SU5416 preparation and administration

To prepare SU5416 solution, 5 mg SU5416 was dissolved in 0.5 mL 0.5% cmc (carboxymethylcellulose). Subsequently, the tube was placed on a plate shaker for ~2 hours to dissolve and homogenize the solution. Within 3 hours after preparation of the SU5416 solution, it was administered in a dose of 25mg/kg subcutaneously in the neck.

Telemetry implantation

Implantation surgery was performed as described previously [12]; a telemetry transmitter TA11PA-C40, (DSI, St. Paul, MN) was implanted by a transdiaphragmatic approach allowing insertion of the tip of the catheter into the RV, while the transmitter body was placed in the abdomen (survival rate was ~90%). Animals were allowed to recover for one week prior the induction of SuHx.

Telemetry acquisition and analyses

The transmitter emits data to a receiver which is connected to the acquisition computer. Throughout the entire study, 1-minute acquisition bins of ~200-400 cardiac beats were collected every hour (DSI, St. Paul, MN). Every data bin used underwent a quality assessment before averaging. The morphology of the blood pressure waveforms was visually assessed **(supplemental figure 1)**. Signals were only used when clear from technical deviations, which can be induced by electrical interference, (e.g. spikes derived from electromagnetic fields from the surroundings), signal dampening (e.g. due to clot formation on the tip of the catheter) and unexpected offset changes (e.g. due to kinking of the fluid filled catheter). When the signal did not meet the quality criteria, data from that time point onwards was excluded from further analysis, as recommended [17, 25, 26]. Parameters derived from telemetric

monitoring were RVSP and Heart Rate (HR). Due to the sensitivity to gravity of the system, which may cause a pressure deviation of maximally four mmHg, RV diastolic pressure is not reported.

To account for circadian rhythm-induced changes, the 10PM bin of RVSP data was used for daily RVSP representation. Animals are active at this time and not affected by the possible stress due to biotechnical handlings. For assessing the reversible component of the RVSP increase, every week animals were briefly (5 minutes) exposed to 60% oxygen plus anesthesia in a small single-rat anesthesia induction chamber. 1% isoflurane was given for animal welfare reasons. The 4 subsequent 1 minute acquisition bins during the hyperoxic exposure were compared with the last RVSP acquisition bin measured prior to hyperoxia exposure (measured in the conscious animal in his 'home cage'). To assess daily circadian ranges in RVSP, maximum and minimum one-minute RVSP averages were determined and their difference, defined as delta RVSP ($\Delta RVSP_{max\text{-}min}$), was averaged over a week. RVSP did not show a clear sinusoid day/night rhythm (7AM to 7PM light/dark cycle), but multiple fluctuations in pressure throughout the day. The incidence of fluctuations was not different between groups (control **(supplemental figure 2A)**, hypoxia **(supplemental figure 2B)** and SuHx **(supplemental figure 2C)**) or between time point (before hypoxia, at end of hypoxia and at end of study). However, the magnitude of the fluctuations was significantly different between groups and time points.

Histological and morphometric analyses

Four µm slides of lung tissue were prepared and stained with Elastica van Gieson, for specific coloration of the elastic laminae, and scanned (3DHISTECH, Budapest, Hungary). A field of ~ 5 billion μm^2 on every slide was fully evaluated. Because media

hypertrophy and neo-muscularisation manifest during the development of PAH, small arteries and arterioles were divided into three classes, based on external diameters. The ranges of these classes were chosen to allow distinction of effect size and generation diameters of the pulmonary arteries in rats, as described by Hislop et al. [15]: <30 µm vessels represent pre-capillaries (intra-acinar, neomuscularisation and intima remodeling), 30-60 µm vessels represent alveolar duct or respiratory bronchiole arteries (intra-acinar, medial hypertrophy and intima remodeling) and 60-100 µm vessels represent terminal bronchiole (axial, pre-acinar) arteries. Concordant to human PAH, we hypothesized no changes in the latter class of vessels [18]. Only vessels with an approximate circular profile were included. Media and intima wall thickness were measured as described previously [4, 9, 16]. In each vessel, the diameter of the external elastic lamina, the diameter of the internal elastic lamina and the diameter of the lumen were determined. These values were used to calculate the relative medial wall thickness and intima thickness. Media thickness was measured *in duplo* by calculating (100*(diameter external elastic lamina– diameter internal elastic lamina))/(diameter external elastic lamina)[16]. Intima thickness was measured twice in each vessel by calculating (100*(diameter internal elastic lamina - diameter lumen)/(diameter external elastic lamina)). Hence, media and intima thickness are represented as averages. Completely obliterated vessels were also observed and included in the analyses. Representative images **(supplemental figure 3)** show vascular lesions in rows during wk0 through wk7.

Necropsy

Animals were exsanguinated under anesthesia after blood was taken to measure hematocrit. Lungs and hearts were weighed after separation of the heart into RV and left ventricle plus septum (LV+S). Tissues were fixed in formalin and embedded in

paraffin. To preserve the integrity of the telemetry catheter, it was not possible to determine the RV/(LV+S) of the animals with a telemetry catheter inserted. From the non-telemetry animals, RVSP was measured using a Millar pressure catheter (Millar,Houston,TX, USA), as published before [12] **(supplemental figure 4)**. The left lung lobe was inflated with low-melt 0.5% agarose on 25 cm H2O pressure, fixed in formalin and embedded in paraffin. Four µm slides of lung tissue were prepared and stained with H&E and EvG and scanned (3DHISTECH, Budapest, Hungary).