Supplemental Materials and Methods

Mouse and human tissues (Pancreas). Mouse experiments were conducted under the guidelines of the NIH animal care and use committee, and an approved animal study protocol (K070-MDB-12). Pancreas and liver from 22 months old wild type (WT) and *Men1*$^{+/-}$ mice (1) was procured. Liver DNA was used to further confirm the mouse genotypes.

Human tumor samples (4 frozen MEN1 and 3 frozen non-MEN1 pancreatic neuroendocrine tumors (PNETs), 1 frozen MEN1 insulinoma and 23 archived formalin-fixed and paraffin embedded (FFPE) sporadic insulinomas) were obtained with informed consent from patients under NIH IRB approved protocols (NCT01005654). All 5 MEN1 patients carry germline mutations in *MEN1*. The diagnosis of insulinomas was based on supervised fasting with all patients having serum glucose levels less than 45 mg/dL within 48 hours of fasting together with elevated plasma insulin, proinsulin and C-peptide levels. All patients had their tumor localized and removed without any recurrence during follow-up. For IHC controls, FFPE pancreas sections of normal human pancreas (n=7) were obtained (Abcam, IHC World, Origene, ProSci, US Biomax, Zyagen and Dr. Michael Emmert-Buck of NCI). For RNA and DNA isolation, normal human pancreatic islet preps (n=1 fresh and n=5 frozen) were obtained (Lonza and University of Alabama Islet resource facility).

Isolation of mouse pancreatic islets. Mice were put to sleep and intraductal collagenase perfusion was performed following a standard protocol (2). Islet isolation was performed using 100 μm and 70 μm strainers. Briefly, the amorphic portions of the pancreas were transferred to a 50 ml centrifuge tube, and incubated at 37˚C for 23 min with shaking at every 5 min intervals to check the digestion by collagenase. Ten ml ice cold HBSS with 1% FBS (FBSS buffer) was added to stop the collagenase reaction and the digested tissue was passed through a 14G needle with a syringe a few times to break the chunks of tissue. The tissue was washed three times with FBSS buffer, passed through a 1.5 mm mesh, followed by 0.8 mm mesh. The flow-through was collected and then passed through a 100 μm strainer followed by a 70

μm strainer. The islets collected in the strainers were pooled, washed with FBSS buffer, dispersed in DMEM medium, and were hand-picked under the dissecting microscope.

Meg3 cDNA cloning. Meg3 cDNA was amplified by RT-PCR using RNA from normal human islets, WT mouse islets, or RNA isolated from 5'-Aza-2'-deoxycytidine (decitabine, Sigma) treated MIN6-4N cells, with human or mouse specific primers located at the beginning of exon-1 and the end of exon-10 (Human and mouse NCBI Reference Sequence: NR_002766.2, and NR_027652.1) (3,4), cloned into pcDNA3.1 (Invitrogen), and sequenced (Genewiz). (Supplemental Fig. S1).

Cell culture, transfection, and stable cell lines. Mouse insulinoma cell lines, MIN6 (5) and MIN6-4N (6), were cultured in low glucose DMEM supplemented with 15% FBS and 1X antibiotic/antimycotic (Invitrogen, Gemini) at 37˚C and 5% CO_2. MIN6-4N is a tetraploid (4N) cell line that was isolated from MIN6 cells that have mixed ploidy (2N and 4N). Stable cell lines were maintained in the above medium containing 500μg/ml of G418 (Life Technologies). Plasmids were transfected using Lipofectamine 2000 (Invitrogen) or by nucleofection with solution-T (Amaxa/Lonza).

Vec and M-27 are MIN6 stable cell lines containing pcDNA3.1 vector and pcDNA3.1-Myc-His-Menin, respectively (7). Vec-4N and M-27-4N are MIN6-4N stable cell lines containing pcDNA3.1 vector and pcDNA3.1-Myc-His-Menin, respectively, derived by single cell isolation from Vec and M-27. Menin expression was determined by western blot probed with rabbit anti-menin (Bethyl Laboratories) and mouse anti-tubulin (Calbiochem) followed by HRP-conjugated secondary antibodies, and visualized using ECL (Amersham).

Vector or mouse Meg3 transfected stable cell lines were generated in MIN6-4N cells as described (7). Meg3 expression was determined by RT-PCR. Vec-3 and Meg-5 are stable cell lines of MIN6-4N containing pcDNA3.1 vector and pcDNA3.1-mMeg3-3, respectively. Vec-9 and Meg-14 are MIN6-4N stable cell lines containing pcDNA3.1 vector and pcDNA3.1-mMeg3-1, respectively. mMeg3-3 was the most abundant cDNA clone (isolated from WT mouse islets) that lacks exon-2b and 4, and mMeg3-1 is

full-length with all 10 exons (Supplemental Fig. S2).

DNA, RNA isolation and quantitative real time-PCR (QRT-PCR). Tissue was micro-dissected from slides of frozen or FFPE pancreas sections, followed by DNA and RNA isolation (QIAamp DNA FFPE tissue kit, RNeasy FFPE kit) (Qiagen). From cells and fresh islets, DNA/RNA was isolated using the DNeasy or RNeasy Mini Kit (Qiagen). DNA/RNA from mouse ESC-WT and ESC-*Men1*-Null was previously described (8). RNA samples were treated with DNaseI (Ambion), and first strand cDNA synthesis was performed by using an oligo-dT primer and Superscript-III (Invitrogen). QRT-PCR was performed with the Brilliant SYBR Green QPCR Master Mix (Qiagen) and Mx3000p thermal cycler (Stratagene). Ct values were normalized to mouse or human Gapdh. Relative gene expression changes were calculated by the $2^{-\Delta ct}$ method, and the fold changes are plotted with respect to the controls.

DNA methylation analysis. Bisulfite treatment of the DNA samples (500 to 1000 ng) was performed with the Epitect bisulfite kit (Qiagen). The *Meg3* promoter region near the transcriptional start site was amplified from untreated and bisulfite-treated DNA, with Meg3-1F and Meg3-1R primers to yield a 251 bp product which was cloned into the PCR2.1 vector using a TA cloning kit (Invitrogen). Five to ten clones with insert were sequenced, and at least 5 clones with good quality sequence were analyzed to detect differences in methylation.

To analyze the methylation status of the CRE-site, PCR product obtained with mouse or human Meg3-1F and Meg3-1R primers was diluted (1:25), and used as the template for nested PCR with mouse or human Meg-1R and a forward primer that would specifically anneal to the methylated or unmethylated CpG in the CRE-site (mouse or human Meg3-MF and Meg3-UF, respectively). The PCR products were run on the same agarose gel, and the relative band intensities were quantitated (ImageJ software).

Meg3 promoter cloning and Luciferase assay. Mouse Meg3 promoter (-560 to +122) and human MEG3 promoter (-539 to +140) was amplified by PCR from mouse or human genomic DNA,

respectively, and cloned into the luciferase reporter vector pGL4.10 (Promega) to generate plasmids pGL4.10-mMeg3p and pGL4.10-hMeg3p.

MIN6-4N cells were seeded in 12-well plates, and transfected with pcDNA3.1 vector or pcDNA3.1-Myc-His-Menin, and the promoter constructs. Cell lysates were prepared 48h post-transfection and processed for measuring luciferase activity (Promega) using a luminometer (Berthold). Menin transfection was confirmed by western blot.

Chromatin immunoprecipitation (ChIP). Chromatin from Vec and M-27 cells was processed for ChIP assay (1.5 x 10^6 cells per ChIP) (Upstate/Millipore). Antibodies used were: rabbit anti-CREB (Cell signaling), rabbit anti-H3K4me3 (Millipore), rabbit anti-Myc-tag (Millipore) for transfected myc-his-menin, and rabbit anti-HA-tag (Abcam) as negative control. ChIP-PCR was performed with a primer pair specific for the CREB binding region at the CRE-site in the Meg3 promoter (Meg3-1F and Meg3-1R). PCR products were detected by agarose gel electrophoresis.

Microarray analysis and validation. DNase-treated total RNA from 3 independent cultures of the vector (Vec-3) and Meg3 transfected (Meg-5) stable clones were used for gene expression microarray analysis with Affymetrix Genechip mouse genome ST 1.0 arrays (Affymetrix). Microarray hybridization and data analysis was performed at the NIDDK genomics core facility. Fifteen up and 36 down-regulated genes (changed >2.5-fold, and $p<0.05$) were validated by QRT-PCR.

Cell proliferation and viability assays. MIN6-4N stable cell lines (2 x 10^5 cells) were seeded in 6-well plates and cultured for 6 days with change of medium (containing G418) every other day. On the 6^{th} day, cells were trypsinized and counted using cell counter slides (Nexcelcom). For the effect of DNA demethylating drugs on MIN6-4N cells, 10^4 cells per well were seeded in 96-well plates, and on the following day medium containing DNA demethylating agents was added: 0, 0.5, 1, 2 μM of 5'-Aza-2'-deoxycytidine (decitabine, Sigma), and L-ascorbic acid (Sigma) - 0, 0.1, 0.5, 1 mM. At different time

points (24h, 48h, 72h, and 96h), viable cells were assessed by the MTT assay (Promega).

Flow cytometry and cell cycle analysis. After 6 days in culture cells were harvested with trypsin and counted, 2 x 10^5 cells were incubated in Vindelov's propidium iodide buffer and analyzed by fluorescence-activated cell sorting to generate cell cycle histograms (FACSCalibur, BD Biosciences). Each sample was analyzed in triplicate for at least 10,000 events. The raw data was subjected to ModFit analysis to determine the percentages of cells in G0/G1, S, and G2/M.

Immunofluorescence (IF) and Immunohistochemistry (IHC). FFPE pancreas sections were stained with rabbit anti-menin (Bethyl, 1:500) and chicken anti-insulin (Abcam, 1:1000), followed by anti-rabbit alexa fluor 488 (green), and anti-chicken alexa fluor 594 (red) using standard double-IF procedures. For IHC, FFPE pancreas sections were processed for insulin and c-MET staining as described (6). The c-MET (Santa Cruz biotechnology) antibody (1:1000) was validated in HeLa cells (ATCC) (Supplemental Fig. S3). Images were captured on the Axiovert 40 CFL (Zeiss) or AxioObserver Z1 (Zeiss).

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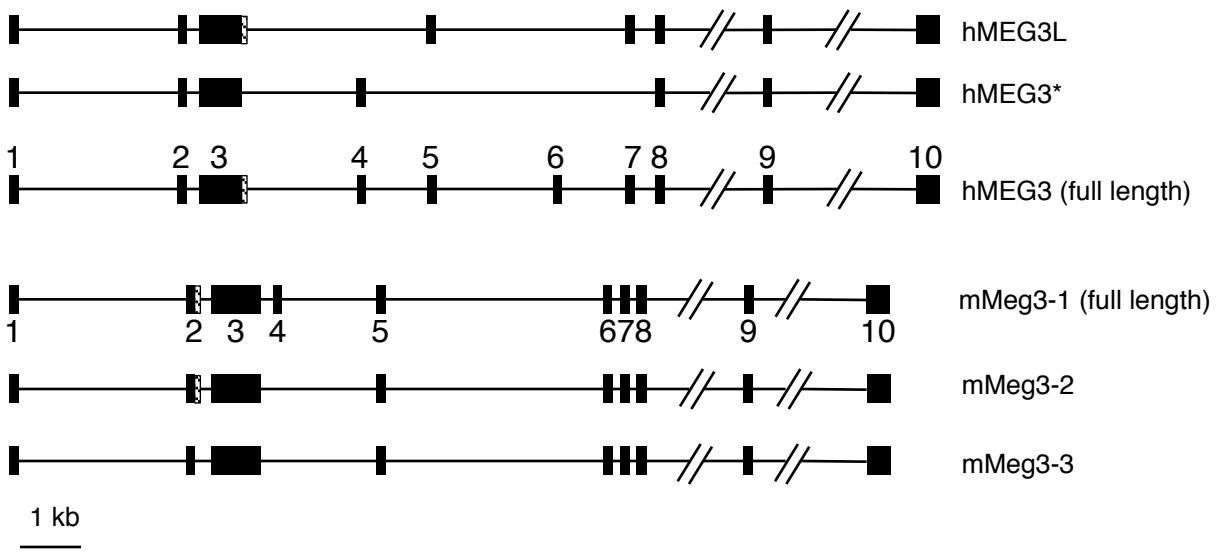


Supplemental Figure 1. Genomic structure of human and mouse *MEG3* .
The genomic structure of full-length human MEG3 mRNA with 10 exons was assembled from the alternatively spliced transcripts variant-2 (lacks exon-3a and exon-7, NCBI Reference Sequence NR_003530.2), variant-10 (lacks exon-6 and exon-7, NCBI Reference Sequence NR_046467.1), and variant-13 (lacks exon-3a, exon-4 and exon-6, NCBI Reference Sequence NR_046470.2). Exon-3a in the human gene diagram is striped which is generated from an alternative splice donor in exon-3. Above human MEG3 (full length) are shown the 2 transcripts of MEG3 that we isolated from normal human pancreatic islets' RNA by PCR using a primer pair located at the beginning of exon-1 and exon-10. hMEG3* is identical to the most abundant transcript in human islets, which also was found to be most abundant in human pituitary *(Zhang et al. 2010 Mol Cell Endocrinol 326:40-47*). Shown above hMEG3* is hMEG3L which was a rare but the longest transcript that we isolated from human islets (lacks exon-4 and exon-6) and not previously reported.

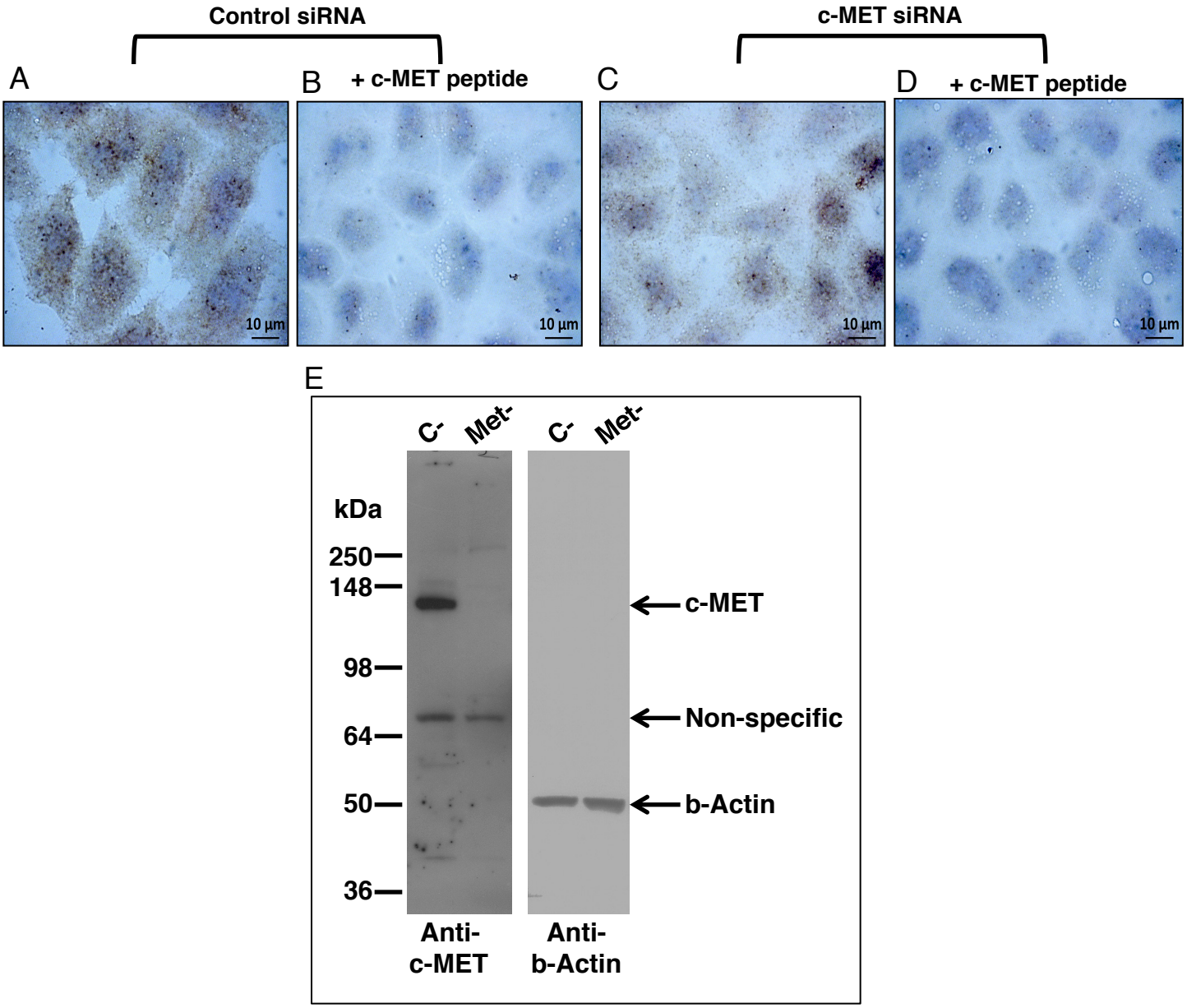
Below the human MEG3 diagram are shown the genomic structures of the 3 mouse Meg3 transcripts that we isolated from pancreatic islets of a wild type mouse, or decitabine-treated MIN6 cells by PCR using a primer pair located at the beginning of exon-1 and exon-10: mMeg3-1 is full-length with 10 exons (identical to variant-3, NCBI Reference Sequence NR_027652.1), mMeg3-2 lacks exon-4, and mMeg3-3 lacks exon-2b and exon-4. Exon-2b in mMeg3-1 is striped which is generated from an alternative splice donor in exon-2. mMeg3-3 was the most abundant transcript in both mouse cell types. Other less abundant human and mouse transcripts from islets are not shown that lacked various exons. Human MEG3 gene spans 35 kb, and mouse Meg3 gene spans 31 kb. The break in the lines after exon-8 and exon-9 indicate the large intron-8 and intron-9 in the human and mouse gene. Scale bar = 1 kb.


1 ACAGAAGACG AAGAGCTGGA ATAGAGCTCG CCTCGGCTCT GCTGGCCTTG GCTGCAGCTC TTCCAGAAAC CCGGGGCGCC CACAGAAGAA TCTCTTACCT
101 GGCTCTCTCT TCAGGGATGA CATCATCGGC TCACACCAGT CTTCCAGGAC CACCTTCTGG ATGCCAAGGC TGCTGCTCGA GTACCTGCTG TGCACCTCTA
201 CCTCCTGGTA CCCCAGCCAC TCCCTCAAGT TGCCTGCATG TTATATGGCC TAGCCAAGGA GCACGGATTC CAGGAACCCA CTACCATACA GAGCAACTCC
301 TTGTGGACCC CCCTGGGATC AGGACAGCGA GGGACAAGCG ACAAAGAGGA TCATCAGTGG CCAGCTAGTT TCTCTGGGGT TCAAACCTTG AACCAGTGCC
401 CTAGTGAGGG GGCACTGGCC ATGGCCCTTG ACCTTTGCTC TGCTTGTGTC TTGAGTCTGA GCCCTTTCCT GTACATCTGT GCTCGTGTTC ATCTGCTAGT
501 GAACTGGAGT GCTGCCCTCC CCGAGGAGGG TCGTCCCTTG TGACTGATCA TGCTGTCCTA ACAATGTCCT GAGCAAAAGG GTCCCTTTGG GAACCTCTCA
601 GGAGGGGGAC CCGGGTCAGG GGCGACCAGC ATCTTGCTGG CAACTCCGTG GGTGGGGTGG GGTGGGGTGC TTCCTTCTGG AATGAGCACG TGGCTGACCC
701 CCCAAGGCAT GTCCCCTCCC CCTCCTCCAC CCACCTTCTC GGAGATGTCC CTTTTGGGGT AGTGGGGACA TTAGGAGCAA CCTCCTAGGG TTGTTGTGAG
801 AATTAAATGA ACTGCAGCAG CCTGAGGCAG GGCTGGGCAG AGACCTCAGC ACATGTTTGT TGAAAGGTTT GCAGGTGGAT CTAGTCCTCC CGTTCATGGC
901 TCATGTGTCT CAACCATTCT CTCGCAGACT CCTGCAGCCC CTATGCCCAG GGCTCTCCTT GCGCCAGAGG TAGGTGGGAA AGAGAACTGG GAGAGCCCGG
1001 ACTCACTCAT GAGATTGAAC TTAAATTCAC ACGGAGGACA CTTGGACTCT TGCCACATTA GCCCCGGCTT CTCGAGGCCT GTCTACACTC GCTGCTTTCC
1101 TTCCTCACCT CCAATTTCCC CTCCAACCCA CTGCTTCCTG ACTCGCTCTT CTCCATCGAA CGGCTCTCGC TCAGGTTTAA GGCTTGGCTT GCTGGCCCTG
1201 GAGATCCCGT CGCCGTCTTC GTCGAACTCG AAATCCTAGC CATCGTCCTT CCCTGGCCTG TCGCGTCTTC CTGTGCCATT TGCTGTTGTG CTCAGGTTCC
1301 ACGAGCTGCC CATCTCCACA GAAGAGCAGC TGGCATTGCC CACCGGCCAT GCCGGCTGAA GAAAAGAAGA CTGAGGACCC CAGGATGCCC AGCGCGAGGA
1401 CCCCAGGAAG CCCAGCGCGA GGACTCCACC CACGACGCCC AGCGCGAGGA CTTCACGCAC AACACGTTGC AACCCTCCTG GATTAGGCCA AAGCCATCAT
1501 CTGGAATCCT GCGTGGGACC CTGGACACAC GGACACAGAC ACCTGCCCCC AGGACCCTCC AACTGTAAAT CCCTCTACAG CCACGGGGAC GCCTTGCACA
1601 TTTCCTGTGG GACATGCTGG ACCCAAGACT CTGGACCCTG GCCTCCCCTT GAGTAGAGAG ACCCACCTAC TGACTGATGA ACTGCGCTGA CCCTGGGGTC
1701 AGGCATGTGG CCTTGATCCC TACCCATGGA CCCTGAGACT TGGGGGGGTG GGAGAAAGGC TGTTGTGTCT TCACTGTTGA GTCTACATCT GTGAAATGGG
1801 CTCAGGTTCC TACCTCACAG GGCTGTTGTG AGGCAGCCGC AATGTGCTTA GAAGCATGGG GCCTAGTGGC TCATGGTGCT TTCAATAAAT TTCTTGTTTT

Supplemental Figure 2. Mouse Meg3 cDNA sequence.

The sequence of the mouse Meg3 full-length cDNA clone mMeg3-1 that contains all 10 exons is shown. Black arrowheads indicate the position of the introns, determined by comparing to the genomic sequence of mouse Meg3 (NCBI Reference Sequence NW_004450261.1). Region to the left of the grey arrowhead (alternative splice donor site) is exon-2a and to the right is exon-2b. The sequence is identical to mouse Meg3 variant-3, NCBI Reference Sequence NR_027652.1 Boxed regions, exon-2b and exon-4, are absent in the mouse Meg3 cDNA clone mMeg3-3. The polyA signal is underlined.

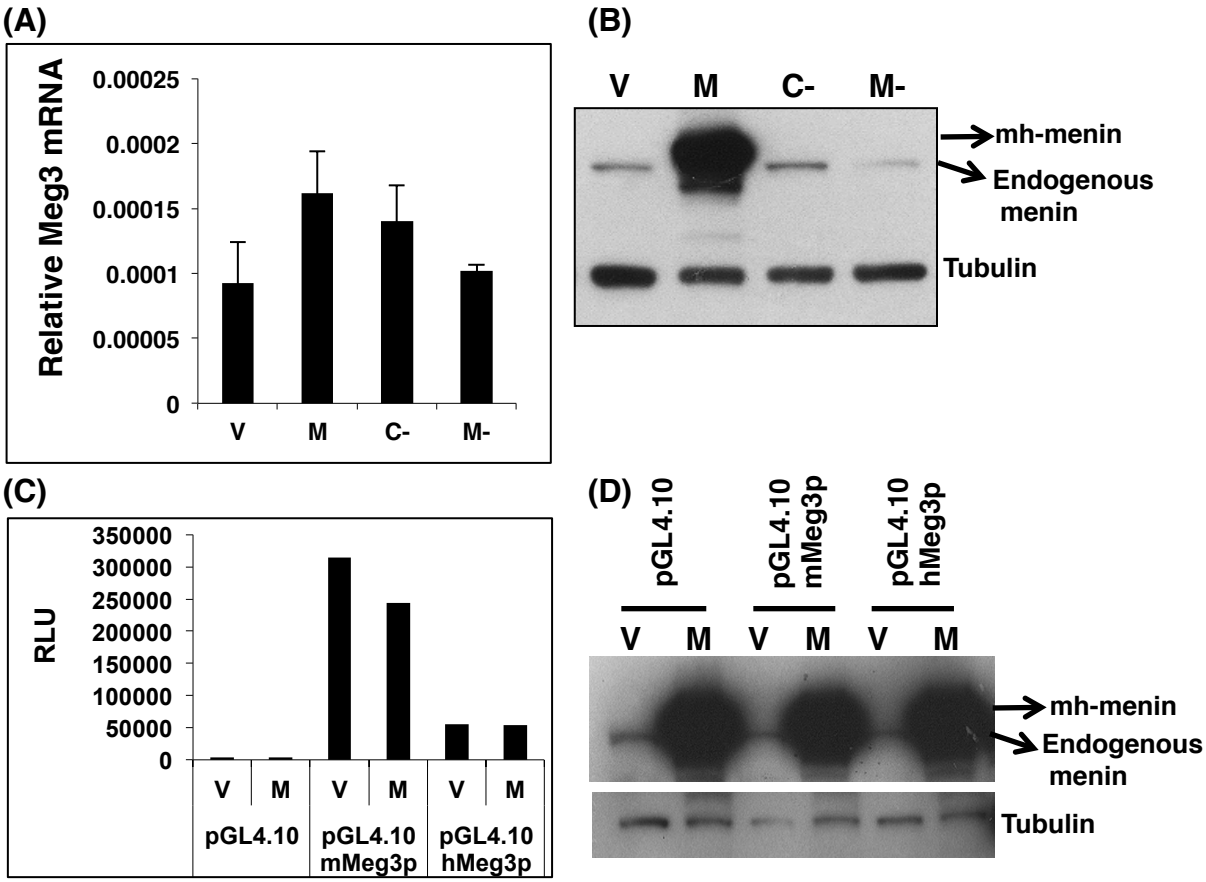
Overall sequence homology of the mouse sequence to the 10 exons of the human full-length MEG3 cDNA sequence is 70%. The region in bold corresponding to exon-5 of the mouse sequence is highly homologous (89% sequence identity) to human exon-4. Sequence homology was determined by ClustalW and DNA Pustell Matrix (MacVector). Sequence from 1-844 is also present as a retro-transposed pseudogene on the mouse X chromosome (mm10 ChrX: 88,759,471-88,760,314). However, this pseudogene is absent in the human genome.



Supplemental Figure 3. Validation of c-MET antibody used for immunohistochemistry (IHC). HeLa cells were transiently transfected with control siRNA (All Stars Negative Control siRNA, Qiagen) or c-MET-specific siRNA (ON-TARGET plus Smart pool, Dharmacon) by nucleofection (Amaxa/Lonza), and the cells were cultured for 48 hours in a chamber slide for IHC, and in a 6-well plate to isolate protein for western blot. IHC was performed as indicated using anti-c-MET (Met C-12, Santa Cruz Biotechnology) without or with c-MET peptide competition (Met C-12P blocking peptide, Santa Cruz Biotechnology).

(A-D) Brightfield microscopy images showing the specificity of the c-MET antibody (magnification = 500X). IHC was performed using anti-c-MET without or with c-MET peptide competition. Brown staining indicates positive c-MET staining which is primarily in the membrane but also seen in the cytoplasm (A). c-MET staining is significantly reduced upon peptide competition (B and D) and upon c-MET knockdown (C). Blue is haematoxylin counterstaining of the nucleus.

(E) Western blot detecting c-MET in HeLa cells used in A-D transfected with control siRNA (C-) or c-MET-specific siRNA (MET-) showing specificity of c-MET antibody and significant reduction in the c-MET-specific band intensity upon c-MET knockdown. Beta-Actin was used as a loading control.



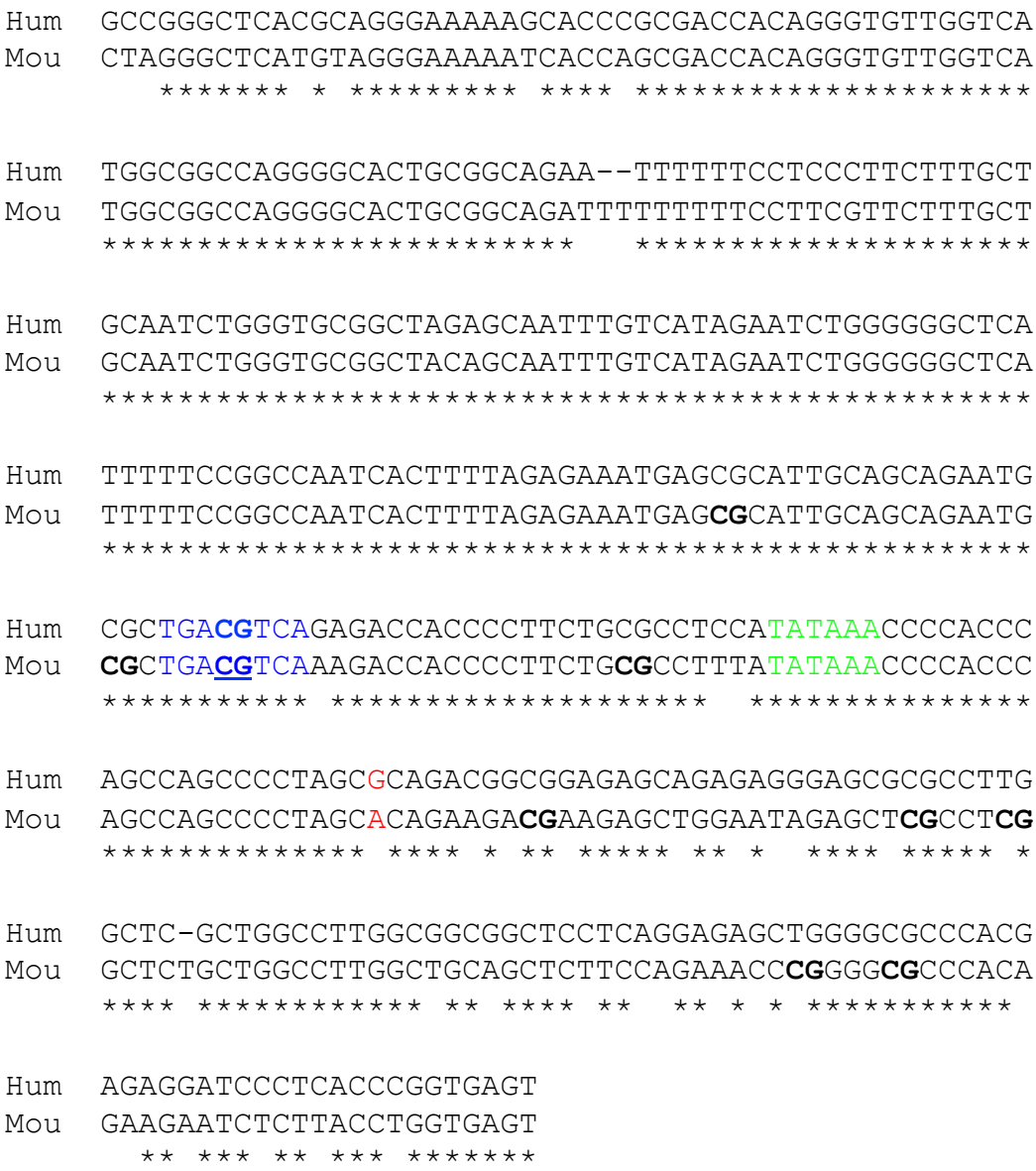
Supplemental Figure 4. Effect of menin on *Me g3* promoter activity.

(A) QRT-PCR of RNA from transiently transfected MIN6-4N cells showing slight increase in Meg3 mRNA when menin is transiently overexpressed. Gapdh was used as the internal control. V = empty vector transfected cells, M = cells transfected with plasmid overexpressing menin (mh-menin), C- = cells transfected with shRNA vector control, and M- = cells with transient knockdown of menin using menin specific shRNA (Men1-shRNA). Note that the endogenous level of Meg3 mRNA is very low in MIN6-4N cells. Cells were analyzed 48 hours post-transfection for V and M, and 96 hours post-transfection for C- and M-.

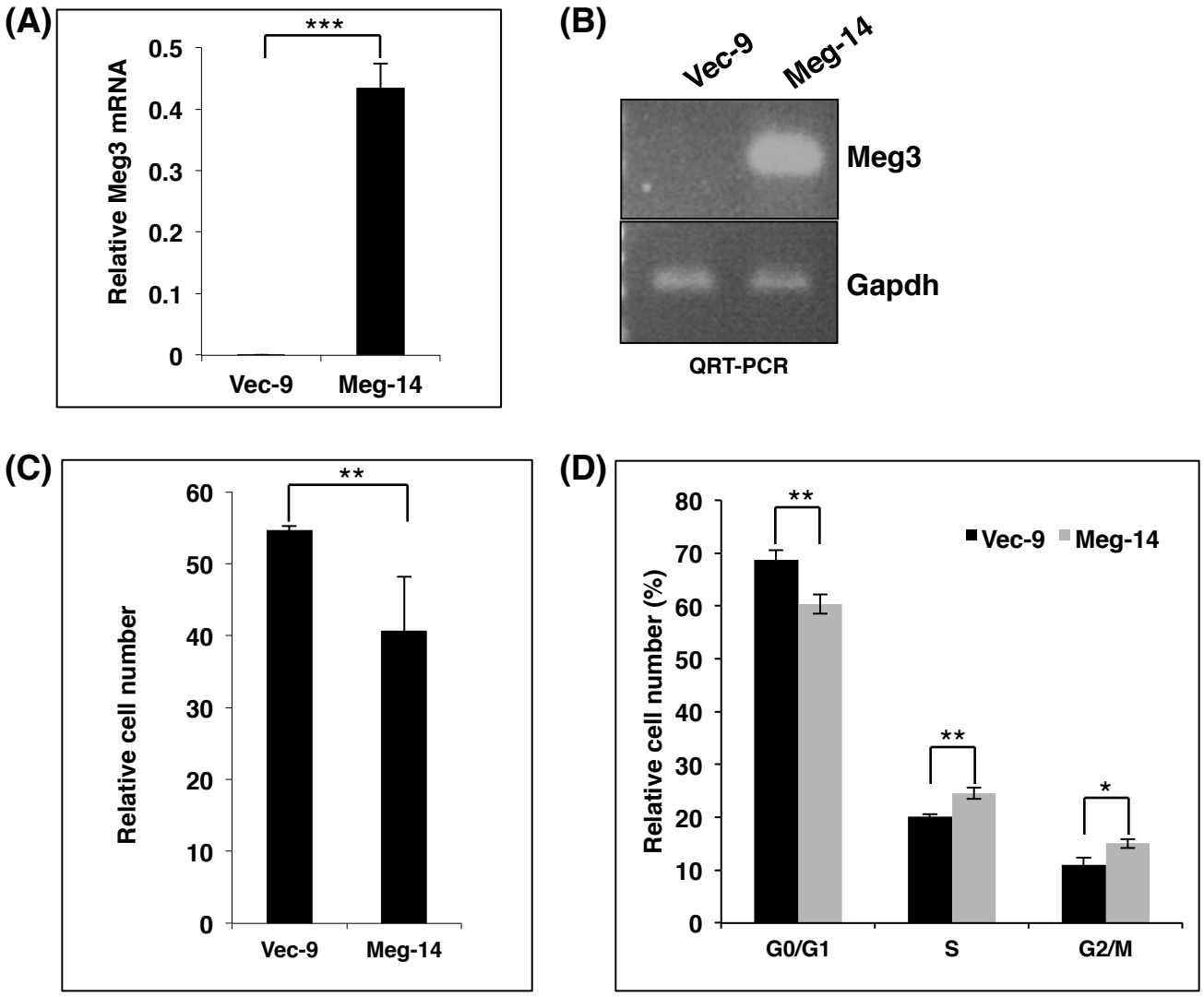
(B) Western blot showing overexpression and knockdown of menin in MIN6-4N cells analyzed in 'A'. Tubulin was used as the loading control.

(C) Luciferase assay data showing no direct effect of menin on the promoter region of mouse *Meg3* or human *MEG3* in transiently transfected MIN6-4N cells. The promoter regions were cloned into the luciferase reporter vector pGL4.10. RLU = relative luciferase units.

(D) Western blot analysis of cell lysates used for luciferase assay in 'C' showing overexpression of menin. Tubulin was used as the loading control.



Supplemental Figure 5. Promoter region of human and mouse *MEG3* .
Nucleotide sequence of the highly homologous region of the *MEG3* promoter in human (Hum) and mouse (Mou) upstream and downstream of the transcriptional site site (red). The TATA box is shown in green. The CREB responsive element (CRE site) located at -60 to -53 is shown in blue with the CpG underlined. The 9 differentially methylated CpGs (bold) in this region that were observed in this study are shown in the mouse sequence. Asterisk indicates identical nucleotides in the human and mouse sequence. Dash indicates gap allowed in the sequence for alignment.



Supplemental Figure 6. Stable expression of full length mouse Meg3 in MIN6-4N cells affects proliferation and cell cycle.

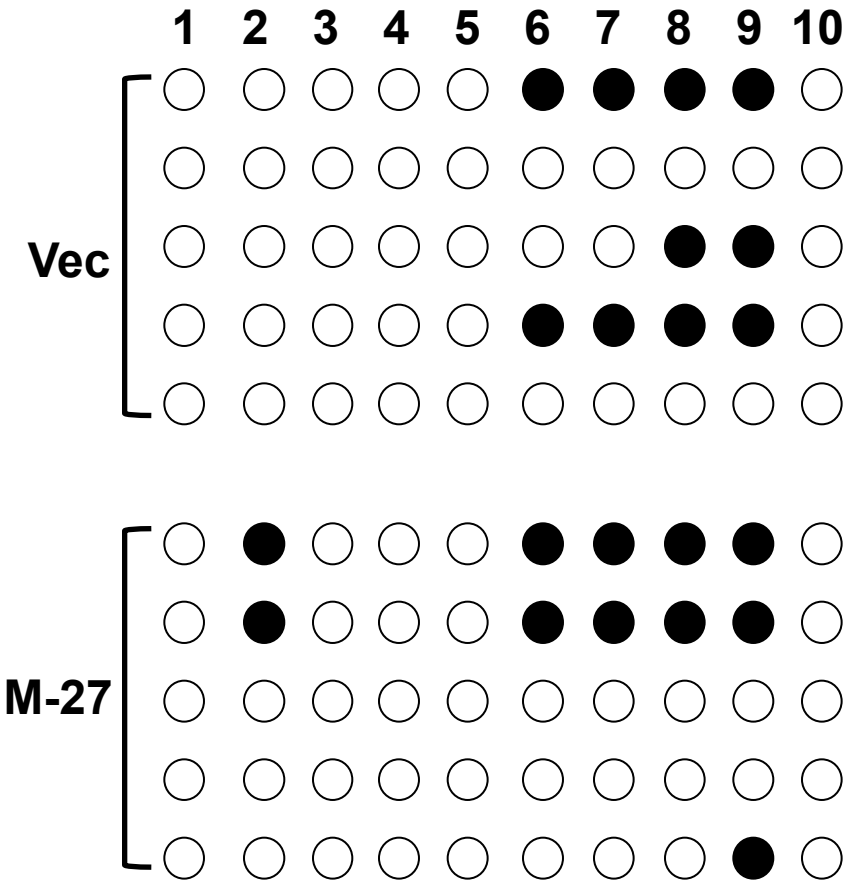
Vec-9 = Stable cell line with empty vector; Meg-14 = Stable cell line overexpressing full length mouse Meg3 cDNA (mMeg3-1).

(A) QRT-PCR data showing overexpression of mouse Meg3 mRNA in Meg-14 cells ($p < 0.0001$). Gapdh was used as the internal control.

(B) Agarose gel showing overexpression of mouse Meg3 mRNA in Meg-14 cells. Gapdh was used as the internal control.

(C) Cell proliferation assay showing significant decrease in cell number ($p < 0.005$) in Meg-14 cells. Cells were cultured for 6 days.

(D) Flow cytometry analysis showing significant decrease in the percentage of cells in G0/G1 ($p < 0.005$) and a significant increase in the percentage of cells in G2/M ($p < 0.05$) in Meg-14 cells.



Supplemental Figure 7. Control experiment for bisulfite sequencing assay (DNA methylation analysis at 10 CpGs in the IG-DMR region of mouse *H19*).
Genomic DNA from MIN6 stable cell lines M-27 (ectopic menin expressing) and Vec (empty vector transfected) was processed for bisulfite conversion. A region in the *H19* IG-DMR previously known to be differentially methylated in some cell types was amplified with primers mH19-DMR-F and mH19-DMR-R, and cloned using the TA-cloning kit. Plasmid preps (n=5) were sequenced and the sequence of the insert was aligned and analyzed for methylated CpGs. Filled circle = "methylated" (C of CpG not converted to T upon bisulfite treatment). Open circle = "unmethylated" (C of CpG converted to T upon bisulfite treatment). Both Vec and M-27 show similar level of methylation in this region confirming good efficiency of bisulfite conversion in our experiments.

Supplemental Table-1			
PRIMER	**FORWARD**	**REVERSE**	**PRODUCT (bp)**
MOUSE			
RT-PCR			
mAnxa2	ttgaagacacctgcccagtatga	tttcggaagtctccagatgtgtca	200
mBC028528	ccacagaaccaagtccaaatca	ccgctgaaccatctctccagtc	210
mCar8	tgcagcgaaggagttacctggat	taggtcggaaattgtctcccaaa	151
mCasp1	ttgccctcattatctgcaacac	aggcagcaaattctttcacctc	171
mCasp4	ttcctgaaggtgcaacaatcat	tggccaagatcacttcttcaa	167
mCd82	acttaaagcgcgtagccagaac	cttgtctgcaagaatccacacc	243
mCd9	ggattgttcttcgggttcctctt	gcaggtgtccgagataaactgct	234
mCdh6	gttgctcaacatggatcgagaa	gaggactcgggggtcttaaact	183
mCeacam10	cgatcataccctaatgcctgtg	caccgtcagctgatgaaatcc	177
mCldn1	cgggcagatacagtgcaaag	gccaatggtggacacaaaga	127
mClldn11	gctggctggggtgctccttatt	cggttttctccaaatgactgtg	204
mcMet	tgccctatatgaagcatggagat	gtggtgaacttctgcgtttgc	316
mCoch	ccctctgatgacattgaggaag	tgcaccagaggcttcacatatt	212
mDach2	tgcaagataatgcccgacttct	ggctctgactcagtcccatttt	181
mDgkb	ggaccatattttgccacctacca	atctgcaaaccttgtccatccat	195
mEgln3	tcaacttcctcctgtccctcatc	tgtaacttggcgtcccaattctt	211
mEnpp3	tagccacagaggagcccatta	tgcgatgagtcaaagcattttt	169
mFam129s	aaggacctggaagggaccat	agctctgactgagttcatctac	232
mFn1	atgcaccgattgtcaacagagt	tgatcagcatggaccacttctt	199
mGapdh	ATCACTGCCACCCAGAAGAC	CAACCTGGTCCTCAGTGTAG	304
mGatm	cttgctttgatgctgctgactt	gcatcgatgtgcattggattgg	166
mGm501	cctgtcagagctcaacacca	ggtgggtggaggacaatagaag	249
mH2-K1	ggagaaacacaggtggaaaagg	gctcacagggaacatcagacac	225
mHect2	tgtttctttacggctgtttcca	ctgccacgtgtgatactcttcc	205
mHoxB9	cagggaggctgtcctgtctaat	tccttctctagctccagcgtct	180
mInsulin	TAGTGACCAGCTATAATCAGAG	ACGCCAAGGTCTGAAGGTCC	289
mKctd12b	gaagctcgatttggcagtacct	tgtacacctggccacctacatt	195
mKlhl1	ccacagatactggctgaccttg	cctgaatccacagatttgttcg	144
mMatn2	cacaatgggcgaaataagtgaaaa	ttcctgaagatttggttgaatgga	200
mMeg3	cttcctgtgccatttgctgttg	tgcaacgtgttgtgcgtgaag	250
mMen1	gtacatgcgctgcgaccgtaag	tcatcctggtagtaggtcttag	270
mMgam	gggaccaggtcttctcatca	cctcgaaggtgaagtccaat	171
mMmd2	cccgaggatctgacaccttt	ccgggactcggtcattcata	173
mMoxd1	ggctttacctatccacctcacg	acccctgcgtcatatctcctta	170
mNrk	tcagattcaattgccttggaga	ctccccacaaagaaccacagta	208
mPcdhb3	aggctgtctccaaaagaccttg	caagagacccacccagaaaaac	164

PRIMER	FORWARD	REVERSE	PRODUCT (bp)
mPcdhb5	agaagctggagagaaatcatcg	gaattgctgtttgtcatggtcag	218
mPhldb2	ccagccagaggcagaagttaat	cccacccattttgatgaggtat	212
mPhldb2	ccagccagaggcagaagttaat	cccacccattttgatgaggtat	212
mPon3	aacaacaacgctctctcatcca	ggccacaactttgacctctttt	209
mPrkd3	gatggtaatgtgcagggtcaaa	gcacggctctccattgaaagtaa	211
mPtprm	aggatggtgtggcatgagaac	tcatgaatgcctctcttttcca	197
mRab3c	cttgggataatgcccaggttatc	aaggtttgcttcacgttgatgtt	157
mRhox2e	gacctgaggaggatgaggaaaa	ttggttgctgttatccatgagg	200
mRiken	gacagcagaaacctcctgtgaa	agaatagggagggcaggtaagg	216
mRiken26	taaagacccgtcatttcgtcc	tccattcagggtgagggttact	206
mRiken49	tcggctatctgaagaaagtaa	cctggatcatgcctctgaatgt	169
mRnasel	atgggagagtcacagatggtca	ccaagtccaaaagaaaggatgg	237
mSamd9I	gttgtttgtcacggacatcttgg	tccagtctttgatcaattttggttg	196
mSntg1	gaaatgcagacatgaggaggtg	ccactgtcacaaagaggagagg	177
mSp110	gctgtggatttctctcccacact	caacgtataacgcccttccagtc	191
mTfpi	accgatttgataccctggaaga	acacgaatcgttcacactgctt	189
mTm4sf4	tttcggatgaggtctggtactt	tgaagcatttaggacccttgtt	240
mTnfsf10	ttgagaacctttcaggacacca	ggcctaaggtctttccatcctt	169
For pcDNA3.1			
mMeg3-cDNA-F-Bam	gccggatccaagacgaagagctggaatagag		1751 and 1890
mMeg3-cDNA-R-Eco		gccgaattcgaaatttattgaaagcaccatgag	
For pGL4.10			
mMeg3p-3F-KpnI	GCCGGTACCtatcaagatagtccgtcagaatc		682 (-560 to +122)
mMeg3p-1R-HindIII		GCCAAGCTTtcaggcaaaggatggctaac	
Bisulfite seq			
mMeg3p-1	ttccggccaatcacttttag	tcaggcaaaggatggctaac	237 (-115 to +122)
mH19-DMR	AGGTTGGAACACTTGTGTTTCTGGAG	AGCATACTCCTATATATCGTGGCCCA	160
MSP			
mMeg3p-1	ttccggccaatcacttttag	tcaggcaaaggatggctaac	237 (-115 to +122)
mMeg3p-MF	AACGCATTACAACAAAATACGCTAACG	tcaggcaaaggatggctaac	210 (-88 to +122)
mMeg3p-UF	AACACATTACAACAAAATACACTAACA	tcaggcaaaggatggctaac	210 (-88 to +122)

PRIMER	FORWARD	REVERSE	PRODUCT (bp)
HUMAN			
RT-PCR			
HcMET-2	caaggttgctgattttggtcttg	agcacgccaaaggaccacacatc	162
hGAPDH	gtgtgaaccatgagaagtatgac	cacagccttggcagcgccagtag	251
hGAPDH-2	gtgtgaaccatgagaagtatgac	ctgggtggcagtgatggcatggac	155
hINS	gaacgaggcttcttctacacacc	acagcattgttccacaatgccacg	156
hMEG3	gacatcatccgtccacctccttg	gtcagtgagtggctgctttgtatg	152
hMEN1	CTTTGAAGTAGCCAATGATG	CAGATGCCGTCGTAGAATCG	171
For pGL4.10			
hMEG3p-F1-KpnI	GCCGGTACCGAACCAGTCAGAAACGCACG		676 (-539 TO +140)
hMEG3p-R1-HindIII		GCCAAGCTTCCTTTTGCACATCCTTTGCG	
Bisulfite seq and MSP			
hMEG3p	ttccggccaatcacttttag	CCTTTTGCACATCCTTTGCG	251 (-110 to +140)
hMEGp3-MF	CATTGCAGCAGAATGCGCTGAC	CCTTTTGCACATCCTTTGCG	220 (-80 to +140)
hMEG3p-UF	TATTGTAGTAGAATGTGTTGAT	CCTTTTGCACATCCTTTGCG	220 (-80 to +140)