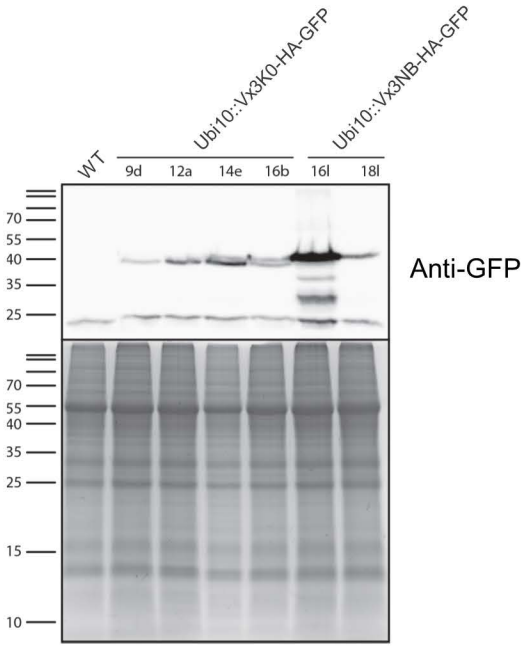

Figure S1. Identification of transgenic lines expressing similar levels of Vx3K0 and Vx3NB proteins. Immunoblots were performed using protein extracts from wild-type (WT) or monoinsertional homozygous sensor lines and probed with anti-GFP antibodies (top). Coomassie staining (bottom) is used as loading control. The protein size in kDa is shown on the left. Lines 12a and 18l were selected for Vx3K0 and Vx3NB, respectively.

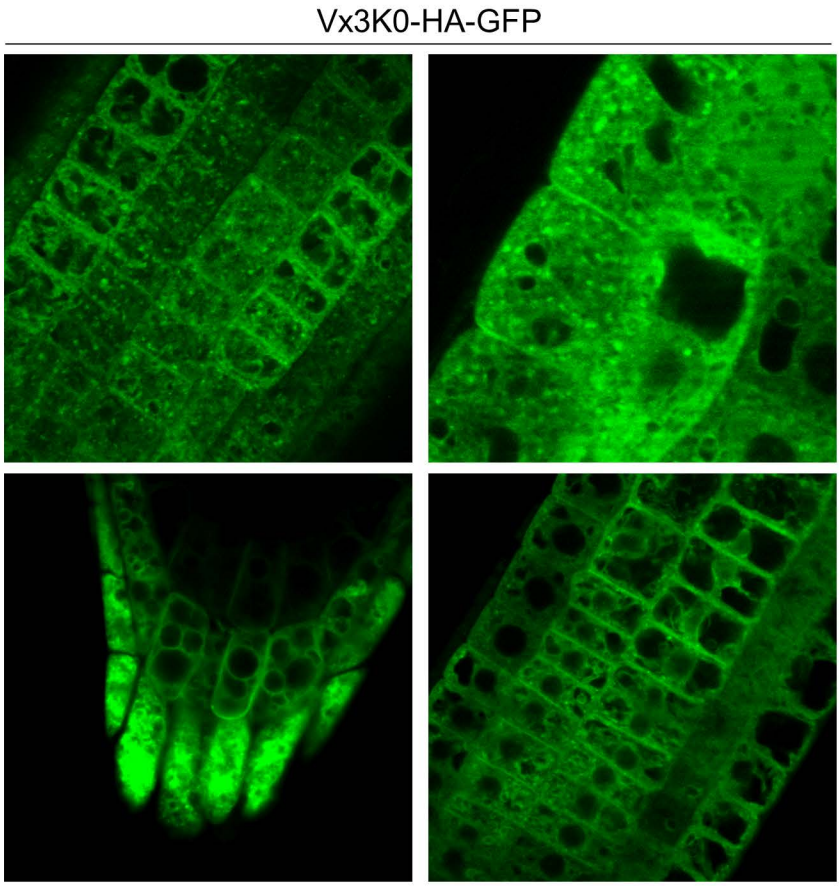

Figure S2. Confocal images showing the enrichment of Vx3K0 in intracellular vesicles, at the plasma membrane, and at the tonoplast.

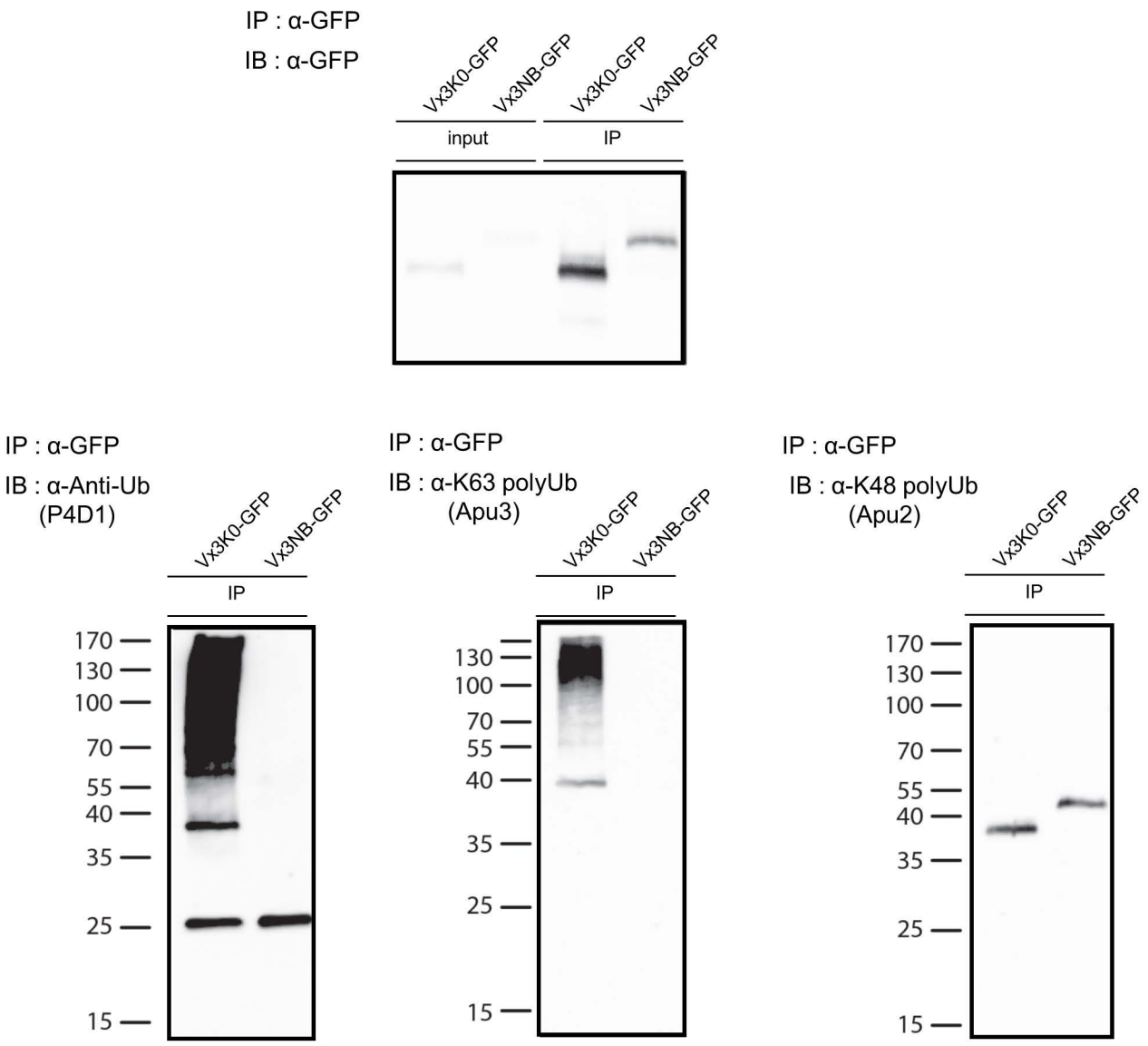

Figure S3. *In vivo* characterization of purified proteins using sensor-based immunoprecipitation of proteins carrying K63 polyubiquitin chains. Immunoprecipitation was performed using anti-GFP antibodies on RIPA buffer-solubilized protein extracts from monoinsertional homozygous plants expressing Vx3K0-HA-GFP and Vx3NB-HA-GFP and subjected to immunoblotting with anti-GFP (top), anti-Ub P4D1 (bottom left), anti-K63polyubiquitin Apu3 (bottom) and anti-K48 polyubiquitin Apu2 antibodies (bottom right). Independent membranes were used to probe with each antibody. IB, immunoblotting; IP, immunoprecipitation. The size of marker proteins in kDa are indicated next to the respective blots.

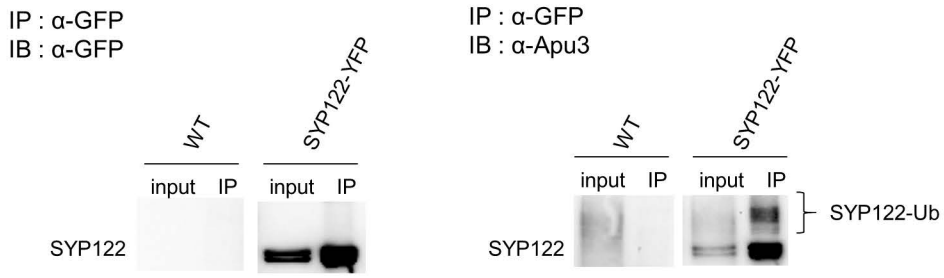

Figure S4. Example of protein identified as K63 polyubiquitinated in the Vx3K0-derived K63 polyubiquitinome and confirmed independently by immunoprecipitation of SYP122-YFP and immunoblotting with anti-GFP (left) and K63 polyubiquitin-specific Apu3 antibodies (right).

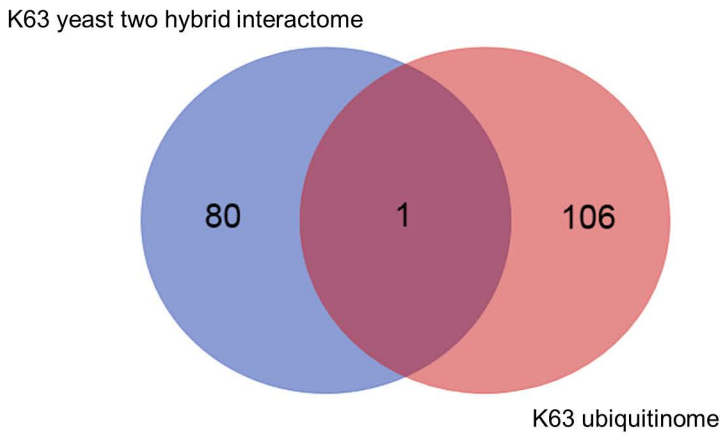

Figure S5. Venn diagram showing the overlap between proteins from the K63 ubiquitinome and Arabidopsis K63 polyubiquitin networks.