Supplementary Table 1. Summary of numbers of RNA edits observed in all RNA-seq experiments

Figure	Cell	BE	gRNA	Sort	Replicate	**A-to-I (for ABE)** or **C-to-U (for CBE)**	Other	A-to-I or C-to-U (%)	Mean Editing Efficiency	Median Editing Efficiency
Fig. 1b	HEK293T	ABEmax (data from Ref[5])	HEK site 2 (ABE site 1)	Top 5%	Rep. 1	**37,059**	87	99.77	22.5	18.8
Fig. 1c & d	HEK293T	ABEmax	HEK site 2 (ABE site 1)	All GFP	Rep. 1	**16,049**	201	98.76	22.1	17.7
					Rep. 2	**27,706**	246	99.12	22.1	18.3
					Rep. 3	**29,597**	193	99.35	21.8	18.2
		miniABEmax	HEK site 2 (ABE site 1)	All GFP	Rep. 1	**10,047**	231	97.75	17.2	15.7
					Rep. 2	**26,552**	251	99.06	18.5	16.9
					Rep. 3	**29,841**	177	99.41	18.4	16.9
		miniABEmax-K20A/R21A	HEK site 2 (ABE site 1)	All GFP	Rep. 1	**1,080**	238	81.94	19.3	17.1
					Rep. 2	**2,203**	383	85.19	20.4	18.2
					Rep. 3	**2,069**	315	86.79	19.6	17.9
		miniABEmax-V82G	HEK site 2 (ABE site 1)	All GFP	Rep. 1	**971**	216	81.80	19.3	17.3
					Rep. 2	**1,654**	333	83.24	20.5	18.2
					Rep. 3	**1,172**	276	80.94	21.3	19.0
	HEK293T	ABEmax	ABE site 16	All GFP	Rep. 1	**28,099**	197	99.30	22.2	18.5
					Rep. 2	**26,571**	238	99.11	22.0	18.3
					Rep. 3	**26,948**	238	99.12	21.8	18.1
		miniABEmax	ABE site 16	All GFP	Rep. 1	**23,187**	216	99.08	18.5	16.9
					Rep. 2	**13,897**	202	98.57	17.7	16.1
					Rep. 3	**19,907**	232	98.85	17.9	16.5
		miniABEmax-K20A/R21A	ABE site 16	All GFP	Rep. 1	**1,376**	292	82.49	20.2	17.9
					Rep. 2	**1,338**	291	82.14	19.5	17.6
					Rep. 3	**1,696**	295	85.18	19.1	17.1
		miniABEmax-V82G	ABE site 16	All GFP	Rep. 1	**936**	243	79.39	19.9	17.6
					Rep. 2	**1,224**	336	78.46	18.9	16.7
					Rep. 3	**1,159**	269	81.16	20.5	18.2
	HEK293T	ABEmax	NT	All GFP	Rep. 1	**15,909**	202	98.75	22.2	17.9
					Rep. 2	**31,521**	229	99.28	22.1	18.4
					Rep. 3	**24,326**	196	99.20	21.6	17.8
		miniABEmax	NT	All GFP	Rep. 1	**8,748**	379	95.85	17.6	16.1
					Rep. 2	**29,540**	244	99.18	18.8	17.1
					Rep. 3	**25,426**	261	98.98	18.4	16.9
		miniABEmax-K20A/R21A	NT	All GFP	Rep. 1	**690**	206	77.01	17.6	15.8
					Rep. 2	**2,102**	325	86.61	20.6	18.2
					Rep. 3	**2,191**	265	89.21	19.2	17.4
		miniABEmax-V82G	NT	All GFP	Rep. 1	**762**	143	84.20	18.3	16.7
					Rep. 2	**1,634**	304	84.31	21.0	18.2
					Rep. 3	**1,582**	282	84.87	18.6	16.7
	HEK293T	GFP	--	All GFP	Rep. 1	**423**	202	67.68	19.3	17.4
					Rep. 2	**270**	175	60.67	17.6	16.0
					Rep. 3	**363**	168	68.36	19.2	17.3
Fig. 3b	HEK293T	GFP	--	MFI-matched to top 5% BE3 expression	Rep. 1	**31**	131	19.14	18.0	15.8
		hA3A-BE3	*RNF2*	Top 5%	Rep. 1	**30,435**	8	99.97	23.4	18.0
					Rep. 2	**27,190**	8	99.97	23.3	18.0
					Rep. 3	**32,402**	11	99.97	23.2	17.8
		eA3A-BE3	*RNF2*	Top 5%	Rep. 1	**98**	101	49.25	19.6	16.1
					Rep. 2	**72**	87	45.28	20.7	17.6
					Rep. 3	**113**	78	59.16	22.2	17.3
		hAID-BE3	*RNF2*	Top 5%	Rep. 1	**45**	201	18.29	17.2	16.7
					Rep. 2	**34**	144	19.10	15.1	15.1
					Rep. 3	**70**	234	23.03	16.1	15.4
		Target-AID	*RNF2*	Top 5%	Rep. 1	**15**	118	11.28	15.7	15.4
					Rep. 2	**19**	132	12.58	17.1	16.3
					Rep. 3	**10**	60	14.29	14.0	14.6
		BE3-R33A (data from Ref[5])	*RNF2*	Top 5%	Rep. 1	**235**	90	72.31	14.4	13.0
					Rep. 2	**355**	111	76.18	17.0	15.4
					Rep. 3	**195**	57	77.38	14.4	13.4
		BE3-R33A/K34A (data from Ref[5])	*RNF2*	Top 5%	Rep. 1	**20**	77	20.62	13.6	15.4
					Rep. 2	**18**	95	15.93	20.3	16.4
					Rep. 3	**7**	42	14.29	17.3	13.6

Supplementary Table 2. Summary of numbers of BE-induced self-edits and gRNA edits

Cell	BE	gRNA	Sort	Replicate	Number of BE self-edits				Number of gRNA edits
					Nonsense	Missense	Synonymous	**Total**	
HEK293T	WT BE3	*RNF2*	All GFP	Rep. 1	11	44	70	**125**	0
				Rep. 2	8	25	51	**84**	0
				Rep. 3	10	38	57	**105**	0
	WT BE3	*EMX1*	All GFP	Rep. 1	6	32	56	**94**	0
				Rep. 2	9	29	45	**83**	0
				Rep. 3	6	31	50	**87**	0
HEK293T	WT BE3	*RNF2*	Top 5%	Rep. 1	14	55	80	**149**	0
				Rep. 2	15	64	98	**177**	0
				Rep. 3	15	57	83	**155**	0
	BE3-R33A	*RNF2*	Top 5%	Rep. 1	0	0	0	**0**	0
				Rep. 2	0	0	0	**0**	0
				Rep. 3	0	0	0	**0**	0
	BE3-R33A/K34A	*RNF2*	Top 5%	Rep. 1	0	0	0	**0**	0
				Rep. 2	0	0	0	**0**	0
				Rep. 3	0	0	0	**0**	0
HepG2	WT BE3	*RNF2*	Top 5%	Rep. 1	10	37	66	**113**	0
				Rep. 2	10	35	56	**101**	0
				Rep. 3	8	45	62	**115**	0
	BE3-R33A	*RNF2*	Top 5%	Rep. 1	0	0	0	**0**	0
				Rep. 2	0	0	0	**0**	0
				Rep. 3	0	0	0	**0**	0
	BE3-R33A/K34A	*RNF2*	Top 5%	Rep. 1	0	0	0	**0**	0
				Rep. 2	0	0	0	**0**	0
				Rep. 3	0	0	0	**0**	0
HEK293T	hA3A-BE3	*RNF2*	Top 5%	Rep. 1	6	5	17	**28**	0
				Rep. 2	6	5	17	**28**	0
				Rep. 3	6	6	19	**31**	0
	eA3A-BE3	*RNF2*	Top 5%	Rep. 1	0	0	0	**0**	0
				Rep. 2	0	0	0	**0**	0
				Rep. 3	0	0	0	**0**	0
	hAID-BE3	*RNF2*	Top 5%	Rep. 1	0	0	0	**0**	0
				Rep. 2	0	0	0	**0**	0
				Rep. 3	0	0	0	**0**	0
	Target-AID	*RNF2*	Top 5%	Rep. 1	0	0	0	**0**	0
				Rep. 2	0	0	0	**0**	0
				Rep. 3	0	0	0	**0**	0
HEK293T	ABEmax	HEK site 2 (ABE site 1)	All GFP	Rep. 1	—	36	3	**39**	0
				Rep. 2	—	54	3	**57**	0
				Rep. 3	—	60	4	**64**	0
	miniABEmax	HEK site 2 (ABE site 1)	All GFP	Rep. 1	—	33	2	**35**	1
				Rep. 2	—	53	4	**57**	1
				Rep. 3	—	61	3	**64**	1
	miniABEmax-K20A/R21A	HEK site 2 (ABE site 1)	All GFP	Rep. 1	—	0	1	**1**	0
				Rep. 2	—	2	1	**3**	0
				Rep. 3	—	1	1	**2**	1
	miniABEmax-V82G	HEK site 2 (ABE site 1)	All GFP	Rep. 1	—	0	0	**0**	1
				Rep. 2	—	0	0	**0**	1
				Rep. 3	—	0	0	**0**	1
HEK293T	ABEmax	ABE site 16	All GFP	Rep. 1	—	51	3	**54**	0
				Rep. 2	—	48	4	**52**	1
				Rep. 3	—	48	2	**50**	0
	miniABEmax	ABE site 16	All GFP	Rep. 1	—	41	3	**44**	1
				Rep. 2	—	33	2	**35**	1
				Rep. 3	—	37	2	**39**	1
	miniABEmax-K20A/R21A	ABE site 16	All GFP	Rep. 1	—	0	1	**1**	1
				Rep. 2	—	0	1	**1**	1
				Rep. 3	—	1	1	**2**	1
	miniABEmax-V82G	ABE site 16	All GFP	Rep. 1	—	0	0	**0**	1
				Rep. 2	—	0	0	**0**	1
				Rep. 3	—	0	0	**0**	1
HEK293T	ABEmax	NT	All GFP	Rep. 1	—	37	2	**39**	0
				Rep. 2	—	54	4	**58**	0
				Rep. 3	—	59	4	**63**	0
	miniABEmax	NT	All GFP	Rep. 1	—	29	2	**31**	1
				Rep. 2	—	63	5	**68**	1
				Rep. 3	—	62	4	**66**	1
	miniABEmax-K20A/R21A	NT	All GFP	Rep. 1	—	1	1	**2**	0
				Rep. 2	—	2	1	**3**	0
				Rep. 3	—	2	1	**3**	0
	miniABEmax-V82G	NT	All GFP	Rep. 1	—	0	0	**0**	1
				Rep. 2	—	0	0	**0**	1
				Rep. 3	—	0	0	**0**	1

Supplementary Note 1.

The ABE 7.10 variant, on which ABEmax is based, was derived through a successive series of engineering steps with different variants numbered sequentially as alterations were made[1]. The second (wild-type) TadA domain was added to ABE 2.6 and then the subsequent 47 iterations of optimization that led to ABE 7.10 were performed on a dimeric TadA architecture (Extended Data Fig. 1 of Ref. 1). ABE 2.1 (which lacked the additional wild-type TadA) possessed comparable editing efficiencies to the heterodimeric architecture of the same variant, ABE 2.10 (Fig. 3a of Ref. 1). Thus, even at this early stage of the engineering process, the additional wild-type TadA domain was not required to enable programmable A-to-G DNA base editing. In addition, the subsequent 47 stages of directed evolution and optimization leading to the final variant ABE 7.10 might have yielded a TadA* variant with greatly improved DNA editing capabilities that might therefore even less dependent on the presence of a linked TadA domain. Finally, because ABEmax is a codon optimized version of the ABE7.10 variant, it is possible that the enhanced expression of this base editor might partially rescue a moderate loss of DNA base editing activity.

Reference:

1. Gaudelli, N. M. *et al.* Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* **551**, 464-471, doi:10.1038/nature24644 (2017).

Supplementary Note 2. FACS gating examples for GFP cell sorting conditions.

Please find attached representative FACS plot data (batch analysis output) demonstrating how control and base editor expressing HEK293T cells were sorted. All data were generated at the MGH Molecular Pathology Flow Cytometry Core Facility on a BD FACSAria II cytometer using BD FACSDiva software (version 6.1.3). We exclusively sorted GFP+ cells.
The depicted dot plots were used for doublet exclusion as well as gating for the cell population and the GFP+ cells being sorted. FITC (x-axis) is a fluorochrome that closely matches the spectral characteristics of GFP.

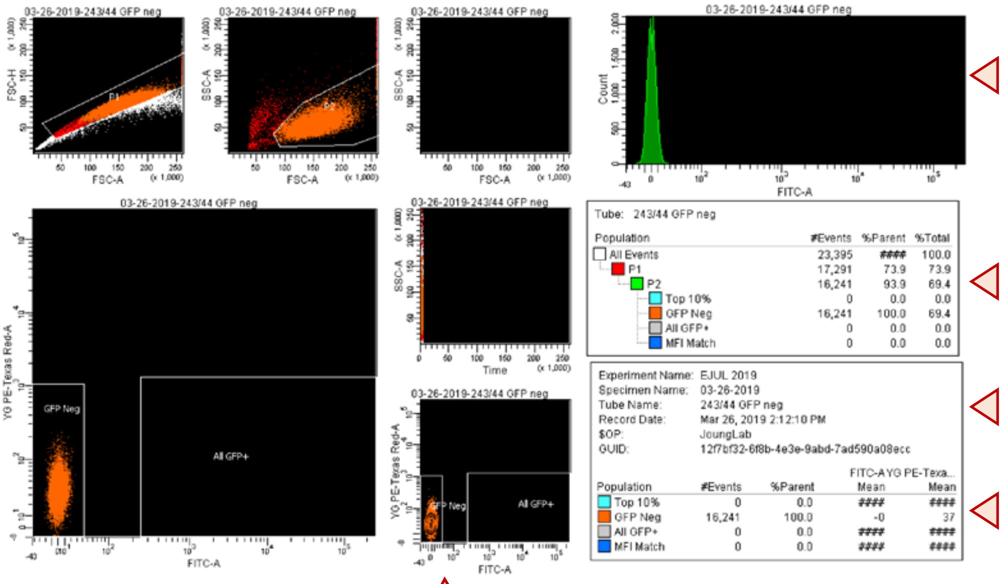
FACS Raw Data Analysis Legends

BD FACSDiva 6.1.3
Batch Analysis

Gating on population

Doublet exclusion

Population histogram



Tube: 243/44 GFP neg

Population	#Events	%Parent	%Total
☐ All Events	23,395	####	100.0
🟥 P1	17,291	73.9	73.9
🟩 P2	16,241	93.9	69.4
☐ Top 10%	0	0.0	0.0
🟧 GFP Neg	16,241	100.0	69.4
☐ All GFP+	0	0.0	0.0
🟦 MFI Match	0	0.0	0.0

Population statistics

Experiment Name: EJUL 2019
Specimen Name: 03-26-2019
Tube Name: 243/44 GFP neg
Record Date: Mar 26, 2019 2:12:10 PM
$OP: JoungLab
GUID: 12f7bf32-6f8b-4e3e-9abd-7ad590a08ecc

Population	#Events	%Parent	FITC-A Mean	YG PE-Texa... Mean
☐ Top 10%	0	0.0	####	####
🟧 GFP Neg	16,241	100.0	-0	37
☐ All GFP+	0	0.0	####	####
🟦 MFI Match	0	0.0	####	####

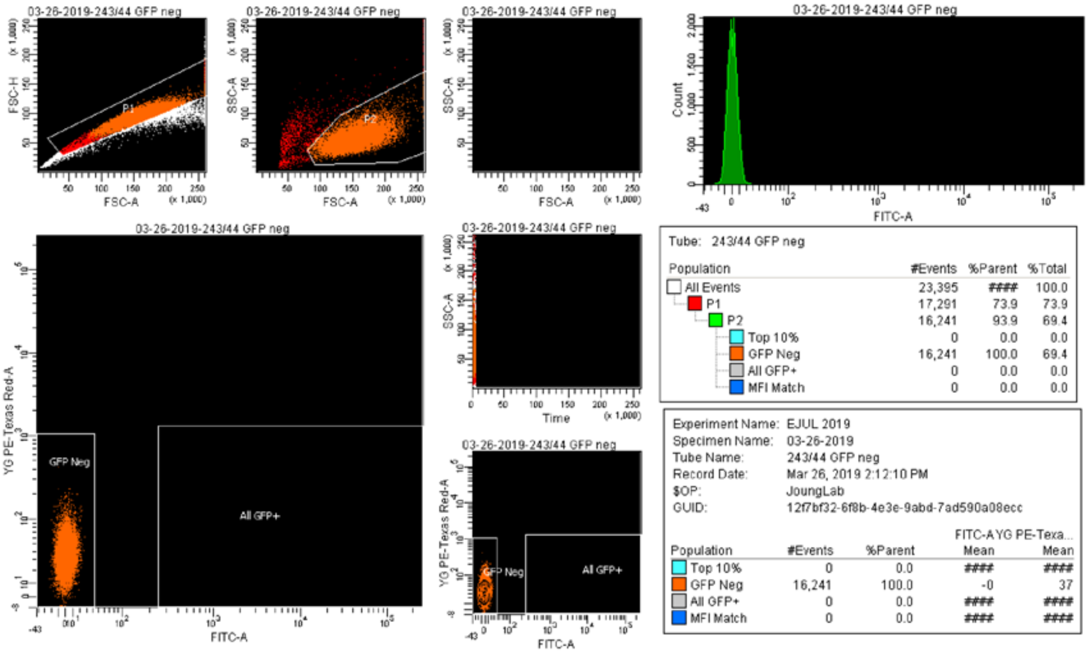
Experiment details/date

Gating for GFP+ cells

Population statistics

Contour plot

HEK293T **Negative Control** (no transfection)



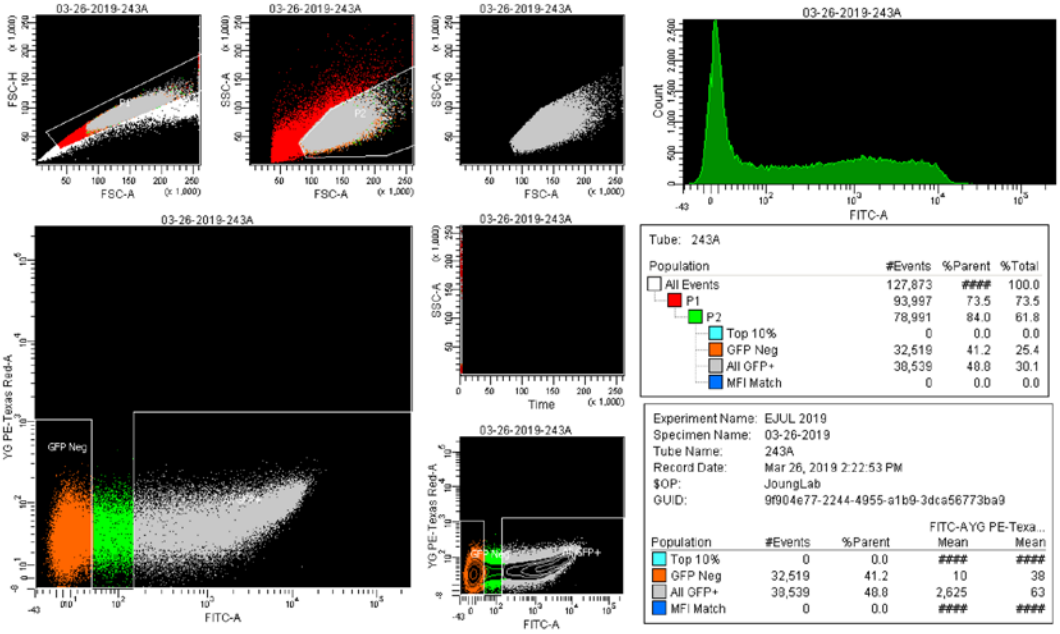
Tube: 243/44 GFP neg

Population	#Events	%Parent	%Total
☐ All Events	23,395	####	100.0
🟥 P1	17,291	73.9	73.9
🟩 P2	16,241	93.9	69.4
🟦 Top 10%	0	0.0	0.0
🟧 GFP Neg	16,241	100.0	69.4
▨ All GFP+	0	0.0	0.0
🟦 MFI Match	0	0.0	0.0

Experiment Name: EJUL 2019
Specimen Name: 03-26-2019
Tube Name: 243/44 GFP neg
Record Date: Mar 26, 2019 2:12:10 PM
$OP: JoungLab
GUID: 12f7bf32-6f8b-4e3e-9abd-7ad590a08ecc

Population	#Events	%Parent	FITC-A Mean	YG PE-Texa... Mean
🟦 Top 10%	0	0.0	####	####
🟧 GFP Neg	16,241	100.0	-0	37
▨ All GFP+	0	0.0	####	####
🟦 MFI Match	0	0.0	####	####

HEK293T **All GFP+**
nCas9 Control for ABE



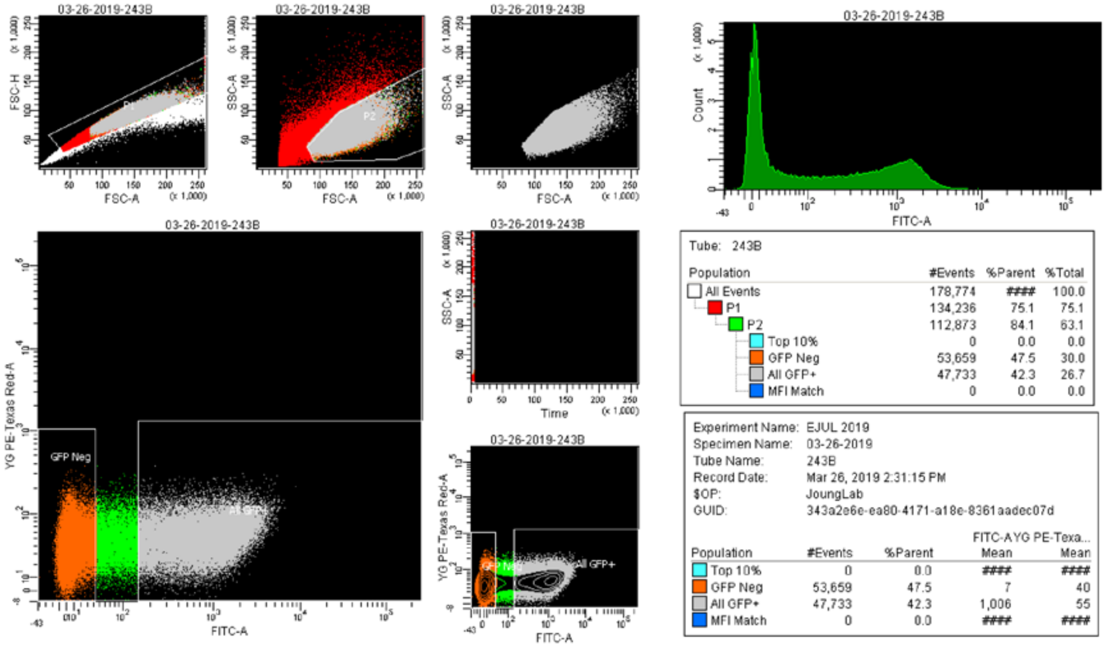
Tube: 243A

Population	#Events	%Parent	%Total
☐ All Events	127,873	####	100.0
🟥 P1	93,997	73.5	73.5
🟩 P2	78,991	84.0	61.8
🟦 Top 10%	0	0.0	0.0
🟧 GFP Neg	32,519	41.2	25.4
▨ All GFP+	38,539	48.8	30.1
🟦 MFI Match	0	0.0	0.0

Experiment Name: EJUL 2019
Specimen Name: 03-26-2019
Tube Name: 243A
Record Date: Mar 26, 2019 2:22:53 PM
$OP: JoungLab
GUID: 9f904e77-2244-4955-a1b9-3dca56773ba9

Population	#Events	%Parent	FITC-A Mean	YG PE-Texa... Mean
🟦 Top 10%	0	0.0	####	####
🟧 GFP Neg	32,519	41.2	10	38
▨ All GFP+	38,539	48.8	2,625	63
🟦 MFI Match	0	0.0	####	####

HEK293T **All GFP+**
ABEmax treated



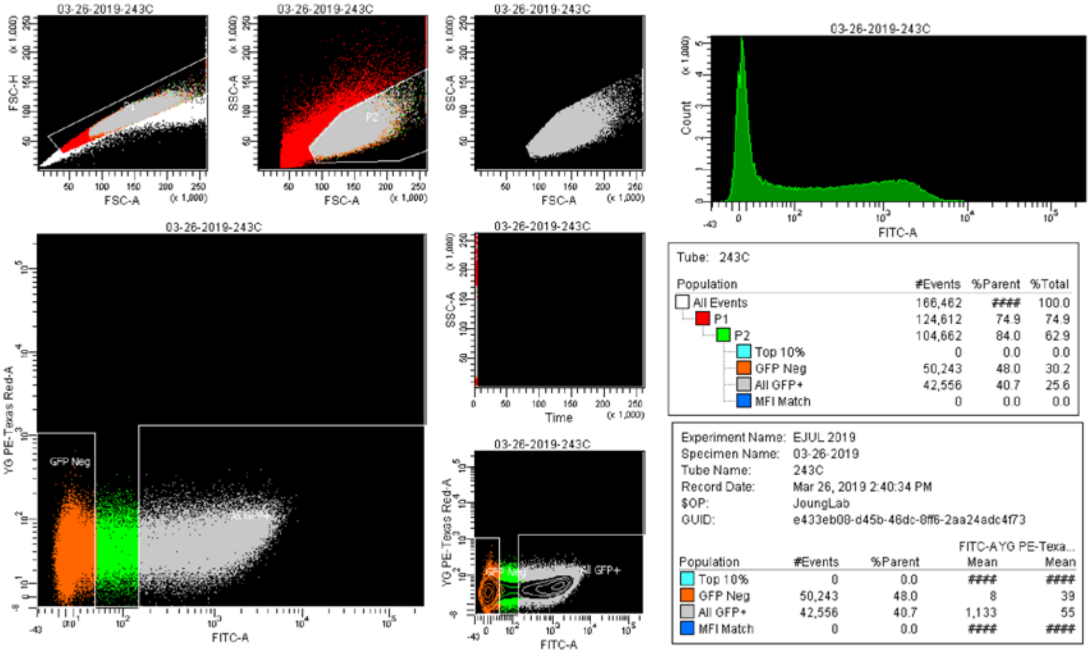
Tube: 243B

Population	#Events	%Parent	%Total
☐ All Events	178,774	####	100.0
🟥 P1	134,236	75.1	75.1
🟩 P2	112,873	84.1	63.1
Top 10%	0	0.0	0.0
🟧 GFP Neg	53,659	47.5	30.0
All GFP+	47,733	42.3	26.7
🟦 MFI Match	0	0.0	0.0

Experiment Name: EJUL 2019
Specimen Name: 03-26-2019
Tube Name: 243B
Record Date: Mar 26, 2019 2:31:15 PM
$OP: JoungLab
GUID: 343a2e6e-ea80-4171-a18e-8361aadec07d

Population	#Events	%Parent	FITC-A Mean	YG PE-Texa... Mean
Top 10%	0	0.0	####	####
🟧 GFP Neg	53,659	47.5	7	40
All GFP+	47,733	42.3	1,006	55
🟦 MFI Match	0	0.0	####	####

HEK293T **All GFP+**
miniABEmax treated



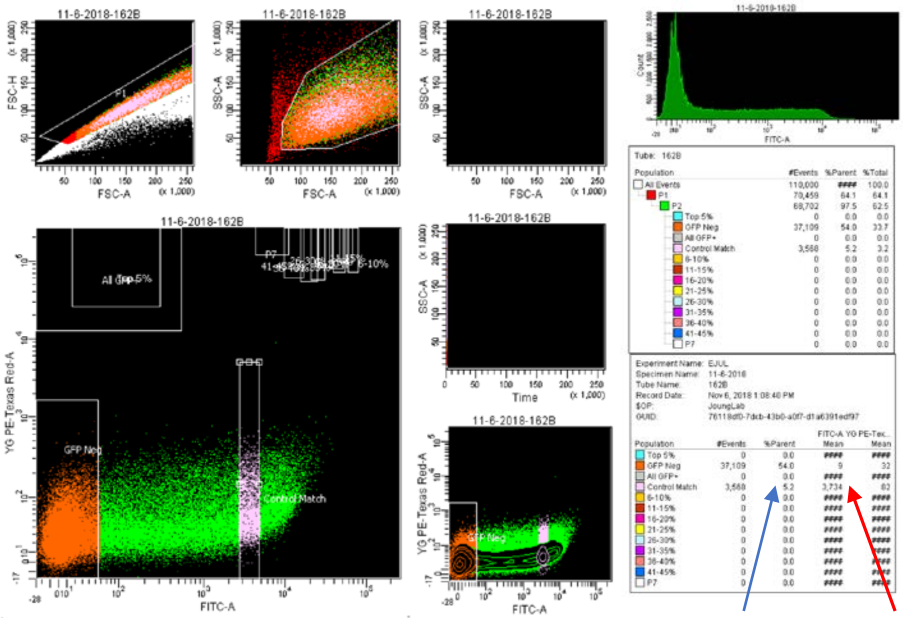
Tube: 243C

Population	#Events	%Parent	%Total
☐ All Events	166,462	####	100.0
🟥 P1	124,612	74.9	74.9
🟩 P2	104,662	84.0	62.9
Top 10%	0	0.0	0.0
🟧 GFP Neg	50,243	48.0	30.2
All GFP+	42,556	40.7	25.6
🟦 MFI Match	0	0.0	0.0

Experiment Name: EJUL 2019
Specimen Name: 03-26-2019
Tube Name: 243C
Record Date: Mar 26, 2019 2:40:34 PM
$OP: JoungLab
GUID: e433eb08-d45b-46dc-8ff6-2aa24adc4f73

Population	#Events	%Parent	FITC-A Mean	YG PE-Texa... Mean
Top 10%	0	0.0	####	####
🟧 GFP Neg	50,243	48.0	8	39
All GFP+	42,556	40.7	1,133	55
🟦 MFI Match	0	0.0	####	####

HEK293T **Top 5%-matched** nCas9 Control for CBE



Tube: 162B

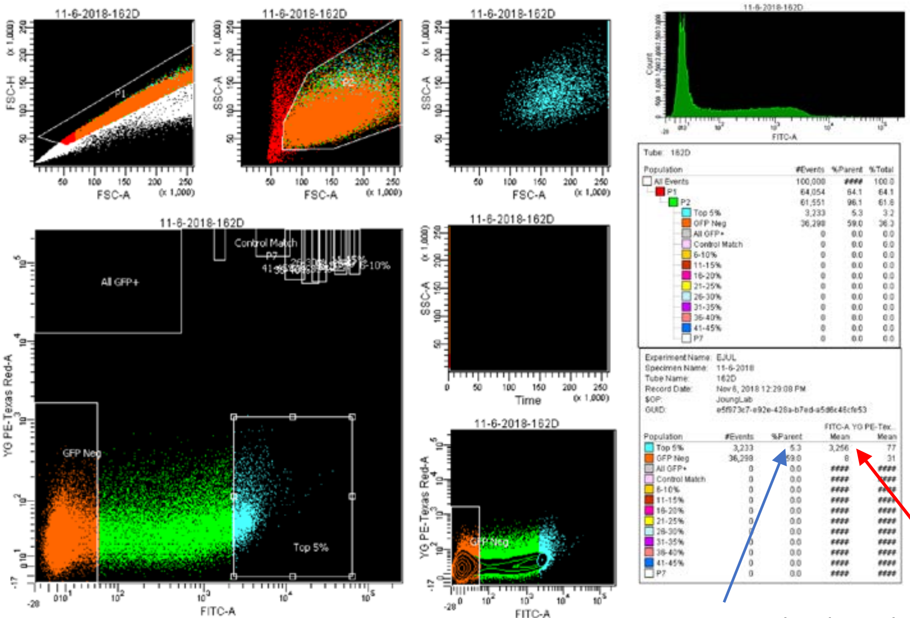
Population	#Events	%Parent	%Total
All Events	110,000	####	100.0
P1	70,459	64.1	64.1
P2	68,702	97.5	62.5
Top 5%	0	0.0	0.0
GFP Neg	37,109	54.0	33.7
All GFP+	0	0.0	0.0
Control Match	3,568	5.2	3.2
6-10%	0	0.0	0.0
11-15%	0	0.0	0.0
16-20%	0	0.0	0.0
21-25%	0	0.0	0.0
26-30%	0	0.0	0.0
31-35%	0	0.0	0.0
36-40%	0	0.0	0.0
41-45%	0	0.0	0.0
P7	0	0.0	0.0

Experiment Name: EJUL
Specimen Name: 11-6-2018
Tube Name: 162B
Record Date: Nov 6, 2018 1:08:40 PM
$OP: JoungLab
GUID: 76118df0-7dcb-43b0-a0f7-d1a6391edf97

Population	#Events	%Parent	FITC-A Mean	YG PE-Tex... Mean
Top 5%	0	0.0	####	####
GFP Neg	37,109	54.0	9	32
All GFP+	0	0.0	####	####
Control Match	3,568	5.2	3,734	82
6-10%	0	0.0	####	####
11-15%	0	0.0	####	####
16-20%	0	0.0	####	####
21-25%	0	0.0	####	####
26-30%	0	0.0	####	####
31-35%	0	0.0	####	####
36-40%	0	0.0	####	####
41-45%	0	0.0	####	####
P7	0	0.0	####	####

5% population with GeoMean (MFI)
matched to top 5% BE-treated cells (see next slide)

HEK293T **Top 5%** CBE-treated



Tube: 162D

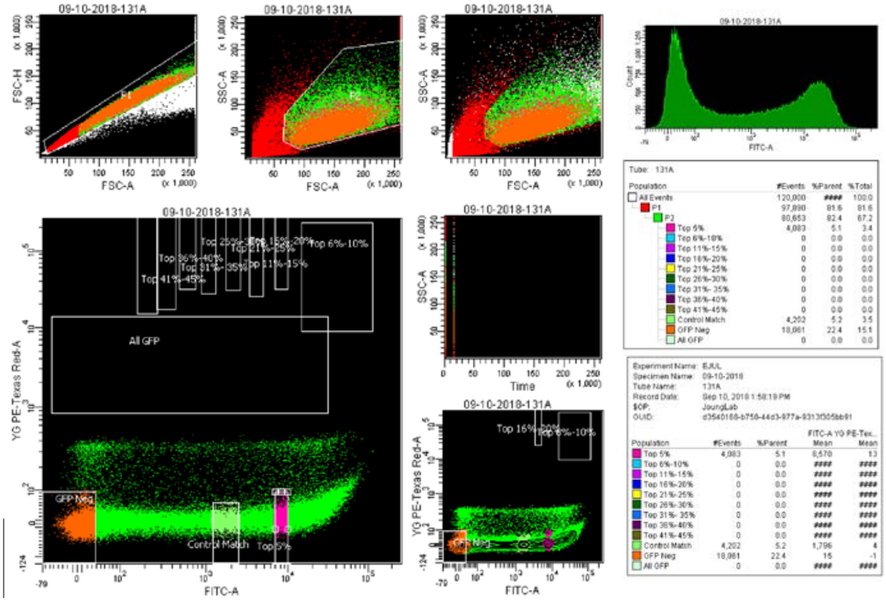
Population	#Events	%Parent	%Total
All Events	100,000	####	100.0
P1	64,054	64.1	64.1
P2	61,551	96.1	61.6
Top 5%	3,233	5.3	3.2
GFP Neg	36,298	59.0	36.3
All GFP+	0	0.0	0.0
Control Match	0	0.0	0.0
6-10%	0	0.0	0.0
11-15%	0	0.0	0.0
16-20%	0	0.0	0.0
21-25%	0	0.0	0.0
26-30%	0	0.0	0.0
31-35%	0	0.0	0.0
36-40%	0	0.0	0.0
41-45%	0	0.0	0.0
P7	0	0.0	0.0

Experiment Name: EJUL
Specimen Name: 11-6-2018
Tube Name: 162D
Record Date: Nov 6, 2018 12:29:08 PM
$OP: JoungLab
GUID: e5f973c7-e92e-428a-b7ed-a5d6c45cfe53

Population	#Events	%Parent	FITC-A Mean	YG PE-Tex... Mean
Top 5%	3,233	5.3	3,256	77
GFP Neg	36,298	59.0	8	31
All GFP+	0	0.0	####	####
Control Match	0	0.0	####	####
6-10%	0	0.0	####	####
11-15%	0	0.0	####	####
16-20%	0	0.0	####	####
21-25%	0	0.0	####	####
26-30%	0	0.0	####	####
31-35%	0	0.0	####	####
36-40%	0	0.0	####	####
41-45%	0	0.0	####	####
P7	0	0.0	####	####

Top 5% signal population & GeoMean (MFI)

HEK293T **Top 5%-matched**
GFP Control



Tube: 131A

Population	#Events	%Parent	%Total
☐ All Events	120,000	####	100.0
🟥 P1	97,890	81.6	81.6
🟩 P2	80,653	82.4	67.2
■ Top 5%	4,083	5.1	3.4
■ Top 6%-10%	0	0.0	0.0
■ Top 11%-15%	0	0.0	0.0
■ Top 16%-20%	0	0.0	0.0
■ Top 21%-25%	0	0.0	0.0
■ Top 26%-30%	0	0.0	0.0
■ Top 31%- 35%	0	0.0	0.0
■ Top 36%-40%	0	0.0	0.0
■ Top 41%-45%	0	0.0	0.0
■ Control Match	4,202	5.2	3.5
■ GFP Neg	18,061	22.4	15.1
■ All GFP	0	0.0	0.0

Experiment Name: EJUL
Specimen Name: 09-10-2018
Tube Name: 131A
Record Date: Sep 10, 2018 1:58:19 PM
$OP: JoungLab
GUID: d3540188-b758-44d3-977a-9313f305bb91

Population	#Events	%Parent	FITC-A Mean	YG PE-Tex... Mean
■ Top 5%	4,083	5.1	8,570	13
■ Top 6%-10%	0	0.0	####	####
■ Top 11%-15%	0	0.0	####	####
■ Top 16%-20%	0	0.0	####	####
■ Top 21%-25%	0	0.0	####	####
■ Top 26%-30%	0	0.0	####	####
■ Top 31%- 35%	0	0.0	####	####
■ Top 36%-40%	0	0.0	####	####
■ Top 41%-45%	0	0.0	####	####
■ Control Match	4,202	5.2	1,796	4
■ GFP Neg	18,061	22.4	15	-1
■ All GFP	0	0.0	####	####