Figure S1

(A) Effect of AAL-R 0.1 mg/kg on peripheral leukocyte cell count

	before treatment	1st day	2nd day	3rd day	7th day
Total WBC	6.72	2.76	4.42	5.61	9.68
Neutrophil	1	1.15	2.78	1.76	1.57
Lymphocyte	5.38	1.3	1.27	3.34	7.63

(B) Effect of FTY720 0.1 mg/kg on peripheral leukocyte cell count

	before treatment	1st day	2nd day	3rd day	5th day	7th day
Total WBC	11.17	7.76	9.99	10.34	11.65	10.38
Neutrophil	1.04	1.02	1.07	0.98	1.81	0.81
Lymphocyte	9.57	6.43	8.39	8.93	9.29	9

(C) Effect of AAL-R 0.3 mg/kg on peripheral leukocyte cell count

	before treatment	1st day	3rd day	5td day	7th day
Total WBC	8.91	3.51	3.26	5.68	4.75
Neutrophil	1.27	1.74	0.88	1.25	1.03
Lymphocyte	7.15	1.32	2	3.89	3.41

(D) Effect of FTY720 0.5 mg/kg on peripheral leukocyte cell count

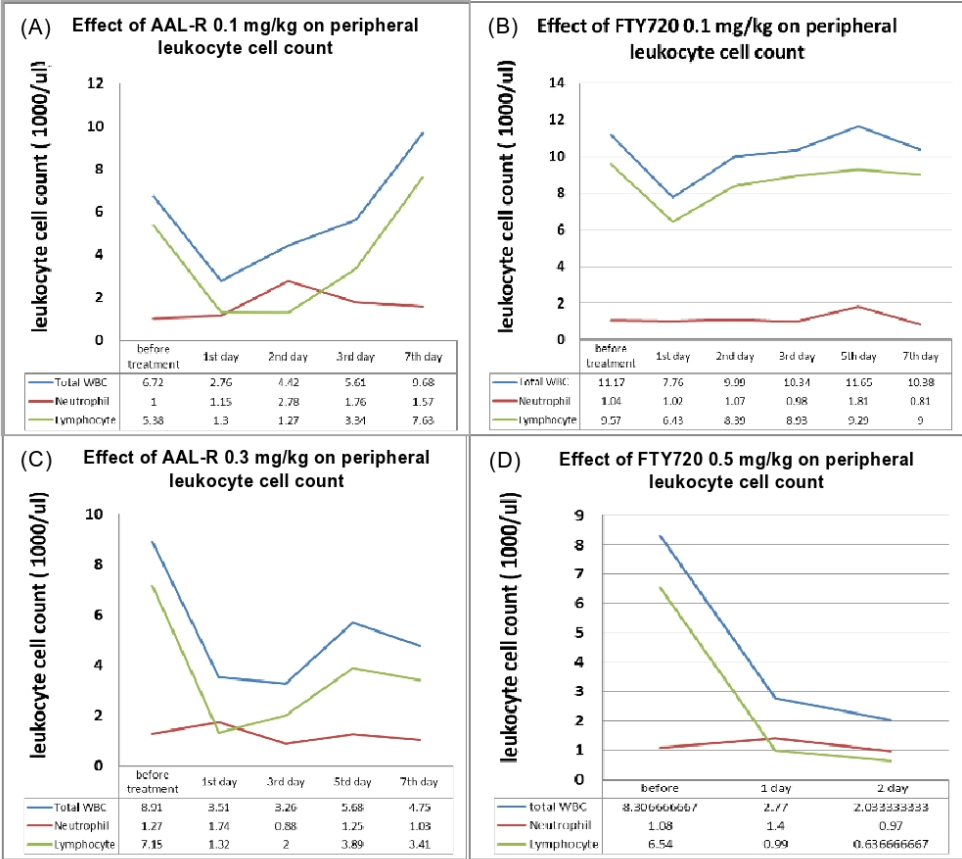|	before	1 day	2 day
total WBC	8.306666667	2.77	2.033333333
Neutrophil	1.08	1.4	0.97
Lymphocyte	6.54	0.99	0.636666667

Supplemental figures

Figure e1. Fingolimod and FTY720 AAL-R analog bioactivity

(A) AAL-R fingolimod S1P agonist and (B) Fingolimod dose-dependently decreased Sprague-Dawley rat blood leukocytes, and had minimal effect on PMN. Lower doses of AAL-R and fingolimod (0.1 mg/kg) decreased total WBC and lymphocyte counts; recovery began after day 1.

(C) A higher dose of AAL-R (0.3 mg/kg) decreased immune cells to below baseline levels through day 7.

(D) High-dose fingolimod (0.5 mg/kg) markedly decreased total WBC and lymphocyte counts, similar to the early effect seen with high-dose AAL-R.
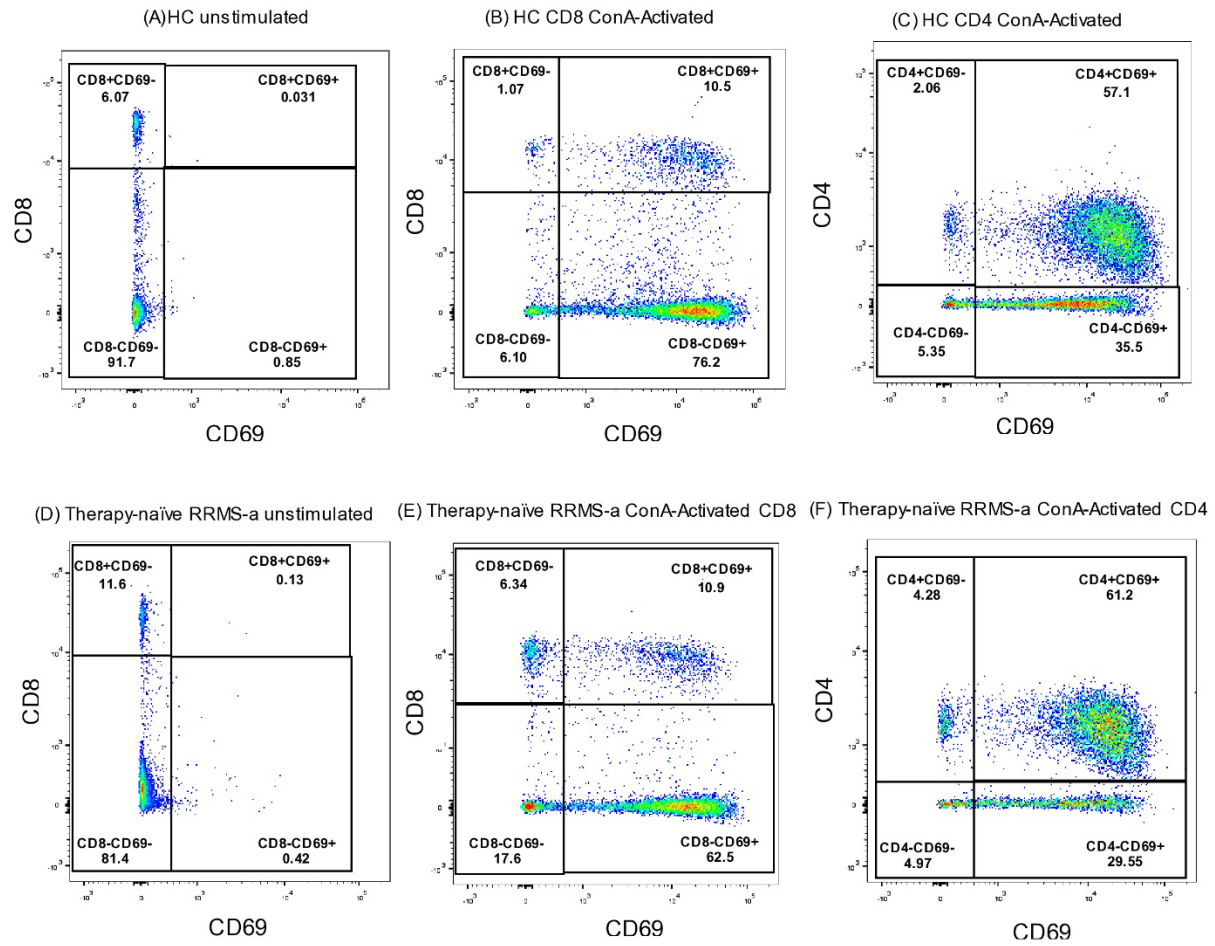
Figure S2
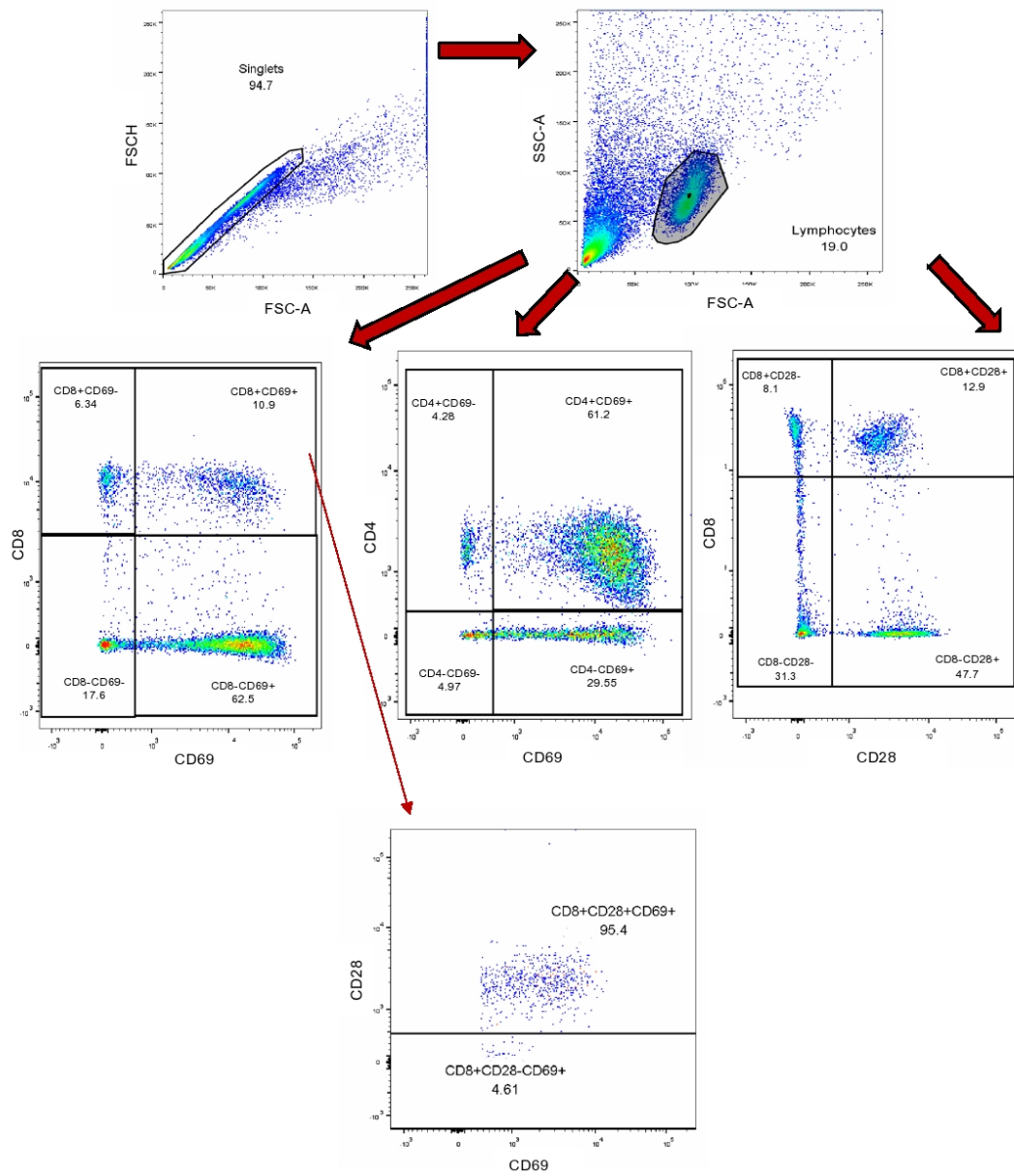
Figure e2. Flow Cytometry Gating Procedures

Singlets were first gated out in order to discriminate between single cells and clumps of cells by removing cells with disproportions between height and area. Lymphocytes were then gated using FSC and SSC using LSRFortessa 4-15 or 4-15 HTS flow cytometers (BD Biosciences). 10,000 lymphocytes were run for each condition, so absolute number parallels percentages within the lymphocyte population. Lymphocyte subset frequency, median fluorescence intensity (MFI), and multi-parameter compensation values were calculated with FlowJo software.
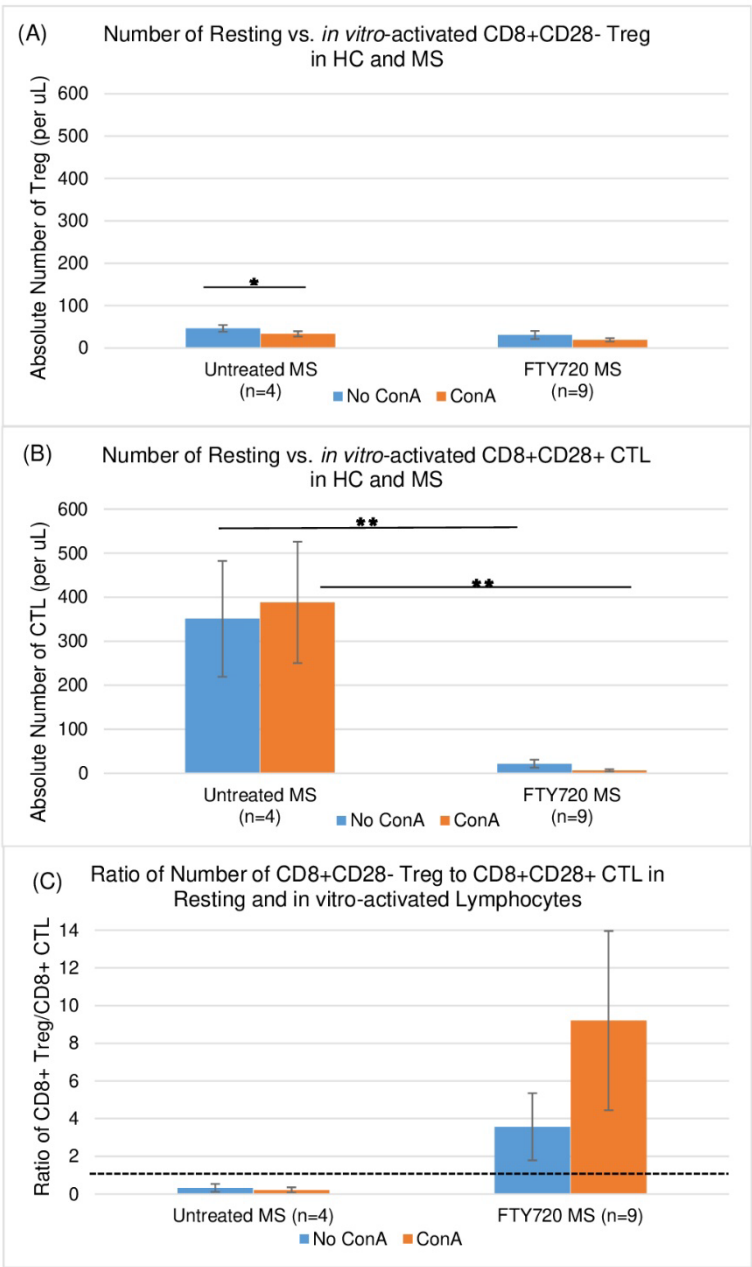
Figure S3

(A) HC unstimulated

CD8+CD69-
6.07

CD8+CD69+
0.031

CD8-CD69-
91.7

CD8-CD69+
0.85

CD8 (y-axis) / CD69 (x-axis)

(B) HC CD8 ConA-Activated

CD8+CD69-
1.07

CD8+CD69+
10.5

CD8-CD69-
6.10

CD8-CD69+
76.2

CD8 (y-axis) / CD69 (x-axis)

(C) HC CD4 ConA-Activated

CD4+CD69-
2.06

CD4+CD69+
57.1

CD4-CD69-
5.35

CD4-CD69+
35.5

CD4 (y-axis) / CD69 (x-axis)

(D) Therapy-naïve RRMS-a unstimulated

CD8+CD69-
11.6

CD8+CD69+
0.13

CD8-CD69-
81.4

CD8-CD69+
0.42

CD8 (y-axis) / CD69 (x-axis)

(E) Therapy-naïve RRMS-a ConA-Activated CD8

CD8+CD69-
6.34

CD8+CD69+
10.9

CD8-CD69-
17.6

CD8-CD69+
62.5

CD8 (y-axis) / CD69 (x-axis)

(F) Therapy-naïve RRMS-a ConA-Activated CD4

CD4+CD69-
4.28

CD4+CD69+
61.2

CD4-CD69-
4.97

CD4-CD69+
29.55

CD4 (y-axis) / CD69 (x-axis)

Figure e3. ConA activation induces CD69 expression on CD4+ and CD8+ T cells from HC and MS patients

(A, D) Resting HC and MS CD8+ and CD8- cells expressed minimal levels of CD69. CD8+CD69+ and CD4+CD69+ percentages in unstimulated PBMC were similar (DNS). ConA activation *in vitro* induced CD69 expression in (B) CD8+ and (C) CD4+ T cell subsets from healthy controls, and in (E) CD8+ and (F) CD4+ T cells from therapy-naïve exacerbating RRMS (MS-a). CD69 expression in CD8+ T cells and CD4+ T cells was equivalent in activated (C) HC and (F) RRMS cells. Figures are representative of 60 samples. Activated CD69 expression in cells of patients treated with fingolimod was similar to that in the therapy-naïve MS group, and was similar between stable MS (MS-s) and MS-a (DNS).

Figure S4

(A) Number of Resting vs. *in vitro*-activated CD8+CD28- Treg in HC and MS

(B) Number of Resting vs. *in vitro*-activated CD8+CD28+ CTL in HC and MS

(C) Ratio of Number of CD8+CD28- Treg to CD8+CD28+ CTL in Resting and in vitro-activated Lymphocytes

Figure e4. Absolute number of CD8+CD28- Treg and CD8+CD28+ CTL, and effect of activation

(A) The absolute number of CD8+CD28- Treg within the lymphocyte population is similar in untreated and fingolimod-treated MS. ConA activation tends to reduce the calculated number of Treg in both groups.

(B) CD8+CD28+ CTL are reduced 16-fold by fingolimod therapy in resting PBMC and 55-fold in activated PBMC. ConA activation has no effect on CTL numbers in untreated MS.

(C) The ratio of the number of Treg:CTL is low in untreated MS but increased 11.67-fold in in resting and 46-fold in ConA-activated fingolimod-treated MS. ConA activation increases the Treg:CTL ratio in fingolimod-treated MS by depleting CTL (seen in B). Dashed line at 1 on y-axis is 50:50 ratio of Treg:CTL. Absolute numbers are calculated from the number of lymphocytes in the complete blood count (CBC) and differential count. ✶ $p \leq 0.05$, ✶✶ $p \leq 0.01$, ✶✶✶ $p \leq 0.001$.

Figure S5

(A) Number of CD8+CD28- Treg Before and After ConA Activation in Young and Old

(B) Number of CD8+CD28+ CTL Before and After ConA Activation in Young and Old
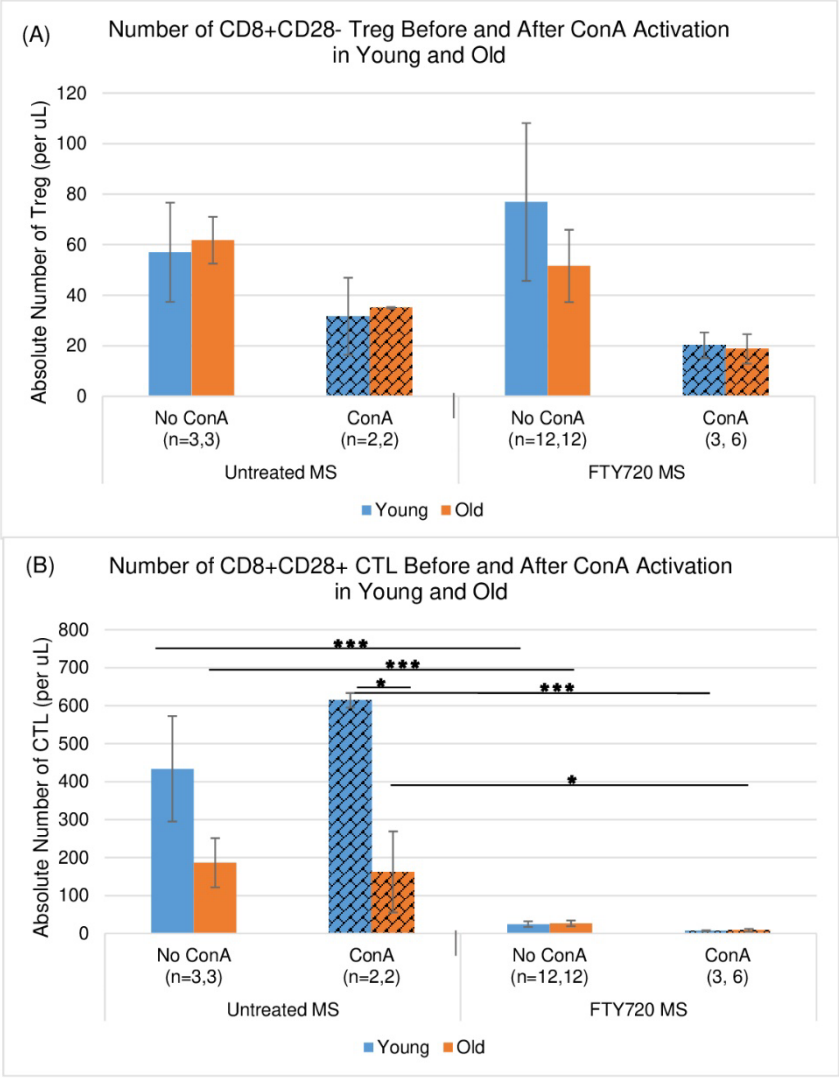
(C) Ratio of Number of CD8+CD28- Treg and CD8+CD28+ CTL Before and After ConA Activation in Young and Old
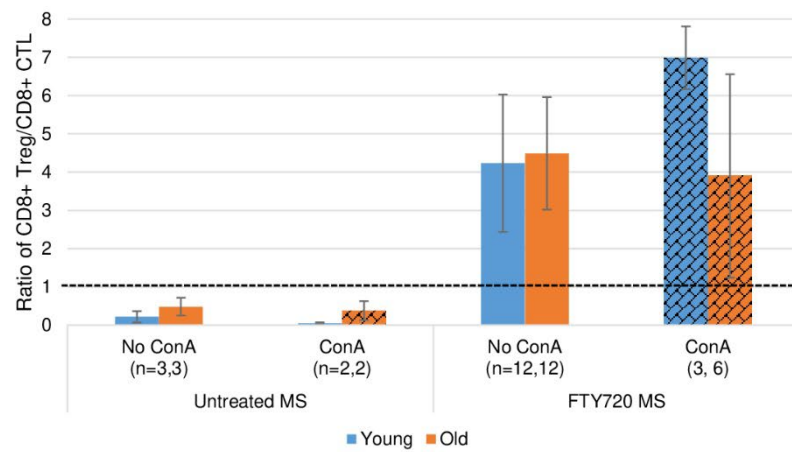
Figure e5. Absolute number of CD8+CD28- Treg and CD8+CD28+ CTL, and effects of age

(A) The absolute number of Treg was equivalent in young and old, untreated and fingolimod-treated MS. The number fell in both groups with ConA activation.

(B) The absolute number of CTL was greater in young than old untreated MS. The number fell 17.3-fold in younger and 6.89-fold in older fingolimod-treated MS. The number fell in both groups with ConA activation. In this paired analysis, the number of CTL did not change with ConA activation. Absolute numbers are calculated from the number of lymphocytes in the complete blood count (CBC) and differential count.

(C) The ratio of the number of Treg:CTL is low in untreated MS but increased 21-fold in younger and 11-fold in older fingolimod-treated MS. The increase is ~2-fold greater in young than old patients. ConA activation increases the Treg:CTL ratio in fingolimod-treated MS by depleting CTL (seen in B). Young *vs.* old are dichotomized by median age of the group (see text). Dashed line at 1 on y-axis is 50:50 ratio of Treg:CTL. Hashing in bars indicates ConA activation. $*$ $p \leq 0.05$, $**$ $p \leq 0.01$, $***$ $p \leq 0.001$.