HYPOXIA INDUCED PULMONARY ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION IS CONTROLLED BY FOXM1

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Online Data Supplement

Materials and methods:

Western Blot Analysis

Equal amounts of protein were loaded onto 10% SDS-PAGE gels, which were transferred to a nitrocellulose membrane. Membranes were blocked with 7.5% non-fat milk and incubated in primary antibody overnight at 4 °C. Membranes were washed and incubated in secondary antibody and developed using SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA). Protein band density was quantified using NIH Image J software. Tubulin was used as loading control.

qRT-PCR Primer sequences used:

FoxM1,GGAGGAAATGCCACACTTAGCG (sense), TAGGACTTCTTGGGTCTTGGGGTG (antisense); HIF-1α, TGAACATAAAGTCTGCAACATGGA (sense),
TGAGGTTGGTTACTGTTGGTATCATATA (antisense); HIF-2α, TGCTCCCACGGCCTGTAC (sense), TTGTCACACCTATGGCATATCACA (antisense); Aurora A kinase,

AATCTGGAGGCAAGGTTCGA (sense), CTGGATTTGCCTCCTGTGAAG (antisense); ribosomal protein L 19 (RPL19), ATCATCCGCAAGCCTGTG (sense), TGACCTTCTCTGGCATTCG (antisense); Cyclin D1(Qiagen, Valencia, CA, USA).