

Supplemental material

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Figure S1. **Comparison of H410C and G343C cyt-DATL PIFE kinetics. (A and B)** Stopped-flow PIFE of WT cyt-DATL labeled with Cy3 on H410C (A) or G343C (B) after addition of excess GTP. **(C)** H410C and G343C PIFE traces overlaid for comparison. For each, cyt-DATL was 2 μ M, and GTP was 1 mM (final concentrations). Traces were normalized (minimum value = 0; maximum value = 1). All traces are the average of three to five individual traces and representative of two independent protein preparations.



Figure S2. **Comparison of WT cyt-DATL PIFE kinetics under single- (limiting GTP) and multiple (saturating GTP)-turnover conditions.** The multiple-turnover condition had 2 μ M cyt-DATL and 1 mM GTP, whereas the single-turnover condition had 15 μ M cyt-DATL and 7.5 μ M GTP. Traces were normalized for ease of comparison (initial value = 0; maximum value = 1).



Figure S3. Atlastin binds mant-dGTP and mant-GTP with similar kinetics. (A and B) Binding of 10 μM mant-dGTP (A) or 10 μM mant-GTP (B) to 100 nM cyt-DATL (black lines) shows similar kinetics. A fit of each to a single exponential decay equation (red lines) shows a lesser fit for mant-GTP (B) that was likely due to the presence of a mixture of 2'- and 3'-OH-labeled nucleotides, whereas mant-dGTP (A) binding fit well.