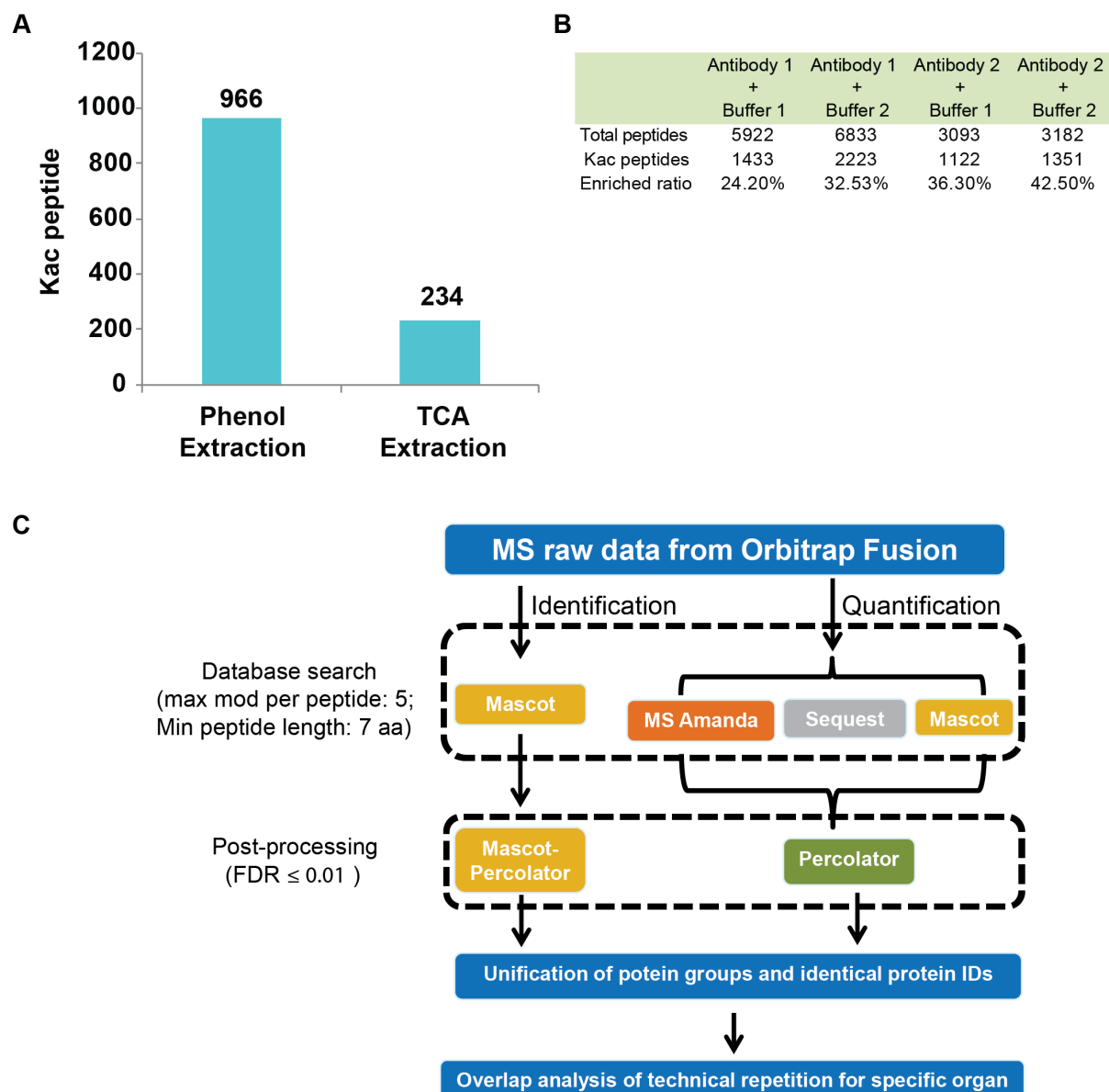
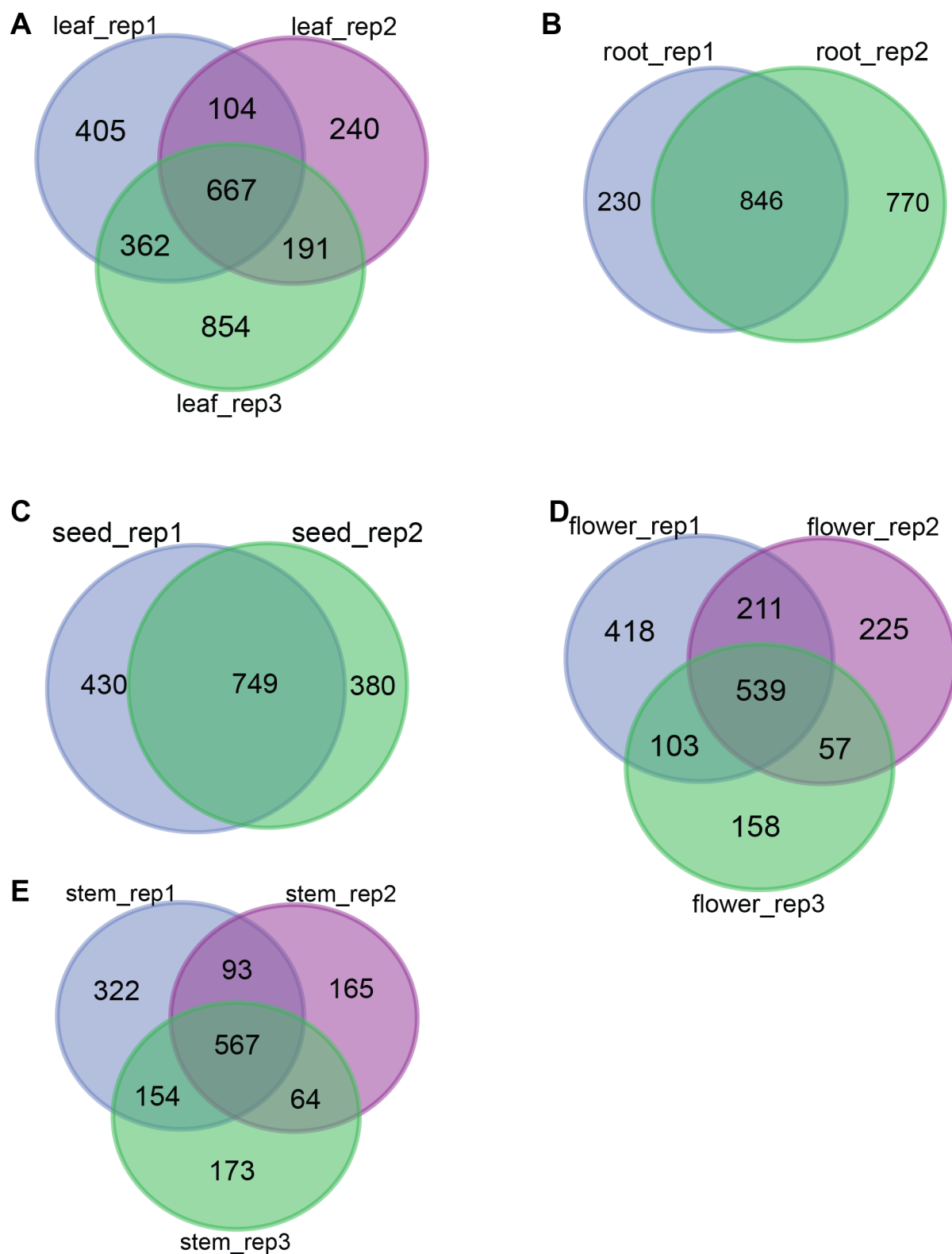
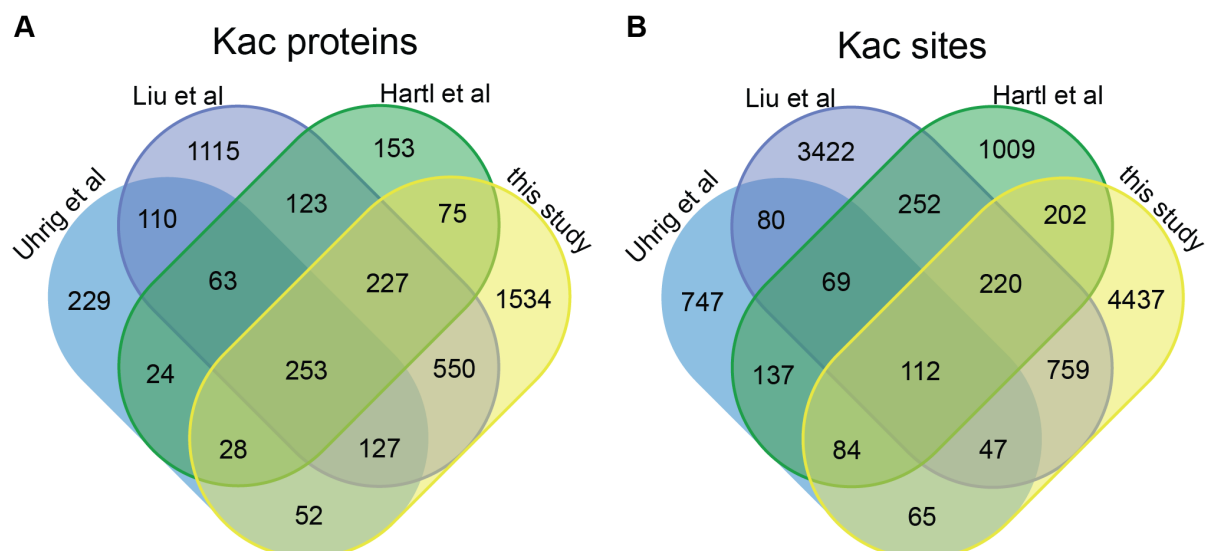




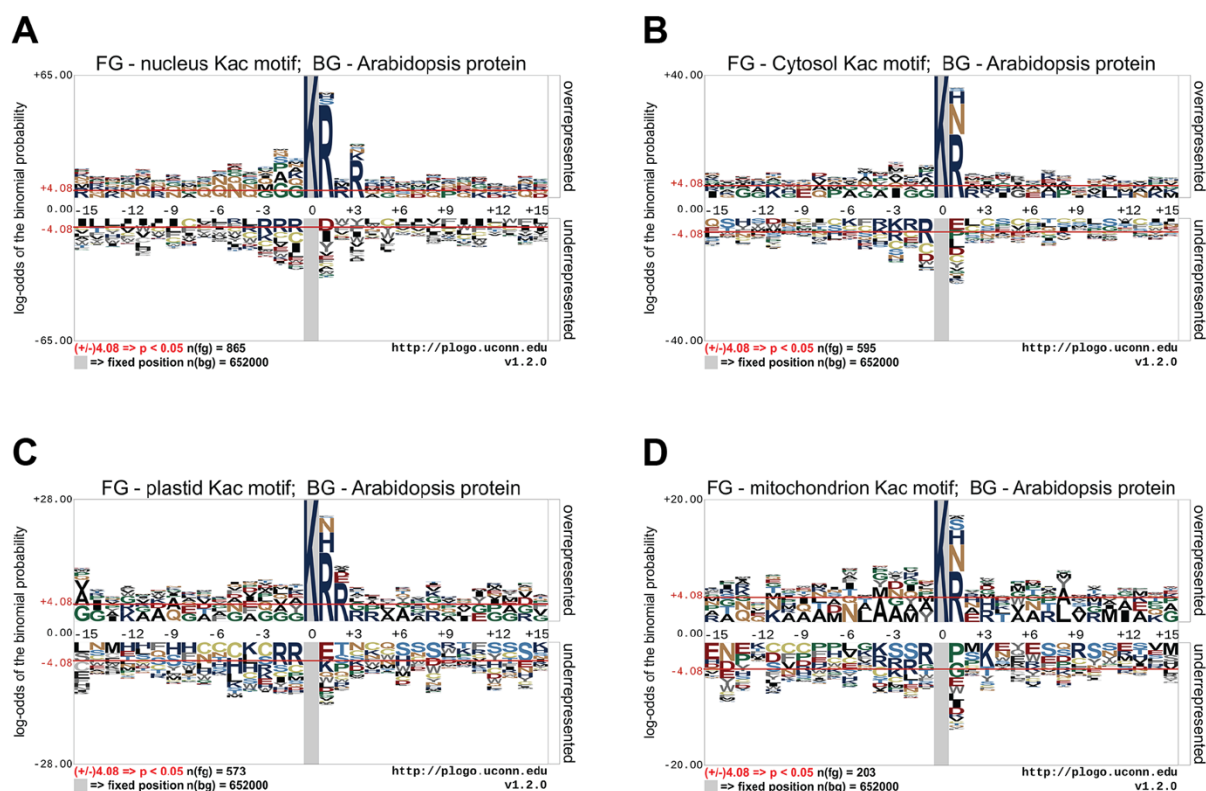
Supplemental Figure 1 Optimization of experiment parameters for the acetylproteomic analysis pipeline. **(A)** Comparison of the number of Kac peptides identified using two different protocols for total protein extraction from (Isaacson et al., 2016) **(B)** Comparison of different combinations of anti-acetyl-lysine antibody and the immunoprecipitation buffer. Antibody 1: Cell Signaling, #9441; Antibody 2: PTM Biolabs, PM104; Buffer 1: 50 mM MOPS pH 7.2, 10 mM Sodium phosphate, 50 mM Sodium chloride (Choudhary et al., 2009); Buffer 2: NETN buffer. **(C)** A flowchart showing data processing procedure after LC-MS/MS.



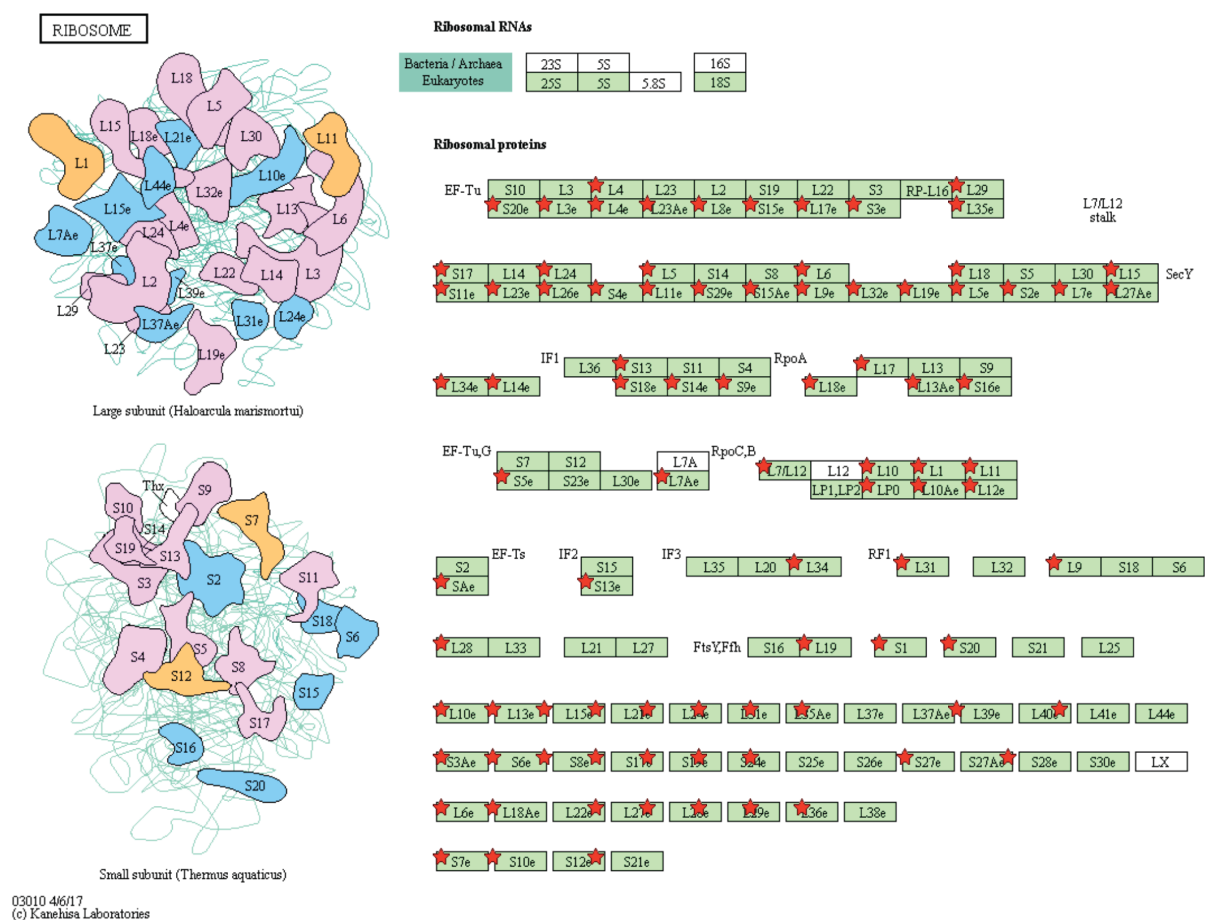
Supplemental Figure 2 Overlap of Kac sites identified between different biological replicates from the same tissue. (A) Leaf. (B) Root. (C) Seed. (D) Flower. (E) Stem.



Supplemental Figure 3 Comparison of lysine acetylation sites (**A**) and acetylated proteins (**B**) identified in this study with previous published datasets (Hartl et al, 2017; Liu et al, 2018; Uhrig et al, 2019).

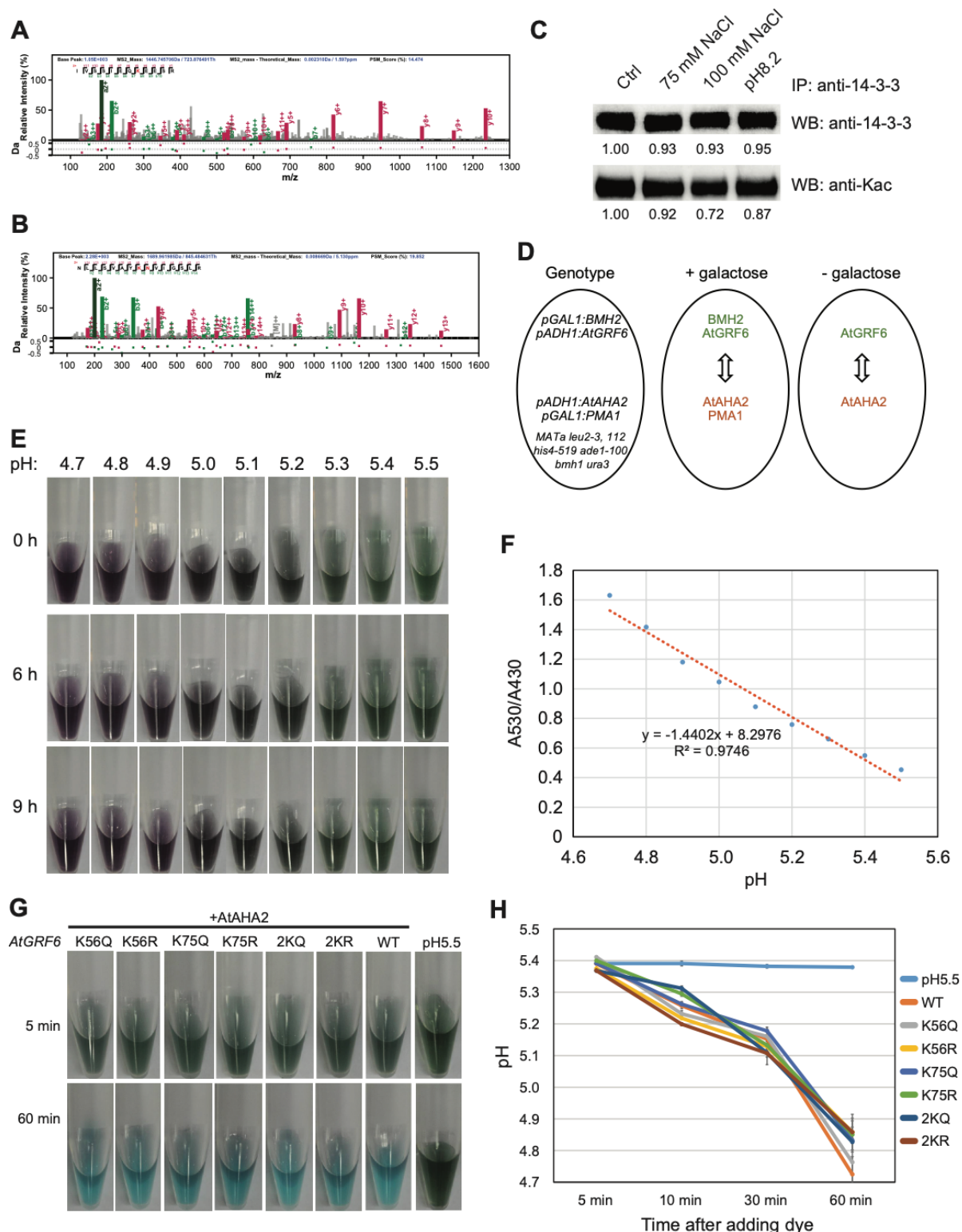


Supplemental Figure 4 Analyses of overrepresented motifs surrounding the acetylation sites of Kac proteins from different organelles. **(A)** Nuclear proteins. **(B)** Cytosolic proteins. **(C)** Plastidal proteins. **(D)** Mitochondria proteins.



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Supplemental Figure 5 Overview of lysine acetylated proteins present in the ribosome. Red stars indicate acetylated proteins.



Supplemental Figure 6 Acetylation of Lys56 in GRF6 negatively regulates alkaline tolerance response. **(A)** and **(B)** MS spectra showing the peptide NLLSVAYKNVIGSLR **(A)** and IVSSIEQKEESR **(B)** containing acetylated lysine at Lys56 and Lys75 of GRF6, respectively. **(C)** Western blot showing the protein and acetylation level of immunoprecipitated 14-3-3 proteins. **(D)** Diagrams of the genotype and the 14-3-3 proteins and proton ATPases expressed in the modified RS72v strain. **(E)** Colors of the methyl purple solutions under different pH. The colors are stable for at least 9 hours after the dye is added. **(F)** Standard curve for pH

measurement. The green color of methyl purple has spectral absorption at 530 nm, and the purple color has spectral absorption at 430 nm. The A530/A430 ratio linearly correlates with the pH from 4.7 to 5.5. **(G)** Methyl purple staining showing the pH changes of yeast cells harboring AtAHA2 and different version of AtGRF6 growing in galactose-containing liquid medium. **(H)** The pH changes of the yeast cells in (G).