Fig 2 – Supplement 1: CMG requires a 3' dT_{40}/ssDNA tail for efficient loading

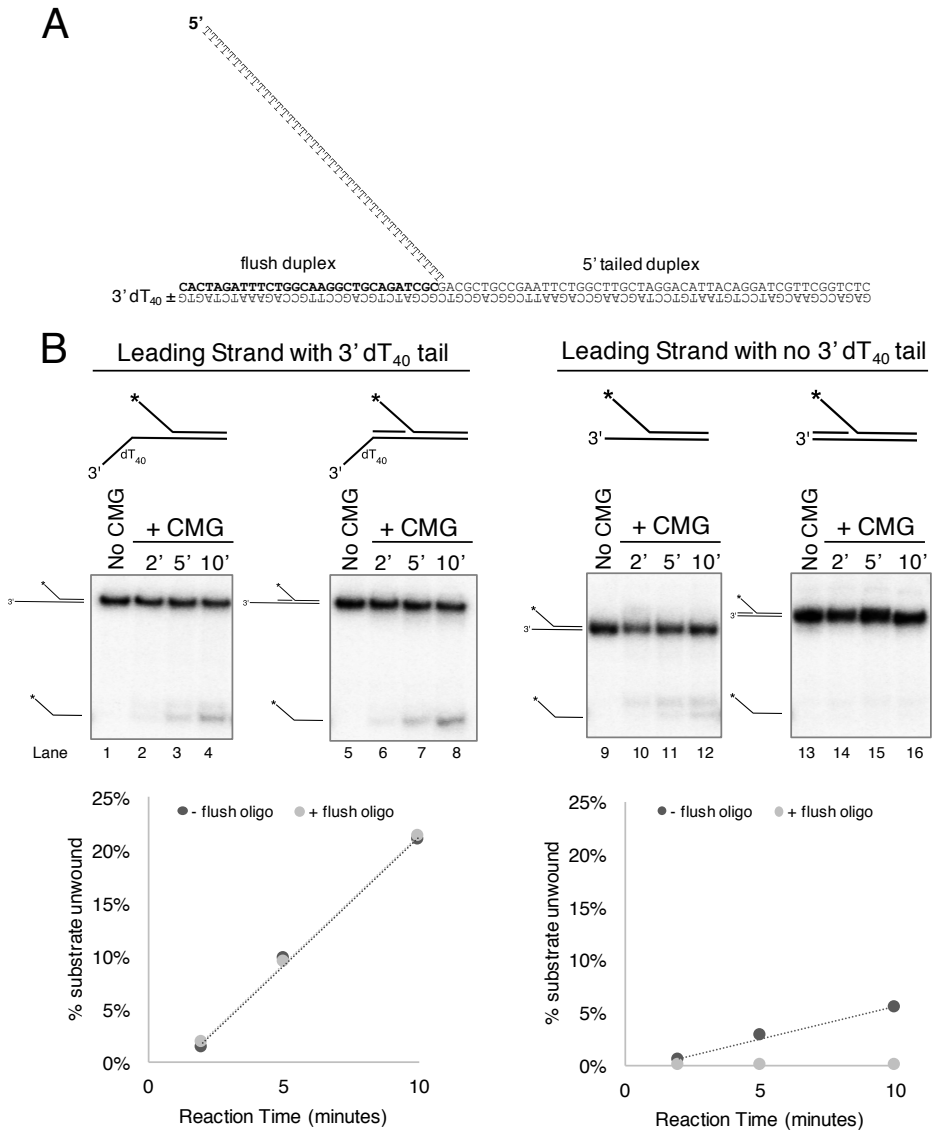

Figure 2 – Supplement 1: **CMG requires a 3' dT_{40}/ssDNA tail for loading.**
(A) Schematic of the substrates used in Figure 2 and in part (B) of this Figure. Further details on the oligos used are in Table I. The flush duplex oligo is named "flush duplex LAG". The 5' tailed oligo is "50duplex LAG". The leading strand template is either "Paired duplex LEAD + 3' tail" or "Paired duplex LEAD no 3' tail".

(B) Control experiments to show the requirement for the 3' dT_{40} tail in CMG loading. At left are two helicase assays like those described in Figure 2 but using the substrate containing a 3' dT_{40} tail with the radiolabel on the 5' tailed duplex. Lanes 1-4 show unwinding in the absence of the flush duplex oligo and lanes 5-8 in the presence of the flush duplex. The % of 5' tailed oligo unwound is quantified in the graph below the gels, showing that the presence of the flush duplex oligo does not affect CMG loading or unwinding. At right is an identical pair of experiments but with a substrate that does not contain the 3' dT_{40} tail. Lanes 9-12 and the quantitation in the graph below show that unwinding is greatly reduced in the absence of the 3' tail (compare lanes 9-12 with lanes 1-4), and in the presence of the flush duplex oligo (lanes 13-16) CMG does not load/unwind at all. These experiments support the conclusion of Figure 2 that CMG translocates over flush duplex without unwinding and requires a 3' dT_{40} tail for efficient loading.