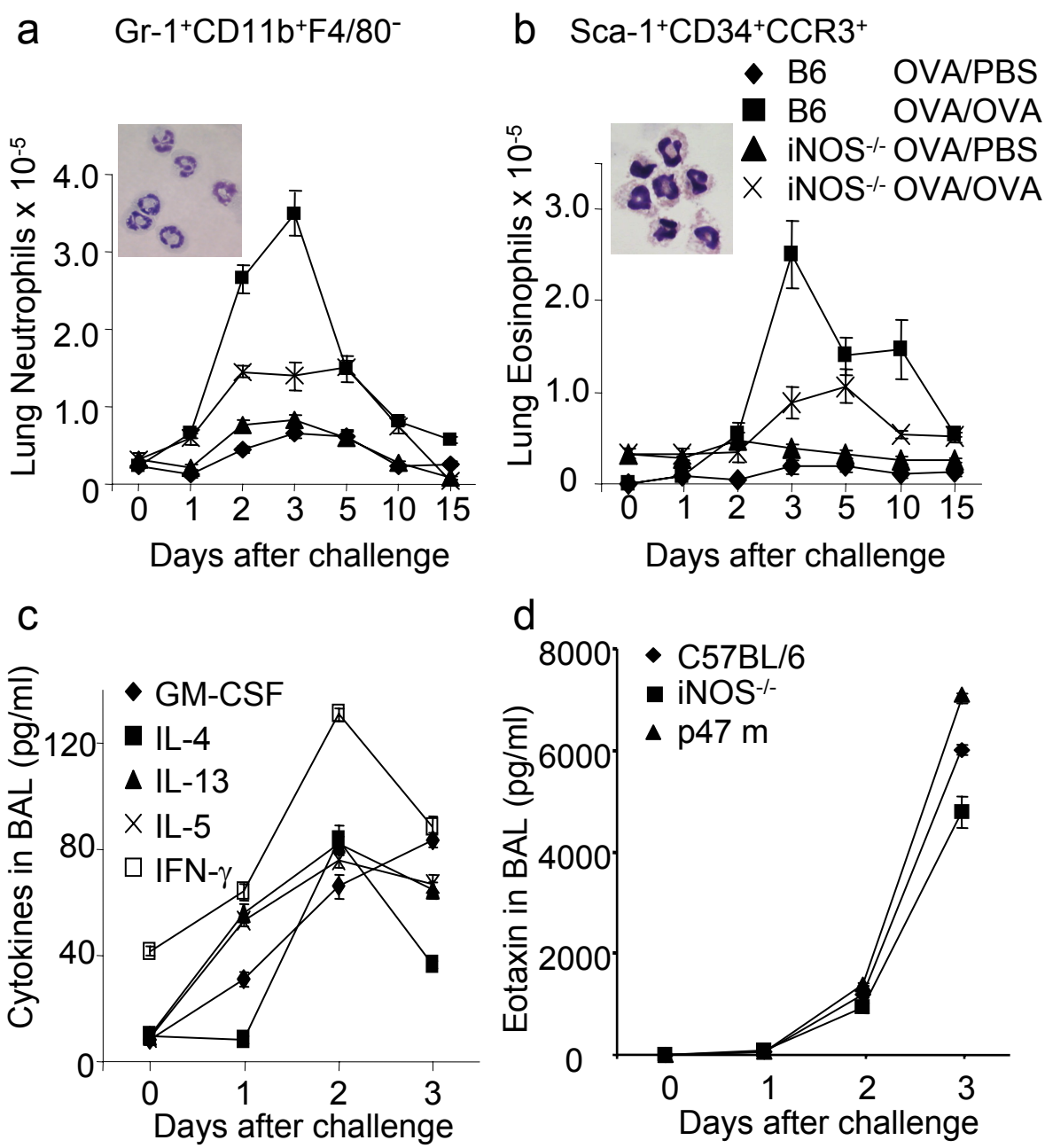
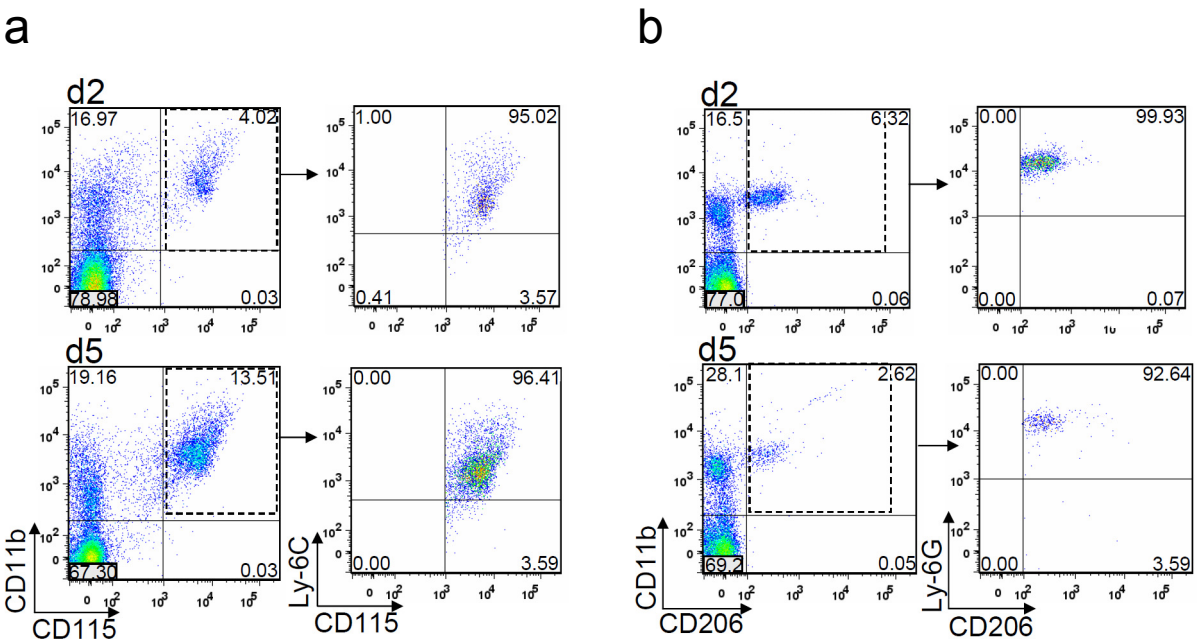
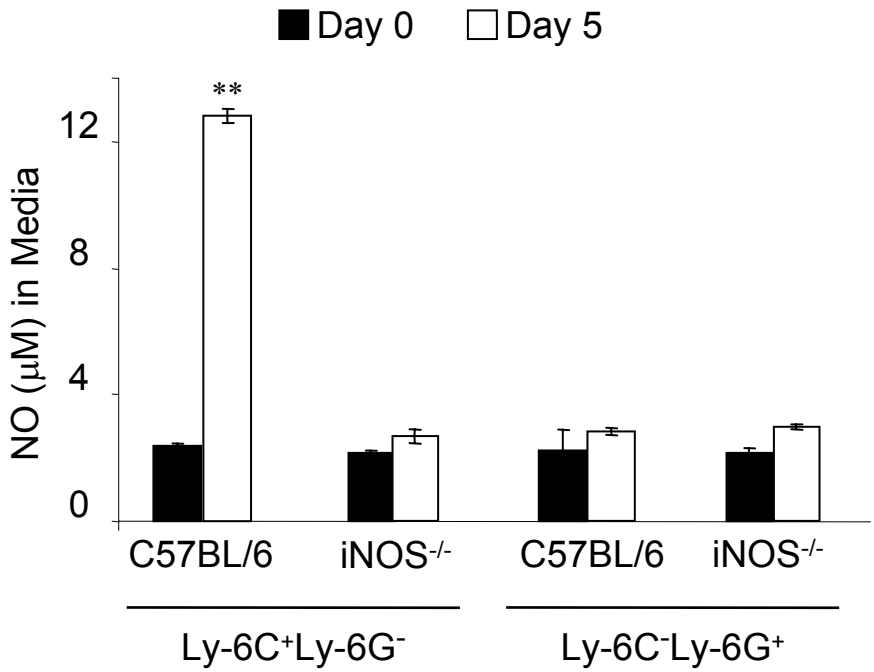


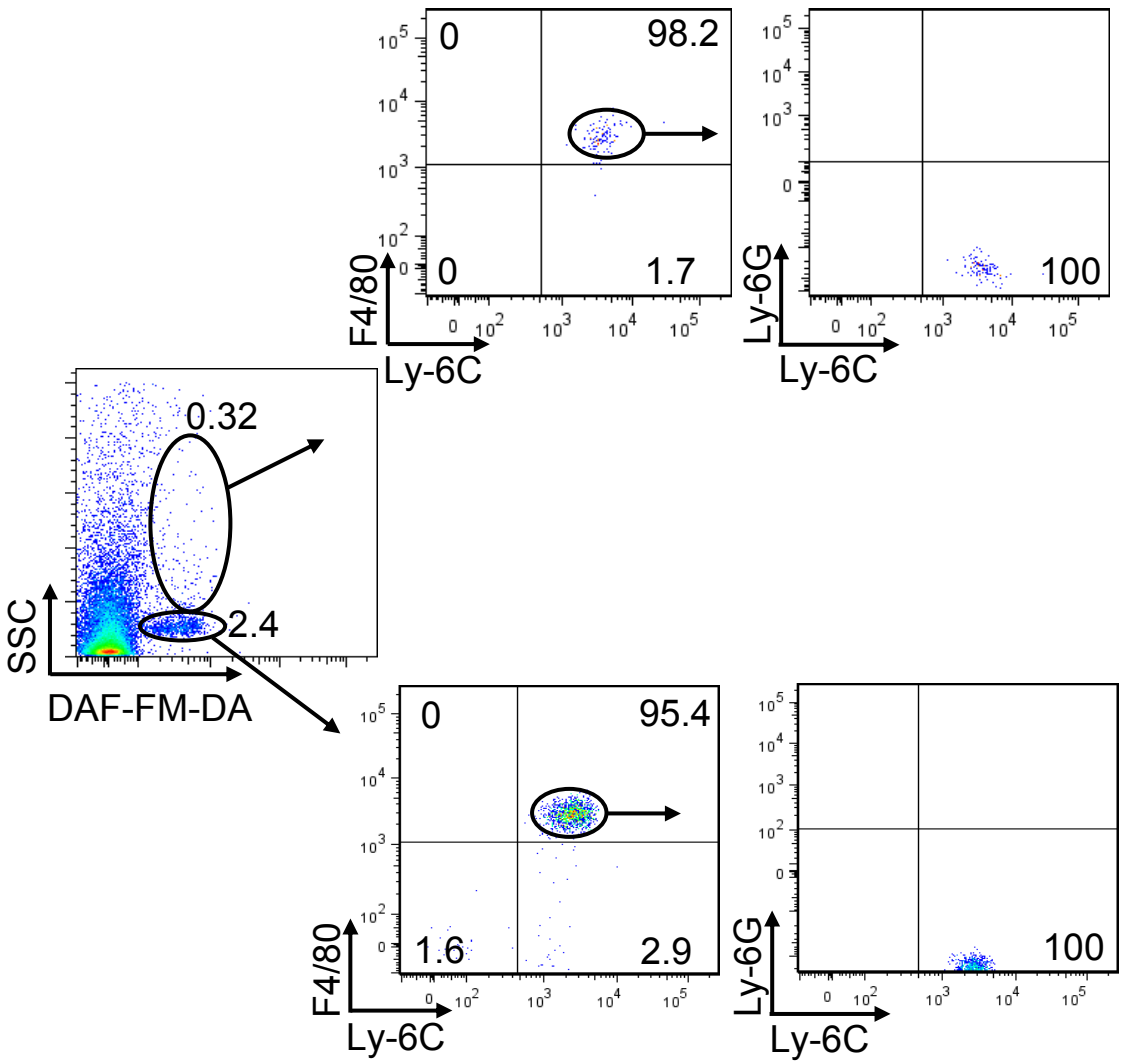
Supplementary Figure 1 Th2 cytokines and eotaxin are elevated in BAL fluid of mice in an experimental model of allergic airway inflammation. (**a**) Numbers of Gr-1$^+$CD11b$^+$F4/80$^-$ neutrophils and (**b**) numbers of Sca-1$^+$CD34$^+$CCR3$^+$ eosinophils were determined by flow cytometry. Insets in (a) and (b) show representative images of FACS purified and Diff Quik stained cells from cytospin preparations. Magnification X1000. Total numbers in (a) and (b) were derived from the product of total numbers of lung leukocytes x percent of total gated cells x percent positive for the indicated surface antigens. (**c**) Cytokines in BAL fluid harvested at the indicated numbers of days after OVA challenge were measured by multiplex cytokine assay. Data are means ± SD (*n* = 3). (**d**) Eotaxin levels in BAL of OVA challenged C57BL/6, iNOS$^{-/-}$ and B6(Cg)Ncf1m1J/J (p47m) mice measured by multiplex cytokine analysis. Data are means ± SD (*n* = 3).



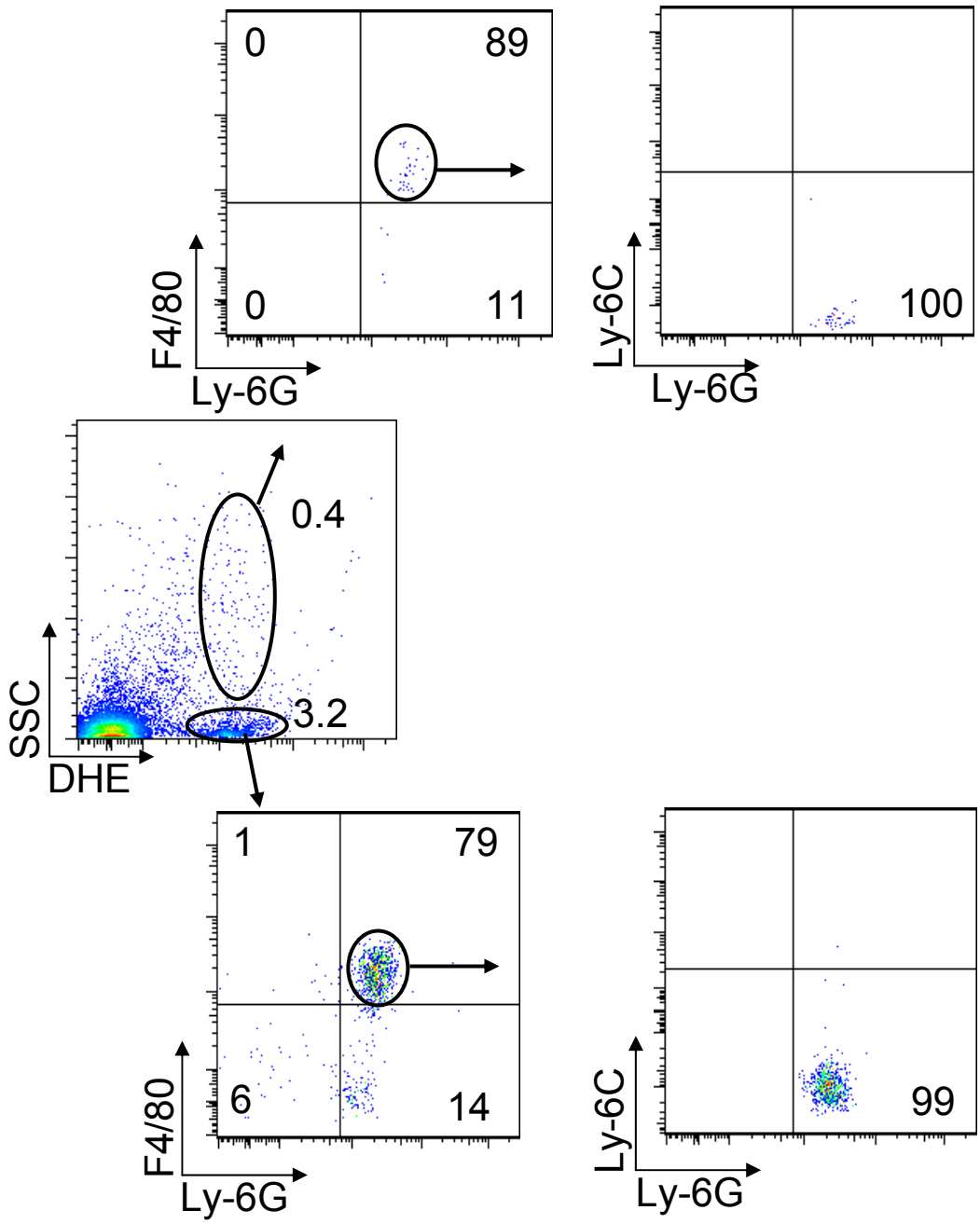
Supplementary Figure 2 Myeloid-derived regulatory cell subsets differentially express CD115 and CD206. (**a**) FACS analysis showing the percent $CD11b^+$ $CD115^+Ly\text{-}6C^+$ cells in lungs of OVA challenged C57BL/6 mice harvested at d2 and d5 after challenge. (**b**) FACS analysis showing the percent $CD11b^+CD206^+Ly\text{-}6G^+$ cells in lungs of OVA challenged C57BL/6 mice harvested at d2 and d5 after challenge. Similar data were obtained in 2 other experiments.



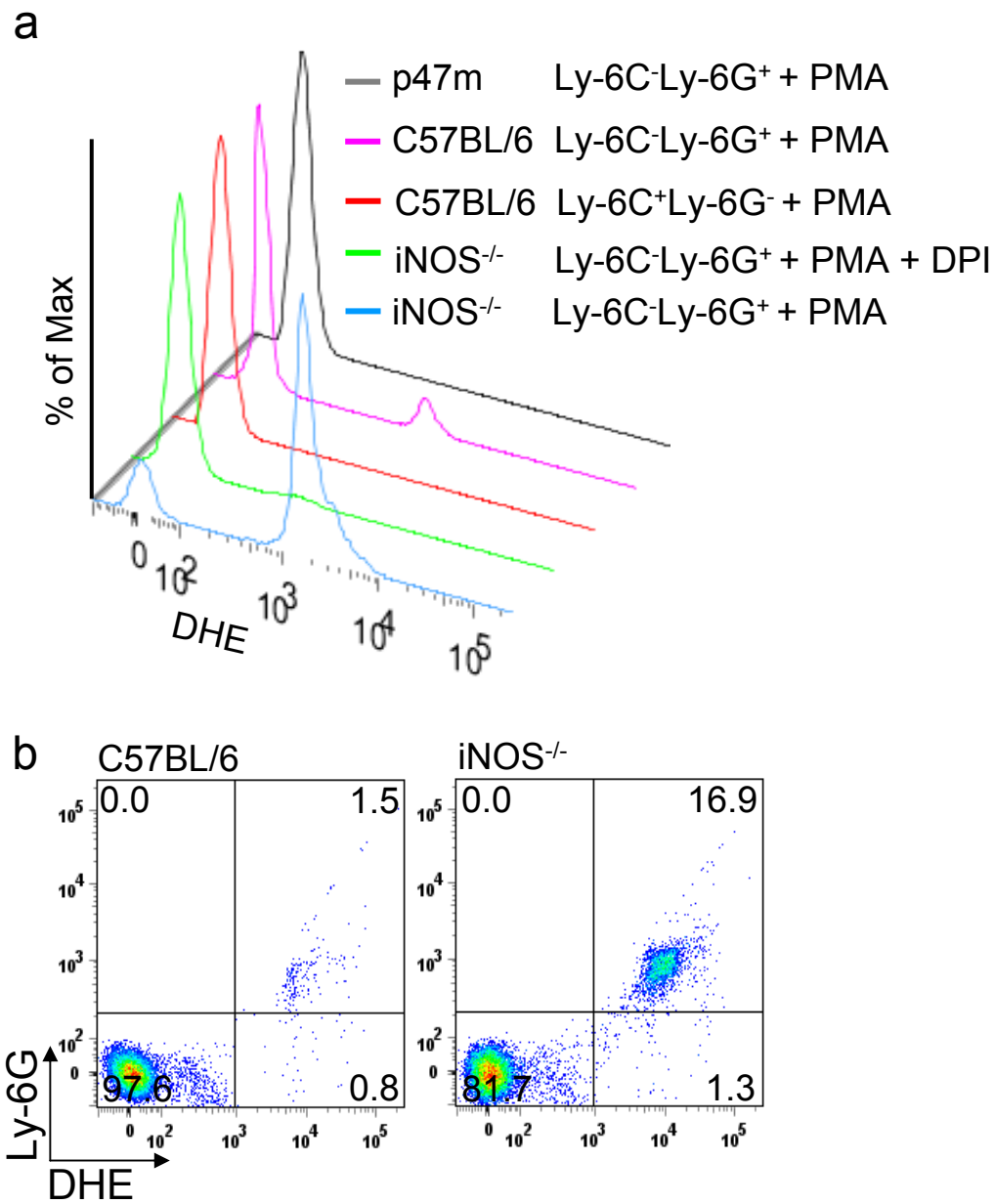
Supplementary Figure 3 NO production by lung Ly-6C^{+}Ly-6G^{-} cells peaks at d5 and is regulated by iNOS. Lung Ly-6C^{+}Ly-6G^{-} cells were isolated at d0 & d5 after challenge and levels of NO were measured by Griess assay in supernatants of 10^4 cells cultured for 48h. Data represent means ± SD for triplicate determinations. ***P*<0.001 compared to B6 Ly-6C^{+}Ly-6G^{-} cells at d0 and compared to B6 and iNOS$^{-/-}$ Ly-6C^{-}Ly-6G^{+} cells at all times shown.



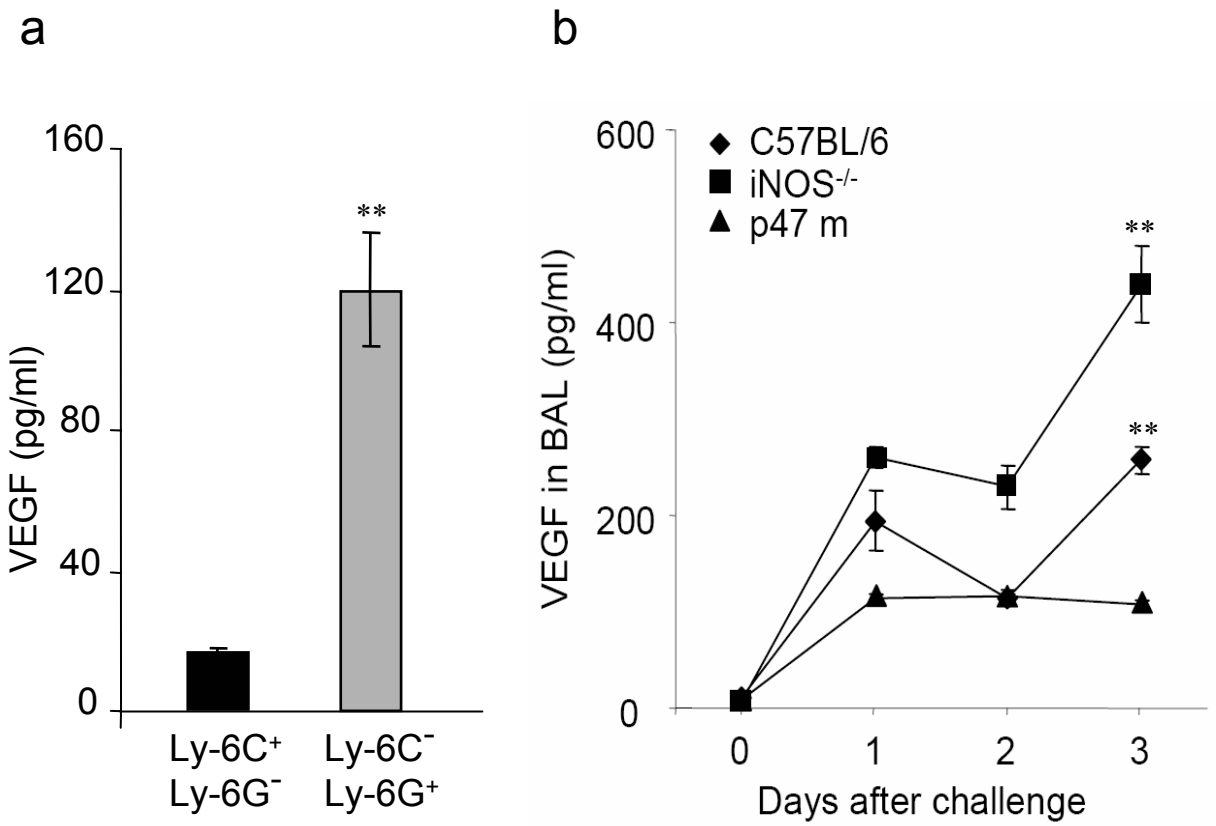
Supplementary Figure 4 The majority of the NO-producing cells in the lungs of OVA challenged wt mice are Ly-6C^{+}Ly-6G^{-}. Representative flow cytometry plots showing characterization of NO-producing cells. Total lung cells harvested from collagenase treated tissue were stained with myeloid specific markers and DAF-FM-DA, the fluorescent indicator for NO. Total lung cells were first gated to exclude dead cells and aggregates. Cells in the DAF-FM-DA^{+} gate were then further analyzed based on expression of F4/80, Ly-6C and Ly-6G as shown.



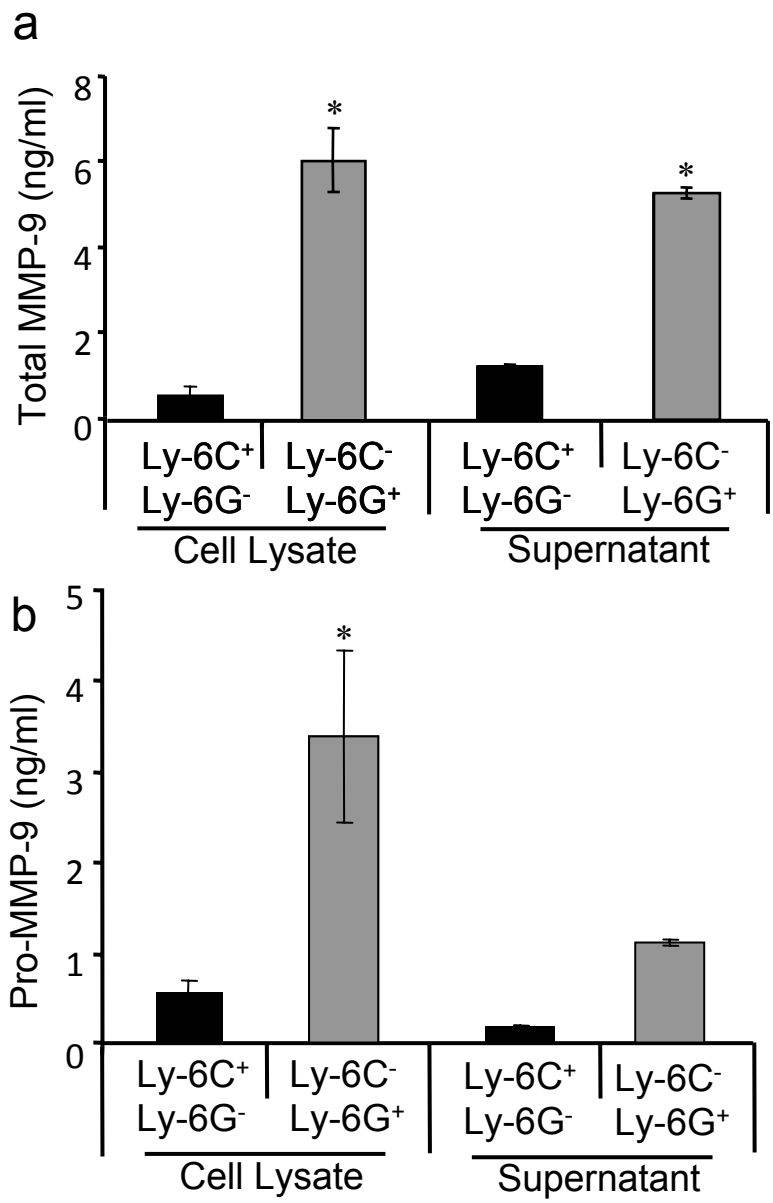
Supplementary Figure 5 The majority of the superoxide-producing cells in the lungs of OVA challenged wt mice are Ly-6C^{-}Ly-6G^{+} cells. Representative flow cytometry plots showing characterization of superoxide-producing cells. Total lung cells harvested from collagenase treated tissue were stained with myeloid specific markers and Dihydroethidium (DHE), the fluorescent indicator for superoxide. Total lung cells were first gated to exclude dead cells and aggregates. Cells in the DHE^{+} gate were then further analyzed based on expression of F4/80, Ly-6C and Ly-6G as shown.



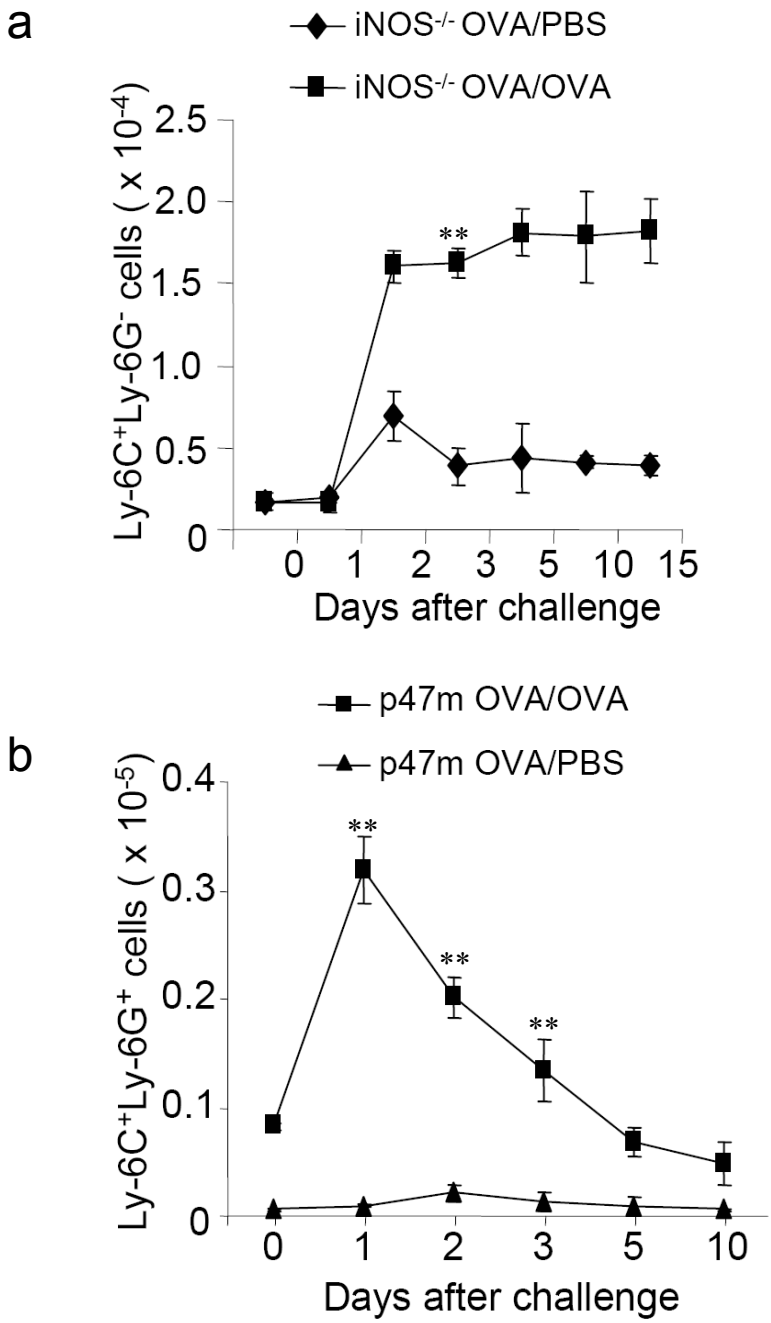
Supplementary Figure 6 Superoxide-producing Ly-6C$^-$Ly-6G$^+$ cells are increased in the iNOS$^{-/-}$ mice. (**a**) Histogram plots showing FACS analysis of sorted Ly-6C$^-$Ly-6G$^+$ and Ly-6C$^+$Ly-6G$^-$ myeloid cells purified as above and activated for 15 min with phorbol myristate acetate (PMA; 1mg/ml) in the presence or absence of diphenyleneiodonium (DPI; 1mM), an inhibitor of intracellular NADPH oxidase, then stained with dihydroxyethidium (DHE), an indicator dye for superoxide ($O_2^{\cdot-}$) and its metabolites. Data are representative of 3 experiments. Data shown in (a) are representative of 3 experiments. (**b**) FACS analysis of BAL cells harvested from wt and iNOS$^{-/-}$ mice stained with anti-Ly-6G antibody and DHE to detect superoxide-producing MDRC.



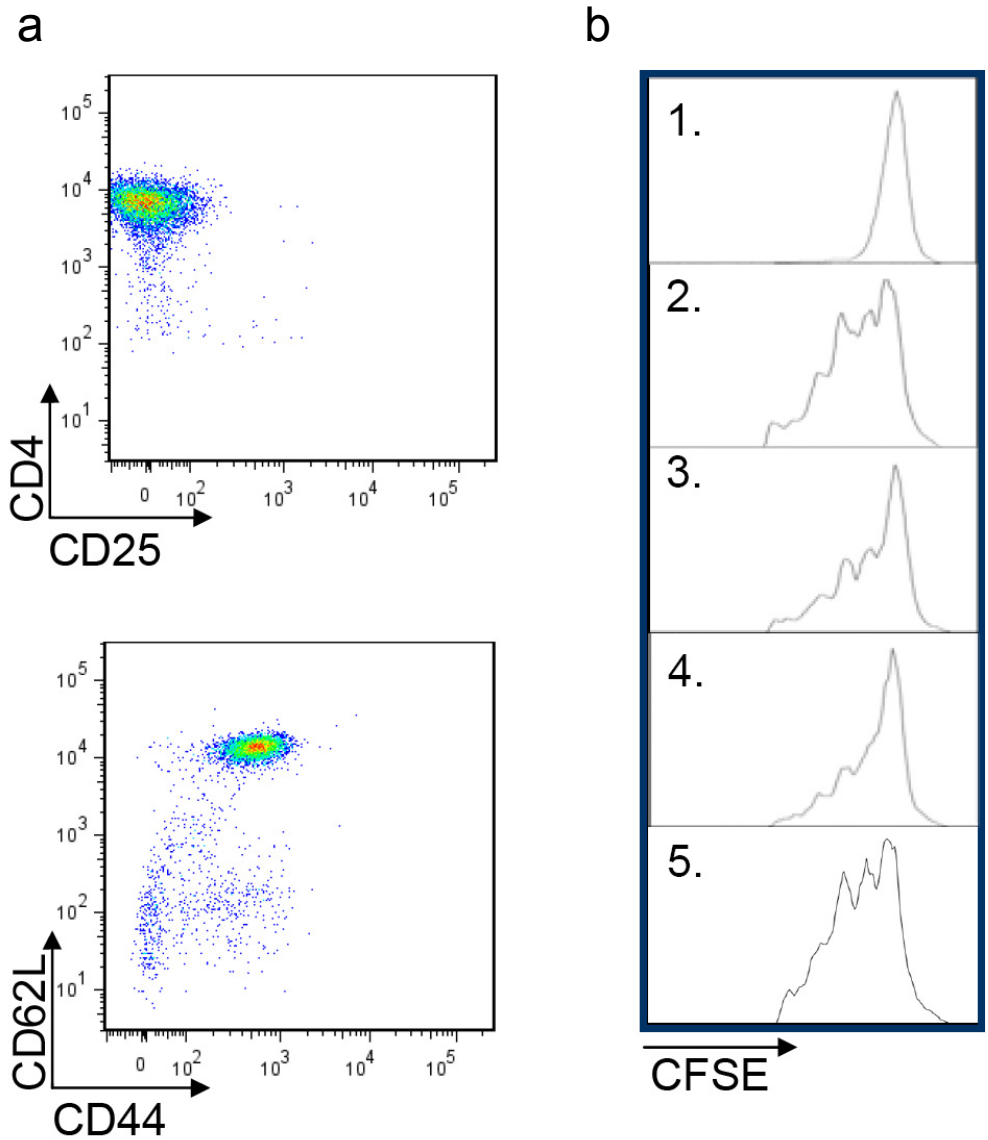
Supplementary Figure 7 VEGF is secreted by Ly-6C$^-$Ly-6G$^+$ cells and is increased in BAL from asthmatic airways of mice.(**a**) Levels of VEGF in culture supernatants from Ly-6C$^+$Ly-6G$^-$ (black bars) and Ly-6C$^-$Ly-6G$^+$ (grey bars) myeloid cells that had been purified by FACS at d2 after challenge from B6 mice and cultured for 48h were determined by ELISA. $^{**}P<0.001$ for Ly-6C$^-$Ly-6G$^+$ at d3 compared to Ly-6C$^+$Ly-6G$^-$ (**b**) VEGF levels in BAL of OVA challenged C57BL/6, iNOS$^{-/-}$ and B6(Cg)Ncf1m1J/J (p47m) mice measured by multiplex cytokine analysis. Data are means ± SD (n = 3). $^{**}P<0.001$ for iNOS$^{-/-}$ at d3 compared to d1 and when compared to C57Bl/6 at d3 and p47 m at d3. $^{**}P<0.001$ for C57BL/6 at d3 compared to p47m at d3.



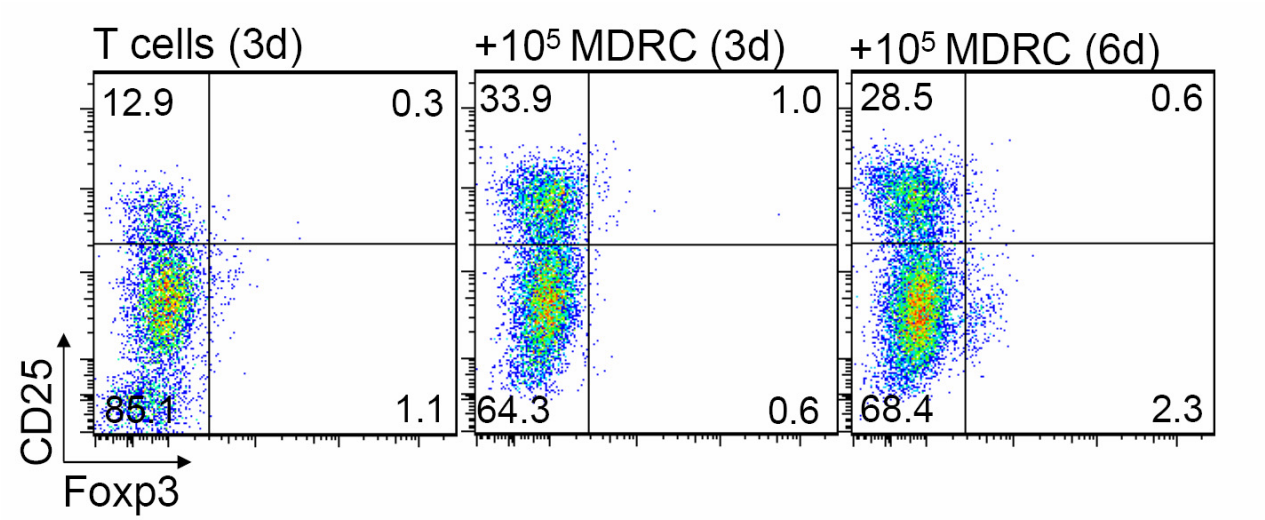
Supplementary Figure 8 MMP-9 is produced by Ly-6C$^-$Ly-6G$^+$ cells that had been purified by FACS at d2 after challenge from B6 mice and cultured for 48h. Total MMP-9 (**a**) and pro-MMP-9 (**b**) levels were determined by ELISA using cell lysates and culture supernatants of 3 x 10^5 sorted Ly-6C$^-$Ly-6G$^+$ and Ly-6C$^+$Ly-6G$^-$ myeloid cells. *P<0.01 compared to Ly-6C$^+$Ly-6G$^-$ cells for (**a**) and (**b**). All data shown are means ± SD (n = 3). Active MMP-9 can be calculated as Total MMP-9 – Pro-MMP-9.



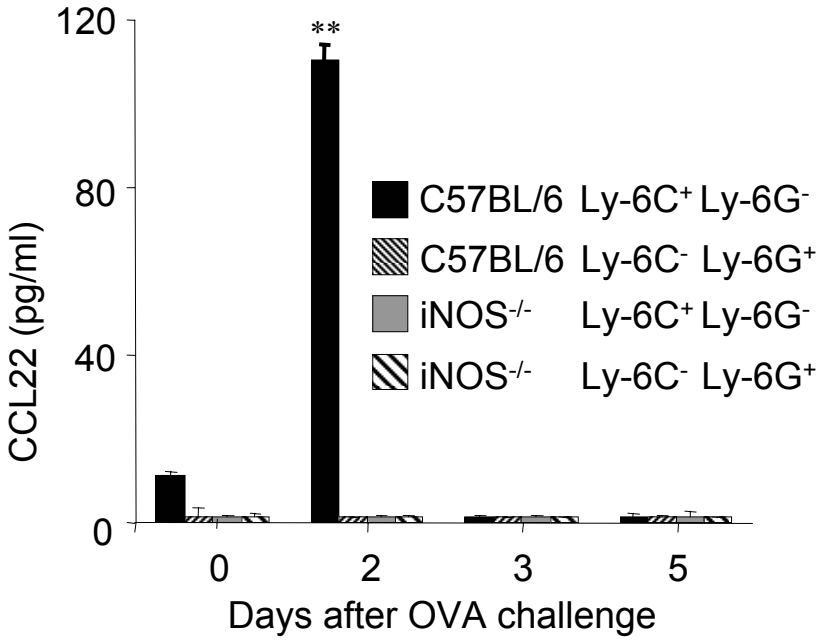
Supplementary Figure 9 (**a**) Low numbers of Ly-6C$^+$Ly-6G$^-$ myeloid cells in the lungs of OVA sensitized and challenged iNOS$^{-/-}$ mice. Numbers of Ly-6C$^+$Ly-6G$^-$ cells in the OVA challenged (OVA/OVA) iNOS$^{-/-}$ mice compared to PBS challenged controls (OVA/PBS) harvested at the indicated days after challenge (total cells in the lung x percent of total gated cells x percent positive). Data are means ± SEM (*n* = 5).
(**b**) Low number of Ly-6C$^+$Ly-6G$^+$ myeloid cells in the lungs of OVA challenged p47m mice. Numbers of Ly-6C$^+$Ly-6G$^+$ cells in the OVA challenged p47m mice compared to PBS controls harvested at the indicated days after challenge. Data are means ± SEM (*n* = 5). $^{**}P$<0.001 compared to control OVA/PBS for (a) and (b).



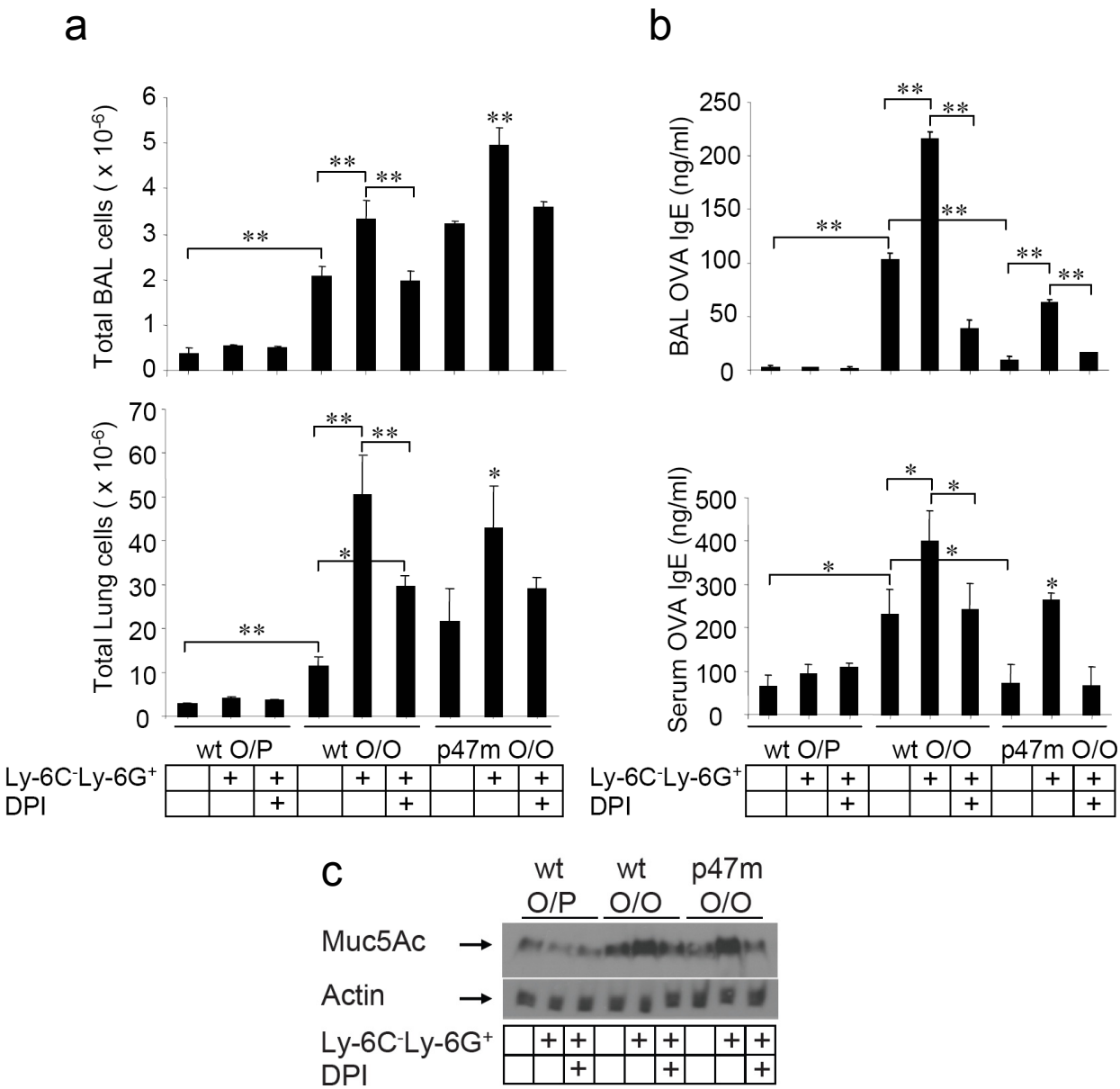
Supplementary Figure 10 Ly-6C^{+}Ly-6G^{-} myeloid cells isolated from wt asthmatic mice suppress proliferation of antigen-specific T cells in a dose-dependent and iNOS-dependent fashion. (**a**) Representative flow cytometry plots showing FACS purified CD4^{+}CD25^{-}CD62L^{hi}CD44^{lo} naïve T cells isolated from spleen that were then used for co-culture experiments as described in Figure 6. (**b**) Naïve OVA-specific CD4^{+} T cells sorted from spleens of OT-II mice were labeled with CFSE and incubated in complete media with or without bone marrow-derived dendritic cells (BMDC) and Ly-6C^{+}Ly-6G^{-} myeloid cells purified by FACS from challenged C57BL/6 or iNOS$^{-/-}$ mice. Co-cultures were carried out using 10^5 T cells: the indicated number of myeloid cells: 10^4 BMDC pulsed with OVA peptide. T cell proliferation was measured as dilution of CFSE using flow cytometry after 72h of co-culture. (**1**) T cells alone; (**2**) T cells + BMDC; (**3**) T cells + BMDC + 10^5 wt Ly-6C^{+}Ly-6G^{-} cells; (**4**) T cells + BMDC + 2 x 10^5 wt Ly-6C^{+}Ly-6G^{-} cells; (**5**) T cells + BMDC + 10^5 iNOS$^{-/-}$ Ly-6C^{+}Ly-6G^{-} cells.



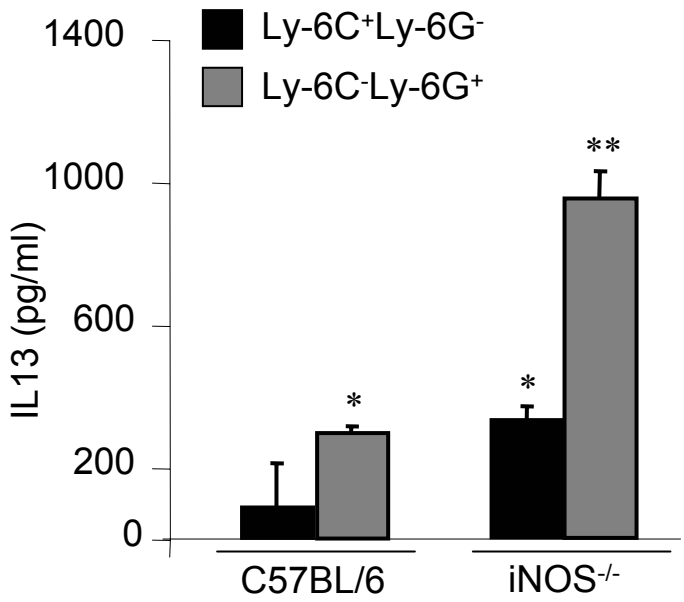
Supplementary Figure 11 Ly-6C$^+$Ly-6G$^-$ cells induce a modest increase in CD25$^+$Foxp3$^+$ T regulatory cells (T_{reg}) *in vitro*. CD4$^+$CD25$^-$CD62L^{hi}CD44^{lo} naïve T cells isolated from spleens of *Foxp3*^{gfp} mice were co-cultured (10^5 cells, 1:1 ratio) with Ly-6C$^+$Ly-6G$^-$ MDRC purified from wt mice at d2 after OVA challenge following activation with anti-CD3 and anti-CD28 antibodies as described in the methods. At 3 and 6 days after co-culture, flow cytometry analysis was carried out. CD4$^+$ cells were gated and frequencies of CD25$^+$Foxp3$^+$ T_{reg} were determined.



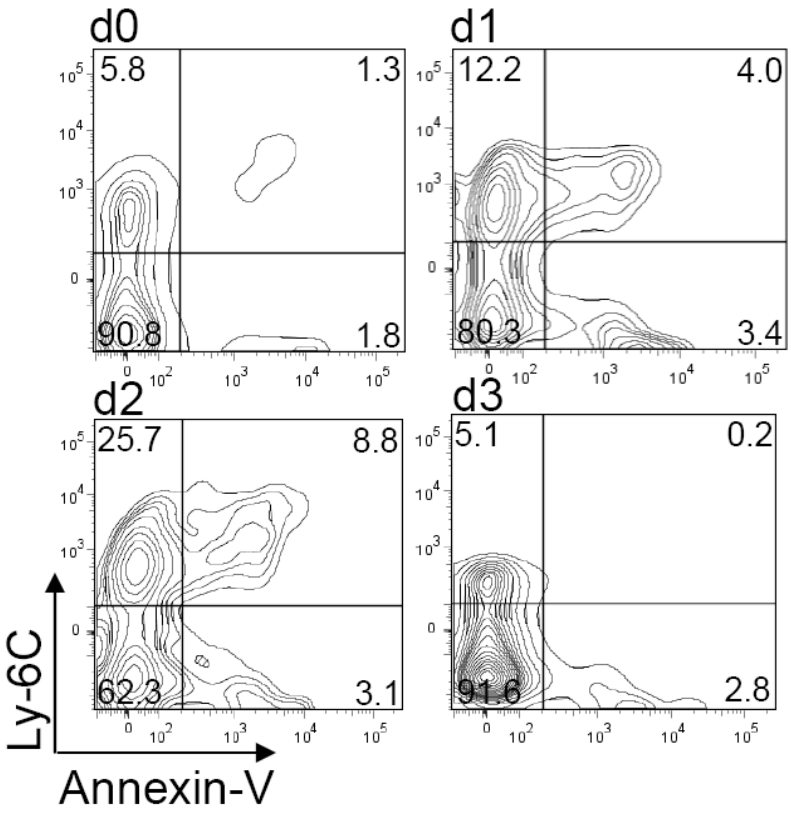
Supplementary Figure 12 Ly-6C$^+$Ly-6G$^-$ cells secrete CCL22, a chemokine that promotes chemotaxis of T_{reg} cells. CCL22 levels were determined by ELISA using culture supernatants from Ly-6C$^+$Ly-6G$^-$ and Ly-6C$^-$Ly-6G$^+$ myeloid cells sorted from collagenase digests of lung tissue harvested from wt and iNOS$^{-/-}$ mice at the indicated number of days after challenge, then cultured for 24h. $^{**}P<0.001$ for Ly-6C$^+$Ly-6G$^-$ lung cells purified from C57BL/6 at d2 compared to d0, d3 and d5 as well as in comparisons with Ly-6C$^-$Ly-6G$^+$ cells purified from both C57BL/6 and iNOS$^{-/-}$ mice.



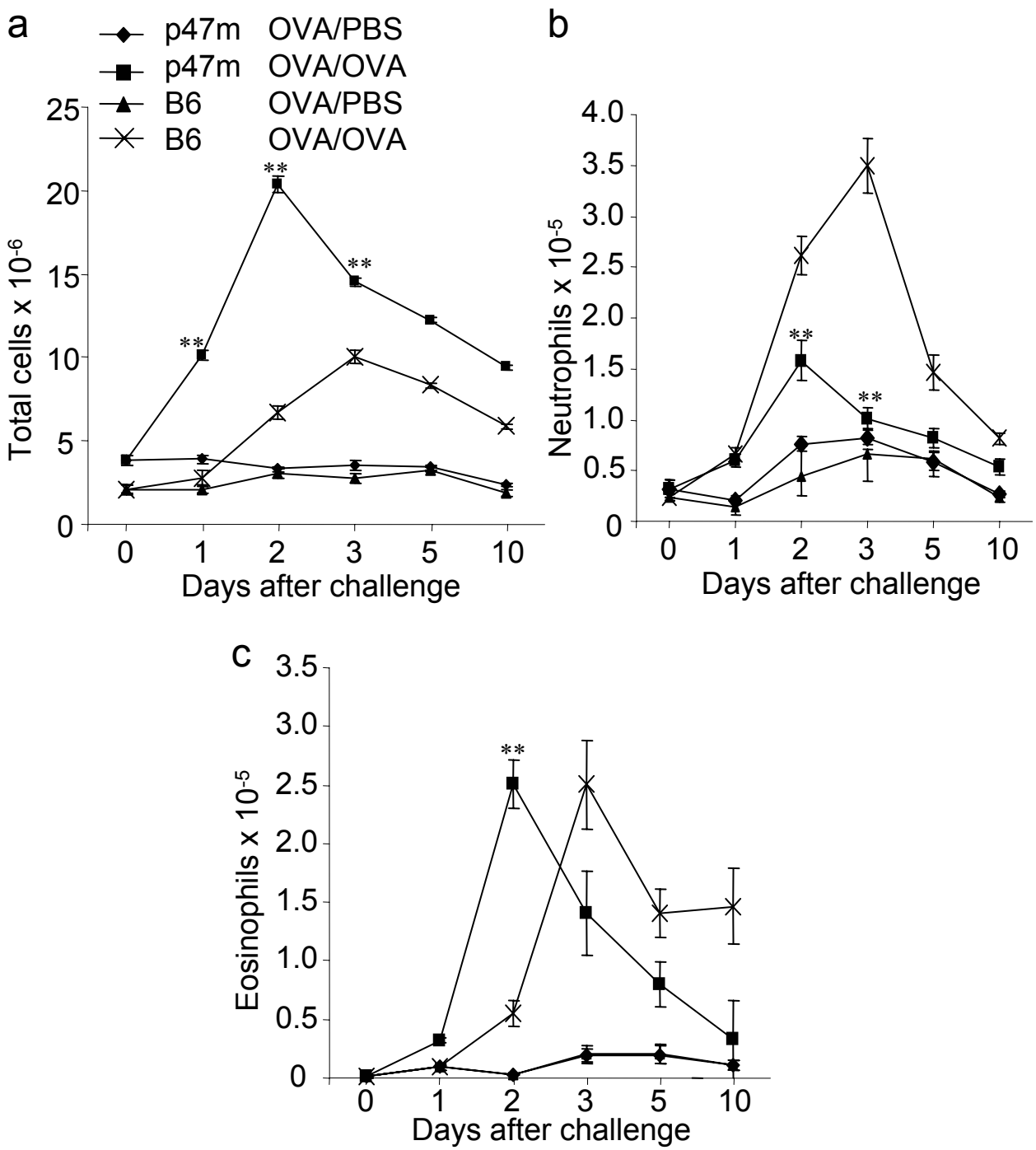
Supplementary Figure 13 Intratracheal (i.t.) transfer of wt Ly-6C$^-$Ly-6G$^+$ cells modulates BAL fluid and serum OVA IgE and Muc5Ac protein levels during airway inflammation**.** O/P represents OVA/PBS and O/O represents OVA/OVA. (**a**) Total BAL cells (top panel) and total lung cells (bottom panel) from wt and p47m mice before and after i.t. transfer of wt Ly-6C$^-$Ly-6G$^+$ cells untreated or treated with the NADPH oxidase inhibitor DPI. (**b**) OVA-specific IgE in BAL fluid (top panel) and serum (bottom panel) from wt and p47m mice before and after i.t. transfer of wt Ly-6C$^-$Ly-6G$^+$ cells untreated or treated with DPI. (**c**) Representative western blot showing Muc5Ac protein levels in lung homogenates from wt and p47m mice before and after i.t. transfer of wt Ly-6C$^-$Ly-6G$^+$ cells untreated or treated with DPI. Samples were fractionated by electrophoresis on a 10% polyacrylamide gel under non-denaturing conditions. Actin was used as a loading control. For (a) and the top panel of (b) $^{**}P$<0.001 and $^{*}P$<0.01. For (b) bottom panel $^{*}P$<0.05.



Supplementary Figure 14 Superoxide producing Ly-6C$^+$Ly-6G$^-$ cells produce IL-13 in an iNOS regulated manner**.** IL-13 levels were determined by multiplex cytokine analysis in supernatants of cultured myeloid cell subsets purified from wt or iNOS$^{-/-}$ mice at d2 after OVA challenge. $^{**}P<0.001$ and $^{*}P<0.01$ compared to wt Ly-6C$^+$Ly-6G$^-$ cells. Data are means ± SD, $n = 3$.



Supplementary Figure 15 Apoptosis of Ly-6C$^+$ myeloid cells in the lung tissue during allergic airway inflammation**.** Contour plots from flow cytometric analyses of collagenase extracted total lung cells from OVA-challenged wt mice at the indicated days after antigen exposure. Cells were stained with anti-Ly-6C antibody and Annexin-V. Representative plots are shown from 3 mice.



Supplementary Figure 16 Airway inflammation in the B6(Cg)Ncf1m1J/J (p47m) mice. (**a**) Total numbers of infiltrating lung cells extracted from collagenase-digested lungs of p47m mice after i.n. OVA challenge compared to PBS controls at the indicated days after challenge. (**b**) Numbers of Gr-1^{+}CD11b^{+}F4/80^{-} neutrophils and (**c**) numbers of Sca-1^{+}CD34^{+}CCR3^{+} eosinophils determined by flow cytometry based on surface expression of the markers. Total numbers in (b) and (c) were calculated from the product of total cells in the lungs x percent of total gated cells x percent positive. Data are means ± SEM (n = 5). $^{**}P$<0.001 comparing p47 m OVA/OVA vs B6 OVA/OVA for (a), (b) & (c).

Supplementary Table I. Levels of CCL22 (pg/ml) in BAL fluid*

	Day 0	Day 2	Day 3
wt OVA/PBS+	ND	ND	ND
B6 OVA/OVA	1.66 ± 0.16	46.94 ± 5.09	ND
p47m OVA/PBS	7.02 ± 2.13	ND	9.16 ± 0.48
p47m OVA/OVA	7.22 ± 2.16	133.05 ± 19.15	64.7 ± 4.09

*Mean ± SD (n=3 mice). CCL22 was undetectable in BAL from iNOS$^{-/-}$ mice.
+OVA/PBS, OVA-sensitized and mock challenged with PBS.
OVA/OVA, OVA-sensitized and challenged with OVA.
ND, Not detected.

Supplementary Methods:

Asthma model and collection and preparation of tissue samples: Contamination of isolated lung cells with blood was reduced by perfusion of the pulmonary circulation with PBS via the right ventricle following euthanasia and thoracotomy. Airway lavage was performed three times with 0.8 ml of PBS each. A 100 µl aliquot from the first collection of the return lavage fluid, after depletion of cells by centrifugation, was used for the determination of levels of metabolites of the arginase and iNOS pathways and for measurement of cytokines. The three aliquots of lavage from each animal were pooled and used for determination of total cell numbers and for analysis of myeloid cell subsets. The total number of viable lavage cells was determined using Trypan blue and a hemacytometer. Differential cell counts were determined using Diff Quik staining of cytospin preparations. Infiltrating leukocytes were isolated from minced lung tissue by treatment with collagenase-B (2 mg/ml, Roche) and DNase I (0.02 mg/ml, Sigma Chemical) in Iscove's modified Dulbecco's medium (IMDM) supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, 10 µg/ml penicillin-streptomycin, 25 µM 2-mercaptoethanol and 0.1 mM non-essential amino acids (Life Technologies) at 37^{o}C for 30 min. This was followed by the addition of an equal volume of IMDM containing 20% FBS. Cell suspensions were passed through a 40-µm cell strainer and washed with PBS before further analyses.

Fluorescent activated cell sorting and analysis: Myeloid cells recovered from the airways by BAL or from collagenase digested lung tissue were sorted based on the expression of myeloid lineage-specific cell surface antigens. Cells were pretreated on ice for 20 min

with 2.0 µg/ml of the mAb 2.4G2 (BD Pharmingen, Franklin Lakes, NJ), then for 30 min with the APC-conjugated anti-Gr-1 mAb RB6-8C5 (Caltag, Burlingame, CA), the PE-Alexa 647-conjugated anti-F4/80 mAb A3.1 (AbD Serotec, Raleigh, NC), the biotinylated anti-Ly-6C mAb AL-21, the PE-conjugated anti-Ly-6G mAb 1A8, and the APC-Cy-7-conjugated anti-CD11b mAb M1/70 (BD Pharmingen). Isotype control and secondary antibodies were also obtained from BD Pharmingen. Streptavidin-PE-Cy-7 (Southern Biotechnology, Birmingham, AL) was used for the detection of cells stained with biotin-conjugated antibodies. The PE-conjugated anti-murine CSF-1 receptor (anti-CD115) and the Alexa-Fluor 647-conjugated anti-mannose receptor c type 1 (anti-CD206) were from AbD Serotec. Cells were washed twice with PBS before analysis using a Becton Dickinson LSR II with FACS Diva software (BD Biosciences, San Jose, CA). Data were further analyzed using FlowJo 7.2 (Tree Star, Ashland, OR). Cells were purified by sorting using a FACSAria (Becton Dickinson).

Measurements of NO: MDRC, sorted as described above, were incubated with 5 µM of the NO indicator (DAF-FM-DA, Molecular Probes, Eugene, OR) (Lacza, Z., Snipes, J.A., Zhang, J., Horvath, E.M., Figueroa, J.P., Szabo, C., and Busija, D.W. 2003, Mitochondrial nitric oxide synthase is not eNOS, nNOS or iNOS. *Free Radic Biol Med* 35:1217-1228; Lepiller, S., Laurens, V., Bouchot, A., Herbomel, P., Solary, E., and Chluba, J. 2007, Imaging of nitric oxide in a living vertebrate using diamino-fluorescein probe. *Free Radic Biol Med* 43:619-627) in PBS for 20 min at RT or treated with the iNOS inhibitor 1400w (Cayman Chemical, Ann Arbor, MI; 500 nM) for 30 min before incubation with DAF-FM-DA. After washing with PBS, the cells were incubated for an additional 15 min in fresh PBS, and stained cells were washed again twice in PBS

before analysis by flow cytometry. 10^4 sorted myeloid cells recovered from collagenase-digested lungs were cultured in serum free media for 48 h and metabolites of NO were measured in culture supernatants or 50 µl BAL fluid (Griess assay, Promega Corporation, Madison, WI).

Measurements of arginase activity: Arginase activity in cultured lung myeloid cells and BAL fluid was estimated by measuring urea with the Quantichrom Urea Assay kit (DIUR-500) (BioAssay Systems, Hayward, CA). 50 µl BAL fluid harvested at different times after OVA challenge of sensitized or control mice or mice treated with or without 1400w (Cayman Chemical) and 50 µl supernatants from 10^4 cultured myeloid cells (48 h) FACS purified from lungs of OVA-sensitized mice at various times after antigen challenge were used for urea measurements.

Measurements of superoxide: $O_2^{\cdot-}$-producing MDRC were detected by flow cytometry after staining with myeloid cell-specific antibodies and incubation for 20 min at RT with dihydroxyethidium (DHE, 10 µM; Molecular Probes, Eugene, OR) (Mahfouz, R., Sharma, R., Lackner, J., Aziz, N., and Agarwal, A. 2009, Evaluation of chemiluminiscence and flow cytometry as tools in assessing production of hydrogen peroxide and superoxide anion in human spermatozoa. *Fertil Steril* 92:819-82. Horvathova, M., Wsolova, L., and Jahnova, E. 2005, Simultaneous flow cytometric evaluation of phagocytosis and oxidative burst in human polymorphonuclear cells. *Bratisl Lek Listy* 106:63-66). The specificity of DHE for $O_2^{\cdot-}$ was validated by inducing a respiratory burst in the sorted myeloid cells by incubation at 37°C for 15 min with phorbol myristate acetate (PMA, 1 µg/ml) or PMA + the NADPH oxidase inhibitor diphenylene iodonium (DPI, 1 µM; Tocris

Bioscience, Ellisville, MO) added 15 min before PMA (Reis, K., Halldin, J., Fernaeus, S., Pettersson, C., and Land, T. 2006, NADPH oxidase inhibitor diphenyliodonium abolishes lipopolysaccharide-induced down-regulation of transferrin receptor expression in N2a and BV-2 cells. *J Neurosci Res* 84:1047-1052). For quantitation of the fold increase in $O_2^{\cdot-}$ production, $O_2^{\cdot-}$-dependent reduction of cytochrome c (Sigma) was assayed. (McCord, J.M., and Fridovich, I. 1968, The reduction of cytochrome c by milk xanthine oxidase. *J. of Biol.Chem.* 243:5753-5760). Briefly, 4 x 10^4 purified MDRC were stimulated with PMA in RPMI 1640 plus 0.5% FBS and the time course of reduction of cytochrome c (10 μM) was monitored at λ= 550 nm, ϵM = 21 $mM^{-1} \cdot cm^{-1}$ using a spectrophotometer (UV-2501PC Shimadzu, Shimadzu, Japan). $O_2^{\cdot-}$ produced by NADPH oxidase was determined by the subtraction of the DPI (1 μM) sensitive rate of cytochrome c reduction from the rate determined in the absence of DPI.

Polarization of T regulatory (T_{reg}) cells: Spleen and lymph node cells were harvested from *Foxp3*^{gfp} mice. Following lysis of red cells using ACK (Quality Biologicals, Inc, Gaithersburg, MD), $CD4^+$ cells were isolated using $CD4^+$ Dynabeads (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. $CD4^+CD25^-$ cells were sorted using the CD25 microbead isolation system (Miltenyi Biotech, Auburn, CA) and then activated in culture with CD3CD28 expander beads (Invitrogen) according to the manufacturer's suggestions in the presence of 5 ng/ml TGF-β1 (R&D Systems, Minneapolis, MN), 50 U/ml IL-2 (R&D Systems), 100 nM retinoic acid (Sigma-Aldrich, St. Louis, MO), 10 μg/ml anti-IFNγ (clone XMG 1.2), 10 μg/ml anti-IL-4 (clone 11B11) in complete R-10 medium. Four days later, live $CD4^+$Foxp3/GFP^+ cells were isolated by

FACS, using 7-amino actinomycin-D (7-AAD; BD Bioscience) to exclude dead cells.

Analysis of T_{reg} cell chemotaxis: Chemotaxis of T_{reg} cells *in vitro* was analyzed as previously described (Deshane J, Garner CC, Sontheimer H. 2003, Chlorotoxin inhibits glioma cell invasion via matrix metalloproteinase-2 (MMP-2). *J. Biol. Chem.* 278:4135-44). $CD4^+CD25^-$Foxp3/GFP^+ T_{reg} cells that had been purified by sorting following *in vitro* polarization were cultured at 10^4 cells/ml in IMDM supplemented with 1% FBS and 0.1% BSA in a transwell plate with 3-µm migration filters (Corning Life Sciences, Lowell, MA). The culture supernatants (200 µl) from 10^4 MDRC that had been sorted from the lungs of OVA sensitized and challenged mice and cultured for 48 hrs in the absence of additional stimulus were used in the wells of a 24 well plate. Chemotaxis was allowed to proceed for 6h at 37ºC. The percent of migrated T_{reg} cells was determined by flow cytometric analysis of the GFP^+ cells in the lower chambers.

ELISA for MMP9: Levels of total MMP-9 and of pro–MMP-9 in Ly-$6C^+$Ly-$6G^-$ and Ly-$6C^-$ Ly-$6G^+$ cell subsets were determined in cell lysates and in cell culture supernatants from 3 x 10^5 sorted cells using ELISA following the manufacturer's recommendations (R&D Systems). Colorimetric detection was performed using a microplate reader (Tecan, NC) set to 450 nm with correction wavelength of 540 nm. The data were normalized using standard curves provided with the kits.

ELISA for detection of cytokines and growth factors: Supernatants were removed after 48 h of cell culture, centrifuged to remove cell debris, and tested for IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and VEGF using ELISA kits according to the manufacturer's instructions (R&D Systems). Cell-free BAL fluid samples were prepared at the indicated

days following airway antigen challenge and were analyzed in a similar fashion.

Multiplex cytokine analysis: In selected experiments, the BioPlex (Millipore) mouse 19-plex cytokine kit was used for analysis of BAL fluid and cell culture supernatants. Standard curves were established using the manufacturer's instructions and reagents. The data were analyzed using the BioPlex Manager software (Bio-Rad).

Adoptive transfer experiments and OVA-specific IgE measurements: In experiments designed to evaluate the direct effects of $O_2^{\bullet-}$ produced by the MDRC on AHR, Ly-6C$^-$Ly-6G$^+$ cells purified from OVA-challenged wt mice at d3 after challenge were first treated with DPI (500nM) or diluent control for 45 minutes. The cells were then washed in PBS several times before 10^5 cells were transferred intratracheally (i.t.) into OVA challenged wt or p47m recipient mice. At 3 days after challenge, AHR was measured and comparisons were made between mice that received transfer of DPI treated and untreated cells and PBS transferred control mice. Numbers of total lung leukocytes and number of BAL cells were determined before transfer, before OVA challenge, and at d3 after challenge. Sera and BAL fluid were collected from mice that received i.t. transfer of wt Ly-6C$^-$Ly-6G$^+$ cells treated or untreated with DPI at d0 after transfer and d3 after OVA challenge. Mouse OVA-specific IgE was measured in sera and BAL fluid using an IgE ELISA kit (AbD Serotec, Oxford, UK) following the manufacturer's recommendations.

Western blot analysis for determination of Muc5Ac protein: Total lung homogenates were prepared at d3 from wt or OVA challenged wt or p47m recipient mice following i.t. transfer of Ly-6C$^-$Ly-6G$^+$ cells as described above as well as from wt sensitized mice before challenge. Samples (50 μg total protein) were electrophoresed on a 10%

polyacrylamide gel under non-denaturing conditions for optimal detection of Muc5Ac protein using monoclonal anti-Muc5Ac antibody (211-M1,1:1000, Abcam, Cambridge, MA). The blot was then stripped and reprobed with rabbit anti- actin polyclonal antibody (Sigma, MI).