Supporting information Figures S1 –S5

Molecular characterisation of HLA class II binding to the LAG-3 T cell co-inhibitory receptor

Authors and Affiliations

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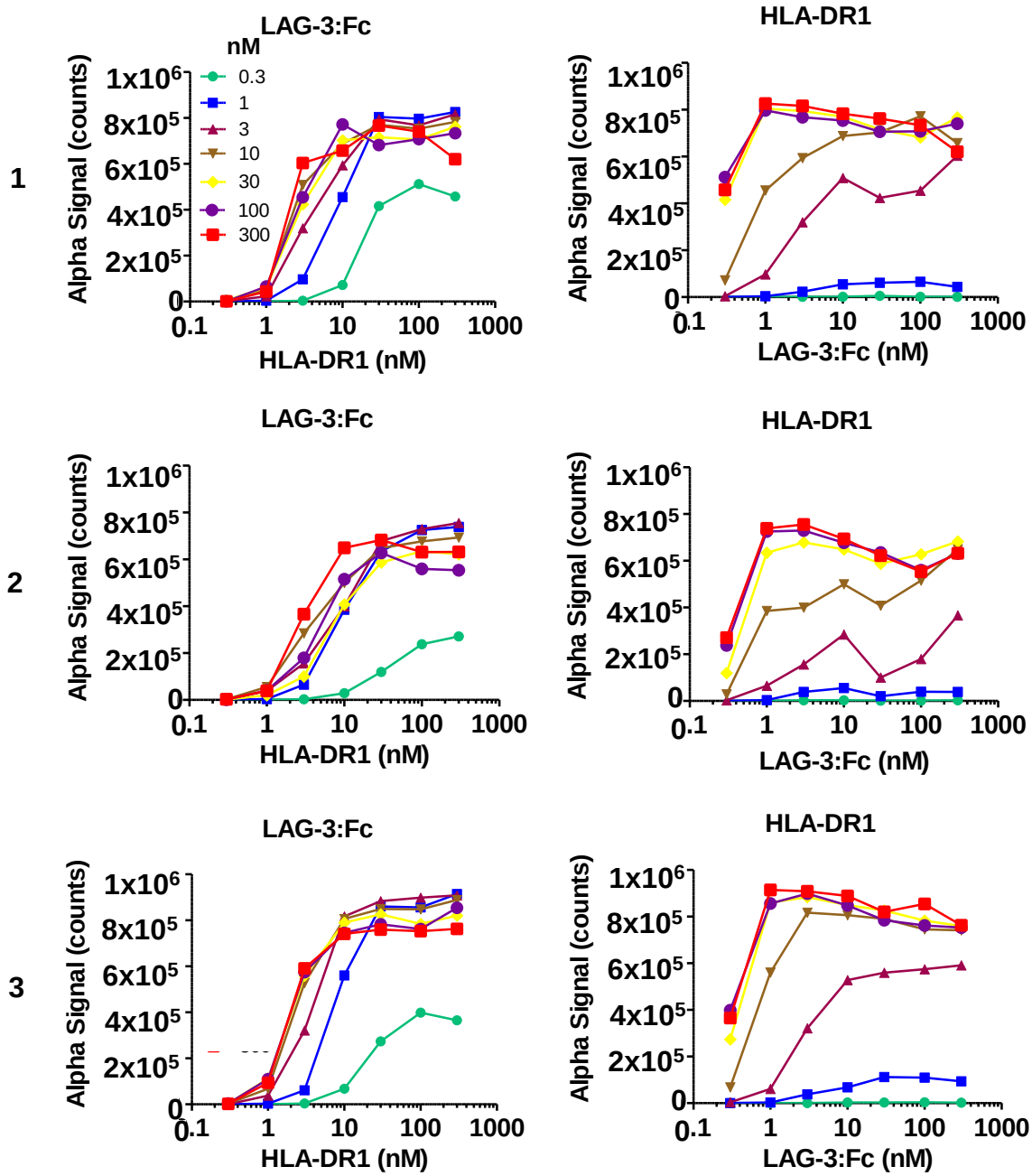
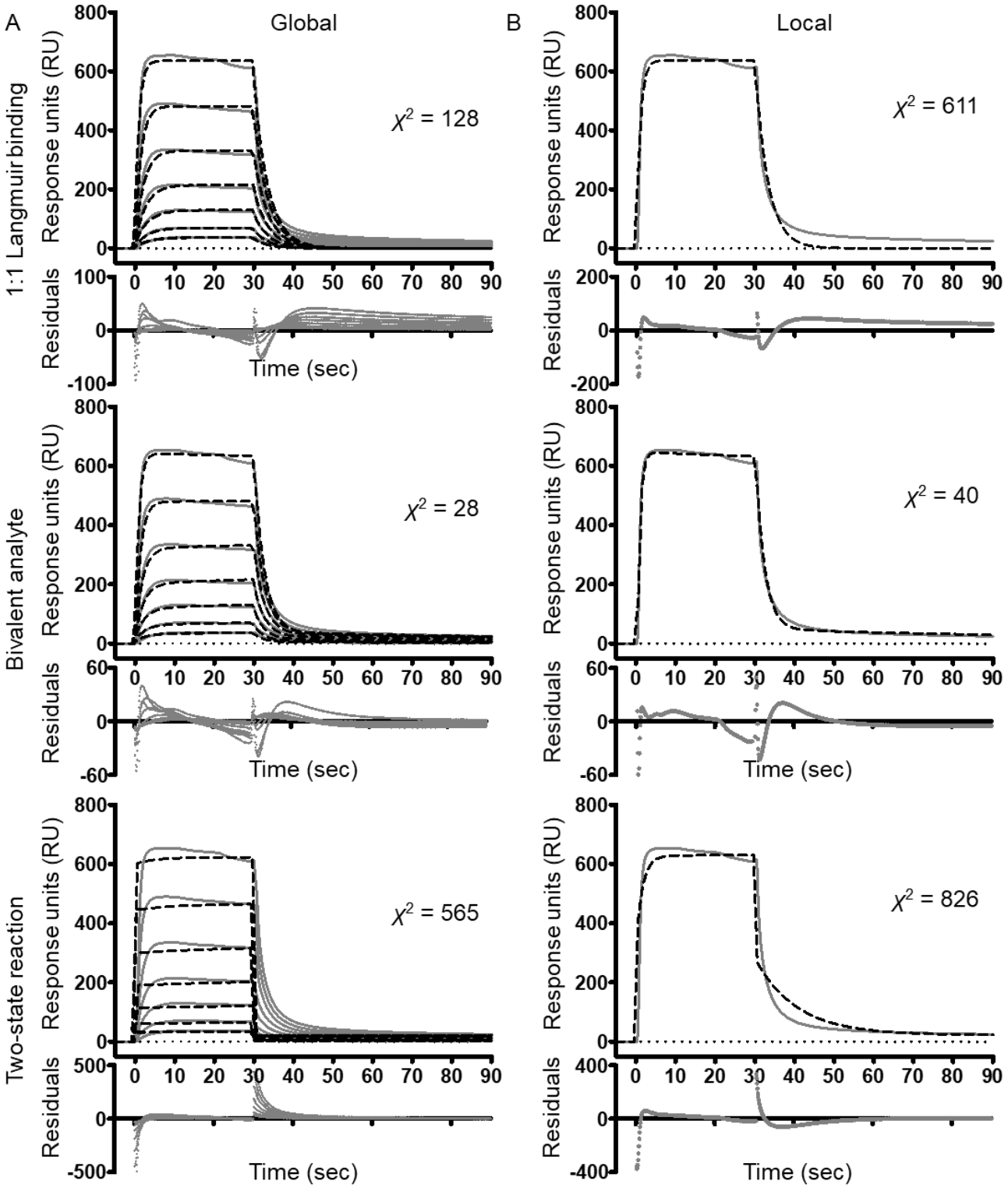
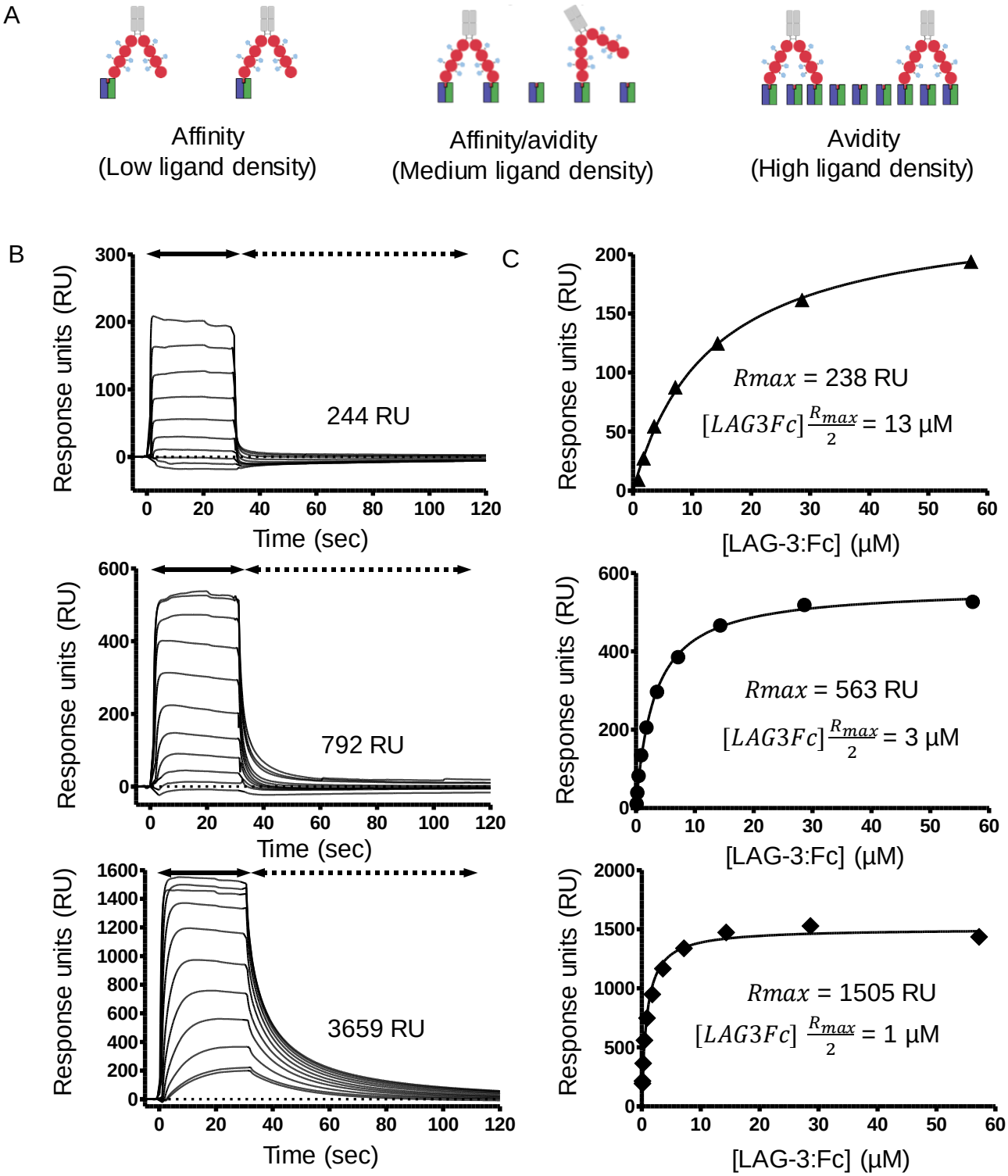


Supplementary Figure S1 – LAG-3:Fc binding detected to multiple pHLA-II alleles. Three replicate protein cross titrations (marked 1-3) using LAG-3:Fc and pHLA-DR1 using a 1 in 3 dilution series from 300 nM to 0.3 nM. All assays were performed using Protein A acceptor beads and streptavidin donor beads at a final bead concentration of 20 µg/mL, in 96 well ½ area opti plates and analysed using an En-vision plate reader at RT.

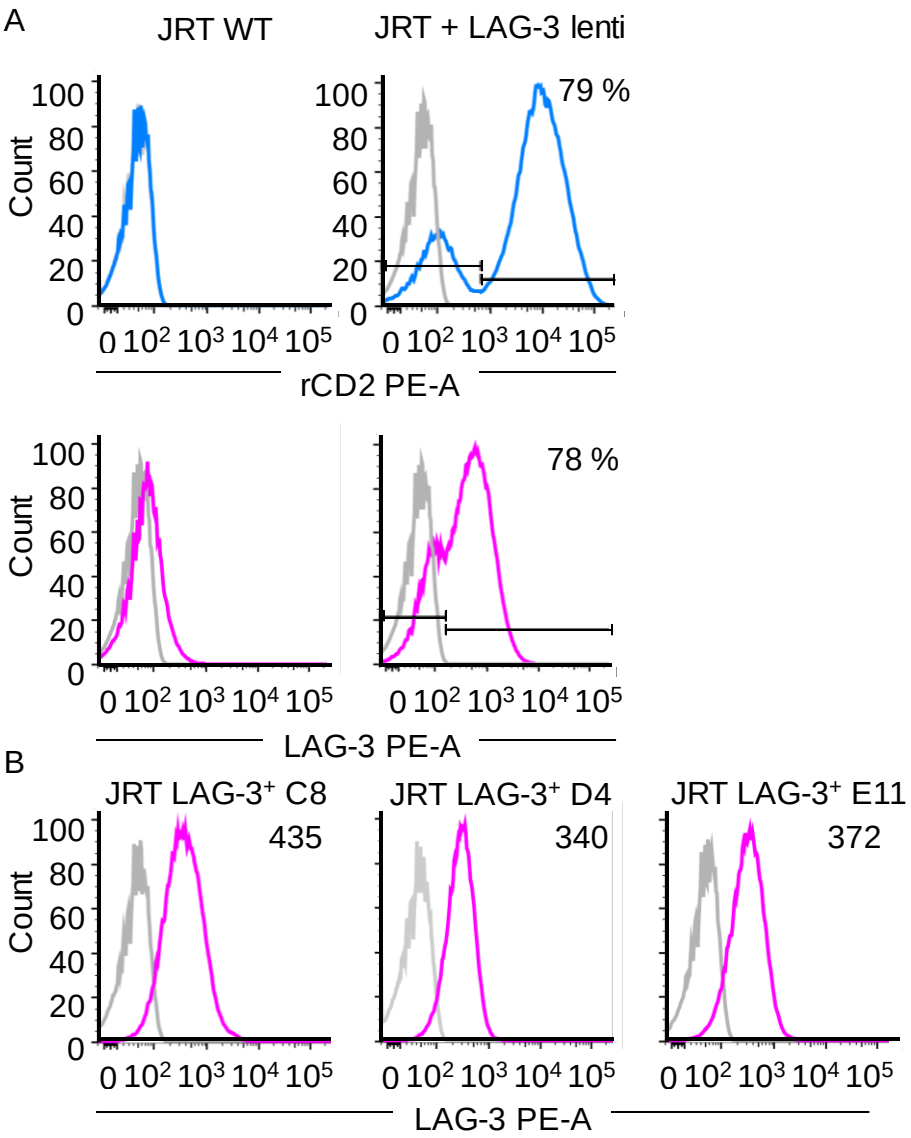


Supplementary Figure S2 – LAG-3:Fc binding to HLA-DR1 exhibits bivalent analyte kinetics.

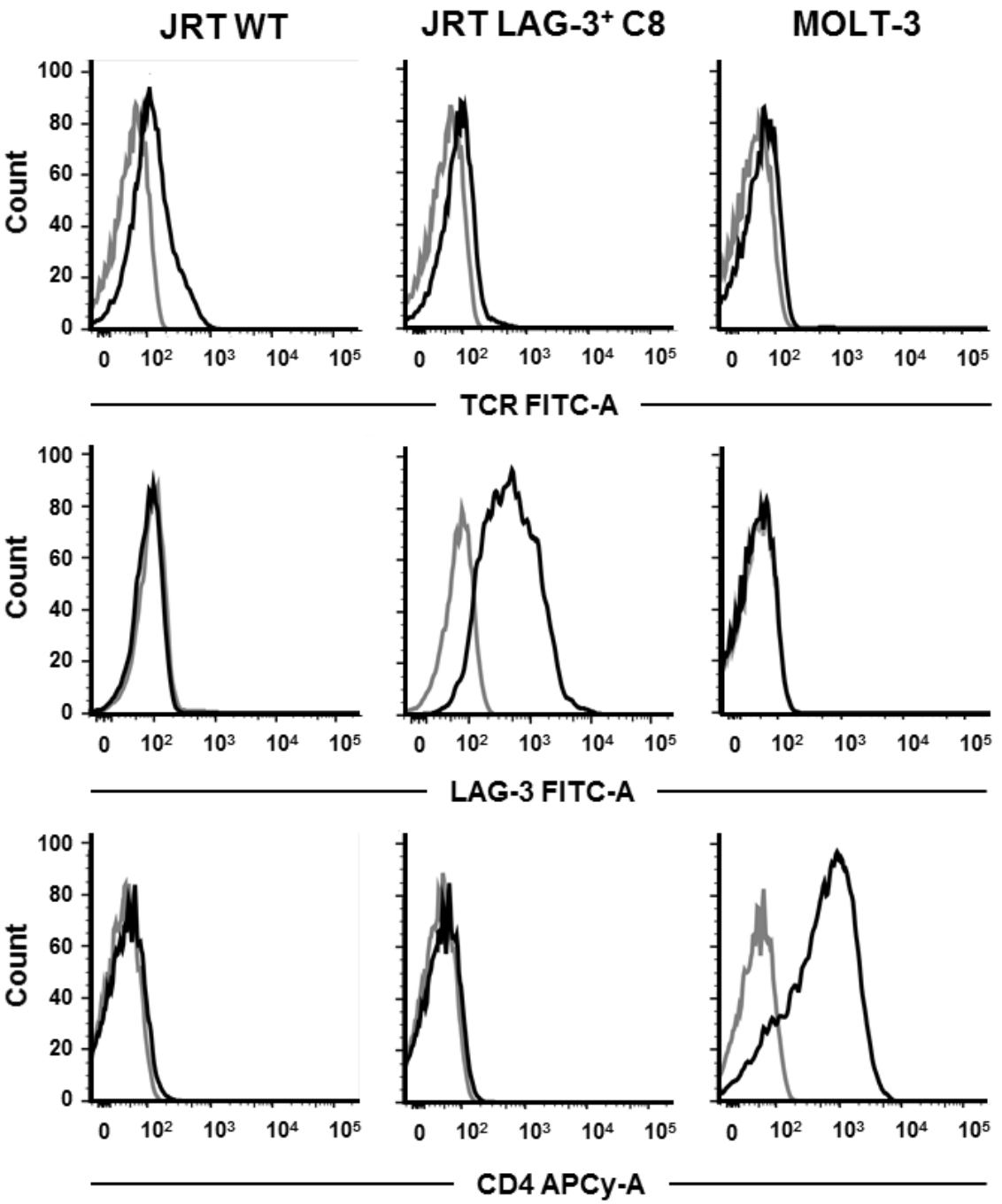
A) Global fit analysis of reference subtracted sensograms of LAG-3:Fc (0.1 – 7 µM) binding to immobilised (525 RU) HLA-DR1 fitted with three candidate models: 1:1 Langmuir binding, bivalent analyte and two-state reaction. Observed sensograms are shown as grey solid lines, fitted curves as black dashed lines with inset χ^2 value and kinetic derived dissociation affinity constant K_D. Corresponding curve fit residual plots are shown below each fit. Data are representative of three independent experimental repeats. **B)** Local fit analysis of the reference subtracted sensogram describing LAG-3:Fc binding at 7 µM to immobilised HLA-DR1. Data are representative of three independent experimental repeats.



Supplementary Figure S3– LAG-3 binds HLA-DR1 with micromolar affinity. A) Schematic overview of the effects of increasing concentration of ligand on the observed binding of LAG-3:Fc in SPR experiments. **B)** SPR analysis of LAG-3:Fc binding to HLA-DR1 immobilised at low (244 RU), intermediate (792 RU) and high (3659 RU) ligand concentrations. **C)** Steady-state analysis of LAG-3:Fc binding to HLA-DR1 at varying ligand concentrations analysed from sensograms as shown in **A** by plotting RU increase from baseline during steady-state (25 seconds into injections) against concentration of LAG-3:Fc. Data are representative of three independent experimental repeats.



Supplementary Figure S4 – Formation of the JRT LAG-3$^+$ C8 cell line via lentiviral delivery. A) Flow cytometry histogram plots of rCD2 and LAG-3 expression in wild type (WT) JRT cells and transduced JRT line following lentiviral delivery (JRT + LAG-3 lenti). rCD2 staining coloured blue, unstained control coloured grey and LAG-3 staining coloured pink. Data are representative of three independent experimental repeats. **B)** Flow cytometry histogram plots of LAG-3 expression in three consequent JRT LAG-3$^+$ clones C8, D4 & E11 generated from the line described in **A**. Unstained control coloured grey and LAG-3 staining coloured pink. Inset numbers represent gMFI of LAG-3 staining. Data are representative of three independent experimental repeats.



Supplementary Figure S5 – Surface characterisation of T cell derived cell lines. Flow cytometry histograms of TCR, LAG-3 and CD4 surface expression on LAG-3- JRT WT, JRT LAG-3+ C8 and MOLT-3 cells. Stained marker = black, FMO/unstained control = grey. Data are representative of three independent experimental repeats.