SUPPLEMENTARY MATERIAL

Ubiquitination Screen Using Protein Microarrays for Comprehensive Identification of Rsp5 Substrates in Yeast

Ronish Gupta[1,2]*, Bart Kus[1,2]*, Christopher Fladd[1,2], James Wasmuth[3], Raffi Tonikian[4,5], Nevan J. Krogan[6], Charlie Boone[4,5], John Parkinson[2,3,5] and Daniela Rotin[1,2#]

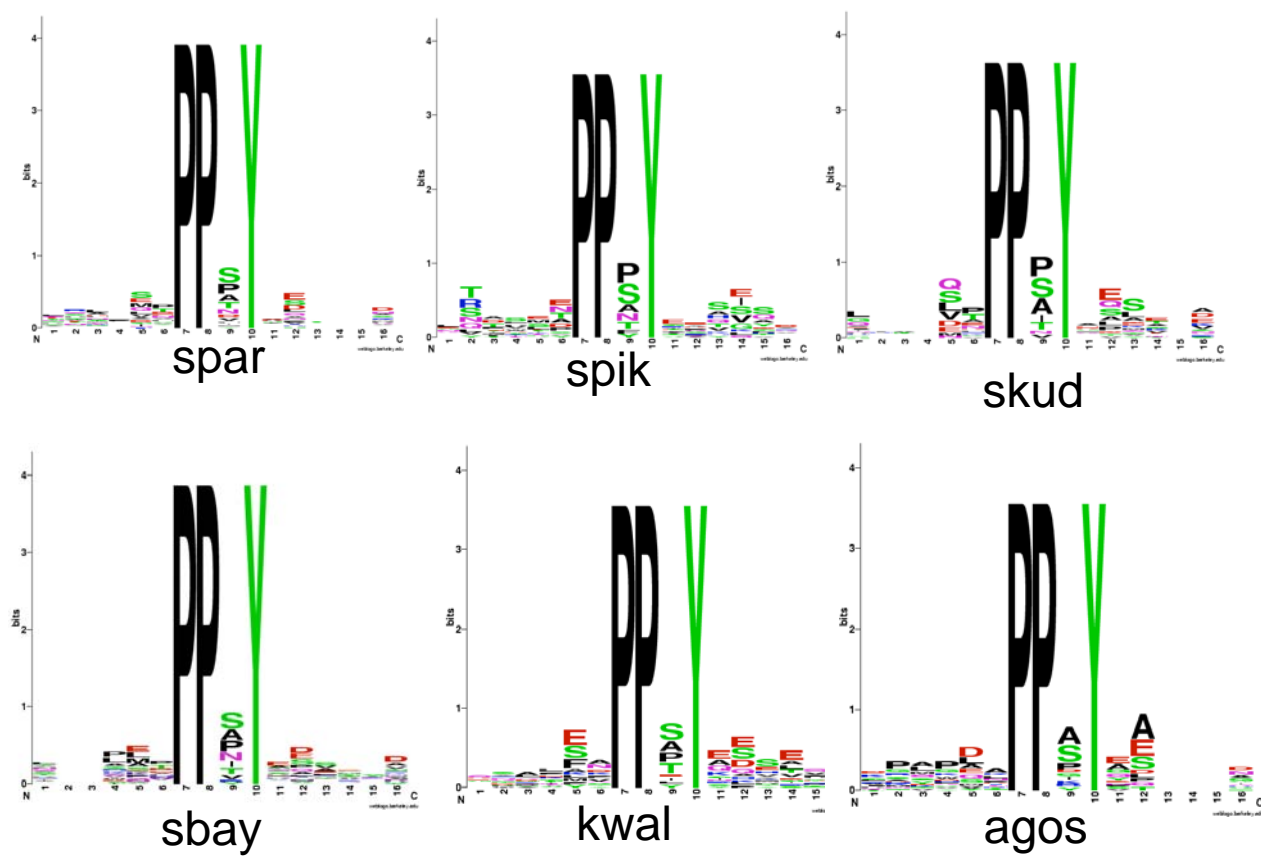**Supplementary Figure S1.   Sequence logos for selected yeast strains.**
A. The PY ((L/P)PxY) motifs from Rsp5 substrate homologues in different yeast strains were aligned. The results from 6 yeast strains are displayed as sequence Logos.
B. Extent of purifying selection of the (L/P)PxY motif. The ratio of non-synonymous and synonymous change (Ka/Ks) was calculated from nucleotide alignments for each group of yeast orthologues. This graph shows the mean ratio across 46 groups, with focus on the (L/P)PxY motif. Lower Ka/Ks ratios are indicative of purifying selection; a desire to prevent nucleotide mutation that changes the encoded for amino acid.
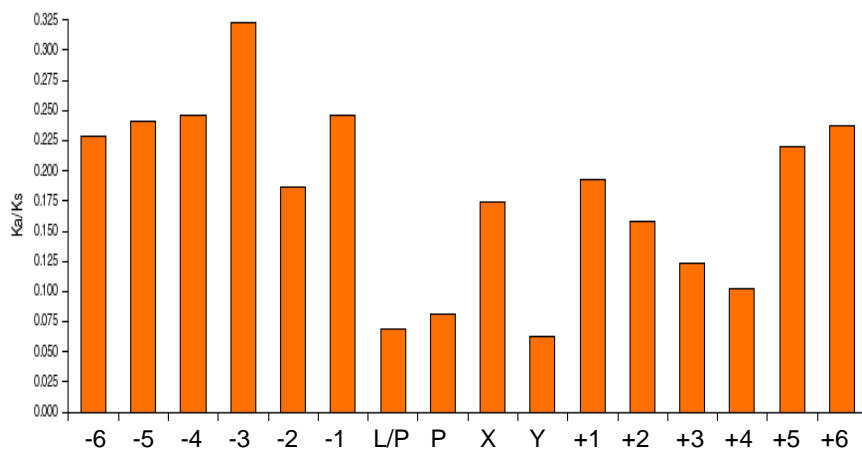C. Homologues of Rsp5 substrates from different strains were aligned. The table lists the related yeast strains, the most (1st) preferred residues and the 2nd most preferred residues at the x position of the (L/P)PxY motifs of the Rsp5 substrate homologues.

A.

spar

spik

skud

sbay

kwal

agos

B.

C.

Preferred residues at 3rd position

	1st	2nd
Ylip	A	T
Spo	S	A
Spar	S	P
Smik	S	P
Skud	S	P
Sklu	S	P
Scer	S	P
Scas	S	A
Sbay	S	A
ncra	S	Q
Kwal	S	A
Klac	S	A
fgra	A	P
Dhan	A	S
Ctro	S	A
Cgla	S	A
anid	P	S
agos	A	S