Supplemental Figure Legends

Figure S1. Time Course of $CD4^+$ T cell expansion in response to intradermal immunization with dmLT or CpG. C57BL/6 mice were immunized with 2W1S-GFP protein plus either CpG or dmLT in the pinna of each ear. At each indicated time point, the mice were assayed the number indicated cells in CLN and spleen: A) Total 2W1S-specific $CD4^+$ cells. B) Total $\alpha4\beta7^+$ 2W1S-specific T cells. Each time point represents three mice and is representative of two independent experiments.

Figure S2. Quantification of total numbers of antigen-specific $CD4^+$ T cells in different tissues of dmLT and CpG immunized mice. Mice immunized with CpG or dmLT plus 2W1S-GFP were assayed nine days later for 2W1S-specific $CD4^+$ T cell responses which were then quantified. A) Total number of 2W1S-specific CD4 T cells in the CLN, MLN, and spleen, B) total number of a4b7+, 2W1S-specific CD4 T cells in the CLN, MLN, and spleen, C) Total number of 2W1S-specific CD4 T cells in the small and large intestine. Five mice per group representative of two independent experiments. Significance was determined by student's two-tailed t-test. Statistical significance is shown on each graph. Error bars represent SEM.

Figure S3. Antigen-specific $CD4^+$ T cell lung migration was comparable between dmLT and CpG immunized mice. Mice immunized with CpG or dmLT plus 2W1S-GFP were assayed nine days later for lung 2W1S-specific $CD4^+$ T cell responses. $Tetramer^+$ cells were magnetically enriched before analysis. A) representative flow cytometry plots and B) quantification of T cell numbers are shown. Representative of two independent experiments with two to four mice per group.

Significance was determined by student's two-tailed t-test. Statistical significance is defined as follows: *, P<0.05; **, P<0.01; ***, P<0.005. Error bars represent SEM.

Figure S4. dmLT induces greater expression of α4β7 and MLN migration compared to Pam3CSK(4). C57BL/6 mice were intradermally immunized with 2W1S-GFP plus either Pam3CSK(4) or dmLT. Nine days post immunization, CLN, MLN, and spleen were harvested and dissociated to yield lymphocytes. Single-cell suspensions were then magnetically enriched for 2W1S-specific $CD4^+$ T cells. A) Representative plots on the left demonstrate the enriched 2W1S-specific fraction of $CD4^+$ T cells. B) To the right are the representative plots showing α4β7 expression on 2W1S-specific $CD4^+$ T cells. The bottom figure is the graphical representation of $\%\alpha4\beta7^+$ 2W1S-specific cells. From one independent experiment of two experiments with three mice per group. Significance was determined by Student's two-tailed test with Holm-Sidak correction for multiple comparisons where *=p<0.05; **=p<0.01; ***=p<0.001.

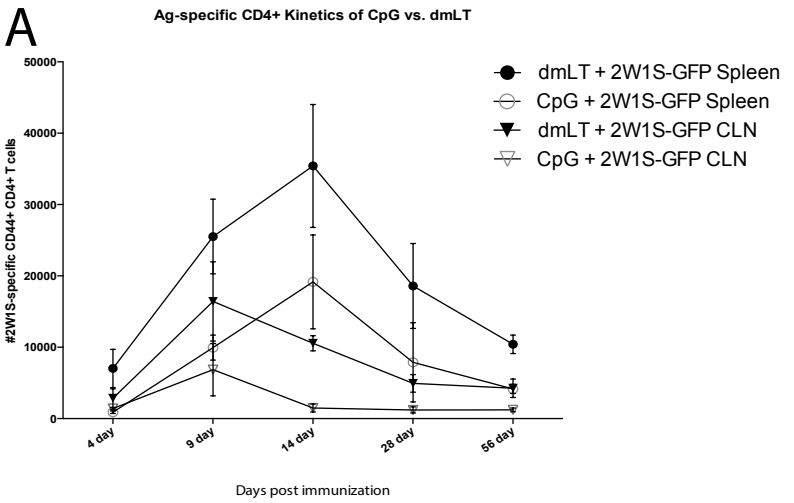
Figure S5. Modifying Route of dmLT Administration Does Not Impact α4β7 Imprinting on 2W1S-specific T cells. C57BL/6 mice were immunized with 2W1S-GFP plus dmLT intradermally in each ear pinna, the flank near the hind leg, or intramuscularly in the hind leg. At nine days post immunization, the draining CLN and MLN were harvested and dissociated to yield lymphocytes. Single-cell suspensions were then magnetically enriched for 2W1S-specific $CD4^+$ T cells. A) Representative plots showing comparison of intradermal ear pinna versus flank injections and B) representative plots showing the comparison between intradermal and intramuscular immunizations. From two independent experiments with two to three mice per group.

Figure S6. dmLT induces IL-17 and IFN-γ following in vivo restimulation with 2W1S peptide in 2W1S-specific CD4$^+$ T cells. C57BL/6 mice were immunized with CpG or dmLT plus 2W1S-GFP and then immunized mice were intravenously pulsed with 100 µg of purified 2W1S peptide nine days after prime. Two hours after peptide stimulation, mice were sacrificed and spleens harvested. Tissues were directly homogenized in media containing Brefeldin A. 2W1S-specific cells were labeled with 2W1S:MHCII and magnetically enriched. Enriched cells were then fixed, permeabilized and stained for cytokines. A) Representative FACS plots of splenic 2W1S-specific CD4$^+$ T cell IL-17A and IFN-γ cytokine staining, B) Graphs of cytokine production for two independent experiments pooled are shown with three mice per group. Significance was determined by Student's two-tailed t test where *=p<0.05; **=p<0.01; ***=p<0.001.

Figure S7. Batf3$^{-/-}$ mice lack CD103 dDCs. Batf3$^{-/-}$ mice and WT mice were intradermally immunized with dmLT. One day after immunization, CLN were harvested and stained for the presence of CD11c$^+$ CD103$^+$ DCs and the absence of CD103$^+$ was confirmed by FACS analysis. Gated events represent CD19- MHCII$^+$ bulk DCs. Representative of three mice.

Figure S1

A


Ag-specific CD4+ Kinetics of CpG vs. dmLT
#2W1S-specific CD44+ CD4+ T cells
50000
40000
30000
20000
10000
0
4 day
9 day
14 day
28 day
56 day
Days post immunization
dmLT + 2W1S-GFP Spleen
CpG + 2W1S-GFP Spleen
dmLT + 2W1S-GFP CLN
CpG + 2W1S-GFP CLN

B

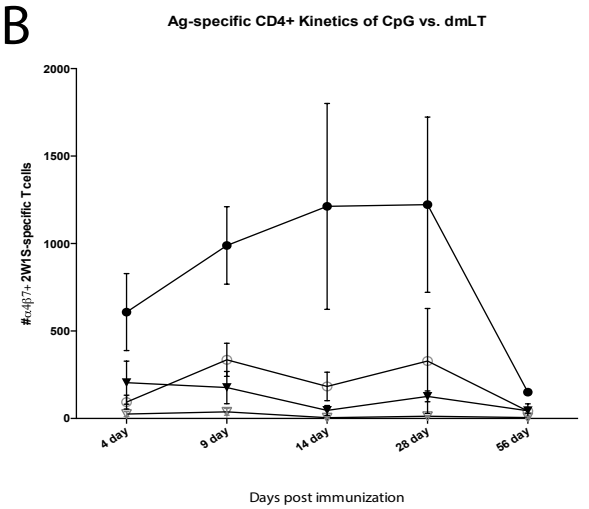

Ag-specific CD4+ Kinetics of CpG vs. dmLT
#α4β7+ 2W1S-specific T cells
2000
1500
1000
500
0
4 day
9 day
14 day
28 day
56 day
Days post immunization

Figure S2

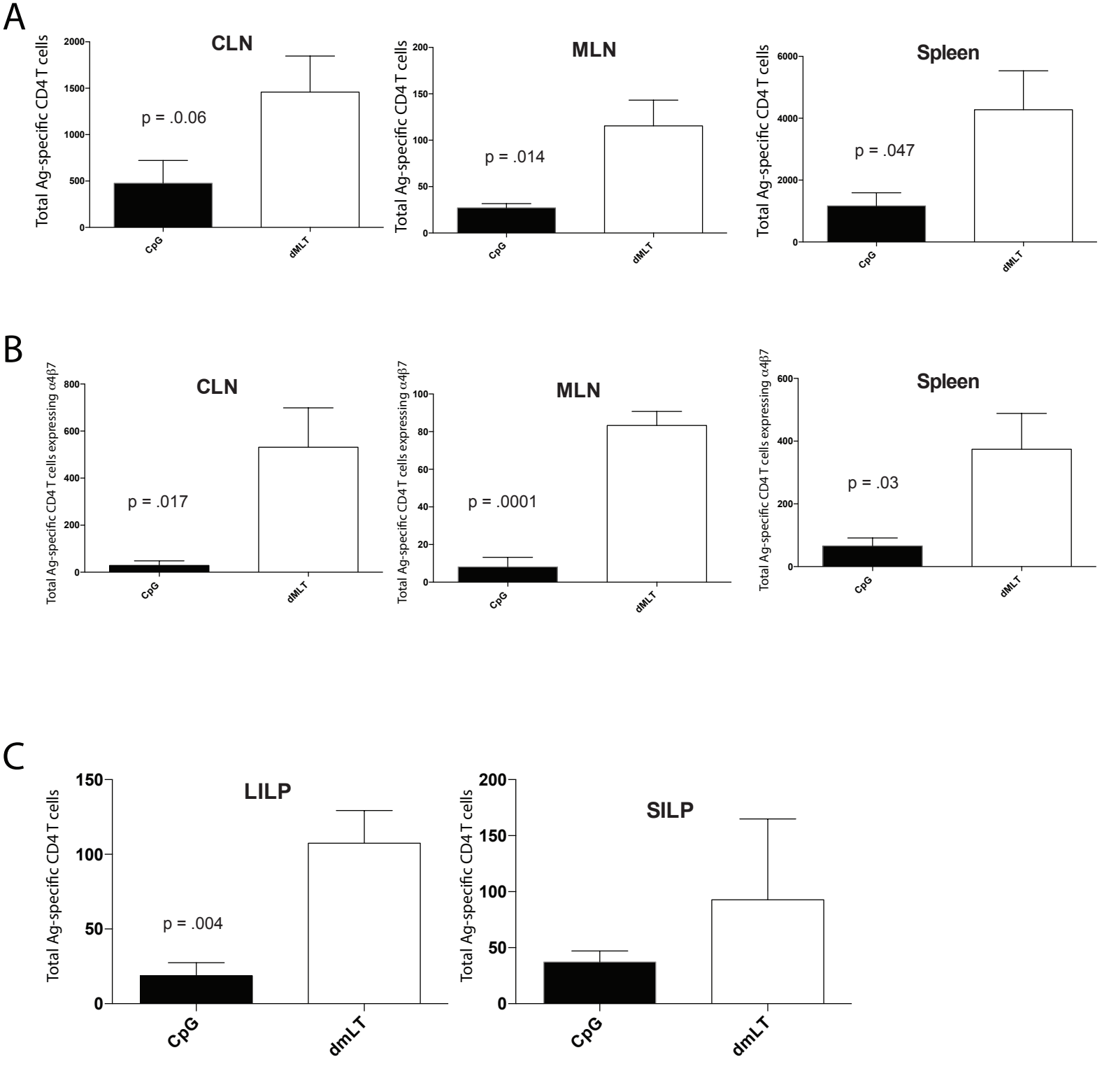

A
CLN
Total Ag-specific CD4 T cells
p = .0.06
CpG
dMLT
MLN
p = .014
Spleen
p = .047
B
CLN
Total Ag-specific CD4 T cells expressing α4β7
p = .017
MLN
p = .0001
Spleen
p = .03
C
LILP
p = .004
SILP
dmLT

Figure S3

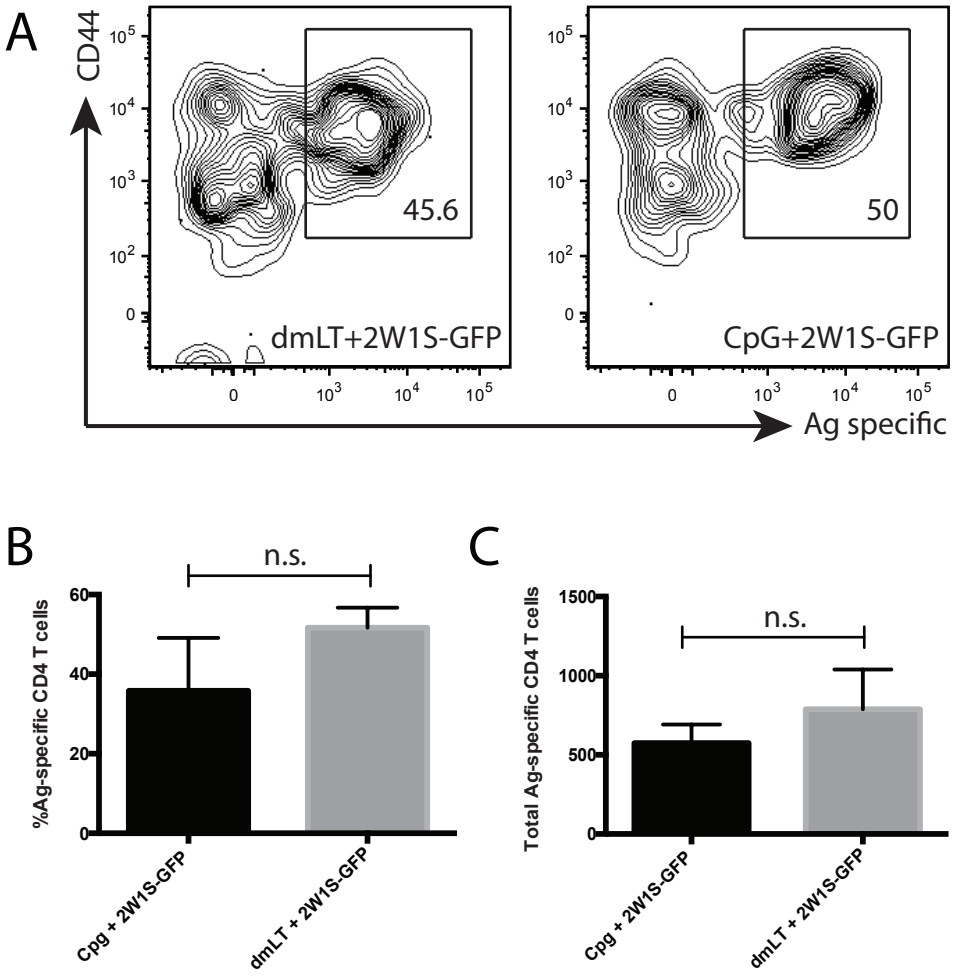
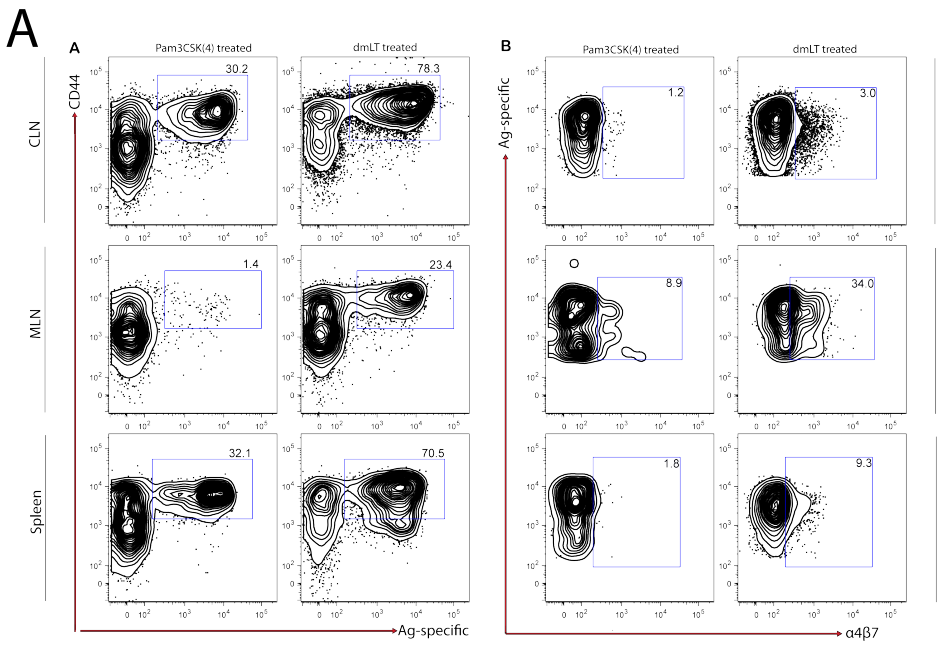

A
CD44
45.6
dmLT+2W1S-GFP
50
CpG+2W1S-GFP
Ag specific
B
n.s.
%Ag-specific CD4 T cells
Cpg + 2W1S-GFP
dmLT + 2W1S-GFP
C
n.s.
Total Ag-specific CD4 T cells
Cpg + 2W1S-GFP
dmLT + 2W1S-GFP

Figure S4


A
A
Pam3CSK(4) treated
dmLT treated
CD44
CLN
MLN
Spleen
30.2
78.3
1.4
23.4
32.1
70.5
Ag-specific
B
Pam3CSK(4) treated
dmLT treated
Ag-specific
1.2
3.0
8.9
34.0
1.8
9.3
α4β7

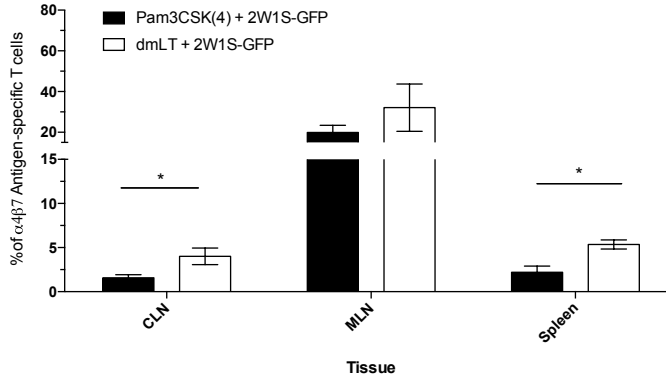

B
Pam3CSK(4) + 2W1S-GFP
dmLT + 2W1S-GFP
%of α4β7 Antigen-specific T cells
80
60
40
20
15
10
5
0
*
*
CLN
MLN
Spleen
Tissue

Figure S5

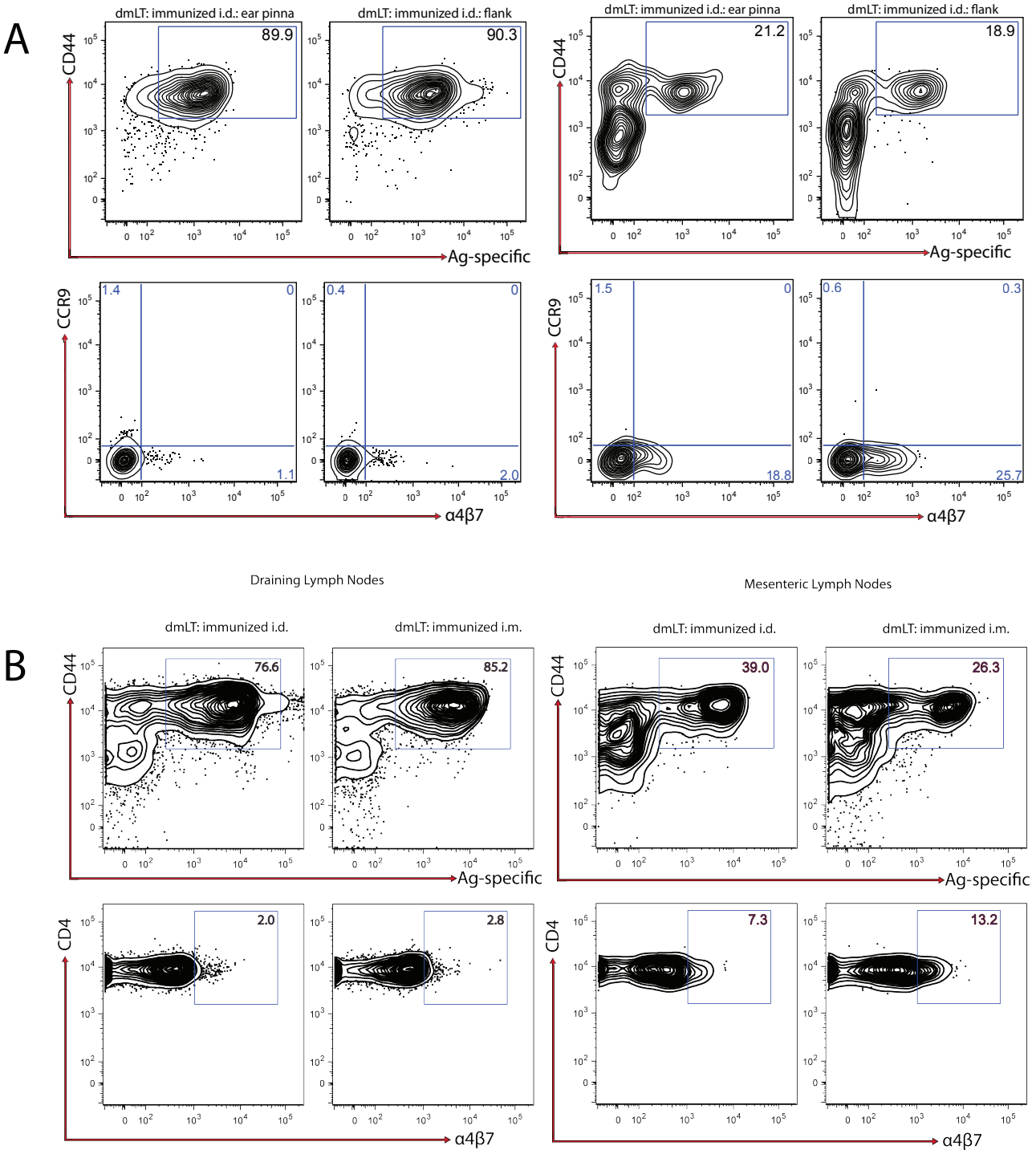
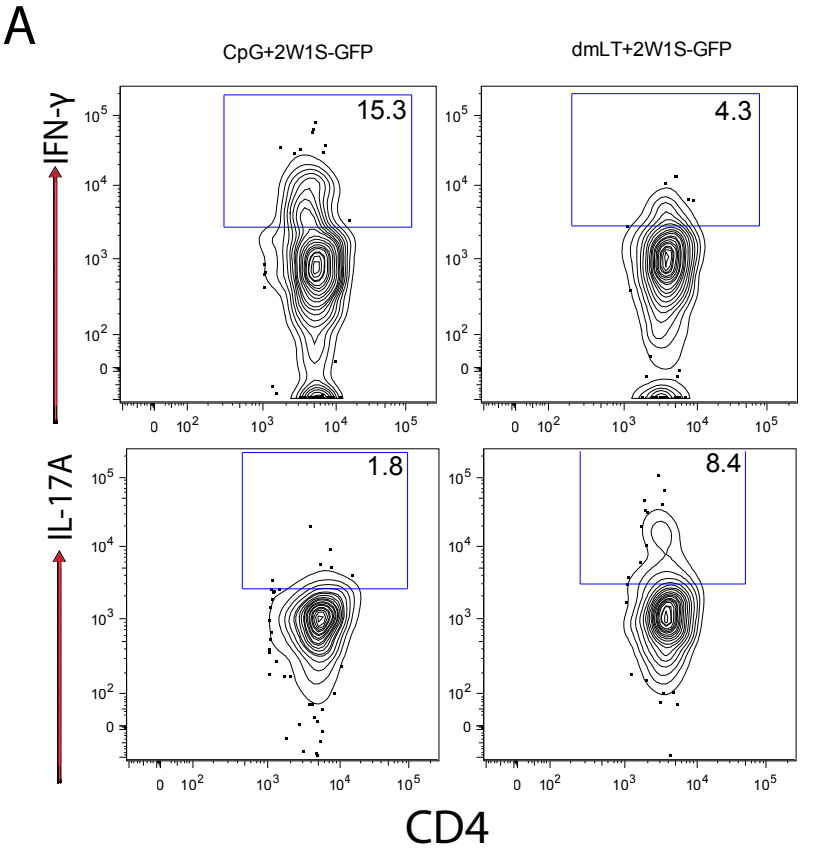

A
dmLT: immunized i.d.: ear pinna
dmLT: immunized i.d.: flank
dmLT: immunized i.d.: ear pinna
dmLT: immunized i.d.: flank
CD44
89.9
90.3
21.2
18.9
Ag-specific
CCR9
1.4
0
1.1
0.4
0
2.0
1.5
0
18.8
0.6
0.3
25.7
α4β7
Draining Lymph Nodes
Mesenteric Lymph Nodes
B
dmLT: immunized i.d.
dmLT: immunized i.m.
dmLT: immunized i.d.
dmLT: immunized i.m.
CD44
76.6
85.2
39.0
26.3
Ag-specific
CD4
2.0
2.8
7.3
13.2
α4β7

Figure S6


A
CpG+2W1S-GFP
dmLT+2W1S-GFP
IFN-γ
15.3
4.3
IL-17A
1.8
8.4
CD4

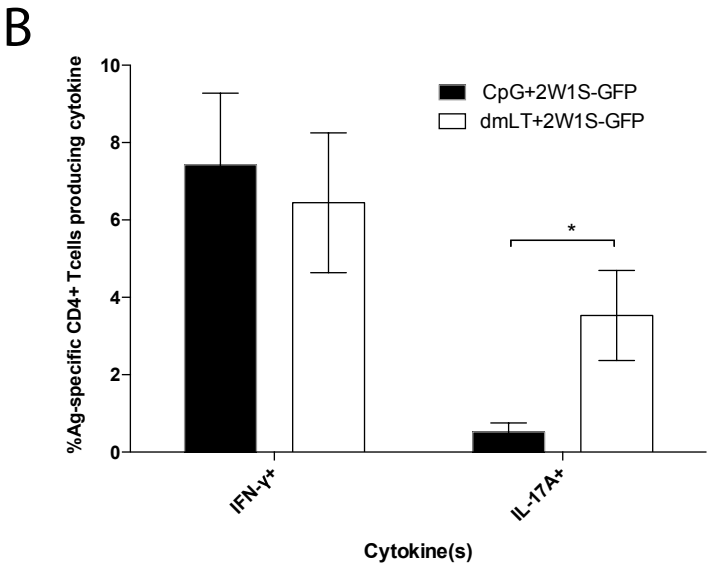

B
%Ag-specific CD4+ Tcells producing cytokine
CpG+2W1S-GFP
dmLT+2W1S-GFP
*
IFN-γ+
IL-17A+
Cytokine(s)

Figure S7