## Supplementary Information

# Disturbance of cardiac gene expression and cardiomyocyte structure predisposes *Mecp2*-null mice to arrhythmias

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**Inventory of supplemental Items**

**-Supplementary Tables**

Related to DNA microarray

- Table S1

Related to Figure 4:

- Table S2
- Table S3

Related to Methods

- Table S4

**-Supplementary Figures and Figure Legends**

Related to Figure 5:

- Supplementary Figure S1
- Supplementary Figure S2

Related to Figure 7 and 8:

- Supplementary Figure S3
- Supplementary Figure S4
- Supplementary Figure S5

**Table S1.**

**Genes upregulated or downregulated in the purified FLK1/CXCR4/ PDGFRα -positive cardiovascular progenitor cells derived from wild-type and *Mecp2*-null ES cells.**

| Accession No. | Description | Fold difference |
|---|---|---|
| NM_009876 | cyclin-dependent kinase inhibitor 1C (P57) (Cdkn1c), transcript variant 2 | 51.1 |
| NM_008006 | fibroblast growth factor 2 (Fgf2) | 10.5 |
| NM_053093 | tachykinin 4 (Tac4) | 7.4 |
| NM_001161832 | brachyury 2 (T2) | 4.5 |
| NM_008501 | leukemia inhibitory factor (Lif), transcript variant 1 | 3.7 |
| NM_008266 | homeobox B1 (Hoxb1) | 3.5 |
| NM_008006 | fibroblast growth factor 2 (Fgf2), | 3.4 |
| NM_177781 | transient receptor potential cation channel, subfamily A, member 1 (Trpa1) | 3.3 |
| NM_001164171 | Myosin, heavy polypeptide 6, cardiac muscle, alpha (Myh6/alphaMHC) | 3.2 |
| NM_008006 | fibroblast growth factor 2 (Fgf2) | 2.9 |
| NM_009723 | ATPase, Ca++ transporting, plasma membrane 2 (Atp2b2), transcript variant 1 | 2.9 |
| NM_013877 | calcium binding protein 5 (Cabp5) | 2.4 |
| NM_008725 | natriuretic peptide type A (Nppa) | 2.3 |
| NM_007706 | suppressor of cytokine signaling 2 (Socs2), transcript variant 1 | 2.3 |
| NM_008589 | Mesoderm posterior 2 (Mesp2) | 2.2 |
| NM_009331 | transcription factor 7, T-cell specific (Tcf7) | 2.2 |
| NM_001024716 | TRIO and F-actin binding protein (Triobp), transcript variant 1 | 2.2 |
| ENSMUST00000090180 | ens\|sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3G | 2.2 |
| NM_009290 | wingless-related MMTV integration site 8A (Wnt8a), | 2.1 |
| NM_001039104 | transient receptor potential cation channel, subfamily M, member 1 (Trpm1), transcript variant 2 | 2.0 |
| NM_009314 | tachykinin receptor 2 (Tacr2) | 2.0 |
| ENSMUST00000102575 | dishevelled 2, dsh homolog (Drosophila) | 2.0 |
| | | |
| NM_010788 | methyl CpG binding protein 2 (Mecp2), transcript variant 2 | 0.04 |
| NM_172632 | mitogen-activated protein kinase 4 (Mapk4) | 0.15 |
| NM_007392 | actin, alpha 2, smooth muscle, aorta (Acta2) | 0.16 |

| | | |
|---|---|---|
| NM_028610 | developmental pluripotency associated 4 (Dppa4), transcript variant 1 | 0.16 |
| NM_009606 | actin, alpha 1, skeletal muscle (Acta1) | 0.18 |
| NM_011532 | Mus musculus T-box 1 (Tbx1), mRNA [NM_011532] | 0.19 |
| NM_011537 | T-box 5 (Tbx5) | 0.21 |
| NM_018865 | WNT1 inducible signaling pathway protein 1 (Wisp1) | 0.21 |
| NM_009311 | tachykinin 1 (Tac1) | 0.21 |
| NM_013693 | tumor necrosis factor (Tnf) | 0.23 |
| NM_175455 | ankyrin repeat domain 34B (Ankrd34b) | 0.24 |
| NM_011097 | paired-like homeodomain transcription factor 1 (Pitx1) | 0.24 |
| NM_011619 | troponin T2, cardiac (Tnnt2), transcript variant 9 | 0.26 |
| NM_027303 | ankyrin repeat domain 60 (Ankrd60), transcript variant 1 | 0.28 |
| NM_013468 | ankyrin repeat domain 1 (cardiac muscle) (Ankrd1) | 0.29 |
| NM_172850 | ankyrin repeat and MYND domain containing 1 (Ankmy1) | 0.29 |
| NM_001164248 | tropomyosin 1, alpha (Tpm1), transcript variant 1 | 0.31 |
| NM_080444 | ankyrin repeat and SOCS box-containing 10 (Asb10) | 0.32 |
| NM_009608 | actin, alpha, cardiac muscle 1 (Actc1) | 0.32 |
| NM_018865 | WNT1 inducible signaling pathway protein 1 (Wisp1) | 0.33 |
| NM_009523 | wingless-related MMTV integration site 4 (Wnt4) | 0.33 |
| NM_009152 | sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A (Sema3a), transcript variant 1 | 0.33 |
| NM_010518 | insulin-like growth factor binding protein 5 (Igfbp5) | 0.34 |
| NM_010094 | left right determination factor 1 (Lefty1) | 0.40 |
| NM_009393 | troponin C, cardiac/slow skeletal (Tnnc1) | 0.41 |
| NM_011352 | sema domain, immunoglobulin domain (Ig), and GPI membrane anchor, (semaphorin) 7A (Sema7a) | 0.41 |
| NM_001024851 | ankyrin repeat domain 34A (Ankrd34a) | 0.44 |
| NM_001160012 | gap junction protein, beta 3 (Gjb3), transcript variant 1 | 0.44 |
| NM_080444 | ankyrin repeat and SOCS box-containing 10 (Asb10) | 0.44 |
| NM_013569 | potassium voltage-gated channel, subfamily H (eag-related), member 2 (Kcnh2) | 0.45 |
| NR_001461 | KCNQ1 overlapping transcript 1 (Kcnq1ot1), non-coding RNA | 0.46 |
| NM_008700 | NK2 transcription factor related, locus 5 (Nkx2.5) | 0.46 |
| NM_001164251 | tropomyosin 1, alpha (Tpm1), transcript variant 5, mRNA | 0.47 |
| NM_145136 | myocardin (Myocd), transcript variant A, mRNA | 0.47 |
| NM_009152 | sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A (Sema3a), transcript variant 1, mRNA | 0.48 |

| | | |
|---|---|---|
| NM_027480 | ankyrin repeat domain 24 (Ankrd24), mRNA | 0.49 |
| NM_016700 | mitogen-activated protein kinase 8 (Mapk8) | 0.49 |
| NM_001008231 | dishevelled associated activator of morphogenesis 2 (Daam2), mRNA | 0.49 |
| NM_146024 | ankyrin repeat domain 40 (Ankrd40), transcript variant 2, mRNA | 0.50 |
| NM_008711 | noggin (Nog), mRNA | 0.50 |
| | | |
| NM_010612 | Kinase insert domain protein receptor (KDR/Flk1) | 1.62 |
| NM_011058 | Platelet derived growth factor receptor, alpha polypeptide (PDGFRa) | 1.03 |
| NM_009911 | Chemokine (C-X-C motif) receptor 4 (CXCR4) | 0.95 |
| NM_010136 | Eomesodermin (Eomes) | 1.07 |
| NM_009309 | Brachyury (T/Bra) | 1.54 |
| NM_008588 | Mesoderm posterior 1 (Mesp1) | 1.73 |
| NM_008092 | GATA binding protein 4 (GATA4) | 0.72 |
| NM_025282 | Myocyte enhancer factor 2C (MEF2c) | 0.59 |
| NM_021459 | ISL1 transcription factor, LIM/homeodomain (Isl1) | 1.15 |
| NM_007989 | Forkhead box H1 (Foxh1) | 1.00 |
| NM_008213 | Heart and neural crest derivatives expressed transcript 1 (Hand1) | 1.51 |
| NM_010402 | Heart and neural crest derivatives expressed transcript 2 (Hand2) | 1.55 |
| NM_080728 | Myosin, heavy polypeptide 7, cardiac muscle, beta (Myh7/betaMHC) | 1.08 |
| NM_001032378 | Platelet/endothelial cell adhesion molecule 1 (Pecam1/CD31) | 0.74 |
| NM_016791 | Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1) | 1.12 |

**Table S2.**

**Electrocardiographic parameters in wild-type and *Mecp2*-null mice**

Electrocardiography was performed on *Mecp2*-null (*Mecp2*$^{-/y}$), wild-type (*Mecp2*$^{flox/y}$), and normal C57BL/6 male (BL6) mice at the age of 6 or 8 weeks. RR, time interval between two consecutive R waves; PQ, interval between the start of P and Q waves; QRS, interval from beginning of Q wave to the end of S wave; QT, interval from beginning of Q wave to end of T wave; QTc, QT interval corrected for heart rate, $QTc = QT / (RR/100)^{0.5}$. The T wave in mice ends when it returns to the isoelectric baseline. Significant differences in these parameters are indicated as follows: $^{*}p < 0.05$ and $^{**}p < 0.01$ for BL6 vs. *Mecp2*$^{-/y}$; $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ for BL6 vs. *Mecp2*$^{flox/y}$; $^{\ddagger}p < 0.05$ and $^{\ddagger\ddagger}p < 0.01$ for *Mecp2*$^{-/y}$ vs. *Mecp2*$^{flox/y}$ mice. All comparisons were evaluated using Student's t-test.

| 6 weeks | RR (msec) | PQ (msec) | QRS (msec) | QT (msec) | QTc (msec) | n |
|---|---|---|---|---|---|---|
| BL6 | 126 ± 15 | 40 ± 2 | 10 ± 2 | 46.9 ± 4.2 | 42.1 ± 3.2 | 11 |
| *Mecp2*$^{flox/y}$ | 109 ± 9 $^{\dagger\dagger}$ | 37 ± 3 $^{\dagger\dagger}$ | 10 ± 1 | 44.2 ± 3.8 | 41.7 ± 2.4 | 12 |
| *Mecp2*$^{-/y}$ | 106 ± 9 $^{**}$ | 33 ± 3 $^{**,\ddagger}$ | 9 ± 1 $^{\ddagger\ddagger}$ | 42.3 ± 4.6 | 41.2 ± 4.1 | 14 |
| 8 weeks | RR (msec) | PQ (msec) | QRS (msec) | QT (msec) | QTc (msec) | n |
| BL6 | 115 ± 10 | 38 ± 3 | 12 ± 3 | 45.1 ± 2.5 | 42.1 ± 3.2 | 10 |
| *Mecp2*$^{flox/y}$ | 115 ± 18 | 37 ± 2 | 10 ± 1 | 45.6 ± 6.4 | 41.7 ± 2.4 | 11 |
| *Mecp2*$^{-/y}$ | 110 ± 8 | 33 ± 2 $^{**,\ddagger\ddagger}$ | 9 ± 1 $^{**,\ddagger\ddagger}$ | 43.8 ± 3.9 | 41.2 ± 4.1 | 10 |

**Table S3.**

**Echocardiographic Parameters in wild-type and *Mecp2*-null mice.**

Echocardiography was performed on *Mecp2*-null (*Mecp2*$^{-/y}$), wild-type (*Mecp2*$^{flox/y}$), and normal C57BL/6 male (BL6) mice at the age of 8 weeks. Left ventricular end-systolic and end-diastolic dimensions (LVDs and LVDd, respectively) were measured from the LV M-mode trace. Percent fractional shortening (%FS) of the LV was calculated as %FS = (LVDd – LVDs)/LVDd × 100. Significant differences in these parameters are indicated as follows: *$p < 0.05$ and **$p < 0.01$ for BL6 vs. *Mecp2*$^{-/y}$; ‡$p < 0.05$ and ‡‡$p < 0.01$ for *Mecp2*$^{-/y}$ vs. *Mecp2*$^{flox/y}$. All comparisons were evaluated using Student's t-test.

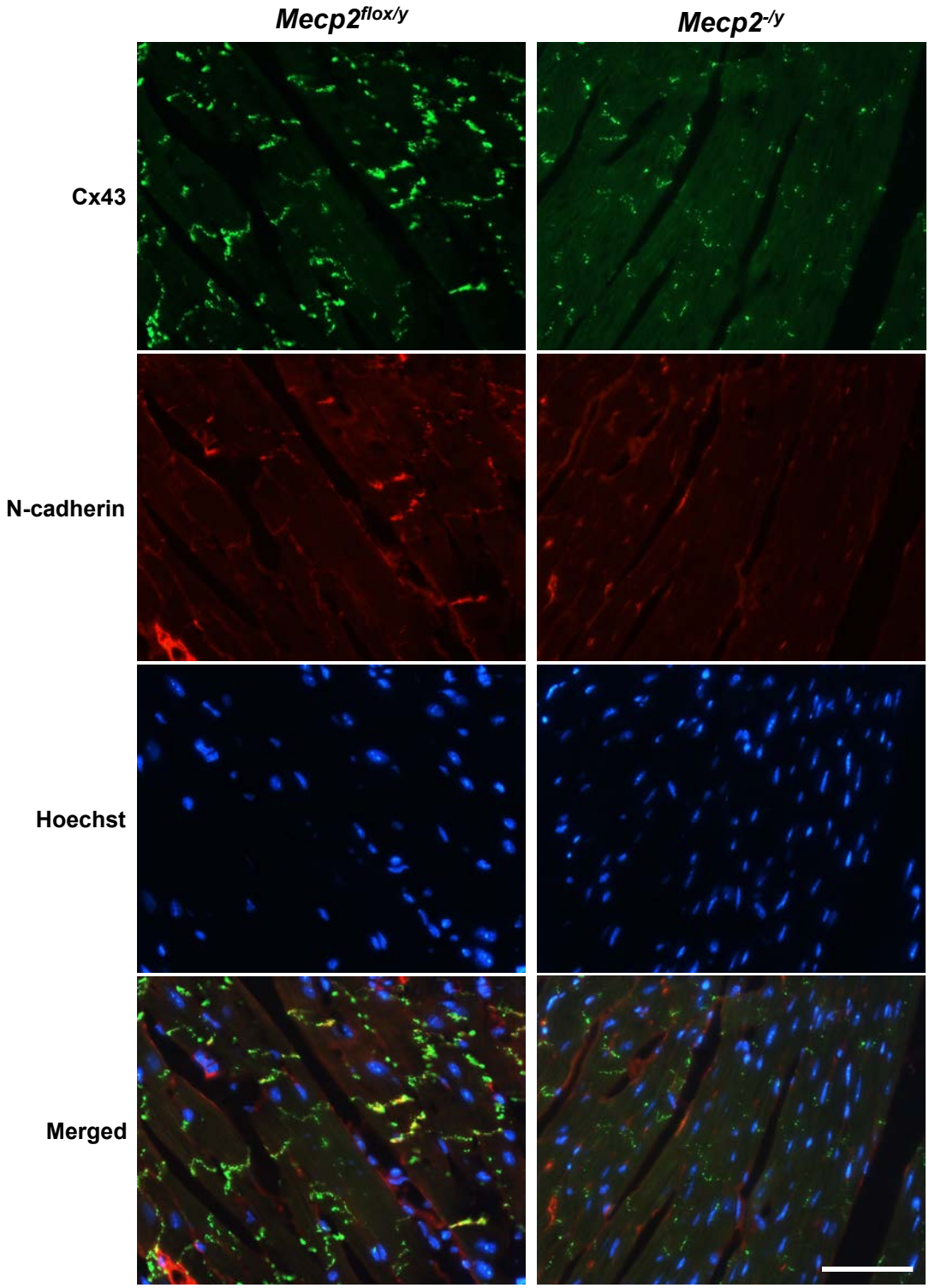
| 8 weeks | IVST (mm) | PWT (mm) | LVDd (mm) | LVDs (mm) | %FS (%) | n |
|---|---|---|---|---|---|---|
| BL6 | 0.72 ± 0.02 | 0.73 ± 0.03 | 3.39 ± 0.15 | 2.01 ± 0.16 | 40.9 ± 2.94 | 11 |
| *Mecp2*$^{flox/y}$ | 0.72 ± 0.03 | 0.74 ± 0.02 | 3.37 ± 0.16 | 2.03 ± 0.17 | 39.7 ± 3.40 | 9 |
| *Mecp2*$^{-/y}$ | 0.56 ± 0.04 **,‡‡ | 0.56 ± 0.05 **,‡‡ | 2.70 ± 0.17 **,‡‡ | 1.55 ± 0.12 **,‡‡ | 42.6 ± 2.31 ‡ | 12 |

## Table S4. Primers used for PCR, quantitative real-time PCR, ChIP, and bisulfite sequencing analysis.

| **PCR and quantitative real time PCR** | | | |
|---|---|---|---|
| Gene | Forward Primer | Reverse Primer | Ta (°C) |
| *Mecp2* | 5'-GGTAAAACCCGTCCGGAAAATG-3' | 5'-TTCAGTGGCTTGTCTCTGAG-3' | 61 |
| *Mesp1* | 5'-GTCTGCAGCGGGGTGTCGTG-3' | 5'-CGGCGGCGTCCAGGTTTCTA-3' | 61 |
| *Mesp2* | 5'-CGCCTGGCCATCCGCTACAT-3' | 5'-ACCCCCAGGACACCCCACTACT-3' | 61 |
| *T* | 5'-ATCAAGGAAGGCTTTAGCAAATGGG-3' | 5'-GAACCTCGGATTCACATCGTGAGA-3' | 60 |
| *Eomes* | 5'-TGTTTTCGTGGAAGTGGTTCTGGC-3' | 5'-AGGTCTGAGTCTTGGAAGGTTCATTC-3' | 60 |
| *Tbx5* | 5'-GCTGATAACAAATGGTCCGTA-3' | 5'-CCACAGGATACTCTTTACTTTGC-3' | 61 |
| *Nkx2.5* | 5'-AAGCAACAGCGGTACCTGTC-3' | 5'-GCTGTCGCTTGCACTTGTAG-3' | 59 |
| *Gata4* | 5'-CTGTCATCTCACTATGGGCA-3' | 5'-CCAAGTCCGAGCAGGAATTT-3' | 57 |
| *Mef2c* | 5'-AGATCTGACATCCGGTGCAG-3' | 5'-TCTTGTTCAGGTTACCAGGT-3' | 55 |
| *Islet1* | 5'-CCACAAGCAGCCGGAGAAGAC-3' | 5'-GAGGGTTGGCGGCATAGCAG-3' | 63 |
| *Flk1* | 5'-GGCGGTGGTGACAGTATCTT-3' | 5'-CTCGGTGATGTACACGATGC-3' | 57 |
| *Cxcr4* | 5'-GTGACCGCCTTTACCCCGATAGC-3' | 5'-TGACCCCCAAAAGGATGAAGGAGT-3' | 60 |
| *Pdgfra* | 5'-CTGGTGCCTGCCTCCTATGAC-3' | 5'-CACGATCGTTTCTCCTGCCTTAT-3' | 60 |
| *Acta2* | 5'-GAGAAGCCCAGCCAGTCG-3' | 5'-CTCTTGCTCTGGGCTTCA-3' | 58 |
| *Pecam1* | 5'-GTCATGGCCATGGTCGAGTA-3' | 5'-TCCTCGGCGATCTTGCTGAA-3' | 57 |
| *Nfatc1* | 5'-CCTGACCACCGATAGCAC-3' | 5'-GCTCGTATGGACCAGAATGT-3' | 57 |
| *Myh6* | 5'-ATCATTCCCAACGAGCGAAAG-3' | 5'-AAGTCCCCATAGAGAATGCGG-3' | 59 |
| Myh7 | 5'-TACCTCATGGGGCTGAACTC-3' | 5'-CCCTTGGTGACGTACTCGTT-3' | 57 |
| *Myl7* | 5'-TGACCCAGGCAGACAAGTTC-3' | 5'-CGTGGGTGATGATGTAGCAG-3' | 60 |
| *Myl2* | 5'-AAGGTGTTTGATCCCGAGGG-3' | 5'-GGGAAAGGCTGCGAACATCT-3' | 60 |
| *Nppa* | 5'-CGGTGTCCAACACAGATCTG-3' | 5'-TCTCTCGAGGTGGGTTGAC-3' | 58 |
| *Ank2* | 5'-AGATTACTGTGCAGCATAACAGG-3' | 5'-TGGTTGTAAAGGAAACACACTCA-3' | 59 |
| *Scn5a* | 5'-GCAGAAGGTGAAGTTCGTGG-3' | 5'-TGAAGACCAAGTTTCCGACC-3' | 60 |
| *Kcnq1* | 5'-CATCGGTGCCCGTCTGAACAGG-3' | 5'-TTGCTGGGTAGGAAGAGCTCAG-3' | 59 |
| *Kcnh2* | 5'-GATCGCCTTCTACCGGAAA-3' | 5'-CATTCTTCACGGGTACCACA-3' | 56 |
| *Kcne1* | 5'-CCCAATTCCACGACTGTTCT-3' | 5'-CAGCTTCTTGGATCGGATGT-3' | 59 |
| *Kcne2* | 5'-CTGGAGGATGCCTTCAAAAA-3' | 5'-TGCCAATCTTCCACGATGTA-3' | 55 |
| *Kcnj2* | 5'-TTGGCTGTGTGTTTTGGTTGAT-3' | 5'-AGTCTGCGTTGTCAATGTCCTG-3' | 60 |
| *Kcnj12* | 5'-ATCATCTTCTGGGTCATTGCTGTC-3' | 5'-CGTCTCGAGGTCCTGACGGCTAAT-3' | 65 |
| *Cacna1c* | 5'-CCCTTCTTGTGCTCTTCGTC-3' | 5'-ACACCCAGGGCAACTCATAG-3' | 60 |
| *Cacna1d* | 5'-ACATTCTGAACATGGTCTTCACAG-3' | 5'-AGGACTTGATGAAGGTCCACAG-3' | 58 |
| *Cacna1g* | 5'-TCCTTGGAGATGCTGCTGAAG-3' | 5'-CACACGCTGATGACCACAATG-3' | 58 |
| *Hcn1* | 5'-CTCTTTTTGCTAACGCCGAT-3' | 5'-CATTGAAATTGTCCACCGAA-3' | 55 |
| *Hcn2* | 5'-TTTCAACGAGGTGCTGGAGG-3' | 5'-TGGCATTCTCCTGGTTGTTG-3' | 61 |
| *Hcn3* | 5'-GACACCCGCCTCACTGATGGAT-3' | 5'-GTTTCCGCTGCAGTATCGAATTC-3' | 61 |
| *Hcn4* | 5'-CGACAGCGCATCCATGACTA-3' | 5'-GCTGGAAGACCTCGAAACGC-3' | 58 |
| *Cx40* | 5'-CAGAGCCTGAAGAAGCCAAC-3' | 5'-CGAGCCTTCCACGTTTAGAG-3' | 60 |
| *Cx43* | 5'-GAGAGCCCGAACTCTCCTTT-3' | 5'-TGGGCACCTCTCTTTCACTT-3' | 60 |
| *Gapdh* | 5'-CCAGAACATCATCCCTGCATC-3' | 5'-CCTGCTTCACCACCTTCTTGA-3' | 57 |

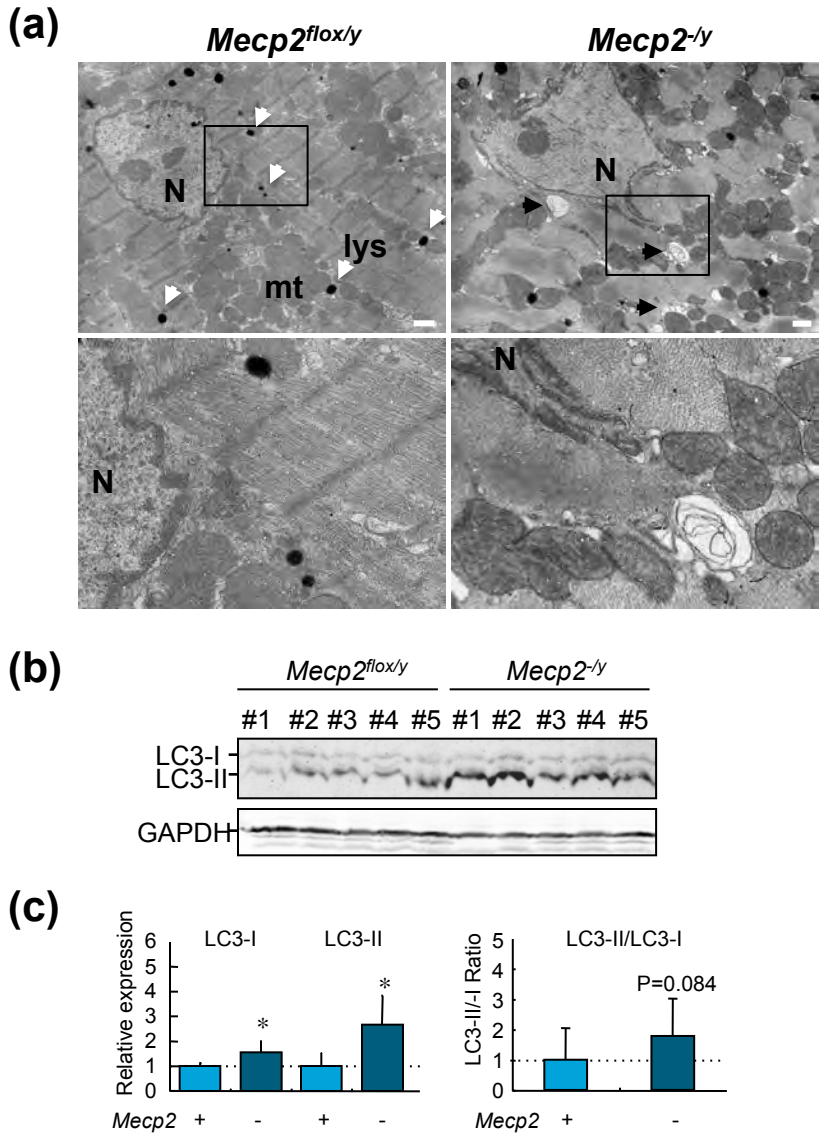
| **ChIP analysis** | | |
|---|---|---|
| Primer name | Sequence | Ta (°C) |
| Mef2c CpG-F1775<br>Mef2c CpG-R1864 | ATCCTGGATCGTGGCTTTTA<br>CGCCCCCTTTAGATTCTCAG | 59 |
| Mef2c TSS-F<br>Mef2c TSS-R | TGAGGAGGGAGCTGCAGTAT<br>CCGTTTCTCTATCCCAACCA | 60 |
| Tbx5 TSS-F4798<br>Tbx5 TSS-R4906 | CTGGCTCAGAGCAGTCAACA<br>CCCCAAACTCTTGAACCACT | 59 |
| Tbx5 CpG1-F<br>Tbx5 CpG1-R | CGCTGTGACTTCGTACCAGA<br>CATGCGTCCACATAAAGCAG | 59 |
| Tbx5 CpG2-F<br>Tbx5 CpG2-R | GCTGCAGTGTAGAGGGAAGG<br>CACGGGTCTGGGTTGTAAA | 59 |
| Mesp1 Tbx-F<br>Mesp1 Tbx-R | TGGGTTCCTTTGGGAGCTGCTTGG<br>CTCTCACCTCTGTTCTGATGGGG | 62 |
| Mesp1 TSS-F<br>Mesp1 TSS-R | TTGGGTCCGCTGTAGCTTTATC<br>GCTATGGTTCAAAGGGTTCAGGC | 62 |
| Mesp1 CpG-F462<br>Mesp1 CpG-R552 | GAGGTTGTCCTCGCTGAGTC<br>CCAGAACCTGACCAAGATCG | 60 |

| **Bisulfite sequencing analysis** | Sequence | Sequence size |
|---|---|---|
| Meth Mef2c-F<br>Meth Mef2c-R | GGTTAAAAGTTTTATATATTTTGGG<br>CTAACAAAAAAATCCTTCTAAAATC | 243 bp, CpG in product:17 |
| Meth Mesp1-F<br>Meth Mesp1-R | GATTTTGGTTAGGTTTTGGT<br>AACAACCCTACACCACCCTAC | 152 bp, CpG in product:28 |
| Meth Tbx5 CpG1-F<br>Meth Tbx5 CpG1-R | AAGGTAAGTTTGAGAGAGTTTTTATT<br>CTATAAAACTATAAACCCACCCCTC | 133 bp, CpG in product:10 |
| Meth Tbx5 CpG2-F<br>Meth Tbx5 CpG2-R | GTTAGAAATTGGGTAAAGTATTTGT<br>AACTCCCTAACATTCTATTCCCTAC | 268 bp, CpG in product:17 |
| Meth HCN1-F<br>Meth HCN1-R | GTTGGGTAGAAGTGGTATAGG<br>CCTAATACTAAAACTAAAACTAAAACTAC | 157 bp, CpG in product:21 |
| Meth HCN2-F<br>Meth HCN2-R | GGGGTTTGGATTATTTATTTTTATAG<br>CCTCTACACACCAAAATCCCTC | 217 bp, CpG in product:21 |
| Meth HCN3-F<br>Meth HCN3-R | GATTTTTATTGTTTTGTGGTTGT<br>CTAAACTACTCTCCCAAAAACTTAC | 294 bp, CpG in product:26 |
| Meth HCN4-F<br>Meth HCN4-R | TTTTAAGAAGTTTAGGTAGGTAGGT<br>CTCCTCTAACTAAACCCAACC | 222 bp, CpG in product:24 |
| Meth Cacna1g-F<br>Meth Cacna1g-R | TTTTTTGTTTTTAGGGGTATGTTGT<br>AACCCCTTAAATCCTACTCCAACTA | 299 bp, CpG in product:25 |




## Figure S1.

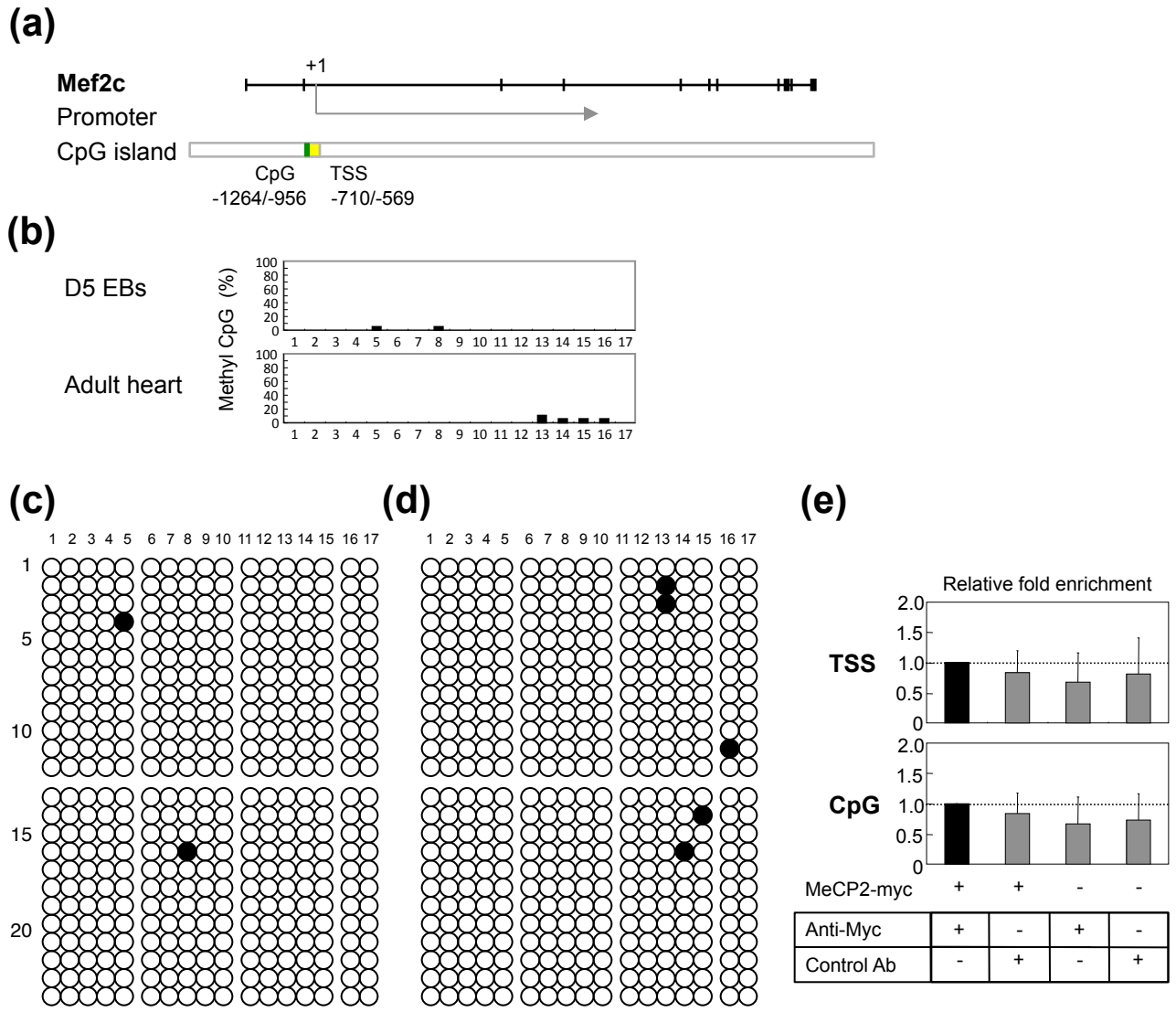
**Representative immunofluorescence images showing the distribution of Cx43 signal in wild-type and *Mecp2*-null left ventricular tissues.** Cryosections of left ventricular tissue obtained from 8-week-old wild-type (left panels) and *Mecp2*-null (right panels) mice were stained for Cx43 (green, upper panels) and N-cadherin (red, upper-middle panels), Hoechst 33342 (blue, lower-middle panels), and merged images (bottom panels). There was an evident change in the pattern of Cx43 staining in *Mecp2*-null hearts, relative to that in wild-type hearts. Scale bars indicate 50 μm.




## Figure S2.

### Transmission electron microscopy of hearts from *Mecp2*-null mice.

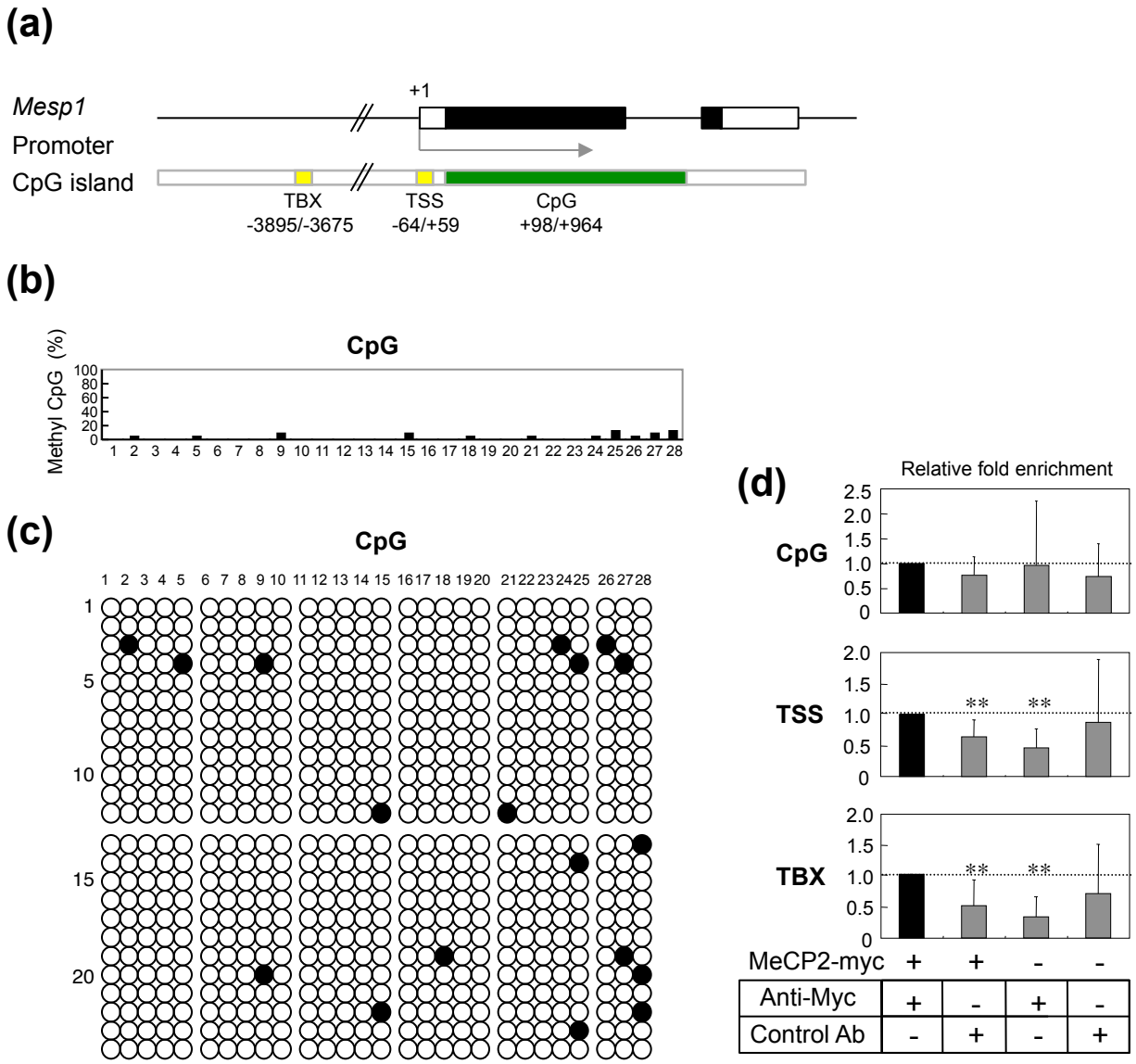
**(a)** Transmission electron microscopy analysis of left ventricular tissues in wild-type (left panells) and *Mecp2*-null mice (right panels) hearts. Electron micrographs show more remarkably hypertrophied nucleus and more abundant autophagic vacuoles (black arrows) in contrast to less abundant lysosomes (Lys, white arrows) suggesting autophagy activation in cardiomyocytes from *Mecp2*-null mice. The area in the rectangle is shown at higher magnification in the lower panels. N, nucleus; mt, mitochondria. Scale bars indicate 1.0 µm. **(b)** Western blot performed on ventricular tissue samples from 8-week-old wild-type and *Mecp2*-null mice. Blots were probed with antibodies against LC3 and GAPDH. Blots were scanned with the Odyssey CLx infrared imaging system (LI-COR), and the band intensities were quantitated using the Image Studio software (LI-COR). **(c)** Graphs show the relative intensity of indicated protein bands (left panel) and the LC3-II/LC3-I ratio (right panel). The intensity of each protein band was normalized against that of the corresponding GAPDH band. All data were further normalized against the corresponding value from wild-type mice, which was defined as 1.0. *$p < 0.05$ versus wild-type ventricular tissue samples (n = 5, each group).




## Figure S3.

### ChIP analysis and DNA methylation in the *Mef2c* gene.

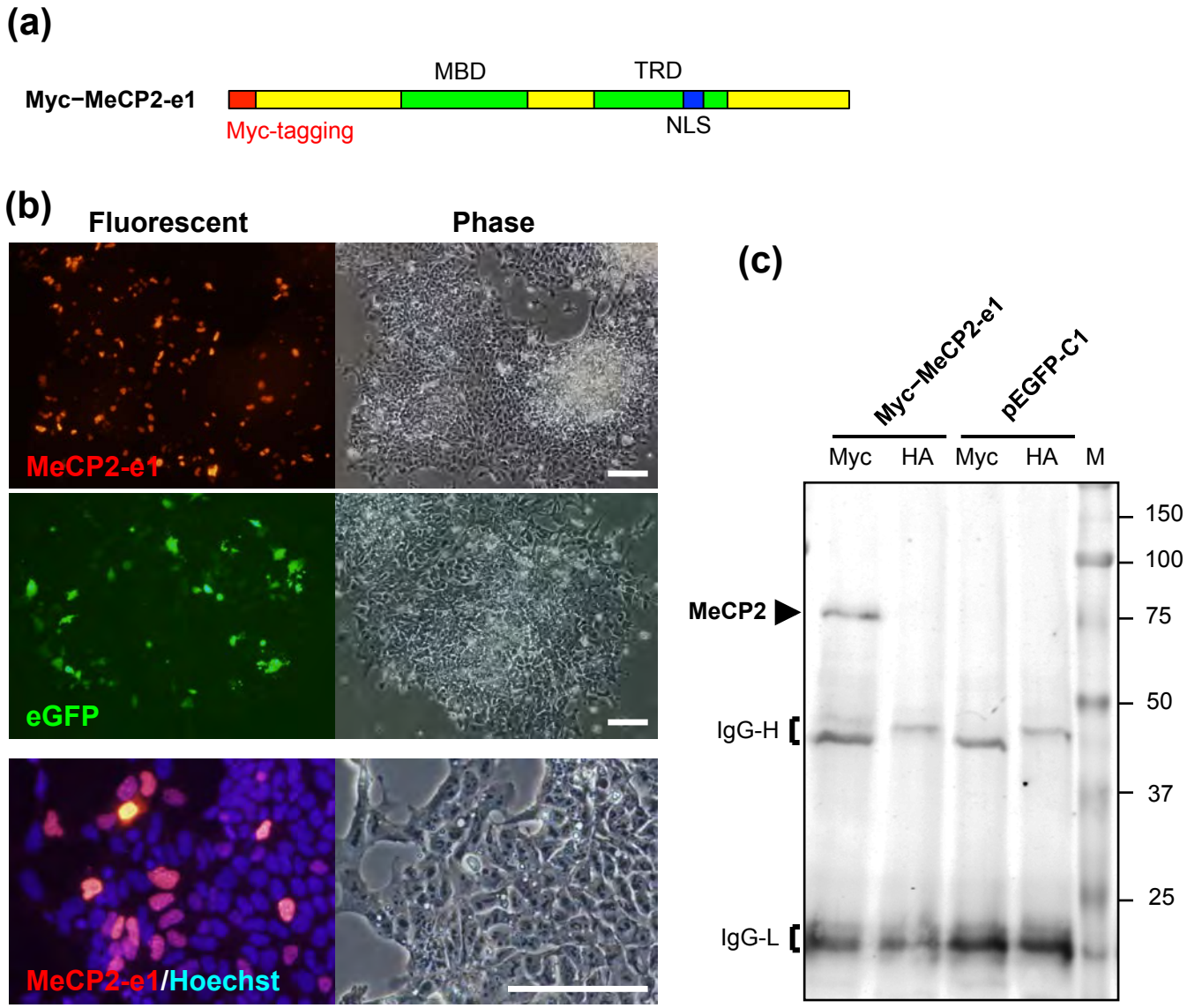
**(a)** Schematic representation of the *Mef2c* locus. Boxes represent exons, and lines connecting boxes represent introns. Black boxes represent coding sequences, whereas white boxes represent untranslated regions. The CpG island is shown as a green rectangle. The yellow box indicates the location of TSS. Numbers below each box represent the genomic location relative to the TSS (+1). **(b)** Percentage of methylated CpG islands from day 5 EBs and adult hearts. Each graph represents the percentage of methylated clones (number of methylated clones among 12 clones from each of two independent EB cultures or hearts, divided by 24, multiplied by 100). **(c** and **d)** DNA methylation patterns at the *Mef2c* locus in EBs and adult hearts. Bisulfite sequencing analysis of DNA methylation; filled circles represent methylated CpG dinucleotides. Numbers across the top indicate specific CpG dinucleotides within the CpG islands. Each row represents methylation data from a single clone of two independent EB cultures (**c**) or adult hearts (**d**), indicated on the left. **(e)** ChIP analysis was performed to investigate MeCP2 enrichment at the *Mef2c* CpG and TSS regions. ChIP assays were performed with chromatin harvested from EBs 24 h after transient transfection with the myc-tagged MeCP2-e1 construct or pEGFP-C1 plasmid. Primers were designed that amplify DNA -1226 to -1137 (CpG) and -710 to -569 (TSS) relative to TSS (+1). Enrichment for MeCP2 marks is shown for primer sets TSS (top) and CpG (bottom). Data are expressed as relative fold enrichment of anti-myc antibody (Anti-myc) in EBs transfected with myc-tagged MeCP2-e1. Data are expressed as means ± SD. *$p < 0.05$, **$p < 0.01$ versus anti-myc antibody (Anti-myc) in EBs transfected with myc-tagged MeCP2-e1, for the same primer set. Control Ab, negative control antibody. As negative controls, immunoprecipitations were performed on myc-tagged MeCP2-e1 or pEGFP-C1–transfected EBs using control mouse IgG, and on pEGFP-C1–transfected EBs using anti-myc antibody.




## Figure S4.

### ChIP analysis and DNA methylation in the *Mesp1* gene.

**(a)** Schematic representation of the *Mesp1* locus. Boxes represent exons, and lines connecting boxes represent introns. Black boxes represent coding sequences, whereas white boxes represent untranslated regions. The yellow boxes indicate the location of TSS and T-box binding site (TBX). Numbers below each box represent the genomic location relative to TSS (+1). **(b)** Percentage of methylated CpG islands in day 5 EBs. Each graph represents the percentage of methylated clones (number of methylated clones among 12 clones from each of two independent EB cultures, divided by 24, multiplied by 100). **(c)** DNA methylation patterns at the *Mesp1* locus in day 5 EBs. Bisulfite sequencing analysis of DNA methylation; filled circles represent methylated CpG dinucleotides. Numbers across the top indicate specific CpG dinucleotides within the CpG islands. Each row represents methylation data from a single clone of two independent EB cultures, indicated on the left. **(d)** ChIP analysis was performed to investigate MeCP2 enrichment at the *Mesp1* TSS, TBX, and CpG regions. ChIP assays were performed on chromatin harvested from EBs 24 h after transient transfection with the myc-tagged MeCP2-e1 construct or pEGFP-C1 plasmid. Primers were designed to amplify DNA -3895 to -3675 (TBX), -64 to +59 (TSS), and +411 to +501 (CpG) relative to the TSS (+1). Enrichment for MeCP2 marks is shown for primer sets CpG (top), TSS (middle), and TBX (bottom). Data are expressed as relative fold enrichment of anti-myc antibody (Anti-myc) in EBs transfected with myc-tagged MeCP2-e1. Data are expressed as means ± SD. *$p < 0.05$, **$p < 0.01$ versus anti-myc antibody (Anti-myc) in EBs transfected with myc-tagged MeCP2-e1, for the same primer set. Control Ab, negative control antibody. As negative controls, immunoprecipitations were performed on myc-tagged MeCP2-e1 or pEGFP-C1–transfected EBs using control mouse IgG, and on pEGFP-C1–transfected EBs using anti-myc antibody.




## Figure S5.

**Expression and immunoprecipitation of epitope-tagged MeCP2 protein.**

**(a)** Schematic representation of the myc-MeCP2-e1 construct used in this study. **(b)** Expression of MeCP2 protein in EBs. EBs were transfected with constructs expressing myc-MeCP2-e1 (upper and bottom panels) or enhanced green fluorescent protein (middle panels). Cells were stained for ectopically expressed MeCP2 protein with anti-myc antibody, and nuclear DNA was counterstained with Hoechst 33342. For these experiments, typical transfection efficiencies were 10–15% (12.87 ± 4.12%). Scale bar, 100 µm. **(c)** Immunoprecipitation of epitope-tagged MeCP2 protein. Epitope-tagged MeCP2-e1 protein expressed in ESCs was immunoprecipitated. The immunoprecipitates were subjected to immunoblots using anti-myc antibody.