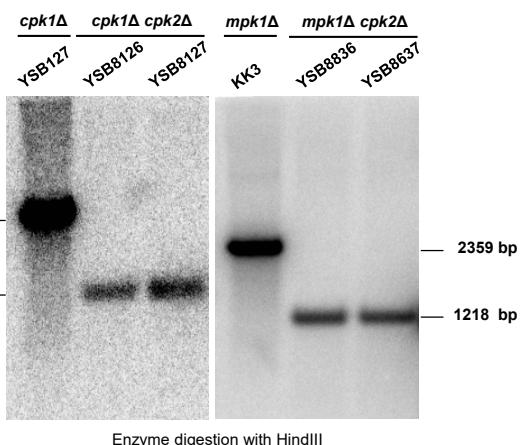
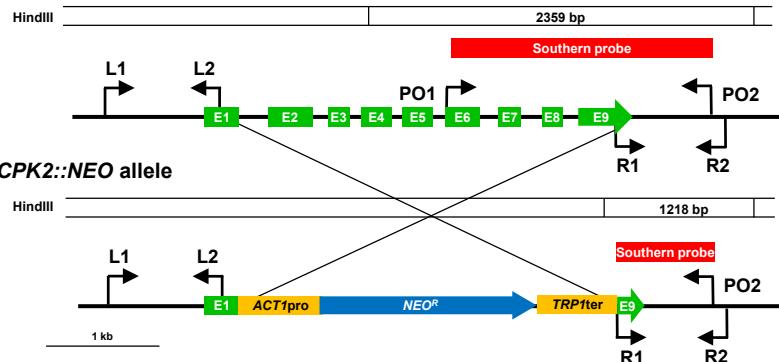


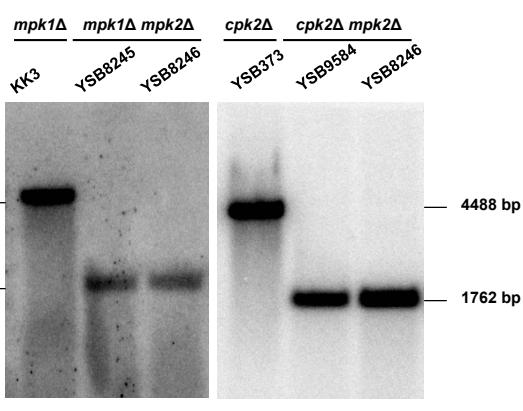
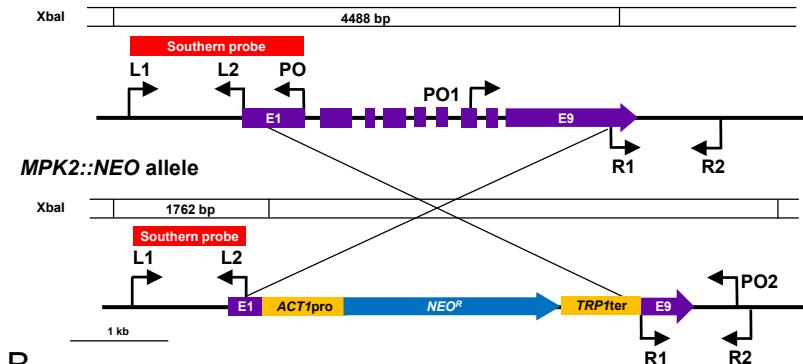
# Jang et al. (Supplementary Figure 1)

A

## *CPK2* WT allele

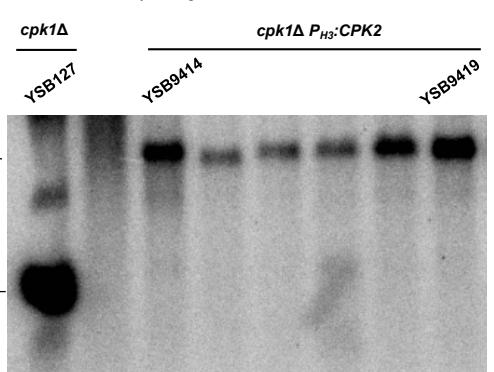
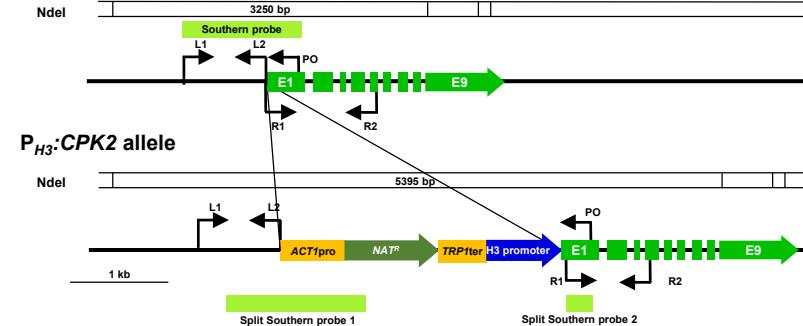


## *MPK2* WT allele

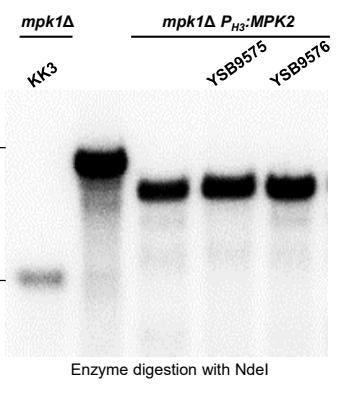
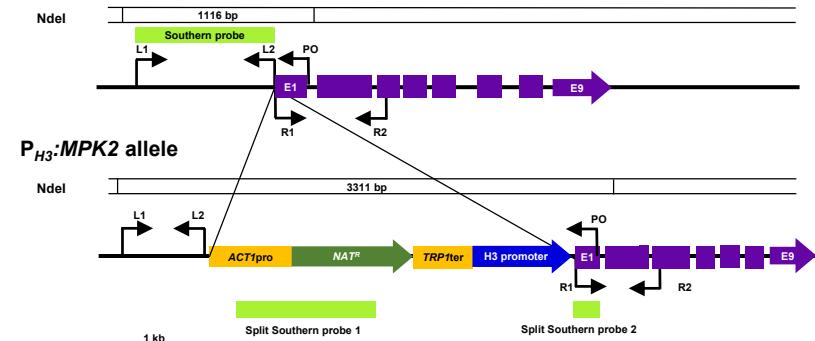


B

## *CPK2* WT allele



## *MPK2* WT allele



(continued)

### Jang et al. (Supplementary Figure 1)

C

#### *MAT2 WT allele*

KpnI 5442 bp

Southern probe L1 L2 PO

E1 E2 E3 E4 E5

R1 R2

#### *MAT2::NAT allele*

KpnI 3944 bp

Southern probe L1 L2

E1 ACT<sub>1</sub>pro NAT<sup>R</sup> TRP1ter E5

R1 R2

1 kb

*MATα*  
*mat2Δ*



#### *MAT2 WT allele*

NsiI 5978 bp

Southern probe L1 L2 PO

E1 E2 E3 E4 E5

R1 R2

#### *MAT2::NEO allele*

NsiI 2800 bp

Southern probe L1 L2

E1 ACT<sub>1</sub>pro NEO<sup>R</sup> TRP1ter E5

R1 R2

1 kb

*MATα*  
*mat2Δ*



#### *MAT2 WT allele*

PstI 3732 bp

Southern probe L1 L2 PO

E1 E2 E3 E4 E5

R1 R2

*cpk1Δ*  
*P<sub>H3</sub>·CPK2*

*cpk1Δ*  
*P<sub>H3</sub>·CPK2 mat2Δ*

#### *MAT2::HYG allele*

PstI 2385 bp

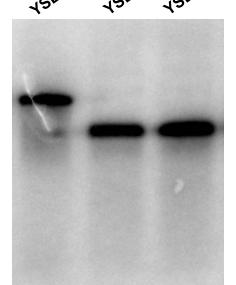
Southern probe L1 L2

E1 ACT<sub>1</sub>pro HYG<sup>R</sup> TRP1ter E5

R1 R2

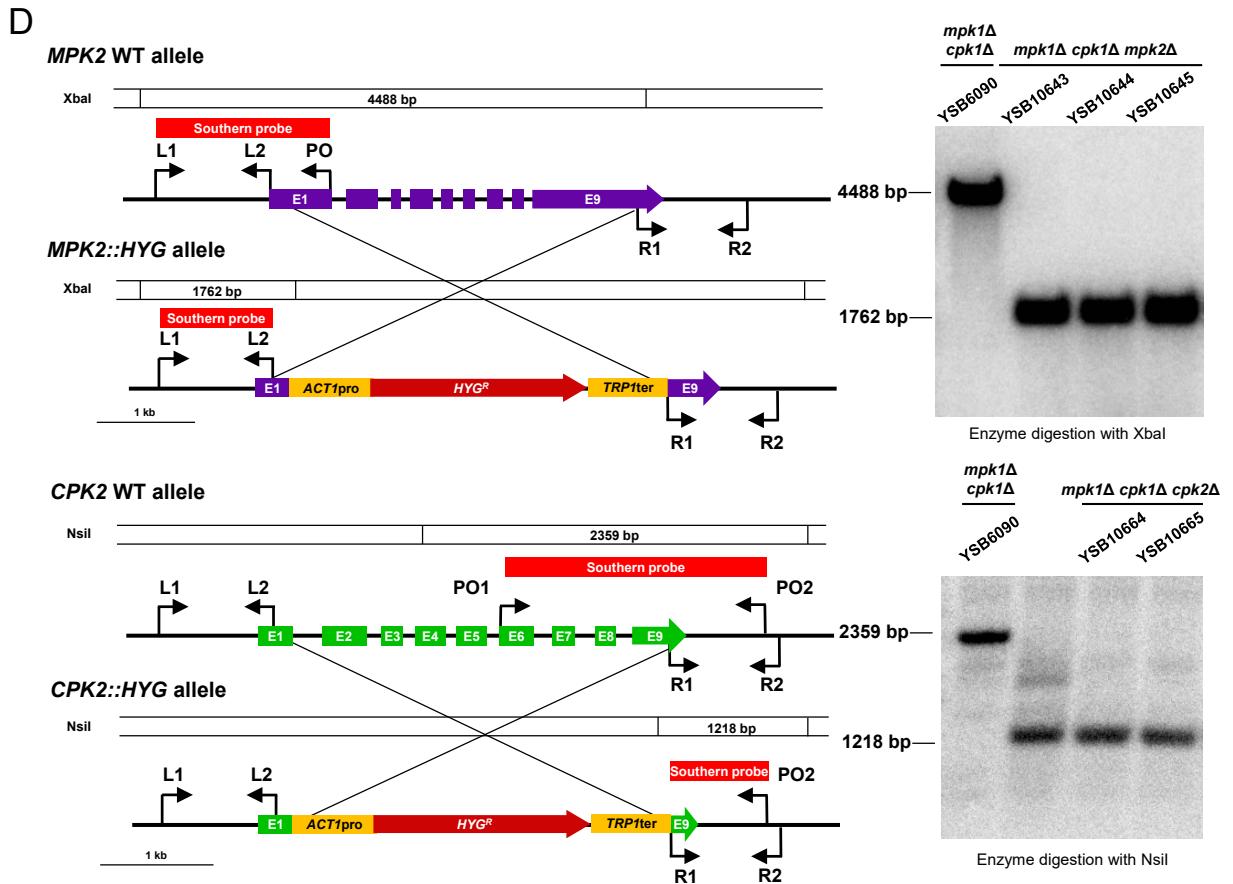
3732 bp

1 kb



(continued)

## Jang et al. (Supplementary Figure 1)



**Figure S1. Construction of mutant strains were established through Southern blot analysis.** The target MAPK genes were replaced with neomycin or hygromycin resistance genes ( $NEO^R$  or  $HYG^R$ ), and the genes were delivered using the gene gun biostatic transformation method (see Material and Method). Graphical representations depict the strategies for homologous recombination between the wild-type gene and the deletion cassette. The newly constructed strains were verified through diagnostic PCR and further validated by Southern blot using dCTP labeled with the radioactive phosphorus  $P^{32}$ . The primer sets used for these procedures are detailed in Table S2.