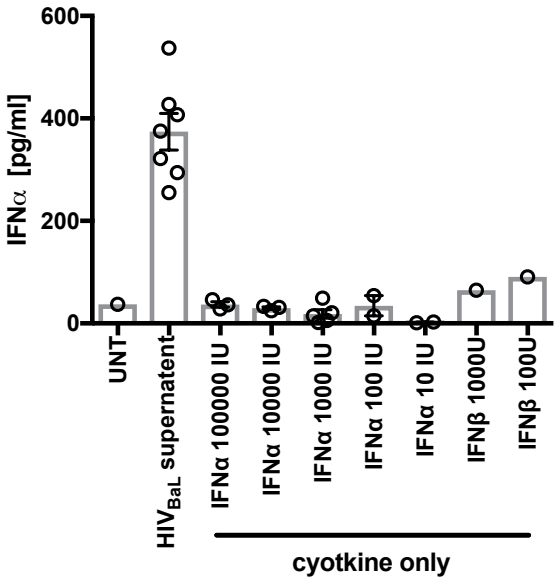
Supplemental Data for Veenhuis *et al.* HIV-antibody complexes enhance plasmacytoid dendritic cell type I interferon production

Table S1. Epitope details for monoclonal antibodies

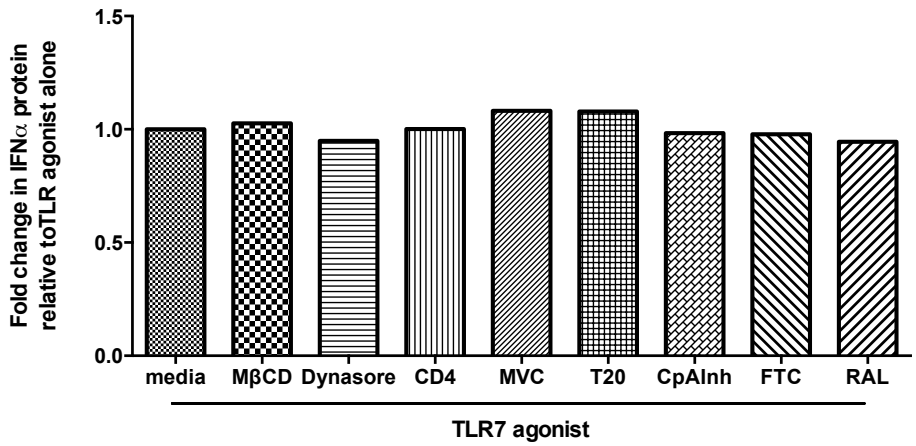
Monoclonal Antibody	Envelope Protein	Epitope specificity	Interferes with CD4 binding	Effect on IFN
b12	**gp120**	**CD4 binding site**	**Yes**	**Suppress**
VRC01 (and variants)	**gp120**	**CD4 binding site**	**Yes**	**Suppress**
PG16	**gp120**	**V1/V2**	**Yes**	**Suppress**
PG9	**gp120**	**V1/V2**	**Yes**	**Suppress**
2G12	**gp120**	**Outer glycans**	**No**	**Minor enhancement**
4E10	**gp41**	**MPER**	**No**	**Major enhancement**
2F5	**gp41**	**MPER**	**No**	**Minor enhancement**
5F3	**gp41**	**CC Loop**	**No**	**Major enhancement**
246-D	**gp41**	**FP/PR**	**No**	**Major enhancement**

Abbreviations: V(1-3), Variable regions (1-3), MPER, membrane proximal external region, FP, fusion peptide, PR, proximal region



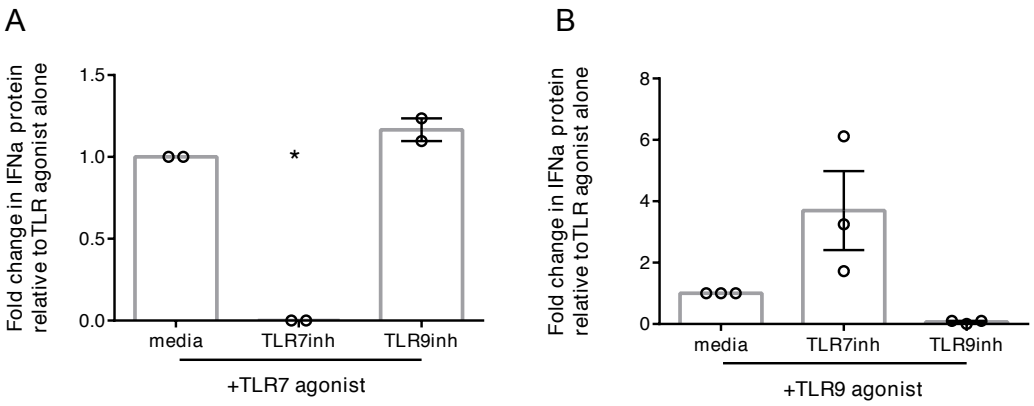
Supplemental Figure 1. The addition of exogenous IFNα or β does not induce IFNα production by pDCs.

Human pDCs were cultured with various concentrations of exogenous IFNα or β for 15h. Supernatants were harvested and assessed for IFNα protein production. Each cytokine condition was repeated 2-5 times. Error bars and gray boxes represent the standard error of the mean and the mean, respectively.



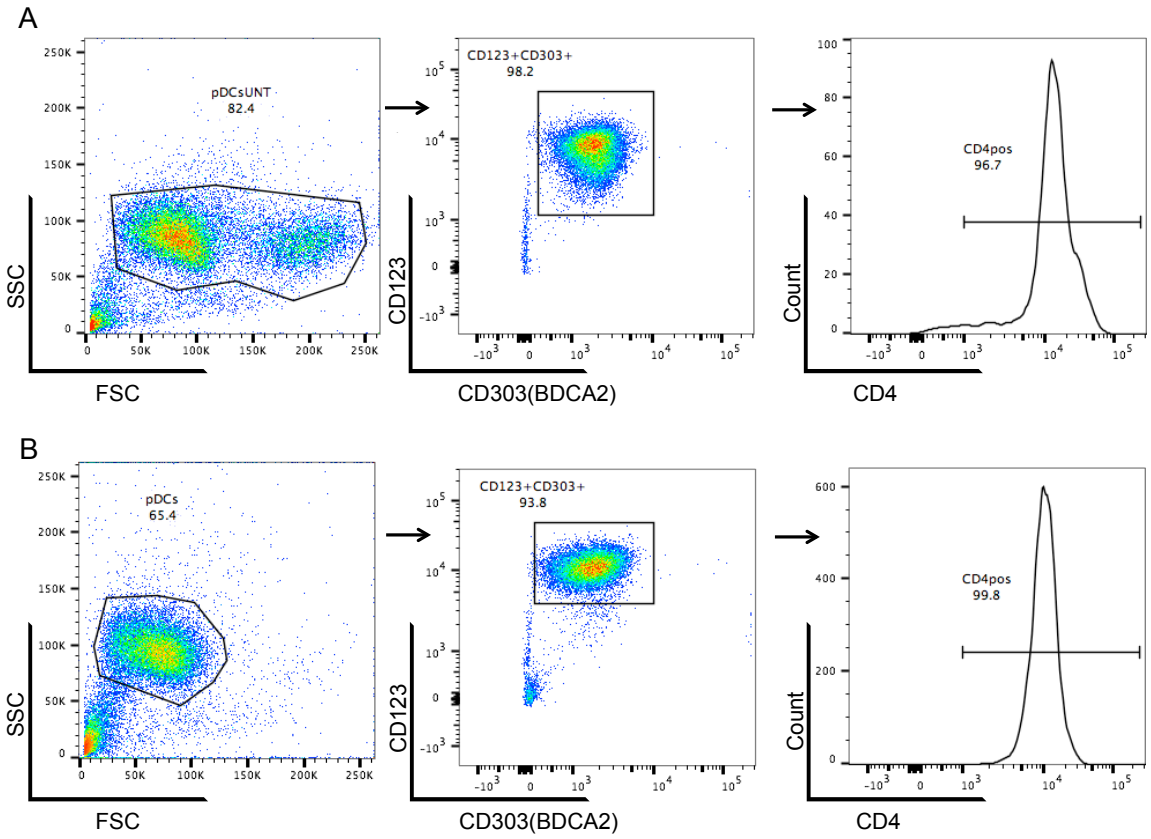
Supplemental Figure 2. pDCs are capable of producing IFNα in response to a TLR7 agonist in the presence of inhibitors

Human pDCs were cultured with endocytosis inhibitors, receptor and co-receptor blockade, and HIV life cycle inhibitors for 1h followed by the addition of TLR7 agonist, Resiquimod for 15h. Supernatants were harvested and assessed for IFNα protein production; representative of one experiment. There was no effect of any treatment on TLR signaling with Resiquimod stimulation.



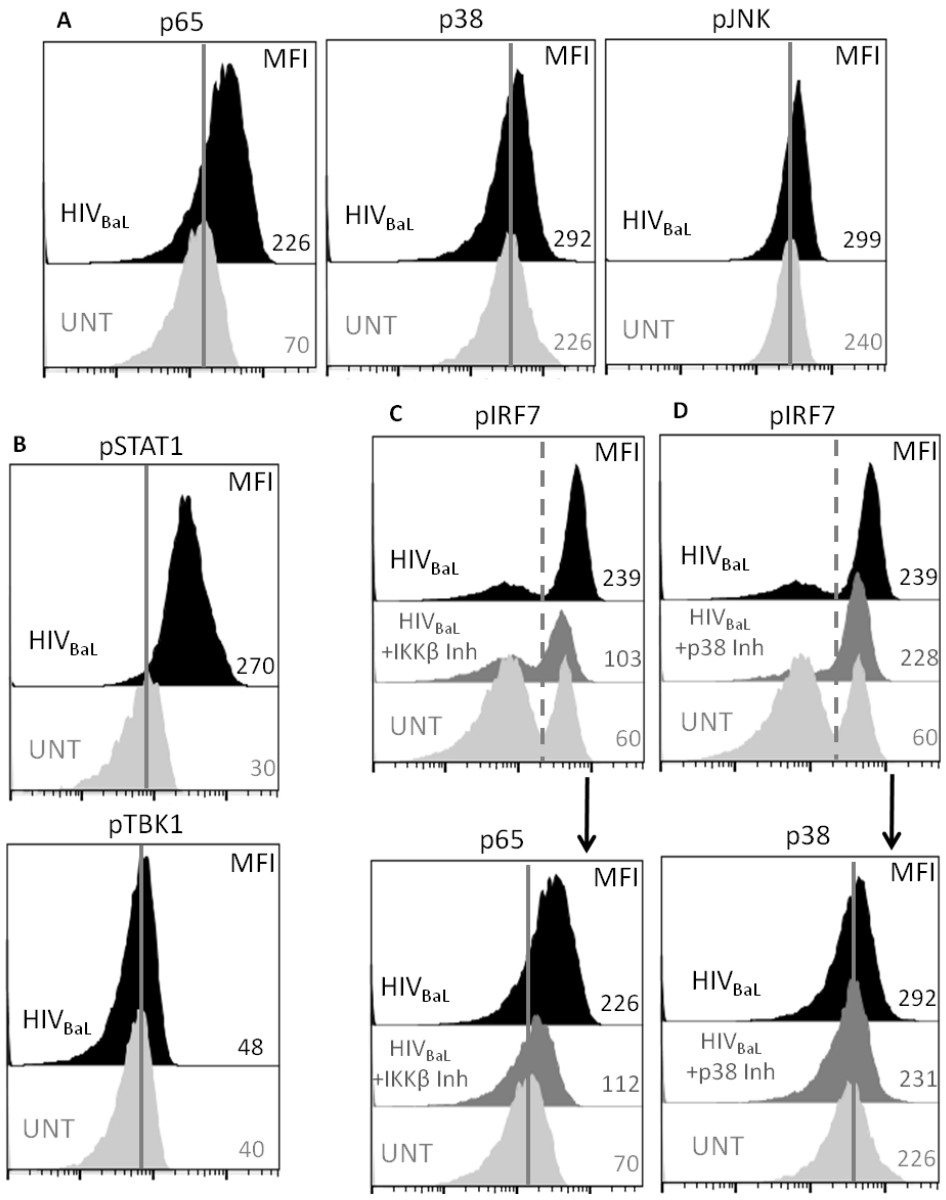
Supplemental Figure 3. Selective Activity of TLR 7 and 9 antagonists

Human pDCs were cultured with TLR inhibitors (**A** and **B**) for 1h followed by the addition of A) a TLR7 agonist (Resiquimod, or B) a TLR9 agonist (ODN2216) for 15h. Supernatants were harvested and assessed for IFNα protein production. Each data point indicates the average IFNα production from one donor's pDCs tested in at least duplicate and normalized to the media alone condition. Error bars and gray boxes represent the standard error of the mean and the mean, respectively. Conditions were compared using a one-way ANOVA with correction for multiple comparisons *$p<0.01$, $n=3$.



Supplemental Figure 4. pDC cell population gating scheme

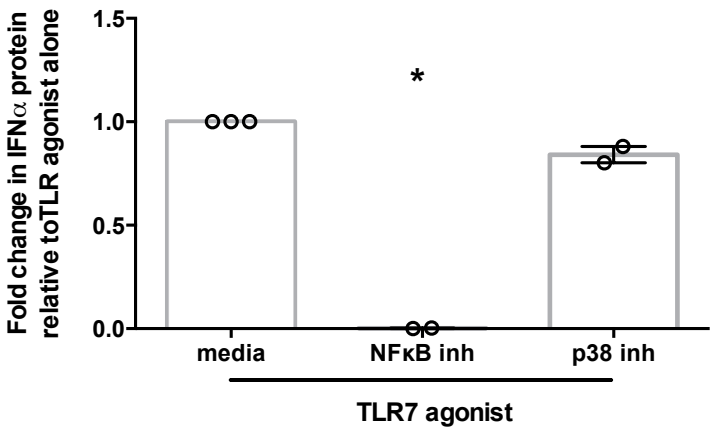
Human pDCs were isolated from healthy donors and cultured for 15h (A) without treatment or (B) with HIV_{BaL}. Cells were then harvested and surface stained for flow cytometry; one representative experiment.



Supplemental Figure 5. Inhibitors block HIV induced phosphorylation of NFκB, p38 MAPK, JNK and STAT1

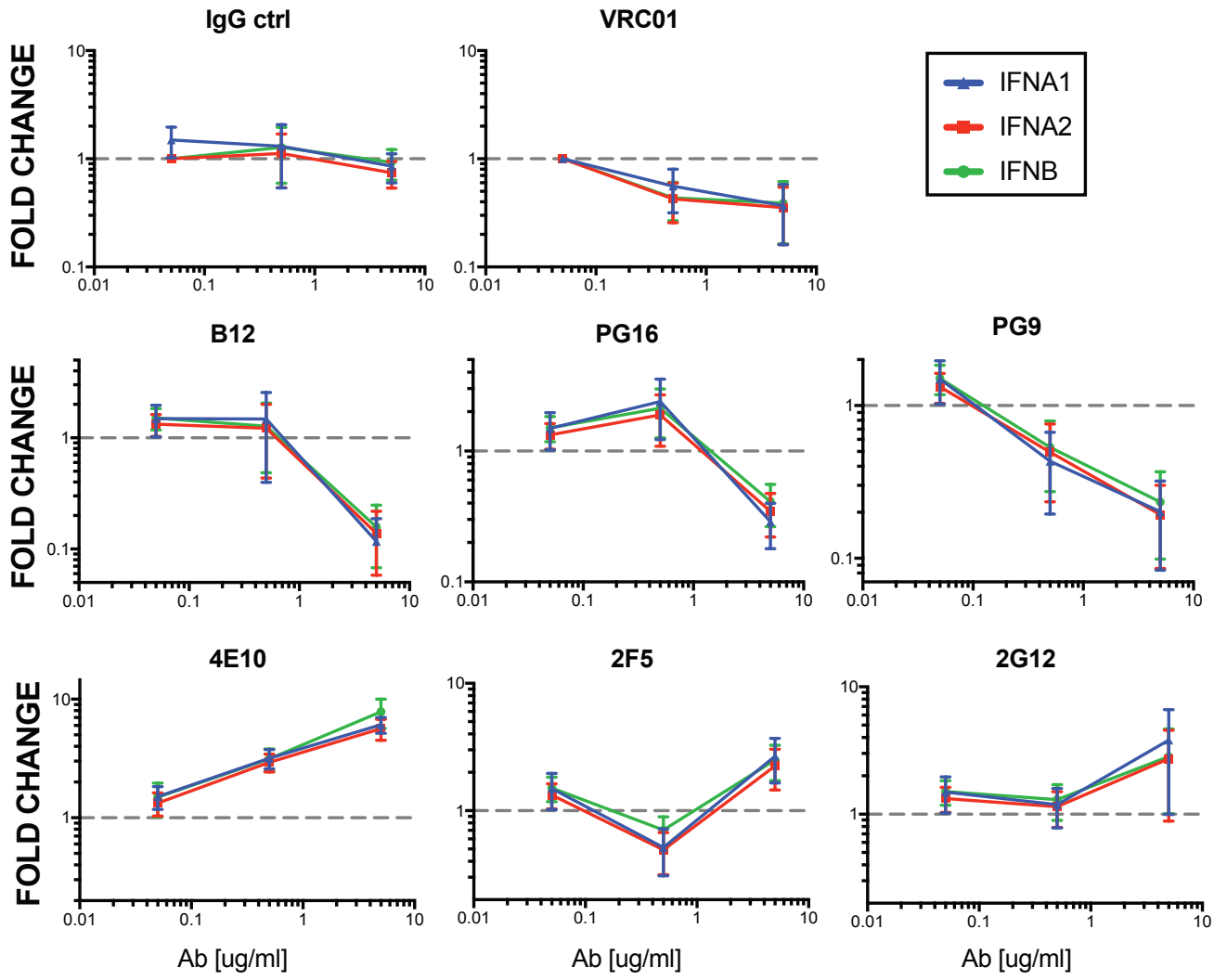
Human pDCs were cultured with HIV_{BaL} only (black histogram) or without HIV_{BaL} (light gray histogram) for 15h (**A** and **B**). Cells were then permeabilized, fixed and stained for phosphorylated NFκB, p38 MAPK, JNK, STAT1 and TBK1. The solid gray line indicates the peak of the untreated histogram. Human pDCs were cultured with a IKKβ **(C**) or a p38 (**D**) inhibitor (dark gray histogram) for 1h followed by the addition of HIV_{BaL} (black histogram) or

media (light gray histogram) for 15h. Cells were then permeabilized and stained for phosphorylated IRF7, NFκB p65 and p38. The dashed gray line indicates the gate cut off for the positive pIRF7 population and the population shown in both of the p65 and p38 panels, the solid gray line indicates the peak of the untreated histogram; one representative experiment.



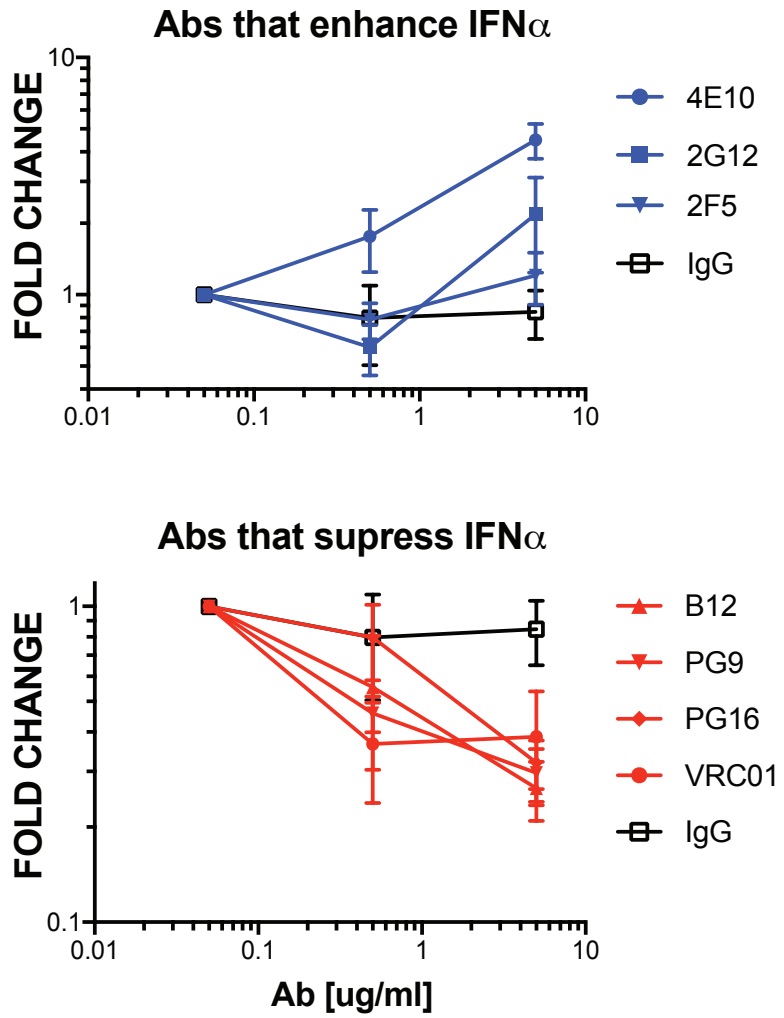
Supplemental Figure 6. pDCs require NFκB, not CREB, signaling to produce IFN in response to a TLR7 agonist

Human pDCs were cultured with signaling inhibitors for 1h followed by the addition of TLR7 agonist, Resiquimod for 15h. Supernatants were harvested and assessed for IFNα protein production. Each data point indicates the average IFNα production from one donor's pDCs tested in at least duplicate and normalized to the media condition. Error bars and gray box represent the standard error of the mean and mean, respectively. *$p<0.001$, $n=2$.



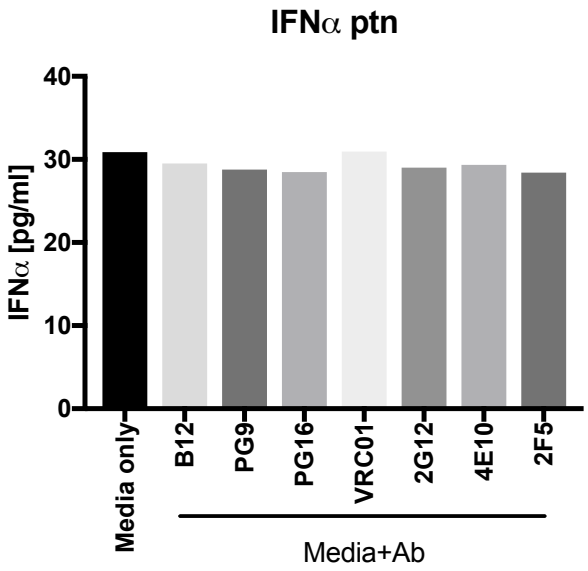
Supplemental Figure 7. Abs have a titratable effect on enhancement or suppression of type I IFN mRNA

HIV_{BaL} was cultured with a titration of HIV specific Abs, for 1-2h and then added to pDCs. Cells were lysed after 15h and assessed for IFNA1 mRNA (blue), IFNA2 mRNA (red), and IFNB (green) expression. Error bars represent the standard error of the mean, n=3.



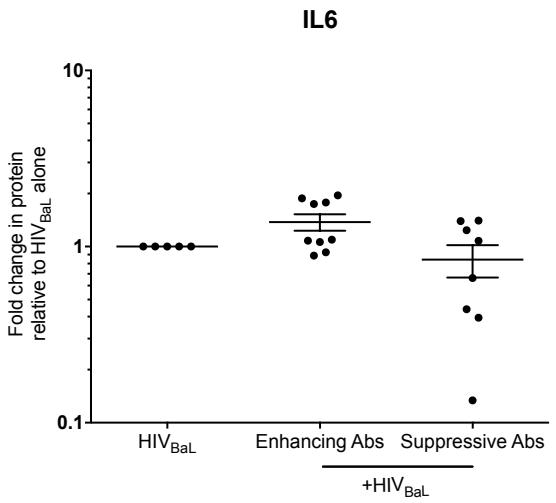
Supplemental Figure 8. Abs have a titratable effect on enhancement or suppression of IFN protein production

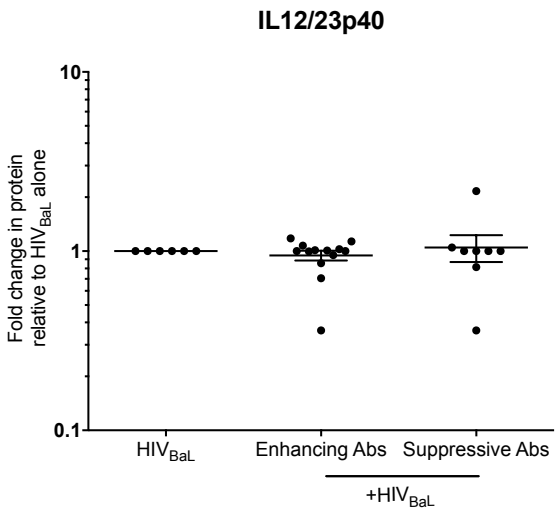
HIV_{BaL} was cultured with a titration of HIV specific Abs, for 1-2h and then added to pDCs. Supernatants were harvested after 15h and assessed for IFNα protein production. Abs that enhance IFNα are shown in blue, Abs that suppress IFNα shown in red. Error bars represent the standard error of the mean, n=3.

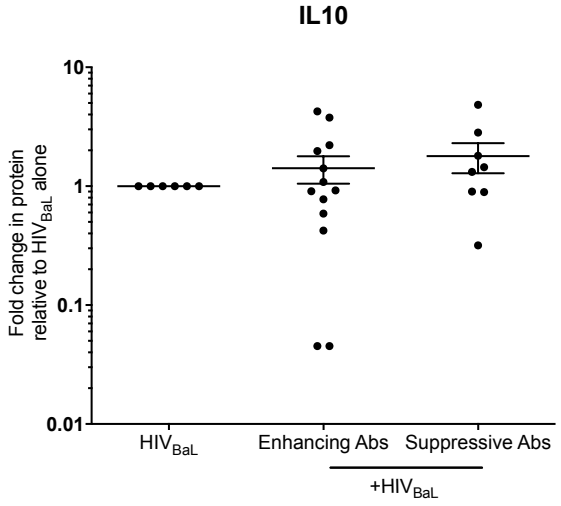


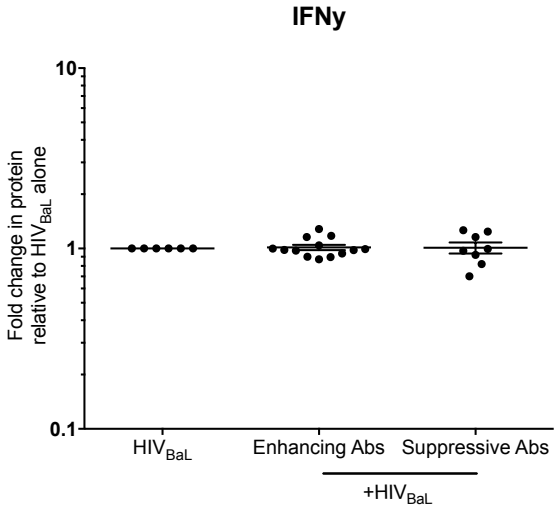
Supplemental Figure 9. mAbs do not induce IFNα protein in the absence of HIV

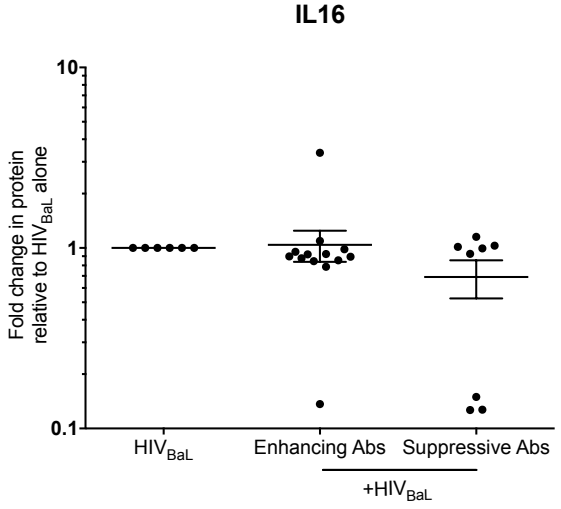
Human pDCs were cultured with HIV specific mAbs without the addition of HIV_{BaL} for 15h. Culture supernatants were harvested and assessed for IFNα protein production; one representative experiment.

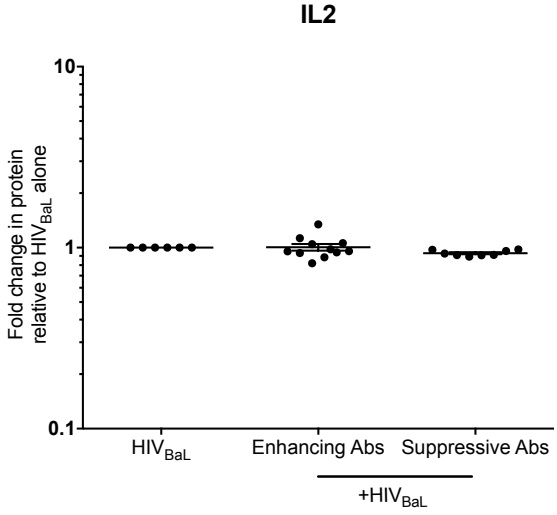

IL6
Fold change in protein relative to HIV$_{BaL}$ alone
10
1
0.1
HIV$_{BaL}$
Enhancing Abs
Suppressive Abs
+HIV$_{BaL}$

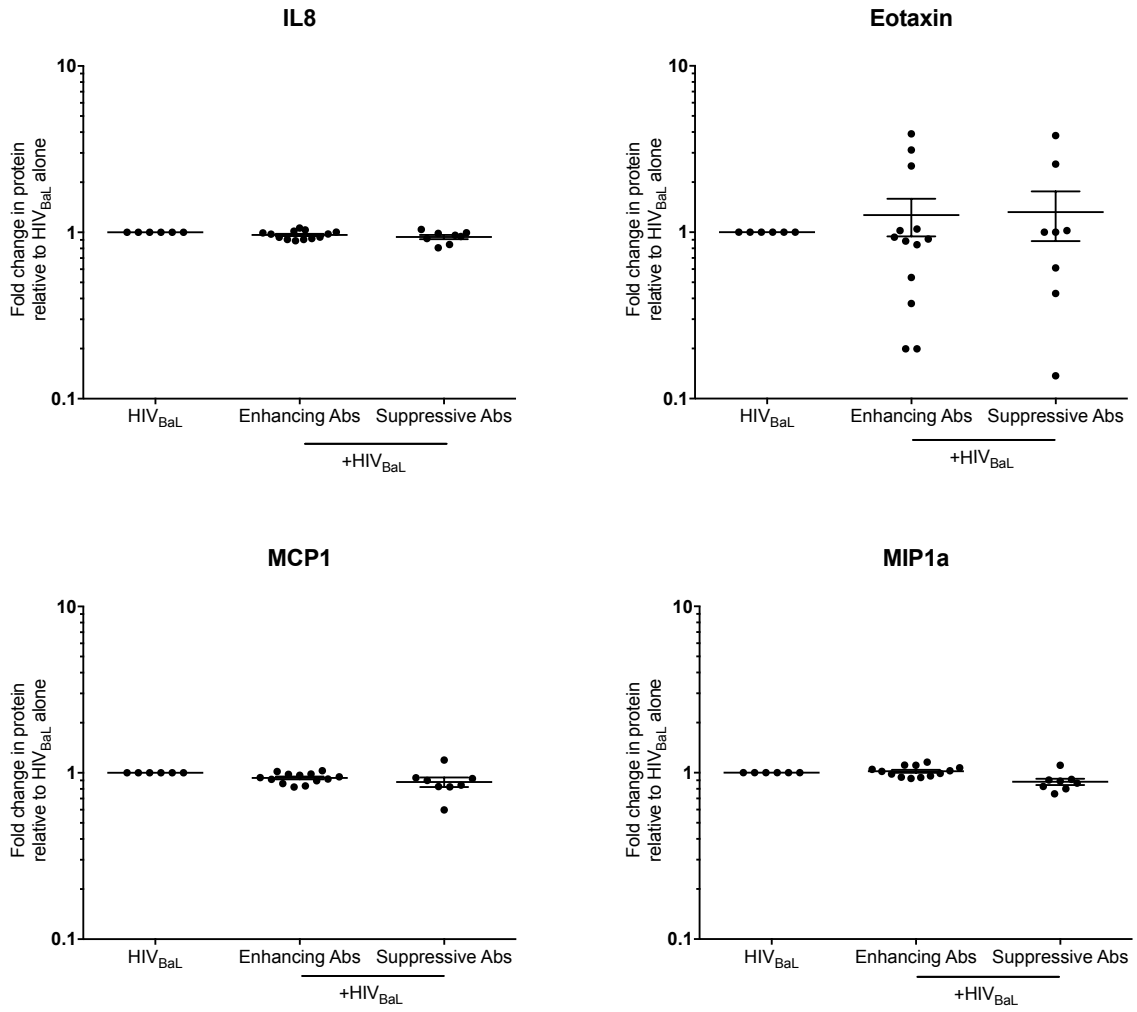

IL12/23p40
Fold change in protein relative to HIV$_{BaL}$ alone
10
1
0.1
HIV$_{BaL}$
Enhancing Abs
Suppressive Abs
+HIV$_{BaL}$


IL10
Fold change in protein relative to HIV$_{BaL}$ alone
10
1
0.1
0.01
HIV$_{BaL}$
Enhancing Abs
Suppressive Abs
+HIV$_{BaL}$


IFNy
Fold change in protein relative to HIV$_{BaL}$ alone
10
1
0.1
HIV$_{BaL}$
Enhancing Abs
Suppressive Abs
+HIV$_{BaL}$

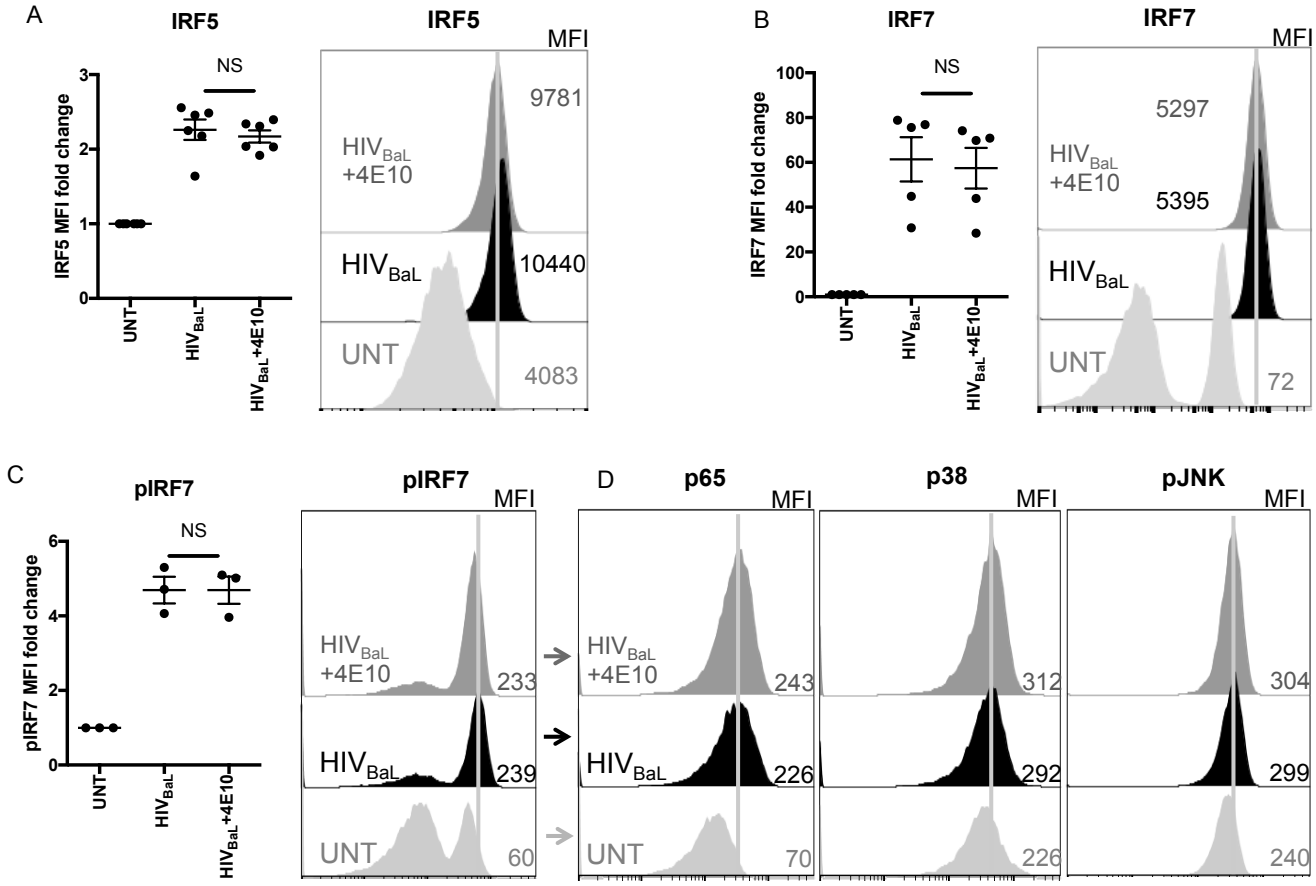

IL16
Fold change in protein relative to HIV$_{BaL}$ alone
10
1
0.1
HIV$_{BaL}$
Enhancing Abs
Suppressive Abs
+HIV$_{BaL}$


IL2
Fold change in protein relative to HIV$_{BaL}$ alone
10
1
0.1
HIV$_{BaL}$
Enhancing Abs
Suppressive Abs
+HIV$_{BaL}$

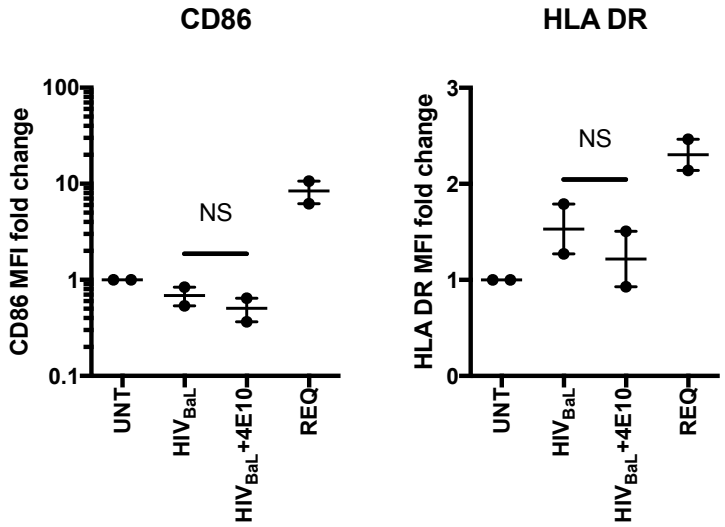


Supplemental Figure 10. The additon of HIV specific Abs does not change 13 additional cytokines tested.

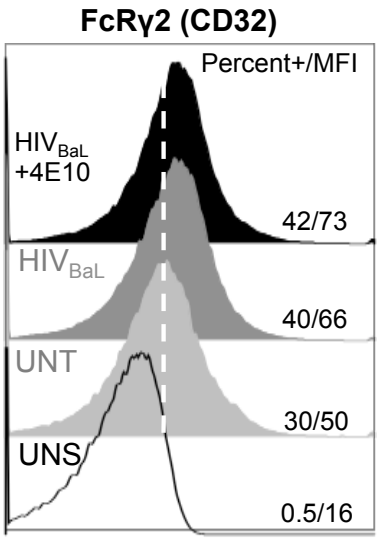
HIV$_{BaL}$ was cultured with a single enhancing Ab (4E10, 2F5, 246-D, 5F3) or a single suppressive Ab (B12, PG9, PG16, VRC01) or no Ab for 1-2h and then added to pDCs. Supernatants were harvested after 15h and assessed for IL6, IL12/23p40, IL10, IFNγ, IL16, IL2, IL8, eotaxin, MCP1, MIP1α, IL1β, IL15, and IL17 protein production. IL1β, IL15 and IL17 were undetectable and therefore not shown. Abs were grouped for analysis based on their ability to enhance or suppress independently. Each data point indicates the protein production from one replicate normalized to the media condition. Supernatants were tested from 3 independent donors, with 2 replicates each. Error bars represent the standard error of the mean and the mean. *$p<0.05$.



Supplemental Figure 11. The addition of 4E10 does not change the level of IRF5, IRF7 expression or phosphorylation of IRF7, NFκB, MAP kinase and JNK. Human pDCs were cultured with HIV_{BaL}+4E10 (dark gray histogram) or HIV_{BaL} only (black histogram) or without HIV_{BaL} and 4E10 (light gray histogram) for 15h cells were then permeabilized and stained for (A and B) IRF5 and IRF7 or (C and D) phosphorylated IRF7, NFκB p65, p38 and JNK. The solid gray line is a reference to visualize the difference in histograms seen between conditions. (A-C) are composed of summary graphs of at least 3 independent experiments and one representative histogram, (D) is one representative histogram. Error bars represent the standard error of the mean and the mean.

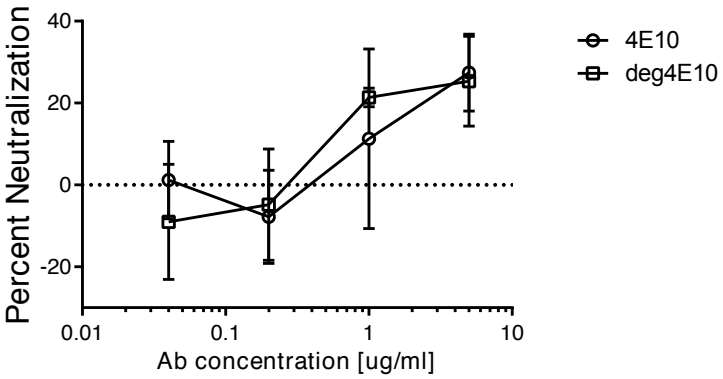


Supplemental Figure 12. 4E10 does not alter CD86 or HLA DR marker expression on pDCs. Human pDCs were cultured with media, HIV_{BaL}, HIV_{BaL} +4E10 or resiquimond (REQ) for 15h. Cell were then harvested and stained to assess CD86 and HLA DR expression levels. Error bars represent the standard error of the mean and the mean, n=2.



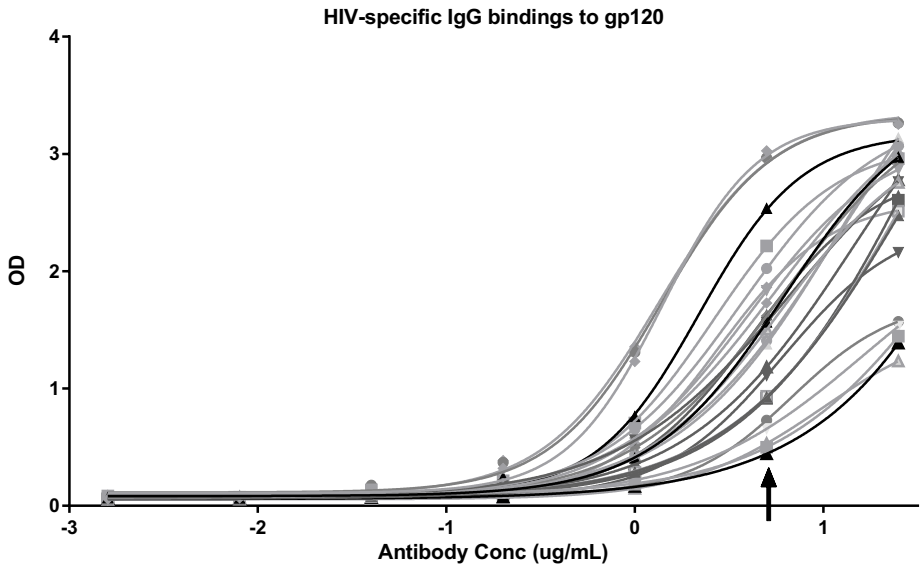
Supplemental Figure 13. CD32 expression on pDCs

Human pDCs were cultured with media (UNT, light gray histogram), HIV_{BaL} (dark gray histogram), or HIV_{BaL} +4E10 (black histogram) for 15h. Cell were then harvested and stained to assess CD32 (FcγR2) expression level before and after activation. The light gray dotted line indicates the positive gate for CD32 as determined by the unstained control (UNS, black line histogram); one representative experiment.



Supplemental Figure 14. Deglycosylated 4E10 maintains the same neutralization capacity as intact 4E10

HIV_{BaL} was cultured with 4E10 and deglycosylated 4E10 for 1-2h following addition to TZM-Bl cells. The cells are incubated for 72h and then assessed for infection by luciferase. Removing the glycans from 4E10 had no significant effect on its ability to neutralize.



Supplemental Figure 15. IgG isolated from HIV infected subjects bind to gp120 lysates with varying affinities

Polyclonal IgG isolated from 13 HIV infected subjects at two time points were assessed for their binding capacity to gp120. Arrow indicates the Ab concentration used in the pDC assay [5ug/ml].