Oral therapy with colonization factor antigen I prevents development of type 1 diabetes in Non-obese Diabetic mice

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Supplemental Table 1. Antibodies used for Flow Cytometry in LL-CFA/I Treg Studies.

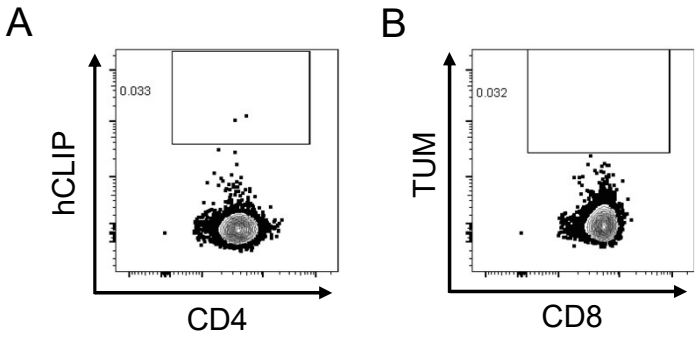
Antibody specificity	Clone	Source	Dilution
CD19	EBio1D3	eBioscience	1/600
CD25	PC61.5	Biolegend	1/500
CD39	24DMS1	eBioscience	1/500
CD4	RM4-5	Biolegend	1/500
CD49B	HMa2	BD	1/400
CD8α	53-6.7	eBioscience	1/500
CTLA-4	UC10-4F10-11	BD	1/500
Foxp3	FJK-16s	eBioscience	1/250
IFN-γ	XMG1.2	BD	1/250
IL-10	JES5-16E3	eBioscience	1/250
Lag-3	C9B7W	Biolegend	1/400
PD-1	RMPI-30	eBioscience	1/500
Tbet	4B10	Biolegend	1/250
TCR-β	H57-597	Biolegend	1/500
TGF-β (Lap)	TW7-16B4	Biolegend	1/400
TIGIT	1G9	Biolegend	1/400
TNF-α	MP6-XT22	Biolegend	1/250

Summary of specificity, clone, and source of antibodies used in flow cytometry analysis.

Supplemental Table 2. Primer sequences for detection of cytokine-specific mRNA.

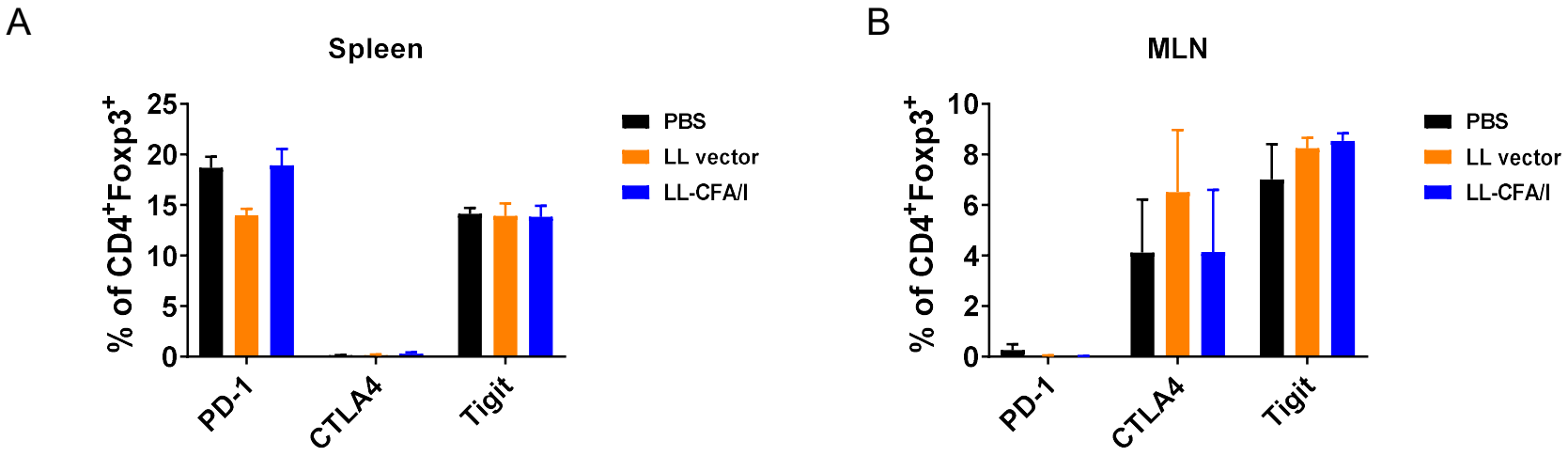
Gene Name	Primer	Size	Sequence (5`-3`)
IL-6	F	23	TAGTCCTTCCTACCCCAATTTCC
	R	21	TTGGTCCTTAGCCACTCCTTC
TNF-α	F	23	CCCTCACACTCAGATCATCTTCT
	R	19	GCTACGACGTGGGCTACAG
IL-10	F	21	GCTCTTACTGACTGGCATGAG
	R	20	CGCAGCTCTAGGAGCATGTG
IL-33	F	21	ACAGATATATGACTTACGGCG
	R	23	AAATGGACCCTCTCTAAAGCAAA
GAPDH	F	20	ACCACAGTCCATGCCATCAC
	R	19	TCCACCACCCTGTTGCTGTA
B-actin	F	22	ATCTACGAGGGCTATGCTCTCC
	R	21	AGCCTCGGTCAGGATCTTCAT

Supplemental Figure 1. Negative Controls for Tetramer Staining



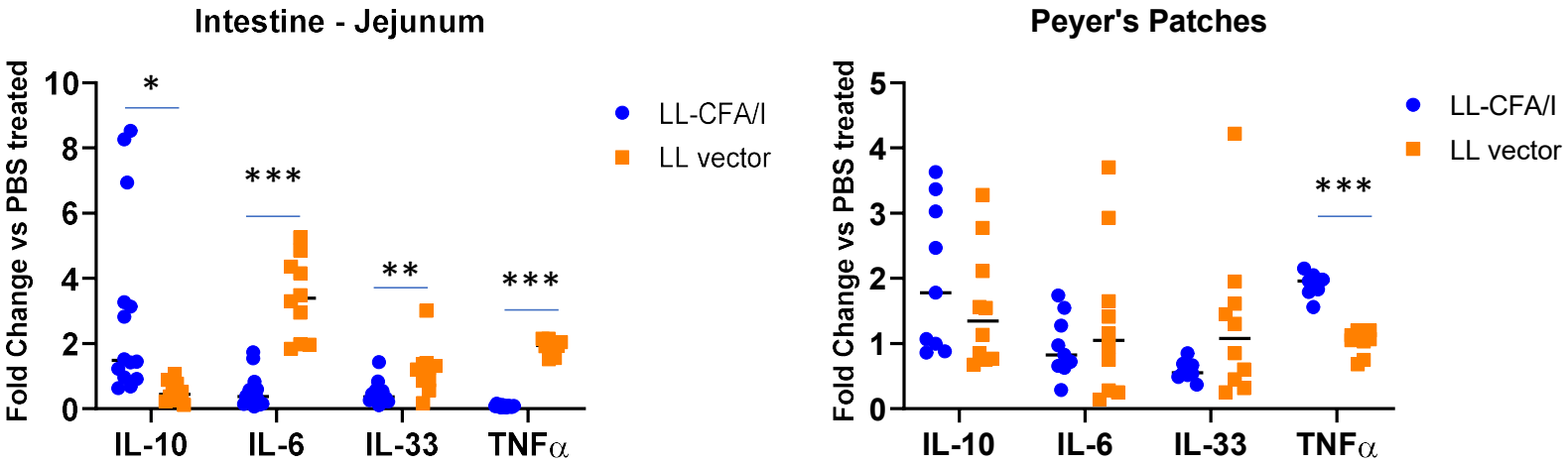
Supplemental Figure 1. Negative controls for tetramer staining. Four wk-old NOD females were orally dosed with $5x10^7$ CFUs of LL-CFA/I, LL vector, or PBS (n=5/group). Additional doses were given every 2 wks. At 11 wks of age, lymphocytes from the PaLNs were isolated and labeled with tetramers specific for (A) human CLIP or (B) TUM peptide.

Supplemental Figure 2. LL-CFA/I does not Induce Negative Regulators in NOD Mice



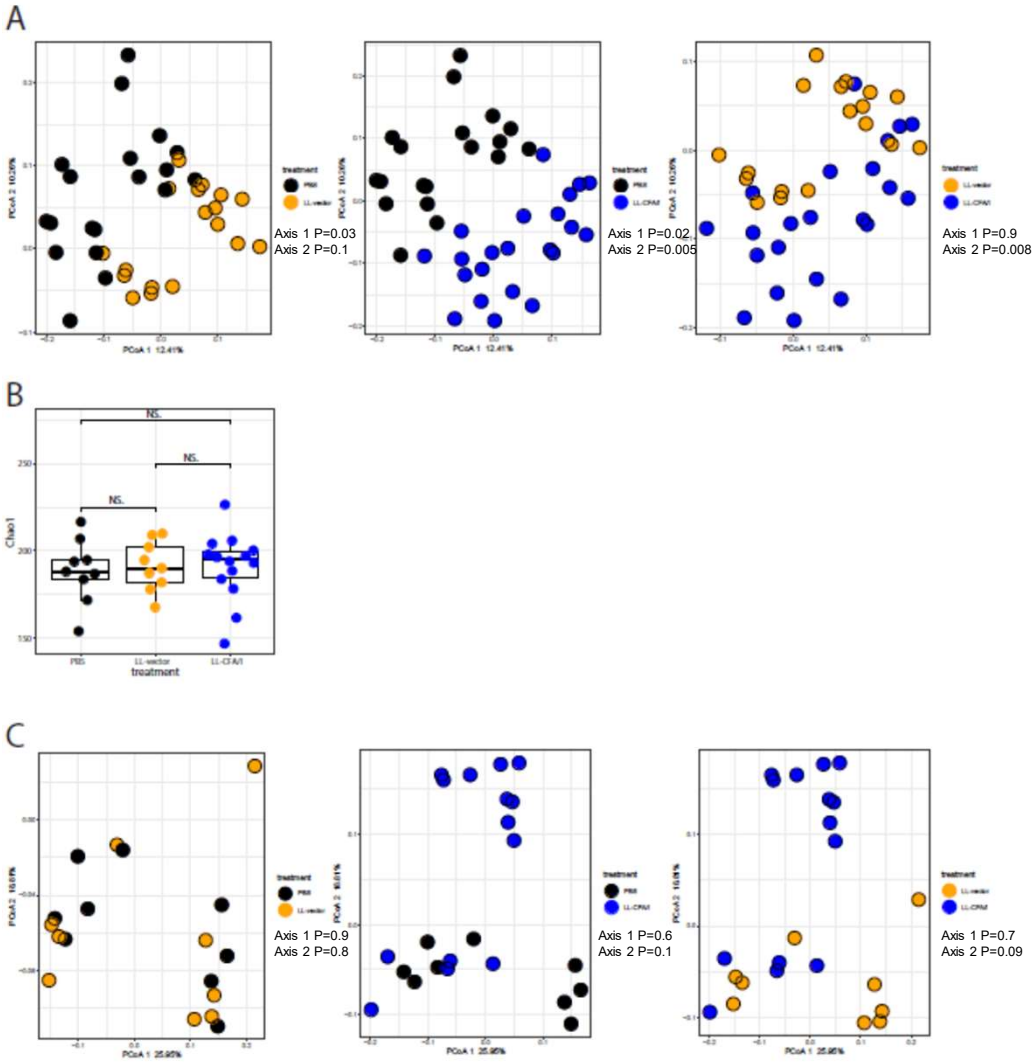
Supplemental Figure 2. LL-CFA/I does not induce negative regulators in NOD mice. Four wk-old NOD females were orally dosed with $5x10^7$ CFUs of LL-CFA/I, LL vector, or PBS (n=5/group). Additional doses were given every 2 wks. At 11 wks of age, $Foxp3^+CD4^+$ Tregs from the (A) spleens and (B) MLNs were examined for expression of PD-1, CTLA-4 and Tigit.

Supplemental Figure 3. LL-CFA/I promotes regulatory environment in the gut.



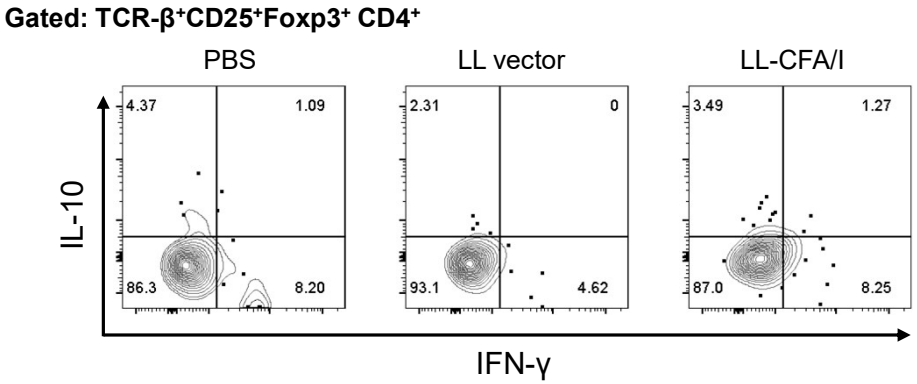
Supplemental Figure 3. LL-CFA/I promotes regulatory environment in the gut. Eight week old BALB/c mice (n=9-14/group) were orally dosed with $2x10^9$ CFUs of LL-CFA/I, LL-vector or PBS. After 1.5 hours, small intestines and Peyer's Patches were collected. Expression of cytokines was analyzed as fold change against the PBS control group. *p<0.05, **p<0.005, and ***p<0.0001 for LL-CFA/I vs LL-vector.

Supplemental Figure 4. Mouse Microbiota are not Significantly Different Between Groups Before LL Treatments Begin.



Supplemental Figure 4. Mouse microbiota are not significantly different between groups before LL treatments begin. (A) Pair-wise comparisons of beta diversity post-treatment (11 weeks old). (B) Alpha diversity of pre-treatment samples at 4 weeks old. (C) PCoA plots of beta diversity in pre-treatment samples. NS, not significant.

Supplemental Figure 5. LL-CFA/I does not Induce Splenic IFN-γ$^+$IL-10$^+$ Foxp3$^+$CD4$^+$ Tr1 cells at 17 weeks



Supplemental Figure 5. LL-CFA/I does not Induce Splenic IFN-γ$^+$IL-10$^+$ Foxp3$^+$CD4$^+$ Tr1 cells at 17 weeks. Four wk-old NOD females were orally dosed with 5x10^7 CFUs of LL-CFA/I, LL vector, or PBS (n=5/group). Additional doses were given every 2 wks from 6 to 16 weeks of age. (A) Splenocytes were stimulated with anti-CD3 and anti-CD28 mAbs and CD25$^+$Foxp3$^+$CD4$^+$Tregs analyzed for expression of IFN-γ and IL-10.