A single point mutation in the C-terminus extension of wheat Rubisco activase dramatically reduces ADP inhibition via enhanced ATP binding affinity

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	TaRca2-α			TaRca2-α K428R		
	V_{max}	K_{half}	K_i	V_{max}	K_{half}	K_i
Competitive	56	238	4.9	63	68	3.4
Non-competitive	58	Ambiguous	138	62	Ambiguous	Ambiguous

Table S1. Comparison of global iteratively fit parameters generated from the competitive and non-competitive models tested for predicting ATP substrate and ADP inhibitor interaction. The Rca variants TaRca2-α and TaRca2-α K428R were analyzed, as presented in Fig. 5. The parameters are: V_{max}, the maximum ATP dependent velocity in ECM min $x10^{-3}$ mol^{-1}; K_{half}, the ATP concentration required to reach half maximal velocity in μM; h, the Hill-slope constant; K_i, the apparent inhibition binding constant of ADP in μM. The modeled equations were, competitive [$K_{half\text{-}Obs}= K_{half}^{h}(1+I/K_i)$] [$V=V_{max}*S^h/(K_{half\text{-}Obs}+S^h)$], and non-competitive [$V_{max\text{-}Inh}=V_{max}/(1+I/K_i)$] [$V=V_{max\text{-}Inh}*S^h/(K_{half}+S^h)$]. S is the ATP substrate concentration and I is the ADP inhibitor concentration in μM. Ambiguous means no discrete or reasonable value was generated by the model (e.g. a K_{half} of $1.1*10^6$).

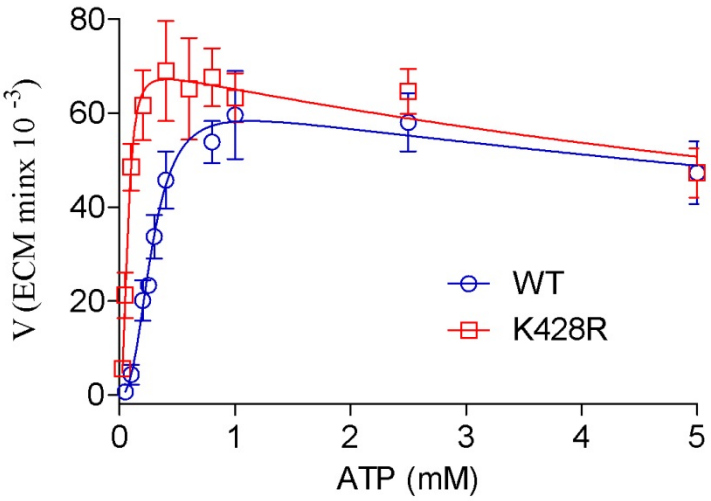

Figure S1. ATP substrate dependent enzyme kinetic curves of the wheat Rca α isoform and the K428R mutant. The enzymatic velocity of Rubisco reactivation by Rca (ECM reactions regenerated per minute per Rca) were plotted against ATP concentration added to assays. Curves are a modified Hill equation, accounting for substrate inhibition [$V=V_{max}*S*^{h}/(K_{half}*S^{h})*(1+S/K_{i})$] and fit with an ordinary least-squares model. V_{max} was calculated as 63±6 and 70±4 mM ATP and K_{half} was calculated as 285±8 and 71±10 µM ATP for TaRca2-α and K428R, respectively. h was set at 2.7 for TaRca2-α and 2.5 for K428R from curves at a lower ATP range. The inhibition constant of the ATP substrate (K_i) was calculated as 16±11 and 13±7 mM ATP. Values are the means and SD of four experimental replicates. Of note, concentrations of ATP at 5 mM or above are unrealistic of *in vivo* conditions but demonstrate an ATP substrate limitation on Rubisco activation by Rca at such high concentrations.

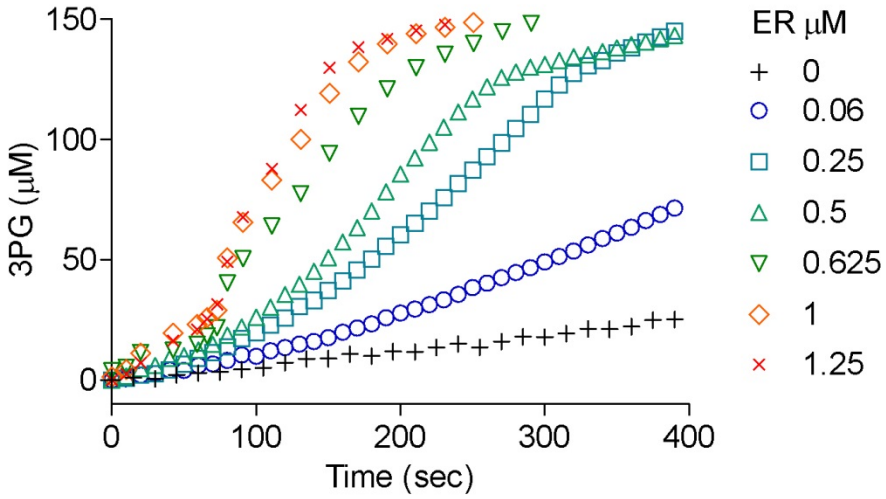

Figure S2. An example of the Rubisco assay with varying Rubisco concentrations and a constant Rca concentration. The production of 3-phosphoglyceric acid (3PG) by Rubisco versus assay time with varying active site concentrations of RuBP substrate inhibited Rubisco (ER) but with a constant application of 0.08 mg ml Rca α isoform corresponding to 1.6 μM of Rca monomer. Note, the concentrations of ER active sites used in all standard experiments presented in this study were at 0.25±0.05, well below the Rubisco dependent V_{max} of Rca. However, this was unavoidable as higher concentrations of ER substrate caused the reaction to run faster than the desired range for reliable calculations using the spectrophotometric assay. Lower concentrations of Rca enzyme was not possible as loss of activity due to the Rca requirement for concentration dependent self-association occurs.

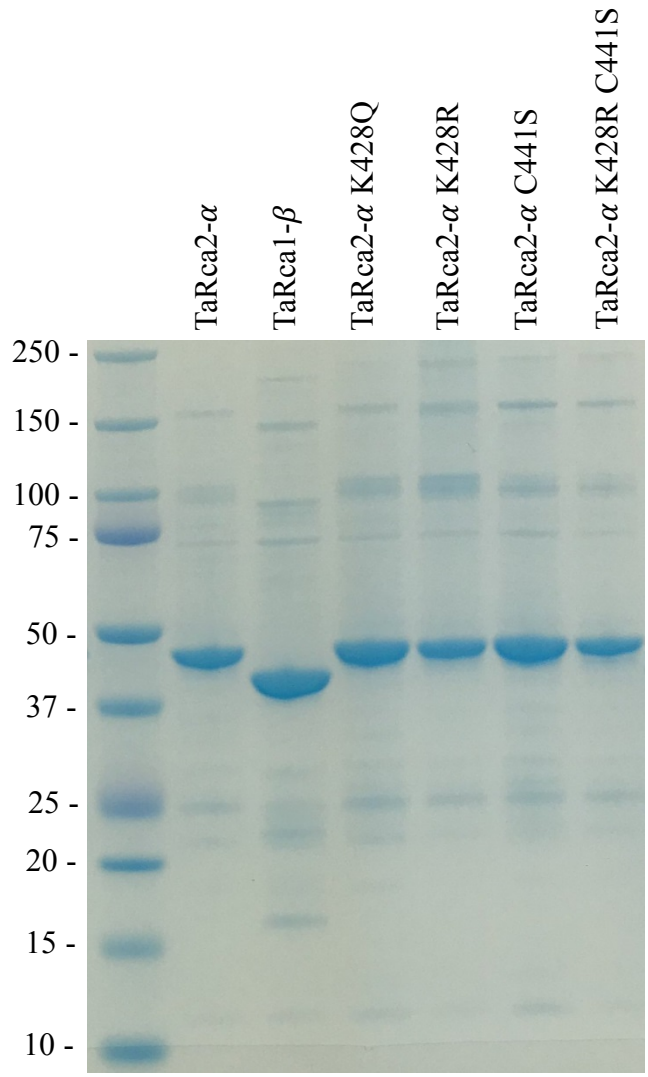

Figure S3. A representative SDS-PAGE gel of the recombinant Rca proteins utilized in this report. Protein was run on a 4-12% Criterion XT Bis-tris gel (Bio-Rad) at a constant 150 volts for 70 min. 8 µg of protein were loaded per sample. Lanes from left to right are: 1, Precision Plus Dual Xtra standards protein ladder (Bio-Rad); 2, TaRca2-α; 3, TaRca1-β; 4, TaRca2-α K428Q; 5, TaRca2-α K428R; 6, TaRca2-α C441S; and 7, TaRca2-α K428R + C441S.

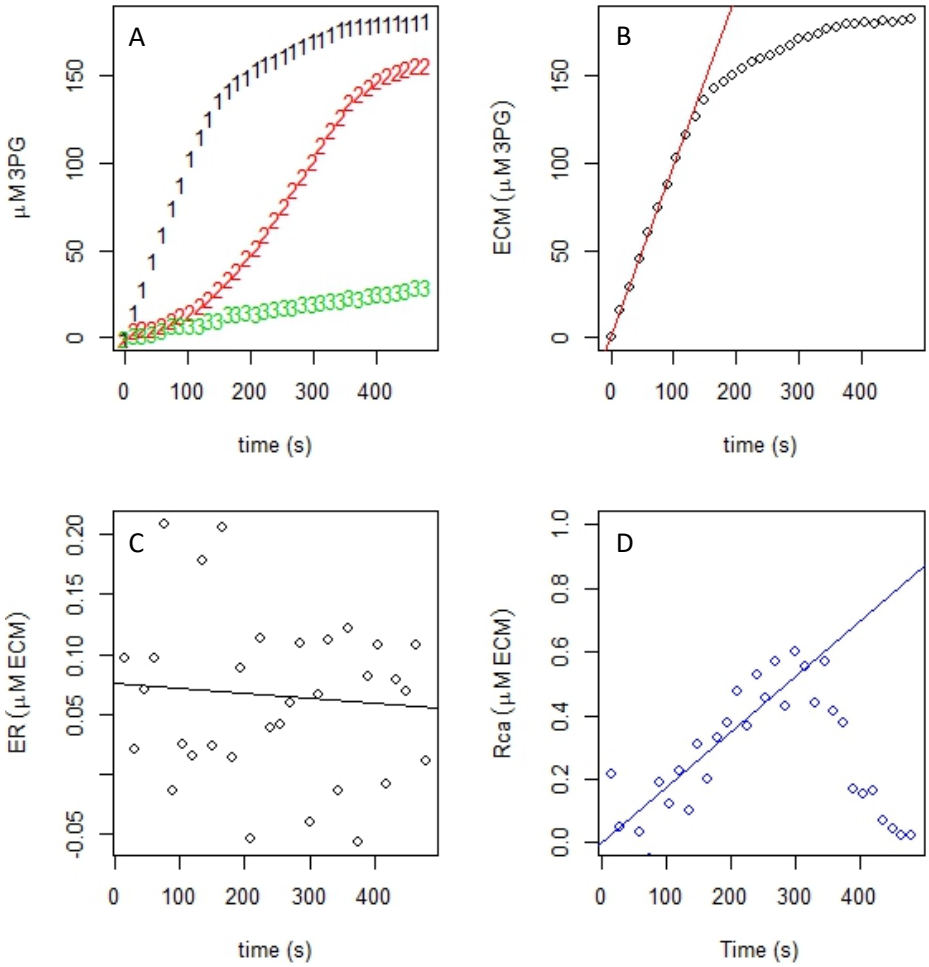

Figure S4. An example of Rca activity measurements. Panel A shows the raw measurements transformed from Abs 340 nm to µM of 3-phosphoglyceric acid product (3PG) using an NADH molar extinction coefficient of 6.211 L $mmol^{-1}$ cm^{-1}. Symbol 1 represents ECM, 2 is ER with Rca, and 3 is ER with no Rca added. Panel B shows the 3PG product for the fully carbamylated Rubisco sample (ECM) fitted with a linear regression through measurements in the first 60 seconds. The slope of this line divided by 2 (as 2 molecules of 3PG per CO_2 fixed) and divided again by 2.1 (the turnover rate of wheat Rubisco at 25°C) was used to calculate the number of Rubisco active sites in the assay. In this and for all experiments 0.25±0.05 µM of Rubisco active sites were added to the assay. Panel C (ER without Rca) and D (ER and Rca) are plots of the slopes (µM ECM) between adjacent values as presented in Panel A and plotted against time. These plotted slopes were then fit with a linear regression restrained through the point of origin and set to the maximum time before values declined (i.e. to 300 sec in Panel D). The slope of this linear regression (D) minus the slope of the ER control (C) provides the number of ECM reactions regenerated by Rca per unit of time. In other words, it is equivalent to the second derivative of the original curves and corresponds to the acceleration in Rubisco product over time generated by newly activated Rubisco.

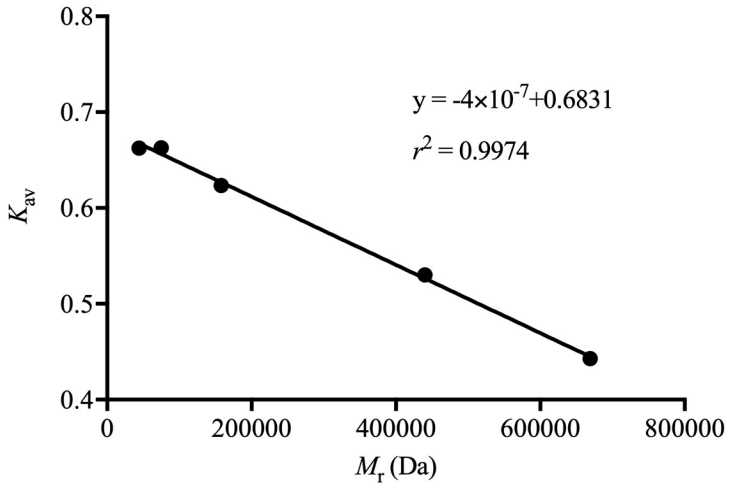

Figure S5. Gel-filtration chromatography standard curve. The partition coefficient (K_{av}) was plotted against the molecular weight in Daltons (M_r) of thyroglobulin (669,000), ferritin (440,000), aldolase (158,000), conalbumin (75,000), and ovalbumin (44,000). K_{av} was calculated as $K_{av}=(V_e-V_o)/(V_c-V_o)$, where V_o is the void volume, V_e is the elution volume, and V_c is the geometric column volume. V_o was determined by the elution volume of blue dextran 2000, and V_c was 24 mL. This standard curve was used to determine the elution volume corresponding to Rca complex sizes based on the TaRca2-α monomer being 46,171 Da.

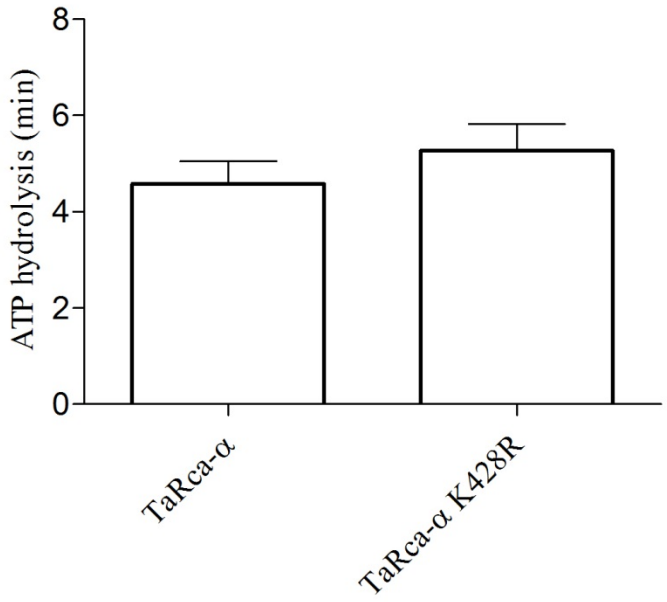

Figure S6. ATP hydrolyzed by TaRca2-α and K428R mutant per minute. Approximately 1.6 μM of Rca was added to each Rubisco activation assay and measurements were taken over 4-min; therefore, for TaRca2-α: 4.6 ATPs hydrolyzed per min * 1.6 μM of Rca * 4-min measurement period = ~30 μM of ATP converted to ADP over a standard assay. This 30 μM ADP concentration was factored into ADP inhibition assays that did not contain a phosphocreatine kinase ATP regeneration system in the reaction mixture.