SUPPLEMENTAL INFORMATION

Identification of galacturonic acid-1-P kinase: a new member of the GHMP kinase super family in plants and comparison with galactose-1-P kinase

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Supplemental Figures

Supplemental Figure 1. **Full ^{1}H-NMR spectrum of UDP-GalA derived from reaction products of GalAK.**
A. Expanded proton NMR spectra that covers the sugar anomeric region (5.5-6 ppm).
B. Expended view of the proton NMR spectra that covers the nucleotide-sugar carbon ring of UDP-GalA between 3.8 and 4.6 ppm.

Supplemental Figure 2. **Full ^{1}H-NMR spectrum of UDP-Gal derived from reaction products of GalK.**
A. Expanded proton NMR spectra that covers the sugar anomeric region (5.5-6 ppm).
B. Expended view of the proton NMR spectra that covers the nucleotide-sugar carbon ring of UDP-Gal between 3.8 and 4.4 ppm.

Supplemental Figure 3. **Expression and purification of recombinant wild type (wt) and mutants of GalK**.
Panel A. SDS-PAGE analyses of total soluble protein (S20) and inclusion bodies (P20) derived from cell expressing WT or mutant GalK altered in the following aa ($^{E62A;\ Y262F;\ A437S;\ S206G}$). Panel B. SDS-PAGE analyses of column-purified wt and mutants GalK recombinant proteins.

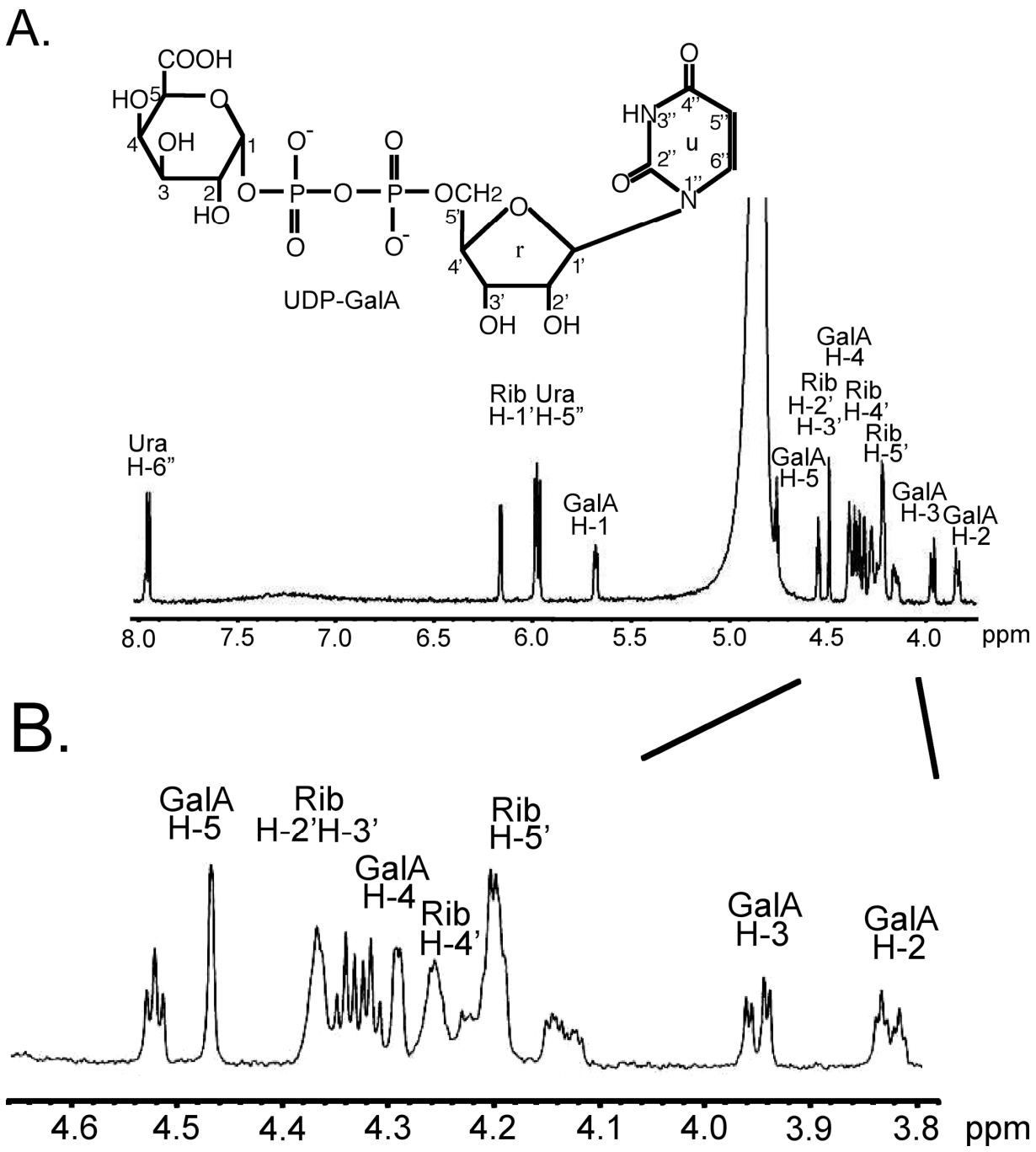
Supplemental Figure 4. **Expression and purification of recombinant wild type (wt) and mutants of GalAK**.
Panel A. SDS-PAGE analyses of total soluble protein (S20) and inclusion bodies (P20) derived from cell expressing WT or mutant GalAK altered in the following aa ($^{A41E;\ Y250F;\ A368S}$). Panel B. SDS-PAGE analyses of column-purified wt and mutants GalAK recombinant proteins.

Supplemental Figure 5. **Selected portions of the ^{1}H-NMR spectra corresponding to α-GalA and β-GalA peaks before and during GalAK conversion of α-GalA to α-GalA-1-P.**
A. Portions of the ^{1}H-NMR spectrum (no enzyme) showing the anomeric proton (H-1) of α-GalA (5.27 ppm), the anomeric proton (H-1) of the β-GalA (4.55 ppm), and the H-5 peak of β-GalA (4.03 ppm). D-GalA (2 mM) was mixed with 50 mM phosphate buffer (pH/pD = 7.6), 5 mM $MgCl_2$ and D_2O and monitored continuously by ^{1}H-NMR spectroscopy. The spectra shown were obtained after 2, 6, and 20 min.
B. After ~ 20 min, ATP (2 mM) was added and monitored continuously by ^{1}H-NMR spectroscopy. The spectra shown (+ATP) were obtained after 2, 6, and 20 min. The panels are showing no change in the α

and β configuration at time 2, 6, and 20 min post addition of ATP. NOTE: signal from the ribose protons (H-2', 3') of ATP are overlap with H-1 of β-GalA (β1).
C. After ~ 20 min in the presence of ATP, the recombinant kinase (GalAK, 3 μg) was added and the reaction monitored continuously by ^{1}H-NMR spectroscopy. The spectra shown (+ enzyme) were obtained after 2, 6, and 20 min, and show the enzymatic phosphorylation of the α-GalA configuration and its conversion to α-GalA-1-P.
Abbreviation of the NMR spectrum are as follows: α1, peak corresponding to H-1 of α-GalA (5.27 ppm); β1, peak corresponding to H-1 of β-GalA (4.55 ppm); β5, peak corresponding to H-5 of β-GalA (4.03 ppm); α1P, peak corresponding to H-1 of α-GalA-1-P, (5.52 ppm). Note the β1 peaks in panels B, overlap with ribose protons of ATP; the β1 peaks in panels C, overlap with the ribose protons from ADP/ATP thus creating additional shift when compared with B.

Supplemental Figure 6. **Selected portions of the ^{1}H-NMR spectra corresponding to α-Gal and β-Gal peaks before and during GalK conversion of α-Gal to α-Gal-1-P.**
A. Portions of the ^{1}H-NMR spectrum (no enzyme) showing the anomeric proton (H-1) of α-Gal (5.24 ppm), the anomeric proton (H-1) of the β-Gal (4.56 ppm), and the H-4 peak of β-Gal (3.90 ppm). D-Gal (2 mM) was mixed with 50 mM phosphate buffer (pH/pD = 7.6), 5 mM $MgCl_2$ and D_2O and monitored continuously by ^{1}H-NMR spectroscopy. The spectra shown were obtained after 2, 6, and 20 min.
B. After ~ 20 min, ATP (2 mM) was added and monitored continuously by ^{1}H-NMR spectroscopy. The spectra shown (+ATP) were obtained after 2, 6, and 20 min. The panels are showing no change in the α and β configuration at time 2, 6, and 20 min post addition of ATP. NOTE: signal from the ribose protons (H-2', 3') of ATP are overlap with H-1 of β-Gal (β1).
C. After ~ 20 min in the presence of ATP, the recombinant kinase (GalK, 3 μg) was added and the reaction monitored continuously by ^{1}H-NMR spectroscopy. The spectra shown (+ enzyme) were obtained after 2, 6, and 20 min, and show the enzymatic phosphorylation of the α-Gal configuration and its conversion to α-Gal-1-P.
Abbreviation of the NMR spectrum are as follows: α1, peak corresponding to H-1 of α-Gal (5.24 ppm); β1, peak corresponding to H-1 of β-Gal (4.56 ppm); β4, peak corresponding to H-4 of β-Gal (3.90 ppm); α1P, peak corresponding to H-1 of α-Gal-1-P, (5.47 ppm). Note the β1 peaks in panels B, overlap with ribose protons of ATP; the β1 peaks in panels C, overlap with the ribose protons from ADP/ATP thus creating additional shift when compared with B.

Supplemental Figure 1



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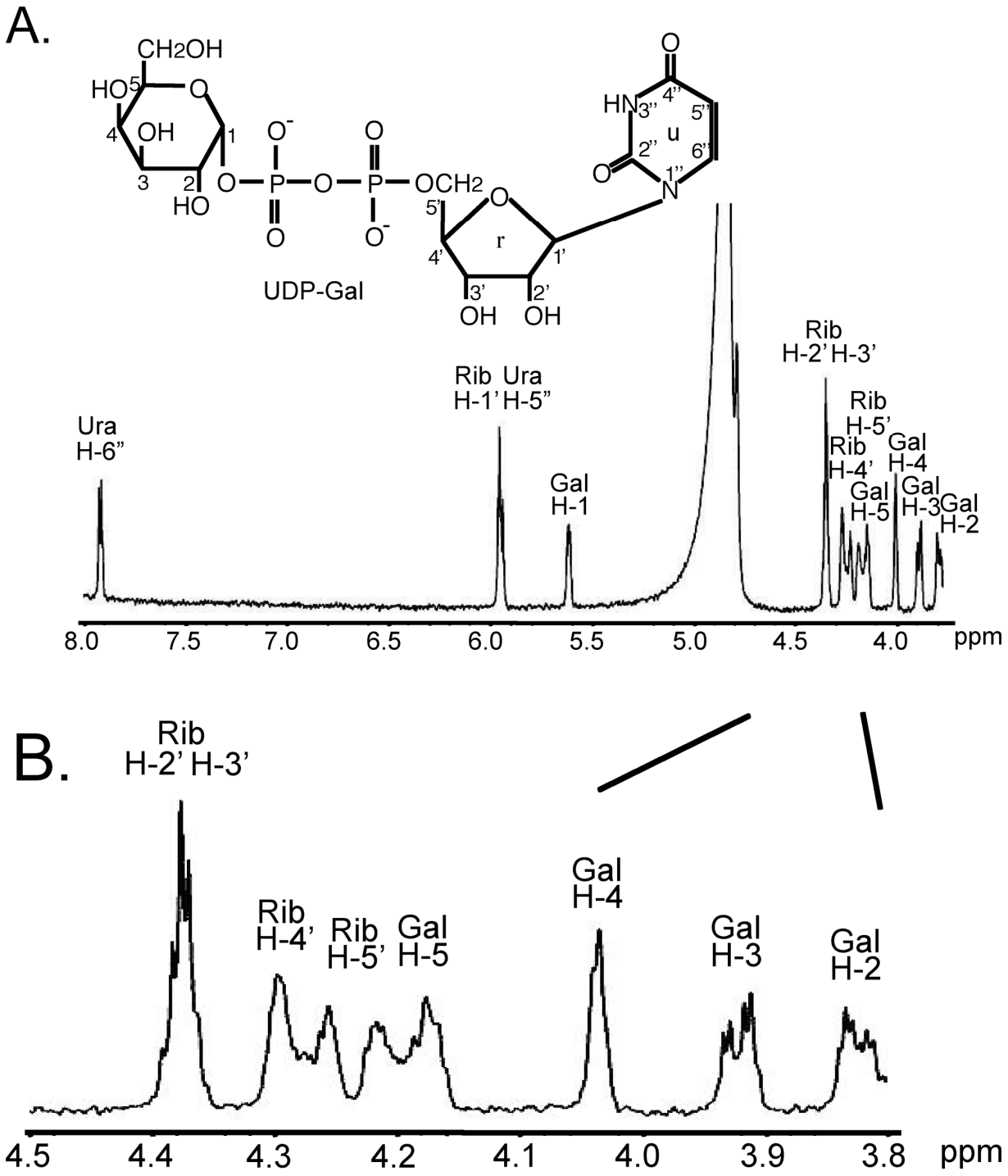
Supplemental Figure 2



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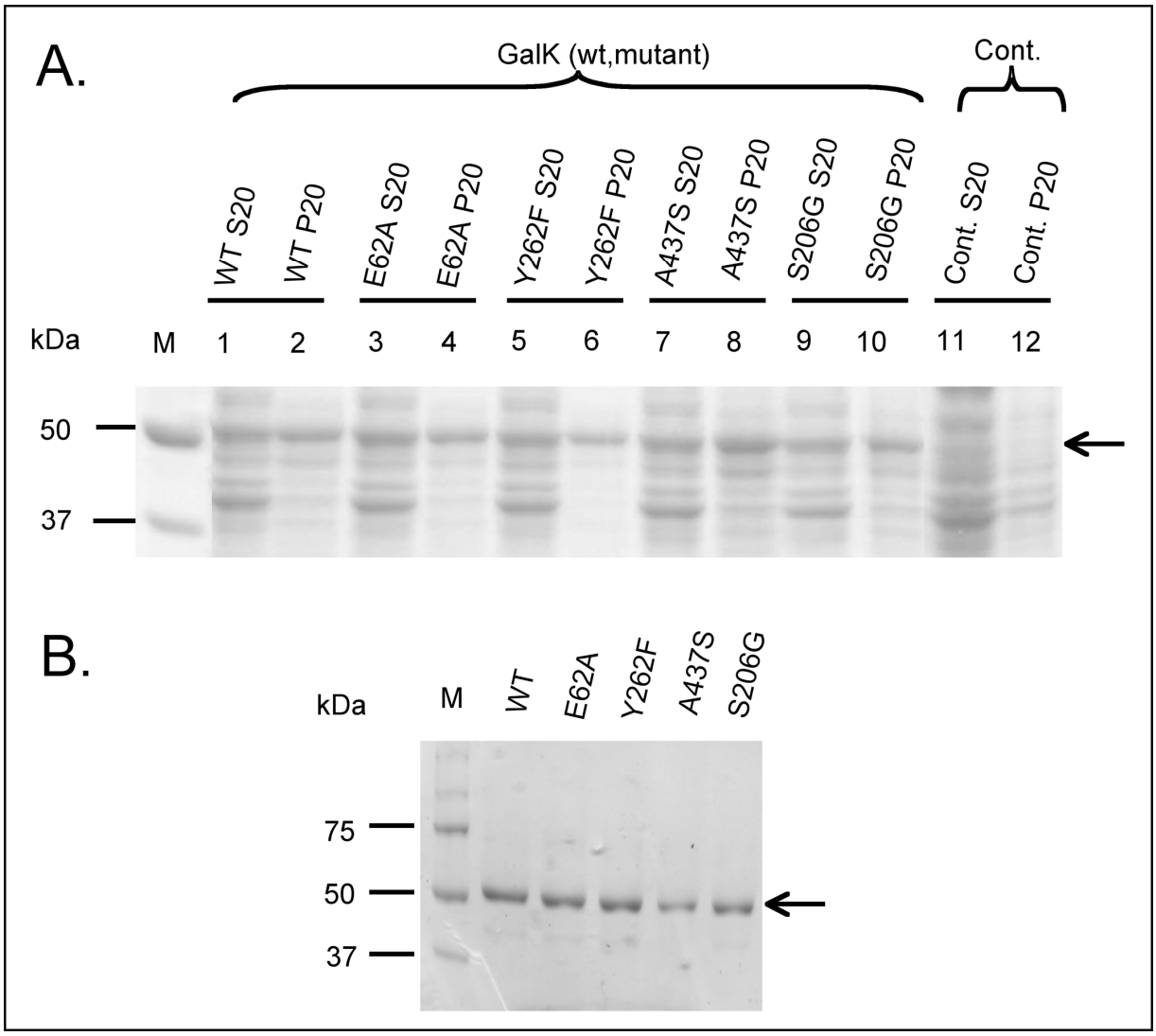

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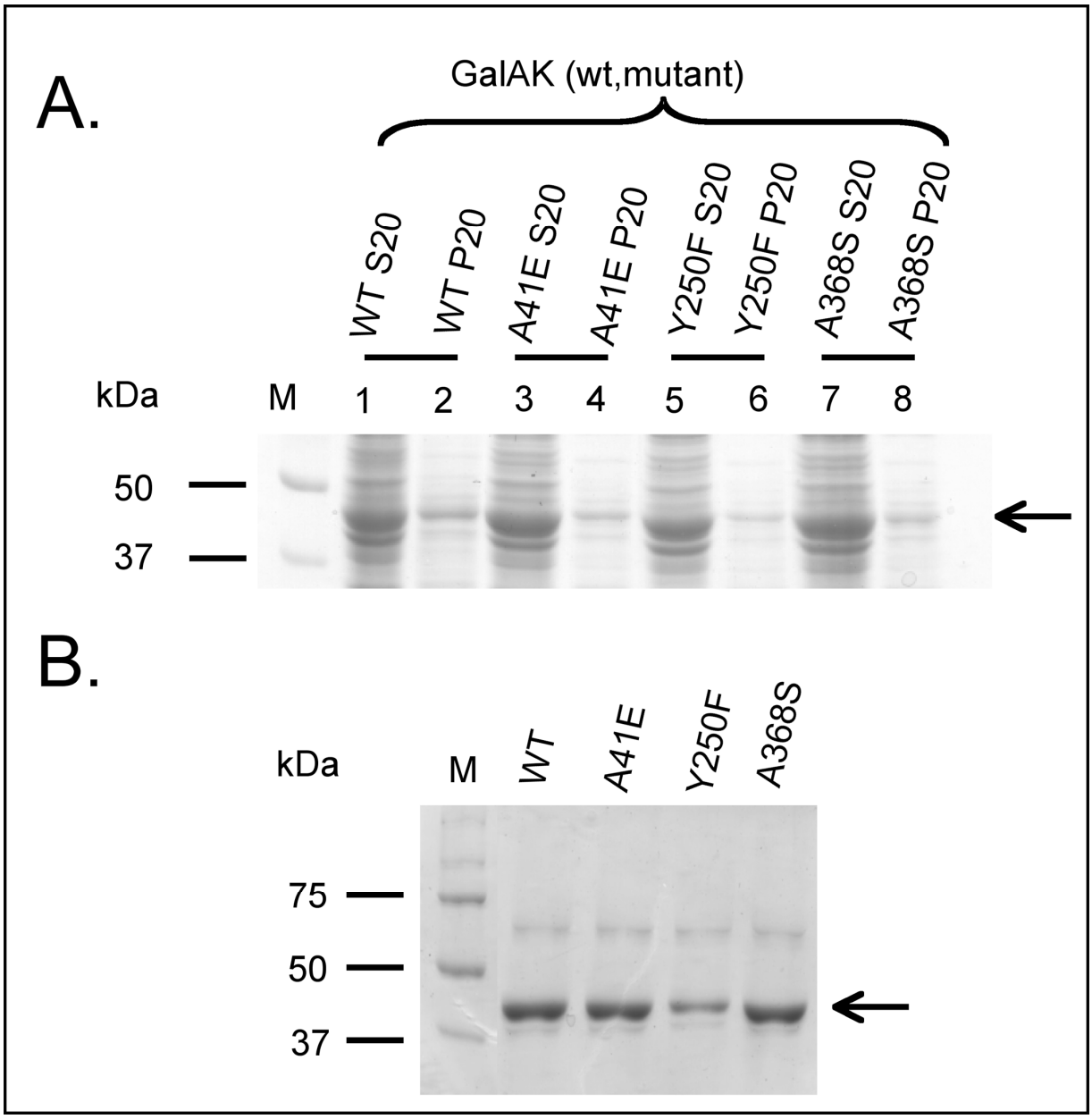

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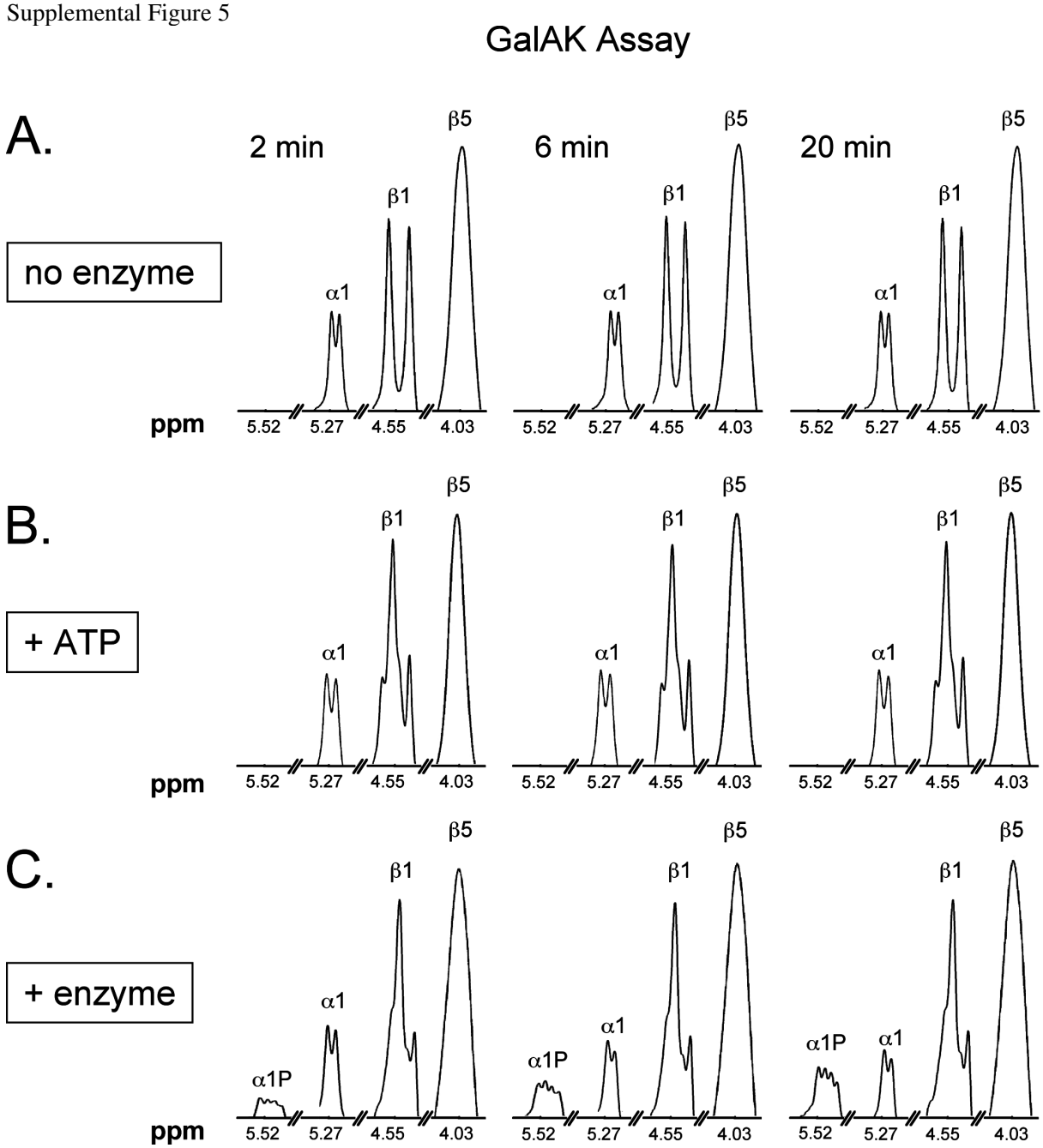
Supplemental Figure 5



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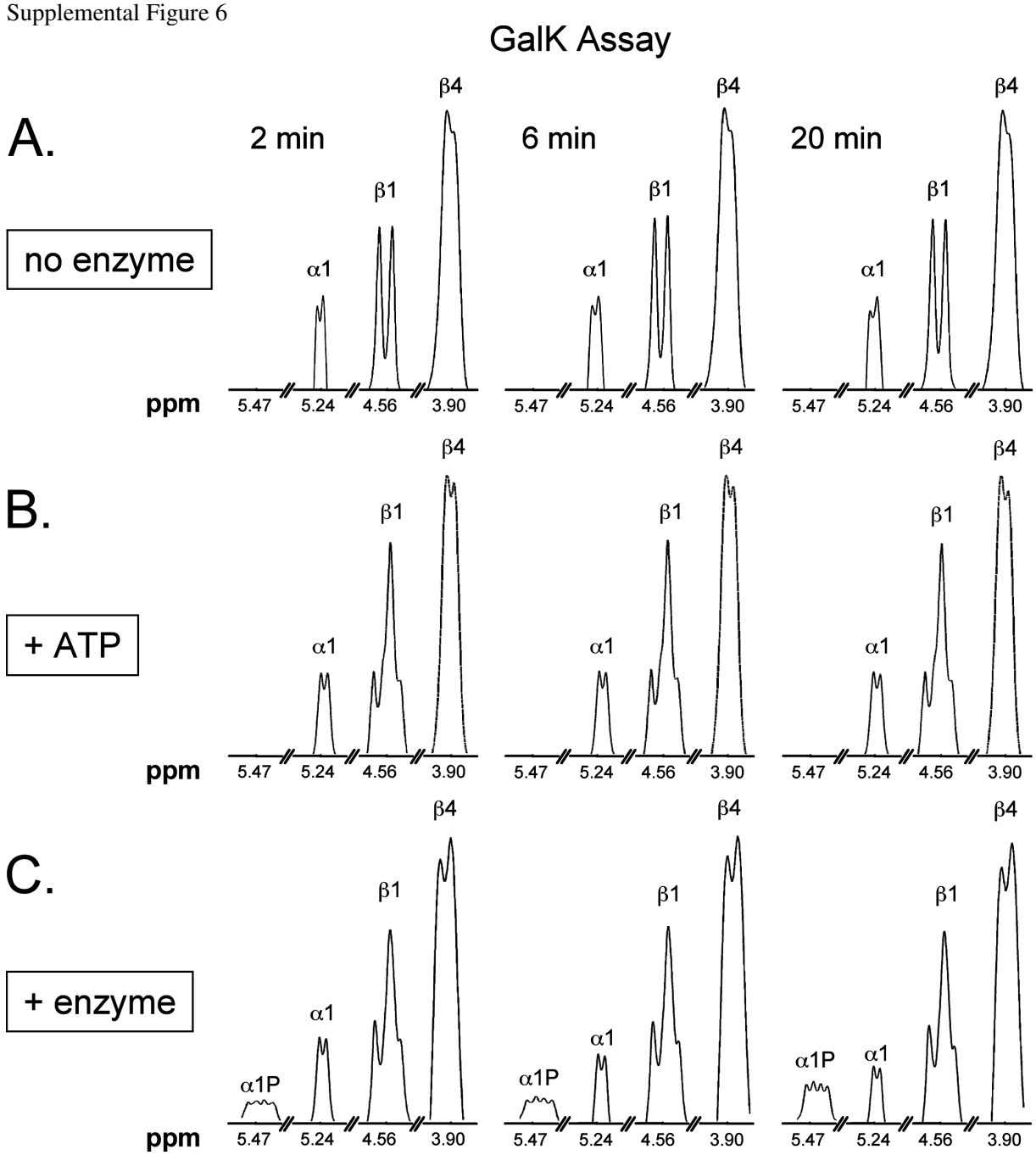
Supplemental Figure 6



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