Supplemental Methods

Yeast Two-Hybrid Analysis

Yeast two hybrid experiments were conducted using the Matchmaker GAL4 Two-Hybrid System 3 with bait and prey vectors (pGBKT7, pGADT7) containing the binding domain (BD) and activation domain (AD), respectively (Clontech, Mountain View, CA). Yeast host strain *Saccharomyces cerevisiae* AH109 (Clontech, Mountain View, CA) was used to take advantage of the three reporters ADE2, HIS3, and MEL1 (MEL1 encodes α-galactosidase, α-GAL). All yeast methods were conducted following Manufacturer's instructions (Clontech, Mountain View, CA).

The coding regions of each cDNA (*AP3, PI, AqvAP3-1, AqvAP3-2, AqvAP3-3* and *AqvPI*) excluding the MADS domain (Yang et al., 2003a) were cloned into pGBKT7 or pGADT7 using NcoI and appropriate 3' restriction sites. All constructs were sequenced to confirm that the binding domain or activation domain was fused N-terminally and in-frame with the coding region of the appropriate locus. Transformation into yeast strain AH109 was performed with single constructs using a lithium acetate/PEG method following manufacturer's instructions (Clontech, Mountain View, CA) or a kit (Zymo Research Corp, Orange, CA). Strains were selected on synthetic dropout (SD) media supplemented with adenine (Ade) and 2% glucose but lacking tryptophan (Trp) for the binding domain constructs or lacking leucine (Leu) for the activation domain constructs.

To check for auto-activation, three independent yeast transformants for each construct were tested for growth on media supplemented with 0 to 30 mM 3-amino-1,2,4-aminotriazole (3AT, Sigma, St. Louis, MO) that lacked either Trp for the BD constructs or lacking Leu for the AD constructs. Reduction in any background auto-activation responded linearly to increasing concentration of 3AT and 20 mM was selected as the optimal concentration. Protein interactions were then tested by transformation of appropriate construct pairs into AH109 and growth on selective media. Selection for interactions were conducted on SD media lacking His, Leu, Trp (-HLT) or Ade, His, Leu, Trp (-AHLT) supplemented 0 to 30 mM 3-AT (Sigma, St. Louis, MO). Fusion protein expression was confirmed by immunoblotting with anti-c-Myc monoclonal and/or HA-Tag polyclonal antibodies (BD Biosciences, San Jose, CA).

Protein interactions were characterized using both growth on selective media and assays of α-GAL activity. For the former, cultures were grown overnight at 30ºC shaking at 270 rpm. Each overnight culture was diluted to an optical density (OD) at 600 nm of 0.8, which represented ~10^5 colony forming units (cfu). Cultures were serially diluted (~10^5, ~10^4 ~10^3 ~10^2 cfu) and two microliters of each dilution were plated onto –HLT or -AHLT SD media supplemented with 0 to 30 mM 3-AT (Sigma, St. Louis, MO). Plates were incubated at 30ºC from 3-8 days. Photos were taken beginning on day 3 (photos for –AHLT plates are not shown). For the test of α-GAL activity, we used the MEL1 marker to conduct α-galactosidase assays. Experiments were conducted on liquid SD media from an overnight culture containing secreted α-GAL and the substrate

p-nitrophenyl α-D-galactopyranoside (Sigma N0877). Methods are detailed in the Yeast Protocol Handbook (Clontech, Mountain View, CA). For each experiment, three replicates per construct-pair were assessed. For each replicate, four yeast colonies grown on -LT plates for maintenance of paired BD and AD constructs were inoculated into 4 ml of liquid SD supplemented with 20 mM 3-AT, Ade and 2% glucose media but lacking His, Leu, and Trp. Cultures were grown overnight at 30ºC shaking at 270 rpm. Each overnight culture was diluted to an optical density (OD) at 600 nm of 0.8. Sixteen microliters of diluted culture was incubated with 48 ul of Assay Buffer for 60 min at 30ºC in darkness. Reaction was terminated with 136 ul of Stop solution. Optical density of the solution was recorded at 410 nm. Activity of secreted α-GAL was determined from the OD and appropriate dilution factors following calculations in the Yeast Protocol Handbook (Clontech, Mountain View, CA). The two-hybrid interactions were monitored with the positive control, p53/T-antigen, and negative control, LAM, and empty vector interactions, while those for the α-GAL assay also included the positive CL1 control. Both growth and α-Gal assays were repeated 2-4 times each with two independent sets of yeast transformants for the entire set of protein interactions.