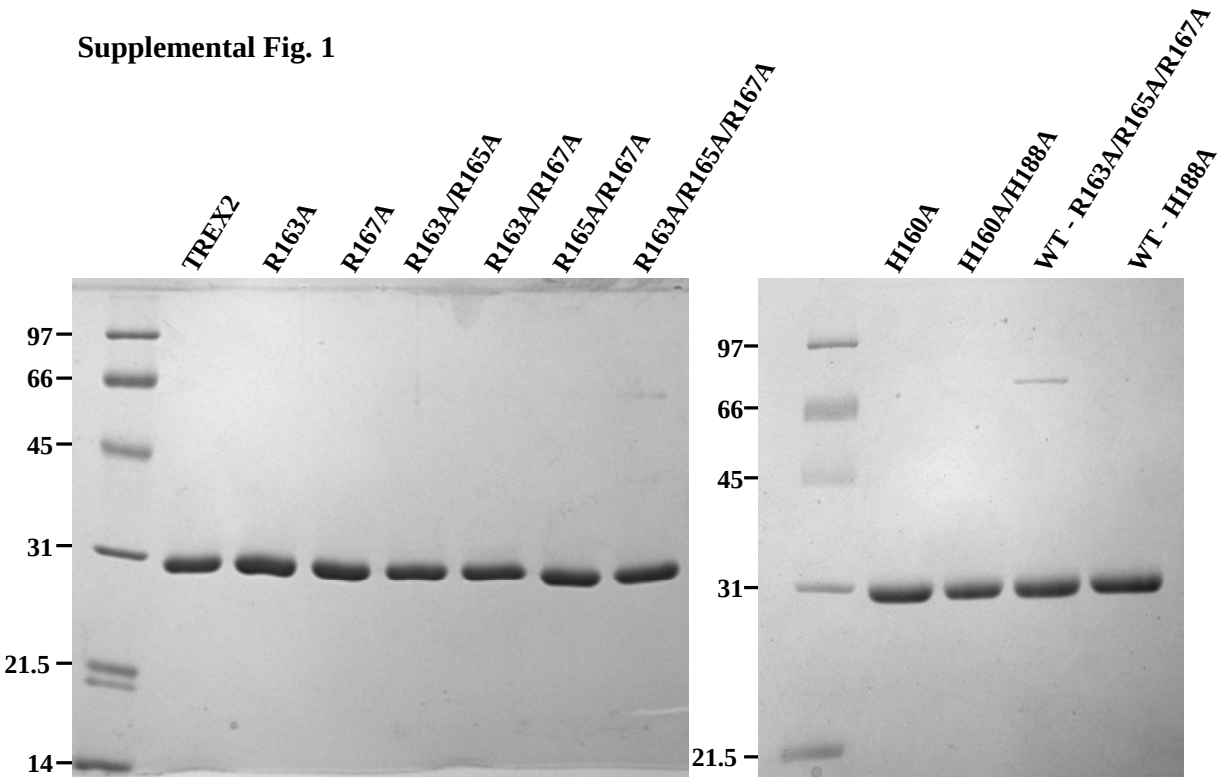
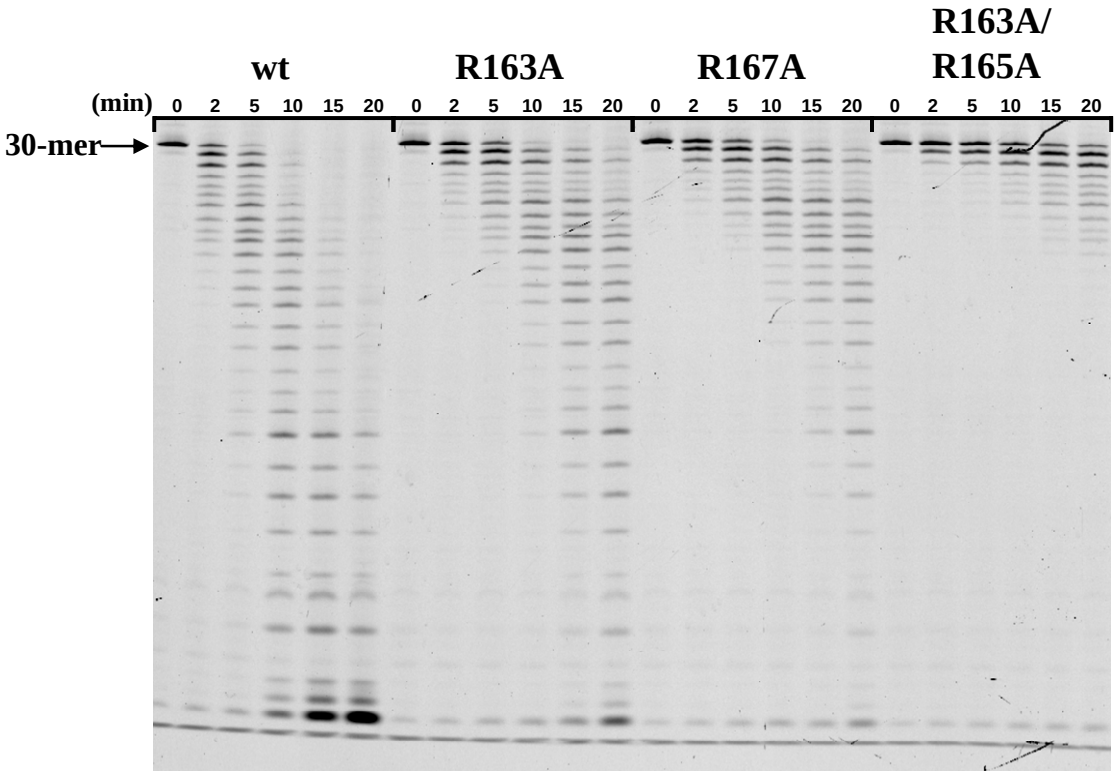
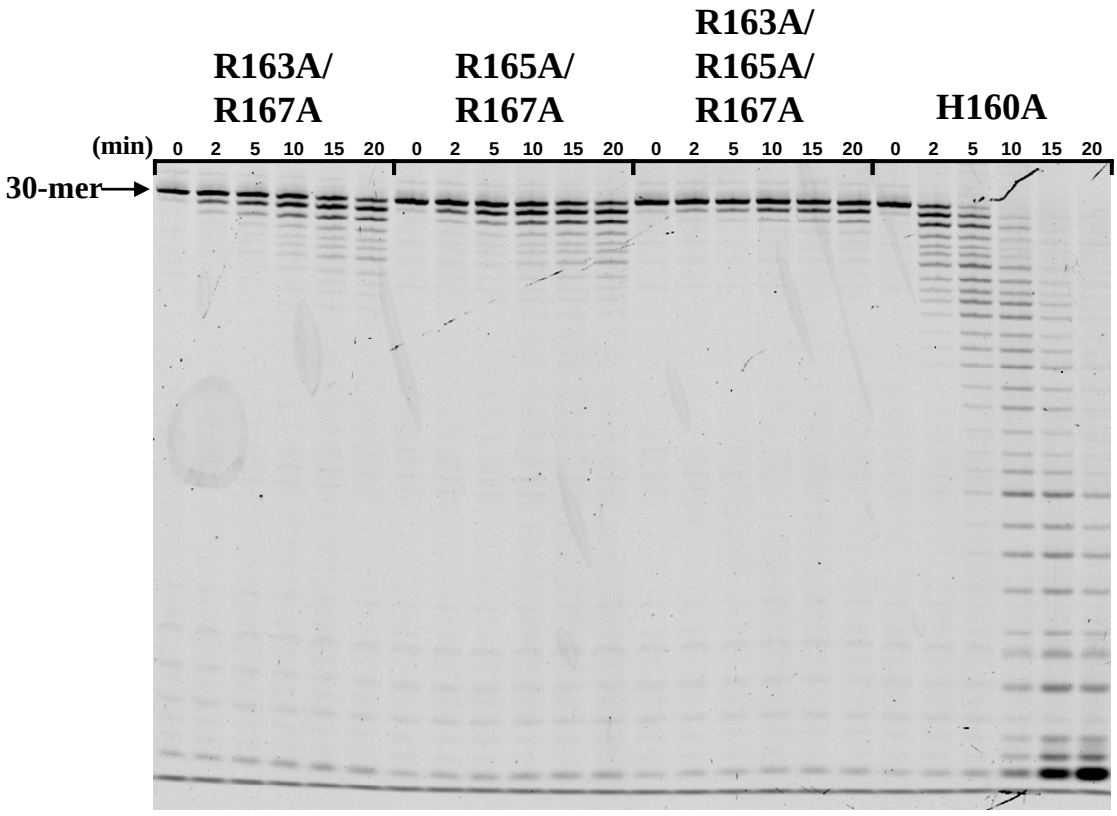
Supplemental Fig. 1



Supplementary Figure 1. Preparation of TREX2 and variants - The plasmid constructs used to express the human TREX2 and variants in *E. coli* and purification of the enzymes has been described (1-3). Enzymes are stored at ~0.5 mg/ml in aliquots at -80 °C. The TREX2 concentrations were determined by A_{280} as described in the text.

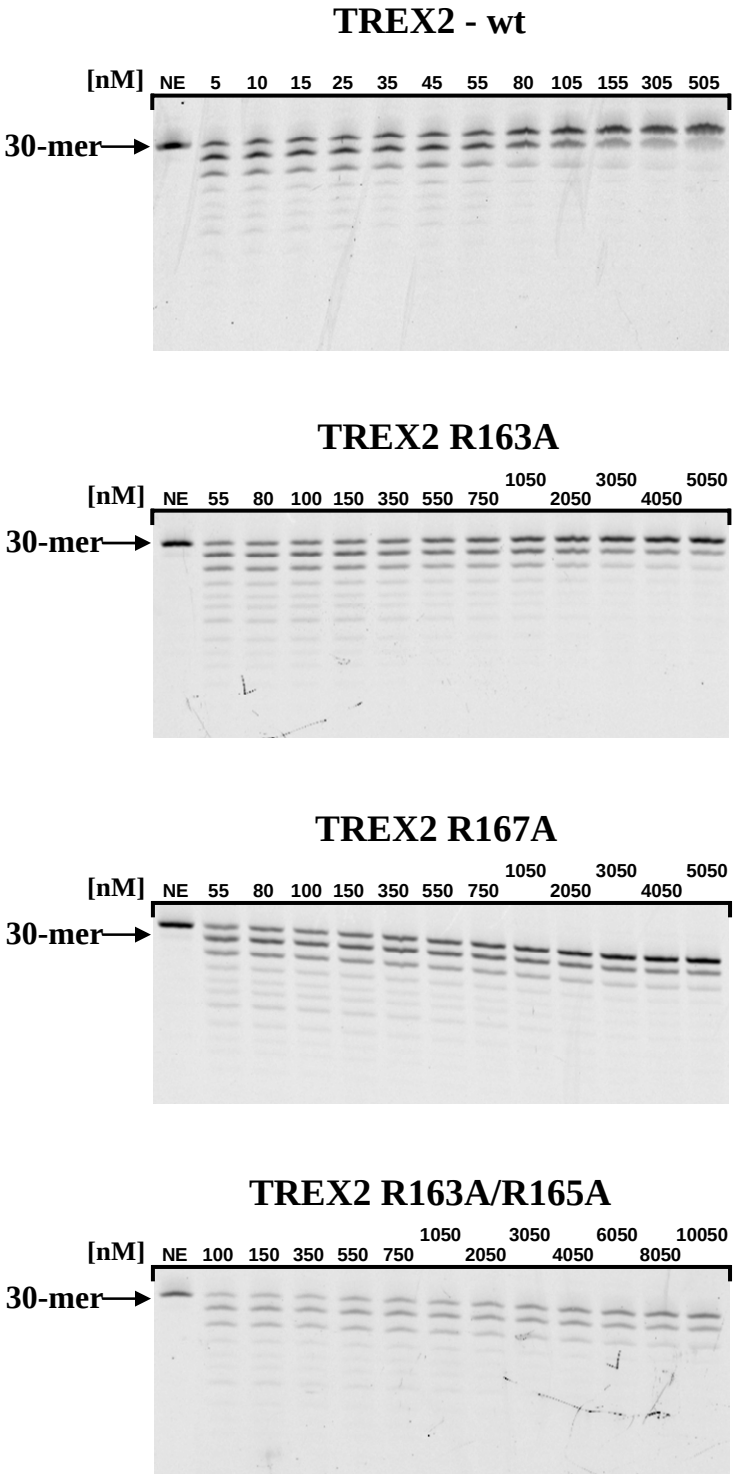
Supplemental Fig. 2



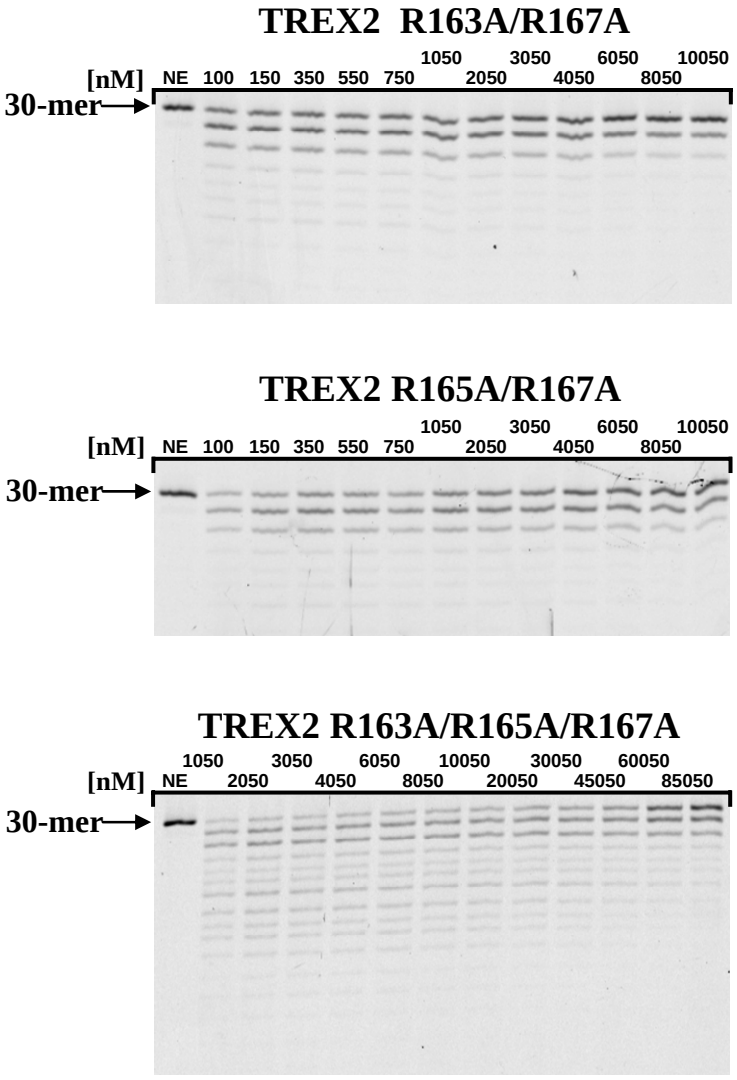


Supplementary Figure 2. Time course reactions (210μl) were prepared as described in the text containing 50 nM FAM-labeled ssDNA 30-mer oligonucleotide and 380 pM TREX2 enzyme. Samples (30 μl) were removed after incubation at 25°C for 2, 5, 10, 15, and 20 minutes, quenched by addition of 90 μl of cold ethanol and dried in vacuo. Pellets were resuspended in 6 μl of formamide, heated to 95°C for 2 min, and separated on 23% denaturing polyacrylamide gels. FAM-labeled oligonucleotide bands were visualized and quantified using a Storm phosphorimager (Molecular Dynamics). The amount of dNMP product was calculated as described in the text. The position of migration of the 30-mer is indicated.

Supplemental Fig. 3



Supplemental Fig. 3 (cont)



Supplementary Figure 3. Steady-state kinetic reactions (10µl or (50µl for wt)) were prepared as described in the text containing the indicated concentration of FAM-labeled ssDNA 30-mer oligonucleotide and 38 pM TREX2^{wt}, 76 pM TREX2^{R163A} or TREX2^{R167A}, 380 pM TREX2$^{R163A-R165A}$, TREX2$^{R163A-R167A}$, or TREX2$^{R165A-R167A}$ and 1500 pM TREX2$^{R163A-R165A-R167A}$ enzyme. Incubations were 20 min at 25°C. Reaction products were processed and quantified as described in the text. Kinetic constants were determined by regression analysis using SigmaPlot 8.02 (SPSS Science, Inc.).

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