# An immunomodulating peptide with potential to suppress tumour growth and autoimmunity

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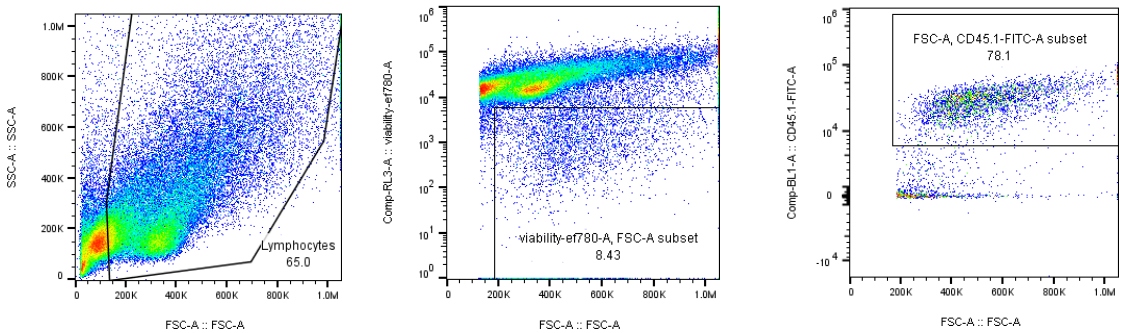
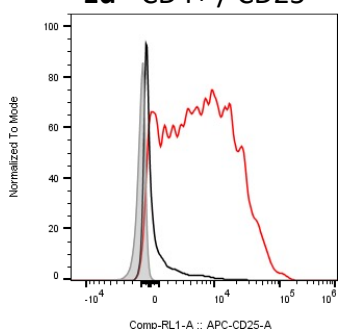
## Supplementary information
## Flow cytometry data

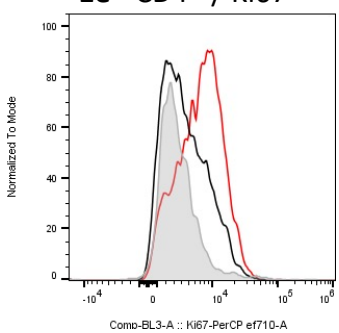
**Figure S1:** Refers to manuscript **Figure 1d and 1e**
CD25 and Ki67 (Splenocyte-derived exhausted CD4+ T cells)




**1d -** CD4+ / CD25



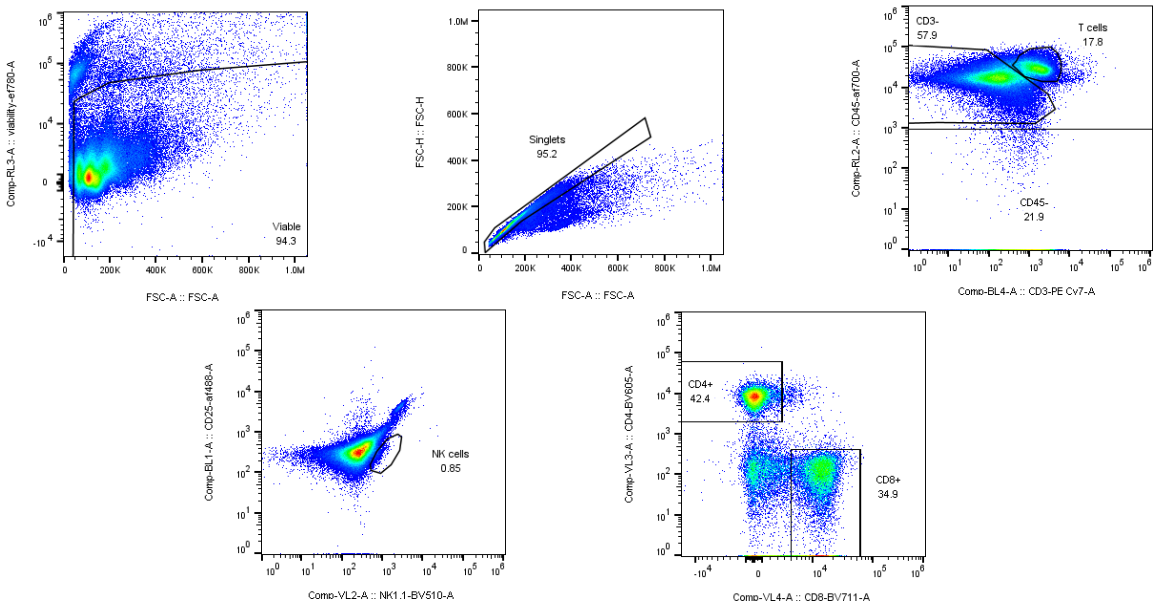

**1e -** CD4+ / Ki67




Grey = isotype
Black = vehicle
Red = IK14004 (1.25 μM)

The Figure shows an exemplar gating strategy from Donor 1. Debris were excluded, then viable (live) and CD45.1+ cells (Tg4 exhausted CD4+ T cells) selected for analysis of CD25 (1d) or Ki67 (1e).

**Figure S2:** Refers to manuscript **Figure 2e – 2k**
CD25, IL-12Rβ1 and IL-12Rβ2 (Splenocytes derived from the LLC xenograft model CD4+ T cells)




The Figure shows an exemplar gating strategy from mouse 2.0 (IK14004). Viable (live) cells selected, doublets excluded, $CD45^{+}CD3^{-}$ and $CD45^{+}CD3^{+}$ cell populations selected. Within $CD3^{-}$ cells, $NK1.1^{+}$ cells were selected and within $CD3^{+}$ cells, $CD4^{+}$ and $CD8^{+}$ cell populations gated.

Viability

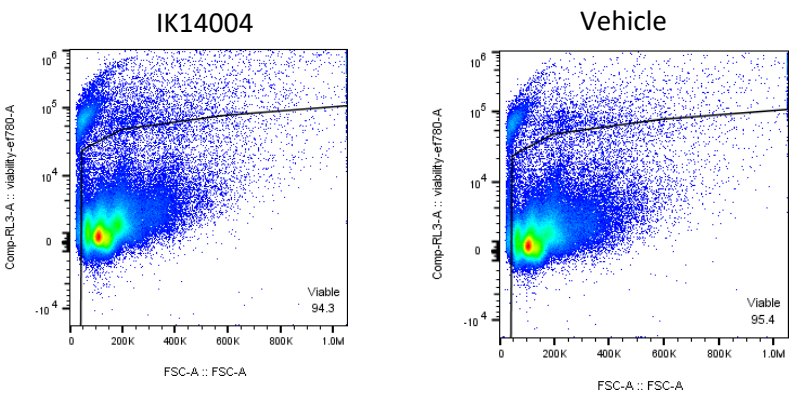
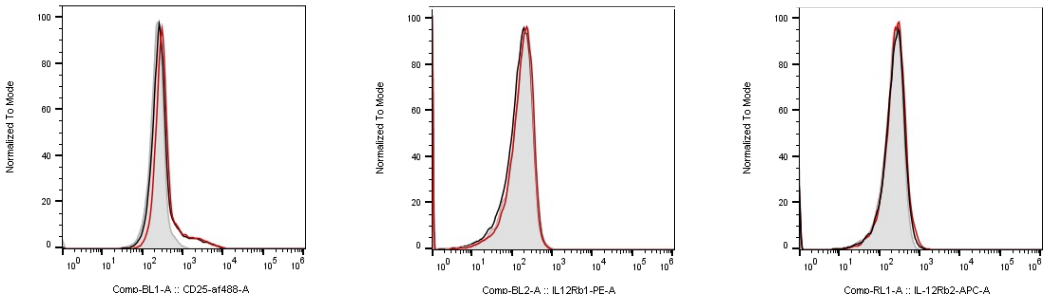


**Figure S2:** Refers to manuscript **Figure 2e – 2k (continued)**
CD25, IL-12Rβ1 and IL-12Rβ2 (Splenocytes derived from the LLC xenograft model CD4+ T cells)

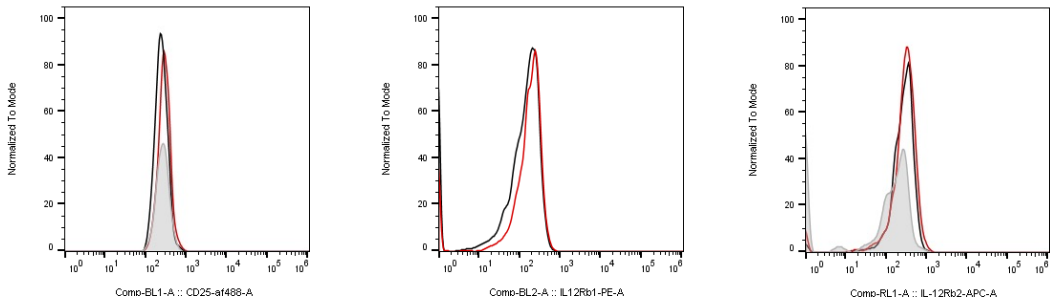
**2f -** CD4+ / CD25 **2g -** CD4+ / IL-12Rβ1 **2h -** CD4+ / IL-12Rβ2




Grey = isotype
Black = vehicle
Red = IK14004

Analysis of CD25, IL-12Rβ1 and IL-12Rβ2 in $CD4^+$ T cells**.**

**2f -** NK1.1+ / CD25 **2g -** NK1.1+ / IL-12Rβ1 **2h -** NK1.1+ / IL-12Rβ2

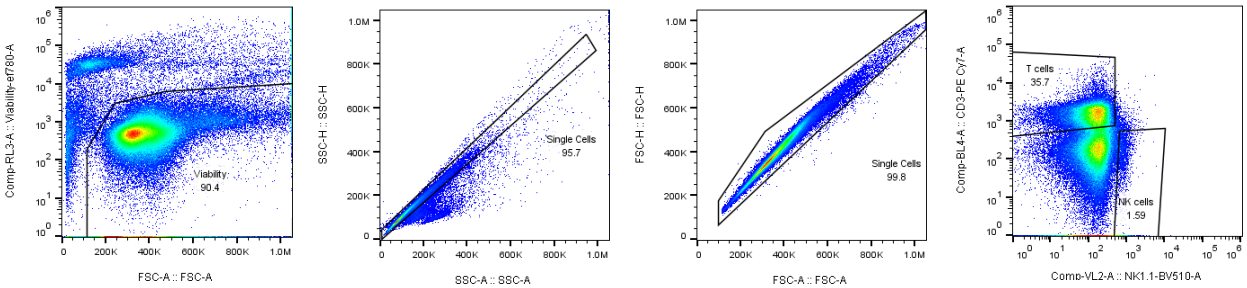



Grey = isotype
Black = vehicle
Red = IK14004

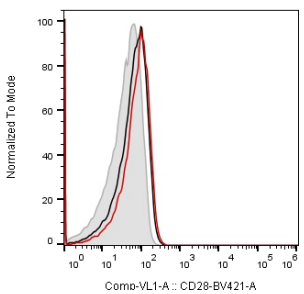
Analysis of CD25, IL-12Rβ1 and IL-12Rβ2 in $NK1.1^+$ cells.

**Figure S3:** Refers to manuscript **Figure 2m**
CD28 (Splenocytes derived from the LLC xenograft model CD4+ T cells)

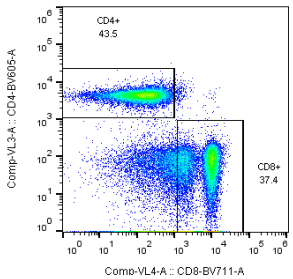
Non-stimulated splenocytes




**2m -** CD4+ / CD28



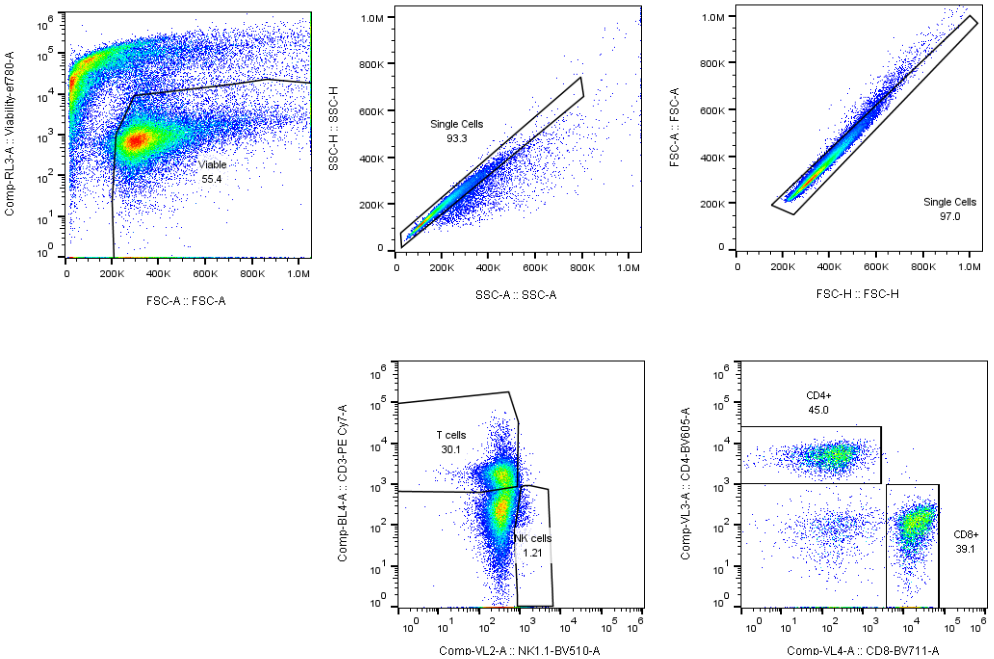

Grey = isotype
Black = vehicle
Red = IK14004




The Figure shows an exemplar gating strategy from mouse 2.0 (IK14004). Viable (live) cells selected, doublets excluded, $CD3^+$ T cell and $CD3^-NK1.1^+$ NK cell populations selected. Within $CD3^+$ cells, $CD4^+$ selected for analysis of CD28.

**Figure S4:** Refers to manuscript **Figure 2n, 2q and 2r**
Viability, IL-12Rβ1 and IL-12Rβ2 (Cultured splenocytes derived from the LLC xenograft model CD4+ T cells)

Viability: cultured splenocytes




**2n -** Viability

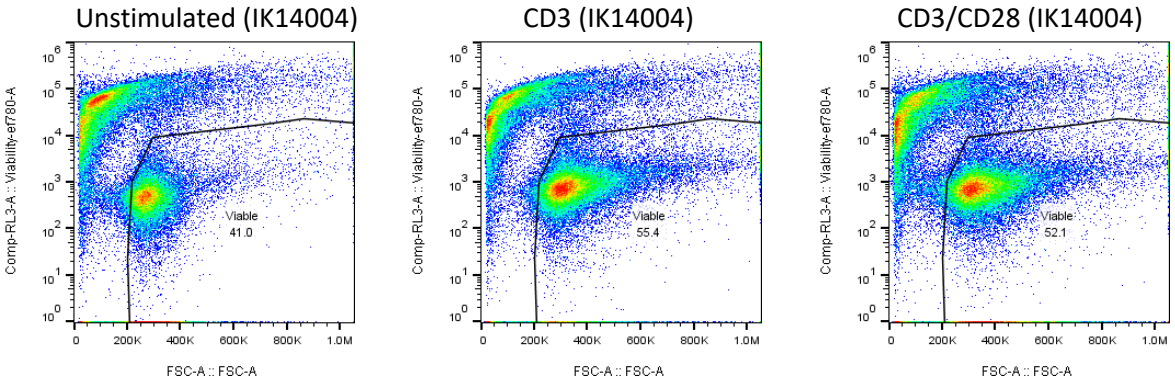
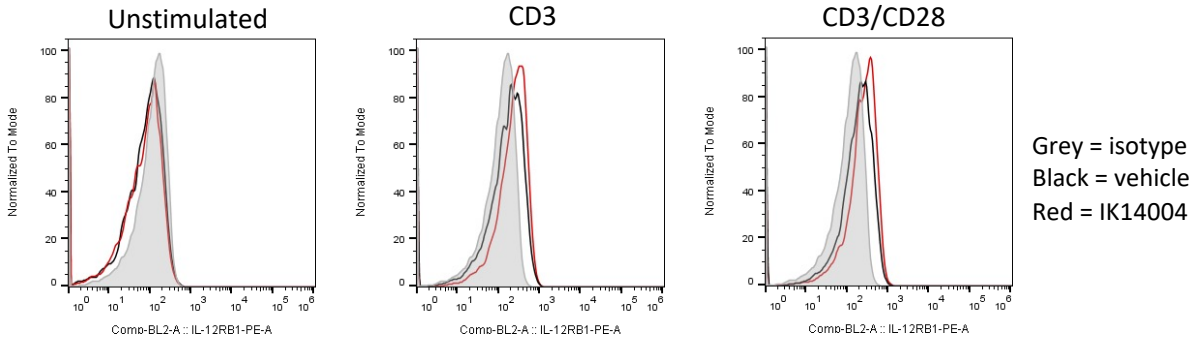
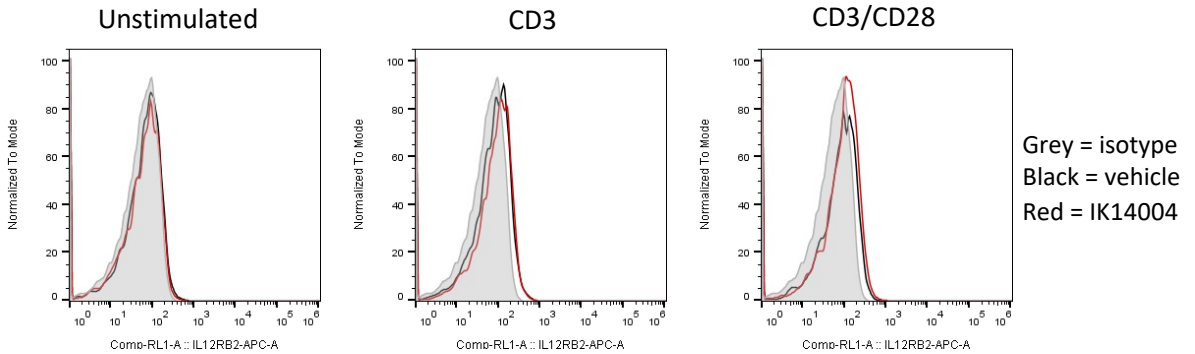


**Figure S4:** Refers to manuscript **Figure 2n, 2q and 2r (continued)**
IL-12Rβ1 and IL-12Rβ2 (Cultured splenocytes derived from the LLC xenograft model CD4+ T cells)

**2q -** CD4+ / IL-12Rβ1

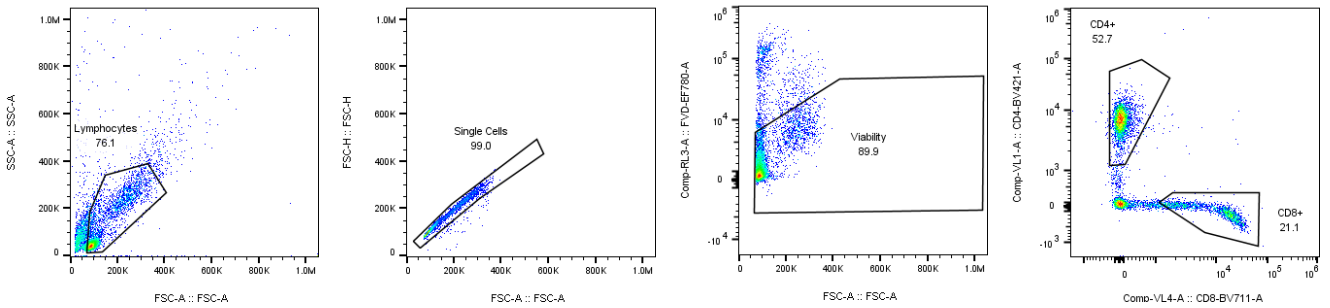



**2r -** CD4+ / IL-12Rβ2

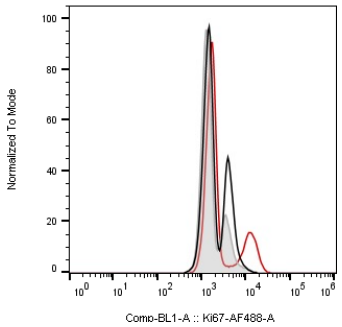



The Figure shows an exemplar gating strategy from anti-CD3 stimulated mouse 2.0 (IK14004). Viable (live) cells selected, doublets excluded, $CD3^+$ T cell and $CD3^-NK1.1^+$ NK cell populations selected. Within $CD3^+$ cells, $CD4^+$ selected for analysis of IL-12Rβ1 and IL-12Rβ2.

**Figure S5:** Refers to manuscript **Figure 3a and 3e**
Ki67 and CD25 (Unstimulated human PBMC cultures)

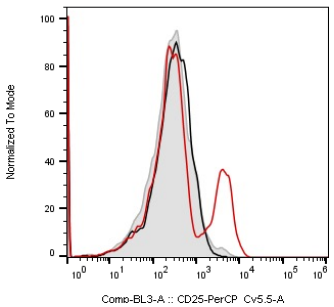



**3a** - CD4+ / Ki67




Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

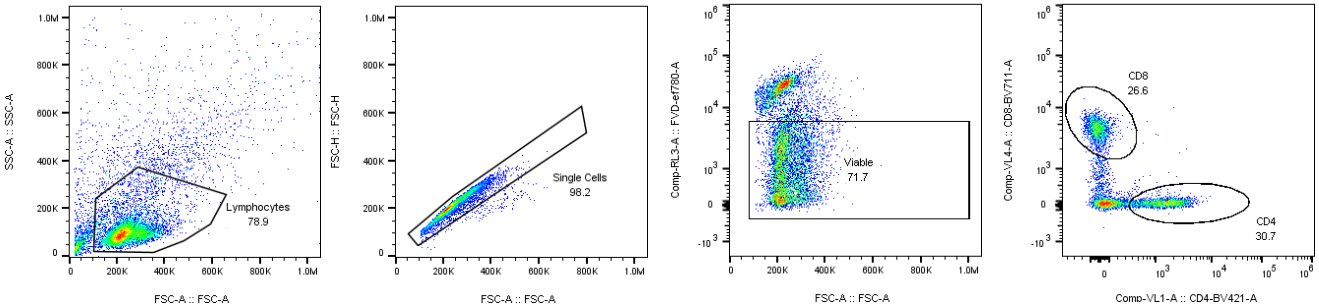
**3e** - CD4+ / CD25



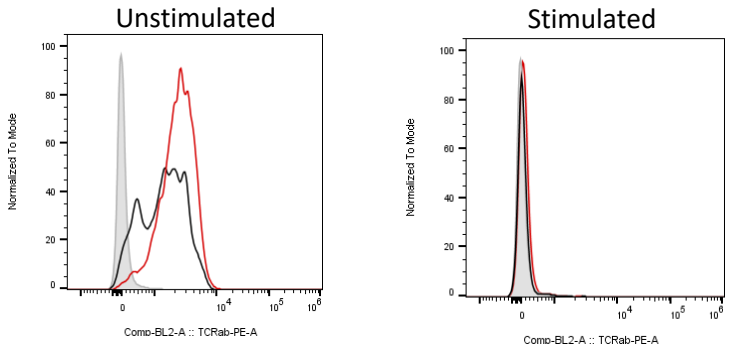

Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 3 unstimulated PBMCs (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, and viable (live) cells selected. Within viable cells, $CD4^+$ T cell selected for analysis of Ki67 and CD25.

**Figure S6:** Refers to manuscript **Figure 3j and 3k**
CD4+/CD8+ **-** TCRα/β (Unstimulated/stimulated human PBMC cultures)

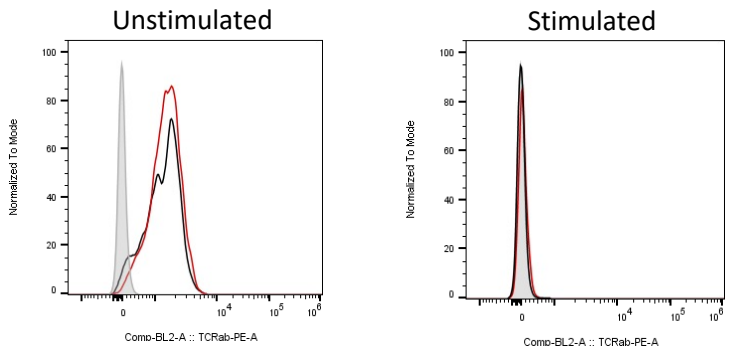



**3j** - CD4+ / TCRα/β




Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

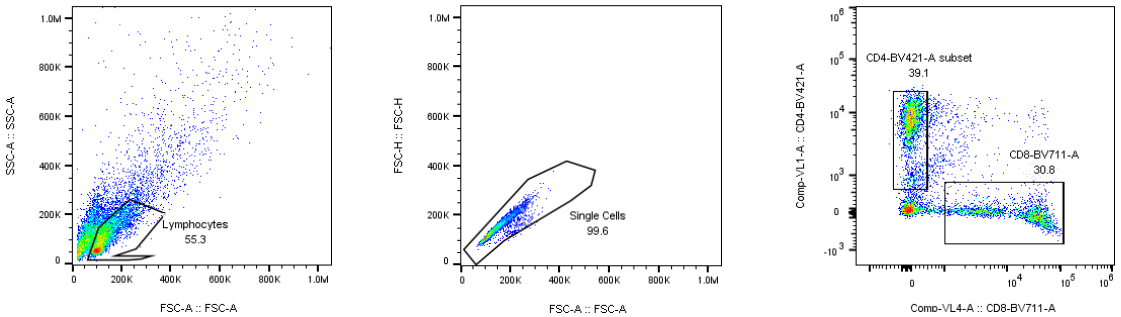
**3k** - CD8+ / TCRα/β




Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

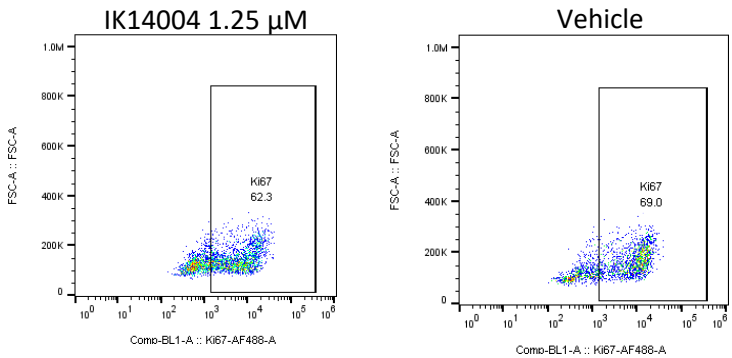
The Figure shows an exemplar gating strategy from Donor 1 unstimulated PBMCs (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion and viable (live) cells selected. Within viable cells, $CD4^+$ and $CD8^+$ T cells selected for analysis of TCRα/β.

**Figure S7:** Refers to manuscript **Figure 4a – 4f**
Ki67, IL-12Rβ1 and IL-12Rβ2 (Stimulated human PBMC cultures: CD4+/CD8+ T cells)

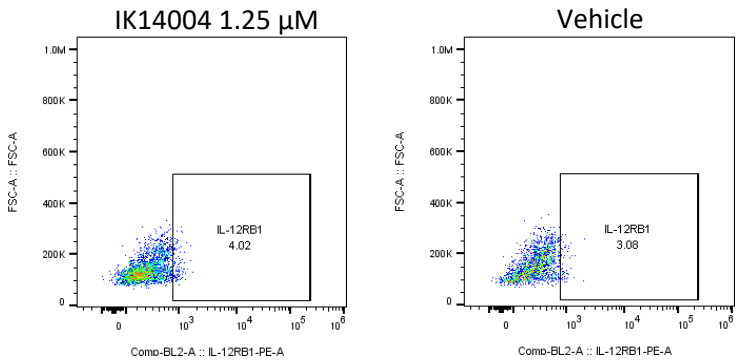



The Figure shows an exemplar gating strategy from Donor 2 stimulated PBMCs (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion and $CD4^+$ and $CD8^+$ T cells selected for analysis of Ki67, IL-12Rβ1 and IL-12Rβ2.

**4a -** CD4+ / Ki67




**4b -** CD4+ / IL-12Rβ1




**4c -** CD4+ / IL-12Rβ2

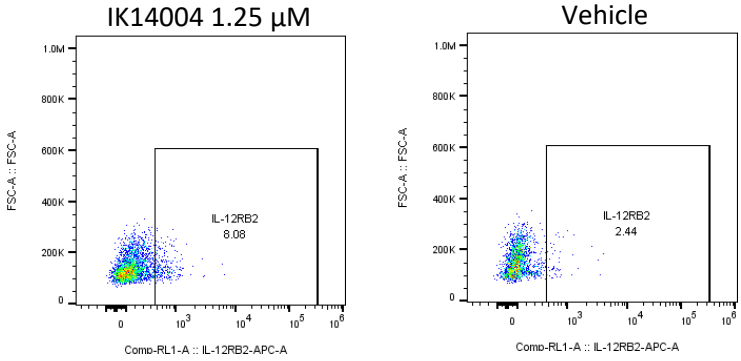
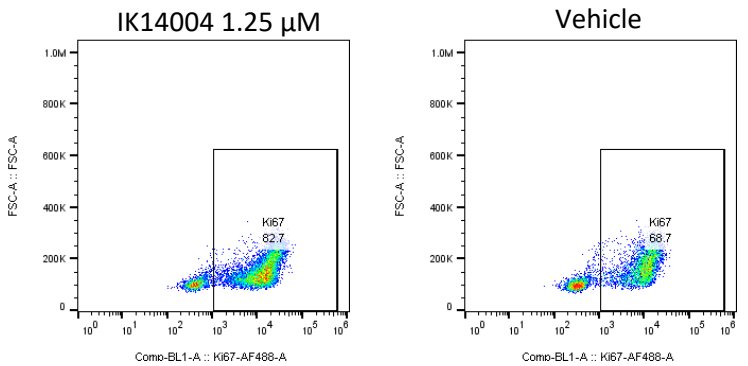
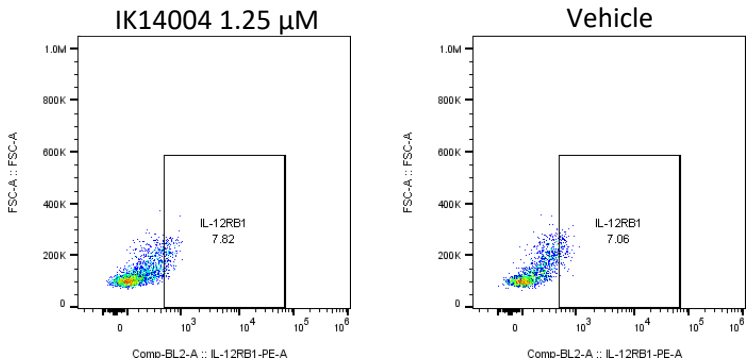


**Figure S7:** Refers to manuscript **Figure 4a – 4f (continued)**
Ki67, IL-12Rβ1 and IL-12Rβ2 (Human PBMC cultures: CD4+/CD8+ T cells)

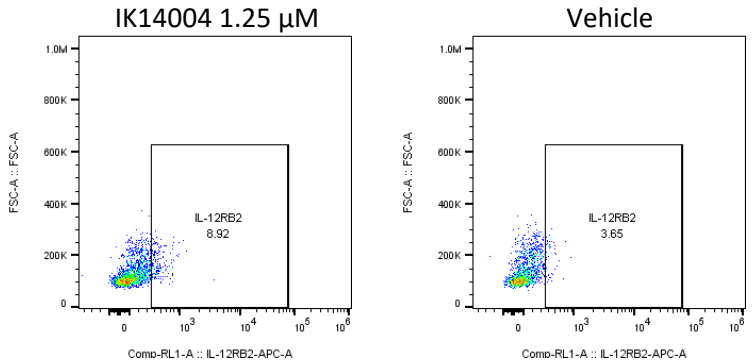
**4d -** CD8+ / Ki67




**4e -** CD8+ / IL-12Rβ1

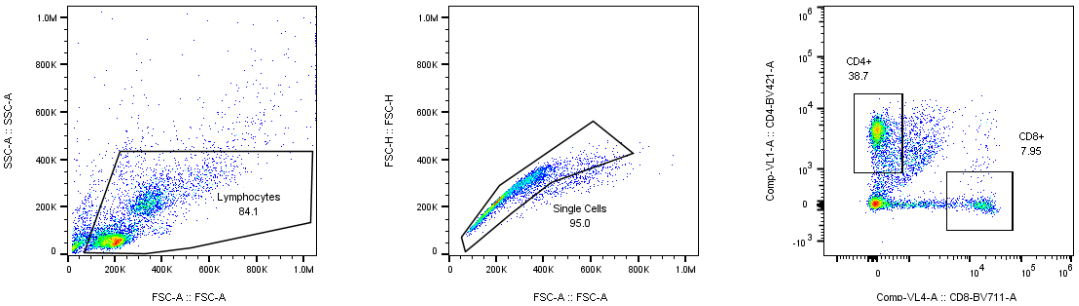



**4f -** CD8+ / IL-12Rβ2

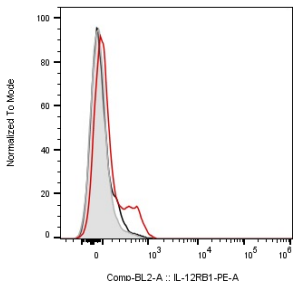



Cells gated to remove debris, doublet cell exclusion and $CD4^+$ and $CD8^+$ T cells selected for analysis of Ki67, IL-12Rβ1 and IL-12Rβ2 (Donor 2 and 3).

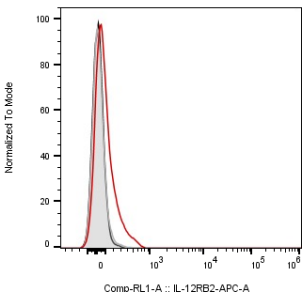
**Figure S8:** Refers to manuscript **Figure 4g – 4j**
IL-12Rβ1 and IL-12Rβ2 (Unstimulated human PBMC cultures: CD4+/CD8+ T cells)




**4g -** CD4+ / IL-12Rβ1

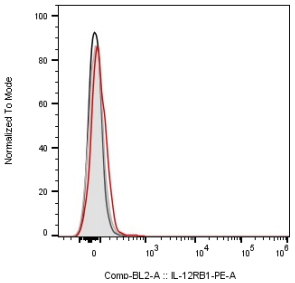



**4h -** CD4+ / IL-12Rβ2

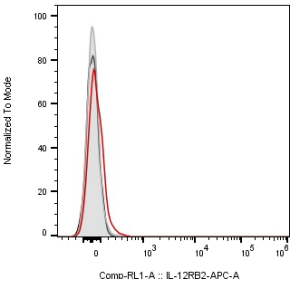



Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

**4i -** CD8+ / IL-12Rβ1



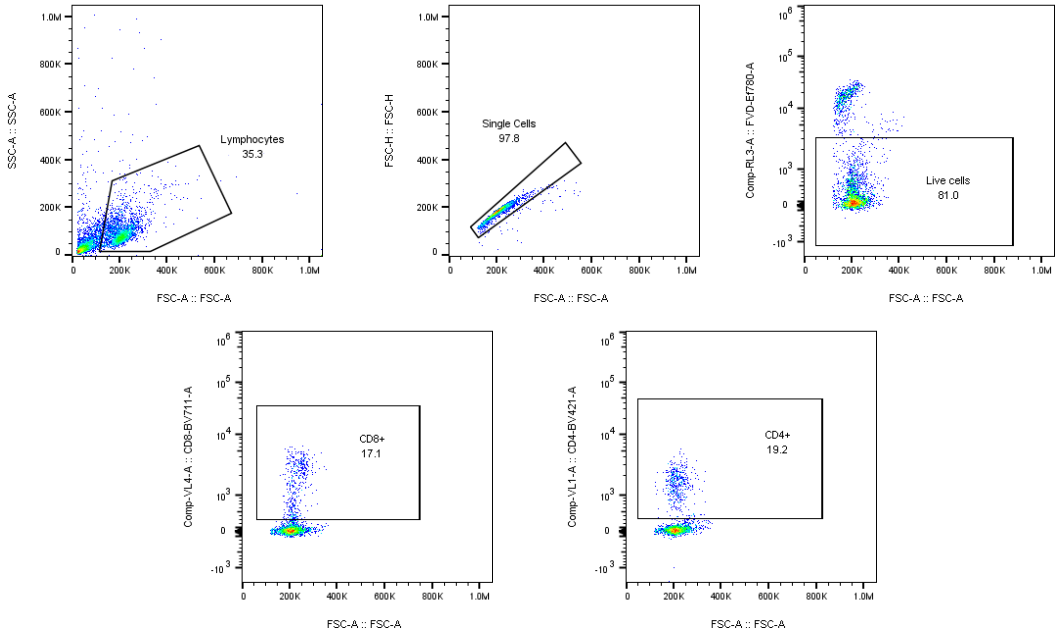

**4j -** CD8+ / IL-12Rβ2



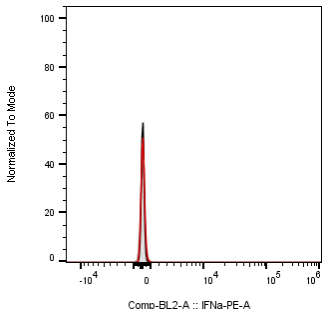

Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 1 unstimulated PBMCs (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion and $CD4^+$ and $CD8^+$ T cells selected for analysis of IL-12Rβ1 and IL-12Rβ2.

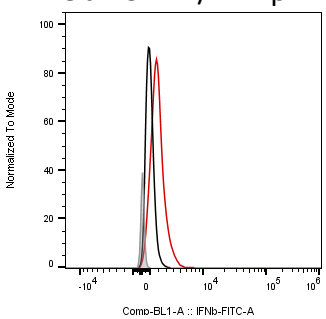
**Figure S9:** Refers to manuscript **Figure 5a - 5d**
IFN-α and IFN-β (Stimulated human PBMC cultures: CD4+/CD8+ T cells)




**5a -** CD4+ / IFN-α

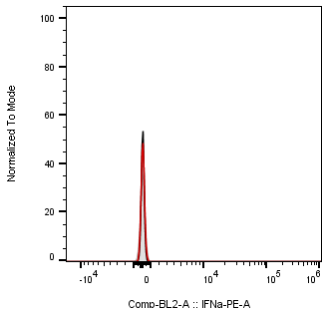



**5b -** CD4+ / IFN-β

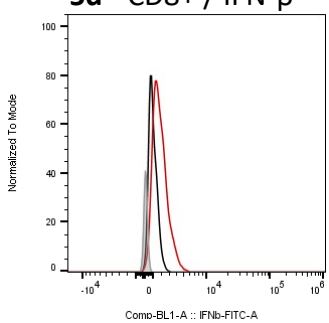



Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

**5c -** CD8+ / IFN-α



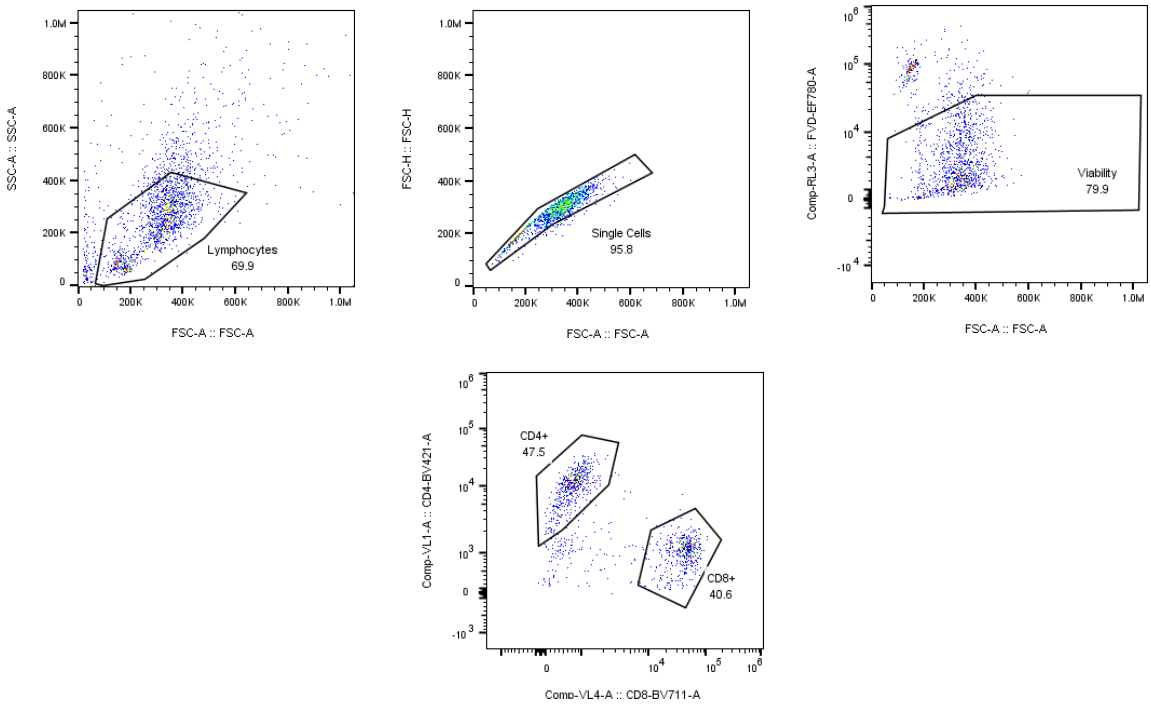

**5d -** CD8+ / IFN-β



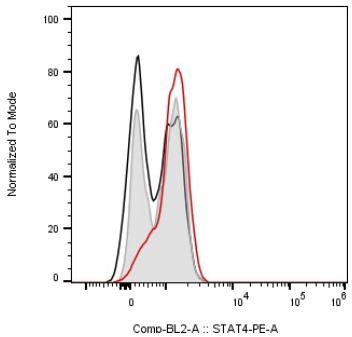

Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 2 stimulated PBMCs (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, viable cells gated and $CD4^+$ and $CD8^+$ T cells selected for analysis of IFN-α and IFN-β.

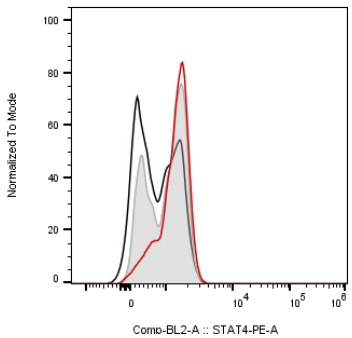
**Figure S10:** Refers to manuscript **Figure 5e and 5f**
pSTAT4 (Isolated, stimulated human CD3+ T cells)




**5e -** CD4+ / pSTAT4



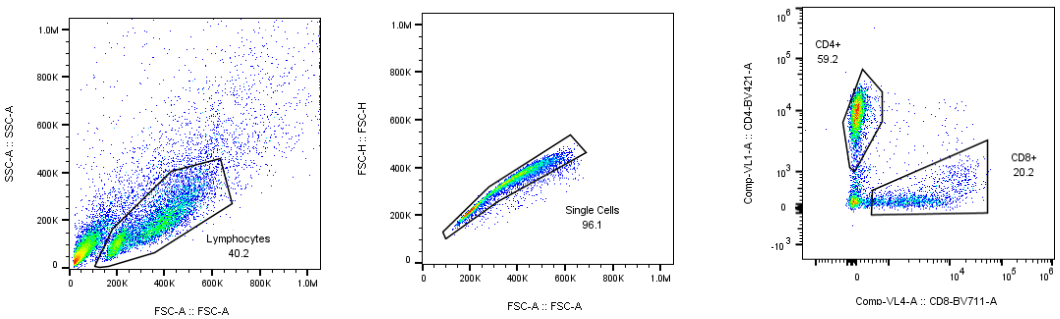

**5f -** CD8+ / pSTAT4



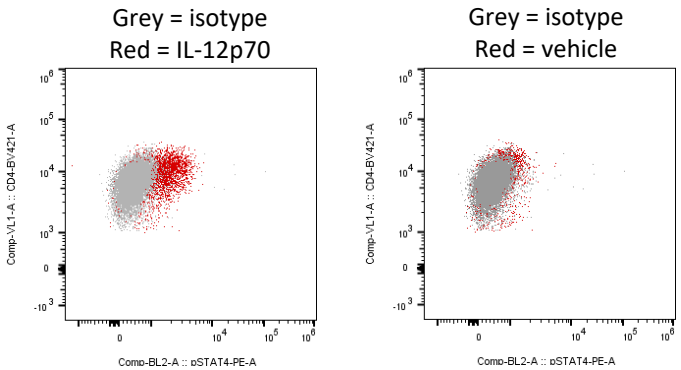

Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 1 stimulated CD3⁺ T cells (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, viable cells gated and CD4⁺ and CD8⁺ T cells selected for analysis of pSTAT4.

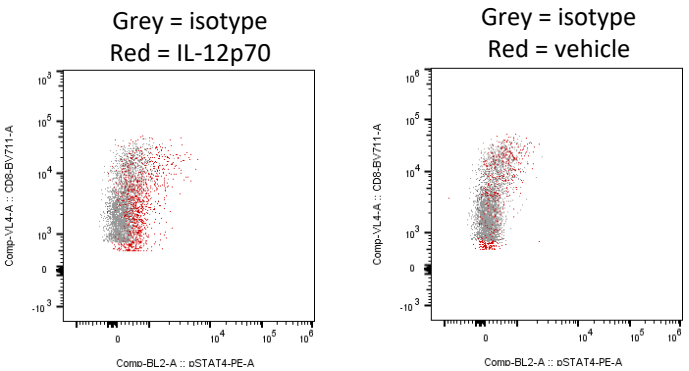
**Figure S11:** Refers to manuscript **Figure 5g and 5h**
pSTAT4 (Stimulated human PBMC cultures: CD4+/CD8+ T cells)




**5g -** CD4+ / pSTAT4

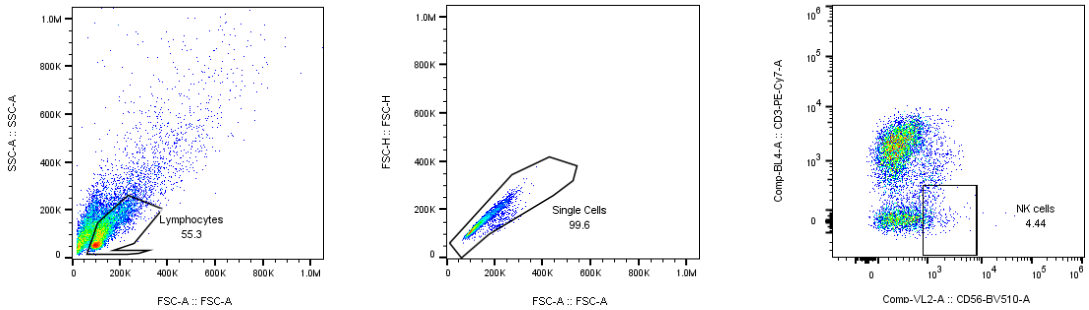



**5h -** CD8+ / pSTAT4

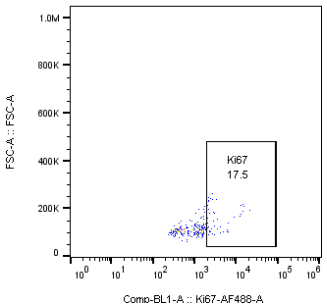



The Figure shows an exemplar gating strategy from Donor 1 stimulated PBMC (IL-12p70). Cells gated to remove debris, doublet cell exclusion, and CD4⁺ and CD8⁺ T cells selected for analysis of pSTAT4.

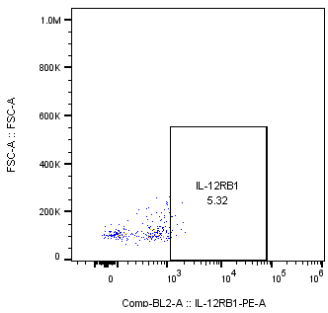
**Figure S12:** Refers to manuscript **Figure 6a - 6c**
Ki67, IL-12Rβ1 and IL-12Rβ2 (Stimulated human PBMC cultures: CD3-CD56+$^{dim}$ NK cells)



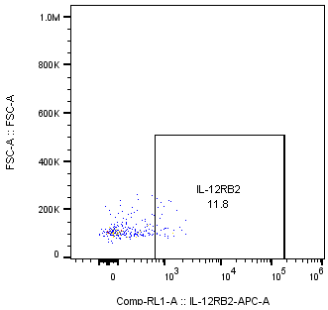

**6a -** CD3-CD56+$^{dim}$ / Ki67




**6b -** CD3-CD56+$^{dim}$ / IL-12Rβ1

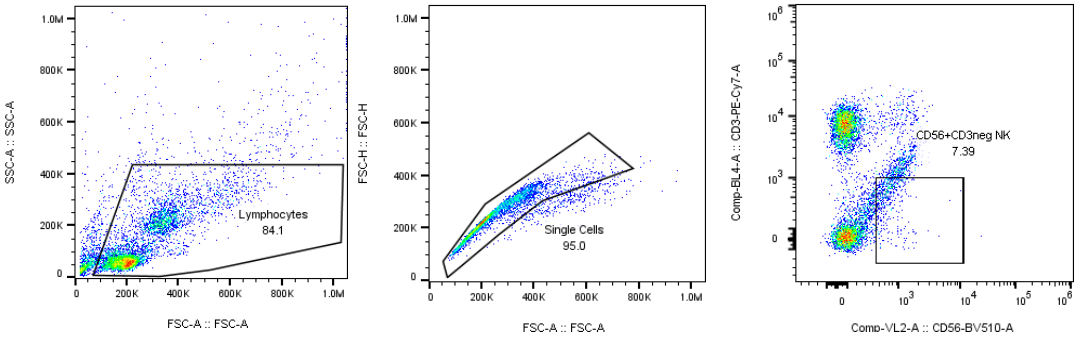



**6c -** CD3-CD56+$^{dim}$ / IL-12Rβ2

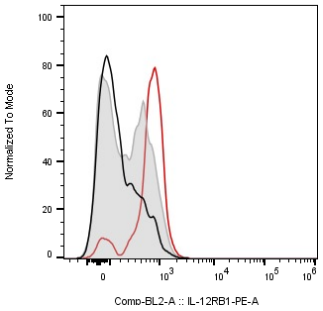



The Figure shows an exemplar gating strategy from Donor 3 stimulated PBMC (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, and CD3-CD56+$^{dim}$ NK cells selected for analysis of Ki67, IL-12Rβ1 and IL-12Rβ2 (IK14004 1.25 µM).

**Figure S13:** Refers to manuscript **Figure 6d and 6e**
IL-12Rβ1 and IL-12Rβ2 (Unstimulated human PBMC cultures: CD3-CD56+$^{dim}$ NK cells)

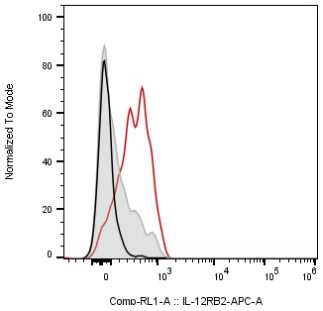



**6d -** CD3-CD56+$^{dim}$ / IL-12Rβ1




Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

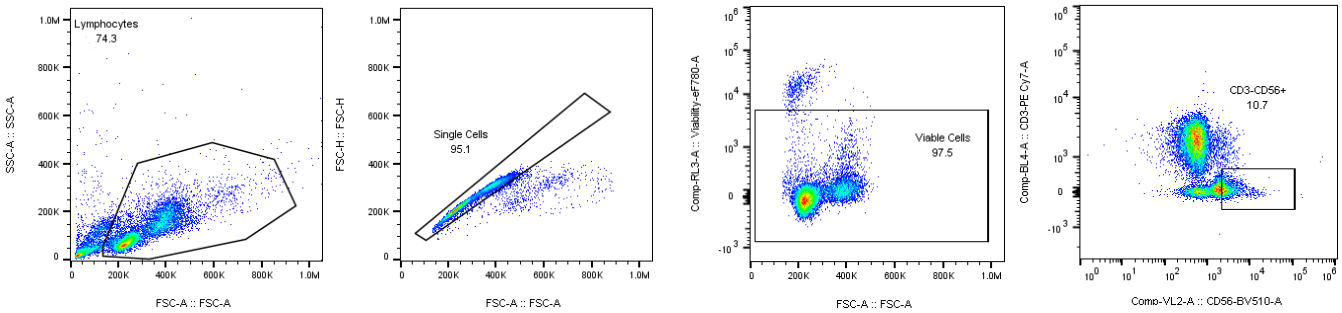
**6e -** CD3-CD56+$^{dim}$ / IL-12Rβ2



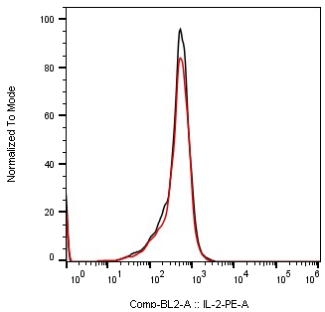

Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 1 unstimulated PBMC (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, and CD3-CD56+$^{dim}$ NK cells selected for analysis of IL-12Rβ1 and IL-12Rβ2.

**Figure S14:** Refers to manuscript **Figure 6f**
Intracellular IL-2 (Unstimulated human PBMC cultures)



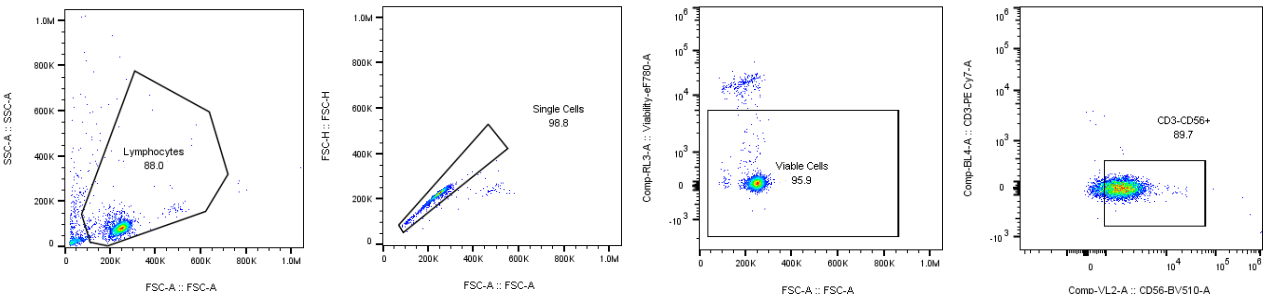

**6f -** CD3-CD56+$^{dim}$ / IL-2



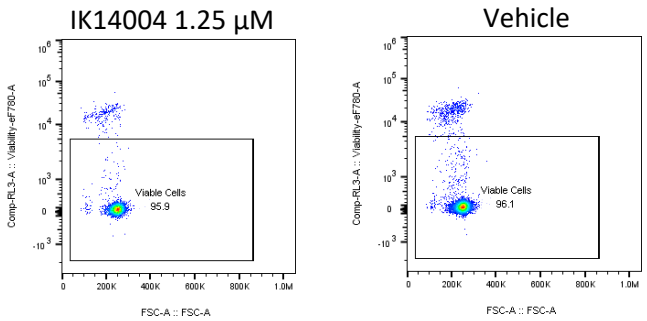

Black = vehicle
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 3 unstimulated PBMC (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, viable cells gated and CD3-CD56+$^{dim}$ NK cells selected for analysis of intracellular IL-2.

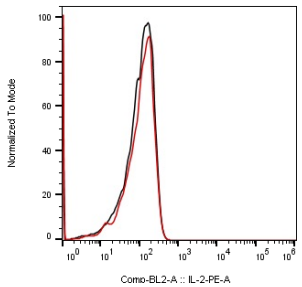
**Figure S15:** Refers to manuscript **Figure 6g and 6h**
Viability and Intracellular IL-2 (Isolated CD3-CD56+$^{dim}$ NK cells)




**6g -** CD3-CD56+$^{dim}$ / Viability



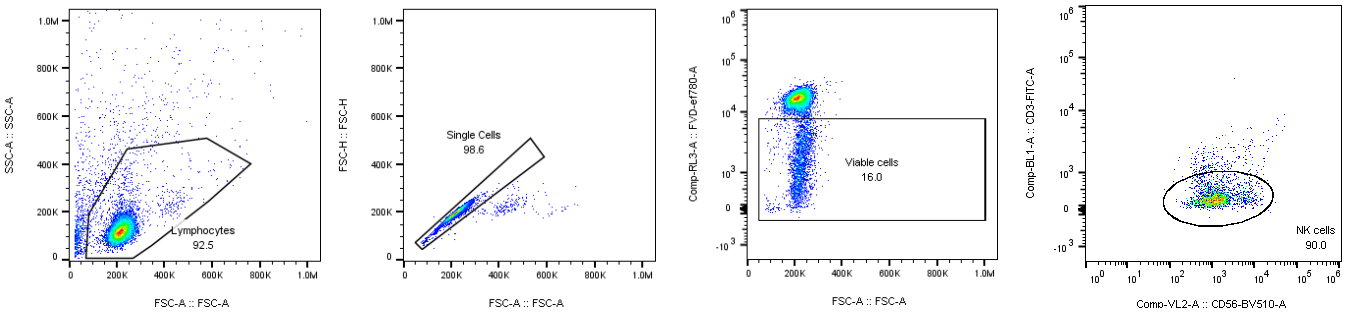

**6h -** CD3-CD56+$^{dim}$ / Intracellular IL-2



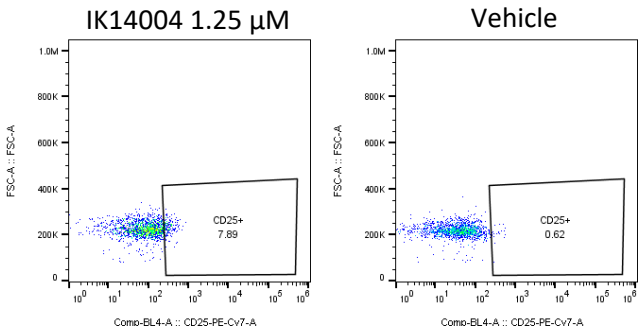

Black = vehicle
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 1 unstimulated NK cells (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, viable cells gated and CD3-CD56+$^{dim}$ NK cells selected for analysis of intracellular IL-2.

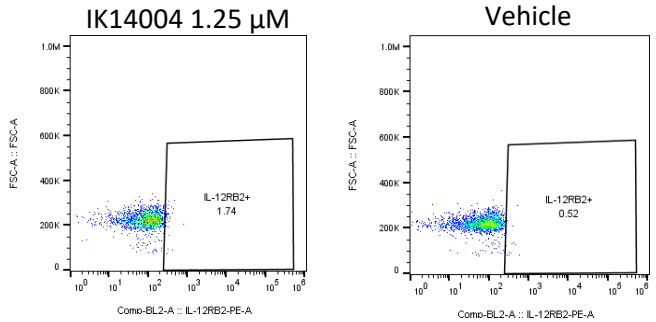
**Figure S16:** Refers to manuscript **Figure 6i and 6j**
CD25 and IL-12Rβ2 (Isolated CD3-CD56+$^{dim}$ NK cells)




**6i -** CD3-CD56+$^{dim}$ / CD25

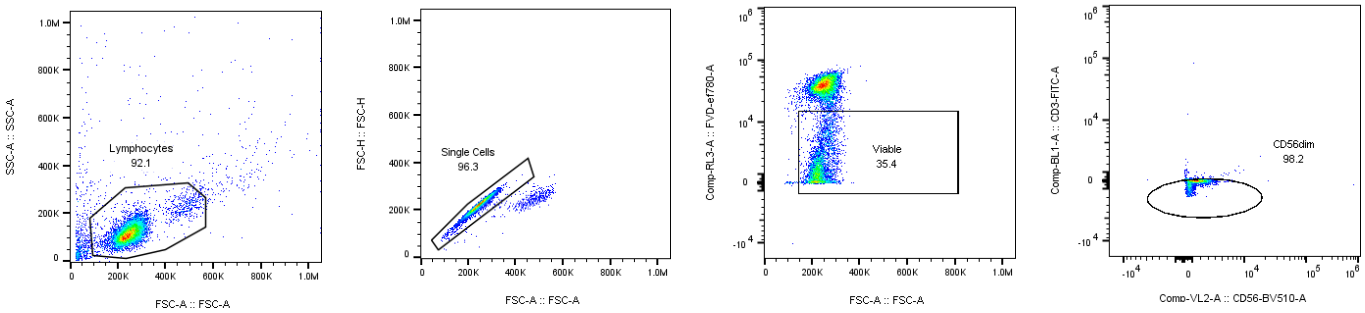



**6j -** CD3-CD56+$^{dim}$ / IL-12Rβ2

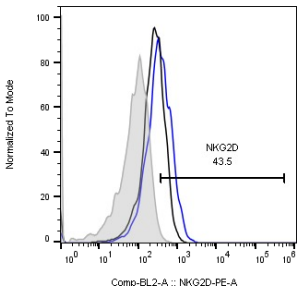



The Figure shows an exemplar gating strategy from Donor 4 unstimulated NK cells (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, viable cells gated and CD3-CD56+$^{dim}$ NK cells selected for analysis of CD25 and IL-12Rβ2.

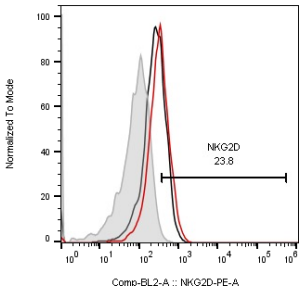
**Figure S17:** Refers to manuscript **Figure 6n - 6p**
NKG2D and NKp44 (Isolated CD3-CD56+$^{dim}$ NK cells)



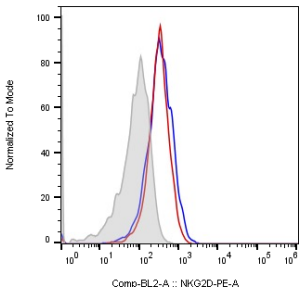

**6n -** CD3-CD56+$^{dim}$ / NKG2D




Grey = isotype
Black = vehicle
Blue = rIL-2

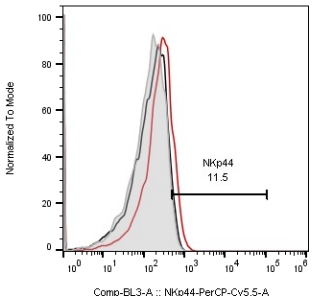



Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM



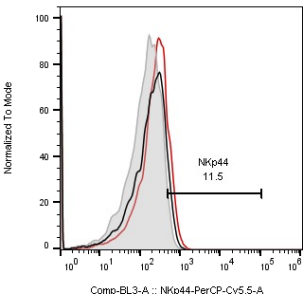

Grey = isotype
Blue = rIL-2
Red = IK14004 1.25 µM

**6o -** CD3-CD56+$^{dim}$ / NKp44




Grey = isotype
Black = vehicle
Red = IK14004 1.25 µM

**6p -** CD3-CD56+$^{dim}$ / NKp44




Grey = isotype
Black = rIL-2
Red = IK14004 1.25 µM

The Figure shows an exemplar gating strategy from Donor 3 unstimulated NK cells (IK14004 1.25 µM). Cells gated to remove debris, doublet cell exclusion, viable cells gated and CD3-CD56+$^{dim}$ NK cells selected for analysis of NKG2D and NKp44.