

Supplementary Material

A CRISPR-Cas and Tat peptide with Fluorescent RNA Aptamer System for Signal Amplification in RNA Imaging

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Table S1. Sequences of TAR-Aptamer. Sequences are showed in the corresponding color of modules in the figures below (TAR in gray, framework in orange, Broccoli in green and Pepper in neon green). Multiple aptamer sequences are connected with 3-basepair-linkers and units of them are indicated as superscript serial-number and underline. DFHBI-1T and HBC530 fluorophores are revealed as green stars.

TAR-Aptamer Sequences (5'-3')	
TAR-Broccoli	<p>UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCC<u>CCCACAUACUCUGAU</u>- GAUCCGAGACGGUCGGUCAGAU<u>AUUCGUAUCUGUCGAGUAGAGUGU</u> GGCUCGGAUCAU<u>UCAUGGCAA</u></p>
TAR-2xBroccoli	<p>UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCC<u>CCCACAUACUCUGAU</u>- GAUCC¹GAGACGGUCGGGUCCAUCU²GAGACGGUCGGGUCCAGAU<u>AUUC</u> GUAUCUGUCGAGUAGAGUGUGGGCUCAGA¹UGUCGAGUAGAGUGUG- GGCUCGGAUCAU<u>UCAUGGCAA</u></p>
TAR-Pepper	<p>UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCC<u>CCCACAUACUCUGAU</u>- GAUCC<u>CCAUCGUGGCGUGUCGGCCUGCUUCGGCAGGCACUGGCGCC</u> GGAUCAU<u>UCAUGGCAA</u></p>
TAR-8xPepper	<p>UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCC<u>CCCACAUACUCUGAU</u>- GAUCC¹CCAUCGUGGCGUGUCGGCCUCUC²CCAUCGUGGCGUGUCGG CCUCUC³CCAUCGUGGCGUGUCGGCCUCUC⁴CCAUCGUGGCGUG- UCGGCCUCUC⁵CCAUCGUGGCGUGUCGGCCUCUC⁶CCAUCGUG- GCGUGUCGGCCUCUC⁷CCAUCGUGGCGUGUCGGCCU- CUC⁸CCAUCGUGGCGUGUCGGCCUCUCUCCGA- GAGGCACUGGCGCCGGAG⁷AGGCACUGGCGCCGGAG⁶AG- GCACUGGCGCCGGAG⁵AGGCACUGGCGCCGGAG⁴AGGCACUGGCGCCG- GAG³AGGCACUGGCGCCGGAG²AGGCACUGGCGCCGGAG¹AG- GCACUGGCGCCGGGAUCAU<u>UCAUGGCAA</u></p>

Modular structures of TAR-Aptamers (TAR in gray, framework in orange, Broccoli in green and Pepper in neon green, DFHBI-1T and HBC530 fluorophores are revealed as green stars).

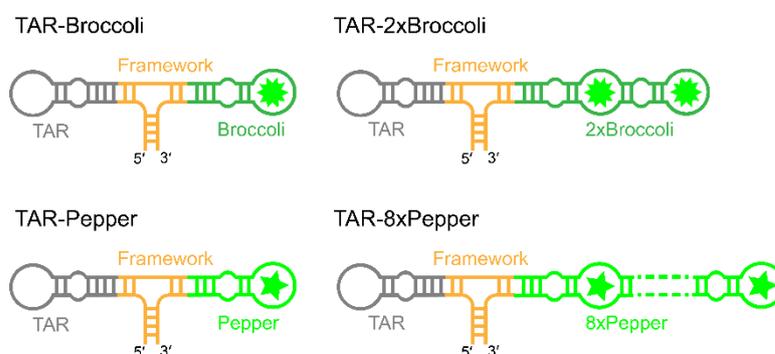


Table S2. Sequences of sgRNA, targeting *GCN4*, *MUC4* and *SatIII*. Sequences are showed in the corresponding color of modules in the figures below (spacer sequences in black, dCas13 sgRNA hairpin in blue, TBP sgRNA hairpin in purple and SLBP sgRNA hairpin in gray).

		sgRNA sequences (5'-3')
gGCN4	dCas13b	GGTGGTAATTCTTTGAAAGCAG GUUGUG-GAAGGUCCAGUUUUGAGGGGCCUAUUACAAC
	CIRTS(3)	GGTGGTAATTCTTTGAAAGCAG GGCCAGATCTGAGCCTGG-GAGCTCTCTGGCC
	CIRTS(10)	GGTGGTAATTCTTTGAAAGCAG CCAAAGGCTCTTCTCAGAGCCACCCA
gMUC4	dCas13b	GTGACCTGTGGATGCTGAGG GUUGUG-GAAGGUCCAGUUUUGAGGGGCCUAUUACAAC
	CIRTS(3)	GTGACCTGTGGATGCTGAGG GGCCAGATCTGAGCCTGG-GAGCTCTCTGGCC
	CIRTS(10)	GTGACCTGTGGATGCTGAGG CCAAAGGCTCTTCTCAGAGCCACCCA
gSatIII	dCas13b	GATTCCAATCCATGCCATTCCAC GUUGUG-GAAGGUCCAGUUUUGAGGGGCCUAUUACAAC
	CIRTS(3)	GATTCCAATCCATGCCATTCCAC GGCCAGATCTGAGCCTGG-GAGCTCTCTGGCC
	CIRTS(10)	GATTCCAATCCATGCCATTCCAC CCAAAGGCTCTTCTCAGAGCCACCCA

Modular structures of sgRNA (spacer sequences in black, dCas13 sgRNA hairpin in blue, TBP sgRNA hairpin in purple and SLBP sgRNA hairpin in gray).

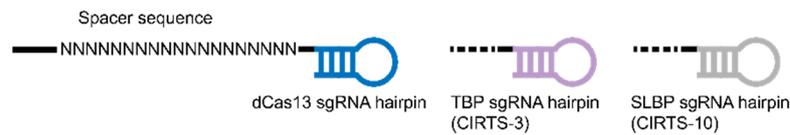
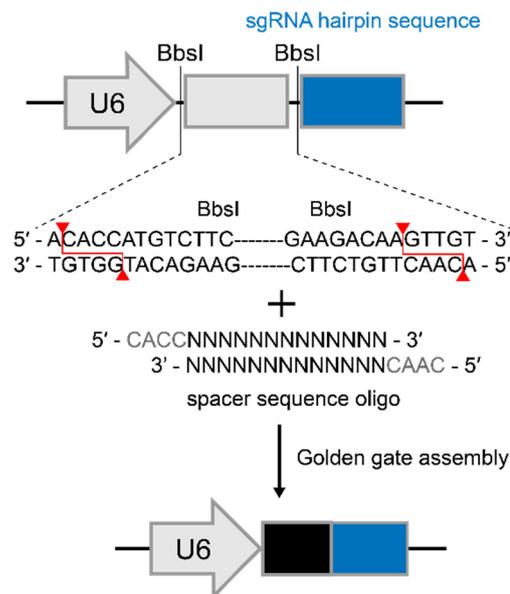


Table S3. *GCN4* smFISH probes (5'-3').

<i>GCN4</i> smFISH probe (5'-3')	
GCN4-sm-1	GCAGTTCTTCTCCACTGCCAGAA
GCN4-sm-2	GGTGGTAATTCTTTGAAAGCAG
GCN4-sm-3	GCTACCTCATTTCCAGGTGG



full sgRNA expression plasmids with spacer and hairpin sequences

Figure S1. Single-step cloning for sgRNA cassettes in Table S2 with guide sequence to label RNA target. Guide sequence oligo (black) was inserted after cutting of BbsI restriction enzyme to generate a seamless cloning of guide sequence in the upstream of sgRNA hairpin sequence (blue).

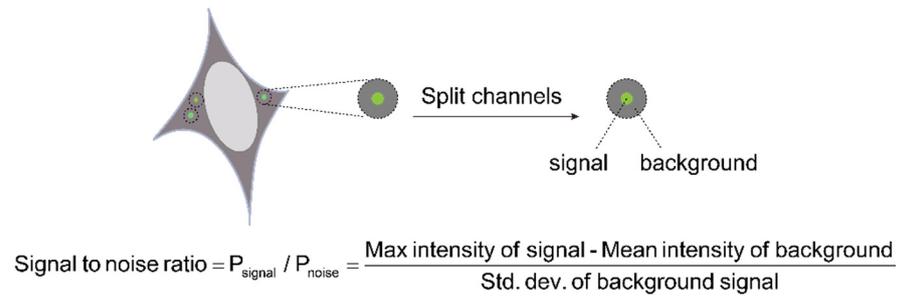


Figure S2. Schematic for the calculation of SNR.

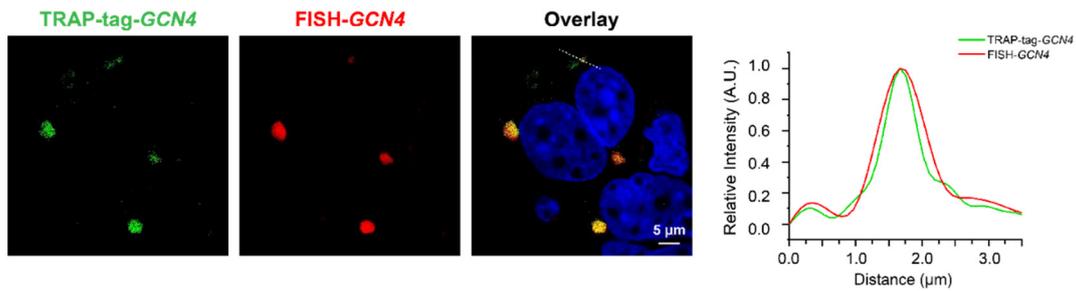


Figure S3. Colocalization analysis of TRAP-tag-GCN4 and smFISH signals in HEK 293T cells. The TRAP-tag composed by dCas13b-10xTat-2xNLS, sgRNA targeting GCN4 and TAR-Broc coli are expressed in the cells to visualize GCN4. Left, image of the GCN4 foci. Right, line scan of the relative fluorescence intensity of the signal indicated as the dotted line in the image on the left.

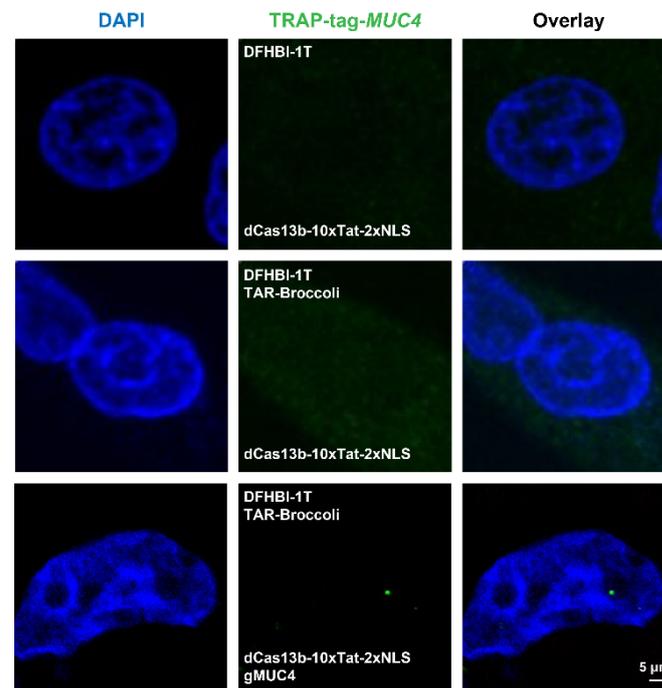


Figure S4. Labeling endogenous *MUC4* mRNAs by the CRISPR TRAP-tag, including control groups of Figure 3b. First row, dPspCas13b-10xTat-2xNLS were transfected to the cells; Second row, the cells were co-transfected with pdPspCas13b-10xTat-2xNLS and pTAR-Broc coli. Bottom row, co-transfection of pdPspCas13b-10xTat-2xNLS, pgMUC4 and pTAR-Broc coli was carried out. The nucleus was dyed by DAPI. Scale bar is shown in the figure. The cell was treated with DFHBI-1T (10 μM) before imaging.

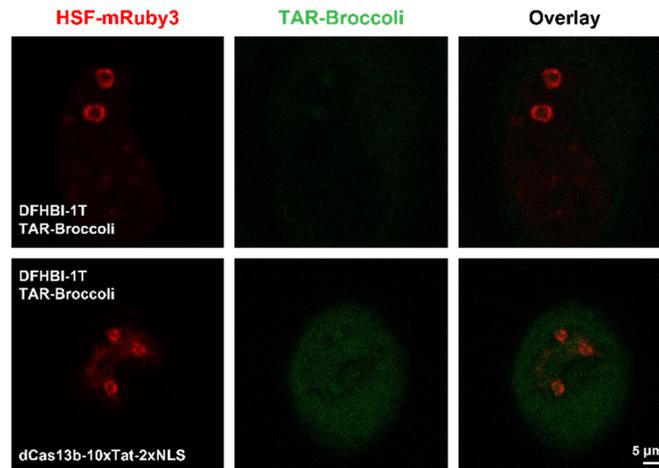


Figure S5. Representative images of HSF1-mRuby3 (red) and TAR-Broccoli (green) in HEK 293T cells upon SA (100 μ M, 6 h) treatment. Top row, cell expressing HSF-mRuby3 is transfected with TAR-Broccoli and treated by DFHBI-1T(10 μ M) to image after SA treatment, as a blank control of dCas protein for the experiment in Figure 3d. Bottom row, dPspCas13b-10xTat-2xNLS and TAR-Broccoli (without sgRNA of dCas13b targeting *SatIII*) were transfected to the cells expressing HSF-mRuby3, followed by SA and DFHBI-1T treatments, as a blank control of sgRNA for the experiment in Figure 3d.

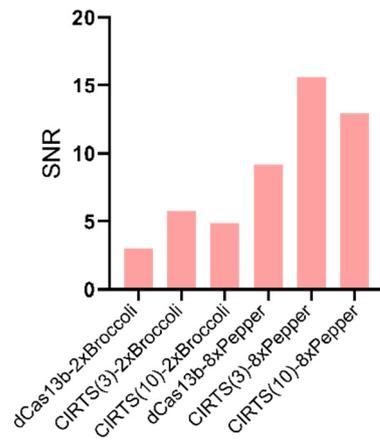


Figure S6. Signal-to-Noise Ratio (SNR) in Figure 6. The labels below the x-axis refer to the components of TRAP-tag, indicating the dCas protein and apta.