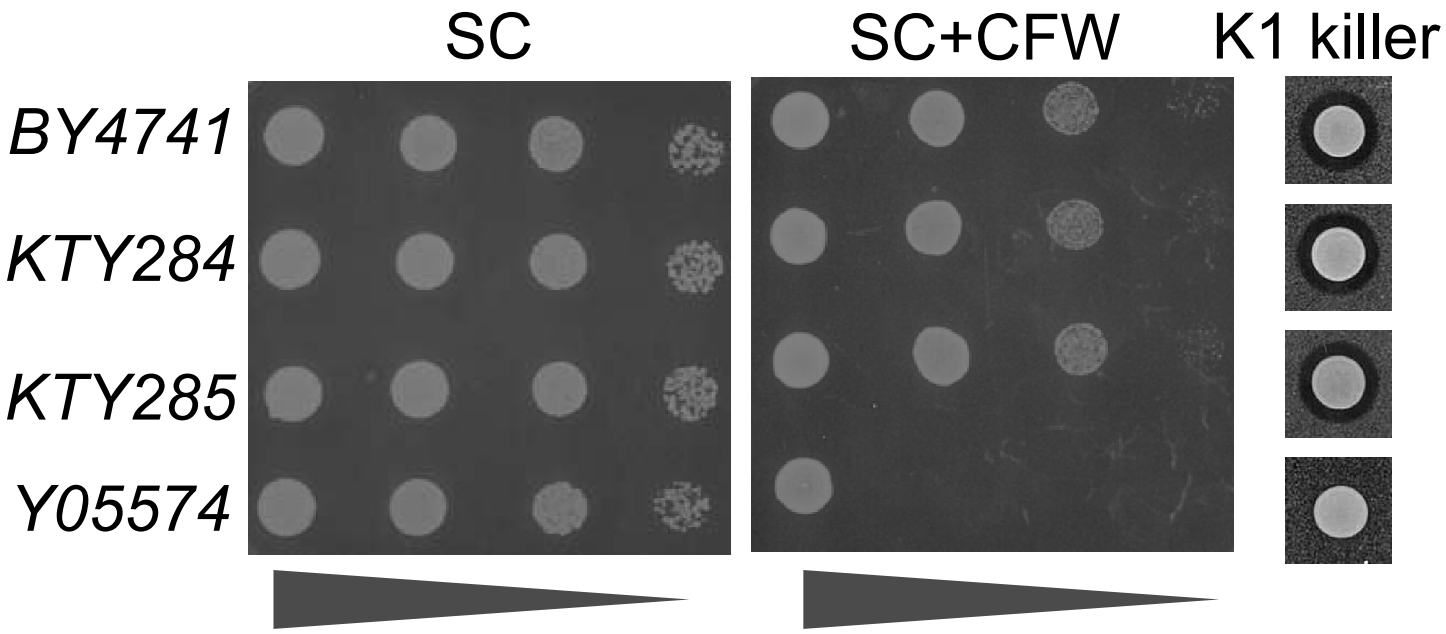
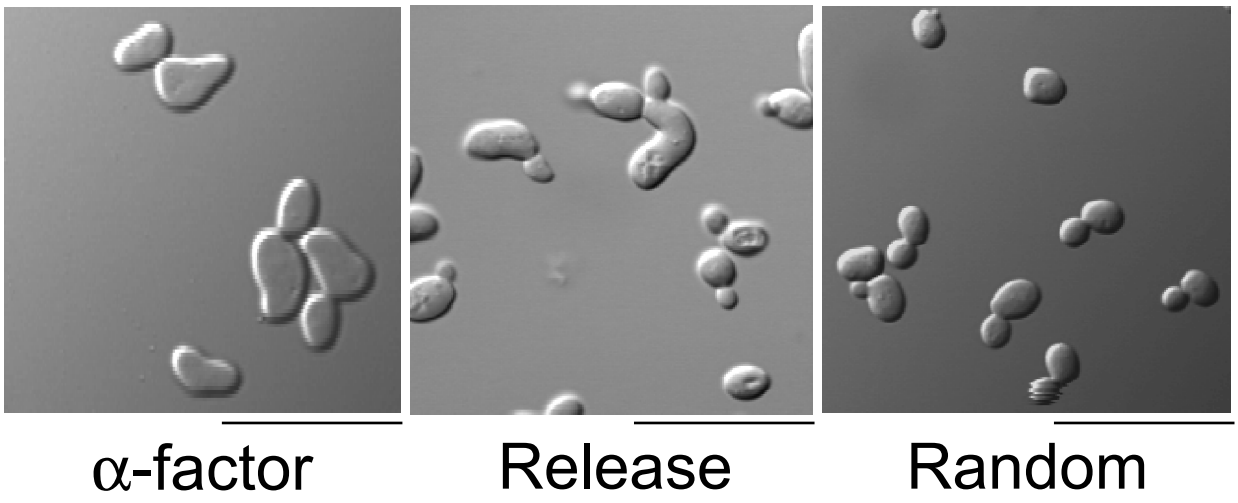
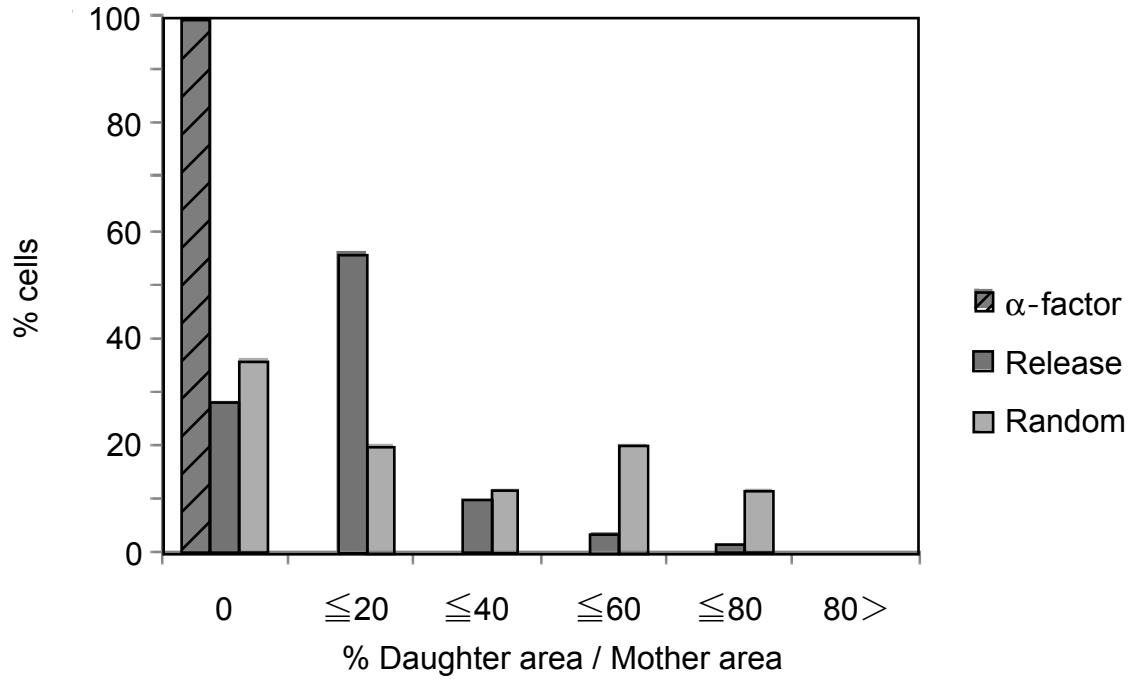
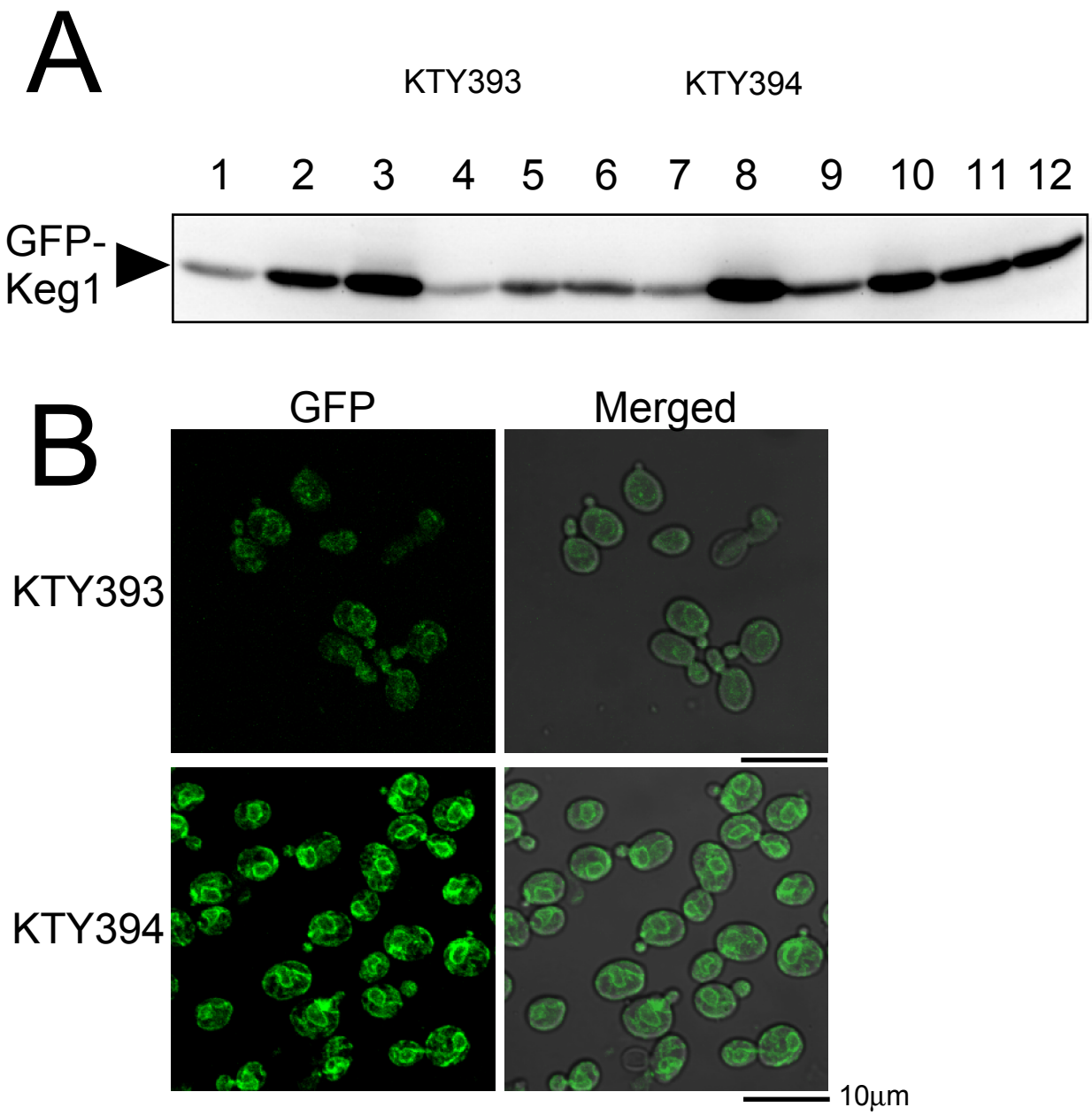


Supplementary Figure 1. **Sensitivity to Calcofluor White and K1 Killer Toxin.** *KRE6* (BY4741), *KRE6-3HA* (KTY284 and KTY285, two independent isolates in strain construction) and Δ*kre6* (Y05574) were examined. For the Calcofluor White sensitivity test, cells were grown in an SC medium until OD_{600nm}=1, diluted 2-fold, and then serially diluted 4 times by ten-fold. Ten μl of these samples were spotted on an SC plate with or without 100 μg/ml Calcofluor White (CFW) and incubated at 25 °C for 3 d. For the K1 killer toxin sensitivity test, cells were grown in YPD at 30 °C until OD_{600nm} = 1.0 and plated on a low-pH YPD plate. Five μl of a fresh overnight culture of the killer producer (NCYC232) grown at 20 °C was spotted, and the plates were incubated at 25 °C for 2 d to show the growth inhibitory zone around the killer producer spot.



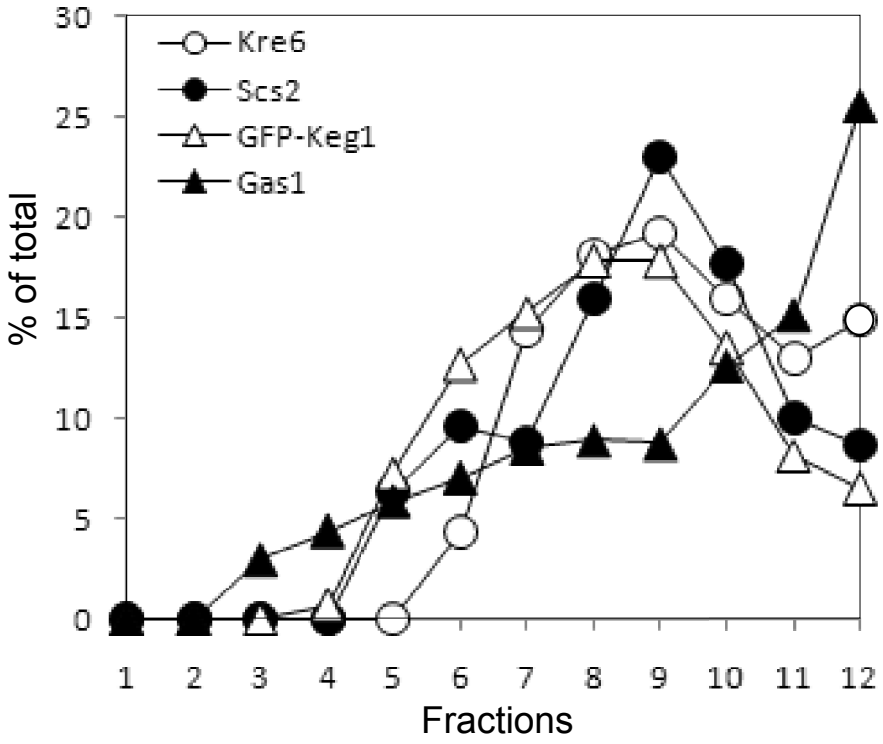


Supplementary Figure 2. **Synchronized culture of KRE6 (BY4741) by the cell cycle block and release using α-factor.** Phase-contrast microscopic observation of the cells in the culture of 3 h after incubation in the presence of α-factor (α-factor), 110 min after release of the block in a fresh YPD medium (Release) and random culture without synchronization (Random). The percentage of the various cell stages are shown.

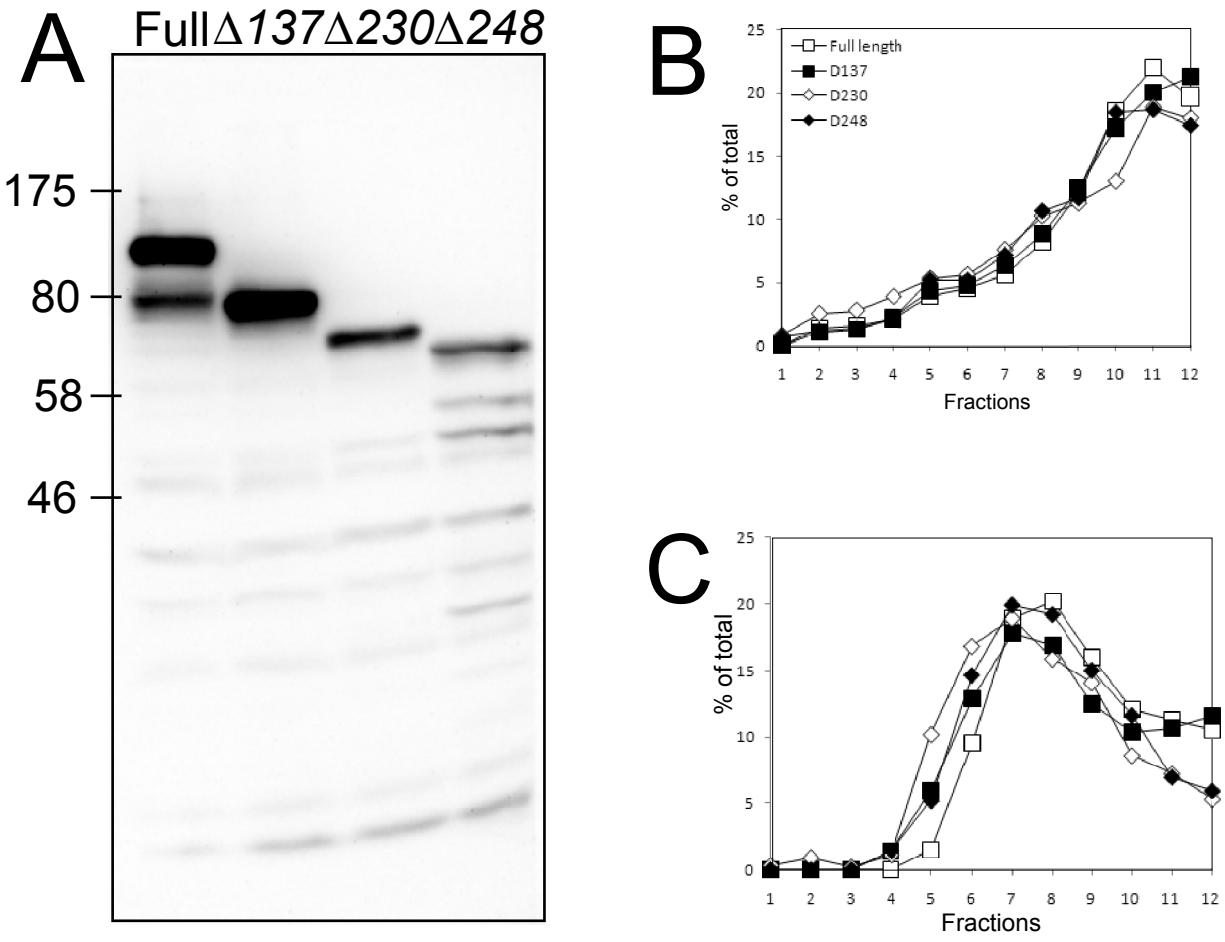


Supplementary Figure 3. **Production of GFP-Keg1 proteins by chromosomal integration of its expression units at the chromosomal ura3-52 locus and their intracellular localization.** *A*, the *YPT1*$_{promoter}$*-GFP-KEG1* expression constructs were integrated by homologous recombination at the ura3-52 locus and productivity of the tagged protein in 12 transformants were determined by Western blotting using anti-GFP antibody. *B*, the GFP fluorescence images without (GFP) or with (Merged) their Nomarski images of the cells of the lowest (KTY393, lane 4 in A) or highest (KTY394, lane 8 in A) expression level.

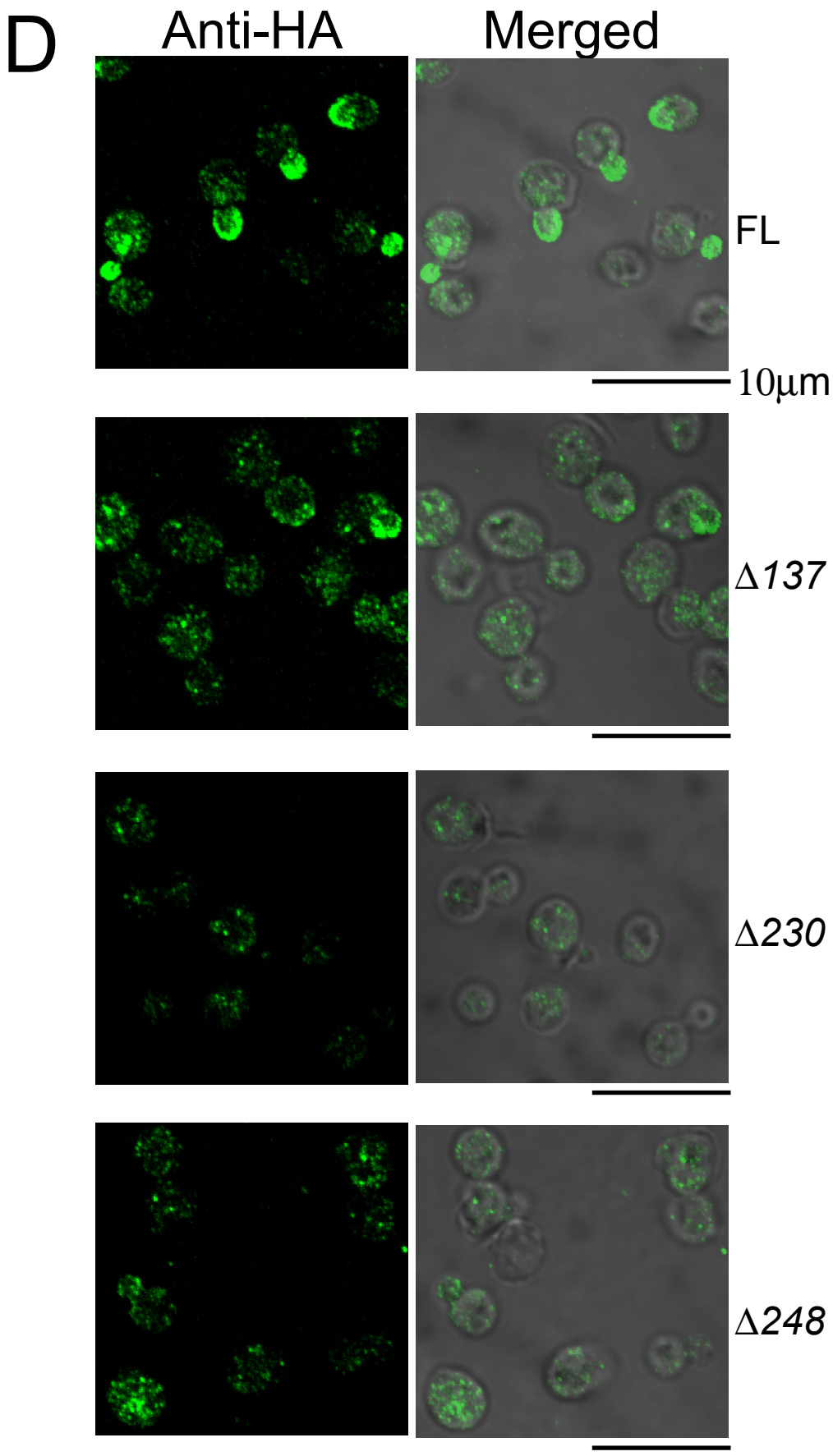
C



Supplementary Figure 3. **Production of GFP-Keg1 proteins by chromosomal integration of its expression units at the chromosomal *ura3-52* locus and their intracellular localization.** *C*, a representative result of the sucrose density gradient fractionation of GFP-Keg1, Kre6, Scs2 and Gas1 in a synchronized culture of KTY634 (as KTY393, but MATa) in the presence of EDTA.
The experiments were done as Figure 4D.



Supplementary Figure 4. **Deletions including possible ER-exit motifs in the cytoplasmic domain and localization of the Kre6 derivatives.** *A*, Western blot indicates the production and stability of the truncated Kre6 proteins using anti-HA mAb. The apparent molecular masses of the main bands are larger than the calculated values probably because of posttranslational modification(s). *B* and *C*, representative results of the sucrose density gradient fractionation of the truncated versions of Kre6 without and with the addition of EDTA, respectively. The experiments were done as Figures 4A and 4B, respectively.



Supplementary Figure 4. **Deletions including possible ER-exit motifs in the cytoplasmic domain and localization of the Kre6 derivatives.**
D, immunofluorescence images without (Anti-HA) or with (Merged) their Nomarski images of the cells with various truncated derivatives of Kre6.