Supplementary Information

Human cell based directed evolution of adenine base editors with improved efficiency

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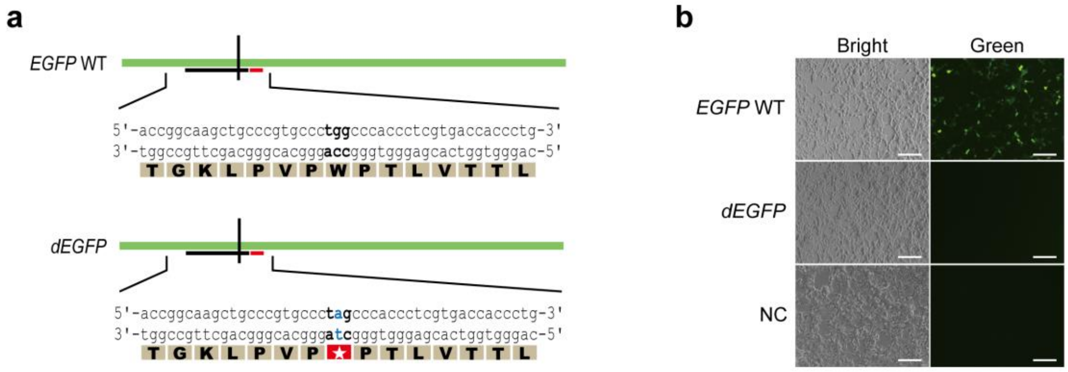
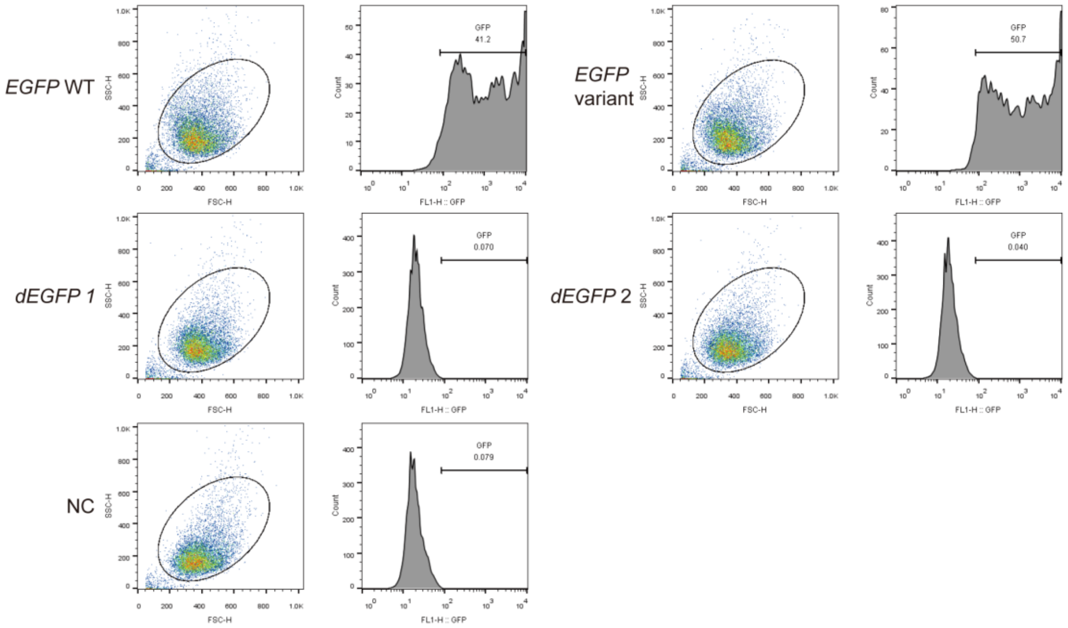


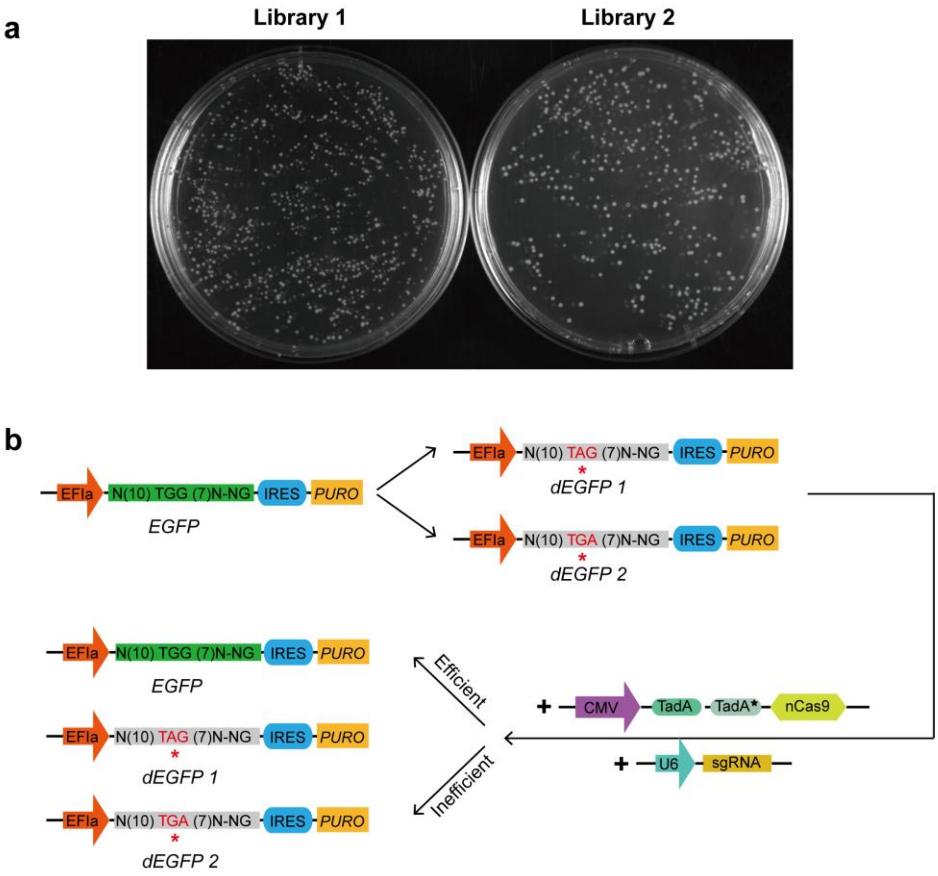
Supplementary Fig. 1 Engineering *EGFP* gene for ABE optimization.

a A single base mutation introducing stop codon (TAG) to inactivate *EGFP*. The stop codon was highlighted with red star. **b** HEK-293 cells were transfected with dead *EGFP* encoding plasmids (200 ng), and images were obtained at 48 hr post-transfection. NC represents the negative control. Scale bar, 10 μm.



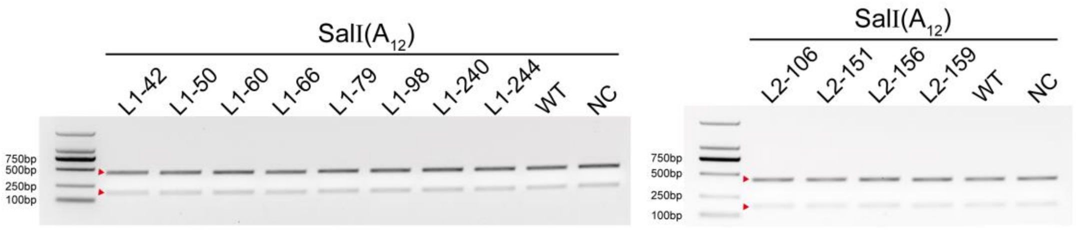
Supplementary Fig. 2 The fluorescence intensity of wild-type and engineered *EGFP*.

The HEK-293 cells were transfected with the plasmids coding for *EGFP* (wild-type) and *EGFP* mutants. The flow cytometry analysis was performed to obtain the percent of *EGFP* positive cells.



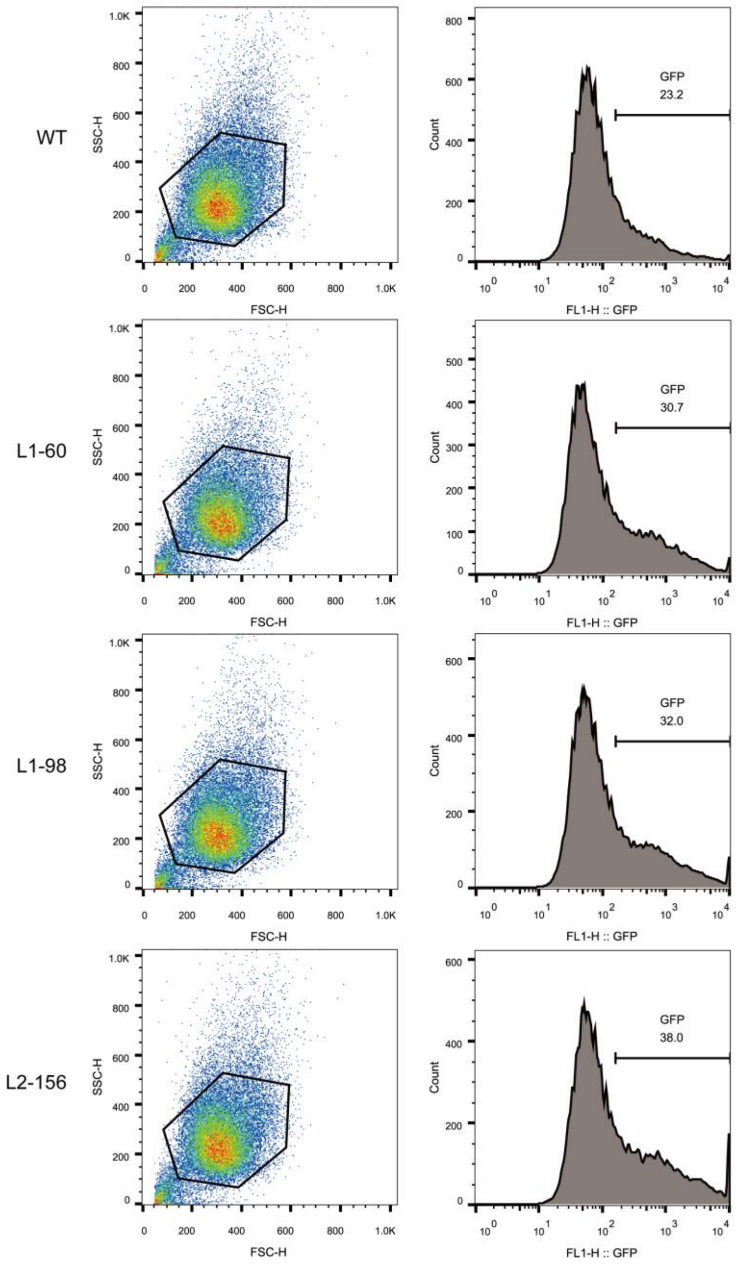
Supplementary Fig. 3 Generation and screening of the libraries.

a There are two libraries (library 1 and library 2) generated in this study. Each library contains two plates; here we only show one plate from each library. **b** The screening strategy of variants.



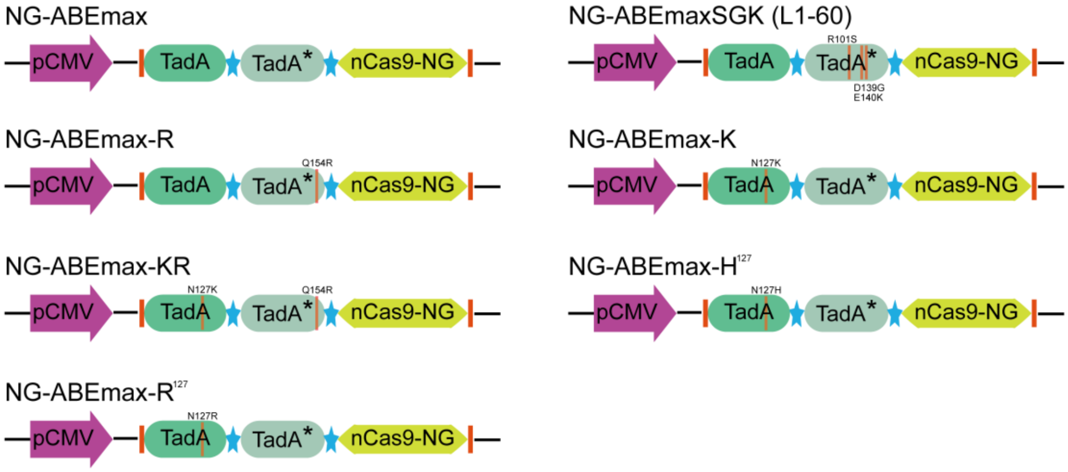
Supplementary Fig. 4 The editing efficiencies of ABE variants at A12.

Agarose gel electrophoresis results of testing editing efficiency of NG-ABEmax variants with SalI. Cleaved bands from SalI are labeled with red triangle. The amplicon is 606-bp. WT and NC represent wild-type NG-ABEmax and no plasmid, respectively.



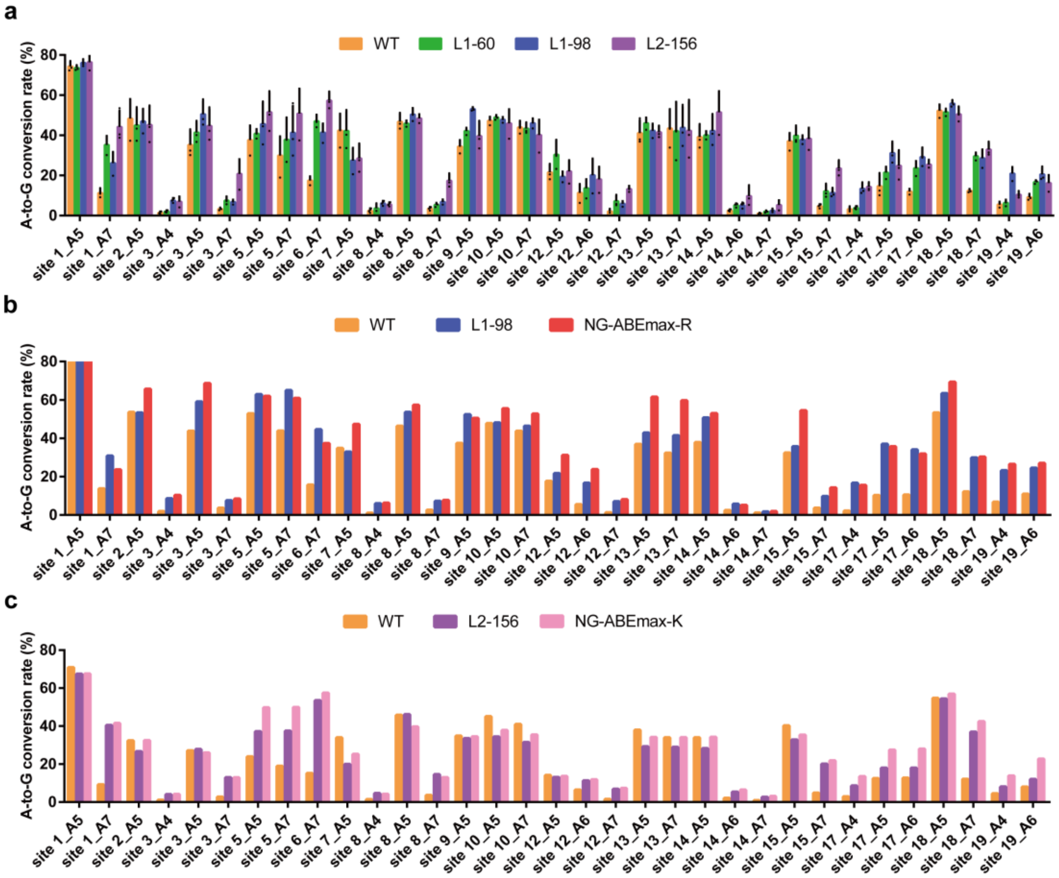
Supplementary Fig. 5 Flow cytometric analysis showing boosted editing efficiency of three ABE variants.

Flow cytometric analysis of three ABE variants at A7. GFP represents the positive signal (percentage).



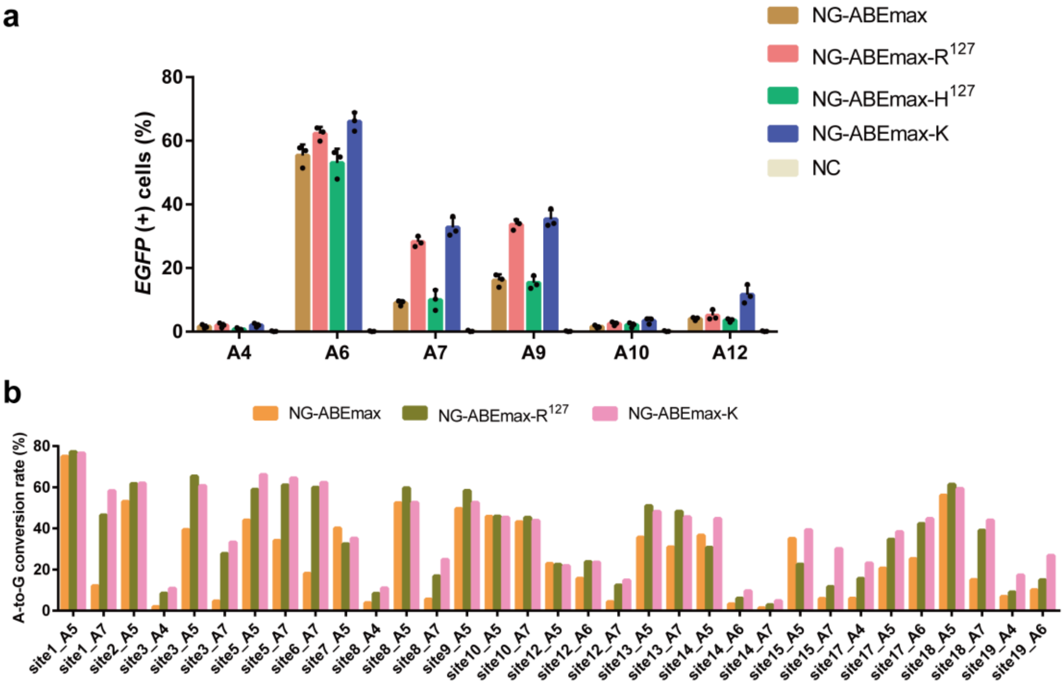
Supplementary Fig. 6 Schematic of NG-ABEmax variants structures.

Two small orange rectangular boxes and pentagrams represent nuclear localization signals and 32-AA linkers, respectively.



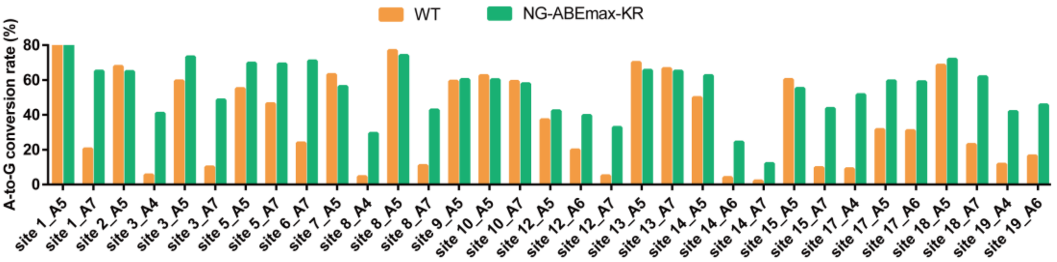
Supplementary Fig. 7 Comparison of the activity of NG-ABEmax and NG-ABEmax variants at endogenous genomic sites.

a Comparison of the activity of NG-ABEmax and NG-ABEmax variants (L1-60, L1-98, L2-156); Error bars indicate mean ± s.d. (*n* = 3 independent experiments). **b** Comparison of the activity of NG-ABEmax, L1-98 and NG-ABEmax-R; **c** Comparison of the activity of NG-ABEmax, L2-156 and NG-ABEmax-K. WT represents wild-type NG-ABEmax.



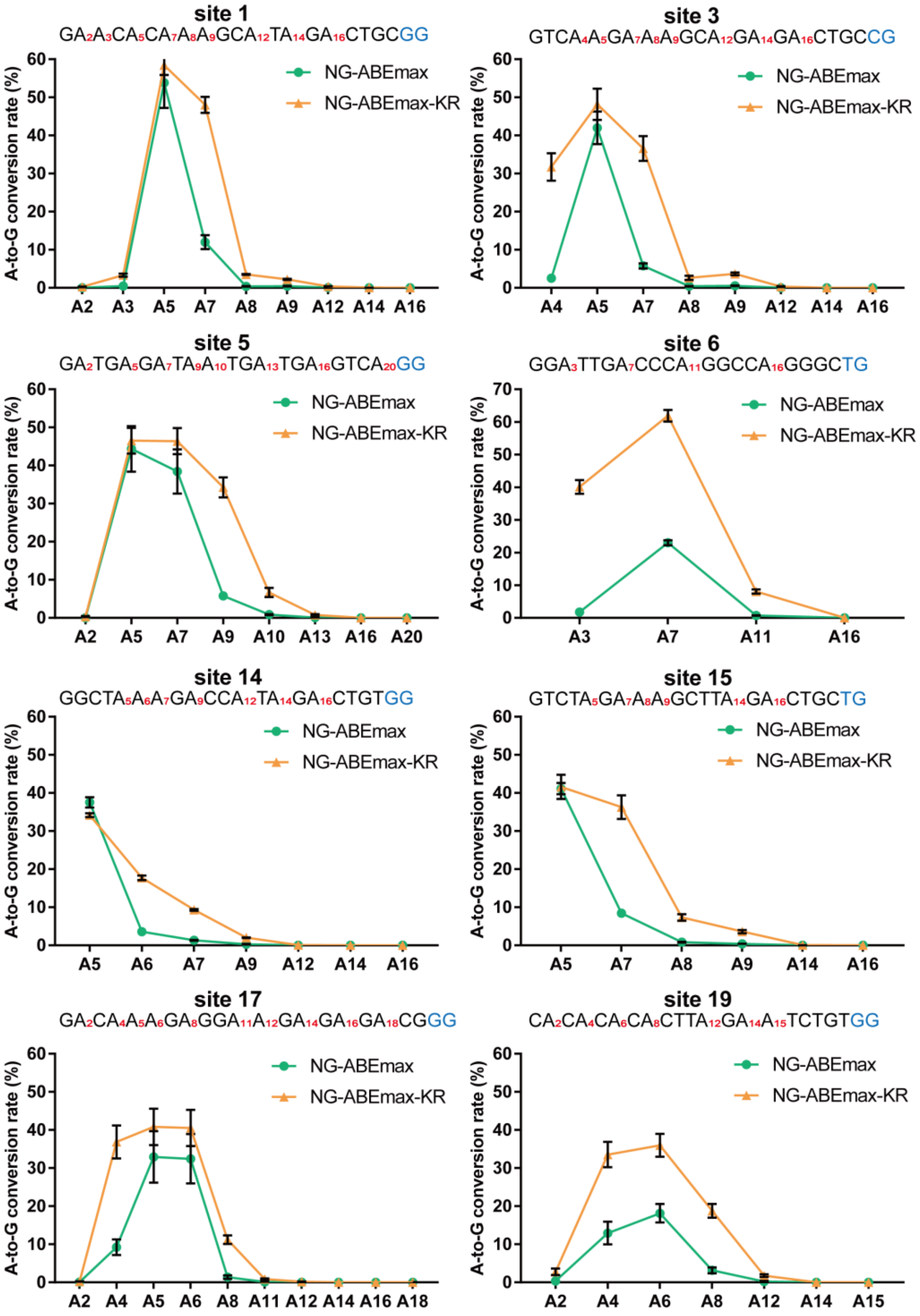
Supplementary Fig. 8 Variants harboring a strong basic residue at position 127 in TadA boosting the editing efficiency.

a The editing efficiencies of three variants harboring different basic residues (H, K and R) at position 127 in TadA via EGFP-based reporter system. EGFP positive cells were quantified by flow cytometry. NC represents the negative control. Error bars indicate mean ± s.d. (*n* = 3 independent experiments). **b** The editing efficiencies of NG-ABEmax, NG-ABEmax-R^{127} and NG-ABEmax-K and at endogenous genomic sites.



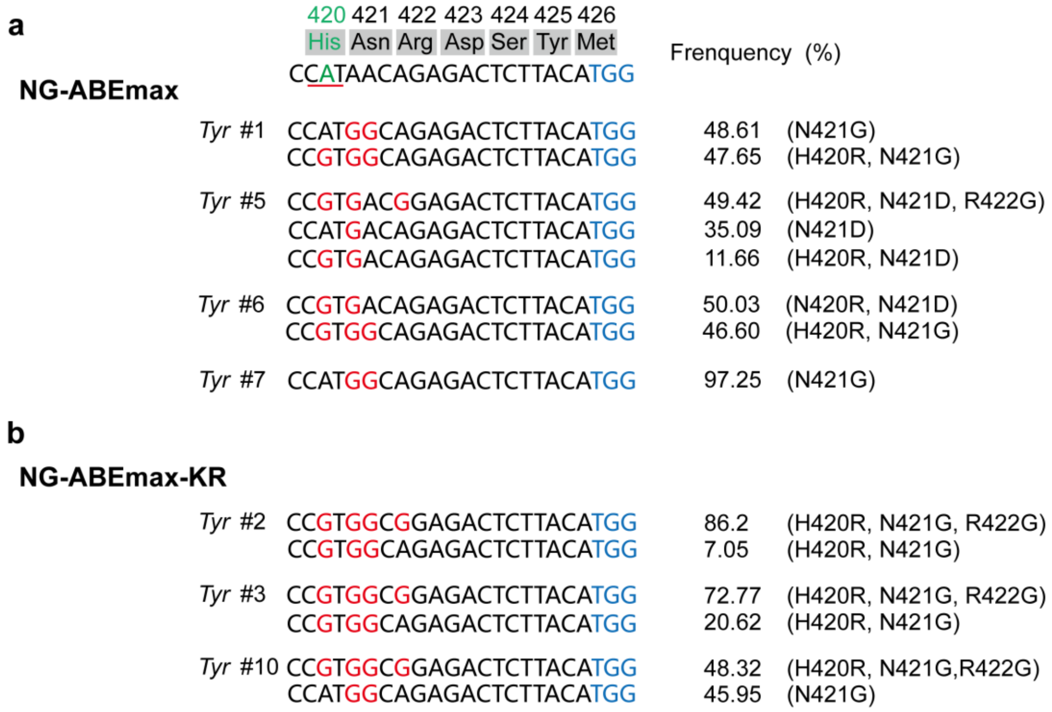
Supplementary Fig. 9 Editing efficiencies of NG-ABEmax-KR.

Boosted editing efficiencies of NG-ABEmax-KR at endogenous genomic sites.



Supplementary Fig. 10 Increased editing window of NG-ABEmax-KR at endogenous genomic sites.

Summary of increased editing window of NG-ABEmax-KR in different endogenous genomic sites. Each A has been highlighted in red. PAM sequence is in blue. Error bars indicate mean ± s.d. ($n = 3$ independent experiments).



a NG-ABEmax

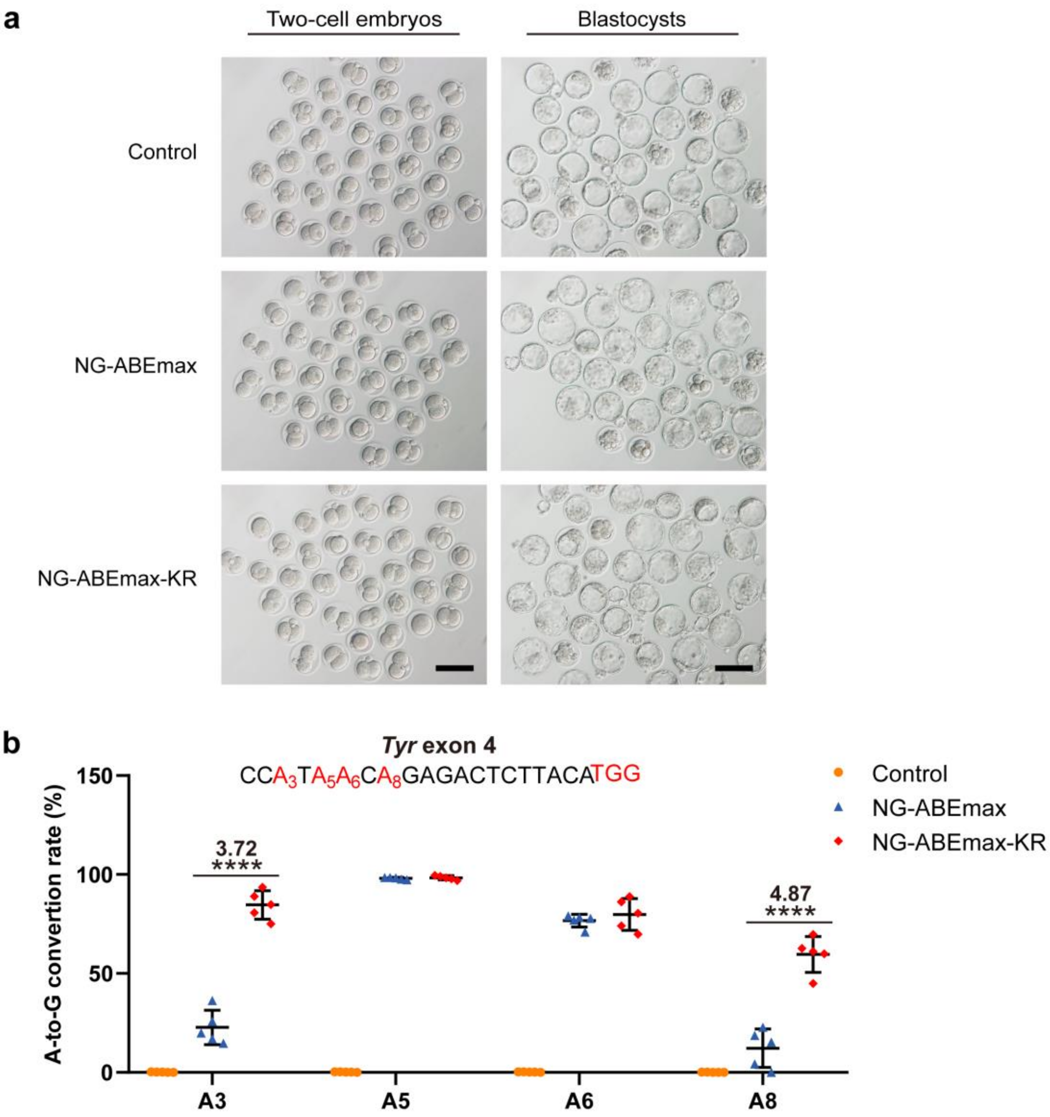
	Sequence	Frenquency (%)	
Tyr #1	CCATGGCAGAGACTCTTACATGG	48.61	(N421G)
	CCGTGGCAGAGACTCTTACATGG	47.65	(H420R, N421G)
Tyr #5	CCGTGACGGAGACTCTTACATGG	49.42	(H420R, N421D, R422G)
	CCATGACAGAGACTCTTACATGG	35.09	(N421D)
	CCGTGACAGAGACTCTTACATGG	11.66	(H420R, N421D)
Tyr #6	CCGTGACAGAGACTCTTACATGG	50.03	(N420R, N421D)
	CCGTGGCAGAGACTCTTACATGG	46.60	(H420R, N421G)
Tyr #7	CCATGGCAGAGACTCTTACATGG	97.25	(N421G)

b NG-ABEmax-KR

	Sequence	Frenquency (%)	
Tyr #2	CCGTGGCGGAGACTCTTACATGG	86.2	(H420R, N421G, R422G)
	CCGTGGCAGAGACTCTTACATGG	7.05	(H420R, N421G)
Tyr #3	CCGTGGCGGAGACTCTTACATGG	72.77	(H420R, N421G, R422G)
	CCGTGGCAGAGACTCTTACATGG	20.62	(H420R, N421G)
Tyr #10	CCGTGGCGGAGACTCTTACATGG	48.32	(H420R, N421G,R422G)
	CCATGGCAGAGACTCTTACATGG	45.95	(N421G)

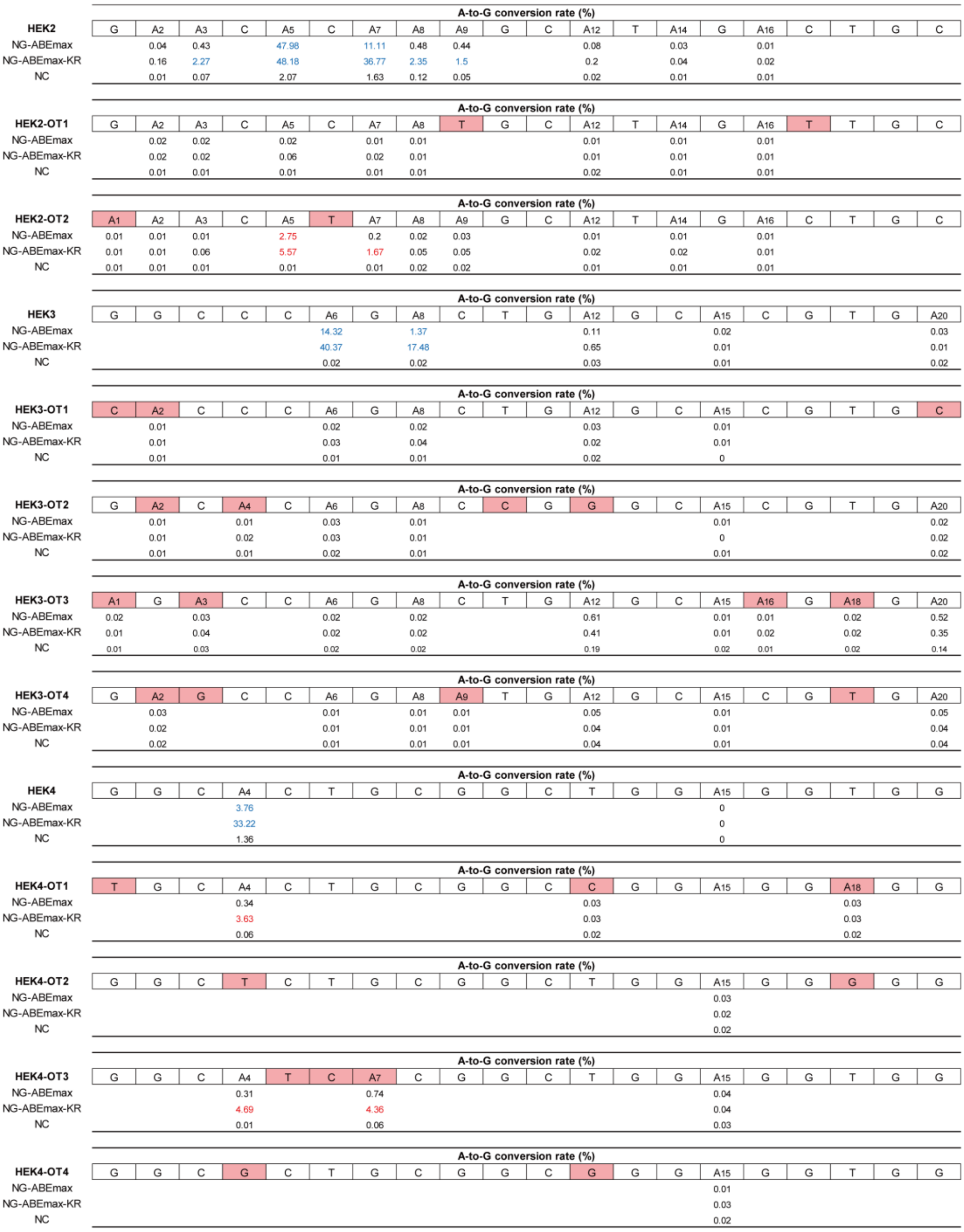
Supplementary Fig. 11 Sequence information of mice generated with NG-ABEmax-KR.

The *Tyr* gene information from each mouse was obtained from next-generation sequencing. The ID of mice was labeled with *Try* #. The predicted partial proteins information has been provided in the brackets. Editing information of NG-ABEmax(**a**) and NG-ABEmax-KR(**b**).



Supplementary Fig. 12 Editing efficiencies of NG-ABEmax or NG-ABEmax-KR in mouse embryos.

a Images of pre-implantation embryos obtained by injection of NG-ABEmax or NG-ABEmax-KR mRNA and sgRNA to target Tyr into zygotes. Control represents only ddH2O injection. Scale bar, 100 μm. **b** Statistical analysis of on-target A-to-G base conversions induced by NG-ABEmax or NG-ABEmaxKR in all blastocysts. Total embryos from each group were randomly divided into five groups; Data are mean ± s.d. for five groups of blastocysts. Each A base was highlighted in red. ****$P < 0.0001$ by Student’s unpaired two-sided t-test. Exact P value of A3 = 0.000002, exact P value of A8 = 0.000044.



A-to-G conversion rate (%)

HEK2	G	A2	A3	C	A5	C	A7	A8	A9	G	C	A12	T	A14	G	A16	C	T	G	C
NG-ABEmax		0.04	0.43		47.98		11.11	0.48	0.44			0.08		0.03		0.01				
NG-ABEmax-KR		0.16	2.27		48.18		36.77	2.35	1.5			0.2		0.04		0.02				
NC		0.01	0.07		2.07		1.63	0.12	0.05			0.02		0.01		0.01				

A-to-G conversion rate (%)

HEK2-OT1	G	A2	A3	C	A5	C	A7	A8	T	G	C	A12	T	A14	G	A16	T	T	G	C
NG-ABEmax		0.02	0.02		0.02		0.01	0.01				0.01		0.01		0.01				
NG-ABEmax-KR		0.02	0.02		0.06		0.02	0.01				0.01		0.01		0.01				
NC		0.01	0.01		0.01		0.01	0.01				0.02		0.01		0.01				

A-to-G conversion rate (%)

HEK2-OT2	A1	A2	A3	C	A5	T	A7	A8	A9	G	C	A12	T	A14	G	A16	C	T	G	C
NG-ABEmax	0.01	0.01	0.01		2.75		0.2	0.02	0.03			0.01		0.01		0.01				
NG-ABEmax-KR	0.01	0.01	0.06		5.57		1.67	0.05	0.05			0.02		0.02		0.01				
NC	0.01	0.01	0.01		0.01		0.01	0.02	0.02			0.01		0.01		0.01				

A-to-G conversion rate (%)

HEK3	G	G	C	C	C	A6	G	A8	C	T	G	A12	G	C	A15	C	G	T	G	A20
NG-ABEmax						14.32		1.37				0.11			0.02					0.03
NG-ABEmax-KR						40.37		17.48				0.65			0.01					0.01
NC						0.02		0.02				0.03			0.01					0.02

A-to-G conversion rate (%)

HEK3-OT1	C	A2	C	C	C	A6	G	A8	C	T	G	A12	G	C	A15	C	G	T	G	C
NG-ABEmax		0.01				0.02		0.02				0.03			0.01					
NG-ABEmax-KR		0.01				0.03		0.04				0.02			0.01					
NC		0.01				0.01		0.01				0.02			0					

A-to-G conversion rate (%)

HEK3-OT2	G	A2	C	A4	C	A6	G	A8	C	C	G	G	G	C	A15	C	G	T	G	A20
NG-ABEmax		0.01		0.01		0.03		0.01							0.01					0.02
NG-ABEmax-KR		0.01		0.02		0.03		0.01							0					0.02
NC		0.01		0.01		0.02		0.01							0.01					0.02

A-to-G conversion rate (%)

HEK3-OT3	A1	G	A3	C	C	A6	G	A8	C	T	G	A12	G	C	A15	A16	G	A18	G	A20
NG-ABEmax	0.02		0.03			0.02		0.02				0.61			0.01	0.01		0.02		0.52
NG-ABEmax-KR	0.01		0.04			0.02		0.02				0.41			0.01	0.02		0.02		0.35
NC	0.01		0.03			0.02		0.02				0.19			0.02	0.01		0.02		0.14

A-to-G conversion rate (%)

HEK3-OT4	G	A2	G	C	C	A6	G	A8	A9	T	G	A12	G	C	A15	C	G	T	G	A20
NG-ABEmax		0.03				0.01		0.01	0.01			0.05			0.01					0.05
NG-ABEmax-KR		0.02				0.01		0.01	0.01			0.04			0.01					0.04
NC		0.02				0.01		0.01	0.01			0.04			0.01					0.04

A-to-G conversion rate (%)

HEK4	G	G	C	A4	C	T	G	C	G	G	C	T	G	G	A15	G	G	T	G	G
NG-ABEmax				3.76											0					
NG-ABEmax-KR				33.22											0					
NC				1.36											0					

A-to-G conversion rate (%)

HEK4-OT1	T	G	C	A4	C	T	G	C	G	G	C	C	G	G	A15	G	G	A18	G	G
NG-ABEmax				0.34								0.03						0.03		
NG-ABEmax-KR				3.63								0.03						0.03		
NC				0.06								0.02						0.02		

A-to-G conversion rate (%)

HEK4-OT2	G	G	C	T	C	T	G	C	G	G	C	T	G	G	A15	G	G	G	G	G
NG-ABEmax															0.03					
NG-ABEmax-KR															0.02					
NC															0.02					

A-to-G conversion rate (%)

HEK4-OT3	G	G	C	A4	T	C	A7	C	G	G	C	T	G	G	A15	G	G	T	G	G
NG-ABEmax				0.31			0.74								0.04					
NG-ABEmax-KR				4.69			4.36								0.04					
NC				0.01			0.06								0.03					

A-to-G conversion rate (%)

HEK4-OT4	G	G	C	G	C	T	G	C	G	G	C	G	G	G	A15	G	G	T	G	G
NG-ABEmax															0.01					
NG-ABEmax-KR															0.03					
NC															0.02					

Supplementary Fig. 13 Off-target of NG-ABEmax and NG-ABEmax-KR at endogenous genomic sites.

There are three target sites (HEK2, HEK3 and HEK4) and corresponding off-target sites (labeled with OT#) were selected for off-target test. On-target and off-target editing efficiencies are in blue and red, respectively. Median values are shown, generated from *n*=3 biological replicate.

	A-to-G conversion rate (%)																			
***Tyr* exon 4**	C	C	A_{3}	T	A_{5}	A_{6}	C	A_{8}	G	A_{10}	G	A_{12}	C	T	C	T	T	A_{18}	C	A_{20}
NG-ABEmax-1			48.91		99.95	99.69		0.16		0.07		0.01						0.01		0.01
NG-ABEmax-2			46.54		99.96	99.71		0.09		0.05		0.01						0.01		0.01
NG-ABEmax-3			99.02		99.02	99.02		57.84		0.98		0						0		0
NG-ABEmax-4			99.84		99.96	50.97		0.04		0.02		0.01						0.01		0.01
NG-ABEmax-5			9.65		99.82	91.38		1.25		0.07		0.01						0.01		0.01
NG-ABEmax-6			63.55		99.95	0.62		51.45		0.04		0.01						0.01		0.01
NG-ABEmax-7			49.25		99.93	5.01		0.91		4.1		0.02						0.01		0.01
NG-ABEmax-8			40.23		99.9	60.04		31.33		0.07		0.07						0.01		0.01
NG-ABEmax-9			16.34		99.92	67.29		0.53		0.04		0.01						0.01		0.01
NG-ABEmax-10			49.22		99.81	99.74		48.91		0.14		0.03						0.01		0.01
NG-ABEmax-KR-1			77.37		99.92	96.04		44.76		21.06		0						0		0
NG-ABEmax-KR-2			99.5		99.87	99.56		99.04		48.37		0.01						0.01		0.01
NG-ABEmax-KR-3			99.09		99.77	99.29		49.7		39		0.01						0.01		0.01
NG-ABEmax-KR-4			99.11		99.84	99.46		82.42		0.09		0.01						0.01		0.01
NG-ABEmax-KR-5			98.05		98.39	94.99		25.51		0.68		0.17						0		0.01
NG-ABEmax-KR-6			99.08		99.18	98.98		52.83		0.26		0.11						0.01		0.01
NG-ABEmax-KR-7			99.15		99.29	99.16		98.64		0.45		0.12						0.01		0.01
NG-ABEmax-KR-8			99.17		99.31	99.15		75.3		0.35		0.08						0.01		0.01
NG-ABEmax-KR-9			25.71		99.02	98.88		98.55		0.44		0.17						0.01		0.01
NG-ABEmax-KR-10			77.94		80.48	80.4		80.11		0.69		0.12						0.01		0.01

	A-to-G conversion rate (%)																			
Off target-1	T	T	A_{3}	T	A_{5}	A_{6}	C	A_{8}	G	A_{10}	G	A_{12}	C	T	C	T	T	A_{18}	C	T
NG-ABEmax-1			0.01		0.01	0.01		0.01		0.01		0.01						0.02		
NG-ABEmax-2			0.01		0.01	0.01		0.01		0.01		0.01						0.03		
NG-ABEmax-3			0.01		0.01	0.01		0.01		0.01		0.01						0.02		
NG-ABEmax-4			0		0.01	0.01		0.01		0.01		0.01						0.02		
NG-ABEmax-5			0.01		41.82	41.84		41.83		0.01		0.01						0.02		
NG-ABEmax-6			0		0.06	0.06		0.05		0.01		0.01						0.02		
NG-ABEmax-7			0.01		0.01	0.02		0.01		0.01		0.01						0.03		
NG-ABEmax-8			0		0.01	0.01		0.01		0.02		0.01						0.03		
NG-ABEmax-9			20.2		51.92	0.03		72.2		0.02		0.01						0.02		
NG-ABEmax-10			28.19		28.32	0.05		28.34		0.01		0.02						0.03		
NG-ABEmax-KR-1			0		0.13	0.01		0.07		0.01		0.01						0.02		
NG-ABEmax-KR-2			0.01		38.21	0.02		38.07		0.01		0.01						0.02		
NG-ABEmax-KR-3			0.01		42.05	0.02		0.05		0.01		0.01						0.02		
NG-ABEmax-KR-4			0		0.01	0.01		0.01		0.01		0.01						0.02		
NG-ABEmax-KR-5			0.01		0.02	0.02		0.02		0.01		0						0.02		
NG-ABEmax-KR-6			0.01		0.01	0.01		0.01		0.01		0.01						0.01		
NG-ABEmax-KR-7			0.01		0.01	4.15		0.01		0.01		0.01						0.02		
NG-ABEmax-KR-8			0.01		0.01	0.01		0.01		0.01		0.01						0.02		
NG-ABEmax-KR-9			0.01		0.02	0.01		39.08		0.01		0.01						0.01		
NG-ABEmax-KR-10			0.01		0.01	0.01		0.02		0		0.01						0.01		

	A-to-G conversion rate (%)																			
Off target-2	C	G	A_{3}	T	T	A_{6}	C	A_{8}	G	A_{10}	G	A_{12}	C	T	C	C	T	A_{18}	C	A_{20}
NG-ABEmax-1			0.01			0.01		0.02		0.01		0.01						0.01		0.01
NG-ABEmax-2			0.01			0.01		0.02		0.01		0.02						0.01		0.01
NG-ABEmax-3			0.01			0.01		0.02		0.01		0.01						0.01		0.01
NG-ABEmax-4			0			0.01		0.02		0.01		0.01						0.01		0.01
NG-ABEmax-5			0.01			0.01		0.02		0.02		0.01						0.01		0.01
NG-ABEmax-6			0			0.01		0.01		0.01		0.01						0.01		0.02
NG-ABEmax-7			0			0.01		0.02		0.01		0.01						0		0
NG-ABEmax-8			0.01			0		0.02		0		0.01						0.01		0.01
NG-ABEmax-9			0.01			0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-10			0.01			0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-KR-1			0.01			0.01		0.02		0.02		0.01						0.01		0.01
NG-ABEmax-KR-2			0.01			0.01		0.02		0.02		0.01						0.01		0.01
NG-ABEmax-KR-3			0			0.01		0.02		0.01		0.01						0.01		0.01
NG-ABEmax-KR-4			0			0.01		0.02		0.02		0.01						0.01		0.01
NG-ABEmax-KR-5			0			0.01		0.01		0.01		0.01						0		0.01
NG-ABEmax-KR-6			0			0.01		0.02		0.01		0.01						0.01		0.01
NG-ABEmax-KR-7			0			0.01		0.02		0.01		0.01						0		0.01
NG-ABEmax-KR-8			0.01			0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-KR-9			0			0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-KR-10			0.01			0.01		0.01		0.01		0.01						0		0.01

	A-to-G conversion rate (%)																			
Off target-3	C	C	A_{3}	T	A_{5}	A_{6}	C	T	G	A_{10}	G	A_{12}	C	T	C	T	G	C	C	A_{20}
NG-ABEmax-1			0.01		0.01	0.01				0.01		0.02								0.01
NG-ABEmax-2			0.01		0.01	0.01				0.02		0.01								0.01
NG-ABEmax-3			0.01		0.01	0.01				0.02		0.01								0.01
NG-ABEmax-4			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-5			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-6			0		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-7			0.01		0.01	0.02				0.02		0.02								0.02
NG-ABEmax-8			0.01		0	0.01				0.01		0.01								0.01
NG-ABEmax-9			0.01		0.01	0.01				0.02		0.01								0.01
NG-ABEmax-10			0.01		0	0.01				0.01		0.01								0.01
NG-ABEmax-KR-1			0.01		0	0.01				0.01		0.01								0.01
NG-ABEmax-KR-2			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-KR-3			0.01		0.01	0				0.02		0.02								0.01
NG-ABEmax-KR-4			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-KR-5			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-KR-6			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-KR-7			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-KR-8			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-KR-9			0.01		0.01	0.01				0.01		0.01								0.01
NG-ABEmax-KR-10			0.01		0.01	0.01				0.01		0.01								0.01

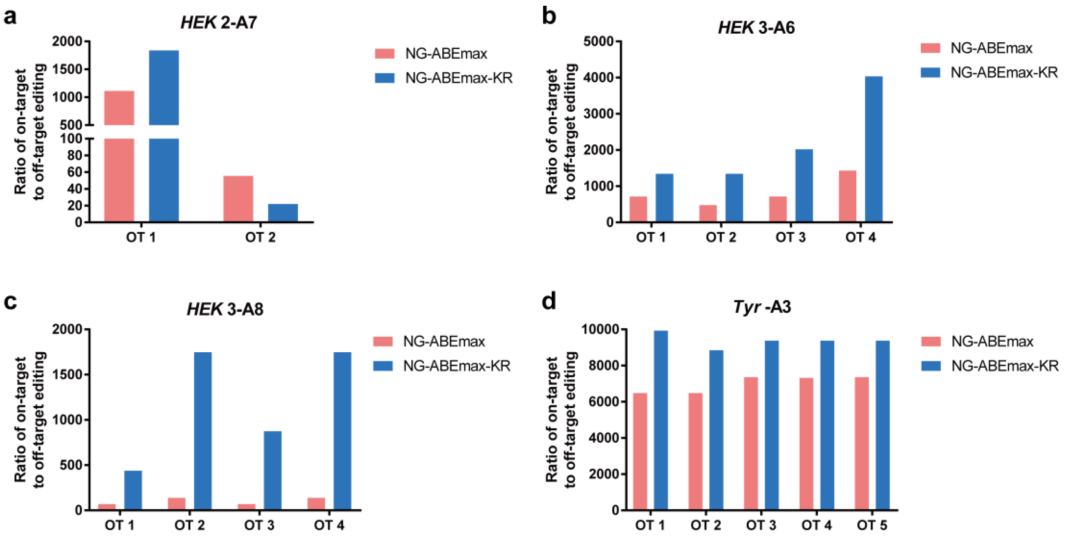
	A-to-G conversion rate (%)																			
Off target-4	C	A_{2}	A_{3}	T	A_{5}	A_{6}	C	A_{8}	G	A_{10}	G	G	T	T	C	T	T	A_{18}	C	A_{20}
NG-ABEmax-1		0	0		0	0		0		0								0		0
NG-ABEmax-2		0.01	0.01		0.01	0.01		0.01		0.01								0.01		0.01
NG-ABEmax-3		0.01	0		0.01	0		0.01		0.01								0.01		0.02
NG-ABEmax-4		0.01	0.01		0.01	0.01		0.01		0.01								0.01		0.01
NG-ABEmax-5		0.01	0.01		0.01	0		0.01		0.01								0.01		0.01
NG-ABEmax-6		0	0.01		0	0		0.02		0.01								0.01		0.02
NG-ABEmax-7		0	0.01		0.01	0.01		0.01		0.01								0.01		0.02
NG-ABEmax-8		0.01	0.01		0.01	0		0.01		0.01								0.01		0.01
NG-ABEmax-9		0.02	0.01		0	0		0.01		0.01								0		0.01
NG-ABEmax-10		0.01	0.01		0.01	0.01		0.02		0.01								0.01		0.02
NG-ABEmax-KR-1		0.01	0.01		0.01	0		0.01		0.01								0.01		0.01
NG-ABEmax-KR-2		0.01	0.01		0.01	0.01		0.01		0.01								0.01		0.01
NG-ABEmax-KR-3		0.01	0.01		0.01	0.01		0.01		0.02								0.01		0.01
NG-ABEmax-KR-4		0.01	0.01		0.01	0.01		0.01		0.01								0.01		0.01
NG-ABEmax-KR-5		0.01	0.01		0.01	0		0.01		0.01								0.01		0.01
NG-ABEmax-KR-6		0.01	0.01		0.01	0.01		0.01		0.01								0.01		0.01
NG-ABEmax-KR-7		0.01	0.01		0.01	0.01		0.01		0.01								0.01		0.01
NG-ABEmax-KR-8		0.01	0.01		0	0		0.01		0.01								0.01		0.01
NG-ABEmax-KR-9		0.01	0.01		0.01	0		0.01		0.01								0.01		0.01
NG-ABEmax-KR-10		0.01	0.01		0.01	0.01		0.01		0.01								0		0.01

	A-to-G conversion rate (%)																			
Off target-5	A_{1}	C	A_{3}	T	A_{5}	G	C	A_{8}	G	G	G	A_{12}	C	T	C	T	T	A_{18}	C	A_{20}
NG-ABEmax-1	0.01		0.01		0.01			0.01				0.01						0.01		0.02
NG-ABEmax-2	0.01		0.01		0.01			0.01				0.01						0.01		0.02
NG-ABEmax-3	0.01		0.01		0.02			0.02				0.01						0.01		0.01
NG-ABEmax-4	0.01		0.01		0.06			0.06				0.01						0.02		0.02
NG-ABEmax-5	0.01		0.01		0.01			0.01				0.01						0.01		0.02
NG-ABEmax-6	0.01		0.01		0.01			0.01				0.01						0.02		0.03
NG-ABEmax-7	0.01		0.01		0.01			0.02				0.01						0.01		0.02
NG-ABEmax-8	0.02		0.01		0.02			0.01				0.01						0.01		0.02
NG-ABEmax-9	0.01		0.01		0.01			0.01				0.01						0.02		0.02
NG-ABEmax-10	0.02		0.01		0.02			0.02				0.01						0.01		0.02
NG-ABEmax-KR-1	0.01		0.01		46.4			46.4				0.01						0.01		0.03
NG-ABEmax-KR-2	0.01		0.01		0.02			0.02				0.01						0.01		0.02
NG-ABEmax-KR-3	0.02		0.01		0.02			0.03				0.01						0.01		0.02
NG-ABEmax-KR-4	0.01		0.01		0.01			0.01				0.01						0.01		0.02
NG-ABEmax-KR-5	0.02		0.03		13.07			0.02				0.01						0.02		0.02
NG-ABEmax-KR-6	0.01		0.01		0.01			0.06				0.01						0.01		0.02
NG-ABEmax-KR-7	0.02		0		0.06			0.02				0.01						0.01		0.03
NG-ABEmax-KR-8	0.01		0.01		25.64			0.02				0.01						0.01		0.02
NG-ABEmax-KR-9	0.03		0.01		0.01			0.02				0.01						0.01		0.02
NG-ABEmax-KR-10	25.81		0.01		25.85			25.81				0.01						0.02		0.02

	A-to-G conversion rate (%)																			
Off target-6	T	C	C	A_{4}	A_{5}	A_{6}	C	A_{8}	G	A_{10}	G	A_{12}	C	T	C	T	T	A_{18}	C	A_{20}
NG-ABEmax-1				0.01	0.01	0		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-2				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.02
NG-ABEmax-3				0.01	0.01	0		0.01		0.01		0.01						0.01		0.02
NG-ABEmax-4				0.01	0.01	0.01		0		0.01		0.02						0.01		0.01
NG-ABEmax-5				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-6				0.01	0.02	0		0.01		0.01		0.01						0.02		0.03
NG-ABEmax-7				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-8				0.01	0.01	0		0.01		0.01		0.01						0.01		0.02
NG-ABEmax-9				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-10				0.01	0.01	0.01		31.56		0.01		0.01						0.01		0.02
NG-ABEmax-KR-1				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.02
NG-ABEmax-KR-2				0.01	0.01	0.01		0.01		0.02		0.01						0.01		0.01
NG-ABEmax-KR-3				0.01	0.02	0.01		0.05		0.02		0.01						0.01		0.02
NG-ABEmax-KR-4				0.01	0.01	3.61		30.05		0.02		0.02						0.01		0.02
NG-ABEmax-KR-5				0.01	0.01	0		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-KR-6				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-KR-7				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.02
NG-ABEmax-KR-8				0.01	0.01	0.01		0		0.01		0.01						0.01		0.02
NG-ABEmax-KR-9				0.01	0.01	0.01		0.01		0.01		0.01						0.01		0.01
NG-ABEmax-KR-10				0.01	0.01	0.01		0.02		0.01		0.01						0.01		0.01

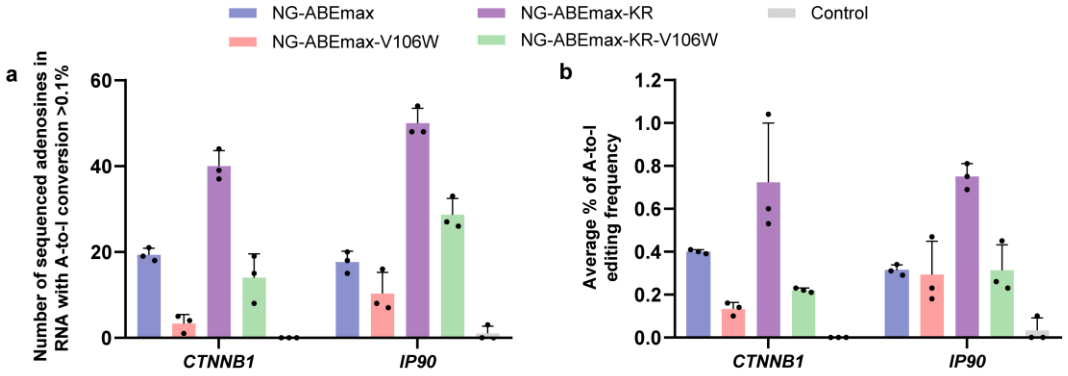
Supplementary Fig. 14 Off-target of NG-ABEmax and NG-ABEmax-KR at mouse *Try* gene.

Four mice harboring the edited A3 base from each group have been selected. Six off-target sites (labeled with Off target-#) were selected for off-target test. Off-target editing efficiencies are in red.



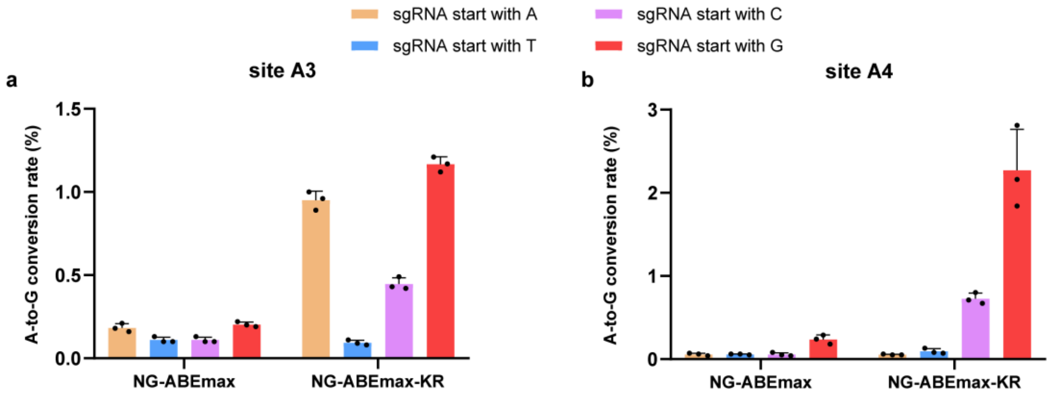
Supplementary Fig. 15 Higher ratio of on-target to off-target editing of NG-ABEmax-KR at human endogenous gene and mouse *Try* gene.

On-target and off-target editing efficiency of NG-ABEmax and NG-ABEmax-KR at different sites were performed. Data from OT site represent the ratio of on-target to off-target editing. The data of sites from human endogenous genes (*HEK* 2, *HEK* 3) are showed (**a, b** and **c**). The *Try* on-target and off-target editing ratio from four mice are summarized (**d**).



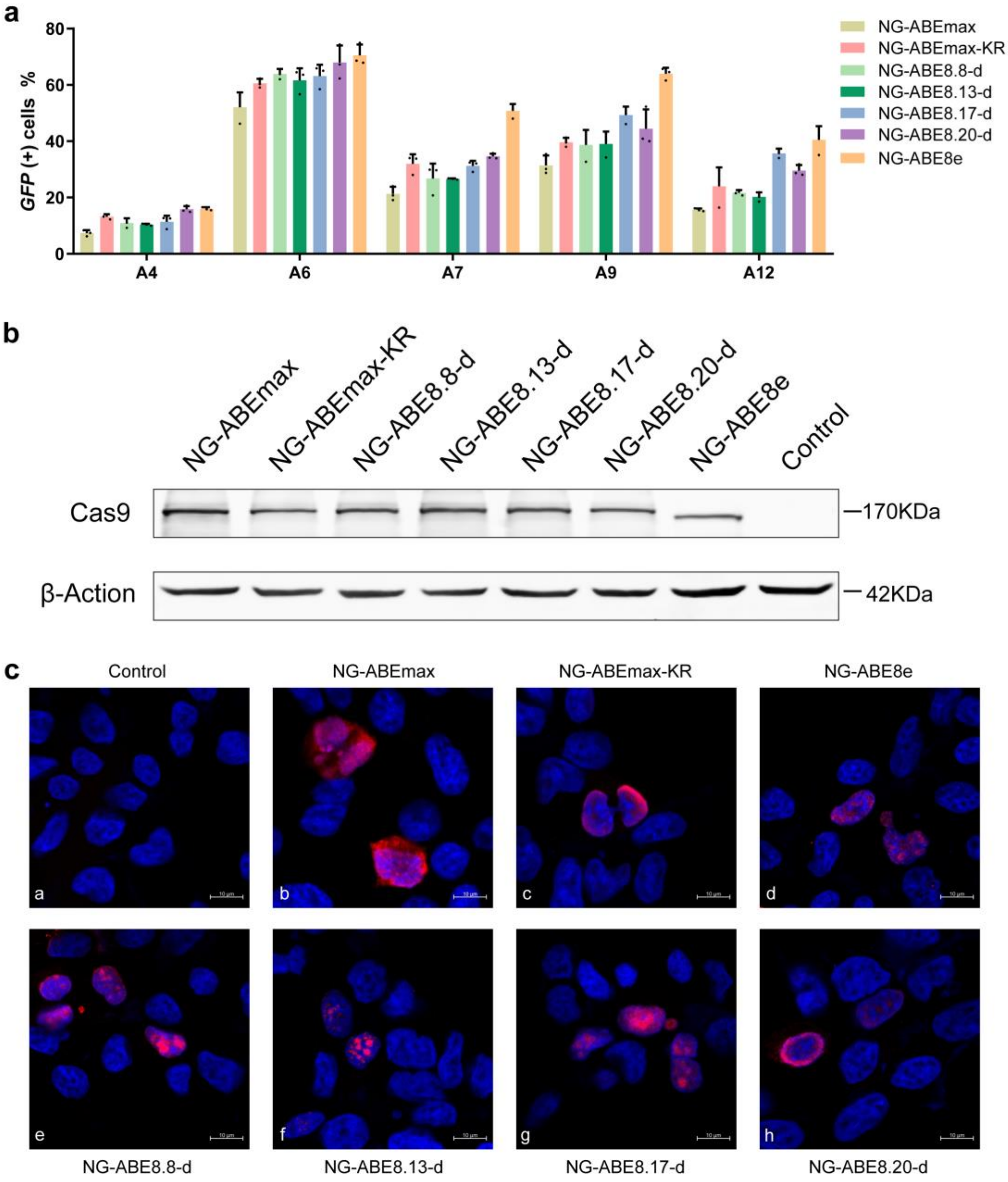
Supplementary Fig. 16 RNA off-target editing of NG-ABEmax-KR and variants with two mutations (E59A in TadA, V106W in TadA*).

a The number of adenosines converted to inosine at a detectable level (>0.1%) of the corresponding mRNA. b. Average A-to-I RNA editing frequencies by NG-ABEmax, NG-ABEmax-KR and variants with AW (E59A in TadA, V106W in TadA*). Error bars indicate mean ± s.d ($n = 3$ independent experiments).



Supplementary Fig. 17 Base editing efficiencies for sgRNA started with different first nucleotide.

a The editing efficiencies of NG-ABEmax and NG-ABEmax-KR with four sgRNA targeted at position A3 in the EGFP (Figure 1d) in HEK-293 cells. **b** The editing efficiencies at position A4 in HEK-293 cells. Error bars indicate mean ± s.d (n = 3 independent experiments).



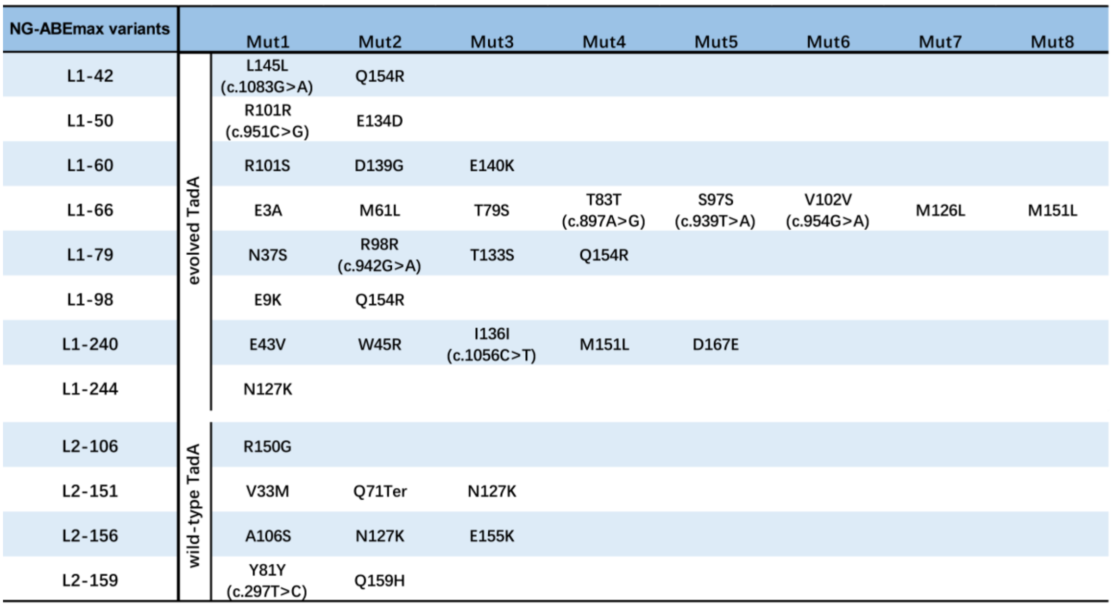
Supplementary Fig. 18 Activity, protein expression levels and nuclear localization of ABE variants.

a Base editing induced by ABEs at different A base in EGFP. Error bars indicate mean ± s.d. (*n* = 3 independent experiments). **b** Western blot analysis of ABE variants via the recognition with specific Cas9 antibody. **c** Nuclear localization of ABE variants. HEK-293 cells were transfected with the plasmids, fixed 24 hours post-transfection and stained with the antibody against Cas9 (red). DAPI (blue) indicated the nucleus. Scale bar is 10 µm.

Supplementary Table. 1 Mutations information of randomly picked variants.

Colony ID	Mutation number/575 bp	Colony ID	Mutation number /730 bp
S-L1-1	2	S-L2-1	1
S-L1-2	3	S-L2-2	6
S-L1-3	Deletion	S-L2-3	4
S-L1-4	1	S-L2-4	2
S-L1-5	2	S-L2-5	5
S-L1-6	5	S-L2-6	6
S-L1-7	4	S-L2-7	2
S-L1-8	1	S-L2-8	1
S-L1-9	2	S-L2-9	3
S-L1-10	4	S-L2-10	3
S-L1-11	1	S-L2-11	1
S-L1-12	1	S-L2-12	3
S-L1-13	3	S-L2-13	3
S-L1-14	3	S-L2-14	3
S-L1-15	1	S-L2-15	6
S-L1-16	5	S-L2-16	Deletion
S-L1-17	5	S-L2-17	8
S-L1-18	5	S-L2-18	1
S-L1-19	1	S-L2-19	6
S-L1-20	5	S-L2-20	3

Supplementary Table. 2 Summary of mutations information.



NG-ABEmax variants		Mut1	Mut2	Mut3	Mut4	Mut5	Mut6	Mut7	Mut8
L1-42	evolved TadA	L145L (c.1083G>A)	Q154R						
L1-50		R101R (c.951C>G)	E134D						
L1-60		R101S	D139G	E140K					
L1-66		E3A	M61L	T79S	T83T (c.897A>G)	S97S (c.939T>A)	V102V (c.954G>A)	M126L	M151L
L1-79		N37S	R98R (c.942G>A)	T133S	Q154R				
L1-98		E9K	Q154R						
L1-240		E43V	W45R	I136I (c.1056C>T)	M151L	D167E			
L1-244		N127K							
L2-106	wild-type TadA	R150G							
L2-151		V33M	Q71Ter	N127K					
L2-156		A106S	N127K	E155K					
L2-159		Y81Y (c.297T>C)	Q159H						

Supplementary Table. 3 No toxic effect of NG-ABEmax-KR for mouse development.

Methods	No. of injected embryos	No. of two-cell embryos (%)	No. of blastocysts (%)	No. of transferred embryos	No. of offsprings (%)
H_2O injection	40	39 (97.5)[a]	30 (76.9)[b]	NA	NA
NG-ABEmax mRNA+ Tyr sgRNA	36	35 (97.2)[a]	26 (74.3)[b]	NA	NA
NG-ABEmax-KR mRNA+ Tyr sgRNA	39	37 (94.9)[a]	34 (91.9)[b]	NA	NA
H_2O injection	56	54 (96.4)[a]	NA	54	21 (38.9)[c]
NG-ABEmax mRNA+ Tyr sgRNA	32	30 (93.8)[a]	NA	30	12 (40)[c]
NG-ABEmax-KR mRNA+ Tyr sgRNA	63	60 (95.2)[a]	NA	60	22 (36.7)[c]

NA: not applicable.

[a] Calculated from the number of injected embryos.

[b] Calculated from the number of developed two-cell stage embryos.

[c] Calculated from the number of transferred embryos.

Supplementary Table. 4 Primers used to generate different *EGFP* variants.

Primer Description	Primer sequence (5’-3’)
fwd_EGFP	CAGGTGTCGTGAGGGAATTCGATTATCGCC
rev_dEGFP1	CGTAGGTCAGCGACGACACCAGCGAGGGCTAGGGCA
fwd_dEGFP1	TAGCCCTCGCTGGTGTCGTCGCTGACCTACGG
rev_dEGFP2	CGACACCAGCGAGGGTCAGGGCACGGG
fwd_dEGFP2	CCCTGACCCTCGCTGGTGTCGTCGCTGA
rev_EGFP-variant	GTAGGTCAGCGACGACACCAGCGAGGGCCAGGGCA
fwd_EGFP-variant	TGGCCCTCGCTGGTGTCGTCGCTGACCTACGG
rev_EGFP	CCCTAGATGCATGCGGATCCTTCGAACTAG

Supplementary Table. 5 Primers used to generate sgRNA plasmids targeting different sites of *dEGFP1* or *dEGFP2*.

Primer Description	Primer sequence (5’-3’)
dEGFP1-A3 F	CACCCTAGCCCTCGCTGGTGTCGT
dEGFP1-A3 R	AAACACGACACCAGCGAGGGCTAG
dEGFP1-A6 F	CACCGCCCTAGCCCTCGCTGGTGT
dEGFP1-A6 R	AAACACACCAGCGAGGGCTAGGGC
dEGFP1-A9 F	CACCCGTGCCCTAGCCCTCGCTGG
dEGFP1-A9 R	AAACCCAGCGAGGGCTAGGGCACG
dEGFP1-A11 F	CACCCCCGTGCCCTAGCCCTCGCT
dEGFP1-A11 R	AAACAGCGAGGGCTAGGGCACGGG
dEGFP1-A12 F	CACCGCCCGTGCCCTAGCCCTCGC
dEGFP1-A12 R	AAACGCGAGGGCTAGGGCACGGGC
dEGFP1-A15 F	CACCGCTGCCCGTGCCCTAGCCCT
dEGFP1-A15 R	AAACAGGGCTAGGGCACGGGCAGC
dEGFP2-A1 F	CACCACCCTCGCTGGTGTCGTCGC
dEGFP2-A1 R	AAACGCGACGACACCAGCGAGGGT
dEGFP2-A4 F	CACCCTGACCCTCGCTGGTGTCGT
dEGFP2-A4 R	AAACACGACACCAGCGAGGGTCAG
dEGFP2-A7 F	CACCGCCCTGACCCTCGCTGGTGT
dEGFP2-A7 R	AAACACACCAGCGAGGGTCAGGGC
dEGFP2-A10 F	CACCCGTGCCCTGACCCTCGCTGG
dEGFP2-A10 R	AAACCCAGCGAGGGTCAGGGCACG
dEGFP2-A13 F	CACCGCCCGTGCCCTGACCCTCGC
dEGFP2-A13 R	AAACGCGAGGGTCAGGGCACGGGC
dEGFP2-A16 F	CACCGCTGCCCGTGCCCTGACCCT
dEGFP2-A16 R	AAACAGGGTCAGGGCACGGGCAGC

Supplementary Table. 6 Primers used to generate sgRNA plasmid targeting MCS site in the HEK293-PME cell line.

Primer Description	Primer sequence (5’-3’)
MCS F	CACCTTCTGCAGTCGACGGTACCG
MCS R	AAACCGGTACCGTCGACTGCAGAA

Supplementary Table. 7 Primers used to generate sgRNA plasmids targeting different genomic loci.

Primer Description	Primer sequence (5’-3’)
site1 (HEK2) F	CACCGAACACAAAGCATAGACTGC
site1 (HEK2) R	AAACGCAGTCTATGCTTTGTGTTC
site2 F	CACCGAGTATGAGGCATAGACTGC
site2 R	AAACGCAGTCTATGCCTCATACTC
site3 F	CACCGTCAAGAAAGCAGAGACTGC
site3 R	AAACGCAGTCTCTGCTTTCTTGAC
site5 F	CACCGATGAGATAATGATGAGTCA
site5 R	AAACTGACTCATCATTATCTCATC
site6 F	CACCGGATTGACCCAGGCCAGGGC
site6 R	AAACGCCCTGGCCTGGGTCAATCC
site7 F	CACCGAATACTAAGCATAGACTCC
site7 R	AAACGGAGTCTATGCTTAGTATTC
site8 F	CACCGTAAACAAAGCATAGACTGA
site8 R	AAACTCAGTCTATGCTTTGTTTAC
site9 F	CACCGAAGACCAAGGATAGACTGC
site9 R	AAACGCAGTCTATCCTTGGTCTTC
site10 F	CACCGAACATAAAGAATAGAATGA
site10 R	AAACTCATTCTATTCTTTATGTTC
site12 F	CACCGTAGAAAAAGTATAGACTGC
site12 R	AAACGCAGTCTATACTTTTTCTAC
site13 F	CACCGAAGATAGAGAATAGACTGC
site13 R	AAACGCAGTCTATTCTCTATCTTC
site14 F	CACCGGCTAAAGACCATAGACTGT
site14 R	AAACACAGTCTATGGTCTTTAGCC
site15 F	CACCGTCTAGAAAGCTTAGACTGC
site15 R	AAACGCAGTCTAAGCTTTCTAGAC
site17 F	CACCGACAAAGAGGAAGAGAGACG
site17 R	AAACCGTCTCTCTTCCTCTTTGTC
site18 F	CACCACACACACACTTAGAATCTG
site18 R	AAACCAGATTCTAAGTGTGTGTGT
HEK3 F	CACCGGCCCAGACTGAGCACGTGA
HEK3 R	AAACTCACGTGCTCAGTCTGGGCC
HEK4 F	CACCGGCACTGCGGCTGGAGGTGG
HEK4 R	AAACCCACCTCCAGCCGCAGTGCC
HBG1/2 F	CACCGTGGGGAAGGGGCCCCCAAG
HBG1/2 R	AAACCTTGGGGGCCCCTTCCCCAC
Tyr F	CACCCCATAACAGAGACTCTTACA
Tyr R	AAACTGTAAGAGTCTCTGTTATGG

Supplementary Table. 8 Primers used to identify key mutations for L1-60, L1-98 and L2-156.

Primer Description	Primer sequence (5'-3')
fwd_BamHI	TCTGGAGGATCTAGCGGAGGATCC
rev_60-1	CAAACACCACGCTGCCGATC
fwd_60-1	GGCAGCGTGGTGTTTGGCGT
rev_60-2	CGGCACATTCACCTGCCAGG
fwd_60-2	GCAGGTGAATGTGCCGCCCT
rev_60-3	CGGCACATTTATCTGCCAGG
fwd_60-3	GCAGATAAATGTGCCGCCCT
rev_60-4	CAAACACCACGCGGCCGATC
fwd_60-4	GGCCGCGTGGTGTTTGGCGT
rev_98-1	TCAGGGCATGTCTCATCCAGTACTTGTGG
fwd_98-1	CCACAAGTACTGGATGAGACATGC
rev_98-2	GCATTGAACACCCGTCTAGGCA
fwd_98-2	TAGACGGGTGTTCAATGCTCAGA
rev_ABEmax	CCTTGGCCATCTCGTTGCTGAAGATCT
fwd_TadA-mut	TCAGATCCGCTAGAGATCCGCGG
rev_W156-1	CCGTGATCCGAACACCACTCT
fwd_W156-1	GTGGTGTTCGGATCACGGGAC
rev_W156-2	GGTGCTTCATGCCGGGGTGGT
fwd_W156-2	ACCCCGGCATGAAGCACCGG
rev_W156-3	TGATCTTCTGTCTCCGCATTC
fwd_W156-3	TGCGGAGACAGAAGATCAAGG
rev_W156-4	TGATCTCCTGTCTCCGCATTC
fwd_W156-4	TGCGGAGACAGGAGATCAAGG
rev_W156-5	TGGTTCATGCCGGGGTGGTGC
fwd_W156-5	CCACCCCGGCATGAACCACC
rev_W156-6	CCGTGCTCCGAACACCACTCT
fwd_W156-6	GTGGTGTTCGGAGCACGGGAC
rev_TadA-mut	TGGGAAAACTCCACCTCAGAGGATCC

Supplementary Table. 9 Primers used to generate NG-ABEmax-R^{127} and NG-ABEmax H^{127}.

Primer Description	Primer sequence (5'-3')
fwd_TadA-mut	TCAGATCCGCTAGAGATCCGCGG
rev_ N127R	GGTGCCTCATGCCGGGGTGGT
fwd_ N127R	ACCCCGGCATGAGGCACCGG
rev_ N127H	GGTGGTGCATGCCGGGGTGGT
fwd_ N127H	ACCCCGGCATGCACCACCGGGTGG
rev_TadA-mut	TGGGAAAACTCCACCTCAGAGGATCC

Supplementary Table. 10 Primers used to generate 2 NG-ABEmax variants libraries.

Primer Description	Primer sequence (5’-3’)
fwd_TadA*-mut	TCCGCCACACCAGAGAGCTCCG
rev_TadA*-mut	TCAGAGCCAGAGGAGCCTCCGCTAGAT
fwd_TadA-mut	TCAGATCCGCTAGAGATCCGCGG
rev_TadA-mut	TGGGAAAACTCCACCTCAGAGGATCC

Supplementary Table. 11 Primers used for fragments amplification.

Primer Description	Primer sequence (5’-3’)
fwd_MCS	CAGGGAGCTCAAAATGGAGG
rev_MCS	TGCAGATGAACTTCAGGGTC
fwd_site1 (HEK2)	GGAGTGAGTACGGTGTGCAGACCTGGCTGAGCTAACTG
rev_site1 (HEK2)	GAGTTGGATGCTGGATGGCCCATCTGTCAAACTGTGCG
fwd_site2	GGAGTGAGTACGGTGTGCGCTTTTCAGCTAGAGGGAACCT
rev_site2	GAGTTGGATGCTGGATGGCTGAAATGCTGTGCGTGTCT
fwd_site3	GGAGTGAGTACGGTGTGCGGTTTCCTGTCTTCCCTTTCCC
rev_site3	GAGTTGGATGCTGGATGGCCGACAGCCAGTGGTTAAGT
fwd_site5	GGAGTGAGTACGGTGTGCAAGGAACTCAGGTCCAGGGAA
rev_site5	GAGTTGGATGCTGGATGGTGAGAGCAGGGACCACATCTA
fwd_site6	GGAGTGAGTACGGTGTGCGCATGCATTTGTAGGCTTGATGC
rev_site6	GAGTTGGATGCTGGATGGAGGAAAAGCTGTCCTGCGAC
fwd_site7	GGAGTGAGTACGGTGTGCTCCACCGCCTTGTTTACTGCT
rev_site7	GAGTTGGATGCTGGATGGTAGGTTTGCATAGACCTGCCC
fwd_site8	GGAGTGAGTACGGTGTGCCCCTGTTCCTAAAGCCCACC
rev_site8	GAGTTGGATGCTGGATGGACTGGTTCTGTTTGTGGCCA
fwd_site9	GGAGTGAGTACGGTGTGCGGCCCAATGACACCACATGA
rev_site9	GAGTTGGATGCTGGATGGGGAACAGGCAGCGTATTGCT
fwd_site10	GGAGTGAGTACGGTGTGCTCCACCTCCCCACTTCTCTT
rev_site10	GAGTTGGATGCTGGATGGGGTGAAATGAGCAAGGCACA
fwd_site12	GGAGTGAGTACGGTGTGCCAGGTGATGGAAAGCATTTGCC
rev_site12	GAGTTGGATGCTGGATGGACCCATGTGCCTGACATAGG
fwd_site13	GGAGTGAGTACGGTGTGCCTTCTCTTTCTCTCCCCCACCC
rev_site13	GAGTTGGATGCTGGATGGTCACTTCAGCCCAGGAGTAT
fwd_site14	GGAGTGAGTACGGTGTGCTCTAACCTGTGTGACACTTGGCA
rev_site14	GAGTTGGATGCTGGATGGGAACCTGAAGCCTTTCCCCA
fwd_site15	GGAGTGAGTACGGTGTGCAACTAGGTGGCTTCAGAGGC
rev_site15	GAGTTGGATGCTGGATGGGCACTCAGCTAGACTTAACTCCC
fwd_site17	GGAGTGAGTACGGTGTGCCCTGATTCCAAGGAGATTGCCA
rev_site17	GAGTTGGATGCTGGATGGTCCCTCCTCTGCGTGAATTTG
fwd_site18	GGAGTGAGTACGGTGTGCGCATTACCTGGGAGCCTGTT
rev_site18	GAGTTGGATGCTGGATGGAACTTCAGCGGGCATCAGAA
fwd_HEK2-off1	GGAGTGAGTACGGTGTGCGTCCAGATAATCAGAGTGTGG
rev_HEK2-off1	GAGTTGGATGCTGGATGGCTTGTGAAACAGAAATGTCAG
fwd_HEK2-off2	GGAGTGAGTACGGTGTGCGGTACTCGAGTGTTATTCAGCC
rev_HEK2-off2	GAGTTGGATGCTGGATGGGAGCAGTGAGTTTGGGCACT
fwd_HEK3	GGAGTGAGTACGGTGTGCTGCATTTGTAGGCTTGATGC
rev_HEK3	GAGTTGGATGCTGGATGGACATACTAGCCCCTGTCTAG
fwd_HEK3-off1	GGAGTGAGTACGGTGTGCTCCCCTGTTGACCTGGAGAA
rev_HEK3-off1	GAGTTGGATGCTGGATGGTACTTGCCCTGACCAGAGGT
fwd_HEK3-off2	GGAGTGAGTACGGTGTGCGAAGGGGAAGGAGACTTAGTG
rev_HEK3-off2	GAGTTGGATGCTGGATGGTTGGTGTTGACAGGGAGCAA

fwd_HEK3-off3	GGAGTGAGTACGGTGTGCTGCTCATCTTAATCTGCTCAGCC
rev_HEK3-off3	GAGTTGGATGCTGGATGGTCCTAGCACTTTGGAAGGTCG
fwd_HEK3-off4	GGAGTGAGTACGGTGTGCAAAGGAGCAGCTCTTCCTGG
rev_HEK3-off4	GAGTTGGATGCTGGATGGGTCTGCACCATCTCCCACAA
fwd_HEK4	GGAGTGAGTACGGTGTGCAAGATGGCTGACAAAGGCCG
rev_HEK4	GAGTTGGATGCTGGATGGCCCCCACTGTAGTCACACA
fwd_HEK4-off1	GGAGTGAGTACGGTGTGCGACTCATAGCTGGGGCTGAA
rev_HEK4-off1	GAGTTGGATGCTGGATGGGTATCACTGTCCCCTTGCACT
fwd_HEK4-off2	GGAGTGAGTACGGTGTGCAAGAGGCTGCCCATGAGAGC
rev_HEK4-off2	GAGTTGGATGCTGGATGGTTTGGCAATGGAGGCATTGG
fwd_HEK4-off3	GGAGTGAGTACGGTGTGCTGGGATGGAATCACCTGCAC
rev_HEK4-off3	GAGTTGGATGCTGGATGGCTCGGTTCCTCCACAACACA
fwd_HEK4-off4	GGAGTGAGTACGGTGTGCGCAGGGGAGGGATAAAGCAG
rev_HEK4-off4	GAGTTGGATGCTGGATGGCACGGGAAGGACAGGAGAAG
fwd_Mouse-Tyr	GGAGTGAGTACGGTGTGCCACCGCCCTCTTTTGGAAGT
rev_Mouse-Tyr	GAGTTGGATGCTGGATGGGGCAACTGAGCTTTACCTGAC
fwd_CTNNB1	GGAGTGAGTACGGTGTGCATTTGATGGAGTTGGACATGGCC
rev_ CTNNB1	GAGTTGGATGCTGGATGGCCAGCTACTTGTTCTTGAGTGAAGG
fwd_IP90	GGAGTGAGTACGGTGTGCCTGGTTGACCAATCTGTGGTGAATAG
rev_IP90	GAGTTGGATGCTGGATGGGTACGTACTCAGGCTCATCATCTAACC

Supplementary Table. 12 Primers used for next-generation sequencing.

Primer	Primer sequence (5`-3`)
1F-1	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGCGTtggagtgagtacggtgtgc
1F-2	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGTAGtggagtgagtacggtgtgc
1F-3	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttACGCtggagtgagtacggtgtgc
1F-4	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttCTCGtggagtgagtacggtgtgc
1F-5	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGCTCtggagtgagtacggtgtgc
1F-6	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttAGTCtggagtgagtacggtgtgc
1F-7	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttCGACtggagtgagtacggtgtgc
1F-8	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGATGtggagtgagtacggtgtgc
1F-9	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttATACtggagtgagtacggtgtgc
1F-10	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttCACAtggagtgagtacggtgtgc
1F-11	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttGTGCtggagtgagtacggtgtgc
1F-12	ACTCTTTCCCTACACGACGCTCTTCCGATCTgcttACTAtggagtgagtacggtgtgc
2F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTT
1R-A	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGCGTtgagttggatgctggatgg
1R-B	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGTAGtgagttggatgctggatgg
1R-C	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtACGCtgagttggatgctggatgg
1R-D	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtCTCGtgagttggatgctggatgg
1R-E	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGCTCtgagttggatgctggatgg
1R-F	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtAGTCtgagttggatgctggatgg
1R-G	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtCGACtgagttggatgctggatgg
1R-H	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgtGATGtgagttggatgctggatgg
2R	CAAGCAGAAGACGGCATACGAGATCGCTGATCGTGACTGGAGTTCAGACGTGTGCTCTT

Supplementary Table. 13 The raw screening data of Library 1.

Mutant	L1-1	L1-2	L1-3	L1-4	L1-5	L1-6	L1-7	L1-8	L1-9	L1-10	L1-11	L1-12	L1-13	L1-14	L1-15	L1-16	L1-17	L1-18	L1-19	L1-20
EGFP(+)%	0.03	2.16	0	0.34	0.62	0.01	2.54	2.28	2.84	3.7	0	0	0	2.15	0.72	1.6	0.14	2.4	0	0
Mutant	L1-21	L1-22	L1-23	L1-24	L1-25	L1-26	L1-27	L1-28	L1-29	L1-30	L1-31	L1-32	L1-33	L1-34	L1-35	L1-36	L1-37	L1-38	L1-39	L1-40
EGFP(+)%	0	2.12	3.68	0	2.47	4.35	0.12	1.6	0	0	0.02	3.6	0.01	0.01	1.47	2.01	2.74	0.12	1.45	0.39
Mutant	L1-41	L1-42	L1-43	L1-44	L1-45	L1-46	L1-47	L1-48	L1-49	L1-50	L1-51	L1-52	L1-53	L1-54	L1-55	L1-56	L1-57	L1-58	L1-59	L1-60
EGFP(+)%	0	9.95	0	3.4	0.62	0.34	0.24	0	0.93	10.18	0	3.46	2.6	1.87	0.14	5.28	5.93	3.24	5.29	12.42
Mutant	L1-61	L1-62	L1-63	L1-64	L1-65	L1-66	L1-67	L1-68	L1-69	L1-70	L1-71	L1-72	L1-73	L1-74	L1-75	L1-76	L1-77	L1-78	L1-79	L1-80
EGFP(+)%	0.48	0	0.11	4.2	4.87	10.97	3.75	5.15	3.89	0.01	4.09	1.08	4.21	0.02	5.29	4.7	4.76	3.22	10.4	0.08
Mutant	L1-81	L1-82	L1-83	L1-84	L1-85	L1-86	L1-87	L1-88	L1-89	L1-90	L1-91	L1-92	L1-93	L1-94	L1-95	L1-96	L1-97	L1-98	L1-99	L1-100
EGFP(+)%	0.03	4.12	0.02	0.02	0.1	5.04	0.03	0	3.34	3.64	0	0	0.64	7.5	0	2.87	0.04	10.84	5.45	0.05
Mutant	L1-101	L1-102	L1-103	L1-104	L1-105	L1-106	L1-107	L1-108	L1-109	L1-110	L1-111	L1-112	L1-113	L1-114	L1-115	L1-116	L1-117	L1-118	L1-119	L1-120
EGFP(+)%	0.01	5.22	4.38	4.28	5.44	0.07	0.02	3.63	0	0	8.25	0.72	0.41	1.48	1.32	0.12	0	1.15	0	2.97
Mutant	L1-121	L1-122	L1-123	L1-124	L1-125	L1-126	L1-127	L1-128	L1-129	L1-130	L1-131	L1-132	L1-133	L1-134	L1-135	L1-136	L1-137	L1-138	L1-139	L1-140
EGFP(+)%	0.86	0.13	0.01	3.47	0.01	1.13	0	4.4	4.42	4.02	0	0.95	2.98	2.1	0	2.79	0.78	0.71	2.68	0
Mutant	L1-141	L1-142	L1-143	L1-144	L1-145	L1-146	L1-147	L1-148	L1-149	L1-150	L1-151	L1-152	L1-153	L1-154	L1-155	L1-156	L1-157	L1-158	L1-159	L1-160
EGFP(+)%	0.1	0	2.86	0.02	0.18	0.08	0	2.17	3.41	0.96	0.05	0.01	0.01	3.63	0.01	0.01	0	0	3.24	0.27
Mutant	L1-161	L1-162	L1-163	L1-164	L1-165	L1-166	L1-167	L1-168	L1-169	L1-170	L1-171	L1-172	L1-173	L1-174	L1-175	L1-176	L1-177	L1-178	L1-179	L1-180
EGFP(+)%	1.42	1.66	2.57	0.03	4.43	0.04	0.56	2.75	1.62	0.08	0.01	2.65	3.19	2.36	2.96	3.05	3.93	4.05	1.19	3.47
Mutant	L1-181	L1-182	L1-183	L1-184	L1-185	L1-186	L1-187	L1-188	L1-189	L1-190	L1-191	L1-192	L1-193	L1-194	L1-195	L1-196	L1-197	L1-198	L1-199	L1-200
EGFP(+)%	0.22	0.77	1.8	0	1.94	0.89	0.34	1.94	0.26	0.4	2.56	2.77	0.15	2.86	2.56	2.45	2.69	0.01	3.3	0.72
Mutant	L1-201	L1-202	L1-203	L1-204	L1-205	L1-206	L1-207	L1-208	L1-209	L1-210	L1-211	L1-212	L1-213	L1-214	L1-215	L1-216	L1-217	L1-218	L1-219	L1-220
EGFP(+)%	2.81	0.01	0.85	0.02	0.02	0.01	0.01	3.11	1.9	2.87	0.04	0.11	0.01	2.97	1.13	0.13	2.64	0.11	3.26	3.41
Mutant	L1-221	L1-222	L1-223	L1-224	L1-225	L1-226	L1-227	L1-228	L1-229	L1-230	L1-231	L1-232	L1-233	L1-234	L1-235	L1-236	L1-237	L1-238	L1-239	L1-240
EGFP(+)%	3.34	0.01	3.53	3.37	1.13	2.4	3.58	2.42	3.67	2.13	3.37	0.01	4.04	0.65	4	0.64	0.05	0.01	3.3	11.46
Mutant	L1-241	L1-242	L1-243	L1-244	L1-245	L1-246	L1-247	L1-248	L1-249	L1-250	L1-251	L1-252	L1-253	L1-254	L1-255	L1-256	L1-257	L1-258	L1-259	L1-260
EGFP(+)%	0	0.17	0	11.41	0.01	4.35	0	0.68	4.24	0	0.03	3.96	2.77	0.76	0.79	8.08	3.39	2.41	2.3	0
Mutant	L1-261	L1-262	L1-263	L1-264	L1-265	L1-266	L1-267	L1-268	L1-269	L1-270	L1-271	L1-272	L1-273	L1-274	L1-275	L1-276	L1-277	L1-278	L1-279	L1-280
EGFP(+)%	2.79	0	4.08	3.75	3.34	2.78	0.01	4.35	1.36	0.28	4.32	5.68	5.19	3.21	1.48	0	0.04	1.68	0.07	0
Mutant	L1-281	L1-282	L1-283	L1-284	L1-285	L1-286	L1-287	L1-288	L1-289	L1-290	L1-291	L1-292	L1-293	L1-294	L1-295	L1-296	L1-297	L1-298	L1-299	L1-300
EGFP(+)%	2.07	0.03	0.45	0.45	4.76	0	2.19	2.06	2.45	2.46	0.02	0.06	3.76	2.22	4.65	3.49	3.94	0	0.01	2.79
Mutant	L1-301	L1-302	L1-303	L1-304	L1-305	L1-306	L1-307	L1-308	L1-309	L1-310	L1-311	L1-312	L1-313	L1-314	L1-315	L1-316	L1-317	L1-318	L1-319	L1-320
EGFP(+)%	1.21	1.21	0	0.71	0	0.03	0	0.02	0.01	2.14	0	0.01	2.25	3.91	0.01	3.61	0.52	3.29	0.26	0.03
Mutant	L1-321	L1-322	L1-323	L1-324	L1-325	L1-326	L1-327	L1-328	L1-329	L1-330	L1-331	L1-332	L1-333	L1-334	L1-335	L1-336	L1-337	L1-338	L1-339	L1-340
EGFP(+)%	3.22	0.02	3.93	4.13	0.01	1.61	2	0.15	1.15	2.5	1.88	0.61	2.35	0.02	3.67	0.25	0.03	0.13	0.37	2.95
Mutant	L1-341	L1-342	L1-343	L1-344	L1-345	L1-346	L1-347	L1-348	L1-349	L1-350	L1-351	L1-352	L1-353	L1-354	L1-355	L1-356	L1-357	L1-358	L1-359	L1-360
EGFP(+)%	0.01	0	1.02	3.75	0.02	0.17	4.17	0.63	0.01	2.81	0.05	0.02	0.03	0.04	1.3	0.32	2.83	0.01	6.32	3.16
Mutant	L1-361	L1-362	L1-363	L1-364	L1-365	L1-366	L1-367	L1-368	L1-369	L1-370	L1-371	L1-372	L1-373	L1-374	L1-375	L1-376	L1-377	L1-378	L1-379	L1-380
EGFP(+)%	0	3	0.52	0.07	0	0.19	2.91	1.18	0.53	2.26	2.34	3.3	2.75	1.98	2.39	2.76	2.17	2.59	2.01	0.02
Mutant	L1-381	L1-382	L1-383	L1-384	L1-385	L1-386	L1-387	L1-388	L1-389	L1-390	L1-391	L1-392	L1-393	L1-394	L1-395	L1-396	L1-397	L1-398	L1-399	L1-400
EGFP(+)%	0.02	1.65	0.66	2.52	0	7.88	0.69	0.02	2.58	0.5	2.48	4.48	6.11	0.06	3.02	0	0	2.38	1.42	1.09
Mutant	L1-401	L1-402	L1-403	L1-404	L1-405	L1-406	L1-407	L1-408	L1-409	L1-410	L1-411	L1-412	L1-413	L1-414	L1-415	L1-416	L1-417	L1-418	L1-419	L1-420
EGFP(+)%	1.56	2.3	0.11	0.4	1.19	2.57	1.02	2.29	0.03	5.73	2.17	2	0.01	0	2.27	0	1.08	1.31	0.31	0.02
Mutant	L1-421	L1-422	L1-423	L1-424	L1-425	L1-426	L1-427	L1-428	L1-429	L1-430	L1-431	L1-432	L1-433	L1-434	L1-435	L1-436	L1-437	L1-438	L1-439	L1-440
EGFP(+)%	0	0.89	1.85	1.61	0.07	2.76	1.61	0	0	0.32	2.62	0	0.01	0.68	2.4	2.64	5.02	1.81	0.04	2.78
Mutant	L1-441	L1-442	L1-443	L1-444	L1-445	L1-446	L1-447	L1-448	L1-449	L1-450	L1-451	L1-452	L1-453	L1-454	L1-455	L1-456	L1-457	L1-458	L1-459	L1-460
EGFP(+)%	1.91	2.39	0	0.01	0.05	2.69	3.44	0	0	0	0	0.07	2.78	0	4.06	0	0.1	1.83	1.65	0.02
Mutant	L1-461	L1-462	L1-463	L1-464	L1-465	L1-466	L1-467	L1-468	L1-469	L1-470	L1-471	L1-472	L1-473	L1-474	L1-475	L1-476	L1-477	L1-478	L1-479	L1-480
EGFP(+)%	0.08	1.24	4.26	5.36	2.89	1.57	3.52	0.02	2.08	0.08	2.64	0.06	0.17	0.04	0	0.05	0.6	3.52	0.36	2.9
Mutant	L1-481	L1-482	L1-483	L1-484	L1-485	L1-486	L1-487	L1-488	L1-489	L1-490	L1-491	L1-492	L1-493	L1-494	L1-495	L1-496	L1-497	L1-498	L1-499	L1-500
EGFP(+)%	0.1	0	0.03	4.19	0.12	0.03	0.06	0	4.66	0	0	0.12	0.16	2.68	0	0	0.39	0.04	6.58	0.05
Mutant	L1-501	L1-502	L1-503	L1-504	L1-505	L1-506	L1-507	L1-508	L1-509	L1-510	L1-511	L1-512	L1-513	L1-514	L1-515	L1-516	L1-517	L1-518	L1-519	L1-520
EGFP(+)%	0.18	3.96	4.89	0.26	0	0.01	0	0	0	0	0	1.86	0.7	0.03	0.04	0.02	0.02	2.12	0	4.48
Mutant	L1-521	L1-522	L1-523	L1-524	L1-525	L1-526	L1-527	L1-528	L1-529	L1-530	L1-531	L1-532	L1-533	L1-534	L1-535	L1-536	L1-537	L1-538	L1-539	L1-540
EGFP(+)%	2.97	0.48	0	2.93	0.18	3.53	0.08	0	0.35	0.45	2.86	3.37	5.07	4.17	1.06	2.34	5.14	0.02	0.01	0.07
Mutant	L1-541	L1-542	L1-543	L1-544	L1-545	L1-546	L1-547	L1-548	L1-549	L1-550	L1-551	L1-552	L1-553	L1-554	L1-555	L1-556	L1-557	L1-558	L1-559	L1-560
EGFP(+)%	3.52	0	1.24	0	0	0.99	0.13	0.11	0.01	2.45	0.05	0.03	1.51	0.01	0.69	2.69	0.27	4.09	2.49	0.61
Mutant	L1-561	L1-562	L1-563	L1-564	L1-565	L1-566	L1-567	L1-568	L1-569	L1-570	L1-571	L1-572	L1-573	L1-574	L1-575	L1-576	L1-577	L1-578	L1-579	L1-580
EGFP(+)%	0	1.32	0	0.33	0.37	0.15	0.01	0	1.37	2.42	0.01	0.01	4.94	4.92	7.25	0.11	3.21	5.34	0.05	0.14
Mutant	L1-581	L1-582	L1-583	L1-584	L1-585	L1-586	L1-587	L1-588	L1-589	L1-590	L1-591	L1-592	L1-593	L1-594	L1-595	L1-596	L1-597	L1-598	L1-599	L1-600
EGFP(+)%	3.41	2.23	4.28	0.01	0.03	4.57	0.03	5.43	3.34	5.58	0.02	0.02	0	1.01	0.07	0.01	2.97	0.59	1.03	0.01
Mutant	L1-601	L1-602	L1-603	L1-604	L1-605	L1-606	L1-607	L1-608	L1-609	L1-610	L1-611	L1-612	L1-613	L1-614	L1-615	L1-616	L1-617	L1-618	L1-619	L1-620
EGFP(+)%	2.21	2.16	0.54	3.79	3.13	0.01	2.52	0.15	0.64	0.21	0.03	0.02	0	0.01	0	3.08	4.69	3.23	4.93	4.51
Mutant	L1-621	L1-622	L1-623	L1-624	L1-625	L1-626	L1-627	L1-628	L1-629	L1-630	L1-631	L1-632	L1-633	L1-634	L1-635	L1-636	L1-637	L1-638	L1-639	L1-640
EGFP(+)%	0.07	4.11	0.49	0.01	5.13	5.93	0.22	2.87	0.03	0.23	1.46	0.05	0	2.52	5.94	6.36	3.07	0.07	2.08	2.4
Mutant	L1-641	L1-642	L1-643	L1-644	L1-645	L1-646	L1-647	L1-648	L1-649	L1-650	L1-651	L1-652	L1-653	L1-654	L1-655	L1-656	L1-657	L1-658	L1-659	L1-660
EGFP(+)%	1.02	2.04	0.01	0.04	0.08	0.01	0.13	0.14	0.01	0.06	0.73	0.17	3.01	3.19	2.91	0	1.41	2.11	0	0
Mutant	L1-661	L1-662	L1-663	L1-664	L1-665	L1-666	L1-667	L1-668	L1-669	L1-670	L1-671	L1-672	L1-673	L1-674	L1-675	L1-676	L1-677	L1-678	L1-679	L1-680
EGFP(+)%	1.88	0.08	2.54	0	0.02	3.08	0	0.08	1.69	2.04	0.25	0.01	0	0.01	0.01	1.83	1.69	3.7	6.37	2.22
Mutant	L1-681	L1-682	L1-683	L1-684	L1-685	L1-686	L1-687	L1-688	L1-689	L1-690	L1-691	L1-692	L1-693	L1-694	L1-695	L1-696	L1-697	L1-698	L1-699	L1-700
EGFP(+)%	1.05	0	0.48	0.01	2.59	0.4	4.08	0	1.78	0.38	0	0.04	4.46	0.03	0.01	3.96	1.59	1.97	0.08	0
Mutant	L1-701	L1-702	L1-703	L1-704	L1-705	L1-706	L1-707	L1-708	L1-709	L1-710	L1-711	L1-712	L1-713	L1-714	L1-715	L1-716	L1-717	L1-718	L1-719	L1-720
EGFP(+)%	0.37	0.1	0.94	5.28	0.2	2.39	4.26	5.85	0.01	5.49	0.08	0.02	2.19	6.05	1.38	0.01	0.71	6.18	0.04	0
Mutant	L1-721	L1-722	L1-723	L1-724	L1-725	L1-726	L1-727	L1-728	L1-729	L1-730	L1-731	L1-732	L1-733	L1-734	L1-735	L1-736	L1-737	L1-738	L1-739	L1-740
EGFP(+)%	2.51	3.7	0.16	1.81	0.51	0.88	2.6	0.08	0	3.81	0.04	6.11	0.02	1.52	3.26	0.06	0	1.01	1.06	1.54
Mutant	L1-741	L1-742	L1-743	L1-744	L1-745	L1-746	L1-747	L1-748	L1-749	L1-750	L1-751	L1-752	L1-753	L1-754	L1-755	L1-756	L1-757	L1-758	L1-759	L1-760
EGFP(+)%	4.09	0.11	0.02	1.73	2.62	4.2	3.2	1.35	4.26	2.82	8.92	4.19	0.19	4.19	0.1	0.11	6.3	1.02	0.12	5.23
Mutant	L1-761	L1-762	L1-763	L1-764	L1-765	L1-766	L1-767	L1-768	L1-769	L1-770	L1-771	L1-772	L1-773	L1-774	L1-775	L1-776	L1-777	L1-778	L1-779	L1-780
EGFP(+)%	7.08	6.01	5.41	0.21	5.65	4.14	2.2	1.2	0.27	0	2.8	0.01	2.32	0.07	1.37	2.78	0.57	0	0.01	0

Mutant	L1-781	L1-782	L1-783	L1-784	L1-785	L1-786	L1-787	L1-788	L1-789	L1-790	L1-791	L1-792	L1-793	L1-794	L1-795	L1-796	L1-797	L1-798	L1-799	L1-800
EGFP(+)%	1.53	3.84	1.25	2.56	0.02	0.6	2	3.82	4.05	0.88	0.05	2.14	3.23	0.05	0	3.46	0.05	3.45	0.02	3.52
Mutant	L1-801	L1-802	L1-803	L1-804	L1-805	L1-806	L1-807	L1-808	L1-809	L1-810	L1-811	L1-812	L1-813	L1-814	L1-815	L1-816	L1-817	L1-818	L1-819	L1-820
EGFP(+)%	1.42	3.86	1.99	1.72	0.04	3.89	0	0.08	1.63	0.1	7.28	3.39	0.33	1.74	5.12	1.39	2.56	0.08	0.01	3.4
Mutant	L1-821	L1-822	L1-823	L1-824	L1-825	L1-826	L1-827	L1-828	L1-829	L1-830	L1-831	L1-832	L1-833	L1-834	L1-835	L1-836	L1-837	L1-838	L1-839	L1-840
EGFP(+)%	3.88	0.02	0.82	0.58	0.02	0	2.26	2.63	3.78	0.03	0.01	3.37	0.74	0.04	3.6	0	3.14	3.17	0.04	5.08
Mutant	L1-841	L1-842	L1-843	L1-844	L1-845	L1-846	L1-847	L1-848	L1-849	L1-850	L1-851	L1-852	L1-853	L1-854	L1-855	L1-856	L1-857	L1-858	L1-859	L1-860
EGFP(+)%	0	4.55	0.81	0.28	1.9	4.27	2.61	0.56	3.52	3.69	3.89	0	2.59	0	0.33	2.11	0	1.26	2.15	0
Mutant	L1-861	L1-862	L1-863	L1-864	L1-865	L1-866	L1-867	L1-868	L1-869	L1-870	L1-871	L1-872	L1-873	L1-874	L1-875	L1-876	L1-877	L1-878	L1-879	L1-880
EGFP(+)%	1.27	0.02	0.22	3.02	3.46	0	0	3.3	3.43	3.34	0.04	1.92	1.36	4.12	2.38	0.13	0	3.54	0	0.04
Mutant	L1-881	L1-882	L1-883	L1-884	L1-885	L1-886	L1-887	L1-888	L1-889	L1-890	L1-891	L1-892	L1-893	L1-894	L1-895	L1-896	L1-897	L1-898	L1-899	L1-900
EGFP(+)%	0	3.36	1.9	0.53	2.63	1.02	0.03	1.62	1.91	6.87	3.15	1.15	2.74	0.13	0	3.73	0.02	0.03	0.15	0.66
Mutant	L1-901	L1-902	L1-903	L1-904	L1-905	L1-906	L1-907	L1-908	L1-909	L1-910	L1-911	L1-912	L1-913	L1-914	L1-915	L1-916	L1-917	L1-918	L1-919	L1-920
EGFP(+)%	0.9	0	0.02	0.01	1.26	2.91	1.15	4.09	0.02	3.12	3.11	2.94	1.38	0.14	1.15	0.01	1.85	2.65	0.11	2.35
Mutant	L1-921	L1-922	L1-923	L1-924	L1-925	L1-926	L1-927	L1-928	L1-929	L1-930	L1-931	L1-932	L1-933	L1-934	L1-935	L1-936	L1-937	L1-938	L1-939	L1-940
EGFP(+)%	0.71	0.05	0.14	1.3	2.45	0.01	2.67	0.25	1.51	2.22	1.15	0.84	1.13	1.47	0.05	1.08	0.17	0	0	0.01
Mutant	L1-941	L1-942	L1-943	L1-944	L1-945	L1-946	L1-947	L1-948	L1-949	L1-950	L1-951	L1-952	L1-953	L1-954	L1-955	L1-956	L1-957	L1-958	L1-959	L1-960
EGFP(+)%	0	0.01	2.39	0.12	1.53	3.91	0.51	0.29	0.39	1.96	0.39	0.01	0	1.05	2.72	3.79	1.67	0	0	0
Mutant	L1-961	L1-962	L1-963	L1-964	L1-965	L1-966	L1-967	L1-968	L1-969	L1-970	L1-971	L1-972	L1-973	L1-974	L1-975	L1-976	L1-977	L1-978	L1-979	L1-980
EGFP(+)%	3.31	0	0.04	0.02	0.24	3.85	0.05	4.32	0.11	0.01	1.61	0.69	4.2	4.36	4.39	4.12	3.83	0.05	0.13	1.47
Mutant	L1-981	L1-982	L1-983	L1-984	L1-985	L1-986	L1-987	L1-988	L1-989	L1-990	L1-991	L1-992	L1-993	L1-994	L1-995	L1-996	L1-997	L1-998	L1-999	L1-1000
EGFP(+)%	4.87	0.01	0	0.99	5.84	0.01	0	0.02	0	0.03	2.06	0.07	4.66	3.61	0.15	0.7	3.58	0.13	3.97	0.21
Mutant	L1-1001	L1-1002	L1-1003	L1-1004	L1-1005	L1-1006	L1-1007	L1-1008	L1-1009	L1-1010	L1-1011	L1-1012	L1-1013	L1-1014	L1-1015	L1-1016	L1-1017	L1-1018	L1-1019	L1-1020
EGFP(+)%	0	4.16	0	1.75	1.91	0.57	0	1.79	0.13	0	0.8	0.02	0.04	0.12	1.84	1.74	0	2.16	0.62	2.21
Mutant	L1-1021	L1-1022	L1-1023	L1-1024	L1-1025	L1-1026	L1-1027	L1-1028	L1-1029	L1-1030	L1-1031	L1-1032	L1-1033	L1-1034	L1-1035	L1-1036	L1-1037	L1-1038	L1-1039	L1-1040
EGFP(+)%	0.64	0	0.02	0	0	6.3	5.36	0.35	0.6	0.02	7.67	8.22	2.66	3.3	4.54	0.14	2.28	1.58	2.11	0.48
Mutant	L1-1041	L1-1042	L1-1043	L1-1044	L1-1045	L1-1046	L1-1047	L1-1048	L1-1049	L1-1050	L1-1051	L1-1052	L1-1053	L1-1054	L1-1055	L1-1056	L1-1057	L1-1058	L1-1059	L1-1060
EGFP(+)%	0.3	0.71	0.01	0.37	0	0.22	0	1.27	0	0	3.89	4.38	0.6	0.05	0	1.41	1.1	0	2.92	0
Mutant	L1-1061	L1-1062	L1-1063	L1-1064	L1-1065	L1-1066	L1-1067	L1-1068	L1-1069	L1-1070	L1-1071	L1-1072	L1-1073	L1-1074	L1-1075	L1-1076	L1-1077	L1-1078	L1-1079	L1-1080
EGFP(+)%	0.07	2.8	0	6.55	1.59	0.11	0.19	3	1.1	1.8	0	0.05	0.07	2.99	0.03	0	2.79	0.45	1.91	2.92
Mutant	L1-1081	L1-1082	L1-1083	L1-1084	L1-1085	L1-1086	L1-1087	L1-1088	L1-1089	L1-1090	L1-1091	L1-1092	L1-1093	L1-1094	L1-1095	L1-1096	L1-1097	L1-1098	L1-1099	L1-1100
EGFP(+)%	2.64	3.98	2.97	4.4	1.68	2.2	0	1.9	5.02	0.84	0.3	4.33	0	0	0	0	3.98	0.06	0.23	1.89
Mutant	L1-1101	L1-1102	L1-1103	L1-1104	L1-1105	L1-1106	L1-1107	L1-1108	L1-1109	L1-1110	L1-1111	L1-1112	L1-1113	L1-1114	L1-1115	L1-1116	L1-1117	L1-1118	L1-1119	L1-1120
EGFP(+)%	1.5	0	0	0	1.72	0	1.95	1.3	0.91	1.71	2.81	2.13	3.93	0	0	3.67	0	0	0	0
Mutant	L1-1121	L1-1122	L1-1123	L1-1124	L1-1125	L1-1126	L1-1127	L1-1128	L1-1129	L1-1130	L1-1131	L1-1132	L1-1133	L1-1134	L1-1135	L1-1136	L1-1137	L1-1138	L1-1139	L1-1140
EGFP(+)%	0	0	0	0	2.59	0	0.07	0.29	0.24	0.08	1119	0.36	0.04	0.05	0.04	0.19	6.77	0.16	5.55	4.81
Mutant	L1-1141	L1-1142	L1-1143	L1-1144	L1-1145	L1-1146	L1-1147	L1-1148	L1-1149	L1-1150	L1-1151	L1-1152	L1-1153	L1-1154	L1-1155	L1-1156	L1-1157	L1-1158	L1-1159	L1-1160
EGFP(+)%	0	0.31	0.39	6.64	0.17	3.08	5.71	3.01	1.43	5.77	1.61	2.15	2.59	1.2	0.26	1.52	0.1	0	2.36	0.62
Mutant	L1-1161	L1-1162	L1-1163	L1-1164	L1-1165	L1-1166	L1-1167	L1-1168	L1-1169	L1-1170	L1-1171	L1-1172	L1-1173	L1-1174	L1-1175	L1-1176	L1-1177	L1-1178	L1-1179	L1-1180
EGFP(+)%	2.49	4.08	0.08	0.01	5.19	0	0.03	4.4	0.52	0	0.03	4.47	0.37	0	0.58	3.31	0.08	0	0.05	0.63
Mutant	L1-1181	L1-1182	L1-1183	L1-1184	L1-1185	L1-1186	L1-1187	L1-1188	L1-1189	L1-1190	L1-1191	L1-1192	L1-1193	L1-1194	L1-1195	L1-1196	L1-1197	L1-1198	L1-1199	L1-1200
EGFP(+)%	0.08	5.88	7.94	0.04	2.24	4.29	1.98	3.91	5.72	2.46	0.37	0.37	0.18	1.31	0.05	3.86	0.01	2.24	0.37	5.52
Mutant	L1-1201	L1-1202	L1-1203	L1-1204	L1-1205	L1-1206	L1-1207	L1-1208	L1-1209	L1-1210	L1-1211	L1-1212	L1-1213	L1-1214	L1-1215	L1-1216	L1-1217	L1-1218	L1-1219	L1-1220
EGFP(+)%	0.03	3.11	3.18	4.27	0.49	1.24	4.01	0	4.08	1.95	3.83	0.09	5.34	0.02	0.05	0.21	3.85	2.75	0.44	0.02
Mutant	L1-1221	L1-1222	L1-1223	L1-1224	L1-1225	L1-1226	L1-1227	L1-1228	L1-1229	L1-1230	L1-1231	L1-1232	L1-1233	L1-1234	L1-1235	L1-1236	L1-1237	L1-1238	L1-1239	L1-1240
EGFP(+)%	2.15	1.48	2.91	0	0	0.09	0	0.06	2.79	0	0	2.92	0	1.41	0	0.02	0.41	0.02	0.88	0.09
Mutant	L1-1241	L1-1242	L1-1243	L1-1244	L1-1245	L1-1246	L1-1247	L1-1248	L1-1249	L1-1250	L1-1251	L1-1252	L1-1253	L1-1254	L1-1255	L1-1256	L1-1257	L1-1258	L1-1259	L1-1260
EGFP(+)%	2.54	0.43	3.93	0.01	2.17	1.46	0.34	0.69	0.04	0	0.06	0.03	4.79	5.31	0.42	0.96	0.01	0.29	6.77	0
Mutant	L1-1261	L1-1262	L1-1263	L1-1264	L1-1265	L1-1266	L1-1267	L1-1268	L1-1269	L1-1270	L1-1271	L1-1272	L1-1273	L1-1274	L1-1275	L1-1276	L1-1277	L1-1278	L1-1279	L1-1280
EGFP(+)%	0.03	4.75	4.72	0.5	0.06	0.04	2.57	0.12	0.01	0.99	1.4	0.99	0.11	0.05	6.66	1.08	0	5.74	5.35	0.15
Mutant	L1-1281	L1-1282	L1-1283	L1-1284	L1-1285	L1-1286	L1-1287	L1-1288	L1-1289	L1-1290	L1-1291	L1-1292	L1-1293	L1-1294	L1-1295	L1-1296	L1-1297	L1-1298	L1-1299	L1-1300
EGFP(+)%	1.92	2.57	0.32	3.92	0.58	3.74	0	0.02	0.1	3.04	0.02	2.82	0.24	0	5.32	3.86	0.38	0.55	0	3.08
Mutant	L1-1301	L1-1302	L1-1303	L1-1304	L1-1305	L1-1306	L1-1307	L1-1308	L1-1309	L1-1310	L1-1311	L1-1312	L1-1313	L1-1314	L1-1315	L1-1316	L1-1317	L1-1318	L1-1319	L1-1320
EGFP(+)%	0.36	1.86	0	2.29	0	0.5	0	0	0	0.11	0.77	0.03	1.79	0	0.04	0	0	0.37	2.07	0.89
Mutant	L1-1321	L1-1322	L1-1323	L1-1324	L1-1325	L1-1326	L1-1327	L1-1328	L1-1329	L1-1330	L1-1331	L1-1332	L1-1333	L1-1334	L1-1335	L1-1336	L1-1337	L1-1338	L1-1339	L1-1340
EGFP(+)%	1.04	0.44	0	0.14	0	1.71	0	3.26	1.79	1	1.34	0	0	0	4.74	3.65	2.61	0.5	1.13	0.33
Mutant	L1-1341	L1-1342	L1-1343	L1-1344	L1-1345	L1-1346	L1-1347	L1-1348	L1-1349	L1-1350	L1-1351	L1-1352	L1-1353	L1-1354	L1-1355	L1-1356	L1-1357	L1-1358	L1-1359	L1-1360
EGFP(+)%	0.02	0	4.32	2.37	0	0.59	0.06	2.04	0.8	0	4.34	2.19	7.57	4.24	3.51	0	0.18	2.71	2.06	0.36
Mutant	L1-1361	L1-1362	L1-1363	L1-1364	L1-1365	L1-1366	L1-1367	L1-1368	L1-1369	L1-1370	L1-1371	L1-1372	L1-1373	L1-1374	L1-1375	L1-1376	L1-1377	L1-1378	L1-1379	L1-1380
EGFP(+)%	2.43	1.16	2.97	0	2.25	0	0.33	0	0	3.44	3.49	0	3.65	2.07	0.25	0	2.96	2.95	0.2	0
Mutant	L1-1381	L1-1382	L1-1383	L1-1384	L1-1385	L1-1386	L1-1387	L1-1388	L1-1389	L1-1390	L1-1391	L1-1392	L1-1393	L1-1394	L1-1395	L1-1396	L1-1397	L1-1398	L1-1399	L1-1400
EGFP(+)%	1.02	1.15	0.03	0.02	0.45	0.1	1.37	0.8	0	0.01	0.41	3.99	2.96	0	0	0.2	0	1.45	1.74	1.1
Mutant	L1-1401	L1-1402	L1-1403	L1-1404	L1-1405	L1-1406	L1-1407	L1-1408	L1-1409	L1-1410	L1-1411	L1-1412	L1-1413	L1-1414	L1-1415	L1-1416	L1-1417	L1-1418	L1-1419	L1-1420
EGFP(+)%	3.04	0	0.8	0	0.08	1.25	1.7	0.22	0	0.92	0	0	1.36	0.02	2.44	0	2.21	1.24	1.08	0.02
Mutant	L1-1421	L1-1422	L1-1423	L1-1424	L1-1425	L1-1426	L1-1427	L1-1428												
EGFP(+)%	0	4.37	3.39	0.54	2.58	3.31	2.08	1.65												

Supplementary Table. 14 The raw screening data of Library 2.

Mutant	L2-1	L2-2	L2-3	L2-4	L2-5	L2-6	L2-7	L2-8	L2-9	L2-10	L2-11	L2-12	L2-13	L2-14	L2-15	L2-16	L2-17	L2-18	L2-19	L2-20
EGFP (+)%	0.48	1.38	2.73	2.73	2.84	5.86	0.46	1.7	5.12	3.59	2.14	4.45	1.64	3.91	3.87	0.54	4.01	3.7	2.4	4.14
Mutant	L2-21	L2-22	L2-23	L2-24	L2-25	L2-26	L2-27	L2-28	L2-29	L2-30	L2-31	L2-32	L2-33	L2-34	L2-35	L2-36	L2-37	L2-38	L2-39	L2-40
EGFP (+)%	3.22	2.86	1.3	4.39	2.68	4.19	0.06	0.94	2.01	3.35	2.37	2.59	2.96	2.81	8.26	2.31	2.36	0.03	2.91	4.32
Mutant	L2-41	L2-42	L2-43	L2-44	L2-45	L2-46	L2-47	L2-48	L2-49	L2-50	L2-51	L2-52	L2-53	L2-54	L2-55	L2-56	L2-57	L2-58	L2-59	L2-60
EGFP (+)%	1.64	4.06	0.32	1.26	1.94	0.02	2.42	0	2.93	2.37	2.35	1.25	2.17	1.37	2.8	1.14	1.35	2.13	5	0.09
Mutant	L2-61	L2-62	L2-63	L2-64	L2-65	L2-66	L2-67	L2-68	L2-69	L2-70	L2-71	L2-72	L2-73	L2-74	L2-75	L2-76	L2-77	L2-78	L2-79	L2-80
EGFP (+)%	3.57	2.7	0.83	0.67	0.56	1.65	0.76	3.91	2.81	2.56	0.15	1.71	0.89	2.49	2.7	2.26	2.41	2.56	2.6	0.69
Mutant	L2-81	L2-82	L2-83	L2-84	L2-85	L2-86	L2-87	L2-88	L2-89	L2-90	L2-91	L2-92	L2-93	L2-94	L2-95	L2-96	L2-97	L2-98	L2-99	L2-100
EGFP (+)%	4.23	4.31	3.57	1.4	2.26	0.59	2.42	3.08	0	3.42	3.34	3.85	2.68	1.32	2.77	2.72	0.04	0.56	1.97	1.81
Mutant	L2-101	L2-102	L2-103	L2-104	L2-105	L2-106	L2-107	L2-108	L2-109	L2-110	L2-111	L2-112	L2-113	L2-114	L2-115	L2-116	L2-117	L2-118	L2-119	L2-120
EGFP (+)%	3.44	0.39	7.89	1.75	3.92	9.06	3.03	2.6	4.83	0.12	2.29	2.61	4.8	4.73	2.85	3.08	1.6	8.9	0.13	0.09
Mutant	L2-121	L2-122	L2-123	L2-124	L2-125	L2-126	L2-127	L2-128	L2-129	L2-130	L2-131	L2-132	L2-133	L2-134	L2-135	L2-136	L2-137	L2-138	L2-139	L2-140
EGFP (+)%	0.45	2.91	0.14	5.37	3.67	3.43	1.5	3.53	1.11	3.56	5.7	1.77	3.81	4.99	6.1	2.63	3.37	2.38	1.42	6.17
Mutant	L2-141	L2-142	L2-143	L2-144	L2-145	L2-146	L2-147	L2-148	L2-149	L2-150	L2-151	L2-152	L2-153	L2-154	L2-155	L2-156	L2-157	L2-158	L2-159	L2-160
EGFP (+)%	3.27	6.26	3.26	1.23	5.1	3.63	0.25	4.27	1.6	4.72	10.71	0.15	4.1	2.1	1.82	11.29	2.84	3.89	10.21	1.79
Mutant	L2-161	L2-162	L2-163	L2-164	L2-165	L2-166	L2-167	L2-168	L2-169	L2-170	L2-171	L2-172	L2-173	L2-174	L2-175	L2-176	L2-177	L2-178	L2-179	L2-180
EGFP (+)%	3.27	7.85	8.6	4.8	1.77	5.05	1.9	8.25	3.4	5.52	8.31	1.52	1.42	2.94	1.03	1.74	3.03	6.59	1.84	0.01
Mutant	L2-181	L2-182	L2-183	L2-184	L2-185	L2-186	L2-187	L2-188	L2-189	L2-190	L2-191	L2-192	L2-193	L2-194	L2-195	L2-196	L2-197	L2-198	L2-199	L2-200
EGFP (+)%	2.95	1.54	2.35	2.13	3.24	2.3	1.6	2.36	2.48	2.5	1.74	1.69	2.17	1.47	2.04	2.15	2.87	1	1.62	2.35
Mutant	L2-201	L2-202	L2-203	L2-204	L2-205	L2-206	L2-207	L2-208	L2-209	L2-210	L2-211	L2-212	L2-213	L2-214	L2-215	L2-216	L2-217	L2-218	L2-219	L2-220
EGFP (+)%	4.02	1.98	0	1.85	3.56	3.58	3.98	1.96	0.75	3.87	2.62	2.57	1.23	2.43	1.87	0.83	1.65	1.15	4.35	1.19
Mutant	L2-221	L2-222	L2-223	L2-224	L2-225	L2-226	L2-227	L2-228	L2-229	L2-230	L2-231	L2-232	L2-233	L2-234	L2-235	L2-236	L2-237	L2-238	L2-239	L2-240
EGFP (+)%	0.98	1.6	3.46	2.2	2.18	2.68	0.49	3.26	1.2	0.21	2.24	0.87	0.34	2	0	3.08	1.07	3.41	2.53	3.27
Mutant	L2-241	L2-242	L2-243	L2-244	L2-245	L2-246	L2-247	L2-248	L2-249	L2-250	L2-251	L2-252	L2-253	L2-254	L2-255	L2-256	L2-257	L2-258	L2-259	L2-260
EGFP (+)%	0	2.54	0.09	2.31	3.6	1.23	3.51	1.32	1.35	3.18	2.34	1.74	3.92	2.91	2.17	0.31	3.32	3.14	0.06	3.26
Mutant	L2-261	L2-262	L2-263	L2-264	L2-265	L2-266	L2-267	L2-268	L2-269	L2-270	L2-271	L2-272	L2-273	L2-274	L2-275	L2-276	L2-277	L2-278	L2-279	L2-280
EGFP (+)%	3.1	2.58	0.26	6.31	2.44	0.32	1.07	2.11	0.69	2.06	1.42	0.13	2.46	1.67	5.51	1.85	2.05	1.56	3.2	1.87
Mutant	L2-281	L2-282	L2-283	L2-284	L2-285	L2-286	L2-287	L2-288	L2-289	L2-290	L2-291	L2-292	L2-293	L2-294	L2-295	L2-296	L2-297	L2-298	L2-299	L2-300
EGFP (+)%	2.1	1.97	0.5	2.56	0.35	2.22	2.98	3.24	4.28	0	1.43	1.73	1.98	0.6	3.28	0.03	3.11	0.22	2.35	4.67
Mutant	L2-301	L2-302	L2-303	L2-304	L2-305	L2-306	L2-307	L2-308	L2-309	L2-310	L2-311	L2-312	L2-313	L2-314	L2-315	L2-316	L2-317	L2-318	L2-319	L2-320
EGFP (+)%	1.58	1.08	3.72	2.41	0.11	1.42	1.58	2.22	0.29	0.27	2.17	0.4	4.75	1.79	0.02	2.25	0.05	2.48	3.15	8.59
Mutant	L2-321	L2-322	L2-323	L2-324	L2-325	L2-326	L2-327	L2-328	L2-329	L2-330	L2-331	L2-332	L2-333	L2-334	L2-335	L2-336	L2-337	L2-338	L2-339	L2-340
EGFP (+)%	0	2.61	0.03	2.23	3.48	5.82	3.43	1.89	1.8	2.1	0	1.87	2.86	1.55	2.29	1.81	0.16	2.3	0.74	2.19
Mutant	L2-341	L2-342	L2-343	L2-344	L2-345	L2-346	L2-347	L2-348	L2-349	L2-350	L2-351	L2-352	L2-353	L2-354	L2-355	L2-356	L2-357	L2-358	L2-359	L2-360
EGFP (+)%	2.43	1.77	3.52	1.73	3.68	3.2	1.88	3.97	4.51	2.37	2.35	0.01	2.87	3.48	2.53	0.99	2.08	0.63	1.16	4.53
Mutant	L2-361	L2-362	L2-363	L2-364	L2-365	L2-366	L2-367	L2-368	L2-369	L2-370	L2-371	L2-372	L2-373	L2-374	L2-375	L2-376	L2-377	L2-378	L2-379	L2-380
EGFP (+)%	3.65	0.14	0.59	1.51	0	2.16	0.72	3.59	3.1	7	1.1	5.35	5.68	3.48	4.44	1.59	4.64	5.09	3.85	3.26
Mutant	L2-381	L2-382	L2-383	L2-384	L2-385	L2-386	L2-387	L2-388	L2-389	L2-390	L2-391	L2-392	L2-393	L2-394	L2-395	L2-396	L2-397	L2-398	L2-399	L2-400
EGFP (+)%	3.8	3	2.2	7.57	0.14	2.95	2.61	3.03	1.87	4.17	0.32	5.29	0.76	1.66	3.51	3.65	0	2.14	7.85	2.13
Mutant	L2-401	L2-402	L2-403	L2-404	L2-405	L2-406	L2-407	L2-408	L2-409	L2-410	L2-411	L2-412	L2-413	L2-414	L2-415	L2-416	L2-417	L2-418	L2-419	L2-420
EGFP (+)%	0.89	2.73	0.05	3.75	3.84	2.18	7.85	3.38	2.7	3.73	2.33	1.67	1.28	3.08	3.3	3.12	0.74	3.96	3.71	2.45
Mutant	L2-421	L2-422	L2-423	L2-424	L2-425	L2-426	L2-427	L2-428	L2-429	L2-430	L2-431	L2-432	L2-433	L2-434	L2-435	L2-436	L2-437	L2-438	L2-439	L2-440
EGFP (+)%	0.58	1.94	0.01	1.39	4.2	0.06	1.86	0.37	2.72	1.35	1.48	1.06	1.57	5.85	2.44	0.08	5.61	2.58	3.42	2.22
Mutant	L2-441	L2-442	L2-443	L2-444	L2-445	L2-446	L2-447	L2-448	L2-449	L2-450	L2-451	L2-452	L2-453	L2-454	L2-455	L2-456	L2-457	L2-458	L2-459	L2-460
EGFP (+)%	2.3	2.13	3.89	1.45	2.73	3.71	1.29	5.34	2.97	0	1.02	2.62	4.84	8.26	7.99	1.05	5.73	0.99	1.11	2.38
Mutant	L2-461	L2-462	L2-463	L2-464	L2-465	L2-466	L2-467	L2-468	L2-469	L2-470	L2-471	L2-472	L2-473	L2-474	L2-475	L2-476	L2-477	L2-478	L2-479	L2-480
EGFP (+)%	0.84	0	1.53	0.53	2.13	2.29	2.37	1.3	1.44	1.67	0.49	4.12	1.44	3.35	6.18	1.97	3.65	4.14	0.83	1.99
Mutant	L2-481	L2-482	L2-483	L2-484	L2-485	L2-486	L2-487	L2-488	L2-489	L2-490	L2-491	L2-492	L2-493	L2-494	L2-495	L2-496	L2-497	L2-498	L2-499	L2-500
EGFP (+)%	0.7	0.01	1.93	2.79	2.83	2.19	0	0	2.43	3.49	2.16	2.04	2.6	0.21	1.02	3.54	4	4.56	2.47	3.7
Mutant	L2-501	L2-502	L2-503	L2-504	L2-505	L2-506	L2-507	L2-508	L2-509	L2-510	L2-511	L2-512	L2-513	L2-514	L2-515	L2-516	L2-517	L2-518	L2-519	L2-520
EGFP (+)%	4.97	2.54	0	0	1.7	1.75	1.72	4.48	2.18	2.54	1.04	2.86	2.59	0	1.88	1.95	3.23	1.54	2.5	2.91
Mutant	L2-521	L2-522	L2-523	L2-524	L2-525	L2-526	L2-527	L2-528	L2-529	L2-530	L2-531	L2-532	L2-533	L2-534	L2-535	L2-536	L2-537	L2-538	L2-539	L2-540
EGFP (+)%	2.74	2.4	3.12	2.47	4.15	1.32	0.05	3.57	0.56	1.46	0	1.33	6.54	5.26	7.8	2.83	5.42	2.53	0.7	4.13
Mutant	L2-541	L2-542	L2-543	L2-544	L2-545	L2-546	L2-547	L2-548	L2-549	L2-550										
EGFP (+)%	3.25	3.23	4.37	10.85	1.54	4.17	4.26	3.94	0.78	1.14										