Table S1 List of strains used in this study. The genotype and source for each strain are indicated.

Figure S1 Phenotypes for *ksp1* deletions and kinase-defective mutants under pseudohyphal growth-inducing conditions of nitrogen limitation and limited glucose availability (SLALD medium). (A) Surface filamentation phenotypes for the indicated strains. Surface filament formation relative to wild type was quantified as described previously. Percentages indicate mean values with standard deviation for four replicate colonies. "+", wild-type filamentation; "-", decreased filament formation relative to wild type. Scale, 500 μm. (B) Cell morphology of wild type and *ksp1* mutant strains. Elongated cells with a length-to-width ratio of greater than 2 were counted per strain. The percentage of such elongated cells is indicated along with the total number of cells counted. *P*-values are generated from the pairwise comparison of elongated cell percentages, treating cell counts for each strain as independent population proportions. Scale, 1 μm.

Figure S2 Pbp1p-GFP puncta and U1A-mCherry-bound RNA puncta are elevated in numbers relative to wild type in a homozygous diploid strain deleted for *KSP1*. (A) Pbp1p-GFP was expressed from its native locus by integration of a cassette encoding GFP in wild type and *ksp1*Δ/Δ. Pbp1p-GFP foci are elevated in *ksp1*Δ/Δ relative to wild type after 24 hours growth in media lacking glucose. The *ksp1*Δ/Δ mutant grew very poorly in media lacking glucose, and stress granules formed abundantly in this growth condition. (B) mRNA puncta were visualized using the U1A-mCherry system recognizing modified *PGK1* transcripts. Wild-type and *ksp1* deletion strains were transformed with plasmids encoding U1A-mCherry and *PGK1* with U1A-binding sites incorporated into its 3'-UTR. Strains were grown to a high cell density as described. U1A-mCherry was imaged by fluorescence microscopy. Asterisks indicate mRNA puncta. Percent of cells with puncta are indicated for each strain along with the total number of cells counted.

Figure S3 The $P_{FLO11\text{-}6/7}$ *lacZ* reporter is responsive to *FLO8* activity. The plasmid-based reporter contains sequence from the *FLO11* promoter (-1 to -1.4 kb upstream of the *FLO11* initiator ATG) with binding sites for PKA-regulated Flo8p. To test the responsiveness of the reporter to *FLO8*, we introduced the reporter plasmid into wild-type yeast and a strain deleted for *FLO8*. β-galactosidase activity was significantly reduced in *flo8*Δ relative to wild type under normal growth conditions in yeast grown to log phase. β-galactosidase activity relative to wild type is shown to the right. Assays were performed in triplicate, and standard deviation is indicated.

Figure S4 Protein levels of mutants with Ala substituted at Ksp1p-dependent phosphorylation sites in (A) Ste20p-HA, Tif4631p-HA, and (B) Pbp1p-HA. Mutant and wild-type alleles were chromosomally tagged at their 3'-termini with a cassette encoding three copies of the HA epitope. Protein extracts were prepared by TCA precipitation from cells grown to log phase under normal conditions. Extracts were blotted and probed with anti-HA antibody. Protein levels were normalized to total protein loaded. Relative levels of the mutant proteins to wild type were determined from duplicate blots by densitometry using Image J software; standard deviations are indicated. The mass of the HA-tagged proteins is indicated. No significant changes in protein levels relative to wild-type were observed for the ste20p-T203A-HA, tif4631p-S176A-HA, or pbp1p-S436A-HA mutants, consistent with levels predicted from fluorescent micrographs of GFP-tagged proteins.

File S1 List of Supplementary Material included in this manuscript.

File S2 Analysis of RNA sequencing data from triplicate wild type and *ksp1*-K47D strains using DESeq2. Statistical analyses of all changes in transcript abundance, both significant and insignificant, for each gene between the *ksp1*-K47D mutant and wild type are provided. Column headings are defined in the "Glossary" sheet in the Excel file. Parameters for DESeq2 analysis are indicated in the "Info" sheet.

File S3 Boxplots generated from DESeq2 analysis indicating non-normalized counts, depth-normalized counts, and regularized Log 2-normalized counts for each RNA-Seq sample sequenced (three biological replicates of the homozygous diploid wild-type strain and three biological replicates of the homozygous diploid *ksp1*-K47D strain) are provided in PDF format.

File S4 Analysis of RNA sequencing data from three biological replicates each of the wild type and *ksp1*-K47D strains using the Tuxedo pipeline. Statistical analyses of all changes in transcript abundance, both significant and insignificant, for each gene between the *ksp1*-K47D mutant and wild type are provided. Column headings are defined in the "Glossary" sheet in the Excel file. Isoform-level differential expression analysis by Cuffdiff (in the Cufflinks package) is provided in the "Isoforms" sheet. Parameters for analysis are indicated in the "Info" sheet.

File S5 Summary boxplots from Tuxedo analysis indicating FPKM (Fragments Per Kilobase of transcript per Million mapped reads) distribution in Log 10 scale for each RNA sample (three biological replicates of the wild-type strain and three biological replicates of the *ksp1*-K47D strain) are provided as a PDF file.

File S6 A listing is provided of the union set of genes identified as undergoing statistically significant changes in transcript abundance between *ksp1*-K47D and wild type by both DESeq2 and Tuxedo analysis. The gene listing is provided as an Excel spreadsheet, and systematic names are indicated for each *S. cerevisiae* gene.

File S7 An Excel file providing the mass spectrometry data from Mascot and MaxQuant analysis for differentially abundant phosphopeptides between *ksp1*-K47D and wild type. The "Experiment" column indicates whether the peptide was observed in the first, second, or third experiment run. The "Target Protein (Systematic)" column indicates the systematic name for the protein to which the peptide matches. The "Target protein (Standard and Aliases)" column provides standard names and aliases for each

protein. The “Number of Phospho(STY)” column indicates the number of predicted phosphorylation sites (Ser, Thr, or Tyr residues) for each indicated peptide. Mascot scores and PTM scores are provided. The “Modified Sequence” is indicated for each peptide, along with the associated probability of phosphorylation at each predicted site (“Phospho(STY) Probabilities”) and accompanying score differences. The ratio of heavy-to-light-labeled peptides after normalization against peptide abundance without phosphopeptide enrichment is provided for each indicated peptide, along with the Significance (A) score.

File S8 The Excel spreadsheet provides the protein-protein interactions extracted from BioGRID for the Dhh1p, Ksp1p, Pbp1p, Ste20p, and Tif4631p proteins used as nodes for the construction of the network presented in Figure 4. The diagram in Figure 4 was generated by the open-source bioinformatics software platform Cytoscape.