Endothelial Apelin-FGF Link Mediated by MicroRNAs 424 and 503 is Disrupted in Pulmonary Arterial Hypertension

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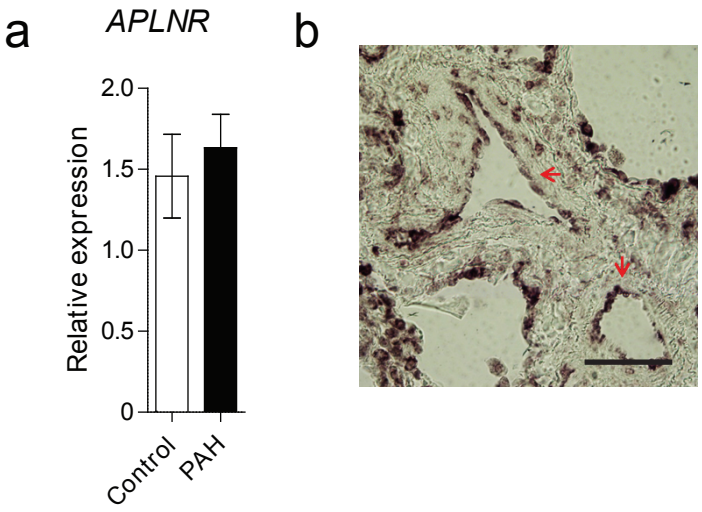
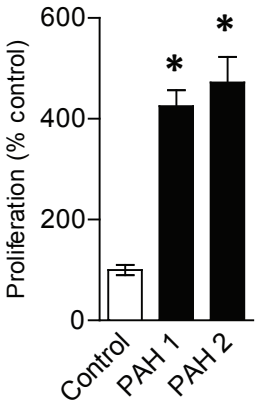
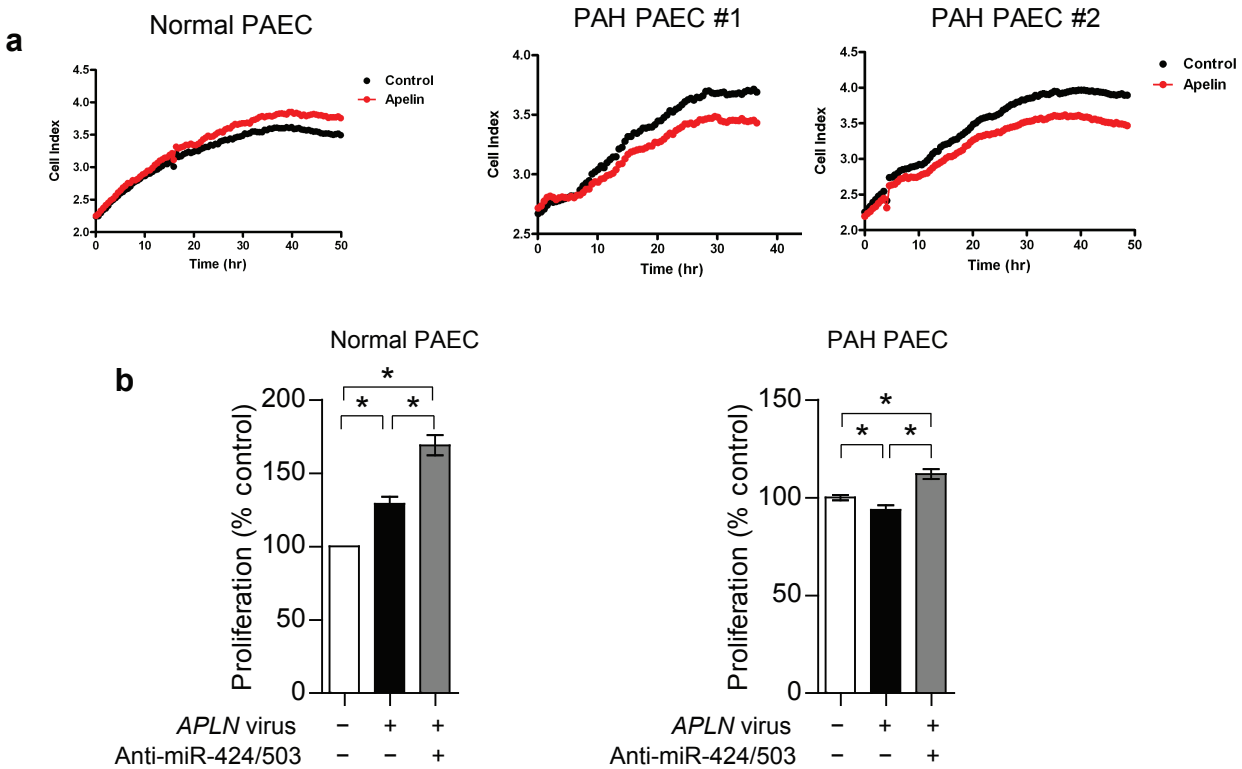


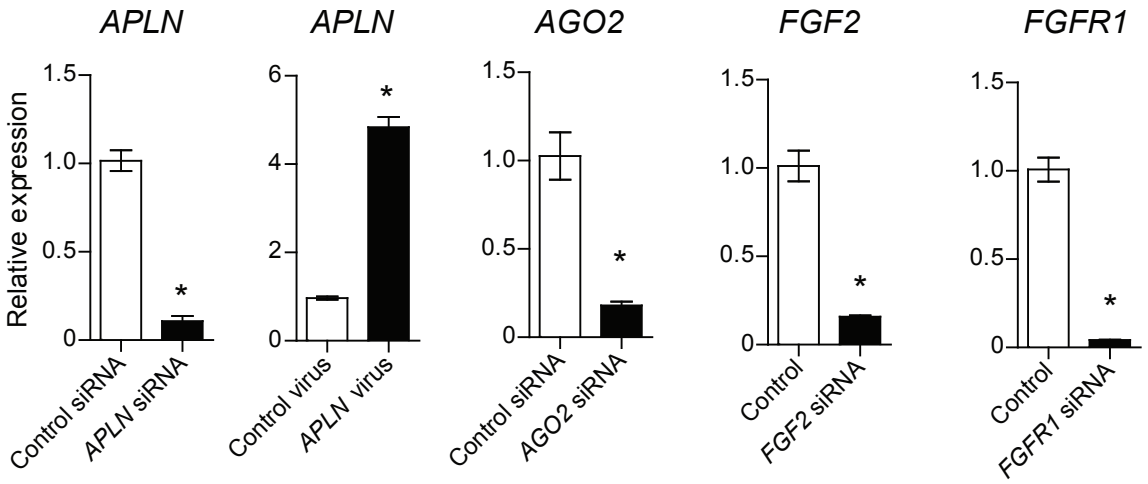
Supplementary Figure 1. a) Total mRNA levels of *APLNR* in normal and PAH PAECs. b) *In situ* hybridization for *APLNR* in a normal lung. Scale bar = 50 μm.



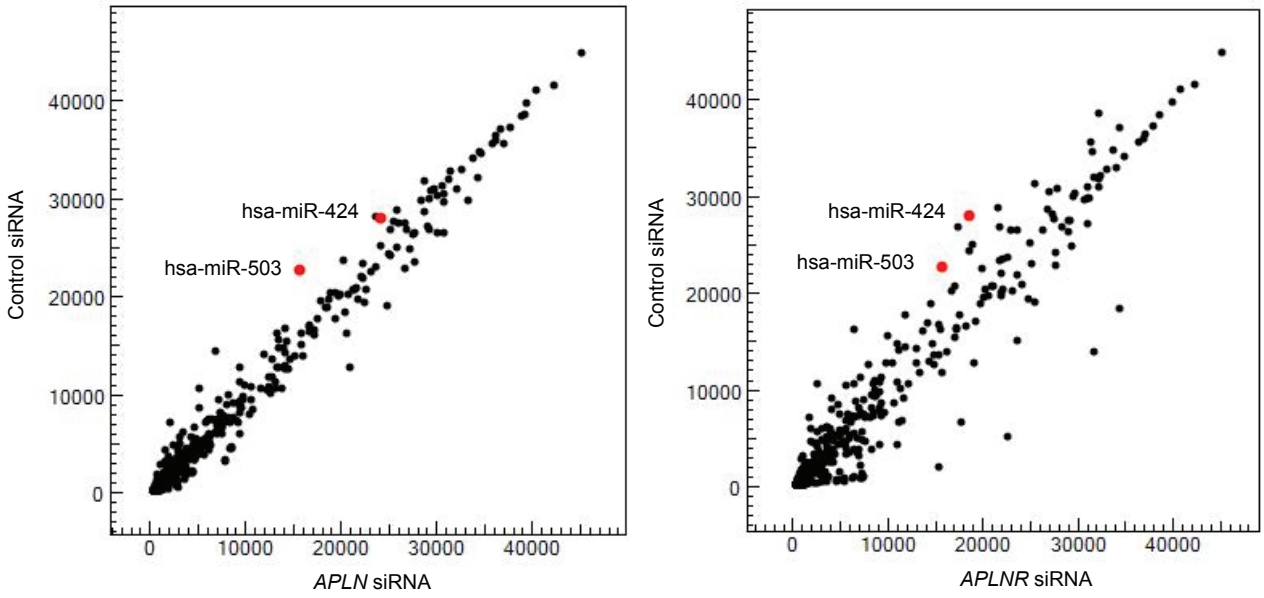
Supplementary Figure 2. Proliferation rate of PAECs from control and PAH patients. * P<0.001.



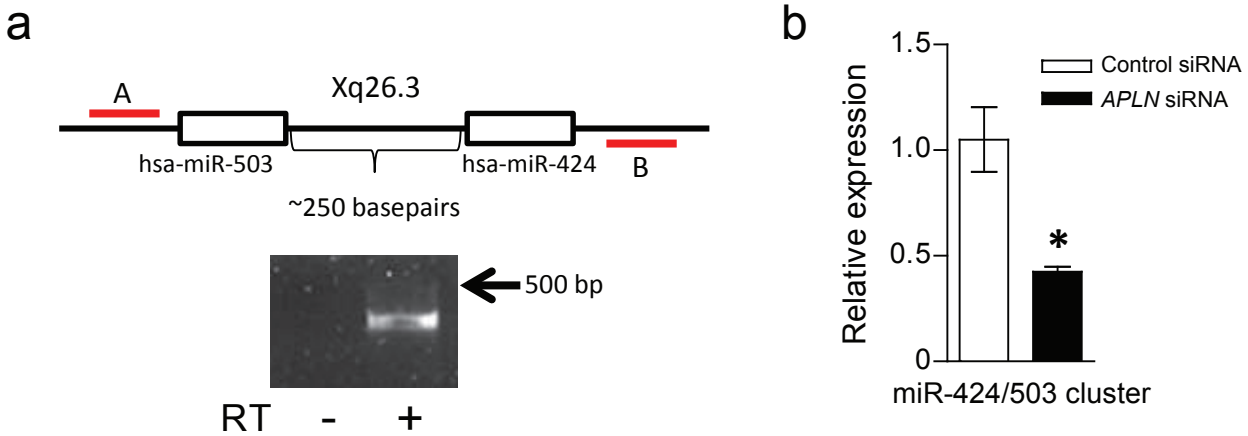
Supplementary Figure 3. a) Proliferation of normal and PAH PAECs in response to stimulation with apelin-13 peptide. b) Proliferation of normal and PAH PAECs in response to *APLN* overexpression in conjunction with concurrent miR-424 and miR-503 inhibition with anti-miR-424/503 transfection. *P < 0.05.



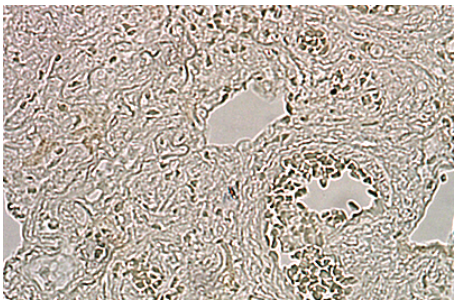
Supplementary Figure 4. Knockdown and overexpression efficacy of *APLN, AGO2, FGF2* and *FGFR1* in PAECs. **P* < 0.001.



Supplementary Figure 5. MicroRNA microarray analysis with *APLN* or *APLNR* knockdown in PAECs. MiR-424 and miR-503 are shown in red.



Supplementary Figure 6. a) Transcription of miR-424 and miR-503 as a single transcript. PCR using human PAEC cDNA and two primers flanking miR-424 and miR-503 (designated A and B). No reverse transcriptase (RT) control is also shown. b) Quantative PCR using primers A and B in PAECs with *APLN* knockdown. **P* < 0.001.



Supplementary Figure 7. Scrambled control for *in situ* hybridizations of human lung sections.

FGF2

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3'       aaguUUUGUACUUAACGACGAc 5' hsa-miR-424
             |:| || |  |||||||
5054:5'  uauuAGAAAUUA--UGCUGCUa 3' FGF2
             |||| |    : ||||||
3'            UCUUGACAAGGGCACGAU 5' hsa-miR-503

3'       aaGUUUUGUACUUAACGACGAc 5' hsa-miR-424
           :||||:||   ||||||||
5587:5'  uuUAAAAUAU--UUUGCUGCUa 3' FGF2
           ||    |  :::|||||||
3'        UCUUGACAA--GGGCGACGAU 5' hsa-miR-503

3'       aagUUUUGUA-CUUAACGACGAc 5' hsa-miR-424
           :|| | |  |: |||||||
5625:5'  gaaGAAUCUUACAGAUGCUGCUa 3' FGF2
                :|    :|||||||
3'            UCUUGACAAGGGCGACGAU 5' hsa-miR-503
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FGFR1

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3'       aaguuUUGUACUUAACGACGAc 5' hsa-miR-424
             ||:|  ||||||||||
872:5'   cccucAAUAAAAAUUGCUGCUg 3' FGFR1
             ||:     ::|||||||
3'            UCUUGACAAGGGCGACGAU 5' hsa-miR-503

3'       aagUUUUGUACUUAACGACGAc 5' hsa-miR-424
           ||||:: |:||||||||||
2039:5'  gggAAAAUG-GGAUUGCUGCUu 3' FGFR1
             || ||    ::|||||||
3'          UCUUGAC-AAGGGCGACGAU 5' hsa-miR-503
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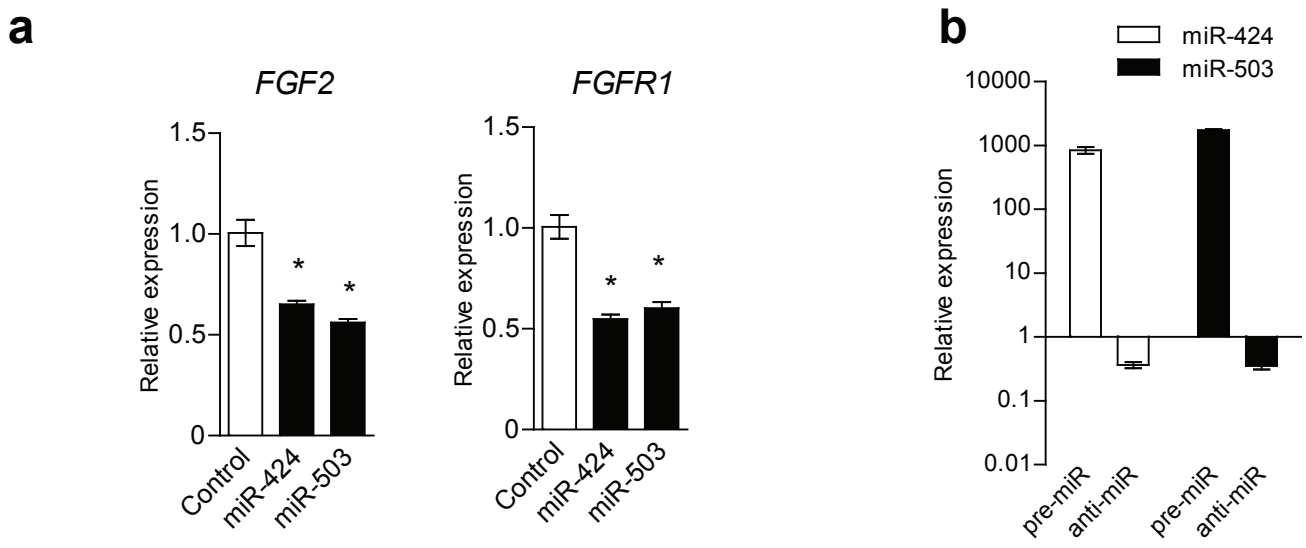
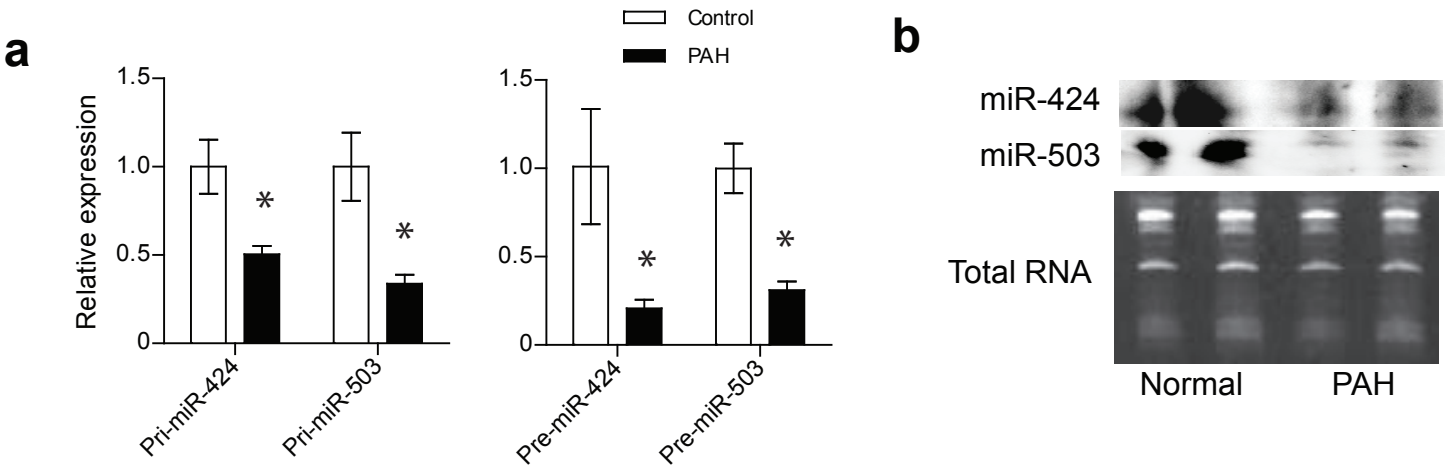
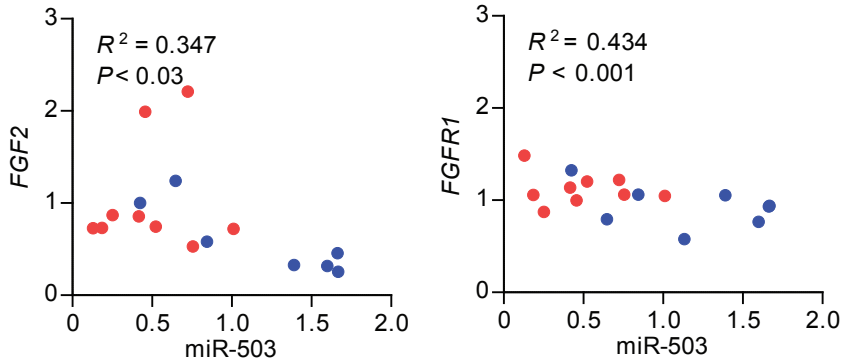
Supplementary Figure 8. Predicted target sequences of *FGF2* and *FGFR1* 3' UTRs targeted by miR-424 and miR-503.



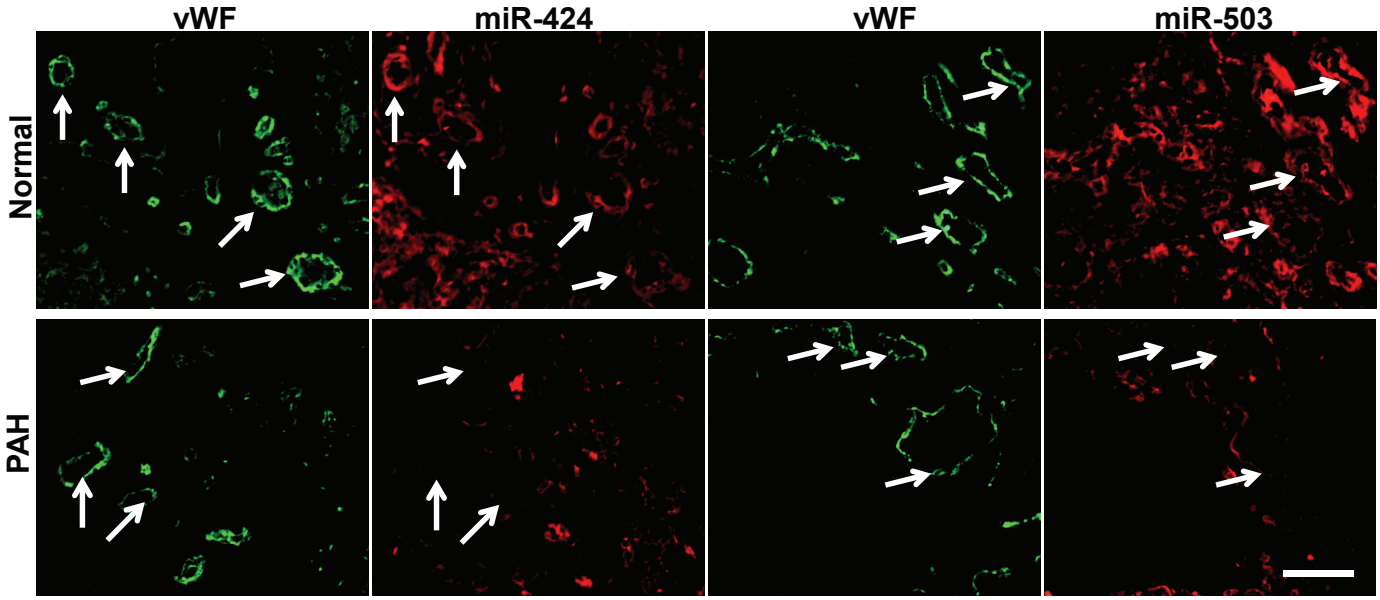
Supplementary Figure 9. a) *FGF2* and *FGFR1* mRNA expression levels in response to miR-424 or miR-503 overexpression in normal PAECs. **P* < 0.01 vs. control. b) Levels of miRNA achieved with overexpression or knockdown in PAECs.



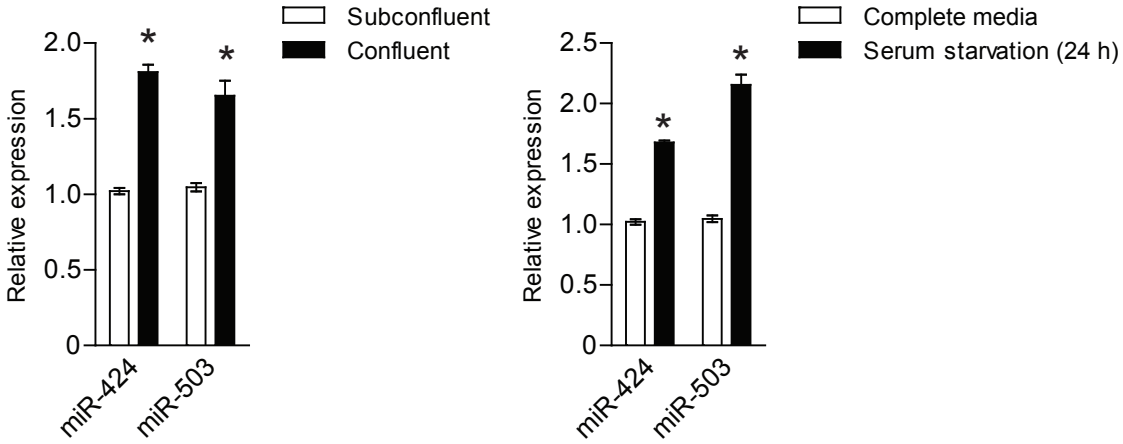
Supplementary Figure 10. a) Transcript levels of pri- and pre- forms of miR-424 and miR-503 in normal and PAH PAECs. b) Northern blots of miR-424 and miR-503 in normal and PAH PAECs. *$P < 0.01$.



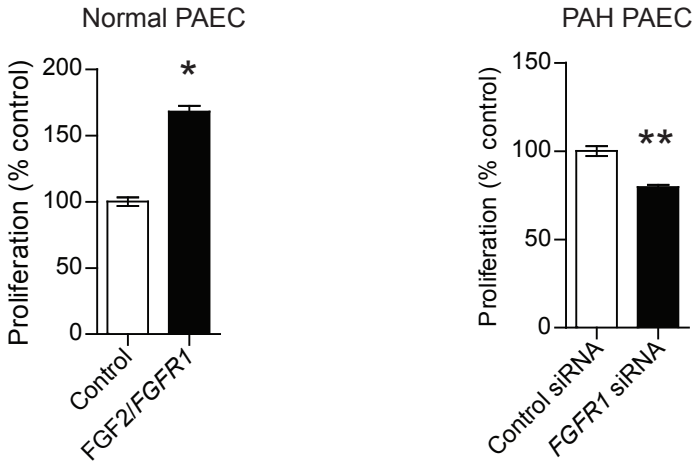
Supplementary Figure 11. Correlation of miR-503 in normal and PAH PAECs with *FGF2* and *FGFR1* mRNA levels.



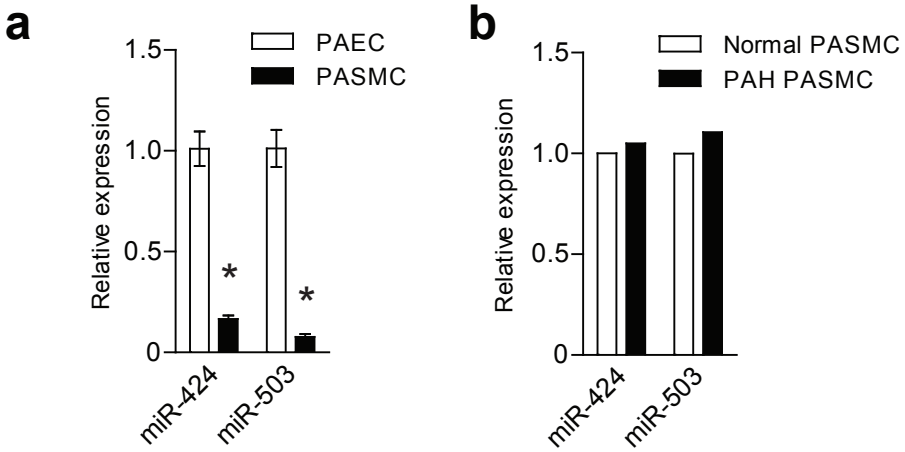
Supplementary Figure 12. Microvascular endothelial expression of miR-424 and miR-503 in normal and PAH patient, as demonstrated by costaining with von Willebrand factor. Endothelial layer is designated by white arrows. Scale bar = 70 μm.



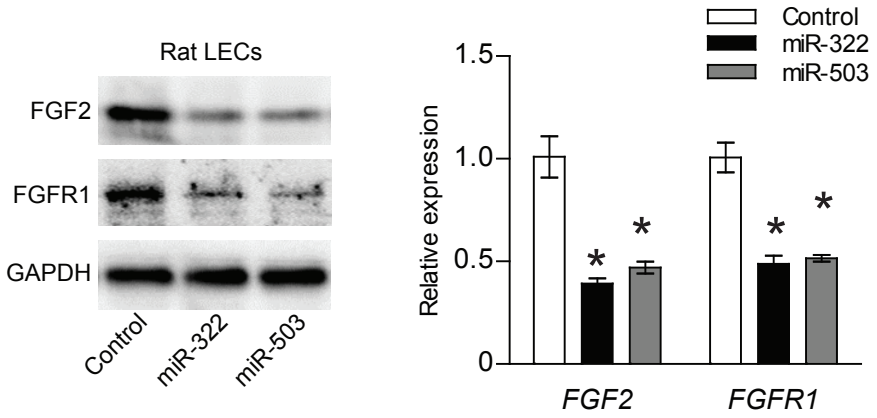
Supplementary Figure 13. Expression levels of miR-424 and miR-503 with varying cell confluent conditions and serum starvation in PAECs. **P* < 0.001.



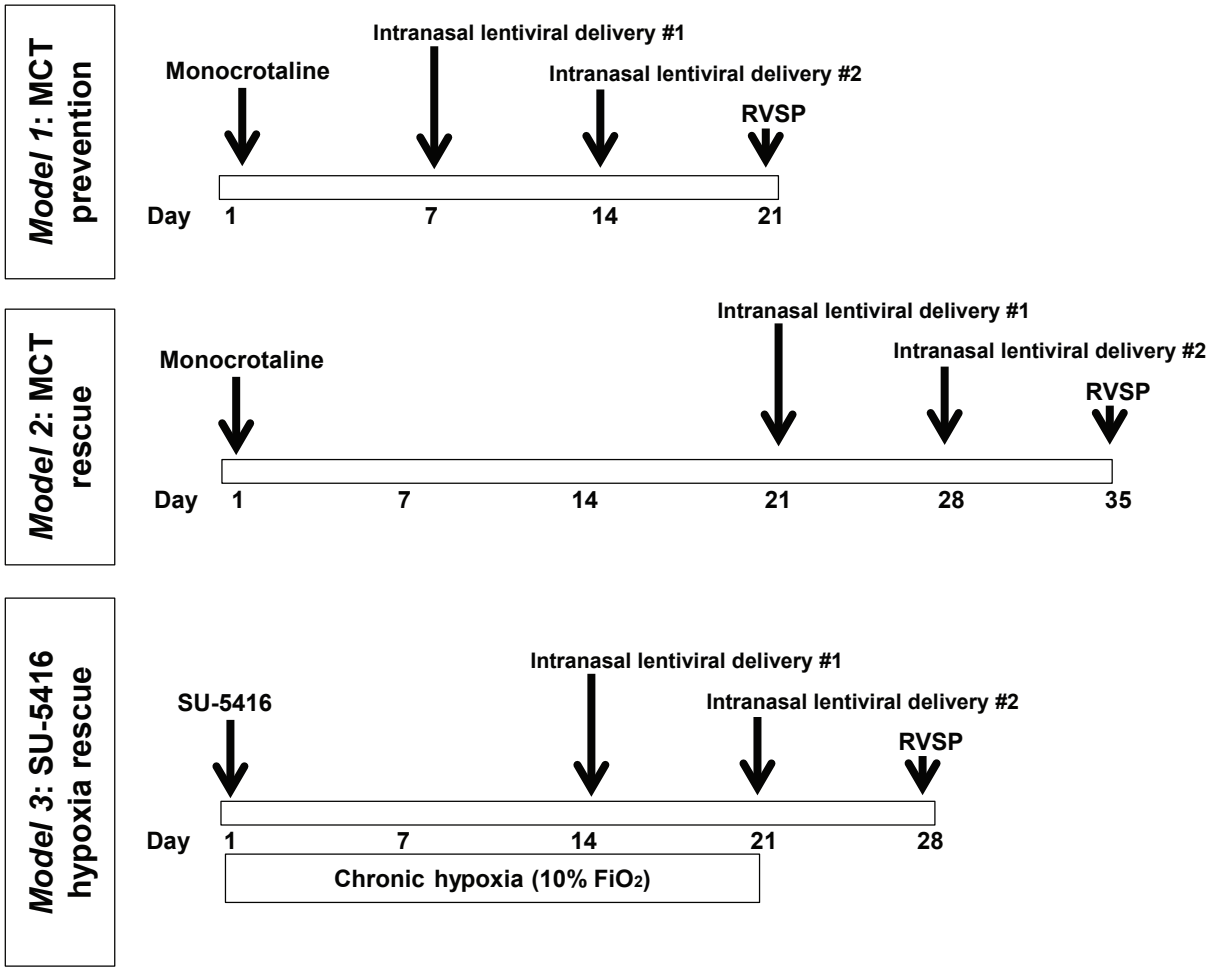
Supplementary Figure 14. Normal PAEC proliferation in response to augmentation of FGF signaling via FGF2 stimulation and *FGFR1* overexpression, and PAH PAEC proliferation in response to knockdown of *FGF2* and *FGFR1*. **P* < 0.001 and ***P* < 0.05.



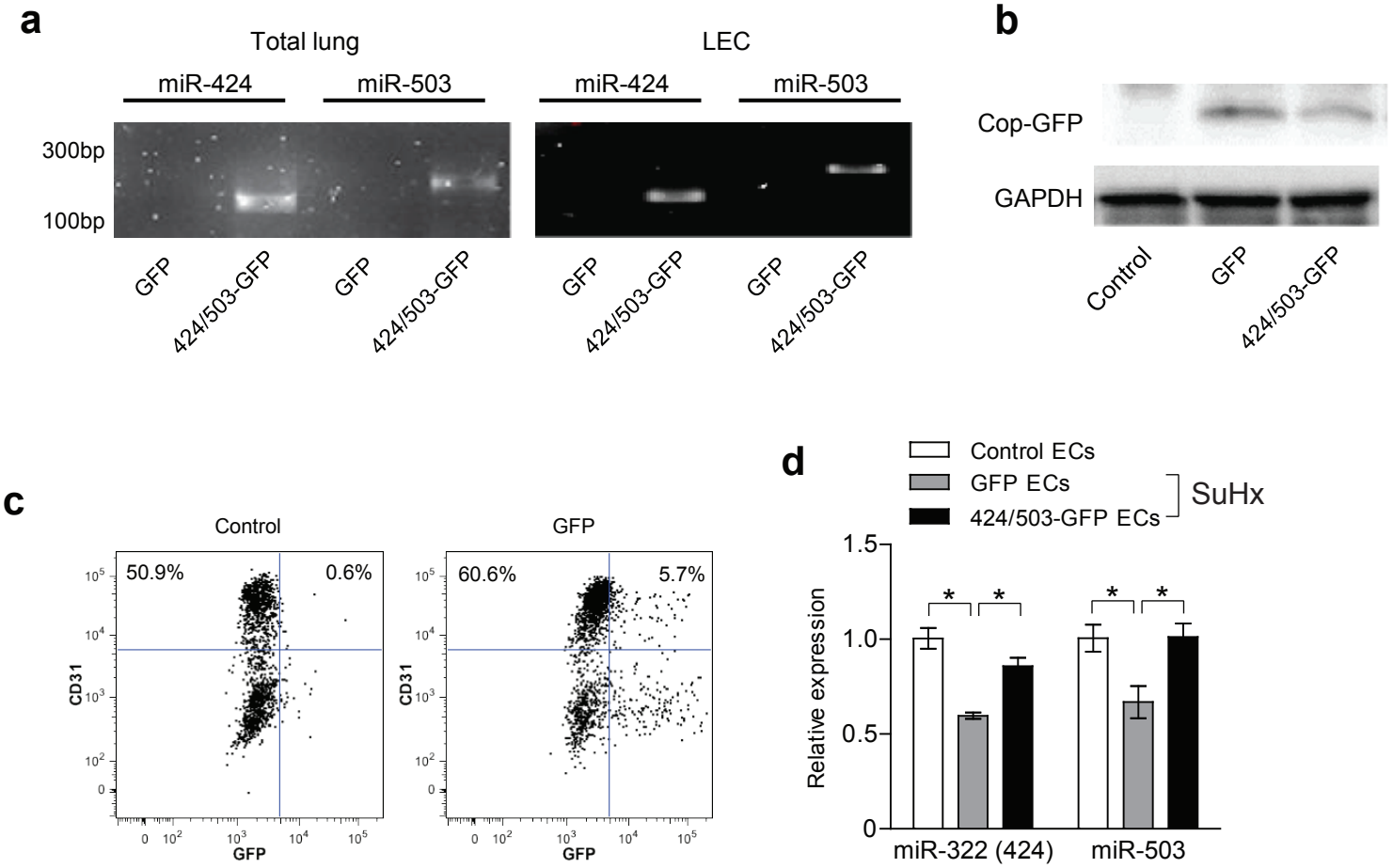
Supplementary Figure 15. a) Expression levels of miR-424 and miR-503 in PAECs and PASMCs. **P* < 0.001. b) Relative expression of miR-424 and miR-503 in PASMCs of normal and PAH patients.



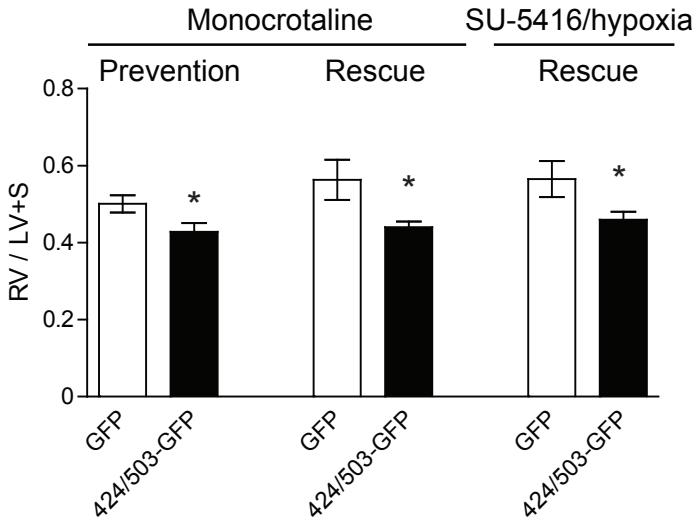
Supplementary Figure 16. FGF2 and FGFR1 protein (left) and mRNA (right) expression in response to overexpression of rat miR-322 or miR-503 mimics in isolated rat LECs. *$P < 0.01$.



Supplementary Figure 17. Schematic of experimental pulmonary hypertension models used.



Supplementary Figure 18. Validation of intranasal lentiviral miRNA delivery. a) PCR analysis of lentivirally-derived miR-424 and miR-503 transcripts in the lungs and isolated LECs of rats receiving the two lentiviral vectors (GFP control or 424/503-GFP). b) Detection of lentivirally expressed copGFP in lung homogenates of GFP or 424/503-GFP groups. c) Flow cytometry for CD31 and GFP demonstrate LECs that are GFP positive. d) Expression levels of miR-322 (hsa-miR-424 + rno-miR-322) and miR-503 in the isolated LECs of rats receiving GFP control or 424/503-GFP with the SU-5416/hypoxia (SuHx) induced pulmonary hypertension. **P* < 0.05.



Supplementary Figure 19. Right ventricle to left ventricle + septum weight ratios in the rats from the three pulmonary hypertension models. **P* < 0.05.

Supplementary Table 1. List of microRNAs significantly upregulated or downregulated in all three conditions: 1) *APLN/APLNR* knockdown, 2) *APLN* knockdown, and 3) *APLNR* knockdown.

Upregulated	Downregulated
hsa-miR-27a*	hsa-miR-23a
	hsa-miR-95
	hsa-miR-139-5p
	hsa-miR-149
	hsa-miR-200a
	hsa-miR-210
	hsa-miR-328
	hsa-miR-424
	hsa-miR-424*
	hsa-miR-450a
	hsa-miR-450b-5p
	hsa-miR-503
	hsa-miR-542-5p
	hsa-miR-551a

Supplementary Table 2. Sequences of oligonucleotide primers used.

	5' Primer	**3' Primer**
***FGF2*- 3'UTR**	TAGGCGATCGCTCGAGCAGACAGATTAATCCAGAAGC	TTGCGGCCAGCGGCCGCGGGAGACAAGAAAACACAAA
***FGFR1*-3'UTR**	TAGGCGATCGCTCGAGATTGAAGGTGACCTCTGCC	TTGCGGCCAGCGGCCGCCTCTCCCAAGGACTTATGAA
Human miR-424 transcript	GGCTTCCTTCAGTCATCCAGT	ACCTTCTACCTTCCCCACGA
Human miR-503 transcript	GGAAGGTAGAAGGTGGGGTC	GGAAACAATACCCCAGAGCA
Human miR-424/503 promoter	TTTCTCTATCGATAGGTACCCCATTTTCGAGTGGAGCC	CCGGAATGCCAAGCTTGAGTCAATGAAGGGGGATC