Supporting Information

Triggering isothermal exponential amplification of miRNA via liposome fusion

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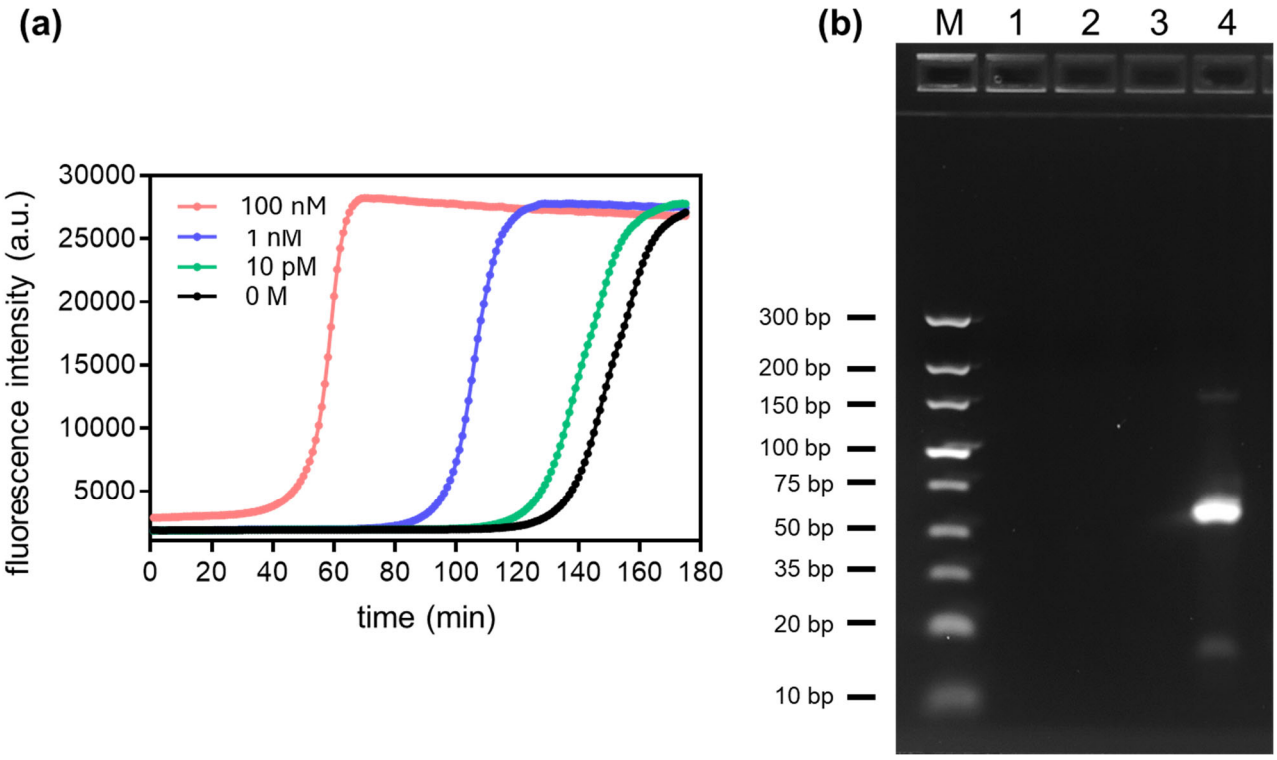

Fig. S1 Result of the in-tube EXPAR. (a) Time-course of the fluorescent intensity, reflecting the amount of cDNA-template complex, started at various miRNA concentrations. (b) Gel-electrophoresis result of EXPAR solution. M: ladder, 1: Before incubation, let-7a (-), 2: Before incubation, 100 nM let-7a (+), 3: After 120 min incubation, let-7a (-), After incubation, 100 nM let-7a (+).

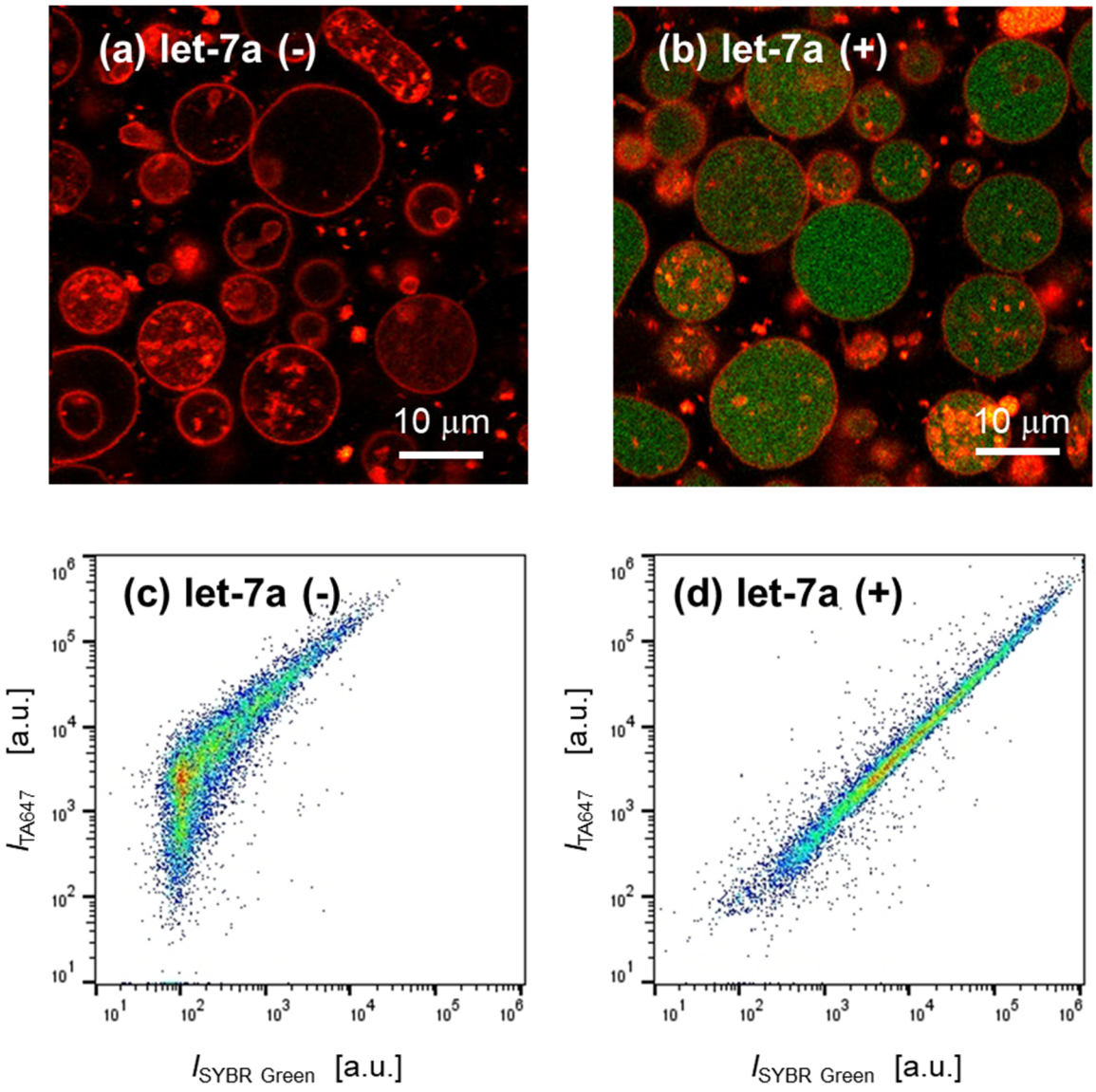

Fig. S2 Encapsulation of post-EXPAR solution into GUVs. Fluorescent confocal laser scanning images of GUVs encapsulating EXPAR solutions, which were incubated for 80 min in a test tube (a) without and (b) with 100 nM let-7a. Red: Membrane marker DiI, Green: cDNA amplification marker (SYBR Green). (c) and (d) are corresponding flow cytometry scatter plots, showing correlation of the fluorescence intensities of the GUV volume marker (TA647, vertical axis) and cDNA amplification marker (SYBR Green, horizontal axis).

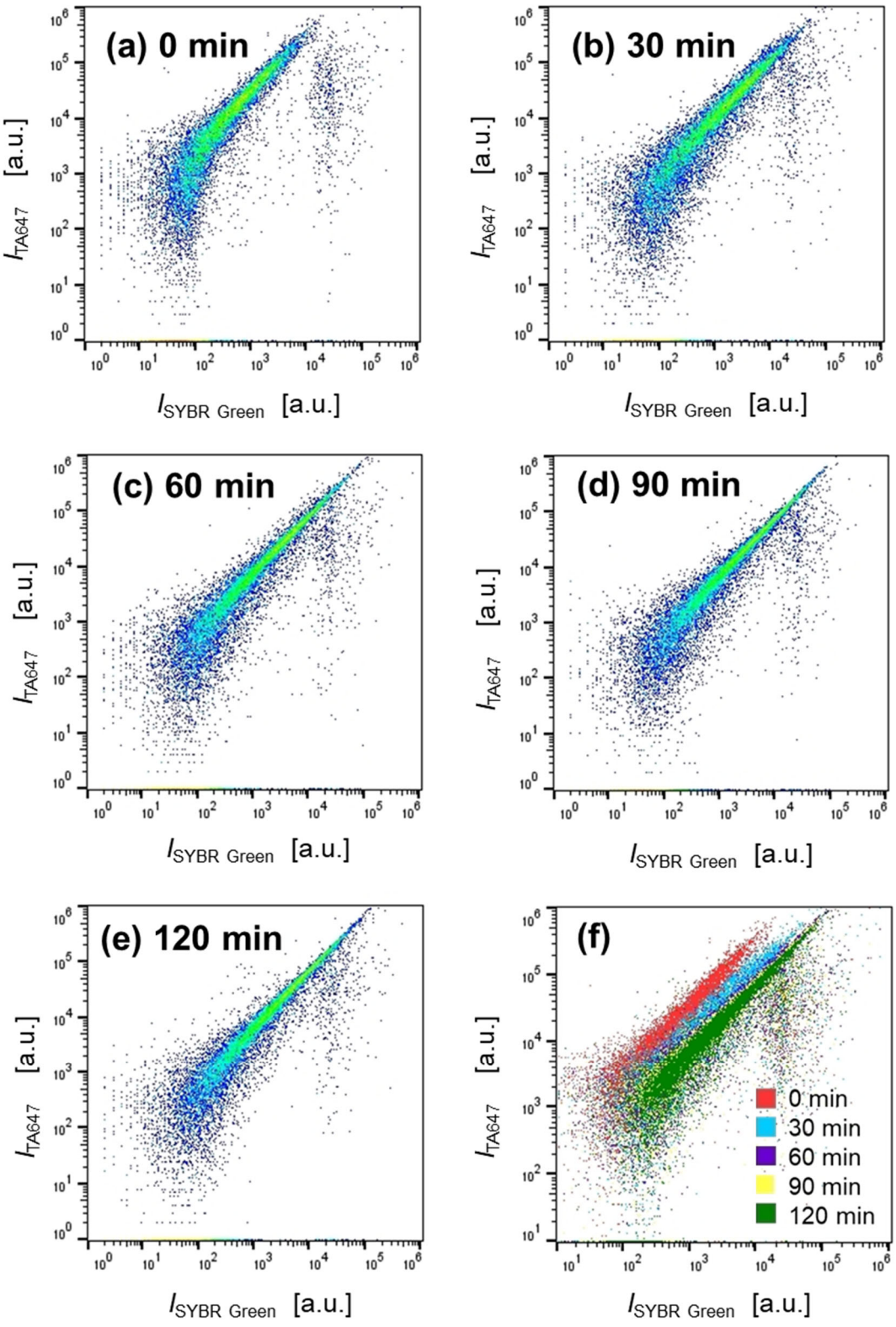

Fig. S3 Flow cytometry scatter plots showing the correlation between the vesicle volume marker in the vertical axis (I_{TA647}) and the amount of amplicon in the horizontal axis ($I_{SYBR\ Green}$), respectively, in the GUV population, in which EXPAR is occurring, sampled at different incubation time. (a) 0 min, (b) 30 min, (c) 60 min, (d) 90 min, (e) 120 min, (f) a plot overlying all time points.

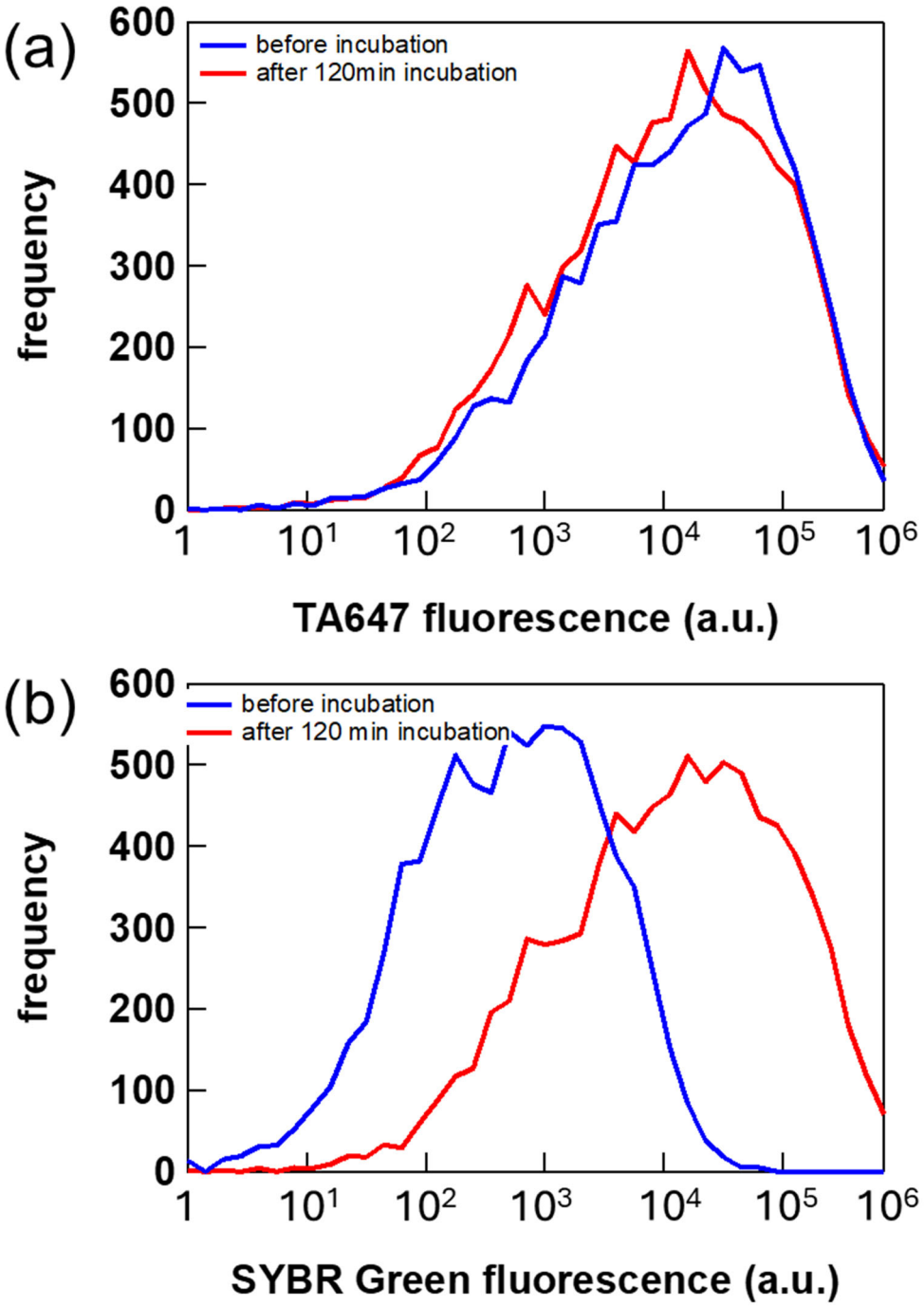

Fig. S4 Frequency distributions of (a) TA647 (volume marker) fluorescent intensity and (b) SYBR Green (cDNA amplification marker) fluorescent intensity in the flow cytometry measurement of GUVs containing EXPAR system and 100 nM let-7a. (Fig. 2B).

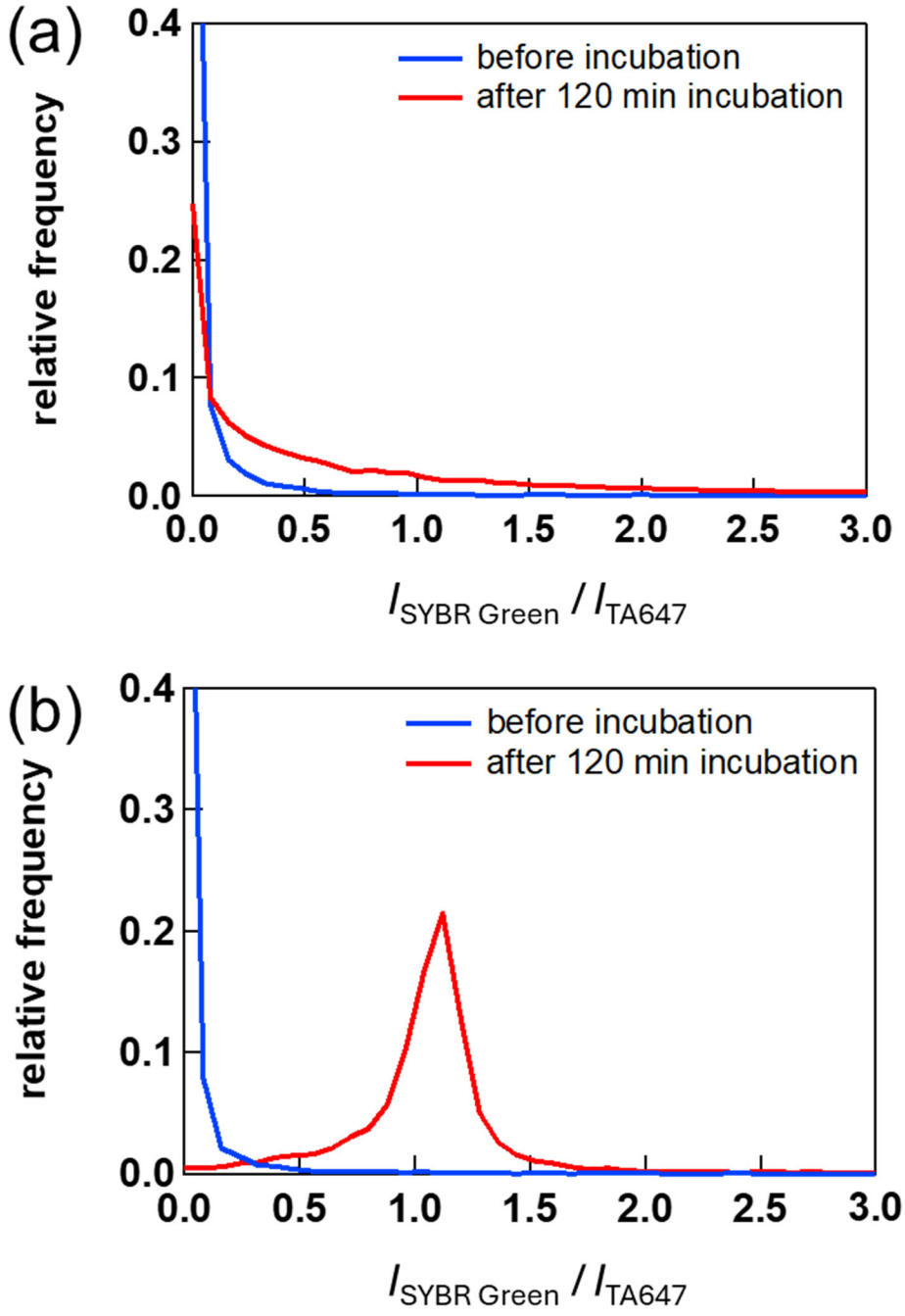

Fig. S5 Relative frequency distributions of the ratio of SYBR Green (cDNA amplification marker) fluorescent intensity over the TA647 (Volume marker) fluorescent intensity, reflecting the amount of amplicon per unit GUV volume. Data was calculated from the FCM data shown in Fig. 2B. (a) Without let-7a, (b) with 100 nM let-7a.

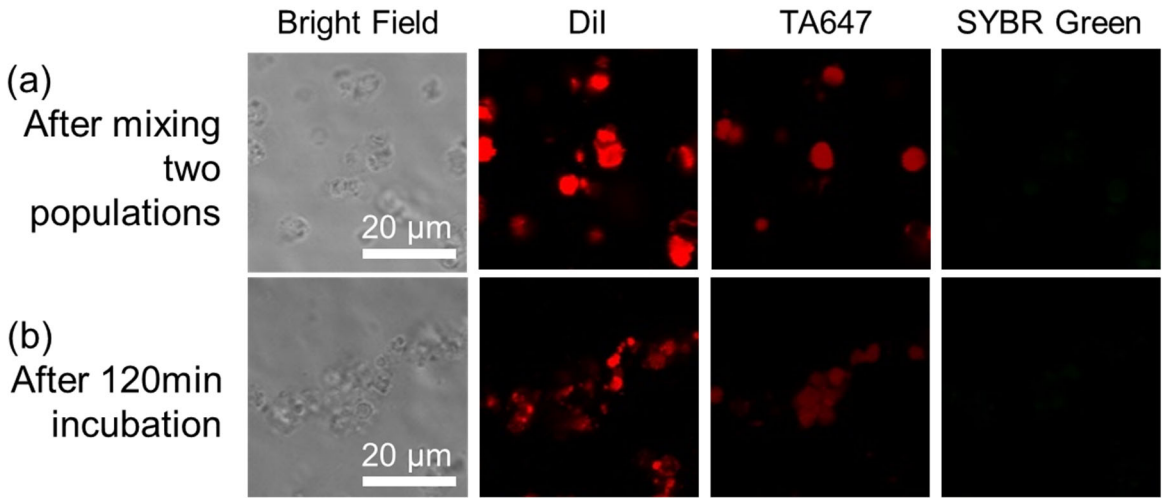

Fig. S6 Fluorescent confocal laser scanning microscopy images of the mixture of two GUV populations, containing EXPAR system (marked with DiI) and miRNA (marked with TA647). Composition and experimental procedure are exactly the same with the experiment shown in Fig. 3 in the main text, except the electrofusion step was omitted. No green signal was observed, excluding the possibility of spontaneous membrane fusion and reaction.

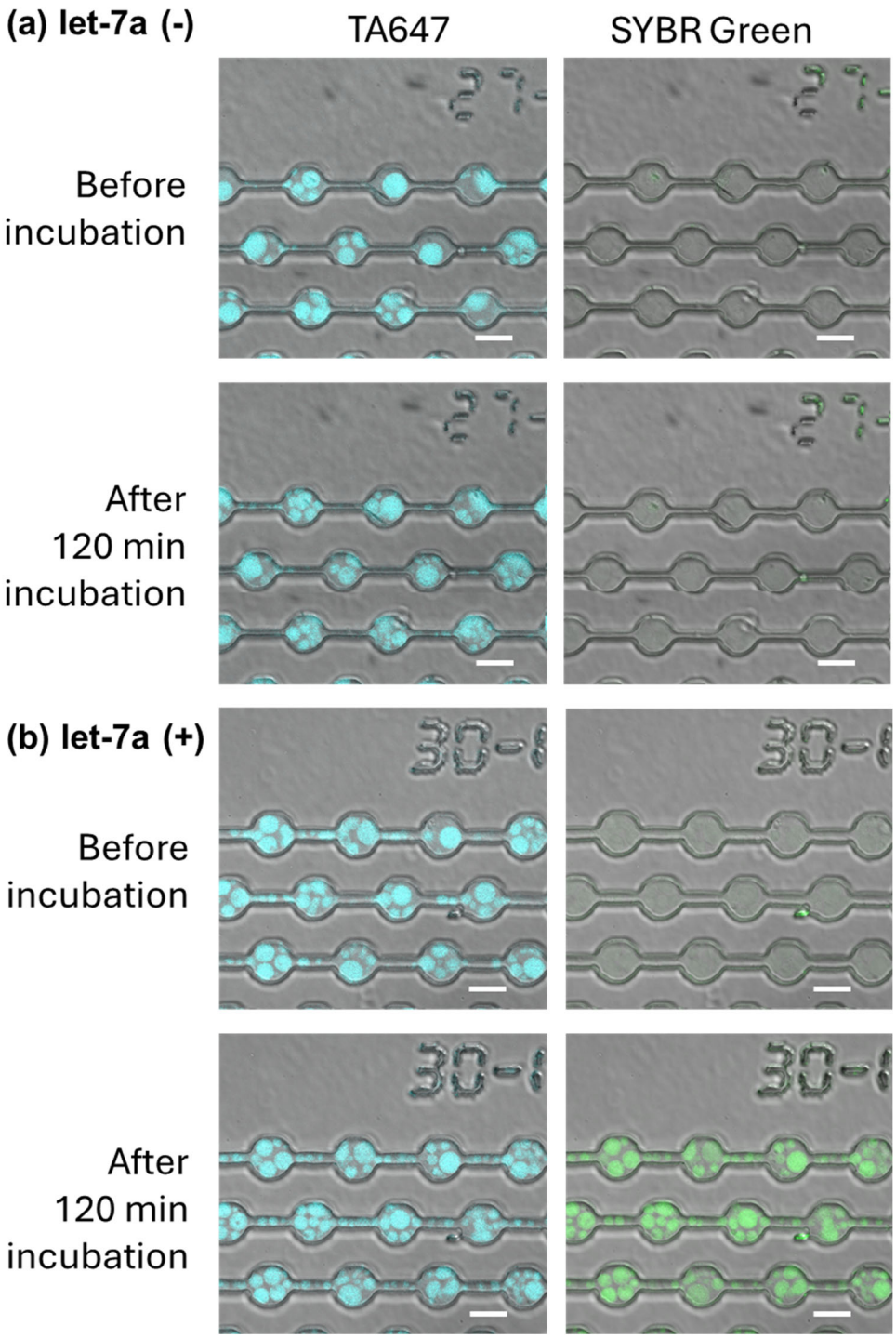

Fig. S7 Test of *in-liposome* EXPAR settled within the microchambers in the custom-made electrofusion device. (a) Without let-7a and (b) with 100 nM let-7a. Amplification was properly proceeded only when the miRNA was present in the mixture encapsulated in GUVs. Scale bars = 20 μm

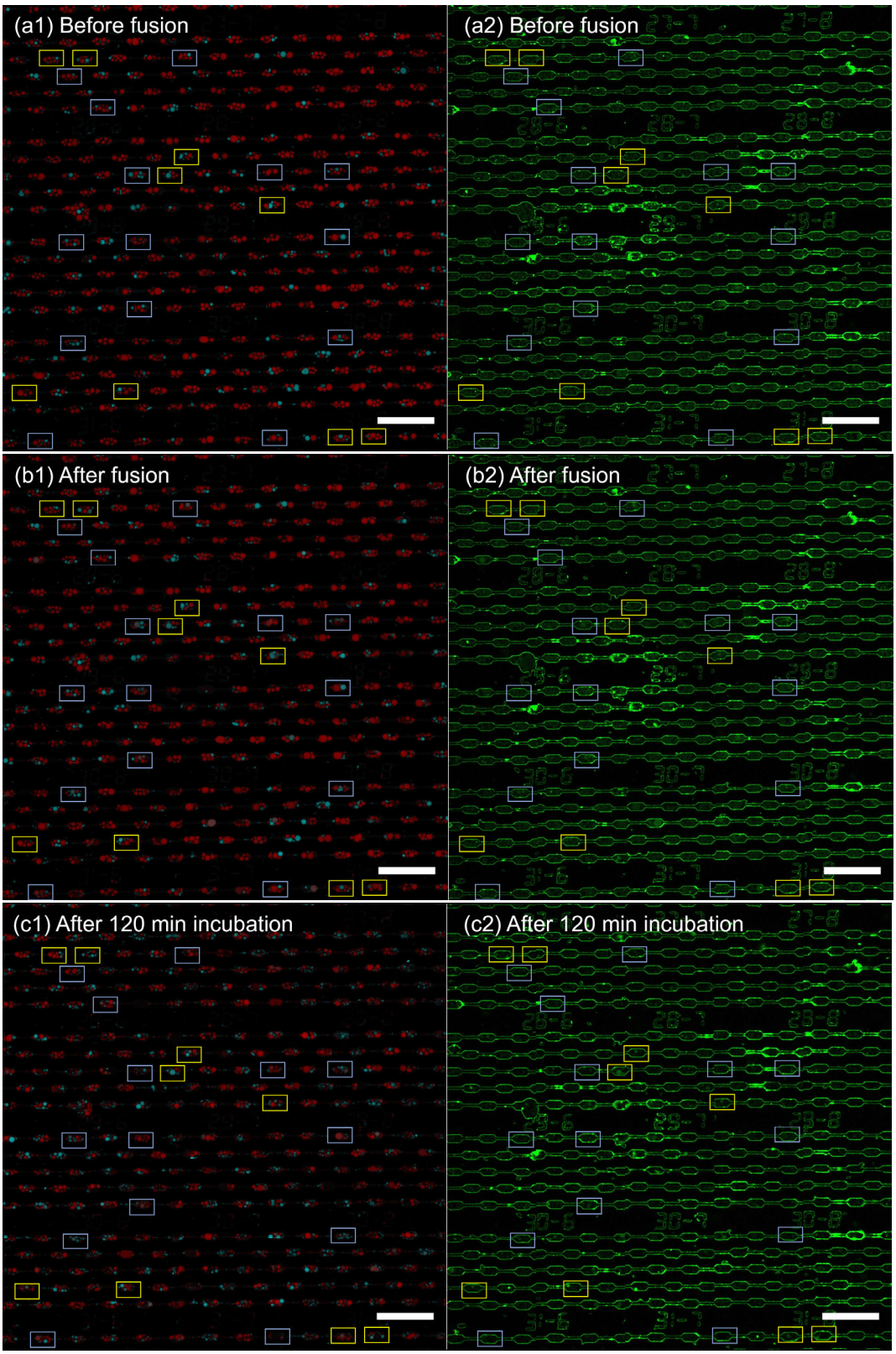

Fig. S8 Wide view of the electrofusion device for GUV-GUV fusion experiment with miRNA. Red (TRITC) GUV and cyan (TA647) GUV contain miRNA (let-7a) and EXPAR system, respectively, in panels a1, b1, and c1. Green (SYBR Green) fluorescens in panels a2, b2, and c2 is the marker of the cDNA amplification. (a) Before applying electric pulse, (b) after applying electric pulse, and (c) after 120 min incubation. In microchambers marked with yellow bexes, two types of GUVs fused, and the green fluorescence was determined to increase by EXPAR. In microchambers marked with blue boxes, two types of GUVs were fused, but green fluorescence did not appear after incubation. Scale bars are 100 μm.

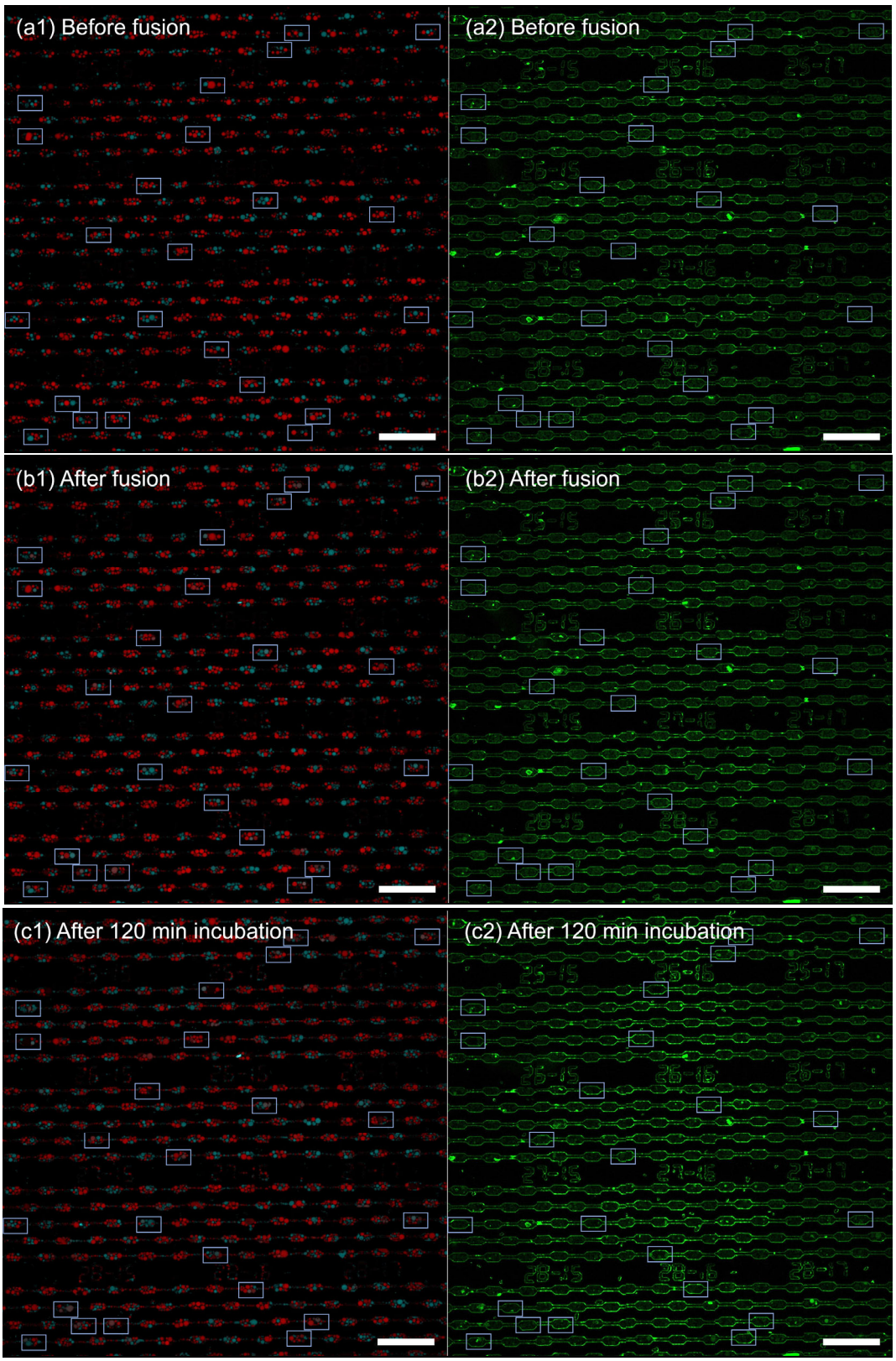

Fig. S9 Wide view of the electrofusion device for GUV-GUV fusion experiment without miRNA. Red (TRITC) GUV and cyan (TA647) GUV contain no miRNA and EXPAR system, respectively, in panels a1, b1, and c1. Green (SYBR Green) fluorescens in panels a2, b2, and c2 is the marker of the cDNA amplification. (a) Before applying electric pulse, (b) after applying electric pulse, and (c) after 120 min incubation. In microchambers marked with blue boxes, two types of GUVs were fused, but green fluorescence did not appear after incubation. Scale bars are 100 μm.

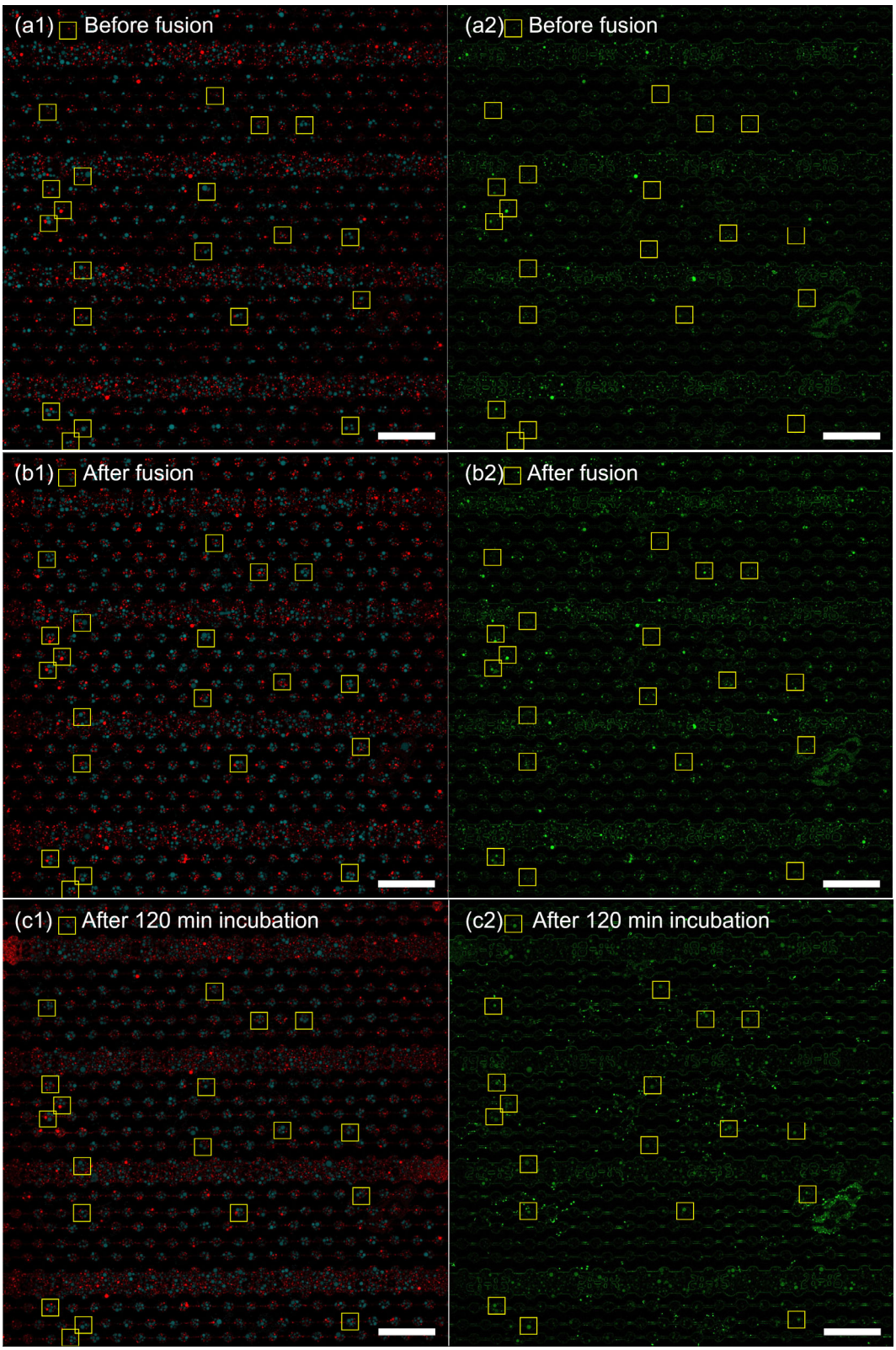

Fig. S10 Wide view of the electrofusion device for GUV-small GUV fusion experiment with miRNA. Red (TRITC) small (extruded) GUV and cyan (TA647) GUV contain miRNA (let-7a) and EXPAR system, respectively, in panels a1, b1, and c1. Green (SYBR Green) fluorescens in panels a2, b2, and c2 is the marker of the cDNA amplification. (a) Before applying electric pulse, (b) after applying electric pulse, and (c) after 120 min incubation. In microchambers marked with yellow boxes, the green fluorescence was determined to increase by EXPAR. Scale bars are 100 μm.

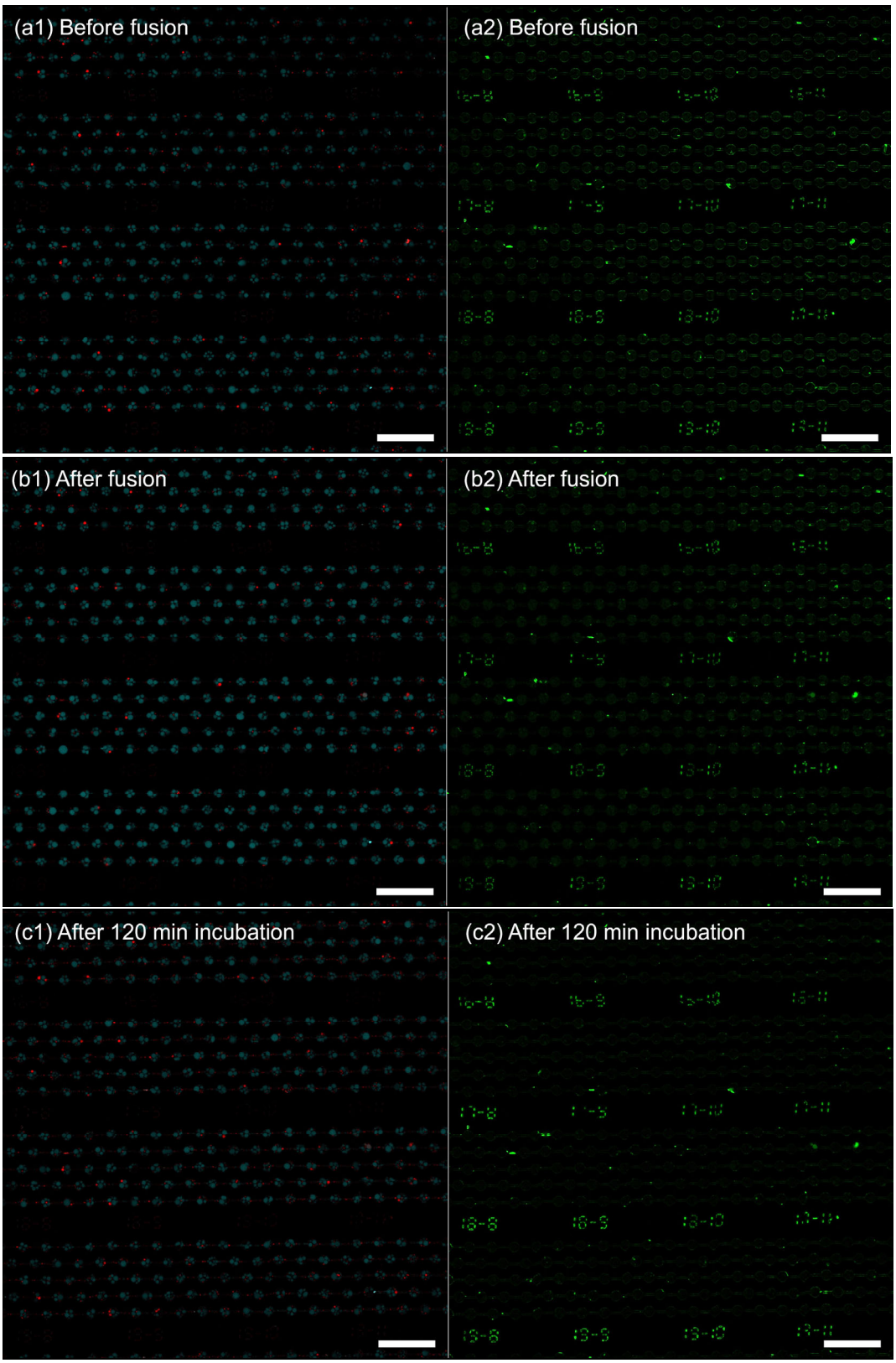

Fig. S11 Wide view of the electrofusion device for GUV-small GUV fusion experiment without miRNA. Red (TRITC) small GUV and cyan (TA647) GUV contain no miRNA and EXPAR system, respectively, in panels a1, b1, and c1. Green (SYBR Green) fluorescens in panels a2, b2, and c2 is the marker of the cDNA amplification. (a) Before applying electric pulse, (b) after applying electric pulse, and (c) after 120 min incubation. Scale bars are 100 μm.

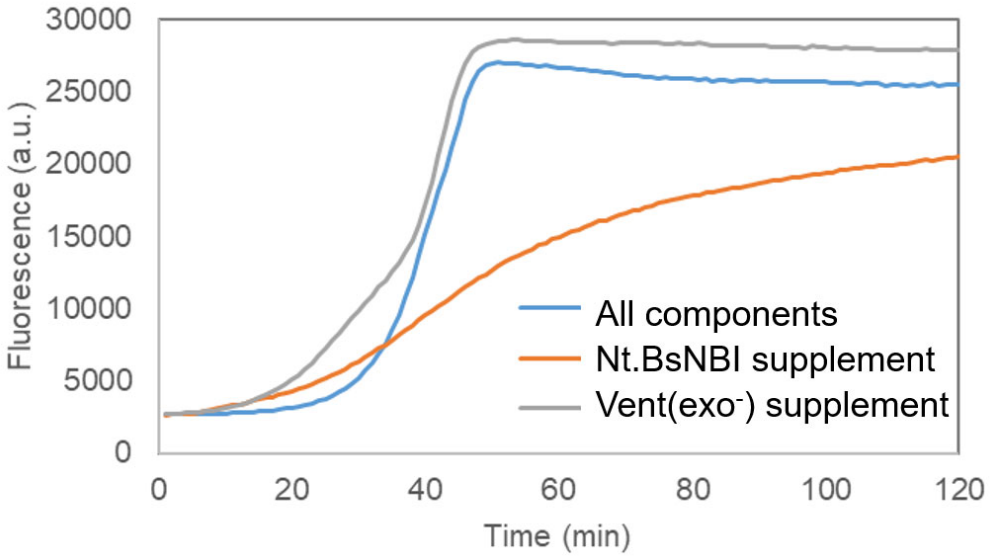

Fig. S12 Time-course of the SYBR Green fluorescent intensities in the test tube. Blue line: a reference reaction curve of EXPAR, in which all components were present including 100 nM let-7a. Gray line: The EXPAR mixture from which Vent (exo-) polymerase was initially excluded, and this solution was subjected to the electric pulse (700 V, 60 μs, three times) in a electrofusion cuvette. Then, Vent (exo-) was supplemented to record fluorescent intensity. Orange line: The EXPAR mixture from which Nt.BsNBI endonuclease was initially excluded, and this solution was subjected to the electric pulse (700 V, 60 μs, three times) in a electrofusion cuvette. Then, Nt.BsNBI was supplemented to record fluorescent intensity. The decreased amplification activity in the last condition indicates that activity of Vent (exo-) was weakened by the electric pulse.

Table S1. Compositions of the internal aqueous solutions for two GUV populations for the bulk GUV-GUV fusion experiment (Fig. 3).

GUV-1 (EXPAR system containing population)

Component	Final Conc.
Sucrose	200 mM
Custom ThermoPol® buffer	1x
NEBuffer3.1	0.5x
RNase inhibitor	0.8 unit/μL
Amplification template	0.2 μM
Nt.BstNBI	0.8 unit/μL
Vent (exo$^-$)	0.1 unit/μL
dNTP	0.5 mM
SYBR Green I	2.5x

GUV-2 (miRNA containing population)

Component	Final Conc.
Sucrose	200 mM
Custom ThermoPol® buffer	1x
NEBuffer3.1	0.5x
RNase inhibitor	0.8 unit/μL
let-7a	0.4μM
dNTP	0.5 mM
SYBR Green I	2.5x
TA647	4 μM

*Membrane of GUV-1 is stained with DiI.

Table S2. Compositions of the internal aqueous solutions for two GUV populations for the GUV-GUV fusion experiment within the electrofusion microdevice (Fig. 4B).

GUV-1 (EXPAR system containing population)

Component	Final Conc.
Sucrose	200 mM
Custom ThermoPol® buffer	1x
NEBuffer3.1	0.5x
RNase inhibitor	0.8 unit/μL
Amplification template	0.4 μM
Nt.BstNBI	0.8 unit/μL
Vent (exo$^-$)	0.1 unit/μL
dNTP	0.5 mM
SYBR Green I	2x
TA647	2 μM

GUV-2 (miRNA containing population)

Component	Final Conc.
Sucrose	200 mM
Custom ThermoPol® buffer	1x
NEBuffer3.1	0.5x
RNase inhibitor	0.8 unit/μL
let-7a	0.4μM
TRITC-conjugated dextran	0.6 mg/mL

Table S3. Compositions of the internal aqueous solutions for two GUV populations for the GUV-small GUV fusion experiment within the electrofusion microdevice (Fig. 4C).

GUV-1 (EXPAR system containing population)

Component	Final Conc.
Sucrose	200 mM
Custom ThermoPol® buffer	1x
NEBuffer3.1	0.5x
RNase inhibitor	0.8 unit/μL
Amplification template	0.4 μM
Nt.BstNBI	0.8 unit/μL
Vent (exo^-)	0.1 unit/μL
dNTP	0.5 mM
SYBR Green I	2x
TA647	2 μM

small GUV-2 (miRNA containing population)

Component	Final Conc.
Sucrose	200 mM
Custom ThermoPol® buffer	1x
NEBuffer3.1	0.5x
RNase inhibitor	0.8 unit/μL
let-7a	50 μM
TRITC-conjugated dextran	0.6 mg/mL