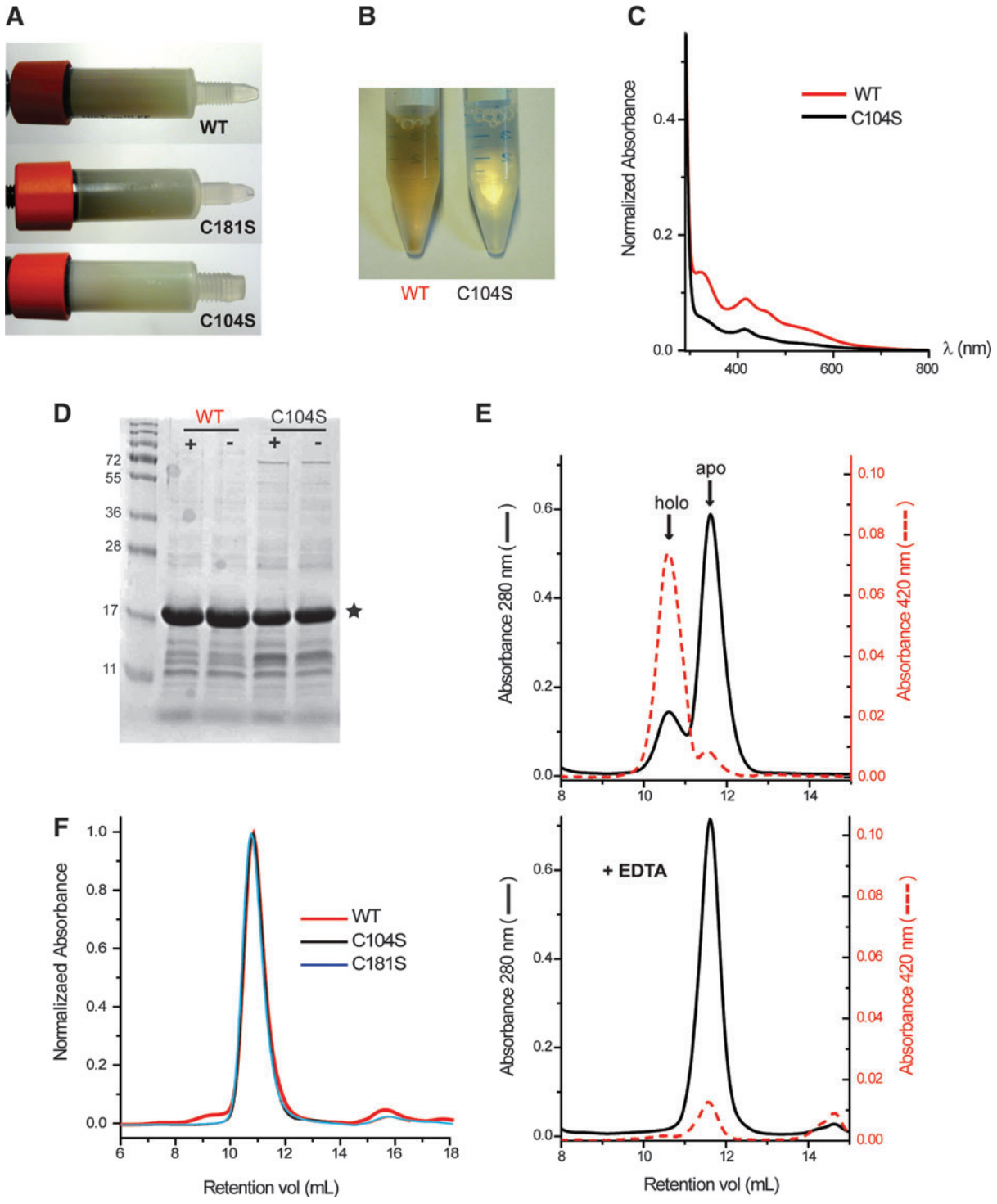

SUPPLEMENTARY FIG. S2. ISC binding of different His-tagged *Tb*1-C-Grx1 species. (A) Ni^{2+}-affinity chromatography columns loaded with *E. coli* lysates containing recombinant WT, C181S, and C104S forms of *Tb*1-C-Grx1. **(B)** Samples of WT and C104S 1-C-Grx1 immediately after elution from Ni^{2+}-affinity chromatography columns. **(C)** At equal protein concentrations, the UV–visible spectrum of the C104S mutant (*black line*) lacks the characteristic chromophore peaks at 320 and 420 nm present in the WT protein (*red line*). **(D)** Freshly prepared WT and C104S 1-C-Grx1 were subjected to SDS-PAGE under reducing (+) and nonreducing (−) conditions (20 μg protein/lane). Both proteins displayed ≥90% purity and run at a mass expected for the His-tagged monomeric protein (17.630 kDa, star). Molecular weight standard bands (kDa) are shown on the left lane. **(E)** Freshly purified WT 1-C-Grx1 **(**see **D)**, was subjected to SEC with online UV–visible detection at 280 nm (*black solid line*) and 420 nm (*red dashed line*) as described in the Materials and Methods section. A small fraction eluted before the main protein peak and presented absorption at 420 nm (*upper chromatogram*). This species disappeared when the protein sample was treated for 30 min with 5 m*M* EDTA before the chromatography (*lower chromatogram*). **(F)** SEC analysis of 500 μg His-tagged WT (*red line*), C181S (*blue line*), and C104S (*black line*) 1-C-Grx1 treated with 5 m*M* DTT for 10 min before injection into the Superdex 75 10/300 GL column equilibrated with 50 m*M* sodium phosphate and 300 m*M* NaCl, pH 7.8. All the proteins presented identical retention volumes. SECs shown in panel E and F were performed in two different Superdex 75 10/300 GL columns calibrated independently. DTT, dithiotreithol; SEC, size-exclusion chromatography; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; WT, wild type.