Supplementary Figure legends

Supplementary Figure 1. Frequencies of ovalbumin-specific FoxP3$^+$ T cells in high or low vitamin A/retinoic acid status after immunization with ovalbumin. The data are shown in a dot plot (A) or graph (B) format. Splenocytes of DO11.10 Rag2 (-/-) mice were injected into Low-A and Hi-A BALB/c mice. The mice were immunized with ovalbumin and sacrificed at day 7. A group of Low-A mice was injected with retinoic acid at multiple times following the immunization. The cells examined were KJ1.26$^+$CD4$^+$ cells present in spleen, peripheral lymph nodes (PLN), mesenteric lymph nodes (MLN), Peyer's patches (PP), small intestine (S-intestine), colon, peritoneal cavity (P. cavity), lung and blood. Combined data of three independent experiments (total n=3) are shown in panel B. *Significant differences from the Low-A group.

Supplementary Figure 2. The impact of Low-A and Hi-A statuses on the frequency of FoxP3$^+$ T cells and CD103$^+$ FoxP3$^+$ T cells in SAMP1/YP mice. (A) Frequencies of FoxP3$^+$ T cells as % of CD4$^+$ cells and (B) frequencies of CD103$^+$ FoxP3$^+$ T cells as % of CD4$^+$ cells in Hi-A, Mid-A and Low-A SAMP1/YP mice are shown. SAMP1/YP mice were fed with Low-A, Mid-A or Hi-A diets for 20 weeks for this study. Averages of five mice with SEM are shown. *Significant differences from Mid-A ($p<0.05$).

Supplementary Figure 3. Surface phenotype of the FoxP3$^+$ T cells induced in three different vitamin A statuses. (A) Expression levels of Treg-associated surface antigens by CD4$^+$ FoxP3$^+$ T cells are shown as dot plots. (B) Expression of CD25 by the FoxP3$^+$ T cells of

the MLN of Low-A, Mid-A or Hi-A mice. A set of representative data out of four independent experiments are shown.

Supplementary Figure 4. The frequencies of IL-17$^+$, IFN-γ$^+$, and IL-4$^+$ CD4$^+$ T cells in Low-A, Mid-A or Hi-A SAMP1/YP mice. A set of representative data out of four independent experiments are shown. The cells are gated on CD4$^+$CD44$^+$ memory/effector T cells. SAMP1/YP mice were fed with Low-A, Mid-A or Hi-A diets for 20 weeks for this study.

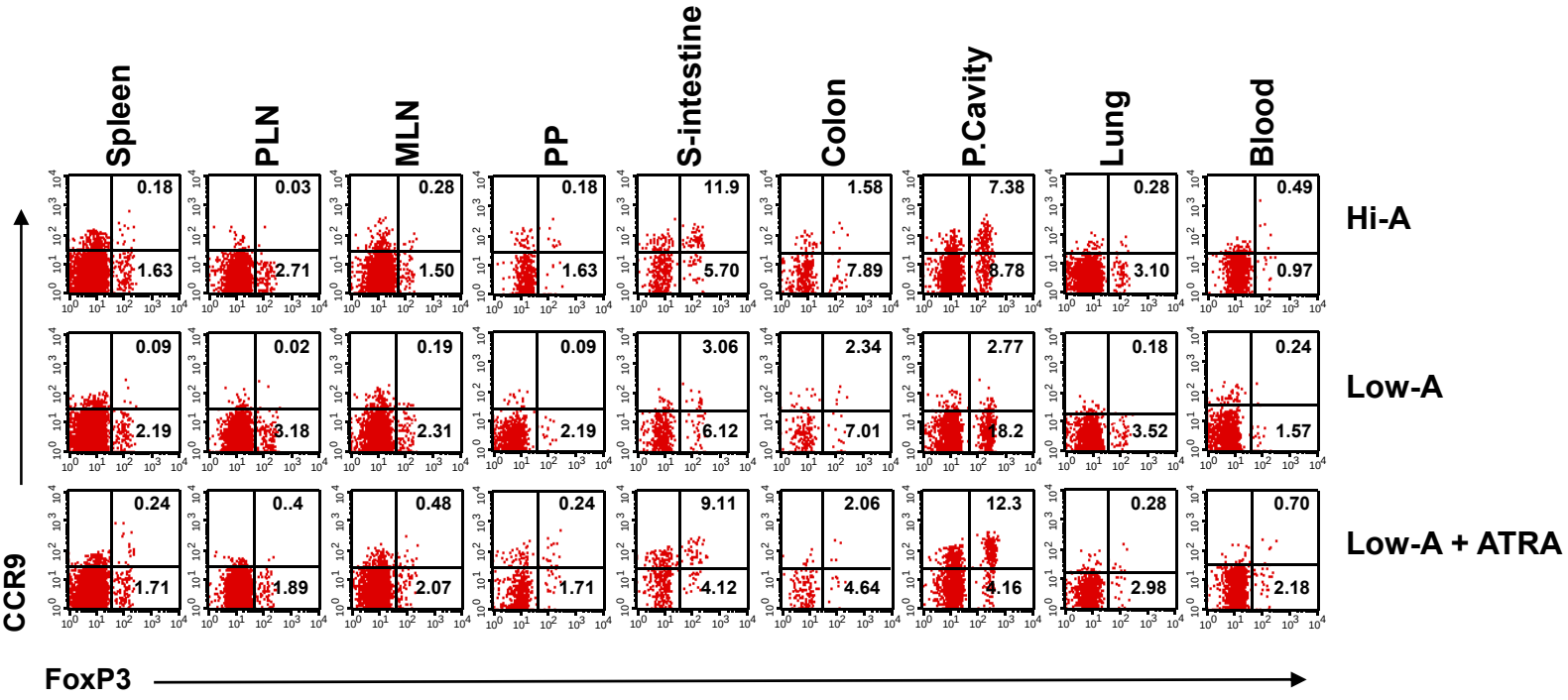
Supplementary Figure 5. In vitro suppressive activity of CD103$^-$ Low-A FoxP3$^+$ T cells. [3H]-thymidine incorporation by CD4$^+$CD25$^-$ target T cells cultured in the presence of indicated numbers of Tregs was measured. Representative data of three independent experiments are shown.

Supplementary Figure 6. Effects of RARα blockade or activation with ATRA on induction of CD103$^+$ FoxP3$^+$ T cells. (A) Induction of CD103$^+$ FoxP3$^+$ T cells by irradiated APC and anti-CD3 in the presence of ATRA or Ro41-5253. Naïve CD4$^+$CD25$^-$ T cells were co-cultured with irradiated APC or CD11c$^+$ DCs for 5-6 days. (B) TGF-β1 (0.2 ng/ml) was added as indicated. (D) Dot plots for cells cultured with thymic APC, spleen DC or MLN DCs. Combined (A) or representative (B and C) data out of 3 independent experiments are shown. Significant difference from the control group (*) or the ATRA group (**).

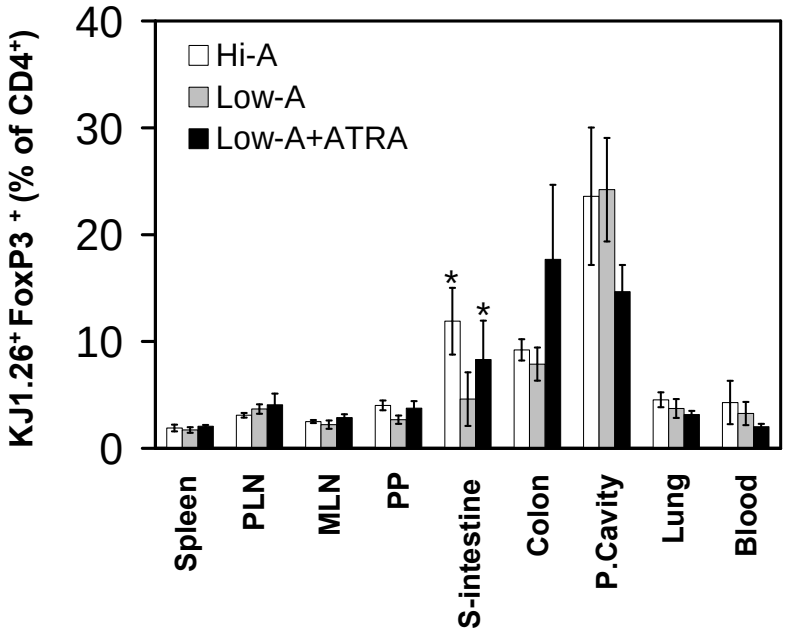
Supplementary Figure 7. Additional features of Low-A and Hi-A APC or mice. (A) Induction of CD103$^+$FoxP3$^+$ T cells by Low-A APC versus Mid-A APC. The culture condition

was similar to that described for Figure 8B**.** Low-A APC was 20-30% more efficient than Mid-A APC in induction of $CD103^{+}FoxP3^{+}$ T cells but there was no statistically significant increase in absolute numbers of total $FoxP3^{+}$ T cells. *Significant differences from the Mid-A group. (B) Surface phenotype of DCs in Mid-A, Low-A and Hi-A mice. Expression of CD40, CD70, CD80, CD86 and CD103 was examined. CD70 expression by the splenic $CD11C^{+}CD11b^{+}$ DCs in Low-A mice was higher than the cells in Hi-A or Mid-A mice. (C) Expression of RALDH1 and RALDH3 was increased in the small intestinal lamina propria (SI-LP) cells of Low-A mice. RALDH2 was decreased in MLN DCs of Low-A mice. Real time PCR analysis was performed with the expression normalized with that of β-actin. All experiments were performed at least 3 times, and combined (A) or representative (B and C) data are shown.

A

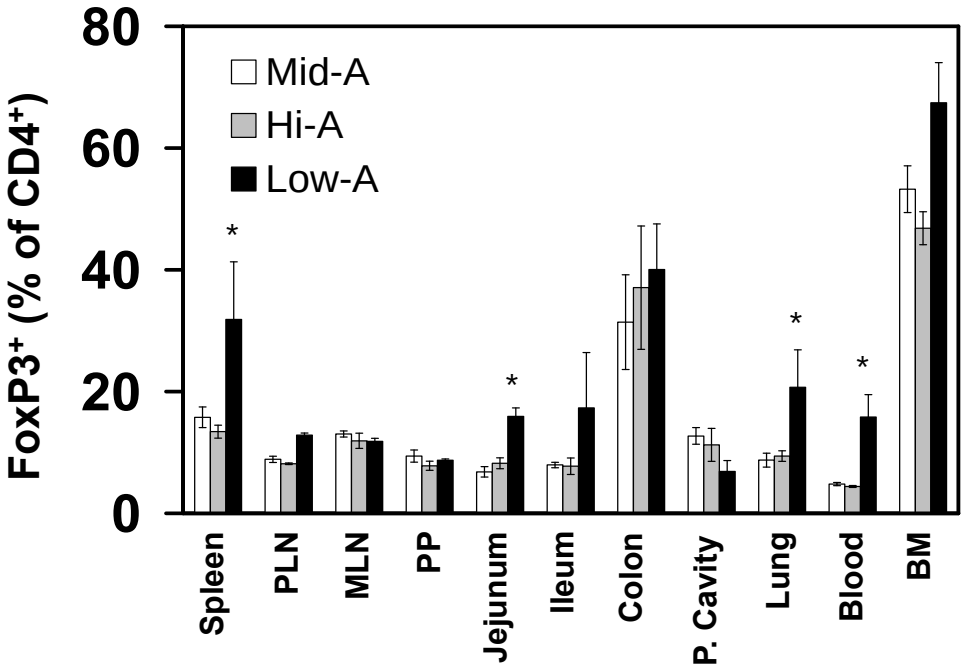

Spleen
PLN
MLN
PP
S-intestine
Colon
P.Cavity
Lung
Blood
Hi-A
Low-A
Low-A + ATRA
CCR9
FoxP3
0.18
1.63
0.03
2.71
0.28
1.50
0.18
1.63
11.9
5.70
1.58
7.89
7.38
8.78
0.28
3.10
0.49
0.97
0.09
2.19
0.02
3.18
0.19
2.31
0.09
2.19
3.06
6.12
2.34
7.01
2.77
18.2
0.18
3.52
0.24
1.57
0.24
1.71
0..4
1.89
0.48
2.07
0.24
1.71
9.11
4.12
2.06
4.64
12.3
4.16
0.28
2.98
0.70
2.18

B

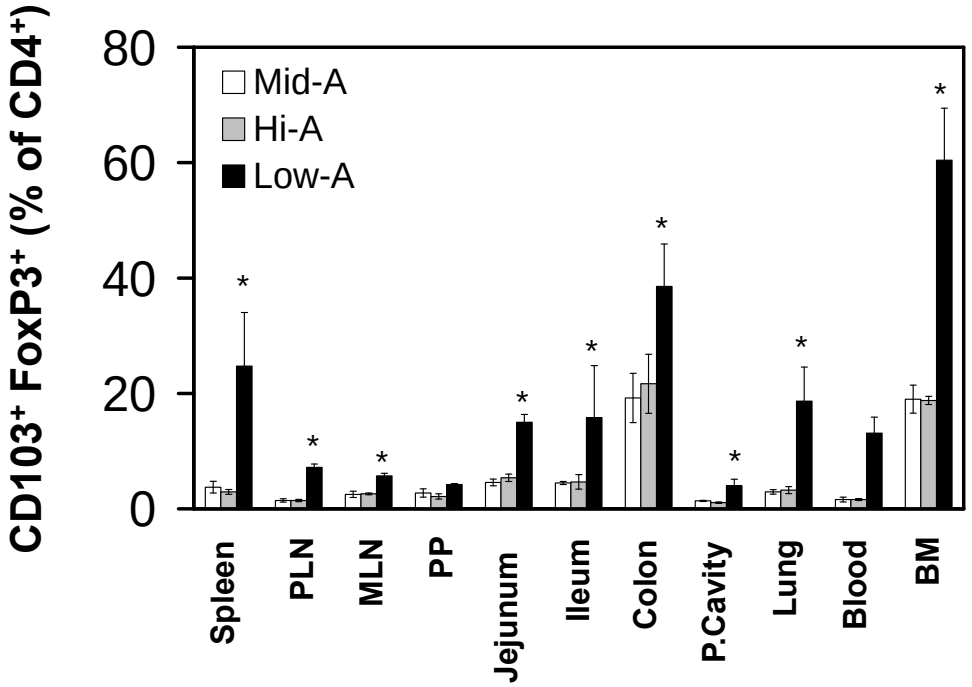

KJ1.26+FoxP3+ (% of CD4+)
Hi-A
Low-A
Low-A+ATRA
0
10
20
30
40
*
*
Spleen
PLN
MLN
PP
S-intestine
Colon
P.Cavity
Lung
Blood

Supp-Fig. 1

A

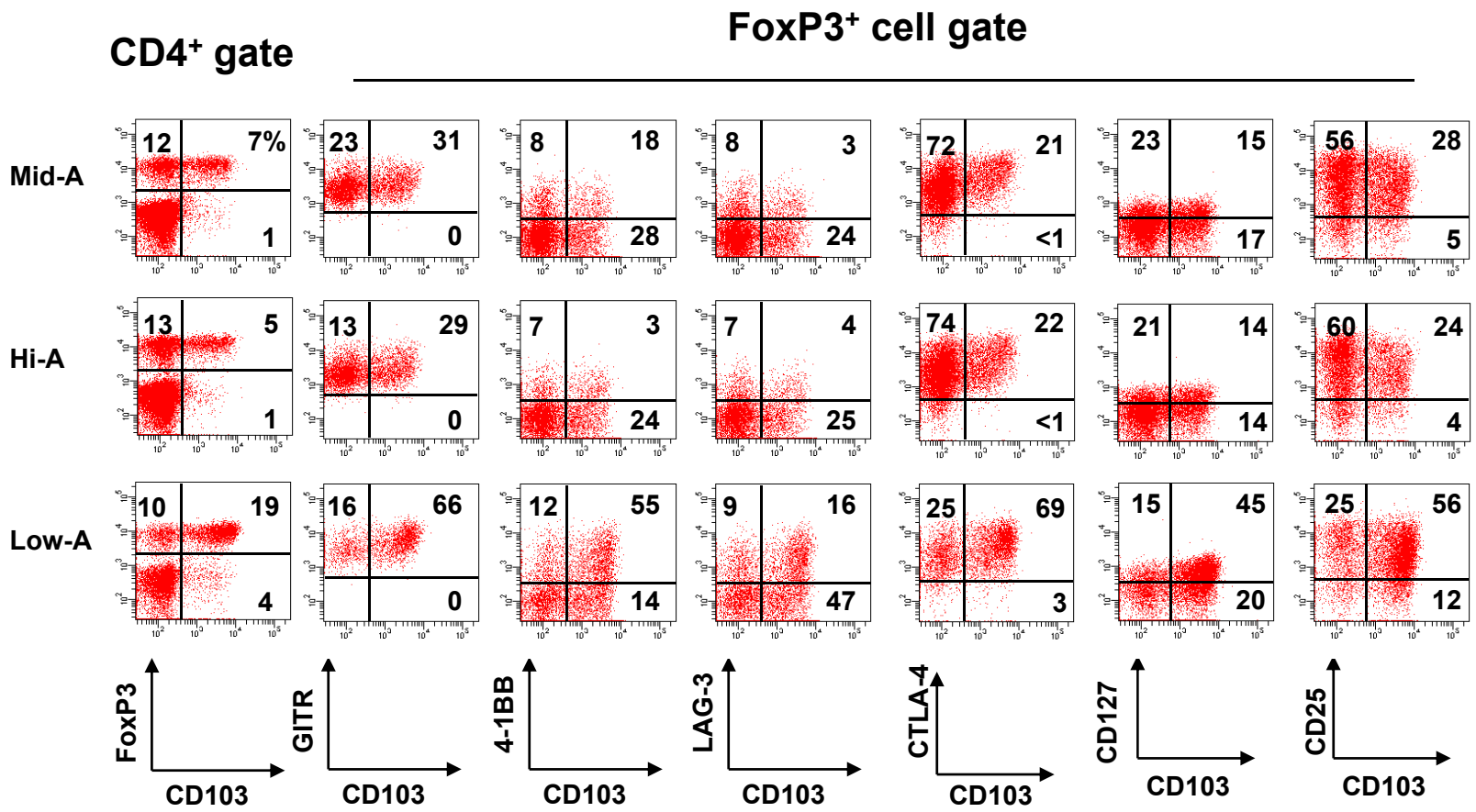

80
60
40
20
0
FoxP3+ (% of CD4+)
Mid-A
Hi-A
Low-A
*
Spleen
PLN
MLN
PP
Jejunum
Ileum
Colon
P. Cavity
Lung
Blood
BM

B

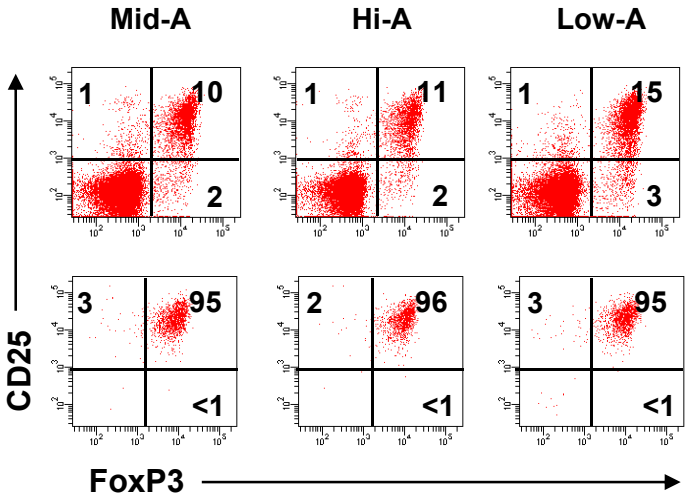

80
60
40
20
0
CD103+ FoxP3+ (% of CD4+)
Mid-A
Hi-A
Low-A
*
Spleen
PLN
MLN
PP
Jejunum
Ileum
Colon
P.Cavity
Lung
Blood
BM

Supp-Fig. 2

A



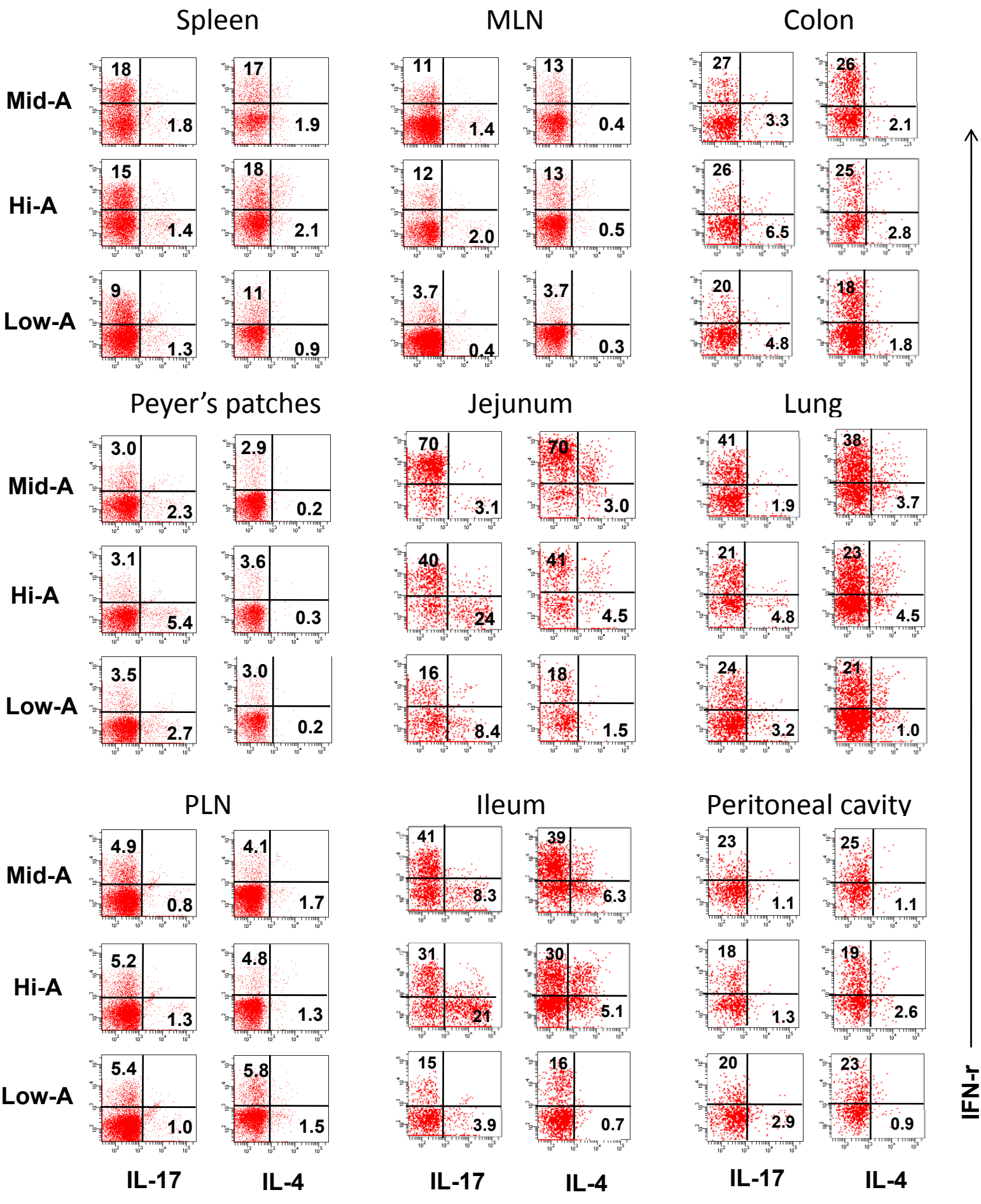
B



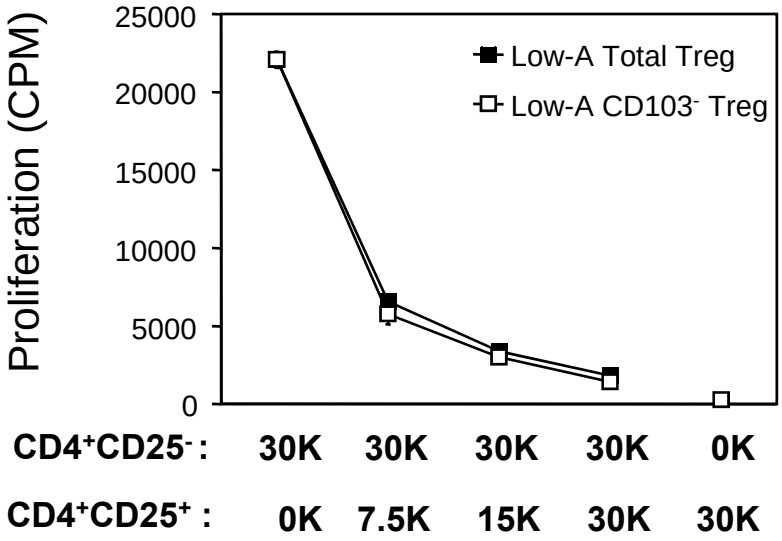
CD4+ cell gate

Isolated Tregs used in Fig 6

Supp-Fig. 3


Spleen
MLN
Colon
Peyer's patches
Jejunum
Lung
PLN
Ileum
Peritoneal cavity
Mid-A
Hi-A
Low-A
18
1.8
17
1.9
11
1.4
13
0.4
27
3.3
26
2.1
15
1.4
18
2.1
12
2.0
13
0.5
26
6.5
25
2.8
9
1.3
11
0.9
3.7
0.4
3.7
0.3
20
4.8
18
1.8
3.0
2.3
2.9
0.2
70
3.1
70
3.0
41
1.9
38
3.7
3.1
5.4
3.6
0.3
40
24
41
4.5
21
4.8
23
4.5
3.5
2.7
3.0
0.2
16
8.4
18
1.5
24
3.2
21
1.0
4.9
0.8
4.1
1.7
41
8.3
39
6.3
23
1.1
25
1.1
5.2
1.3
4.8
1.3
31
21
30
5.1
18
1.3
19
2.6
5.4
1.0
5.8
1.5
15
3.9
16
0.7
20
2.9
23
0.9
IL-17
IL-4
IL-17
IL-4
IL-17
IL-4
IFN-r

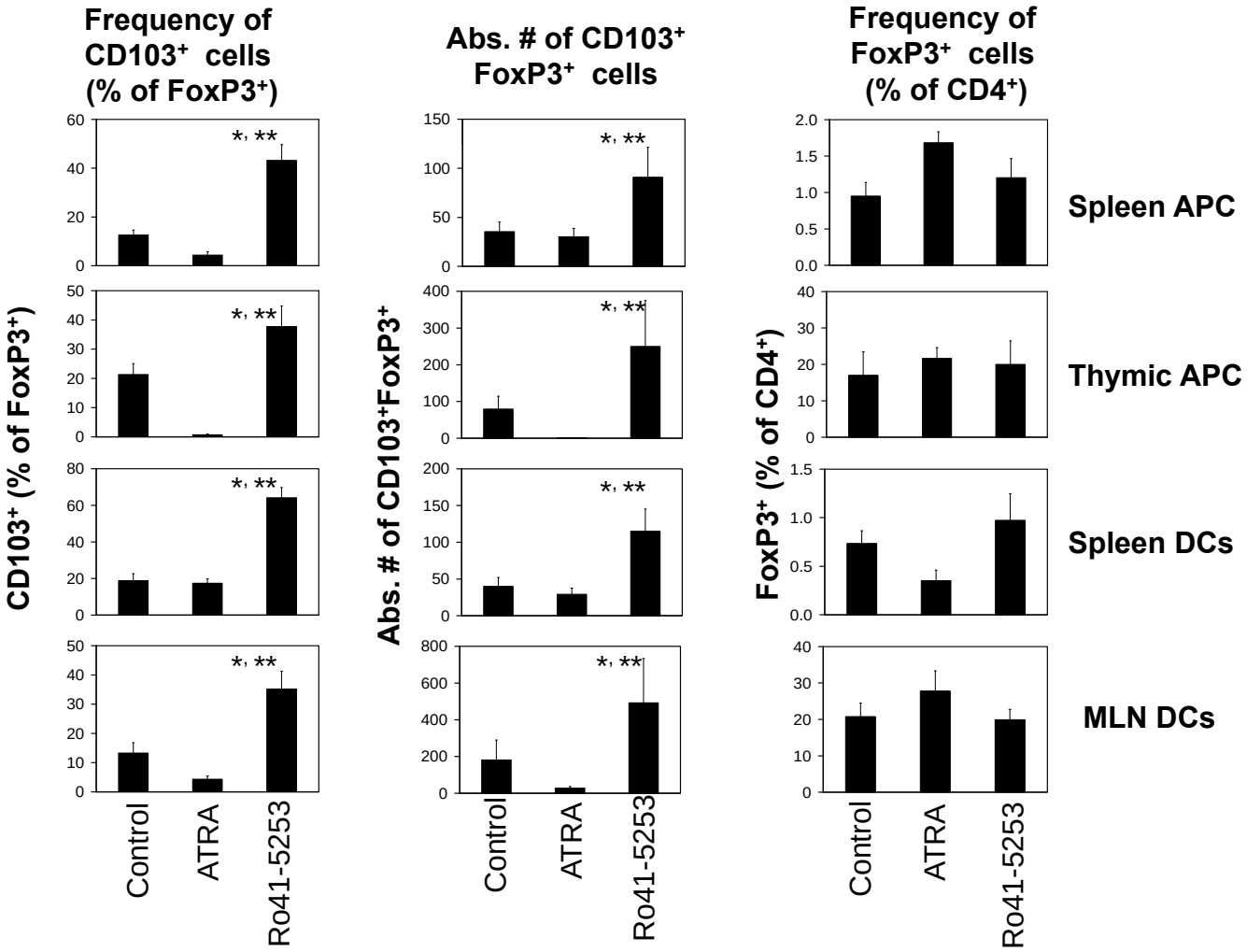
Supp-Fig. 4


Proliferation (CPM)
25000
20000
15000
10000
5000
0
Low-A Total Treg
Low-A CD103- Treg
CD4+CD25- : 30K 30K 30K 30K 0K
CD4+CD25+ : 0K 7.5K 15K 30K 30K

Supp-Fig. 5

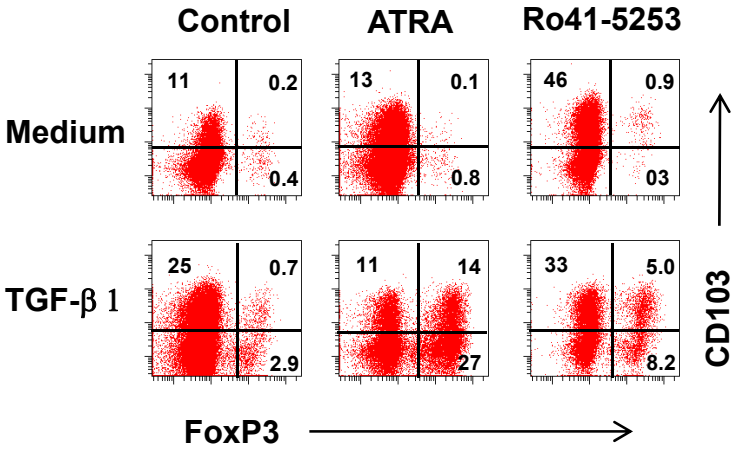
With APC & anti-CD3 activation

A

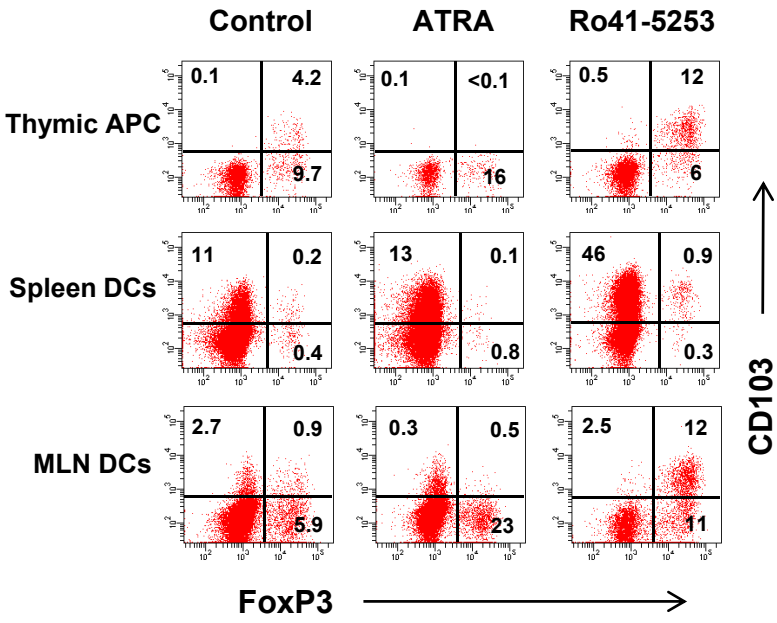


B

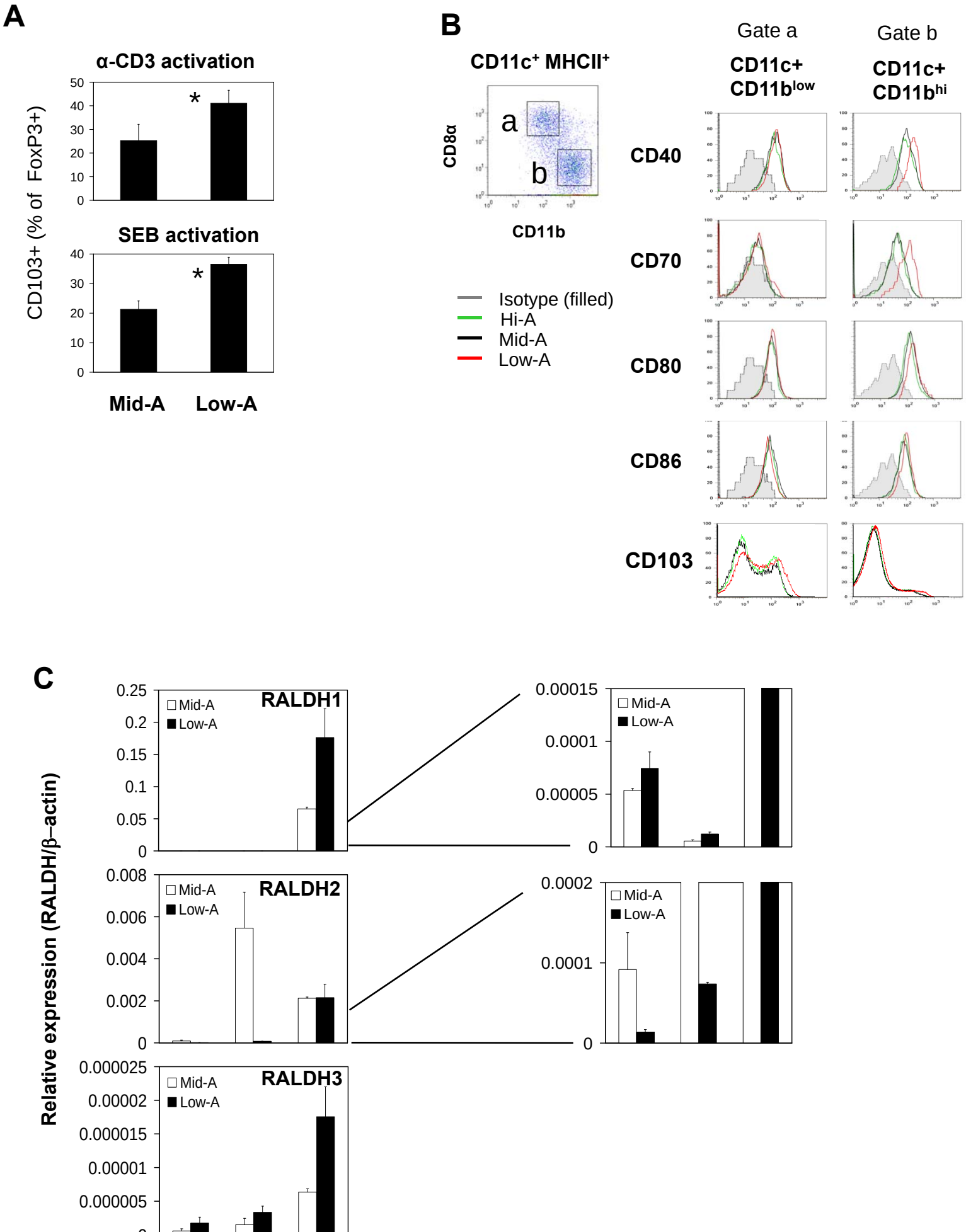
With spleen APC & anti-CD3



C



Supp-Fig. 6


A
α-CD3 activation
SEB activation
CD103+ (% of FoxP3+)
Mid-A
Low-A
B
CD11c+ MHCII+
CD8α
CD11b
a
b
Isotype (filled)
Hi-A
Mid-A
Low-A
Gate a
CD11c+ CD11blow
Gate b
CD11c+ CD11bhi
CD40
CD70
CD80
CD86
CD103
C
Relative expression (RALDH/β–actin)
RALDH1
RALDH2
RALDH3
Mid-A
Low-A
Spleen DC
MLN DC
SI-LP cells

Supp-Fig. 7