



**Figure S5.** Presence of a C-terminal mCherry tag or G domain mutation G29D reduces Cdc11 function providing additional sensitized genetic backgrounds to assess Shs1 functionality. (A) GFY-160, GFY-161 and GFY-162 were grown overnight in SD-Ura at 30°C and spotted onto medium without or with 5-FOA (to remove the covering *CDC11*-expressing plasmid). Each Cdc11 construct contained a C-terminal mCherry tag and each Shs1 construct contained a C-terminal eGFP tag. (B) Strains (GFY-532, GFY-533 and GFY-534) otherwise identical to those in (A) were constructed in which the mCherry tag was replaced with the *ADH1(term)::Hyg<sup>R</sup>* cassette inserted immediately after the *CDC11* ORF (indicated by an asterisk) and first propagated on 5-FOA medium (to select against the covering *CDC11*-expressing plasmid), then grown overnight in rich medium at 25°C, and finally spotted on plates and incubated at either 25°C or 37°C, as indicated. (C) Derivatives (GFY-724, GFY-725 and GFY-726) lacking the *SHS1* gene were generated from the same strains in (B) and then were tested as in (A). (D) Strains (GFY-681, GFY-694 and GFY-675) in which the *CDC11* alleles shown in (B) also harbored a G29D mutation (*cdc11-7*) within the GTP-binding domain (NAGARAJ *et al.* 2008; WEEMS *et al.* 2014), and in which the *SHS1* locus lacked any fluorescent tag, were grown and spotted as in (A).