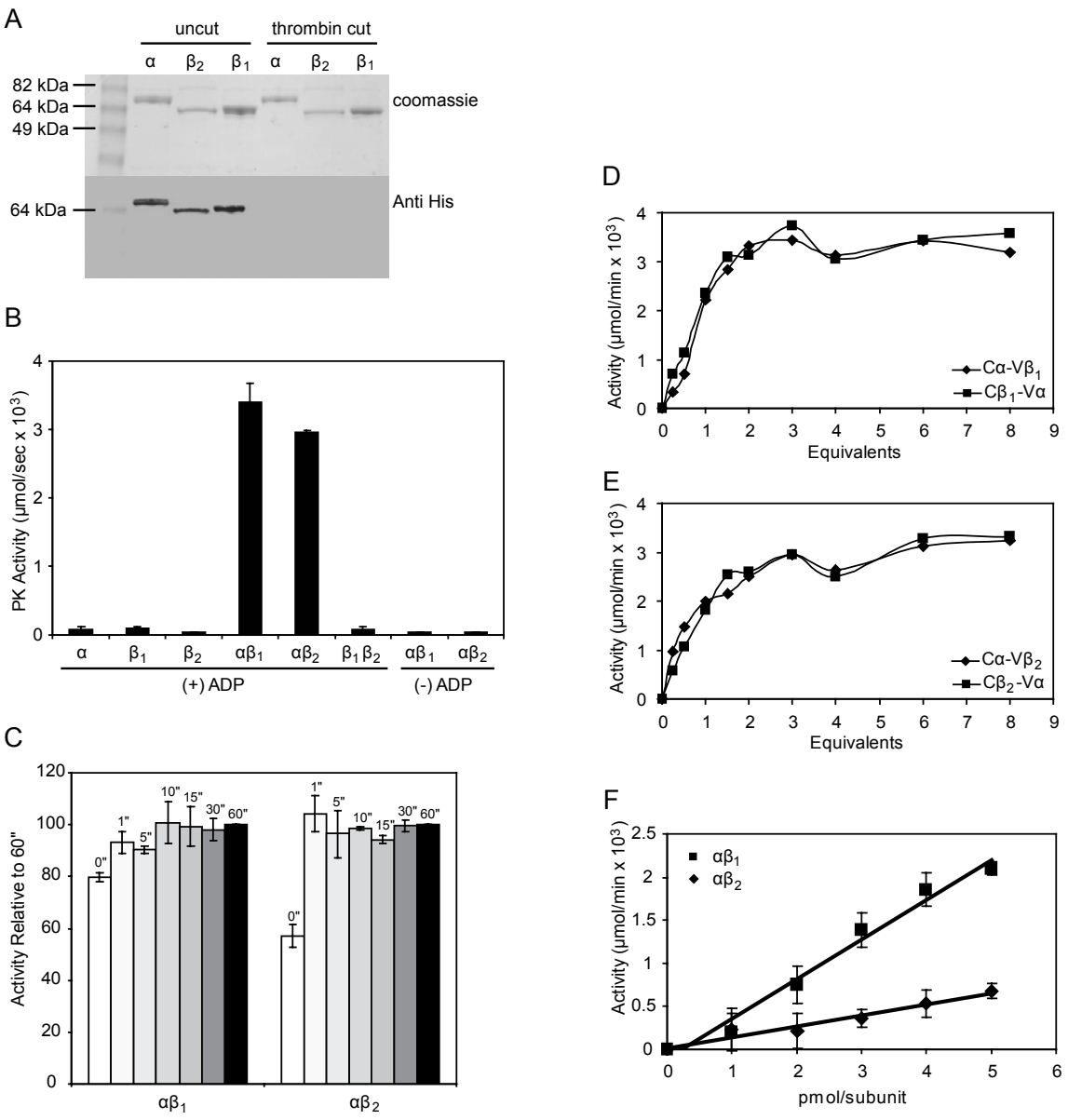

Supplemental Figure 1. PK_p protein purification and subunit association biochemistry

(A) SDS-PAGE and anti-His immunblot of affinity purified PK_p subunits. Individual subunits were purified and subjected to no treatment or treatment with thrombin to remove the His tag. 500 ng protein was loaded per well. Immunoblot shows His tag has been removed after treatment with thrombin.

(B) Pyruvate kinase activity of various subunit mixtures. Equal volumes of purified subunits were assayed alone or in combination and tested for PK activity in liquid assays. Approximately 0.1 ng of each subunit was used in each assay with saturating substrate

concentrations. Only the $\alpha\beta_1$ and $\alpha\beta_2$ mixtures are active. No PEP phosphatase activity was observed in control reactions without ADP.

(C) Time course of pyruvate kinase activity after mixing subunits. Numbers above bars represent the time (in minutes) incubated prior to assay. 2 pmol of each subunit was used per assay under saturating substrate conditions. Activity is expressed relative to the 60 minute sample.

(D) Subunit titration curve with PK_p-α and PK_p-β_1. For Cα-Vβ_1, the α subunit was held constant and the β_1 subunit was variable. For Cβ_1-Vα, the β_1 subunit was held constant and the α subunit was variable. 2.5 pmol of the constant subunit was used per reaction in saturating substrate conditions. Equivalents refers to the molar ratio of the variable subunit to the fixed one.

(E) Subunit titration curve with PK_p-α and PK_p-β_2. For Cα-Vβ_2, the α subunit was held constant and the β2 subunit was variable. For Cβ_2-Vα, the β_2 subunit was held constant and the α subunit was variable. Equivalents refers to the molar ratio of the variable subunit to the fixed one.

(F) Pyruvate kinase activity relative to protein concentration. Equal amounts of either α and β_1 or α and β_2 were mixed and assayed under sub-saturating substrate conditions.