



Supplemental Figure 5. Primer extension from mismatched substrates requires exonuclease activity.

(A) Primer extension from mismatched substrates by wild-type Mtb DnaE1 and *E. coli* PolIII $\alpha+\epsilon$ is blocked by a phosphorothioate linkage (denoted by -S-) that is resistant to exonuclease activity. In contrast, a matched primer with a terminal phosphorothioate linkage can be extended normally. **(B)** Addition of *E. coli* ϵ -exonuclease in *trans* allows Mtb DnaE1 PHP mutants to extend from mismatched DNA substrates.