**Supporting Information Figure S1** Proteins Used. **(A)** Diagram and partial domain map of hexahistidine tagged TRF1, TRF2, TRF2∆B and TRF2∆M compared against endogenous TRF2 (Q15554.2) and endogenous TRF1 (NP\_059523.2), partially adapted from*40*. **(B)** Coomassie stained SDS-PAGE gel containing 2 μg of each of purified Rad51, TRF2, TRF2∆B, TRF2∆M, TRF1, and BSA.

**Supporting Information Figure S2** TRF2-mediated D-loop formation requires telomeric homology while Rad51 does not. **(A)** Rad51 promotes telomeric D-loop formation between pBB and T90 (top left), but not between pGL GAP and T90. Rad51 can promote non-telomeric D-loop formation between D1 and both pGL GAP and pBB. **(B)** Quantification of (A). T90+pGL GAP complex formation was too low to accurately quantify and is omitted. **(C)** TRF2 promotes D-loop formation only between T90 and pBB. **(D)** Quantification of (C). D1+pBB, D1+pGL GAP and T90+pGL GAP complex formation were too low to accurately quantify and are omitted.

**Supporting Information Figure S3** TRF2 inhibits Rad51-mediated telomeric D-loop formation only when added early in D-loop reactions. **(A)** Diagram of order of addition experiment. Complex formation was assessed at 3-hrs and 4-hrs. **(B)**  TRF2 inhibits Rad51-mediated telomeric D-loop formation when added early in the reactions (T0 and T0+10min vs no TRF2) but not when added late in the reaction (T0+3hrs). **(C)** Quantification of (B), error bars shown 95% confidence intervals from three independent experiments. (\*) significant difference between indicated samples, paired samples t-test α=0.05. (NS) No significant difference.

**Supporting Information Figure S4** TRF2 inhibits Rad51-mediated telomeric D-loop formation optimally when pre-incubated with dsDNA. **(A)** Diagram of order of addition experiment. **(B)** Telomeric D-loop reactions containing 750 nM Rad51 and 0 or 125 nM TRF2 added as described in (A). **(C)** Quantification of (B), error bars shown 95% confidence intervals from three independent experiments. (\*) significant difference between indicated samples, paired samples t-test α=0.05.

**Supporting Information Figure S5** Area under the curve (AUC) calculation procedures (C% = Complex %). **(A)** A representative activity trace from data shown in Figure 1C. AUC is calculated as (Complex% × [TRF2] nM) for all regions of the activity trace. **(B)** A representative activity trace from data shown in Figure 2A. AUC is calculated as (Complex% × [Rad51] nM) for 500-750 nM [Rad51].

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**Supporting Information Figure S1**

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**Supporting Information Figure S2**

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**Supporting Information Figure S3**

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**Supporting Information Figure S4**

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**Supporting Information Figure S5**

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