

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	UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCCCCACAUACUCUGAU- GAUCCGAGACGGUGCGGUCAGAUUAUUCGUAUCUGUCGAGUAGAGUGU GGGCUCGGAUCAUUC AUGGCAA
TAR-2xBroccoli	UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCCCCACAUACUCUGAU- GAUCC ¹ GAGACGGUGCGGUGCCAUCU ² GAGACGGUGCGGUGCCAGAUUAUUC GUAUCUGUCGAGUAGAGUGUGGGCUCAGA ¹ UGUCGAGUAGAGUGUG- GGCUCGGAUCAUUC AUGGCAA
TAR-Pepper	UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCCCCACAUACUCUGAU- GAUCCCAUUCGUGGCGUGUCGGCCUGCUUCGGCAGGCACUGGCGCC GGAUCAUUC AUGGCAA
TAR-8xPepper	UUGCCAUGUGUAUGUGGGGGCUCGUGUAGCUCAU- UAGCUCCGAGCCCCACAUACUCUGAU- GAUCC ¹ CAAUCGUGGCGUGUCGGCCUCUC ² CAAUCGUGGCGUGUCGG CCUCUC ³ CAAUCGUGGCGUGUCGGCCUCUC ⁴ CAAUCGUGGCGUG- UCGGCCUCUC ⁵ CAAUCGUGGCGUGUCGGCCUCUC ⁶ CAAUCGUG- GCGUGUCGGCCUCUC ⁷ CAAUCGUGGCGUGUCGGCCU- CUC ⁸ CAAUCGUGGCGUGUCGGCCUCUCUUCGGA- GAGGCACUGGCGCCGGAG ⁷ AGGCACUGGCGCCGGAG ⁶ AG- GCACUGGCGCCGGAG ⁵ AGGCACUGGCGCCGGAG ⁴ AGGCACUGGCGCCG- GAG ³ AGGCACUGGCGCCGGAG ² AGGCACUGGCGCCGGAG ¹ AG- GCACUGGCGCCGGGAUCAUUC AUGGCAA

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).

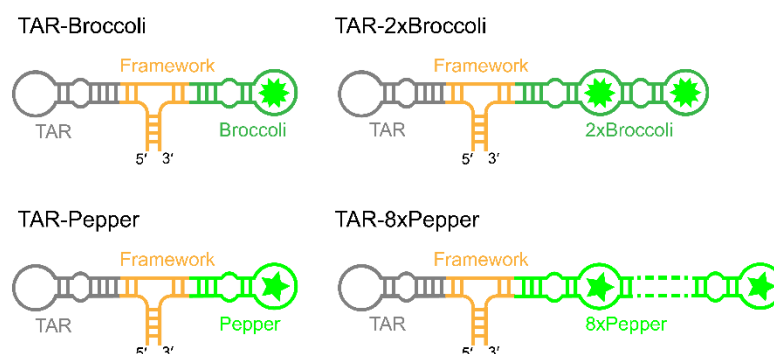


Table S2. Sequences of sgRNA, targeting *GCN4*, *MUC4* and *SatIII*. Sequences are showed in the corresponding color of modules in the figures below (spacer sequences in black, dCas13 sgRNA hairpin in blue, TBP sgRNA hairpin in purple and SLBP sgRNA hairpin in gray).

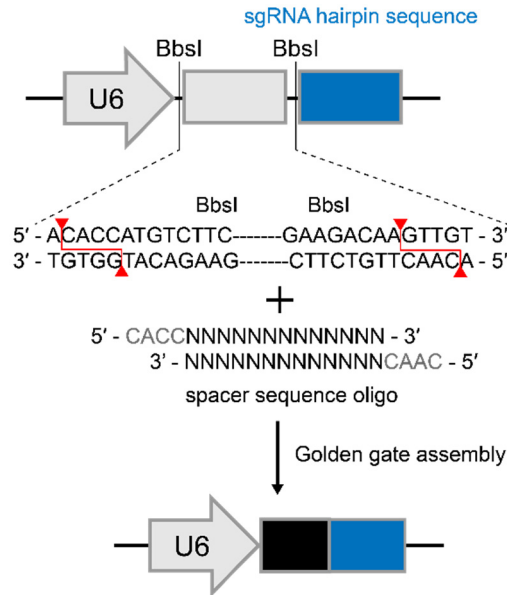
sgRNA sequences (5'-3')	
dCas13b	GGTGGTAATTCTTTGAAAGCAGGUUGUG- GAAGGUCCAGUUUUGAGGGGCCUAUUACAAC
gGCN4	CIRTS(3) GGTGGTAATTCTTTGAAAGCAGGGCCAGATCTGAGCCTGG- GAGCTCTCTGGCC
	CIRTS(10) GGTGGTAATTCTTTGAAAGCAGCCAAAGGCTCTTCTCAGAGCCACCCA
	dCas13b GTGACCTGTGGATGCTGAGGGUUGUG- GAAGGUCCAGUUUUGAGGGGCCUAUUACAAC
gMUC4	CIRTS(3) GTGACCTGTGGATGCTGAGGGGCCAGATCTGAGCCTGG- GAGCTCTCTGGCC
	CIRTS(10) GTGACCTGTGGATGCTGAGGGCCAAAGGCTCTTCTCAGAGCCACCCA
	dCas13b GATTCCAATCCATGCCATTCCACGUUGUG- GAAGGUCCAGUUUUGAGGGGCCUAUUACAAC
gSatIII	CIRTS(3) GATTCCAATCCATGCCATTCCACGGCCAGATCTGAGCCTGG- GAGCTCTCTGGCC
	CIRTS(10) GATTCCAATCCATGCCATTCCACCCAAAGGCTCTTCTCAGAGCCACCCA

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').

GCN4 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).

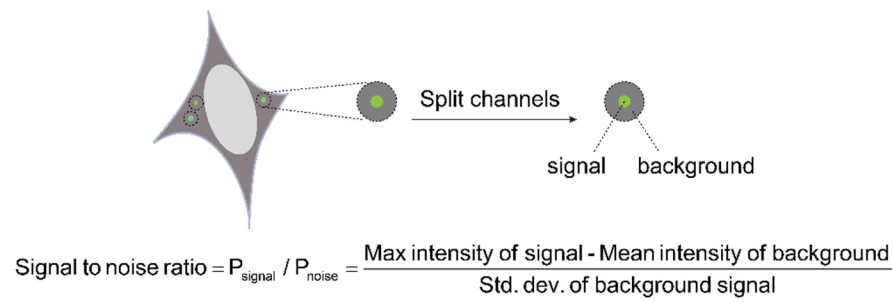


Figure S2. Schematic for the calculation of SNR.

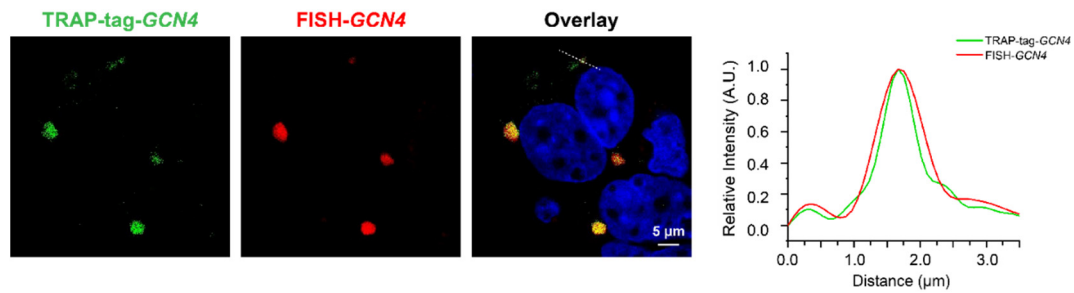


Figure S3. Colocalization analysis of TRAP-tag-GCN4 and smFISH signals in HEK 293T cells. The TRAP-tag composed by dCas13b-10xTat-2xNLS, sgRNA targeting GCN4 and TAR-Broccoli are expressed in the cells to visualize GCN4. Left, image of the GCN4 foci. Right, line scan of the relative fluorescence intensity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

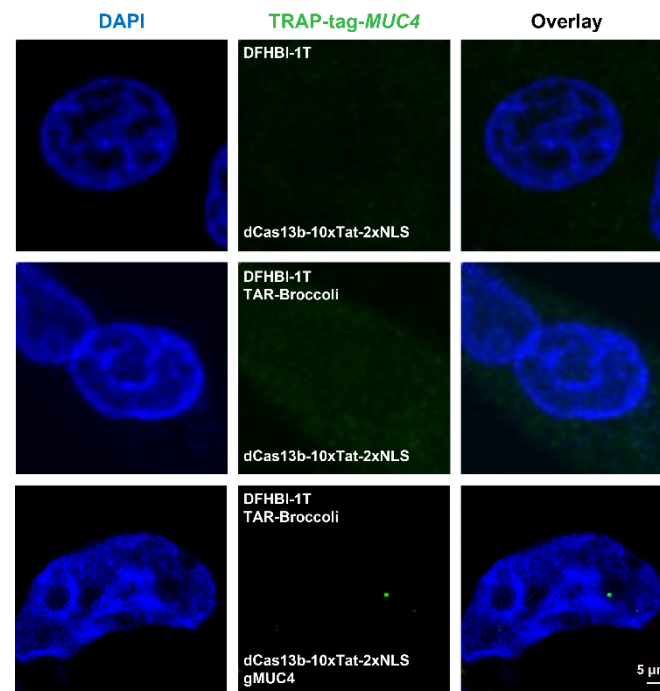


Figure S4. Labeling endogenous *MUC4* mRNAs by the CRISPR TRAP-tag, including control groups of Figure 3b. First row, dPspCas13b-10xTat-2xNLS were transfected to the cells; Second row, the cells were co-transfected with pdPspCas13b-10xTat-2xNLS and pTAR-Broccoli. Bottom row, co-transfection of pdPspCas13b-10xTat-2xNLS,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

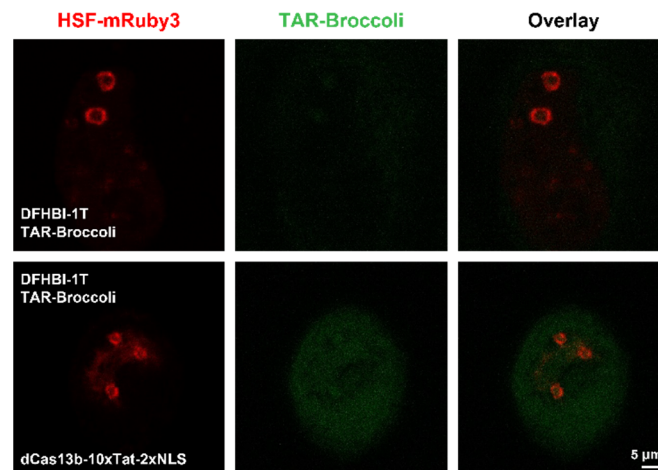


Figure S5. Representative images of HSF1-mRuby3 (red) and TAR-Broccoli (green) in HEK 293T cells upon SA (100 μ M, 6 h) treatment. Top row, cell expressing HSF1-mRuby3 is transfected with TAR-Broccoli and treated by DFHBI-1T(10uM) to image after SA treatment, as a blank control of dCas protein for the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

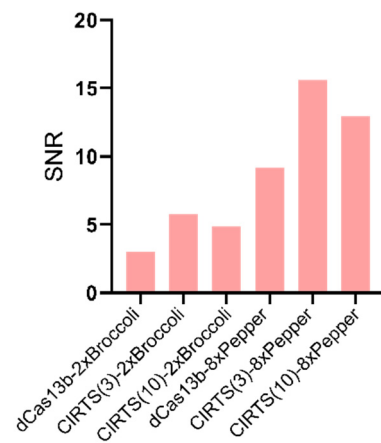


Figure S6. Signal-to-Noise Ratio (SNR) in Figure 6. The labels below the x-axis refer to the components of TRAP-tag, indicating the dCas protein and apta.