COUPLED TRANSPORT OF ARABIDOPSIS p24 PROTEINS AT THE ER-GOLGI INTERFACE

Juan Carlos Montesinos*[1], Silke Sturm*[2], Markus Langhans[2], Stefan Hillmer[2], María Jesús Marcote[1], David G. Robinson[2] and Fernando Aniento[1,#]

SUPPLEMENTARY MATERIAL

Figure S1. Nt- and Ct-sequences of Arabidopsis p24 proteins.

Sequence of the N-terminus and the C-terminus of the 11 members of the p24 family in Arabidopsis, including those from the delta subfamily (p24δ3-p24δ11) and those from the beta subfamily (p24β2-p24β3). Sequences used to obtain peptide antibodies are underlined.

Figure S2. Characterization of T-DNA insertion mutants.

A. RT-PCR analysis of *p24δ5-1*, *p24δ4-1* mutants to show the absence of *p24δ5 and p24δ4* mRNA, respectively. Total RNA from leaves of the T-DNA insertion mutant and wild-type plants were used for the RT-PCR. In the PCRs, gene specific primers were used. *Actin7* (*ACT7*) was used as a control. B. *p24δ5-1*, *p24δ4-1* and *p24δ4δ5* mutant seedlings grown on vertical agar plates for 6 days did not show a phenotype different from that of wild-type (Col-0)

plants. C. Three-week-old *p24δ5-1*, *p24δ4-1* and *p24δ4δ5* mutant plants did not show a phenotype different from that of wild-type (Col-0) plants.

Figure S3. Localization of RFP-p24δ5 and deletion mutants and colocalization between GFP-p24β2 and RFP-p24δ5 or RFP-p24δ5(ΔCC) in *Arabidopsis* protoplasts.

A-L. Transient gene expression in *Arabidopsis* protoplasts. A-C. RFP-p24δ5 (A) shows a typical ER pattern and colocalizes with the ER marker GFP-HDEL (B) (merged image in C). D-F. RFP-p24δ5 deletion mutants lacking the coiled-coil domain (ΔCC) (D), the GOLD domain (ΔGOLD) (E) or both (TMCT) (F) show a typical ER pattern and colocalize with the ER marker GFP-HDEL. G-I. GFP-p24β2 (G) and RFP-p24δ5 (H) colocalize in punctate structures (see merged images in I). J-L. GFP-p24β2 (J) and RFP-p24δ5 (ΔCC) (K) do not colocalize (see merged image in L). Scale bars = 5 μm.

Figure S4. RFP-p24δ5 and RFP-p24δ5 (ΔCC) localize to the ER at different expression levels.

A-P. Transient gene expression in tobacco mesophyl protoplasts using the indicated concentrations of DNA (from 0.3 to 10 μg). A-H. RFP-p24δ5 shows a typical ER pattern at all DNA concentrations and both at 5 (A-D) or 24 (E-H) hours post-electroporation. I-P. RFP-p24δ5(ΔCC) shows a typical ER pattern at all DNA concentrations and both at 5 (I-L) or 24 (M-P) hours post-electroporation. Scale bars = 5 μm.

Figure S5. Co-expression of RFP-p24δ5 and different DNA concentrations of GFP-p24β2.

A-I. Transient gene expression in tobacco mesophyll protoplasts. An increased ratio in the concentrations of GFP-p24β2 (A, 5 μg; D, 25 μg; G, 50 μg) vs RFP-p24δ5 (35 μg, B, E and H) induces a progressive change in the localization of RFP-p24δ5, from a mainly reticulate pattern (B) to a mostly punctate one (H), and increased colocalization between both proteins (merged images in C, F and I). Scale bars = 5 μm.

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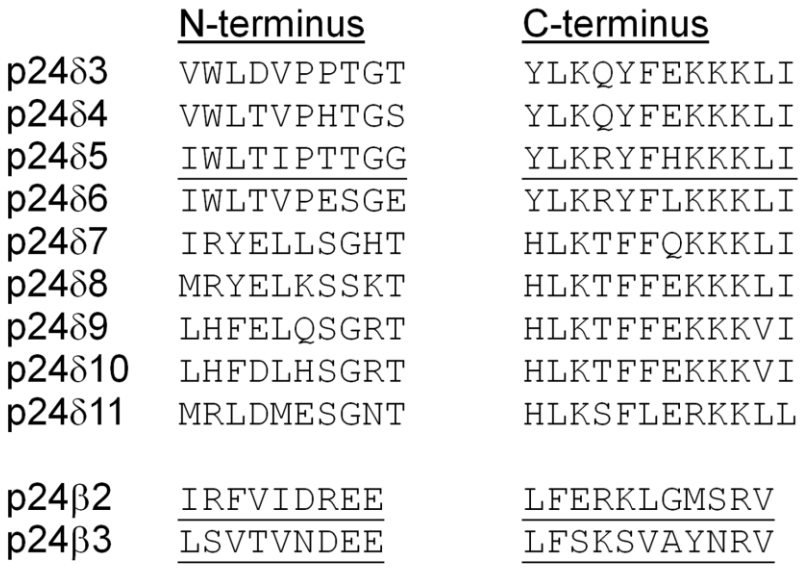
Figure S7. 2 h BFA treatment redistributes RFP-p24δ5 and GFP-p24β2 to the ER.

A-C. Transient gene expression in tobacco mesophyll protoplasts. Treatment with BFA (90 μM, 120 min) after coexpression of GFP-p24β2 (A) and RFP-p24δ5 (B) may induce a complete relocalization of both proteins to the ER (merged image in C). Scale bars = 5 μm.

Figure S8. A comparison between frame scan and line scan modes for colocalization studies. A-L. Transient gene expression in tobacco mesophyll protoplasts. A-F. GFP-p24β2 (A, D) colocalizes partially with the Golgi marker Man1-RFP (B, E) in punctate structures (merged images in C and F), both in the frame scan mode (A-C) and in the line scan mode (D-F). G-L. GFP-p24β2 (G, J) punctae do not colocalize with the PVC marker ARA6-RFP (H, K) (merged images in I and L), neither in the frame scan mode (G-I) nor in the line scan mode (J-L).

Figure S1



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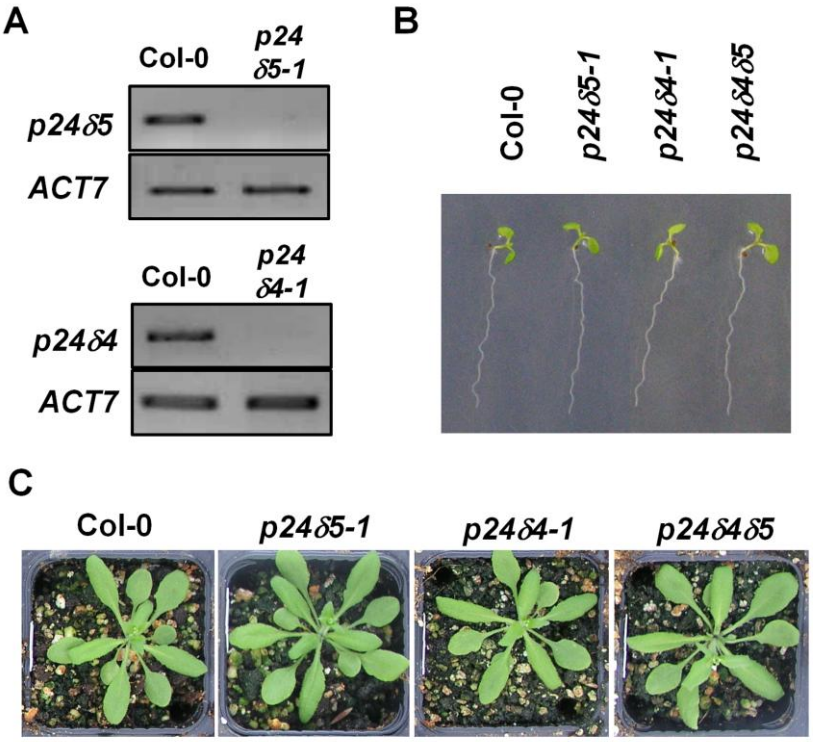

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Figure S3

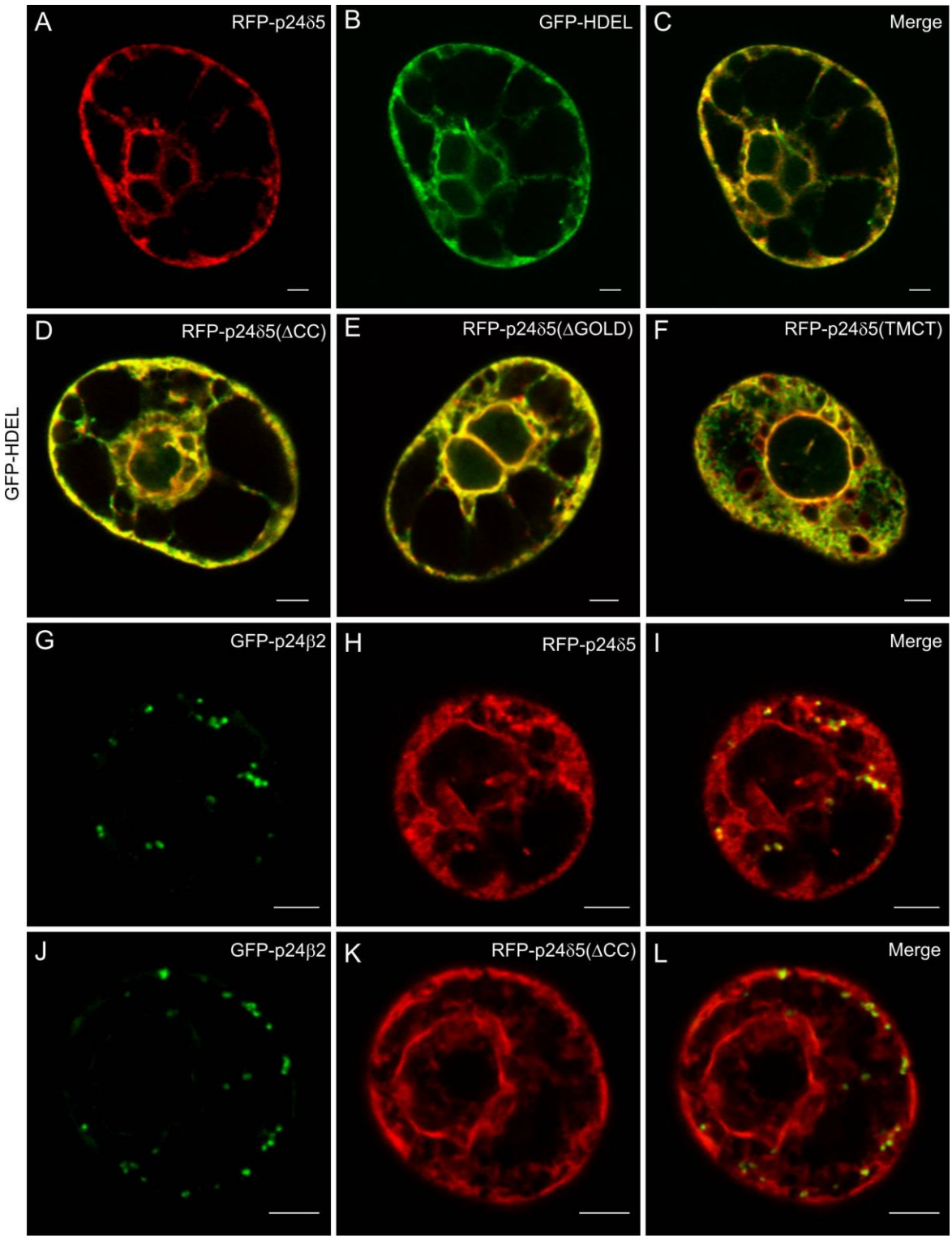
A
RFP-p24δ5
B
GFP-HDEL
C
Merge
D
RFP-p24δ5(ΔCC)
E
RFP-p24δ5(ΔGOLD)
F
RFP-p24δ5(TMCT)
GFP-HDEL
G
GFP-p24β2
H
RFP-p24δ5
I
Merge
J
GFP-p24β2
K
RFP-p24δ5(ΔCC)
L
Merge

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Figure S4

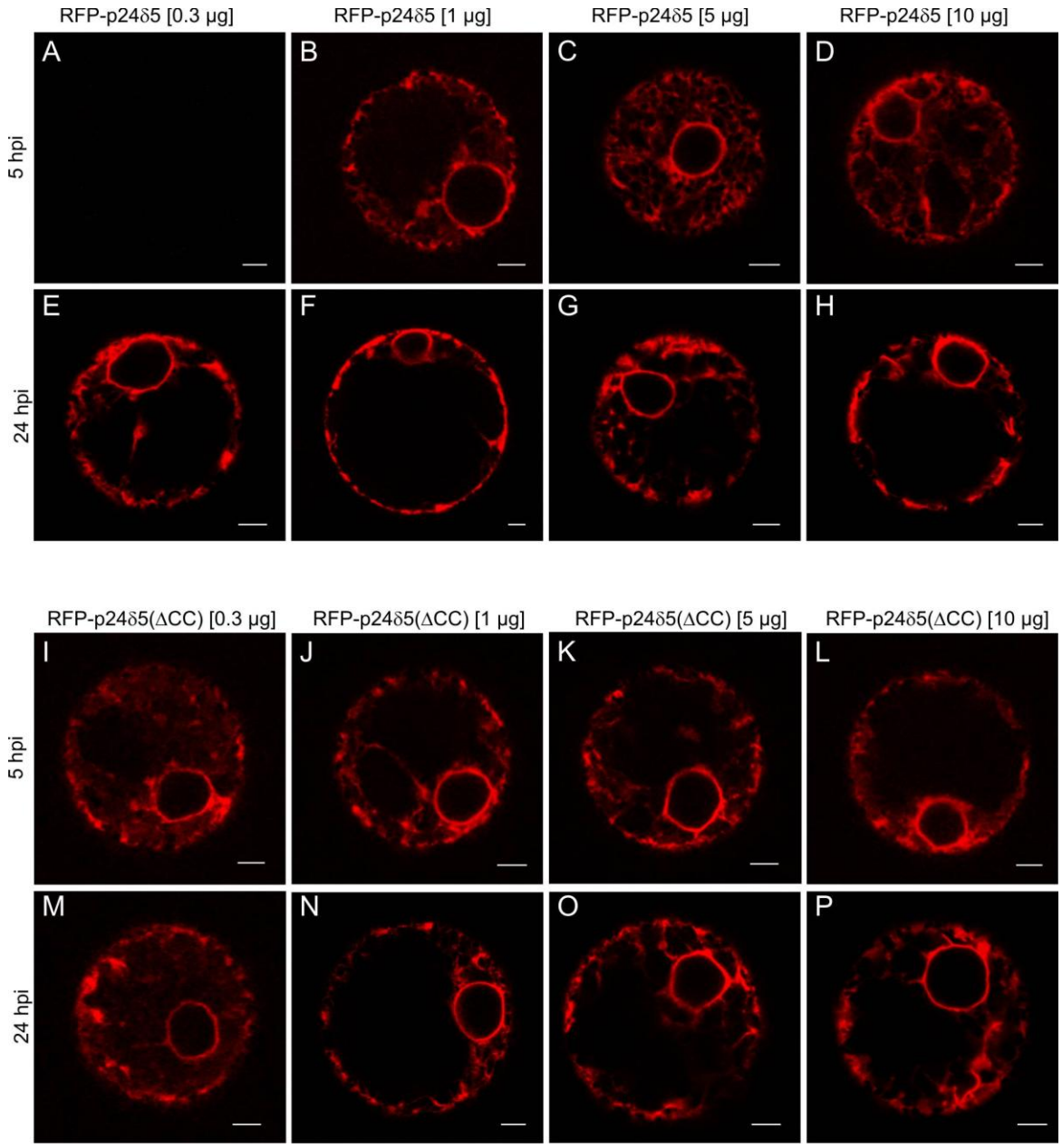

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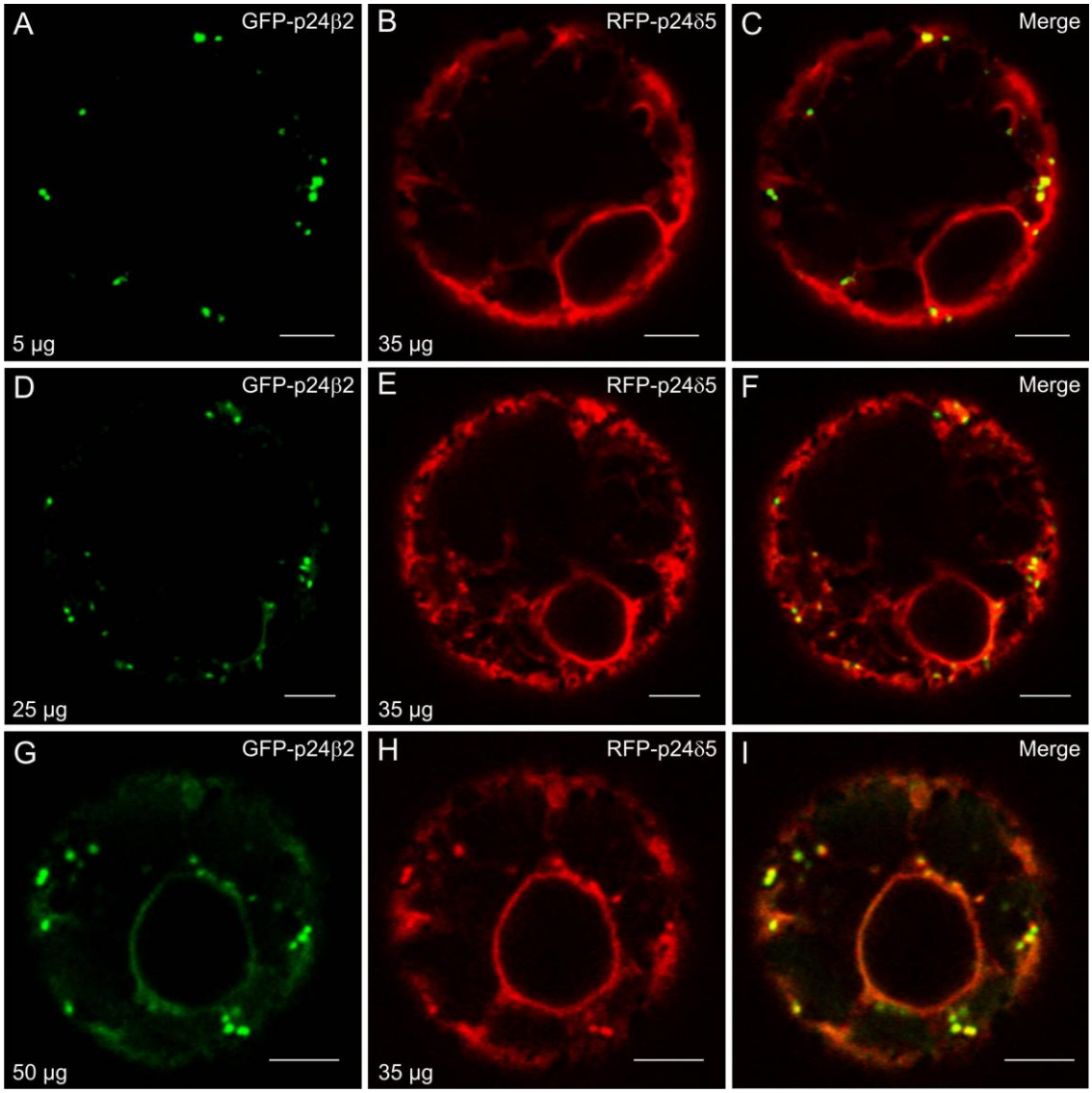
Figure S5



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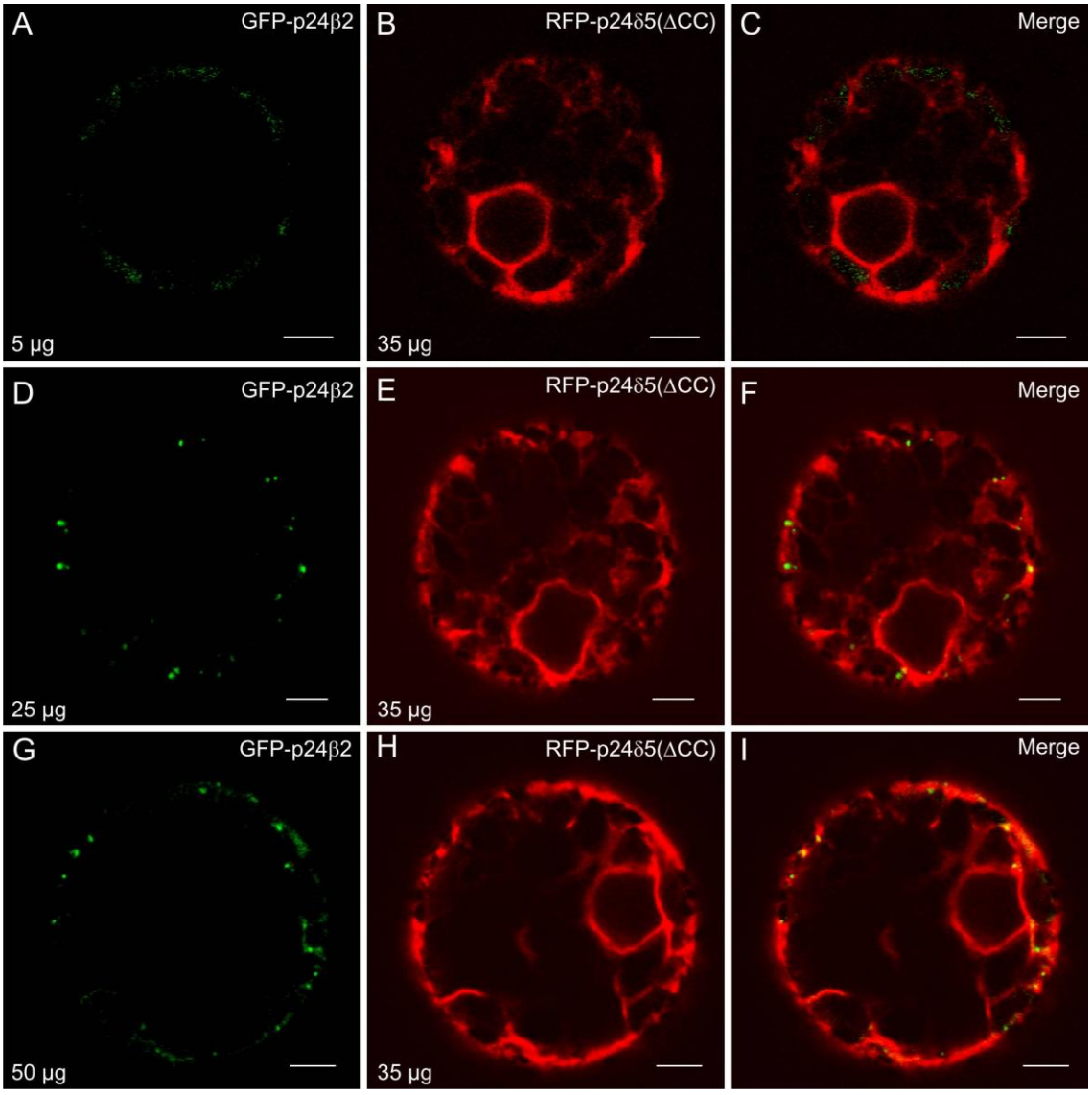

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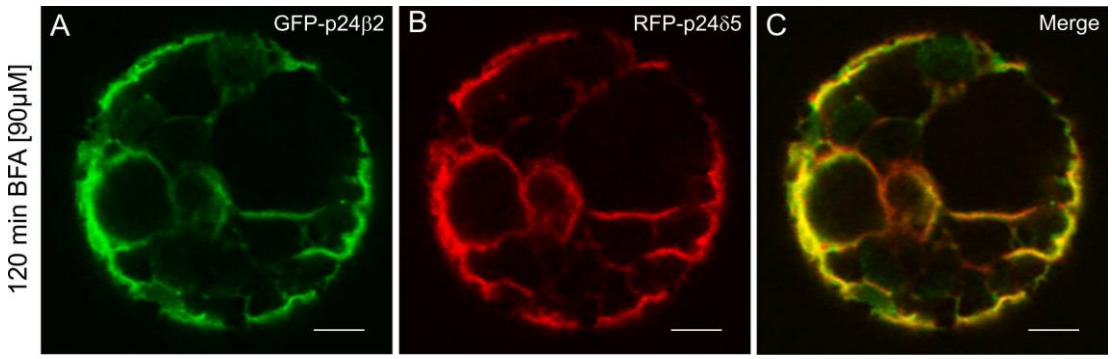

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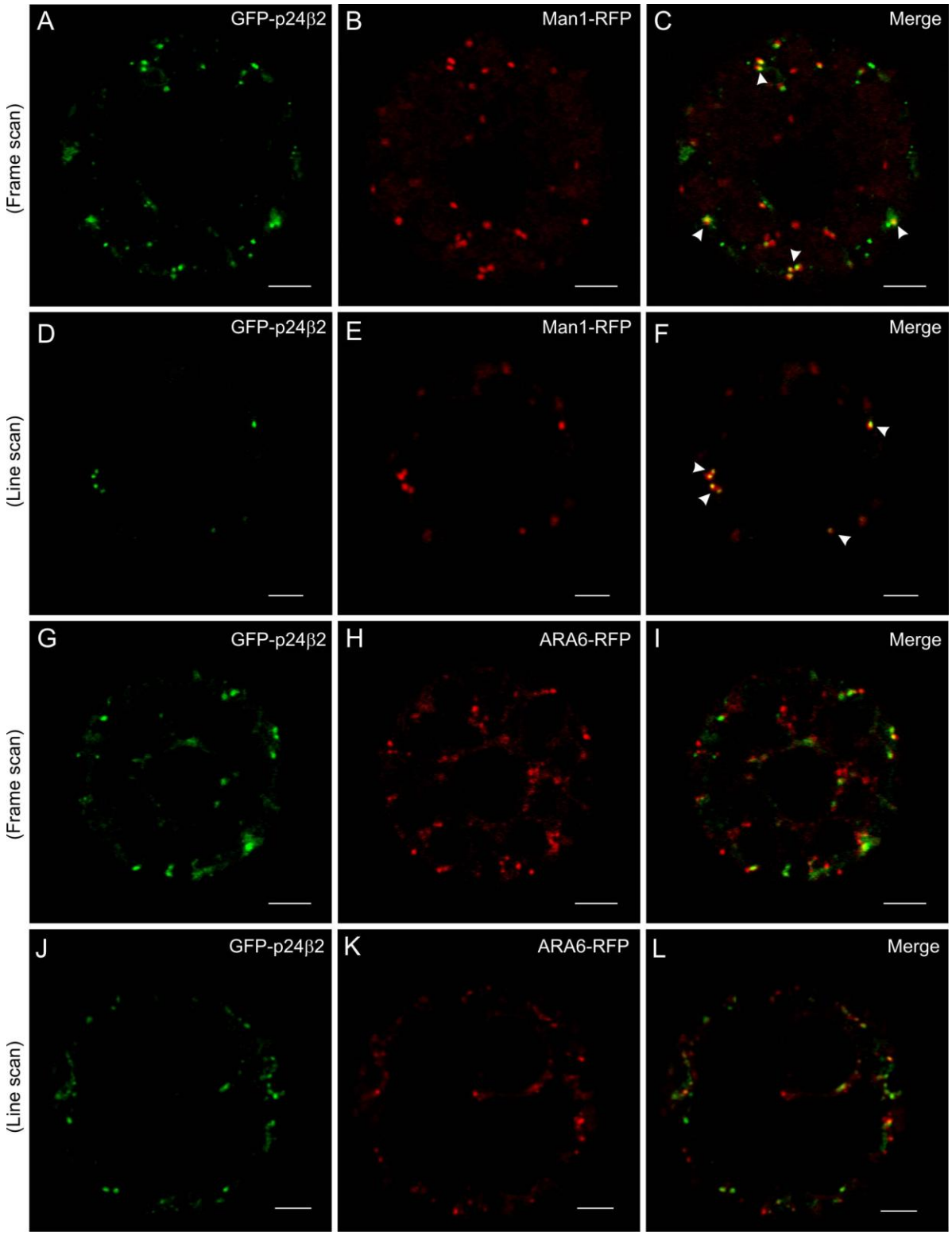
A
GFP-p24β2
B
Man1-RFP
C
Merge
(Frame scan)
D
GFP-p24β2
E
Man1-RFP
F
Merge
(Line scan)
G
GFP-p24β2
H
ARA6-RFP
I
Merge
(Frame scan)
J
GFP-p24β2
K
ARA6-RFP
L
Merge
(Line scan)

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