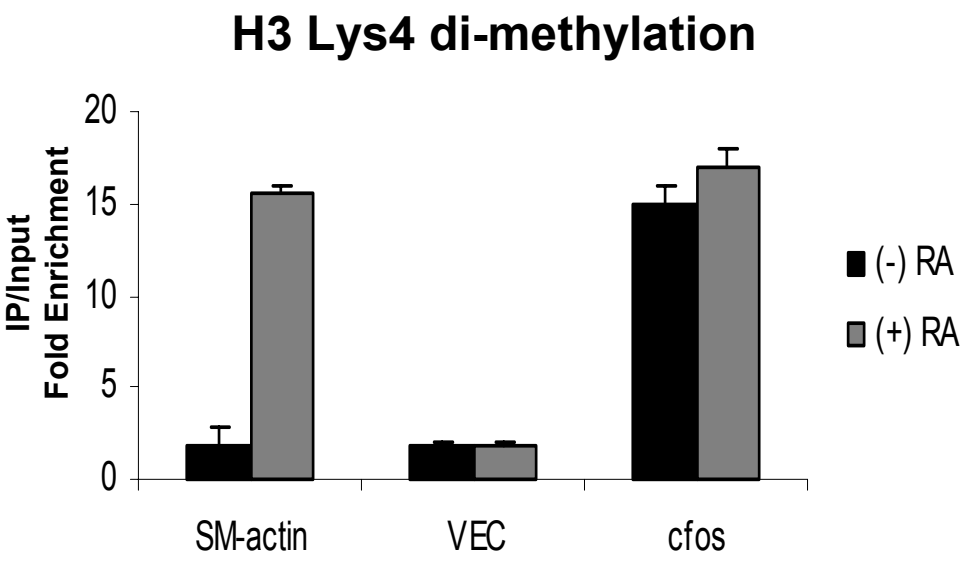
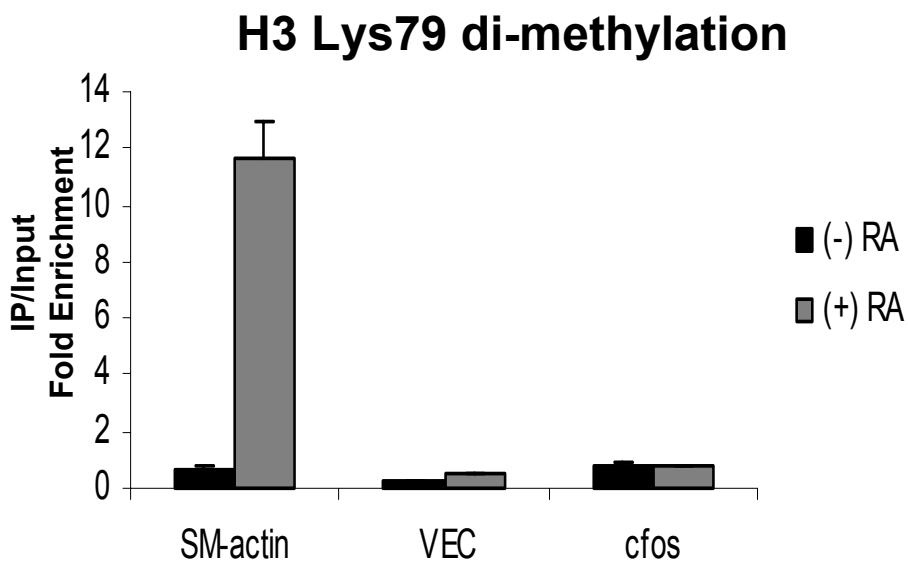
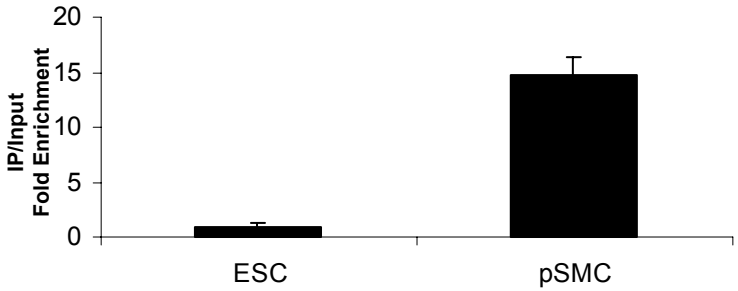
**Supplementary Figure 1A**
**A404 Cells +/- Retinoic Acid**



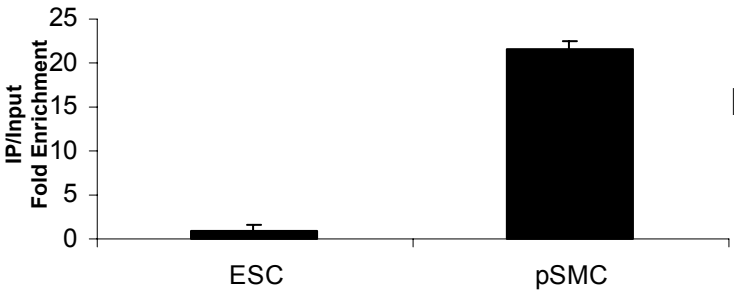



**Supplementary Figure 1B**
**5'-CArG Region of SM α-actin**
**Mouse SMCs Purified from Embryoid Bodies**

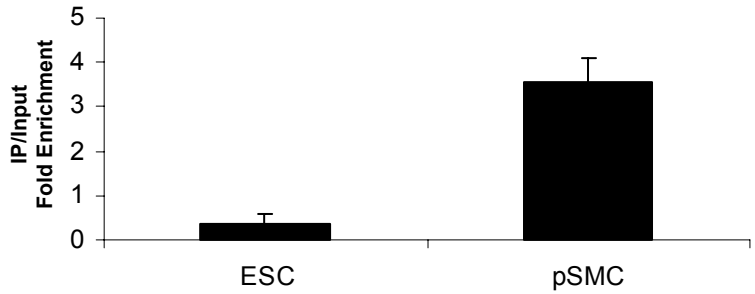
**SRF Binding**



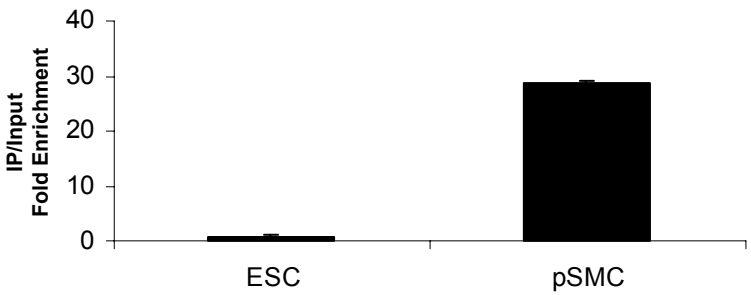

**H3 Lys4 Di-methyl**



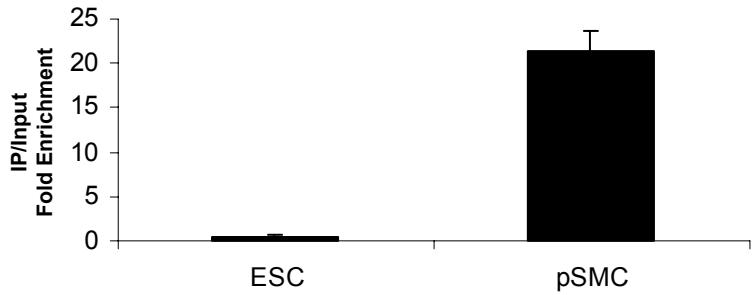

**H3 Lys79 Di-methyl**



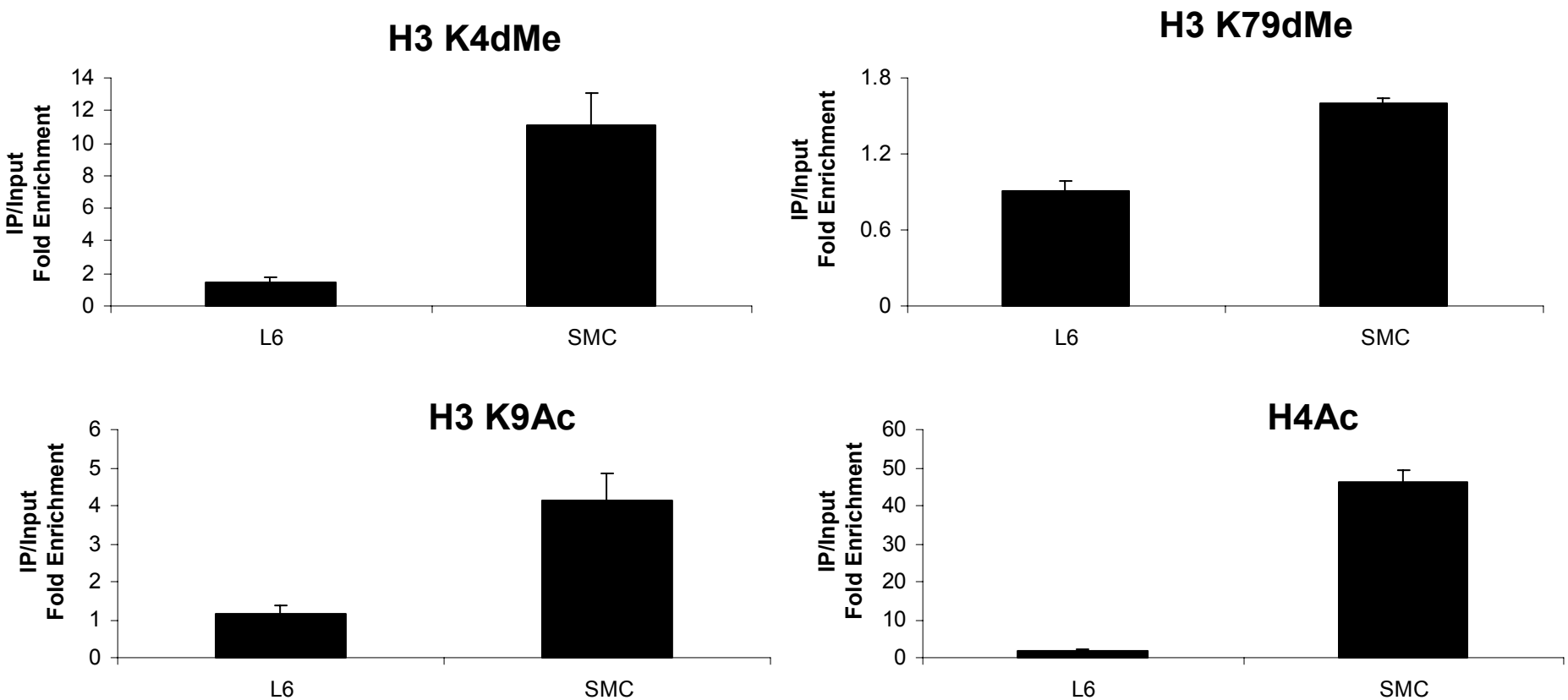

**H3 Lys9 Acetyl**



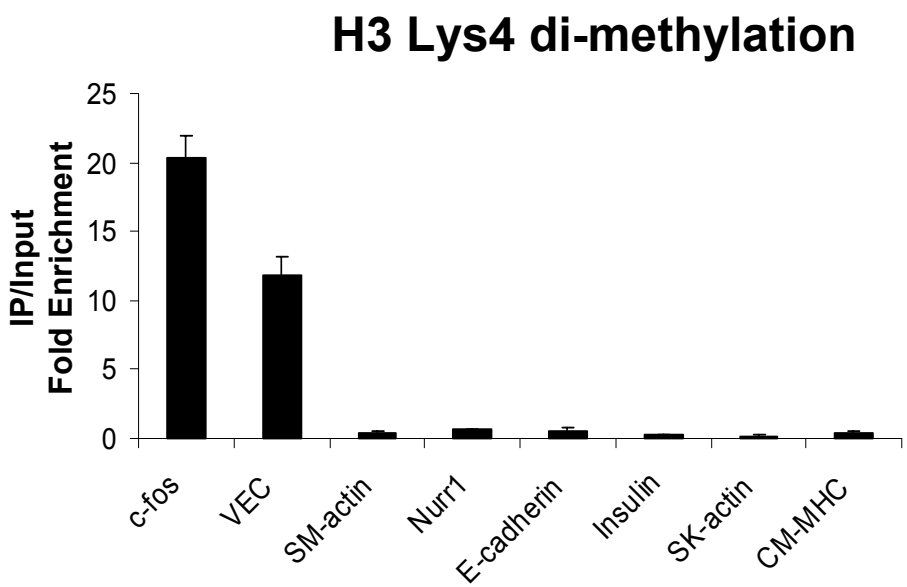

**H4 Acetyl**



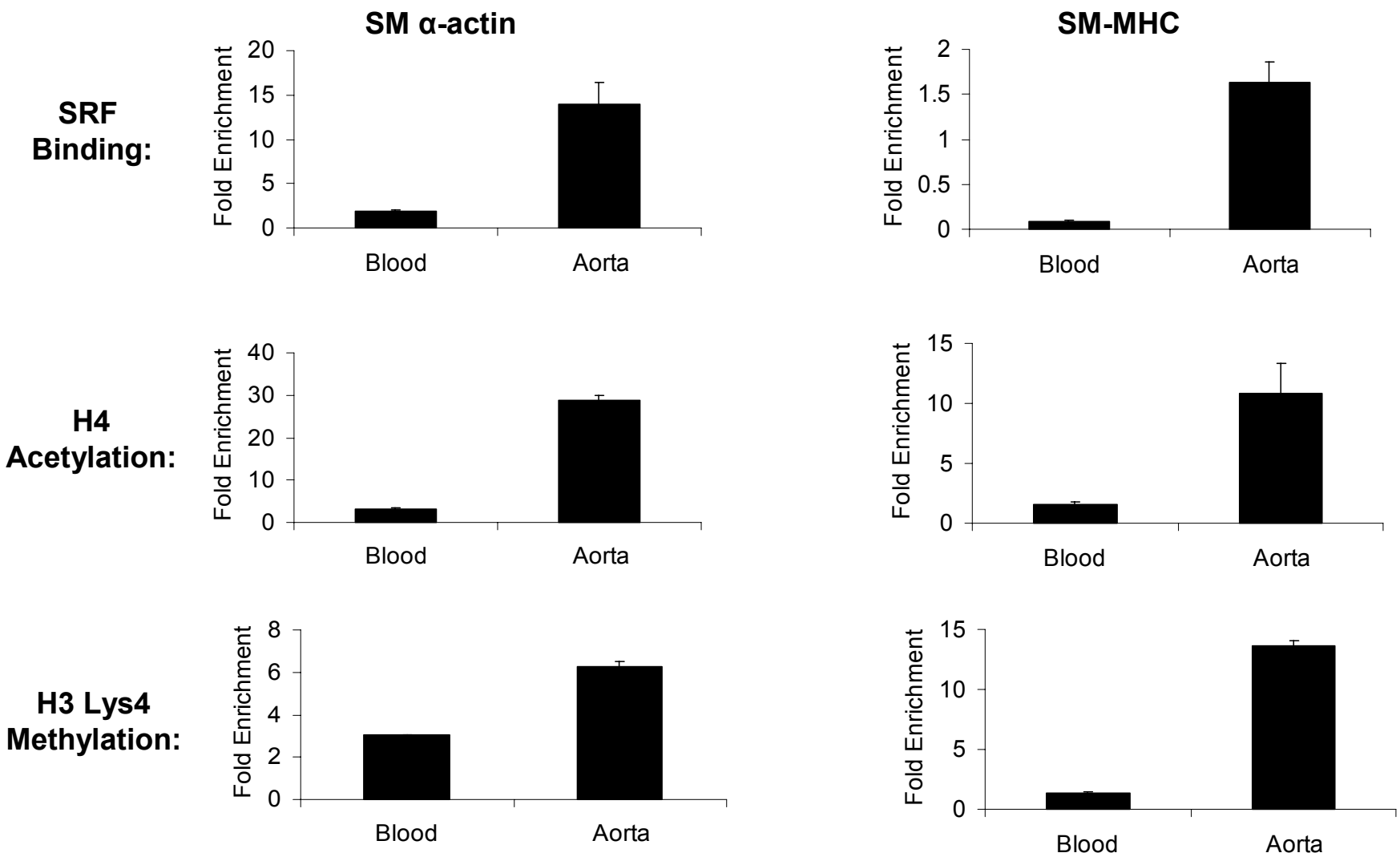
**Supplementary Figure 1C**
**SM-MHC 5'-CArG Boxes in SMCs verses L6 Skeletal Muscle Myotubes**



**Supplementary Figure 1D**
**Cultured Rat Aortic ECs**

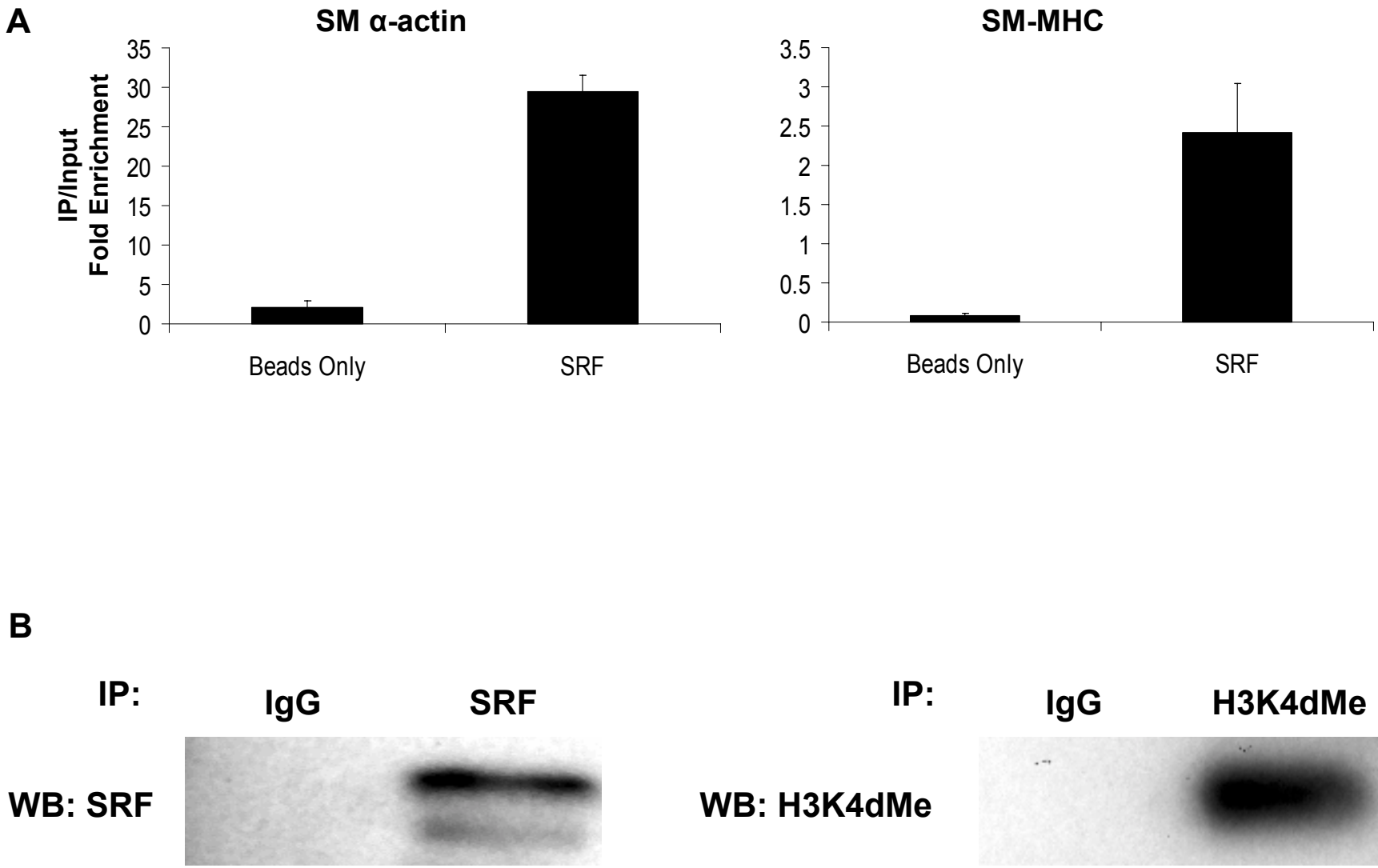


**Supplementary Figure 1E**
**5'-CArG Regions From Rat Tissues**



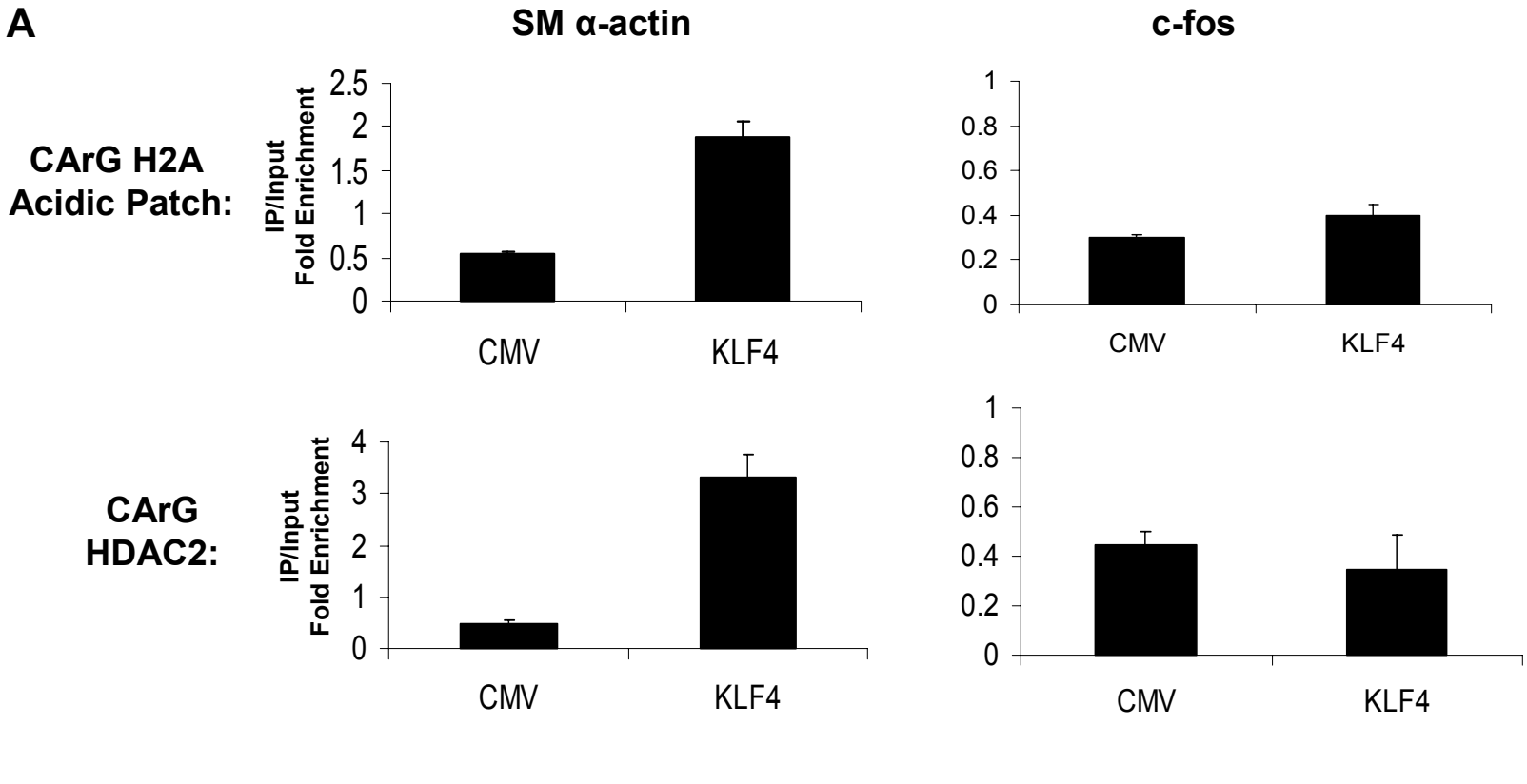
**Supplementary Figure 1.A**) Levels of H3K4dMe and H3 K79 methylation were measured by ChIP in A404s cells treated with or without retinoic acid, which induces expression of SM α-actin. Our previous studies showed that RA treatment induced histone H3/H4 acetylation and the appearance of SRF association at CArG boxes of SM α-actin. B) Levels of SRF and histone modifications were measured in mouse embryonic stem cells compared to SMCs purified from mouse embryoid bodies at SM α-actin CArG boxes. Similar results were obtained for SM-MHC (data not shown). C) SRF binding and histone modifications were measured at CArG boxes of SM-MHC in rat L6 skeletal muscle myotubes as compared to rat aortic SMCs. SMCs were enriched for SRF and modifications associated with activation (green in Table 1), whereas L6s were not enriched with these modifications. D) Histone modifications associated with gene activation (H3 Lys4 di-methylation) were measured at several cell lineage-specific promoters in rat aortic ECs. Promoters: c-fos: ubiquitously expressed, VEC: EC-specific, SM-actin: SMC-specific, Nurr1: neuron-specific, E-cadherin: epithelial-specific, insulin: pancreatic β-cell-specific, SK-actin: skeletal muscle-specific, CM-MHC: cardiac muscle-specific. E) Levels of SRF, and H4K4dMe and H4Ac (shown to be upstream of SRF in Figure 3A) were measured by ChIP from chromatin isolated from rat aorta and compared to chromatin isolated from rat blood cells.

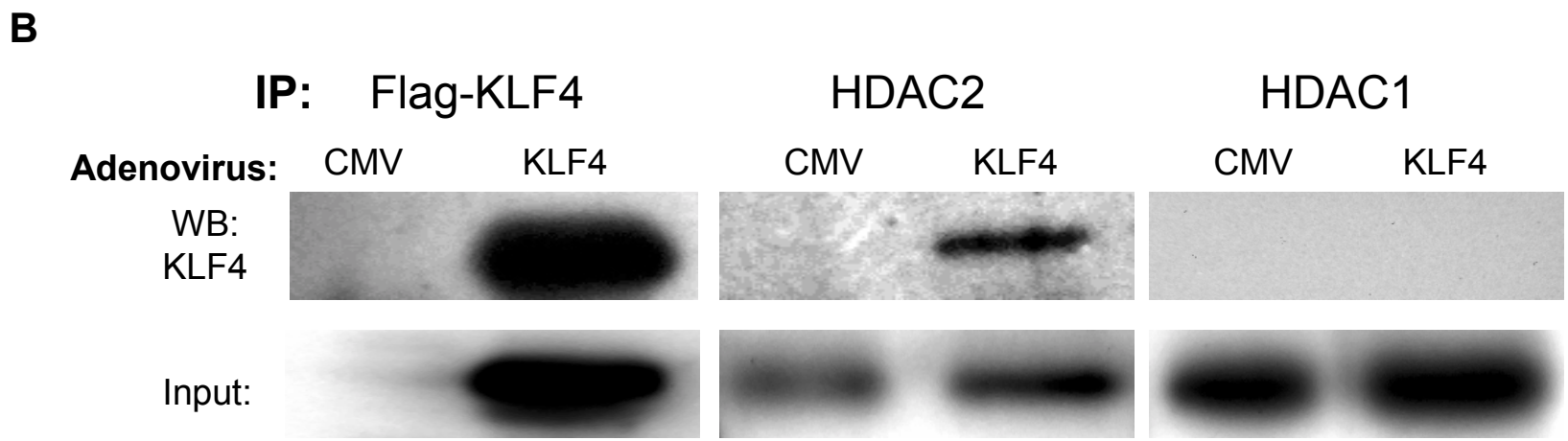
**Supplementary Figure 2**



**Supplementary Figure 2.** No antibody/Beads only and non-immune IgG do not pull down factors assayed for in ChIP or protein assays. Data is representative of all ChIP and protein assay experiments. A) The no antibody/Beads-only negative controls did not immunoprecipitate DNA from the SM α-actin or SM-MHC 5'-CArG loci, as compared to SRF. We consistently observed that the beads-only control immunoprecipitated very low amounts of DNA, and PCR amplified at much higher cycle numbers, than samples that were incubated with antibody. B) Non-immune IgG did not immunoprecipitate amounts of SRF or H3K4dMe protein that were comparable to protein immunoprecipitated by antibodies to these factors. 200ug aliquots were taken from the same SMC nuclear extract and incubated with the corresponding antibodies, and immunoprecipitates were western blotted for SRF or H3K4dMe.

**Supplementary Figure 3**



**Supplementary Figure 3.** A) Adenoviral expression of KLF4 in cultured rat aortic SMCs increased the concentration of the acidic patch domain of histone H2A accompanied by the appearance of HDAC2 at the CArG boxes of SM α-actin but not c-fos, compared to control cells. B) HDAC2, but not HDAC1, was able to immunoprecipitate flag-tagged KLF4 from SMCs infected with KLF4 adenoviruses compared to control cells.

**Real-time PCR Primer Sequences (Sybergreen or FAM-probes)**

**I. ChIP Primers**

1. α-SMA

*A) 5'-CArG Box (rat, mouse, human)*

F: AGC AGA ACA GAG GAA TGC AGT GGA AGA GAC

R: CCT CCC ACT CGC CTC CCA AAC AAG GAG C

*B) 5'-CArG Box (rat-specific) Tm: 60*

F (2551): CCT GTT TCG AGA GCA GAG

R (2735): GGT TAT ATA GCC CCC TGG

*C) 5'-CarG Box (mouse-specific) Tm: 60*

F (2551): CCT GTT TCG GGA GCA GAA

R (2735): GGT TAT ATA GCC CCC TGG

*D) Epimapping Primers (rat) Tm: 60 (Figure 2)*

F (-100): AGA TGC CCC AAT GGG TAC AAT GCA TGC

R (-100): AAC CCA GTA CTT TCT GAT TCA GCT CAC C

F (-400): ATG TGT CCA GTG GCT ACT GCA TCA GG

R (-400): TCT GGT CCA GAG ATG ACT TAG CAT GG

F (-800): CTC AGT GGA GCT CTG TAA ACT TGT ACG

R (-800): ATG TGG TTG CTG GGA TTT GAA CTC TGG

F (-1200): CAA ATC CTC CTT GGT TAC TTC AGA TTG C

R (-1200): TTG TCT TGG TCA TTT CAA GAT GCT AAT TCC

F (-1600): CAG ACA ATG TAT TTC TAG TTG GTG AAA CC

R (-1600): ATG CTC TTG ATT AAT GGT GTC CAC CTG C

F (-2000): CAT AAA CAC GGA GGA GGA TGA GCA GG

R (-2000): GGT ACA GTC CAC GTG CAT GTT GTT GG

F (Ebox): GCA TCT TCT GAG GAA TGT G

R (Ebox): GGT CTC TTC CAC TGC ATT C

F (+2800/+1): GCT CCT TGT TTG GGA AGC GAG TGG GAG G

R (+2800/+1): TCA TTC AGA TTA CTA CAG ACA ACG TCT GG

F (+3200/+400): TTG TCT ATG GTT CAG ACT TAG TGT GAG G

R (+3200/+400): GCA ACG CAG TCC AGT CAG CCA AAA CC

F (+3600/+800): GAA ACG GAG TTT TCT CGA TAA GAT TTT CC

R (+3600/+800): CAG CAC AGT GGA GCT CAT CTT GTG C

F (+4000/+1200): CTA AGG CAC AAC CTT AAG TCA TCC TGC

R (+4000/+1200): AGA GTC TAC AGG AAG CCT GCT GTC C

F (+4400/+1600): TCT CTG GGT AAA GAC AAT AGT CAC ATG G

R (+4400/+1600): CTT GAC TAA CTG AGC ACA TAC TCT TTT GG

F (+4800/+2000): GGA CTT GGT AGC CTT TCA ACT TGT GAT CC

R (+4800/+2000): CTA CCA CTT GGG AAG CTG AGG CAG G

F (+5200/+2400): TAG ACC TGT GTT CTC CAC TCA GGA CC

R (+5200/+2400): ACA AGC CCT CAG CAC AAA GAG CAG G

*E) Integrase Primers (Figure 3A)* Tm=60

a) Wild-type experiments

F (Wt Endogenous): GCC TGC GTC TGC TTC ATG

F (Wt Transgene): GCC TGC GTC TGC TTC ACA

R (both): GGT TAT ATA GCC CCC TGG

b) CArG Mutant Experiments

R (Endogenous): CCA CTC GCT TCC CAA ACA AGG

R (Mutant Transgene): CCA CTC GCT TCT TAA ACA ATT

F (both): CCT GTT TCG AGA GCA GAG

2. SM-MHC

*5'-CArG Boxes (rat, mouse, human)*

F: CTG CGC GGG ACC ATA TTT AGT CAG GGG GAG

R: CTG GGC GGG AGA CAA CCC AAA AAG GCC AGG

3. c-fos

*A) 5'-CArG Box (rat, mouse)*

F: CGG TTC CCC CCC TGC GCT GCA CCC TCA GAG

R: AGA ACA ACA GGG ACC GGC CGT GGA AAC CTG

*B) 5'-CArG Box (human)*

F: CCG CCT CCC CCC GCA CTG CAC CCT CGG TG

R: CAG GGC TAC AGG GAA AGG CCG TGG AAA CCT G

4. Cardiac Muscle $\alpha$-MHC

*A) 5'-CArG Boxes (rat) Tm=60*

F: ACC TTT CAT GGG CAA ACC TCA G

R: CCT GCT GCC TAA ATT TGG AGT CA

Probe: CCC TCC CTC CCA TCC CTC CTG TCC

*B) 5'-CArG Boxes (mouse)*

F: ATC TGC CCA TCG GCC CTT TGG G

R: ACA GCA GGG CCC CAA GGT TTG C

5. Vascular Endothelial Cadherin

*A) 5'-Promoter Region (rat)*

F: ACA AAG GAA CAA TAA CAG GAA ACC ATC

R: CTT TGT GTA GAG CTC TGT TGA TTG C

*B) 5'-Promoter Region (mouse)*

R: GTC CAG GGC CGA GCT TTG TGG

*C) 5'-Promoter Region (human)*

R: CCT GTC AGC CGA CCG TCT TTG G

6. Skeletal Muscle Alpha Actin

*A) 5'-CArG Boxes (rat, mouse)*

F: CTG AGA ACC AGC CGA AGG AAG GGA CTC TAG

R: ACC TCC ACC CTA CCT GCT GCT CTG ACT CTG

7. Nurr1

*5'-Promoter Region (rat)*

F: CCA CCC AAG TGG GCT ACC AAG GTG AAC

R: AAT CGC CGA GCC GCC GCG CTC

8. E-Cadherin

*5'-Promoter Region (rat)*

F: GAA GTC TCC TAA GCC CGG CCC

R: GCT GCG GCC GCC AGG TGA GC

9. Insulin

*5'-Promoter Region (rat)*

F: GCC AAA ACT CTA GGG ACT TTA GGA AGG ATG

R: GCC GGG CAA CCT CCA GTG CCA AGG TCT GAA GAT C

10. Fibroblast-specific Protein 1 (FSP-1, S100A)

*5'-Promoter Region (rat)*

F: TCA CTA CTT GAT TGT GCC TGC TAG

R: TAT ACC CTG CCA TGC CCT GCC C

**II. RT-PCR Primers (Tm = 60˚C)**

1. α-SMA

*Mouse*

F: CGCTGTCAGGAACCCTGAGA

R: CGAAGCCGGCCTTACAGA

Probe: CAGCACAGCCCTGGTGTGCGA

*Rat*

F: AGTCGCCATCAGGAACCTCGAG

R: ATCTTTTCGATGTCGTCCCAGTTG

Probe: CAGCACAGCTCTGGTGTGTGAC

2. SM-MHC

*Mouse*

F: TGGACACCATGTCAGGGAAA

R: ATGGACACAAGTGCTAAGCAGTCT

Probe: AGAACACTAAACGACAGCAGAGCCCAGC

*Rat*

F: CAGTTGGACACTATGTCAGGGAAA

R: ATGGAGACAAATGCTAATCAGCC

Probe: CAAAATACCAAATGACAGCAAAGCCCAGC

3. Myocardin

*Mouse and Rat*

F: CGGATTCGAAGCTGTTGTCTT

R: AAACCAGGCCCCCTCCC

Probe: ACTCTGACACCTTGAGATCATCCAGGTTTGG

4. KLF4

*Rat*

F: CTTTCCTGCCAGACCAGATG

R: GGTTTCTCGCCTGTGTGAGT

Probe: ATTATCAAGAGCTCATGC

5. 18S

*Mouse and Rat*

F: CGGCTACCACATCCAAGGAA

R: AGCTGGAATTACCGCGGC

Probe: TGCTGGCACCAGACTTGCCCTC