

ONLINE SUPPLEMENT

Epigenetic Up-regulation of Large-Conductance Ca²⁺-Activated K⁺ Channel Expression in Uterine Vascular Adaptation to Pregnancy

By

Man Chen, Chiranjib Dasgupta, Fuxia Xiong and Lubo Zhang

Center for Perinatal Biology, Division of Pharmacology, Department of Basic Sciences, Loma
Linda University School of Medicine, Loma Linda, CA, 92350

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Author for correspondence:

Lubo Zhang, Ph.D.
Center for Perinatal Biology
Division of Pharmacology
Department of Basic Sciences
Loma Linda University
School of Medicine
Loma Linda, CA 92350
Tel: 909-558-4325
Fax: 909-558-4029
Email: lzhang@llu.edu

Table S1. Primers used in quantitative methylation-specific PCR

Primer Name	Primer sequence (5'→3')	Orientation	Status
SP1 ₋₃₈₀ UMF	GGGTTTTGGTTGTTGGGT	Forward	U
SP1 ₋₃₈₀ UMR	ATTTCTTCTTCACTTAATCTCTCTAAAC	Reverse	U
SP1 ₋₃₈₀ MF	GGGTTTTGGTTGTTGGGC	Forward	M
AP1 ₋₆₅₂ UMF	AGTTTGTTTGGTTTAGTGATTTTTT	Forward	U
AP1 ₋₆₅₂ UMR	TTTTAATCCTTCCAACAACCAC	Reverse	U
AP1 ₋₆₅₂ MF	AGTTTGTTTGGTTTAGTGATTTTTTC	Forward	M
AP1 ₋₈₇₉ UMF	GAGTTTTAGATTTAGTTTTTTTAATTGATT	Forward	U
AP1 ₋₈₇₉ UMR	CATAACTTCTCTCTACTCTTTCATC	Reverse	U
AP1 ₋₈₇₉ MF	GAGTTTTAGATTTAGTTTTTTTAATTGATC	Forward	M
AP1 ₋₁₁₉₇ UMF	AATTTGGTAGGATTCGTTTTGAT	Forward	U
AP1 ₋₁₁₉₇ UMR	CTCCTCTATCCATAAAATTCTCC	Reverse	U
AP1 ₋₁₁₉₇ MF	AATTTGGTAGGATTCGTTTTGAC	Forward	M

F, forward; R, reverse; M, methylated; U, unmethylated.

GCTAGCCCTCCCCTCTATAAAAGGGGTAGGACGAGCTCTACCTGAATATGACTGTGAGCCTGCAGTGCTTATTACGTTATGGG
 GTTTTTGAAGTTTGAAGTCAGGCAAGCAGTTTTGTGATCTCTAACCCCTCTGTGCCAACGCAGAACACCAGTCTTTATCAGTGT
 AACATCGTTGTTTCCTTCCTTCCTACAATCATCTGCCCCACACGAACCCACAGCCAGCCAGGGAGCAGGGTTTCCACCCG
 CATACCCTCAAGAGACAGGCATGTCAAAGCATTGTTGGCAAGAGTTGCTTCGATGCAGCAGAAAGATATACCAGCCCTGTCT
 GTTCCCATTTTCCAAGGGGAGAATACTTGGGAAGAAGGCTTGTGTTGCTTATAAAGTCCAATGTGTAACCTCCTAATACCTTTA
 GTCAGTTACAAGCATGTAGCCCTTTAGCCATACTTTAAAAGACTGTCTGTTGTAAGTACTGAGGGTCTCTCCCCGAAAGGAGAAAG
 AAGGATTTTTGCACTAGATCACAGGCAGGGATCTTCTGGCTCCTGCGTGGGCCAGGACCATCTTGGAAAATCACCCCTCTGGG
 ATTCATTCTTGGCCATGTCCTTCACCCGCAATGCTGCATCTGCAAGGTCTGAGGCCCTTTAGACACCCCTTGGCTATGGTGCC
 CGTTAAACAGACGATTTACTCTCCTGTTAATAGGATTCAATTACGAAAAGGGGTGGGGGAAGGGAGCTCACTGCTGGGGAGA
 GTTTAGGGCCTGAGCACTGCTCTCGTTTTATGGCCGATAAGCTGGTTGCCTCATAGCAACAGAGGCAGAAGTGAGGAGGG
 TCCAGAACTCTTACAGTTGGCAGGGCTGAGCTGTGCCACCTCCCCAGGAGCTCTAAAATATCAAGTGGCGAGTGTCTGCACA
 ACAGAAGTGAGCTTTCAGGTCTTAAAGCAGCACCAGGCTCAGGAGAGAGAGCCAGGCTCAGCAGAGAGAGCCCCAGCTCTG
Ap1₋₁₁₉₇ TAATCTGGCAGGACCCGCTTTGAGCGCTGGCTCCTGCACTTGGGGAACCCCCAGTGGCTCAGTGGTGAAGAGTCCGCCTGCCA
 GTGCAAGAGACCTGGGTTCTGTCCCTGAGTCGGGGAGTCCCTCTGGAATAGGAAATGGCAGCCCACTCCAGTATTCTTGCCTG
 GAGAATCCCATGGACAGAGGAGCCTGGCGGGCTACAGTCCACGGGGTGCAGAGAGTTCGGATGCGACTGAGCGCGCACGCG
 CATGCATGCATCCATGCAGCCCCGCTTAGAGCAGCCAATGTGGGAGAGCAATTTAAGGCTTCTGAGCTTCAGATTCAAGTTT
Ap1₋₈₇₉ CTTCAACTGACCGTTGAGTAAACTGAGGAGCAGAGACGCTAGGATCATTCTGATACTTAGGGAAGGCCCTATGCTCGTGTTT
 ATTTTTGTCACTGCTTTTTAATCCTTCCAACAACCACTTTAAGGATGAAAGAGTAGAGAGAAGTTATGTTTTTGCCTTCAAATG
 TTATTTCTCACTTGTCTTCTATTGATGGGAACAAATCTCTGCCATGCGCCAAACTGTACAGTTTGACCA**CGGGAGGTCA** **Ap1**₋₆₅₂
 CTGGGCCAAGCAGGCTTCTTATAGATCTGGCCAGCTGGAACAGCCGTGAAGCTCTCACTGCTGCCCGCTTCTCCTTGGTTGG
 TCTTTAAAAGGTGAGTTTCTTTTTCTGCCCCAAATCTCCGCCATTTCTAACTCTTATCACTACTCTGTACAAAGTAAGGTTTA
 AAAACCCCAAGAGGCTCAGAGAGGCATCGTGACCTGCCTGGGGTACACAGTACACAGTTGTCAAAGGCTGAGGGTTTTGGC
Sp1₋₃₈₀ TGCTGGGCGGGTTGGAAATGCCAGCCCCCTCCAGCTGCTGATGAGCCCCACCCCTTAGAGCTTCCACTCCTCCTCCCTCCCTCC
 GTCAGCAAAAAGAACTCATTTCATCCAGACTTCTCTGCCAGCCAGAGATTAAGTGAAGAAGAAATGTCTCCAAAC
 ATACCGAGATGGTCTTTCCTGGCTTCTCAGATTTGCTGTCTGTGGTTTGAACCTTTTGGCCAGTAGATTCTGGGAATTGCAGG
 GCAGGGCCTGGGTAACAGCTGCCAGCCCCCTGGGCTGGGTACCTGCCCTCTCGAGTTTGTGTCTGGGGCTGTGTGCTCAGAC
 TTGACCATGTTTGGGCATGGAGGCTCGTCTCAGACGAGAACCAAAGGCCGCT
 mRNA of BKβ1
 TCCCCAGAGACGGGCTGGGAGCCCAA**ACTTCTGCTGCAGCTCGGGCAGCTTCTTTTGGGTGGGGGCTGGGGACCAG**
GAAGAAAAGGTCTGCCGAAG AAGCTT

Figure S1. Mapping of the ovine BKβ1 promoter sequence and transcription factor binding sites. Transcription factor consensus binding sites, including Ap1 and Sp1 binding sites were underlined and CpG units were marked bold. Arrow, transcription start site.

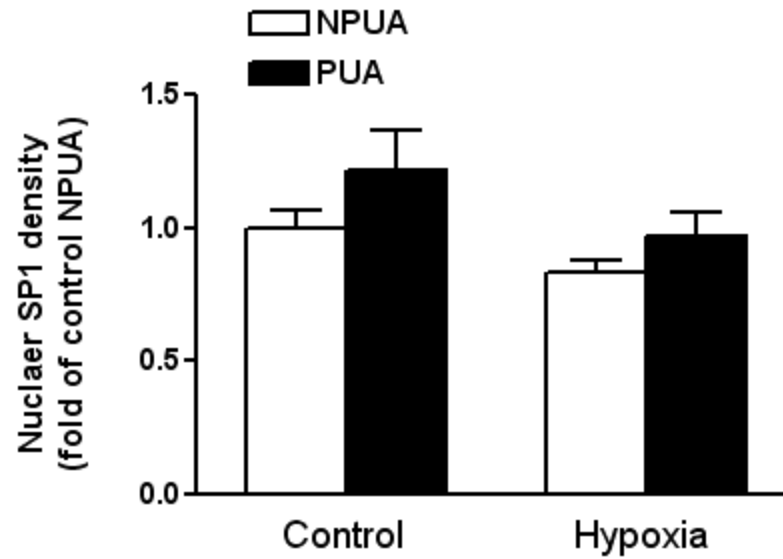


Figure S2. Effect of pregnancy and chronic hypoxia on Sp1 abundance in nuclear extracts. Nuclear extracts were isolated from uterine arteries of nonpregnant (NPUA) and pregnant (PUA) sheep treated with normoxia (Control) and long-term high-altitude hypoxia (Hypoxia). Sp1 abundance was determined by Western blot assay. Data are means \pm SEM. n = 5