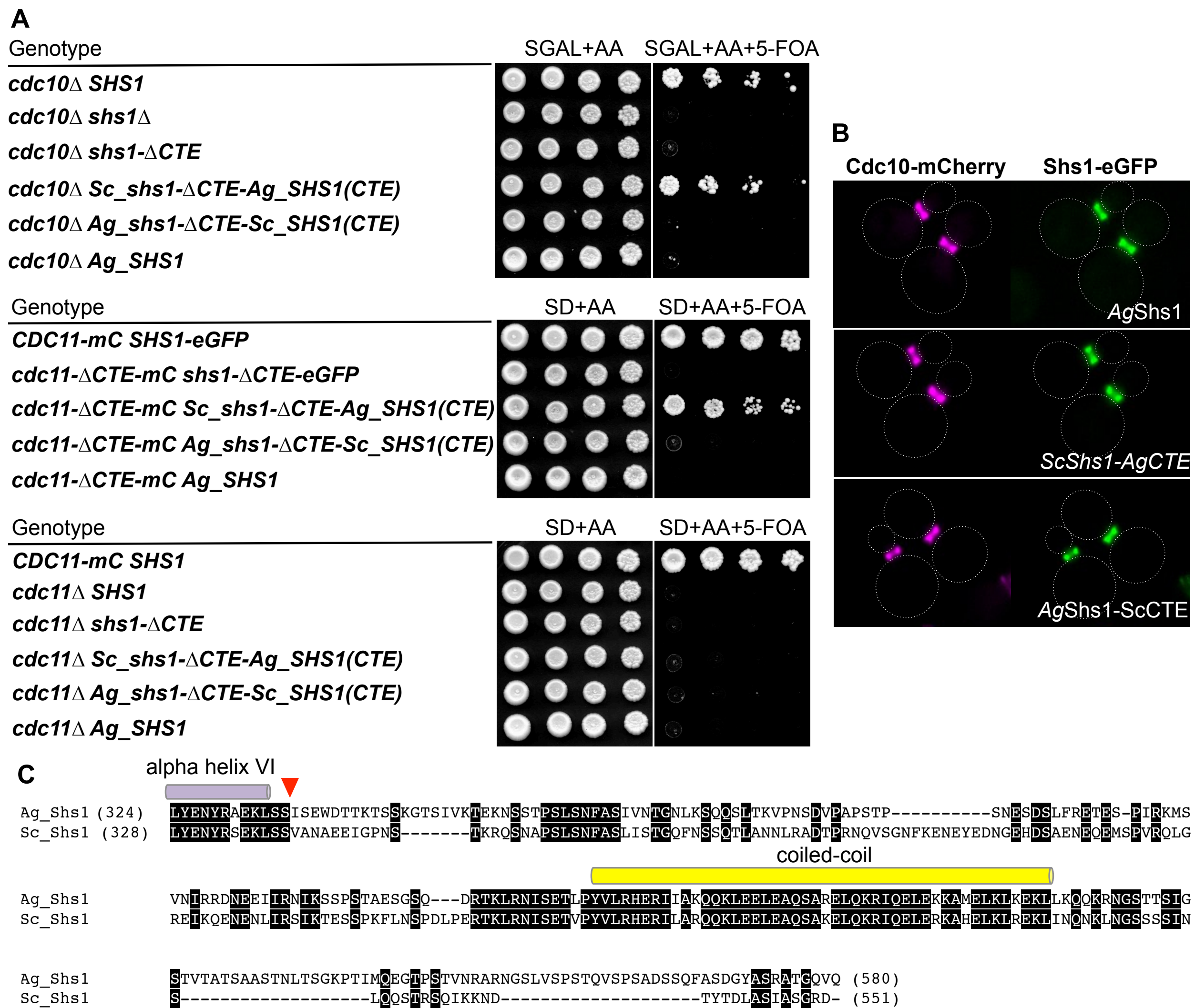

Figure S6. *A. gossypii* Shs1 localizes to the bud-neck, but does not functionally replace *S. cerevisiae* Shs1. (A) Functionality of intact Ag Shs1 and two chimeric protein derivatives (one in which the CTE of Ag Shs1 was replaced with the CTE of Sc Shs1, and the other in which the CTE of Sc Shs1 was replaced with the CTE of Ag Shs1), each tagged at its C-terminus with eGFP and expressed from the endogenous Sc *SHS1* promoter, were tested in two Shs1-dependent genetic backgrounds described in this study, *cdc10Δ* (*upper panel*) and *cdc11-CTEΔ-mCherry* (*middle panel*). For the former, overnight cultures of GFY-87, GFY-137, GFY-94, GFY-644, GFY-655 and GFY-643 were grown in YPGal at 25ºC before spotting onto Gal medium without and with 5-FOA (to select against the covering *URA3*-marked *CDC10*-expressing plasmid). For the latter, GFY-160, GFY-162, GFY-615, GFY-571 and GFY-614 were grown overnight in SD-Ura at 30ºC before spotting onto medium without and with 5-FOA (to select against the covering *URA3*-marked *CDC11*-expressing plasmid). *Lower panel*, expression of *SHS1* in cells lacking Cdc11 is lethal (because Shs1 caps the ends of Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12 hexamers) (MCMURRAY *et al.* 2011). Likewise, each of the Ag Shs1 constructs was unable to support the growth of *cdc11Δ* cells, indicating that all three were able to form a G-interface with Cdc12. For this test, GFY-160, GFY-147, GFY-149, GFY-639, GFY-637 and GFY-683 were grown and spotted as for the middle panel. (B) GFY-643, GFY-644 and GFY-655 were transformed with a vector expressing *CDC10-mCherry* (pGF-preIVL6), selected twice on medium containing 5-FOA to remove the covering *URA3*-marked plasmid expressing WT *CDC10*-expressing vector, grown to mid-exponentially phase in SD-Leu medium, and viewed by fluorescence microscopy. Cell perimeter, white dotted lines. (C) Alignment of the CTEs of Ag Shs1 and Sc Shs1, as in Fig. S4A, with the position chosen as the breakpoint for the CTE domain swaps indicated by the red triangle.