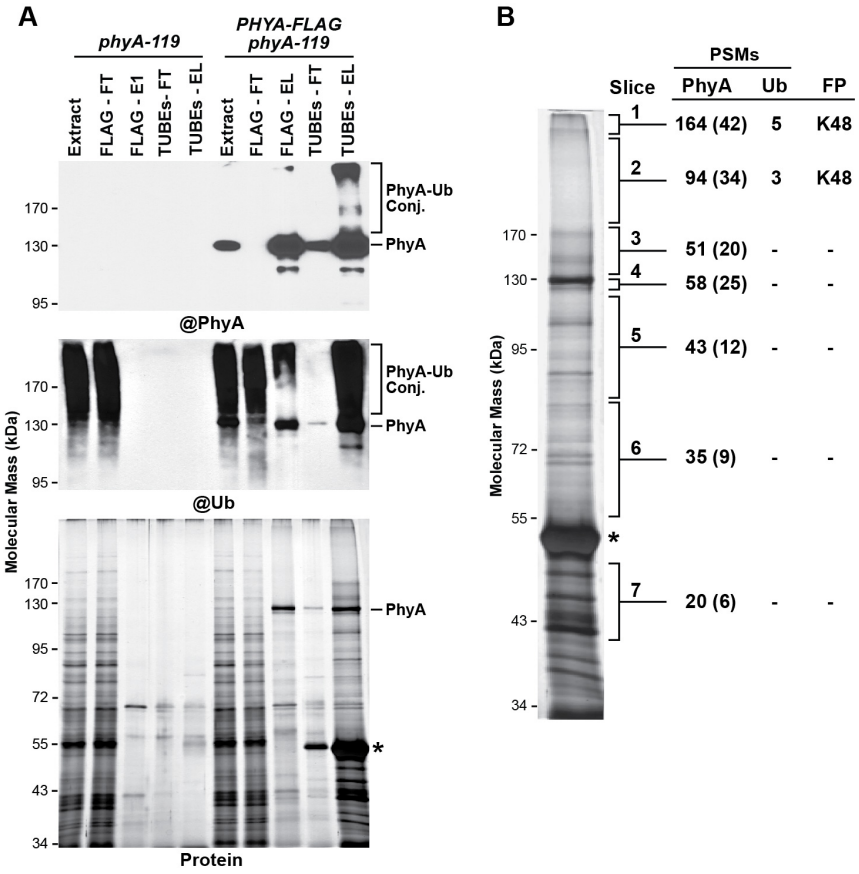

Supplemental Figure 5. Isolation of Ubiquitylated PhyA and Detection of PhyA Poly-Ubiquitylation by MS. PhyA-FLAG was affinity purified by sequential anti-FLAG and TUBEs affinity steps from etiolated *pPHYA:PhyA-FLAG phyA-119* seedlings exposure for 1 hr to red light.

(A) SDS-PAGE analysis of the samples during the various purification steps. The gels were either immunoblotted with the anti-PhyA monoclonal antibody 073D or anti-Ub (@Ub) antibodies, or stained for protein with silver (Protein). FL and EL represent flow through and elution fractions for each step, respectively. The migration positions of PhyA and PhyA-Ub conjugates are indicated. Purification using the control seedlings (*phyA-119*) was included for comparison.

(B) MS analysis of PhyA preparations. Following SDS-PAGE and protein staining, the indicated gel slices were trypsinized and subjected to tandem MS. The total number of PSMs in each fraction that could be assigned to PhyA and Ub are indicated; the numbers in parentheses indicate the number of unique peptides for each protein. FP, detection of Ub footprint peptides on Ub; the residue number of the modified Ub lysine (K48) is indicated. Astericks locate the GST-TUBEs fusion.