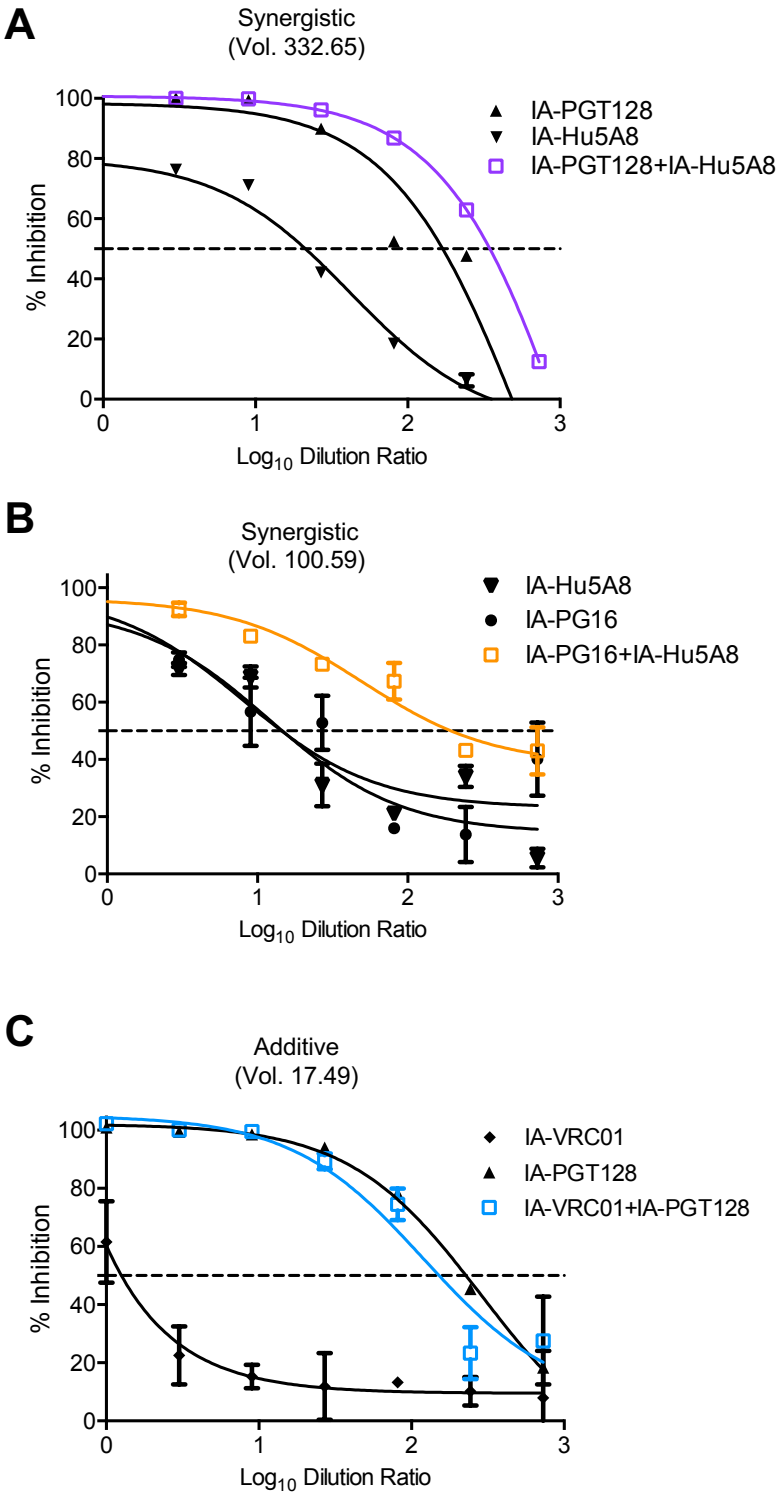
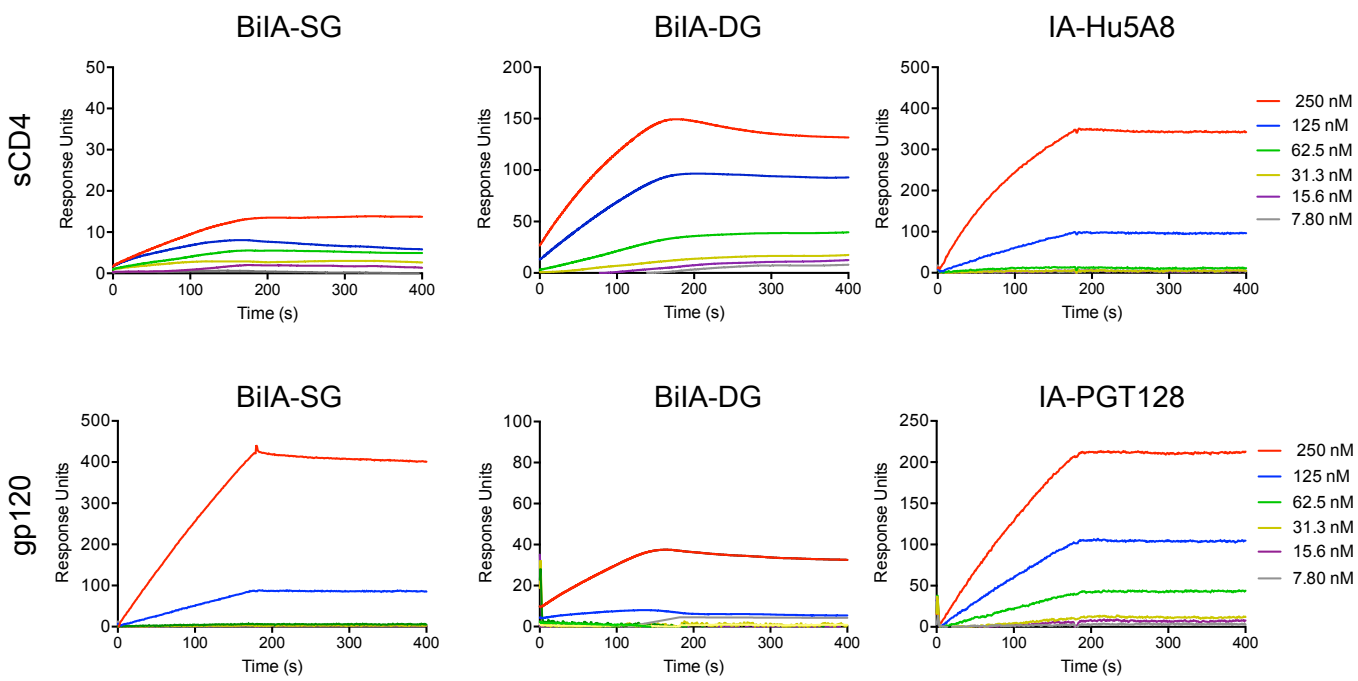


Supplemental Figure 1. Graphic plots of synergistic (A and B) and additive (C) effects in paired IAs checkerboard synergy assay. Data represent mean ± SEM of experiment with duplicates. All experiments were repeated twice.



Supplemental Figure 2. Surface plasmon resonance analysis of BiIA-SG binding to gp120 and soluble CD4 compared with IA-PGT128 and IA-Hu5A8. Soluble CD4 (top) or HIV-1 gp120 (bottom) was immobilized on CM5 chip. BiIA-SG, BiIA-DG, IA-PGT128 and IA-Hu5A8 were flowed over the chip as analyte at 2-fold serial increasing concentrations ranging from 7.8 to 250nM, respectively. The 3 min association and 8 min dissociation phases were measured at a flow rate of 30 µl/min. Each analyte was tested in duplicates. The Ka, Kd and KD values were generated automatically by the Biacore X100 machine.

Virus	subtype	infection stage	tropism	Risk factor	IC_{50}					IC_{90}				
					BiIA-SG	BiIA-DG	PGT128	Hu5A8	VRC01	BiIA-SG	BiIA-DG	PGT128	Hu5A8	VRC01
320008	**01AE**	**Acute T/F**	**R5**	**MSM**	0.003	0.013	0.032	0.178	/	0.030	0.178	0.465	/	/
320040	**01AE**	**Acute T/F**	**R5**	**MSM**	0.032	0.039	0.099	0.312	0.267	0.098	0.479	1.555	/	1.027
CNE3	01AE	Chronic	R5	IDU	0.280	0.030	3.360	0.010	0.260	1.087	0.520	/	0.140	/
CNE55	01AE	Chronic	R5	IDU	0.104	2.166	/	0.023	0.197	1.080	/	/	0.622	/
CNE107	01AE	Chronic	X4	IDU	0.431	/	/	0.026	0.999	/	/	/	0.215	/
CNE8	01AE	Chronic	R5	IDU	0.039	0.137	0.058	0.054	1.366	0.139	0.699	0.295	0.232	/
CNE26	01AE	Chronic	R5	IDU	0.025	0.056	0.021	0.041	/	0.098	0.250	0.123	0.293	/
CH106	**B**	**Acute T/F**	**R5**	**MSMW**	0.343	/	/	0.123	/	/	/	/	1.700	/
62357_14	B	Fiebig II T/F	R5	SPD	0.190	0.230	/	0.070	0.470	0.760	0.920	/	1.780	/
B3(PVO.4)	B	Fiebig III T/F	R5	MSM	0.020	0.100	0.050	0.090	1.000	0.140	0.500	0.220	0.760	/
B4(TRO.11)	B	Fiebig III T/F	R5	MSM	0.170	3.000	2.270	0.090	0.400	1.150	/	/	1.160	2.000
SC422661.8	B	Fiebig IV T/F	R5	MSMW	0.150	3.000	2.000	0.020	0.370	1.040	/	/	0.170	1.950
SF162	B	Chronic	R5	MSM	0.010	0.020	0.010	0.035	0.110	0.130	0.160	0.056	/	1.288
JRCSF	B	Chronic	R5	MSM	0.020	0.120	0.027	0.015	0.129	0.060	0.510	0.094	0.201	1.456
JRFL	B	Chronic	R5	MSM	0.040	0.070	0.060	0.040	0.008	0.140	0.340	0.240	/	0.129
CNE1	B'	Chronic	X4	IDU	0.126	0.310	/	0.020	0.030	1.527	/	/	0.140	0.060
CNE4	B'	Chronic	R5	IDU	0.546	/	/	/	2.062	/	/	/	/	/
CNE6	B'	Chronic	R5	IDU	0.011	0.035	0.050	0.020	/	0.031	0.190	0.160	0.290	/
CNE9	B'	Chronic	R5	HSX	0.050	0.799	0.061	0.142	/	0.374	/	0.346	/	/
CNE14	B'	Chronic	R5	HSX	0.017	0.100	0.130	0.030	0.650	0.099	0.380	1.000	0.340	2.790
CNE57	B'	Chronic	X4	MSM	0.013	0.050	0.080	0.040	0.700	0.067	0.720	0.380	0.110	/
CNE64	B'	Chronic	R5	Blood	0.020	0.060	0.060	0.100	/	0.060	0.130	0.600	0.710	/
CNE12	B'	Chronic	R5	HSX	0.005	0.010	0.052	0.037	2.600	0.031	0.010	0.184	0.131	/
CNE15	B'C	Chronic	R5	IDU	0.025	0.070	0.078	0.040	0.380	0.068	0.244	0.353	0.161	2.644
CNE7	B'C	Chronic	R5	IDU	0.030	0.180	0.260	0.050	0.320	0.410	1.080	3.470	0.270	/
CNE16	B'C	Chronic	R5	IDU	0.179	/	/	0.450	0.420	1.024	/	/	/	2.820
CNE18	B'C	Chronic	R5	IDU	0.030	0.011	0.011	0.067	0.300	0.210	0.045	0.092	0.618	/
CNE20	07BC	Chronic	R5	HSX	0.010	0.049	0.010	0.025	/	0.060	0.607	0.035	0.206	/
CNE40	07BC	Chronic	R5	IDU	0.480	/	/	/	0.370	/	/	/	/	3.190
CNE68	07BC	Chronic	R5	HSX	0.990	3.090	/	0.300	0.440	/	/	/	/	/
CNE46	08BC	Chronic	R5	IDU	0.150	0.330	0.599	0.330	1.230	1.820	1.520	/	/	/
CNE47	08BC	Chronic	R5	IDU	0.380	1.570	/	0.130	/	1.960	/	/	/	/
CNE49	08BC	Chronic	X4	IDU	1.030	/	/	0.420	0.490	/	/	/	/	2.360
CH198	**C**	**Acute T/F**	**R5**	**HSX**	0.016	0.062	0.057	0.018	0.205	0.088	0.481	0.543	/	1.001
CNE2	C	Chronic	R5	IDU	0.010	0.010	0.010	0.010	0.700	0.070	0.160	0.060	0.080	2.620
CNE17	C	Chronic	R5	IDU	0.030	0.340	0.480	1.400	0.930	0.800	2.790	/	/	/
CNE23	C	Chronic	R5	IDU	0.100	0.440	0.770	0.120	1.890	1.000	/	/	0.400	/
CNE30	C	Chronic	R5	IDU	0.200	0.835	1.070	0.150	1.280	2.200	/	/	1.520	/
CNE31	C	Chronic	R5	IDU	0.760	0.790	/	0.011	1.150	/	/	/	0.129	/
CNE53	C	Chronic	R5	IDU	0.231	0.140	0.082	0.096	0.150	0.541	0.810	0.231	/	1.230
MuLV					/	/	/	/	/	/	/	/	/	/

(μg/ml)
<0.01
0.01-0.1
0.1 - 1
1 - 3.5
/ >3.5

Supplementary Figure 3. Neutralization activity of BiIA-SG, BiIA-DG, parental antibodies and VRC01 against the HKU panel of 40 pseudoviruses with various HIV-1 subtypes. Data represent mean IC_{50} and IC_{90} values tested in duplicates. HIV-1 resistance to:IA-PGT128 (light grey), IA-Hu5A8 (dark grey) and both (light green). All experiments were repeated twice.

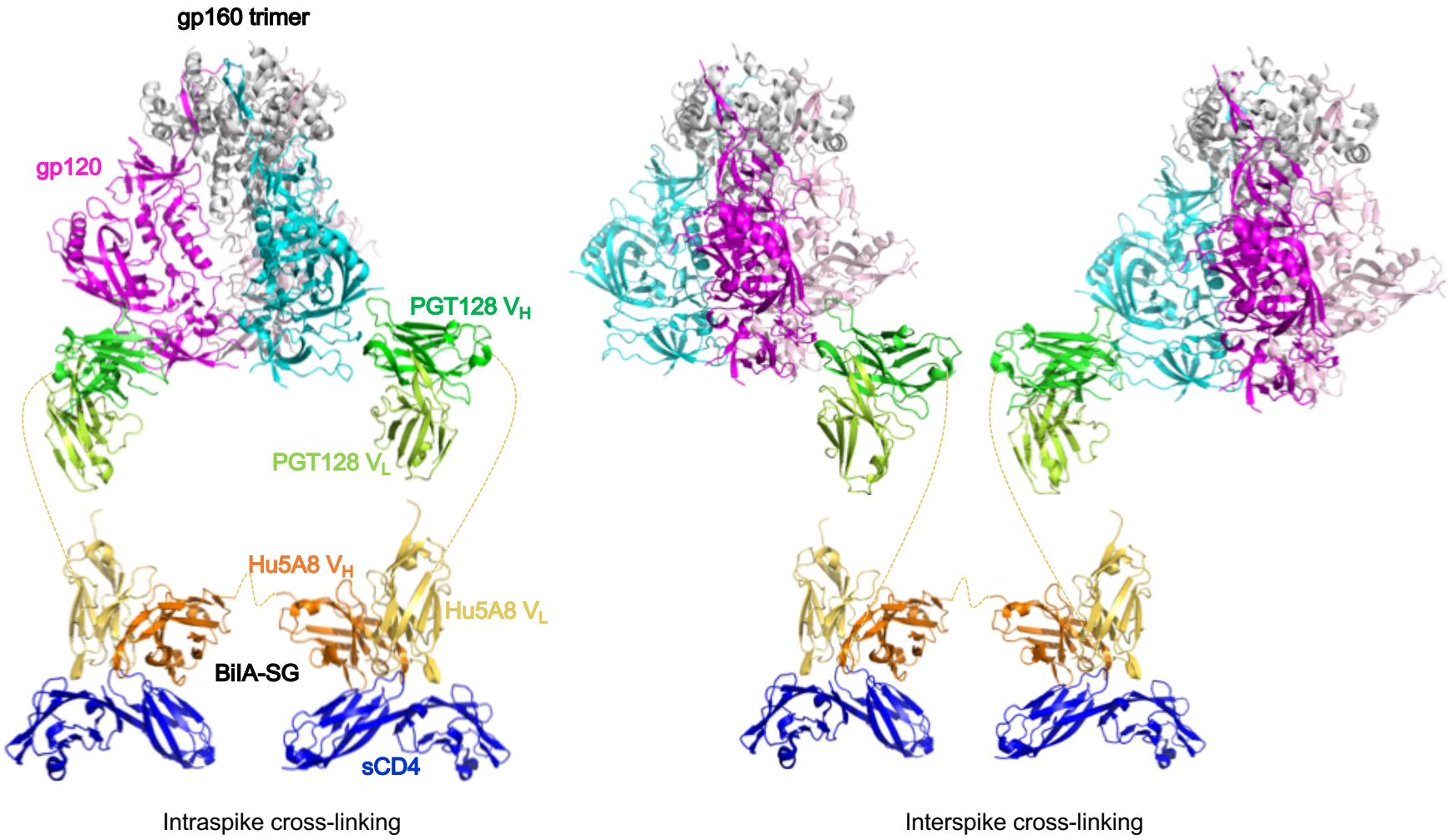
Virus			IC_{50}		IC_{90}	
Subtype	Name	Stage	V3-CD4 BilA-SG	CD4bs VRC01	V3-CD4 BilA-SG	CD4bs VRC01
A	398F1	*Unk	0.0053	0.1841	0.0064	2.669
AC	246F3	Fiebig VI	0.0022	0.1253	0.0075	1.5178
CRF01_AE	CNE8	Chronic	0.007	1.09	0.068	/
	CNE55	Chronic	0.0385	0.2765	0.2371	2.983
	CNE3	Chronic	0.0487	2.9175	0.1166	/
	CNE5	Chronic	0.0208	0.1942	0.0774	/
	CNE59	Chronic	0.1968	0.7721	0.2316	/
	CNE107	Chronic	0.1413	1.245	0.3104	/
	MG03(BJOX5)	Fiebig I/II	0.0395	2.0465	0.2078	/
	BJOX09	Fiebig IV	0.0001	1.4855	0.0006	/
	MG07(BJOX10)	Fiebig I/II	0.0008	/	0.0059	/
	BJOX15	Fiebig I/II	0.0441	0.5041	0.0832	/
	BJOX17	Fiebig VI	0.0002	/	0.0014	/
	MG10(BJOX18)	Fiebig IV	0.0212	/	0.1049	/
	MG11(BJOX21)	Fiebig VI	<0.0001	/	<0.0001	/
	BJOX23	Fiebig IV	0.0006	1.1605	0.0036	/
	MG15.33(BJOX25.01)	Fiebig I/II	0.0656	1.983	0.2955	/
	MG15.36(BJOX25.05)	Fiebig I/II	0.0253	2.8457	0.2092	/
	MG18(BJOX28.10)	Fiebig I/II	0.0005	0.1195	0.0041	1.0648
	BJOX28.34	Fiebig I/II	0.0565	0.1606	0.0993	1.394
	BJOX28.43	Fiebig I/II	0.0162	0.494	0.0278	/
	BJOX31	Fiebig V	0.0606	0.9298	0.073	/
	P08	Fiebig IV	0.0422	1.736	0.1018	/
	P11	Fiebig IV	0.055	3.131	0.1443	/
B	TRO11	Fiebig III	0.0088	0.4943	0.0435	/
	X2278	Fiebig V/VI	0.0059	0.1691	0.0295	0.4082
	MG01(BJOX3)	Fiebig I/II	0.0466	0.1124	0.125	0.9256
	MG02(BJOX4)	Fiebig VI	0.0045	3.08	0.0966	/
	MG08(BJOX6)	Fiebig IV	0.0607	0.689	0.1241	/
	MG04(BJOX7)	Fiebig V	<0.0001	0.1907	<0.0001	1.8137
	BJOX14	Fiebig V	<0.0001	0.7657	<0.0001	/
	MG09(BJOX20)	Fiebig I/II	0.0486	2.0815	0.1079	/
	MG13(BJOX22)	Fiebig IV	0.1963	0.3645	0.2479	3.037
	MG23(BJOX41)	Fiebig V	0.0104	/	0.0392	/
	BJOX46	Fiebig V	<0.0001	0.547	0.0004	/
	JR-FL	Chronic	0.0426	0.0145	0.2835	0.1951
	SF162	Chronic	0.1728	0.0848	0.1919	1.1685

Virus			IC_{50}		IC_{90}	
Subtype	Name	Stage	V3-CD4 BilA-SG	CD4bs VRC01	V3-CD4 BilA-SG	CD4bs VRC01
B'	CNE1	Chronic	0.0316	0.0101	0.1674	0.0649
	CNE4	Chronic	0.0464	1.289	0.4121	/
	CNE6	Chronic	0.0435	/	0.0933	/
	CNE9	Chronic	<0.0001	/	0.0009	/
	CNE10	Chronic	0.0413	1.0622	0.0722	/
	CNE11	Chronic	0.052	0.5238	0.0722	/
	CNE14	Chronic	0.0521	0.3581	0.3916	/
	CNE57	Chronic	0.0485	0.9678	0.2543	/
CRF07_BC	CNE19	Chronic	0.0915	0.1862	0.45	/
	CNE20	Chronic	0.0219	1.206	0.0306	/
	CNE21	Chronic	0.3707	0.2493	0.3706	/
	CNE40	Chronic	0.5708	0.5761	0.6525	/
	CNE68	Chronic	0.1888	0.6502	0.2233	/
	CNE23	Chronic	0.0314	/	0.1217	/
	CNE30	Chronic	<0.0001	1.1075	0.0004	/
	CNE46	Chronic	0.0511	0.8708	0.2963	/
	CNE47	Chronic	<0.0001	/	<0.0001	/
	CNE49	Chronic	0.0538	0.1397	0.8491	/
	CNE53	Chronic	0.0183	0.0174	0.0327	1.9306
	BJOX11	Fiebig V	0.003	0.365	0.0155	/
	BJOX16	Fiebig VI	0.0661	0.2937	0.0752	2.695
	BJOX19	Fiebig I/II	0.003	1.496	0.0127	/
	M14(BJOX27)	Fiebig V	<0.0001	0.1738	<0.0001	1.3745
	MG17(BJOX29)	Fiebig V	0.0015	0.4456	0.0059	/
	BJOX37	Fiebig V	<0.0001	2.2275	0.0002	/
	CH119	Chronic	0.0115	0.2741	0.0919	/
	BJOX2000	Fiebig I/II	0.0172	/	0.1034	/
C	CNE2	Chronic	0.022	0.2513	0.0313	3.4345
	CNE17	Chronic	0.0412	1.2144	0.2978	/
	CNE58	Chronic	0.1218	0.0262	0.7748	1.8234
	CNE65	Chronic	<0.0001	1.8585	0.0001	/
	CE0217	Fiebig V/VI	0.0177	0.1058	0.0721	0.2857
	CE1176	Fiebig I/II	0.0095	1.248	0.0299	/
	25710	Fiebig V	0.0102	0.5594	0.0215	0.8704
G	X1632	Chronic	0.0835	0.1063	0.6242	0.2197

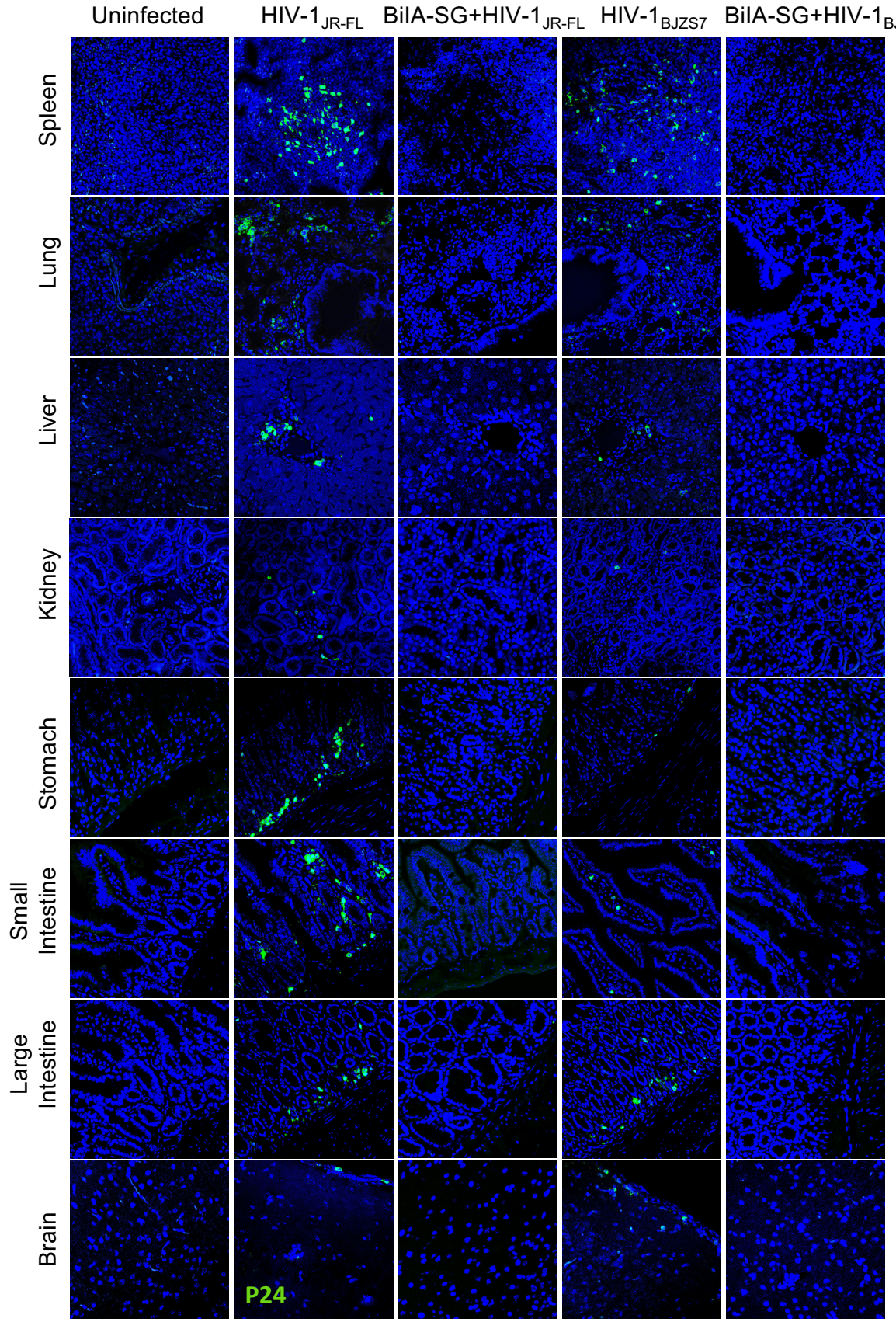
*Unk: Unknown

(μg/ml)
<0.01
0.01-0.1
0.1 - 1
1 - 3.5
/ >3.5

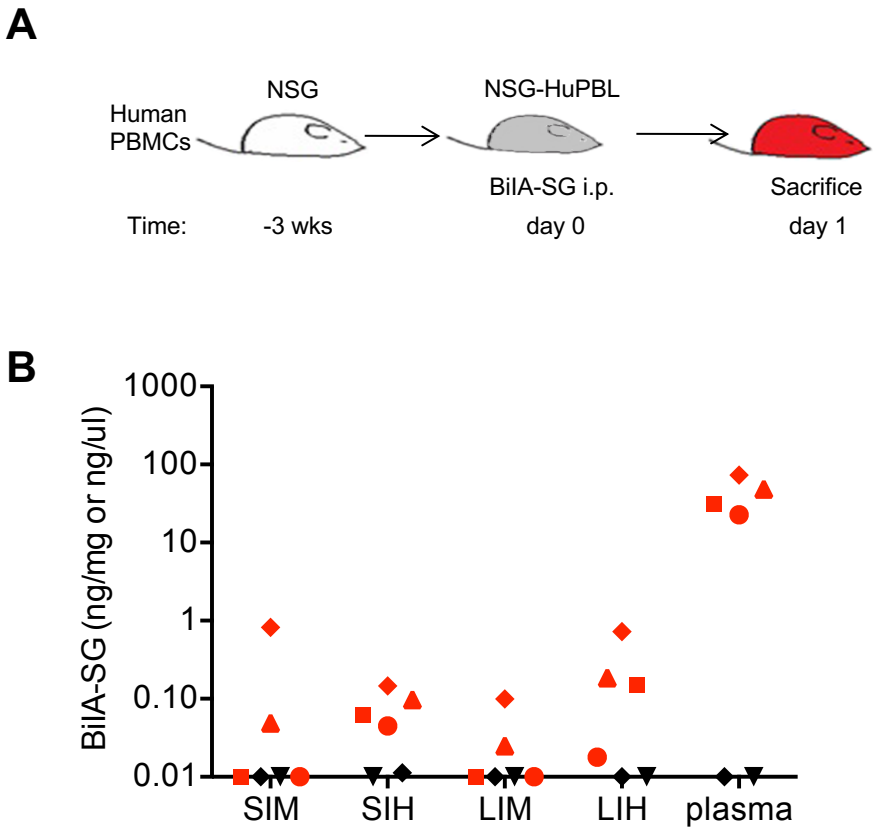
Supplementary Figure 4. Neutralization activity of BiIA-SG and VRC01 against the Tsinghua panel of 72 pseudoviruses of various HIV-1 subtypes. Data represent mean IC_{50} and IC_{90} values tested in duplicates. All experiments were repeated twice.



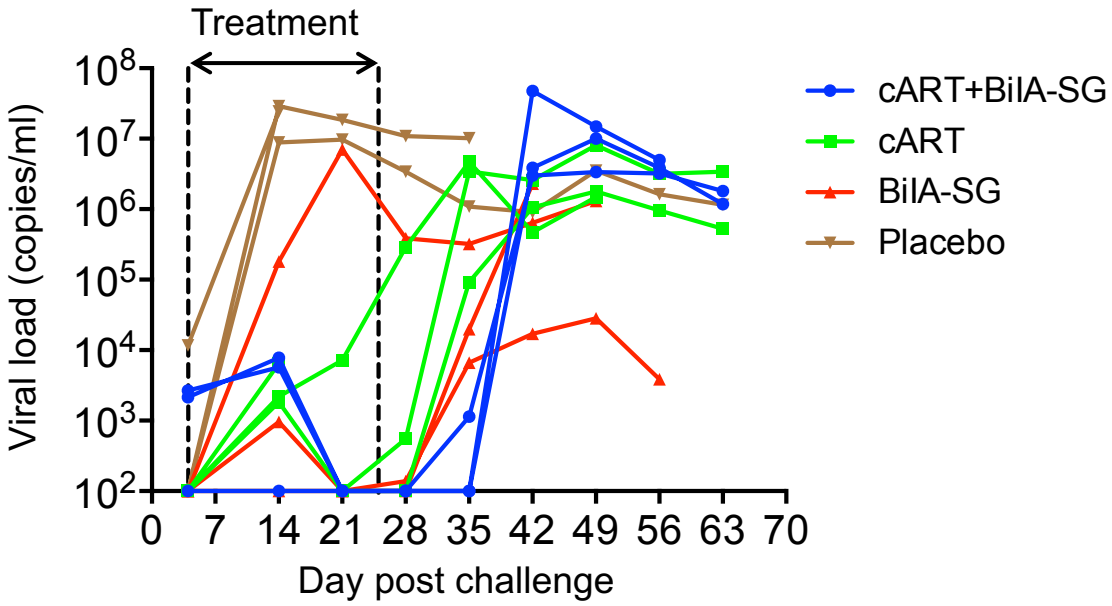
Supplementary Figure 5. Proposed structures of BiIA-SG in complex with gp120 domains and soluble CD4. Left, intraspike cross-linking interaction. Right, interspike cross-linking interaction. These models are predicted based on the crystal structures of PGT128 and HIV-1 gp160 trimer (PDB 5ACO, cyan, fuchsia, and pale red for gp120 monomer, grey for gp41 trimer, dark and light green for PGT128 V_H and V_L chain,), and of Hu5A8 and soluble CD4 (PDB 3O2D, orange and yellow for Hu5A8 V_H and V_L chain, blue for sCD4) via 20mer linkers (dash lines).



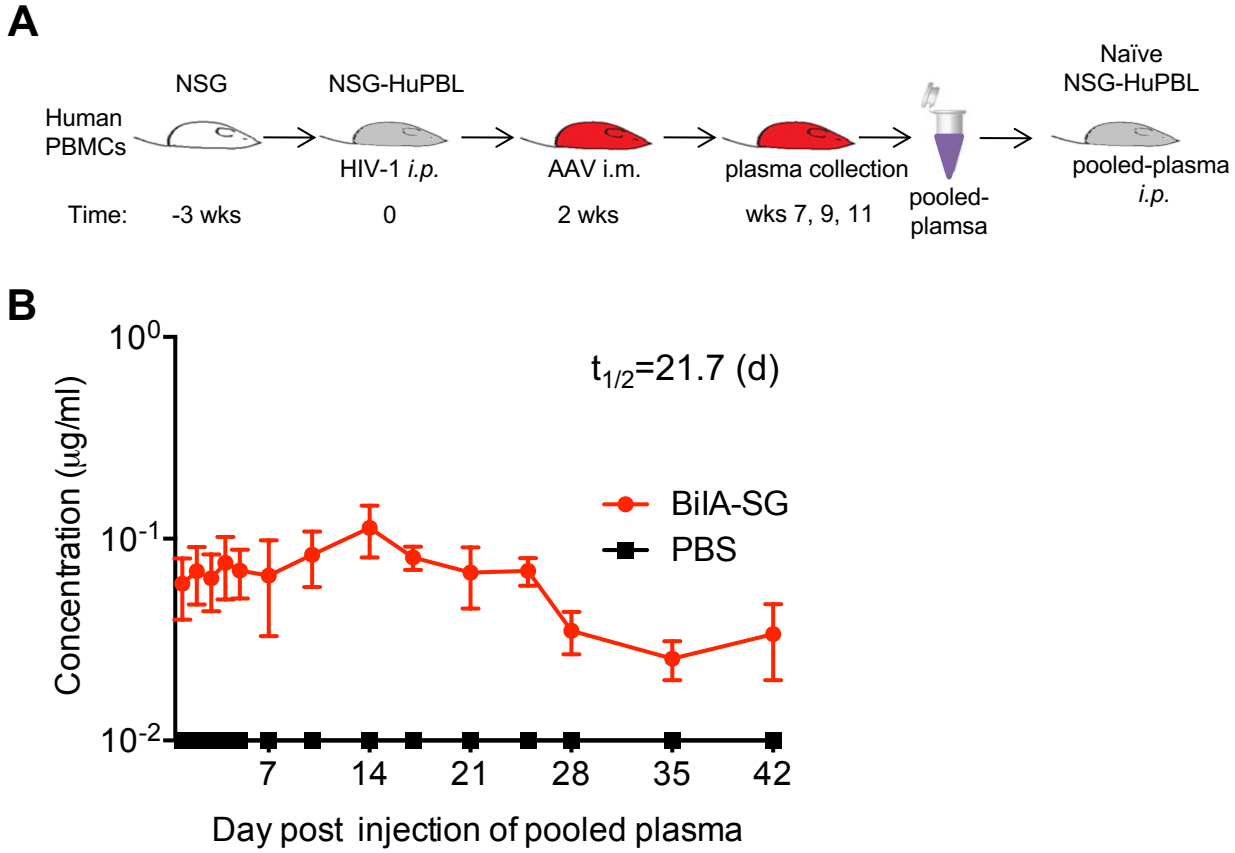
Supplemental Figure 6. BiIA-SG confers complete protection against systemic infection of two diverse live HIV-1 strains in NSG-HuPBL mice. Tissue sections were extensively analyzed using antibodies specific for HIV-1 P24 protein among the same five groups of mice as described in Figure 5. Productively infected HIV-1 P24^{+} T cells (green) were not detected in any animals that received 10 mg/kg BiIA-SG by IFA at day 14 *p.i.*. Representative tissue sections were derived from the spleens, lungs, livers, kidneys, stomachs, small intestines, large intestines and brains of the NSG-HuPBL mice tested. Tissue sections were observed at 200X magnification.



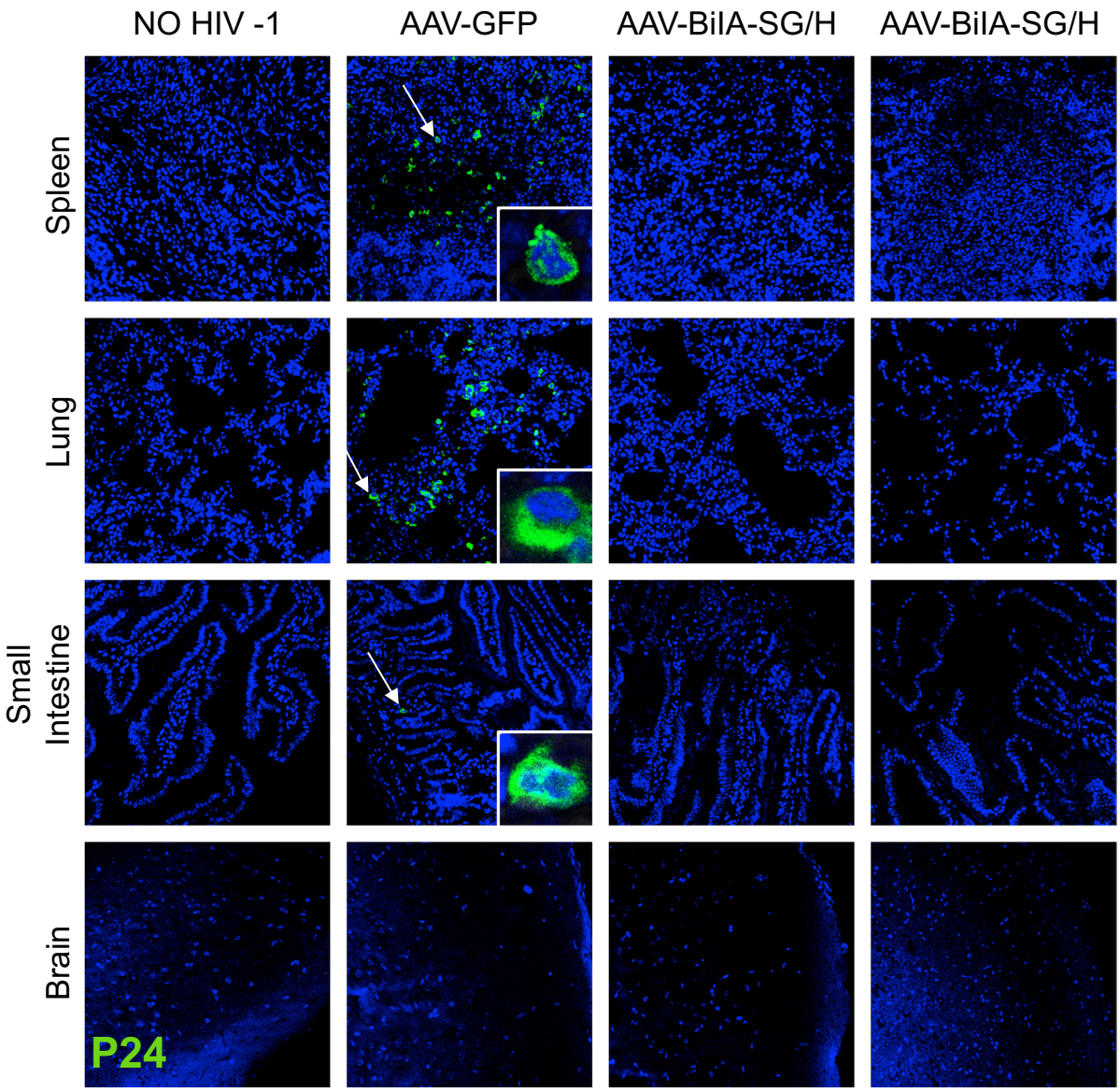
Supplemental Figure 7. BiIA-SG penetrance in mucosal sites of NSG-HuPBL mice. (**A**) Experiment schedule. 10mg/kg BiIA-SG was injected 24 hours before sacrifice. (**B**) BiIA-SG levels were detected by ELISA in small intestine mucus (SIM, ng/µl), small intestine homogenate (SIH, ng/mg), large intestine mucus (LIM, ng/µl), large intestine homogenate (LIH, ng/mg) and plasma (ng/µl). BiIA-SG treated (n=4, 2M+2F) and untreated mice (n=2, 1M+1F) are color-coded in red and black, respectively. Each symbol represents one mouse.



Supplemental Figure 8. Therapeutic efficacy of BiIA-SG, cART or cART +BiIA-SG combination in NSG-HuPBL mice pre-infected with live R5-tropic HIV-1_{BJZS7}. Plasma viral loads were measured among four color-coded groups of NSG-HuPBL mice including cART+BiIA-SG combination (blue, n=3, 2M+1F), cART (green, n=3, 3M), BiIA-SG (red, n=3, 1M+2F) and Placebo (brown, n=3, 3M). Data represent mean ± SEM with samples tested in triplicates.



Supplemental Figure 9. Bioavailability and the half-life of BiIA-SG in NSG-HuPBL mice adoptively transferred with pooled plasma derived from AAV-BiIA-SG injected mice. (**A**) Experimental schedule. Briefly, a total of 2 ml pooled-plasma was collected from 11 NSG-HuPBL mice at week 7, 9 and 11 after the AAV-BiIA-SG injection. The BiIA-SG concentration was 11.75 ng/μl in the pooled plasma. 400μl-pooled plasma (~4.7μg) into each naïve NSG-HuPBL mice through *i.p.* injection. (**B**) Plasmas concentration was measured over time. Data represent mean ± SEM. n=5, 3M+2F in the BiIA-SG group.



Supplemental Figure 10. Single high dose AAV-vectored BiIA-SG monotherapy eliminates HIV-1 infected lymphocytes in NSG-HuPBL mice. Tissue sections were extensively analyzed using antibodies specific for HIV-1 P24 protein among the mice as described in Figure 6. Infected mice treated with middle (4/5) or high dose (5/8) of AAV-BiIA-SG had undetectable $P24^+$ T cells in their spleens, lungs, intestines and brains by immunofluorescence staining 11 wpi. Representative tissue sections were derived from the spleens, lungs, small intestines and brains of two AAV-BiIA-SG high dose treated mice. Tissue sections were observed at 200X magnification. The insets are enlarged images of individual cells pointed by corresponding arrows.