A novel immunomodulating peptide with potential to complement oligodeoxynucleotide-mediated adjuvanticity in vaccination strategies

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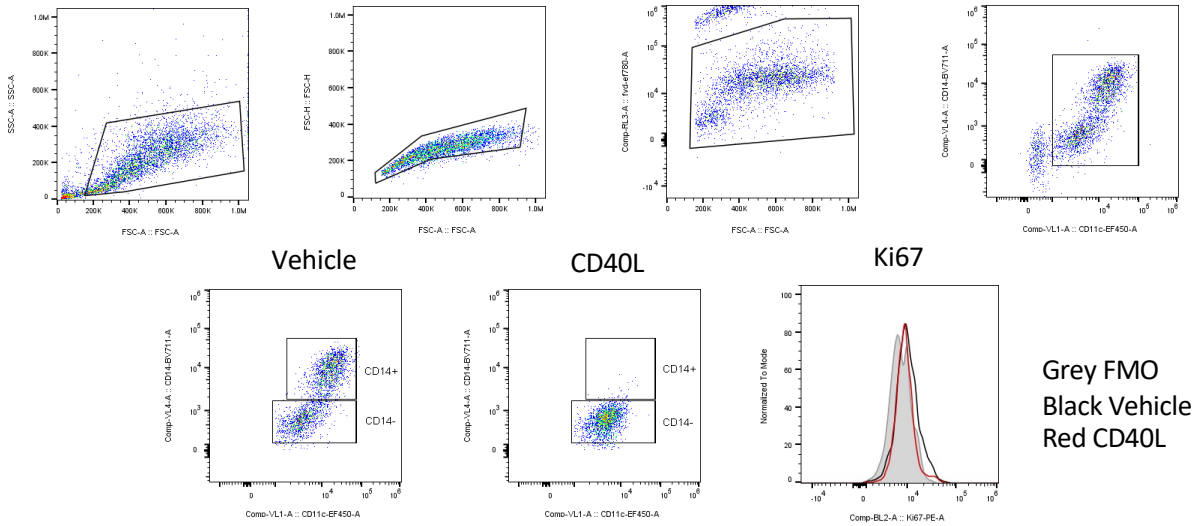
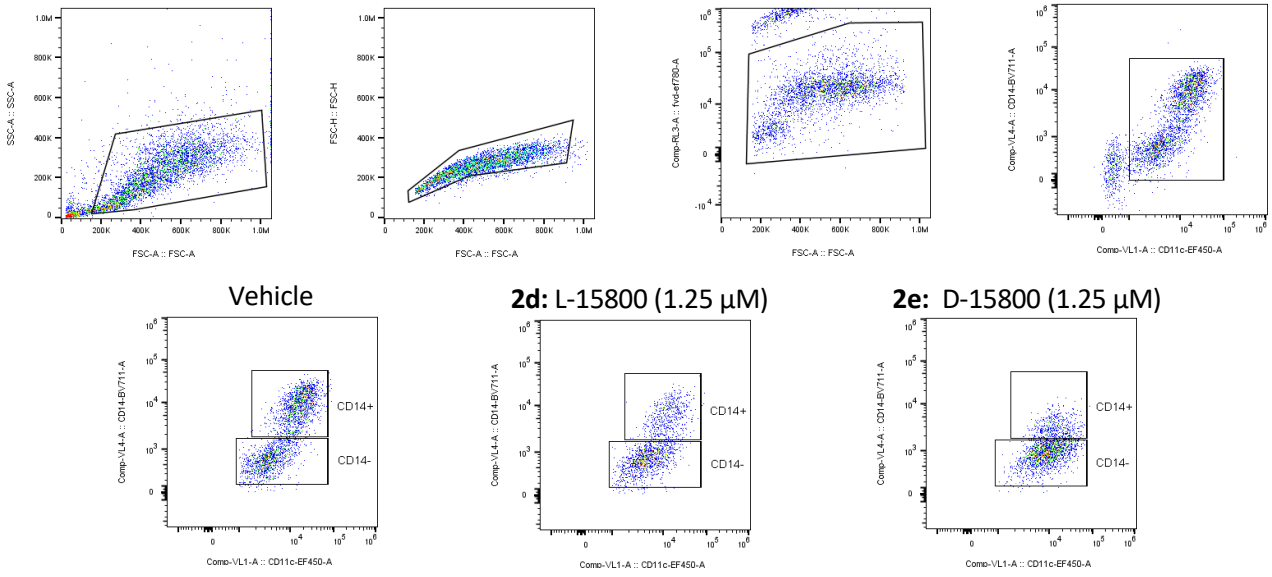
Supplementary information

Figure S1: Refers to manuscript **Figure 1a and 1b**
CD14^{+}/CD14^{neg} (Monocyte-derived Dendritic Cells)



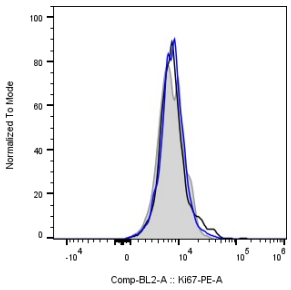
Debris and multicellular events were excluded, then viable (live) cells gated and CD11c^{+} cells identified. Within the CD11c^{+} population, CD14^{+} and CD14^{neg} cell populations were identified.

Figure S2: Refers to manuscript **Figure 1c and 1d**
CD14^{+}/CD14^{neg} (Monocyte-derived Dendritic Cells)

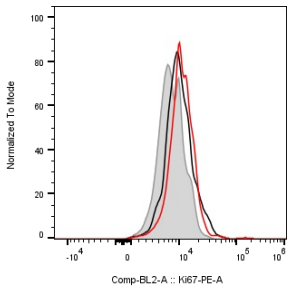


Debris and multicellular events were excluded, then viable (live) cells gated and CD11c^{+} cells identified. Within the CD11c^{+} population, CD14^{+} and CD14^{neg} cell populations were identified.

Figure S3: Refers to manuscript **Figure 1e**
Ki67 (Monocyte-derived Dendritic Cells)

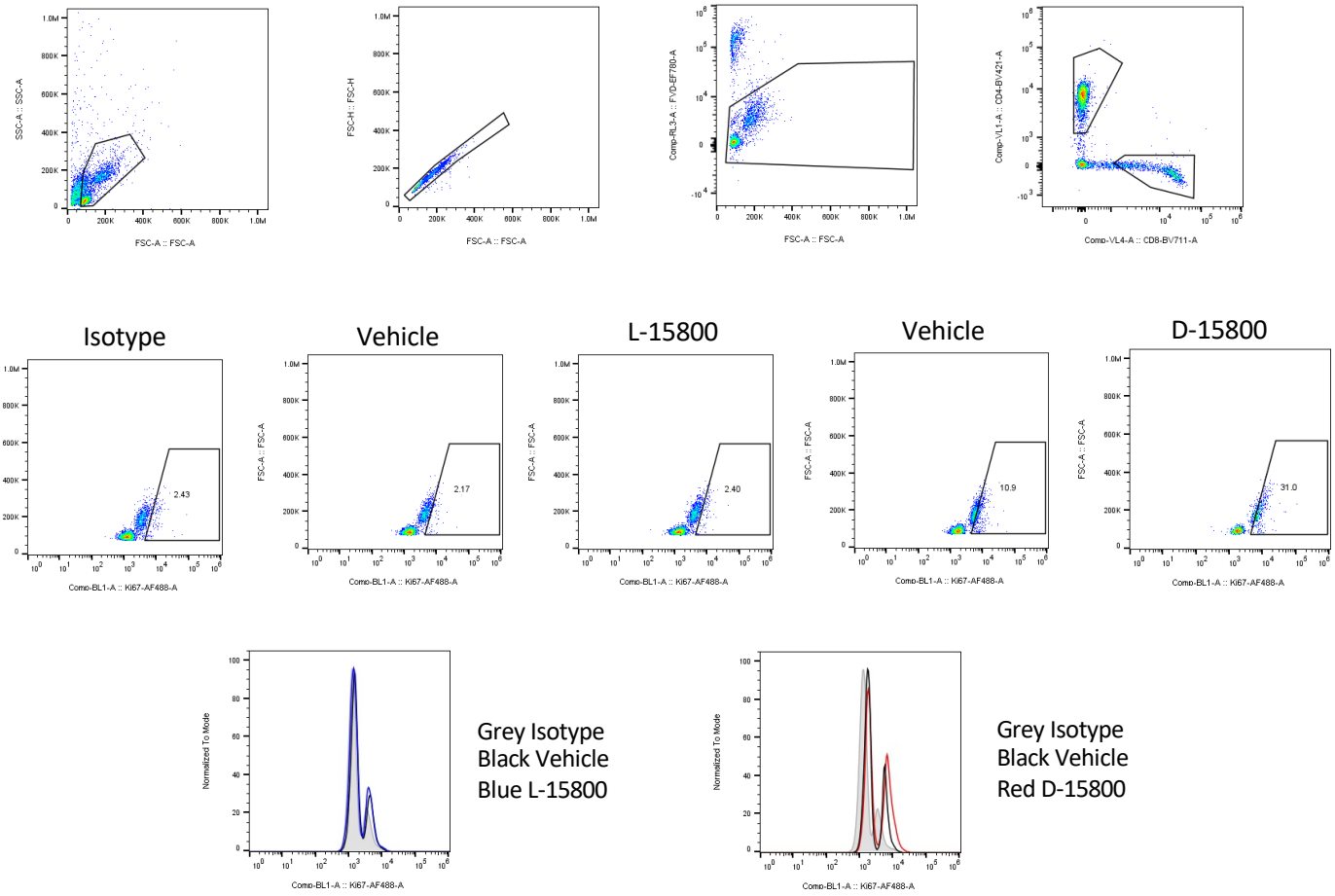


Grey FMO
Black Vehicle
Blue L-15800



Grey FMO
Black Vehicle
Red D-15800

Figure S4: Refers to manuscript **Figure 2c and 2d**
Ki67 (Unstimulated human PBMC cultures: CD4⁺ T cells)

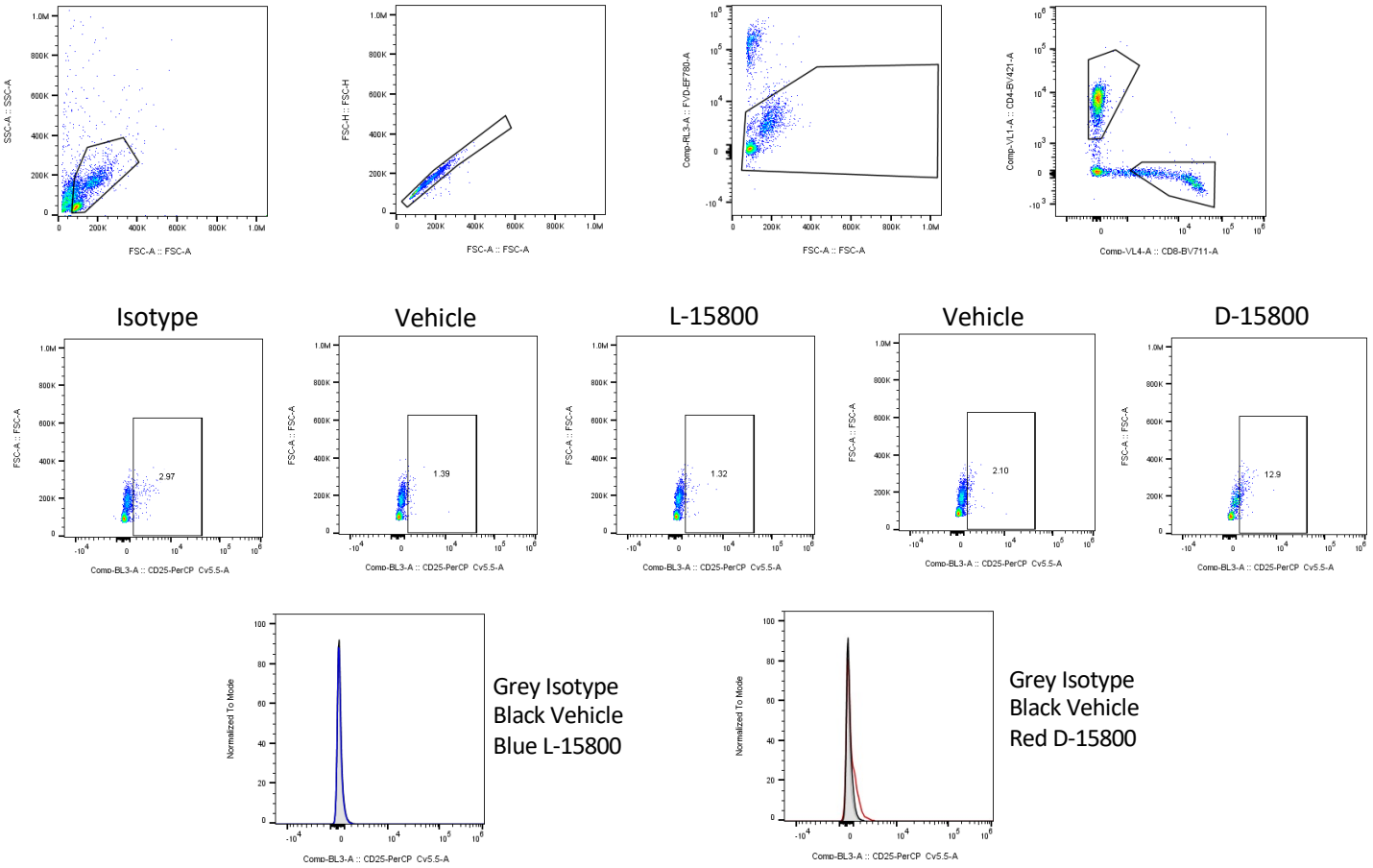


Grey Isotype
Black Vehicle
Blue L-15800

Grey Isotype
Black Vehicle
Red D-15800

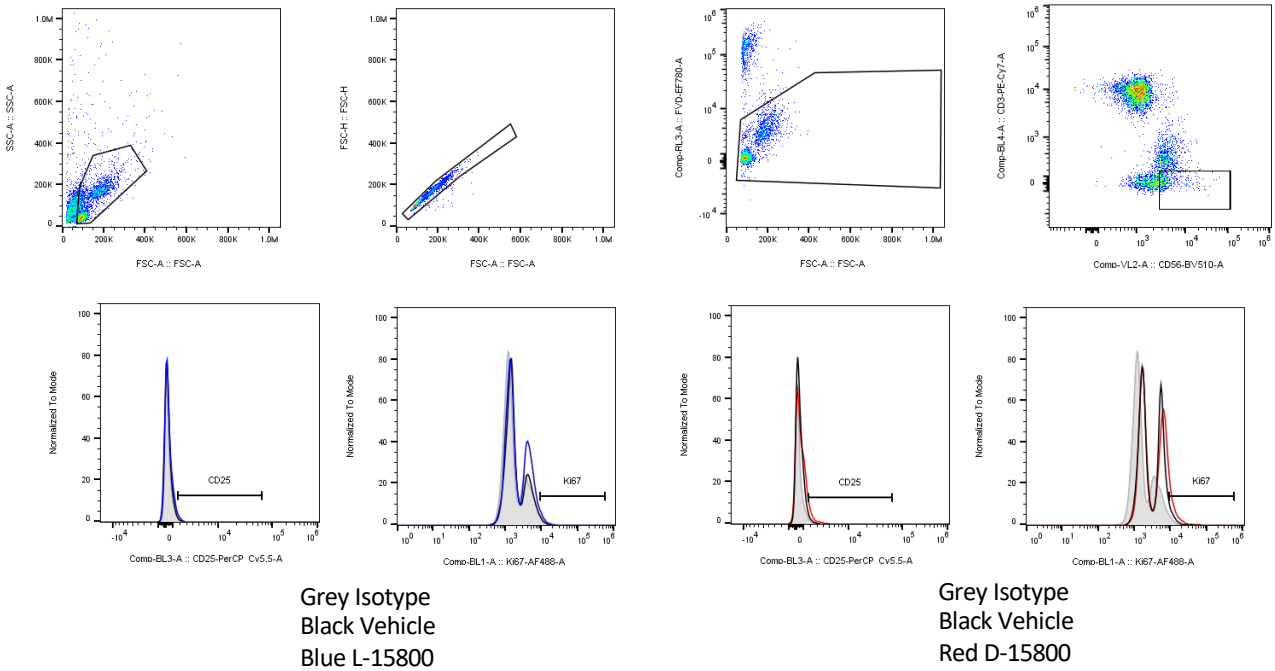
Debris and multicellular events were excluded, then viable (live) cells gated. CD4⁺ cells selected for analysis of Ki67. L-15800 and D-15800 treatment groups were cultured across different plates, with vehicle control groups included on each plate.

Figure S5: Refers to manuscript **Figure 2e and 2f**
CD25 (Unstimulated human PBMC cultures: CD4⁺ T cells)



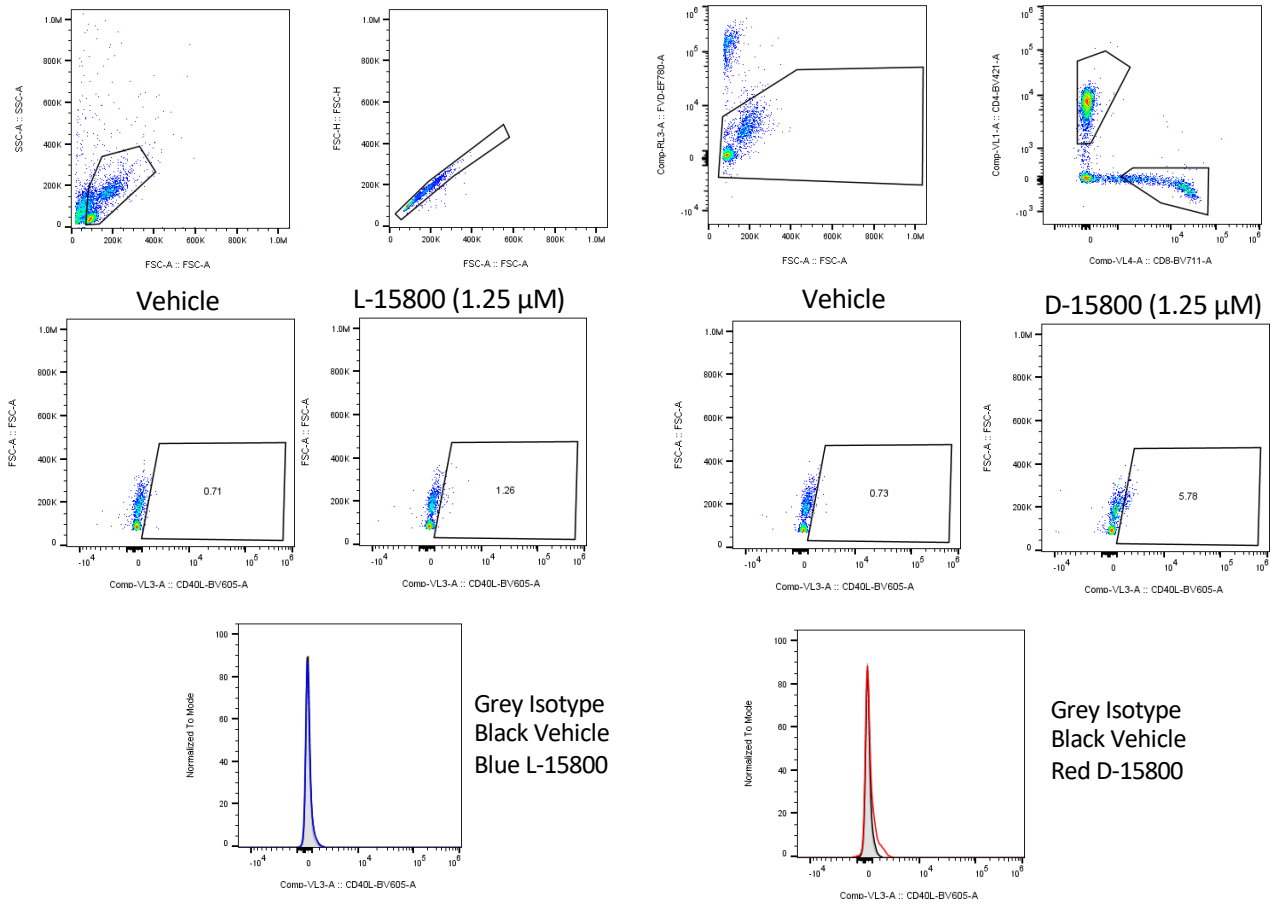
Debris and multicellular events were excluded, then viable (live) cells gated. CD4⁺ cells selected for analysis of CD25. L-15800 and D-15800 treatment groups were cultured across different plates, with vehicle control groups included on each plate.

Figure S6: Refers to manuscript **Figure 2g – 2j**
Ki67 and CD25 (Unstimulated human PBMC cultures: $CD3^{neg}$ $CD56^{+}$ cells)



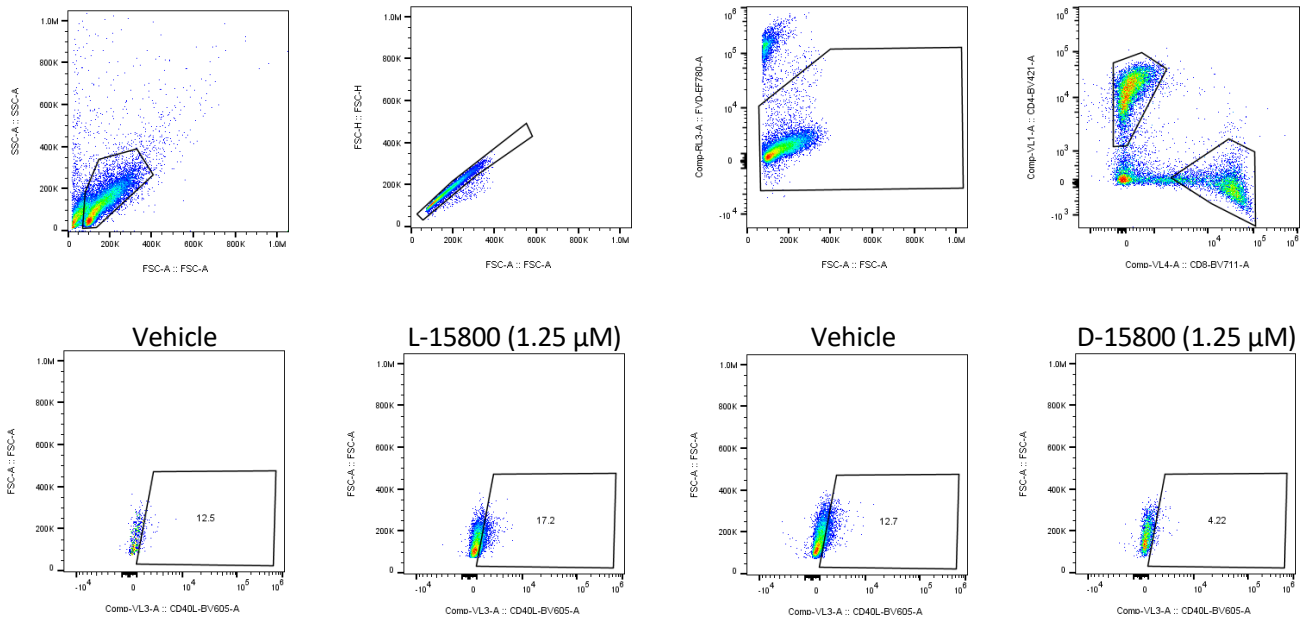
Debris and multicellular events were excluded, then viable (live) cells gated. $CD3^{neg}$ $CD56^{+}$ cells selected for analysis of Ki67 and CD25.

Figure S7: Refers to manuscript **Figure 2k and 2l**
CD40L (Unstimulated human PBMC cultures: CD4⁺ T cells)



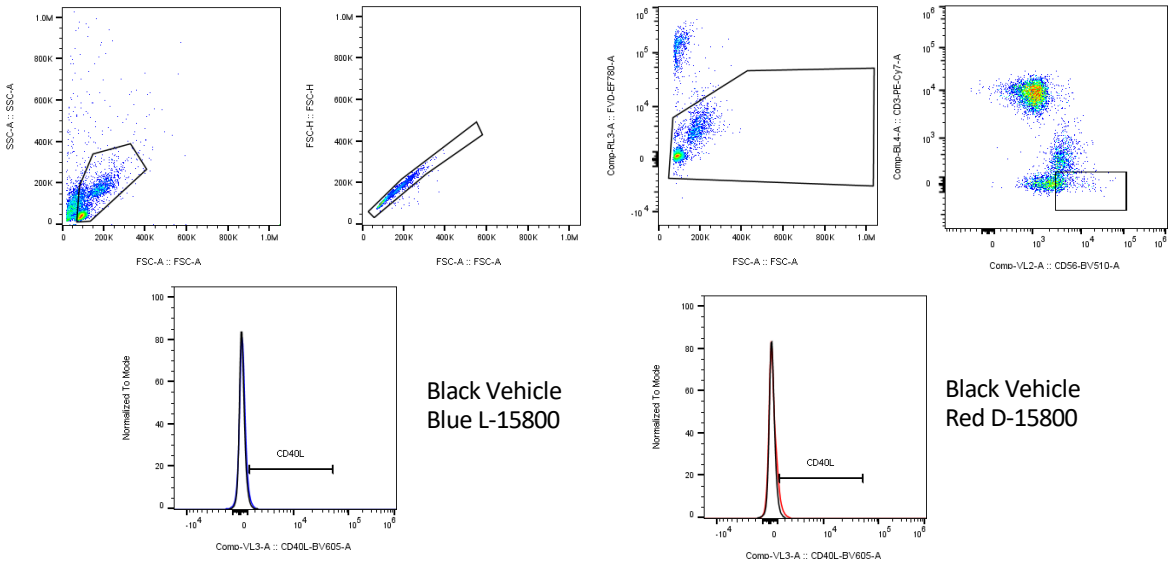
Debris and multicellular events were excluded, then viable (live) cells gated and CD4⁺ cells selected for analysis of CD40L. L-15800 and D-15800 treatment groups were cultured across different plates, with vehicle control groups included on each plate.

Figure S8: Refers to manuscript **Figure 2m**
CD40L (Stimulated human PBMC cultures: CD4⁺ T cells)



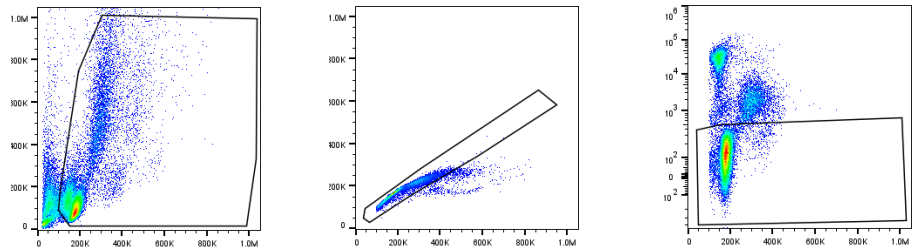
Debris and multicellular events were excluded, then viable (live) cells gated. CD4⁺ T cells selected for analysis of CD40L. L-15800 and D-15800 treatment groups were cultured across different plates, with vehicle control groups included on each plate.

Figure S9: Refers to manuscript **Figure 2n**
CD40L (Unstimulated human PBMC cultures: $CD3^{neg}$ $CD56^{+}$ cells)



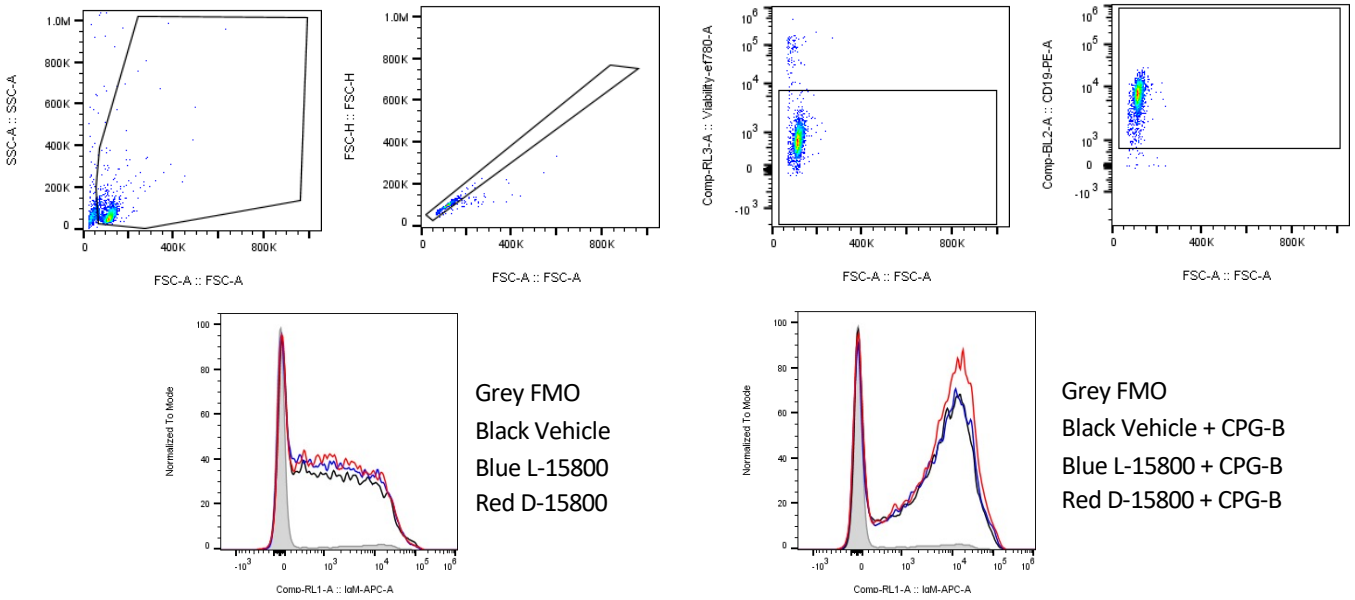
Debris and multicellular events were excluded, then viable (live) cells gated and $CD3^{neg}CD56^{+}$ cells selected for analysis of CD40L. L-15800 and D-15800 treatment groups were cultured across different plates, with vehicle control groups included on each plate.

Figure S10: Refers to manuscript **Figure 3b**
Cell viability (unstimulated PBMCs)



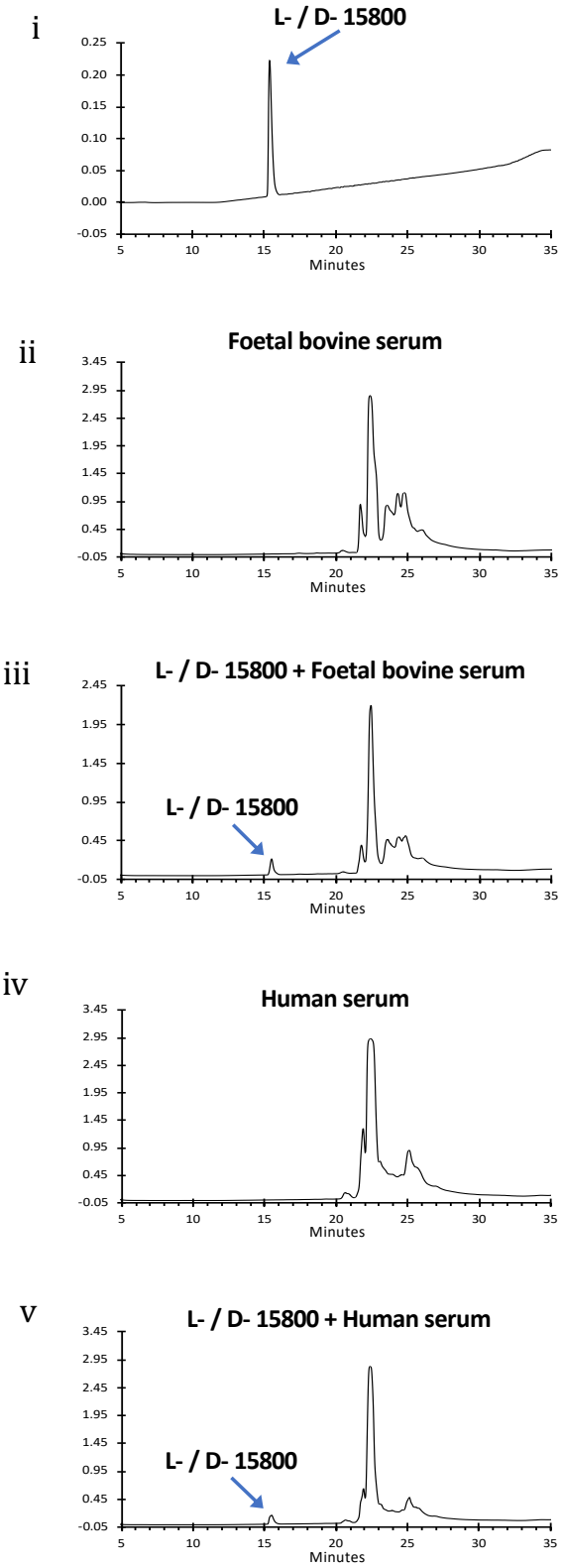
Debris and multicellular events were excluded, then viable (live) cells gated.

Figure S11: Refers to manuscript **Figure 4a and Figures 4e - 4h**
Cell viability and IgM^{+} (Isolated $CD19^{+}$ expressing B cells)



Debris and multicellular events were excluded, viable (live) cells selected and $CD19^{+}$ cells identified for analysis of IgM expression.

Figure S12 – Refers to manuscript **Figure 5a and 5b:**
Elution profile of 15800 peptide isomers and sera from a Phenomenex™ Jupiter 5 µm, C4, 300Å column



The percentage and rate of degradation of the L-15800 and D-15800 peptide isomers in both human serum (HS) and foetal bovine serum (FBS) at 37°C over 48 hours were determined by area under the curve (AUC) analysis of the integrated peak area observed at T=0 with those observed at the T=1, 4, 24 & 48 h time points.
i, Elution profile of L- / D- 15800 peptide isomers. **ii,** Elution profile of FBS. **iii,** Elution profile of L- / D- 15800 peptide isomers combined with FBS. **iv,** Elution profile of HS. **v,** Elution profile of L- / D- 15800 peptide isomers combined with HS.