**S1 Fig. Monitoring the gene expression program along the cell cycle. (A)** DNA staining profiles along the cell cycle in the two repeats of *S. cerevisiae, S. paradoxus* and the hybrid**. (B)** Correlation matrices of periodic transcripts between the two repeats. Note high correlation along the diagonal and high correlation between first and second cycle. **(C)** Expression of periodic transcripts. Each gene was normalized to its median expression, plotted are all periodic genes ordered by their expression peak time. **(D)** Bar graphs showing the percentage of genes with significant correlation to the respected module (corrected p-value < 0.05, rho>0.4). **(E)** Periodic co-expression of cycling genes.Interspecies (left) and within hybrid (right) correlations between mean expression of cell cycle modules, classified to 12 groups. Note high correlations between early G1 to late genes in *S. paradoxus*, indicating rapid activation of S-phase genes. **(F)** Fold changes in expression levels of periodic genes. Left: shown are fold change (FC) in absolute levels at expression peak between species, hybrid and *trans* (Species-hybrid). Genes ordered by high expression differences in *S. cerevisiae*. Right: distribution of top changing genes in *S. cerevisiae* (top) and *S. paradoxus* (bottom).

**S2 Fig. Downregulation of ACE2 targets in *S. paradoxus*. (A)** Median FC (indicated in Z-scores) of TFs targets defined by ChIP-seq in another dataset ((MacIsaac et al. 2006)). Number indicates the number of targets genes in the respected group. **(B)** Absolute expression levels of Ace2 targets (as defined by (Voth et al. 2007)) along the cell cycle in the two species.

**S3 Fig. Changes in Ace2 binding does not result from changes in expression of protein sequence. (A)** Pearson correlation matrices of normalized sum signal on promoters between repeats of Ace2 and Swi5, in *S. cerevisiae, S. paradoxus* and hybrid (*S. cerevisiae* genome). **(B)** Meta gene profiles of Ace2 and Swi5. All genes were aligned according to their transcription start site (TSS), and the signal was averaged across all genes. **(C)** Normalized sum of signal on each promoter of Ace2 and Swi5 in the hybrid, comparing binding of the two orthologs of each factor to the S. cerevisiae genome within the hybrid. **(D)** Ploidy and expression levels of Ace2 do not affect binding patterns. plotted is Ace2 normalized sum of signal on each promoter. Pearson correlation values of top 100 promoters are shown. Left: *S. cerevisiae* haploid vs *S. cerevisiae* hemizygote diploid expressing only one copy of ACE2. Middle: *S. cerevisiae* haploid vs diploid. Right: *S. paradoxus* haploid vs diploid. **(E)** Ace2 binding on AMN1 promoter, notable *cis* effect as a result of changes in Ace2 motifs. **(F)** Ace2 binding on CLN3 promoter. Shown are the sequence changes leading to loss or gain of Ace2 binding site. **(G)** Effect of introducing CLN3 SNP of *S. paradoxus* to *S. cerevisiae* on the cell cycle. Left: DNA staining profile of *S. cerevisiae* strain carrying the mutation of *S. paradoxus* in CLN3 promoter. Right: quantification of % cells with 1N DNA content. Shown are the mean and standard error of 4 repeats. **(H)** Quantification of G1 duration in daughter cells in live microscopy (see methods). Reduced budding size indicates reduces cell size control. Asterisks represent significant p-value (two-sample t-test).

**S4 Fig. Fkh1 and Fkh2 binding profiles (A)** Pearson correlation matrices of normalized sum signal on promoters between repeats of Fkh1 and Fkh2 **(B)** Meta gene profiles of Fkh1 and Fkh2. All genes were aligned according to their transcription start site (TSS), and the signal was averaged across all genes. **(C)** Fkh1 and Fkh2 Motif scores of all 7-mers between species. Marked in red is the known consensus motif . **(D)** Sequence logos based on top 10 motifs.

**S5 Fig. Fkh1 and Fkh2 have direct effect on Ace2 binding. (A)** Over-expression of CLB2 doesn’t restore Ace2 binding to cell separation genes. Shown are sum signal on promoters of Ace2 in WT vs *fkh1*Δ*fkh2*Δ, WT vs *fkh1*Δ*fkh2*Δ CLB2-O.E, and *fkh1*Δ*fkh2*Δ vs *fkh1*Δ*fkh2*Δ CLB2-O.E. **(B)** ACE2 relative expression levels in WT and in *fkh1*Δ*fkh2*Δ, as measured by RT-qPCR. **(C)** *S. paradoxus*’s FKH2 is sufficient to restore yeast-like growth in *S. cerevisiae.* Shown are images of *S. cerevisiae* *fkh1*Δ*fkh2*Δ and *S. cerevisiae* *fkh1*Δwith *S. paradoxus Fkh2*