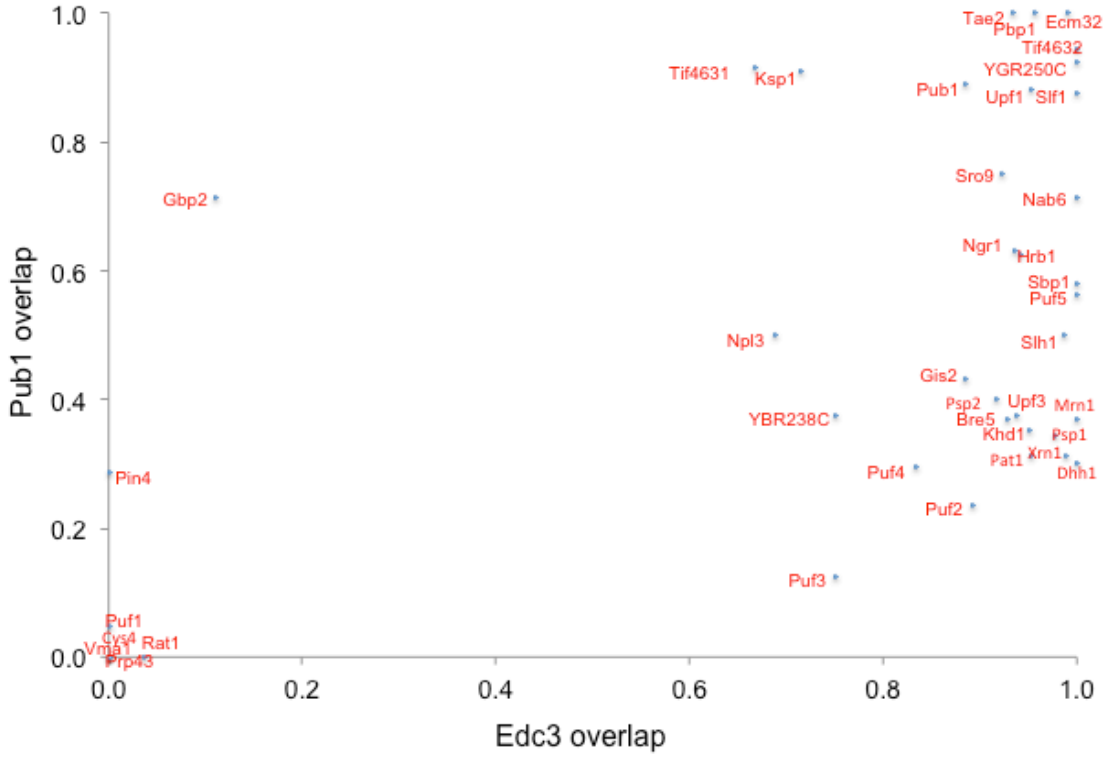
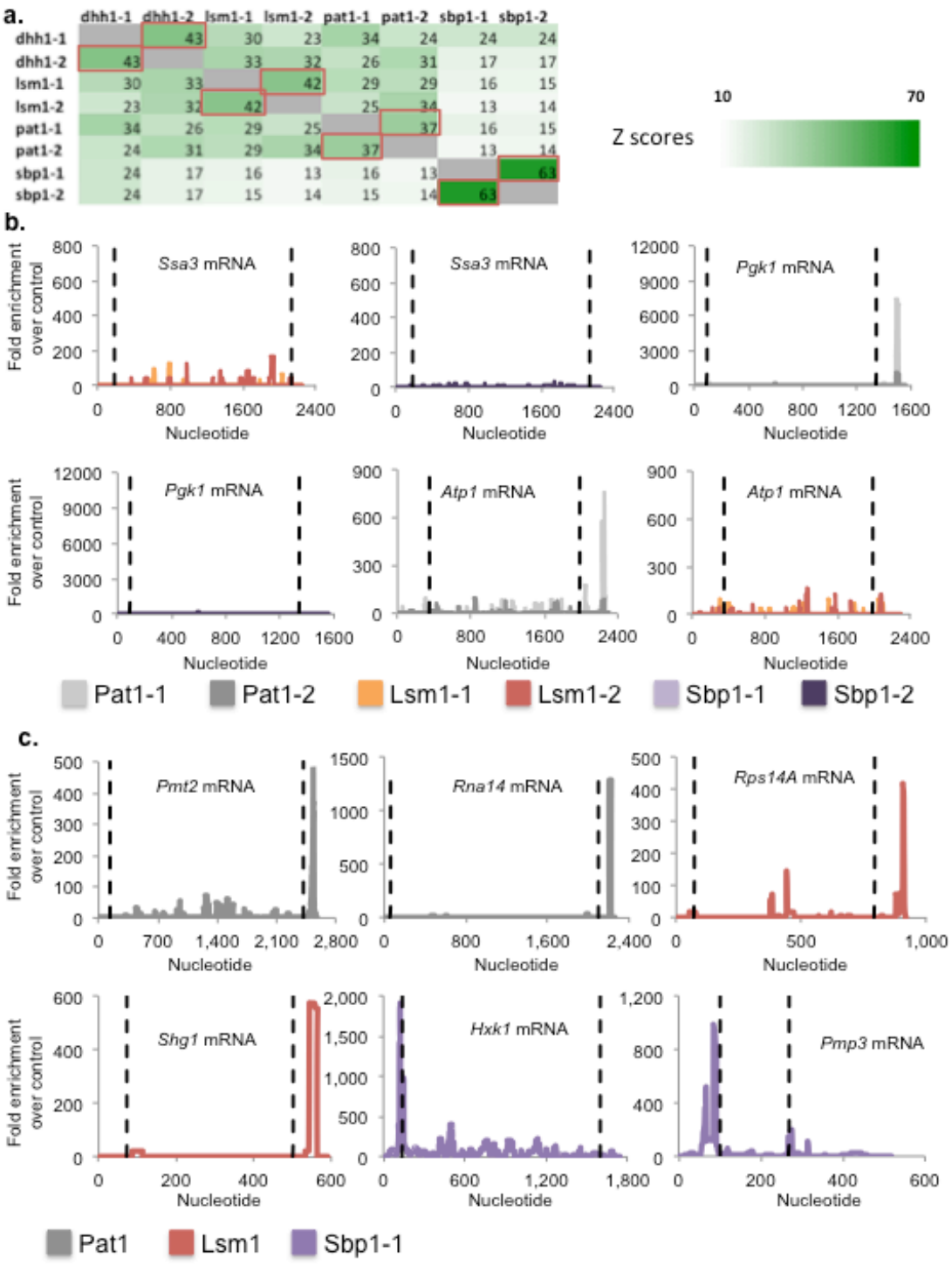
Supplementary Materials

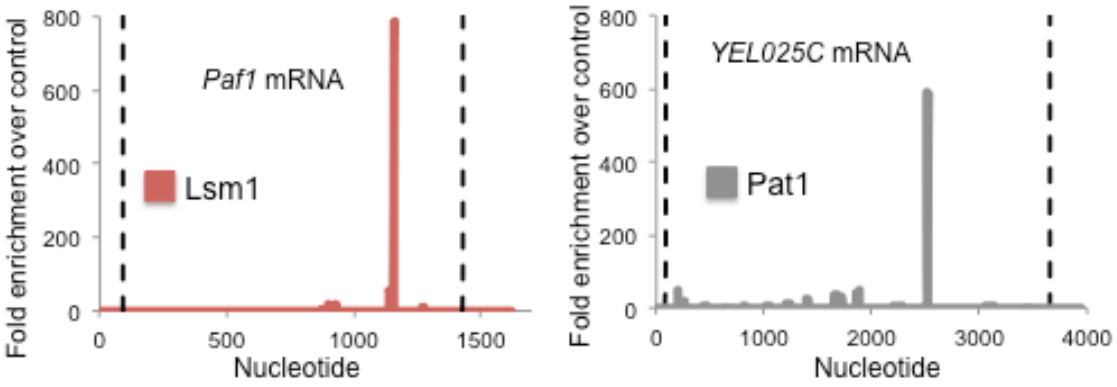


Supplementary Figure 1 Plot of the degree of overlap between GFP foci and mCherry labeled Pub1 (y-axis) and Edc3 (x-axis) as measured by fluorescence microscopy. Proteins with fraction of overlap with Edc3 greater than 0.67 are considered to be either stress granule or P-body components. Gbp2, a known stress granule component[3] seems to be an outlier, however it is possible that insufficient time was given for this factor to enter granules (J.R. Buchan and R. Parker, unpublished). Components that demonstrate greater than 0.67 fractional overlap with Edc3 have been plotted on a heat map in Figure 3b to qualify their stress granule or P-body-like character.



Supplementary Figure 2 P-body proteins bind an overlapping set of mRNA with positional specificity. (**a**) Z-scores indicating the significance of the overlap between mRNA targets identified by CLIP for the indicated proteins. Replicates

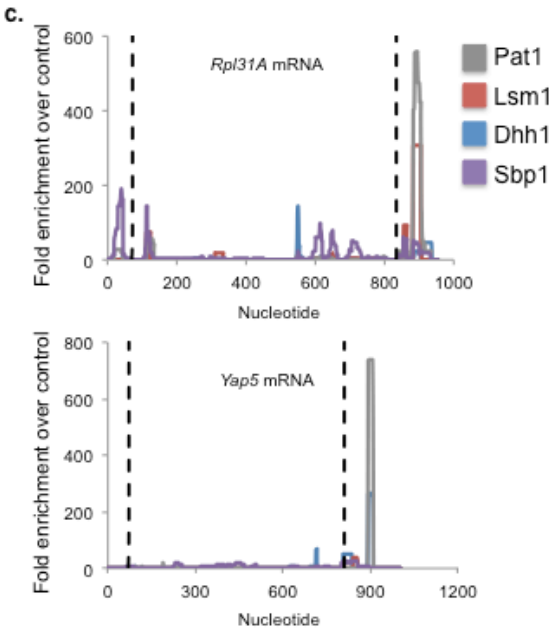
of CLIP experiments are highlighted in red boxes. Experiments 1 and 2 are indicated by -1 and -2 respectively. Cells are colored based on Z-scores as shown in the color bar. (**b**) CLIP data for the mRNA shown in Figure 4d showing the enrichment of sequence reads for proteins not shown in the main text. Lsm1 is shown in orange, Pat1 in gray and Sbp1 in purple. Replicate traces are shown in darker shades. (**c**) Additional example mRNA are shown to demonstrate the positional localization of Pat1, Lsm1 and Sbp1. Pat1 data is shown in gray for *Pmt2* and *Rna14* mRNA, Lsm1 in red for *Shg1* and *Rps14A* mRNA, and Sbp1 is shown in purple for *Hxk1* and *Pmp3* mRNA. For both (**b**) and (**c**), dashed black lines indicate the limits of the open reading frame.

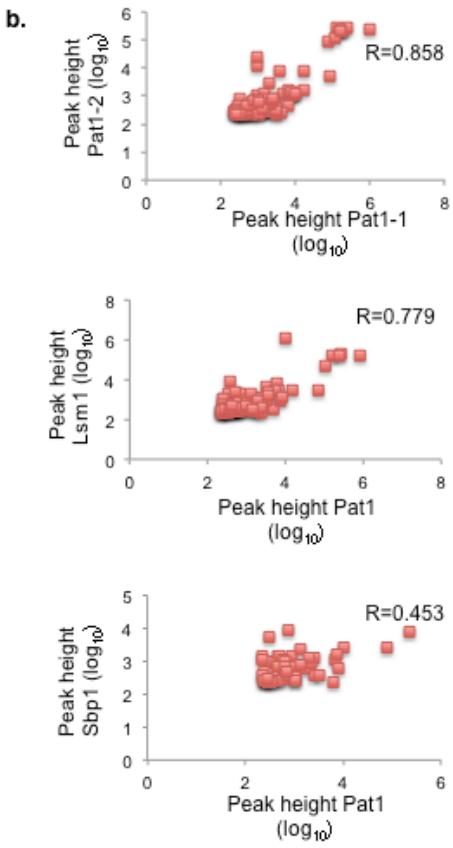


Supplementary Figure 3 Analysis of positional specificity in P-body protein binding to mRNA. Examples of CLIP data showing Pat1 and Lsm1 binding sites outside of the 3′ UTR. From left: *Paf1* mRNA demonstrates Lsm1 binding in the ORF. *YEL025C* mRNA demonstrates Pat1 binding to the ORF.

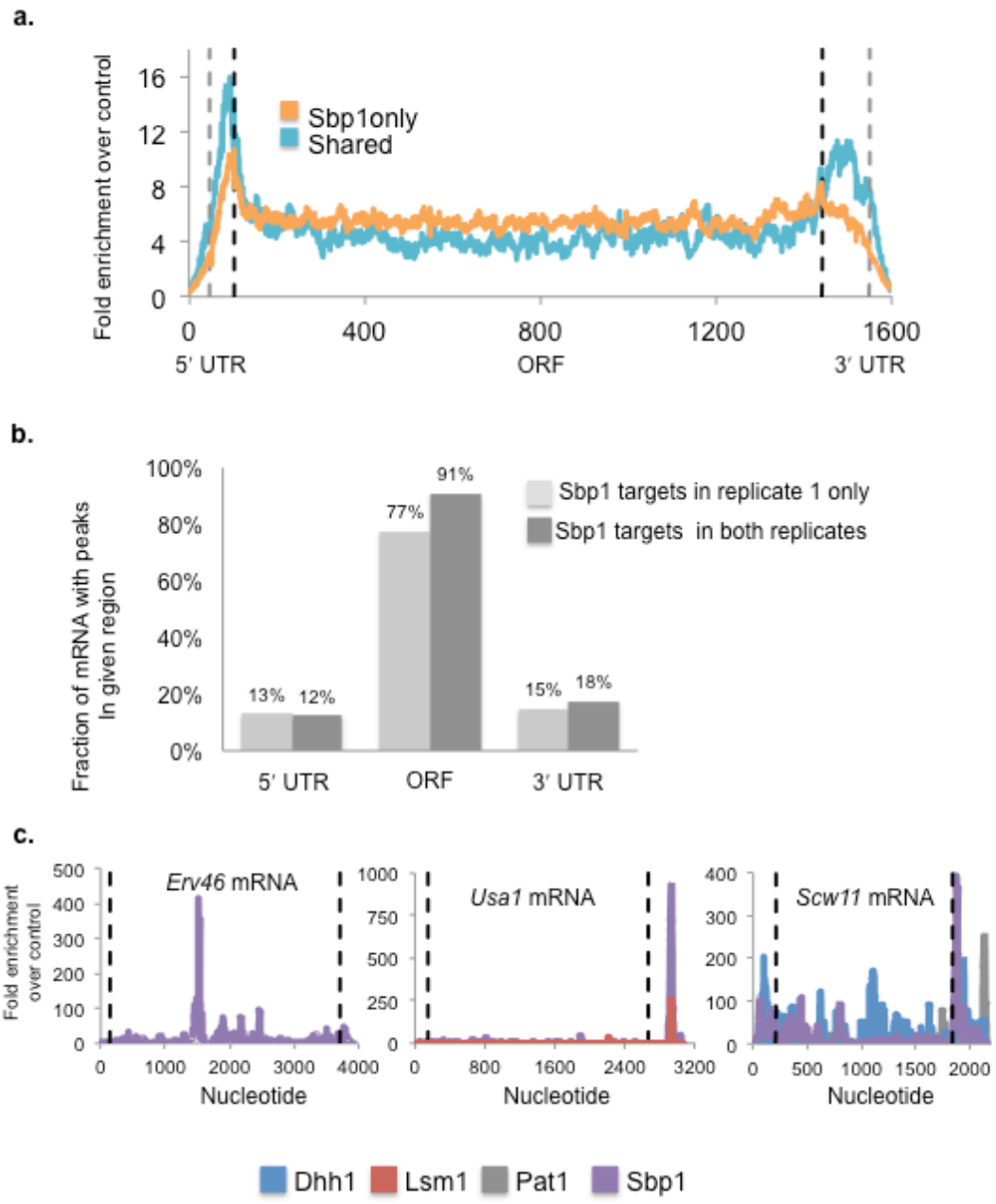
a.

	Lsm1	Pat1	Dhh1	Sbp1
Lsm1	0.886	0.925	0.784	0.515
Pat1	0.925	0.95	0.88	0.628
Dhh1	0.784	0.88	0.954	0.696
Sbp1	0.515	0.628	0.696	0.971





Supplementary Figure 4 P-body proteins co-localize on mRNA. (**a**) A chart showing the Pearson correlation coefficients as calculated from peak positions of mRNA bound by both proteins (see methods). (**b**) Example plots of comparisons of sequence reads for co-localized peaks between replicate data sets (top) and between proteins. (**c**) Plots showing enrichment over the control sequence data for individual mRNAs. Dhh1 data is shown in blue, Lsm1 in red, Pat1 in green and Sbp1 in purple. Dashed black lines indicate the limits of the open reading frame.



Supplementary Figure 5 Effects of other P-body proteins on the binding sites of Sbp1. (**a**) Average plot of enrichment of CLIP sequence tags over the control sequence for Sbp1 target mRNAs are shown. Those that bind only to Sbp1 are shown in orange, those that also bind to Dhh1, Lsm1 and/or Pat1 are shown in aqua. (**b**) The effect of other proteins on Sbp1 distribution is not due to internal biases in the Sbp1 data sets: Bar graph showing the distribution of peaks across

the transcript for mRNA identified as Sbp1 targets in replicate 1 only (light gray) or in both replicates of the Sbp1 CLIP experiment (dark gray). No statistically significant difference was observed in the distribution of Sbp1 peaks in the UTRs, unpaired Student's t-test was used, n=363 mRNA for replicate 1 only and 671 mRNA for overlap between replicates. (**c**) Individual CLIP traces showing examples of Sbp1 peak position when bound to mRNA with and without binding sites of other P-body proteins. *Erv46* mRNA demonstrates Sbp1 binding in the ORF when binding sites for none of the other tested proteins are present. *Scw11* mRNA demonstrates Sbp1 binding in the 3' UTR in the presence of Pat1 and Dhh1 binding sites. *Usa1* mRNA demonstrates Sbp1 binding to the 3' UTR in the presence of Lsm1 binding sites.

Supplementary Table 1: A list of all 120 proteins identified as enriched by the in vivo capture of RBPs. Supplementary_Table1.xlsx

Supplementary Table 2: mRNA binding proteins conserved in mammals[1,2]

Yeast Protein	Homologue/ Related Human Protein	Identified in Castello et al.	Identified in Baltz et al.
Bms1	Bms1	YES	YES
Dbp1	DDX3X	YES	YES
Ded1	DDX3X	YES	YES
Dhh1	DDX6	YES	YES
Ebp2	EBNA1BP2	YES	YES
Gbp2	HNRNPM	YES	YES
Gis2	CNBP	YES	YES
Mak21	CEBPZ	YES	YES
Mex67	NXF1	YES	YES
Mrd1	RBM19	YES	YES
Nab2	ZC3H14	YES	YES
Nam8	TRNAU1AP	YES	YES
Nan1	WDR75	YES	YES
Ngr1	TRNAU1AP	YES	YES
Noc2	NOC2L	YES	YES
Nop4	RBM28	YES	YES
Nop56	NOP56	YES	YES
Nop58	NOP58	YES	YES
Nop7	PES1	YES	YES
Nop9	C14orf21	YES	YES
Npl3	SRSF4/SFRS4	YES	YES
Nsr1	NCL	YES	YES
Pab1	PABPC1	YES	YES
Pap2	PAPD5	YES	YES
Pat1	PATL1	YES	YES
Prp43	DHX16	YES	YES
Prp8	PRPF8	YES	YES
Pub1	TIA1	YES	YES
Puf3	PUM1	YES	YES
Puf4	PUM2	YES	YES
Puf6	KIAA0020	YES	YES
Pus1	PUS1	YES	YES
Rat1	XRN2	YES	YES

Rpb2	POLR2B	YES	YES
Rps20	RPS20	YES	YES
Rrp12	RRP12	YES	YES
Scp160	HDLBP	YES	YES
Sen1	HELZ	YES	YES
Slh1	SNRNP200	YES	YES
Sof1	DCAF13	YES	YES
Spt5	SUPT5H	YES	YES
Tif3	EIF4B	YES	YES
Tif32	EIF3A	YES	YES
Tif4631	EIF4G1	YES	YES
Tif4632	EIF4G2	YES	YES
Tma46	ZC3H15	YES	YES
Ubp3	USP10	YES	YES
Upf1	UPF1	YES	YES
Urb1	URB1	YES	YES
Utp20	UTP20	YES	YES
Xrn1	XRN1	YES	YES
Ygr250c	CELF1	YES	YES
Ylr419w	DHX36	YES	YES
Cpr1	PPIA	YES	YES
Nip1	EIF3C	YES	YES
Pwp2	PWP2	YES	YES
Cbf5	DKC1	YES	NO
Dbp9	DDX56	YES	NO
Hek2	PCBP3	YES	NO
Mlp1	TPR	YES	NO
Mtr4	SKIV2L2	YES	NO
Nug1	GNL3L	YES	NO
Pbp2	PCBP3	YES	NO
Rrp5	PDCD11	YES	NO
Sup35	GSPT1	YES	NO
Tys1	YARS	YES	NO
Dus3	DUS3L	NO	YES
Hrb1	MYEF2	NO	YES
Hrp1	MSI1	NO	YES
Hts1	HARS2	NO	YES
Sto1	NCBP1	NO	YES

Supplementary Table 3 :A list of mRNAs (Tier1 and Tier2) bound by Dhh1, Lsm1, Pat1 and Sbp1. Supplementary_Table3.xlsx

Supplementary Note

Yeast Strains and Growth Conditions

For in vivo capture of RBPs, yeast strain BY4741 was grown to 0.5–0.6 OD_{600} in YEPD with 2% glucose at 30°C. Strains for microscopy were grown in minimal medium with the appropriate amino acids and 2% glucose to an OD_{600} of 0.4–0.6. Yeast strains were transformed using standard chemical transformation techniques.

Statistical Analysis

Two methods were applied to assess the similarity of peaks between replicate data sets and CLIP data sets for different proteins:

A) Signal strength of co-localized peaks (Fig. 4a and Supplementary Fig. 4c): For all peaks from the two data sets that are within 30 nucleotides (the approximate length of our sequence reads), the number of sequence reads after normalization (see above) were plotted and Pearson correlation coefficients calculated from the scatter plots.

B) Proximity of peaks (Supplementary Fig. 5b): For each mRNA found in both data sets, the peak positions for each peak in data set 1 and the closest corresponding peak in data set 2 were plotted on a 2-D scatter plot as distance from transcriptional start site. Pearson correlation coefficients were calculated from the scatter plots.

References

1. Castello, A. *et al.* Insights into RNA Biology from an Atlas of Mammalian mRNA-Binding Proteins. *Cell* **149**, 1393–1406 (2012).
2. Baltz, A. G. *et al.* The mRNA-Bound Proteome and Its Global Occupancy Profile on Protein-Coding Transcripts. *Molecular Cell* **46**, 674–690 (2012).
3. Buchan, J. R., Muhlrad, D. & Parker, R. P bodies promote stress granule assembly in Saccharomyces cerevisiae. *J Cell Biol* **183**, 441–455 (2008).