

Distinct region-specific neutralization profiles of contemporary HIV-1 clade C against best-in-class broadly neutralizing antibodies

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Short title: Neutralization profiles of contemporary HIV-1 clade C

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Abstract

Broadly neutralizing antibodies (bnAb) have been clinically proven to be an excellent choice for HIV-1 prevention. However, the relative effectiveness of best-in-class bnAbs against regionally relevant circulating HIV-1 forms is not clear. In the present study, we compared the degree of neutralization sensitivity of contemporary HIV-1 Indian clade C with that of South African origin. Phylogenetic analysis revealed that these clade C viruses continue to evolve distinctly from one another. Env-pseudotyped viruses prepared using contemporary HIV-1 clade C *env* genes (N=115) obtained from nine geographically distinct sites in India (between 2020-2023) were found to be most sensitive to V3-directed bnAbs 10-1074 and BG18, and second generation CD4 binding site (CD4bs) directed bnAbs (VRC07, N6 and 1-18), however they were found to be significantly resistant to V1/V2 apex directed bnAbs. Moreover, we observed that the degree of sensitivity varied between contemporary Indian and South African clade C viruses. Differences in degree of neutralization susceptibility were associated with differences observed in key residues that form bnAb contact sites, gp120 loop lengths and the number of N-linked glycans in the V4 hypervariable region. Interestingly, the second generation CD4bs bnAbs (VRC07, N6, 1-18) showed neutralization of VRC01 and 3BNC117 resistant viruses but with 2-7-fold reduced potency compared to the VRC01 sensitive counterparts, likely due to the enrichment of resistance associated residues observed in loop D. Predictive analysis indicated that combination of BG18, N6 and PGDM1400 can provide over 95% neutralization coverage at 1µg/mL of contemporary India clade C, an observation found to be distinct to that reported for the Africa clade C viruses. Taken together, we found distinct neutralization patterns and *env* signatures associated with resistance to key bnAbs. Our study highlights that towards achieving clinical effectiveness, both the complementarity of bnAb classes and the regionally relevant HIV forms need to be considered.

Author summary

While the development of vaccines to prevent HIV infection remains a global priority, their potential effectiveness is limited by the extraordinarily diversified circulating forms of HIV-1. The prospect of best-in-class bnAbs as potential prevention option has been demonstrated in several studies including the Phase II Antibody Mediated Prevention (AMP) trial; however, to be broadly applicable, bnAbs will need to overcome the substantial variability of HIV *env*. The present study highlights that the contemporary HIV-1 clade C viruses are evolving to be less sensitive to the best-in-class bnAbs and HIV-1 clade C that predominates in India and South Africa vary in their degree of susceptibility to best-in-class clinically relevant bnAbs. This indicates differences in the antigenic properties between globally circulating HIV-1 clade C at a population level. Overall, the outcome of this study highlights the need for periodic assessment of sequence and neutralization profiles of the circulating regionally relevant HIV-1 forms towards prioritizing the bnAb combination suitable for effective intervention.

Introduction

HIV with complex and evolving diversity [1] remains a global health priority with over 39 million people currently infected globally, 2.4 million of which reside in India making India the third largest HIV epidemic globally [2, 3]. The high genetic variability of globally circulating HIV, both between and within an individual has been a major roadblock in designing an effective preventive intervention despite significant efforts [4]. While antiretroviral therapy has been successful in treatment of HIV and slowing down the spread of the initial pandemic, rising global resistance to available antiretrovirals necessitates expansion of available therapeutics and has reinvigorated efforts to design effective vaccines [5]. In the absence of an efficacious vaccine against HIV, passively administered broadly neutralizing monoclonal antibodies (bnAbs) along with effective antiretroviral drugs (ARV) could play a significant role in reducing incidence in high risk groups and key populations [6]. While efforts towards developing vaccine immunogens capable of inducing broadly neutralizing antibodies (bnAb) are ongoing, the recently conducted Phase 2B Antibody-Mediated Prevention (AMP) efficacy trial (HVTN 704/HPTN 085) demonstrated that a passively administered bnAb could prevent infection by bnAb sensitive viruses [7, 8]. The study also highlighted that combination of bnAbs would be required for optimal coverage of globally circulating HIV-1 subtypes towards capturing those that are resistant to one bnAb class but sensitive to another. HIV-1 clade C, which is the major globally circulating form, also forms the bulk of infections in South Africa and India, although genetic and functional data pertaining to contemporary HIV-1 forms from India are very limited. HIV-1 evolution over time is believed to contribute to changes in *env* sequence, the sole target of bnAbs, that will likely impact the consistencies with the breadth and potency of bnAbs with clinical relevance to effectively tackle currently circulating forms globally [9-11]. This is particularly important as HIV-1 evolution over time is expected to bring in changes in *env* sequence, the sole target of neutralizing antibodies which would impact the degree of susceptibility of contemporary viruses to bnAbs that are relevant for clinical use [9-11].

Previous studies provided evidence of HIV-1 clade B and non-India C viruses becoming increasingly resistant to select bnAbs over time [9, 11-15]. Our previous study using limited historical

(obtained prior to 2014) HIV-1 clade C of Indian origin [10] indicated significant variation in their susceptibility to bnAbs and also indicated that evolving viruses were becoming increasingly resistant to key bnAbs such as CAP256-VRC26.25. In the present study, we examined neutralization profiles of the HIV-1 clade C as pseudoviruses encoding full length *env* (*gp160*) of India isolated between 2020-2023 (contemporary) from nine geographically distinct regions in India, compared with that from South Africa and examined the best-in-class bnAbs that would provide optimal neutralization coverage of contemporary India clade C viruses. The *env* sequence diversity and neutralization profiles of contemporary India clade C were also compared with that of South African origin.

Results

Phylogenetic profiles of contemporary HIV-1 clade C from different geographical regions of India.

We first examined the phylogenetic relationship of the *env* gene of the contemporary viruses from different geographical regions. We obtained unique full length *env* (*gp160*) sequences from 232 individuals from nine geographically distinct sites in India between 2020 and 2023 (**Fig 1A**). These include sequences obtained from ART naïve early seroconverter and from individuals on ART (**Table S1**). The PCR amplified products were processed for high throughput deep sequencing using Oxford Nanopore technology (ONT) and Illumina based NGS platforms to obtain long and short read sequences. A consensus *env* sequence representative of the major circulating variant for each individual was constructed based on multiple alignment of both short and long read sequences to ensure inclusion of the accurate dominant *env* sequences for further analysis. Phylogenetic analysis was performed using these contemporary Indian *env* sequences along with 17 HIV-1 group M reference sequences (hiv.lanl.gov), shown in **Fig.1B**. No region-specific phylogenetic clustering was observed. Interestingly, we identified five subtype A1, one subtype B and four A1/C recombinants (**Table S1**). Furthermore, *pol* gene sequencing of HIV+ RNA obtained from the therapy naïve individuals showed that 11% of them contain major (>50% variant frequency in deep sequencing data) drug resistance associated mutations (DRM) with reverse transcriptase (RT) associated DRM found to be more prevalent compared to protease (PR) and integrase inhibitor associated DRMs (**Table S1**). Our observation provides evidence of establishment of infection by the drug-resistant HIV-1.

Contemporary HIV-1 India clade C demonstrated significant resistance to V1/V2 directed antibodies compared to those that target CD4 binding and V3 supersites.

Next, we examined the neutralization profiles of the contemporary viruses against a large panel of bnAbs with distinct specificities. We randomly selected 115 unique sequences representative all the nine geographically distinct sites in India and prepared pseudoviruses expressing these unique *env* sequences (**Table 1**), which were further examined against 14 bnAbs of distinct epitope specificities in viral Env. All of these envelopes were found to be CCR5 tropic (**Table 1**). As shown in **Fig. 2**,

pseudoviruses expressing contemporary clade C *envs* showed broad sensitivity to bnAbs targeting CD4bs and V3 glycan supersite, compared to ones that target V1/V2 apex. Among bnAbs targeting CD4bs directed bnAbs, N6, 1-18 and VRC07 showed >90% of viruses neutralized with IC50/IC80 values <25µg/mL respectively and over 78% and 65% of the panel viruses neutralized with IC50/IC80 values <1µg/mL respectively) (**Fig.2; Table S2**). Among the V3 glycan supersite directed bnAbs, 10-1074 and BG18 were observed to demonstrate maximal neutralization breadth (>80% and >70% of the panel viruses neutralized with IC50 and IC80 values <25µg/mL by these bnAbs while over 81% and 74% of the panel viruses neutralized with IC50 and IC80 values <1µg/mL). Amongst all the bnAbs examined, N6 (demonstrated >94% coverage with IC80 of 0.44 µg/mL) and 10-1074 (demonstrated >80% coverage with IC80 of 0.63 µg/mL) were found to be most broad while BG18 (IC80 of 0.29 µg/mL) was found to be most potent. While we identified several clade C viruses with class-specific resistance (**Fig.S1**), V1/V2 directed bnAb class-specific resistant viruses were relatively common (**Table S2**). A total of 45% and 40% contemporary viruses were found to be resistant to CAP256-VRC26.25 and PGDM1400 respectively. We also identified viruses with resistance to best-in-class bnAbs that target the CD4bs (VRC01, 3BNC117, VRC07, N6 and 1-18) and V3 supersite (PGT121, BG18, 10-1074). Specifically, we identified *env* sequence isolated from four unique donors, which when expressed as pseudoviruses (**Table S2; Fig.S1**) demonstrated complete resistance to all the CD4s-directed bnAbs tested in our study; VRC01, VRC07, N6 and 1-18. For V3 glycan supersite directed bnAbs, we identified eight unique donors, *envs* which demonstrated complete resistance to PGT121, 10-1074 and BG18 (**Table S2; Fig.S1**) Moreover, amongst these individuals, we identified *env* sequences from six individuals which when expressed as pseudovirus demonstrated very broad resistance to the majority of the class-specific bnAbs tested in this study (**Table 2**).

We next compared the neutralization profile of the contemporary and historic (isolated prior to 2014) HIV-1 India clade C against CAP256-VRC26.25, PGDM1400, VRC01, VRC07 and PGT121. Contemporary viruses were found to become significantly more resistant to CAP256-VRC26.25 ($p<0.005$) and more sensitive to PGT121 ($p<0.01$) when compared with historic viruses (collected before 2014) (**Fig.3A**). While not reaching statistical significance, a trend in contemporary viruses

becoming resistant to PGDM1400 and VRC01 was observed. Conversely, a significant increase in sensitivity of contemporary viruses to PGT121 ($p < 0.0005$) was observed. Interestingly, VRC07 was found to demonstrate comparable neutralization of both historical and contemporary Indian clade C, which is in contrast to that observed with contemporary African clade C viruses [9].

By comparing the *env* sequences of contemporary and historical-Indian clade C viruses, we found that overall, they significantly differ in their PNLG site content in *gp120*, specifically in the V1/V2 domain and in their net charge in the V1 hypervariable region. (**Fig.3B**). These features may contribute to reduced sensitivity of contemporary viruses to CAP256-VRC26.25 and PGDM1400, but increased sensitivity to PGT121.

Neutralization profiles of India and South Africa clade C viruses differ against multiple bnAb classes.

We next made a head-to-head comparison of contemporary India and South Africa HIV-1 clade C *envs* (*gp160*) to examine (a) their phylogenetic relatedness and (b) their sensitivity to bnAbs. For the phylogenetic analysis, we examined 232 and 73 clade C *envs* of Indian and South African origin. Of 73 HIV-1 clade C *env* sequences of African origin, 41 were obtained from individuals enrolled in the FRESH cohort [16] and the rest (32) [9] were obtained from the placebo arm of the phase 2 HVTN 704/HPTN 085 Antibody Mediated Prevention (AMP) prevention trial (South Africa 19, Malawi 7, Zambia 4 and 1 each from Mozambique and Botswana. [7]. As shown in **Fig 4A**, we observed distinct clustering of India and South Africa viruses, consistent with our previous observation [17]. This indicates that *env* genes are genetically distinct and continue to evolve independently in the two geographic regions.

We next compared neutralization sensitivity of contemporary Indian (N=115) and South African clade C (N=47, obtained from AMP Placebo group) *envs* against 14 bnAbs described above. We observed significant differences in their neutralization susceptibility to N6 ($p < 0.05$), 10-1074 ($p < 0.0005$) and BG18 ($p < 0.005$) (**Fig.4B**), with Indian HIV-1 clade C being significantly more sensitive to these three bnAbs than African viruses. In general, we observed that except for VRC01 and 3BNC117, comparable neutralization sensitivity to VRC07, 1-18 and N6 observed between Indian and

South Africa clade C viruses. However, India clade C viruses demonstrated increased sensitivity to the V3 glycan supersite directed bnAbs examined. Notably, both India and African contemporary clade C viruses were found to show poor susceptibility to CAP256-VRC26.25 and PGDM1400 compared to other bnAbs. However, the degree of resistance to CAP256-VRC26.25 and PGDM1400 were found to differ between India and South Africa clade C (**Fig.4C**). Furthermore, while both Indian and African clade C viruses are broadly resistant to CAP256-VRC26.25, Indian clade C viruses were found to be more resistant to CAP256-VRC26.25 compared to that observed with African viruses as determined by their mean IC₅₀ values Overall, Indian and African HIV-1 clade C vary significantly in their bnAb neutralization profiles, highlighting the divergence that can occur, even within the same clade.

Diversity in sequence characteristics that differentiate neutralization sensitive and resistant envelopes.

Next, we examined the amino acids in Env that form bnAb contact sites. We created sequence logos to examine the distribution of amino acids and performed statistical tests to assess enrichment of resistance associated signatures. Since the majority of the contemporary viruses were resistant to the V1/V2 directed bnAbs (CAP256-VRC26.25 and PGDM1400), we first examined the distribution of relevant amino acid residues within these epitopes. As shown in **Fig. 5A**, for CAP256-VRC26.25 resistant viruses, we saw signals at positions 160, 166, 169, 170, 200, 332, 632 and 775. We observed significant increases in the frequency of R169, Q170, T332 and decreased frequency of N160, K169 in CAP256-VRC26.25 resistant viruses when compared with the CAP256-VRC26.25 sensitive viruses. For PGDM1400, we observed enrichment of resistance associated residues at positions 160, 169, 172, 275, 332 (**Fig.5B**). Variation in V1/V2 loop length has been shown to modulate sensitivity to V2 apex directed neutralizing antibodies [18-20]. The CAP256-VRC26.25 resistant viruses were also found to have significantly lower net charge in V2 region ($P=0.006$) compared to their sensitive counterparts (**Fig.5C**). Unlike South African clade C viruses, no significant differences were seen in V1 loop length of CAP256-VRC26.25 sensitive and resistant India clade C viruses (**Fig.5C**; **Fig.S2**), nor was there a significant difference in V2 loop length and net charge between PGDM1400 sensitive and resistant India clade C viruses (**Fig.5C**; **Fig.S2**). For V3 directed bnAbs, we examined residues associated with

190 resistance to PGT121, 10-1074 and BG18. We observed higher variation/entropy within such residues
 191 in PGT121 resistant viruses followed by 10-1074 and BG18 resistant viruses (**Fig.S3**). For PGT121
 192 resistant viruses, significant enrichment and reduction of key residues at position 137, 139, 140, 328,
 193 332, and 334 was observed (**Fig.S3A**). In 10-1074 resistant viruses, there was significant enrichment
 194 of A137, E322, K328, Y330, T332 and N334 compared with sensitive viruses (**Fig.S3A**). While most
 195 contemporary viruses were potently neutralized by BG18, the few that showed resistance were
 196 significantly enriched for Y330 and N/T330 (**Fig.S3A**). For PGT121 resistant viruses, we observed low
 197 net charge in the V1/V2 hypervariable region compared to the PGT121 sensitive viruses ($P=0.04$). We
 198 also observed significant differences in V4 loop length ($P=0.02$) and net charge in the V1 loop ($P=0.04$)
 199 in the 10-1074 resistant viruses (**Fig.S3B**). With respect to BG18 resistant viruses, we observed
 200 significant differences in the net charges in the V1 loop ($P=0.03$), V4 loop length ($P=0.01$) and PNLG
 201 content in V4 loop ($P=0.01$) when compared with BG18 sensitive viruses (**Fig.S3B**). Amongst CD4bs
 202 directed bnAbs examined, contemporary viruses showed least susceptibility to 3BNC117 (26.08% were
 203 found to be resistant) followed by VRC01 (23.47%), 1-18 (10.43%), VRC07 9.56%) and N6 (7.82%)
 204 (**Fig.8A**). We observed significant enrichment of E279, S280, R282, F318, V371, E455 and significant
 205 reduction in the occurrence of N280, Y318, S365, I371, R456, G459, G471 in viruses resistant to
 206 3BNC117 (**Fig.S4**). Overall, in VRC01 resistant viruses, we observed enrichment of aspartic (D) and
 207 glutamic acid (E) residues at position 97 in C1 region of the envelope inner domain, polymorphisms at
 208 279 and 281 positions in the loop D and enrichment of glutamic acid (E) and/or leucine (L) at 455
 209 position, tryptophan (W) at 456 position, aspartic acid (D) at asparagine (N) at the positions 455, 456
 210 and 474 positions in the β 23/loop- β 24/V5 region of the viral Env protein. Similarly, in 3BNC117
 211 resistant viruses, we observed significant polymorphism at positions 279, 280, 282, 318, 371, 455, 459
 212 and 471 on viral Env protein that are associated with modulation of sensitivity to 3BNC117
 213 (www.hiv.lanl.gov). Interestingly, we observed enrichment of several amino acid residues at positions
 214 279, 280, 281, 355, 365, 456, 459, 463 and 471 in the N6 resistant viruses (**Fig.S4**) around CD4bs
 215 region. Significant differences observed between the variable region of viruses sensitive and resistant
 216 to VRC01, VRC07 and N6 are shown in **Fig.S5**.

217 When we compared the sequence features of the contemporary HIV-1 clade C *env* of Indian
 218 and South African origins, we observed a significant difference in their *gp160* (both *gp120* loop and
 219 *gp41*) lengths (**Fig.6A**). In particular, we found a significant difference in the V4 loop length between
 220 the contemporary viruses from these two geographically distinct regions with longer loops observed
 221 with Indian contemporary viruses. Moreover, contemporary India and Africa clade C *envs* also
 222 significantly differed in their V1/V2 net charge and PNLGs in the V4 loop (**Fig.6A**). We compared the
 223 *env* sequences of India and Africa viruses that showed resistance to V1/V2 directed bnAbs. For
 224 CAP256-VRC26.25 and PGDM1400 resistant viruses, we found differences at sites 160, 166, 169 and
 225 170 (**Fig.6B**). Interestingly, except for differences in net charge in V2 loop between PGDM1400
 226 resistant viruses of India and South Africa clade C, no differences in loop lengths and PNGs observed
 227 between CAP256-VRC26.25 and PGDM1400 resistant India and South Africa clade C viruses
 228 suggestive of similar mechanisms of resistance across the two regions (**Fig.S6**). We also observed
 229 differences in frequencies of contact residues targeted by CD4bs (N6) and V3 glycan directed
 230 (PGT121, 10-1074, BG18) bnAbs between India and South Africa clade C viruses (**Fig.S7**) which may
 231 explain the differences in their sensitivity (**Fig. 4B**). While the above analysis was carried out using the
 232 *env* sequences which were expressed and tested against the selected bnAbs as pseudoviruses, we
 233 analyzed additional contemporary clade C *env* sequences (not used for preparing pseudoviruses)
 234 isolated from nine geographically distinct regions of India (as described above) and from South Africa
 235 (sourced from FRESH cohort). While both datasets displayed enrichment of resistant signatures for
 236 CAP256-VRC26.25 bnAb at positions 165, 166 and 169, statistically significantly different
 237 enrichment of resistant signature was observed at residue position 166 (**Fig.S8**). Analysis of
 238 PGDM1400 contact residues indicated a trend of differential abundance at position 160 with significant
 239 differences at positions 130 and 161 as well as 211. N332, the target site for PGT121, 10-1074 and
 240 BG18 bnAbs was more conserved in Indian sequences compared to those from South Africa (**Fig.S8**).
 241 Overall, our data indicated that the differential sensitivity of India and Africa clade C contemporary
 242 viruses bnAb classes is associated with distinct sequence features including those in bnAb contact
 243 residues.

244

245 **Viruses resistant to V1/V2 directed antibodies remain well neutralized by CD4bs directed** 246 **antibodies.**

247 We next examined the ability of other bnAbs to neutralize V1/V2 directed bnAb resistant viruses. As
248 shown in **Fig.7**, CAP256-VRC26.25 resistant viruses were found to be best neutralized by CD4bs
249 directed bnAbs (82.25% breadth) over the V3 directed bnAbs (74.19%). Amongst the CD4bs directed
250 bnAbs, N6 and 1-18 demonstrated best breadth (91.93%) and 3BNC117 was found to be least broad
251 amongst all (58.06%). As for V3 directed bnAbs, CAP256-VRC26.25 resistant viruses were found to
252 be best neutralized by 10-1074 (80.64%), followed by BG18 (75.80%) and PGT121 (66.13%). When
253 we analyzed viruses that demonstrated complete resistance to all the V1/V2 apex directed bnAbs, we
254 again found that compared to V3 directed bnAbs (65.38% breadth), they are best neutralized by CD4bs
255 directed bnAbs (81.53%) with both N6 and 1-18 demonstrating maximum breadth (92.30% breadth in
256 both), however N6 was found to be more potent with IC50 of 0.35µg/mL over 1-18 with IC50 of 1.01
257 µg/mL. Our data indicates that while contemporary Indian clade C viruses showed poor susceptibility
258 to V1/V2 directed bnAbs, they remain broadly sensitive to lead CD4bs and V3 specific bnAbs.

260 **VRC01 & 3BNC117 resistant viruses are neutralized by second generation CD4bs bnAbs but** 261 **with reduced potency.**

262 While CD4bs directed antibodies were found to demonstrate best neutralization coverage of the
263 contemporary viruses, we next examined whether resistance to CD4bs bnAbs VRC01 and 3BNC117
264 conferred decreased sensitivity to second generation CD4bs bnAbs. As shown in **Fig.8A**, amongst all
265 the CD4bs bnAbs tested, contemporary Indian clade C showed greater resistance to VRC01 and
266 3BNC117 (23.48% and 26.08% respectively) compared to those that showed resistance to VRC07
267 (9.56%), N6 (7.82%) and 1-18 (10.43%). We next examined the extent of neutralization of VRC01 and
268 3BNC117 resistant contemporary viruses by other CD4bs bnAb classes. We observed that N6
269 neutralized (77.77%) most of the VRC01 resistant viruses, while 1-18 could neutralize (75%) most of
270 the 3BNC117 resistant contemporary viruses (**Fig.8B**). Interestingly, when compared with VRC01 and
271 3BNC117 sensitive viruses, VRC07, N6 and 1-18 were found to neutralize VRC01 and 3BNC117

272 resistant viruses with reduced potency by over 2-fold (**Fig.8C**). The reduced potencies could likely be
273 due to enrichment of resistance associated amino acid residues observed when we compared the VRC01
274 and 3BNC117 sensitive and resistant viruses. Overall, we found that in addition to clinically relevant
275 V1/V2 bnAb resistant viruses, the second generation CD4bs bnAbs such as VRC07, N6 and 1-18 are
276 capable of neutralizing viruses that are resistant to first generation CD4bs bnAbs VRC01 and
277 3BNC117.

278

279 **Combination of BG18, N6 and PGDM1400 is predicted to provide optimal neutralization of India**
280 **clade C.**

281 Towards identifying the most optimal combination of bnAbs capable of comprehensively neutralizing
282 contemporary clade C viruses, we included bnAbs that demonstrated neutralization breadth >50% with
283 IC80 of <25µg/mL. Also, in order to perform a head-to-head comparison, we assessed the extent of
284 neutralization coverage of the contemporary clade C viruses from Africa (N=40) by the same set of
285 bnAbs. The CombiNAber analysis

286 (<https://www.hiv.lanl.gov/content/sequence/COMBINABER/combinaber.html>) was carried out for
287 both set of viruses (of India and Africa origins) at the target concentration of 1µg and 10µg/mL
288 respectively using the Bliss-Hill model. At 10 µg/mL, Indian contemporary viruses were observed to
289 be most effectively neutralized by N6 and BG18 (**Fig.9A**). Both of these provided 91% and 72%
290 coverage at the target concentration with potency (IC80) of 0.44 and 0.30 µg/mL respectively. 1-18
291 and 10-1074 were the next best two CD4bs and V3 directed bnAbs with breadth of 83 and 80 and
292 potency (IC80) of 0.59 and 0.63 µg/mL respectively. With respect to the contemporary clade C viruses
293 from Africa, N6 was observed to be the most effective bnAb with 90% coverage and potency (IC80)
294 of 0.51 µg/mL. PGDM1400 and BG18 were comparably next most effective bnAbs with neutralization
295 breadth of 57.7% and 56.6% and potency (IC80) of 1.59 and 1.54 µg/mL respectively. When we
296 assessed 3 bnAb combination prediction, BG18 + N6 + PGDM1400 appear to provide best
297 neutralization coverage of Indian contemporary viruses with 99.13% breadth with IC80 predicted to be
298 at 0.03 µg/mL. However, the coverage drops to 79% when considering at least two active bnAbs. For
299 the HIV-1 clade C from South Africa, the combination of BG18 + PGDM1400 + 1-18 appear to be the

300 best combination with 100% coverage at 0.03 ug/mL IC80. The coverage drops to mere 81.81% when
 301 considering at least two active bnAbs. At 1 µg/mL, Indian contemporary clade C viruses appeared to
 302 be most effectively neutralized by BG18 and N6 (**Fig.9B**) as above. However, they showed 64 and
 303 66% neutralization coverage at the target concentration with potency (IC80) of 0.30 and 0.44 µg/mL
 304 respectively. Similarly, as with 10µg/mL concentration, 1-18 and 10-1074 were the next best bnAbs
 305 found with predicated neutralization coverage of 64% and 66% and potency (IC80) of 0.59 and 0.63
 306 µg/mL respectively. For the contemporary clade C viruses from South Africa, the most effective single
 307 mAbs were found to be 1-18 and BG18. These two bnAbs were observed to provide 65 and 43%
 308 coverage respectively, with potency of 0.53 and 1.54 ug/mL respectively. When we assessed
 309 neutralization coverage by three antibody combination, BG18 + N6 + PGDM1400 observed to provide
 310 93.91% coverage of Indian contemporary clade C viruses at IC80 of 0.03 ug/mL. This neutralization
 311 coverage, however drops to 58% when at least two active bnAbs were considered. Conversely, we
 312 observed BG18 + PGDM1400 + 1-18 combination to provide 95.45% coverage of African
 313 contemporary viruses at IC80 of 0.03 ug/mL and which drops significantly to 45.45% when considering
 314 at least two active bnAbs. Overall, our predictive data indicates that no combination could provide
 315 100% coverage for the clade C viruses from India and African origin at any of the considered target
 316 concentrations. The data further indicated that while a combination of V3, V2 apex and CD4bs directed
 317 bnAbs was effective across both regions, the clade C viruses from India and Africa are distinctly
 318 sensitive to different bnAbs of clinical relevance.

319

Discussion

While HIV-1 clade C is the major globally circulating form, evolutionary pattern may vary across different geographical regions representing ethnically diversified populations that may contribute to differential susceptibility to class-specific bnAbs. For example, there has been significant association between HIV evolution at population level and increased resistance to serum and bnAb mediated neutralization observed in HIV-1 clade B infected individuals [11-13]. Moreover, intra-clade diversity has been predicted to have better neutralization advantage in geographical regions with lower viral diversity compared to regions with substantial intra-clade diversities[21]. Therefore, it is unclear whether same combination of select bnAbs would stand fit to comprehensively provide neutralization coverage of the globally circulating and evolving HIV at the population level. It is therefore important to understand whether globally evolving HIV at a geographically and ethnically distinct population level can influence antigenic properties. Little information is available for contemporary HIV-1 clade C viruses predominantly circulating across India.

In the present study, we examined how *env* sequence diversity of contemporary HIV-1 Indian clade C (isolated between 2020 and 2023) differentiates them from historical viruses as well as contemporary HIV-1 clade C of South African origin. To encompass contemporary HIV-1 of Indian origin at the population level, we obtained samples as source of HIV from nine geographically distinct origins representing different risk group. Although region-specific numbers of viruses were moderate, perhaps accounting for the fact that we saw no region-specific clustering, to the best of our knowledge, this is first such study of genetic and neutralization profiles of contemporary viruses from geographically distinct regions in India. A larger sample size of region-specific circulating forms would provide more precise phylogenetic details.

Although the Indian contemporary clade C *envs* continue to cluster with historical viruses, we found a significant drift in the degree of their sensitivity to CAP256-VRC26.25 and PGDM1400, the two clinically relevant bnAbs that target V1/V2 apex region of the viral Env protein. Over 45% and 40% of the contemporary viruses were found to be resistant to CAP256-VRC26.25 and PGDM1400 respectively. Our observation is consistent with our earlier study [10] and that of South African clade C viruses [9]. Conversely, the contemporary India viruses showed increased sensitivity to PGT121

which is in contrast to previous observation in South African clade C viruses [9]. These differences could be due to increased predicted glycosylation in *gp120*, particularly in V1/V2, as previously described [9, 22, 23] and possibly also differences in net V1 charges as observed in our study. The resistance to CAP256-VRC26.25 and PGDM1400 is also likely due to enrichment of resistance associated amino acid residues in the key contact sites on viral envelope protein; such enrichment of K166, Q169 and/or K169 residues in CAP256-VRC26.25 resistant viruses and D160, Q169 and K275 in PGDM1400 resistant viruses.

Mkhize *et al.* [9] recently also reported a trend in decreasing sensitivity of Africa clade C (obtained from the placebo arm of the AMP trial participants) to VRC01 and VRC07, an observation that was not noted with Indian clade C viruses tested in this study. These observations along with other *env* sequence features such as loop length, glycosylation and net charges that differentiated contemporary India and Africa HIV-1 clade C clearly indicates that they continue to evolve independently and distinctly at population level.

A notable observation made was that the second generation CD4bs bnAbs (N6, 1-18 and VRC07) were able to neutralize contemporary Indian clade C viruses with significantly better breadth and potency compared to the first generation CD4bs bnAbs (VRC01 and 3BNC117). They also were found to neutralize majority of the contemporary viruses that showed resistance to the V1/V2 apex directed bnAbs (CAP256-VRC26.25 and PGDM1400) and VRC01 and 3BNC117. Such observation indicate that in comparison to V1/V2 directed bnAbs, the key contact sites and epitopes for N6, 1-18 and VRC07 are evolutionarily preserved. The poor neutralization breadth conferred by VRC01 and 3BNC117 could possibly be because of the substitutions of amino acid residues resulting due to selection pressure during the course of natural infection at one or more of their key contact sites that were reported to be essential for their ability to neutralize efficiently [24-26]. Interestingly, the second generation CD4bs bnAbs (N6, 1-18 and VRC07) were found to neutralize the VRC01 and 3BNC117 resistant viruses with over 2-fold lower potency than what was observed with their corresponding sensitive viruses. This could possibly due the following reasons observed with few VRC07, N6 and 1-18 resistant viruses: (a) increased net charge in V1/V2 hypervariable regions (VRC07), differences in

375 PNGS content in V1/V2 region and increased V1/V2 net charge in V1/V2 (N6) and/or (b) enrichment
376 of resistance associated residues.

377 Combination of best-in-class bnAbs with distinct specificities have been reported to improve
378 the optimal neutralization coverage of the HIV-1 diversity both by prediction and real-world
379 application in experimental trials [27-31]. Emergence of HIV-1 clade C variants that showed broad
380 resistance to major clinically relevant bnAbs was an interesting observation to note. Although few
381 identified in this study, their presence in early infected individuals may imply that such resistant viruses
382 can transmit and establish infection. Moreover, more such broadly resistant viruses are likely to evolve
383 over time at the population level. It is therefore important to identify bnAbs that can be included in the
384 antibody cocktail that can suitably compensate the inability of the existing best-in-class bnAbs to
385 neutralize such viruses. Identification of viruses that are broadly resistant to existing best-in-class
386 bnAbs also provides an opportunity to isolate new class of antibodies with new target specificities that
387 are capable of neutralization evolving viruses that are broadly resistant to the existing bnAbs. Although
388 we have predicted, based on individual virus neutralization data that BG18+N6+PGDM1400 would
389 provide maximal coverage, however this needs to be validated by neutralization assays.

390 One of the interesting observations made in this study is identification of mutations in *pol* gene
391 associated with drug resistance in isolates obtained from over 10% ART naïve donors (**Table S1**). This
392 indicates the ability of establishment of infection by drug resistant viruses [32, 33] which can
393 potentially minimize the efficacy of antiretroviral therapy post exposure. Such observation further
394 justifies the importance of the using next generation bnAbs as prevention strategy. One of the
395 limitations of this study is that we examined neutralization properties of the cross-sectionally collected
396 HIV+ samples and it will be useful to monitor longitudinally followed up cohort to periodically assess
397 how HIV evolution overtime influences effectivity of the clinically relevant bnAbs. Moreover, several
398 bnAbs that are under clinical development were isolated long ago and emerging reports indicate
399 including our present study indicates several of them becoming irrelevant for intervention. Therefore,
400 the need for periodic assessment of sequence and neutralization profiles of the regionally relevant
401 contemporary HIV-1 forms against engineered optimized clinically relevant bnAbs is required for
402 prioritizing and development of effective bnAbs as product for prevention.

403

404

Materials and Methods

Ethics statement.

406 All clinical samples from nine different sites in India were obtained following approval from respective
407 institutional ethical committees. Written informed consent forms in English and local languages were
408 provided and duly signed by all the recruited study participants. Experiments at respective institutions
409 were initiated post approval of institutional ethics committee. All experiments were carried out at the
410 THSTI, Faridabad post approval of institutional ethics committee (IEC) and institutional biosafety
411 committee.

412

Study participants.

414 HIV-1 infected individuals were recruited from nine geographically distinct clinical sites in India. They
415 are from Eastern (Kolkata), Western (Mumbai, Belagavi), Northern (Delhi, Bhopal), Southern (Nellore,
416 Hyderabad, Vizag) and North Eastern (Aizawl). following approvals from the institutional ethics
417 committee at all participating institutions. A total of 232 study participants were recruited from nine
418 different geographical sites as indicated in **Table 1**. Clinical parameter data such as CD4 counts, viral
419 load and antiretroviral therapy status were obtained for each study participant.

420

Plasmids, antibodies and cells.

422 Plasmids encoding full length codon optimized *gp160* of Indian origin synthesized at GenScript Inc.
423 were used for preparing pseudoviruses. Plasmids encoding HIV-1 clade C *env* genes of South African
424 origin from AMP placebo arm reported earlier [9] were used to prepare pseudoviruses for the
425 neutralization assay. pSG3ΔEnv was obtained from the NIH AIDS Reagent and Reference Program.
426 Plasmids encoding heavy and light chain immunoglobulins of CAP256-VRC26.25 was provided by
427 Prof Lynn Morris and ones with VRC01, VRC07, N6, 1-18, PGDM1400, 3BNC117, BG18, 10-1074,
428 10E8, VRC34.1 were provided by the IAVI Neutralizing Antibody Center. HEK 293T, TZM-bl, were
429 obtained from the American Type Culture Collection (ATPC) and GHOST-Hi5, GHOST-CXCR4 cells

were obtained from the NIH AIDS Reagents & Reference Program respectively. GHOST-CCR8 cells were kindly provided by Paul Clapham. Expi293 cells were purchased from Thermo Inc.

Isolation of viral and genomic DNA and cDNA synthesis.

Viral RNA was isolated from plasma using the High Pure viral RNA kit (Roche) as per manufacturer's instruction as described earlier[34]. Genomic DNA was isolated from peripheral blood mononuclear cells (PBMC) using QIAmp blood DNA mini kit (Qiagen) as per the manufacturer's instructions and as described earlier [34]. Plasma isolated RNA was primed with EnvR1 oligo (5'-GCACTCAAGGCAAGCTTTATTGAGGCT-3') proximal to 3' end of the HIV RNA genome (HXB2: 9605-9632) and Aenvseq4 (5'-CAAGCTTGTGTAATGGCTGAGG-3') binding downstream of the *pol* gene (HXB2: 6817-6838). Synthesis of cDNA was performed using Superscript III first strand synthesis kit (Invitrogen) following the protocol provided by the manufacturer.

Amplification of full length *gp160* and *pol*.

Full length *env* (*gp160*) genes were PCR amplified from HIV+ plasma samples with slight modification as described previously [34]. *Rev-env gp160* cassette were amplified from the cDNA product using La Taq high fidelity DNA polymerase in the 1st round (Takara Bio Inc.) and PrimeSTAR GXL high fidelity DNA polymerase (Takara Bio Inc.) in the second round. The primers used for the 1st round were EnvF1: 5'-AGARGAYAGATGGAACAAGCCCCAG-3' (HXB2: 5550–5574) and EnvRP2: 5'-GTGTGTAGTTCTGCCAATCAGGGAA-3' (HXB2: 9157–9181) while for the second round were Env IF: 5'-CACCGGCTTAGGCATCTCCTATGGCAGGAAGAA-3' (HXB2: 5950–5982) and EnvIR: 5'-TATCGGTACCAGTCTTGAGACGCTGCTCCTACTC-3' (HXB2: 8882–8915). PCR condition followed for both rounds were: initial denaturation of 94 °C for 2 min followed by 15 cycles of 94 °C for 10 secs, 60 °C for 30 secs, 68 °C for 3 min, 20 cycles of 94 °C for 10 secs, 55 °C for 30 s, 68 °C for 3 min with final extension of 68 °C for 10 min. The *gp160* amplicons were purified and subsequently subjected to short (Illumina) and short read deep sequencing to obtain dominant sequences as described below which were subjected to codon optimization, synthesized and cloned into pcDNA3.1 expression vector. Few *env* clones (**Table 1**) were cloned in-house in

458 pcDNA3.1/V5-His-TOPO (Invitrogen Inc.) vector as described before [34]. The primers used for the
 459 1st round towards pol amplification were Pro5F: 5'- AGAAATTGCAGGGCCCCTAGGAA -3'
 460 (HXB2: 1996-2018) and PolR1: 5'- GGTACCCCATAATAGACTGTRACCCACAA -3' (HXB2:
 461 6324-6352) while for the second round were Pro3F: 5'- AGANCAGAGCCAACAGCCCCACCA -3'
 462 (HXB2: 2143-2166) and PolR2: 5'- CTCTCATTGCCACTGTCTTCTGCTC -3' (HXB2: 6207-6231).
 463 PCR condition followed in the both rounds were initial denaturation of 94 °C for 2 min followed by 15
 464 cycles of 94 °C for 10 secs, 65 °C for 30 secs, 68 °C for 3 min, 20 cycles of 94 °C for 10 secs, 55 °C
 465 for 30 s, 68 °C for 3 min with final extension of 68 °C for 10 min.

466

467 **Next generation deep sequencing and construction of *env* sequences.**

468 Env amplicons were sequenced using both long read Oxford Nanopore (ON) and short read Illumina
 469 (IL) platforms. Next generation sequencing was performed for 5' fragments using the Illumina platform
 470 while 3' fragments were sequenced using both Illumina and Oxford nanopore platforms. The raw data
 471 obtained from the nanopore sequencing was converted to Fastq files using Guppy basecaller (v6.3.7).
 472 Raw reads were further filtered for quality and read length using Prowler (Flags: -l 1500 -q 12 -c "LT"
 473 -g "F1" -m "S") [35]. The reads were aligned to the HIV-1 subtype C reference sequence (GenBank
 474 ID: AF067155.1) [36] using Minimap2 [37, 38] and processed for read sorting and filtration with
 475 samtools [39]. Reads encompassing the entire gene were extracted from the binary alignment maps
 476 using Picard tools (<https://broadinstitute.github.io/picard/>). Reads were further clustered and corrected
 477 using isONclust and isONcorrect respectively into quasispecies clusters [40, 41]. Consensus sequences
 478 were generated for each quasispecies clusters using iVar [42]. Quasispecies thus constructed were
 479 further corrected with the help of Illumina reads using Pilon [43].

480

481 **Preparation of Env-pseudoviruses.**

482 Pseudotyped viruses were prepared as described previously [44]. Briefly 293T cells were co-transfected
 483 by plasmid DNA encoding *gpl60* and pSG3ΔEnv plasmid (having a premature stop codon at the
 484 beginning of *env*) into 293T cells in 6-well tissue culture plates using FuGENE6 transfection reagent
 485 kit (Promega Inc.). Cell culture supernatants containing pseudotyped viruses were harvested at 48 h

post transfection and subsequently stored at -80° C until use. The virus infectivity was measured using TZM-bl reporter cells by addition of pseudoviruses containing DEAE-dextran (25 µg/ml) in 96-well microtiter plates, and the viral titers were determined by measuring the luciferase activity using Britelite luciferase substrate (PerkinElmer Inc.) with a Victor X2 luminometer (PerkinElmer Inc.).

Coreceptor usage.

Coreceptor preference of contemporary envelopes was examined by cell-cell fusion assay as described before [45]. Briefly, 293T cells expressing individual *env* was mixed with GHOST-Hi5, GHOST-CXCR4 and GHOST-CCR8 post 24 hours of transfection and further incubated for additional day at 37°C in a CO₂ incubator. Syncytia forming giant cells were identified by staining with chilled methanol containing 1% methylene blue and 0.25% basic fuchsin. 293T cells expressing 16055-2.3 for GHOST-Hi5 [46], NARI-VB105 [45] for GHOST-CXCR4 and NARI-VB52 for GHOST-CCR8 [47] were used as positive controls.

Pseudovirus Neutralization assay.

Neutralization assays were carried out using TZM-bl cells as described before [44]. Briefly, Env-pseudotyped viruses were pre-incubated in 96-well tissue culture plates with various concentrations of bnAbs (IgG) for an hour at 37°C in a CO₂ incubator under humidified conditions. Subsequently, 1 × 10⁴ TZM-bl cells were added to the mixture in the presence of 25 µg/ml DEAE-dextran (Sigma, Inc.). The plates were further incubated for 48 h. The degree of virus neutralization was assessed by measuring reduction in relative luminescence units (RLU) in a luminometer (Victor X2; PerkinElmer Inc.). The IC₅₀ and IC₈₀ values were calculated using R using the DRC statistical package (analysis of dose response curves).

ARV resistance mutations prediction.

Illumina FASTQ reads were filtered for quality (>Q30) using Trimmomatic (v0.39). All the reads were aligned to the HXB2 genome using bwa-mem (v0.7.17-r1188). BAM files were filtered for quality

using samtools. Variant calling was performed for the *pol* gene using iVar pipeline. Drug resistance mutation prediction was then performed for the variants obtained using Stanford drug resistance database HIVdB (<https://hivdb.stanford.edu/hivdb/by-patterns/>). Resistance patterns were recorded only for variants with frequency greater than 10%.

Phylogenetic analysis.

Phylogenetic trees were generated for 249 HIV-1 envelope amino acid sequences that included 232 contemporary sequences from India and 17 HIV-1 group M subtype reference sequences; and 594 HIV-1 envelope amino acid sequences that included 233 contemporary and 132 historical sequences from India, 74 contemporary and 138 historical sequences from Africa along with 17 HIV-1 group M subtype reference sequences. These sequence datasets were aligned using MAFFT and the alignment was manually curated in BioEdit v7.2.5. The tree was constructed with IQ-TREE under HIVb model [48, 49] with estimated γ parameters and number of invariable sites. The robustness of the tree topology was further assessed by SH-aLRT as well as 1000 ultrafast bootstrap replicates implemented in IQ-TREE as described earlier [17].

Variable region characteristics and prediction of pNLG.

Variable region characteristics such as loop length, charge and number of pNLG sites were assessed for all envelope sequences using the ‘variable characteristics tool’ hosted at the Los Alamos National Laboratory HIV database (LANL-HIVDB, https://www.hiv.lanl.gov/content/sequence/VAR_REG_CHAR/index.html). Potential N linked glycosylation sites prediction was performed with the tool N-Glycosite at LANL-HIVDB (<https://www.hiv.lanl.gov/content/sequence/GLYCOSITE/glycosite.html>).

bnAb contact site assessment.

For bnAbs CAP256-VRC26.25, PGDM1400, PGT145 (V2 apex directed), PGT121, BG18, 10-1074 (V3g supersite directed), VRC01, VRC07, 1-18, N6, 3BNC117 (CD4 binding site) and 10E8 (MPER directed), specific epitope contact positions as well as documented sensitivity/resistance imparting

541 variants at each position were retrieved from CATNAP database
 542 (<https://www.hiv.lanl.gov/components/sequence/HIV/neutralization/main.comp>). Each of the
 543 sequences were then assessed for presence of sensitive/resistant/undefined mutation at each of these
 544 positions using custom bash scripts. In sequence logos, O has been used to differentiate potential N
 545 linked glycosylated Asparagine from potentially unglycosylated Asparagine (N).

546

547 **CombiNAber analysis.**

548 Optimal combination prediction was performed with the CombiNAber tool at LABL-HIVDB
 549 (<https://www.hiv.lanl.gov/content/sequence/COMBINABER/combinaber.html>). CombiNAber
 550 predictions were made with the IC50 and IC80 neutralization data using the bliss hill model at target
 551 concentrations of 10ug/mL and 1ug/mL for 3 distinct specificity bnAb combinations as well as active
 552 coverage by at least 2 bnAbs.

553

554 **Statistical analyses and data presentation.**

555 Phylogenetic trees were annotated using the ‘ggtree’ package in R. Sequence logos were constructed
 556 with ‘ggseqlogo’ package in R. All plots were prepared using the R package ggplot2. Statistical
 557 comparison of variable region characteristics with Mann-Whitney test. Fisher’s test for abundance of
 558 bnAb resistance associated residues was performed through R statistical computing software (v3.4.0)
 559 and R studio v1.0.143. Statistical analysis for neutralization breadth and potency were done using
 560 GraphPad Prism version 10 for Windows, GraphPad Software.

561

562 **Data availability.**

563 Novel *env* and *pol* nucleotide sequences obtained from Indian donors are being submitted to GenBank.
 564 *env* nucleotide sequences from FRESH cohort has been submitted to GenBank.

565

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575

Individual contributions.

577 JB with help of VP, KGM conceptualized the study and supervised the entire study, was responsible
578 for funding acquisition and resources for the conduct of the present study; JS, PJ, RM along with JB
579 planned major experiments; JS, PJ, RM carried out majority of the experiments and carried out
580 sequence and neutralization data analysis; JS led the next generation sequencing based experiments
581 along with SB, PK, SS, NP, constructed consensus sequences used for synthesis of constructs used for
582 preparation of pseudoviruses; NN, VP, PD, NK, SB, VB; MA, CVK, TRD helped with viral gene
583 amplification and sequencing; PJ, JS, RM, SD carried out molecular cloning; SC, ST, BS, SD helped
584 with preparation of pseudoviruses and neutralization assays; SM, NS, CP, NK prepared purified IgGs
585 and carried out QA/QC for ensuring quality and bnAb specificities before they used in neutralization
586 assays; AKS, JS, SM, BB, SKG contributed in creation of cohorts in different sites in India and
587 supervised sample preparation, clinical investigations; DK, DB, DP, RSP, SM, H, RD, AK helped with
588 separation of plasma and PBMCs, assessment of immunological parameters; DKit, HK, NNM carried
589 out majority of neutralization assays and sequence analysis of HIV-1 clade C viruses of South African
590 origin; BN and KG helped with providing HIV-1 clade C *env* sequence information obtained from
591 FRESH acute cohort, South Africa; PLM and TN helped with data analysis, supervision of experiments
592 carried out at individual laboratories in South Africa; DS helped with experimental design and data
593 analysis; JB with assistance from JS, PJ, RM, VP, KGM, PLM and TN wrote the manuscript.

594

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 605

Table Legends

Table 1. Source, coreceptor usage and other properties of contemporary HIV-1 clade C functional clones.

Table 2. Neutralization profiles of pseudoviruses bearing contemporary *envs* broadly resistant to bnAbs with distinct specificities.

Figure legends.

Fig.1. Phylogenetic relatedness of the contemporary HIV-1 India clade C at the population level.

A. Surveillance sites built and samples collected across different geographical sites in India between 2020-2023 **B.** Phylogenetic relatedness of HIV-1 clade C Env proteins representing circulating forms in different geographic regions. Phylogenetic trees were generated for 249 HIV-1 envelope amino acid sequences that included 232 contemporary (obtained between 2020 and 2023) from India and 17 HIV-1 group M reference sequences. These sequences were aligned using MAFFT and the alignment was manually curated in BioEdit v7.2.5. The phylogenetic tree was constructed with IQ-TREE under HIVb model with estimated γ parameters and number of invariable sites. The robustness of the tree topology was further assessed by SH-aLRT as well as 1000 ultrafast bootstrap replicates implemented in IQ-TREE.

Fig.2. Neutralization profiles of contemporary HIV-1 India clade C to best-in-class existing

bnAbs. Pseudoviruses expressing 115 contemporary *envs* obtained from individuals representing nine geographically distant regions in India and comprising distinct risk groups were assessed for their degree of susceptibility to 14 bnAbs as indicated having distinct epitope specificities on viral Env. IC50 and IC80 refers to the IgG concentrations [$\mu\text{g/mL}$] at which pseudoviruses demonstrated 50% and 80% neutralizations respectively. Pseudoviruses that were not neutralized up to $25\mu\text{g/mL}$ of IgG were considered as resistant viruses. Neutralization assay was carried out at least 3 times in duplicates and average was used to plot the graph. Neutralization breadth of each bnAb expressed as percent neutralization by IgG up to $25\mu\text{g/mL}$ are shown on top of each graph (upper and lower panel).

Fig.3. Comparison of neutralization sensitivity to key bnAbs between historic and

contemporary India clade C. A. Degree of neutralization susceptibility of historical (N=124; obtained before 2014) and contemporary viruses (N=115; obtained between 2020-2023)

assessed by pseudovirus neutralization assay. IC₅₀ value of 25 µg/mL was considered as neutralization sensitivity threshold. Statistical analysis to assess significance (P values) of differences in neutralization sensitivity to a given bnAb by pseudoviruses expressing both historical and contemporary *envs* was performed by Mann-Whitney test. Neutralization assay was repeated at least 3 times in duplicates and average was used to plot the graph. **B.** *gp120* variable loop characteristics of historical and contemporary *env* sequences were assessed using the ‘variable characteristics tool’ hosted at the Los Alamos National Laboratory HIV database (LANL-HIVDB, https://www.hiv.lanl.gov/content/sequence/VAR_REG_CHAR/index.html). Potential N linked glycosylation sites prediction was performed with the tool N-Glycosite at LANL-HIVDB (<https://www.hiv.lanl.gov/content/sequence/GLYCOSITE/glycosite.html>). Statistical significance was assessed by Mann-Whitney test. P values between 0.05-0.01, 0.01-0.001, <0.001 and <0.0001 are depicted as ‘*’, ‘**’, ‘***’ and ‘****’ respectively.

Fig.4. Comparison of phylogenetic and head-to-head neutralization profiles between contemporary India and South African clade C. **A.** Phylogenetic relatedness of *env* genes obtained from contemporary HIV-1 clade C of India (N=232) and Africa (N=73) origins as well as historical India (N=132) and Africa (N=138) origins and 17 HIV-1 group M reference sequences. South Africa clade C *envs* comprised those obtained from FRESH cohort (N=41) and AMP placebo arm (N=32). **B.** Comparison of the degree of neutralization susceptibility of pseudoviruses expressing contemporary HIV-1 clade C *envs* of Indian (N=115) and South African (N=40; AMP placebo arm) origins to 12 best-in-class bnAbs with distinct epitope specificities on viral Env. Env expressed as pseudovirus that showed IC₅₀ value >25 µg/mL against a particular bnAb was considered as resistant. Statistical analysis to assess significance (P values) of differences in neutralization sensitivity to a given bnAb by pseudoviruses expressing *envs* of India and South African origins was assessed by Mann-Whitney test. **C.** Comparison of the magnitude of neutralization sensitivity India and South Africa clade C viruses to select clinically relevant bnAbs. The neutralization breadth of each bnAb tested against India and South Africa clade C envelopes is expressed in the Y-axis as percent

neutralization at given concentration of corresponding antibody (IgG) concentration given in X-axis. The values in X-axis are the geometric mean of the IC80 values ($\mu\text{g/mL}$) calculated for each bnAb. Neutralization assay was carried out in duplicate replicates at least 3 times and average values were used to plot the graph.

Fig.5. Diversity in *gp120* sequence features and contact sites polymorphism between contemporary India clade C Envs sensitive and resistant to V1/V2 apex clinically relevant bnAbs. Frequency of contact sites associated with CAP256-VRC26.25 and PGDM1400 sensitivity were compared between CAP256-VRC26.25 sensitive and resistant pseudoviruses (A) and PGDM1400 sensitive and resistant viruses (B). The gp160 position (based on HXB2 numbering) of the key amino acids in the sequence logo are shown in X-axis and their relative abundance expressed as probability in Y axis. O has been used to differentiate potential N linked glycosylated Asparagine from potentially unglycosylated Asparagine (N). Residues underscored in purple line are direct Ab contact sites. Residues showing statistically significant changes in abundance following a Fisher's exact test are highlighted with yellow arrows. C. Variable loop length, PNGs and net charges of sensitive and resistant envelopes.

Fig.6. Comparison of *env* sequence features of contemporary India and South Africa clade C viruses. A. The amino acid sequences of complete *envs* (*gp120* and *gp41*) of India and South Africa contemporary HIV-1 clade C were analyzed to compare their average variable loop lengths, PNLGs and net charges in *gp120* as well as the length of *gp41*. These are analyzed using 'Variable region characteristics' tool available at the Los Alamos HIV database (https://www.hiv.lanl.gov/content/sequence/VAR_REG_CHAR/index.html) and N-Glycosite (<https://www.hiv.lanl.gov/content/sequence/GLYCOSITE/glycosite.html>). B. Comparison of key amino acid residues on India and South Africa clade C *envs* that are linked with CAP256-VRC26.25 and PGDM1400 resistance is shown in Sequence logos. The statistically significant enrichment of key residues for viruses sensitive and resistant to CAP256-VRC26.25 and

PGDM1400 are shown in Y-axis. O has been used to differentiate potential N linked glycosylated Asparagine from potentially unglycosylated Asparagine (N). Amino acid residues underscored in purple line are direct Ab contact sites for respective bnAbs. Residues showing statistically significant changes in abundance following a Fisher's exact test are highlighted with yellow arrows

Fig.7. Neutralization of V1/V2 apex bnAb resistant contemporary Indian clade C viruses by

CD4bs and V3 glycan supersite directed bnAbs. A. Sensitivity of pseudoviruses expressing contemporary Indian clade C *envs* which were fully resistant to all V1/V2 directed bnAbs to CD4bs (VRC01, VRC07, 3BNC117, N6 and 1-18) and V3 glycan supersite (PFT121, 10-1074 and BG18). Left panel shows percent neutralization of pseudoviruses that were resistant to all V1/V2 directed bnAbs tested (CAP256-VRC26.25, PGDM1400, PG9) (N=26) by CD4bs and V3 glycan directed bnAbs. Right panel shows same but only to pseudoviruses resistant CAP256-VRC26.25 resistant envelopes (N=62). Percent neutralization breadth conferred by CD4bs and V3 glycan directed bnAbs was calculated by the number of resistant viruses showed IC80 values <25 μ g/mL. **B.** Magnitude of neutralization of V1/V2 directed bnAb resistant pseudoviruses conferred by each of the CD4bs and V3 glycan directed bnAbs. The magnitude of virus neutralization equivalent to potency was measured as the lowest geometric mean titer (GMT) of the conferred by each bnAb IgG (μ g/mL) that demonstrated 80% neutralization of pseudovirus. Neutralization assay was carried out in duplicate replicates at least 3 times and average values were used to plot the graph.

Fig.8. Neutralization efficiency of VRC01 and 3BNC17 resistant contemporary viruses by

second generation CD4bs directed bnAbs. A. Proportion of contemporary viruses (N=115) that were found to be resistant to first (VRC01 and 3BNC117) and second (VRC07, N6, 1-18) generation CD4bs directed bnAbs. Pseudoviruses with neutralization score of IC80 >25 μ g/mL to respective bnAbs were considered resistant. **B.** Proportion of VRC01 and 3BNC117 resistant

contemporary pseudoviruses that demonstrated sensitivity to second generation CD4bs bnAbs (VRC07, N6, 1-18). Note that both VRC01 and 3BNC117 resistant viruses were least neutralized by 3BNC117 (11.11%) and VRC01 (17.85%) compared to VRC07, N6 and 1-18, indicating that they viruses resistant to both of them lacks common key residues that are essential for both VRC01 and 3BNC117 for comprehensive neutralization. All the second generation CD4bs bnAbs showed better neutralization (over 50%) with 1-18 demonstrated most (>74%) of VRC01 and 3BNC117 resistant viruses. C. Comparison of magnitude of neutralization of VRC01 and 3BNC117 sensitive and resistant viruses by second generation CD4bs bnAbs. Left panel shows the differences in the magnitudes of neutralization of VRC01 sensitive and resistant viruses by all the three CD4bs bnAb (VRC07, N6, 1-18) and right panel same with 3BNC117 sensitive and resistant viruses. The fold difference in magnitude of neutralization was obtained by calculating the average (GMT) of IC80 ($\mu\text{g/mL}$) for each paired set. GraphPad Prism was used to plot all the graphs.

Fig.9. Predictive neutralization coverage of contemporary India clade C viruses by clinically relevant bnAbs. Cumulative neutralization coverage of pseudoviruses carrying contemporary HIV-1 clade C *envs* by bnAb combination was assessed using CombiNAber tool using the Bliss-Hill statistical model. (<https://www.hiv.lanl.gov/content/sequence/COMBINABER/combinaber.html>). CombiNAber analysis of 115 contemporary viruses from India against BG18 + N6 + PGDM1400 and 45 Contemporary viruses from Africa against BG18 + 1-18 + PGDM1400 as well as the same Combinations with at least 2 active bnAbs have been plotted for target bnAb concentrations of 1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$ respectively. Predicted IC80 ($\mu\text{g/mL}$) combinations have been plotted on the X axis while the cumulative breadth of the viruses has been depicted on the Y axis.

Supporting data

Supporting Tables:

Table S1. Demographics details of nine different geographical regions in India, risk groups and ART status of HIV+ donors.

Table S2. Neutralization breadth and potency (IC50) of pseudoviruses expressing contemporary India clade C *envs*.

Supporting Figures:

Fig.S1. Hierarchal clustering using heatmap depicting the magnitude (<https://www.hiv.lanl.gov/content/sequence/HEATMAP/heatmap.html>) of neutralization sensitivity of contemporary HIV-1 India clade C viruses against 14 bnAbs with distinct epitope specificities. Heatmap was prepared using IC50 and IC80 values (ug/mL).

Fig.S2. Comparison of Env variable loop, PNGS and net charges between South Africa clade C viruses that showed sensitivity and resistance to CAP256-VRC26.25 and PGDM1400.

Fig.S3. Sequence features of India clade C viruses encoding contemporary *envs* sensitive and resistant to V3 glycan supersite directed bnAbs. A. Sequence logos of PGT121, 10-1074 and BG18 sensitive and resistant viruses. B. Variable loop, PNGs and net charge characteristics between sensitive and resistant viruses.

Fig.S4. Sequence logo of India clade C viruses encoding contemporary *envs* sensitive and resistant to the CD4bs directed bnAbs.

Fig.S5. Comparison of variable loop length, PNLGs and net charge of contemporary Indian clade C

viruses sensitive and resistant to CD4bs directed bnAbs.

Fig.S6. Comparison of variable loop length, PNLGs and net charge between Indian and South Africa

clade C viruses resistant to CAP256-VRC26.25 and PGDM1400.

Fig.S7. Comparison of frequency of key contact residues on *envs* that when expressed as

pseudoviruses of India (N=115) and South Africa (N=40) origins showed resistance to V3

glycan directed bnAbs.

Fig.S8. Abundance of CAP256-VRC26.25/PGDM1400 sensitivity/resistance associated residues in

sequences from India (N=118) and South Africa (N=41) that have not been tested through *in*

vitro neutralization assays. X axes define amino acid positions in gp160 whereas Y axes

define percent abundance of residues. The tables indicate the residues associated with

sensitivity or resistance. Und: undefined.

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Table 1. Source, coreceptor usage and other properties of contemporary HIV-1 clade C functional clones.

	<i>env (gp160)</i>	Subtype	Region	Source	Vector	Coreceptor usage
1	TSG-EHI6	A1	Nellore	PLASMA	pCDNA3.1	CCR5
2	TSG-EHI9	C	Nellore	PLASMA	pCDNA3.1	CCR5
3	TSG-EHI28	C	Nellore	PLASMA	pCDNA3.1	CCR5
4	TSG-EHI32	C	Nellore	PLASMA	pCDNA3.1	CCR5
5	TSG-EHI38	C	Nellore	PLASMA	pCDNA3.1	CCR5
6	TSG-EHI39	C	Nellore	PLASMA	pCDNA3.1	CCR5
7	TSG-EHI41	C	Nellore	PLASMA	pCDNA3.1	CCR5
8	TSG-EHI42	C	Nellore	PLASMA	pCDNA3.1	CCR5
9	TSG-EHI44	C	Nellore	PLASMA	pCDNA3.1	CCR5
10	TSG-EHI45	C	Nellore	PLASMA	pCDNA3.1	CCR5
11	TSG-EHIPre8	C	Nellore	PLASMA	pCDNA3.1	CCR5
12	TSG-EHI13D6	C	Nellore	PLASMA	pCDNA3.1	CCR5
13	TSG-EHI17B14	C	Nellore	PLASMA	pCDNA3.1	CCR5
14	TSG-EHI8	C	Nellore	PLASMA	pCDNA3.1	CCR5
15	TSG-EHI14	C	Nellore	PLASMA	pCDNA3.1	CCR5
16	TSG-EHI40-C18	C	Nellore	PLASMA	pCDNA3.1	CCR5
17	TSG-EHI11	C	Nellore	PLASMA	pCDNA3.1	CCR5
18	TSG-EHI21	C	Nellore	PLASMA	pCDNA3.1	CCR5
19	TSG-EHI26	A1	Nellore	PLASMA	pCDNA3.1	CCR5
20	TSG-EHI30	C	Nellore	PLASMA	pCDNA3.1	CCR5
21	TSG-EHI18	C	Nellore	PLASMA	pCDNA3.1	CCR5
22	TSG-EHI22	C	Nellore	PLASMA	pCDNA3.1	CCR5
23	TSG-EHI29	C	Nellore	PLASMA	pCDNA3.1	CCR5
24	TSG-EHI35	C	Nellore	PLASMA	pCDNA3.1	CCR5
25	TSG-EHI37	C	Nellore	PLASMA	pCDNA3.1	CCR5
26	TSG-EHI7	C	Nellore	PLASMA	pCDNA3.1	CCR5
27	TSG-EHI12	C	Nellore	PLASMA	pCDNA3.1	CCR5
28	TSG-EHI20	C	Nellore	PLASMA	pCDNA3.1	CCR5
29	TSG-EHI23	C	Nellore	PLASMA	pCDNA3.1	CCR5
30	TSG-EHI27	C	Nellore	PLASMA	pCDNA3.1	CCR5
31	TSG-EHI33	C	Nellore	PLASMA	pCDNA3.1	CCR5
32	TSG-EHI55	C	Nellore	PLASMA	pCDNA3.1	CCR5
33	TSG-EHI60	C	Nellore	PLASMA	pCDNA3.1	CCR5
34	TSG-EHI61	C	Nellore	PLASMA	pCDNA3.1	CCR5
35	TSG-EHI62	C	Nellore	PLASMA	pCDNA3.1	CCR5
36	TSG-EHI63	C	Nellore	PLASMA	pCDNA3.1	CCR5
37	TSG-EHI25	C	Nellore	PLASMA	pCDNA3.1	CCR5
38	TSG-EHI50	C	Nellore	PLASMA	pCDNA3.1	CCR5
39	TSG-EHIPre15	C	Nellore	PLASMA	pCDNA3.1	CCR5
40	TSG-EHI51	C	Nellore	PLASMA	pCDNA3.1	CCR5
41	TSG-EHI16	C	Nellore	PLASMA	pCDNA3.1	CCR5
42	TSG-EHI36	C	Nellore	PLASMA	pCDNA3.1	CCR5
43	TSG-EHI34	C	Nellore	PLASMA	pCDNA3.1	CCR5
44	TSG-EHI53	C	Nellore	PLASMA	pCDNA3.1	CCR5
45	TSG-EHI57	C	Nellore	PLASMA	pCDNA3.1	CCR5
46	TSG-EHI58	C	Nellore	PLASMA	pCDNA3.1	CCR5

47	TSG-EHI59	C	Nellore	PLASMA	pCDNA3.1	CCR5
48	TSG21Y02A0012	C	Delhi	PLASMA	pCDNA3.1	CCR5
49	TSG21Y02E0018-D18	C	Delhi	PLASMA	pCDNA3.1	CCR5
50	TSG21Y02E0024-D24	C	Delhi	PLASMA	pCDNA3.1	CCR5
51	TSG22Y02E0035-DE35	C	Delhi	PLASMA	pCDNA3.1	CCR5
52	TSG22Y02E0037-DE37	C	Delhi	PBMC	pCDNA3.1	CCR5
53	TSG21Y02E0011-D11	C	Delhi	PLASMA	pCDNA3.1	CCR5
54	TSG21Y02E0014-D14	C	Delhi	PLASMA	pCDNA3.1	CCR5
55	TSG21S01A0008_STMART07	C	Kolkata	PLASMA	pCDNA3.1	CCR5
56	TSG21S01A0013-STMART12	C	Kolkata	PLASMA	pCDNA3.1	CCR5
57	TSG21S01A0003-STMART02	C	Kolkata	PLASMA	pCDNA3.1	CCR5
58	TSG21S01A0014-STM13ART	C	Kolkata	PLASMA	pCDNA3.1	CCR5
59	TSG21S01E0017-STM16E	C	Kolkata	PLASMA	pCDNA3.1	CCR5
60	TSG21S01E0001-STM01E	C	Kolkata	PLASMA	pCDNA3.1	CCR5
61	TSG21S01A0002-STMART01	C	Kolkata	PBMC	pCDNA3.1	CCR5
62	TSG21S01E0033-C33	C	Kolkata	PBMC	pCDNA3.1	CCR5
63	TSG21S01E0018-STM17E	C	Kolkata	PLASMA	pCDNA3.1	CCR5
64	TSG21S01A0004-C4	C	Kolkata	PLASMA	pCDNA3.1	CCR5
65	TSG21S01A0005-C5	C	Kolkata	PLASMA	pCDNA3.1	CCR5
66	TSG21N01N017	C	Mumbai	PLASMA	pCDNA3.1	CCR5
67	TSG21N01N011-C10	C	Mumbai	PLASMA	pCDNA3.1	CCR5
68	TSG21N01N029	C	Mumbai	PLASMA	pCDNA3.1	CCR5
69	TSG21N01N018	C	Mumbai	PLASMA	pCDNA3.1	CCR5
70	TSG21N01S028	C	Mumbai	PLASMA	pCDNA3.1	CCR5
71	TSG21N01N010	C	Mumbai	PLASMA	pCDNA3.1	CCR5
72	TSG21N01N014	C	Mumbai	PLASMA	pCDNA3.1	CCR5
73	TSG21N01N023	C	Mumbai	PLASMA	pCDNA3.1	CCR5
74	TSG21N01N031	C	Mumbai	PLASMA	pCDNA3.1	CCR5
75	TSG21N01F003	C	Mumbai	PLASMA	pCDNA3.1	CCR5
76	TSG21N01N001CM	C	Mumbai	PBMC	pCDNA3.1	CCR5
77	TSG21N01N013	C	Mumbai	PLASMA	pCDNA3.1	CCR5
78	TSG21N01N015	C	Mumbai	PLASMA	pCDNA3.1	CCR5
79	TSG21N01N025	A1C	Mumbai	PLASMA	pCDNA3.1	CCR5
80	TSG21N01N026	C	Mumbai	PLASMA	pCDNA3.1	CCR5
81	TSG21N01S007	C	Mumbai	PLASMA	pCDNA3.1	CCR5
82	TSG21N01S027	C	Mumbai	PLASMA	pCDNA3.1	CCR5
83	TSG21N01S030	C	Mumbai	PLASMA	pCDNA3.1	CCR5
84	TSG21N01S100	C	Mumbai	PLASMA	pCDNA3.1	CCR5
85	TSG21N01S055	C	Mumbai	PLASMA	pCDNA3.1	CCR5
86	TSG21N01S062	C	Mumbai	PLASMA	pCDNA3.1	CCR5
87	TSG22Y03E0010-B10	C	Bhopal	PLASMA	pCDNA3.1	CCR5
88	TSG22Y03A0015-B15	A1	Bhopal	PBMC	pCDNA3.1	CCR5
89	TSG22Y03A0022-B22	C	Bhopal	PLASMA	pCDNA3.1	CCR5
90	TSG22Y03A0031-B31	C	Bhopal	PLASMA	pCDNA3.1	CCR5
91	TSG22Y03A0032-B32	C	Bhopal	PLASMA	pCDNA3.1	CCR5
92	TSG22Y03E0019-B19	C	Bhopal	PLASMA	pCDNA3.1	CCR5
93	TSG22Y03E0023-B23	C	Bhopal	PBMC	pCDNA3.1	CCR5
94	TSG23Y07A0012-A12	C	Aizawl	PBMC	pCDNA3.1	CCR5
95	TSG23Y07A0013-A13	C	Aizawl	PBMC	pCDNA3.1	CCR5

96	TSG23Y07A0015-A15	C	Aizawl	PLASMA	pCDNA3.1	CCR5
97	TSG23Y06A012-V12	C	Vizag	PBMC	pCDNA3.1	CCR5
98	TSG23Y06A015-V15	C	Vizag	PBMC	pCDNA3.1	CCR5
99	TSG23Y06E001-V1	C	Vizag	PLASMA	pCDNA3.1	CCR5
100	TSG23Y06A003-V3	C	Vizag	PBMC	pCDNA3.1	CCR5
101	TSG23Y06A004-V4	C	Vizag	PBMC	pCDNA3.1	CCR5
102	TSG22Y04A0011-H11	C	Hyderabad	PBMC	pCDNA3.1	CCR5
103	TSG22Y04A0015-H15	C	Hyderabad	PBMC	pCDNA3.1	CCR5
104	TSG22Y04A0016-H16	C	Hyderabad	PBMC	pCDNA3.1	CCR5
105	TSG22Y04A0020-H20	C	Hyderabad	PBMC	pCDNA3.1	CCR5
106	TSG22Y04A0002-H2	C	Hyderabad	PBMC	pCDNA3.1	CCR5
107	TSG22Y04A0003-H3	C	Hyderabad	PBMC	pCDNA3.1	CCR5
108	TSG22Y04A0004-H4	C	Hyderabad	PBMC	pCDNA3.1	CCR5
109	TSG22Y04A0005-H5	C	Hyderabad	PBMC	pCDNA3.1	CCR5
110	TSG22Y05A0018-BL18	A1	Belagavi	PBMC	pCDNA3.1	CCR5
111	TSG22Y05A0021-BL21	C	Belagavi	PLASMA	pCDNA3.1	CCR5
112	TSG22Y05A0002-BL2	C	Belagavi	PBMC	pCDNA3.1	CCR5
113	TSG22Y05E0033-BL33	C	Belagavi	PBMC	pCDNA3.1	CCR5
114	TSG22Y05E0043-BL43	C	Belagavi	PBMC	pCDNA3.1	CCR5
115	TSG22Y05A0006-BL6	C	Belagavi	PBMC	pCDNA3.1	CCR5

Table 2. Neutralization profiles of pseudoviruses bearing contemporary *envs* broadly resistant to bnAbs with distinct specificities.

PSV ID	V1/V2 apex directed			CD4bs directed					V3g directed			MPER	Fusion peptide
	CAP256-VRC26.25	PGDM1400	PG9	VRC01	VRC07	3BNC117	N6	1-18	PGT121	10-1074	BG18	10E8	VRC34.01
TSG21N01N017_c18	>25	>25	>25	>25	>25	>25	>25	>25	>25	2.23	>25	17.0	>25
TSG22Y05A0018-BL18	>25	5.44	>25	>25	>25	>25	>25	>25	>25	>25	>25	6.45	>25
TSG.EHI_27	>25	>25	>25	>25	>25	>25	0.85	0.75	>25	>25	0.88	2.36	0.67
TSGNO31G	>25	>25	>25	0.84	0.78	>25	0.12	0.04	>25	>25	>25	5.60	>25
TSG.EHI_17	>25	>25	0.87	>25	>25	>25	1.67	0.14	0.42	0.04	0.20	>25	>25
TSG22Y05A0006-BL6	>25	>25	2.73	>25	>25	>25	>25	>25	0.02	<0.01	1.83	1.13	>25

Values indicate IC80 ($\mu\text{g/mL}$) as determined by TZM-bl pseudovirus neutralization assay. Viruses resistant to select bnAbs up to $25\mu\text{g/mL}$ are highlighted.

CD4bs refers to CD4 binding site-directed bnAbs;

V3g refers to V3 glycan supersite-directed bnAbs;

MPER refers to membrane proximal external region of gp41

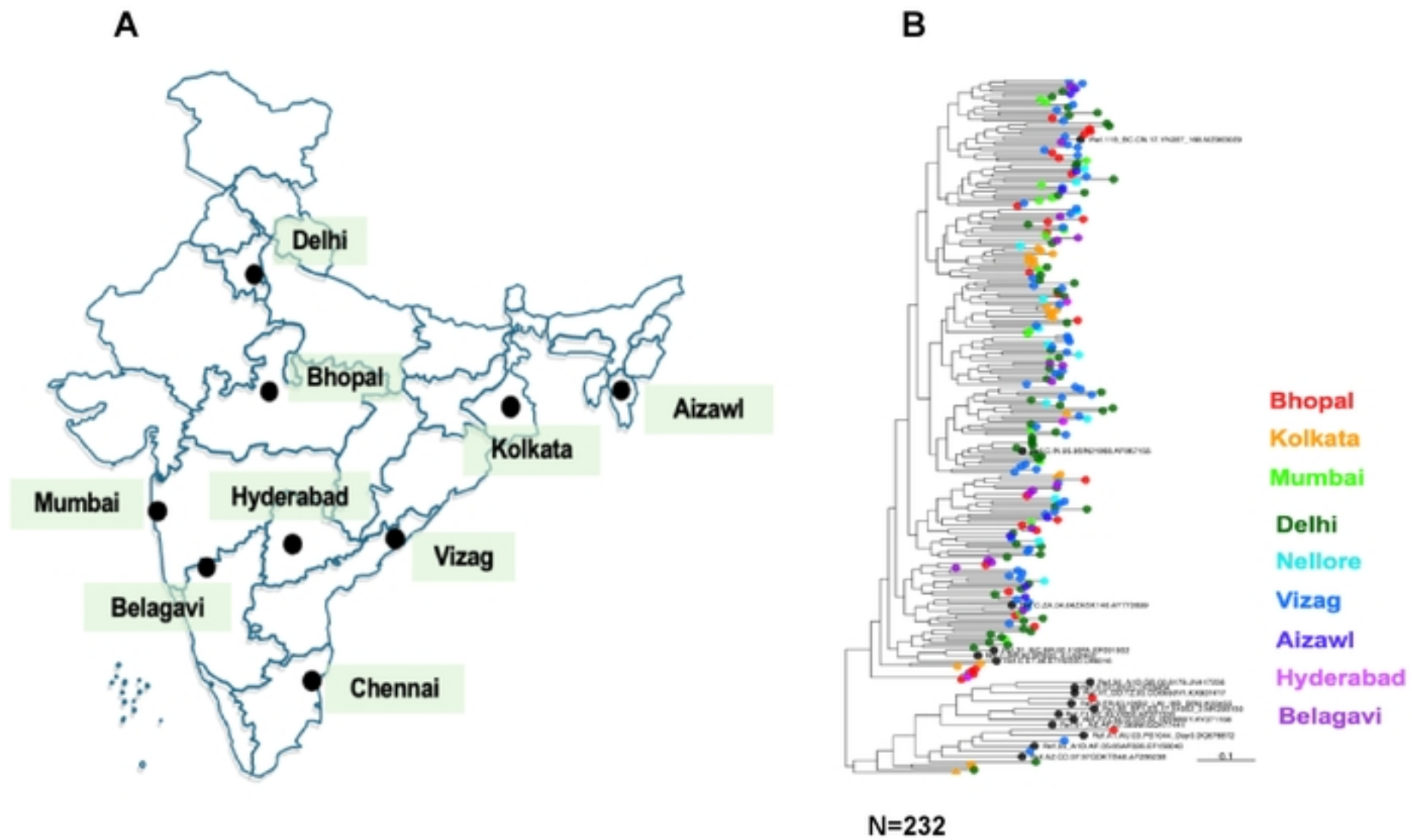


Fig.1

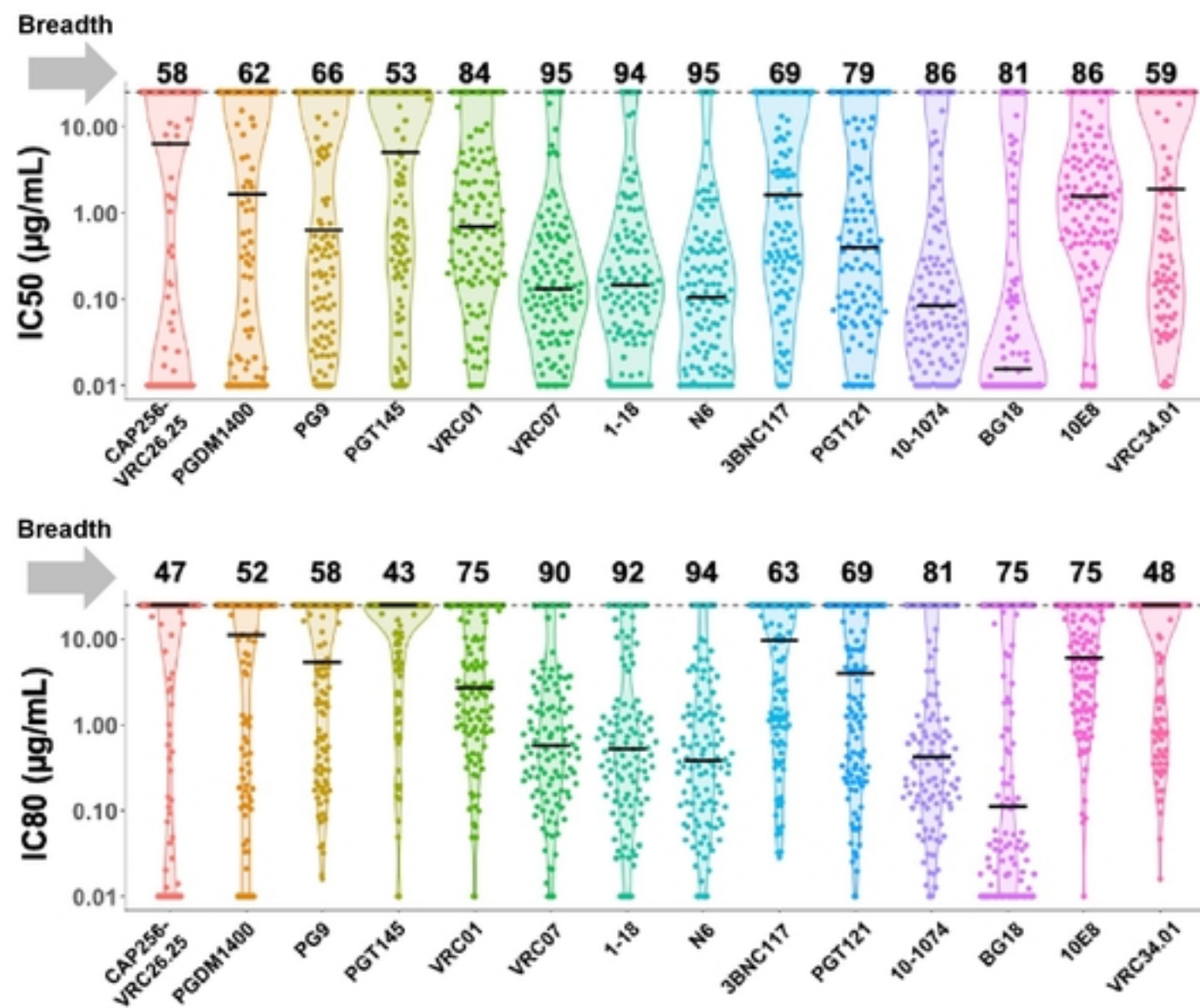


Fig.2

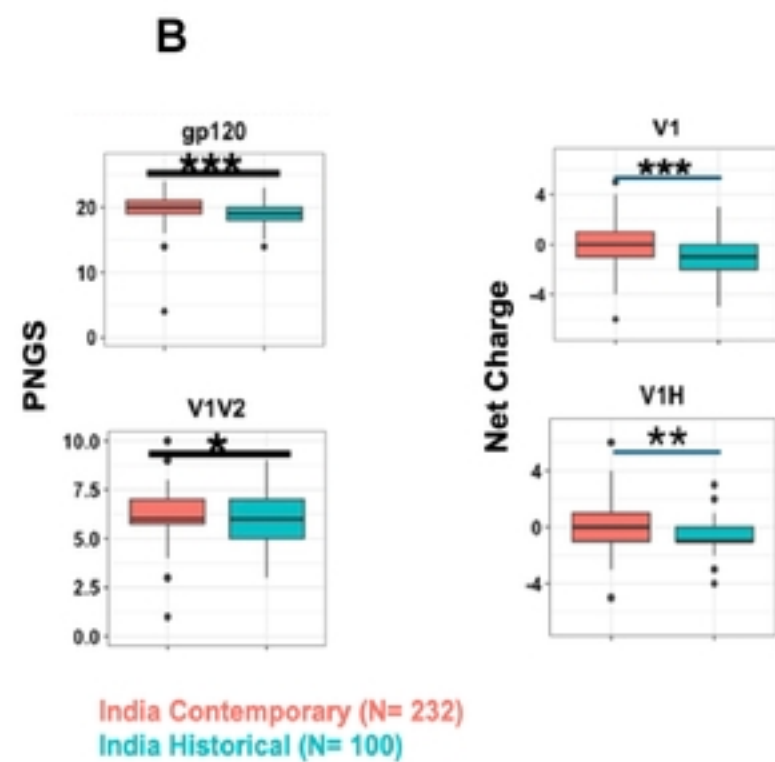
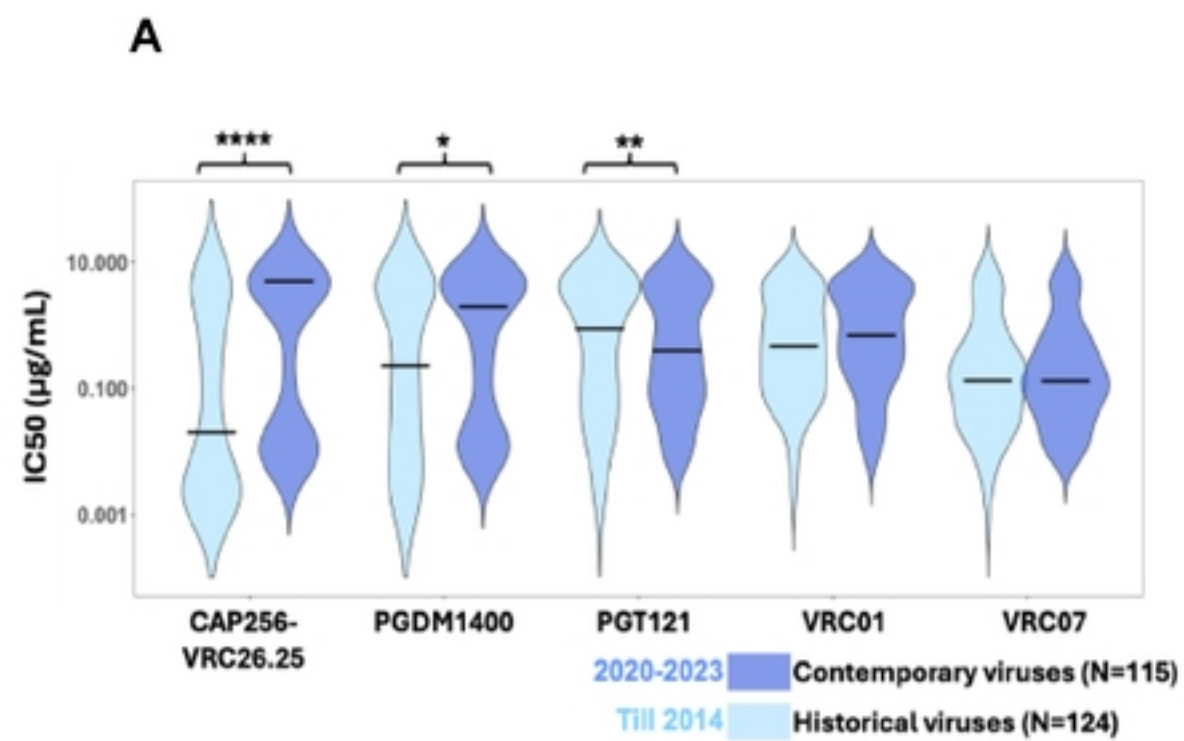


Fig.3

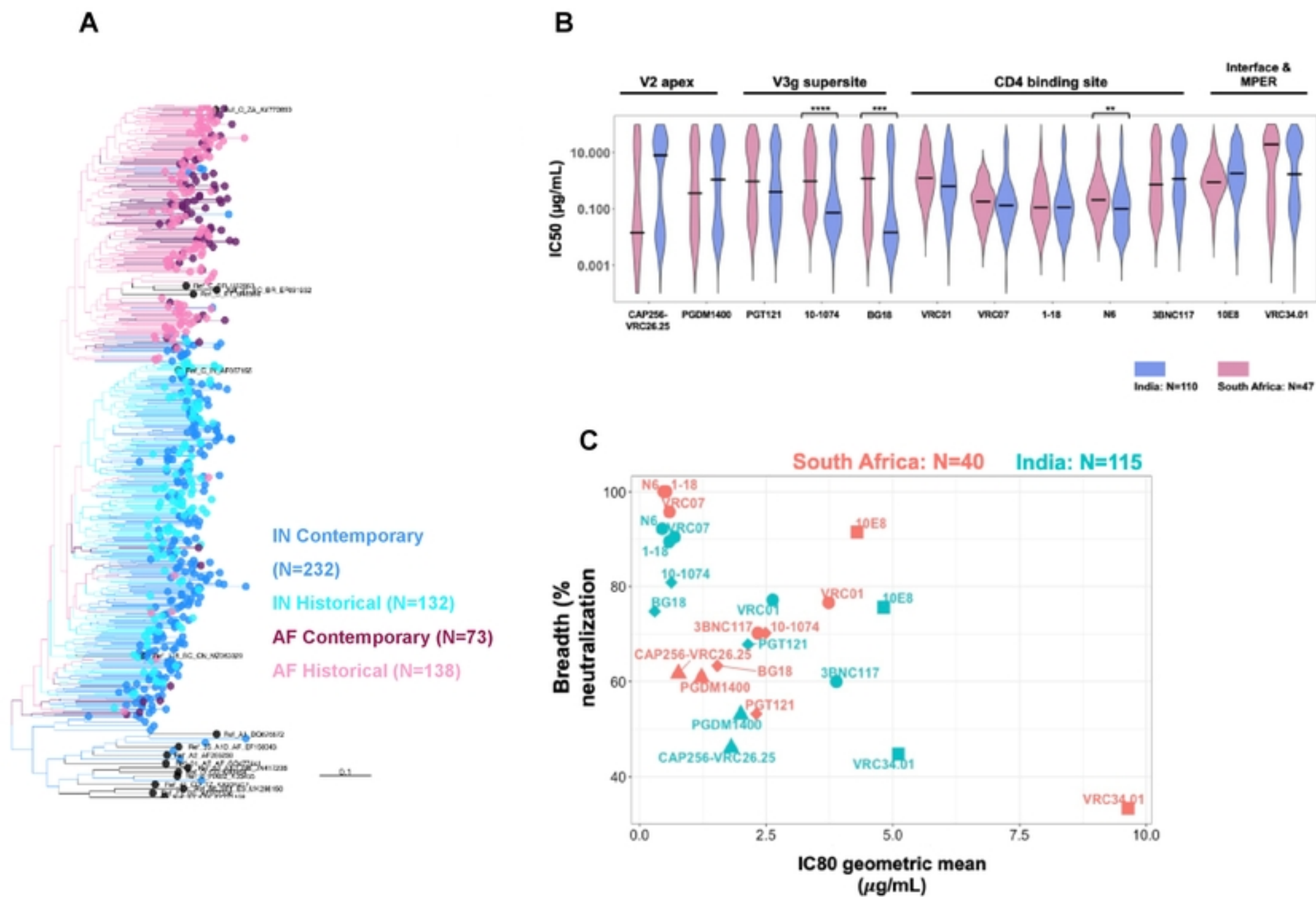
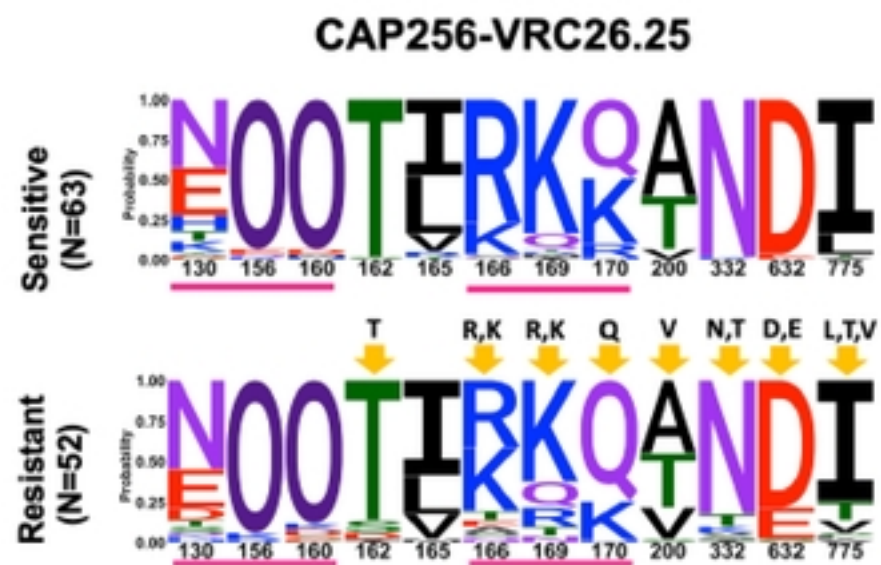
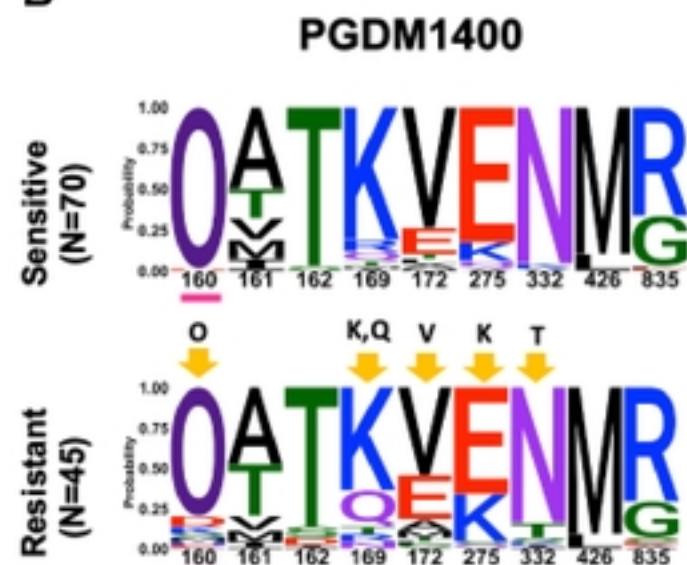


Fig.4

A



B



C

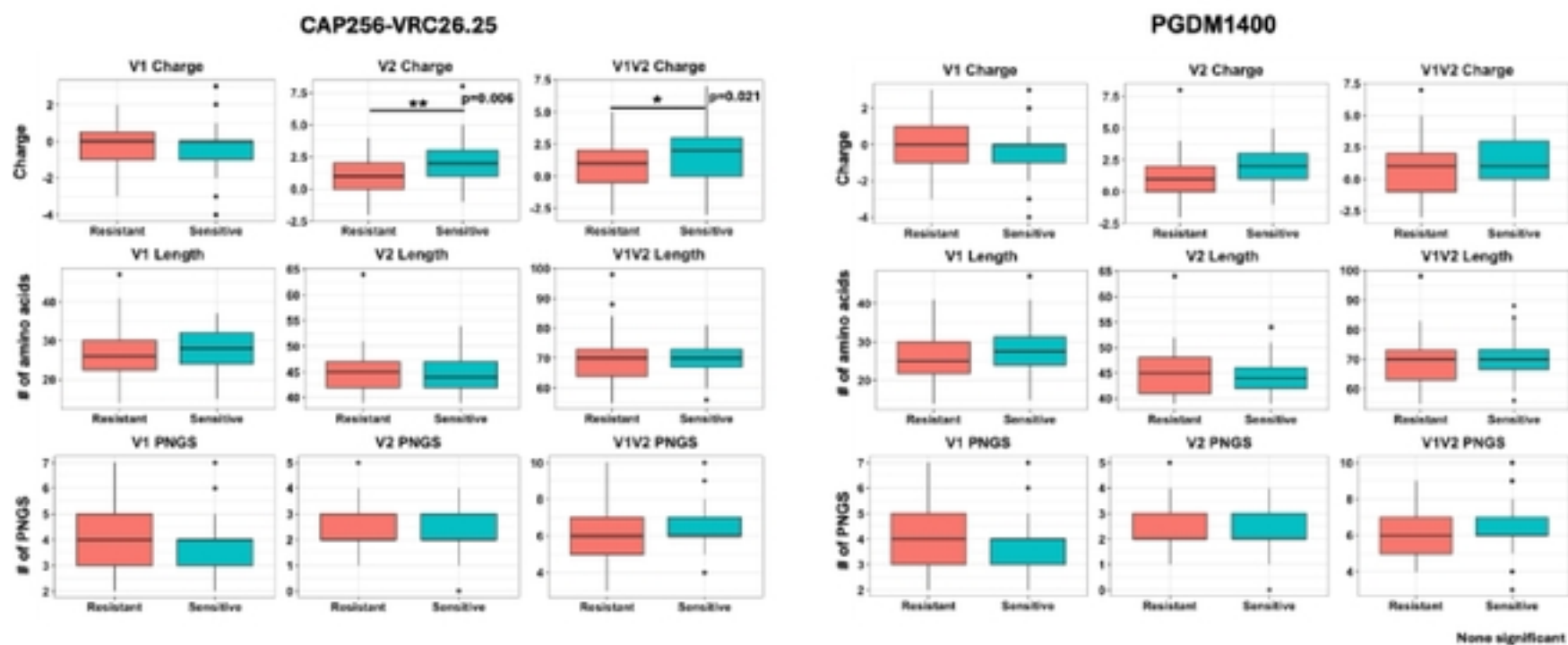
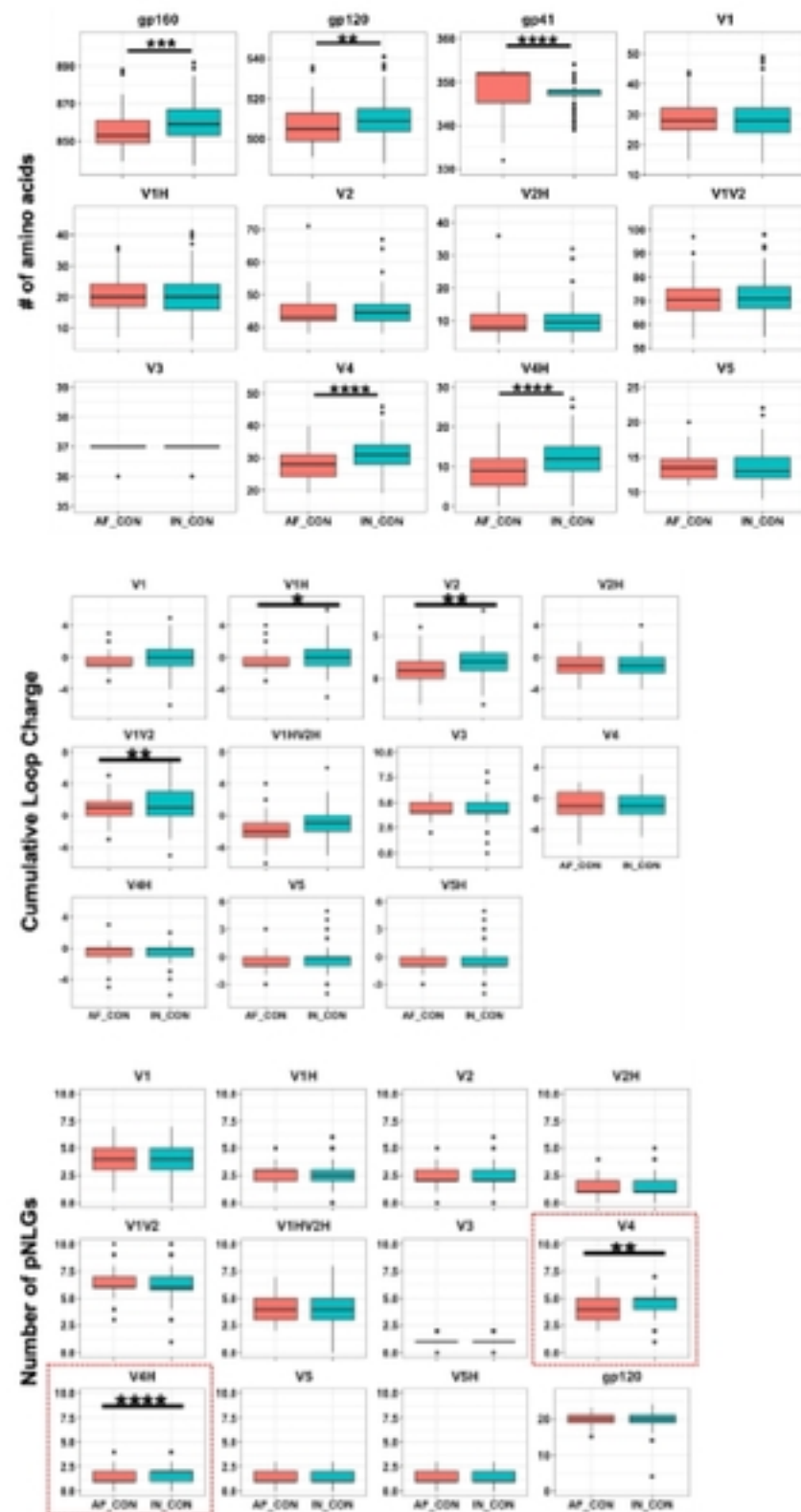


Fig.5

A



Contemporary India vs Africa

B

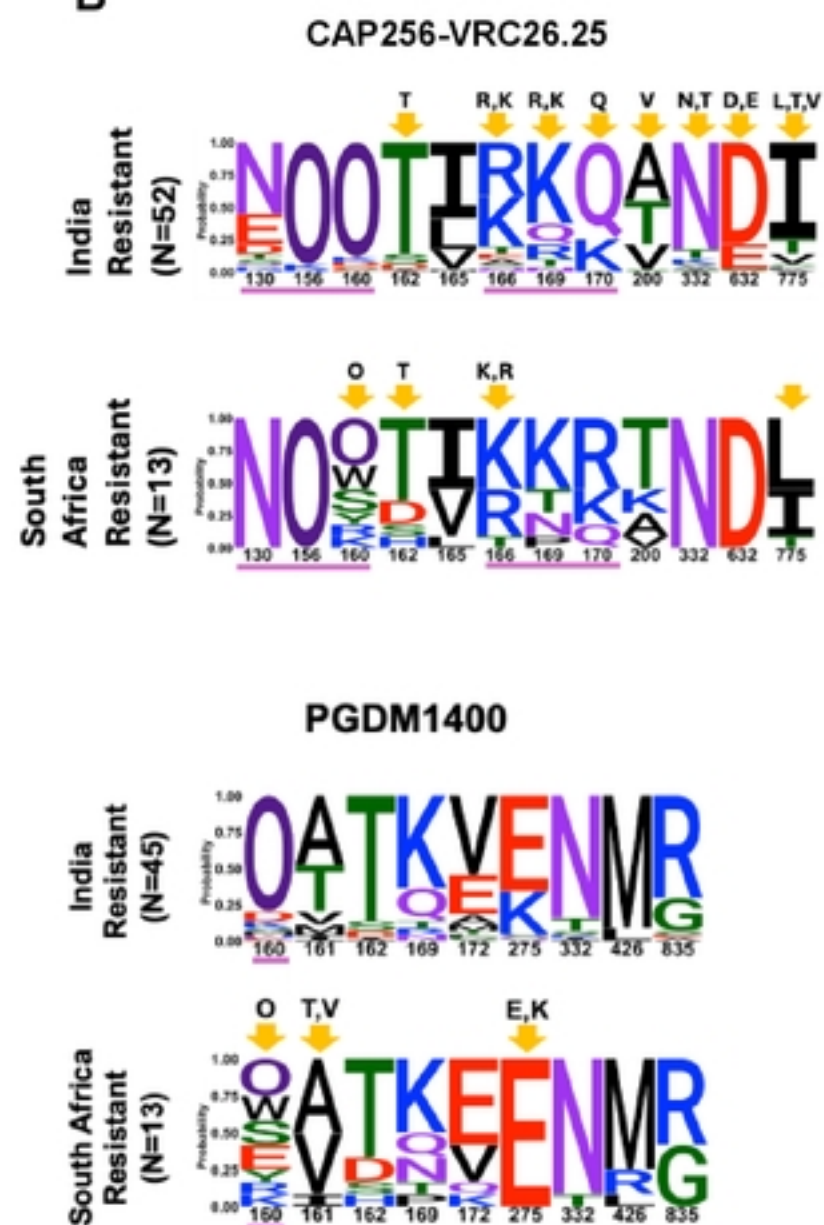
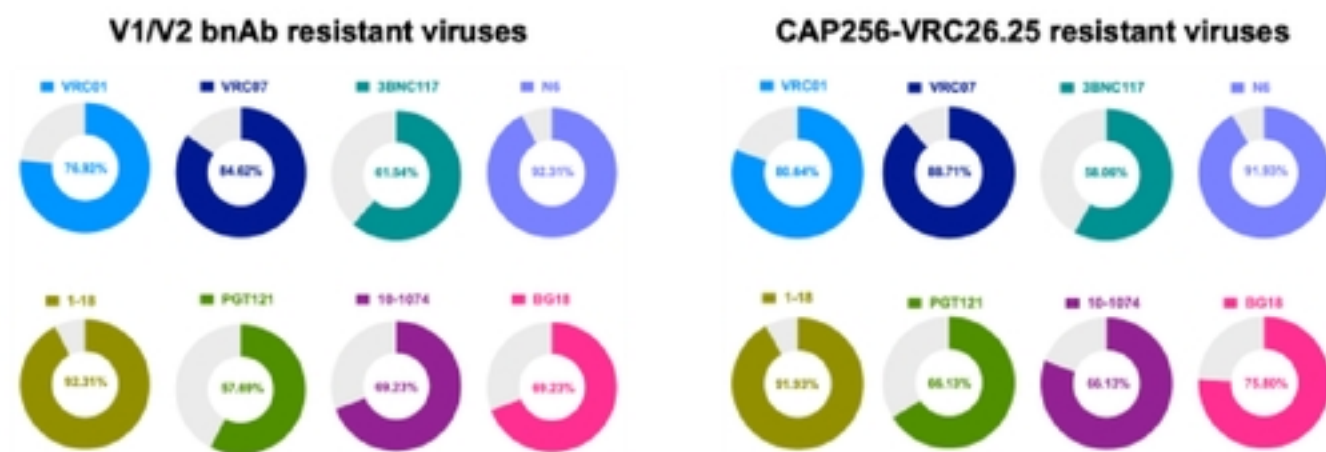


Fig.6

A



B

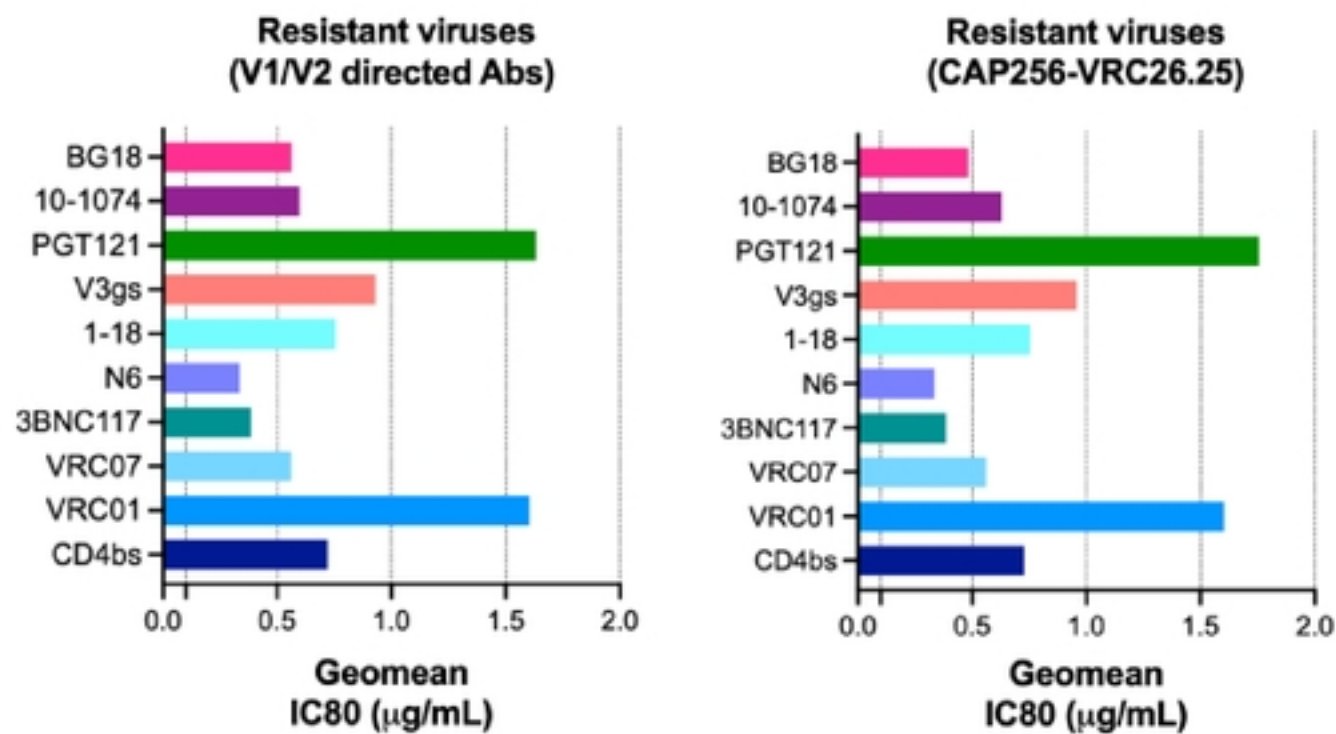


Fig.7

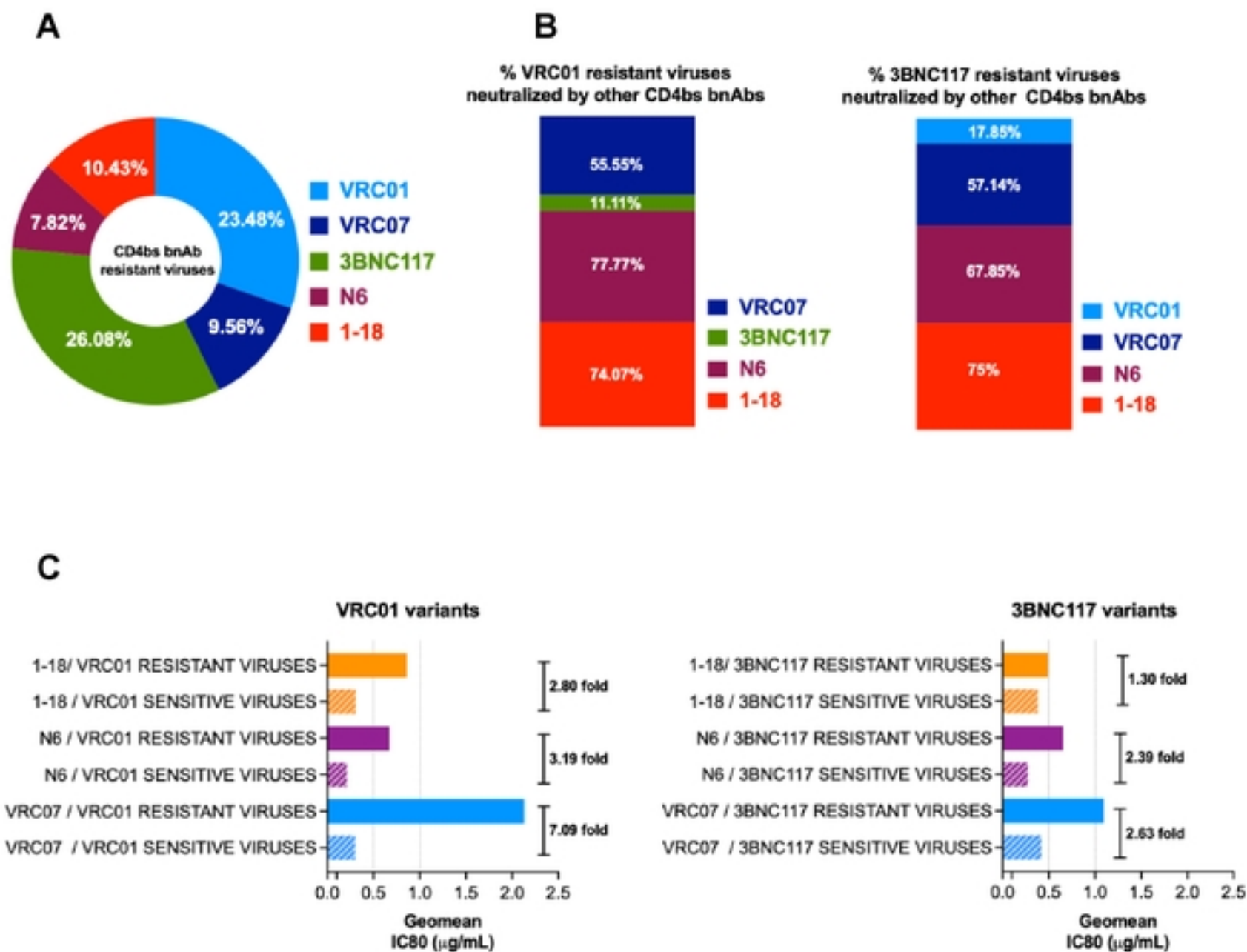


Fig.8

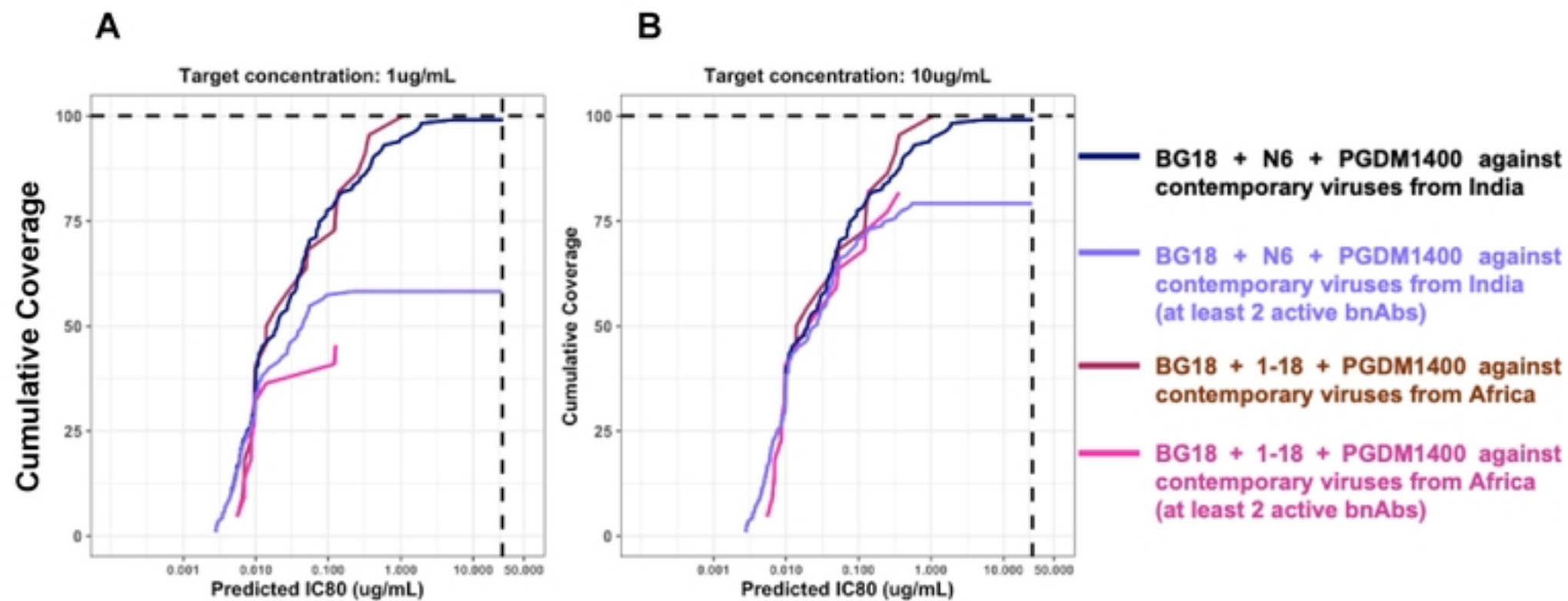


Fig.9