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Supplemental Information

A novel regulatory mechanism network mediated by lncRNA *TUG1* that induces the impairment of spiral artery remodeling in preeclampsia

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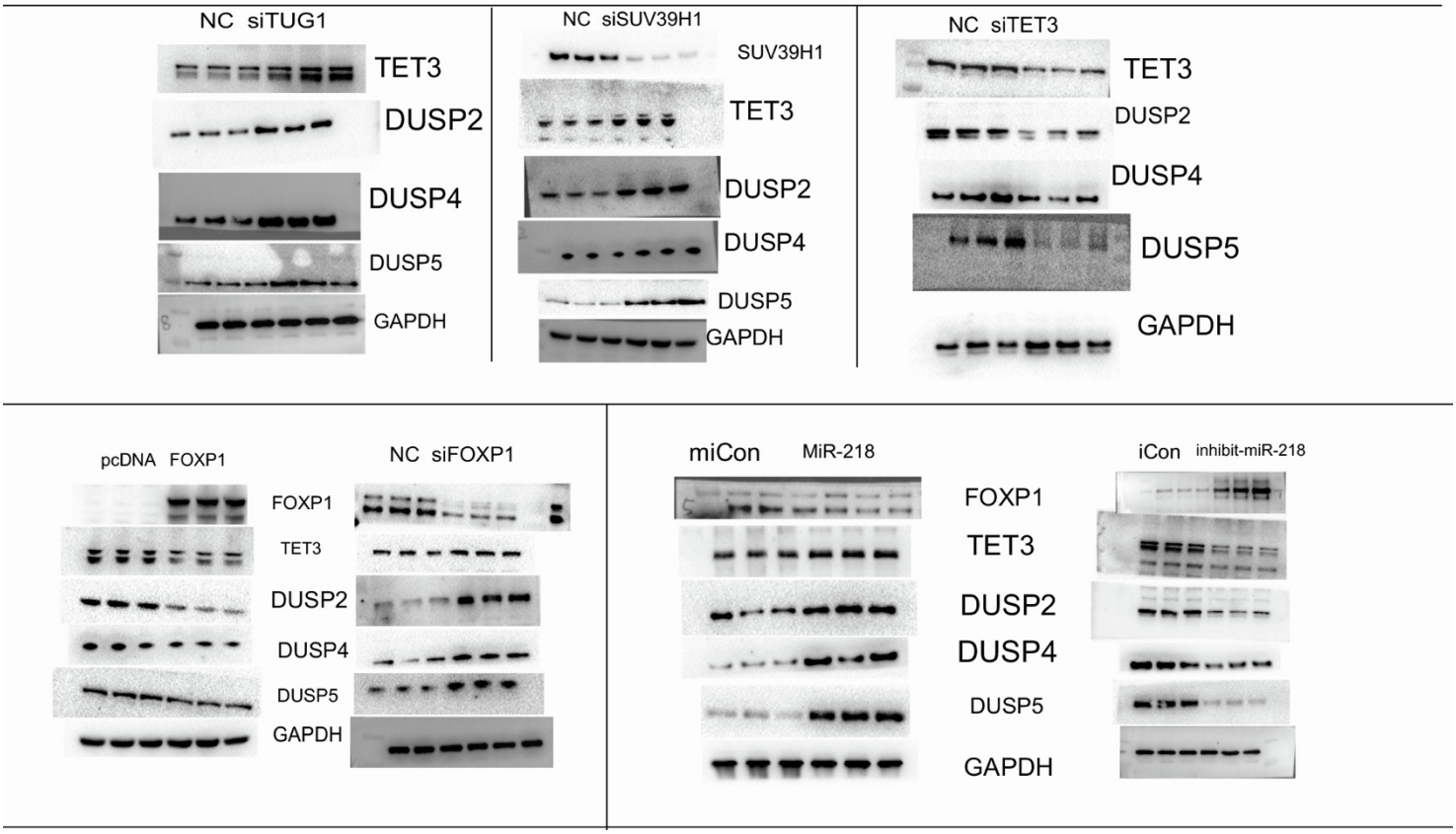
Supplementary file 1：The list of PCR / ChIP-PCR primers and siRNAs sequences

Real-time PCR primer sequences (human)		
Gene	Forward Primer	Reverse Primer
GAPDH	5’ - CTTTGTCAAGCTCATTTCCTGG -3’	5’ - TCTTCCTCTTGTGCTCTTGC -3’
ACTB	5’- ATCAAGATCATTGCTCCTCCTGAG-3’	5’- CTGCTTGCTGATCCACATCTG-3’
TET1	5’ - GCAGCGTACAGGCCACCACT -3’	5’ - AGCCGGTCGGCCATTGGAAG -3’
TET2	5’ - TTCGCAGAAGCAGCAGTGAAGAG-3’	5’ - AGCCAGAGACAGCGGGATTCCTT-3’
TET3	5’ -GACGAGAACATCGGCGGCGT -3’	5’ - GTGGCAGCGGTTGGGCTTCT-3’
TUG1	5’ -TAGCAGTTCCCCAATCCTTG -3’	5’ -CACAAATTCCCATCATTCCC -3’
DUSP1	5′ - AGTACCCCACTCTACGATCAGG -3’	5′ - GAAGCGTGATACGCACTGC -3′
DUSP2	5’ - TACTTCCTGCGAGGAGGCTT -3’	5’ - TAGACAGGAGCCCTGGAGTC -3’
DUSP4	5’ - AGTGGAAGATAACCACAAGG -3’	5′ - GCTTAACGAACTCGAAGG -3′
DUSP5	5′ -TGTCGTCCTCACCTCGCTA -3’	5′ - GGGCTCTCTCACTCTCAATCTTC -3′
DUSP6	5′ - GATCACTGGAGCCAAAAC -3’	5′ - CAAGCAATGTACCAAGACAC -3′
DUSP7	5’-CCAAGAAGTGTGGTGTCCTG -3’	5’-ACAAAGTCGTAGGCGTCGTT-3’
DUSP9	5’- CAGCCGTTCTGTCACCGTC -3’	5’- CAAGCTGCGCTCAAAGTCC -3’
DUSP10	5′ - TTTGAAGAGGCTTTTGAGTT -3’	5′ - GGGAGATAATTGGTCGTTT -3′
DUSP12	5’ - gcagccaggattgtatttcg-3’	5’ - agtaggtccgtctcgggttt -3’
DUSP13	5’ - ATCTTGCCCTTCCTGTTCTT-3’	5’ - CTTCCCACACTGGTGAGCTT-3’
FOXP1	5’ - GTTGCAGTCCTGTGGCATTA-3’	5’ - TGACCGCCGCACTCTAGTAA -3’
CHIP-PCR primer sequences (human)		
TUG1 CHIP primer-1	5’-CATGAACACTGTATGTTTCTGTGGA-3’	5’-CTGGCAGATTGCTCACATCC-3’
TET3 CHIP primer-1	5’-AGCCTCCTTATTTCGCACCC-3’	5’-TTGCTAGAGGAGGAAGCCCT-3’
TET3 CHIP primer-2	5’-CTGAGCGCAATGTGAATGGG-3’	5’-GCGCCCAAGATAAAACAGCC-3’
TET3 CHIP primer-3	5’-CTCACCAATGCAGGTCCACA-3’	5’-ACACTGTTGGATGCTGCTGA-3’
DUSP2 CHIP primer-1	5’- TGACACCACTCCCCCATCTC-3’	5’- CAGCACCTGGGTCTCAAACT-3’
DUSP2 CHIP primer-2	5’ - GTATGTCCCGGTGTGTCCTC-3’	5’- ACCCAGTCTGCAAGGGAGAT-3’
DUSP4 CHIP primer-1	5’ - GTCCCTTCTTAGCTCTCGCC-3’	5’- CATCTCCCCGACTCCAGCTA-3’
DUSP4 CHIP primer-2	5’- GGCGTCCCTTCTTAGCTCTC-3’	5’- GACTCCTTCCCGTGCCAATA-3’
DUSP5 CHIP primer-1	5’- CCGGCCAAAGACTAAGACCC-3’	5’- GCGTACTGGAGGTATGTGGG-3’
DUSP5 CHIP primer-2	5’- CGCGGAAGAGAGAACGAAGA-3’	5’- ATGTGAATGAGTGCCGTCCG-3’
U6	5′ - CTCGCTTCGGCAGCACA -3’	5′ - AACGCTTCACGAATTTGCGT -3′
Ago2	5’ - CCCGCATCATCTTCTACCGC -3’	5’ - GCTTGTCCCCCGCTCGTT -3’
hsa-miR-218-5p inhibitor (human		
sense 5’ - UUGUGCUUGAUCUAACCAUGU -3’		
antisense 5’ - ACAUGGUUAGAUCAAGCACAA -3’		
TUG1 siRNA sequence (human)		
1# sense 5’ -UAGAAUUGUUCUCUGGCUAUAUCCC-3’ , antisense 5’ - GGGAUAUAGCCAGAGAACAAUUCUA-3’		
2# sense 5’ -GCUUGGCUUCUAUUCUGAAUCCUUU-3’ ,		

antisense 5’ - AAAGGAUUCAGAAUAGAAGCCAAGC-3’
3# sense 5’ -CAGCUGUUACCAUUCAACUUCUUAA-3’ , antisense 5’ -UUAAGAAGUUGAAUGGUAACAGCUG-3’
TET3 siRNA sequence (human)
1# sense 5’ -CAGCAACUCCUAGAACUGAtt-3’ , antisense 5’ - UCAGUUCUAGGAGUUGCUGga -3’
2# sense 5’ - CGAUUGCGUCGAACAAAUAtt-3’ , antisense 5’ - UAUUUGUUCGACGCAAUCGca -3’
TET1 siRNA sequence (human)
1# sense 5’ - CCCAAGUCAUGCAGCCCUAtt-3’ , antisense 5’ -UAGGGCUGCAUGACUUGGGcg -3’
2# sense 5’ - GCUAUACGCUUAAUUACCAtt -3’ , antisense 5’ -UGGUAAUUAAGCGUAUAGCat -3
TET2 siRNA sequence (human)
1# sense 5’ - CCCAAUCUCUCCAAUCAAAtt-3’ , antisense 5’ - UUUGAUUGGAGAGAUUGGGtt
2# sense 5’ - GAGUUGUCCUGUGAGAUCAtt-3’ , antisense 5’ -UGAUCUCACAGGACAACUCat -3’
SAHH siRNA sequence (human)
1# sense 5’ - CAAGCUAACUGAGAAGCAAtt-3’ , antisense 5’ - UUGCUUCUCAGUUAGCUUGgt -3’
FXOP1 siRNA sequence (human)
1# sense 5’ - CTCAGTCCACACTCCCAAA -3’
2# sense 5’ - CCACAGAGCTTACCTCATA -3’
3# sense 5’ - CTGGTTCACACGAATGTTT -3’
SUV39H1 siRNA sequence (human)
1# sense 5’ - GCATCACTGTAGAGAATGA -3’
2# sense 5’ - GGGTCCGTATTGAATGCAA -3’
3# sense 5’ - GGGCCTTCGTGTACATCAA -3’

Supplemental file 2: Uncut western gel imagines.

Uncut Western gel images



Supplemental file 3: The binding sites of TUG1 with FOXP1.

TUG1 promoter region（2000bp）

Binding to FOXP1 Prediction

>NC_000022.11:30967211-30969211 Homo sapiens chromosome 22, GRCh38.p12 Primary Assembly

（-2000）

AGAACCATGAACACTGTATGTTTCTGTGGAAATTTTGGGGAAAGACTATCTAAATA（-1943）GAGATATAAACAAGT（-1929）

TGTGCCGTTTCCTGTTTAGCATATACAAATAATATTGGAT

GTGAGCAATCTGCCAGGAAGATAATGCCAACTTCTGAAAAAATAAAAATTTTCTTCTTGAA

AGTTGTCATTTAAATGTCTTCTGTTTTCTTGTT（-1796）AGAAAGTAAACACTT（-1782）

GTTATAAACCTAACTGTTTAGGACGTTGTCACTACAGAGGGGAAACGGTTGATACTAAA

ATAGTCCAACATTTGGCGATCCAATAGAGCAGTATTTGTTGGATTCCTATAAACACTTGAA
TGAAACAACTCAACATATTGACTTCAAATAACTAAAGTTTGCATTTAGAAAATTTTG（-
1604）ATCCAATAAACAAAA（-1590）ATCAGTGATCTCAGTTCTTCAATACAGAGCT
CAAGATATCCAGTGACACATTTCACTCCAGTGGGATACATCTCAAAAAGTGAAAAAGCAA
AATTTTCTGGGGAAACGATTTCCATATAATATCAAGGAACGAGTTACTGGTTACCTCAGT
GGCACCTAGAGGATACTTACGAATTTGTGTGCAGATATTCAAAGGTTAGCTGAGCTCGAT
TCAGACTGCTGTAATTTGTGAAAGCCATGACTGCAATAAGGTCTCCAGCCCTTCACCCG
CTAACTGGGAAATATAACCTTATAATGATATCGATTTCCCAGGATTCTGTCCAGTAAAGCT
TCAGTAAGTCTGTGCTCCTTAATGACAGTTAAAGTAACCTAGTAGCTATCCAAAATATATG
CAGAGATGTTTAAAACTACAATTTCTTCAAGTGTTTTTTTTTAATCTTCTCAATGATTTATG
ATGTAATATTTTGGAAGGAACTATGTCATAAATCTGTAGCTTTAAGGAGCTTTTAGCATTA
AGTTGCGATAGCTCAATTCAGACAATCTGAGTCTCGTGTGGATGGTCCTGAAGGAGATG
GTCCTTGAGTGGGTGGTTTATGACTGCTCCATCTTCATGTTCTTCCAGCCTTTTTTGGTTC
CCCGAAATTTCCTGTCAGGAAATTTACCAACGCGGAAATTTACCCACTTCTGTCAGAAAA
CTGATCAGCCATGTCTTCGCCACACGTGATGCCAGGATTCTTAAACAGGCCCAAGACCG
GAGACCAAGATGTCGACGGAGTGTTATATGGCGCAAGTTGCAGCTTCACCACCTCCCAA
GTGAAAAAGCTCCTAGGCACTCACGATTTCTTTTTTCAAAGCCGGACCAAACCCAGTTCA
GGGTGGTGGTCAGATTACCTCACACTTGGCCCACATAAACACCTCACAAATGACCCGCC
CACAGCACCTCGGGCAGGCCGCGCCCCGCCCCCACGTCCCTCAGACAACAGCCTCAG
CCTCCCAGGCCCTTCCCGGGCCTCGGTCCCGTGGCCGCCTCCCCACGAAGAGGAGCTA
CTCCCGGCTTCCAAGGACCGGATCGAGGGCAGTGGCGAGCGCACCACCTGACCGGGC
ACTACCCGGATCTCACAACTGCCTTTGTCCCTCCCCGCGGAATTGGAACCCAACAGCCG
CAGAGCGCTCTTGGAACCATGTGCTGCTGCCGCCTCCGCCGCCGCCGCCGCTGCCTCC
GCCGCCGCCCGCGCGCCGCCCGCGCGCCGCGCTAGTTGAGATGGTGACAGGATTCAG
CAACACGAAATTCGGCGTTTAGAGCTGCTCTGCCGCCGCCGTCACAGACACCACAGCC
ACCGCTTTGTCTCCGATAGTGCACACAGCCCCGGCCACCTCGCACACTCCCCGACGCC
CCAAGCCCCCGAGGCCTCCGCGGAGCATTCAGCCAATCACAAAGCTGCAGCTCCTCCC
GCCCCCGCCCTGATGTACAGCCTCCTGATTGGCGGGCTGCGTGTCCCCATGTGACCGG
ATCTTGGTTGGCCGGTCCCGCCCCTGTCACGTGACAGCGTGCGCCTCTCTT （-1）

Yellow：The position of the representative locus in the promoter region.
Red：The corresponding base sequence site.

6 putative sites were predicted with these settings (80%) in sequence named NC_000022.11:30967211-30969211

Model ID	Model name	Score	Relative score	Start	End	Strand	predicted site sequence
MA0481.1	FOXP1	10.085	0.836492339754999	57	71	1	GAGATATAAACAAGT
MA0481.1	FOXP1	14.567	0.909936734368791	189	203	-1	AACAAGAAAACAGAA
MA0481.1	FOXP1	13.030	0.884750649022651	204	218	1	AGAAAGTAAACACTT
MA0481.1	FOXP1	13.467	0.891911559430109	396	410	1	ATCCAATAAACAAAA
MA0481.1	FOXP1	10.182	0.838081832454137	861	875	-1	TAAAAAAAAACACTT
MA0481.1	FOXP1	8.556	0.811437346590232	863	877	-1	ATTAAAAAAAAACAC

Comment: This type of analysis has a high sensitivity but abysmal selectivity. In other words: while true functional will be detected in most cases, most predictions will correspond to sites bound in vitro but with no function in vivo. A number of additional contraints of the analysis can improve the prediction; phylogenetic footprinting is the most common. We recommend using the ConSite service, which uses the JASPAR datasets.

The review Nat Rev Genet. 2004 Apr;5(4):276-87 gives a comprehensive overview of transcription binding site prediction

Supplemental file 4: Detailed process of methyltarget sequencing.

Acknowledgement
We acknowledged the technical support from the Shanghai Genesky Biotechnology Company (Shanghai, China).

Detailed process of methyltarget sequencing.

CpG islands selected

CpG islands located in the proximal promoter of DUSP2, DUSP4 and DUSP5 were selected for measurement according to the following criteria: (1) 200 bp minimum length; (2) 50% or higher GC content; (3) 0.60 or higher ratio of observed/expected dinucleotides CpG.

Bisulfite conversion and multiplex amplification

DNA methylation level was analysis by MethylTarget® (Genesky Biotechnologies Inc., Shanghai, China), an NGS-based multiple Targeted CpG methylation analysis method. Specifically, the genomic regions of interest were analyzed and transformed to bisulfite-converted sequences by geneCpG software. PCR primer sets were designed with the Methylation Primer software from bisulfate converted DNA.

Genomic DNA (400ng) was subjected to sodium bisulfite treatment using EZ DNA Methylation™-GOLD Kit (Zymo Research) according to manufacturer's protocols. Multiplex PCR was performed with optimized primer sets combination. A 20 µl PCR reaction mixture was prepared for each reaction and included 1x reaction buffer (Takara), 3 mM Mg^{2+}, 0.2 mM dNTP, 0.1 µM of each primer, 1U HotStarTaq polymerase (Takara) and 2 µl template DNA. The cycling program was 95ºC for 2 min; 11 cycles of 94ºC for 20 s, 63ºC for 40s with a decreasing temperature step of 0.5ºC per cycle, 72ºC for 1 min; then followed by 24 cycles of 94ºC for 20 s, 65ºC for 30 s, 72ºC for 1 min; 72ºC for 2 min.

Index PCR

PCR amplicons were diluted and amplified using indexed primers. Specifically, a 20 µl mixture was prepared for each reaction and included 1x reaction buffer (NEB Q5™), 0.3 mM dNTP, 0.3 µM of F primer, 0.3 µM of index primer, 1 U Q5™ DNA polymerase (NEB) and 1 µL diluted template. The cycling program was 98ºC for 30 s; 11 cycles of 98ºC for 10 s, 65ºC for 30 s, 72ºC for 30 s; 72ºC for 5 min. PCR amplicons (170bp-270bp) were separated by agarose electrophoresis and purified using QIAquick Gel Extraction kit (QIAGEN).

Sequencing

Libraries from different samples were quantified and pooled together, followed by sequencing on the Illumina MiSeq platform according to manufacturer's protocols. Sequencing was performed with a 2x150bp paired-end mode.

Data analysis

FLASH (Fast Length Adjustment of SHort reads) is an accurate and fast tool, which was used to merge paired-end reads[1]. Fastq to fasta format step was then processed using the Fastx toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html). Reads with fasta format were mapped to targeted Bisulfite Genome (hg19) by Blast [2]. Unmapped reads were filtered and mapped reads with coverage greater than 90% and identity greater than 95% were kept as effective reads and were used for following statistics. Sequencing depth for each amplicon per sample was calculated by blasting the effective reads against the targeted genome region. Reads less than 10-fold were removed and overall sequencing depth for each sample were evaluated. Methylation and haplotype were analyzed using Perl script. Statistics were performed by t-test and ANOVA.

1 Magoc T , Salzberg S L . FLASH: fast length adjustment of short reads to improve genome assemblies[J]. Bioinformatics, 2011, 27(21):2957-2963.
2 Camacho C , Coulouris G , Avagyan V , et al. BLAST+: architecture and applications[J]. BMC

Bioinformatics, 2009, 10(1):421-0.

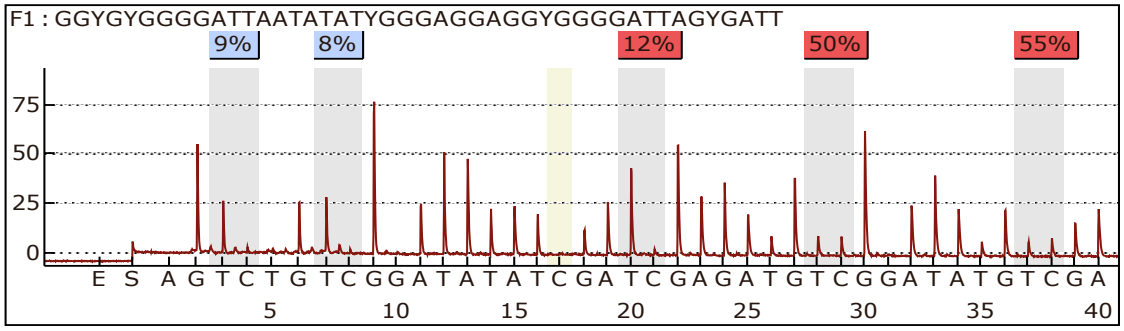
Supplemental file 5: Promoter demethylation levels of DUSP2, DUSP4 and DUSP5 genes detected by pyrosequencing.

DUSP2

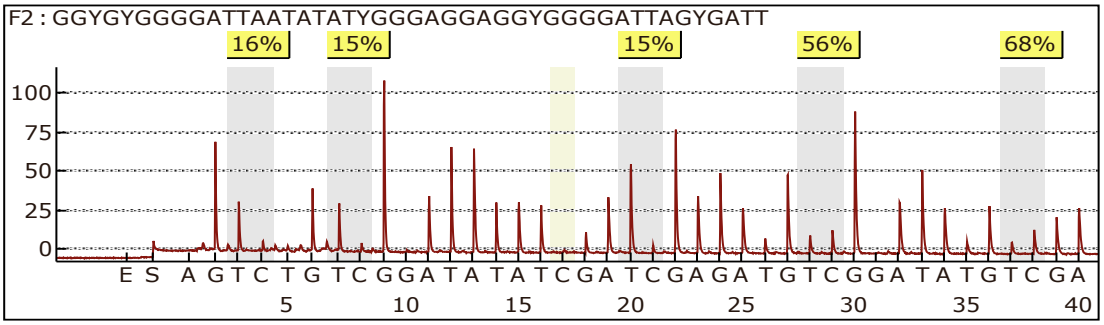
Assay Name: PM403-1FS

Sample ID: 1-1

siCon



siTET3

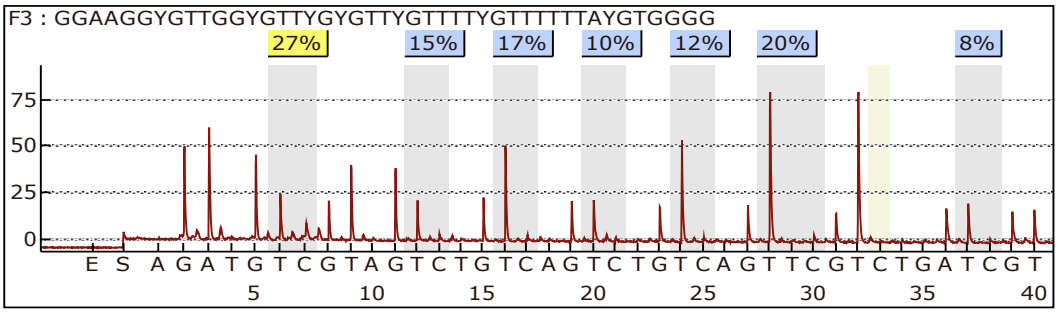


DUSP2

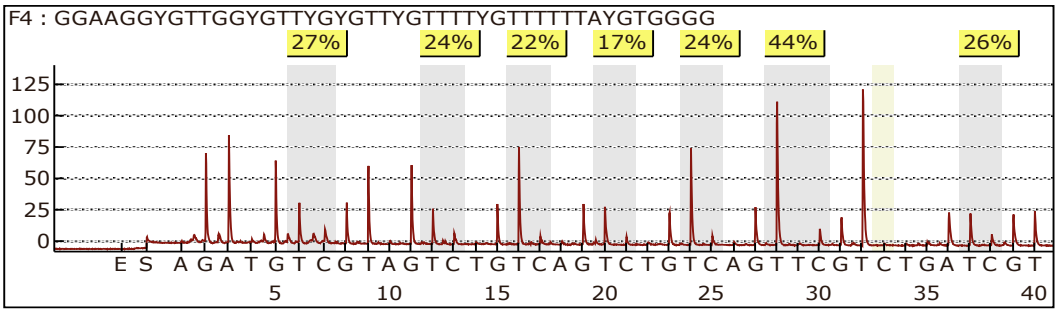
Assay Name: PM403-2FS

Sample ID: 2-1

siCon



siTET3

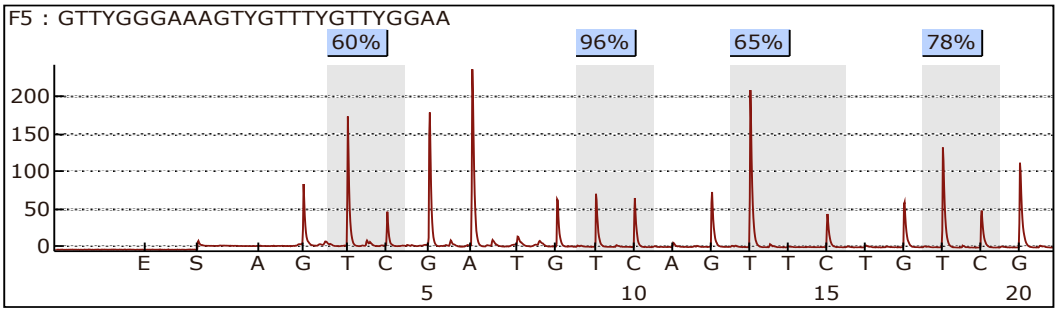


DUSP4

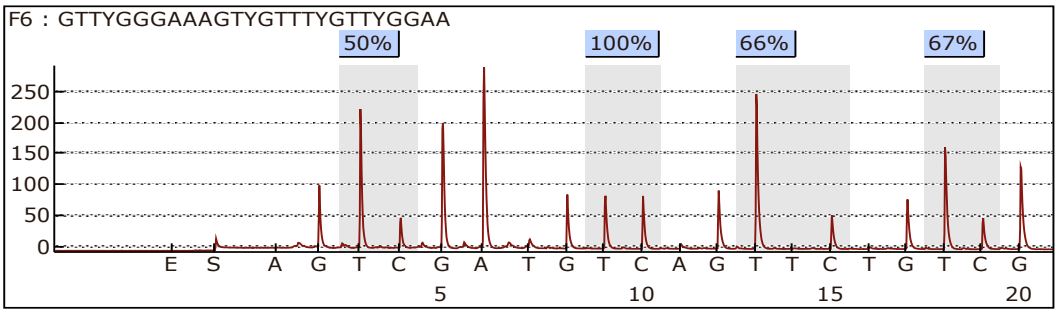
Assay Name: PM43-3FS

Sample ID: 3-1

siCon



siTET3

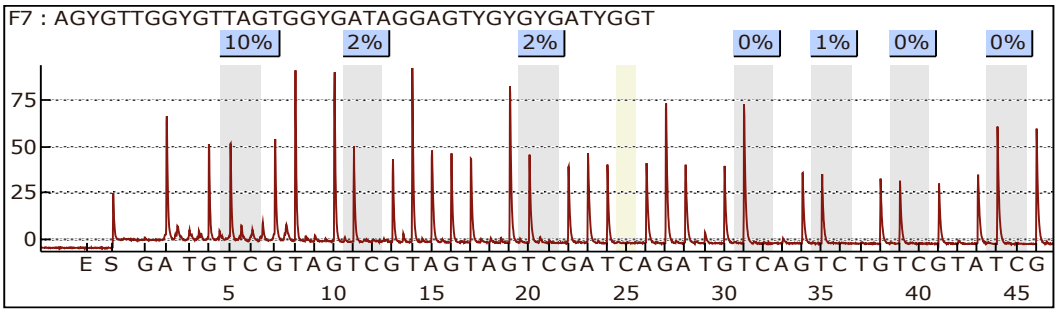


DUSP4

Assay Name: PM403-4FS

Sample ID: 4-1

siCon



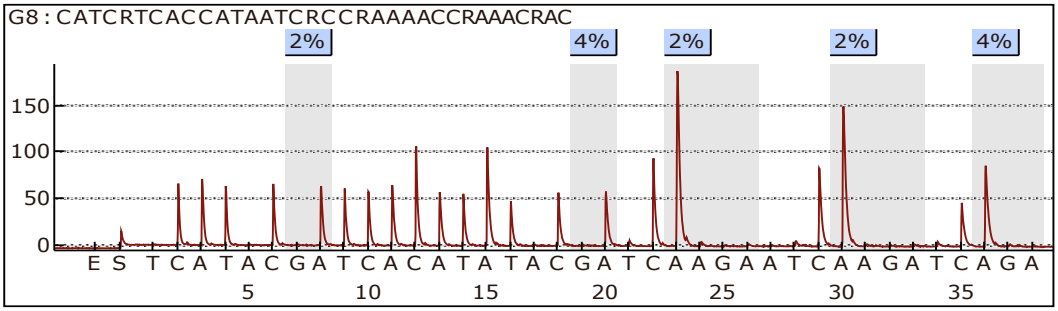
siTET3

DUSP4

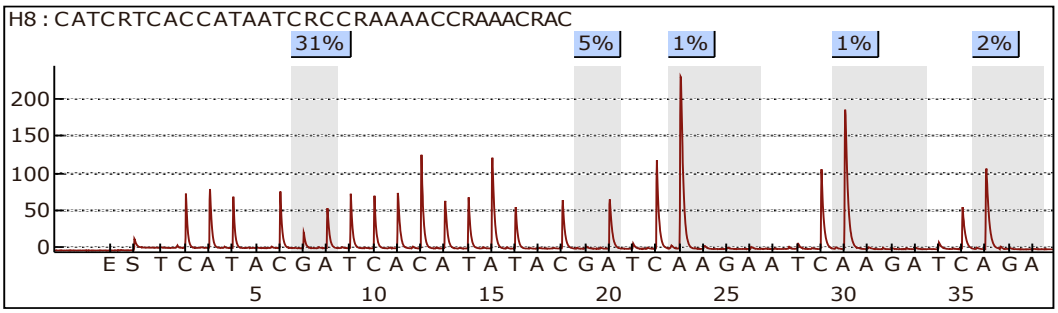
Assay Name: PM403-5FS

Sample ID: 1

siCon



siTET3

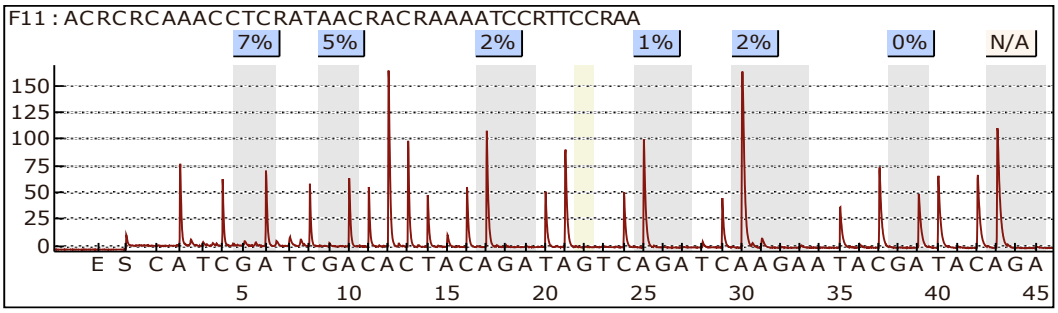


DUSP5

Assay Name: PM403-6RS

Sample ID: 6-1

siCon



siTET3

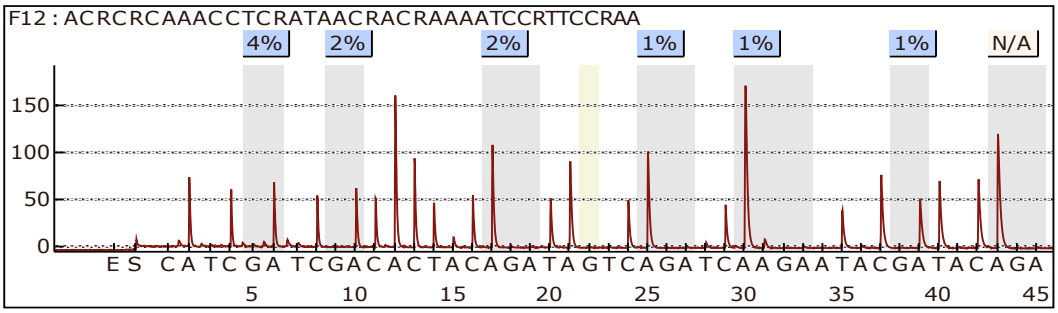


Figure legends

HTR-8/SVneo cells were transfected with siTET3 or siCon. After 48-h post-transfection, Promoter demethylation levels of DUSP2, DUSP4 and DUSP5 genes detected by pyrosequencing. The methylation levels of the CpG sites in the *DUSP2*, *DUSP4*, and *DUSP5* genes are shown.

Supplemental file 6: Single-nucleotide resolution genome-wide DNA methylation profiling (GEO: GSE117190) after TET3-knockdown in primary human leiomyoma cells.

DUSP2

（+1）

TTAACCCGGGCCGCCGCGGAGGGCGCCCGGAGTCGACCGCTCGGGCAGCGCCACCGCCACGAGAGCCCG
GGACGCGGGAAAGACCGAAAGGAAGAGGAAGAGGCACCGGTGGCCATGGGGCTGGAGGCGGC（10%-
23%）GCGCGAGCTGGAGTGC（0%-23%）GCGGC（0%-15%）
GCTGGGCACGCTGCTGCGGGATCCGCGGGAGGCGGAACGCA（+193）

DUSP4

（-578）AAACCTACGGGGCTGTCACGCGGGGAAGC（52%-87%）

GCGAAGGTGCCAAGGGATGAAAGCTCAAACCCGAGCCCTGGCCTCCTCAGC（11%-33%）

CGGCTATTTCCTTTGGCGCCGCCCGCCTAGCGGCGGGGTGCAGCGGCGGCACAGGTGCCGGTGTCGGGC

TGGAGGCGCGGCGCAGGCTGGGCCCGCGGGTAGACGGCGAAAGGC（77%-91%）

GCCGCGCGCTCCATTCACAAAGTCCGGGCGCTGCCCGCCGCTGGCGGCGGGTC（0%-11%）

GGAGGCCGCCTCCCTCTTCCTCTCGGCCTCGGTTTTATGAATGGGCCTGATG（-280）

DUSP5

（-671）

TCACCGCCGACCCCCAACCCCCGTTTTTACTTTTACAAACTTTATTATGAAAAATATCAAACATACAGA
AAAGTGGAGAAAACAGTATAACGAATCCGGCCACCCATCACCCAGCCTCAGCGATGACCAACCCGCGCC
TGACCTCTCCAGTCATCTATACCCCACCCCTCGAGGCAAATCTCC（0%-18%）GAC（0%-12%）
GTCCTCTCCTTACACGC（0% -10% ）GCAGACCTCGGTAGCGACGGGAATCC（0%-9%）
GTTCCGGACTCCTCTGGGGCCCTGGCCGGCTCCCTCGGCCCTGCTC（-397）

Figure legends

Sequences of promoter region of DUSP2, DUSP4, and DUSP5. The differentially methylated CpGs are highlighted in red. Purple numbers mark percentage of methylation in siCon (left) or siTET3 (right) transfected leiomyoma cells as determined by genome-wide single-nucleotide-resolution methylation analysis. Blue numbers indicate positions relative to the transcriptional start sites (+1). ChIP-qPCR amplified regions are underlined.

Supplemental file 7: Immunohistochemistry staining (IHC) using antibodies against FOXP1, TET3 and DUSP5 in rat model placenta.

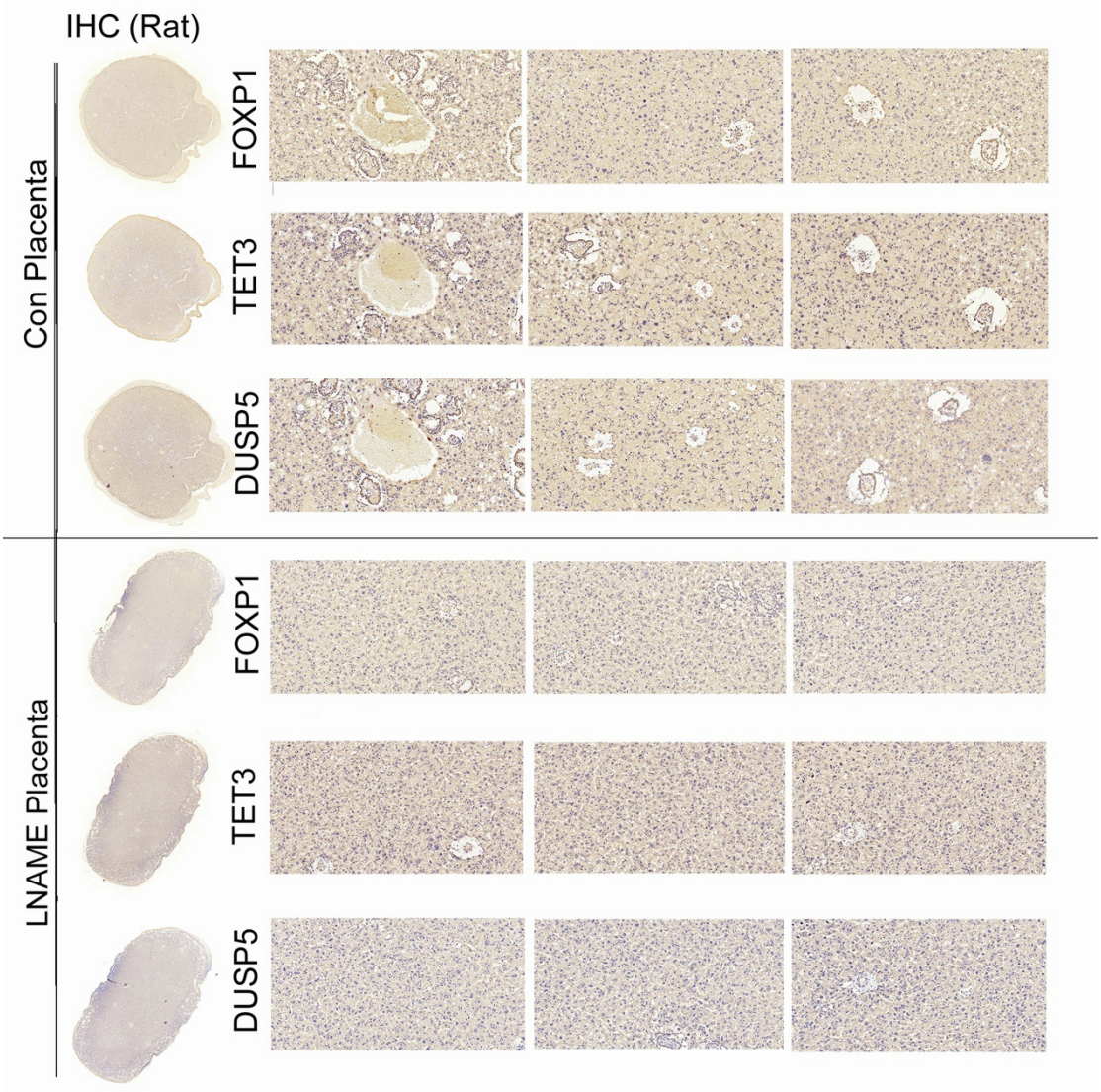


Figure legend:
Six images on the left show representative photomicrographs of hematoxylin-eosin-stained placenta sections of saline- or L-NAME-treated rats on GD 18.5. Eighteen images on the right show immunohistochemistry staining of placental tissues using antibodies against FOXP1, TET3, and DUSP5. Magnification, 20x; Scale bar, 100 μm. The abundance of FOXP1, TET3, and DUSP5protein was detected by immunohistochemistry

Supplemental file 8: EDITORIAL CERTIFICATE.

51runse

EDITORIAL CERTIFICATE

This is to certify that the paper titled:

A novel regulatory network mediated by lncRNA TUG1 that induces the impairment of spiral artery remodeling in preeclampsia

has been edited by professional English editors at 51runse, for proper English language, grammar, spelling, punctuation and academic style, while the intent of the authors' message was not altered in any way during the editing process. Documents receiving this certification should be English-ready for publication, with the assumption that the edited changes have been accepted by the receivers.

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Certificate Number: O202107022047223016

Chief Editor: David C. Gilbert

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Supplemental file 9: Detailed process of RNA pulldown assays.

RNA pulldown assay

1. Plasmid templates Linearization

Prepare reagent supplies： Sense RNA Plasmid、Antisense RNA Plasmid、Restriction Enzyme Digestion、Ultrapure water、Buffer

1. Thaw the frozen reagents

2. Assemble transcription reaction at room temp

Component	Amount
Ultrapure water	to 50 μL
Buffer	5 μL
Plasmid	1 μg
Restriction Enzyme Digestion	1 μL

3. Mix thoroughly，Incubate at 37°C, 2 hr

4. Agarose gel electrophoresis confirmed adequate digestion

5. Expand the optimized reaction system

6. Heat inactivation 65°C for 20 min

7. Proteinase K、0.5% SDS for 30 min at 50°C，follow this with phenol/chloroform extraction (using an equal volume).

8. 2 volumes of ethanol precipitation. Mix well and chill at –20°C for at least 15 min.

9. Then pellet the DNA for 15 min in a microcentrifuge at top speed. Remove the supernatant, Resuspend in dH2O or TE buffer at a concentration of 0.5–1 µg/µL.

2. RNA Transcription in Vitro

Prepare reagent supplies：mMESSAGE mMACHINE® Kit AM1344、DNA template、EP tube

1. Thaw the frozen reagents
   Place the RNA Polymerase Enzyme Mix on ice, it is stored in glycerol and will not
   be frozen at –20°C.
   Vortex the 10X Reaction Buffer and the 2X NTP/CAP until they are completely in solution. Once thawed, store the ribonucleotides (2X NTP/CAP) on ice, but keep the 10X Reaction Buffer at room temperature while assembling the reaction.
   All reagents should be microfuged briefly before opening to prevent loss and/or contamination of material that may be present around the rim of the tube.

2. Assemble transcription reaction at room temp
   The following amounts are for a single 20 µL reaction. Reactions may be scaled up
   or down if desired

Component	Amount
Nuclease-free Water	to 20 µL
2X NTP/CAP	10 µL
10X Reaction Buffer	2 µL
(optional) [α-32P]UTP as a tracer	(1 µL)
linear template DNA†	0.1–1 µg
Enzyme Mix	2 µL

3. Mix thoroughly

Gently flick the tube or pipette the mixture up and down gently , and then microfuge tube briefly to collect the reaction mixture at the bottom of the tube.

4. Incubate at 37°C, 2 hr
Typically，80% yield is achieved after a 1 hr incubation. For maximum yield, we recommend a 2 hr incubation. Since SP6 reactions are somewhat slower than T3 and T7 reactions, they especially may benefit from the second hour of incubation.

5. (optional) Add 1 µL TURBO DNase, mix well and incubate 15 min at 37°C
This DNase treatment removes the template DNA. For many applications it may not be necessary because the template DNA will be present at a very low concentration relative to the RNA.

a. Add 1 µL TURBO DNase, and mix well.
b. Incubate at 37°C for 15 min.

3. Purification for RNA Transcription Reactions

Prepare reagent supplies：MEGAclear™ Kit AM1908

1. Bring the RNA sample to 100 µL with Elution Solution. Mix gently but thoroughly

2. Add 350 µL of Binding Solution Concentrate to the sample. Mix gently by pipetting.

3. Add 250 µL of 100% ethanol to the sample. Mix gently by pipetting.

4. Pipet the RNA mixture onto the Filter Cartridge. Centrifuge for ~15 sec to 1 min, or until
the mixture has passed through the filter. Centrifuge at RCF 10,000–15,000 × g (typically 10,000–14,000 rpm).

5. Wash with 2 × 500 µL Wash Solution.

6. Elute RNA from the filter with 50 µL Elution Solution using one of the methods
described below; they are equivalent in terms of RNA recovery.

a. Pre-heat 110 µL of Elution Solution per sample to 95° C.
b. Apply 50 µL of the pre-heated Elution Solution to the center of the Filter Cartridge, close the cap of the tube and centrifuge for 1 min at room temperature (RCF 10,000–15,000 x g) to elute the RNA.
c. To maximize RNA recovery , repeat this elution procedure with a second pre-

heated 50 µL aliquot of Elution Solution. Collect the eluate into the same Collection/Elution Tube.

7. (optional) Precipitate with 5 M Ammonium Acetate. To concentrate the RNA,
precipitate as follows:

a. Add 1:10 volume of 5 M Ammonium Acetate (NH4Ac) to the purified RNA.
Note: If the sample was eluted with 100 µL Elution Solution as suggested,
this will be 10 µL of 5 M NH4Ac.
b. Add 2.5 volumes of 100% ethanol (275 µL if the RNA was eluted in 100 µL).
Mix well and incubate at –20°C for 30 min.
c. Microcentrifuge at top speed for 15 min at 4°C or room temperature (RT).
d. Carefully remove and discard the supernatant.
e. Wash the pellet with 500 µL 70% cold ethanol, centrifuge again and remove
the 70% ethanol.
f. To remove the last traces of ethanol, quickly re-spin the tube, and aspirate any residual fluid with a very fine tipped pipette, or with a syringe needle.
g. Air dry the pellet. Resuspend the pellet using the desired solution and volume.

4. Pierce RNA 3' End Desthiobiotinylation

Prepare reagent supplies： Pierce RNA 3' End Desthiobiotinylation Kit、1-50pmol of RNA for labeling、Heated mixer/chiller for incubation at 37°C/16°C、Chloroform:isoamyl alcohol (24:1)、Nuclease-free pipette tips and tubes、5M NaCl、Ultrapure water、100% ethanol, ice-cold、70% ethanol, ice-cold

1. Thaw all kit components except the PEG 30% and DMSO on ice. Thaw DMSO at room temperature and warm the PEG 30% at 37°C for 5-10 minutes until volume is fluid.

2. Adjust the heating block to 85°C.

3. Transfer 5µL of the Non-labeled RNA Control to a microcentrifuge tube. Heat the RNA for 3-5 minutes at 85°C. Place RNA immediately on ice.

Note: The RNA may require heating to relax the secondary structure. Also, heating the RNA in the presence of ~25% DMSO may increase efficiency for RNA with significant secondary structure.

4. Prepare the labeling reaction for the control system or test RNA by adding components in the order listed in Table 1.

Note: The last added reagent is PEG 30%. Carefully pipette the PEG 30% into the reaction mixture. Use a new pipette tip to mix the ligation reaction after the PEG 30% addition.

Component	Volume (µL)	Final Concentration
Nuclease-free Water	3	---
10X RNA Ligase Reaction Buffer	3	1X
RNase Inhibitor	1	40U
Non-labeled RNA Control or Test RNA	5	50pmol
Biotinylated Cytidine Bisphosphate	1	1nmol
T4 RNA Ligase	2	40U
PEG 30%	15	15%
Total	**30**	---

5. Incubate the reactions at 16°C for 2 hours for the control RNA. Ligation may require overnight incubation to increase efficiency.

6. Add 70µL of nuclease-free water to the ligation reaction.

7. Add 100µL of chloroform:isoamyl alcohol to each reaction to extract the RNA ligase. Vortex the mixture briefly, then centrifuge 2-3 minutes at high speed in a microcentrifuge to separate the phases. Carefully remove the top (aqueous) phase and transfer to a nuclease-free tube.

8. Add 10µL of 5M NaCl, 1µL of glycogen and 300µL of ice-cold 100% ethanol. Precipitate for ≥ 1 hour at -20°C.
9. Centrifuge at ≥ 13,000 × g for 15 minutes at 4°C. Carefully remove the supernatant, taking care not to disturb the pellet.

10. Wash the pellet with 300µL of ice-cold 70% ethanol. Carefully remove ethanol and air-dry the pellet (~5 minutes).

11. Resuspend the pellet in 20µL of nuclease-free water or buffer of choice.

5. Pierce Magnetic RNA-Protein Pull-Down

Prepare reagent supplies： Pierce Magnetic RNA-Protein Pull-Down Kit、Target RNA for labeling、Chloroform:isoamyl alcohol (24:1)、Ethanol, absolute、Cell Lysis Buffer (for preparation of cell lysate)、Magnetic separation stand

A. Pre-Washing Streptavidin Magnetic Beads (Optional)

1. Resuspend the beads in the original vial by gentle swirling or rotation.
2. Remove the amount to be treated and transfer to a nuclease-free tube.
3. Place tube on a magnetic stand to collect the beads against the sides of the tube.
4. Wash the beads twice with a 2X volume of 0.1M NaOH, 50mM NaCl (nuclease-free).
5. Wash the beads once in 100mM NaCl.
6. Continue with equilibration of magnetic beads for RNA capture (Section D).

B. Preparation of Cell Lysate

1.Cell lysates may be prepared using standard lysis buffers
2. Ensure the cell lysate protein concentration is greater than 2mg/mL, such that there is significant dilution into the Binding Reaction Buffer.

C. Binding of Labeled RNA to Streptavidin Magnetic Beads

Note: Use a range of 25-100pmol of RNA per 20-50µL of magnetic beads. The instructions below use a scale of 50pmol of RNA to 50µL of beads.

1. Add 50µL of streptavidin magnetic beads to a 1.5mL microcentrifuge tube.
2. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
3. Wash with an equal volume of 20mM Tris (pH 7.5). Resuspend beads by pipetting or vortexing.
4. Repeat Steps 2 and 3.
5. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
6. Add an equal volume of 1X RNA Capture Buffer. Resuspend beads by pipetting or vortexing.
7. Add 50pmol of labeled RNA to the beads. Mix gently by pipetting.
8. Incubate for 15-30 minutes at room temperature with agitation.

D. Binding of RNA-Binding Proteins to RNA

1. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
2. Wash with an equal volume of 20mM Tris (pH 7.5). Resuspend beads by pipetting or vortexing.
3. Repeat Steps 1 and 2.
4. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
5. Dilute 10X Protein-RNA Binding Buffer to 1X (i.e., 10µL into 90µL of ultrapure water for each reaction).
6. Add 100µL of 1X Protein-RNA Binding Buffer to the beads and mix well.
7. Prepare a Master Mix of RNA-Protein Binding Reaction (Table 2).

Reagent	Volume (µL) per 100µL reaction for control	Range
10X Protein-RNA Binding Buffer	10	5-20µL
50% glycerol	30	0-50µL
Lysate (protein conc. > 2mg/mL	1-30	20-200µg
Nuclease-free water	to 100	to 100µL

8. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
9. Add 100µL of Master Mix to the RNA-bound beads. Mix by pipetting or gentle vortexing.
10. Incubate 30-60 minutes at 4°C with agitation or rotation.

E. Washing and Elution of RNA-Binding Protein Complexes

1. Place the tube into a magnetic stand to collect the beads against the side of the tube. Transfer the supernatant to a tube for later analysis.
2. Wash with equal volume of 1X wash buffer (100µL).
3. Repeat Steps 1 and 2 two additional times. Save wash supernatants for analysis, if desired.
4. Place the tube into a magnetic stand to collect the beads against the side of the tube. Transfer the supernatant to a tube for later analysis.
5. Add 50µL of Elution Buffer to the beads and mix well by vortexing. Incubate 15-30 minutes at 37°C with agitation.
6. Place the tube into a magnetic stand to collect the beads against the side of the tube.
7. Remove supernatant for downstream analysis.

8. If the downstream application is Western blotting, add reducing sample buffer to samples to 1X.

F. Western Blot Analysis

Supplemental table 1: The detailed clinicopathological features of normal pregnancy and preeclampsia

Clinical characteristics of preeclamptic and normal pregnancies.

Variable	PE (N=64)	Normal (N=64)	*P* value[a] Normal vs P
Maternal age (year)	32±4.95375	34.2968±3.5307	p>0.05
Maternal weight (kg)	76.226±11.1376	74.0703±8.0602	p>0.05
Smoking	1	0	p>0.05
Systolic blood pressure (mm Hg)	162.6718±14.9443	116.1406±7.9758	p<0.01
Diastolic blood pressure (mm Hg)	105.9687±10.8173	71.5843±8.6279	p<0.01
Proteinuria (g/day)	>0.3g	<0.3g	p<0.05
Body weight of infant (g)	2291.9375±9089.06	3385.15±364.243	p<0.05
Gestational age (week)	34.06±3.231	38.375±0.9677	p<0.05

Supplemental table 2: Related antibody information and description.

GAPDH	5% nonfat milk	5% nonfat milk	1:2000	CMC TAG,AT0002	5% milk	1:3000		90min	
FOXP1	5% nonfat milk	5% nonfat milk	1:750	PROTEINTECH GROUP, 22051-1-AP	5% milk	Anti-Rabbit IgG, 1:3000		0.22um 250mA, 100min	Wash 3 times after 1st and 2nd Abs, 10 min each time
TET3	5% nonfat milk	5% nonfat milk	1:500	GENE Tex 121453	5% milk	Anti-Rabbit IgG, 1:3000		0.22um 250mA, 120min	Wash 3 times after 1st and 2nd Abs, 5 min each time
DUSP2	5% nonfat milk	5% nonfat milk	1:750	PROTEINTECH GROUP, 27327-1-AP	5% milk	Anti-Rabbit IgG, 1:3000		0.22um 250mA, 90min	Wash 3 times after 1st and 2nd Abs, 5 min each time
DUSP4	5% nonfat milk	5% nonfat milk	1:750	PROTEINTECH GROUP, 66349-1-Ig	5% milk	Anti-Mouse IgG 1:3000		0.22um 250mA, 90min	Wash 3 times after 1st and 2nd Abs, 5 min each time
DUSP5	5% nonfat milk	5% nonfat milk	1:500	abcam ab 200708	5% milk	Anti-Rabbit IgG, 1:3000		0.22um 250mA, 90min	Wash 3 times after 1st and 2nd Abs, 5 min each time
foxp2	5% nonfat milk	5% nonfat milk	1:750	PROTEINTECH GROUP, 20529-1-AP	5% milk	Anti-Rabbit IgG, 1:3000		0.22um 250mA, 100min	Wash 3 times after 1st and 2nd Abs, 5 min each time
TET1	5% nonfat milk	5% nonfat milk	1:500	abcam ab 191698	5% milk	Anti-Rabbit IgG, 1:3000		0.22um 250mA, 120min	Wash 3 times after 1st and 2nd Abs, 5 min each time
SUV39H1	5% nonfat milk	5% nonfat milk	1:500	abcam ab 12045	5% milk	Anti-Mouse IgG 1:3000		0.22um 250mA, 100min	Wash 3 times after 1st and 2nd Abs, 5 min each time

Supplemental table 3: Bioinformatics analysis (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) predicted the binding sites of miR-218 in the 3'-UTR of FOXP1

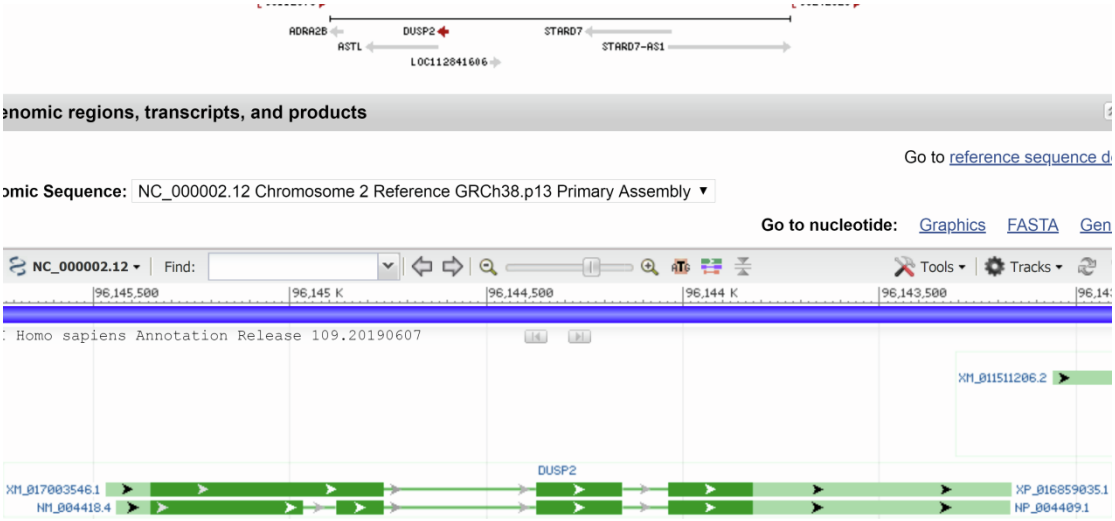
miRNA	MIMATid	Gene	EntrezID	RefseqID	miRWalk	Microt4	miRanda	mirbridge	miRDB	miRMap	miRNAMap
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001244808	1	1	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_032682	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264740	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264742	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001244813	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264731	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001244812	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264739	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264741	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264735	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264736	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264733	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001244815	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001244814	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001244816	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264737	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264738	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264732	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264729	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264734	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001244810	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	XM_005264730	1	0	0	0	0	1	0
hsa-miR-218-5p	MIMAT0000	FOXP1	27086	NM_001012505	0	0	0	0	0	1	0

Supplemental table 4: Target_Primer in methyltarget sequencing.

Target	Chr	Gene	mRNA	mRNA_Strand	TSS	TES	Start	End	Length	Target_Strand	Distance2TSS	PrimerF
DUSP2_04	chr2	DUSP2	NM_004418	-	96145440	96143168	96145482	96145675	194	+	-42	GGTTTTYGGGGTATATATAAGGGTAGA
DUSP4_18	chr8	DUSP4	NM_057158	-	29348805	29333061	29348363	29348097	267	-	442	TTGAGYGGGGTTAGTTTTGGTTA
DUSP5_21	chr10	DUSP5	NM_004419	+	1.1E+08	1.11E+08	1.1E+08	1.1E+08	259	+	-100	GTTYGAGGGGYGGGAAATA

Supplemental table 5: Pyrosequencing related primer data.

1. DUSP2



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/db_xref="MIM:603068"
ORIGIN

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61 gagagccCGg gaCGCGggaa agacCGaaag gaagaggaag aggcacCGgt ggccatgggg

NOTES: -2000bp+5 'UTR+some CDSsequences (ATG are marked in green), and methylation islands are marked with horizontal lines

Homo sapiens chromosome 2, GRCh38.p13 Primary Assembly

NCBI Reference Sequence: NC_000002.12

GenBank Graphics

>NC_000002.12:c96147468-96143166 Homo sapiens chromosome 2, GRCh38.p13 Primary Assembly

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CCTTCTGCACGCTCTGTAATTTGCCCCTGGAATAAGCATTAAGTCATTTGGAGGCCGATGCCAAAGATA
AGGGCTCATATCAGCCTTTGGGATCTGTGGCCCAGACTCCCTGGCTCTGTTGTGGGCTTTGCTGCCTCA
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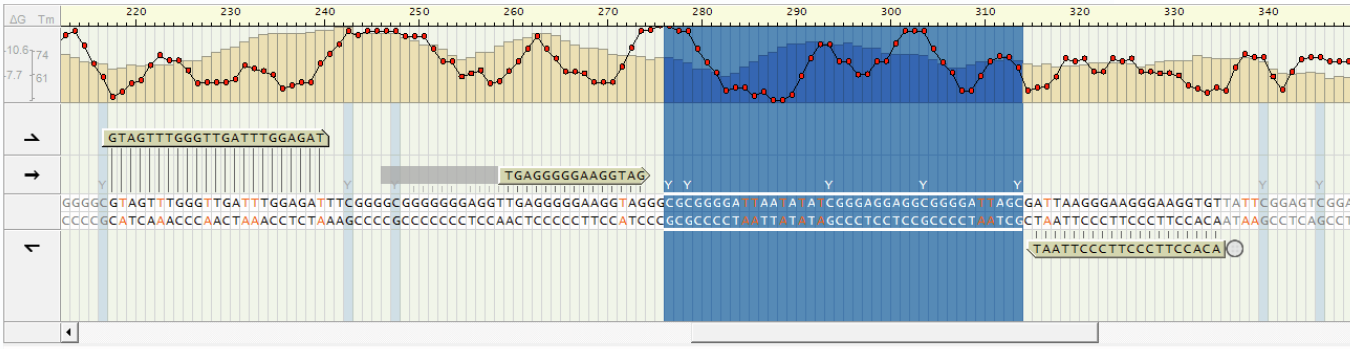
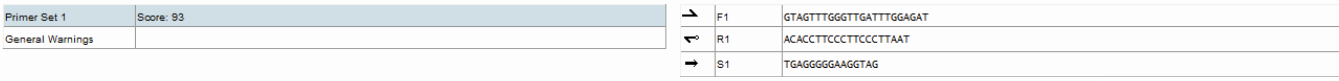
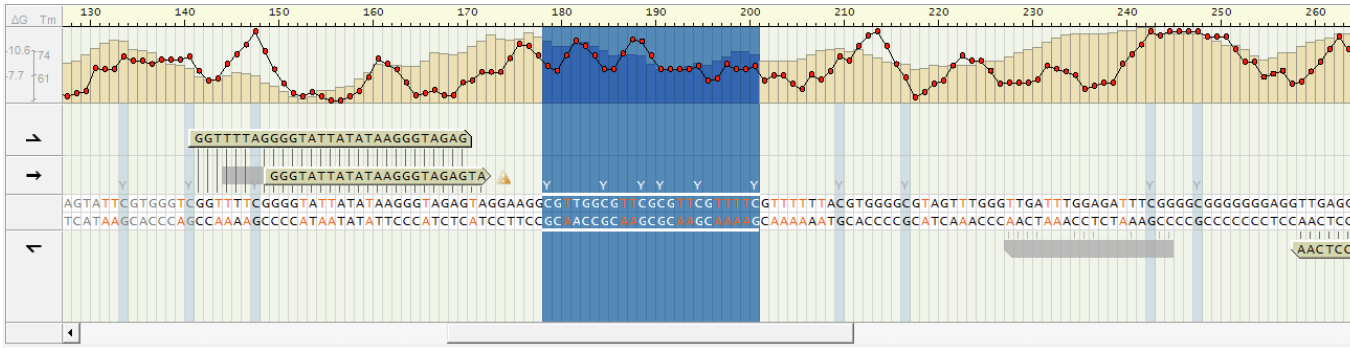


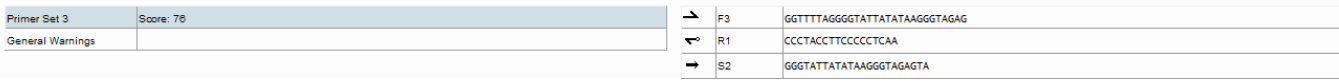


Primer Set 1	Score: 93
General Warnings	

F1	GTAGTTTGGGTTGATTTGGAGAT
R1	ACACCTTCCCTTCCCTTAAT
S1	TGAGGGGGAAGGTAG

	PCR Product	Forward PCR Primer, F1	Reverse PCR Primer, R1	Sequencing Primer, S1
Length, nt	118	23	20	15
Position, 5'- 3'		217 - 239	334 - 315	259 - 273
Warnings				
Tm, ºC		59.7	61.1	49.1
%GC	50.8	39.1	45.0	60.0
Sequence to Analyze	GGYGYGGGGA TTAATATATY GGGAGGAGGY GGGGATTAGY GATTAAGGGA AGGGAAGGTG TTATT			





Primer Set 3	Score: 76
General Warnings	

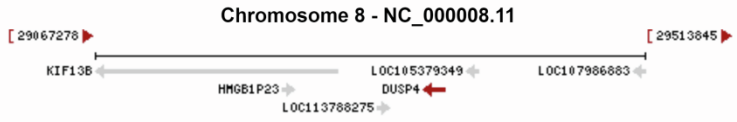
F3	GGTTTTAGGGGTATTATATAAGGGTAGAG
R1	CCCTACCTTCCCCCTCAA
S2	GGGTATTATATAAGGGTAGAGTA

	PCR Product	Forward PCR Primer, F3	Reverse PCR Primer, R1	Sequencing Primer, S2

Length, nt	135	29	18	23
Position, 5'- 3'		141 - 169	275 - 258	149 - 171
Warnings				A homopolymer is dete adjacent to polymorphi
Tm, ºC		58.8	58.7	45.9
%GC	45.2	37.9	61.1	34.8
Sequence to Analyze	GGAAGGYGTT GGYGTTYGYG TTYGTTTTYG TTTTTTAYGT GGGG			

2. DUSP4

109.20190607	current	GRCh38.p13 (GCF_000001405.39)	8	NC_000008.11 (29333062..29350684, complement)
105	previous assembly	GRCh37.p13 (GCF_000001405.25)	8	NC_000008.10 (29190579..29208267, complement)

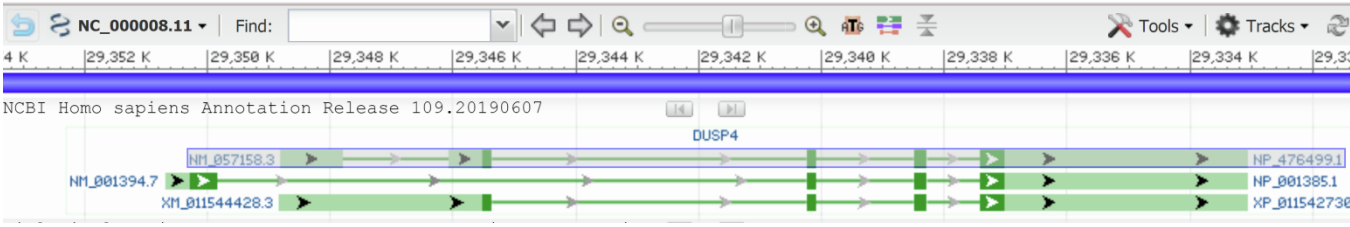


Genomic regions, transcripts, and products

Go to reference sequence

Genomic Sequence: NC_000008.11 Chromosome 8 Reference GRCh38.p13 Primary Assembly

Go to nucleotide: Graphics FASTA Ge

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Homo sapiens chromosome 8, GRCh38.p13 Primary Assembly

NCBI Reference Sequence: NC_000008.11

GenBank Graphics

>NC_000008.11:c29352684-29333062 Homo sapiens chromosome 8, GRCh38.p13 Primary Assembly

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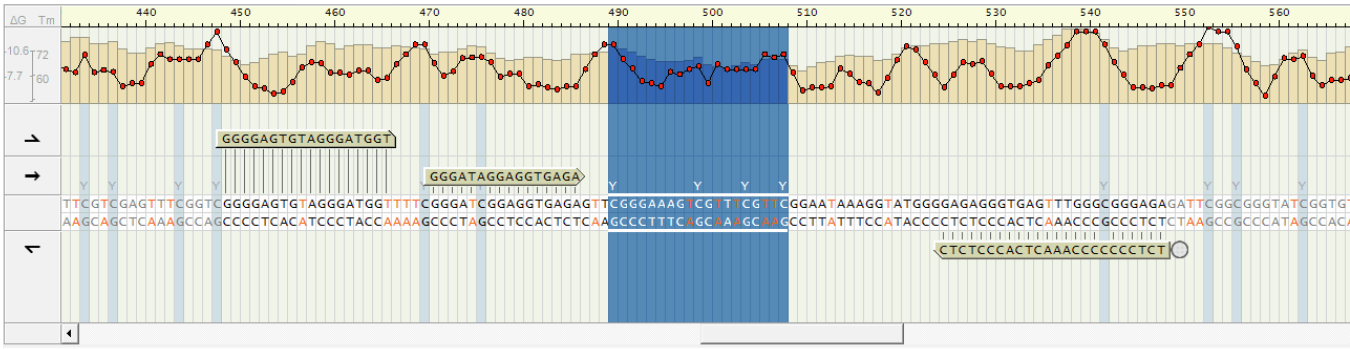

Primer Set 1	Score: 89
General Warnings	

→	F1	GGGGAGTGTAGGGATGGT
←	R1	TCTCCCCCCCAAACTCACCCTCTC
→	S1	GGGATAGGAGGTGAGA

	PCR Product	Forward PCR Primer, F1	Reverse PCR Primer, R1	Sequencing Primer, S1
Length, nt	100	18	24	16
Position, 5'- 3'		448 - 465	547 - 524	470 - 485
Warnings				
Tm, ºC		62.1	63.1	42.8
%GC	49.0	61.1	62.5	56.3
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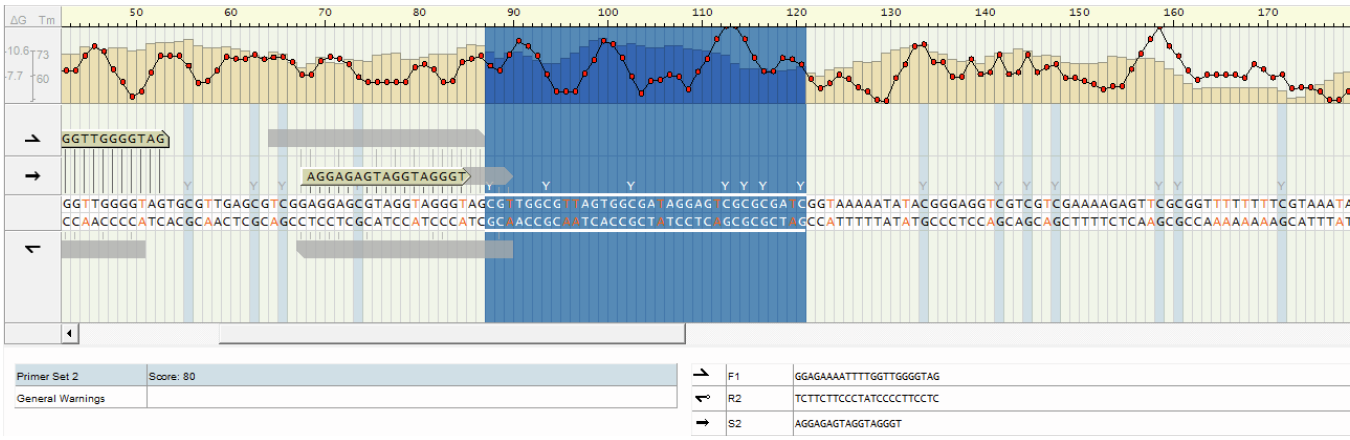
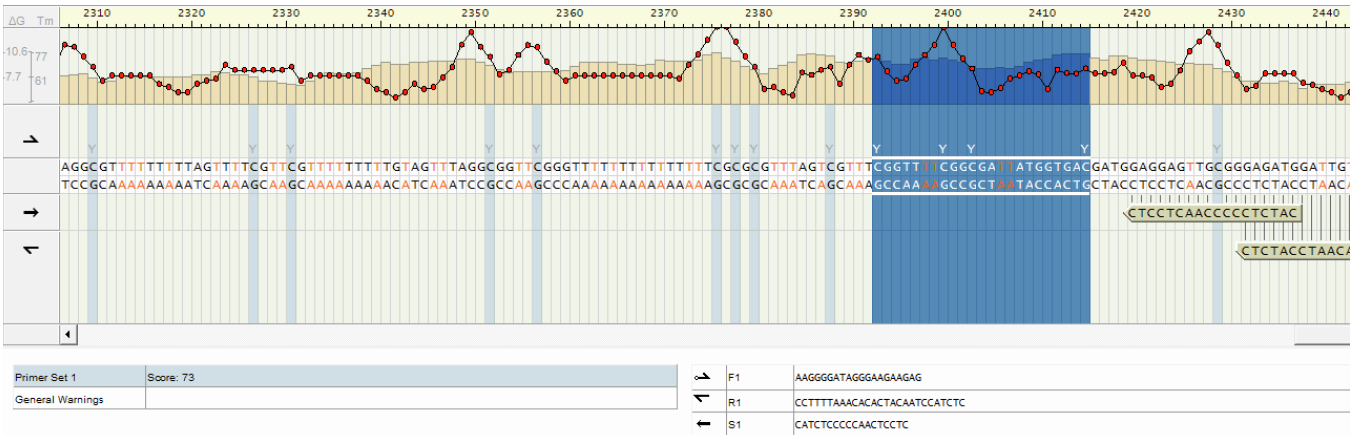


Primer Set 2	Score: 80
General Warnings	

F1	GGAGAAAATTTTGGTTGGGGTAG
R2	TCTTCTTCCCTATCCCCTTCCTC
S2	AGGAGAGTAGGTAGGGT

	PCR Product	Forward PCR Primer, F1	Reverse PCR Primer, R2	Sequencing Primer, S2
Length, nt	267	23	23	17
Position, 5'- 3'		30 - 52	296 - 274	68 - 84
Warnings	Deviation from optimal amplicon size			
Tm, ºC		60.8	62.7	46.1
%GC	36.7	43.5	52.2	52.9
Sequence to Analyze	AGYGTTGGYG TTAGTGGYGA TAGGAGTYGY GYGATYGGTA AAAATATA			



Primer Set 1	Score: 73
General Warnings	

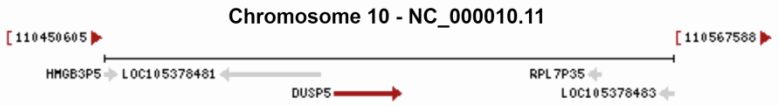
F1	AAGGGGATAGGGAAGAAGAG
R1	CCTTTTAAACACACTACAATCCATCTC
S1	CATCTCCCCCAACTCCTC

	PCR Product	Forward PCR Primer, F1	Reverse PCR Primer, R1	Sequencing Primer, S1
Length, nt	219	20	27	18
Position, 5'- 3'		2239 - 2258	2457 - 2431	2436 - 2419

Warnings				
Tm, °C		59.9	60.3	45.7
%GC	32.4	50.0	37.0	61.1
Sequence to Analyze	CATCRTCACC ATAATCRCCR AAAACCRAAA CRACTAAAC			

3. DUSP5

105 previous assembly GRCh37.p13 (GCF_000001405.25) 10 NC_000010.10 (112257625..112271302



Genomic regions, transcripts, and products

Go to reference seque

Genomic Sequence: NC_000010.11 Chromosome 10 Reference GRCh38.p13 Primary Assembly

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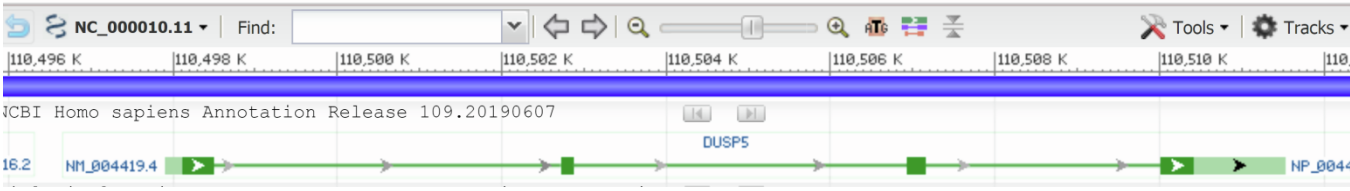


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Homo sapiens chromosome 10, GRCh38.p13 Primary Assembly

NCBI Reference Sequence: NC_000010.11

GenBank Graphics

>NC_000010.11:110495907-110511533 Homo sapiens chromosome 10, GRCh38.p13 Primary Assembly

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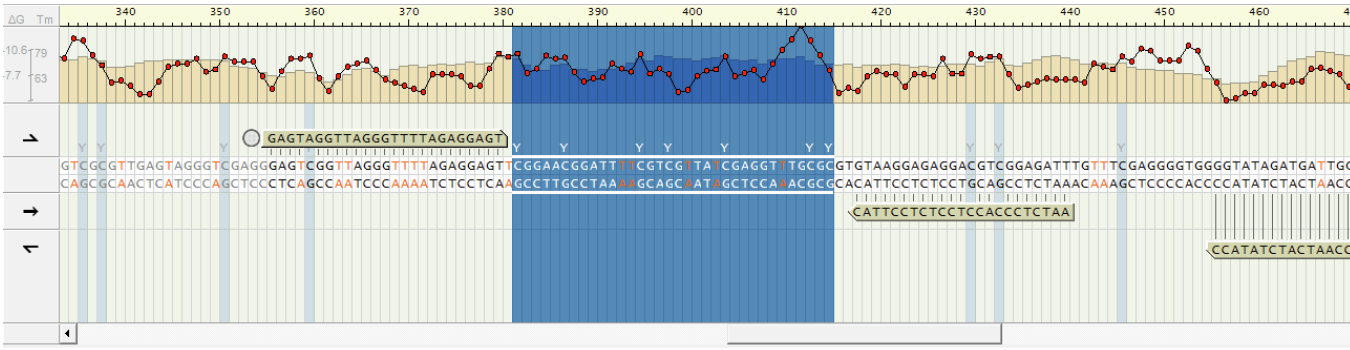
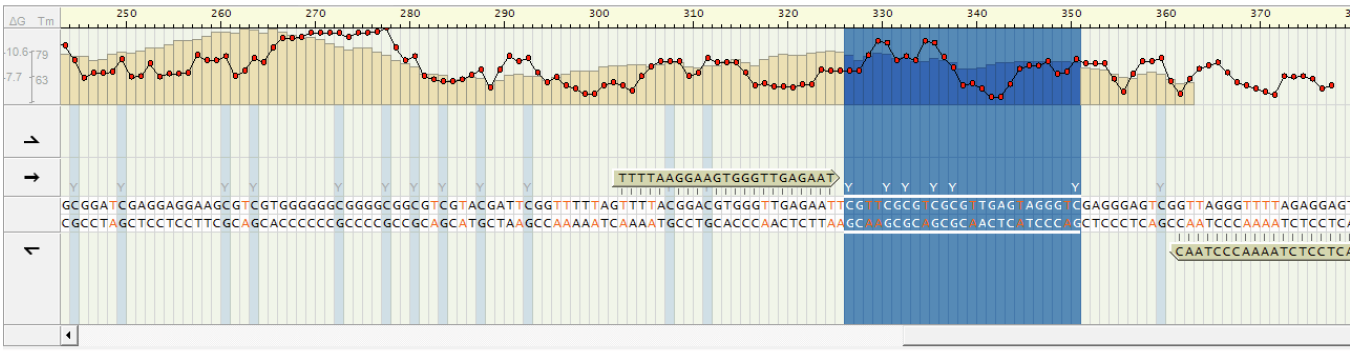


Primer Set 1	Score: 89
General Warnings	

F1	GAGTAGGTTAGGGTTTTAGAGGAGT
R1	AACCTCTCCAATCATCTATACC
S1	AATCTCCCACCTCCTCTCCTTAC

	PCR Product	Forward PCR Primer, F1	Reverse PCR Primer, R1	Sequencing Primer, S1
Length, nt	122	25	22	23
Position, 5'- 3'		355 - 379	476 - 455	439 - 417
Warnings				
Tm, ºC		59.9	57.9	44.3
%GC	41.0	44.0	40.9	52.2
Sequence to Analyze	ACRCRCAAAC CTCRATAACR ACRAAAATCC RTTCCRAACT CCTCTAAAAC CCTAACC			



Primer Set 1	Score: 76
General Warnings	

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R1	AACTCCTCTAAAACCCTAAC
S1	TTTTAAGGAAGTGGGTTGAGAAT

	PCR Product	Forward PCR Primer, F1	Reverse PCR Primer, R1	Sequencing Primer, S1
Length, nt	269	22	20	23
Position, 5'- 3'		112 - 133	380 - 361	302 - 324

Warnings	Deviation from optimal amplicon size			
Tm, ºC		57.5	55.8	44.1
%GC	39.4	50.0	40.0	34.8
Sequence to Analyze	TYGTTYGYGT YGYGTTGAGT AGGGTYGAGG GAGTYGGTTA GGGTTTTAGA G			

Supplemental table 6 and Supplemental table 7: The mRNA variation abundance for TUG1-knockdown in HTR-8/SVneo cells, which can be found online at Xu et al[1]

[1]Xu, Y., Ge, Z., Zhang, E., Zuo, Q., Huang, S., Yang, N., Wu, D., Zhang, Y., Chen, Y., Xu, H., Huang, H., et al. (2017). The lncRNA TUG1 modulates proliferation in trophoblast cells via epigenetic suppression of RND3. Cell Death Dis *8*, e3104. 10.1038/cddis.2017.503.