

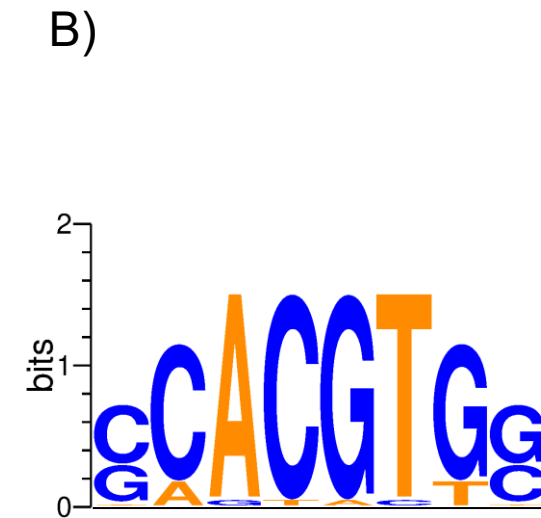
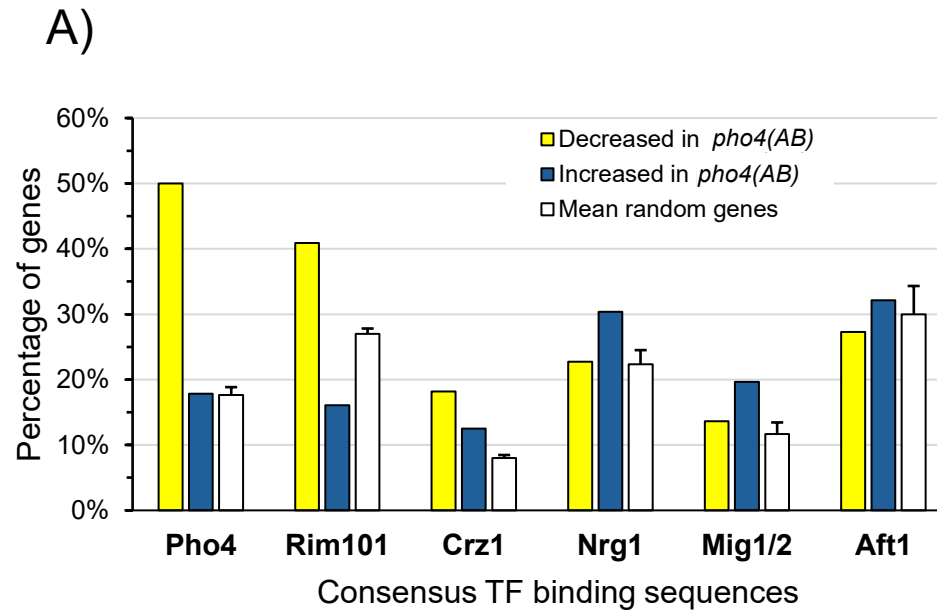


Figure S1. Enrichment in consensus binding sequences for selected TFs in genes whose response to alkaline pH is affected by the *pho4(A) pho4(B)* mutation. A) The matrix-scan algorithm was used to scan the -800/-1 upstream regions of the 22 and 56 genes whose response to alkalization decreased or increased, respectively, in the *pho4(A) pho4(B)* mutant (see Materials and Methods). Three sets of 100 randomly selected genes were analyzed for comparison and the mean $\pm$ SEM is plotted. B) Graphical representation of the 20 Pho4 consensus sequences found in the upstream regions of 11 out 22 *pho4(AB)*-dependent genes. The logo was generated with WebLogo 3.9.1 (<https://weblogo.berkeley.edu/>).

Figure S2. Position of consensus sequences for selected transcription factors in the promoters of the 22 genes whose induction by alkaline pH is reduced in the *pho4(A) pho4(B)* mutant

Gene_ID	Gene name	Transcription Factor					
		Pho4	Crz1	Rim101	Nrg1	Mig1/2	Aft1
PAS_c121_0015	<i>PHO81</i>	-108/-101	-784/-779	-785/-779; - 681/-675			-614/-605
PAS_chr1-1_0135							-324/-315
PAS_chr1-4_0040	<i>FTR1</i>						
PAS_chr1-4_0355	<i>HRP1</i>			-745/-739; - 615/-609			
PAS_chr2-1_0103	<i>PHO5</i>	-323/-316					
PAS_chr2-1_0177	<i>PHO4(B)</i>	-678/-671; -241/-234					
PAS_chr2-1_0235	<i>PHO89</i>	-585/-578; -440/-433; -415/-408; -409/-402	-417/-412	-546/-540; -537/-531; -417/-411; -165/-159	-365/-358		
PAS_chr2-1_0579				-742/-736			
PAS_chr2-1_0580	<i>IDP2</i>					-36/-31	-164/-155
PAS_chr2-1_0668	<i>VTC2</i>	-84/-77					
PAS_chr2-1_0685		-148/-141; -126/-119	-309/-304				
PAS_chr2-1_0787	<i>FET3</i>						
PAS_chr2-2_0420	<i>VTC4</i>	-91/-84					
PAS_chr3_0505						-637/-632	
PAS_chr3_0886							
PAS_chr4_0005	<i>OPT1-1</i>			-337/-331			
PAS_chr4_0077	<i>TYE7</i>	-355/-348; -247/-240; -238/-231; -150/-143		-492/-486; -430/-424			-643/-634
PAS_chr4_0185	<i>NSR1</i>			-629/-623; -132/-126			
PAS_chr4_0290	<i>VTC1</i>	-85/-78	-743/-738		-480/-473		
PAS_chr4_0337	<i>PHO84-1</i>	-373/-366; -98/-91		-212/-206			-369/-360
PAS_chr4_0399	<i>FRE2-1</i>				-327/-320		
PAS_chr4_0570	<i>PHO86</i>	-109/-102		-797/-791; -713/-707; -334/-328	-575/-568	-678/-673; -552/-547	-463/-454

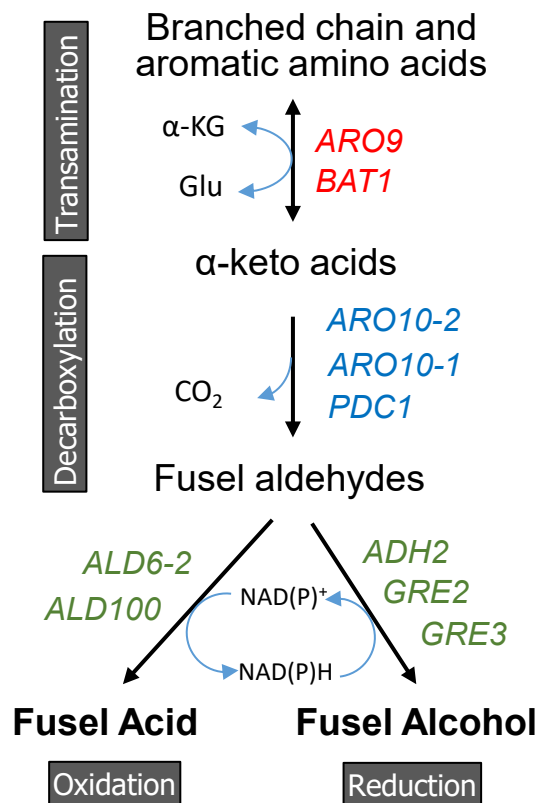
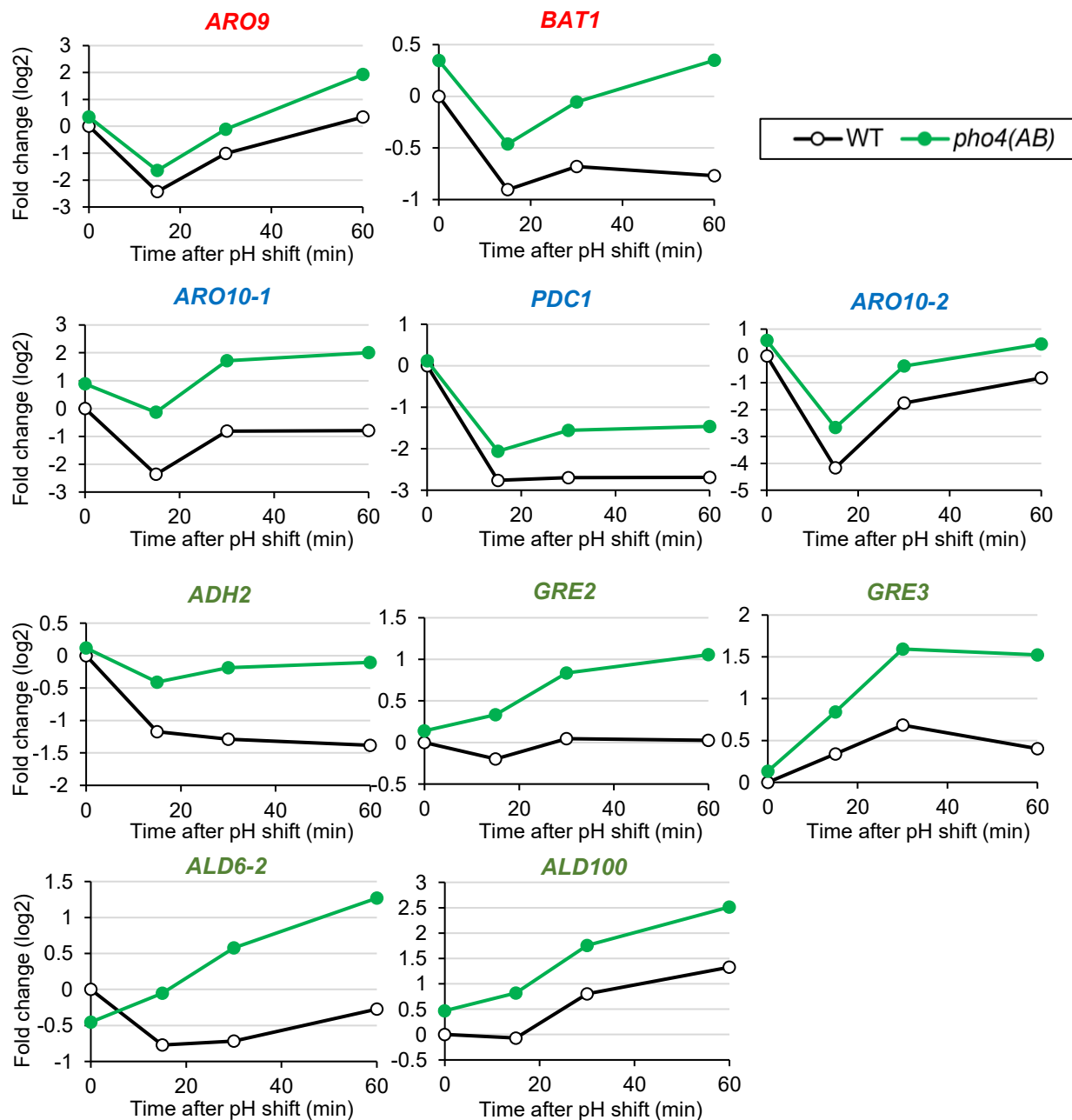


Figure S3. Impact of the *pho4(A) pho4(B)* mutation on amino acid catabolism through the Ehrlich pathway in cells subjected to alkaline stress. Changes in expression of diverse genes encoding enzymes required for degradation of branched chain and aromatic amino acids through the Ehrlich pathway in response to alkalinization in wild-type (WT) and *pho4(A) pho4(B)* mutants. The three steps that constitute the pathway and the genes assigned to each step are shown in the cartoon on the left. α -KG, α -ketoglutarate.



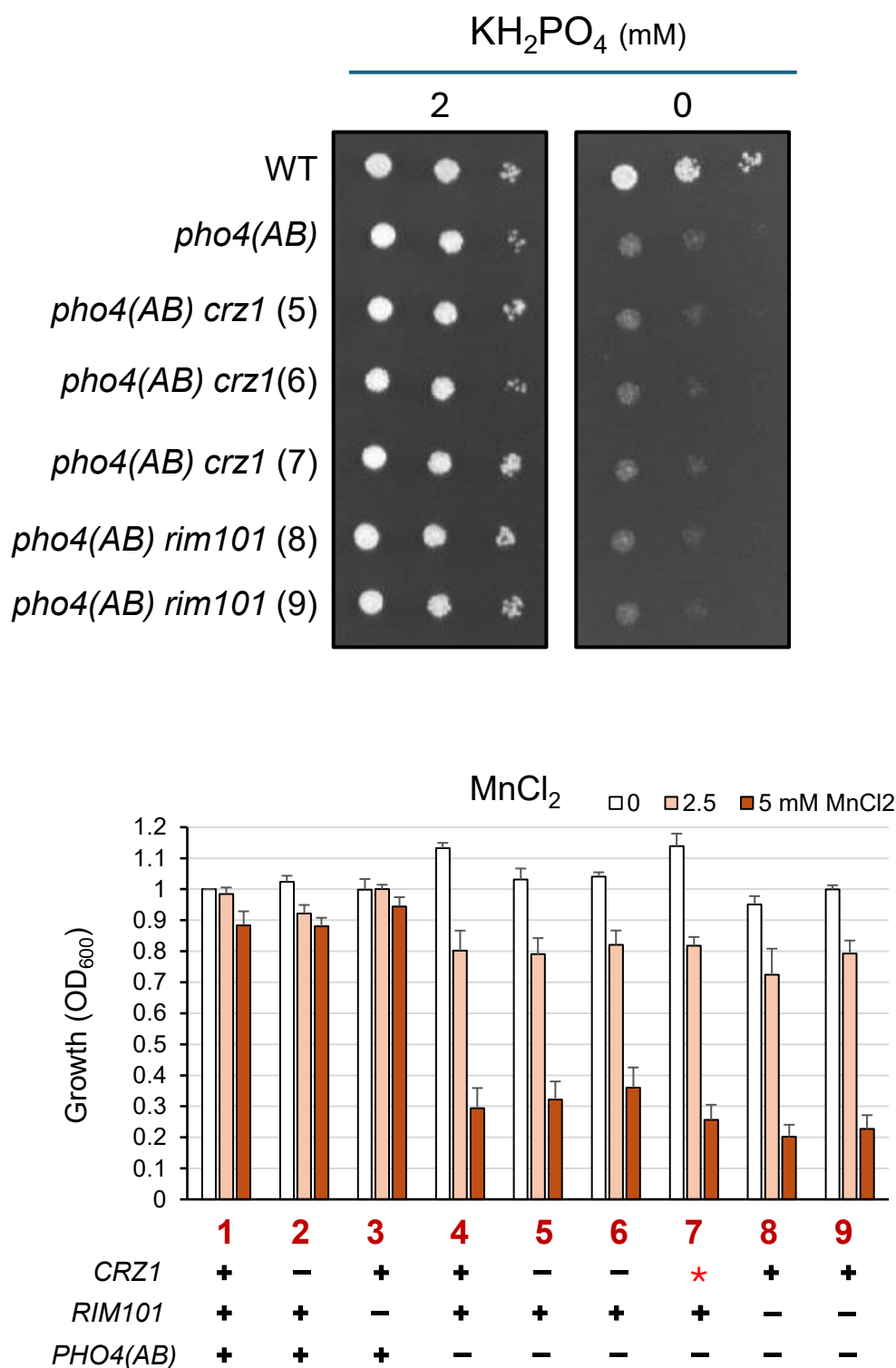


Figure S4. Phenotypic characterization of *CRZ1*, *RIM101*, and *PHO4(AB)* mutants, and of strains carrying combination of these mutations. The wild-type strain X-33 (1, WT) and its isogenic derivatives PJA-001 (2, *crz1*), PJA-007 (3, *rim101*), PJA-026 (4, *pho4(AB)*), PPC-001 to PPC-003 (5 to 7, *pho4(AB) crz1*), PPR-001 and PPR-003 (8 and 9, *pho4(AB) rim101*) were grown on A) Synthetic medium plates containing the indicated amounts of KH_2PO_4 , or B) YPGly medium containing the indicated amounts of MnCl_2 , as described in Methods. The asterisk (*) denotes a mutation that removes specific residues ($^{185}\text{K}^{186}$) in strain 7 but does not interrupt the polypeptide. Growth in liquid cultures was recorded after 19-20 h, and the mean $\pm$ SEM from nine independent cultures is presented. Purified agar (Condalab, Cat. #1806) was used for testing growth on Pi limitation and plates were documented after 3 days at 28 °C.