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

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<sup>5</sup> DNA Services Facility, Research Resources Center, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL USA

<sup>6</sup> Department of Pharmacology, Department of Physiology, Rush University Medical Center, Chicago, IL USA 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 comapred 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 devided into diabetic and nondiabetic 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-L1on 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





B

			Age	week 4	week 5	week 6	week 7	week 8 w	veek 9	week 10	week 11	week 12	week 13	week 14	week 15	week 16	week 17	week 18	week 19	week 20	week 21	week 22	week 23	week 24	week 25	week 26	week 27	week 28	week 29	week 30
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4	Α	13		0	0	0	0	0 0	)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1
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7	Α	16		0	0	0	0	0 0	)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Α	17		0	0	0	0	0 0	)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	Α	18		0	0	0	0	0 0	)	0	0	0	0	0	0	0	0	0	0	0	0	4	4							
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Figure S3





Fold Change Scale (log 10)











Figure S9

