The *Arabidopsis* pho2 mutant, which is defective in a ubiquitin-conjugating E2 enzyme, displays Pi toxicity as a result of enhanced uptake and root-to-shoot translocation of Pi. To elucidate downstream components of the PHO2-dependent regulatory pathway, we identified two pho2 suppressors as carrying missense mutations in *PHO1*, which has been implicated in Pi loading to the xylem. We found that the protein level of PHO1 is increased in pho2, whereas such accumulation is ameliorated in both pho2 suppressors. PHO1 and PHO2 are partially co-localized and physically interact in the endomembranes, where the ubiquitin conjugase activity of PHO2 is required for PHO1 degradation. We further showed that PHO1 degradation is PHO2-dependent and involves multivesicular body (MVB)-mediated vacuolar proteolysis. In addition, we presented a functional association between xylem loading and acquisition of Pi. Together, our findings uncover a pivotal molecular mechanism by which PHO2 modulates the degradation of PHO1 in the endomembranes to maintain Pi homeostasis in plants.