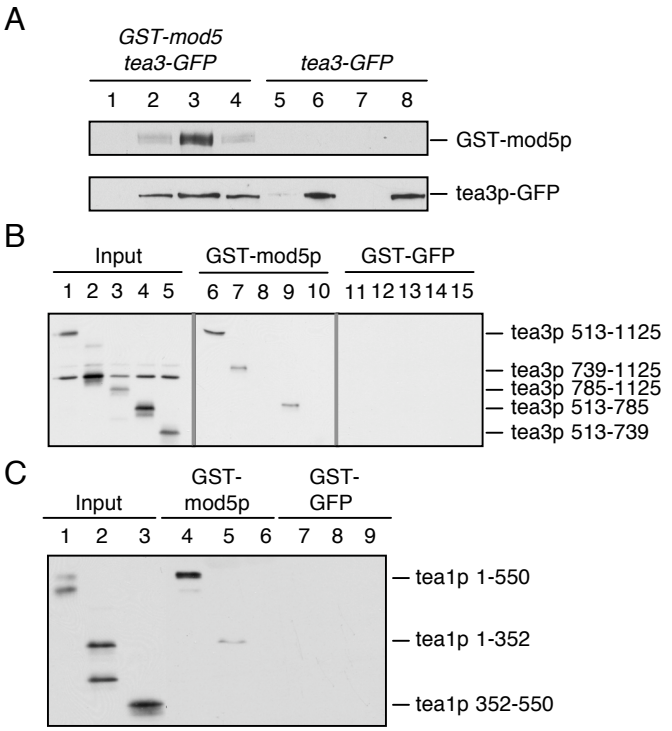
Snaith, Samejima and Sawin, 2005: Supplementary Figure 1



Supplementary Figure 1: Interactions between tea1p, tea3p and mod5p
(A) To confirm the two-hybrid interaction between mod5p and tea3p we carried out GST-pulldown assays from fission yeast extracts. Extracts of insoluble (lanes 1, 5) and soluble (lanes 2, 6) material were prepared from cells expressing GST-mod5p and tea3p-GFP (lanes 1-4) or from cells expressing tea3p-GFP only (lanes 5-8). GST-mod5p was pulled down from soluble extracts using GSH-agarose beads (lanes 3, 7), and unbound supernatant fractions were also analyzed (lanes 4, 8). Each sample was probed for GST-mod5p and tea3p-GFP. **(B)** To further define the region of tea3p required for binding to mod5p, we performed GST-pulldown assays using *in vitro*-translated fragments of tea3p and bacterially expressed GST-mod5p. Fragments of tea3p containing at least amino acids 739 to 785 bound successfully to GST-mod5p, whereas fragments lacking these amino acids were unable to bind. Amino acids 513-1125 (lanes 1, 6 and 11), 739-1125 (lanes 2, 7, and 12), 785-1125 (lanes 3, 8 and 13), 513-785 (lanes 4, 9 and 14), and 513-739 (lanes 5, 10 and 15) of tea3p were generated by *in vitro* translation. Input is shown in lanes 1-5. Each tea3p-HA fragment was incubated with either GST-mod5p (lanes 6-10) or GST-GFP (lanes 11-15) and the resulting precipitates analyzed by western blotting. GST-pulldown fractions were loaded 4 x relative to input. **(C)** To analyze the region of tea1p required for binding to mod5p, we performed GST-pulldown assays using *in vitro*-translated fragments of tea1p and bacterially expressed GST-mod5p. Fragments of tea1p containing at least amino acids 1-352 bound successfully to GST-mod5p. Amino acids 1-550 (lanes 1, 4 and 7), 1-352 (lanes 2, 5, and 8) and 352-550 (lanes 3, 6 and 9) of tea1p were generated by *in vitro* translation. Input is shown in lanes 1-3. Each tea1p-HA fragment was incubated with either GST-mod5p (lanes 4-6) or GST-GFP (lanes 7-9) and the resulting precipitates analyzed by western blotting. GST-pulldown fractions are loaded 4 x relative to input.

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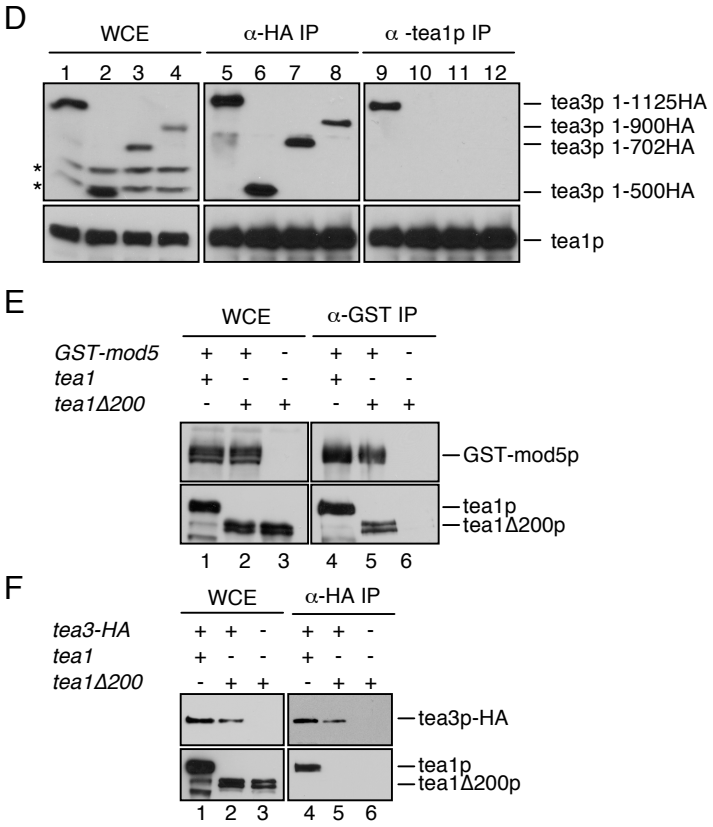

Supplementary Figure 1: Interactions between tea1p, tea3p and mod5p cont.
(D) To analyze the region of tea3p required for binding to tea1p we constructed a series of HA-tagged C-terminal truncations of tea3p in fission yeast. Immunoprecipitations were performed with either anti-HA (lanes 5-8) or tea1p (lanes 9-12) antibodies from whole cell extracts (WCE, lanes 1-4) expressing tea3p1-1125HA (lanes 1, 5, 9), tea3p1-500HA (lanes 2, 6, 10), tea3p1-702HA (lanes 3, 7, 11) or tea3p1-900HA (lanes 4, 8, 12), and the resulting precipitates analyzed by western blotting. Asterisks show position of background bands. **(E)** To determine whether GST-mod5p could bind tea1Δ200p, GST-mod5p was immunoprecipitated from soluble protein extracts expressing GST-mod5p with either wild-type tea1p (lanes 1, 4) or tea1Δ200p (lanes 2, 5), or cells expressing tea1Δ200p only (lanes 3, 6), and the resulting immunocomplexes analyzed for GST-mod5p and tea1p. Whole cell extract (WCE) fractions are shown in lanes 1-3 and immunoprecipitates in lanes 4-6. Immunoprecipitates were loaded 30 x relative to WCE fractions. **(F)** To test whether tea3p-HA could bind tea1Δ200p, tea3p-HA was immunoprecipitated from soluble protein extracts expressing tea3p-HA with either wild-type tea1p (lanes 1, 4) or tea1Δ200p (lanes 2, 5), or from cells expressing tea1Δ200p only (lanes 3, 6), and the resulting immunocomplexes analyzed for tea3p-HA and tea1p. WCE fractions are shown in lanes 1-3 and immunoprecipitates in lanes 4-6. Immunoprecipitates were loaded 20 x relative to WCE fractions.