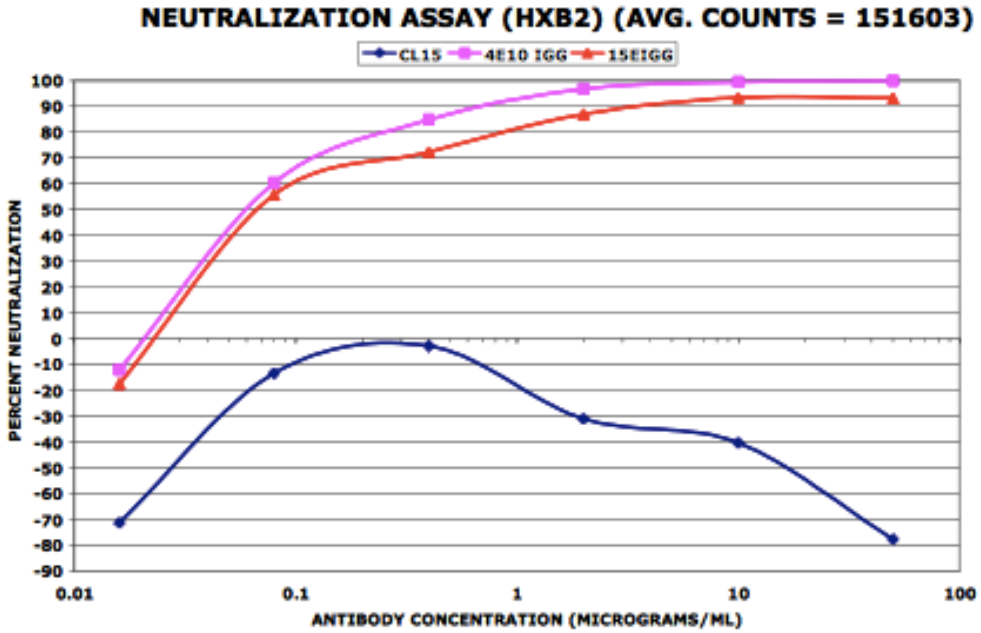
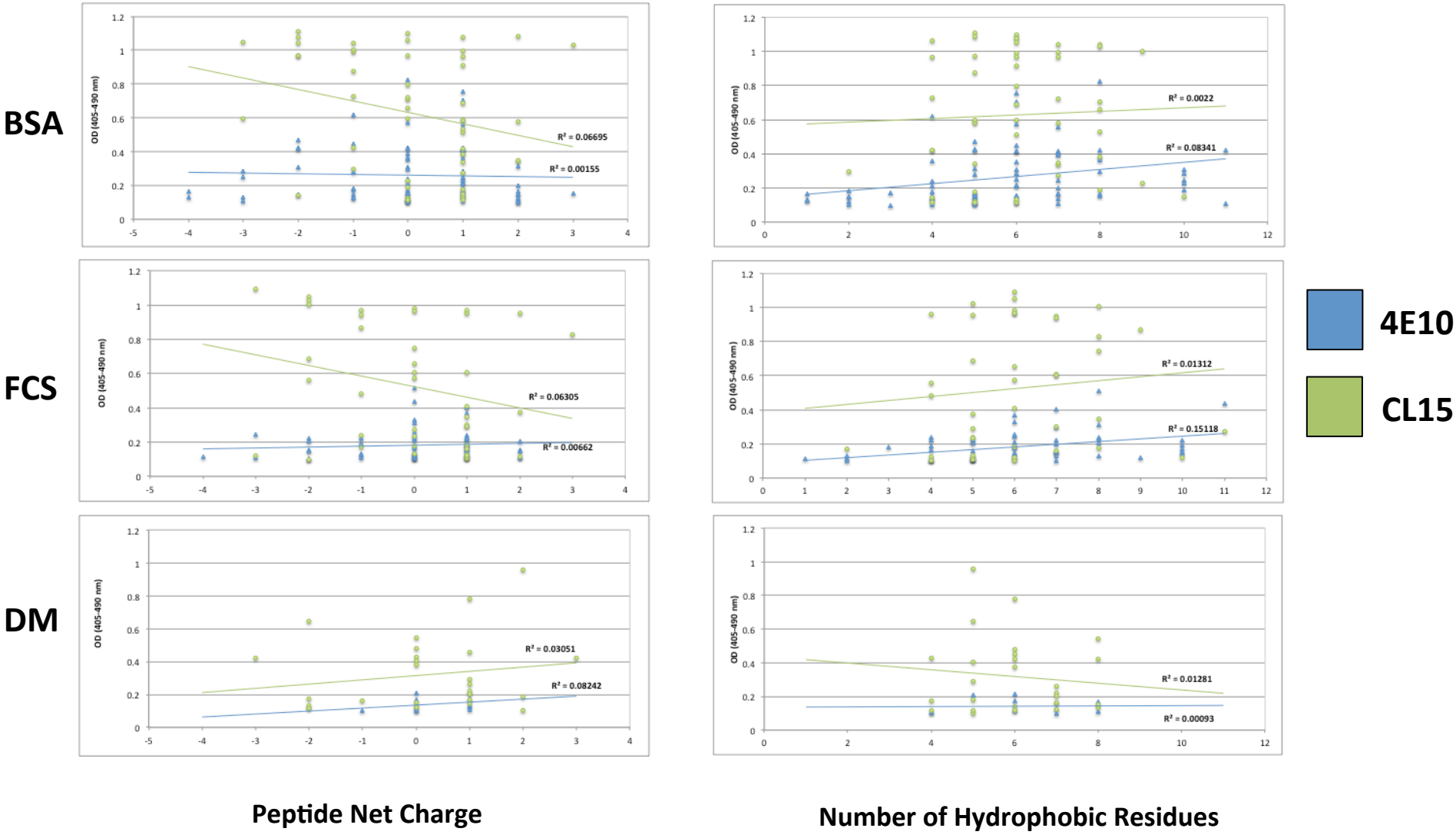
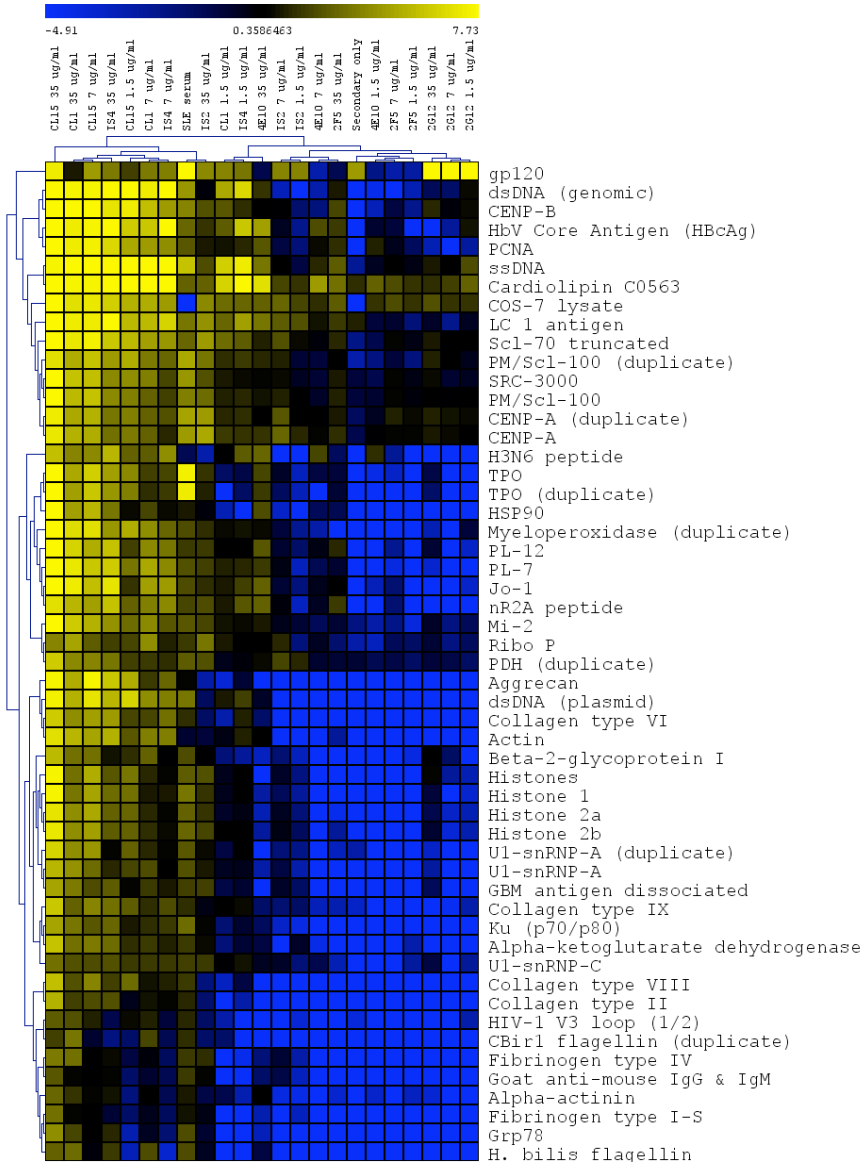


Supplemental Figure S1. Neutralization of HIV-1 strain HxB2 by MAbs 2F5, 4E10 and CL15. Recombinant HIV-1_{HxB2} virions, competent for a single round of infection, were generated using the luciferase reporter plasmid pNL4-3.Luc.R-E-, as described previously [2, 8]. The pseudotyped virus was assayed for neutralization using U87.CD4.CXCR4 target cells. MAbs were diluted and added (1:1 by volume) to HIV-1, and pre-incubated for 1 hour at 37°C. The mixture of MAb and HIV-1 was then added (1:1 by volume) to the target cells, and the assay was developed using luciferase reagent (Promega) following a 72 hour incubation at 37°C. The degree of virus neutralization was determined as a percentage reduction of viral infectivity against an antibody-free control. The experiment was performed in triplicate.

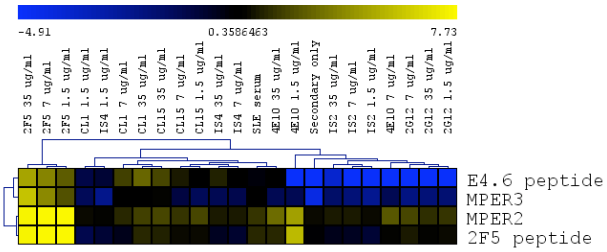


Supplemental Figure S2. Linear regression analysis of ELISA reactivity of MAbs 4E10 and CL15 with HIV-1 MN Env peptides (text Fig. 2) *vs.* peptide net charge (left) and number of hydrophobic amino acids (right). In 5% bovine serum albumin (BSA) and 50% fetal calf serum (FCS), MAb CL15 binding (but not MAb 4E10 binding) is associated with peptide net negative charge; in 5% dried milk (DM) there is no such association. For the purposes of this figure, the set of hydrophobic amino acids includes C, F, I, L, M, V and W. Only peptides bound by each MAb with O.D. > 0.1 were included in the analysis.

(A)

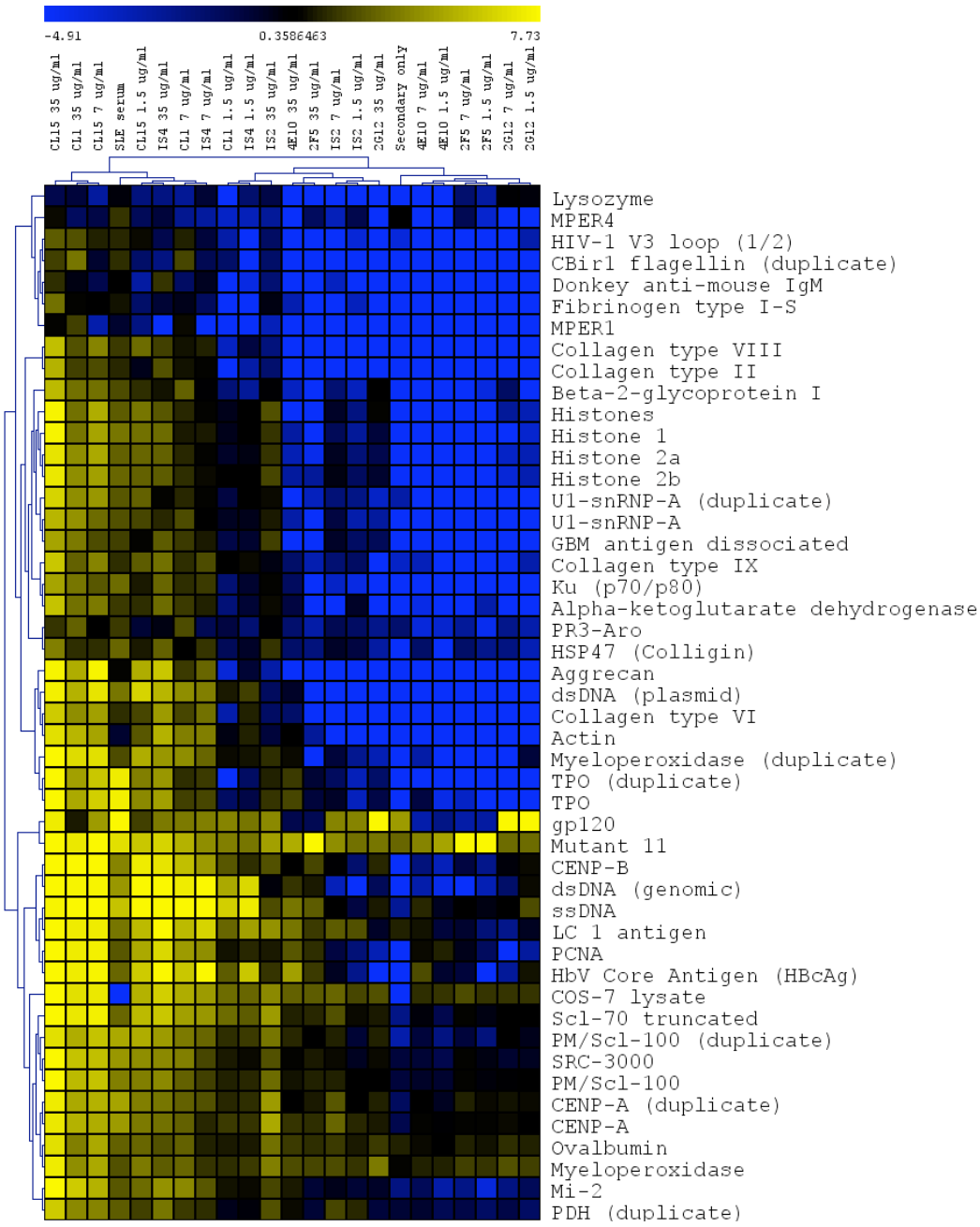


(B)

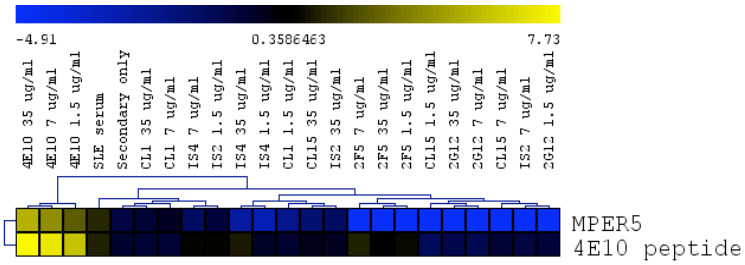


Supplemental Figure S3. Hierarchical clustering of the reactivity of all MAbs with only those antigens bound significantly differently by MAbs CL15 and 2F5. (A) CL15 > 2F5 significantantigens. (B) 2F5 > CL15 significant antigens. The order of antigens is based on overall reactivity similarities across all MAbs.

(A)

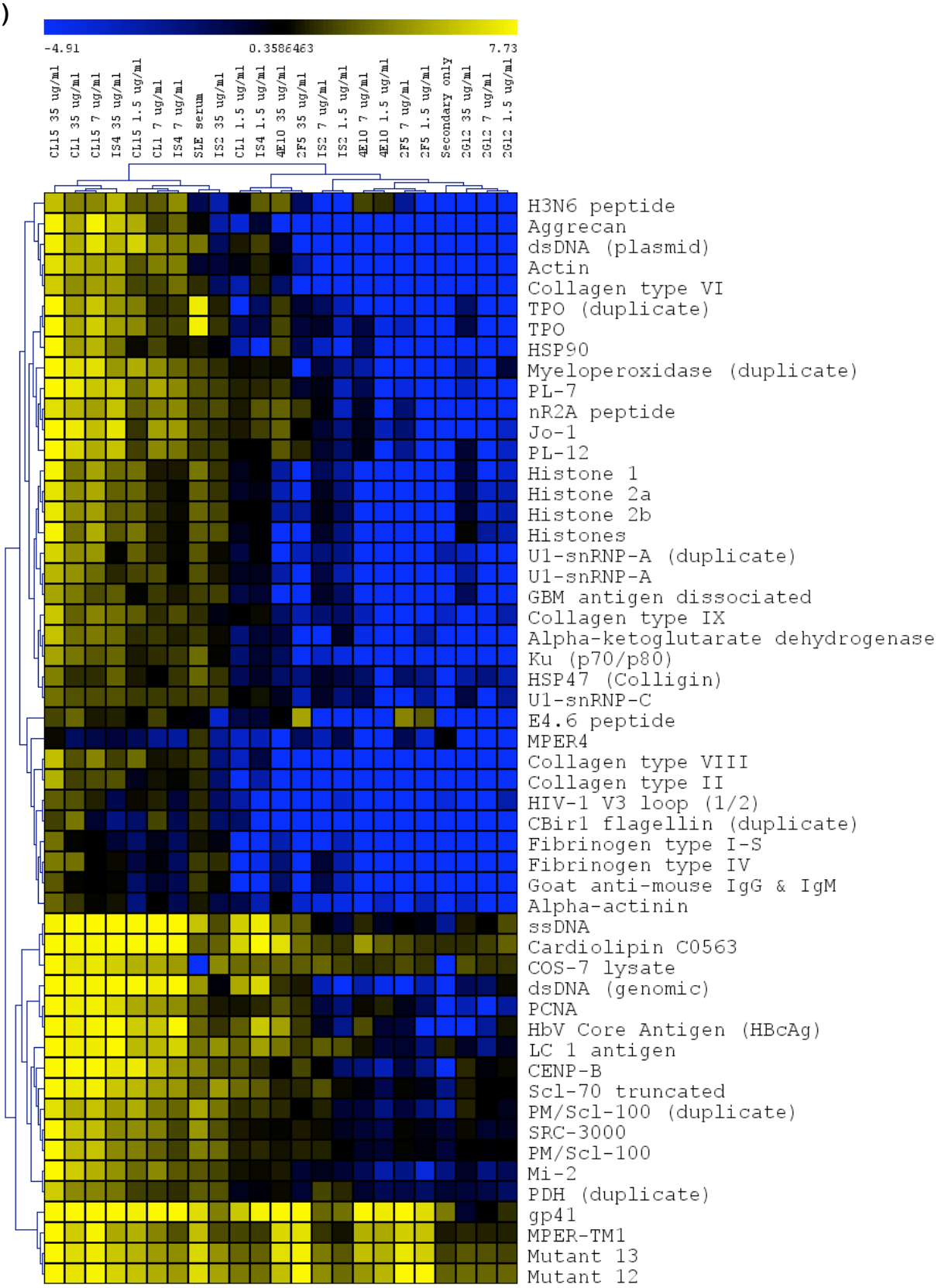


(B)



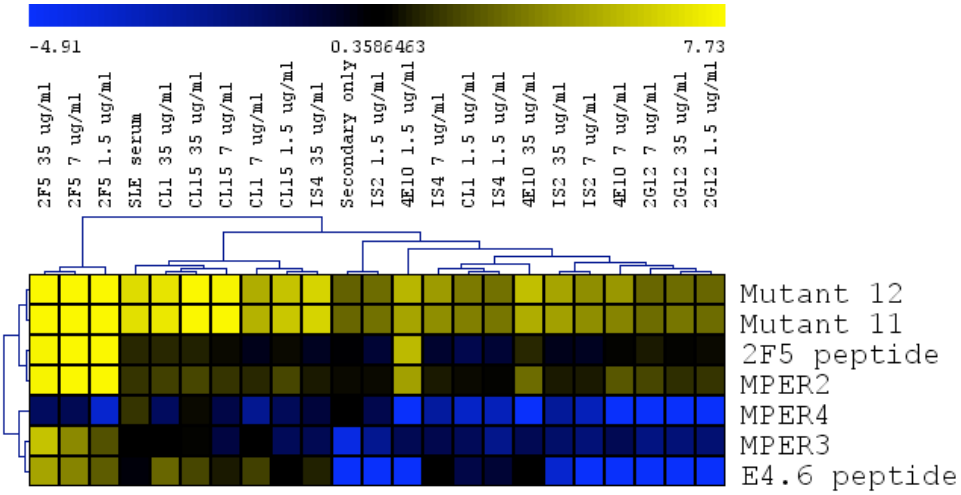
Supplemental figure S4. Hierarchical clustering of the reactivity of all MAbs with only those antigens bound significantly differently by MAbs CL15 and 4E10. (A) CL15 > 4E10 significantantigens. (B) 4E10 > CL15 significant antigens. The order of antigens is based on overall reactivity similarities across all MAbs.

(A)

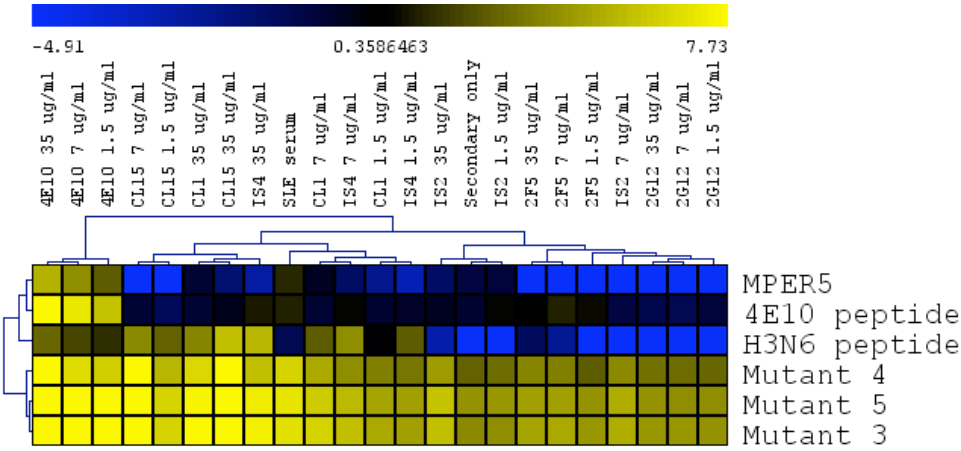


Supplemental figure S5. Hierarchical clustering of the reactivity of all MAbs with only those antigens bound significantly better by MAb CL15 in comparison with MAb 2G12. The order of antigens is based on overall reactivity similarities across all MAbs.

(A)

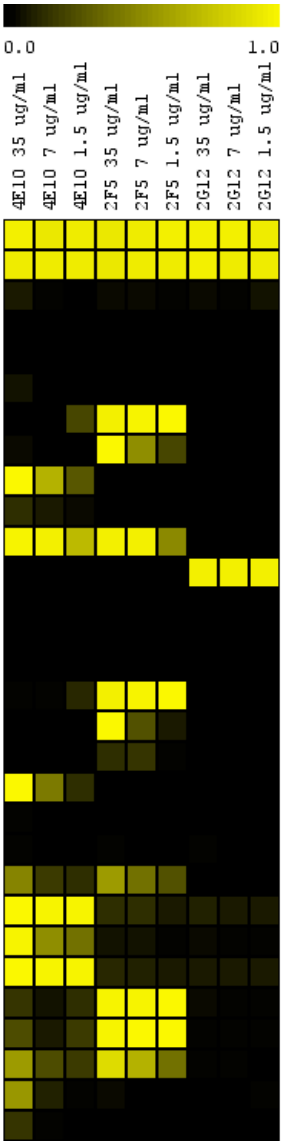


(B)



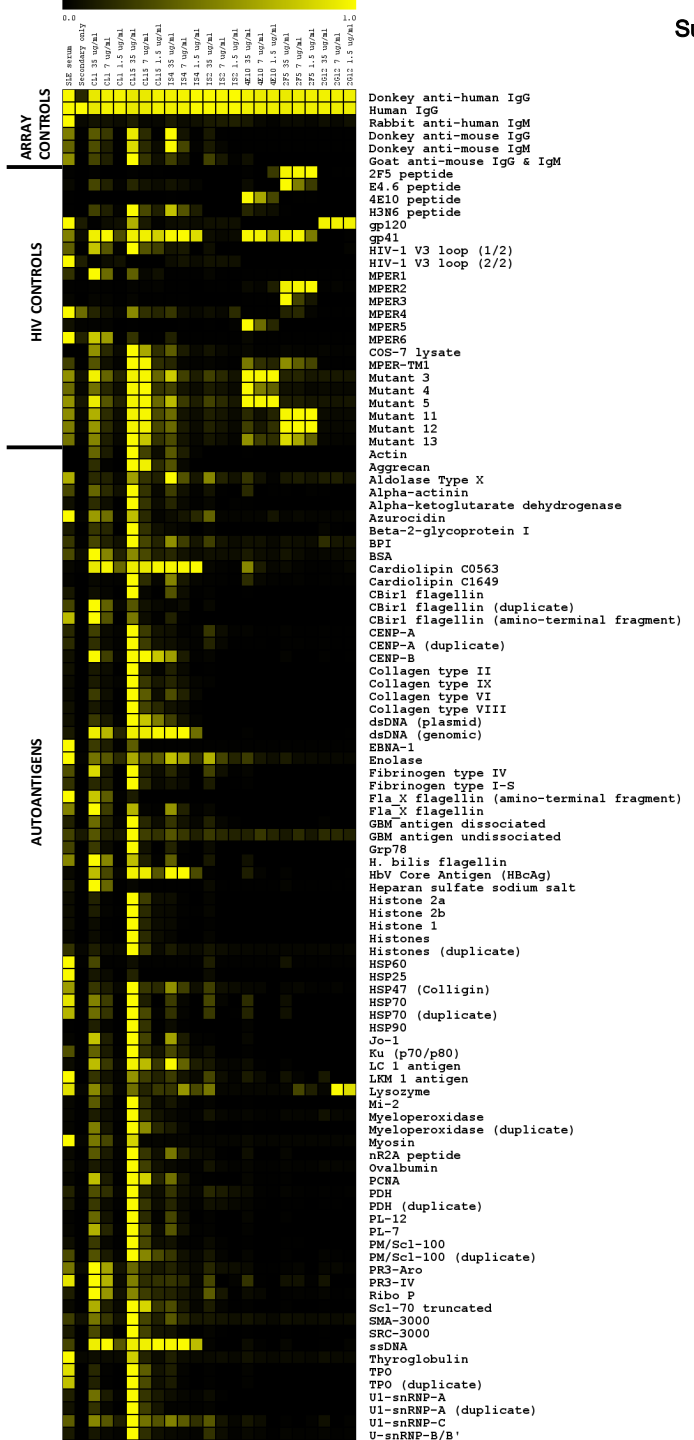
Supplemental figure S6. Hierarchical clustering of the reactivity of all MAbs with only those antigens bound significantly differently by MAbs 2F5 and 4E10. (A) 2F5 > 4E10 significantantigens. (B) 4E10 > 2F5 significant antigens. The order of antigens is based on overall reactivity similarities across all MAbs.

Supplemental Figure S7A. Color version of text Figure 3A.

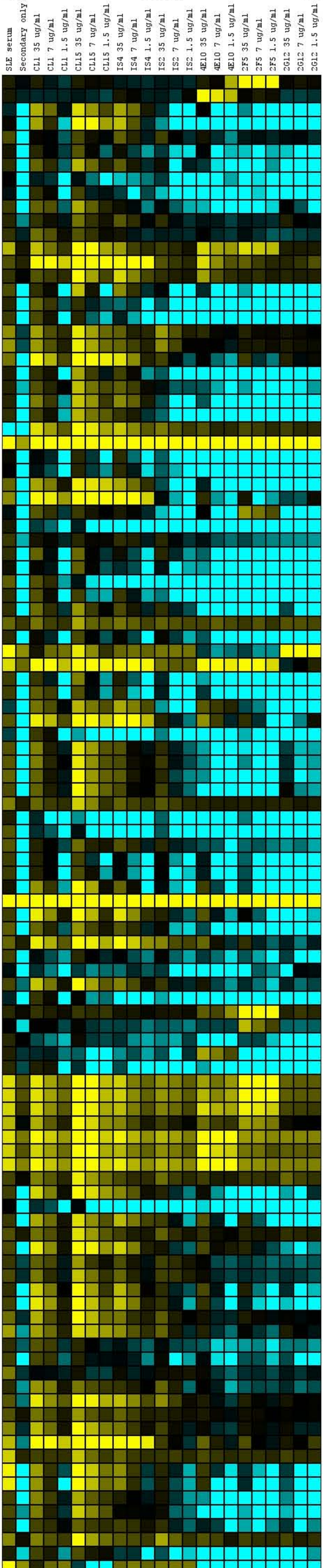


	Peptide/Construct Sequence:
Donkey anti-human IgG	
Human IgG	
Rabbit anti-human IgM	
Donkey anti-mouse IgG	
Donkey anti-mouse IgM	
Goat anti-mouse IgG & IgM	
2F5 peptide	EQELLELDKWASLWSGK(Bio)GC
E4.6 peptide	LHEESMDKWSNLMQCCTAEGK(Bio)
4E10 peptide	SLWNWFDITNWLWYISGK(Bio)GC
H3N6 peptide	AEPAENNWFMLTYFLAAEGC
gp41	
gp120	
HIV-1 V3 loop (1/2)	CTRPNYNKRKRIHIG
HIV-1 V3 loop (2/2)	NYNKRKRIHIGPGRA
MPER1	QQEKNEQELLELDKW
MPER2	NEQELLELDKWASLW
MPER3	LLELDKWASLWNWFD
MPER4	DKWASLWNWFDITNW
MPER5	SLWNWFDITNWLWYI
MPER6	WFDITNWLWYIKIFI
COS-7 lysate	
MPER-TM1	…ELLELDKWASLWNWFDITNWLWYIK…
Mutant 3	…ELLALDKWASLWNWFDITNWLWYIK…
Mutant 4	…ELLEADKWASLWNWFDITNWLWYIK…
Mutant 5	…ELLELAKWASLWNWFDITNWLWYIK…
Mutant 11	…ELLELDKWASLWAWFDITNWLWYIK…
Mutant 12	…ELLELDKWASLWNAFDITNWLWYIK…
Mutant 13	…ELLELDKWASLWNWADITNWLWYIK…
Cardiolipin C0563	
Cardiolipin C1649	

Supplemental Figure S7B. Color version of text Figure 3B.



Supplemental figure S8. Raw log2-transformed median antigen fluorescence intensities.



Supplemental Table S1. Description and sources of microarray antigens.

Antigen	Description	Source	Reference
Donkey anti-human IgG	Secondary antibody produced in donkey	Jackson	
Human IgG	Human IgG purified from serum	Jackson	
Rabbit anti-human IgM	Secondary antibody produced in rabbit	Jackson	
Donkey anti-mouse IgG	Secondary antibody produced in donkey	Jackson	
Donkey anti-mouse IgM	Secondary antibody produced in donkey	Jackson	
Goat anti-mouse IgG & IgM	Secondary antibody produced in goat	Jackson	
2F5 peptide	Peptide bearing 2F5 gp41 epitope (sequence EQELLELDKWASLWSGK(Bio)GC)	NeoMPS	5
E4.6 peptide	Peptide selected by MAb 2F5 from phage-displayed library	NeoMPS	5
4E10 peptide	Peptide bearing 4E10 gp41 epitope (sequence SLWNWFDITNWLWYISGK(Bio)GC)	UBC NAPS Unit	1
H3N6 peptide	Peptide selected by MAb 4E10 from phage-displayed library	EZBiolab	1,7
gp120	HIV envelope glycoprotein 120, recombinant	ImmunoDiagnostics	
gp41	HIV envelope glycoprotein 41, recombinant	ImmunoDiagnostics	
HIV-1 V3 loop (1/2)	Peptide from HIV strain MN gp120 (sequence CTRPNYNKRKRIHIG; catalogue number 6284)	NIH AIDS Research & Reference Reagent Program	
HIV-1 V3 loop (2/2)	Peptide from HIV strain MN gp120 (sequence NYNKRKRIHIGPGRA; catalogue number 6285)	NIH AIDS Research & Reference Reagent Program	
MPER	Peptide composed of aa 652-666 of HIV strain MN gp41 (sequence QQEKNEQELLELDKW)	NIH AIDS Research & Reference Reagent Program	
MPER2	Peptide composed of aa 656-670 of HIV strain MN gp41 (sequence NEQELLELDKWASLW)	NIH AIDS Research & Reference Reagent Program	
MPER3	Peptide composed of aa 660-674 of HIV strain MN gp41 (sequence LLELDKWASLWNWFD)	NIH AIDS Research & Reference Reagent Program	
MPER4	Peptide composed of aa 664-678 of HIV strain MN gp41 (sequence DKWASLWNWFDITNW)	NIH AIDS Research & Reference Reagent Program	
MPER5	Peptide composed of aa 668-682 of HIV strain MN gp41 (sequence SLWNWFDITNWLWYI)	NIH AIDS Research & Reference Reagent Program	
MPER6	Peptide composed of aa 672-686 of HIV strain MN gp41 (sequence WFDITNWLWYIKFI)	NIH AIDS Research & Reference Reagent Program	
COS-7 lysate	COS-7 cell lysate (African green monkey)	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
MPER-TM1	Lysate from COS-7 cells transfected with DNA construct encoding aa 643-732 of HIV gp41	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
Mutant 3	Lysate from COS-7 cells transfected with DNA construct encoding aa 643-732 of HIV gp41 with E662A mutation	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
Mutant 4	Lysate from COS-7 cells transfected with DNA construct encoding aa 643-732 of HIV gp41 with L663A mutation	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
Mutant 5	Lysate from COS-7 cells transfected with DNA construct encoding aa 643-732 of HIV gp41 with D664A mutation	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
Mutant 11	Lysate from COS-7 cells transfected with DNA construct encoding aa 643-732 of HIV gp41 with N671A mutation	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
Mutant 12	Lysate from COS-7 cells transfected with DNA construct encoding aa 643-732 of HIV gp41 with W672A mutation	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
Mutant 13	Lysate from COS-7 cells transfected with DNA construct encoding aa 643-732 of HIV gp41 with F673A mutation	Gift from Dr. Marinieve Montero (Simon Fraser University)	6
Actin	Rabbit muscle actin	Molecular Probes	
Aggrecan	Bovine articular cartilgae aggrecan	Sigma	
Aldolase Type X	Rabbit muscle aldolase	Sigma	
Alpha-actinin	Chicken gizzard alpha-actinin	Sigma	
Alpha-ketoglutarate dehydrogenase	Alpha-ketoglutarate dehydrogenase purified from porcine heart	Sigma	
Azurocidin	Human azurocidin from whole blood	The Binding Site	
Beta-2-glycoprotein I	Human β2-glycoprotein I, recombinant, His-tagged	Diarect	
BPI	Human bactericidal permeability-increasing protein purified from whole blood	The Binding Site	
BSA	Purified bovine serum albumin	Sigma	
CBir1 flagellin	Flagellin clone from enteric bacteria of the *Clostridium coccoides* group	Gift from Dr. Charles Elson (University of Alabama-Birmingham)	3
CBir1 flagellin (duplicate)	Flagellin clone from enteric bacteria of the *Clostridium coccoides* group	Gift from Dr. Charles Elson (University of Alabama-Birmingham)	3
Cardiolipin C0563	Cardiolipin sodium salt from bovine heart	Sigma	
Cardiolipin C1649	Cardiolipin solution in ethanol from bovine heart	Sigma	
CBir1 flagellin (amino-terminal fragment)	Flagellin clone from enteric bacteria of the *Clostridium coccoides* group	Gift from Dr. Charles Elson (University of Alabama-Birmingham)	3
CENP-A	Human centromere protein A, recombinant, His-tagged	Diarect	
CENP-A (duplicate)	Human centromere protein A, recombinant, His-tagged	Diarect	
CENP-B	Human centromere protein B, recombinant, His-tagged	Diarect	
Collagen type II	Bovine collagen type II from tracheal cartilage	Sigma	
Collagen type IX	Human collagen type IX from placenta	Sigma	
Collagen type VI	Human collagen type VI from placenta	Sigma	
Collagen type VIII	Human collagen type VIII from placenta	Sigma	
dsDNA (plasmid)	3.4 kb *E. coli* plasmid DNA	Diarect	
dsDNA (genomic)	Salmon testes genomic DNA	Sigma	
EBNA-1	Epstein-Barr nuclear antigen 1 (synthetic peptide comprising aa 398-412, sequence PPPGRRPFFHPVGEA)	Sigma	
Enolase	Rabbit muscle enolase	Sigma	

(*continued*)

Antigen	Description	Source	Reference
Fibrinogen type IV	Fibrinogen type IV purified from bovine plasma	Sigma	
Fibrinogen type I-S	Fibrinogen type I purified from bovine plasma	Sigma	
Fla_X flagellin (amino-terminal fragment)	Flagellin clone from enteric bacteria of the *Clostridium coccoides* group	Gift from Dr. Charles Elson (University of Alabama-Birmingham)	3
Fla_X flagellin	Flagellin clone from enteric bacteria of the *Clostridium coccoides* group	Gift from Dr. Charles Elson (University of Alabama-Birmingham)	3
GBM antigen dissociated	Dissociated human glomerular basement membrane antigen, recombinant, His-tagged	Diarect	
GBM antigen undissociated	Undissociated human glomerular basement membrane antigen, recombinant, His-tagged	Diarect	
Grp78	Hamster glucose-regulated protein 78, recombinant	Stressgen	
H. bilis flagellin	Flagellins purified from *Helicobacter bilis* cultures	Gift from Dr. Charles Elson (University of Alabama-Birmingham)	4
HbV Core Antigen (HBcAg)	Hepatitis B virus core antigen, recombinant	Sigma	
Heparan sulfate sodium salt	Heparan sulfate, from bovine kidney	Sigma	
Histone 2a	Histone 2a from bovine thymus tissue	Immunovision	
Histone 2b	Histone 2b from bovine thymus tissue	Immunovision	
Histone 1	Histone 1 from bovine thymus tissue	Immunovision	
Histones	Whole histones purified from chicken red blood cells	Immunovision	
Histones (duplicate)	Whole histones purified from chicken red blood cells	Immunovision	
HSP60	Human heat shock protein 60, recombinant	Stressgen	
HSP25	Murine heat shock protein 25, recombinant	Stressgen	
HSP47 (Colligin)	Human heat shock protein 47, recombinant	Stressgen	
HSP70	Human heat shock protein 70, recombinant	Stressgen	
HSP70 (duplicate)	Human heat shock protein 70, recombinant	Stressgen	
HSP90	Human heat shock protein 90, from HeLa cells	Stressgen	
Jo-1	Human histidyl-tRNA synthetase (Jo-1), recombinant, His-tagged	Diarect	
Ku (p70/p80)	Human Ku, recombinant, His-tagged	Diarect	
LC 1 antigen	Human formiminotransferase cyclodeaminase (liver cytosol antigen type 1), recombinant	Diarect	
LKM 1 antigen	Human cytochrome P450 2D6 (liver kidney microsomal antigen 1), recombinant	Diarect	
Lysozyme	Human lysozyme from whole blood	The Binding Site	
Mi-2	Human Mi-2β nuclear antigen, recombinant	Diarect	
Myeloperoxidase	Myeloperoxidase (pANCA antigen) from human promyelocytic cell line	ImmunoVision	
Myeloperoxidase (duplicate)	Myeloperoxidase (pANCA antigen) from human neutrophils	Biodesign	
Myosin	Myosin, calcium-activated, from rabbit muscle	Sigma	
nR2A peptide	NR2A (glutamate/N-methyl-D-aspartic acid (NMDA) receptor subunit) peptide, sequence SVSYDDWDYSLEARV	Sigma	
Ovalbumin	Albumin from chicken egg white	Sigma	
PCNA	Human proliferating cell nuclear antigen, recombinant, His-tagged	Diarect	
PDH	Pyruvate dehydrogenase purified from porcine heart	Sigma	
PDH (duplicate)	Pyruvate dehydrogenase purified from porcine heart	Sigma	
PL-12	Human alanyl-tRNA synthetase, recombinant, His-tagged	Diarect	
PL-7	Human threonyl-tRNA synthetase, recombinant, His-tagged	Diarect	
PM/Scl-100	Human PM/Scl-100, recombinant, His-tagged	Diarect	
PM/Scl-100 (duplicate)	Human PM/Scl-100, recombinant, His-tagged	Diarect	
PR3-Aro	Proteinase 3 antigen (cANCA antigen) from human neutrophils	Arodia Arotech Diagnostics	
PR3-IV	Proteinase 3 antigen (cANCA antigen) from human promyelocytic cell line	Immunovision	
Ribo P	Human ribosomal phosphoprotein P0, recombinant, His-tagged	Diarect	
Scl-70 truncated	Human DNA topoisomerase I (sclerodoma antigen 70), recombinant, His-tagged	Diarect	
SMA-3000	Smith antigen from bovine thymus and/or spleen	Immunovision	
SRC-3000	Smith antigen/ribonucleoprotein complex from rabbit and calf thymus	Immunovision	
ssDNA	Single-stranded DNA from calf thymus	Sigma	
Thyroglobulin	Human thyroglobulin, native	Diarect	
TPO	Human thyroid peroxidase, recombinant, His-tagged	Diarect	
TPO (duplicate)	Human thyroid peroxidase, recombinant, His-tagged	Diarect	
U1-snRNP-A	Human small nuclear ribonucleoprotein complex A, recombinant, His-tagged	Diarect	
U1-snRNP-A (duplicate)	Human small nuclear ribonucleoprotein complex A, recombinant, His-tagged	Diarect	
U1-snRNP-C	Human small nuclear ribonucleoprotein complex C, recombinant, His-tagged	Diarect	
U-snRNP-B/B′	Human small nuclear ribonucleoprotein complex B/B′, recombinant, His-tagged	Diarect	

Supplemental References

1. Bahr SL. Peptide markers for the HIV-1 neutralizing antibody 4E10 [M.Sc. thesis]. Burnaby: Simon Fraser University; 2004.
2. Connor RI, Chen BK, Choe S, Landau NR. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology* 1995,**206**:935-944.
3. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR*, et al.* Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 2004,**113**:1296-1306.
4. Maggio-Price L, Shows D, Waggie K, Burich A, Zeng W, Escobar S*, et al.* Helicobacter bilis infection accelerates and H. hepaticus infection delays the development of colitis in multiple drug resistance-deficient (mdr1a-/-) mice. *Am J Pathol* 2002,**160**:739-751.
5. Menendez A, Chow KC, Pan OC, Scott JK. Human immunodeficiency virus type 1-neutralizing monoclonal antibody 2F5 is multispecific for sequences flanking the DKW core epitope. *J Mol Biol* 2004,**338**:311-327.
6. Montero M. Antigenicity and immunogenicity of the membrane proximal external region of the HIV-1 envelope protein gp41 [Ph.D. thesis]. Burnaby: Simon Fraser University; 2007.
7. van Houten NE, Henry KA, Smith GP, Scott JK. Engineering filamentous phage carriers to improve focusing of antibody responses against peptides. *Vaccine* 2010,**28**:2174-2185.

8. Zwick MB, Kelleher R, Jensen R, Labrijn AF, Wang M, Quinnan GV, Jr.*, et al.* A novel human antibody against human immunodeficiency virus type 1 gp120 is V1, V2, and V3 loop dependent and helps delimit the epitope of the broadly neutralizing antibody immunoglobulin G1 b12. *Journal of virology* 2003,**77**:6965-6978.