

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1 (related to Fig.3).

(A) Overview of the simultaneous repression of *pab1* under the thiamine-regulated *p41nmt1* promoter and degradation of the protein under the mini version of the auxin-inducible degron (here referred to as *3PK-mAID-pab1* in the genetic background *Padh15-skp1-At-Tir1-2NLS-Padh15-skp1-Os-Tir1*). While thiamine leads to the repression of transcription, the addition of auxin leads to the recognition of 3PK-mAID-Pab1 by Tir1 and its subsequent ubiquitination and proteasome-mediated degradation.

(B) Representative images of DAPI and blankophor-stained ethanol-fixed cells of *p41nmt1slp1 41nmt1-3PK-miniAID-pab1* under the presence of auxin but not thiamine (+A/-T). The same cells grown in EMM (-T/-A) were used as a control (left).

(C) FACS profile of *p41nmt1slp1* and *pnmt41slp1 par1Δ* ethanol-fixed cells stained with propidium iodide during a time course after thiamine addition. Wild type cells were used as a control (left).

Supplementary Figure S2 (related to Fig. 6).

(A) Principal component analysis (PCA) of the samples shows high reproducibility of replicates. The dimension 1 separates treatment and control and dimension 2 between time points. PCA was performed based on the proteins with significant differential expression determined by an ANOVA test using an FDR cut-off < 0.05 (**Table S3**).

(B) Heatmap of the differential expression levels between thiamine-treated samples vs the non-treated sample at 0 hours with a fold-change > 2-fold up or down (n= 1403). Red indicates up-regulated differential expression and blue indicates down-regulated expression at each indicated time point.

(C) PPI network of the significant proteins with changes in the dynamic of expression after *slp1* repression clustered in **A**. Proteins are clustered based on biological functions. Four clusters represented by one single GO-term that was chosen based on semantic similarity between multiple top-go terms from each cluster.

Supplementary Figure S3 (related to Fig. 6).

(A, C) Volcano plot. The log₂ FC indicates the mean expression level for each protein. Each dot represents one protein. The line represents a cut-off of FDR < 0.01 with a

S0= 0.5. Beyond the line, black dots represent significant hits between EMM T0 and EMM +T 3 h **(A)** and +T 4 h **(C)**. Dots are coloured based on GO annotations: purple, cell cycle; green, chromosome segregation; orange, cytokinesis and septation and blue, 1-3 β -glucanase activity.

(B, D, E) Top GO terms for the proteins upregulated after 3 h **(B)**, 4 h **(D)** and 6 h **(E)** of thiamine addition (See **Fig. 6E** for the related volcano plot). Enrichment analysis was performed on proteins with a differential expression >3x and an FDR adjusted p-values < 0.05. Metascape; All GO annotations, background gene list (**Supplementary Table S2**), and a hypergeometric test with Benjamini–Hochberg correction. All GO terms are listed in **Supplementary Table S5**.

(F) Venn diagram. The numbers indicate the overlap between the list of significant proteins up-regulated at 6 h with thiamine and the proteins containing a Slp1 recognition motif (Destruction box (DB, RxxLxxxxN) and the KEN box (KENxxxN)). List of proteins that contain these motifs in their sequence were retrieved from Pombase (<https://www.pombase.org/>).