

# Structural insights into the nuclear import of human adenovirus fiber proteins

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# 1. Introduction

Human adenoviruses (HAdVs) cause respiratory, ocular, and gastrointestinal disease (1). As viral DNA replication and capsid assembly occur in the host nucleus, efficient nuclear import of viral proteins is essential. The fiber protein, critical for host cell attachment and cellular tropism (Figure 1), contains predicted nuclear localization signals (NLSs) that mediate import via host importins (Figure 2) (2). Our study investigates how HAdV fiber NLSs interact with importin  $\alpha$  isoforms, revealing isoform-specific binding.

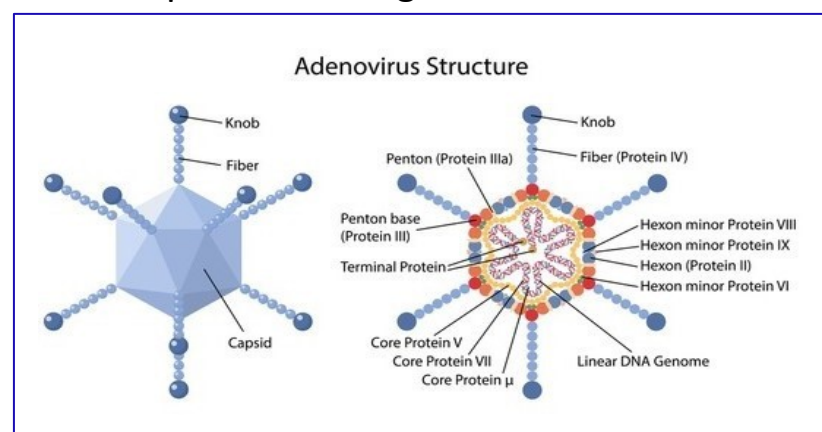


Figure 1: Structure of HAdV virion (3)

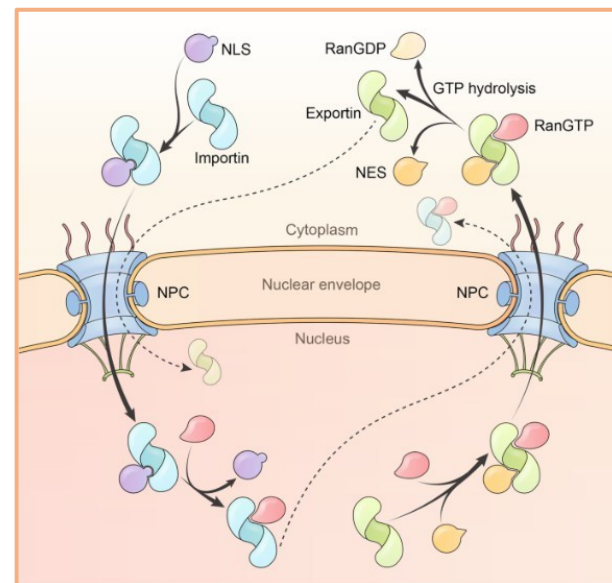
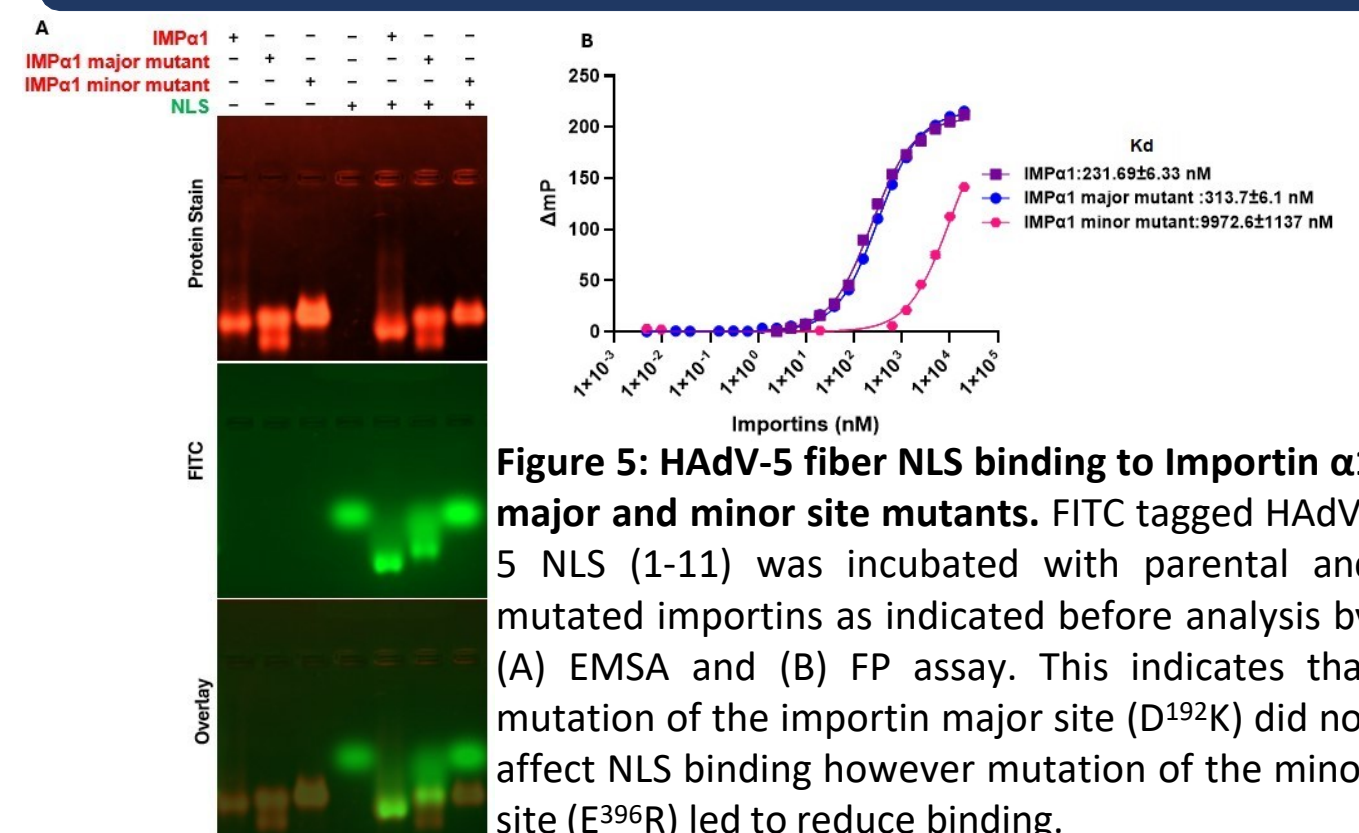


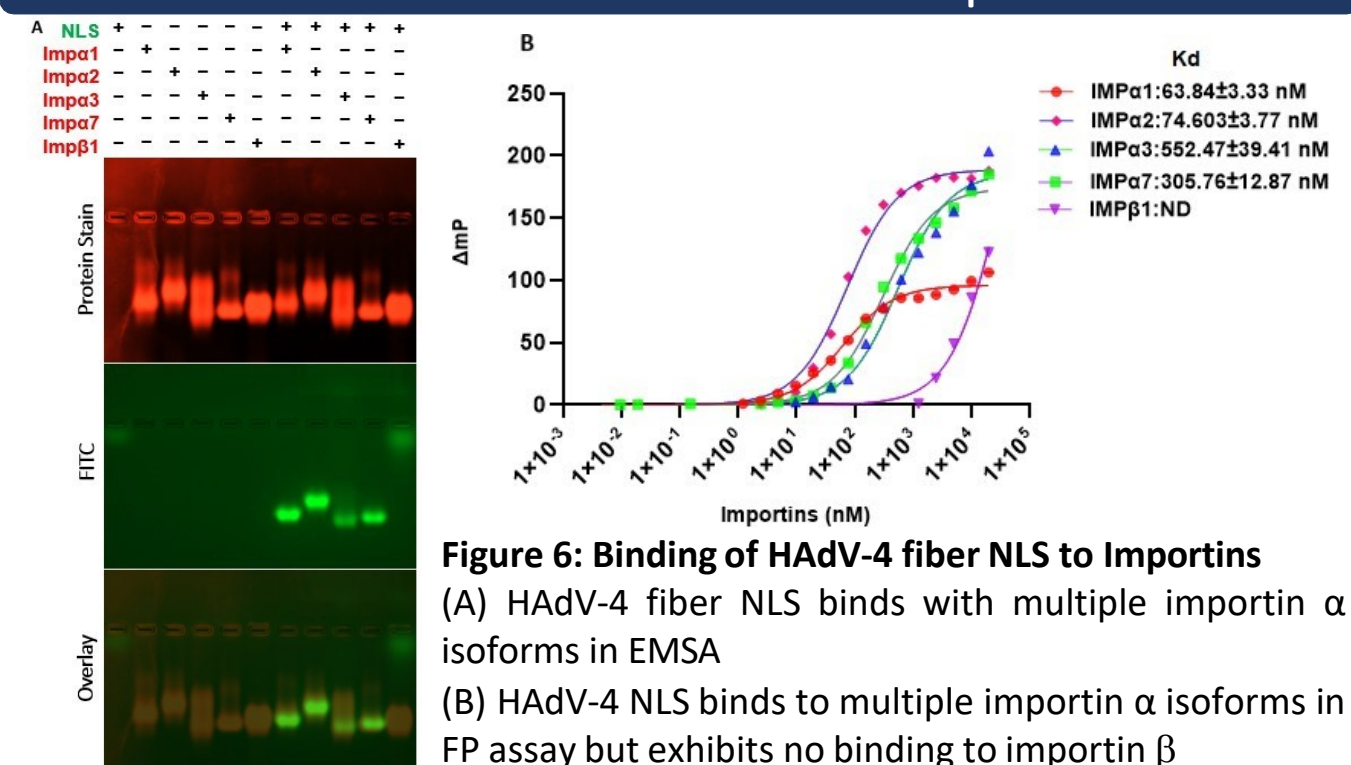
Figure 2: The classical nuclear import cycle (4)

#### 4. HAdV-5 fiber NLS interaction with importin $\alpha$ 1 major and minor mutants



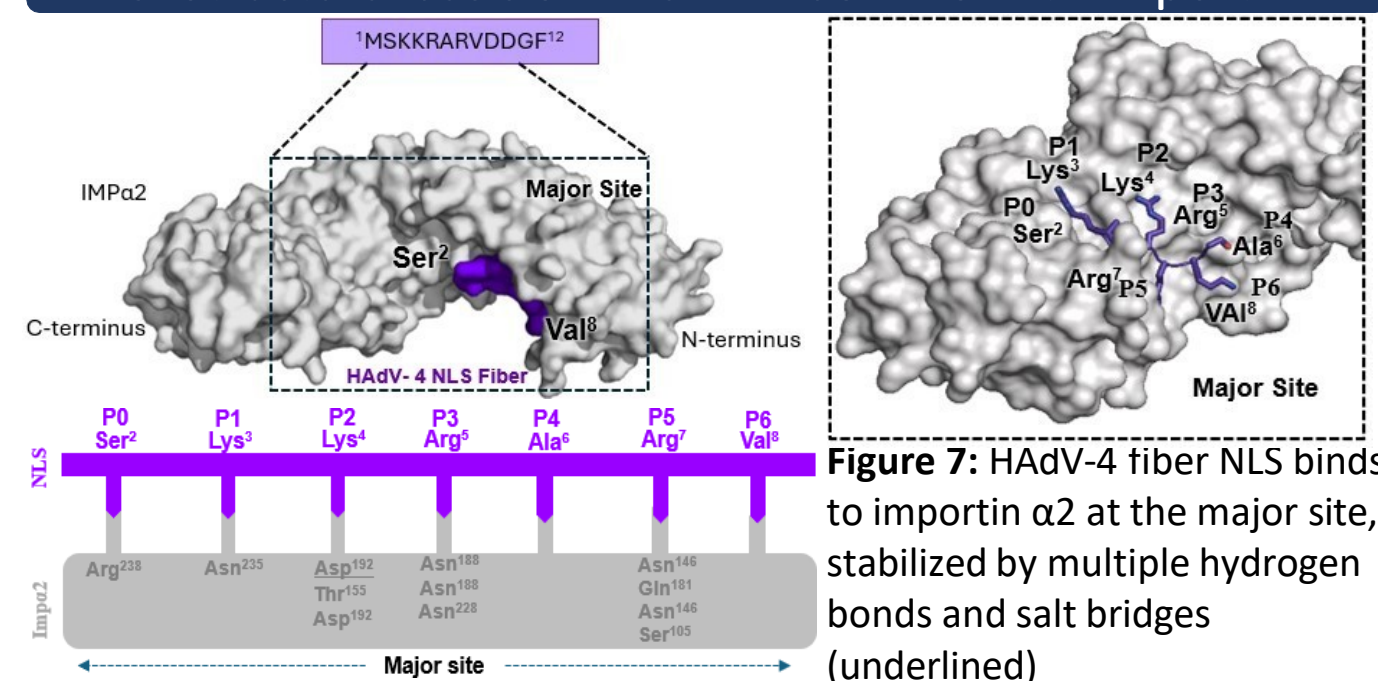
**Figure 5: HAdV-5 fiber NLS binding to Importin  $\alpha 1$  major and minor site mutants.** FITC tagged HAdV-5 NLS (1-11) was incubated with parental and mutated importins as indicated before analysis by (A) EMSA and (B) FP assay. This indicates that mutation of the importin major site (D<sup>192</sup>K) did not affect NLS binding however mutation of the minor site (E<sup>396</sup>R) led to reduce binding.

## 5. HAdV-4 fiber NLS binds to human importin isoforms



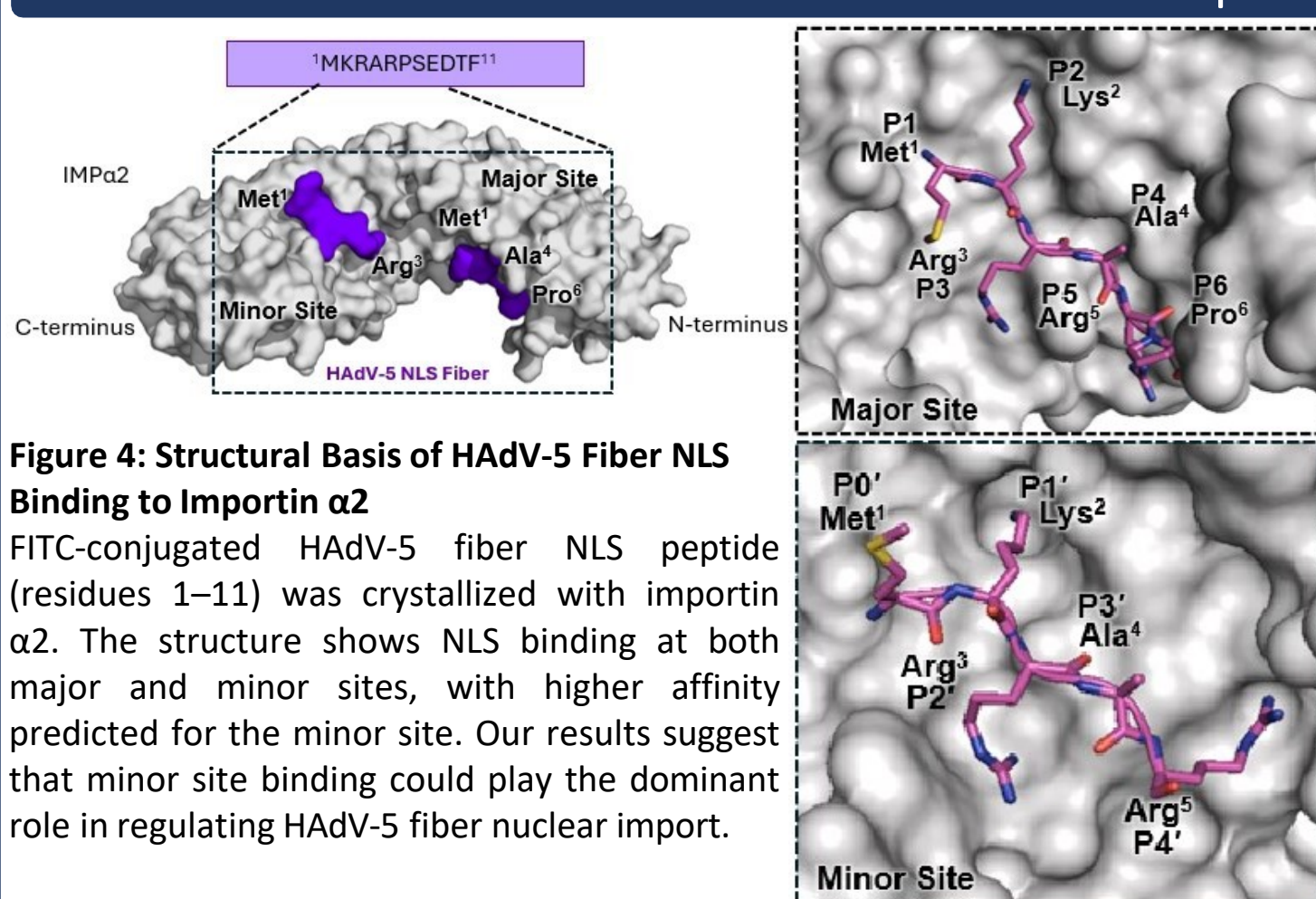
**Figure 6: Binding of HAdV-4 fiber NLS to Importins**  
(A) HAdV-4 fiber NLS binds with multiple importin  $\alpha$  isoforms in EMSA  
(B) HAdV-4 NLS binds to multiple importin  $\alpha$  isoforms in FP assay but exhibits no binding to importin  $\beta$

## 6. Structural basis of HAdV-4 fiber NLS with importin



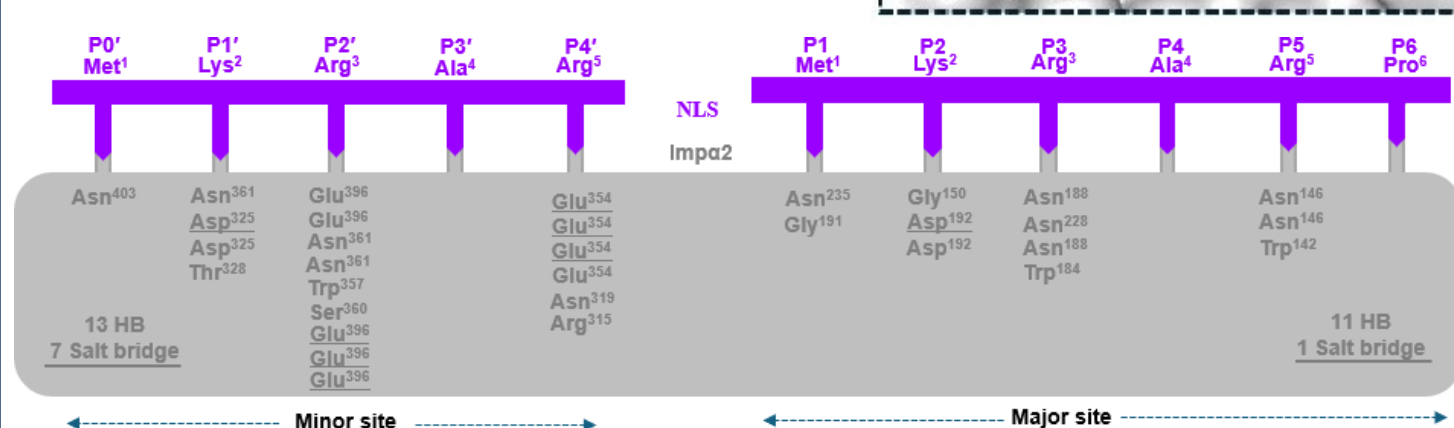
**Figure 7:** HA $\Delta$ V-4 fiber NLS binds to importin  $\alpha$ 2 at the major site, stabilized by multiple hydrogen bonds and salt bridges (underlined)

### 3. Structural basis of HAdV-5 fiber NLS interaction with importin



### Figure 4: Structural Basis of HAdV-5 Fiber NLS Binding to Importin $\alpha 2$

FITC-conjugated HAdV-5 fiber NLS peptide (residues 1–11) was crystallized with importin  $\alpha 2$ . The structure shows NLS binding at both major and minor sites, with higher affinity predicted for the minor site. Our results suggest that minor site binding could play the dominant role in regulating HAdV-5 fiber nuclear import.



## 7. Conclusion

- The NLS of the HAdV-5 fiber protein preferentially interacts with the minor binding site of importin  $\alpha$ .
- Structural analysis of the HAdV-4 fiber NLS: importin interface indicates binding at the importin major site.
- This finding highlights the intricate nature of adenovirus-host interactions and demonstrates that viruses use selective engagement of nuclear import receptors to influence intracellular trafficking.
- Elucidating these mechanisms enhances our understanding of HAdV biology and may reveal novel targets for antiviral intervention.

## References

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