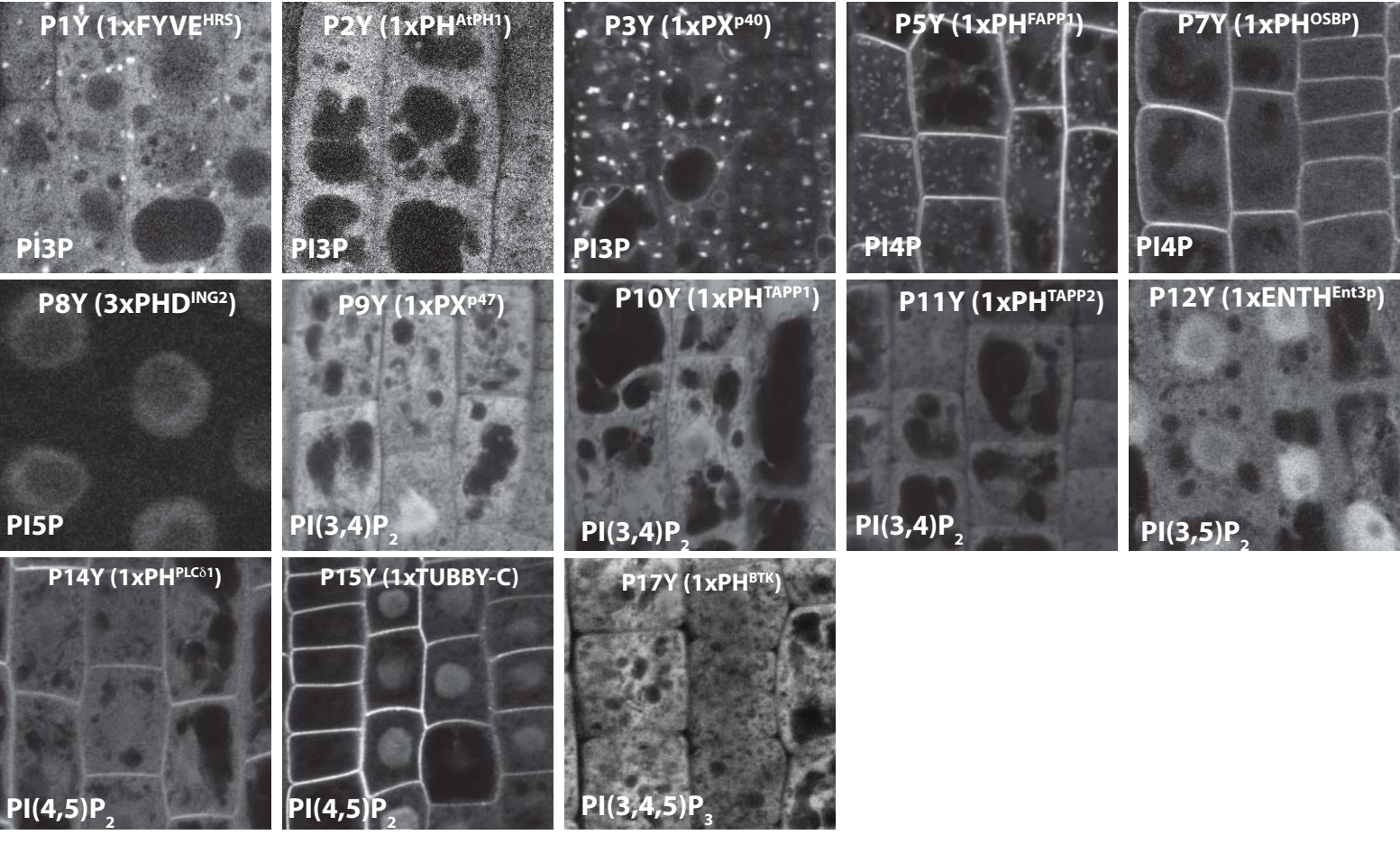

Figure S1: Identification of phosphoinositide biosensors targeted to the endomembrane system and/or plasma membrane in transgenic Arabidopsis roots.

Confocal pictures of Arabidopsis root epidermal cells expressing various CITRINE-tagged LBDs. The phosphoinositide targeted by each LBD is indicated in the bottom left corner of each image and the respective PIPline name is indicated in the top left corner.

Supplementary text of Figure S1: We initially included markers for PI(3,4)P_2 and PI(3,4,5)P_3. The Arabidopsis genome lacks a canonical type I or type II PI(3)-kinase, however non-canonical phosphoinositide biosynthetic pathways have been found in animal (Shin et al., 2005). Accordingly to previous biochemical studies suggesting their absence in plants (Meijer & Munnik, 2003; Munnik & Nielsen, 2011), markers for PI(3,4)P_2 and PI(3,4,5)P_3 were found in the cytosol. We detected the PI5P biosensor exclusively in the nucleus. This result is compatible with the presence of PI(5)P in plants (Meijer et al., 2001) and with the reported localisation of PI5P in the nucleus of mammalian cells (Gozani et al., 2003). However, we could not exclude that this LBD is localised in the nucleus in the absence of nuclear PI5P, possibly because of its small size or the presence of a cryptic nuclear localisation signal. Therefore, we excluded it in subsequent analyses. PI(3,5)P_2 have been reported to be localised in late endosomal compartments in yeasts (Eugster et al., 2004; Friant et al., 2003). In Arabidopsis, there are four orthologs of FAB1, the kinase involved in PI(3,5)P_2 synthesis in yeast and animals (Hirano et al., 2011). Genetic invalidation of two FAB1 genes (FAB1A and FAB1B) is lethal during male gametogenesis, suggesting the existence and functional importance of PI(3,5)P_2 in plant cells (Hirano et al., 2011). Unfortunately, the biosensors that we used were either not stably expressed or not associated with any membranous compartments. Therefore, we cannot conclude for the localisation of PI(3,5)P_2 with the available domains known to interact with this lipid.

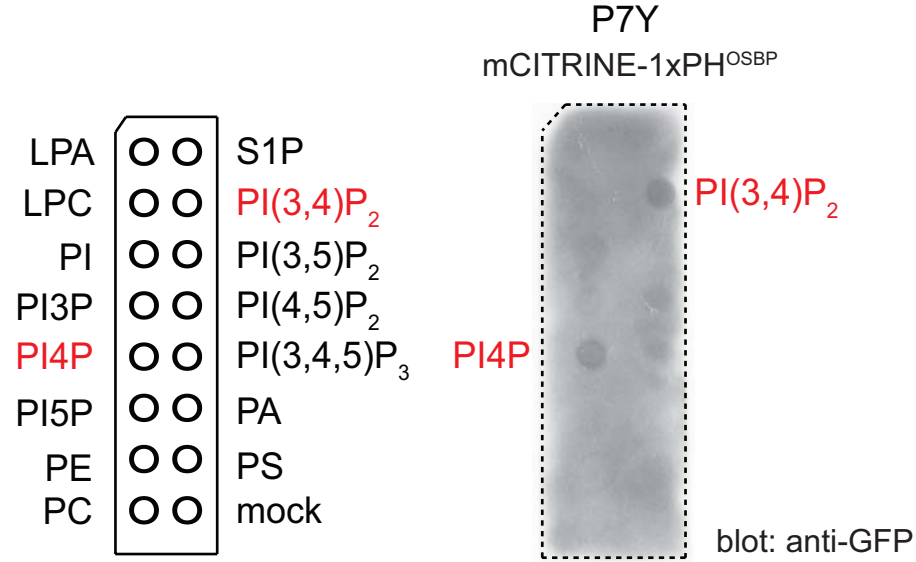

Figure S2. mCITIRNE-1xPH^{OSBP} interacts with PI4P and PI(3,4)P_2 in protein-lipid overlay assay. Lipid overlay assay with mCITRINE-1xPH^{OSBP} proteins extracted from P7Y transgenic plants. The position of each lipid is indicated on the map at the left.

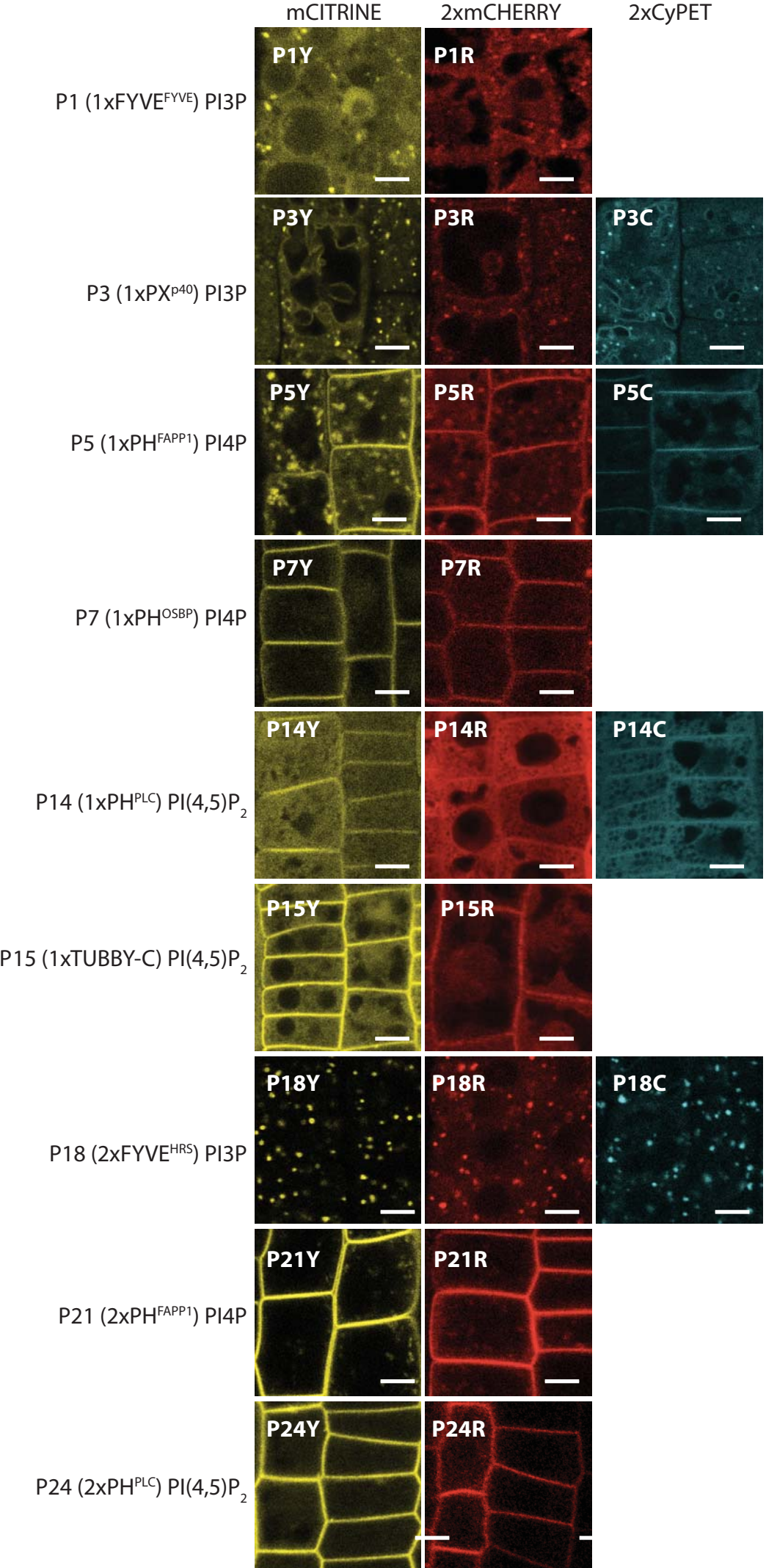

Figure S3. Multi-colour imaging of the PIPline collection. Confocal pictures of Arabidopsis root epidermal cells expressing the various PIPline constructs. Left, mCITRINE-tagged PIPlines, middle 2xCHERRY-tagged PIPlines and right 2xCyPET-tagged PIPlines. Scale bar 5 µm.

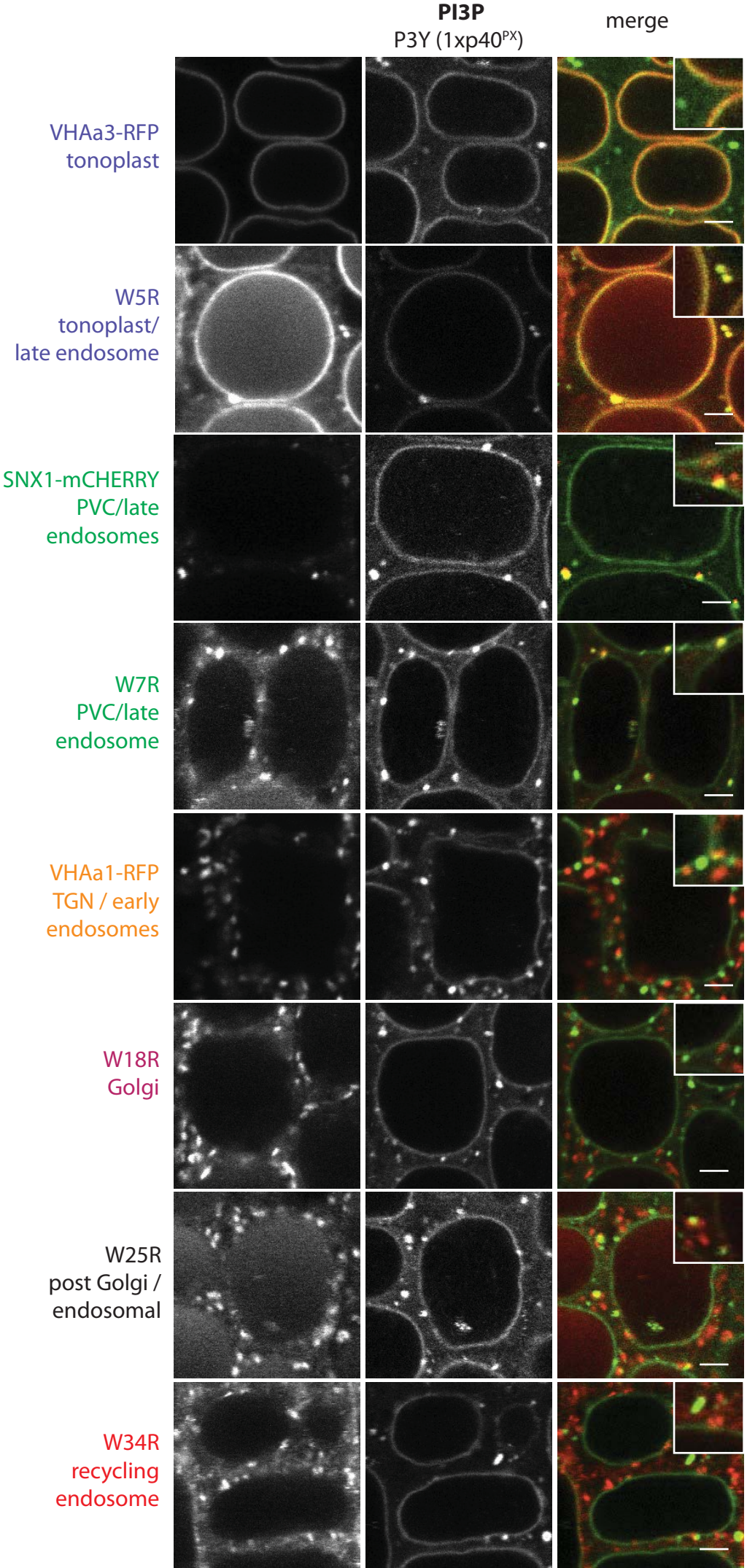

Figure S4. 1xPX^{p40}-CITRINE localises to late endosomes and tonoplast in Arabidopsis root epidermis. Confocal pictures of root epidermal cells co-expressing 1xPX^{p40}-CITRINE with intracellular compartment markers fused with a red fluorescent protein. Left pictures correspond to the compartment markers, middle pictures correspond to 1xPX^{p40}-CITRINE (both depicted in grey scale for increased contrast), while the right pictures correspond to the overlay of both channels with the compartment markers in red and the 1xPX^{p40} sensor in green. Scale bar 5 µm.

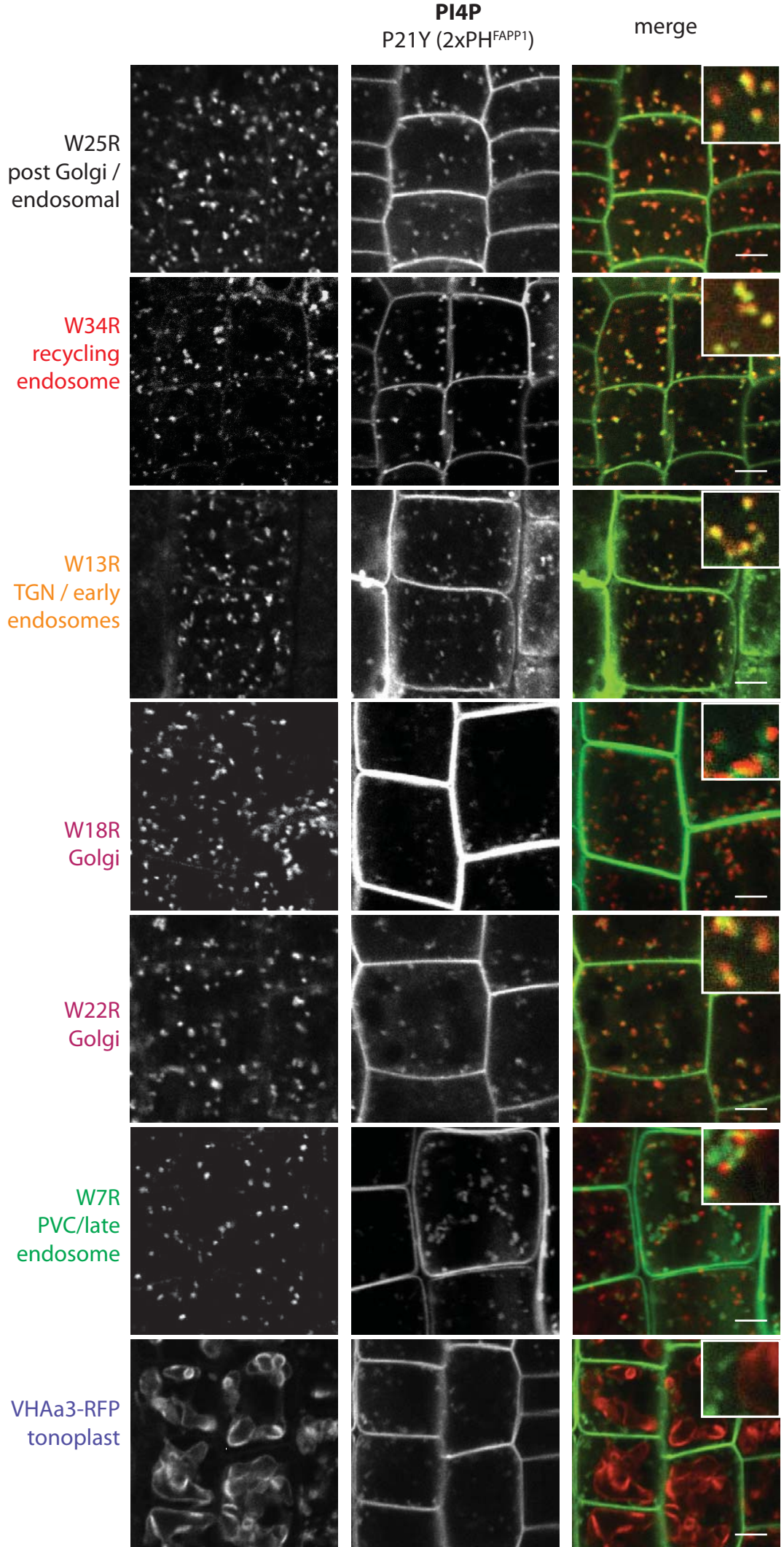

Figure S5. Intracellular CITRINE-2xPH^{FAPP1} mainly accumulates to post-Golgi/endosomal compartments in Arabidopsis root epidermis. Confocal pictures of root epidermal cells co-expressing CITRINE-2xPH^{FAPP1} with intracellular compartment markers fused with a red fluorescent protein. Left pictures correspond to the compartment markers, middle pictures correspond to CITRINE-2xPH^{FAPP1} (both depicted in grey scale for increased contrast), while the right pictures correspond to the overlay of both channels with the compartment markers in red and the 2xPH^{FAPP1} sensor in green. Scale bar 5 µm.