

Supplemental material

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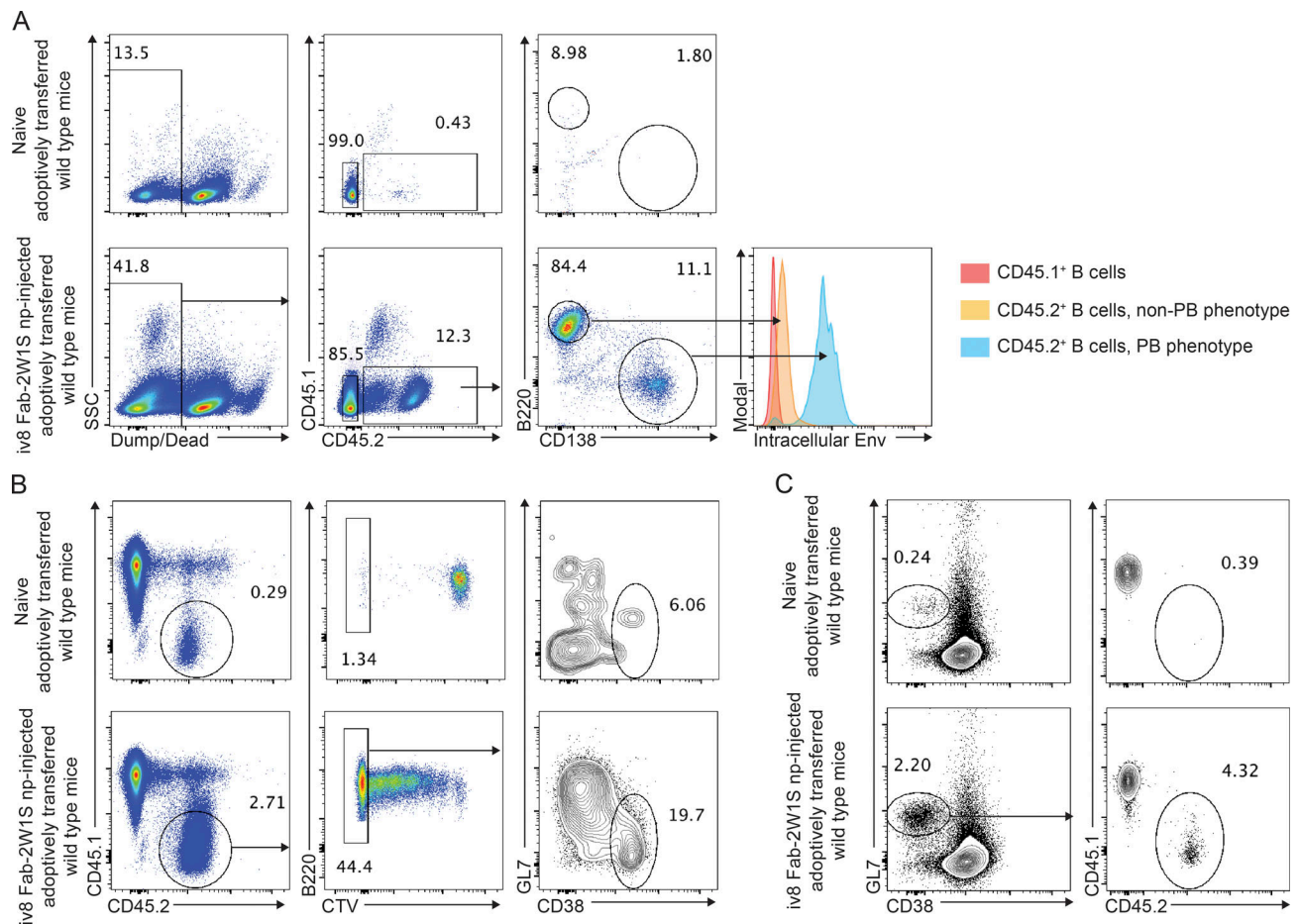


Figure S1. **Flow cytometric gating strategies for enumeration of B cells after injection of iv8 Fab-2W1S and N276D-2W1S nanoparticles. (A)** Gating strategy for enumeration of 3BNC60^{SI} plasmablasts (PB; CD45.2⁺, CD138⁺, B220⁻, intracellular Env^{high}) in adoptively transferred wild-type mice 5 d after injection of iv8 Fab-2W1S and N276D-2W1S nanoparticles. Cells were pregated on live lymphocytes and singlets. SSC, side scatter. **(B)** Gating strategy for enumeration of 3BNC60^{SI} early memory B cells (mB; CD45.2⁺, CTV⁻, CD38⁺, GL7⁻) in adoptively transferred wild-type mice 5 d after injection of iv8 Fab-2W1S and N276D-2W1S nanoparticles. Cells were pregated on lymphocytes, singlets, and live B220⁺ B cells. **(C)** Gating strategy for enumeration of 3BNC60^{SI} GC cells (CD45.2⁺, CD95⁺, CD38⁻) in adoptively transferred wild-type mice. Cells were pregated on lymphocytes, singlets, and live B220⁺ B cells.

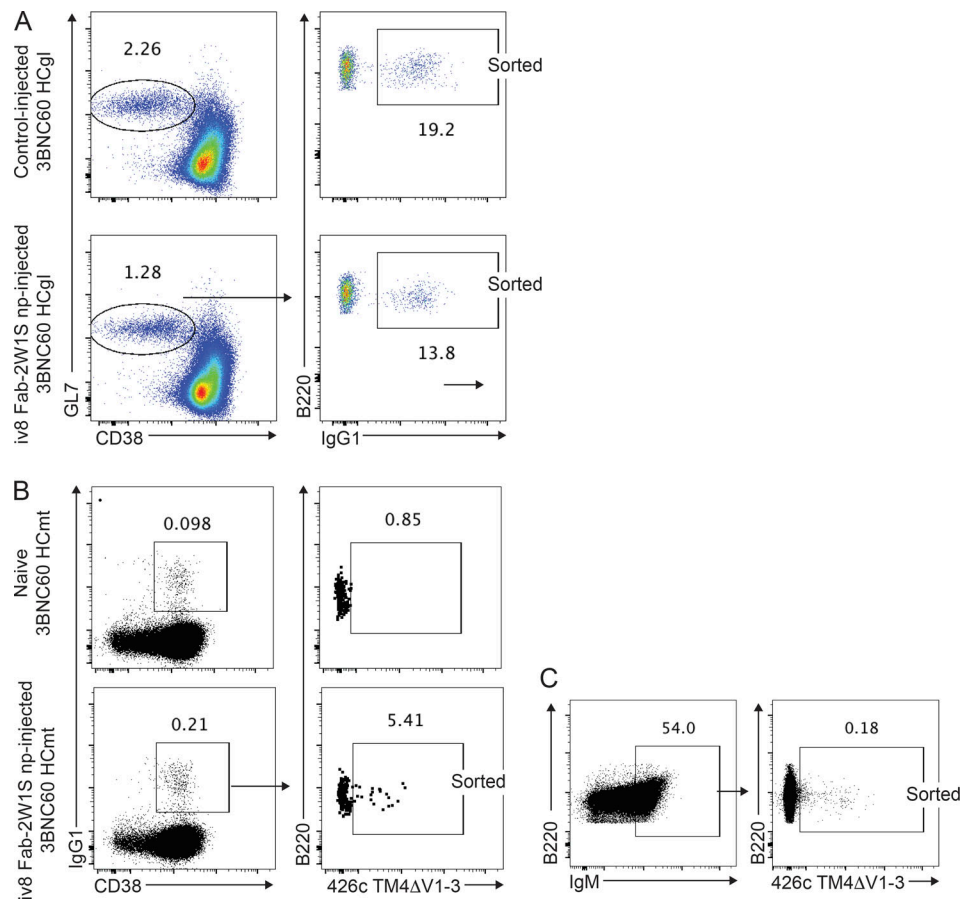


Figure S2. **Flow cytometry gating strategy for single-cell sorting of heavy-chain knock-in mice. (A)** Flow cytometry gating strategy for single-cell sorting of IgG1⁺ GC B cells 14 d after injection of 3BNC60 HCgl mice with control iv1 Fab-2W1S nanoparticles (top panels) or iv8 Fab-2W1S nanoparticles (bottom panels). Cells were pre-gated on lymphocytes, singlets, or live B220⁺ B cells. **(B)** Flow cytometry gating strategy for the identification of Env-specific (426c TM4ΔV1-3) memory B cells in naive (top panels) or iv8 Fab-2W1S nanoparticle-injected 3BNC60 HCmt mice (bottom panels) 42 d after injection. Cells were pre-gated on lymphocytes, singlets, live B cells, or IgM⁻. **(C)** Flow cytometry gating for the isolation of single naive B cells from 3BNC60 HCmt mice binding to HIV-1 Env (426c TM4ΔV1-3). Cells were pre-gated on lymphocytes, singlets, or live B cells.

Table S1. **Data collection and refinement statistics for iv8scFv + gIVRC01scFv**

Data collection	
Space group	P1
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	51.583, 107.937, 116.961
α , β , γ (°)	85.489, 80.276, 84.172
Resolution (Å)	50–2.419 (2.506–2.42) ^a
<i>R</i> _{sym} or <i>R</i> _{merge}	0.059 (0.169) ^a
<i>I</i> / <i>s</i>	10.2 (2.8) ^a
Completeness (%)	92.0 (50.2) ^a
Redundancy	1.7 (1.4) ^a
CC1/2	(0.952) ^a
Refinement	
Resolution (Å)	49.78–2.419 (2.506–2.419) ^a
No. reflections	87,138 (5,909)
<i>R</i> _{work} / <i>R</i> _{free} (%)	17.43/22.40 (21.65/27.10) ^a
No. atoms	
Protein	14,355
Water	1,152
Ligand	184
B-factors (Å ²)	
Protein	33.35
Water	36.56
Ligand	53.41
Root mean square deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.96
Ramachadran favored %	96.98
Ramachadran outliers %	0.00
MolProbity all-atoms clashscore	3.25
Protein Data Bank ID	6OL7

$R_{\text{sym}} = R_{\text{merge}} = [\sum_h \sum_i |I_h - I_{hi}| / \sum_h \sum_i I_{hi}]$ where I_h is the mean of I_{hi} observations of reflections h . $R_{\text{work}} = \sum ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum |F_{\text{obs}}|$ for 95% of recorded data. $R_{\text{free}} = \sum ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum |F_{\text{obs}}|$ for 5% of recorded data. *I*/*s*, signal to noise ratio.

^aStatistics for the highest resolution shell are shown in parentheses.

Table S2. Detailed interactions between gIVRC01 and iv8

a. Detailed interactions of gIVRC01 light chain with iv8 heavy chain

gIVRC01 Light Chain				iv8 Heavy Chain				Hydrogen Bonds		
	HSDC	ASA	BSA		HSDC	ASA	BSA		Dist. [Å]	
L:SER 28	H	89.59	23.35	A:ALA 33		24.26	2.51	L:ASP 50[O]	3.17	A:ASN 52[ND2]
L:VAL 29		64.20	0.49	A:ASN 52	H	43.98	36.57	L:SER 52[OG]	2.88	A:ASN 52[ND2]
L:SER 30		96.21	31.64	A:ASN 52A	H	56.14	32.65	L:ASN 53[OD1]	2.86	A:ASN 52A[ND2]
L:SER 31		30.91	12.67	A:ASN 53	H	120.69	37.55	L:SER 52[O]	2.69	A:ASN 53[ND2]
L:TYR 32	H	165.01	68.50	A:GLY 55	H	72.55	54.07	L:GLY 68[O]	2.87	A:TYR 56[OH]
L:TYR 49		102.29	0.78	A:TYR 56	H	120.29	71.62	L:GLY 66[O]	3.39	A:THR 57[N]
L:ASP 50	H	60.26	34.24	A:THR 57	H	53.73	28.53	L:SER 28[OG]	2.99	A:HIS 58[NE2]
L:ALA 51		18.98	11.80	A:HIS 58		90.31	47.65	L:ASN 53[ND2]	3.13	A:ASN 52A[OD1]
L:SER 52	H	66.81	51.92	A:TYR 59		23.23	1.61	L:GLY 66[N]	2.79	A:GLY 55[O]
L:ASN 53	H	55.62	43.51	A:TYR 97	H	154.18	24.79	L:GLY 68[N]	2.99	A:THR 57[O]
L:GLY 64		8.20	0.61	A:TRP 98		213.44	100.02	L:TYR 32[OH]	3.77	A:TYR 97[O]
L:SER 65		62.92	11.05							
L:GLY 66	H	43.27	34.64							
L:SER 67		84.67	17.84							
L:GLY 68	H	65.72	57.00							
L:THR 69		40.69	0.84							
L:PHE 71		20.20	10.01							
L:TYR 91		130.01	19.46							

b. Detailed interactions of gIVRC01 light chain with iv8 light chain

gIVRC01 Light Chain				iv8 Light Chain				Hydrogen Bonds		
	HSDC	ASA	BSA		HSDC	ASA	BSA		Dist. [Å]	
L:ILE 2		10.48	4.53	Q:GLN 27		87.15	5.15	L:SER 30[N]	2.85	Q:TYR 92[O]
L:GLN 27		101.49	28.48	Q:TYR 27D	H	94.17	77.59	L:SER 30[OG]	2.76	Q:PHE 91[O]
L:SER 28	H	89.59	56.79	Q:SER 27E		97.78	3.27	L:TYR 91[OH]	2.65	Q:TYR 32[OH]
L:VAL 29		64.20	57.48	Q:GLY 27F		58.07	6.89	L:GLU 96[N]	3.32	Q:TYR 27D[OH]
L:SER 30	H	96.21	64.57	Q:ASN 28		60.44	8.80	L:SER 28[O]	3.13	Q:TYR 94[N]
L:SER 31		30.91	0.58	Q:TYR 32	H	34.96	19.92	L:SER 30[OG]	2.73	Q:TRP 96[NE1]
L:TYR 91	H	130.01	58.16	Q:PHE 91	H	45.37	7.31	L:GLU 96[OE2]	2.72	Q:TYR 27D[OH]
L:GLU 96	H	135.97	33.54	Q:TYR 92	H	76.06	58.04			
L:PHE 97		44.92	12.23	Q:PHE 93		60.83	37.01			
				Q:TYR 94	H	169.30	68.44			
				Q:TRP 96	H	122.56	7.44			

c. Detailed interactions of gIVRC01 heavy chain with iv8 light chain

gIVRC01 Heavy Chain				iv8 Light Chain				Hydrogen Bonds		
	HSDC	ASA	BSA		HSDC	ASA	BSA		Dist. [Å]	
H:TRP 47		64.81	4.53	Q:TYR 27D		94.17	16.58	H:ASN 58[ND2]	2.70	Q:SER 27E[O]
H:TRP 50	H	51.04	30.75	Q:SER 27E	H	97.78	11.85	H:ASN 58[ND2]	3.02	Q:GLY 27F[O]
H:THR 57		55.30	7.13	Q:GLY 27F	H	58.07	51.18	H:TRP 50[NE1]	2.75	Q:GLY 27F[O]
H:ASN 58	H	67.19	47.27	Q:ASN 28		60.44	23.07			
H:TRP 100B		114.31	47.15	Q:GLN 29		147.98	46.23			

d. Detailed interactions of gIVRC01 heavy chain with iv8 heavy chain

gIVRC01 Heavy Chain				iv8 Heavy Chain				Hydrogen Bonds		
	HSDC	ASA	BSA		HSDC	ASA	BSA		Dist. [Å]	
H:ASP 99	H	130.70	31.05	A:TYR 97	H	154.18	52.44	H:ASP 99[O]	2.49	A:TYR 97[OH]
H:TYR 100		123.36	33.83	A:TRP 98		213.44	20.27			

HSDC, hydrogen/disulphide bond, salt bridge or covalent link; ASA, accessible surface area, Å²; BSA, buried surface area, Å²; ||||, buried area percentage, one bar per 10%.

Table S3. Kinetics of iv8-binding to 3BNC60^{S1} determined by BLI

K_D (M)	k_{on} ($M^{-1} s^{-1}$)	k_{on} error	k_{off} (s^{-1})	k_{off} error
2.11×10^{-9}	1.26×10^5	9.10×10^2	2.66×10^{-4}	2.02×10^{-6}

K_D , dissociation constant; k_{on} , association rate; k_{off} , dissociation rate.

Tables S4–S7 are provided online as separate Excel files. Table S4 list IgL sequences from two single cell-sorted control-injected 3BNC60 HCgl knock-in mice. Table S5 lists IgL sequences from two single cell-sorted iv8 Fab-2W1S-injected 3BNC60 HCgl mice. Table S6 lists IgL sequences from one single cell-sorted naive 3BNC60 HCmt mouse. Table S7 lists IgL sequences from one single cell-sorted iv8 Fab-2W1S-injected 3BNC60 HCmt mouse.