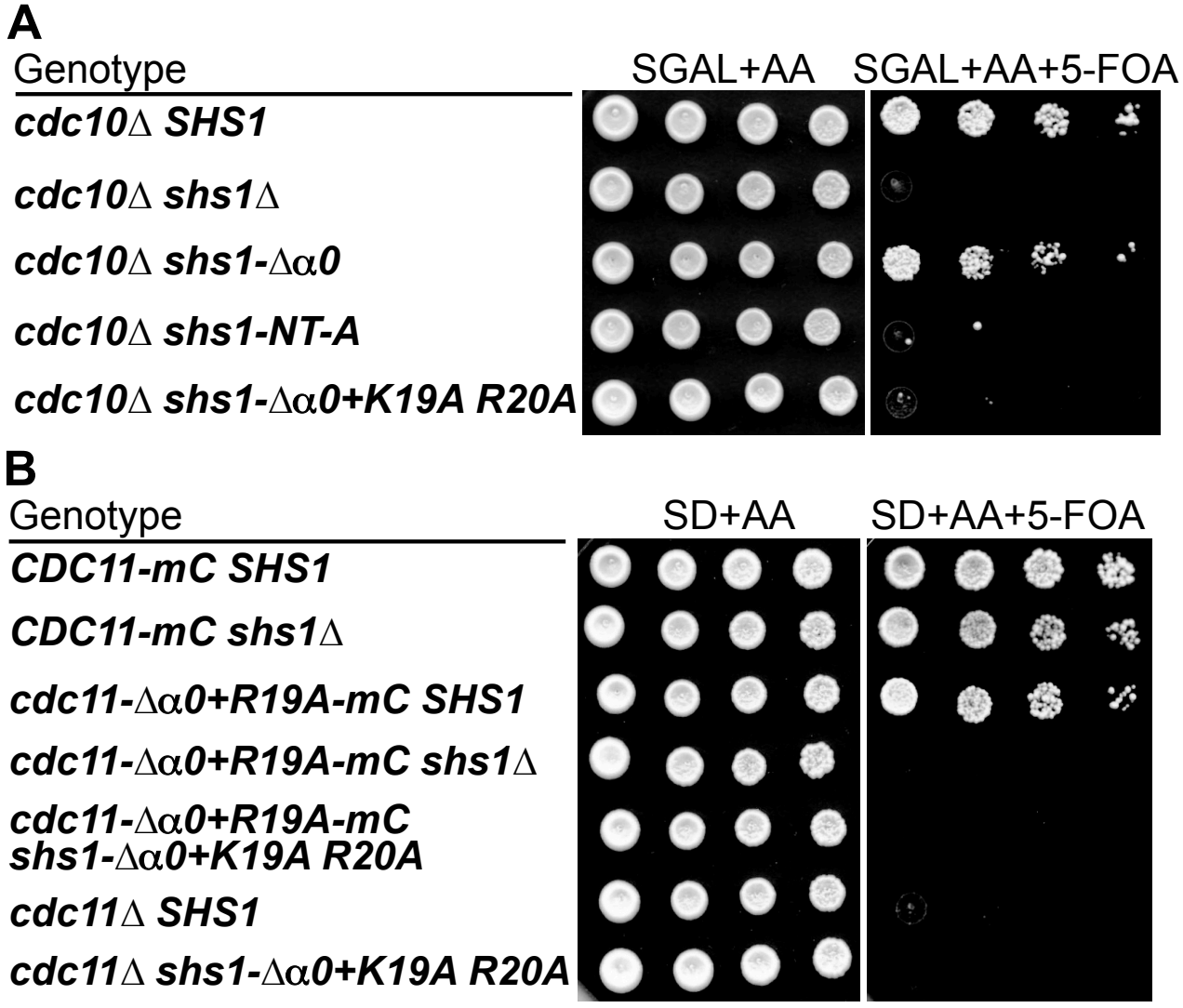

Figure S3. Basic residues are a critical functional element of the N-terminal α0 helix in both Shs1 and Cdc11. (A) Functionality of four endogenously-expressed *SHS1* mutants (each tagged with C-terminal eGFP)— a null allele (*shs1*Δ), a deletion of α0 [*shs1(Δ2-18)*], Ala substitution of all basic residues in α0 [*shs1(R13A R14A K15A K16A K19A R20A)* ("*shs1-NT-A*")], and the α0 deletion combined with Ala substitution of the remaining adjacent basis residues [*shs1(Δ2-18; K19A R20A)*] —were tested in one of the Shs1-dependent genetic backgrounds described in this study by growing the corresponding strains (GFY-87, GFY-137, GFY-93, GFY-249 and GFY-1021) overnight in YPGal at 25ºC and spotting onto Gal medium in the absence of presence of 5-FOA (to select against the covering *CDC10*-expressing plasmid). (B) As indicated, combinations of an α0 deletion (residues 2-18Δ for both Cdc11 and Shs1) without and with Ala substitution mutation of the remaining adjacent basic residue(s), R19 in Cdc11 and K19 and R20 in Shs1 (see Fig. S2A), were tested for function by growing the corresponding strains (GFY-58, GFY-164, GFY-1024, GFY-1062, GFY-1061, GFY-147 and GFY-1022) overnight in SD-Ura medium at 30ºC and then spotting onto medium in the absence or presence of 5-FOA (to select against the covering *CDC11*-expressing plasmid). The *SHS1* derivatives in these strains contained a C-terminal eGFP tag.