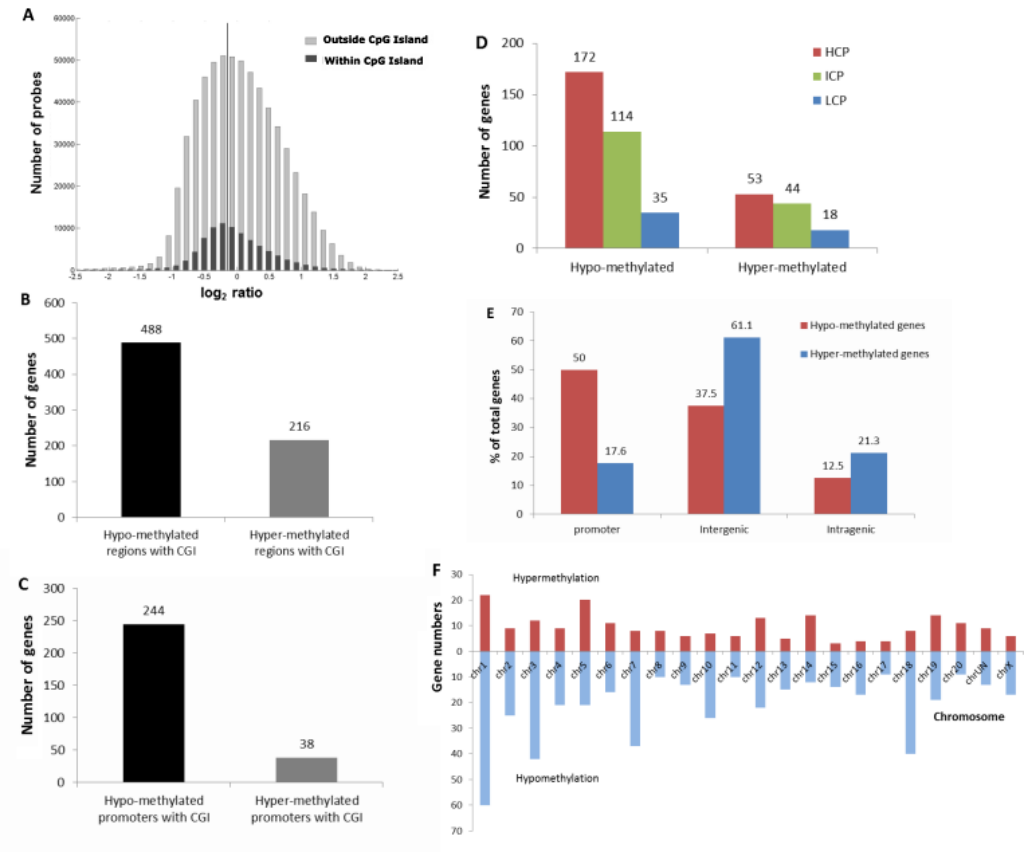

Fig.S1. mDIP-CHIP Assay to Profile the Promoter Methylation Pattern in pulmonary vascular endothelial cells

(A) All probes for the array are classified as either inside or within 50 bp of a CpG island (black) or outside a CpG island (gray) and then grouped by their average log2 ratio. The solid line is placed at log2 ratio of +0.2 value to annotate the proportion of methylated gene promoters. ***(B)*** The numbers of differentially Methylated regions (DMRs) associated with CpG islands. ***(C)*** The number of DMRs in the both gene promoters and CpG islands. ***(D)*** CpG Density of hypo-methylated and hyper-methylated promoter. Classification of all promoters by DMRs between EUGR and control rats, hypo-methylated promoters, or hyper-methylated promoters with high (HCP), intermediate (ICP), and low (LCP) CpG content. ***(E)*** The distribution of DMRs associated with genes. Most of the identified hypo-methylated regions associated with genes were mapped to gene promoters, and most of the identified hyper-methylated regions associated with genes were mapped to intergenic. ***(F)*** The chromosomal distribution of 216 promoter hypermethylated genes and 60 promoter hypomethylated genes.

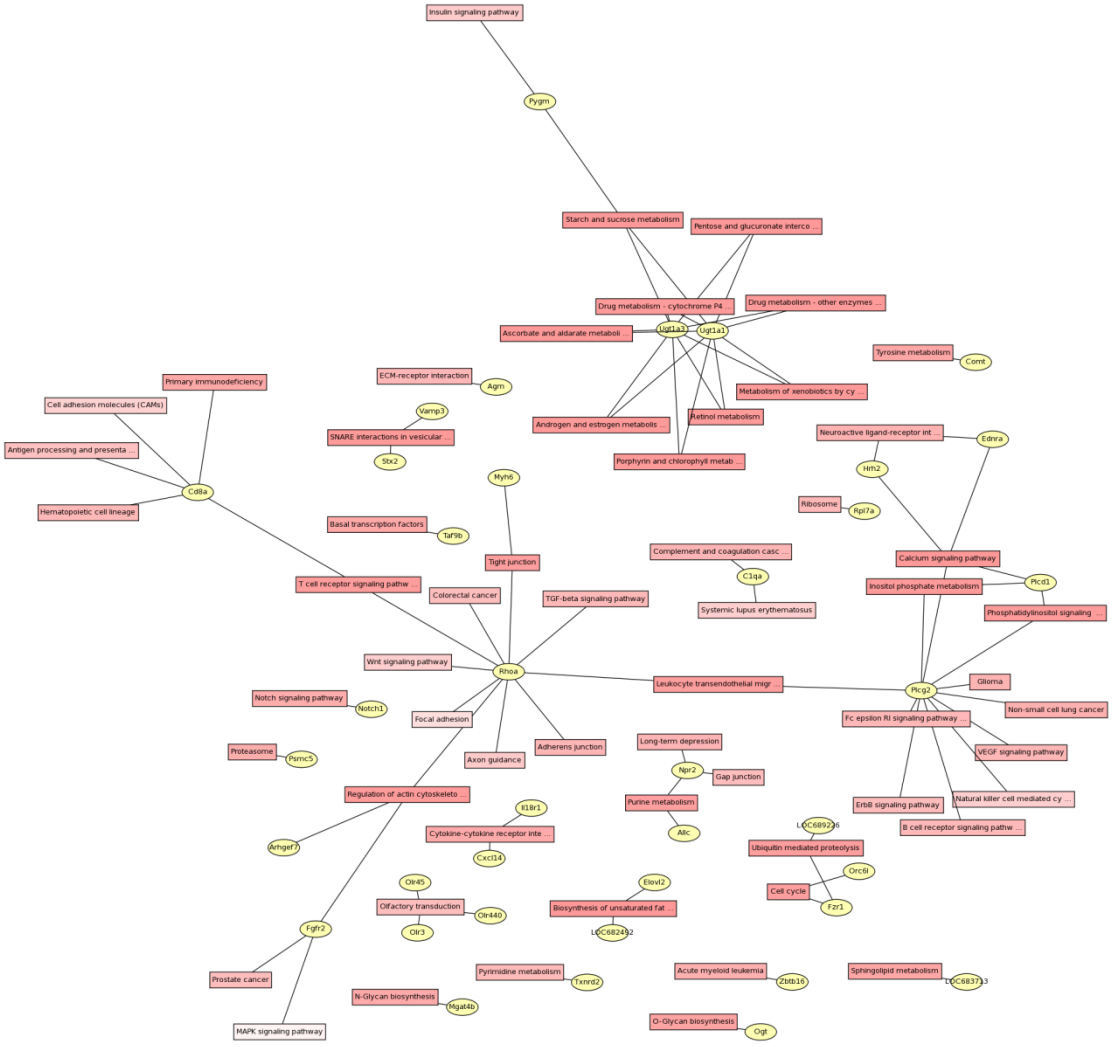

Fig.S2. Pathway analysis of genes showing differentially Methylated regions (DMRs) between male EUGR and Control PVECs at d 63.

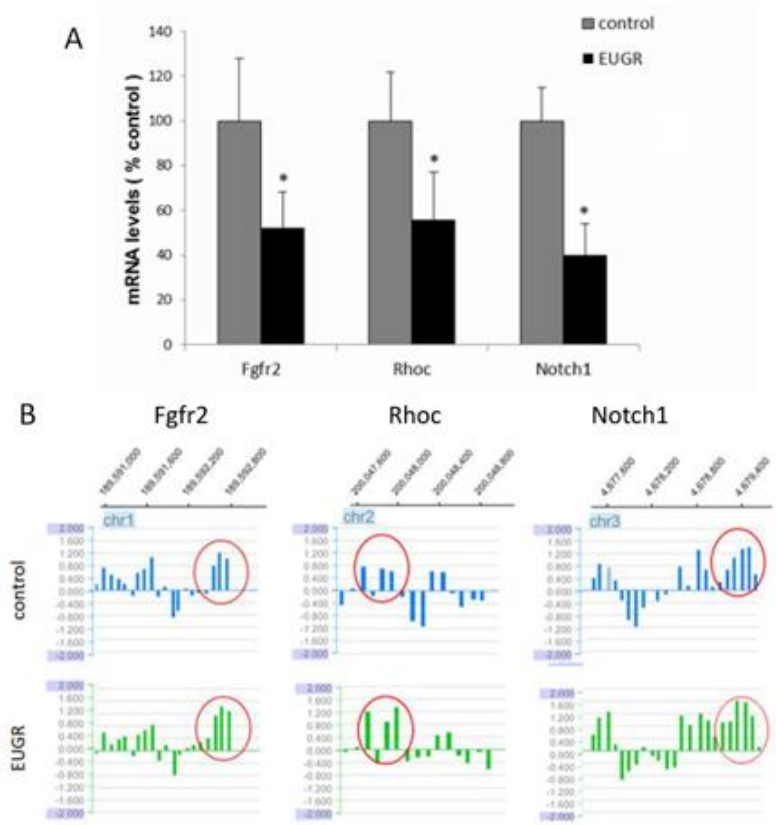

Fig.S3. Quantitative RT-PCR shows changes in mRNA expression concordant with the cytosine methylation changes. Primers were designed to target mRNA transcripts for three loci, these three genes associated with differential hypermethylation (Fgfr2, Rhoc and Notch1). ***(A)*** Average $\log_2$ ratio (±SE.) of EUGR expression data relative to controls was calculated for each gene. n =7-8, $^*P < 0.05$. ***(B)*** The methylation array profiles of three genes showed that promoter hypermethylation of these three genes in EUGR samples relative to controls. Fgfr2, Fibroblast growth factor receptor 2; Rhoc, Ras homolog gene family, member C.

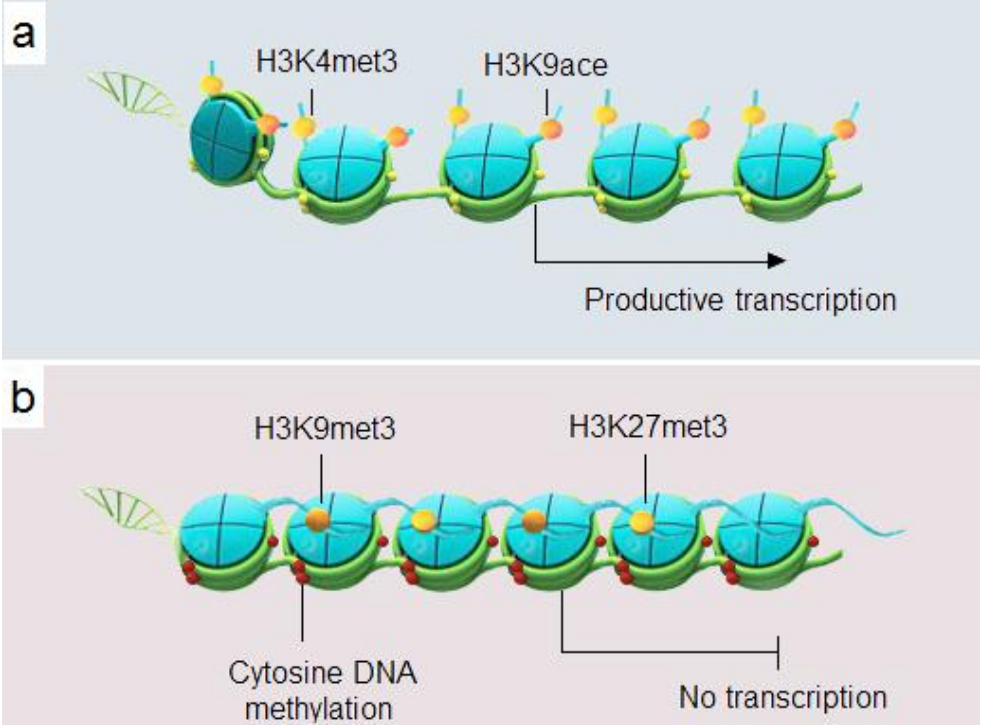

Fig.S4.The schematic diagram shows the role of epigenetic regulation in vascular endothelial gene expression. Histone modification and cytosine methylation changes of the genes that are related to lung development and pulmonary vascular tone regulation in later life. (*a*) Histone tails are decorated with activating posttranslational modifications, including H3K4met3 and H3K9ace. The chromatin architecture assumes an open configuration accessible to relevant transcription factors. (*b*) The gene is transcriptionally silent and demonstrates a contrasting epigenetic landscape. CpG dinucleotides of the gene promoter are densely methylated. Histone tails are decorated with repressive posttranslational marks including H3K9met3 and H3K27met3. The chromatin architecture is closed and RNA polymerase II is not recruited to the gene promoter.

Supp. Tables:

Supp. Table 1.Primer sequences for real-time PCR

	PRIMERS	
Gene name	Forward	Reverse
eNOS	GACATCACCGCAGACAAAC	ACCGATTACACGACATTGAG
PPARr	ACACCATGCTGGCCTCCCTGA	AAGCCTGGGCGGTCTCCACT
IGF-1	CACCTCAGACAGGCATTGTG	TCTGAGTCTTGGGCATGTCA
Fgfr2	CTGATGGGACCACACTTTCCA	CATCGGAGGCTATAAGGTACGAA
RhoC	GCCTACAGGTCCGGAAGAAT	GCACCAACCTAGTTCCCAGA
Notch1	CACCCATGACCACTACCCAGTT	CCTCGGACCAATCAGAGATGTT
GAPDH	CAAGATGGTGAAGGTCGGTGT	CAAGAGAAGGCAGCCCTGGT
beta-actin	GCCAACCGTGAAAAGATG	TGCCAGTGGTACGACCAG

Supp. Table 2. Primer sets for ChIP analysis

	PRIMERS	
Gene name	Forward	Reverse
eNOS promoter A1	CTCTTCAAGTTGCCCATGCCAG	GTGGGCATGAAGCCGAGGTTTT
eNOS promoter A2	CCTGGACCCCTTGCTCTCTTCT	ATGCGTCATTTGACAGGATTGG
eNOS promoter A3	GTCAAATGACGCATGTTTCCCT	TCCCACTCCTGTACACCCAAAT
eNOS promoter A4	CTGTGTCTGTCCTGGCAGTAGC	CAAAGTAAACTCTGTCCCCCAA
PPARr 5' of P1	TCGACGGCTTTCTGAATGTG	CTTGCCCTCTTTCAGCTCTTTC
PPARr P1	AAAAACAAACTTCTGCGTGACAGT	GGTCCCACGTTCCTCAGACA
PPARr P2	CCAAGTCTTGCCAAAGAAGCA	GATTGAGAGCCAGCTGTGACAA
PPARr Exon 4	CCATCAGGTTTGGGCGAAT	GATCTCCGCCAACAGCTTCT
PPARr 3' End	CGCCAAGGTGCTCCAGAA	CTGCACGTGCTCTGTGACAA
IGF P1	CAGGTCTGGCTCATTTCCATC	GCGCTTTCCATGGCTGTC
IGF P2	GCCGGAGGGCTTAATTCATAA	GGAAGCATTTGAAAGCAGCAC
IGF Exon 5	GCCCCTATCGACACACAAGAA	TCCTGGGTGTGCCTTTGAC
IGF Proximal 3' UTR	GACCTACAGAATGTAGGAGGAGCC	GATGTTTTGCAGGTTGCTCAAG
IGF Distal 3' UTR	ACAATAGAGGTGGCCTTCTCCA	CACTGGGAATCATCGAAGCC
GAPDH promoter	CAGGACCCAGAAACCAGAAAGCTG	TGTGTCACTACCGAAGAACAACGA

Supp. Table 3. Primer sets for the quantitative analysis of methylation (The Sequenom Mass ARRAY platform)

Gene name	PRIMERS
IGF-1_1F	aggaagagagGATTGGGTAAGTGGTTGGTAGTATG
IGF-1_1R	cagtaatacgactcactatagggagaaggctTTAACCACACAAAAAATTAAACCAAA
IGF-1_2F	aggaagagagGTTTGGTTTAATTTTTTGTGTGGTT
IGF-1_2R	cagtaatacgactcactatagggagaaggctCCTCCCACCACTAATATATCTTCAAA
eNOS_F	aggaagagagTTGAGATAGAAGAGAGTAAGGGGTTT
eNOS_R	cagtaatacgactcactatagggagaaggctCACTCTTCAAATTACCCATACCAAC
PPAR gamma_1F	aggaagagagGGGTTATGGGTATTTGTTTGA
PPAR gamma_1R	cagtaatacgactcactatagggagaaggctTCCTATCTAAATATAACTTCTCCTCAAC
PPAR gamma_2F	aggaagagagATAAGGTGATTAGGTTGAGGGGA
PPAR gamma_2R	cagtaatacgactcactatagggagaaggctATCACCTAAACAATCAAATCCAATC
PPAR gamma_3F	aggaagagagGGGATGAGGAGAAGTTATTTTTTGT
PPAR gamma_3R	cagtaatacgactcactatagggagaaggctAAAACATTCACCTTATCAATCACACT
PPAR gamma_4F	aggaagagagGAGTGTGATTGATAAGGTGAATGTTT
PPAR gamma_4R	cagtaatacgactcactatagggagaaggctCTACCTACTCCAATTAACCCTACCC

Supp. Table 4. The target DNA regions Length and Number of CpGs for the quantitative analysis of methylation

Gene name	Target Length	Number of CpGs
IGF-1_1	473	9
IGF-1_2	269	5
NOS3	438	8
PPAR gamma_1	481	53
PPAR gamma_2	193	8
PPAR gamma_3	348	15
PPAR gamma_4	449	30