Supporting Information

Guan *et al.* 10.1073/pnas.0813392106

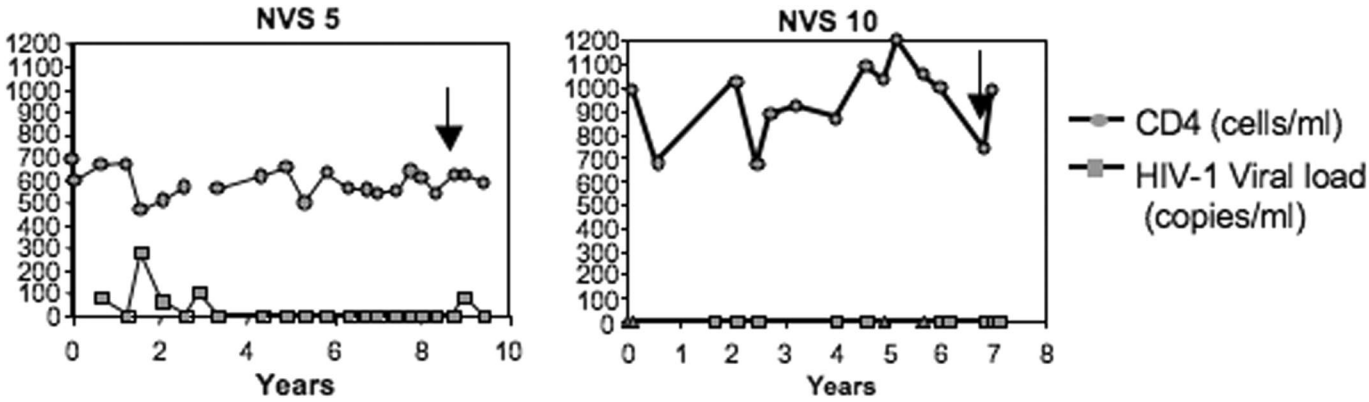

Fig. S1. Viral loads and CD4 counts for NVS5 and NVS10. All donors had stable CD4 count (circles, cells/mL). Viral load (squares) detection limit was 75 copies/mL. A less-sensitive viral load assay (<400 copies/mL) was used at several time points for NVS10 (triangles). Arrows indicate the time point of sampling for this study. Specimens for NVS9 were collected in year 3 (third collection) after enrollment (1).

1. Sajadi MM, *et al.* (2009) Epidemiologic characteristics and natural history of HIV-1 natural viral suppressors. *J AIDS*, in press.

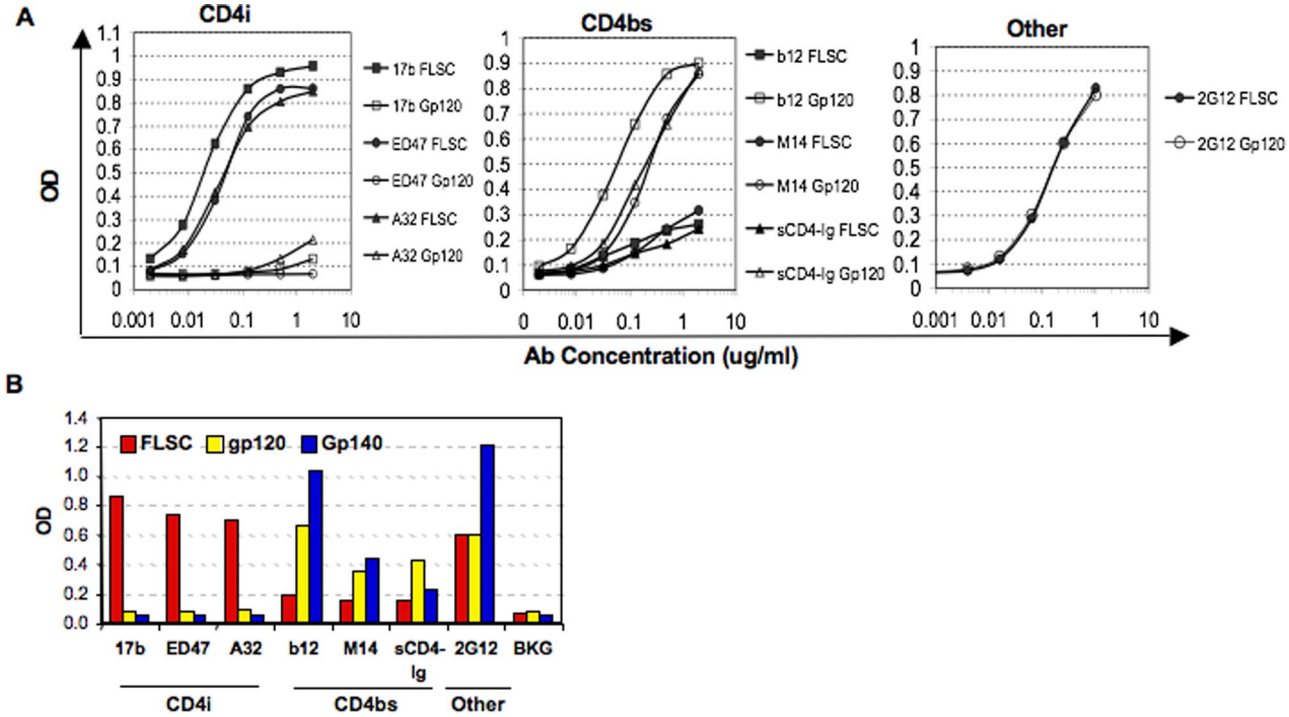

Fig. S2. Identification of CD4i and CD4bs mAbs by ELISA. Representative CD4i and CD4bs mAbs were tested in 3 formats of HIV-1 Env ELISAs as described in *Materials and Methods*. (*A*) *Right*, *Middle*, and *Left* charts show results for CD4i mAbs (17b, ED47, and A32), CD4bs mAbs (b12, M14, and sCD4-Ig), and mAb 2G12, respectively. ELISA curves for reactivity to FLSC (filled symbols) and to $gp120_{Ba\text{-}L}$ (open symbols) are shown. (*B*) OD values of ELISAs for all these representative mAbs at 0.1 μg/mL. CD4i mAbs are identified by their strong binding to FLSC and weak binding to $gp120_{Ba\text{-}L}$ or $gp140_{Ba\text{-}L}$, whereas CD4bs mAbs are identified by comparison of their strong binding to $gp120_{Ba\text{-}L}$ or $gp140_{Ba\text{-}L}$ and their weak binding to FLSC. MAbs other than CD4i and CD4bs are denoted as ''Other.'' One of the ''Other'' mAb, 2G12, binds equally well to FLSC and $gp120_{Ba\text{-}L}$ and therefore is used to standardize the ELISAs.

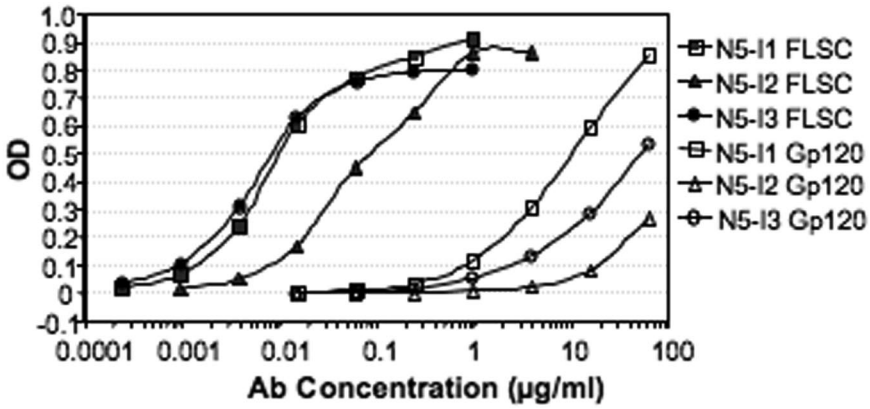

Fig. S3. Isolation of three novel CD4i mAbs. Three mAbs were cloned from CD4i Ab positive wells of NVS5 as described in Materials and Methods. ELISA curves for selective reactivity of the mAbs to FLSC (filled symbol) relative to $gp120_{Ba\text{-}L}$ (open symbol) are shown.

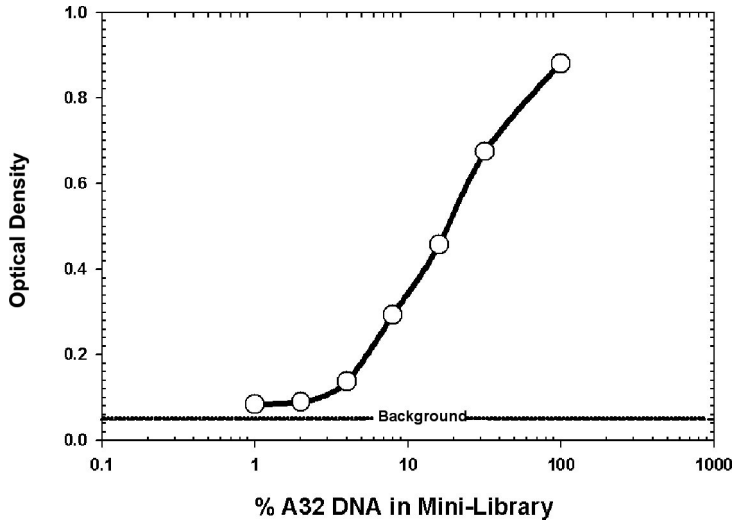

Fig. S4. Modeling study to determine limit of detection for the correct VH/VL pair in a minilibrary. The heavy and light chain genes were cloned for the A32 mAb and inserted into IgG1 or lambda chain eukaryotic expression vectors, respectively. An equate-molar mixture of the two vectors was then mixed with a minilibrary mixture from a culture that did not have an Env-reactive B_{Mem} at the percentages indicated on the x axis of and used to transfect 293T cells. Supernatants were collected three days after transfection and the binding to FLSC was determined by ELISA. Background for supernatants collected from cells transfected with the Env-negative minilibrary only is indicated by the dashed line. This modeling study indicates that a positive signal is obtained when the functional VH/VL pair is present at the level of ≈1% or greater in a population of irrelevant clones. In practice, we have found this to consistently be the case in the isolation of 33 new mAbs from NVS volunteers (in preparation).