Switched Fluorogenic CRISPR-tDeg System for RNA Imaging in Living Cells

Huimin Zhong[1,2], Jiayan Zhou[1,2], Fujun Qin[2], Xian-En Zhang[3,4,*], Minghai Chen[2,*]

[1] College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

[2] State Key Laboratory of Quantitative Synthetic Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

[3] Faculty of Synthetic Biology, Shenzhen University of Advanced Technology, Shenzhen 518107, China

[4] National Laboratory of Biomacromolecules, Key Laboratory of Biomacromolecules (CAS), Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

[*]Corresponding author: Minghai Chen, Email: mh.chen1@siat.ac.cn or Xian-En Zhang, Email: zhangxe@ibp.ac.cn

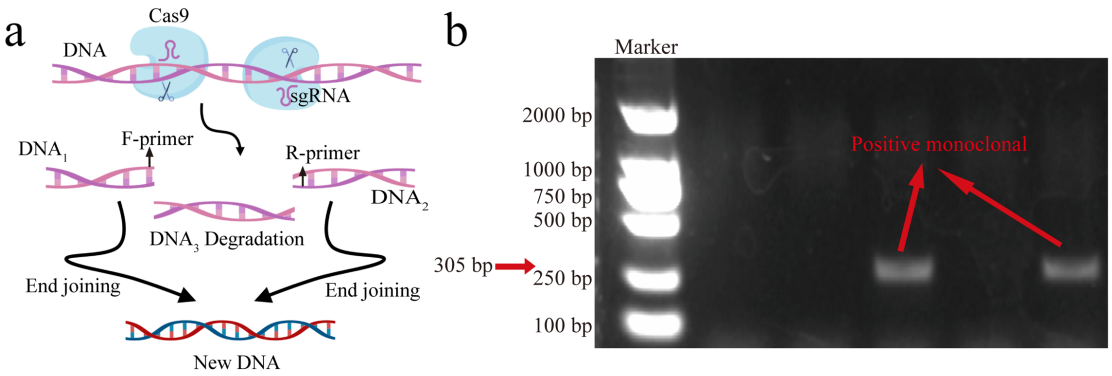

Fig. S1. The construction of the gene knockout (KO) cell lines. (a) Schematic diagram of nonhomologous end joining (NHEJ). (b) PCR gel plot showing positive KO monoclonal in (a).

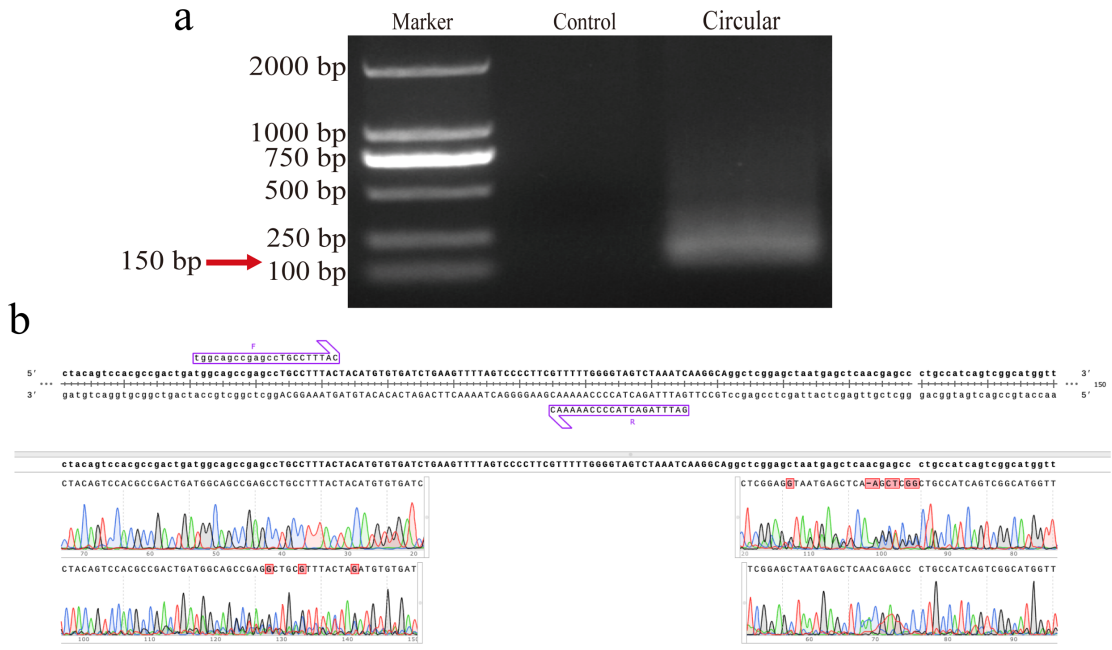

Fig. S2. The development of the circular switched sgRNA. (a) Gel electrophoresis to demonstrate the engineered sgRNA circular structure. (b) Sequencing results confirming engineered sgRNA circular structure.

Original tDeg	S	G	P	R	P	R	G	T	R	G	K	G	R	R	I	R	R	R	G								
Y1	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	H	Y	C	F	G								
Y2	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	H	Y	K	K	V	G	T	M	A	A	G	
Y3	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	H	Y	K	K	V	G	T	M	R	G	R	G	L
Y4	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	N	Y	K	S	G								
Y5	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	*	Y	*	*	N								
Y6	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	*	Y	*	R	G								
Y7	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	*	Y	*	R	G	N							
Y8	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	H	Y	K	K	V	G	M	G	R	K			
I1	*	*	*	*	*	*	*	*	*	*	*	*	*	L	*	*	Y	T	Y	C	G	L						
I2	*	*	*	*	*	*	*	*	*	*	*	*	*	L	*	*	V	T	Y	C	G	L						
I3	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	L	L	G	*									
I4	*	*	*	*	*	*	*	*	*	*	*	*	*	K	*	A	A	E	*									
g1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	M	G	G							
g2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	V	Q	W	G							
g3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	W	*	R	R	G						
g4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	P	C	Q	R	G						
g5	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	P	R	Q	P	S	R	D	G		
g6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	P	S	S	G	G					
g7	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	S	*	L	R	R	R	R	H	R	G	E
g8	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	L	T	G	F	S	G	M	K	G		
g9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	Q	R	S	R	D	G					
g10	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	H	W	R	G	Q	E	G				
G1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	L								
G2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	L	L	*	G								
G3	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	H	G	G										
G4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	M	*	G									
G5	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	P	*	K	Q	G								
G6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	V	Q	W	G									
G7	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	W	G	R	R	G								
G8	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	P	C	Q	R	G								
G9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	P	R	Q	P	S	R	D	G				
G10	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	P	S	S	G	G							
G11	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	P	S	S	G	G						
G12	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	S	G	L	R	R	R	R	H	R	G	E		
G13	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	L	T	*	F	S	G	M	K	G				
G14	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	Q	*	S	R	D	G							
G15	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	H	W	R	G	Q	E	G						
G16	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	A	E	*									

Fig. S3. Sequence information of various mutant variants of tDeg.

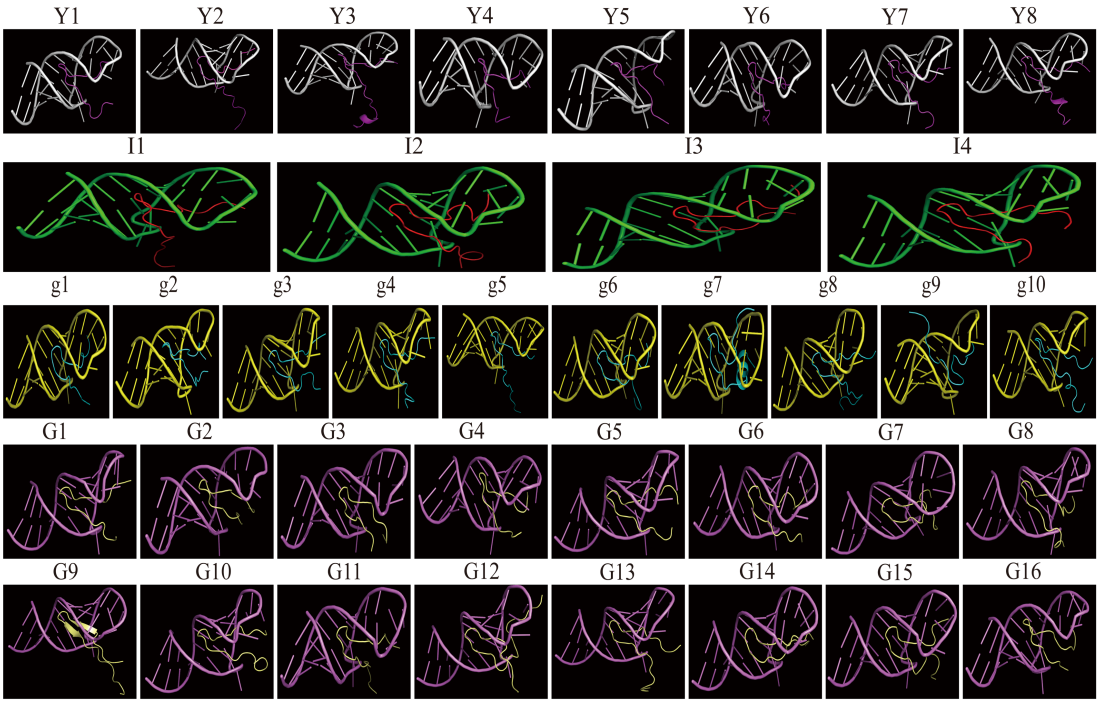

Fig. S4. Prediction the structure of mutant variants of tDeg in combination with pepper using AlphaFold3.

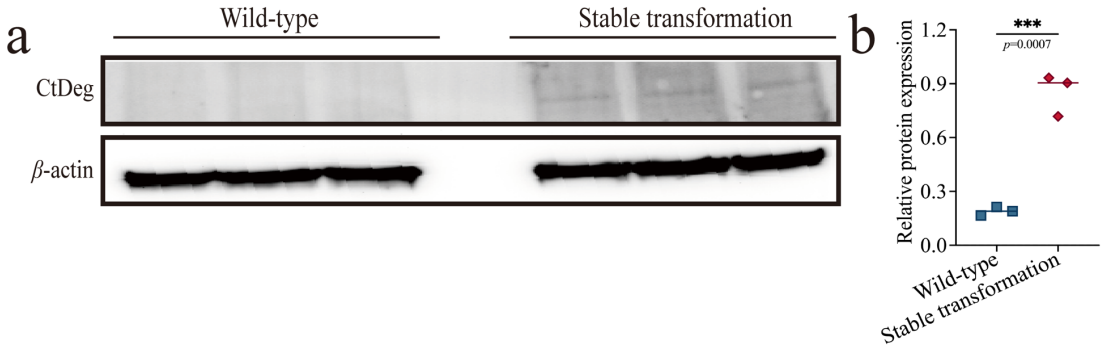

Fig. S5. Construction of the CtDeg expressing stable cell lines. (a) Western blotting analysis demonstrates the expression of the target protein (CMV-GFP $_{(1\text{-}10)}$-MiniCMV-GFP$_{11}$-sfGFP-d*Lwa*Cas13a-tDeg (G3)) in stable cell lines (Vero E6). (b) Statistics of target protein expression in wild type cells and stably transformed cells. Statistical analyses were performed using a t-test. *** indicates *p*-value < 0.001.

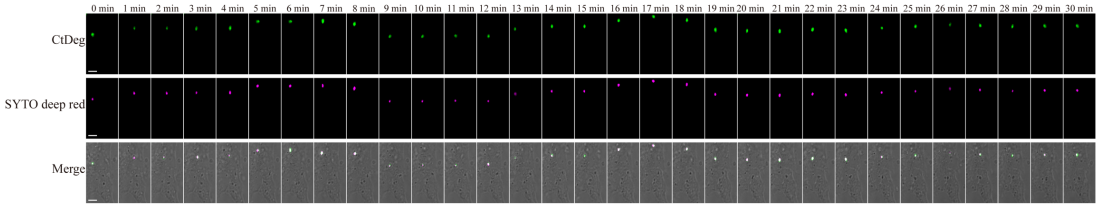

Fig. S6. Real-time microscopic images show the position of SARS-CoV-2 genomic RNA at different time points, captured at 1-minute intervals. Scale bars: 5 μm.

Video S1. Fusion/kiss and run events of paraspeckles labeled by CtDeg were tracked over an 11-minute period at 1-minute intervals.

Video S2. The motion events of SARS-CoV-2 genomic RNA in the cytoplasm labeled by CtDeg and SYTO deep red was tracked over a 30-minute period at 1-minute intervals.

Video S3. The motion events of SARS-CoV-2 genomic RNA in the cytoplasm labeled by CtDeg and SYTO deep red was tracked over a 15-minute period at 3-minute intervals.