



Supplemental Figure S4. Assessment of Catalytic Activity of Src42 Mutations Identified *in vivo*. S2 cells were left alone (-) or transfected with the indicated of P<sub>yo</sub>-tagged Src42 variants (0.4 – 0.6  $\mu\text{g}$ ). Expression of p<sub>yo</sub>-Src42 constructs was induced 48 hr post-transfection and the cells were harvested 24 hr later. Cell lysates were immunoprecipitated using  $\alpha$ -PYO and Src42 kinase activity was detected in the immunoprecipitates as described in the Experimental Procedures. Src42 levels were monitored by immunoblots. The W241R mutant has an activity comparable to wild-type Src42 in its ability to phosphorylate enolase ( $^{32}\text{P}$ -enolase) or to autophosphorylate ( $^{32}\text{P}$ -Src42). In contrast, the D370V mutant is completely inert as the catalytically-inactivated K276R mutant.