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Title	Membrane Protein Rim21 Plays a Central Role in Sensing Ambient pH in Saccharomyces cerevisiae
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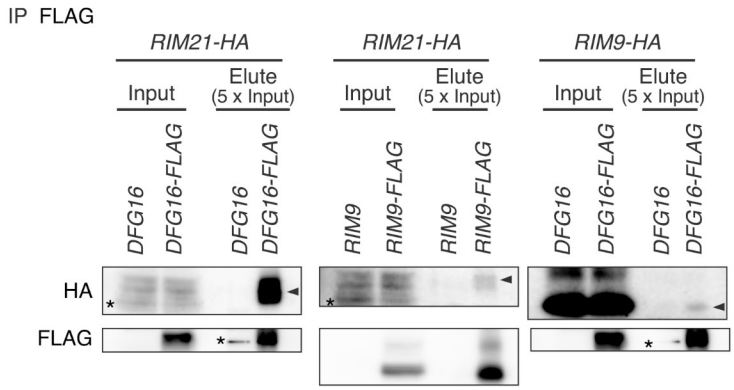


FIGURE S1. Interaction profile among Rim21, Dfg16, and Rim9 does not change upon alkaline treatment. After 5-min alkaline treatment, total lysates were prepared form YOK2540 (*RIM21-HA*), YOK2546 (*RIM21-HA DFG16-FLAG*), YOK2547 (*RIM21-HA RIM9-FLAG*), YOK2542 (*RIM9-HA*), and YOK2548 (*RIM9-HA DFG16-FLAG*) cells. Immunoblotting of co-precipitated proteins with anti-FLAG M2-agarose were performed with anti-HA and anti-FLAG antibodies. IB, immunoblot; IP, immunoprecipitation; arrowheads, co-immunoprecipitates; asterisks, non-specific bands.

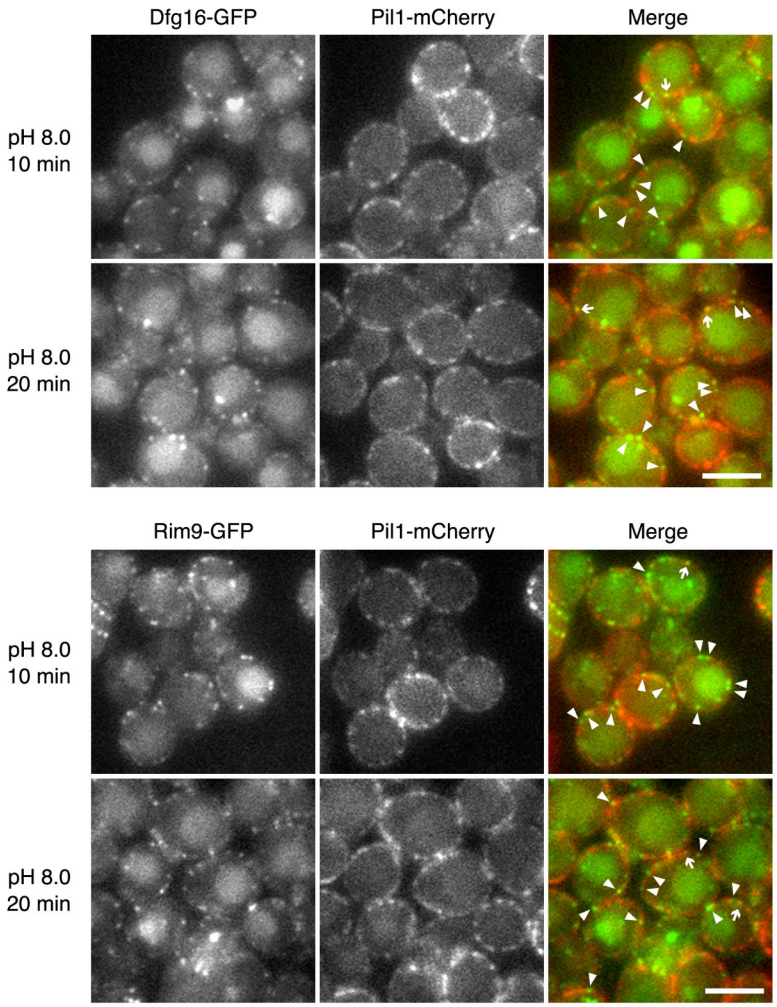


FIGURE S2. External alkalization does not facilitate eisosome localization of Dfg16 and Rim9. After 10- and 20-min alkaline treatments, YOK2729 (*DFG16-EGFP PIL1-mCherry*) and YOK2731 (*RIM9-EGFP PIL1-mCherry*) cells were subjected to fluorescence microscopy. Arrows, Dfg16-EGFP or Rim9-EGFP that co-localized with Pil1-mCherry; arrowheads, Dfg16-EGFP or Rim9-EGFP that did not co-localize with Pil1-mCherry; bars, 5 μm.

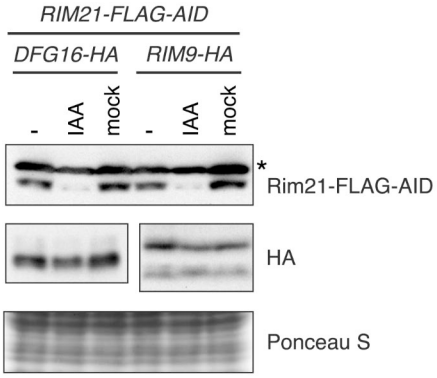


FIGURE S3. Dfg16 and Rim9 are not degraded in *RIM21-FLAG-AID* cells upon IAA treatment. After treatment with 500 μM IAA or ethanol (mock) for 30 min, total lysates were prepared from YOK2891 (*RIM21-FLAG-AID DFG16-HA*) and YOK2892 (*RIM21-FLAG-AID RIM9-HA*) cells. After Endo-H treatment, immunoblotting was performed with anti-FLAG and anti-HA antibodies. To demonstrate uniform protein loading, proteins were stained with Ponceau S after immunoblotting. Asterisk, non-specific band.

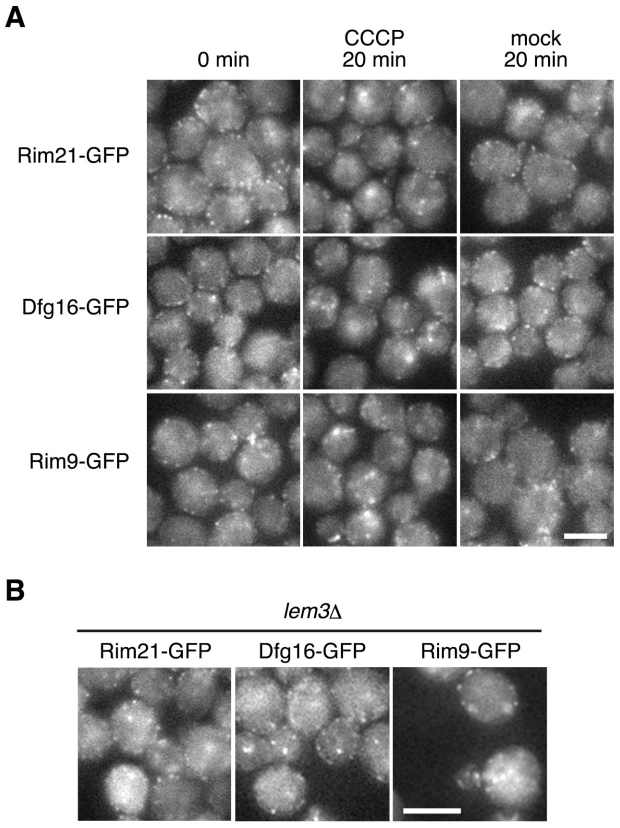


FIGURE S4. Rim21, Dfg16, and Rim9 are internalized upon CCCP treatment. A. YOK3208 (*RIM21-2xEGFP*), YOK2210 (*DFG16-EGFP*), and YOK2229 (*RIM9-EGFP*) cells were subjected to fluorescence microscopy before and 20 min after CCCP- or mock-treatment. Bar, 5 μm. B. YOK3211 (*RIM21-2xEGFP lem3*Δ), YOK2214 (*DFG16-EGFP lem3*Δ), and YOK2218 (*RIM9-EGFP lem3*Δ) cells were subjected to fluorescence microscopy. Bar, 5 μm.