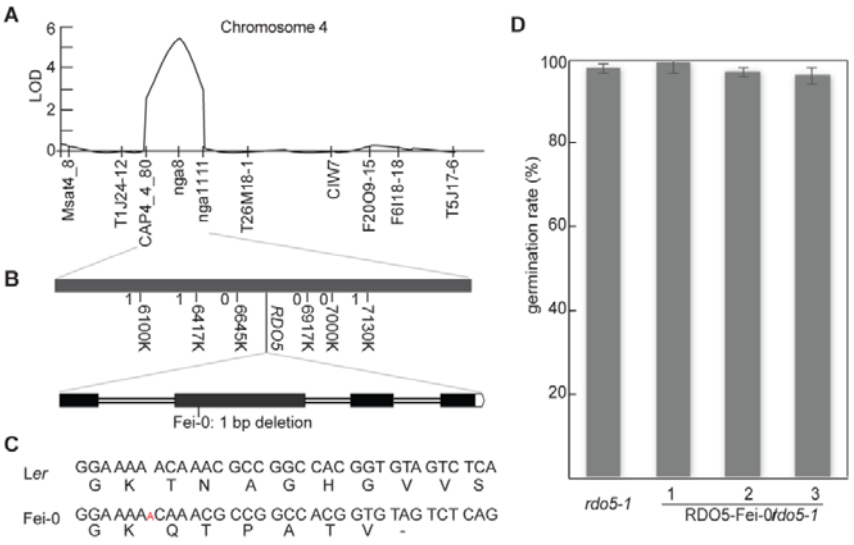
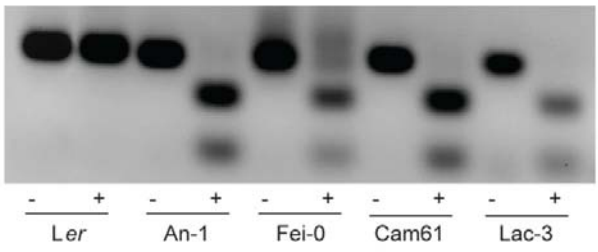
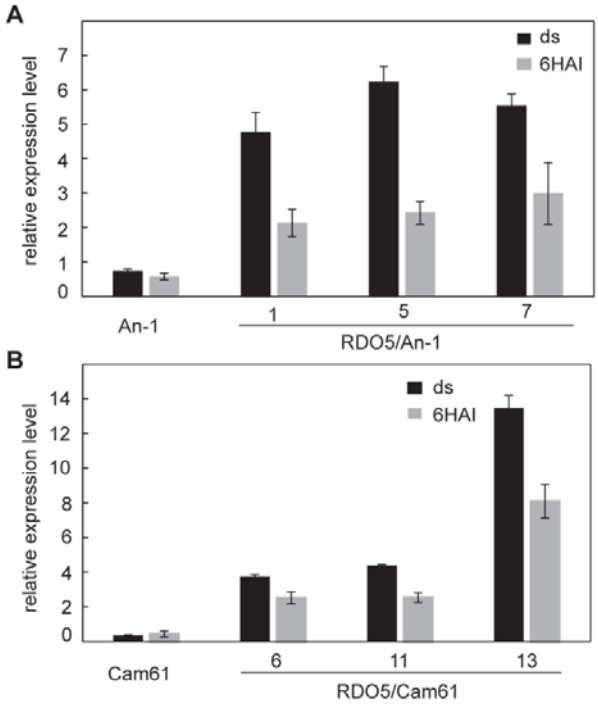
Supplemental Files



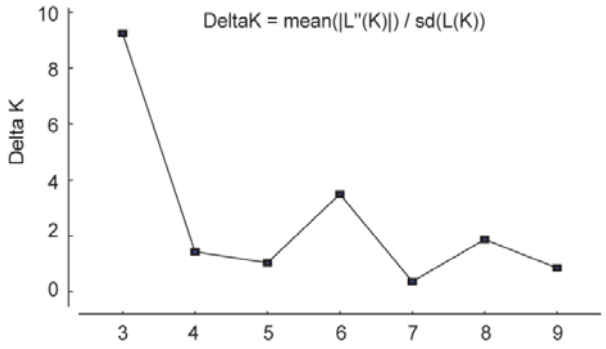
Supplemental Figure 1. Map based cloning of *DOG18*.
(A) QTL likelihood map for DSDS50 values of the L*er*/Fei-0 RILs. The Y-axis corresponds to the genetic map of chromosome 4.
(B) Fine mapping narrowed the position of *DOG18* down to a 700 kb interval, containing the *RDO5* gene, between markers 6417K and 7130K. The numbers between the markers indicate number of recombinants. The lower part of the figure shows the *RDO5* gene (with exons in black) and the location of a 1 bp deletion in Fei-0.
(C) The 1 bp deletion in Fei-0 causes a frame shift and premature stop codon in *RDO5*.
(D) Germination of freshly harvested seeds from the *rdo5-1* mutant and trangenic *rdo5-1* lines containing the *DOG18* Fei-0 allele. Shown are means ± SE of six to eight independent batches of seeds for each genotype.



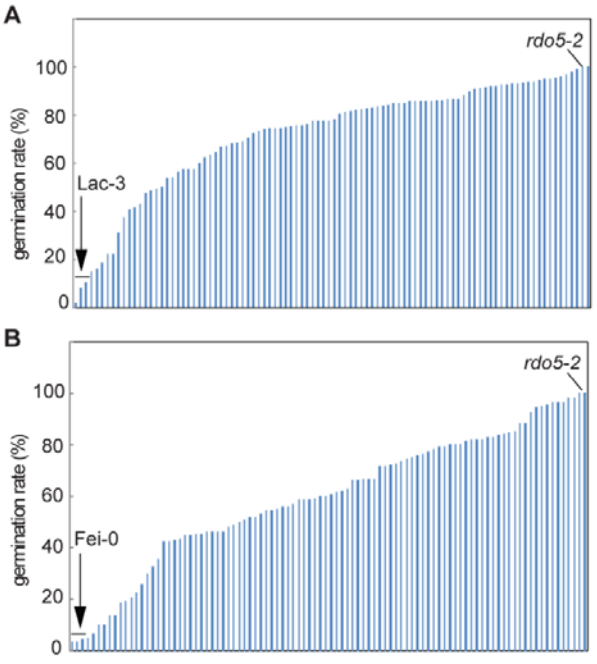
Supplemental Figure 2. *DOG18* Fei-0 allele screening.
DOG18 Fei-0 allele screen of Arabidopsis hapmap accessions. A dCAPs marker is used for genotyping. A 124bp PCR product was amplified. DOG18 Fei-0 like alleles can be digested into 84bp and 40bp by Xmn1 (NEB, USA). -/+ indicate before/after Xmn1 digestion.



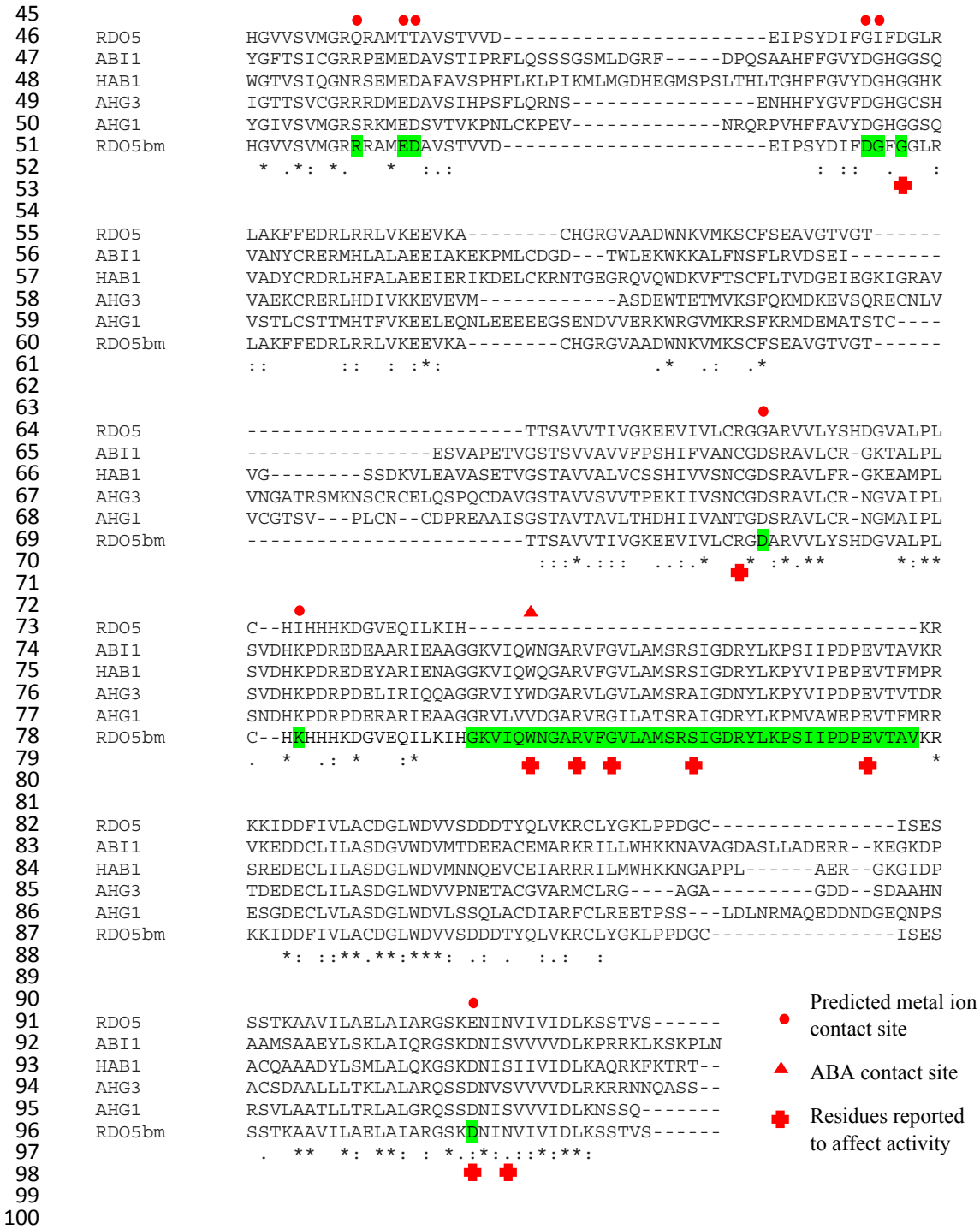
Supplemental Figure 3. *RDO5* transcript level detection in transgenic lines.
qRT-PCR analysis of *RDO5* transcript levels in dry and 6HAI seeds from transgenic lines in An-1 (A) and Cam61 (B) background. The expression values were normalized using *ACT8* as control. n = 3 biological replicates; error bars represent SE.



Supplemental Figure 4. Structure estimation of populations for K ranging from one to fourteen by delta K-values.

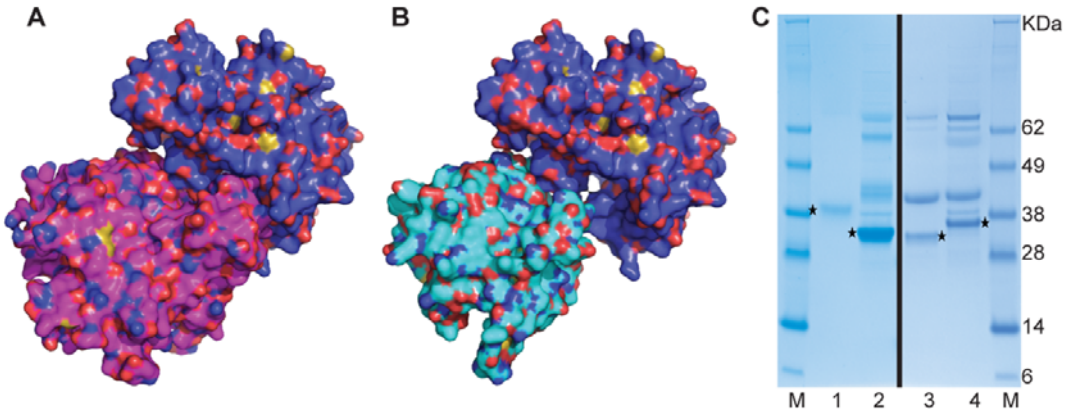


Supplemental Figure 5. Germination of *rdo5* segregating populations.
(A) and (B) Germination percentage of freshly harvested seeds from individuals of segregating F2 progenies from the crosses *rdo5-2* × Lac-3 (A) and *rdo5-2* × Fei-0 (B). 96 independent lines were analyzed including 4 Lac-3 (A) or Fei-0 (B) and 2 *rdo5-2* plants as control. X-axis shows F2 progeny.



Supplemental Figure 6. RDO5 sequence analysis.

Sequence alignment of RDO5 and four Arabidopsis PP2C clade A phosphatases. The back-mutated RDO5 protein (RDO5bm) is added at the bottom for comparison. Red circles indicate the amino acids important for metal ions coordination. Red arrows indicate the reported amino acid residues required for PP2C activity and triangle points the conserved W (W385 in HAB1) in PP2C clade A that is needed for ABA sensing and stable interaction with ABA receptor. The alignment was performed using online ClusterW2 tools with default settings (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Asterisks indicate fully conserved residues. Colons and periods indicate strong and weak similarity, respectively.

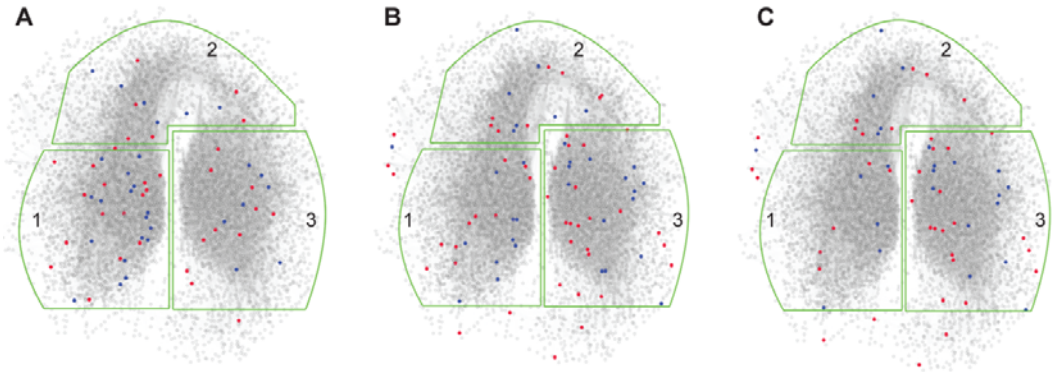


Supplemental Figure 7. RDO5 topology structure analysis and SDS PAGE gel of purified proteins.

(A) Crystal structure of the HAB1/SnRK2.6 complex (PDB: 3ujg). Figures were made using PyMOL (http://pymol.sourceforge.net/) and protein surface are shown.

(B) 3-D Model of the RDO5 obtained by using Swiss-model (http://swissmodel.expasy.org). The RDO5 model was superimposed on the known structure of HAB1/SnRK2.6 (PDB: 3ujg) using PDBeFold (http://www.ebi.ac.uk/msd-srv/ssm/) to look at the possibility for interaction with SnRK2.6. The RDO5 model surface is shown in light blue and the surfaces of HAB1 and SNRK2.6 are shown in magenta and blue respectively.

(C) SDS PAGE gel showing purified AHG3 and RDO5 related proteins. M, Invitrogen SeeBlue Plus2 Pre-stained Protein Standard; 1, purified AHG3 with HIS tag; 2, purified RDO5-L*er* with HIS tag; 3, purified RDO5-L*er*; 4, purified back mutated RDO5 (RDO5bm). Stars indicate target proteins. The 42 KDa proteins in line 3-4 are His-MBP tags obtained after cleavage by TEV protease after purification.



Supplemental Figure 8. Localization of differently expressed proteins in the SeedNet network after 6 hours imbibition.

Wild-type NIL DOG1 (A), *rdo5-1* (B) and potential RDO5 specific targets (C). The regions outlined in green correspond to clusters associated with dormancy (region 1) or germination (regions 2 and 3). The red dots represent the proteins with decreased levels, and the blue dots represent the proteins with increased levels.

Supplemental Table 1. Information of 42 loss-of-function *DOG18* accessions including mutation type, accession name, ID, country, origin, habitat, latitude and longitude of collection site.

Mutation Type	Abbreviated Name	Stock ID	Latitude	Longitude	Country	Location	Habitat
Non-sense mutation	POG-0	N28650	49.2655	-123.2060	Canada	Point Grey, British Columbia	field border
Non-sense mutation	CATS-6	N76760	50.7900	2.6900	France	Mont des Cats	perennial meadow
Non-sense mutation	Com-1	N28193	49.4160	2.8230	France	Compiegne	garden
Non-sense mutation	CON-7	N76781	47.2364	4.4314	France	Conforgien	river bank
Non-sense mutation	GEN-8	N76877	50.5900	3.3000	France	Genech	cultivated field
Non-sense mutation	TueV13	N76407	48.5232	9.5198	Germany	Tubingen - Volksbank	
Non-sense mutation	Tu-KB-6		48.5200	9.0500	Germany	Tubingen (central)	stone paved road
Non-sense mutation	Tu-Wa1-2	N76405	48.5342	9.0345	Germany	Tubingen - Wanne	
Non-sense mutation	Abd_0	N76429	57.1539	-2.2207	United Kingdom	Aberdeen, Kingswells	
Non-sense mutation	Gol-2	N28974	57.9672	-3.9672	United Kingdom	Golspie, Scotland	
Non-sense mutation	HR-10	N28355	51.4083	-0.6383	United Kingdom	HR Ascot	
Non-sense mutation	NFA-10	N28533	51.4083	-0.6383	United Kingdom	Ascot	
Non-sense mutation	NFA-8	N28532	51.4083	-0.6383	United Kingdom	Ascot	
Non-sense mutation	UKNW06-403	N78797	54.7000	-3.4000	United Kingdom	Cockermouth	
Non-sense mutation	UKSE06-118	N78799	51.3000	0.5000	United Kingdom	East Malling Research Station	
Non-sense mutation	UKSW06-179		50.4000	-4.9000	United Kingdom	St Columb	
Non-sense mutation	UKSW06-226		50.4000	-4.9000	United Kingdom	St Dennis	
Non-sense mutation	UKSW06-285	N78811	50.3000	-4.9000	United Kingdom	St Stephens	
Non-sense mutation	UKSE06-470	N78804	51.2000	0.4000	United Kingdom	Paddock Wood	
Non-sense mutation	UKSE06-533	N78806	51.3000	1.1000	United Kingdom	Canterbury	house garden
Non-sense mutation	Vind-1	N28803	54.9902	-2.3671	United Kingdom	Vindolanda	near Vindolanda restaurant
Non-sense mutation	LP3413.41	N77053	41.6862	-86.8513	USA	Michigan City	
Non-sense mutation	Cnt-1.5726		51.3000	1.1000	United Kingdom	Canterbury	
Indels	An-1	N28015	51.2167	4.4000	Belgium	Antwerpen	Botanic Garden
Indels	Ri-0		49.1632	-123.1370	Canada	Ri	
Indels	Cam-61	N76108	48.2667	-4.5833	France	CAM	

Indels	Lac-3	N76157	47.7000	6.8167	France	LAC	
Indels	WAV-8		50.6500	2.9900	France	Wavrin	garden
Indels	Sp-0	N28743	52.5339	13.1810	Germany	Berlin/Spandau	
Indels	ICE163	N76353	46.3716	11.2376	Italy	Altenburg	stark
Indels	Fei-0	N28250	40.9200	-8.0400	Portugal	St. Maria d. Feiria	
Indels	Oemoe2-1		56.1481	15.8199	Sweden	OeMoe2	
Indels	11C1	N76640	55.8877	3.2107	United Kingdom	Hillend	Sandy loam with pine litter
Indels	Durh-1	N28215	54.7761	-1.5733	United Kingdom	Durham -bot garden	botanical garden
Indels	UKNW06-003		54.5000	-3.0000	United Kingdom	Dove Cottage,Wordsworth Trust, nr Grasmere	
Indels	UKNW06-481		54.4000	-2.9000	United Kingdom	Windemere	
Deletions	HOV3-2		56.1000	13.7400	Sweden	Hov3	
Deletions	Love-1		62.8010	18.0790	Sweden	Loev	
Deletions	Vaar2-6	N28799	55.5800	14.3340	Sweden	Varhallarna, S Sweden (Skane)	
Deletions	Tny-04		62.9600	18.2844	Sweden	TNY	
Gene loss	Fr-2	N28266	50.1102	8.6822	Germany	Frankfurt	Botanic Garden
Splicing site mutations	Boot-1	N28091	54.4000	-3.2667	United Kingdom	Boot, Eskdale	parking lot pub

Supplemental Table 2. Overview of quantitative mass spectrometry results. Number of quantified protein groups in total extract as well as phosphoprotein groups, phosphopeptides and phosphorylation sites are given for each condition separately and for all samples combined. Proteins and phosphosites were required to be quantified in at least two replicates of at least one condition. High confidence phosphosites were filtered for a localization probability > 0.75.

	Total extract protein groups	TiO_2 enriched fractions			
		Phospho-Protein groups	Phospho-peptides	Phosphosites Total	Phosphosites High confidence
NIL-DOG1 ds	4949	854	1264	1458	1253
NIL-DOG1 6HAI	4941	691	952	1075	929
rdo5-1 ds	4950	858	1279	1479	1258
rdo5-1 6HAI	4925	667	919	1033	896
All samples	5017	875	1308	1527	1290

Supplemental Table 3. Overview of regulated phosphosites and phosphorylated protein groups. Fold changes greater than 2 (log2 ratio > 1 for upregulated or < -1 for downregulated sites or proteins, respectively) were required. Significantly regulated sites or proteins either had a t-test p value < 1% or where quantified in at least two replicates of one condition and not quantified in any replicate of the other condition. High confidence phosphosites were filtered for a localization probability > 0.75. Reliably regulated phosphosites are both significantly regulated localized with high confidence.

		Protein groups		Phosphosites		Phosphoprotein groups	
	Log2 ratio	Total	Significant	Total	Reliable	Total	Significant
NIL-DOG1 6HAI vs ds	> 1	197	41	124	26	63	21
	< -1	234	65	832	380	491	238
rdo5-1 6HAI vs ds	> 1	197	48	75	23	49	17
	< -1	243	84	1135	529	629	285

Supplemental Table 4. Primers used in this study.

Name	Primer sequence	Purpose
*DOG18*F(Xmn1)	GGAGATGTTGGGACATCAGAAGGAACACACGTTTTAGTCGATGCAC	dCAPs marker for *DOG18* Fei-0 allele
*DOG18*R(Xmn1)	TGTCGTCCCATGACTGAGACTACACCGTGGCCGGCGaaTGTTT	
Q18-6100K-F(BamH1)	TGTTGAAAACATCGAAATTGTTG	dCAPs marker for *DOG18* map based cloning
Q18-6100K-R(BamH1)	GTCCTATTTTTCAACTAAACGAGTG	
Q18-6410K-F(Dde1)	TTCAGCTTTAACACTTTCCATGC	
Q18-6410K-R(Dde1)	ATGTGGTGTGTGATGGGAAAA	
Q18-7000K-F(HincII)	TTCGAATTCAGACCCGAAAC	
Q18-7000K-R(HincII)	TGCCTCAAGGGATGAGGTAT	
Q18-7130K-F(HindIII)	CTTGTTGGAGCACCTTCTGG	
Q18-7130K-R(HindIII)	CATCATGTTGCTATTCTCTGCTG	
Q18-6917K-F(Ssp1)	TGTCGGTGCTTTCGTTCCA	
Q18-6917K-R(Ssp1)	CTATGGCACAAACGTTTGGGA	
Q18-6645K-F(Bcc1)	TTTTTGGACACAAAACCACAA	
Q18-6645K-R(Bcc1)	GAGATTTTACACTCATCGTTCCAA	
RDO5-F	AAAAAGCAGGCTTCATGAAAACGGATACTACTCTACCCA	*RDO5* cDNA amplify from L*er* and Fei-0 for protein expression
RDO5-R	AGAAAGCTGGGTAAAACTAAGAAACGGTAGAGCTTTTG	
RDO5TEV-attBF	AAAAAGCAGGCTATGAAAACCTGTATTTTCAGGGCAAAACGGATACTACTCTACCCA	*RDO5* cDNA amplify for protein expression (TEV protease clevage site indicate with underline)
RDO5TEV-attBR	AGAAAGCTGGGTAAAACTAAGAAACGGTAGAGCTTTTG	
AHG3-F	AAAAAGCAGGCTATAATTCAAATTCTGTTACG	*AHG3* cDNA amplify for protein expression
AHG3-R	AGAAAGCTGGGTACACTAATTATTAAGACGACG	
RDO5 promoter-F	AAAAAGCAGGCTTCTCGCAATCAGTTGGAGTAAA	*RDO5* cloning with native promoter for DOG18-Fei-0 complementation
RDO5 promoter-R	GTTTTCATCTTGTCATCGTCATCCTTGTAGTCCATGATAACAACTCCTCTTTA	
RDO5 cDNA-F	GACGATGACAAG ATGAAAACGGATACTACTCT	
RDO5 cDNAHA -R	AGAAAGCTGGGTCTAAGCGTAATCTGGAACATCGTATGGGTAAGAAACGGTAGAGCT	

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