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Supporting Information

The smallest near-infrared fluorescence complementation system for imaging protein-protein and RNA-protein interactions

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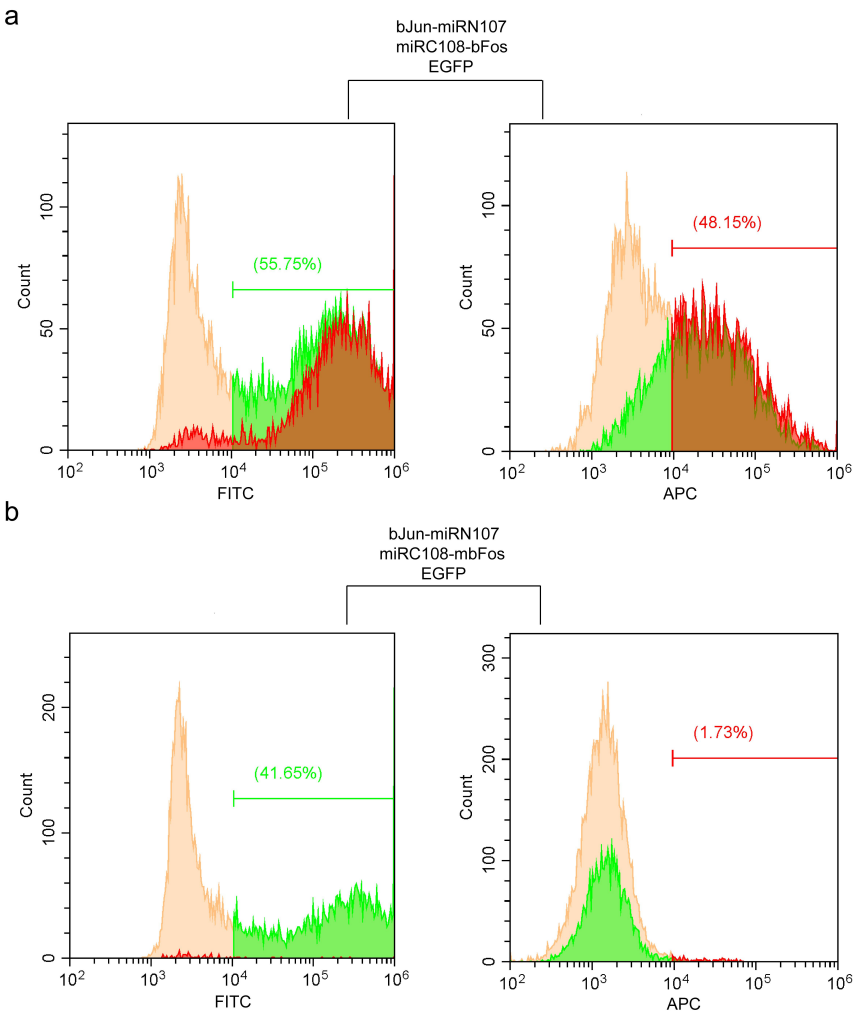

Fig. S1. Flow cytometry analysis was carried out to characterize the miRFP670nano-BiFC system for imaging PPIs in living cells. (a) bJun-bFos interaction was analyzed with flow cytometry in HEK 293T cells. (b) bJun-mbFos interaction was selected as the negative control and analyzed with flow cytometry in HEK 293T cells. EGFP was co-expressed in the cells as an internal control. EGFP fluorescence was detected by FITC channel and miRFP670nano fluorescence was detected by APC channel. This experiment was repeated three times.

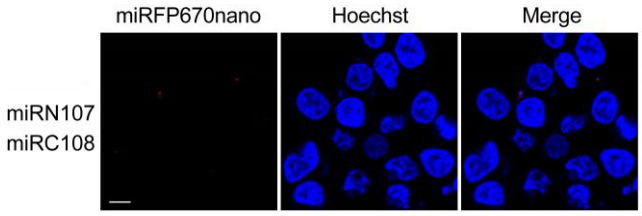

Fig. S2. No miRFP670nano complementary fluorescence signal was detected when co-expression of the two free miRFP670nano split fragments without fusion to the bJun-bFos heterodimer. The nuclei were stained with Hoechst 33342. Scale bar: 10 μm

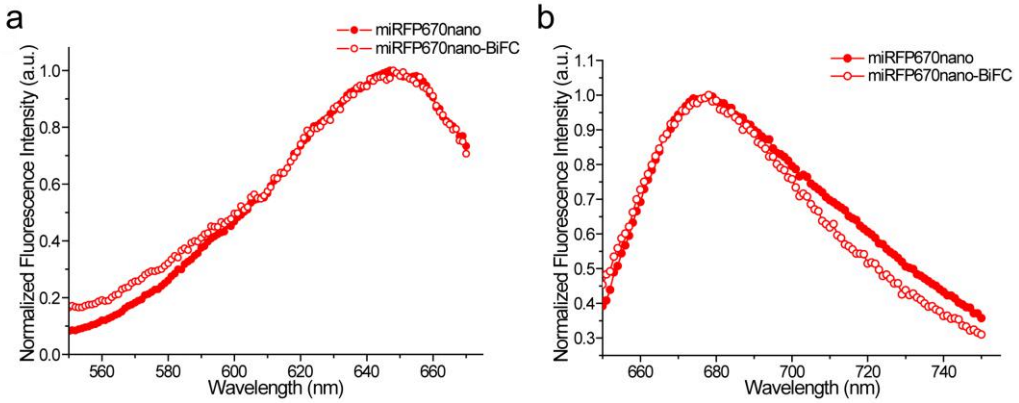

Fig. S3. The excitation and emission spectra of native (red filled circle) and BiFC-reconstituted (red open circle) miRFP670nano. (a) The excitation spectra of native (red filled circle) and BiFC-reconstituted (red open circle) miRFP670nano. (b) The emission spectra of native (red filled circle) and BiFC-reconstituted (red open circle) miRFP670nano. The excitation spectra of miRFP670nano were taken with emission at 700 nm and collected from 550 nm to 670 nm. The emission spectra of miRFP670nano were taken with excitation at 620 nm and collected from 650 nm to 750 nm.

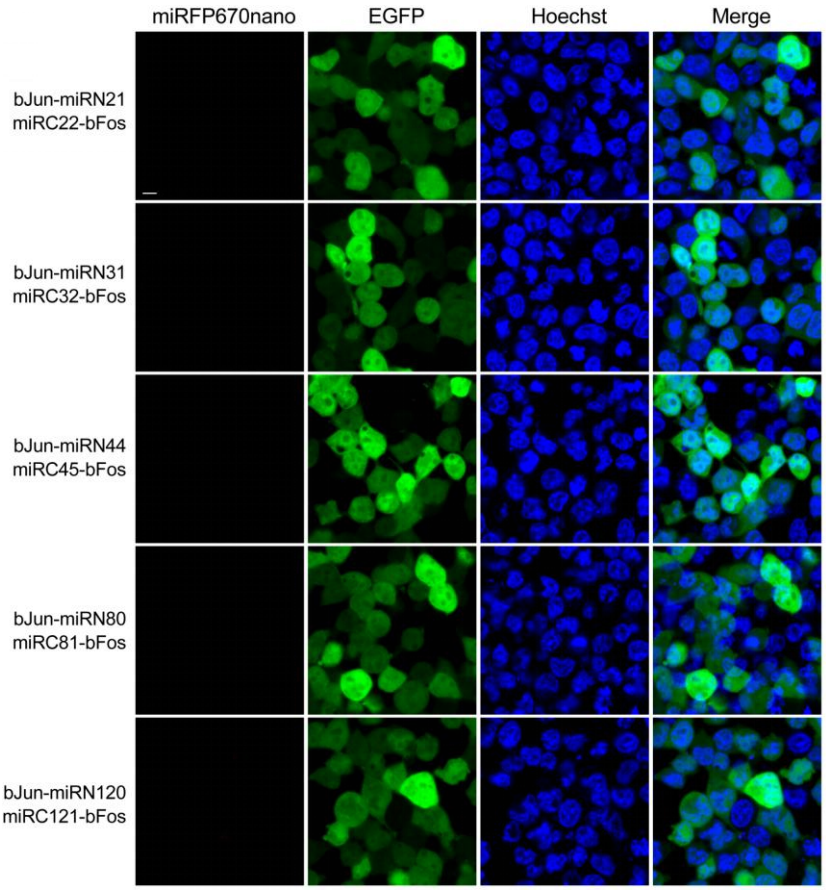

Fig. S4. No observable BiFC signal was detected when miRFP670nano was split into fragments at other five split sites. Nuclei were stained with Hoechst 33342. Scale bar: 10 μm.

bJun-miRN107 (591 bp)

ATGAAGGCGGAGAGGAAGCGCATGAGAAACCGCATCGCTGCCTCCAAGTGC
CGGAAAAGGAAGCTGGAGCGGATCGCCCGGCTAGAGGAAAAAGTGAAAACC
TTGAAAGCGCAAAACTCCGAGCTGGCGTCCACGGCCAACATGCTCAGGGAA
CAGGTGGCACAGCTTAAACAGAAAGTCATGAACCACGTTAACAGTGGGTGCC
AACTCATGCTAACGCAGCAGTTGCAAACGTTTGGAGGTGGCGGGAGTGGAG
GTGGCGGGAGTATGGCAAACCTGGACAAGATGCTGAATACCACAGTAACAGA
GGTGCGGCAGTTCCTGCAGGTGGACAGAGTGTGCGTGTTCCAGTTTGAGGA
GGATTATAGCGGAGTGGTGGTGGTGGAGGCCGTGGACGATAGGTGGATCTC
CATCCTGAAGACCCAGGTGCGGGATAGATACTTCATGGAGACAAGGGGCGAG
GAGTATTCTCACGGCCGCTACCAGGCCATCGCCGACATCTACACCGCAAACC
TGACAGAGTGCTACAGGGATCTGCTGACACAGTTTCAGGTGAGAGCAATCCT
GGCCGTGCCCATCCTGCAGGGCTAA

miRC108-bFos (438 bp)

ATGAAGAAGCTGTGGGGCCTGTTGGTGGCACACCAGCTGGCGGCCCCTAGA
CAGTGGCAGACCTGGGAGATCGACTTTCTGAAGCAGCAGGCCGTGGTGGTG
GGCATCGCCATCCAGCAGAGCGGAGGTGGCGGGAGTGGAGGTGGCGGGAG
TGGTCGTGCGCAGTCCATCGGTCGTCGCGGTAAAGTTGAACAACTGTCCCCG
GAAGAGGAAGAGAAACGTCGCATCCGCCGTGAACGTAACAAAATGGCGGCA
GCGAAATGCCGTAACCGCCGTCGTGAACTGACCGACACCCTGCAGGCGGAA
ACCGACCAGCTGGAAGACGAAAAATCCGCGCTGCAAACCGAAATCGCGAAC
CTGCTGAAAGAAAAAGAAAAGCTGGAGTTCATCCTGGCGGCACACCGTCCG
GCGTGCAAAATCCCGAACGACCTGGGTTAA

Fig. S5. The gene sequences for the new constructs of miRFP670nano-BiFC system.

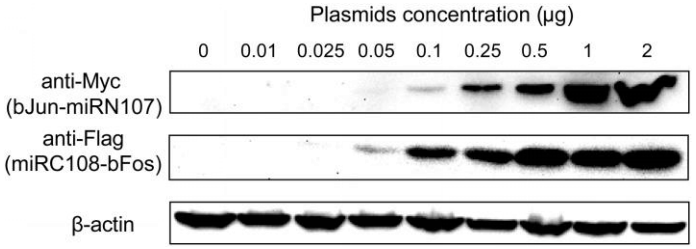

Fig. S6. The corresponding protein expression in **Fig. 1f** was determined by western blotting with anti-Myc or anti-Flag antibody. β-actin was used as the loading control.

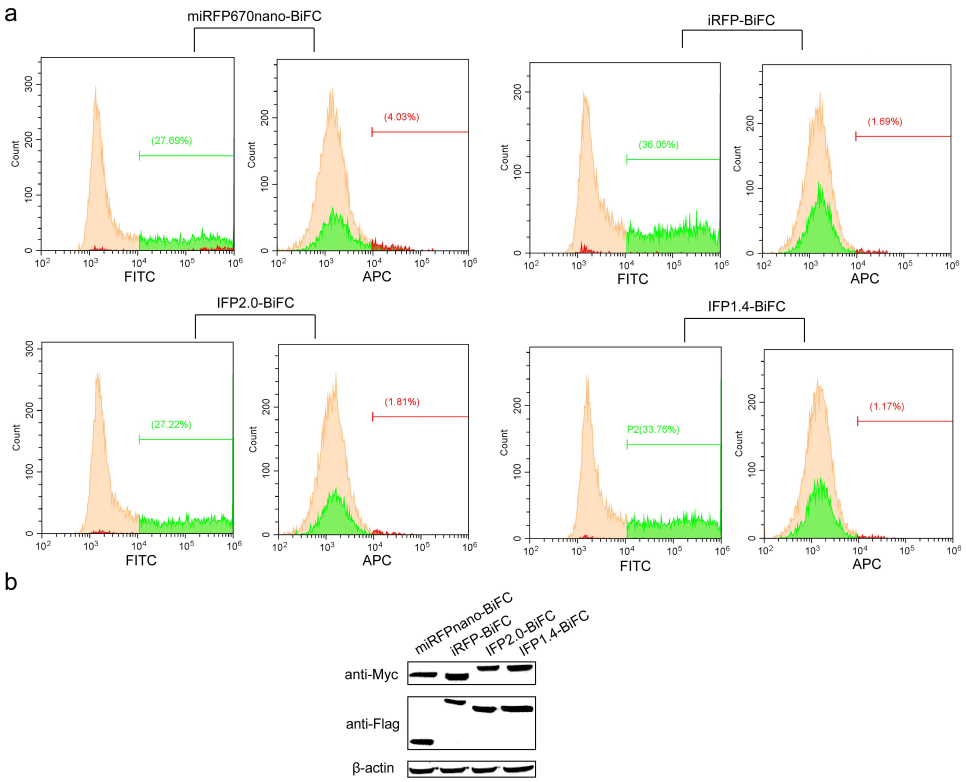

Fig. S7. Characterizing the maturation of miRFP670nano-BiFC and the reported near-infrared BiFC systems in living cells. (a) Different near-infrared BiFC systems were analyzed with flow cytometry in HEK 293T cells. EGFP was co-expressed in the cells as an internal control. EGFP fluorescence was detected by FITC channel and reconstructed near-infrared fluorescence was detected by APC channel. This experiment was repeated three times. (b) Expression of different near-infrared BiFC systems fusion proteins was determined by western blotting with anti-Myc or anti-Flag antibody. β-actin was used as the loading control. The N-terminal split fragments of different near-infrared BiFC systems were fused with Myc tag, and the C-terminal split fragments of different near-infrared BiFC systems were fused with Flag tag.

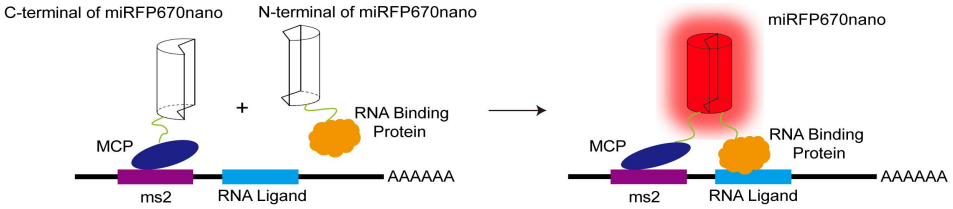

Fig. S8. The Schematic illustration of the miRFP670nano-TriFC constructs. MCP: bacteriophage MS2 coat protein; ms2: stem-loop RNA operator. The two fragments of miRFP670nano could come together and reconstitute to produce a TriFC signal based on the RNA binding protein/RNA ligand interaction.

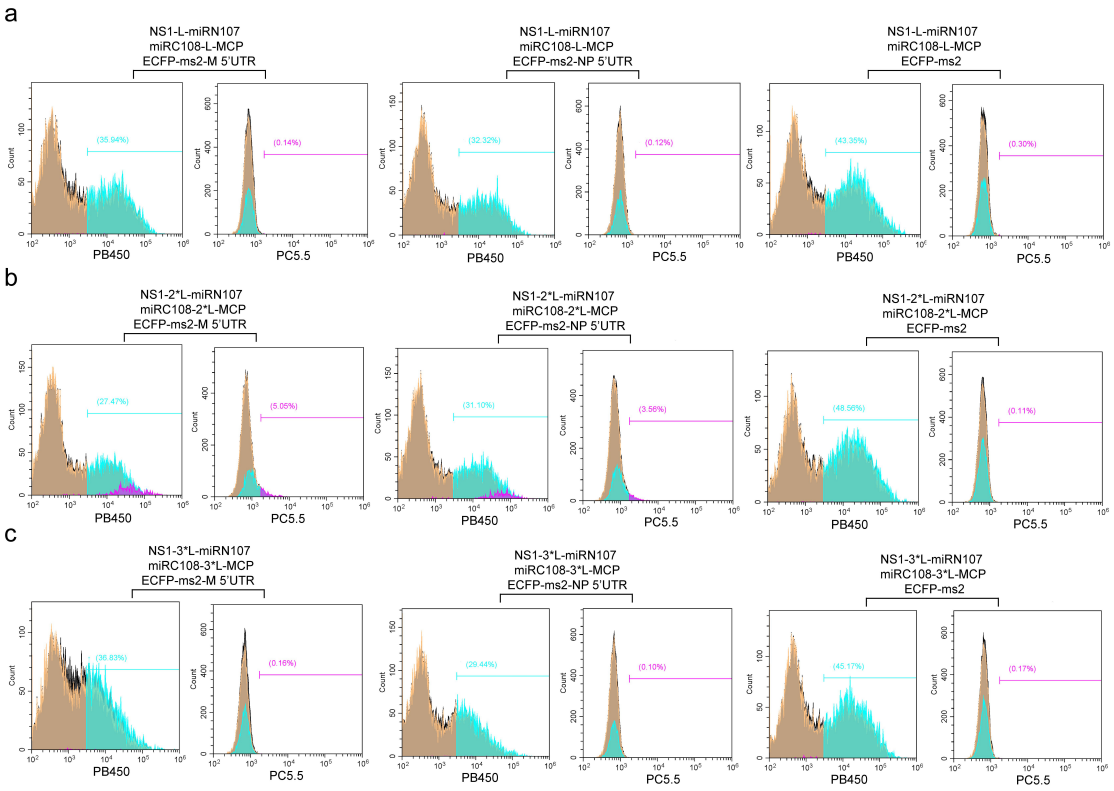

Fig. S9. Flow cytometry analysis was carried out to characterize the miRFP670nano-TriFC systems for imaging RNA-protein interaction in living cells. (a) The miRFP670nano-TriFC system with "L" as the linker was analyzed with flow cytometry in HEK 293T cells. (b) The miRFP670nano-TriFC system with "2*L" as the linker was analyzed with flow cytometry in HEK 293T cells. (c) The miRFP670nano-TriFC system with "3*L" as the linker was analyzed with flow cytometry in HEK 293T cells. ECFP fluorescence was detected by PB450 channel and miRFP670nano fluorescence was detected by PC5.5 channel. This experiment was repeated three times.

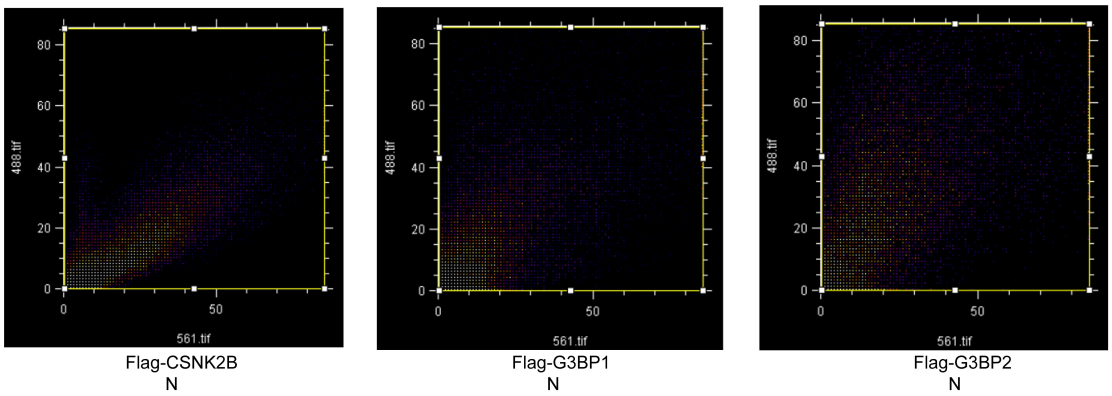

Fig. S10. The co-localization analysis of cellular stress granule proteins with N protein. The 488 channel represents the Flag-tagged cellular stress granule proteins, and 561 channel represents the N protein.

Table S1. The information of the split fragments of miRFP670nano to develop the BiFC assay.

Split sites	N-terminal split reporter	C-terminal split reporter
21-22	miRN21	miRC22
31-32	miRN31	miRC32
44-45	miRN44	miRC45
80-81	miRN80	miRC81
107-108	miRN107	miRC108
120-121	miRN120	miRC121

Table S2. Properties of the newly developed miRFP670nano-BiFC system compared with native miRFP670nano.

Name	Ex (nm)	Em (nm)	Extination coefficient ($M^{-1}cm^{-1}$)	Quantum yield (%)	Brightness in mammalian cells vs miRFP670nano (%)	Ref.
miRFP670nano	645	670	95000	10.8	100	1
miRFP670nano-BiFC	647	674	41800	6.3	25	This work

Table S3. Sequences of primers used in this study

Primers	Sequences (5′-3′)
bJun-NheI-F	CTAGCTAGCGCCACCATGAAGGCGGAGAGGAAGCGCATG AGAAACCGC
Linker-1-R	ACTCCCGCCACCTCCACTCCCGCCACCTCCAAACGTTTGC AACTGCTGCGTTAG
Linker-2-F	GGAGGTGGCGGGAGTGGAGGTGGCGGGAGTATGGCAAAC CTGGACAAGATGCTG
miRN21-HindIII-R	CCCAAGCTTTTACACCTGCAGGAACTGCCGCACCTC
miRC22-NheI-F	CTAGCTAGCGCCACCATGGACAGAGTGTGCGTGTTCCAGT TTG
Linker-3-R	ACTCCCGCCACCTCCACTCCCGCCACCTCCGCTCTGCTGG ATGGCGATGCCCACCAC
Linker-4-F	GGAGGTGGCGGGAGTGGAGGTGGCGGGAGTGGTCGTGCG CAGTCCATCGGTCG
bFos-HindIII-R	CCCAAGCTTTTAACCCAGGTCGTTCGGGATTTTGCACGCC GGACGG
miRN31-HindIII-R	CCCAAGCTTTTACTCCTCAAACTGGAACACGCACAC
miRC32-NheI-F	CTAGCTAGCGCCACCATGGATTATAGCGGAGTGGTGGTGG TGGAGG
miRN44-HindIII-R	CCCAAGCTTTTAATCGTCCACGGCCTCCACCACCAC
miRC45-NheI-F	CTAGCTAGCGCCACCATGAGGTGGATCTCCATCCTGAAGA CC
miRN80-HindIII-R	CCCAAGCTTTTAGGTGTAGATGTCGGCGATGGCCTG
miRC81-NheI-F	CTAGCTAGCGCCACCATGGCAAACCTGACAGAGTGCTACA GG
miRN107-HindIII-R	CCCAAGCTTTTAGCCCTGCAGGATGGGCACGGCC
miRC108-NheI-F	CTAGCTAGCGCCACCATGAAGAAGCTGTGGGGCCTGTTGG TG
miRN120-HindIII-R	CCCAAGCTTTTACGCCAGCTGGTGTGCCACCAACAG
miRC121-NheI-F	CTAGCTAGCGCCACCATGGCCCCTAGACAGTGGCAGACCT GG

Flag-miRC108-1-NheI-F	CTAGCTAGCGCCACCATGGATTATAAAGATGACGACGATAAAAAG
Flag-miRC108-2-F	TTATAAAGATGACGACGATAAAAAGAAGCTGTGGGGCCTGTTGGTG
miRN107-KpnI-F	GGGGTACCATGGCAAACCTGGACAAGATGCTG
miRN107-XbaI-R	TGCTCTAGATTAGCCCTGCAGGATGGGCACGGCC
miRC108-HindIII-R	CCCAAGCTTGCTCTGCTGGATGGCGATGCCCACCAC
miRN107-2*L-KpnI-F	GGGGTACCGGAGGTGGCGGGAGTGGAGGTGGCGGGAGTATGGCAAACCTGGACAAGATG
miRC108-2*L-HindIII-R	CCCAAGCTTACTCCCGCCACCTCCACTCCCGCCACCTCCGCTCTGCTGGATGGCGATGC
miRN107-3*L-KpnI-F	GGGGTACCGGAGGTGGCGGGAGTGGAGGTGGCGGGAGTGGAGGTGGCGGGAGTGGAGGTGGCGGGAGTATGGCAAACCTGGACAAGATGCTG
miRC108-3*L-HindIII-R	CCCAAGCTTACTCCCGCCACCTCCACTCCCGCCACCTCCACTCCCGCCACCTCCACTCCCGCCACCTCCGCTCTGCTGGATGGCGATGCCCACCAC
CSNK2B-NheI-F	CTAGCTAGCGCCACCATGAGCAGCTCAGAGGAGGTGTCCTGGATTTCC
CSNK2B-KpnI-R-1	GGGGTACCGCGAATCGTCTTGACTGGGCTCTTGAAGTTGCTGGCG
G3BP1-NheI-F	CTAGCTAGCGCCACCATGGTGATGGAGAAGCCTAGTCCCCTGC
G3BP1-HindIII-R-1	CCCAAGCTTCTGCCGTGGCGCAAGCCCCCTTCCCACTC
G3BP2-NheI-F	CTAGCTAGCGCCACCATGGTTATGGAGAAGCCCAGTCCGCTGC
G3BP2-KpnI-R-1	GGGGTACCGCGACGCTGTCCTGTGAAGCGGCCCTCC
Flag-CSNK2B-NheI-F	CTAGCTAGCGCCACCATGGATTATAAAGATGACGACGATAAAATGAGCAGCTCAGAGGAGGTGTCC
CSNK2B-KpnI-R-2	GGGGTACCTTAGCGAATCGTCTTGACTGGGCTCTTGAAG
Flag-G3BP1-NheI-F	CTAGCTAGCGCCACCATGGATTATAAAGATGACGACGATAAAATGGTGATGGAGAAGCCTAGTCC
G3BP1-HindIII-R-2	CCCAAGCTTTTACTGCCGTGGCGCAAGCCCCCTTCC

Flag-G3BP2-NheI-F	CTAGCTAGCGCCACCATGGATTATAAAGATGACGACGATAAAATGGTTATGGAGAAGCCCAGTCCG
G3BP2-KpnI-R-2	GGGGTACCTTAGCGACGCTGTCCTGTGAAGCGGCCC
CSNK2B-F	CCTGGTTCTGTGGGCTCCGTGGCAATG
CSNK2B-R	CCATAAAGCATCTCGGCTGCCTGCTC
G3BP1-F	GGATCAAAGAGTGCGAGAACAACG
G3BP1-R	CTCCACCACGTTTCCATAACTTTG
G3BP2-F	GTTGGTAACTTGCCACATGATATTG
G3BP2-R	CACATTTAAACGTACTTCCCCTCG
GAPDH-F	AGAAGGCTGGGGCTCATTTG
GAPDH-R	AGGGGCCATCCACAGTCTTC
N-BamHI-F	CGGGATCCATGTCTGATAATGGACCCCAAAATCAG
N-NotI-R	ATTTGCGGCCGCTTAGGCCTGAGTTGAGTCAGCACTGCTCATGG
N(1-246)-NotI-R	ATTTGCGGCCGCTTAGACAGTTTGGCCTTGTTGTTGTTGGCC
N(247-419)-BamHI-F	CGGGATCCATGACTAAGAAATCTGCTGCTGAGGCTTCTAAG
Myc-bJun-NheI-F	CTAGCTAGCGCCACCATGGAGCAGAAACTCATCTCTGAAGAGGATCTGATGAAGGC
bJun-2-F	CTCATCTCTGAAGAGGATCTGATGAAGGCGGAGAGGAAGCGCATGAGAAAC
bJun-R	ACTCCCGCCACCTCCACTCCCGCCACCTCCAAACGTTTGCAACTGCTGCGTTAG
miRN107-F	GGAGGTGGCGGGAGTGGAGGTGGCGGGAGTATGGCAAACCTGGACAAGATGCTG
miRN107-HindIII-R	CCCAAGCTTTTAGCCCTGCAGGATGGGCACGGCC
iRN97-F	GGAGGTGGCGGGAGTGGAGGTGGCGGGAGTATGGCTGAAGGATCCGTCGCCAGGC
iRN97-HindIII-R	CCCAAGCTTTTACATCGTGAAGCCGACAGTGATCGGTGCTC

IFP2.0-IFN132-F	GGAGGTGGCGGGAGTGGAGGTGGCGGGAGTATGGCTCGGGACCCTCAACCTTTC
IFP2.0-IFN132-HindIII-R	CCCAAGCTTTTACCATGCCTCAGTAGGCTCGAATTCCAG
IFP1.4-IFN132-F	GGAGGTGGCGGGAGTGGAGGTGGCGGGAGTATGGCTCGGGACCCTCTGCCATTCTTTC
IFP1.4-IFN132-HindIII-R	CCCAAGCTTTTACCAGGCCTCGGTAGGTTCGAACTCCAGGATC
Flag-iRC98-NheI-F	CTAGCTAGCGCCACCATGGATTATAAAGATGACGACGATAAACG
iRC98-2-F	GATTATAAAGATGACGACGATAAACGAAAGGACGCAGGCTTCATCGGCTCC
iRC98-R	ACTCCCGCCACCTCCACTCCCGCCACCTCCCTCTTCCATCACGCCGATCTGCCAGG
bFos-F	GGAGGTGGCGGGAGTGGAGGTGGCGGGAGTGGTCGTGCGCAGTCCATCGGTCG
bFos-HindIII-R	CCCAAGCTTTTAACCCAGGTCGTTCGGGATTTTGCACGCCGGACGG
Flag-IFP2.0-IFC133-NheI-F	CTAGCTAGCGCCACCATGGATTATAAAGATGACGACGATAAAGAC
IFP2.0-IFC133-2-F	GATTATAAAGATGACGACGATAAAGACTCTATTGGACCCCACGCTCTGAG
IFP2.0-IFC133-R	ACTCCCGCCACCTCCACTCCCGCCACCTCCGGCTTCTTTCCTCTGCACCTGCAGGG
Flag-IFP1.4-IFC133-NheI-F	CTAGCTAGCGCCACCATGGATTATAAAGATGACGACGATAAAGAC
IFP1.4-IFC133-2-F	GATTATAAAGATGACGACGATAAAGACAGCATTGGCCCTCACGCCCTGAG
IFP1.4-IFC133-R	ACTCCCGCCACCTCCACTCCCGCCACCTCCTTTATACAGCTCGTCCATTCCGGC
His-miRFP670nano-NheI-F	CTAGCTAGCGCCACCATGCACCACCACCACCACCACCACCACATGGCAAACCTGGAC
His-miRFP670nano-2-F	CACCACCACCACCACCACATGGCAAACCTGGACAAGATGCTGAATACC
miRFP670nano-HindIII-R	CCCAAGCTTTTAGCTCTGCTGGATGGCGATGCCCACCAC

His-bJun-NheI-F	CTAGCTAGCGCCACCATGCACCACCACCACCACCACCACC ACAAGGCGGAGAG
His-bJun-2-F	CACCACCACCACCACCACCACCACAAGGCGGAGAGGAAG CGCATGAGAAAC
His-miRC108-NheI-F	CTAGCTAGCGCCACCATGCACCACCACCACCACCACCACC ACAAGAAGCTGTGG
His-miRC108-2-F	CACCACCACCACCACCACCACCACAAGAAGCTGTGGGGC CTGTTGGTG

Forward primer; R, Reverse primer

1. O. S. Oliinyk, A. A. Shemetov, S. Pletnev, D. M. Shcherbakova and V. V. Verkhusha, *Nat Commun*, 2019, **10**, 279.