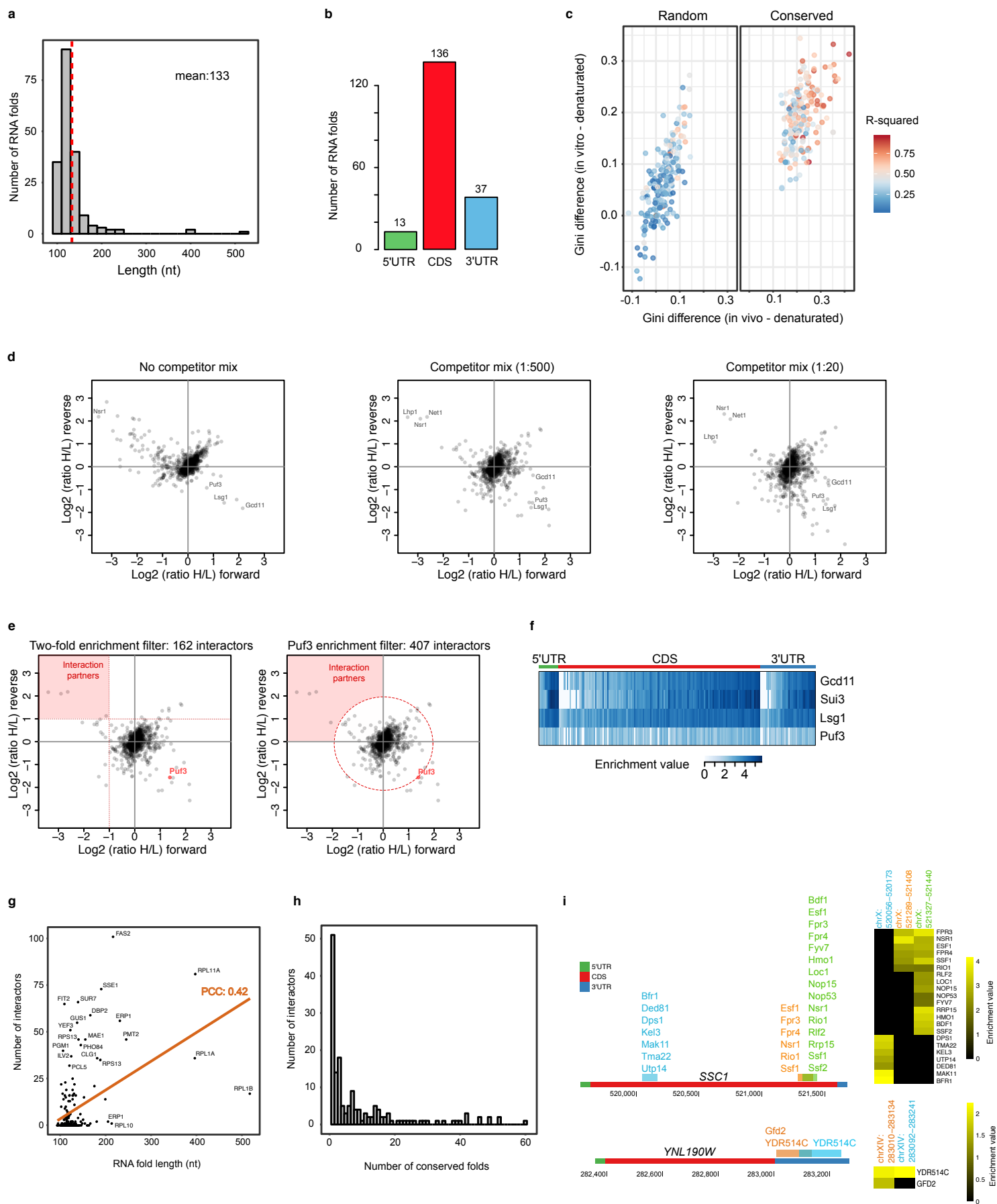


Supplementary Information

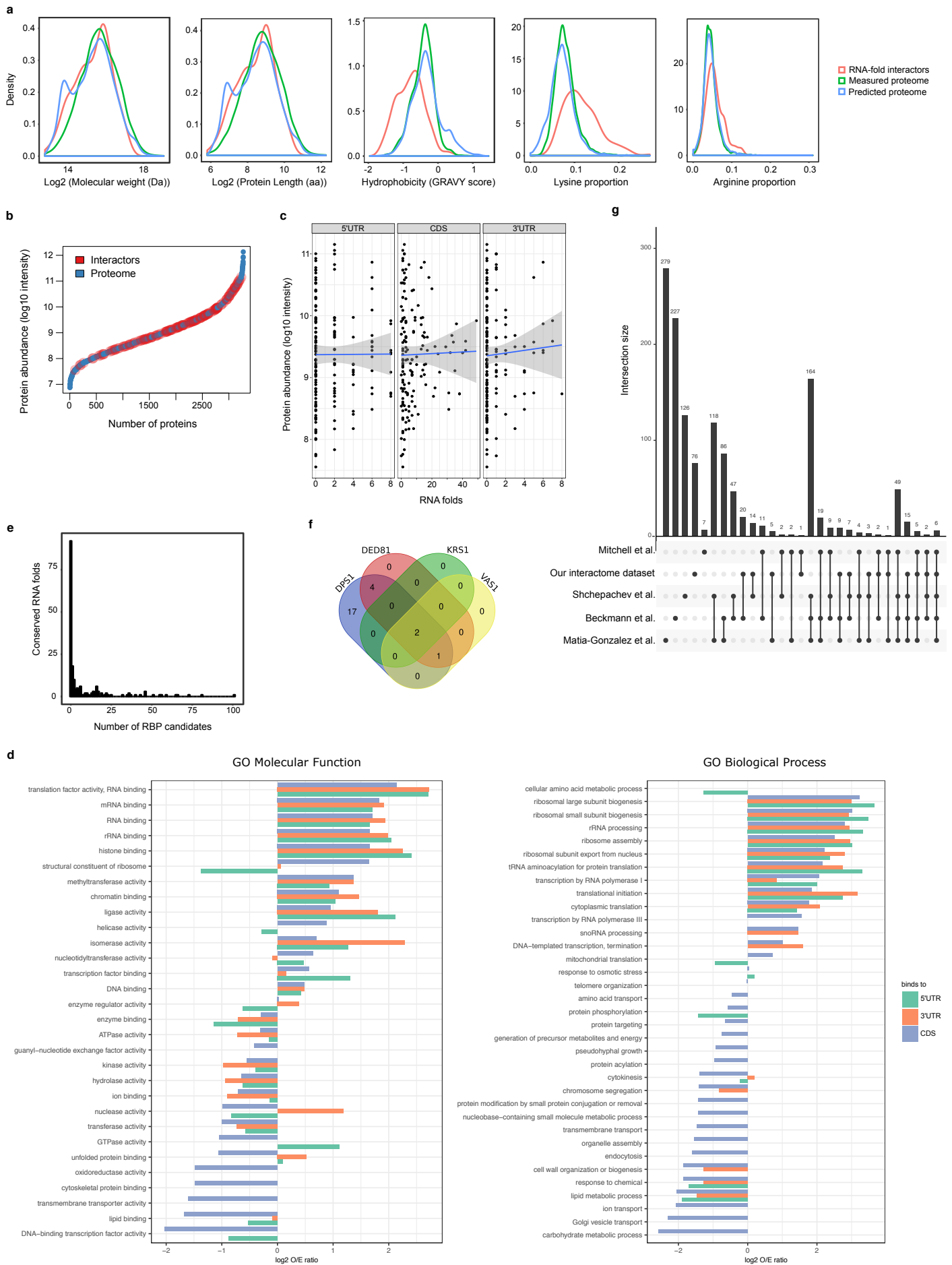
The RNA fold interactome of evolutionary conserved RNA structures in *S. cerevisiae*

Casas-Vila et al.



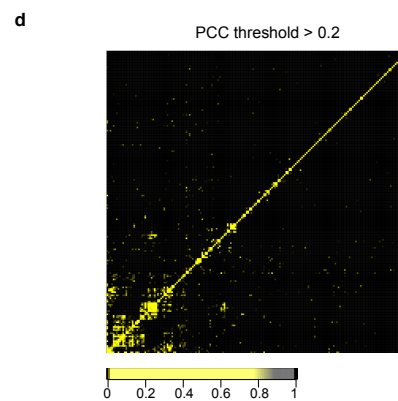
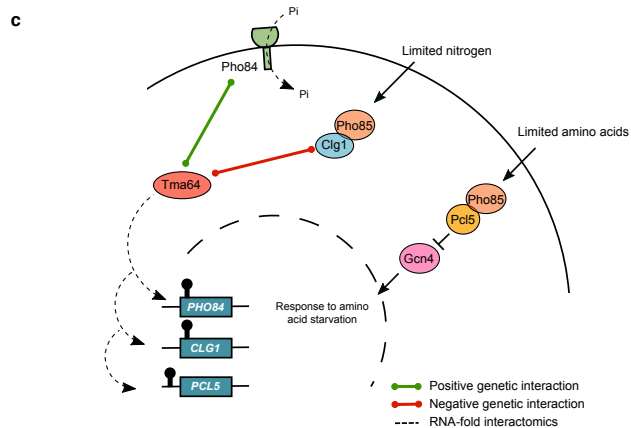
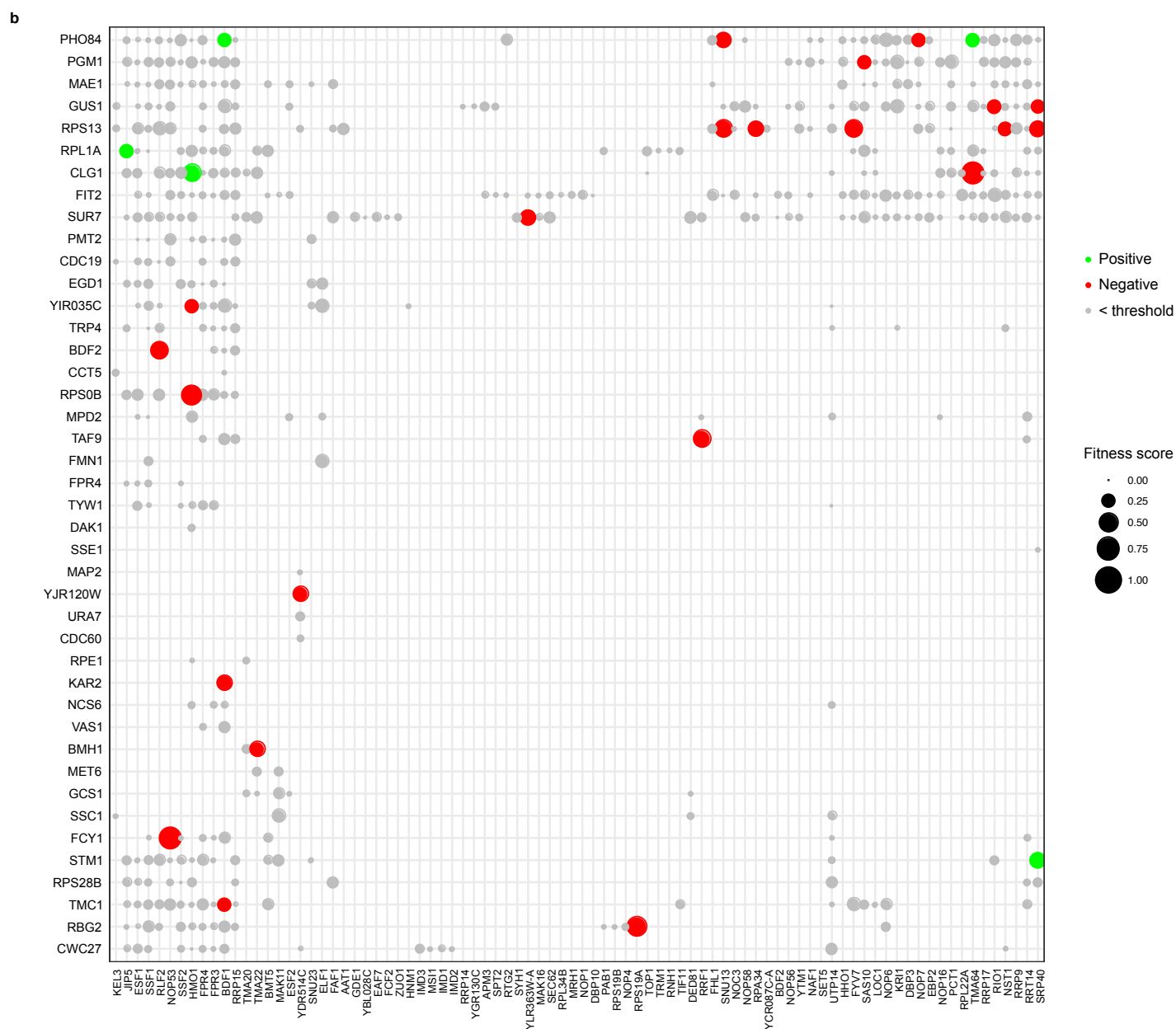
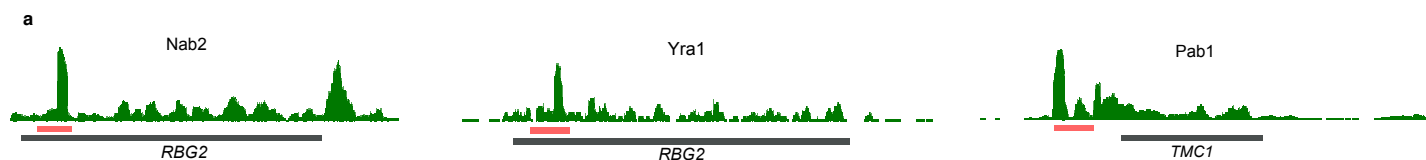
Supplementary Figure 1. RNA fold features and technical aspects of our RNA fold interactome.

- (a)** Length (nt) distribution of the 188 evolutionary conserved RNA folds. Red-dashed line indicates mean value.
- (b)** Distribution of the conserved RNA folds according to location (5'UTR, CDS, 3'UTR).
- (c)** Structure analysis based on *in vitro* and *in vivo* DMS-Seq data comparing 188 random genomic regions to the set of 188 evolutionary conserved RNA structures. Folds are colored according to *in vitro/in vivo* DMS-Seq correlations. R-squared coloring according to the degree of correlation between *in vivo* and *in vitro* conditions.
- (d)** Two-dimensional interaction plot showing protein enrichment for one RNA fold using different competitor mix concentrations (0, 1:500 and 1:20 dilutions).
- (e)** Examples of two different filtering criteria to score for interaction partners. Red area indicates interaction partners.
- (f)** Enrichment values for the four control proteins Puf3, Gcd11, Sui3 and Lsg1 at the *COX17* UTR in all 186 pull-down experiments.
- (g)** Correlation plot on number of RBP candidates binding one structure vs. structure length. PCC: Pearson Correlation Coefficient.
- (h)** Histogram showing the number of RBP interactors binding to each RNA fold.
- (i)** Schematics of RNA fold localization on the *SSC1* and *YNL190W* mRNAs harboring multiple folds and the corresponding interactors. Enrichment values for each interaction are shown in the heatmap.



Supplementary Figure 2. Characterization of our RNA fold interactors and comparison to other studies.

- (a)** Density plots showing biochemical features of our RNA fold interactome (orange), measured proteome (green) and predicted proteome (blue).
- (b)** Ranked protein abundance plotted against enriched interactors reveals that interactors (red) cover the full proteome abundance range.
- (c)** Protein abundance grouped by fold type (5'UTR, CDS, 3'UTR). Shaded area represents confidence interval (0.95) around the linear model.
- (d)** GO term enrichment analysis on molecular function and biological process of interactors differentiated by location within the target RNA (5'UTR, CDS, 3'UTR). The log2 values of the observed/expected ratio for the top25 GO terms of ranked by fold type are shown.
- (e)** Histogram showing the number of conserved folds bound by each RBP candidate.
- (f)** Overlap of target genes for the four identified tRNA synthetases.
- (g)** Comparison of our RBP candidates with other existing interactome capture studies in *S. cerevisiae*.



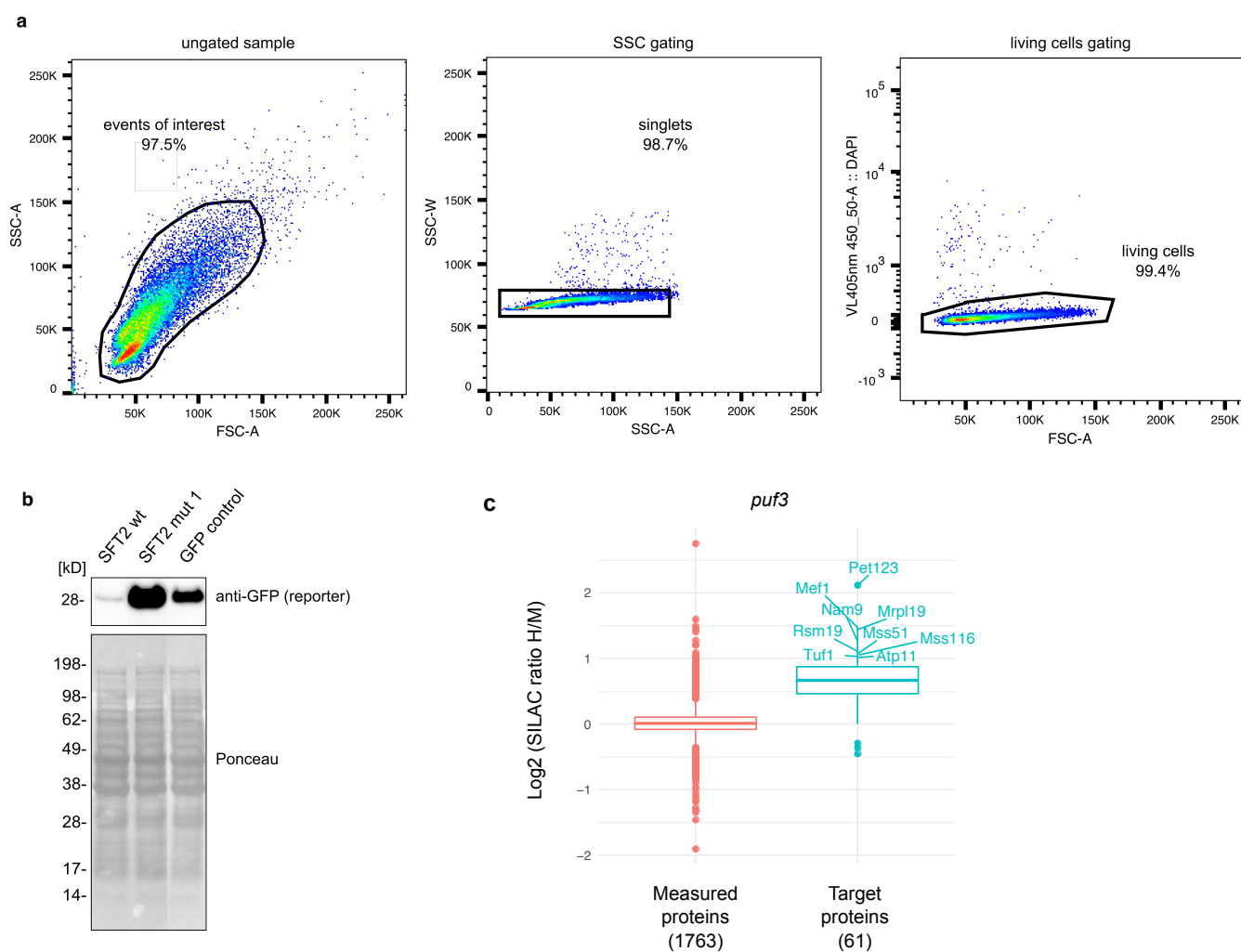
Supplementary Figure 3. RNA fold-protein interaction validation and genetic interaction data integration.

(a) PAR-CLIP data for Pab1, Yra1 and Nab2 validates binding to the investigated RNA folds. The location of the corresponding evolutionary conserved RNA fold is indicated in salmon.

(b) Complete matrix of genetic interactions described for our RBP (x-axis) and RNA fold (y-axis) interacting pairs. Genetic interactions with a fitness score > 0.08 are colored according to positive (green) and negative (red) interactions and the remaining are shown in grey. The circle size is proportional to the fitness score of the double knock-out strain of the two relevant genes.

(c) Integration of literature knowledge of Tma64 and functional evidences based on our interactomics screen and genetic interaction data.

(d) Distance correlation of genetic interaction profiles of all genes (interactors and mRNAs). Correlations higher than 0.2 are highlighted in yellow and filtered for similar genetic interaction profiles.



Supplementary Figure 4. Technical aspects of the reporter screen and positive control for pulsed SILAC.

(a) Example of gating used for GFP fluorescence intensities quantification by flow cytometry.

(b) Immunostaining shows changes in GFP levels upon fusion of the wild-type or the mutant 5'UTR *SFT2* loop as previously validated⁷, and examined in two independent biological experiments.

(c) Pulsed SILAC box plots for *puf3* knock-out compared to a wild-type strain. Known target genes (blue) are upregulated compared to all measured proteins (salmon)⁴⁰. Boxes show median (center) and interquartile ranges (ends), lower whisker representing the smallest observation greater than or equal to 1.5 times the interquartile range and upper whisker representing the largest observation less than or equal to 1.5 times the interquartile range.