Daniela Fera
GUP3 Application - Figures and Preliminary Results

A. **B.**

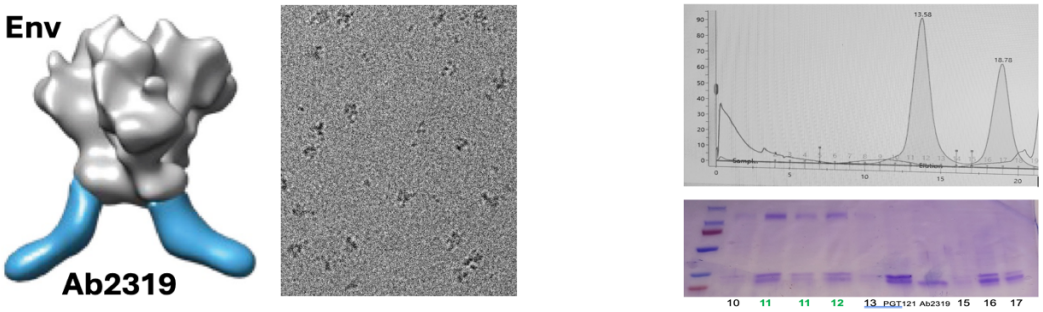

Figure 1. Antibody 2319 Bound to HIV Spike. (A) (left) Negative stain EM model of antibody (blue) binding BG505 HIV spike (gray) at the base. (right) Preliminary cryo-EM micrograph obtained of the same antibody in complex with RC1 HIV spike using the Titan Krios. **(B)** (top) SEC chromatogram of Ab2319 with HIV Spike and a stabilizing antibody (PGT121). Left peak denotes ternary complex and right peak are excess Fabs. (bottom) SDS-PAGE gel showing the purity of the sample. Fractions in green were concentrated to >3 mg/mL.

A. **B.**

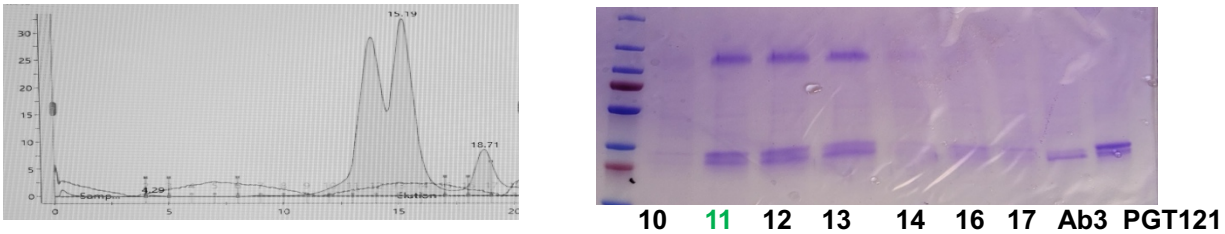

Figure 2. Antibody 3 Bound to HIV Spike. (A) SEC chromatogram of Ab3 with HIV Spike and PGT121. Left doublet peak is of complex – left side is likely the ternary complex and the right side likely has only PGT121 or fewer copies of Ab3. Right peak is excess Fabs. **(B)** SDS-PAGE gel showing the purity of the sample. Fraction in green (corresponding to left part of doublet peak) was concentrated for cryo-EM studies.

A. **B.** **C.** **D.**

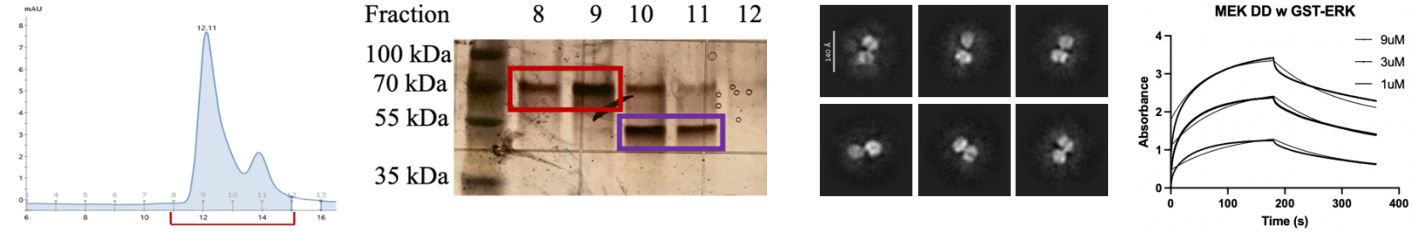

Figure 3. MEK-ERK Analysis. (A) SEC of GST-ERK + MEK. **(B)** Silver stain of GST-ERK + MEK from SEC fractions showing no co-elution (GST-ERK boxed in red and MEK DD in purple) but high purity of individual proteins. **(C)** Cryo-EM images obtained using a Titan Krios show uncomplexed GST-ERK dimer. **(D)** Biolayer interferometry showing binding between GST-ERK and MEK DD even down to 1uM.

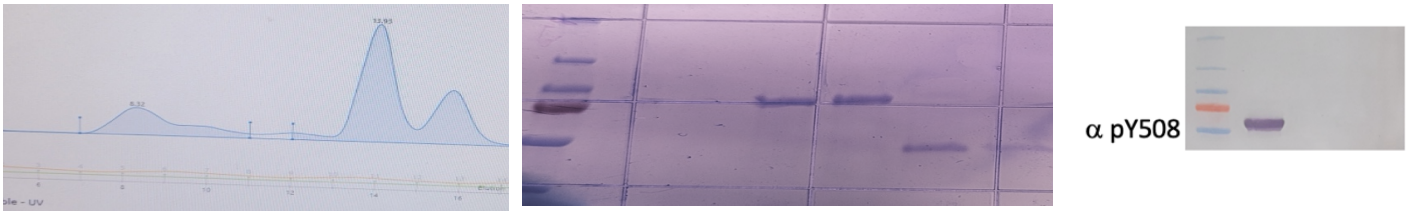

Figure 4. Autoinhibited Lyn. (left) SEC of Lyn Y397F mutant with SH3, SH2, and kinase domains. (middle) SDS-PAGE of fractions (two highest bands correspond to largest SEC peak and are of >95% purity). (right) Western blot showing C-terminal Tyr phosphorylated, maintaining inhibitory (compact) state.