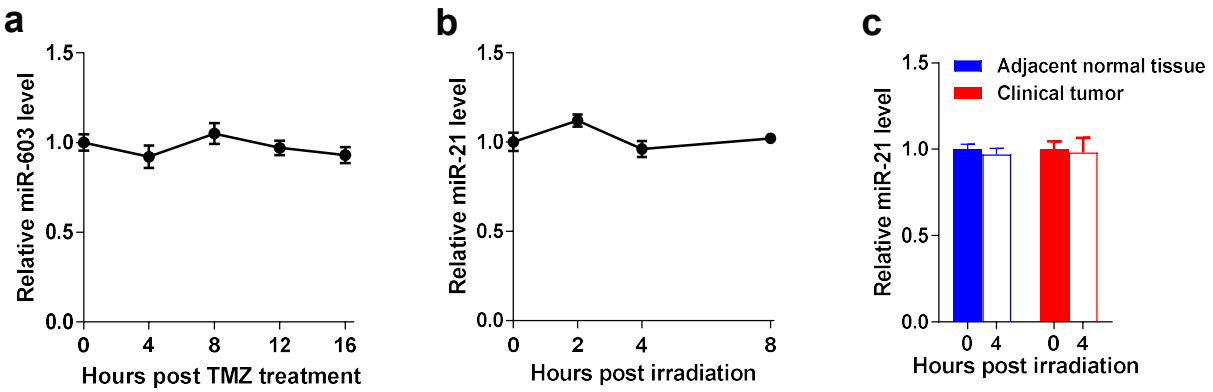


Fig S1. Related to Figure 1. Effects of ionizing radiation and TMZ treatment on miR-603 level in glioblastoma.

(a) TMZ treatment did not alter miR-603 level in glioblastoma cells. Effect of TMZ on the level of miR-603 in LN340 cell line was detected using qPCR at the indicated times.

(b) Ionizing radiation did not alter miR-21 level in glioblastoma cells. Effect of IR on the level of miR-603 in LN340 cell line was detected using qPCR at the indicated times.

(c) IR did not change miR-21 level in glioblastoma tissue compared to adjacent normal tissue. miR-21 level was measured using qPCR in freshly resected human glioblastoma specimens or adjacent normal tissue after receiving 2 Gy radiation. Error bars represent SE.

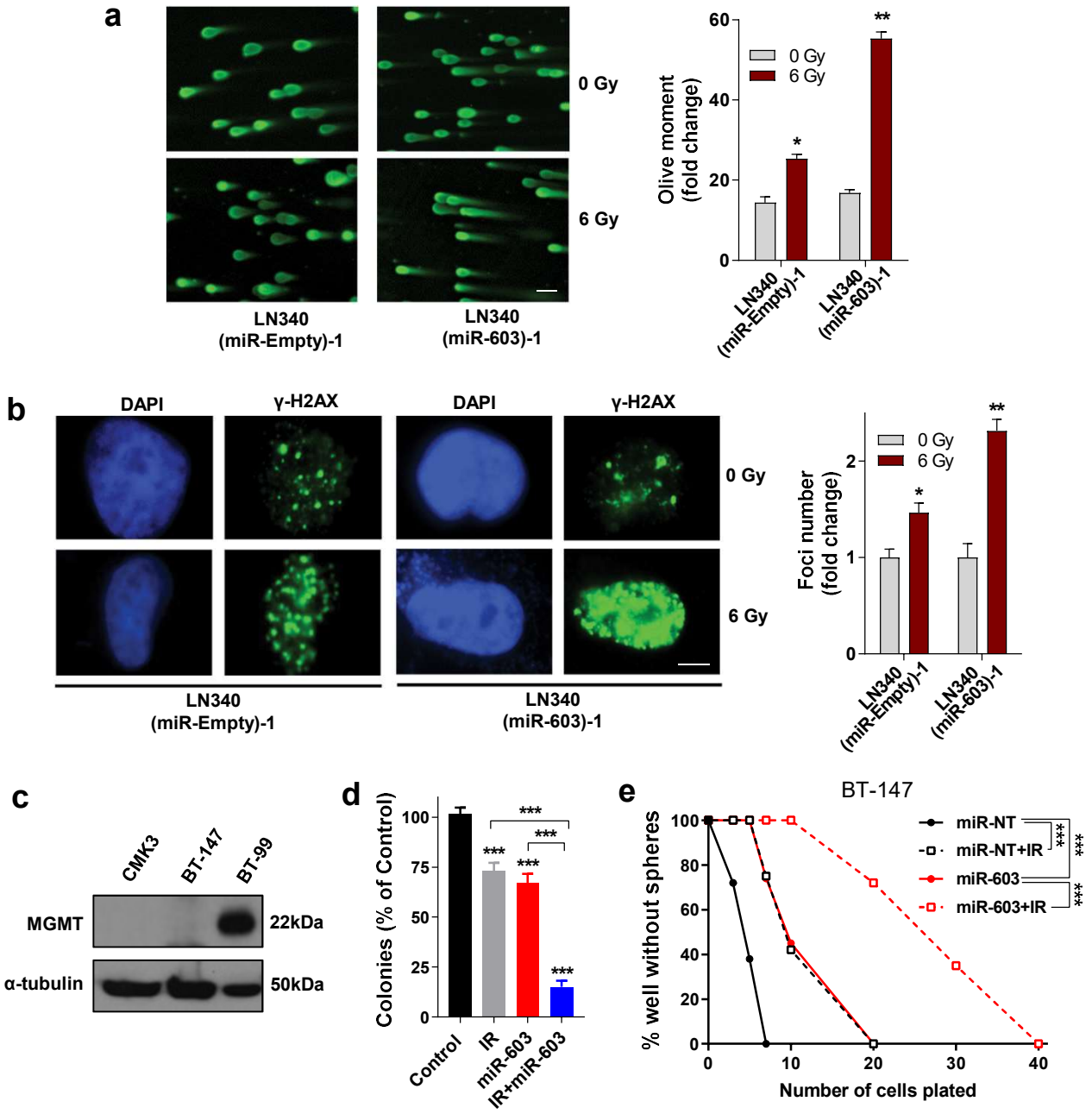


Fig S2. Related to Figure 3. Ectopic miR-603 expression increases radiation sensitivity in GBM cells.

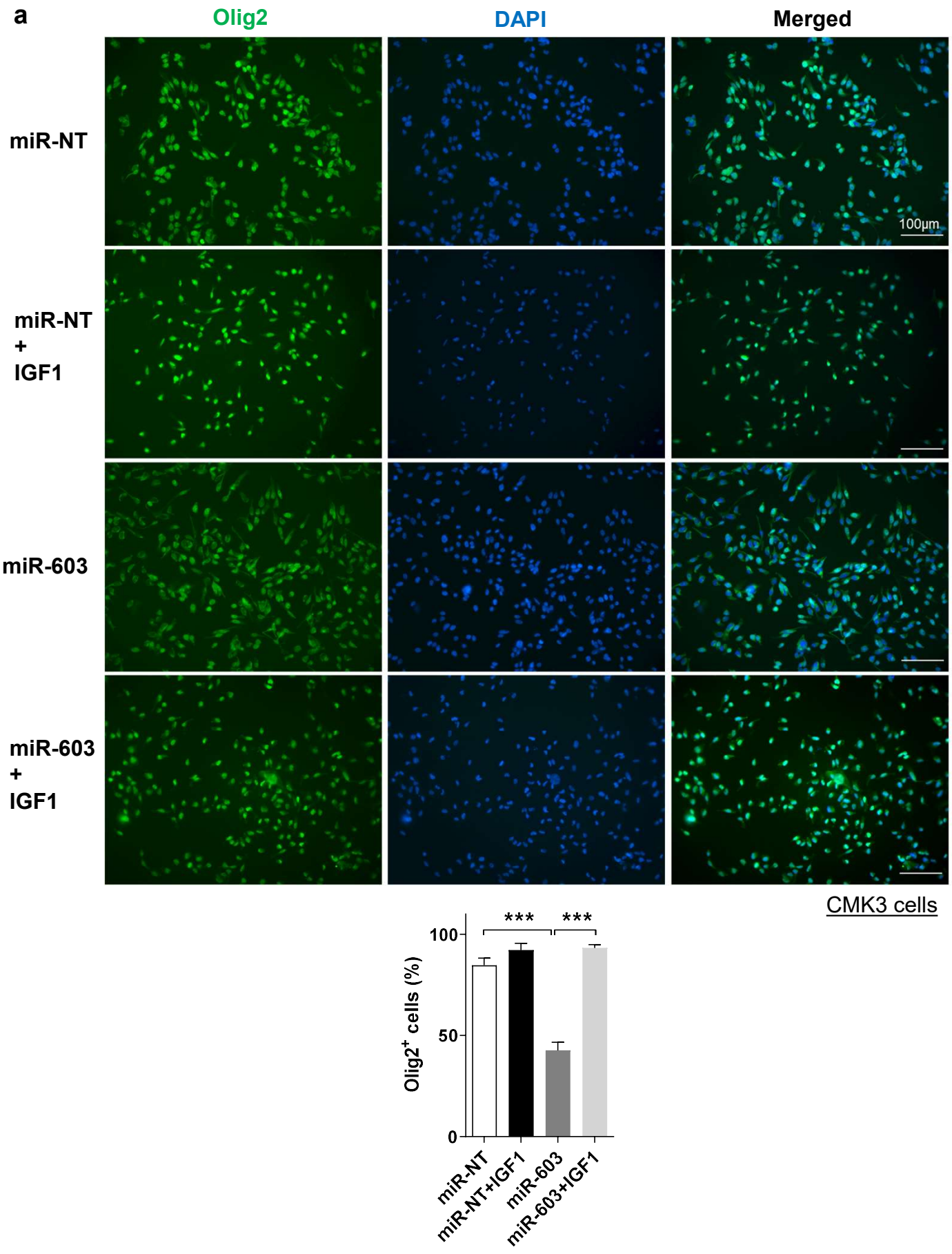
(a) Comet assay was performed in LN340(miR-Empty)-1 and LN340(miR-603)-1 cells (unirradiated or received 6 Gy radiation). Representative images of olive moments (left panel) and olive moments quantification (right panel) are shown. *$p < 0.05$ and **$p < 0.01$ indicate statistically significant differences compared to 0 Gy control group in the same cell line (Student's *t* test) . Scale bar, 50 μm.

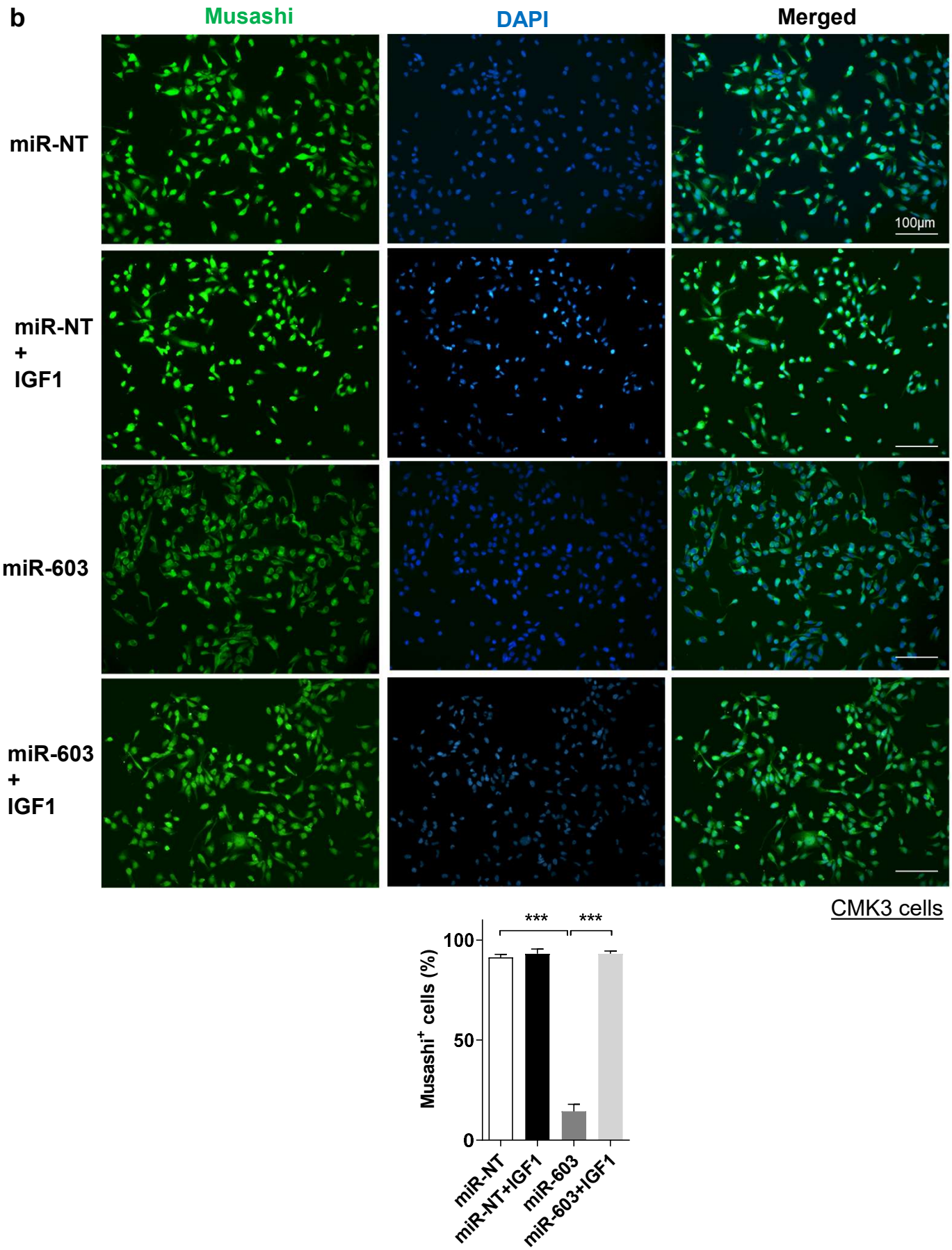
(b) Representative immunofluorescence staining images of γ-H2AX foci in LN340(miR-Empty)-1 and LN340(miR-603)-1 cells with or without 6 Gy radiation (left panel). γ-H2AX foci in those cells were quantified (right panel). *$p < 0.05$ and **$p < 0.01$ indicate statistically significant differences compared to 0 Gy control group in the same cell line (Student's *t* test). Scale bar, 5 μm.

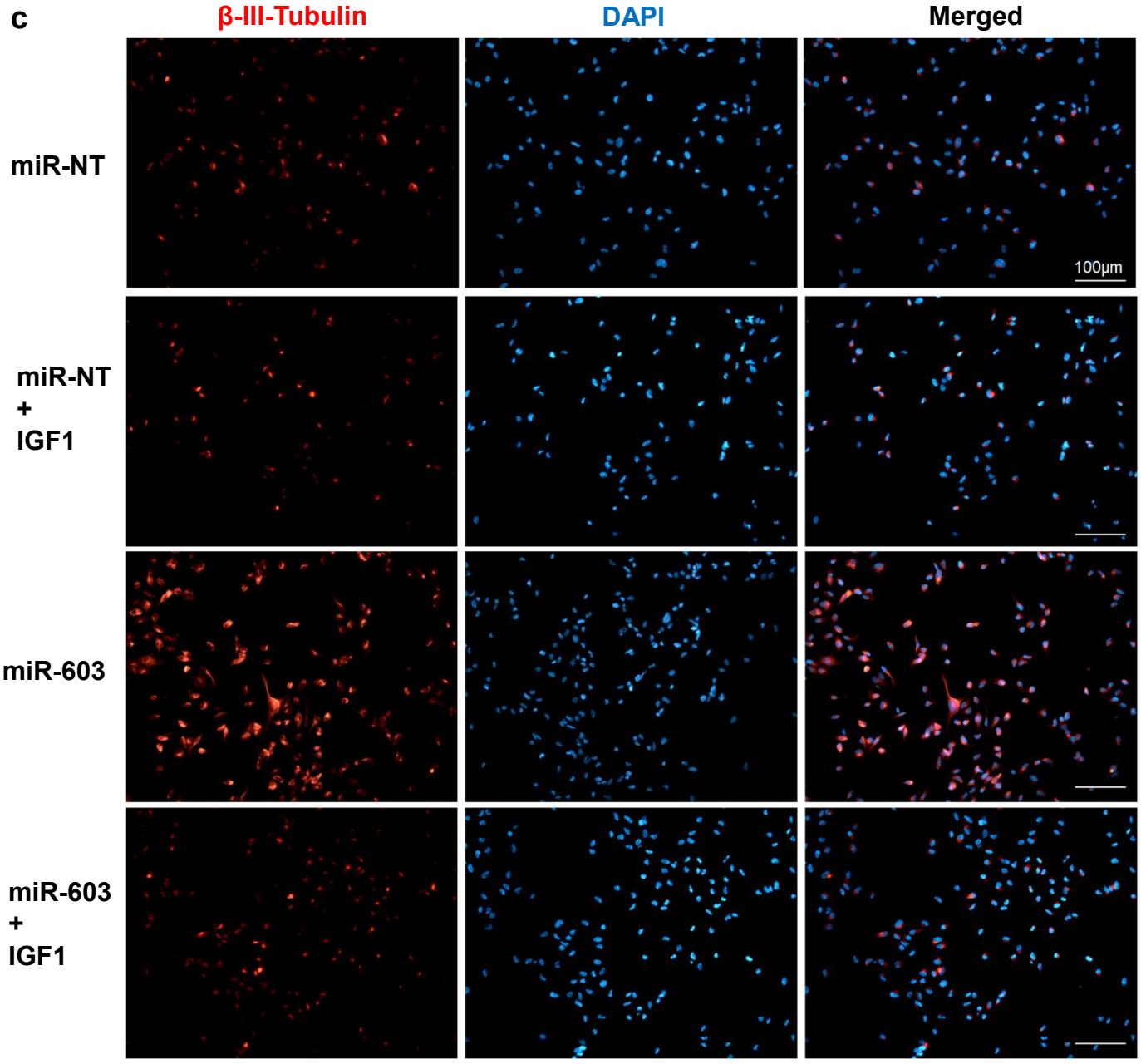
(c) MGMT protein expression levels were measured by western blot in CMK3, BT-147, and BT-99 cells. α-Tubulin was used as the loading control.

(d) Colony formation assay was performed in U87MG cells transfected with miR-603 mimic or the mimic control. ***$p < 0.001$ between indicated groups (one-way ANOVA).

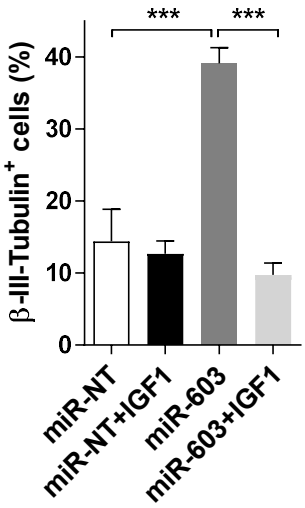
(e) miR-603 sensitized patient-derived glioblastoma cells to IR. Clonogenic potential of BT-147 cells (propagated as neurospheres) was determined using limiting dilution assay. BT-147 cells were transfected with human miR-603 mimic or non-targeting control miRNA before IR treatment. ***$p < 0.001$ between indicated groups (Chi-square test).


a
Olig2
DAPI
Merged
miR-NT
miR-NT + IGF1
miR-603
miR-603 + IGF1
100μm
CMK3 cells
***
***
100
50
0
Olig2+ cells (%)
miR-NT
miR-NT+IGF1
miR-603
miR-603+IGF1


b
Musashi
DAPI
Merged
miR-NT
miR-NT
+
IGF1
miR-603
miR-603
+
IGF1
100μm
CMK3 cells
***
***
100
50
0
Musashi+ cells (%)
miR-NT
miR-NT+IGF1
miR-603
miR-603+IGF1


c
β-III-Tubulin
DAPI
Merged
miR-NT
miR-NT + IGF1
miR-603
miR-603 + IGF1
100μm

CMK3 cells


***
***
β-III-Tubulin+ cells (%)
0
10
20
30
40
miR-NT
miR-NT+IGF1
miR-603
miR-603+IGF1

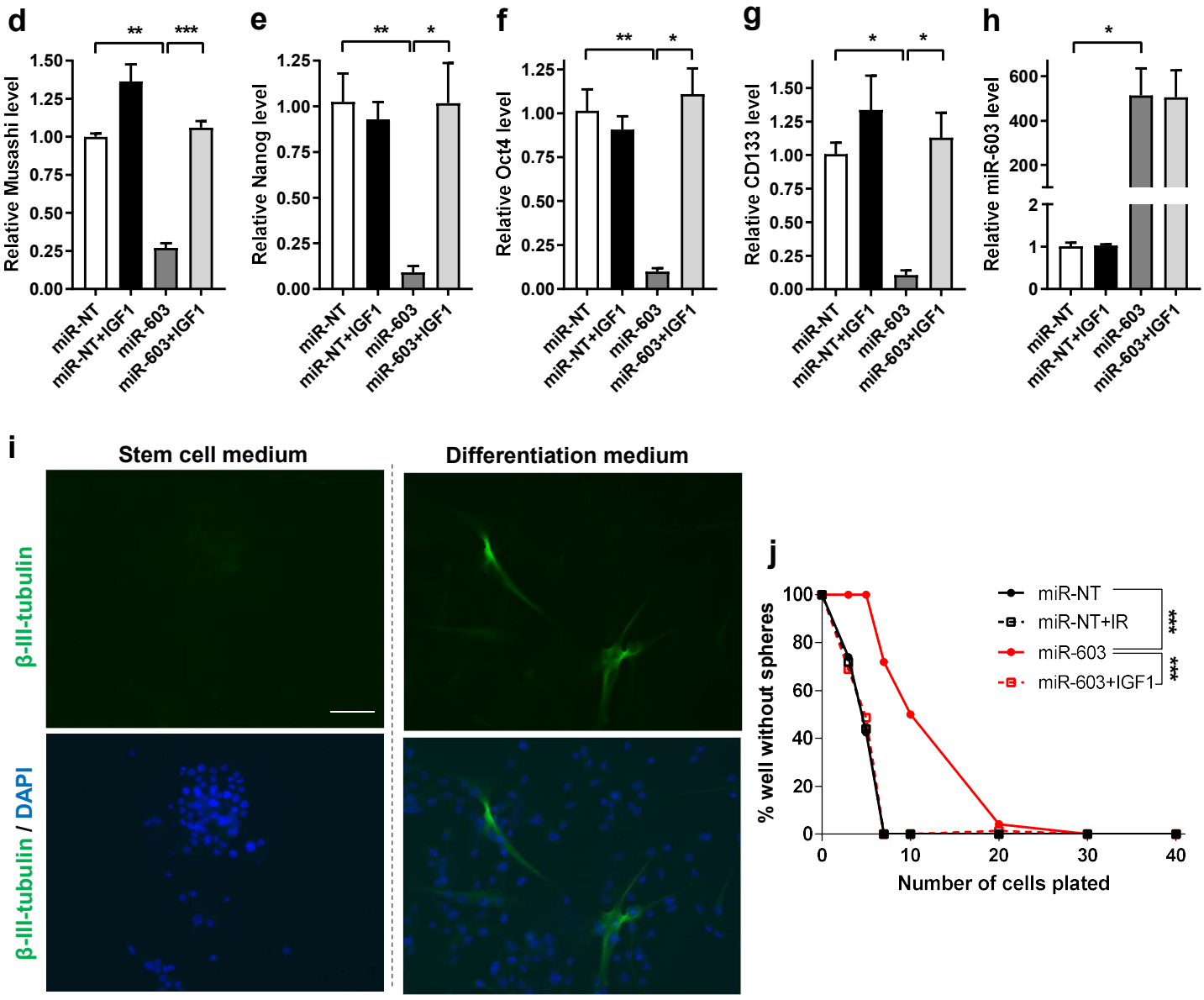


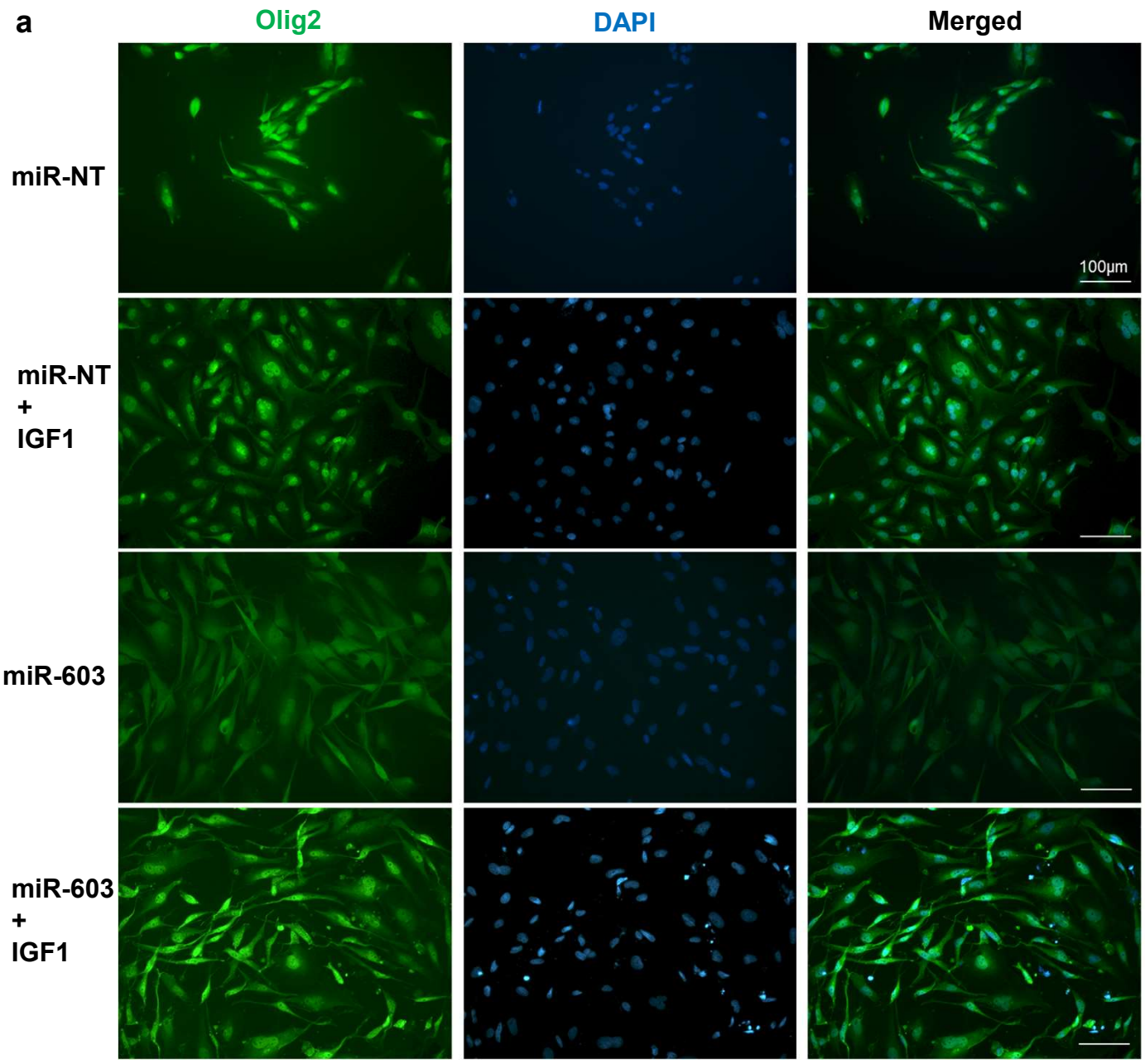
Fig S3. Related to Figure 4. Detection of GBM stem-cell markers and differentiation marker in CMK3 cells transfected with miR-603 mimic.

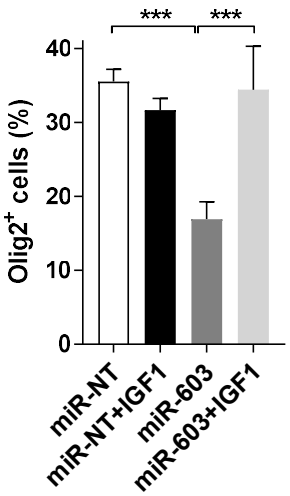
(a-c) CMK3 cells were transfected with non-targeting control miRNA or miR-603 mimic in the presence or absence of IGF1 treatment. The expression of Olig2 (a), Musashi (b), and β-III-Tubulin (c) was analyzed by immunofluorescence staining. Quantifications of staining positive cells are provided. ***$p < 0.001$ between indicated groups (one-way ANOVA). Scale bar is 100 μm.

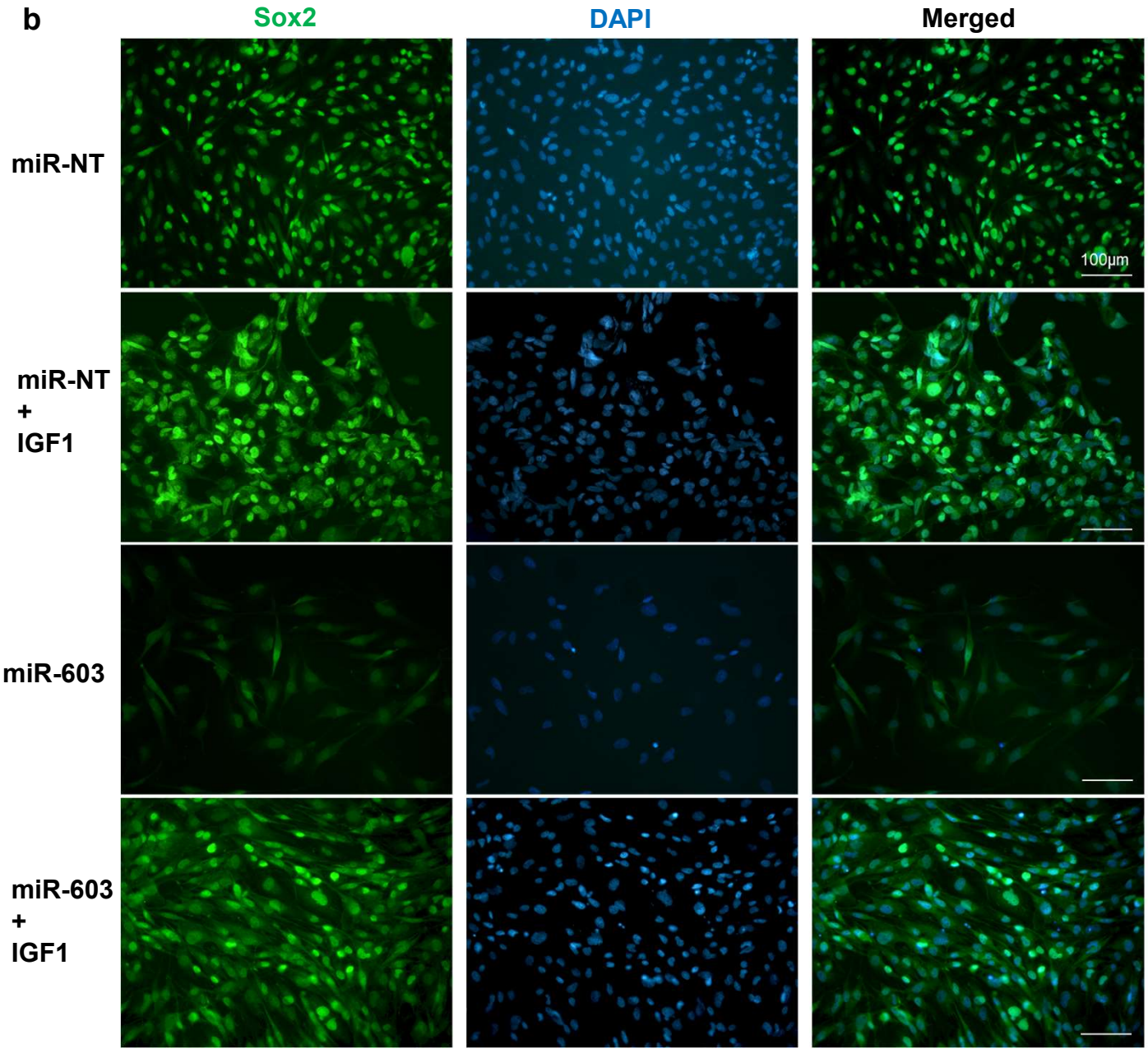
(d-h) The mRNA levels of stem cell markers: Musashi (d), Nanog (e), Oct4 (f), CD133 (g) were suppressed by miR-603 transfection (h) in CMK3 cells, which was reversed by exogenous addition of IGF1. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ between indicated groups (one-way ANOVA).

(i) Fluorescent staining of differentiation marker β-III-tubulin in BT-99 cells grown in stem cell medium or differentiation medium. Scale bar, 50 μm.

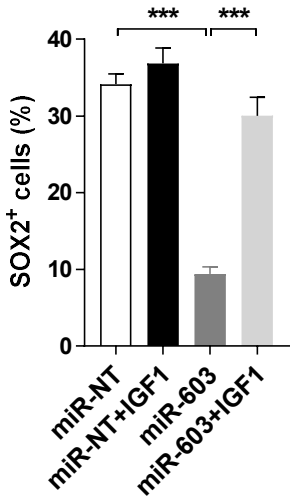
(j) *In vitro* limiting dilution assay in CMK3 cells transfected with miR-603 or miR-NT in the presence or absence of IGF1 treatment. ***$p < 0.001$ between indicated groups (Chi-square test).

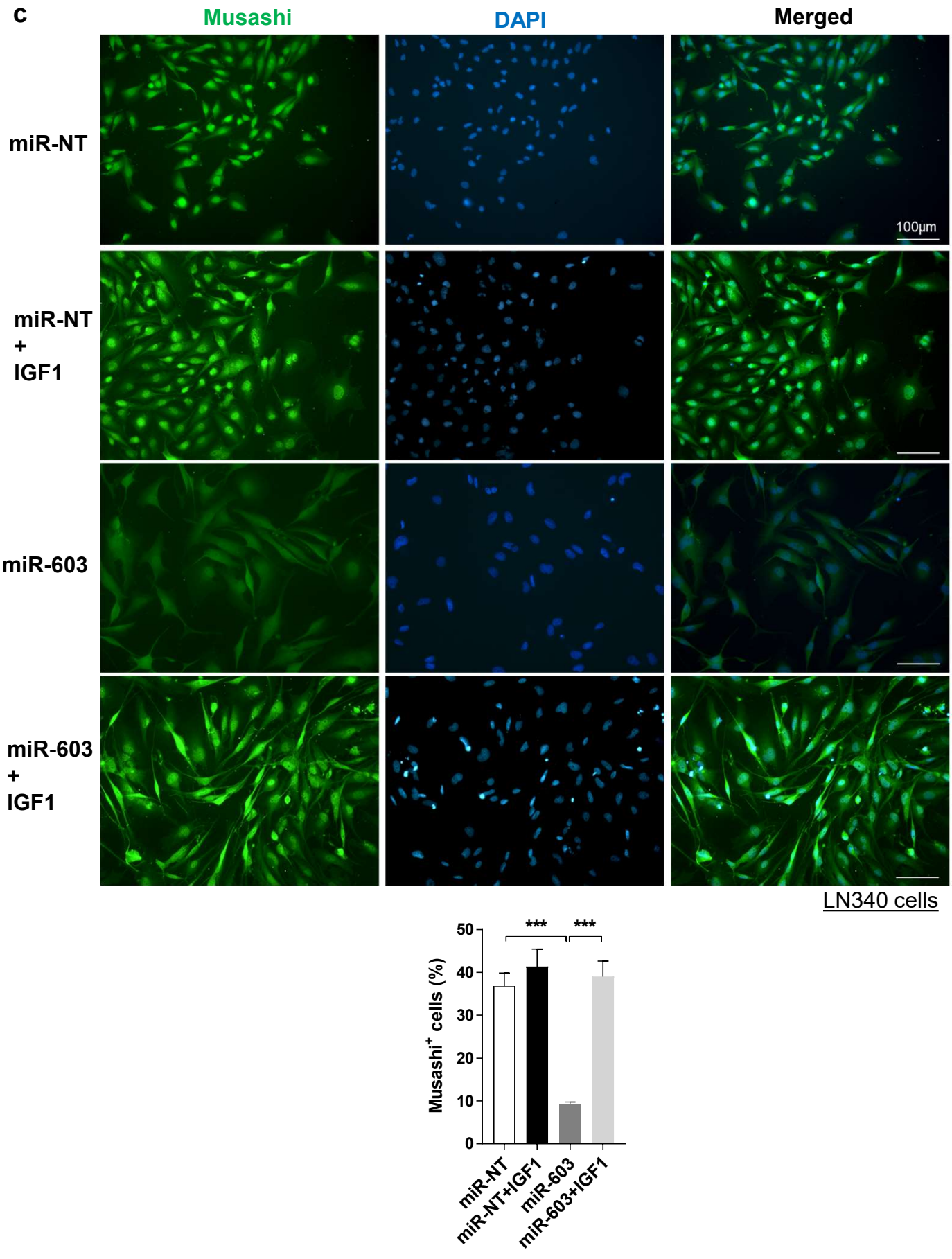

a
Olig2
DAPI
Merged
miR-NT
miR-NT + IGF1
miR-603
miR-603 + IGF1
100μm
LN340 cells

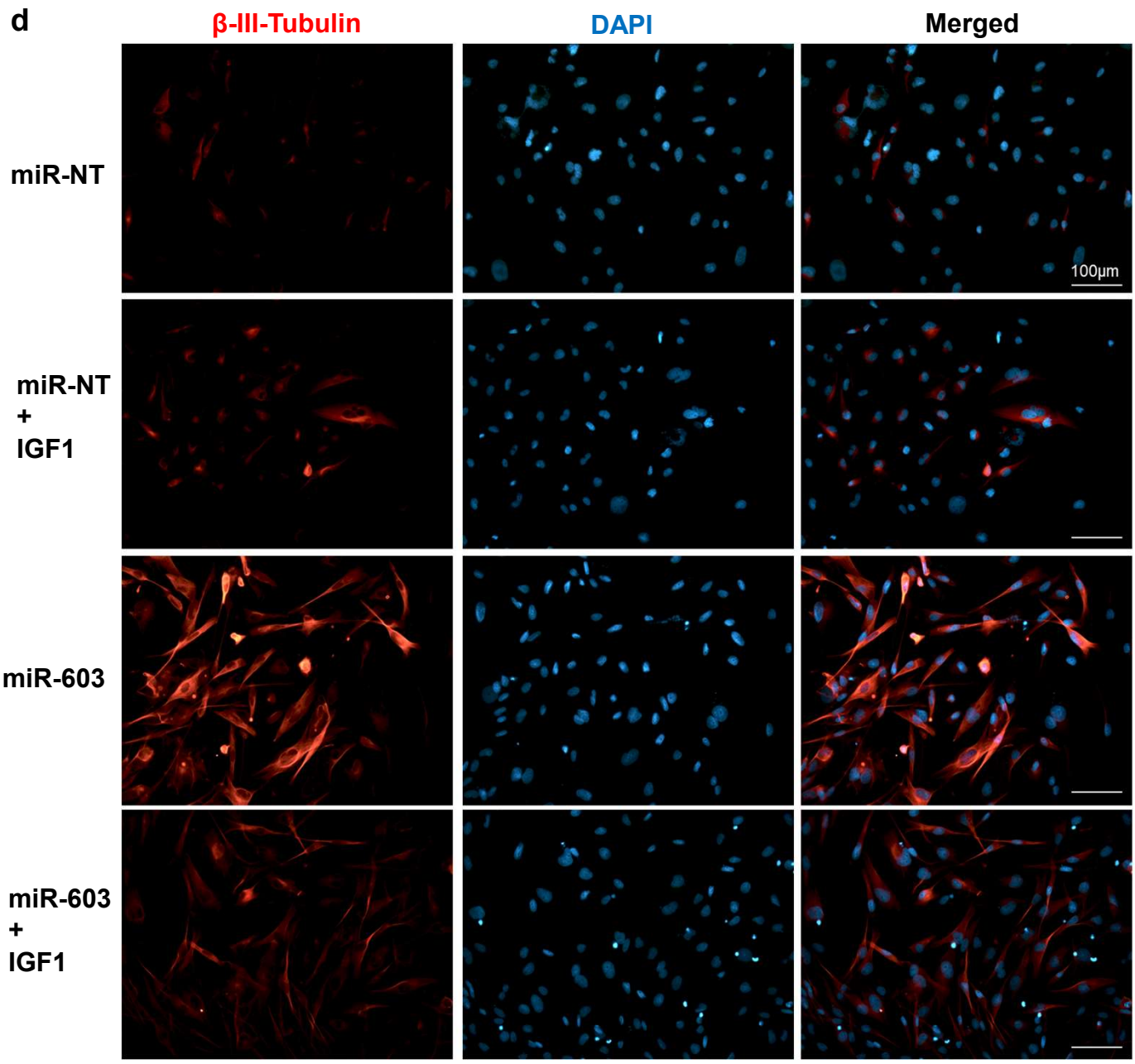

***
***
40
30
20
10
0
Olig2+ cells (%)
miR-NT
miR-NT+IGF1
miR-603
miR-603+IGF1


b
Sox2
DAPI
Merged
miR-NT
miR-NT + IGF1
miR-603
miR-603 + IGF1
100μm

LN340 cells


***
***
40
30
20
10
0
SOX2+ cells (%)
miR-NT
miR-NT+IGF1
miR-603
miR-603+IGF1


c
Musashi
DAPI
Merged
miR-NT
miR-NT + IGF1
miR-603
miR-603 + IGF1
100μm
LN340 cells
Musashi+ cells (%)
50
40
30
20
10
0
***
***
miR-NT
miR-NT+IGF1
miR-603
miR-603+IGF1


d
β-III-Tubulin
DAPI
Merged
miR-NT
miR-NT + IGF1
miR-603
miR-603 + IGF1
100μm

LN340 cells

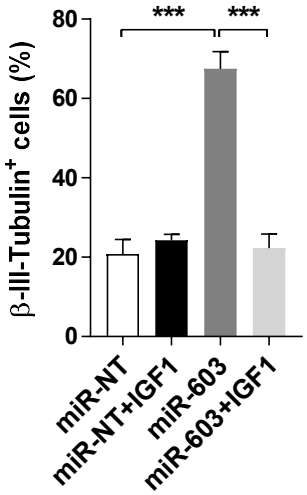

β-III-Tubulin+ cells (%)
80
60
40
20
0
***
***
miR-NT
miR-NT+IGF1
miR-603
miR-603+IGF1

Fig S4. Related to Figure 4. Detection of GBM stem-cell markers and differentiation markers in LN340 cells transfected with miR-603 mimic.
LN340 cells were transfected with non-targeting control miRNA or miR-603 mimic in the presence or absence of IGF1 treatment. The expression of Olig2 (a), Sox2 (b), Musashi (c), and β-III-Tubulin (d) was analyzed by immunofluorescence staining. Quantifications of staining positive cells are provided. *** p <0.001 between indicated groups (one-way ANOVA). Scale bar is 100 μm.

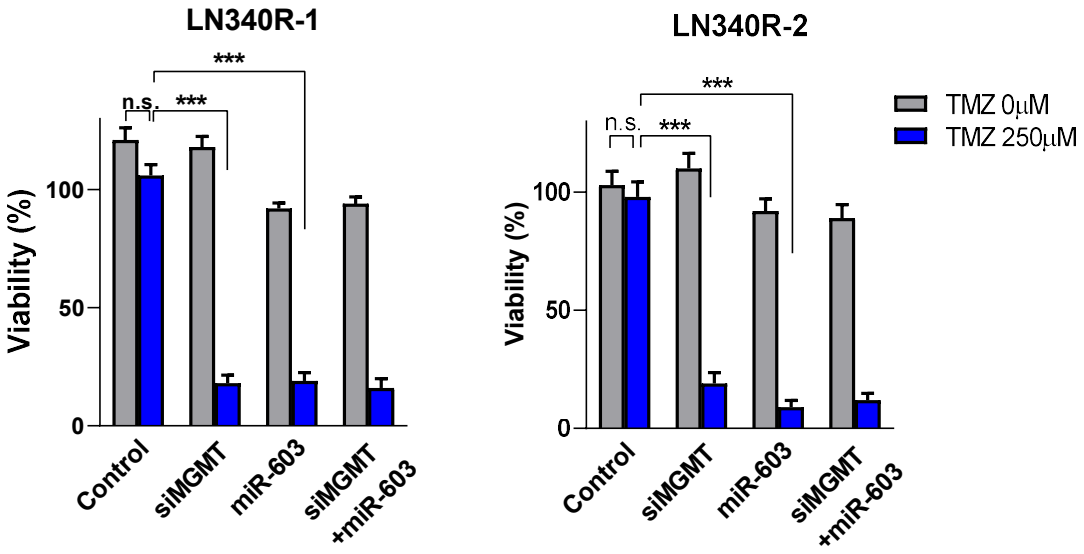
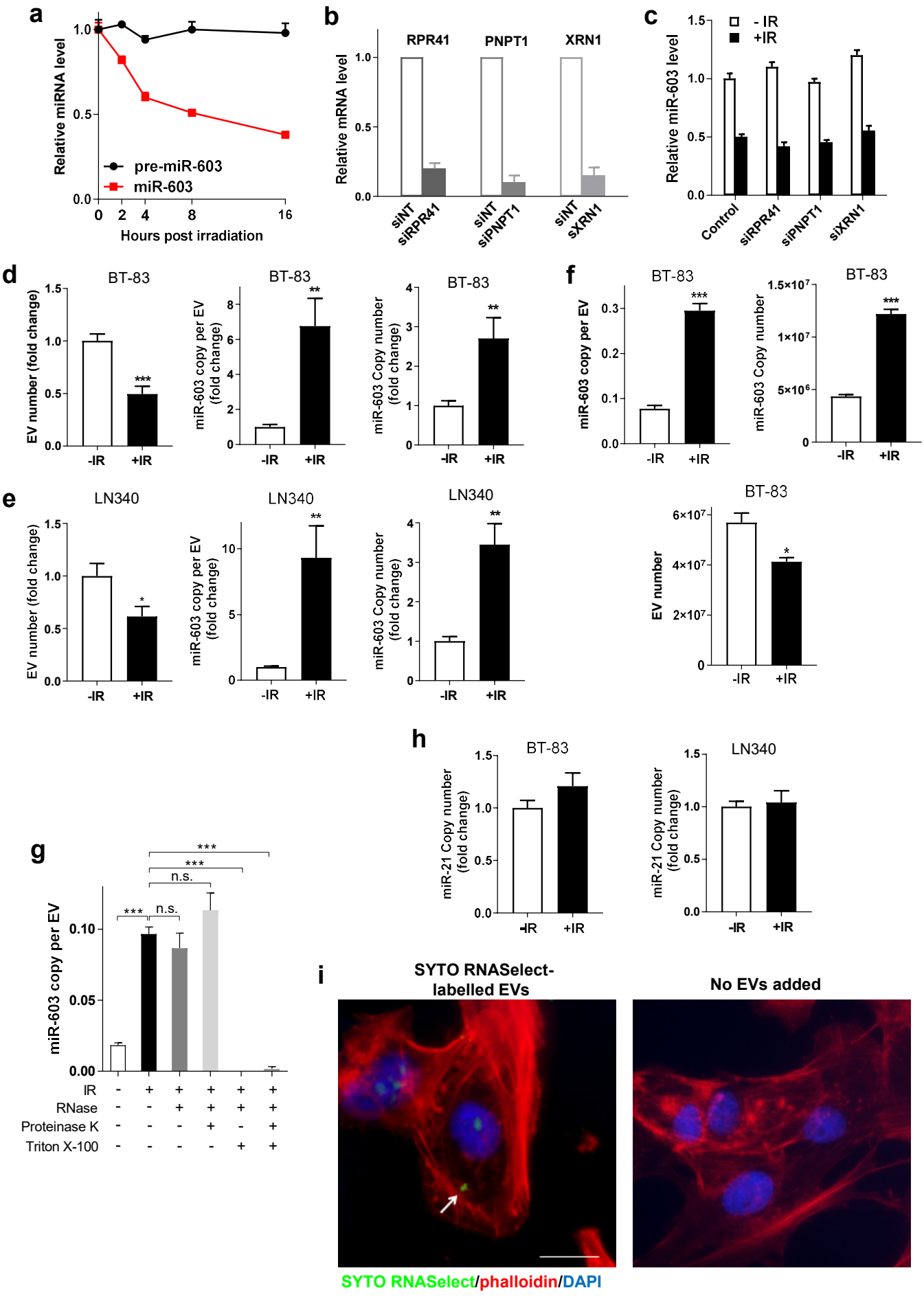


Fig S5. Related to Figure 5. Ionizing radiation decreases miR-603 level to induce cross-resistance to TMZ. IR caused cross-resistance to TMZ through upregulating MGMT. LN340R-1 and LN340R-2 cells were transfected with control, siMGMT, or miR-603 24 hours prior to TMZ (250 μM) treatment. Cell viability was measured 7 days after TMZ treatment and normalized to untreated LN340 without TMZ treatment. ***$p < 0.001$, $^{n.s.}p > 0.05$ between indicated groups (one-way ANOVA).


a
Relative miRNA level
1.0
0.5
0.0
pre-miR-603
miR-603
0
2
4
8
16
Hours post irradiation
b
Relative mRNA level
RPR41
PNPT1
XRN1
siNT
siRPR41
siNT
siPNPT1
siNT
siXRN1
c
Relative miR-603 level
- IR
+IR
Control
siRPR41
siPNPT1
siXRN1
d
BT-83
EV number (fold change)
-IR
+IR
miR-603 copy per EV (fold change)
miR-603 Copy number (fold change)
f
miR-603 copy per EV
miR-603 Copy number
e
LN340
EV number (fold change)
miR-603 copy per EV (fold change)
miR-603 Copy number (fold change)
EV number
g
miR-603 copy per EV
n.s.
IR
RNase
Proteinase K
Triton X-100
h
miR-21 Copy number (fold change)
i
SYTO RNASelect-labelled EVs
No EVs added
SYTO RNASelect/phalloidin/DAPI

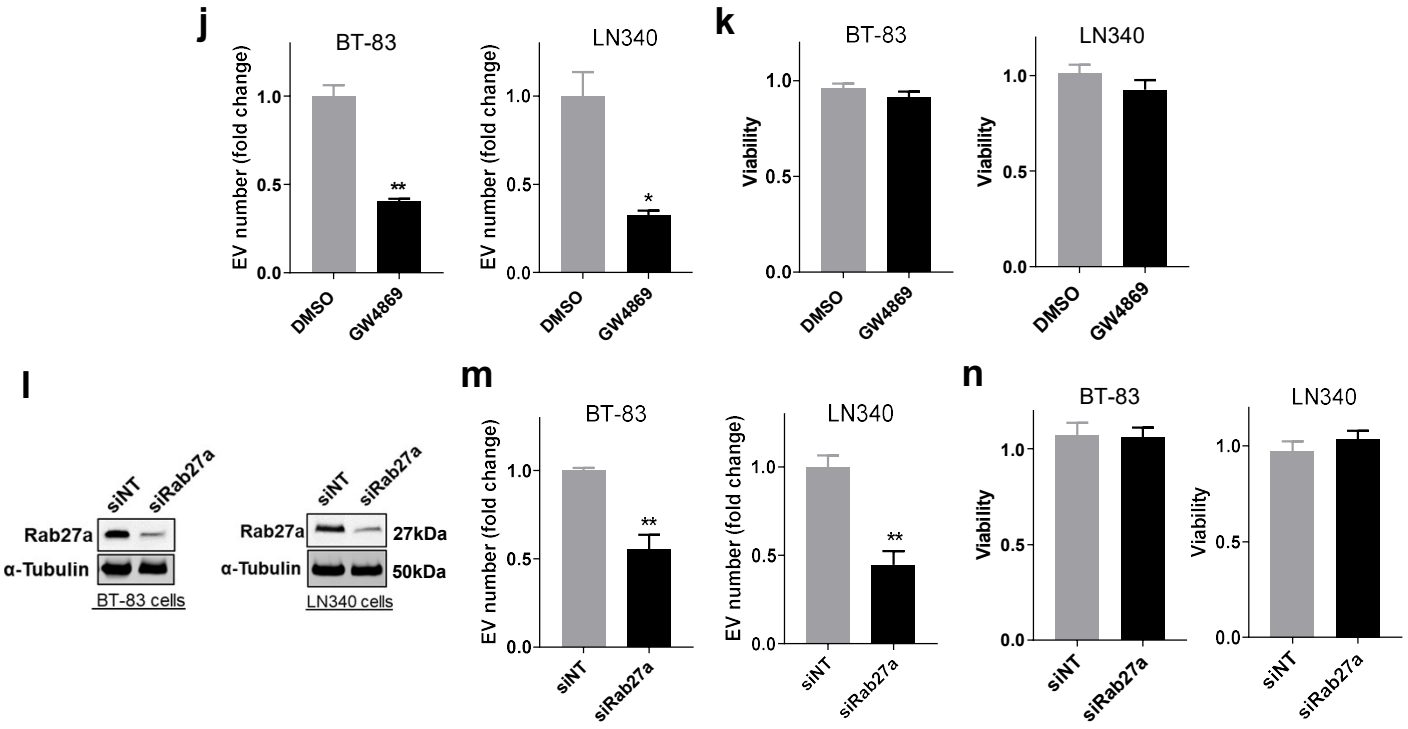


Fig S6. Related to Figure 6. IR does not promote extracellular export of miR-21 via EVs.

(a) Ionizing radiation decreased cellular level of miR-603, but not pre-miR-603 in LN340 cells. qPCR was performed to measure the expression of miR-603 or pre-miR-603 at indicated time points in LN340 cells treated with or without 6 Gy IR.

(b) qPCR analysis of RPR41/PNPT1/XRN1 mRNA expression following siRNA-mediated silencing in LN340 cells.

(c) Silencing of microRNAses (RPR41, PNPT1, XRN1) did not significantly impact the effect of IR on miR-603 level in LN340 cells.

(d and e) EVs secreted by irradiated-or unirradiated BT-83 cells (d) or LN340 cells (e) were isolated using Total Exosome Isolation Reagent. RNA was extracted from isolated EVs and subject to absolute quantification of miR-603. The differences of EV number, miR-603 copy number and miR-603 copy number per EV between those two groups was expressed as fold changes. $^{**}p < 0.01$ indicate statistically significant difference versus unirradiated control group (Student's *t* test).

(f) EVs secreted by irradiated-or unirradiated BT-83 cells were isolated using Qiagen exoEasy Maxi kit and quantified by NTA. RNA was extracted from isolated EVs and subject to absolute quantification of miR-603. The values of miR-603 copy number, EV number and miR-603 copy number per EV in an representative experiment were shown. $^{*}p < 0.05$, $^{***}p < 0.001$ versus unirradiated control group (Student's *t* test).

(g) EVs secreted by irradiated- or unirradiated LN340 cells were isolated using differential ultracentrifugation (dUC) and incubated with the indicated treatments before isolating RNA and measuring the levels of miR-603. The EV pellets were incubated with RNase A (0.5 µg/µl) alone or were incubated with proteinase K (0.4 µg/µl) prior to RNase treatment in the presence or absence or 2% Triton X-100. $^{***}p < 0.001$, $^{n.s.}p > 0.05$ between indicated groups (one-way ANOVA).

(h) miR-21 secreted through EVs was not changed after IR treatment in both BT-83 and LN340 cells.

(i) Representative fluorescence microscopy images for SYTO RNASelect-labelled EVs uptake by human microglia HMC3 cells after 6 hours incubation. Green: SYTO RNASelect stain; red: Alexa Fluor 594 phalloidin; blue: DAPI. Scale bar: 10 µm.

(j) Fold change of EV secretion in BT-83 and LN340 cells with or without GW4869 treatment. $^{*}p < 0.05$, $^{**}p < 0.01$ versus DMSO control group (Student's *t* test).

(k) Cell viability fold changes of DMSO-treated and GW4869-treated BT-83 or LN340 cells.

(l) Protein expression of Rab27a was measured in negative control siRNA(siNT)- or siRab27a-transfected BT-83 or LN340 cells by western blot assay. α-Tubulin was used as the loading control.

(m) Fold change of EV secretion in BT-83 and LN340 cells transfected with siNT or siRab27a. $^{**}p < 0.01$ versus siNT-transfected group (Student's *t* test).

(n) Cell viability fold changes of siNT and siRab27a-transfected BT-83 or LN340 cells.

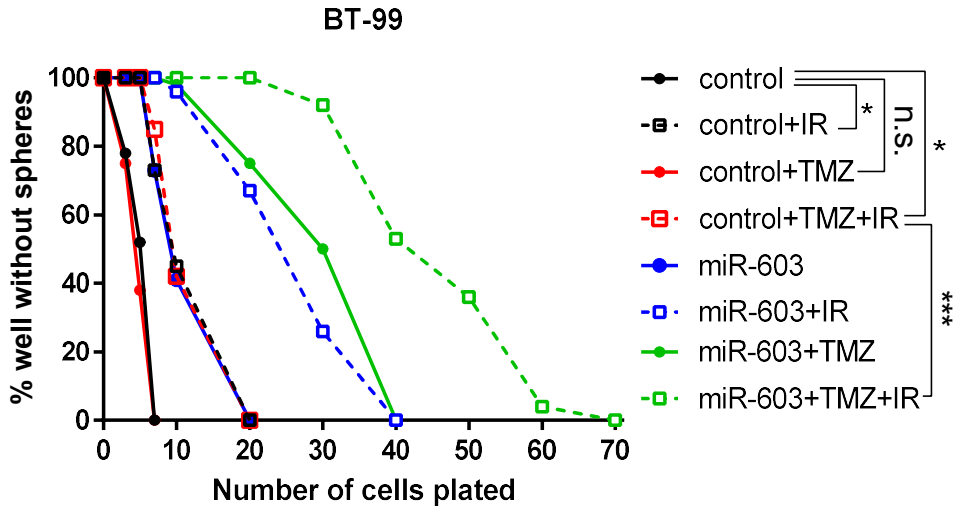


Fig S7. Related to Figure 7. miR-603 synergized with IR (6 Gy) and TMZ treatment (100 µM) in BT-99 *in vitro*.

Limiting dilution assay was performed using BT-99, an MGMT promoter unmethylated glioblastoma with high MGMT expression, with or without stable miR-603 expression. *$p < 0.05$; ***$p < 0.001$; $^{n.s.}p > 0.05$ between indicated groups (Chi-square test)