Electronic Supplementary Information

Mismatched fluorescent probes with enhanced strand displacement reaction rate for intracellular long noncoding RNA imaging

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Experimental section

Materials and Reagents

LncRNA HOTAIR, lncRNA MALAT1, RNase inhibitor and RNase-free water were purchased from TaKaRa Bio. Inc. (Dalian, China). All DNA oligonucleotides (Table S1) were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China) and purified by HPLC. Human lung adenocarcinoma cell line (A549 cells), human breast cancer cell line (MCF-7 cells) and human bronchial epithelial cell line (HBE cells) were bought from Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). All other reagents were of analytical grade and used as received without further purification.

Cell culture and preparation of total RNA

MCF-7 cells, A549 cells and HBE cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C with 5% CO_2. The number of cells was measured by Countstar cell counter. The total RNA was obtained by universal extraction kit (GeneDotech, Shenzhen, China) according to the manufacturer's procedure, and quantified by using NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, U.S.A.).

In vitro fluorescence detection of lncRNA

The capture probe and reporter probe were diluted to 10 μM in 1× reaction buffer (10 mM Tris-HCl, 50 mM NaCl, pH 8.0), respectively, and then mixed and incubated at 95 °C for 5 min, followed by slowly cooling to the room temperature to form the mismatched probe and the matched probe. For lncRNA assay, 1.5 μL of mismatched probe (10 μM) or matched probe (10 μM), different concentrations of lncRNA / total RNA samples, 20 U of RNase inhibitor were added in the 2 μL of 10× TDT buffer (100 mM $Mg(Ac)_2$, 200 mM Tris-Ac, 500 mM KAc, pH 7.9) with a final volume of 20 μL. The mixture was incubated at 37 °C for 3 h in the dark to release the reporter probes. The fluorescence signals were measured by Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) with an excitation wavelength of 488 nm. The emission spectra were scanned in the range from 500 to 650 nm, and the emission intensities at 520 nm were used for data analysis.

The qRT-PCR assay

Total RNA obtained from different cell lines was reverse transcribed to cDNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) according to the manufacturer's instructions. The lncRNA levels were quantified by RT-PCR using TB Green Premix Ex Taq II (Tli RNaseH Plus) (TaKaRa, Dalian, China) in a BIO-RAD CFX connect Real-Time system. The results were analyzed by using the ΔΔCT method with HBE cells as the

control.

Imaging of lncRNA in living cells

MCF-7 cells, A549 cells and HBE cells were plated in 20-mm glass-bottom cell culture dish and incubated overnight in DMEM medium containing 10% fetal bovine serum. The cells were washed twice with 1× PBS. Transfection assays were performed using Lipofectamine 3000 reagent according to the manufacturer's instructions. Briefly, 7.5 μL of Lipofectamine 3000 was diluted in 250 μL of Opti-MEM. The 18.7 μL of mismatched probe (10 μM) or matched probe (10 μM) and 10 μL of P3000 were diluted in 250 μL of Opti-MEM. The Opti-MEM transfection mixtures were prepared by mixing the above solutions and incubated at room temperature for 15 min. The cells were incubated with the Opti-MEM transfection mixtures in a humidified incubator containing 5% CO_2 for 3 h at 37 °C. After the transfection, the cells were washed five times with 1× PBS, and the dishes were filled with fresh DMEM medium containing 10% fetal bovine serum. The cell images were obtained on an inverted Olympus IX71 microscope (Olympus, Japan) with 10× objective. For data analysis, Image J software was used to select regions of interest of 300 × 300 pixels. The average fluorescence intensity was obtained by calculating six frames.

For imaging of lncRNA in single MCF-7 cell and HBE cell, the cells were plated in 20-mm glass-bottom cell culture dish, respectively, and incubated overnight in DMEM medium containing 10% fetal bovine serum. The 7.5 μL of Lipofectamine 3000 was diluted in 250 μL of Opti-MEM. The 8 μL of capture probe (10 μM) and 10 μL of P3000 were diluted in 250 μL of Opti-MEM. The cells were incubated with the Opti-MEM transfection mixtures in a humidified incubator containing 5% CO_2 for 1.5 h at 37 °C. After the transfection, the cell images were obtained on inverted microscope with 60× objective. For data analysis, Image J software was used to select the regions of interest of 300 × 300 pixels. The average fluorescence intensity was obtained by calculating six frames.

Table S1. Sequences of the oligonucleotides

note	sequence (5′-3′)
lncRNA HOTAIR	GCA ACU CUA UAA UAU GCU UAU AUU AGG UCU AGA AG
capture probe	BHQ1-T* C*T*A* G*AC CTA ATA TAA GCA TAT TAT AGA G*T*T *G*C
reporter probe 1	A*T* G*CT TAT **T**TT AGG **A**CT *A*G*A-FAM
reporter probe 2	G*C*T *TAT ATT AGG TCT* A*G*A-FAM
reporter probe 3	G*C*T* TAT ATT **T**GG TCT *A*G*A-FAM
reporter probe 4	C*T* T*ATA TTA GGT CT*A *G*A-FAM
reporter probe 5	G*C*T* TAT **T**TT AGG **A**CT *A*G*A-FAM
reporter probe 6	T* T*A*T ATT AGG TCT *A*G*A-FAM
reporter probe 7	G*C*T* T**T**T ATT **T**GG TC**A** *A*G*A-FAM
reporter probe 8	T*A*T* ATT AGG TCT *A*G*A-FAM
reporter probe 9	G*C*__A__* TAT ATT AGG TCT *__A__*G*A-FAM
reporter probe 10	G*C*T* __A__AT ATT AGG TC__A__ *A*G*A-FAM
reporter probe 11	G*C*T* T**T**T ATT AG**C** TCT *A*G*A-FAM
reporter probe 12	G*C*T* TAT AT__A__ AGG __A__CT *A*G*A-FAM
reporter probe 13	G*C*T* TAT AT__A__ __T__GG TCT *A*G*A-FAM
reporter probe 14	G*C*T* TAT ATT A**CC** TCT *A*G*A-FAM
scrambled capture probe	BHQ1-A*T*T* G*T*C TAC TTA TAC GAT TAC ACG ATA TA*G *G*A*A
scrambled reporter probe	A*T*C* GTA TAA GTA GAC* A*A*T-FAM
let-7a	UGA GGU AGU AGG UUG UAU AGU U
miR-486-5p	UCC UGU ACU GAG CUG CCC CGA G
lncRNA MALAT1	UAA GAU UUC CCA AGC AGA CAG CCC GUG CUG CUC CG
HOTAIR-forward primer	GGG ACA GAA GGA AAG CCC TC
HOTAIR- reverse primer	TTG AGA GCA CCT CCG GGA TA
GAPDH-forward primer	ATC TCT GCC CCC TCT GCT GA
GAPDH-reverse primer	GAT GAC CTT GCC CAC AGC CT

[a] The asterisk indicates the phosphorothioate modification. The underlined boldface bases represent the mismatched bases.

Supplementary Results

Altering the position of mismatched bases in mismatched probe 5

We altered the position of two mismatched bases in mismatched probe 5 to obtain mismatched probe 9, mismatched probe 10, mismatched probe 11, mismatched probe 12, mismatched probe 13, and mismatched probe 14, respectively. As shown in Fig. S1, the F/F_0 value obtained by using mismatched probe 5 is higher than those obtained by using mismatched probe 9-14, where F and F_0 are the fluorescence intensity in the presence and absence of HOTAIR lncRNA, respectively.

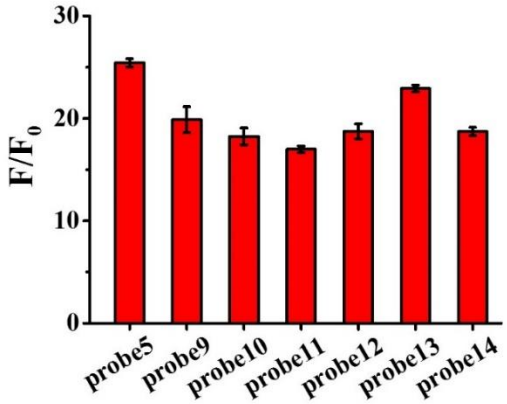

Fig. S1 Variance of the F/F_0 value with different mismatched probes. F and F_0 are the fluorescence intensity in the presence and absence of lncRNA, respectively. The concentration of lncRNA HOTAIR is 1 μM. Error bars show the standard deviation of the three experiments.

Comparation of the initial reaction rate of mismatched fluorescent probe 5 with that of matched fluorescent probe 6

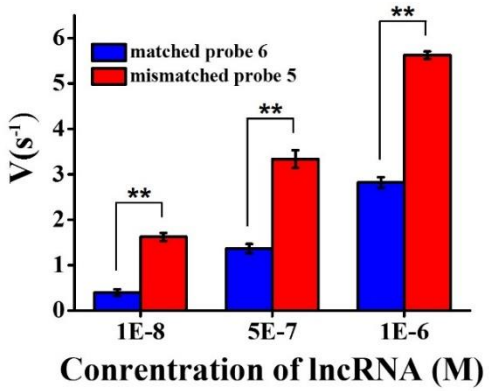

Fig. S2 Initial reaction rates of mismatched fluorescent probe 5 and matched fluorescent probe 6 in response to different-concentration HOTAIR lncRNA. $^{**}p < 0.01$ compared with matched fluorescent probe 6. Error bars indicate

the standard deviation of three experiments.

Strand displacement reaction induced by mismatched fluorescent probes and matched fluorescent probes.

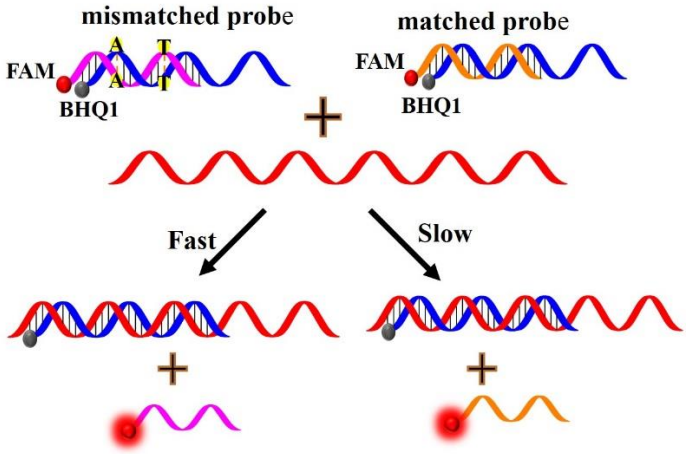

Fig. S3 Strand displacement reaction induced by mismatched fluorescent probes and matched fluorescent probes.

Optimization of reaction time

We optimized the reaction time of strand displacement reaction. As shown in Fig. S4, in the presence of lncRNA HOTAIR, the F/F_0 value increases with reaction time, and reaches the maximum value at 3 h. Thus, the reaction time of 3 h is used in the subsequent experiments.

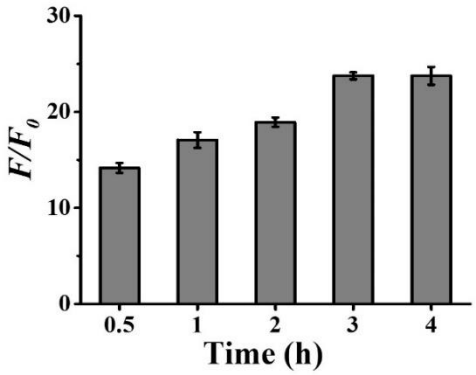

Fig. S4 Variance of the F/F_0 value with reaction times of strand displacement reaction. F and F_0 are the fluorescence intensity in the presence and absence of lncRNA, respectively. The concentration of lncRNA HOTAIR is 1 μM. Error bars show the standard deviation of the three experiments.

Measurement of lncRNA HOTAIR using mismatched probe 5

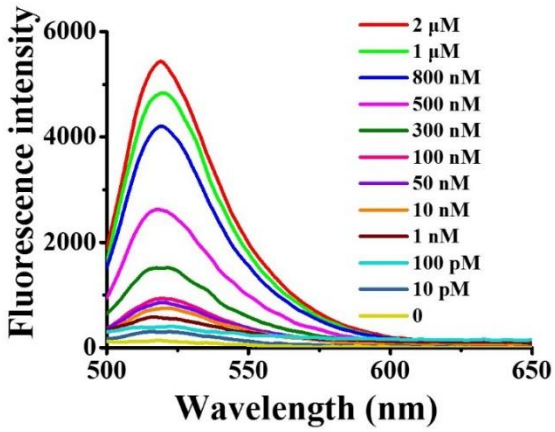

Fig. S5 Use of mismatched probe 5 for the detection of lncRNA HOTAIR.

Measurement of lncRNA HOTAIR using matched probe 6

Under the optimal reaction time (Fig. S4), we investigated the sensitivity of matched probes 6 for lncRNA assay. As shown in Fig. S6a, fluorescence signal enhances with the increasing concentration of target HOTAIR lncRNA from 25 nM to 2 μM. In Fig. S6b, a linear relationship is obtained between the fluorescence intensity and target lncRNA concentrations in the range from 2.5×10^{-8} to 8×10^{-7} M, and the limit of detection (LOD) is measured to be 9.8 nM in terms of 3 times deviation over the blank response rule.

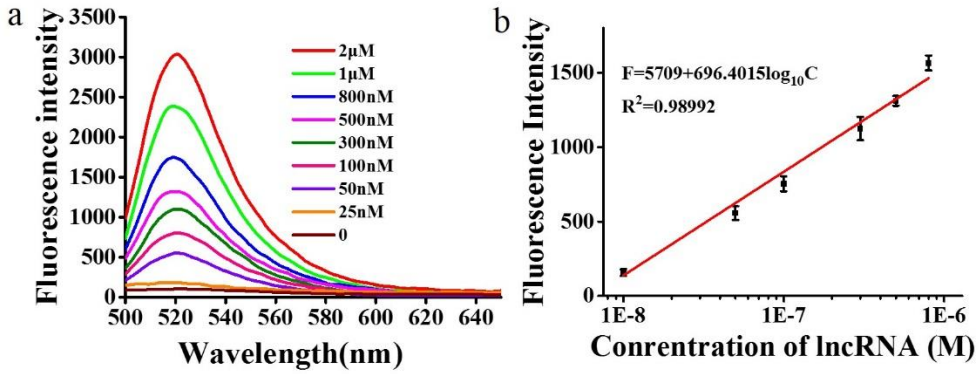

Fig. S6 (a) Use of matched probe 6 for the detection of lncRNA HOTAIR in the range from 0 to 2 μM. (b) Fluorescence intensity exhibits a linear relationship with the logarithm of lncRNA HOTAIR concentration in the range from 25 nM to 800 nM. Error bars show the standard deviation of the three experiments.

Detection specificity of matched probe 6 towards HOTAIR lncRNA

We evaluated the detection specificity of matched probe 6 towards HOTAIR lncRNA. As shown in Fig. S7, MALAT1 lncRNA, miRNA486-5P and let-7a exhibit very low fluorescence signals, close to that of the control with only

reaction buffer. In contrast, a higher fluorescence signal is generated in response to HOTAIR lncRNA, suggesting good selectivity of matched probe 6 towrads lncRNA assay.

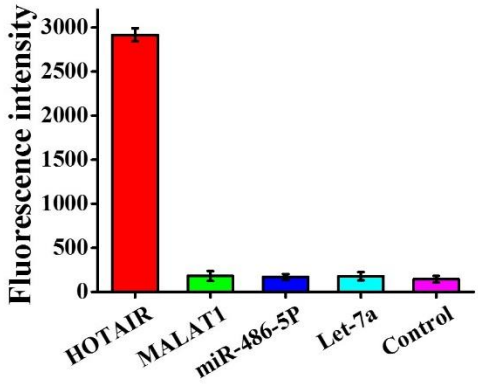

Fig. S7 Measurement of fluorescence intensity by using matched probe 6 in response to 1 μM lncRNA HOTAIR (red column), 1 μM lncRNA MALAT1 (green column), 1 μM miRNA-486-5P (blue column), 1 μM miRNA let-7a (cyan column) and the control with only reaction buffer (magenta column), respectively. Error bars show the standard deviation of the three experiments.

The qRT-PCR measurement

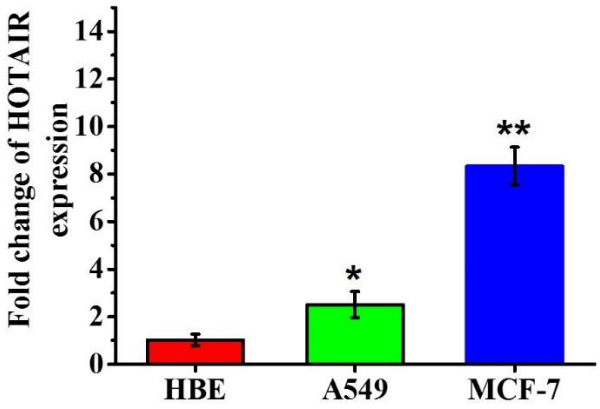

Fig. S8 Detection of lncRNA HOTAIR level in MCF-7 cells, A549 cells and HBE cells by RT-PCR. The results are analyzed by using the ΔΔCT method with HBE cells as the control. *$p < 0.05$ and **$p < 0.01$ compared with the normal HBE cells. Error bars show the standard deviation of the three experiments.

Imaging of lncRNA HOTAIR in MCF-7 cells using mismatched probe 5 and matched probe 6

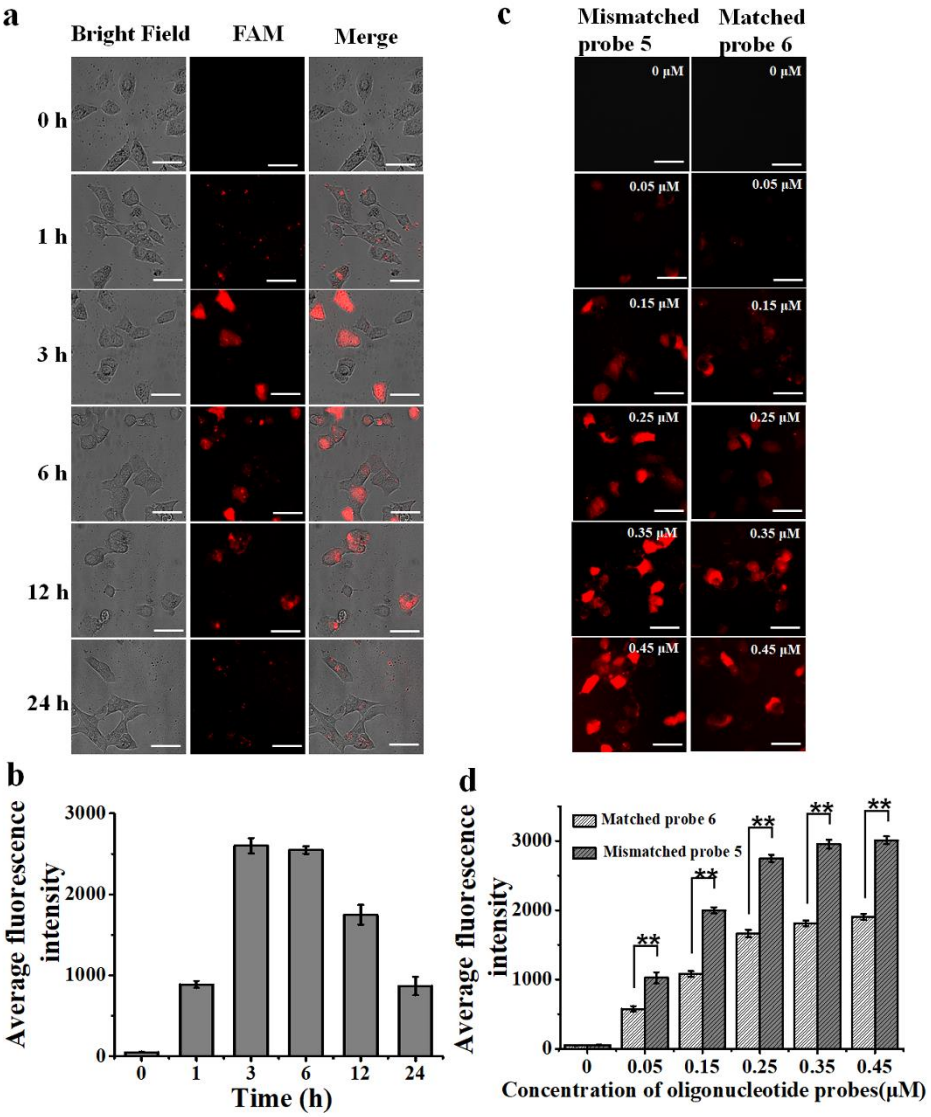

Fig. S9 (a) Time-dependent fluorescence images of MCF-7 cells using mismatched probe 5 (0.25 μM). The scale bar is 50 μm. (b) Variance of average fluorescence intensity with reaction time. (c) Dosage-dependent fluorescence images of MCF-7 cells after treatment with mismatched probe 5 (left panel) and matched probe 6 (right panel) for 3 h, respectively. The scale bar is 50 μm. (d) Variance of average fluorescence intensity with the probe concentration. **p < 0.01 compared with matched probe 6. Error bars indicate the standard deviation of three experiments.

Imaging of lncRNA HOTAIR in MCF-7 cells using scrambled fluorescent probe

To prove that the fluorescence signal is specifically induced by target lncRNA, we designed a scrambled fluorescent probe containing one scrambled capture probe (33 nt) and one scrambled reporter probe (18 nt) (Table S1) as a negative control. Several bases at the terminals of the sequences are modified with phosphorothioate to prevent the

nonspecific digestion by endogenous nuclease. The scrambled capture probe can partly hybridize with the scrambled reporter probe to form the scrambled fluorescent probe. As shown in Fig.S10, in the presence of mismatched probe 5, MCF-7 cells generate higher intracellular fluorescence signals, but no significant intracellular fluorescence signal is observed in the presence of scrambled fluorescent probe, suggesting that the fluorescence signal is specifically induced by target lncRNA and the phosphorothioate modification can prevent the nonspecific digestion induced by endogenous nuclease.

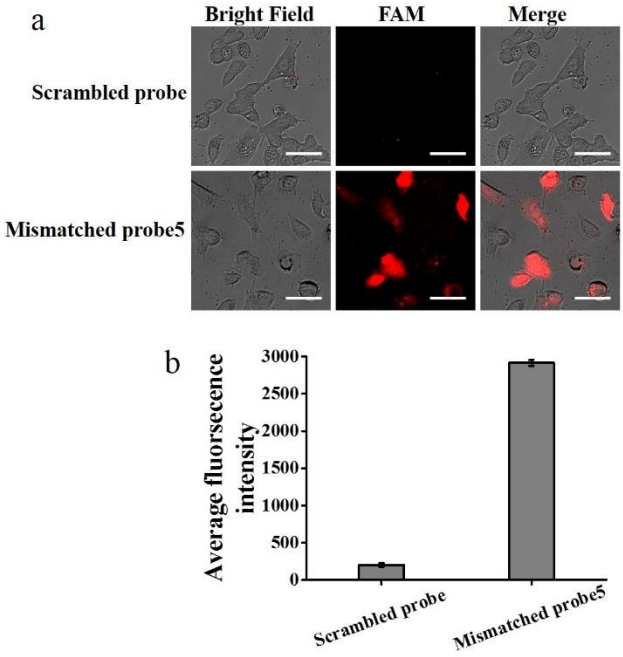

Fig. S10 (a) Fluorescence images of HOTAIR lncRNA in MCF-7 cells after incubation with mismatched probe 5 (0.25 μM) and scrambled fluorescent probe (negative control, 0.25 μM) for 3 h, respectively. The scale bar is 50 μm. (b) Average fluorescence intensity in MCF-7 cells after incubation with mismatched probe 5 (0.25 μM) and scrambled fluorescent probe (negative control, 0.25 μM) for 3 h, respectively.