Supplementary Information

Figure S1

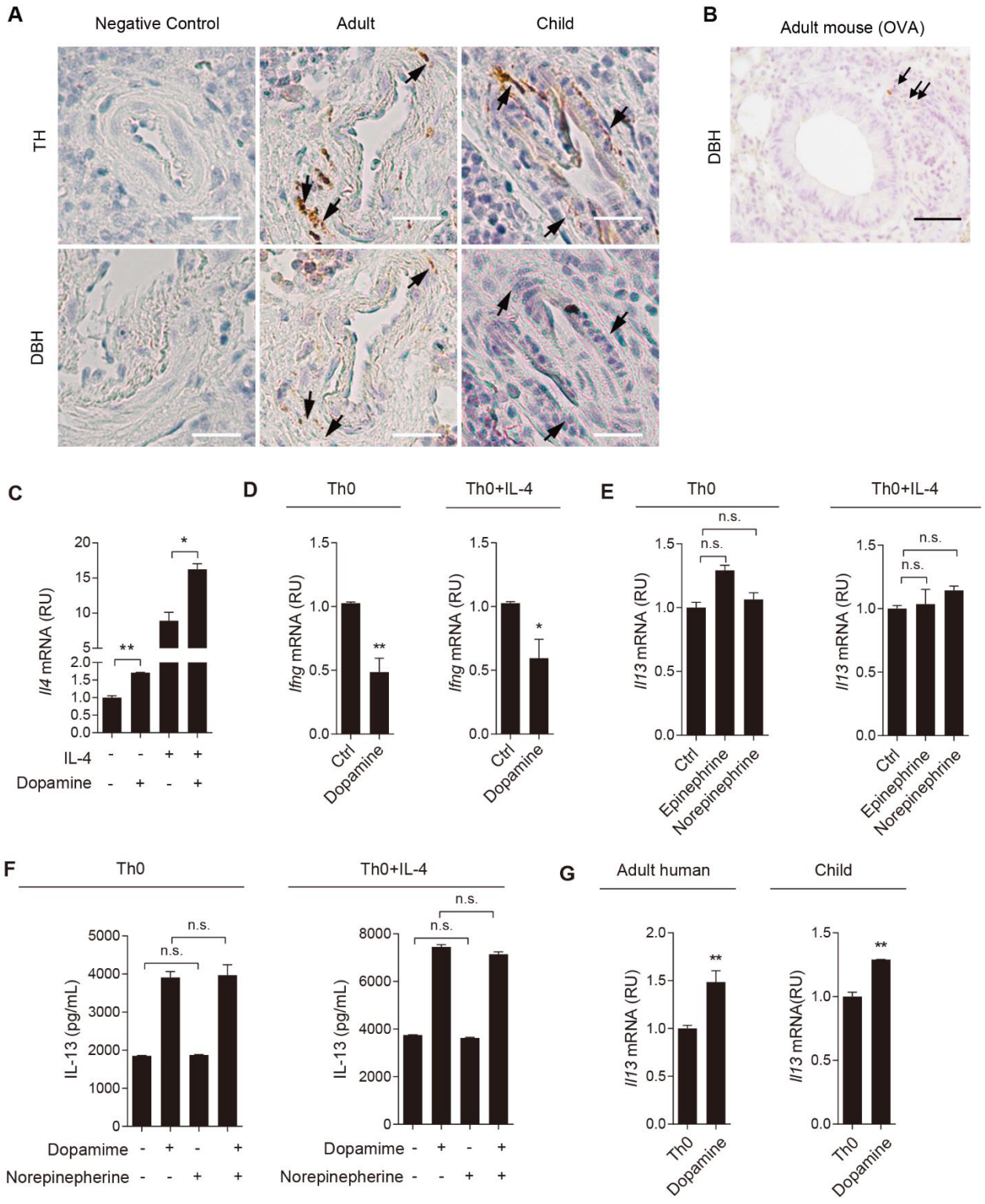

Figure S1. DBH is selectively expressed by nerves innervating human lymph nodes and mouse lungs; dopamine, but not norepinephrine and epinephrine, promotes a Th2 phenotype in CD4$^+$ T cells. Related to Figures 1 and 2. **(A)** Representative images of TH and DBH staining of adjacent tissue sections of

lymph nodes associated with donor lungs from adults (40-65 years of age) and children (0-13 years of age). Arrows point to TH^+ and DBH^+ nerves. Scale bars, 50 μm. Similar results were found in 3 individuals in each age group. (**B**) Representative DBH staining in the adult mouse lung following OVA exposure. Arrows mark DBH^+ labelled nerves. Scale bars, 50 μm. For (**C**-**G**), cultures were treated with neurotransmitters at a concentration of 10 μM and analyzed at day 4 for gene expression by qPCR and for IL-13 production by ELISA. (**C, D**) The expression of *Il4* and *Ifng* genes in mouse Th0 and Th0+IL-4 cultures with and without dopamine treatment. (**E**) *Il13* gene expression in mouse Th0 and Th0+IL-4 cultures with and without the treatment of epinephrine and norepinephrine. (**F)** IL-13 production in mouse Th0 and Th0+IL-4 cultures treated with dopamine and norepinephrine, alone and in combination. (**G**) *Il13* gene expression in human $CD4^+$ T cells in Th0 cultures with and without the treatment of dopamine. Naïve $CD4^+$ T cells were purified from the lymph nodes associated with donor lungs in adults (40-65 years of age) and children (0-13 years of age). For all the bar graphs, data present mean ± SEM of the results in triplicates in each experiment and from at least 3 independent experiments. *$p<0.05$; **$p<0.01$ by two-tailed student's t test. n.s., not significant.

Figure S2

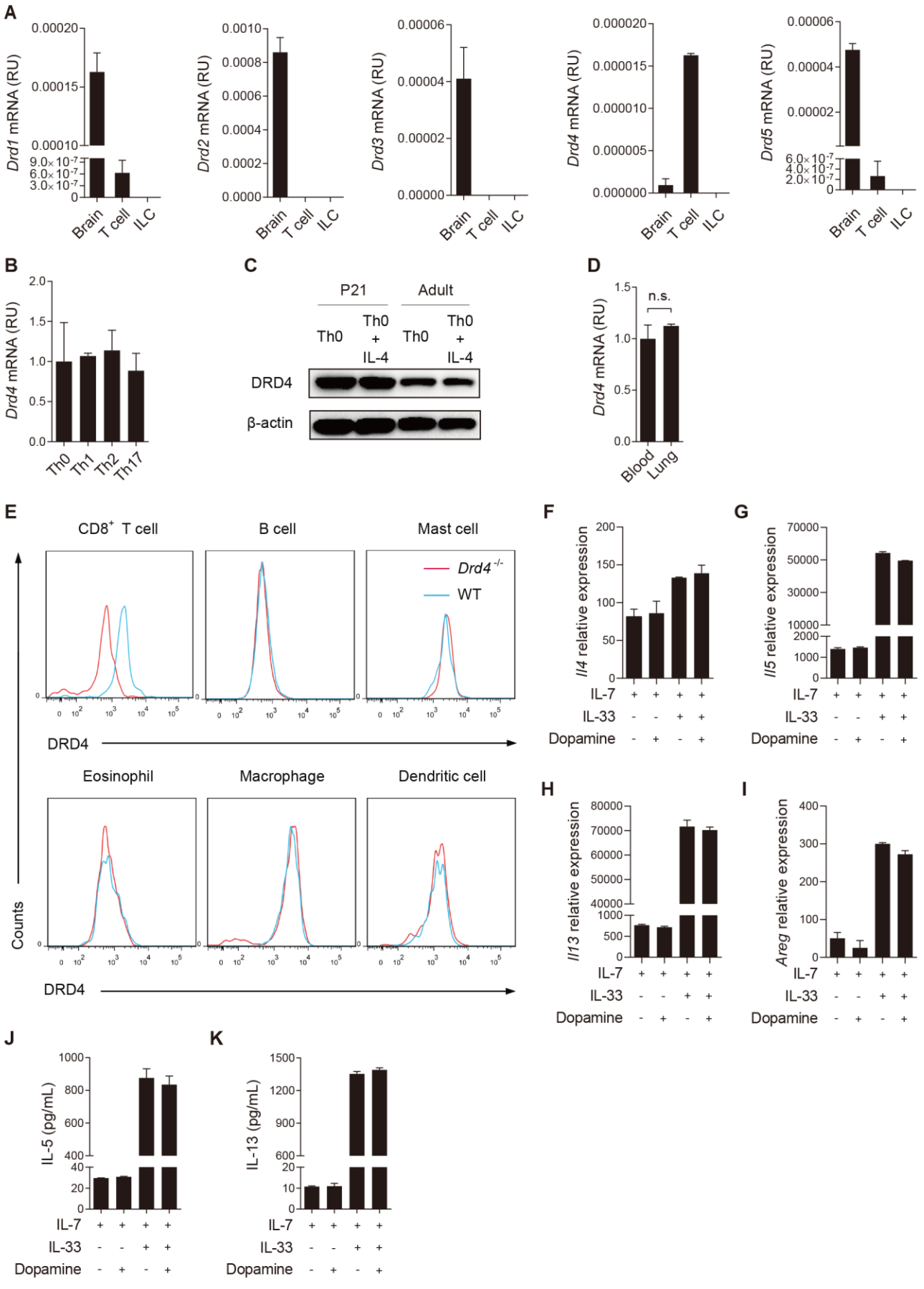
A
Drd1 mRNA (RU)
Drd2 mRNA (RU)
Drd3 mRNA (RU)
Drd4 mRNA (RU)
Drd5 mRNA (RU)
Brain
T cell
ILC
B
Drd4 mRNA (RU)
Th0
Th1
Th2
Th17
C
P21
Adult
Th0
Th0 + IL-4
DRD4
β-actin
D
Drd4 mRNA (RU)
n.s.
Blood
Lung
E
CD8+ T cell
B cell
Mast cell
Drd4 -/-
WT
DRD4
Eosinophil
Macrophage
Dendritic cell
Counts
F
Il4 relative expression
G
Il5 relative expression
H
Il13 relative expression
I
Areg relative expression
IL-7
IL-33
Dopamine
J
IL-5 (pg/mL)
K
IL-13 (pg/mL)

Figure S2. CD4$^+$ T cells and a subset of CD8$^+$ T cells, but no ILCs, express DRD4. Related to Figures 3 and 7. (**A**) qPCR analyses of *Drd1-Drd5* gene expression in purified naïve CD4$^+$ T cells from mouse spleen and ILCs from mouse lung. Mouse brain was positive control. For the analyses in T cells, the results represent mean ± SEM of 3 independent experiments. For the analyses of ILCs, the results represent mean ± SEM of 2 independent experiments. (**B**) *Drd4* gene expression in Th0, Th1, Th2 and Th17 cultures at day 4. Data represent mean ± SEM of 3 independent experiments. (**C**) DRD4 expression in Th0 and Th0+IL-4 cultures prepared from P21 and adult mice by Western blot. β-actin is loading control. Each lane represents one sample. Data represent the results in 3 independent experiments. (**D**) *Drd4* gene expression in CD4$^+$ T cells isolated from blood and lung-associated lymph nodes. Cells were isolated from samples pooled from 5 mice for each experiment. Data represent mean ± SEM of 2 independent experiments. (**E**) Flow cytometry analyses of DRD4 expression in CD8$^+$ T cells, B cells, mast cells, eosinophils, macrophages, dendritic cells. B cells were isolated from mouse spleens and all other immune cells were isolated from mouse lungs. Samples prepared from *Drd4*$^{-/-}$ mice were negative control for the specificity of the DRD4 antibody. Similar results were obtained from 3 independent experiments. (**F**-**K**) ILCs were isolated from 5 mouse lungs by cell sorting before cells were maintained in media with IL-7 (20 ng/mL), IL-7 + dopamine (10 μM), IL-7 + IL-33 (200 ng/mL) or IL-7 + IL-33 + dopamine. The expression of type 2 cytokine and *amphiregulin* (*Areg*) genes was analyzed at day 3 in culture. Data represent mean ± SEM of cultures in duplicates.

Figure S3

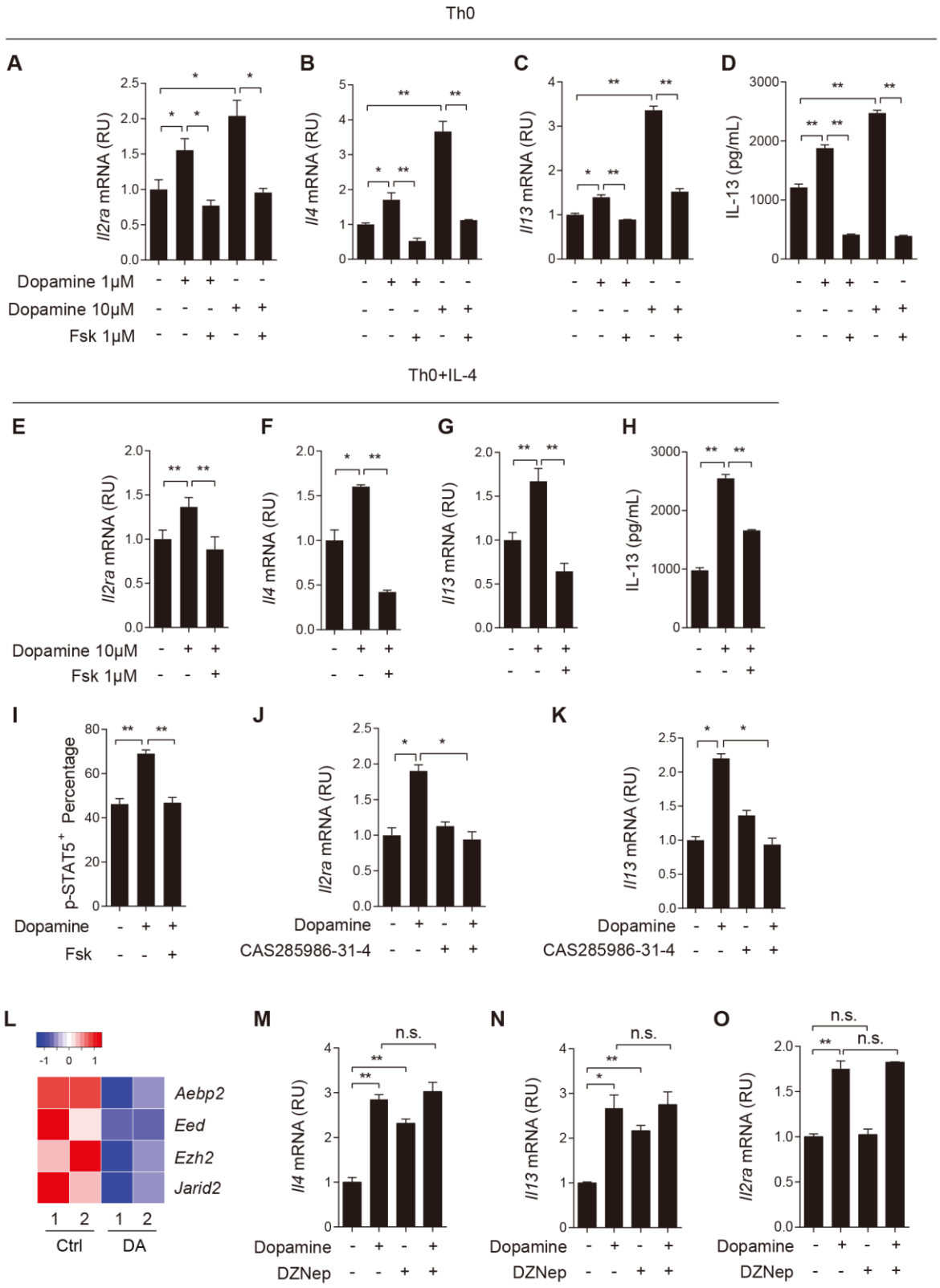

Figure S3. Dopamine signaling downregulates the cAMP and EZH2 pathways. Related to Figures 3 and 4. (**A-C**) Relative expression of *Il2ra*, *Il4 and Il13* genes in mouse Th0 cultures with and without dopamine and Fsk (1μM) treatment. (**D**) The production of IL-13 in each group was measured by ELISA. (**E-H**) *Il2ra*,

Il4 and Il13 gene expression and IL-13 production in Th0+IL-4 cultures with and without dopamine and Fsk treatment. (**I**) Quantification of the abundance of p-STAT5^{+} cells in Th0+IL-4 cultures treated with dopamine alone and a combination of dopamine and Fsk. Cultures were analyzed by flow cytometry. (**J**, **K**) Relative expression of *Il2ra* and *Il13* genes in Th0 cultures treated with dopamine and CAS285986-31-4 (20 μM), alone and together. (**L**) Heat map of differential gene expression of *Ezh2* and other PRC2 component genes in Th0 cultures treated with dopamine (DA). Data are from the results of RNA-seq. (**M-O**) Relative expression of *Il4, Il13 and Il2ra* genes in mouse Th0 cultures treated with dopamine and DZNep (7.5 nM), alone and in combination. Data were normalized to untreated baseline. All bar graphs represent mean ± SEM of 3 independent experiments. *$p<0.05$; **$p<0.01$ by two-tailed student's t test. n.s., not significant.

Figure S4

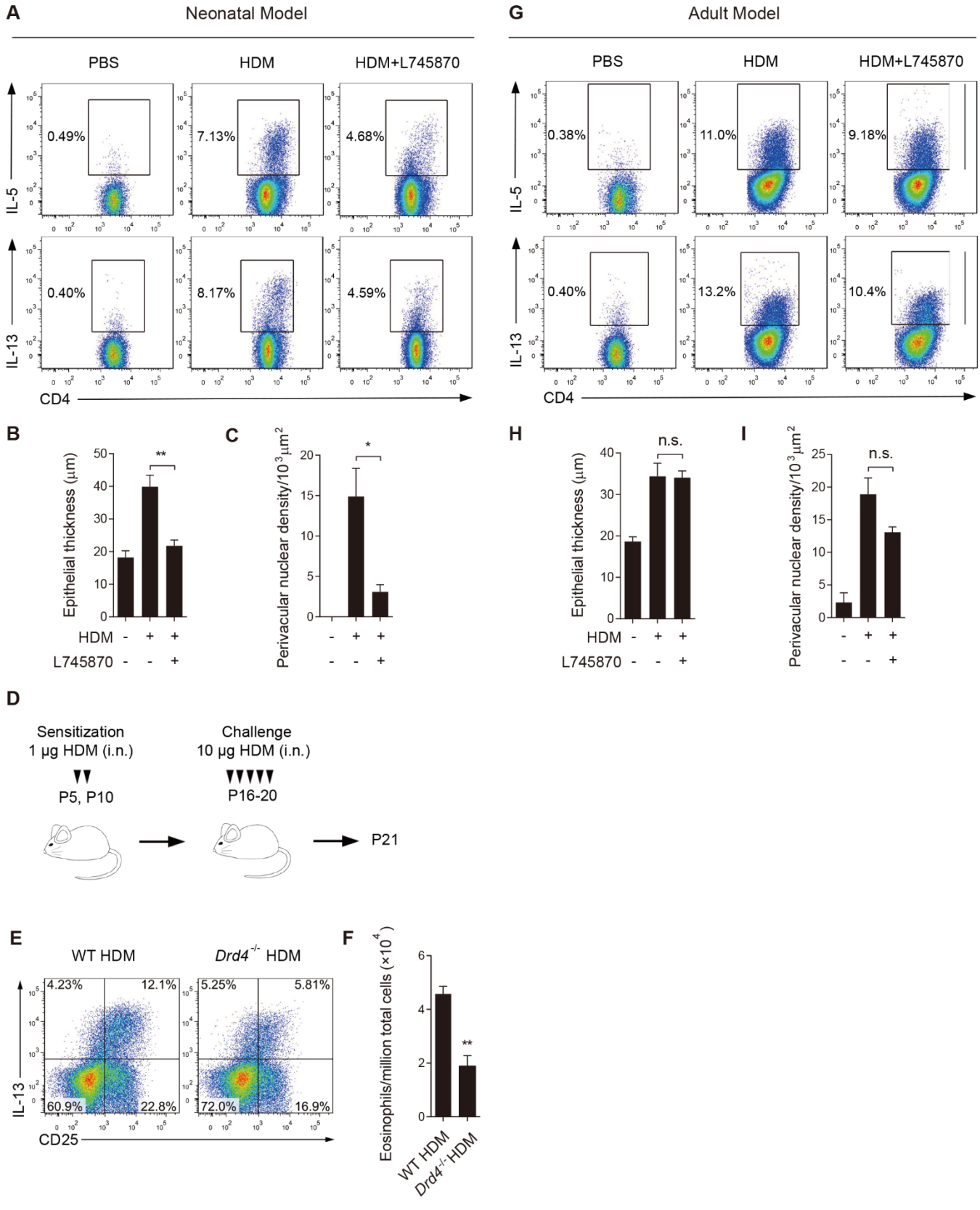

Figure S4. Functional disruption of DRD4 by L745870 and genetic deficiency ameliorates Th2 inflammation in neonatal mice following HDM exposure. Related to Figure 5. (**A**) Flow cytometry

analyses of IL-5^{+}CD4^{+} and IL-13^{+}CD4^{+} T cells in the lung following HDM exposure with and without L745870 treatment in neonates. Results were representative of 3 independent experiments. (**B**, **C**) Pathological measurements of epithelial thickness and peri-vascular nuclear density in each group. Data represent mean ± SEM of 3 non-overlapping histological areas in each mouse, 6 mice in each group from 3 independent experiments. (**D**) Experimental scheme of HDM exposure in WT and *Drd4*$^{-/-}$ neonatal mice. Mice were sacrificed 1 day after the last challenge. (**E**) Representative flow cytometry analyses of IL-13^{+}CD25^{+}CD4^{+} T cells in WT and *Drd4*$^{-/-}$ mice at P21. Similar results were obtained in 3 independent experiments. (**F**) The abundance of eosinophils in the lung of WT and *Drd4*$^{-/-}$ mice following HDM exposure at P21. Data represent mean ± SEM of 6 mice of each genotype in 3 independent experiment. (**G-I**) Assessment of Th2 inflammation in adult mice following HDM exposure by flow cytometry and pathological measurements as described in (**A**-**C**) for neonatal mice. N=5 adult mice from 2 independent experiments. *$p<0.05$; **$p<0.01$ by two-tailed student's t test. n.s., not significant.

Figure S5

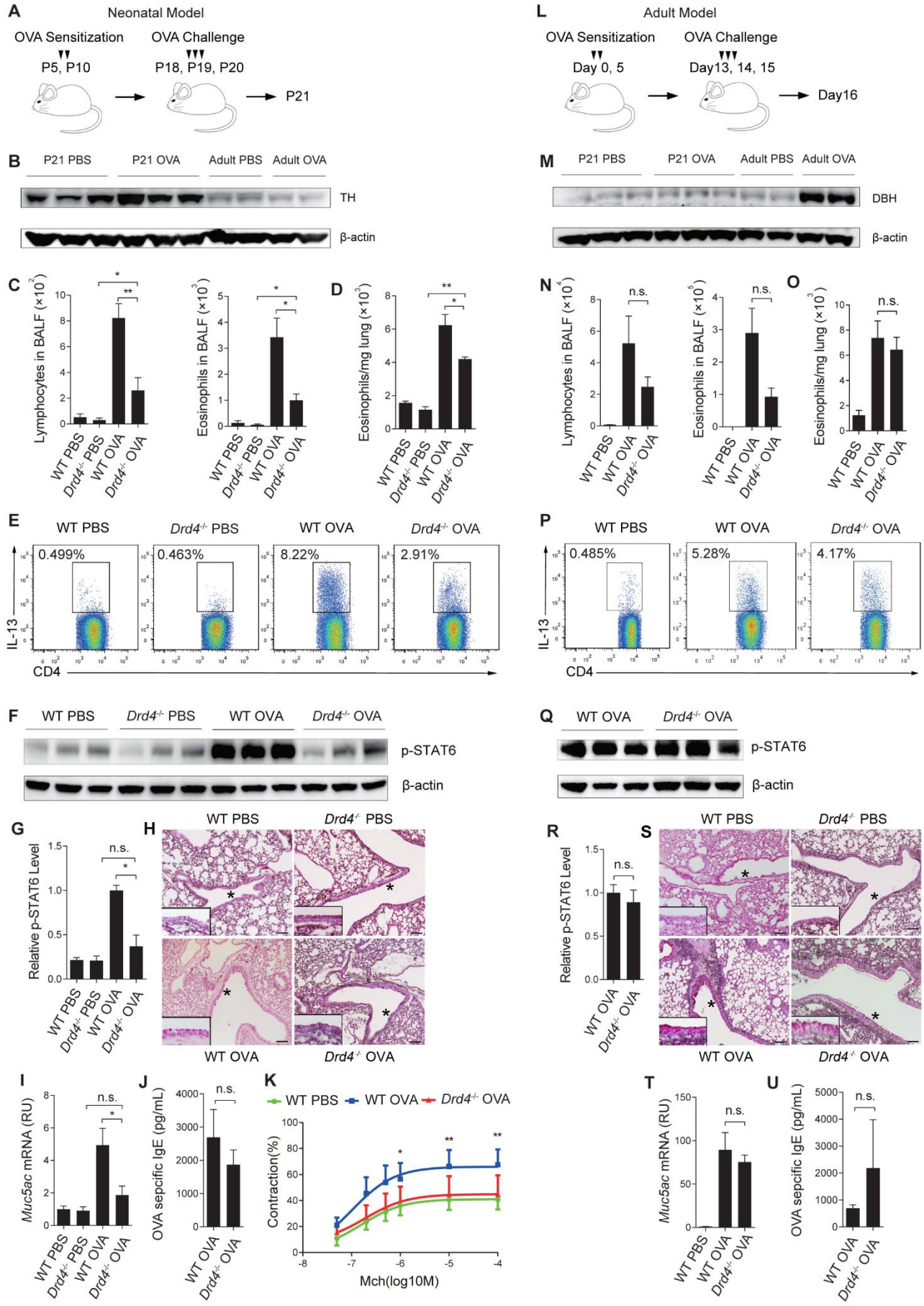
A
Neonatal Model
OVA Sensitization
P5, P10
OVA Challenge
P18, P19, P20
P21
L
Adult Model
OVA Sensitization
Day 0, 5
OVA Challenge
Day13, 14, 15
Day16
B
P21 PBS
P21 OVA
Adult PBS
Adult OVA
TH
β-actin
M
DBH
C
Lymphocytes in BALF (×10^2)
Eosinophils in BALF (×10^3)
D
Eosinophils/mg lung (×10^3)
WT PBS
Drd4-/- PBS
WT OVA
Drd4-/- OVA
N
Lymphocytes in BALF (×10^4)
Eosinophils in BALF (×10^5)
O
n.s.
E
0.499%
0.463%
8.22%
2.91%
IL-13
CD4
P
0.485%
5.28%
4.17%
F
p-STAT6
β-actin
Q
G
Relative p-STAT6 Level
H
R
S
I
Muc5ac mRNA (RU)
J
OVA sepcific IgE (pg/mL)
K
Contraction(%)
Mch(log10M)
T
U

Figure S5. DRD4 deficiency ameliorates Th2 inflammation in neonatal mice, but not in adult mice, following OVA exposure. Related to Figure 5. (**A** and **L)** Experimental scheme of OVA sensitization and challenge in neonatal mice (**A**) and adult mice (**L**). Mice were sacrificed 1 day after the last challenge. **(B** and **M)** The amount of tyrosine hydroxylase and DBH in saline and OVA-exposed lungs of WT neonatal and adult mice. β-actin was loading control. Each lane represents one sample. Similar results were obtained in 3 independent experiments. (**C**) Flow cytometry analyses of lymphocytes and eosinophils in BALF of WT and *DRD4*$^{-/-}$ mice following OVA exposure at P21. N=6 in 3 independent experiments. (**D**) The abundance of eosinophils in the lung of WT and *Drd4*$^{-/-}$ mice at P21. Results were normalized to lung weight. N=5 in 3 independent experiments. (**E**) Representative cytometry results of IL-13^{+}CD4^{+} T cells in P21 lungs of each group. Similar results were obtained from 3 independent experiments. (**F**) Western blot analyses of p-STAT6 in the lung of WT and *Drd4*$^{-/-}$ mice at P21. β-actin was loading control. Each lane represents one sample. Similar results were obtained in 3 independent experiments. Data were quantified in **(G**). (**H**) Representative images of PAS staining of tissue sections in P21 lungs from each group. The area of the airway epithelium marked by * is enlarged and shown by the insert. Similar results were obtained in 3 independent experiments. Scale bars, 100 μm. (**I**) Mucus production in WT and *Drd4*$^{-/-}$ P21 lungs assayed by *Muc5ac* qPCR. N=5 for P21 *Drd4*$^{-/-}$ PBS group and N=10-12 for other groups in 3 independent experiments. (**J**) The amount of OVA-specific IgE in serum of WT and *Drd4*$^{-/-}$ mice at P21. N=12 in 3 independent experiments. (**K**) Assessment of airway hypercontractility following OVA exposure in WT and *Drd4*$^{-/-}$ mice at P21 using precision cut lung slices. Airway contractility in response to methacholine was measured by the percentage of reduction in the luminal size from the baseline. N=15-20 airways from 3 mice of each group in 2 independent experiments. (**N**-**U**) Assessment of Th2 inflammation in adult, WT and *Drd4*$^{-/-}$ mice following OVA exposure using flow cytometry, immunological and pathological assays as described for neonatal mice in (**C**-**J)**. N=5-7 for adult WT groups and N=5 for *Drd4*$^{-/-}$ group in 3 independent experiments. For the results in (**K**), data were analyzed by one-way ANOVA for multiple comparisons between treatment and methacholine dosage. For all other panels, data were analyzed by two-tailed student's t test. *$p<0.05$; **$p<0.01$; n.s., not significant.

We noted significant variations in OVA-specific IgE in the neonatal OVA models in (**J**). The variation may contribute to the statistically insignificant difference in OVA-specific IgE between WT and *Drd4*$^{-/-}$ mice.

Figure S6

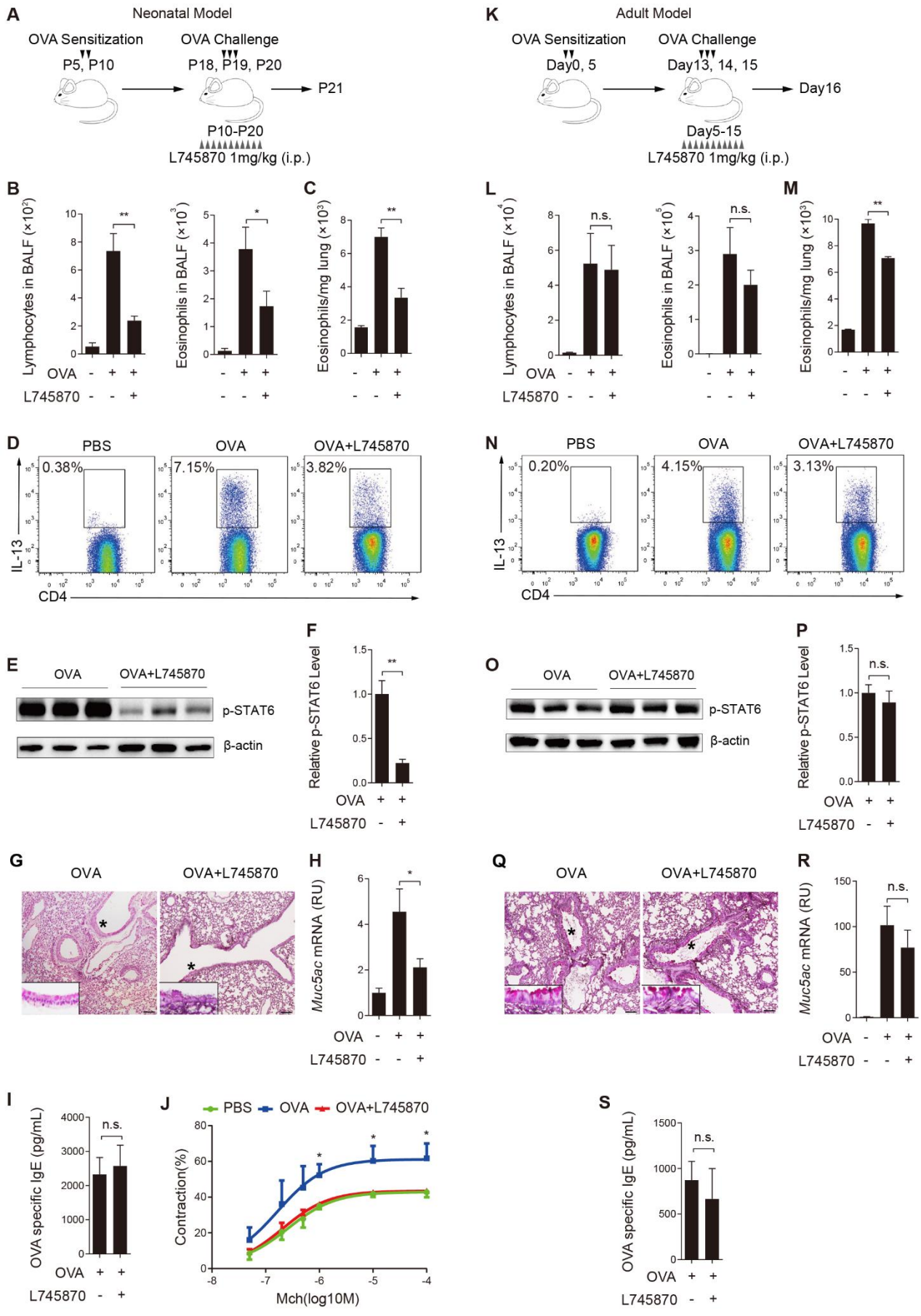
A
Neonatal Model
OVA Sensitization
P5, P10
OVA Challenge
P18, P19, P20
P21
P10-P20
L745870 1mg/kg (i.p.)
K
Adult Model
OVA Sensitization
Day0, 5
OVA Challenge
Day13, 14, 15
Day16
Day5-15
L745870 1mg/kg (i.p.)
B
Lymphocytes in BALF (×10²)
Eosinophils in BALF (×10³)
C
Eosinophils/mg lung (×10³)
L
Lymphocytes in BALF (×10⁴)
n.s.
Eosinophils in BALF (×10⁵)
n.s.
M
Eosinophils/mg lung (×10³)
OVA
L745870
D
PBS
OVA
OVA+L745870
0.38%
7.15%
3.82%
IL-13
CD4
N
PBS
OVA
OVA+L745870
0.20%
4.15%
3.13%
IL-13
CD4
E
OVA
OVA+L745870
p-STAT6
β-actin
F
Relative p-STAT6 Level
O
OVA
OVA+L745870
p-STAT6
β-actin
P
Relative p-STAT6 Level
n.s.
G
OVA
OVA+L745870
H
Muc5ac mRNA (RU)
Q
OVA
OVA+L745870
R
Muc5ac mRNA (RU)
n.s.
I
OVA specific IgE (pg/mL)
n.s.
J
PBS
OVA
OVA+L745870
Contraction(%)
Mch(log10M)
S
OVA specific IgE (pg/mL)
n.s.

Figure S6. Pharmaceutical blockade of DRD4 signaling ameliorates Th2 inflammation in neonatal mice, but not adult mice, following OVA exposure. Related to Figure 5. (**A**) Experimental scheme of L745870 treatment during OVA sensitization and challenge in neonatal mice. L745870 was given intra-peritoneally at 1 mg/kg body weight daily between P10-P20. Mice were analyzed one day after the last challenge at P21. (**B**) Flow cytometry analyses of lymphocytes and eosinophils in BALF of each group at P21. N=5 for P21 PBS group, N=7 for P21 OVA group and N=7 for P21 OVA+L745870 group in 3 independent experiments. (**C**) The abundance of eosinophils in the lung of each group at P21. Results were normalized to lung weight. N=5 for P21 PBS group, N=7 for P21 OVA group and N=7 for P21 OVA+L745870 group in 3 independent experiments. (**D**) Representative flow cytometry analyses of IL-$13^{+}CD4^{+}$ T cells in P21 lungs of each group. Similar results were obtained from 3 independent experiments. (**E**, **F**) Quantification of p-STAT6 in P21 mouse lungs with and without L745870 treatment by Western blot. β-actin was loading control. Each lane represents one sample. Similar results were obtained from 5-7 mice in 3 independent experiments. (**G**, **H**) Mucus production in different experimental groups assayed by PAS staining and *Muc5ac* qPCR. The area of the airway epithelium marked by * is enlarged and shown in the insert in (**G**). N=9 in 3 independent experiments. Scale bars, 100 μm. (**I**) The amount of OVA-specific IgE in serum from mice at P21 in each group. N=17 in the OVA group and N=12 in OVA+L745870 group from 3 independent experiments. (**J**) Assessment of airway hypercontractility in OVA-exposed mice with and without L745870 treatment at P21 using precision cut lung slices. Airway contractility in response to methacholine was measured by the percentage of reduction in the luminal size from the baseline. N=15-20 airways from 3 mice in 2 independent experiments for each group. (**K**) Experimental scheme of L745870 (1 mg/kg body weight) treatment during OVA sensitization and challenge in adult mice. Adult lungs were analyzed one day after the last challenge at experimental day 16. (**L**-**S**) Assessment of Th2 inflammation in adult mice with and without L745870 treatment following OVA exposure using similar assays as described in (**B**-**I**) for neonatal mice. For BALF assays in (**L**), N=7 for each group in 3 independent experiments. For assays in (**M**, **N**), N=5-6 mice in 3 independent experiments. For results in (**O**, **P**), N=3 in 2 independent experiments. For results in (**Q**, **R**), N=8 in 3 independent experiments. For results in (**S**), N=6 in 3 independent experiments. For airway contractility measurement in (**J**), data were analyzed by one-way ANOVA for multiple comparisons between treatment and methacholine dosage. For all other results, data were analyzed by two-tailed student's t test. *$p<0.05$; **$p<0.01$; n.s., not significant.

We noted significant variations in OVA-specific IgE in the neonatal OVA models in (**I**). The variation may explain why the amount of OVA-specific IgE was not statistically significant between WT and *Drd4*$^{-/-}$ mice at P21, although Th2 inflammation was diminished. For panel (**M**), the reduction in eosinophilic infiltrates in the adult lung reached statistical significance in L745870-treated model. However, in other adult exposure

experiments (Figures 5**K** and S5**O**), there was a consistent trend of mild reduction in the number of eosinophils by DRD4 deficiency without reaching statistical significance. The discrepancy may be contributed by technical variations among multiple repeats of the experiment. It is important to note that comparing neonatal and adult mice (Figures S6**C** and S6**M**), L745870 treatment caused a greater reduction in the number of eosinophils in neonates than adults. These findings support our conclusion that the dopamine-DRD4 pathway contributes significantly to the Th2 inflammation in the early lung.

Figure S7

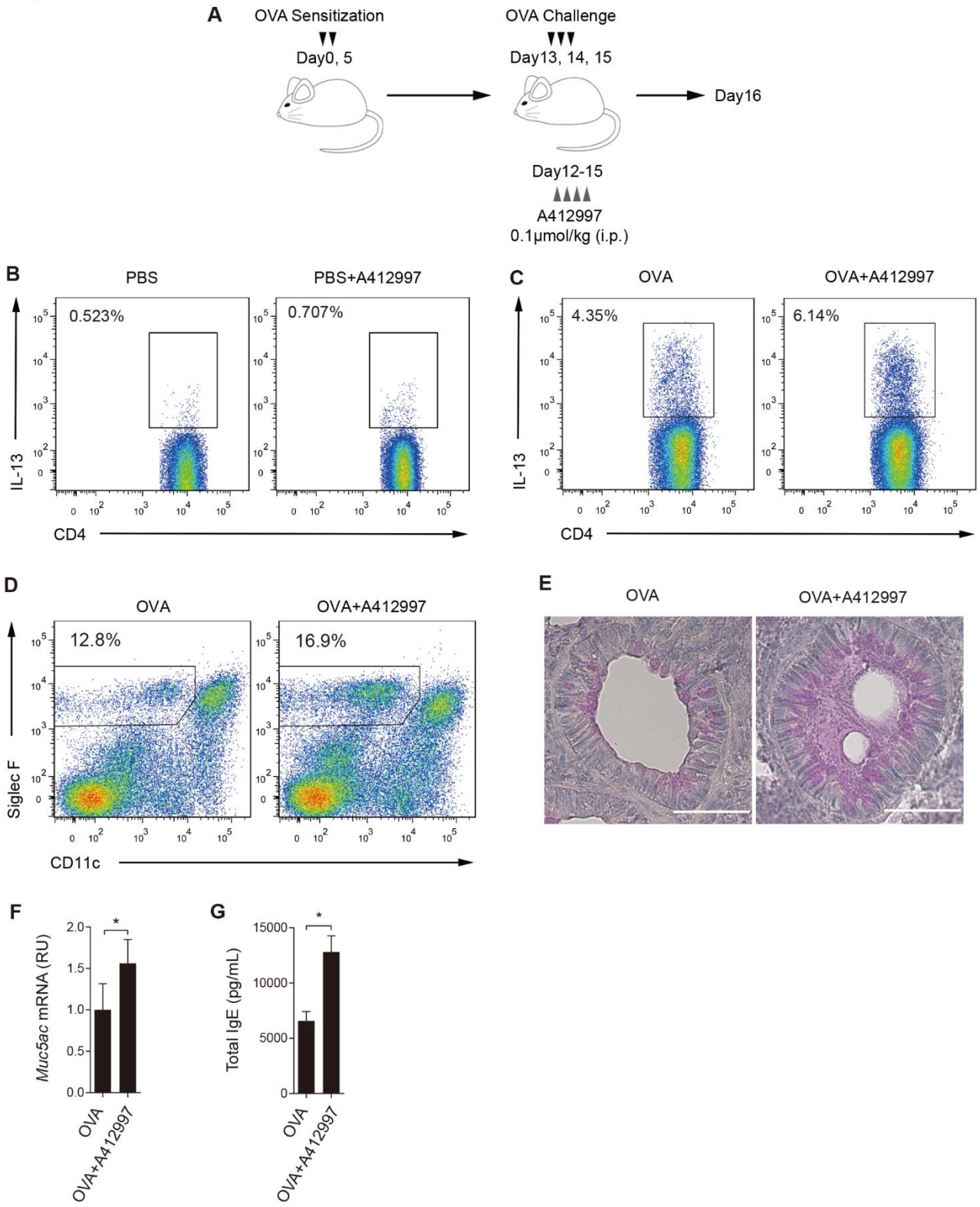

Figure S7. Activation of DRD4 in adult mice by an agonist A412997 worsens Th2 inflammation following OVA exposure. Related to Figure 6. (**A**) Experimental scheme of A412997 treatment during OVA sensitization and challenge in adult mice. A412997 was given intraperitoneally to adult mice between

experimental day 12 -15. A412997 was given once per day at a dose of 1 μmol/kg body weight. Mice were analyzed one day after the last challenge on experimental day 16. (**B**-**D**) Representative flow cytometry analyses of IL-13^{+}CD4^{+} T cells and eosinophils in the lung of saline- and OVA-exposed mice with and without A412997 treatment. Similar results were obtained from 3 independent experiments. (**E, F**) Mucus overproduction assayed by PAS staining and *Muc5ac* gene expression in OVA-exposed mice with and without A412997 treatment. N=6 in 3 independent experiments. Scale bars, 50 μm. (**G**) The amount of total IgE in serum of OVA-exposed mice with and without A412997 treatment. N=6 in 3 independent experiments. *$p<0.05$ by two-tailed student's t test.