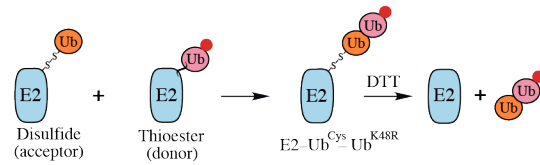


A



B

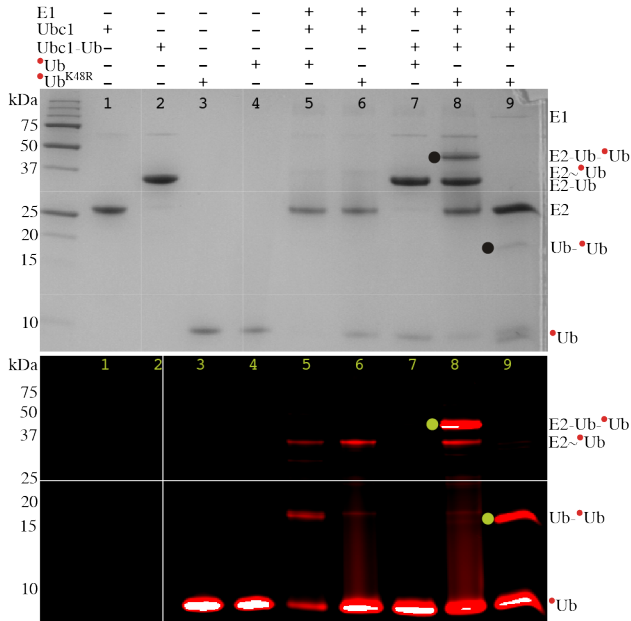


Figure S1. Ubc1 activity gels showing ubiquitin transfer from the Alexa-680 labeled Ubc1~Ub^{K48R} thioester onto the Ubc1-Ub^{Cys} disulfide. (A) Mechanism detailing Ubc1~Ub^{K48R} thioester reaction with the Ubc1-Ub^{Cys} disulfide to produce Ubc1-Ub^{Cys}-Ub^{K48R} (Ubc1-Ub₂). (B) Ubc1 enzyme reactions performed with Alexa-labeled Ub and Ub^{K48R} were visualized with Coomassie (top) or 700 nm fluorescence (bottom). Reactions were conducted as described in the text for 4 h at 37 °C. Reducing agent was only added to lane 9 (10 mM DTT and 10 mM TCEP) to break the disulfide linkage. Reaction products are indicated by the filled black / yellow circles. Molecular weight standards and protein species are listed to the left and right of each gel respectively.