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# Potential new therapeutic targets: pUL77 and pUL93, partner proteins of HCMV packaging complex



## packaging complex

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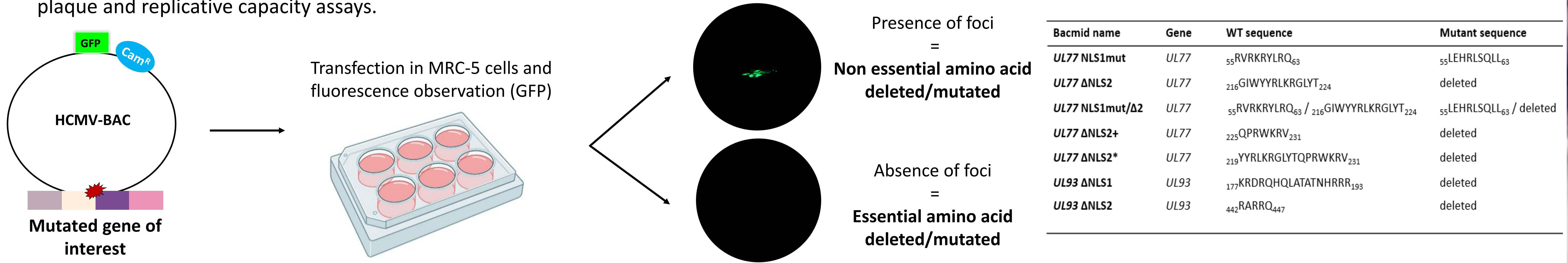
## Introduction

Human cytomegalovirus (HCMV) is responsible for life-threatening infections in immunocompromised individuals<sup>1</sup> and can cause serious congenital malformations<sup>2</sup>. Available antivirals target the viral polymerase pUL54 but are subject to cross-resistance and toxicity<sup>3</sup>. New antivirals targeting other replication steps and inducing fewer adverse effects are therefore needed. An inhibitor of the terminase complex (pUL56-pUL89-pUL51), the letermovir<sup>4</sup> was developed and approved in 2017<sup>5</sup> but resistance mutations were already reported<sup>6</sup>. pUL77 and pUL93 are essential to the packaging of viral genome<sup>7</sup> by stabilizing neoformed capsids and interacting with the terminase complex. For both proteins, two putative nuclear localisation signals (NLSs) were already described<sup>8-9</sup>.

→ The aim of this study was to highlight essential domains into pUL77 and pUL93 to identify potential new antiviral targets

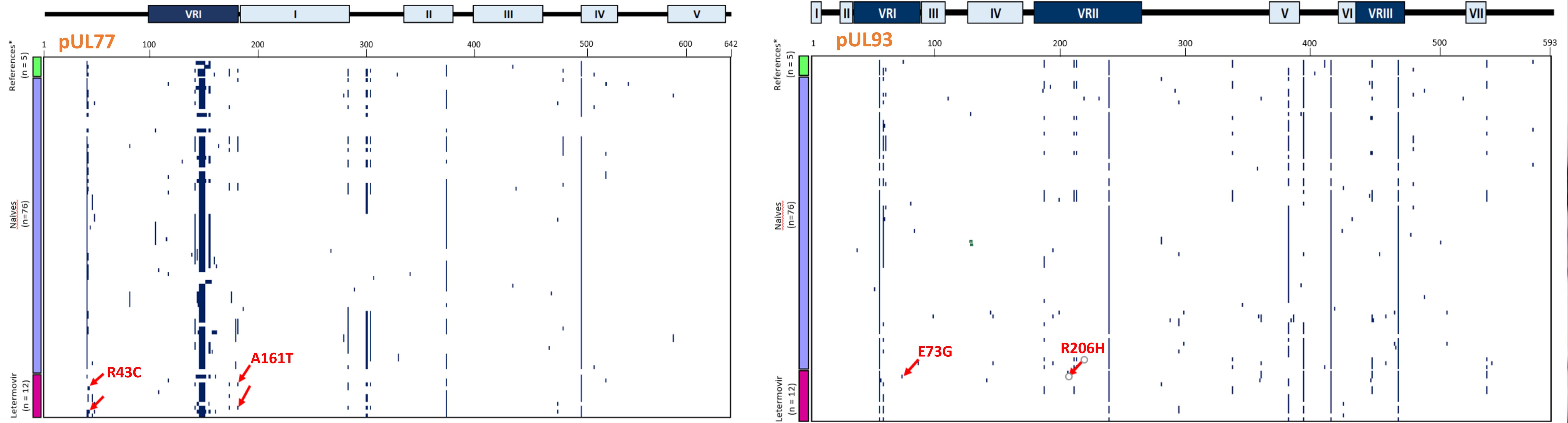
## Material and methods

- Conserved and variable regions were defined by 17 herpesvirus homolog protein alignments of pUL77 and pUL93 proteins. Polymorphism study have been done by sequencing genes encoding UL77 and UL93 of several HCMV strains (from congenital CMV infection and from transplant patients treated or not by letermovir).
- Protein 3D modelling done with AlphaFold IA software and RMSD alignment with respective HSV1 homologs (pUL17 and pUL25) achieved with VMD software.
- Mutation/deletion in pUL77 and pUL93 proteins were studied with recombinant HCMV-BAC constructed by "en passant" mutagenesis<sup>10</sup> and transfected in MRC-5 cells for plaque and replicative capacity assays.



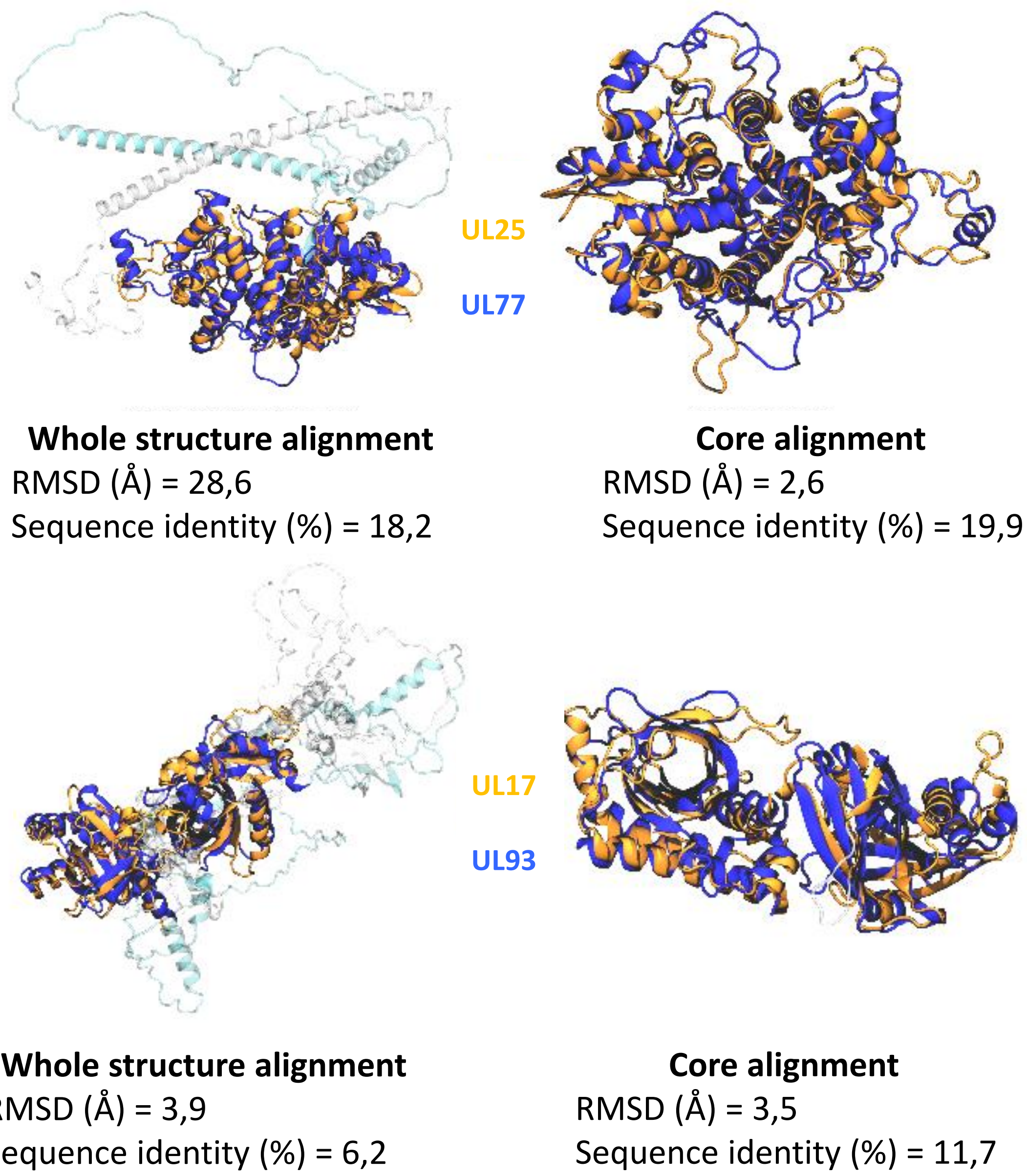
## Results

### A – Conserved domains of pUL77 and pUL93



- None of the new mutations found conferred letermovir resistance.
- pUL77 and pUL93 are **highly polymorphic proteins** with variable and conserved regions (dark blue and light blue respectively).

### B - Protein 3D modelling with respective HSV1 homologs (pUL17 and pUL25)



- pUL77 and pUL93 are structurally similar to their homologues according to the core alignment even if sequence identities are very low.

### C - Transfection of deleted/mutated NLS HCMV-BAC mutants and study of viral replication after 11 days.

| Gene | Mutation | D11 viral replication |
|------|----------|-----------------------|
| UL93 | WT       | +++                   |
| UL93 | ΔNLS1    | +++                   |
| UL93 | ΔNLS2    | +++                   |

- Deletion/mutation of pUL77 NLSs inhibits viral replication.
- pUL93 NLSs are positioned in variable regions.
- Deletion of pUL93 NLSs doesn't impact HCMV replication but the simultaneous deletion of both NLSs has to be done to assess if it is a bipartite NLS.

## Conclusion

pUL77 and pUL93 are highly polymorphic proteins with little sequence identities with their homologues. The new mutations found under letermovir selective pressure are polymorphism mutations suggesting that letermovir does not interact directly with pUL93 and pUL77 although these proteins interact with the terminase complex.

With regards to the study of the NLSs, our results suggest that pUL77 NLSs are true and that the region <sub>225</sub>QPRWKRV<sub>231</sub> of the second NLS is sufficient for the nuclear localisation function. Fluorescence microscopy will be used to evaluate and validate the function of these domains. These results suggest that both proteins could be targets of interest for new therapeutic strategies.

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**References:** <sup>1</sup>Snydman et al., 2005; <sup>2</sup>Leruez-Ville et Ville., 2017; <sup>3</sup>Billat et al., 2016; <sup>4</sup>Ligat et al., 2018; <sup>5</sup>Marty et al., 2017; <sup>6</sup>Chou et al., 2023; <sup>7</sup>Borst et al., 2016; <sup>8</sup>DeRussy et al., 2016; <sup>9</sup>Koppen-Rung et al., 2017; <sup>10</sup>Borst et al., 1999