

INTRODUCTION

- Human coronaviruses can cause thrombosis with serious clinical consequences, such as deep vein thrombosis, pulmonary embolism and stroke.
- HCoV-229E is a prevalent cold virus, giving inflammation and possible pneumonia.
- Enveloped viruses acquire host proteins as they egress from the cell (Fig. 1A)

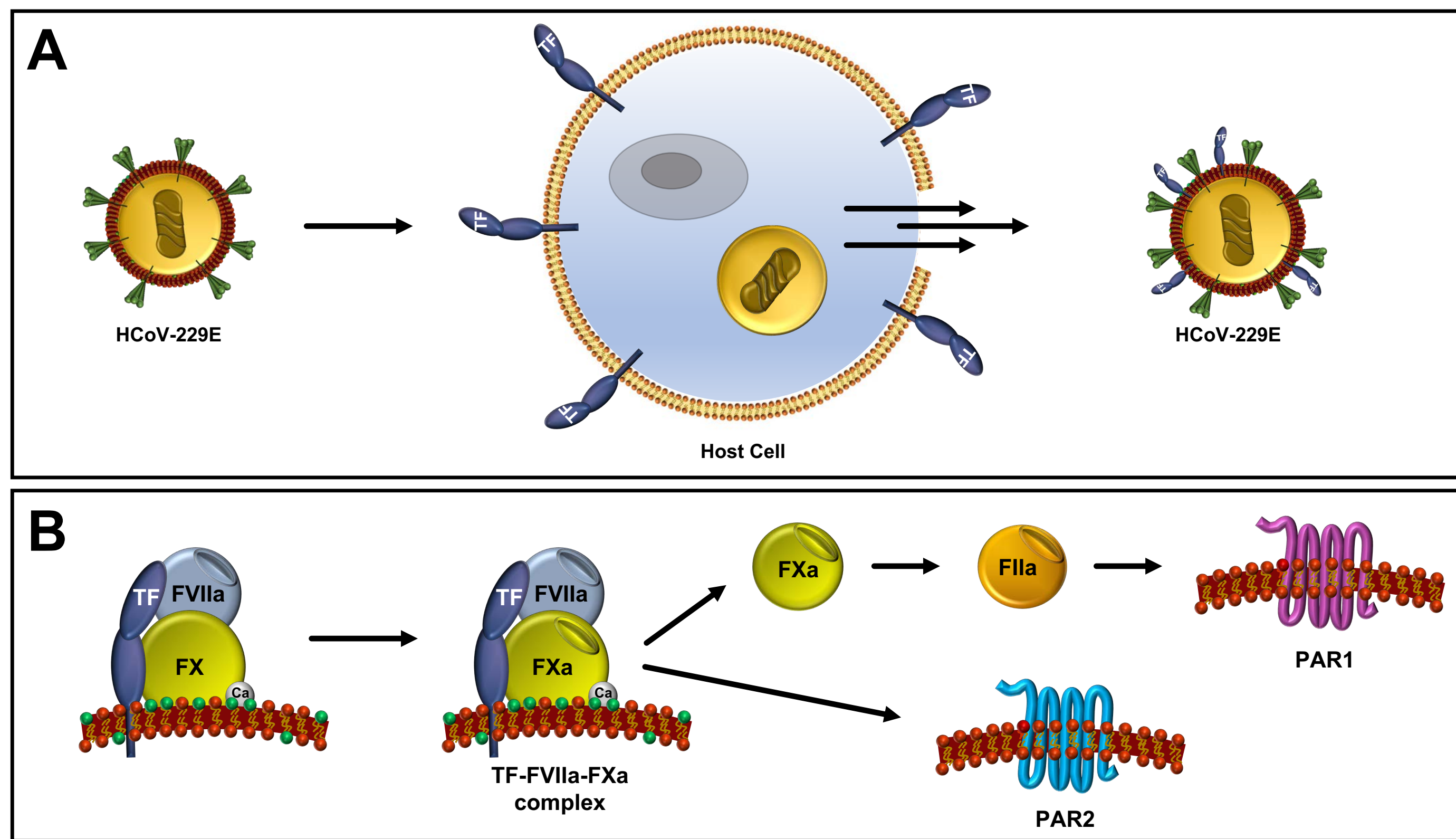


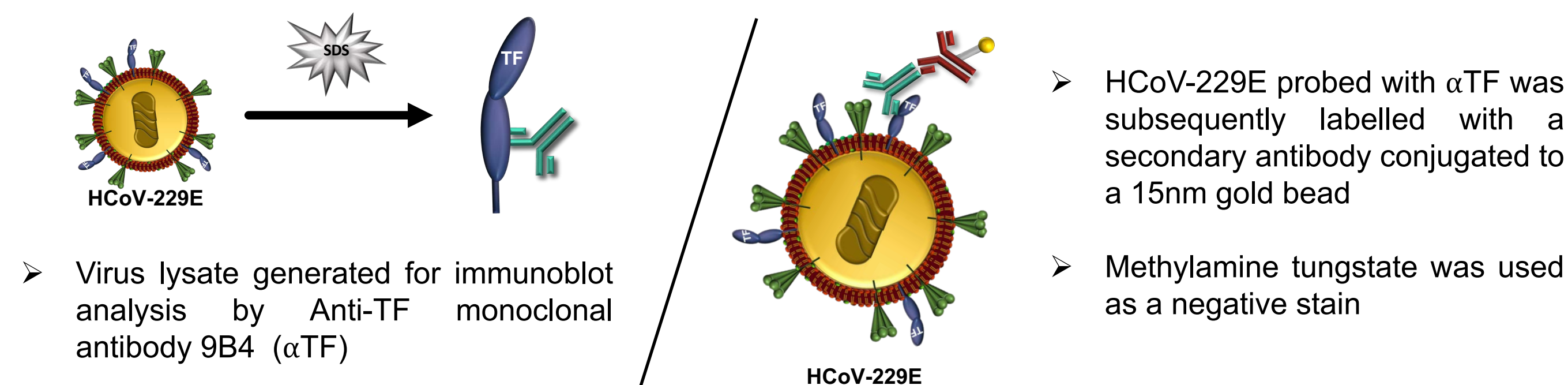
Figure 1. A) HCoV-229E egression **B)** Protease Activated Receptor (PAR) signaling by tissue factor (TF) and clotting proteases.

- TF, a host cellular protein, is a coagulation cofactor that accelerates factor VIIa (FVIIa)-mediated activation of factor X (FX) to factor Xa (FXa) (Fig. 1B)
- TF also signals cells through PAR2 from within the TF/FVIIa/FXa complex or through PAR1 by downstream-generated thrombin (FIIa) (Fig. 1B)

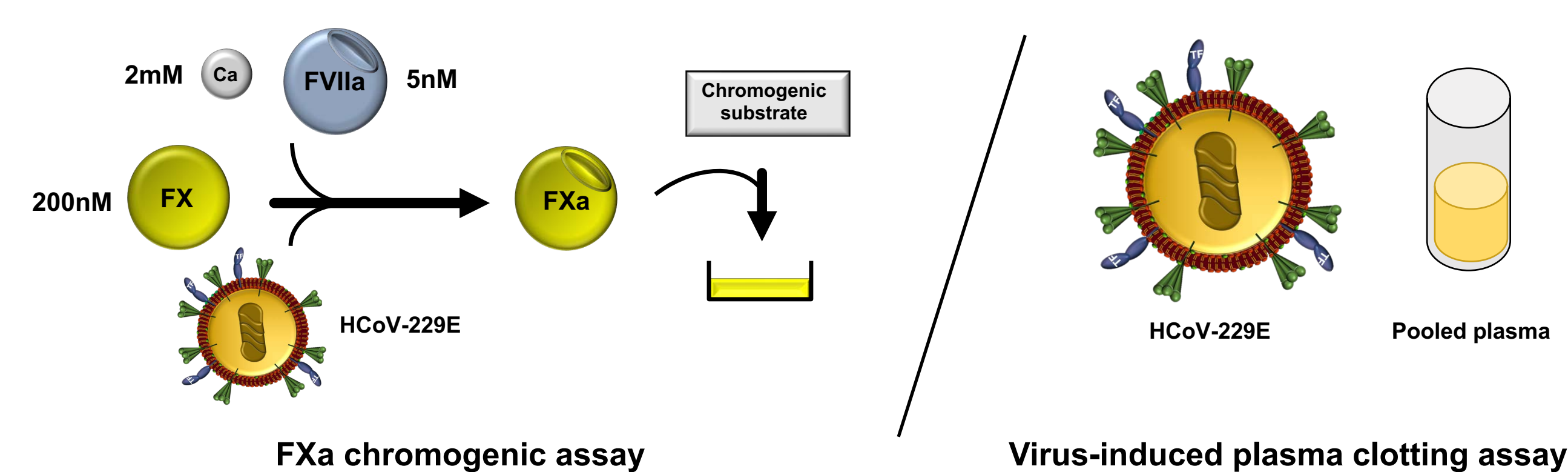
We hypothesize that, when replicated in TF-bearing cells, HCoV-229E can acquire TF and that viral TF will be functional.

AIMS AND METHODS

1: Identify TF antigen on HCoV-229E by immunoblot / electron microscopy.



2 + 3: Functional assays to characterize HCoV-229E TF activity.



1: DETECTION OF TF ANTIGEN

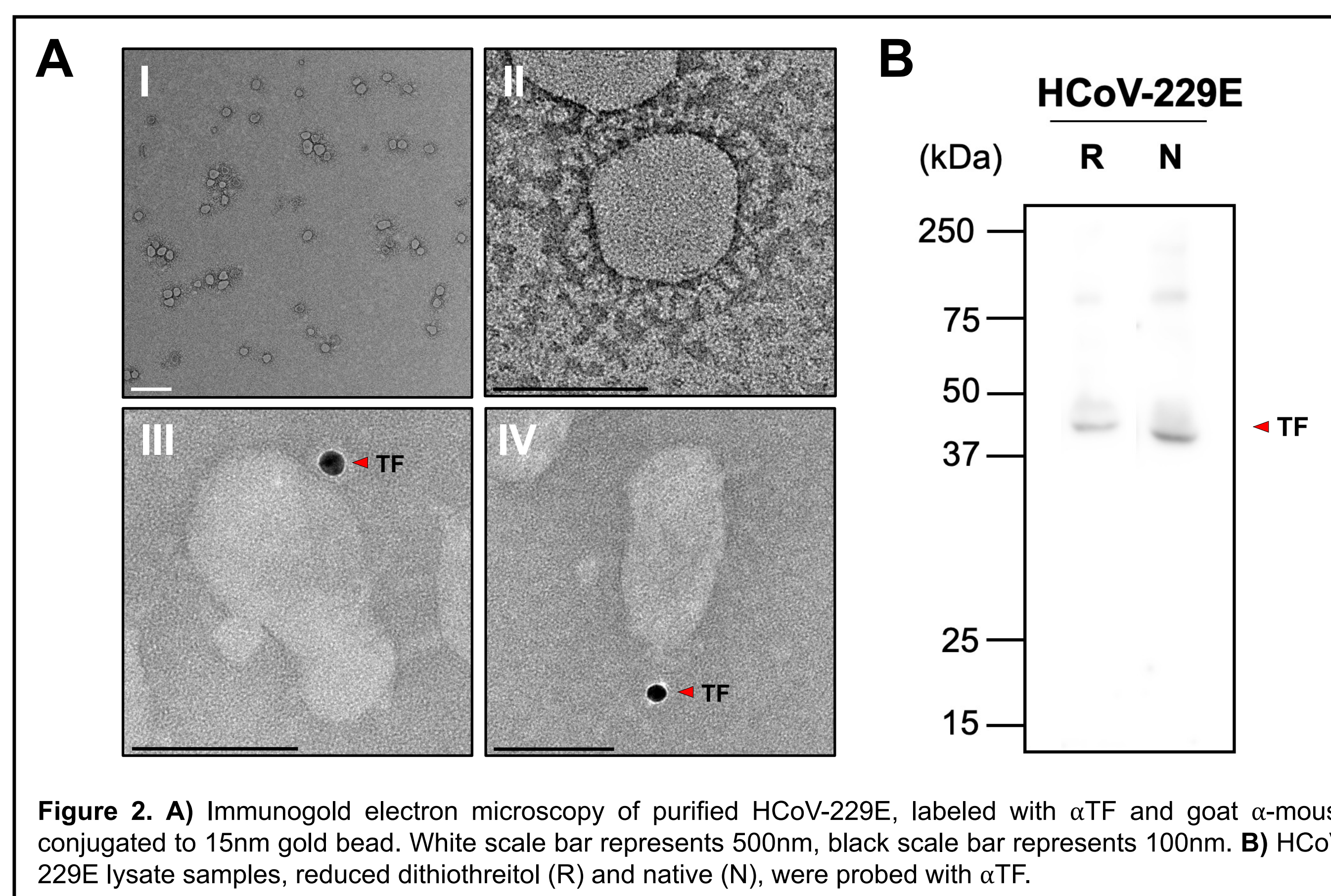


Figure 2. A) Immunogold electron microscopy of purified HCoV-229E, labeled with α TF and goat α -mouse conjugated to 15nm gold bead. White scale bar represents 500nm, black scale bar represents 100nm. **B)** HCoV-229E lysate samples, reduced dithiothreitol (R) and native (N), were probed with α TF.

- Fig 2A: TF is labeled on purified HCoV-229E (III and IV), unlabeled HCoV-229E is identifiable by the distinct spike "corona" (II)
- A distinct TF band is observed at approximately 47kDa by immunoblot analysis

The quality and purity of the HCoV-229E is confirmed and TF antigen is on purified HCoV-229E.

2: DOES VIRAL TF ACCELERATE FVIIa?

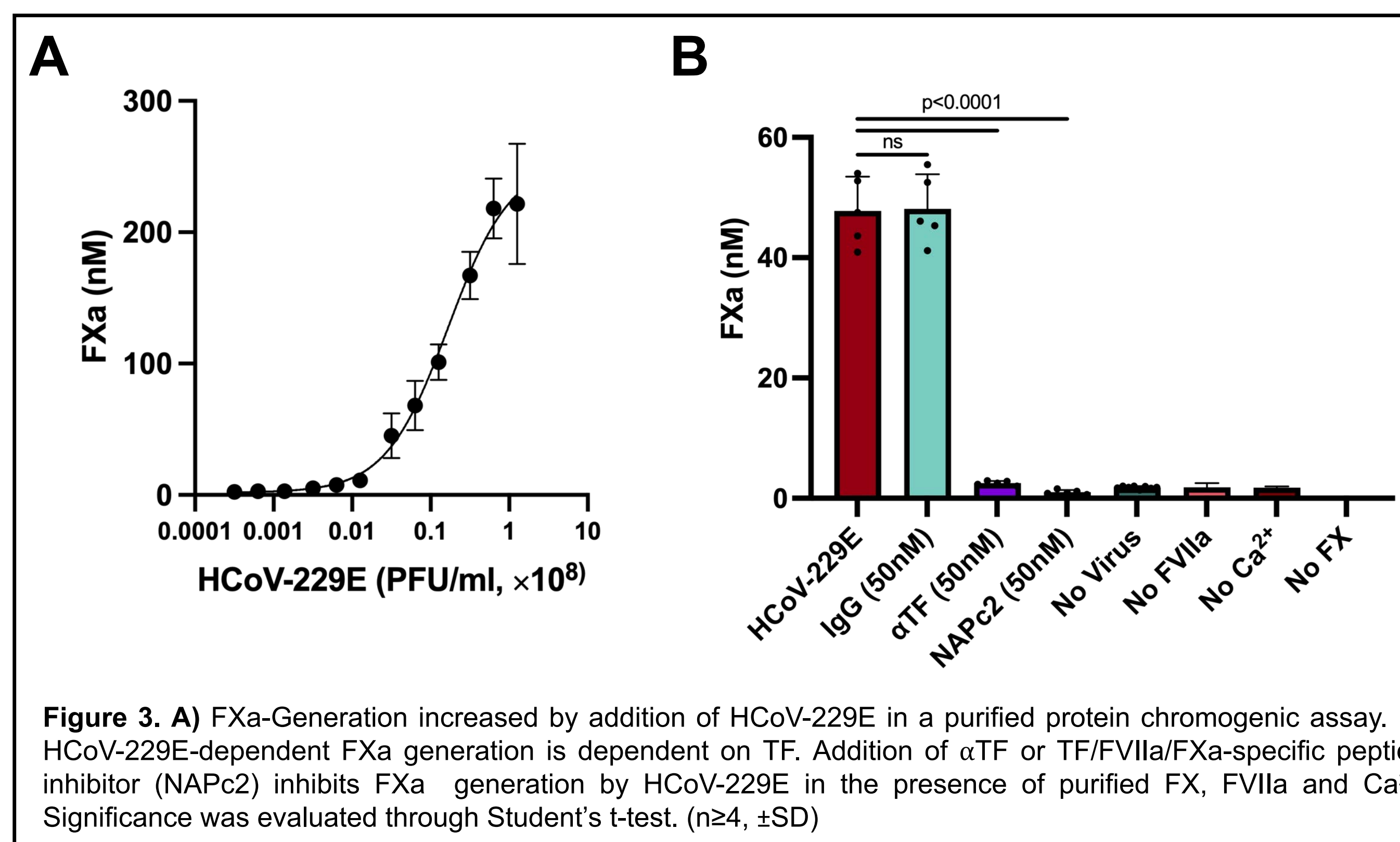


Figure 3. A) FXa-Generation increased by addition of HCoV-229E in a purified protein chromogenic assay. **B)** HCoV-229E-dependent FXa generation is dependent on TF. Addition of α TF or TF/FVIIa/FXa-specific peptide inhibitor (NAPc2) inhibits FXa generation by HCoV-229E in the presence of purified FX, FVIIa and Ca^{2+} . Significance was evaluated through Student's t-test. (n \geq 4, \pm SD)

- Fig. 3A: Purified HCoV-229E is sufficient to generate procoagulant FXa species
- Fig. 3B: HCoV-229E FXa generation is TF-dependent and can be reduced by two different types of TF-specific inhibitors

TF on HCoV-229E has cofactor activity, allowing for the production of FXa from purified FX. FXa generated by HCoV-229E may facilitate procoagulant activity and cell signaling functions.

3: DOES TF ON HCoV-229E MAKE PLASMA CLOT?

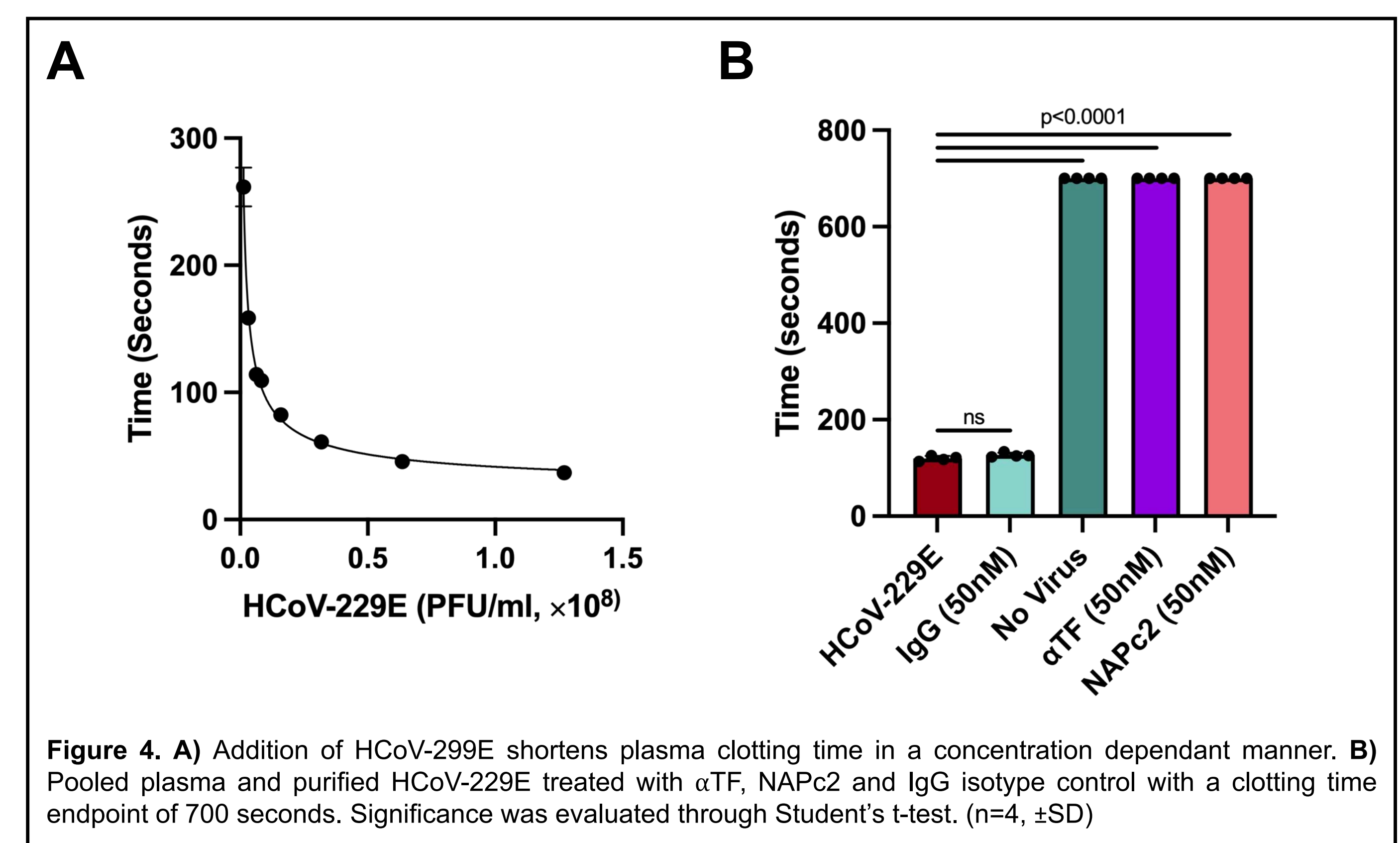


Figure 4. A) Addition of HCoV-229E shortens plasma clotting time in a concentration dependant manner. **B)** Pooled plasma and purified HCoV-229E treated with α TF, NAPc2 and IgG isotype control with a clotting time endpoint of 700 seconds. Significance was evaluated through Student's t-test. (n=4, \pm SD)

- Fig. 4B: Clot formation is dependent on HCoV-229E
- Fig. 4B: HCoV-229E clotting activity is inhibited by α TF or NAPc2 (50nM)

HCoV-229E has TF-dependent plasma clotting activity. This further supports chromogenic data that TF on the virus surface acts to generate FXa and FIIa giving fibrin clot formation.

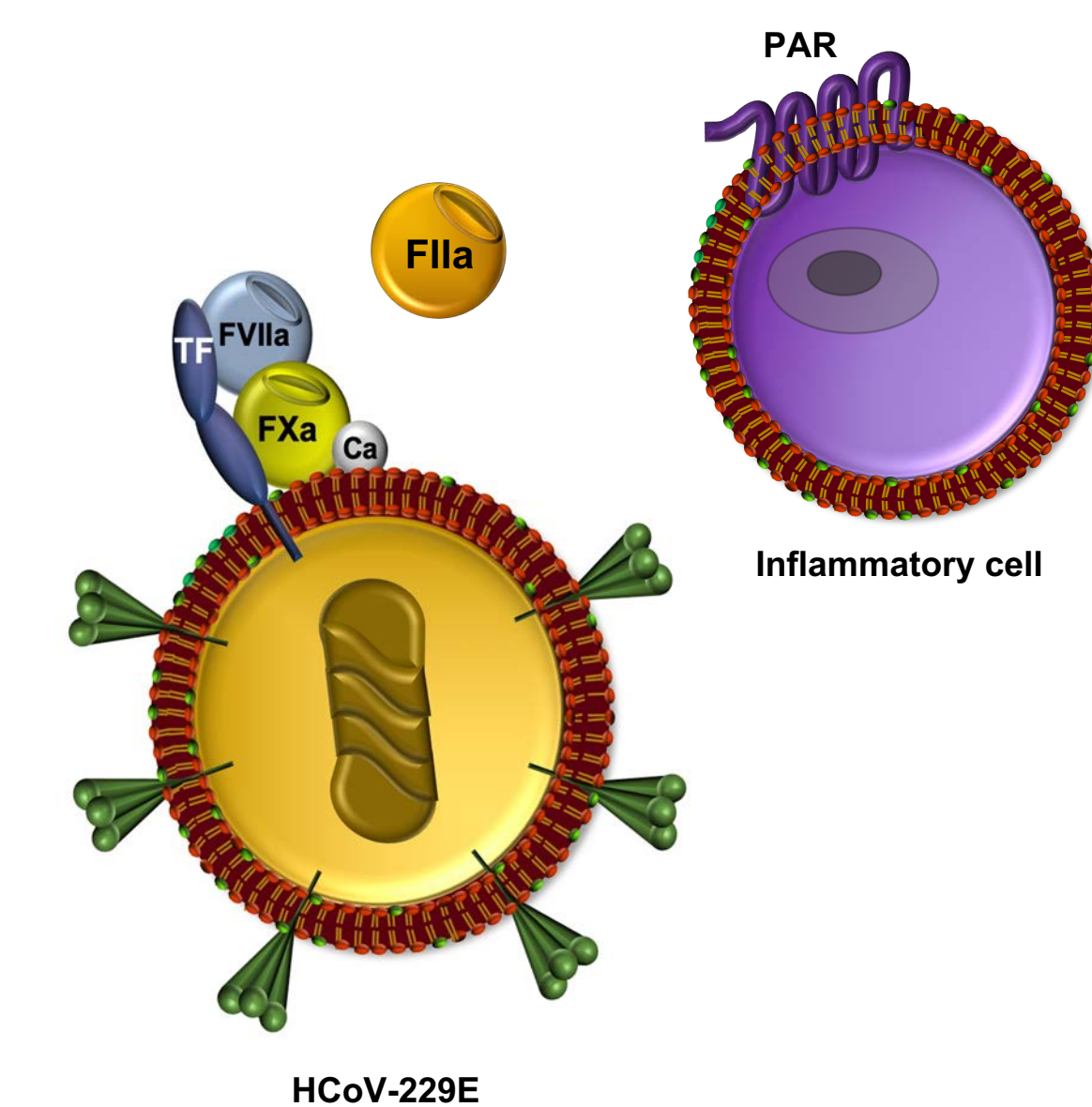
CONCLUSIONS

Summary:

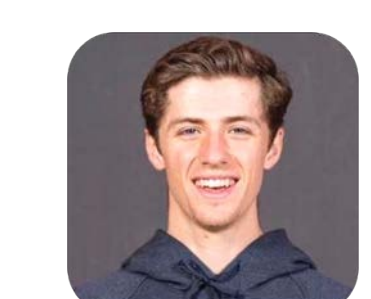
- TF antigen is on purified HCoV-229E
- TF on HCoV-229E has the expected FXa-generating FVIIa co-factor activity, inhibited by a TF-specific antibody and specific inhibitor, NAPc2
- The TF-dependent clotting activity of HCoV-229E shortens plasma clotting time in a concentration-dependent manner

Future directions and implications:

- The involvement of viral TF and protease activation on cultured cell infectivity will be assessed using plaque formation assays, with the goal of identifying TF as an anti-viral target
- HCoV-229E adds to the families of enveloped viruses identified with surface TF, indicating a broad-spectrum antiviral target for pathology and infectivity.



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