

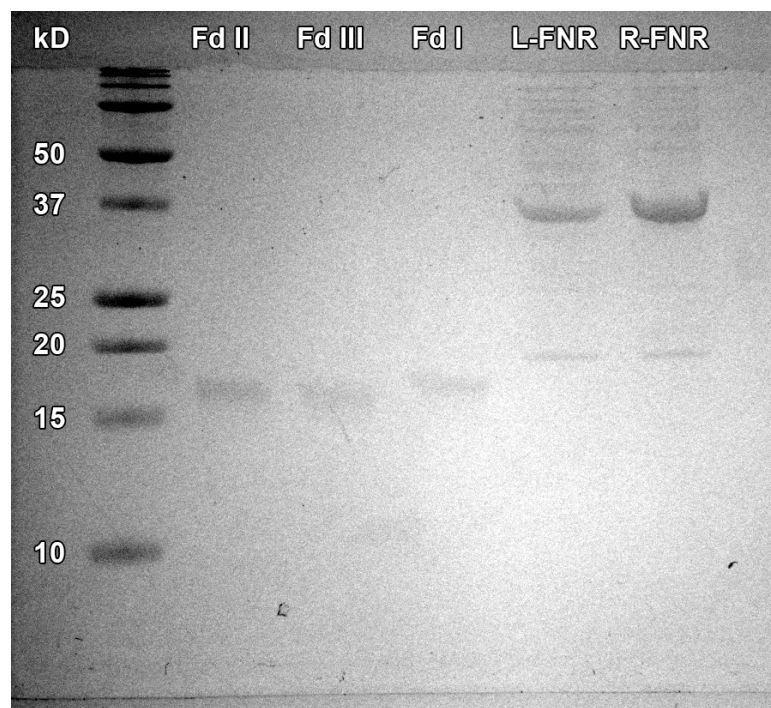
## Supplementary Materials

**Table S1.** List of primers used to amplify and clone *Fd* and *FNR* genes from first-strand cDNA into a pSBET expression vector. Plastidial targeting sequences are excluded to generate amplicons that correspond to the native proteins.

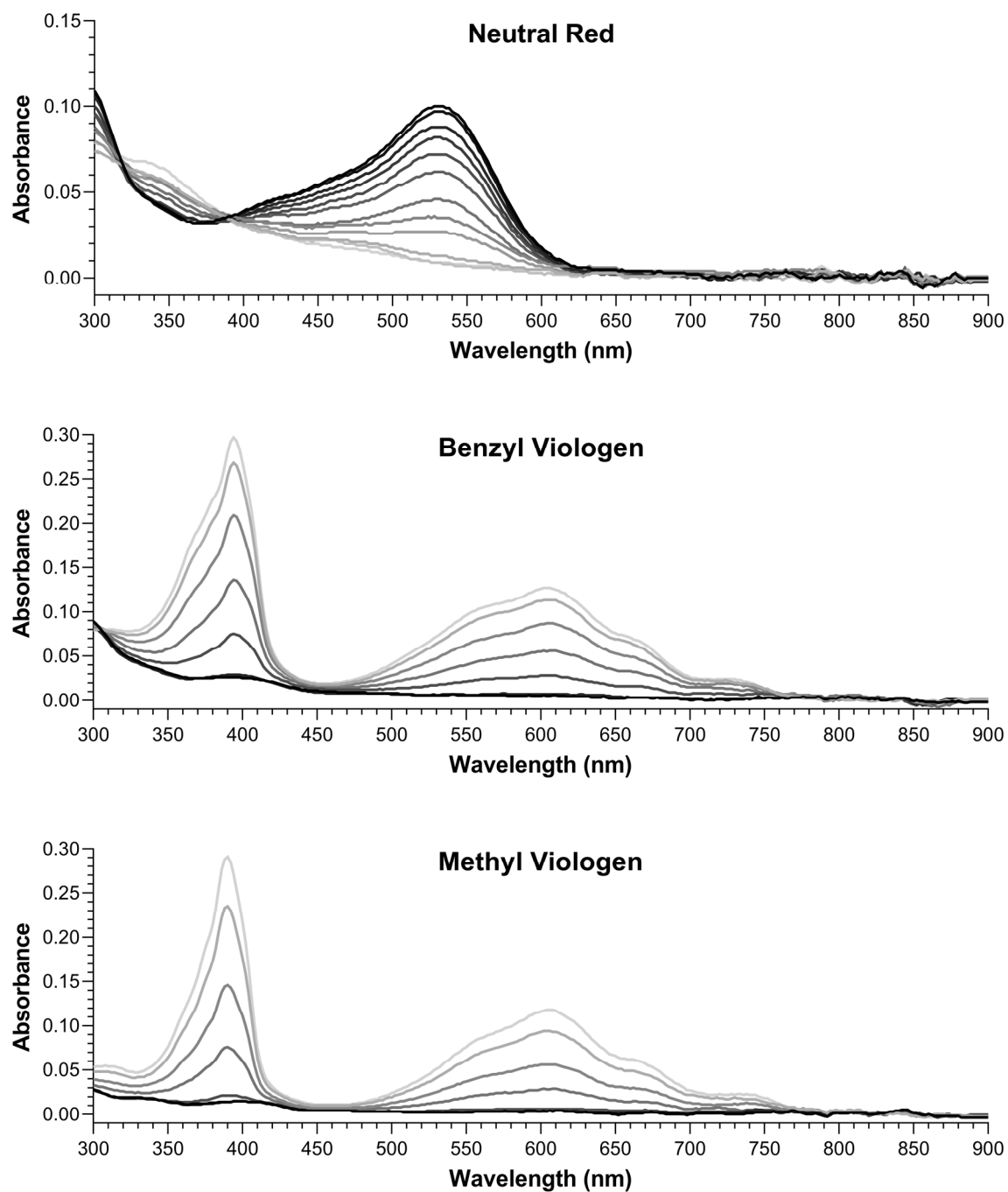
Gene	Primer ID	Primer Forward (5'-3')	Primer Reverse (5'-3')
FNR (Leaf)	L-FNR-A	TGGCACAGGTAACCACAGAAGCAC	CTCAGTACACTTCCACATTCCACTG
FNR (Leaf)	L-FNR-B	TATGGCACAGGTAACCACAGAAGCAC	GATCCTCAGTACACTTCCACATTCCACTG
FNR (GT/Root)	R-FNR-A	TGTCACTGCAACAAGCTAGC	CTTAGTAGACTTCAACATGCCA
FNR (GT/Root)	R-FNR-B	TATGTCACTGCAACAAGCTAGC	GATCCTTAGTAGACTTCAACATGCCA
Fd I (Leaf)	Fd-1-A	TGGCTAGCTACAAGGTGAAGCTG	CTCAAGCAATATCATCCTCCTTGTGCTG
Fd I (Leaf)	Fd-1-B	TATGGCTAGCTACAAGGTGAAGCTG	GATCCTCAAGCAATATCATCCTCCTTGTGCTG
Fd II (Major GT/Root)	Fd-2-A	TGGCTGTATACAAGGTGAAAC	CTCAGTAGAGATCACCTCC
Fd II (Major GT/Root)	Fd-2-B	TATGGCTGTATACAAGGTGAAAC	GATCCTCAGTAGAGATCACCTCC
Fd III (Minor GT/Root)	Fd-3-A	TGGCCACATACAAGGTGAAATTG	CTCAATACAAATCACTTTCCTTGTG
Fd III (Minor GT/Root)	Fd-3-B	TATGGCCACATACAAGGTGAAATTG	GATCCTCAATACAAATCACTTTCCTTGTG

**Table S2.** List of peppermint genes and their respective primers that were used in the qPCR analysis.

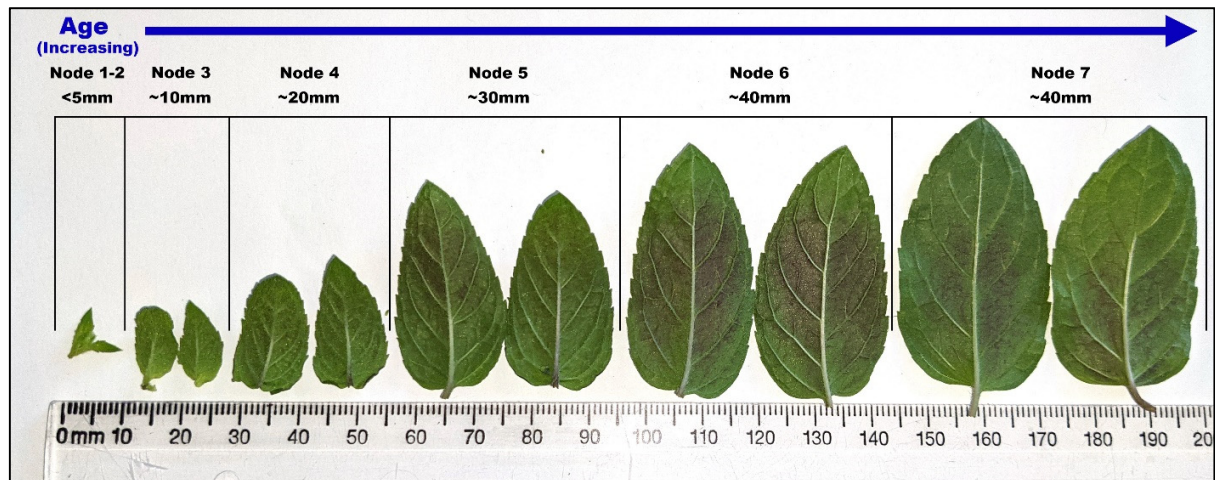
Gene	Type	Name	qPCR FWD (5'-3')	qPCR REV (5'-3')
GAPDH	Reference	Glyceraldehyde-3-Phosphate Dehydrogenase	AGGCATCCTTCGGTCTAAATG	TGTGTCGCCGTTGCTTTA
ACT	Reference	Actin	TGTCAGCAACTGGGATGATATG	CGATTGGCCTTGGGATTAAGA
CYP	Reference	Cyclophilin	CGGAAGGATCGTGATGGAG	GAAGCACGATCCCTTGTAGT
EIF3E	Reference	Eukaryotic Translation Initiation Factor 3E	TCGCACTTGAGGAGCTTAAC	CGTTGTCATGGTTGAAGAAGATG
PP2A	Reference	Serine/threonine-protein phosphatase 2A	CTGGAGGTCATGTACCAGAAAC	CCACGTAGGAGAGTAATGTTAGC
$\alpha$ -TUB	Reference	Alpha Tubulin	TGACACGTCTGTGCGAATTG	CCAGTCCTAACTTCGTCGATAAC
Fd III + PT	Experimental	Ferredoxin III (Minor) (with plastidial targeting sequence)	TCACGACTGCACCTCTGA	TGGCCATTGCAGAAGCTC
Fd II + PT	Experimental	Ferredoxin II (Major) (with plastidial targeting sequence)	CAAAAGTGCTCCTCAAAGGC	GGCAGTTGCTTTGAAATCGG
Fd I	Experimental	Ferredoxin I (Leaf)	ACAAGGTGAAGCTGGTGAC	AGGTGGAGCAGGATCCG
R-FNR	Experimental	Ferredoxin NADP-Reductase (from GTs / roots)	CACCCTTAGCAGAGAACAGAAG	CTTGAGCCACAGAAGTAGATG
L-FNR	Experimental	Ferredoxin NADP-Reductase (from leaves)	GCTCCTTTCGCTCTTTCTT	GCTCGAATTCCTCCTTGTAGAG



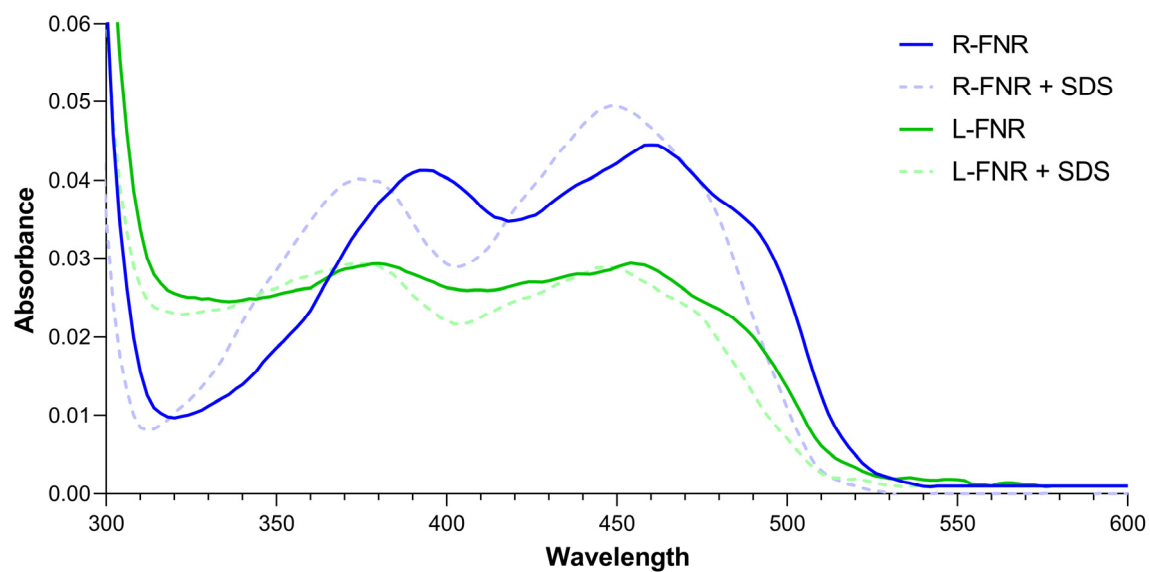
**Figure S1.** Tricine SDS-PAGE (16 %) of purified recombinant Fd and FNR isoforms from peppermint. All experimental lanes contain 20  $\mu$ g of protein as determined by the Bradford protein assay. The ladder lane (left) contains 3.5  $\mu$ l of Precision Plus Protein Standard (Cat. No. 161-0373, Bio-Rad, Hercules, CA, USA).



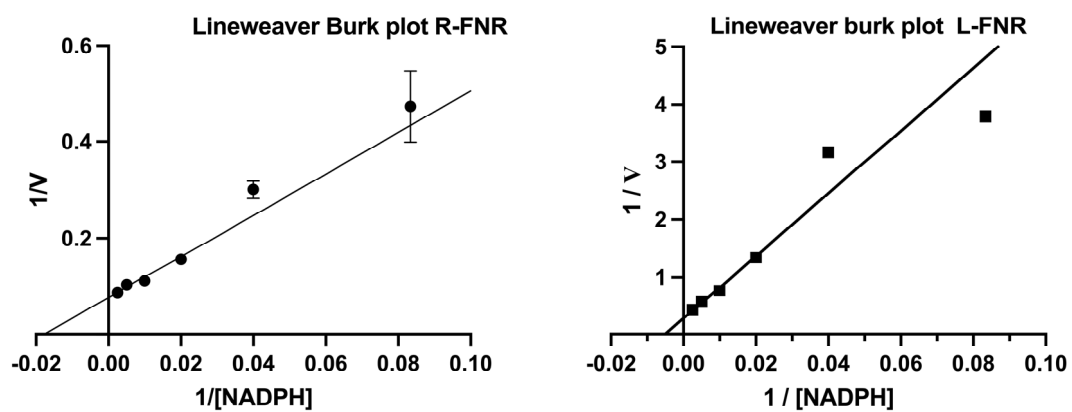
**Figure S2.** Absorbance spectrum of individual redox dyes subjected to photoreduction for determination of isosbestic points. Black lines are most oxidized and light-gray lines are most reduced. Top panel, neutral red; middle panel, benzyl viologen; bottom panel, methyl viologen.



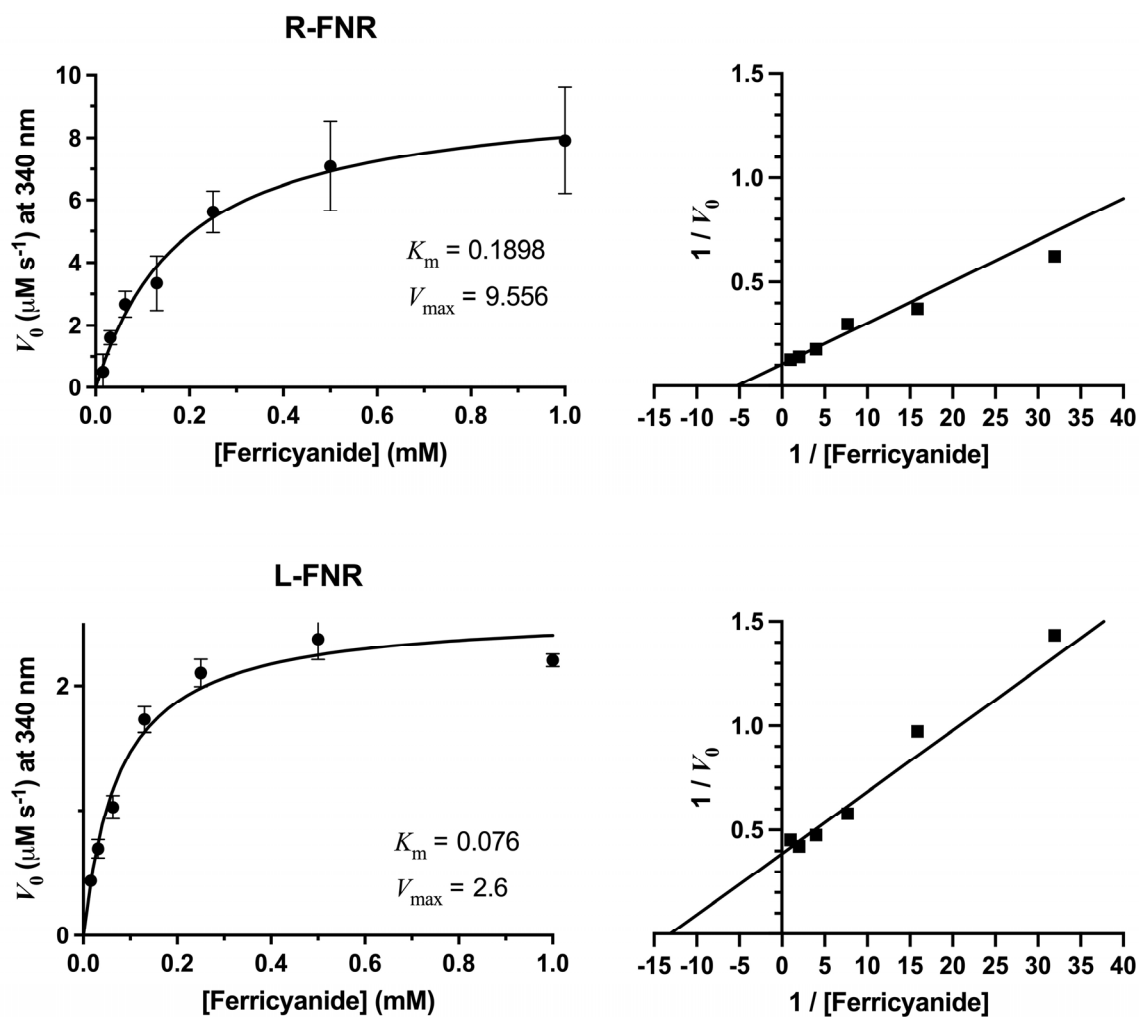
**Figure S3.** Peppermint leaves in order of increasing developmental age and their respective leaf blade lengths (measured from the tip of the leaf to point of petiole attachment). Leaves are grouped in pairs based on their stem attachment node and increasing distance from the meristem.



**Figure S4.** Characteristic absorbance spectra of purified, recombinant FNR isoforms from peppermint (diluted 1:10 from stock in 10 mM HEPES buffer at pH 7.0). Solid lines show FNR isoforms without SDS denaturation with  $\lambda_{max}$  at 392 nm and 460 nm for R-FNR (blue) and 380 nm and 456 nm for L-FNR (green). Dashed lines show FNR isoforms denatured by the addition of SDS up to 0.2%, demonstrating the characteristic absorbance spectra of the now dissociated FAD cofactor with  $\lambda_{max}$  shifted to 375 nm and 450 nm for both isoforms.



**Figure S5.** Potassium ferricyanide diaphorase Lineweaver-Burk plots for 0.05  $\mu$ M R-FNR and 0.05  $\mu$ M L-FNR from peppermint paired with variable concentrations of NADPH.

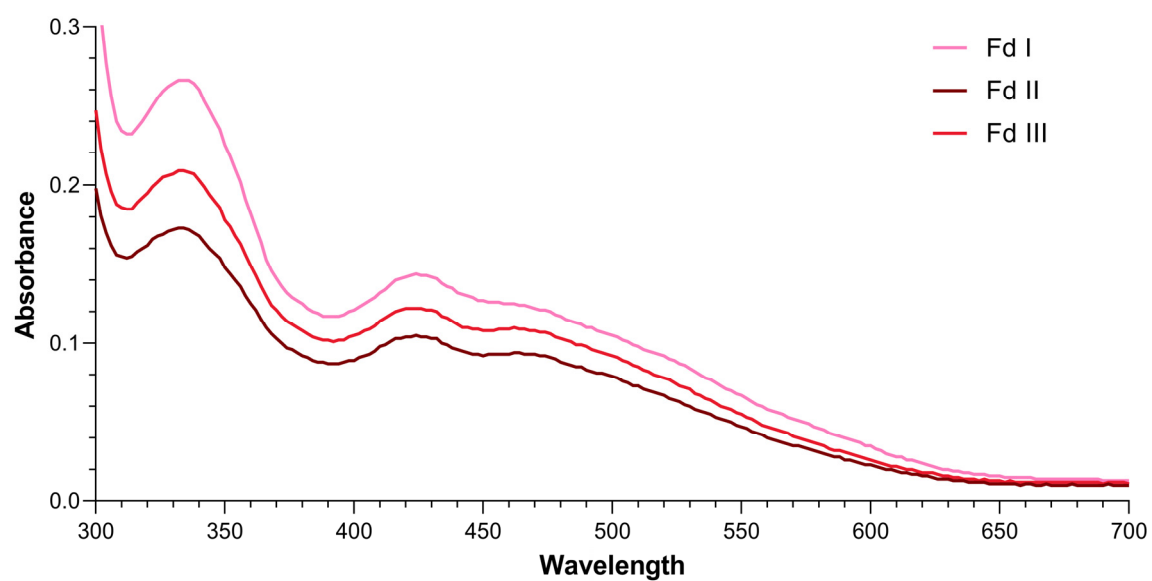


**Figure S6.** Potassium ferricyanide diaphorase Michaelis-Menten curves (left side) and Lineweaver-Burk plots (right side) for 0.05  $\mu\text{M}$  R-FNR and 0.05  $\mu\text{M}$  L-FNR from peppermint paired with variable concentrations of potassium ferricyanide.

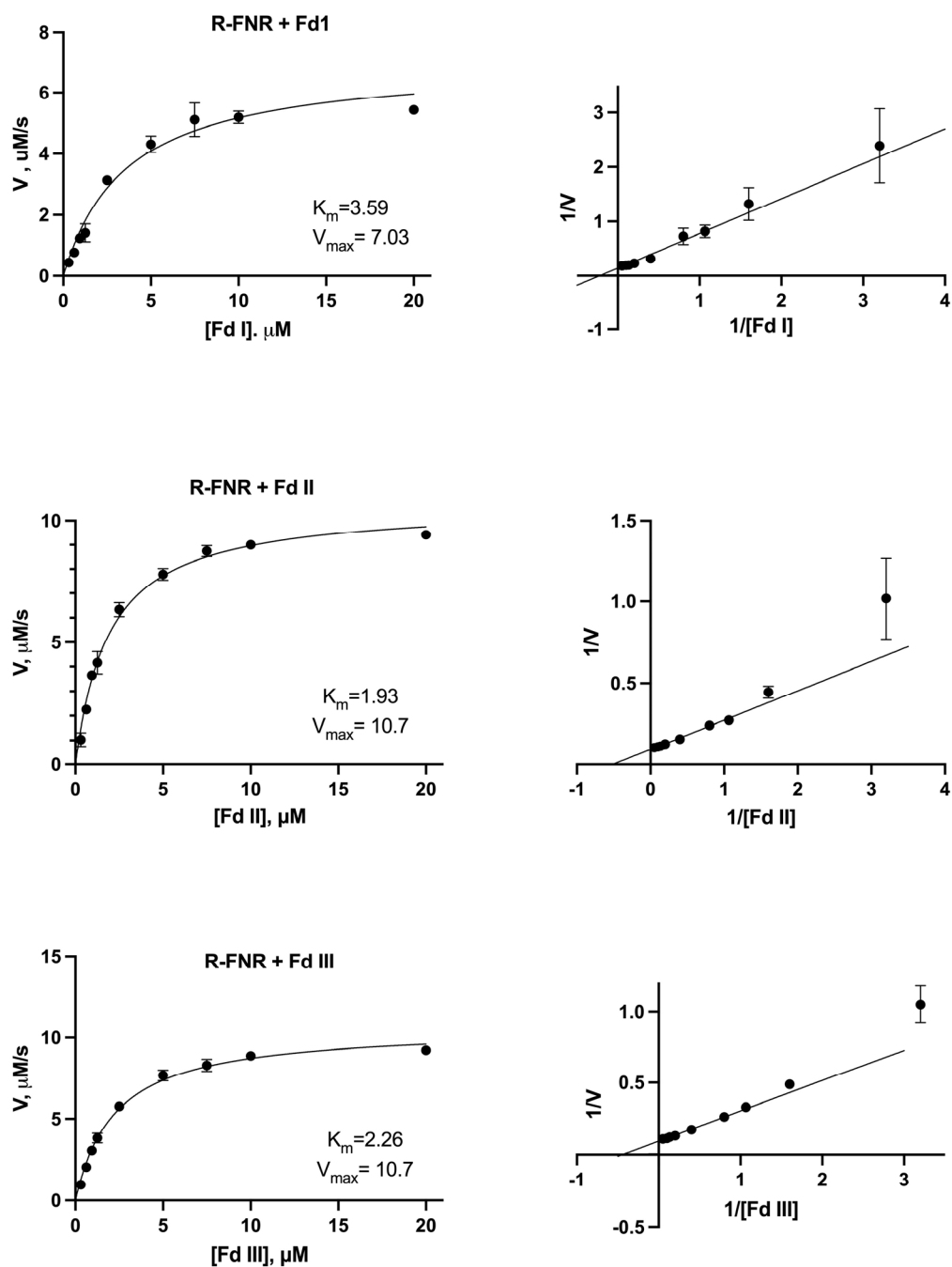
		10	20	30	40	50	60	
R-FNR	MAHSVLSQVP	VAVSVNNDVS	LRK-SVFKSN	HVSFHEKSW	SSLSMDFRIS	SFQSKGRPT-		58
L-FNR	MTAAVSAAVS	LPSSKSTSLT	ART-SIVSPE	RIHFNK----	--FPLHYRNV	AK---IVPIR		50
L-FNR-spinach	MTTAVTAAVS	FPSTKTTSLS	ARSSSVISPD	KISYKK----	--VPLLYRNV	SATGKMGPPIR		54
Clustal Consensus	*: *: *	. : . . . . .	*. * . . : : : :	. : *: :				23
		70	80	90	100	110	120	
R-FNR	--VCM SVQQA	SKPKVAVSPL	SLEDADPPL	HLFKNKEPYT	GTIVSVVERIV	GENAPGETCH		116
L-FNR	AQVT-----	TEAPAKAEKI	HKKQEEGIIV	NKFRPKPEYV	GRCLLNVRILT	GDDAPGETWH		104
L-FNR-spinach	AQIASDVEAP	PPAPAKVEKH	SKKMEEGITV	NKFKPKTPYV	GRCLLNTKIT	GDDAPGETWH	(59)	114
Clustal Consensus	:	. .	: : . : *	* * *	* : : .	* : * : *		46
		130	140	150	160	170	180	
R-FNR	IVIDHDKVP	YWEGQSYGII	PPGENPKPG	NPHNVRLYSI	ASTRYGDFFD	GKTASFVRR		176
L-FNR	MVFSTEGEVP	YREGQSIGII	PDGID--KNG	KPHKLRLYSI	ASSALGDFGD	SKTVSLCVKR		162
L-FNR-spinach	MVFSHEGEIP	YREGQSVGVI	PDGED--KNG	KPHKLRLYSI	ASSALGDFGD	AKSVSLCVKR	(117)	172
Clustal Consensus	*: . : *: *	* * * * *	* * : *	* * : * : * : *	** : * * *	* . : * : * : *		92
		190	200	210	220	230	240	
R-FNR	AVYYDPETGK	EDPSKKGVCS	NFLCDSPGD	KVQITGPSGK	IMLLPENDPN	ATHIMLATGT		236
L-FNR	LIYTND----	AGEVVKGVCS	NFLCDLKAGA	EVKITGPVGK	EMLMPK-DPN	ATIIMLGTTG		217
L-FNR-spinach	LIYTND----	AGETIKGVCS	NFLCDLPGA	EVKLTGPVGK	EMLMPK-DPN	ATIIMLGTTG	(172)	227
Clustal Consensus	* :	. * * * *	* * * * *	* : * : * * *	* : * : * * *	* * * : * * *		132
		250	260	270	280	290	300	
R-FNR	GVAPYRGYLR	RMFMENVPNF	KFGGLAWLFL	GVANKDSLLY	DEEFSNYLQE	YPDNFRFDRT		296
L-FNR	GIAPFRSFLW	KMFFEEDHEDY	KFNGLAWLFL	GVPTGSSLLY	KEEFELMKKEK	NPENFRLDFA		277
L-FNR-spinach	GIAPFRSFLW	KMFFEKHDDY	KFNGLAWLFL	GVPTSSSLLY	KEEFELMKKEK	APDNFRLDFA	(232)	287
Clustal Consensus	*: * : * : *	*: * : *	: : * * * * * *	* * . * * *	. * * * .	* : * * * : *		176
		310	320	330	340	350	360	
R-FNR	LSREQNKSG	GKMYVQDKIE	EYSDEIFKLL	DN-GAHYIFC	GLKGMPGFIQ	DTLKKVAELR		355
L-FNR	VSREQTNAKG	EKMYIQTRMA	EYAEELWEML	KKDNTFVYMC	GLKGMEKGID	DIMVSLAARD		337
L-FNR-spinach	VSREQTNEKG	EKMYIQTRMA	QYAVELWEML	KKDNTFYFMC	GLKGMEKGID	DIMVSLAAAE	(292)	347
Clustal Consensus	* * * * *	* * * : *	: : * : * : * *	: : . . . * : *	* * * * *	* * : * : *		216
		370	380					
R-FNR	GESWEEKLSQ	LKKNKQWVE	VY	377				
L-FNR	GIDWIEYKRQ	LKKAEQWNVE	VY	359				
L-FNR-spinach	GIDWIEYKRQ	LKKAEQWNVE	VY	369	(314)			
Clustal Consensus	* . * *	* * * : * : *	* * *	231				

**Figure S7.** Alignment of FNR sequences. Residue numbers in parentheses are counted after removal of the plastidial targeting sequence. Legend for consensus: "\*" means that the residues in that column are identical in all sequences in the alignment; ":" means that conserved substitutions have been observed; "." means that semi-conserved substitutions are observed. Conserved means the amino acid is replaced by one having similar characteristics.

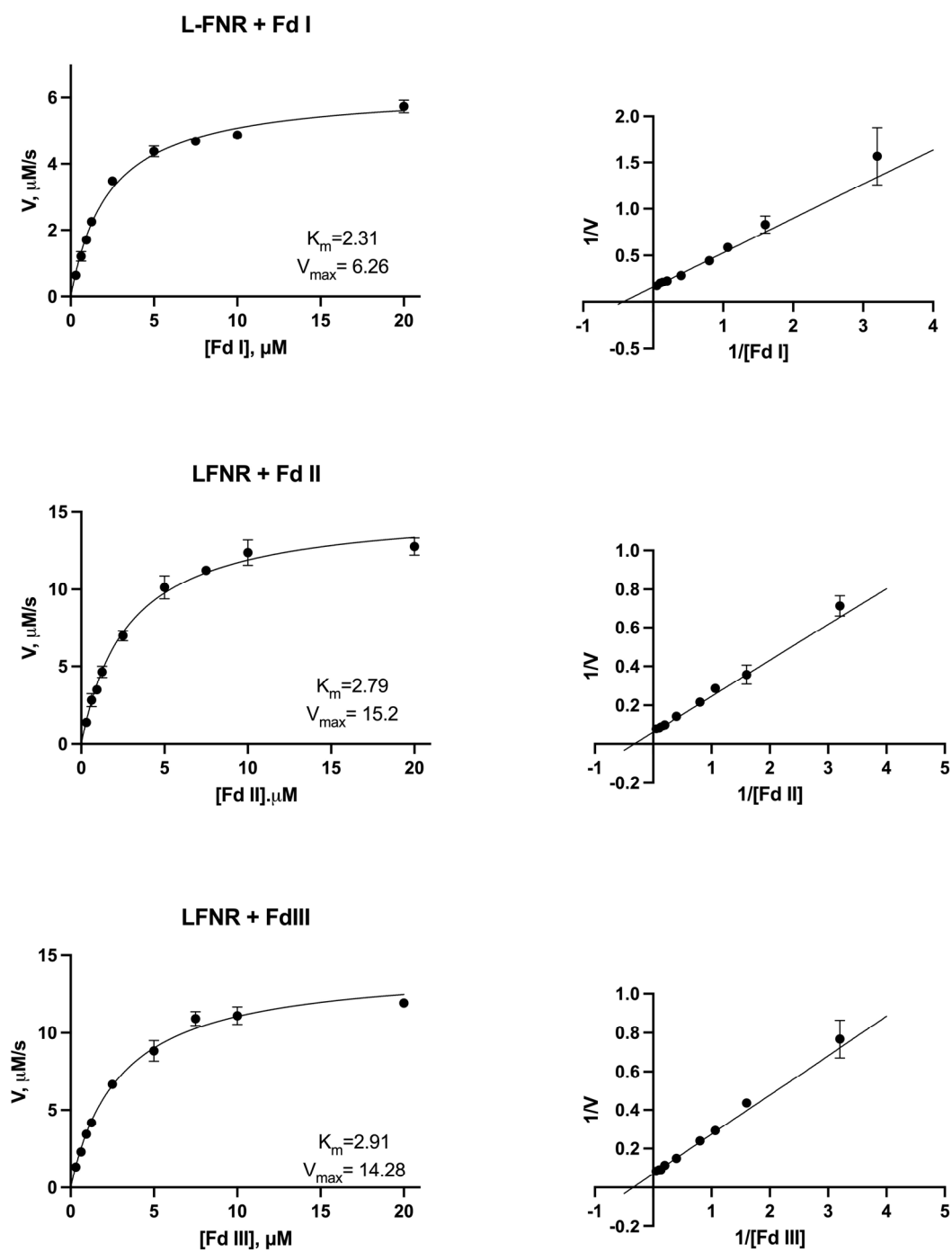




**Figure S8.** Characteristic absorbance spectra of purified recombinant Fd isoforms from peppermint (diluted 1:20 from stock in 50 mM Tris buffer at pH 7.5) with  $\lambda_{max}$  at 330 nm and 420 nm for all isoforms.



**Figure S9.** Cytochrome c reduction Michaelis-Menten curves (left side) and Lineweaver-Burk plots (right side) for 0.025  $\mu\text{M}$  R-FNR paired with variable concentrations of Fd I, Fd II, and Fd III from peppermint.



**Figure S10.** Cytochrome c reduction Michaelis-Menten curves (left side) and Lineweaver-Burk plots (right side) for 0.05  $\mu\text{M}$  L-FNR paired with variable concentrations of Fd I, Fd II, and Fd III from peppermint.

	10	20	30	40	50	60	
Maize_FdIII	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	
Maize_FdIII	MSTS----	TF ATSC	TLGNV	RTQASQTAV	KSPSSL	SFFS QVTK	---VPS LKTSKKLDVS 53
Peppermint_FdII	MAAA----	SL PTIC	MFKSAP	QRQ-TT	GAFV KIPSSL	GSVK STSR	---IFG LKAKPDFKAT 52
Peppermint_FdIII	MATA----	RL PSNC	VITTAP	LNKKTASAFT	RGSISL	GSVK SITK	---TFG LKAKLDFRAS 53
Maize_FdI	MATVLGSPRA	PA-FFFSSSS	L-----	RAAPAPTAVA	LPAAKVGIMG	RSASSRRRLR	50
Peppermint_FdI	MATLSSTM-F	NR-AFLTRKP	L-----	AGATSLRSVN	Q-AALFGL--	KSSSGGGRV	46
Clustal Consensus	*::	:		:	.	:	:: 7
	70	80	90	100	110	120	
Maize_FdIII	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	
Maize_FdIII	AMAVYKVKLV	GPEGEEHFD	APDDAYILDA	AETAGVELPY	SCRAGACSTC	AGKIESGSVD	113
Peppermint_FdII	AMAVYKVKLI	GPDGDETEFE	APDDCYILDS	AESAGVELPY	SCRAGACSTC	AGKMEKGTVD	112
Peppermint_FdIII	AMATYKVKLI	GADGEECEFE	APDDCYILDS	AETAGVELPY	SCRAGACSTC	AGKMGVGSVD	113
Maize_FdI	AQATYNVKLI	TPEGE-VELQ	VPDDVYILDQ	AEEDGIDLPI	SCRAGSCSSC	AGKVVSGSVD	109 (60)
Peppermint_FdI	CMASYKVKLL	TPEGE-VEFD	CPDDMYIVDK	AEEEGVDLPY	SCRAGSCSSC	AGKVVSGSVD	105
Clustal Consensus	. * *::***:	:*:	*::	*** **:*	** *::***	*****::**	***: .*:** 53
	130	140	150	160			
Maize_FdIII	.... ....	.... ....	.... ....	.... ....	.		
Maize_FdIII	QSDGSFLDDG	QEEGYVLTC	VSYPKSDCVI	HTHKEGDLY	-	152	
Peppermint_FdII	QSDGSFLDDK	QMEEGYLLTC	VSYPKSDCVI	HTHKEGDLY	-	151	
Peppermint_FdIII	QSDGSFLDDN	QMEQGYLLTC	VSYPKSDCVI	HTHKEGDLY	-	152	
Maize_FdI	QSDQSYLDDG	QIADGWVLTC	HAYPTSDVVI	ETHKEEELTG	A	150 (101)	
Peppermint_FdI	QSDGSFLDDE	QVAEGWVLTC	VAYPTSDVVI	ATHKEDDIA	-	144	
Clustal Consensus	*** *::***	* :*:***	:*::*	*** :		82	

**Figure S11.** Alignment of Fd sequences. Residue numbers in parentheses are counted after removal of the plastidial targeting sequence. Legend for consensus: "\*" means that the residues in that column are identical in all sequences in the alignment; ":" means that conserved substitutions have been observed; "." means that semi-conserved substitutions are observed. Conserved means the amino acid is replaced by one having similar characteristics.