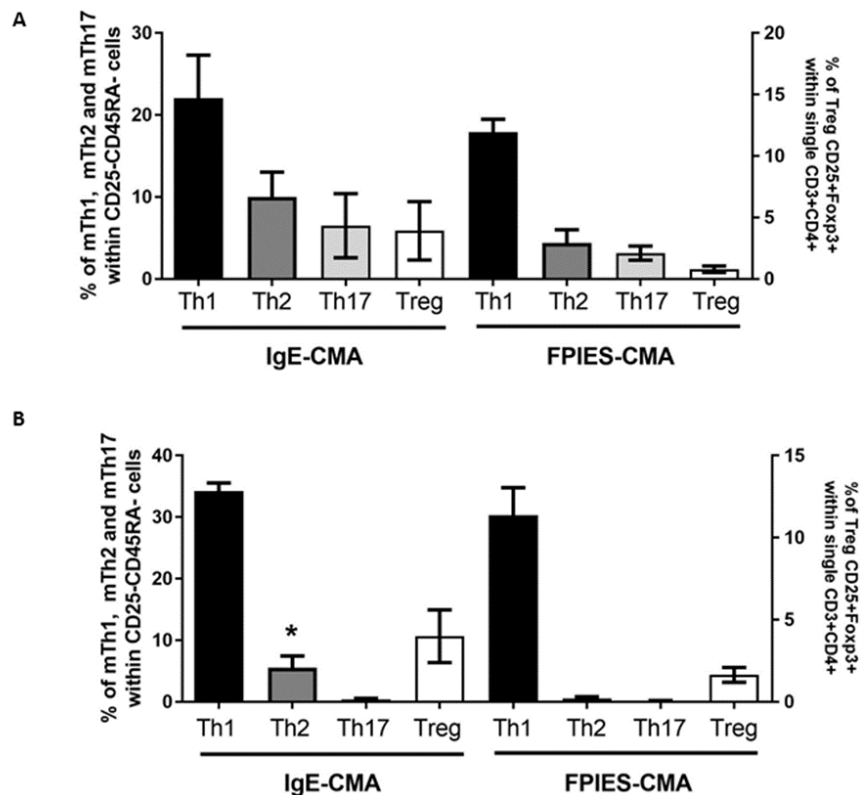


Additional file 2 : Analysis of memory cells within PBMC before and after reactivation using PHA mitogen

Memory Th1, Th2, Th17 and Treg cells in non-stimulated (A) and PHA-activated (B) PBMC. *Analysis of T helper memory cells:* after 6 days of culture, PBMC were collected and labelled extracellularly with anti-human CD4, CD25, CD45RA, CCR6, CXCR3 and CCR4. Gating strategies were performed as described in [1], to analyse memory Th1, Th2, Th17 and Th1* cells. Within CD4⁺ single cells, CD25⁻CD45RA⁻ memory cells were selected, and further analysed for CCR6, CXCR3 and CCR4 expression. Th1 memory cells (mTh1, ■) correspond to CXCR3⁺CCR4⁻ cells within CCR6⁻ population, Th2 memory cells (mTh2, ■) correspond to CXCR3⁻CCR4⁺ cells within CCR6⁻ population, Th17 memory cells (mTh17, ■) to CXCR3⁻CCR4⁺ cells within CCR6⁺ population and non-conventional Th1 memory cells (mTh1*) to CXCR3⁺CCR4⁻ cells within CCR6⁺. This latter population was not numerous enough to be analysed (not shown). Results are expressed as percentage of corresponding cells within the selected CD25⁻CD45RA⁻ population of CD4⁺ single cells. *Analysis of Treg cells:* after 6 days of culture, PBMC were collected and labelled extracellularly with anti-human CD3, CD4, CD25 and intracellularly with Foxp3. Treg cells (□) were defined as CD25⁺Foxp3⁺ population within CD3⁺CD4⁺ single cells.

* indicates a significant difference between IgE-CMA and FPIES-CMA patients ($p < 0.05$, Mann Whitney test).



1. Becattini S, Latorre D, Mele F, Foglierini M, De GC, Cassotta A, Fernandez B, Kelderman S, Schumacher TN, Corti D *et al*: T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines. *Science*. 2015; 347(6220):400-406.