**E.** 14-3-3ζ is required for the cleavage of Phldb2 by Lem8. HA-Phldb2-Flag was expressed in HEK293T cells, immunoprecipitated with a Flag-specific antibody, and eluted with 3×Flag peptides. Purified Phldb2 was incubated with His6-Lem8 or each of the mutants in reactions with or without His6-14-3-3ζ. Total proteins of all samples were resolved with SDS-PAGE, and probed by immunoblotting with a HA-specific antibody, a Flag-specific antibody and a His-specific antibody.