

**Sequence of human heavy chain constant region**

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**Sequence of human light chain constant region**

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD  
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**iMab-heavy chain**

QVQLQQSGPEVVKPGASVKMSCKASGYTFTSYVIHWVRQKPGQGLDWIGYINPYNDGT  
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**iMab-Light chain**

DIVMTQSPDSLAVSLGERVTMNCKSSQSLLYSTNQKNYLAWYQQKPGQSPKLLIYWASTR  
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**10E8-heavy chain**

EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIKSKTDGGT  
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**10E8-Light chain**

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**PGT121-heavy chain**

QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYP  
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**PGT121-Light chain**

SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVYDDSDRPSGIPERF

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#### **PGDM1400-heavy chain**

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#### **PGDM1400-light chain**

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#### **Recombinant CD4 protein** (aa 26-396, sequence as following:

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#### **CCR5 protein** (aa 1-352, sequence as following:

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#### **Whole genome sequence of HIV Shenzhen BC**

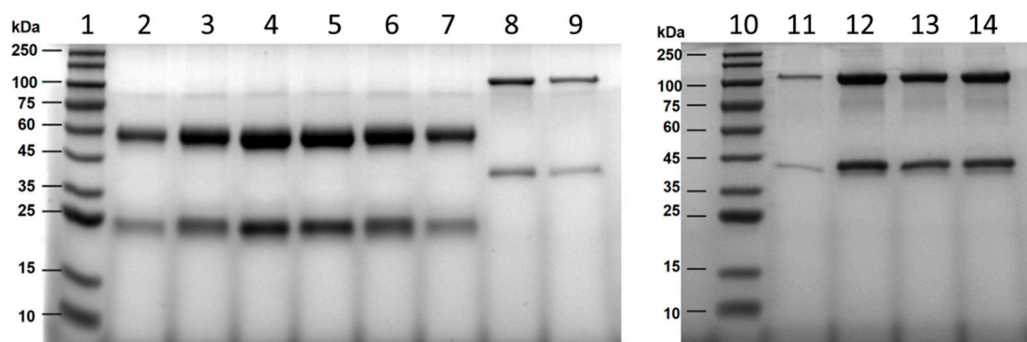
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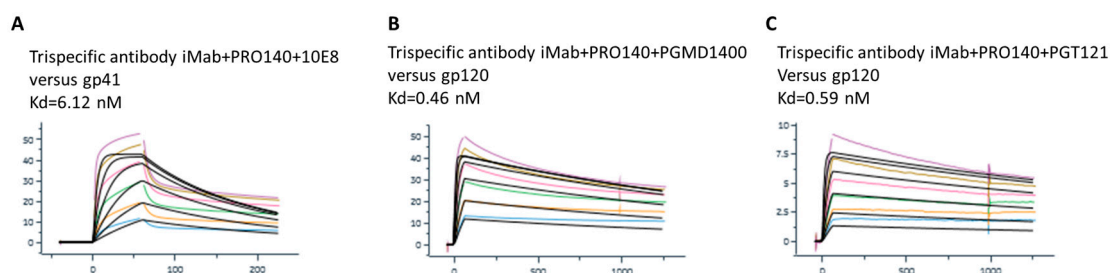
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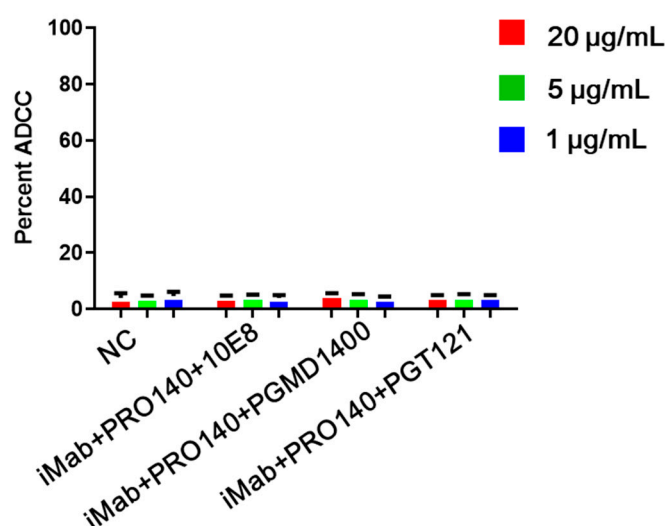
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**Figure S1. SDS-PAGE analysis of monoclonal antibodies iMab, PRO140, 10E8, PGT121, PGDM1400, VRC01, trispecific antibodies iMab+PRO140+10E8, iMab+PRO140+PGMD1400, and iMab+PRO140+PGT121 expression and purification.** Lane 1: Marker; Lane 2. iMab (5  $\mu$ g); Lane 3. PRO140 (5  $\mu$ g); Lane 4. 10E8 (5  $\mu$ g); Lane 5. PGMD1400 (5  $\mu$ g); Lane 6. PGT121 (5  $\mu$ g); Lane 7. VRC01 (5  $\mu$ g); Lane 8. Trispecific antibody iMab+PRO140+10E8 (1  $\mu$ g); Lane 9. Trispecific antibody iMab+PRO140+PGMD1400 (1  $\mu$ g); Lane 10. iMab+PRO140+PGT121 (1  $\mu$ g); Lane 11. Trispecific antibody iMab+PRO140+10E8 (5  $\mu$ g); Lane 12. Trispecific antibody iMab+PRO140+PGMD1400 (5  $\mu$ g); Lane 13. iMab+PRO140+PGT121 (5  $\mu$ g).



**Figure S2. Binding curves for reported  $K_d$  values for binding of three trispecific antibodies to HIV-1 antigens.** The other curves are the experimental traces from testing various concentrations of antibodies in SPR experiments. (A) Trispecific antibody iMab+PRO140+10E8 versus gp41. (B) Trispecific antibody iMab+PRO140+PGMD1400 versus gp120. (C) Trispecific antibody iMab+PRO140+PGT121 versus gp120.



**Figure S3 Antibody-dependent cell-mediated cytotoxicity (ADCC) activity of three HIV-1 trispecific antibodies against CD4<sup>+</sup> CCR5<sup>+</sup> TZM-BL cells.** ADCC values for iMab+PRO140+10E8, iMab+PRO140+PGMD1400 and iMab+PRO140+PGMD1400 (1, 2, and 20  $\mu\text{g}/\text{mL}$ ) against TZM-BL cell, data represent the mean  $\pm$  SEM.at 20  $\mu\text{g}/\text{mL}$ , 2  $\mu\text{g}/\text{mL}$  and 1  $\mu\text{g}/\text{mL}$ .

**Table S1. Summary of monoclonal antibodies and their recognition targets used in this study.**

Antibody	Target
iMab	CD4 receptor
PRO140	CCRS co-receptor
10E8	Membrane-proximal external region of gp41 of HIV
PGMD1400	V2 apex of HIV
PGT121	N332 glycan supersite of HIV
VRC01	CD4 binding site of HIV

**Table S2. Statistical analysis (t-test) of IC50 values for neutralizing HIV-1 in each antibody.**

\*p < 0.05, \*\*p < 0.05, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

T-test	Significance	P value summary	P value
iMab vs. PRO140	No	ns	0.2246
iMab vs. 10E8	Yes	*	0.0243
iMab vs. PGDM1400	No	ns	0.127
iMab vs. PGT121	Yes	**	0.0047
iMab vs. iMab+ PRO140+10E8-Cocktail	Yes	****	<0.0001
iMab vs. iMab+ PRO140+PGDM1400-Cocktail	Yes	****	<0.0001
iMab vs. iMab+ PRO140+ PGT121-Cocktail	Yes	****	<0.0001
iMab vs. iMab+ PRO140+10E8	Yes	****	<0.0001
iMab vs. iMab+ PRO140+PGDM1400	Yes	****	<0.0001
iMab vs. iMab+ PRO140+ PGT121	Yes	****	<0.0001
iMab vs. VRC01	Yes	**	0.0063
PRO140 vs. 10E8	No	ns	0.2842
PRO140 vs. PGDM1400	No	ns	0.998
PRO140 vs. PGT121	No	ns	0.0937
PRO140 vs. iMab+ PRO140+10E8-Cocktail	Yes	****	<0.0001
PRO140 vs. iMab+ PRO140+PGDM1400-Cocktail	Yes	****	<0.0001
PRO140 vs. iMab+ PRO140+ PGT121-Cocktail	Yes	****	<0.0001
PRO140 vs. iMab+ PRO140+10E8	Yes	****	<0.0001
PRO140 vs. iMab+ PRO140+PGDM1400	Yes	****	<0.0001
PRO140 vs. iMab+ PRO140+ PGT121	Yes	****	<0.0001
PRO140 vs. VRC01	No	ns	0.1154
10E8 vs. PGDM1400	No	ns	0.4499
10E8 vs. PGT121	No	ns	0.537
10E8 vs. iMab+ PRO140+10E8-Cocktail	Yes	****	<0.0001
10E8 vs. iMab+ PRO140+PGDM1400-Cocktail	Yes	****	<0.0001

10E8 vs. iMab+ PRO140+ PGT121-Cocktail	Yes	****	<0.000 1
10E8 vs. iMab+ PRO140+10E8	Yes	****	<0.000 1
10E8 vs. iMab+ PRO140+PGDM1400	Yes	****	<0.000 1
10E8 vs. iMab+ PRO140+ PGT121	Yes	****	<0.000 1
10E8 vs. VRC01	No	ns	0.6079
PGDM1400 vs. PGT121	No	ns	0.172
PGDM1400 vs. iMab+ PRO140+10E8-Cocktail	Yes	****	<0.000 1
PGDM1400 vs. iMab+ PRO140+PGDM1400-Cocktail	Yes	****	<0.000 1
PGDM1400 vs. iMab+ PRO140+ PGT121-Cocktail	Yes	****	<0.000 1
PGDM1400 vs. iMab+ PRO140+10E8	Yes	****	<0.000 1
PGDM1400 vs. iMab+ PRO140+PGDM1400	Yes	****	<0.000 1
PGDM1400 vs. iMab+ PRO140+ PGT121	Yes	****	<0.000 1
PGDM1400 vs. VRC01	No	ns	0.8776
PGT121 vs. iMab+ PRO140+10E8-Cocktail	No	ns	0.227
PGT121 vs. iMab+ PRO140+PGDM1400-Cocktail	No	ns	0.1935
PGT121 vs. iMab+ PRO140+ PGT121-Cocktail	No	ns	0.2048
PGT121 vs. iMab+ PRO140+10E8	Yes	****	<0.000 1
PGT121 vs. iMab+ PRO140+PGDM1400	Yes	****	<0.000 1
PGT121 vs. iMab+ PRO140+ PGT121	Yes	****	<0.000 1
PGT121 vs. VRC01	No	ns	0.2066
iMab+ PRO140+10E8-Cocktail vs. iMab+ PRO140+PGDM1400-Cocktail	Yes	**	0.0018
iMab+ PRO140+10E8-Cocktail vs. iMab+ PRO140+ PGT121-Cocktail	Yes	**	0.0088
iMab+ PRO140+10E8-Cocktail vs. iMab+ PRO140+10E8	No	ns	0.167
iMab+ PRO140+10E8-Cocktail vs. iMab+ PRO140+PGDM1400	Yes	****	<0.000 1
iMab+ PRO140+10E8-Cocktail vs. iMab+ PRO140+ PGT121	Yes	****	<0.000 1
iMab+ PRO140+10E8-Cocktail vs. VRC01	Yes	****	<0.000 1

iMab+ PRO140+PGDM1400-Cocktail vs. iMab+ PRO140+ PGT121-Cocktail	No	ns	0.5834
iMab+ PRO140+PGDM1400-Cocktail vs. iMab+ PRO140+10E8	No	ns	0.0683
iMab+ PRO140+PGDM1400-Cocktail vs. iMab+ PRO140+PGDM1400	Yes	***	0.0002
iMab+ PRO140+PGDM1400-Cocktail vs. iMab+ PRO140+ PGT121	Yes	*	0.0295
iMab+ PRO140+PGDM1400-Cocktail vs. VRC01	Yes	****	<0.000 1
iMab+ PRO140+ PGT121-Cocktail vs. iMab+ PRO140+10E8	No	ns	0.1973
iMab+ PRO140+ PGT121-Cocktail vs. iMab+ PRO140+PGDM1400	Yes	****	<0.000 1
iMab+ PRO140+ PGT121-Cocktail vs. iMab+ PRO140+ PGT121	Yes	**	0.0071
iMab+ PRO140+ PGT121-Cocktail vs. VRC01	Yes	****	<0.000 1
iMab+ PRO140+10E8 vs. iMab+ PRO140+PGDM1400	Yes	****	<0.000 1
iMab+ PRO140+10E8 vs. iMab+ PRO140+ PGT121	Yes	****	<0.000 1
iMab+ PRO140+10E8 vs. VRC01	Yes	****	<0.000 1
iMab+ PRO140+PGDM1400 vs. iMab+ PRO140+ PGT121	No	ns	0.0818
iMab+ PRO140+PGDM1400 vs. VRC01	Yes	****	<0.000 1
iMab+ PRO140+ PGT121 vs. VRC01	Yes	****	<0.000 1