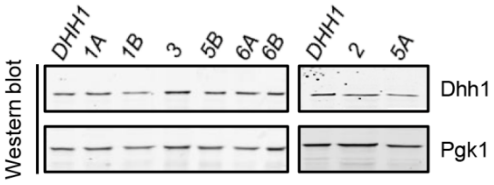
SUPPLEMENTAL FIGURES

Supplemental_Fig_S1

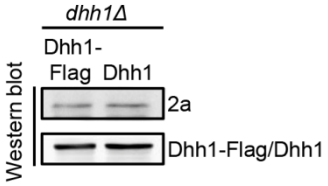
A

Name	motif	mutation
1A	1	R89A
1B	1	R89A K91A
2	2	E196A
3	3	S226A T228A
5A	5	R345A
5B	5	R345A G346A
6A	6	H369A
6B	6	R370A

B



C



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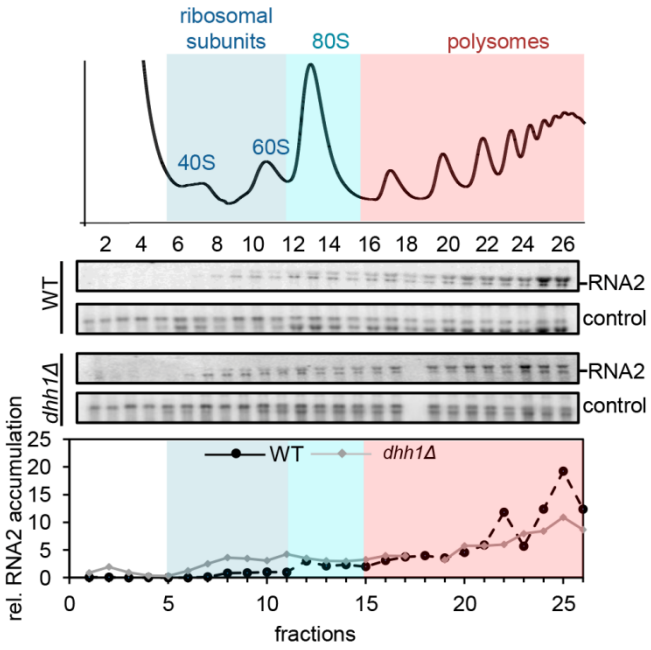
TLS

Supplemental Fig. S1:

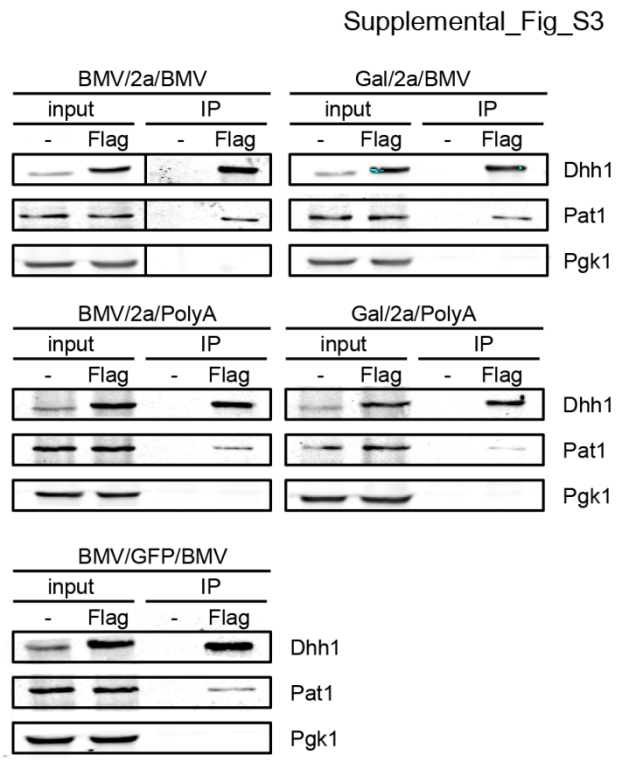
(A) Table of Dhh1 point mutants. (B) Analysis of Dhh1 mutant protein expression levels by western blot. (C) Dhh1-Flag is functional in promoting BMV RNA2 translation. Western Blot analysis of protein 2a expression in the presence of Dhh1-Flag and Dhh1. (D) Dhh1 CRAC of BMV RNA2. 5' and 3'UTR are marked in blue, the tRNA-like structure (TLS) is marked in bold. Sites bound by Dhh1 are underlined.

Supplemental_Fig_S2



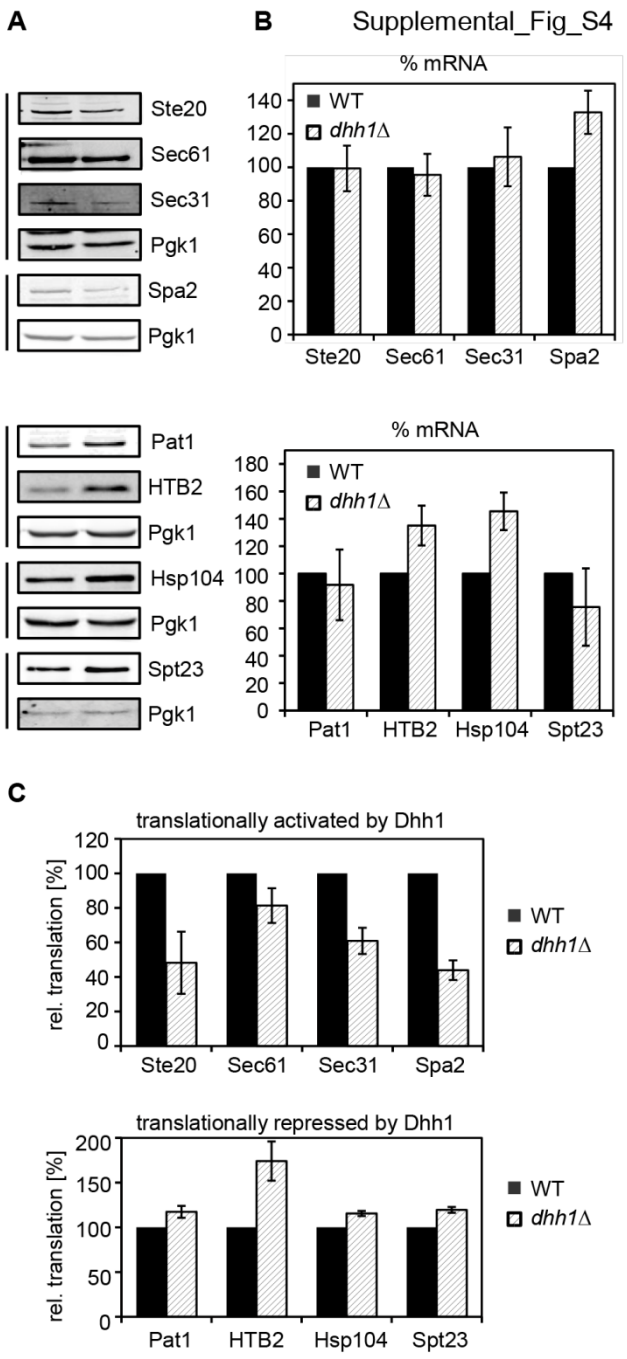
Supplemental Fig. S2:

Deletion of Dhh1 shifts BMV RNA2 out of the polysomal fractions towards single ribosomal subunits. Aligned to a representative UV absorbance profile is in WT and *dhh1Δ* cells the Northern Blot analysis of RNA2 and of an externally added RNA control to monitor sample quality. Diagram below shows the relative amount of RNA2 normalized to the control RNA in each fraction and is representative for the obtained results.



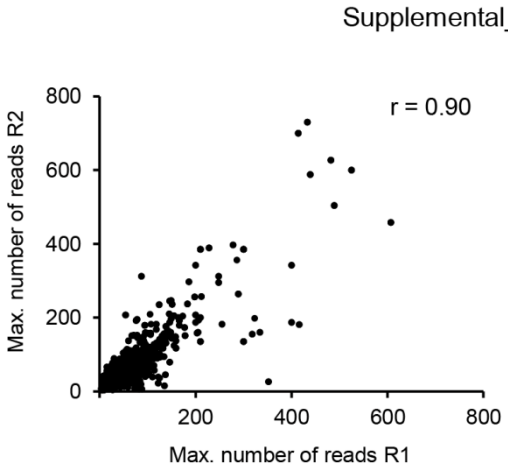
Supplemental Fig. S3:

Corresponding Western blot analysis from co-immunoprecipitation assays of Fig. 3B. Input corresponds to 100 µg and "IP" to output after co-immunoprecipitation. "-" indicates Dhh1 was expressed, "Flag" indicates Dhh1-Flag was expressed.



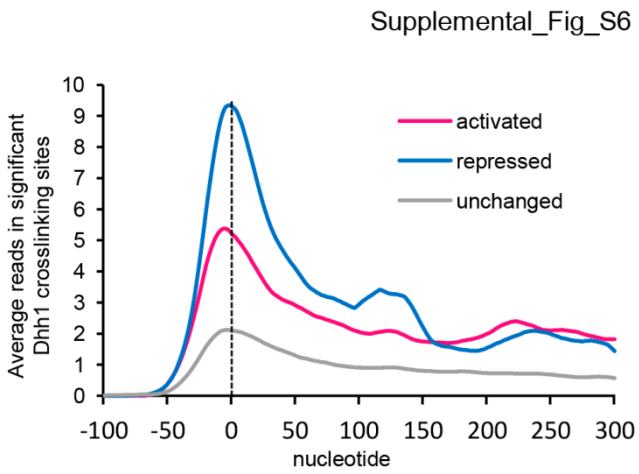
Supplemental Fig. S4:

Validation of expression changes by Western blot and qPCR analysis for some of the genes identified by ribosome profiling. Experiments have been carried out at least three times independently. (A) Western Blot analysis. Vertical lines indicate that same membrane was used. As control for equal sample size Pgk1 protein levels were examined. (B) qPCR analysis. mRNA amount in WT cells was set to 100. *ACT1* mRNA levels were examined as a control for equal sample size. (C) Relative translation of the different genes based on the results from (A) and (B), Translation in WT was set to 100%. For all analyzed genes the changes between WT and *dhh1*Δ cells were significant ($p < 0.05$; TTEST).



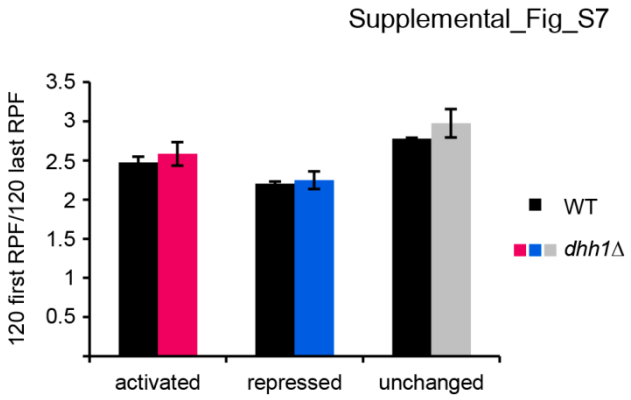
Supplemental Fig. S5:

CRAC replicates scatter plot and correlations. Values are maximum overlapping reads in overlapping significant peaks.



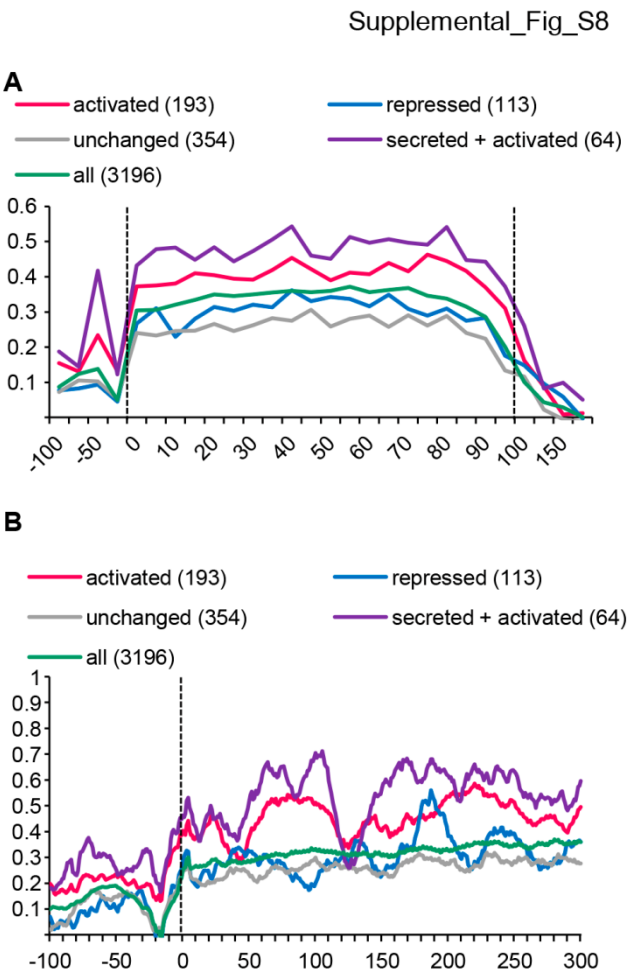
Supplemental Fig. S6:

Distribution of Dhh1 crosslinking sites in the first 300 nucleotides for genes translationally activated, repressed or not affected by Dhh1. Y-axis shows average number of reads in significant Dhh1 crosslinking sites (peaks) in the corresponding region. Reads that were not part of a significant peak are not considered.



Supplemental Fig. S7:

Ribosome density of the first 120 nucleotides versus the last 120 nucleotides in WT and *dhh1*Δ cells for the different subsets of mRNAs.



Supplemental Fig. S8:

(A) Metagene PARS score analysis of the different sets of genes. Y-axis shows the average of smoothend PARS score on the corresponding region. Smoothing is achieved by calculating the average PARS scores over a window of size 20 centered on the corresponding nucleotide. Numbers in the legend indicate the number of analyzed genes in each subset. (B) Like in (A) but for the 5'UTR and the first 300 nucleotides.

SUPPLEMENTAL TABLE TITLES

Supplemental Table S1: Ribosome profiling analysis – Related to Figure 4

Supplemental Table S2: CRAC analysis – Related to Figure 4

Supplemental Table S3: Significance of PARS score differences – Related to Figure 5 C-D.

SUPPLEMENTAL TABLE LEGENDS:

Supplemental Table S1: Ribosome profiling analysis. (A) Lists of genes translationally activated and repressed by Dhh1 including the log-fold change and the significance of RPF and mRNA levels between WT and *dhh1*Δ strain for each gene. (B) Overlap of ribosome profiling and CRAC data. List of genes translationally activated and bound by Dhh1 and of genes translationally repressed and bound by Dhh1.

Supplemental Table S2: CRAC analysis. List of all genes bound by Dhh1, genes bound in the CDS, genes bound in the 5'UTR and genes bound in the 3'UTR.

Supplemental Table S3: Significance of PARS score differences. The significance was calculated using a standard independent two-sample test that assumes equal population variances. (A) Significance of metagene PARS score differences between the subsets (Fig. 5C) indicated for each position (e.g. position "5" means from 5%-10%). (B) Significance of PARS score differences per nucleotide between the subsets (Fig.5D) indicated for each nucleotide beginning 100 nucleotides before the "AUG". Positions with a p-value < 0,05 are marked in red. If a region contains several nucleotides with p-values < 0,05 the position is marked in bold.