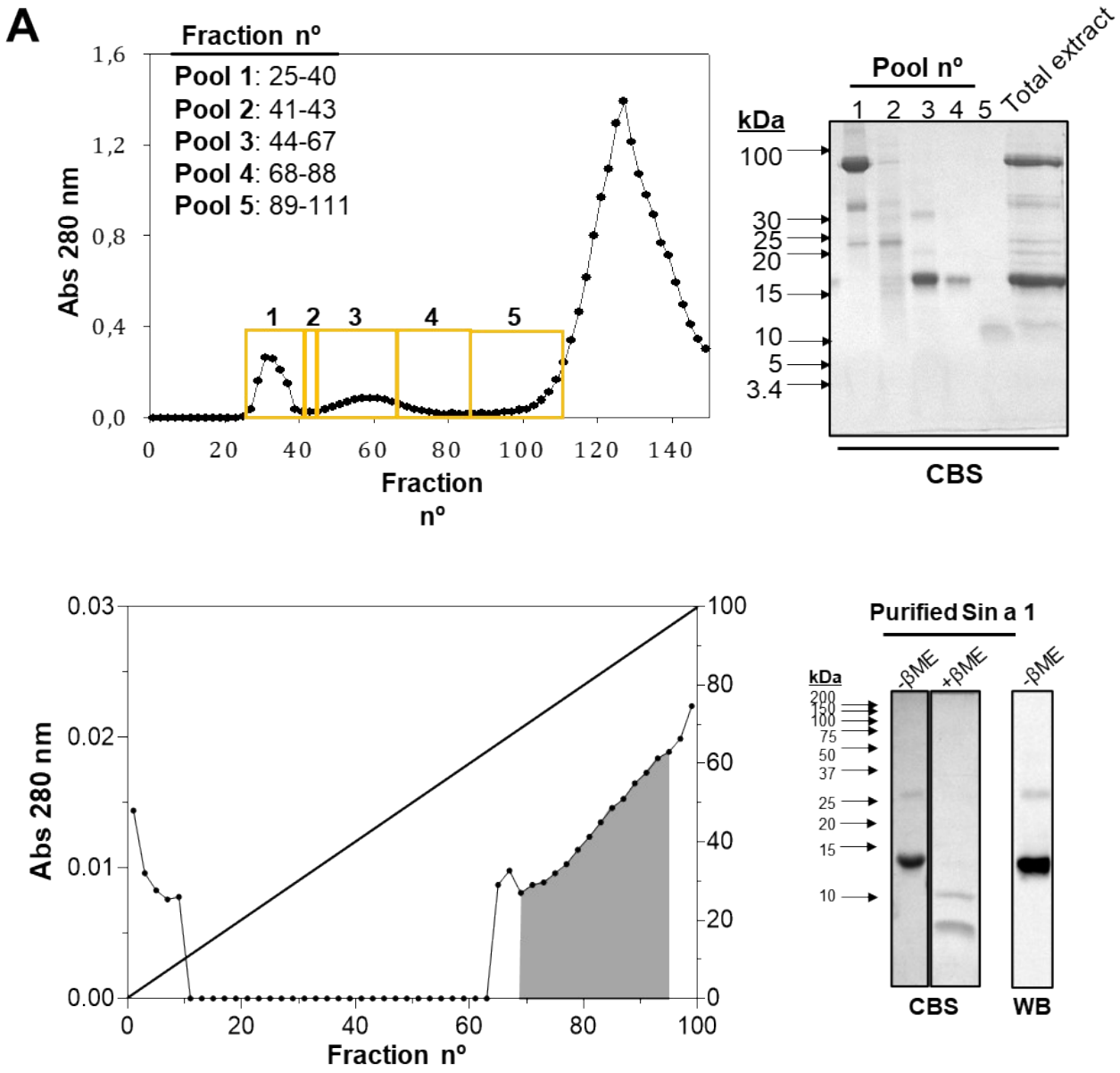
Electronic Supplementary Material (ESI) for Food & Function.

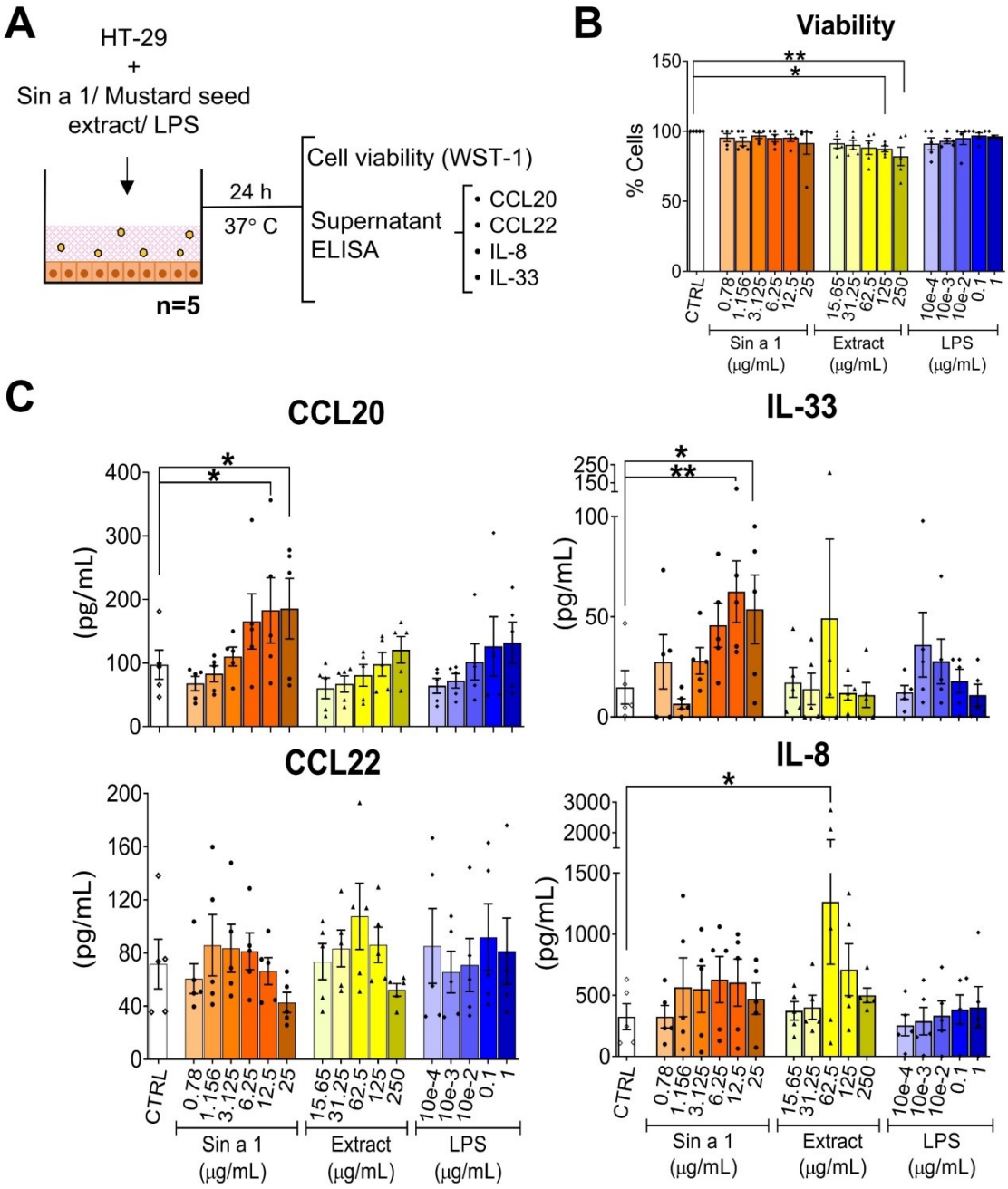
Supplementary information

Cytokine or immunoglobulin	Source	Cat Number
IFNγ	Invitrogen	88-7316-88
IL-4	Invitrogen	88-7046-88
IL-8	Invitrogen	88-8086-88
IL-10	Invitrogen	88-7106-88
IL-12p70	Invitrogen	88-7126-88
IL-13	Invitrogen	88-7439-88
IL-15	Biolegend	435104
IL-17	Invitrogen	88-7176-88
IL-25	R&D systems	DY1258-05
IL-33	R&D systems	DY3625B
TGFβ	Invitrogen	88-50390-88
TNFα	Invitrogen	88-7346-88
TSLP	Invitrogen	88-7497-88
IgE	Invitrogen	88-50610-88
IgG	Invitrogen	88-50550-88
CCL20	R&D systems	DY360
CCL22	R&D systems	DY336

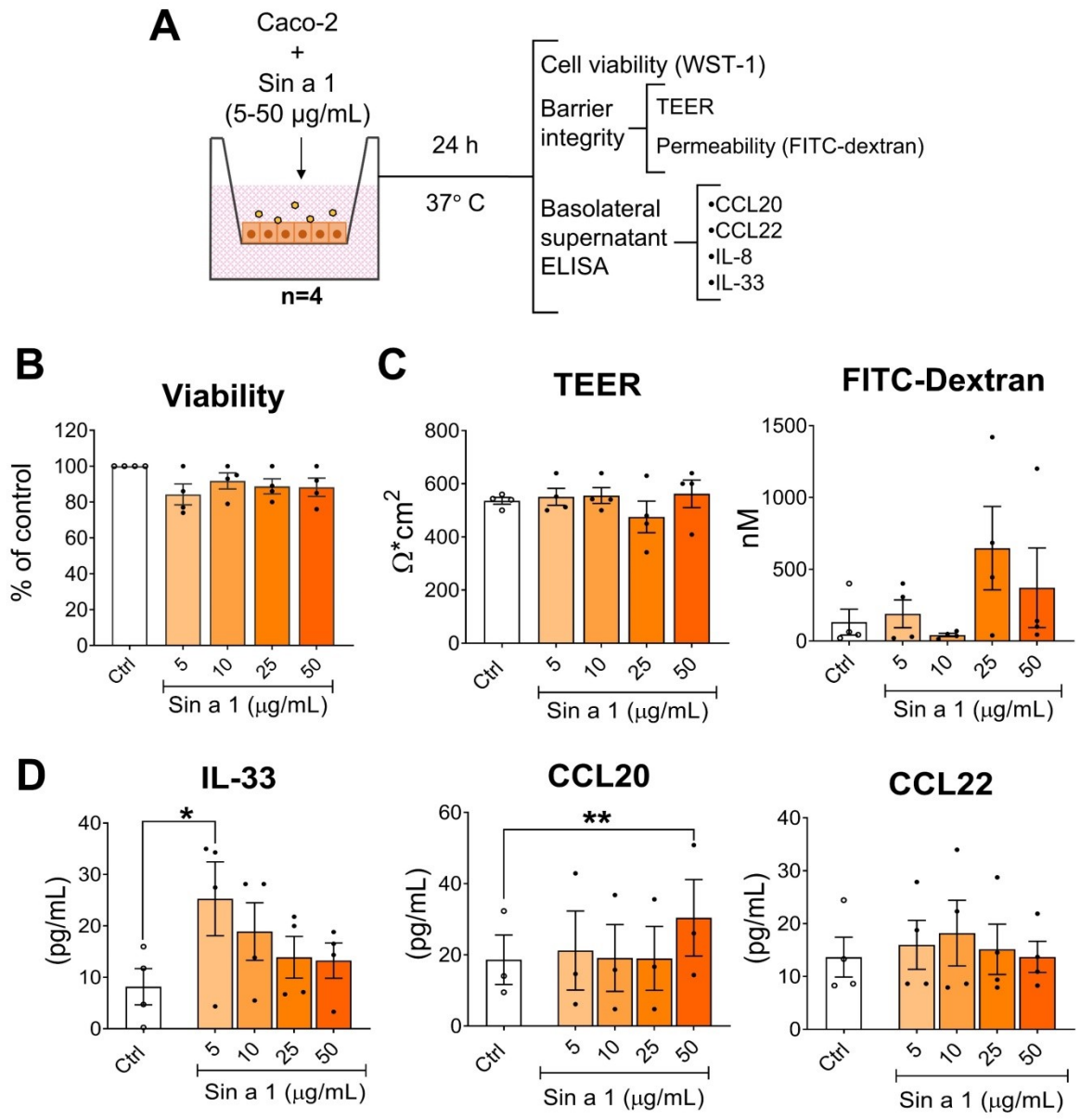
Table 1 Supplementary. ELISA kits employed to determine cytokine concentrations.



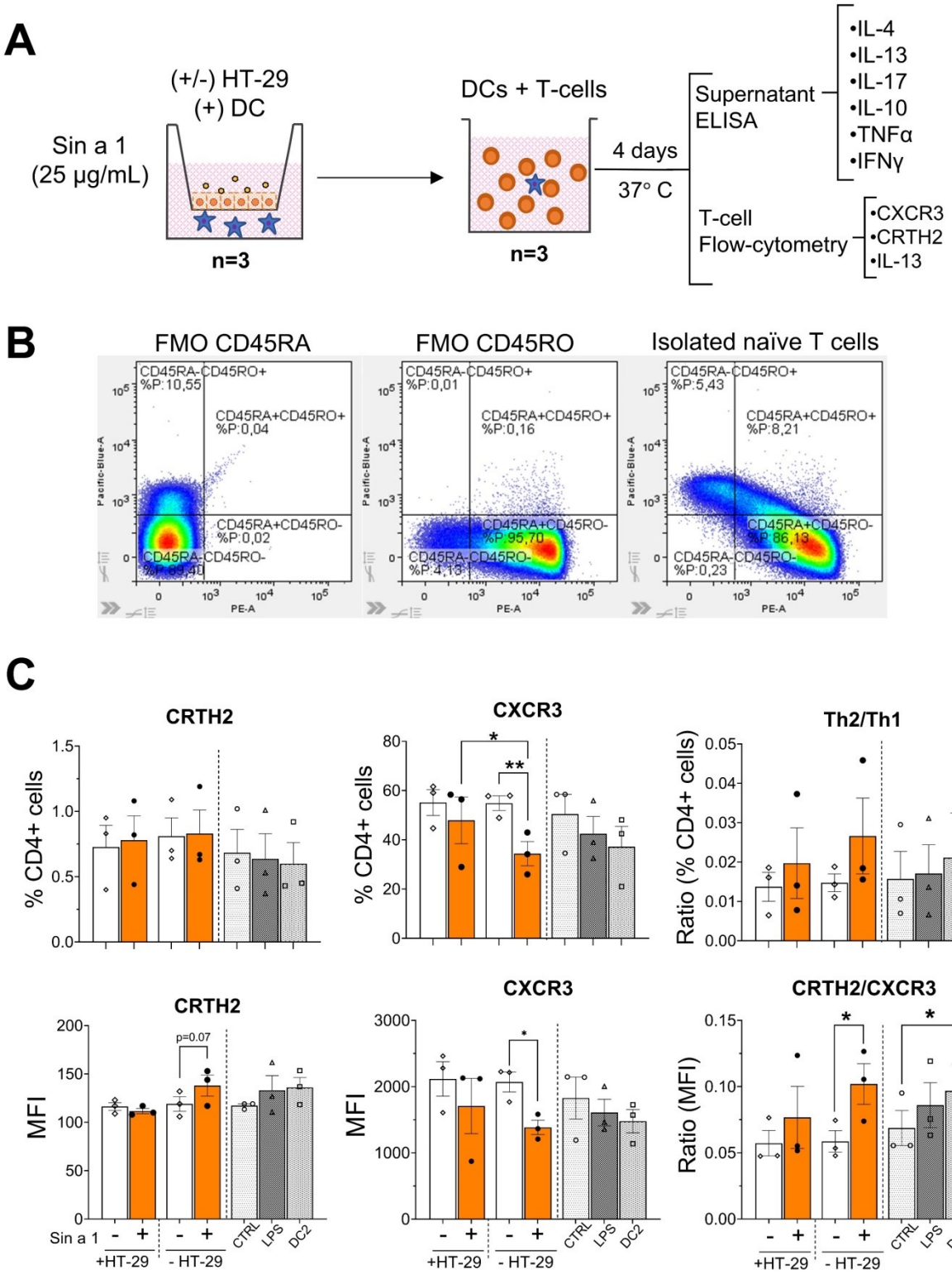
Supplementary Figure 1. Sin a 1 isolation from yellow mustard seed extract. Two consecutive chromatographic steps were employed for Sin a 1 isolation from total protein mustard seed extract: a size exclusion chromatography (A), and an ion-exchange chromatography of pools 3 and 4 from previous step (B).



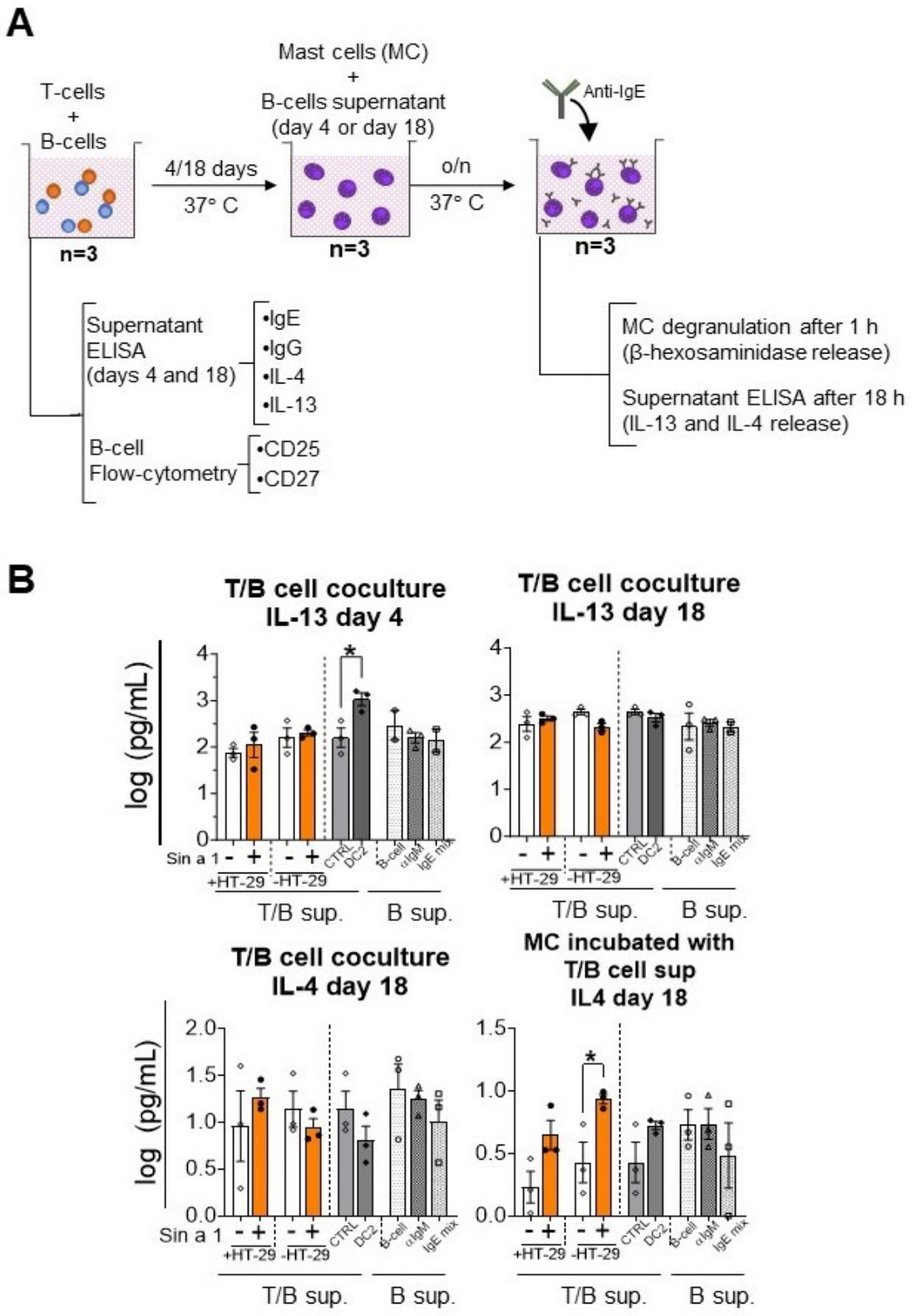
Supplementary Figure 2. Exposure of HT-29 to Sin a 1, mustard seed extract and LPS. (A) HT-29 cell line was seeded in flat-bottom 48 well-plates and incubated with increasing doses of Sin a 1, total mustard seed or LPS, for 24 h (n=5 independent experiments). After that, cell viability was determined using WST-1 reagent (B), while cell supernatants were employed for cytokine analysis by ELISA. (C) Cytokine profile of cell supernatants after incubation with Sin a 1. Data was analysed by one-way ANOVA and Bonferroni post hoc test with selected pairs, mean ± SEM * p<0.05, ** p<0.01, *** p<0.001).



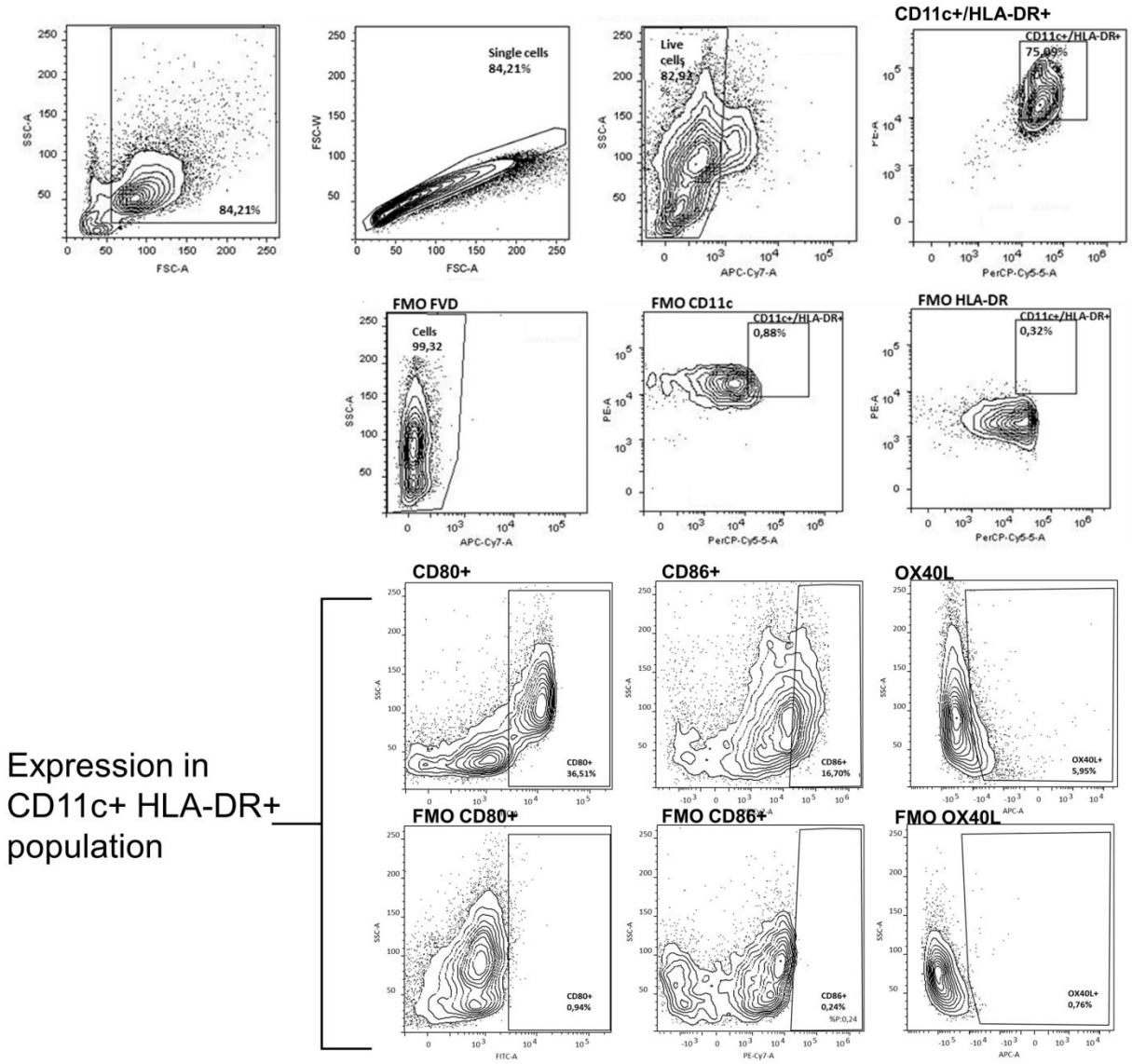
Supplementary Figure 3. Caco-2 activation in presence of Sin a 1. (A) Caco-2 cell line was seeded in 12 well transwell insert plates and, once confluence was reached cells were polarized for 14-21 days, after which they were incubated with increasing doses of the mustard seed major allergen, Sin a 1, for 24 h (n=4 independent experiments). After that, cell viability (B), barrier integrity (C) was determined, and cytokine analysis was performed by ELISA (D) were performed. Data was analysed by one-way ANOVA and Bonferroni post hoc test with selected pairs, mean ± SEM * p<0.05, ** p<0.01, *** p<0.001).



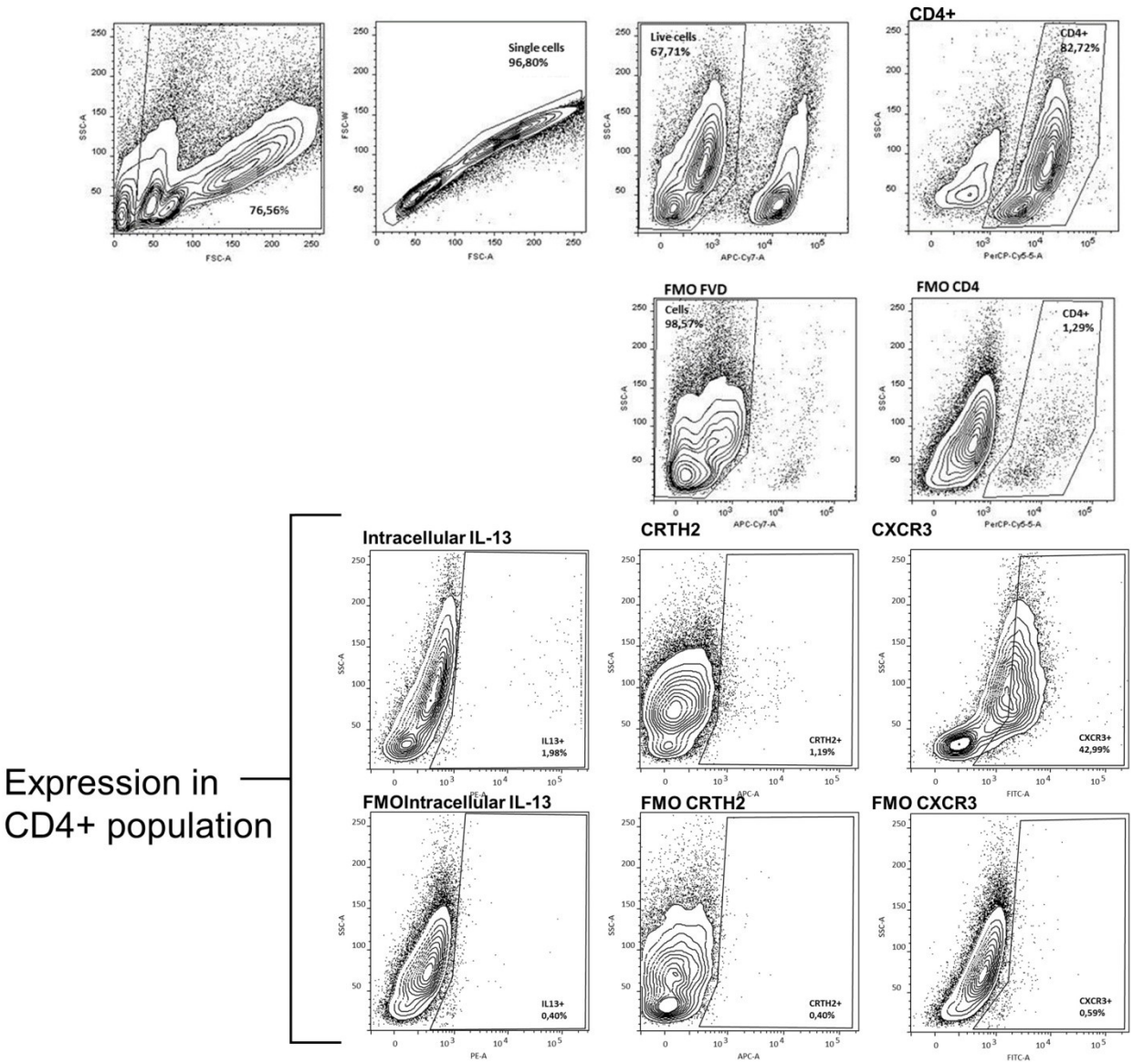
Supplementary Figure 4. Co-culture of Sin a 1 primed IEC-DC or DC with naïve T-cells results in a type-2 immune response. (A) DC from the previous step were cocultured with allogenic naïve T-cells for 96 h (n=3 independent donors). Naïve T-cell isolation (CD45RA+RO-) was confirmed by flow cytometry after MACS isolation step (B), and T-cell phenotype was determined after co-culture with DC (CRTH2 marker Th2 cells, CXCR3, marker for Th1 cells; % cells, median MFI and ratio Th2/Th1) (C). Controls were LPS and DC2 type cytokine mix (DC2) matured DC. Data was analysed by one-way ANOVA and Bonferroni post hoc test with selected pairs, mean ± SEM (* p<0.05, ** p<0.01, *** p<0.001).



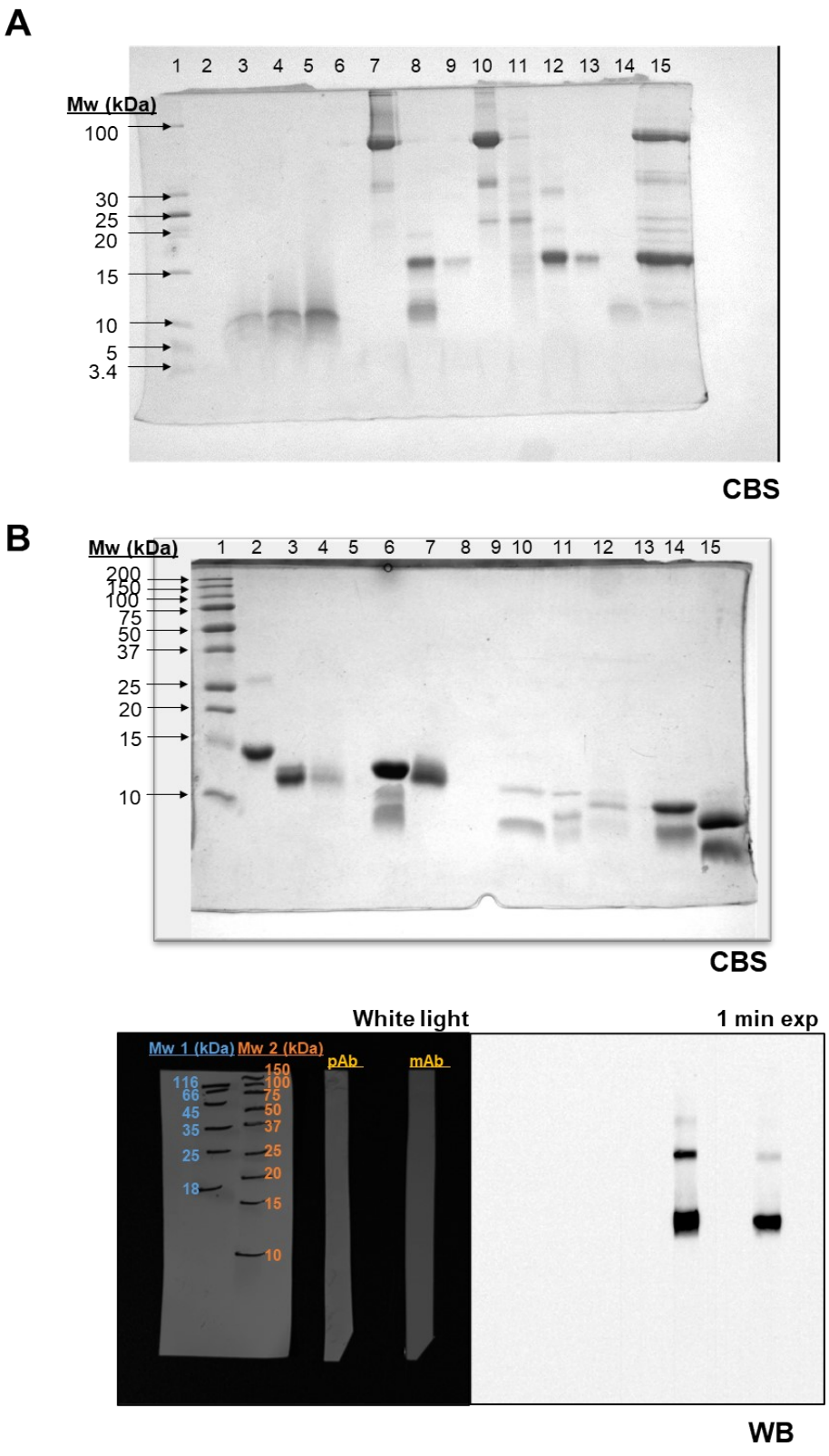
Supplementary Figure 5. T/B-cell cocultures and MC exposed to B-cell supernatants (n=3 independent donors) (A). IL-4 and IL-13 concentrations were determined after 4 days (not-detected) and 18 days in the supernatant of the autologous T/B-cell coculture, and in supernatants from MC exposed to these supernatants (only IL-4 was detectable when exposed to 18 days supernatant) (B). Controls are B-cells alone, medium-exposed (B-cell), anti-IgM exposed (aIgM), or anti-IgM+antiCD40+IL-4 (IgE mix). Data was analysed by one-way ANOVA and Bonferroni post hoc test with selected pairs, or by paired t-test for CTRL vs DC2, mean ± SEM (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).



Supplementary Figure 6. Gating strategy of moDC. First, CD11c+HLA-DR+ DC were selected within the live cell population. Then, CD80, CD86 and OX40L expression markers were analysed within the double-positive DC population. FMO (isotype) controls of each marker are shown to confirm specificity of the obtained results.



Supplementary Figure 7. Gating strategy of T cells. First, CD4+ (T helper) were selected within the viable cell population. Then intracellular IL-13, CRTH2 and CXCR3 expression markers were analysed within the CD4+ population. FMO (isotype) controls of each marker are shown to confirm specificity of the obtained results



Supplementary Figure 8. **Originals of SDS-PAGE and western blotting**. **A.** SDS-PAGE stained in CBS of pools obtained from size-exclusion chromatography (lanes 10-14) and total protein extract of yellow mustard seed. Lanes 2-13 correspond to other proteins no related to the present study. **B.** SDS-PAGE in CBS of 2S albumins from different sources under non-reducing (lanes 2-7) and reducing (lanes 10-15) conditions. Sin a 1 was loaded in lanes 2 and 8. Under CBS, original pictures of western blotting in non-reducing conditions of Sin a 1 using a policlonal (pAb) and a monoclonal (mAb) antibody.