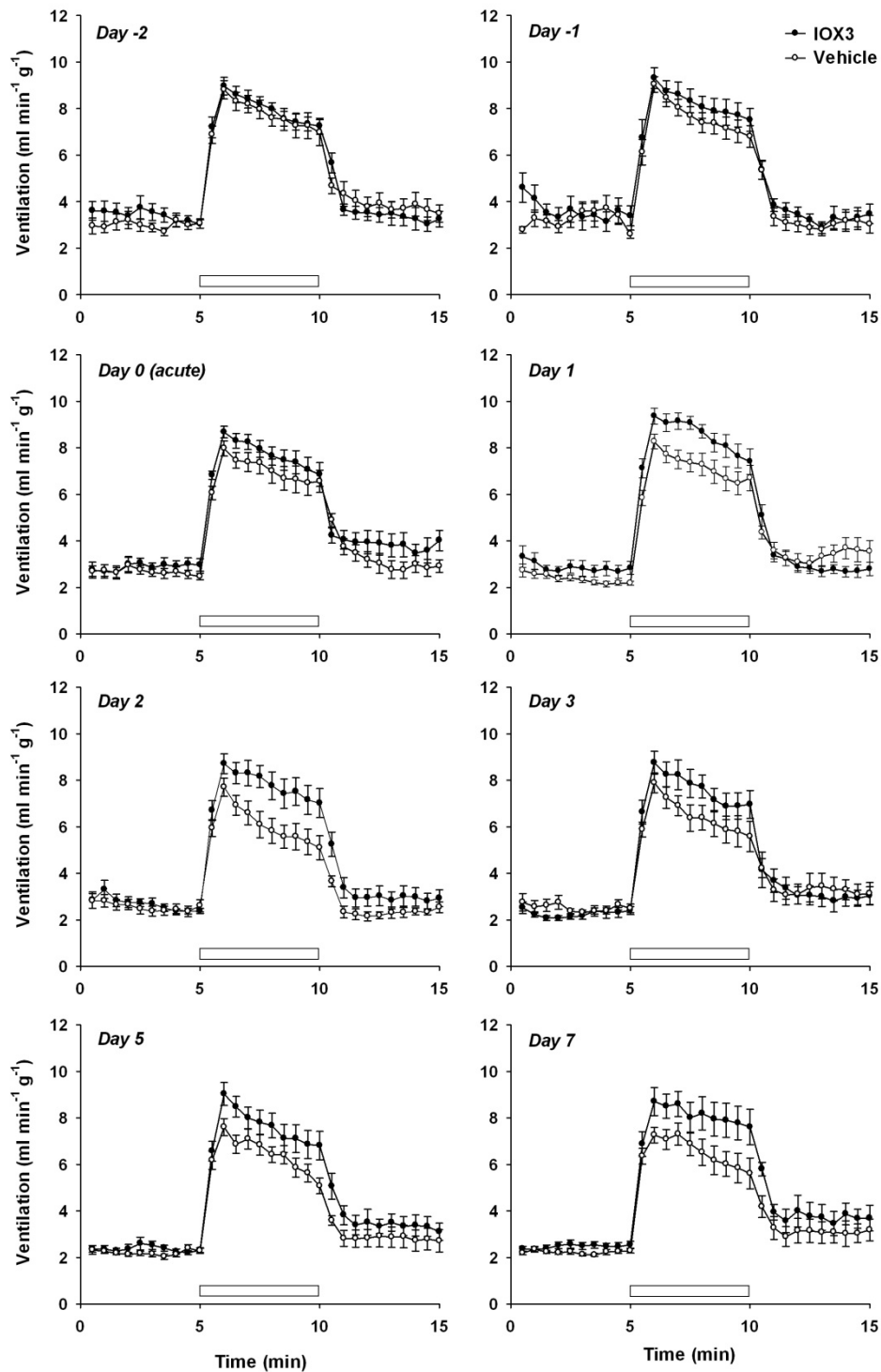


Supplementary figure 1: Dose-dependent effect of IOX3 on HIF α protein levels in the liver. IOX3 (or vehicle) was administered to male wild-type C57BL/6 mice by intraperitoneal injection at 15 – 60 mg kg⁻¹ and livers harvested 1 hr after treatment for immunoblotting with HIF-1 α and HIF-2 α antibodies (Novus Biologicals; PM9) as well as with β actin. HIF-1 α and HIF-2 α induction was near maximal at 30 mg kg⁻¹. This dose was chosen for twice daily injections over the 7 day period as the 60 mg kg⁻¹ dose was not tolerated by mice after multiple injections.



Supplementary figure 2: Effect of pharmacological PHD inhibition on ventilatory responses to acute hypoxia. Baseline responses were measured before IOX3 administration (days -1 and -2), within 90 min of the first dose of IOX3 (day 0) and on days 1, 2, 3, 5 and 7 after the onset of intraperitoneal administration of IOX3 at a dose of 30 mg kg⁻¹ twice daily, or vehicle control. On each day of study, mice breathed air within the chamber for at least 30 min before measurements; baseline ventilation was then measured over the next 5 min. Mice were then exposed to 5 min of 10% oxygen with 3% carbon dioxide (open bar), before returning to breathing air for a further 5 min. Values are mean \pm S.E.M.; n = 11 C57BL/6 animals in each group.

Baseline ventilation

	Pre-treatment (day -1)		Post-treatment (day 7)	
	Hypoxia	Normoxia	Hypoxia	Normoxia
Minute ventilation (ml min ⁻¹ g ⁻¹)	4.3 ± 0.4	4.8 ± 0.5	5.8 ± 0.3**	3.7 ± 0.3
Tidal volume (μl g ⁻¹)	15.0 ± 0.8	15.8 ± 0.9	15.9 ± 0.5	14.1 ± 1.0
Respiratory rate (breaths min ⁻¹)	278 ± 23	253 ± 18	373 ± 17**	275 ± 17

Hypoxic ventilatory response (HVR) to 10% oxygen

	Pre-treatment HVR (day -1)		Post-treatment HVR (day 7)		Change in HVR	
	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia
Minute ventilation (ml min ⁻¹ g ⁻¹)	1.0 ± 0.5	1.0 ± 0.5	4.0 ± 0.3**	1.7 ± 0.4	3.0 ± 0.5**	0.7 ± 0.6
Tidal volume (μl g ⁻¹)	3.5 ± 0.9	4.0 ± 0.8	8.0 ± 0.8*	5.1 ± 0.7	4.5 ± 1.3*	1.1 ± 0.7
Respiratory rate (breaths min ⁻¹)	15 ± 16	29 ± 34	38 ± 22	8 ± 34	23 ± 34	-20 ± 45

Hypoxic ventilatory response (HVR) to 10% oxygen, 3% carbon dioxide

	Pre-treatment HVR (day -1)		Post-treatment HVR (day 7)		Change in HVR	
	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia
Minute ventilation (ml min ⁻¹ g ⁻¹)	5.5 ± 0.4	4.5 ± 0.7	10.8 ± 0.3**	4.1 ± 0.7	5.3 ± 0.7**	-0.3 ± 0.9
Tidal volume (μl g ⁻¹)	12.0 ± 1.0	11.0 ± 1.0	20.6 ± 1.1**	10.9 ± 1.7	8.6 ± 1.1**	-0.2 ± 2.0
Respiratory rate (breaths min ⁻¹)	84 ± 38	47 ± 35	138 ± 12*	43 ± 31	54 ± 41	-5 ± 37

Supplementary table 1: Effect of chronic hypoxia on hypoxic ventilatory response (HVR). Tidal volume and respiratory rate were measured in awake animals using individual whole body plethysmographs. Baseline ventilation and HVR was measured before (day -1) and after (day 7) exposure to 7 days of 10% oxygen or room air (normoxia). On each day of study, mice breathed air within the chamber for at least 30 min before measurements; baseline ventilation was then measured over the next 5 min, before mice were exposed to 5 min of 10% oxygen, with or without the addition of 3% carbon dioxide. HVR is the difference between the relevant ventilatory parameter (minute ventilation, tidal volume or respiratory rate) during the 1 min prior to onset of hypoxia and the first 1 min of stable hypoxia (i.e. excluding the first 30 seconds after switching to hypoxic gas delivery). Values are mean ± S.E.M.; n = 8 C57BL/6 animals in each group. Comparisons between chronic hypoxia and normoxic control group were made with unpaired *t*-tests **p*<0.05, ***p*<0.01.

Baseline ventilation

	<i>PHDI</i> ^{-/-}	Wild-type	<i>PHD2</i> ^{+/-}	Wild-type	<i>PHD3</i> ^{-/-}	Wild-type
Minute ventilation (ml min ⁻¹ g ⁻¹)	3.6 ± 0.4	3.1 ± 0.2	5.4 ± 0.6	4.7 ± 0.4	2.9 ± 0.4	3.8 ± 0.4
Tidal volume (μl g ⁻¹)	12.6 ± 0.5	12.1 ± 0.7	16.2 ± 1.1	13.7 ± 0.7	13.1 ± 1.2	12.8 ± 0.6
Respiratory rate (breaths min ⁻¹)	300 ± 38	275 ± 15	341 ± 41	359 ± 30	227 ± 18	317 ± 44

Hypoxic ventilatory response (HVR) to 10% oxygen

	<i>PHDI</i> ^{-/-}	Wild-type	<i>PHD2</i> ^{+/-}	Wild-type	<i>PHD3</i> ^{-/-}	Wild-type
Minute ventilation (ml min ⁻¹ g ⁻¹)	–	–	2.7 ± 0.7	1.3 ± 0.5	–	–
Tidal volume (μl g ⁻¹)	–	–	6.7 ± 0.8	5.3 ± 0.6	–	–
Respiratory rate (breaths min ⁻¹)	–	–	11 ± 35	-35 ± 38	–	–

Hypoxic ventilatory response (HVR) to 10% oxygen, 3% carbon dioxide

	<i>PHDI</i> ^{-/-}	Wild-type	<i>PHD2</i> ^{+/-}	Wild-type	<i>PHD3</i> ^{-/-}	Wild-type
Minute ventilation (ml min ⁻¹ g ⁻¹)	4.9 ± 0.3	4.2 ± 0.4	8.4 ± 0.7**	5.0 ± 0.8	3.1 ± 0.6	3.8 ± 0.7
Tidal volume (μl g ⁻¹)	10.3 ± 0.6	10.6 ± 0.9	18.8 ± 1.3*	12.9 ± 2.0	6.4 ± 1.0	9.0 ± 0.9
Respiratory rate (breaths min ⁻¹)	140 ± 6*	58 ± 29	97 ± 45	47 ± 42	79 ± 44	36 ± 47

Supplementary table 2: Effect of genetic PHD deficiency on hypoxic ventilatory response (HVR). Tidal volume and respiratory rate were measured in awake animals using individual whole body plethysmographs. Mice breathed air within the chamber for at least 30 min before measurements; baseline ventilation was then measured over the next 5 min, before mice were exposed to 5 min of 10% oxygen, or 10% oxygen with 3% carbon dioxide. HVR is the difference between the relevant ventilatory parameter (minute ventilation, tidal volume or respiratory rate) during the 1 min prior to onset of hypoxia and the first 1 min of stable hypoxia (i.e. excluding the first 30 seconds after switching to hypoxic gas delivery). Values are mean ± S.E.M.; n = 5-7 littermate pairs in each group. *PHD2*^{+/-} mice were on a pure C57BL/6 genetic background. *PHDI*^{-/-} and *PHD3*^{-/-} mice were on a mixed Swiss/129SvEv background. Comparisons between *PHD*-deficient and wild-type groups were made with unpaired *t*-tests **p*<0.05, ***p*<0.01.

Baseline ventilation

	Pre-treatment (day -1)		Post-treatment (day 7)	
	Hypoxia	Normoxia	Hypoxia	Normoxia
Minute ventilation (ml min ⁻¹ g ⁻¹)	5.9 ± 0.3	5.7 ± 0.5	5.7 ± 0.2	4.8 ± 0.4
Tidal volume (μl g ⁻¹)	15.3 ± 0.3	14.9 ± 0.4	17.4 ± 0.1	15.2 ± 0.6**
Respiratory rate (breaths min ⁻¹)	400 ± 16	395 ± 33	326 ± 13	329 ± 26

Hypoxic ventilatory response (HVR) to 10% oxygen

	Pre-treatment HVR (day -1)		Post-treatment HVR (day 7)		Change in HVR	
	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia
Minute ventilation (ml min ⁻¹ g ⁻¹)	2.0 ± 1.0	2.2 ± 1.1	3.2 ± 0.8	1.7 ± 0.5	1.2 ± 0.8	-0.5 ± 0.8
Tidal volume (μl g ⁻¹)	6.7 ± 0.6	7.1 ± 1.0	5.2 ± 0.8	5.9 ± 0.6	-1.5 ± 0.5	-1.2 ± 0.8
Respiratory rate (breaths min ⁻¹)	-33 ± 58	-15 ± 53	68 ± 35	-38 ± 28*	101 ± 54	-22 ± 66

Hypoxic ventilatory response (HVR) to 10% oxygen, 3% carbon dioxide

	Pre-treatment HVR (day -1)		Post-treatment HVR (day 7)		Change in HVR	
	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia
Minute ventilation (ml min ⁻¹ g ⁻¹)	7.9 ± 0.6	8.3 ± 0.8	8.3 ± 0.6	6.6 ± 0.7	0.4 ± 0.8	-1.7 ± 1.0
Tidal volume (μl g ⁻¹)	17.7 ± 0.9	19.1 ± 1.1	17.0 ± 0.4	16.9 ± 1.2	-0.7 ± 1.2	-2.2 ± 1.6
Respiratory rate (breaths min ⁻¹)	66 ± 30	98 ± 44	67 ± 33	45 ± 39	1 ± 47	-52 ± 65

Supplementary table 3: Effect of chronic hypoxia on hypoxic ventilatory response (HVR) in PHD2 deficient animals. Tidal volume and respiratory rate were measured in awake *PHD2*^{+/±} animals using individual whole body plethysmographs. Baseline ventilation and HVR were measured before (day -1) and after (day 7) exposure to 7 days of 10% oxygen or room air (normoxia). On each day of study, mice breathed air within the chamber for at least 30 min before measurements; baseline ventilation was then measured over the next 5 min, before mice were exposed to 5 min of 10% oxygen, with or without the addition of 3% carbon dioxide. HVR is the difference between the relevant ventilatory parameter (minute ventilation, tidal volume or respiratory rate) during the 1 min prior to onset of hypoxia and the first 1 min of stable hypoxia (i.e. excluding the first 30 seconds after switching to hypoxic gas delivery). Values are mean ± S.E.M.; n = 5 C57BL/6 animals in each group. Comparisons between chronic hypoxia and normoxic control group were made with unpaired *t*-tests **p*<0.05, ***p*<0.01.

Baseline ventilation

	Pre-treatment (day -1)		Post-treatment (day 0)		Post-treatment (day 7)	
	IOX3	Vehicle	IOX3	Vehicle	IOX3	Vehicle
Minute ventilation (ml min ⁻¹ g ⁻¹)	4.5 ± 0.5	3.8 ± 0.3	3.6 ± 0.3	2.8 ± 0.2	3.0 ± 0.3	3.0 ± 0.3
Tidal volume (μl g ⁻¹)	12.7 ± 0.5	12.5 ± 0.5	12.2 ± 0.3	11.5 ± 0.5	12.0 ± 0.4	11.4 ± 0.3
Respiratory rate (breaths min ⁻¹)	358 ± 37	317 ± 23	302 ± 23	251 ± 20	252 ± 21	271 ± 26

Day 0: Hypoxic ventilatory response (HVR) to 10% oxygen, 3% carbon dioxide

	Pre-treatment HVR (day -1)		Post-treatment HVR (day 0)		Change in HVR	
	IOX3	Vehicle	IOX3	Vehicle	IOX3	Vehicle
Minute ventilation (ml min ⁻¹ g ⁻¹)	5.5 ± 0.4	5.7 ± 0.3	5.5 ± 0.4	5.2 ± 0.3	0.0 ± 0.7	-0.5 ± 0.4
Tidal volume (μl g ⁻¹)	13.5 ± 0.9	13.5 ± 1.0	13.4 ± 1.1	12.3 ± 1.2	-0.1 ± 1.1	-1.2 ± 1.2
Respiratory rate (breaths min ⁻¹)	68 ± 30	96 ± 15	83 ± 22	100 ± 19	15 ± 45	5 ± 18

Day 7: Hypoxic ventilatory response (HVR) to 10% oxygen, 3% carbon dioxide

	Pre-treatment HVR (day -1)		Post-treatment HVR (day 7)		Change in HVR	
	IOX3	Vehicle	IOX3	Vehicle	IOX3	Vehicle
Minute ventilation (ml min ⁻¹ g ⁻¹)	5.5 ± 0.4	5.7 ± 0.3	6.1 ± 0.6	4.9 ± 0.3	0.6 ± 0.5*	-0.9 ± 0.3
Tidal volume (μl g ⁻¹)	13.5 ± 0.9	13.5 ± 1.0	13.5 ± 1.1	11.2 ± 0.9	0.0 ± 0.7*	-2.3 ± 0.7
Respiratory rate (breaths min ⁻¹)	68 ± 30	96 ± 15	129 ± 14	115 ± 9	61 ± 35	19 ± 15

Supplementary table 4: Effect of pharmacological PHD inhibition on hypoxic ventilatory response (HVR). Tidal volume and respiratory rate were measured in awake animals using individual whole body plethysmographs. Baseline ventilation and HVR were measured before administration of the PHD inhibitor IOX3 (day -1), within 90 min of the first dose of IOX3 (day 0) and after 7 days of intraperitoneal IOX3 at a dose of 30 mg kg⁻¹ twice daily, or vehicle control. On each day of study, mice breathed air within the chamber for at least 30 min before measurements; baseline ventilation was then measured over the next 5 min before mice were exposed to 5 min of 10% oxygen with 3% carbon dioxide. HVR is the difference between the relevant ventilatory parameter (minute ventilation, tidal volume or respiratory rate) during the 1 min prior to onset of hypoxia and the first 1 min of stable hypoxia (i.e. excluding the first 30 seconds after the switch to hypoxic gas delivery). Values are mean ± S.E.M.; n = 11 C57BL/6 animals in each group. Comparisons between IOX3 and vehicle-treated groups were made with unpaired *t*-tests **p*<0.05, ***p*<0.01.