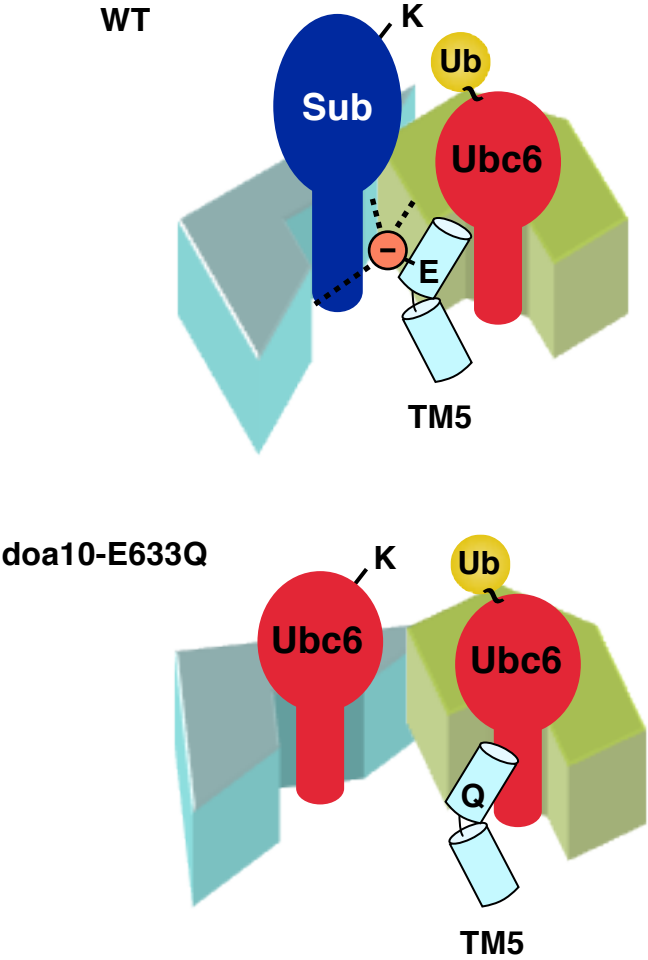**Supplementary Figure Legends**

Suppl. Fig. S1. Model for Doa10 TM5 function in coordinating Ubc6 activity. In this speculative cartoon, the TM5 Glu633 forms a network of interactions within Doa10 to create a gate between the Ubc6 "E2 site" (green, on right) and the membrane protein "substrate site" (light blue, on left) of Doa10. Normally, only a small fraction of Ubc6 can slip laterally into the substrate binding site, which might require the ubiquitin (Ub) thiolester form of Ubc6. In the *doa10-E633Q* mutant, the gate is perturbed, allowing frequent Ubc6 movement into the substrate site, ubiquitylation of this Ubc6 molecule by a second Ubc6 protein present in the E2 site, and rapid Ubc6 degradation. K, lysine residue that becomes modified by Ub.

Suppl. Fig. S1. Kreft & Hochstrasser

Table S1. Kreft & Hochstrasser

Table S1. Yeast strains used in this study

Strain	Genotype	Source
MHY500	a *his3-200 leu2-3,112 ura3-52 lys2-801 trp1-1*	Chen et al., 1993
MHY1310	a *his3-200 leu2-3,112::LEU2-Deg1-lacZ ura3-52 lys2-801 trp1-1*	M.H. lab, unpublished
MHY1685	MHY500 with *doa10Δ::HIS3*	Swanson et al. (2001)
MHY3118	MHY500 with *doa10-E633Q*	This study
MHY3191	MHY1310 with *doa10::CORE cassette* integrated at ORF pos. 1914	This study
MHY3255	MHY1310 with *doa10-E633Q*	This study
MHY3280	MHY1310 with *doa10-G642V*	This study
MHY3283	MHY1310 with *doa10-G642A*	This study
MHY3297	MHY1310 with *doa10-P638A*	This study
MHY3299	MHY1310 with *doa10-P638A/G642A*	This study
MHY3333	MHY1310 with *ubc7Δ::LEU2*	This study
MHY3335	MHY1310 with *ubc7Δ::LEU2 doa10-E633Q*	This study
MHY3339	MHY1310 with *doa10-C39S*	This study
MHY3340	MHY1310 with *doa10-C39S/E633Q*	This study
MHY3367	MHY1310 with *ubc6-Δ1::HIS3*	This study
MHY3368	MHY1310 with *ubc6-Δ1::HIS3 doa10-E633Q*	This study
MHY3416	MHY1310 with *doa10-E633A*	This study
MHY3417	MHY1310 with *doa10-E633K*	This study
MHY3443	MHY1310 with *doa10-E633R*	This study
MHY3481	MHY500 with *doa10Δ::HIS3*	This study
MHY3612	MHY1310 with *DOA10-yEGFP::his5+*	Deng and Hochstrasser, 2006
MHY3693	MHY1310 with *doa10-E633Q-yEGFP::his5+*	Deng and Hochstrasser, 2006
MHY4268	MHY1310 with *doa10-G636R*	This study
SKY166	MHY1310 with *doa10Δ::hphMX4*	This study
SKY212	MHY500 with *doa10-E633Q ubc6-Δ1::HIS3*	This study
SKY218	MHY500 with *ubc6-Δ1::HIS3*	Sommer and Jentsch, 1993
SKY225	MHY500 with *doa10-G636R*	This study
SKY254	MHY1310 with *doa10-E633D*	This study
SKY263	MHY1310 with *doa10-D646A*	This study
SKY263	MHY1310 with *doa10-D646N*	This study
SKY290	MHY1310 with *doa10-E633D ubc6-Δ1::HIS3*	This study
SKY293	MHY1310 with *doa10-G642A ubc6-Δ1::HIS3*	This study