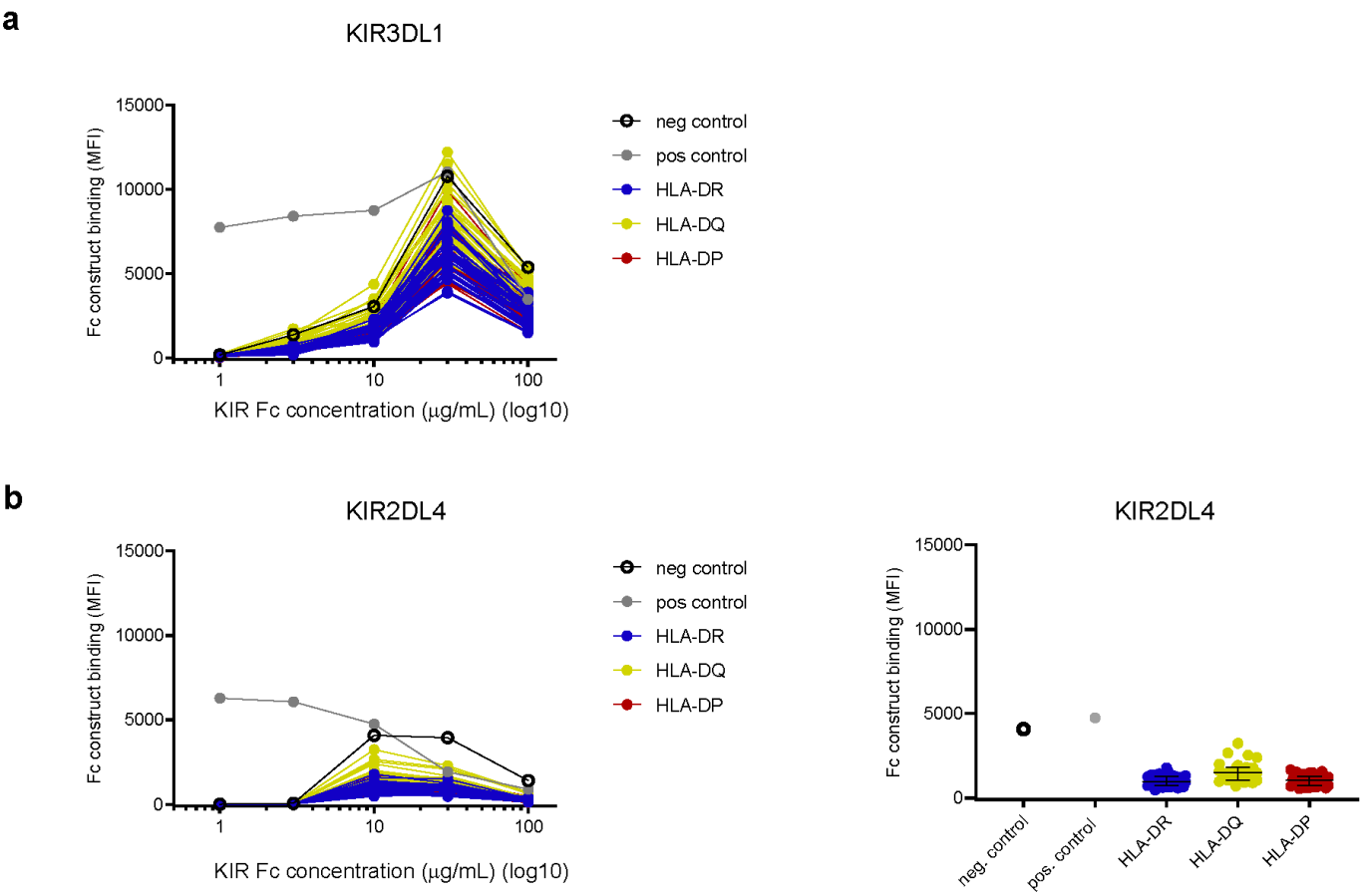
# SUPPLEMENTARY INFORMATION

## Supplementary Figure 1

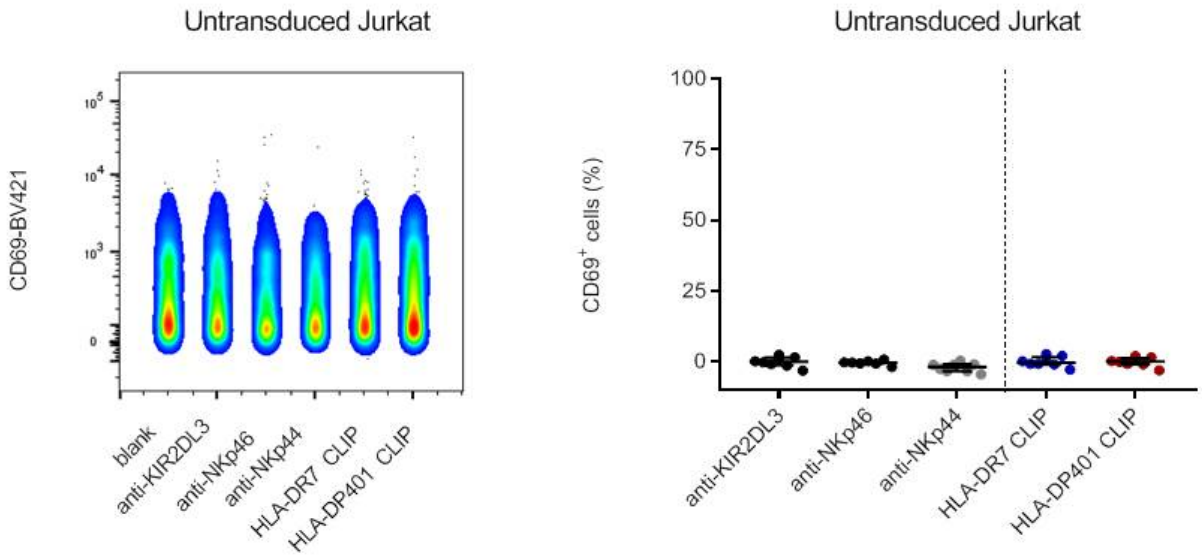



**Supplementary Figure 1**

**KIR3DL1 and KIR2DL4 Fc-constructs exhibit unspecific binding to HLA class II coated beads at high concentrations.**

**a** Binding of KIR3DL1 Fc construct at various concentrations (1-100 µg/mL) to HLA-DR (blue), HLA-DQ (yellow) and HLA-DP (red) coated beads as well as positive (grey) and negative (black) control beads is plotted as median fluorescence intensity (MFI). Each dot represents the measured MFI for binding of the KIR3DL1 Fc construct at a specific concentration to a certain HLA class II coated bead or negative/positive control beads. Lines connect matching MFI values for one specific HLA class II allotype or negative/positive control beads measured at different KIR3DL1 Fc construct concentrations. Data is representative for one single experiment (n=1). **b** MFI values of KIR2DL4 Fc construct binding at varying concentrations (1-100 µg/mL; left panel) and at 10µg/mL to HLA-DR (blue), HLA-DQ (yellow) and HLA-DP (red) coated beads as well as positive (grey) and negative (black) control beads (right panel) are depicted. Each dot in both panels represents one HLA class II molecule. Lines in the left panel connect matching MFI values for one specific HLA class II allotype or negative/positive control beads measured at different KIR2DL4 Fc construct concentrations. Horizontal line in right panel indicates median, error bars indicate interquartile range. Data is representative for one single experiment (n=1).

## Supplementary Figure 2

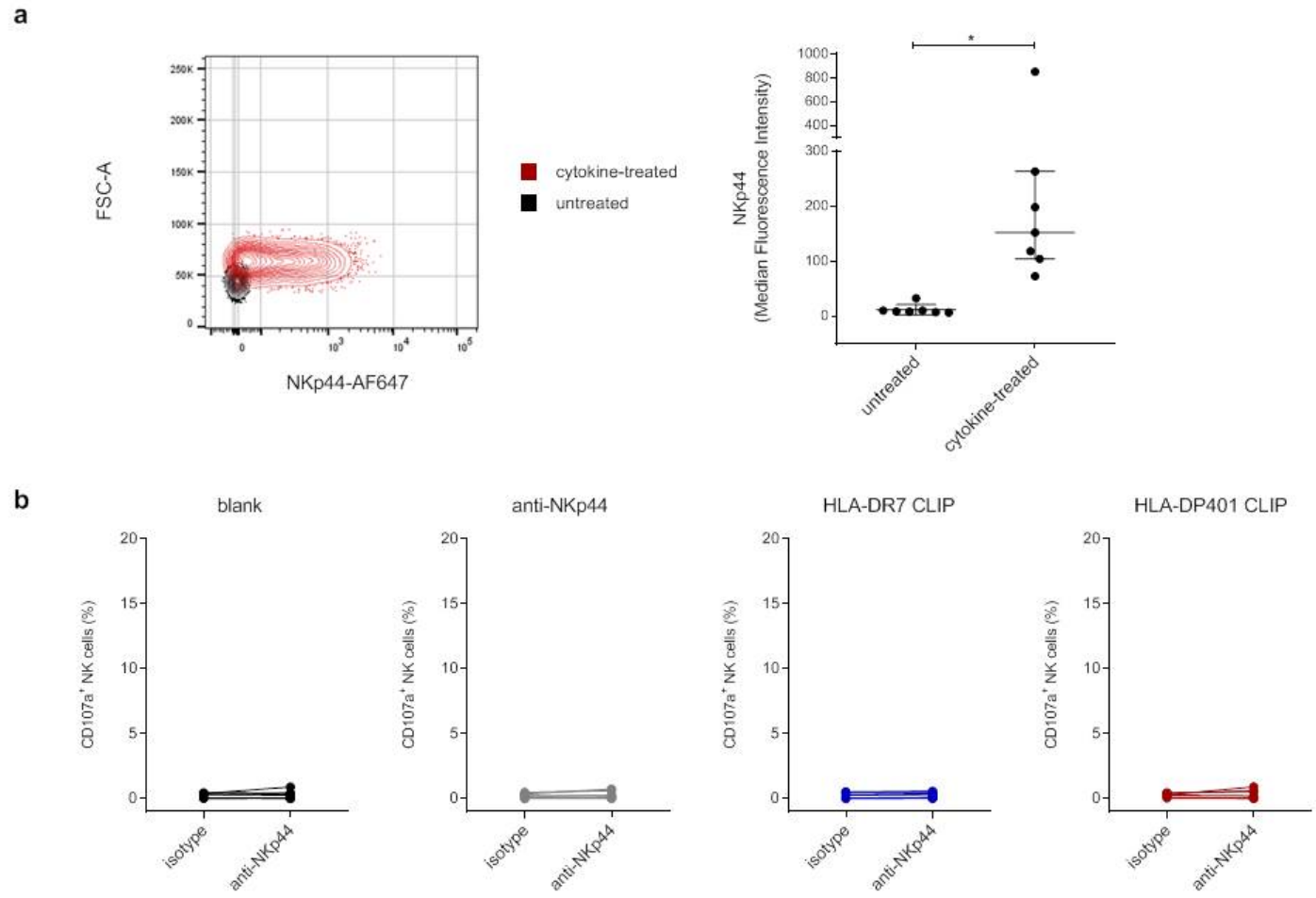



**Supplementary Figure 2**

**Untransduced Jurkat cells do not show functional responses to HLA class II molecules.**

Activation of untransduced Jurkat cells in response to anti-KIR2DL3, anti-NKp46, anti-NKp44 as well as HLA-DR7 and HLA-DP401 CLIP monomers was assessed by the expression of CD69. Plot represents one of eight independent experiments (left panel). The percentage of $CD69^+$ cells following incubation on non-coated wells (blank) was subtracted from all samples. Corrected values are illustrated as median with interquartile range as determined in eight independent biological replicates (n=8).

## Supplementary Figure 3

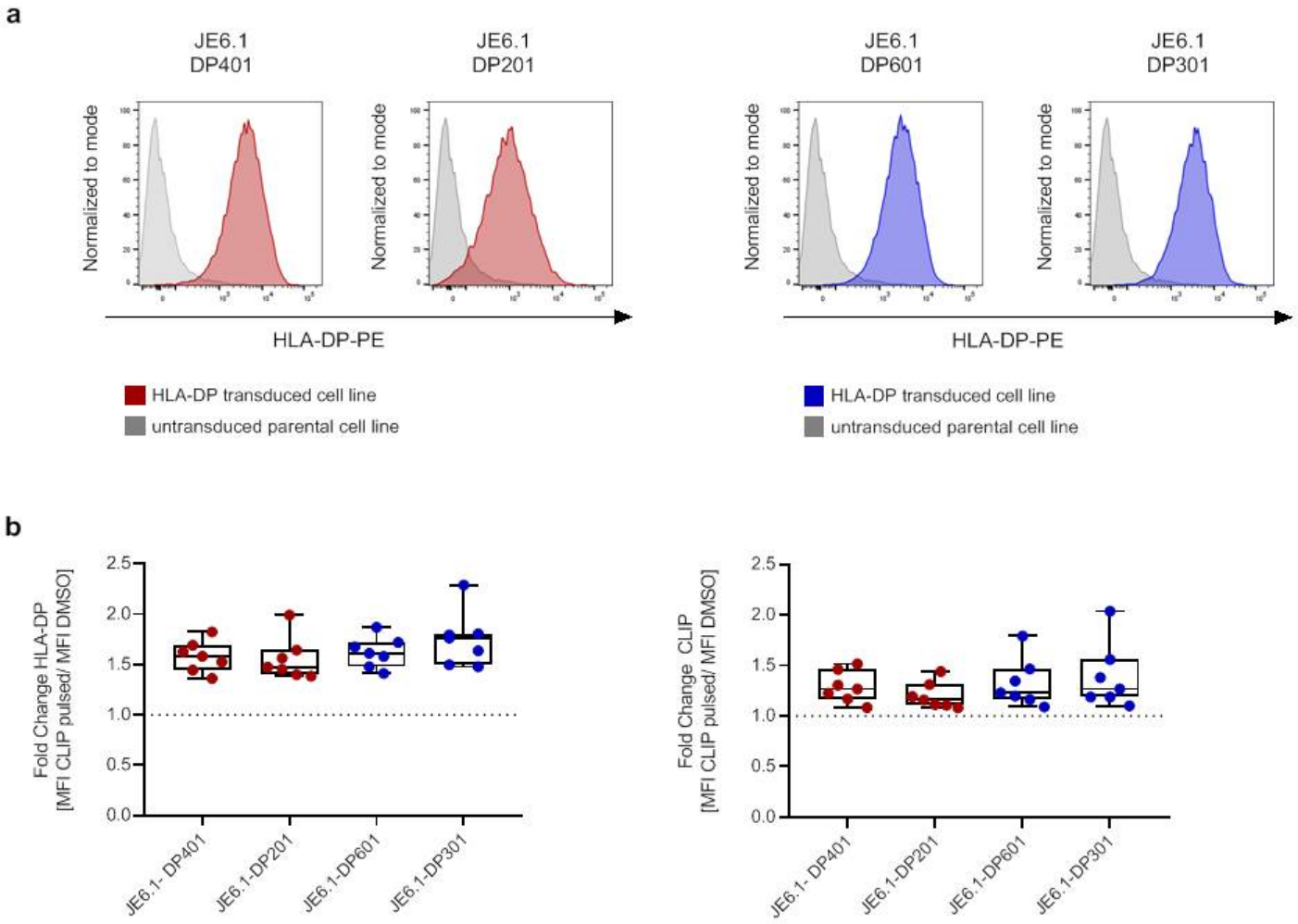



**Supplementary Figure 3**

**Unstimulated NK cells do not express NKp44 and do not degranulate upon co-incubation with HLA-DP401.**

**a** Surface expression of NKp44 was determined in freshly isolated untreated (black) and IL-2 plus IL-15 treated (red) primary NK cells. Plot represents one of seven individual donors. MFI of NKp44 expression from untreated and cytokine-treated primary NK cells was determined in seven individual donors (n=7). Each dot represents one donor. Horizontal line indicates the median, error bars display interquartile range. Two-tailed Wilcoxon matched-pairs signed rank test was used to assess differences in the surface expression of NKp44 between untreated and cytokine-treated primary NK cells. **p=0.02*. **b** Freshly isolated unstimulated NK cells were isolated from seven individual donors and co-incubated with plate-coated anti-NKp44, HLA-DR7 CLIP, HLA-DP401 CLIP or non-coated wells (blank) in the presence of purified mouse IgG1 isotype or purified anti-human NKp44 antibody (both at a final concentration of 10 µg/mL). The percentage of $CD107a^+$ cells was determined. Each dot represents one individual donors (n=7) and lines connect responses from one individual donor.

## Supplementary Figure 4




**Supplementary Figure 4**

**HLA-DP surface expression of JE6.1-DP transduced cell lines is increased by CLIP pulsing.**

**a** HLA-DP surface expression of HLA-DP transduced cell lines (red and blue histograms) is depicted. The HLA-DP expression of the untransduced parental cell line is displayed in grey. Plots represent one of seven individual experiments. **b** HLA-DP (left panel) and CLIP (right panel) surface expression following CLIP pulsing in comparison to DMSO-treated cells is depicted as fold change in MFI [MFI CLIP pulsed/MFI DMSO] for the four indicated JE6.1-DP expressing cell lines. Each dot represents one individual biological replicate as determined in seven independent experiments (n=7). Boxes represent 25$^{th}$ to 75$^{th}$ percentiles. Whiskers indicate minimum and maximum values, horizontal line indicates the median.

**Supplementary Table 1 Sequences of peptides loaded on HLA class II molecules**

| Allele | Peptide | Sequence |
|---|---|---|
| DPB1*04:01-DPA1*01:03 | human CLIP 87-101 | PVSKMRMATPLLMQA |
| DPB1*04:01-DPA1*01:03 | human CTAG1 157-170 | SLLMWITQCFLPVF |
| DPB1*04:01-DPA1*01:03 | HIV-1 env 31-45 | TEKLWVTVYYGVPVW |
| DPB1*04:01-DPA1*01:03 | *C. tetani* TT 948-968 | FNNFTVSFWLRVPKVSASHLE |
| DPB1*04:01-DPA1*01:03 | human MAGE3 243-258 | KKLLTQHFVQENYLEY |
| DPB1*04:01-DPA1*01:03 | human oxytocinase 272-284 | KKYFAATQFEPLA |
| DRB1*07:01-DRA1*01:01 | human CLIP 87-101 | PVSKMRMATPLLMQA |
| DRB1*07:01-DRA1*01:01 | HIV-1 gag 293-312 | FRDYVDRFYKTLRAEQASQE |

**Supplementary Table 2 Antibodies used in the study**

| Antibody | Clone | Fluorophore | Manufacturer |
|---|---|---|---|
| anti-CD107a | H4A3 | BV785 | Biolegend |
| anti-CD16 | 3G8 | FITC | Biolegend |
| anti-CD3 | UCHT1 | BUV737 | BD Biosciences |
| anti-CD3 | SK7 | BV510 | Biolegend |
| anti-CD56 | HCD56 | BV605 | Biolegend |
| anti-CD69 | FN50 | BV421 | Biolegend |
| anti-CLIP | CerCLIP | FITC | BD Biosciences |
| anti-HLA-ABC | W6/32 | APC | Biolegend |
| anti-HLA-DP | B7/21 | APC | Leinco Technologies |
| anti-HLA-DP | B7/21 | PE | Leinco Technologies |
| anti-KIR2DL3 | REA147 | PE | Miltenyi Biotec |
| anti-KIR2DL3 biotin | REA147 | Unconjugated | Miltenyi Biotec |
| anti-NKp44 | P44-8 | PE | Biolegend |
| anti-NKp44 | P44-8 | AF647 | Biolegend |
| anti-NKp44 biotin | 2.29 | Unconjugated | Miltenyi Biotec |
| anti-NKp44 biotin | P44-8 | Unconjugated | Biolegend |
| anti-NKp46 | 9E2 | PE | BD Biosciences |
| anti-NKp46 biotin | 9E2 | unconjugated | Miltenyi Biotec |
| LEAF™ purified anti-NKp44 | P44-8 | Unconjugated | Biolegend |
| LEAF™ purified Mouse IgG1, Isotype Ctrl antibody | MOPC-21 | Unconjugated | Biolegend |
| LIVE/DEAD™ Fixable Near-IR | | | Invitrogen™ |

| | | | |
|---|---|---|---|
| Dead Cell Stain Kit | | | |
| Zombie NIR™ Fixable Viability Kit | | | Biolegend |
| Zombie Aqua™ Fixable Viability Kit | | | Biolegend |

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