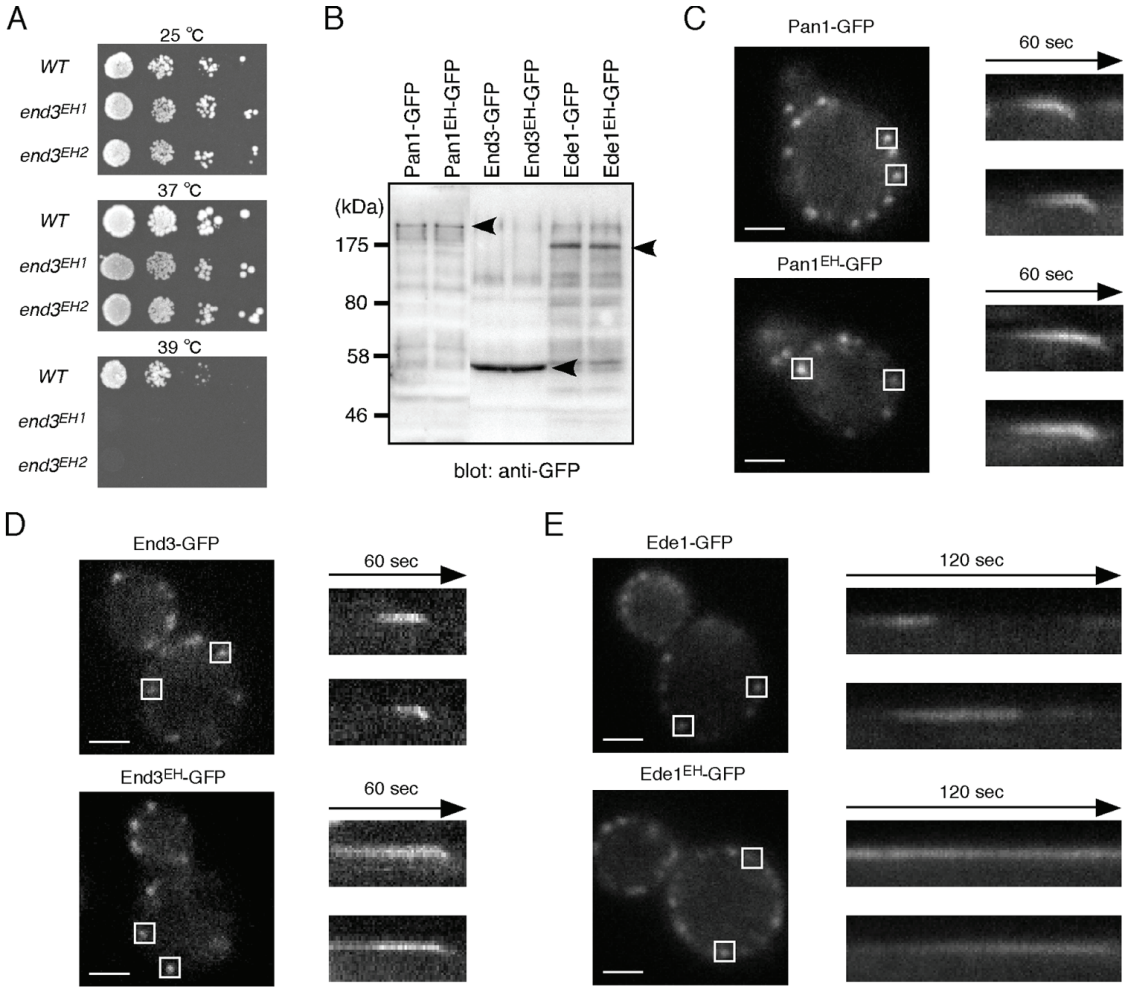
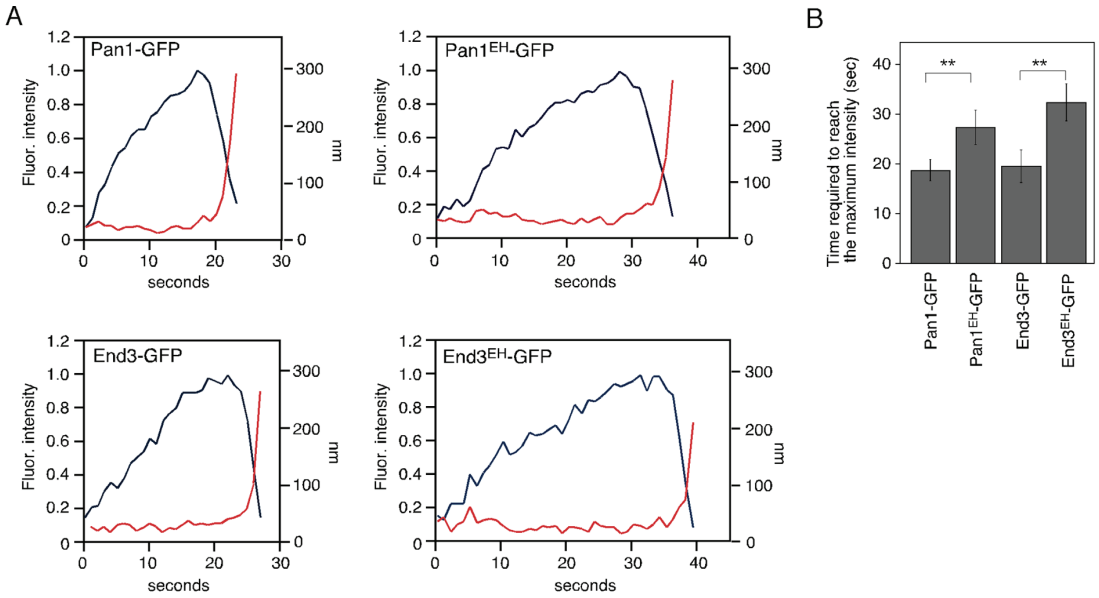


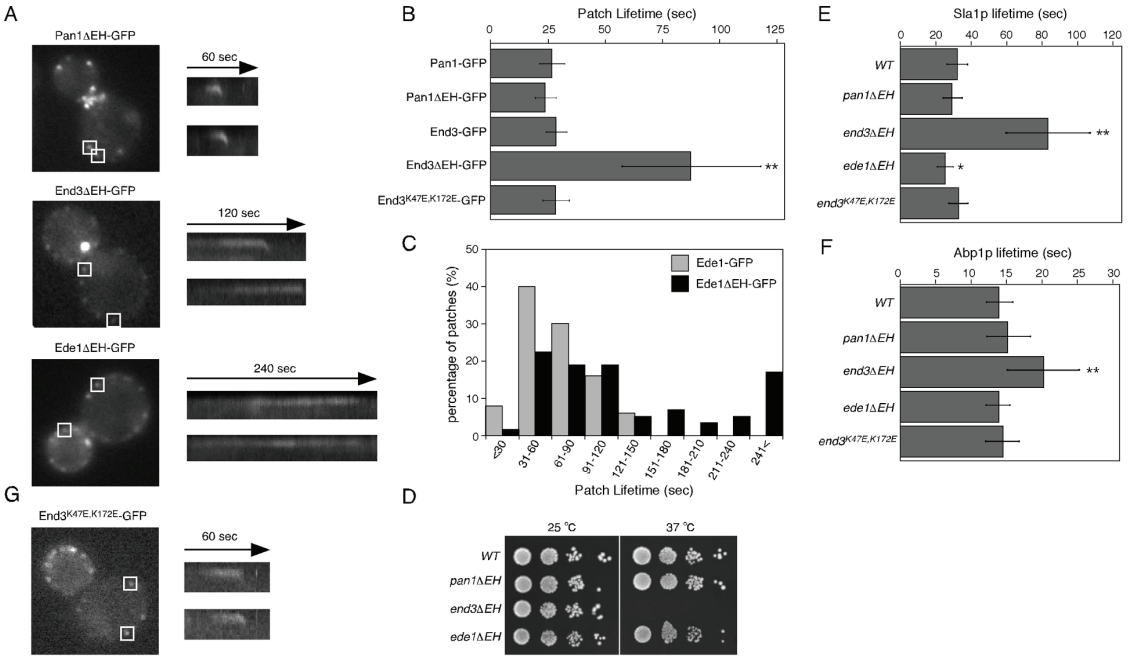
Supplemental Figure S1. Viability of *end3*^{EH} mutants, and localization of the Pan1p, End3p, Ede1p patches in EH domain mutants.

(A) The *end3*^{EH1} and *end3*^{EH2} mutants were temperature sensitive for growth at 39°C, respectively. A dilution series of cells was plated on YPD medium and incubated for the indicated temperatures. (B) Immunoblots of GFP-tagged wild-type and EH mutant proteins. Each 50 μg of lysates from the indicated cells was immunoblotted with anti-GFP antibody. (C-E) Localization of Pan1-GFP and Pan1^{EH}-GFP (C), End3-GFP and End3^{EH}-GFP (D), or Ede1-GFP and Ede1^{EH}-GFP (E) in a single cross-sectional plane. Movies were taken for 1~2 min with a 1-s frame interval. Kymographs from the same movies are shown in the right-hand panels. Scale bars, 2 μm.

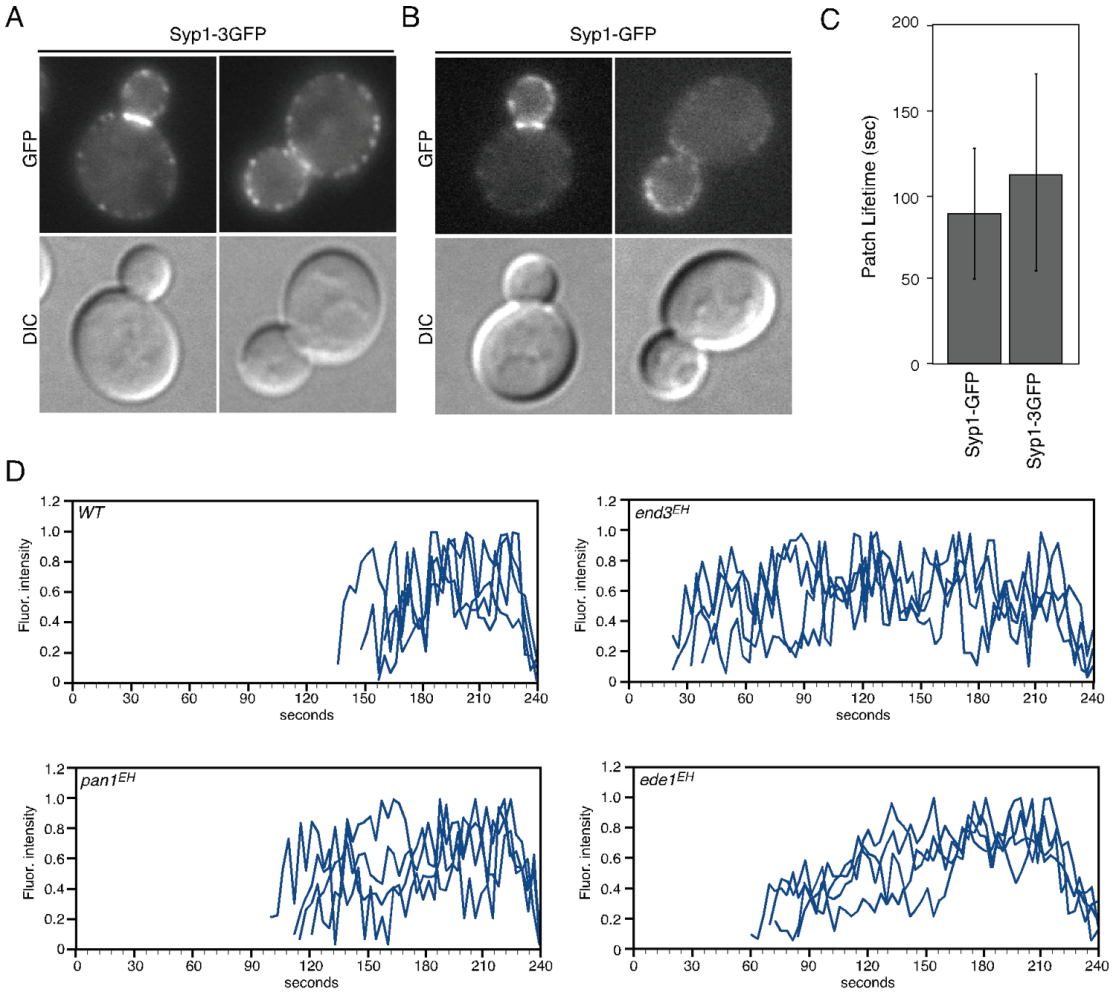


Supplemental Figure S2. Dynamics of averaged fluorescence intensity of Pan1p and End3p in wild-type cells and EH domain mutants.

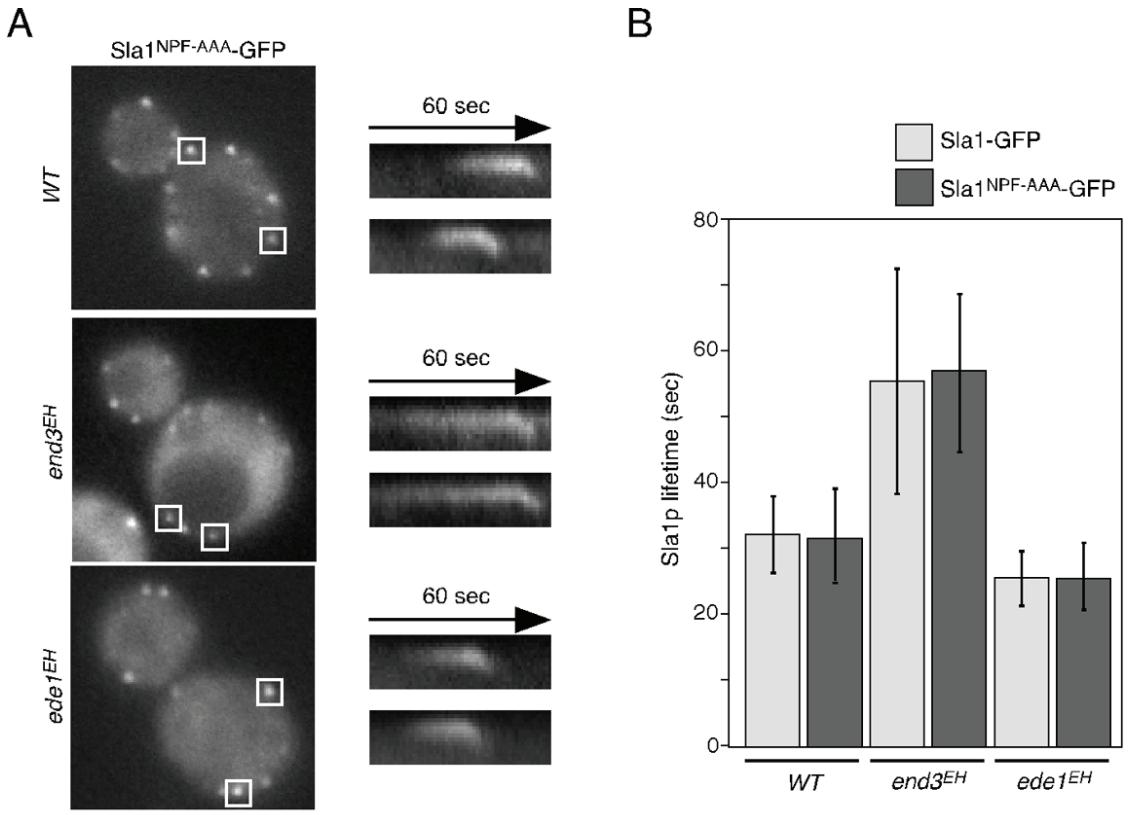
(A) Quantification of fluorescence intensity (red) and distance from site of patch formation (blue) for indicated GFP-tagged proteins. Data from at least ten patches were averaged using one-color videos of GFP-tagged proteins. Fluorescent intensity over time was corrected for photobleaching. (B) Average times ± standard deviation required to reach the maximum fluorescence intensity. Data were taken from at least ten patches analyzed in (A). **, p value < 0.001.



Supplemental Figure S3. Characterization of EH domain deletion mutants in Pan1p, Ede1p and End3p. (A) Localization of Pan1ΔEH-GFP, End3ΔEH-GFP, and Ede1ΔEH-GFP in a single cross-sectional plane. Movies were taken for 1~3 min with a 1-s frame interval. Kymographs from the same movies are shown in the right-hand panels. (B) Average lifetimes of Pan1-GFP and End3-GFP ± standard deviation in wild-type and mutant cells. Data were taken from 2-min movies with a 1-s frame interval. n = 50 patches for each strain. *, p value < 0.01, **, p value < 0.001. (C) Distribution of Ede1-GFP and Ede1ΔEH-GFP patch lifetimes. Movies were taken with a 1-s frame interval. (D) The *end3ΔEH* mutant was temperature-sensitive for growth at 37^{o}C. A dilution series of cells was plated on YPD medium and incubated at 25, or 37^{o}C. (E, F) Average lifetimes of Sla1-GFP and Abp1-mRFP ± standard deviation for indicated strains. n = 50 patches for each strain. *, p value < 0.01, **, p value < 0.001. (G) Localization and its kymographs of End3$^{K47E, K172E}$-GFP, in a single cross-sectional plane.



Supplemental Figure S4. Localization and patch liferimes of 3GFP- or GFP-tagged Spy1p in wild-type cell or EH domain mutants. (A, B) Localization of Syp1-3GFP (A) and Syp1-GFP (B) in wild-type cell. Wild-type cells expressing Syp1-3GFP or Syp1-GFP were grown to early to mid-logarithmic phase in YPD medium at 25^{o}C. (C) Average lifetimes of Syp1-3GFP and Syp1-GFP ± standard deviation in wild-type cells. Data were taken from 10 min movies with a 3-s frame interval. (D) Quantification of fluorescence intensity for individual Syp1-GFP patches over time. Each curve represents data from one patch. Behavior of four independent patches was plotted for each strain. Fluorescence intensity was corrected for photobleaching. Movies were taken with 3-s frame intervals.



Supplemental Figure S5. Localization and patch liferimes of Sla1p$^{NPF-AAA}$ in wild-type cell or EH domain mutants. (A) Localization of Sla1$^{NPF-AAA}$-GFP in wild-type, *end3*^{EH} or *ede1*^{EH} mutant cells. Each cells expressing Sla1$^{NPF-AAA}$-GFP were grown to early to mid-logarithmic phase in YPD medium at 25^{o}C. (B) Average lifetimes of Sla1$^{NPF-AAA}$-GFP ± standard deviation. Data were taken from 1- or 2-min movies with a 1-s frame interval.