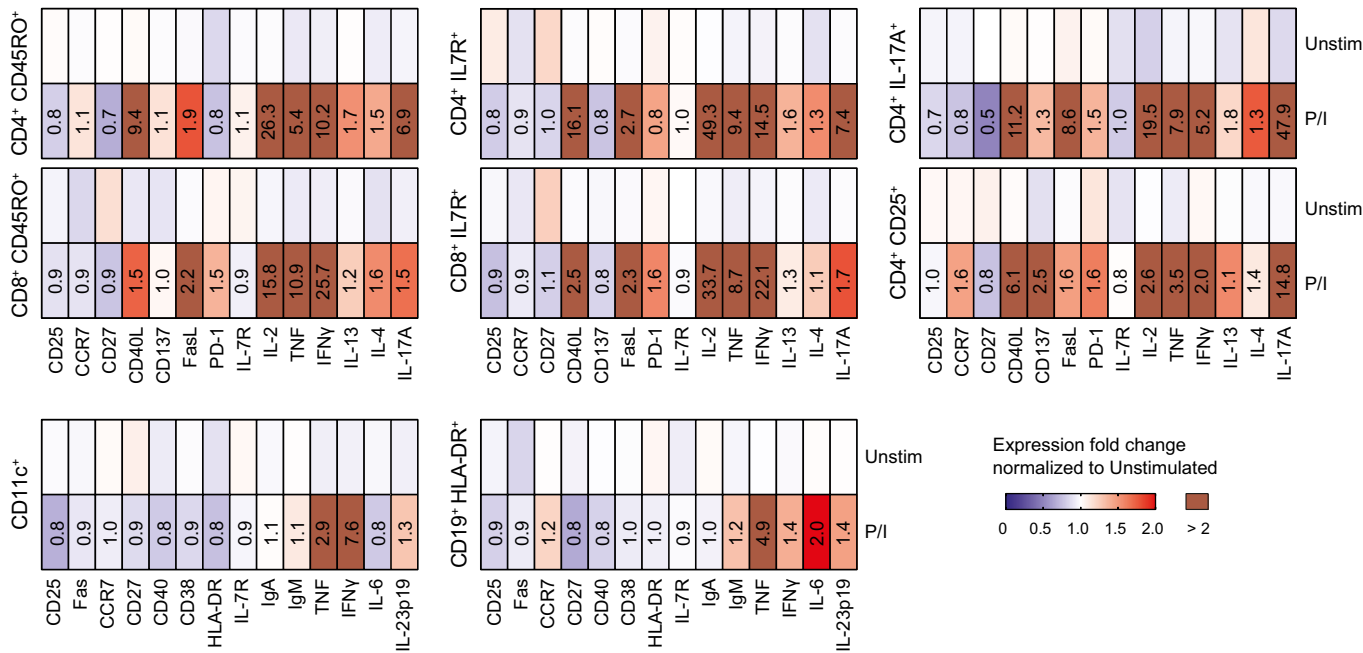


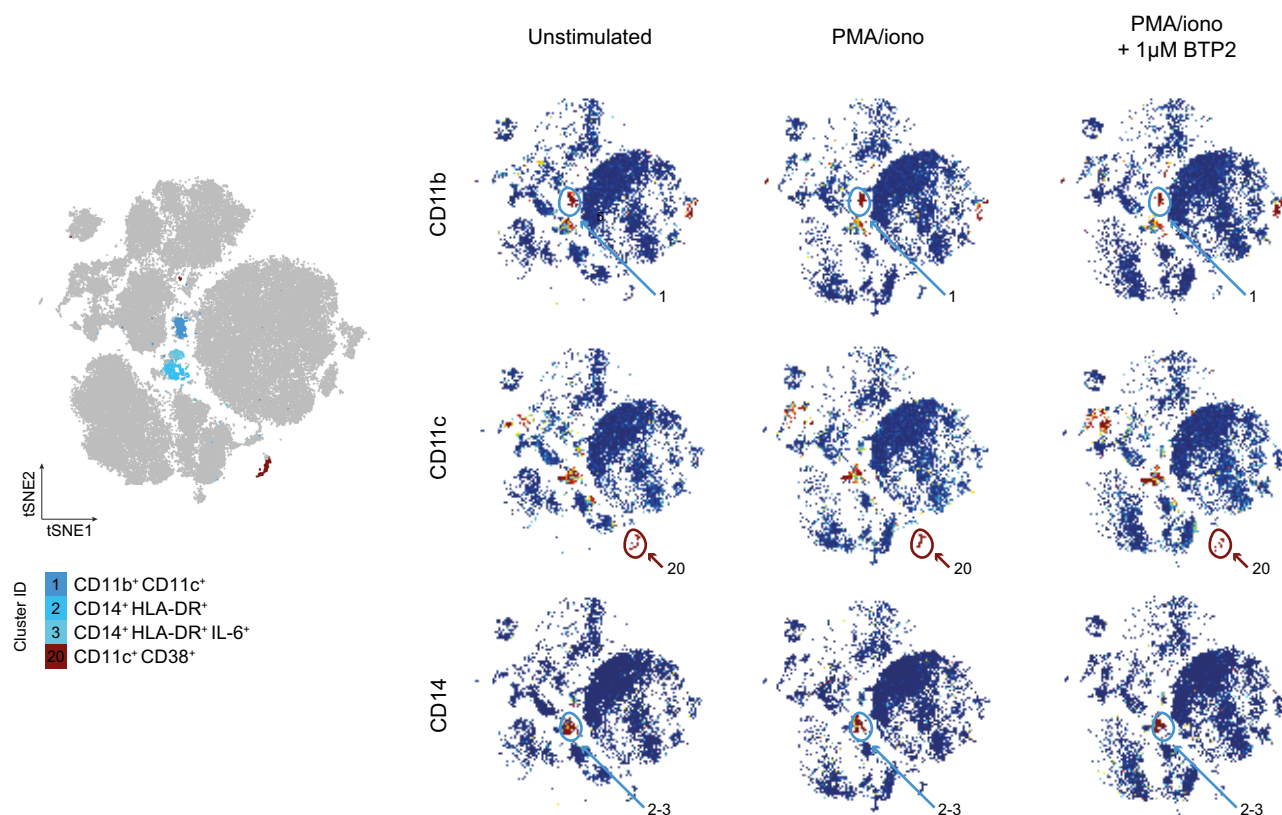
Expanded View Figures



**Figure EV1. Effects of PMA/ionomycin stimulation on protein expression of human lamina propria (LP) immune cells.**

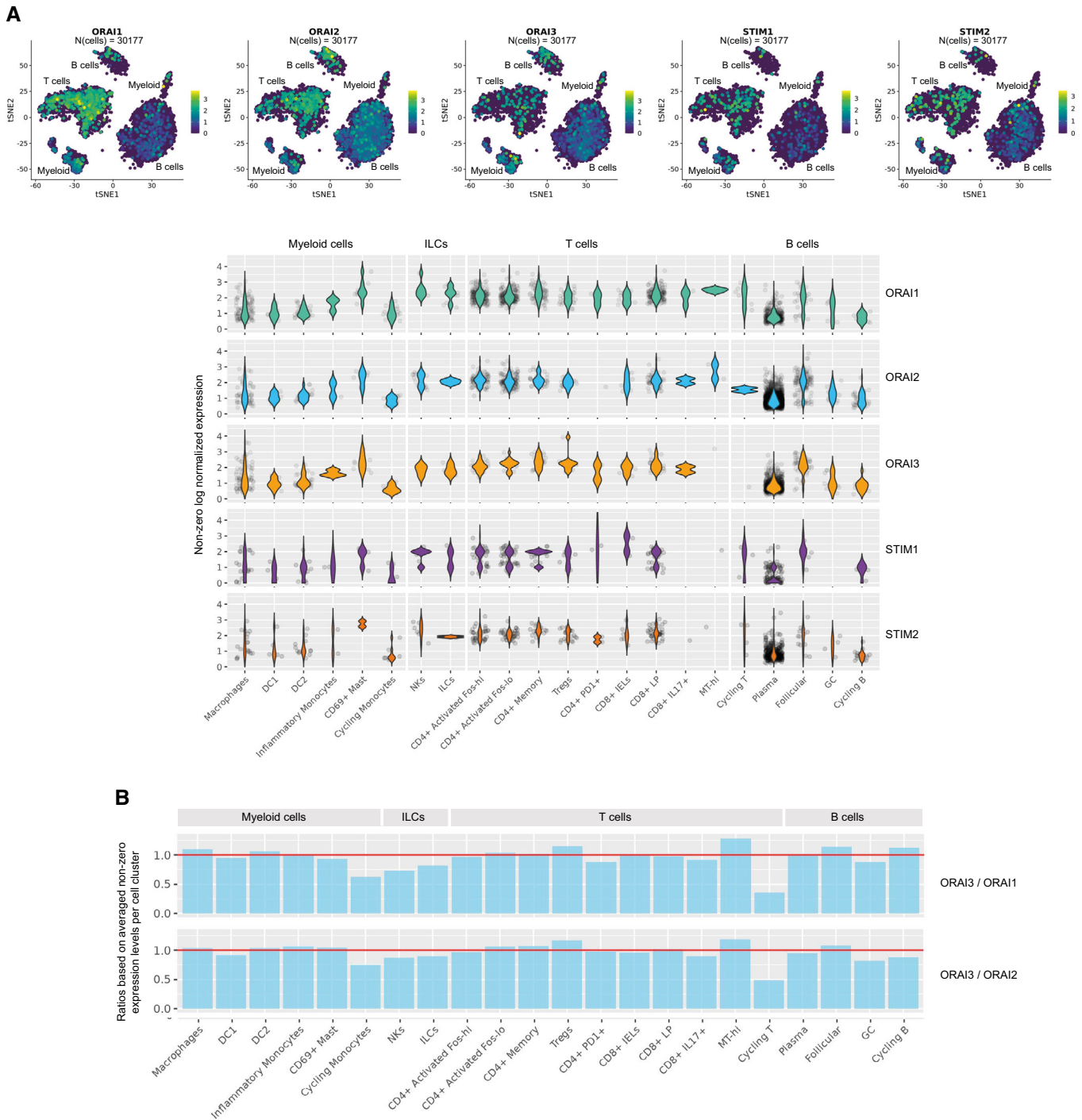
Heatmaps representing the median fold change (FC) of cytokine and surface marker expression in CD45<sup>+</sup>CD3<sup>+</sup> T cells (top row) and CD45<sup>+</sup>CD3<sup>+</sup> immune cells (bottom row) isolated from colon lamina propria of five patients with Crohn's disease. Cells were treated with 20 ng/ml PMA and 1 μg/m (P/I) or DMSO (unstimulated) for 4 h *in vitro*, and protein expression was measured by mass cytometry (CyTOF). FC values are relative to unstimulated samples.

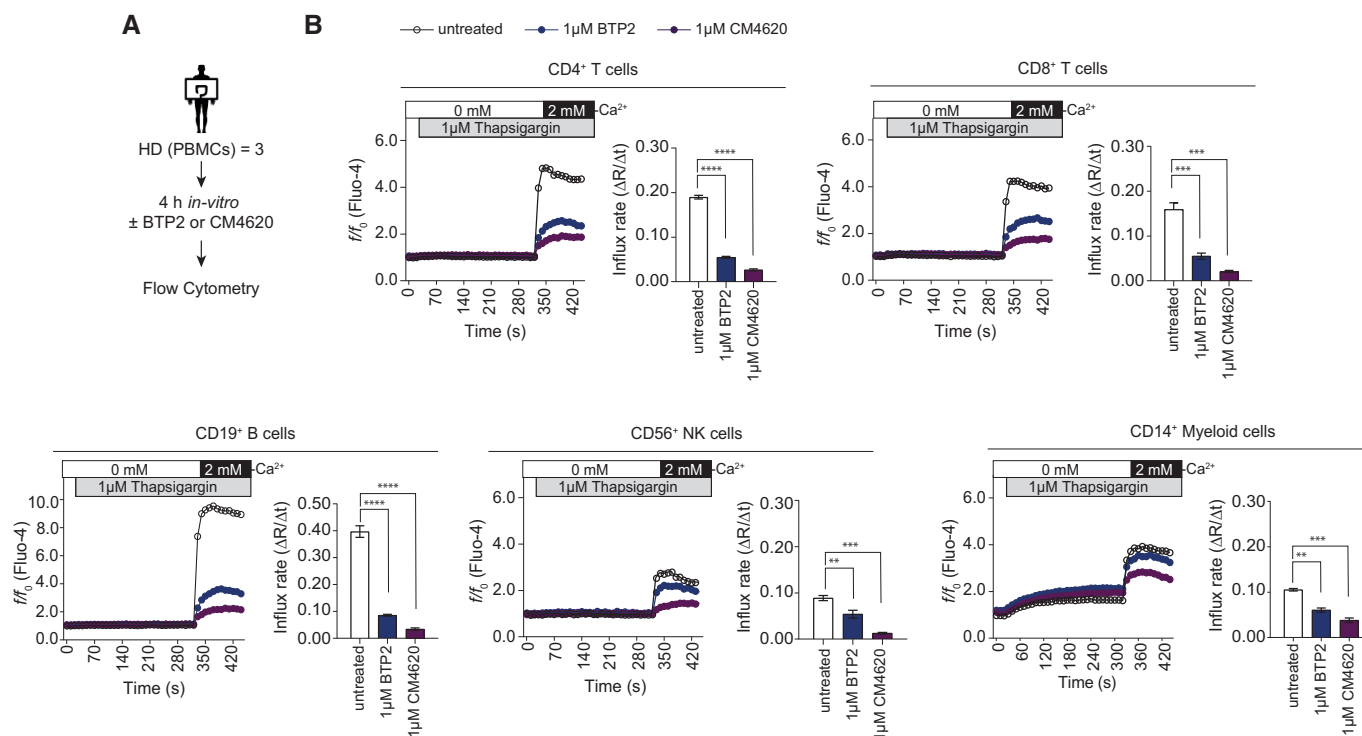
Source data are available online for this figure.



**Figure EV2. Four clusters of myeloid cells in the colon lamina propria (CLP) of IBD patients and effects of SOCE inhibition.**

(Left) FlowSOM plot of merged FCS files from unstimulated IBD samples and samples treated with PMA/ionomycin  $\pm$  1  $\mu$ M BTP2 (non-inflamed:  $n = 4$ , CD:  $n = 6$ , UC:  $n = 6$ ). Colors indicate myeloid cell clusters within CD45<sup>+</sup>CD3<sup>-</sup> LPMCs. (Right) viSNE plots of one exemplary CD patient. Colors indicate expression levels of cell surface markers (blue: low; red: high) in cells left unstimulated, stimulated with PMA/ionomycin or stimulated in the presence of 1  $\mu$ M BTP2.





**Figure EV4. Similar effects of CRAC channel inhibition with CM4620 and BTP2 on  $\text{Ca}^{2+}$  influx in human immune cell subsets.**

A Experimental design for  $\text{Ca}^{2+}$  influx measurements in PBMCs of three healthy donors (HD) by flow cytometry.

B  $\text{Ca}^{2+}$  influx rates were measured in CD4<sup>+</sup> or CD8<sup>+</sup> T cells, CD19<sup>+</sup> B cells, CD56<sup>+</sup> NK cells and CD14<sup>+</sup> myeloid cells following pre-incubation with 1 μM BTP2 or 1 μM CM4620 for 4 h. Cells were stimulated with thapsigargin in  $\text{Ca}^{2+}$  free buffer followed by addition of 2 mM  $\text{Ca}^{2+}$  Ringer solution. Bar graphs show the mean values of influx rates after addition of 2 mM  $\text{Ca}^{2+}$  from one experiment (samples of  $n = 3$  HD run in technical triplicates). Statistical significance was calculated with repeated measures (RM) one-way ANOVA test. \*\*\*\* $P < 0.0001$ ; \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

Source data are available online for this figure.

**Figure EV5. Suppression of cytokine production in LPMCs of IBD patients by CM4620.**

A Experimental setup for  $\text{Ca}^{2+}$  influx measurements of LPMCs from three IBD patients by flow cytometry.

B Human lamina propria CD4<sup>+</sup> and CD8<sup>+</sup> T cells were pretreated with 250 nM or 1,000 nM BTP2 or CM4620 for 4 h *in vitro* and added to the cell suspension until acquisition. Cells were stimulated with 1 μM thapsigargin (TG) in  $\text{Ca}^{2+}$  free Ringer solution followed by the readdition of 2 mM  $\text{Ca}^{2+}$  Ringer solution. Bar graphs show the mean ± SEM of  $\text{Ca}^{2+}$  influx rates after the addition of 2 mM  $\text{Ca}^{2+}$  Ringer solution from one experiment ( $n = 3$  IBD patients). Statistical significance was calculated with repeated measures (RM) one-way ANOVA test. \*\*\*\* $P < 0.0001$ ; \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

C–F Mass cytometry analysis of LPMCs from six IBD patients. (C) Experimental design. (D) viSNE plots of CD45<sup>+</sup>CD3<sup>+</sup> LPMCs stimulated with PMA/ionomycin in the presence of various (v) concentrations (250 or 1,000 nM) of BTP2 or CM4620 with unstimulated samples serving as controls. Colors indicate the expression level of IL-2, IL-17, IFN $\gamma$ , and TNF $\alpha$  (blue: low, red: high) and are representative of one CD patient. (E) Heatmaps representing the median fold change of cytokines and cell surface molecules on CD45<sup>+</sup>CD3<sup>+</sup> or CD45<sup>+</sup>CD3<sup>−</sup> LPMCs that were stimulated with PMA/ionomycin for 4 h *in vitro*. Data are normalized to unstimulated samples. (F) Heatmaps representing the median fold change of cytokine and cell surface marker expression in CD45<sup>+</sup>CD3<sup>+</sup> and CD45<sup>+</sup>CD3<sup>−</sup> LPMCs stimulated with PMA/ionomycin for 4 h in the presence or absence of 250 nM or 1,000 nM BTP2 or 250 nM or 1,000 nM CM4620. Data were normalized to samples treated with PMA/ionomycin alone. Statistical significance in (E, F) was calculated using a one-tailed paired Wilcoxon matched-pairs signed rank test. \* $P < 0.05$ .

Source data are available online for this figure.

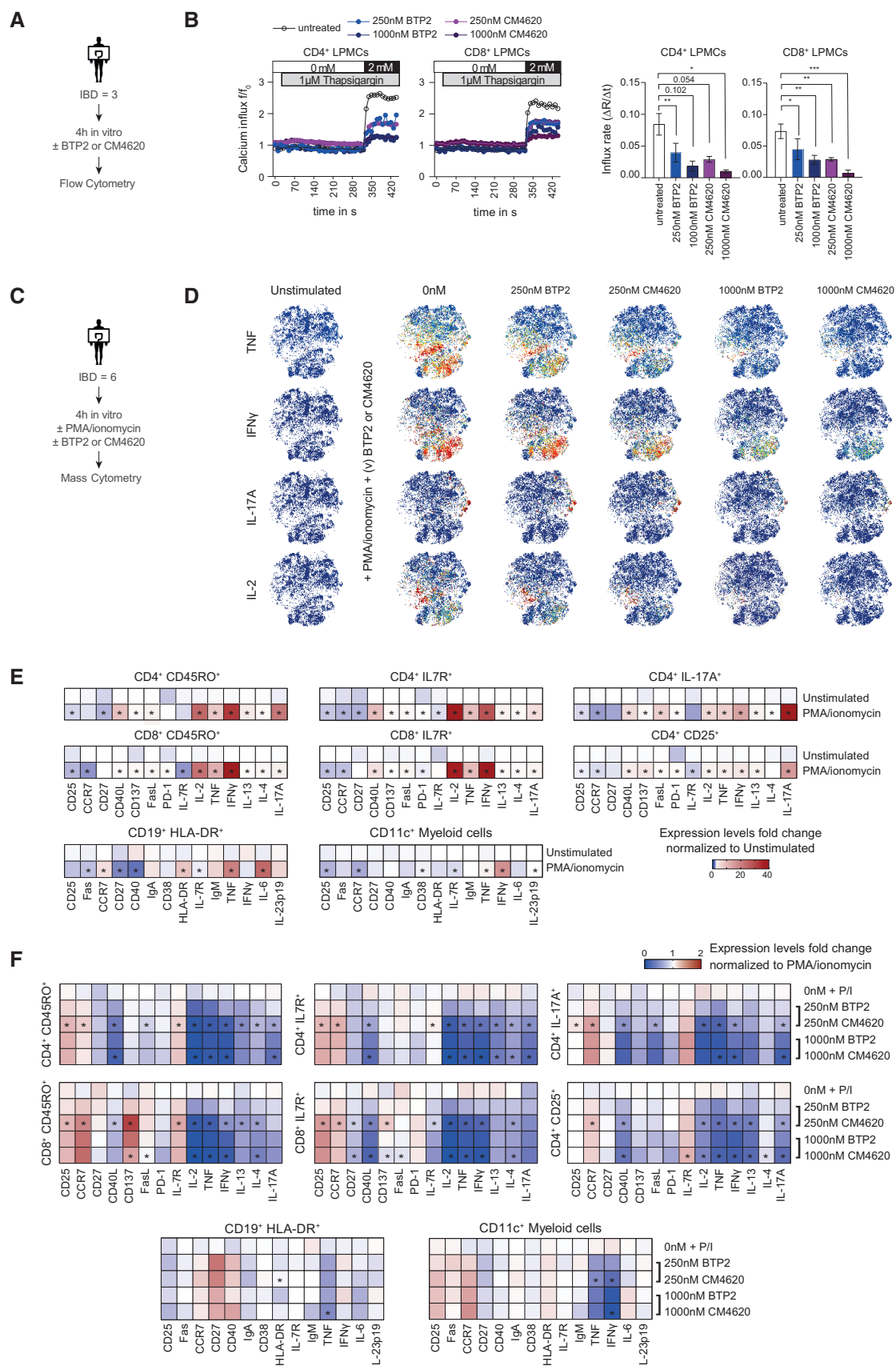
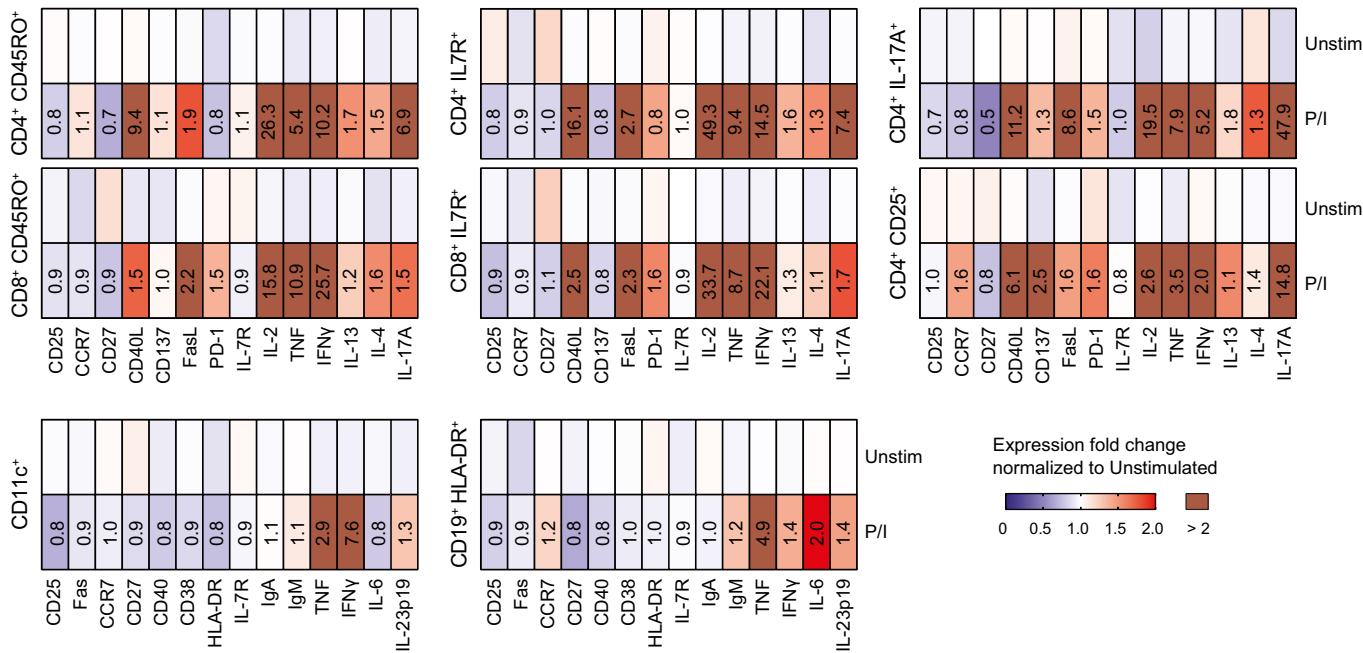


Figure EV5.

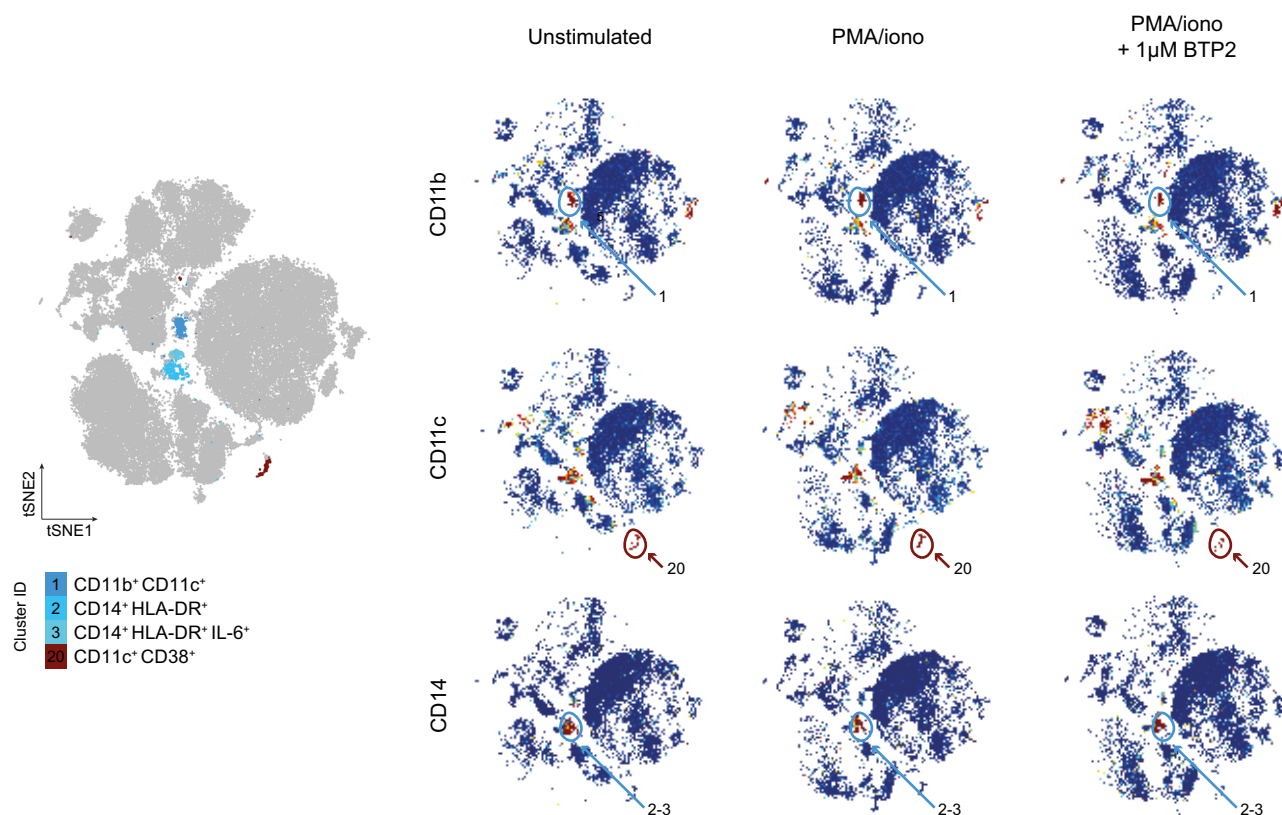
Expanded View Figures



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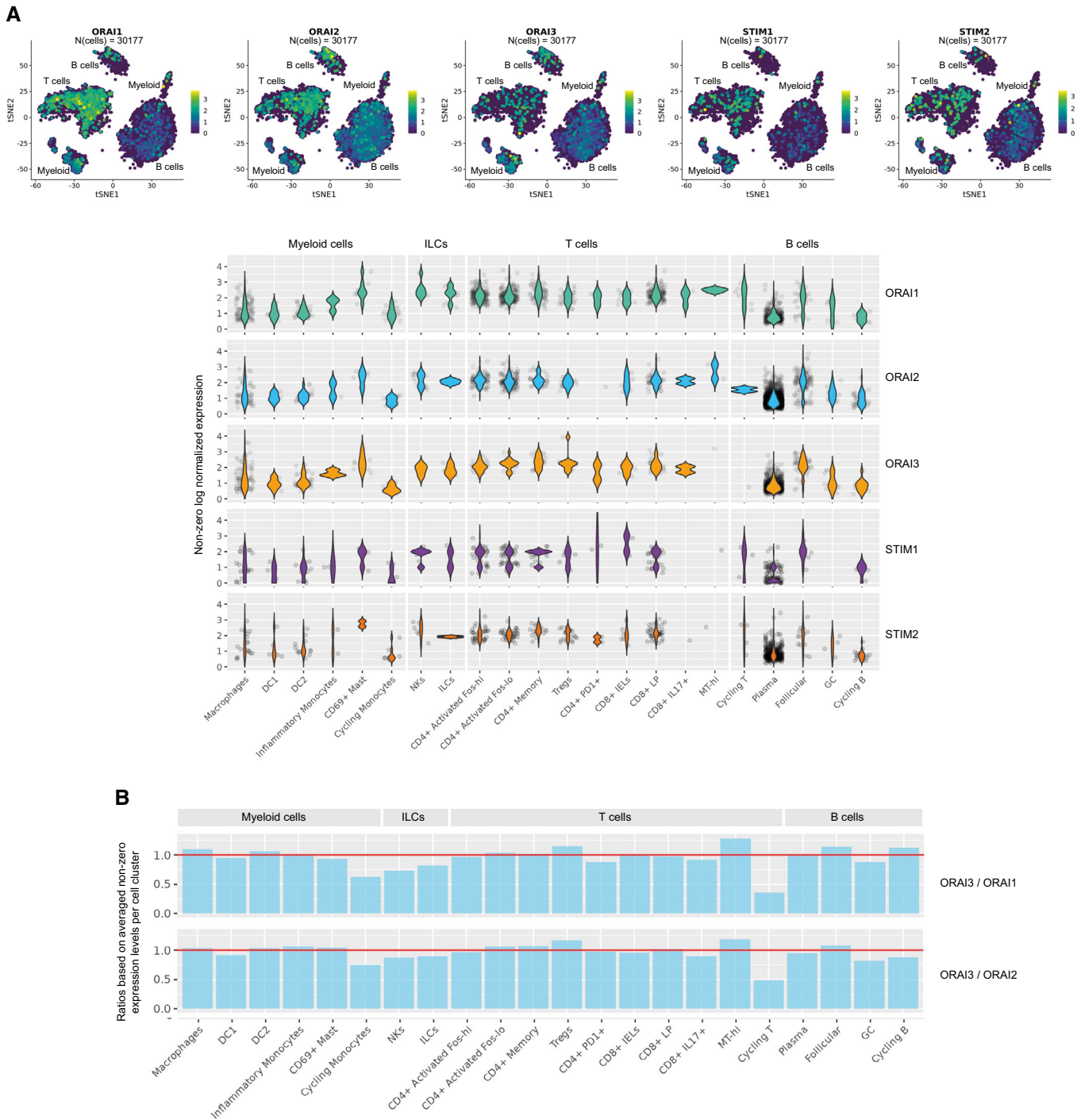
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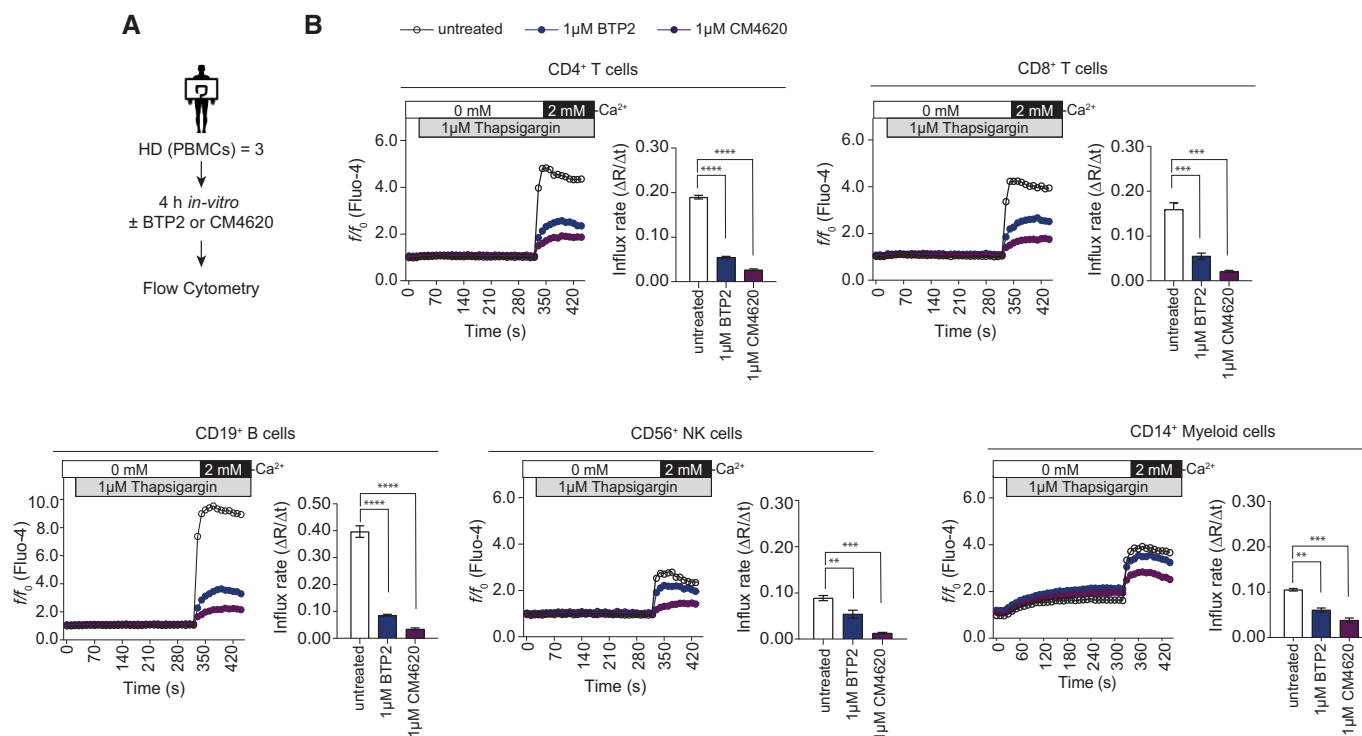
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(Left) FlowSOM plot of merged FCS files from unstimulated IBD samples and samples treated with PMA/ionomycin  $\pm$  1  $\mu$ M BTP2 (non-inflamed:  $n = 4$ , CD:  $n = 6$ , UC:  $n = 6$ ). Colors indicate myeloid cell clusters within CD45<sup>+</sup>CD3<sup>-</sup> LPMCs. (Right) viSNE plots of one exemplary CD patient. Colors indicate expression levels of cell surface markers (blue: low; red: high) in cells left unstimulated, stimulated with PMA/ionomycin or stimulated in the presence of 1  $\mu$ M BTP2.









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A Experimental design for  $\text{Ca}^{2+}$  influx measurements in PBMCs of three healthy donors (HD) by flow cytometry.

B  $\text{Ca}^{2+}$  influx rates were measured in  $\text{CD4}^{+}$  or  $\text{CD8}^{+}$  T cells,  $\text{CD19}^{+}$  B cells,  $\text{CD56}^{+}$  NK cells and  $\text{CD14}^{+}$  myeloid cells following pre-incubation with 1 μM BTP2 or 1 μM CM4620 for 4 h. Cells were stimulated with thapsigargin in  $\text{Ca}^{2+}$  free buffer followed by addition of 2 mM  $\text{Ca}^{2+}$  Ringer solution. Bar graphs show the mean values of influx rates after addition of 2 mM  $\text{Ca}^{2+}$  from one experiment (samples of  $n = 3$  HD run in technical triplicates). Statistical significance was calculated using repeated measures (RM) one-way ANOVA test. \*\*\*\* $P < 0.0001$ ; \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

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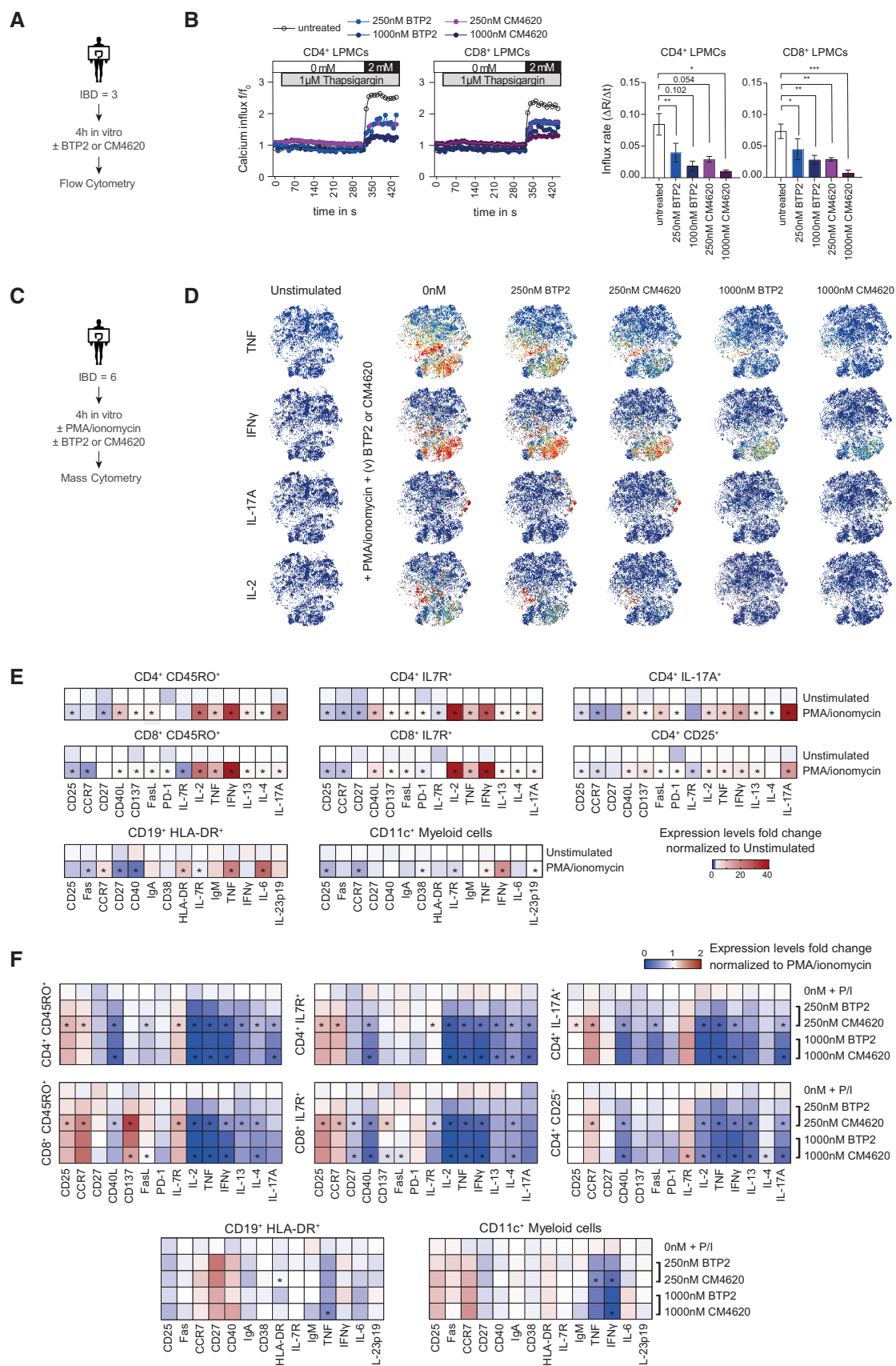
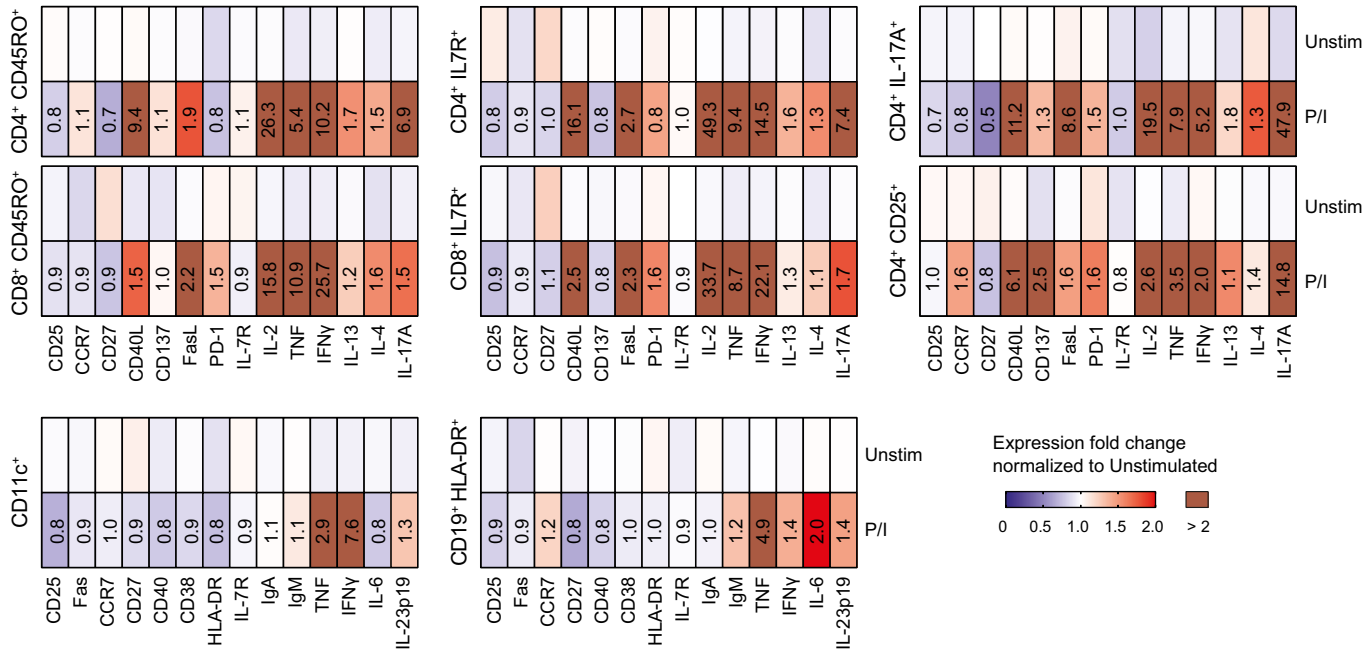


Figure EV5.

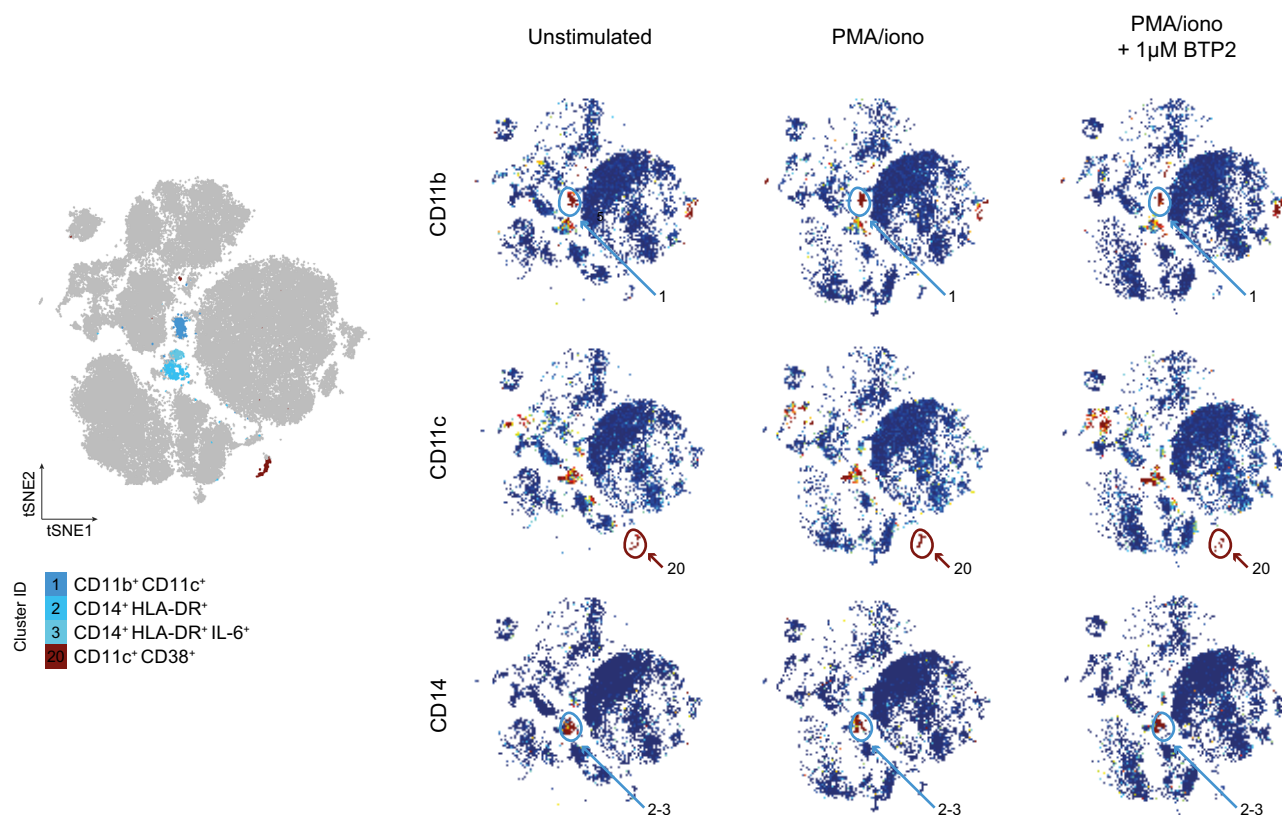
Expanded View Figures



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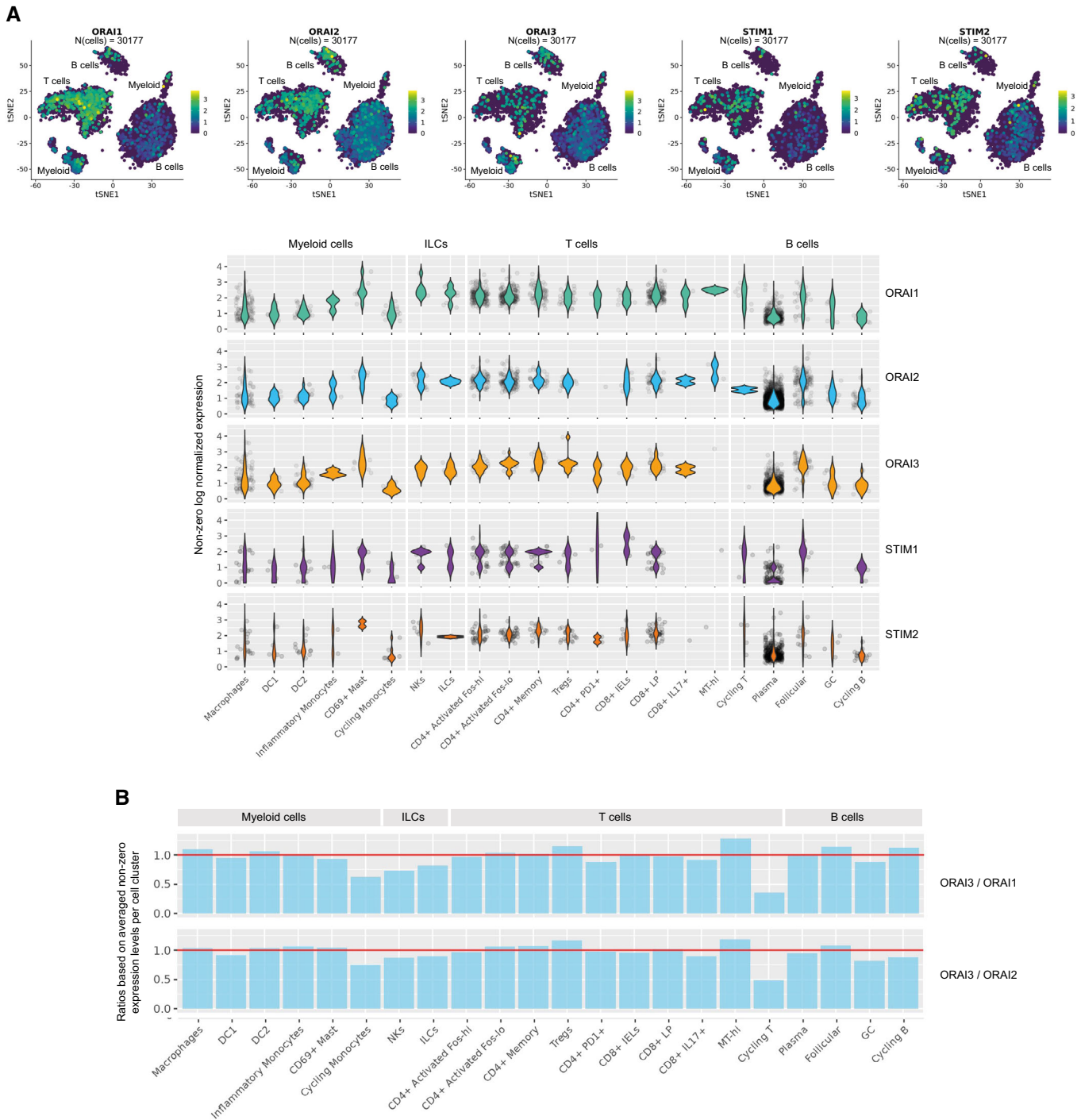
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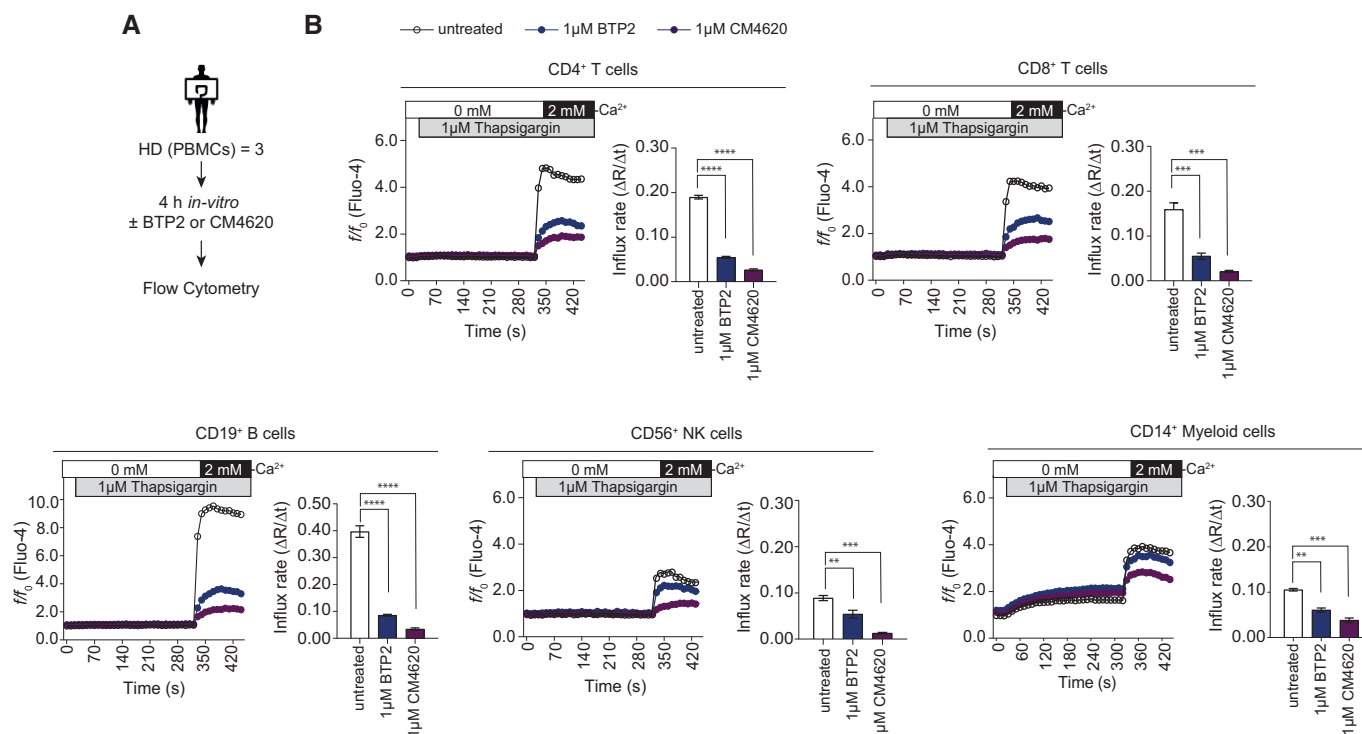
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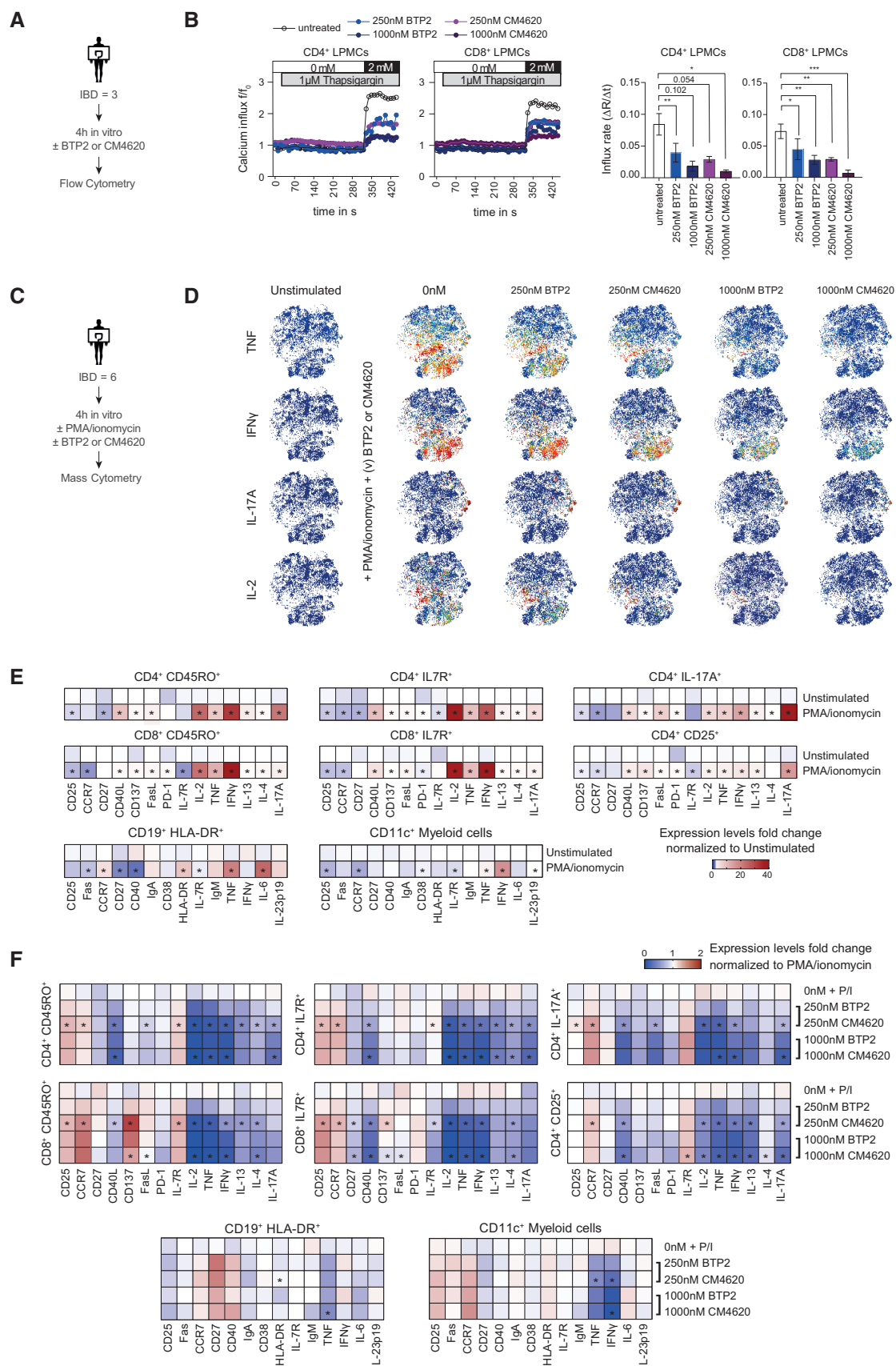


Figure EV5.