## **Supplemental Information Inventory**

## **Supplemental Figures**

- Figure S1. Optimization of NFL linker length and expression, related to Figure 1.
- Figure S2. Negative stain EM of JRFL gp140-foldon and 16055 gp140 NFL2P along with thermodynamic analysis of JRFL and BG505 gp120 by ITC, related to Figure 3.

Figure S3. Antigenicity of NFL2P trimers by ELISA and BLI, related to Figure 4.

- Figure S4. EM 3D reconstructions of 2G12-liganded BG505 gp140 NFL2P and BG505 SOSIP.664 trimers and 2D class averages of PGT151-bound to the uncleaved trimers, related to Figures 5-6 and Table S2.
- Figure S5. Projection matching and Fourier Shell Correlation graphs, related to Figure 5-6 and Table S2.
- Table S1. Binding kinetics of trimer-specific bNAbs to JRFL and BG505 gp140 NFL2P, SOSIP, and foldon trimers as observed by BLI, related to Figure 4 and S3.
- Table S2. Binding stoichiometries of bNAbs to JRFL and BG505 gp140 NFL2P trimers as observed by negative stain EM and number of particles used for 3D EM reconstructions, related to Figures 5-6 and S4-5.

## **Supplemental Experimental Methods**

## **Supplemental References**