ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

A scalable 3D-printed brain tumour model for high-throughput screening glioblastoma biology and drug response

Supplementary File

Table S1. Sequences of PCR primers and TaqMan probes used for ddPCR assays in this study. Probes were labelled with 6-FAM at 5' end

Target miRNA	Mature miRNA sequence	Stem-loop sequence
hsa-miR-490-5p	CCAUGGAUCUCCAGGUGGGU	UGGAGGCCUUGCUGGUUUGGAAAGUUCAUUGUUCGACAC CAUGGAUCUCCAGGUGGGUCAAGUUUAGAGAUGCACCAAC CUGGAGGACUCCAUGCUGUUGAGCUGUUCACAAGCAGCGGA CACUUCCA
hsa-miR-137-5p	ACGGGUAUUCUUGGGUGGAUAAU	GGUCCUCUGACUCUCUUCGGUGACGGGUAUUCUUGGGUG GAUAAUACGGAUUACGUUGUUAUUGCUUAAGAAUACGCGUA GUCGAGGAGAGUACCAGCGGCA
hsa-miR-876-3p	UGGUGGUUUACAAAGUAAUUCA	UGAAGUGCUGUGGAUUUCUUUGUGAAUCACCAUAUCUAAG CUAAUGUGGUGGUGGUUUACAAAGUAAUUCAUAGUGCUUCA
hsa-miR-876-5p	UGGAUUUCUUUGUGAAUCACCA	UGAAGUGCUGUGGAUUUCUUUGUGAAUCACCAUAUCUAAG CUAAUGUGGUGGUGGUUUACAAAGUAAUUCAUAGUGCUUCA
hsa-miR-448	UUGCAUAUGUAGGAUGUCCCAU	GCCGGGAGGUUGAACAUCCUGCAUAGUGCUGCCAGGAAAUC CCUAUUUCAUAUAAGAGGGGGCUGGCUGGUUGCAUAUGUAG GAUGUCCCAUCUCCCAGCCCACUUCGUCA
hsa-miR-501-3p	AAUGCACCCGGGCAAGGAUUCU	GCUCUUCCUCUCUAAUCCUUUGUCCCUGGGUGAGAGUGCU UUCUGAAUGCAAUGCACCCGGGCAAGGAUUCUGAGAGGGU GAGC
Endogenous control (miR-191-5p)	CAACGGAAUCCCAAAAGCAGCUG	CGGCUGGACAGCGGGCAACGGAAUCCCAAAAGCAGCUGUU GUCUCCAGAGCAUUCCAGCUGCGCUUGGAUUUCGUCCCCUG CUCUCCUGCCU

Table S2. The amount of extracted total RNAs

Sample	Elution volume (μL)	Concentration (ng/μL)	Total RNA yield (ng)	A260/A280
KNS42 control	11	126.31	1389.41	1.69
KNS42 treated with 16 μM TMZ	11	151.69	1668.59	1.67
KNS42 treated with 32 μM TMZ	11	130.49	1435.39	1.70
KNS42 treated with 64 μM TMZ	11	93.57	1029.27	1.62
KNS42 treated with 125 μM TMZ	11	120.35	1323.85	1.77
KNS42 treated with 250 μM TMZ	11	98.68	1085.48	1.65
KNS42 treated with 500 μM TMZ	11	92.72	1019.92	1.66
KNS42 treated with 1000 μM TMZ	11	58.92	648.12	1.61
SF188 control	10	44.17	441.70	1.61
SF188 treated with 16 μM TMZ	10	48.49	484.90	1.59
SF188 treated with 32 μM TMZ	10	42.11	421.10	1.60
SF188 treated with 64 μM TMZ	10	50.09	500.90	1.61
SF188 treated with 125 μM TMZ	10	53.12	531.20	1.60
SF188 treated with 250 μM TMZ	10	22.86	228.60	1.59
SF188 treated with 500 μM TMZ	10	25.61	256.10	1.66
SF188 treated with 1000 μM TMZ	10	13.01	130.10	1.75

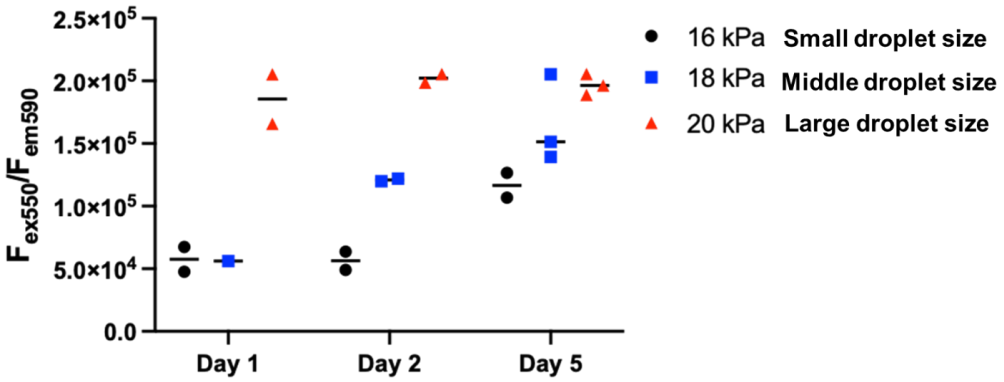

Figure S1. Growth of paediatric high-grade glioma SF188 tumoroids bioprinted with 5% (w/v) GelMA hydrogel. SF-188 cell-laden GelMA droplets were printed at extrusion pressures of 16, 18 and 20 kPa to produce small, medium and large droplets, respectively. The growth of printed GBM cells was evaluated by PrestoBlue™ assay on days 1, 2 and 5 cultures.

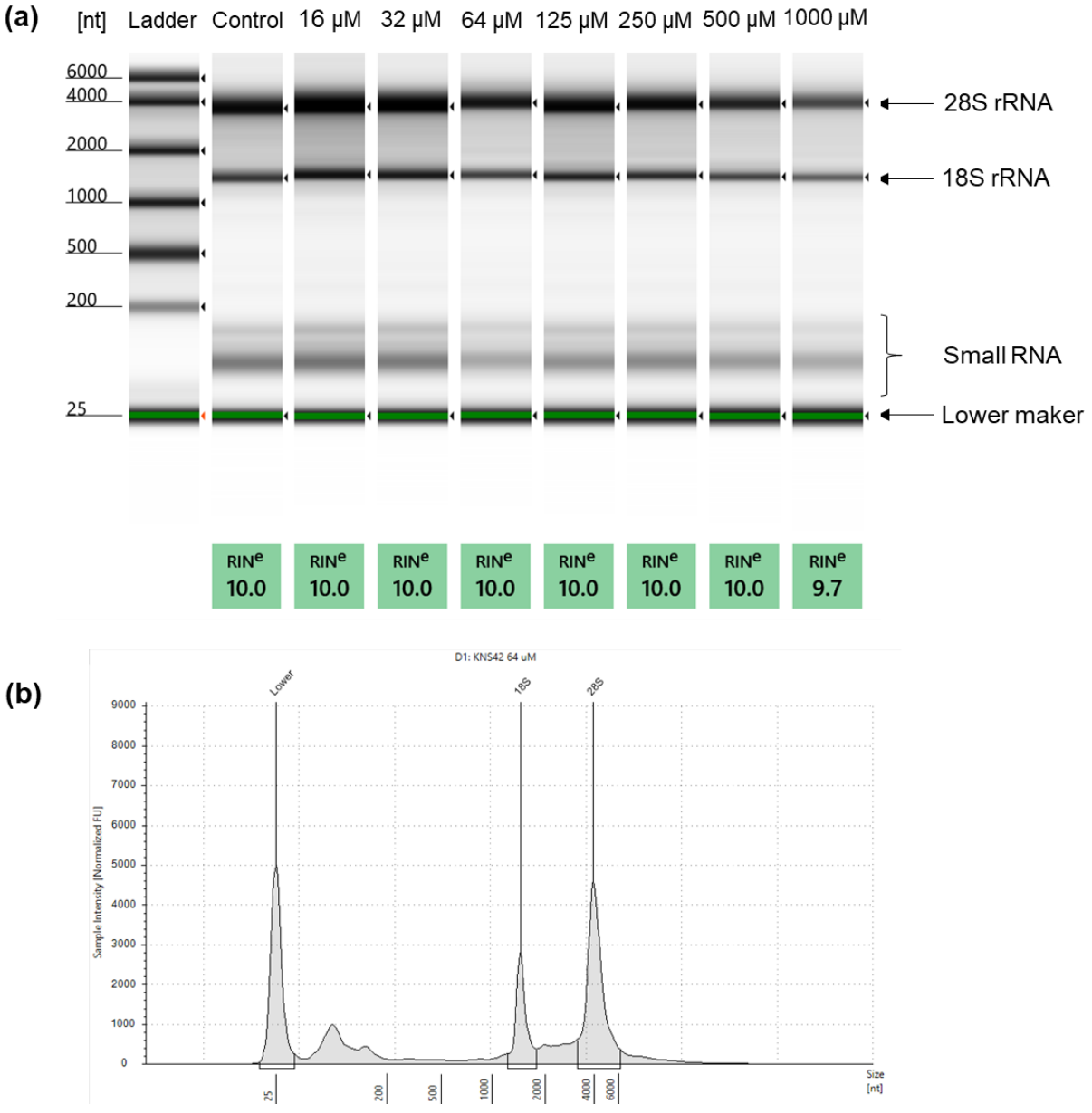

Figure S2. Assessment of the quality of RNA extracted from KNS42 cells embedded in GelMA hydrogel for 48 days, followed by treatment with TMZ at various concentrations using the Agilent 4200 TapeStation system. (a) The gel image presents the separation profile of each sample along with RIN values ranging from 1 to 10, where 10 indicates the highest quality RNA and 1 signifies degraded RNA. The electropherogram displays ribosomal RNA peaks of 28S and 18S, including annotations, as well as small RNA and the lower marker. (b) A representative electropherogram of total RNA extracted from KNS42 cells treated with 64 μM TMZ.

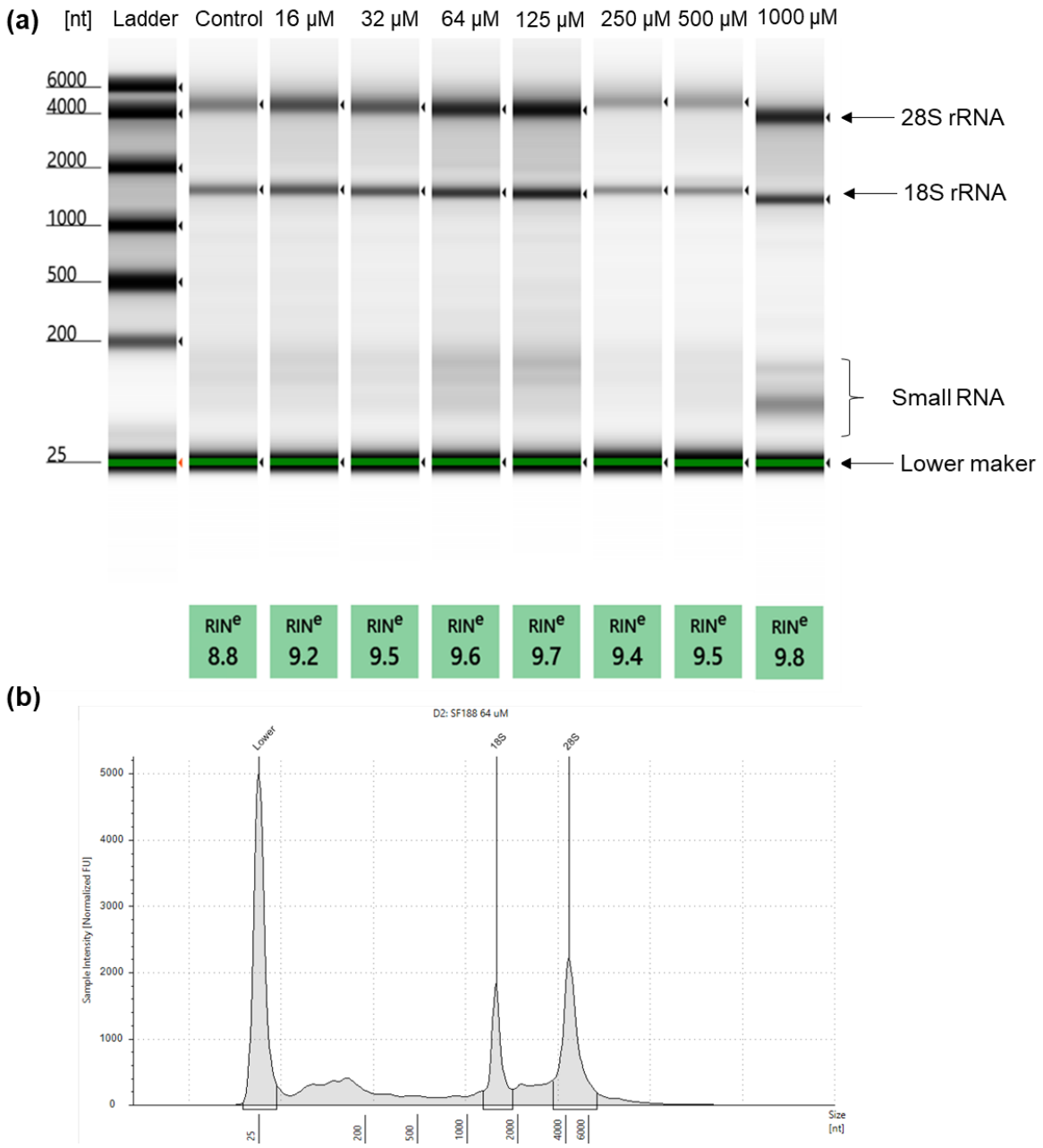

Figure S3. Assessment of the quality of RNA extracted from SF188 cells embedded in GelMA hydrogel for 48 days, followed by treatment with TMZ at various concentrations using the Agilent 4200 TapeStation system. (a) The gel image displays the separation profile of each sample along with RIN values ranging from 1 to 10, where 10 indicates the highest quality RNA and 1 signifies degraded RNA. The electropherogram illustrates ribosomal RNA peaks of 28S and 18S with annotations, along with small RNA and the lower marker. (b) A representative electropherogram of total RNA extracted from SF188 cells treated with 64 μM TMZ.

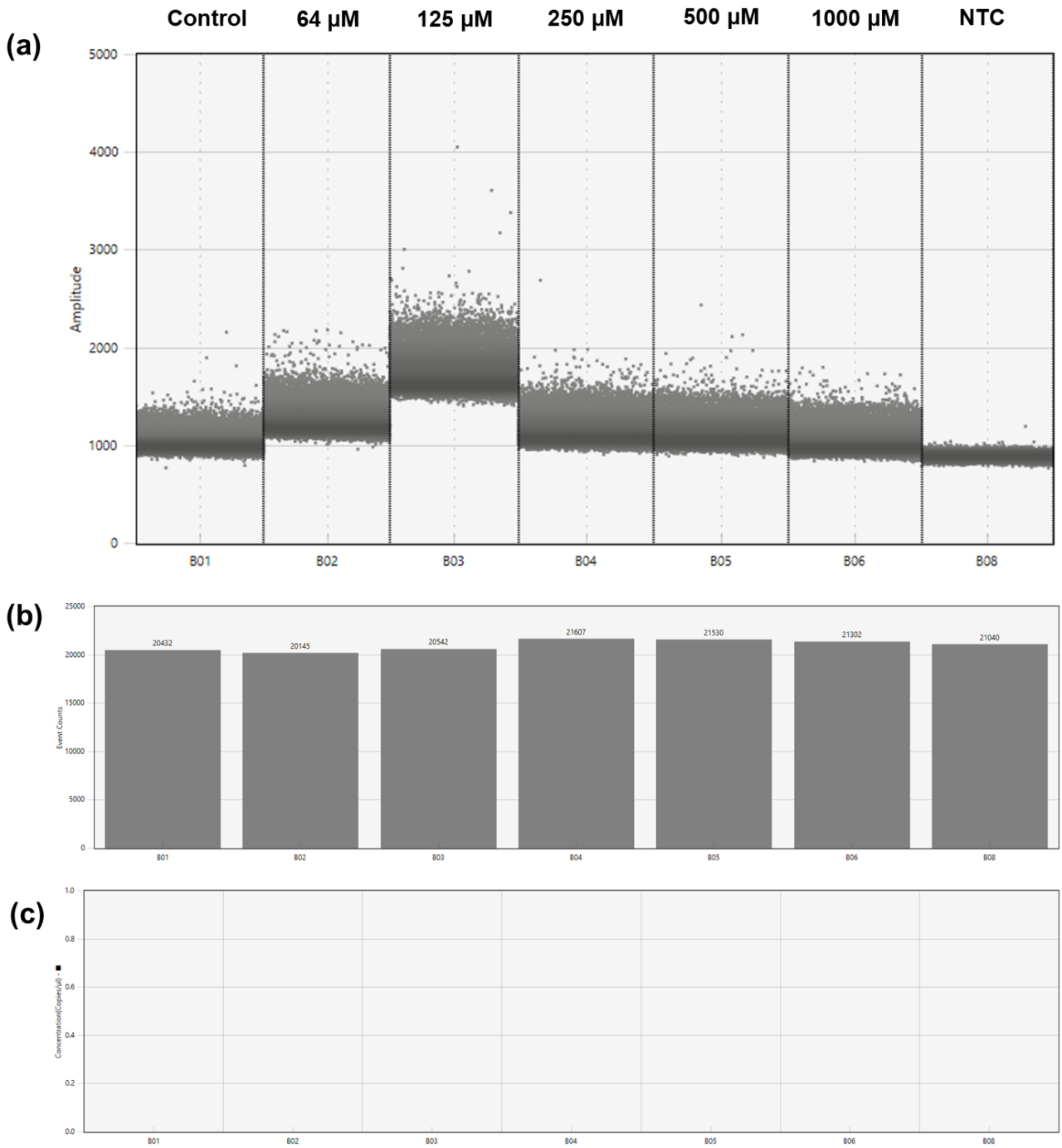

Figure S4. ddPCR of miRNA-876-3p expression of KNS42 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-876-3p (copies/μL) detected in each condition.

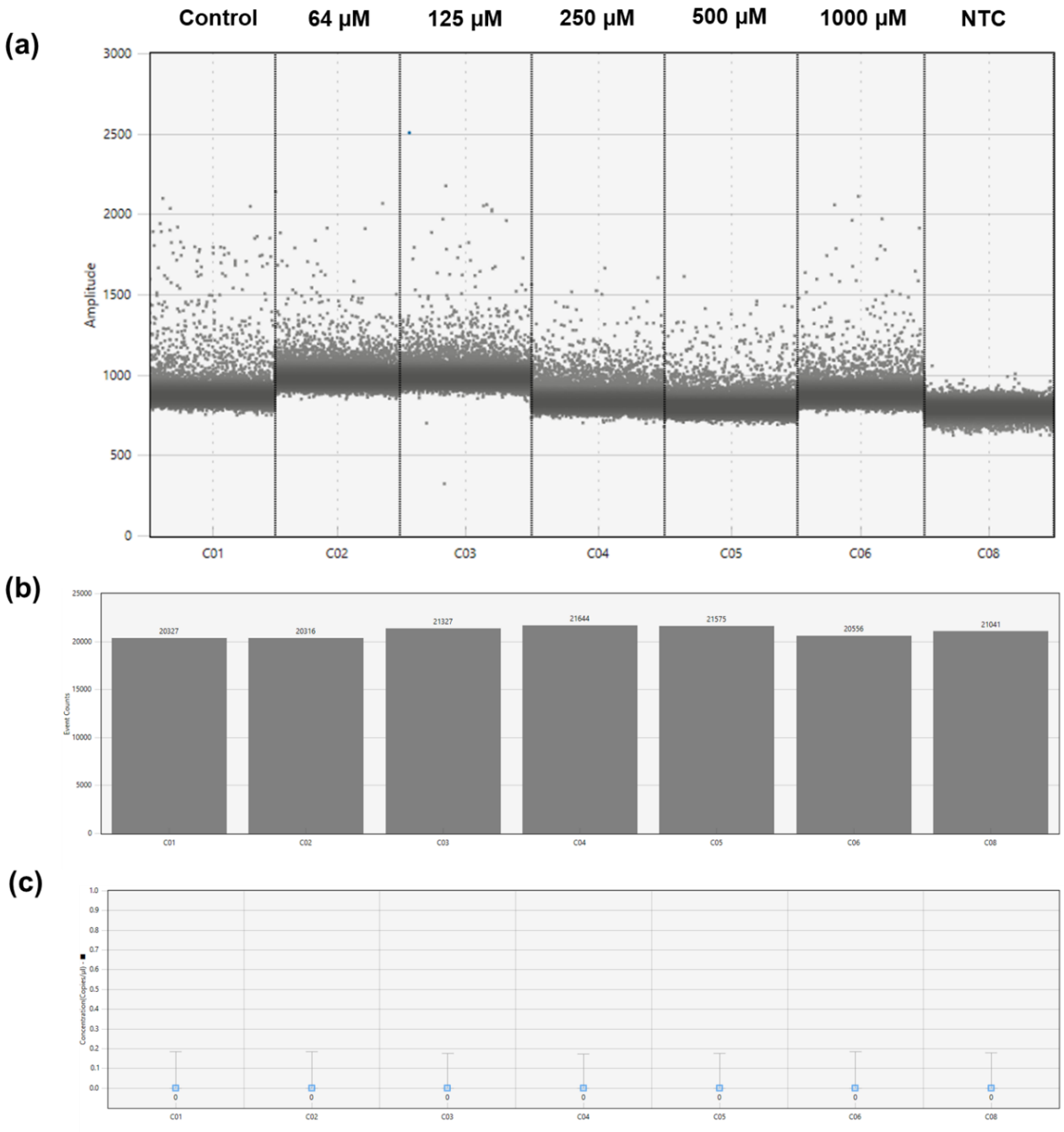

Figure S5. ddPCR of miRNA-876-5p expression of KNS 42 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-876-5p (copies/μL) detected in each condition.

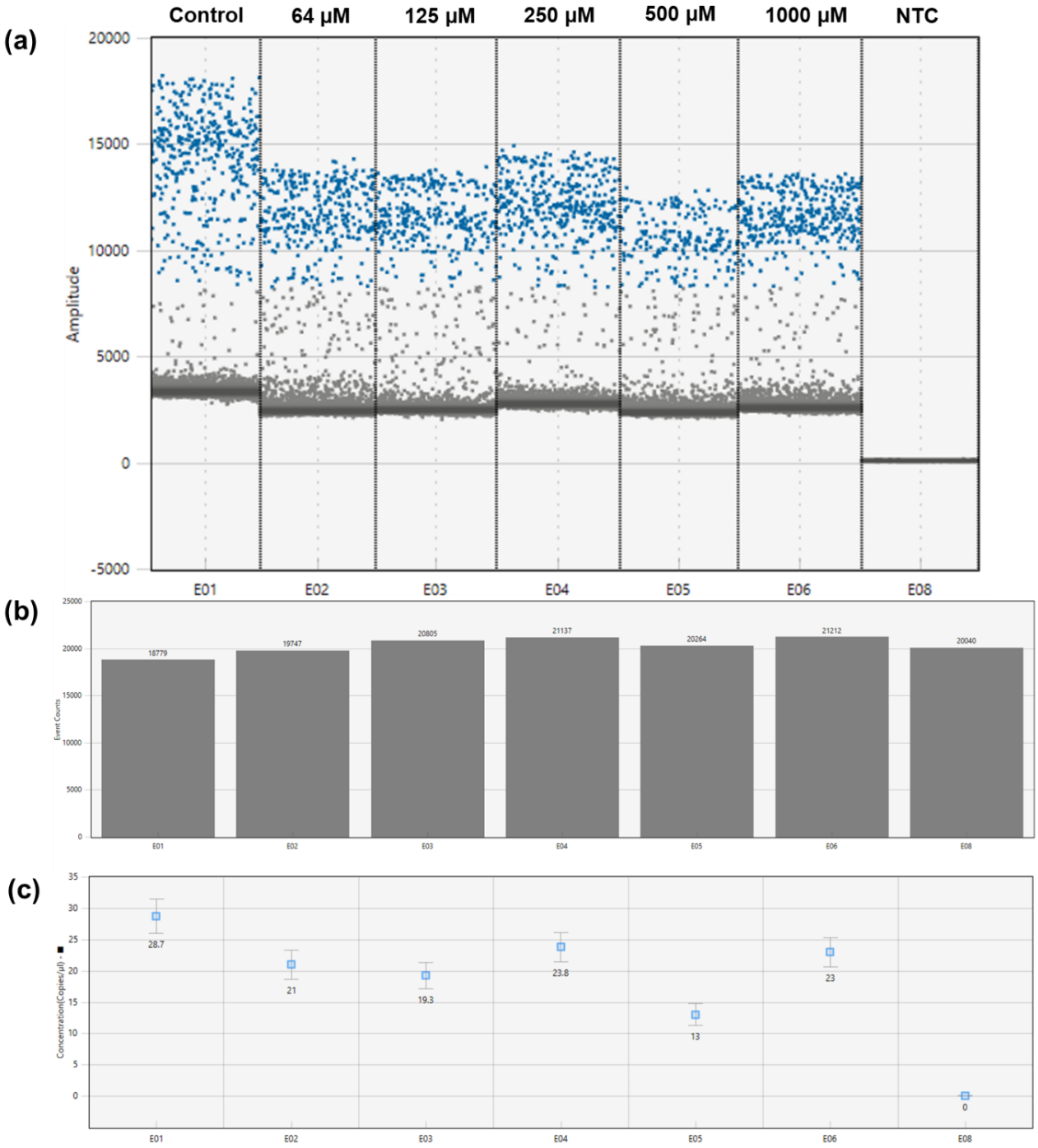

Figure S6. ddPCR of miRNA-137 expression of KNS 42 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 µM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-137 (copies/µL) detected in each condition.

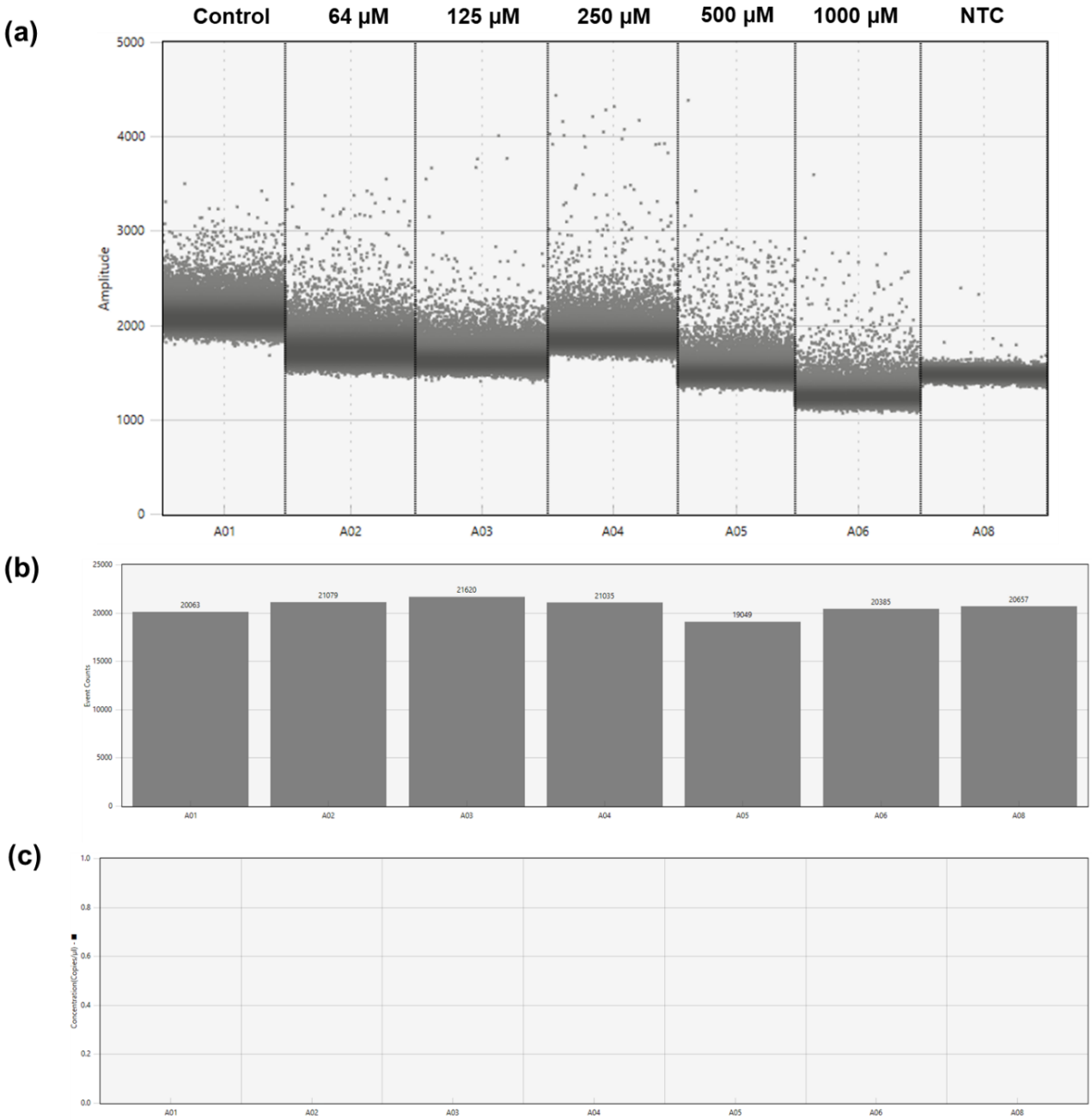

Figure S7. ddPCR of miRNA-490 expression of KNS 42 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 µM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-490 (copies/µL) detected in each condition.

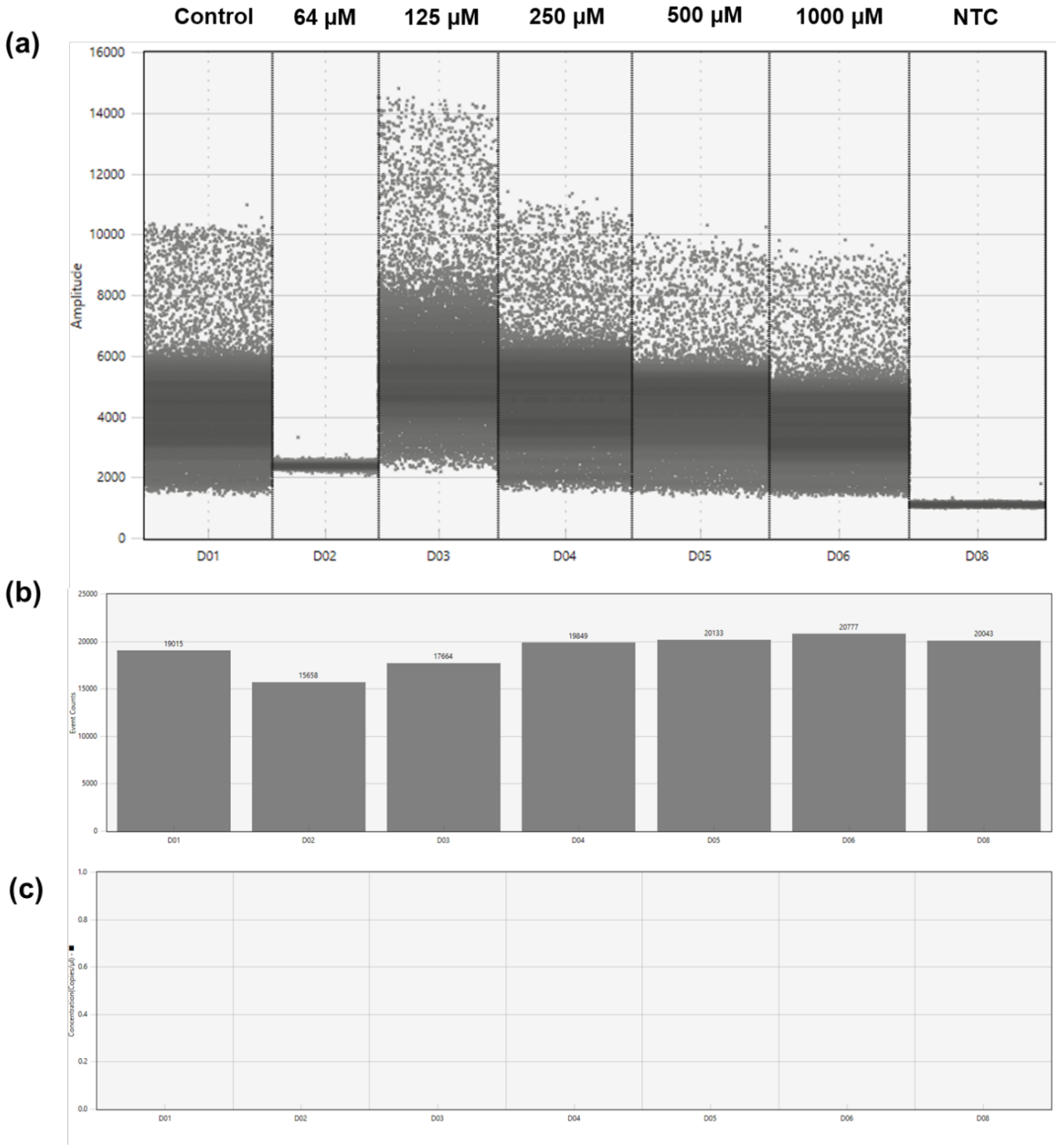

Figure S8. ddPCR of miRNA-448 expression of KNS 42 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 17,000 total droplets detected, and a minimum number of positive droplets of ≥3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-448 (copies/μL) detected in each condition.

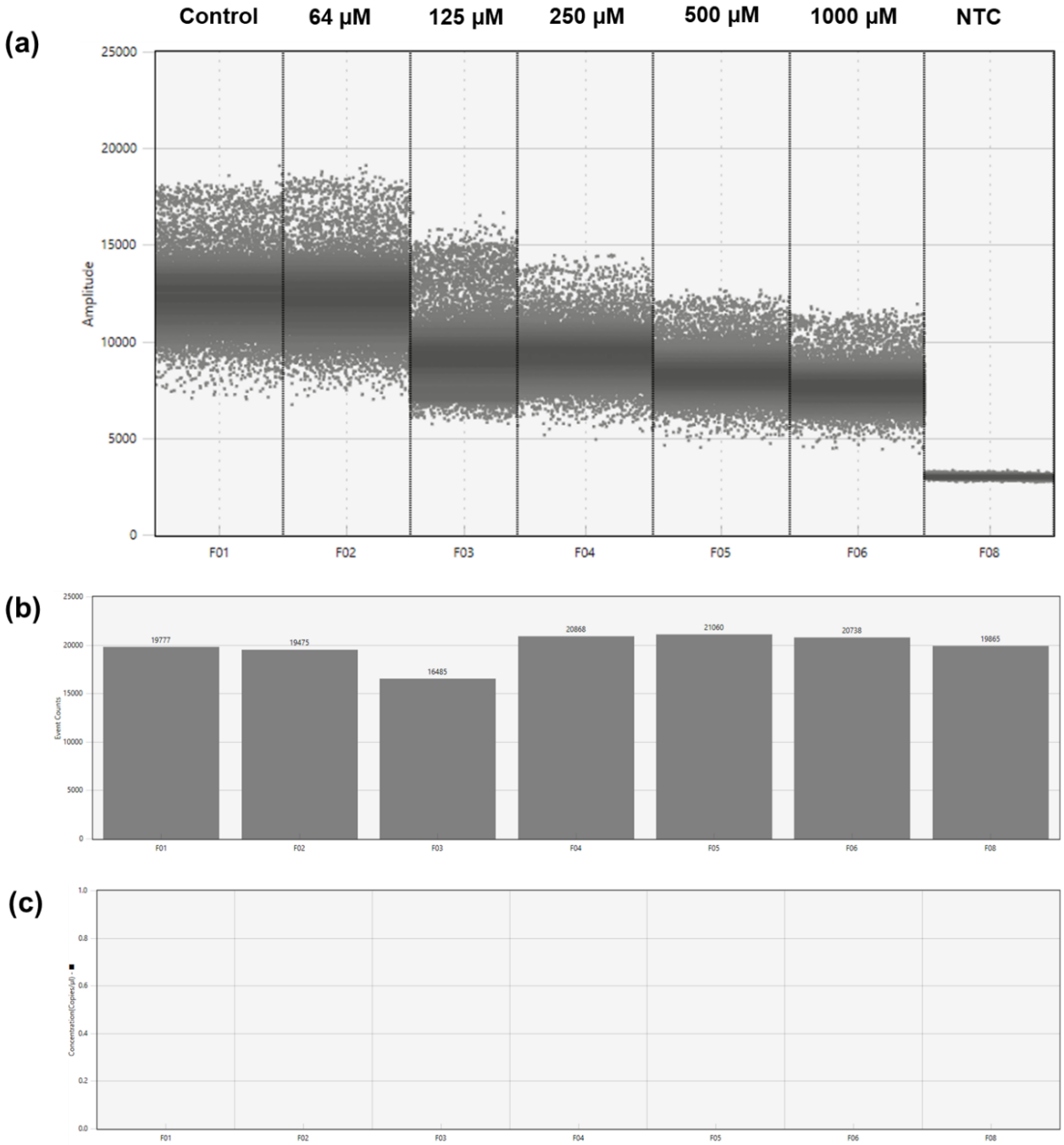

Figure S9. ddPCR of miRNA-501-3p expression of KNS 42 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 17,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-501-3p (copies/μL) detected in each condition.

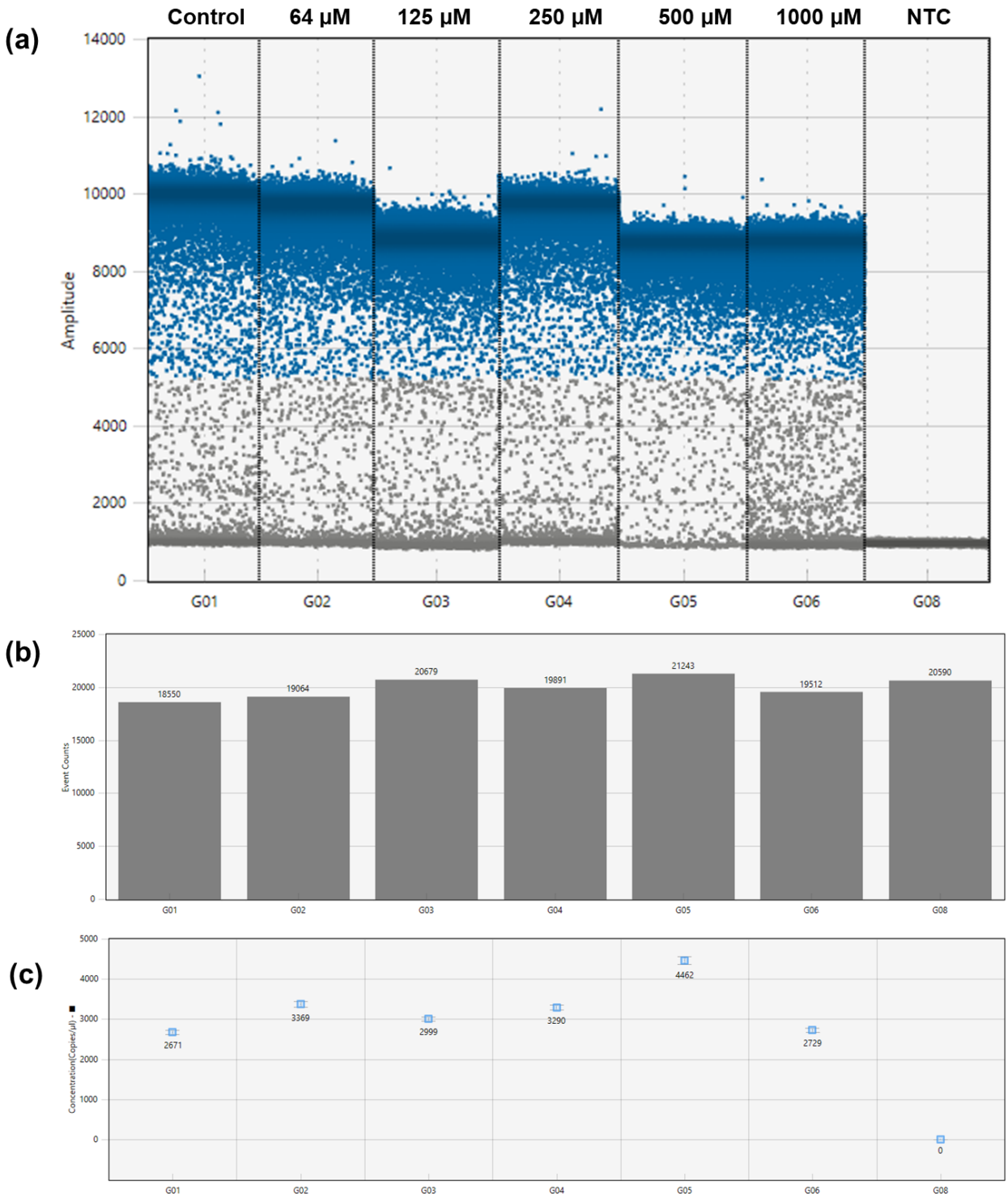

Figure S10. ddPCR of miRNA-191-5p expression (endogenous control) of KNS 42 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 µM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 20,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-191-5p (copies/µL) detected in each condition.

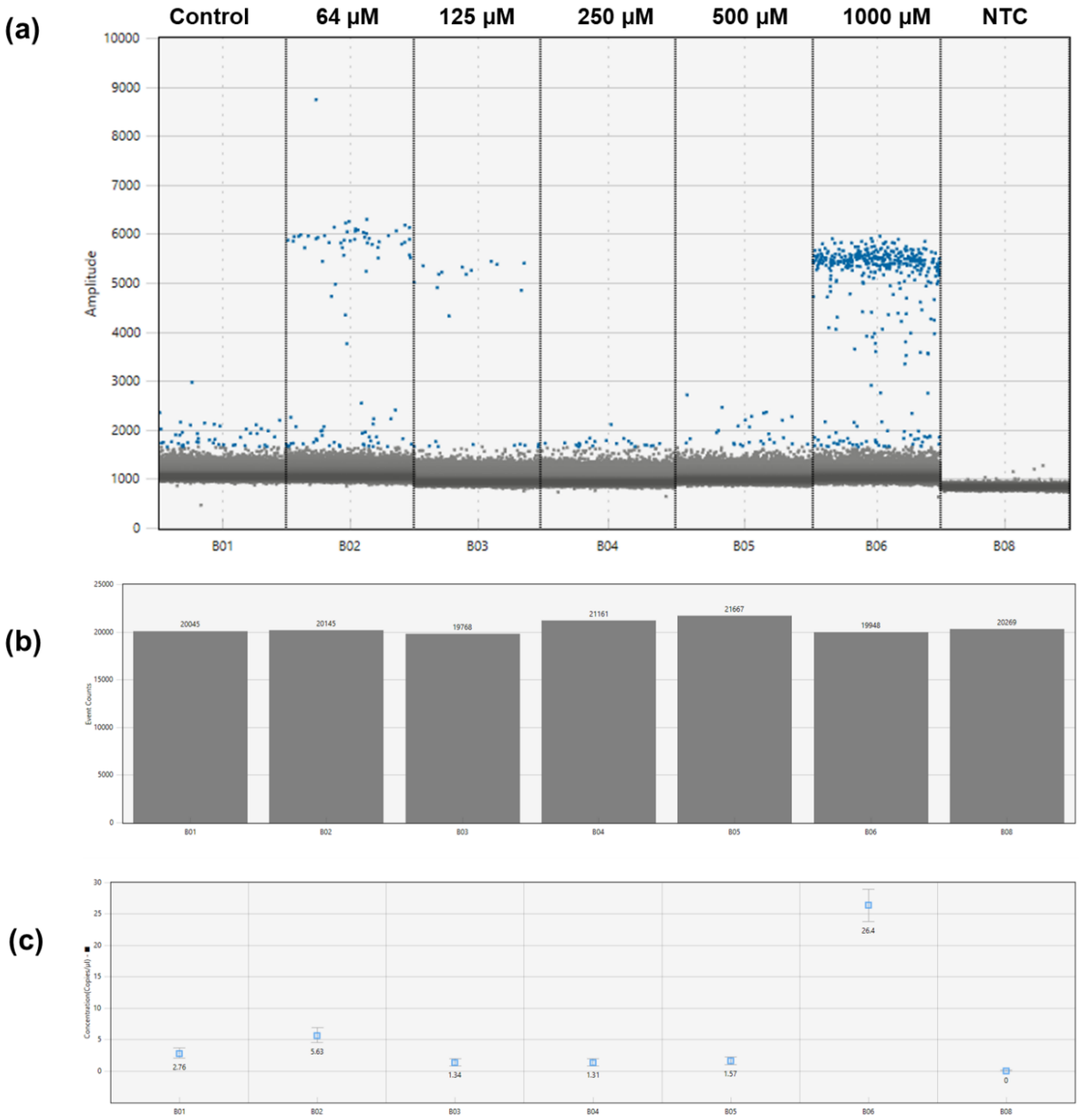

Figure S11. ddPCR of miRNA-876-3p expression of SF188 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-876-3p (copies/μL) detected in each condition.

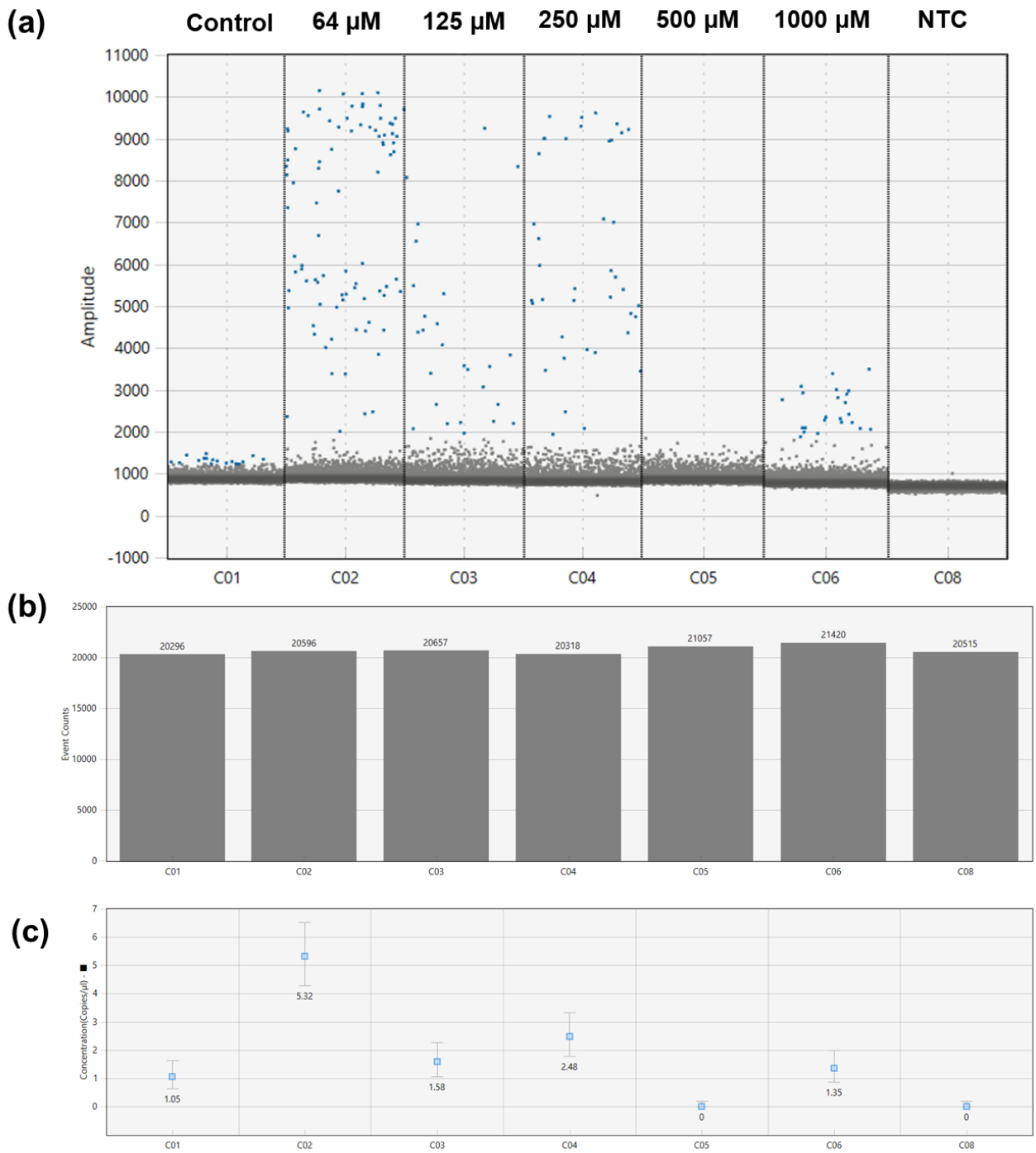

Figure S12. ddPCR of miRNA-876-5p expression of SF188 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-876-5p (copies/μL) detected in each condition.

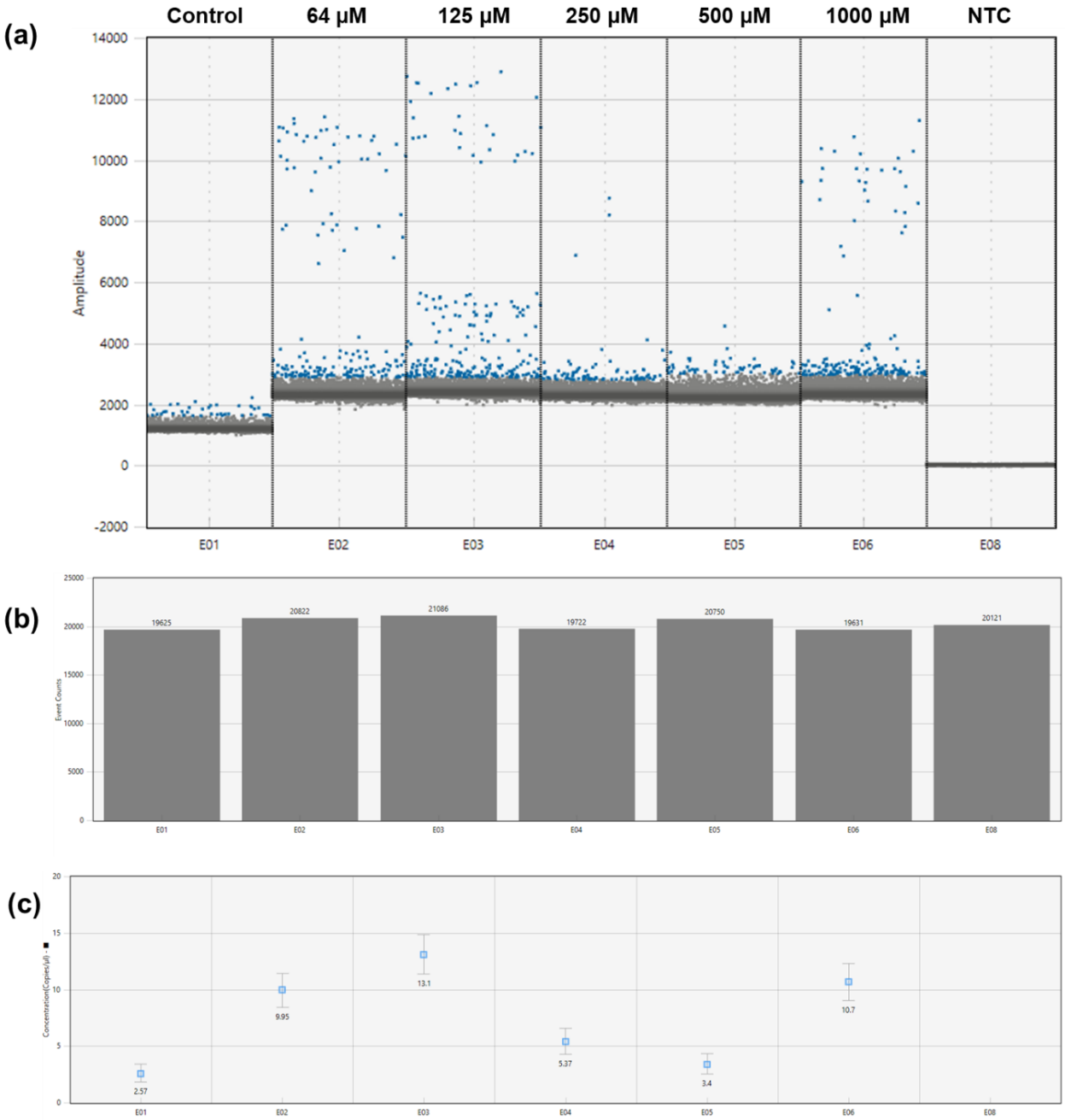

Figure S13. ddPCR of miRNA-137 expression of SF188 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-137 (copies/μL) detected in each condition.

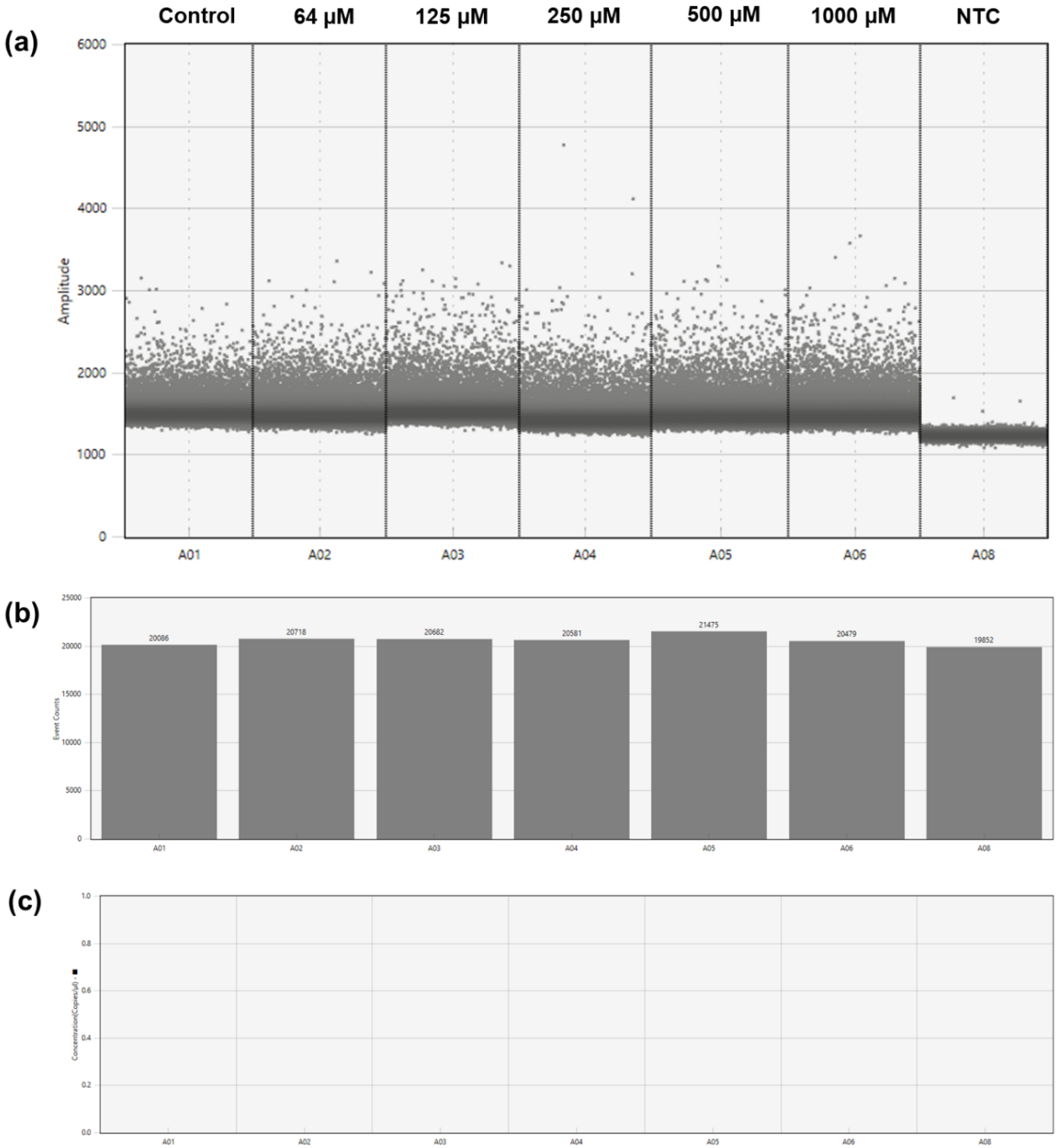

Figure S14. ddPCR of miRNA-490 expression of SF188 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 18,000 total droplets detected, and a minimum number of positive droplets of ≥3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-490 (copies/μL) detected in each condition.

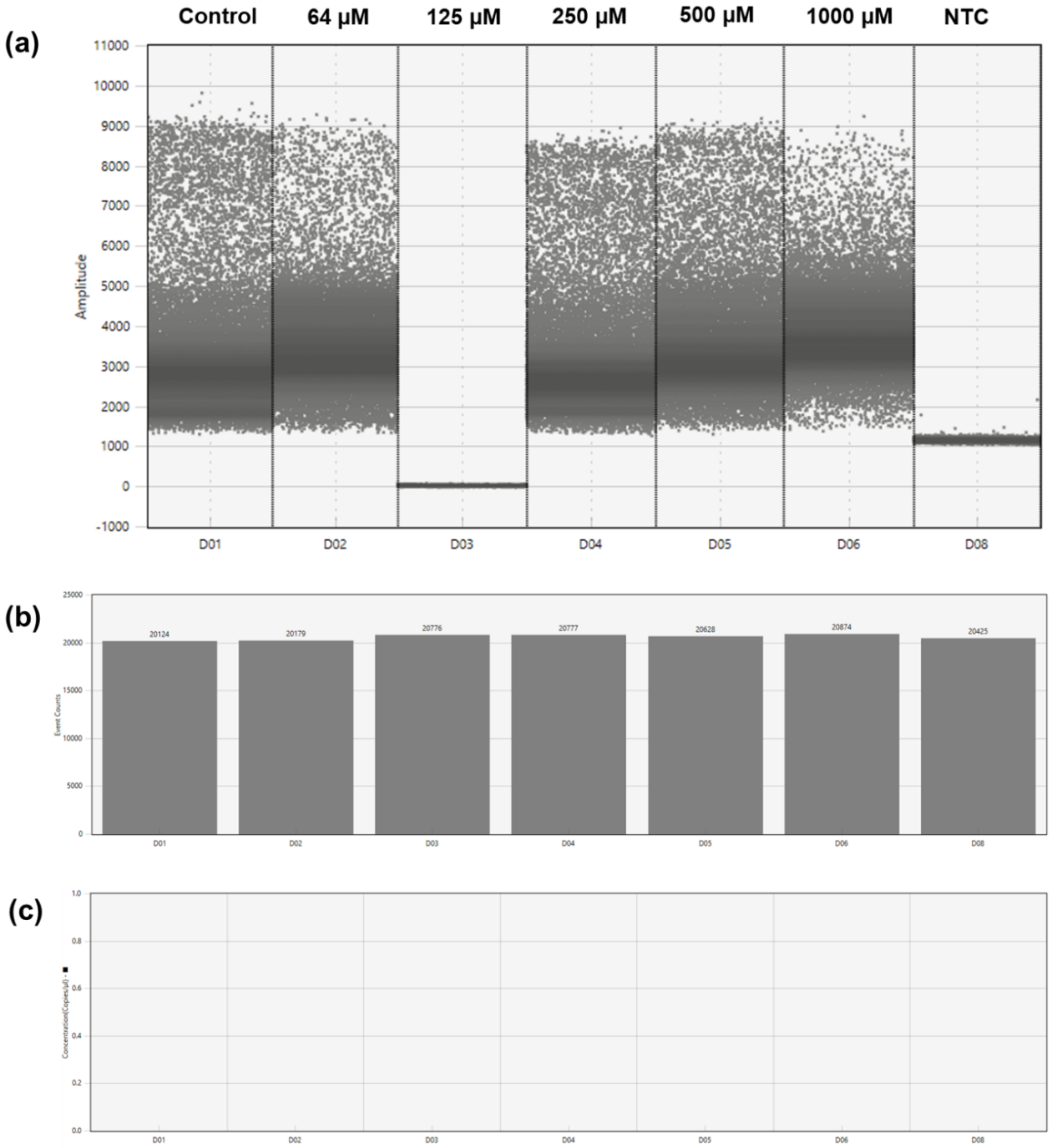

Figure S15. ddPCR of miRNA-448 expression of SF188 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 17,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-448 (copies/μL) detected in each condition.

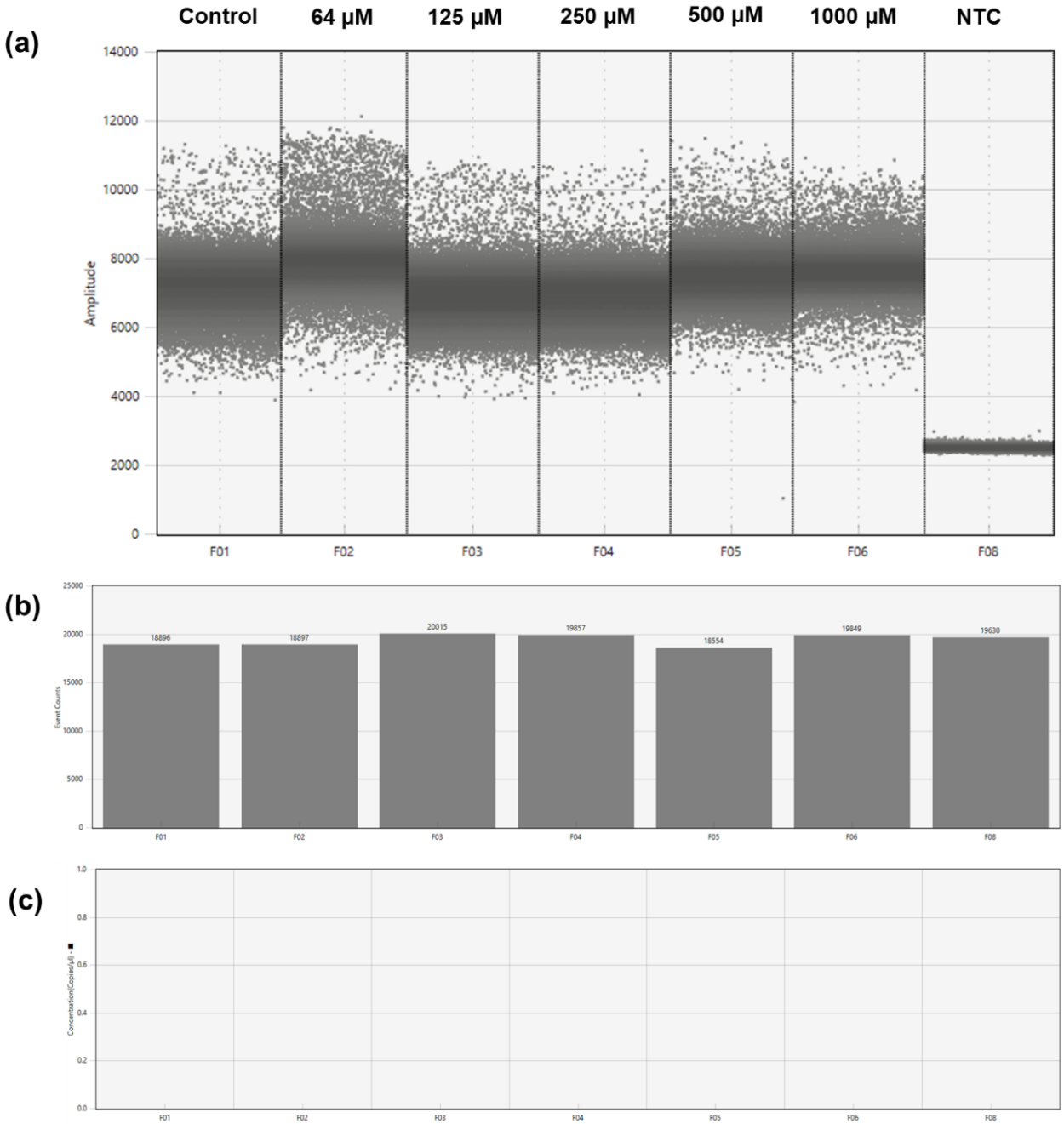

Figure S16. ddPCR of miRNA-501-3p expression of SF188 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 17,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-501-3p (copies/μL) detected in each condition.

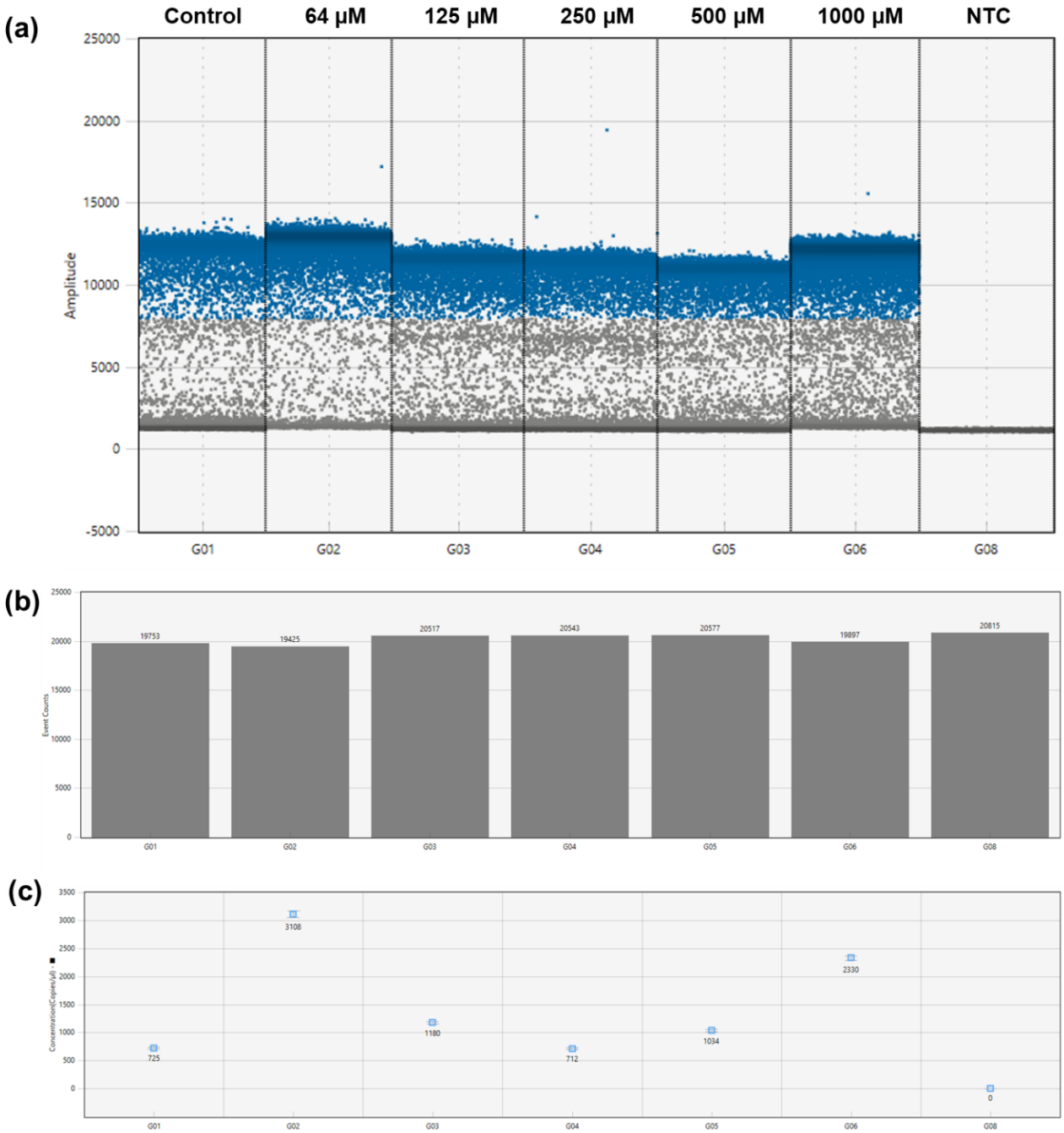

Figure S17. ddPCR of miRNA-191-5p expression (endogenous control) of SF188 GBM tumouroids treated with TMZ at different concentrations (0, 16, 64, 125, 250, 1000 μM) for 96 hours. (a) A representative 1D plot showing the expression of miRNAs, with blue and grey data points indicating positive (high amplitude) and negative (low amplitude) droplets, respectively. As expected, no positive droplets were measured for no template control (NTC). (b) All samples had more than 20,000 total droplets detected, and a minimum number of positive droplets of ≥ 3 was considered a positive sample for miRNA detection. (c) Absolute quantification of miR-191-5p (copies/μL) detected in each condition.