Supplementary data for

Overexpression of the *Arabidopsis* NF-YA transcription factor family leads to opposing abscisic acid responses during seed germination

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AGI#	Construct	Plant line#	Homozygous or Hemizygous
At5g12840	p35S::NF-YA1-CFP/HA	1	Homozygous
		2	Homozygous
At3g05690	p35S::NF-YA2-CFP/HA	1	Homozygous
		2	Homozygous
At1g72830	p35S::NF-YA3-CFP/HA	1	Homozygous
		2	Homozygous
At2g34720	p35S::NF-YA4-CFP/HA	1	Homozygous
		2	Hemizygous
At1g54160	p35S::NF-YA5-CFP/HA	1	Hemizygous
		2	Hemizygous
At3g14020	p35S::NF-YA6-CFP/HA	1	Hemizygous
		2	Hemizygous
At1g30500	p35S::NF-YA7-CFP/HA	1	Homozygous
		2	Homozygous
At1g17590	p35S::NF-YA8-CFP/HA	1	Homozygous
		2	Homozygous
At3g20910	p35S::NF-YA9-CFP/HA	1	Homozygous
		2	Homozygous
At5g06510	p35S::NF-YA10-CFP/HA	1	Hemizygous
		2	Homozygous

Table S1 List of homozygous and hemizygous *p35S::NF-YA* plant lines.

	1 10	20	30	40	50	60	70	80	90	100	110
Consensus	MEQYTANSNSS	reqivvqagqiqq	ονχχχχχχχχχ	ххххххххх	XXXXXXXXXXX	XXXXXXXXXXX	****	XXXXXXXXXX	******	XXXXXXXXX	****
At NF-YA6			MOEFHSSK	SLPC	PATSWDN	SV-FTNSNVO	G S- S-SLTDNN	TLSLTME	MKOTGFOMOHY	DSSSTQ	STGGES-YSEVASL
At NF-YA5			MQVFQRKE	S	SWGN	SMPTTNSNIQ	G S ESFSLTKDM	IMSTTQLPA	MKHSGLQLQN	QDSTSSQ	STEEESGGGEVASF
At NF-YA8			MDKKVSFT	S S VAHSTPPY	LSTSISWG-	LPTKSN	GV-TESLSLKV	VDARPE-RI	INTKNISFQD	QDSSSTL	SS-AQS-SNDVTSS
At NF-YA3			MMHQMLNKK	SATHSTLPY	LNTSISWGV	VPTDSVANRR	GS-AESLSLK-	VDSRPG-HI	QTTKQISFQDQ	QDSSSTQ	ST-GQS-YTEVASS
At NF-YA7											MTSSIHEL
At NF-YA4											MTSSVHEL
At NF-YA9			MGIEDMHSKS	S GG -	NKVDS	EVHGTVSSSI	NSLNPWHRA	AAAC-NANS	SVEAGDKSSK	5IALALE	SNGSKSPSN
At NF-YA1			MQSKP	GRE	NEEEV	NNHHAVQQPM	MYAEPWWKNNS	FGVVPQARE	SGIPSNSSSLI	CPNGSE	SNDVHSASEDGALN
At NF-YA10						MQT EEL	LSPPQTPWWNA	FGSQPLTTE	SLSGEASDSF	GVKAVT	TEAEQG-VVDKQT-
At NF-YA2						MAMQTVREGL	FSAPQTSWWTA	FGSQPLAPE	SLAGD-SDSF1	A GVK - VG	SVGETGQRVDKQSN
HS NF-YA	MEQYTANSNSS	reqivvqagqiqq	<u>ov</u> o g oplmvovs	GGQLITSTGÇ	2PIMVQAVPG	GQGQ T IMQVP	VSGTQGLQQIQ	LVPPGQIQI	QGGQAVQVQG	QQQQTQQII	IQQPQTAVTAGQTQ
-	120 13	30 140	150	160	170	180	190	200	210	220	230
Consensus	XXXXXXXXXXXXXXXX	****	****	*********	XXXXXXXXXXX	XXXXXXXXXXX	****	XXXXXXXXXX	*****	XXXXXXXXXX	XXX L XXXXXXZXXX
At NF-YA6	SEPTNRYGHNI	VVTHLSGYKEN	PENPIGSH S I	SKVSQDS	SVV-LPIEAA	-SWPLHG	NVT P HFNGFLS	FP-YASQHI	-VQHPQIRGL-	VPS	RMPLP-HNIPENEP
At NF-YA5	GE-YKRYGCSIV	VNNNLSGYIEN	LGKPIENYTK S I	FTSSMVSQDS	SVFPAPTSGQ	ISWSLQC	AETSHFNGFLA	PE-YASTPI	ALPHLEMMGL-	VSS	RVPLP-HHIQENEP
At NF-YA8	GDDNPSRQISFI	LAH S DVCKGFEET	QRKRFAIK S G	SSTAGIAI	DIHSSPSKAN	FSFH Y	AD- P HFGGLMP	AA-YLPQAI			RVPLP-FDLIENEP
At NF-YA3	GDDNPSRQISF	SAK S GSEIT	QRKGFASNPKQG	SMT-GF P 1	NIHFAPAQAN	FSFH Y	AD-PHYGGLLA	AT-YLPQAE	P-TCNPQMVSM-	IPG	RVPLP-AELTETDP
At NF-YA7	SDNIGSHEKQE	2RD S HFQ	PPIPSARNYE S I	VTSLVYSDPO	GTTNSMAPGQ	YPY	PD- P Y Y RSIFA	P P P Q P Y	TGVHLQLMGV-	QQQ	GVP L P-SDAVE-EP
At NF-YA4	SDNNESHAKKEI	RPD S QTR	PQVPSGRSSE S I	OTNSVYSE P -	MAHGL	YP Y	PD- P Y Y RSVFA	QQAYLPHPY	PGVQLQLMGM-	QQP	GVPLQ-CDAVE-EP
At NF-YA9	RDNT-VNKESQV	/TT S PQSA	GDYS-DKNQE S L	H - HGIT - QPB	PPHP-QLVGH	TVGWASSNPY	QD - PYYAGVMG	AYGHHE	P-LGFVPYGGM-	PHS	RMPUP-PEMAQ-EP
At NF-YA1	GENDGTWKDSQA	AATSSRSV	DNHGMEGNDPAL	SIRNMHDQPI	JVQPPELVGH	YIACVP-NP¥	QD-PYYGGLMG	AYGHQQ)-LGFRPYLGM-	PRE	RTALP-LDMAQ-EP
At NF-YAIU	STTLFTFSPGGI	KSSRDVP	KPH-VAFAMQ S A	JFEFGFAQ P -	MMYTK	HPH	VEQ-YYG-VVS	AYGSQF	CSSGRVM		- IPLK-METEBDGT
	SATHLAFSLGD	A DECOUTIVE	APHGATESMUSP ODVNADCETIOO	UTUDUCCMT			GEQQI X G-VVS	AIGSQ- TIDVACNVI			-LPLN-MET-PDST
HS NF-TA	TQQQTAVQGQQ	250 260 260		VIVPVSGMII		QIVQIGANIN 200	1155GQG1V1V 210	ILPVAGNVV		AGSVFALQ	KINDPGALMINE
Conconsus			vvvvvvvvvvvvv			CCDEVVVVV	. A A A A A A A A A A A A A A A A A A A	520 I VVVVVVVVV		1 1 7 7 7 7 7 7 7 7 7 7 7	
Consensus						GGREAAAAAA					
At NF-YA6	IFVNAKQYQAII	LRRRERRAKLEA-	QNKLI-KVRK	PYLHESRHI	HALKRVRGS	GGRFLN TKK-	-HQESNSS	LSPPFLIPE	PHVFKNSP-GKI	RQMDISRG	GVVSSVSTTSCSDI
	I FVNAKQYHALI WRVNAKORHALI		QNKLI-KCRK	- PYLHESRHI	JHALKRARGS	GGRFLNTKK-	-LQESSNS	LCSSQMANG	GONFSMSPHG	-GGSGIG	SSSISPSSNS
AL NE VA2	VFVNAKQFHALI	ARREQURARLEA-	ONKLI-KARK		HALKRPRGS	GGRFLNTKK-	-LQEST	DPRQDMPIQ	QQUATGNMERI	VLIQLQ-N	NKD BEEMMECHDI
AL NE-VA7	VEVNAKOVHCTI	PPPOSPAPIES_	ONKUT-KARK			CCRELNAKK		RAEDGG	QVNKKINHSKI	SACKSA	
At NE-YA4	VEVNAKOVHGTI	RRROSRAKLEA-			HATRRPREC	GGRFLNAKK -	ENGD	HKEEEEATS	DENTSEASSSI	RSEKLA	MATSGPNGRS
At NF-YA9	VEVNAKOYOATI	RRRDARAKAEL	EKKLT-KSRK	PYTHESRH		GGRFAKKTN-	-TEASKRKAEE	KSNGHVTOS	PSSSNSDOGE	WNGDYR	TPOGDEMOSSA
At NF-YA1	VYVNAKOYEGII	RRRKARAKAEL -		PYTHESRH	HAMRRARAS	GGRFAKKSE-	-VEAGE	DAGGR	DRERGS	TN	SSGSE-0
At NF-YA10	IYVNSKQYHGI	RRRDSRAKA	EKLS-RCRK	PYMHHSRH	HAMRRPRGS	GGRFLNTK T-	ADA	AK	QSKPSNS	SQSSEVF	HPENETINSSR
At NF-YA2	IYVNSKQYHGI	RRRDSRAKAAAV	LDQKKLSSRCRK	PYMHHSRHI	HALRRPRGS	GGRFLNTKSQ	NLENSGTNA	KKGDGSMQI	QSQPKPQQSNS	QNSEVV	HPENGTMNLS-
Hs NF-YA	LY VNAKQ YNR I	LKRRQARAKLEA-	EGKIP-KERR	- K <mark>YLHESRH</mark> F	RHAMARKRGE	GGRF FSPKE-	KD S P	HMQDPN	QADEEAMTQI	RVS	
	360	370	380 390	40	0 405						
Consensus	**********	XXXXXXXXXXXXXXX	xxxxxxxxxxx	ХХХХХХВХХХ	XXXXX						
At NF-YA6	T = -GNNNDMFOO	ONPOFR FS GY P SN	HHVSVLM								
At NF-YA5	NCINMFO	NPOFRFSGYPST	HHASALMSGT								
At NF-YA8	TSASDSVNLFG	-HSEFLISDCPSO	TNPTMYVHGOSN	OMHGGRNTHE	IFSVH I						
At NF-YA3	TSVSDGADIFG-	-HTEFQ FS GF P TP	INRAMLVH G QS N	OMHGGG D MHH	HFSVHI						
At NF-YA7											
At NF-YA4											
At NF-YA9	YKRREEGECSG	QQWNSLS S NH P SQ	ARLAIK								
At NF-YA1	VETD-SN1	ETLNS S GAP		_	_						
At NF-YA10	EANESNLSDSAV	/TSMDYFLSSSAY	SPGGMV M PIKW N	AAAM D IGC	CCKLNI						
At NF-YA2	NGLNVSGSEV	/TSMNYFLSSPVH	SLGGMV M PSKWI.	AAAAAM D NGC	CCNFKT						
hs NF-YA											

Fig. S1 Multiple sequence alignment of full-length NF-YA protein sequences. At, *Arabidopsis thaliana*; Hs, *Homo sapiens*. The conserved core domain is underlined in black. The three black boxes correspond to nuclear localization signal in human.

	AtNF-YA2	AtNF-YA10	AtNF-YA1	AtNF-YA9	AtNF-YA5	AtNF-YA6	AtNF-YA3	AtNF-YA8	AtNF-YA4	AtNF-YA7	HsNF-YA
AtNF-YA2	$>\!$	63.2%	23.0%	24.0%	22.3%	21.4%	21 .6%	1 9.4%	27.7%	26.5%	1 9.6%
AtNF-YA10	63.2%	$>\!$	21.5%	20.9%	18.8%	18.5%	20.6%	18.5%	27.5%	27.0%	20.2%
AtNF-YA1	23.0%	21.5%	$>\!$	41.6%	22.2%	18.8%	22.4%	20.0%	26.8%	28.6%	20.4%
AtNF-YA9	24.0%	20.9%	41.6%	$>\!$	22.8%	25.0%	23.1%	21.8%	29.8%	31.0%	20.3%
AtNF-YA5	22.3%	18.8%	22.2%	22.8%	$>\!$	56.3%	34.4%	33.7%	30.5%	32.1%	21.3%
AtNF-YA6	21.4%	18.5%	18.8%	25.0%	56.3%	$>\!$	35.9%	33.7%	30.1%	30.6%	18.6%
AtNF-YA3	21.6%	20.6%	22.4%	23.1%	34.4%	35.9%	$>\!$	63.9%	33.3%	33.0%	21.3%
AtNF-YA8	19.4%	18.5%	20.0%	21.8%	33.7%	33.7%	63.9%	$>\!$	32.3%	31.5%	1 9.7%
AtNF-YA4	27.7%	27.5%	26.8%	29.8%	30.5%	30.1%	33.3%	32.3%	$>\!$	64.5%	23.0%
AtNF-YA7	26.5%	27.0%	28.6%	31.0%	32.1%	30.6%	33.0%	31.5%	64.5%	$>\!$	25.7%
HsNF-YA	19.6%	20.2%	20.4%	20.3%	21.3%	18.6%	21.3%	19.7%	23.0%	25.7%	$>\!$

b

	AtNF-YA1	AtNF-YA9	AtNF-YA4	AtNF-YA7	AtNF-YA3	AtNF-YA8	AtNF-YA5	AtNF-YA6	AtNF-YA2	AtNF-YA10	HsNF-YA
AtNF-YA1	$>\!$	78.9%	64.9%	70.2%	66.7%	64.9%	70.2%	66.7%	57.4%	63.2%	64.9%
AtNF-YA9	78.9%	$>\!$	71.9%	77.2%	73.7%	75.4%	73.7%	75.4%	59.0%	63.2%	68.4%
AtNF-YA4	64.9%	71.9%	\geq	86.0%	74.1%	75.9%	74.1%	72.4%	61.3%	63.8%	63.8%
AtNF-YA7	70.2%	77.2%	86.0%	\geq	75.9%	77.6%	77.6%	75.9%	61.3%	63.8%	65.5%
AtNF-YA3	66.7%	73.7%	74.1%	75.9%	\geq	93.1%	82.8%	81.0%	64.5%	63.8%	63.8%
AtNF-YA8	64.9%	75.4%	75.9%	77.6%	93.1%	$>\!$	86.2%	84.5%	61.3%	60.3%	65.5%
AtNF-YA5	70.2%	73.7%	74.1%	77.6%	82.8%	86.2%	\geq	91.4%	64.5%	63.8%	67.2%
AtNF-YA6	66.7%	75.4%	72.4%	75.9%	81.0%	84.5%	91.4%	\geq	61.3%	60.3%	65.5%
AtNF-YA2	57.4%	59.0%	61.3%	61.3%	64.5%	61.3%	64.5%	61.3%	\geq	83.9%	51.6%
AtNF-YA10	63.2%	63.2%	63.8%	63.8%	63.8%	60.3%	63.8%	60.3%	83.9%	$>\!$	55.2%
HsNF-YA	64.9%	68.4%	63.8%	65.5%	63.8%	65.5%	67.2%	65.5%	51.6%	55.2%	$>\!\!\!\!>\!\!\!\!>\!\!\!\!<$

Fig. S2 Identity matrix of the NF-YA proteins. a) Full-length protein. b) Conserved domain. At, *Arabidopsis thaliana*; Hs, *Homo sapiens*. Note that pairs of Arabidopsis NF-YA paralogs demonstrate high identity across the full-length protein sequence and the conserved domain.

а



Fig. S3 Overexpression of the *NF-YA* gene family leads to growth defects. Quantitative measurements of (a) plant height (b) rosette width (c) number of siliques on primary bolt (d) length of siliques.



Fig. S4 Quantifications of the cotyledon-like leaves in *p35S::NF-YA5*. (a) Leaf area (b) number of trichomes (c) chlorophyll content. Asterisks represent significant differences derived from Student's t-tests.



Fig S5. Abiotic stress responses of *p35S::NF-YA5* seedlings. Response to a) 6% mannitol b) 150 mM NaCl. Asterisks represent significant differences derived from Student's t-tests.







Fig. S6 Confirmation of nuclear localization of NF-YA proteins. (a) Hoechst 33342 (blue) labels nuclei and CFP signal (green) shows localization of NF-YA7. Merged images demonstrate co-localization of DNA label and CFP fluorescence. Scale - 15 μ m. (b) Differential Interference Contrast (DIC) imaging (grey) of root cell nuclei (red arrows point to the nuclei) with CFP signal (cyan) showing localization of NF-YA7. Merged images indicate CFP fluorescence is localized to the root cell nuclei. Scale - 5 μ m.



Fig. S7 Overexpression of select *NF-YA* genes leads to ABA hypersensitivity during root elongation. The percent root elongation in growth medium with ABA was compared to the unsupplemented medium for Col-0, *p35S::NF-YA7*, *p35S::NF-YA8*, and *p35S::NF-YA9*. Asterisks represent significant differences derived from one-way ANOVA (P < 0.05) followed by Dunnett's multiple comparison post hoc test against Col-0



Fig. S8 Publically available microarray data demonstrate that *NF-YA* genes are expressed in seeds. Data was obtained from the Arabidopsis e-FP browser at http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi.



Fig. S9 qPCR analysis of gene expression in *p35S::NF-YA8* and *p35S::NF-YA9* seeds. (A) *NF-YA8* (B) *NF-YA9*. Asterisks represent significant differences derived from twoway ANOVA (p<0.05), in which genotype and seed growth media are the two variables, followed by Bonferroni multiple comparisons post hoc test against Col-0 on B5 media or on B5 + 1 μ M ABA.



Fig. S10 ABA-induction of gene expression in *p35S::NF-YA8* and *p35S::NF-YA9* seeds. Expression of (A) *ABF3* (B) *AIA* (C) *HAB1*. Asterisks represent significant differences derived from two-way ANOVA (p<0.05), in which genotype and seed growth media are the two variables, followed by Bonferroni multiple comparisons post hoc test against Col-0 on B5 media or B5 + 1 μ M ABA.