Online Data Supplement

PKA REGULATED ASSEMBLY OF A MEF2/HDAC4 REPRESSOR COMPLEX CONTROLS C-JUN EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS

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Running Head: MEF2/HDAC4 repress c-Jun in VSMC

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Supplemental Figure Legends

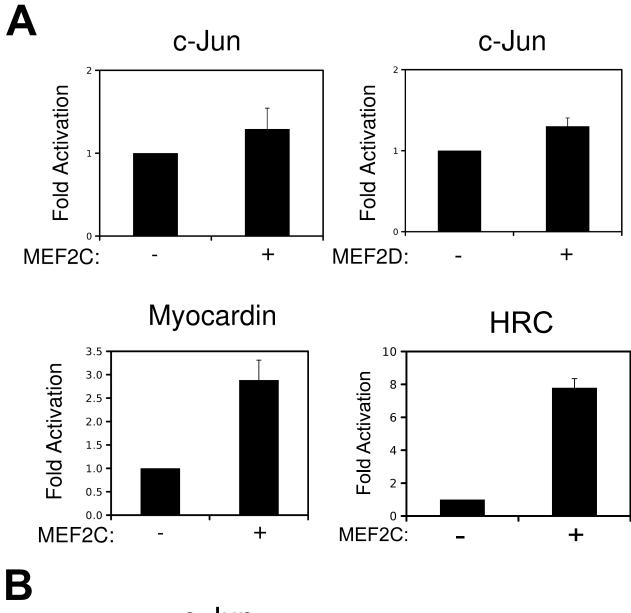
Figure S1. MEF2 activates of the myocardin reporter gene but not *c-jun* in VSMCs. A) A10 cells were transfected with the *c-jun*, HRC, or myocardin enhancer reporter-genes and either empty pcDNA3 or MEF2C, or MEF2D. Cells were harvested for luciferase 24 hours after recovery. B) A10 cells transfected with the *c-jun* reporter gene were treated overnight with TGF- β 1 (2 ng/mL) and harvested for luciferase assay.

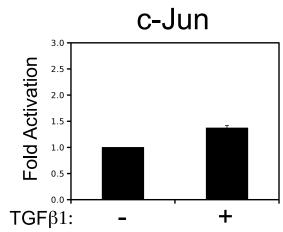
Figure S2. HDAC5 does not inhibit the *c-jun* promoter and HDAC4 does not inhibit the HRC promoter.

A) A10 cells were transfected with the HRC reporter-gene and pMT2-MEF2A and/or Flag-HDAC4, or Flag-HDAC5, as indicated. Cells were harvested for luciferase 24 hours after recovery. B) A10 cells were transfected with the myocardin-enhancer reporter gene (MyE-luc), a myocardin-enhancer with the MEF2 binding site mutated (MyEmut-luc), the HRC promoter (HRC-luc), or the HRC promoter with a mutation in the MEF2 binding site (HRCmut-luc). Cells were harvested for luciferase assay.

Figure S3. Effect of PKA on c-Jun expression and the MCP-1 promoter. A) Quiescent A10 cells were pretreated with isoproteronol (1 μ M), milrinone (10 μ M), Rp-cAMPS (10 μ M), or H89 (10 μ M), as indicated, and stimulated with PDGF (20ng/mL) for 1 hour. Protein extracts were subjected to immunoblotting. B) A10 cells were transfected with the MCP-1 reporter-gene and pFC-PKA, as indicated. Cells were harvested for luciferase 24 hours after recovery. C) A10 cells were transfected with a MEF2 reporter gene (MEF2-luc), pMT2-MEF2A, or pFC-PKA, as indicated. Cells were harvested for luciferase assay.

Figure S1.





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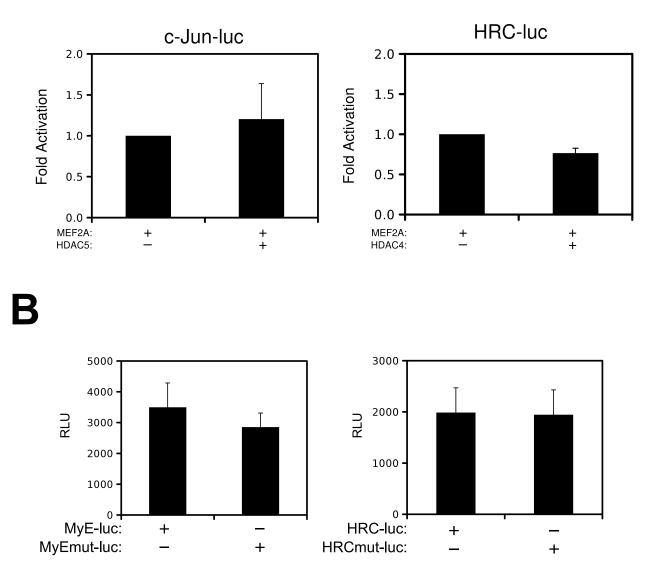


Figure S3.

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