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Electronic Supplementary Information (ESI)

Chemical labeling achieves 8-oxo-7,8-dihydroguanine mapping in microRNA transcriptome

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Materials and Methods

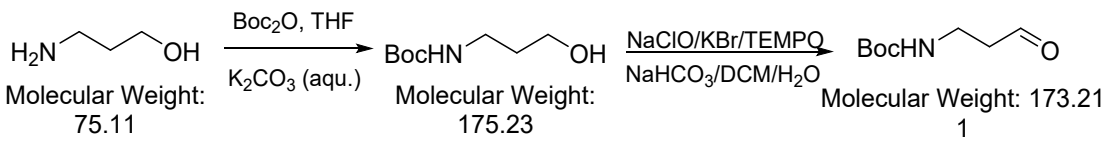
Synthesis of N-(3-azidopropyl)-spermine-5-carboxamide (APSC)

The N-(3-azidopropyl)-spermine-5-carboxamide (APSC) was designed, and then custom-synthesized in Chemily Glycoscience (Georgia, USA) that provided the synthesis process as follows:

Synthesis of Compound 1

A solution of 3-amino-1-propanol (0.750 mL, 737 mg, 9.82 mmol) in tetrahydrofuran (THF, 7 mL) was added with di-tert-butyldicarbonate (Boc_2O, 2.35 g, 10.8 mmol) and then a solution of sodium carbonate (2.08 g, 19.6 mmol) in H_2O (5 mL). After vigorously stirring for 2 h, the reaction mixture was poured over ethyl acetate (EtOAc). The organic phase was washed with minimum amount of saturated sodium bicarbonate ($NaHCO_3$) aqueous solution, separated, dried over $MgSO_4$, filtered and concentrated under reduced pressure to provide a crude pale yellow oil (2 g, ~100%) that was used without further purification.

This crude alcohol (1.05 g, 6 mmol) was dissolved in dichloromethane (CH_2Cl_2, DCM, 7 mL), and followed by adding 3 mL of KBr (722 mg, 6.07 mmol) aqueous solution and 2,2,6,6-tetramethylpiperidinooxy (TEMPO, 9 mg, 0.06 mmol), and the mixture was cooled to 0 °C. After stirring for 10 min, a solution of NaOCl (492 mg, 6.61 mmol) and $NaHCO_3$ (1.84 g, 21.9 mmol) in H_2O (9 mL) was added dropwise. After stirring for 1.5 h, additional NaOCl (104 mg, 1.4 mmol) was added, and thin-layer chromatography (TLC) analysis indicated that the starting material was consumed within 20 min. The product was extracted with DCM for two times. The combined organic phase was separated, dried over anhydrous magnesium sulfate ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified via column chromatography to afford the desired compound 1 (792 mg, 76 %).

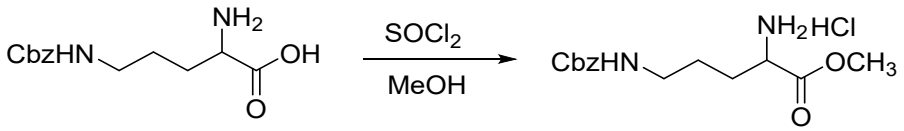


Molecular Weight: 75.11 | Molecular Weight: 175.23 | Molecular Weight: 173.21

Synthesis of Compound 2

Thionyl chloride (2.24 g, 18.8 mmol) was added into a solution of N'-

benzyloxycarbonyl-L-ornithine (N'-Cbz-L-ornithine, 1.00 g, 3.76 mmol) in anhydrous methanol (MeOH, 25 mL) at 0 °C. After completing the addition, the reaction mixture was stirring for 18 hours at room temperature. The reaction system was then concentrated under reduced pressure to obtain a yellowish solid. Recrystallization with MeOH:ethyl acetate (EtOAc) (1:6) afforded the desired compound 2 as a white solid (600 mg, 0.190 mmol). The filtrate and EtOAc washings were cooled for 48 h at -20°C to provide additional product (177mg, 0.561 mmol). The two batches (total: 777 mg, 65 %) were combined for further use.

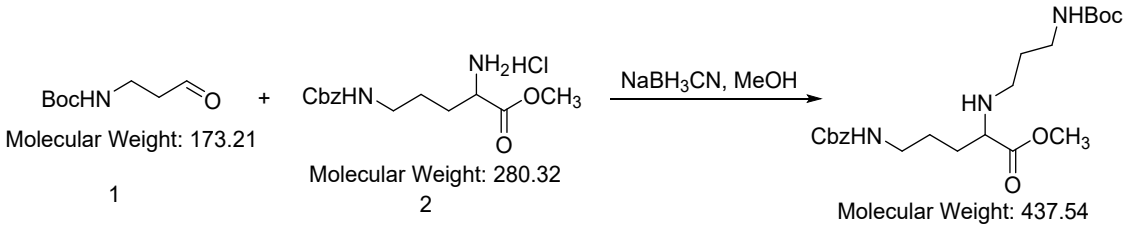


Molecular Weight: 266.29 Molecular Weight: 280.32

2

Synthesis of Compound 3

Compound 1 (988 mg, 5.7 mmol) was added into the solution of Compound 2 (1.6 g, 5.7 mmol) in anhydrous MeOH (30 mL). The reaction mixture was stirred for 10 min at room temperature, followed by addition of $NaBH_3CN$ (359 mg, 5.7 mmol). The reaction was monitored by liquid chromatography mass spectrometry (LC-MS) until starting material disappeared. The reaction was then quenched by saturated K_2CO_3 aqueous solution and extracted with chloroform ($CHCl_3$). After removing solvent, the residue was purified by the flash chromatography (EtOAc/Hexane 0 to 3/1) to afford pure compound 3. Yield is always about 60%.



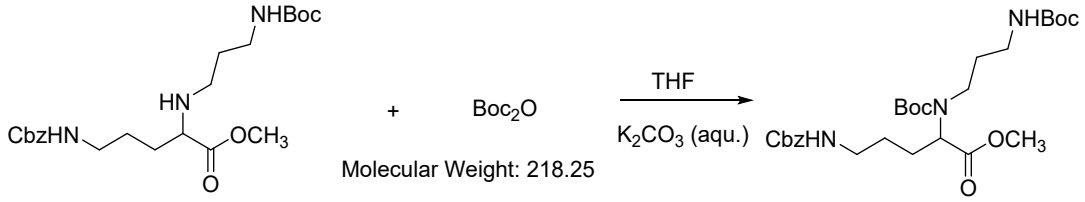
Molecular Weight: 173.21 Molecular Weight: 280.32 Molecular Weight: 437.54

1 2 3

Synthesis of Compound 4

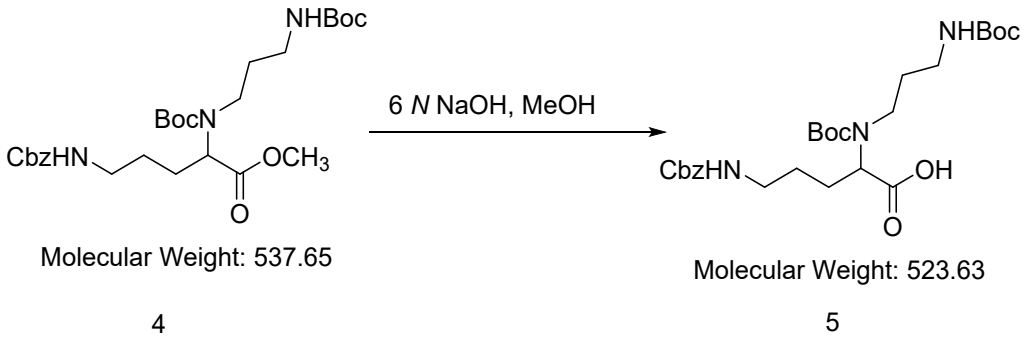
Compound 3 (1.618 g, 3.7 mmol) was dissolved in tetrahydrofuran (THF, 20 mL) and saturated K_2CO_3 (766 mg in 10 mL water) was added. And then, Boc2O (1.21 g, 5.55

mmol) was added under stirring. After stirring overnight, the reaction system was diluted with EtOAc and the organic layers were washed by saturated $NaHCO_3$. After drying over anhydrous $MgSO_4$, the solution was concentrated under reduced pressure, the residue was then purified by the flash chromatography (EtOAc/Hexane 0 to 1/1) to afford the pure compound.



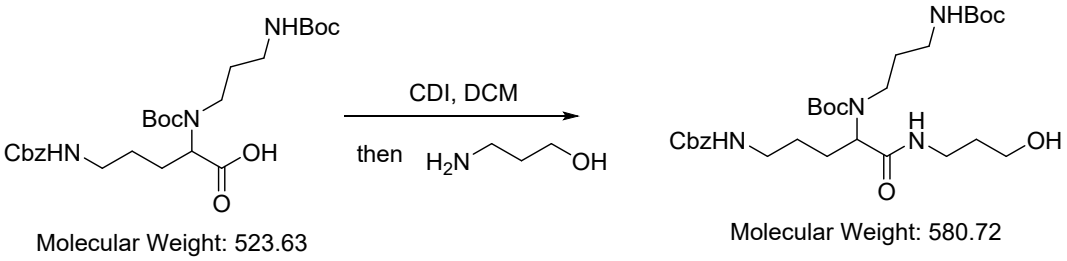
Synthesis of Compound 5

Compound 4 (1.99 g, 3.7 mmol) was dissolved in MeOH (10 mL), and NaOH (4 mL, 6 M) was added. The reaction was monitored by LC-MS. After quenching with HCl (25 mL, 1M), the product was extracted with $CHCl_3$, and then the solvent was removed. The residue was used without further purification.



Synthesis of Compound 6

Compound 5 (1.94 g, 3.7 mmol) was dissolved in anhydrous DCM (30 mL) and N,N'-carbonyldiimidazole (CDI, 600 mg, 3.7 mmol) was added. The reaction was monitored by TLC. After 1 hour, 3-aminopropan-1-ol (2 mL) was added. The reaction was then monitored by LC-MS until the reaction was complete. The reaction mixture was then diluted by $CHCl_3$ and washed by saturated K_2CO_3. The organic solvent was then removed under reduced pressure. The residue was purified by flash chromatography to afford pure compound 6 (1.51 g, 2.88 mmol).



Molecular Weight: 523.63

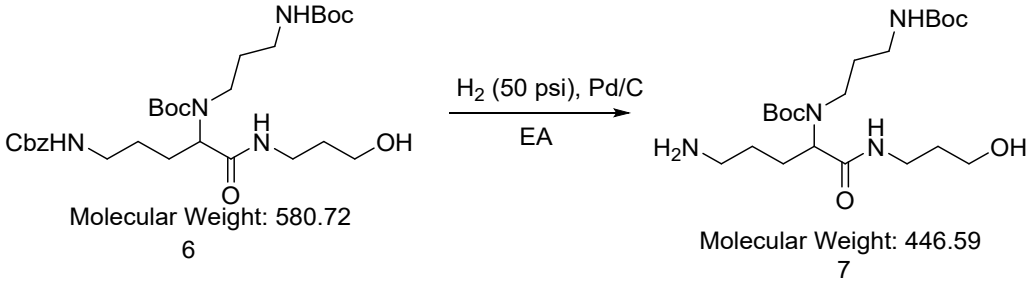
5

Molecular Weight: 580.72

6

Synthesis of Compound 7

Compound 6 (1.51 g, 2.88 mmol) was dissolved in EtOAc (20 mL) and Pd/C (150 mg, 10% w/w) was added. The mixture was shaken at the atmosphere of H_2 (50 psi) and reaction was monitored by LC-MS. The mixture was filtered through celite. The filtrate was then concentrated to afford crude compound 7 which was used without further purification.



Molecular Weight: 580.72

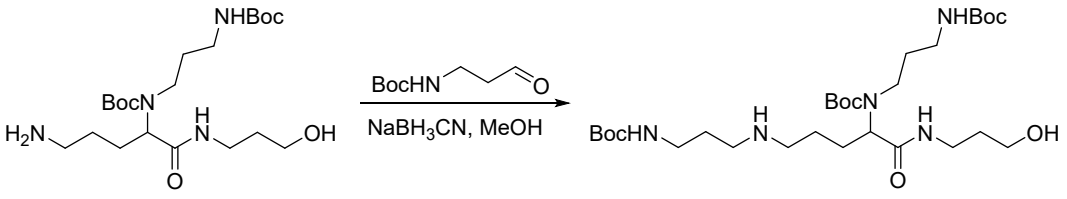
6

Molecular Weight: 446.59

7

Synthesis of Compound 8

Compound 7 (1.286 g) was dissolved in anhydrous MeOH (30 mL). Compound 1 (498 mg, 2.88 mmol) was then added. The reaction was then monitored by LC-MS until the starting material disappeared. The reaction was then quenched by saturated K_2CO_3 and extracted with $CHCl_3$. The solvent was then removed and the residue was used in the next step without further purification.



Molecular Weight: 446.59

7

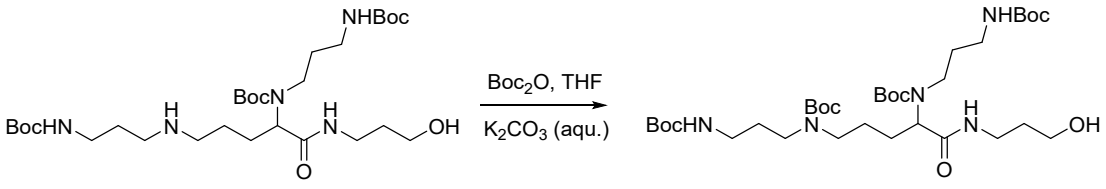
Molecular Weight: 603.80

8

Synthesis of Compound 9

Compound 8 (1.74 g) was dissolved in THF (20 mL) and saturated K_2CO_3 (597 mg in

5 mL water) was added. Then Boc_2O (942 mg, 4.32 mmol) was added. The reaction was monitored by LC-MS. The reaction system was diluted with EtOAc and the organic layers were combined and washed with saturated $NaHCO_3$. The solvent was concentrated under reduced pressure. The residue was purified by the flash chromatography (EtOAc/Hexane 0 to 1/1) to afford the pure compound 9 (751 mg, 1.067 mmol).

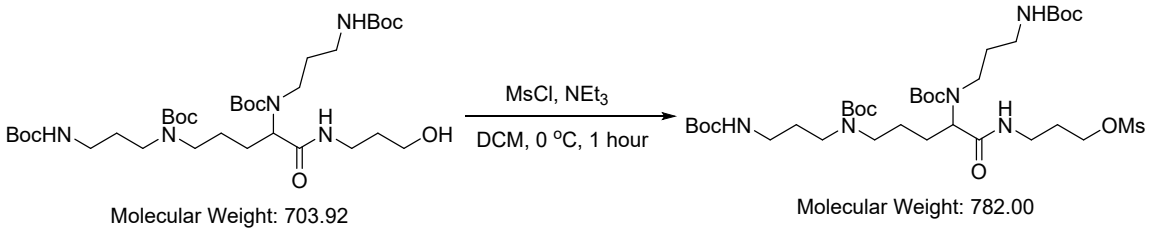


Molecular Weight: 603.80 — 8

Molecular Weight: 703.92 — 9

Synthesis of Compound 10

Compound 9 (751 mg, 1.067 mmol) was dissolved in anhydrous DCM (15 mL) and the solution was cooled to 0 °C. Triethylamine (NEt_3, 0.22 mL, 1.6 mmol) was added and followed by the slow addition of methanesulfonyl chloride (MsCl, 90 uL, 1.05 mmol). The reaction was then stirred at 0 °C for 1 hour when TLC indicated that all the starting material disappeared. The reaction was quenched by brine and extracted by $CHCl_3$. The combined organic layers was evaporated under reduced pressure. The residue was used in the next step without further purification.



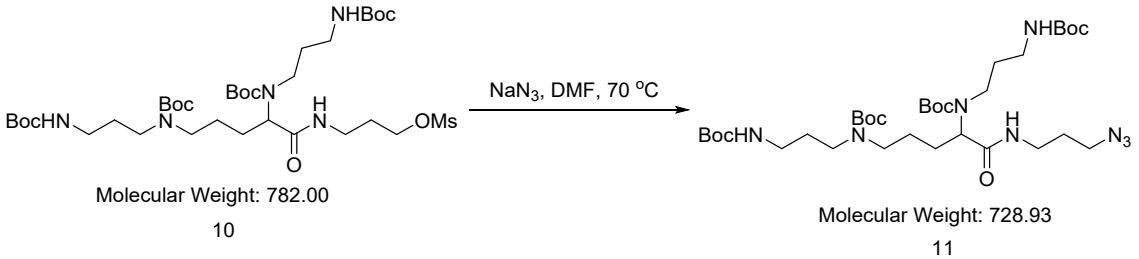
Molecular Weight: 703.92 — 9

Molecular Weight: 782.00 — 10

Synthesis of Compound 11

Compound 10 (835 mg, 1.067 mmol) was dissolved in anhydrous DMF (15 mL). NaN_3 (104 mg, 1.6 mmol) was added and the reaction was heated to 70 °C. After the reaction was complete indicated with LC-MS, the reaction system was cooled down to room temperature. Brine was added and the mixture was extracted by methyl tert-butyl ether. The solvent was then removed under reduced pressure and the residue was then

subjected to flash chromatography to afford pure compound 11 (450 mg, 0.617 mmol).



Molecular Weight: 782.00

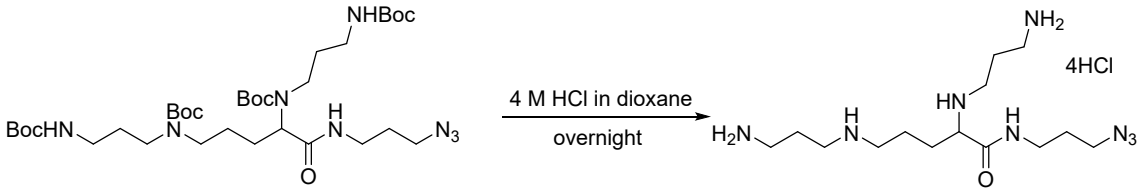
10

Molecular Weight: 728.93

11

Synthesis of APSC hydrochloride

Compound 11 (450 mg, 0.617 mmol) was dissolved in a solution of 4M HCl in dioxane (10 mL) and stirred overnight. The reaction mixture was subjected to ultrasonic treatment for 2 hours, during which a lot of light yellow precipitate formed. The solvent was removed and the solid was collected to afford light yellow solid (292 mg), namely APSC hydrochloride.



Molecular Weight: 728.93

11

Molecular Weight: 328.47

APSC

8-oxoGua labeling with APSC

The 10-nt DNA or RNA templates with or without an 8-oxoGua (15 μM) was incubated with APSC (0.3 mM) in 18 μL of sodium phosphate buffer (SPB, 20 mM, pH 7.5), and 2 μL of $K_3Fe(CN)_6$ (6 mM) or Na_2IrCl_6 (1 mM) was added.[1, 2] After gentle mixing with a pipette, the reaction was carried out for 30 min at 25 °C and then quenched by adding 5 μL of EDTA solution (20 mM, pH 8.0). The DNA/RNA was precipitated in 1.5 volumes of 100% isopropanol after adding 2 μL of glycogen (10 mg/mL, Thermo) at -20 °C overnight. The DNA/RNA pellet was obtained through centrifuging for 10 min at 12000 rpm at 4 °C and washed with 80% ethanol, which was dissolved in deionized water.

Considering the fact that APSC has two primary amine groups, two different isomers of adducts may be formed during the oxidation/addition step. However, no matter which adduct isomer form is formed, it will not affect the subsequent pull-down of 8-oxoGua-containing nucleic acids.

The 10-nt DNA used in this experiment is presented below:

5'-CAGTAXTGAC-3' X = 8-oxoGua

The 10-nt RNA used in this experiment is presented below:

5'-CAGUAXCGAC-3' X = 8-oxoGua

The reactions of APSC with synthetic DNA containing 8-BrGua, 8-oxoAde, or 5-fCyt and RNA containing 8-oxoGua

The 10-mer DNAs (15 μM) containing an 8-BrGua, 8-oxoAde, or 5-fCyt and 21-nt RNA containing an 8-oxoGua were reacted with $K_3Fe(CN)_6$ (0.6 mM) in the presence of APSC (0.3 mM) in 20 μL of SPB (20 mM, pH 7.5) treated with diethyl pyrocarbonate (DEPC) (named as RNAse-free SPB), respectively. After incubation for 30 min at 25 ^oC, the reaction was quenched with EDTA (1mM, pH 8). The DNA was precipitated in isopropanol and washed with 80% ethanol. They were dissolved in 10 μL of RNAse-free water DNA.

The 10-mer DNA templates (Takara) used in this experiment are presented below:

5'-CAGTAXTGAC-3' X = dG

5'-CAGTAYTGAC-3' Y = 8-BrGua

5'-GTACGACXTG-3' X = 8-oxoAde

5'-GTACGAYATG-3' Y = 5-fCyt

The 21-nt RNA template (Takara) used in this study is presented below.

5’-CGAUAUCGUAUCXCGUAUCGC-3’ X = 8-oxoGua

Cell Culture

4T1 breast carcinoma cells were obtained from Dr. Peipei Shan, and cultured in the RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Biological Industries), 100 μM nonessential amino acids, 100 μM sodium pyruvate, 100 μg/ml streptomycin-100 unit/ml penicillin in a 5% CO2 incubator at 37 °C.

Human aortic vascular smooth muscle cells (VSMCs) were originally purchased from ATCC. They were cultured in the Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco) supplemented with 10% FBS, and 100 units/mL penicillin-100 μg/mL streptomycin (Hyclone) at 37 ^{o}C, 5% CO_2 atmosphere. Cells were passed at 70-80% of confluency by using the solution of trypsin (0.25%)-EDTA (0.02%) in DMEM. For H_2O_2 treatment, the VSMCs were cultured in the medium supplemented with 100 μM H_2O_2 for 12 h.[3]

Preparation of cellular DNA and small RNAs

Genomic DNA was extracted from VSMCs by using genomic DNA extraction kits (Tiangen Biotech, Beijing, China), in which 2-mercaptoethanol (100 mM) was supplemented in the solutions. VSMC genomic DNA and rat genomic DNA (obtained from Dr. Tao Xu) was dissolved in 50 μL of Tris-HCl buffer (10 mM, pH 8.0), respectively, and the concentration was determined with a Nanodrop (Thermo Fisher Scientific).

50 μg of genomic DNA was diluted with 100 μL of Tris-HCl buffer (10 mM, pH 8.0) and then sonicated into 150-300 bp with a focused-ultrasonicator (M220, Covaris). To inhibit adventitious formation of 8-oxoGua, the antioxidant, 2-mercaptoethanol (100 mM), was used in the steps for DNA extraction and fragmentation by sonication. The fragmented DNA was precipitated with isopropanol in the presence of glycogen at -20 ^{o}C overnight. After centrifugation, the DNA pellet was washed twice with 80% ethanol, and dissolved in 40 μL of SPB (20 mM, pH 7.5).

The small RNAs (< 200 bp in length) were extracted from the VSMCs and 4T1 cells (obtained from Dr. Peipei Shan) with miRNA isolation kit (DP501, Tiangen Biotech), in which 2-mercaptoethanol (100 mM) was supplemented in solutions, respectively. They were recovered and purified with twice ethanol precipitation in the presence of glycogen. The RNA pellet was re-suspended in 50 μL of the RNAse-free SPB (20 mM, pH 7.5); its concentration was measured by using the Nanodrop, and about 40 μg for each group was obtained.

8-oxoGua labeling and pull-down enrichment

8-oxoGua tagging with APSC was carried out as described above. Synthetic DNA template (1 nmol) was reacted with APSC (0.3 mM) and $K_3Fe(CN)_6$ (0.6 mM) in 50 μL of reaction mixture. For genomic DNA, 40 μg of the DNA fragments (150-300 bp) was also labeled in the 50 μL of SPB (20 mM, pH 7.5) containing 0.3 mM of APSC and 0.6 mM of $K_3Fe(CN)_6$. For small RNA, the 8-oxoGua labeling was carried out in 50 μL of RNAse-free SPB (20 mM, pH 7.5) containing 20 μg of RNAs, 0.3 mM of APSC, and 0.6 mM of $K_3Fe(CN)_6$. All the reactions were carried out for 30 min at 25

°C, and NA samples were then precipitated with isopropanol in the presence of glycogen at -20°C overnight, respectively.

The resultant 8-oxoGua-N_3 was purified via twice precipitation with isopropanol in the presence of glycogen, and then dissolved in 40 uL of 10 mM Tris-HCl buffer (pH 7.4), respectively. The click chemistry was carried out by adding the biotin-terminated functional linker with disulfide and DBCO (200 μM) and followed by incubating at 37 °C for 3 hours at 800 rpm in the Eppendorf thermomixer.[4] The sample was purified by twice isopropanol precipitation and washing with 80% ethanol.

The pull-down of biotinylated NAs (namely 8-oxoGua-N3-S-S-Biotin) was carried out with the streptavidin-coated magnetic beads (65001, Invitrogen) according to manufacturer's instructions with minor modifications. Tween-20 was added into the washing and binding buffer at final concentration of 0.01% and 0.02% (v/v), respectively. 40 μL of magnetic bead suspension was used for binding of 8-oxoGua-N_3-S-S-Biotin in each sample. After washing the beads at least six times, 50 μL of dithiothreitol (DTT) solution (60 mM) in RNAse-free water was employed to release 8-oxoGua-containing NA fragments (namely 8-oxoGua-N3-SH) by incubating at 25 °C for 1 h under vortexing at 1200 rpm. The solution of 8-oxoGua-N_3-SH was collected after separating the magnetic beads with a magnet, recovered via isopropanol precipitation, and then dissolved in 20 μL of RNAse-free water.

A 12-mer DNA template (Takara) used in this experiment is presented below:

5’-CCGXAATTGGCC-3’ X = 8-oxoGua

Amplification assay of DNA containing 8-oxoGua-N_3-SH using polymerase chain reaction (PCR)

The 100-mer single-stranded DNA (100 ng) containing an 8-oxoGua was subjected to 8-oxoGua pull-down protocol. The enriched 8-oxoGua-N3-SH was acted as the template to perform PCR using various commercial DNA polymerases, including Deep Vent® (exo-) DNA Polymerase (NEB), TruePrep Amplify Enzyme (Vazyme), Pfu DNA Polymerase (Promega), Q5® High-Fidelity DNA Polymerases (NEB), according to the manufacturer's instructions, respectively. Besides, as the control, the 8-oxoGua pull-down protocol was also carried out on the model DNA except APSC tagging, followed by PCR with Deep Vent® (exo-) DNA Polymerase (NEB). Reaction mixtures were incubated at 95 °C for 5 minutes, followed by 30 cycles of 15 s at 95 °C, 30 s at 55 °C, and 15 s at 72 °C. The PCR products were analyzed by electrophoresis on 1.5% agarose gels and imaged under UV light after staining with 1×Ultra GelRed Nucleic Acid Stain (Vazyme). The 100 bp DNA Ladder (Vazyme) was used as a size marker. The 100-mer single-stranded DNA (Takara) used in this study is presented below:

5’-

CCTCACCATCTCAACCAATATTATATTACGCGTATATCGCGTATACXCGTA

TATCGCGTATCGCGTATTTCGCGTTATAATATTGAGGGAGAAGTGGTGA-3’

X = 8-oxoGua or G

The sequences of PCR primers (Sangon Biotech Co., Ltd, Shanghai, China) were as follows:

Forward primer: 5’-CCTCACCATCTCAACCAATA-3’

Reverse primer: 5’-TCACCACTTCTCCCTCAATA-3’

Fluorescence labeling of 8-oxoGua

As described above, synthetic 35-bp synthetic DNA and 21-nt RNA templates with or without 8-oxoGua (1 nmol), VSMC DNA (5 μg), and VSMC RNA (5 μg) was reacted with APSC (0.3 mM) in 50 μL of SPB (20 mM, pH 7.5) in the presence of $K_3Fe(CN)_6$ (0.6 mM), respectively. The resultant 8-oxoGua-N_3 was obtained by isopropanol precipitation, and then dissolved in 40 uL of 10 mM Tris-HCl buffer (pH 7.4) containing FITC-DBCO (200 μM). The reaction was carried out at 37 °C for 3 hours, and purified by twice precipitation in isopropanol. The FITC-labeled samples were dissolved in 10 mM Tris-HCl buffer (pH 7.4).

The 35-bp DNA template (Takara) used in this study is presented below (The complementary strand is not listed):

5’-AATTCGAGCTGGCGCGXACGTATGAGGAGCGGTAC-3’ X = 8-oxoGua

Quantification of 8-oxoGua by fluorescence assay

DNA content was determined using a Nanodrop. The fluorescence intensity of FITC-labeled sample was measured, respectively, at an excitation wavelength of 495 nm and emission wavelength of 525 nm in a 96-well plate with 50 μL of solution per well using a micro-plate reader. For the 35-bp DNA templates, the graph was plotted with the fluorescence intensity versus corresponding 8-oxoGua concentration, which was also served as standard curve. The 8-oxoGua content of VSMC DNA (100 ng) after adding DNA templates, including 52 nM, 104 nM, or 156 nM, was also determined. In

addition, the fluorescence intensity was measured for calculation of 8-oxoGua content in DNA and RNA of the VSMCs treated with or without H_2O_2, respectively.

Dot-blot assays

The synthetic DNA templates with or without 8-oxoGua, cellular DNAs and RNAs were labeled with FITC, and purified, respectively. Each of them (60 ng/μL) was spotted on a positively charged nylon membrane (INYC00010, Millipore). They were observed under a fluorescence imaging system (Tanon, 5200 Multi).

Spike-in study and qPCR validation of 8-oxoGua enrichment

The spike-in and qPCR experiments were used to validate the specificity and efficiency of the protocol for labeling and pull-down of 8-oxoGua-containing NAs. 100 pg of synthetic 100-bp DNA template (Takara) with or without an 8-oxoGua was spiked into the 5 μg of rat genomic DNA fragments, respectively, followed by 8-oxoGua labeling and enrichment. In addition, the enrichment protocol was also performed on 4T1 cell-derived small RNAs (5 μg) spiked with synthetic 21-nt RNA containing an 8-oxoGua (2 ng).

All qPCR reactions were performed in triplicate using a CFX96 Real-Time PCR System (Bio-Rad). For DNA, 2 ng of the input (genomic DNA fragments spiked with model DNA) or pull-down DNA was used in a 20 μL of qPCR reaction containing 1× IQ SYBR Green Supermix (Bio RAD), forward primer (0.5 μM), and reverse primer (0.5 μM). Reaction mixtures were incubated at 95 °C for 5 minutes, followed by 40 cycles of 15 s at 95 °C, 30 s at 55 °C, and 15 s at 72 °C.

The 100-bp DNA template (Takara) containing an 8-oxoGua used in this study is presented below (The complementary strand is not listed):

5'-

CCTCACCATCTCAACCAATATTATATTACGCGTATATCGCGTATACXCGTA

TATCGCGTATCGCGTATTTCGCGTTATAATATTGAGGGAGAAGTGGTGA-3'

X = 8-oxoGua

The sequences of primers (Sangon Biotech Co., Ltd, Shanghai, China) were as follows:

Forward primer: 5'-CCTCACCATCTCAACCAATA-3'

Reverse primer: 5'-TCACCACTTCTCCCTCAATA-3'

For small RNA, cDNA was synthesized from the small RNAs (extracted from 4T1 cells) spiked with model RNA (input) and enriched RNAs, respectively, using the 21-nt RNA-specific stem-loop primer (Sangon Biotech Co., Ltd, Shanghai). Typically, 50 ng of RNA sample was respectively reverse-transcribed into cDNA in a 20 μL of reaction mixture containing 100 nM stem-loop primer, 1× PrimeScript Buffer 2 (Takara), and 1 μL PrimeScript RT Enzyme Mix I (Takara) using a T10 thermal cycler (Bio-Rad) at 37 °C for 15 min, 85 °C for 15 sec, and 4 °C for 5 min. The cDNA solution was diluted to a final volume of 100 μL. 2 μL of diluted cDNA was used in each 20 μL of qPCR reaction containing 1× IQ SYBR Green Supermix (Bio RAD), forward primer (0.5 μM), and reverse primer (0.5 μM). Reaction was run with an initial denaturation at 20 °C for 5 min, followed by 40 cycles of 15 s at 95 °C, 30 s at 55 °C, and 15 s at 72 °C. The sequences of primers (Sangon Biotech Co., Ltd, Shanghai) were as follows:

Stem-loop primer: 5’-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGG-ATACGACGCGATAC-3’

Forward primer: 5’-GCAGGGTCCGAGGTATTC-3’

Reverse primer: 5’-CGATATCGTATCGCGTATCG-3’

Fold enrichment was calculated as 2^-dC_t, where $dC_t = C_t$(enriched) - C_t(Input).

Synthesis of DNA containing 5-hydroxycytosine (5-OHCyt) and RNA containing 5-hydroxyuracil (5-OHUra)

The 5-OHCyt-containing DNA is synthesized by PCR amplification using a modified deoxyribonucleoside triphosphate (dNTP), namely 5-hydroxy-2'-deoxycytidine-5'-triphosphate (5-hydroxy-dCTP, B8102, APExBIO), and three conventional dNTP, i.e. 2'-deoxyadenosine-5'-triphosphate (dATP), 2’-deoxyaguanosine-5’-triphosphate (dGTP), and 2'-deoxythymidine-5'-triphosphate (dTTP). The plasmid containing Mfn2 gene and Hieff® Taq DNA Polymerase (Yeasen, China) was used as the PCR template and DNA polymerase, respectively. The primers for synthesis of 5-OHCyt-containing DNA were listed below. The PCR product was purified with DNA clean beads (N411, Vazyme Biotech Co. Ltd, China), and examined by agarose gel. Besides, the control DNA template is synthesized with the same primer and conventional dNTPs, including dATP, dTTP, dCTP, and dGTP.

For preparation of the 5-OHUra-containing RNA, the DNA template containing T7 promoter at the 5'-end is first synthesized by using the plasmid containing Mfn2 gene as the template. The primers for synthesis of the DNA containing T7 promoter were listed below. The 5-OHUra-containing RNA is synthesized using T7 high yield RNA

transcription kit (TR101, Vazyme Biotech Co. Ltd, China) after replacing uridine-5'-triphosphate (UTP) with 5-hydroxy-UTP (B8060, APExBIO), purified with RNA clean beads (N412, Vazyme Biotech Co. Ltd, China), and examined by agarose gel. Besides, the control RNA template is synthesized with conventional NTPs, namely ATP, TTP, CTP, and GTP.

Primers for synthesis of 5-OHCyt-containing DNA:

Forward primer: 5'-CAAGGTCAGGGGAATCAGCG-3'

Reverse primer: 5'-GCTAGCTGGTTCACGGTCTT-3'

Primers for synthesis of the DNA containing T7 promoter:

Forward primer: 5'-TAATACGACTCACTATAGGGCAAGGTCAGGGGAATCAG-CG-3'

Reverse primer: 5'-GCTAGCTGGTTCACGGTCTT-3'

Pull-down yield assay

8-oxoGua labeling and enrichment were carried out on synthetic model DNA and RNA for assessing their enrichment yields, respectively. 1 μg of the 100-bp DNA or 21-nt RNA containing an 8-oxoGua was treated with 8-oxoGua labeling, and then pulled down by using 50 μL of streptavidin magnetic beads (65001, Invitrogen), respectively. The DNA or RNA was recovered via isopropanol precipitation, followed by washing with 80% ethanol. After dissolving in 20 μL of RNAse-free water, their concentrations were measured with a Nanodrop. 721.3 ± 81.0 ng of DNA-8-oxoGua and 757.3 ± 110.2 ng of RNA-8-oxoGua was harvested. As expected, almost no DNA and RNA without 8-oxoGua modification was collected. In addition, the same labeling and pull-down

protocol was also performed on 4 μg of 5-OHUra-containing RNA and 5-OHCyt-containing DNA, respectively. 0.014 ± 0.003 μg of RNA (without 5-OHUra), 0.137 ± 0.019 μg of RNA-5-OHUra, 0.011 ± 0.004 μg of DNA (without 5-OHCyt), and 0.048 ± 0.004 μg of DNA-5-OHCyt was obtained.

Native polyacrylamide gel electrophoresis (PAGE)

Samples were suspended in 1×DNA loading buffer and then loaded onto a 20% polyacrylamide gel (19:1) (Solarbio, Beijing, China). After electrophoresis, the gel was stained with SYBR Gold (Invitrogen) and visualized under UV light.

Electrospray ionization-ion trap mass spectrometry (ESI-ITMS) analysis

DNA and RNA samples were analyzed by electrospray ionization ion trap mass spectrometry (ESI-ITMS) in negative ion mode. The sample was dissolved in water and the final concentration is 2-5 μM, and which was infused into the mass spectrometer (LCQ Deca XP, Thermo Finnigan) at a flow rate of 10 μL/min. The source and desolvation temperatures were 110 ^{o}C and 350 ^{o}C respectively. Capillary voltage and cone voltage was set to 4.5 kV and 46 V, respectively. The data was processed by using the ProMass deconvolution software (Thermo Fisher Scientific).

Library preparation and deep sequencing

The 8-oxoGua enrichment described above as well as subsequent sequencing was termed as APSC-8-oxoGua-seq. For DNA sample, 50 ng of DNA fragments or pull-down 8-oxoGua-containing DNA fragments were used to prepare DNA sequencing-library with NEBNext Ultra II DNA Library Preparation Kit for Illumina (E7645, NEB) and NEBNext Multiplex Oligos for Illumina (E7600, NEB) according to

manufacturer's instructions. After adaptor ligation, cleanup of adaptor-ligated DNA was performed to remove adaptor contamination using 1.1 volumes of AMPure XP Beads. DNA library fragments of 200-400 bp were size selected with AMPure XP Beads after PCR amplification. The purified DNA libraries were paired-end sequenced on the Illumina NovaSeq platform (Berry Genomics, Beijing, China).

For small RNA, 40 ng of small RNAs (input) or pull-down 8-oxoGua-containing small RNAs was employed as starting material to construct small RNA sequencing-library by using VAHTS Small RNA Library Prep Kit for Illumina (NR801, Vazyme Biotech) and VAHTS Small RNA Index Primer Kit for Illumina (N813, Vazyme Biotech) following manufacturer's instructions. They were ligated with RNA 3' and 5' adapters, and then the reverse transcription was performed with the primer to the 3' adapter. After PCR amplification, the products were purified gel electrophoresis, and then sequenced on an Illumina HiSeq 2500 (LC-Sciences, Hangzhou, China).

G>T transversion test

To evaluate G>T transversion at 8-oxoGua sites after the pull-down protocol and PCR, a 100-bp synthetic DNA template containing two 8-oxoGua was subjected to APSC-based 8-oxoGua labeling, enrichment, and sequencing (namely APSC-8-oxoGua-seq). This 100-bp synthetic DNA template (Takara) containing two 8-oxoGua is presented below (The complementary strand is not listed):

5'-

CCGCTCGAGATCGATCGATCGATCGATCGAATCXATCGATCGATCGATCG

ATCATCGTAGCATCXACATGATCGATCGAATCGATCGATCGAGGTACCCA-3’ X = 8-oxoGua

Bioinformatics analysis

For DNA sequencing data, the raw FASTQ data were trimmed by using Trim_Galore (version 0.6.6, parameters: --phred33 --length 36 --stringency 5 --paired) and were aligned to human reference genome, hg38, by using bowtie2 (version 2.3.5.1). Peak calling was performed using MACS2 (version 2.1.0) with the input sample as control ($p < 0.01$, fold-enrichment >3). Peak annotation was performed using HOMER.

For small RNA data, the raw sequencing reads were subjected to the ACGT101-miR program (LC Sciences, Houston, Texas, USA). The resultant unique sequences with length of 18-26 nt were aligned to precursors/mature miRNA in miRBase 22.0 to identify known miRNAs. The unmapped sequences were aligned to genome sequence of human (Homo sapiens, Ensembl_v96) and the hairpin RNA structures containing sequences were predicted with the RNAfold software to identify potentially novel miRNAs. Total miRNA read counts were normalized and the relative expression was calculated. The differential expression was analyzed by using Fisher exact test and Chi-squared 2×2 test, where $p < 0.001$ was considered statistically significant.

G>T transversion was analyzed with an in-house Python script containing Bowtie. For model DNA template, random 30× reads were selected for drawing the heatmap graphics using $\log_{10}$(reads number). For cellular miRNA, the target genes were predicted using online TargetScan and miRanda databases on the wild-type sequence and the sequences after the substitution of 8-oxoGua with U at 8-oxoGua sites,

424 respectively. The differential target genes were analyzed using the OmicStudio tools
425 (https://www.omicstudio.cn/tool) for Gene Ontology (GO) analysis as well as Kyoto
426 Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

427 **Statistical analysis**

428 The results were expressed as means ± standard deviation (SD) with at least three
429 parallel samples. The results of two groups were statistically analyzed with Student's
430 t-test, and a significant difference was defined as $p < 0.05$ unless stated otherwise.

431 **Data availability**

432 Sequencing data of this study have been deposited into the National Center for
433 Biotechnology Information (NCBI) under accession number PRJNA692633.

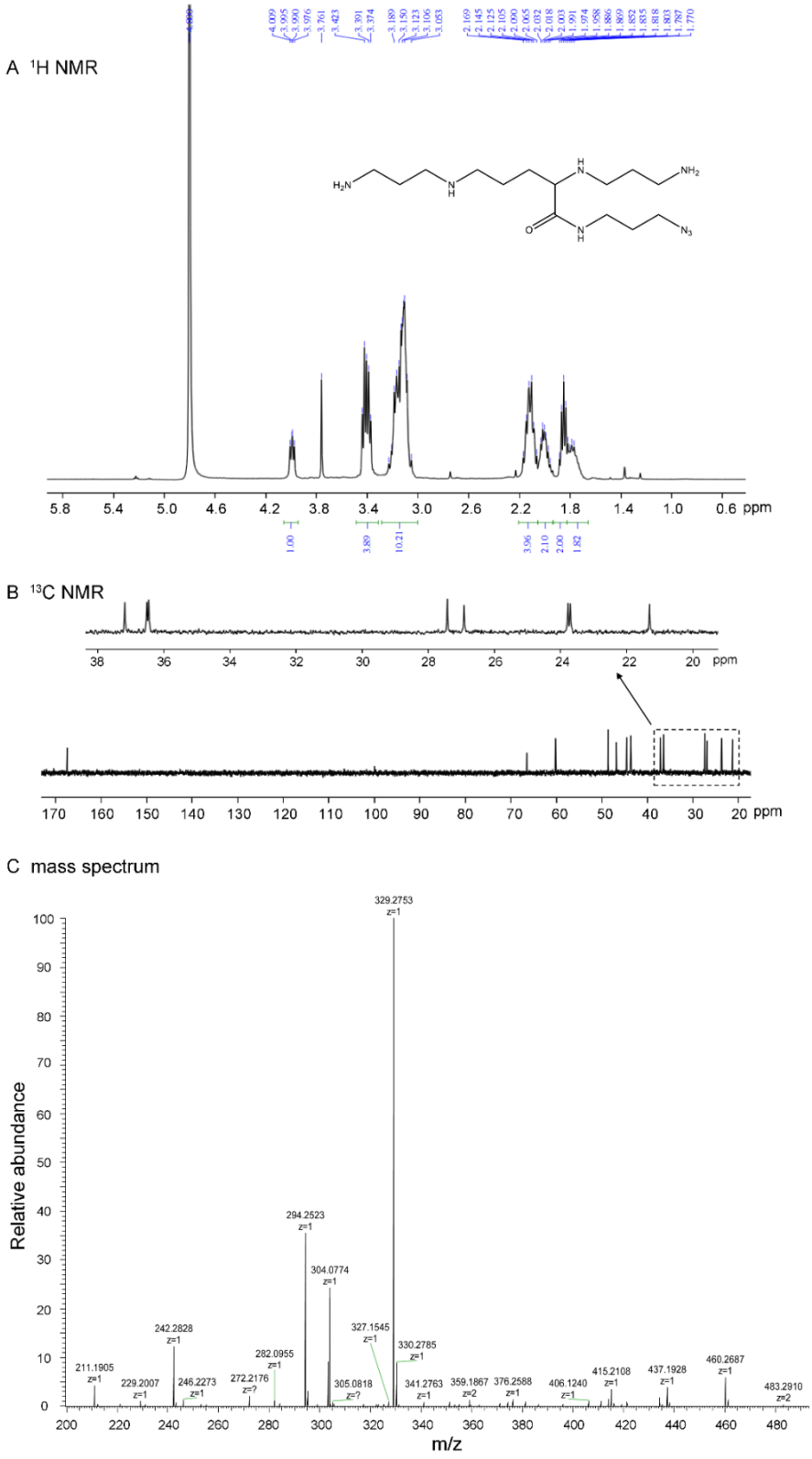


Fig. S1. NMR and mass spectroscopy characterization of APSC. (A) ^{1}H NMR spectrum, (B) ^{13}C NMR spectrum, (C) mass spectrum.

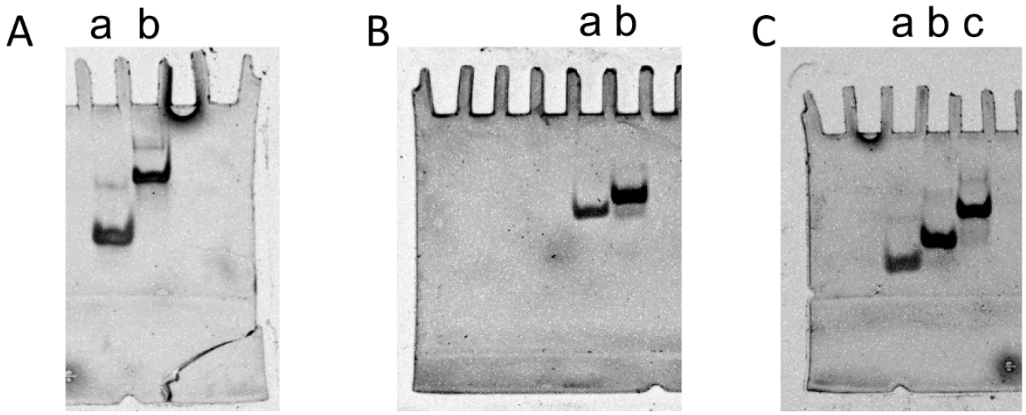


Fig. S2. Native polyacrylamide gel electrophoresis (PAGE) analysis for APSC adduct formation with 8-oxoGua-containing 10-mer DNA (A) and 21-nt RNA (B) as well as for enrichment process of 8-oxoGua-containing 12-mer DNA. (A) Lane a: 10-mer DNA containing an 8-oxoGua, Lane b: 10-mer DNA containing an 8-oxoGua with APSC and $K_3Fe(CN)_6$. (B) Lane a: 21-nt RNA containing an 8-oxoGua, Lane b: 21-nt RNA containing an 8-oxoGua with APSC and $K_3Fe(CN)_6$. (C) Lane a: 12-mer DNA containing an 8-oxoGua, Lane b: 12-mer DNA-8-oxoGua-N_3, Lane c: 12-mer DNA-8-oxoGua-SH. The crude reaction mixtures are directly analysed by PAGE.

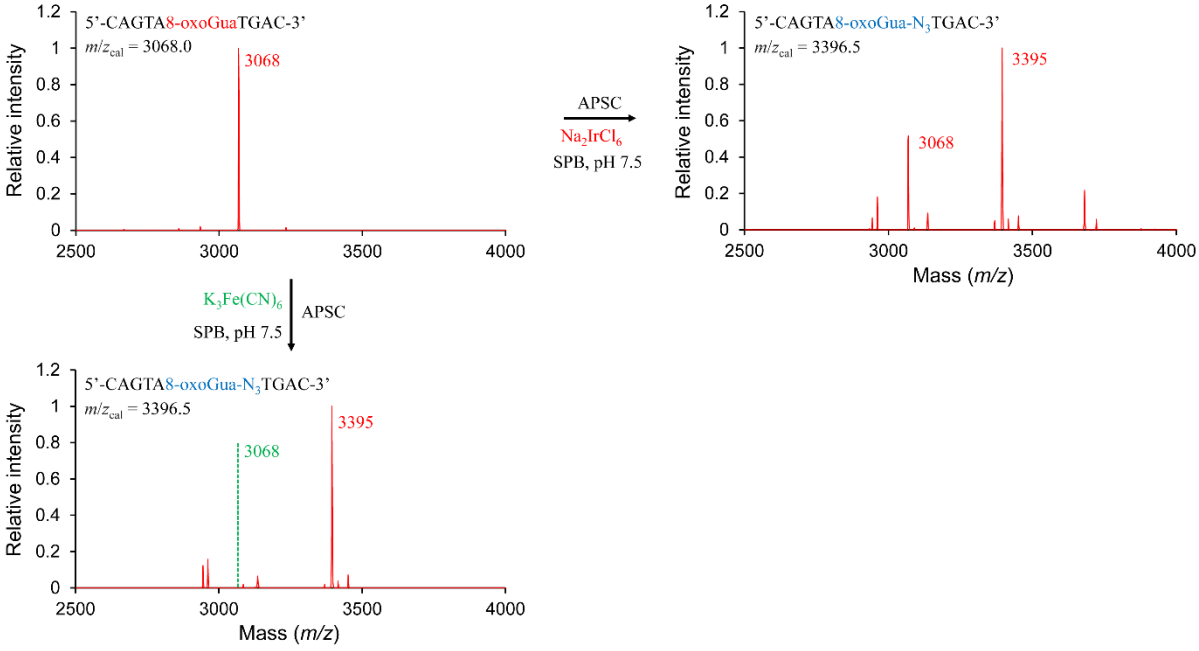
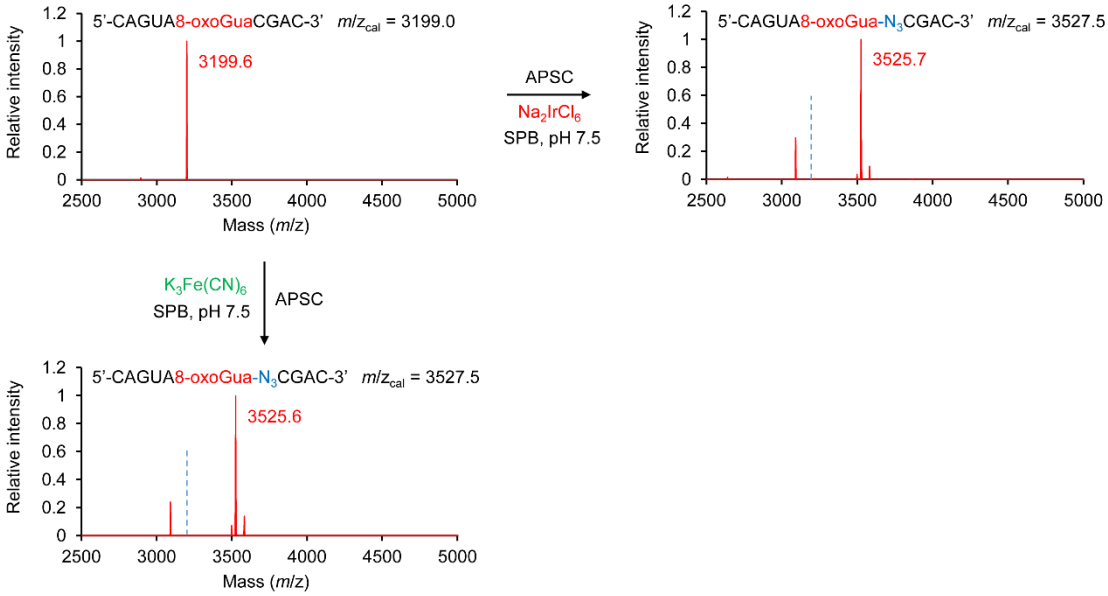


Fig. S3. The formation of APSC adduct in a 10-mer DNA containing an 8-oxoGua at the presence of $K_3Fe(CN)_6$ or Na_2IrCl_6 in sodium phosphate buffer (SPB, 20 mM, pH 7.5), respectively. The higher reaction efficiency was observed under oxidation with $K_3Fe(CN)_6$.



453

454 **Fig. S4.** The formation of APSC adduct in a 10-mer RNA containing an 8-oxoGua at
455 the presence of $K_3Fe(CN)_6$ or Na_2IrCl_6 in SPB (20 mM, pH 7.5), respectively. The same
456 high reaction efficiency (almost 100%) was observed under both conditions.

457

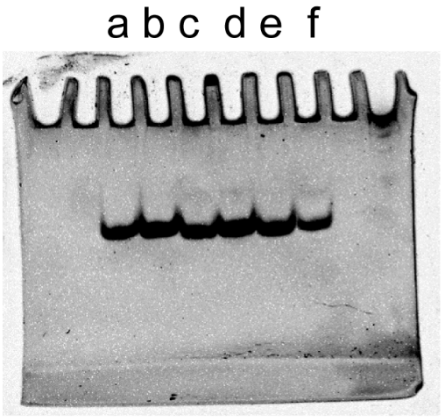


Fig. S5. PAGE analysis for the reaction between APSC and 10-mer DNA containing 8-BrGua, 8-oxoAde or 5-fCyt, respectively, upon oxidation with $K_3Fe(CN)_6$. Lane a: 8-BrGua, Lane b: 8-BrGua with APSC, Lane c: 8-oxoAde, Lane d: 8-oxoAde with APSC, Lane e: 5-fCyt, Lane f: 5-fCyt with APSC. No APSC adducts were formed in these reactions.

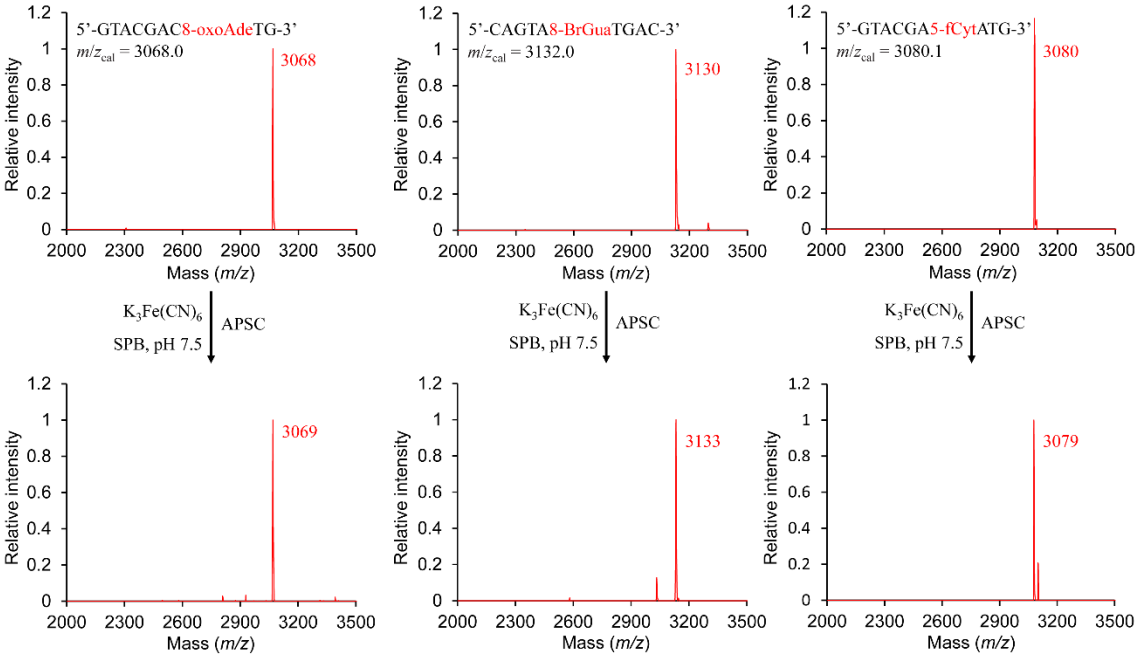


Fig. S6. Electrospray ionization-ion trap mass spectrometry (ESI-ITMS) analysis for the reaction between APSC and 10-mer DNA containing 8-BrGua, 8-oxoAde or 5-fCyt, respectively. No APSC adducts were detected.

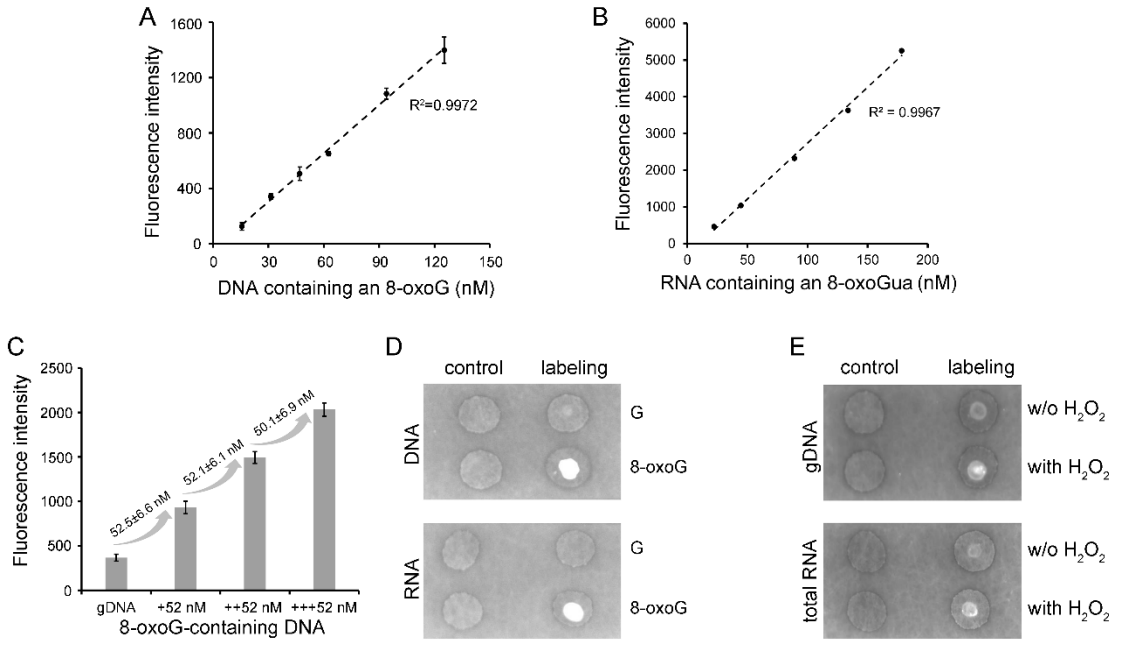
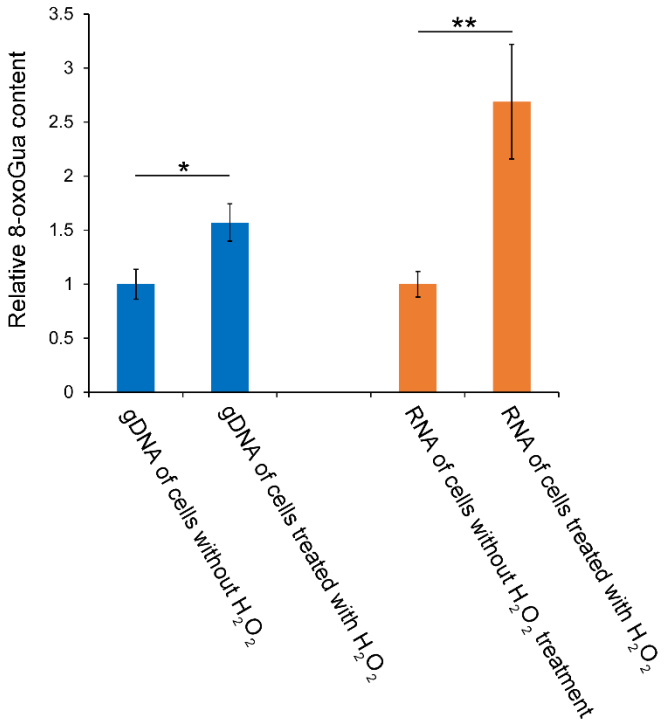
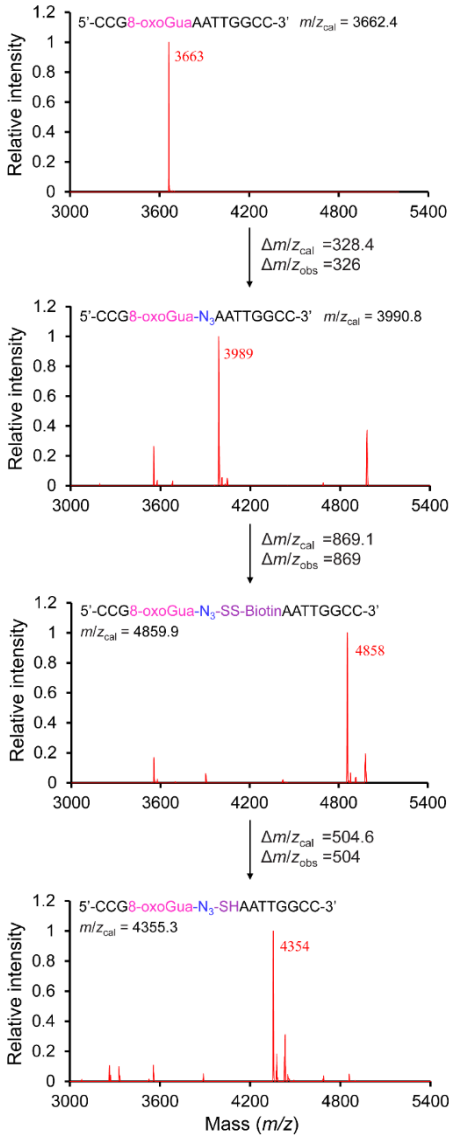


Fig. S7. Efficient labeling of 8-oxoGua in DNA and RNA, respectively, with APSC and subsequent DBCO-FITC. Fluorescence intensity of FITC-labeled (A) DNA and (B) RNA containing an 8-oxoGua is linearly related to DNA and RNA concentration, respectively. (C) Determination of the increased concentration of 8-oxoGua-containing DNA added into gDNA solution. The measured increase of 8-oxoGua concentration (indicated with arrow) is in agreement with actual increase of 8-oxoGua concentration. (D) Dot-blot assay of DNA or RNA templates labeled with APSC and DBCO-FITC. Row G: The template without 8-oxoGua. Row 8-oxoGua: The template with an 8-oxoGua. Lane control: The starting materials. Lane labeling: FITC-labeled samples. (E) Dot-blot assay of gDNA and total RNA extracted from the VSMCs treated with or without (w/o) H2O2, respectively. Lane control: The starting materials. Lane labeling: FITC-labeled samples.



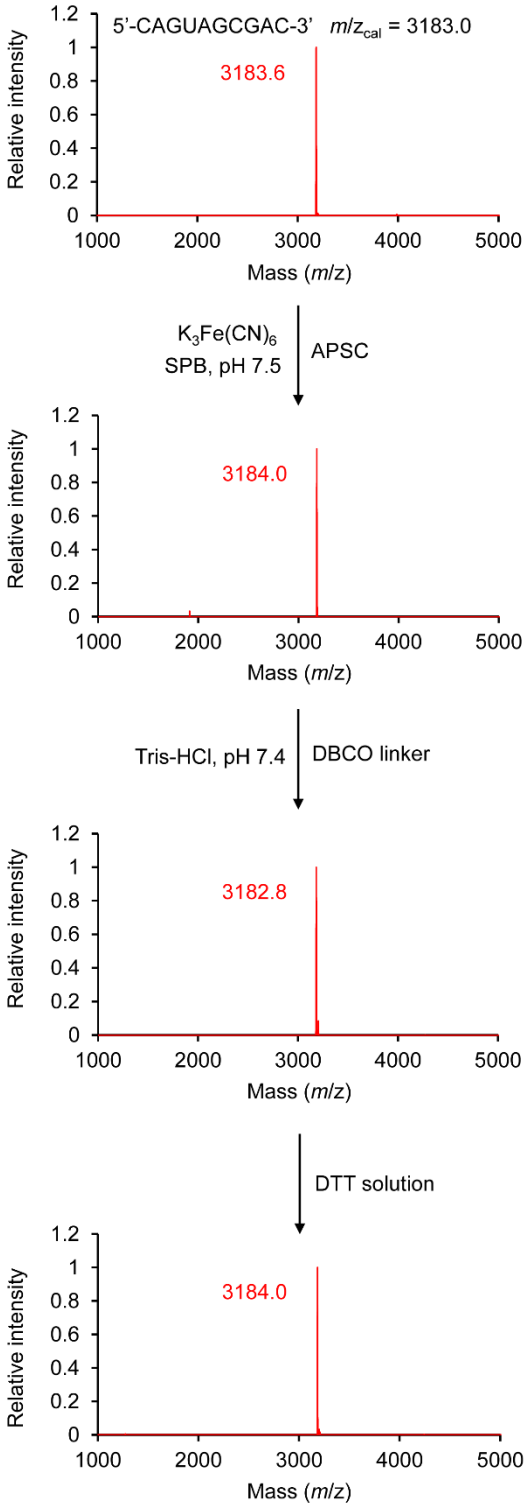
484

485 **Fig. S8.** Relative 8-oxoGua content in DNA and RNA of the VSMCs treated with or
486 without H_2O_2, respectively, measured after FITC labeling. Statistical significance is
487 indicated with * ($p \leq 0.05$) and ** ($p \leq 0.01$).
488



489

490 **Fig. S9.** ESI-ITMS characterization of the 12-nt DNA templates containing an 8-
491 oxoGua, 8-oxoGua-N3, 8-oxoGua-N3-SS-Biotin, and 8-oxoGua-N3-SH, respectively.
492 The molecular weight is indicated with calculated value (m/z_{cal}) and observed value
493 (m/z_{obs}), respectively.
494



495

496 **Fig. S10.** ESI-ITMS characterization of the 10-nt RNA templates (without 8-oxoGua)
497 undergoing APSC tagging reaction, click reaction with DBCO linker, and treatment
498 with DTT. The molecular weight has not changed throughout the process. This result
499 indicates the APSC adduct is not formed with the natural RNA without 8-oxoGua and
500 the RNA chain is not degraded.
501

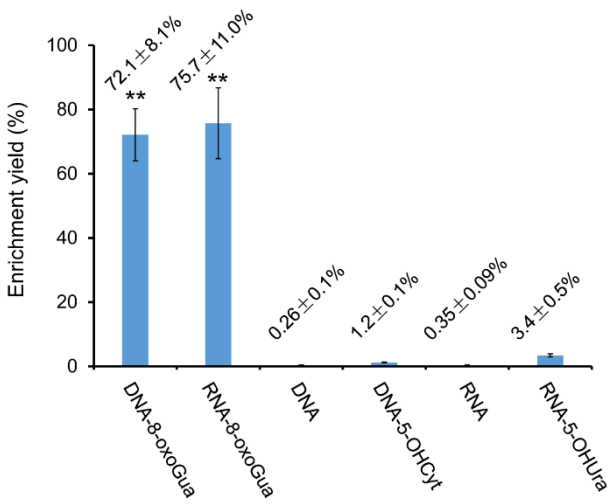


Fig. S11. Assessment for pull-down enrichment yield of the DNA and RNA templates, respectively. Statistical significance is indicated with ** ($p \leq 0.01$) between DNA-8-oxoGua and DNA-5-OHCyt as well as between RNA-8-oxoGua and RNA-5-OHUra, respectively.

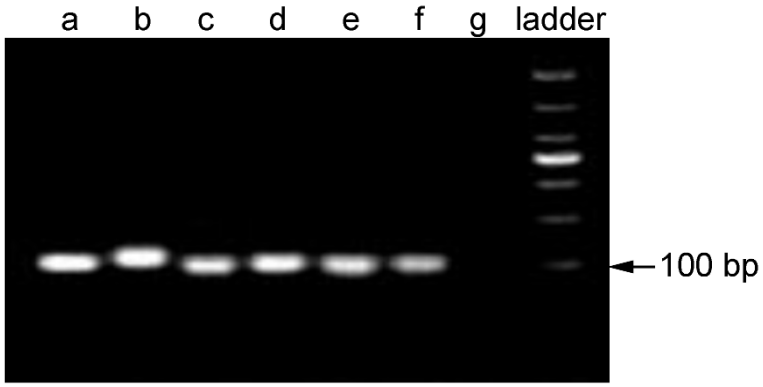


Fig. S12. Agarose gel electrophoresis analysis of PCR product. Lane a: 100-bp DNA template. Lane b: 100-bp DNA containing 8-oxoGua-N3-SH. Lane c: PCR product with Deep Vent® (exo-) DNA Polymerase (NEB). Lane d: PCR product with TruePrep Amplify Enzyme (Vazyme). Lane e: PCR product with Pfu DNA Polymerase (Promega). Lane f: PCR product with Q5® High-Fidelity DNA Polymerases (NEB). Lane g: PCR product of the enriched sample using 8-oxoGua enrichment lacking APSC labeling, under Deep Vent® (exo-) DNA Polymerase (NEB). No PCR product indicated almost no nonspecific enrichment of DNA.

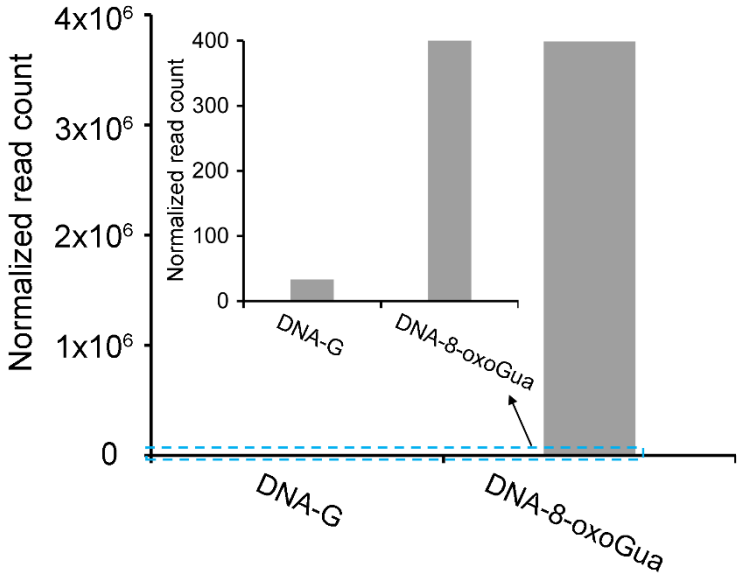


Fig. S13. Next-generation sequencing tests for enrichment of the DNA with 8-oxoGua (DNA-8-oxoGua) than the DNA without 8-oxoGua (DNA-G) that were spiked in genomic DNA fragments.

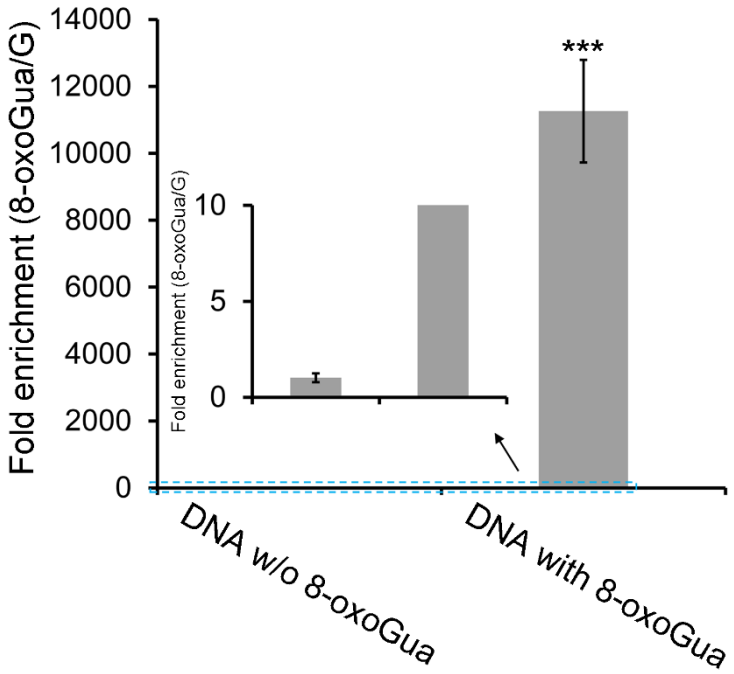
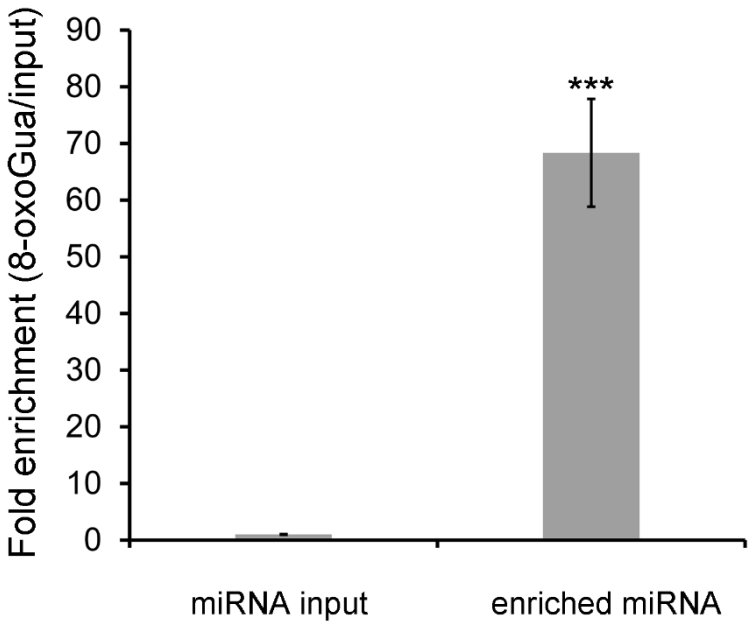


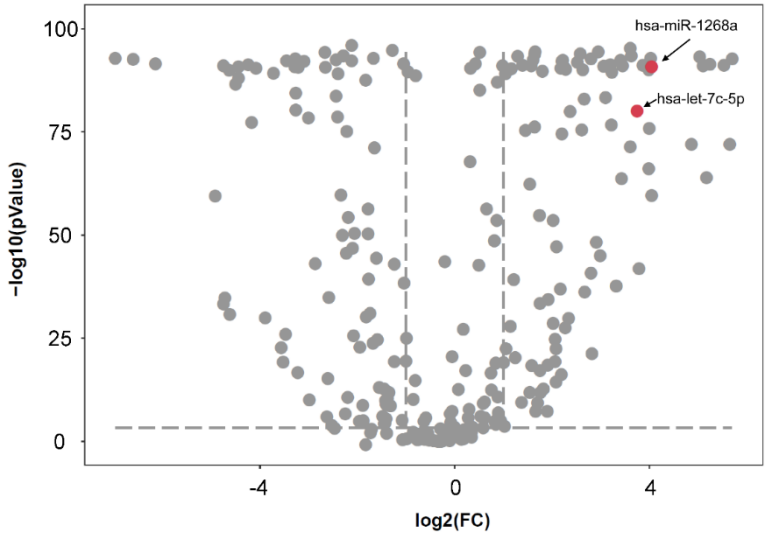
Fig. S14. qPCR verification for enrichment of the spiked DNA with or without (w/o) an 8-oxoGua.



528

529 **Fig. S15.** qPCR verification for enrichment of the spiked 8-oxoGua-containing RNA.

530



531

532 **Fig. S16.** The results of APSC-8-oxoGua-seq for miRNAs of the VSMCs without H_2O_2
533 treatment. The red dot indicated the typical miRNAs with 8-oxoGua modification in
534 seed region.

535

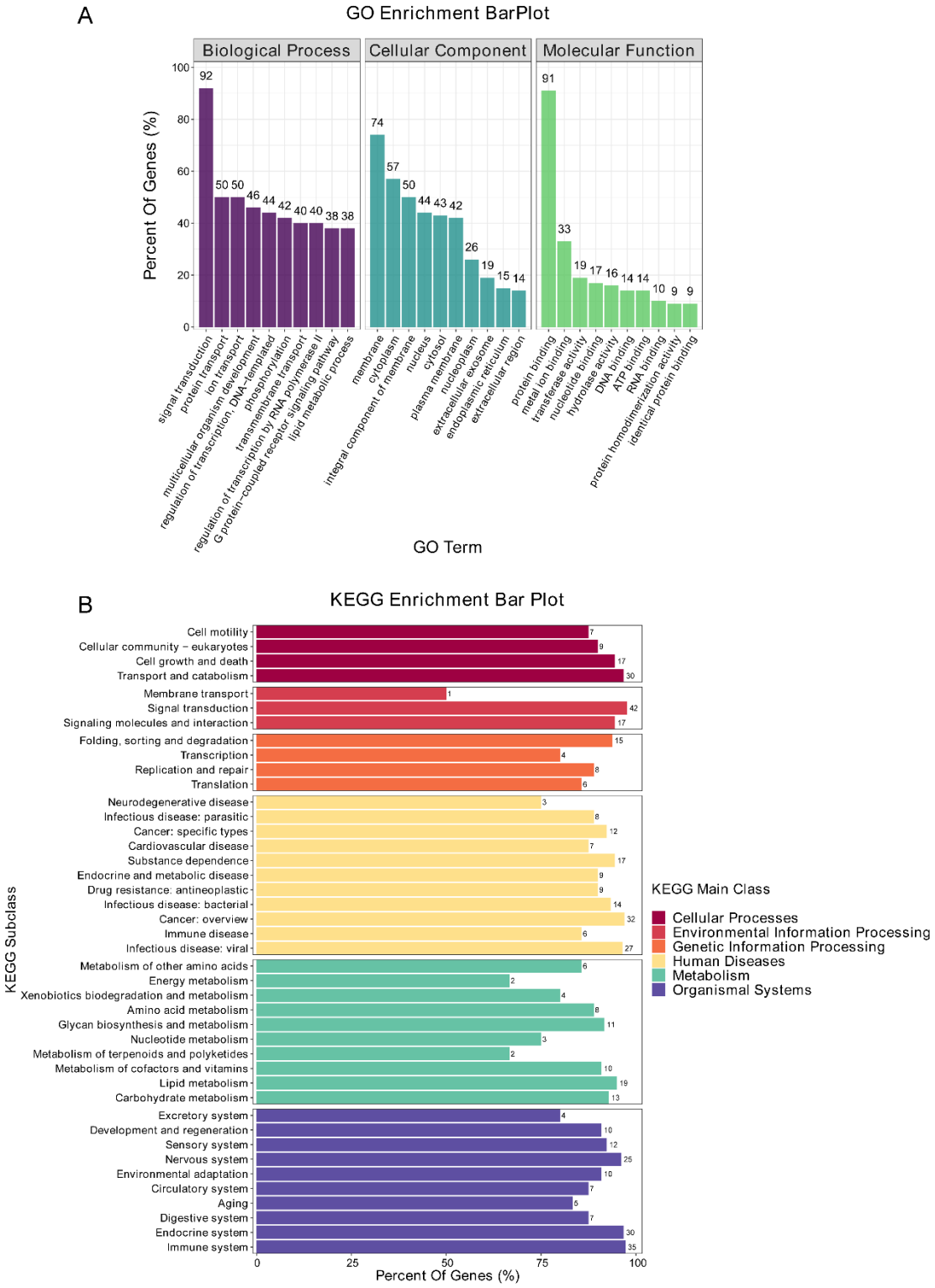


Fig. S17. GO analysis (A) and KEGG analysis (B) for the differential target genes after substituting 8-oxoGua with U at position 2 of miR-1268a sequence.

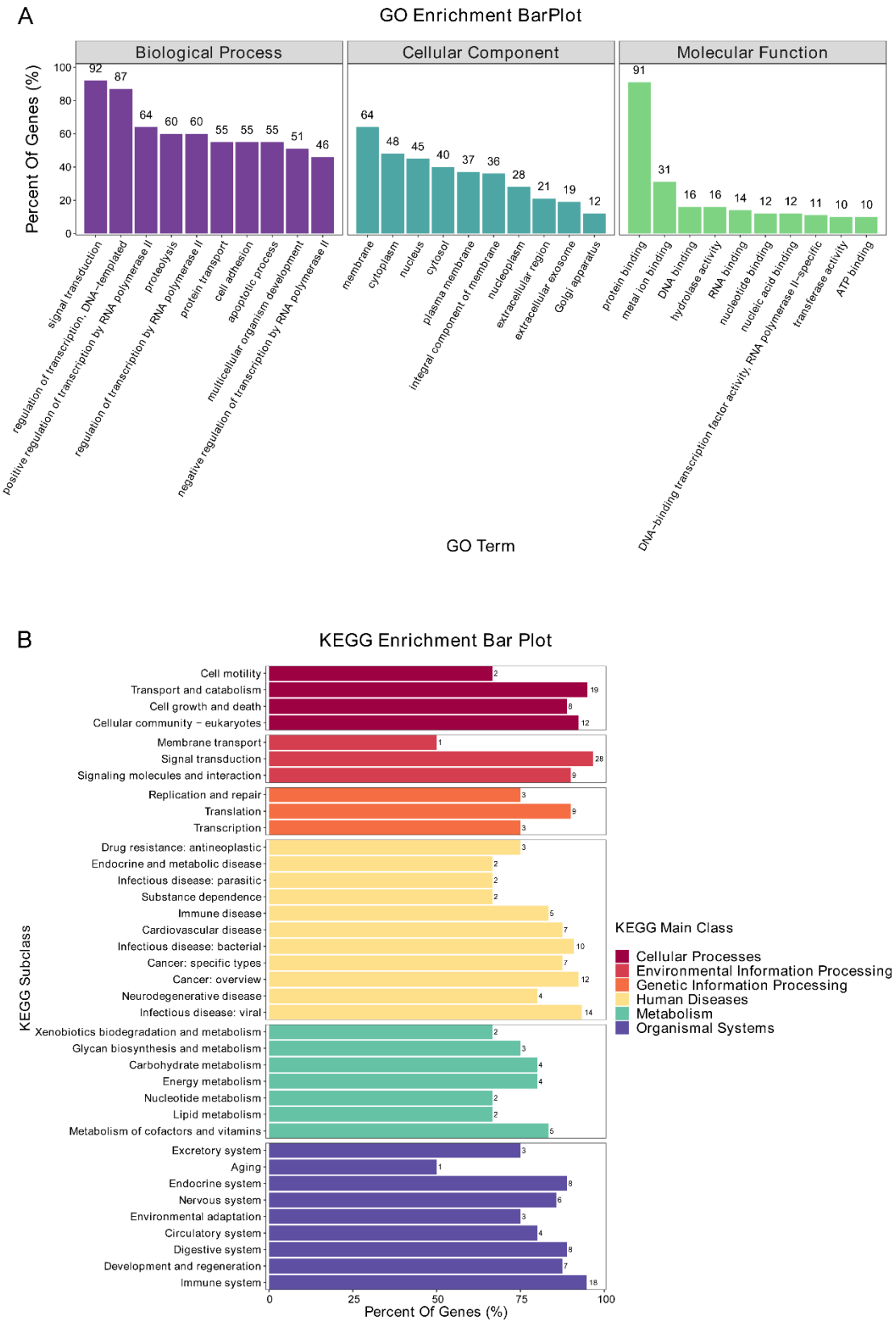


Fig. S18. GO analysis (A) and KEGG analysis (B) of the differential target genes after substituting 8-oxoGua with U at position 8 of miR-1268a sequence.

544 **Table S1.** The overlapped oxidized miRNAs.

Name	Sequence (5'-3')
pal-miR-9993a-3p	ATCTCGGTGGGACCTCCA
hsa-miR-3168_R+1_1ss14AC	GAGTTCTACAGTCCGACA
hsa-miR-320d_R-1	AAAAGCTGGGTTGAGAGG
bta-miR-2478_L-2	ATCCCACTTCTGACACCA
hsa-miR-320a-3p	AAAAGCTGGGTTGAGAGGGCGA
hsa-miR-139-5p	TCTACAGTGCACGTGTCTCCAGT
hsa-miR-320b_R-2	AAAAGCTGGGTTGAGAGGGC
hsa-miR-3620-5p_R-2	GTGGGCTGGGCTGGGCTGGG
mmu-miR-3968_L-3_1ss14AT	ATCCCACTCCTGACACCA
cgr-mir-1285-p3_1ss1TC	CACTGCACTCCAGCCTGGGC
oan-miR-1386	CTCCTGGCTGGCTCGCCA
hsa-miR-92b-5p_R+2	AGGGACGGGACGCGGTGCAGTGTT
pal-miR-9226-5p_L-3	AGTCCCTGTTCGGGCGCCA
eca-miR-296_L-1R-3_1ss10GT	AGGGTTGGTTGGAGGCTT
pal-miR-9993b-3p_1ss9GT	ATCTCGCTTGGGCCTCCA
hsa-miR-1275_R+1	GTGGGGGAGAGGCTGTCG
PC-3p-31279_64	AGCAGGACTGTGGCCATGGAAGT
hsa-mir-1303-p5	CTGGGCAACATAGCGAGAC
hsa-miR-486-5p	TCCTGTACTGAGCTGCCCCGAG
hsa-miR-125a-5p	TCCCTGAGACCCTTTAACCTGTGA
hsa-miR-320c_R-1	AAAAGCTGGGTTGAGAGGG
hsa-miR-483-5p_L-1R+3	AGACGGGAGGAAAGAAGGGAGTGG
hsa-miR-4484_L+2R-4	AAAAAAGGCGGGAGAAGC
hsa-miR-23a-5p	GGGGTTCCTGGGGATGGGATTT
hsa-miR-375-3p	TTTGTTCGTTCGGCTCGCGTGA
eca-miR-296_L-1R-2	AGGGTTGGGTGGAGGCTTT
pal-miR-9993b-3p	ATCTCGCTGGGGCCTCCA
oan-miR-1386_1ss7GA	CTCCTGACTGGCTCGCCA
hsa-miR-574-5p	TGAGTGTGTGTGTGTGAGTGTGT
hsa-miR-122-5p_R-1	TGGAGTGTGACAATGGTGTTT
hsa-mir-5100-p3_1ss17TC	ATCCCAGCGGTGCCTCCA
mmu-mir-6240-p3_1ss22GT_1	ATTTCTGCCCAGTGCTCTGAATG
efu-mir-9277-p5_1ss1CG	GGTGGCCGAGTGGTTAAG

hsa-miR-150-5p	TCTCCCAACCCTTGTACCAGTG
hsa-miR-99a-5p_R-1	AACCCGTAGATCCGATCTTGT
hsa-let-7c-5p	TGAGGTAGTAGGTTGTATGGTT
PC-3p-14138_148	AAAAGGGGGCTGAGGTGGAGG
hsa-miR-1268a	CGGGCGTGGTGGTGGGGG
hsa-miR-126-3p	TCGTACCGTGAGTAATAATGCG
hsa-miR-205-5p	TCCTTCATTCCACCGGAGTCTG
hsa-miR-145-5p	GTCCAGTTTTCCCAGGAATCCCT
hsa-miR-143-3p_R+1	TGAGATGAAGCACTGTAGCTCT
hsa-miR-203a-3p	GTGAAATGTTTAGGACCACTAG
hsa-miR-126-5p	CATTATTACTTTTGGTACGCG
mmu-mir-1983-p3_1ss2GA	TAATGCCGAGGTTGTGAGTT

Table S2. APSC-8-oxoGua-seq result of a 100-bp DNA template containing two 8-oxoGua modification.

Position-base[a]	A[b]	C[b]	G[b]	T[b]
1C	0	30	0	0
2C	0	29	1	0
3G	0	0	30	0
4C	0	30	0	0
5T	0	0	0	30
6C	0	30	0	0
7G	0	0	30	0
8A	30	0	0	0
9G	0	0	30	0
10A	30	0	0	0
11T	0	0	0	30
12C	0	30	0	0
13G	0	0	30	0
14A	30	0	0	0
15T	0	0	0	30
16C	0	30	0	0
17G	0	0	30	0
18A	30	0	0	0
19T	0	0	0	30
20C	0	30	0	0
21G	0	0	30	0
22A	30	0	0	0
23T	0	0	0	30

24C	0	30	0	0
25G	0	0	30	0
26A	30	0	0	0
27T	0	0	0	30
28C	0	30	0	0
29G	0	0	30	0
30A	30	0	0	0
31A	30	0	0	0
32T	0	0	0	30
33C	0	30	0	0
8-oxoGua	0	0	3	27
35A	30	0	0	0
36T	0	0	0	30
37C	0	30	0	0
38G	0	0	30	0
39A	30	0	0	0
40T	0	0	0	30
41C	0	30	0	0
42G	0	0	30	0
43A	30	0	0	0
44T	0	0	0	30
45C	0	30	0	0
46G	0	0	30	0
47A	30	0	0	0
48T	0	0	0	30
49C	0	30	0	0
50G	0	0	30	0
51A	30	0	0	0
52T	0	0	0	30
53C	0	30	0	0
54A	30	0	0	0
55T	0	0	0	30
56C	0	30	0	0
57G	0	0	30	0
58T	0	0	0	30
59A	30	0	0	0
60G	0	0	30	0
61C	0	30	0	0
62A	30	0	0	0
63T	0	0	0	30
64C	0	30	0	0
8-oxoGua	0	0	5	25
66A	30	0	0	0
67C	0	30	0	0

68A	30	0	0	0
69T	0	0	0	30
70G	0	0	30	0
71A	30	0	0	0
72T	0	0	0	30
73C	0	30	0	0
74G	0	0	30	0
75A	30	0	0	0
76T	0	0	0	30
77C	0	30	0	0
78G	0	0	30	0
79A	30	0	0	0
80A	30	0	0	0
81T	0	0	0	30
82C	0	30	0	0
83G	0	0	30	0
84A	30	0	0	0
85T	0	0	0	30
86C	0	30	0	0
87G	0	0	30	0
88A	30	0	0	0
89T	0	0	0	30
90C	0	29	0	1
91G	0	0	30	0
92A	29	0	1	0
93G	0	0	30	0
94G	0	0	30	0
95T	0	0	0	30
96A	30	0	0	0
97C	0	30	0	0
98C	0	30	0	0
99C	0	30	0	0
100A	30	0	0	0

[a] Position and pre-designed base type in positive-sense strand of DNA from 5' to 3'.
[b] The number of base (namely A, C, G, T) in sequencing reads at each position.

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