SUPPLEMENTAL MATERIAL AND METHODS:

Measurement of the $\Delta\Psi m$ in live PASMCs (37°C) was performed using a FV1000 confocal microscope equipped with a live cell apparatus (Olympus, Center Valley, PA). Mitochondrial membrane potential was determined using 10nM tetramethylrhodamine methyl-ester perchlorate (TMRM, Invitrogen Branchburg, NJ, USA). Nuclei were stained using 50 nM Hoechst 33342 (Invitrogen Branchburg, NJ, USA). Between 100 and 150 cells were measured by experiment, in 3 experiments.

Proliferation and apoptosis measurements: Apoptosis was measured using Apoptag apoptosis detection kit (TUNEL; Millipore, Temecula, CA). Proliferation was measured by the proliferating cell nuclear antigen PCNA antibody from (1/400, DAKO, Carpinteria, CA) and Ki67 antibody (1/400, Millipore) according to the manufacturer's instructions[16, 34]. The percentage of positive PASMCs for TUNEL or Ki67 was determined by the number of cells showing nuclear staining over the total number of cell. The percentage of PCNA positive cell was determined using a threshold based on the intensity obtained in control-PASMCs. This threshold was applied in all the other groups and cells showing a nuclear fluorescent intensity superior to the threshold were considered as positive. Between 100 and 150 cells were measured by experiment, in 3 experiments by cell line, in 5 control- and 3 PA-PASMCs cell lines.

Nuclear translocation assay: In PASMCs, protein nuclear localizations were measured in at least 100 to 150 cells by experiment, in 3 experiments. The percentage of positive cell was determined using a threshold based on the intensity obtained in control-PASMCs. This threshold was applied in all the other groups and cells showing a nuclear fluorescent intensity superior to the threshold were considered as positive. The same analysis was performed in rats, in 10 distal arteries (<100μm) /rat in 5 rats per groups.

Cell migration assays were performed using the QCM^{TM} 24-Well Insert Colorimetric Cell Migration Assay kit from Millipore following manufacturer instructions. Cells were transfected with siRNA or treated with PF228, PP2 (or the appropriate control PP3) 48 hours before performing the assay. Then, cells were trypsinized and 2.10^5 cells were plated in each insert in absence of FBS. 10% FBS in the lower chamber was used as chemoattractant. Plates were incubated 24h at 37°C, 4-6% CO2. After 24h, media was removed and inserts were then placed in a staining solution.

Inserts were washed and non-migratory cells layers from the interior of inserts were removed using a cotton-tipped swab. The stain was extracted from the underside by gently tilting inserts back and forth several times in an extraction buffer during 15 min. Optical densities (absorption) of the stained solutions were measured at 560nm and are relative to the number of cells on the underside, i.e having migrated.

Wound healing assay by scratch test: Cells were plated (2.10^5 cells by well) in a 24 well plate and transfected with siRNA or treated with PF228, PP2 (or the appropriate control PP3) 48 hours before performing the assay. Cell monolayers were wounded using a 10uL pipette tip. Wells were photographed just after scraping and 24h after. Pictures were analyzed using ImageJ software to define the number of invasive cell over the wound area.

Immunoblots were performed as previously described[16, 34]. Protein expression of PY705-STAT3, STAT3, PY419-Src, PY576-FAK, PY397-FAK, FAK (1/1000 Cell Signaling), c-Src (1/1000, Santa Cruz) were quantified and normalized to smooth muscle actin (1/500, Santa Cruz) as previously described[16]. Phosphorylated forms were obtained from the same membranes after 30min stripping at 50 degrees.

Hemodynamic measurements:

Rats were initially anesthetized with 3–4% isoflurane and maintained with 2% during procedures. All rats underwent hemodynamic and echocardiography studies as previously described[16, 35]. Right heart catheterizations (closed chest) were performed using Sciscence catheters to directly measure PA pressures. Longitudinal and non-invasive evaluations of PAH was performed by echocardiography (using Vevo 2100 VisualSonics equipment) as previously described[16 35]. Euthanasia was performed by exsanguinations.

Treadmill:

Exercise capacity has been tested by measuring maximal distance run on a motorized treadmill (Simplex II Instrument; Columbus Instruments, Columbus, OH). The initial treadmill speed was 10 m/min and increased 2m/min every 3 min until the rat fatigued.

Histology measurements: % media wall thickness was assessed as previously described[16]. Briefly, lungs slides were stained with H&E, and distal PAs <400μm wall

thickness was measured using image Proplus software (Media Cybernetics, Inc USA). Two measurements by artery, in at least in 5 to 10 distal arteries per rat, in 5 rats, were performed.

Muscularization: Lung sections (5µm) were stained for smooth muscle actin and von Willebrand Factor (endothelial cell maker). Vessels (<50µm) were classified as fully (100%), partially, or nonmuscularized (0%), based on the percentage of vWF surrounded by SMA in each vessel as previously described[35].

SUPPLEMENTAL TABLE 1
Table S1. Patients providing tissue

	Tissues	Patient type	Sex	Age	Mean PA pressure (mmHg)	Medications	PVR (dyne*sec)/cm^5
1	Lung	Control	M	35	ND	None	ND
2	Lung	Control	F	43	ND	None	ND
3	Lung	Control	F	47	ND	None	ND
4	Lung	Control	F	50	ND	None	ND
5	Lung	PAH group1	M	72	39	Lasix/Sitaxsentan	11.7
6	Lung	PAH group1	F	68	37	Lasix/coumadin/Norvasc	544
7	Lung	PAH group1	F	51	41	Lasix	990
8	Lung	PAH group1	F	51	51	Lasix/coumadin	1199

SUPPLEMENTAL FIGURES LEGEND:

Supplemental Figure S1: (A) FAK is hyperphosphorylated in PAH-PASMCs.

FAK activation and phosphorylation on Y397 and Y576 (showing FAK full kinase activity) have been evaluated by immunofluorescence in PAH_PASMCs compared to control-PASMCs *in vitro*. 100 and 150 cells were measured by experiment, in 3 experiments by cell line. Significance $p<0.001$ ***.

(B) Transfection efficiencies.

siRNA were transfected at 20nmol.L^{-1} final concentration with $CaCl_2$. Western Blot showing efficiencies of transfection are presented for both siFAK and siSTAT3.

Spplemental Figure S2: PY576-FAK is increased in distal PAs of human PAH.

Levels of PY576-FAK were studied in human PAH- and Control-patients (4 of each) using immuno-staining on lung section. No significative differences were observed between controls (n=20 to 30 arteries by patient) but an increased of PY576-FAK has

been observed in all PAH-patients compare to controls (n=4 in each group). Moreover, PY576-FAK levels (n=20 to 30 arteries by patient) are also increased with the severity of the disease assessed by pulmonary vascular resistances (PVR). Significance $p<0.01$ by ** and $p<0.001$ by ***.

Supplemental Figure S3: PY397 and PY576-FAK are increased in resistance PAs of MCT-PAs rats and inhibited by siFAK or PF-228 treatments.

Levels of PY397 (Green, top panel) and PY576-FAK (Green, bottom panel) phosphorylations were evaluated by immuno-staining on lung rat sections. Arteries were stain by SMA (Pink). Mean data of fluorescence intensities (fluorescent units FU) measured in arteries (n=10 to 20 arteries per rat, in 5 rats) are presented on graphs on the right. FAK inhibition by either siRNA or PF-228 significantly decreased levels of FAK phosphorylation compare to MCT-PAH or MCT-PAH+siSCR rats. Significance $p<0.01$ by ** and $p<0.001$ by ***.

Supplemental Figure S4: PY397 and PY576-FAK are increased in PAs of MCT-PAs rats and inhibited by siFAK or PF-228 treatments.

Levels of PY397 and PY576-FAK phosphorylations were evaluated by immuno-blots performed on proteins extracted from isolated PAs. Representative pictures and ratio phosphorylated forms/total forms normalized to SM-Actin are presented (n=3 rats in each groups). Significance $p<0.05$ by * and $p<0.01$ by **.

Supplemental Figure S5:

(A) FAK inhibition decreases STAT3 activation in vivo in distal PAs- Western Blot on PAs.

STAT3 activation was evaluated by immunoblots performed on rats PAs extracts with the ratios phosphorylated forms/total forms and PY705-STAT3/STAT3 normalized to SM-Actin (n=3 in each groups). Significance $p<0.05$ by * and $p<0.01$ by **.

B) FAK inhibition decreases STAT3 activation in vivo in distal PAs - Immunostainning on lung section.

STAT3 inhibition by the therapeutic intervention was further confirmed by nuclear translocation assay, showing a decrease in nuclear PY705-STAT3 (n=10 to 20 arteries per rats in 5 rats in each groups). Significance $p<0.01$ by ** and $p<0.001$ by ***.

Supplemental Figure S6: FAK inhibition increased PAH-PASMCs mitochondrial dependent apoptosis and decreased PAH-PASMCs proliferation

Histology corresponding to mean data presented in Figure 6. FAK inhibition by both siRNA, PF228 and PP2 in PAH-PASMC reverses ΔΨm hyperpolarization (TMRM, 1st row), decreased resistance to apoptosis in serum starved PAH-PASMCs (TUNEL positive cells, 2d row) and decreased PAH-PASMC proliferation (Ki67 3rd row and PCNA 4th row).

Supplemental Figure S7: FAK inhibition treatments decreased PAECs proliferation in vivo.

Improvement of PAH condition in rats treated with FAK inhibitors is not associated with significant changes in proliferation (% Ki67 (green) positive cells) or apoptosis (TUNEL) of PAECs (stained in red by vWF). (n=10 to 20 arteries per rats in 5 rats in each groups). Significance $p<0.05$ by *.