Comprehensive mapping of neutralization escape across HIV Envelopes from divergent strains

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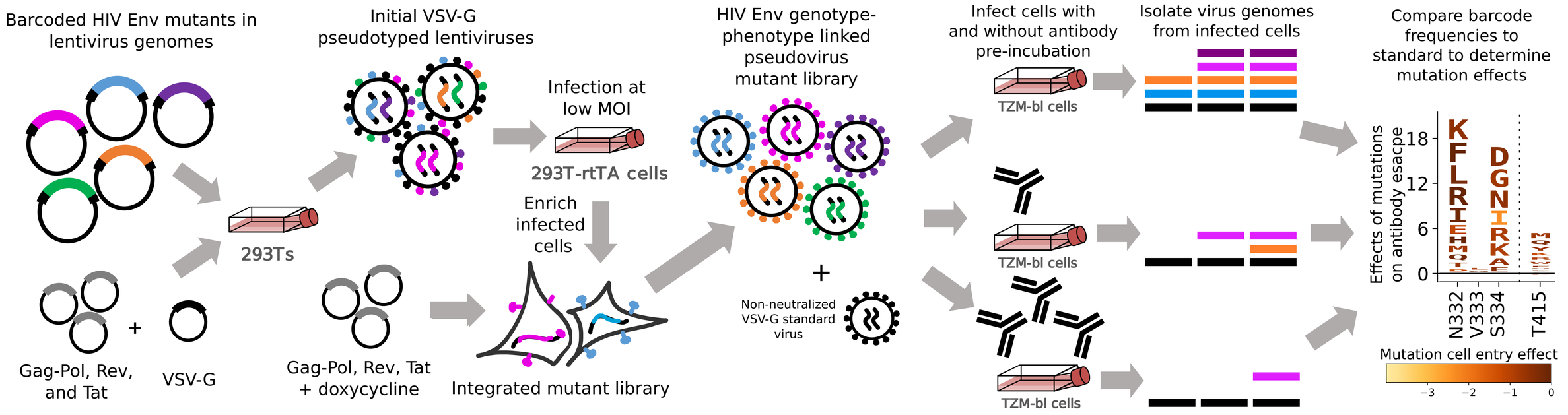
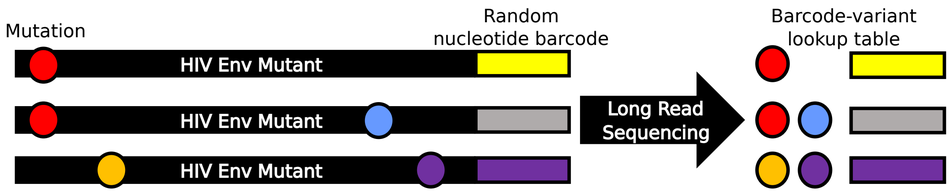
Methods: We have developed a lentiviral pseudovirus-based deep mutational scanning platform capable of safely measuring the effects of all possible HIV Envelope (Env) mutations on neutralization by antibodies or sera

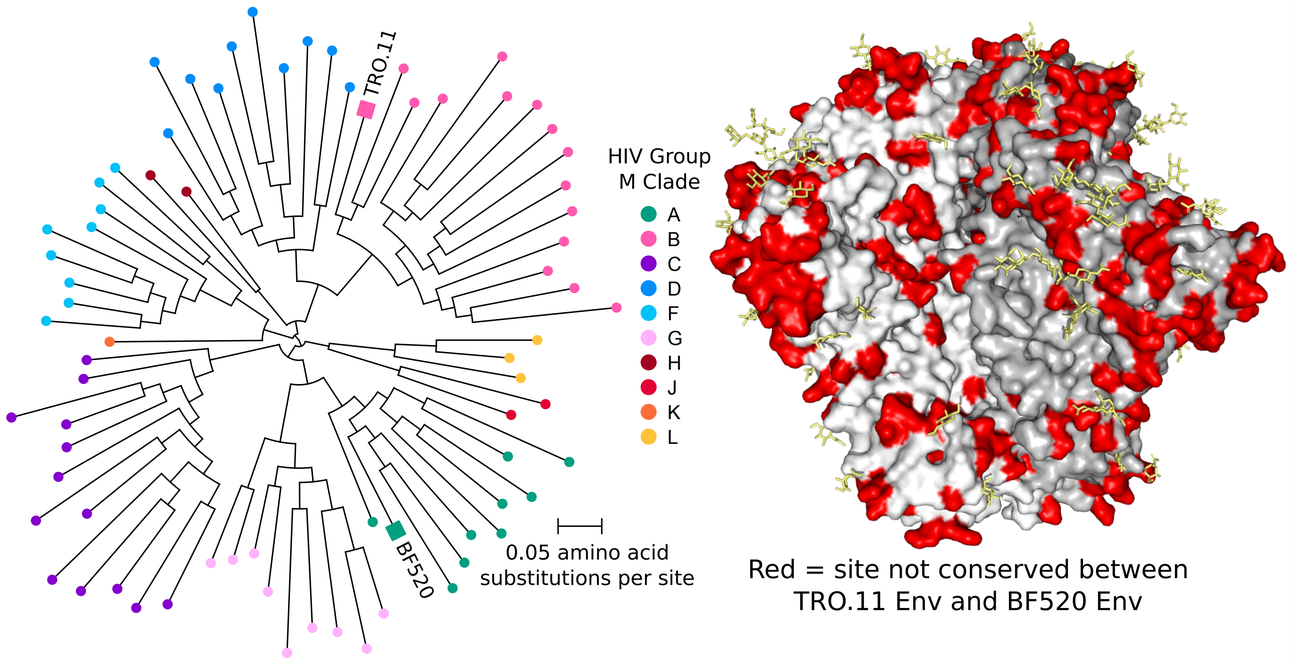


Each lentivirus genome contains one HIV Env mutant and one short random nucleotide barcode. An initial infection is used to integrate one genome per cell into a cell line.



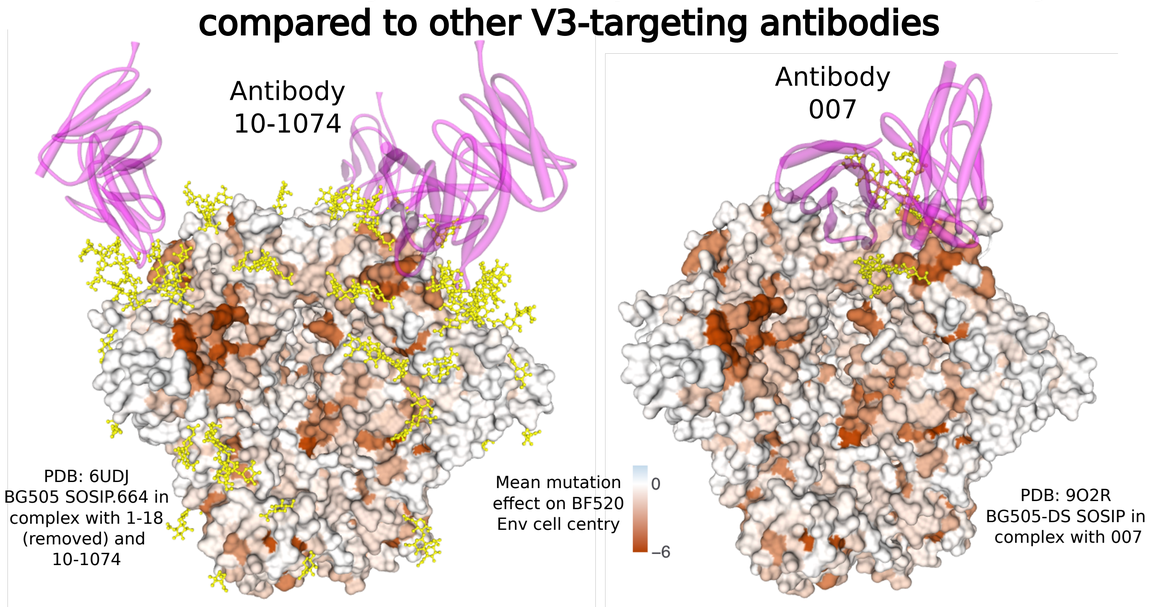
In this way, genotype-phenotype linked pseudoviruses can be produced from those cells without mutant-barcode scrambling by lentiviral recombination, and inexpensive Illumina sequencing can be used to read the barcode frequencies after experiments

Library Design: We are using mutant Env libraries of clade A BF520 Env and clade B TRO.11 Env to map escape from neutralization across strains

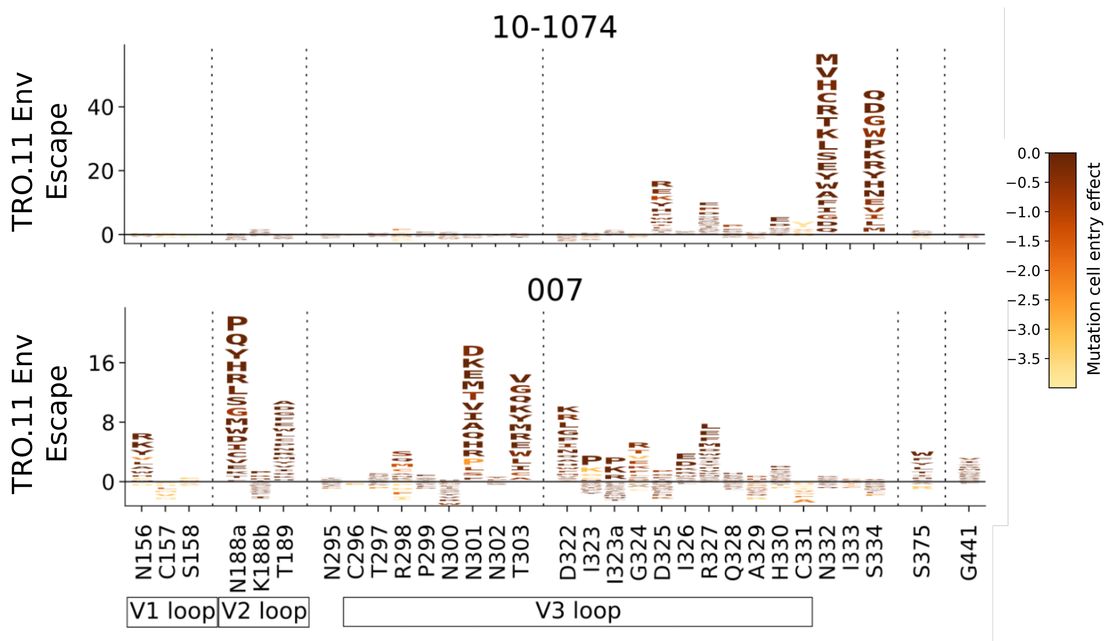


Red = site not conserved between TRO.11 Env and BF520 Env

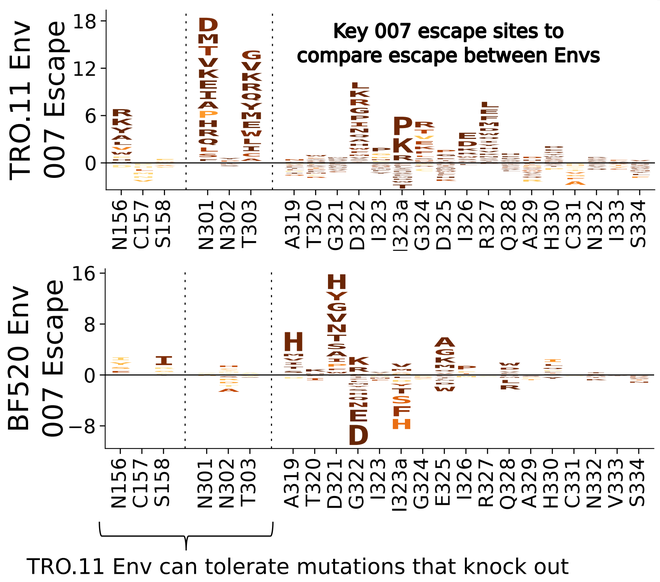
Results: Antibody 007 has unique neutralization activity compared to other V3-targeting antibodies



DMS shows how escape from 007 is driven by mutations outside of the N332 glycosylation motif, unlike other V3-targeting anitbodies like 10-1074

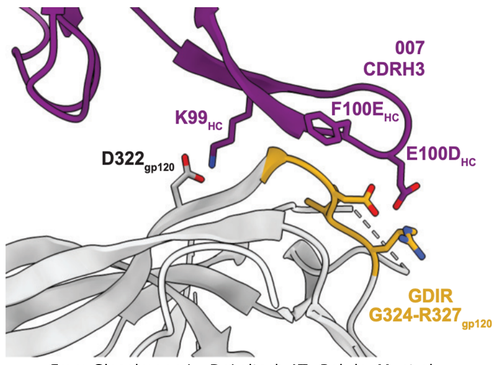


Patterns of mutation effects conserved between TRO.11 Env and BF520 Env can reveal antibody-Env interactions



TRO.11 Env can tolerate mutations that knock out the N156 and N301 glycosylation motifs, but BF520 Env cannot tolerate the majority of these mutations

Mutations that remove negatively charged amino acid D or that introduce positively charged amino acids K/R/H at sites 319-324 in both strains cause escape. The structure of 007 in complex with Env reveals this is likely due to charge-charge interactions in this region.



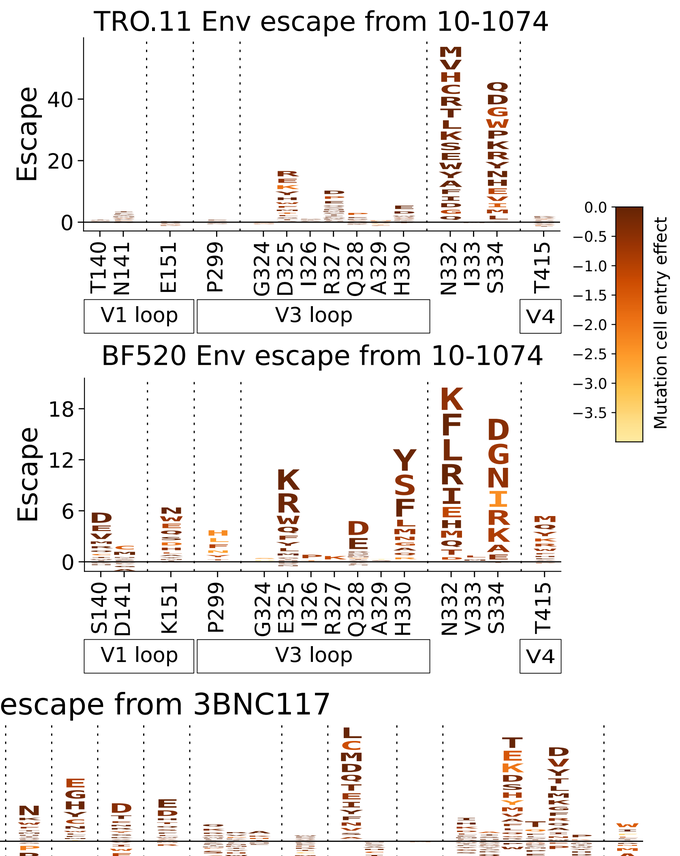
From Gieselmann L., DeLaitsch AT., Rohde, M. et al.

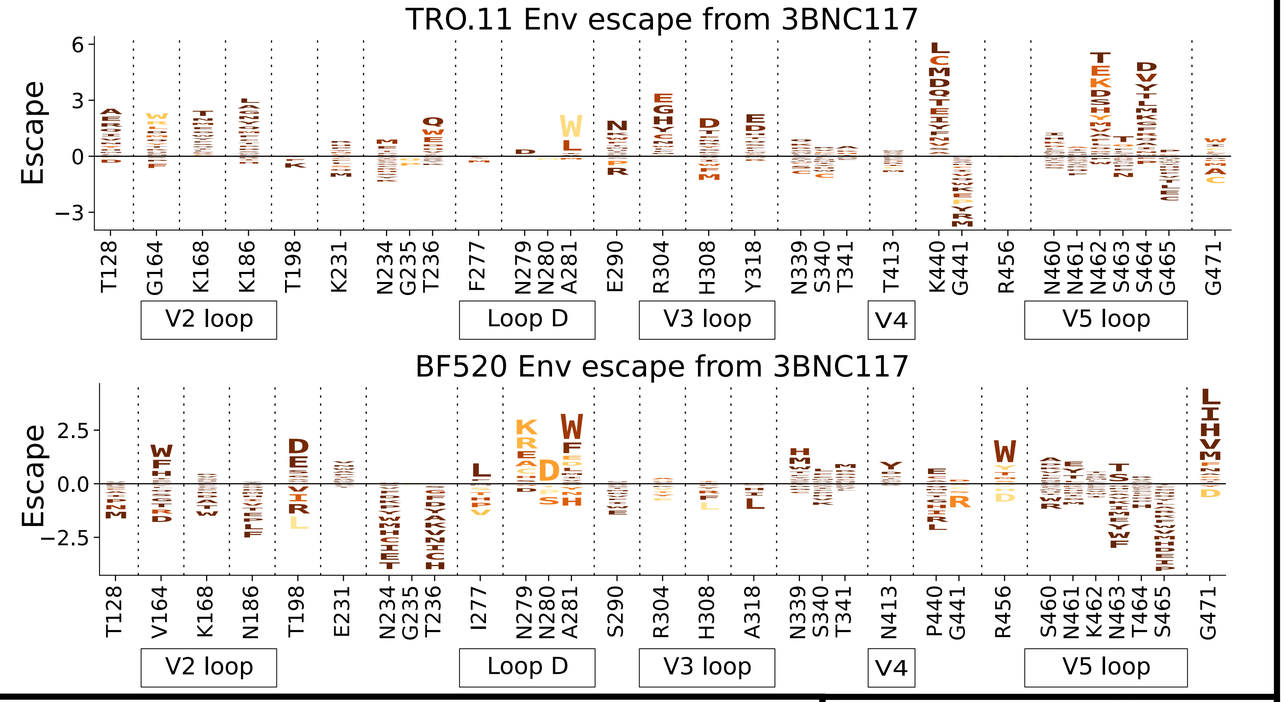
Results: Conservation of effects of mutations on escape between strains differs for antibodies 10-1074 and 3BNC117

Understanding escape from potential therapeutic antibodies like 10-1074 and 3BNC117 is critical for designing antibody cocktails and deploying them against the uneven geographic diversity of HIV.

Effects of mutations on escape from 10-1074 are similar bteween Envs, with mutations that knock out the N332 glyosylation motif and mutations in or around the 324-GD/EIR-327 motif causing escape.

Escape is less conserved between Envs for 3BNC117, with the effects of mutations on escape differing between Envs across many regions.





Next steps: We have shown the degree to which the effects of mutations on escape from neutralization can be generalized between divergent Envs differs among antibodies, but that some effects are conserved between Envs and can reveal antibody-Env interactions. To further investigate this, we are currently making a new mutant Env library with contemporary clade C Env strain 1952B.

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References:
Radford, CE. et al. Mapping the neutralizing specificity of human anti-HIV serum by deep mutational scanning. Cell Host & Microbe, Volume 31, Issue 7, 1200 - 1215.e9
Radford CE, Bloom JD. Comprehensive maps of escape mutations from antibodies 10-1074 and 3BNC117 for Envs from two divergent HIV strains. 2025. J Virol 99:e00195-25.
Gieselmann L., DeLaitsch AT., Rohde, M. et al. Identification of a broad and potent V3 glycan site bNAb targeting an N332 gp120 glycan-independent epitope. In Press.