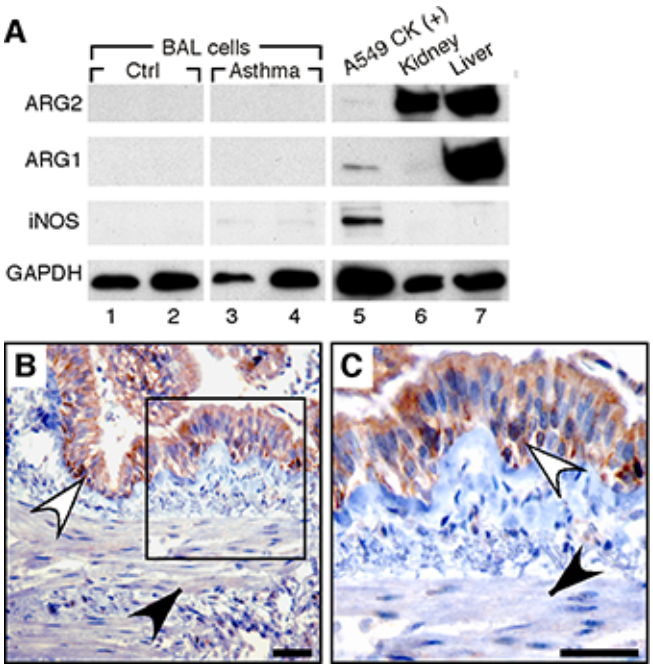
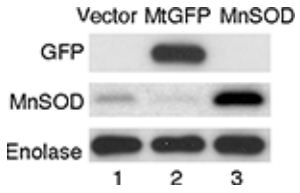
Supplemental Figures and Figure Legends



Supplemental Figure 1. Expression of ARG2 in BAL cells and airway smooth muscle cells. (**A**) Western blot analyses of ARG2, ARG1 and iNOS expression in mononuclear cells from BAL freshly obtained from asthmatics (lanes 3 – 4) and controls (lanes 1 –2). A549 cells exposed to cytokine mixture (CK)(lane 5) as positive control for iNOS expression, mouse kidney tissue as positive control for ARG2 and mouse liver tissue as positive control for ARG1. GAPDH as a loading control. Images representative of 3 asthmatics and 2 controls. (**B** − **C**) Immunohistochemistry of ARG2 in lung of asthmatic individual. Expression of ARG2 is abundant in bronchial epithelium (white arrowheads) but low to undetectable in airway smooth muscle cells (black arrowheads). Images representative of multiple sections from open lung biopsies of 4 asthmatics. **C** is close-up view of **B**. Scale bars: 40 μm.



Supplemental Figure 2. Western blot of GFP and MnSOD in BET1A cells transfected with empty vector, MtGFP or MnSOD. *n* = 3 replicate experiments.

Supplemental Tables

Supplemental Table 1. Features of study participants

Characteristics	**Control (*n* = 60)**	**Asthma (*n* = 79)**	***P****
Mean age, yr.	37 ± 1	39 ± 1	0.5
BMI, kg/m^2	27.4 ± 0.8	29.1 ± 0.6	0.11
Gender, M/F	22/38	31/48	0.7
Ethnicity, C/AA/other	29/26/5	40/31/8	0.5
Blood pressure, mmHg			
Systolic	118 ± 3	124 ± 2	0.19
Diastolic	73 ± 1	77 ± 1	0.06
FEV_1 % predicted	96 ± 1	83 ± 2	<0.0001
FEV_1/FVC	0.80 ± 0.01	0.74 ± 0.01	<0.0001
IgE, IU/ml	82 ± 20	310 ± 46	<0.0001
% Atopy	53	92	0.0001
PC_{20}, mg/ml	NR[#]	4.0 ± 0.9	0.0000
F_ENO (ppb)	19 ± 1	43 ± 5	<0.0001

Mean ± SEM; Definition of abbreviations: M, male; F, female; C, Caucasian; AA, African American; FEV_1, Forced expiratory volume in 1 second; FVC, Forced vital capacity; PC_{20}, provocative concentration of methacholine causing a 20% fall in FEV_1; F_ENO**,** fractional exhaled nitric oxide; ***P* value, asthma vs. controls; [#]non-reactive.

Supplemental Table 2. Differential cell count of bronchoalveolar lavage (BAL) cells

BAL, %	**Control (*n* = 16)**	**Asthma (*n* = 30)**	***P****
Macrophages	91± 1	91 ± 1	0.9
Neutrophils	2.9 ± 0.5	3.0 ± 0.7	0.9
Eosinophils	0.04 ± 0.03	0.63 ± 0.21	0.01
Lymphocytes	5.1 ± 1.1	4.4 ± 0.7	0.6

Mean ± SEM; ***P* value, asthma vs. controls.

Supplemental methods

Stable isotopic studies and de novo arginine synthesis. Patients were admitted to the Clinical Research Unit at the Cleveland Clinic after an 8-hour overnight fast. All participants had an intravenous catheter placed in the antecubital vein for isotope infusions and in a superficial hand vein of the contralateral arm for blood sampling. After a baseline blood sample was obtained, primed, constant rate intravenous infusions of guanidine-[$^{15}N_2$]-arginine (prime = 8 μmol·kg^{-1}, infusion = 8 μmol·kg^{-1}·h^{-1}), [^{13}C,$^{2}H_4$]-citrulline (prime = 1.5 μmol·kg^{-1}, infusion = 1 μmol·kg^{-1}·h^{-1}), [^{13}C]-urea (prime = 80 μmol·kg^{-1}, infusion = 8 μmol·kg^{-1}·h^{-1}), and [$^{15}N_2$]-ornithine (prime = 2 μmol·kg^{-1}, infusion = 2 μmol·kg^{-1}·h^{-1}) were started and maintained for 6 hours. A bolus infusion of [^{15}N]-citrulline (0.16 μmol·kg^{-1}) was administered to prime the secondary citrulline pool in order to achieve a steady state early. Blood samples, 6 ml each, were obtained every 30 minutes between 4.5 and 6 hours of the tracer infusion.

Tracer/tracee ratios of plasma arginine, citrulline, and ornithine were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The amino acids were converted into their 5-dimethylamino-1-hapthalene sulfonamide derivatives, and the transitions monitored were precursor ion *m/z* 410 to product ion 392 for arginine, precursor ion *m/z* 414 to 397 for citrulline, and precursor ion *m/z* 601 to product ion 170 for ornithine. The tracer/tracee ratio of plasma urea was determined by electron impact ionization (EI) GC-MS of its 2-pyrimidinol-tertbutyldimethylsilane derivative at m/z 153 to 155. Rate of appearance (Ra) or total flux (Q) and endogenous flux of arginine, citrulline, ornithine, and urea, the rate of conversion of citrullline to arginine, and the rate of NO synthesis were calculated.

Rate of appearance or total flux (Q) of arginine, citrulline, ornithine, and urea were calculated from the steady state equation

$$Q(\mu mol \cdot kg^{-1} \cdot h^{-1}) = (E_{inf}/E_{plat}) \times i$$

Where E_{inf} is the isotopic enrichment of the infusate, E_{plat} is the isotopic enrichment in plasma at isotopic steady state, and i is the infusion rate of the tracer in $\mu mol \cdot kg^{-1} \cdot h^{-1}$.

Because arginine is converted to citrulline and nitric oxide (NO) in a 1:1 ratio, the rate of conversion of [$^{15}N_2$]-arginine to [^{15}N]-citrulline is an index of NO synthesis. Similarly, the conversion of [^{13}C,$^{2}H_4$]-citrulline to [^{13}C,$^{2}H_4$]-arginine is a measure of de novo arginine synthesis, and the conversion of [$^{15}N_2$]-arginine to [$^{15}N_2$]-urea is an index of arginase activity. The conversion of precursor to product was determined by

$$Q_{pre \rightarrow prod} (\mu mol \cdot kg^{-1} \cdot h^{-1}) = Q_{prod} \times E_{prod}/E_{pre}$$

Where Q_{prod} is the flux of the product (citrulline, arginine, or urea), E_{prod} is the plasma enrichment of the product (m+1 citrulline, m+5 arginine, or m+2 urea), and E_{pre} is the plasma enrichement of the precursor (m+2 arginine, m+5 citrulline, or m+2 arginine).

Western analyses. Antibodies included rabbit anti-ARG1 (sc-20150), ARG2 (sc-20151), iNOS (sc-651), ASS (sc-99178), Enolase sc-15343), MnSOD (sc-3008), CuZnSOD (sc-11407), STAT6 (sc-981), GFP (sc-8334)(Santa Cruz Biotechnology, Santa Cruz, CA), ASL (H00000435-M01, Abnova Corporation, Taiwan), VDAC (#4661), phospho-Stat6 (pSTAT6, #9361)(Cell Signaling Technology, Danvers, MA), CAT2 (NBP1-59872), HIF-1α (NB100-479), HIF-2α (NB100-122), Carbonic anhydrase IX (CAIX, NB100-417)(Novus Biologicals, LLC Littleton, CO), CAT3 (70R-8576, Fitzgerald, Acton, MA), and Peroxiredoxin-SO_3 (Prx-SO_3, LF-PA0004, Thermo Scientific) polyclonal Ab, Goat anti-CAT1 (sc-33087) and Lamin B (sc-6216)(Santa Cruz Biotechnology) polyclonal Ab, and mouse anti-Complex I (A21344), II (A11142), III-1 (A21362), III-2 (A11143), IV-4 (A6403)(Molecular Probes, Inc., Eugene, OR), Cytokeratin (M3515, DAKO North America, Inc., Carpinteria, California), OAT (10R-1173), GAPDH (10R-

G109a)(Fitzgerald), and Prx3 (LF-MA0044)(Thermo Scientific). Secondary Ab included anti-rabbit (NA9340) or anti-mouse Ab (NA931)(GE Healthcare Life Sciences, Marlborough, MA).

Ultrastructural analyses. Ultrastructure of airway epithelial cells and BET1A cells was studied using Tecnai G2 Spirit BioTWIN Transmission Electron Microscope (FEI Company, Hillsboro, OR). For stereologic quantitation of mitochondria in airway epithelial cells, electron microscopic images (anonymized by random coding) were scanned and transformed to jpeg files using Adobe Photoshop. Overall, there were 3 to 10 images per group (mean: 5.8). They comprised two sets. The first set had an original resolution of 1024×1280 pixels; these images were adjusted to a final resolution of 1178×1166 pixels; the second set of images, with an original resolution of 1358×1016 pixels were adjusted to a resolution of 1178×900 pixels, both using Adobe Photoshop so to fit into the analyses screen of Stepanizer (http://www.stepanizer.com/). Stereological analysis was performed in the setting of 1024×1280; the contrast was optimized for improved recognition of mitochondria during stereological analyses. For the stereological analyses of the first set of EM images, the test grid consisted of 128 lines and 256 points (lines/point = 29 pixels); the second set was of 324 lines and 648 points. The testing grid was optimized to have between a minimum of 100 to 200 line intercepts in mitochondria; overall, mitochondria intercepts/group ranged between 137 to 595 (mean: 363); mitochondria-hitting points/group, 103-403 (mean: 234); cell-hitting points/group, 1000-2727 (mean: 1895). Mitochondria surface (Sm)/ cellular volume (Vc) was determined by counting the line intersects with mitochondria and points hitting the reference tissue (cells). The final surface/volume assessment was performed using the stereological formula:

$$\frac{Sm}{Vc} = \left[2 \times \sum(\text{mitochondria intersects})\right] / \left[\left(\frac{L}{P}\right) \times \sum(\text{points in cells})\right]$$

The volume density of mitochondria (Vm) /cell was determined by the formula:

$$\frac{\mathrm{Vm}}{\mathrm{Vc}} = \sum(\text{points in mitochondria}) / \sum(\text{points in cells})$$