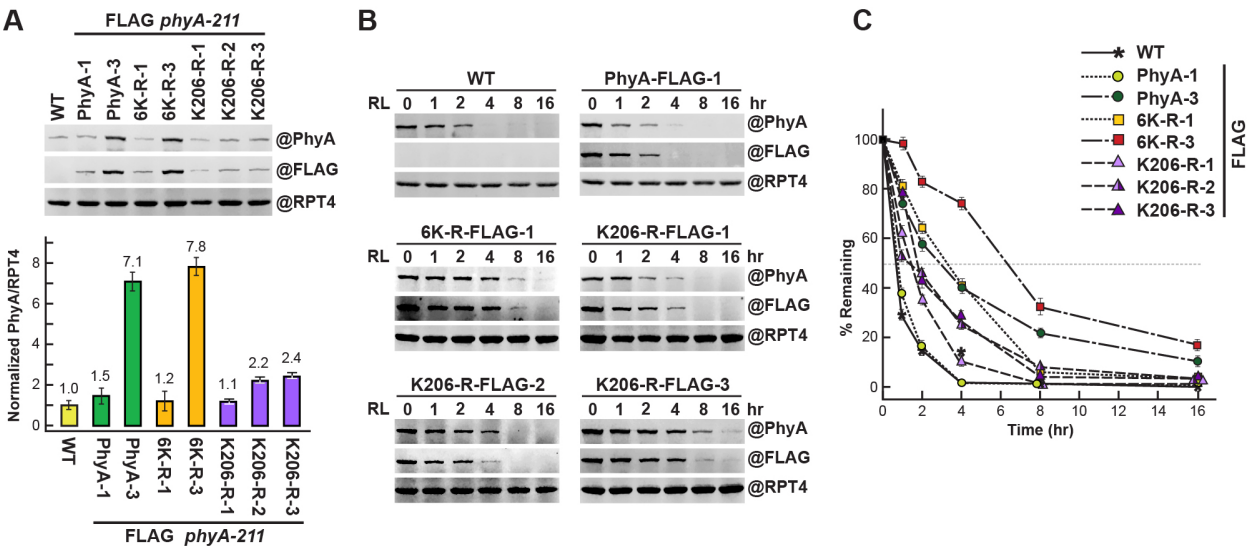

Supplemental Figure 7. Preventing Ubiquitylation at Lysine-206 in *Arabidopsis* PhyA Slows but Does not Block Degradation as Pfr.

Either the normal PhyA sequence (PhyA), the 6K-R mutant, or one in which K206 was substituted for arginine (K206-R) were expressed with a FLAG tag in the *phyA-211* background. **(A)** Levels of PhyA in 4-d-old etiolated seedlings before irradiation. Clarified extracts from WT, *phyA-211*, or *phyA-211* seedlings transformed with an empty vector or the PhyA, 6K-R, or K2-6-R transgenes were subjected to SDS-PAGE and immunoblotting with either the 073D anti-PhyA or anti-FLAG monoclonal antibody. Three independent transformants were analyzed for K206-R. The PhyA and 6K-R lines were described in Figure 7. Near equal protein loading was confirmed by immunoblotting with anti-RPT4 antibodies. Quantification of PhyA levels by densitometric scans of the anti-PhyA antibody immunoblots is shown below. The values were normalized to the PhyA level in WT.

(B) Degradation of PhyA in red light (R). Four-d-old etiolated seedlings were irradiated with 50 μmol m^{-2} sec^{-1} R for the indicated times and harvested. Clarified extracts were subjected to immunoblotting as in panel (A).

(C) Quantification of PhyA degradation rates by densitometric scans of the anti-PhyA antibody immunoblots shown in panel (B). Each point was normalized to the value for PhyA at t=0. Dashed lines highlight the time when 50% of PhyA was degraded.