

**HISTONE CODE MODIFICATIONS REPRESS GLUCOSE TRANSPORTER 4  
EXPRESSION IN THE INTRA-UTERINE GROWTH RESTRICTED OFFSPRING**

**Nupur Raychaudhuri, Santanu Raychaudhuri, Manikkavasagar Thamotharan  
and Sherin U. Devaskar\***

Division of Neonatology and Developmental Biology and the Neonatal Research Center,  
Department of Pediatrics, David Geffen School of Medicine UCLA, Los Angeles, CA 90095-  
1752

**Supplemental Materials**

**Table 1**

Sequences of primers used for cloning and mutational analysis of GLUT4 promoter elements

Primers

DNA Sequences

GLUT4-For	5'-gca cag gta ccg agg ctc agg act tca gag-3'
GLUT4-Rev	5'-gca tca agc ttt aaa aga aga ctc agg cac tgc-3'
<u>Deletional mutant constructs (-723 bp to -610 bp)</u>	
Δ CpG-I-For	5'-gtg agc acc tgt ccc ttg gtt cga gcg gaa gtt att ggt cc-3'
Δ CpG-I-Rev	5'-gga cca ata act tcc gct cga acc aag gga cag gtg ctc ac-3'
<u>Deletional mutant constructs (-574 bp to -476 bp)</u>	
Δ CpG-II-For	5'-gtc cct tgg gtc atc tcc ttg acg tgg gag cta aaa ata gc-3'
Δ CpG-II-Rev	5'-gct att ttt agc tcc cac gtc aag gag atg acc caa ggg ac-3'
<u>Deletional mutant constructs (-453 bp to -424 bp)</u>	
Δ CpG-III-For	5'-cgt ggg agc taa aaa tag cca cac aca cac aca c-3'
Δ CpG-III-Rev	5'-gtg tgt gtg tgt gtg gct att ttt agc tcc cac g-3'
<u>Deletional mutant constructs (-744 bp to -723 bp)</u>	
Δ MyoD-I-For	5'-gag ggt gat gtg acc ggc cct cca gga acc aat gta gag-3'
Δ MyoD-I-Rev	5'-ctc tac att ggt tcc tgg agg gcc ggt cac atc acc ctc-3'
<u>Deletional mutant constructs (-156 bp to -145 bp)</u>	
Δ MyoD-II-For	5'-gaa cct tag ggg cgt gtc att aat ctt agg gtt ggg-3'
Δ MyoD-II-Rev	5'-ccc aac cct aag att aat gac acg ccc cta agg ttc-3'
<u>Deletional mutant constructs (-473 bp to -453 bp)</u>	
Δ MEF2-For	5'-gac att tgg cgg agc cta act ccg ggt tac ttc ggg-3'
Δ MEF2-Rev	5'-ccc gaa gta acc cgg agt tag gct ccg cca aat gtc-3'

**Table 2**

Primers used in the gelshift assays

Primers

DNA sequence

GLUT4-MEF2-For	5'-ccctttaaggctccatctcccttgc-3'
GLUT4-MEF2-Rev	5'-aatggctatttttagctccca-3'
GLUT4-MyoDI-For	5'-gcacaggtaccgaggctcaggacttc-3'
GLUT4-MyoDI-Rev	5'-ggaccaataactccgctcgaaccaagg-3'
GLUT4-MyoDII-For	5'-ccaggatttgggtggcggg-3'
GLUT4-MyoDII-Rev	5'-gcccccaaccctaagattaatgccagc-3'

## Histone Code and GLUT4 Expression

**Table 3**

Primers used for creating probes employed in gelshift competition assays

Primers	DNA Sequence
MEF2- Sense	5'-gtgggagctaaaaatagccatt-3'
MEF2-Anti-sense	5'-aatggctatttttagctcca-3'
MyoD-I Sense	5'-gtgagcacctgtcccttggtt-3'
MyoD-I Anti-sense	5'-accaaggacaggtgctcac-3'

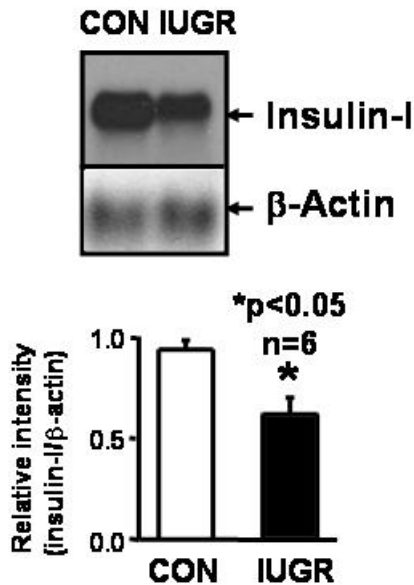
**Table 4**

Primers used in CHIP assays

Primers	DNA Sequence	Position
GLUT4-(MyoDI-MEF2)-For	5'-ccaaaacaggagctgactctg-3'	-836 bp
GLUT4-(MyoDI-MEF2)-Rev	5'-aatggctatttttagctcca-3'	-452 bp
GLUT4-(MEF2-MyoDII)-For	5'-gtgggagctaaaaatagccatt-3'	-473 bp
GLUT4-(MEF2-MyoDII)-Rev	5'-cacctcctcactcccgcccc -3'	-45 bp
GAPDH-For	5'- ccggaattcgaaggctcgggtcaacggattgg -3'	+1 bp
GAPDH-Rev	5'-cacacctgcagcctggaagatggtgatgggttcc -3'	+230 bp

**Figure S.1**

### Adult Studies



**Figure S.1. Adult Studies:** Pancreatic insulin I mRNA. Representative Northern blots demonstrating pancreatic insulin I mRNA (top panel) and  $\beta$ -actin mRNA (internal control) (bottom panel) of 60d old adult female CON and IUGR rats are shown. Quantification of insulin I mRNA is depicted in relative intensity as a ratio to  $\beta$ -actin. Inter-group differences were established by the Student's t-test (\*).

# Histone Code and GLUT4 Expression

## Figure S.2

-792 \* MyoD-I

R -----AGCACATG C-CTG-----GA GGCTCAGGACTTCAG AGAGGGTGATGTGAC **CGGC-GTGAGCACCT**

M AAGCTTGTGCGCCACGCGCCAGCACATG C-CTG-----GA GGCTCAGGGACTTCA GGGAGGGTGGTGTGA CTGGCGTGAG**CACCT**

H -----CGTTCACGCGCCAGCATATG CTCAGAGACCTCAGA GGCTCAGAGACCTCA GGGCTGGTGGTGTGG TCGGTGTGAC**CACCTT**

1 2 3

-734 <-----**CpG-I**----->

R **GTCCCTTGGTTCCC**- TCCAGGAACCAATGT AGAGAAATGTGTGGA GGGGATGGGCC**-GTA** -GACTGTGCAC**CGCC** CAGAAGTG**CG**TGGAA

M **GTCCCTTGGGTCCCC** TCCAAGAACCAGTGT AGAGAC-TATGTGGA GGGGATGGGCCAG-T AGGCAGCG**CGCC** CAGAGATG**CG**TGGAA

H **GTCCCTCGGACCGGC** TCCAGGAACCAACCT GGGGAA-TGTGTGTA GGGGAAGGG**CGGGAT** AGACAGTGCC**CGGAG** CAGGGAGG**CG**TGAA

4 5 6 7 8 9 10

-----**CpG-I**-----> **PU.1** <-----**CpG-II**-----<

R AGATAGGAC**CGACCAG** ACAC**CG**TTCTCAGAC ACAC**CG**--GGAG**CGG** **AAGT**TATTGGTCCCT TGGTTCATCTCCTTG TGGGAAG**CGAGT****CGC**

M AGAAAGGAC**CGATCAG** GCATGGTCTCCAGAT AACTA--GGA**ACCGG** **AAGT**TATTGGTCCCT TGGATCATCTCCT**CG** TGGGAAG**CGTGT****CGC**

H AGACAGGACCAAGCA GCC**CG**GCCACCAGAC **CCG**TGTGGG**AA****CGG** **AATT**TCTTGCCCCC AGGGCCACACT**CGCG** TGGGAAGCATGT**CGC**

11 12 13 14

-----**CpG-II**----->

R **GGACCCTTTAAGGCT** CCATCTCCCTTGCCC TCC---CC**CG**CCTGG GACAGGCTGGGACAC **CGGGACCTGACATT** TGG**CGGAGC**TAA**CG**

M **GGACCCTTTAAGGCT** CCATCTCCTTTGCCC TCC---CC**CG**CCTGG GACAGGCTGGGACAC **CGGGACCTGACATT** TGG**CGGAGC**-TAA**CG**

H **GGACCCTTTAAGGCG** TCATCTCCCTGTCTC TCC**CG**CCCC**CG**CCTGG GACAGG**CGGGACGC** **CGGGACCTGACATT** TGGAGGCTCCCAA**CG**

15 16

-472 **MEF2** <-----**CpG-III**-----> **WT-1 Repeat (C<sup>m</sup>A)**

R TGGGAGCT**AAAAATA** GCCATTC**CGGGTTAC** TTCGGGGCATTGTTT CTGACACACACACAC ACACACACACACACA CACACACACACACAC

M TGGGA**ACTAAAAATA** GCCACTC**CGGGTTAC** TTCGGGGCAT----- -ACA CACATACACACACAC

H TGGGAGCT**AAAAATA** GCAGCCC**CGGGTTAC** TTTGGGGCATTG--- ----- CTC-----CTCTCCC

17 \* 18

-382

R ACACACACACACACA CC**CG**CAGGCTCTGTG TCACCCTGCTGGAGT TACCC-**GT**ACCCTGG CAAGTACACCTAGCC CATACCCTCCTCTTC

M ACACACACACACACA CA**CG**CGGGCTCTATG TCATCCTGCTGGAGT TACCCCGTACCCTGG CAAGTACATGTAGCC CATACCCTCCTCTTC

H A-**ACCCGCGCGCCGG** CT**CG**CGAGCC--GTC TCAGG**CG**CCTGGAGT TTCC-----**CGGG** CAAGTACACCTGGCC CGTCTCTCCTCTCA

19 20

-293

R CACCTCTCAGGGGA CCAGTGC**ACT**-AAC TCTTTAAGAAAT**TTT** -GCAGTCCAGGATTT TGGGTGG**CGGGAAGA** G-----

M GACCTTT**CAGGGGA** CCAGTGC**ACT**CAAT TCTTT**CAGAAATTT** -GCAGTCCAAGATTT TGGATGG**CGGGAAGA** G-----

H GACCCACT---GT CCAGACCCGC--AG AGTTAAGATGCTTC TGACCCCGGGATCC TAGCTGGTGGGCGGA GTCCTAACACGTGGG

21 22 MyoD-II

-219

R -----CCTTTT GTTCCAAGGACCCCA CTTTGA**AA**TCC---- ---CAGAGGCAGG**CG** GGAACCTTAGGGG**CG** TGTC**TCCC****CAGCTG**-

M -----CCTTTT GTTCCAAGGACCC**TA** CTTTGA**AA**ACT---- ---CAGAAGCAGG**CG** GGAACCTTAGGGG**CG** TGTC**TCCC****CAGCCA**-

H TGGGCGGGCC**TTTT** GTTCCAGGACTCTT TTCT**CA**AAACT**TCCC** AGTCGGAGGCTGG**CG** GGAACCCGAGAGG**CG** TGTC**TCC****CAGCCAC**

23 24

-146

R GC----- -ATT----- -AATCTTAG G-GTTGGGG**CG**TGG CCTTTTGGGGTGTG**C**

M GC----- -ACT----- -AGGGCTAG G-GGTGGGG**CG**TGG CCTTTTGGGGTGTG**C**

H **CGG**AGGGG**CG**TGGC CTCAT**TGG**CC**CG**CCC CACCAACTCCAGCCA AACTCTAAACCC**CAG** GCGAGGGGG**CG**TGG CCTTCTGGGGTGTG**C**

## Histone Code and GLUT4 Expression

```

      NF-1
-104  GGGCTCCTGGCCAAT GGGTGTGTGAAGGG CGTGGCC-ATGGCGG GGCGGGAGTGAGGAG GTGGCTTCAGCTCTC CGCATCTTTCCCCCT
GGGCTCCTGGCCAAT GGGTGTGTGAAGGG CGTGTCCTATGGCGG GGCGGGAGTGGGGAG GTGGCTTCAGCTCTC CGCATCTTTCCCCCT
GGGCTCCTGGCCAAT GGGTGTGTGAAGGG CGTGGCC--CGCGGG GGCAGGAGC---GAG GTGGCGGGGGCTTCT CGCGTCTTTTCCCC-

      SP-1
      25      26      27      28

-15  R CAAGCCCATCTCATT AGATCCCGGAGAGCC TTGGTGTCTCCGGT TCCTTGGGTTGTGGC AGTGAGTCCCACCAG ACCCGCCCTTTGCAC
M CAAGCGGGTCTCACT AGATCCCGGAGAGCC TTGGTGTCTCCGGT TCCGTGGGTTGTGGC AGTGAGTCCCACCAG ACCCGCCCTTTGCAC
H CAGCCCGCTCCACA AGATCCCGcGGAGCC CCACTGTCTCCGGA TCCTTGGCTTGTGGC TGTGGTCCCATCGG GCCCGCCCTC-GCAC

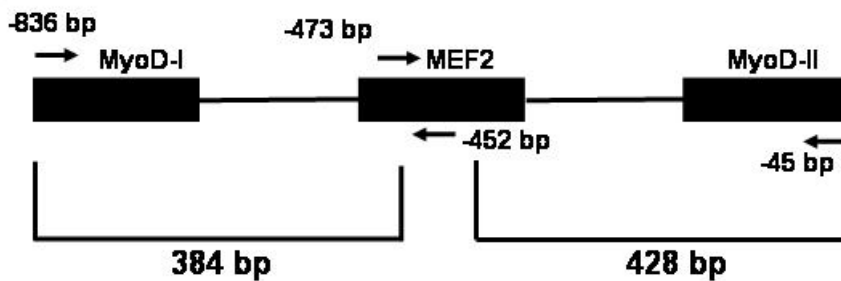
+76  R ACCACTTCCGAAGGC CGG-GGTCTTC---- ----TGCCCGCCAGG CCGGGACACTATACC CTAT--TCATTTTTT T---ATTGCAGTGCC
M ACGGCTTCCGAACGC CGG-GGTCTCG---- ----TGCCCGGCCAGG CCCGGACCCTATACC CTAT--TCATTTTTT TCTTATTGCAGCGCC
H GTCACTCCGGGACC CCGcGGCCTCCCGCAG GTTCTGCGCCTCCAGG CCGGAGTCAGAGACT CCAGGATCGGTTCTT TC--AT-----

+152
R TGAGTCTTCTTTTAA AACAAGATG
M TGAGTCTTTTCTTCT T-----
H -----

```

**Figure S.2.** 5'-upstream region of GLUT4 DNA - DNA sequence alignment (CLUSTAL-W) of rat (R) mouse (M) and human (H) GLUT4 upstream region. Transcription factor binding sites are shown in bold type based on the computer bioinformatic search. CpG islands are shown between `\*' asterisk marks based on the `CpG islander' computer search and are labeled as CpG-I, CpG-II, and CpG-III. The dashed arrow ( $\leftarrow\text{---}\rightarrow$ ) is shown for the CpG deletions that were separately created. The conserved CpGs are depicted in underlined large and bold type, while the nearly conserved CpGs are shown in large and bold type. The non-conserved CpGs are shown only in bold type. Bold ATG (translational start site) and +1 (transcriptional start site) are shown. The Cs in the WT-1 repeat sequences are highly hypermethylated and shown as (C<sup>m</sup>A). All the CpGs numbered 1 to 28 are hypomethylated as determined by bisulfite conversion, TOPO-TA cloning and DNA sequencing. CpGs in the 5'-UTR of GLUT4 (+1 to ATG) that range from 29 to 35 in number have not been examined for their methylation status.

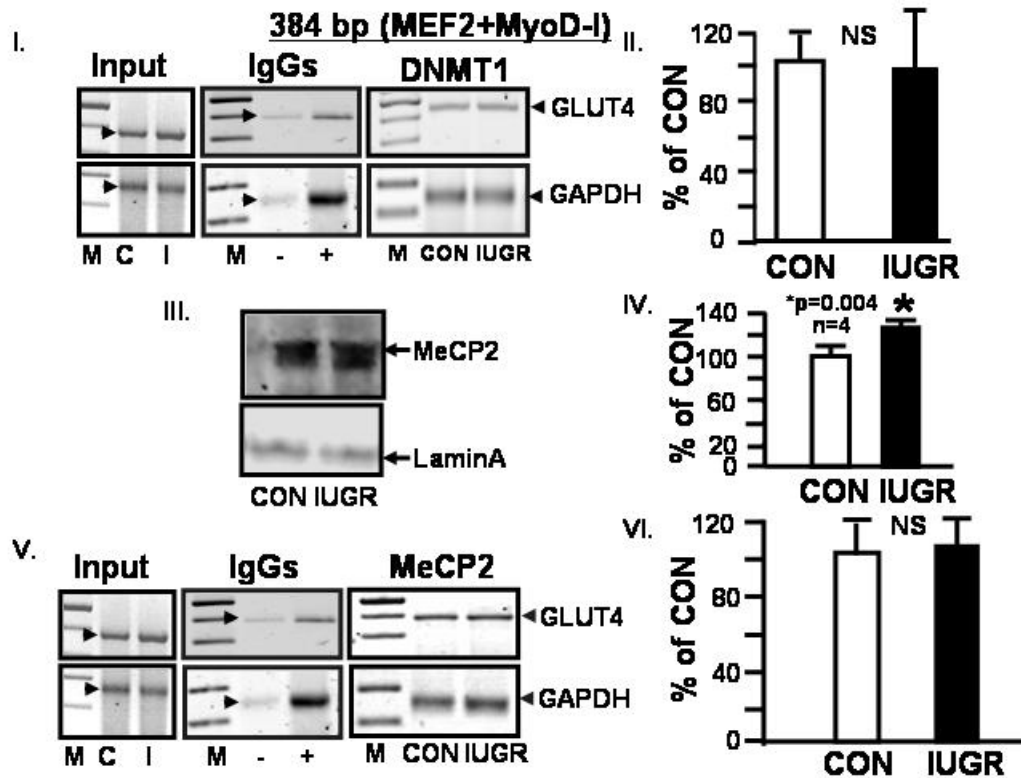
### Figure S.3



**Figure S.3.** Schematic representation of the GLUT4 promoter region demonstrating the MEF2, MyoD-I and MyoD-II sites and the primers employed for PCR amplification in chromatin immunoprecipitation (ChIP) assays that generate 384 bp and 428 bp size amplification products.

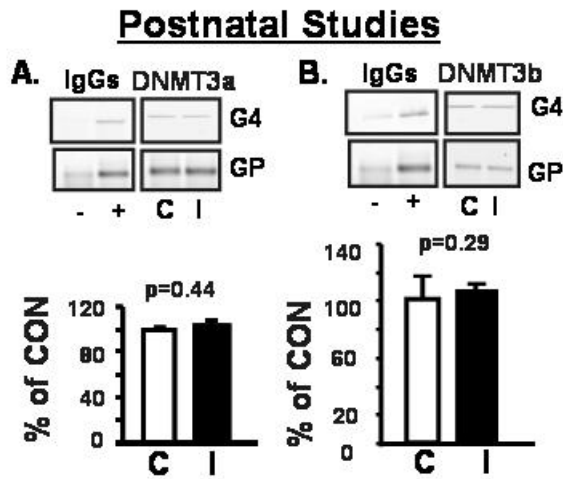
**Figure S.4**

**Adult Studies**



**Figure S.4. Adult Studies:** DNA methyl transferase 1, Methyl CpG binding protein 2 (MeCP2) and GLUT4 5'-upstream region. **A. I.** Representative 2% agarose gels demonstrate the input PCR GLUT4 and GAPDH control without an antibody (left panel) (M = DNA size markers, C = Control and I = IUGR), in the presence of negative (-) (non-specific IgG) and positive (+) (anti-polymerase II IgG) IgGs (middle panel), and ChIP assay demonstrating the 384 bp PCR GLUT4 DNA amplification product which contains the MEF2 and MyoD-I binding sites and CpG-I and CpG-II regions (arrow heads) and the 230 bp PCR GAPDH DNA amplification product (served as an internal control) (arrow heads) obtained from DNMT1 nuclear IPs from control (CON) and IUGR (right panel). **II.** Quantification of the amplified GLUT4 DNA product as a ratio to that of GAPDH, corrected for the input control and expressed as a percent of control (CON) (right panel). M = DNA size markers, NS = not significant. **III.** Representative Western blot demonstrating total nuclear MeCP2 protein concentrations and the internal control nuclear marker Lamin A (left panel). **IV.** Quantification of MeCP2 protein is depicted as a ratio to Lamin A protein intensity and expressed as a percent of CON. Difference between the two groups was assessed by Student's t-test (\*). **V.** Representative 2% agarose gels demonstrating the input PCR GLUT4 and GAPDH control without an antibody (left panel) (M = DNA size markers, C = Control and I = IUGR), in the presence of negative (-) (non-specific IgG) and positive (+) (anti-polymerase II IgG) IgGs (middle panel), and ChIP PCR amplification products of GLUT4 (384 bp; arrow heads) and GAPDH (230 bp; arrow heads; internal control) within MeCP2 nuclear IPs obtained from CON and IUGR (right panel). **VI.** Quantification of MeCP2 immunoprecipitated GLUT4 is shown as a ratio to that of the GAPDH DNA product corrected for the input control and expressed as a percent of CON. M = DNA molecular markers; NS = not significant.

**Figure S.5**



**Figure S.5. Postnatal studies A-B.** Left panels: Representative 2% agarose gels demonstrate the input chromatin PCR amplified GLUT4 (G4) and GAPDH (GP) control without an antibody (not shown), in the presence of non-specific (-) and anti-polymerase II (+) IgGs and ChIP assay demonstrating the PCR amplification product of the 384 bp GLUT4 DNA (MEF2, MyoD-I, CpG-I & CpG-II) or 230 bp GAPDH DNA (internal control) from 2d CON (C) and IUGR (I) skeletal muscle chromatin in the presence of either anti-DNMT3a (A) or anti-DNMT3b (B) IgG. Right panels: Quantification of the PCR GLUT4 amplification product in either the DNMT3a (A) or DNMT3b (B) chromatin IP as a ratio to the GAPDH DNA product corrected for the input control and expressed as a percent of CON. Inter-group difference was established by the Student's t-test (\*).