Supplementary Methods

ILC1 and ILC3 isolation and culture

Following cell sorting, we confirmed that the recovered cells belonged to the ILC1, ILC2 and ILC3 subsets. For this purpose we performed intracellular flow cytometry for the transcription factors *t-bet*, *gata3* and *rorγt*, and assessed production of IFN-γ, IL-13 and IL-22 (Supp. Figs. 4A-G). To confirm that the $CD56^+$ ILC3 population was not lost following the NK combo pre-enrichment protocol, we evaluated CD56 and CD161 expression on the cell surface of NK cells and ILCs when pre-enriched with three different protocols: NK enrichment, ILC2 enrichment and NK combo. The latter separation, decreased the amount of contaminating NK cells prior to cell sorting but did not exclude the $CD56^+$ expressing population in the ILC3 lineage (Supp. Fig. 4H).

For cell culture of ILC1 and ILC3, all cytokines were added to the culture at 50 ng/mL, and media was topped up every 2/3 days (table 2). Cells were cultured at 37°C, 5%CO_2.

Supplementary Results

Group 1 and group 3 ILCs can be isolated from blood and activated in culture

Despite our focus being on ILC2s throughout this study, our method of isolation also yielded high purity ILC1 and ILC3 populations. We evaluated the phenotype of these cells via flow cytometry and assessed their effector function through the ability to produce cytokines, in the same way as for ILC2s. As with ILC2s, both ILC1s and ILC3s demonstrated increased survival in SFEM II relative to IMDM media, but unlike ILC2s, their survival or proliferation was unaffected by the addition of cytokines (Supp. Fig. 5A,B). Evaluation of surface phenotype revealed that both ILC1s and ILC3s maintained CD127 expression and that ILC3s remained $CD117^+$ and ILC1s $CD117^-$ (Supp. Fig 5D, E). ILC1s did show some modulation of CD161, with expression increasing marginally upon stimulation with IL-2 and IL-12 (Supp. Fig. 5F).

Stimulation of ILC1 and ILC3 increased their production of effector cytokines significantly. Although neither produced IL-13, ILC1s produced high amounts of IFN-γ as expected (Supp. Fig. 6A) and ILC3s produced high levels of IL-22, TNF-α, GM-CSF (Supp. Fig. 4D,G and Supp. Fig 6B) and moderate levels of IL-8 and IL-10 (Supp. Fig 4E,F). No IL-17A was detected in the supernatants of the ILC3 sub-set cultures, however we did observe intracellular staining for both IL-22 (around 80% of cells) and IL-17 (around 50%) following activation with IL-23 and IL-1β , even in the absence of PMA/ionomycin activation (Supp. Fig. 6B). It is possible that ILC3s were producing IL17-A, but that the levels of this cytokine were below the lower limit of quantitation in the ELISA, due to the limited number of cells per well, together with a lack of extensive proliferation. Granzyme B production by these subsets was also evaluated, and although we found ILC1s to produce granzyme B at baseline and up-regulate it following IL-2+IL-12 activation, due to the lack of positive markers in the isolation of this population it is still unclear whether this is a true feature of the ILC1 population or if there is some NK cell contamination in the culture (or even potentially the presence of ILC2s that could have acquired an ILC1 phenotype prior to cell sorting). We did not find evidence of CD94 or CD56 expression in this population after cell isolation or culture (data nor shown). ILC3s could also express granzyme B but only in the presence of IL-2, as stimulation with IL-23+IL-1β only showed a minor effect (Supp. Fig. 6D).

Supplementary Figure 1. Evaluation of different protocols for the selection of methodology providing the highest enrichment for all three ILC populations. Several protocols were utilised to optimise conditions of enrichment for ILCs prior to cell sorting. (A) Representative figure showing dot-plots for gating on all ILC populations based on their canonical markers and table comparing percentages of all populations using each protocol. (B) Percentage of cells obtained after Easysep^{TM} enrichment for each protocol, calculated based on the initial numbers of total PBMCs and (C) purity of ILC2 or ILC1/ILC3 per experiment. The lower the percentage of recovery from initial PBMCs, the higher the purity of the ILC populations. Each number on the x-axis represents one individual experiment, and each symbol represents one donor. (D) Number of ILCs obtained post-sort, as counted by trypan blue dye exclusion (each symbol represents one donor).(E) Purity check was performed after cell sorting by evaluating the percentage of cells within the lineage^{-}CD127^{+} gate in the top dot-plots and confirming ILC1,2 and 3 phenotype by their expression of CD117 (y-axis) and CRTh2 (x-axis); bottom dot-plots. Data are representative of up to 7 independent experiments, with n=2/3 donors per experiment.

Supplementary Figure 2. Comparative phenotype of ILC2s cultured in SFEM II media. ILC2s were cultured in SFEM II and their cell surface phenotype was evaluated by flow cytometry. (A) Percentage of CD117^{+}CD161^{+} ILC2s in SFEM II culture and mean fluorescence intensity for (B) CD127 and (C) CRTh2. (D) Percentage of cells double positive for IL-17BR and TSLPR and (E) percentage of ST2^{+} ILC2s. Data is depicted as mean±SEM from two independent experiments, with n=2/3 donors each. Significance was calculated using one way ANOVA, corrected for multiple comparisons, to compare all treatment groups to each other where *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001. Statistics above bars represent comparison to media baseline; comparison between samples is represented by connecting line.

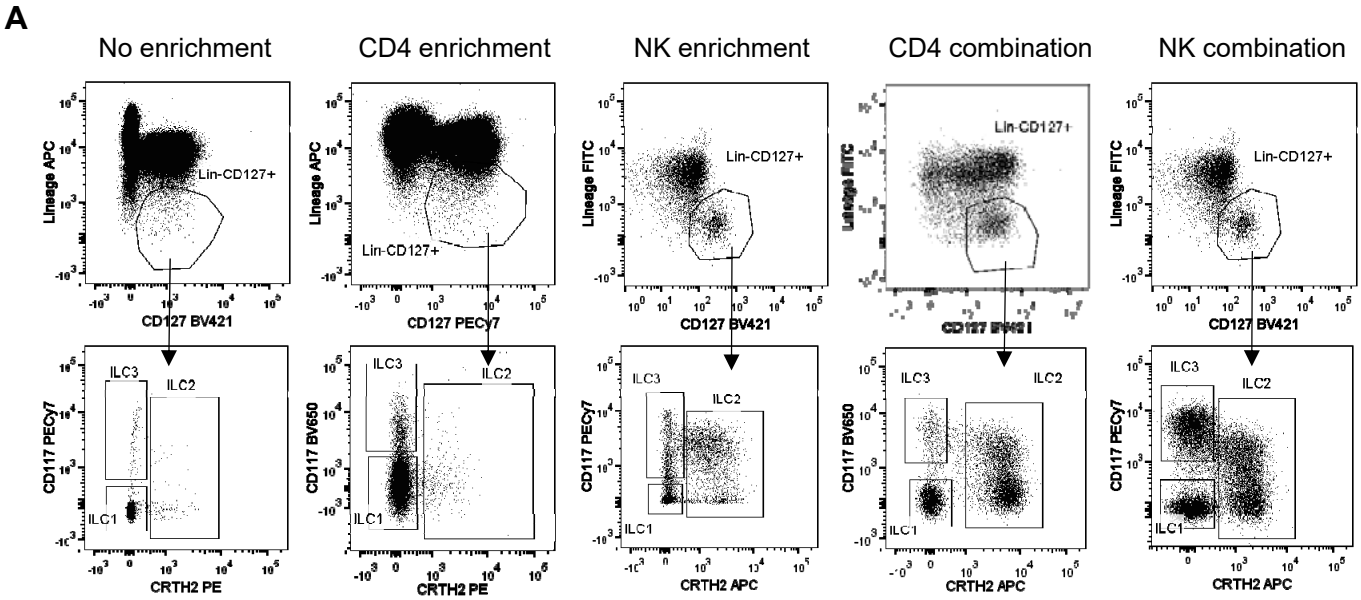
Supplementary Figure 3. Dose response of ILC2s to cytokine stimulation. ILC2s were cultured for five days in the presence of epithelial cytokines alone, in double or triple combination or with IL-2+IL-1β using a 1:10 serial dilution. (A) IL-13 and (B) IL-5 were measured in cell supernatants of ILC2s cultured with different cytokine cocktails. Data is depicted as mean±SEM from two independent experiments, with n=2 donors each. Significance was calculated using one way ANOVA, corrected for multiple comparisons, to compare all treatment groups to each other where *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001. Statistics above bars represent comparison to media baseline; comparison between samples is represented by connecting line. (C) IL-13 and (D) IL-5 were measured in the cell supernatants following serial dilution of cytokines in order to evaluate an optimal concentration of stimulating cytokines. Data are representative of two independent experiments, n=1 donor per experiment.

Supplementary Figure 4. Phenotypic analysis of PBMC-derived ILC populations Transcription factor staining for ILC1, ILC2, ILC3 and NK cells following pre-enrichment protocol. ILCs were stained for transcription factors *rorγt, gata3* and *t-bet* by flow cytometry. (A) Dot plots showing the gating strategy for all three ILC populations and for NK cells, and histograms showing their relative expression of each transcription factor. Representative data for two experiment, n=2 donors. ILC1, ILC2 and ILC3 were then isolated and stimulated *in vitro* for 5 days, before supernatants were analysed for the cytokines: (B) IL-13, (C) IFN-γ, (D) TNF-α, (E) IL-8, (F) IL-10 and (G) IL-22. Data are shown as mean±SEM from two independent experiments, with n=2/3 donors each. Significance was calculated using one way

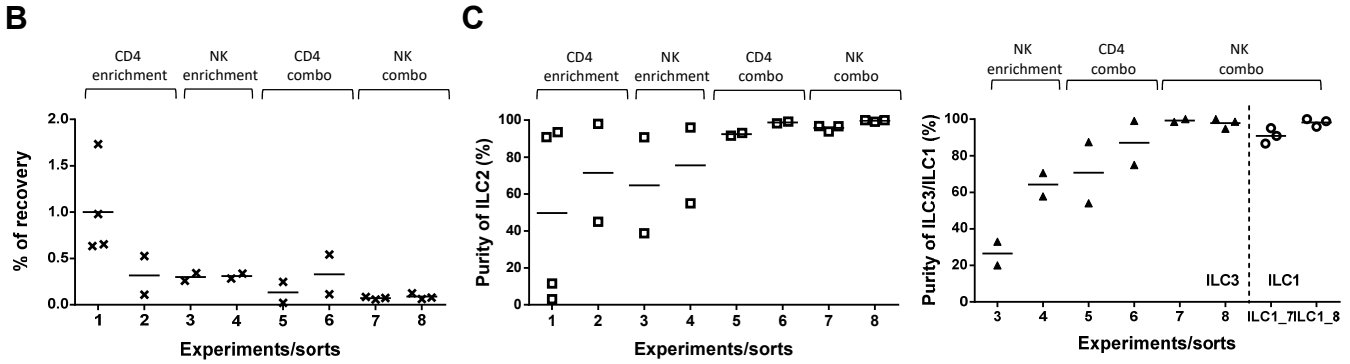
ANOVA followed by correction for multiple comparisons, where *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Statistics above bars represent comparison to media baseline; comparison between samples is represented by connecting line. (H) Dot plots showing the gating strategy for both PBMC-derived NK cells and ILCs and comparative expression of the CD161 and CD56 markers between three different pre-enrichment protocols. Data is representative of two independent experiments (n=2 donors per experiment).

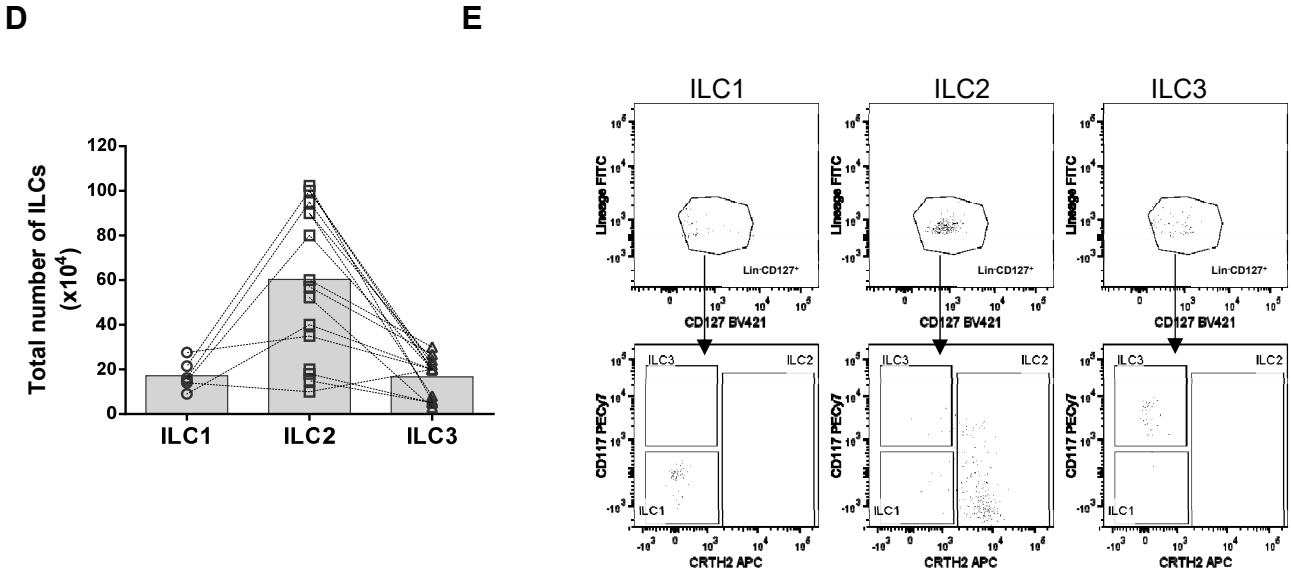
Supplementary Figure 5. Phenotypic changes following *in vitro* culture of ILC1s or ILC3s. Cell sorted ILC1s and ILC3s were cultured for five days in the presence of IL-2+IL-12, or IL-23+IL-1β, respectively, in IMDM or SFEM II. Survival was measured for (A) ILC1 and (B) ILC3 in both IMDM or SFEM II and (C) cell number was calculated for both populations. Cell surface phenotype was also evaluated for (D) CD127, (E) CD117 and (F) CD161 by quantification of the mean fluorescence intensity as compared to isotype control. Data are shown as mean±SEM from two independent experiments, with n=2 or 3 donors each. Significance was calculated using one way ANOVA followed by correction for multiple comparisons, where *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Statistics above bars represent comparison to media baseline; comparison between samples is represented by connecting line.

Supplementary Figure 6. Modulation of ILC1 and ILC3 effector function following activation *in vitro*. Following isolation, ILC1s and ILC3s were cultured for 5 days *in vitro* and stimulated with different cytokine cocktails, as indicated, in IMDM baseline media. (A) IL-13 and IFN-γ and, (B) IL-22 and IL-17A expression were evaluated by intracellular flow cytometry, after incubation of ILC1s and ILC3s with brefeldin A±PMA/ionomycin. (D) Granzyme B production was also evaluated for both cell types after intracellular staining and a 4 hour incubation with brefeldin A only. Data is representative of two independent experiments (n=2 donors/experiment).

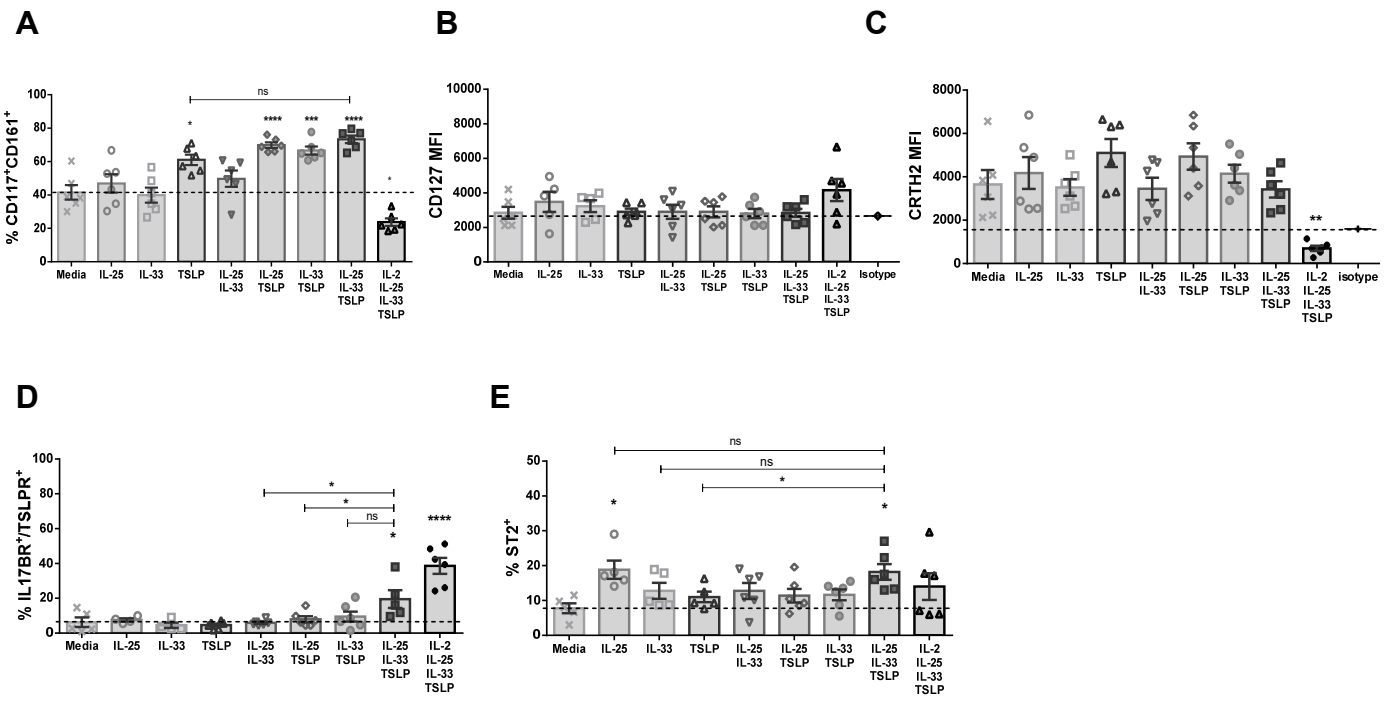


	No enrichment (n=10)	CD4 enrichment (n=5)	NK enrichment (n=4)	CD4 combo (n=3)	NK combo (n=10)
% total ILCs (live CD45⁺)	0.14±0.07	0.36±0.17	0.37±0.19	1.01±0.67	3.83±1.33
ILC1 (% of total ILC)	78±8	56±26	25±16	45±24	22±6
ILC2 (% of total ILC)	14±6	25±17	50±11	42±23	59±9
ILC3 (% of total ILC)	6±3	10±8	21±9	7±4	16±5

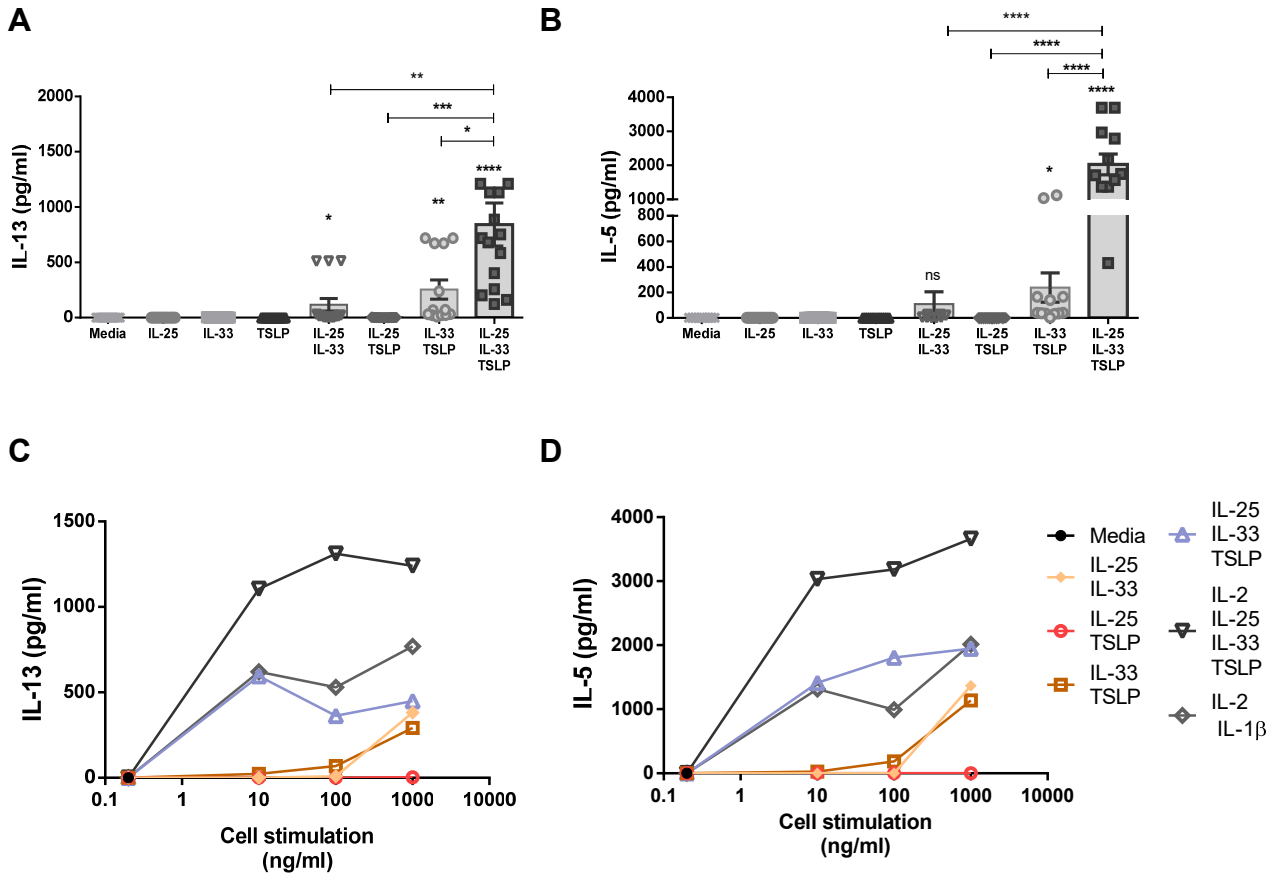




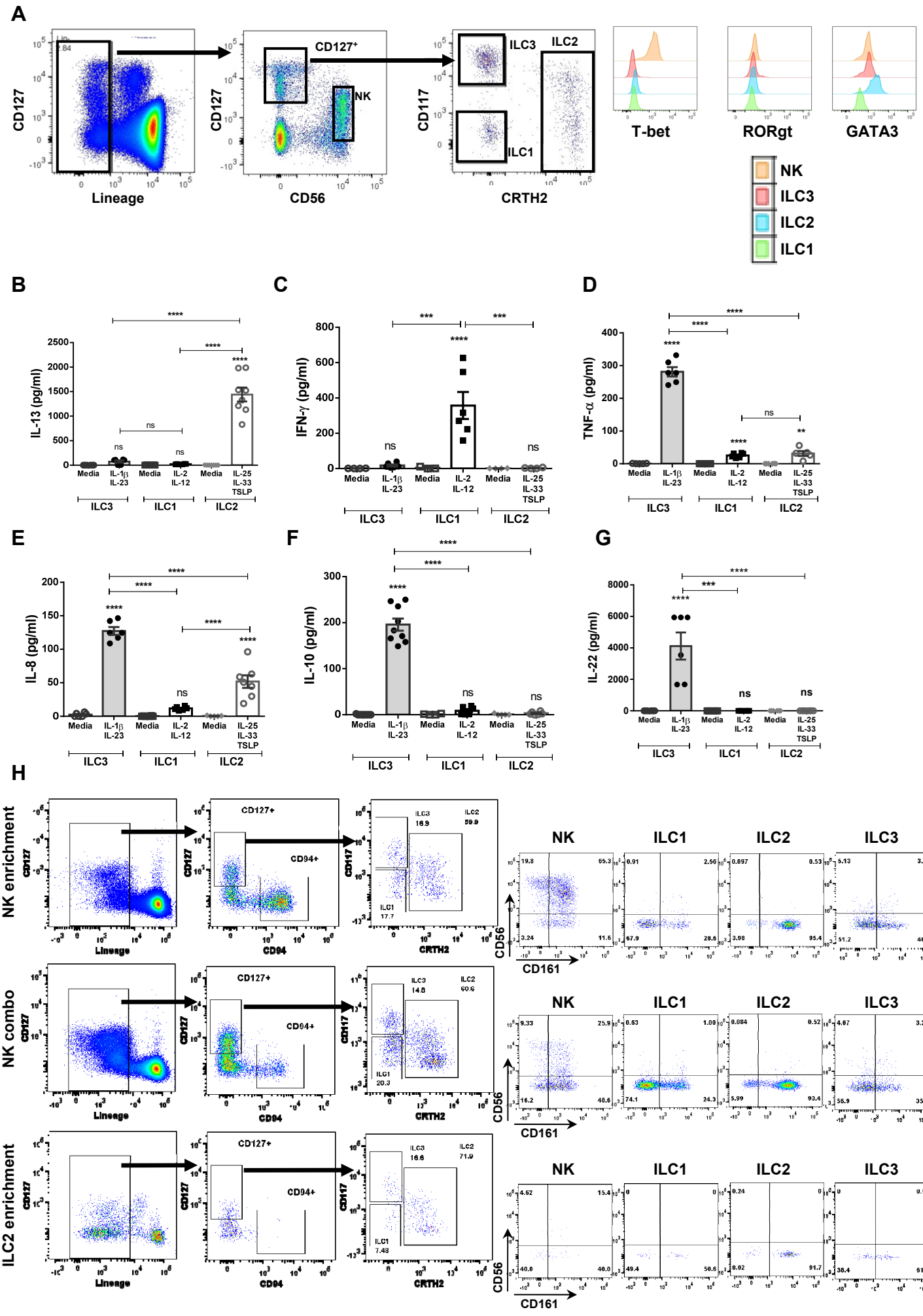
Supplementary Figure 1



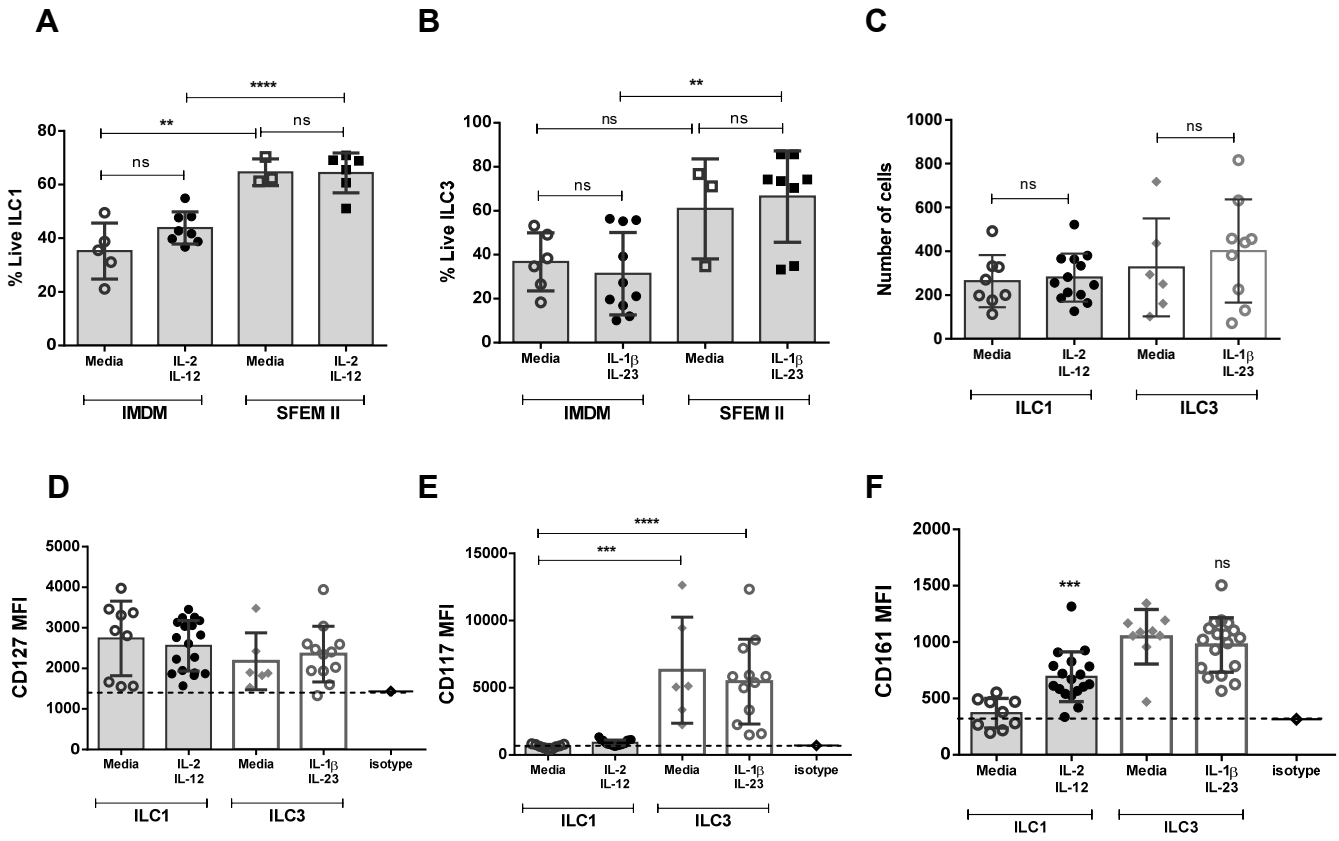
Supplementary Figure 2



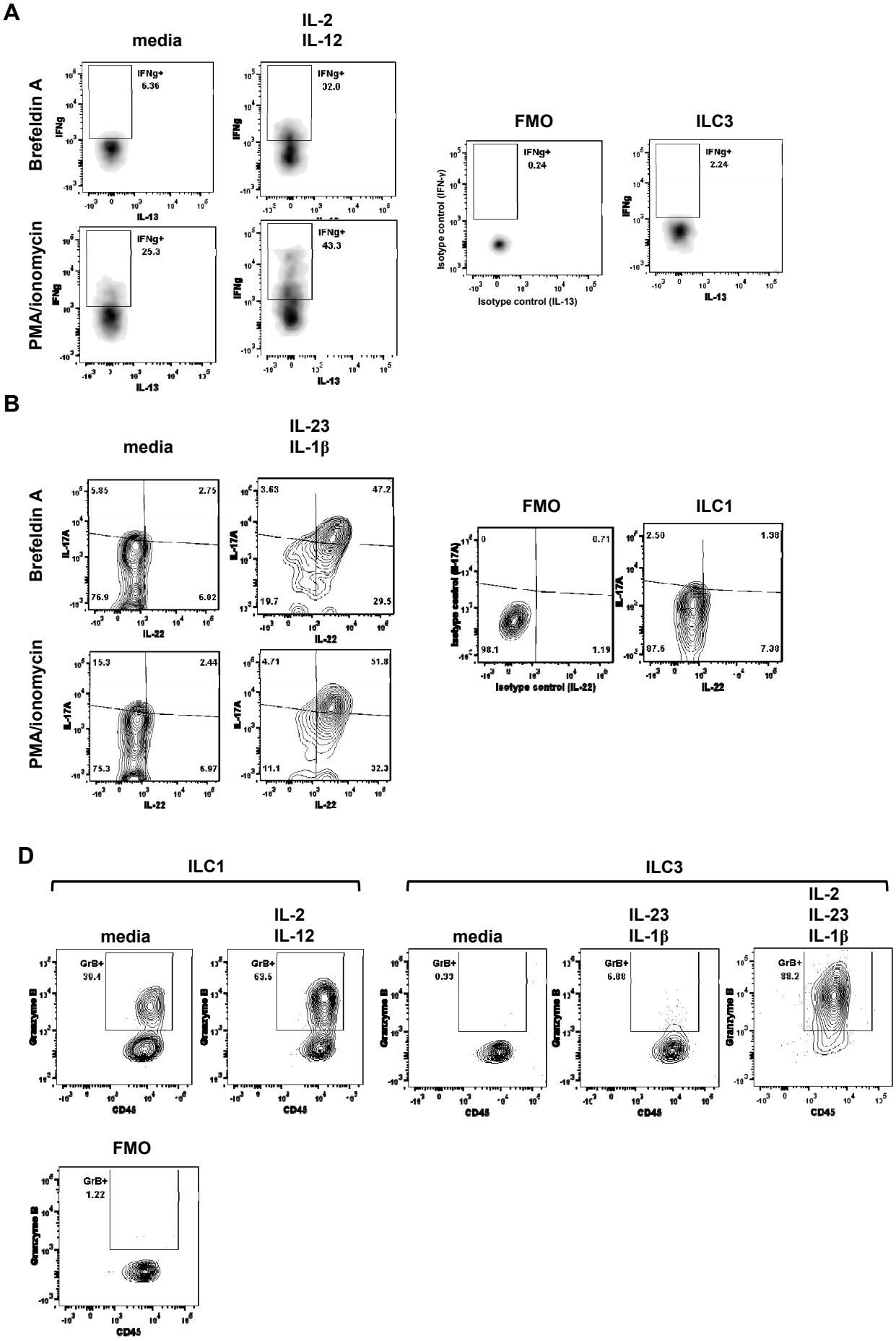
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6