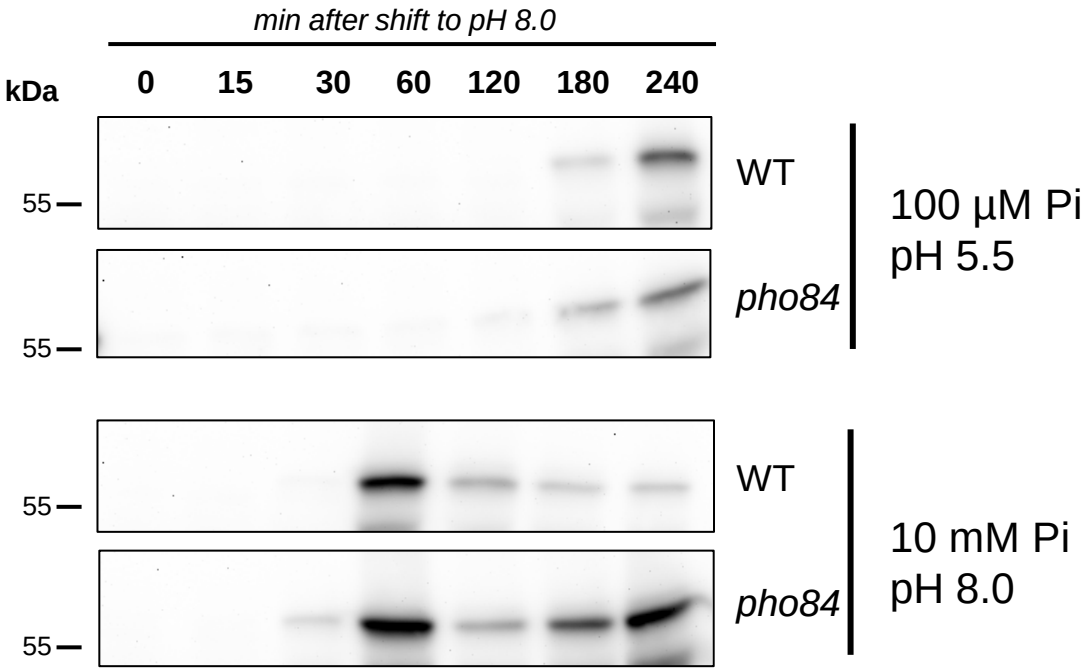
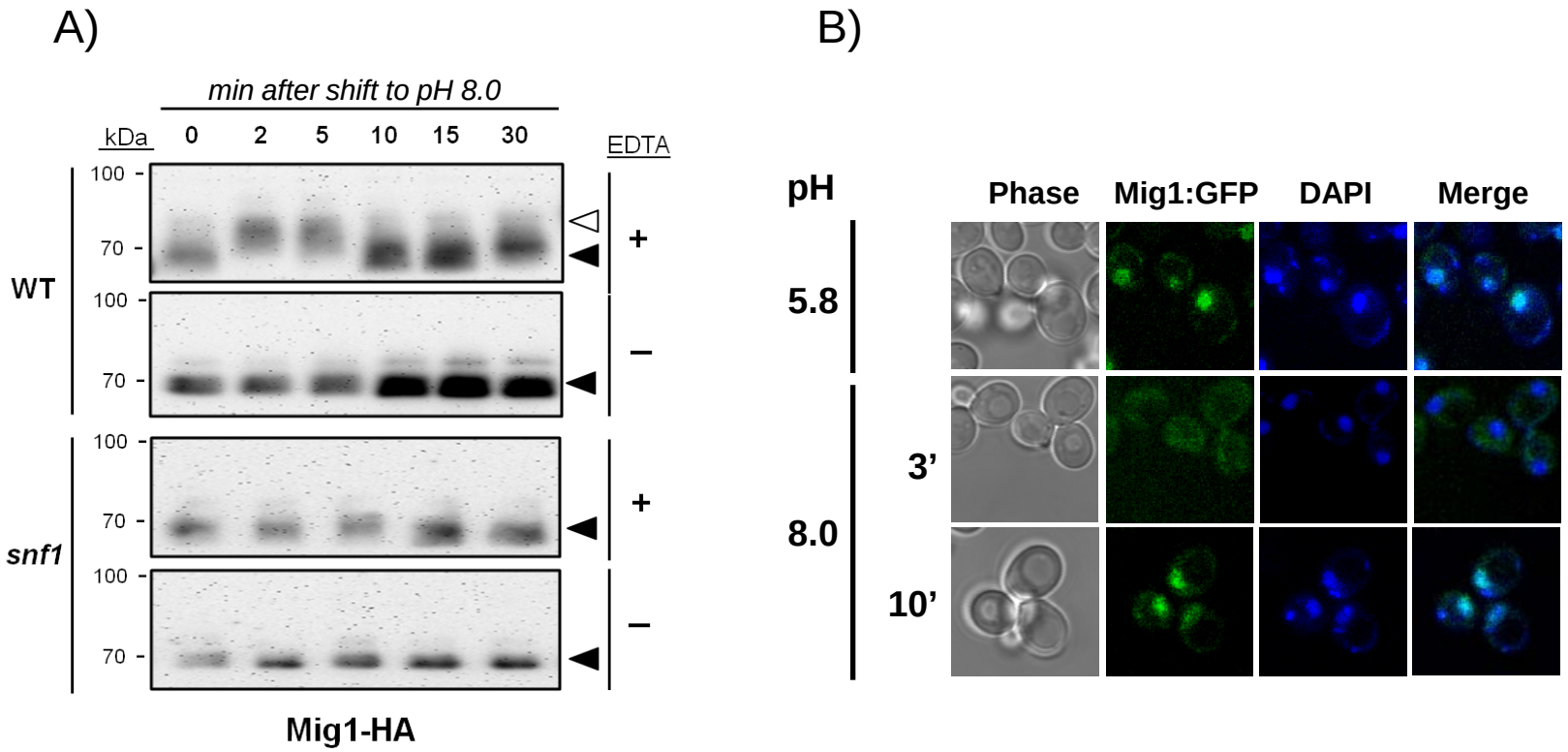
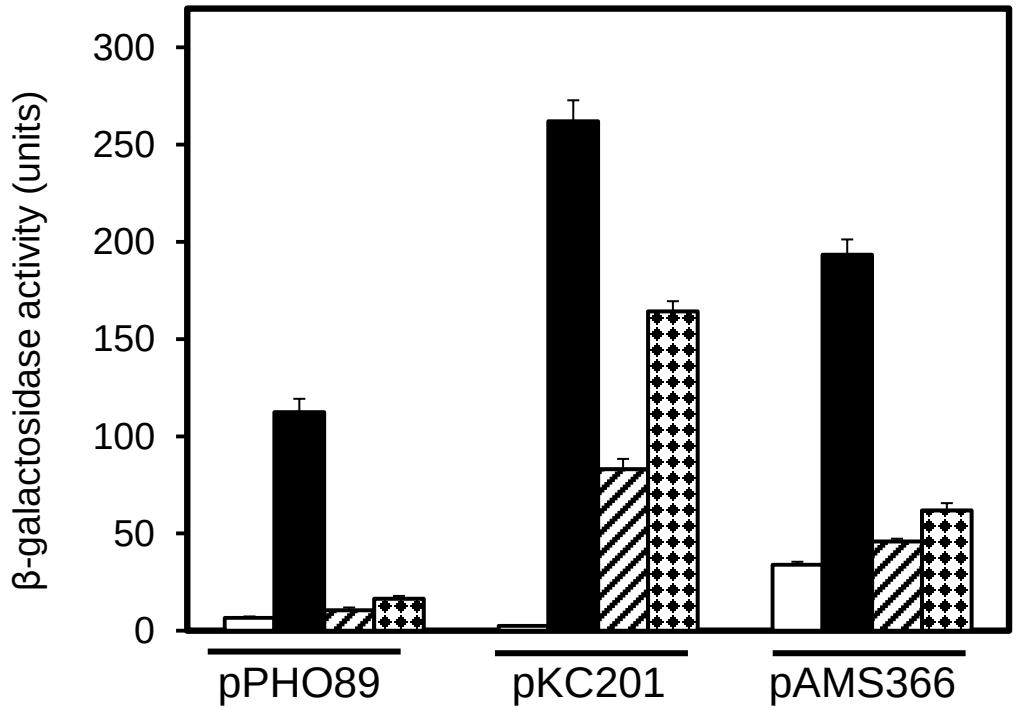


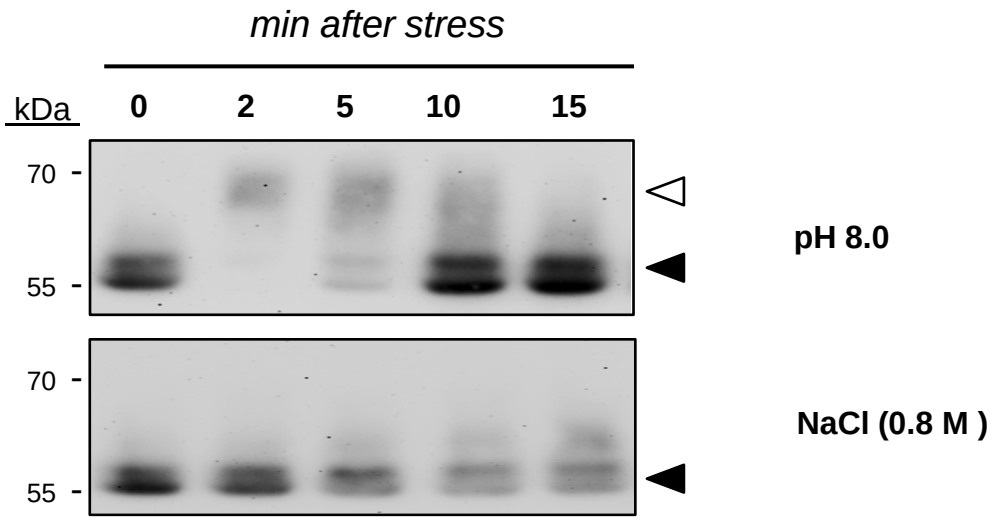
Supplemental Figure 1. **Effect of the *pho84* mutation on Pho89 expression under low Pi or high pH stress**. DBY476 wild type strain (WT) the *pho84* were transformed with pMM17-PHO89 plasmid carrying the *PHO89* gene fused with the 3x-HA epitope. Cells were grown in synthetic high P_i medium (10 mM potassium phosphate) to exponential phase (OD 0.6) and subjected to high pH or low phosphate stresses for the indicated times. Proteins (30 µg) were resolved by SDS-PAGE (10% gels) and electroblotted on PVDF filters (Immobilon-P, Millipore). Pho89-HA was detected by the use of anti-HA antibody as described in Materials and Methods.



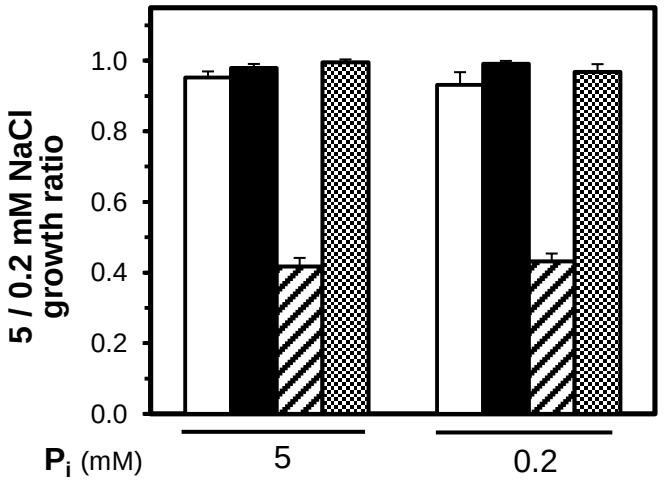
Supplemental Figure 2. **High pH stress induces transient phosphorylation and nuclear-cytoplasmic shift and Mig1.** A) A chromosomal copy of Mig1 including a C-terminal 3xHA epitope tag was introduced in wild type BY4741 cells and in its *snf1* derivative. After exposing cells to pH 8.0 for the indicated times, extracts were prepared and subjected to SDS-PAGE (8% polyacrylamide gels), prior treatment with alkaline phosphatase in the absence (-) or the presence (+, to prevent the action of the phosphatase) of 50 mM EDTA. Immunoblots were performed using anti-HA antibodies. Empty triangles denote slower (more phosphorylated) species. B) Strain SP048, containing chromosomally encoded C-terminal fusions of GFP with Mig1, were shifted to pH 8.0 and the localization of the repressor followed by fluorescence confocal microscopy. Nuclei were stained with DAPI to illustrate nuclear co-localization of the GFP and DAPI signals (merged).



Supplemental Figure 3. Wild type strain DBY746 was transformed with pPHO89-LacZ, pKC201 (carrying the entire *ENA1* promoter fused to LacZ) or pAMS366 (in which LacZ is driven by a 4x-CDRE tandem). Cells were subjected to high pH stress for 90 min (pH 8.0, closed bars), 0.4 M NaCl for 60 min (stripped bars) or 0.8 M NaCl for 90 min (dotted bars), prior determination of β-galactosidase activity. Data correspond to the mean ± SEM for 6 determinations.



Supplemental Figure 4. A chromosomal copy of Mig2 including a C-terminal 3x-HA epitope tag was introduced in wild type BY4741 cells. After exposing cells to pH 8.0 or 0.8 M NaCl for the indicated times, extracts were prepared and subjected to 8% SDS-PAGE. Immunoblots were performed using anti-HA antibodies. The empty triangle denotes slow migrating (phosphorylated) species



Supplemental Figure 5. Strains ONA1 (*pho84,* empty bars), RH16.6 (*ena1*, closed bars), ASC17 (*ena1 pho84*, stripped bars), and ASC30 (*ena1 pho89*, crossed bars) were grown in liquid media (adjusted to pH 7.2) containing the indicated concentrations of P_i and either 0.2 or 5 mM NaCl. The OD_{650} was determined after 47 h (due to the slow growth of the strains) and the 5 mM / 0.2 mM NaCl absorbance ratio calculated. Data are mean ± SEM from 5 to 9 experiments.