



**Supplemental Figure 1.** Experimental Setup to Analyze by Tandem MS the Pool of Ubiquitylated Proteins that Accumulate in Etiolated *Arabidopsis* Seedlings.

(A) Protocol for treating 7-d-old etiolated seedlings grown in liquid culture with MG132 and red light (R) and the subsequent two-step affinity purification of ubiquitylated proteins before LC-ESI-MS/MS.

(B-D) Pictures of the seedling cultures and representative seedlings either kept in darkness (Etiolated) or exposed to continuous white light (Green). S, shoot. R, root.

(E) Comparisons of the free Ub and Ub conjugate profiles in WT and 6His-UBQ seedlings either kept in darkness or exposed to R for the indicated times. Clarified seedling extracts were subjected to SDS-PAGE and either subjected to immunoblotting with anti-Ub antibodies (@Ub) or stained for protein with silver (Protein). Ub conjugates and the Ub and 6His-Ub monomers and Ub and 6His-Ub dimers are indicated.