

Journal of Experimental Botany, Involvement of microRNA-related regulatory pathways in the glucose-mediated control of Arabidopsis early seedling development.

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Supplementary Table S1.

Target	AGI	Primer Sequences		Reference
		Forward (5' - 3')	Reverse (5' - 3')	
<i>PAP1/AtMYB75*</i>	AT1G56650	GGTTGAACTATTTGAAGCCA	GCAATTAAGACCACCTATTC	Luo <i>et al.</i> , 2011
<i>TAS4</i>	AT3G25795	CGACCTCGATCCTTACCT	ATTTTCTAGACCTGCATTGTTAT	
<i>AT2G28390</i>	AT2G28390	AACTCTATGCAGCATTGATCCACT	TGATTGCATATCTTTATCGCCATC	
<i>AT5G15710</i>	AT5G15710	AAGTTCGCCAGCAAACATGTC	GCTGACCAGGTCTTCATTAACACTG	
<i>PP2A/PDF2</i>	At1g13320	TAGATCGCTCGGAACCTTGAAA	CCTCACAAAACCTCAAATCACTCC	
<i>Pri-miR156d</i>	AT5G10945	CAGAAGAGAGTGAGCACAAAAGGG	GTGAGCACGAAAAGCAACCATATAC	
<i>Pri-miR156f</i>	AT5G26147	TGGTGAGGAATTGATGGTGACA	CCTTCAAATATGCAAGAAAGCCAC	
<i>Pri-miR159b</i>	AT1G18075	GGAGGGTTTAGCAGGGTGAAGTAAAG	CCAAAGAAGAGTGAAGCCATTAAGGG	
<i>Pri-miR166c</i>	AT5G08712	AGTGTGAGAGGATTGTTGTCTGGC	GAATGAAGCCTGGTCCGAGAATCATC	
<i>Pri-miR169a</i>	AT3G13405	AAAGTAACATGATCGCAAGTTGTCC	GCGACACAAAGTAACGTGTAGCC	Pant <i>et al.</i> , 2009
<i>Pri-miR390a</i>	AT2G38325	TAGCGCATGATGATCACATTC	AATGAAACTCAGGATGGATAGCG	
<i>Pri-miR399c</i>	AT5G62162	CATCTTTCTATTGGCAGGCGACTTGG	AAGCAGTGACAGGGCAACTCTCC	
<i>Pri-miR773</i>	AT1G35501	AGGAGGCAATAGCTTGAGCAAA	AGGTGACAGCTTTAGTCGATGGA	
<i>Pri-miR775</i>	AT1G78206	CATTGAAACTGTCTTTCAACATCCCA	TGGCACTGCTAGACATCGAAAAT	
<i>Pri-miR823</i>	AT3G13724	CCATTTAGTTCTAGTGGTGGTGAT	GATATGTTTCACTGTTACCATTACCAATCT	
<i>Pri-miR827</i>	AT3G59884	TCCTTGTGTTGATCGATTGGTTTA	CGATGCAAAACCACGAAAGAG	
<i>Pri-miR828</i>	AT4G27765	AAATGATTCACTCACTCGTAT	GATATTAATAGTCCCACTTCC	
<i>UBQ10</i>	AT4G05320	GGCCTTGATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGAAACATAGT	
<i>ABI3*</i>	AT3G24650	GCAGTGCCGCCCTCAATTAC	TTCTGGTTTCCATCCCTGCC	
<i>ABI4</i>	AT2G40220	GAGGTGGCGTTAGGGCAGG	GGTGGATGAGTTATTGATAGAC	
<i>ABI5*</i>	AT2G36270	GCAAGAAAACAAGCATATACAG	TTCTCTTCTCTCCAACCTC	
<i>ARF3*</i>	AT2G33860	CTGTCTCTGAGGGGATTCTG	GGCTCCACCATCCGAACAAG	
<i>AT1G53290*</i>	AT1G53290	TATCGAAGAGGAGTACAGTAAG	TAGCAGAGAGAGTCGATCTG	
<i>CMT3*</i>	AT1G69770	TCAGTTTCAATCAAAGTCC	AATTCGCTCCCTTTCTCTTGG	This Work
<i>MET2/DMT2*</i>	AT4G14140	AGGTTTACGCTATGATGCTGG	GTAGTCCATACTCTTTGTTTAT	
<i>MYB33*</i>	AT5G06100	GCACGTATGGCTGCACATTTG	CACTCAAGTGCCTCAACATGC	
<i>NF-YA5*</i>	AT1G54160	AATGCCGTAACCGTACCTTC	GCTTCTTTGTATTGAGGAAACG	
<i>PHV/ATHB9*</i>	AT1G30490	AACATGAAGAGTTTCTCGAAG	AGCACAACTCCAACCACATT	
<i>SPL13A*</i>	AT5G50570	ACAATGCAGCAGTTTCATG	GACGACCGATATGTTACAGGC	
<i>TAS3</i>	AT3G17185	AAACATAACCTCCGTGATGC	GCTCAGATAGGATAACACCG	

Note. Primer pairs spanning exon-exon junctions are marked with an asterisk.

Supplementary Table S1. Continued.

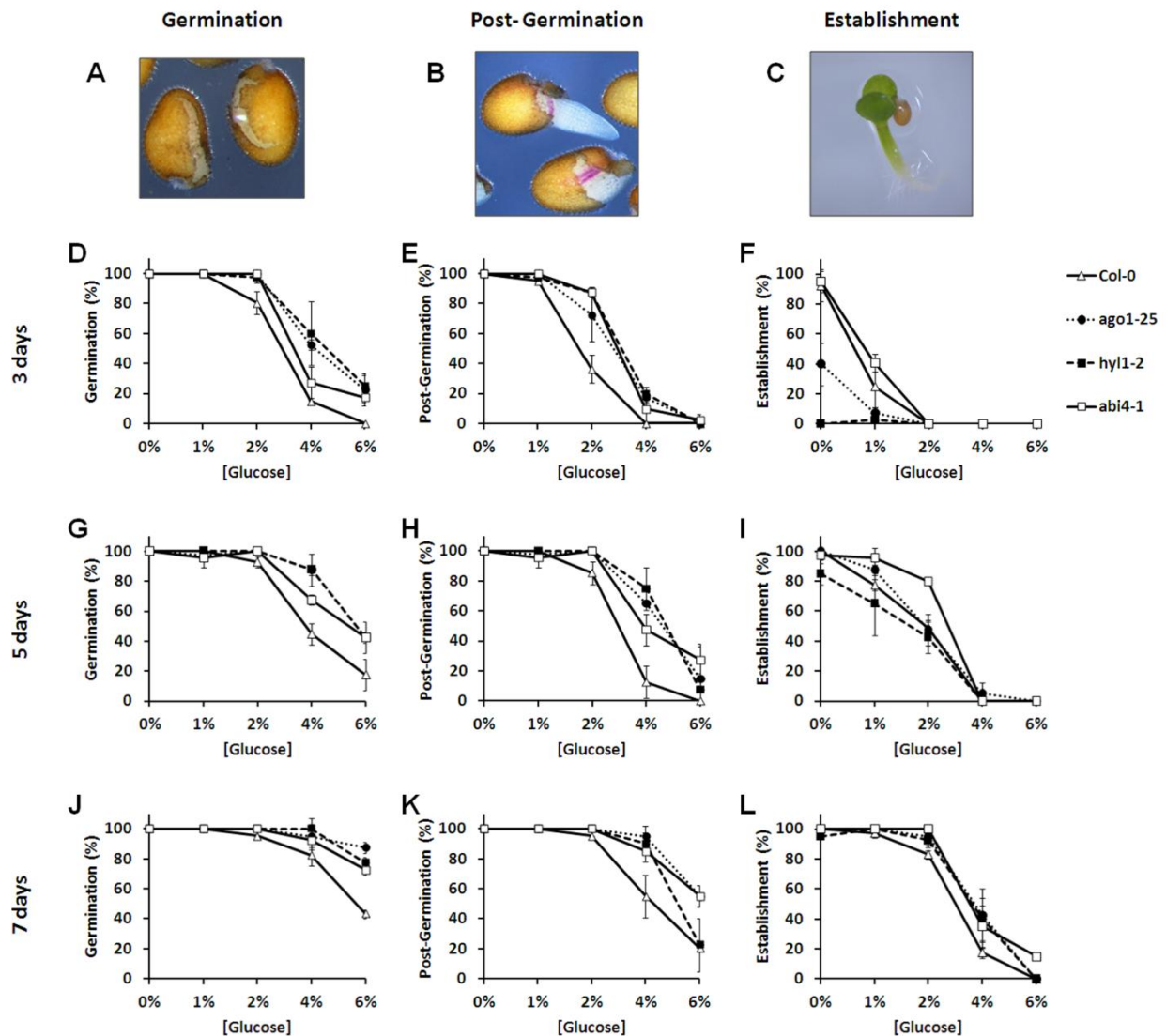
miRNA Primer Sequences			
miRNA	Amplification Forward (5' - 3')	Reverse Transcription Primer (5' - 3')	Reference
miR159	CGGCGGTTTGGATTGAAGGGA	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAGAGC	This Work
miR166	CTCGCTTCGACCAGGCTTCA	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGGGAA	
miR169	CGTGAGCAGCCAAGGATGACT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCGGCA	
miR390	GGACGGAAGCTCAGGAGGGAT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGCGCT	
miR773	CGATGCGTTTGCTTCCAGCTTT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAGACA	
miR775	GCGGCGGTTTCGATGTCTAGCA	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGGCAC	
miR823	GGACGGTGGGTGGTGATCATA	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATCTTA	
miR828	CGGCGGTCTTGCTTAAATGAGT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGGAAT	
miR156	GCGGCGGTGACAGAAGAGAGT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTGCTC	
miRuniversal-R*	-	GTGCAGGGTCCGAGGT	

Note. The miRuniversal is the amplification reverse primer for all miRNAs.

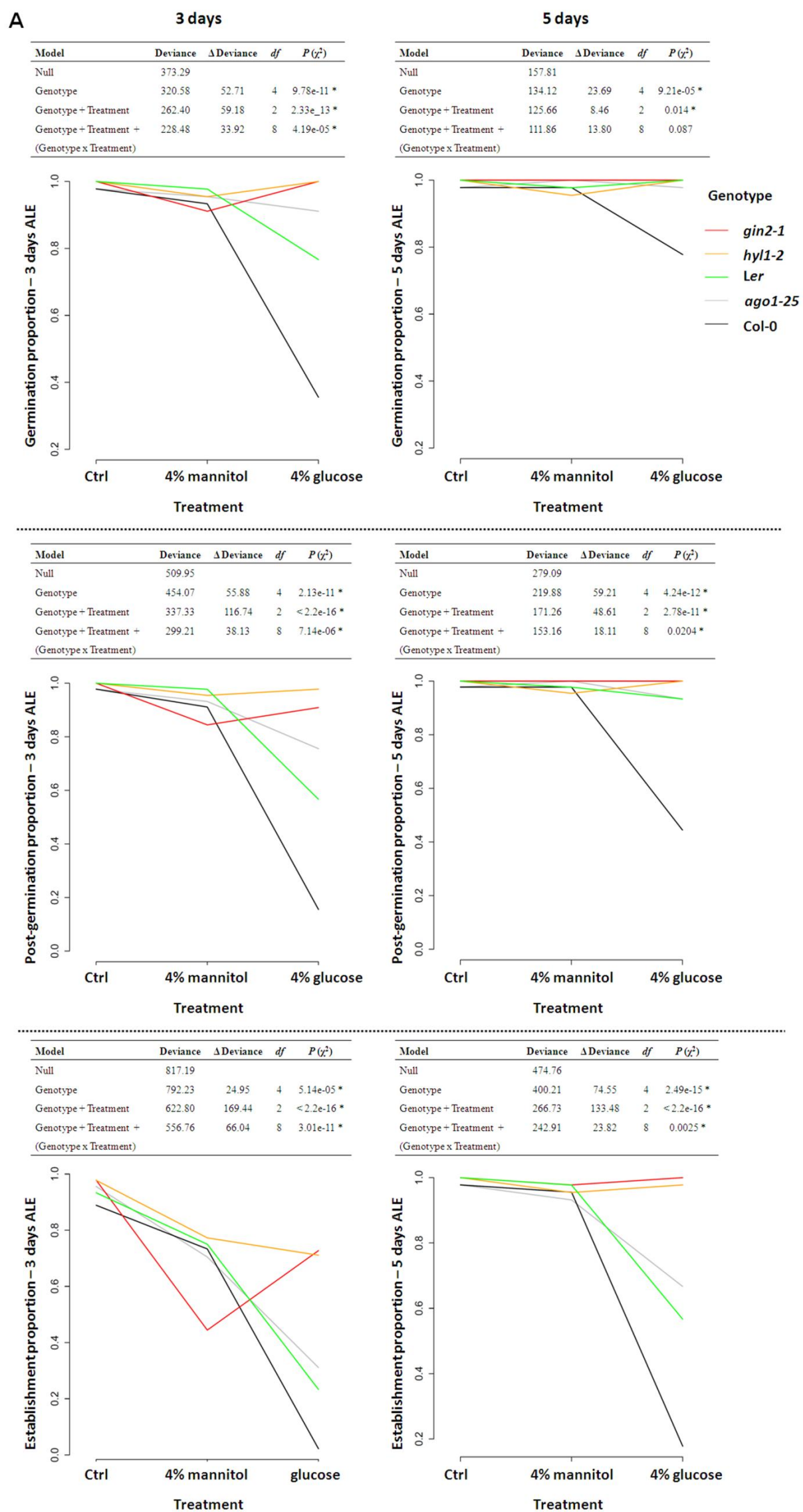
Supplementary Table S2. Glucose-regulated pri-miRs and corresponding miRNAs targets.

miRNA Family	4% Glucose-responsive pri-miRNAs	4% Mannitol fold 4% Glucose fold	Target Family	Target Genes
156/157	<u>156d</u> <u>156f</u> 157a 157c	1.24 -1.67 -1.02 -7.62 1.24 -2.83 1.95 -2.50	SPL	AT1G27360, AT1G27370, AT1G53160, AT1G69170, AT2G33810*, AT2G42200, AT3G15270*, AT3G57920, AT5G43270, AT5G50570, AT5G50670
158	158b	1.34 3.42	PPR	-
159	159b	3.24 6.70	MYB	AT2G26950, AT2G32460, AT4G26930, AT5G55020
161	161a	1.89 4.90	PPR	AT1G06580, AT1G62590, AT1G62670, AT1G62860, AT1G62910, AT1G62930, AT1G63070, AT1G63080, AT1G63130, AT1G63150, AT1G63230, AT1G63330, AT1G63400, AT1G64580, AT2G41720, AT3G16710, AT4G26800, AT5G16640, AT5G41170, AT5G65560
163	163a	1.86 -1.87	SAMT	AT1G66690, AT1G66700, AT1G66720, AT3G44860, AT3G44870
164	164a	1.15 -2.59	NAC	AT1G56010, AT3G12977, AT3G15170, AT5G07680, AT5G53950, AT5G61430
165/166	165a 165b 166c	1.17 3.68 1.63 3.36 3.55 -1.61	HD-ZIP III	AT1G30490, AT1G52150, AT2G34710, AT4G32880, AT5G60690
167	<u>167d</u>	2.72 -3.38	ARF	-
169	169a	1.90 4.37	HAP2	AT1G17590, AT1G54160, AT1G72830, AT3G20910, AT5G12840
173	173a	1.64 5.61	TAS1,TAS2	AT1G50055, AT2G27400, AT2G39675, AT2G39681
390	390a	1.29 9.88	TAS3	AT3G17185
394	394a	-1.06 5.51	F-Box	AT1G27340
395	395a	-1.21 4.67	APS,AST	AT3G22890, AT4G14680, AT5G10180, AT5G43780
399	399a 399c	2.45 5.03 -17.71 8.00	E2-UBC	AT2G33770
402	402	1.10 2.52	ROS1-Like	AT4G34060
413	413	1.55 4.96	-	-
773	773	4.72 17.45	MET2	AT4G14140
775	775	4.00 22.28	GT	AT1G53290
777	777	1.23 3.25	CIP4.1-like	AT1G30060
779	779	2.84 7.39	-	AT5G53890
823	823	4.40 17.06	CMT3	AT1G69770
825	<u>825</u>	1.22 -3.61	-	-
827	827	-1.53 3.39	SPX	AT1G02860
828	828	1.41 3.93	MYB	AT1G66370
829	829	2.85 10.91	-	-
850	850	-1.31 -3.90	-	-
856	856	6.67 13.39	CHX	AT5G41610
861	861	1.43 -2.31	-	-
863	863	-1.12 -3.39	-	-
865	865	-1.08 2.03	-	-
3932	<u>3932b</u>	-1.09 -9.87	-	-
4221	4221	2.35 7.06	-	-

Note. Red denotes induction by glucose after three days of light exposure; green represents repression. Mannitol and glucose fold values are in comparison to the pri-miR expression in non-treated Col-0 seedlings. Potential HXK1-dependent pri-miRs (Figure 2) are underlined. SPL genes marked with an asterisk are exclusive targets of miR156. Target gene families are based on miRBase (Kozomara and Griffiths-Jones, 2011) and TarBase (Vergoulis *et al.*, 2011) databases.

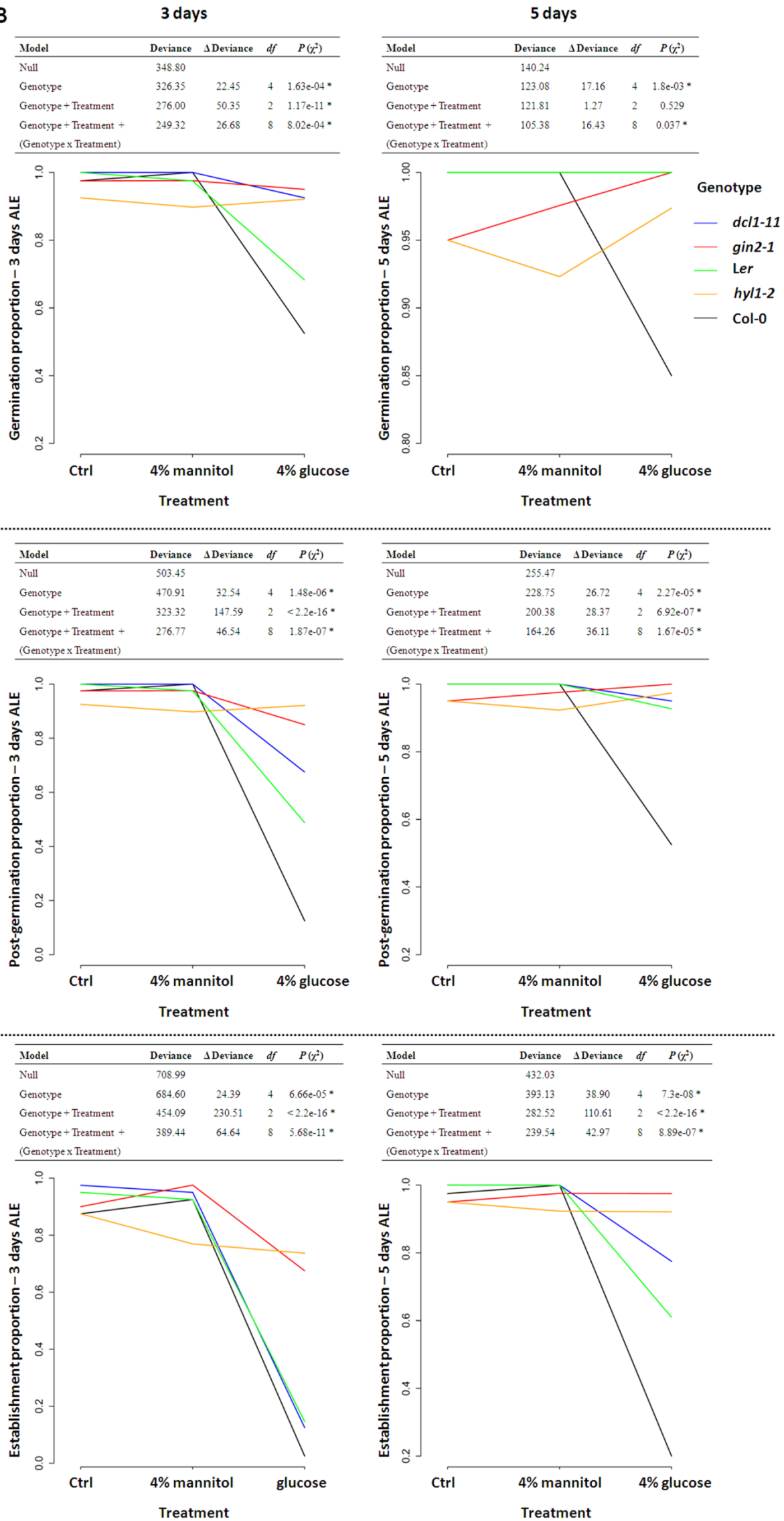


Supplementary Fig. S1. Effects of different concentrations of glucose on early seedling development of wild-type Col-0, miRNA-deficient mutants *ago1-25* and *hyl1-2*, and glucose-insensitive mutant *abi4-1/gin6*. (A) Germination (*testa* rupture), (B) post-germination (radicle emergence and elongation), and (C) establishment (cotyledons expansion and greening) were scored three days (D-F), five days (G-I) and seven days (J-L) after light exposure. Seeds of each genotype were sown in MS/2 plates supplied with the indicated sugar concentration, kept for two days at 4°C in the dark for stratification and transferred to continuous light ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 24°C. Results presented are the means \pm SD of two independent experiments each of which including 20 seeds.



Supplementary Fig. S2. Analyses of deviance and interaction plots used to compare the germination and development efficiencies of each genotype grown in the following conditions: **(A)** Three or five days after light exposure in control media or media supplied with 4% glucose or 4% mannitol (Fig. 1 samples); **(B)** Three or five days after light exposure in control media or media supplied with 4% glucose or 4% mannitol (Supplementary Fig. S3 samples); **(C)** Five days after light exposure in control media or media supplied with 0.5-5 μ M of ABA (Supplementary Fig. S4 samples). The null model considered no effect of treatment or genotype in germination or development efficiencies, nor interaction between these variables. Significant p values ($P < 0.05$) are marked with an asterisk.

B

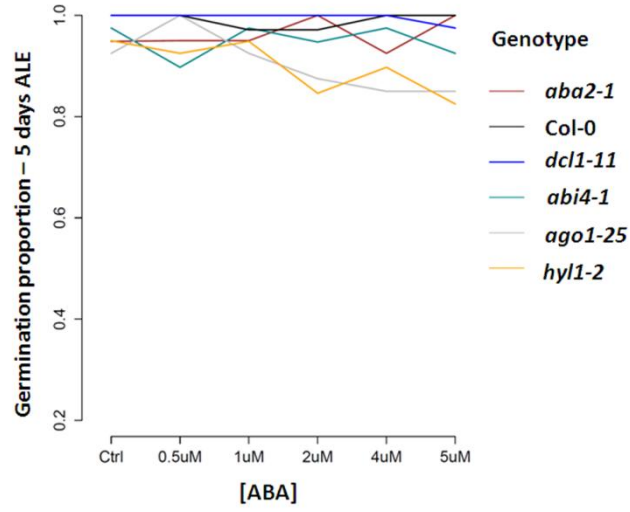


Supplementary Fig. S2. Continued.

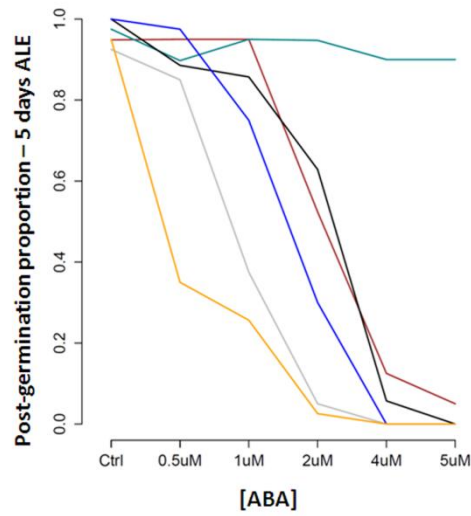
C

5 days

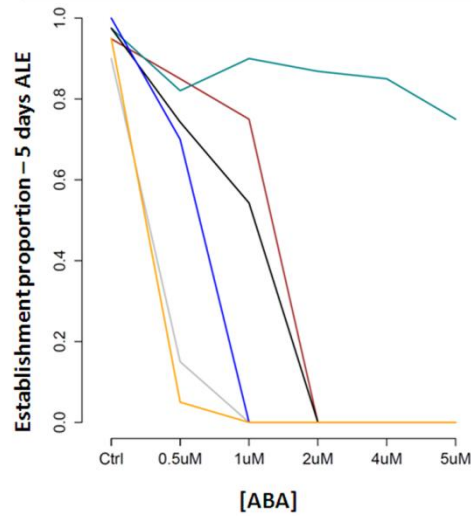
Model	Deviance	Δ Deviance	df	$P(\chi^2)$
Null	348.80			
Genotype	326.35	22.45	4	1.63e-04 *
Genotype + Treatment	276.00	50.35	2	1.17e-11 *
Genotype + Treatment + (Genotype x Treatment)	249.32	26.68	8	8.02e-04 *

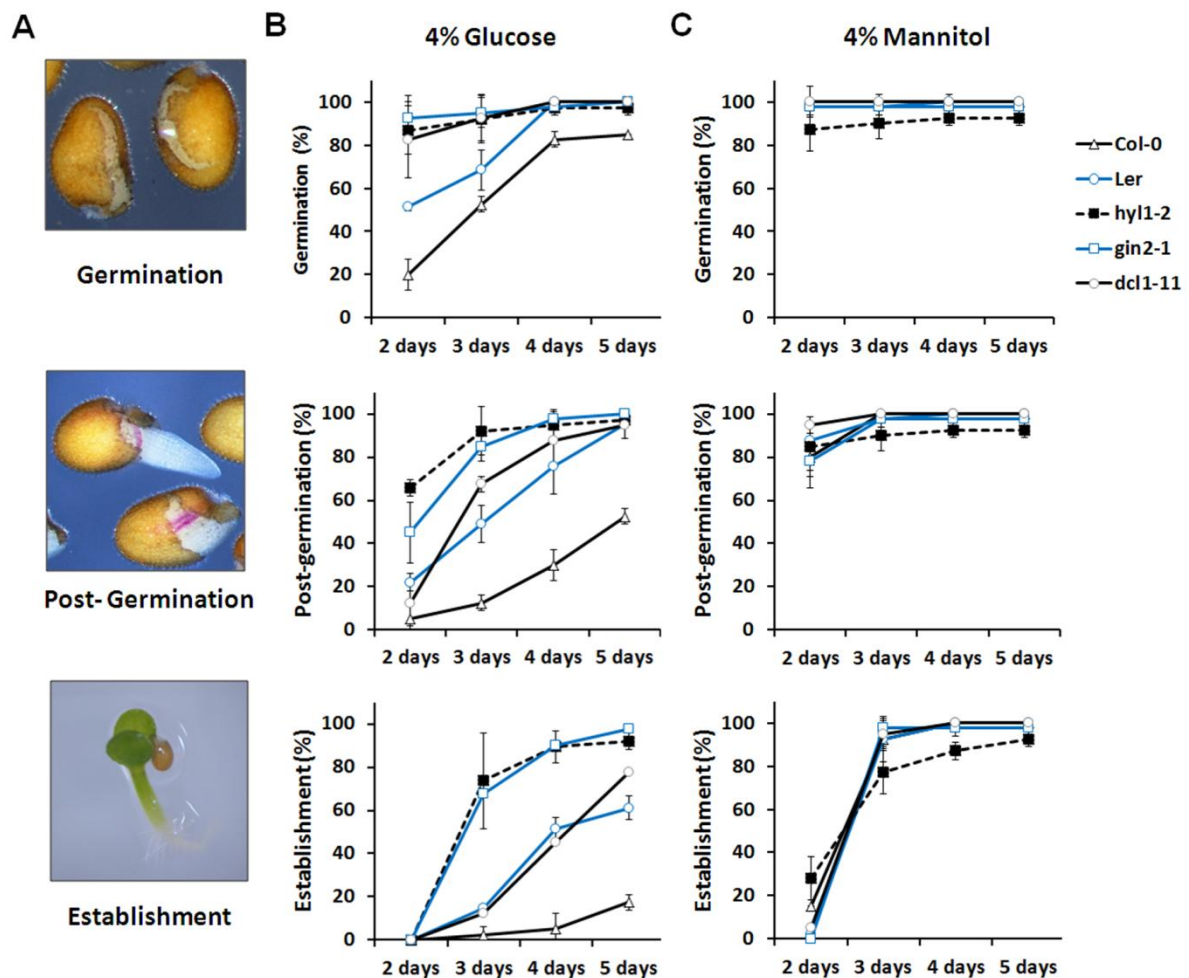


Model	Deviance	Δ Deviance	df	$P(\chi^2)$
Null	503.45			
Genotype	470.91	32.54	4	1.48e-06 *
Genotype + Treatment	323.32	147.59	2	<2.2e-16 *
Genotype + Treatment + (Genotype x Treatment)	276.77	46.54	8	1.87e-07 *

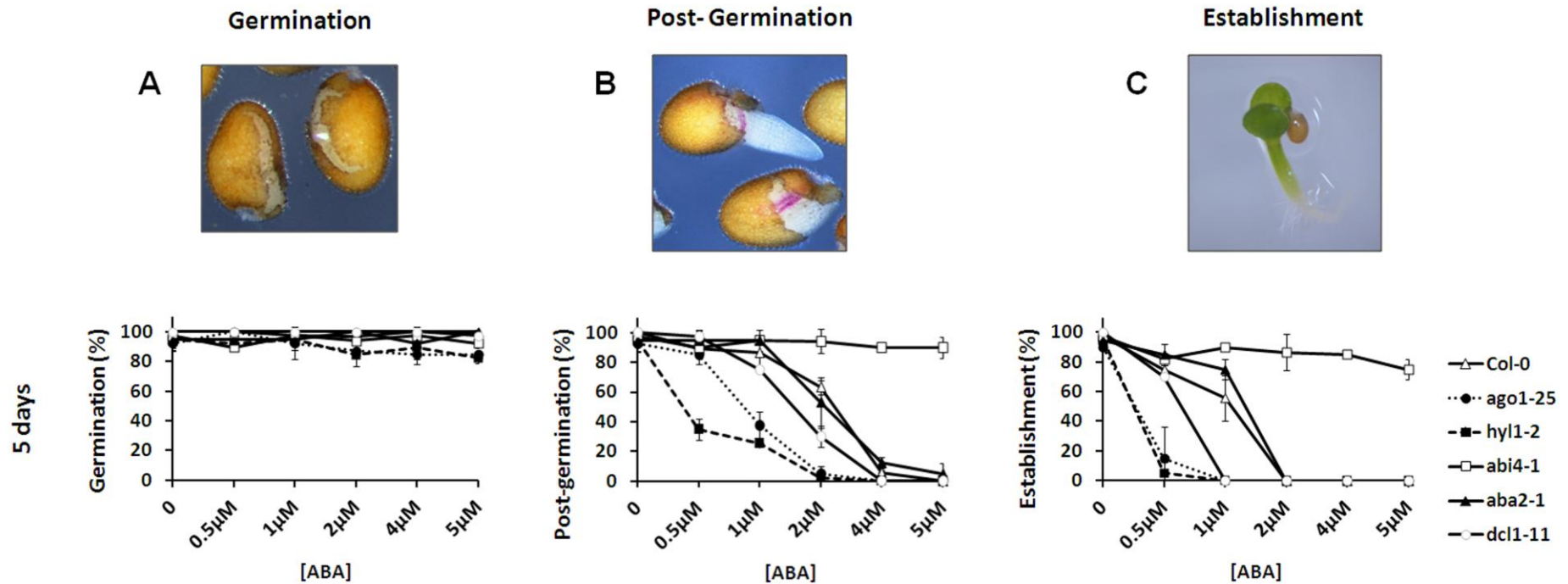


Model	Deviance	Δ Deviance	df	$P(\chi^2)$
Null	708.99			
Genotype	684.60	24.39	4	6.66e-05 *
Genotype + Treatment	454.09	230.51	2	<2.2e-16 *
Genotype + Treatment + (Genotype x Treatment)	389.44	64.64	8	5.68e-11 *

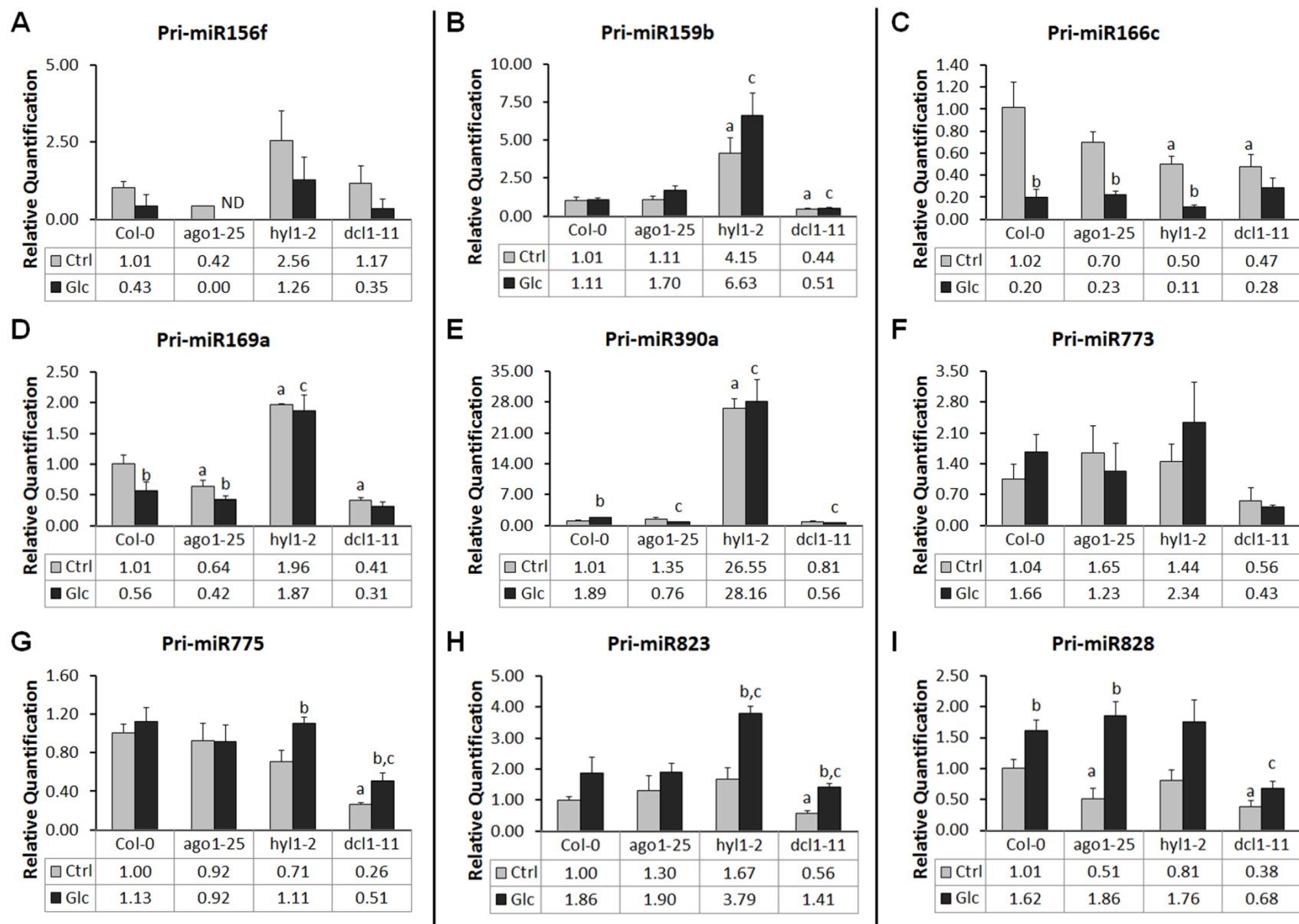




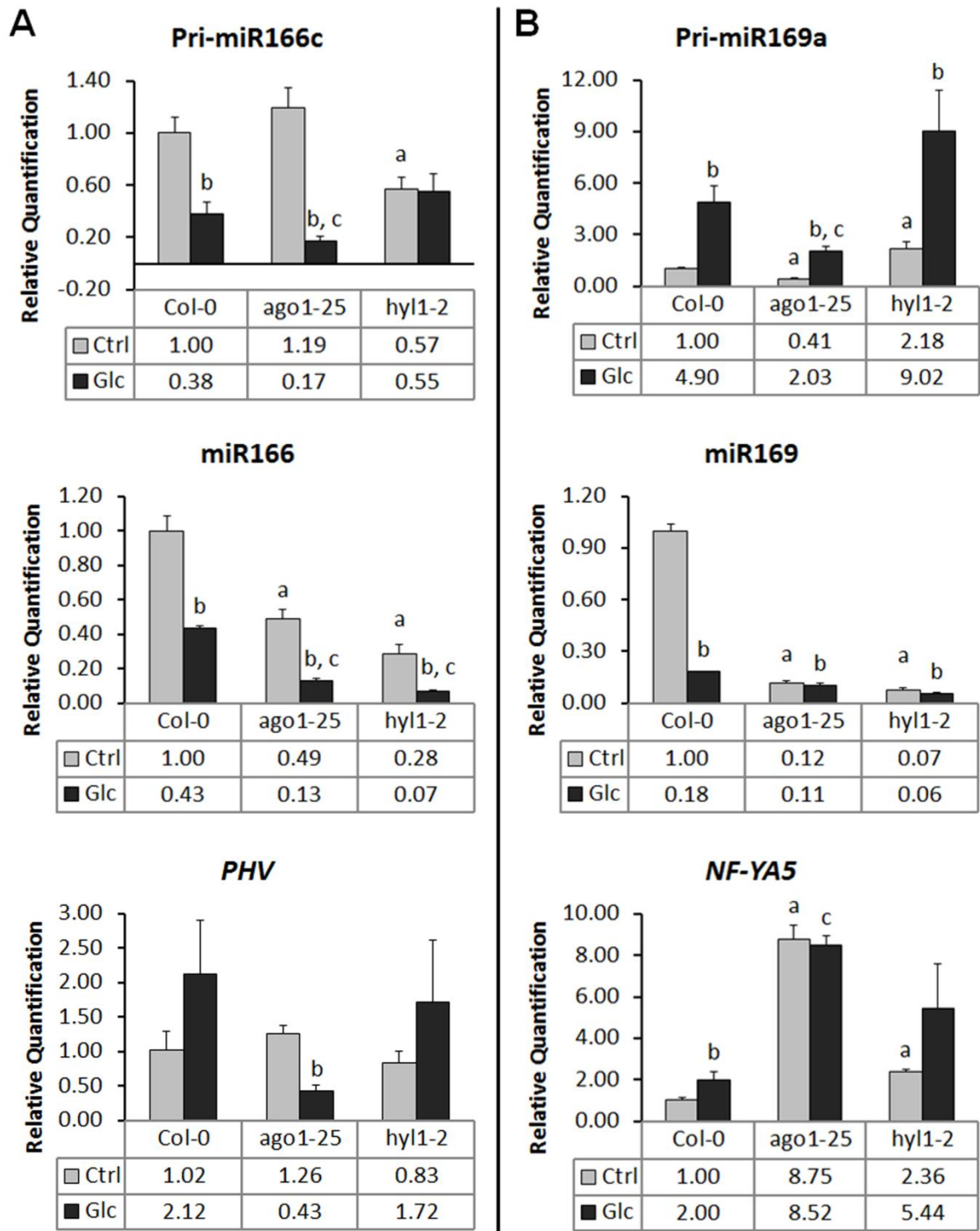
Supplementary Fig. S3. Glucose-induced delay of early seedling development is dependent upon miRNA machinery activity (A) Developmental phases that were monitored; Germination (*testa* rupture), post-germination (radicle emergence and elongation) and establishment (cotyledons expansion and greening); (B) Effects of 4% glucose on germination and development of miRNA-deficient mutants *dcl1-11* and *hyl1-2* was less severe than for wild-type Col-0 and *Ler*, and was similar to the glucose insensitive *gin2-1*. (C) Osmotic control 4% mannitol could not reproduce the delay observed for glucose. In media not supplied with sugar, all seeds reached post-germination stage after two days, and establishment within three days after light exposure (Supplementary Fig. S2B). In glucose-supplied media, growth arrest was not observed before seedling establishment stage was reached. Seeds of each genotype were sown in MS/2 plates supplied or not with the indicated sugar, kept for two days at 4°C in the dark for stratification and transferred to continuous light ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 24°C. Germination, post-germination, and establishment were scored from two to five days after light exposure. Results presented are the means \pm SD of three independent experiments each of which including 20 seeds.



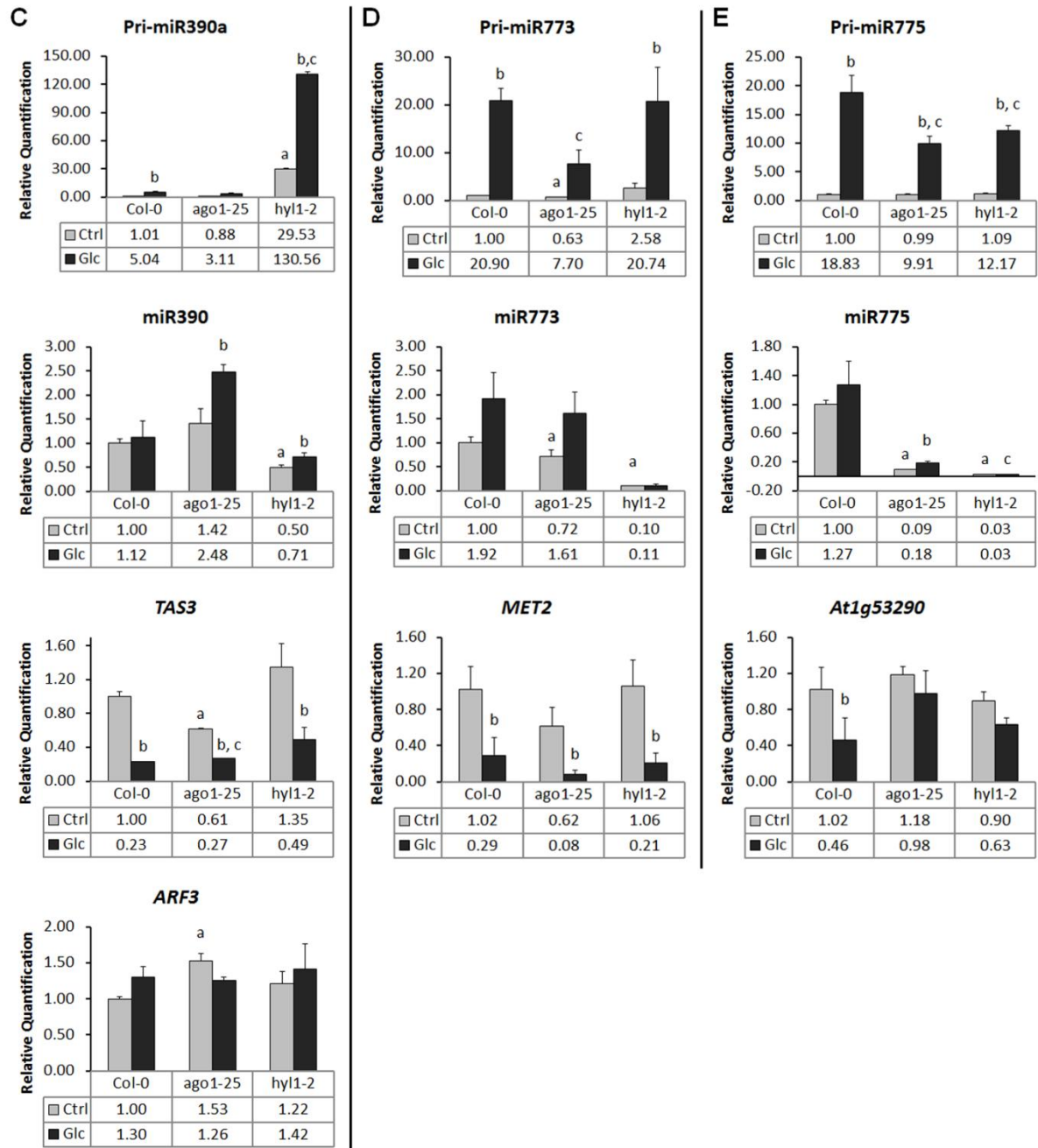
Supplementary Fig. S4. Effects of different ABA concentrations on early seedling development of wild-type Col-0, miRNA-deficient mutants *ago1-25*, *dcl1-11* and *hyl1-2*, ABA-insensitive mutant *abi4-1*, and ABA-biosynthesis mutant *aba2-1*. (A) Germination (*testa* rupture), (B) post-germination (radicle emergence and elongation) and (C) establishment (cotyledons expansion and greening) were scored five days after light exposure. Except for the ABA-insensitive mutant *abi4-1*, all genotypes had the development arrested before establishment within five days (Supplementary Fig. S2C). Seeds of each genotype were sown in MS/2 plates supplied with the indicated ABA concentration, kept for two days at 4°C in the dark for stratification and transferred to continuous light ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 24°C. Results presented are the means \pm SD of two independent experiments each of which including 20 seeds.



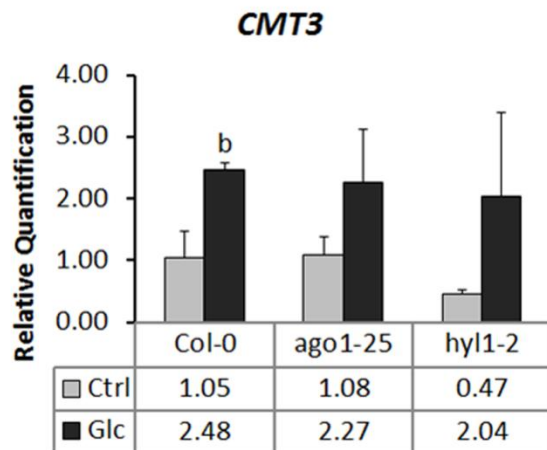
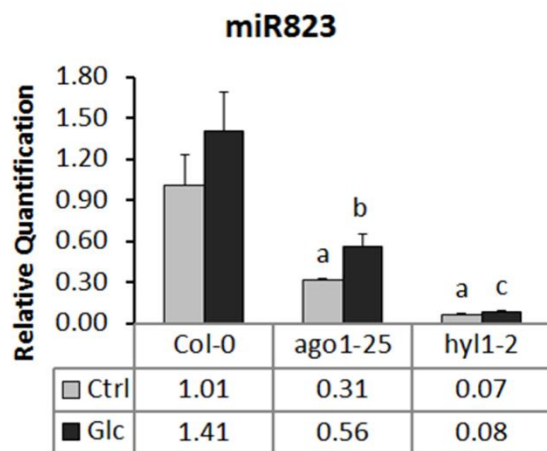
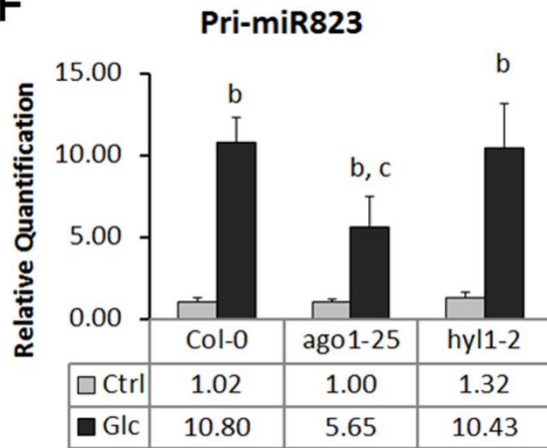
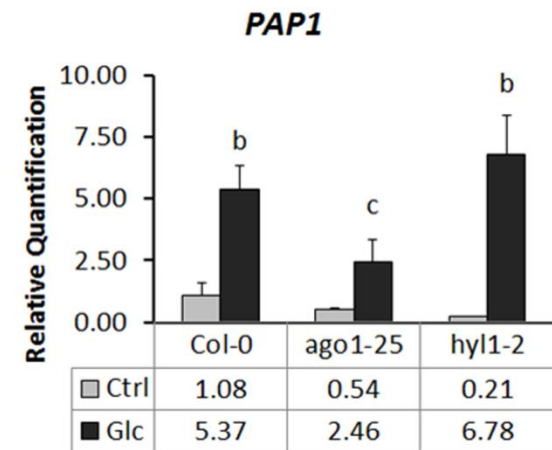
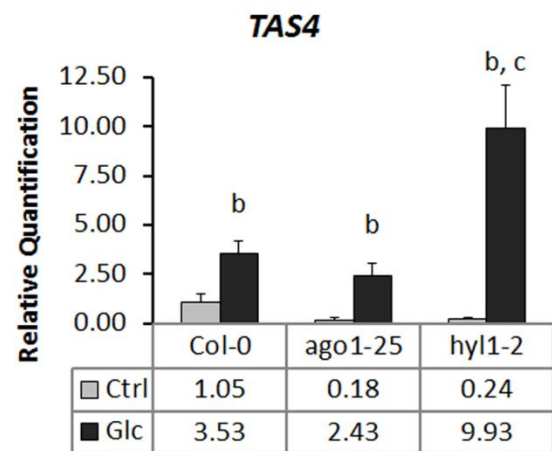
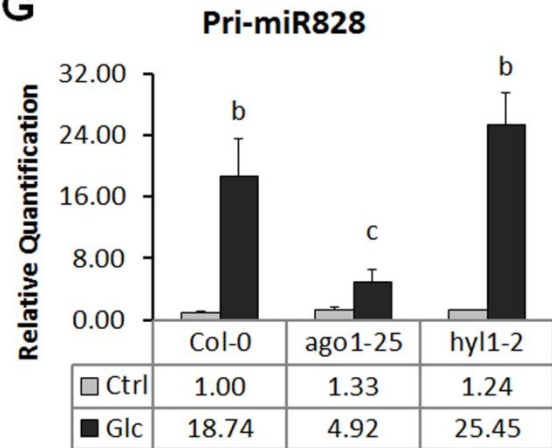
Supplementary Fig. S5. Accumulation of (A) pri-miR156f, (B) pri-miR159b, (C) pri-miR166c, (D) pri-miR169a, (E) pri-miR390a, (F) pri-miR773, (G) pri-miR775, (H) pri-miR823, and (I) pri-miR828 in Col-0, *ago1-25*, *dcl1-11* and *hyl1-2* seedlings grown in control media for three days of light exposure and treated for 4h with 4% glucose. All expression values are in comparison to untreated Col-0. The values are means of three biological replicates \pm SD. Below each graph is given the relative transcript abundance. Changes in transcript accumulation were considered significant for differences with fold change \geq |1.5| and according to Student's *t* test ($p < 0.05$) for the following comparisons: **a.** untreated *ago1-25*, *dcl1-11* or *hyl1-2* vs untreated Col-0; **b.** glucose vs untreated samples (same genotype); **c.** glucose-treated mutant vs glucose-treated.



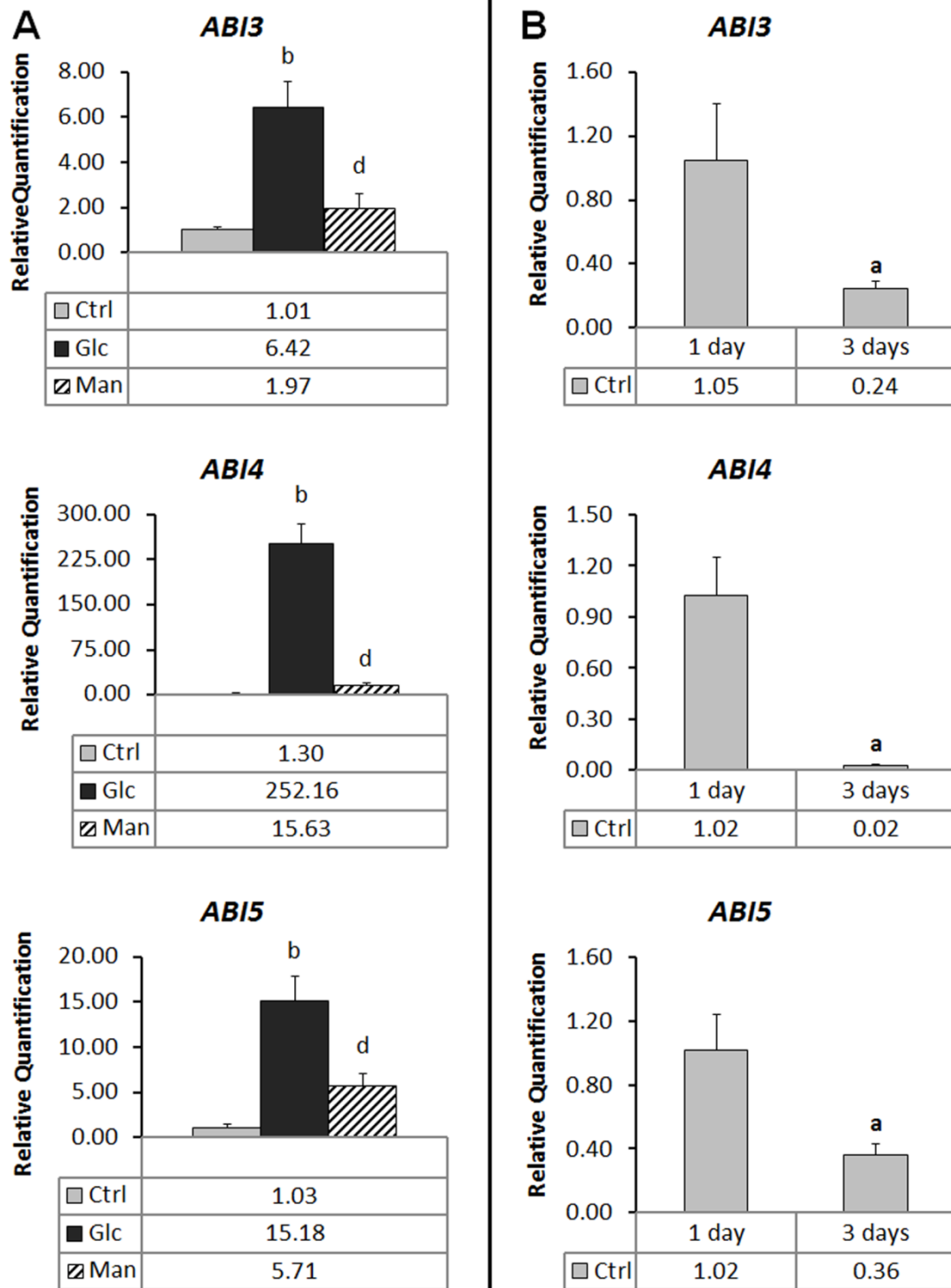
Supplementary Fig. S6. Accumulation of (A) pri-miR166c, (B) pri-miR169a, (C) pri-miR390a, (D) pri-miR773, (E) pri-miR775, (F) pri-miR823, (G) pri-miR828 and their corresponding mature miRNA family and respective targets in Col-0, *ago1-25* and *hyl1-2* seedlings grown in 4% glucose or control media after three days of light exposure. All expression values are in comparison to untreated Col-0. The values are means of three biological replicates \pm SD. Below each graph is given the relative transcript abundance. Changes in transcript accumulation were considered significant for differences with fold change $\geq |1.5|$ and according to Student's *t* test ($p < 0.05$) for the following comparisons: **a.** untreated *ago1-25* or *hyl1-2* vs untreated Col-0; **b.** glucose vs untreated samples (same genotype); **c.** glucose-treated mutant vs glucose-treated Col-0. miR828 did not yield a specific amplification.



Supplementary Fig. S6. Continued.

T**G**

Supplementary Fig. S6. Continued.



Supplementary Fig. S7. Validation of glucose-promoted changes on *ABI3*, *ABI4* and *ABI5* accumulation. **(A)** Relative expression of *ABI3*, *ABI4* and *ABI5* in Col-0 seedlings grown in 4% glucose or 4% mannitol in comparison to untreated samples three days after light exposure (ALE). **(B)** Relative expression of *ABI3*, *ABI4* and *ABI5* in untreated Col-0 seedlings with one day ALE in comparison to three days ALE samples. The values are means of three biological replicates \pm SD. Below each graph is given the relative transcript abundance. Changes in transcript accumulation were considered significant for differences with fold change $\geq |1.5|$ and according to Student's *t* test ($p < 0.05$) for the following comparisons: **a.** untreated one day ALE vs three days ALE samples; **b.** glucose vs untreated samples; **d.** mannitol- vs glucose-treated samples.