Supplementary Figure I



Supplementary figure I: Both forms of CX3CL1 (chemokine domain and full-length) show equivalent induction of receptor internalisation. Monocytes were incubated in serum-free medium +/- 50 nM chemokine domain (CKD) or full-length (FL) CX3CL1 for 4 hours then stained with rat anti-human CX3CR1(or isotype control) and analysed by flow cytometry. A single representative donor is shown.



Supplementary figure II: CCL2 but not CX3CL1 induces human monocyte migration measured in an xCelligence real-time chemotaxis assay. A) Primary human monocytes (400,000) per well were loaded in the upper chamber of a 16 well CIM plate and allowed to migrate for 3 hours through a filter with 8 µm pores towards chemotaxis buffer (vehicle; black line), CCL2 (10 nM; red line) or CX3CL1 (10 nM; blue line). Migrating cells adhering to the underside of the chemotaxis filter impede current flow which is measured as a change in cell index. Data show the mean of 2-3 replicate wells from 1 donor. B) Using xCelligence software, the area under the curve over the entire 3 hour time course was quantified as a measure of cell migration. Data are mean + SEM of 3 independent donors, each derived from 2-3 replicate wells. Data analysed with one-way ANOVA and Dunnett's posthoc test, *p<0.05 relative to vehicle.



Supplementary figure III: Sample primary data from CellROX green experiments. Primary human monocytes were loaded for 30 mins with CellROX green reagent, then incubated for 30 mins with SFM (dashed line), 10% FCS (dotted line) or 100 nM CX3CL1 (solid line) then analysed by flow cytometry. Traces show responses in 3 independent donors.