

Supplementary Materials:

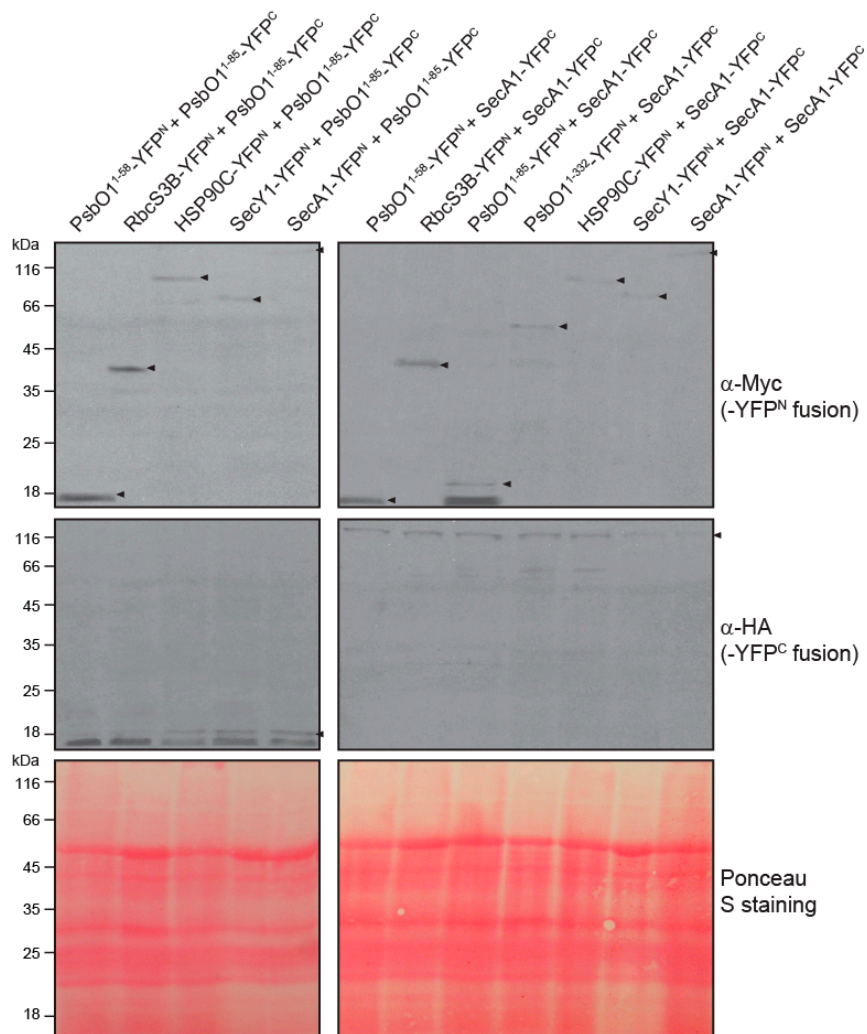


Figure S1: Immunoblot of transiently expressed soluble half-YFP fusion proteins in tobacco leaf. Protein bands of interest are marked by the arrows, and anti-Myc and anti-HA were used to detect YFP N- and C-terminal half-fusion proteins, respectively.

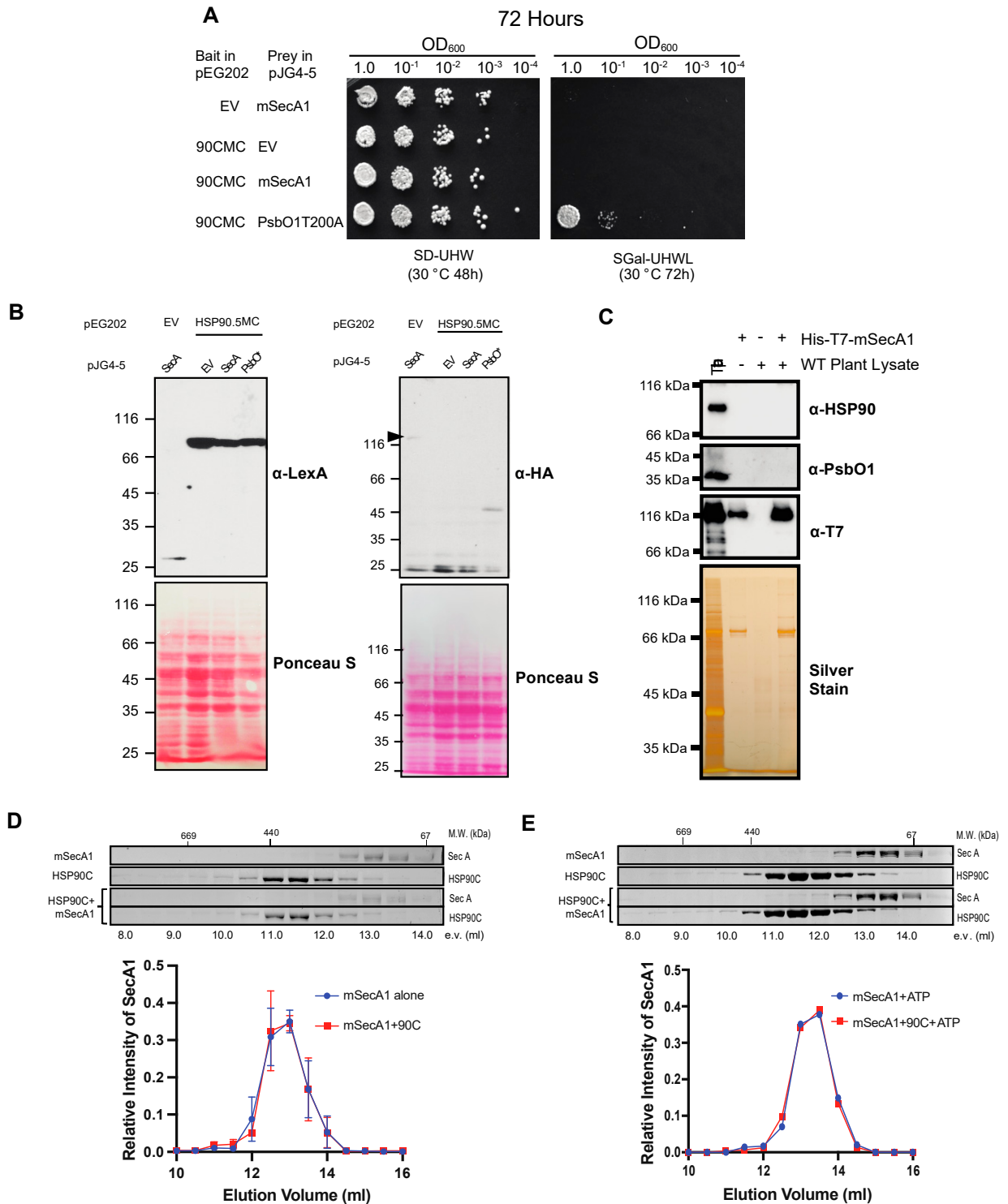


Figure S2: Lack of significant direct interaction between HSP90C and SecA1 explored through in vitro and heterologous expression assays. **(A)** Yeast-two-hybrid assay between SecA1 and the HSP90C middle and C-terminal domain. **(B)** Immunoblot confirmation of the presence of bait and prey proteins from the yeast two-hybrid assay using anti-LexA and anti-HA antibodies. **(C)** Immunoblot analysis of the pull-down assay using immobilized mSecA1 protein and wild-type plant lysate prey. **(D)** Assessment of the elution profiles obtained from size exclusion chromatography analysis using 100 µg of purified mSecA1 and HSP90C. **(E)** Assessment of the elution profiles obtained from size exclusion chromatography analysis using 100 µg of purified mSecA1 and HSP90C when pre-treated with 5 mM ATP.

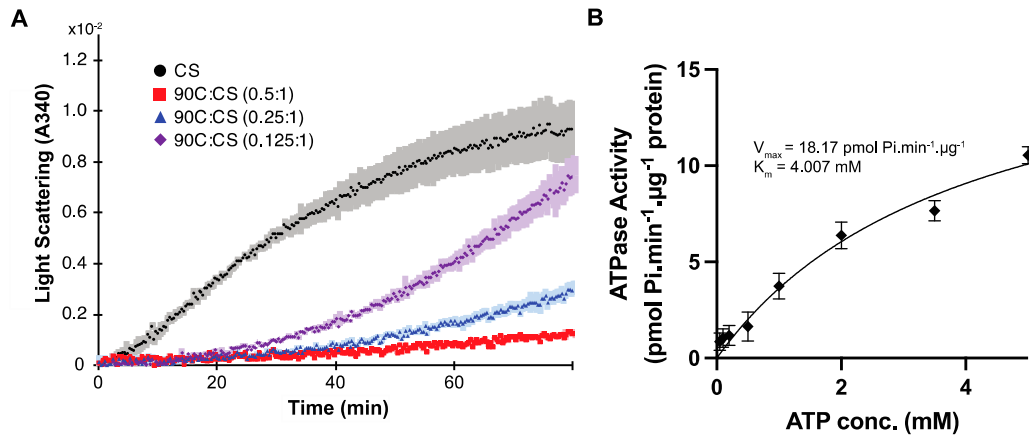


Figure S3: Verification of HSP90C's chaperone activity. (A) Citrate synthase (CS, 1 μM) was incubated in the presence or absence of different amounts of HSP90C (90C). The molar ratios of CS:HSP90C are indicated in parentheses. The shaded areas represent the SEM from three trials for each molar ratio of the chaperone used. (B) Kinetics assay of HSP90C in hydrolyzing ATP. The specific activity was measured as Radicolol-sensitive activity and error bars represent SD from six experimental repeats for each ATP concentration.