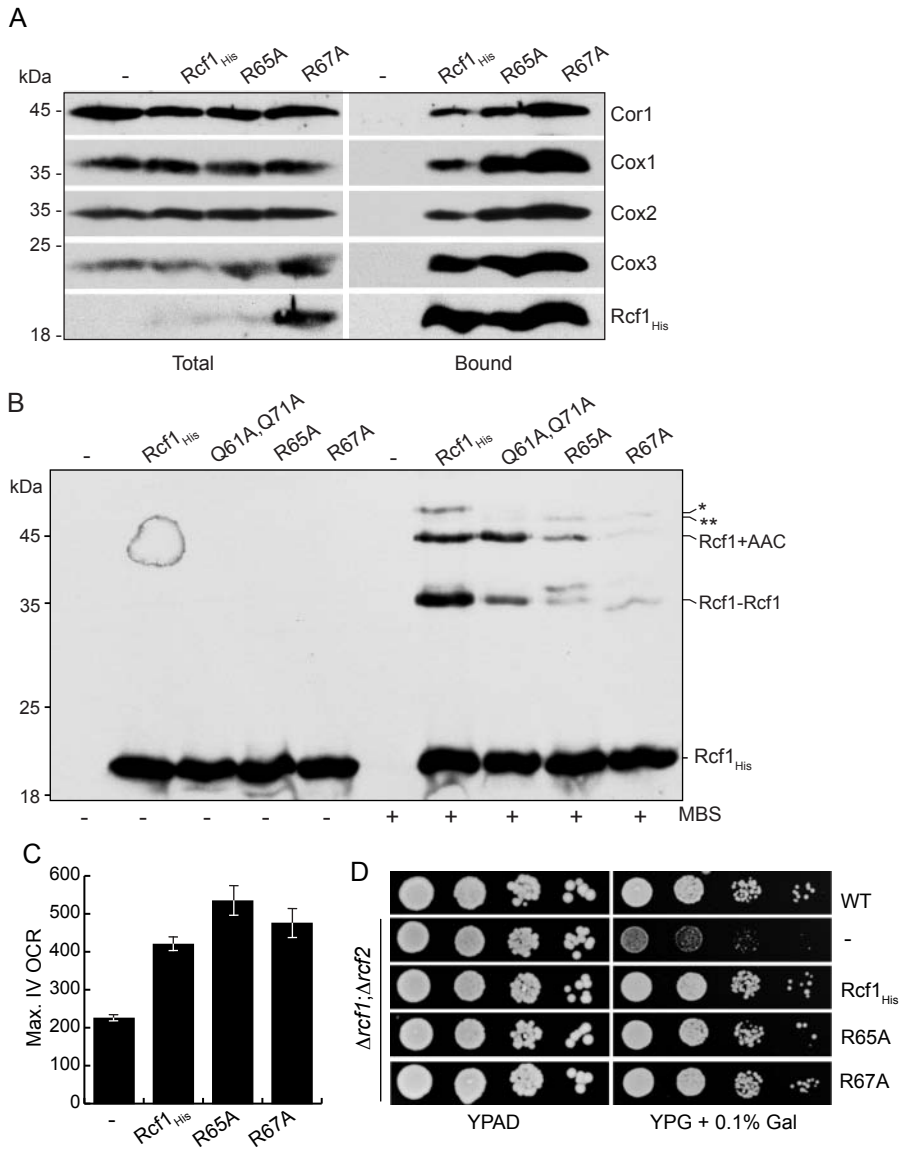

FIGURE S1. Mutation of R65 residue alters Rcf1's association with complex IV and AAC, but does not perturb Rcf1's ability to support assembly of an active complex IV. A, affinity purification of $Rcf1_{His}$, $rcf1_{His}^{R65A}$ and $rcf1_{His}^{R67A}$ derivatives following solubilization with 1% digitonin was performed, followed by SDS-PAGE, Western blotting and immunedecoration, as indicated. Total, 5% of solubilized material; Bound, 100% of affinity purified material on the Ni-NTA beads. B, chemical cross-linking using MBS was performed on mitochondria isolated from Δ*rcf1*;Δ*rcf2* strain harboring either the wild type $Rcf1_{His}$, $rcf1_{His}^{Q61A,Q71A}$, $rcf1_{His}^{R65A}$ or $rcf1_{His}^{R67A}$ mutant derivatives, as indicated. Following SDS-PAGE, and Western blotting, decoration with His-tag epitope antiserum was performed. The positions of the dominant 45 kDa (Rcf1-AAC) and 36 kDa (Rcf1-Rcf1) Rcf1-containing adducts are indicated. The position of a less abundant Rcf1-50 kDa (Rcf1-Cox2) adduct detected in the $rcf1_{His}^{R65A}$ and $rcf1_{His}^{R67A}$ mitochondria is indicated by **. Note a slightly larger (52 kDa), as yet uncharacterized Rcf1 adduct is also observed in the $Rcf1_{His}$ mitochondria is indicated by *. D, maximal oxygen consumption rate (OCR) of bioenergetically isolated complex IV was measured in mitochondria isolated from the Δ*rcf1*;Δ*rcf2* strain and the Δ*rcf1*;Δ*rcf2* strain harboring the $Rcf1_{His}$, $rcf1_{His}^{R65A}$ or $rcf1_{His}^{R67A}$ derivatives, following addition of ascorbate/TMPD and CCCP. E, serial 10-fold dilutions of wild type (WT), and Δ*rcf1*;Δ*rcf2* expressing $Rcf1_{His}$, $rcf1_{His}^{R65A}$, $rcf1_{His}^{R67A}$ derivatives or not, as indicated, were spotted on YP plates containing glucose (YPAD) or glycerol supplemented with 0.1 % galactose (YPG +0.1% Gal) and grown at 30 oC.