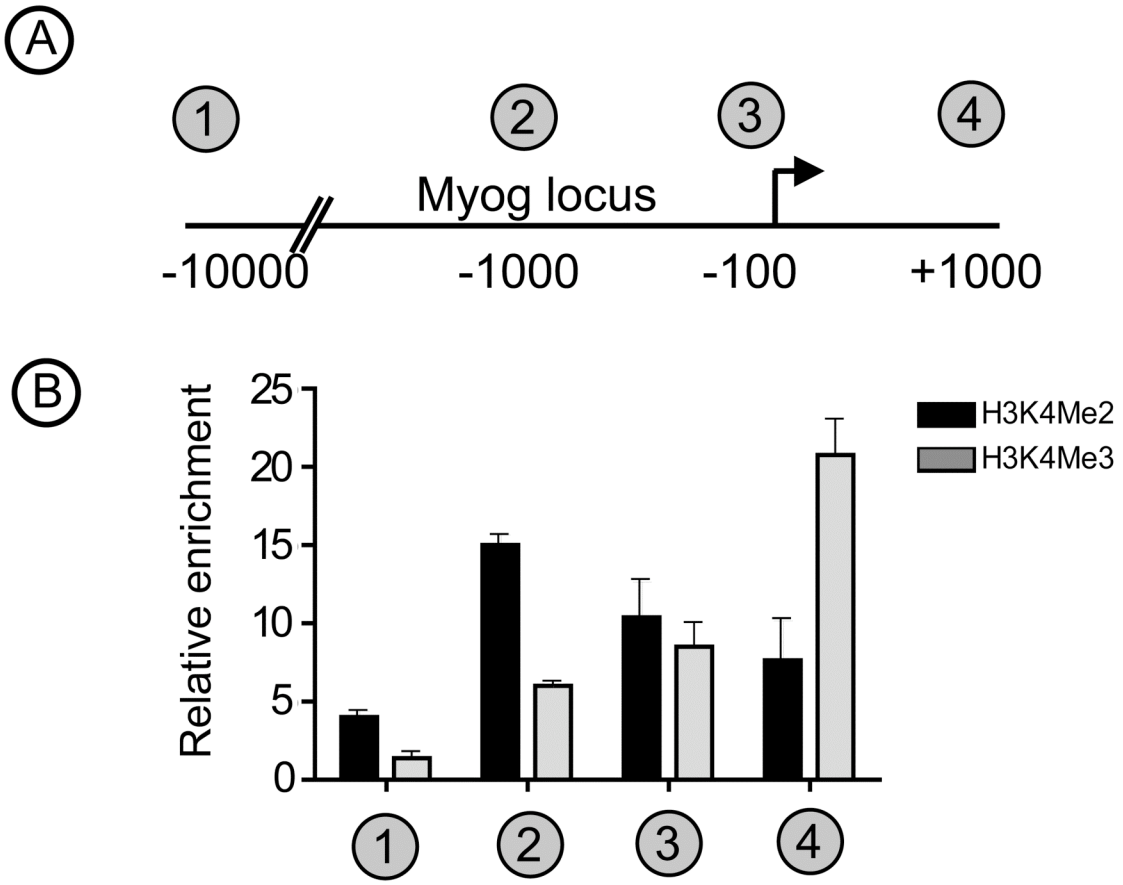
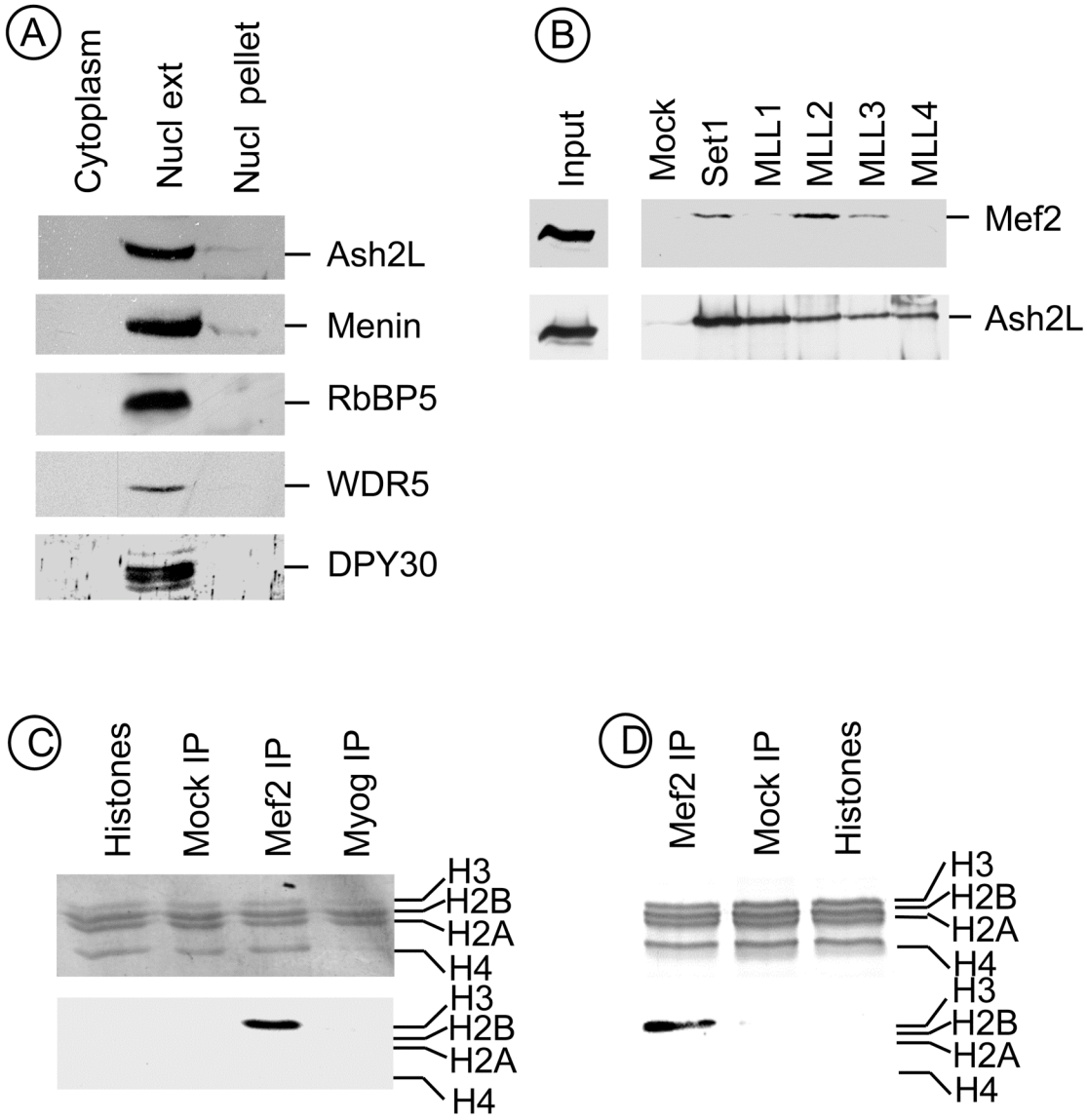
# p38 MAPK signaling regulates recruitment of Ash2L-containing methyltransferase complexes to specific genes during differentiation

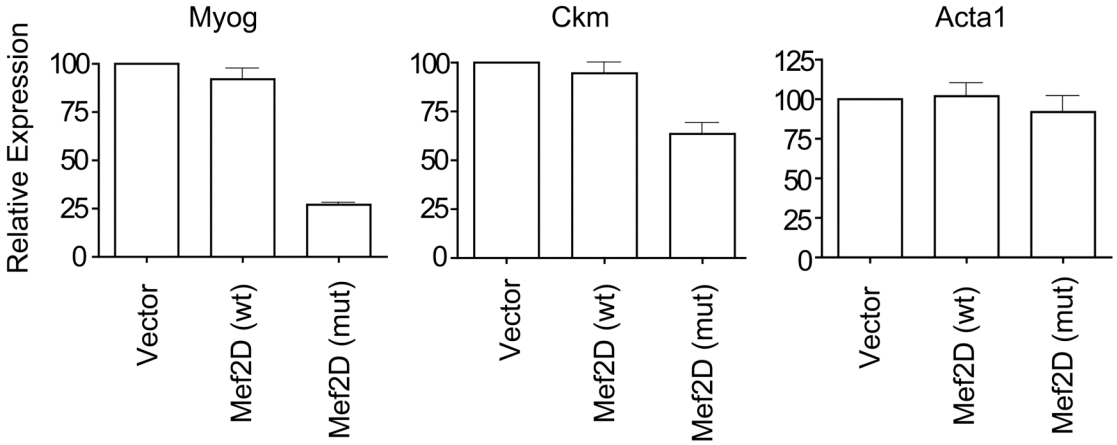
Shravanti Rampalli, LiFang Li, Esther Mak, Kai Ge, Marjorie Brand, Stephen J. Tapscott, and F. Jeffrey Dilworth



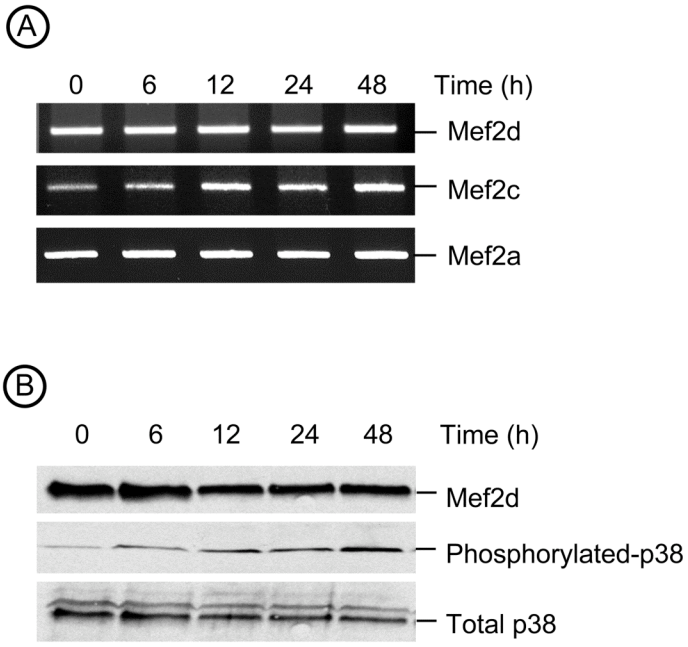

**Supplemental Figure 1. Distribution of H3K4me2 and H3K4me3 across the Myog locus.** A) Schematic representation of the Myog gene locus. The numbers 1 through 4 represent the position of primer sets used in the ChIP studies. B) Native ChIP analysis was used to measure relative enrichment of H3K4me2 and H3K4me3 at various locations along the Myog locus in differentiating (48 h) C2C12 cells as described above. Resolution of the native ChIP was ~550 bp after micrococcal nuclease digestion. Relative enrichment is expressed as the signal observed on the Myog gene with respect to that observed at the inactive Igh gene. Average values of duplicate qPCR reactions are displayed with error bars corresponding to ± s.d. Each experiment was performed at least twice independently.



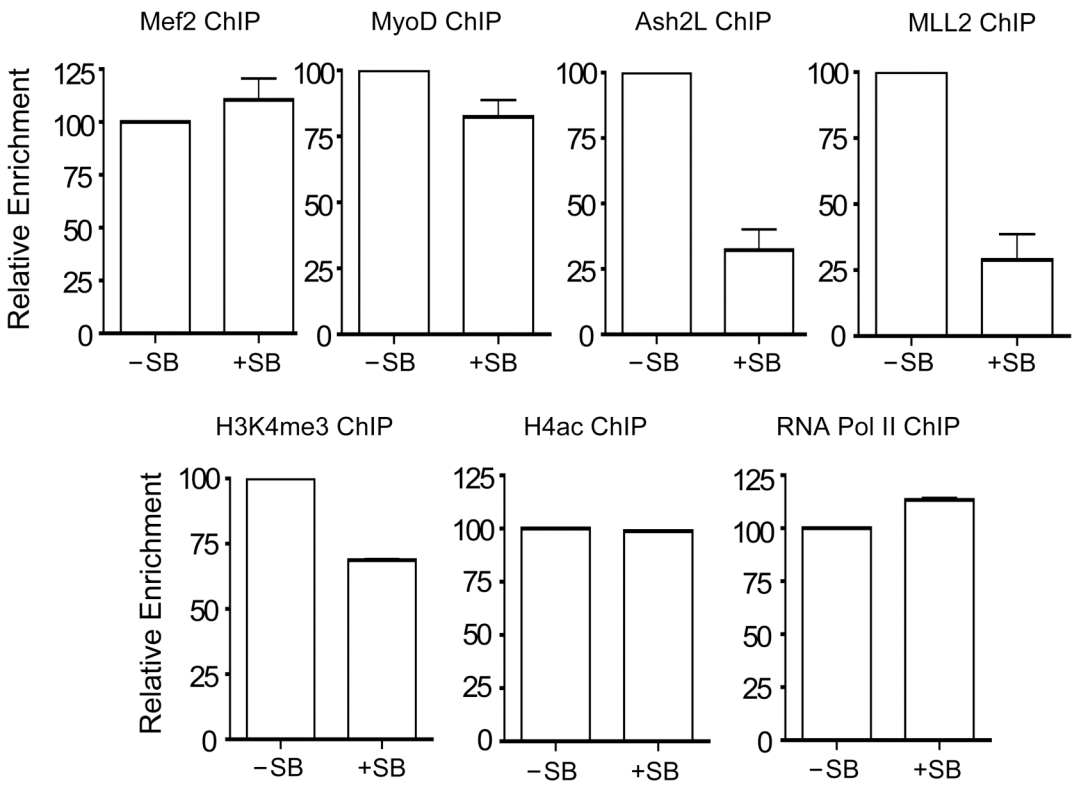

**Supplemental Figure 2. Mef2 interacts with multiple Ash2L-containing methyltransferase complexes.** A) The Ash2L complex is expressed in C2C12 cells. Extracts were prepared from C2C12 cells corresponding to the cytoplasm, nucleus, or nuclear pellet. Extracts were analyzed by western blot using indicated antibodies. B) Mef2 interacts with the MLL2, MLL3, and Set1 containing Ash2L-complexes. Nuclear extracts prepared from differentiating C2C12 cells (48 h) were subjected to immunoprecipitation using anti-MLL, -MLL2, -MLL3, -MLL4, -Set1 or control Rabbit IgG antibodies. Immunopreciptated proteins were analyzed by western blot using either Mef2 or Ash2L antibodies. C) Mef2 associates with an H3K4 methyltransferase activity in C2C12 cells. Mef2, Myog, and mock immunoprecipitates from differentiating (48 h) C2C12 cells were incubated with *S*-[methyl-$^{3}$H]-adenosyl-*L*-methionine in the presence of purified core histones. Reactions were split into 2 fractions, separated by SDS-PAGE, and either stained with coommassie blue, or transferred to PVDF membrane and exposed to autoradiographic film. D) Mef2 and mock immunoprecipitates from differentiating (48 h) C2C12 cells were incubated with 10 $\mu$M *S*-adenosyl-*L*-methionine in the presence of purified core histones. Reactions were split into 2 fractions, separated by SDS-PAGE, and either stained with coommassie blue, or analyzed by western blotting using antibodies directed against H3K4me3.



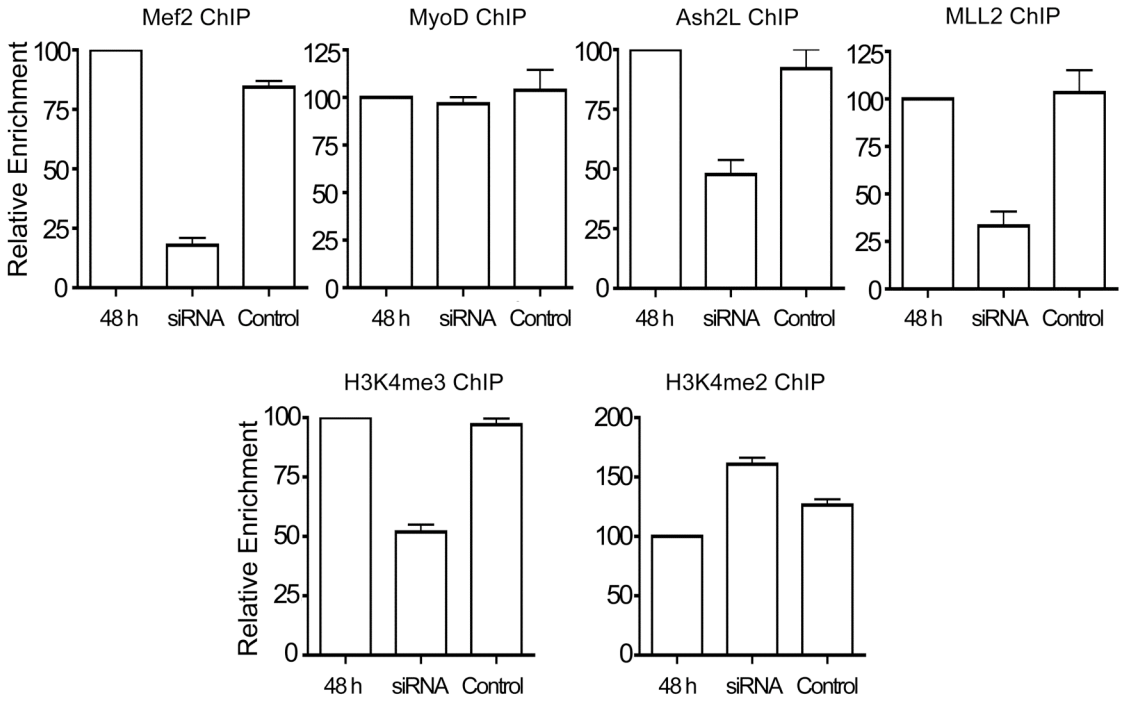

**Supplementary Figure 3. A mutant Mef2d that is not phosphorylated by p38 acts in a dominant negative manner to inhibit Myog and Ckm expression.** C2C12 cells were transfected with pBABE (Vector), pBABE-Mef2d(wt), or pBABE-Mef2d(mut), and allowed to differentiate. After 48 h, RNA was extracted and subjected to reverse transcription and duplex qPCR analysis using Taqman probes. Expression of Myog, Acta1, and Ckm is reported relative to the control 18S RNA signal. Average values of triplicate RT-qPCR reactions are displayed with error bars corresponding to ± s.d. Each experiment was performed at least twice independently.




**Supplementary Figure 4. Phosphorylation of p38 is increased during differentiation.** A) RNA isolated from C2C12 cells at different stages of differentiation was reverse transcribed and subjected to semi-quantitative PCR analysis using primers specific for the genes indicated. B) Whole cell proteins extracts prepared from C2C12 cells at different stages of differentiation were analyzed by western blot using indicated antibodies




**Supplementary Figure 5. Inhibition of p38 activity prevents recruitment of Ash2L and prevents H3K4me3 at the Ckm promoter.** ChIP was used to measure relative enrichment of the proteins indicated to the Ckm promoter in C2C12 cells differentiated (48 h) in the presence or absence of SB203580. After deproteination, immunopurified DNA was quantitated by Real-Time PCR using Taqman probes. Average values of duplicate qPCR reactions are displayed with error bars corresponding to ± s.d. Each experiment was performed at least twice independently.




**Supplementary Figure 6. Knock-down of Mef2d and Mef2c in C2C12 cells leads to reduced recruitment of the Ash2L complex to the Ckm gene promoter.** Prior to differentiation, C2C12 cells were transfected with siRNA targeting both Mef2c and Mef2d (such that both family members would be knocked down), or an untargeted control siRNA. Both transfected and untransfected cells were then differentiated for 48 h, and further analyzed by ChIP for enrichment of H3K4 methylation at the Ckm promoter. After deproteination, immunopurified DNA was quantitated by Real-Time qPCR using Taqman probes. Average values of duplicate qPCR reactions are displayed with error bars corresponding to ± s.d. Each experiment was performed at least twice independently.

## Supplementary Table 1. Primers Used for PCR analysis.

| | |
|---|---|
| Myog Coding (+1000) | |
| Probe | 5'FAM-ACCCTGGTAACATGGCTCAAATCCCT-3'BHQ1 |
| Forward | 5'-TCCCAACCCAGGAGGTAAGTGAAT-3' |
| Reverse | 5'-AAGGGTGATTGGGACAGGGCTTAT-3' |
| Myog Start Site (-145) | |
| Probe | 5'FAM-CTTGATGTGCAGCAAC-3'BHQ1 |
| Forward | 5'-TCACATGTAATCCACTGGAAACG-3' |
| Reverse | 5'-CCTGAGCCCCCCTCTAAGC-3' |
| Myog Promoter (-1000) | |
| Probe | 5'FAM-TCGACCCTTCTACAGAAAGGAAAGAGTCA-3'BHQ1 |
| Forward | 5'-GCCCAGGACAGACAAATGATGCAA-3' |
| Reverse | 5'-AATGCCTTCTGGCACTAGAACCGT-3' |
| Myog Upstream (-10000) | |
| Probe | 5'FAM-TCCTTTGGCTCTCACCGTGTCTACAT-3'BHQ1 |
| Forward | 5'-CTCTCTTCTGAATGGCGTTTGCGT-3' |
| Reverse | 5'-TTTGAAAGAGCTCCAGCTTTGGGC-3' |
| Ckm Enhancer (-1340) | |
| Probe | 5'FAM-TCTAGGCTGCCCATGTAAGGAGGCAA-3'BHQ1 |
| Forward | 5'-GCTCCTGTCATATTGTGTCCTGCT-3' |
| Reverse | 5'-TTATAACCAGGCATCTCGGGTGTC-3' |
| Ckm Intron 1 (+915) | |
| Probe | 5'FAM-AGCAAGGAGGGAGGACAGGT-3'BHQ1 |
| Forward | 5'-CAATGCTGACTTAGTGCAAGGCGA-3' |
| Reverse | 5'-AGACAGAGACCCAAAGCCCTTGAA-3' |
| Acta1 Promoter (-317) | |
| Probe | 5'FAM-CCTTTGGCCCAGCACAGCCCT-3'BHQ1 |
| Forward | 5'-ACCAGCGGTCAAAGCAGTG-3' |
| Reverse | 5'-CTCCCAACTGGCTCCAAGG-3' |
| Cdkn1a Promoter (-297) | |
| Probe | 5'FAM-ATAGATGTATGTGGCTCTGCTGGTGC-3'BHQ1 |
| Forward | 5'-AGTTGGTCAGGGACAGACCCATAA-3' |
| Reverse | 5'-ACACCTGGGCTATTCTCTTGTCAC-3' |
| Igh Enhancer (+4367) | |
| Probe | 5'FAM-CGCCTCTGAGCCTGGGCAAGG-3'BHQ1 |
| Forward | 5'-GCCTGGGCTTGCTTTGTCT-3' |
| Reverse | 5'-TGACCCCTTTCGCTCATTG-3' |
| Myog cDNA | |
| Probe | 5'FAM-ATGGTGCCCAGTGAATGCAACTCCCA-3'BHQ1 |
| Forward | 5'-CATCCAGTACATTGAGCGCCTACA-3' |
| Reverse | 5'-AGCAAATGATCTCCTGGGTTGGGA-3' |
| Ckm cDNA | |
| Probe | 5'FAM-TCAACCACGAGAACCTCAAGGGTGGA-3'BHQ1 |
| Forward | 5'-ACCCACAGACAAGCATAAGACCGA-3' |
| Reverse | 5'-AGGCAGAGTGTAACCCTTGATGCT-3' |
| Acta1 cDNA | |
| Probe | 5' FAM-TATGTGGCCCTGGACTTCGAGAATGA-3'BHQ1 |
| Forward | 5'-TTGTGCGCGACATCAAAGAGAAGC-3' |
| Reverse | 5'-GAAACGCTCATTGCCGATGGTGAT-3' |

| | |
|---|---|
| Mef2a cDNA | |
| Forward | 5'-ATTCTCCAATTGTGCTTGGCCGAC-3' |
| Reverse | 5'-AGGAAGTGCCAGACTGGTCTGTTT-3' |
| Mef2c cDNA | |
| Forward | 5'-TCAACAGCACCAACAAGCTGTTCC-3' |
| Reverse | 5'-ACCTGTTATGGCTGGACACTGGGA-3' |
| Mef2d cDNA | |
| Forward | 5'-TTCAGGCGCTATGGGTCATCTGTT-3' |
| Reverse | 5'-AGGCTCCATTAGCACTGTTGAGGT-3' |
| Cdkn1a cDNA | |
| Forward | 5'-TGTCCAATCCTGGTGATGTCC-3' |
| Reverse | 5'-TCAGACACCAGAGTGCAAGAC-3' |
| Gapdh cDNA | |
| Forward | 5'-AGCCACATCGCTCAGACACC-3' |
| Reverse | 5'-GTACTCAGCGGCCAGCATCG-3' |