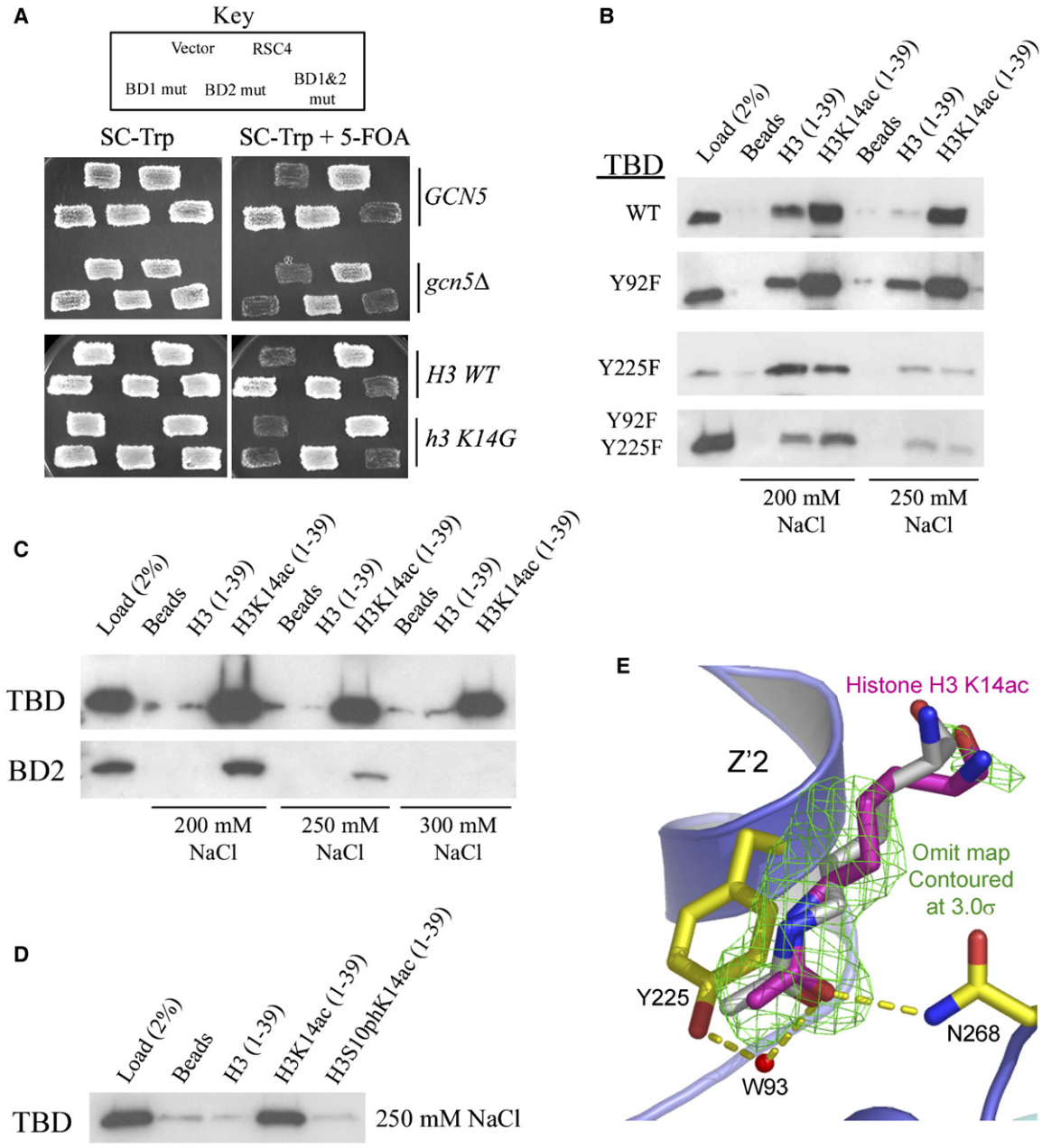

Figure 2. Rsc4 BD2 Interacts Genetically and Biochemically with Histone H3K14ac

(A) Complementation of *rsc4* BD mutants in WT (YBC627 and YBC2499), *gcn5Δ* (YBC2352), and histone *h3*K14G (YBC2501) strains. Patches were incubated for 2 days at 30°C. The *RSC4* construct location in the groups of five is provided by the key at top: vector, pRS314; RSC4, pRS314.*RSC4* (p1060); BD1 mutant, pRS314.*rsc4* Y92A Y93A (p1462); BD2 mutant, pRS314.*rsc4* Y225A Y226A (p1463); BD1&BD2 mutant, pRS314.*rsc4* Y92A Y93A Y225A Y226A (p1471). Strains were grown on selective media with or without 5-FOA, as 5-FOA enforces the loss of the pRS316.*RSC4* plasmid (right panels).

(B) Rsc4 BD2 is required for acetyl-specific binding to the H3K14 peptide. The binding of purified Rsc4(46–334) derivatives to biotinylated histone H3 tail peptides conjugated to streptavidin beads was examined by western blot. The H3 peptides extend from amino acid 1 to 39 of the H3 sequence and are either unmodified or acetylated at K14 as indicated. Binding avidity was further examined by increasing salt stringency in the wash buffers, as indicated. Western blots were probed with anti-Rsc4 polyclonal antibodies.

(C) Rsc4 BD2 is sufficient to bind to the H3K14ac peptide. Western blot analysis of purified Rsc4(36–321) TBD and Rsc4(157–321) BD2, as in (B).

(D) Binding by Rsc4 TBD and BD2 to H3K14ac is inhibited by H3S10ph. Western blot analysis of purified Rsc4 TBD bound to the H3 tail peptides, as in (B).

(E) Acetyl-lysine bound within the BD2 pocket of Rsc4(36–340) following soaking with H3(6–18) K14ac peptide. Difference density ($F_o - F_c$), contoured at 3.0 × rmsd, was phased on protein model refined prior to inclusion of the ligand. The closely overlapping acetyl lysine side chains from a Gcn5 bromodomain:peptide complex structure (Owen et al., 2000) are shown colored white following superposition of the protein structures.