



**Figure S6. A-D)** Titration curves (upper panels) and binding isotherms (lower panels) of eIF5B interactions with GDP or GTP. **A)** Ct-eIF5B(517C) with GDP at 20 °C in the presence of  $\text{MgCl}_2$ ; **B)** Ct-eIF5B(517C) with GDP at 25 °C in the absence of  $\text{MgCl}_2$ ; **C)** Ct-eIF5B(517C) with GTP at 20 °C in the presence of  $\text{MgCl}_2$ ; **D)** Ct-eIF5B(517-852) with GTP at 20 °C in the presence of  $\text{MgCl}_2$ .