**SUPPLEMENTARY INFORMATION**

**Cell-Free Synthetic Biosensors Based on CRISPR/Cas Mediated Cascade Signal Amplification for Precise RNA Detection**

Chao Zhang1,2\*, Penghui Zhang1,2\*, Hui Ren2,3\*, Pengpeng Jia1,2, Jingcheng Ji1,2, Lei Cao1,2, Peiwei Yang1,2, Yuxin Li1,2, Jie Liu1,2, Zedong Li1,2, Minli You1,2, Xiaoman Duan1,2, Jie Hu4, Feng Xu1,2#

*1 MOE Key Laboratory of Biomedical Information Engineering, School of Life Science and Technology, Xi’an Jiaotong University, Xi’an 710049, P.R. China*

*2 Bioinspired Engineering and Biomechanics Center (BEBC), Xi’an Jiaotong University, Xi’an 710049, P.R. China*

*3 Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, P.R. China*

*4 Diyinan Biotech Company, Suzhou 215000, PR China*

*\* The authors contributed equally*

*# Corresponding author:* [*fengxu@mail.xjtu.edu.cn*](mailto:fengxu@mail.xjtu.edu.cn)

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**Supplementary Note 1**

**SHARK reaction optimization**

Prior to optimization of the SHARK formulation, the buffer of the reaction system was evaluated. SHARK reaction buffer was optimized based on the cell-free protein expression system and Cas13a cleavage system. We modified the concentrations of Tris-HCl to 50 mM, and removed dithiothreitol due to its negative effect on enzyme activity. We added dextran sulfate to achieve better hybridization between target RNA and crRNA in isothermal condition.

We further tested several reaction conditions and additives to establish the optimal condition for SHARK reaction. First, temperature optima were determined by assaying G6PD expression in pre-optimized buffers at 4-50 ℃ (**Supplementary Fig. 19**). The ratio of intensities compared to control group was highest at 37 ℃.

Then, components of SHARK system were properly optimized. A wide concentration range of plasmids was tested. A high amount of plasmids can accelerate the reactions, while unnecessarily high volume can inhibit the Cas13a cleavage reaction rate. Finally, the plasmids concentration was adjusted to pET23a-G6PD plasmid DNA 6 nM; pIX-Invertase plasmid DNA 10 nM; pFN6A-Luciferase plasmid DNA 2 nM. pET30-RLuc-YFP plasmid DNA 0.1 nM. The NTPs concentration was adjusted to be 2.5 mM for higher transcription efficiency. In summary, optimized SHARK reaction contained 50 nM LbuCas13a, 50 nM crRNA, 50 mM Tris-HCl, 10 mM MgCl2, 2.5 mM NTPs, 1 μL DSS (50 ng/μL) and 11 μL T7 S30 Extract with S30 Premix Plus.

**Supplementary Note 2**

**miRNA screening based on support vector machine and implementation on python**

Support vector machine (SVM) learning is a powerful classification tool that has been used for cancer classification or subtyping. We constructed the identification tree and tested phase SVM process for miRNA screening of NSCLC. miRNAs expression data from NSCLC and healthy individuals in TCGA were used for differential expression analysis with a desired accuracy. The learning process was summarized:

import numpy as np

import pandas as pd

from copy import deepcopy

import matplotlib.pyplot as plt

import seaborn as sns

from sklearn.svm import SVC

from sklearn.manifold import TSNE

from sklearn.ensemble import ExtraTreesClassifier

from sklearn.metrics import accuracy\_score, precision\_score, recall\_score, f1\_score, confusion\_matrix

def readfile(path):

feature\_name = []

with open(path, 'r') as f:

lines = f.readlines()

title = lines[0].split()

person = {}

for id in range(1, len(title)):

person[id] = [title[id], []]

for line in range(1, len(lines)):

micrna = lines[line].split()

feature\_name.append(micrna[0])

for id in range(1, len(micrna)):

person[id][1].append(micrna[id])

return person, feature\_name

path0 = 'GSE40738 1924miRNA.txt'

path1 = 'GSE40738 1924miRNA.txt'

path2 = 'GSE40738 1924miRNA.txt'

file0, feature\_name0 = readfile(path0)

file1, feature\_name1 = readfile(path1)

file2, feature\_name = readfile(path2)

data0 = np.array([file0[x][1] for x in file0]).astype(float) # NSCLC

data1 = np.array([file1[x][1] for x in file1]).astype(float) # NSCLC

data2 = np.array([file2[x][1] for x in file2]).astype(float) # Healthy

dataX = np.concatenate((data0, data1, data2), axis=0)

dataY = np.append(np.zeros(len(data0)+len(data1)), np.ones(len(data2)))

# normalization

dataX\_min = np.min(dataX, axis=0)

dataX\_max = np.max(dataX, axis=0)

dataX = (dataX - dataX\_min) / (dataX\_max - dataX\_min)

# shuffle

np.random.seed(101)

state = np.random.get\_state()

np.random.shuffle(dataX)

np.random.set\_state(state)

np.random.shuffle(dataY)

k = 905

testX = dataX[-k:, ]

testY = dataY[-k:, ]

trainX = dataX[:-k, ]

trainY = dataY[:-k, ]

# Feature extraction

tr = ExtraTreesClassifier()

fit = tr.fit(trainX, trainY)

# output important features

d = list(enumerate(fit.feature\_importances\_))

d.sort(key=lambda a: a[1], reverse=True)

rna = []

d\_max = 30

for index, item in enumerate(d[:d\_max]):

print(item[0], "th\t", feature\_name[item[0]], item[1])

rna.append(item[0])

# visualization

tsne\_X = dataX.take(rna[:30], 1)

tsne\_Y = deepcopy(dataY)

tsne = TSNE(n\_components=2, init='pca', random\_state=0)

tsne\_obj = tsne.fit\_transform(tsne\_X)

tsne\_min, tsne\_max = tsne\_obj.min(0), tsne\_obj.max(0)

tsne\_obj = (tsne\_obj - tsne\_min) / (tsne\_max - tsne\_min)

tsne\_df = pd.DataFrame({'X': tsne\_obj[:, 0],

'Y': tsne\_obj[:, 1],

'digit': tsne\_Y})

print(tsne\_df.head())

sns.scatterplot(x="X", y="Y",

hue="digit",

palette=['red', 'blue'],

legend='full',

data=tsne\_df)

plt.savefig('micro\_rna.png')

plt.show()

feature\_num\_list = [1, 3, 5, 7, 10, 15, 20, 25, 30]

columns = ['number of features', 'accuracy', 'precision', 'recall', 'f1-score', 'true positive', 'false positive', 'true negative', 'false negative', 'bias'] + [feature\_name[d[i][0]] for i in range(d\_max)]

info\_sum = []

for feature\_num in feature\_num\_list:

info = {}.fromkeys(columns)

info['number of features'] = feature\_num

clf = SVC(kernel='linear')

svm\_X = trainX.take(rna[:feature\_num], 1)

svm\_Y = trainY

clf.fit(svm\_X, svm\_Y)

svm\_test\_X = testX.take(rna[:feature\_num], 1)

svm\_test\_Y = testY

score = clf.score(svm\_test\_X, svm\_test\_Y)

# print(score)

pre = clf.predict(svm\_test\_X)

w = clf.coef\_

b = clf.intercept\_

m = w \* 5

n = b \* 5

m = np.rint(m)

n = np.rint(n)

# pred\_y = m \* x + n

pred\_y = np.dot(m, svm\_test\_X.T) + n

pred\_y = pred\_y.flatten()

pred\_y[pred\_y < 0] = 0

pred\_y[pred\_y > 0] = 1

info['accuracy'] = accuracy\_score(testY, pred\_y)

info['precision'] = precision\_score(testY, pred\_y)

info['recall'] = recall\_score(testY, pred\_y)

info['f1-score'] = f1\_score(testY, pred\_y)

info['true positive'] = confusion\_matrix(testY, pred\_y)[0][0]

info['false positive'] = confusion\_matrix(testY, pred\_y)[0][1]

info['true negative'] = confusion\_matrix(testY, pred\_y)[1][0]

info['false negative'] = confusion\_matrix(testY, pred\_y)[1][1]

info['bias'] = n[0]

for i in range(feature\_num):

info[feature\_name[d[i][0]]] = (m[0][i])

info\_sum.append(info)

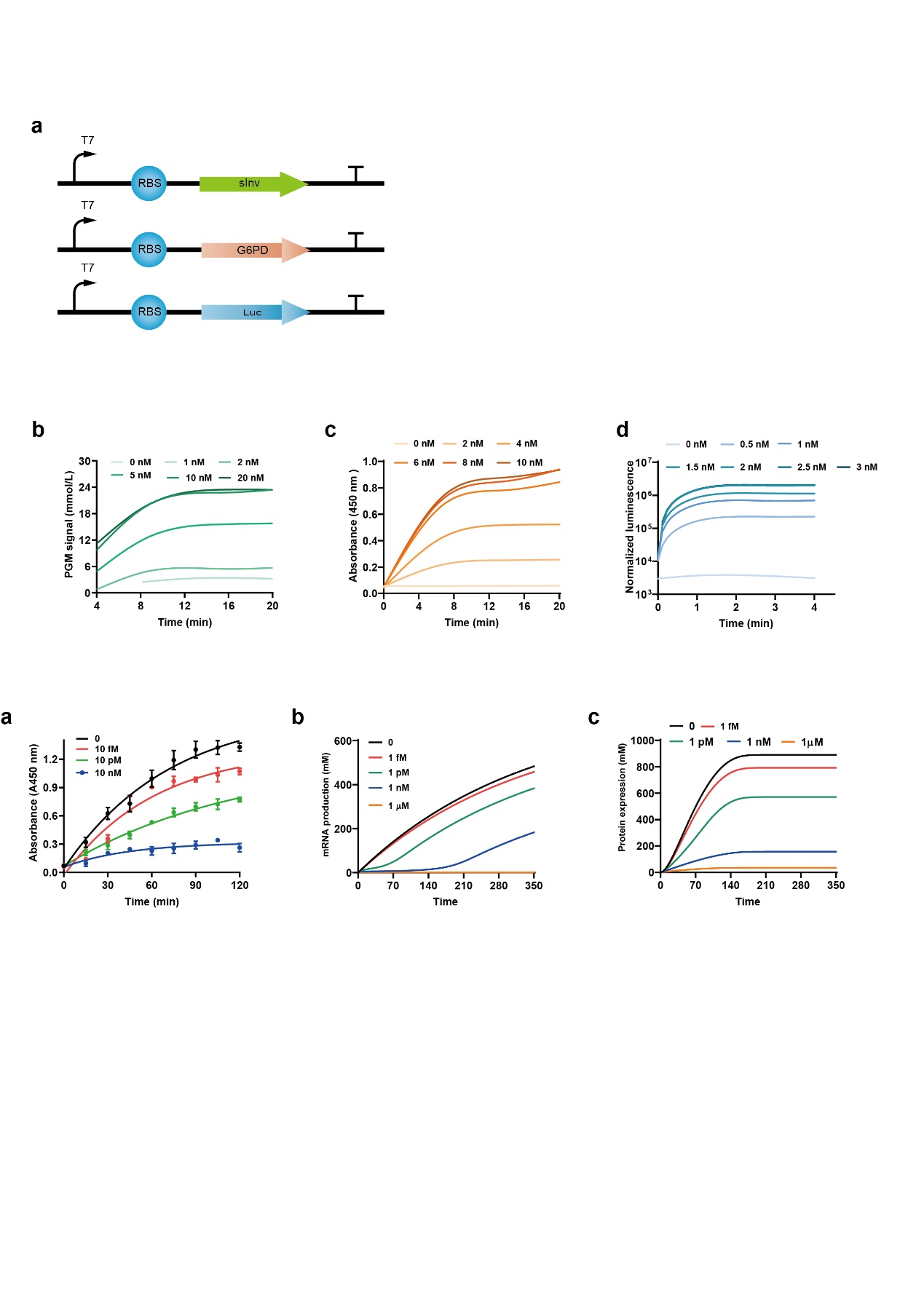
rna\_pd = pd.DataFrame(info\_sum, columns=columns)

rna\_pd.to\_csv('rna\_pd.csv', index=None, encoding='gb18030')

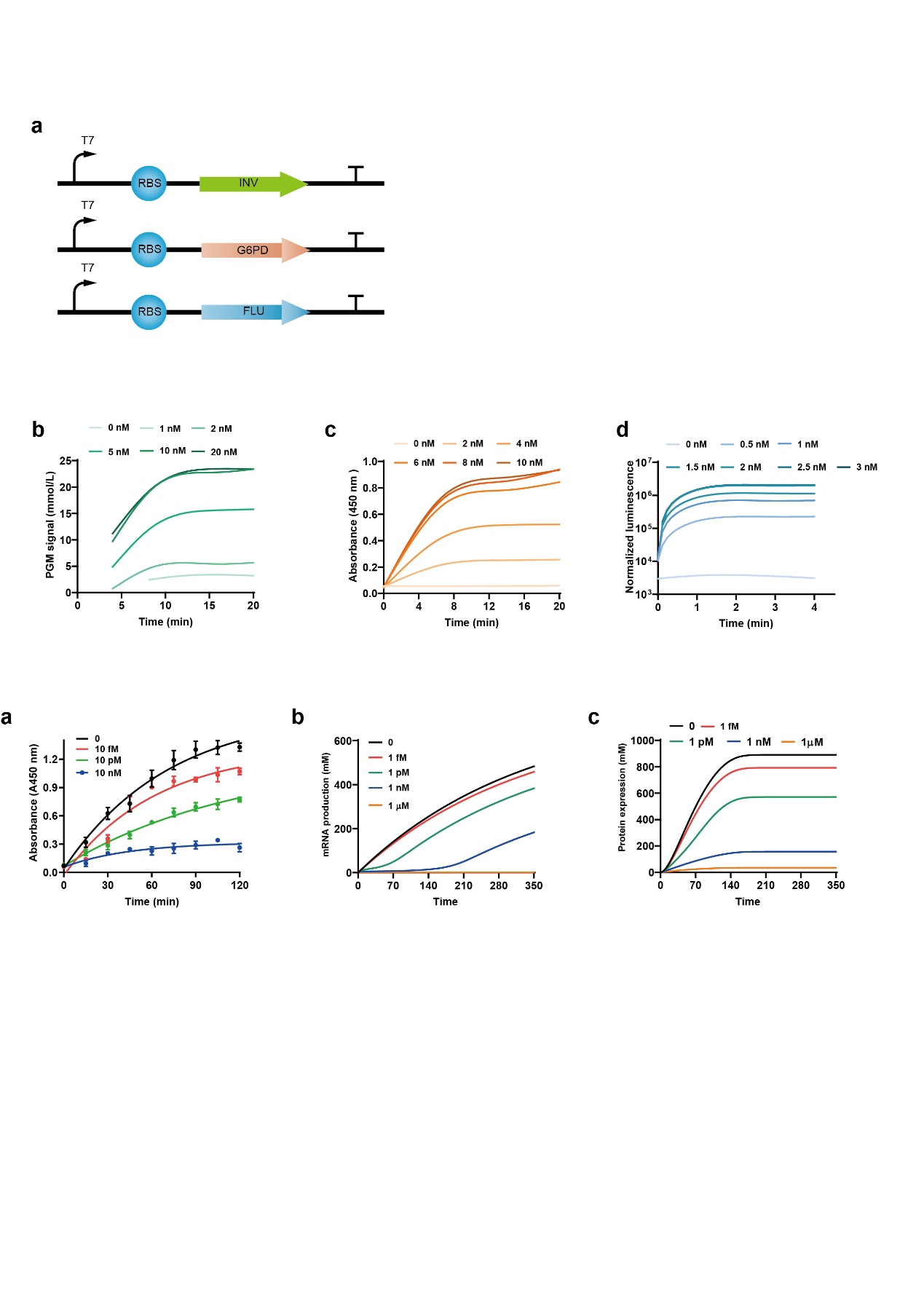
**Supplementary Note 3**

**Cell-free biosensor based on Cas9:RsgRNA for RNA detection**

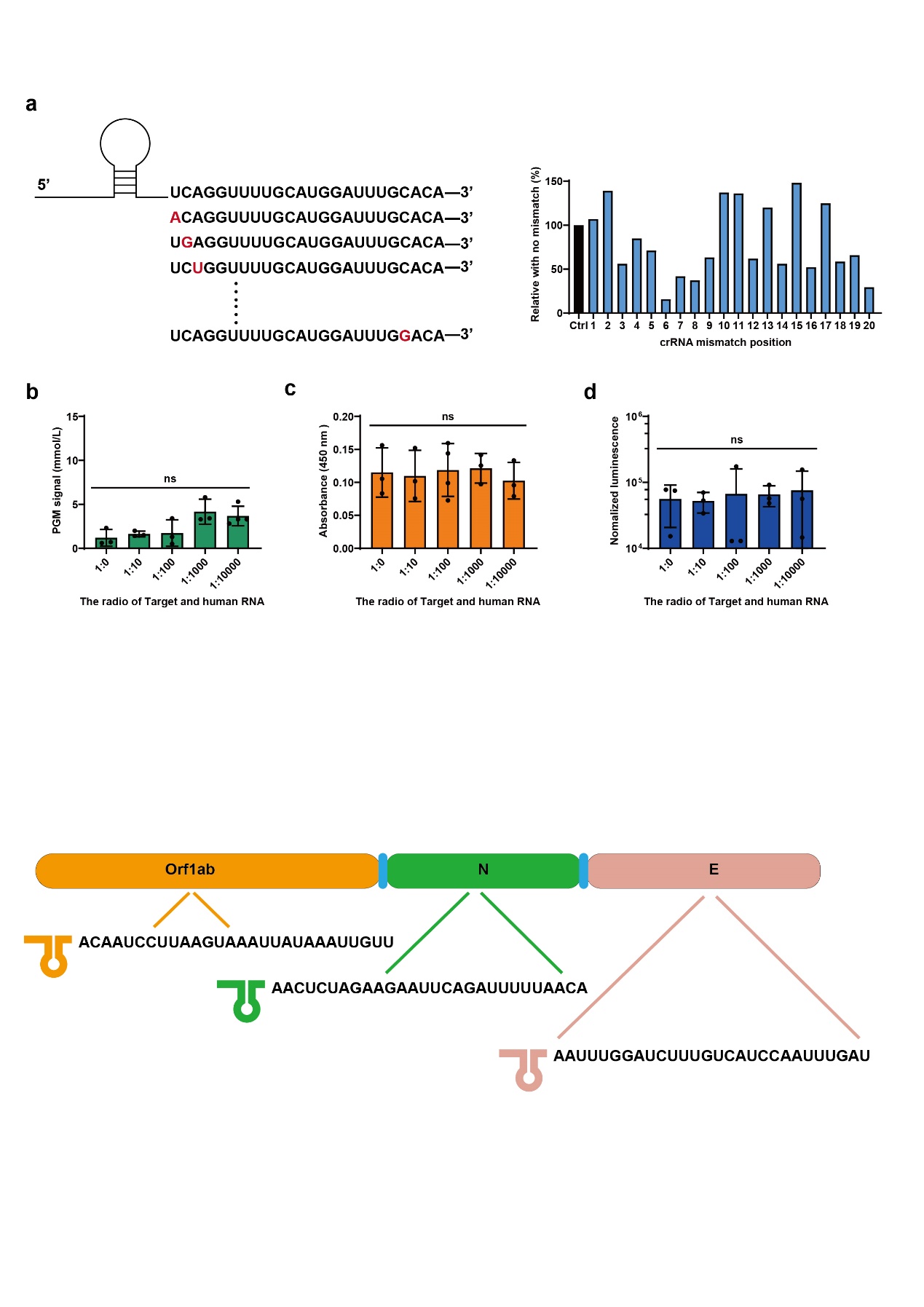
In order to employ down-regulated miRNAs for biological computing, we established a turn-on system based on programmable Cas9-sgRNA for regulating enzyme expression by degrading plasmids. We synthesized programmable repress sgRNA (RsgRNA), which was formed by making a single stranded 5' extension and repressed by binding a complementary RNA (**Supplementary Fig. 12b**). Without target RNA, RsgRNA could guide Cas9 to specifically cut plasmid DNA, enabling transcription regulation. When the 5'-RsgRNA binding to the target RNA, the cleavage ability of Cas9-RsgRNA was inhibited, and enzymes could be expressed (**Supplementary Fig. 12c, d**), thereby generating a signal output.



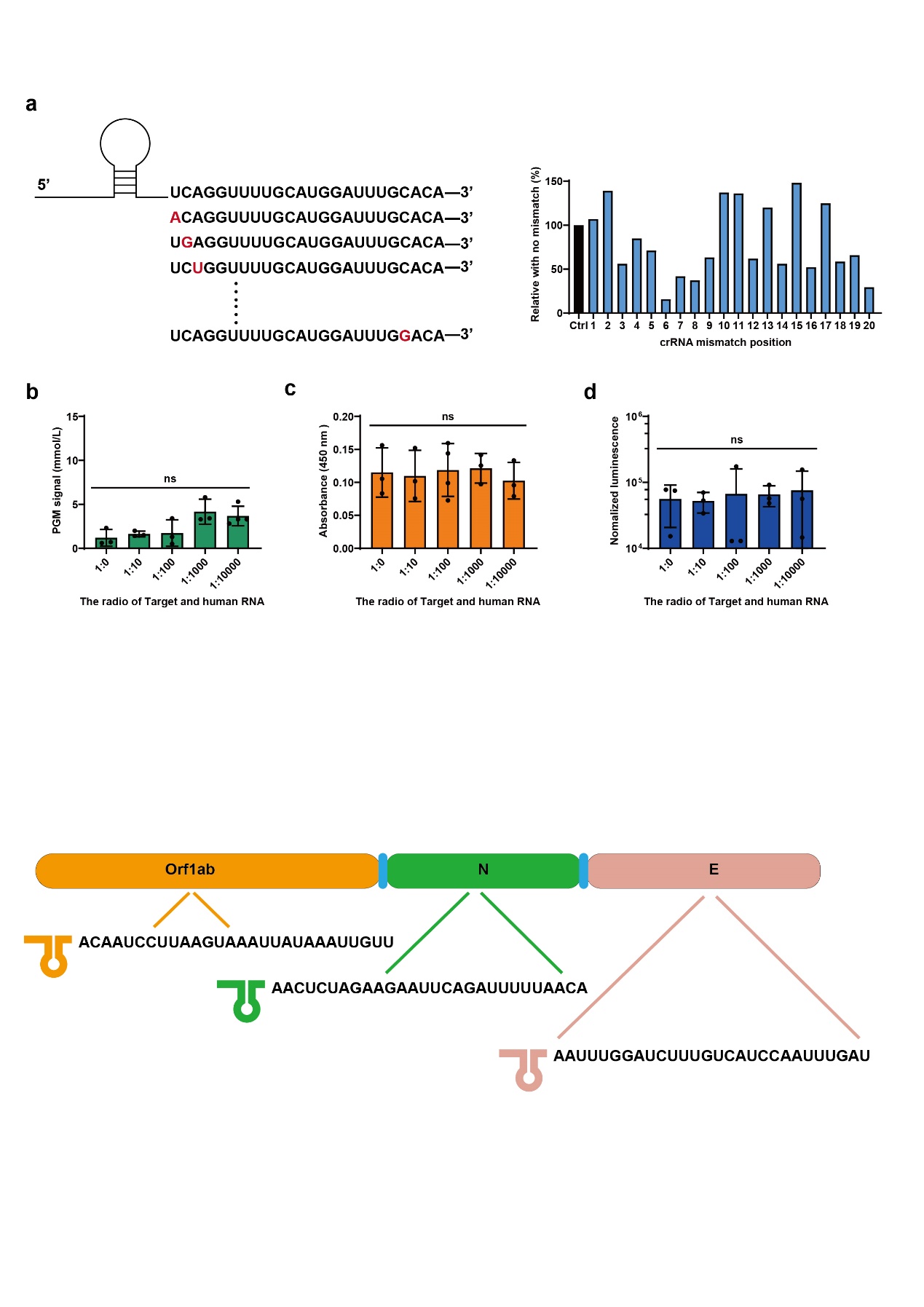
**Supplementary Fig. 1 Construction of enzyme expression plasmids. a** Construction of sInv, G6PD and Luc expression plasmids. T7: T7 promoter, RBS: Ribosome binding site. **b-d** The enzymatic kinetic curves of different plasmid concentrations, (**b**) sInv, (**c**) G6PD, (**d**) Luc. All curves were fitted.



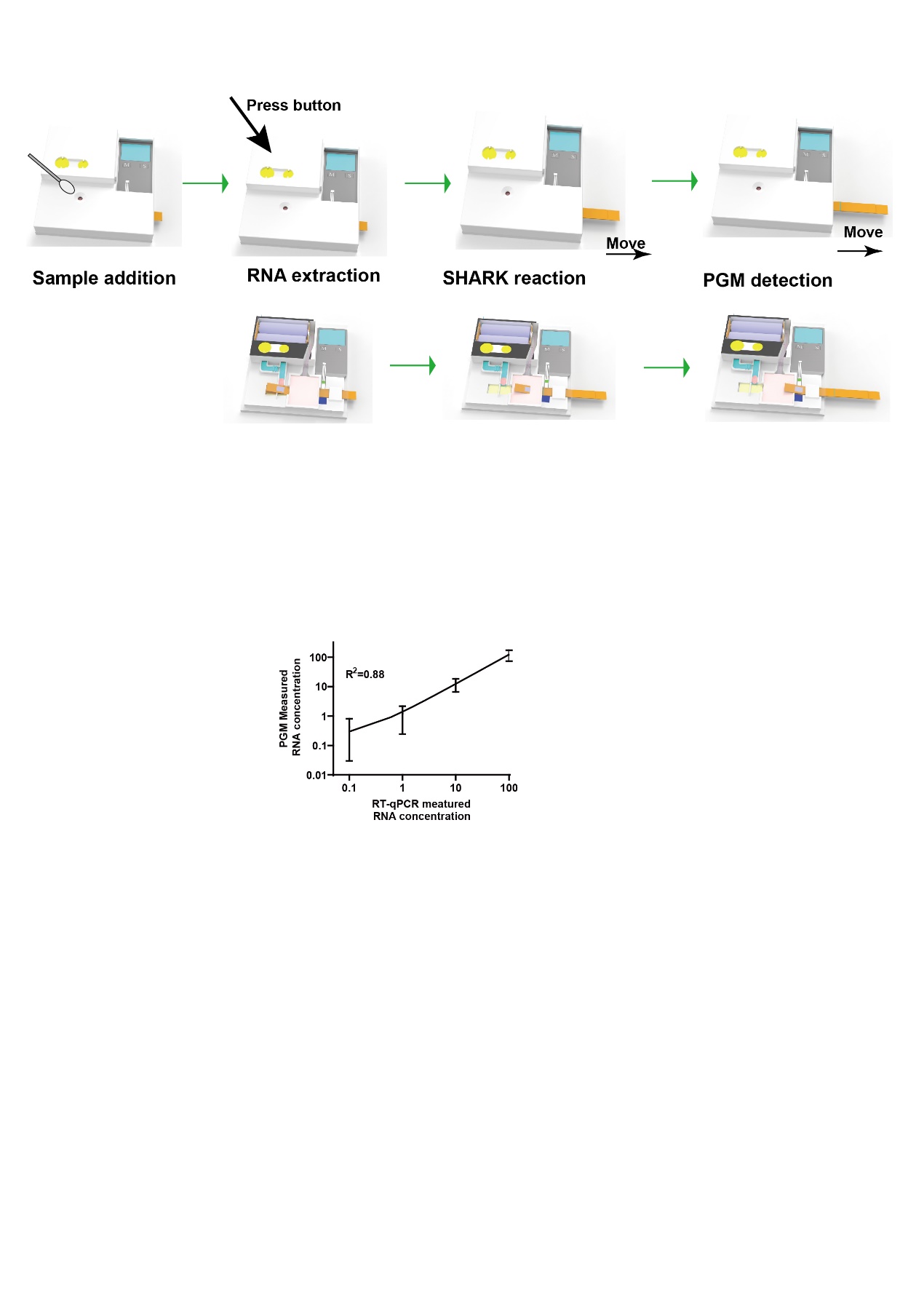
**Supplementary Fig. 2 The Michaelis-Menten enzyme kinetics of SHARK**. **a** Apparent enzymatic rates of SHARK across a range of target RNA concentration. Normalized reporter signal curves were fitted.The results are shown for one representative of n = 3 independent biological replicates. **b**, **c** Using the Michaelis-Menten enzyme kinetics model, limit of detection was simulated by testing 10-9, 10-6, 10-3, 1 μM trigger RNA. The turn-off assay for mRNA (**b**) and protein (**c**) are related to the target concentrations.



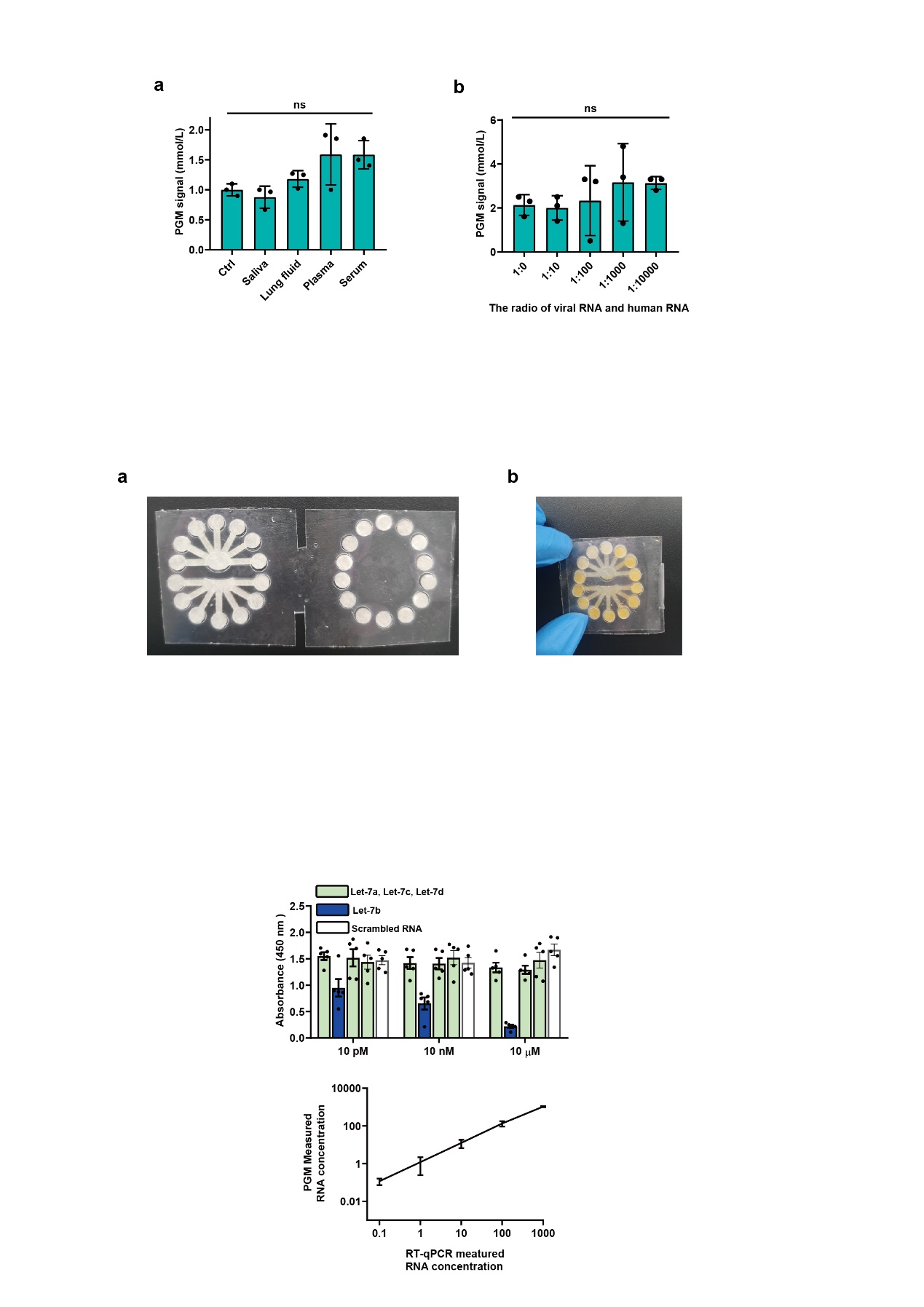
**Supplementary Fig. 3 The specificity of the SHARK. a** Mismatches occurring at positions of 6-9 at the 5' end of crRNA significantly reduce collateral cleavage of Cas13a activity. **b-d** Presence of human total RNA contamination didn’t affect the stability of the system with PGM (**b**), colorimetric (**c**), and bioluminescence (**d**) signal output. Difference from control assessed by one-way ANOVA and Dunnett’s multiple comparisons test. All data are represented as mean ± s.d. (n=3 independent experiments). n.s. indicates not significant.



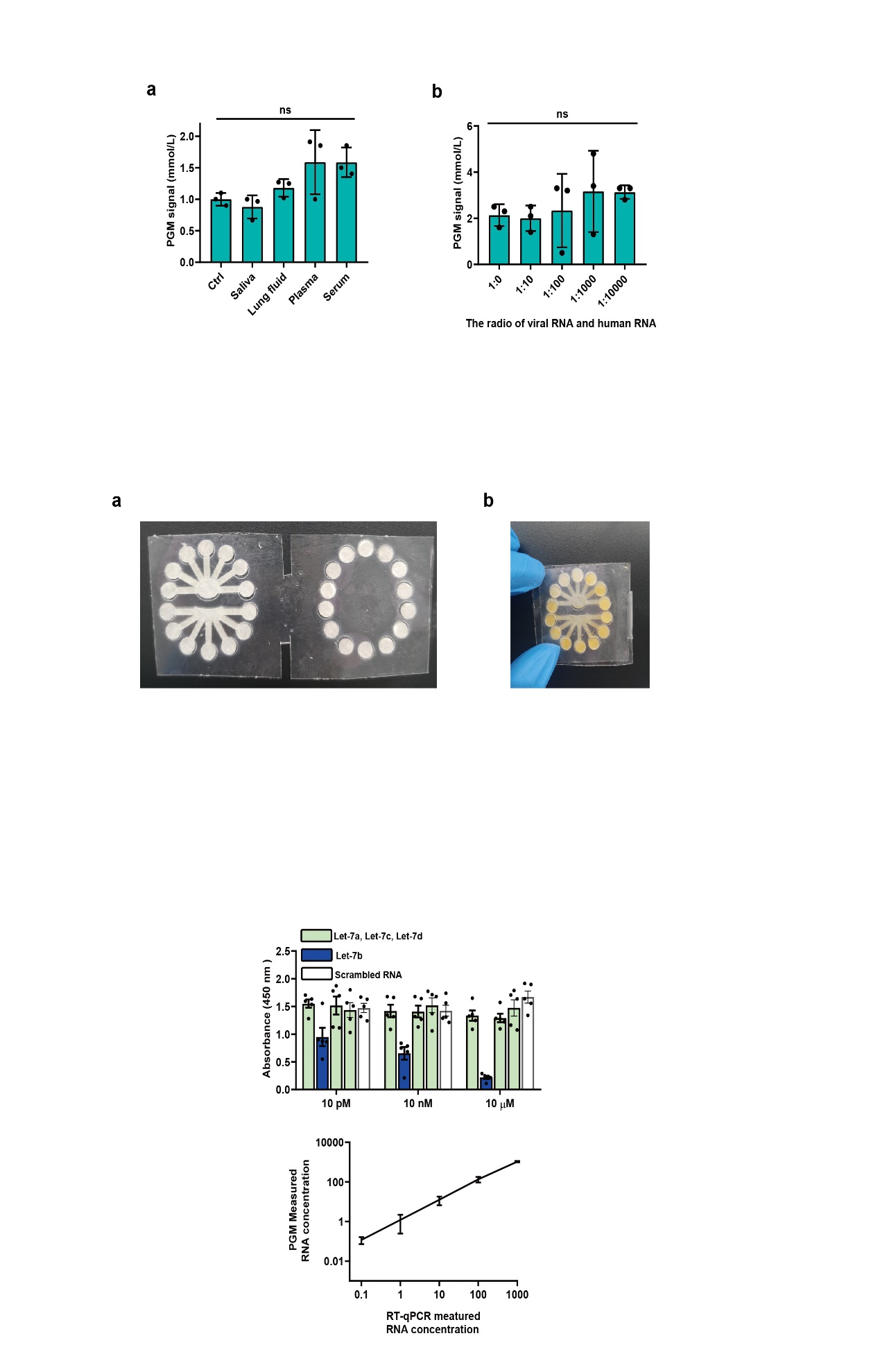
**Supplementary Fig. 4 Design of crRNAs for contrived SARS‑CoV‑2**. Schematic of the contrived SARS-CoV-2 gene and the corresponding locations of each crRNA spacer region. One in Orf1ab gene, one in N gene and one in E gene of contrived SARS-CoV-2 genome map were selected for sInv-SHARK detection. Selected regions and complementary hybrid crRNAs were annotated.



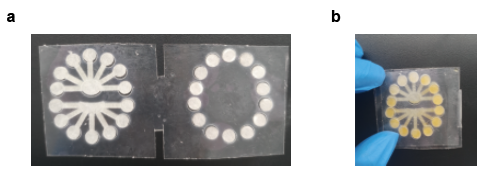
**Supplementary Fig. 5 The whole operation process of SARS‑CoV‑2 detection device based on paper-loaded sInv-SHARK.** At the beginning of the detection process, the SARS-CoV-2 in the throat swab or sputum sample was lysed and released, and then, the released RNA hybridized with the specific SARS-CoV-2 crRNAs to activate SHARK assay. The entire detection process involves one pot sInv-SHARK assay (step i) and PGM analysis (step ii).



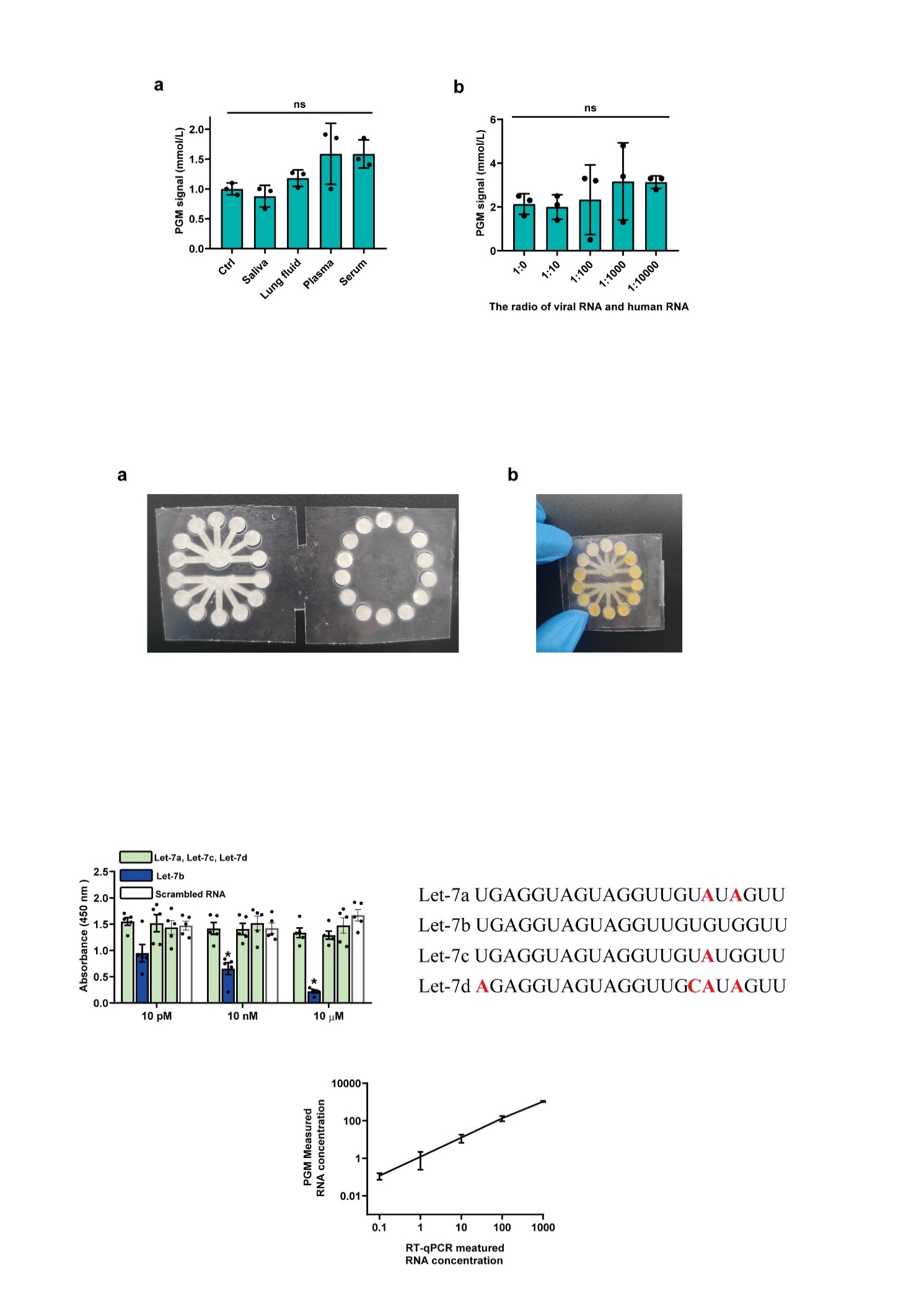
**Supplementary Fig. 6 Performance of sInv-SHARK assay and qRT-PCR assay.** We calculated the quantitative curve of target RNA concentration and PGM reading on the basis reaction kinetics, and then we confirmed coincidence between the sInv-SHARK and qRT-PCR methods.

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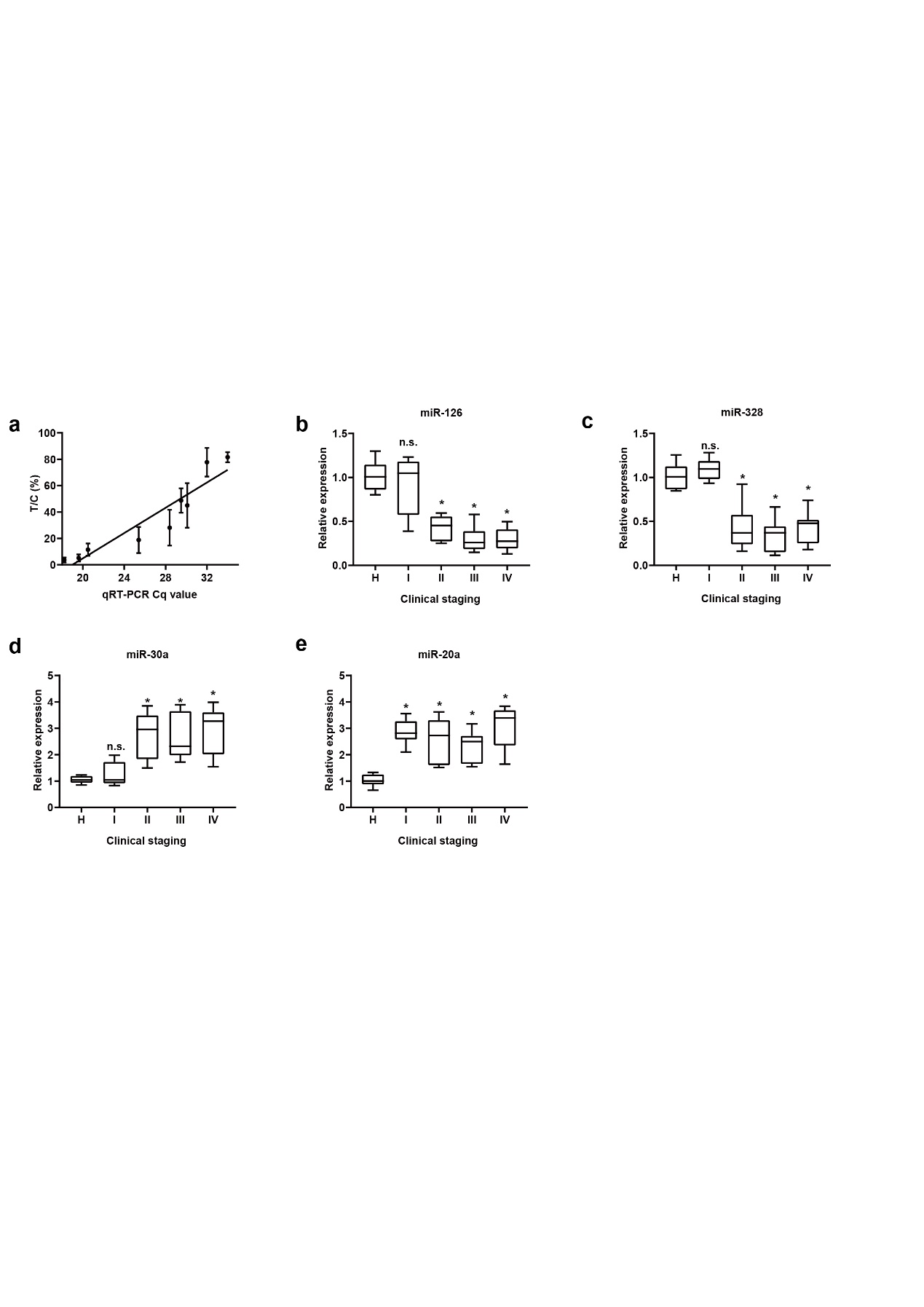
**Supplementary Fig. 7 Correcting matrix effect in sInv-SHARK assay. a** No significant change of sInv-SHARK signal results in the presence of different matrix. **b** System stability in the presence of RNA contamination. The presence of human total RNA contamination didn’t affect the stability of sInv-SHARK assay. Difference from control was assessed by one-way ANOVA and Dunnett’s multiple comparisons test. All data are represented as mean ± s.d. (n=3). n.s. indicates not significant.



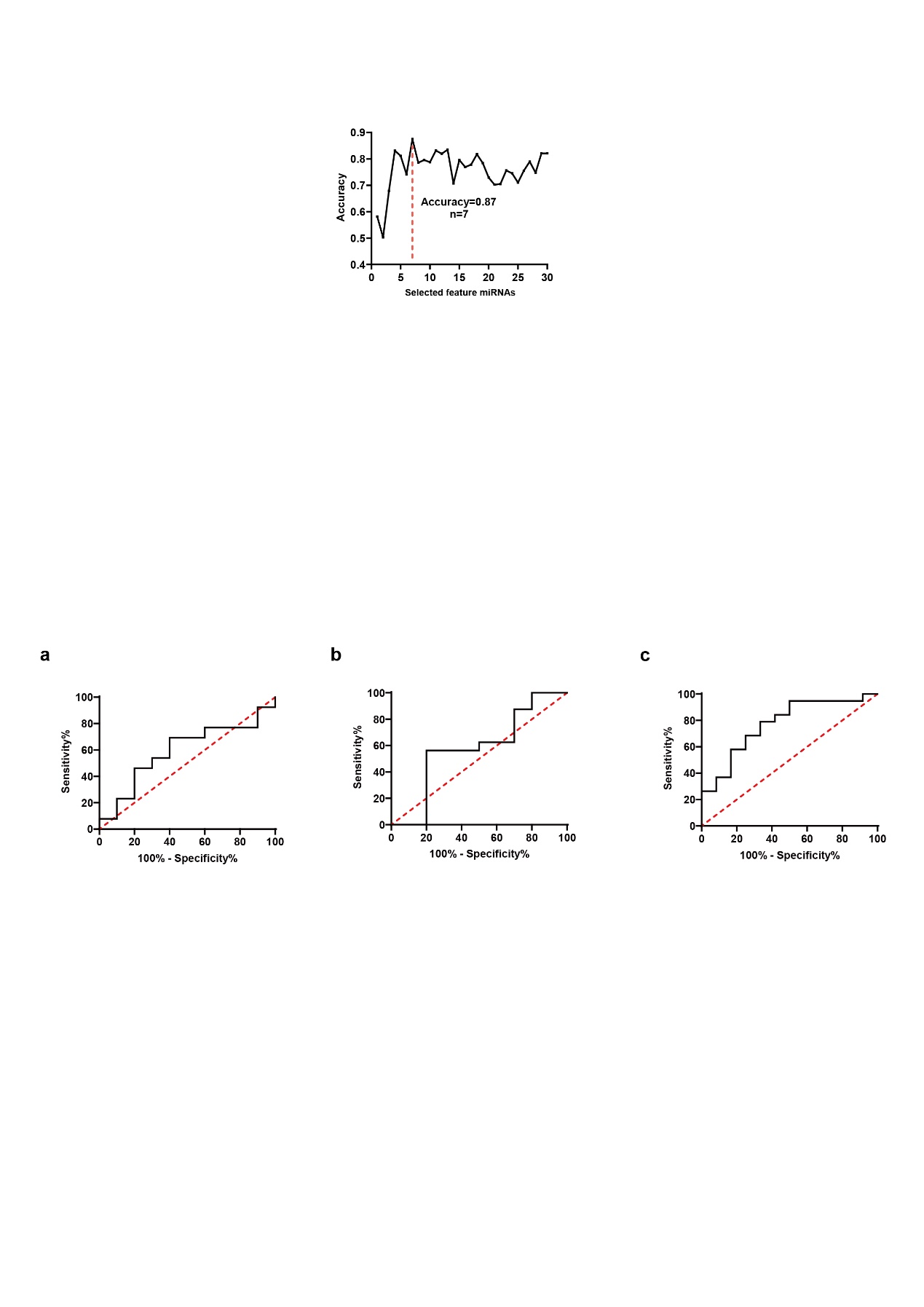
**Supplementary Fig. 8 Design of multiplex PAD with G6PD-SHARK assay. a** Picture of the unfolded PAD. Sample dispersion and G6PD-SHARK assay occurred in the left layer. The substrate of G6PD was freeze-dried in the right layer for field deployment, thereby creating sensors that are activated on rehydration with the sample of interest. **b** Colorimetric reaction occurred after folding.



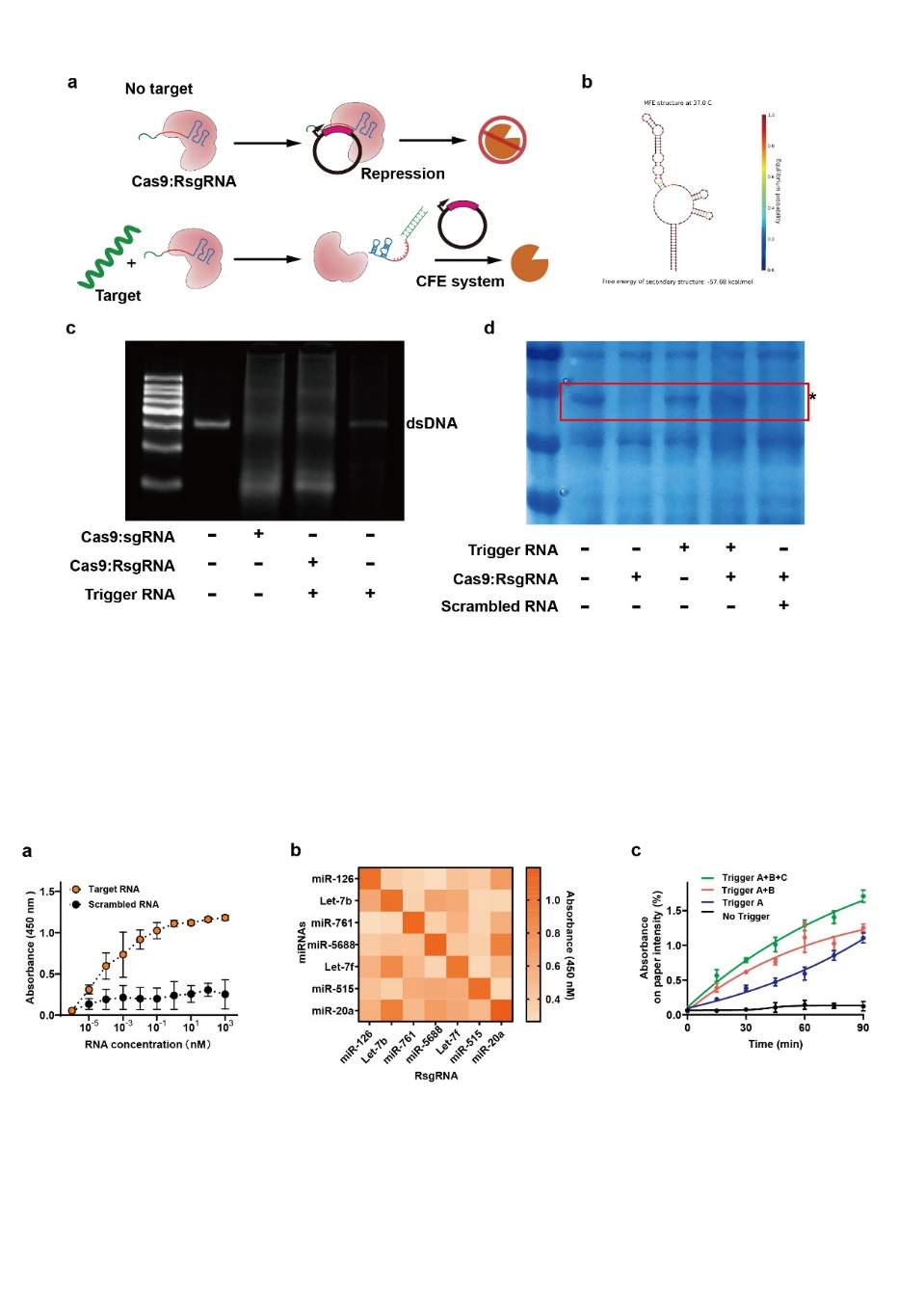
**Supplementary Fig. 9 G6PD-SHARK assay to specifically detect the let-7 family.** ThecrRNA of G6PD-SHARK assay was designed to have high affinity for let-7b. crRNA target region had a weak ability to hybridize with other members of the let-7 family. Mismatches in the Let-7a, Let-7c and Let-7d are highlighted in red. Difference from control was assessed by one-way ANOVA and Dunnett’s multiple comparisons test. All data are represented as mean ± s.d. (n=5). \* *P*<0.05.



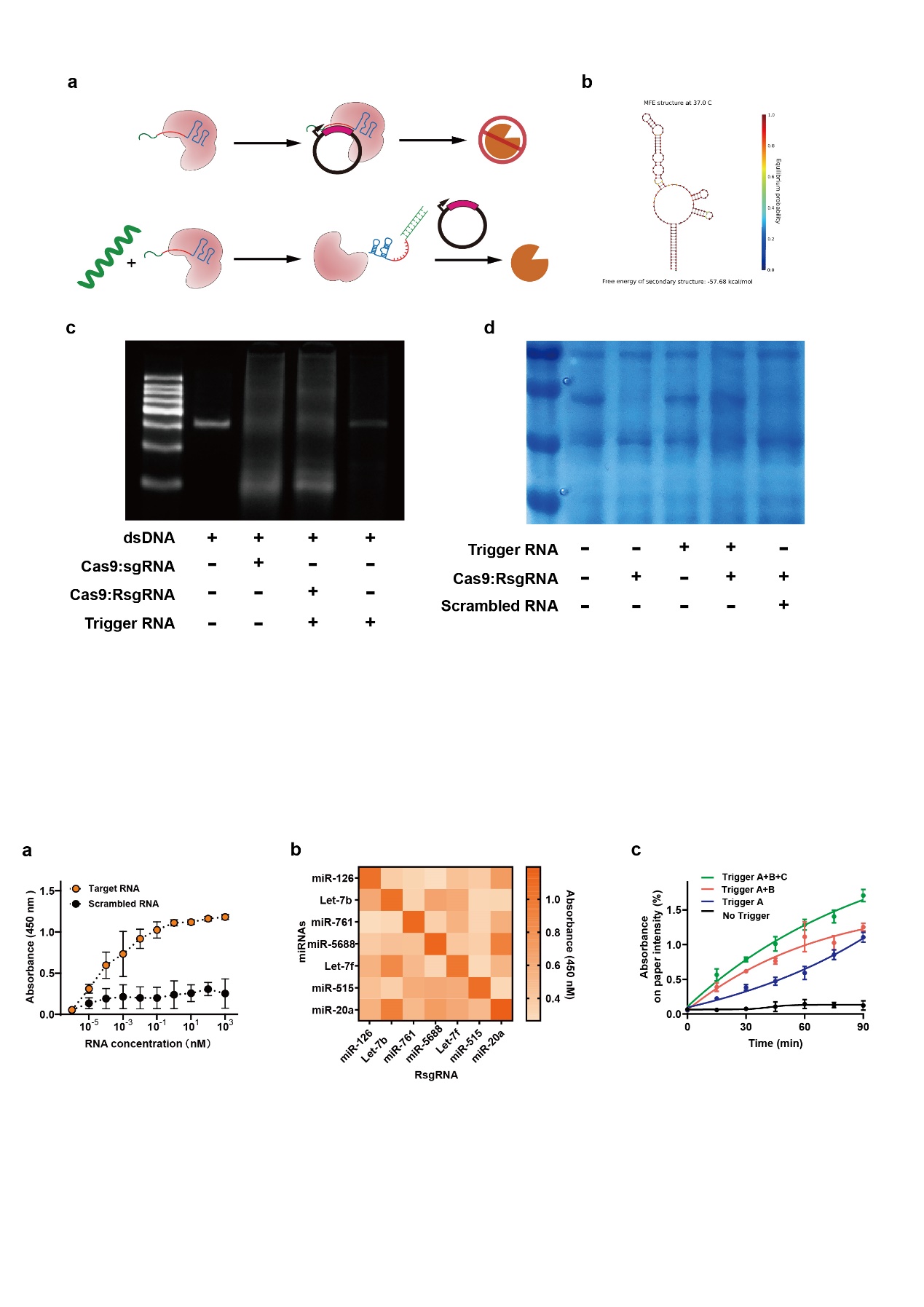
**Supplementary Fig. 10 Discrimination of advanced stage NSCLC patients from healthy individuals by detecting miRNAs using G6PD-SHARK. a** Consistence evaluation of SHARK and qRT-PCR. Clinical samples were analyzed by G6PD-SHARK platform and RT-qPCR. **b-e** Box plots showed miRNAs (miR-126, miR-328, miR-30a, miR-20a) expression at all stages compared with healthy people. Difference from control was assessed by one-way ANOVA and Dunnett’s multiple comparisons test. All data are represented as mean ± s.d. \* P<0.05.

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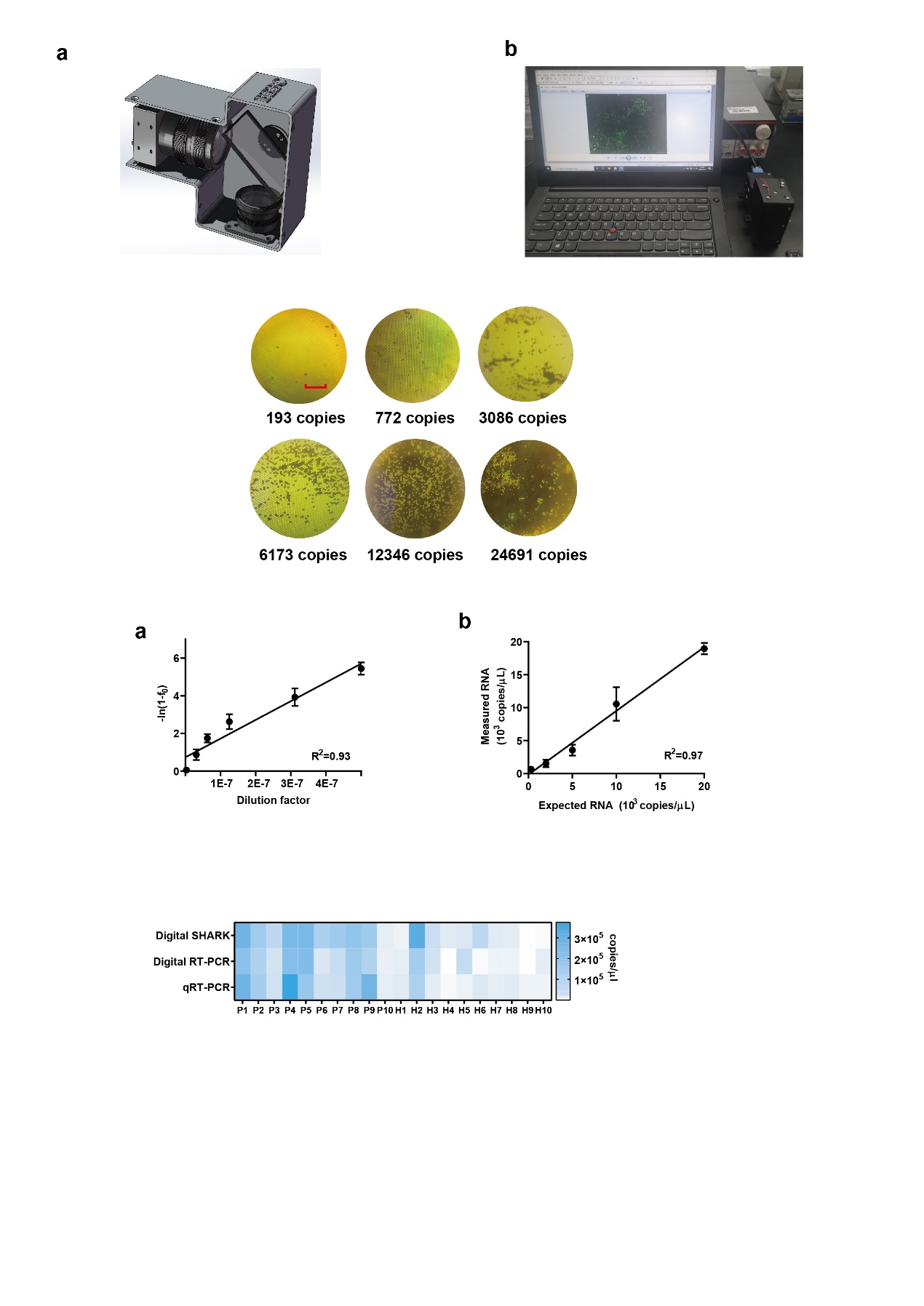
**Supplementary Fig. 11 Accuracy of biocomputing using different miRNA features.** miRNAs features were identified to attain optimal accuracy in training and test dataset by support vector machine-recursive feature elimination.

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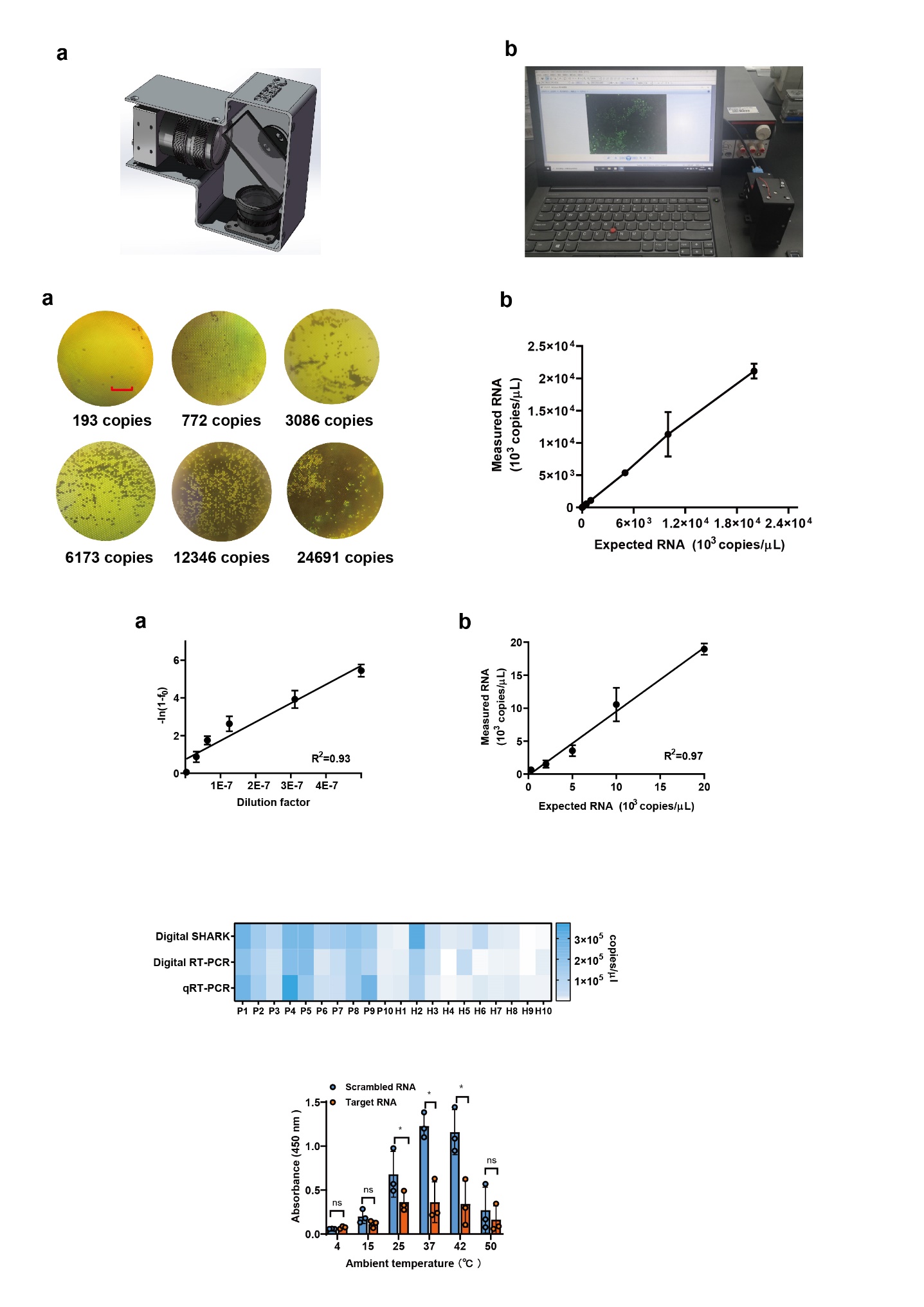
**Supplementary Fig. 12 Cas9:RsgRNA system controlling enzyme expression for RNA detection. a** Schematic diagram of Cas9:RsgRNA system for controlling dsDNA cleavage.In the absence of an analyte, transcription process was blocked, whereas, in the presence of target RNA,the enzyme was produced. **b** The secondary structure changes of RsgRNA were affected by the trigger predicted by NUPACK. **c** Gel images for dsDNA cleavage by trigger RNA controlled Cas9:RsgRNA system controlling **d** Demonstration of Cas9: RsgRNA activity controlled by trigger RNA to produce G6PD.

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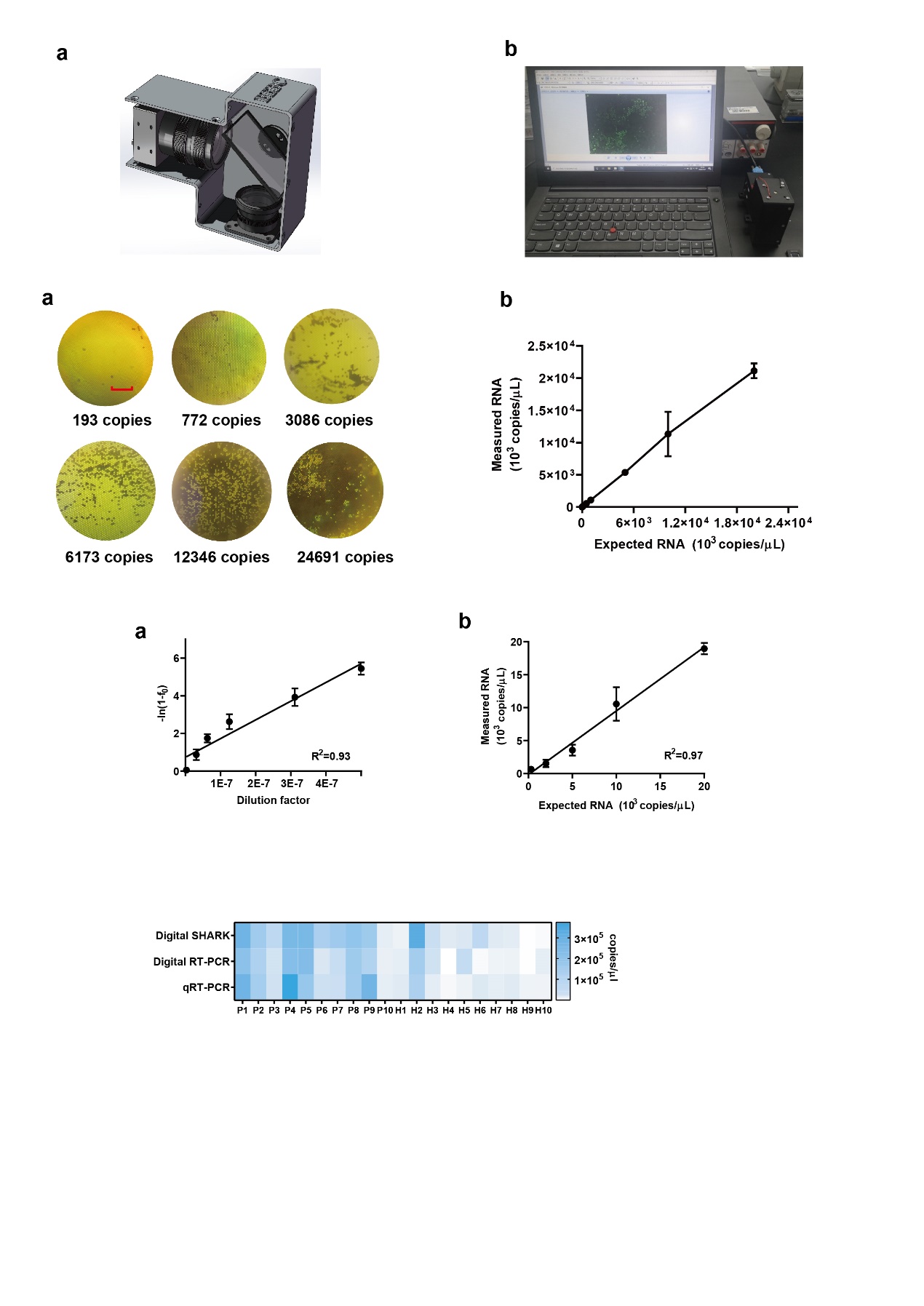
**Supplementary Fig. 13 Cell free synthetic biosensor based on Cas9:RsgRNA with high sensitivity and specificity. a** Visual assay was detected after addition of 100 nM trigger RNA or scrambled RNA. The kinetics of G6PD by Cas9:RsgRNA coupling with CFE was initiated by the introduction of specific target RNA. The results showed that Cas9:RsgRNA system was capable to sensitively detect miRNA.All data are represented as mean ± s.d. (n=3). **b** A subset of Cas9 system was tested to evaluate cross reactivity with different miRNAs. **c** Quantification of absorbance over time by single or combined RsgRNA for miRNA detection array. All data are represented as mean ± s.d. (n=3).

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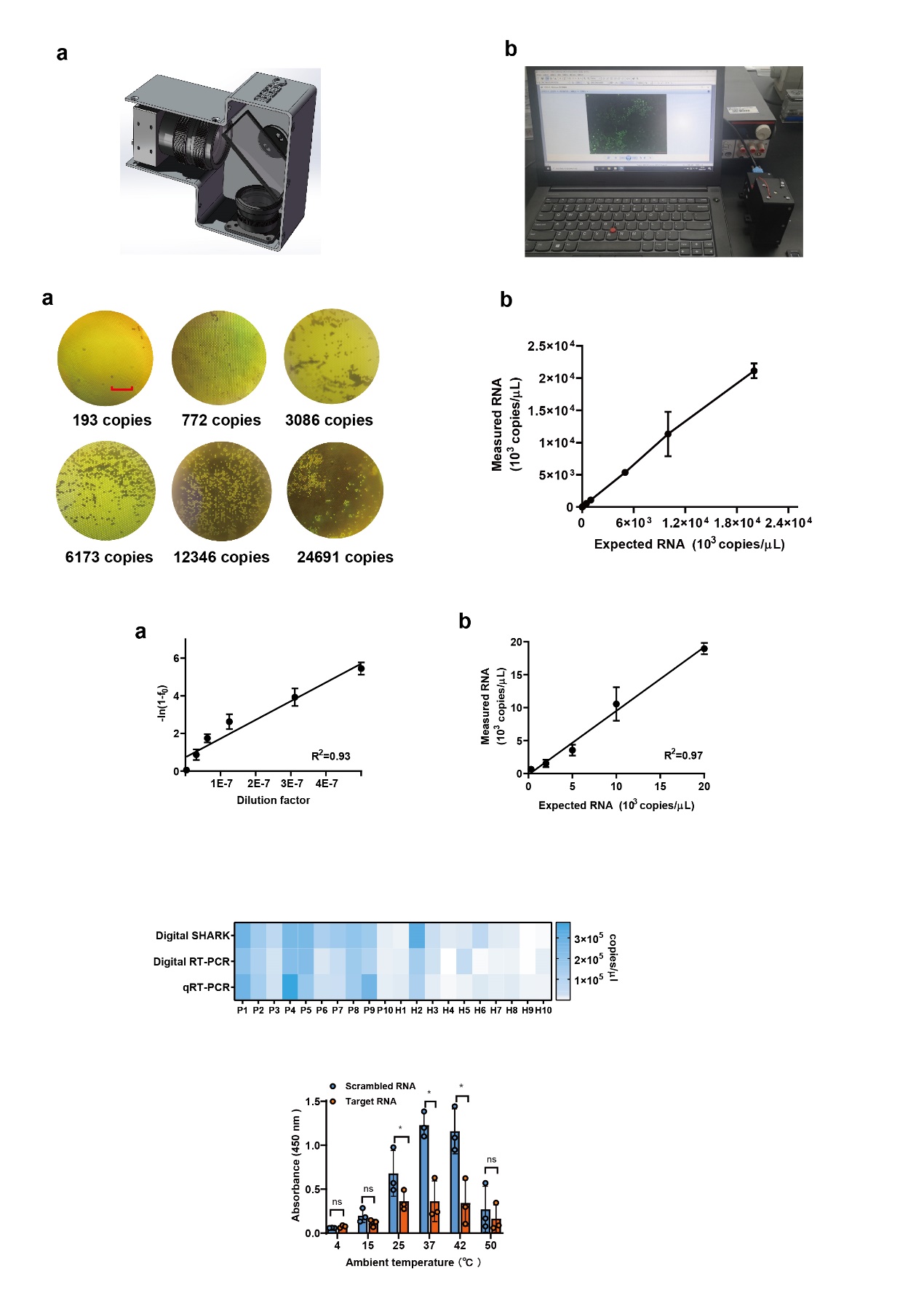
**Supplementary Fig. 14 Design and verification of handheld luminescence illustrator. a** 3D rendering of the handheld digital SHARK device. **b** Image of the handheld reader developed to read bioluminescence output from digital-SHARK reactions held in a chip. Data were collected every 10 seconds and processed by IC capture**.**



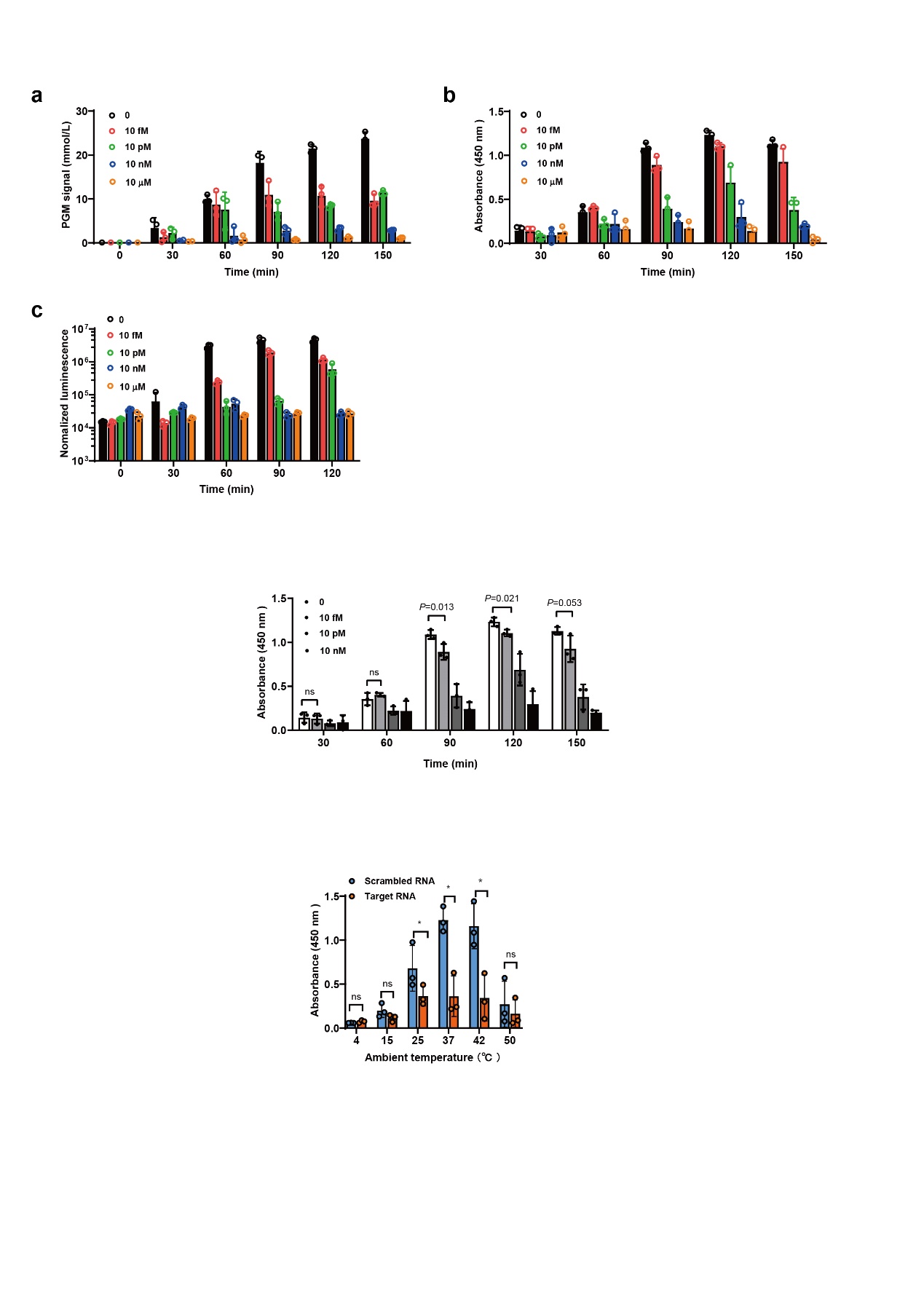
**Supplementary Fig. 15 Quantitation performance of digital SHARK. a** According to the scatter plot of -ln (1-f0) and the dilution factor, the distribution of scatter plot was well-fit by an Prison value distribution. Then the copy number concentration could be calculated on the basis of the regression equation. **b** Correlation using digital SHARK with diluted RNA concentration. All data are represented as mean ± s.d. (n=3).



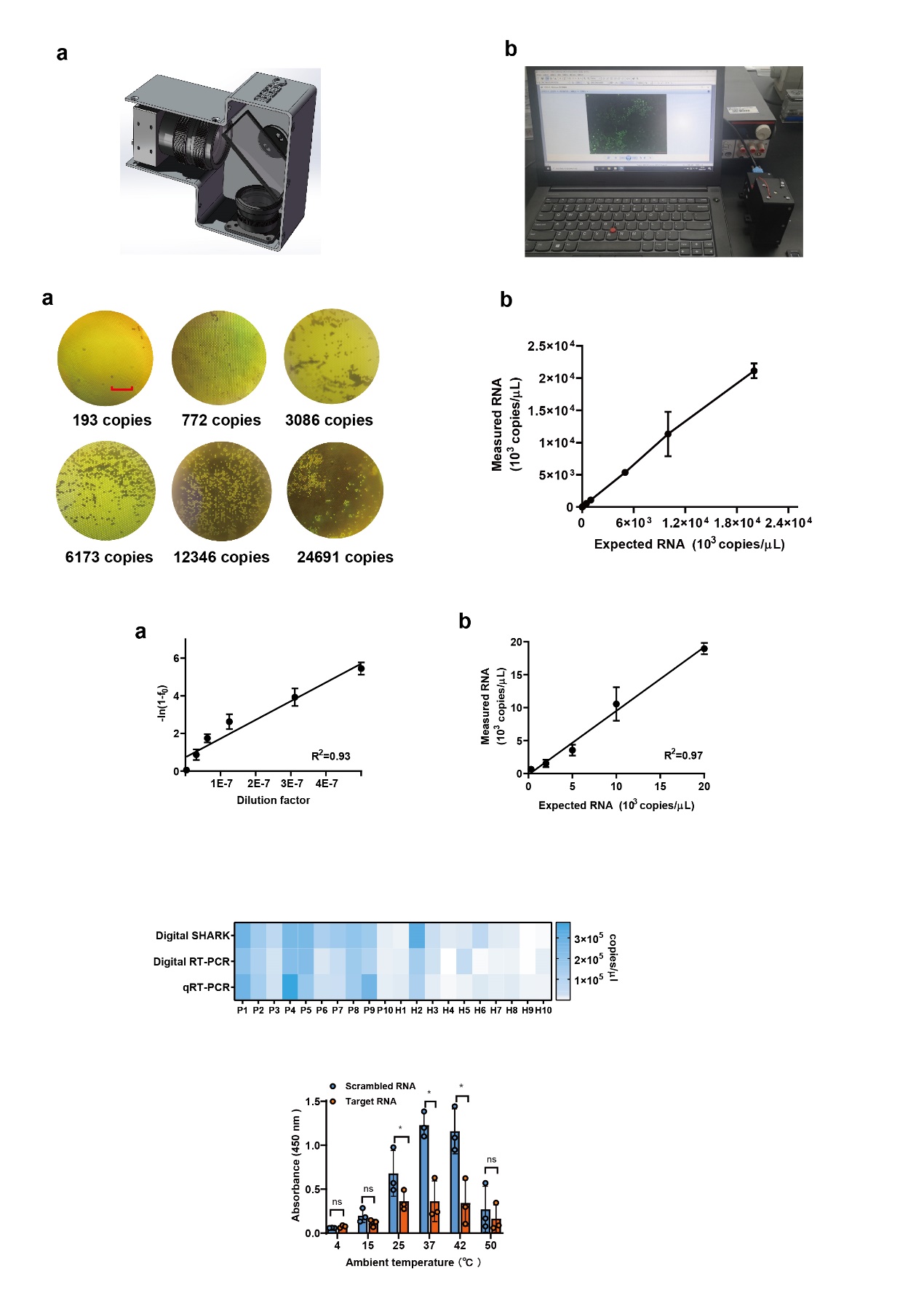
**Supplementary Fig. 16 Full view of the chip under digital-SHARK device to detect diluted miR-20a. a** Chips were imaged with handheld device. A 10×magnification objective lens is capable of imaging 0.785 mm2 areas, allowing almost 15000 micro-wells to be captured in a picture frame. **b** Quantification range of the digital SHARK based on handheld analysis device. All data are represented as mean ± s.d. (n=3).

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**Supplementary Fig. 17 Evaluation of miR-20a expression in clinical sample using qRT-PCR, digital RT-PCR and digital SHARK.** 10 clinical specimens collected from NSCLC patients and 10 healthy individuals were tested.

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**Supplementary Fig. 18 Optimization of detection time for SHARK.** RNA samples were incubated with SHARK for different time. The colorimetric results showed that 60-90 min was sufficient to detect 10 fM RNA. Difference from control was assessed by one-way ANOVA and Dunnett’s multiple comparisons test. All data are represented as mean ± s.d. (n= 3 independent reactions). n.s. indicates not significant.



**Supplementary Fig. 19 Optimization of temperature for SHARK.** Detection performance of SHARK reaction was investigated at 4 °C, 15 °C, 25 °C, 37 °C and 42 °C temperature. Asterisks indicate significant difference *versus* the inactivated SHARK assay (n.s., not significant; \* p<0.05; Student’s two-tailed t-test).

**Supplementary Table 1 | Consistence of sInv-SHARK and qRT-PCR Testing for SARS-CoV-2.**

|  |  |  |  |
| --- | --- | --- | --- |
| **sInv-SHARK** | **qRT-PCR** | | |
| **Positive** |  | **Negative** |
| **Positive** | 27 |  | 0 |
| **Negative** | 2 |  | 15 |
| **Sensitivity** | 93% | | |
| **Specificity** | 100% | | |
| **Accuracy** | 94.5% | | |

**Supplementary Table 2 Clinical characteristics of subjects in NSCLC patients and healthy individuals.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | **Patient** | **Healthy** |  |
|  | **n=38** | **n=26** | ***p*-value** |
| **Age, median** | 59 | 55 | 0.54 |
| **Gender, n (%)** |  |  |  |
| **Male** | 22 (58%) | 12 (44%) |  |
| **Female** | 16 (42%) | 14 (56%) | 0.86 |
| **Stage, (%)** |  |  |  |
| **Stage I** | 8 (21%) | NA |  |
| **Stage II** | 7 (18%) | NA |  |
| **Stage III** | 10 (26%) | NA |  |
| **Stage IV** | 13 (34%) | NA |  |
|  |  |  |  |
| **Histology, n (%)** |  |  |  |
| **Adenocarcinoma** | 30 (79%) | NA |  |
| **Squamous cell carcinoma** | 8 (21%) | NA |  |

**Supplementary Table 3 Results and weights of miRNAs identification for NSCLC using SVM algorithms.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Number of features** | **3** | **5** | **7** | **10** | **13** | **15** |
| **Accuracy** | 0.678 | 0.821 | 0.857 | 0.785 | 0.795 | 0.729 |
| **Precision** | 1 | 0.819 | 0.818 | 0.797 | 0.797 | 0.787 |
| **Recall** | 0.182 | 0.727 | 0.818 | 0.636 | 0.636 | 0.712 |
| **F1-Score** | 0.307 | 0.791 | 0.828 | 0.710 | 0.700 | 0.700 |
|  | **Weight** | | | | | |
| **miR-126** | -6 | -4 | -5 | -4 | -5 | -5 |
| **Let-7f** | -6 | -4 | -3 | -5 | -7 | -3 |
| **miR-5688** | 10 | 11 | 9 | 8 | 10 | 7 |
| **miR-20a** |  | 8 | 1 | 4 | 4 | 4 |
| **miR-30a** |  | 8 | 4 | 6 | 6 | 7 |
| **miR-761** |  |  | -2 | -4 | -3 | -3 |
| **Let-7b** |  |  | 6 | 11 | 9 | 10 |
| **miR-548** |  |  |  | 8 | 2 | 9 |
| **miR-515** |  |  |  | 3 | 3 | 3 |
| **miR-200a** |  |  |  | -8 | -7 | -3 |
| **miR-4685** |  |  |  |  | 3 | 4 |
| **miR-4302** |  |  |  |  | -3 | -6 |
| **miR-1294** |  |  |  |  | 5 | 4 |
| **miR-1278** |  |  |  |  |  | 8 |
| **miR-3610** |  |  |  |  |  | -2 |

**Supplementary Table 4 Sequences of synthetic target RNAs used in this work.**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5’-3’)** | **Used in** |
| Target RNA 1 | CAAGCACCUGUGCAAAUCCAUGCAAAACUGAGAGGCGGCGACUUGAACCCAC | Fig. 1, Fig. 2,  S2, S3, S18, S19 |
| Contrived SARS-CoV-2 (ORF1) | ATCGTGTTGTCTGTACTGCCGTTGCCACATAGATCATCCAAATCCTAAAGGATTTTGTGACTTAAAAGGTAAGTATGTACAAATACCTACAACTTGTGCTAATGACCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAGGTTATGGCTGTAGTTGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAATCGTTTTTAAACGGGTTTGCGGTGTAAGTGCAGCCCGTCTTACACCGTGCGGCACAGGCACTAGTACTGATGTCGTATACAGGGCTTTTGACATCTACAATGATAAAGTAGCTGGTTTTGCTAAATTCCTAAAAACTAATTGTTGTCGCTTCCAAGAAAAGGACGAAGATGACAATTTAATTGATTCTTACTTTGTAGTTAAGAGACACACTTTCTCTAACTACCAACATGAAGAAACAATTTATAATTTACTTAAGGATTGTCCAGCTGTTGCTAAACAT | Fig. 3, S4, S6, S7 |
| Contrived SARS-CoV-2 (N) | ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAG | Fig. 3, S4, S6, S7 |
| Contrived SARS-CoV-2 (E) | ATGTACTCATTCGTTTCGGAAGAGACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATATTGTTAACGTGAGTCTTGTAAAACCTTCTTTTTACGTTTACTCTCGTGTTAAAAATCTGAATTCTTCTAGAGTTCCTGATCTTCTGGTCTAA | Fig. 3, S4, S6, S7 |
| Homo sapiens miR-126-3p | UCGUACCGUGAGUAAUAAUGCG | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| Homo sapiens miR-328-3p | CUGGCCCUCUCUGCCCUUCCGU | Fig.4, S10, S11, S12, S13 |
| Homo sapiens miR-19b-3p | UGUGCAAAUCCAUGCAAAACUGA | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| Homo sapiens miR-141-5p | CAUCUUCCAGUACAGUGUUGGA | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| Homo sapiens Let-7b-5p | UGAGGUAGUAGGUUGUGUGGUU | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| Homo sapiens miR-20a-3p | CUUUCAGUCGGAUGUUUGCAGC | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13, S15, S16, S17 |
| Homo sapiens miR-30a-5p | UAAAGUGCUUAUAGUGCAGGUAG | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| Homo sapiens miR-761 | GCAGCAGGGUGAAACUGACACA | Fig. 5, |
| Homo sapiens Let-7f-1-3p | CUAUACAAUCUAUUGCCUUCCC | Fig. 5 |
| Homo sapiens miR-5688 | UAACAAACACCUGUAAAACAGC | Fig. 5 |

**Supplementary Table 5 Sequences of crRNA used in this work.**crRNAs were designed with online software and optimized through experiments. We designed crRNAs using CRISPR-RT (<http://bioinfolab.miamioh.edu/CRISPR-RT/>)

|  |  |  |
| --- | --- | --- |
| **Name** | **sequence** | **Used in** |
| Trigger RNA crRNA | CCACCCCAAUAUCGAAGGGGACUAAAACUCAGUUUUGCAUGGAUUUGCACA | Fig. 1, Fig. 2,  S2, S3, S18, S19 |
| Orf1a/b crRNA | CCACCCCAAUAUCGAAGGGGACUAAAACACAAUCCUUAAGUAAAUUAUAAAUUGUU | Fig. 3, S4, S6, S7 |
| E crRNA | CGACCCCAAUAUCGAAGGGGACUAAAACAACUCUAGAAGAAUUCAGAUUUUUAACA | Fig. 3, S4, S6, S7 |
| N crRNA | GCACCCCAAUAUCGAAGGGGACUAAAACAAUUUGGAUCUUUGUCAUCCAAUUUGAU | Fig. 3, S4, S6, S7 |
| MERS crRNA 1 | CCACCCCAAUACGAAGGGGACUAAAACUAUAGAUUGUUCUUUUAAUGUGAUGAAGC | Fig. 3 |
| MERS crRNA 2 | CCACCCCAAUACGAAGGGACUAAAACCUAUAAGAUUGUUUCUUUUAAUGUGAUGA | Fig. 3 |
| MERS crRNA 3 | CCACCCCAAUACGAAGGGGACUAAAACUAAGAUAACAAUUAUUAUCACUCAAUUU | Fig. 3 |
| SARS crRNA 1 | CCACCCAAUACGAAGGGGACUAAAACACGACAGUAAAAUUUAUUAUUGUUUAUA | Fig. 3 |
| SARS crRNA 2 | CCACCCCAAUACGAAGGGGACUAAAACUGUUCCAGUUUUGUUCAUAAUCUUCAAU | Fig. 3 |
| SARS crRNA 3 | CCACCCCAAUACGAAGGGGACUAAAACUCAAAAACACCUGUAAUGAGAAAUUUUGA | Fig. 3 |
| IVA crRNA 1 | CCACCCCAAUACGAAGGGGACUAAAACAAAUAAGUUUUGUAGAUUUUUGGAUAAU | Fig. 3 |
| IVA crRNA 2 | CCACCCCAAUACGAAGGGGACUAAAACUUCAAAUUCCAUUUUAUUGUAUAGCAUU | Fig. 3 |
| IVA crRNA 3 | AUAACCCCACCCGAAGGGGACUAAAACUUUUUAAACUAUUCGACACUAAUUGAUG | Fig. 3 |
| HKU1 crRNA 1 | CCACCCCAAUACGAAAGGGGACUAAAACAAUAAUACUAAAAUCUUCUUUAUUAUUA | Fig. 3 |
| HKU1 crRNA 2 | CCACCCCAAUACGAAGGGGACUAAAACAAUUUAAAAUUUUGAAAUUAUUAAAUCAC | Fig. 3 |
| HKU1 crRNA 3 | CCACCCCAAUACGAAGGGGACUAAAACUUAUAUAUAUAGAUUUUAAUAGAAAAAUCAU | Fig. 3 |
| 16s RNA crRNA 1 | CCACCCCAAUACGAAGGGGACUAAAACGUGGUAACCGUCCCCCUUGC | Fig. 3 |
| 16s RNA crRNA 2 | CCACCCCAAUACGAAGGGGACUAAAACAGCACUCGGGAGGAAAGAAG | Fig. 3 |
| 16s RNA crRNA 3 | CCACCCCAAUACGAAGGGGACUAAAACUGAAUAAGGUUAUUAACCUC | Fig. 3 |
| miR-126 crRNA | CGACCCCAAUAUCGAAGGGGACUAAAACCGCAUUAUUACUCACGGUACGA | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| miR-328 crRNA | CCACCCCAAUAUCGAAGGGGACUAAAACACGGAAGGGCAGAGAGGGCCAG | Fig.4, S10, S11, S12, S13 |
| miR-19b crRNA | GGACCCCAAUAUCGAAGGGGACUAAAACUCAGUUUUGCAUGGAUUUGCACA | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| miR-141 crRNA | CGACCCCAAUAUCGAAGGGGACUAAAACUCCAACACUGUACUGGAAGAUG | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| Let-7b crRNA | CCACCACCCCAAAAAUGAAGGGGACUAAAACAAGGCAGUAGGUUGUAUAG | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| miR-20a crRNA | GGACCCCAAUAUCGAAGGGGACUAAAACCUACCUGCACACUAUAAGCACUUUA | Fig.4, Fig.5, Fig.6, S10, S11, S12, S13, S15, S16, S17 |
| miR-30a crRNA | CGACCCCAAUAUCGAAGGGGACUAAAACGCUGCAAACAUCCGACUGAAAG |  |
| Fig.4, Fig.5, Fig.6, S10, S11, S12, S13 |
| miR-761 crRNA | GCACCACCCCAAAAAUGAAGGGGACUAAAACGUAGUUUCACCCUGCUGC | Fig. 5 |
| Let-7f-1 | GCACCCCAAUAUCGAAGGGGACUAAAACAACUAUACAAUCUACUACCUCA | Fig. 5 |
| miR-5688 crRNA | GGACCACCCCAAAAAUGAAGGGGACUAAAACGUUUUACAGGUGUUUGUUA | Fig. 5 |

**Supplementary Table S6 | Reference values for SHARK assay.**

|  |  |  |
| --- | --- | --- |
| **Description** | **Kinetic parameters** | **Value** |
| Michaelis constant of transcription | *K*S | 8.5 nM |
| Michaelis constant of translation | *K*l | 65.8 nM |
| Michaelis constant of TIR | *K*TIR | 6e-5 M |
| Michaelis constant of Cas13a | *K*m | 35 nM |
| Transcription scaling factor | *k*cs | 1.1e-2 min-1 |
| TlR depletes rate | δTIR | 4.5e-3 min-1 |
| Transcription rate | *k*ts | 18.2 nM/min |
| Translation rate | *k*tl | 16.1 nM/min |
| Maturation rate | *k*mat | 0.2 min-1 |
| Cas13a cleavage | *kcas* | 6000 μM/s |
| G6PD mRNA length | *l*G | 1458 nt |
| Invertase mRNA length | *l*I | 1650 nt |
| Luciferase mRNA length | *l*L | 1653 nt |
| G6PD protein length | *L*g | 486 aa |
| Intvertase protein length | *L*i | 550 aa |
| Luciferase protein length | *L*l | 551 aa |

**Supplementary Table 7 Sequences of RsgRNA used in this work.**

Based on G6PD expression plasmid, we determined the effects of RsgRNA region on blast off-targets and folding correct.

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence** | **Used in** |
| Trigger RNA RsgRNA | AGUUUUGCAUGGAUUUGCAGAGCCGGCACUAGAGGGAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU | Fig. 5, S12, S13 |
| Mutant1 RNA RsgRNA | AGUUUAGCAUGGAUUUGCAGAGCCGGCACUAGAGGGAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU | S13 |
| Mutant2 RNA RsgRNA | AGUUUAGCUUGGAUUUGCAGAGCCGGCACUAGAGGGAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU | S13 |
| Mutant3 RNA RsgRNA | AGUUUAGCAUAGAUUUGCAGAGCCGGCACUAGAGGGAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU | S13 |
| miR-761 RsgRNA | GUAGUUUCACCCUGCUGCGAGCCGGCACUAGAGGGAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU | Fig.5, S13 |
| Let-7f-1 RsgRNA | AAGGCAGUAGGUUGUAUAGGAGCCGGCACUAGAGGGAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU | Fig.5, S13 |
| miR-126  RsgRNA | CGCAUUAUUACUCACGGUACGAGAGCCGGCACUAGAGGGAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU | Fig.5, S13 |

**Supplementary Table 8 Sequences of primers used in this work.**

The hybridization sequences were systematically selected using the nucleic acid design software Primer-BLAST and NUPACK to minimize structure formation and maximize hybridization with target RNA.

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence** | **Used in** |
| ORF1 a/b Forward | CCCTGTGGGTTTTACACTTAA | Fig. 3, S6 |
| ORF1 a/b Reverse | ACGATTGTGCATCAGCTGA | Fig. 3, S6 |
| E Gene Forward | ACAGGTACGTTAATAGTTAATAGCGT | Fig. 3, S6 |
| E Gene Reverse | CAGACATTTTGCTCTCAAGCTG | Fig. 3, S6 |
| N Gene Forward | GGGGAACTTCTCCTGCTAGAAT | Fig. 3, S6 |
| N Gene Reverse | CAGACATTTTGCTCTCAAGCTG | Fig. 3, S6 |
| miR-126 stem loop | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCGCAT | Fig. 4 |
| miR-126 Forward | TCGGCAGGTCGTACCGTGAGTA | Fig. 4 |
| miR-126 Reverse | CTCAACTGGTGTCGTGGA | Fig. 4 |
| miR-328 stem loop | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAACGGAA | Fig. 4 |
| miR-328 Forward | TCGGCAGGCTGGCCCTCTCTGC | Fig. 4 |
| miR-328 Reverse | CTCAACTGGTGTCGTGGA | Fig. 4 |
| miR-19b stem loop | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGTTTT | Fig. 4 |
| miR-19b Forward | TCGGCAGGTGTGCAAATCCATG | Fig. 4 |
| miR-19b Reverse | CTCAACTGGTGTCGTGGA | Fig. 4 |
| miR-141 stem loop | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCCAACA | Fig. 4 |
| miR-141 Forward | TCGGCAGGATCTTCCAGTACA | Fig. 4 |
| miR-141 Reverse | CTCAACTGGTGTCGTGGA | Fig. 4 |
| Let-7b stem loop | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTAT | Fig. 4 |
| Let-7b Forward | TCGGCAGGTGAGGTAGTAGA | Fig. 4 |
| Let-7b Reverse | CTCAACTGGTGTCGTGGA | Fig. 4 |
| miR-30a stem loop | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCTGCA | Fig. 4 |
| miR-30a Forward | TCGGCAGGCTTTCAGTCGGATG | Fig. 4 |
| miR-30a Reverse | CTCAACTGGTGTCGTGGA | Fig. 4 |
| miR-20a stem loop | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTACCT | Fig. 4, S17 |
| miR-20a Forward | TCGGCAGGTAAAGTGCTTATAGT | Fig. 4, S17 |
| miR-20a Reverse | CTCAACTGGTGTCGTGGA | Fig. 4, S17 |