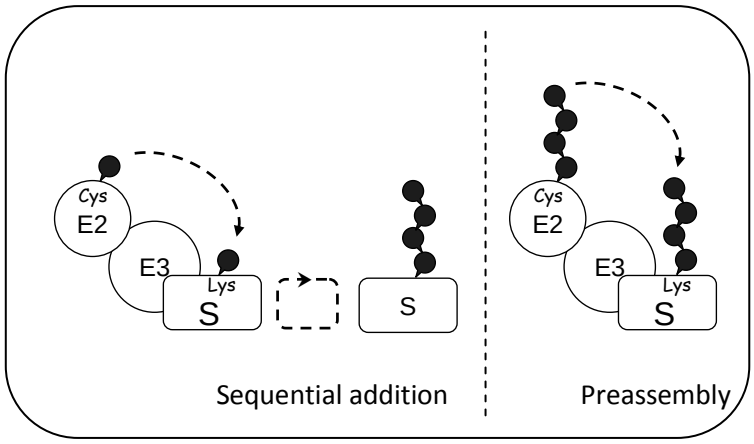


Figure1. Proposed models for substrate poly-ubiquitylation

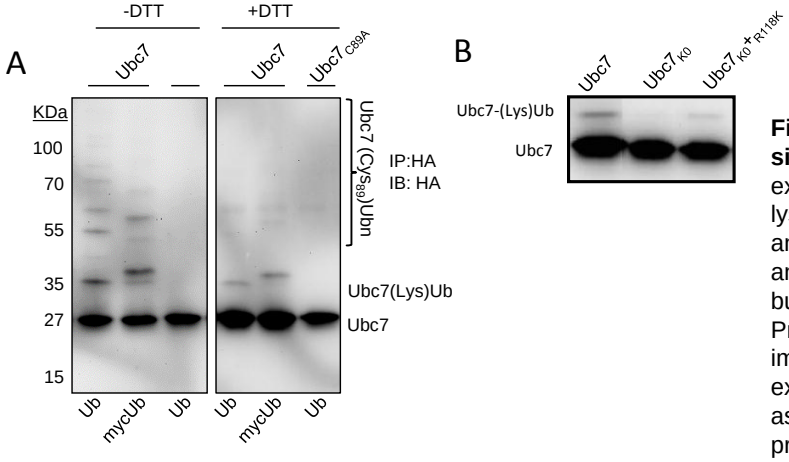


Figure 2 Ubc7 is self-ubiquitylated on its active-site cysteine as well as on Lys$_{118}$. A. Cells co-expressing Ubc7-HA and either Ub or MycUb were lysed and proteins were precipitated using anti HA antibodies. Ubc7 was detected with anti HA antibodies after extracting the proteins in a sample buffer with or without the reducing agent DTT. Proteins were separated on a SDS-PAGE and immunoblotted with anti HA antibodies. B. Cells expressing the indicated Ubc7 molecules were lysed as above and Ubc7conjugates were detected in the presence of DTT.
K0- lysineless Ubc7

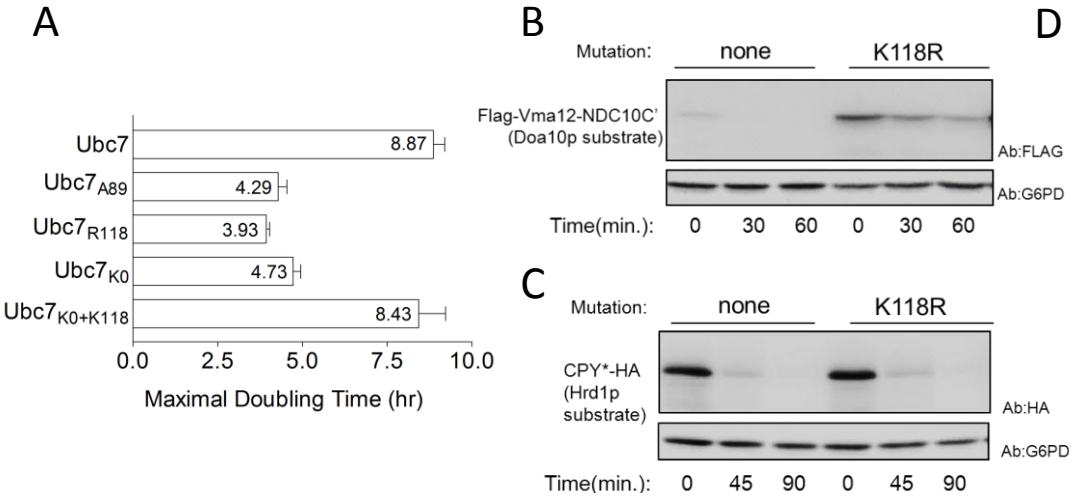


Figure3. A role for Lys$_{118}$ on Ubc7 in Doa10-dependent degradation. A. Doubling time values of cells expressing the indicated Ubc7 variants grown in in SD-Ura media. B, C. CHX-chase analyses of the indicated substrates in cells expressing wild type Ubc7 and Ubc7$_{R118}$.

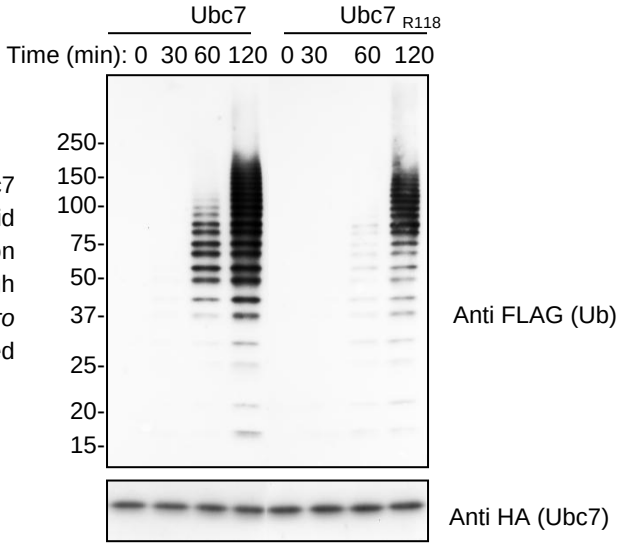
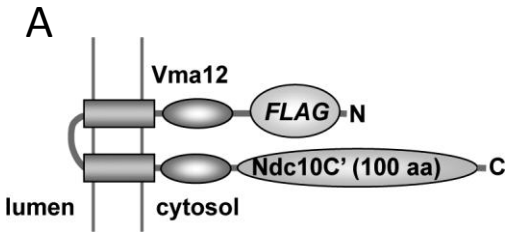
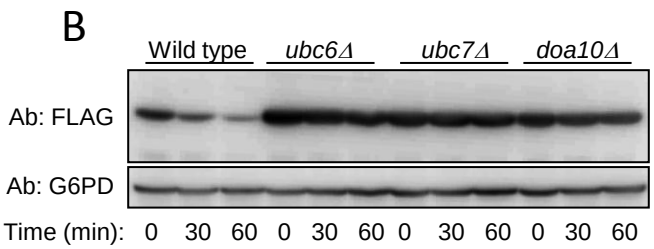


Figure 4. **.** *In vitro* analysis of Ubc7 activity. Wild type Ubc7 Lys[118] mutant, co-expressed with Cue1 in a bi-citronic plasmid were purified from bacteria using an intein tag that binds on chitin column. Proteins were released from the column through treatment with DTT, dialyzed and then were subjected to *in vitro* ubiquitylation assay for the indicated time periods, as described by Bazirgan and co-workers (Bazirgan et al. 2006).





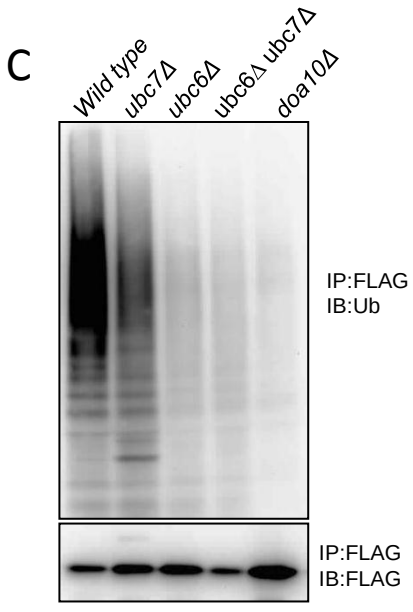


Figure 5. Ubc6 and Ubc7 play different roles in the ubiquitylation of Doa10 substrates. A. Schematic presentation of the topology of the Doa10 substrate Vma12-Ndc10C'. B. CHX-chase analysis of the degradation of Vma12-Ndc10C', expressed at the indicated knockout strains. C. 10 OD equivalents of the indicated strains, expressing Vma12-Ndc10C' were lysed and proteins were precipitated using anti FLAG affinity gel, followed by immunoblotting with anti Ub and anti-FLAG Abs.