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Supplemental Data

Deletion of *STAT5a/b* in Vascular Smooth Muscle Abrogates the Male-Bias in Hypoxic Pulmonary Hypertension in Mice: Implications in the Human Disease

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Supplementary Table S1. Primers used in the qPCR analyses of tail DNA (as in Fig. S1).

Name of Primer	Primer sequence 5′→3′	Primer type
STAT5 flox primer 1	5′-GAA AGC ATG AAA GGG TTG GAG-3′	For wild type mice
STAT5 flox primer 2	5′-AGC AGC AAC CAG AGG ACT AC-3′	Common primer (for wt & floxed)
STAT5 flox primer 3	5′-AAG TTA TCT CGA GTT AGT CAG G-3′	For STAT5 floxed allele
Cre 1 (forward)	5′-GCG GTC TGG CAG TAA AAA CTA TC-3′	For Cre transgene
Cre 2 (reverse)	5′-GTG AAA CAG CAT TGC TGT CAC TT-3′	For Cre transgene
Cre 3 (internal control forward)	5′-CTA GGC CAC AGA ATT GAA AGA TCT-3′	Control for positive PCR reaction
Cre 4 (internal control reverse)	5′-GTA GGT GGA AAT TCT AGC ATC ATC C-3′	Control for positive PCR reaction

STAT5 primers were from Cui *et al.* (25). Cre primers were as designated by The Jackson Labs.

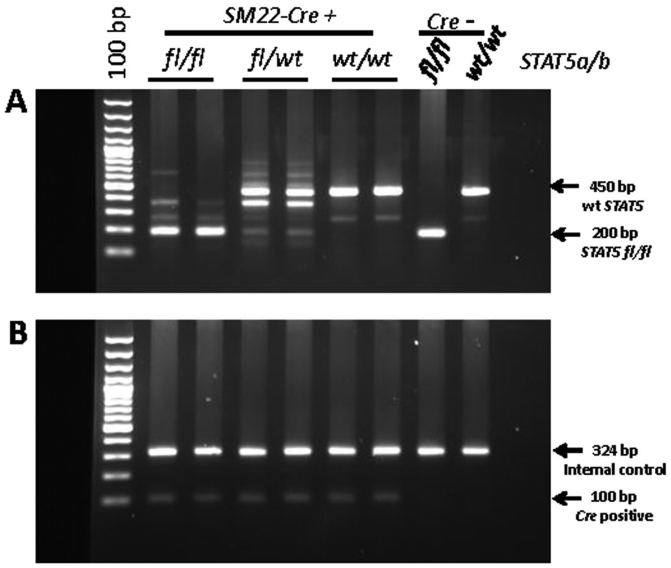
Supplementary Table S2. Patient materials from PHBI.

Lab ID#		Cell lines[1]	Age (year)	STAT5 in art/lesions	PAP S/D/M[2]
Lung sections: MALES					
5	CTRL		27	+	
6	CTRL		18	+	
7	CTRL		60	+	
8	CTRL		54	+	
21	CTRL	EC #107	21	+	
22	CTRL	SMC #102	12	+	
1	IPAH		7	↓	143/82/105
2	IPAH	SMC #73	56	↓	100/45/65
3	IPAH		24	↓	113/70/88
4	IPAH		18	↓	76/61/67
23	IPAH	SMC #74	14	+/-	106/48/72
24	IPAH	SMC #66	27	+/-	90/51/68
9	HPAH	c.1285_1286insGATTG	35	↓	65/34/48
10	HPAH	no BMPR2 mutation	47	↓	82/49/63
Lung sections: FEMALES					
15	CTRL		29	+	
16	CTRL		52	+	
17	CTRL		44	+	
18	CTRL	SMC #54	1	+	
25	CTRL	SMC #109	51	+	
26	CTRL	SMC EC #101	60	NC[3]	
11	IPAH	SMC EC #36	46	↓	102/32/62
12	IPAH		29	↓	60/25/37
13	IPAH		48	↓	84/43/56
14	IPAH		26	↓	134/56/87
27	IPAH	SMC #25	39	↓	76/40/47
19	HPAH	no BMPR2 mutation	56	+/-	110/55/75
20	HPAH	R491W	34	+/-	87/44/61
ADDITIONAL CELLS FROM:					
MALES					
	CTRL	SMC EC #99	27		
	CTRL	SMC EC #103	26		
	CTRL	SMC EC #104	26		
	IPAH	SMC EC #90	41		114/55/76
	IPAH	SMC #82	26		94/42/59
	IPAH	SMC EC #95	40		118/49/73
	IPAH	SMC EC #100	51		41/19/30
FEMALES					
	CTRL	SMC EC #98	37		
	CTRL	EC #106	56		
	CTRL	SMC #41	28		
	IPAH	SMC EC #105	26		69/33/47
	IPAH	SMC #85	57		83/39/57
	IPAH	SMC #89	28		95/50/65

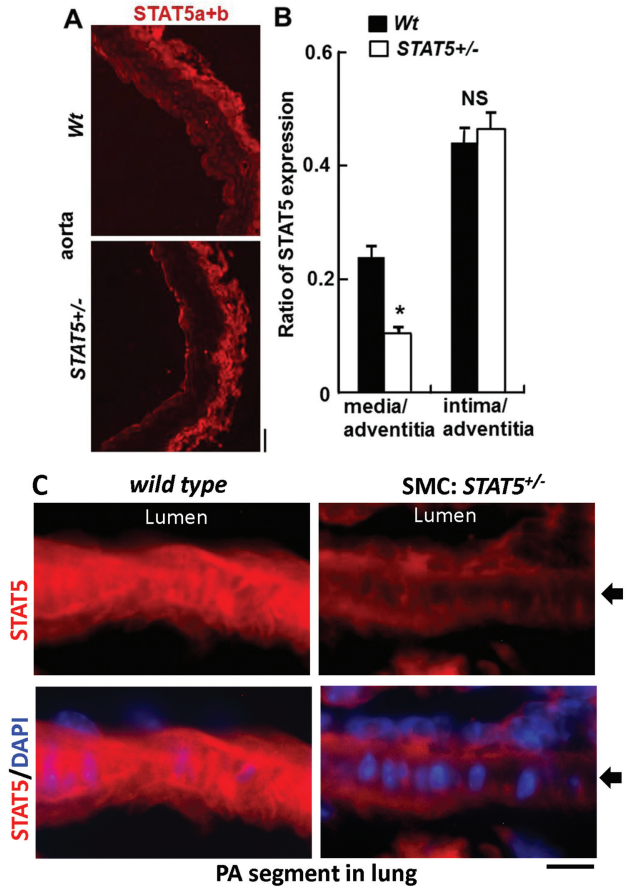
[1]Derived SMC and EC cell lines as designated, and *BMPR2* status in HPAH; [2]Pulmonary artery pressure: systolic/diastolic/mean in mm Hg; [3]NC, not clear.

Supplementary Table S3. Primers used in the real-time RT-PCR analyses of RNA transcripts (as in Fig. S3C).

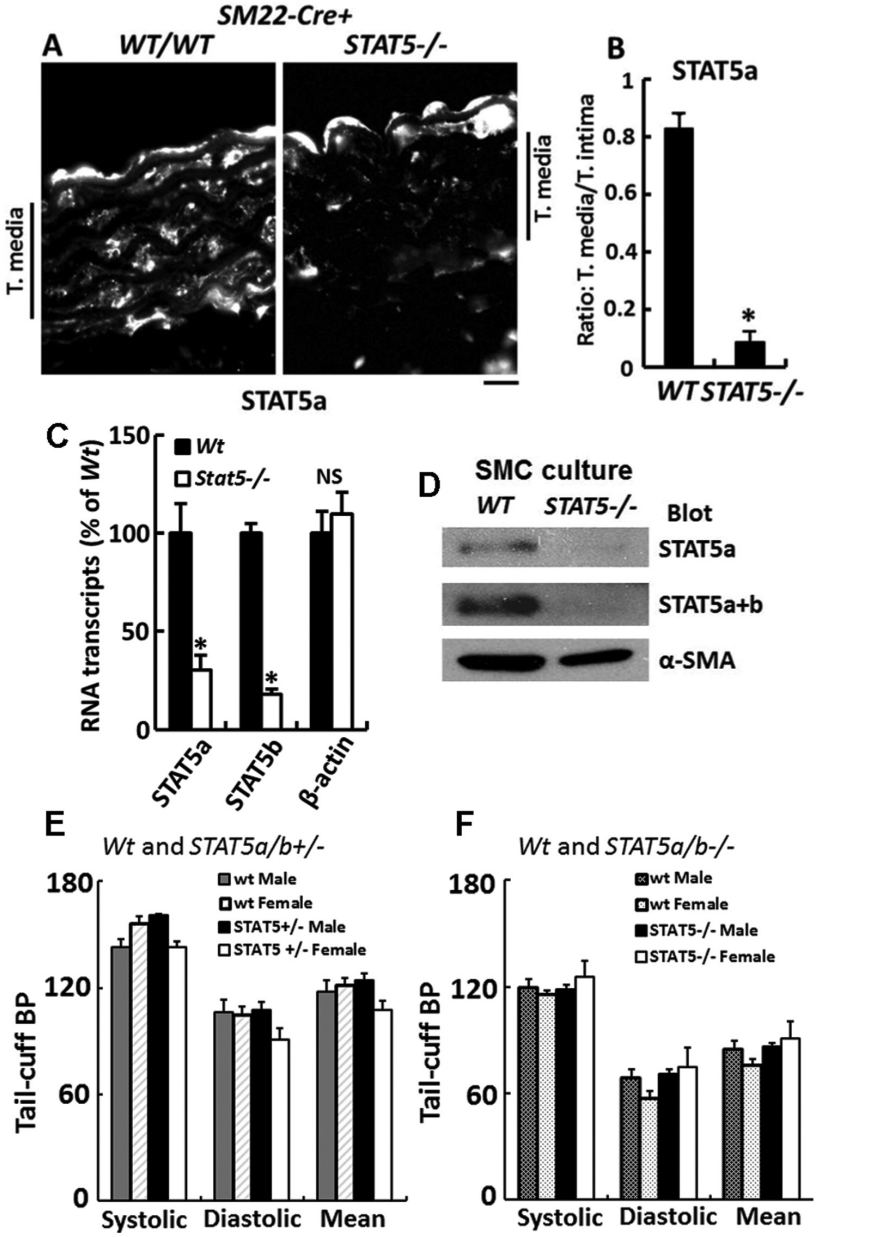
Name	Primer	Product length	Annealing temp
mStat5a Forward	5′-GTTTGTGGCTAAAGCGGTGG-3′	180 bp	60°C
mStat5a Reverse	5′-ACTTAGTTGCCTAAACCTGCCT-3′		
β-actin Forward	5′-ACCCAGATCATGTTTGSGAC-3′	221 bp	55-60°C
β-actin Reverse	5′- TGAGGTAGTCAGTCAGGTCC-3′		
GAPDH Forward	5′-AAC CTG CCA AGT ATG ATG AC-3′	191 bp	55-60°C
GAPDH Reverse	5′-ATA CCA GGA AAT GAG CTT GA-3′		



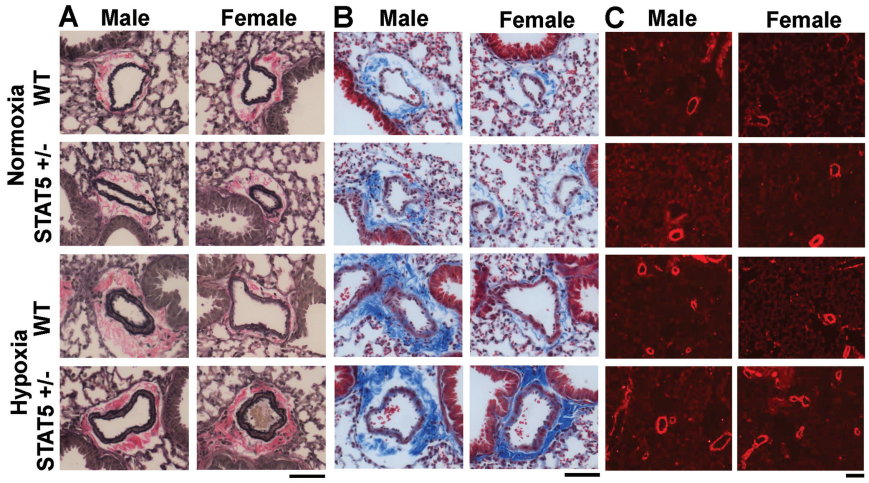
Supplementary Figure S1. Examples of genotyping by PCR analyses of respective mouse tail DNA samples isolated from crosses between *STAT5a/b*$^{wt/fl}$ and *SM22α-Cre*$^{+/-}$ males and females. (A) shows genotyping of the DNA samples (0.5 μg/reaction) for *STAT5a/b* floxed alleles using the three STAT5 allele-related primers listed in Table S1 which give a 450-bp band for the wt allele and a 200-bp band for the floxed allele. (B) shows a simultaneous analysis using the same DNA isolates (0.5 μg/reaction) and the four Cre-related primers listed in Table S1 which give a 100-bp band for the Cre positive allele and a 324-bp band as an internal control. The deduced genotypes are indicated at the top of the lanes. The *SM22α-Cre*$^{+}$*, STAT5a/b* $^{fl/wt}$ genotype is abbreviated as the heterozygous *STAT5+/-* genotype, and the *SM22α-Cre*$^{+}$*, STAT5* $^{fl/fl}$ genotype is abbreviated as the homozygous *STAT5a/b-/-* genotype.



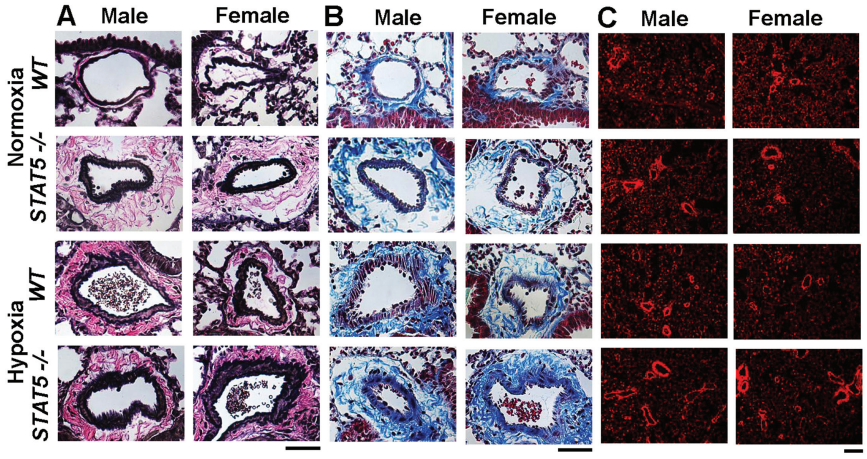
Supplementary Figure S2. Tissue-specific reduction in STAT5a/b in the tunica media of the aorta (A and B) and pulmonary artery segments in lung sections (C) derived from heterozygous *STAT5+/-* mice compared to *wt/wt* mice. Immunofluorescence analyses were carried out for STAT5a+b (using C-17 pAb). (B) shows quantitation of data from multiple regions similar to that illustrated in (A) (n = 6 images; mean ± SE; * $P < 0.05$; pair-wise Student *t* test). Black arrow in (B) points to tunica media. Scale bars = 50 μm in (A) and = 20 μm in (C).



Supplementary Figure S3. Tissue-specific reduction in STAT5a/b expression in the tunica media/smooth muscle cells of the aorta (A–D) in *STAT5-/-* mice but without changes in systemic blood pressure (E and F). (A and B): STAT5a immunofluorescence (L-20 pAb) analyses of aortic segments; n= 6 images quantitated; mean ± SE; * $P < 0.05$. Scale bar = 10 μm. (C): Quantitation of a real-time RT-PCR analysis for the expression of STAT5a, STAT5b and β-actin transcripts in RNA isolated from aortic segments from the respective wild-type and knockout mice normalized to the level of G6PD transcripts (3 mice per group; mean ± SE, * $P < 0.01$). (D): Western blot analyses of for STAT5a (L-20 pAb), STAT5a+b (C-17 pAb) and α-SMA.in extracts of aortic SMCs derived from respective mice (similar cultures were verified to contain >90% SMA-positive cells by immuno-fluorescence). (E and F): Summary of systemic blood pressure measurements on groups of 8-wk-old mice of the indicated gender and genotype using the tail-cuff method (mm Hg). Pair-wise Student *t* test in (B and C), and multiple group ANOVA in (E and F).



Supplementary Figure S4. Pulmonary vascular remodeling in lungs of *STAT5+/-* mice exposed to hypoxia. Figure illustrates a compilation of the histology of representative lung sections derived from mice in the experiment in Figure 3. The sections were stained respectively with Verhoeff Van Gieson elastin stain (A), Mallory's Trichrome stain (B), or for α-SMA immunofluorescence. Scale bars = 65 μm.



Supplementary Figure S5. Pulmonary vascular remodeling in lungs of *STAT5-/-* mice exposed to hypoxia. Figure illustrates a compilation of the histology of representative lung sections derived from mice in the experiment in Figure 4. The sections were stained respectively with Verhoeff Van Gieson elastin stain (A), Mallory's Trichrome stain (B), or for α-SMA immunofluorescence. Scale bars = 45 μm.