Expanded View Figures

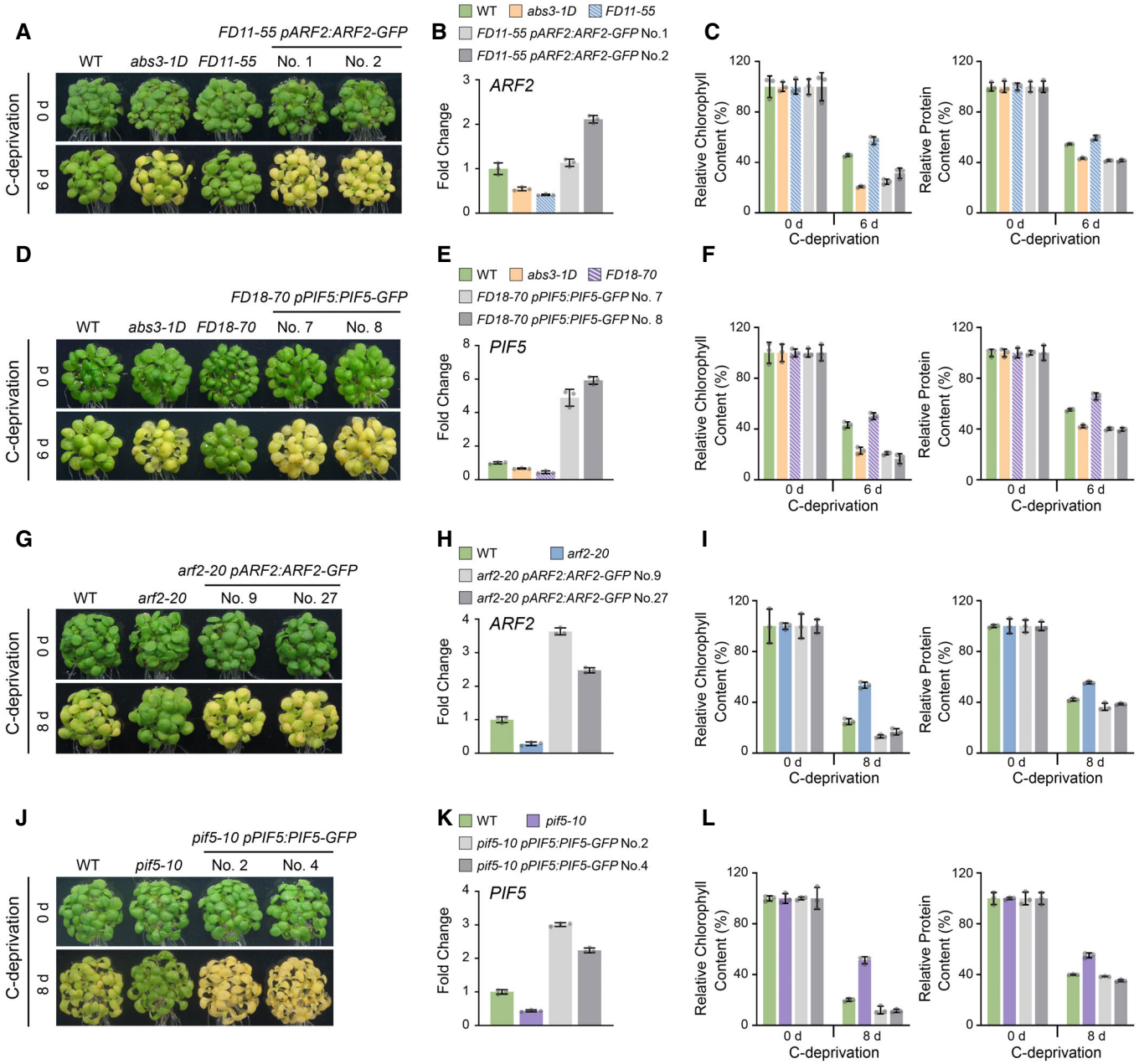

Figure EV1.

Figure EV1. Complementation of *FD11-55*, *arf2-20*, *FD18-70*, and *pif5-10*.

A Seedlings of WT, *abs3-1D*, *FD11-55*, and two representative complementation lines expressing *pARF2:ARF2-GFP* in the *FD11-55* background before and after 6 days C-deprivation.
B RT-qPCR analysis of *ARF2* expression levels in 7-day-old seedlings of the same genotypes as shown in (A).
C Chlorophyll and protein content reduction in genotypes shown in (A) after 6 days C-deprivation. Color legends in (C) are the same as in (B).
D Seedlings of WT, *abs3-1D*, *FD18-70*, and two representative complementation lines expressing *pPIF5:PIF5-GFP* in the *FD18-70* background before and after 6 days C-deprivation.
E RT-qPCR analysis of *PIF5* expression levels in 7-day-old seedlings of the same genotypes as shown in (D).
F Chlorophyll and protein content reduction in genotypes shown in (D) after 6 days C-deprivation. Color legends in (F) are the same as in (E).
G Seedlings of WT, *arf2-20*, and two representative complementation lines expressing *pARF2:ARF2-GFP* in the *arf2-20* background before and after 8 days C-deprivation.
H RT-qPCR analysis of *ARF2* expression levels in 7-day-old seedlings of the same genotypes as shown in (G).
I Chlorophyll and protein content reduction in genotypes shown in (G) after 8 days C-deprivation. Color legends in (I) are the same as in (H).
J Seedlings of WT, *pif5-10*, and two representative complementation lines expressing *pPIF5:PIF5-GFP* in the *pif5-10* background before and after 8 days C-deprivation.
K RT-qPCR analysis of *PIF5* expression levels in 7-day-old seedlings of the same genotypes as shown in (J).
L Chlorophyll and protein content reduction in genotypes shown in (J) after 8 days C-deprivation. Color legends in (L) are the same as in (K).

Data information: In B, E, H, and K, fold changes were calculated with respect to the expression level in the WT. In B, C, E, F, H, I, K, and L, data are means ± s.d. of three biological replicates.
Source data are available online for this figure.

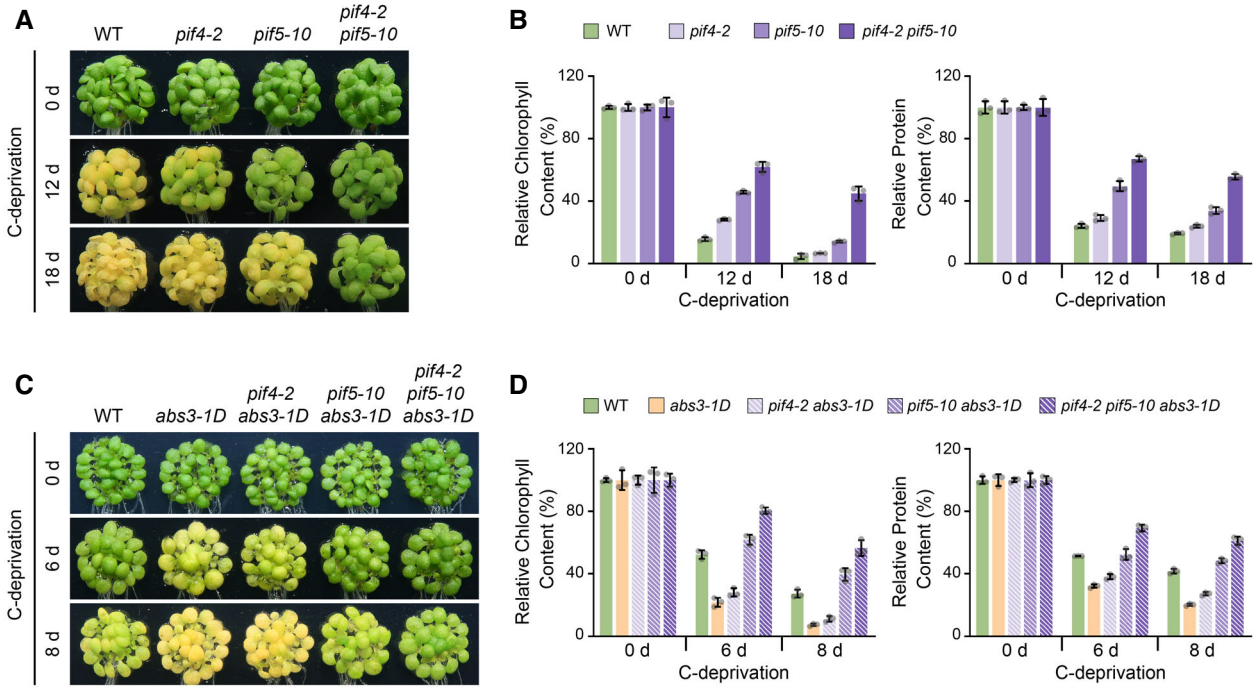

Figure EV2. PIF5 and PIF4 share redundant roles in the ABS3-mediated senescence pathway.

A Seedlings of WT, *pif4-2*, *pif5-10*, and *pif4-2 pif5-10* before and after 12 days and 18 days C-deprivation.
B Chlorophyll and protein content reduction in genotypes shown in (A) after 12 days and 18 days C-deprivation.
C Seedlings of WT, *abs3-1D*, *pif4-2 abs3-1D*, *pif5-10 abs3-1D*, and *pif4-2 pif5-10 abs3-1D* before and after 6 days and 8 days C-deprivation.
D Chlorophyll and protein content reduction in genotypes shown in (C) after 6 days and 8 days C-deprivation.

Data information: In B and D, data are means ± s.d. of three biological replicates.
Source data are available online for this figure.

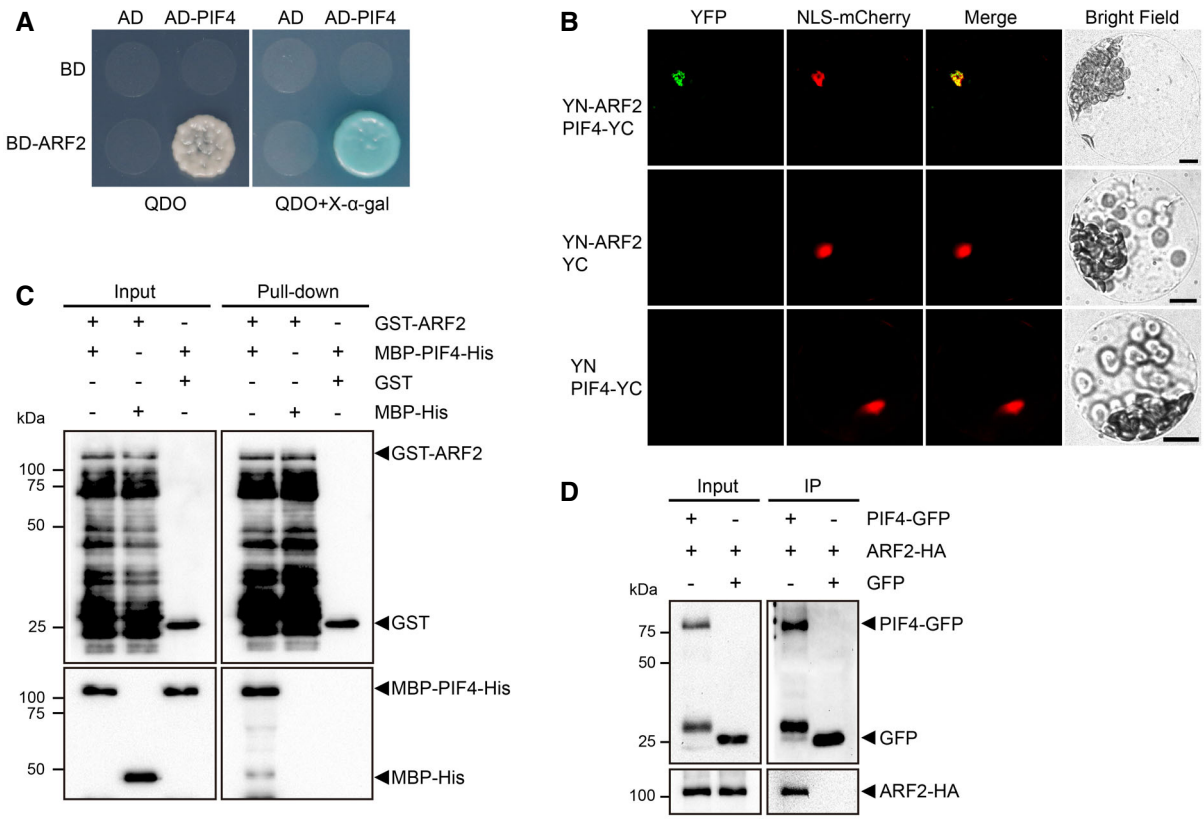

Figure EV3. ARF2 interacts directly with PIF4.

A Interaction between ARF2 and PIF4 in the yeast two-hybrid assay. Growth of yeast cells co-transformed with indicated BD and AD vectors was monitored on quadruple dropout medium (QDO, SD-Ade/-His/-Leu/-Trp) and QDO medium containing X-α-gal.

B Interaction between ARF2 and PIF4 in protoplast BiFC assays. YN-ARF2 and PIF4-YC were coexpressed with the nuclear marker NLS-mCherry in *Arabidopsis* mesophyll protoplasts. Coexpression of YN-ARF2 and YC alone or YN alone with PIF4-YC served as negative controls.

C Direct interaction between GST-ARF2 and MBP-PIF4-His in *in vitro* GST pull-down assays.

D Co-immunoprecipitation of ARF2-HA with PIF4-GFP *in vivo*. ARF2-HA was coexpressed with PIF4-GFP or GFP alone in protoplasts. Proteins co-immunoprecipitated by GFP-Trap beads were immunoblotted with anti-GFP and anti-HA antibodies.

Source data are available online for this figure.

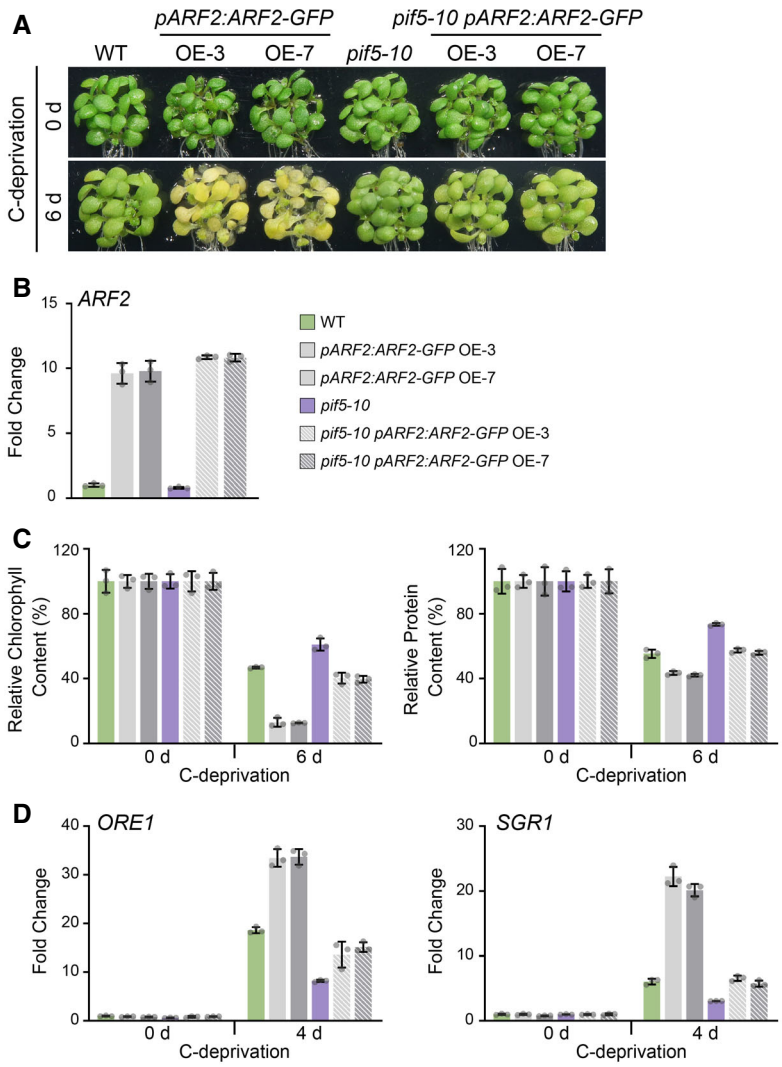

Figure EV4. ARF2 requires PIF5 to promote senescence.

A Seedlings of WT, *pARF2:ARF2-GFP* OEs in the WT background, *pif5-10*, and *pARF2:ARF2-GFP* OEs in the *pif5-10* background before and after 6 days C-deprivation.

B RT-qPCR analysis of *ARF2* expression levels in 7-day-old seedlings of the same genotypes as shown in (A). Fold changes were calculated with respect to the expression level in the WT.

C Chlorophyll and protein content reduction in genotypes shown in (A) after 6 days C-deprivation.

D RT-qPCR analysis of *ORE1* and *SGR1* expression levels in genotypes shown in (A) before and after 4 days C-deprivation. Fold changes were calculated with respect to the expression level in the WT before C-deprivation. Color legends in (C) and (D) are the same as in (B).

Data information: In B-D, data are means ± s.d. of three biological replicates.
Source data are available online for this figure.

Figure EV5. *ORE1* and *SGR1* are direct output of the ARF2-PIF5 functional module.

A Seedlings of WT, *abs3-1D*, *ore1-101*, and *ore1-101 abs3-1D* before and after 6 days C-deprivation.
B Chlorophyll and protein content reduction in genotypes shown in (A) after 6 days C-deprivation.
C RT-qPCR analyses of *ABS3* expression levels in 7-day-old seedlings of the same genotypes as shown in (A).
D RT-qPCR analysis of *ORE1* and *SGR1* expression levels in genotypes shown in (A) before and after 4 days C-deprivation. Color legends in (C) and (D) are the same as in (B).
E Seedlings of WT, *abs3-1D*, and *sgr1-101 abs3-1D* before and after 6 days C-deprivation.
F Chlorophyll and protein content reduction in genotypes shown in (E) after 6 days C-deprivation.
G RT-qPCR analysis of *ABS3* expression levels in 7-day-old seedlings of the same genotypes as shown in (E).
H RT-qPCR analysis of *ORE1* and *SGR1* expression levels in genotypes shown in (E) before and after 4 days C-deprivation. Color legends in (G) and (H) are the same as in (F).
I Seedlings of WT, *mateq*, and two representative lines expressing *pORE1:ORE1-GFP* in the *mateq* background before and after 9 days C-deprivation.
J Chlorophyll and protein content reduction in genotypes shown in (I) after 9 days C-deprivation.
K RT-qPCR analysis of *ORE1* and *SGR1* expression levels in genotypes shown in (I) before and after 4 days C-deprivation. Color legends in (K) are the same as in (J).
L Seedlings of WT, *mateq*, and two representative lines expressing *pSGR1:SGR1-GFP* in the *mateq* background before and after 9 days C-deprivation.
M Chlorophyll and protein content reduction in genotypes shown in (L) after 9 days C-deprivation.
N RT-qPCR analysis of *ORE1* and *SGR1* expression levels in genotypes shown in (L) before and after 4 days C-deprivation. Color legends in (N) are the same as in (M).

Data information: In C and G, fold changes were calculated with respect to the expression level in the WT. In D, H, K, and N, fold changes were calculated with respect to the expression level in the WT before C-deprivation. In B–D, F–H, J, K, M, and N, data are means ± s.d. of three biological replicates.
Source data are available online for this figure.

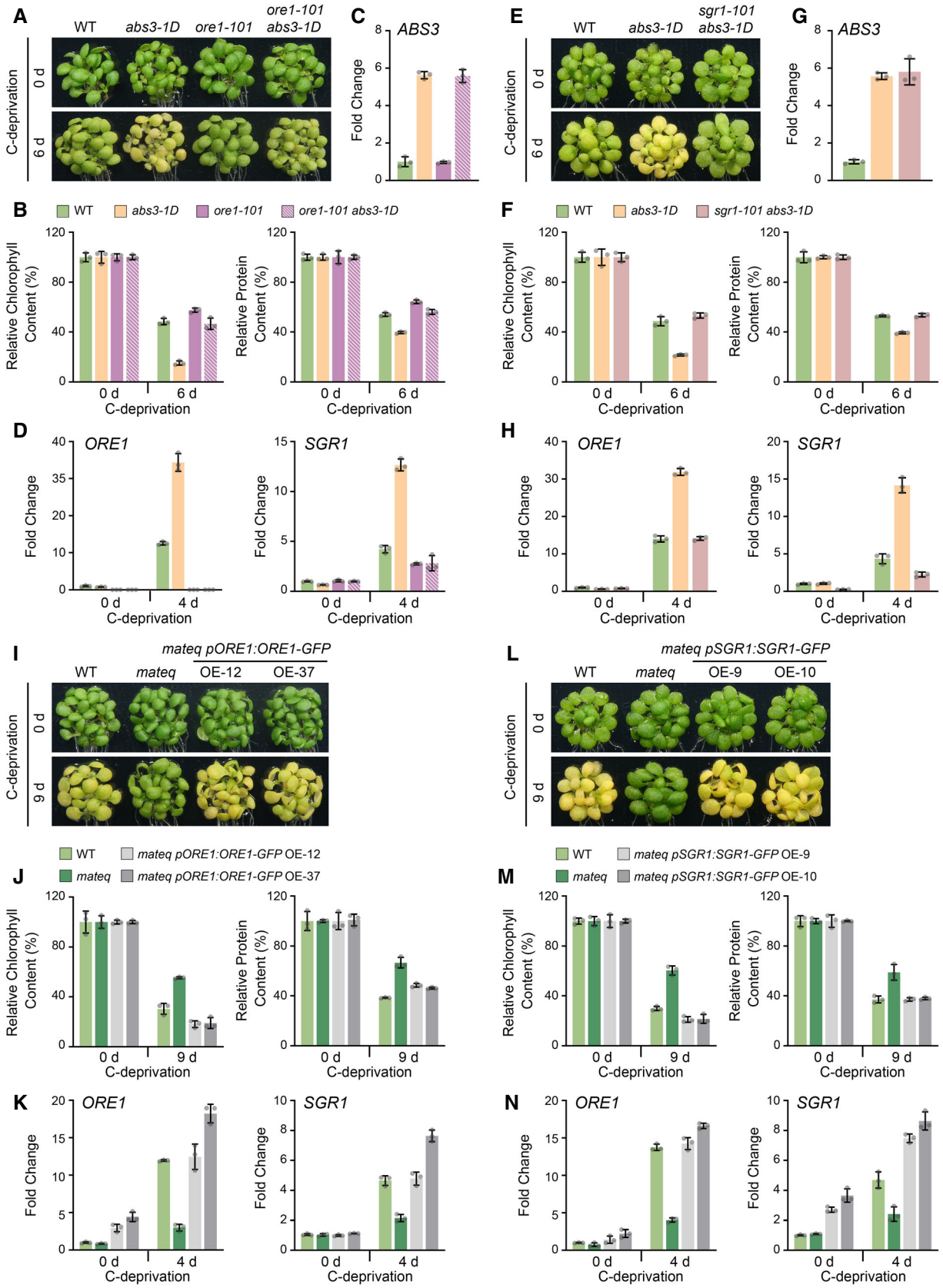
A
WT
abs3-1D
ore1-101
ore1-101 abs3-1D
C-deprivation
0 d
6 d
C
ABS3
Fold Change
E
WT
abs3-1D
sgr1-101 abs3-1D
G
ABS3
B
WT
abs3-1D
ore1-101
ore1-101 abs3-1D
Relative Chlorophyll Content (%)
Relative Protein Content (%)
C-deprivation
F
WT
abs3-1D
sgr1-101 abs3-1D
D
ORE1
SGR1
Fold Change
4 d
H
I
mateq pORE1:ORE1-GFP
mateq
OE-12
OE-37
9 d
L
mateq pSGR1:SGR1-GFP
OE-9
OE-10
J
WT
mateq pORE1:ORE1-GFP OE-12
mateq
mateq pORE1:ORE1-GFP OE-37
M
WT
mateq pSGR1:SGR1-GFP OE-9
mateq
mateq pSGR1:SGR1-GFP OE-10
K
N

Figure EV5.