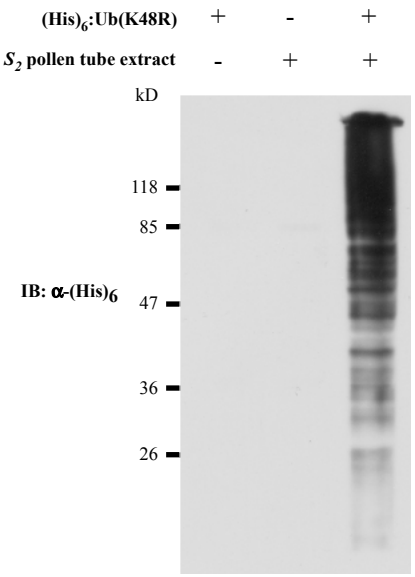
Hua and Kao, Supplemental Figure 7



Supplemental Figure 7. Ubiquitination assay of total protein in *S2* pollen tube extract. $(His)_6$:Ub(K48R), expressed and purified from *E. coli* BL21(DE3)pLysS (Novagen), was used in the assay. The replacement of Lys-48 by Arg in Ub(K48R) blocked the formation of poly ubiquitin chains but still allowed the attachment of mono ubiquitin units to target proteins. Five micrograms of *S2* pollen tube extract were incubated in 30 µL ubiquitin reaction buffer (50 mM Tris-HCl, pH 7.4, 2 mM ATP, 2 mM DTT, 5 mM $MgCl_2$, ~4 µg creatine phosphokinase [Calbiochem], 10 mM creatine phosphate [Calbiochem], 1 mM PMSF) for 1 hr at 30°C in the presence or absence of 30 µg of $(His)_6$:Ub(K48R). The reactions were stopped by the addition of 7 µL 5 × SDS reducing sample buffer and subsequent heating at 95° C for 5 min. The samples were analyzed by immunoblotting using an anti-$(His)_6$ tag antibody. The strong smear signals detected by the antibody indicate that many proteins in the pollen tube extract were ubiquitinated.