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Supplemental Information

BEON: A Functional Fluorescence Reporter for Quantification and Enrichment of Adenine Base-Editing Activity

Peipei Wang, Li Xu, Yandi Gao, and Renzhi Han

Supplementary Information

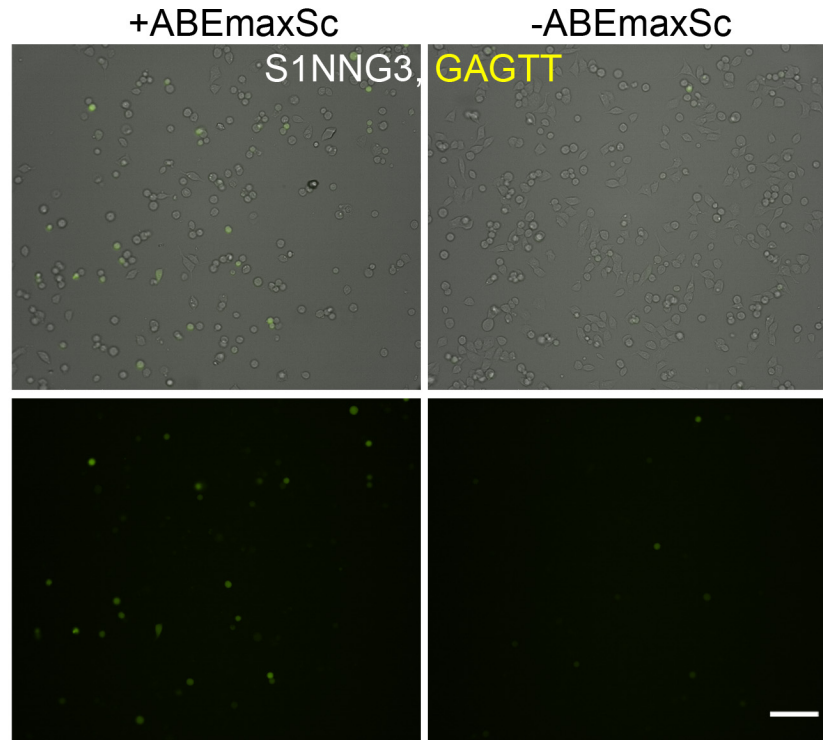
Supplementary Table I. Oligos for PCR and construction of gRNAs and reporters

Purpose	Name	Sequences (5' → 3')
gRNAs	S1gRNA-F	ACCGATGACAGGCAGGGGCACCG
	S1gRNA-R	AAACCGGTGCCCCTGCCTGTCAT
	DYSF_Q605X_gF	ACCGATCCTACAGCATGGTGGCTG
	DYSF_Q605X_gR	AAACCAGCCACCATGCTGTAGGAT
	DYSF_R377X_gF	ACCGCCTCACAGGGCTACGCCTGT
	DYSF_R377X_gR	AAACACAGGCGTAGCCCTGTGAGG
	DYSF_Q1278X_gF	ACCGTCTCTAGATGAGCTCAAAG
	DYSF_Q1278X_gR	AAACCTTTTGAGCTCATCTAGAGA
	CAR-gF	ACCGCTTTAATGCGCTGACTTGTG
	CAR-gR	AAACCACAAGTCAGCGCATTAAG
	mTmem5-gF	ACCGACTTACGTTGCTACACCTAA
	mTmem5-gR	AAACTTAGGTGTAGCAACGTAAGT
PCR	S1-F	TTCCAGTGGTTC AATGGTCA
	S1-R	CTTTCAACCCGAACGGAGAC
	puro-F	AGTGGTCTCCGAAACCTCCGCGCCCCGCAAC
	EGFP_R	GTAGGTCAGGGTGGTCACGA
	CAR-F	CCCTCTGTTATGCCACCAGT
	CAR-R	ACTCAGGAGGCTGAAGTGGA
	mTmem5-F	GAAGAGGGCAAATCCAACA
	mTmem5-R	TTGCTTGAAATGAGCACTG
	Reporters	S1-repF
S1-repR		GATCCACTCCGCGGTGCCCCTGCCTGTCATCCG
S1NNG-F		AATTCGGATGACAGGCAGGGGCACCGAAGTTTG
S1NNG-R		GATCCAAACTTCGGTGCCCCTGCCTGTCATCCG
S1NNG2-F		AATTCGGATGACAGGCAGGGGCACCGCAGTTTG
S1NNG2-R		GATCCAAACTGCGGTGCCCCTGCCTGTCATCCG
S1NNG3-F		AATTCGGATGACAGGCAGGGGCACCGGAGTTTG
S1NNG3-R		GATCCAAACTCCGGTGCCCCTGCCTGTCATCCG
S1NNG4-F		AATTCGGATGACAGGCAGGGGCACCGATGTTTG
S1NNG4-R		GATCCAAACATCGGTGCCCCTGCCTGTCATCCG
S1NNG5-F		AATTCGGATGACAGGCAGGGGCACCGCTGTTTG
S1NNG5-R		GATCCAAACAGCGGTGCCCCTGCCTGTCATCCG
S1NNG6-F		AATTCGGATGACAGGCAGGGGCACCGGTGTTTG
S1NNG6-R		GATCCAAACACCGGTGCCCCTGCCTGTCATCCG
S1NNG7-F		AATTCGGATGACAGGCAGGGGCACCGTTGTTTG
S1NNG7-R		GATCCAAACAACGGTGCCCCTGCCTGTCATCCG
S1NNG8-F		AATTCGGATGACAGGCAGGGGCACCGACGTTTG
S1NNG8-R		GATCCAAACGTCGGTGCCCCTGCCTGTCATCCG
S1NNG9-F		AATTCGGATGACAGGCAGGGGCACCGCCGTTTG
S1NNG9-R		GATCCAAACGGCGGTGCCCCTGCCTGTCATCCG
S1NNG10-F	AATTCGGATGACAGGCAGGGGCACCGGCGTTTG	

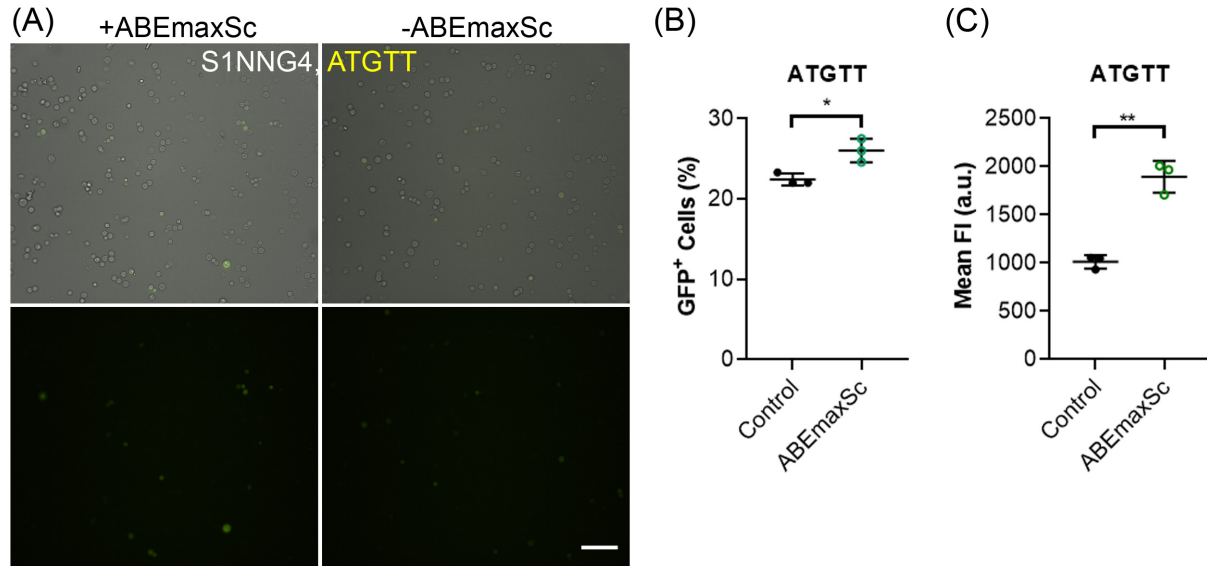
S1NNG10-R	GATCCAAACGCCGGTGCCCCCTGCCTGTCATCCG
S1NNG11-F	AATTCGGATGACAGGCAGGGGCACCGTCGTTTG
S1NNG11-R	GATCCAAACGACGGTGCCCCCTGCCTGTCATCCG
DYSF_Q605X_repF	AATTCTACTCAGCCACCATGCTGTAGGATGTGG
DYSF_Q605X_repR	GATCCCACATCCTACAGCATGGTGGCTGAGTAG
DYSF_R377X_repF	AATTCGGGCCACAGGCGTAGCCCTGTGAGGAGCCG
DYSF_R377X_repR	GATCCGGCTCCTCACAGGGCTACGCCTGTGGGCCGG
DYSF_Q1278X_repF	AATTCCTGGCCTCTTTTGAGCTCATCTAGAGAGAGG
DYSF_Q1278X_repR	GATCCCTCTCTCTAGATGAGCTCAAAAGAGGCCAGG
CAR-rep-F	AATTCCTTTAATGCGCTGACTTGTGTGGGG
CAR-rep-R	GATCCCCCACACAAGTCAGCGCATTAAAGG
mTmem5-rep-F	AATTCTGGCCTTTAGGTGTAGCAACGTAAGTACAAG
mTmem5-rep-R	GATCCTTGTACTIONTACGTTGCTACACCTAAAGGCCAG

Supplementary Table II. List of plasmids used in this study

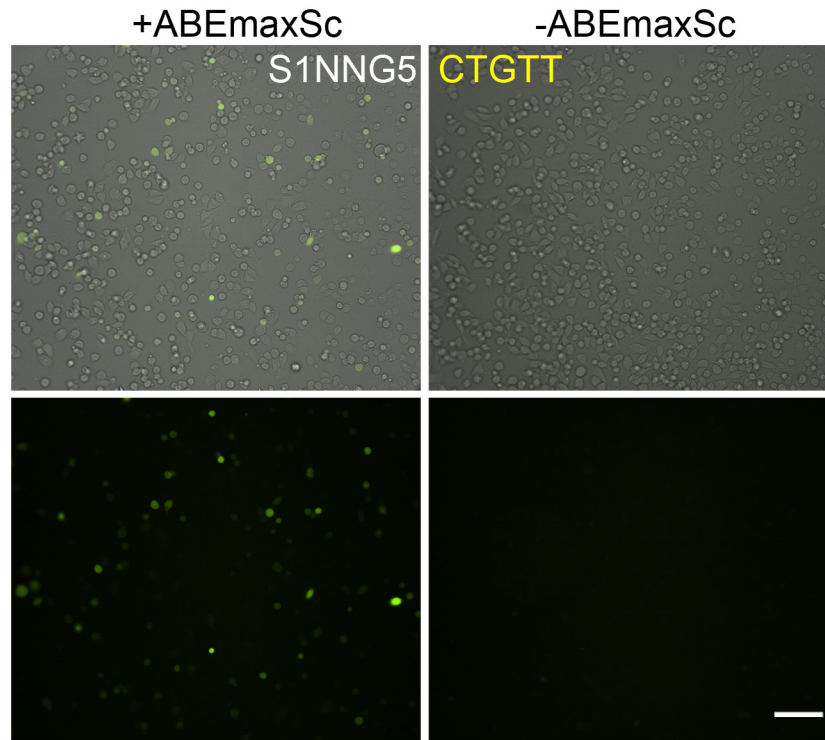
Purpose	Name	Information
gRNAs	pLenti-S1-ogRNA	S1 optimized gRNA
	pLenti-Q605X-ogRNA	Q605X optimized gRNA
	pLenti-R377X-ogRNA	R377X optimized gRNA
	pLenti-Q1278X-ogRNA	Q1278X optimized gRNA
	pLenti-CAR-ogRNA	CAR optimized gRNA
	pLenti-mTmem5-ogRNA	mTmem5 optimized gRNA
ABEs	ABE7.10	ABE based on SpCas9
	ABEmax	ABE based on codon optimized SpCas9
	xABE	ABE based on xCas9(3.7)
	ScCas9-ABE	ABE based on codon optimized ScCas9
	ABE-NG	ABE based on codon optimized SpCas9-NG
Reporters	pLKO-puro-E2A-GFP	Empty reporter
	pLKO-puro-S1-E2A-EGFP	S1 reporter
	pLKO-puro-Q605X-E2A-EGFP	Dysferlin Q605X reporter
	pLKO-puro-R377X-E2A-EGFP	Dysferlin R377X reporter
	pLKO-puro-Q1278X-E2A-EGFP	Dysferlin Q1278X reporter
	pLKO-puro-S1NNG(1-11)-E2A-EGFP	S1NNG (1-11) reporters
	pLKO-puro-CAR-E2A-EGFP	CAR reporter
pLKO-puro-mTmem5-E2A-EGFP	mTmem5 reporter	



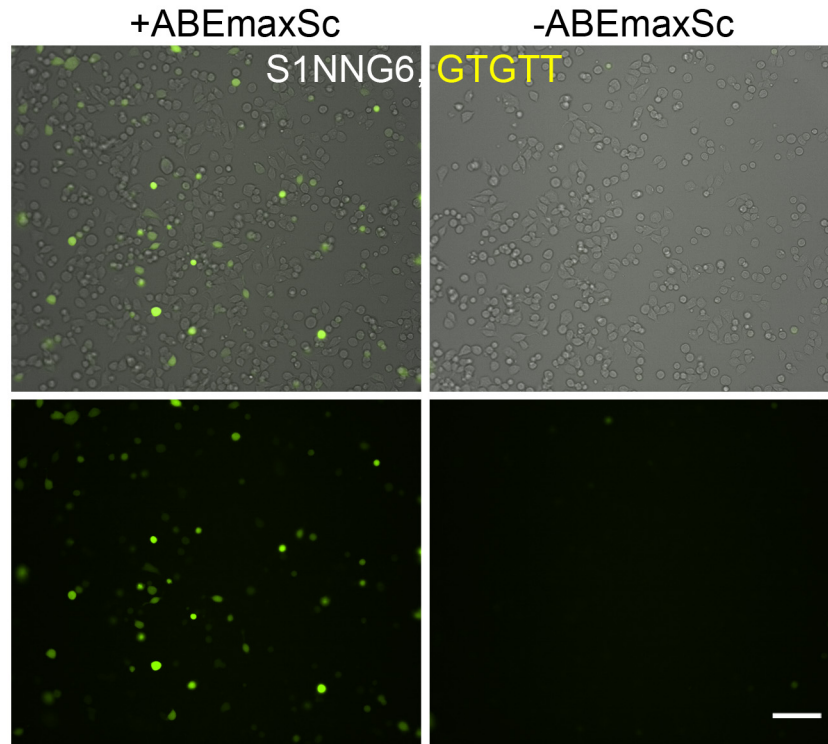
Supplementary Figure S1. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG3 (GAG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG3 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.



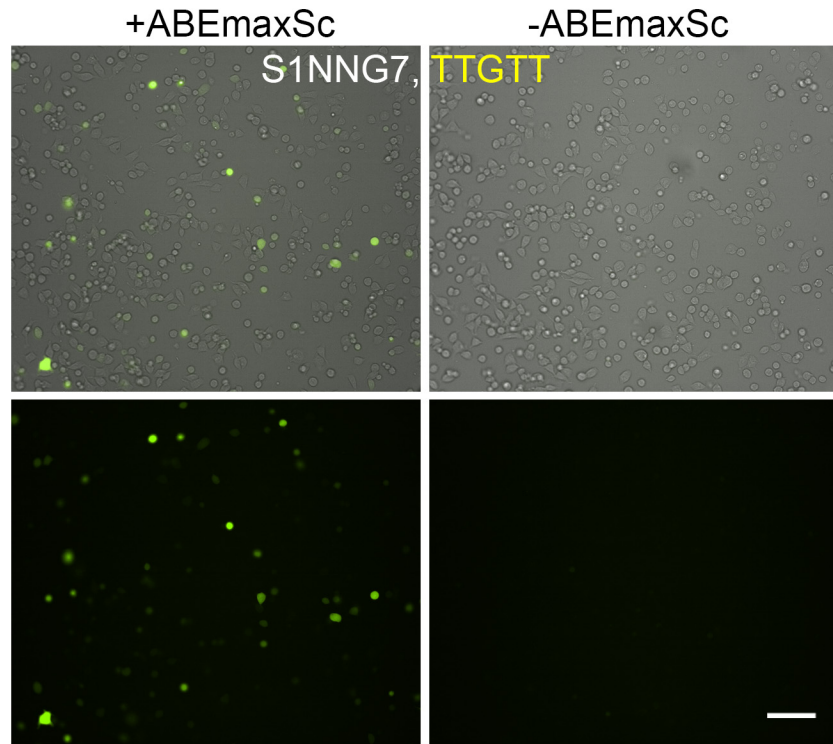
Supplementary Figure S2. (A) Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG4 (ATG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG4 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m. (B) FACS quantification of GFP⁺ cells for S1NNG4 reporter with or without ScCas9-ABE editing. * p <0.05. (C) FACS quantification of the geometric mean of GFP fluorescence intensity in GFP⁺ cells (out of a total 1×10^5 cells) for S1NNG4 reporter with or without ScCas9-ABE editing. ** p <0.01.



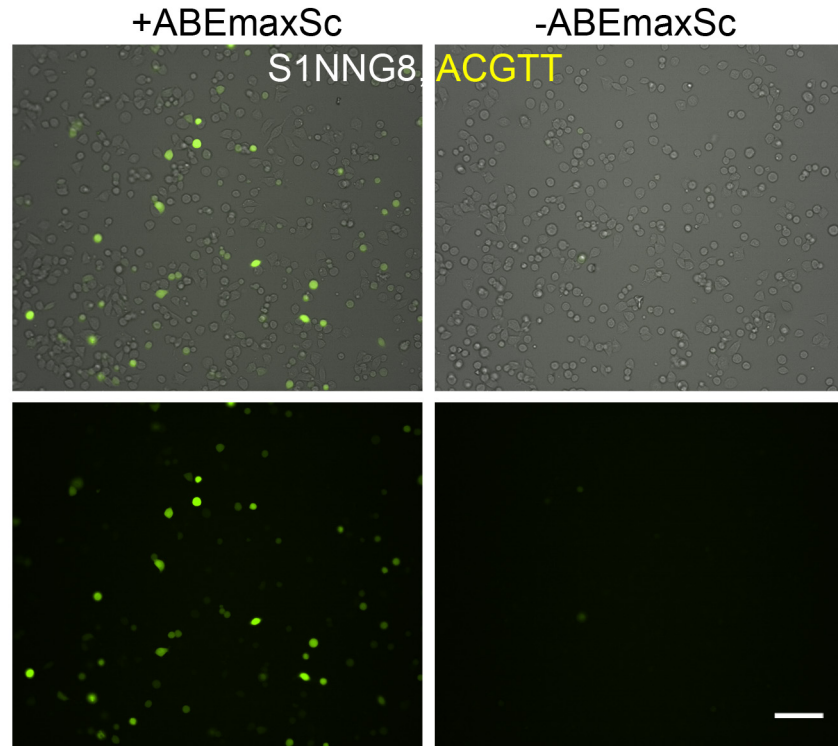
Supplementary Figure S3. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG5 (CTG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG5 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.



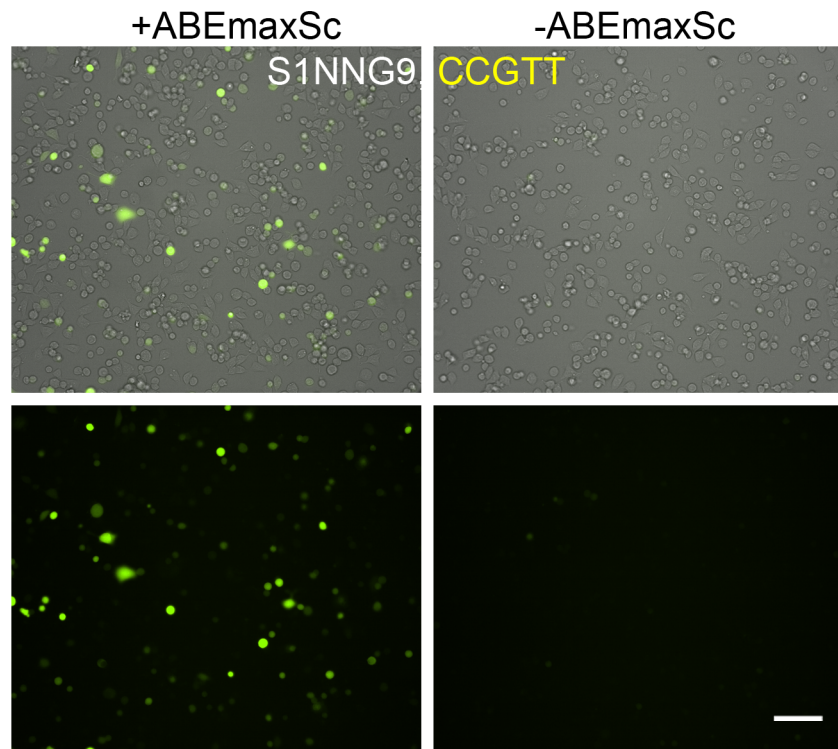
Supplementary Figure S4. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG6 (GTG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG6 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.



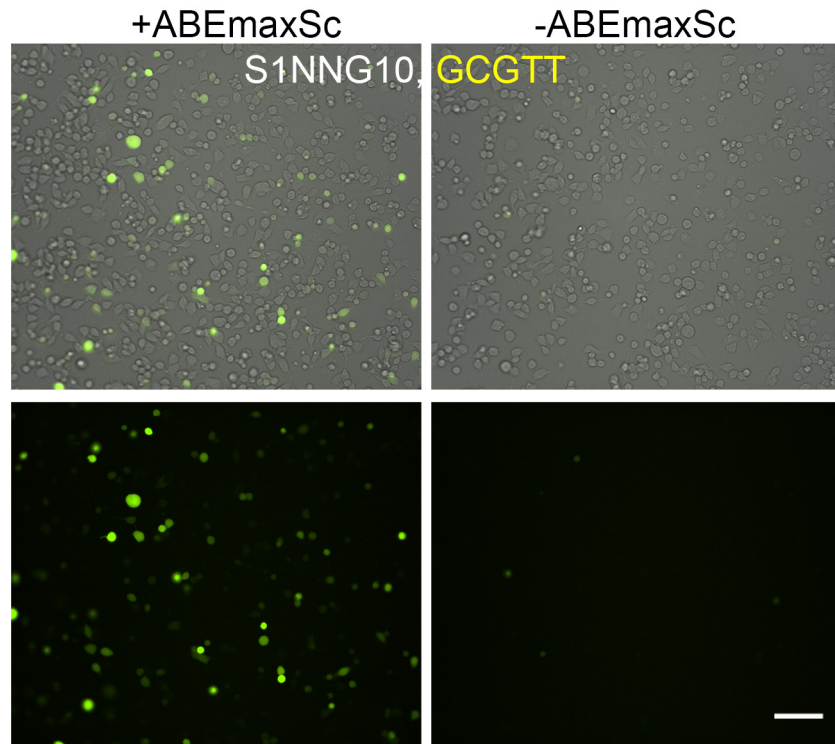
Supplementary Figure S5. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG7 (TTG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG7 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.



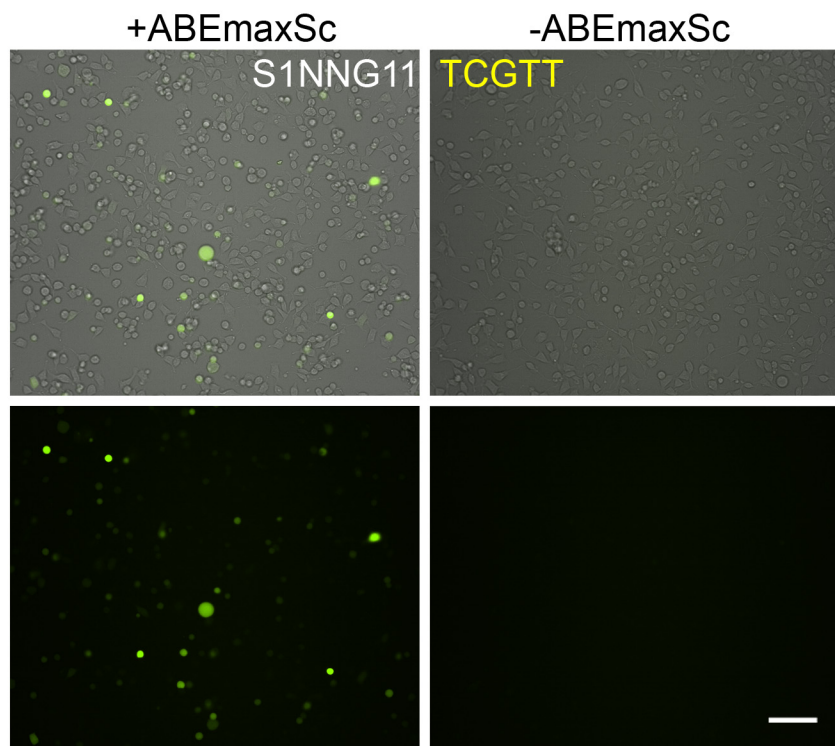
Supplementary Figure S6. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG8 (ACG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG8 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.



Supplementary Figure S7. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG9 (CCG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG9 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.



Supplementary Figure S8. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG10 (GCG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG10 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.



Supplementary Figure S9. Representative fluorescence and bright-field (merged) images of HEK293 cells transfected with the S1NNG11 (TCG)-reporter alone, or co-transfected with S1-gRNA, ScCas9-ABE and S1NNG11 reporter. The PAM sequences are listed in yellow. Scale bar: 100 μ m.