

Electronic Supplementary Material

Low ambient oxygen prevents atherosclerosis

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Supplemental Material

Online Methods

List of PCR primers

Mouse mRNA RT-PCR:

EIF3F (TIF)

F 5'-CTGAGGATGTGCTGTCTGGGAA-3'

R 5'-CCTTTGCCTCCACTTCGGTC-3'

IL10

F 5'-GACCCTCAGGATGCGGCTGAG-3'

R 5'-CTAGGTCCTGGAGTCCAGCAGAC-3'

IFNG

F 5'-AGCCAAGACTGTGATTGCGG-3'

R 5'-CATTCGAGTGCTGTCTGGCCTG-3'

IL4

F 5'-GTCACAGGAGAAGGGACGCCATG-3'

R 5'-GTGCAGCTTATCGATGAATCCAG-3'

IL-17

F 5'-CCTCAGACTACCTCAACCGTTCC-3'

F 5'-GAAGGGGCAGCTCTCAGGCTC-3'

FOXP3

F 5'-CAGTGCGGAGGCACCTCTGG-3'

F 5'-GCTCAGGTTGTGGCGGATGG-3'

VEGF

F 5'-GTGACAAGCCAAGGCGGTGAG-3'

R 5'-GGCGAATCCAGTCCCACGAGG-3'

Human mRNA RT-PCR:

EIF3F (TIF)

F 5'-GACACAAGTCTCCAGAACGGC-3'

R 5'-TGGTCTCAAAGTCATCGGGAA-3'

IL10

F 5'-CCTGGGTTGCCAAGCCTTGTCTG-3'

R 5'-CGATGACAGCGCCGTAGCCTC-3'

Mouse ChIP PCR:

Nonspecific control (APOE)

F 5'-GCCTAGCCGAGGGAGAGCCG-3'

R 5'-TGTGACTTGGGAGCTCTGCAGC-3'

IL10_HRE1

F 5'-GAGTGTACCCTCTACATGGGTC-3'

R 5'-GTACCCTGGGCAAGCAACTAC-3'

IL10_HRE2

F 5'-GAACTCTCCTCTGACCAACTGCC-3'

R 5'-GTCACCTTAGCACTCAGTTACC-3'

IL10_HRE3

F 5'-CGGACGTTCAACCCAGGTTGAG-3'

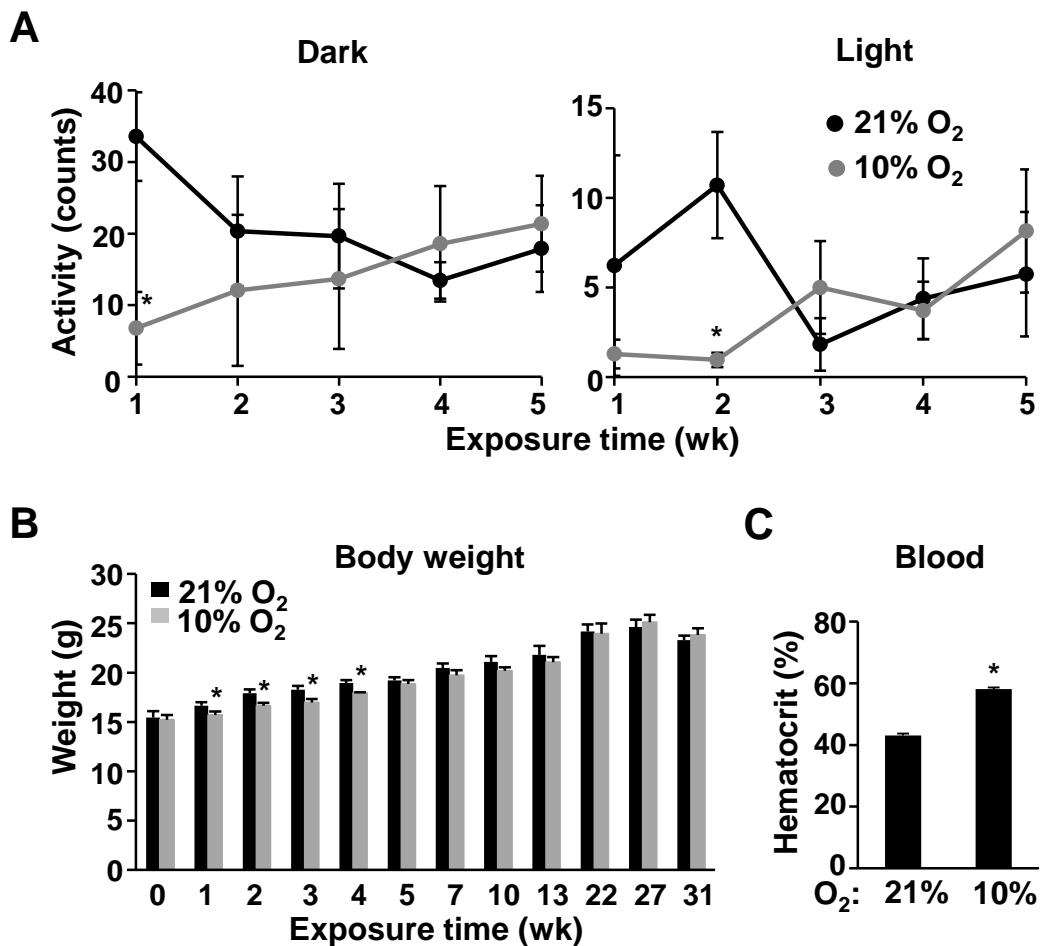
R 5'-CTGAGGCAGACAGCTGTTCTATG-3'

IL10_HRE4

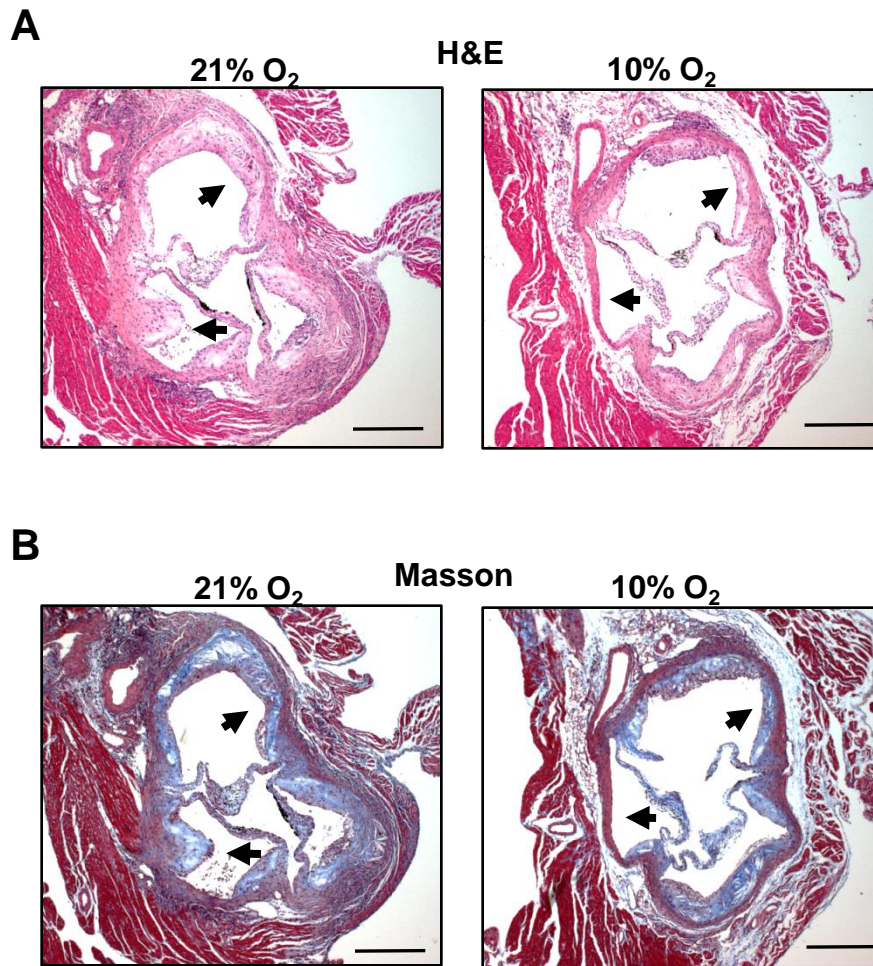
F 5'-GCATACCTTGTTTCGATTGGAG-3'

R 5'-CAGATTGCCCTACATGTAACAGC-3'

Supplemental Fig. 1

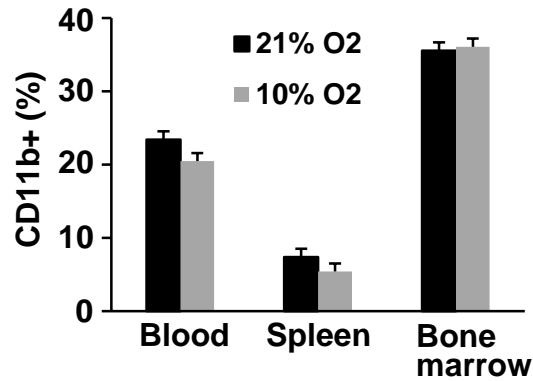


Supplemental Fig. 1. Acclimation of mice to low ambient oxygen. (A) Telemetry transmitters were surgically implanted into 14-wk old *ApoE*^{-/-} mice and allowed to recover for 2 wk after which they were maintained in either 10% or 21% O₂ ambient oxygen condition. Their average activity levels (counts) were monitored over a 5 wk acclimation period in the hypoxia chamber ($n=4$). (B) Mice (5 wk old) were chronically exposed to the indicated oxygen concentration and their body weights monitored over time ($n=4-20$). (C) Blood hematocrit levels were determined in mice exposed to 10% or 21% O₂ for 22 wk ($n=4$). Values shown as mean \pm SEM, $*p < 0.05$.



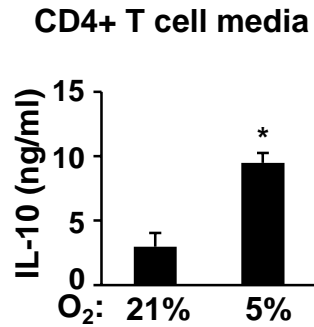
Supplemental Fig. 2. Effect of low ambient oxygen (10% O₂) compared to room air (21% O₂) on atherosclerotic plaque development. (A) H&E and (B) Masson trichrome collagen stained cross sections of the aortic root are shown at the valve leaflet insertion level. Note the decreased neointimal layer (arrowheads) under the 10% O₂ condition. Scale bars: 200 μ m.

Supplemental Fig. 3

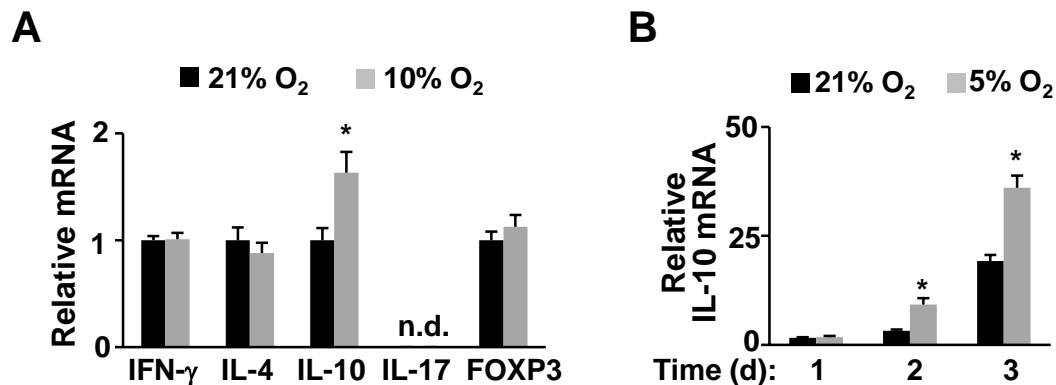


Supplemental Fig. 3. Low ambient oxygen has no significant effect on myeloid cell population. *ApoE*^{-/-} mice were exposed to the indicated ambient oxygen condition for 22-29 wk. Cell suspension were stained with myeloid cell marker CD11b and analyzed by flow cytometry ($n=10$). Values shown as mean \pm SEM.

Supplemental Fig. 4

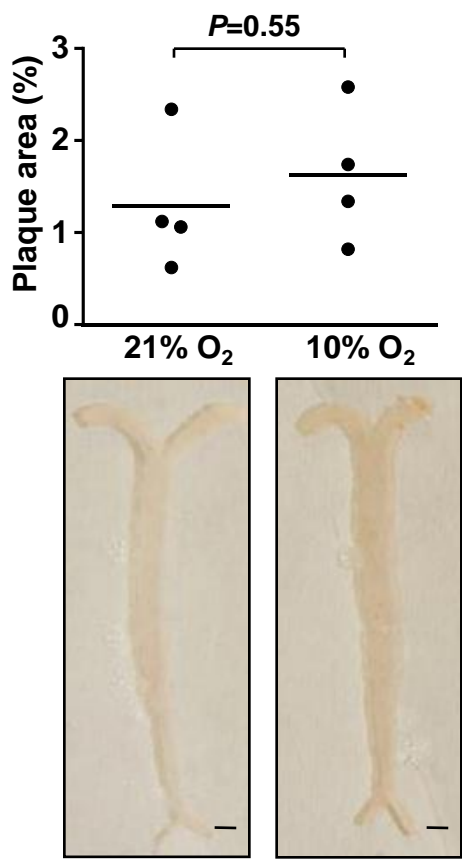


Supplemental Fig. 4. IL-10 released by *ApoE*^{-/-} mouse CD4+ T cells under low ambient oxygen *in vitro*. IL-10 released into media by CD4+ T cell cultured under 21% or 5% O₂ was measured by immunoassay ($n=6$). * $p < 0.05$.



Supplemental Fig. 5. Increased IL-10 expression in *ApoE*^{+/+} CD4⁺ T cells exposed to low oxygen *in vivo* and *in vitro*. (A) Mouse CD4⁺ T cells were isolated from spleens of *ApoE*^{+/+} mice exposed to the indicated ambient oxygen condition for 26 wk and the mRNA levels were measured by RT-PCR ($n=4$). IL-17 mRNA was not detectable by this assay (n.d.). (B) Effect of hypoxia (5% O₂, 1 to 3 d exposure) on IL-10 mRNA levels in cultured *ApoE*^{+/+} CD4⁺ T cells ($n=3$). Values shown as mean \pm SEM, * $p < 0.05$.

Supplemental Fig. 6



Supplemental Fig. 6. Effect of 3 wk exposure to low ambient oxygen. *ApoE*^{-/-} mice (6 wk old) were maintained in room air (21% O₂) or placed in a hypoxia chamber (10% O₂) for 3 wk prior to analyses. The aortas were longitudinally dissected and atherosclerotic plaque areas were quantified after staining with Sudan IV (*n*=4). Scale bars: 1 mm.