

Supporting Information

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SI Materials and Methods

Htc Adjustments. *Wild type + wtNESP.* NESP [Aranesp (darbepoietin alfa); Amgen] of 3.125 $\mu\text{g}/\text{kg}$ to 12.5 $\mu\text{g}/\text{kg}$ (wtNESP) or saline (0.9%) was s.c. injected twice a week to increase and maintain the Htc levels. To this end, the animals were anesthetized with 7–8% sevoflurane (Sevorane; Abbot) in pure O_2 to avoid uncontrolled moving of the blood pressure catheter (1). After the injections, blood samples (10 μL) were taken from the tail vein. **tg6 + tg6PHZ.** Htc was adjusted to a range between 0.3 and 0.9 by s.c. administration of freshly prepared phenylhydrazine (PHZ) that causes chemical hemolysis (2, 3). PHZ hydrochloride (Sigma P6926) was prepared as described in ref. 2. Tg6 animals received two PHZ injections (0.125–1.2 mg/10 g of body weight; tg6PHZ) or saline (0.9%) separated by 2 days after the first injection to decrease the Htc. To maintain lower Htc levels, PHZ was injected at a concentration between 0.065 and 0.5 mg/10 g of body weight every third day. Mice were anesthetized and blood samples were taken after as described above.

To exclude organ failure due to PHZ administration, organs of tg6 and tg6PHZ mice were morphologically evaluated in a preliminary study. No cardiac, gastric, hepatic, renal, muscular, neuronal, or pulmonary degeneration was found.

Surgical Procedures: Splenectomy (tg6 Mice Only) and Implantation of Telemetric Transmitter. Inhalation anesthesia was induced as described above. Anesthesia was maintained with 3.5–4% sevoflurane (1). Preliminary experiments showed that decreased Htc levels in our tg6 mice recovered within days after the PHZ injection. Compared to wild-type mice, tg6 mice showed that massive extramedullary erythropoiesis occurred in the spleen (4, 5). To maintain constant Htc levels, the spleens of 3-week-old tg6 males were removed by left-side-abdominal laparotomy as described in ref. 5. One week later, blood pressure sensors were implanted in tg6 and wild-type mice of the same age. After shaving and disinfecting the neck, the left common carotid artery was isolated (6). The transmitter's catheter was inserted into the artery and pushed forward until the tip just entered the thoracic aorta. The transmitter body was fixed under the skin. Splenectomy and blood pressure transmitter implantation were carried out under aseptic conditions. Mice were allowed to recover for 2 weeks. Measurements were performed by using a TA11PA-C10 transmitter (DataSciences International). Data were generated by using Dataquest A.R.T 3.0 software (DataSciences International).

Measurements. Exercise tests. The exercise tests were performed on an Instrument Simplex II metabolic rodent treadmill (Columbus Instruments) connected to an OxyMax gas analyzer (Columbus Instruments). This system enables the measurement of $\dot{V}\text{O}_2$ and carbon dioxide production ($\dot{V}\text{CO}_2$). Respiratory exchange ratio (RER) was calculated as $\dot{V}\text{CO}_2/\dot{V}\text{O}_2$. Before performing each exercise test, the gas analyzer was calibrated with high-precision standard gas mixture. Mice were gently encouraged to run on the belt until exhaustion with the use of a mild electric shock from the shock grid at the end of the treadmill (0.2 mA, pulse 200 ms, 1 Hz).

To determine $\dot{V}\text{O}_{2\text{max}}$, systolic blood pressure, mean arterial blood pressure, and heart rate were telemetrically monitored at rest and during exercise. Stroke volume was assessed indirectly by using O_2 pulse that—at least in healthy humans—correlates closely with the stroke volume during effort (7, 8). O_2 pulse was calculated as the ration of $\dot{V}\text{O}_{2\text{max}}$ divided by heart rate. As an indicator of myocardial $\dot{V}\text{O}_2$, rate pressure product (RPP) was

calculated from the multiplication of heart rate and systolic blood pressure.

Ten-microliter blood samples were taken 3 h before initiation of the exercise test from the tail vein for the Htc determination. Mice were placed on individual treadmill lines. After acclimation to their surroundings for at least 1 h, basal heart rate, blood pressure, $\dot{V}\text{O}_2$, and $\dot{V}\text{CO}_2$ were monitored over the last 5 min of this period. Mice began running at 2.5 m/min and 0° inclination for 10 min. The intensity was then increased by 2.5 m/min and 2.5° every 3 min thereafter until exhaustion. Exhaustion was defined as the inability to continue regular treadmill running despite the repeated stimulus to the mice. $\dot{V}\text{O}_{2\text{max}}$ was achieved when $\dot{V}\text{O}_2$ did not increase in spite of an increase in workload. For reported values at maximal exercise, 1-min averages of blood pressure and heart rate were taken during the last minute of exercise, whereas for $\dot{V}\text{O}_{2\text{max}}$ the highest 1-min interval was considered. From the maximal value rate, pressure product and O_2 pulse were calculated. After 24 h at rest, all mice performed a constant workload exercise test to exhaustion. The workload was set to 80% of the maximal attained workload of the incremental exercise set. Before performing the time-to-exhaustion test, 10- μL blood samples were taken at rest for the Htc analysis and the animal was warmed up for 10 min at 20% followed by an additional 10 min at 40% of the maximal attained power output of the $\dot{V}\text{O}_{2\text{max}}$ test.

Terminal measurements. The day after performing the constant workload test, mice were anesthetized with a s.c. injection of a mixture of 100 mg/kg ketamine (Ketasol-100; Graebub), 20 mg/kg xylazine (Rompun; Bayer), and 3 mg/kg acepromazine (Sedalin; Chassot). Catheters were introduced into the left femoral artery and vein. Arterial blood was collected in a heparinized capillary 35 min after the injection of the anesthesia, and the arterial acid-base status and SaO_2 was immediately measured. Plasma volume and blood volume were measured by the injection of Evans blue directly after blood sampling for the arterial acid-base status and SaO_2 determination. Twenty minutes later, animals were bled to death to measure blood viscosity, blood volume, plasma volume, Htc, total hemoglobin mass, and [Hb].

Blood analysis. Htc of heparinized blood was measured in duplicate by using a microcentrifuge (Autokrit II; Pharmap). [Hb] were determined by using Abbott Cell-Dyn 3500 (Abbott Diagnostic). Total hemoglobin mass was calculated from the [Hb] and blood volume. SaO_2 was evaluated by using a gas analyzer (AVL Compact 3; AVL List).

Quantification of blood volume has been described (5). Briefly, 10 μL of Evans blue solution (1% in saline) was injected into a femoral vein catheter. Twenty minutes after the injection, 10- μL samples of blood were drawn into heparinized capillaries. Absorbance of the dye in the plasma volume was read at 620 nm with a NanoDrop spectrometer. Evans blue concentrations were derived from a calibration curve and used to calculate plasma volume. Blood volume was calculated from plasma volume and Htc.

Blood viscosity was measured in heparinized blood samples with a rotation viscosimeter DV-II+PRO (Brookfield Engineering Laboratories) and Rheocalc software (Brookfield Engineering Laboratories) as described in ref. 5. However, because of the non-Newtonian fluid characteristics of blood, only blood temperature at 37°C and shear rates of 450 s^{-1} were compared.

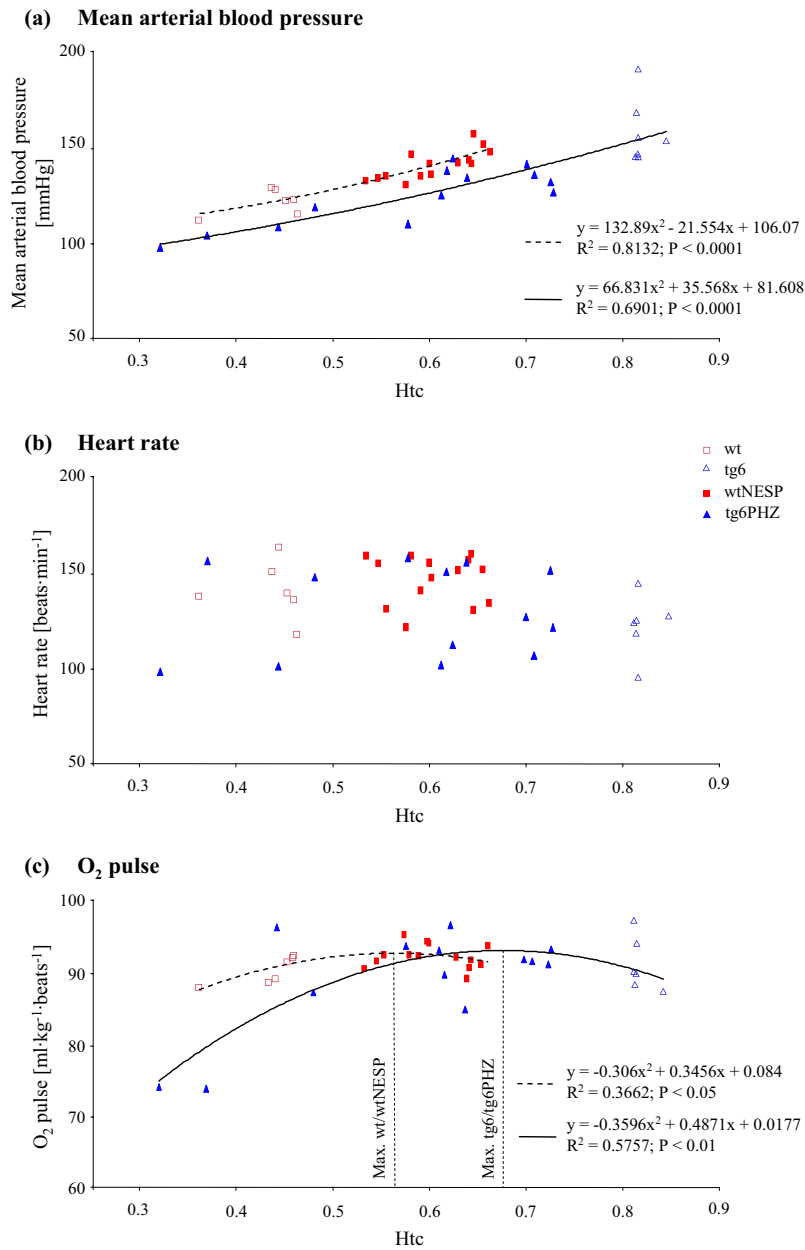


Fig. S3. Relationship between hematocrit (Htc) and mean arterial blood pressure (a), heart rate (b), and O₂ pulse (c) at maximal O₂ uptake (VO_{2max}) in wtNESP and tg6PHZ mice. Singles prints represent individual values. —, regression plot of wild-type (wt)/wtNESP; —, regression plot of tg6/tg6PHZ. Also depicted are the maximal O₂ pulse values of wt/wtNESP (Max. wt/wtNESP) and tg6/tg6PHZ (Max. tg6/tg6PHZ).

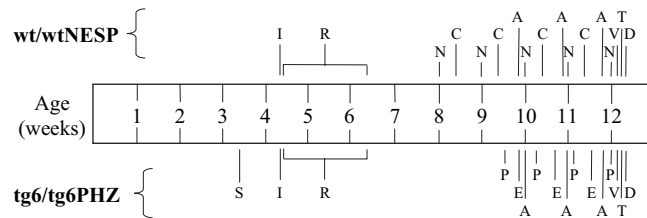


Fig. S4. Timeline of animal age for the experimental set-up. S, splenectomy; I, implantation of telemetric blood pressure transmitter; R, postoperative recovery; N, NESP injection and blood sampling; C, NESP injection; P, phenylhydrazine injection and blood sampling; E, phenylhydrazine injection; A, treadmill adaptation; V, incremental exercise test; T, constant workload test; D, terminal measurements; wt, wild type.

Table S1. Weight, age, and cardiac and metabolic parameters at baseline before performing an incremental exercise test

Type	Weight, g	Age, d	Htc, mL·kg ⁻¹ ·min ⁻¹	$\dot{V}O_2$	RER	MAP, mmHg	Heart rate, beats·min ⁻¹
wt/wtNESP	25.0 ± 1.5	84.1 ± 5.2	0.46 ± 0.03	48.1 ± 2.7	0.81 ± 0.01	108.3 ± 6.7	513.8 ± 38.8
tg6/tg6PHZ	24.9 ± 1.6	86.9 ± 4.0	0.78 ± 0.06	50.1 ± 3.5	0.82 ± 0.01	103.4 ± 14.7	525.6 ± 55.0

Note that, routinely, the body weight was measured before hematocrit (Htc) manipulation. Values represent means ± SD. MAP, mean arterial blood pressure; RER, respiratory exchange ratio; $\dot{V}O_2$, O₂ uptake; wt, wild type.