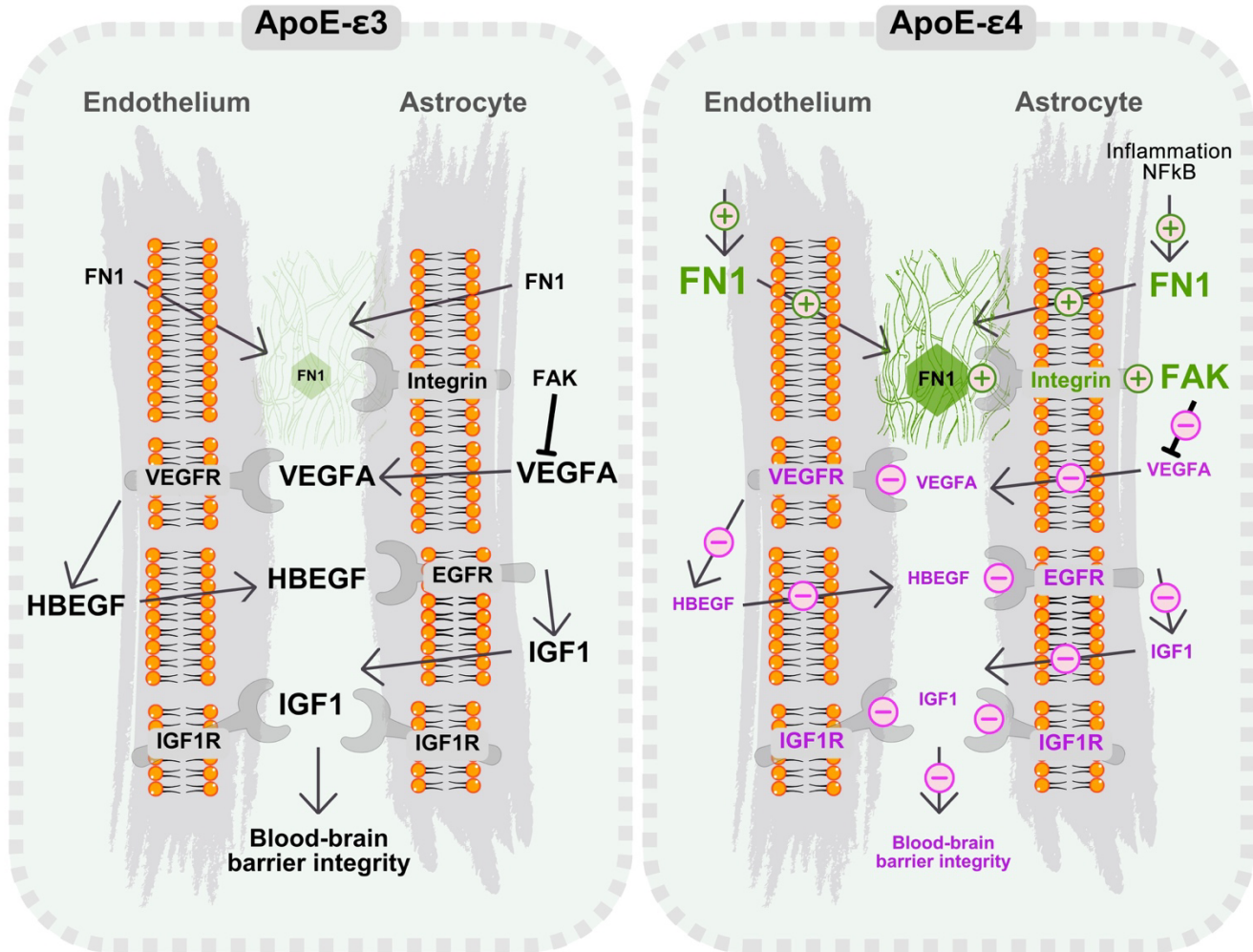
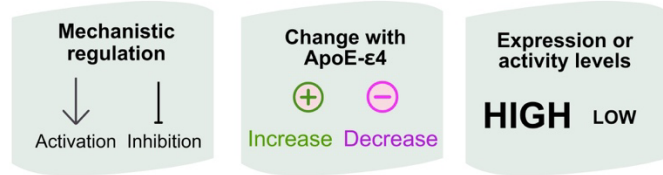
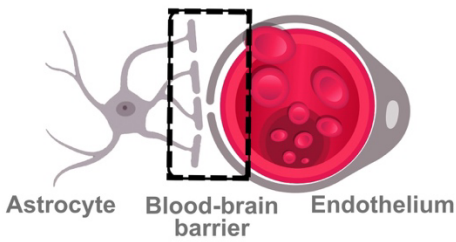


Fibronectin mediates APOE4-driven blood–brain barrier dysfunction in Alzheimer’s disease

In the format provided by the
authors and unedited



Supplementary Figure 1: Summary of APOE4-mediated FN1 pathology at the BBB. Under physiological conditions (APOEε3, left), coordinated signaling between astrocytes and endothelial cells maintains blood-brain barrier (BBB) integrity through paracrine pathways involving VEGFA, HBEGF, and IGF1. These interactions support balanced growth factor receptor activation (VEGFR, EGFR, IGF1R) and stable barrier properties. In the APOEε4 context (right), elevated inflammatory NF-κB activity in astrocytes induce FN1 expression, which in turn activates FAK and integrin signaling pathways, leading to reduced VEGFA, HBEGF, and IGF1 signalling. Together, these lead to impaired receptor activation, and disrupted BBB maintenance. Green symbols indicate increased and red symbols decreased expression or activity with APOEε4 relative to APOEε3. The schematic summarizes key molecular and cellular changes contributing to BBB dysfunction in APOEε4 carriers.

Case	Age	Sex	Braak Stage	Arteriolosclerosis score (NYBB)	Category
2	58	Man	VI	ATS1	AD
7	72	Woman	VI	ATS1	AD
14	81	Woman	VI	ATS1	AD
20	89+	Man	IV	ATS2	AD
23	89+	Man	V	ATS1	AD
5	65	Woman	VI	ATS1	AD
8	77	Woman	VI	ATS2	AD
6	71	Woman	VI	ATS4	AD+CVP
9	77	Man	VI	ATS3	AD+CVP
10	79	Man	VI	ATS3	AD+CVP
12	80	Man	V	ATS3	AD+CVP
18	89+	Woman	VI	ATS3	AD+CVP
1	41	Woman	0	None	Control
3	62	Man	I	ATS1	Control
11	80	Woman	0	ATS2	Control
19	89+	Man	II	ATS2	Control
21	89+	Woman	0	ATS2	Control
22	89+	Man	II	ATS1	Control
4	63	Man	II	ATS3	CVP
13	81	Woman	II	ATS4	CVP
15	86	Woman	III	ATS4	CVP
16	89+	Woman	IV	ATS4	CVP
17	89	Man	V (no dementia)	ATS4	CVP

Supplementary Table 1: Demographics of human brains used.

Trait	Locus	rsid	CHR	POS	Function	Nearest Gene	EA	NEA	EA_Freq	Beta	SE	p
Any ischemic stroke	2q35	rs55942719	2	216307134	intergenic	FN1;LOC102724849	A	G	0,25	-0,03	0,01	2,23E-04
Large artery ischemic stroke	2q35	rs1250203	2	216248305	intronic	FN1	T	G	0,88	0,12	0,03	3,35E-04
Any stroke	2q35	rs55942719	2	216307134	intergenic	FN1;LOC102724849	A	G	0,25	-0,03	0,01	3,78E-04
Perivascular spaces in hippocampus	2q35	rs1250229	2	216304384	intergenic	FN1;LOC102724849	T	C	0,26	0,01	0,00	5,67E-04
Any stroke	2q35	rs56137176	2	216288841	intronic	FN1	T	C	0,74	0,03	0,01	7,24E-04
Perivascular spaces in hippocampus	2q35	rs1250232	2	216306635	intergenic	FN1;LOC102724849	T	C	0,74	-0,01	0,00	8,87E-04
Large artery ischemic stroke	2q35	rs1968510	2	216258251	intronic	FN1	A	G	0,10	-0,12	0,04	9,56E-04

CHR: Chromosome; POS: Position (GRCh37); EA: Effect allele; NEA: Non-effect allele; EA Freq: Effect allele frequency; Beta: Effect estimate; SE: Standard error.

Supplementary Table 2: Association of variants in or near FN1 gene with the cerebral small vessel disease and

Trait	rsid	CHR	POS	EA	NEA	EA FREQ	N	Beta	SE	LOG10P	p
FN1 protein level in blood (Olink, UK Biobank)	rs1250203	2	216248305	T	G	0,89	33995	-0,13	0,01	24,69	2,06E-25
FN1 protein level in blood (Olink, UK Biobank)	rs1968510	2	216258251	G	A	0,91	33995	-0,13	0,01	21,37	4,26E-22
FN1 protein level in blood (Olink, UK Biobank)	rs56137176	2	216288841	C	T	0,25	33995	0,20	0,01	112,71	1,97E-113
FN1 protein level in blood (Olink, UK Biobank)	rs1250229	2	216304384	C	T	0,74	33995	0,44	0,01	581,68	0
FN1 protein level in blood (Olink, UK Biobank)	rs1250232	2	216306635	T	C	0,74	33995	0,44	0,01	585,90	0
FN1 protein level in blood (Olink, UK Biobank)	rs55942719	2	216307134	A	G	0,25	33995	0,18	0,01	98,44	3,62E-99

CHR: Chromosome; POS:
 Position (GRCh37); EA: Effect
 allele; NEA: Non-effect allele; EA
 Freq: Effect allele frequency;
 Beta: Effect estimate; SE:
 Standard error; LOG10P: -
 log10(p)

Supplementary Table 3: Association of FN1 variants with protein plasma levels in UK Biobank. Statistics: linear

Exposure	Outcome	method	N SNPs	Mendelian Randomization					Egger intercept			MR STEIGER					
				Beta	SE	p	Q	p(Q)	Beta	SE	p	snp_r2.exposure	snp_r2.outcome	correct	causal_direction	p	
FN1_Fibronectin	Any stroke	Inverse variance weighted	3	-0.13	0.04	0.0015	0.93	0.6275				0.01	8.83E-06		TRUE		3.77E-109
FN1_Fibronectin	Any stroke	MR Egger	3	-0.08	0.07	0.4968	0.24	0.6209	-0.007	0.008	0.5593						
FN1_Fibronectin	Any stroke	Weighted mode	3	-0.12	0.04	0.1157											
FN1_FN1_3	Any stroke	Inverse variance weighted	3	-0.13	0.04	0.0028	1.88	0.3900				0.01	8.61E-06		TRUE		3.46E-93
FN1_FN1_3	Any stroke	MR Egger	3	-0.07	0.10	0.6302	1.30	0.2544	-0.008	0.011	0.6240						
FN1_FN1_3	Any stroke	Weighted mode	3	-0.13	0.05	0.1042											
FN1_FN1_4	Any stroke	Inverse variance weighted	4	-0.12	0.04	0.0059	0.47	0.9262				0.01	6.52E-06		TRUE		6.87E-103
FN1_FN1_4	Any stroke	MR Egger	4	-0.17	0.10	0.2429	0.17	0.9194	0.006	0.011	0.6397						
FN1_FN1_4	Any stroke	Weighted mode	4	-0.13	0.05	0.0726											
FN1_Fibronectin	Any ischemic stroke	Inverse variance weighted	3	-0.12	0.04	0.0066	1.97	0.3737				0.01	7.51E-06		TRUE		1.74E-109
FN1_Fibronectin	Any ischemic stroke	MR Egger	3	-0.04	0.08	0.6965	0.72	0.3956	-0.010	0.009	0.4649						
FN1_Fibronectin	Any ischemic stroke	Weighted mode	3	-0.10	0.05	0.1800											
FN1_FN1_3	Any ischemic stroke	Inverse variance weighted	3	-0.12	0.06	0.0371	3.03	0.2203				0.01	7.73E-06		TRUE		2.24E-93
FN1_FN1_3	Any ischemic stroke	MR Egger	3	-0.02	0.12	0.9113	1.62	0.2026	-0.013	0.014	0.5233						
FN1_FN1_3	Any ischemic stroke	Weighted mode	3	-0.12	0.05	0.1423											
FN1_FN1_4	Any ischemic stroke	Inverse variance weighted	4	-0.10	0.05	0.0266	0.77	0.8569				0.01	4.80E-06		TRUE		1.96E-103
FN1_FN1_4	Any ischemic stroke	MR Egger	4	-0.12	0.11	0.3927	0.73	0.6929	0.002	0.013	0.8687						
FN1_FN1_4	Any ischemic stroke	Weighted mode	4	-0.12	0.05	0.1125											
FN1_FN1_4	Large artery ischemic stroke	Inverse variance weighted	4	-0.29	0.15	0.0447	3.35	0.3409				0.01	2.31E-05		TRUE		1.62E-92
FN1_FN1_4	Large artery ischemic stroke	MR Egger	4	-0.19	0.49	0.7320	3.27	0.1946	-0.013	0.061	0.8500						
FN1_FN1_4	Large artery ischemic stroke	Weighted mode	4	-0.25	0.16	0.2087											
FN1_Fibronectin	Small vessel ischemic stroke	Inverse variance weighted	3	-0.25	0.12	0.0336	0.06	0.9683				0.01	9.89E-06		TRUE		5.31E-104
FN1_Fibronectin	Small vessel ischemic stroke	MR Egger	3	-0.21	0.23	0.5192	0.02	0.8997	-0.006	0.026	0.8619						
FN1_Fibronectin	Small vessel ischemic stroke	Weighted mode	3	-0.24	0.12	0.1903											
FN1_FN1_3	Small vessel ischemic stroke	Inverse variance weighted	3	-0.27	0.13	0.0327	0.02	0.9896				0.01	9.89E-06		TRUE		2.15E-88
FN1_FN1_3	Small vessel ischemic stroke	MR Egger	3	-0.26	0.27	0.5192	0.02	0.9010	-0.002	0.029	0.9530						
FN1_FN1_3	Small vessel ischemic stroke	Weighted mode	3	-0.27	0.14	0.1997											
FN1_FN1_4	Small vessel ischemic stroke	Inverse variance weighted	4	-0.29	0.13	0.0185	0.45	0.9292				0.01	1.33E-05		TRUE		1.72E-96
FN1_FN1_4	Small vessel ischemic stroke	MR Egger	4	-0.27	0.28	0.4353	0.45	0.8001	-0.002	0.030	0.9431						
FN1_FN1_4	Small vessel ischemic stroke	Weighted mode	4	-0.27	0.14	0.1472											

P-values in bold are nominally significant (p<0.05). P-values in blue are significant after correction for multiple testing (p<0.005).

Beta: Effect estimate; SE: Standard error; p: p-value; snp_r2.exposure: variance of the exposure explained by all SNPs.

snp_r2.outcome: variance of the outcome explained by all SNPs.

correct_causal_direction: TRUE if causal direction is exposure - outcome and FALSE otherwise.

Supplementary Table 4: Association of genetically predicted FN1 proteins with inverse-variance weighted analyses and MR-Egger regression. Statistics: Inverse-variance weighted (IVW) meta-analysis, two-sided Wald test and

Causal Mediation Analysis							
predictor > mediator > trait							
Test whether gene expression of mediator mediates the association between gene expression of predictor and trait using ROSMAP DLPFC samples							
<i>predictor</i>	<i>mediator</i>	<i>trait</i>	<i>cov</i>	<i>b</i>	<i>CL</i>	<i>CU</i>	<i>p</i>
HBEGF	IGF1	Pathologic AD	ACME	0,04077024	0,01585596	0,07011354	0
HBEGF	IGF1	Amyloid	ACME	0,08642473	0,03646649	0,14330112	0
HBEGF	IGF1	Tau	ACME	0,06850653	0,01018233	0,13431917	0,022

Supplementary Table 5: Causal mediation analyses between HBEGF and IGF1 for AD traits in ROSMAP brain

1. **fn1-/- single cell sequencing experiment FACS gatings**

FN1_WT-gating control

Measurement Settings

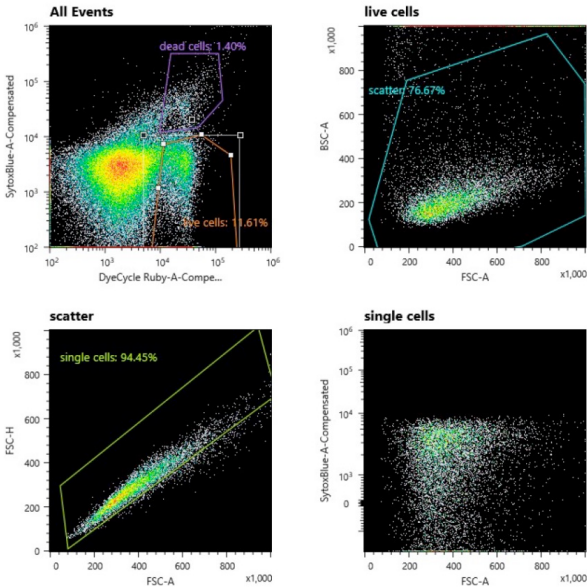
Parameter Settings		Acquisition Select		
Marker	Fluorochrome	Area	Height	Width
FSC	---	✓	✓	✓
BSC	---	✓		
FL1	EGFP			
FL2	PE			
FL3	PE-Texas Red			
FL4	PerCP-Cy5.5			
FL5	PE-Cy7			
FL6	SytoxBlue	✓		
FL7	Brilliant Violet 510			
FL8	Brilliant Violet 570			
FL9	Brilliant Violet 605			
FL10	DyeCycle Ruby	✓		
FL11	Alexa Fluor 700			
FL12	APC-Alexa Fluor 750			

Compensation Settings

Spillover Matrix (%)			Detector
	SytoxBlue	DyeCycle Ruby	
SytoxBlue	100.00	0.00	
DyeCycle Ruby	0.00	100.00	
Fluorochrome			

Negative Value			Detector
Area	0	0	
Height	0	0	

Worksheet



Gates and Statistics			
Name	Events	%Parent	%Total
All Events	98,520	0.00%	100.00%
live cells	11,441	11.61%	11.61%
scatter	8,772	76.67%	8.90%
single cells	8,285	94.45%	8.41%
dead cells	1,376	1.40%	1.40%

FN1_WT-PBS

Tube Information

Name	WT-PBS
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00413370-0000-7090
Nozzle Size	100 µm

Sample Stop Condition

None

Recording Setting

Type	Event Count
Value	100,000

Instrument Setting

Laser	Threshold
405nm On	Channel: FSC
488nm On	Value: 5.00%
561nm Off	
638nm On	

Sensor Gain

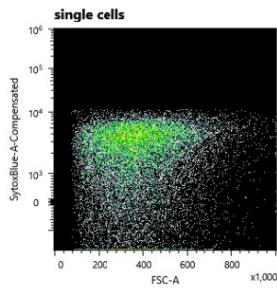
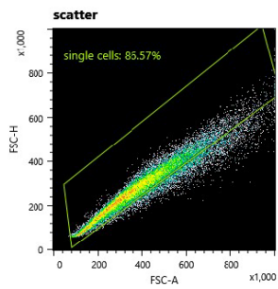
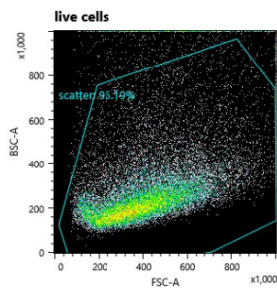
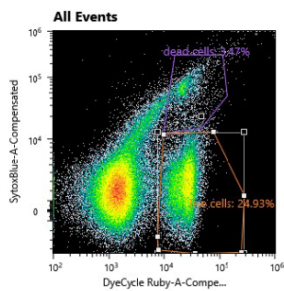
FSC	13	BSC	37.5%
FL1	37.0%	FL2	40.0%
FL3	40.0%	FL4	40.0%
FL5	40.0%	FL6	53.5%
FL7	40.0%	FL8	40.0%
FL9	40.0%	FL10	48.5%
FL11	40.0%	FL12	40.0%

Auto Parameters

Droplet Clock	29,700 Hz
Droplet Drive	15.91
Sort Delay	23
Sort Phase	140 deg
Charge	80.0 %
Deflection FarLeft	-1,064
Deflection Left	-504
Deflection Right	390
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 9

Worksheet



Gates and Statistics

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
live cells	24,929	24.93%	24.93%
scatter	23,729	95.19%	23.73%
single cells	20,542	86.57%	20.54%
dead cells	3,466	3.47%	3.47%

FN1_WT-Ab42

Tube Information

Name	WT-Ab42
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00413370-0000-7090
Nozzle Size	100 µm

Sample Stop Condition

None

Recording Setting

Type	Event Count
Value	100,000

Instrument Setting

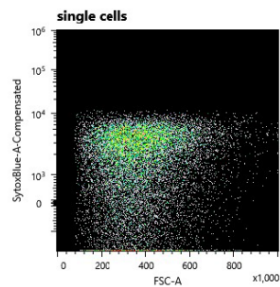
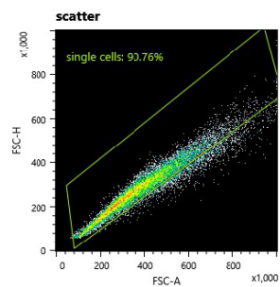
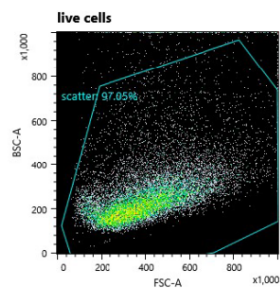
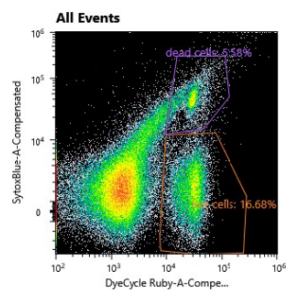
Laser	Threshold
405nm On	Channel: FSC
488nm On	Value: 5.00%
561nm Off	
638nm On	
Sensor Gain	
FSC 13	BSC 37.5%
FL1 37.0%	FL2 40.0%
FL3 40.0%	FL4 40.0%
FL5 40.0%	FL6 53.5%
FL7 40.0%	FL8 40.0%
FL9 40.0%	FL10 48.5%
FL11 40.0%	FL12 40.0%

Auto Parameters

Droplet Clock	29,700 Hz
Droplet Drive	16.08
Sort Delay	23
Sort Phase	140 deg
Charge	80.0 %
Deflection FarLeft	-1,064
Deflection Left	-504
Deflection Right	390
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 9

Worksheet



Gates and Statistics

Name	Events	%Parent	%Total
All Events	103,000	0.00%	100.00%
live cells	16,683	16.68%	16.68%
scatter	15,191	97.05%	16.19%
single cells	14,695	90.76%	14.70%
dead cells	5,580	5.58%	5.58%

FN1_KO-gating control

Measurement Settings

Parameter Settings		Acquisition Select			
	Marker	Fluorochrome	Area	Height	Width
FSC	---	---	✓	✓	✓
BSC	---	---	✓		
FL1		EGFP			
FL2		PE			
FL3		PE-Texas Red			
FL4		PerCP-Cy5.5			
FL5		PE-Cy7			
FL6		SytoxB	✓		
FL7		Brilliant Violet 510			
FL8		Brilliant Violet 570			
FL9		Brilliant Violet 605			
FL10		DyeCycle Ruby	✓		
FL11		Alexa Fluor 700			
FL12		APC-Alexa Fluor 750			

Compensation Settings

Spillover Matrix (%)

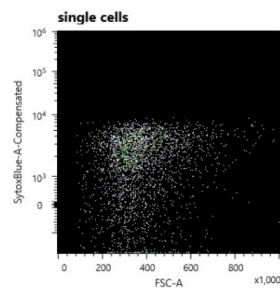
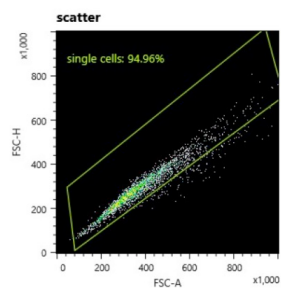
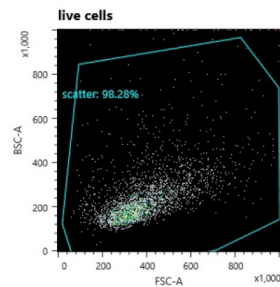
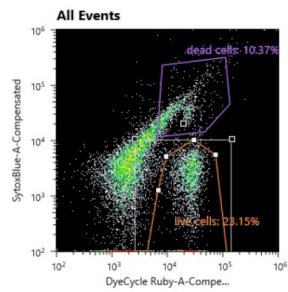
	SytoxBlue	DyeCycle Ruby
SytoxBlue	100.00	0.00
DyeCycle Ruby	0.00	100.00

Fluorochrome

Negative Value

	Area	Height
Area	0	0
Height	0	0

Detector

Worksheet

Gates and Statistics

Name	Events	%Parent	%Total
All Events	11,773	0.00%	100.00%
live cells	2,725	23.15%	23.15%
scatter	2,678	98.28%	22.75%
single cells	2,543	94.96%	21.60%
dead cells	1,221	10.37%	10.37%

FN1_KO-PBS

Tube Information

Name	PBS fn1-/-
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00437355-0000-7075
Nozzle Size	100 µm

Sample Stop Condition

None

Recording Setting

Type	Event Count
Value	10,000,000

Instrument Setting

Laser	Threshold
405nm On	Channel: FSC
488nm On	Value: 5.00%
561nm Off	
638nm On	

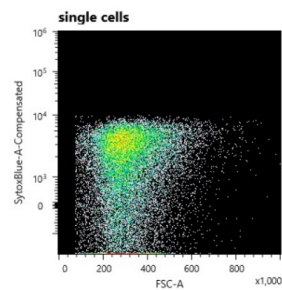
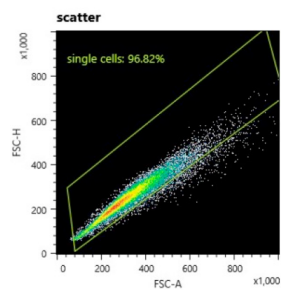
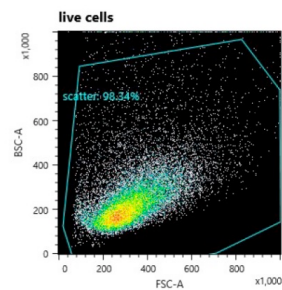
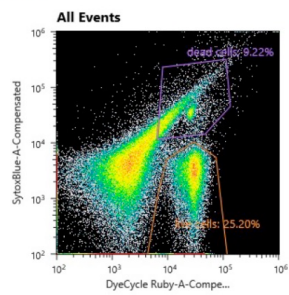
Sensor Gain	
FSC 13	BSC 37.5%
FL1 37.0%	FL2 40.0%
FL3 40.0%	FL4 40.0%
FL5 40.0%	FL6 53.5%
FL7 40.0%	FL8 40.0%
FL9 40.0%	FL10 48.5%
FL11 40.0%	FL12 40.0%

Auto Parameters

Droplet Clock	30,800 Hz
Droplet Drive	13.70
Sort Delay	28
Sort Phase	340 deg
Charge	80.0 %
Deflection FarLeft	-994
Deflection Left	-438
Deflection Right	420
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 9

Worksheet



Gates and Statistics

Displaying 100,000 / 232,885 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
live cells	25,198	25.20%	25.20%
scatter	24,780	98.34%	24.78%
single cells	23,991	96.82%	23.99%
dead cells	9,224	9.22%	9.22%

FN1_KO-Ab42

Tube Information

Name Ab42 fn1-/-
Sample ID1
Sample ID2
Sample ID3
Sample ID4

Basic Information

Description
Model Type LE-MA900FP
Serial Number 716004
Chip Type Sorting Chip
Chip ID 00437355-0000-7075
Nozzle Size 100 µm

Sample Stop Condition

None

Recording Setting

Type Event Count
Value 10,000,000

Instrument Setting

Laser Threshold
405nm On Channel: FSC
488nm On Value: 5.00%
561nm Off
638nm On

Sensor Gain

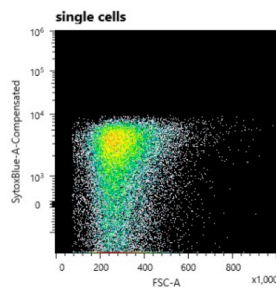
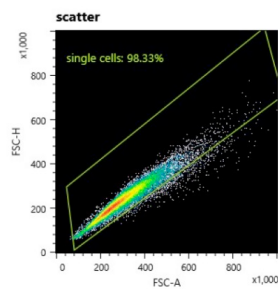
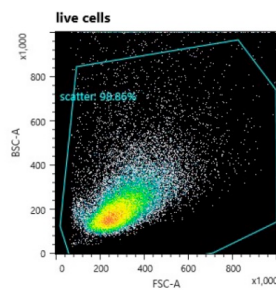
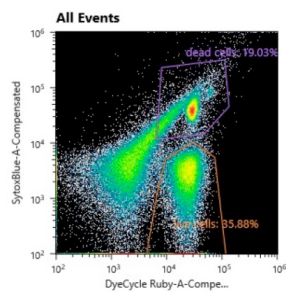
FSC	13	BSC	37.5%
FL1	37.0%	FL2	40.0%
FL3	40.0%	FL4	40.0%
FL5	40.0%	FL6	53.5%
FL7	40.0%	FL8	40.0%
FL9	40.0%	FL10	48.5%
FL11	40.0%	FL12	40.0%

Auto Parameters

Droplet Clock	30,800 Hz
Droplet Drive	13.66
Sort Delay	28
Sort Phase	340 deg
Charge	80.0 %
Deflection FarLeft	-994
Deflection Left	-438
Deflection Right	420
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 9

Worksheet



Gates and Statistics

Displaying 100,000 / 178,644 events

Name	Events	%Parent	%Total	
All Events	100,000	0.00%	100.00%	
live cells	35,880	35.88%	35.88%	
scatter	35,472	98.86%	35.47%	
single cells	34,881	98.33%	34.88%	
dead cells	19,026	19.03%	19.03%	

2. VEGFR Inhibitor treatment single cell sequencing experiment FACS gatings

WT-gating control - 1

Measurement Settings

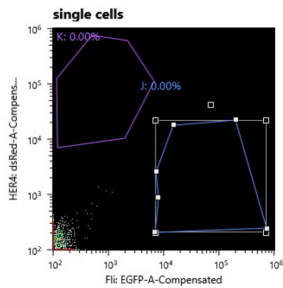
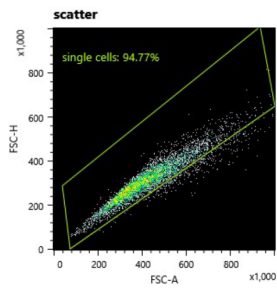
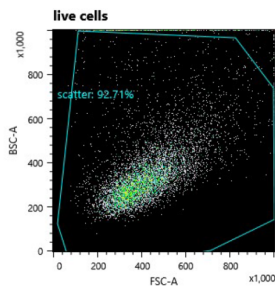
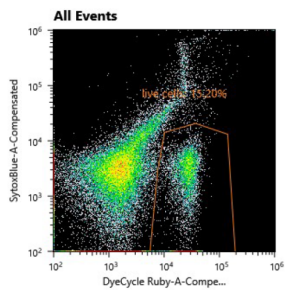
Parameter Settings			Acquisition Select		
	Marker	Fluorochrome	Area	Height	Width
FSC	---	---	✓	✓	✓
BSC	---	---	✓		
FL1	Fli	EGFP	✓		
FL2	HER4	dsRed	✓		
FL3		PE-Texas Red			
FL4		PerCP-Cy5.5			
FL5		PE-Cy7			
FL6		SytoxBlue	✓		
FL7		Brilliant Violet 510			
FL8		Brilliant Violet 570			
FL9		Brilliant Violet 605			
FL10		DyeCycle Ruby	✓		
FL11		Alexa Fluor 700			
FL12		APC-Alexa Fluor 750			

Compensation Settings

Spillover Matrix (%)					Detector
	Fli: EGFP	HER4: dsRed	SytoxBlue	DyeCycle Ruby	
Fli: EGFP	100.00	5.14	0.00	0.00	
HER4: dsRed	0.00	100.00	0.00	0.00	
SytoxBlue	0.00	0.00	100.00	0.00	
DyeCycle Ruby	0.00	0.00	0.00	100.00	

Negative Value					Detector
Area	0	0	0	0	
Height	0	0	0	0	

Worksheet



Gates and Statistics

Name	Events	%Parent	%Total	
☐ All Events	43,918	0.00%	100.00%	
☒ live cells	6,677	15.20%	15.20%	
☒ scatter	6,190	92.71%	14.09%	
☒ single cells	5,866	94.77%	13.36%	
☒ GFP +ve	8	0.14%	0.02%	
☒ J	0	0.00%	0.00%	
☒ K	0	0.00%	0.00%	

dTg-gating control - 1

Tube Information

Name	DTG
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00334310-0000-7070
Nozzle Size	100 µm

Sample Stop Condition

None

Instrument Setting

Laser	Threshold
405nm On	Channel: FSC
488nm On	Value: 5.00%
561nm On	
638nm On	

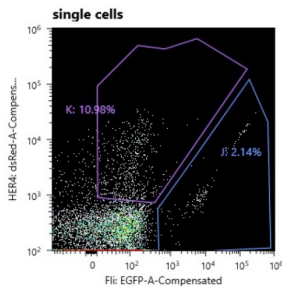
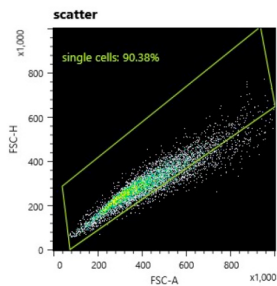
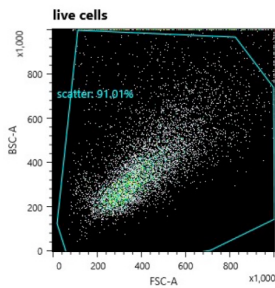
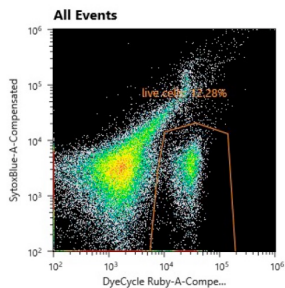
Recording Setting

Type	Event Count
Value	10,000,000

Sensor Gain	
FSC 13	BSC 37.5%
FL1 37.0%	FL2 43.0%
FL3 40.0%	FL4 40.0%
FL5 40.0%	FL6 53.5%
FL7 40.0%	FL8 40.0%
FL9 40.0%	FL10 48.5%
FL11 40.0%	FL12 40.0%

Sample Pressure 8

Worksheet



Name	Events	%Parent	%Total
All Events	53,547	0.00%	100.00%
live cells	6,577	12.28%	12.28%
scatter	5,986	91.01%	11.18%
single cells	5,410	90.38%	10.10%
GFP +ve	3,650	67.47%	6.82%
J	116	2.14%	0.22%
K	594	10.98%	1.11%

dTg-Control DMSO - 1

Tube Information

Name	Control
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00334310-0000-7070
Nozzle Size	100 µm

Sample Stop Condition

None

Recording Setting

Type	Event Count
Value	10,000,000

Instrument Setting

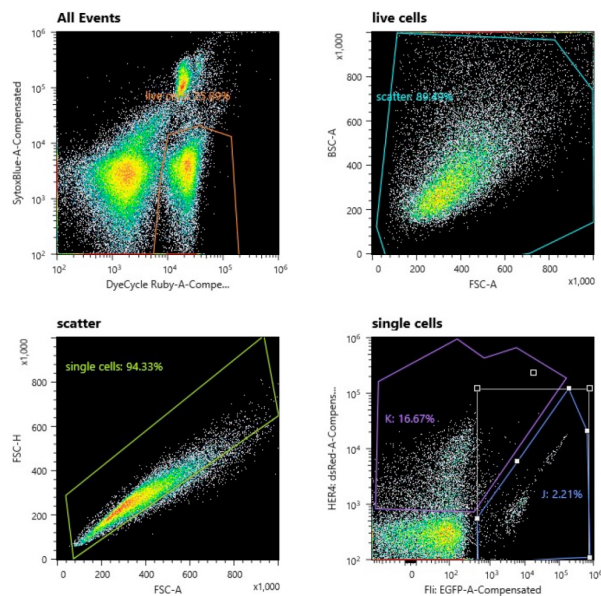
Laser	Threshold
405nm On	Channel: FSC
488nm On	Value: 5.00%
561nm On	
638nm On	
Sensor Gain	
FSC 13	BSC 37.5%
FL1 37.0%	FL2 43.0%
FL3 40.0%	FL4 40.0%
FL5 40.0%	FL6 53.5%
FL7 40.0%	FL8 40.0%
FL9 40.0%	FL10 48.5%
FL11 40.0%	FL12 40.0%

Auto Parameters

Droplet Clock	30,000 Hz
Droplet Drive	17.67
Sort Delay	24
Sort Phase	100 deg
Charge	80.0 %
Deflection FarLeft	-1,064
Deflection Left	-493
Deflection Right	421
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 8

Worksheet



Gates and Statistics				Displaying 100,000 / 2,168,742 events
Name	Events	%Parent	%Total	
☐ All Events	100,000	0.00%	100.00%	
☒ live cells	25,894	25.89%	25.89%	
☒ scatter	23,172	89.49%	23.17%	
☒ single cells	21,857	94.33%	21.86%	
☒ GFP +ve	14,839	67.89%	14.84%	
☒ J	484	2.21%	0.48%	
☒ K	3,644	16.67%	3.64%	

dTg-Inhibitor treatment (TMT) - 1

Tube Information

Name	TMT
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00334310-0000-7070
Nozzle Size	100 µm

Sample Stop Condition

None	
Recording Setting	
Type	Event Count
Value	10,000,000

Instrument Setting

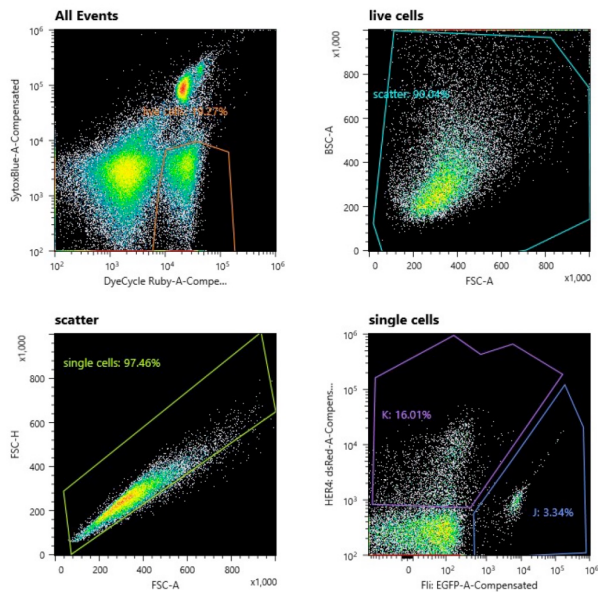
Laser	Threshold	
405nm On	Channel:	FSC
488nm On	Value:	5.00%
561nm On		
638nm On		
Sensor Gain		
FSC 13	BSC	37.5%
FL1 37.0%	FL2	43.0%
FL3 40.0%	FL4	40.0%
FL5 40.0%	FL6	53.5%
FL7 40.0%	FL8	40.0%
FL9 40.0%	FL10	48.5%
FL11 40.0%	FL12	40.0%

Auto Parameters

Droplet Clock	30,000 Hz
Droplet Drive	17.28
Sort Delay	24
Sort Phase	100 deg
Charge	80.0 %
Deflection FarLeft	-1,064
Deflection Left	-493
Deflection Right	421
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 9

Worksheet



Gates and Statistics				Displaying 100,000 / 2,295,510 events
Name	Events	%Parent	%Total	
All Events	100,000	0.00%	100.00%	
live cells	19,265	19.27%	19.27%	
scatter	17,346	90.04%	17.35%	
single cells	16,906	97.46%	16.91%	
GFP +ve	11,168	66.06%	11.17%	
J	565	3.34%	0.57%	
K	2,706	16.01%	2.71%	

WT-gating control – 2

Measurement Settings

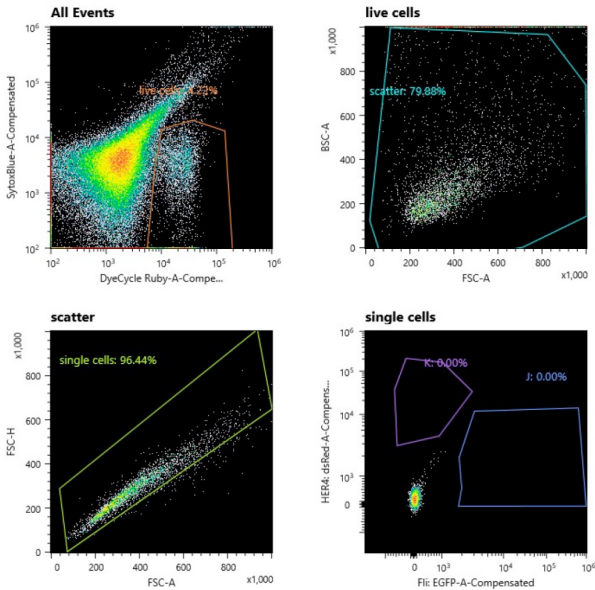
Parameter Settings			
Marker	Fluorochrome	Acquisition Select	Area Height Width
FSC	---	---	✓ ✓ ✓
BSC	---	---	✓
FL1	Fli	EGFP	✓
FL2	HER4	dsRed	✓
FL3		PE-Texas Red	
FL4		PerCP-Cy5.5	
FL5		PE-Cy7	
FL6		SytoxBlue	✓
FL7		Brilliant Violet 510	
FL8		Brilliant Violet 570	
FL9		Brilliant Violet 605	
FL10		DyeCycle Ruby	✓
FL11		Alexa Fluor 700	
FL12		APC-Alexa Fluor 750	

Compensation Settings

Spillover Matrix (%)					Detector
	Fli: EGFP	HER4: dsRed	SytoxBlue	DyeCycle Ruby	
Fli: EGFP	100.00	5.14	0.00	0.00	
HER4: dsRed	0.00	100.00	0.00	0.00	
SytoxBlue	0.00	0.00	100.00	0.00	
DyeCycle Ruby	0.00	0.00	0.00	100.00	

Negative Value					Detector
Area	0	0	0	0	
Height	0	0	0	0	

Worksheet



Gates and Statistics					Displaying 100,000 / 109,220 events
Name	Events	%Parent	%Total		
All Events	100,000	0.00%	100.00%		
live cells	4,220	4.22%	4.22%		
scatter	3,371	79.88%	3.37%		
single cells	3,251	96.44%	3.25%		
J	0	0.00%	0.00%		
K	0	0.00%	0.00%		

dTg-gating control – 2

Tube Information

Name dTg
Sample ID1
Sample ID2
Sample ID3
Sample ID4

Basic Information

Description
Model Type LE-MA900FP
Serial Number 716004
Chip Type Sorting Chip
Chip ID 00374726-0000-7006
Nozzle Size 100 µm

Sample Stop Condition

None

Recording Setting

Type Event Count
Value 10,000,000

Instrument Setting

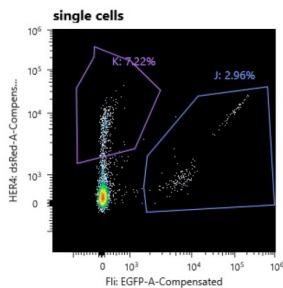
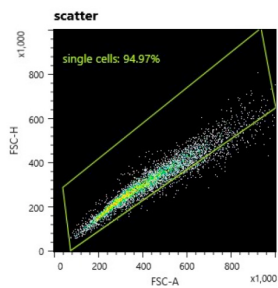
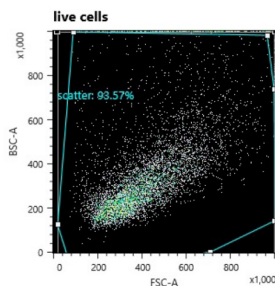
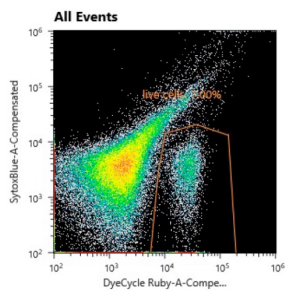
Laser Threshold
405nm On Channel: FSC
488nm On Value: 5.00%
561nm On
638nm On

Sensor Gain

FSC 13 BSC 37.5%
FL1 37.0% FL2 43.0%
FL3 40.0% FL4 40.0%
FL5 40.0% FL6 53.5%
FL7 40.0% FL8 40.0%
FL9 40.0% FL10 48.5%
FL11 40.0% FL12 40.0%

Sample Pressure 10

Worksheet



Gates and Statistics

Name	Events	%Parent	%Total	
All Events	85,291	0.00%	100.00%	
live cells	5,968	7.00%	7.00%	
scatter	5,584	93.57%	6.55%	
single cells	5,303	94.97%	6.22%	
J	157	2.96%	0.18%	
K	383	7.22%	0.45%	

dTg-Control DMSO – 2

Tube Information

Name	Ctrl
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00374726-0000-7006
Nozzle Size	100 μm

Sample Stop Condition

None	
Recording Setting	
Type	Event Count
Value	10,000,000

Instrument Setting

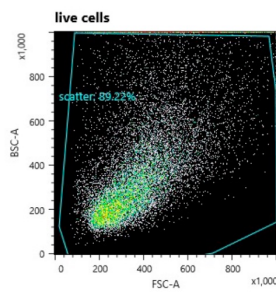
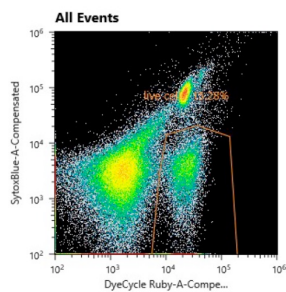
Laser	Threshold	
405nm On	Channel:	FSC
488nm On	Value:	5.00%
561nm On		
638nm On		
Sensor Gain		
FSC 13	BSC	37.5%
FL1 37.0%	FL2	43.0%
FL3 40.0%	FL4	40.0%
FL5 40.0%	FL6	53.5%
FL7 40.0%	FL8	40.0%
FL9 40.0%	FL10	48.5%
FL11 40.0%	FL12	40.0%

Auto Parameters

Droplet Clock	29,600 Hz
Droplet Drive	9.33
Sort Delay	24
Sort Phase	140 deg
Charge	80.0 %
Deflection FarLeft	-1,064
Deflection Left	-501
Deflection Right	412
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 8

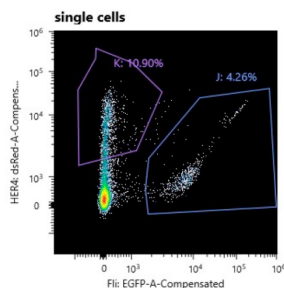
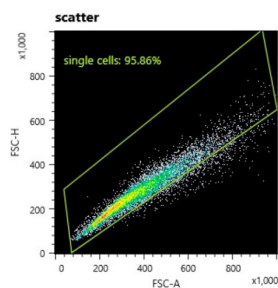
Worksheet



Gates and Statistics

Displaying 100,000 / 920,023 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
live cells	15,275	15.28%	15.28%
scatter	13,628	89.22%	13.63%
single cells	13,064	95.86%	13.06%
J	557	4.26%	0.56%
K	1,424	10.90%	1.42%



dTg-Inhibitor Treatment (TMT) – 2

Tube Information

Name	Tmt
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00374726-0000-7006
Nozzle Size	100 µm

Sample Stop Condition

None	
Recording Setting	
Type	Event Count
Value	10,000,000

Instrument Setting

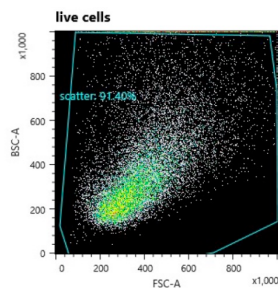
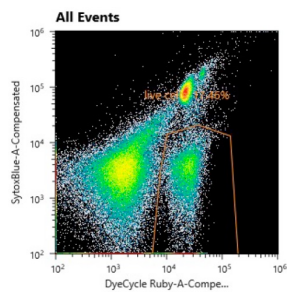
Laser	Threshold	
405nm On	Channel:	FSC
488nm On	Value:	5.00%
561nm On		
638nm On		
Sensor Gain		
FSC 13	BSC	37.5%
FL1 37.0%	FL2	43.0%
FL3 40.0%	FL4	40.0%
FL5 40.0%	FL6	53.5%
FL7 40.0%	FL8	40.0%
FL9 40.0%	FL10	48.5%
FL11 40.0%	FL12	40.0%

Auto Parameters

Droplet Clock	29,600 Hz
Droplet Drive	9.41
Sort Delay	24
Sort Phase	140 deg
Charge	80.0 %
Deflection FarLeft	-1,064
Deflection Left	-501
Deflection Right	412
Deflection FarRight	1,004
Enabled Control Breakoff	☒

Sample Pressure 8

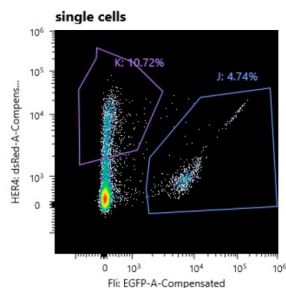
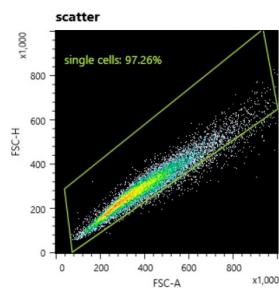
Worksheet

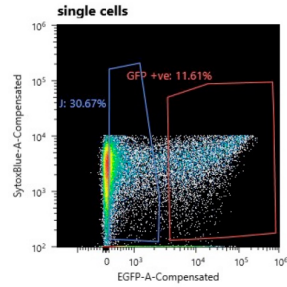
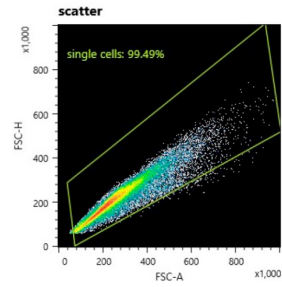
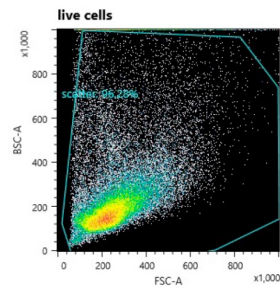
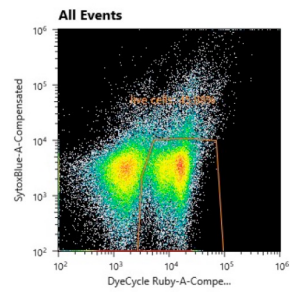


Gates and Statistics

Displaying 100,000 / 1,302,967 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
live cells	17,462	17.46%	17.46%
scatter	15,960	91.40%	15.96%
single cells	15,522	97.26%	15.52%
J	735	4.74%	0.74%
K	1,664	10.72%	1.66%





Gates and Statistics

Displaying 100,000 / 366,673 events

Name	Events	%Parent	%Total	
All Events	100,000	0.00%	100.00%	
live cells	45,876	45.88%	45.88%	
scatter	44,170	96.28%	44.17%	
single cells	43,943	99.49%	43.94%	
GFP +ve	5,100	11.61%	5.10%	
J	13,478	30.67%	13.48%	

Tg(Her4:GFP)_HB-EGF injected

Measurement Settings

Parameter Settings		Acquisition Select		
Marker	Fluorochrome	Area	Height	Width
FSC	---	✓	✓	✓
BSC	---	✓		
FL1	EGFP	✓		
FL2	PE			
FL3	PE-Texas Red			
FL4	PerCP-Cy5.5			
FL5	PE-Cy7			
FL6	SytoxBlue	✓		
FL7	Brilliant Violet 510			
FL8	Brilliant Violet 570			
FL9	Brilliant Violet 605			
FL10	DyeCycle Ruby	✓		
FL11	Alexa Fluor 700			
FL12	APC-Alexa Fluor 750			

Compensation Settings

Spillover Matrix (%)				Detector	
Fluorochrome		EGFP	SytoxBlue	DyeCycle Ruby	
	EGFP	100.00	0.00	0.00	
	SytoxBlue	0.00	100.00	0.00	
	DyeCycle Ruby	0.00	0.00	100.00	
Negative Value				Detector	
Area		0	0	0	
Height		0	0	0	

Tube Information

Name	injected
Sample ID1	
Sample ID2	
Sample ID3	
Sample ID4	

Basic Information

Description	
Model Type	LE-MA900FP
Serial Number	716004
Chip Type	Sorting Chip
Chip ID	00443859-0000-7019
Nozzle Size	100 µm

Sample Stop Condition

None

Recording Setting

Type	Event Count
Value	100,000,000

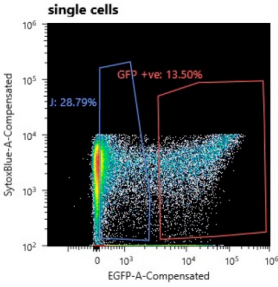
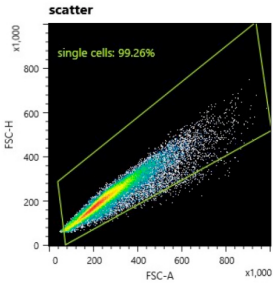
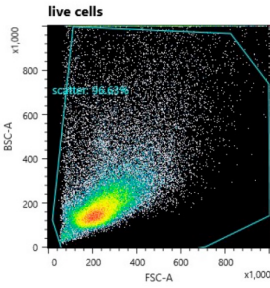
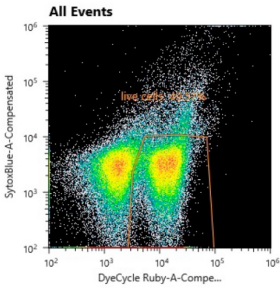
Instrument Setting

Laser			Threshold	
	405nm	On	Channel:	FSC
	488nm	On	Value:	5.00%
	561nm	Off		
	638nm	On		
Sensor Gain				
FSC	13	BSC	37.5%	
FL1	37.0%	FL2	40.0%	
FL3	40.0%	FL4	40.0%	
FL5	40.0%	FL6	53.5%	
FL7	40.0%	FL8	40.0%	
FL9	40.0%	FL10	48.5%	
FL11	40.0%	FL12	40.0%	

Auto Parameters

Droplet Clock	30,900 Hz
Droplet Drive	13.90
Sort Delay	29
Sort Phase	320 deg
Charge	80.0 %
Deflection FarLeft	-984
Deflection Left	-432
Deflection Right	455
Deflection FarRight	1,024
Enabled Control Breakoff	☒

Sample Pressure 9



Gates and Statistics

Displaying 100,000 / 390,754 events

Name	Events	%Parent	%Total	
All Events	100,000	0.00%	100.00%	
live cells	49,569	49.57%	49.57%	
scatter	47,901	96.63%	47.90%	
single cells	47,545	99.26%	47.55%	
GFP +ve	6,419	13.50%	6.42%	
J	13,686	28.79%	13.69%	