Figure S1

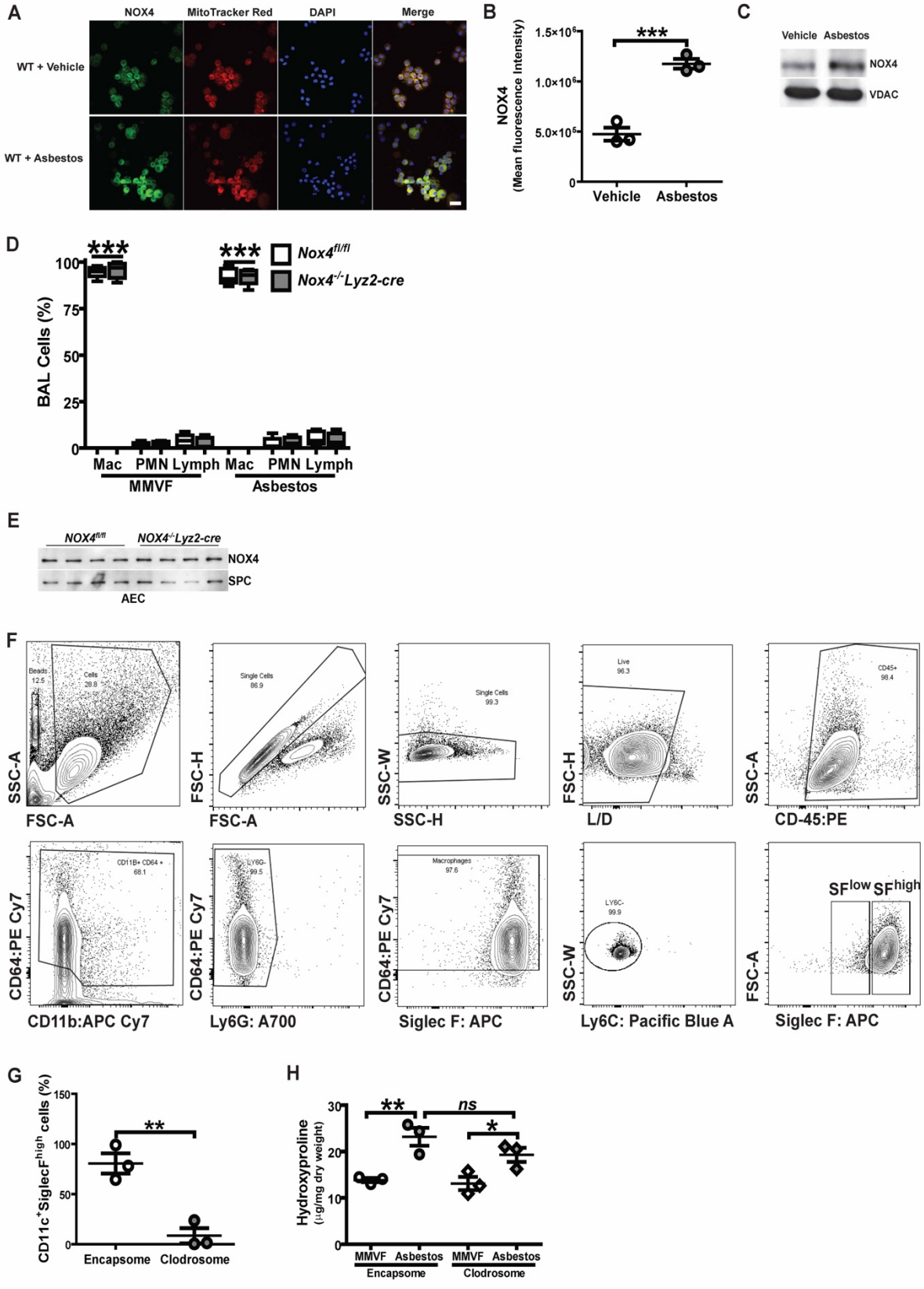
A
NOX4
MitoTracker Red
DAPI
Merge
WT + Vehicle
WT + Asbestos
B
NOX4 (Mean fluorescence Intensity)
1.5×10^6
1.0×10^6
5.0×10^5
0
***
Vehicle
Asbestos
C
Vehicle
Asbestos
NOX4
VDAC
D
BAL Cells (%)
100
75
50
25
0
***
***
Nox4fl/fl
Nox4-/-Lyz2-cre
Mac
PMN
Lymph
Mac
PMN
Lymph
MMVF
Asbestos
E
Nox4fl/fl
Nox4-/-Lyz2-cre
NOX4
SPC
AEC
F
Beads 12.5
Cells 28.8
SSC-A
FSC-A
Single Cells 86.9
FSC-H
FSC-A
Single Cells 99.3
SSC-W
SSC-H
Live 96.3
FSC-H
L/D
CD45+ 98.4
SSC-A
CD-45:PE
CD11B+ CD64 + 68.1
CD64:PE Cy7
CD11b:APC Cy7
LY6G- 99.5
CD64:PE Cy7
Ly6G: A700
Macrophages 97.6
CD64:PE Cy7
Siglec F: APC
LY6C- 99.9
SSC-W
Ly6C: Pacific Blue A
SFlow SFhigh
FSC-A
Siglec F: APC
G
CD11c+SiglecFhigh cells (%)
150
100
50
0
**
Encapsome
Clodrosome
H
Hydroxyproline (μg/mg dry weight)
30
20
10
0
**
ns
*
MMVF
Asbestos
MMVF
Asbestos
Encapsome
Clodrosome

Figure S1: NOX4 is expressed in monocyte-derived macrophages and mediates fibrosis.
(**A**) NOX4 expression is determined by confocal microscopy of lung macrophages from wildtype mice exposed to either MMVF or chrysotile asbestos. Scale bars, 20 μm. (**B**) Quantification of NOX4 fluorescence in lung macrophages. (**C**) Immunoblot for mitochondrial NOX4 in MH-S lung macrophages exposed to asbestos for 30 min. (**D**) Cell differential in bronchoalveolar lavage (BAL) cells from *Nox4*$^{fl/fl}$ and *Nox4*$^{-/-}$*Lyz2-cre* mice (n = 7) exposed to either MMVF or chrysotile asbestos. (**E**) Immunoblot of NOX4 in isolated alveolar epithelial cells from *Nox4*$^{fl/fl}$ and *Nox4*$^{-/-}$*Lyz2-cre* mice. (**F**) Gating strategy to separate monocytes-derived macrophages and tissue-resident macrophages in BAL. (**G**) Numbers of CD11c^{+}SiglecF^{high} cells in BAL (n = 3) after either encapsome (control) or clodrosome administration. (**H**) WT mice exposed to either encapsome or clodrosome first and then MMVF or asbestos 3 days later. Hydroxyproline analysis of homogenized lung (n = 3) from lung at day 21 from MMVF or asbestos exposure. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. Values shown as mean ± S.E.M. Two-tailed t-test or one-way ANOVA followed by Tukey's multiple comparison test was utilized. Each dot represents one human subject, one animal or one sample. All in vitro experiments including western blotting was repeated independently thrice and representative blots are shown.

Figure S2

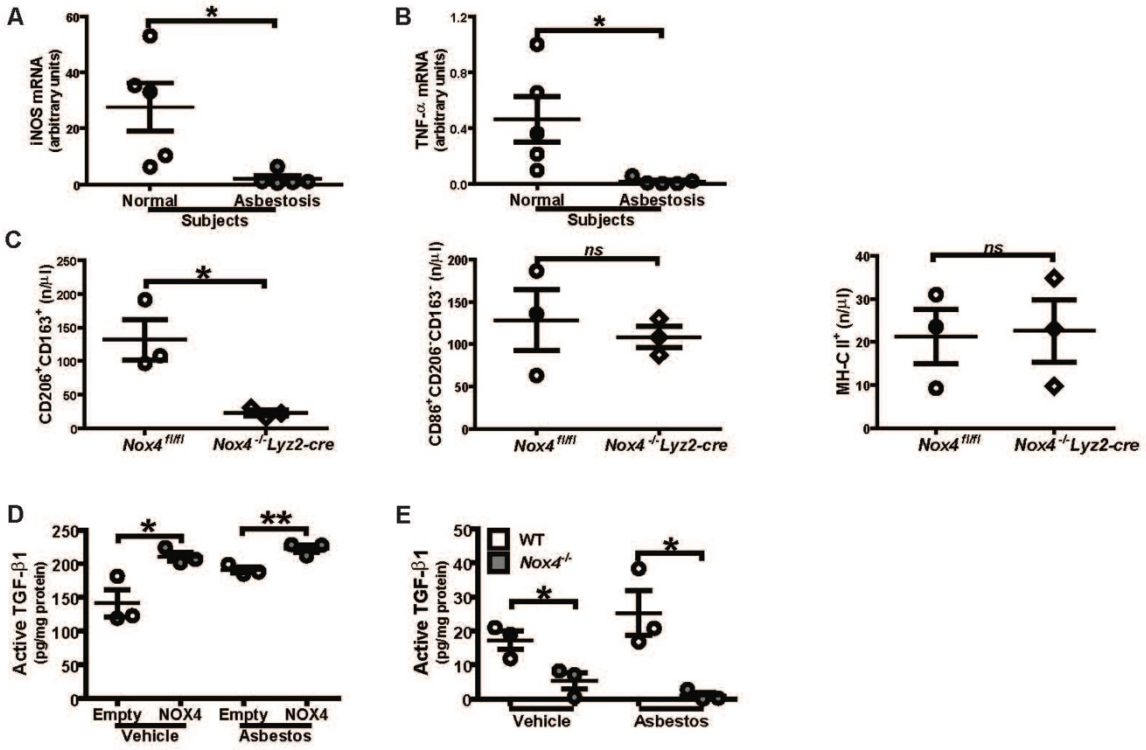

Figure S2: NOX4 regulates macrophage profibrotic polarization.
mRNA expression in BAL cells from normal (n = 5) or asbestosis subjects (n = 5) for (**A**) iNOS and (**B**) TNF-α. (**C**) Lung macrophages in BAL (n = 3) from *Nox4*$^{fl/fl}$ and *Nox4*$^{-/-}$*Lyz2-cre* mice exposed to chrysotile asbestos were analyzed by flow cytometry for CD206, CD163, CD68 and MHC-II expression. (**D**) Active TGF-β1 level in conditioned medium of MH-S lung macrophages expressing empty or NOX4. (**E**) Active TGF-β1 level in conditioned medium of bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. Values shown as mean ± S.E.M. Two-tailed t-test or one-way ANOVA followed by Tukey's multiple comparison test was utilized. Each dot represents one human subject, one animal or one sample. All in vitro experiments including western blotting was repeated independently thrice and representative blots are shown.

Figure S3

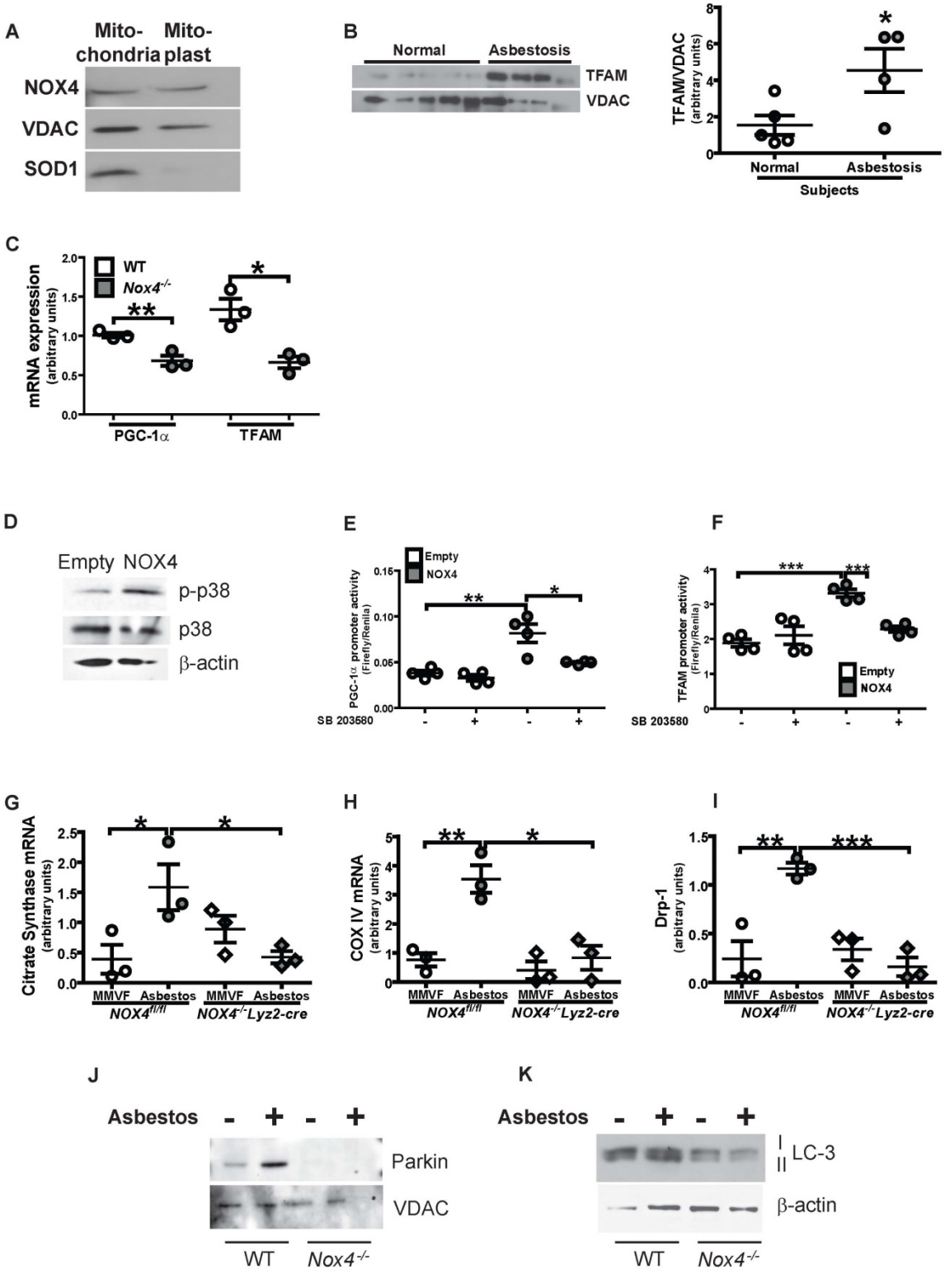
A
Mito-chondria
Mito-plast
NOX4
VDAC
SOD1
B
Normal
Asbestosis
TFAM
VDAC
TFAM/VDAC (arbitrary units)
Normal
Asbestosis
Subjects
C
WT
Nox4-/-
mRNA expression (arbitrary units)
PGC-1α
TFAM
D
Empty
NOX4
p-p38
p38
β-actin
E
Empty
NOX4
PGC-1α promoter activity (Firefly/Renila)
SB 203580
F
TFAM promoter activity (Firefly/Renila)
Empty
NOX4
SB 203580
G
Citrate Synthase mRNA (arbitrary units)
MMVF
Asbestos
NOX4fl/fl
NOX4-/-Lyz2-cre
H
COX IV mRNA (arbitrary units)
I
Drp-1 (arbitrary units)
J
Asbestos
Parkin
VDAC
WT
Nox4-/-
K
Asbestos
LC-3
I
II
β-actin
WT
Nox4-/-

Figure S3: NOX4 regulates mitochondrial biogenesis.
(**A**) Immunoblot of NOX4 in isolated mitochondrial compartment. (**B**) Immunoblot and quantification of TFAM in mitochondria of lung macrophages from normal (n = 5) or asbestosis subjects (n = 4). (**C**) PGC-1α and TFAM mRNA analysis of bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice. (**D**) Immunoblot of phos-p38, total p38 and β-actin in MH-S lung macrophages expressing either an empty or NOX4 vector. MH-S lung macrophages were transfected with (**E**) PGC-1α firefly luciferase vector or (**F**) TFAM firefly luciferase vector and either an empty or NOX4 vector and treated with SB203580 for 1 hr. Firefly and *Renilla* luciferase activities were measured. Results are shown as firefly luciferase normalized to *Renilla* luciferase. (**G**) Citrate synthase, (**H**) COX IV and (**I**) Drp-1 mRNA analysis of lung macrophages from *Nox4*$^{fl/fl}$ and *Nox4*$^{-/-}$*Lyz2-cre* mice (n = 3) exposed to either MMVF or chrysotile asbestos. (**J**) Immunoblot of Parkin and VDAC in bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice in the presence or absence of asbestos. (**K**) Immunoblot of JC-3 and β-actin in bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice in the presence or absence of asbestos.*, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. Values shown as mean ± S.E.M. Two-tailed t-test or one-way ANOVA followed by Tukey's multiple comparison test was utilized. Each dot represents one human subject, one animal or one sample. All in vitro experiments including western blotting was repeated independently thrice and representative blots are shown.

Figure S4

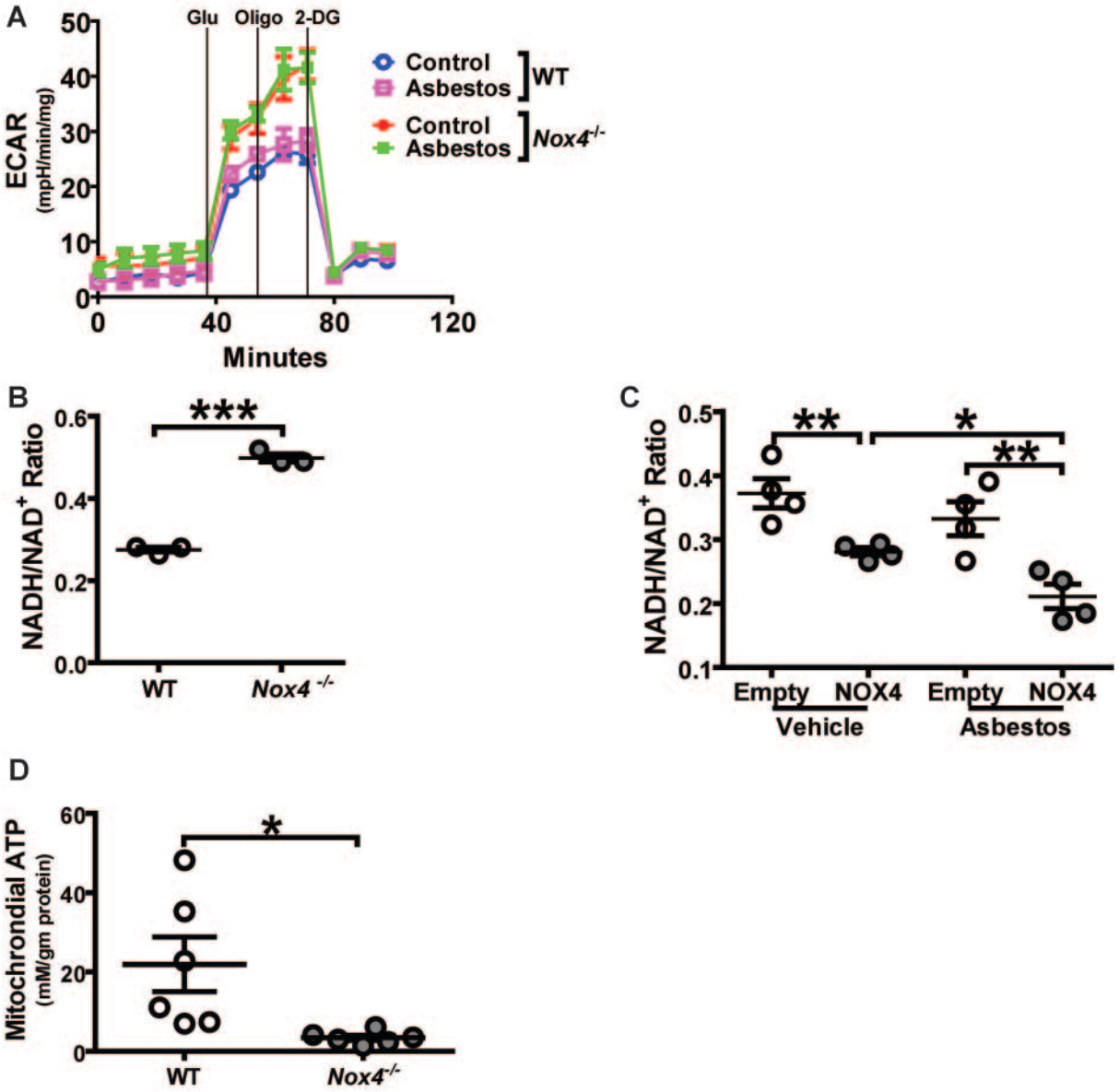

Figure S4: NOX4 regulates bioenergetics and mitochondrial homeostasis.
(**A**) Extracellular acidification rate (ECAR) tracing of bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice in the presence or absence chrysotile asbestos (n = 5). (**B**) NADH/NAD^{+} ratio measured in bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice. (n = 3) (**C**) NADH/NAD^{+} ratio measured in MH-S lung macrophages expressing either an empty or NOX4 vector. (**D**) ATP production in isolated mitochondria from bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice (n = 6). *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. Values shown as mean ± S.E.M. Two-tailed t-test or one-way ANOVA followed by Tukey's multiple comparison test was utilized. Each dot represents one human subject, one animal or one sample. All in vitro experiments including western blotting was repeated independently thrice and representative blots are shown.

Figure S5

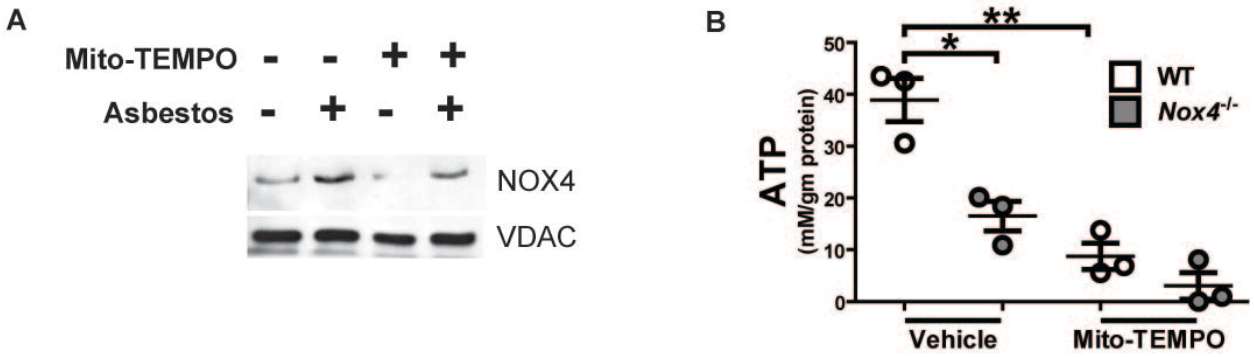

Figure S5: NOX4 is regulated in a redox-dependent manner.

(**A**) Immunoblot for mitochondria NOX4 in MH-S lung macrophages treated with Mito-TEMPO first and then exposed to chrysotile asbestos. (**B**) ATP production in isolated mitochondria from bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice in the presence or absence of Mito-TEMPO. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. Values shown as mean ± S.E.M. Two-tailed *t*-test or one-way ANOVA followed by Tukey's multiple comparison test was utilized. Each dot represents one human subject, one animal or one sample. All in vitro experiments including western blotting was repeated independently thrice and representative blots are shown.

Figure S6

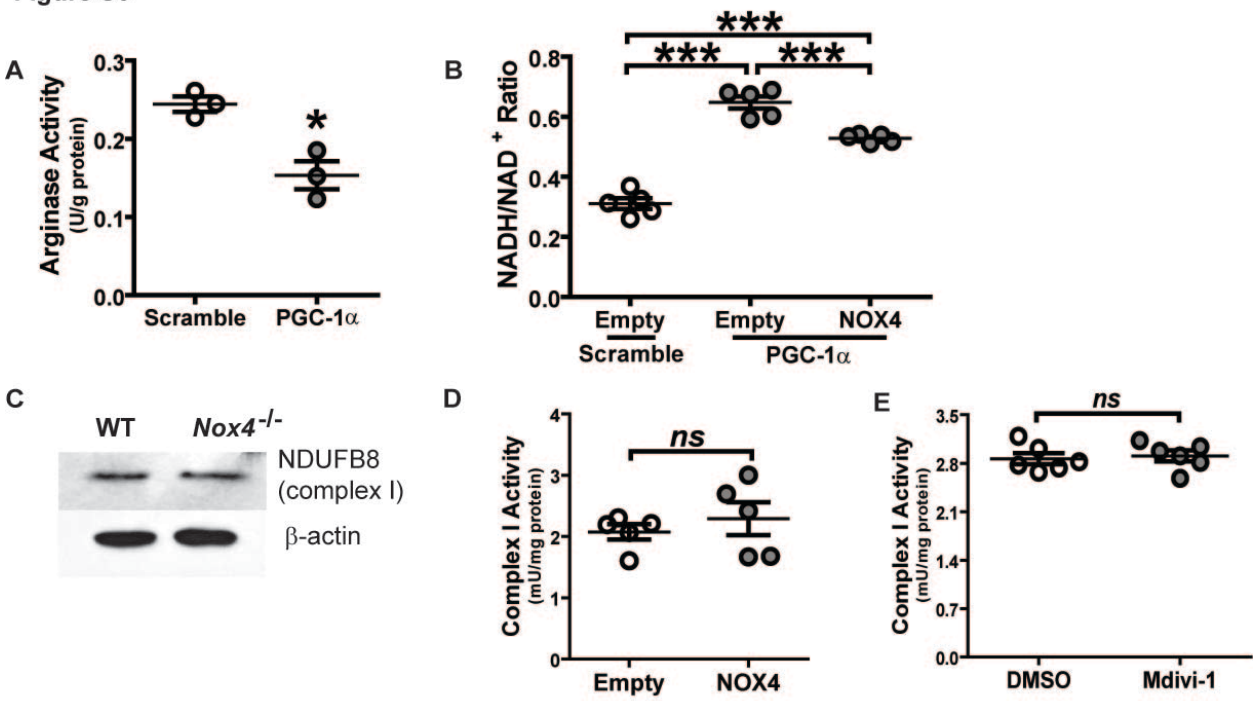

Figure S6: Modulation of mitochondrial biogenesis reprograms macrophage polarization.
(**A**) Arginase activity in MH-S macrophages transfected with either scramble or PGC-1α siRNA. (**B**) NADH/NAD^+ ratio in macrophages transfected with either scramble or PGC-1α siRNA together with empty or NOX4 vector. (**C**) Immunoblot for NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8 (NDUFB8) in bone marrow-derived macrophages from wildtype and *Nox4*$^{-/-}$ mice. (**D**) Complex I activity in MH-S lung macrophages expressing either an empty or NOX4 vector. 1 U complex I activity equals 1 mmol DCIP reduced per min. **(E)** Complex I activity in MH-S lung macrophages treated with or without 20 μM Mdivi-1. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. Values shown as mean ± S.E.M. Values shown as mean ± S.E.M. Two-tailed *t*-test or one-way ANOVA followed by Tukey's multiple comparison test was utilized. Each dot represents one human subject, one animal or one sample. All in vitro experiments including western blotting was repeated independently thrice and representative blots are shown.