## SUPPLEMENTAL FIGURES

Supplemental Figure 1

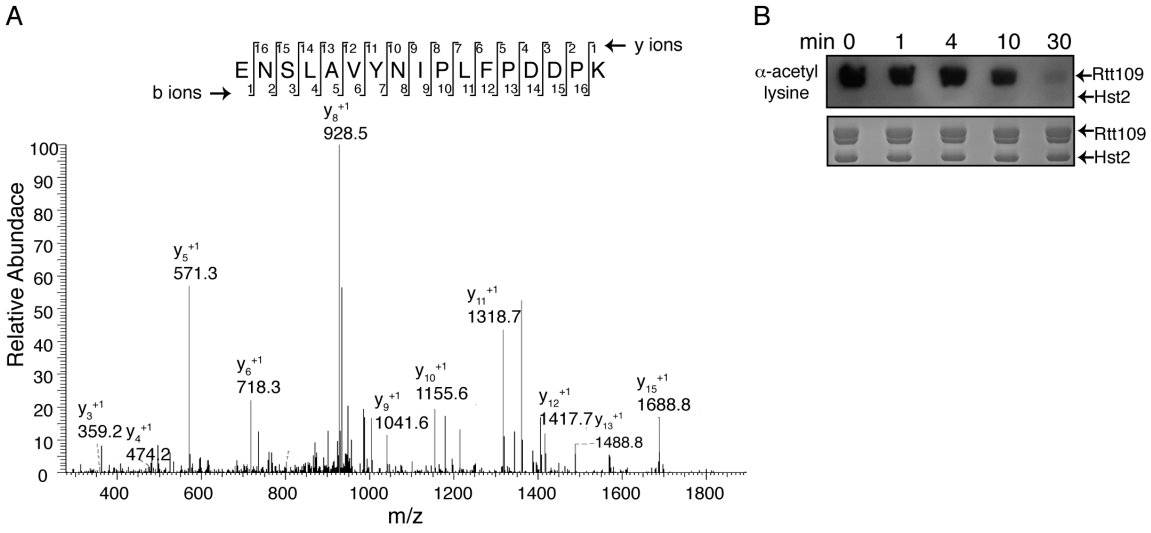


Fig. S1. **Deacetylation of Rtt109 by Hst2.** *(A)* A representative MS/MS chromatogram depicts deacetylated K290, with singly charged y-series ions labeled (inset: theoretical b and y fragment ions). *(B)* Acetyl-lysine immunoblots (top) and SDS-PAGE coomassie stained gels (bottom) of the Hst2 (5 μM) catalyzed deacetylation of Rtt109 (10 μM) reaction at various time points.

Supplemental Figure 2

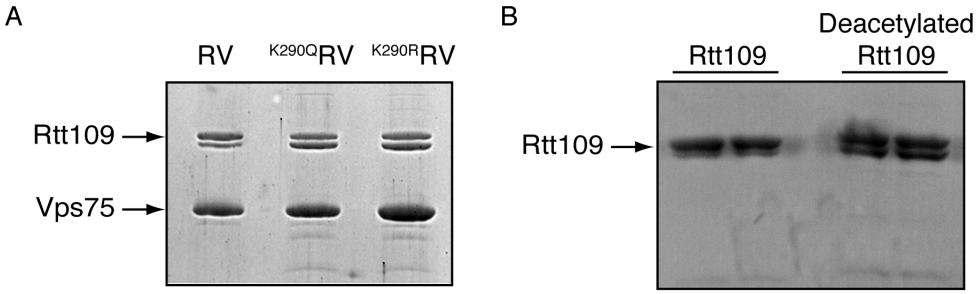


Fig. S2. **SDS-PAGE gel of proteins used in thermal denaturation assay**s. *(A)* Gel of various Rtt109-Vps75 complexes. *(B)* Gel of Rtt109 and deacetylated Rtt109.

Supplemental Figure 3

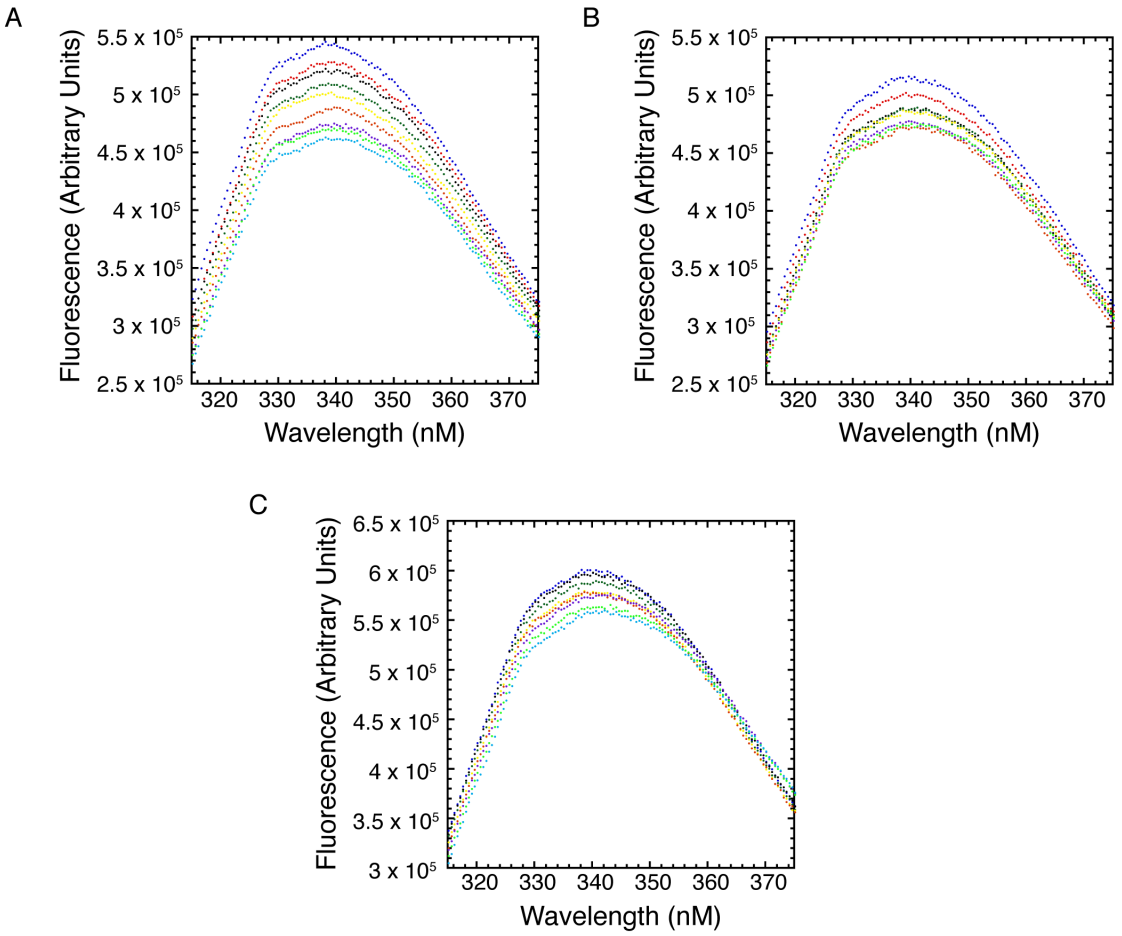


Fig. S3. **Intrinsic protein fluorescence measurements with RV mutants and acetyl-CoA.** Representative emission spectra of the fluorescence reduction due to acetyl-CoA binding to RV and K290 mutants.*(A)* **RV:** 0 µM (blue), 0.44 µM (red), 0.89 µM (black), 1.78 µM (green), 3.56 µM (yellow), 7.11 µM (orange), 14.22 µM (purple), 28.44 µM (light green) and 56.90 µM (light blue) of acetyl-CoA was titrated into RV (0.5 µM). *(B)* **$^{K290Q}$RV**: 0 µM (blue), 14.22 µM (red), 28.44 µM (black), 42.67 µM (green), 56.89 µM (yellow), 85.33 µM (orange), 113.78 µM (purple) and 170.67 µM (light green) of acetyl-CoA was titrated into $^{K290Q}$RV (0.5 µM). *(C)* **$^{K290R}$RV:** 0 µM (blue), 28.44 µM (black), 42.67 µM (green), 56.89 µM (yellow), 85.33 µM (orange), 113.78 µM (purple), 170.67 µM (light green) and 227.56 µM (light blue) of acetyl-CoA was titrated into $^{K290R}$RV (0.5 µM).