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# Human PD-1 agonist treatment alleviates neutrophilic asthma by reprogramming T cells

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**Figure Legends:**

*Figure E1:* Dexamethasone treatment does not reduce neutrophilic lung inflammation in WT and PD-1 KO mice

(A) WT and PD-1 KO mice were sensitized via *s.c.* tail base injection of HDM (200 μg) mixed in CFA (1:1 v/v). After 13 days, mice were *i.n.* challenged with HDM (100 μg) and were treated or not at day 14 with Dexamethasone (1 mg/Kg) via the intraperitoneal route. On day 15, BAL was collected.

(B) Absolute count of $CD45^+$ cells, (C) T cells, and (D) neutrophils.

Data are representative of at least 2 independent experiments and are presented as means ± SEM (n=5, n.s. non-significant).

*Figure E2:* Gating strategy of main $CD45^+$ cells in the BAL and lungs.

(A) Representative flow cytometry plots showing the gating strategy of neutrophils, alveolar macrophages, eosinophils, T and B cells in the BAL.

(B) Representative flow cytometry plots showing the gating strategy of neutrophils, macrophages and DCs in the lungs.

*Figure E3:* The lack of PD-1 potentiates the production of IL-5 and IL-13 in the lungs

WT and PD-1 KO mice were sensitized via *s.c.* tail base injection of HDM (200 μg) mixed in CFA (1:1 v/v). After 13 days, mice were *i.n.* challenged with HDM (100 μg). Control mice received PBS only. On day 14, the BAL was collected to measure cytokines.

(A) Levels of IL-5 and (B) IL-13 quantified in the BAL using the LEGENDplex bead-based immunoassay.

Data are representative of at least 2 independent experiments and are presented as means ± SEM (two-tailed Student's t-test; n=5; *P < .05, **P < .01, ***P < .001, n.s. non-significant).

*Figure E4:* HDM challenge induces the expression of PD-1 on a population of pulmonary immune cells.

BALB/cByJ mice (WT) mice were sensitized via subcutaneous (*s.c.*) tail base injection of HDM (200 μg) mixed in CFA (1:1 v/v). After 13 days, mice were intranasally (*i.n.*) challenged with HDM (100 μg). Control mice received PBS only. On day 14, lungs were collected after euthanasia for flow cytometry staining. The figure shows representative flow cytometry plots of PD-1 induction on pulmonary $CD45^+$ cells from WT mice.

*Figure E5:* PD-1 agonist treatment reduces the levels of IL-17A and Th2 cytokines in the lungs

(A) Humanized PD-1 mice were sensitized via *s.c.* tail base injection of HDM (200 μg) mixed in CFA (1:1 v/v). After 13 days, mice were *i.n.* challenged with HDM (100 μg) and received 500 μg of PD-1 agonist via the intraperitoneal route or the corresponding isotype, while the dose was reduced to 250 μg via the intravenous route on day 14. Naïve mice were not sensitized, challenged, or treated. On day 15, BAL and lungs were collected after euthanasia.

(A) Levels of IL-4, (B) IL-5 and (C) IL-13 quantified in the BAL using the LEGENDplex bead-based immunoassay.

(D) Levels of IL-17A, (E) G-CSF, (F) GM-CSF, and (G) IL-10 quantified in the lung lysates.

Data are representative of at least 2 independent experiments and are presented as means ± SEM (two-tailed Student's t-test; n=5; *P < .05, **P < .01, ***P < .001).

*Figure E6*: PD-1 agonist treatment reduces the number of main innate immune cells in the lungs.

Humanized PD-1 mice were sensitized via *s.c.* tail base injection of HDM (200 μg) mixed in CFA (1:1 v/v). After 13 days, mice were *i.n.* challenged with HDM (100 μg) and received 500 μg of PD-1 agonist via the intraperitoneal route or the corresponding isotype, while the dose was reduced to 250 μg via the intravenous route on day 14. Naïve mice were not sensitized, challenged, and neither treated. On day 15, lungs were washed and collected after euthanasia.

(A) Absolute count of eosinophils, (B) macrophages, and (C) DCs quantified in lungs using the count precision beads.

Data are representative of at least 2 independent experiments and are presented as means ± SEM (two-tailed Student's t-test; n=4; *P < .05, **P < .01).

*Figure E7:* PD-1 is highly expressed on $CD44^+$ $CD4^+$ T cells in the lungs.

Humanized PD-1 mice were sensitized via *s.c.* tail base injection of HDM (200 μg) mixed in CFA (1:1 v/v). After 13 days, mice were *i.n.* challenged with HDM (100 μg). On day 15, lungs were washed and collected after euthanasia.

(A) Representative flow cytometry plots showing the gating of $CD44^-$ and $CD44^+$ $CD4^+$ T cells.

(B) PD-1 expression on $CD44^-$ and $CD44^+$ $CD4^+$ T cells, represented as the MFI.

(C) The percentage of $PD\text{-}1^+$ cells in $CD44^-$ and $CD44^+$ $CD4^+$ T cells.

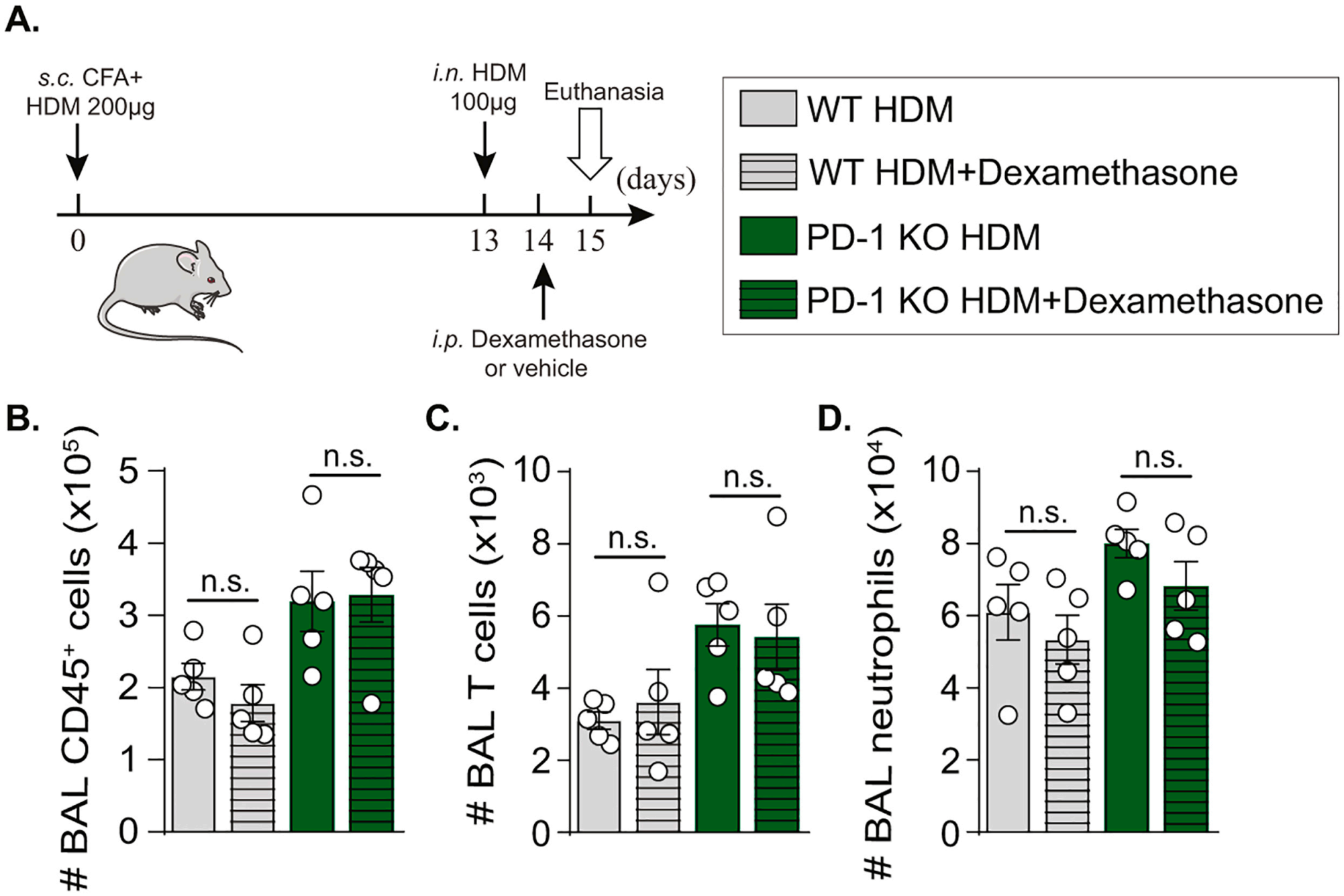
(D) The gating strategy of Treg identified as $CD4^+$ $CD25^+$ $FOXP3^+$ $CD4^+$ cells.

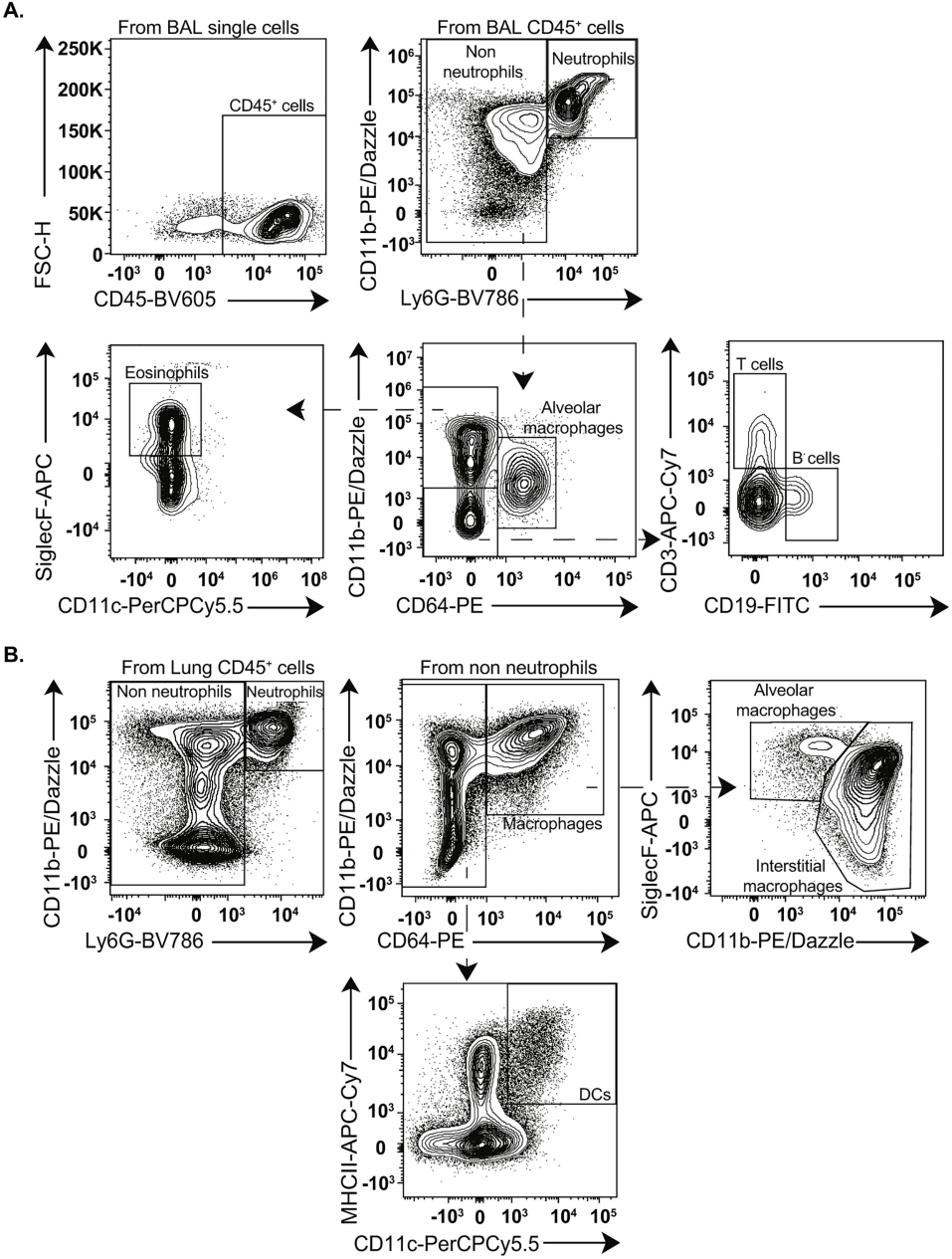
Data are representative of at least 2 independent experiments and are presented as means ± SEM (two-tailed Student's t-test; n=4; ***P < .001).

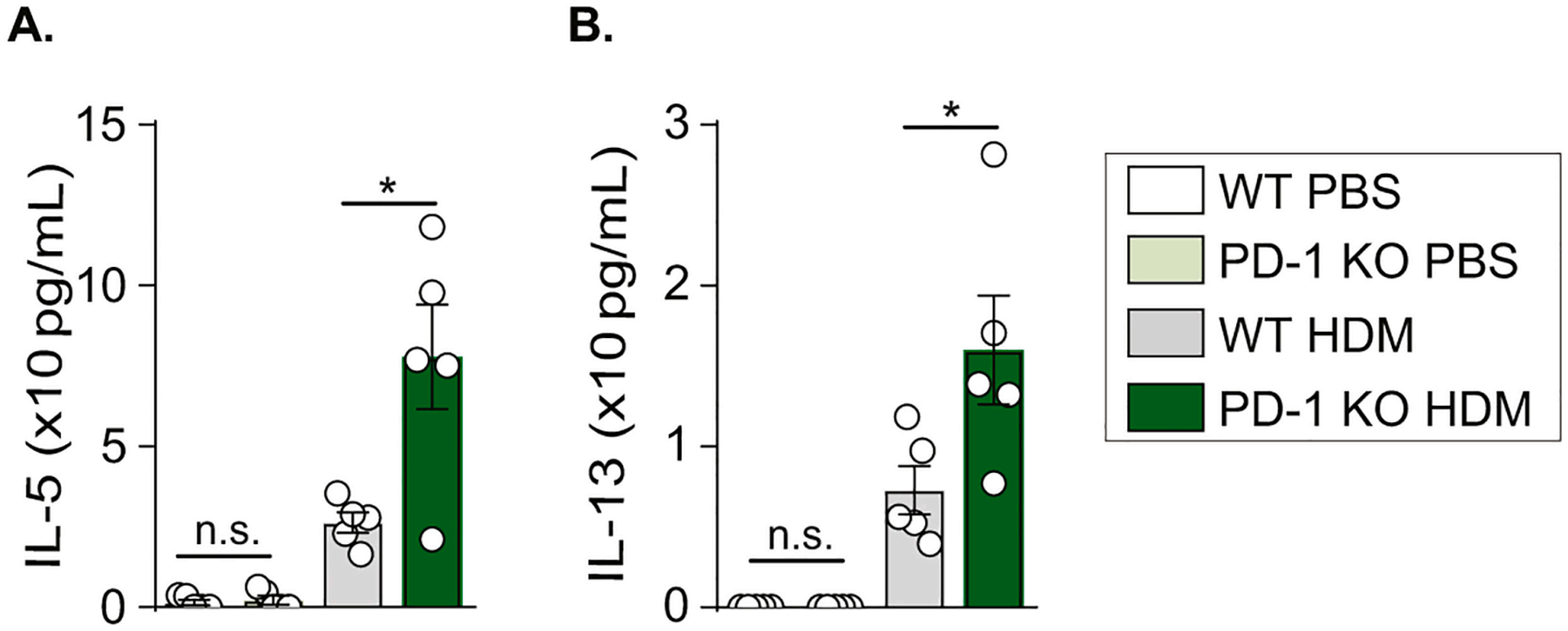
*Figure E8:* PD-1 agonist does not affect the distribution of $CD4^+$ T cells in the spleen.

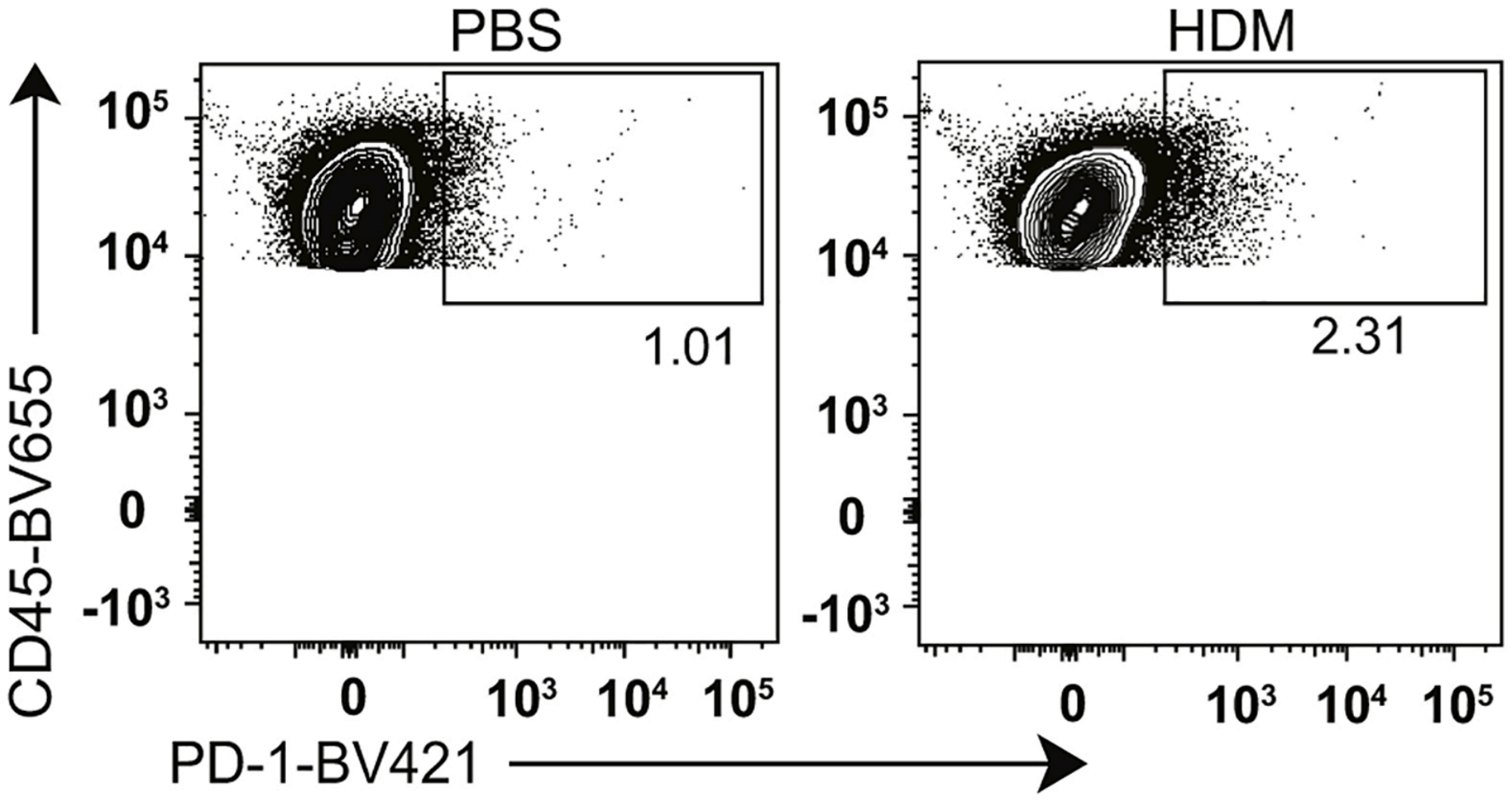
(A) Percentages of effector memory $CD4^+$ T cells, (B) central memory $CD4^+$ T cells, (C) naïve $CD4^+$ T cells, and (D) Teff:Treg ratio in the spleen.

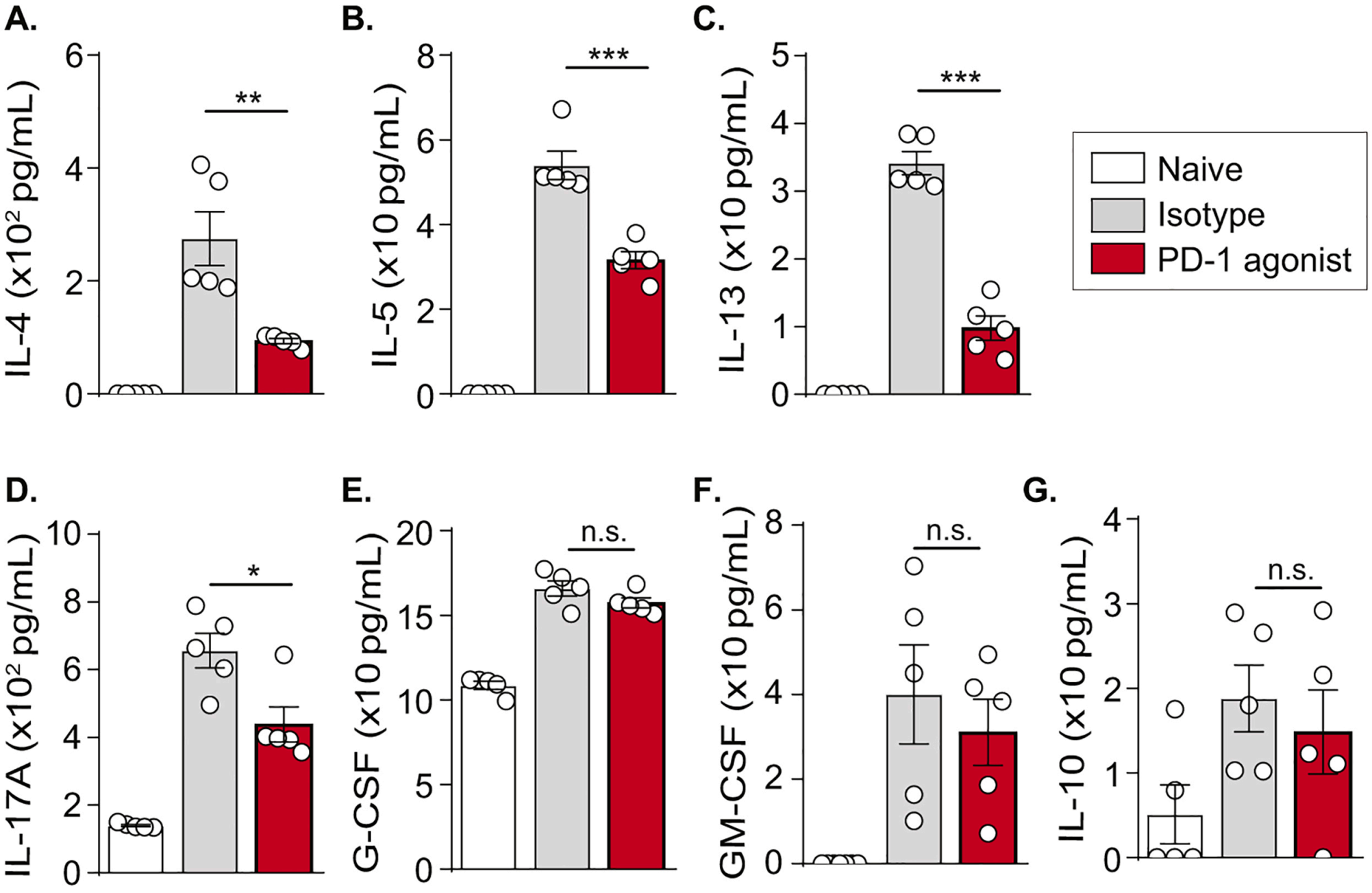
Data are representative of at least 2 independent experiments and are presented as means ± SEM (two-tailed Student's t-test; n=4; n.s. non-significant).

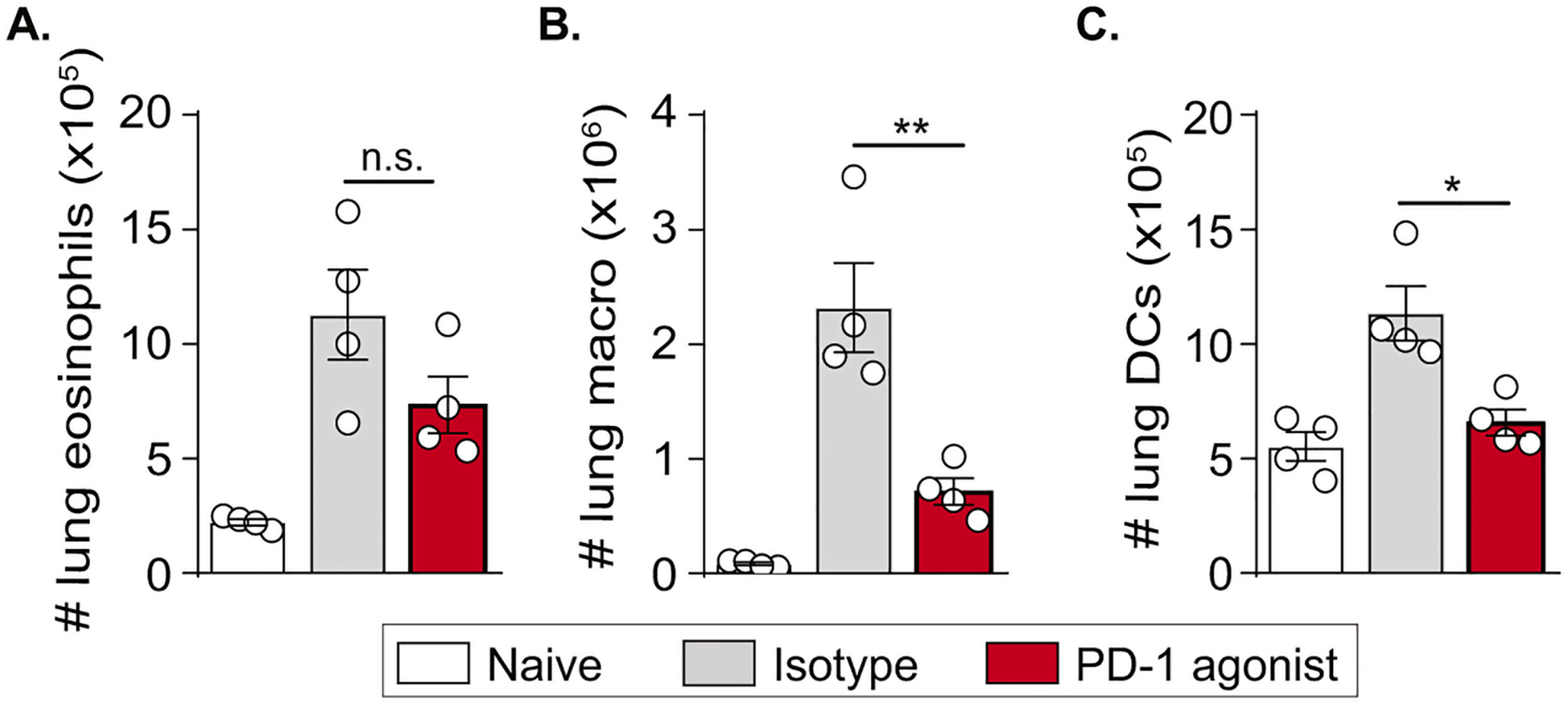

A.
s.c. CFA+
HDM 200µg
i.n. HDM
100µg
Euthanasia
(days)
0
13
14
15
i.p. Dexamethasone
or vehicle
WT HDM
WT HDM+Dexamethasone
PD-1 KO HDM
PD-1 KO HDM+Dexamethasone
B.
# BAL CD45+ cells (x10^5)
n.s.
n.s.
C.
# BAL T cells (x10^3)
n.s.
n.s.
D.
# BAL neutrophils (x10^4)
n.s.
n.s.

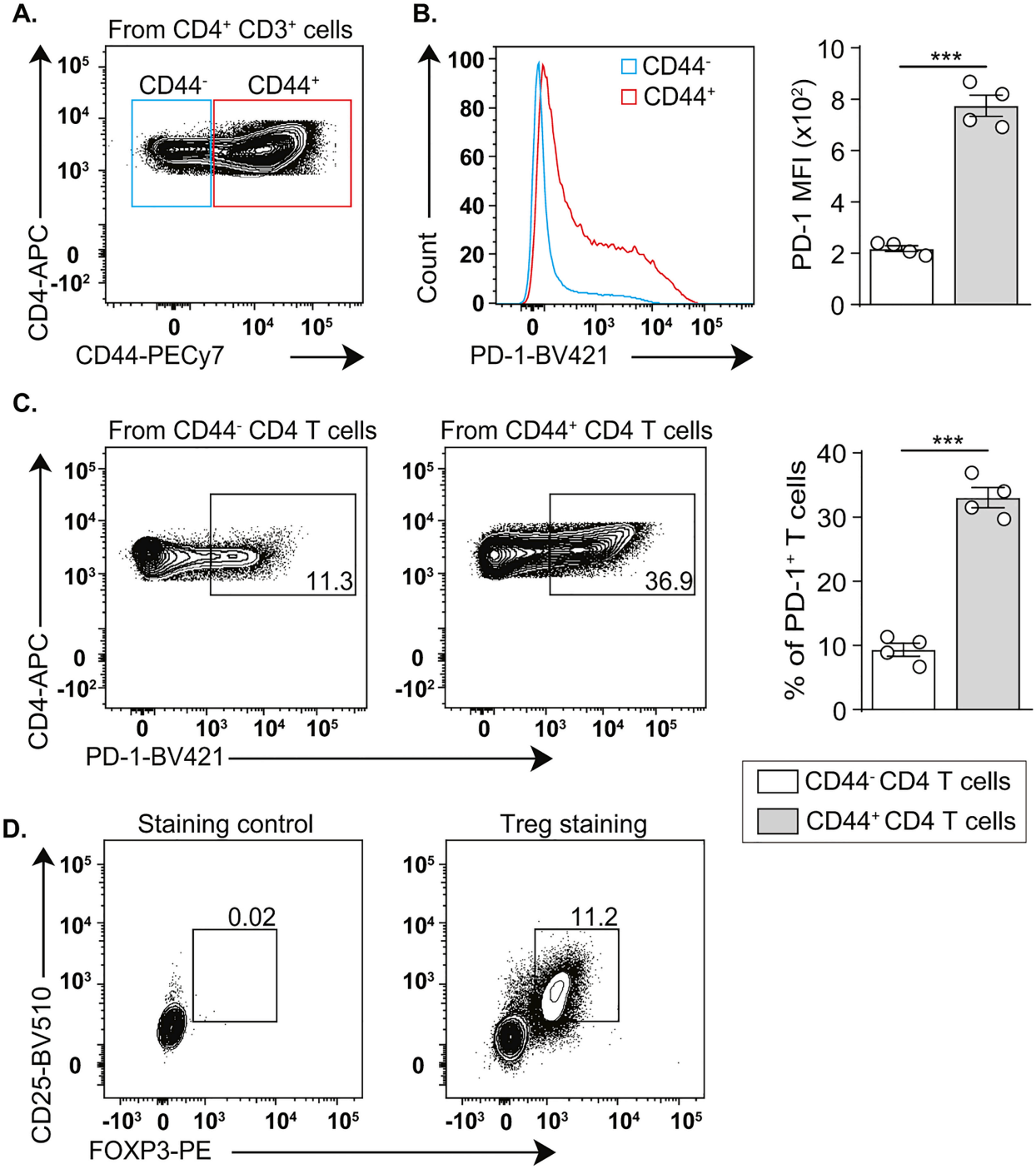

A.
From BAL single cells
CD45+ cells
FSC-H
CD45-BV605
From BAL CD45+ cells
Non neutrophils
Neutrophils
CD11b-PE/Dazzle
Ly6G-BV786
Eosinophils
SiglecF-APC
CD11c-PerCPCy5.5
Alveolar macrophages
CD64-PE
T cells
B cells
CD3-APC-Cy7
CD19-FITC
B.
From Lung CD45+ cells
Non neutrophils
Neutrophils
From non neutrophils
Macrophages
Alveolar macrophages
Interstitial macrophages
DCs
MHCII-APC-Cy7

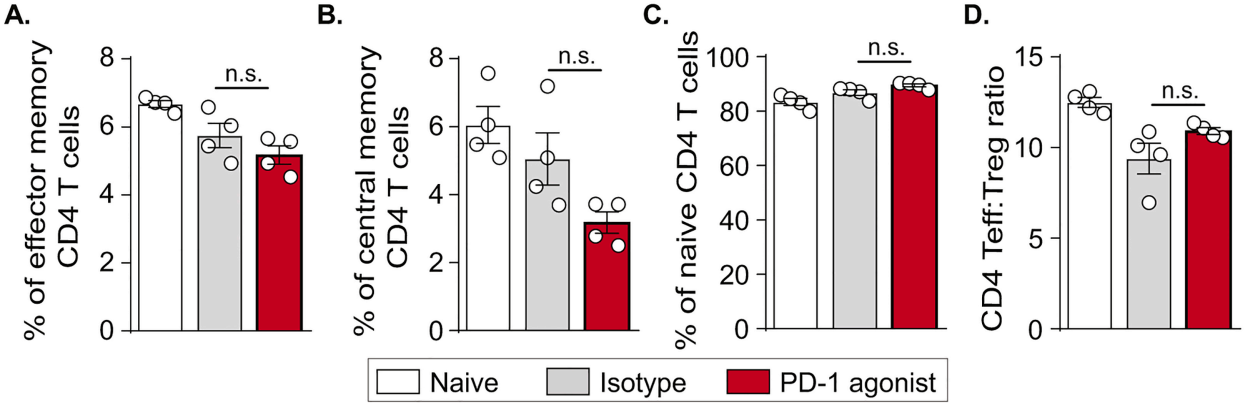

A.
B.
IL-5 (x10 pg/mL)
IL-13 (x10 pg/mL)
15
10
5
0
3
2
1
0
n.s.
n.s.
*
*
WT PBS
PD-1 KO PBS
WT HDM
PD-1 KO HDM


PBS
HDM
1.01
2.31
$10^5$
$10^4$
$10^3$
0
$-10^3$
0
$10^3$
$10^4$
$10^5$
CD45-BV655
PD-1-BV421


A.
B.
C.
D.
E.
F.
G.
IL-4 (x10² pg/mL)
IL-5 (x10 pg/mL)
IL-13 (x10 pg/mL)
IL-17A (x10² pg/mL)
G-CSF (x10 pg/mL)
GM-CSF (x10 pg/mL)
IL-10 (x10 pg/mL)
**
***
***
*
n.s.
n.s.
n.s.
Naive
Isotype
PD-1 agonist


A.
B.
C.
# lung eosinophils (x10^5)
# lung macro (x10^6)
# lung DCs (x10^5)
n.s.
**
*
0
5
10
15
20
1
2
3
4
Naive
Isotype
PD-1 agonist


A.
From CD4+ CD3+ cells
CD44-
CD44+
CD4-APC
CD44-PECy7
B.
CD44-
CD44+
Count
PD-1-BV421
PD-1 MFI (x10²)
***
C.
From CD44- CD4 T cells
From CD44+ CD4 T cells
11.3
36.9
CD4-APC
PD-1-BV421
% of PD-1+ T cells
***
CD44- CD4 T cells
CD44+ CD4 T cells
D.
Staining control
Treg staining
0.02
11.2
CD25-BV510
FOXP3-PE


A.
% of effector memory CD4 T cells
8
6
4
2
0
n.s.
B.
% of central memory CD4 T cells
8
6
4
2
0
n.s.
C.
% of naive CD4 T cells
100
80
60
40
20
0
n.s.
D.
CD4 Teff:Treg ratio
15
10
5
0
n.s.
Naive
Isotype
PD-1 agonist