Supplementary Material

Purification of maize nucleotide pyrophosphatase/phosphodiesterase casts doubt on the existence of zeatin *cis-trans* isomerase in plants

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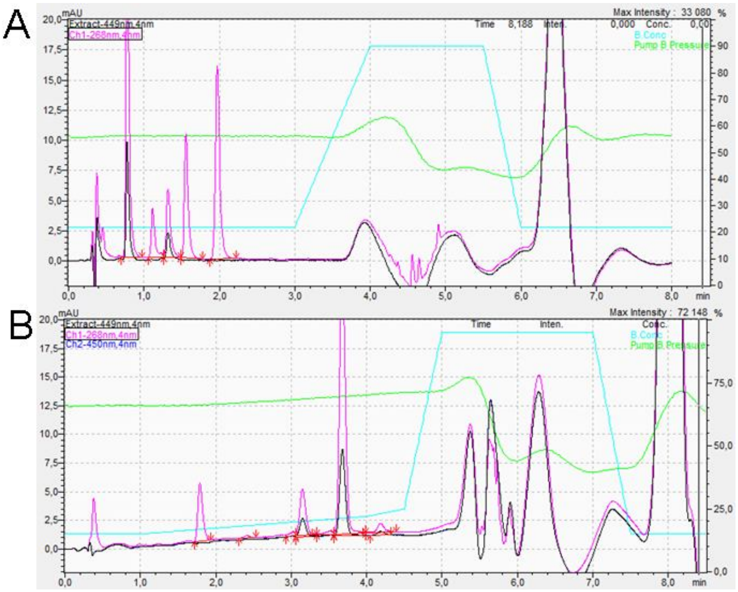
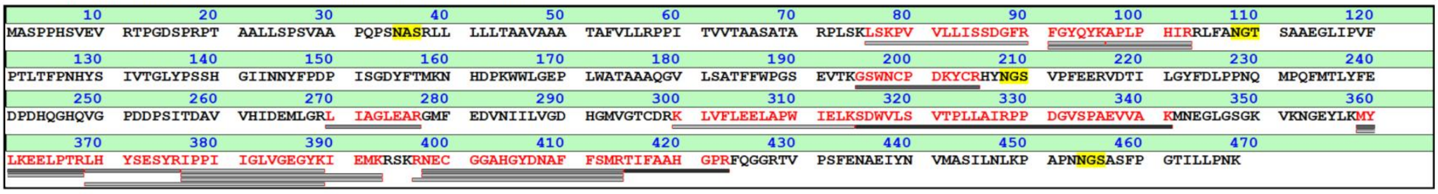
Supplementary Figure S1. Typical UFLC chromatograms of enzymatic reactions. (A) shows the reaction of cis-zeatin isomerization with FAD at 0.75 min, FMN at 1.30 min, tZ at 1.55 min and cZ at 1.95 min. (B) shows typical chromatogram of FAD hydrolysis with FMN peak at 3.15 min and FAD at 3.65 min. Blue line indicates gradient of methanol, green line pressure in UFLC system, purple and black line absorbance at 268 and 449 nm, respectively.



Supplementary Figure S2. Results of MALDI-TOF/TOF MS identification of protein band with zeatin *cis-trans* isomerase activity.

Protein 1: LOC100280465 [Zea mays]
Accession: gi|226531542
Database: NCBInr
Seq. Coverage [%]: 32.30 %
Score: 131.00
MW [kDa]: 51.10
pI: 6.39
No. of Peptides: 19
Modification(s): Carbamidomethyl, Oxidation



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       130        140        150        160        170        180        190        200        210        220        230        240
PTLTFPNHYS IVTGLYPSSH GIINNYFPDP ISGDYFTMKN HDPKWWLGEP LWATAAAQGV LSATFFWPGS EVTKGSWNCP DKYCRHYNGS VPFEERVDTI LGYFDLPPNQ MPQFMTLYFE
       250        260        270        280        290        300        310        320        330        340        350        360
DPDHQGHQVG PDDPSITDAV VHIDEMLGRL IAGLEARGMF EDVNIILVGD HGMVGTCDRK LVFLEELAPW IELKSDWVLS VTPLLAIRPP DGVSPAEVVA KMNEGLGSGK VKNGEYLKMY
       370        380        390        400        410        420        430        440        450        460        470
LKEELPTRLH YSESYRIPPI IGLVGEGYKI EMKRSKRNEC GGAHGYDNAF FSMRTIFAAH GPRFQGGRTV PSFENAEIYN VMASILNLKP APNNGSASFP GTILLPNK
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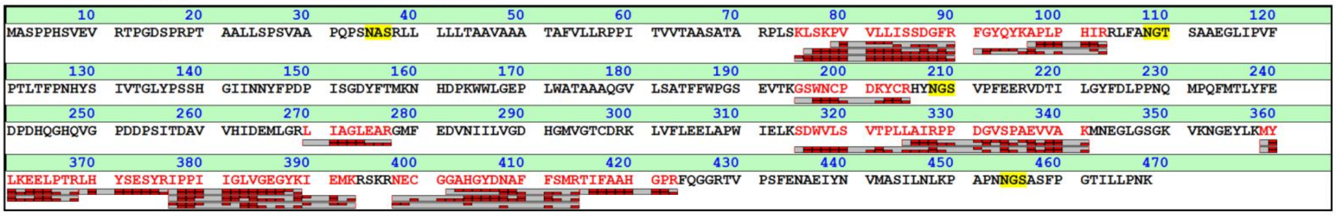
m/z meas.	Δ m/z [ppm]	z	P	Range	Sequence	Modification
1630.9577	3.102	1	0	76-90	LSKPVVLLISSDGFR	
805.3849	-3.721	1	0	91-96	FGYQYK	
1589.8623	2.282	1	1	91-103	FGYQYKAPLPHIR	
803.4890	0.4263	1	0	97-103	APLPHIR	
1442.5965	1.727	1	1	195-205	GSWNCPDKYCR	Carbamidomethyl: 5, 10
842.5132	4.535	1	0	270-277	LIAGLEAR	
1828.0556	-3.451	1	1	300-314	KLVFLEELAPWIELK	
2816.5555	-0.07056	1	0	315-341	SDWVLSVTPLLAIRPPDGVSPAEVVAK	
1279.6700	-1.122	1	1	359-368	MYLKEELPTR	
1295.6715	3.933	1	1	359-368	MYLKEELPTR	Oxidation: 1
1054.4965	1.202	1	0	369-376	LHYSESYR	
2391.2725	0.7624	1	1	369-389	LHYSESYRIPPIIGLVGEGYK	
1355.7942	0.6609	1	0	377-389	IPPIIGLVGEGYK	
1857.0556	0.1071	1	1	377-393	IPPIIGLVGEGYKIEMK	
1873.0493	-0.5566	1	1	377-393	IPPIIGLVGEGYKIEMK	Oxidation: 16
2088.8738	-1.189	1	1	397-414	RNECGGAHGYDNAFFSMR	Carbamidomethyl: 4
1932.7761	0.4818	1	0	398-414	NECGGAHGYDNAFFSMR	Carbamidomethyl: 3

m/z meas.	Δ m/z [ppm]	z	P	Range	Sequence	Modification
1948.7639	-3.170	1	0	398-414	NECGGAHGYDNAFFSMR	Carbamidomethyl: 3; Oxidation: 16
969.5245	-1.992	1	0	415-423	TIFAAHGPR	

Supplementary Figure S3. Results of MALDI-TOF-TOF MS/MS identification of protein band with zeatin *cis-trans* isomerase activity.

Protein 1:	LOC100280465 [Zea mays]		
Accession:	gi|226531542	**Score:**	1345.46
Database:	NCBInr	**MW [kDa]:**	51.10
Seq. Coverage [%]:	29.10 %	**pI:**	6.39
		No. of Peptides:	19
Modification(s):	Carbamidomethyl, Oxidation		



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       130        140        150        160        170        180        190        200        210        220        230        240
PTLTFPNHYS IVTGLYPSSH GIINNYFPDP ISGDYFTMKN HDPKWWLGEP LWATAAAQGV LSATFFWPGS EVTKGSWNCP DKYCRHYNGS VPFEERVDTI LGYFDLPPNQ MPQFMTLYFE
       250        260        270        280        290        300        310        320        330        340        350        360
DPDHQGHQVG PDDPSITDAV VHIDEMLGRL IAGLEARGMF EDVNIILVGD HGMVGTCDRK LVFLEELAPW IELKSDWVLS VTPLLAIRPP DGVSPAEVVA KMNEGLGSGK VKNGEYLKMY
       370        380        390        400        410        420        430        440        450        460        470
LKEELPTRLH YSESYRIPPI IGLVGEGYKI EMKRSKRNEC GGAHGYDNAF FSMRTIFAAH GPRFQGGRTV PSFENAEIYN VMASILNLKP APNNGSASFP GTILLPNK
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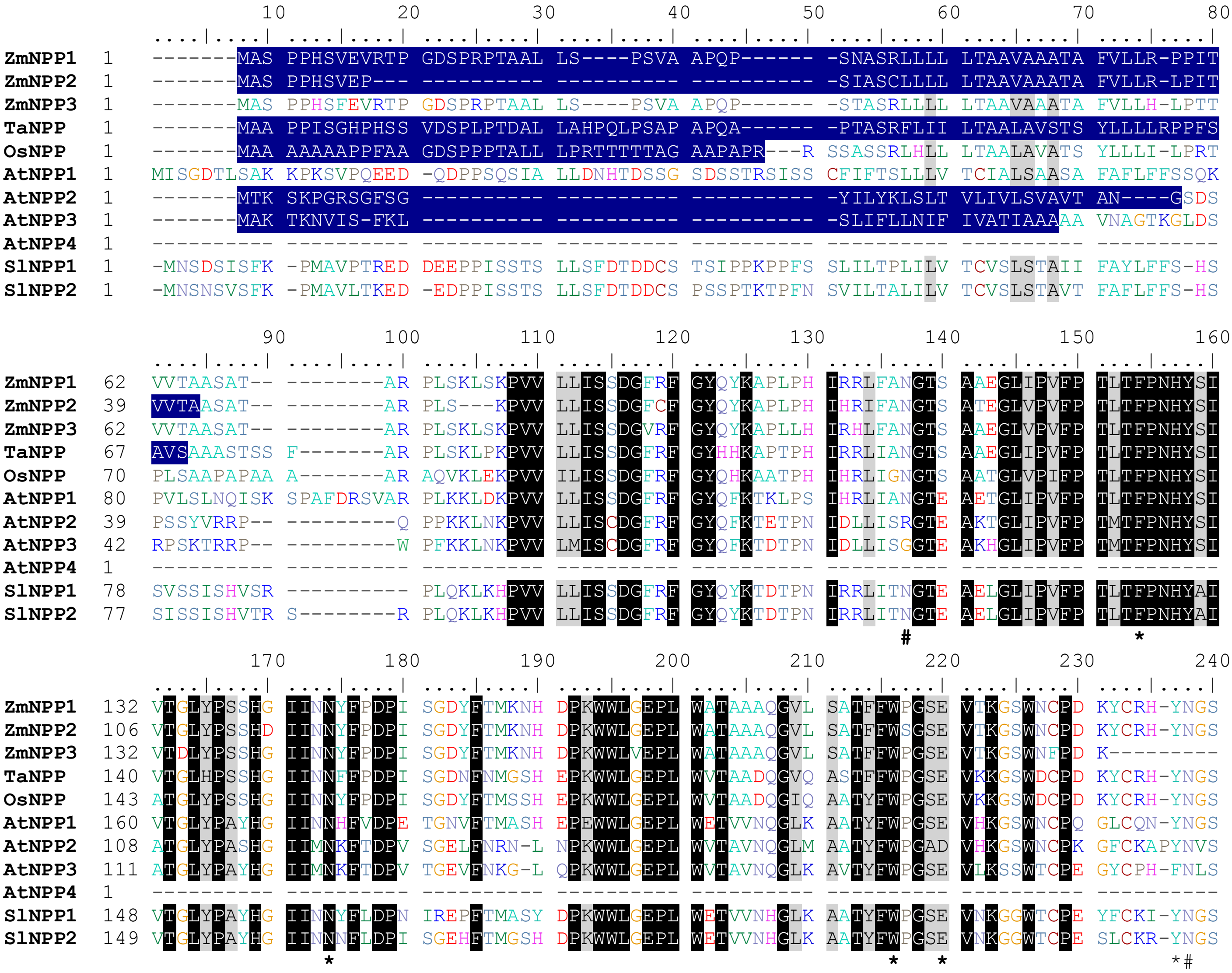
Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
	1	1759.0511	1.99	1		130.9	0	75-90	S.KLSKPVVLLISSDGFR.F	
	1	1630.9552	1.57	1		131.8	0	76-90	K.LSKPVVLLISSDGFR.F	
	1	1302.7463	3.59	1		37.6	0	79-90	K.PVVLLISSDGFR.F	
	1	1589.8526	-3.87	1		23.3	0	91-103	R.FGYQYKAPLPHIR.R	
	1	803.4888	0.19	1		43.7	0	97-103	K.APLPHIR.R	
	1	1442.5953	0.92	1		57.6	0	195-205	K.GSWNCPDKYCR.H	Carbamidomethyl: 5, 10
	1	842.5075	-2.30	1		58.0	0	270-277	R.LIAGLEAR.G	
	1	2816.5538	-0.66	1		112.1	0	315-341	K.SDWVLSVTPLLAIRPPDGVSPAE VVAK.M	
	1	1718.9823	1.36	1		73.0	0	325-341	L.LAIRPPDGVSPAEVVAK.M	
	1	1279.6730	1.17	1		66.8	0	359-368	K.MYLKEELPTR.L	
	1	1295.6672	0.60	1		51.0	0	359-368	K.MYLKEELPTR.L	Oxidation: 1
	1	1054.4941	-1.05	1		68.9	0	369-376	R.LHYSESYR.I	

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
	1	1355.7946	0.94	1		90.9	0	377-389	R.IPPIIGLVGEGYK.I	
	1	1873.0452	-2.73	1		37.7	0	377-393	R.IPPIIGLVGEGYKIEMK.R	Oxidation: 16
	1	1857.0562	0.43	1		74.5	0	377-393	R.IPPIIGLVGEGYKIEMK.R	
	1	1932.7772	1.05	1		109.7	0	398-414	R.NECGGAHGYDNAFFSMR.T	Carbamidomethyl: 3
	1	1948.7717	0.80	1		45.7	0	398-414	R.NECGGAHGYDNAFFSMR.T	Carbamidomethyl: 3; Oxidation: 16
	1	1472.6410	2.31	1		46.2	0	402-414	G.GAHGYDNAFFSMR.T	
	1	969.5270	0.52	1		70.2	0	415-423	R.TIFAAHGPR.F	

Supplementary Figure S4. Alignment of plant nucleotide pyrophosphate/phosphodiesterase protein sequences. Identical and similar amino acids are shaded in black and gray, respectively. Amino acids important for substrate binding are marked with asterisk (numbering as in ZmNPP1): Phe 125, Asn 145, Trp 187 (part of WPG motif), Glu 191 (that is homologus to Asp 308 in mouse NPP1), Tyr 207 and Asp 243. Asparagine residues predicted to be glycosylated are marked with #: Asn 108, Asn 208, Asn 454 and asparagine in AtNPP2 to 4 corresponding to Lys 393; asparagine residues 35, 33 and 34 in ZmNPP1, AtNPP1 and AtNPP2 are not marked. Predicted signal peptides giving a reliable prediction are shaded with dark blue.



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ZmNPP2  1    -------MAS PPHSVEP--- ---------- ---------- ---------- -SIASCLLLL LTAAVAAATA FVLLR-LPIT
ZmNPP3  1    -------MAS PPHSFEVRTP GDSPRPTAAL LS----PSVA APQP------ -STASRLLLL LTAAVAAATA FVLLH-LPTT
TaNPP   1    -------MAA PPISGHPHSS VDSPLPTDAL LAHPQLPSAP APQA------ -PTASRFLII LTAALAVSTS YLLLLRPPFS
OsNPP   1    -------MAA AAAAAPPFAA GDSPPPTALL LPRTTTTTAG AAPAPR---R SSASSRLHLL LTAALAVATS YLLLI-LPRT
AtNPP1  1    MISGDTLSAK KPKSVPQEED -QDPPSQSIA LLDNHTDSSG SDSSTRSISS CFIFTSLLLV TCIALSAASA FAFLFFSSQK
AtNPP2  1    -------MTK SKPGRSGFSG ---------- ---------- ---------- -YILYKLSLT VLIVLSVAVT AN----GSDS
AtNPP3  1    -------MAK TKNVIS-FKL ---------- ---------- ---------- -SLIFLLNIF IVATIAAAAA VNAGTKGLDS
AtNPP4  1    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SlNPP1  1    -MNSDSISFK -PMAVPTRED DEEPPISSTS LLSFDTDDCS TSIPPKPPFS SLILTPLILV TCVSLSTAII FAYLFFS-HS
SlNPP2  1    -MNSNSVSFK -PMAVLTKED -EDPPISSTS LLSFDTDDCS PSSPTKTPFN SVILTALILV TCVSLSTAVT FAFLFFS-HS

                     90        100        110        120        130        140        150        160
             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ZmNPP1  62   VVTAASAT-- --------AR PLSKLSKPVV LLISSDGFRF GYQYKAPLPH IRRLFANGTS AAEGLIPVFP TLTFPNHYSI
ZmNPP2  39   VVTAASAT-- --------AR PLS---KPVV LLISSDGFCF GYQYKAPLPH IHRIFANGTS ATEGLVPVFP TLTFPNHYSI
ZmNPP3  62   VVTAASAT-- --------AR PLSKLSKPVV LLISSDGVRF GYQYKAPLLH IRHLFANGTS AAEGLVPVFP TLTFPNHYSI
TaNPP   67   AVSAAASTSS F-------AR PLSKLPKPVV LLISSDGFRF GYHHKAPTPH IRRLIANGTS AAEGLIPVFP TLTFPNHYSI
OsNPP   70   PLSAAPAPAA A-------AR AQVKLEKPVV ILISSDGFRF GYQHKAATPH IHRLIGNGTS AATGLVPIFP TLTFPNHYSI
AtNPP1  80   PVLSLNQISK SPAFDRSVAR PLKKLDKPVV LLISSDGFRF GYQFKTKLPS IHRLIANGTE AETGLIPVFP TLTFPNHYSI
AtNPP2  39   PSSYVRRP-- ---------Q PPKKLNKPVV LLISCDGFRF GYQFKTETPN IDLLISRGTE AKTGLIPVFP TMTFPNHYSI
AtNPP3  42   RPSKTRRP-- ---------W PFKKLNKPVV LMISCDGFRF GYQFKTDTPN IDLLISGGTE AKHGLIPVFP TMTFPNHYSI
AtNPP4  1    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SlNPP1  78   SVSSISHVSR ---------- PLQKLKHPVV LLISSDGFRF GYQYKTDTPN IRRLITNGTE AELGLIPVFP TLTFPNHYAI
SlNPP2  77   SISSISHVTR S--------R PLQKLKHPVV LLISSDGFRF GYQYKTDTPN IRRLITNGTE AELGLIPVFP TLTFPNHYAI
                                                                          #                  *

                    170        180        190        200        210        220        230        240
             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ZmNPP1  132  VTGLYPSSHG IINNYFPDPI SGDYFTMKNH DPKWWLGEPL WATAAAQGVL SATFFWPGSE VTKGSWNCPD KYCRH-YNGS
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ZmNPP3  132  VTDLYPSSHG IINNYFPDPI SGDYFTMKNH DPKWWLVEPL WATAAAQGVL SATFFWPGSE VTKGSWNFPD K---------
TaNPP   140  VTGLHPSSHG IINNFFPDPI SGDNFNMGSH EPKWWLGEPL WVTAADQGVQ ASTFFWPGSE VKKGSWDCPD KYCRH-YNGS
OsNPP   143  ATGLYPSSHG IINNYFPDPI SGDYFTMSSH EPKWWLGEPL WVTAADQGIQ AATYFWPGSE VKKGSWDCPD KYCRH-YNGS
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AtNPP2  108  ATGLYPASHG IIMNKFTDPV SGELFNRN-L NPKWWLGEPL WVTAVNQGLM AATYFWPGAD VHKGSWNCPK GFCKAPYNVS
AtNPP3  111  ATGLYPAYHG IIMNKFTDPV TGEVFNKG-L QPKWWLGEPL WVTAVNQGLK AVTYFWPGSE VLKSSWTCPE GYCPH-FNLS
AtNPP4  1    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
SlNPP1  148  VTGLYPAYHG IINNYFLDPN IREPFTMASY DPKWWLGEPL WETVVNHGLK AATYFWPGSE VNKGGWTCPE YFCKI-YNGS
SlNPP2  149  VTGLYPAYHG IINNNFLDPI SGEHFTMGSH DPKWWLGEPL WETVVNHGLK AATYFWPGSE VNKGGWTCPE SLCKR-YNGS
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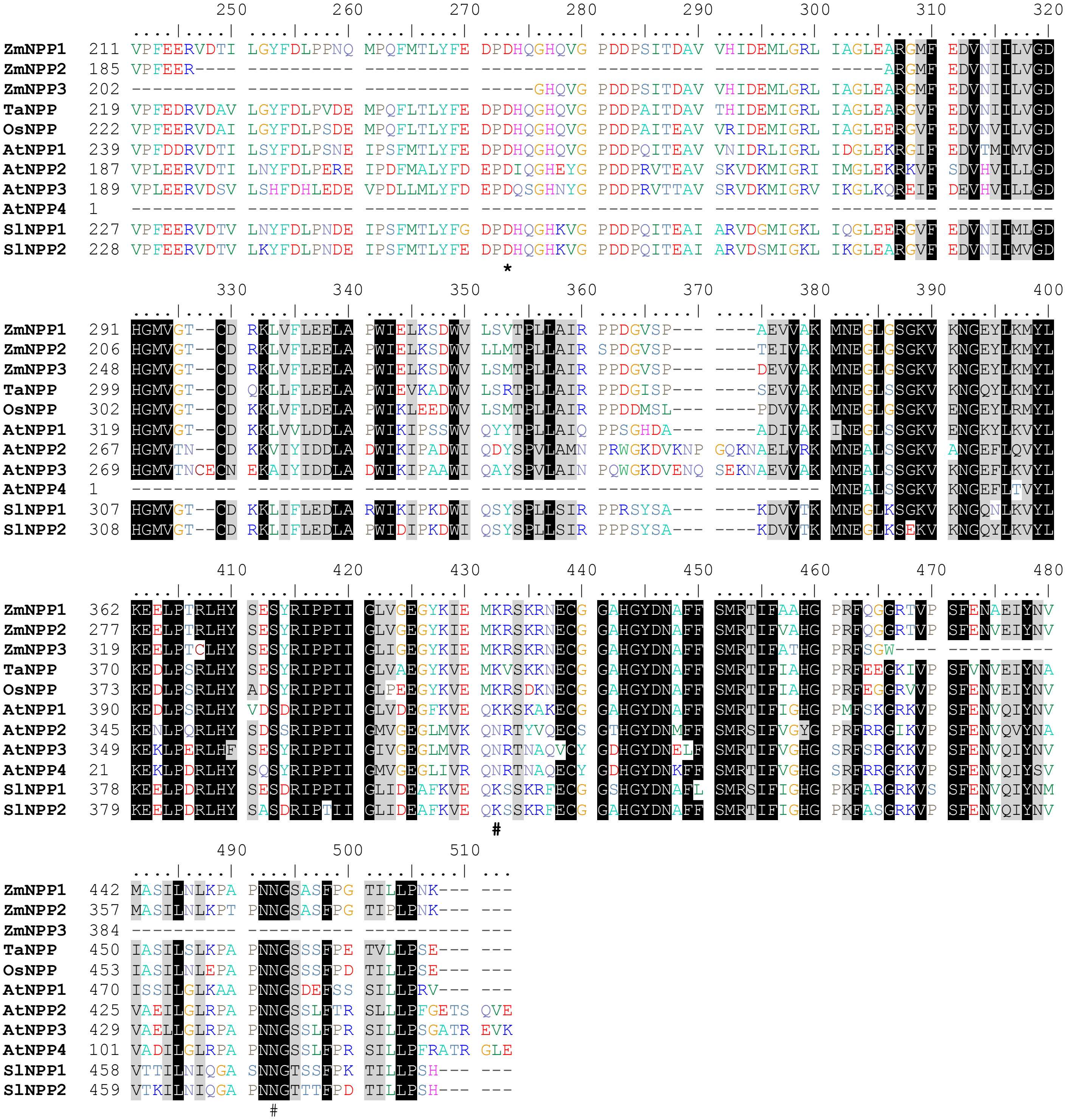
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ZmNPP2 185 VPFEER---- ---------- ---------- ---------- ---------- ---------- -----ARGMF EDVNIILVGD
ZmNPP3 202 ---------- ---------- ---------- -----GHQVG PDDPSITDAV VHIDEMLGRL IAGLEARGMF EDVNIILVGD
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OsNPP 222 VPFEERVDAI LGYFDLPSDE MPQFLTLYFE DPDHQGHQVG PDDPAITEAV VRIDEMIGRL IAGLEERGVF EDVNIILVGD
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AtNPP2 187 VPLEERVDTI LNYFDLPERE IPDFMALYFD EPDIQGHEYG PDDPRVTEAV SKVDKMIGRI IMGLEKRKVF SDVHVILLGD
AtNPP3 189 VPLEERVDSV LSHFDHLEDE VPDLLMLYFD EPDQSGHNYG PDDPRVTTAV SRVDKMIGRV IKGLKQREIF DEVHVILLGD
AtNPP4 1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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*
330 340 350 360 370 380 390 400
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AtNPP2 267 HGMVTN--CD KKVIYIDDLA DWIKIPADWI QDYSPVLAMN PRWGKDVKNP GQKNAELVRK MNEALSSGKV ANGEFLQVYL
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AtNPP4 1 ---------- ---------- ---------- ---------- ---------- ---------- MNEALSSGKV KNGEFLTVYL
SlNPP1 307 HGMVGT--CD KKLIFLEDLA RWIKIPKDWI QSYSPLLSIR PPRSYSA--- ----KDVVTK MNEGLKSGKV KNGQNLKVYL
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410 420 430 440 450 460 470 480
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ZmNPP2 277 KEELPTRLHY SESYRIPPII GLVGEGYKIE MKRSKRNECG GAHGYDNAFF SMRTIFVAHG PRFQGGRTVP SFENVEIYNV
ZmNPP3 319 KEELPTCLHY SESYRIPPII GLIGEGYKIE MKRSKRNECG GAHGYDNAFF SMRTIFATHG PRFSGW---- ----------
TaNPP 370 KEDLPSRLHY SESYRIPPII GLVAEGYKVE MKVSKKNECG GAHGYDNAFF SMRTIFIAHG PRFEEGKIVP SFVNVEIYNA
OsNPP 373 KEDLPSRLHY ADSYRIPPII GLPEEGYKVE MKRSDKNECG GAHGYDNAFF SMRTIFIAHG PRFEGGRVVP SFENVEIYNV
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AtNPP3 349 KEKLPERLHF SESYRIPPII GIVGEGLMVR QNRTNAQVCY GDHGYDNELF SMRTIFVGHG SRFSRGKKVP SFENVQIYNV
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SlNPP1 378 KEELPDRLHY SESDRIPPII GLIDEAFKVE QKSSKRFECG GSHGYDNAFL SMRSIFIGHG PKFARGRKVS SFENVQIYNM
SlNPP2 379 KEELPDRLHY SASDRIPTII GLIDEAFKVE QKSSKRFECG GAHGYDNAFF SMRTIFIGHG PKFASGRKVP SFENVQIYNV
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490 500 510
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ZmNPP2 357 MASILNLKPT PNNGSASFPG TIPLPNK--- ---
ZmNPP3 384 ---------- ---------- ---------- ---
TaNPP 450 IASILSLKPA PNNGSSSFPE TVLLPSE--- ---
OsNPP 453 IASILNLEPA PNNGSSSFPD TILLPSE--- ---
AtNPP1 470 ISSILGLKAA PNNGSDEFSS SILLPRV--- ---
AtNPP2 425 VAEILGLRPA PNNGSSLFTR SLLLPFGETS QVE
AtNPP3 429 VAELLGLRPA PNNGSSLFPR SILLPSGATR EVK
AtNPP4 101 VADILGLRPA PNNGSSLFPR SILLPFRATR GLE
SlNPP1 458 VTTILNIQGA SNNGTSSFPK TILLPSH--- ---
SlNPP2 459 VTKILNIQGA PNNGTTTFPD TILLPSH--- ---
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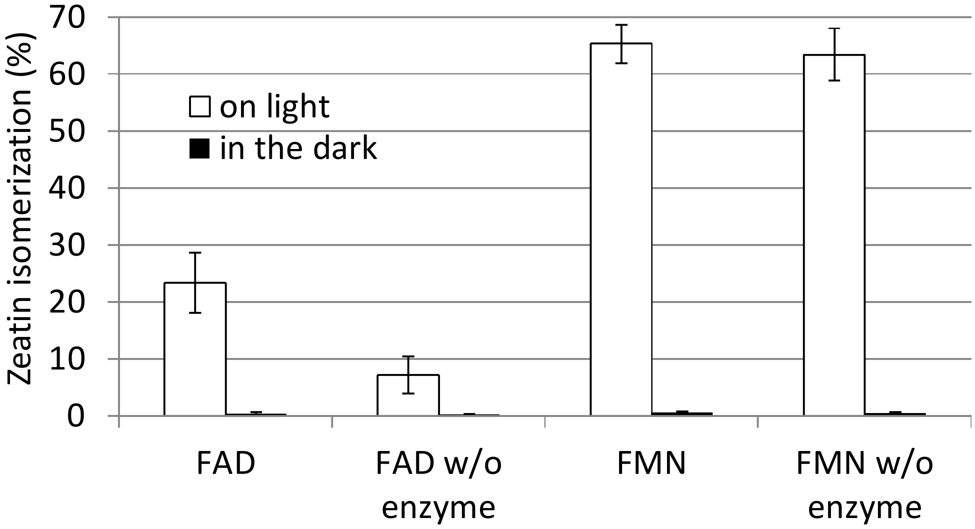
Supplementary Figure S5. Comparison of *cis*-to-*trans* conversion of zeatin isomers (0.2 mM) in a presence of FAD and FMN. Reactions were set up with or without purified recombinant ZmNPP enzyme and incubated in 100 mM McIlvaine buffer containing 20 mM $MgCl_2$, 0.1 mM FAD/FMN, at 37°C under white fluorescent light or in the dark for 20 minutes; determined by UFLC; graph depicts average of three technical replicates with standard deviation.



Supplementary Figure S6. Level of *trans*-zeatin and *cis*-zeatin in green (A) and senescent (B) leaves of Arabidopsis of wild type or expressing ZmNPP. None of the results was significantly different (Student's t-test; $p < 0.05$; n = 4) between the wild type and ZmNPP plants.