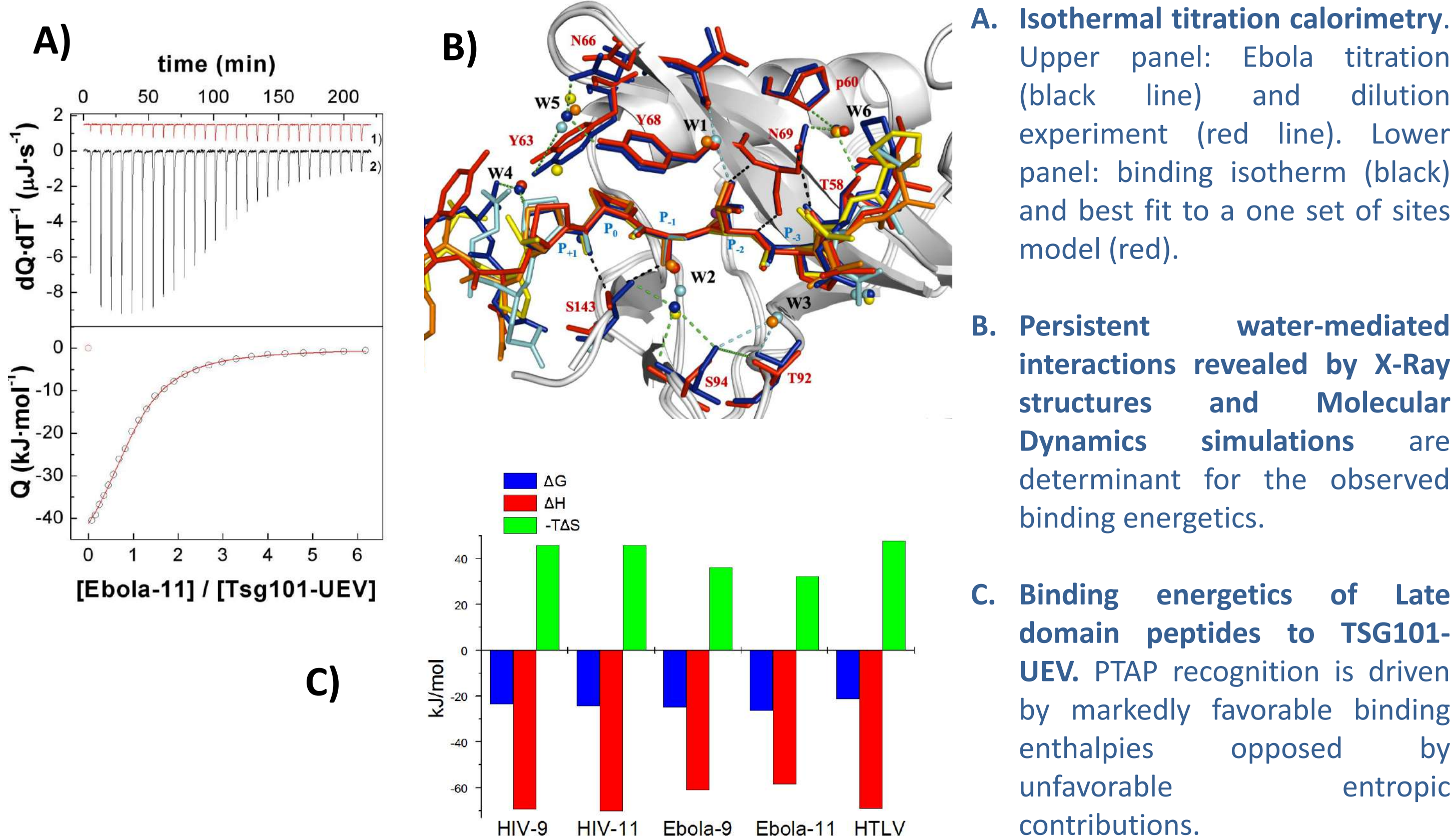


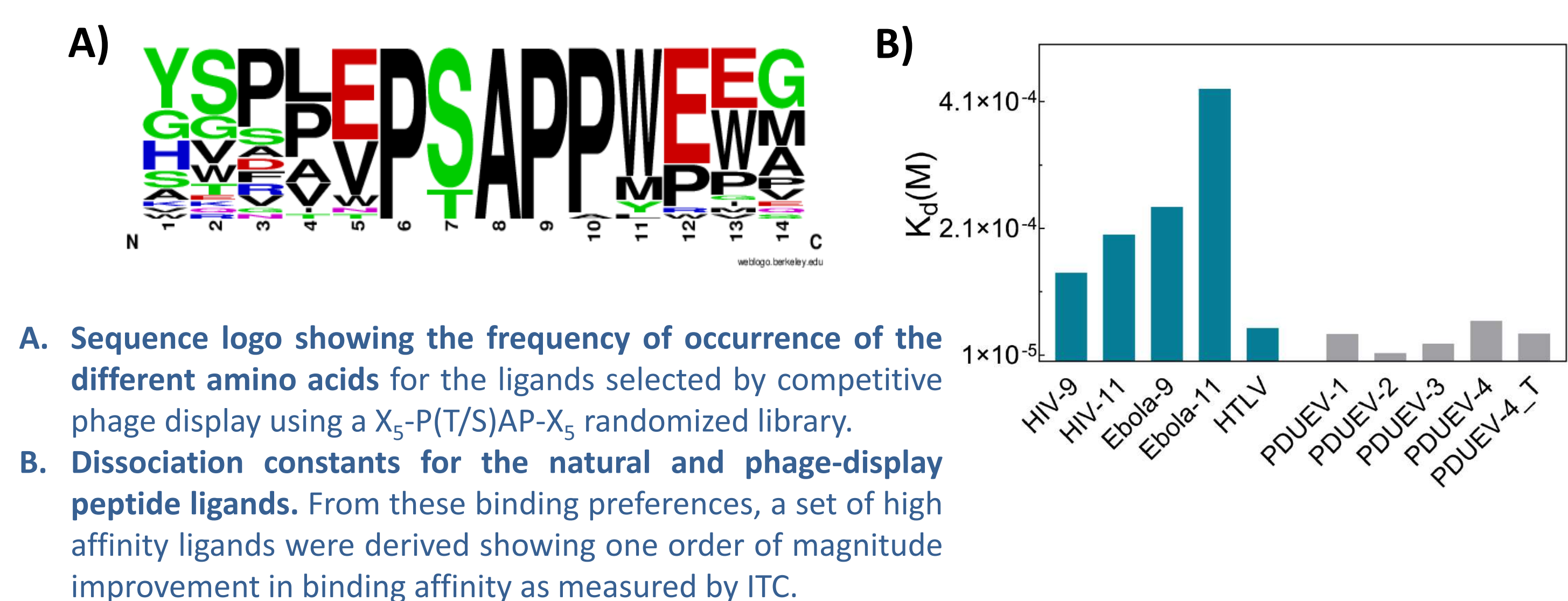
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**ABSTRACT.-** The recognition of PTAP viral Late domains by the UEV domain of the human Tumor Susceptibility Gene 101 (TSG101-UEV) is essential for the budding of many viruses such as Ebola, HIV or HTLV. Blocking TSG101-UEV/Late domain interactions has been shown to stop virus release. We have set-up a multidisciplinary approach combining biophysical studies with phage display and high-throughput screening methodologies in search for ligands of TSG101-UEV with potential as novel broad-spectrum antivirals. We present here a detailed structural, thermodynamic and molecular dynamics study of the binding of TSG101-UEV to a set of peptide ligands derived from the Late domains of HIV, Ebola and HTLV and a set of high-affinity peptides obtained by phage display, which has provided a good understanding of the molecular determinants of these interactions. Also, to explore the druggability of the TSG101-UEV binding interface, high-throughput screening Thermofluor and AlphaScreen miniaturized assays have been developed. The screening of different compound and drug-repurposing libraries have allowed the identification of set of small-molecule ligands that bind TSG101-UEV with IC50 values in the low micromolar range. High-content bimolecular complementation assays in human cells, Ebola and Marburg virus-like particle assays and antiviral activity assays against VSV and HIV-1 validated some of these compounds as broad-spectrum inhibitors TSG101-UEV/Late domain interactions. These results show that targeting TSG101-UEV interactions is a promising strategy toward the development of host-oriented, broad-spectrum antivirals.

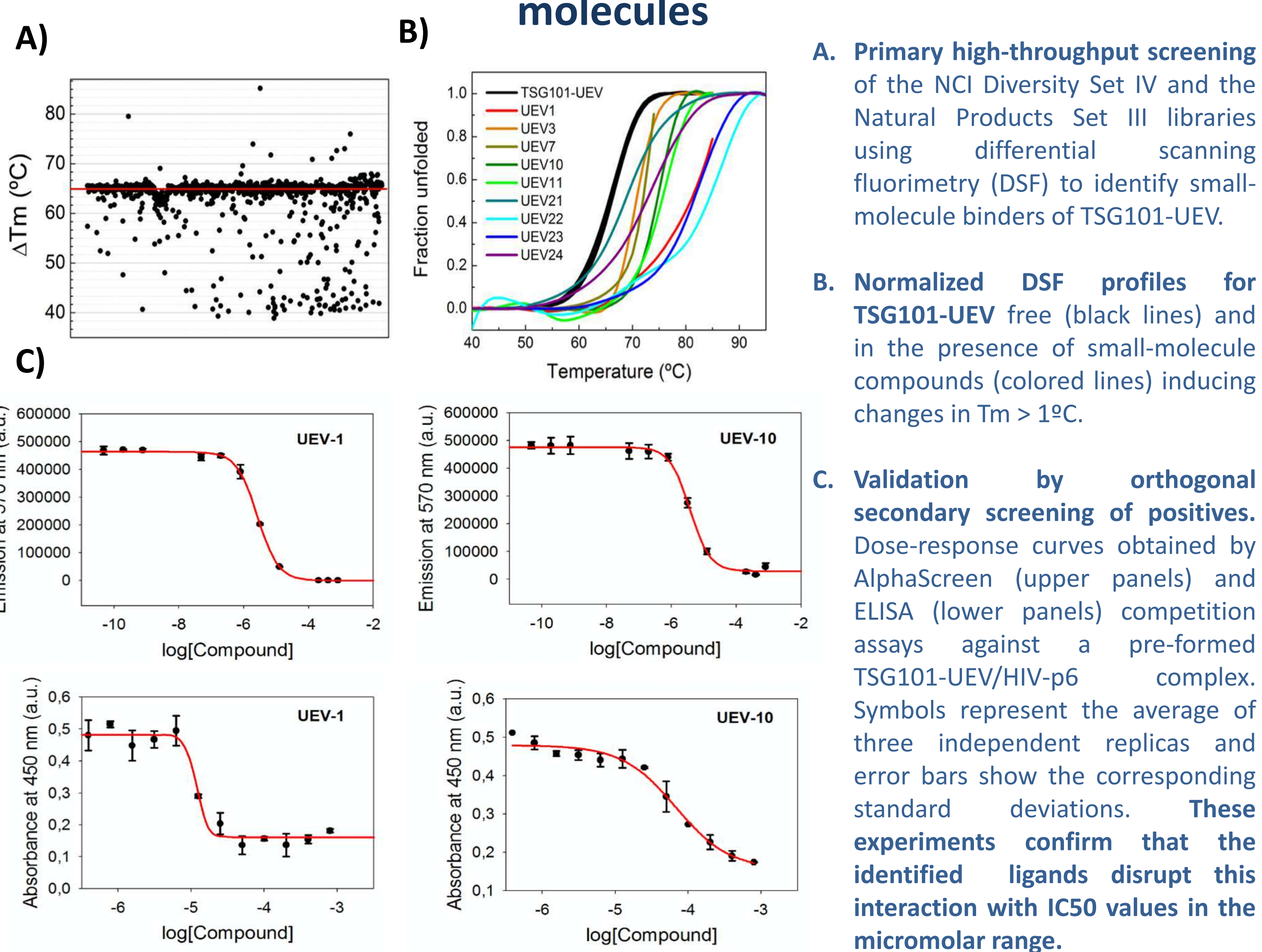
## 1.- PTAP viral Late domains are recognized by TSG101-UEV with low binding affinities and favorable binding enthalpies, determined by water-mediated interactions



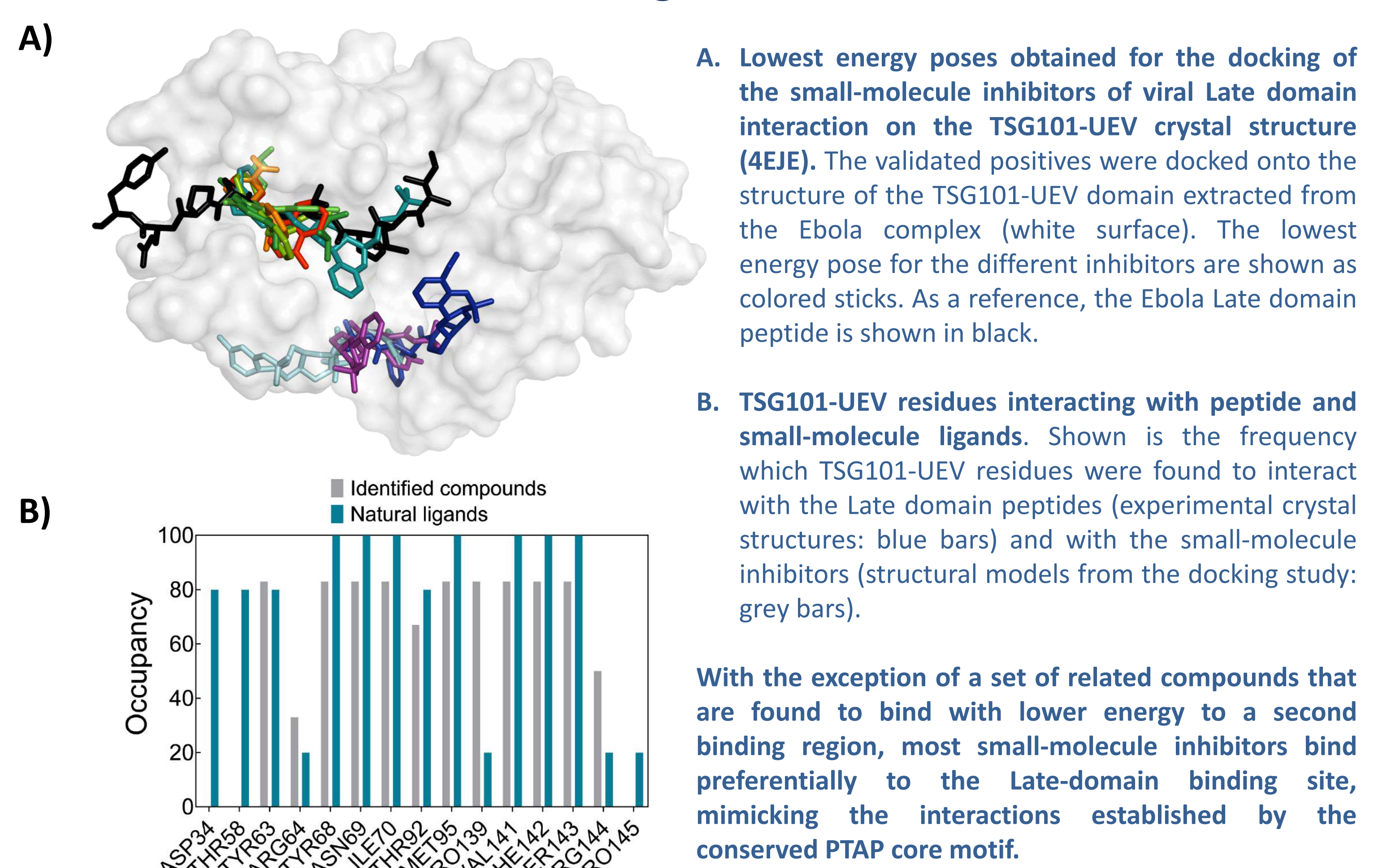
## 2.- Phage display shows that higher binding affinity is attainable for small peptide ligands of TSG101-UEV



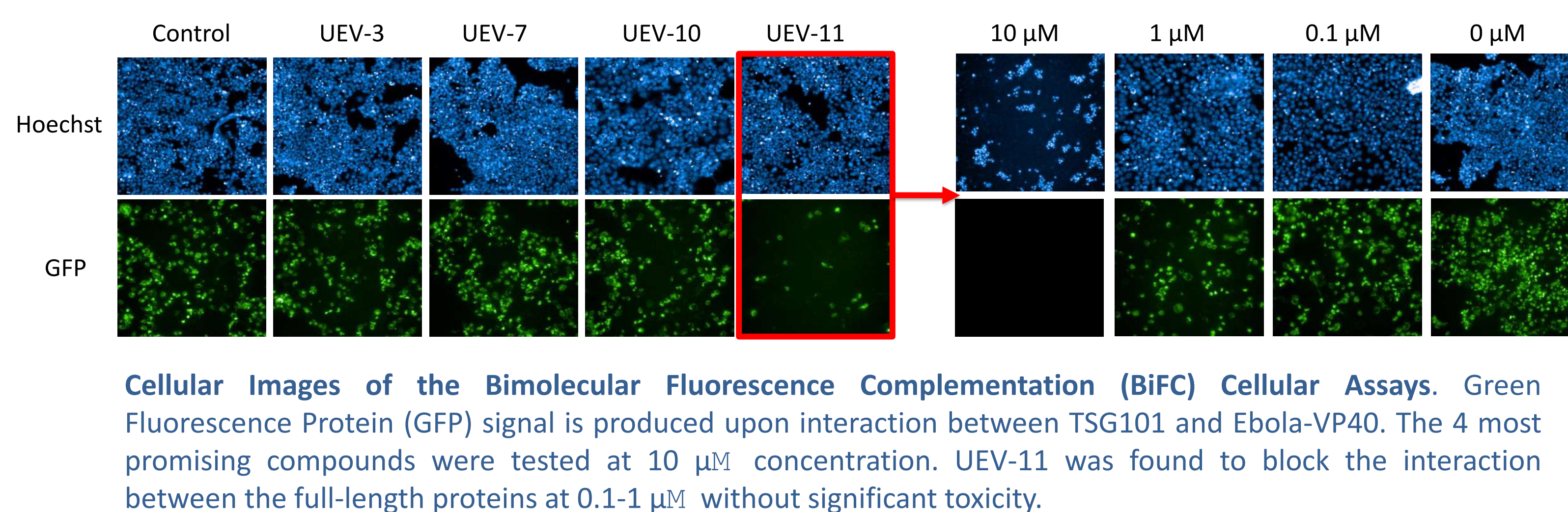
## 3.- The TSG101-UEV binding interface is druggable: TSG101-UEV/Late domain interactions can be disrupted by small drug-like molecules



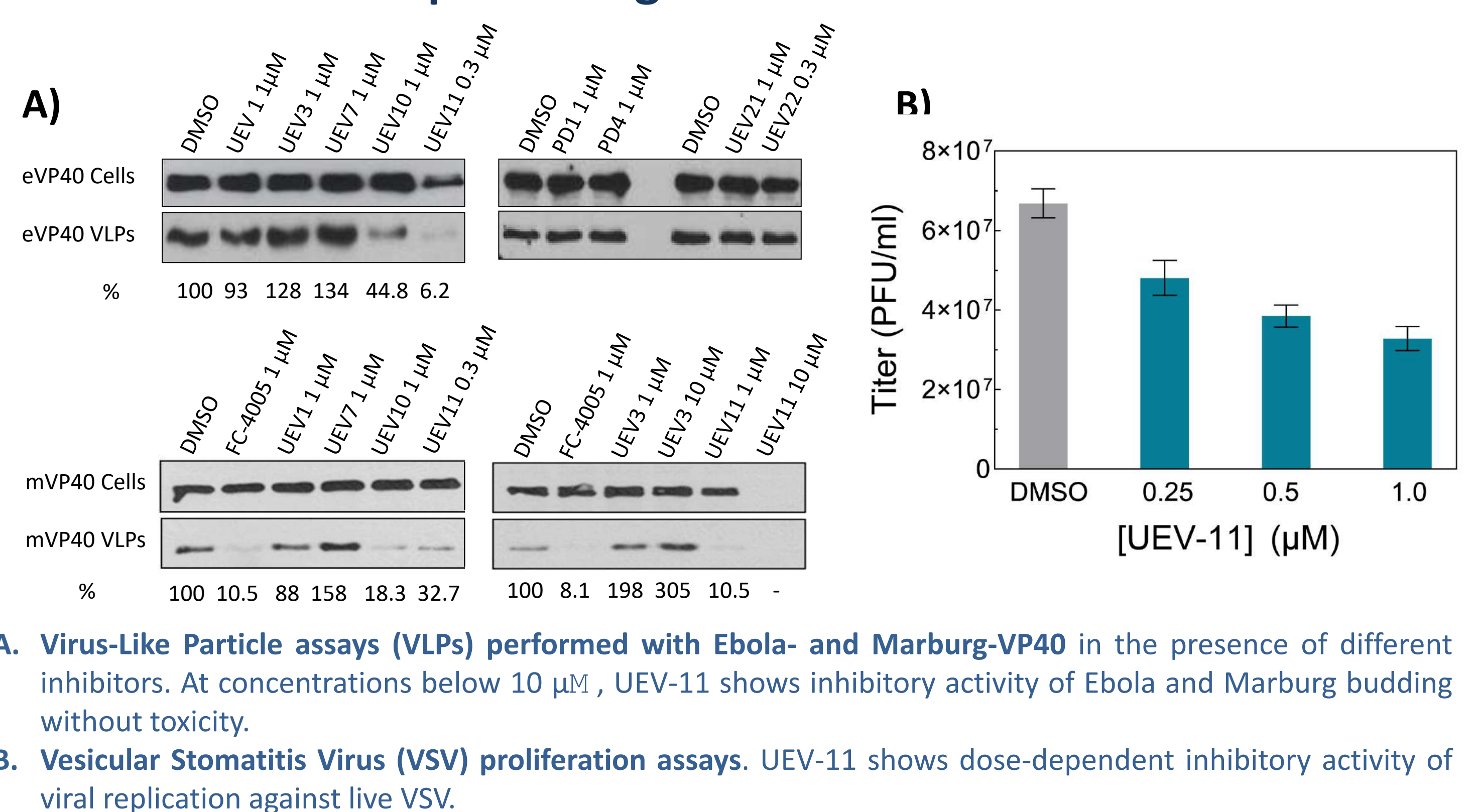
## 4.- The small-molecule inhibitors and the Late-domain peptide ligands interact with the same binding site residues in TSG101-UEV



## 5.- Small-molecule inhibitors can block the interaction between full-length TSG101-UEV and Ebola-VP40 in a cellular context



## 6.- Small-molecule inhibitors of TSG101-UEV/Late domain interactions can block particle egress for different viruses



**Conclusion:** The multidisciplinary approach presented here, combining biophysical characterization, phage display and High-Throughput screening methodologies has allowed the identification of novel compounds blocking the interactions between TSG101-UEV and viral Late domains and disrupting budding and viral proliferation in several encapsulated virus, of interest as potential novel broad-spectrum antivirals.

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