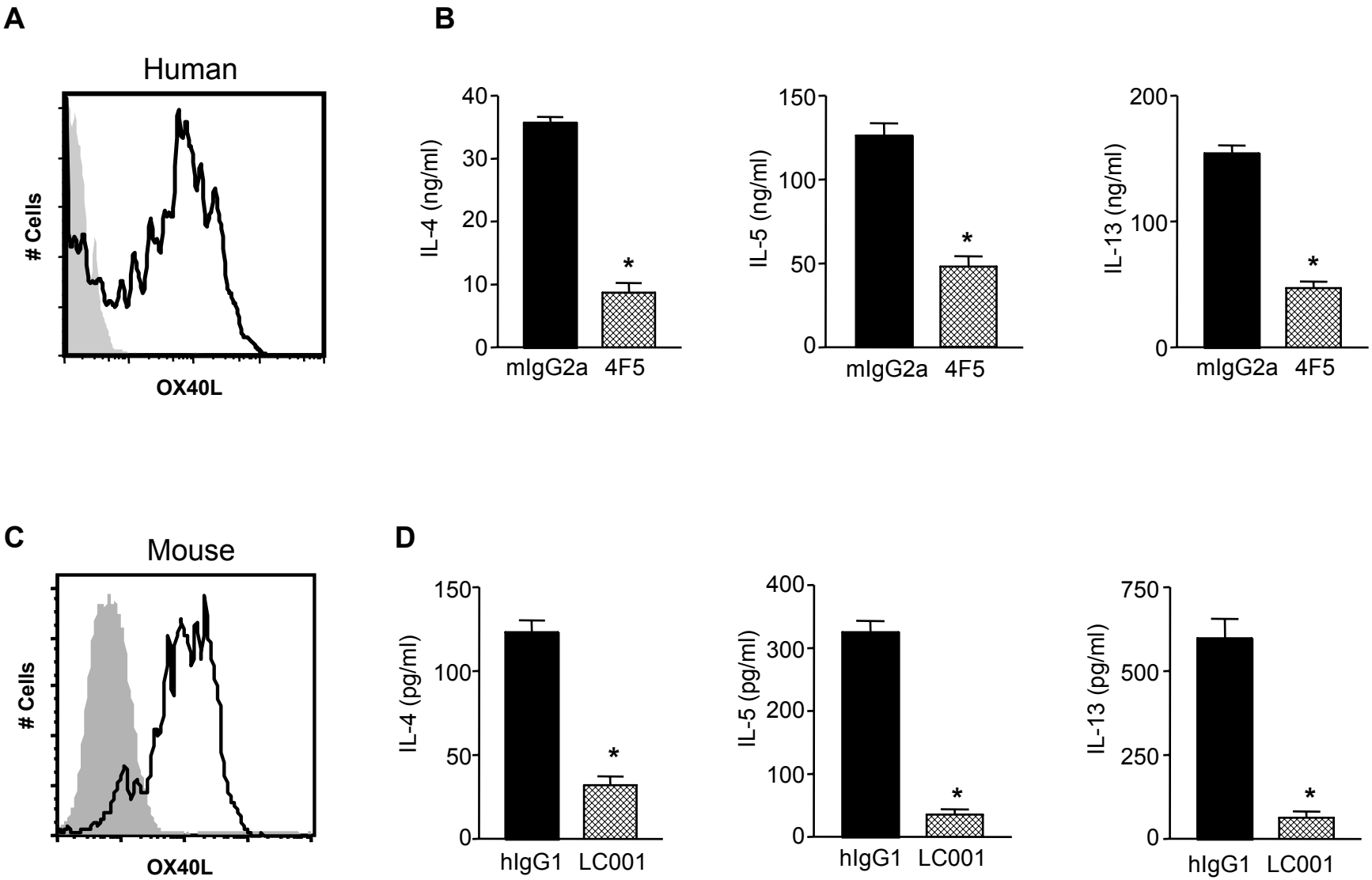
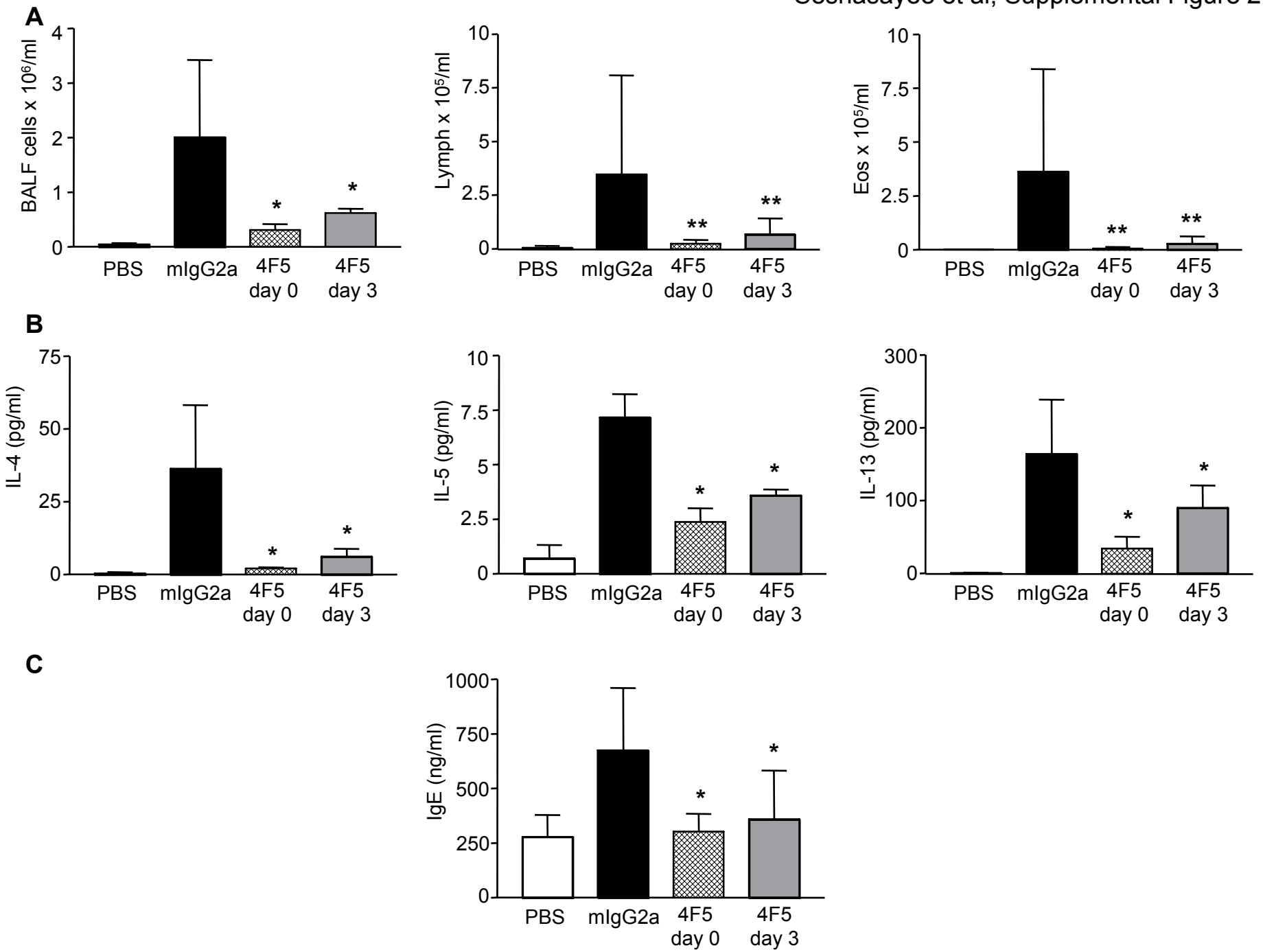
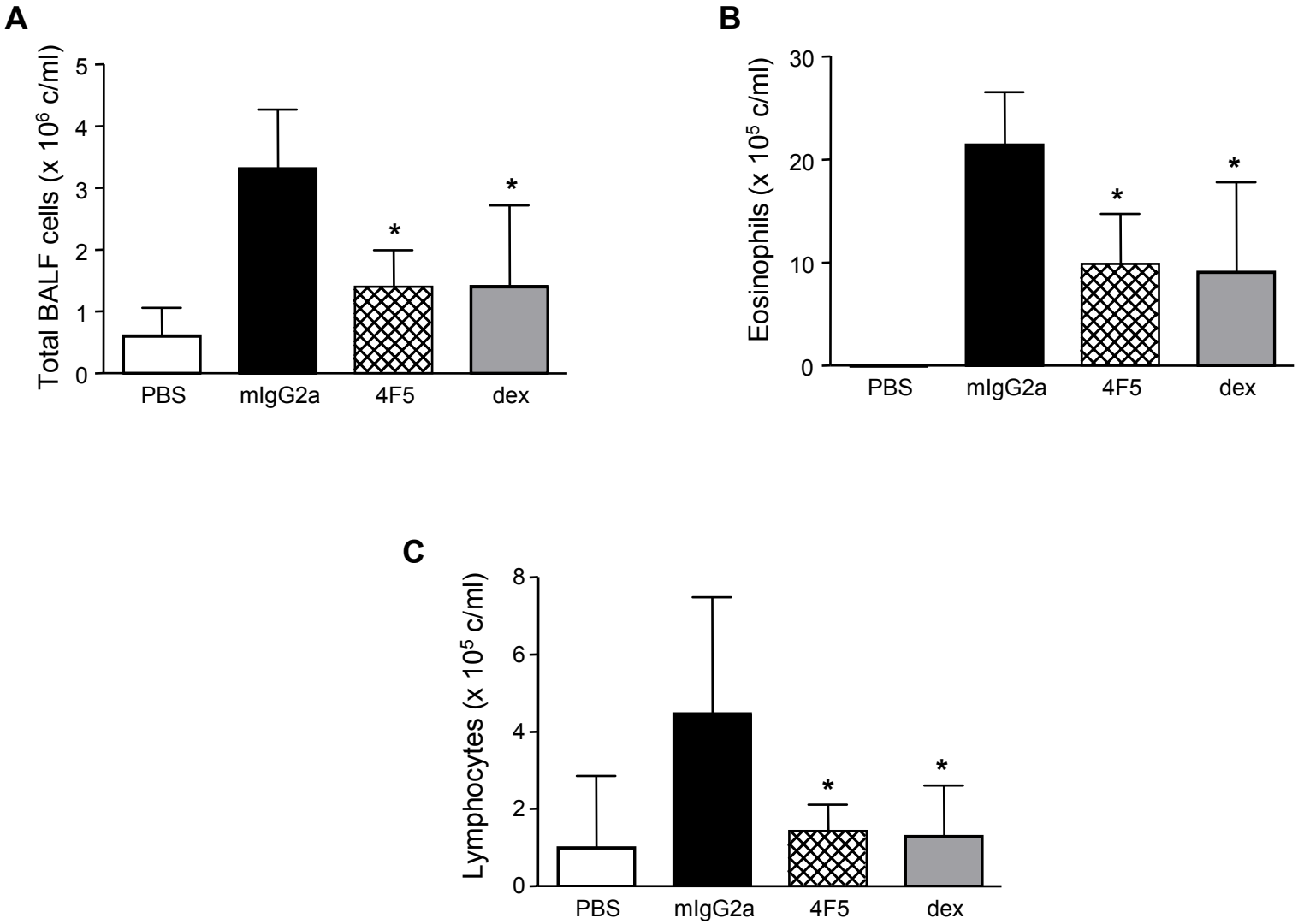
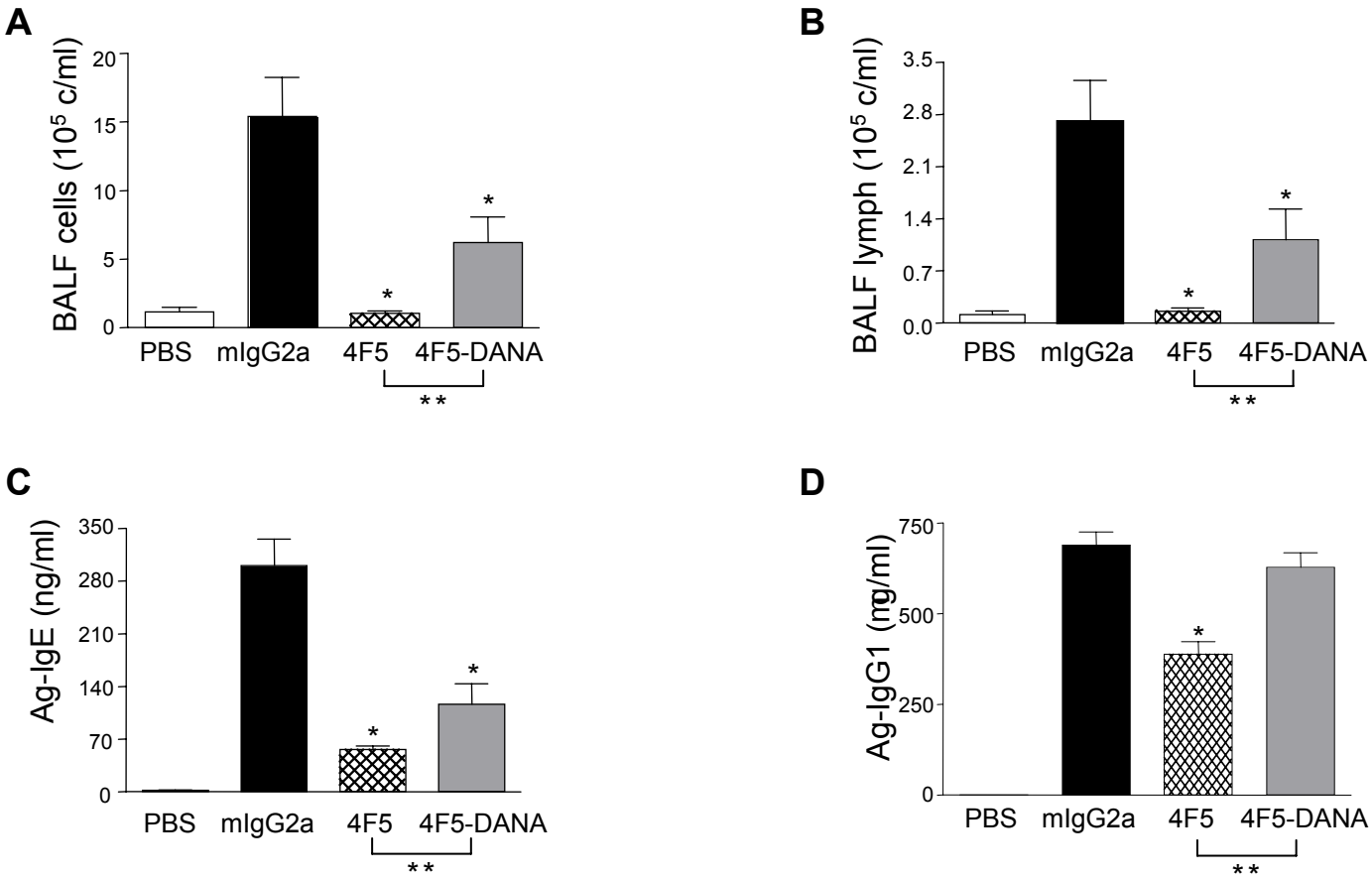
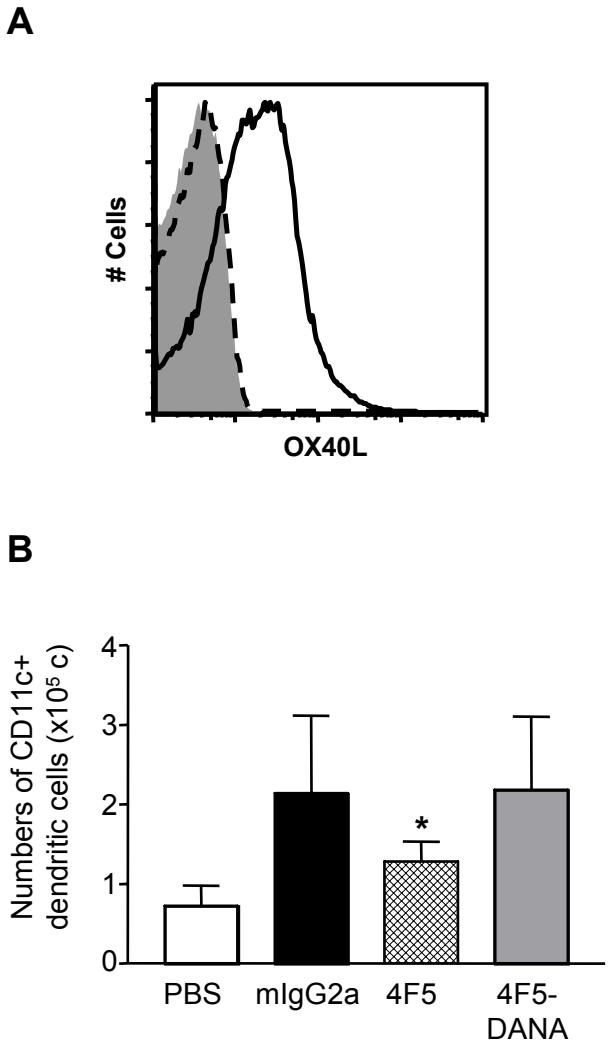
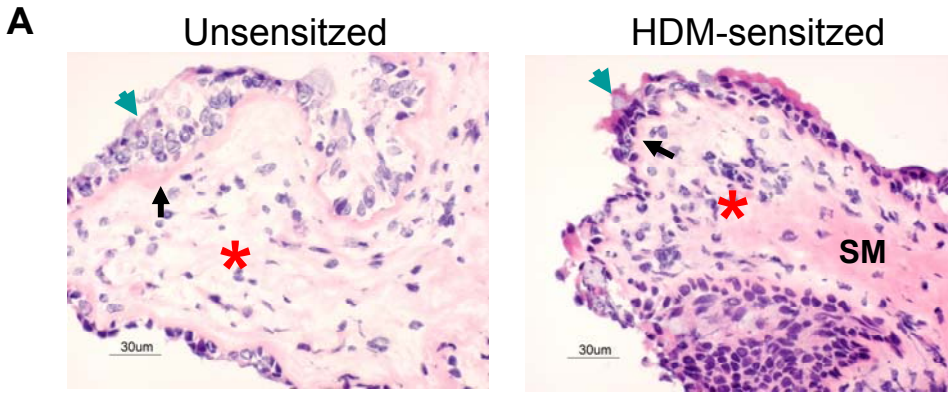

A
Human
Cells
OX40L
B
IL-4 (ng/ml)
40
30
20
10
0
mIgG2a
4F5
IL-5 (ng/ml)
150
100
50
0
mIgG2a
4F5
IL-13 (ng/ml)
200
100
0
mIgG2a
4F5
C
Mouse
Cells
OX40L
D
IL-4 (pg/ml)
150
100
50
0
hIgG1
LC001
IL-5 (pg/ml)
400
300
200
100
0
hIgG1
LC001
IL-13 (pg/ml)
750
500
250
0
hIgG1
LC001

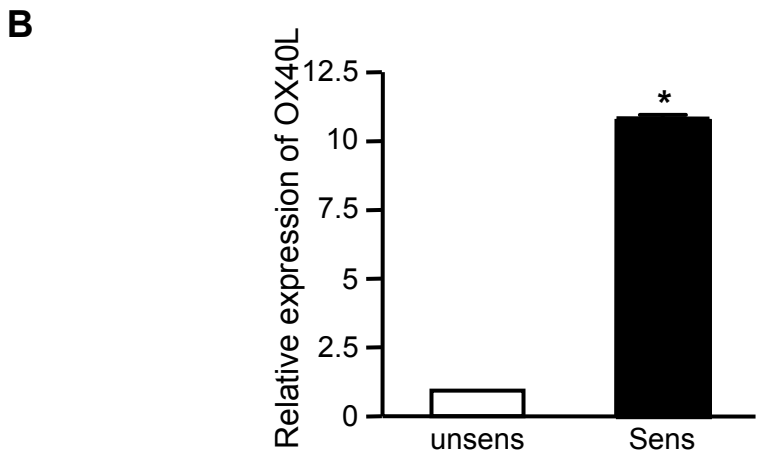

A
BALF cells x 10^6/ml
Lymph x 10^5/ml
Eos x 10^5/ml
PBS
mIgG2a
4F5 day 0
4F5 day 3
B
IL-4 (pg/ml)
IL-5 (pg/ml)
IL-13 (pg/ml)
C
IgE (ng/ml)


A
Total BALF cells (x 10^6 c/ml)
0
1
2
3
4
5
*
*
PBS
mIgG2a
4F5
dex
B
Eosinophils (x 10^5 c/ml)
0
10
20
30
*
*
PBS
mIgG2a
4F5
dex
C
Lymphocytes (x 10^5 c/ml)
0
2
4
6
8
*
*
PBS
mIgG2a
4F5
dex


A
BALF cells (10^5 c/ml)
20
15
10
5
0
PBS
mIgG2a
4F5
4F5-DANA
*
**
B
BALF lymph (10^5 c/ml)
3.5
2.8
2.1
1.4
0.7
0.0
C
Ag-IgE (ng/ml)
350
280
210
140
70
0
D
Ag-IgG1 (mg/ml)
750
500
250
0


A
Cells
OX40L
B
Numbers of CD11c+ dendritic cells (x10^5 c)
0
1
2
3
4
*
PBS
mIgG2a
4F5
4F5-DANA


A
Unsensitzed
HDM-sensitzed
30um
SM
30um


B
Relative expression of OX40L
12.5
10
7.5
5
2.5
0
*
unsens
Sens

Supplemental figure 1.

TSLP induces OX40L expression on dendritic cells and anti-OX40L mAb blocks TSLP-induced Th2 polarization. Human myeloid $CD11c^+$ dendritic cells (A) or mouse splenic $CD11c^+$ dendritic cells (C) were stimulated with TSLP (25 ng/ml) for 48 hrs and analyzed for OX40L expression. Un-treated cells are shown by the grey histogram and TSLP-stimulated cells by the solid black histogram. For TSLP-induced human Th2 polarization assays (B), $CD11c^+$ dendritic cells were activated with TSLP for 3 days, washed and cultured with naive $CD4^+$ T cells in the presence of Th2 polarizing conditions and control mIgG2a/4F5 antibodies for 7 days. Cells were then washed, rested for 3 days and re-stimulated with α-CD3/ α-CD28 for 2 days. Cell culture supernatants were assayed for levels of IL-4, IL-5 and IL-13. For mouse TH2 polarization assays, splenic $CD11c^+$ DCs were stimulated with TSLP for 3 days, washed and cultured with DO11.10 CD4+ T cells in the presence of OVA peptide, Th2 polarizing conditions and control mIgG2a/4F5 antibodies for 5 days. Cells were then washed, rested and re-stimulated as above. Results are the mean number of cells + SD. * indicates a p value < 0.01 (Dunnett's test).

Supplemental figure 2.

α-OX40L mAb blocks TSLP-induced inflammation in lung. Experimental design for treatment of TSLP-induced lung inflammation with a-OX40L ab is shown (A). BALB/c mice (8 per group) were administered 500 ng TSLP (or control PBS)

intra-nasally on days 0, 1 and 2. Mice administered TSLP were treated with 150 μg control mIgG2a and α-OX40L 4F5-WT mAbs i.p starting on day 0 or day 3, with additional doses on days 6, 9 and 13. Mice were sacrificed on day 15. Total number of cells, lymphocytes and eosinophils per ml of BAL fluid are shown (B). Levels of IL-4, IL-5 and IL-13 in the BAL fluid were measured by ELISA and are shown (C). Results are the mean number of cells ± SD. * indicates a p value < 0.01 (Dunnett's test).

Supplemental figure 3.

α-OX40L mAb inhibits lung inflammation in an acute model of OVA-induced asthma. Total numbers of cells (A), eosinophils (B) and lymphocytes (C) infiltrating into the BALF in an acute model of OVA-induced lung inflammation are shown. BALB/c mice were sensitized with OVA-alum on day 0 and challenged with 50 μg OVA administered i.n daily on days 25-28. Mice were treated with 150 μg control mIgG2a, α-OX40L 4F5 antibodies or dexamethasone (2 mg/kg) i.p, on days 26 and 27. Results are mean ± SD. * indicates a p value < 0.01 (Dunnett's test).

Supplemental figure 4.

α-OX40L mAb with effector function is significantly better at inhibition of OVA-induced inflammation compared to the DANA form lacking effector function. Total numbers of cells (A), lymphocytes (B) infiltrating into the BALF, and serum levels of antigen-specific IgE (C) and IgG1 (D) in an acute model of OVA-induced lung

inflammation are shown. BALB/c mice were sensitized with OVA-alum on day 0 and challenged with 50 μg OVA administered i.n daily on days 25-28. Mice were treated with 150 μg control mIgG2a, α-OX40L 4F5 antibodies or dexamethasone (2 mg/kg) i.p, on days 26 and 27. Results are mean ± SD. * and ** indicate a p value < 0.01 (Dunnett's test).

Supplemental figure 5.

α-OX40L mAb can deplete OX40L-expressing dendritic cells in the popliteal and inguinal lymph nodes. BALB/c mice (5 per group) were treated with 1 μg TSLP (or control PBS) in the footpad on days 0, 1 and 2. TSLP-treated mice were administered mIgG2a/4F5/4F5-DANA mAbs on days 0 and 2. On day 3, popliteal and inguinal lymph nodes were harvested and OX40L expression was assayed on CD11c+ dendritic cells (A). Isotype is shown in grey, control PBS mice are represented by a dotted line and TSLP-treated mice in bold. Total numbers of $CD11c^+$ dendritic cells in the lymph nodes for the groups are shown in (B). All analyses were performed on live cells (PI excluded). Results are the mean number of cells ± SD. * indicates a p value < 0.01 (Dunnett's test).

Supplemental figure 6.

Increased inflammatory infiltrate and OX40L expression is observed in BALF of HDM-sensitized rhesus monkeys. (A) H&E staining of lung biopsies from un-sensitized (left panel) and HDM-sensitized (right panel) shows the presence of an inflammatory infiltrate in antigen-challenged monkeys. The respiratory epithelium is marked with a green arrowhead, the sub-epithelial basement membrane with a black arrow and the lamina propria with a red star. (B) BAL fluid from un-

sensitized and HDM-sensitized (vehicle group) monkeys was harvested at week 89, and RNA isolated from cells in the BAL. Levels of OX40L transcripts were assessed by quantitative RT-PCR and are displayed as fold induction in sensitized over control un-sensitized monkeys. Average Ct values for OX40L for the un-sensitized and sensitized monkeys were 30.5 and 27.1 respectively. * indicates a p value < 0.01 (Dunnett's test).