Supplementary Data

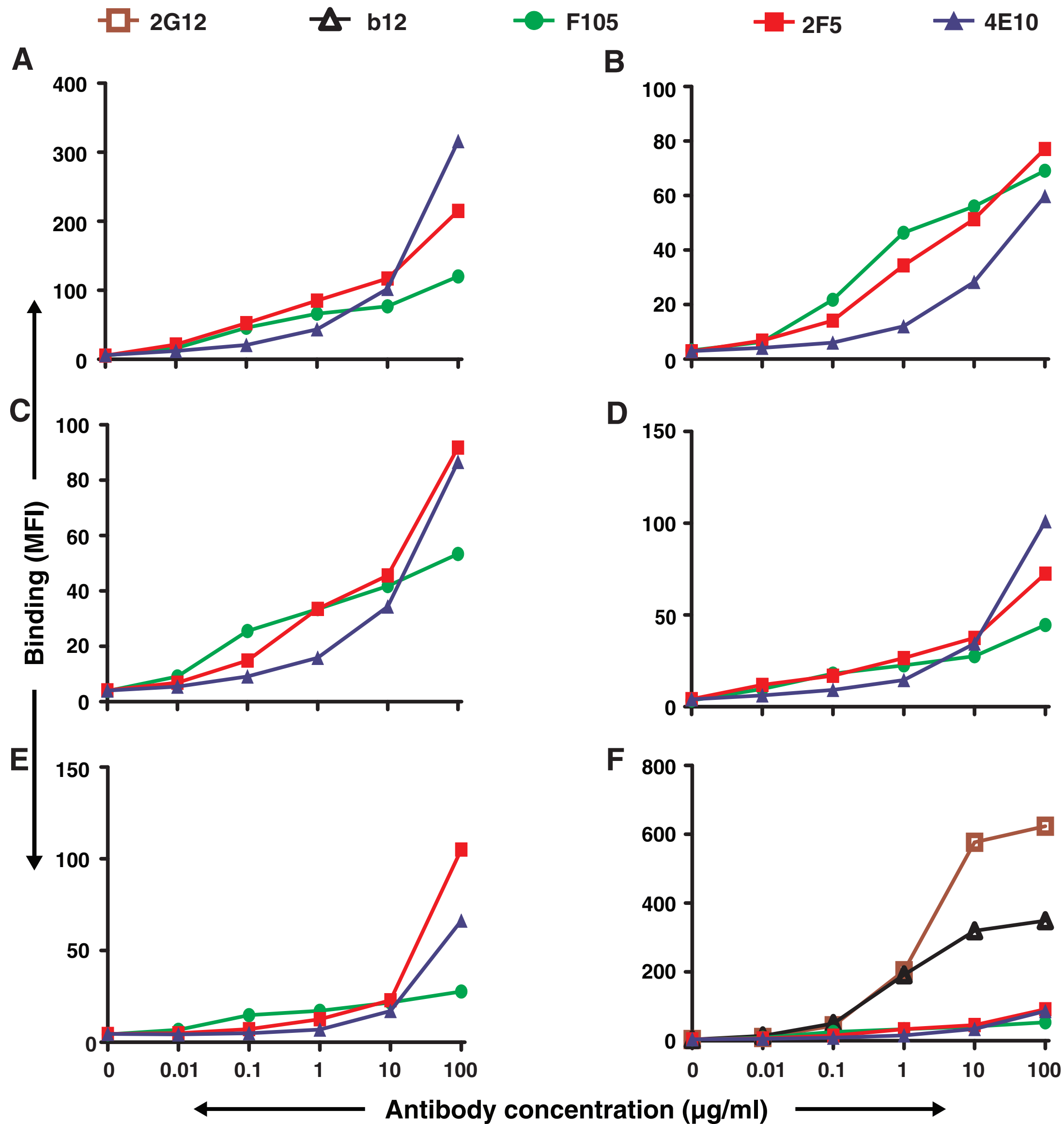

FIG. S1. FACS-based binding curves derived by antibody binding to cleavage-competent cell-surface JR-FL Env. (**A–E**) MFI values derived from five independent experiments utilizing the gp41-directed neutralizing antibodies (2F5 and 4E10) and the gp120-directed nonneutralizing antibody (F105) are shown. (**F**) MFI values of both the neutralizing (2G12, b12, 2F5, and 4E10) and the nonneutralizing antibody (F105) to cleavage-competent JR-FL Env are shown. Note the different scale in panel (**F**) to accommodate all MFI values on the same graph.

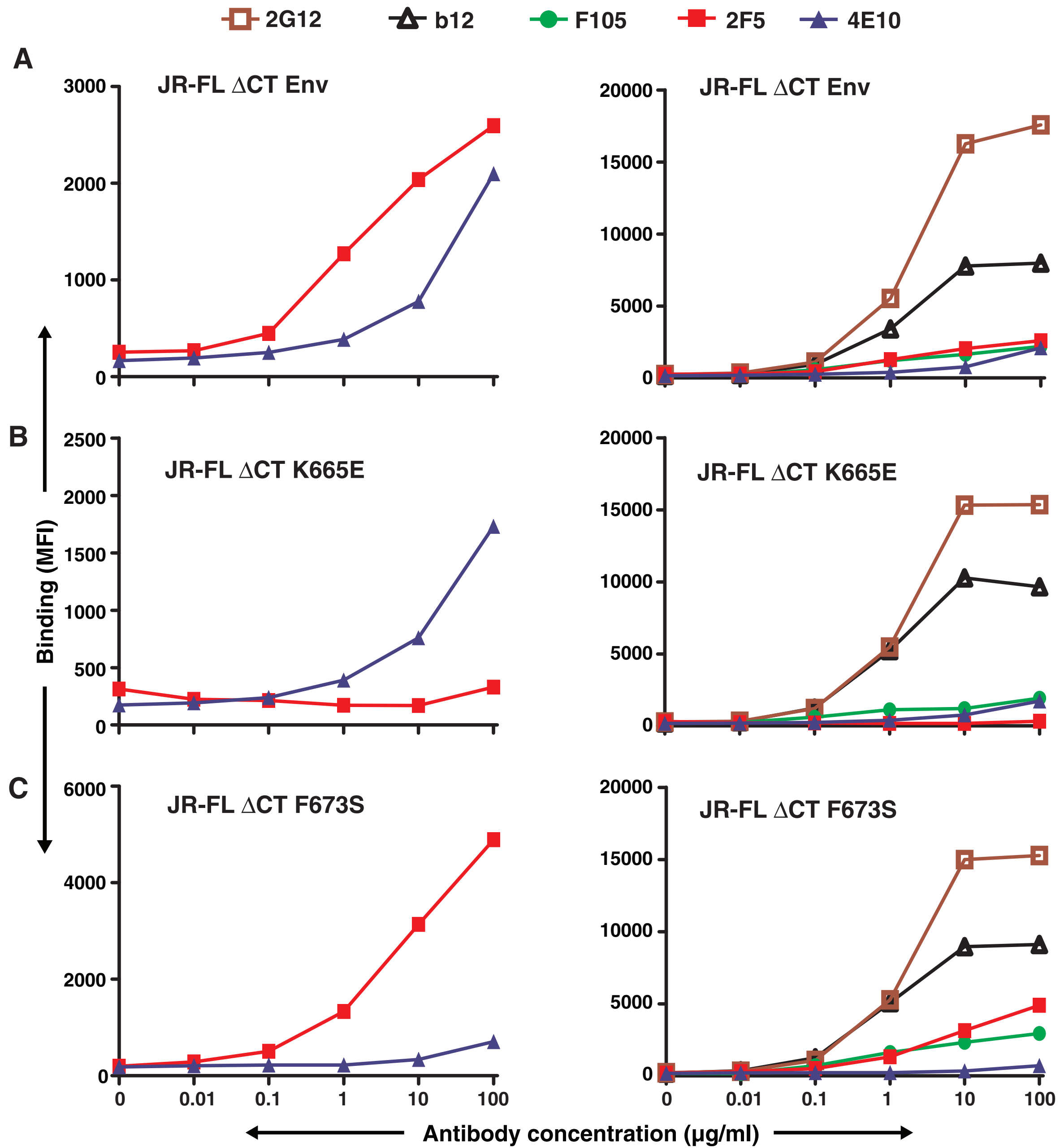

FIG. S2. FACS-based curves (BD LSR-II) derived from antibody binding to variants of the cleavage-competent, cell-surface, JR-FL ΔCT Env to confirm 2F5 and 4E10 binding specificity. (**A**) Left, the binding of 2F5 and 4E10 antibodies to WT JR-FL ΔCT Env; right, binding of all antibodies analyzed. (**B**) Left, the binding of 2F5 and 4E10 antibodies to JR-FL ΔCT K665E mutant, which is not recognized by 2F5 as expected; right, the binding of all antibodies analyzed. (**C**) Left, the binding of 2F5 and 4E10 antibodies to JR-FL ΔCT F673S mutant, which is not recognized by 4E10 as expected; right, the binding of all antibodies analyzed.

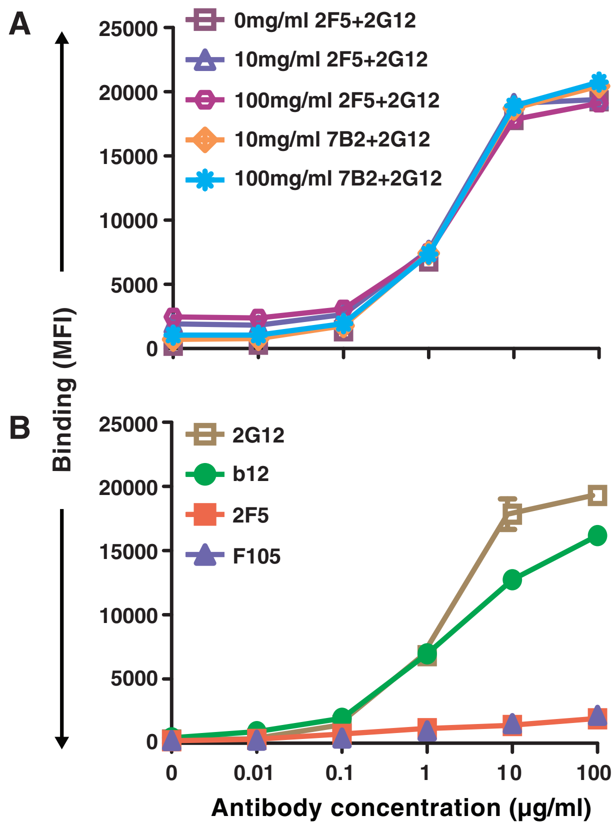

FIG. S3. FACS-based cell surface binding of 2G12 to cleavage-competent JR-FL Env (**A**) MFI values of 2G12 binding following preincubation (and washing) of 2F5 and 7B2 antibodies to confirm that the Env spikes remain intact. (**B**) MFI values of b12, F105, 2G12, and 2F5 control antibodies to untreated Env.

Fluorescence-activated cell sorting (FACS) staining of cell-surface HIV-1 Env. FACS staining was performed as previously described.[54] Forty-eight hours following transfection, the cells were harvested and washed in FACS buffer (PBS, 5% HIFBS, 0.02% azide) and stained with a panel of monoclonal antibodies that was also used in viral neutralization assays. The monoclonal antibody–cell mixture was washed extensively in FACS buffer and antihuman phycoerythrin (PE) (Sigma) at a 1:200 dilution was added for 1 h, followed by extensive washing to remove unbound secondary antibody. To study the effect of 2F5 and 7B2 antibodies on the binding of selected antibody, 2G12, either 2F5 or 7B2 at concentrations of 10 μg/ml or 100 μg/ml was added to the transfected cells and incubated for 1 h at room temperature (RT) with occasional shaking. The transfected cells without antibody were kept as negative controls. The mixture was washed with FACS buffer and incubated with 2G12 for 1 h at RT. The stained cells were analyzed by FACS on a BD LSR-II Instrument.

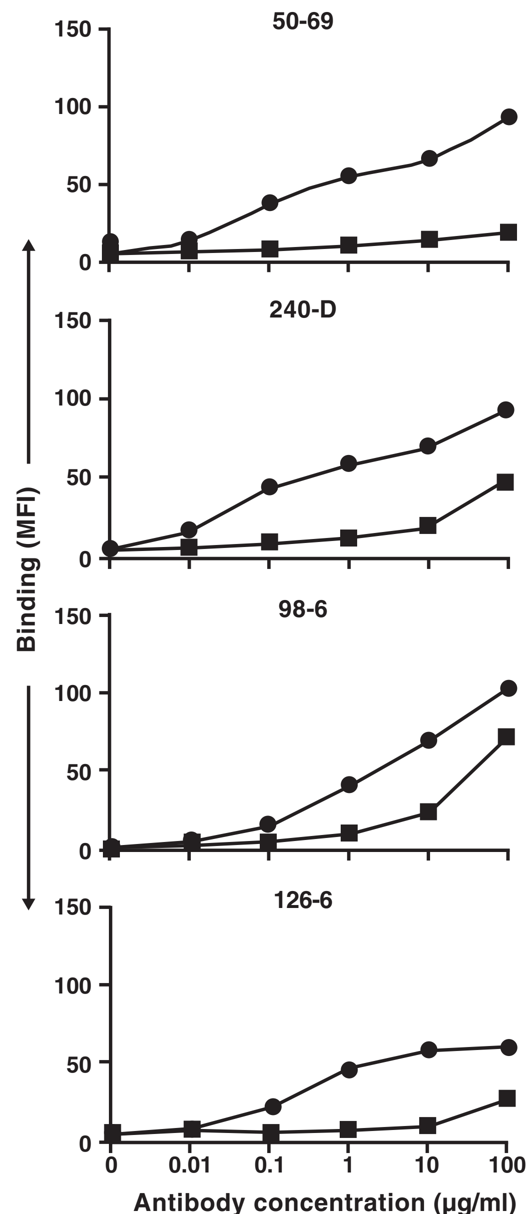

FIG. S4. Effects of sCD4 on the binding of cluster 1 and 2 antibodies to cell cleavage-competent JR-FL Env expressed on the cell surface. MFI curves derived from the binding of the gp41-directed cluster 1 and cluster 2 antibodies (50-69, 240-D, 98-6, and 126-6) to cleavage-competent JR-FL Env without sCD4 (■) and with sCD4 (●).