

Figure S1.

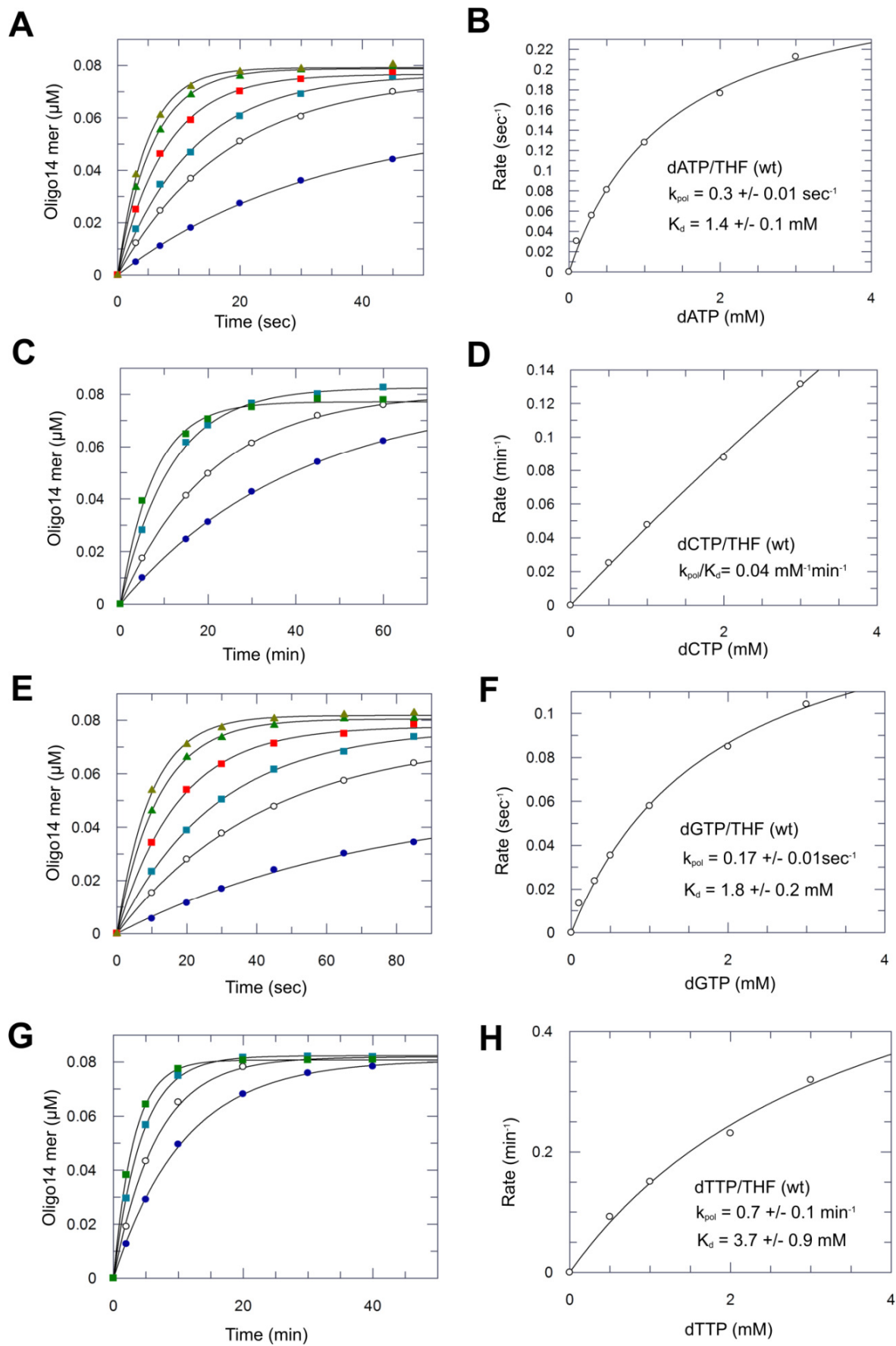


Figure S2.

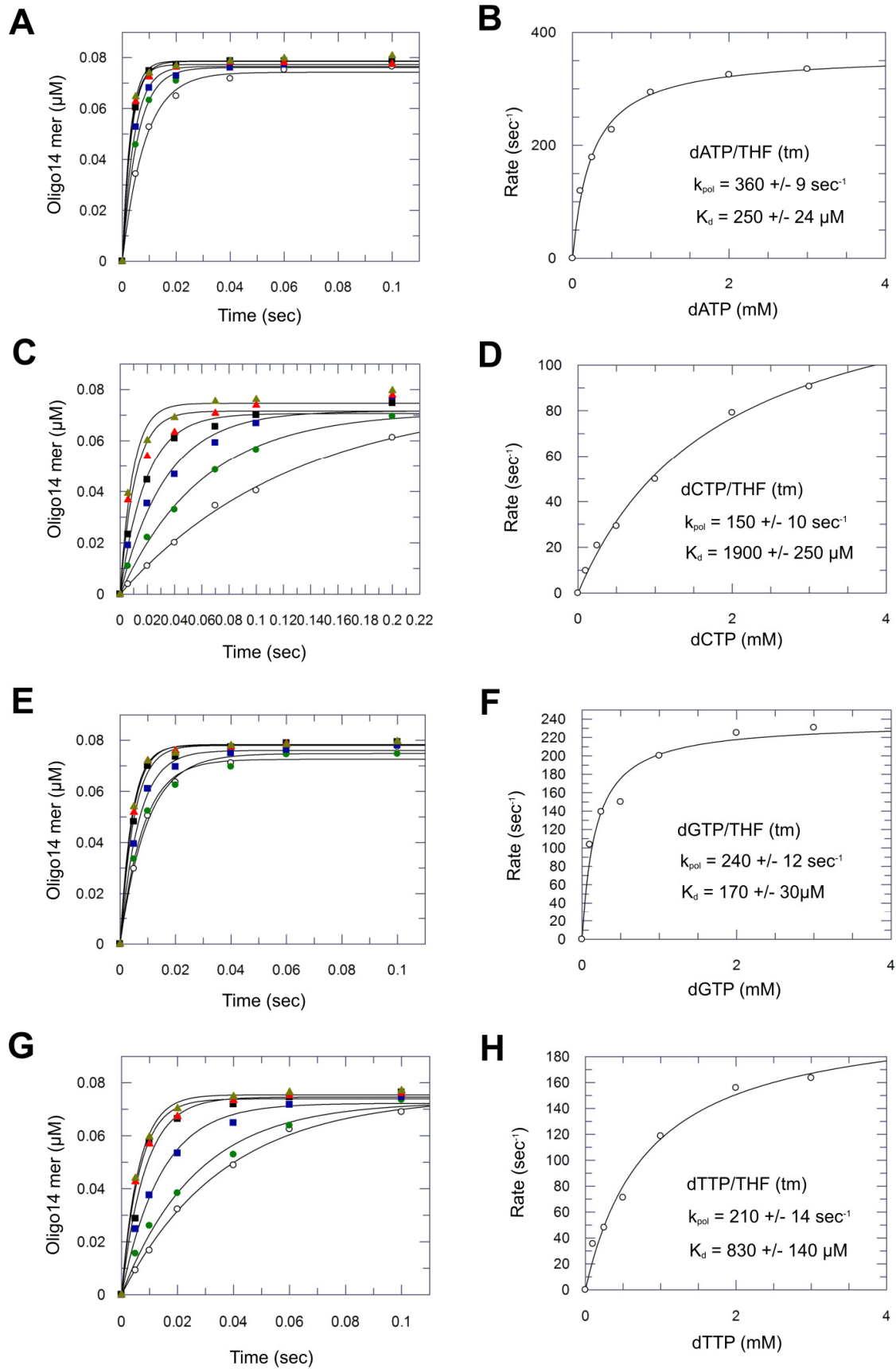


Figure S3.

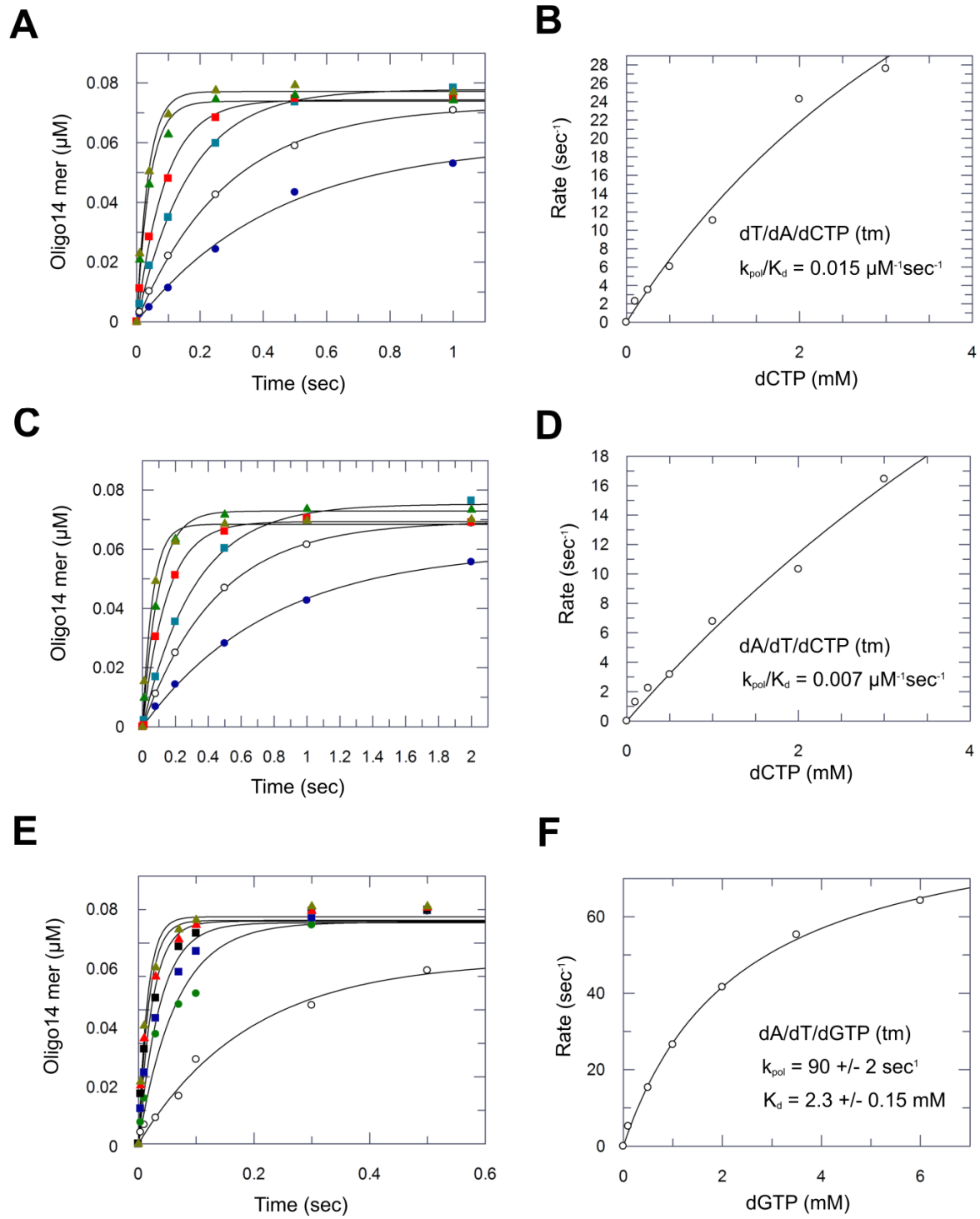


Figure S4.

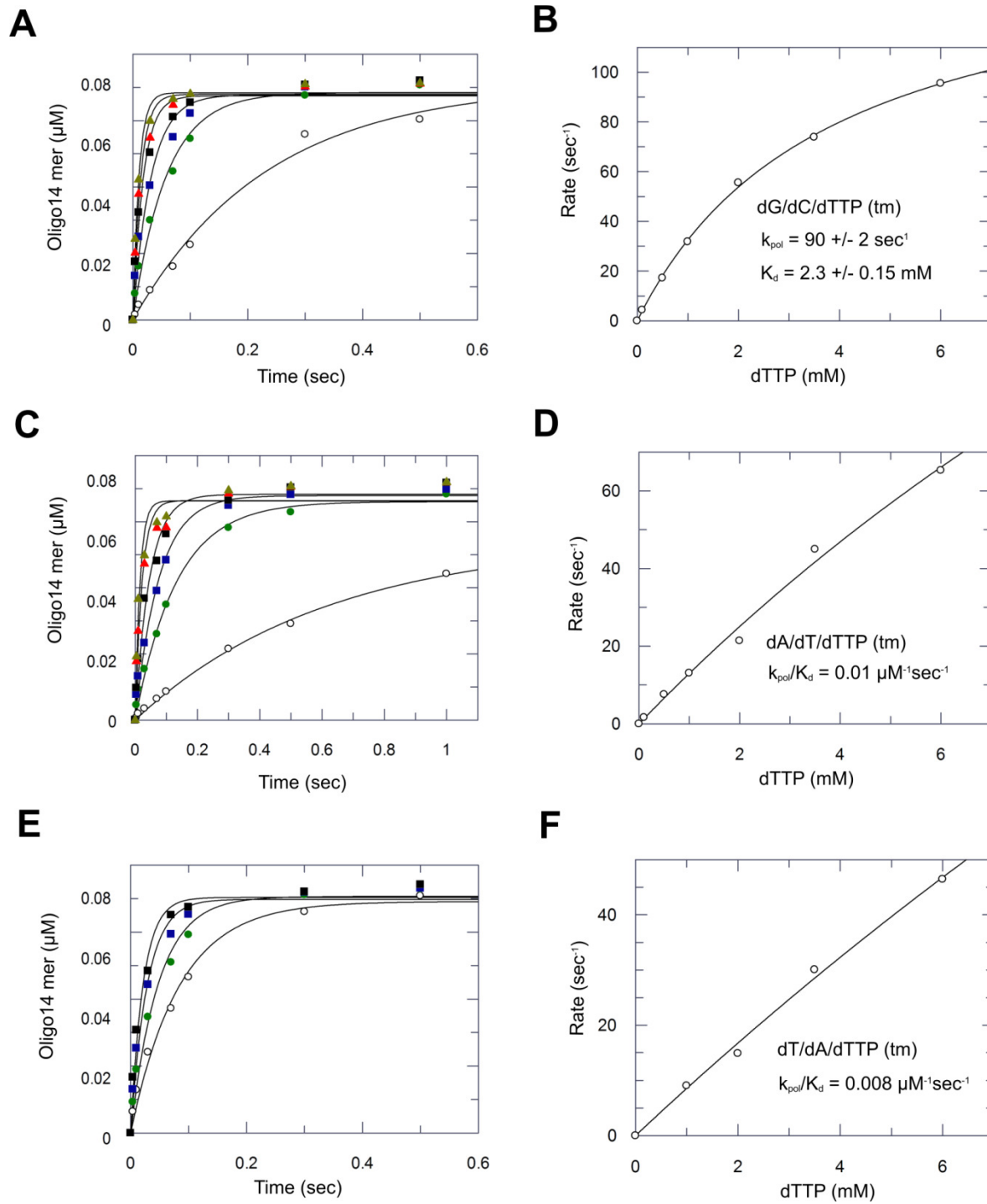


Figure S5.

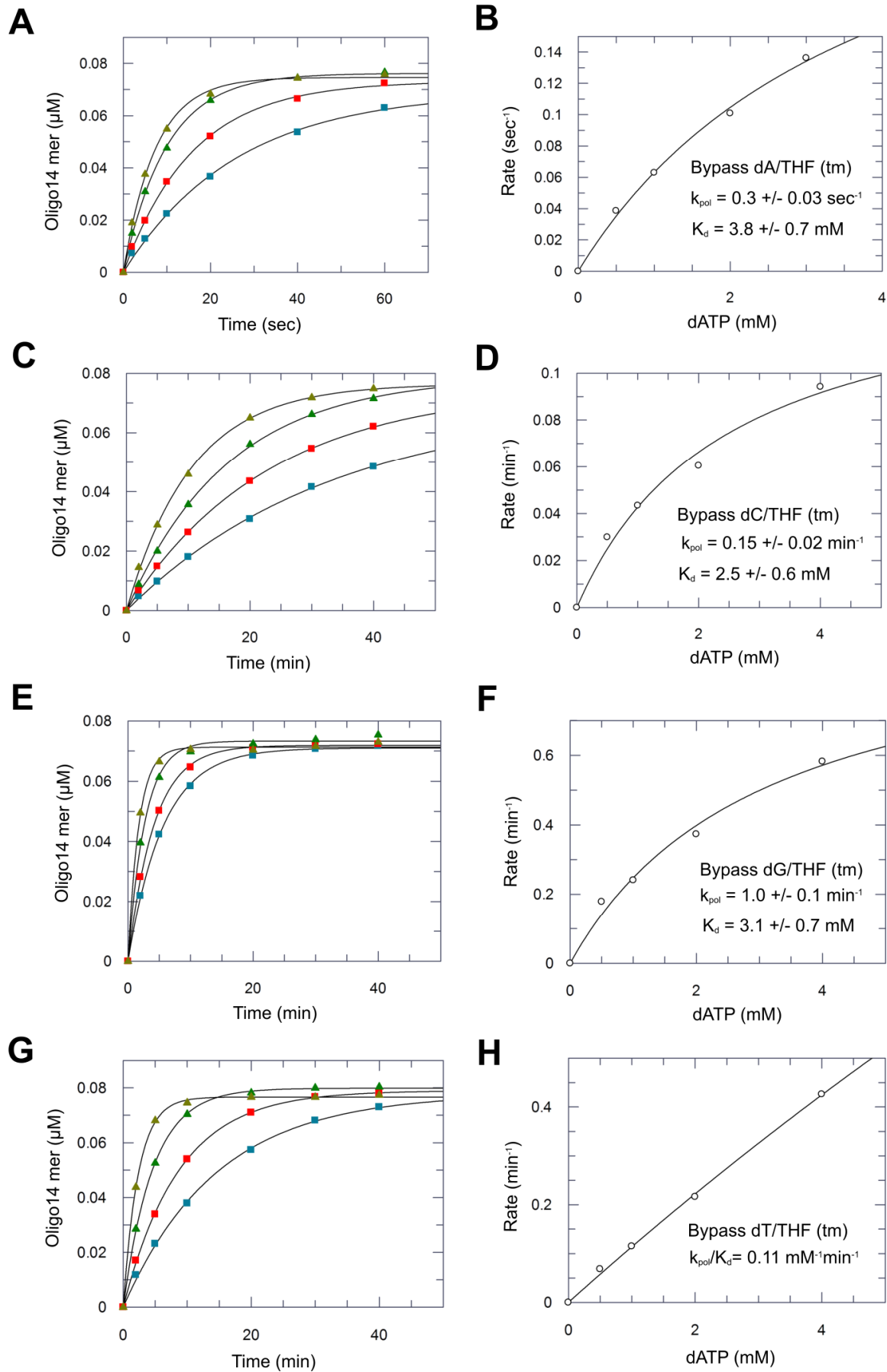


Figure legends:

Sup Fig 1. Kinetic insertion of dNMP opposite THF by wt RB69 pol. (A) Progress curves at various dATP concentrations, 0.1, 0.3, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (B) Plot of  $k_{obs}$  vs [dATP] fit to hyperbola equation to yield  $k_{pol}$  and  $K_{d,app}$ . (C) Progress curves at various dCTP concentrations, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (D)  $k_{pol}/K_{d,app}$  is estimated by the slope of the plot of  $k_{obs}$  vs [dCTP]. (E) Progress curves at various dGTP concentrations, 0.1, 0.3, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (F) Same as panel B using the results depicted in panel E. (G) Progress curves at various dTTP concentrations, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (H) Same as panel B using the results depicted in panel G.

Sup Fig 2. Kinetic insertion of dNMP opposite THF by the tm RB69 pol. (A) Progress curves at various dATP concentrations, 0.1, 0.25, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (B) Plot of  $k_{obs}$  vs [dATP] fit to hyperbola equation to yield  $k_{pol}$  and  $K_{d,app}$ . (C) Progress curves at various dCTP concentrations, 0.1, 0.25, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (D) Same as panel B using the results depicted in panel C. (E) Progress curves at various dGTP concentrations, 0.1, 0.25, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (F) Same as panel B using the results depicted in panel E. (G) Progress curves at various dTTP concentrations, 0.1, 0.25, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations. (H) Same as panel B using the results depicted in panel G.

Sup Fig 3. Kinetic insertion of dNMP opposite THF by the tm RB69 pol with different Penultimate Base-Pairs (PBP). (A) Progress curves at various dCTP concentrations, 0.1, 0.25, 0.5, 1, 2, and 3mM (from bottom to top) with dT/dA as the PBP, fit to single-exponential equations. (B) Plot of  $k_{obs}$  vs [dCTP] fit to hyperbola equation to yield  $k_{pol}$  and  $K_{d,app}$ . (C) Progress curves at various dCTP concentrations, 0.1, 0.25, 0.5, 1, 2, and 3mM (from bottom to top) with dA/dT as the PBP, fit to single-exponential equations. (D)  $k_{pol}/K_{d,app}$  is estimated by the slope of the plot of  $k_{obs}$  vs [dCTP]. (E) Progress curves at various dGTP concentrations, 0.1, 0.5, 1, 2, 3.5 and 6mM (from bottom to top) with dA/dT as the PBP, fit to single-exponential equations. (F) Same as panel B using the results depicted in panel E.

Sup Fig 4. Kinetic insertion of dTMP opposite THF by the tm RB69 pol with different Penultimate Base-Pairs (PBP). (A) Progress curves at various dTTP concentrations, 0.1, 0.5, 1, 2, 3.5 and 6mM (from bottom to top) with dG/dC as the PBP, fit to single-exponential equations. (B) Plot of  $k_{obs}$  vs [dTTP] fit to hyperbola equation to yield  $k_{pol}$  and  $K_{d,app}$ . (C) Progress curves at various dTTP concentrations, 0.1, 0.5, 1, 2, 3.5 and 6mM (from bottom to top) with dA/dT as the PBP, fit to single-exponential equations. (D)  $k_{pol}/K_{d,app}$  is estimated by the slope of the plot of  $k_{obs}$  vs [dTTP]. (E) Progress curves at various dTTP concentrations, 1, 2, 3.5 and 6mM (from

bottom to top) with dT/dA as the PBP, fit to single-exponential equations. (F)  $k_{\text{pol}}/K_{\text{d,app}}$  is estimated by the slope of the plot of  $k_{\text{obs}}$  vs [dTTP].

Sup Fig 5. Kinetic insertion of dAMP opposite dT bypassing a dN/THF lesion by the tm RB69pol. (A) Progress curves at various dATP concentrations, 0.5, 1, 2, and 3mM (from bottom to top) bypassing dA/THF, fit to single-exponential equations. (B) Plot of  $k_{\text{obs}}$  vs [dATP] fit to hyperbola equation to yield  $k_{\text{pol}}$  and  $K_{\text{d,app}}$ . (C) Progress curves at various dATP concentrations, 0.5, 1, 2, and 4mM (from bottom to top) bypassing dC/THF, fit to single-exponential equations. (D) Plot of  $k_{\text{obs}}$  vs [dATP] fit to hyperbola equation to yield  $k_{\text{pol}}$  and  $K_{\text{d,app}}$ . (E) Progress curves at various dATP concentrations, 0.5, 1, 2 and 4mM (from bottom to top) bypassing dG/THF, fit to single-exponential equations. (F) Plot of  $k_{\text{obs}}$  vs [dATP] fit to hyperbola equation to yield  $k_{\text{pol}}$  and  $K_{\text{d,app}}$ . (G) Progress curves at various dATP concentrations, 0.5, 1, 2, and 4mM (from bottom to top) bypassing dT/THF, fit to single-exponential equations. (H)  $k_{\text{pol}}/K_{\text{d,app}}$  is estimated by the slope of the plot of  $k_{\text{obs}}$  vs [dATP].