Fig 3 – Supplement 6: Monovalent Streptavidin (SA) tetramer also inhibits CMG unwinding of substrate with dual biotin on the lagging strand

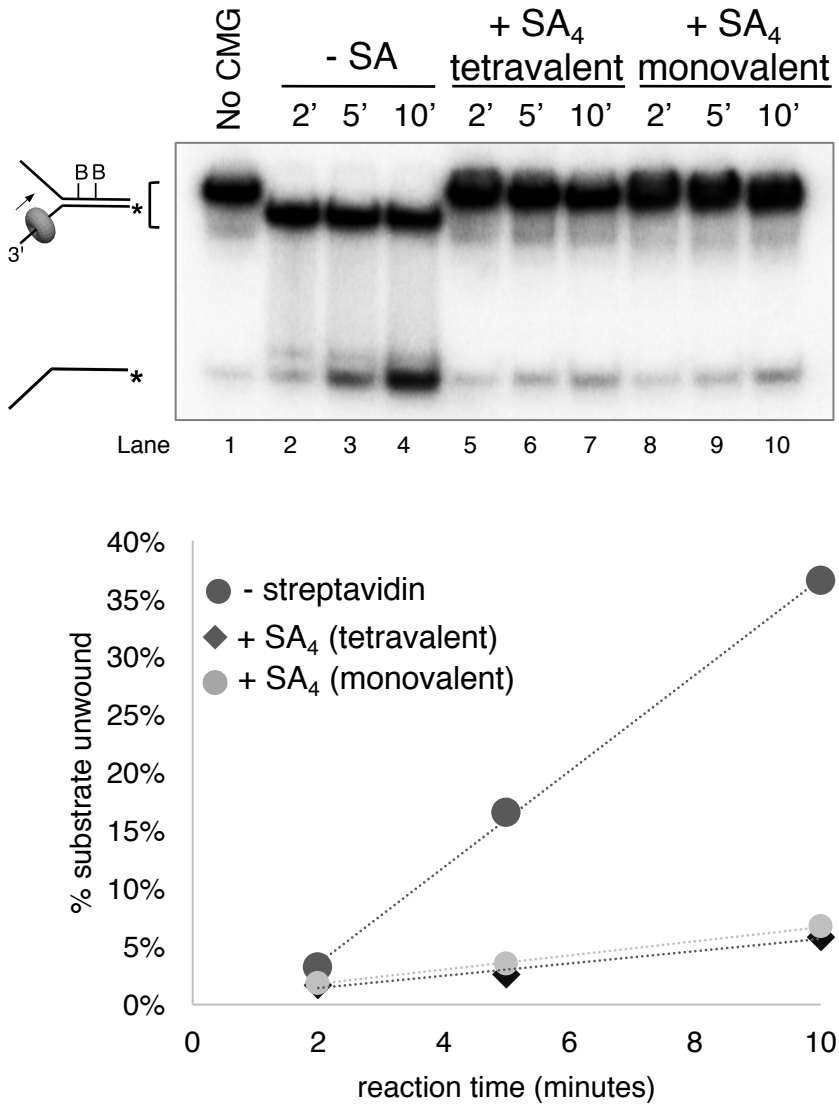

Figure 3 – Supplement 6: **Monovalent streptavidin (SA) tetramer inhibits CMG unwinding to the same extent as tetravalent streptavidin on the substrate with dual biotin on the lagging strand.**
Three helicase assays were performed under otherwise identical conditions comparing CMG unwinding in the absence of streptavidin (lanes 2-4); in the presence of the tetravalent streptavidin used in the experiments of Figures 3-6 (lanes 5-7); and in the presence of a monovalent form of streptavidin in which only one of the four protomers of the streptavidin tetramer binds to biotin while retaining the high affinity of the tetravalent form (Howarth et al., 2006). As shown in the graph below the gel, monovalent streptavidin inhibits CMG unwinding to the same extent as the standard tetravalent form, indicating that inhibition of the helicase is not attributable to distortion of the duplex from "cross-linking" of two nearby biotins by a single streptavidin tetramer. In these experiments, each reaction contained 20 nM CMG in a total reaction volume of 45 µl with 12 µl samples stopped with EDTA/SDS at the times indicated in the Figure. For the reaction in lanes 5-7, 4 µg/ml (final concentration) tetravalent streptavidin was incubated with the substrate at 30°C for 5' and then on ice for 10' before addition of CMG. For the reactions in lanes 8-10, 0.5 µg/ml (final oncentration) of monovalent streptavidin was used instead.