Figure S1. Influence of oral administration of antigen on the antigen-specific $CD8^+$ T-cell response in *B7*$^{-/-}$ mice

WT mice, *Cd80/Cd86*$^{-/-}$ mice, *B7h1*$^{-/-}$ mice, *B7dc*$^{-/-}$ mice, and *B7h2*$^{-/-}$ mice (5 per group) were fed PBS (none) or OVA protein, and then systemically immunized with OVA protein 7 days after the oral priming. Subsequently, Sp $CD8^+$ T cells were collected from each group of mice 14 days after systemic immunization. Proliferative response of Sp $CD8^+$ T cells to WT Sp $CD11c^+$DCs in the presence of OVA protein was measured by [^{3}H]thymidine incorporation. **P* < 0.01 compared with non-fed mice. Data are the mean ± SD, and the results are representative of 4 independent experiments.

Figure S2. Influence of oral tolerance on the frequency of $CD4^+Foxp3^+T_{regs}$ in *B7*$^{-/-}$ mice

(A,B) The proportion of $CD4^+Foxp3^+nT_{regs}$ among gated $CD4^+$ T cells (A) and the absolute number of $CD4^+Foxp3^+nT_{regs}$ (B) in Sp and MLNs under steady-state conditions was analyzed by flow cytometry. **P* < 0.01 compared with WT mice. Data are the mean ± SD, and the results are representative of 4 independent experiments. (C,D) WT mice and *B7*$^{-/-}$ mice (5 per group) were fed PBS (none) or OVA protein, and then systemically immunized with OVA protein 7 days after oral priming. At 14 days after systemic immunization, the proportion of $CD4^+Foxp3^+T_{regs}$ among gated $CD4^+$ T cells in Sp (C) and MLNs (D) was analyzed by flow cytometry. **P* < 0.01 compared with non-fed mice. Data are the mean ± SD, and the results are representative of 4 independent experiments.

Figure S3. Blockade of oral tolerance by the depletion of $CD4^+Foxp3^+T_{regs}$

WT mice (5 per group) that had received control Ig or anti-CD25 mAb were fed PBS (none) or OVA protein, and then systemically immunized with OVA protein 7 days after oral priming. Subsequently, serum and $CD4^+$ T cells in Sp and MLNs were collected from each group of mice 14 days after systemic immunization. (A,B) The proportion of $CD4^+Foxp3^+T_{regs}$ among gated $CD4^+$ T cells in Sp and MLNs on the day of the systemic immunization (day 0) (A) and 14 days (B) after systemic immunization was analyzed by flow cytometry. (C) Serum OVA-specific IgG_1 production was measured by ELISA. (D) Proliferative response of Sp $CD4^+$ T cells to Sp $CD11c^+$DCs in the presence or absence of OVA protein was measured by [^{3}H]thymidine incorporation. **P* < 0.01 compared with non-fed mice that received control Ig. Data are the mean ± SD, and the results are representative of 4 independent experiments.

Figure S4. Flow cytometric analysis of systemic and MLN CD11c$^+$DCs in *B7*$^{-/-}$ mice
Expression of the indicated molecules on Sp or MLN CD11c$^+$DCs in *B7*$^{-/-}$ mice was analyzed by flow cytometry. Data are represented by a histogram, and numbers represent mean fluorescence intensity. Data are representative of 4 independent experiments.

Figure S5. Analysis of CD4$^+$ T cells in *Foxp3*^{EGFP}DO11.10 mice and *Foxp3*^{EGFP}*Rag2*$^{-/-}$DO11.10 mice
Sp CD4$^+$ T cells (A), Sp CD4$^+$CD25$^+$*Foxp3*$^{EGFP+}$ T cells (B), and Sp CD4$^+$CD25$^-$*Foxp3*$^{EGFP-}$ T cells (C) obtained from *Foxp3*^{EGFP}DO11.10 mice, and Sp CD4$^+$ T cells obtained from *Foxp3*^{EGFP}*Rag2*$^{-/-}$ DO11.10 mice (D) were analyzed by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion among gated CD4$^+$ T cells (upper panel) and KJ1-26$^+$ T cells (lower panel) in each quadrant. Data are representative of 4 independent experiments.

Figure S6. Influence of the inflammatory stimulation on the ability of Sp and MLN CD11c$^+$DCs to generate antigen-specific CD4$^+$Foxp3$^+$iT$_{regs}$ from CD4$^+$Foxp3$^-$ T cells
Generation of KJ1-26$^+$*Foxp3*$^{EGFP+}$ T cells from Sp KJ1-26$^+$CD25$^-$*Foxp3*$^{EGFP-}$ T cells by Sp or MLN CD11c$^+$DCs obtained from WT mice in neutral conditions in the presence or absence of TGF-β1 (A,B) or TGF-β1 plus RA (A,C) in combination with IL-6 or CpG ODN was analyzed by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion of *Foxp3*$^{EGFP+}$cells among gated CD4$^+$ T cells in each quadrant (A) and are the percentage of positive cells (B,C). *$P < 0.01$ compared with CD11c$^+$DCs plus TGF-β1 (B) or CD11c$^+$DCs, TGF-β1 plus RA (C). Data are the mean ± SD, and the results are representative of 4 independent experiments.

Figure S7. Influence of the blockade of PD-1 on the ability of Sp and MLN CD11c$^+$DCs to prime antigen-specific CD4$^+$Foxp3$^-$ T cells to form CD4$^+$Foxp3$^+$iT$_{regs}$.
(A) Proliferative response of Sp KJ1-26$^+$CD25$^-$*Foxp3*$^{EGFP-}$ T cells to Sp or MLN CD11c$^+$DCs obtained from WT mice in combination with OVAp in the presence or absence of control Ig or anti–PD-1 mAb was measured by [^{3}H]thymidine incorporation. *$P < 0.01$ compared with CD11c$^+$DCs alone. Data are the mean ± SD, and the results are representative of Data are representative of 4 independent experiments. (B–D) Generation of KJ1-26$^+$*Foxp3*$^{EGFP+}$ T cells

from Sp KJ1-26^{+}CD25^{-}*Foxp3*$^{EGFP-}$ T cells by Sp or MLN CD11c^{+}DCs obtained from WT mice in neutral conditions in the presence or absence of control Ig, anti–PD-1 mAb, TGF-β1 (B,C) or TGF-β1 plus RA (B,D) was analyzed by flow cytometry. Data are represented by a dot plot, and numbers represent the proportion of *Foxp3*$^{EGFP+}$cells among gated CD4^{+} T cells in each quadrant (B) and are the percentage of positive cells (C,D). *$P < 0.01$ compared with CD11c^{+}DCs plus control Ig. Data are the mean ± SD, and the results are representative of 4 independent experiments.

Figure S8. Abrogation of oral tolerance by in vivo application of CpG ODN

WT mice (5 per group) that had been treated with CpG ODN were fed PBS (none) or OVA protein, and then systemically immunized with OVA protein 7 days after oral priming. Subsequently, serum and CD4^{+} T cells in Sp and MLNs were collected from each group of mice 14 days after systemic immunization. (A) The proportion of CD4^{+}Foxp3^{+}T$_{regs}$ among gated CD4^{+} T cells in Sp and MLNs was analyzed by flow cytometry. (B) Serum OVA-specific IgG$_1$ production was measured by ELISA. (C) Proliferative response of Sp CD4^{+} T cells to Sp CD11c^{+}DCs in the presence or absence of OVA protein was measured by [^{3}H]thymidine incorporation. *$P < 0.01$ compared with non-fed mice. Data are the mean ± SD, and the results are representative of 4 independent experiments.

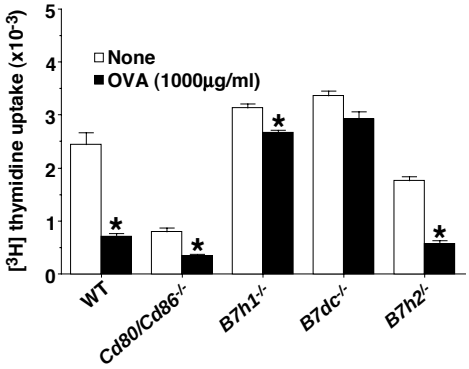
[3H] thymidine uptake (x10-3)
5
4
3
2
1
0
None
OVA (1000µg/ml)
*
*
*
*
WT
Cd80/Cd86-/-
B7h1-/-
B7dc-/-
B7h2-/-

Figure S1

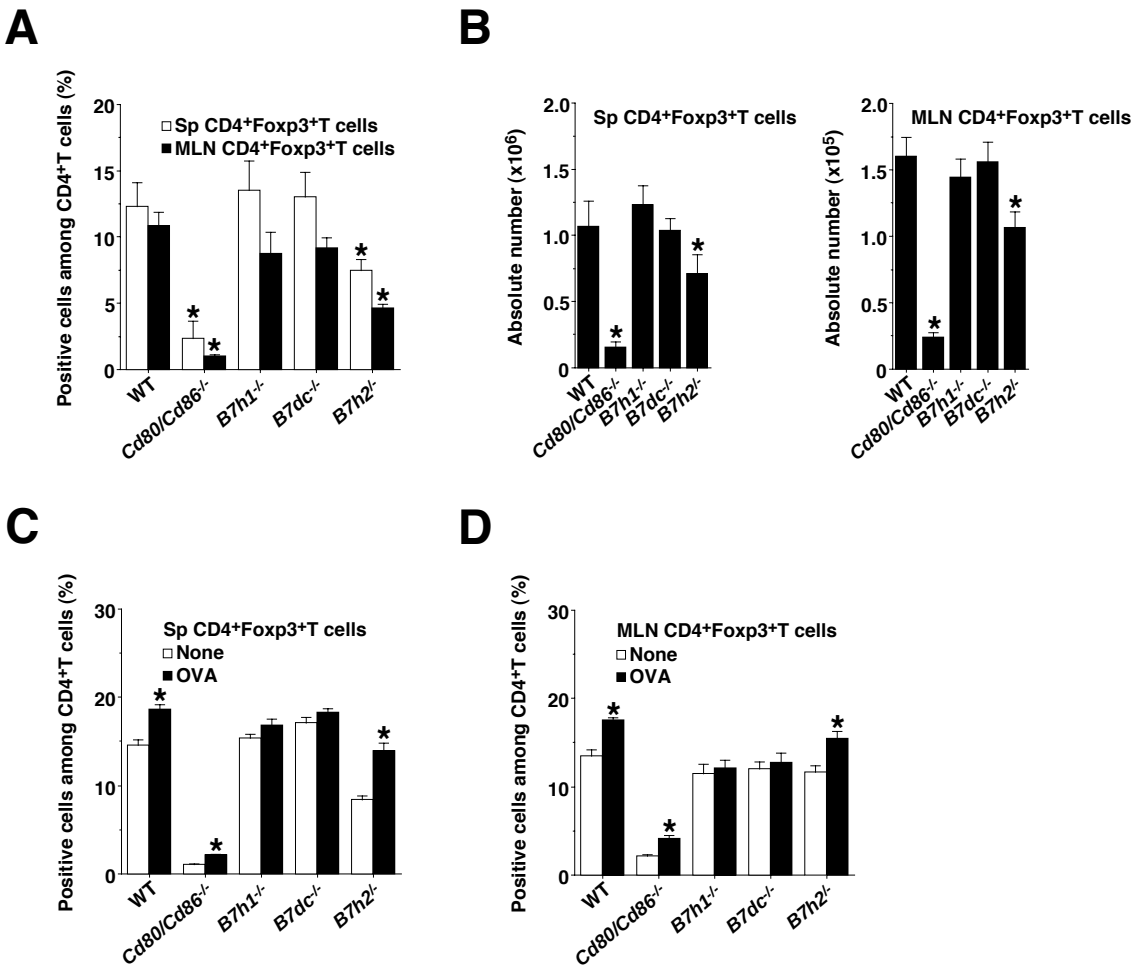
A
B
C
D
Positive cells among CD4+T cells (%)
Sp CD4+Foxp3+T cells
MLN CD4+Foxp3+T cells
Absolute number (x10^6)
Absolute number (x10^5)
None
OVA
WT
Cd80/Cd86-/-
B7h1-/-
B7dc-/-
B7h2-/-

Figure S2

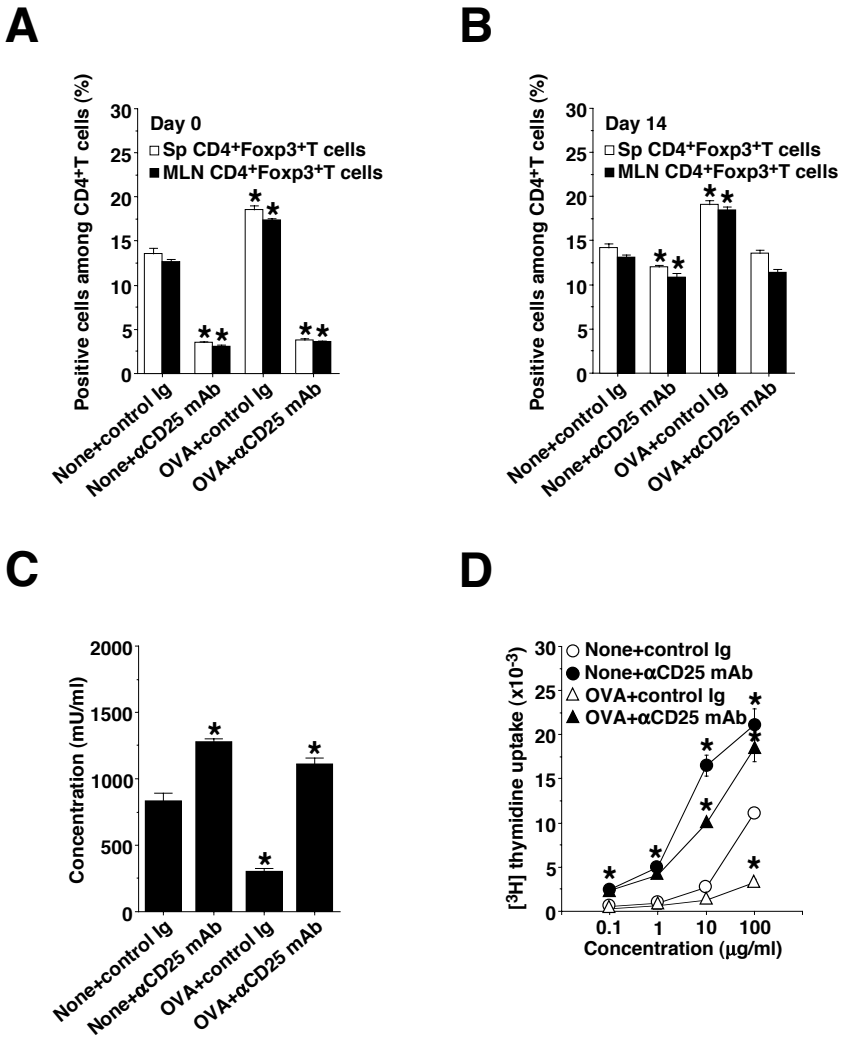
A
Day 0
□Sp CD4+Foxp3+T cells
■MLN CD4+Foxp3+T cells
Positive cells among CD4+T cells (%)
30
25
20
15
10
5
0
None+control Ig
None+αCD25 mAb
OVA+control Ig
OVA+αCD25 mAb
B
Day 14
□Sp CD4+Foxp3+T cells
■MLN CD4+Foxp3+T cells
Positive cells among CD4+T cells (%)
C
Concentration (mU/ml)
2000
1500
1000
500
0
D
○ None+control Ig
● None+αCD25 mAb
△ OVA+control Ig
▲ OVA+αCD25 mAb
[3H] thymidine uptake (x10-3)
0.1
1
10
100
Concentration (μg/ml)

Figure S3

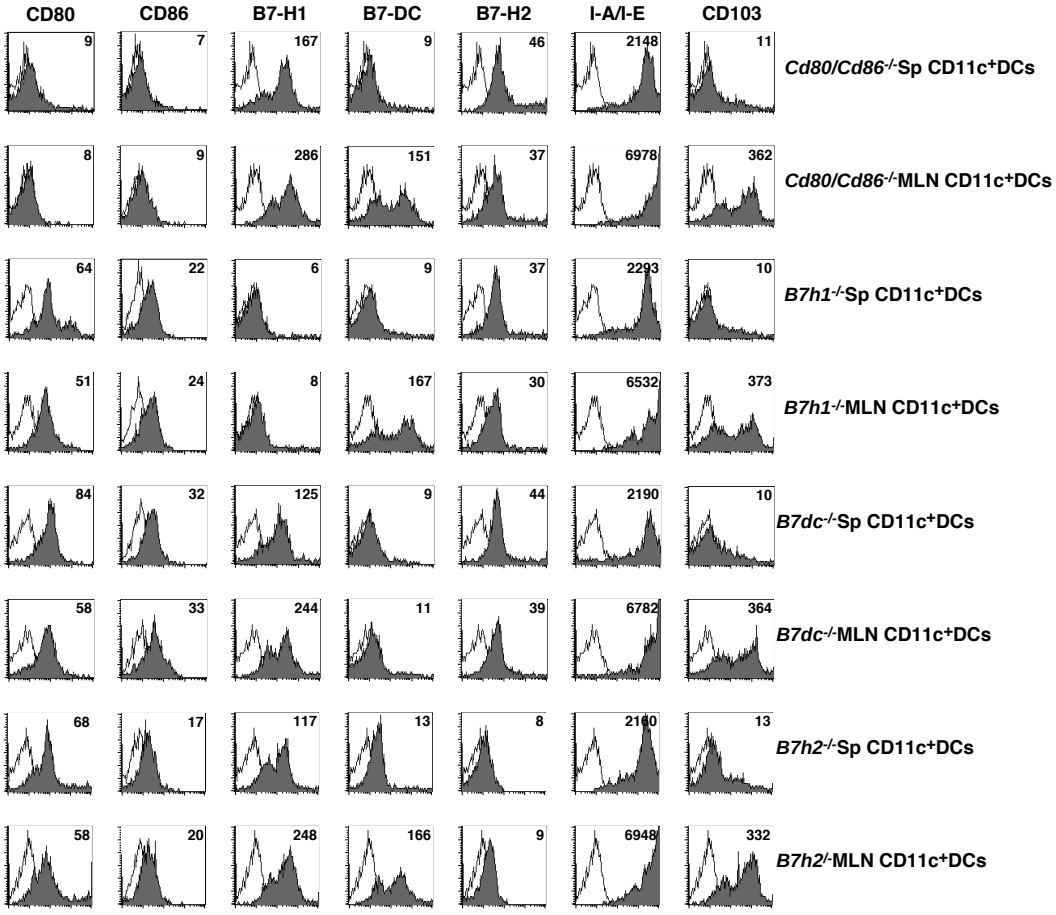
CD80
CD86
B7-H1
B7-DC
B7-H2
I-A/I-E
CD103
9
7
167
9
46
2148
11
Cd80/Cd86-/- Sp CD11c+DCs
8
9
286
151
37
6978
362
Cd80/Cd86-/- MLN CD11c+DCs
64
22
6
9
37
2293
10
B7h1-/- Sp CD11c+DCs
51
24
8
167
30
6532
373
B7h1-/- MLN CD11c+DCs
84
32
125
9
44
2190
10
B7dc-/- Sp CD11c+DCs
58
33
244
11
39
6782
364
B7dc-/- MLN CD11c+DCs
68
17
117
13
8
2160
13
B7h2-/- Sp CD11c+DCs
58
20
248
166
9
6948
332
B7h2-/- MLN CD11c+DCs

Figure S4

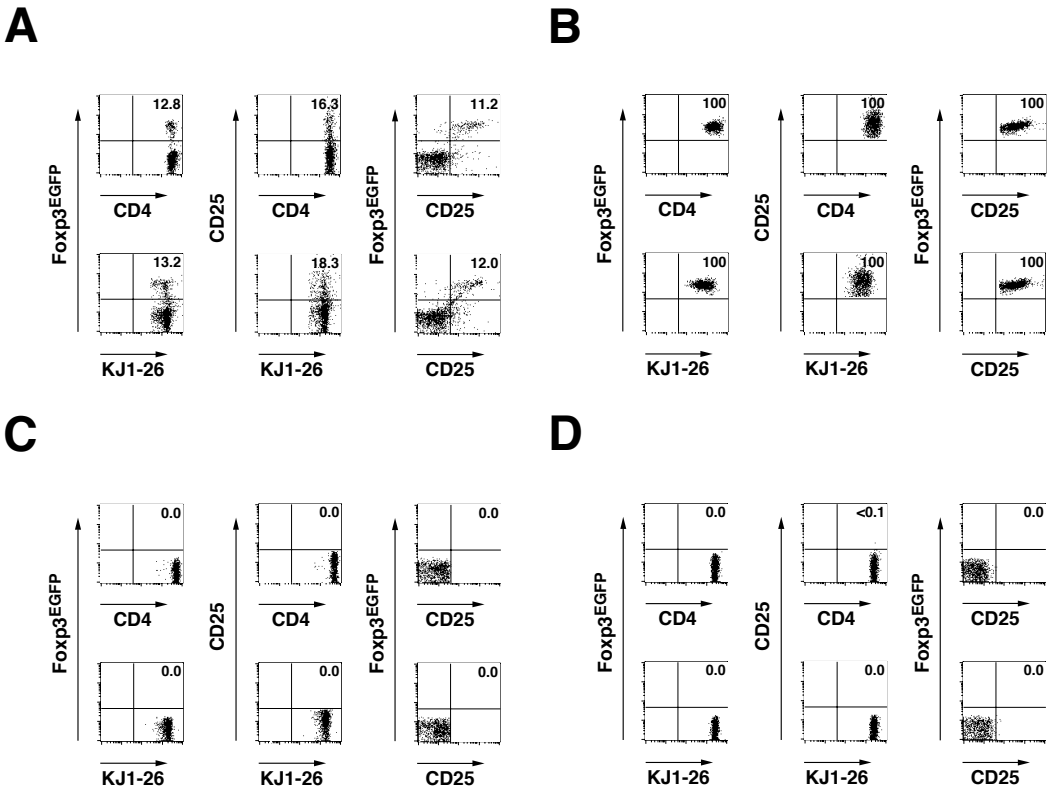
A
B
C
D
Foxp3EGFP
CD4
CD25
KJ1-26
12.8
13.2
16.3
18.3
11.2
12.0
100
0.0
<0.1

Figure S5

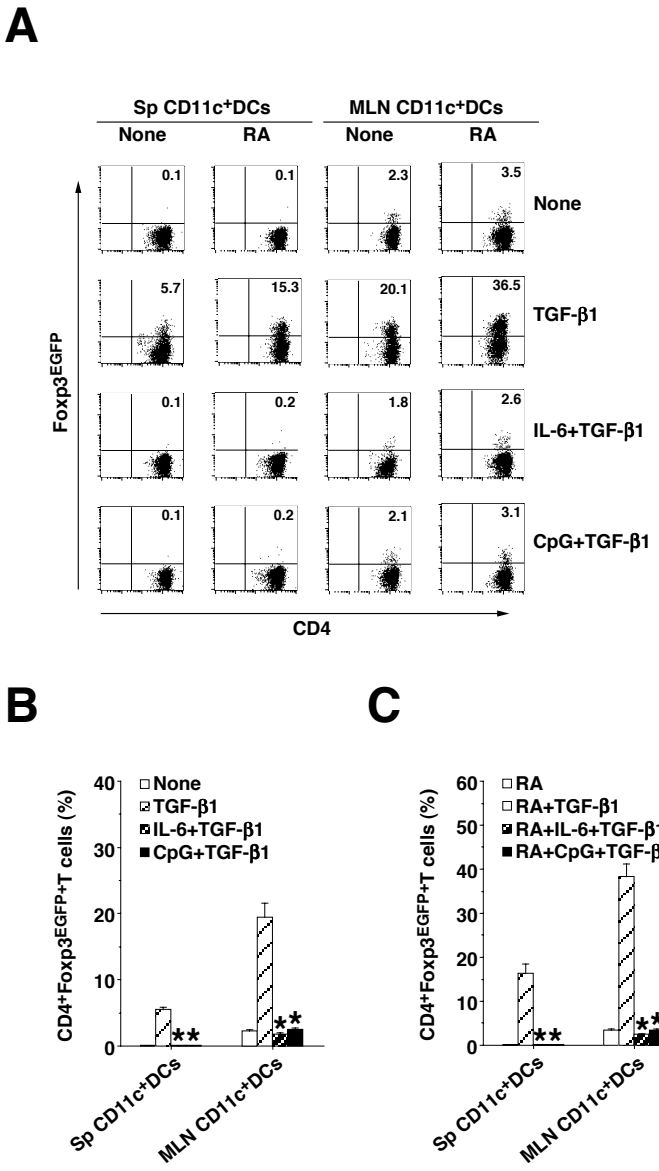
A
Sp CD11c+DCs
MLN CD11c+DCs
None
RA
None
RA
0.1
0.1
2.3
3.5
None
5.7
15.3
20.1
36.5
TGF-β1
0.1
0.2
1.8
2.6
IL-6+TGF-β1
0.1
0.2
2.1
3.1
CpG+TGF-β1
Foxp3EGFP
CD4
B
C
CD4+Foxp3EGFP+T cells (%)
None
TGF-β1
IL-6+TGF-β1
CpG+TGF-β1
RA
RA+TGF-β1
RA+IL-6+TGF-β1
RA+CpG+TGF-β1
Sp CD11c+DCs
MLN CD11c+DCs

Figure S6

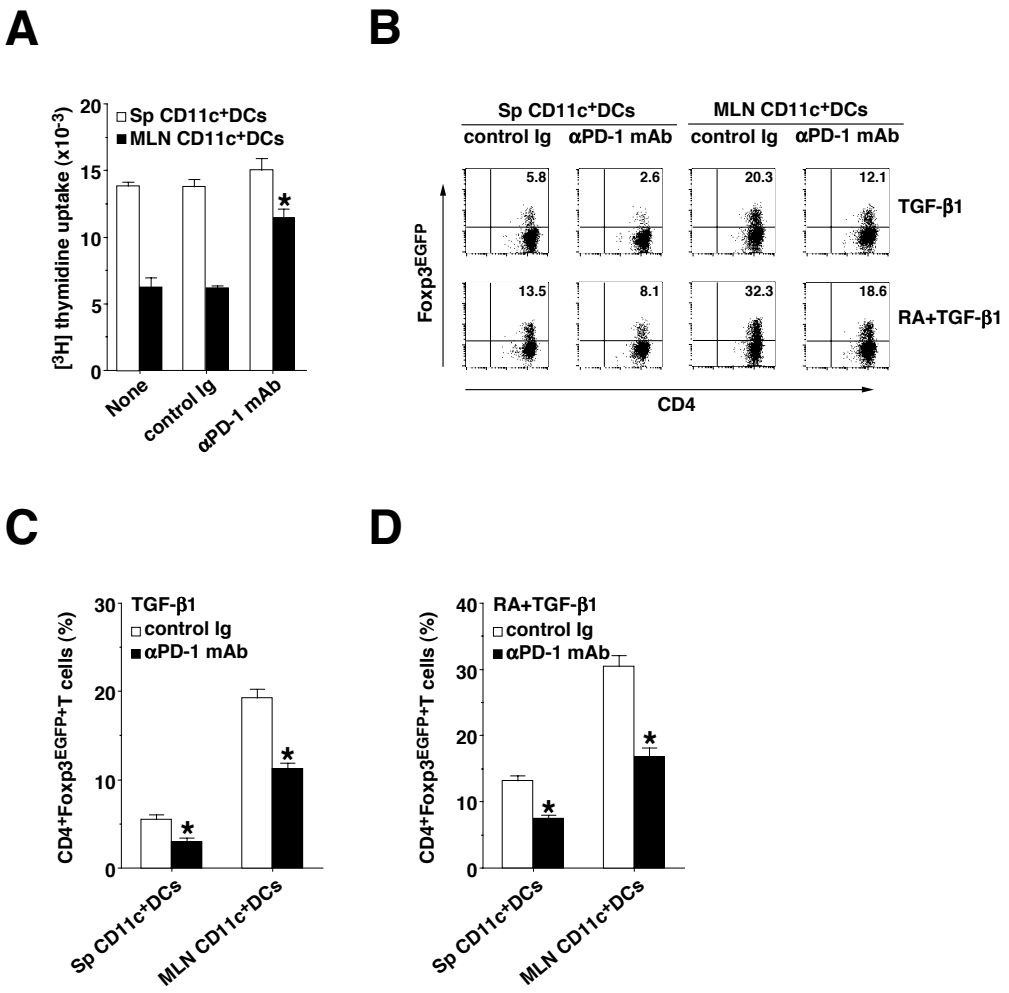
A
B
Sp CD11c+DCs
MLN CD11c+DCs
[3H] thymidine uptake (x10-3)
None
control Ig
αPD-1 mAb
Sp CD11c+DCs
MLN CD11c+DCs
control Ig
αPD-1 mAb
control Ig
αPD-1 mAb
5.8
2.6
20.3
12.1
TGF-β1
13.5
8.1
32.3
18.6
RA+TGF-β1
Foxp3EGFP
CD4
C
D
TGF-β1
control Ig
αPD-1 mAb
CD4+Foxp3EGFP+T cells (%)
Sp CD11c+DCs
MLN CD11c+DCs
RA+TGF-β1
control Ig
αPD-1 mAb
CD4+Foxp3EGFP+T cells (%)
Sp CD11c+DCs
MLN CD11c+DCs

Figure S7

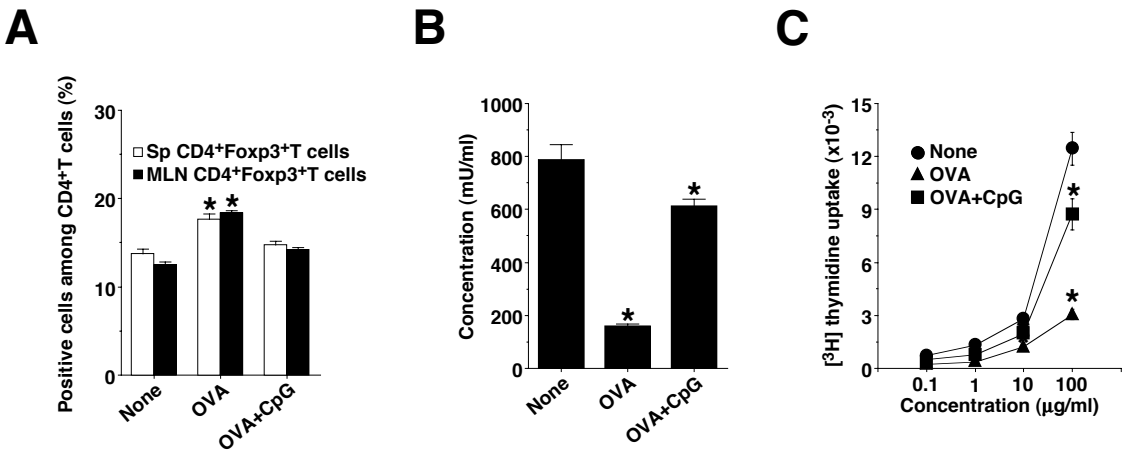
A
B
C
Positive cells among CD4+T cells (%)
30
20
10
0
□Sp CD4+Foxp3+T cells
■MLN CD4+Foxp3+T cells
*
*
None
OVA
OVA+CpG
Concentration (mU/ml)
1000
800
600
400
200
0
*
*
None
OVA
OVA+CpG
[3H] thymidine uptake (x10-3)
15
12
9
6
3
0
None
OVA
OVA+CpG
*
*
0.1
1
10
100
Concentration (μg/ml)

Figure S8