**SUPPLEMENTARY MATERIALS for Chan, Prasad, and Matsudaira, "Genetic selection based on a Ste6\*C-HA-Ura3 substrate identifies new cytosolic quality control alleles in *Saccharomyces cerevisiae*"**


**TABLE S1**

| Strain | Genotype | Source |
|---|---|---|
| W303 WT | *MAT**a**, leu2-3,112, his3-11, trp1-1, ura3-1, can1-100, ade2-1* | P. Walter (UCSF) |
| SNY106 | *MAT**a***, pRP37, W303 background | This study |
| SNY105 | *MAT**a**, ubr1::KANMX*, pRP37, W303 background | This study |
| RPY454 | *MAT**a**, san1::KANMX*, pRP37, W303 background | This study |
| SNY329 | *MAT**a**, san1::KANMX, ubr1::KANMX*, pRP37, W303 background | This study |
| RPY145 | *MAT**a**, pRP22*, W303 background | Prasad et al., 2012 |
| RPY205 | *MAT**a**, pRP42*, W303 background | Prasad et al., 2010 |
| SNY726 | *MAT**a**, pDN431*, W303 background | This study |
| SNY736 | *MAT**a**, pDN1002*, W303 background | This study |
| SNY1128 | *MAT**a**, pSM1083*, W303 background | This study |
| SNY1261 | *MAT**a**, pRP37, pRS314*, W303 background | This study |
| SNY1246 | *MAT**a**, pRP37, pSN87*, W303 background | This study |
| SNY1262 | *MAT**a**, pRP37, pSN59*, W303 background | This study |
| SNY1145 | *MAT**a**, pRP22, pRS314*, W303 background | This study |
| SNY1146 | *MAT**a**, pRP22, pY109*, W303 background | This study |
| SNY1249 | *MAT**a**, pRP22, pSN87*, W303 background | This study |
| SNY1405 | *MAT**a**, pRP22, pSN59*, W303 background | This study |
| SNY1333 | *MAT**a**, pRP42, pRS314*, W303 background | This study |
| SNY1150 | *MAT**a**, pRP42, pY109*, W303 background | This study |
| SNY1247 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pRP37, pRS314*, W303 background | This study |
| SNY1248 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A*, pRP37, *pSN87*, W303 background | This study |
| SNY672 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pRP22*, W303 background | This study |
| SNY674 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pRP42*, W303 background | This study |
| SNY843 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pDN431*, W303 background | This study |
| SNY1319 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pDN1002*, W303 background | This study |
| SNY1320 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pSM1083*, W303 background | This study |
| SNY1250 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pRP22, pRS314*, W303 background | This study |
| SNY1251 | *MAT**a**, pup2-L101P, lcl-K2E, rpc31-G10A, pRP22, pSN87*, W303 background | This study |
| SNY1045 | *MAT**a**, Δdoa4::KANMX*, W303 background | This study |
| SNY1260 | *MAT**a**, Δdoa4::KANMX, pRP37*, W303 background | This study |
| SNY1078 | *MAT**a**, Δdoa4::KANMX, pRP37, pSN59*, W303 background | This study |
| SNY1058 | *MAT**a**, Δdoa4::KANMX, pRP22*, W303 background | This study |
| SNY1059 | *MAT**a**, Δdoa4::KANMX, pRP42*, W303 background | This study |
| SNY1063 | *MAT**a**, Δdoa4::KANMX, pDN431*, W303 background | This study |
| SNY1064 | *MAT**a**, Δdoa4::KANMX, pDN1002*, W303 background | This study |

| | | |
|---|---|---|
| SNY1129 | *MAT**a**, Δdoa4::KANMX, pSM1083*, W303 background | This study |
| SNY1147 | *MAT**a**, Δdoa4::KANMX, pRP22, pRS314*, W303 background | This study |
| SNY1148 | *MAT**a**, Δdoa4::KANMX, pRP22, pY109*, W303 background | This study |
| SNY1406 | *MAT**a**, Δdoa4::KANMX, pRP22, pSN59*, W303 background | This study |
| SNY1151 | *MAT**a**, Δdoa4::KANMX, pRP42, pRS314*, W303 background | This study |
| SNY1152 | *MAT**a**, Δdoa4::KANMX, pRP42, pY109*, W303 background | This study |
| SNY1263 | *MAT**a**, doa4-Q626*, tcb3-N1211L, prp38-C174Y, pRP37, pRS314*, W303 background | This study |
| SNY1264 | *MAT**a**, doa4-Q626*, tcb3-N1211L, prp38-C174Y, pRP37, pSN59*, W303 background | This study |
| SNY901 | *MAT**a**, doa4-Q626*, tcb3-N1211L, prp38-C174Y, pRP22*, W303 background | This study |
| SNY902 | *MAT**a**, doa4-Q626*, tcb3-N1211L, prp38-C174Y, pRP42*, W303 background | This study |
| SNY1265 | *MAT**a**, doa4-S329*, ino1-C19G, pRP37, pRS314*, W303 background | This study |
| SNY1266 | *MAT**a**, doa4-S329*, ino1-C19G, pRP37, pSN59*, W303 background | This study |
| SNY941 | *MAT**a**, doa4-S329*, ino1-C19G, pRP22*, W303 background | This study |
| SNY942 | *MAT**a**, doa4-S329*, ino1-C19G, pRP42*, W303 background | This study |
| SMUra#38 | *MAT**a**, ubr1-A580S, pRP37*, W303 background | This study |
| SMUra#114 | *MAT**a**, Spontaneous mutant #1, pRP37,* W303 background | This study |
| SMUra#125 | *MAT**a**, Spontaneous mutant #2, pRP37,* W303 background | This study |
| SMUra#22 | *MAT**a**, Spontaneous mutant #3, pRP37,* W303 background | This study |
| SMUra#54 | *MAT**a**, Spontaneous mutant #4, pRP37,* W303 background | This study |
| SNY638 | *MAT**a**, rpt5-E282D, chm7-I238M, pRP37, pRP22*, W303 background | This study |
| SNY640 | *MAT**a**, rpt5-E282D, chm7-I238M, pRP37, pRP42*, W303 background | This study |
| SNY545 | *MAT**a**, rvb2-M175R, pre7-Y24S, tos1-E121G, pRP22*, W303 background | This study |
| SNY563 | *MAT**a**, rvb2-M175R, pre7-Y24S, tos1-E121G, pRP42*, W303 background | This study |
| SNY634 | *MAT**a**, rpt3-V209D, pRP22*, W303 background | This study |
| SNY636 | *MAT**a**, rpt3-V209D, pRP42*, W303 background | This study |

**TABLE S1: YEAST STRAINS USED IN THIS STUDY.**

**TABLE S2**

| Plasmid | Encoded protein | Promoter | Vector | Source |
|---------|-----------------|----------|--------|--------|
| pRP37 | Ste6*C-HA-Ura3 | *PRC1* | pRS313 | This study |
| pRP22 | Ste6*C-HA | *TDH3* | pRS313 | Prasad et al., 2012 |
| pRP42 | ΔssPrA-HA | *TDH3* | pRS313 | Prasad et al., 2010 |
| pDN431 | CPY*-HA | *PRC1* | Ycp50 | Ng et al., 2000 |
| pDN1002 | Sec61-2-HA | *SEC61* | pRS315 | Vashist et al., 2001 |
| pSM1083 | Ste6*-HA | *STE6* | *CEN URA3* | Loayza et al., 1998 |
| pSN87 | Pup2 | *PUP2* | pRS314 | This study |
| pSN59 | Doa4 | *DOA4* | pRS314 | This study |
| pY109 | Ub | *TDH3* | pRS314 | Davis Ng Lab Collection |

**TABLE S2: PLASMIDS USED IN THIS STUDY.**

**TABLE S3**

| Primer | Plasmid | Sequence (5' → 3') |
|--------|---------|---------------------|
| RP205 | pRP37 | GTCAGGATCCATGATACCCGATATAAGTAGAGGC |
| RP52 | pRP37 | GCGCCCGGGAGCGTAATCTGGAACATCATATGGGTA |
| SN427 | pSN87 | GATAAGCGGCCGCGTAGGTGAGAAAGTTTCTTGTTGGTC G |
| SN428 | pSN87 | GATGCGTCGACCGATTGATCCAATGACTATTCCTGGC |
| SN295 | pSN59 | GATAAGCGGCCGCCACTCCAAGACAATTGATGCGTAAATCG |
| SN296 | pSN59 | GATGCCTCGAGGATTTGCAACTGGACCCGTTTGAC |

**TABLE S3: OLIGONUCLEOTIDE PRIMERS USED IN THIS STUDY.**

**TABLE S4**

| Elimination method | Number of Spontaneous mutants |
|---|---|
| False positives | Total: 61 |
| Transformation of known CytoQC genes | UBR1: 41 |
| | RPN11: 1 |
| | UMP1: 1 |
| | Total number identified: 43 |

| Whole genome sequencing method | Number of Spontaneous mutants |
|---|---|
| Unidentified true positives | Total: 13<br>• Temperature sensitive: 4<br>• Non-temperature sensitive: 9<br>**Identified: 6**<br>• PUP2: 1<br>• DOA4: 2<br>• RPT3: 1<br>• RPT5: 1<br>• PRE7: 1 |

**TABLE S4: TABULATION OF SPONTANEOUS MUTANT SELECTION ISOLATES**
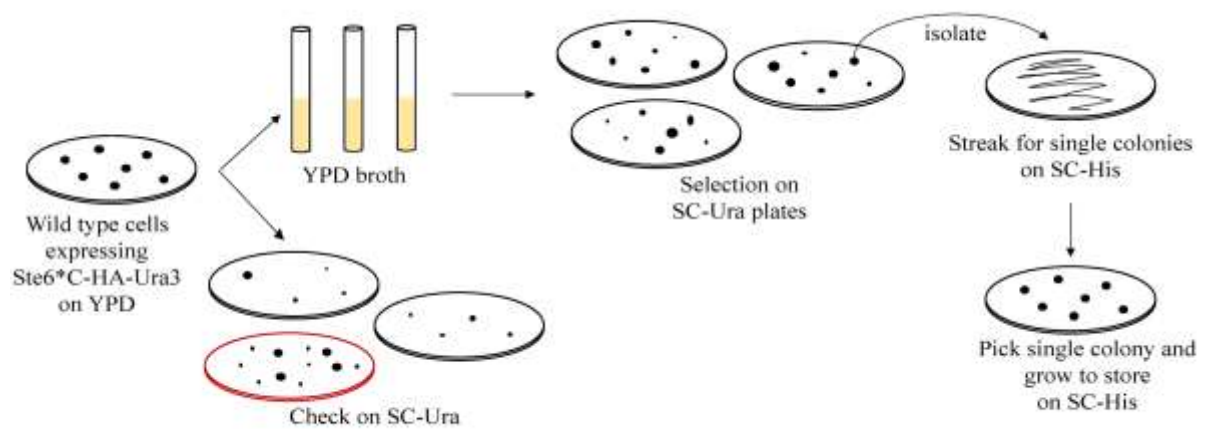
**Figure S1**: Schematic diagram of the genome-wide selection based on spontaneous mutations. Selection is based on the observation of the CytoQC-defective phenotype displayed by colonies that grow on the synthetic complete (SC) selection plates lacking uracil (SC-Ura). Colonies growing on SC-Ura are further isolated and streaked on SC-His and SC-Ura plates for confirmation.
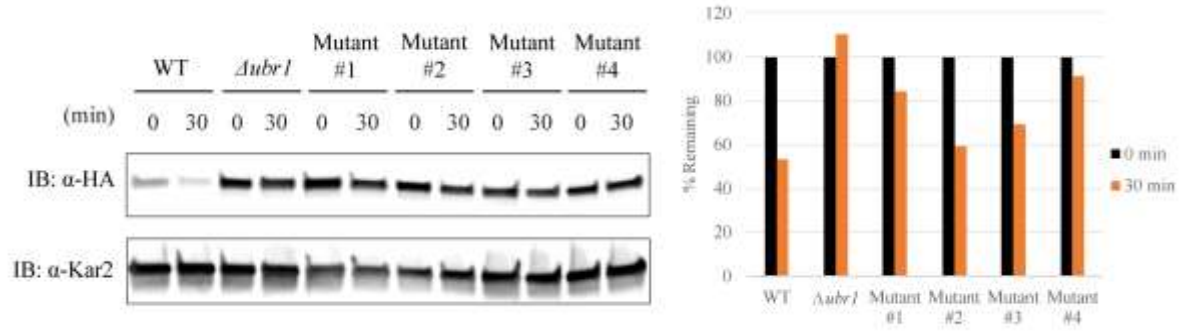
**Figure S2**: Stabilization of the reporter substrate in CytoQC-defective mutants. Cells were treated with 200µg/ml of cycloheximide and chased for the indicated timepoints. Lysates harvested were resolved on SDS-PAGE and analyzed by immunoblotting (IB) with anti-HA antibody. Kar2 was probed as loading control. Graph on the right shows the quantification of the substrate bands in the immunoblot. Intensity of substrate bands are normalized to the loading control of each sample.
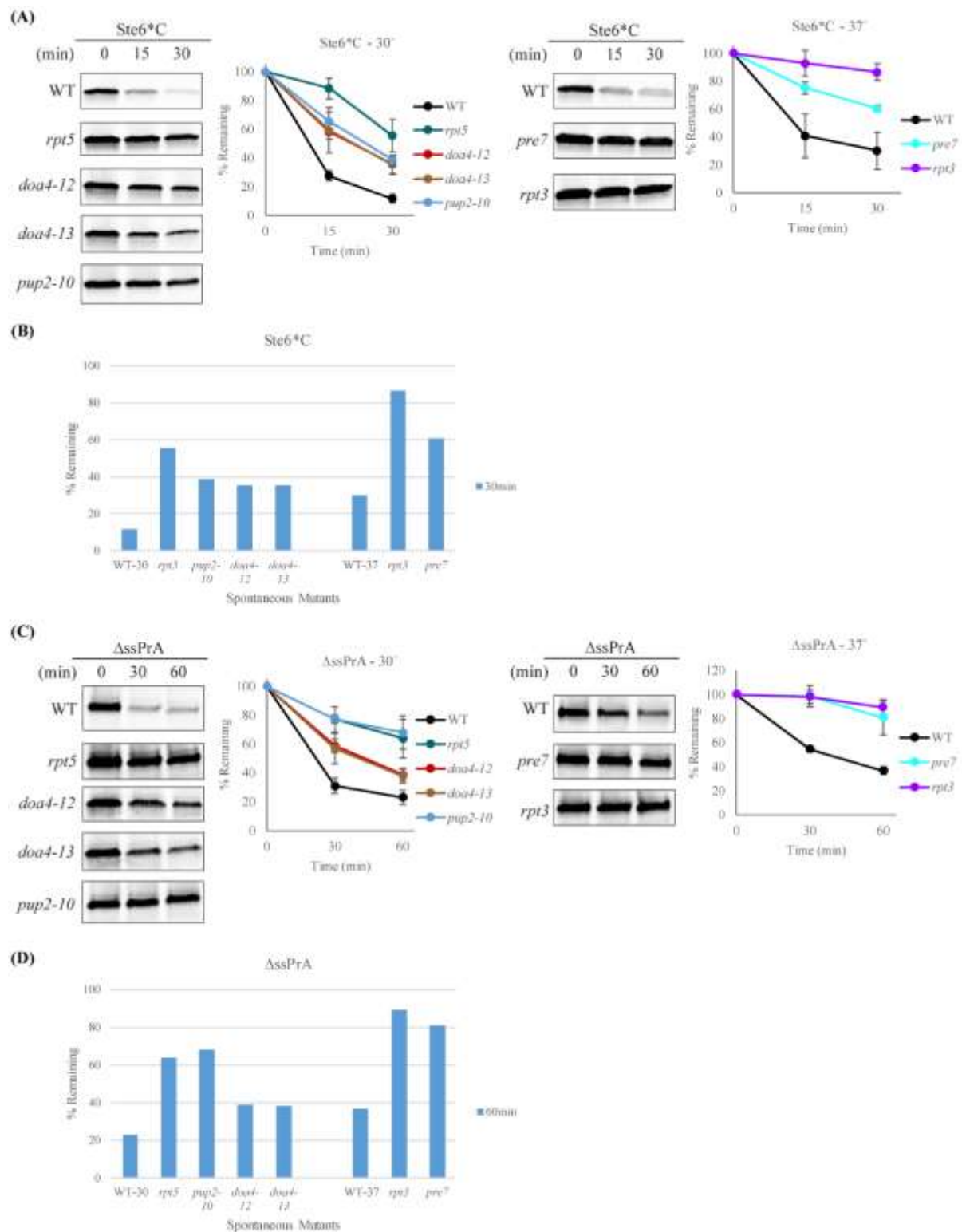
**Figure S3**: Degradation of CytoQC substrates is delayed in the CytoQC mutants. Selected spontaneous mutants from initial characterization assays were transformed with plasmid expressing CytoQC substrates Ste6*C or ΔssPrA. (A and C) All the selected spontaneous mutants stabilize both CytoQC substrates albeit to varying degrees. Strains were pulsed with 35S-Met/Cys for 5min for Ste6*C and 10min for ΔssPrA, followed by chase for the indicated time points. (B and D) Graph of the relative amount of CytoQC substrate remaining in each spontaneous

mutant at the last time point (30min for Ste6*C and 60min for ΔssPrA). WT-30 refers to WT strain pulse-chased at 30° and WT-37 refers to WT strain at 37°. Non-temperature sensitive mutants (left) were pulse-chased at 30° while temperature-sensitive mutants (right) were pulse-chased at 37°.
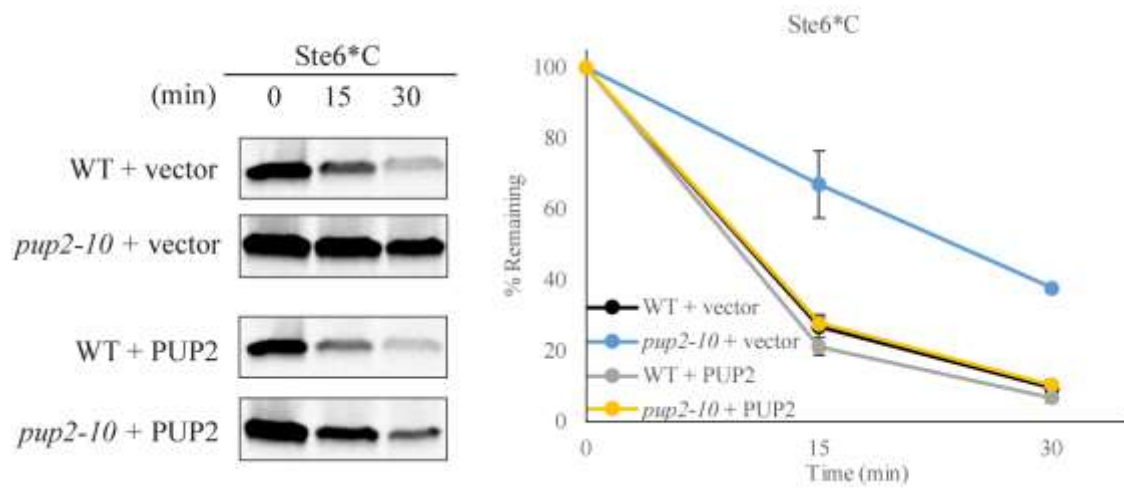
**Figure S4**: Rescue of the degradation delay of Ste6*C in the *pup2-10* mutant. The empty vector (vector) or PUP2 was transformed into WT and *pup2-10* strains expressing CytoQC substrate Ste6*C. Strains were pulsed with 35S-Met/Cys for 5min followed by chase for the indicated time points.
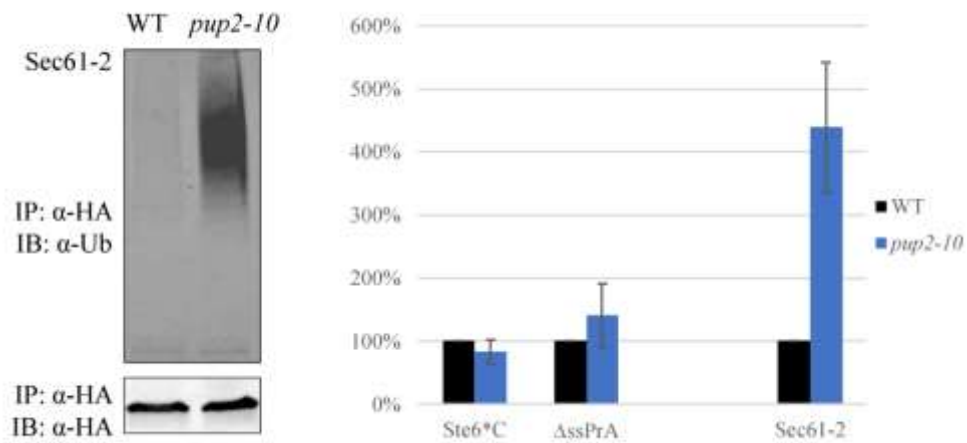
**Figure S5**: An accumulation of polyubiquitinated ERAD substrate Sec61-2 is observed in *pup2-10*. Quantification of polyubiquitinated CytoQC (from Figures 2F and 4F) and ERAD substrates in WT and *pup2-10*. Misfolded substrates expressed in WT and *pup2-10* were immunoprecipitated (IP) by anti-HA antibody, resolved by SDS-PAGE and analyzed by immunoblotting (IB) with anti-ubiquitin antibody to detect polyubiquitinated substrates.
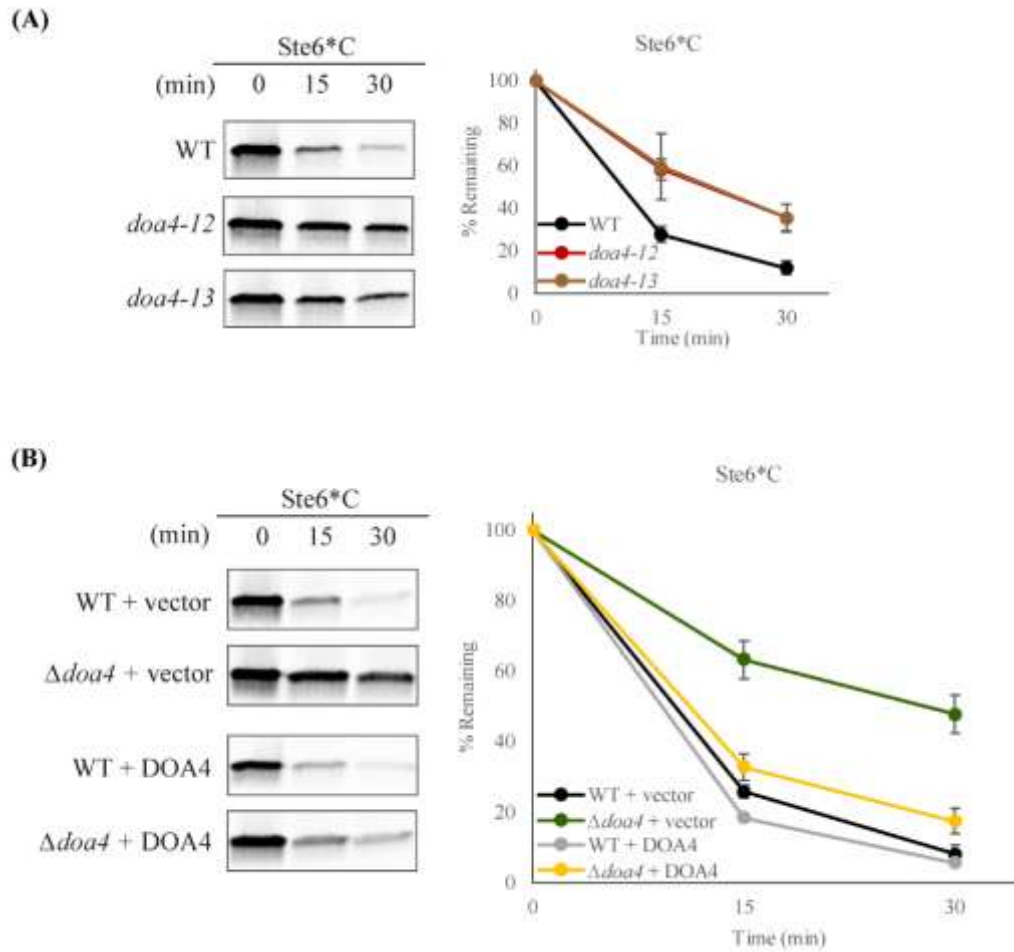
**Figure S6**: Degradation kinetics of Ste6*C in *doa4* mutants. (A) Stabilization of CytoQC substrate Ste6*C was observed in the *doa4* spontaneous mutants, *doa4-12* and *doa4-13*, compared to WT. (B) Rescue of degradation delay of Ste6*C in Δ*doa4*. The empty vector (vector) or DOA4 was transformed into WT and Δ*doa4* strains expressing CytoQC substrate Ste6*C. Strains were pulsed with 35S-Met/Cys for 5min followed by chase for the indicated time points.
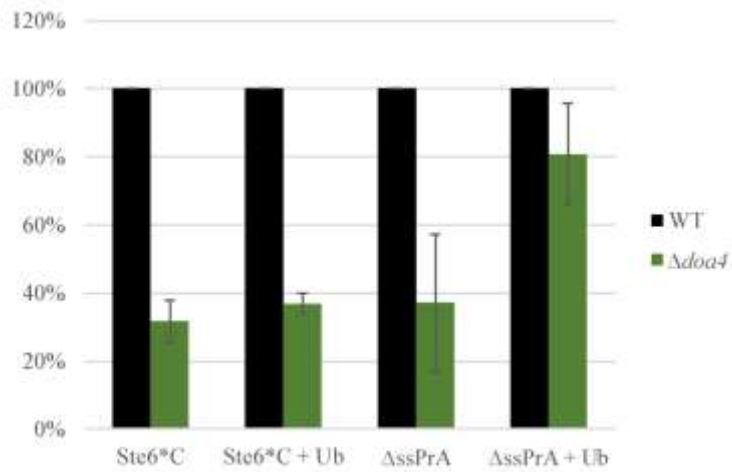
**Figure S7**: Quantification of polyubiquitinated CytoQC substrates in WT and Δ*doa4* in the absence and presence of ubiquitin (Ub) overexpression from Figures 2F, 4F and 5B. Quantification by the Odyssey infrared imaging system and ImageJ.