

Supplementary Figure 1 | qRT-PCR expression analysis of *NLP8* with and without KNO₃ during germination.

Seeds of Col-0 were harvested from plants grown at 16°C, stored for 2 months, imbibed for indicated time periods in water with 1 mM KCl (black line) or KNO₃ (red line). qRT-PCR was performed with three independent biological replicates. Expression levels were normalized by that of At1g13320. Averages of relative expression are shown with SD (n=3).



Supplementary Figure 2 | Construction of the near isogenic line, *nlp8-2/*Cvi.

Location of genetic markers is shown on the five Arabidopsis chromosomes (From I to V). The *nlp8-2* mutant in Col background was crossed to Cvi four times to obtain the *nlp8-2* mutant in Cvi background. The *nlp8-2*/Cvi line was confirmed that all tested markers, except for NLP8, showed the Cvi/Cvi homozygote. The *nlp8-2* locus is indicated by red, while other genetic markers are indicated by blue. The method to create genetic markers distinguishing between Col and Cvi genomic regions is described in Methods, and marker information is in Supplementary Data 2.



Supplementary Figure 3 | The effect of stratification on germination of the *nlp8* mutant.

Seeds of Col-0, *nlp8-1*, *nlp8-2*, *nlp8-2nlp9-1* mutants were harvested from plants grown at 16°C, stored for 2 months. Germination test was performed using biological triplicates, and radicle protrusion through endosperm was counted 7 days after sowing. Percentage of germination in water with KCl without stratification (white bar), with KNO₃ without stratification (lined bar), and with KCl with stratification for 4 days (blue bar) is shown with SD (n=3).

Col nlp8	Annotation	Col	nlp8	Col	nlp8	Annotation	Col	nlp8
	AT1G37130 NIA2	1.72	0.035			AT5G49640 -	-2.135	0.005
	AT1G35560 TCP23	1.655	0.435			AT1G17090	-1.45	-0.61
	AT2G29090 CYP707A2	1.55	0.29			AT5G24352	-1.435	-0.115
	AT4G28460	1.545	0.18			AT3G28956 -	-1.395	-0.51
	AT5G09960 -	1.535	0.255			AT2G42740 RPL16A	-1.3	0.825
	AT2G15620 NIR1	1.495	1.05			AT2G23180 CYP96A1	-1.29	-0.07
	AT5G65140 TPPJ	1.47	0.46			AT5G66450 PPepsilon2	-1 225	0.315
	AT1G69760	1.44	-0.04			AT5G65920	-1 215	0.04
	AT5G47560 SDAT	1.39	0.075			AT1C12450	1 105	0.04
	AT3G02800 PFA-DSP3	1.385	-0.1			AT2G30505	1 1 85	0.515
	AT5G53270	1.38	0.405			AT4C04200	4 4 6 5	-0.313
	AT4G30620	1.345	0.285			AT1057540	-1.105	-0.39
	AT1G01640	1.335	0.39			AT1037340 -	-1.10	0.04
	A14G21/40	1.325	-0.03			AT2G03870 LSM7	1 115	0.343
	AT2G22470 AGP2	1.315	-0.22			A12G46000 -	-1.115	-0.4
	AT1G30510 RFNR2	1.31	0.895			AT1G26750 -	-1.11	-0.13
	AT1G29965 -	1.29	-0.415			AT3G29170 -	-1.11	0.19
	AT1G70410 BCA4	1.285	-0.08		_	AT3G62410 CP12	-1.095	-0.69
	AT4G21610 LOL2	1.275	0.16			AT4G10300 -	-1.085	0.195
	A15G62900	1.26	0.46			AT1G30190	-1.065	0.02
	AT3G46430 -	1.245	0.040			AT5G55460 -	-1.065	0.18
	AT 1077700 NIAT	1 195	0.455			AT4G12850 -	-1.06	-0.165
	AT 1G32920 -	1 175	-0.145			AT3G01435 -	-1.055	0.02
	AT3024000 E302	1.175	0.145			AT3G60810 -	-1.055	0.01
	AT4G27657	1 145	0 4 2 5			AT1G78915 -	-1.055	-0.195
	AT1C14455	1 14	0.355			AT1G18340 -	-1.04	-0.035
	AT5G59340 WOX2	1.135	0.12			AT5G56230 PRA1.G2	-1.01	0.075
	AT3G07910	1,105	0.17			AT3G20110 CYP705A20	-1.01	0.04
	AT1G25550	1.09	0.165			ATMG00630 ORF110B	-1.005	-0.185
	AT1G70782 CPuORE28	1.08	0.24			AT2G26070 AtRTE1	-1.005	0.15
	AT4G05390 RFNR1	1.075	0.205					
	AT4G37300 MEE59	1.075	-0.015					
	AT5G08260 SCPL35	1.07	0.05					
	AT2G46390 SDH8	1.06	0.405					
	AT1G07150 MAPKKK13	1.06	0.01					
	AT1G27330 -	1.055	-0.13					
	AT4G27950 CRF4	1.05	-0.205					
	AT2G43520 ATTI2	1.05	-0.14					
	AT1G25422	1.05	-0.18			0.00.0.00		
	AT5G13110 G6PD2	1.035	0.46	-2.14	1	-0.33 0.33	1.72	2
	AT5G06270 -	1.03	0.02					
	AT3G17609 HYH	1.03	0.21					
	AT2G46690	1.025	-0.07					
	AT5G60840	1.01	-0.085					
	AT2G27830	1	0.105					
	AT5G59840	1	0.16					

Supplementary Figure 4 | Nitrate-regulated genes in 6-hour imbibed seeds in Col-0 or *nlp8-2* mutant.

Seeds were imbibed in water with 1mM KCl or KNO₃ for 6 hours and RNA were extracted for RNA-seq. The readouts from KNO₃-treated seeds were normalized by those from KCl control and a mean of two biological repeats was used to generate a heatmap.

ABA metabolism and signaling

GA metabolism and signaling



Supplementary Figure 5 | Expression of ABA- and GA-related genes in 6-hour imbibed Col-0 and *nlp8* mutant seeds.

The numbers in the heatmap indicate the expression values from the RNA-seq data.



Supplementary Figure 6 | qRT-PCR expression analysis of misexpressed genes in 6-hour imbibed *nlp8* mutant seeds

Seeds of Col-0 and *nlp8-2* mutant were harvested from plants grown at 16°C, stored for 2 months, imbibed for 6 hours in water with 1 mM KCl (white bar) or KNO₃ (lined bar). qRT-PCR was performed with four biological replicates. Expression levels were normalized by that of At1g13320. Averages of relative expression relative to those in Col-0 seeds treated with KCl are shown with SD (*n*=4). * and ** indicate P <0.05 and P <0.01 (Student's *t*-test), respectively.



Supplementary Figure 7 | qRT-PCR expression analysis of nitrate responsive genes in 6-hour imbibed Col-0 and *nlp8* mutant seeds.

Seeds of Col-0 and *nlp8-2* mutant were harvested from plants grown at 16°C, stored for 2 months, imbibed for 6 hours in water with 1 mM KCl (white bar) or KNO₃ (lined bar). qRT-PCR was performed with three independent biological replicates. Expression levels were normalized by that of At1g13320. Averages of relative expression of nitrate up-regulated gene (HYH) and down-regulated genes (At5g49640, At5g24352, and At2g42740) are shown with SD (n=3). * and ** indicate P <0.05 and P <0.01 (Student's *t*-test), respectively.



Supplementary Figure 8 | Promoter deletion analysis to identify the region responsible for NLP8-dependent gene expression

The deletion series of *CYP707A2* promoters were fused to the LUC reporter gene, and the promoter activities were examined in a protoplast system. The top graph indicates the promoter activities of deleted *CYP707A2* promoters when transiently expressing *NLP8* under KCl treatment (white bar) and nitrate treatment (lined bar), while the bottom graph indicates the promoter activities transiently expressing NLP8 (lined bar) and the control ACT2 (white bar) under nitrate treatment. The promoter length of each construct is as follows: A2, 1887-bp; d1, 1661-bp; d1.2, 1638-bp; d1.3, 1615-bp; d1.4, 1589-bp; d1.5, 1549-bp; d3, 741-bp. 35S:GUS construct was co-introduced with NLP8 or ACTIN2, and the LUC activities were normalized by GUS activities. Three independent experiments were performed, and averages of relative expression are shown with SD. ** indicates the P value <0.01 by Student's *t*-test.



Supplementary Figure 9 | The RWP-RK domain of NLP8 binds to the NRE of the CYP707A2 promoter.

(a) A biotin-labeled probe containing 2 copies of d1-d1.5 (1.5ng) and RWP-RK domain of NLP8 (200 ng) were used for each EMSA reaction and separated in a 5% native PAGE gel. Competitors were 2 copies of d1-d1.5 fragment without biotin labeling. KNO3 was added to 10 mM as a final concentration. A red star indicates the shifted band, while the black stars indicate the signal from free probe. (b) The full image of the blot shown in Fig. 5f.



Supplementary Figure 10 | The effect of 35S::NLP8 on the chl1-5 mutation during germination.

Seeds of Col-0, *chl1-5*, *nlp8-2*, *chl1-5nlp8-2* mutants and *35S::NLP8 chl1-5* line were harvested from plants grown at 16°C, stored for 2 months. Germination test was performed using biological triplicates, and radicle protrusion through endosperm was counted 7 days after sowing. Percentage of germination under indicated KNO₃ concentrations is shown with SD. The numbers of biological replicates analyzed were as follows: Col-0, *n*=6; *chl1-5*, *n*=6; *35S::NLP8chl1-5*, *n*=6; *nlp8-2*, *n*=4; *chl1-5nlp8-2*, *n*=4.



Supplementary Figure 11 | Nitrate-induced reporter expression.

GUS staining of transgenic lines harboring both effector (NLP8N-LexA_DB-YFP) and reporter (LexAOP-35Smini::GUS). Seven-day-old seedlings grown on KCl plate were transferred to liquid medium containing 3 mM KCl or KNO₃ for 4 hours and subjected to GUS staining. A bar indicates 5 mm.



Supplementary Figure 12 | Expression of *NLP8* in 35S::NLP8-GFP lines.

Seeds of 35S::NLP8-GFP were harvested from plants grown at 16°C, stored for 2 months, imbibed for 6 hours in water with 1 mM KCl. qRT-PCR was performed with three biological replicates. Expression levels were normalized by that of At1g13320. Three lines showed similar results when analyzed. Data for line OX#6 is shown in the main figures. Averages of relative expression are shown with SD (n=3). * and ** indicate P <0.05 and P <0.01 (Student's *t*-test), respectively.



Supplementary Figure 13 | Expression stability of 4 reference genes.

qRT-PCR expression analysis was performed on 4 Arabidopsis reference genes published previously with cDNA from Col-0 and *nlp8-2* seeds imbibed in KCl or KNO₃ for 6 hours. Expression stability was analyzed by geNORM algorithm. Less M values indicate more stable as reference genes.