

## File S3

### Supplementary Materials and Methods

#### Yeast strains

SDY 356 and SDY427 (*MAT $\alpha$  ura3-52, leu2-3,112 his3- $\Delta$ 200, trp1- $\Delta$ 901, lys2-801, suc2- $\Delta$ 9 GAL - MEL *chc1-521::URA3 UBX3-GFP::HIS3*) were employed for imaging in Figure 2C. SDY500 (*MAT $\alpha$  his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0, trp1- $\Delta$ 901 *ubx3 $\Delta$ ::URA3*) was created, mated with various strains from the GFP library and subsequently sporulated and subjected to tetrad dissection to generate haploid strains SDY586 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, MUP1-GFP::HIS3*), SDY567 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, EDE1-GFP::HIS3*), SDY569 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, ENT2-GFP::HIS3*), SDY570 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, RVS167-GFP::HIS3*), and SDY585 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, SYP1-GFP::HIS3*), SDY667 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, END3-GFP::HIS3*), SDY668 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, LAS17-GFP::HIS3*), SDY669 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, MYO5-GFP::HIS3*), and SDY670 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, ABP1-GFP::HIS3*). These strains were utilized for fluorescence microscopy in Figure 4A. SDY584 (*MAT $\alpha$  his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0, *EDE1-RFP::KANMX4*) was mated with the Syp1-GFP strain from the GFP library (*MAT $\alpha$  his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *SYP1-GFP::HIS*) to create diploid strain SDY595 (*MAT $\alpha$ /MAT $\alpha$  his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *EDE1-RFP::KANMX4 SYP1-GFP::HIS*) which was subjected to sporulation and tetrad dissection, and resulting haploid segregant mated with SDY500. The resulting diploid strain was subjected to tetrad dissection to create SDY596 (*MAT his3 $\Delta$ 1, leu2 $\Delta$ 0, met15- $\Delta$ 0, ura3  $\Delta$ 0 *ubx3 $\Delta$ ::URA3, EDE1-RFP::KANMX4, SYP1-GFP::HIS3*). SDY622 (*MAT $\alpha$  ura3-52, his3 $\Delta$ 1, leu2 $\Delta$ 0, trp1- $\Delta$ 901 *EDE1-RFP::KANMX4, SLA1-*****************

*GFP::TRP1*) was mated with SDY500, sporulated and subjected to tetrad dissection to create SDY633 (*MATa ura3-52, his3Δ1, leu2Δ0, trp1-Δ901 Δubx3::URA3, EDE1-RFP::KANMX4, SLA1-GFP::TRP1*). Strain harboring a deletion of the UBX domain in the endogenous *UBX3* gene was created, SDY648 (*MATa his3Δ1, leu2Δ0, met15-Δ0, ura3 Δ0 EDE1-GFP::HIS3 ubx3<sup>ΔUBXd</sup> ::URA3*). Strains expressing Ubx1-7 –GFP from the endogenous locus were obtained from the GFP library.