

**Table S6.** Plasmids used in this study

| <b>Plasmids used in RNAi experiments</b>                              |               |                                                                                                                                                   |                         |
|-----------------------------------------------------------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| <b>Plasmid</b>                                                        | <b>Strain</b> | <b>Description</b>                                                                                                                                | <b>Reference/Source</b> |
| pDONR/Zeo                                                             | BAS223        | Donor vector for Gateway cloning system; Zeo <sup>R</sup>                                                                                         | Invitrogen              |
| pFANTAi4                                                              | BAS445        | Destination vector with pH2AB divergent promoter driving PaURA5 and an RNAi sentinel system with GFP; PaURA5; hyg <sup>R</sup> ; kan <sup>R</sup> | (1)                     |
| pVN69                                                                 | BAS611        | Integrating RNAi control plasmid, lacks <i>ccdB</i> gene and Gateway recombination sites; PaURA5; hyg <sup>R</sup> ; kan <sup>R</sup>             | (2)                     |
| pSB23                                                                 | BAS538        | Destination vector with pH2AB divergent promoter driving PaURA5 and an RNAi sentinel system with GFP; PaURA5; kan <sup>R</sup>                    | This Study              |
| pCR186                                                                | BAS446        | Episomal RNAi control plasmid, lacks <i>ccdB</i> gene and Gateway recombination sites; PaURA5; kan <sup>R</sup>                                   | Dr. Chad A. Rappleye    |
| pSB7                                                                  | BAS522        | pDONR/Zeo::RYP4-RNAi-E1; Zeo <sup>R</sup>                                                                                                         | This Study              |
| pSB8                                                                  | BAS523        | pDONR/Zeo::RYP4-RNAi-E5; Zeo <sup>R</sup>                                                                                                         | This Study              |
| pSB18                                                                 | BAS533        | pFANTAi4::RYP4-RNAi-E1; PaURA5; hyg <sup>R</sup> ; kan <sup>R</sup>                                                                               | This Study              |
| pSB19                                                                 | BAS534        | pFANTAi4::RYP4-RNAi-E5; PaURA5; hyg <sup>R</sup> ; kan <sup>R</sup>                                                                               | This Study              |
| pSB30                                                                 | BAS545        | pSB23::RYP4-RNAi-E1; PaURA5; kan <sup>R</sup>                                                                                                     | This Study              |
| pSB31                                                                 | BAS546        | pSB23::RYP4-RNAi-E5; PaURA5; kan <sup>R</sup>                                                                                                     | This Study              |
| <b>Plasmids used in Yeast-two-Hybrid experiments</b>                  |               |                                                                                                                                                   |                         |
| <b>Plasmid</b>                                                        | <b>Strain</b> | <b>Description</b>                                                                                                                                | <b>Reference/Source</b> |
| pEG202                                                                | BAS735        | Empty bait plasmid for yeast-two-hybrid assays; <i>HIS3</i> ; Amp <sup>R</sup>                                                                    | (3)                     |
| pJSC401                                                               | BAS736        | Empty prey plasmid for yeast-two-hybrid assays; <i>TRP1</i> ; Amp <sup>R</sup>                                                                    | (4)                     |
| pSB73                                                                 | BAS791        | pEG202::RYP2; <i>HIS3</i> ; Amp <sup>R</sup>                                                                                                      | This Study              |
| pSB74                                                                 | BAS792        | pEG202::RYP3; <i>HIS3</i> ; Amp <sup>R</sup>                                                                                                      | This Study              |
| pSB79                                                                 | BAS797        | pJSC401::RYP2; <i>TRP1</i> ; Amp <sup>R</sup>                                                                                                     | This Study              |
| pSB80                                                                 | BAS798        | pJSC401::RYP3; <i>TRP1</i> ; Amp <sup>R</sup>                                                                                                     | This Study              |
| pSB86                                                                 | BAS826        | pEG202::RYP2 (1-624 bp); <i>HIS3</i> ; Amp <sup>R</sup>                                                                                           | This Study              |
| pSB87                                                                 | BAS827        | pEG202::RYP2 (595-1215bp); <i>HIS3</i> ; Amp <sup>R</sup>                                                                                         | This Study              |
| pJW1                                                                  | BAS1210       | pJSC401::RYP2 (1-624 bp); <i>TRP1</i> ; Amp <sup>R</sup>                                                                                          | This Study              |
| pJW2                                                                  | BAS1211       | pJSC401::RYP2 (595-1215 bp); <i>TRP1</i> ; Amp <sup>R</sup>                                                                                       | This Study              |
| <b>Plasmids used <i>in vivo</i> transcriptional activation assays</b> |               |                                                                                                                                                   |                         |
| <b>Plasmid</b>                                                        | <b>Strain</b> | <b>Description</b>                                                                                                                                | <b>Reference/Source</b> |
| p413TEF                                                               | BAS872        | P <sub>TEF</sub> -(empty vector)-t <sub>CYC1</sub> ; <i>HIS3</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                  | (5)                     |
| p414TEF                                                               | BAS873        | P <sub>TEF</sub> -(empty vector)-t <sub>CYC1</sub> ; <i>TRP1</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                  | (5)                     |
| pSB93                                                                 | BAS925        | p413TEF::RYP1; <i>HIS3</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                                        | This Study              |
| pSB94                                                                 | BAS926        | p414TEF::RYP1; <i>TRP1</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                                        | This Study              |
| pSB95                                                                 | BAS927        | p414TEF::RYP2; <i>TRP1</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                                        | This Study              |
| pSB97                                                                 | BAS929        | p413TEF::RYP3; <i>HIS3</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                                        | This Study              |
| pSB98                                                                 | BAS930        | p414TEF::RYP3; <i>TRP1</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                                        | This Study              |
| pSB99                                                                 | BAS931        | p414TEF::RYP4; <i>TRP1</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                                        | This Study              |
| pSB115                                                                | BAS950        | p413TEF::RYP2-RYP3; <i>HIS3</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                                   | This Study              |
| pSB135                                                                | BAS1209       | p414TEF::RYP2 (1-624 bp); <i>TRP1</i> ; <i>CEN</i> ; Amp <sup>R</sup>                                                                             | This Study              |
| P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i>                                  | BAS878        | Empty <i>lacZ</i> reporter plasmid (no motif); <i>URA3</i> ; 2μ; Amp <sup>R</sup>                                                                 | (6)                     |
| p228                                                                  | BAS933        | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif A/Wor1 motif cloned in forward orientation (AAAAATTAAGTTTTTTTAT); <i>URA3</i> ; 2μ; Amp <sup>R</sup> | (7)                     |

|        |        |                                                                                                                                                              |            |
|--------|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| p230   | BAS934 | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif A/Wor1 knock-out motif cloned in forward orientation (AAAAATACAAGACTTTTAT); <i>URA3</i> ; 2μ; Amp <sup>R</sup>  | (7)        |
| pSB101 | BAS935 | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif A/Wor1 motif cloned in reverse orientation (ATAAAAAAAGCTTTAATTTTT); <i>URA3</i> ; 2μ; Amp <sup>R</sup>          | This Study |
| pSB102 | BAS936 | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif A/Wor1 knock-out motif cloned in reverse orientation (ATAAAAAGTCTTGTATTTTT); <i>URA3</i> ; 2μ; Amp <sup>R</sup> | This Study |
| pSB107 | BAS941 | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif B cloned in forward orientation (ACTAGGTTCCATGGTTC); <i>URA3</i> ; 2μ; Amp <sup>R</sup>                         | This Study |
| pSB108 | BAS942 | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif B cloned in reverse orientation (GAACCATGGAACCTAGT); <i>URA3</i> ; 2μ; Amp <sup>R</sup>                         | This Study |
| pSB109 | BAS943 | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif B knock-out cloned in forward orientation (ACTAGGTTTTCTTTTCC); <i>URA3</i> ; 2μ; Amp <sup>R</sup>               | This Study |
| pSB110 | BAS944 | P <sub>CYC1</sub> -ΔUAS- <i>lacZ</i> ::Motif B knock-out cloned in reverse orientation (GGAAAAGAAAACCTAGT); <i>URA3</i> ; 2μ; Amp <sup>R</sup>               | This Study |

#### Plasmids used to express and purify Ryp proteins for EMSAs

| Plasmid | Strain  | Description                                                  | Reference/Source |
|---------|---------|--------------------------------------------------------------|------------------|
| pSB122  | BAS1164 | pCR2.1-TOPO:: <i>RYP1</i> -6XHis; Amp <sup>R</sup>           | This Study       |
| pSB124  | BAS1166 | pCR2.1-TOPO:: <i>RYP1</i> (1-801 bp)-6XHis; Amp <sup>R</sup> | This Study       |
| pSB128  | BAS1170 | pCR2.1-TOPO:: <i>RYP2</i> -6XHis; Amp <sup>R</sup>           | This Study       |
| pSB130  | BAS1172 | pCR2.1-TOPO:: <i>RYP3</i> -6XHis; Amp <sup>R</sup>           | This Study       |

#### References:

1. Krajaeun T, Gauthier GM, Rappleye CA, Sullivan TD, Klein BS (2007) Development and application of a green fluorescent protein sentinel system for identification of RNA interference in *Blastomyces dermatitidis* illuminates the role of septin in morphogenesis and sporulation. *Eukaryotic Cell* 6(8):1299-1309.
2. Nguyen VQ, Sil A (2008) Temperature-induced switch to the pathogenic yeast form of *Histoplasma capsulatum* requires Ryp1, a conserved transcriptional regulator. *Proc Natl Acad Sci USA* 105(12): 4880-4885.
3. Golemis EA, Serebriiskii I, Finley RL, Kolonin MG, Gyuris J, Brent R (2001) Interaction Trap/Two-Hybrid System to Identify Interacting Proteins. *Curr Protoc Mol Biol*. 46:20.1.1–20.1.40
4. Stanley SA, Raghavan S, Hwang WW, Cox JS (2003) Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system. *Proc Natl Acad Sci USA* 100(22):13001-13016.
5. Mumberg D, Müller R, Funk M (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* 156(1):119-122.
6. Rupp S, Summers E, Lo HJ, Madhani H, Fink G. (1999) MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene. *EMBO J* 18(5):1257-1269.
7. Lohse MB, Zordan RE, Cain CW, Johnson AD (2010) Distinct class of DNA-binding domains is exemplified by a master regulator of phenotypic switching in *Candida albicans*. *Proc Natl Acad Sci USA* 107(32): 14105-14110.