

Strong functional constraint at residues in HIV-1 Gag are predicted by measures of evolutionary rather than population-level genetic conservation.

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Background

HIV variation remains a central barrier to an effective vaccine. Immune escape contributes significantly to intra- and inter-individual HIV diversity (1). Accumulation of certain escape mutations at the population level has been suggested by i. HLA allele associations with subtype consensus residues (2) ii. Correlation between HLA allele frequency and frequency of associated viral escape mutants across diverse populations (3) and iii. Persistence of high fitness escape mutants following transmission (4). Some conserved residues may thus represent “immunologically silent” sites not subject to immune selection, rather than sites of high structural/functional constraint. We utilized known phylogenetic relationships between HIV-1 and SIVs in their natural primate hosts to identify possible sites of deeper evolutionary conservation (“high EvC”) that are distinct from sites of HIV-1/human specific conservation only (Figure 1).

We hypothesized that “high EvC” residues are likely to reflect inherent constraint rather than lack of immune targeting and will demonstrate high replicative cost when mutated. In contrast, residues that are conserved among HIV-1 population isolates but not conserved among phylogenetically-related lentiviruses (“low EvC/high PopC”) may represent sites of early population-level fixation of HLA- or other human-driven adaptations unique to HIV. This latter type of site may not be substantially mutationally constrained; furthermore, population-level HLA adaptation at these sites may render them poorly immunogenic *in vivo*. (Figure 2).

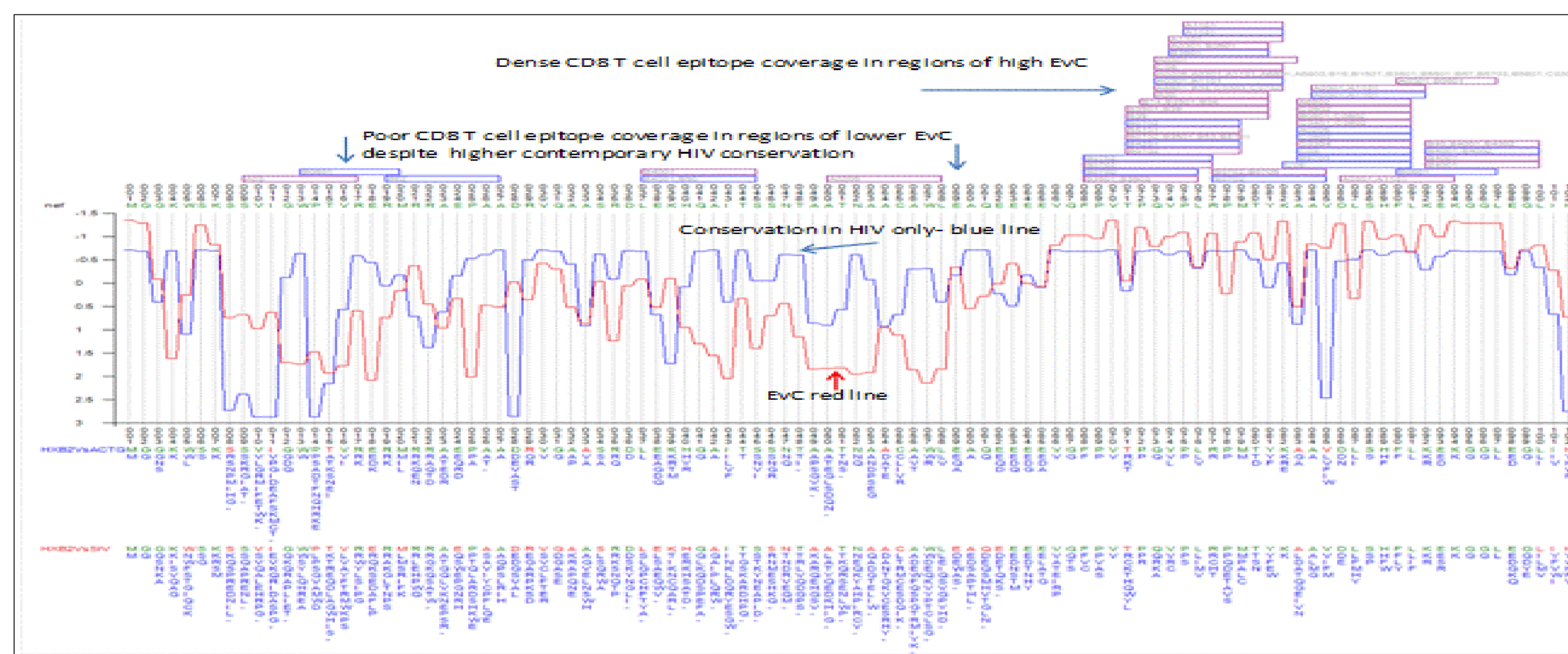


Figure 1: Plot of evolutionary conservation (EvC) of residues in HIV Nef residues 1-106 relative to HIV/SIV phylogeny. Sites with 100% EvC and region associated with high density of known and putative CD8 T cell epitopes indicated. Superimposed EvC (red line) versus HIV-1 subtype B population conservation (blue line). Loss of EvC in the first part of the Nef region corresponds to reduced density of CD8 T cell epitopes. Epitopes mapped here include those in the LANL “A-list” and novel epitopes mapped as a result of large scale epitope mapping in Australian and US populations (5). EvC scores computed in ConSeq server (<http://conseq.tau.ac.il/>), which builds a phylogeny using maximum likelihood or Bayesian methods from a multiple sequence alignment of phylogenetically related primate lentiviruses. Single site scores computed in the context of the entire protein, and based on the site evolutionary rate estimated from this phylogeny, adjusted for varying numbers of protein homologues and protein length distributions.

HIGH EvC+HIGH HIV conservation =high constraint =high replicative capacity cost if mutates	LOW EvC+HIGH HIV conservation =Adaptation of HIV to HLA at population (inter-patient) level or other human host specific adaptation =little or no replicative capacity cost if mutates
HIGH EvC+LOW HIV conservation =adaptation of HIV within individuals (intra-patient) =variable replicative capacity cost <i>in-vitro</i>	LOW EvC+ LOW HIV conservation =low constraint, adaptation to different host species and adaptation of HIV to polymorphic traits within individuals

Figure 2: Predicted characteristics of amino acid residues in HIV-1 based on EvC.

Methods

“High EvC” sites in p24^{Gag} (L188, mutated to nonconservative substitution L188D and conservative substitution L188I) and p7^{Gag} (A431L), and a “low EvC/high PopC” site in p17^{Gag} (E106L) (Figure 3) were selected based on structure information and modeling methods and engineered into an HIV-1 NL4-3 reference strain backbone. VsVg-pseudotyped HIV-1 stocks were generated in HEK-293 cells and their p24^{Gag} levels assessed by ELISA. Infectivity and replication capacity of wild-type NL4.3 and mutant viruses were assessed using an established GFP-reporter T-cell assay.

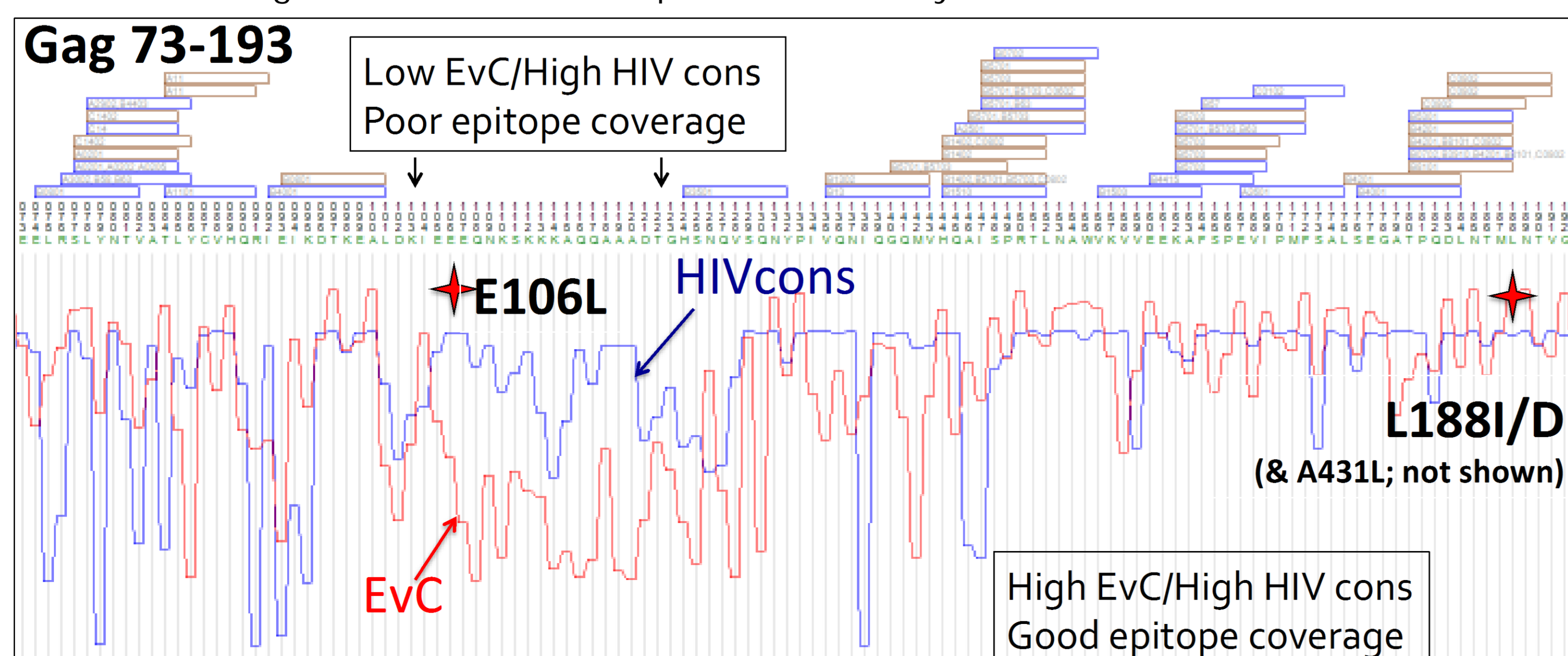


Figure 3: Plot of EvC of residues in HIV gag 73-193 relative to HIV/SIV phylogeny. Superimposed EvC (red line) versus HIV-1 subtype B population conservation (blue line). Sites 106 and 188 indicated.

Results - EvC predicts *in-vitro* replicative capacity

“High EvC” L188 and A431 sites are 100% conserved in the SIV/HIV phylogeny. At codon 188, we engineered L188I and L188D as conservative and non-conservative substitutions respectively, and at A431 we engineered A431L. We hypothesized that any change (even conservative ones) at high EvC sites would be costly to replication. In contrast, E106L is a site conserved among all subtype B HIV-1 isolates but variable across SIVs, suggesting human host-specific conservation only. We hypothesized no or minor effects on replication capacity compared to wtNL4.3. Assessments of p24 production (Figure 4A) and infectivity (Figure 4B) were performed in triplicate with consistent results. Replication capacity experiments using VsVg pseudotyped virions (Figure 4C, 4D) in CEM-GXR cells were performed in duplicate. Overall we observe that:

1. E106L (low EvC/high PopC) was broadly comparable to wildtype NL4-3 with respect to particle formation (measured by p24), viral infectivity and spread.
2. L188I (high EvC, conservative change) was substantially impaired for particle formation, infectivity and viral spread.
3. L188D and A431L (high EvC, non-conservative changes) were essentially non-viable, exhibiting minimal particle formation, negligible infectivity and no viral spread

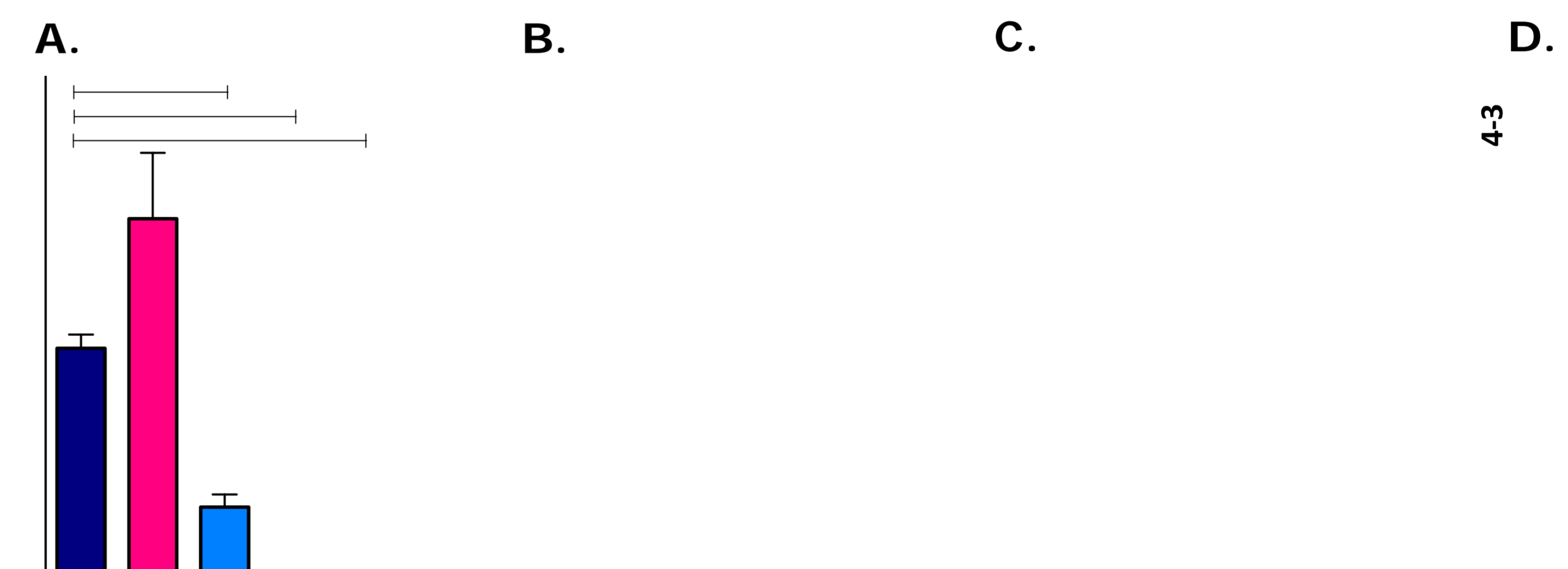


Figure 4: A: P24 ELISA, B: Infectivity, C: and D: Raw and NL4-3-normalized Replicative Capacity using VsVg pseudotyped viral stocks, duplicate cultures initiated at 2% MOI on day 2.

Comparison of EvC values at sites tested using mutagenesis studies (6) in many instances reflect characteristics predicted in the schema shown in Figure 2 (Figure 5).

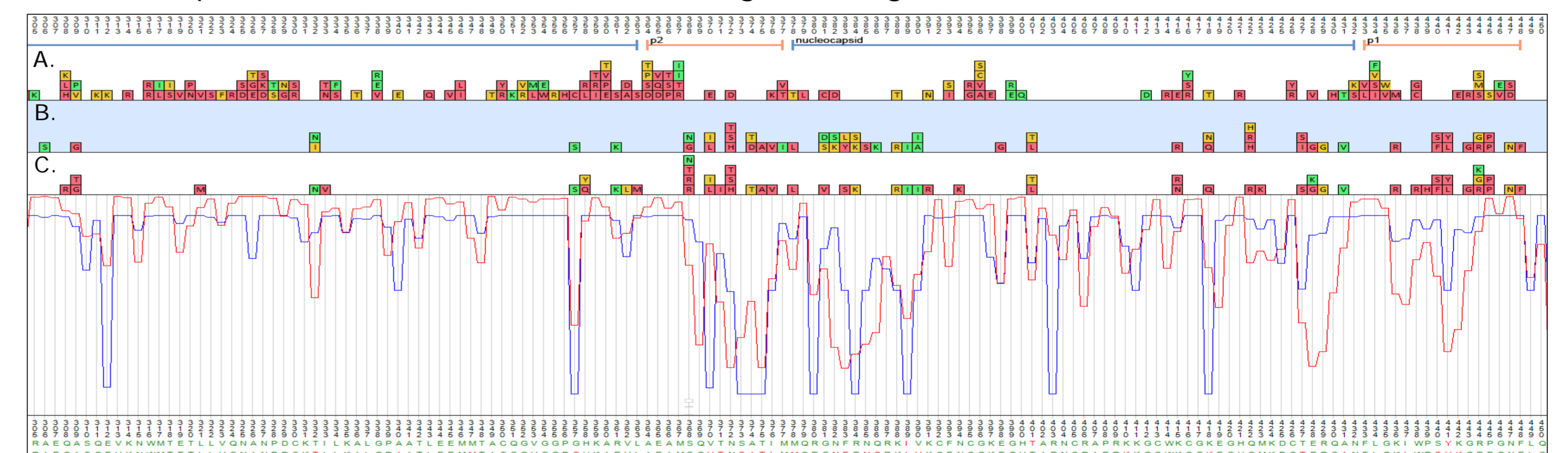


Figure 5: EvC mapped against replicative capacity effects at specific sites in published HIV-Gag mutagenesis experiments (6). Red denotes severe lethal change, yellow intermediate and green comparable to wildtype. Note many sites not previously examined and frequently the specific mutations tested (A) are not those observed either in population HIV (B) or SIV sequences (C).

Discussion

In all tested cases, high EvC correctly predicted a lethal or near lethal effect of mutation whereas absolute population-level conservation alone did not appear to confer any significant constraint. No structural or functional information was used *a priori* to select these sites or specific substitutions.

The initial sites tested support the hypothesis that EvC can be used as a probe to identify virological constraint and/or potential host adaptation early in the pandemic. In this case, E106L falls in an area that is poorly covered by known T-cell epitopes and may represent a site of HIV adaptation to human-specific immunity at the population-level.

Using EvC in a systematic way to understand functional aspects of HIV proteomes and host-specific selection pressures may be applied in the development of novel HIV immunogens.

References

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