Supplementary Information

Materials and Methods

Preparation of Yeast Actin

A DNAse1 affinity column was prepared by coupling Dnase1 (Roche, grade II) to active ester agarose (Bio-Rad, Affigel 10 Gel). 200 mg Dnase1 was dissolved in 10 ml of coupling buffer (0.1M HEPES pH 7.2, 80mM CaCl2, 1mM PMSF) and dialysed overnight against 1 L coupling buffer. 25 ml Affigel –10 resin was washed five times with 30 ml ice cold dH2O and twice with ice cold coupling buffer in a scintered glass filter funnel attached to a vacuum line, taking care not to let the gel dry out. The washed resin was scooped into the Dnase1 solution and incubated on a rocker for 3hrs at 4^{o}C. The resin was poured into a column and washed with 15 column volumes of coupling buffer. For storage 0.2% sodium azide was added to the final column volume. Before use, the column was equilibrated with 5 volumes of GY-buffer (10 mM Tris pH 7.5, 0.2 mM CaCl2, 2 mM DTT, 0.5 mM ATP).

Yeast actin was purified from *Saccharomyces cerevisiae*. 400g of fresh yeast (Sainsburys plc) was washed in 400 ml cold dH2O and collected by centrifugation at 7,000x*g* for 10 mins. The yeast pellet was then resuspended in 400 ml GY-buffer with protease inhibitors (Roche complete EDTA-free protease inhibitor cocktail tablets) and passed once through a cell disrupter (Constant Cell Disruption Systems) at 35 psi. The broken yeast was then frozen in droplets in N2(liq) and kept at –80^{o}C until needed. To purify actin, 100g of broken yeast cells were thawed in 100ml G-buffer (2 mM Tris pH8.0, 0.2 mM ATP, 0.5mM DTT, 0.2mM CaCl2) with protease inhibitors, and spun at 158,000x*g* for 30mins. The supernatant was removed, avoiding as much white lipid material as possible, and loaded directly onto a 25 ml Dnase1 column, previously equilibrated with G-buffer. The column was washed with 50 ml G-buffer at 5 ml/min, and then with 50 ml of each of the following buffers: G-buffer containing 10% formamide, G-buffer containing 10% formamide and 0.2M NH4Cl, G-buffer. Yeast actin was eluted with G-Buffer and 50% formamide. Fractions containing actin were identified by SDS-PAGE. These fractions were dialysed against G-buffer overnight, and then

polymerized by the addition of 1/9 volume of KMEI (1M KCl, 20mM MgCl2, 10mM EGTA, 0.2M Imidazole-Hcl pH 7.0) and incubation at room temperature for 1 hr. Polymerised actin was pelleted by ultracentrifugation at 380,000xg for 1hr. Pelleted actin was resuspended in G-buffer, and frozen in aliquots and stored at –80^{o}C until required.

Figure legends

Supplementary figure 1. Comparison of YAB domains

(A) CLUSTAl W alignment of ysc84 actin binding (YAB) domains from a range of organisms. *S. cerevisiae* Ysc84 NP_011880; *S. cerevisiae* Lsb3 NP_219497; *S.pombe* NP_593048; *A.nidulans* XP_408305.1; *G.zeae* XP_389673.1; *N. crassa* XP_323207; *A.thaliana* NP_189909 *O.sativa* NP_001060279; *D.rerio* TC271701; *X.laevis* NP_001086532.1; *G.gallus* XP_419926; *M.musculus* SH3yl-1 CAG33642, *H.sapiens* SH3yl-1 AAH08375 (Thompson et al, 1994). (B) Phylogenetic tree generated by TreeView from sequences listed above (Page, 1996).

Supplementary Figure 2

Ysc84 interacts with forming but not pre-formed actin filaments

Actin (10 μM) was polymerised 2 hr. at room temperature. Ysc84 (5 and 10 μM) was then added to the preformed filaments for 2 hours. Assays were then spun at high speed (see materials and methods) to determine whether Ysc84 is able to bind actin filaments. Lanes 1 -Actin only; 2 - Ysc84Nt 5 μM only; 3 - Ysc84Nt 10 μM only; 4 - Ysc84 5 μM + actin; 5- Ysc84 10 μM + actin. As shown, Ysc84 shows no significant binding to preformed filaments.

Supplementary Figure 3

Additional 2 hybrid interaction studies

(A) The interaction between Ysc84 and Las17 requires the SH3 domain of Ysc84 Strains were generated carrying a plasmid pKA251 (Ysc84ΔSH3 fused to Gal4 binding domain). These were crossed with strains carrying Las17(292-536) fused

to Gal4 activation domain. Resulting diploids were assayed and compared to full length Ysc84 interactions.

(B) Lsb3 interacts with Las17 polyproline region

Strains were generated carrying a plasmid pKA597 (Lsb3 fused to Gal4 Binding domain). These were crossed with strains carrying Las17 fragments fused to Gal4 activation domain. Resulting diploids were assayed for beta galactosidase activity as a measure of the interaction between Gal4 binding and activation domain fusion interactions.

Supplementary figure 4

Ysc84 interaction with yeast actin

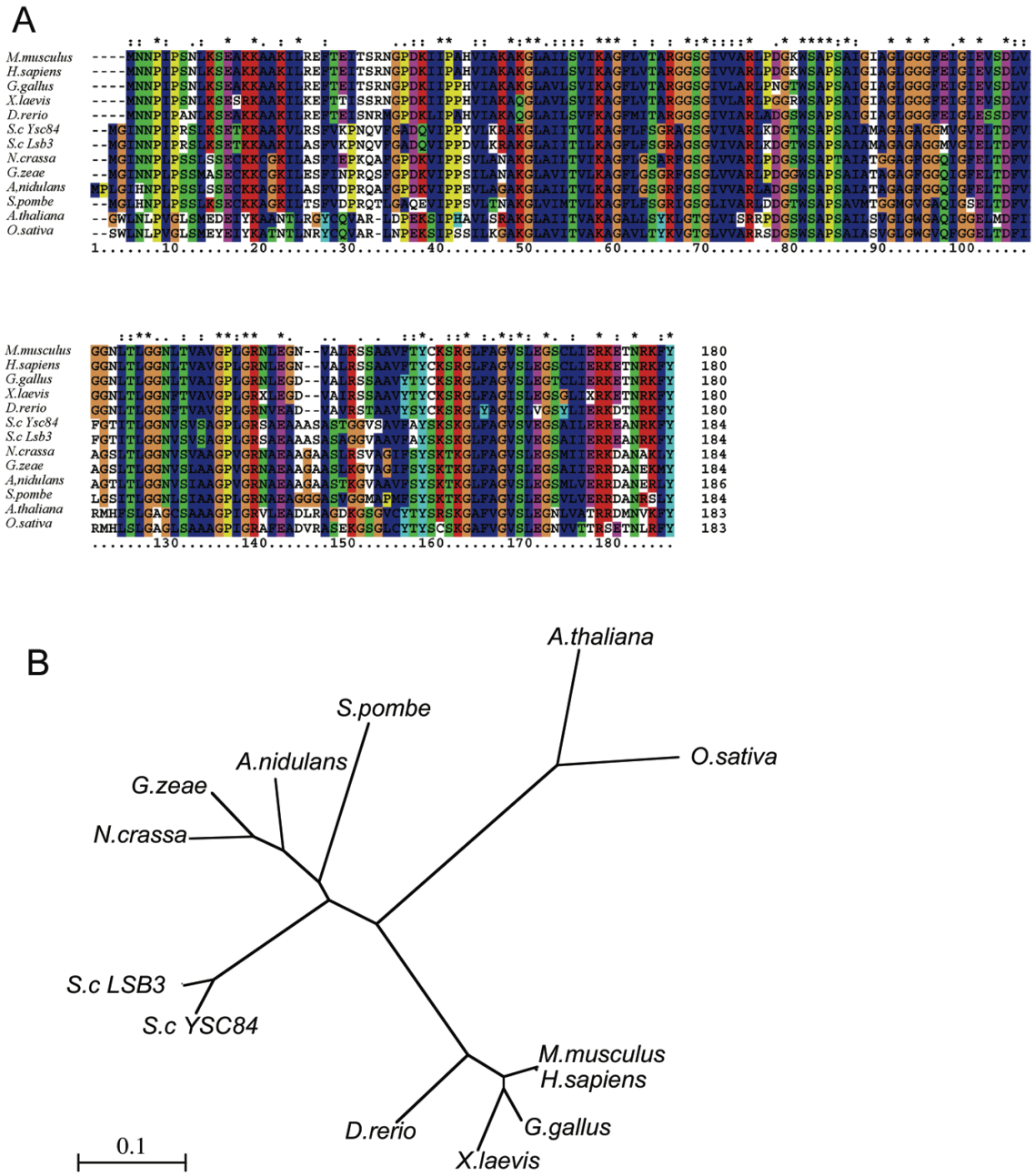
(A) Ysc84Nt binds yeast actin in a high speed pelleting assay. (B). Full length Ysc84 does not bind yeast actin and pellet in a high speed assay except in the presence of the las17 fragment (amino acids 300-422). The Las17 fragment alone appears to bind to actin and prevent it polymerising. This effect is blocked in the presence of Ysc84. (C) Low speed pelleting was performed to assess whether Ysc84 and Las17 are able to bundle actin filaments. As shown in lane 6, a proportion of actin is able to bundle in the presence of bothLas17 and Ysc84 but not in the presence of either protein alone. (D) Electron microscopy of negatively stained yeast actin filaments in the absence and presence of ysc84Nt. Bundles are clearly present in the filaments co-polymerised with Ysc84Nt.

Supplementary references

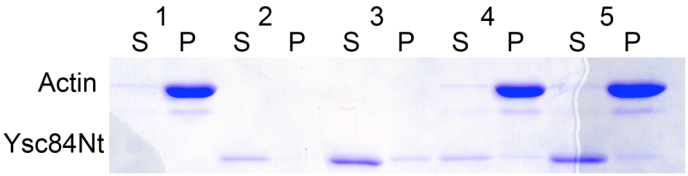
Thompson, J.D, Higgins, D.G. Gibson, T.J. (1004). Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. *22*: 4673-4680

Page, R.D.M. (1996) TreeView: An Application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. *12*, 357-358

Supplementary Figure 1

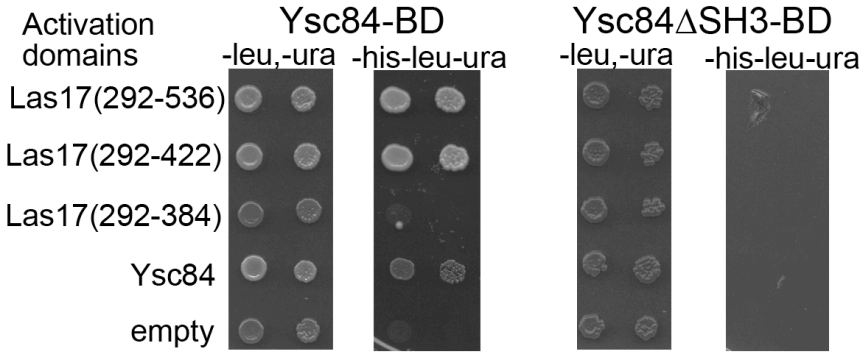


Supplementary Figure 2

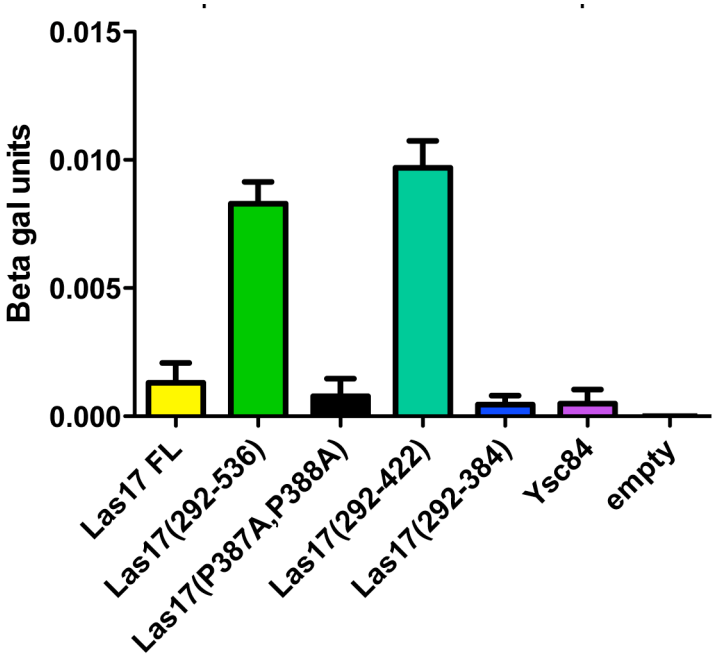


Supplementary Figure 3

(A)

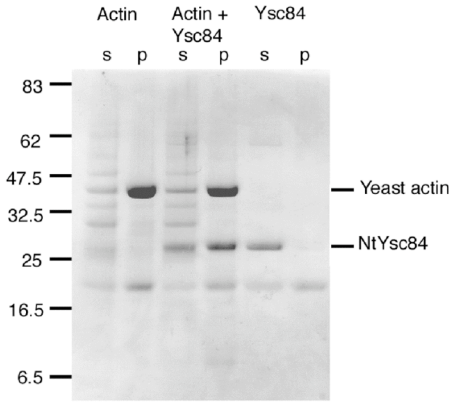


(B) Gal4Binding domain Lsb3 interactions

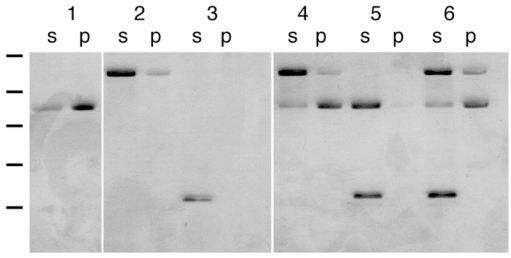


Supplementary Figure 4

A NtYsc84binds to Yeast F-Actin

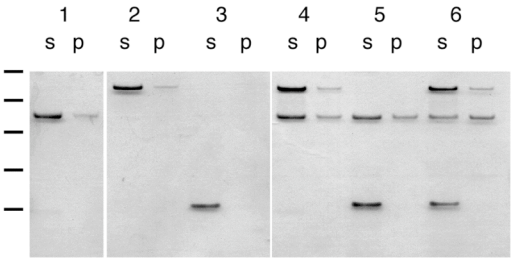


B High speed pelleting assay of FlYsc84 and Las17 with yeast actin



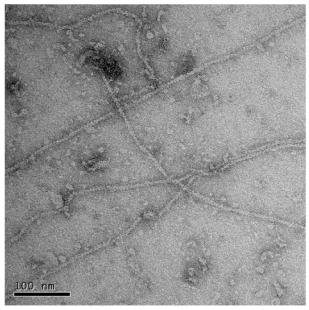
1 Yeast actin only
2 FlYsc84 only
3 Las17 only
4 Yeast actin + FlYsc84
5 Yeast actin + Las17
6 Yeast actin + FlYsc84 + Las17

C Low speed pelleting assay of FlYsc84 and Las17 with Yeast actin



1 Yeast actin only
2 FlYsc84 only
3 Las17only
4 Yeast actin + FlYsc84
5 Yeast actin + Las17
6 Yeast actin + FlYsc84 + Las17

D Yeast actin



Yeast actin + Ysc84Nt