

**Human milk oligosaccharides protect against the development of autoimmune diabetes
in NOD-mice**

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Figure S1. (A) Average body weight of NOD mice fed with HMOS or control diet overtime. Dietary intervention with HMOS does not change the body weight compared with non-treatment. (B) Occurrence of diabetes. Upper table shows the mice from control group and lower table shows the mice from HMOS group. Red highlights represent mice diagnosed as diabetic at specific week, mice were sacrificed once diagnosed as diabetic.

Figure S2. The spectrum of HMOS

Figure S3. Ratio of Firmicutes/Bacteroidetes in diabetic and non-diabetic mice

Figure S4. The Bray Curtis dissimilarity index. Samples from NOD-mice were divided into diabetic and non-diabetic subgroups. There is greater dissimilarity and variability in the microbial community in Week 30 HMOS Non-Diabetic mice. Broad microbial variability at Week 30 HMOS Non-Diabetic mice could indicate that HMOS is not specific to just one taxa, but HMOS could be altering a greater number of taxa in the overall microbial community.

Figure S5. PICRUSt-predicted metagenomes between the ratio of HMOS non-diabetic to Control non-diabetic mice fecal samples. Feces represent different log₁₀ value fold change functional pathways identified by PICRUSt software ($P < 0.05$); 3 pathways were higher abundant in HMOS non-diabetic relative to control non-diabetic NOD-mice.

Figure S6. SCFAs production in cecum content.

Figure S7. The gating strategy used to identify expression of surface markers on DCs.

Figure S8. Effect of polymyxin B on HMOS induced surface markers expression (A) and IL-10 secretion (B). HMOS containing medium was pre-incubated with 10ug/ml polymyxin B for 2h before adding to iDCs for 24h. The expression of CD86, MHC-II, PD-L1 on DCs and IL-10 secretion on the DCs supernatant were analyzed. No significant difference was observed between HMOS and pre-treated HMOS. The histograms shown were from one representative experiment and cytokines results were from two independent experiments.

Figure S9. The gating strategy used to identify the different CD4⁺ T-cell subpopulations.

Figure S10. The gating strategy used to determine CD4 T cells proliferation

Figure S2

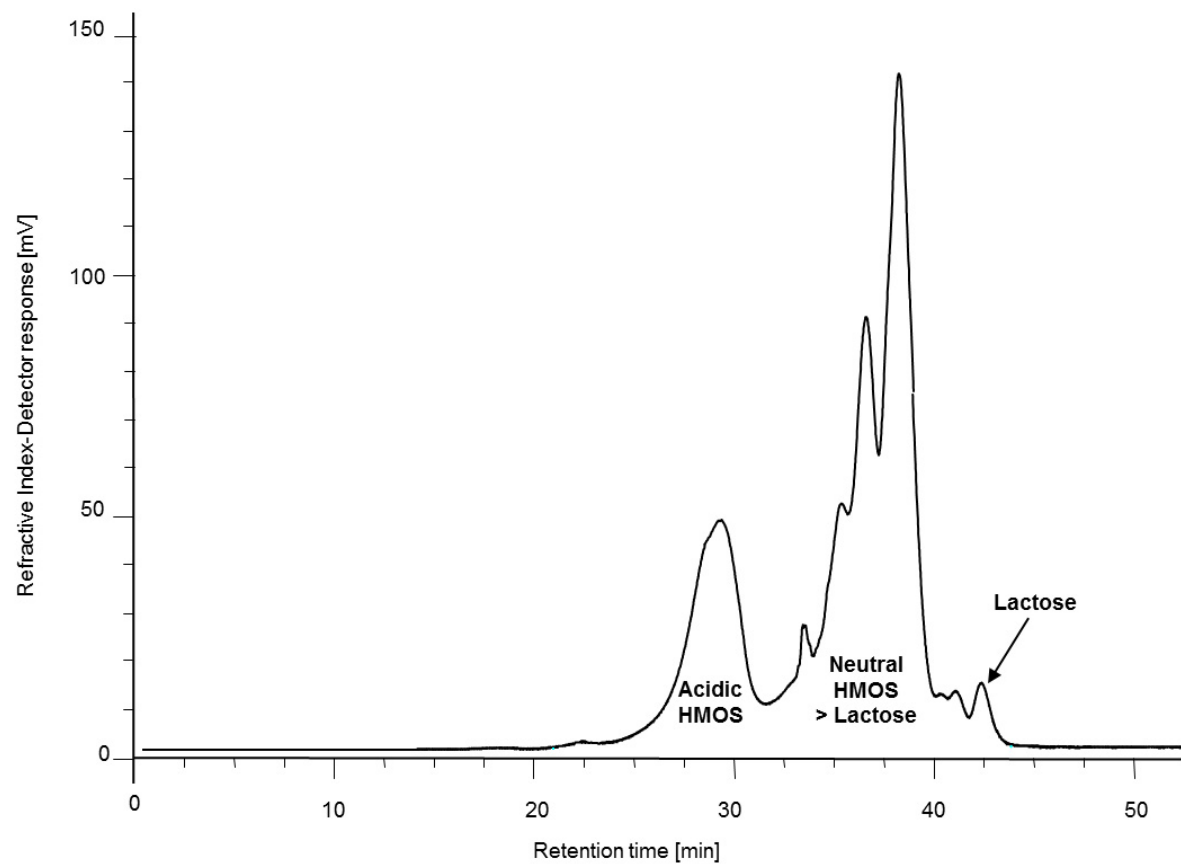


Figure S3

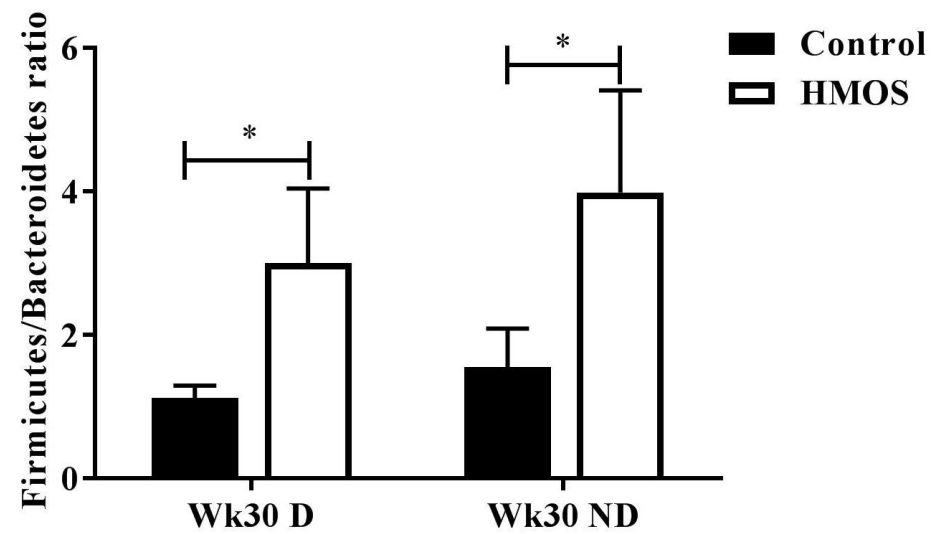


Figure S4

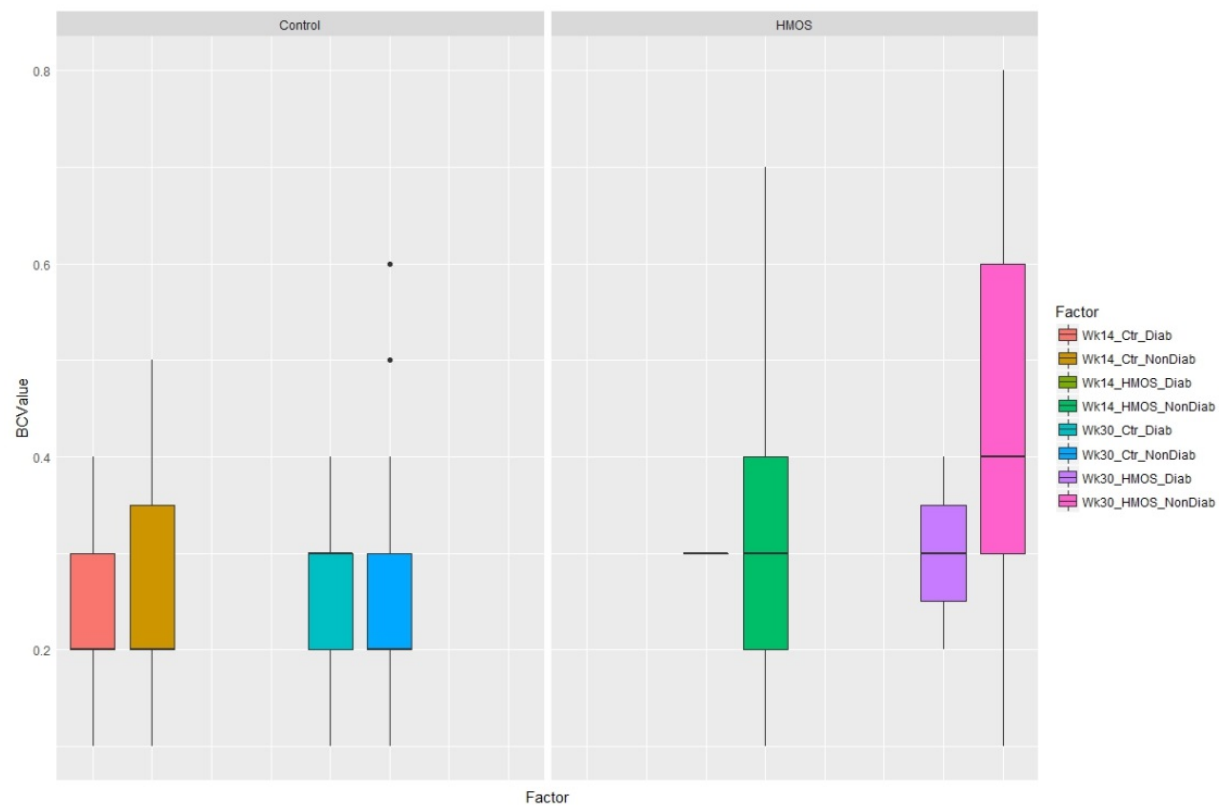


Figure S5

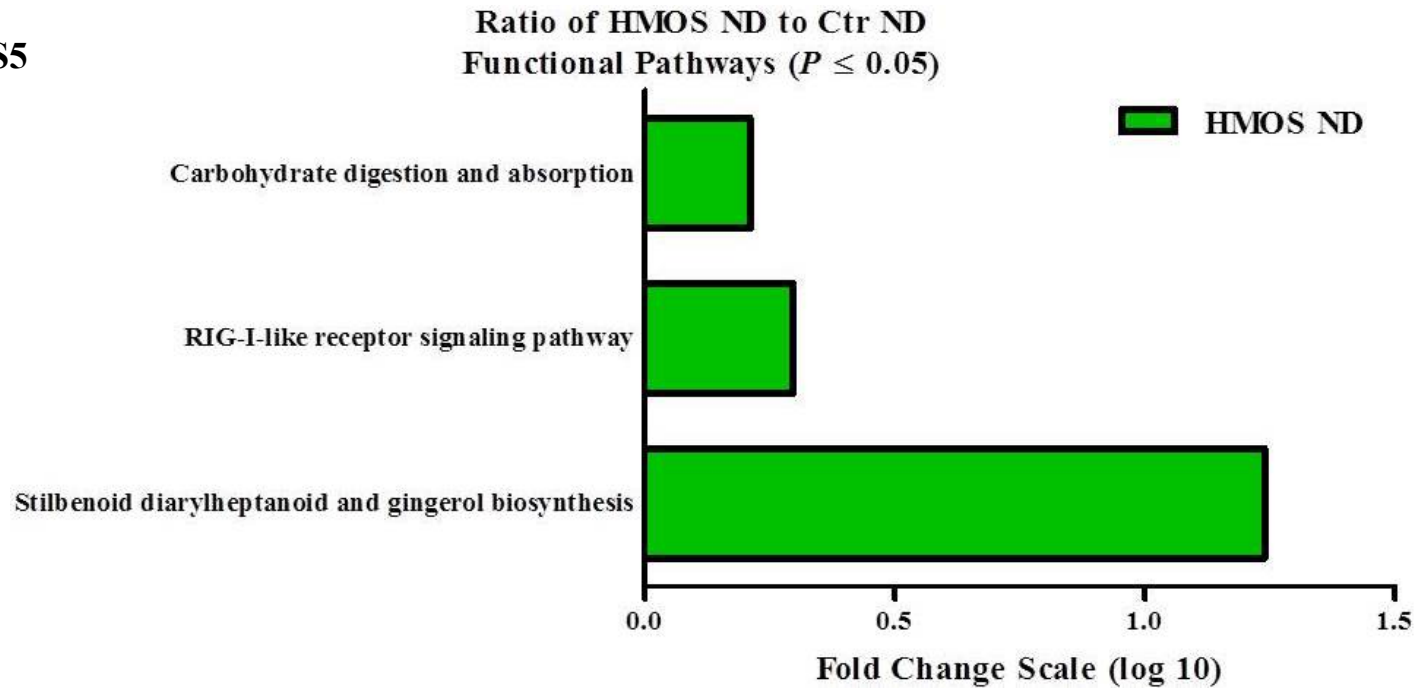


Figure S6

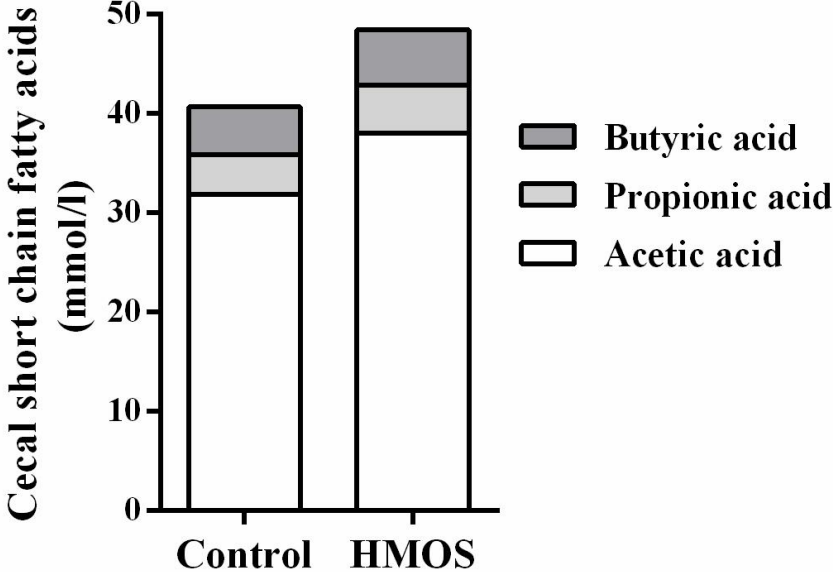


Figure S7

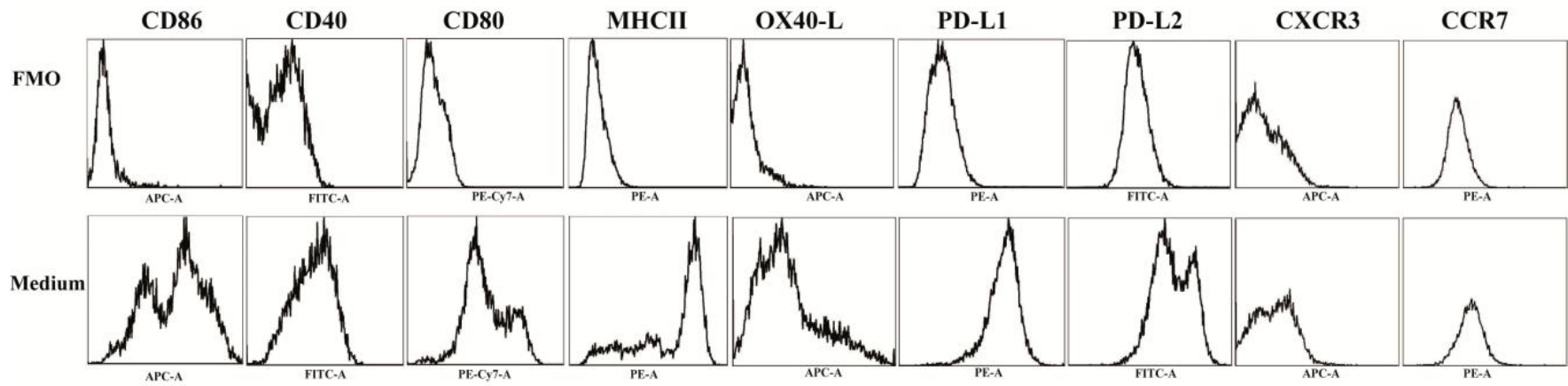
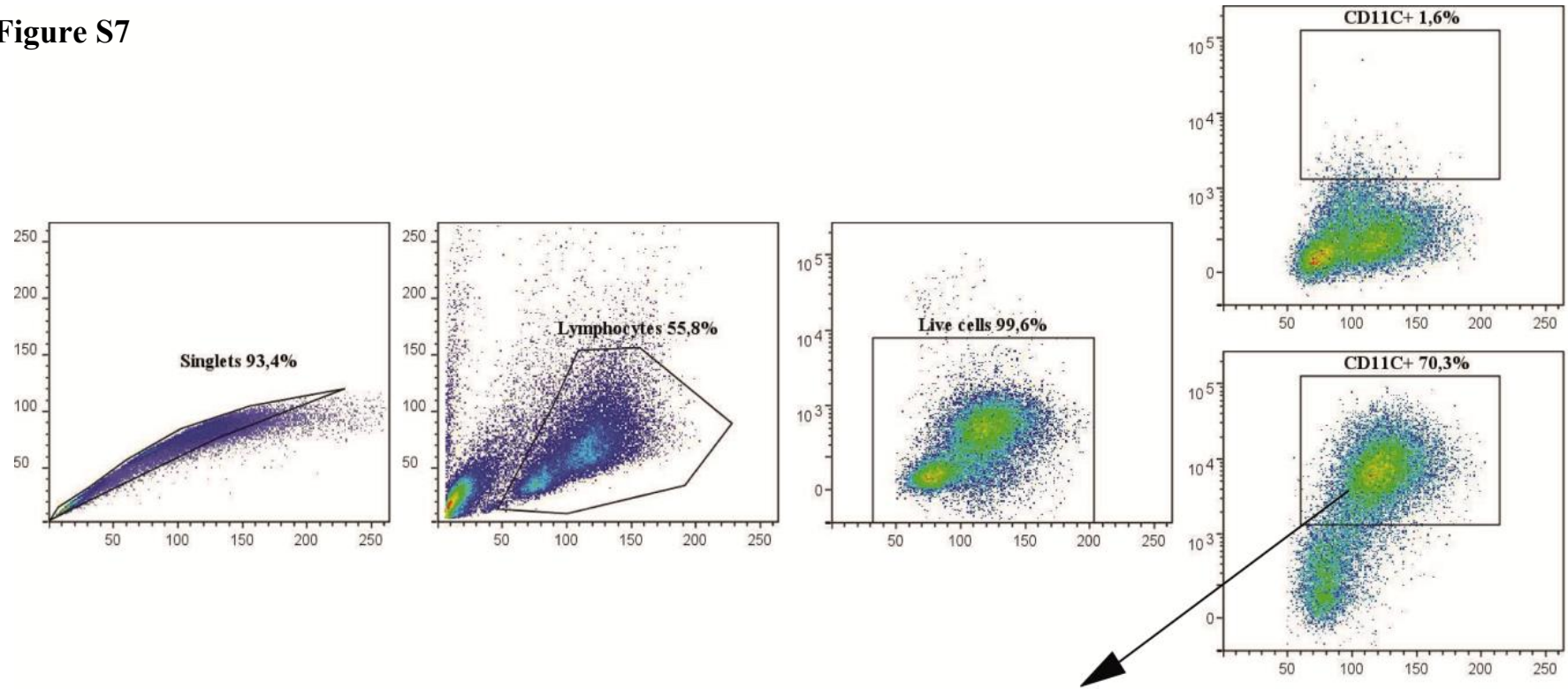


Figure S8

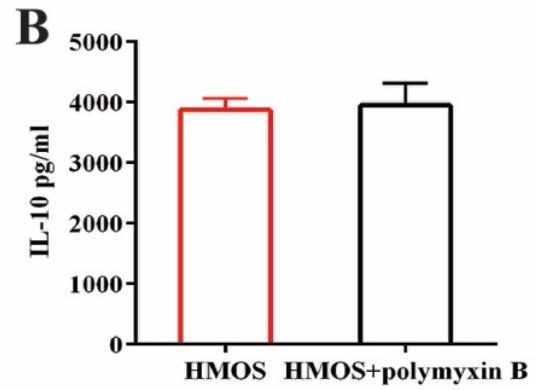
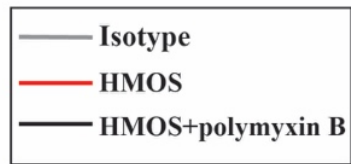
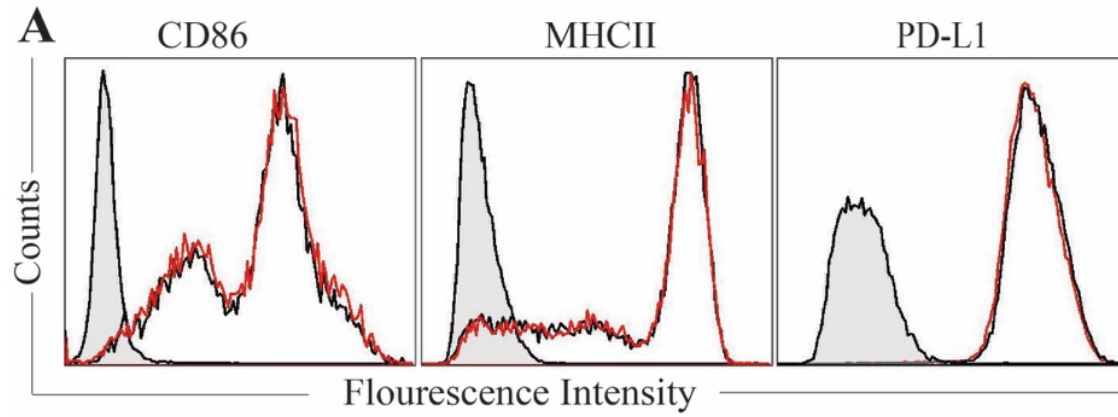


Figure S9

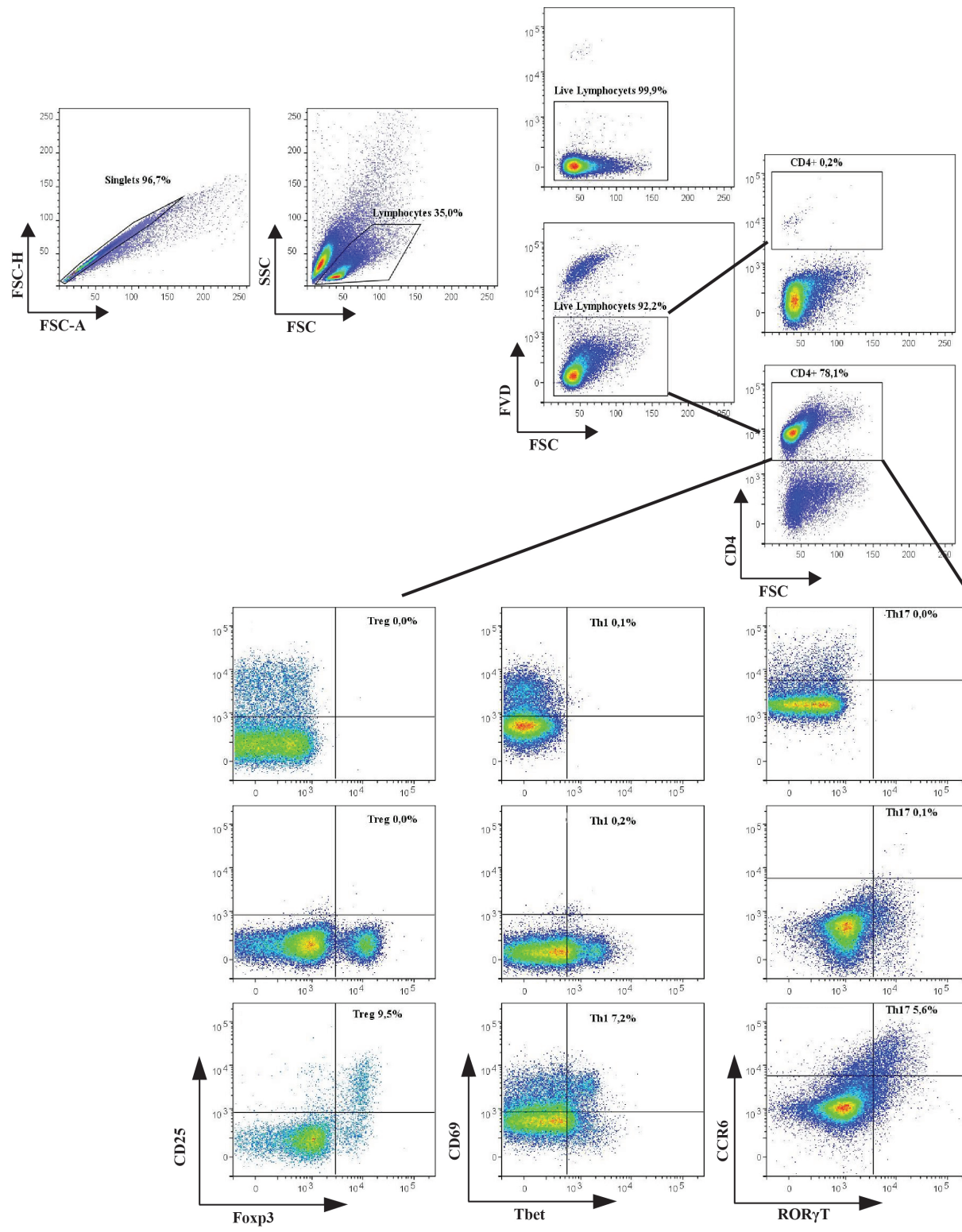


Figure S10

