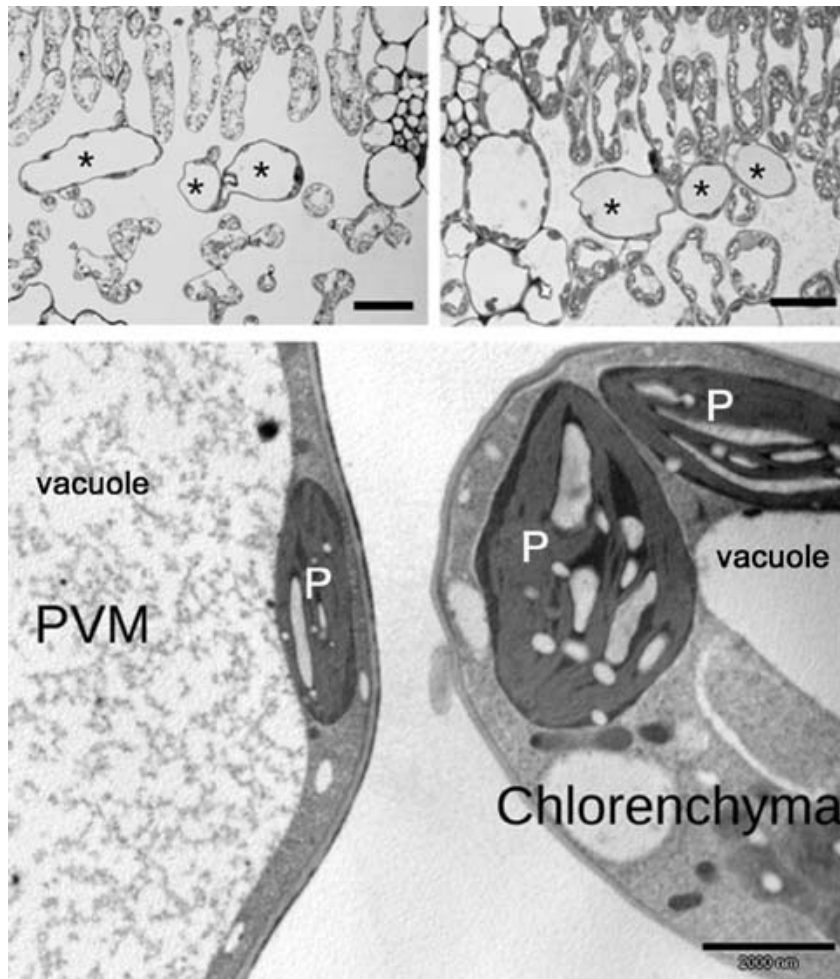


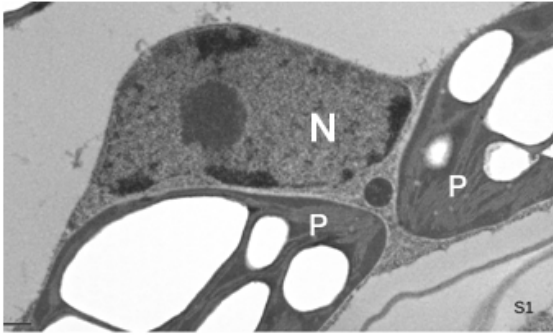
Accessory Publication

Immuno-labelling patterns of Vlx isoforms in soybean leaves

Images A1–A3 represent electron micrographs illustrating soybean leaf architecture. A1. Cross section of a mature leaf from an untreated control plant shows laterally expanded paraveinal mesophyll (PVM; indicated by asterisks) between the palisade and spongy mesophyll chlorenchyma (MC) (bar = 35 μm). A2. Cross section of a mature leaf from a Detip-treated soybean plant (time point D15; for details see main manuscript, Fig. 1). PVM cells (asterisks) in this specimen are denser in appearance than those of controls (bar = 40 μm). A3. Electron micrograph comparing a PVM and an MC cell in a Detip-treated soybean leaf (time point D15). The chloroplast (P) of the PVM cell is evidently smaller than that of the mesophyll chlorenchyma (MC) cell, and its central vacuole (V) contains evident flocculent material that is absent from the MC cell (bar = 2 μm).

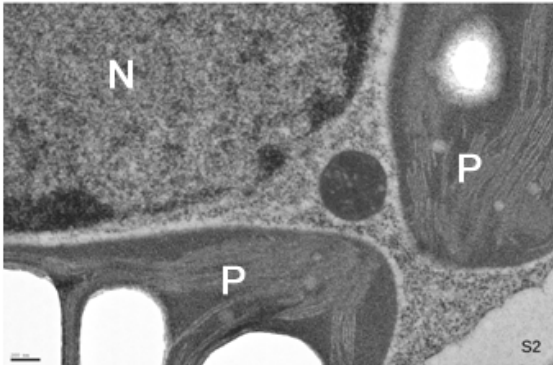


Images S1–S4 represent mesophyll chlorenchyma cells of control samples (C4 time point; corresponding to 34 d after germination) labeled with anti-VlxD antibodies. Acronyms: MC, mesophyll chlorenchyma; PVM paraveinal mesophyll.



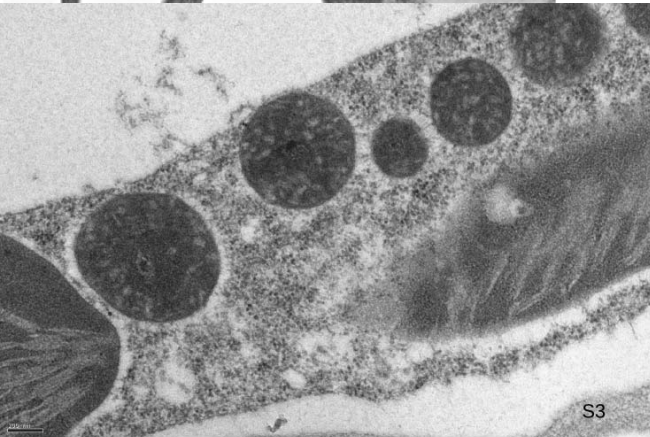
S1. Low magnification of a mesophyll chlorenchyma cell after attempted immuno-gold labeling with the anti-VlxD antibody. Labeling was found to be absent from control samples. P = chloroplasts N = nucleus.

Bar = 0.5 μ m.



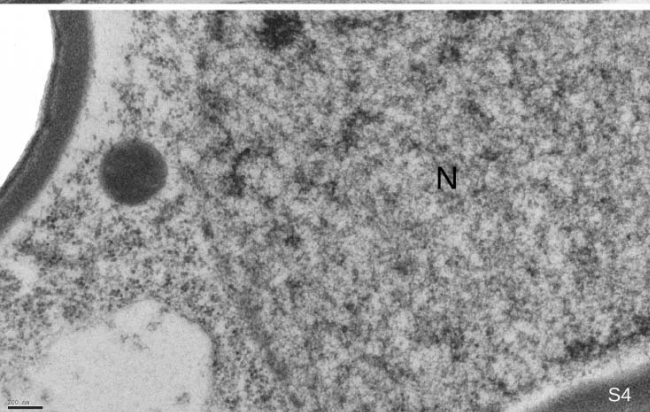
S2. High magnification of the same cell as shown in S1. Immuno-gold label is absent.

Bar = 200 nm.



S3. Cortical cytoplasm with mitochondria and chloroplasts. Immunolabeling for anti-VlxD is absent.

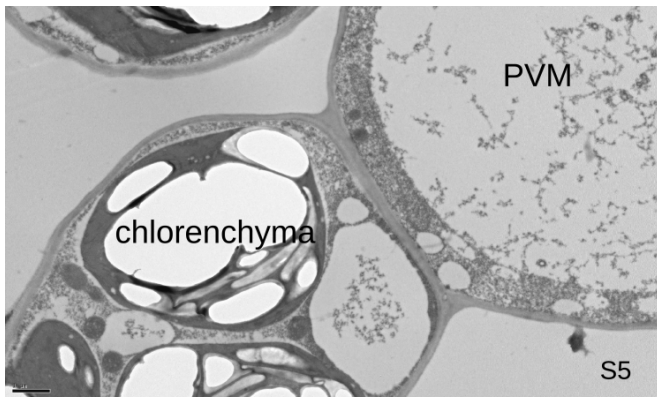
Bar = 200 nm.



S4. Nucleus of a mesophyll chlorenchyma cell lacking immuno-gold label for VlxD.

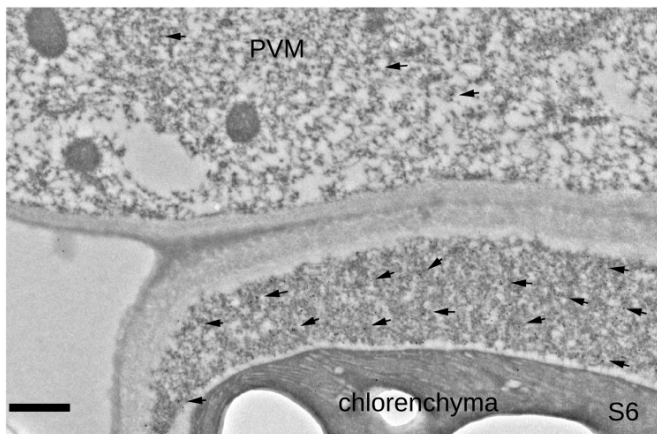
Bar = 200 nm.

Images S5–S9 represent MC and PVM cells of Detip/Retip-treated plants (D15 time point; corresponding to day 45 d after germination) labeled with anti-VlxD antibodies. Labeling is abundant in nuclei and cytoplasm of MC and nearly absent from PVM cells. Images S10-12 represent appropriate control samples (C15 time point).



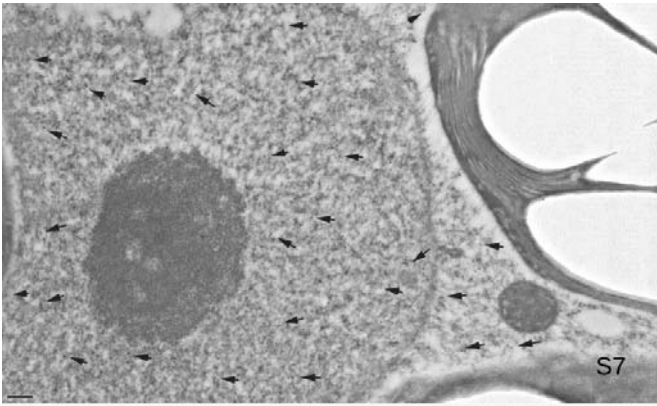
S5. Low magnification of anti-VlxD labeling in a section with an MC and an adjoining PVM cell.

Bar = 1.0 μ m.



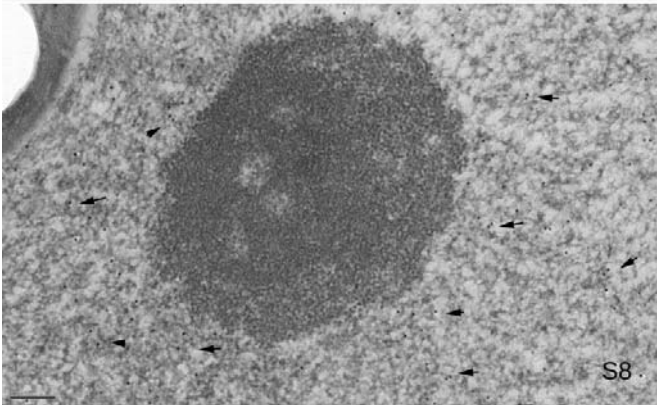
S6. High magnification image of anti-VlxD immuno-labeled MC and PVM cells. The arrows indicate representative immuno-gold label. Label was abundant in the MC but very sparse (essentially background level) within the PVM cell.

Bar = 200 nm.



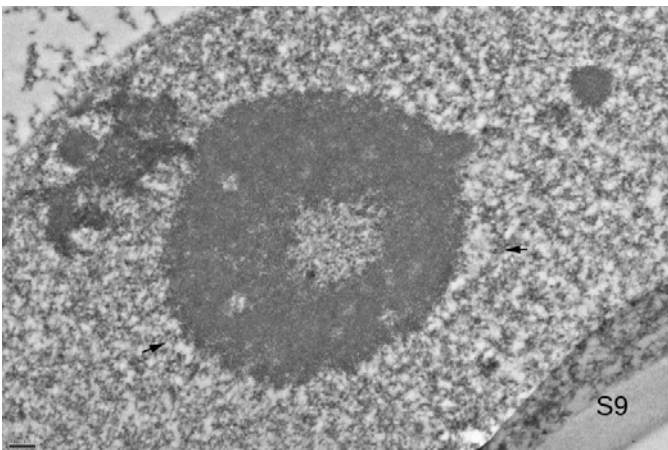
S7. The nucleoplasm and cytoplasm of an anti-VlxD immunolabeled MC cell contain abundant immunogold label (arrows) while plastids and mitochondria are unlabeled.

Bar = 200 nm.



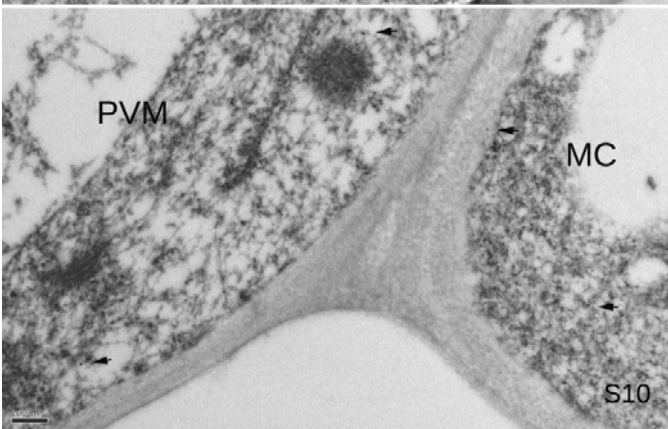
S8. High magnification of the nucleus shown in S7, illustrating abundant immunogold labeling (arrows) of the nucleoplasm of this MC cell with anti-VlxD antibodies.

Bar = 200 nm.



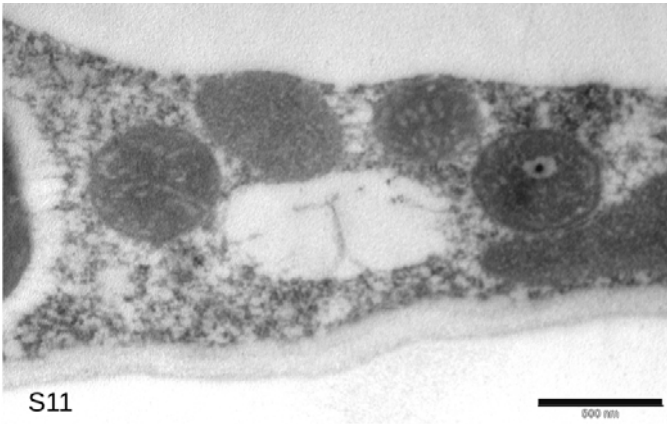
S9. An unlabeled nucleus of a PVM cells from an anti-VlxD immunolabeled section. Compare with S8 (image of an anti-VlxD immunolabeled MC cell).

Bar = 200 nm.



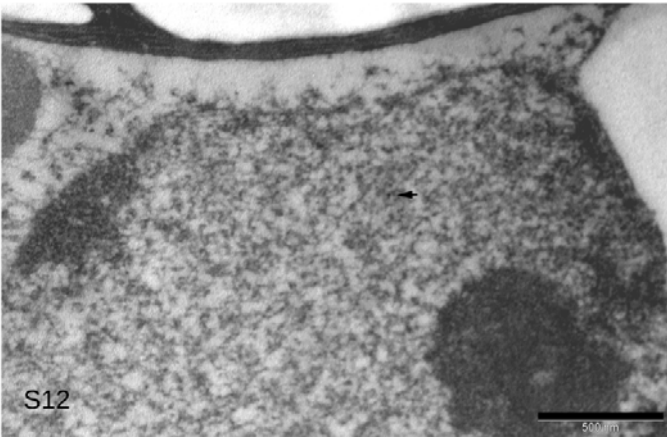
S10. Rabbit serum control. PVM and MC cells labeled with protein A-purified IgG from normal rabbit serum, showing only background levels of staining.

Bar = 200 nm.



S11. MC cells from a control plant (C15) stained anti-VlxD antibodies. Immuno-labeling is absent from the cytoplasm.

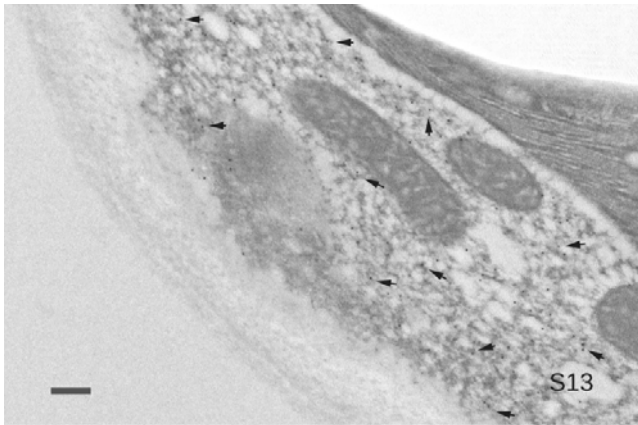
Bar = 500 nm.



S12. Nucleus of an MC cell from a control plant (C15) plant stained anti-VlxD antibodies. Immuno-gold label is absent.

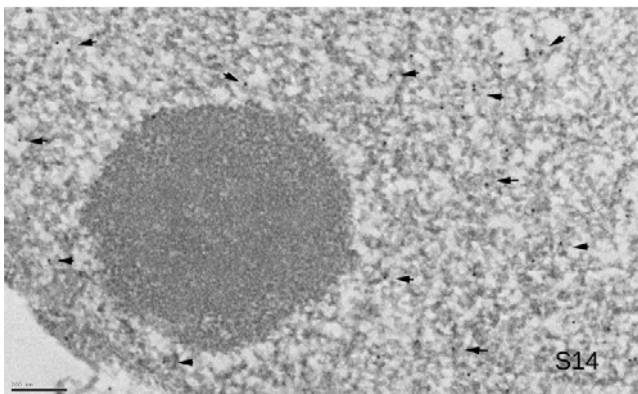
Bar = 500 nm.

Images S13–S16 compare MC and PVM cells of Detip/Retip-treated plants (R25 time point; corresponding to 55 d after germination) labeled with anti-VlxD antibodies. Images S17–S18 show control specimens (R20 time point; corresponding to 50 d after germination) labeled with anti-VlxC antibodies.



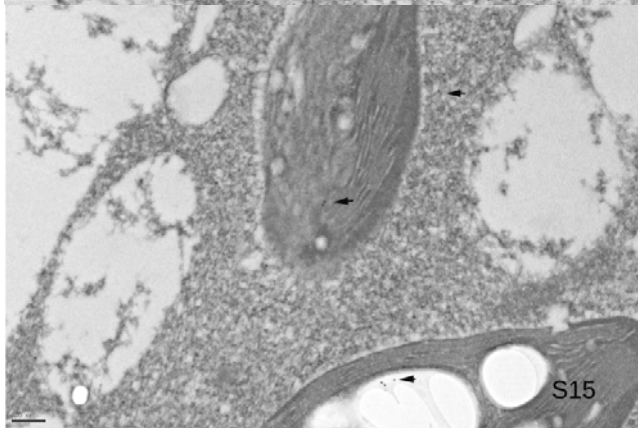
S13. Cortical cytoplasm of an MC cell labeled with anti-VlxD antibodies. This region has an abundance of smooth endoplasmic reticulum, and the immuno-gold label (arrows) often appears to be associated with ER membranes.

Bar = 200 nm.



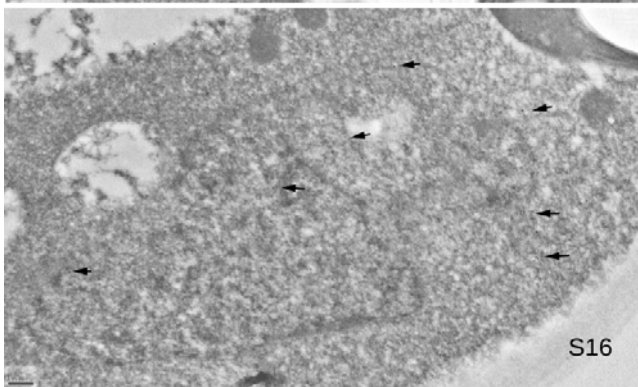
S14. Nucleus of an MC cell labeled with anti-VlxD antibodies (arrows). Labeling is abundant in the nucleoplasm.

Bar = 200 nm.



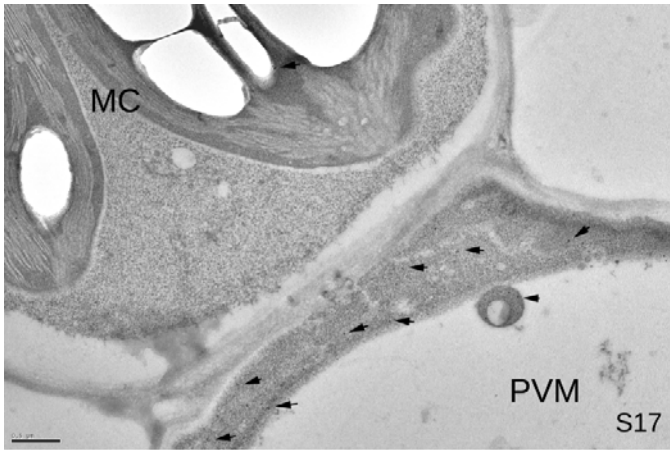
S15. PVM cell stained with anti-VlxD antibodies (arrows). Staining is very sparse and appears to follow a pattern reminiscent of non-specific background staining.

Bar = 200 nm.



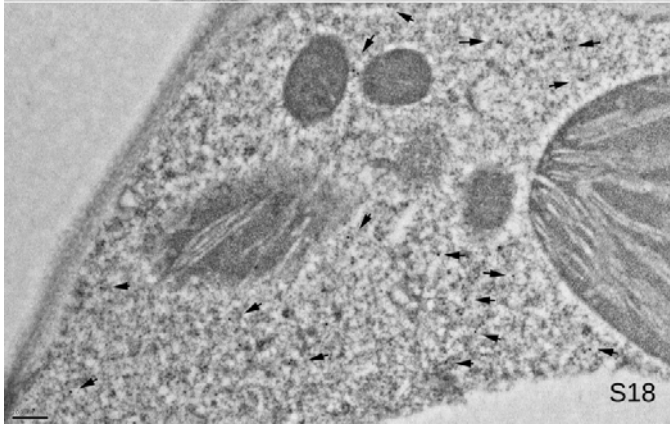
S16. PVM cell (different from that shown in S15) with a portion of a nucleus. Some staining (arrows) appears to be present within the nucleus, but at a much lower level than in the nucleus of MC cells (S14).

Bar = 200 nm.



S17. Portions of adjoining MC and PVM cells. The PVM cell (lower right) shows abundant labeling with VlxC antibodies (arrows), while the MC cell upper left lacks labeling.

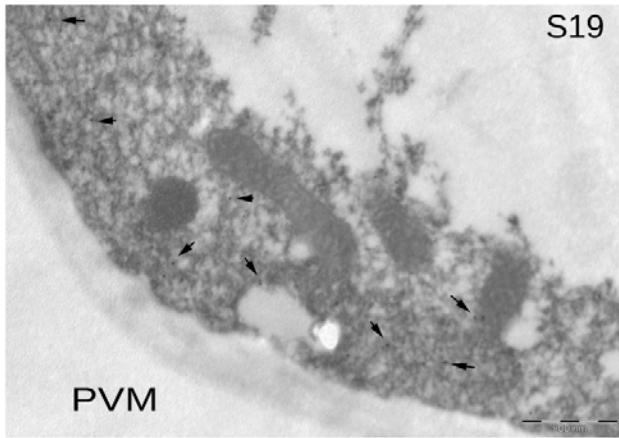
Bar = 500 nm.



S18. High magnification of a PVM cell showing a region of cytoplasm with abundant anti-VlxC immuno-labeling (arrows) in the cortical cytoplasm.

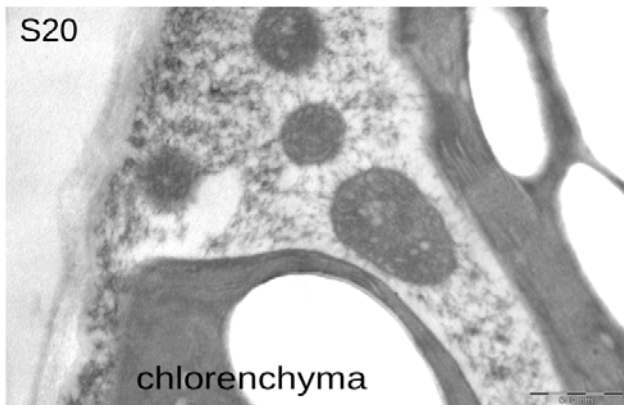
Bar = 200 nm.

Images S19–S21 show detipped specimens at time-course day 45 (detip day 15) labeled with anti-VlxC antibodies. S22 represents a detipped time-course day 45 (detip day 15) specimen stained with normal rabbit serum IgG.



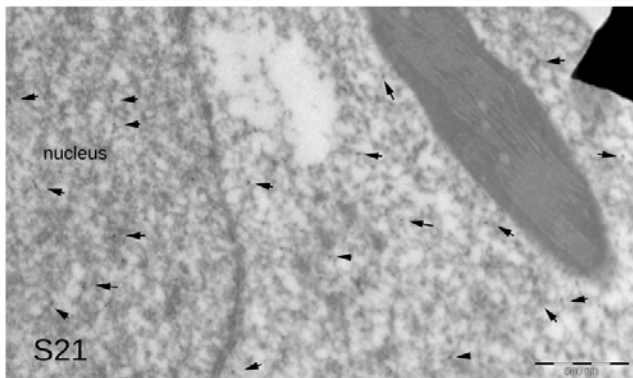
S19. A PVM cell with abundant cytoplasmic labeling (arrows). Label is absent from the flocculant material in the central vacuole.

Bar = 500 nm.



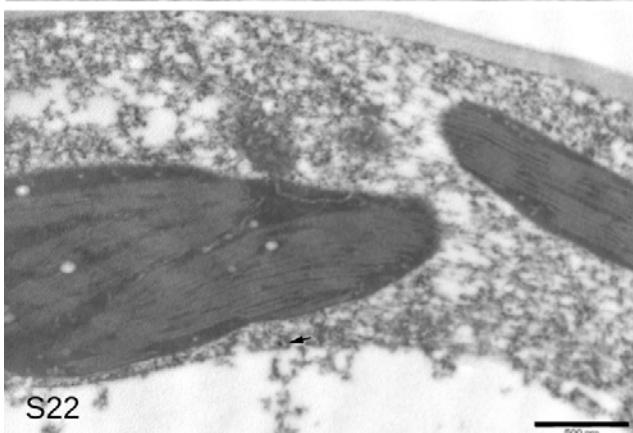
S20. MC cell similarly stained with anti-VlxC antibodies lacks labeling for VlxC.

Bar = 500 nm.



S21. PVM cell showing abundant immuno-labeling (arrows) with anti-VlxC antibody in the nucleoplasm and cytoplasm.

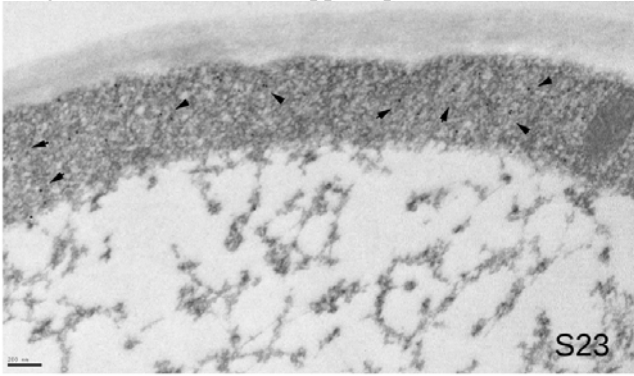
Bar = 500 nm.



S22. PVM cell stained with normal rabbit serum IgG. The cytoplasm lacks immuno-labeling.

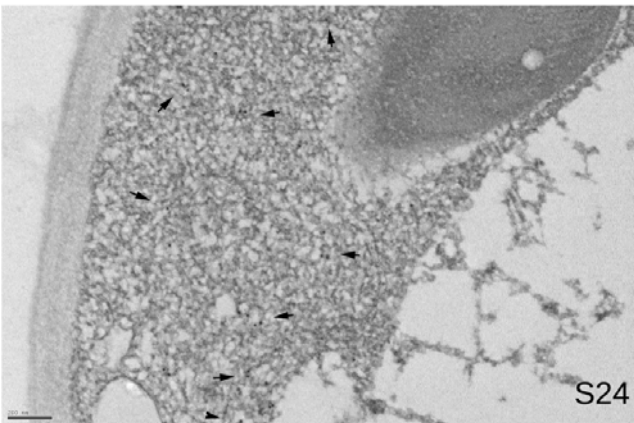
Bar = 500 nm.

Images S23–S24 show detipped specimens at time-course day 56 (retip day 11) labeled with anti-VlxC antibodies.



S23. Cytoplasm and central vacuole of PVM cell with abundant cytoplasmic immuno-gold labeling (arrows) with anti-VlxC antibodies. Labeling is absent from the central vacuole.

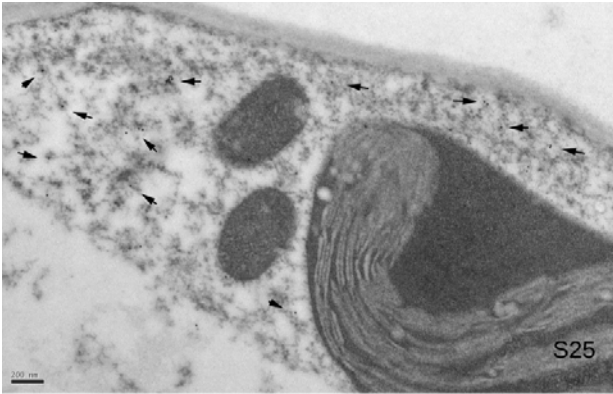
Bar = 200 nm.



S24. Another PVM cells immuno-labeled with anti-VlxC antibodies (arrows). Immuno-labeling occurs throughout the cytoplasm, often appearing to be associated with ER, but is absent from the plastid and the vacuole.

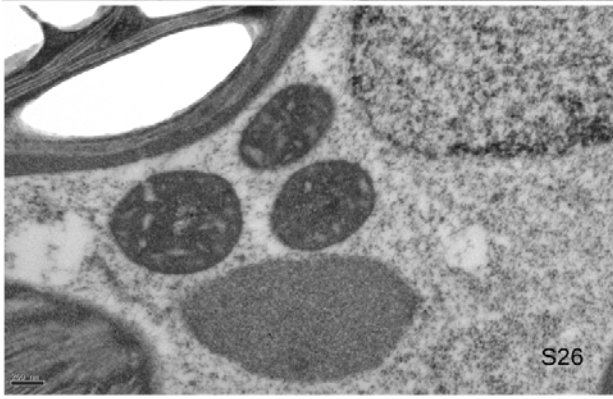
Bar = 200 nm.

Images S25–S26 compare PVM and MC cells from control samples (C4 time point; corresponding to 34 d after germination) labeled with anti-VlxB antibodies. Arrows indicate immuno-gold label.



S25. PVM cell with anti-VlxB labeling restricted to the cytoplasm.

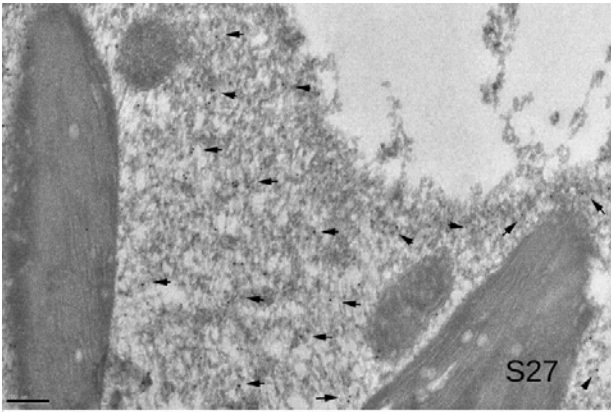
Bar = 200 nm.



S26. MC cell lacks VlxB immuno-gold labeling.

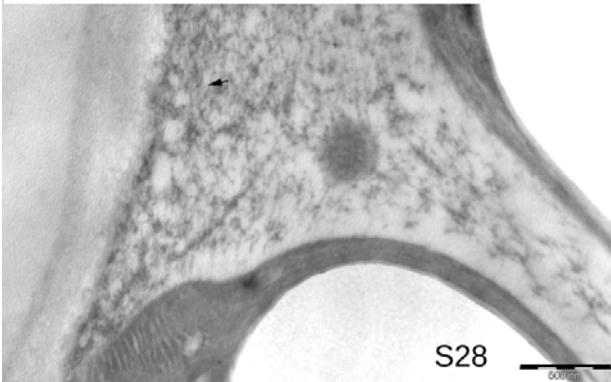
Bar = 200 nm.

Figures S27–S28 compare PVM and MC cells from Detip/Retip-treated samples (D15 time point; corresponding to 45 d after germination) labeled with anti-VlxB antibodies.



S27. PVM cell is strongly labeled with anti-VlxB antibodies. Immuno-gold labeling (arrows) is restricted to the cytoplasm.

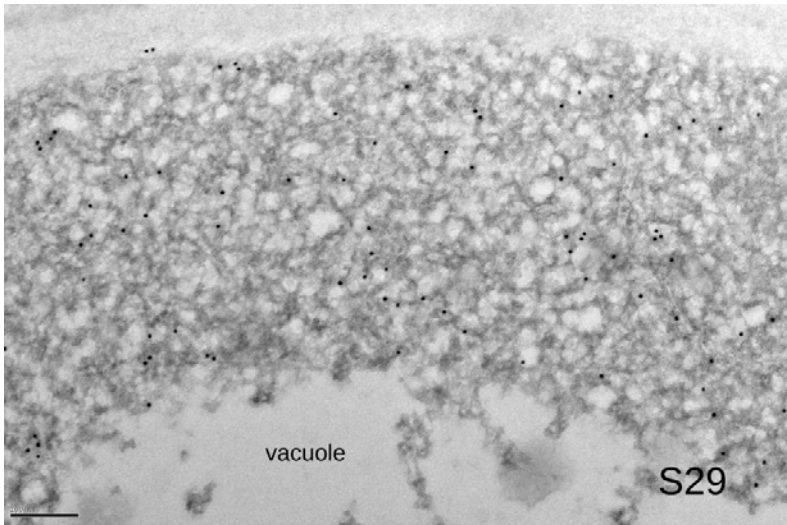
Bar = 200 nm.



S28. MC cell showing no significant labeling after staining with anti-VlxB antibodies.

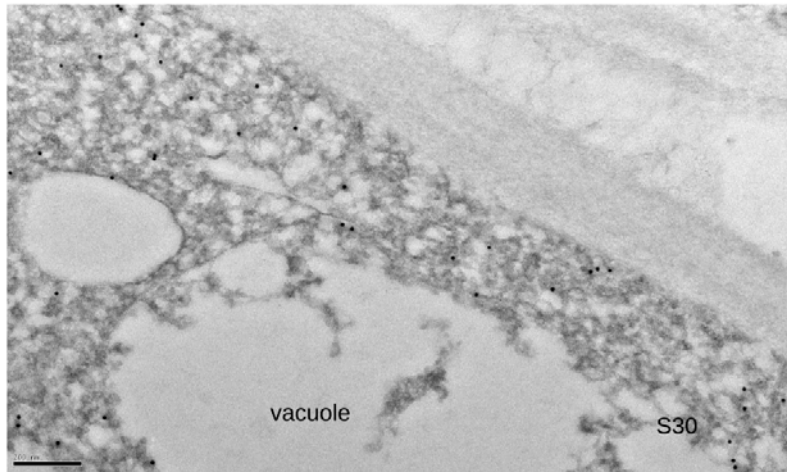
Bar = 500 nm.

Figures S29–S30 show strong anti-VlxB immuno-gold labeling of PVM cells in Detip/Retip-treated samples (R20 time point; corresponding to 50 d after germination).



S29. Immuno-gold labeling in a region of PVM cell cytoplasm with abundant smooth ER.

Bar = 200 nm.



S30. Another PVM cell showing a cytoplasmic distribution of anti- VlxB label that appears to be associated with smooth ER. Vacuolar contents remain unlabeled.

Bar = 200 nm.