## Supplemental Methods

### Intracellular staining of Th2 cytokines in lung cells

To identify the cellular sources of Th2 cytokines in the airways, lung cells were isolated as previously described, by collagenase digestion (64). Lung leukocytes were purified by 35% Percoll gradient centrifugation and counted. After 6 hours of stimulation with PMA/ionomycin/brefeldin A, cells were examined for intracellular cytokine staining after cell-surface staining with allophycocyanin-conjugated anti-CD4 mAb (eBioscience), followed by fixation with 4% paraformaldehyde and permeabilization with 0.1% saponin buffer.

Phycoerythrin-conjugated anti-IL-4, anti-IL-5 or anti-IL-13 mAbs (eBioscience) were used for intracellular cytokine staining and flow cytometry data using a FACSCalibur were analyzed with FlowJo software (TreeStar, Ashland, OR).

### Eosinophils numbers and peroxidase content in peripheral blood from WT and IL-10$^{-/-}$ mice

To determine whether numbers of eosinophils in blood of IL-10$^{-/-}$ mice differed from those of WT mice, peripheral blood was collected with some modification of methods described previously (65). Briefly, 100 μl of peripheral blood was collected into 1 mL of ice-cold 1X-PBS containing 2% FCS and 20 U/mL of heparin. The tubes were centrifuged and cell pellets were collected. Pellets were suspended in 1 mL of ice-cold distilled water for 1 minute to lyse red blood cells (RBC). After addition of 10X PBS to stop RBC lysis, tubes were centrifuged to collect white blood cells. Cytospin slides were made and stained with Wright-Giemsa to

differentiate cell composition. The levels of eosinophil peroxidase (EPO) in the cell pellets were measured by ELISA as previously described (66).

**Lung tissue distribution of eosinophils following bone marrow reconstitution**

To identify eosinophils in the lung tissue of PHIL mice following bone marrow cell transfer, immunohistochemistry with anti-mouse major basic protein (MBP) monoclonal antibody was performed. Lung tissue sections from mice sensitized and challenged to OVA 7 times. Compared were WT or PHIL mice following no treatment or PHIL recipients of bone marrow cells from WT or IL-$10^{-/-}$ mice. Sections were analyzed as described previously (67). Images of stained lung tissue sections were captured by digital camera and the numbers of MBP-positive cells around airways were quantified with NIH Image J software.

**Generation of bone marrow-derived eosinophils and determination of IL-10 production**

To determine the capability of IL-10 production by eosinophils under different conditions, eosinophils were generated from bone marrow cells and examined *in vitro*. Bone marrow cells were isolated from femurs and tibias of WT and IL-$10^{-/-}$ mice, and cultured with recombinant mouse stem cell factor and Flt3-ligand (both from PeproTech, Rocky Hill, NJ) followed by culture with recombinant mouse IL-5 (PeproTech) according to the methods described by Dyer KD et al. (68). At the end of the culture period, cells were differentiated by Wright-Giemsa staining on cytospin slides; eosinophils comprised more than 90% of the cells. The cells were then cultured in complete RPMI 1640 medium ($4x10^6$ cells/ml) and stimulated

with PMA/ionomycin/brefeldin A for 24 hrs. Supernatants were collected and levels of IL-10 determined by ELISA (eBioscience). EPO levels in bone marrow-derived eosinophils were measured by ELISA as described above.

## Supplemental Figure Legends

**Figure E1**. Numbers of IL-4-, IL-5-, or IL-13-positive $CD4^+$ T cells in lungs. To identify the source of Th2 cytokines in sensitized and 7 times OVA challenged WT or PHIL mice, intracellular cytokine staining was carried out. WT mice which received sham sensitization followed by 7 times OVA challenge served as controls. Isolated lung leukocytes were activated with PMA/ionomycin and then stained with anti-CD4 followed by intracellular cytokine staining. Numbers of IL-4-, IL-5-, or IL-13-positive $CD4^+$ T cells in lungs were calculated and expressed as histograms. n=5. *$p<0.05$ vs. WT PBS/OVA-7 group.

**Figure E2**. Circulating eosinophil numbers in WT and IL-$10^{-/-}$ mice were shown to be similar by either (A) cell differential assessment of white blood cells following Wright's-Giemsa staining or by (B) ELISA assessments of eosinophil peroxidase levels (arbitrary EPX units/1x$10^6$ of eosinophils) in unfractionated white blood cell pellets (n=5 mice/group).

**Figure E3**. Localization of eosinophils in the lung tissue following bone marraw cell transfer. Lung tissues were stained with anti-mouse major basic protein (MBP) antibody. (A) Representative photomicrographs of lung tissue from (a) vehicle-treated WT mice after sensitization and 7 OVA challenges, (b) vehicle-treated PHIL mice after sensitization and 7 OVA challenges, (c) PHIL mice which received bone marrow cells from WT mice after sensitization and 7 OVA challenges, (d) PHIL mice which received bone marrow cells from IL-$10^{-/-}$ mice after sensitization and 7 OVA challenges, and (B) numbers of MBP-positive cells. n=6. *$p<0.05$ vs. PHIL OVA/OVA-7+vehicle group.

**Figure E4**. Characterization of bone marrow-derived eosinophils.. Following stimulation with PMA/ionomycin, levels of (A) IL-10 (ng/mL), or (B) eosinophil peroxidase (arbitrary EPX units/mL) in cell culture supernatants from eosinophils derived from WT and IL-$10^{-/-}$ mice were measured by ELISA. (n=5 mice/group). *$p<0.05$

**Figure E1**

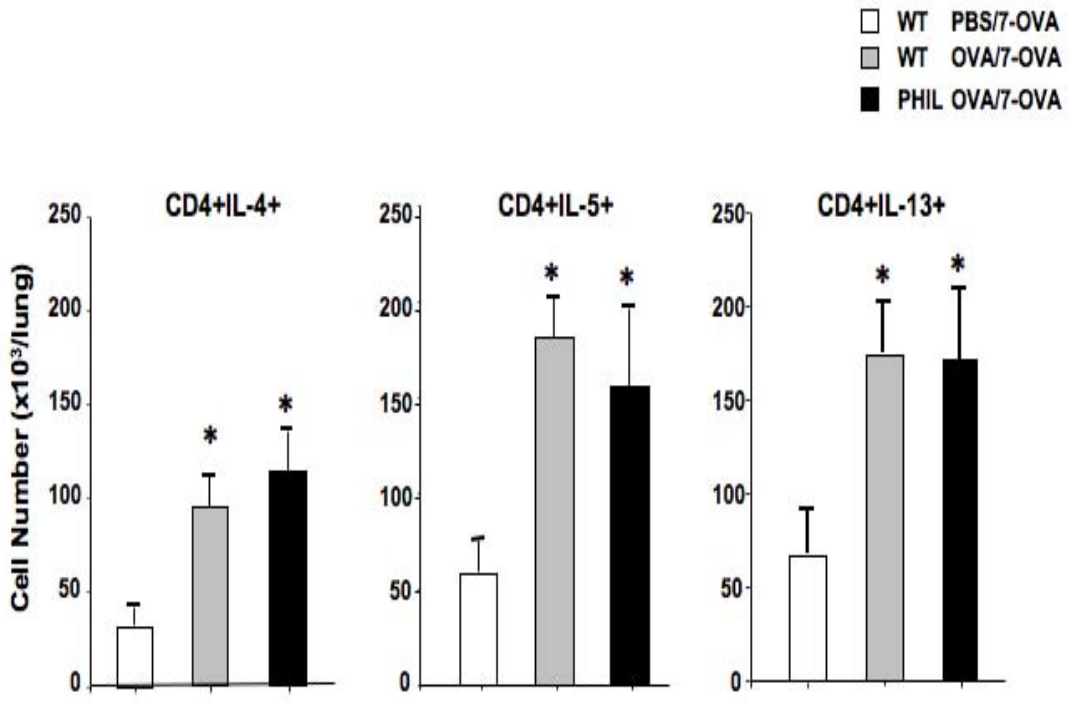
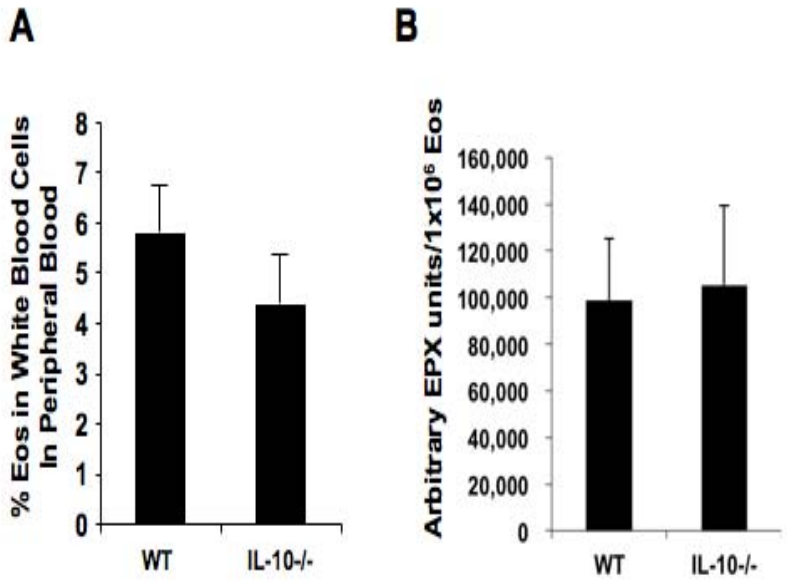

WT PBS/7-OVA
WT OVA/7-OVA
PHIL OVA/7-OVA
CD4+IL-4+
CD4+IL-5+
CD4+IL-13+
Cell Number (x10³/lung)
250
200
150
100
50
0
*

Figure E2


A
% Eos in White Blood Cells In Peripheral Blood
8
7
6
5
4
3
2
1
0
WT
IL-10-/-
B
Arbitrary EPX units/1x10⁶ Eos
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
WT
IL-10-/-

# Figure E3

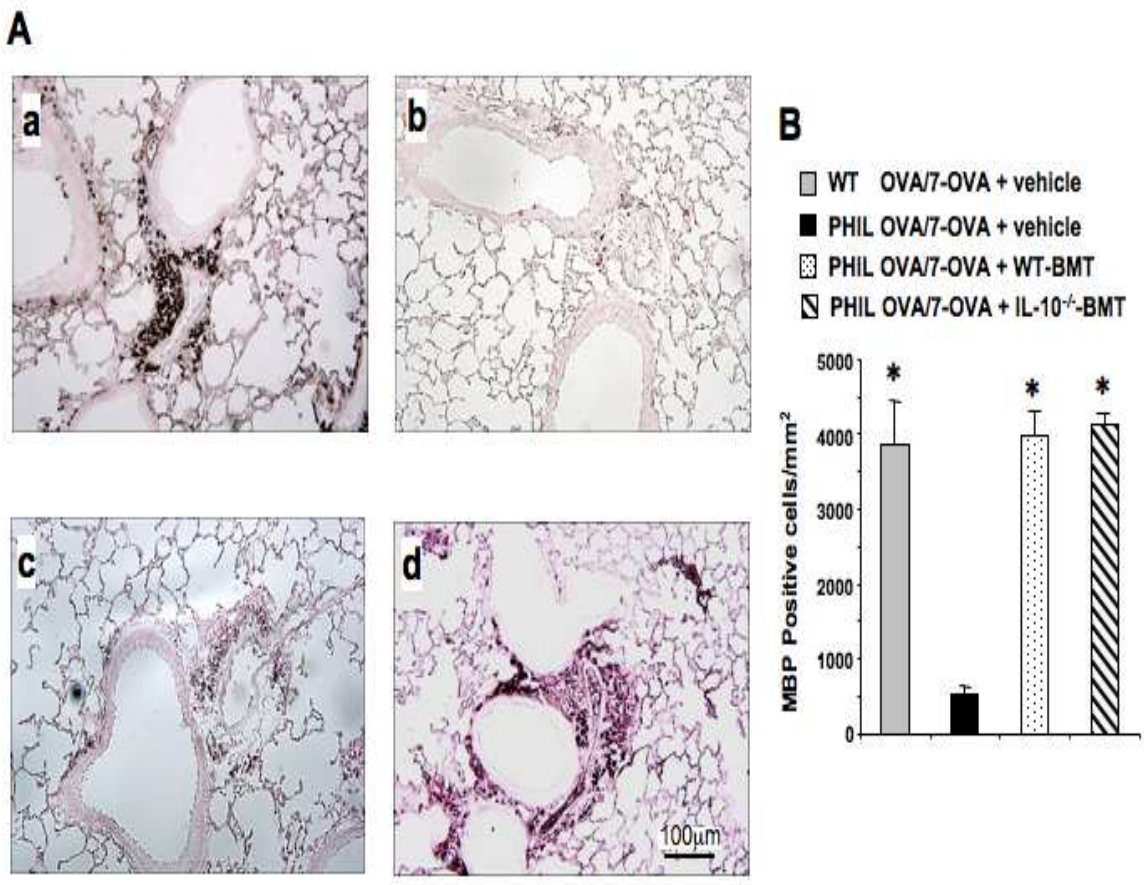
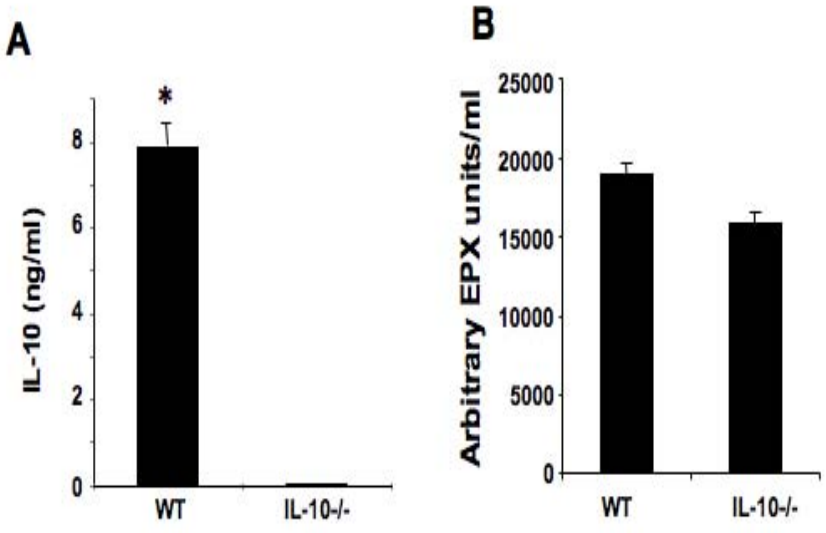

A
a
b
c
d
100μm
B
WT OVA/7-OVA + vehicle
PHIL OVA/7-OVA + vehicle
PHIL OVA/7-OVA + WT-BMT
PHIL OVA/7-OVA + IL-10-/--BMT
MBP Positive cells/mm2
5000
4000
3000
2000
1000
0
*
*
*

**Figure E4**


A
*
IL-10 (ng/ml)
8
6
4
2
0
WT
IL-10-/-
B
Arbitrary EPX units/ml
25000
20000
15000
10000
5000
0
WT
IL-10-/-