Supplementary Figure 1

Map of pCT-VHVL-K1 native $V_H$:V_L display vector.

Natively-paired $V_H$:V_L sequences are cloned en masse into this vector for human antibody repertoire mining, and their corresponding Fabs are expressed on the yeast cell surface via galactose induction.
Supplementary Figure 2

Flow cytometry analysis of a panel of human anti-HA antibodies before and after display optimization, and of the 2 anti-EBOV antibodies and 1 anti-HIV-1 bNAb in the optimized system.

(a) Display of the six anti-HA antibodies listed in Figure 1c that did not functionally display in EBY100 (upper) and in AWY101 with LZ-forced Fab dimerization (lower). (b) Anti-EBOV antibodies c13c6 and KZ52 and anti-HIV-1 bNAb VRC34.01 displayed in the optimized system. For anti-HA antibodies, 100nM recombinant A/California/07/2009 HA was used to stain D1 H1-2, D1 H1-3/H3-3, D1 H1-53, D1 H1-12, and D1 H1-17/H3-14, and 100nM recombinant B/Brisbane/60/2008 HA was used to stain D1 Vic-8/Yama-20. 23 nM GPΔmuc-APC was used to stain c13c6 and KZ52; 50nM VRC34-epitope scaffold-FP-APC was used to stain VRC34.01. A representative profile from 5 (a) or 3 (b) independent experiments for each antibody is shown.
Supplementary Figure 3

Representative FACS gating strategy for EBOV library sorts.

Yeast cells were stained with 2 μg/ml anti-FLAG-FITC and 23 nM GP_{Δmuc}-APC.
Supplementary Figure 4

Flow cytometry antigen binding profiles of monoclonal yeast populations expressing EBOV.YD.09-EBOV.YD.11, which were identified by single colony picking.

Yeast cells were stained with 2 μg/ml anti-FLAG-FITC and 23 nM GP_{Δmuc}^+APC.
Supplementary Figure 5

Biolayer interferometry response curves for human anti-EBOV antibodies from the plasmablast cognate \( V_H:V_L \) repertoire.

Binding was assessed against \( \text{GP}_{\text{Delta}} \). Global analyses were carried out using nonlinear least-squares fitting allowing a single set of binding parameters to be obtained simultaneously for all concentrations used in each experiment.
Supplementary Figure 6

Neutralization of EBOV GP pseudotype infection by human anti-EBOV antibodies.

% Infection is shown relative to the negative control antibody VRC01. Data are reported as average ± standard deviation for three technical replicates.
Supplementary Figure 7

Representative FACS gating strategy for HIV-1 library sorts.

Yeast cells were stained with 2 μg/ml anti-FLAG-FITC, 50 nM VRC34-epitope scaffold-FP-APC, and 50 nM VRC34-epitope scaffold-KO-PE for the isolation of HIV-1 fusion peptide-specific antibodies.
Supplementary Figure 8

Biolayer interferometry response curves for HIV-1 FP-specific antibodies from the B cell repertoire of an HIV-1 slow progressor.

The FP-scaffold protein was immobilized on the biosensor chip. Global analyses were carried out using nonlinear least-squares fitting allowing a single set of binding parameters to be obtained simultaneously for all concentrations used in each experiment.
Supplementary Figure 9

HIV-1 neutralization IC$_{50}$ potency for natively paired VRC34 family antibodies discovered via yeast display.

Neutralization was determined against a panel of 22 viruses.
Supplementary Figure 10

Sequence alignments of HIV-1 FP-specific antibodies from the peripheral B cell repertoire of donor N123.

Mutations are colored in red.
Supplementary Figure 11

Representative FACS gating strategy for flu library screening.

Yeast cells were stained with 2 μg/ml anti-FLAG-FITC, and either 40nM A/Solomon Islands/3/2006 H1 HA (upper panel) or 40nM A/Wisconsin/67/2005 H3 HA (lower panel) for the isolation of H1 and H3-specific antibodies, respectively.
Supplementary Figure 12

Surface plasmon resonance binding curves for anti-HA antibodies from the B cell cognate $V_H$:$V_L$ repertoire.

Representative binding curves from 3 independent experiments for each antibody are shown.
Supplementary Figure 13

Neutralization profiles of anti-HA antibodies isolated via yeast display.

CR9114 and CR6261 were included as positive and negative controls, respectively. Data are reported as average ± standard deviation from three technical replicates.