SUPPLEMENTAL INFORMATION

Supplemental Text. All amino acid sequences of the Arabidopsis (57), maize (47), rice (47) and *E. coli* (5) RH proteins used for phylogenetic analysis in supplemental Figure 1.

Supplemental Table 1. Protein accession numbers, nomenclature, annotation and clade assignment for the RH3 DEAD box helicase family in Arabidopsis, maize, rice used for phylogenetic analysis.

Supplemental Table 2. Primer sequences used for verification of Arabidopsis T-DNA insertions and RNA analysis.

Supplemental figure 1. Phylogenetic tree of the RH3 DEAD box helicase family in Arabidopsis, maize, rice and *E. coli* based on the core domains with major gaps removed. Different clades are assigned and proteins have modified DEAD domains are marked.

Supplemental figure 2. Multiple sequence alignment of ZmRH3A,B and AtRH3. The alignment was generated in ClustalW and BoxShade. Identical residues are shaded in black, and similar residues are shaded in gray. A predicted transit peptide cleavage site by stromal processing protease in AtRH3 is shown in black arrow. The conserved domains (Q-VI) are marked. The C-terminal GUCT and G-R-S enriched domains are showed in black lines with arrows. The red box represents the antigen for maize anti-RH3A. ZmRH3A corresponds to GRMZM2G415491_P01; ZmRH3B corresponds to GRMZM2G163072_P01; and AtRH3 corresponds to At5g26742. Blue: Identical residues. Yellow: Similar residues.

Supplemental figure 3. An albino seedling phenotype of hetero allelic *emb1138/rh3-4*. (A) Wild type (WT), *rh3-4,* and non-complementary plants *emb1138*/*rh3-4* were grown on 1/2 MS medium containing 2% sucrose for 21 days. The *emb1138*/*rh3-4* seedling is white. Bars = 5 mm. (B) PCR genotyping the progeny of the cross between *emb1138* and *rh3-4* confirms that the T-DNA insertion in *AtRH3* gene causes the pigment deficient phenotype. Primers used for the genotyping are shown top panel in (A). Genomic DNA was isolated from albino or wild-type phenotype resulted from cross between *rh3-4* and EMB1138/*emb1138* crossing. All of albino seedlings have T-DNA from both parents, whereas, wild-type phenotype has a T-DNA only from *rh3-4*.

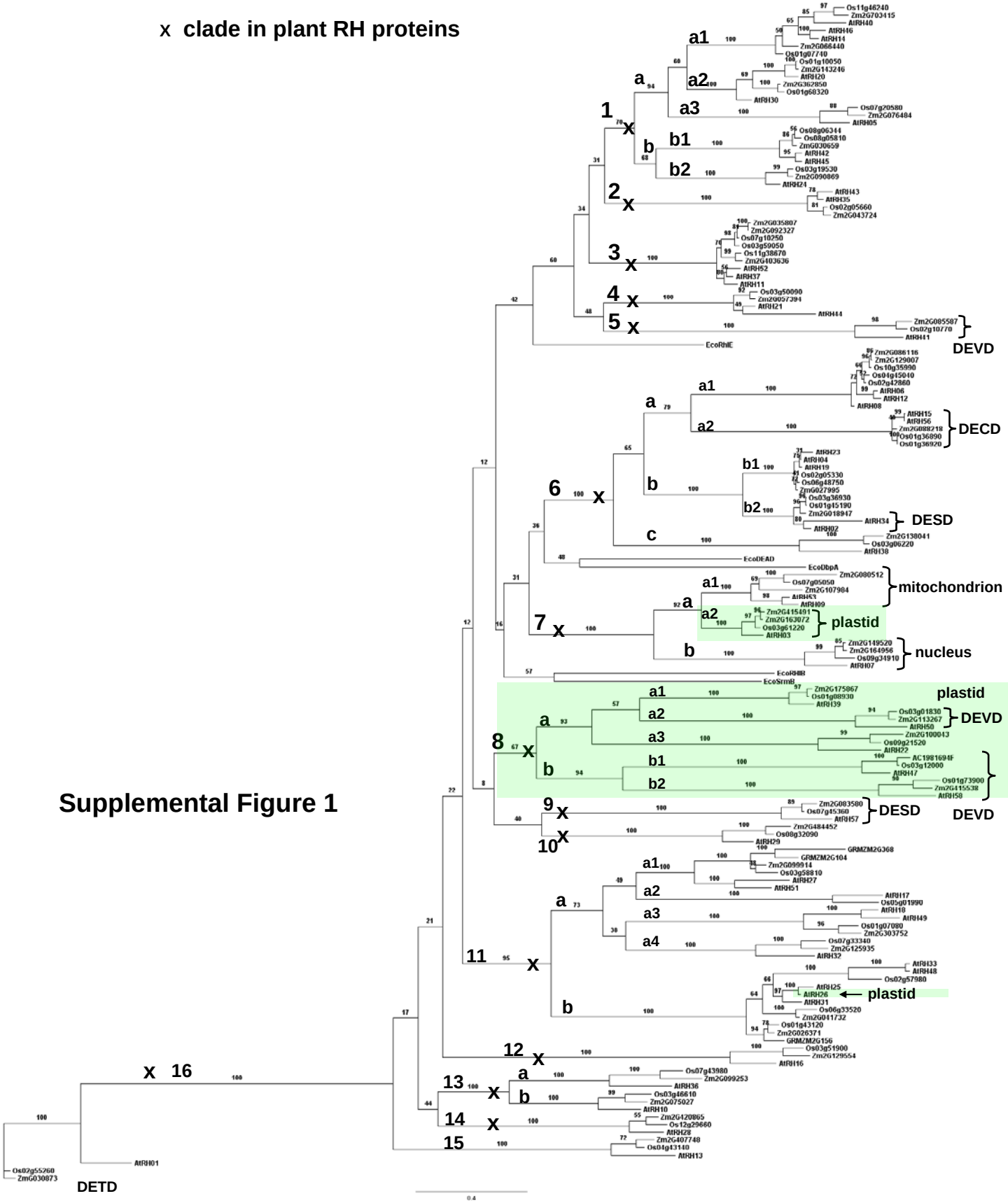
Supplemental figure 4. PCR genotype of double knock down mutant rh3-4/clpr2-1.Primer pairs used for PCR genotyping are shown at the top panels with black arrows. Genomic DNA was isolated from albino, pale-green, wild type (WT) progenies from F2 generation. All of albino plants are double homozygous to *rh3-4* and *clpr2-1,* whereas, pale-green mutants are homozygous to a single mutant. None of wild-type phenotypes are homozygote.

Supplemental figure 5. RH3 in wild type and *clpr2-1* mutant are in the same fraction in sucrose gradients. Arabidopsis wild type (WT) or *clpr2-1* stroma was sedimented through 10-40 % sucrose gradient. An equal portion of each fraction was detected by immunoblots by anti-RH3 antiserum. The blots stained with Ponceau S are showed the place of Rubisco at 550 kDa.

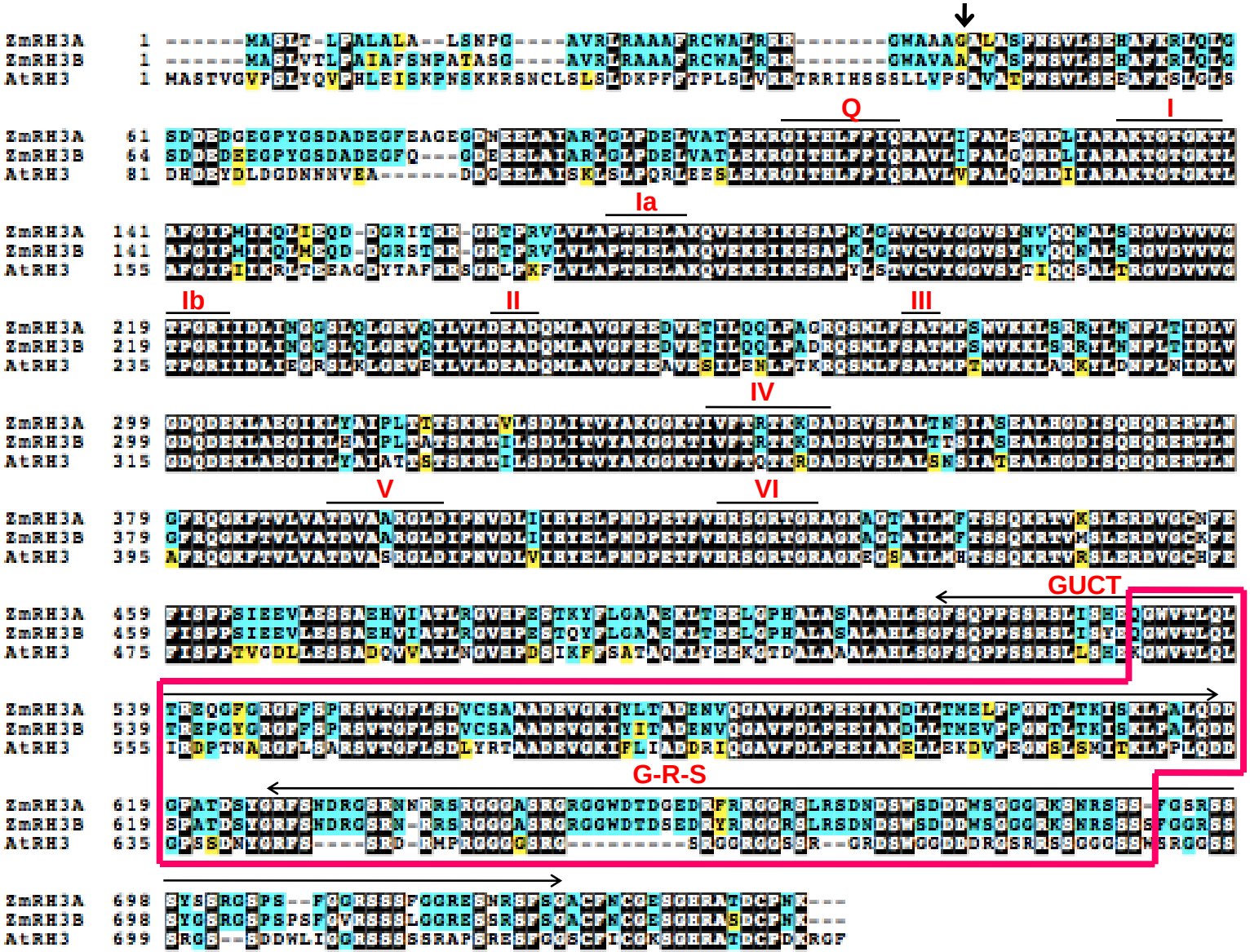
Supplemental Figure 6. RH3 functions and chloroplast splicing factors and their intron target in land plants. Introns in land plant chloroplasts are categorized to group I, subgroups IIA and IIB according to (Michel et al., 1989). RH3 functions both in group IIA intron splicing and ribosome biogenesis. Those found in *Arabidopsis* but not in maize are marked with asterisks. Nucleus-encoded splicing factors are annotated with their conserved domains. Solid arrows mark interactions supported by either genetic or co-IPs data, but not in both. Updated from (Barkan, 2011).

56 Arabidopsis & 46 maize & 47 rice & 5 *E. coli*.
Core domain (minus gaps) with some C-term extension

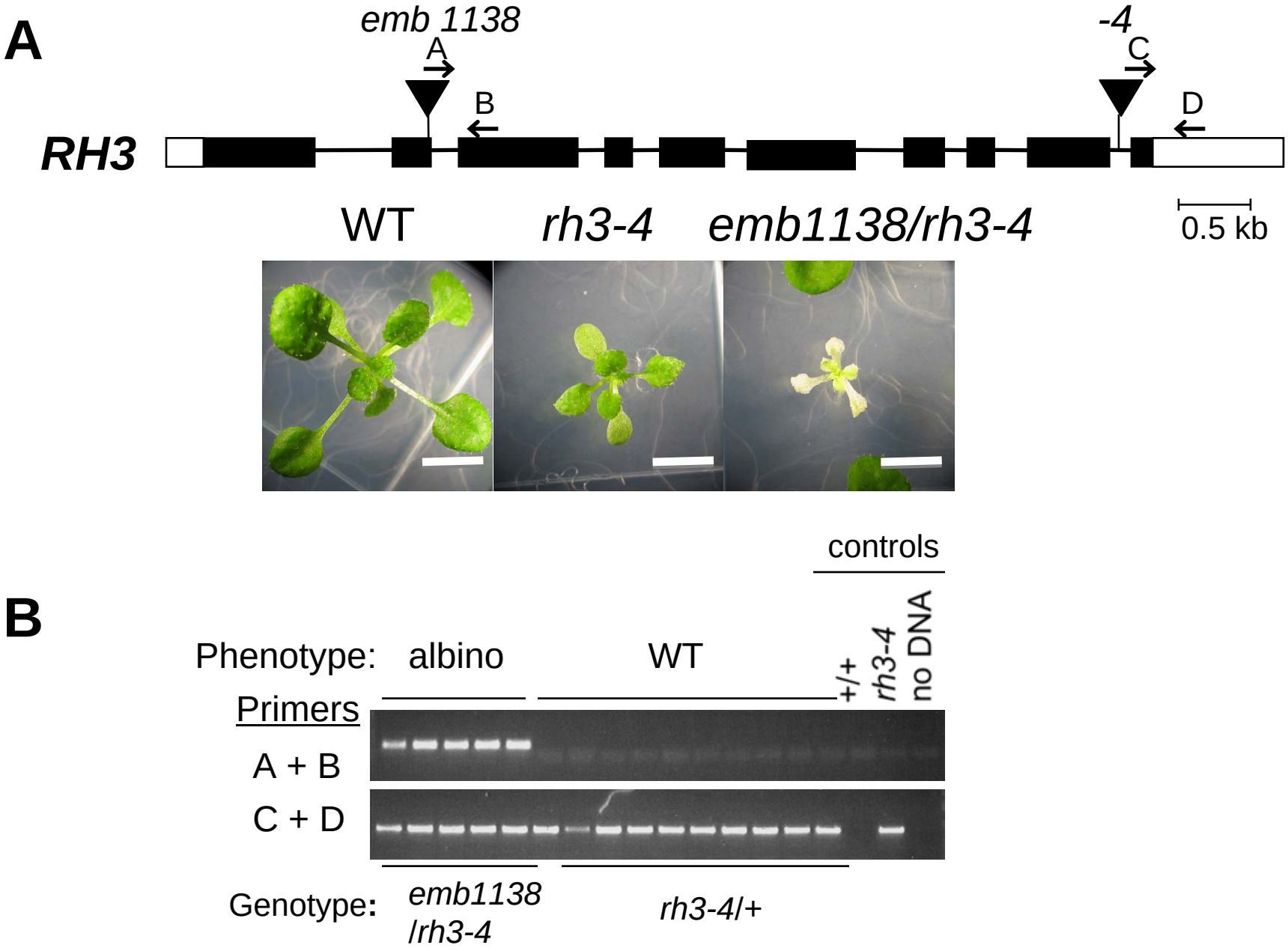
x **clade in plant RH proteins**



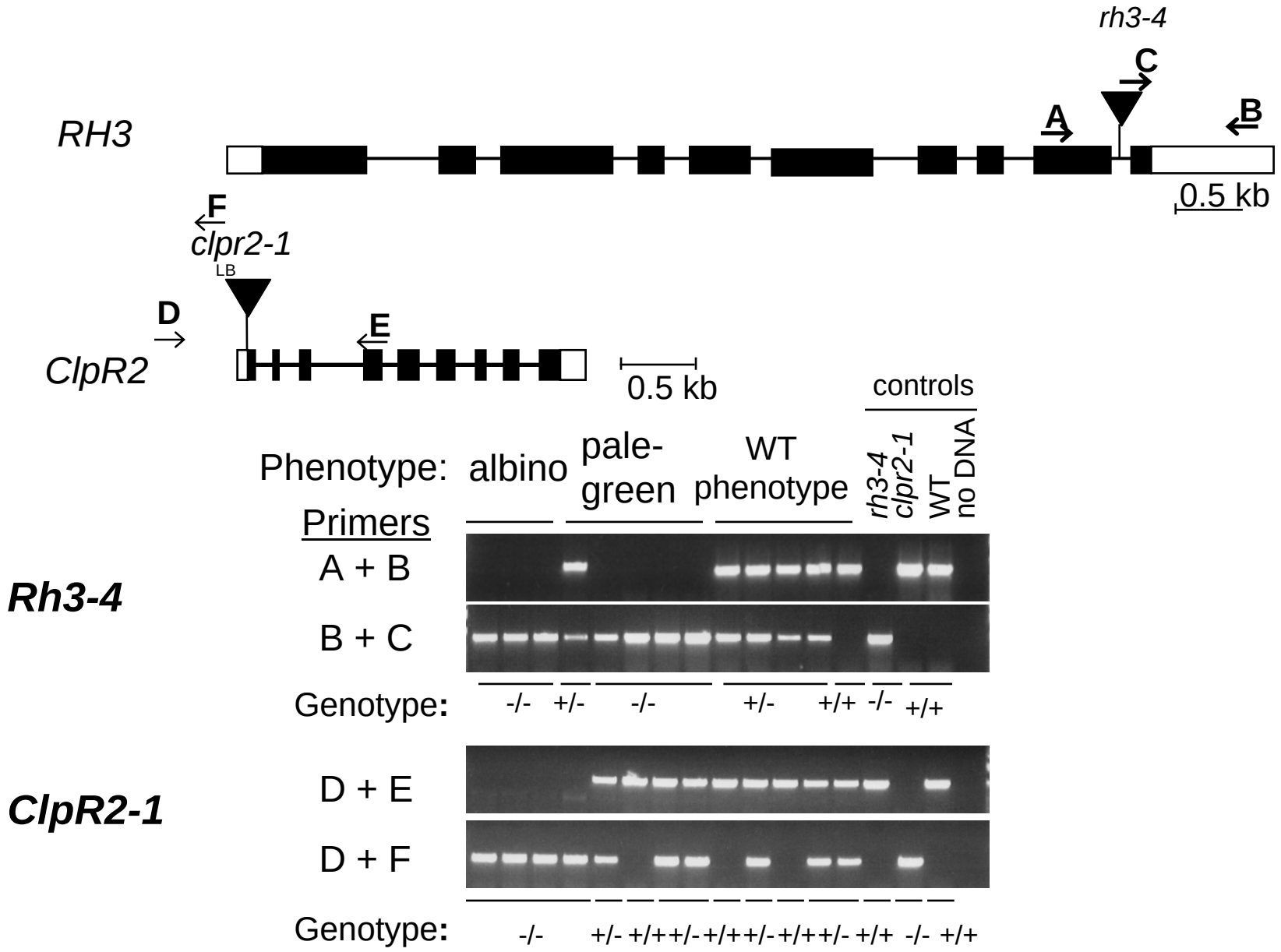
Supplemental Figure 1



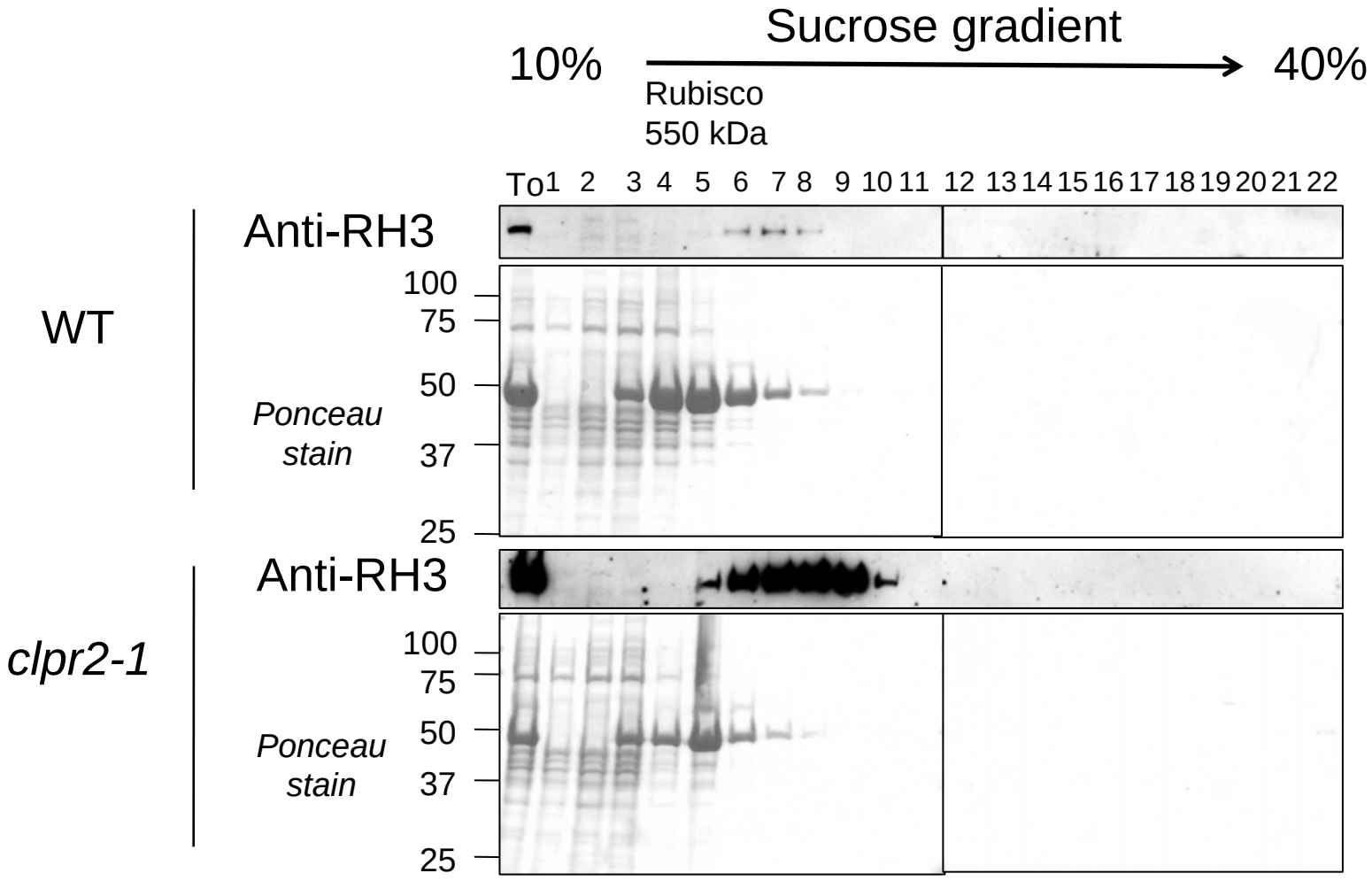
Suppl. Fig.2


A
emb 1138
A
B
-4
C
D
RH3
WT
rh3-4
emb1138/rh3-4
0.5 kb
B
controls
Phenotype:
albino
WT
+/+
rh3-4
no DNA
Primers
A + B
C + D
Genotype:
emb1138
/rh3-4
rh3-4/+

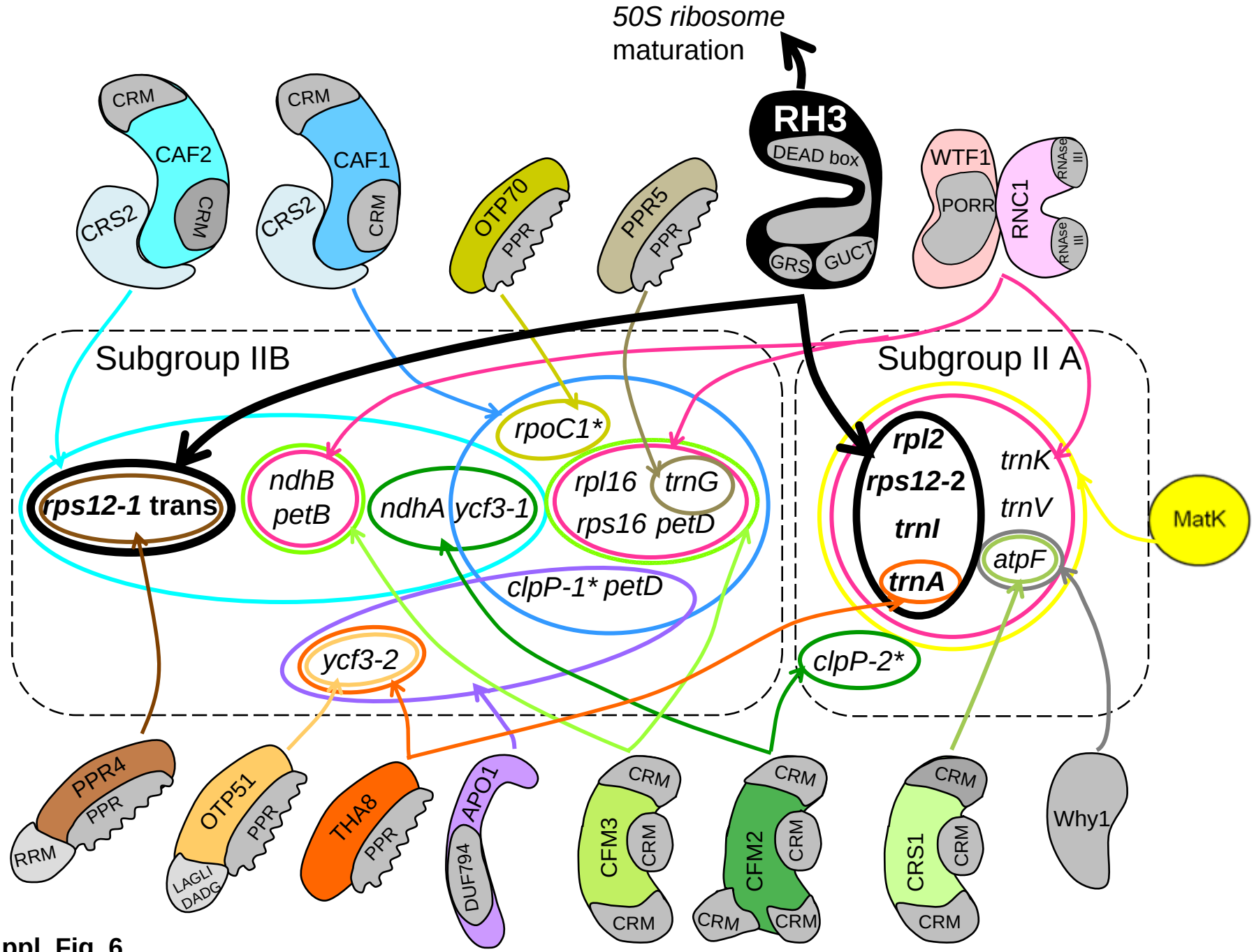
Suppl. Fig. 3


rh3-4
C
RH3
A
B
0.5 kb
F
clpr2-1
LB
D
E
ClpR2
0.5 kb
controls
Phenotype:
albino
pale-green
WT phenotype
rh3-4
clpr2-1
WT no DNA
Primers
A + B
Rh3-4
B + C
Genotype:
-/- +/- -/- +/- +/+ -/- +/+
D + E
ClpR2-1
D + F
Genotype:
-/- +/- +/+ +/- +/+ +/- +/+ +/- +/+ -/- +/+

Suppl Fig. 4


Sucrose gradient
10%
40%
Rubisco
550 kDa
To 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
WT
Anti-RH3
100
75
50
37
25
Ponceau stain
clpr2-1
Anti-RH3
100
75
50
37
25
Ponceau stain

Suppl. Fig. 5


50S ribosome maturation
RH3
DEAD box
GRS
GUCT
CRM
CAF2
CRS2
CAF1
OTP70
PPR
PPR5
WTF1
PORR
RNC1
RNAse III
Subgroup IIB
Subgroup II A
rps12-1 trans
ndhB petB
ndhA ycf3-1
rpoC1*
rpl16 trnG rps16 petD
clpP-1* petD
ycf3-2
rpl2 rps12-2 trnI trnA
trnK trnV atpF
clpP-2*
MatK
PPR4
RRM
OTP51
LAGLI DADG
THA8
APO1
DUF794
CFM3
CFM2
CRS1
Why1

Suppl. Fig. 6