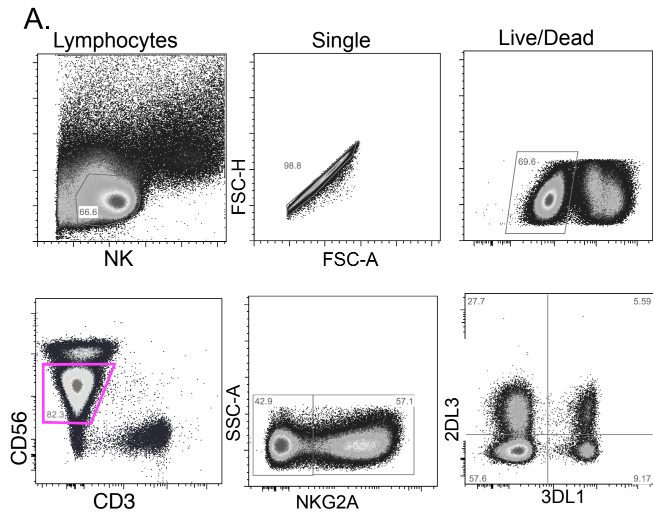
Figure S1



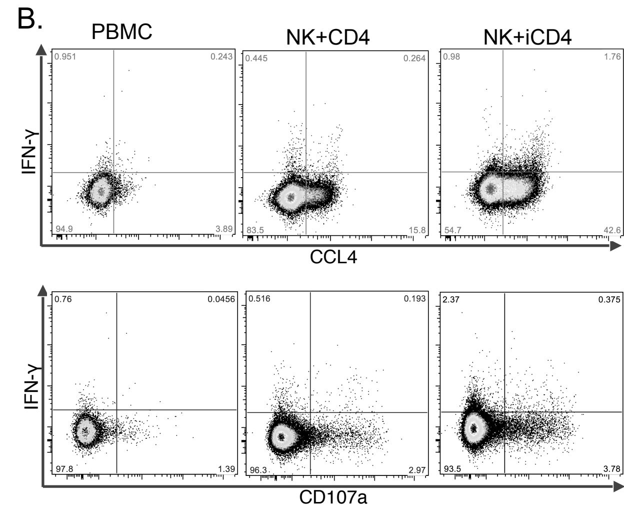
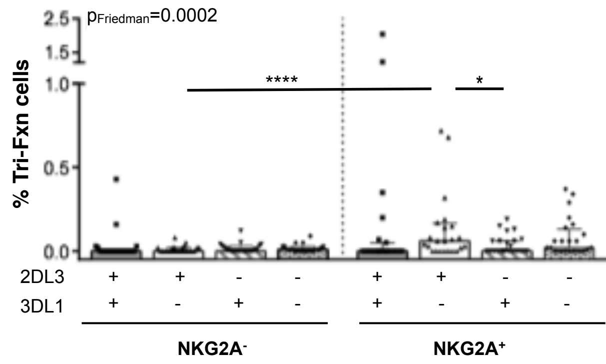


Figure S2

A.



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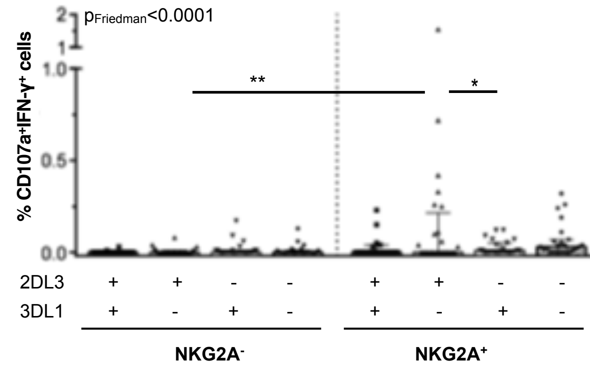


C.

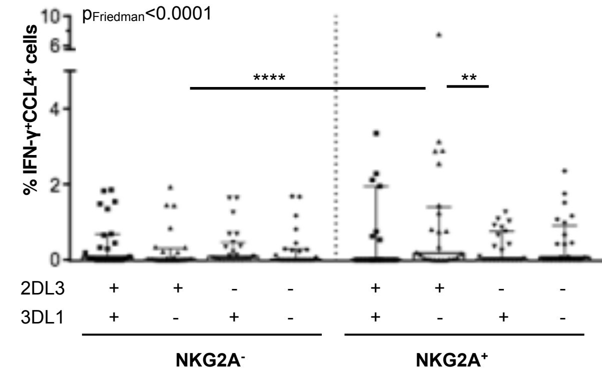


Figure S3

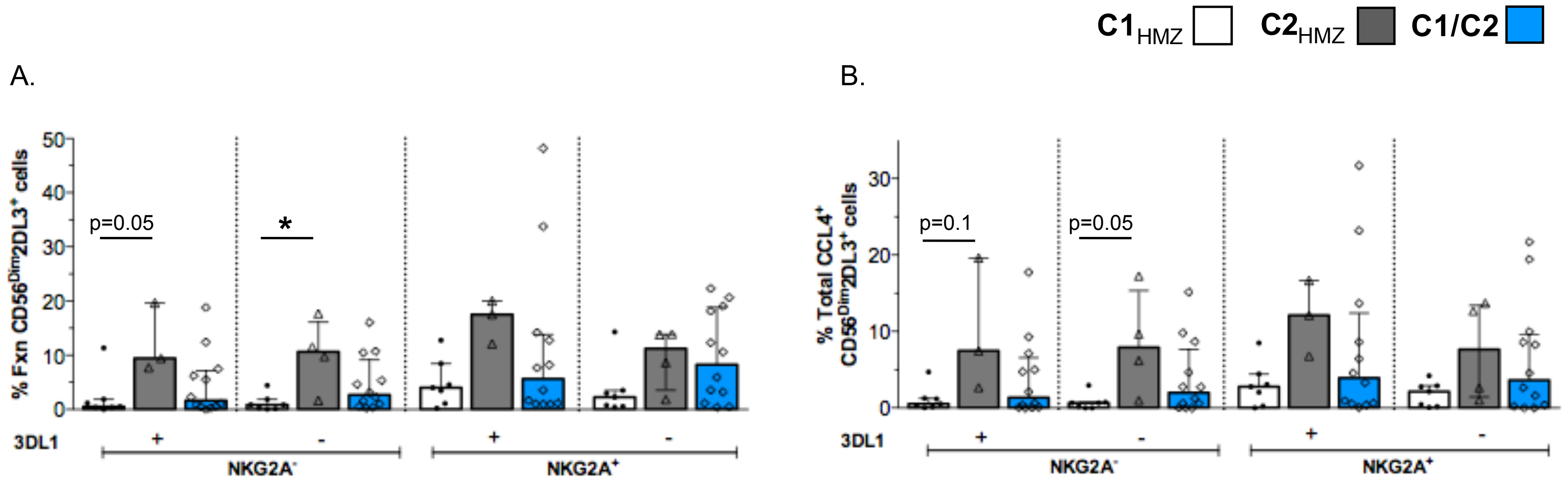


Figure S4

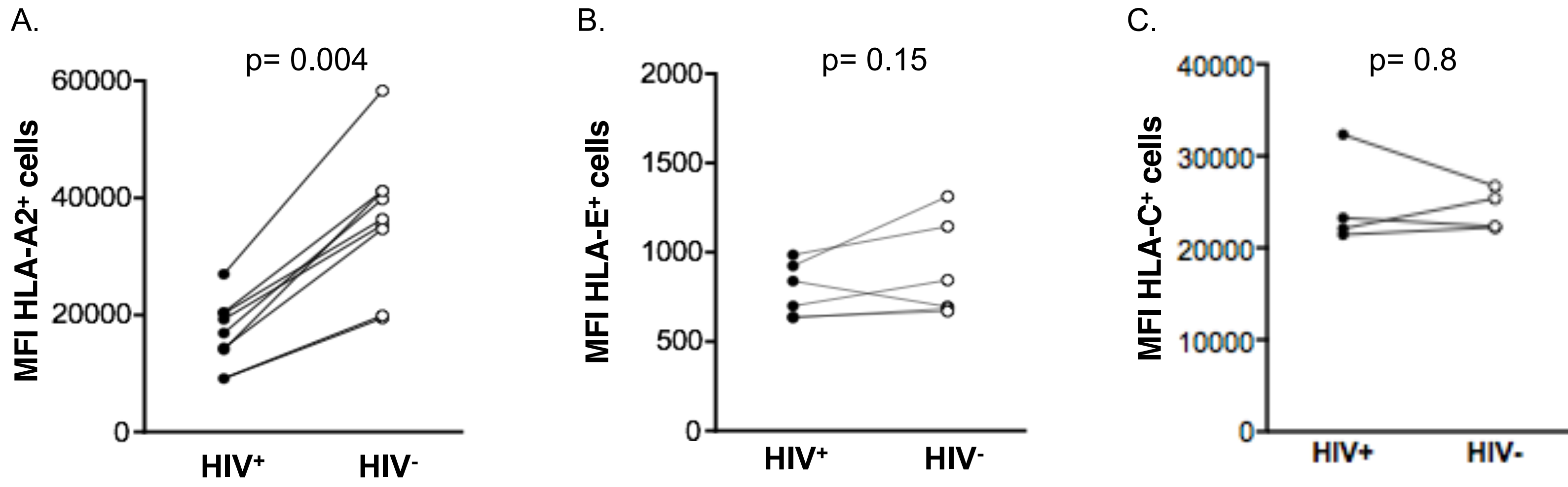
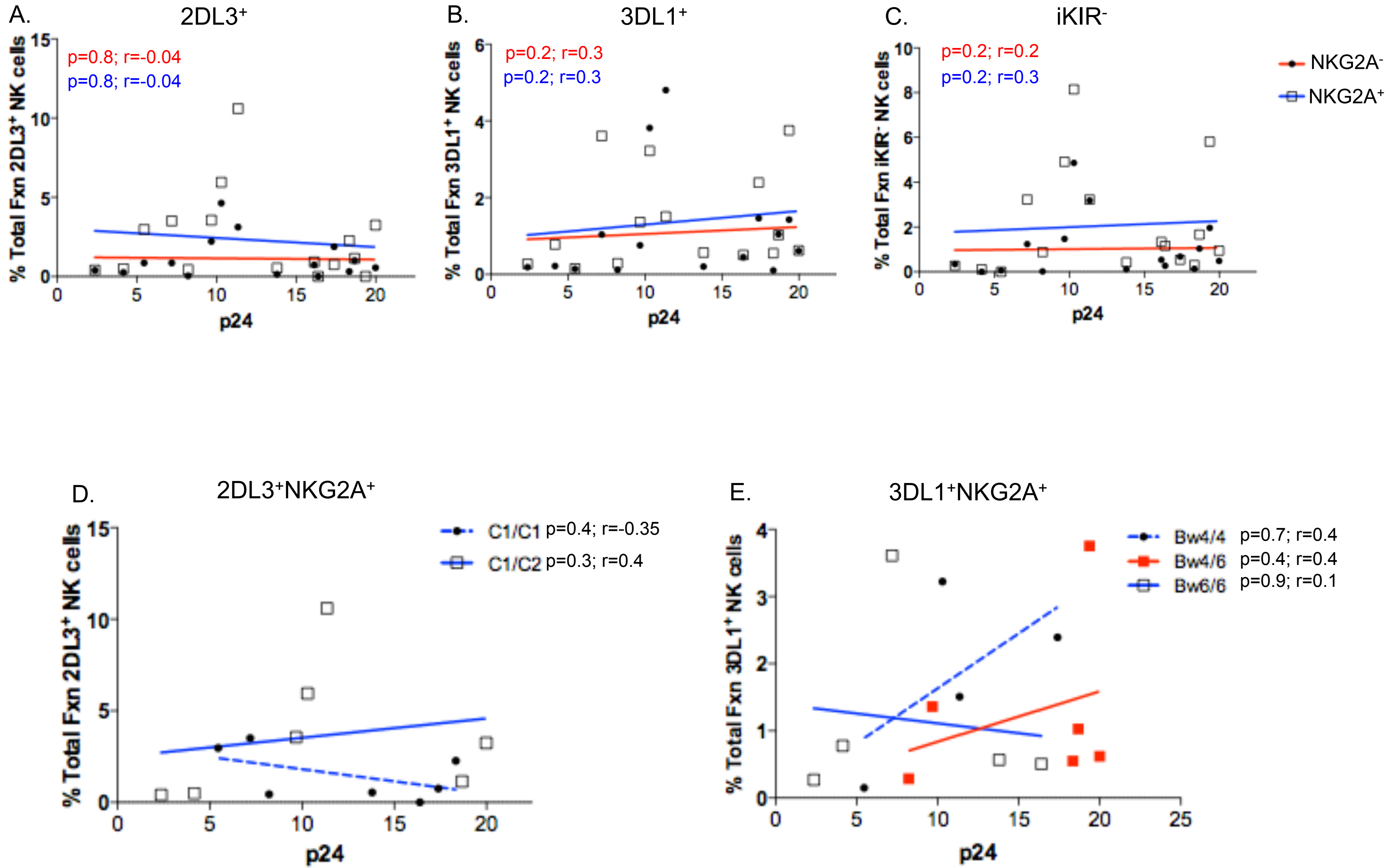


Figure S5



Supplementary Figure 1. The gating strategy used to identify iNKR population and functional subsets. (A). Cells were gated on the live singlet lymphocyte population. NK cells were defined as $CD3^{-}CD56^{+}$ and those that were $CD56^{Dim}$ were distinguished from $CD56^{bright}$. NK cells positive for the iNKR NKG2A, 2DL3 and/or 3DL1 were analyzed from the $CD56^{Dim}$ gate. (B) Functional gates for IFN-γ, CCL4 and CD107a were set on gated $CD56^{Dim}$NK cells from HIV uninfected peripheral blood mononuclear cells (PBMC) (left hand panels). The background for NK cells stimulated with iCD4 (NK+iCD4) (right hand panels) was NK cells co-cultured with autologous uninfected CD4 cells (NK+CD4) (middle panels). The background for the NK+CD4 is higher than the PBMC gating control due to the media required to culture purified NK cells, R10 supplemented with IL-2, as described in the *Materials and methods* section.

Supplementary Figure 2. iCD4 stimulation of functional subsets contributing to the differential responsiveness of $CD56^{Dim}NKG2A^{+}2DL3^{+}$ NK cell populations. The frequency of iCD4 stimulated NK cell populations characterized by trifunctional (A), $CD107a^{+}IFN\text{-}\gamma^{+}$ (B) and $IFN\text{-}\gamma^{+}CCL4^{+}$ (C) response profiles are shown in the y-axis for each $CD56^{Dim}NKG2A^{+/-}2DL3^{+/-}3DL1^{+/-}$ population. Bar height represents the median IQR for each group. Data from 24 individuals analyzed in duplicate were used to generate these results. Friedman ($p_{Friedman}$) and Wilcoxon (*) tests were used to determine significance between data sets. P-values for between group comparisons are shown over lines linking the two groups being compared.

Supplementary Figure 3. HIV iCD4 stimulation of functional NK cell subsets contributing to the differential responsiveness of $CD56^{Dim}NKG2A^{+/-}2DL3^{+/-}3DL1^{+/-}$ NK cell populations stratified by HLA-C groups. The frequency of iCD4 stimulated $CD56^{Dim}NKG2A^{+/-}2DL3^{+}3DL1^{+/-}$ NK cells from *C1* homozygotes ($C1_{HMZ}$; n=7), *C2* homozygotes ($C2_{HMZ}$; n=4) and heterozygous (C1/C2; n=12) characterized by total responsiveness (A) and total CCL4 secretion (B) response profiles are shown on the y-axis. Bar heights and error bars represent the median and IQR for each group. Data from 23 individuals positive for a *2DL3* allele, analyzed in duplicate, are plotted. Kruskal-Wallis with Dunn's post tests were used to determine the significance of between group differences. "*" = $p<0.05$. The data obtained were corrected for background using results obtained following stimulation with uninfected CD4 cells. All data points are displayed, including significant outliers.

Supplementary Figure 4. Surface HLA levels following *in vitro* HIV infection of purified and stimulated CD4 cells. The MFI of HLA-A*02 (A) and HLA-E (B) are shown on the y-axis following *in vitro* HIV infection, as described in materials and methods. Individuals tested for HLA-E were negative by allotyping for HLA-B*27, B*40, C*17 and C*04:03 alleles that have been shown to cross-react with 3D12 antibody. Wilcoxon tests were used to determine significance between data sets. P-values for between group comparisons are shown over lines linking the two groups being compared. HIV+: HIV-infected CD4 cells. HIV-: HIV-uninfected CD4 cells.

Supplementary Figure 5. Correlation between percentage of p24^{+}iCD4 cells used for stimulation and total responsiveness of NK cell populations. The frequency of total responsiveness of 2DL3^{+} (A), 3DL1^{+} (B) and iKIR^{-} (C) NK cells (y-axis) following stimulation with autologous iCD4 was correlated with the frequency of p24^{+} iCD4 (x-axis). Both iNKR^{+} populations were positive for only the indicated iNKR (i.e. 2DL3^{+}3DL1^{-} [A] or 2DL3^{-}3DL1^{+} [B]). In panel C both the NKG2A^{+} (blue) and NKG2A^{-} (red) sub-populations of NK cells were 2DL3^{-}3DL1^{-}. Correlations between the frequency of iCD4 induced functional NKG2A^{+}2DL3^{+} (D) and NKG2A^{+}3DL1^{+} (E) NK cells with the frequency of p24^{+} iCD4 were examined in cells from individuals who expressed 1 or 2 copies of the cognate ligand for 2DL3 (D) or who expressed 1 or 2 copies or no cognate ligand for 3DL1 (E). Spearman tests were used to obtain correlation coefficients and two-tailed p-values for the strength of the correlations between the parameters examined.