

## Supplementary Figures

### Identification of a Small Interface between the Methyltransferase and RNA Polymerase Domain of NS5 that is Essential for Zika Virus Replication

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#### Supplementary Figure 1 Comparison of strain-specific RdRp RNA synthesis activities.

**a**, Denaturing SDS gels showing purified RdRp encoded by Brazilian (SPH2015) and Asian (PRVABC59) strains. **b**, Schematic of RNA elongation assay (top). Denaturing gels showing a time course of single nucleotide incorporation by the indicated RdRp proteins. Template sequence indicated. (left). Plot showing % extension over time (right). **c**, Schematic of *de novo* RNA synthesis assay. Template sequence indicated. (left). Denaturing gel showing *de novo* synthesis performed by the indicated RdRp proteins in quadruplicate (middle). Bar chart showing relative intensities of *de novo* synthesis generated by the indicated RdRp proteins from middle gel. *p* value determined by non-paired, two-tailed student's *t*-test. Data represent mean,  $n = 4$ ,  $\pm$  s.d. RU = relative units. Uncropped and variable contrast gel images are presented in Supplementary Figure 6.

#### Supplementary Figure 2 Controls for *de novo* RNA synthesis.

**a**, Non-denaturing gel showing lack of RNA 3' terminal nucleotidyl transferase activity by NS5 in the presence of ATP. **b**, Schematic of initiation assay (left). Denaturing gel showing *de novo* synthesis for 30 min by NS5 wild-type and mutant proteins on indicated template with 10  $\mu$ M NTPs (middle). Bar chart showing average *de novo* synthesis products generated after 30 min by NS5 wild-type and NS5 mutant enzymes. Data represent mean,  $n = 4$ ,  $\pm$  s.d. RU = relative units (right). Uncropped and variable contrast gel images are presented in Supplementary Figure 6.

#### Supplementary Figure 3

**a**, Uncropped scan of denaturing gel image of Figure 1d (left) and high-contrast scan of Figure 1d (right) in quadruplicate. **b**, Uncropped scan of denaturing gel image scan of Figure 1e (left) and high-contrast scan of Figure 1e in quadruplicate.

#### Supplementary Figure 4

**a**, Uncropped scan of denaturing gel image of Figure 2a (left) and high-contrast scan of Figure 2a. (right) in quadruplicate **b**, Uncropped scan of denaturing gel image scan of Figure 2b (left) and high-contrast scan of Figure 2b (right) in quadruplicate.

### **Supplementary Figure 5**

**a**, Uncropped scan of denaturing gel image of Figure 3b. **b**, Uncropped scan of denaturing gel image of Figure 4c in triplicate. **c**, Uncropped scan of denaturing gel image scan of Supplementary Figure 2 in triplicate (left) and Figure 4d (right).

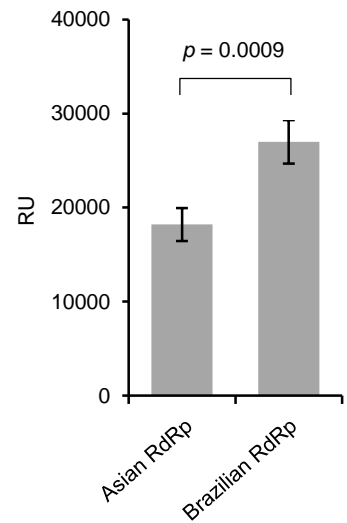
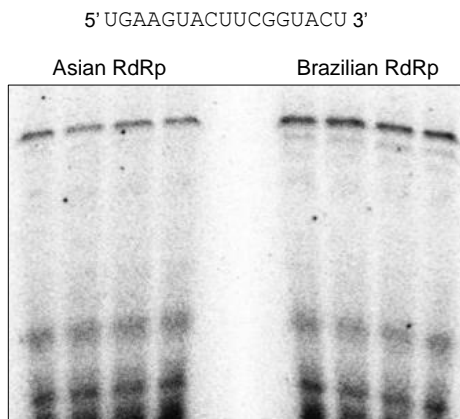
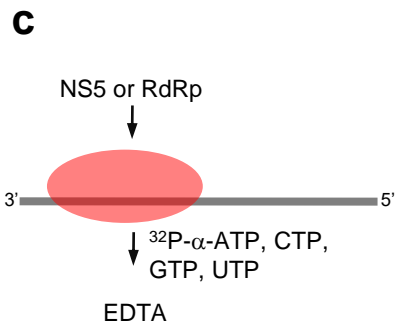
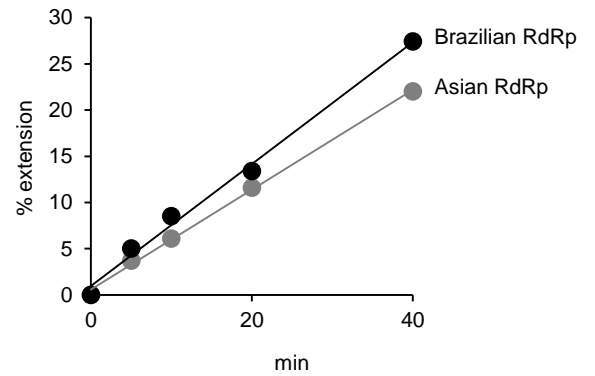
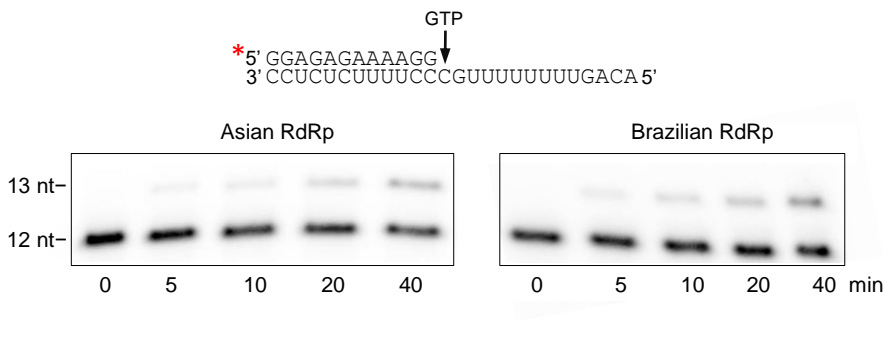
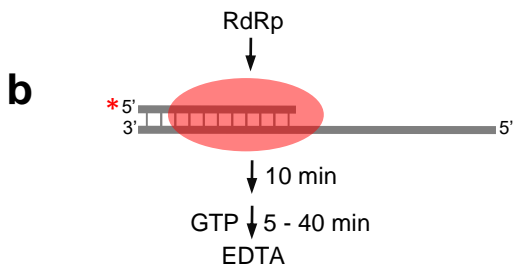
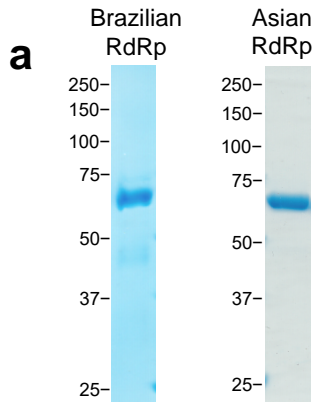
### **Supplementary Figure 6**

**a**, Uncropped scan of denaturing gel image of Supplementary Figure 1b. **b**, Uncropped scan of denaturing gel image scan of Supplementary Figure 1c in quadruplicate.

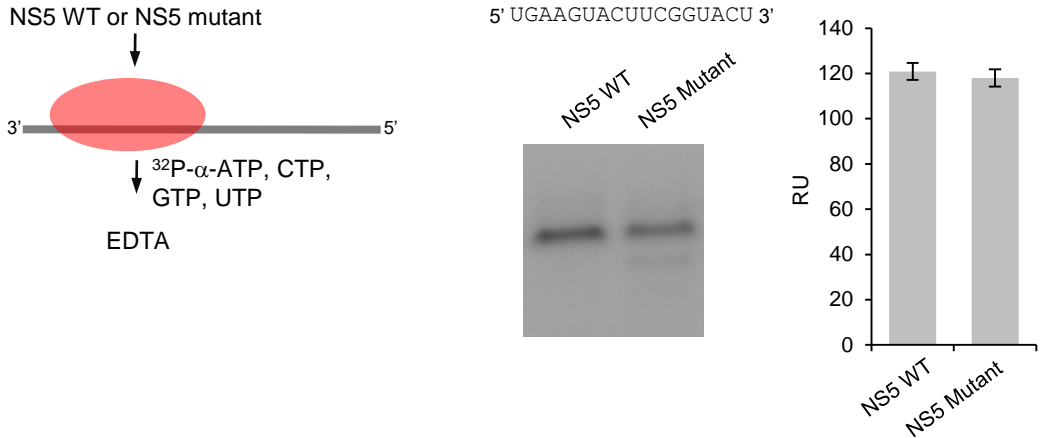
### **Supplementary Figure 7**

**a**. Denaturing gel showing time course of single nucleotide incorporation by Asian RdRp on the indicated template (left). Scatter plot showing % extension over time by RdRp (right). Data represent mean.  $n = 3, \pm$  s.d. **b**. Denaturing SDS gels showing Asian NS5 and Asian RdRp after the indicated incubation period at 37° C in RNA elongation buffer lacking BSA, RNA template and NTPs.

# Supplementary Fig. 1

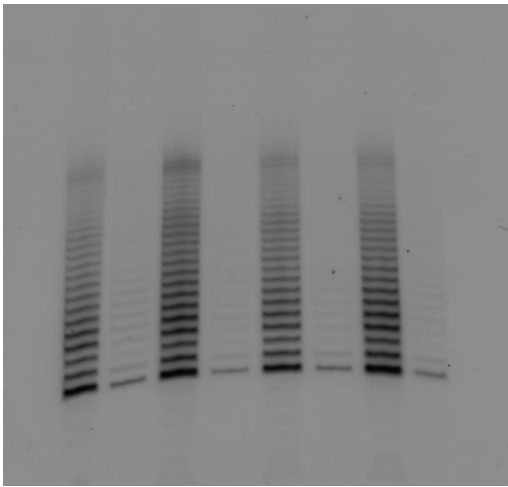


# Supplementary Fig 2

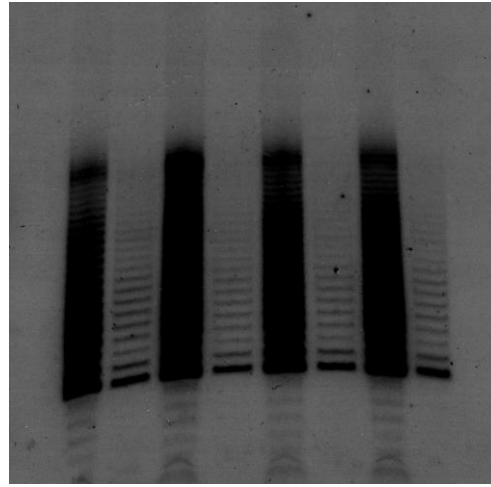


# Supplementary Fig. 3

**a**

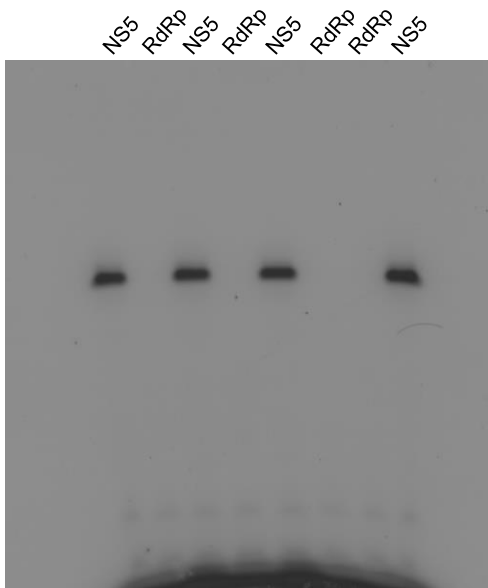


Full-length gel - Fig. 1d

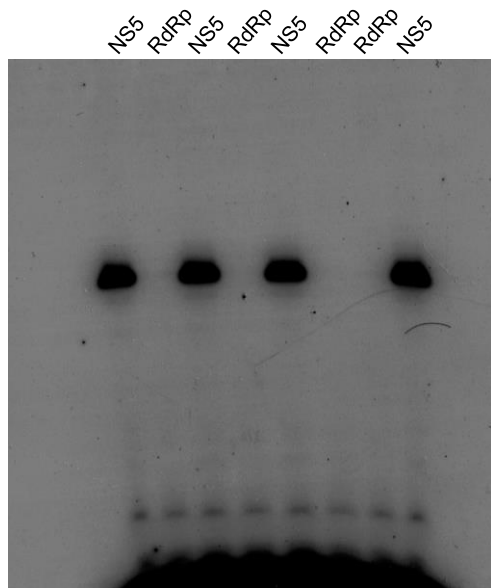


High contrast gel - Fig. 1d

**b**



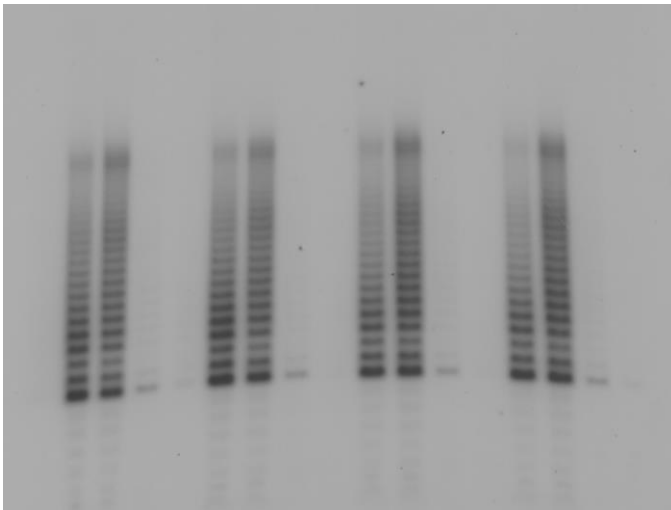
Full-length gel - Fig. 1e



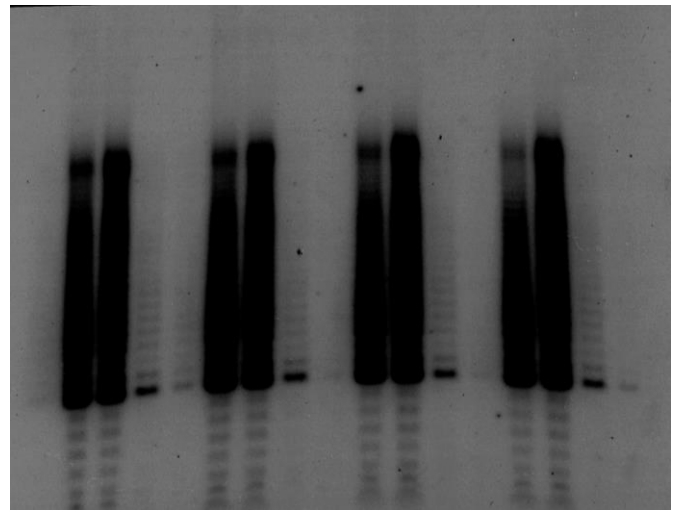
High contrast gel - Fig. 1e

# Supplementary Fig. 4

**a**

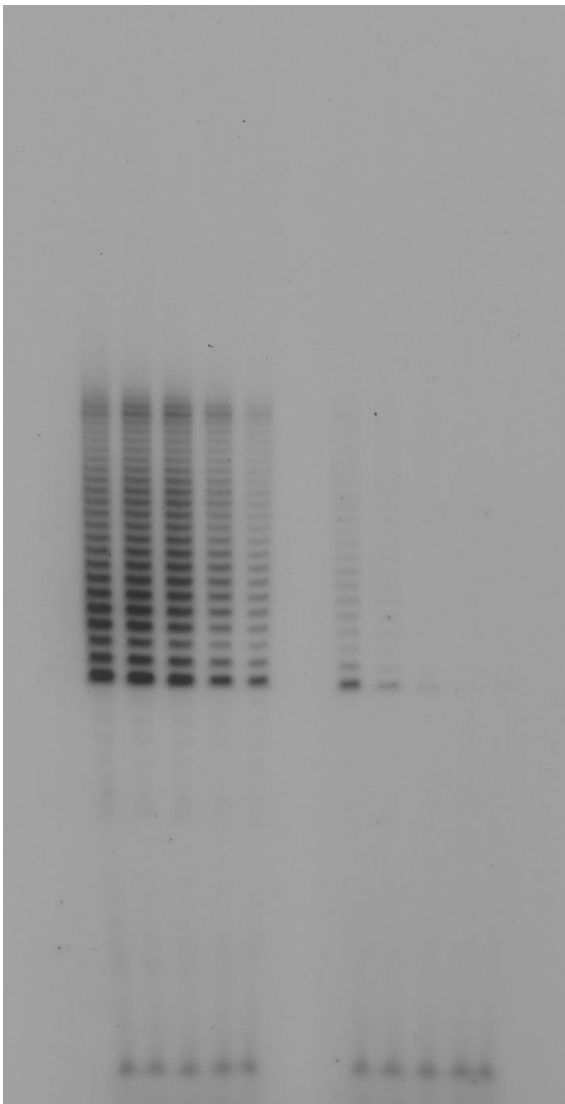


Full length gel - Fig. 2a

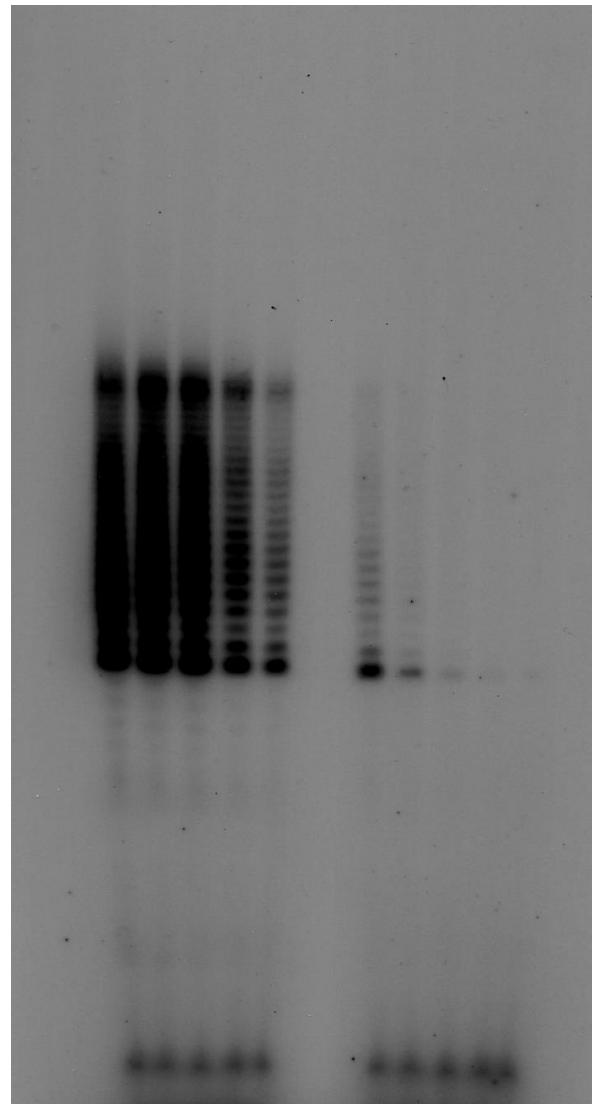


High contrast gel - Fig. 2a

**b**



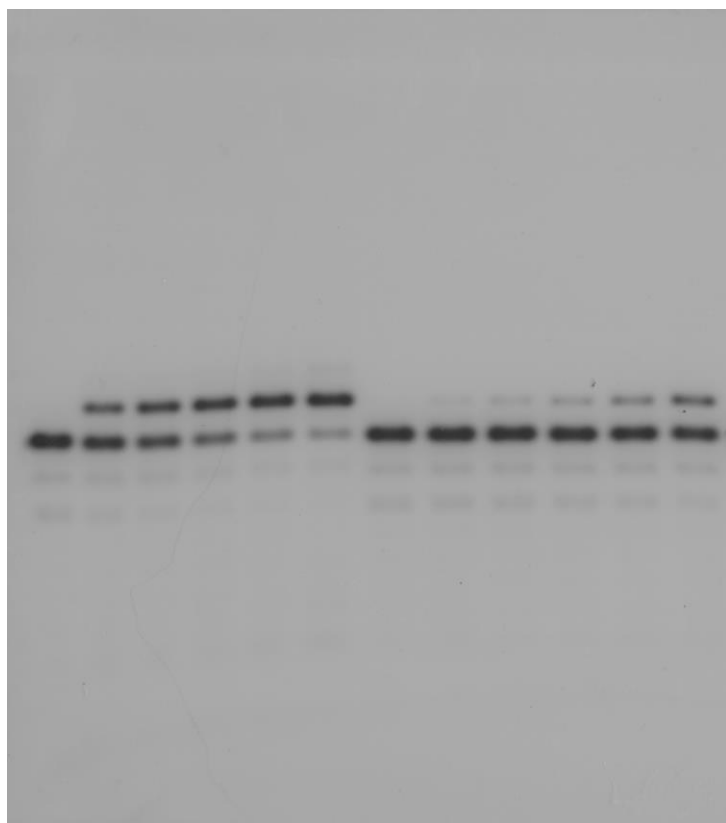
Full length gel - Fig. 2b



High contrast gel - Fig. 2b

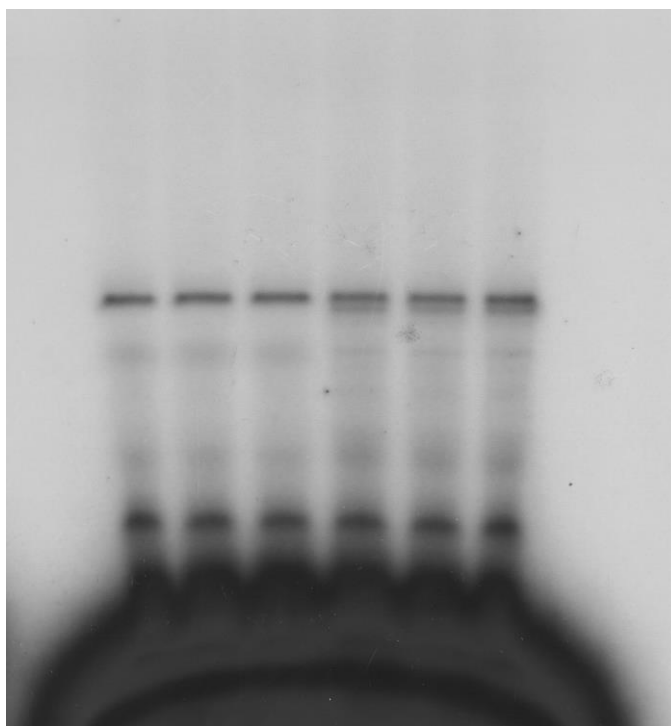
# Supplementary Fig. 5

**a**



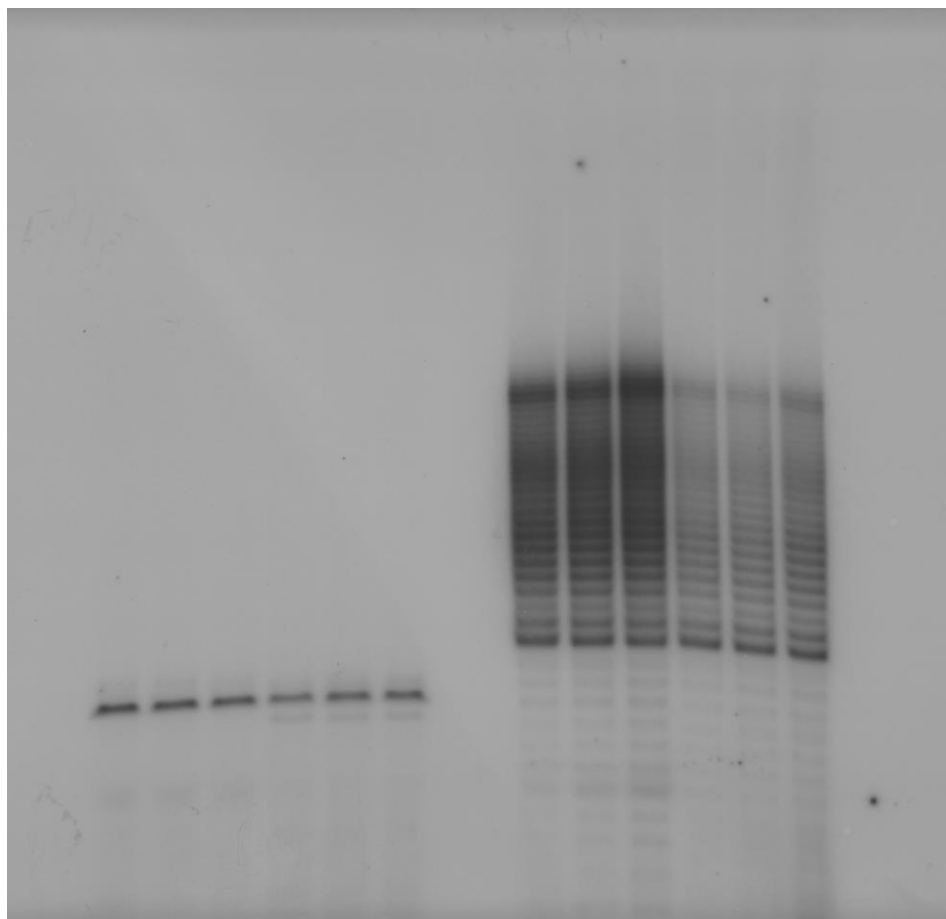
Full length gel - Fig. 3b

**b**



Full length gel – Fig. 4c

**c**

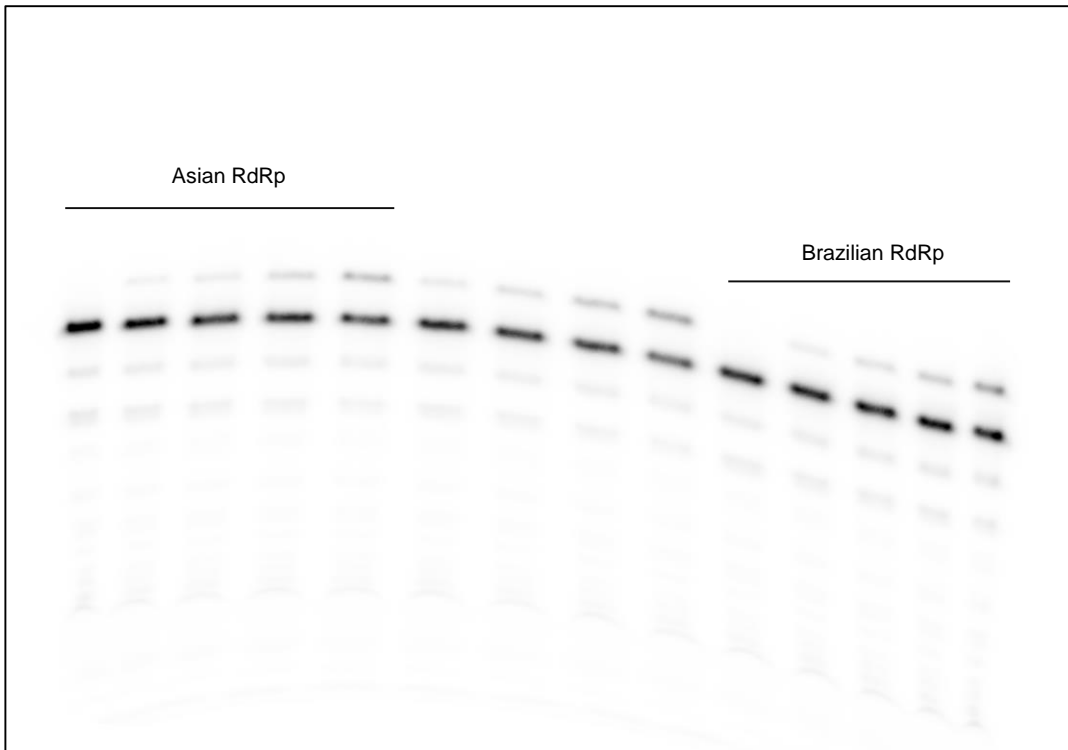


Full length gel – Supplementary Fig. 2

Full length gel – Fig. 4d

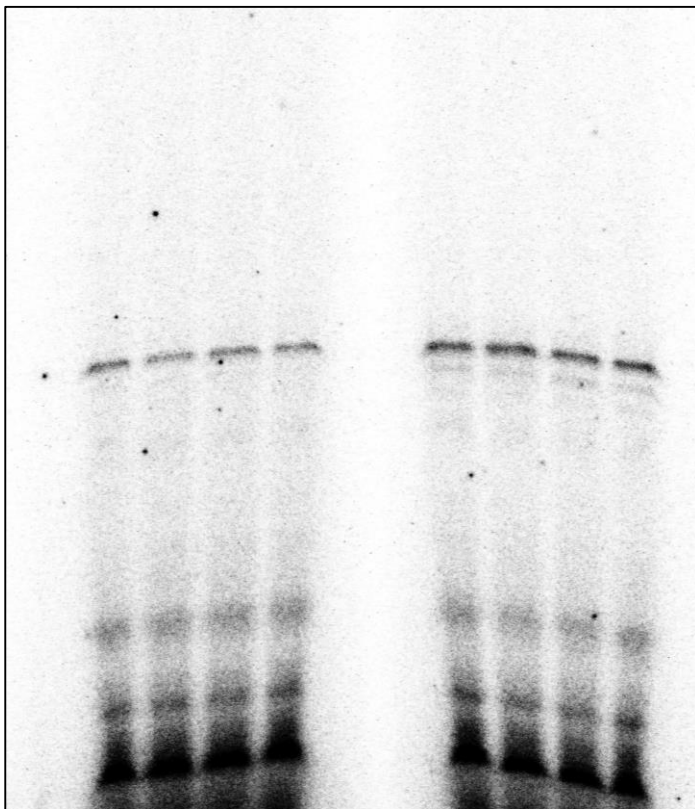
# Supplementary Fig. 6

**a**



Full length gel – Supplementary Fig. 1b

**b**

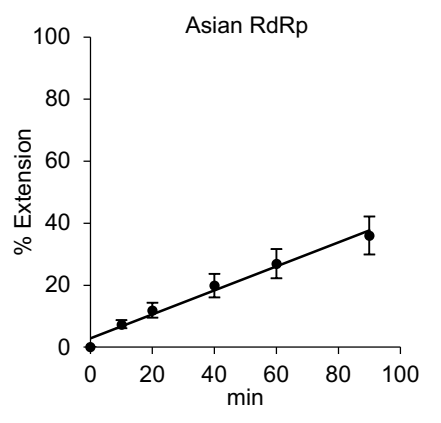
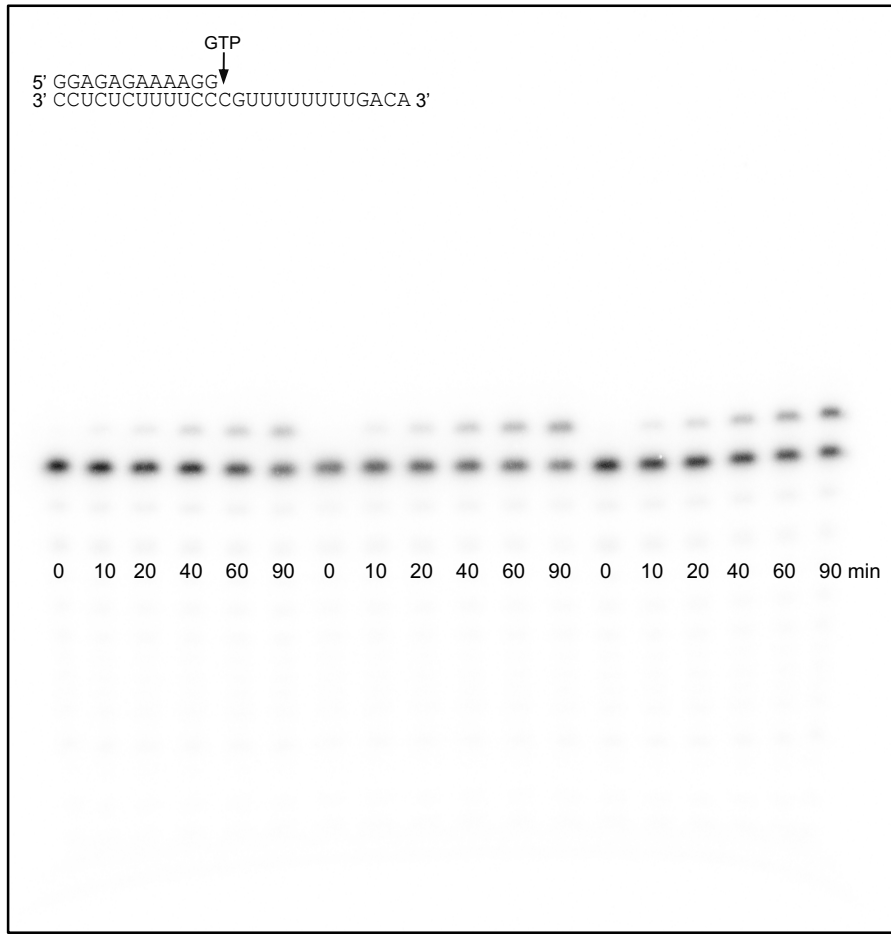


Full length gel – Supplementary Fig. 1c



# Supplementary Fig. 7

**a**



**b**

