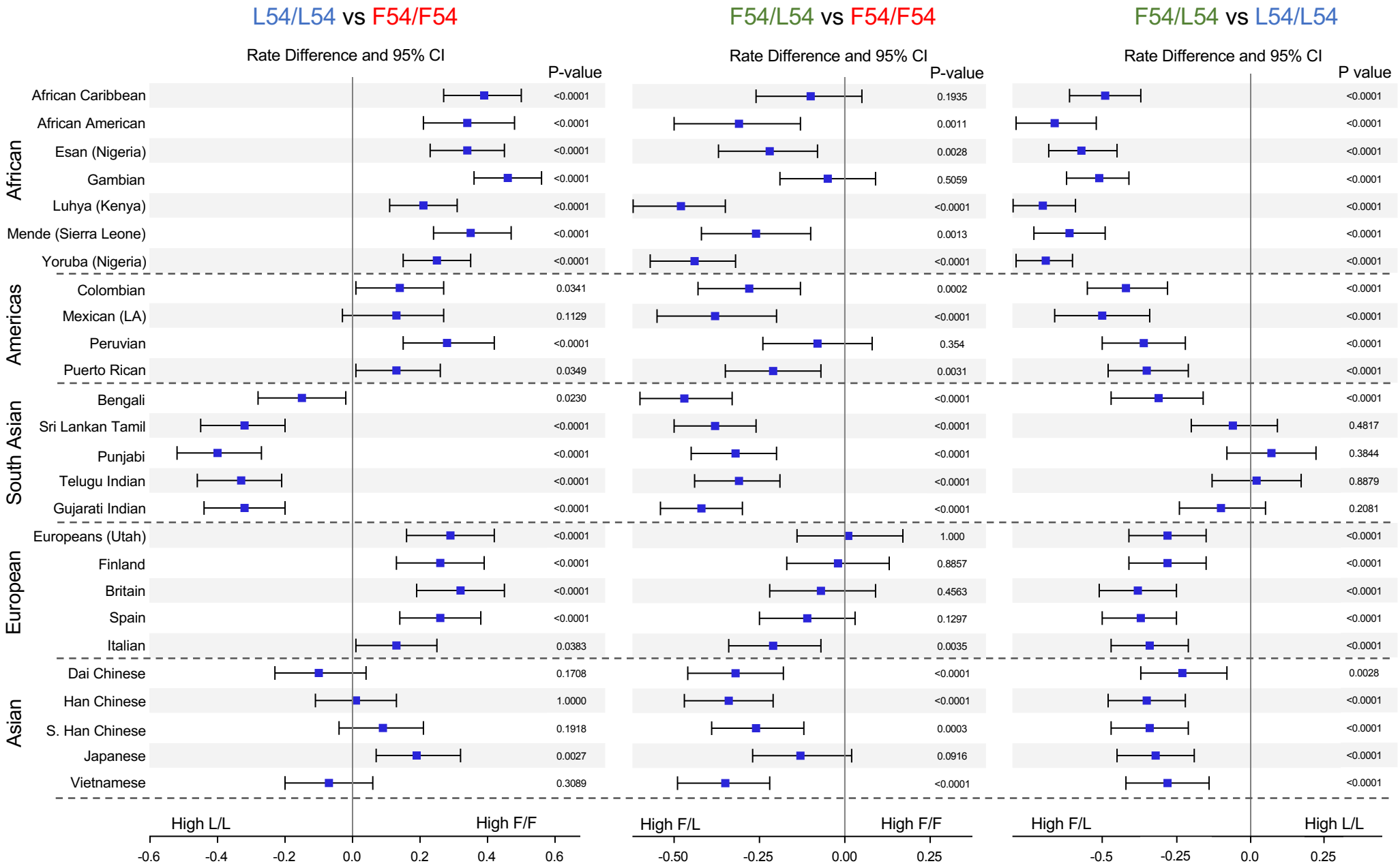

Figure S1. Global distribution of F54 and L54 IGHV1-69 alleles - related to Figure 2. Pairwise comparison of homozygous IGHV1-69 L54/L54 vs F54/F54, homozygous and heterozygous F54/L54 vs F54/F54 and F54/L54 vs L54/L54 individuals across the 26 subpopulations using data mined from the 1000 genomes project (Avnir *et al.*, 2016; Genomes Project *et al.*, 2010). Presented are forest plots with upper and lower confidence limits, in which we compare the difference in proportions of these genotypes (P values from Chi-square test).

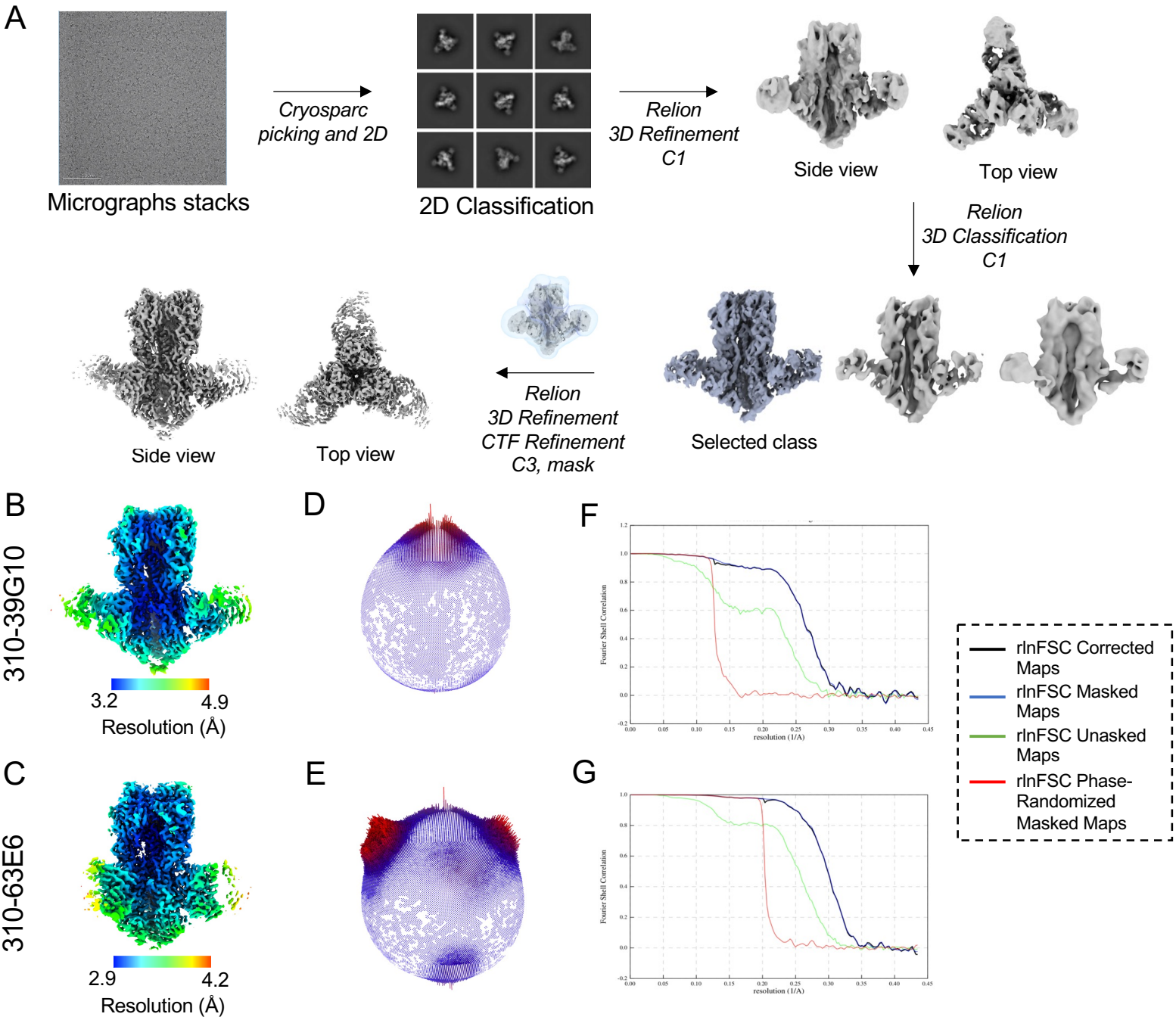

Figure S2. Cryo-EM processing, classification and refinement of HA complexed with 310-39G10 Fab or 310-63E6 Fab - related to Figure 1. (A) Schematic representation used for data processing. (B-C) Local resolution maps calculated by the locres program in Relion/3.0. (D-E) Angular distributions of the particles were obtained in Relion/3.0 after the final step of 3D refinement. Only one third of the sphere is shown because C3 symmetry was applied. (F-G) FSC plots were generated in Relion/3.0.

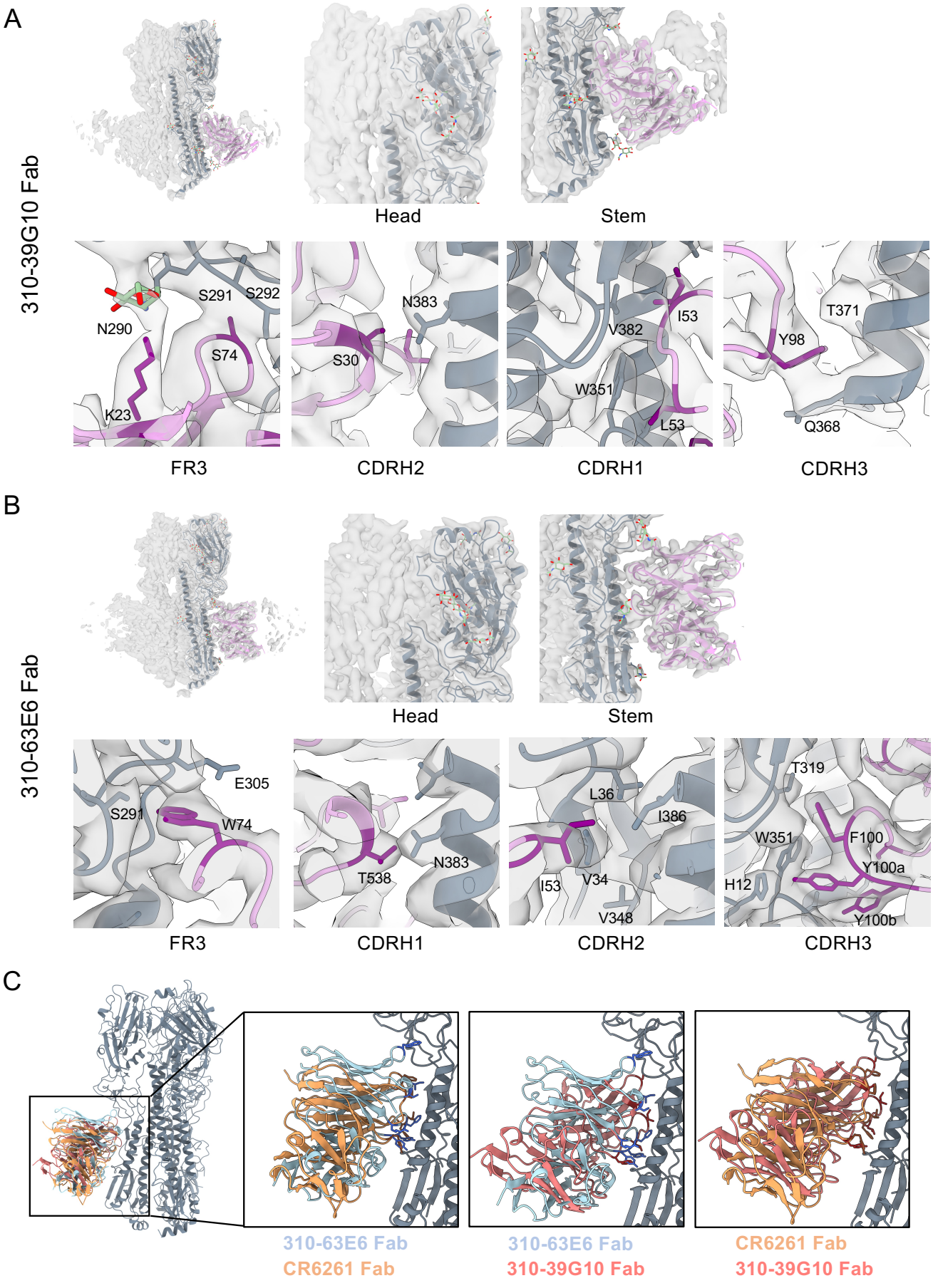

Figure S3. Models-to-map fit of HA in complex with L54 bnAbs - related to Figure 1. 310-39G10 (A) 310-63E6 (B) antibodies. A close-up view of the epitope/paratope region is shown. HA trimers are shown in gray and antibodies are colored in purple. Relevant CDRs and FRs as well as amino acids that interact with the HA trimer are labelled. Ribbon and stick representations are used to display structural components. (C) Superimposition of the different antibodies in complex with HA is shown.

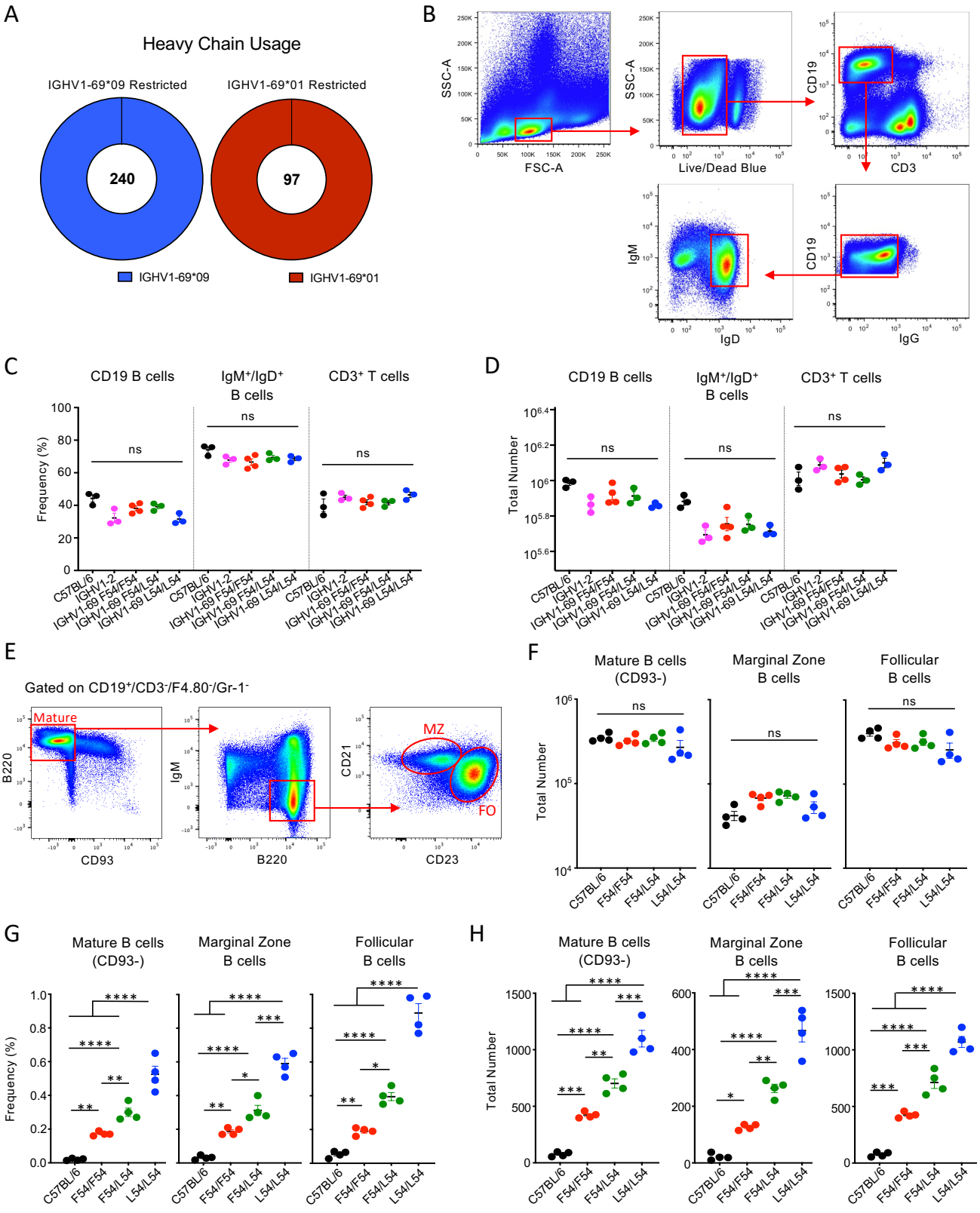

Figure S4. Immune cell phenotypes in the HC2 mice - related to Figure 2. (A) Antibody V_H restriction was confirmed by sequencing single BCRs from homozygous IGHV1-69*09 (L54/L54 IGHV1-69 - 240 BCRs) and IGHV1-69*01 (F54/F54 IGHV1-69 – 97 BCRs). (B) Gating strategy for naive B cells $CD19^+/CD3^-/IgM^+/IgD^+/IgG^-$ splenocytes. (C-D) Frequency and cell numbers of total $CD19^+$ B cells, $CD3^+$ T cells and $IgM^+/IgD^+/IgG^-$ B cells in C57BL/6, IGHV1-2, F54/F54 IGHV1-69, F54/L54 IGHV1-69, and L54/L54 IGHV1-69 mice (mean and SEM, n= 3-4 mice per genotype, P>0.05 ANOVA with Tukey's test). (E) Gating strategy to further separate B cells into functional subsets in the spleen: mature; marginal zone; or follicular. (F) Cell numbers in each subset (mean and SEM, n= 4 mice per genotype, P>0.05 ANOVA with Tukey's test). (G-H) Quantification of on-target B cells within the functional subsets using the SS-np and SSΔstem-np probes (see Figure 2E-F for gating strategy). Data are presented as mean and SEM for n=4 mice per genotype (*P<0.04, **P<0.01, ***P<0.0005 ****P<0.0001, ANOVA with Tukey's test).

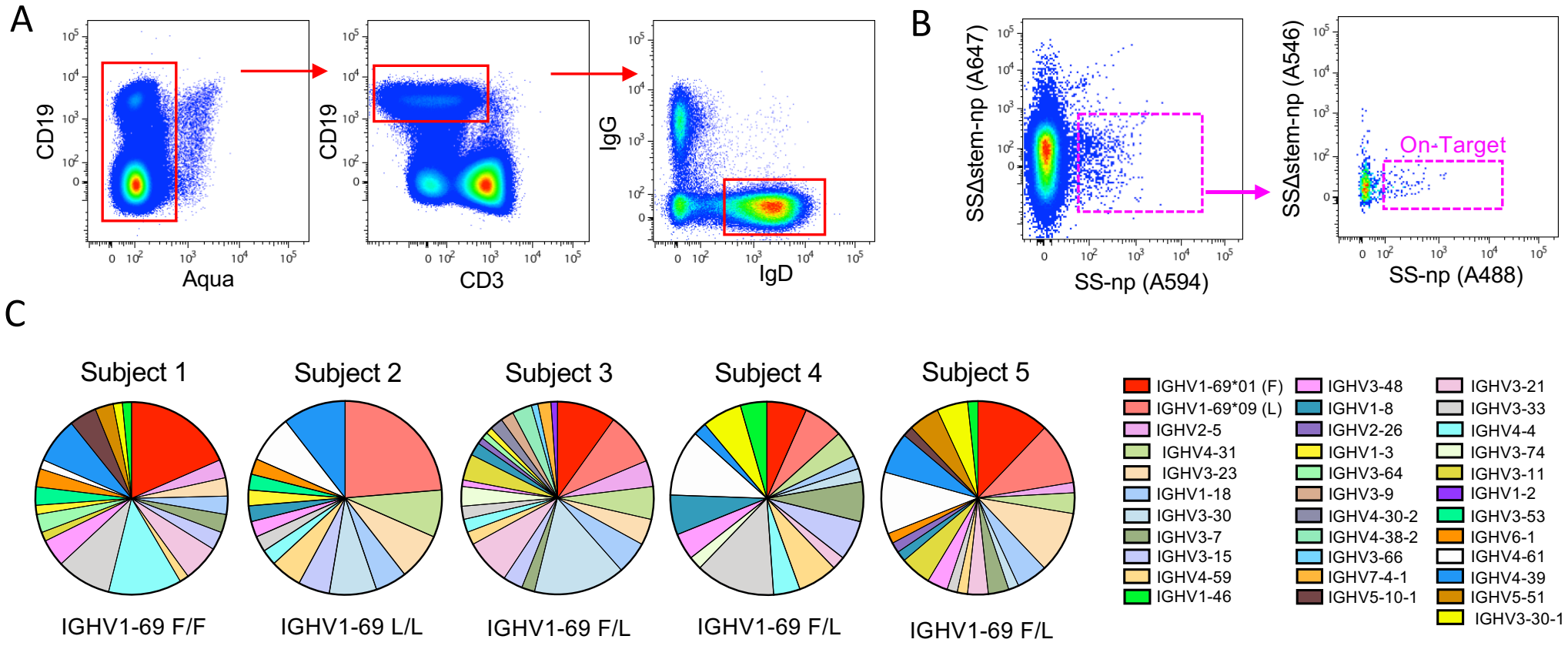

Figure S5. Germline IGHV1-69*09 (L54) and IGHV1-69*01 (F54) stalk targeting antibodies from humans - related to Figures 2 and 4. (A) Gating strategy for FACS isolation of $CD19^{+}/CD3^{-}/IgG^{-}/IgD^{+}$ B cells from human PBMCs. (B) Designation of SS-np on-target (purple box) B cells in human PBMCs. (C) On-target antibody V_H-gene usage in B cells isolated from n=5 human donors with the following genotypes: IGHV1-69*01 (F54)/IGHV1-69*01 (F54) homozygous (Subject 1); IGHV1-69*09 (L54)/IGHV1-69*09 (L54) homozygous (Subject 2); or IGHV1-69*01 (F54)/IGHV1-69*09 (L54) heterozygous (Subject 3-5).

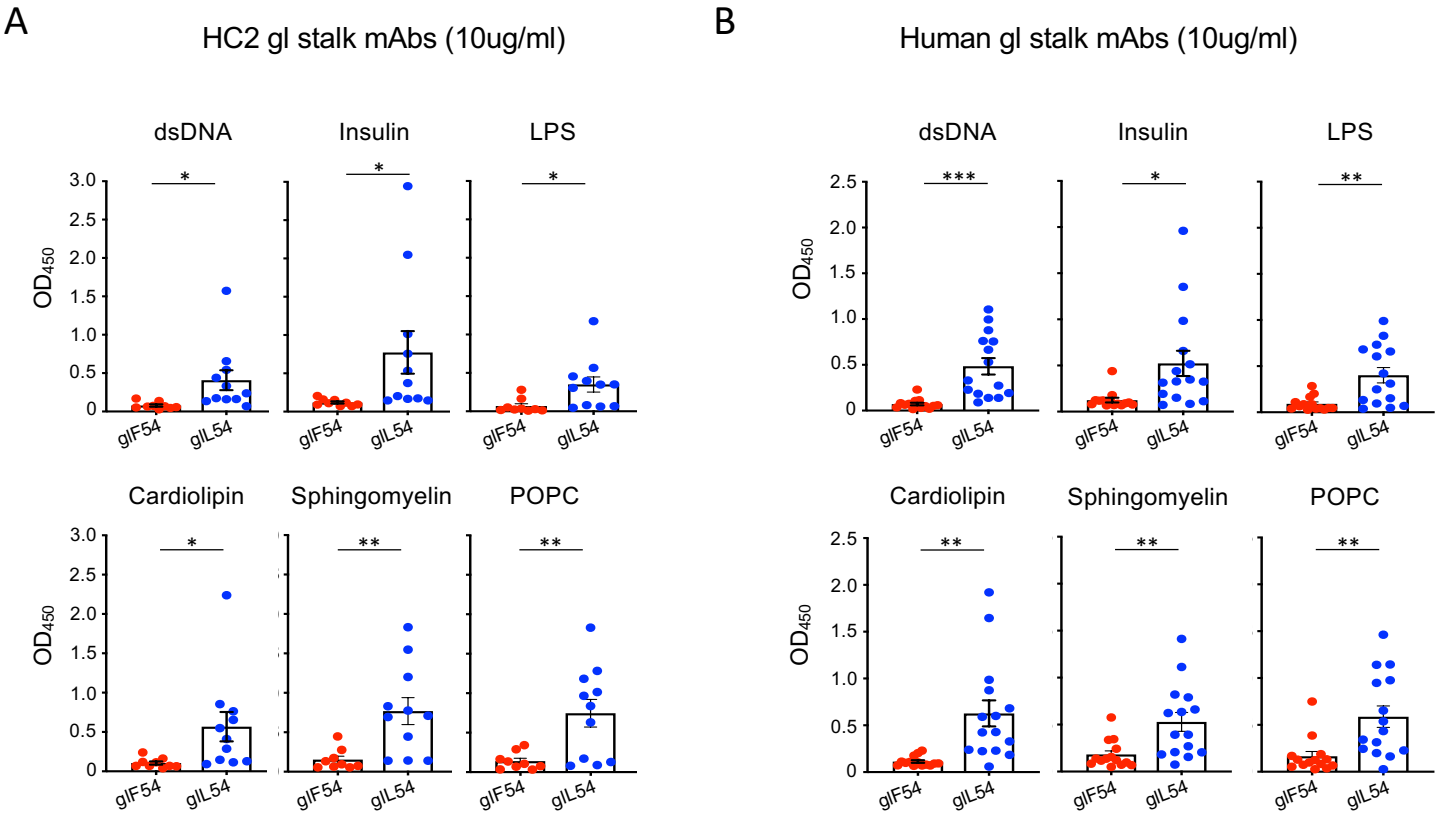

Figure S6. Polyreactivity and autoreactivity of HC2 mouse and human L54 germline stalk mAbs - related to Figure 4. (A) HC2 mouse and (B) human F54 and L54 on-target germline stalk antibody reactivity to polyreactive and autoreactive antigens tested at 10 µg/ml mAb (in contrast to in Figure 4A, D where mAbs were at 200 µg/ml) (mean and SEM, n=3 replicates, *P<0.05, **P<0.007, ***P<0.0003, T-test).

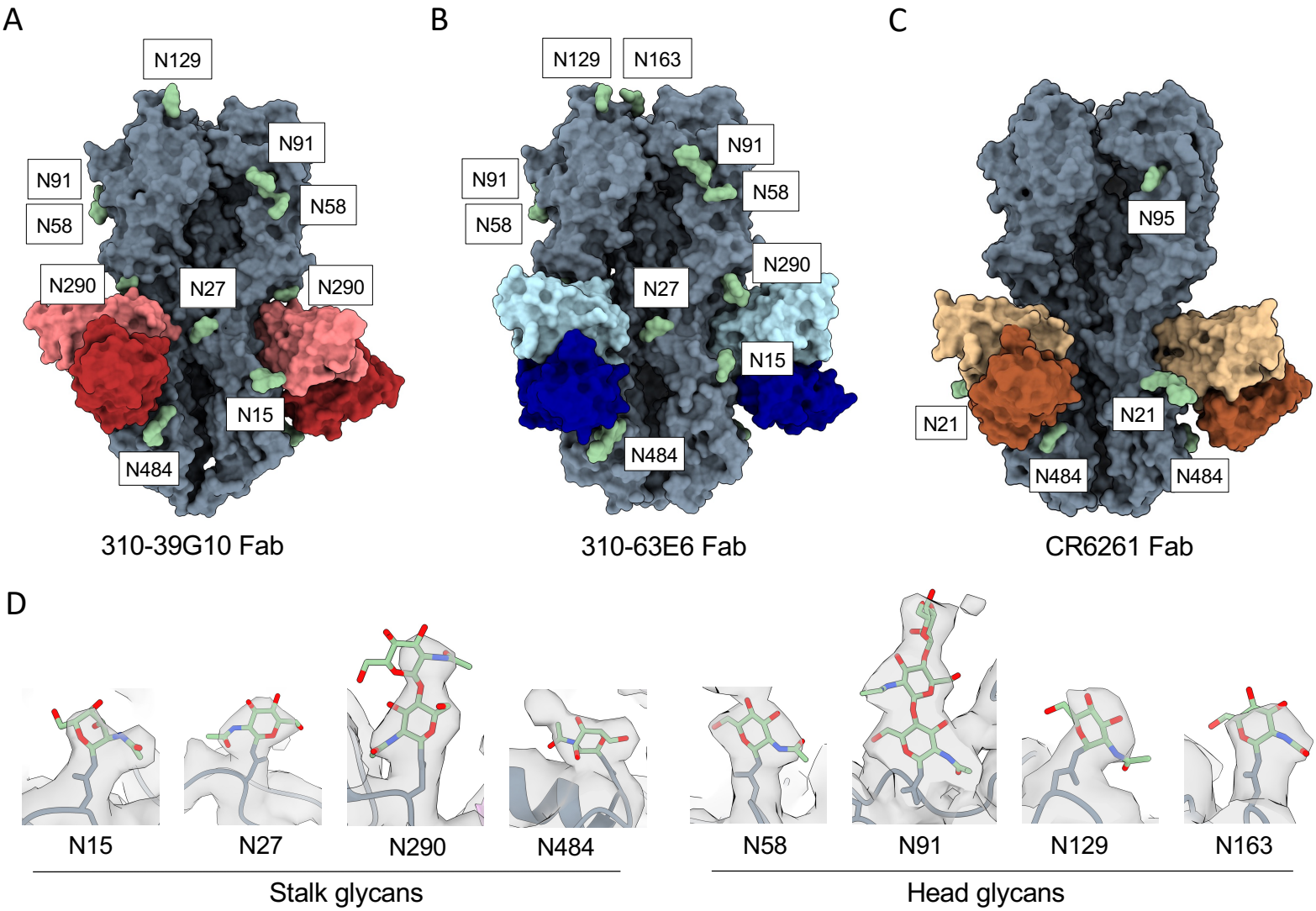

Figure S7. Glycosylation analysis of HA bound to 310-39G10 Fab, 310-36E6 Fab and CR6261 Fab - related to Figure 4. (A-C) Surface representation of HA model showing the glycan sites observed by cryo-EM (A-B) and X-ray crystallography (PDB ID: 3gbm, C) in green with their respective Asn residues. (D) Glycan densities (green) are shown at a threshold of 0.2 in ChimeraX. The unsharpened maps were used to generate the visuals. HA trimer is shown in gray. (E) Binding by poly/autoreactive L54 germline stalk mAbs (HC2 mouse and human) to denatured SS-np, before and after deglycosylation by PNGase F (***P<0.0009, Paired T-test).