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## Supplemental Information

# Sex-Based *Mhrt* Methylation Chromatinizes MeCP2 in the Heart

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## Transparent Methods

### Preparation of mouse heart left ventricular tissue

Hearts isolated from 6-weeks old C57BL/6 male and female mice were used in this study. The ventricular base was carefully dissected from the atrial compartment using sterile iris scissors. Excessive blood from ventricles was removed by washing with ice-cold PBS. Left ventricular (LV) and right ventricular (RV) tissues were then carefully dissected by cutting down the tricuspid valve interface.

### Total RNA extraction and qRT-PCR

LVs isolated from male and female mice were diced and suspended in ice-cold PBS. Total RNA was isolated using Trizol (Invitrogen) reagent by following the manufacturer's protocol. For first strand cDNA synthesis, 1 µg of total RNA was reverse transcribed using High capacity cDNA Reverse Transcription Kit (Life Technologies). Real-time PCR (qPCR) was performed using the ABI Prism 7500 instrument and template amplification was assessed by melt-curve analysis. Gene expression data between male and female LVs are expressed relative to endogenous GAPDH. Oligonucleotide sequences used for qPCR are listed in (Table 1). For strand-specific quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) of *MHC* genes, forward and reverse primers were included in separate reactions, and cDNA synthesis was performed at 60°C using Thermoscript cDNA preparation system (Invitrogen) and real-time quantification was performed using Fast SYBR-Green qPCR system (Applied Biosystems). To ensure strand-specific cDNA synthesis, negative primer controls and negative enzyme controls were included and assessed for negligible non-specific amplification. Samples analyzed included $n \geq 5$ in male and female and are normalized against GAPDH gene and the data are represented as mean ±SEM.

## Chromatin Immunoprecipitation (ChIP)

Male and female LVs were finely chopped in to tiny pieces in ice-cold PBS and chromatin was fixed immediately by incubation for 10 minutes at room temperature using 1% formaldehyde. Excessive formaldehyde was quenched using 0.1M glycine by incubation for 10 minutes at room temperature. Tissues were washed with ice-cold PBS to remove residual glycine, resuspended in fresh ice-cold PBS and homogenized to a clear solution in presence of protease inhibitor cocktail. Homogenates were incubated on ice for 30 minutes in SDS lysis buffer containing 1% SDS, 10mM EDTA, 50mM Tris-HCl, pH 8, and protease inhibitor cocktail. Lysates were separated into 300 μL aliquots and sonication of chromatin was achieved using the bioruptor (Diagenode, Belgium) with constant power settings. Soluble chromatin fractions were collected by centrifugation and chromatin shearing was ensured to be in the range of 300-500bp fragments using MultiNA capillary electrophoresis system (Shimadzu). Approximately 10 μg of chromatin was suspended in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8, 167 mM NaCl) and immunopurification was carried out using antibodies specific to HDAC2 (3159, Sigma), MeCP2 (9317, Sigma), REST (07-579, Upstate), MBD1 (ab2846, Abcam), MBD2 (ab38646, Abcam), MBD3 (ab157464, Abcam). Non-specific IgG was incubated simultaneously to each reaction as control. The antibody-bound chromatin fraction was precipitated using dynabeads coated with protein A/G (Invitrogen). Antibody-chromatin-protein A/G conjugates were washed sequentially with low salt buffer, high salt buffer, lithium chloride, Tris-EDTA and Tris-EDTA + 0.01% SDS. Formaldehyde reverse crosslinking was carried out using ChIP elution buffer (20 mM Tris-HCl pH7.5, 5 mM EDTA, 50 mM NaCl, 1% SDS) and incubation in presence of proteinase K (Invitrogen) for 2 hours at 62$^{o}$C in a thermomixer at 1400rpm (Eppendorf). Input chromatin was included at this reverse crosslinking stage. ChIP enriched DNA was purified using DNA binding columns according to the manufacturer's protocol (Nucleospin, Macherey-Nagel) and eluted in nuclease free water. Relative enrichment of DNA in input, ChIP and IgG samples was quantified using Fast SYBR Green qPCR system (Applied Biosystems). Primer sequences used to amplify the cardiac myosin heavy chain genomic sequences

are provided in Table 2. Percentage input (% input) was calculated for each ChIP experiment, and results are expressed as relative to input chromatin between male and female LV tissues. Samples were analyzed as n=4 for male and female and the percentage of input was calculated, and the data are presented as mean ±SEM.

**RNase treatment of immunopurified chromatin**

ChIP using anti-MeCP2 antibody from male and female LV was carried out as described above. The protein A/G bound conjugates were resuspended in 100 µl of either RNase A reaction buffer (10 mM Tris-HCl, 300 mM NaCl, 5 mM EDTA, pH 7.5) or RNase H reaction buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl2, 10 mM DTT, pH 8.0). RNase A (Sigma), RNase H and RNase V1 (New England Biolabs) were added to corresponding tubes and incubated at 37$^{o}$C for one hour. Sequential washing of beads was carried out as described above. DNA was purified using DNA binding columns according to the manufacturer's protocol (Nucleospin, Macherey-Nagel) and eluted in nuclease free water. Amplification of genomic DNA was carried out using real time qPCR and enrichment of DNA sequences were calculated as % input and statistics was obtained between control, RNase treated samples as described above.

**RNA Chromatin Immunoprecipitation (RNA-ChIP)**

Male and female LV chromatin was prepared by the lysis of tissues in SDS buffer and shearing by sonication to optimal size (300-500 bp long). To ensure RNA integrity and to protect chromatin-bound RNA from RNase degradation, only freshly prepared buffers containing RNase inhibitors were used. Tissue homogenates were sonicated in the size optimized for RNA ChIP and residual DNA from sheared chromatin was removed by mild DNase treatment. Immunoprecipitation of chromatin was carried out at 4°C overnight using anti-MeCP2 antibody (9317, Sigma) and a control IgG antibody. Chromatin-bound RNA was purified using RNeasy columns (Qiagen) followed by removal of residual DNA (Ambion). Relative enrichment of *Mhrt* was assessed using SYBR Green qPCR and

chromatin binding was expressed relative to input (% Input) chromatin in male and female LV tissues. Samples were analysed as n=6 for male and female and the percentage of input was calculated, and the data are presented as mean ±SEM.

**Genomic DNA methylation and sequencing analysis**

About 500 ng of genomic DNA isolated from male and female LV tissues were used per bisulphite reaction. Bisulphite conversion was performed according to the manufacturers protocol (EpiTect, Qiagen). The genomic sequence comprising MHC CpG island was amplified using the primers as listed in Table 3. Between 5 and 50 ng of amplicon DNA was used to prepare sequencing libraries using the NEBNext® DNA Library Prep Reagent Set for Illumina (New England Biolabs, NEB). Enzymatic reaction cleanups were performed using QIAquick PCR Purification Kit (Qiagen). Size selection of the libraries was performed by agarose gel excision and QIAquick Gel Extraction Kit (Qiagen). Each amplicon was given a unique barcode using the NEBNext® Multiplex Oligos for Illumina (Index Primers Set 1 and Set 2) (NEB). Library DNA was quantified on the MultiNA bioanalyzer (Shimadzu) and pooled in equimolar amounts. Pooled library DNA was diluted to 4 nM and underwent sequencing on the MiSeq system using MiSeq Reagent Kit v2 500 cycle (Illumina) at a concentration of 8 pM.

***In vitro* DNA/RNA/Protein binding assay**

Full-length *Mhrt* transcript was amplified using the primers (Fw: AAGAGCCCTACAGTCTGATGAACA / Rev: CCTTTACTCTCTGCTTCATTGCCT) and cloned into pCRII-TOPO cloning vector (Invitrogen). Full-length *Mhrt* RNA was synthesized by the HiScribe T7 Quick Kit (NEB). *In vitro* dsDNA/RNA binding assay was performed as described previously (Mathiyalagan et al., 2014). The dsDNA/RNA hybrids (*Mhrt* full length, CG8-9, CG17-19 or methylated CG17-19) were incubated with rMeCP2 protein at 37°C for 30 mins in the presence or absence of RNaseH. This complex was immunopurified with MeCP2 antibody (9317, Sigma) in

the presence of protein A dynabeads for 30 mins at 25°C (Supplemental Fig. S4). DNA was purified using DNA binding columns according to the manufacturer's protocol (Nucleospin, Macherey-Nagel). Relative enrichment of input DNA and the bound samples were amplified by qPCR to check the binding of MeCP2 to the promoter DNA of pri-miRNA-208b.

**RNA methylation analysis**

Total RNA extracted from male and female LV was used for 5-methyl cytosine (5-mC) methylation analysis using EZ RNA Methylation Kit (Zymo Research). 1 µg RNA was subjected to deamination. Removal of bisulphite, desulphonation and RNA washing were performed in RNA spin columns as recommended by the manufacturer. Deaminated RNA was eluted in Nuclease-free water and used for cDNA conversion using High Capacity cDNA Reverse Transcription Kit (Life Technologies). Methylation efficiency and analysis in male and female LV RNA extract was confirmed using primers specific for deaminated sequences in mAsp tRNA as a positive control (Table 3). To assess bisulphite converted RNA, *Mhrt* was amplified using five individual primer pairs denoted as primers A-E listed in Table 3. This is because bisulphite-qPCR facilitates efficient amplification of shorter amplicons of the transcript. Following bisulphite conversion, we assessed 6-8 clones of each amplicon (i.e. for each of the five regions designated A-E) using Sanger sequencing. Therefore 30-40 clones per mouse combining the five regions designated A-E were analysed. To distinguish male and female differences in *Mhrt* methylation, we examined n=5 mice.

**Methylation of synthetic oligomers and *Mhrt*:MeCP2 binding assay**

Biotin-tagged three synthetic RNA oligomers of 45 nucleotides in length (Fig. 4D) were used in this experiment (Sigma). RNA oligomers were modified for CG sequences as described in the text. For in vitro methylation, oligomers were methylated using *Sss1* methylase (NEB) according to the manufacturers protocol. *Mhrt* oligomer, diluted stock of SAM (S-Adenosyl methionine) and *Sss1* methylase (4U/µl) were incubated at 37°C for 1 hr. The reaction was stopped by heating at 65°C for

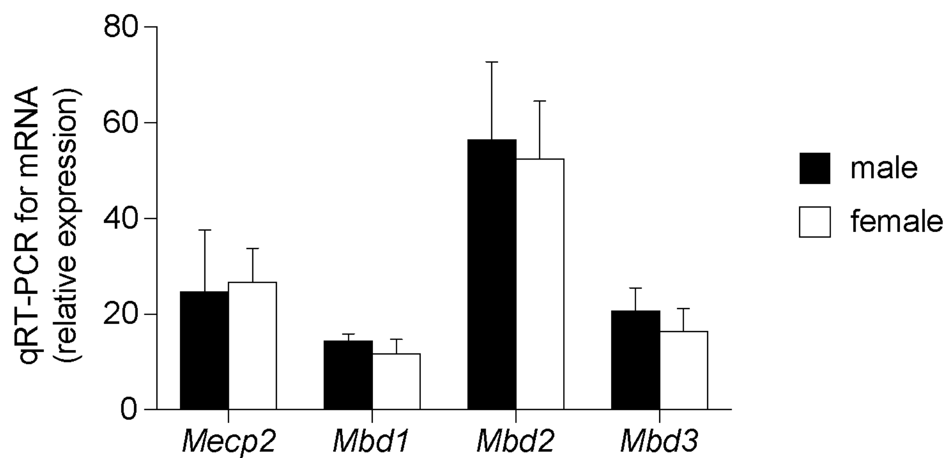
20 mins. In vitro binding assay was carried out in the presence of recombinant proteins MeCP2 or SET7 (negative control). The methylated and unmethylated probes were incubated in the presence of a binding buffer (20mM Tris-HCl, 180mM NaCl, 0.1% NP-40, 1.5mM $MgCl_2$ and 13.2% glycerol) with rMeCP2 or rSET7 proteins for 30 mins at 25°C. Protein:RNA complexes were immunopurified using streptavidin beads and treated for +/- RNAse for 10 mins at 37°C followed by detection by western blotting using MeCP2 (9317, Sigma) and SET7 (NB100-93288, Novus Biologicals) antibodies and quantitation was performed by Odyssey assay. All the assays were done in triplicates and the quantitation was done by normalizing against the input protein.

**Data analysis and statistics**

Data are presented as ±SEM in each group. All data were evaluated with a two-tailed, unpaired Student's t-test. Statistical significance was obtained by comparing male LV and female LV. A value of $P < 0.05$ was considered statistically significant between groups.

**References**

Mathiyalagan, P., Okabe, J., Chang, L., Su, Y., Du, X.J., and El-Osta, A. (2014). The primary microRNA-208b interacts with Polycomb-group protein, Ezh2, to regulate gene expression in the heart. Nucleic Acids Res *42*, 790-803.




**Supplemental Figure S1, related to Figure 2**. mRNA expression of methyl CpG binding proteins (*Mecp2*, *Mbd1*, *Mbd2* and *Mbd3*) examined by qRT-PCR. n=5 mice per group. Data are represented as mean ±SEM.

1 2 3 4 5

TCTGACCCTCTGTGTCCCTGGCT**AGGCACGCATCGAGGAGCTGGAGGAGGAGCTGGAGGC**CGAGCGCACAGCCCGGGCCAAGGT

Rest site

6 7 8 9

GGAGAAGCTG**CGCTCTGACCTGTCCCGGGAGCT**GGAGGAGATCAGTGAAAGGCTGGAGGAGGCAGGCGGGGCCACATCCGTGCA

miR-208b TSS

10 11 12 13 14 15 16 17

GATAGAGATGAACAAGAAG**CGCG**AGGC**CG**AGTTCCAGAAGATG**CG**G**CG**GGACCTGGAGGAGGCCA**CG**CTGCAGCA**CG**AGGCCA**CG**

18 19 20 21 22 23 24 25 26

G**CG**G**CG**GCCCTG**CG**CAAGAAGCATGC**CG**ACAG**CG**TGG**CG**GAGCTGGG**CG**AGCAGAT**CG**ACAACCTCCAG**CG**GGTGAAGCAGAAG

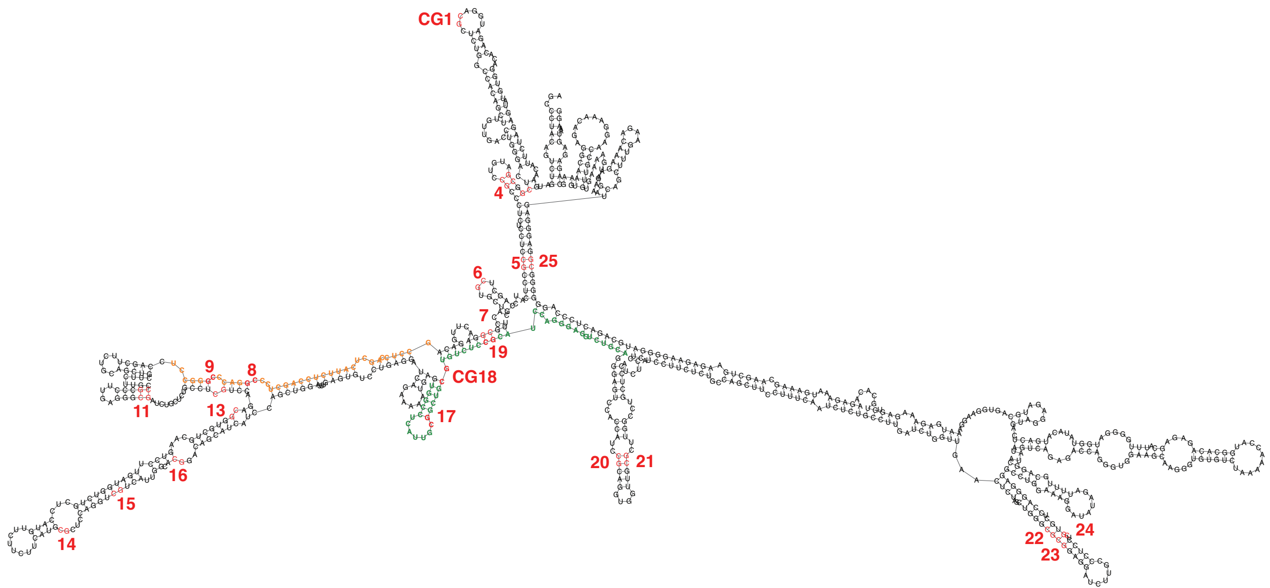
27 28

CTGGAGAAAGAGAAAAG**CG**AGTTCAAGCTGGAGCTGGATGA**CG**TCACCTCCAACATGGAGCAGATCATCAAGGCCAAGGTGGGC

MeCP2 CG-AT4 site

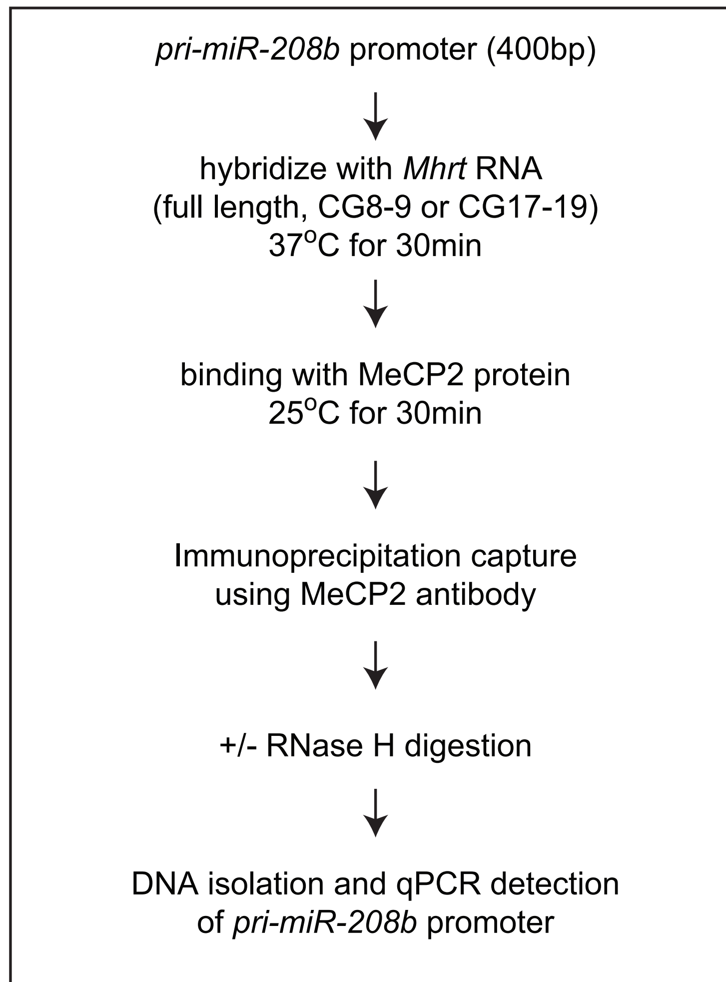
**Supplemental Figure S2, related to Figure 3**. In silico prediction of transcription factor binding revealed MeCP2 and Rest corepressor complex binding sequences at the CGI within *pri-miR-208b* promoter. Transcription start site is shown here as the TSS.

Predicted secondary structure mouse *Mhrt* lncRNA (minimal free energy)

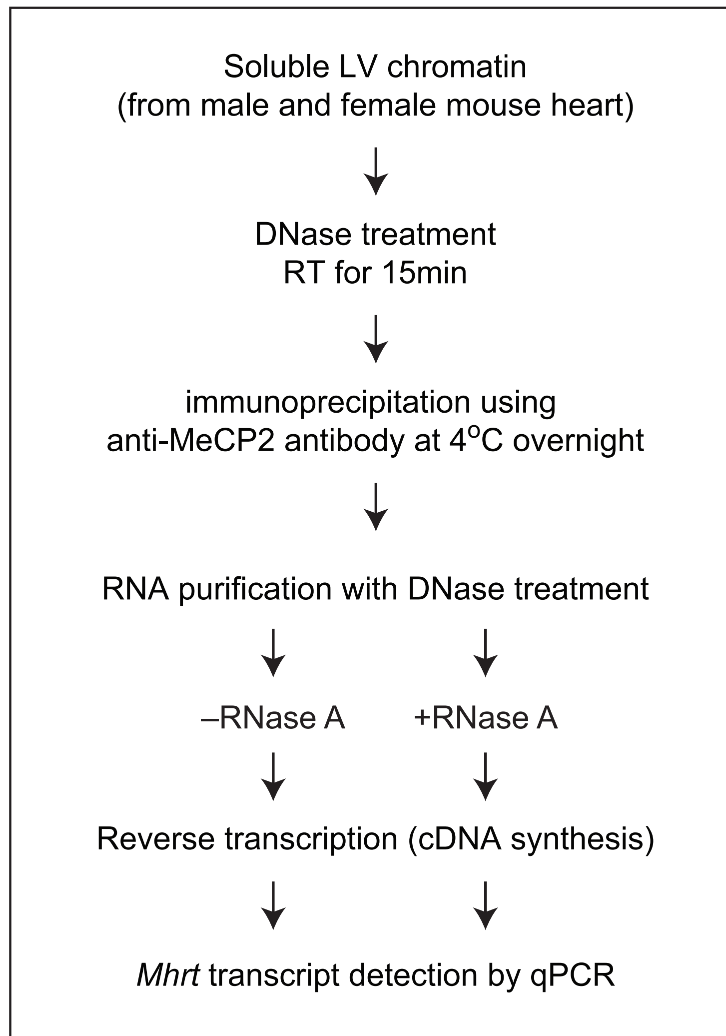



AGCCCTACAGTCTGATGAACATTCTAGAGTATGTGGACACAGATGGACGCTCTGGCCACAGCTTGTTGACCTGGGACTCGGCGATGTCCGCCC
TCTCCTCCGCCTCATCCAGCTCGTGCTGCACCTTGCGGAACTTGGACAGCCTCCAGCTCATTCTCCAGCTCCCGGACCCGGGCCTCCAGCTTC
TGCAGCTGCTTCTTGCCGCCCTTGAGGGCGATCTGCTCTGCCTCGTCCAGACGGTGCTGCAAGTCCTTGATGGTCTGCTCCATGTTCTTCTTC
ATGCGCTCCAGGTCGTCATTGGCACGGACAGCATCATCCAGCTGGATTTGAGTGTCCTGAGGATCAGAAAAATGAGTGGCCTCATTGCGGCTG
CGTGTCTCCGCATCCAGGGAGGTCTGCAGGGAGTCCACCATCCGCAGGTGGTTGCGCTTGGCCTGCTCCATCTCCTCATCCTTCTCTGCCAGC
TTCCTTTCAATCTCTGCCTTGATCTGGTTGAACTCTAGCTGGGCGCGGAGGATCTTGCCCTCCTCGTGCTCCAGGGAGGCCTGGAAAGGATAT
AGATTTTGCAGGCATATAGTCAGAGACCAGGGTGGAAGCAAGGGTGTGTCTAAAAACCATGGCACAGAGAGCATTTGGGGATGGTATACATGA
CTCAGTAGGAGATGCAGTGGAAGGAAATGAGAAAGAGTGTGCACAAGAGAAATGAAAGCAAGCTGAAGAGAAGGGGATGCAGACTCCCAGGGG
GGCGGAGGGAGTCAGCTTTGAAGACAAAGAGGAAAATGAAAAGTGTTGCCAAGGAAACAGAGGCAATGAAGCAGAGAGTAAAGG

**Supplemental Figure S3, related to Figure 3C**. Illustration of predicted secondary structure of full length *Mhrt* using RNAfold Webserver (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). Predicted Mhrt RNA/DNA hybrid sequence at the *pri-miR-208b* promoter shown in orange. The positions of CG dinucleotides shown in red.




**Supplemental Figure S4, related to Figures 3E and 4F**. Protocol for the binding assay using dsDNA (*pri-miR-208b* promoter), *Mhrt* RNA (*full length, CG8-9, CG17-19* or *methylated CG17-19*) and rMeCP2 in presence or absence of RNase H.




**Supplemental Figure S5, related to Figure 4A**. Procedure for RNA-ChIP assay using anti-MeCP2 antibody and soluble LV chromatin.

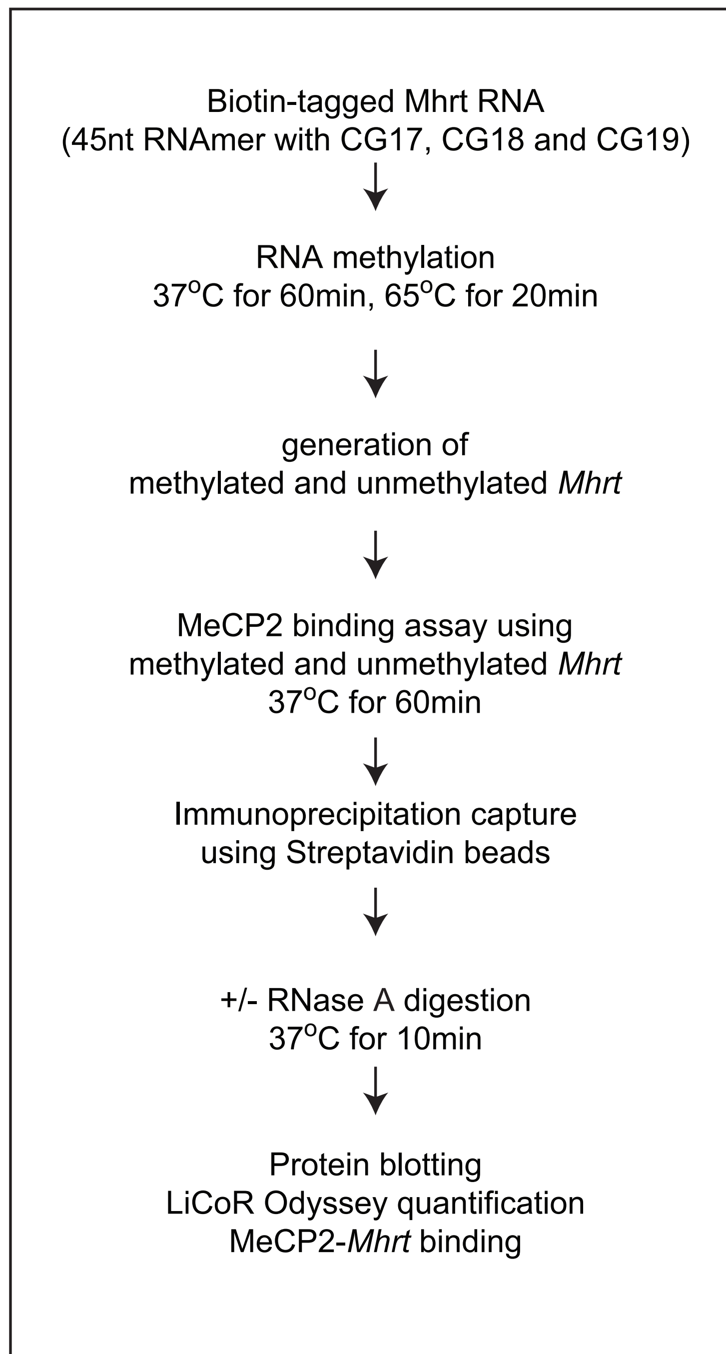
Human *MHRT* sequence

CUGGAGCUGGGACAGGUCAGCAUCCAUCUUCUUCUUCUGGUUGAUGAGGCUGCUGC
UCACCUGGGAAUGCAGCAGCUGCACCCGCUCACUAGUCUCAAUCAGCUCCUGCUCCG
CCAGCUUCCGGGACCGCUCUGUCUGCUCCACCACGGCACGCAACUCCUCCAGCUCAG
CCUGCAGCAGGUUGUUGCGCCGCUCCACGAUGGCGAUGUUCUCCUUCAGGUCGUCG
UUGGCACGGACUGCAUCGUCCAGCUGAAUCUGGGUUACCUUCAACAAGCUCUGGAG
GCUCUUGACUUGCUUCUGGGCCUCGGCGGCCAUGCGGUUGGCGUGGCUGAGCUGGA
UCUCCAUCUCAUUGAGGUCUCCUUCCAUCUUCUUCUUCACCCUCAGGGCCUCGUUGC
GGCUGCGUGUCUCUGCGUCCAGGGAGGUCUGCAGCGAGUCCACCACCCGCAGGUGG
UUGCGCUUGGCCUGUUCCAUCUCCUCGUCCUUCUCUGCCAGCUUCCGCUCGAUCUCU
GCCUUGAUCUGGUUGAACUCCAGCUGGGCCCGGAGGAUCUUGCCCUCCUCCUGCUUU
CGGACCUUCUCCAGCUCAUGGAUAGUCUUUCCGCUGGAACCCAACUGCUCAGUCAAG
UCGGAGAUCUCCUCUGUGUGGGGAACACGGCGUUCUUGAGUUUGAAGAGCUCUGUG
CUGAGGGAGCGAGCCUCCUUCUGCGAGGACUCCAGCUCCGACUGCGACUCCUCAUAC
UUCUGCUUCCACUCGGCCAGGAUCUGCCCGGGGACAAGGCUCACUCUUCAGCCCCCC
AGCCUCAGCCCCAUGUCCAGGGGCUGCAGCAGCAGCAUUGGAGCGCUCUACGUCCAC
CAUCAAGUCCUCGAUCUCAUUCUGUAG

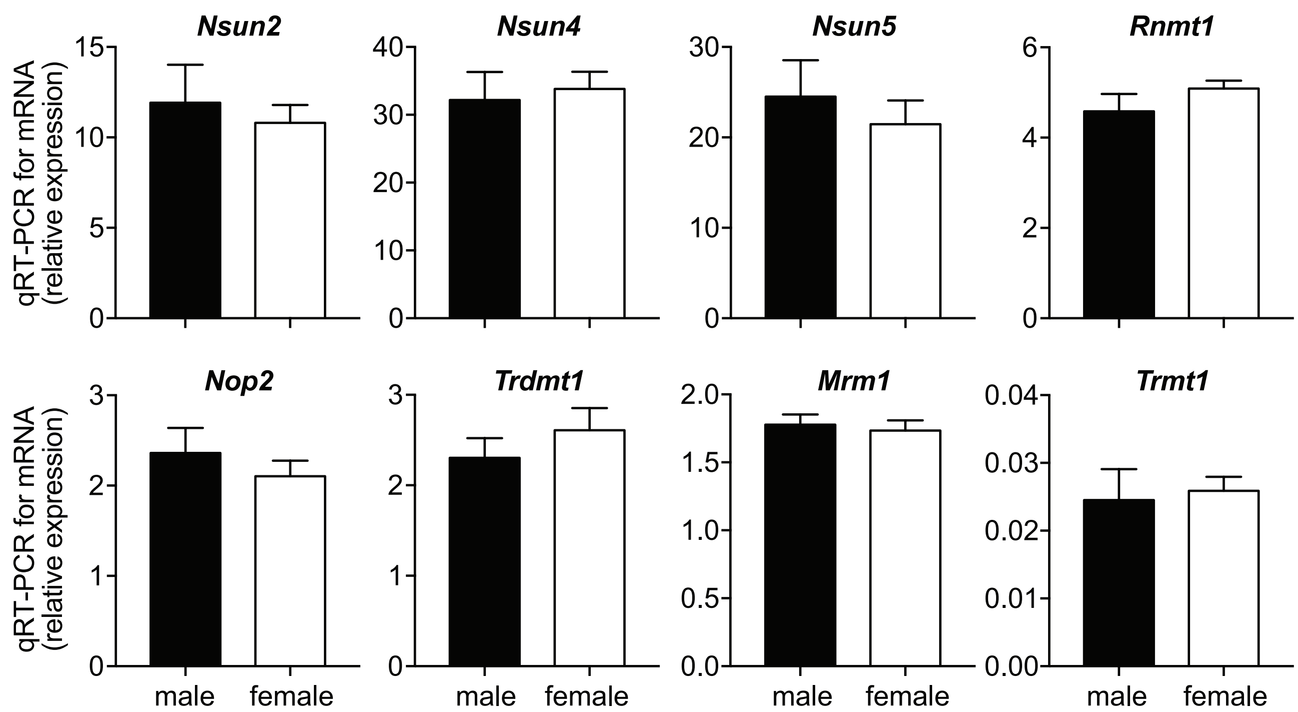
Mouse *Mhrt* sequence

AGCCCUACAGUCUGAUGAACAUUCUAGAGUAUGUGGACACAGAUGGACGCUCUGGC
CACAGCUUGUUGACCUGGGACUCGGCGAUGUCCGCCCUCUCCUCCGCCUCAUCCAGC
UCGUGCUGCACCUUGCGGAACUUGGACAGCCUCCAGCUCAUUCUCCAGCUCCCGGAC
CCGGGCCUCCAGCUUCUGCAGCUGCUUCUUGCCGCCCUUGAGGGCGAUCUGCUCUGC
CUCGUCCAGACGGUGCUGCAAGUCCUUGAUGGUCUGCUCCAUGUUCUUCUUCAUGC
GCUCCAGGUCGUCAUUGGCACGGACAGCAUCAUCCAGCUGGAUUUGAGUGUCCUGA
GGAUCAGAAAAAUGAGUGGCCUCAUUGCGGCUGCGUGUCUCCGCAUCCAGGGAGGU
CUGCAGGGAGUCCACCAUCCGCAGGUGGUUGCGCUUGGCCUGCUCCAUCUCCUCAUC
CUUCUCUGCCAGCUUCCUUUCAAUCUCUGCCUUGAUCUGGUUGAACUCUAGCUGGG
CGCGGAGGAUCUUGCCCUCCUCGUGCUCCAGGGAGGCCUGGAAAGGAUAUAGAUUU
UGCAGGCAUAUAGUCAGAGACCAGGGUGGAAGCAAGGGUGUGUCUAAAAACCAUGG
CACAGAGAGCAUUUGGGGAUGGUAUACAUGACUCAGUAGGAGAUGCAGUGGAAGGA
AAUGAGAAAGAGUGUGCACAAGAGAAAUGAAAGCAAGCUGAAGAGAAGGGGAUGC
AGACUCCCAGGGGGGCGGAGGGAGUCAGCUUUGAAGACAAAGAGGAAAAUGAAAAG
UGUUGCCAAGGAAACAGAGGCAAUGAAGCAGAGAGUAAAGG

**Supplemental Figure S6, related to Figure 4B and 4D**. CG18 and flanking sequence-is conserved between mouse and human *Mhrt* transcripts.




**Supplemental Figure S7, related to Figure 4E**. Assay designed for assessment of *Mhrt*-MeCP2 binding. Biotin-tagged 45nt long *Mhrt* oligomer was methylated using *Sss1* methyltransferase followed by incubation with recombinant MeCP2. Streptavidin immunoprecipitation of *Mhrt* followed by protein blotting for MeCP2 was quantified by LiCoR Odyssey.




**Supplemental Figure S8, related to Figure 4**. mRNA expression of known RNA methyltransferases (*Nsun2, Nsun4, Nsun5, Rnmt1*, *Nop2, Trdmt1, Mrm1, Trmt1*) examined by qRT-PCR. n=5 mice per group. Data are represented as mean ±SEM.

**Table 1**. Transcript Detection Primers

| | |
|---|---|
| *α-MHC* Forward | CCACCTGGGCAAGTCTAACAA |
| *α-MHC* Reverse | TGTAGTCCACGGTGCCAGC |
| *β-MHC* Forward | GATGTTTTTGTGCCCGATGA |
| *β-MHC* Reverse | ACCGTCTTGCCATTCTCCG |
| *Mhrt* Forward | GCTCTGGCCACAGCTTGTTGACCTG |
| *Mhrt* Reverse | CGTGCCAATGACGACCTGGAGCGC |
| *Pri-miR-208b* | Taqman Pri-miRNA assay (Mm03308667_pri) |
| *Mecp2 forward* | ACCTTGCCTGAAGGTTGGAC |
| *Mecp2 Reverse* | AAGCTTTTCCCTGGGGATTGA |
| *Mbd1 Forward* | CCTGGTTGCCAAGGCTACATA |
| *Mbd1 Reverse* | CAGGTTCAGCTTTTCAGCCA |
| *Mbd2 Forward* | CCAAATTCACGAACCACCCG |
| *Mbd2 reverse* | CCCAGAAAAGCTGACGTGGT |
| *Mbd3 Forward* | TGGGAAAGGGAAGAAGTGCC |
| *Mbd3 Reverse* | TCCCGCTGGGGCTATAGTAA |
| *Nsun2 Forward* | AGTGGTTTGCAGACTGGCAT |
| *Nsun2 Reverse* | CAGGATTCGAAGGCATCGCT |
| *Nsun4 Forward* | GACGGGTTGTTGCCGTAATC |
| *Nsun4 Reverse* | ACTCGGACTTGGTTCCCTTC |
| *Nsun5 Forward* | GTCGTAGAGCGCTTGAGGC |
| *Nsun5 Reverse* | GCTTCAGGTTCTGGAAGTTGC |
| *Rnmt Forward* | GTGGATGACTGTGTGGTGTC |
| *Rnmt Reverse* | GACTCTGGGTCAGAAGCGAC |
| *Nop2 Forward* | GCTCTGATCAATCGTGGGGT |
| *Nop2 Reverse* | CTCAGGGGTAGCGCCAATAG |
| *Trdmt1 Forward* | GGTTGCGAGAGGATGGAAC |
| *Trdmt1 Reverse* | TGCAGGGATATGACTTTCTCGC |
| *Mrm1 Forward* | AGAGAAACAGCTGTCCGCTTA |
| *Mrm1 Reverse* | AAAAACCGGGCAGGTCAGGA |
| *Trmt1 Forward* | TTGTCATGTGATTGGCCCGC |
| *Trmt1 Reverse* | TCAACCCGCCCGGACAAG |

**Table 2** ChIP Primers

| | |
|---|---|
| *Pri-miR-208b* promoter Forward | TCTGACCCTCTGTGTCCCTGGCT |
| *Pri-miR-208b* promoter Reverse | GCCCACCTTGGCCTTGATGA |
| Intergenic promoter Forward | GAGCCTCAAGTGACCTCCAG |
| Intergenic promoter Reverse | CTCCAAGGGACCTGATTCAA |

**Table 3** Bisulphite-Specific Primers

| | |
|---|---|
| *Pri-miR-208b* promoter Forward | TTTGATTTTTTGTGTTTTTGGTTAG |
| *Pri-miR-208b* promoter Reverse | ACCCACCTTAACCTTAATAATCTACTC |
| *Mhrt* Region A Forward | GTAGTGGAAGGAAATGAGAAAGAGTGTG |
| *Mhrt* Region A Reverse | CCTCTTTATCTTCAAAACTAACTCCC |
| *Mhrt* Region B Forward | GTTTTGATTTGGTTGAATTTTAGTTGGG |
| *Mhrt* Region B Reverse | CTATATCCTTTCCAAACCTCCCT |
| *Mhrt* Region C Forward | GGATTTGAGTGTTTTGAGGATTAGAAAAATGAGTGG |
| *Mhrt* Region C Reverse | CCCAACTAAAATTCAACCAAATCA |
| *Mhrt* Region D Forward | GAATTTGGATAGTTTTTAGTTTATT |
| *Mhrt* Region D Reverse | CCACTCATTTTTCTAATCCTCAAAACACTC |
| *Mhrt* Region E Forward | GAATATTTTAGAGTATGTGGATATAGATGG |
| *Mhrt* Region E Reverse | AACTAAAAAATAAACTAAAAACTATCC |
| *mAsp* Forward | TGTTAGTATAGTGGTGAGTAT |
| *mAsp* Reverse | CTCCCCATCAAAAAATTA |