCRE	
Ginger			MRG	SIPFSCYPEN	CTLASAAVLR	RNLFLIKCLD	GFCCRSHSQR	MQGTQS---AA	VPAA-EADAE	KTIQMIPAL	DPRIYKQAG	KIAVIGGCRE
Cotton			MFVKHGMNS	GFSS-----	--LASSAVLR	RQKFLIRSLK	GYSV-SDKIR	MEAVKC---LS	GGASLEANA	SVLRAITPPL	DPTRHKGQAG	KIAVIGGCRE
Cocoa			MIVKHMNS	GFSS-----	--LASSAVLR	RQQFLIRSLR	GYSDHTHQKR	MEGMKC---FS	GGASLEADSE	NVLRAIIPSL	DPTRHKGQAG	KIAVIGGCRE
Grape			MIKNGVSL	LHSPNNR---	-MLASSAVFR	RQEFLIRCLG	VGGQSQQFNR	-KSIPR----	-TMALAEADAE	NILRAITPPL	DLARHKGQAG	KIAVIGGCRE
Lotus			MNSQLPL	LRCANTC---	VLLASSPVFR	RQKLLIRSLG	SGIDYHNHNS	SENMQR----	-MRSVEADAE	SIIRAITPAL	DSTRHKGQAG	KIAVIGGCRE
Rice			MW	AASPAFRRRL	FLLRSLSPSP	CAALPGNAAC	SSPSPSTSR	VNAMSA----	SGPVYEADAE	AVVRRITPPL	DRARHKGQAG	KIAVIGGCRE

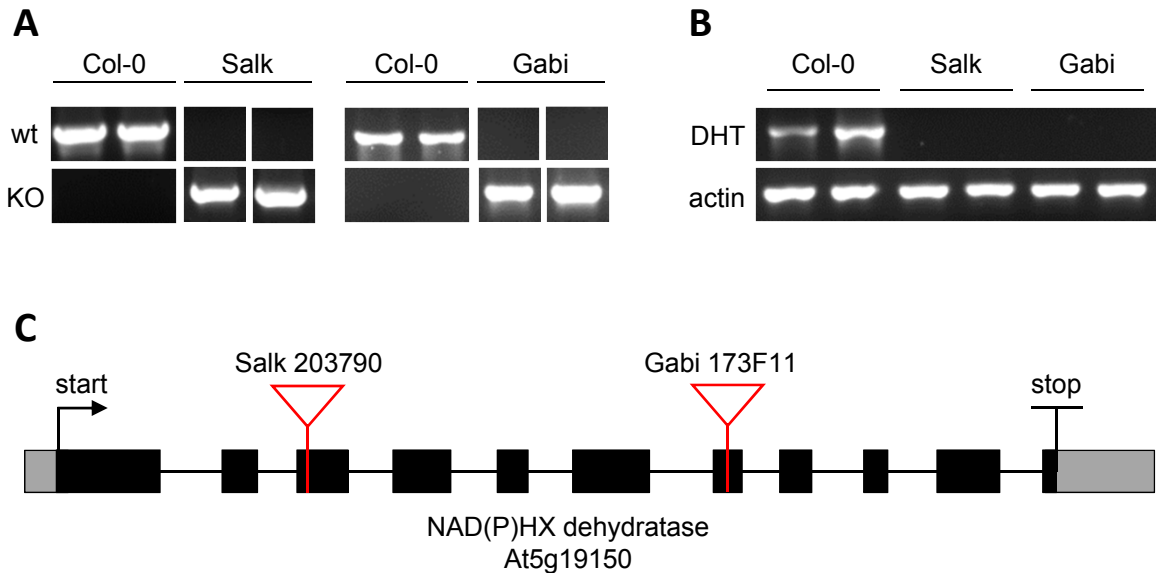
B

Arabidopsis			MRNVIR	RVTMTFTFL	LQSPPLPISP	SPPQFSLSS	PLSKTQRFIT	PSQGSRLRTL	CTKVIIPN--	----MQDSGS	PP--LSYLTQ	REAAEIDETL	
Maize	MLAAVAS	RTRNKSATTV	LSLAMPFLLS	AASASTTNP	HPLSPFPRSQ	NNSRVHRLQL	PARRLPSFPA	VAPGASFAPL	PQRLGMAS--	----LAASAA	AAAEVTHLTQ	RDAAEIDEQL	
Radish				MTFSTL	LQ-SPLPISP	K-PLLSYEQH	RYSLSLLPL	PTHFRLLRT	FCTKSIPN--	----MQDSST	S---VSYLSQ	REAAEIDETL	
Canola			MRTVIR	RRVTRMISAF	LFQQPLPISP	K---HQYSL	PLTTTQRFLT	ASHGFR---T	LSTKSIPN--	----MQDSAS	---VSYLSQ	REAAEIDETL	
Lime	MWSVIRK	SRRMSMTCSL	LAQSLTLAYH	YNSSPAIKST	CNPFSPFVSN	QSSSSVFLR	PVSAPAIRAF	CSKSDAM	S---SAQNSD	----VSHLTQ	REAAEIDETL		
Cotton			MICSLLSQ	APVSPAYYR	SSPSIYKPK	SNRFSWGFLA	PIPSPAIRVF	CSKPGEVGG	----MASSVQ	NQGSISYLTQ	REAAEIDEVL		
Cocoa			MICS	LLTQAPFPLA	YCSSPSSIYE	AKPSGYLP--	--RFSSGF	PISSCAIRAL	CSKSGKVG	----MASSLQ	NQGSITYLTQ	REAAEIDEIL	
Apple			MW	SLARNTRKMT	RLFLNHSLS	SNPIKPPIS	NAYTSFHIPP	SSLSTSALFQ	SVRSPAIRAF	CSNPSGAAGV	GSVHAAPSMQ	NPGDISYLTQ	REAAEIDEVL
Rice	MLTVAASALR	NNKKSTAAAC	FSLAMPFLLS	SPTPPPPPHS	SSL---PRSP	--SPRRLPL	PPPRRAALVT	AAQDPRW---	--RRAMASLA	V---SASASA	SGEEVTHLAQ	REAAEIDEQL	
Sorghum	MLAVA	SRTRNKSATT	VTLAMPFLLS	AANASATTNS	RPLSPYPRSQ	NNSRAHRLQL	PARRLPSFPA	AAPGAPFAPL	PQRRGMASLA	A---SAAAAA	AAE-VTHLTQ	RDAAEIDEQL	
Orchid			MSSCXLS	RSIKLLIPPP	TATVTVPADT	AASLCVPPRG	ITRHSVPLS	IFRRPAYLSL	SRRPPSRRCF	----SLGAQM	AEESVSYLSQ	KEAAEIDEIL	

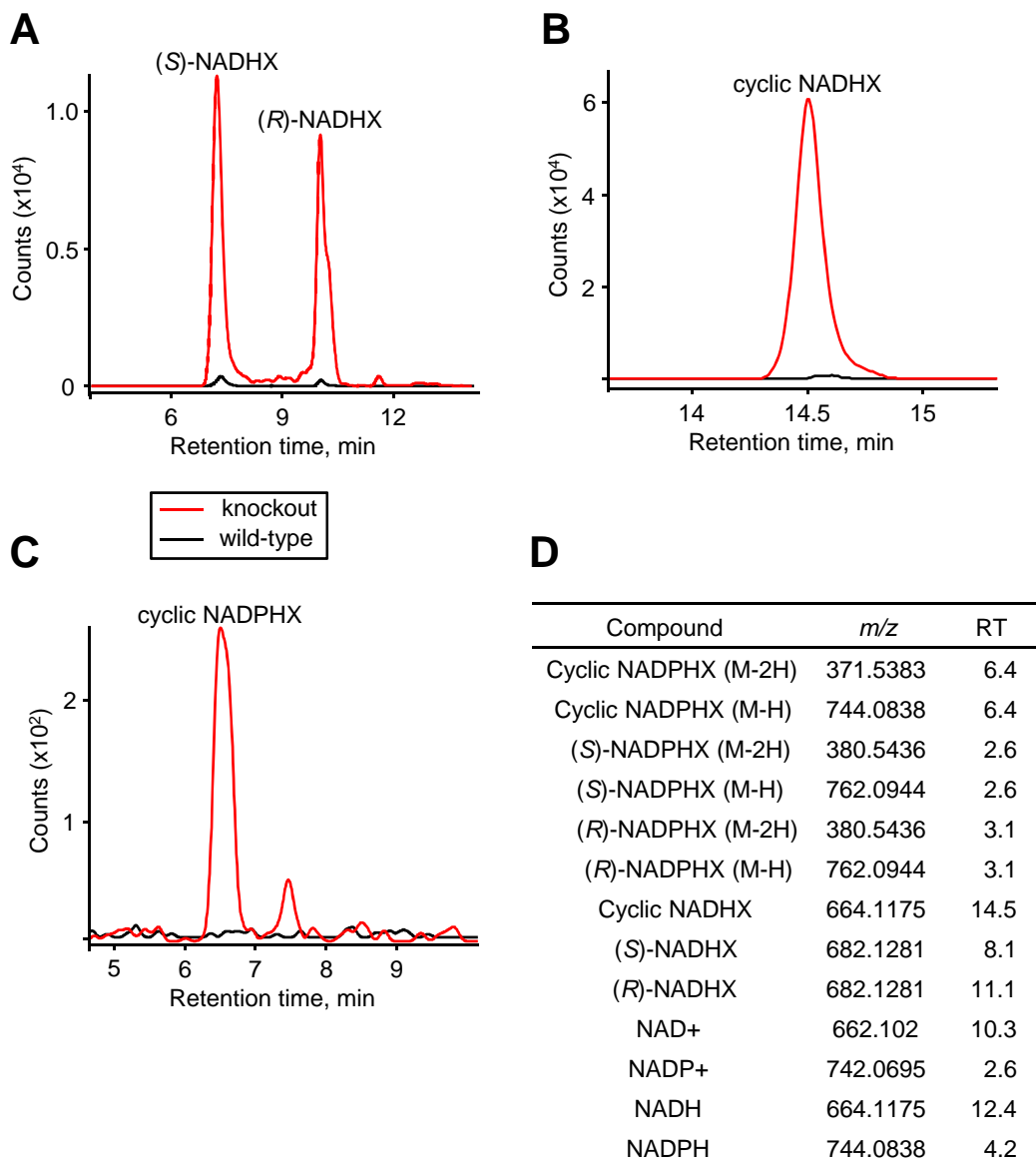
Supplemental Figure 1. Amino acid alignment of various plant NAD(P)HX dehydratase (A) and NAD(P)HX epimerase-PPOX (B) amino-terminal sequences that are supported by EST evidence. Completely conserved and highly conserved residues are colored red and blue, respectively. Dashes are gaps introduced to maximize alignment. Predicted alternative start methionine residues are highlighted yellow. The mature protein is predicted to start close to the beginning of high sequence conservation.



Supplemental Figure 2. Purification to near-homogeneity of recombinant proteins used in this study. Five μg of Ni-affinity purified *Arabidopsis* NAD(P)HX dehydratase (AD), *Arabidopsis* NAD(P)HX epimerase:PPOX (AE), maize NAD(P)HX dehydratase (ZD), maize NAD(P)HX epimerase:PPOX (ZE), *Arabidopsis* NAD(P)HX epimerase separate domain (ED), and *Arabidopsis* PPOX separate domain (PX) were analyzed by SDS-PAGE (10%) with Coomassie staining. Each protein preparation was estimated to be $\geq 90\%$ pure.



Supplemental Figure 3. Confirmation that both *Arabidopsis* NAD(P)HX dehydratase T-DNA insertion lines are knockouts. A, Genotyping the Salk 203790 and Gabi 173F11 lines. Genomic DNA from each line and the corresponding wild-type, Columbia-0 (Col-0) was used as template for PCR reactions with wild-type allele primers (wt, Salk_LP and Salk_RP or Gabi-LP and Gabi-RP, respectively) or T-DNA insertion allele primers (KO, Salk_LBb1.3 and Salk_RP or Gabi_LBb and Gabi_RP, respectively). Amplicons were analyzed by agarose-gel electrophoresis. Results are shown for two wild-type plants and two plants homozygous for the T-DNA insertion. B, Testing for the presence of the NAD(P)HX dehydratase transcript. Total RNA was used for first-strand cDNA synthesis, followed by PCR with primers designed to amplify a fragment of the NAD(P)HX dehydratase transcript (AtDHT 1ex2 F and AtDHT 11ex10 R) or a fragment of the actin-7 transcript (actin-7 F and actin-7 R). Amplicons were analyzed by agarose-gel electrophoresis. C, T-DNA insertion sites in the NAD(P)HX dehydratase gene. PCR amplicons obtained with the T-DNA insertion allele primers in part A above were cloned and sequenced. The gene model is drawn to scale with exons as black boxes, introns as black lines, and 5'- and 3'-UTRs as gray boxes. The T-DNA insertion sites in exon 3 (Salk) and exon 7 (Gabi) are indicated in red. The positions of the first potential start codon and the stop codon are indicated.



Supplemental Figure 4. Targeted metabolomics analysis of NAD(P)HX in *Arabidopsis* seedlings. A, B, and C, Extracted ion chromatograms at m/z 682.1281 (A, NADHX), 664.1175 (B, cyclic NADHX), and 744.0838 (C, cyclic NADPHX) for representative dehydratase knockout (red trace) and wild-type (black trace) samples. D, Molecular ion signals (m/z) and retention times (RT, min) used to monitor NAD(P)HX forms and oxidized or reduced pyridine nucleotides.

A

atgctggccgcccgttgcgagtcgtaccgtaataaaaagtgctaccaccggttctgtccctggctatgccg
tttctgctgtccgctgctagcgcacatctaccacgaccaaccgcatccgctgagcccgtttccgctagt
cagaacaattcccgtgtgcaccgctgcaactgccggcgctcgctgccgagcttcccggcgggtgcc
ccgggtgcttcttcgctccgctgccgcaacgctctgggtatggcttcgctggctgcaagtGCGGCCGCC
GCGGCCGAGGT

B

atgctggccgcccgttgcgagtcgtaccgtaataaaaagtgctaccaccggttctgtccctggctatgccg
tttctgctgtccgctgctagcgcacatctaccacgaccaaccgcatccgctgagcccgtttccgctagt
cagaacaattcccgtgtgcaccgctgcaactgccggcgctcgctgccgagcttcccggcgggtgcc
ccgggtgcttcttcgctccgctgccgcaacgctctgggtatggcttcgctggctgcaagtGCGGCCGCC
GCGGCCGAGGTgacgcacctcacgcagcgcgacgcggcggagatcgacgagcagctcatgggcccgctc
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tggggtggctacaaattgacaccgacactttttgagttctggcaaggacaacagctctcgactgcatgac
cggttacaatactcgcagagagaagtagatgggagcacagtggtggcacatcgagaggttggtccccttga

Supplemental Figure 5. Sequence data for maize epimerase/PPOX (GRMZM2G061988). A, Nucleotide sequence of the recoded 5'-region. B, Nucleotide sequence of the assembled complete open reading frame. Recoded nucleotides are in red, native maize epimerase/PPOX sequence is in black, and overlapping sequence used to splice the sequences is in uppercase.

Table S1. Predicted localization of *Arabidopsis* and maize NAD(P)HX epimerase and dehydratase proteins

Targeting of the full length *Arabidopsis* (At) and maize (Zm) NAD(P)HX dehydratase and NAD(P)HX epimerase/PPOX sequences was predicted using TargetP (<http://www.cbs.dtu.dk/services/TargetP/>), Predotar (<http://urgi.versailles.inra.fr/predotar/predotar.html>), WoLF PSORT (<http://wolfpsort.org/>), and the COSSMOSS ambiguous targeting algorithm (<http://www.cossmoss.org/bm/ATP>). Predicted locations: P, plastids; M, mitochondria; N, nucleus. In some cases the prediction was weak (<).

Protein	TargetP	Predotar	WoLF PSORT	COSSMOSS
At Dehydratase	P	P	P,M	<(P,M)
Zm Dehydratase	M	<M	P,<M	<(P,M)
At Epimerase/PPOX	P	<M	P,<N	P,M
Zm Epimerase/PPOX	P	<M	M,<P	no dual targeting

Table S2. Oligonucleotide primers used in this study.

Restriction enzyme sites are capitalized.

Primer	Name	Sequence
Maize epimerase assembly		
1	Epm assemble1F	gcggccgcgcggcgagggtgacgc
2	Epm assemble1R	acctccgcgcggcgaggccgcaacttgc
3	Zm Epm FL NdeI F	ggaattcCATATGctggccgcggttgcgagtcg
4	Zm Epm EcoRI R	gGAATTCTcaaggggacaacctctcgatgtgcc
Protein overexpression in <i>E. coli</i>		
5	At Dht m NdeI F	ggaattcCATATGagttccacatcagaggccg
6	At Dht EcoRI R	gGAATTCttaagatgcagggcagatatcc
7	At Epm m NdeI F	ggaattcCATATGcaagattcaggatcaccacc
8	At Epm EcoRI R	gGAATTCtcatggggccaatctatgaattttcc
9	Zm Dht m NdeI F	ggaattcCATATGcggcgctccgcgggacgg
10	Zm Dht EcoRI R	gGAATTCctagtgtctcggcagggcagatatc
11	Zm Epm m NdeI F	ggaattcCATATGgcttcgctggctgcaagtgc
12	At ED EcoRI R	gGAATTCctagacatagttcactctcatagcag
13	At PPOX NdeI F	ggttttcCATATGtgtgttagaattggtaaacc
IVT and dual import		
14	Dht FL EcoRI F	gGAATTCctgAtgttgggaagcccagatcatc
15	Dht T EcoRI F	gGAATTCaccatgagttccacatcagaggccg
16	Dht XbaI R	gcTCTAGAttaagatgcagggcagatatcc
17	Dht M2L F	gggtcaccaatcatcagagcattgagttccacatcagaggccg
18	Dht M2L R	cggcctctgatgtggaactcaatgctctgatgatgggtgaccc
19	Epm FL EcoRI F	gGAATTCtaaagagggaatgtgatacgcagagtc
20	Epm T EcoRI F	gGAATTCaccatgcaagattcaggatcaccacc
21	Epm XbaI R	gcTCTAGAtcatggggccaatctatgaattttcc
22	Epm M2L F	gtaccaaagtcatcataccaacttgcaagattcaggatcaccacc
23	Epm M2L R	gggtggatcctgaatcttgcaagttggatgatgactttggtac
GFP fusions		
24	Dht FL BamHI F	cgcGGATCCctgatgttgggaagcccagatcatc
25	Dht T BamHI F	cgcGGATCCatgagttccacatcagaggccgacg
26	Dht BamHI R	cgcGGATCCagatgcagggcagatatcctcc
27	Epm FL BamHI F	cgcGGATCCtaaagagggaatgtgatacgcagagtc
28	Epm T BamHI F	cgcGGATCCatgcaagattcaggatcaccacc
29	Epm BamHI R	cgcGGATCCtggggccaatctatgaattttcc
<i>Arabidopsis</i> knockouts		
30	Salk LP	tcattcacatttggggttaagac
31	Salk RP	cgattgacttctcattcaccttc
32	Salk LBb1.3	atthttgcccatttcggaac
33	Gabi LP	attgagacgaaatgtttttgtgg
34	Gabi RP	caaaaagtccaatgttctctttg
35	Gabi LBb	atattgaccatcatactcattgc
36	At DHT 1ex2 F	caaaggccaagctggaagatagc
37	At DHT 11ex10 R	ggcagatatcctccaaactctccc
38	At actin-7 F	atggccgatgggtgaggat
39	At actin-7 R	gtctcaaacatgatctgagtc