A clear majority of land plants establish symbioses in their roots with Glomeromycota fungi. This fruitful collaboration permits the exchange of sugars and lipids from the plant and mineral nutrients from the fungus. This transaction occurs between the membrane of a tree-shaped fungal structure called “arbuscule” and an extension of the plant plasmalemma termed “peri-arbuscular membrane”. Both structures are critical, their presence allowing the molecular traffic between the organisms. We know since 2010 that no arbuscules nor peri-arbuscular membranes are formed in mutant plants lacking the VAPYRIN protein. Little is known about the function of this symbiosis-specific protein. However, it has been shown that VAPYRIN localizes to small spherical structures (Vapyrin-bodies) that exhibit an intriguing “stop-and-go” movement. In this study, we compare the localization of VAPYRIN with a wide range of subcellular marker proteins. To quantify the spatial relation of VAPYRIN with different organelles or endomembrane compartments, we developed a semi-automated bioinformatics pipeline for the analysis of fluorescent confocal microscopy image series. This helped to characterize the VAPYRIN trafficking network precisely. Also, it permitted the distinction between two types of related localization: strict colocalization of marker proteins with VAPYRIN and close association of VAPYRIN-bodies with other subcellular compartments. Finally, we showed that both parts of the protein are localized to the same place as the entire peptide.