Science Advances
AAAS

Supplementary Materials for

Bypassing mitochondrial complex III using alternative oxidase inhibits acute pulmonary oxygen sensing

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**This PDF file includes:**

Figs. S1 to S4
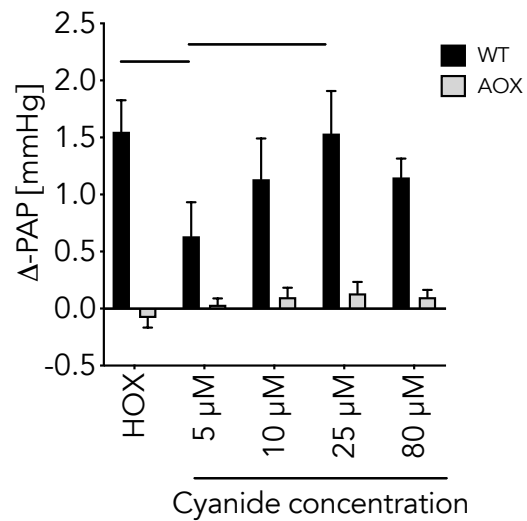
Fig. S1 (relates to Fig. 1). Pulmonary artery pressure (PAP) response of isolated, buffer-perfused WT and AOX murine lungs. HOX, hypoxia (1%), cyanide concentrations as indicated. Data are shown as mean ± SEM of n = 6 experiments. Horizontal bars denote statistically significant differences with *P* < 0.05 for comparison as indicated analyzed by 2way ANOVA and Tukey's multiple comparisons test.

A

| Biomarker	ID	Binding	Wavenumber	Red↑/Oxy↓	Reference
	1	v15	750 cm^{-1}	↑	(23-25)
	4	v22	1127 cm^{-1}	↑	(23, 24)
	6	v21	1313 cm^{-1}	↑	(23)
Cytochrome *c*	8	v4	1369 cm^{-1}	↓	(25)
	9	v3	1505 cm^{-1}	↑	(23)
	10	v37	1638 cm^{-1}	↓	(23)
Cytochrome *b*	7		1337 cm^{-1}	↑	(21)
Ubiquinol (QH$_2$)	5		1167 cm^{-1}	↑	(24)
NAD$^+$	3		1033 cm^{-1}	↑	(22)
NADH	2		1000 cm^{-1}	↓	(22)

B
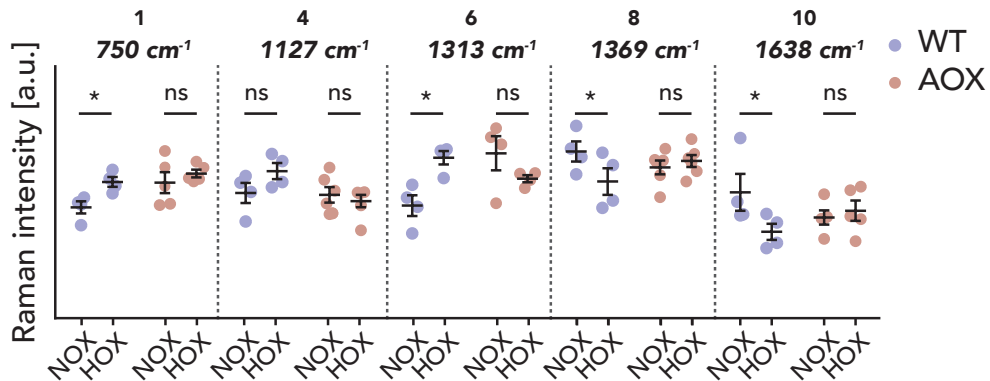
Fig. S2 (relates to Fig. 3). Redox state of mitochondrial biomarkers using Raman spectroscopy. (**A**) Table with assignment of the main peaks for the biomarkers as depicted in the Raman spectra in Fig. 3G. An upward arrow (↑) indicates reduced forms of the biomarkers, whereas a downward arrow (↓) highlights the oxidized form. (**B**) Statistical analysis over Raman intensity for the remaining wavenumbers associated with cytochrome c. Intensities shown as mean ± SEM of n ≥ 4 experiments. ns: not significant; *$P < 0.05$ analyzed by 2way ANOVA and uncorrected Fisher's LSD. Note, 1369 cm^{-1} and 1638 cm^{-1} are associated with the oxidized form of cytochrome c, which decreases upon hypoxic challenge in WT PASMC.
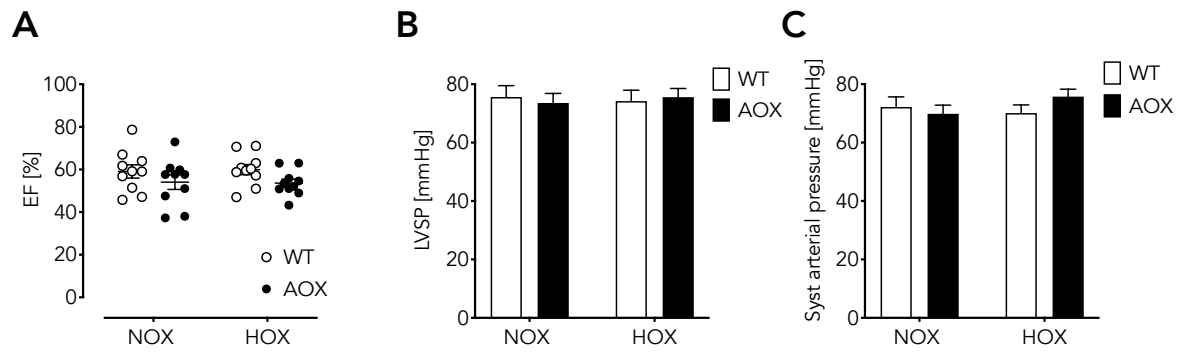
Fig. S3 (relates to Fig. 4). Systemic and left ventricular adaptations upon chronic hypoxia exposure. (A) Left-ventricular (LV) ejection fraction (EF). Data are shown as mean ± SEM of n = 10 experiments. **(B)** Left ventricular (LV) systolic pressure (SP). Data are shown as mean ± SEM of n = 7 experiments. **(C)** Systemic (Sys) arterial pressure. Data are shown as mean ± SEM of n = 8 experiments.
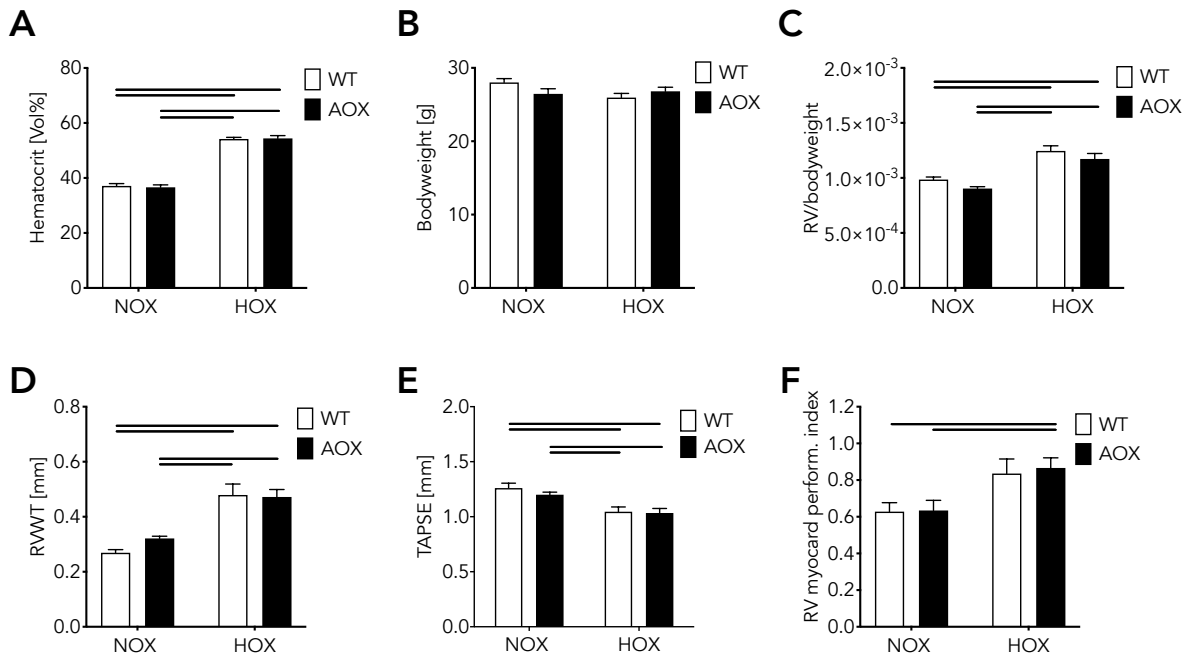
Fig. S4 (relates to Fig. 4). Systemic and right ventricular adaptations upon chronic hypoxia exposure. (**A**) Hematocrit to describe the volume percentage (Vol%) of red blood cells in blood. Data are shown as mean ± SEM of n = 10 experiments. Horizontal bars indicate significant difference with *P* < 0.05 analyzed by 2way ANOVA and Tukey's multiple comparisons test. (**B**) Bodyweight in gram. Data are shown as mean ± SEM of n = 10 experiments. (**C**) Right ventricular (RV) weight per bodyweight to describe the RV hypertrophy due to increased pulmonary blood pressure and lung remodeling. Data are shown as mean ± SEM of n = 10 experiments. Horizontal bars indicate significant difference with *P* < 0.05 analyzed by 2way ANOVA and Tukey's multiple comparisons test. (**D**) Right ventricular wall thickness (RVWT) in mm. Data are shown as mean ± SEM of n = 10 experiments. Horizontal bars indicate significant difference with *P* < 0.05 analyzed by 2way ANOVA and Tukey's multiple comparisons test. (**E**) Tricuspid annular plane systolic excursion (TAPSE) in mm. Data are shown as mean ± SEM of n ≥ 9 experiments. Horizontal bars indicate significant difference with *P* < 0.05 analyzed by 2way ANOVA and Tukey's multiple comparisons test. (**F**) Right-ventricular (RV) myocardial performance index. Data are shown as mean ± SEM of n ≥ 9 experiments. Horizontal bars indicate significant difference with *P* < 0.05 analyzed by 2way ANOVA and Tukey's multiple comparisons test.