Figure S1, related to Figure 2. Sera from uninfected individuals do not enhance recognition of HIV-1-infected cells by anti-cluster A antibodies upon CD4mc addition.

Cell-surface staining of primary CD4+ T cells either mock-infected or infected with NL4.3 GFP expressing the primary R5 ADA Env with Alexa-Fluor 647-conjugated (AF647) anti-cluster A A32 in the presence or not of HIV-negative sera, in the presence of CD4mc JP-III-48 or equivalent volume of DMSO. Shown in (a) are histograms depicting representative staining and (b) the mean fluorescence intensities (MFI) obtained for four HIV-negative sera. Error bars indicate mean ± SEM. Statistical significance was tested using a Kruskal-Wallis with a Dunn's post-test (ns: non-significant).

Figure S2, related to Figure 4. Co-receptor binding site antibody N12-i2 facilitates A32 recognition of HIV-1-infected cells in the presence of CD4mc.

Cell-surface staining of primary CD4+ T cells either mock-infected or infected with CH58 T/F virus with A32-AF647 in presence or not of Fab, Fab'2 or full N12-i2 antibody with CD4mc JP-III-48 or equivalent volume of DMSO. Shown in (a) are histograms depicting representative staining and (b) MFI obtained for 6 different staining. Error bars indicate mean ± SEM. Statistical significance was tested using an Ordinary one-way ANOVA with a Holm-Sidak's post-test (**** $p<0.0001$, ns: non-significant).

Figure S3, related to Figure 4. CD4mc JP-III-48 but not sCD4 facilitates A32 recognition of HIV-1-infected cells in the presence of the CoRBS 17b antibody.

Cell-surface staining of primary CD4+ T cells infected with CH58 T/F virus with A32-AF647 in (a) absence or (b) presence of 5 ug/ml of the CoRBS 17b antibody, with 10 ug/ml of soluble CD4

(sCD4), 50 μM JP-III-48 or equivalent volume of their respective vehicles (PBS or DMSO). Shown are MFI obtained for 3 different staining. Error bars indicate mean ± SEM.

Figure S4, related to Figure 4. Isolation and characterization of the co-receptor binding site C2 antibody. (a) B cell gating and sorting from 9 million PBMCs of an HIV-1 clade B infected individual by flow cytometry. SSC, side scatter; FSC, forward scatter. (b) Phylogenetic analysis of the heavy chain (left) and light chain (right) variable region sequences of 20 clonal variants of the C2 antibody. Boxed sequences have been selected for cloning and expression. (c) The ability of C2 to bind monomeric gp120 variant proteins (wt, R419D, D368R, delta V1V2, delta V3, delta V1V2V3) was compared to HIV+ sera and other anti-Env mAbs (VRC01, A32, 17b) by immunoprecipitation, as previously described (Coutu and Finzi, 2015). Shown are binding obtained in 2 different experiments. Error bars indicate mean ± SEM .The binding profile of C2 was similar to that of the well-established co-receptor binding site 17b antibody. (d) Primary CD4+ T cells infected with NL4.3 ADA GFP virus were stained with the C2 antibody, ± 17b Fab fragment, in the presence of DMSO or CD4mc JP-III-48. An anti-human FC Alexa-Fluor 647-conjugated was used as secondary antibody. Shown are MFI obtained for 3 different experiments. Error bars indicate mean ± SE. Addition of the 17b Fab fragment reduced recognition of HIV-1-infected cells by C2 in the presence of JP-III-48, suggesting that C2 recognizes an epitope overlapping that of 17b; as such, C2 may represent a new CoRBS antibody.

Figure S5, related to Figure 5. JP-III-48 enhances recognition of HIV-1-infected cells by the CoRBS 17b antibody.

Cell-surface staining of primary CD4+ T cells infected with NL4.3 GFP expressing the primary R5 ADA Env with 17b ± 50μM of JP-III-48 or equivalent volume of DMSO. Shown are MFI obtained in 6 different stainings. Error bars indicate mean ± SEM. Statistical significance was tested using an unpaired Student T test.

Figure S6, related to Figure 5. CD4-mimetic BNM-III-170 sensitizes HIV-1-infected cells to ADCC-mediated by the A32 antibody in the presence of the 17b antibody.

Primary CD4 T cells infected with the CH58 T/F virus were used as target cells and autologous PBMC as effector cells in our FACS-based ADCC assay. Shown are the percentages of ADCC-mediated killing obtained with serial dilutions of A32 Abs (0.3125, 0.625 and 1.25 ug/ml) alone or in combination with 17b, in the presence of BNM-III-170 or equivalent volume of DMSO for 3 different experiments. Error bars indicate mean ± SEM.

References

Coutu, M., and Finzi, A. (2015). HIV-1 gp120 dimers decrease the overall affinity of gp120 preparations for CD4-induced ligands. J Virol Methods *215-216*, 37-44.

Figure S1

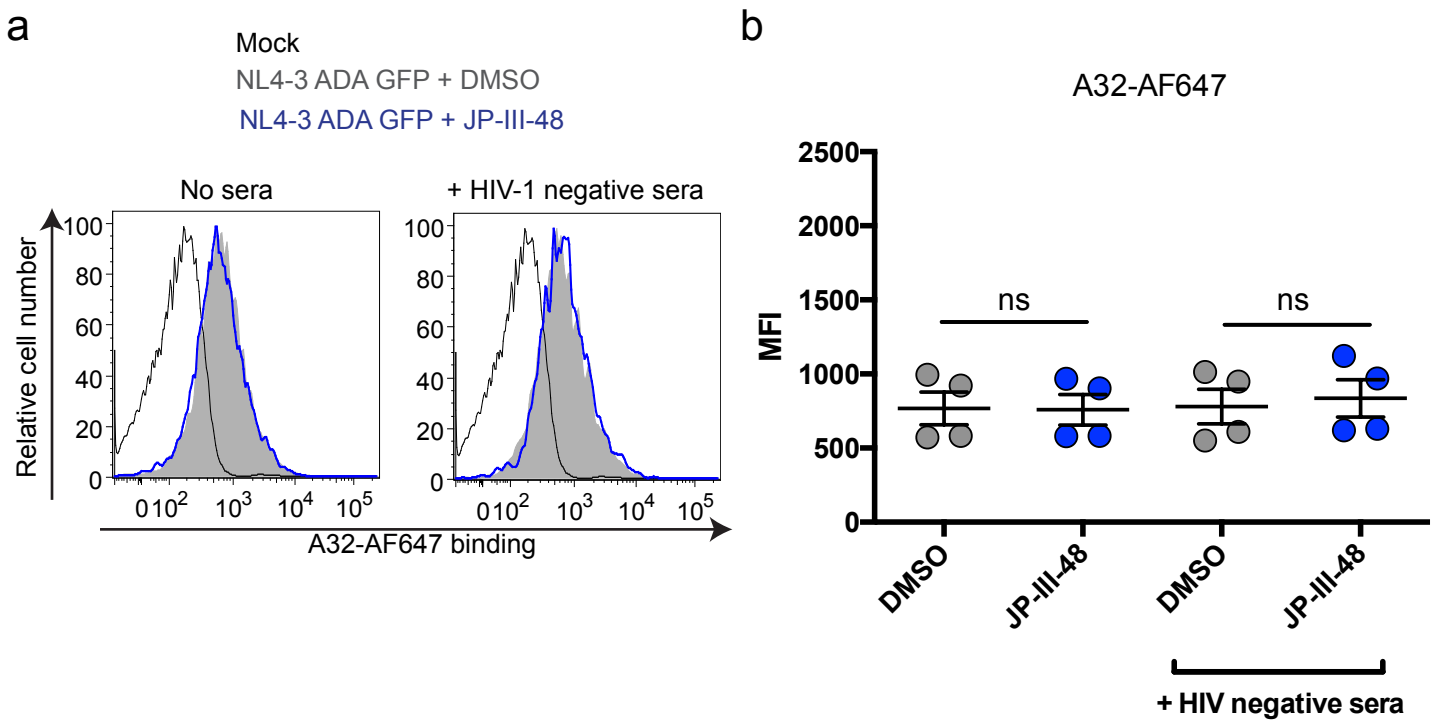

a
Mock
NL4-3 ADA GFP + DMSO
NL4-3 ADA GFP + JP-III-48
No sera
+ HIV-1 negative sera
Relative cell number
100
80
60
40
20
0
0 10^2 10^3 10^4 10^5
A32-AF647 binding
b
A32-AF647
MFI
2500
2000
1500
1000
500
0
ns
ns
DMSO
JP-III-48
DMSO
JP-III-48
+ HIV negative sera

Figure S2

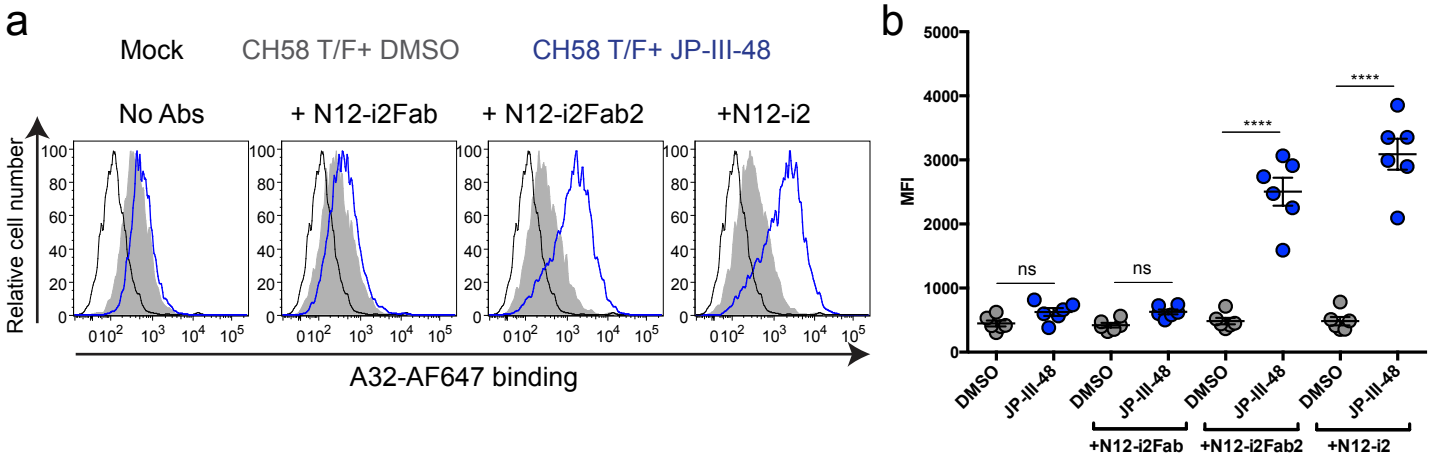

a
Mock
CH58 T/F+ DMSO
CH58 T/F+ JP-III-48
No Abs
+ N12-i2Fab
+ N12-i2Fab2
+N12-i2
Relative cell number
A32-AF647 binding
b
MFI
ns
ns
****
****
DMSO
JP-III-48
+N12-i2Fab
+N12-i2Fab2
+N12-i2

Figure S3

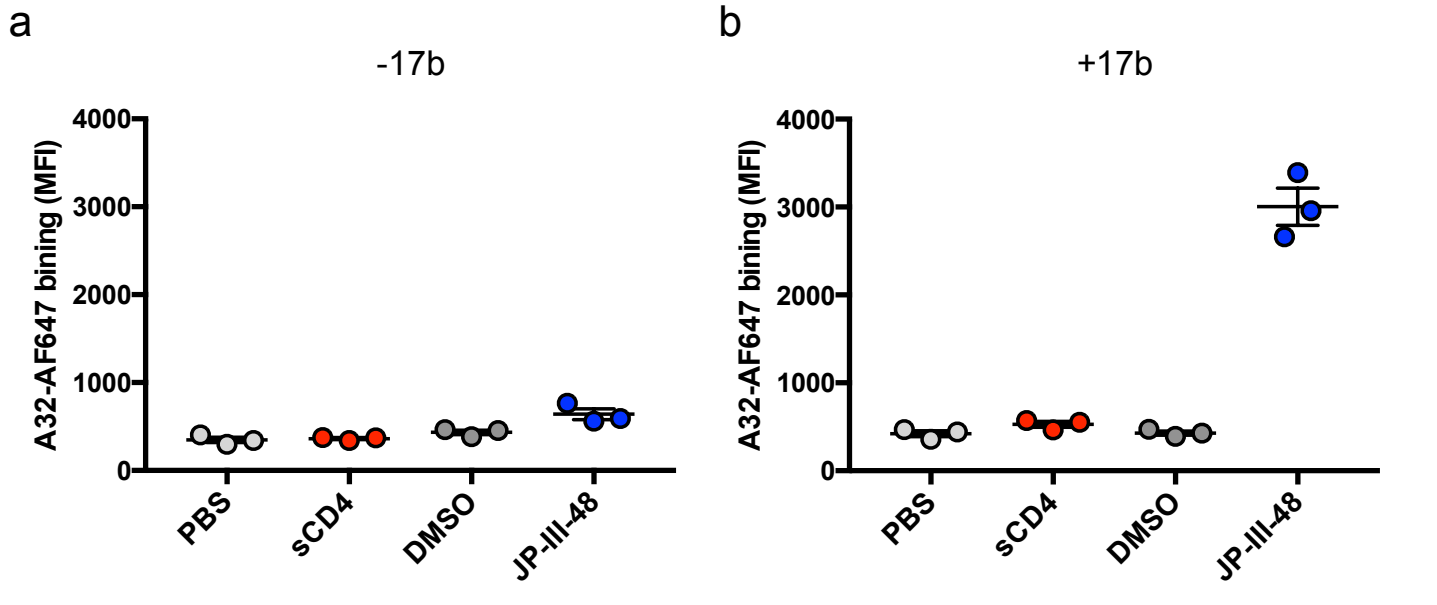

a
-17b
A32-AF647 bining (MFI)
4000
3000
2000
1000
0
PBS
sCD4
DMSO
JP-III-48
b
+17b
A32-AF647 bining (MFI)
4000
3000
2000
1000
0
PBS
sCD4
DMSO
JP-III-48

Figure S4

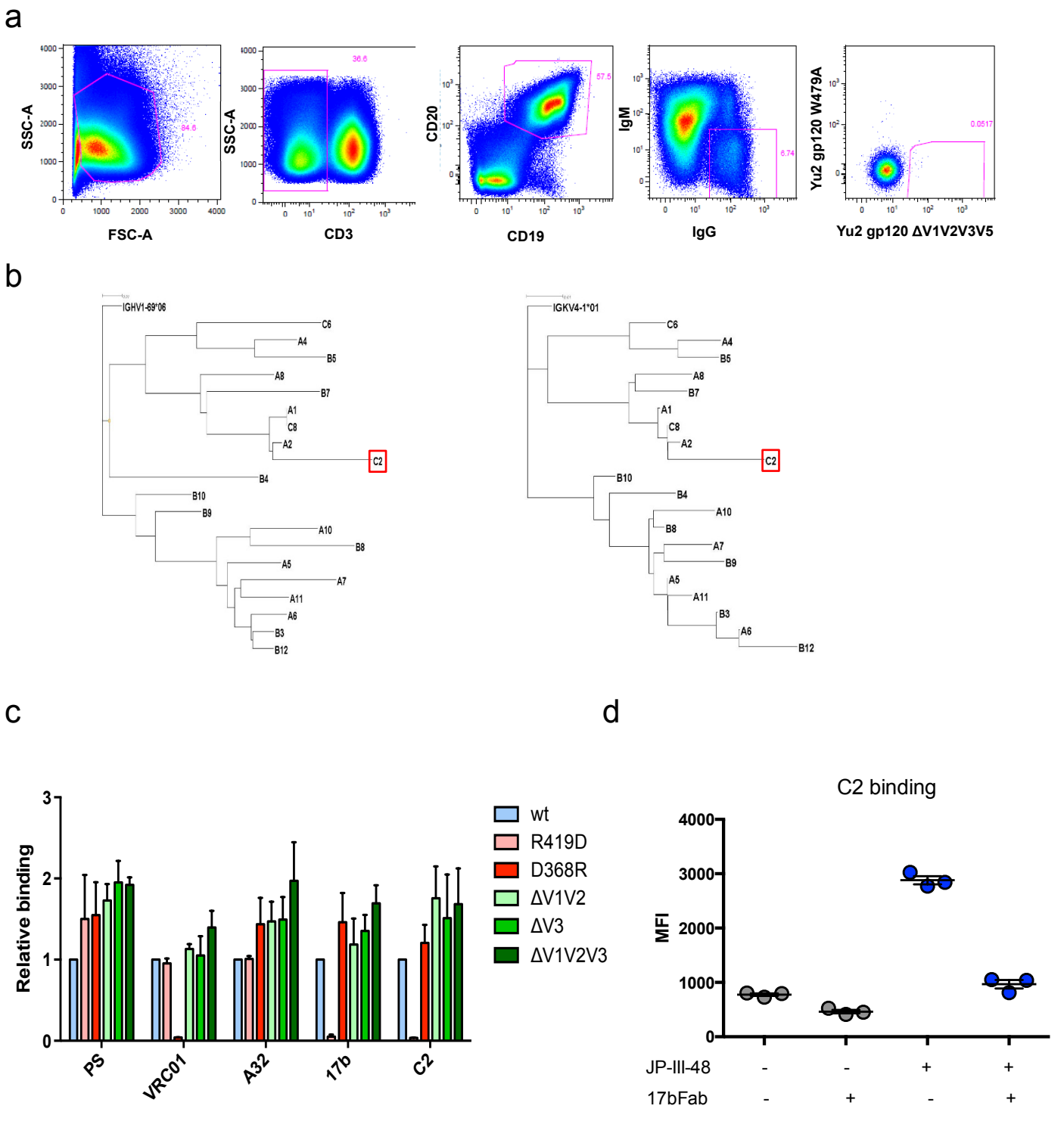

a
SSC-A
FSC-A
84.6
36.6
CD3
CD20
CD19
57.5
IgM
IgG
6.74
Yu2 gp120 W479A
Yu2 gp120 ΔV1V2V3V5
0.0517
b
IGHV1-69*06
IGKV4-1*01
C6
A4
B5
A8
B7
A1
C8
A2
C2
B4
B10
B9
A10
B8
A5
A7
A11
A6
B3
B12
c
Relative binding
PS
VRC01
A32
17b
C2
wt
R419D
D368R
ΔV1V2
ΔV3
ΔV1V2V3
d
C2 binding
MFI
JP-III-48
17bFab

Figure S5

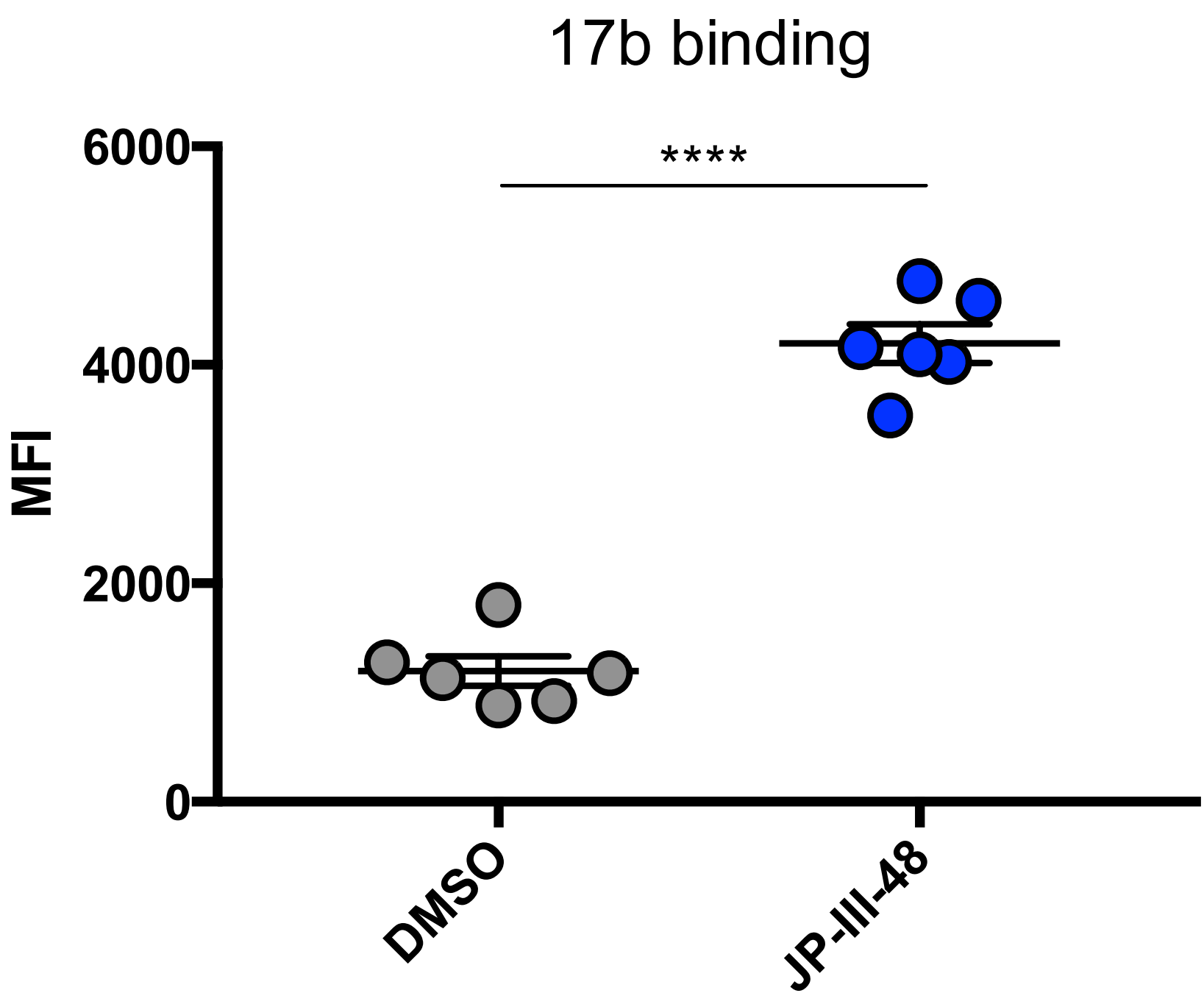
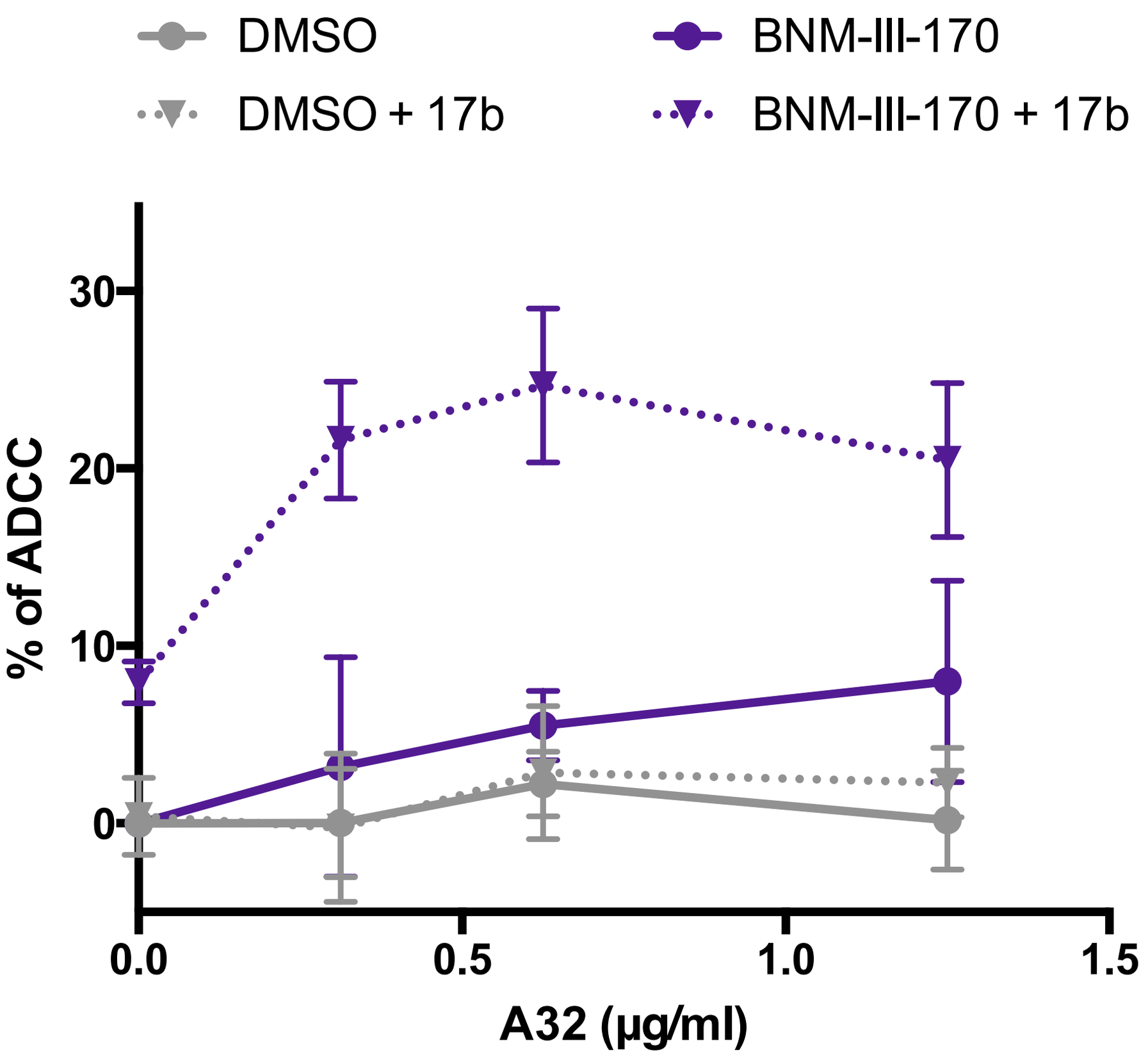


Figure S6


DMSO
BNM-III-170
DMSO + 17b
BNM-III-170 + 17b
% of ADCC
30
20
10
0
0.0
0.5
1.0
1.5
A32 (µg/ml)