Electronic Supplementary Information

Accurate identification of 8-oxoguanine in RNA at single-nucleotide resolution using ligase-dependent qPCR

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Supplementary tables and figures

Table S1. The sequences of the nucleic acids used in this assay.

Name	Sequence (5′-3′ direction)
RNA-1-G	CUUGGUUUUGUUUGAAUGCAUGUAUAUAGUCAU
RNA-1-o^{8}G	CUUGGUUUUGUUUGAAUo^{8}GCAUGUAUAUAGUCAU
Probe L	/PO$_4$/ATTCAAACAAAACCAAGCTCTATGGGCAGTCGGTGATATCGACCTGTACCTCT
Probe R	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTATGACTATATACATrGrC
DNA Probe R	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTATGACTATATACATGC
Probe L2	/PO$_4$/AAAACCAAGCTCTATGGGCAGTCGGTGATATCGACCTGTACCTCT
Probe R2	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTATGACTATATACATGCATTCAArArC
miR-124 probe L1	/PO$_4$/TTACTCTATGGGCAGTCGGTGATATCGACCTGTACCTCT
miR-124 probe R1	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTGGCATTCACCGCGTGrCrC
miR-124 probe L2	/PO$_4$/CGCGTGCCTTACTCTATGGGCAGTCGGTGATATCGACCTGTACCTCT
miR-124 probe R2	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTGGCATTCrArC
Let7a probe L1	/PO$_4$/TCACTCTATGGGCAGTCGGTGATATCGACCTGTACCTCT
Let7a probe R1	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTAACTATACAACCTACTArCrC
Let7a probe L2	/PO$_4$/CTACTACCTCACTCTATGGGCAGTCGGTGATATCGACCTGTACCTCT
Let7a probe R2	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTAACTAT

	ACArArC
miR-29a probe L1	/PO_4/TACTCTATGGGCAGTCGGTGATATCGACCTGTACCTCT
miR-29a probe R1	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTTAACCG ATTTCAGATGGTrGrC
miR-29a probe L2	/PO_4/AGATGGTGCTACTCTATGGGCAGTCGGTGATATCGACCT GTACCTCT
miR-29a probe R2	CCATCTCATCCCTGCGTGTCCATCATCCGTACGTGTTAACCG ATTrTrC
universal primer F	CCATCTCATCCCTGCGTGTC
universal primer R	AGAGGTACAGGTCGATATCA

1 Optimization of the ligation ligation-based qPCR method.

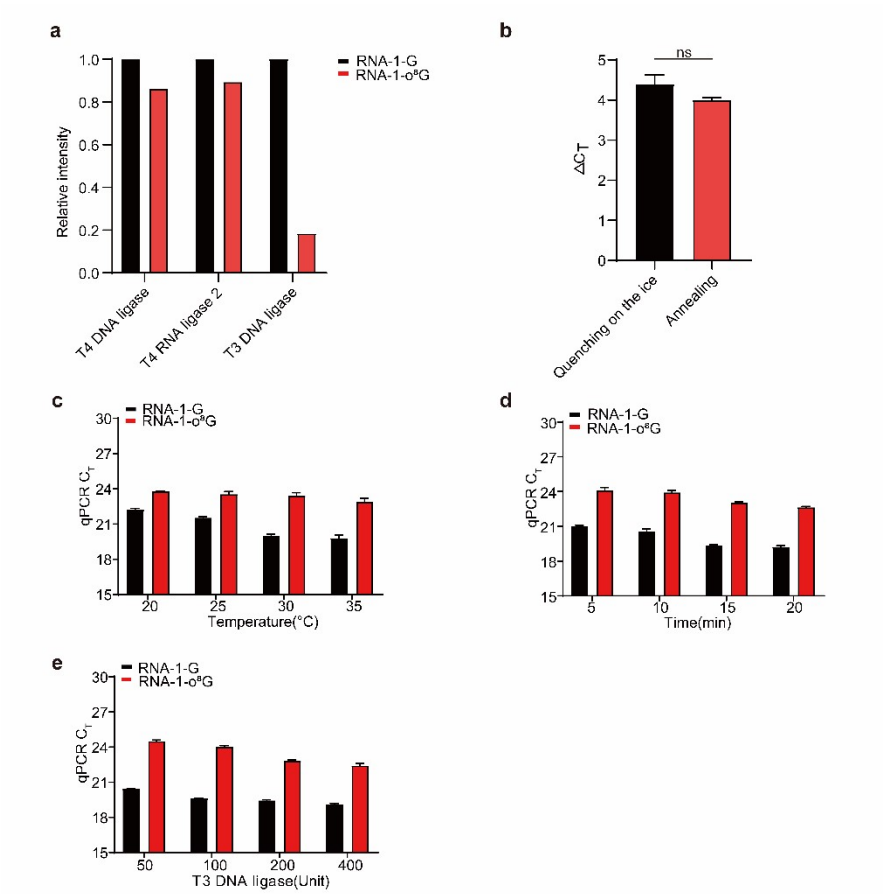

Figure. S1 Optimization of the ligation ligation-based qPCR method.
(**a**) The relative ratios of pixel intensities between the RNA-1-G segment and the RNA-1-o^{8}G segment, determined using different ligases. (**b**) Comparison of two conditions prior to the ligation process. **(c-e)** Optimization of ligation reaction parameters, including temperature **(c)**, ligation time **(d)**, and T3 DNA ligase concentration **(e)**, for the ligation-dependent qPCR assay. The bar graph displays the qPCR threshold cycle (C_T) values between RNA-1-o^{8}G and RNA-1-G under varied reaction conditions.

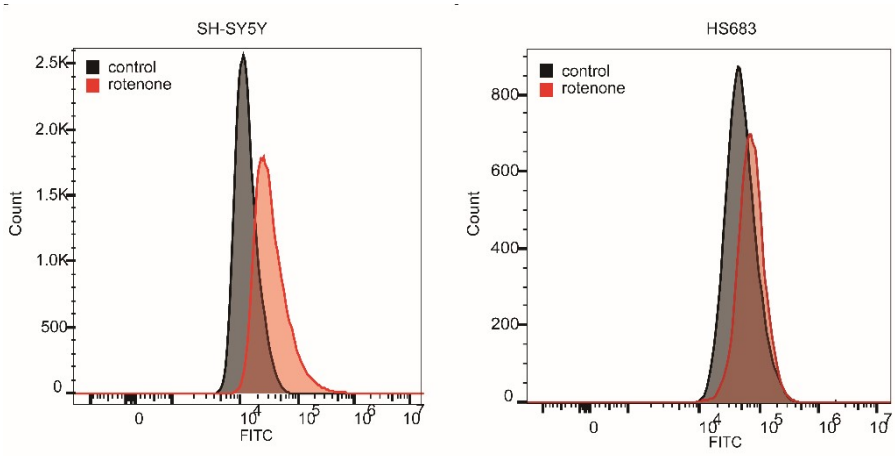

Figure S2 The ROS levels are increased by rotenone treatment in neuronal cells.
(a-b) Flow cytometry analysis depicting ROS levels in SH-SY5Y cells **(a)** and HS683 cells **(b)** following treatment with DMSO (vehicle control) and 5 μM rotenone.

3 Identification of miRNA o^{8}G sites in HS683 cells with ROS damage

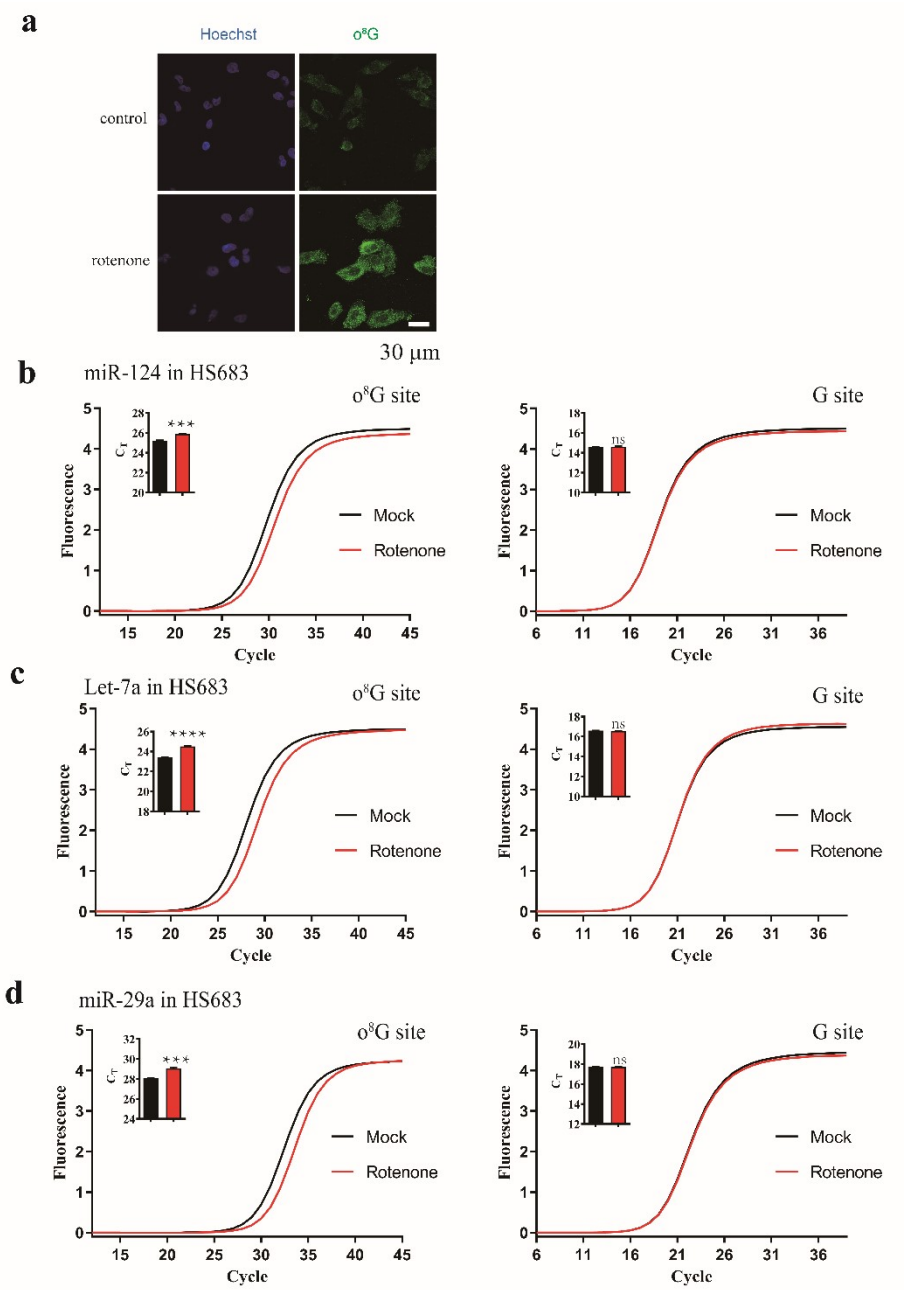

Figure S3 Identification of miRNA o^{8}G sites in HS683 cells with ROS damage.
(a) Fluorescence immunostaining images displaying the presence of o^{8}G in control HS683 cells and cells treated with 5 μM rotenone. The scale bar represents 30 μm. **(b-d)** Real-time qPCR amplification curves and corresponding bar graphs showing the threshold cycle (C_T) values for the detection of o^{8}G and G sites (used as input control) in miR-124 **(b)**, let-7a **(c)**, and miR-29a **(d)** using the ligation-based qPCR assay.

4 Rotenone treatment decreases viability in SH-SY5Y cells

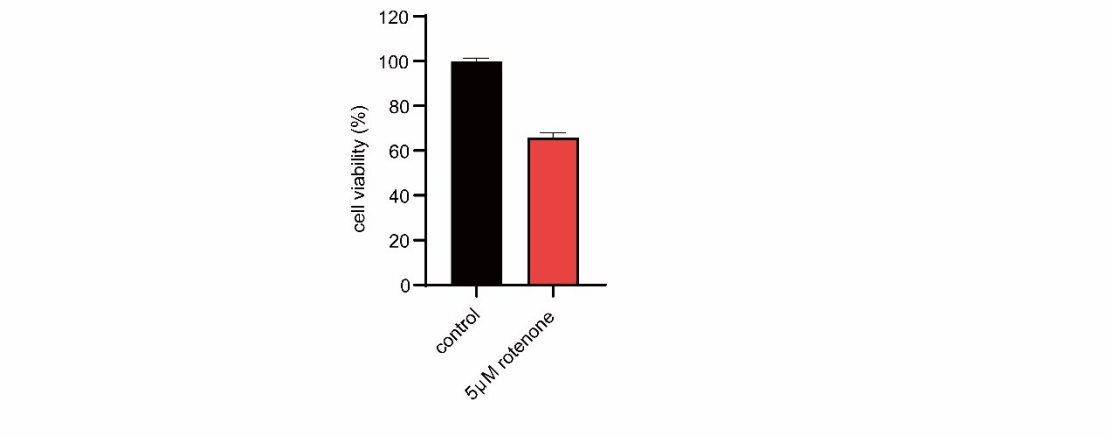

Figure S4 Rotenone treatment decreases viability in SH-SY5Y cells.