Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Size-selective DNA Nanocage-based Activatable CRISPR-Cas12a for Sensitive and Accurate Detection of Mature MicroRNA

Xiaoyi Fu,[a,†] Yao Yin,[a,†] Meng Zhang,[a] Fangqi Peng,[a] Yuyan Shi,[a] Yan Liu,[b] Yin Tan,[a] Zilong Zhao,[a] Xia Yin,[1] Jie Song,[b] Guoliang Ke,[a,*] Xiao-Bing Zhang [a]

[a]Molecular Sciences and Biomedicine Laboratory, State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, China

[b]Institute of Nano Biomedicine and Engineering, Department of Instrument Science and Engineering, School of Electronic Information and Electrical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China.

[†]These authors contributed equally to this work.

*Corresponding author: glke@hnu.edu.cn

Table of Contents

Experimental Section

Materials and Apparatus. All DNA oligonucleotides (Table S1 in Supporting Information) were purchased from Sangon Biotech Co. Ltd. (Shanghai, China) and HIPPOBIO Bio-Technology Co.Ltd (Beijing, China). The modified oligonucleotides and short strands were purified by high-performance liquid chromatography (HPLC), while other oligonucleotides were purified by ultrapage. All miRNA sequences were purchased from Shanghai GenePharma (Shanghai, China). AsCas12a Nuclease was purchased from Tolo Biotech., and NEB buffer 2.1 was purchased from NEB (New England Biolabs, Ipswich, MA, U.S.A.). Other reagents not specifically mentioned were analytical grade and used without further treatment. Ultrapure water was obtained from a Milli-Q system (Billerica, MA, USA). The quantitative analysis of oligonucleotides was performed by Thermo Scientific NanoDrop 2000c Spectrophotometer (Waltham, MA, USA). The fluorescence measurements were measured on an Edinburgh FS5 spectrofluorometer (Livingston, UK) at room temperature. Human blood serum from Xiangya Hospital Central South University.

Preparation and Characterization of DNA-nanocage encapsulated Ts. The DNA nanocage encapsulated Ts were self-assembled based on previous reports with slight modification.[1] In brief, for the encapsulation of Ts for miRNA (we called Ts-miRNA) detection in the DNA nanocages, the Ts-miRNA, linker strands (named as c-linker) and framework strands were mixed as molar ratio of 10:5:1 in Tris-acetic acid-magnesium (TAE-Mg^{2+}) buffer (20 mM Tris, 2 mM EDTA, 20 mM MgCl2, pH 7.4) and annealed from 95 ℃ to 4 ℃. For dsTs-miRNA, Ts-miRNA out of the cage (called Cage-out-Ts-miRNA), and different size of Cage-in-Ts-miRNA were assembled as above. Table S1 shows all sequences used in this work. The DNA nanocages were characterized by 5% native polyacrylamide gel electrophoresis (N-PAGE) with 1×TAE-Mg^{2+} buffer at 110 V for 2 h at room temperature and analyzed by a fluorescence image scanner (FLA-3000G, Fuji, Tokyo, Japan).

Fluorescence Analysis. Fluorescence spectra were determined using an Edinburgh FS5 spectrofluorometer. The fluorescence emission spectrum of FAM was collected from 510 to 650 nm with excitation at 488 nm. For investigation of the encapsulation effect, the inactive Cas12a/crRNA complex (1.5 nM/1.5 nM) in NEB buffer 2.1 was incubated with DNA nanocage probes for 30 min at 37 ℃, followed by addition of 500 nM ssDNA-FQ reporter for 30 min at 37 ℃. For miRNA sensing, DNA nanocage probes were first incubated with a series of concentrations of miRNA for 1h at 37 ℃. Then, the inactive Cas12a/crRNA complex was added to the above reaction product for another 30 min at 37 ℃, followed by addition of 500 nM ssDNA-FQ for 30 min at 37 ℃. To distinguish between miRNA and pre-miRNA, the concentrations of miRNA and pre-miRNA were1 nM and 10 nM, respectively. For the selectivity experiments, the concentrations of miRNA-21 and other control groups were the same 1 nM.

Quantification of miRNA-21 in Serum Samples with INSPIRE-Cas12a. The biological serum samples were obtained from the Xiangya Hospital Central South University (Changsha, China). Approval was obtained from the local agency review board and informed consent was obtained from all subjects for serum sampling. Serum samples were collected by standard surgical procedures. The serum sample was pretreated by centrifugation at 4 ℃(10,000×g, 10 min). Then, 10 μL of supernatant was mixed with 90 μL TAE-Mg^{2+} buffer, and the solution was heated to 95 ℃ for 10 min to deactivate deoxyribonuclease. Finally, different concentrations of miRNA-21 were added to the serum sample by the standard addition method, and detected by the INSPIRE-Cas12a system. The reaction and fluorescence detection were performed using the above-mentioned procedures. The stability of reporter DNA strand in the treated serum was also studied using an Edinburgh FS5 spectrofluorometer.

AFM imaging. 2 μL of DNA nanocage sample (100 nM) was dropped on freshly cleaved mica. After 2 min binding, 100 μL 1×TE/Mg^{2+} buffer (20 mM Tris, 2 mM EDTA, 20 mM $MgCl_2$, pH 7.4) was added. At last, 2 μL 1M $NiCl_2$ was added to increase the binding efficiency of DNA nanocage on mica surface. AFM imaging was performed in liquid mode using Multimode VIII system (Bruker Corporation, Santa, Barbara, CA).

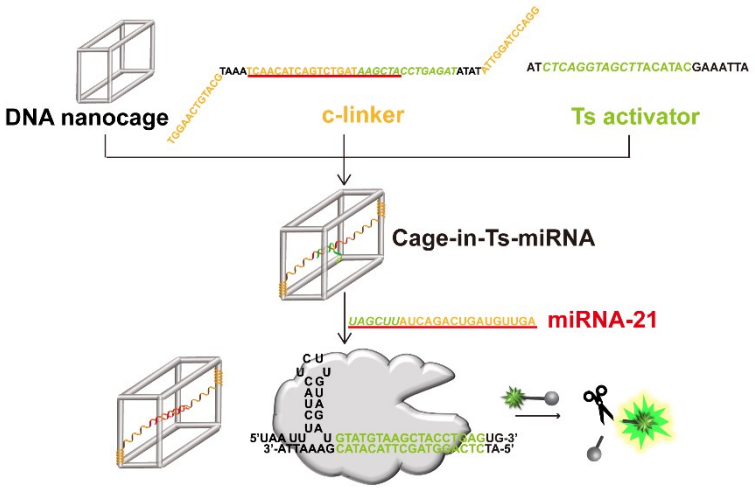

Figure S1. The design of Ts-miRNA encapsulated in the DNA nanocage (Cage-in-Ts-miRNA), which can release Ts to activate CRISPR/Cas12a activity in the presence of miRNA.

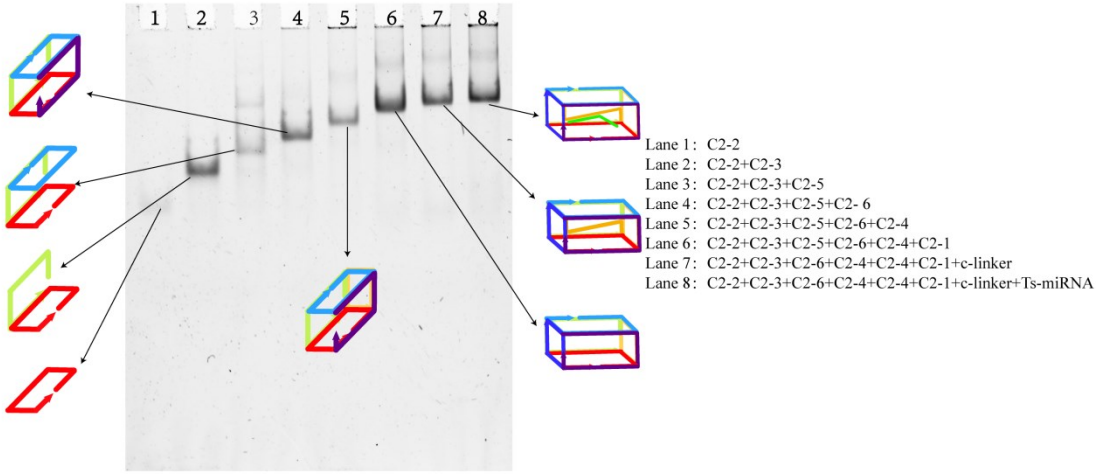

Figure S2. N-PAGE analysis of Cage-in-Ts-miRNA. 5% native-PAGE analysis of the stepwise assembly of DNA nanocage (lane 1 to lane 6) and the hybridization of c-linker (lane 7) and Ts-miRNA (lane 8) (Cage scaffold strands were the same as those of Cage 2).

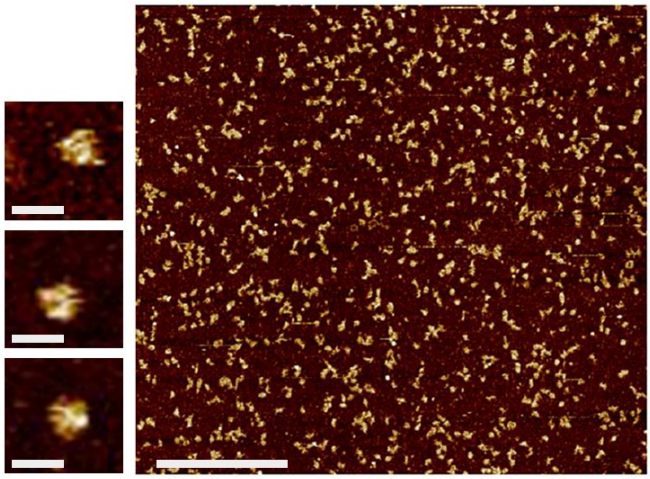

Figure S3. The atomic force characterization of Cage-Ts-miRNA. The scale bars are 500 and 10 nm in large and small imaging, respectively.

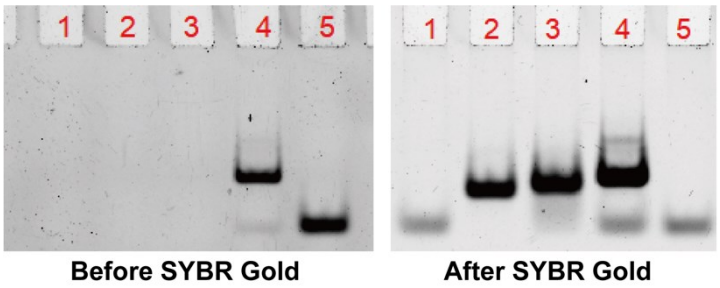

Figure S4: N-PAGE characterization of the binding of FAM-labeled miRNA-21 with c-linker. Lane 1: Ts-miRNA; Lane 2: c-linker; Lane 3: Ts-miRNA + c-linker; Lane 4: Ts-miRNA + c-linker + miRNA-21-FAM; Lane 5: miRNA-21-FAM (Left: fluorescence imaging of FAM-labeled miRNA-21 without SYBR Gold staining; Right: all nucleic acid strand staining with SYBR Gold). As the result shown, we can observe that the miRNA-21 can bind with c-linker to release Ts-miRNA (lane 4).

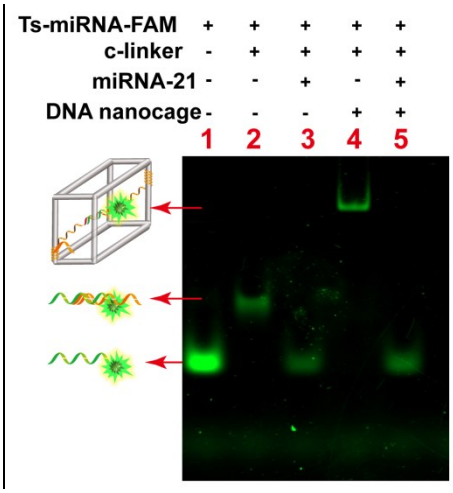

Figure S5. N-PAGE characterization of the release of FAM-labeled Ts-miRNA from Cage-Ts-miRNA in the presence of miRNA. Lane 1: Ts-miRNA-FAM; lane 2: dsTs-miRNA-FAM; lane 3: dsTs-miRNA-FAM with miRNA-21; lane 4: Cage-in-Ts-miRNA-FAM; lane 5: Cage-in-Ts-miRNA-FAM with miRNA-21. As shown in the result, the Ts-miRNA-FAM (lane 1) can hybridize with c-linker (to form dsTs-miRNA-FAM, lane 2) and be encapsulated in the DNA nanocage (lane 4). In the presence of miRNA-21, Ts-miRNA-FAM can be released from c-linker because of the strand displacement reaction between miRNA and c-linker (lane 3). Similarly, the addition of miRNA-21 can also trigger the release of Ts-miRNA-FAM from the DNA nanocage (lane 5). These results confirmed the efficient encapsulation and miRNA-triggered release of Ts-miRNA, thus providing the basis of the INSPIRE-Cas12a system.

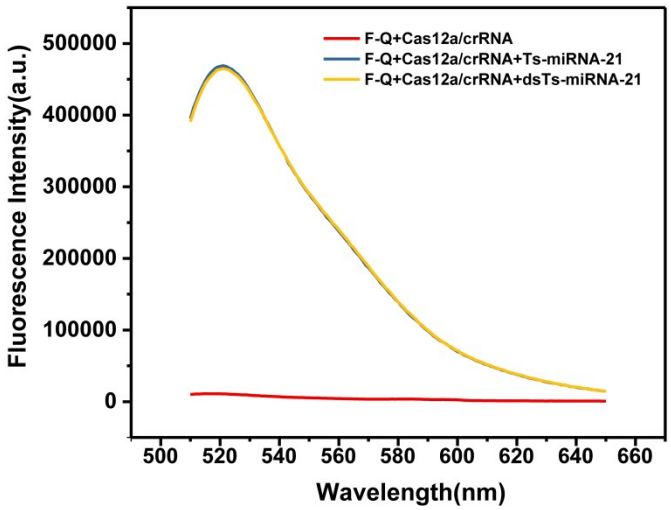

Figure S6. Investigation of Cas12a/crRNA (6 nM/3 nM) collateral activity to Ts-miRNA (2 nM) and dsTs-miRNA (Ts-miRNA hybridized with c-linker, 2 nM). The reporter is a pre-quenched 5 nt-ssDNA with a FAM and BHQ1 labeled on opposite termini, which can be digested to create an enhanced fluorescence signal by the collateral cleavage activity of active Cas12a/crRNA. Obvious fluorescence increase was observed both in the presence of Ts-miRNA and dsTs-miRNA, indicating the feasibility of Ts design.

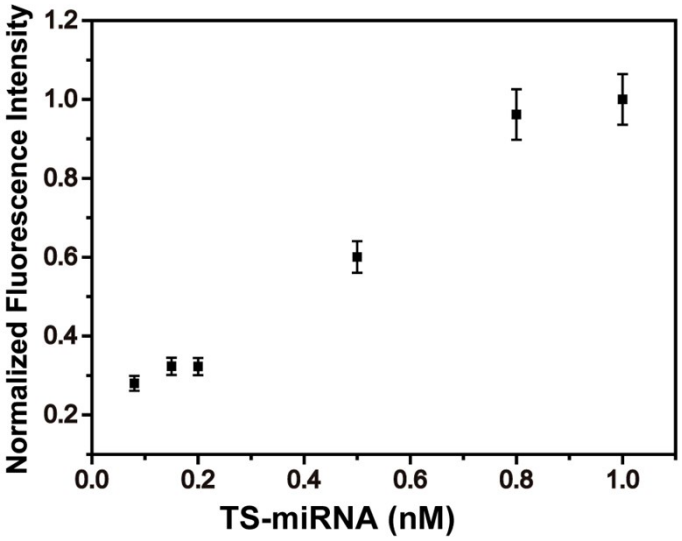

Figure S7. Investigation of Cas12a/crRNA activity (0.5 nM/1.5 nM) to different Ts concentrations (The average fluorescence intensity of 1 nM Ts-miRNA was set as 1). The fluorescence intensity increased with increasing of Ts-miRNA concentration (Figure S3), thus confirming the important role of the Ts-miRNA for Cas12a activation and the concentration dependence of enzyme activity. Since the concentration change exhibited significant influence on the activity at 0.5 nM of Ts, the Ts-miRNA concentration for future experiments was chosen as 0.5 nM.

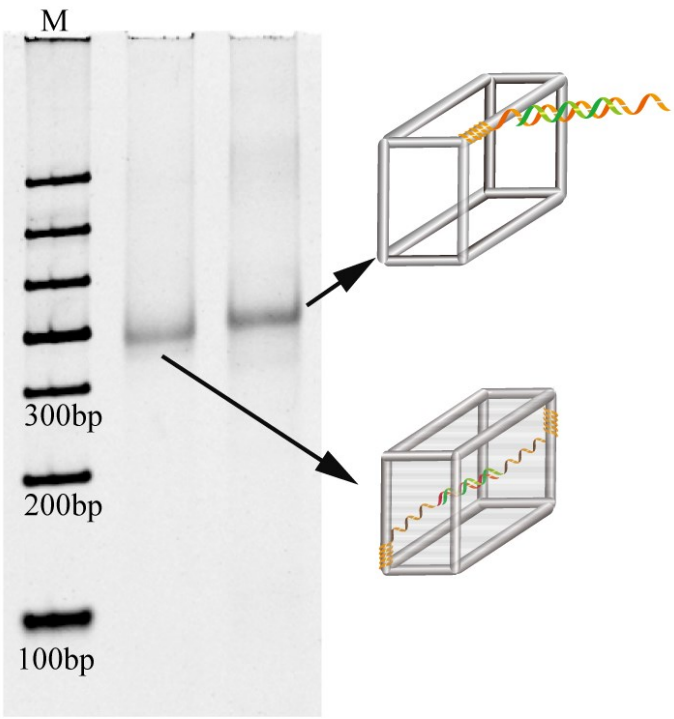

Figure S8. Analysis of Cage-in-Ts-miRNA and Cage-out-Ts-miRNA assembly by 5% N-PAGE. Cage scaffold strands were the same as those of Cage 2.

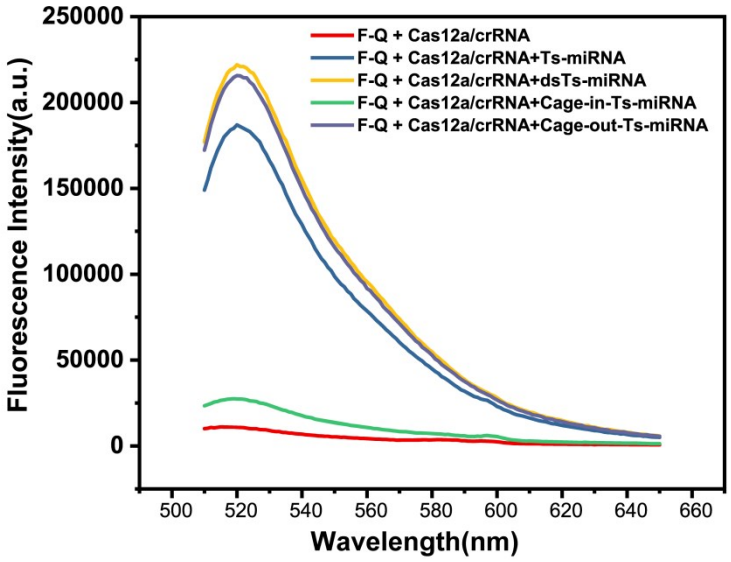

Figure S9. Fluorescence spectral of Ts-miRNA, dsTs-miRNA, Cage-in-Ts-miRNA and Cage-out-Ts-miRNA to activate the collateral cleavage activity of Cas12a/crRNA on reporter.

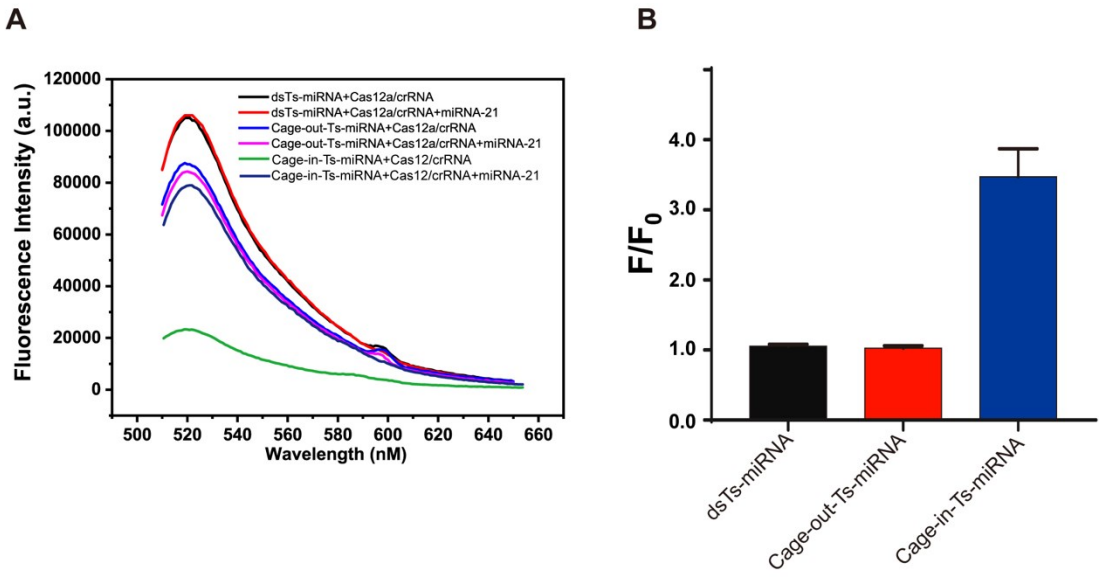

Figure S10. Fluorescence response of Cas12a/crRNA of different forms of Ts-miRNA after treatment with miRNA-21. (A) Fluorescence spectral of dsTs-miRNA, Cage-out-Ts-miRNA and Cage-in-Ts-miRNA after treatment with miRNA-21. (B) Fluorescence ratio of dsTs-miRNA, Cage-out-Ts-miRNA and Cage-in-Ts-miRNA with (F) and without (F_0) target miRNA-21 (10 nM).

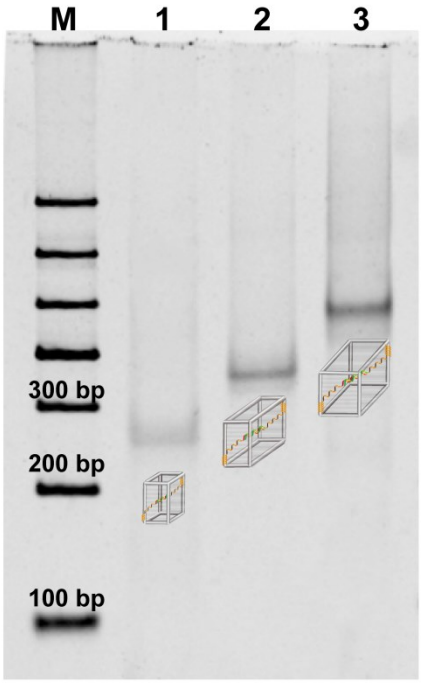

Figure S11. N-PAGE characterization of DNA nanocages from Cage-in-1-Ts-miRNA (lane 1) to Cage-in-3-Ts-miRNA (lane 3). Sort of size : Cage 1 (3.4 nm × 7.1 nm × 3.4 nm) < Cage 2 (3.4 nm × 7.1 nm × 7.1 nm) < Cage 3 (7.1 nm × 7.1 nm × 7.1 nm).

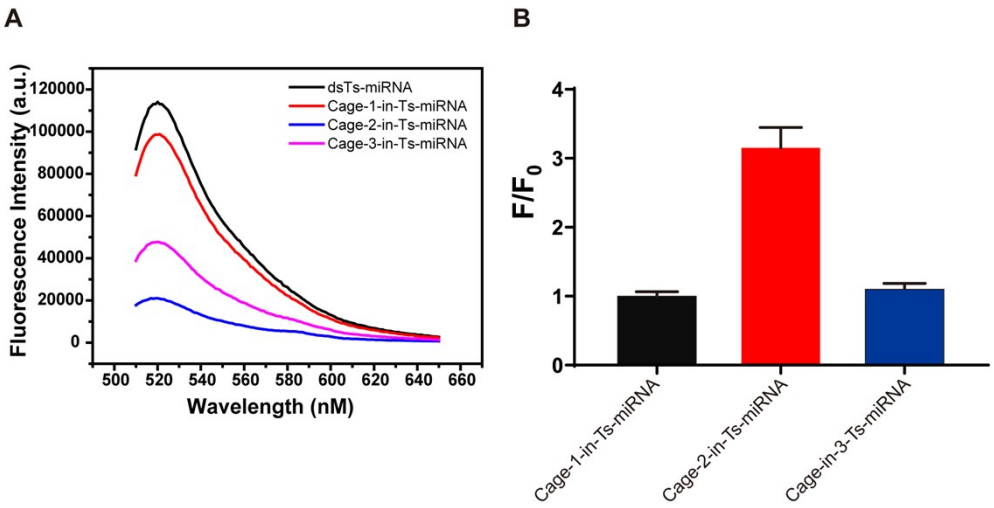

Figure S12. (A) Fluorescence spectra response of Cas12a/crRNA to different size of Cages. (B) Fluorescence ratio of Cage-in-Ts-miRNA with Cas12a/crRNA in different sizes with (F) and without (F_0) target miRNA-21 (10 nM).

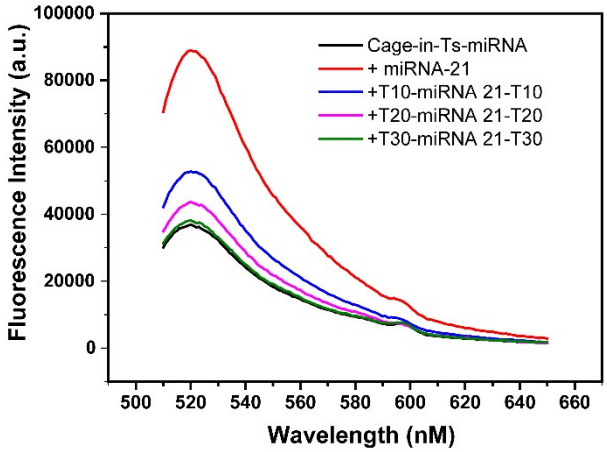

Figure S13. Fluorescence response of INSPIRE-Cas12 to the nucleic acid targets with different lengths (1 nM).

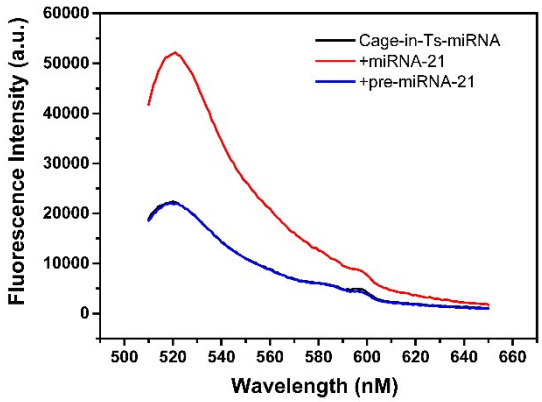

Figure S14. Fluorescence response of INSPIRE-Cas12 to miRNA-21 (1 nM) and pre-miRNA-21 (10 nM).

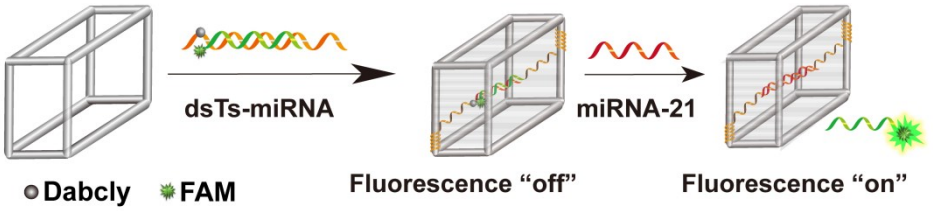

Figure S15. Schematic illustration of Cage-in-Ts-miRNA without CRISPR/Cas12a system for miRNA-21 detection.

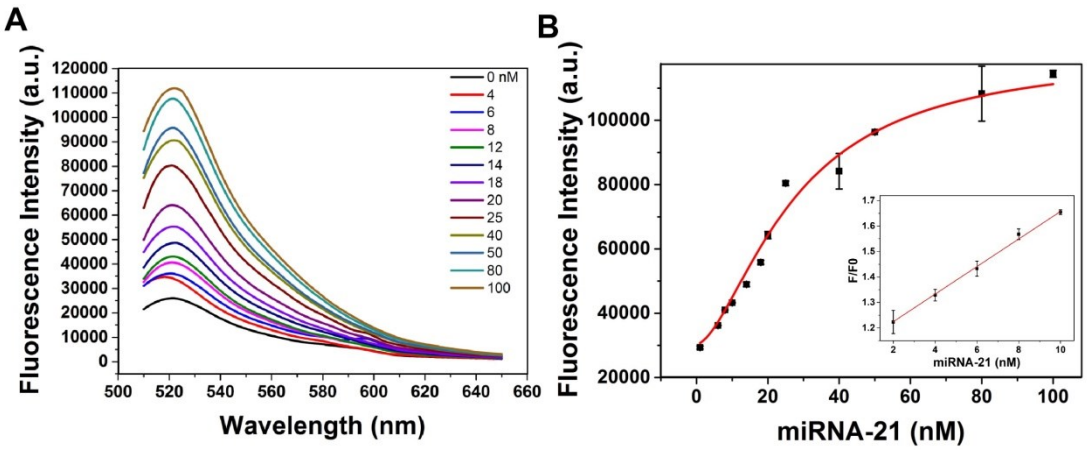

Figure S16. (A) Fluorescence response of Cage-in-Ts-miRNA without Cas12a/crRNA in the presence of different concentrations of miRNA-21, ranging from 0 nM to 100 nM. (B) Standard curve for detection of miRNA-21 from 2 nM to 10 nM. (the detection limit was estimated to be 2.5 nM according to the 3 σ rule.

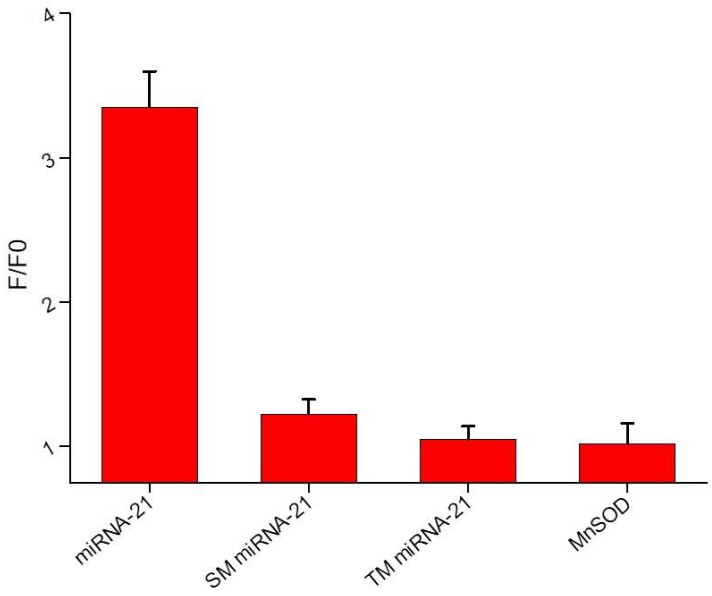

Figure S17. Selectivity studies of INSPIRE-Cas12a. The concentration of miRNA-21 and its analogues was 10 nM.

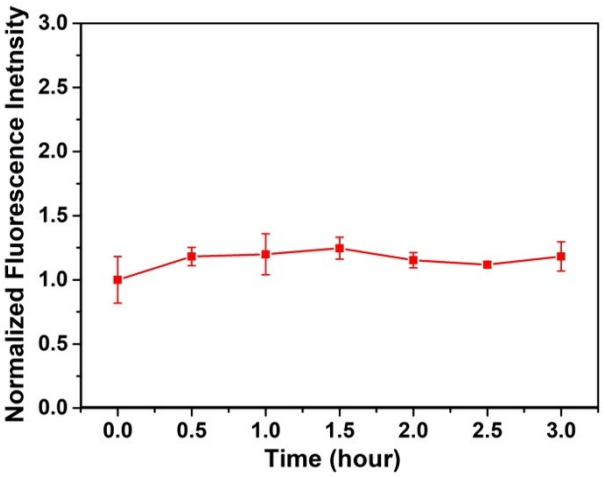

Figure S18. Fluorescence intensity of reporter treated with serum for different time (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 h, respectively). The result indicates the good stability of the reporter in the human serum.

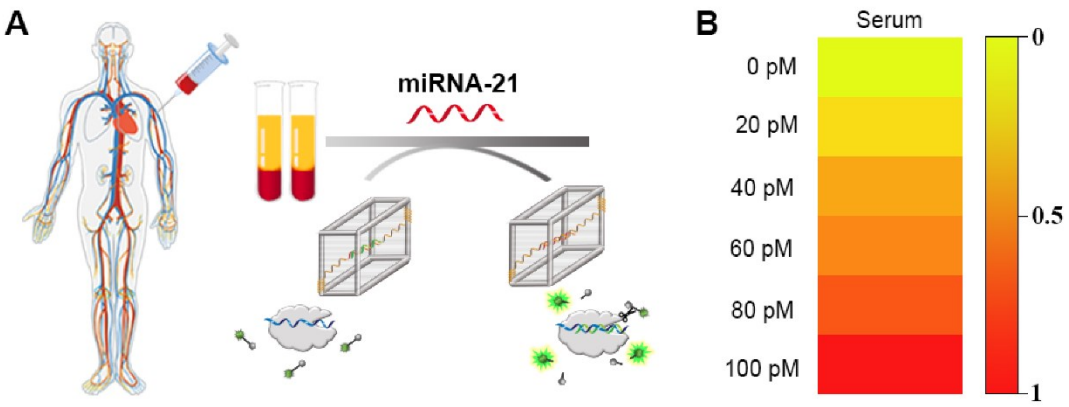

Figure S19. (A) Schematic detection of miRNA-21 in serum samples by INSPIRE-Cas12a. (B) A heat map analysis of different concentration of miRNA-21 in serum samples.

Table S1. Sequences of oligonucleotides used in this work.

Scaffold strands of Cage 1-Cage 3		
Cage 1	C2-1	CCAGCCGCCGTTCCTGGATCCAAGGCTCTAGGTGTATTCAGGTAAGT GGCCATCCAAGCTGCGATCCGAC
	C1-2	CCACTCTGCTTTCTGGGATGCCATGACACAGTGATATTACCTGAAT
	C1-3	GCCCCAGCATTGATGGTCTGCTTGTCGGATCGCAGCTTGGATGGTTT CACTGTGTC
	C2-4	TCTTCAGAGACAGCCAGGAGAATAAACAGAGGCCATGCTGGGGCCG TACAGTTCCAAAGGCATCCCAG
	C1-5	GCCTCTGTTTTTCCGTATATTCTTCGGCGGCTGGTTGCAGACCATC
	C1-6	GAATATACGGTATCTCCTGGCTGTCTCTGAAGATTAGCAGAGTGGTT ACCTAGAGCC
Cage 2	C2-1	CCAGCCGCCGTTCCTGGATCCAAGGCTCTAGGTGTATTCAGGTAAGT GGCCATCCAAGCTGCGATCCGAC
	C2-2	CCACTCCCGTTTCTGGGATGCCATACTCTAACTCAGATTCGCTGATA TTACCTGAATTTTAGCGTTGGCT
	C2-3	GCCCCAGCATTGATAAGGATTTAGGTCAGCCCTTGTCGGATCGCAG CTTGGATGGTTTCAGCGAATCTGAGTTAGAGT
	C2-4	TCTTCAGAGACAGCCAGGAGAATAAACAGAGGCCATGCTGGGGCCG TACAGTTCCAAAGGCATCCCAG
	C2-5	AATCCTTATCTTGCCTCTGTTTTTCCGTATATTCACGAAAAGGAGTTC GGCGGCTGGTTGGGCAGACCTA
	C2-6	CTCCTTTTCGTGAATATACGGTATCTCCTGGCTGTCTCTGAAGATTAC GGGAGTGGAGCCAACGCTATTACCTAGAGCC
Cage 3	C3-1	GCTTGCCGTGGTGTCGGTCTGTTCCTGGATCCAAGGCTCTAGGTGT ATTCAGGTAATGGACCCATAGGTGGCCATCCAAGCTGCGATCCGAC
	C3-2	CCACTCCCGTTTGTCCTCGCTCTCGTTGTCCTGATACTCTAACTCAG ATTCGCTGATACTATGGGTCCATTACCTGAATTTTAGCGTTGGCT
	C2-3	GCCCCAGCATTGATAAGGATTTAGGTCAGCCCTTGTCGGATCGCAG CTTGGATGGTTTCAGCGAATCTGAGTTAGAGT
	C3-4	TCTTCAGAGACAGCCAGGAGAATATAGACTAGGCATCACAGTACCAT GCTGGGGCCGTACAGTTCCAAACAGGACAACGAGAGCGAGGAC
	C3-5	AATCCTTATCTTGTACTGTGATGCCTAGTCTATTTCCGTATATTCACG AAAAGGAGTTCAGACCGACACCACGGCAAGCTTGGGCAGACCTA
	C2-6	CTCCTTTTCGTGAATATACGGTATCTCCTGGCTGTCTCTGAAGATTAC GGGAGTGGAGCCAACGCTATTACCTAGAGCC
[a]The encapsulated c-linker		TGGAACTGTACGTAAATCAACATCAGTCTGATAAGCTACCT GAGATATATATTGGATCCAGG
The encapsulated c-linker with DABCLY		TGGAACTGTACGTAAATCAACATCAGTCTGATAAGCTACCT GAGAT/**iDabclydT**/ATATATTGGATCCAGG
Scaffold strands of Cage-out-Ts-miRNA		

C2-1	CCAGCCGCCGTTCCTGGATCCAAGGCTCTAGGTGTATTCAGGTAAGTGGCCAT CCAAGCTGCGATCCGAC
C2-2	CCACTCCCGTTTCTGGGATGCCATACTCTAACTCAGATTCGCTGATATTACCTG AATTTTAGCGTTGGCT
C2-3	GCCCCAGCATTGATAAGGATTTAGGTCAGCCCTTGTCGGATCGCAGCTTGGAT GGTTTCAGCGAATCTGAGTTAGAGT
C2-4	TCTTCAGAGACAGCCAGGAGAATAAACAGAGGCCATGCTGGGGCCGTACAGT TCCAAAGGCATCCCAG
C2-5-out-1	AATCCTTATCTTGCCTCTGTTTTTCCGTATATTCA
C2-5-out-clinker	CGAAAAGGAGTTCGGCGGCTGGTTGGGCAGACCTATTTTTTCAACATCAGTCT GATAAGCTACCTGAGAT
C2-6	CTCCTTTTCGTGAATATACGGTATCTCCTGGCTGTCTCTGAAGATTACGGGAGT GGAGCCAACGCTATTACCTAGA
C-7-2	TGGAACTGTACGTACCGAATTCAGTTCAGAATTCATCCATTGGATCCAGG
Encapsulated Ts-miRNA	
Ts-miRNA	ATCTCAGGTAGCTTACATACGAAATTA
Ts-miRNA-FAM	FAM-ATCTCAGGTAGCTTACATACGAAATTA
crRNA for AsCas12a	
As-crRNA	*UAAUUUCUACUCUUGUAGAU*GUAUGUAAGCUACCUGAGUG
miRNA-21 mimicry of sequences	
miRNA-21	TAGCTTATCAGACTGATGTTGA
miRNA-21-FAM	FAM-TAGCTTATCAGACTGATGTTGA
T10-miR21-T10	TTTTTTTTTTTAGCTTATCAGACTGATGTTGATTTTTTTTTT
T20-miR21-T20	TTTTTTTTTTTTTTTTTTTTTAGCTTATCAGACTGATGTTGATTTTTTTTTTTTTTTT
T30-miR21-T30	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGCTTATCAGACTGATGTTGATTTTTT TTTTTTTTTTTTTTTTTTTTT
The target RNA sequence of miRNA-21 and pre-miRNA-21	
Mature miRNA-21	UAGCUUAUCAGACUGAUGUUGA
Pre-miRNA-21	UGUCGGAUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACAC CAGUCGAUGGGCUUACUGACA
The control microRNA	
[b]SM miRNA-21	TAACTTATCAGACTGATGTTGA
TM miRNA-21	AAACTAATCAGACTGATGTTGA
MnSOD	GTAATCAACTGGGAGAATGTAACTG
Reporter strands for Cas12a/crRNA system	
reporter	FAM-TTATT-BHQ1

Note: The DNA strands of “The frame of the Cage” are the fixed frame of different cages. For the Ts-miRNA modified on the surface frame of DNA (Cage-out-Ts-miRNA), we split the C2-5 into two DNA strands (C2-5-1, C2-5-out-c-linker), and designed the C-7-2 to maintain the rigidity of the cage.

[a] The bases in green are the sequences of Ts; The underlined bases are the related sequences for miRNA-21.

[b] The bases in red are the mismatched sequences for miRNA-21.

Table S2. Some CRISPR/Cas12a-based biosensors

NO.	Detection limit	Signal interference by pre-miRNAs	References
1	10 pM	without the signal interference by pre-miRNAs	This work
2	50 pM	N/A	Angew Chem Int Ed 2019, 58, 17399-17405.
3	30 pM	N/A	ACS Sens 2020, 5, 557-562.
4	40 pM	N/A.	Anal Chem 2019, 91, 11510-11513.
5	10 pM	N/A	Emerg Microbes Infect 2020, 9, 1140-1148.
6	9 fM	N/A	Chem Comm 2020, 56, 10038-10041
7	1 fM (10 zmol in 10 ml buffer)	N/A	Chem Comm 2020, 56, 13445-13448.

Note: “N/A” stands for “not applicable”.

Ethics Statement

Serum samples of healthy people were collected from the Xiangya Hospital Central South University (Hunan, Changsha). All experiments were performed in accordance with the Guidelines of Clinical Sample Management Rules of Hunan Cancer Hospital and Xiangya Hospital of Central South University, which were reviewed and approved by the Ethics Committee at Hunan Cancer Hospital and Xiangya Hospital of Central South University. Informed consents were received from the blood donors of this project.

Reference

1 X. Fu, G. Ke, F. Peng, X. Hu, J. Li, Y. Shi, G. Kong, X.-B. Zhang and W. Tan, *Nat. Commun.*, 2020, **11**, 1518.