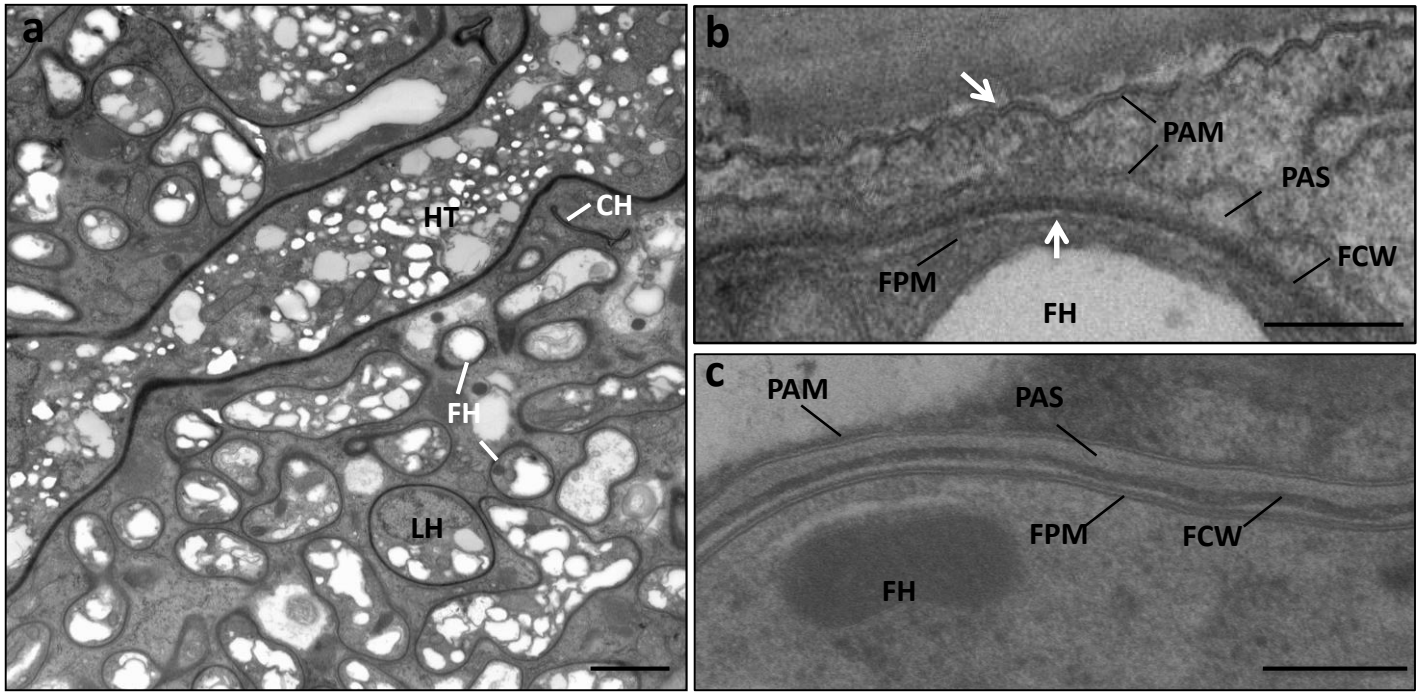
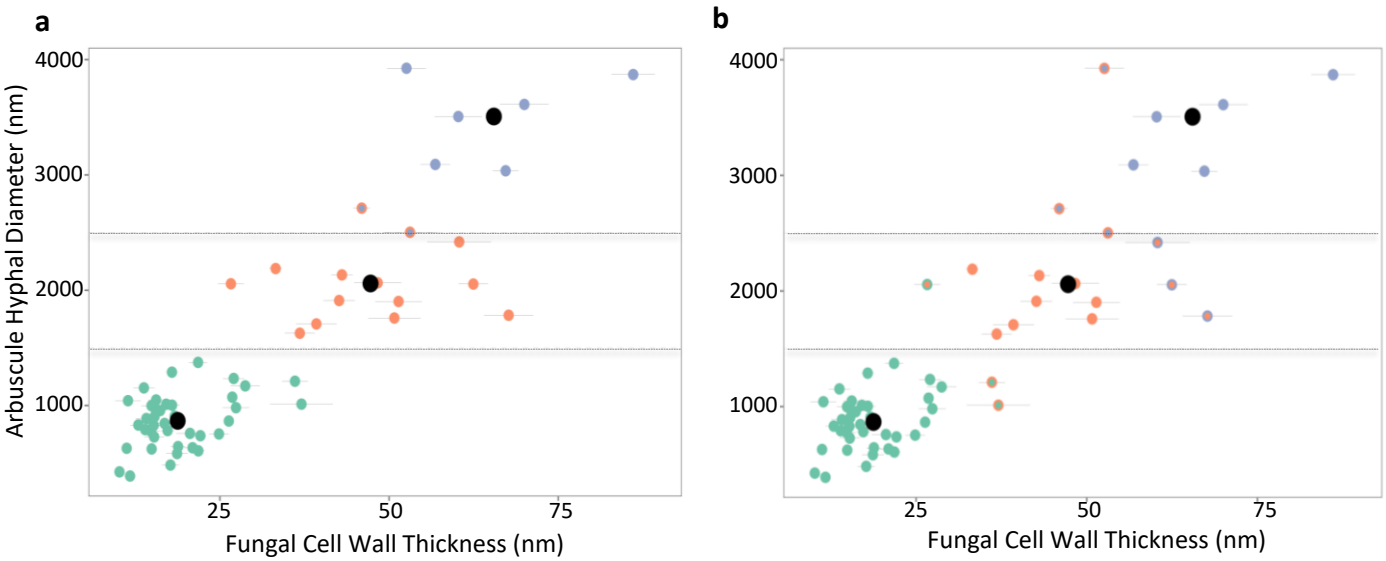
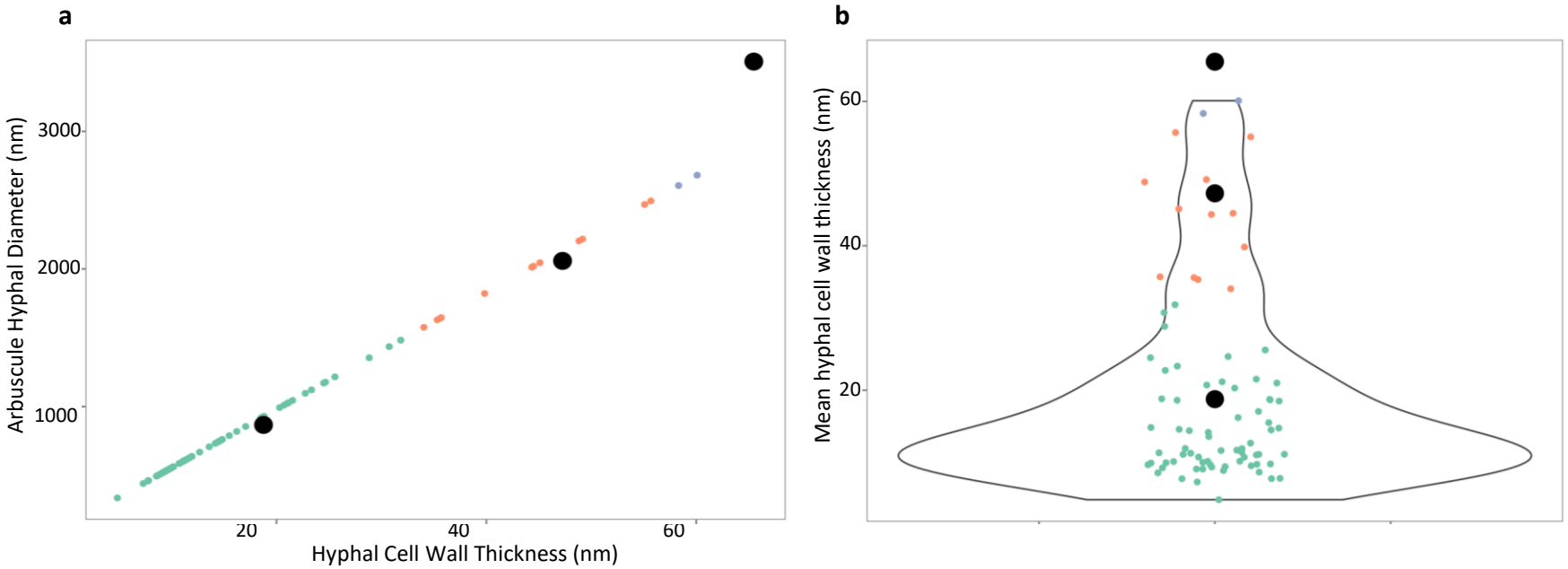


Supplementary Fig. 1. Transmission electron micrographs (TEM) of rice roots colonized by *R. irregularis.*

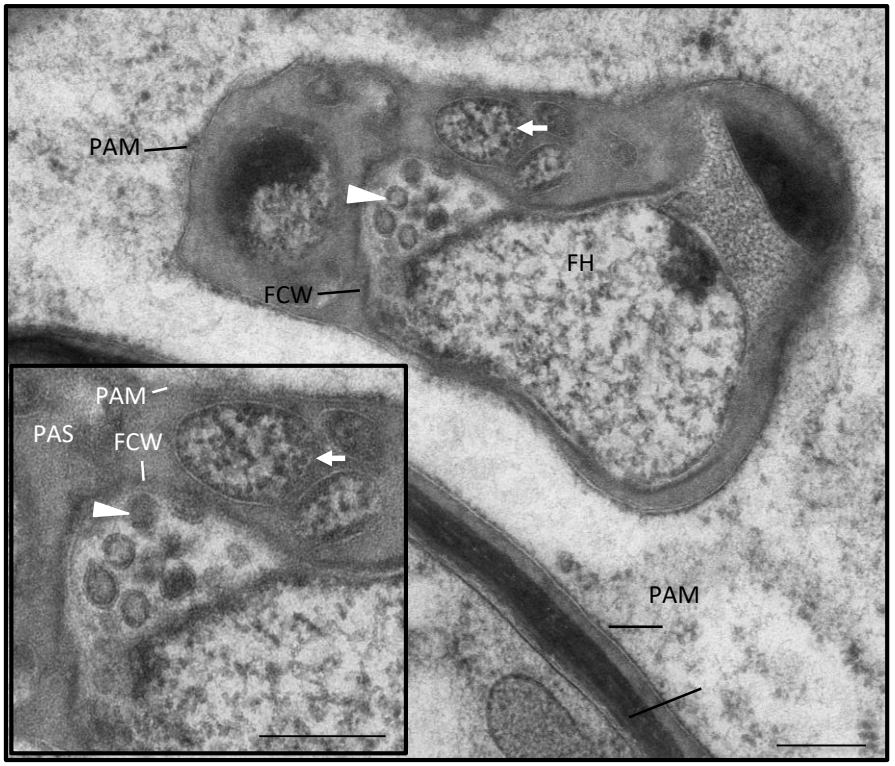
a. Ultrathin section through an arbuscule shows fungal structures corresponding to the hyphal trunk (HT), large hyphae (LH), fine hyphae (FH) and collapsed hyphae (CH). Scale bar, 2µm. **b.** Chemical fixation causes plant and fungal membrane undulations (arrows). Scale bar, 200nm. **c.** Micrograph of high pressure freezing (HPF) and freeze substitutted (FS) material shows preservation of the fungal plasma membrane (FPM) and peri-arbuscular membrane (PAM). Scale bar 200nm Peri-arbuscular space (PAS), fungal hypha (FH), fungal cell wall (FCW).



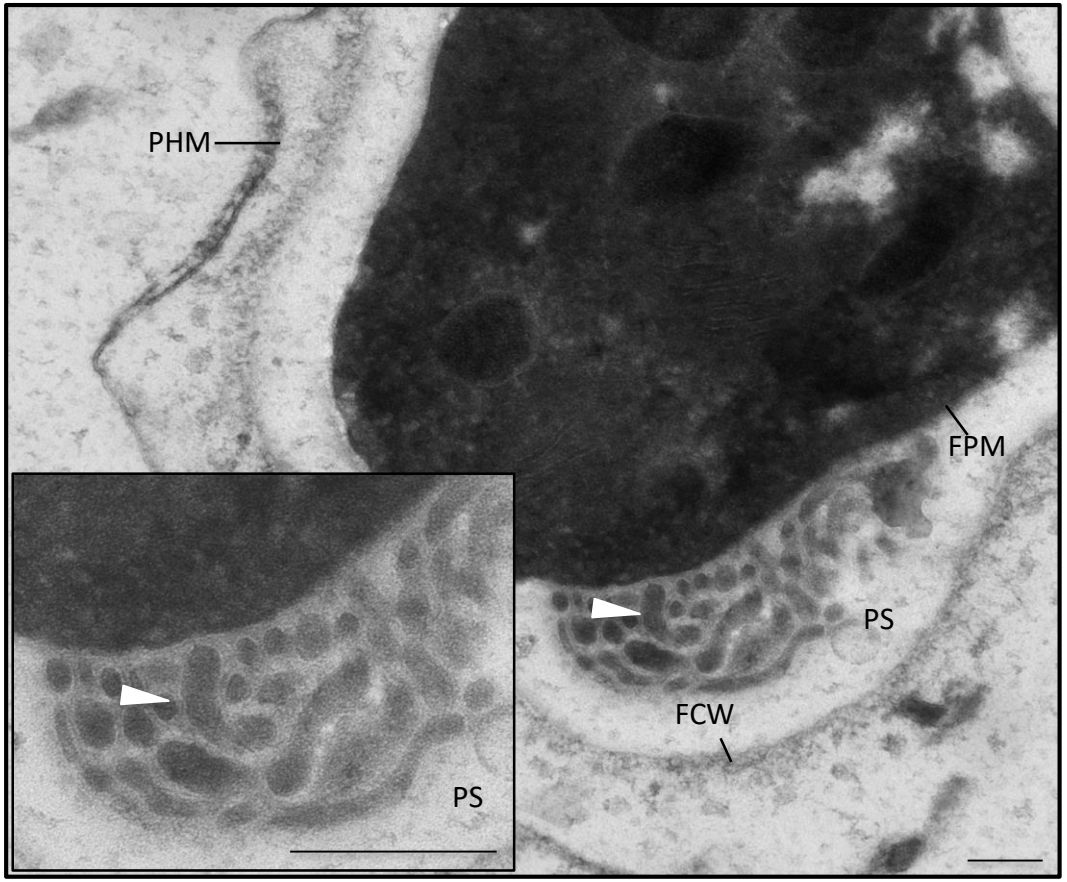
Supplementary Fig. 2. Correlation between fungal hyphal diameter and cell wall thickness. Each point represents measurements for a separate hypha (R^2=0.88, n=61 hyphae) and provided the training dataset to develop a linear model. Unsupervised K-means testing grouped the data into three main clusters (green, orange and blue) with cluster centers indicated by black dots. **a.** Correlation with Rand index of 0.961 was obtained between predicted clusters and visual selection of hyphal classes (dashed lines) using both parameters**. b.** Correlation with Rand index of 0.751 was obtained between predicted clusters and visual selection of hyphal classes using FCW thickness to predict hyphal diameter. Large dots are predicted values while smaller dots are based on observed data. Horizontal error bars represent standard error of FCW thickness (n=5) for each hypha.



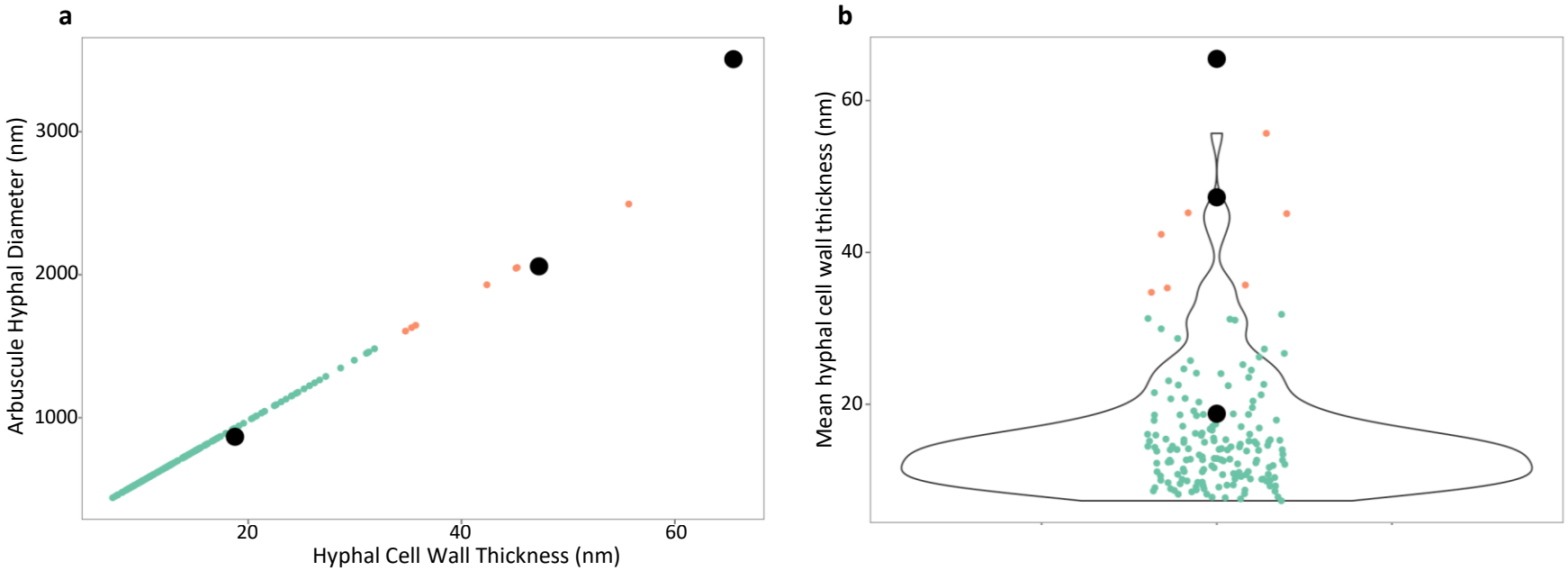
Supplementary Fig. 3. Abundance of fungal memtubs associated with fine, large and trunk hyphal branches. **a.** Linear regression plot of hyphal cell wall thickness and predicted hyphal diameters obtained from a trained linear model**. b.** Violin plot shows the cluster prediction of hyphae associated with memtubs. Rand index 0.923; n=80 hyphae.



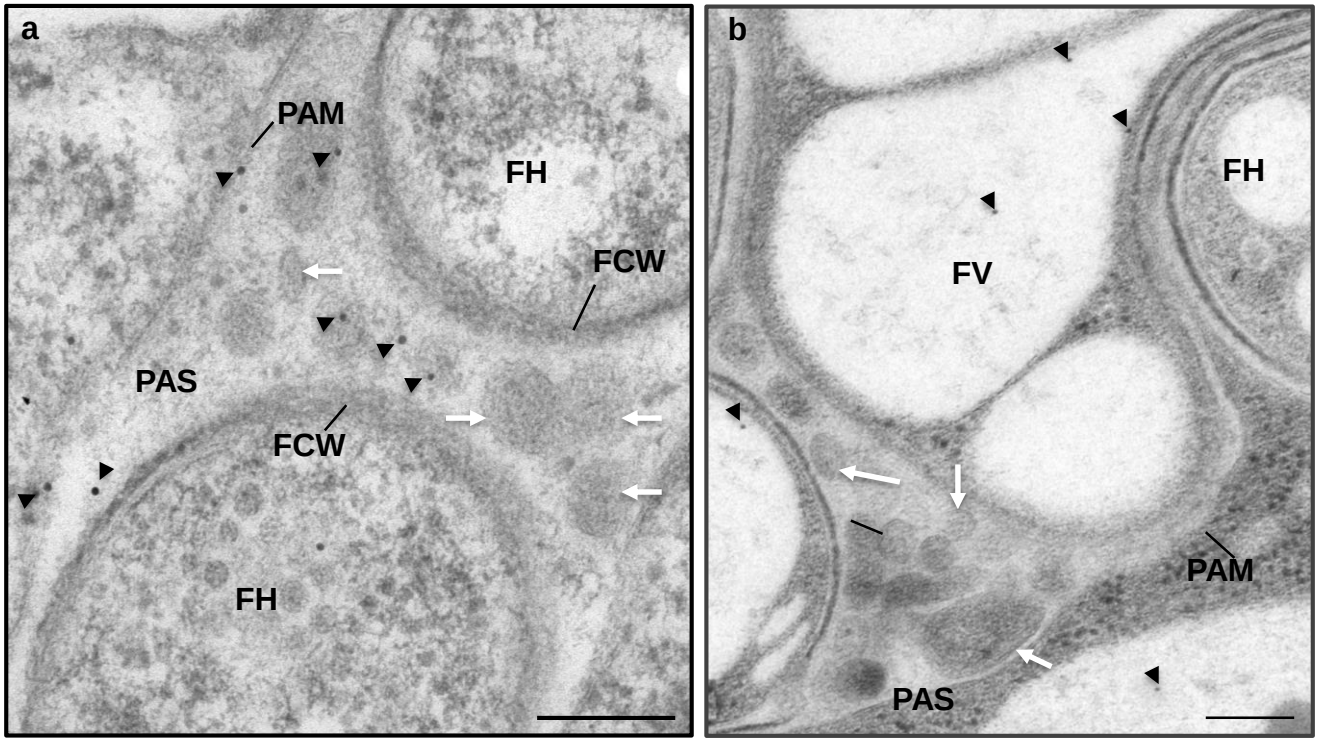
Supplementary Fig. 4. Transmission electron micrographs of a *Gigaspora rosea* arbuscule fine hypha in a rice cortex cell. Paramural fungal memtubs (arrow head) and peri-arbuscular space membrane-bound vesicular bodies (arrow head) are depicted. Inset shows higher magnification of memtubs and PAS-contained structures, both delineated by a lipid bilayer. Scale bar, 200nm. FH, fungal hypha, FCW, fungal cell wall; PAM, peri-arbuscular membrane; PAS, peri-arbuscular space



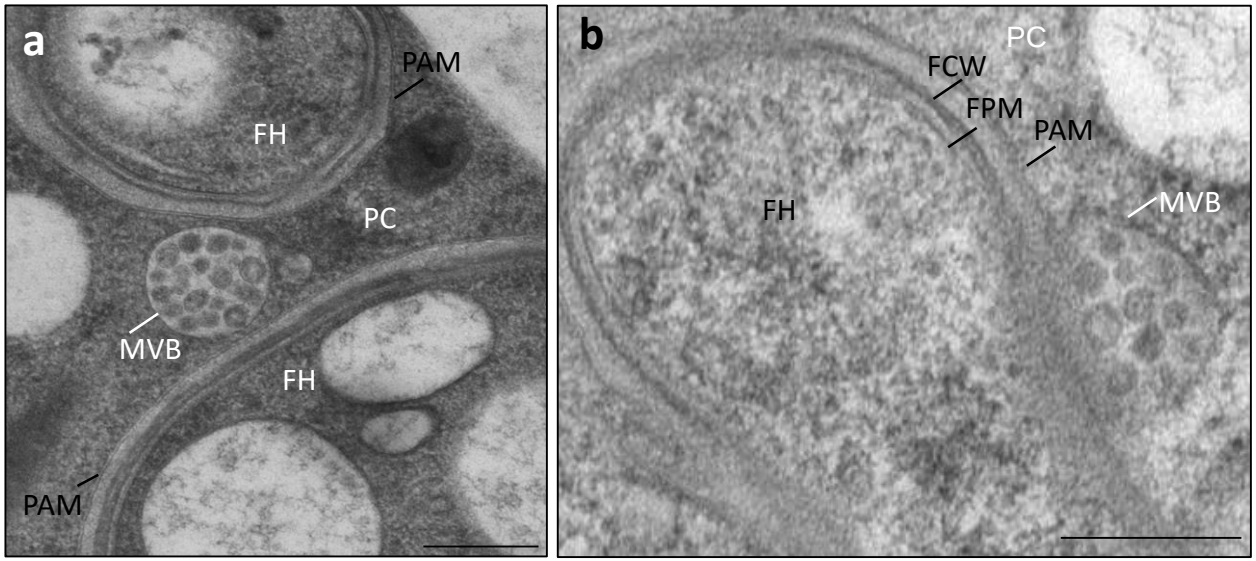
Supplementary Fig. 5. Transmission electron micrographs of a *Ustilago maydis* hypha in a maize leaf sheath cell. At one day post infection memtub-like structures (arrowhead) are present in the paramural space (PS) between the fungal cell wall (FCW) and fungal plasma membrane (FPM). Inset shows memtubs at higher magnification. PHM, peri-hyphal membrane. Scale bar, 200nm.



Supplementary Fig 6. Abundance of PAS-internal membrane-bound vesicular structures, associated with fine, large and trunk hyphal branches. **a.** Linear regression plot of hyphal cell wall thickness and predicted hyphal diameters obtained from our trained linear model**. b.** Violin plot shows the cluster prediction of hyphae associated with vesicular structures. Rand index 0.924; n=164 hyphae



Supplementary Fig. 7. Immunogold-labeling using an anti-GFP anti-body against PT11-GFP. a. On rice roots expressing PT11-GFP, immunogold particles (black arrow heads) localize to the PAM and vesicular bodies inside the PAS (white arrows) with some vesicular structures not labeled (white arrows). **b.** Negative control of IGL on *R. irregularis*-colonised non-transformed rice cells shows an unspecific distribution pattern of immuno-gold particles (black arrow heads), absent from PAS-internal vesicular structures (white arrow heads). Scale bar, 200nm.



Supplementary Fig. 8. Transmission electron micrographs showing plant multivesicular bodies (MVB) in cytoplasm arbusculated rice cells. a, Plant MVB close to fungal hypha. Scale bar, 200nm. **b,** MVB appears to fuse with the PAM. Scale bar, 250nm. FH, fungal hypha; FCW, fungal cell wall, FPM, fungal plasma membrane, PAM, Peri-arbuscular membrane, PC, Plant Cytosol.