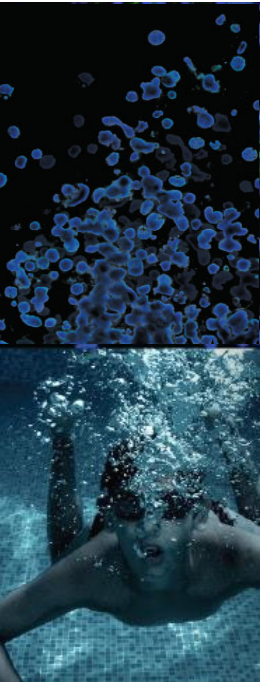


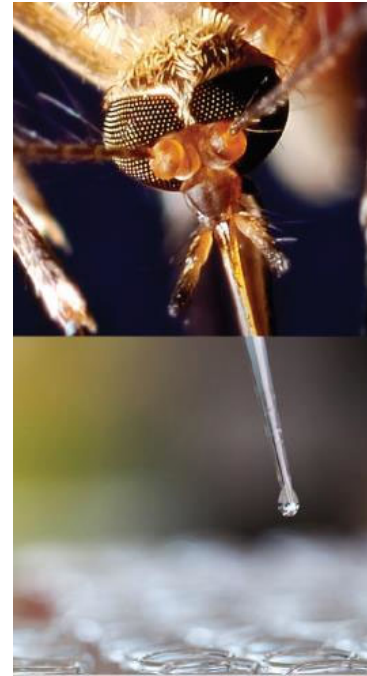


Give Your Virus and Viral Vaccine Production a Boost with CRISPR/Cas9-Engineered Host Cells

Liz Turner Gillies, PhD



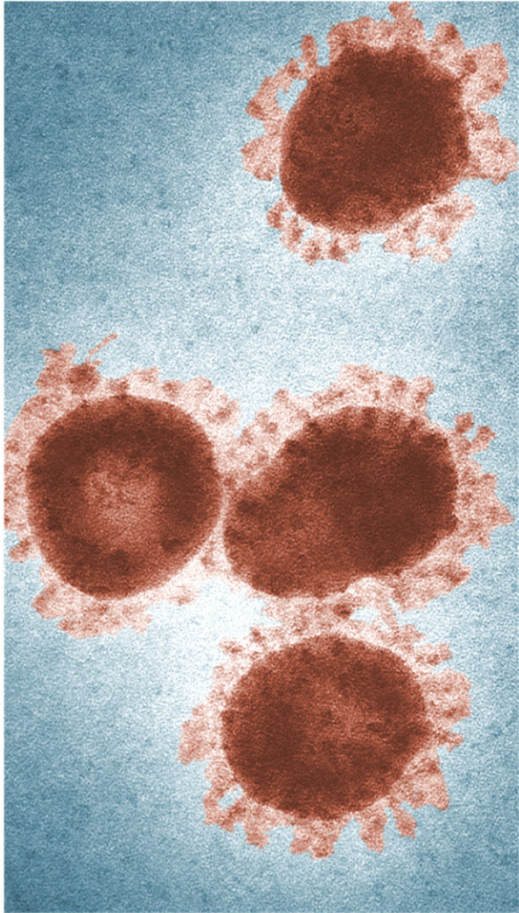
Credible Leads to Incredible™



About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbes – the “*gold standard*”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

Outline: CRISPR-Cas9 Engineered Viral Host Cells

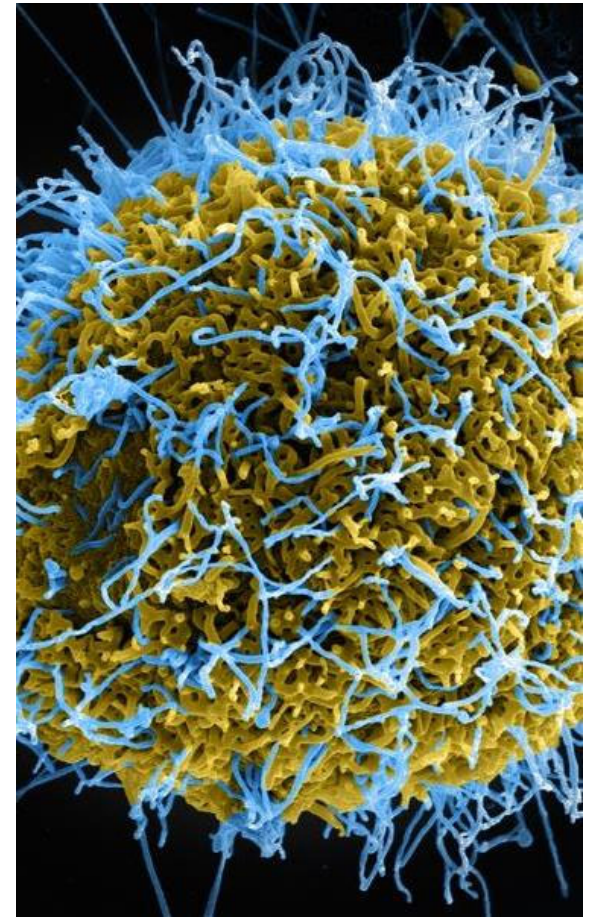


- I. Introduction to viral vaccine production and CRISPR/Cas9 gene editing
- II. Gene editing of Vero cells for enhanced viral production
- III. Gene editing of MDCK cells for enhanced viral production

Coronavirus, image courtesy of CDC Public Health Image Library

I. Introduction to Vaccine Production and Gene Editing

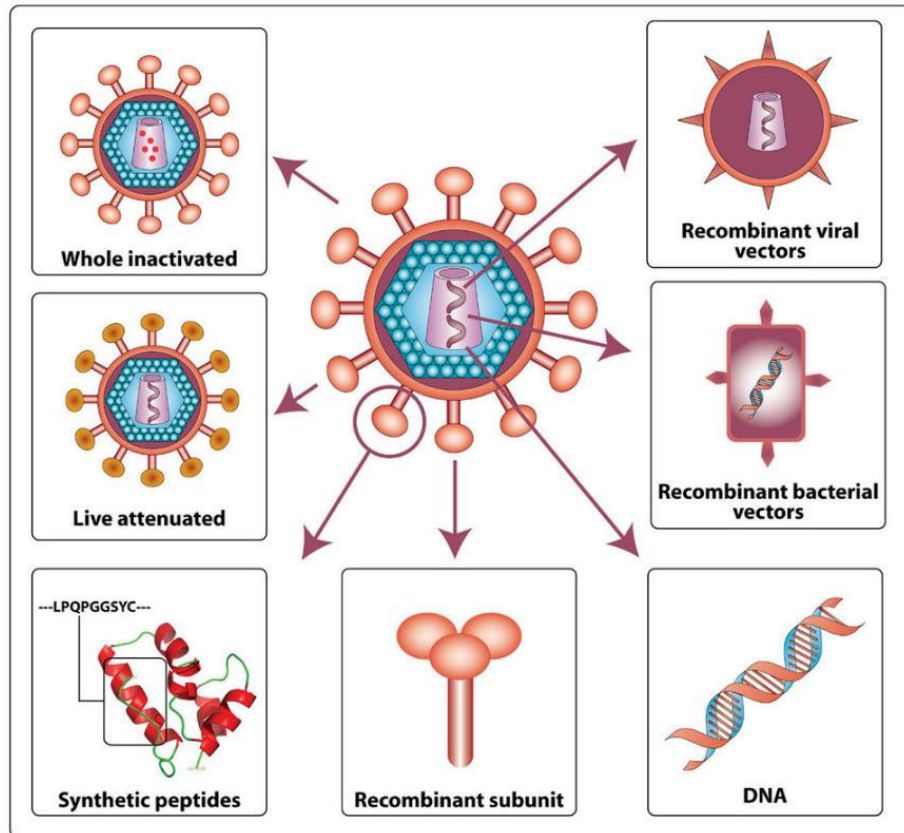
- Overview of viral vaccines
- Manufacturing of viral vaccines in host cell culture
- Cell lines used for viral vaccine manufacturing
- Opportunity for improvement of viral production cells
- Technology for cell line modification
- Host cell anti-viral response
- Use of NHEJ repair mechanism for gene knockout
- Development of enhanced viral production cell lines



Ebola virus infecting Vero cell, image courtesy of NIAID

Overview of Viral Vaccines

Vaccines against viral infections are designed to introduce your immune system to that virus without actually making you sick



en.wikipedia.org/wiki/HIV_vaccine

Examples of Viral Vaccines

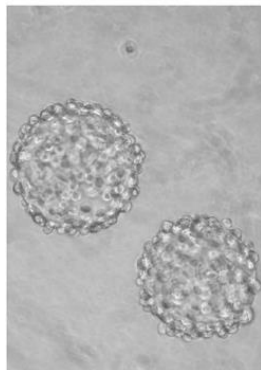
- Flu shot (Influenza)
- MMR (Measles, mumps, rubella)
- Varicella (Chickenpox)
- Polio vaccine
- HPV (Human papillomavirus)
- HepA/B/C (Viral hepatitis)
- Rotavirus vaccine
- Rabies vaccine

Manufacturing of Viral Vaccines in Host Cell Culture

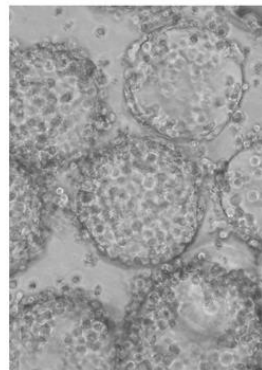
Viral products for vaccines are primarily manufactured in large-scale tissue culture systems



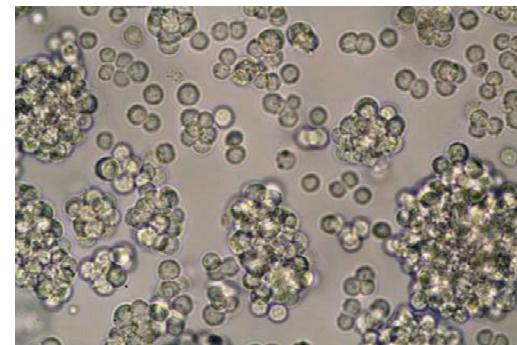
Adherent cells on micro-carrier beads
Vero cells



MRC-5 cells



Carrier-free suspension culture



Cell Lines Used for Viral Vaccine Manufacturing

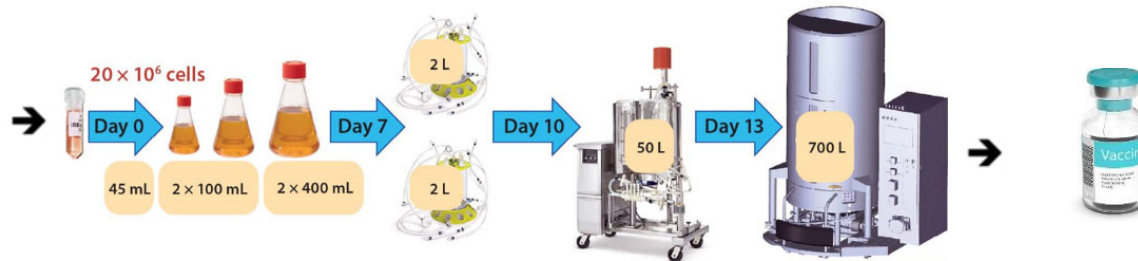
A handful of traditional cell lines are used for viral vaccine manufacturing

Name	ATCC® No.	Species	Cell type	Year Isolated	Vaccines
MDCK	CCL-34™	<i>Canis familiaris</i> (domestic dog)	Kidney epithelial	1958	Influenza A, influenza B (seasonal)
Vero	CCL-81™	<i>Cercopithecus aethiops</i> (green monkey)	Kidney epithelial	1962	Rotavirus, vaccinia, polio, rabies, Japanese encephalitis, dengue, Zika, Chikungunya
WI-38	CCL-75™	<i>Homo sapiens</i> (human)	Lung fibroblast	1961	Adenoviruses, rubella, measles, mumps, varicella zoster, polio, Hep A, rabies
MRC-5	CCL-171™	<i>Homo sapiens</i> (human)	Lung fibroblast	1966	Zoster, polio, hepatitis A, hepatitis B, Varicella, measles, mumps, rubella, rabies

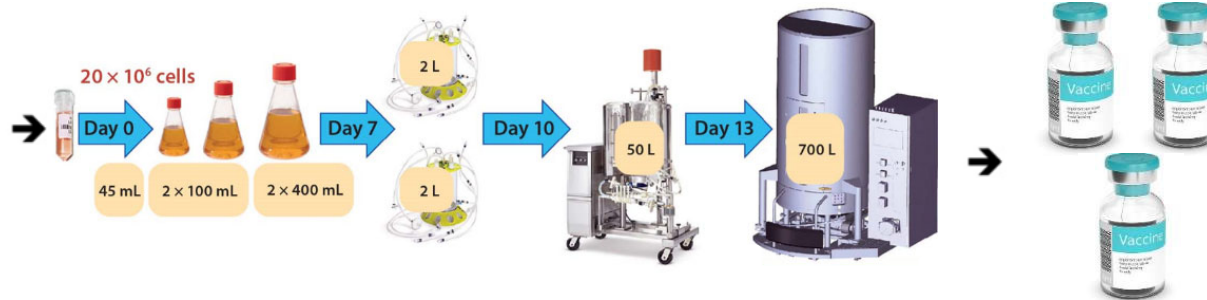
Opportunity for Improvement of Viral Production Cells

Can cell lines be enhanced to produce viruses more efficiently?

Current vaccine production cell line



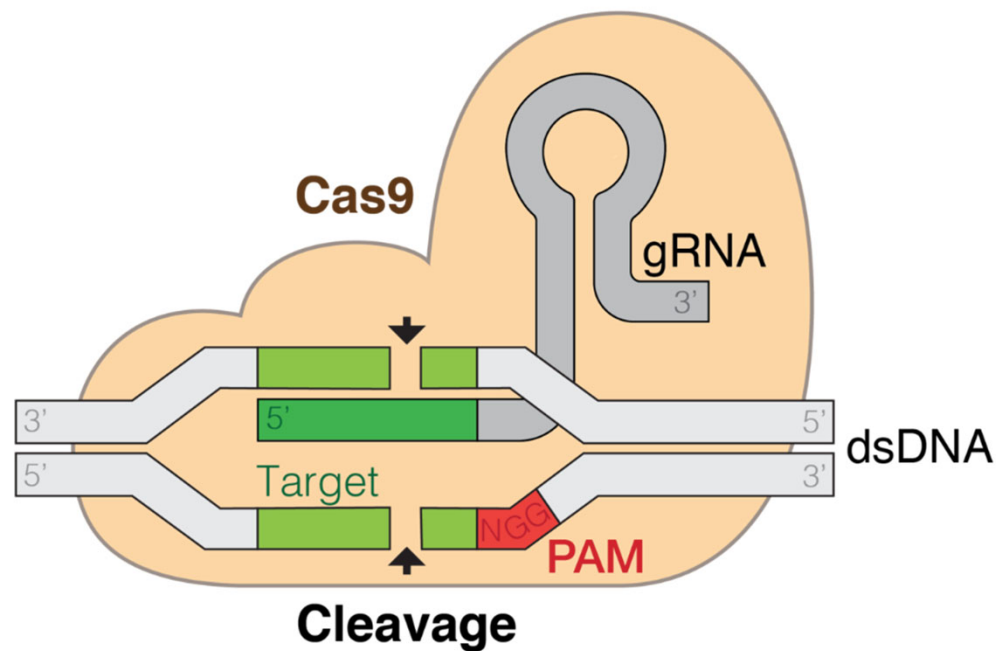
Improved vaccine production cell line



- Faster virus production
- Smaller production scale required to meet manufacturing goals
- Lower production costs
- Fewer regulatory hurdles for using new version of approved cell line than for a new cell line

Technology for Cell Line Modification

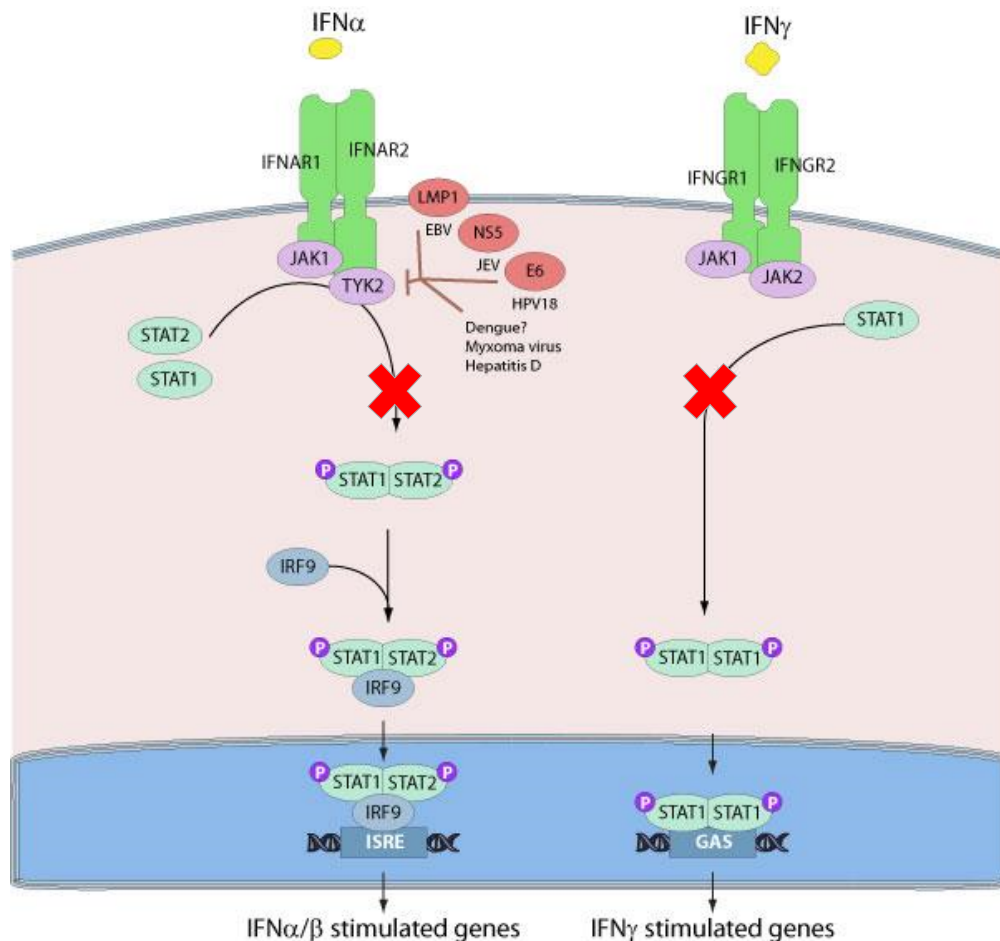
CRISPR-Cas9 can be used to permanently change the genetics and characteristics of a cell line



CRISPR-Cas9 can be used to:

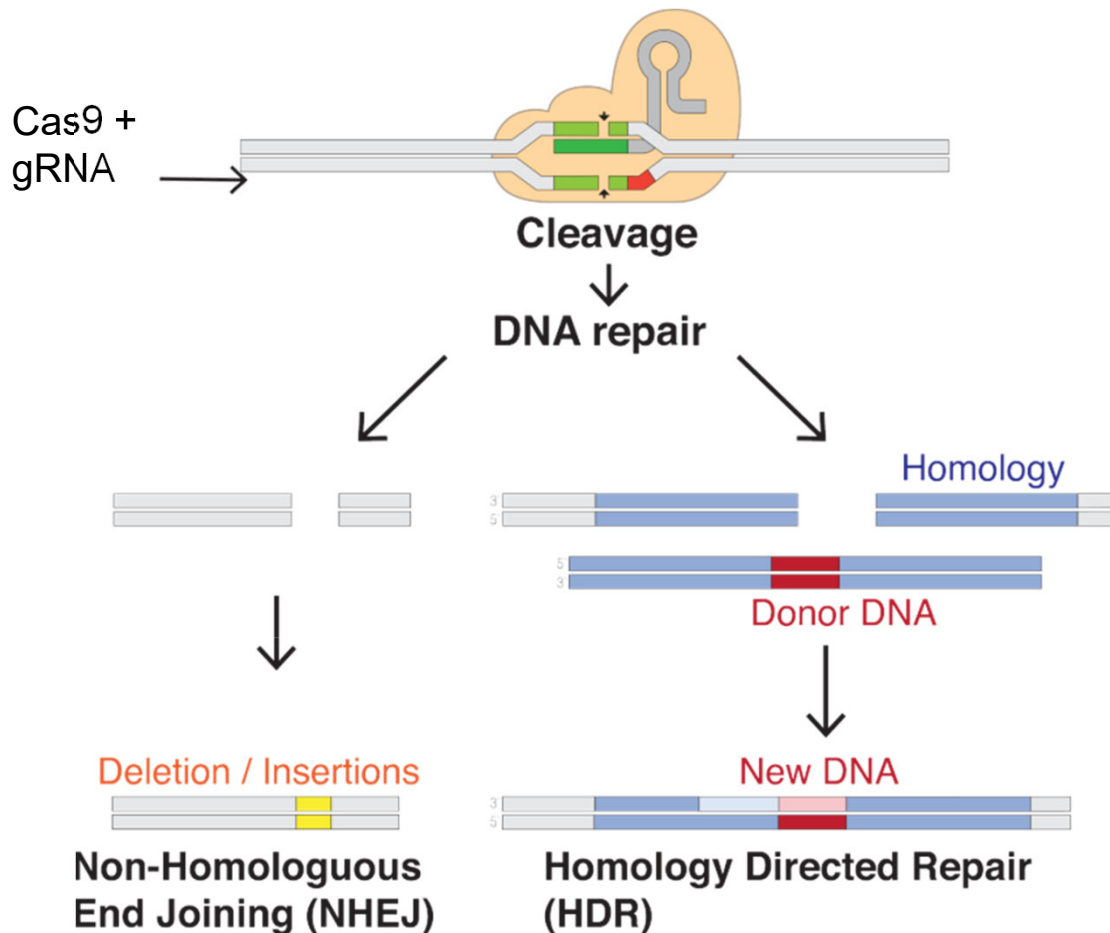
- Locate specific gene sequences inside the nucleus of a cell
- Cut the genomic DNA at that specific location
- Change the genetic sequence at the cut site (mutation)
- Add new genetic sequence to the cut site (insertion)
- Remove genetic sequence from the cut site (deletion)
- Permanently change the characteristics of the cell controlled by the modified gene

STAT1 Signaling Controls Host Cell Anti-viral Response



- Cell lines used in viral production have anti-viral interferon response
- STAT1 protein is essential for anti-viral interferon response
- Strategy – use CRISPR/Cas9 to disrupt STAT1 gene in cells used for viral production
- No STAT1 protein is produced when STAT1 gene is disrupted
- Virus production is enhanced in STAT1 deficient cell lines

Use of NHEJ Repair Mechanism for Gene Knockout



- Design and construct CRISPR/Cas9 reagents to target STAT1 gene
- Transient transfection of Cas9 and STAT1 gRNA constructs into viral production cells
- CRISPR/Cas9 creates double-strand break (DSB) in STAT1 gene using
- DSB is repaired by non-homologous end joining, an error-prone cellular DNA repair mechanism
- NHEJ results in small insertions and deletions at repair site
- Out-of-frame insertions and deletions near the start of STAT1 gene result in a functional protein knockout

Development of Enhanced Viral Production Cell Lines



Evaluate host cell lines



Select target genes



Edited cell pool test

- Editing efficiency
- Functional test

Single clone screening

- Sequencing
- Bio-functional screening

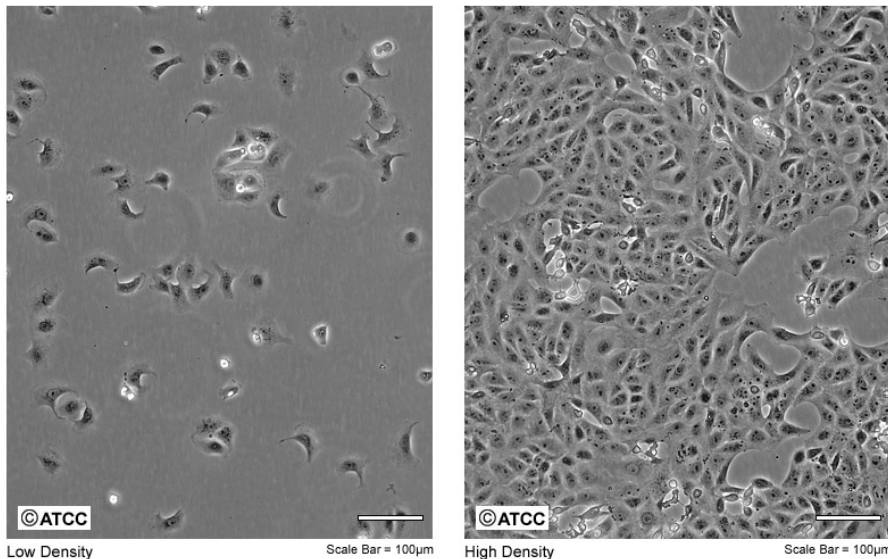
Select top clones

- Sequence verification
- Transcript testing
- Protein expression
- Off target edit evaluation
- Stability testing
- Morphology and cell growth
- Bio-function evaluation

Enhanced
vaccine
production
cell models

Viral Vaccine Production in Vero Cells

ATCC Number: **CCL-81**
Designation: **Vero**



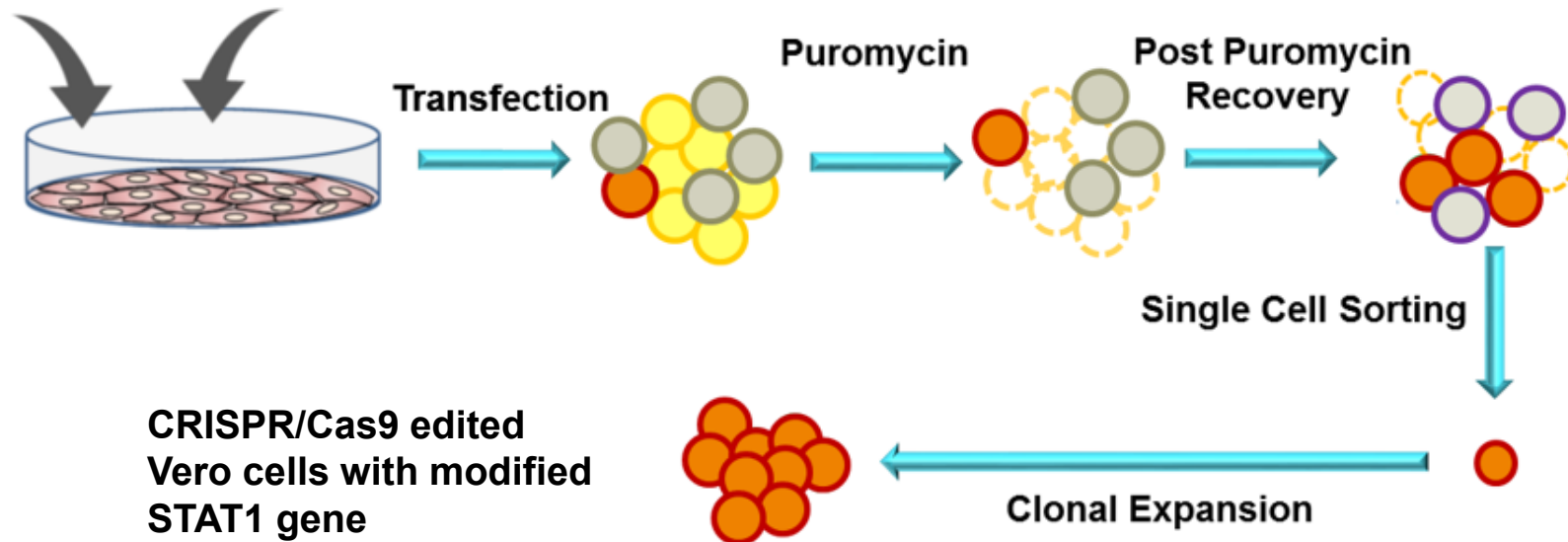
- Vero is an adherent epithelial cell line established from the kidney of a normal adult African green monkey in 1962.
- Vero cells are unusually permissive to infection by a wide variety of animal viruses.

- Vero remains one of the most commonly used cell lines for cell-based viral vaccine production.
- Vero cell line has received worldwide regulatory acceptance.
- Abundant studies on the production of a variety of viruses using Vero cells, such as:
 - Rotavirus
 - Influenza
 - Vaccinia
 - Polio
 - Rabies virus
 - Japanese encephalitis virus
 - Dengue
 - Zika
 - Chikungunya.

Workflow of CRISPR/Cas9 Gene Editing

Used to modify the STAT1 gene of Vero cells

Cas9-Puro and guide RNA targeting STAT1 gene



Cas9 constructs are expressed transiently and are not integrated into gene edited clones

STAT1 KO in CRISPR-Cas9 Edited Vero Cell Clone

Selected STAT1 KO Vero cell clone has a 199 nt deletion in both chromosomal copies of STAT1

```
Vero WT Reference.
Alignment to >TCAATTGTATTTGCTGAATGAAGAAAACCTGCCTTCCATCAACATGAGAACATTTCAACTAAAACACAAAAACCAGGTCATACCTGAAGATTACGCTTGCT>391
Vero WT RV.ape-- >TCAATTGTATTTGCTGAATGAAGAAAACCTGCCTTCCATCAACATGAGAACATTTCAACTAAAACACAAAAACCAGGTCATACCTGAAGATTACGCTTGCT>396
9-H1 E4 p2.ape-- >TCAATTGTATTTGCTGAATGAAGAAAACCTGCCTTCCATCAACATGAGAACATTTCAACTAAAACACAAAAACCAGGTCATACCTGAAGATTACGCTTGCT>391
9-H1 E4 p18.ape-- >TCAATTGTATTTGCTGAATGAAGAAAACCTGCCTTCCATCAACATGAGAACATTTCAACTAAAACACAAAAACCAGGTCATACCTGAAGATTACGCTTGCT>400
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```
Vero WT Reference.
Alignment to >TTTCCTTATGTTATGCTGTAGCAAGAAGTTATTCTCCAAGAAAAGCGACTATATTGATCATCCAGCTGTGACAGGAGGTCATGAAAACGGATGGTGGCA>491
Vero WT RV.ape-- >TTTCCTTATGTTATGCTGTAGCAAGAAGTTATTCTCCAAGAAAAGCGACTATATTGATCATCCAGCTGTGACAGGAGGTCATGAAAACGGATGGTGGCA>496
9-H1 E4 p2.ape-- >TTTCCTTATGTTATGCT [redacted] TTATT [redacted] >412
9-H1 E4 p18.ape-- >TTTCCTTATGTTATGCT [redacted] TTATT [redacted] >421
```

```
Vero WT Reference.
Alignment to >AATGAAACATCATTGGCAGCGTGCTCCCTAGGAGATTTAACATTTACATACTTCATTCTAGAACTAAATGTCTACGTAAAACAGACAAAACAAATGTTGG>591
Vero WT RV.ape-- >AATGAAACATCATTGGCAGCGTGCTCCCTAGGAGATTTAACATTTACATACTTCATTCTAGAACTAAATGTCTACGTAAAACAGACAAAACAAATGTTGG>596
9-H1 E4 p2.ape-- >AAT [redacted] >415
9-H1 E4 p18.ape-- >AAT [redacted] >424
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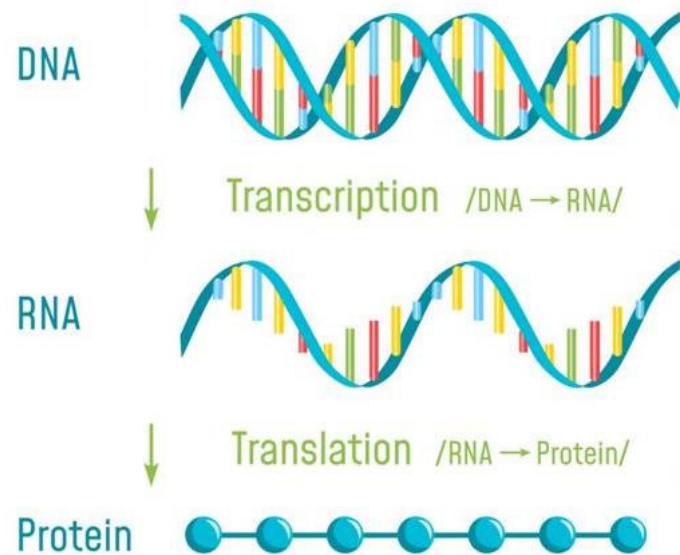
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Vero WT Reference.
Alignment to >TTTCCTTATTGAAATTATTAAAAACCATTTAAGTATTTTCTTAGGTTTATTTTCTTCTTCTGAAACTGATACTGCTTTTAGCAGTAGT-TTATATGGTAT>690
Vero WT RV.ape-- >TTTCCTTATTGAAATTATTAAAAACCATTTAAGTATTTTCTTAGGTTTATTTTCTTCTTCTGAAACTGATACTGCTTTTAGCAGTAGT-GTTATATGGGN>696
9-H1 E4 p2.ape-- > [redacted] ACCATTTAAGTATTTTCTTAGGTTTATTTTCTTCTTCTGAAACTGATACTGCTTTTAGCAGTAGT-TTATATGGTAT>491
9-H1 E4 p18.ape-- > [redacted] ACCATTTAAGTATTTTCTTAGGTTTATTTTCTTCTTCTGAAACTGATACTGCTTTTAGCAGTAGT-N-TTATATGGTAT>500
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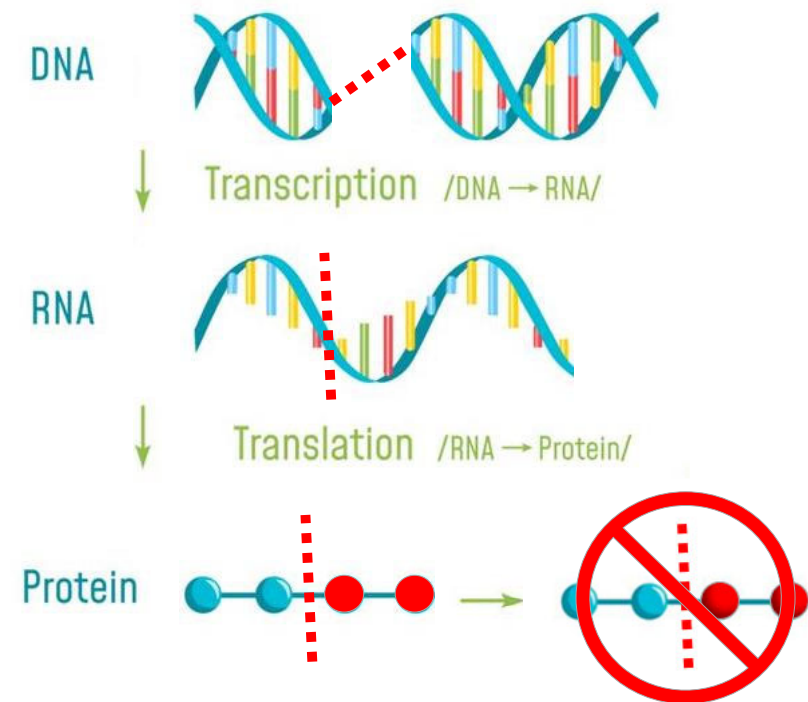
Sequence Deletion Results in Functional STAT1 Knockout

Edited STAT1 gene produces a truncated, non-functional protein that is rapidly degraded

Production of STAT1 Protein in Unmodified Vero Cells

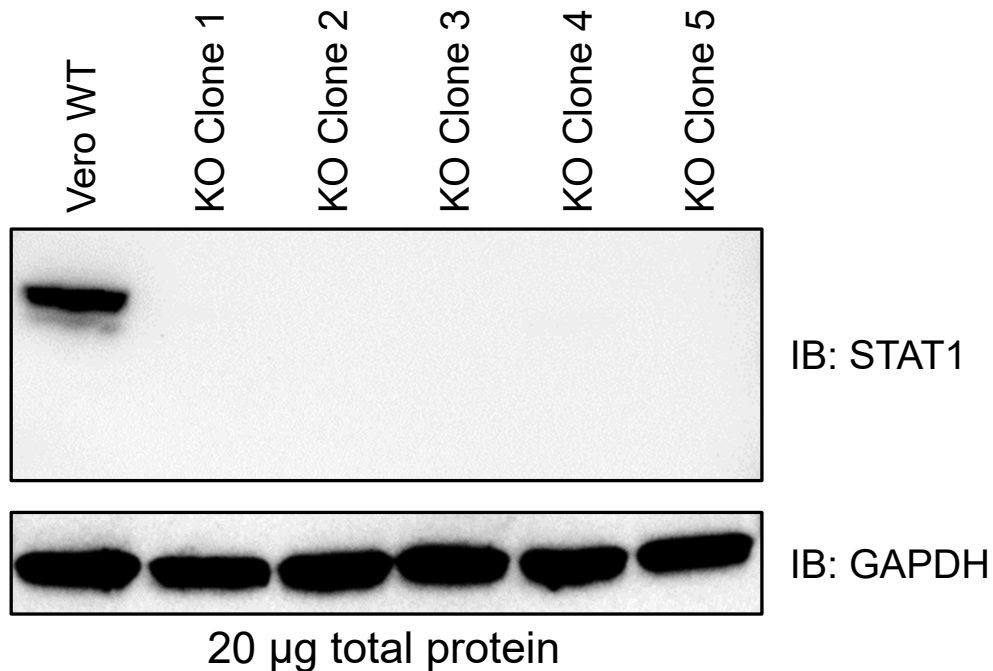


Production of STAT1 Protein in Modified Vero Cells



Confirmation of STAT1 Protein Knockout

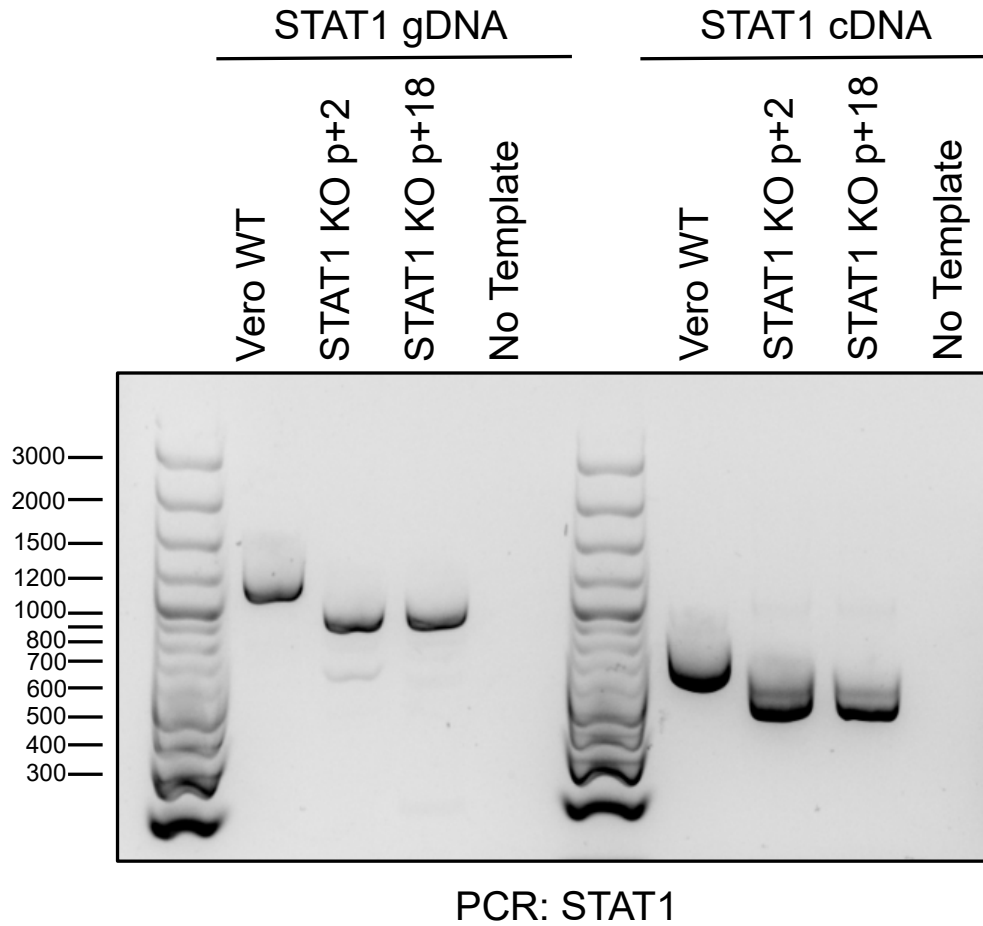
No STAT1 protein detected in selected STAT1 KO Vero clones



Protein Immunoblotting:

- Extract total protein from modified Vero cells
- Separate cellular proteins by size (PAGE gel)
- Transfer separated cellular proteins to blotting paper (PVDF membrane)
- Apply anti-STAT1 antibody to blotting membrane
- Label STAT1 antibody with illuminating dye
- Image to visualize STAT1 protein

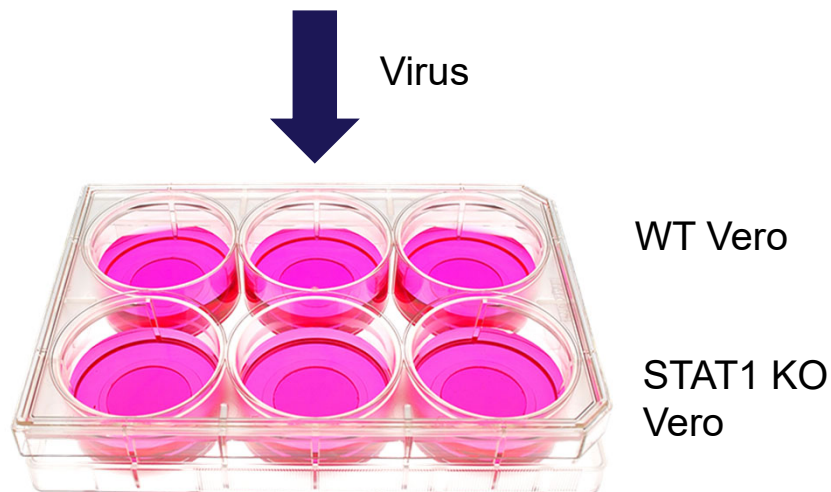
Confirmation of STAT1 Gene Disruption



TCID50 Measurement of Virus Concentration

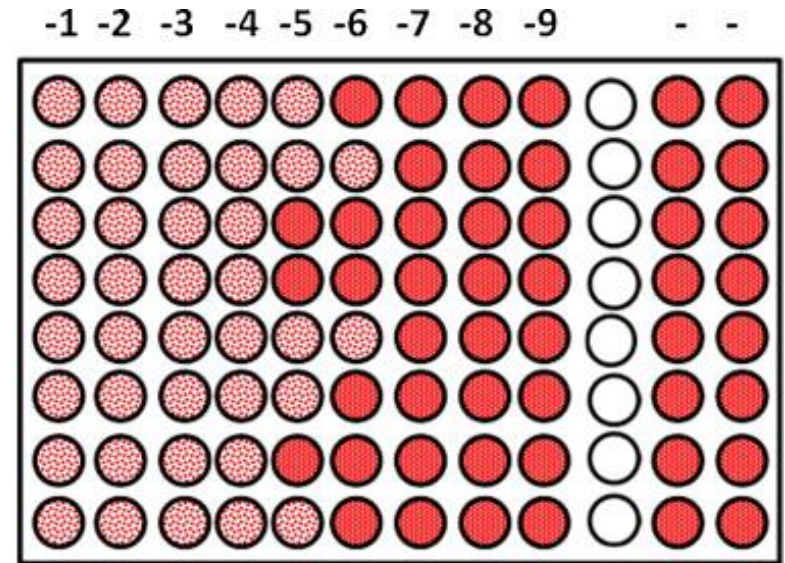
TCID50 – endpoint dilution assay that measures 50% Tissue Culture Infective Dose

1.



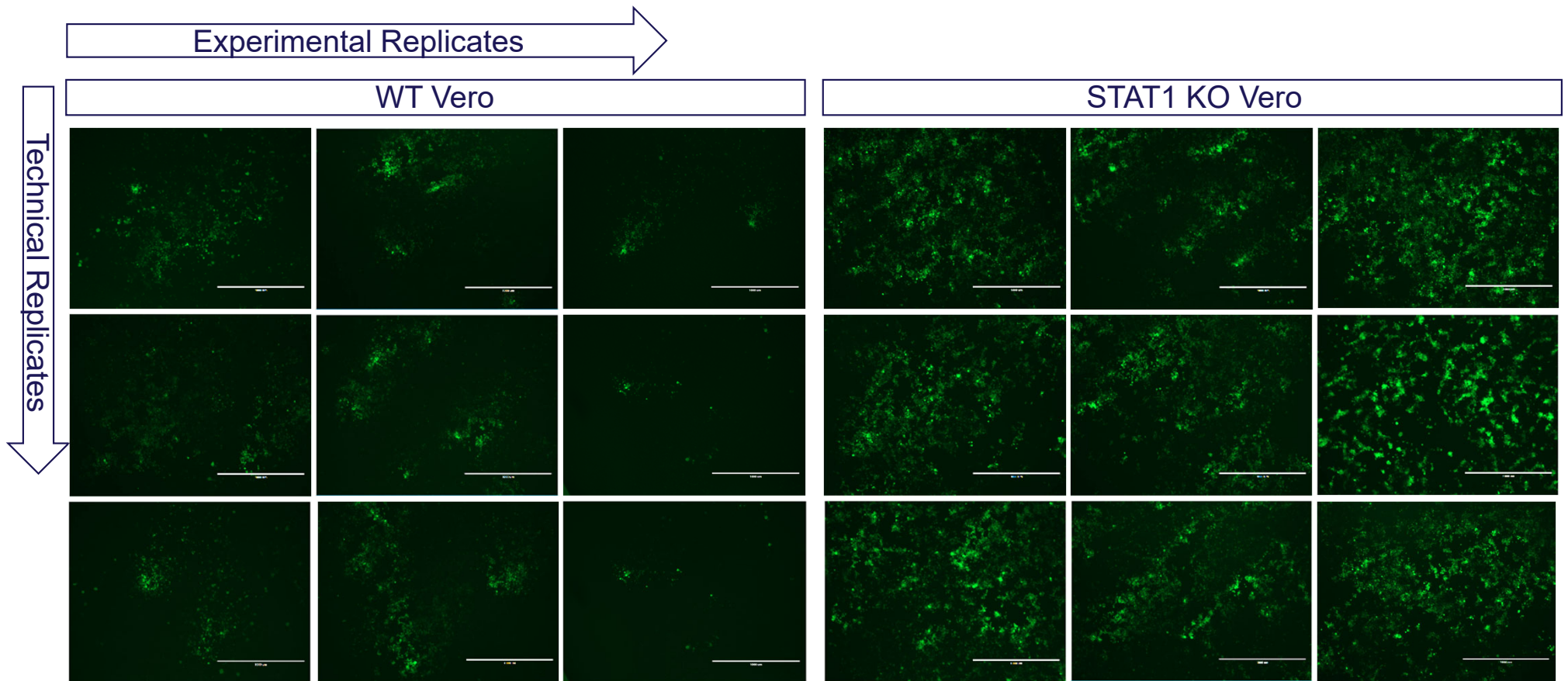
Infect WT and STAT1 KO Vero cells with virus of interest, harvest viral supernatants throughout the course of the viral infection.

2.



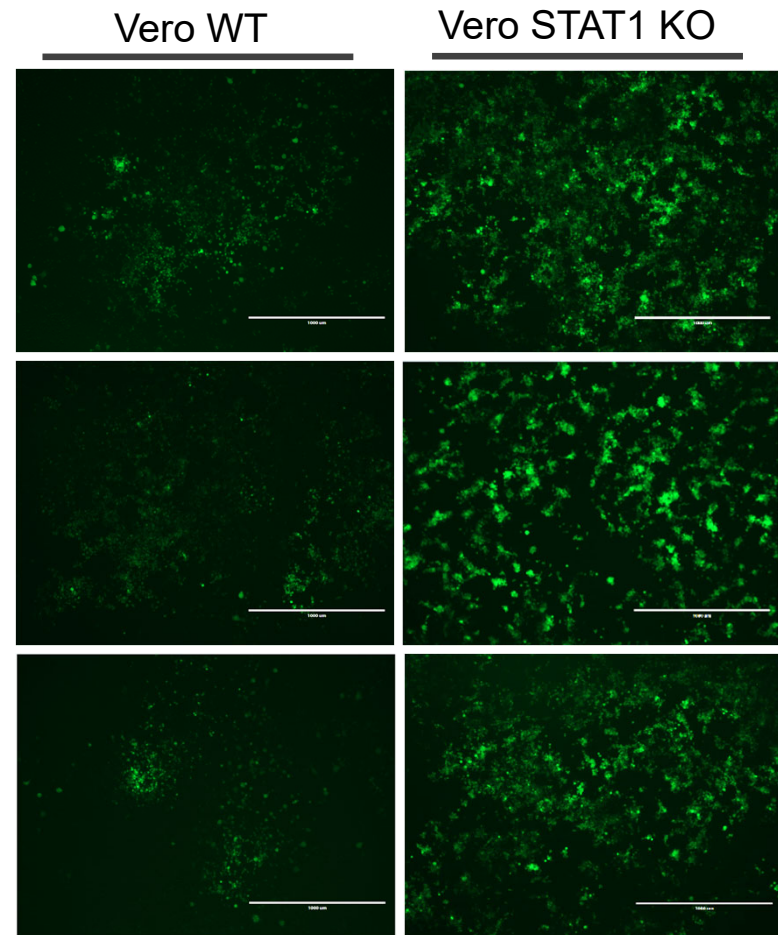
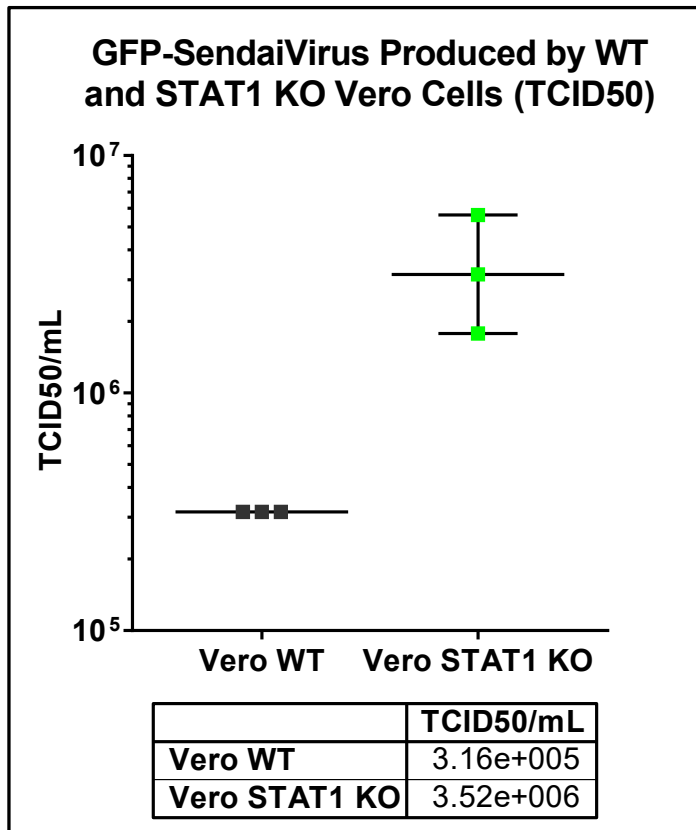
Infect uniformly seeded 96 well replicate plates of WT Vero cells with serial dilutions of experimental viral supernatants. Score wells as positive or negative for infection. Calculate TCID50.

Increased Reporter Virus Production in STAT1 KO Vero



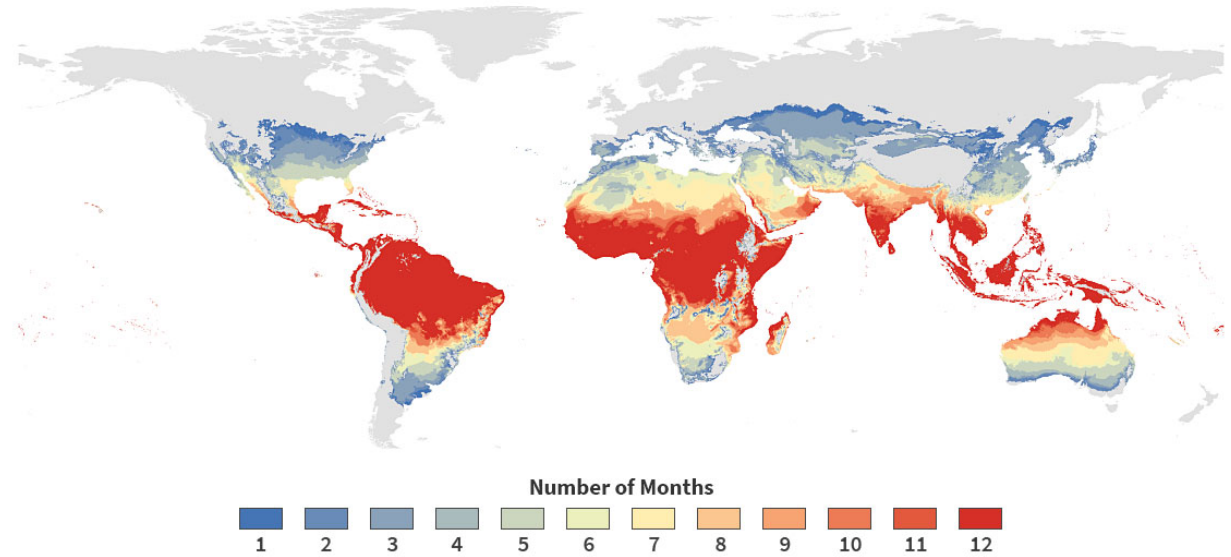
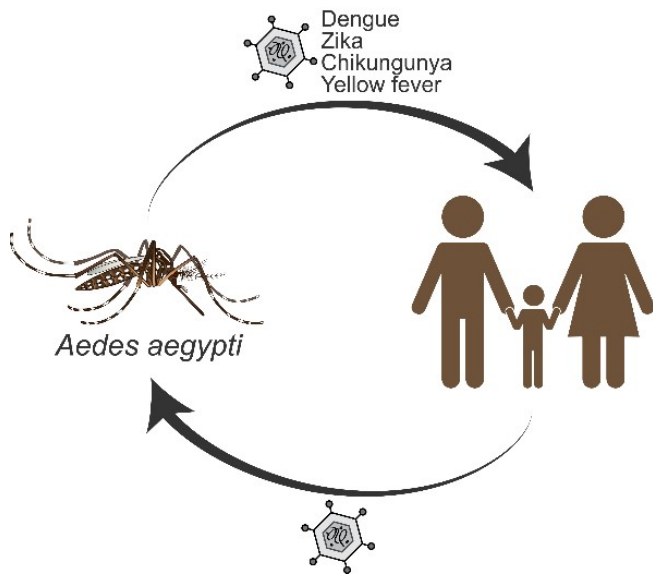
GFP-SeV Production in WT and STAT1 KO Vero Cells

STAT1 KO Vero cells show 10-fold enhancement in GFP-SeV production at 72 HPI

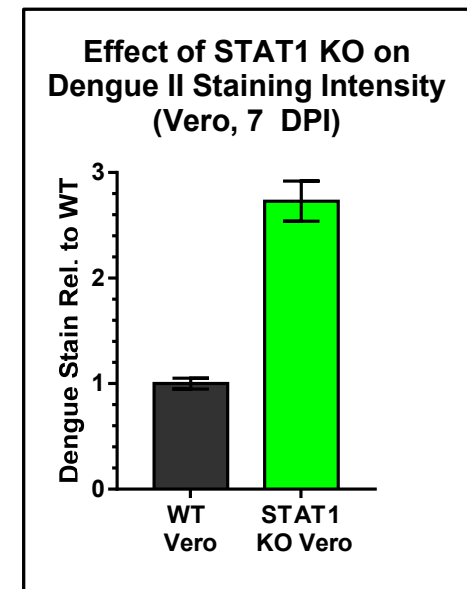
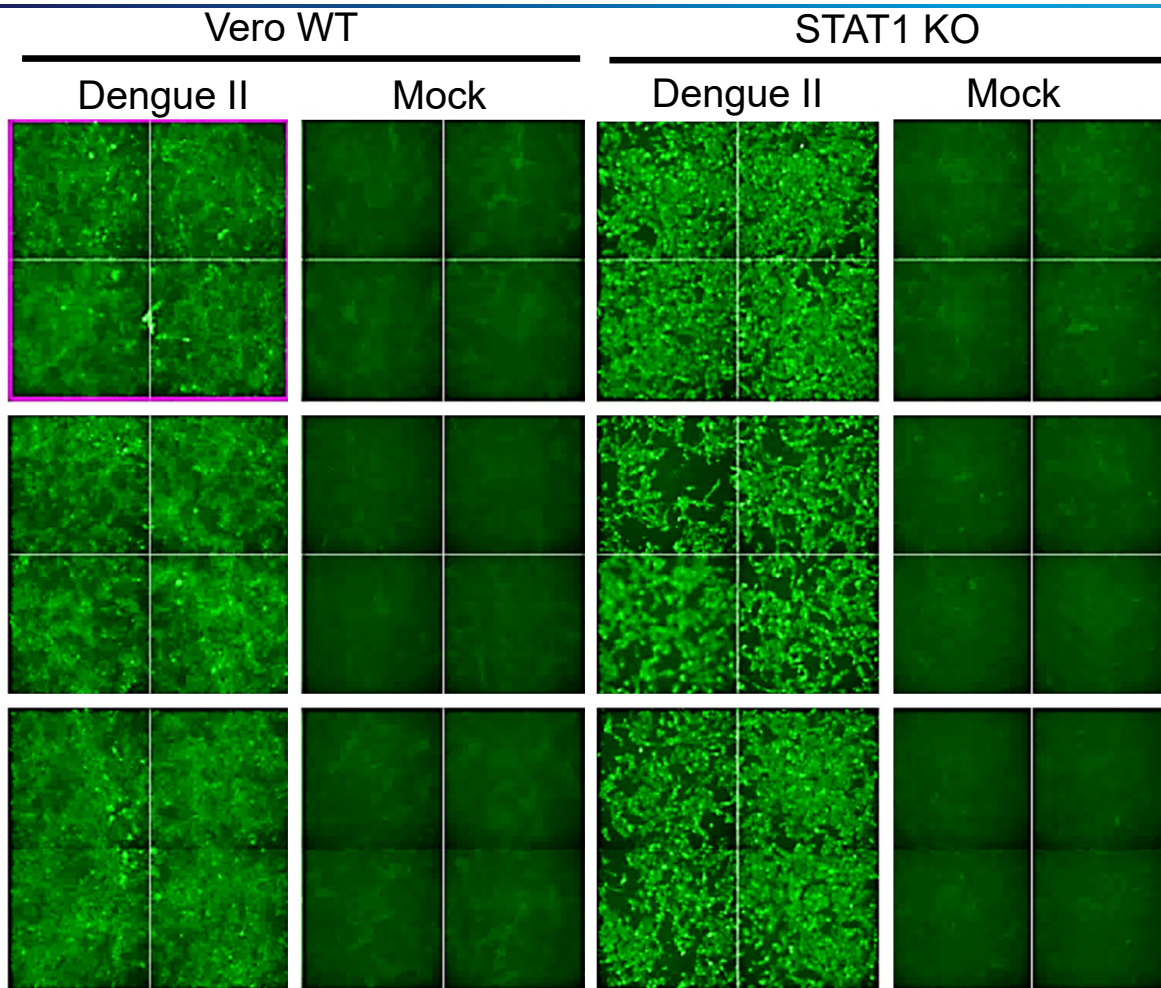


Vaccines Made in Vero Cells: Dengue Virus

Expansion of *Aedes aegypti* habitat is increasing the demand for Dengue vaccine

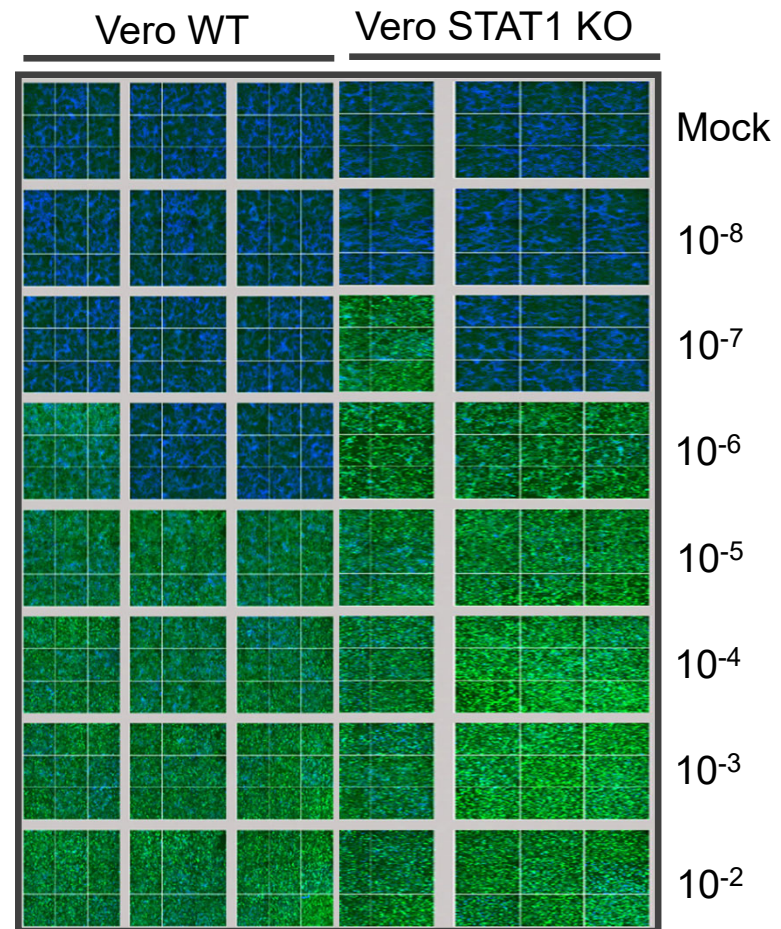
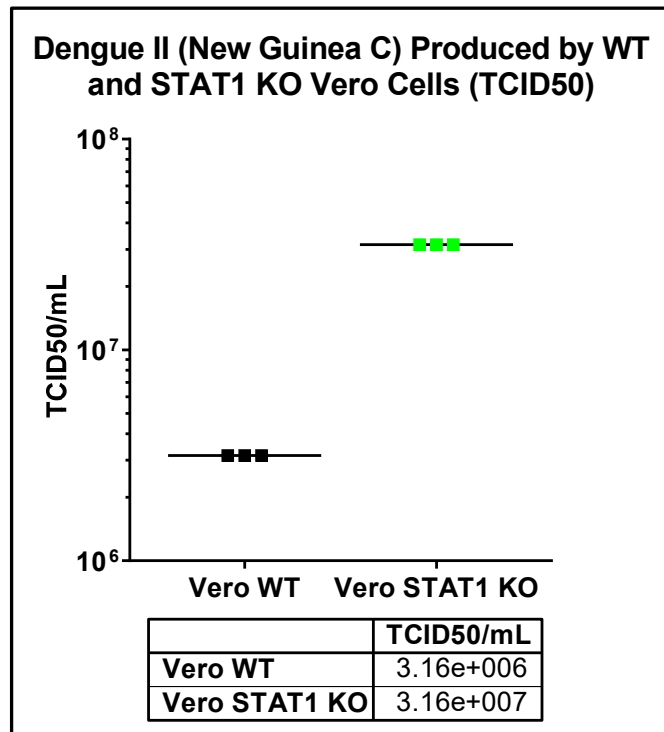


Evaluation of Dengue II Production in STAT1 KO Vero



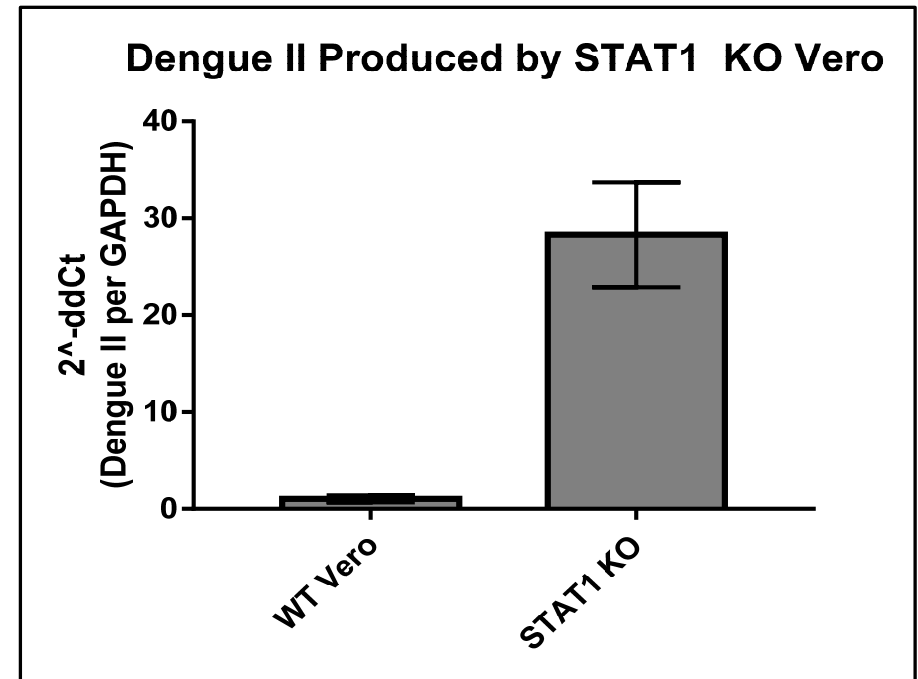
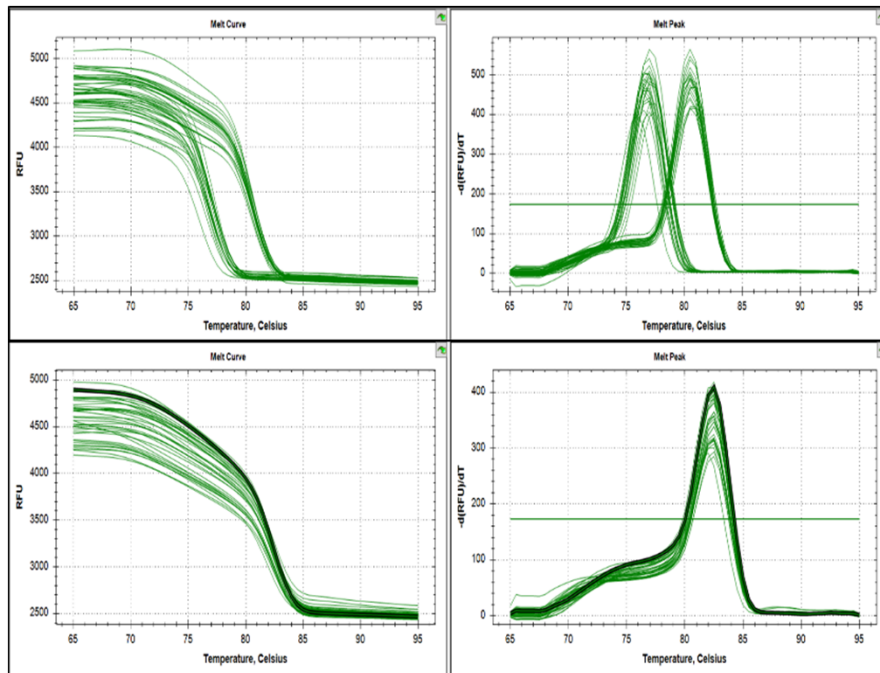
Enhanced Dengue Virus Production – TCID50

Dengue II staining of TCID50 of viral supernatants from WT and STAT1 KO Vero

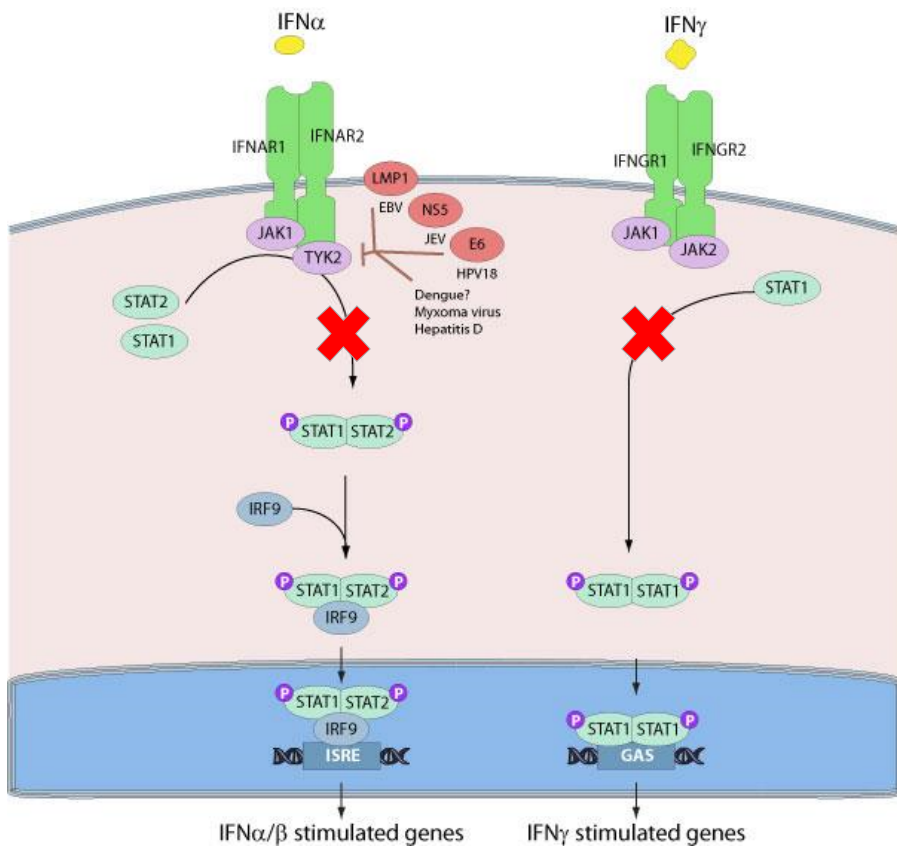


Enhanced Dengue Replication – RT-PCR

RT-PCR shows 30-fold increase in Dengue II viral genomes produced in STAT1 KO Vero cells

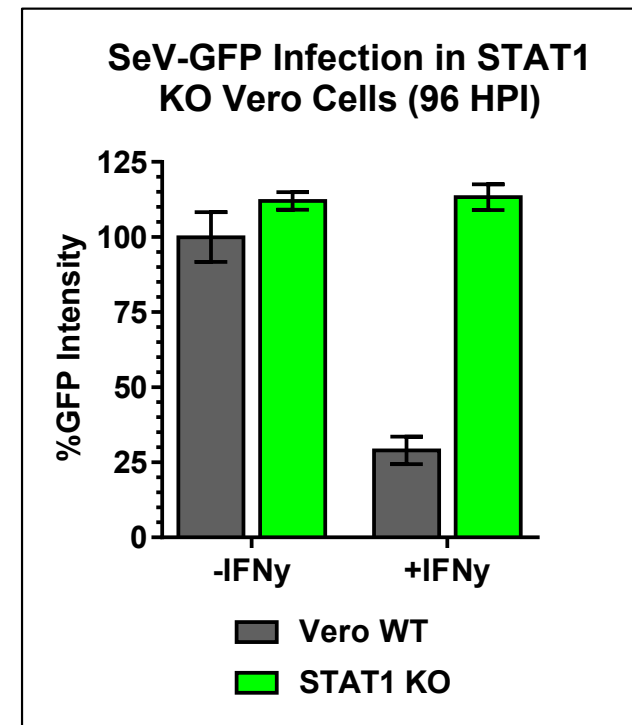
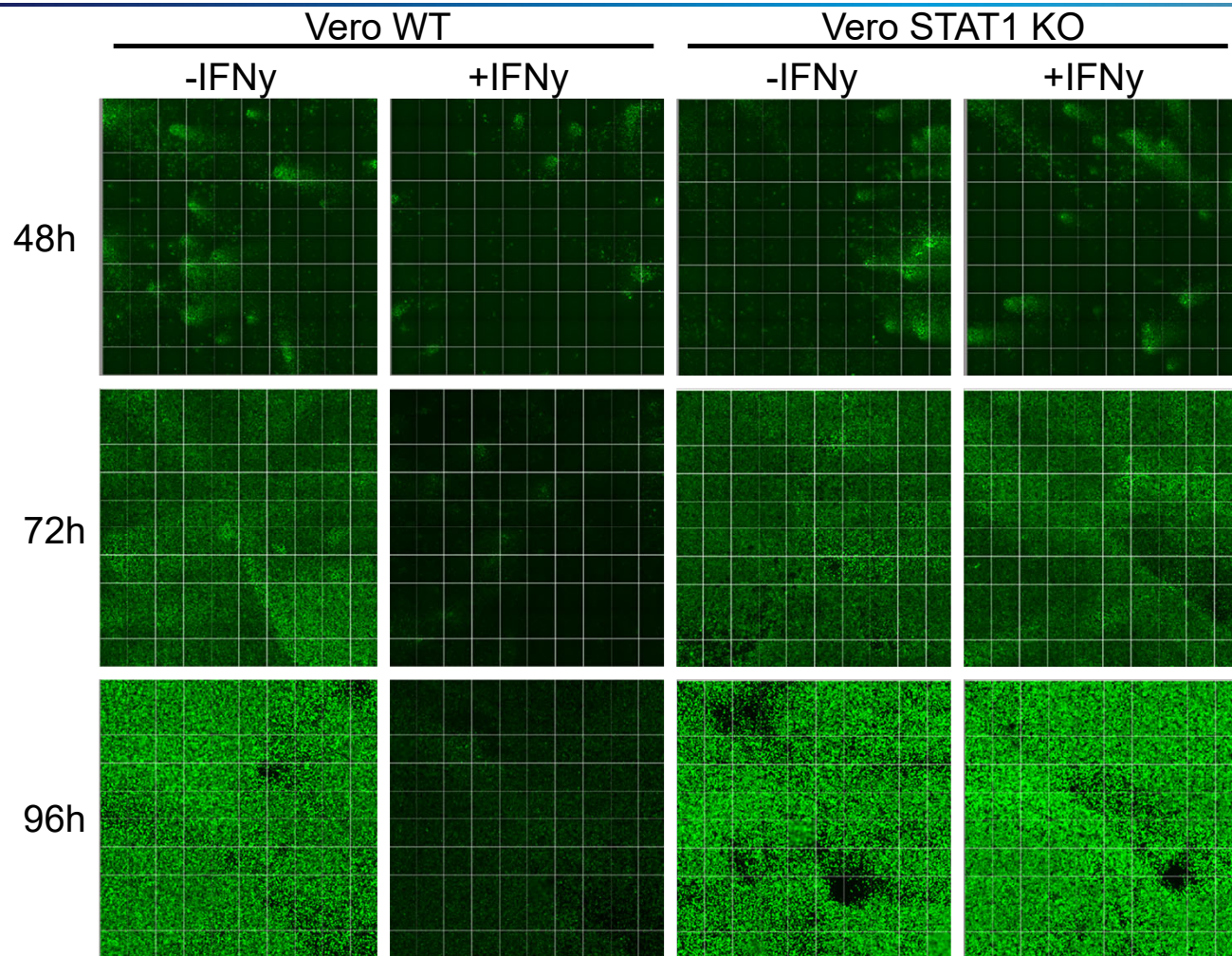


STAT1 KO Vero Cells Show No Response to Interferon



	IFN γ	IFN γ
	+	+
WT Vero	WT Vero	KO Vero
+	+	+
GFP-SeV	GFP-SeV	GFP-SeV
↓	↓	↓
Typical GFP-SeV infection	Reduced GFP-SeV infection	Typical GFP-SeV infection

No Response to Interferon in STAT1 KO Vero Cells



Screening for Off-Target Mutations

Cas9 has the potential to create mutations in sequences that resemble the targeted sequence

	Gene	Gene ID	Also Known As	Target Coordinates	Target Sequence	Type	MM	CFD	WT	KO
	STAT1	103217529	Vero G2	chr10:76532444-76532466:-	TCATGACCTCCTGTACACAGC TGG	Exon	0	N/A		
1	EML6	103220249	EMAPL6	chr14:52084054-52084076:+	TCAT CAC TGCCTGTACACAGC CAG	Exon	3	0.1103		
2	NPB/PCYT2	103243738/ 103243739	N/A	chr16:73777880-73777902:+	TC TGC ACCTCCTG CC CACAGC TGG	Exon	4	0.099		
3	ZNF462	103219061	ZFPIP, Zfp462	chr12:32612133-32612155:-	TCA AGT CCTCCT ATA ACAGC CGG	Exon	4	0.0698		
4	EPHX1	103230002	MEH, EPHX, EPOX, HYL1	chr25:3945289-3945311:-	TCA AC ACCTGCTGTACAC CC AGG	Exon	4	0.0652		
5	SYTL1	103225294	JFC1, SLP1	chr20:105436957-105436979:-	TC GGG ACCTCCTG CC ACTGC GGG	Exon	4	0.0546		
6	CCDC180	103219164	C9orf174	chr12:42077653-42077675:+	TCATGACCTG TGG TACACAG A TGG	Exon	4	0.0299		
7	TG	103237469	TGN, AITD3	chr8:127328236-127328258:-	TCATGACT TACTCT CACAG G CGG	Exon	4	0.0188		
8	ECH1	103234640	HPXEL	chr6:33441150-33441172:-	TCAT CACCG CCTGT GACATC CGG	Exon	4	0.0162		
9	GSDMA	103243525	GSDM, FKSG9, GSDM1	chr16:66196251-66196273:+	CCACG ACCTCCT TTG ACAGC AGG	Exon	4	0.0083		
10	NRXN2	103233117	N/A	chr1:9583866-9583888:-	TCATG GCCTCC CAT CACAGC TGG	Intron	3	0.6246		
11	ASIC4	103217917	ACCN4, BNAC4	chr10:105414648-105414670:-	GCATGACTTCC AA TACAGC CGG	Intron	4	0.6181		
12	FHOD3	103222526	FHOS2, Formactin2	chr18:44299974-44299996:-	CCAGG ACCT ACTG ACACAGC AGG	Intron	4	0.2874		

Example of Off-Target Mutation Evaluation

Sanger sequencing of off-target site shows no mutation across multiple gene-edited clones

```
st8_TG_11C8_TG2_FW.txt-- Matches:398; Mismatches:15; Gaps:31; Unattempted:0
st7_TG_10C12_TG2_FW.txt-- Matches:402; Mismatches:11; Gaps:30; Unattempted:0
st6_TG_9H1_TG2_FW.txt-- Matches:398; Mismatches:14; Gaps:32; Unattempted:0
st5_TG_8F3_TG2_FW.txt-- Matches:401; Mismatches:11; Gaps:291; Unattempted:0
st4_TG_2E10_TG2_FW.txt-- Matches:399; Mismatches:14; Gaps:369; Unattempted:0
st3_TG_2C7_TG2_FW.txt-- Matches:401; Mismatches:12; Gaps:866; Unattempted:0
st2_TG_2B11_TG2_FW.txt-- Matches:400; Mismatches:11; Gaps:296; Unattempted:0
st1_TG_WT_TG2_FW.txt-- Matches:400; Mismatches:11; Gaps:167; Unattempted:0
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* * * * *
22>GTCAGGCCCAAGCCTCTGCAATGTGCTAAAGAGTGGAGTCTCTCCAGGAGAGTTGGCTCAGGCTATGTCCCAGCCTGCAGGGGAAGAGGATGGGGCCTTTTCCCCAGTGCAATGCGACCAGGCCAGGGCAGCTGCTGGTGTGTACGGGA>171
1>~NNG---NNNNNNNNNGC-ATGTGCT-AAGAGTGGAGTCTCTCCAGGAGAGTTGGCTCAGGCTATGTCCCAGCCTGCAGGGGAAGAGGATGGGGCCTTTTCCCCAGTGCAATGCGACCAGGCCAGGGCAGCTGCTGGTGTGTACGGGA>143
1>~NNNNNNNNNNNNNNGC-ATGTGCT-AAGAGTGGAGTCTCTCCAGGAGAGTTGGCTCAGGCTATGTCCCAGCCTGCAGGGGAAGAGGATGGGGCCTTTTCCCCAGTGCAATGCGACCAGGCCAGGGCAGCTGCTGGTGTGTACGGGA>143
1>NNNNNNNNNNNNNG-----NATGTGCTNA-NAGTGGAGTCTCTCCAGGAGAGTTGGCTCAGGCTATGTCCCAGCCTGCAGGGGAAGAGGATGGGGCCTTTTCCCCAGTGCAATGCGACCAGGCCAGGGCAGCTGCTGGTGTGTACGGGA>142
1>NNNNNNNNNNNNGC-----ATGTGCT-AAGAGTGGAGTCTCTCCAGGAGAGTTGGCTCAGGCTATGTCCCAGCCTGCAGGGGAAGAGGATGGGGCCTTTTCCCCAGTGCAATGCGACCAGGCCAGGGCAGCTGCTGGTGTGTACGGGA>142
1>~NNNNNNNNNNNNNGC-ATGTGCT-NANAGTGGAGTCTCTCCAGGAGAGTTGGCTCAGGCTATGTCCCAGCCTGCAGGGGAAGAGGATGGGGCCTTTTCCCCAGTGCAATGCGACCAGGCCAGGGCAGCTGCTGGTGTGTACGGGA>143
1>~NNNNNNNNNNNNNNGC-ATGTGCT-AAGAGTGGAGTCTCTCCAGGAGAGTTGGCTCAGGCTATGTCCCAGCCTGCAGGGGAAGAGGATGGGGCCTTTTCCCCAGTGCAATGCGACCAGGCCAGGGCAGCTGCTGGTGTGTACGGGA>143
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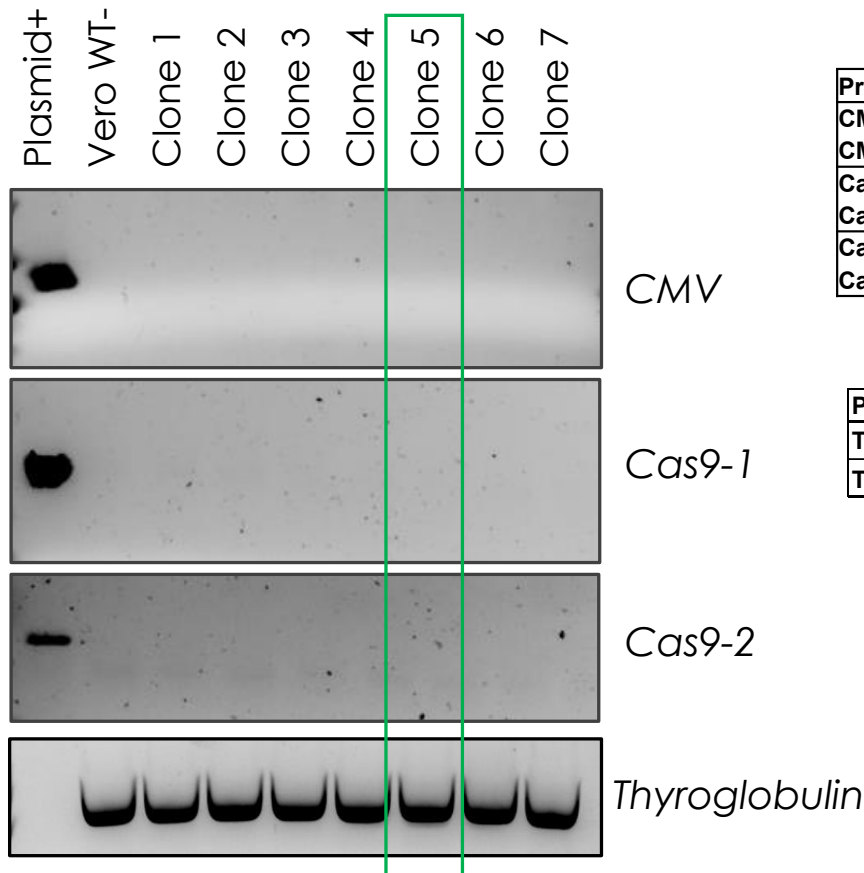
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142>CAGTGGAGAAGAGGTGCCTGAGACGCGTGTGGCCGGGAGCCAGCCCGCTGTGAGAGTAAGTCATGACCCCCCTGGGGGAAAGACAAGGCCTGCATATCTGTTCTTTGATCCAGACTGAGTGAAGTTCTAGAGAAACTGGGAAGGCCGGGG>291

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 Off-target cut site location

Cas9/CMV Plasmid Integration Assessment

No Cas9/CMV integration in selected STAT1 KO Vero clone



Primer	Sequence (5' to 3')	Tm (Q5)	Anneal	Product
CMV-FW	TGGCTCTAGAGGTACCCGTTACATAAC	69	70	342
CMV-RV	AGATGGGGAGAGTGAAGCAGAAC	69		
Cas9-1-FW	CTATAAGGACCACGACGGAGACTACAAG	69	70	323
Cas9-1-RV	TTCTTCTGGCGGTCTCTTCAGC	69		
Cas9-2-FW	TGTCTGCCAGACTGAGCAAGAG	69	70	300
Cas9-2-RV	TCTCGGTGTTCACTCTCAGGATGT	69		

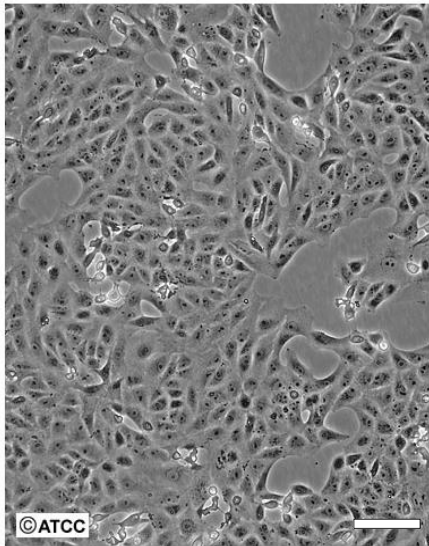
Primer	Sequence (5' to 3')	Tm (Q5)	Anneal	Product
TG FW	GCTCATCTGGCTTGTCTCTGTGT	69°C	70°C	441 bp
TG RV	CCCAGGCTCTTTCTGACTTCAGTTC	69°C		

Selected Clone

Morphology and Growth Kinetics of STAT1 KO Vero Cells

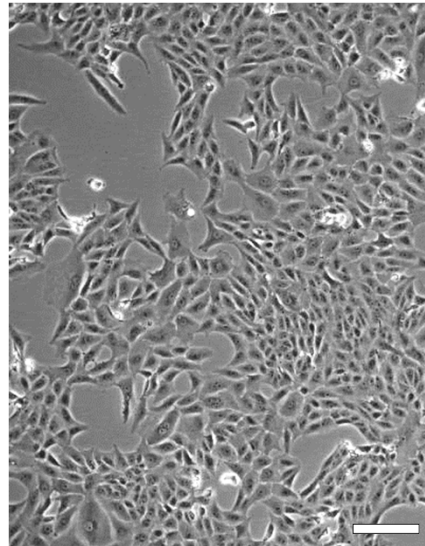
Modified cell line has similar morphology and grows slower than parental Vero cell line

WT Vero



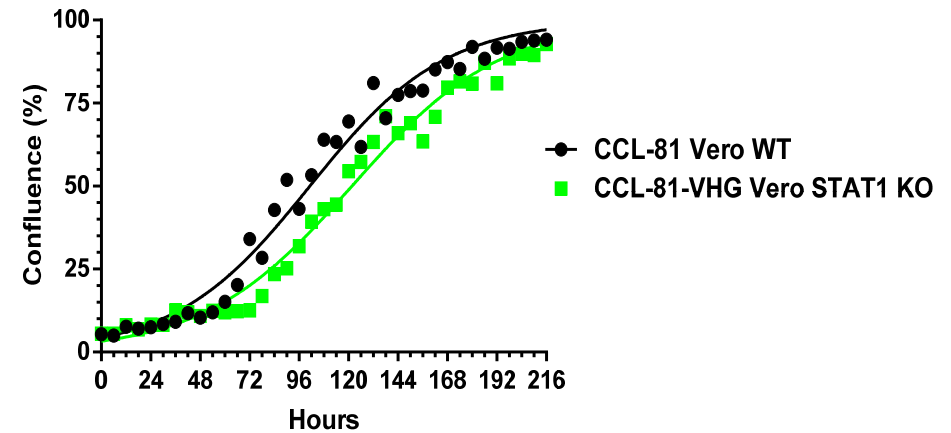
High Density Scale Bar = 100µm

STAT1 KO Vero



High Density Scale Bar = 100µm

Growth Kinetics of CCL-81-VHG STAT1 KO Vero



	Doubling Time (h)
CCL-81 Vero	22.5
CCL-81-VHG Vero STAT1 KO	28.2

Summary: Enhanced Viral Production Vero Cells

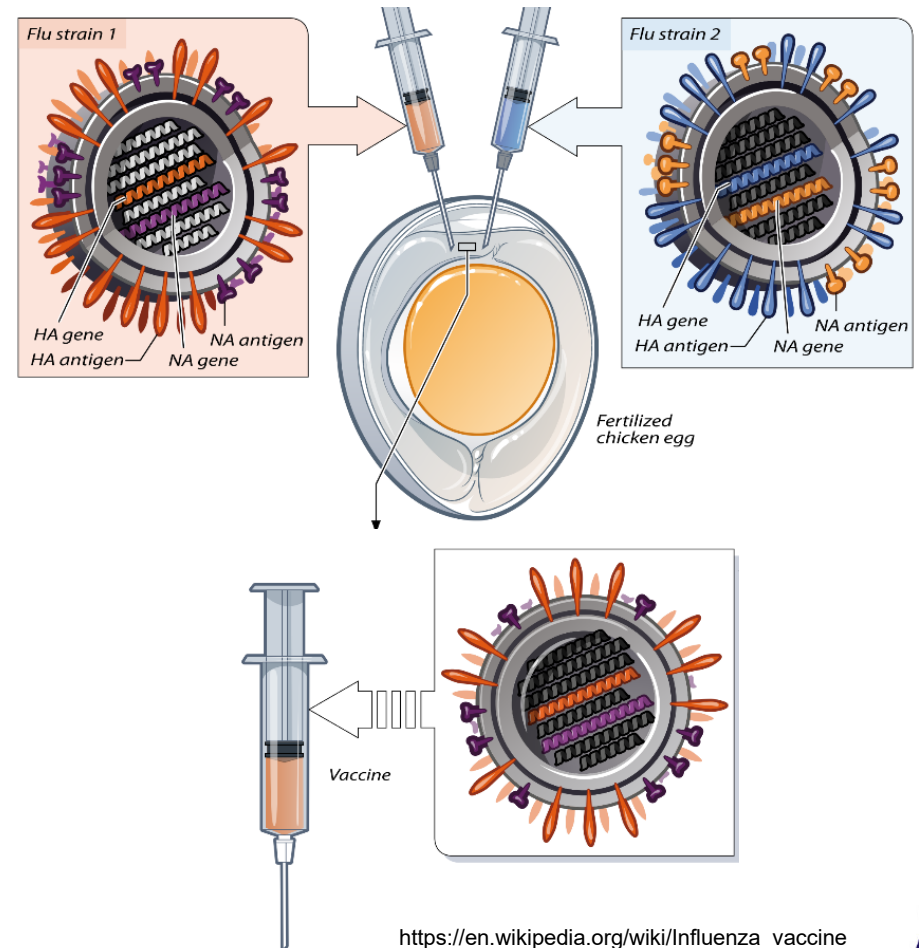
- Successful STAT1 knockout in Vero cells using CRISPR/Cas9
- 10-fold higher yield of both reporter virus and model clinical virus (Dengue II) in STAT1 KO Vero cells relative to unmodified cells
- 30-fold increase in Dengue viral genome replication in STAT1 KO Vero cells
- STAT1 KO Vero cells show no anti-viral response to interferon treatment
- STAT1 knockout genotype is stable over time in selected Vero clone
- No Cas9 plasmid integration detected in selected STAT1 KO Vero clone
- No mutations detected at selected Cas9 off-target cut sites
- STAT1 KO Vero cells have similar morphology and a slower doubling time than unmodified Vero cells



Ebola virus budding from a Vero cell, image courtesy of NIAID

MDCK Cells for Influenza Vaccine Production

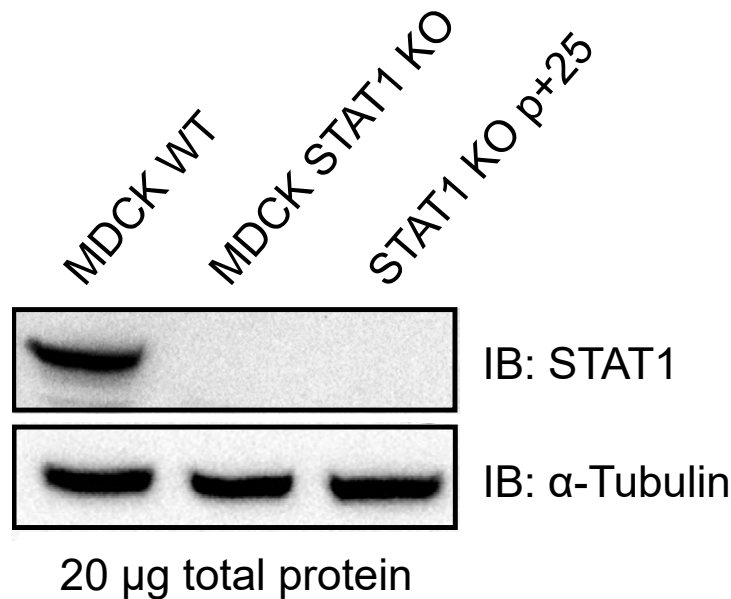
- Over 20% of the human population is infected with the influenza virus each year.
- Traditional production of flu vaccines involves infection and subsequent harvest of embryonated chicken eggs.
 - Time constraints, high production cost
 - High risk of microbial contamination
 - Allergic reactions in some recipients
- Mammalian cell culture-based Influenza vaccines as alternatives to egg-based vaccines.
- A need for rapid and large-scale production of flu vaccine for pandemic preparedness.
- 2012, FDA approved Flucelvax as the first mammalian cell-based (MDCK-produced) Influenza vaccine in US.



https://en.wikipedia.org/wiki/Influenza_vaccine

STAT1 Protein Knockout in MDCK Cells

Stability of STAT1 KO MDCK clone confirmed by immunoblot

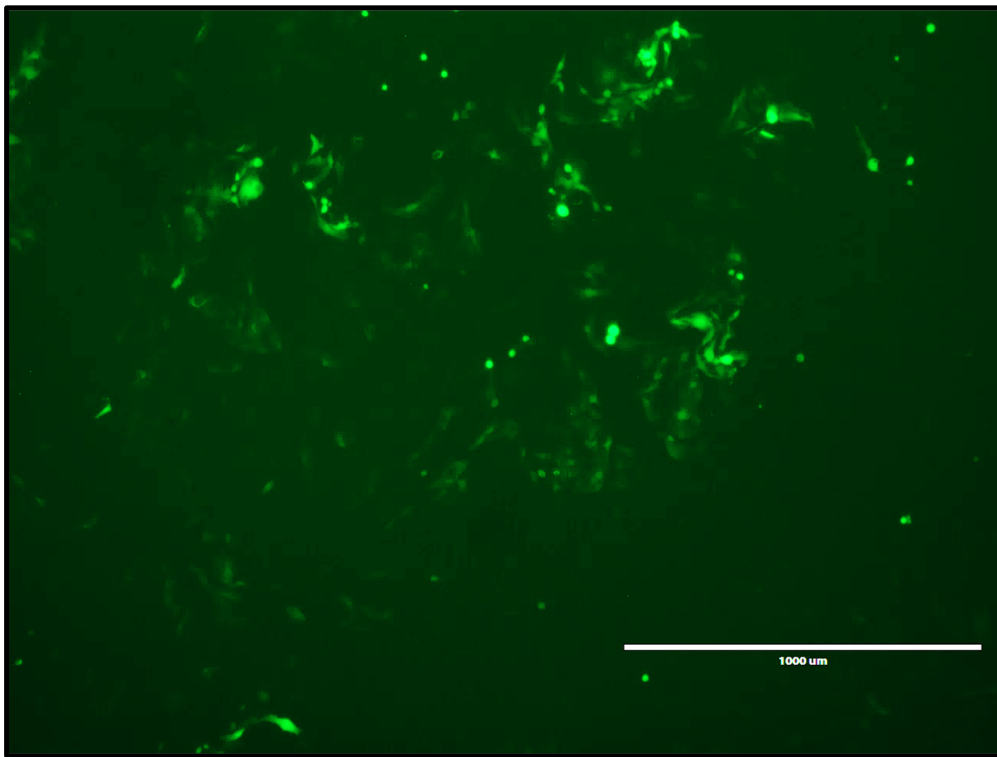


- Short nucleotide insertions and deletions near the beginning of the STAT1 gene were made using CRISPR/Cas9.
- The modified STAT1 gene produces truncated, non-functional STAT1 protein.
- Truncated STAT1 protein is rapidly degraded, resulting in a functional STAT1 protein knockout.
- Lack of functional STAT1 protein results in a lack of anti-viral interferon signaling.

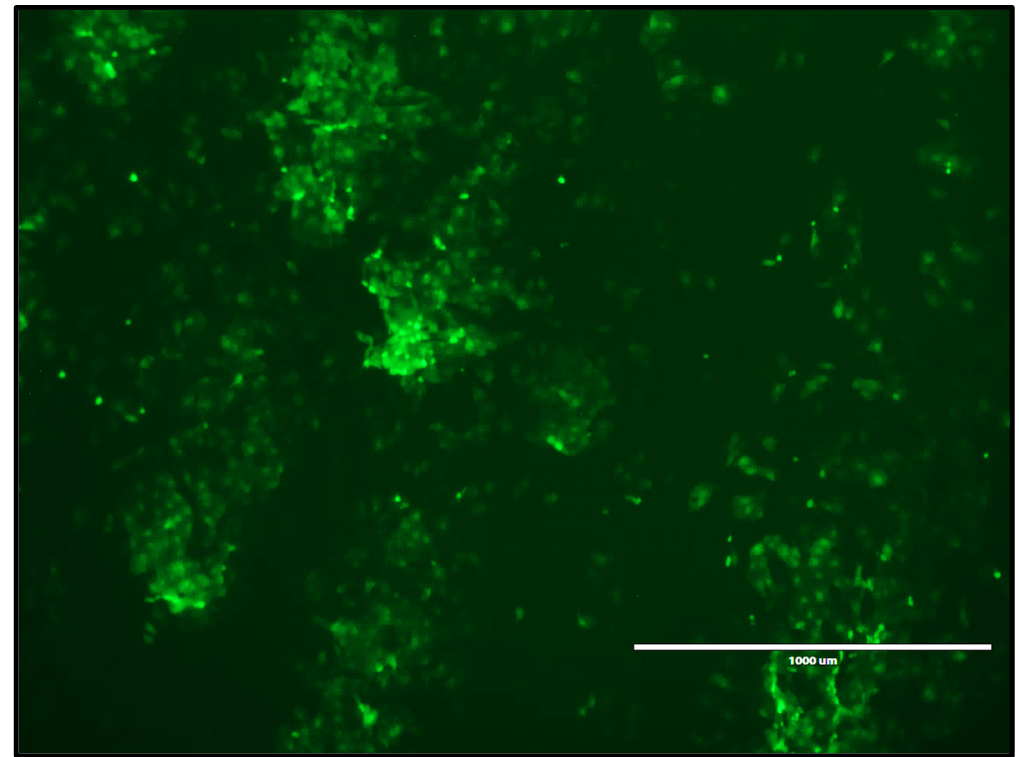
GFP-SeV Infection in WT and STAT1 KO MDCK Cells

GFP reporter virus replicates more quickly in STAT1 KO MDCK cells than in WT MDCK cells

MDCK WT + GFP-SeV (48 HPI)



MDCK STAT1 KO + GFP-SeV (48 HPI)



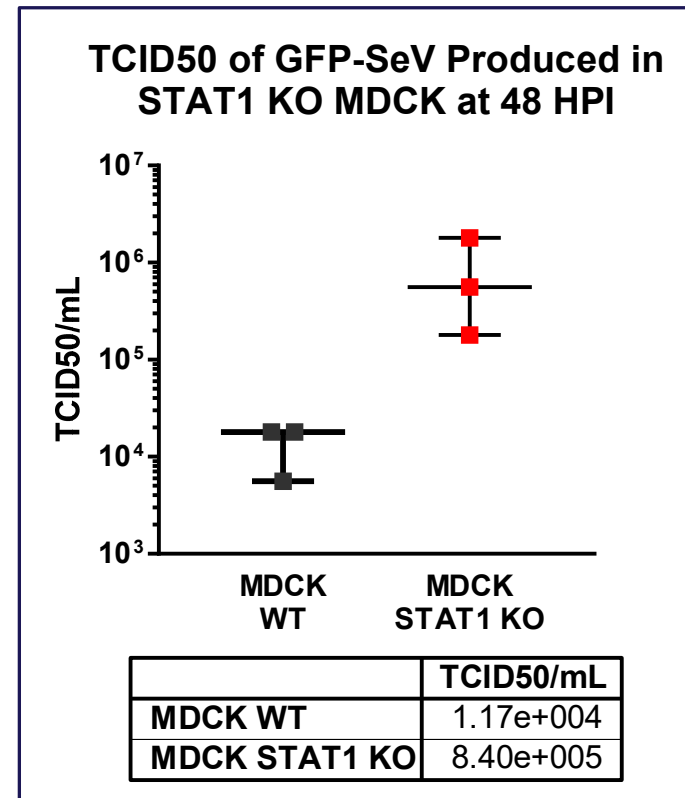
GFP-SeV Production in WT and STAT1 KO MDCK Cells

>50-fold increase in GFP-SeV production in STAT1 KO MDCK

Dilution	MDCK WT			MDCK STAT1 KO		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
-7	-	-	-	-	-	-
	-	-	-	-	-	-
	-	-	-	-	-	-
-6	-	-	-	-	-	-
	-	-	-	-	-	-
	-	-	-	-	-	-
-5	-	-	-	-	-	+
	-	-	-	-	+	+
	-	-	-	-	+	+
-4	-	-	-	+	+	+
	-	-	-	+	+	+
	-	-	-	-	+	+
-3	+	+	+	+	+	+
	-	-	+	+	+	+
	+	-	-	+	+	+
-2	+	+	+	+	+	+
	+	+	+	+	+	+
	+	+	+	+	+	+
TCID50/mL	1.78E+04	5.62E+03	1.78E+04	1.78E+05	5.62E+05	1.78E+06
Average	1.37E+04			8.40E+05		

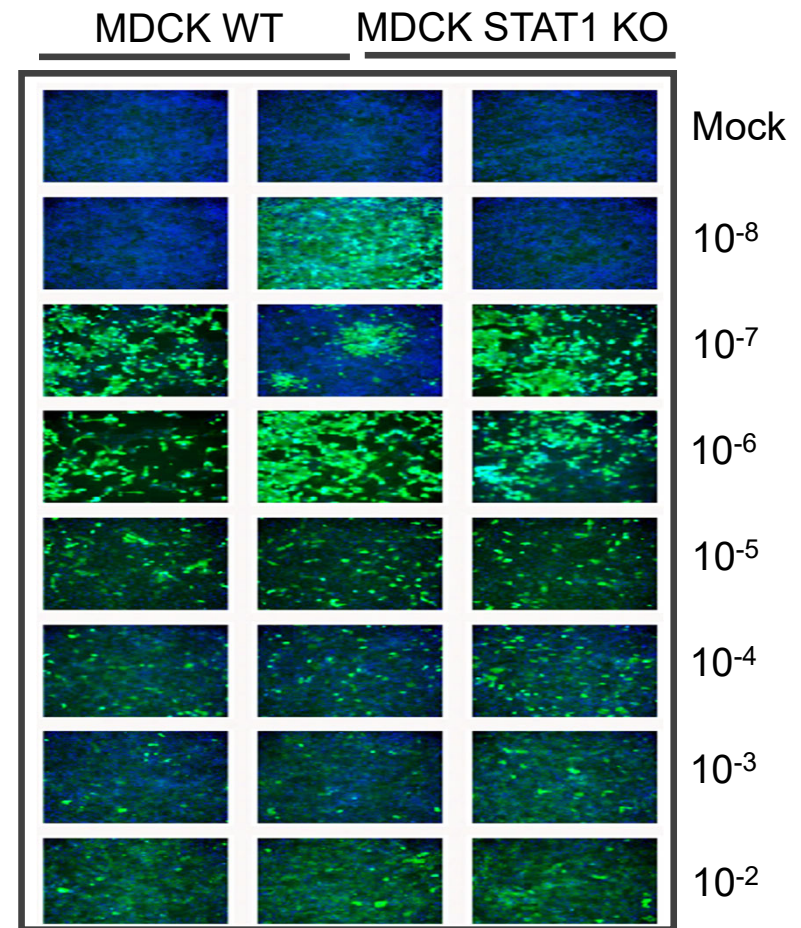
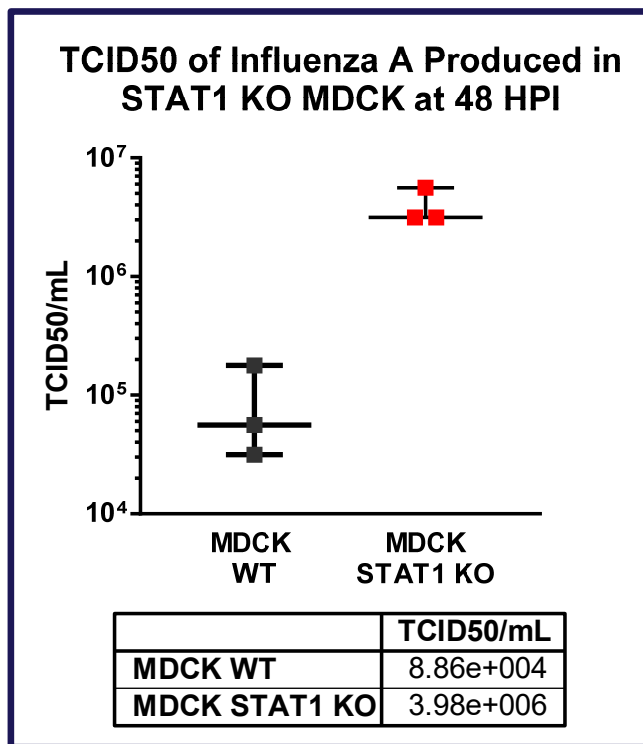
Technical Replicates (vertical arrow on left)

Experimental Replicates (horizontal arrow at bottom)



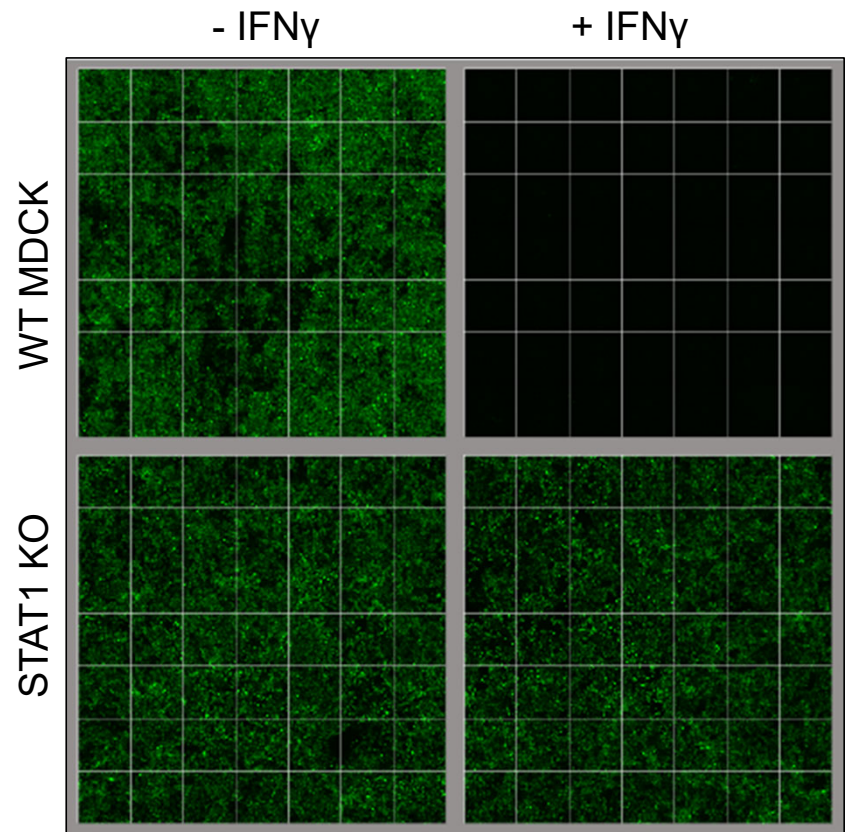
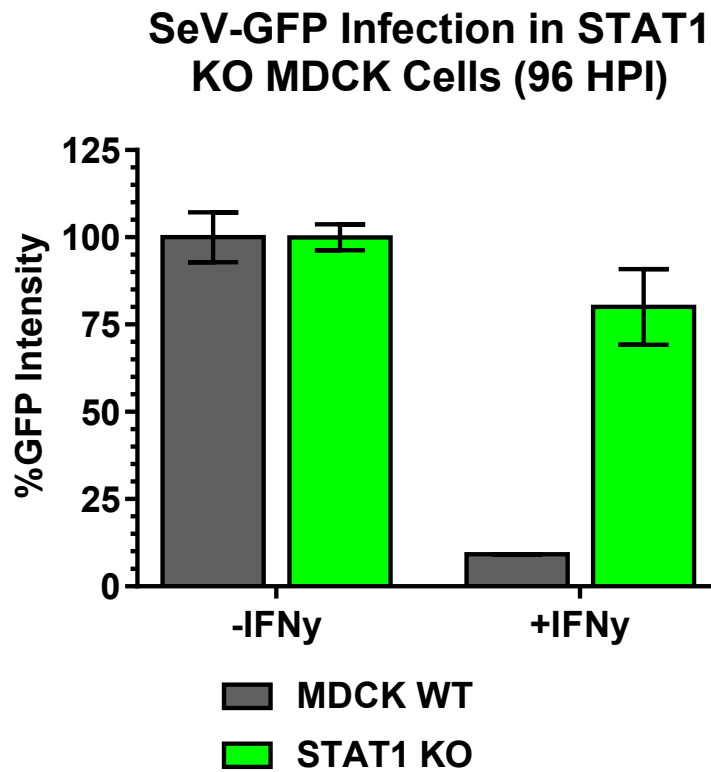
Production of Influenza A in STAT1 KO MDCK

Staining of TCID50 of viral supernatants from STAT1 KO MDCK shows 30-fold increase in viral production



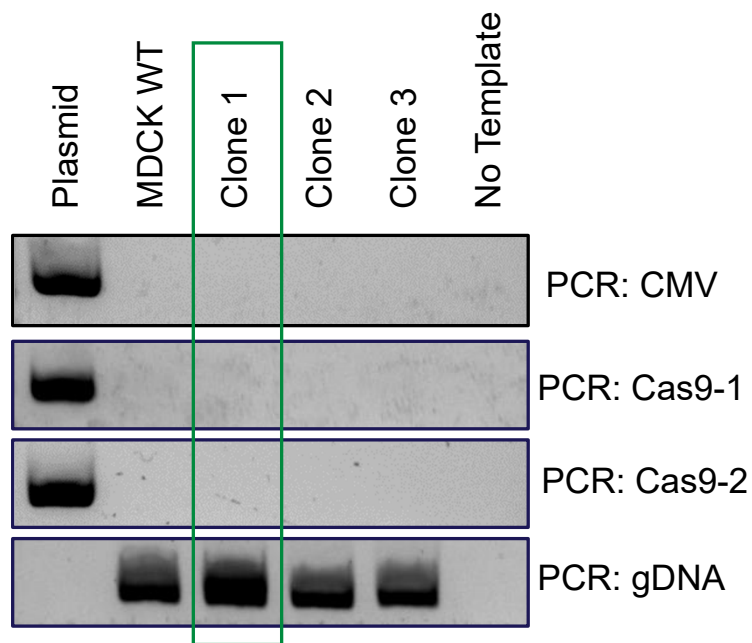
No Response to Interferon in STAT1 KO MDCK Cells

MDCK cells were treated with recombinant canine interferon gamma then infected with reporter virus



Cas9/CMV Plasmid Integration Assessment

No Cas9/CMV integration in STAT1 KO MDCK clone



Primer	Sequence (5' to 3')	Tm (Q5)	Anneal	Product
CMV-FW	TGGCTCTAGAGGTACCCGTTACATAAC	69	70	342
CMV-RV	AGATGGGGAGAGTGAAGCAGAAC	69		
Cas9-1-FW	CTATAAGGACCACGACGGAGACTACAAG	69	70	323
Cas9-1-RV	TTCTTCTGGCGGTTCTCTTCAGC	69		
Cas9-2-FW	TGCTGCCAGACTGAGCAAGAG	69	70	300
Cas9-2-RV	TCTCGGTGTTCACTCTCAGGATGT	69		
cfSTAT1 E2 FW3	GGCTTCTTGAATAATTTTCATAAGGAAAGCA	65	66	247
cfSTAT1 E2 RV3	CTTATGCTTGGGAACATTTTGGC	65		

Selected Clone

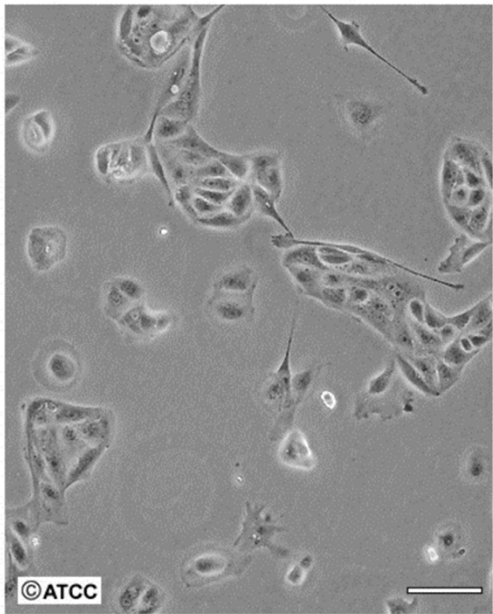
Screening for Off-Target Mutations in STAT1 KO MDCK

No mutation detected in Cas9 off-target screen

	Gene	Gene ID	Gene Description	Target Sequence	Type	MM	CFD	Tm (Q5)	Anneal:	Product	STAT1 KO
	STAT1	488449	signal transducer and activator of transcription 1	GAGGTCGTGAAAACGGATGG TGG	Exon	0	N/A	65°C 65°C	66°C	247 bp	
1	CDH13	489689	cadherin 13	GAAGTCATGAAAATGGATGA AGG	Intron	4	0.516	66°C 68°C	67°C	331 bp	
2	MYRIP	485603	myosin VIIA and Rab interacting protein	GAGGTCCAGAAAACAGATGA GGG	Intron	4	0.485	67°C 68°C	68°C	442 bp	
3	ASIC2	491150	acid sensing ion channel subunit 2	GAGGTCCAGGAAAGCAGATGG GGG	Intron	4	0.450	68°C 67°C	68°C	413 bp	
4	CCDC170	476248	coiled-coil domain containing 170	AAAGTTC TGAAAACGGATGG TGG	Intron	4	0.431	68°C 68°C	69°C	618 bp	
5	PAK5	485772	p21 (RAC1) activated kinase 5	AAGGTCC TGAAAGATGGATGG AGG	Intron	4	0.328	67°C 67°C	68°C	537 bp	
6	SLAMF6	100684044	SLAM family member 6	GGGGCGTGGAAACAGATGG GGG	Intron	4	0.268	69°C 67°C	68°C	795 bp	

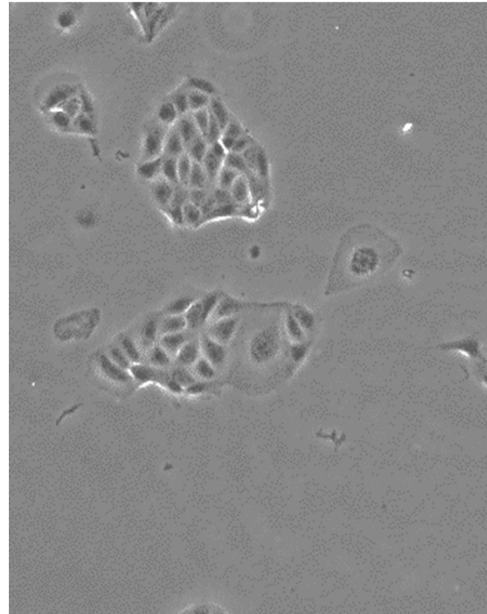
MDCK Morphology and Growth Kinetics

Modified cell line has similar morphology and grows slightly slower than parental MDCK cell line

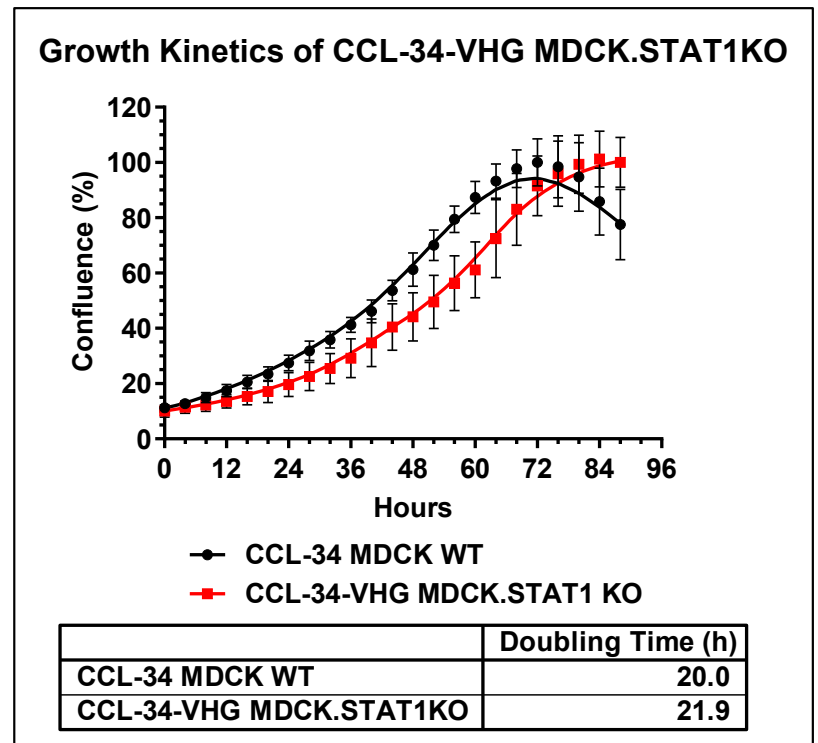


Low Density Scale Bar = 100µm

CCL-34 MDCK

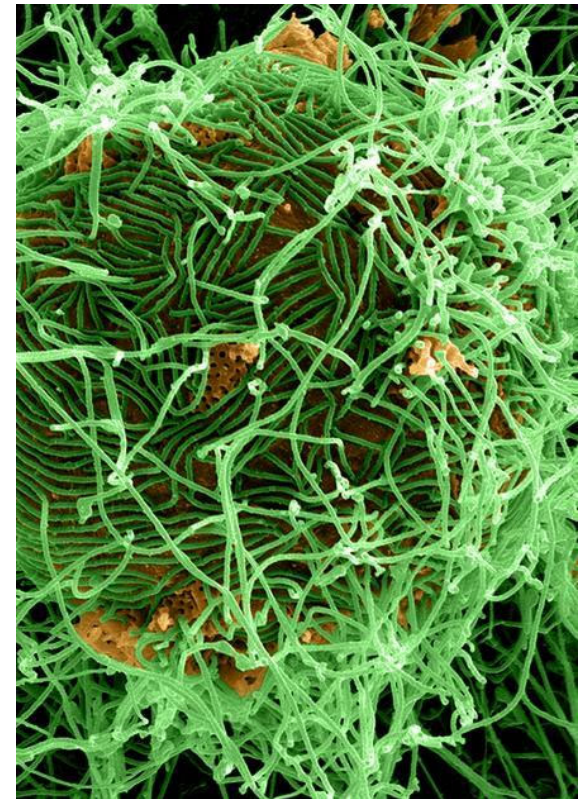


CCL-34-VHG MDCK.STAT1KO



Summary - Enhanced Viral Host Cells

- CRISPR-Cas9 is a powerful technology for precise genetic modification in various organisms. ATCC is broadly utilizing CRISPR across our portfolio of cell lines to develop enhanced products to meet critical customer needs.
- Vero and MDCK cells have been modified by CRISPR-Cas9 to have a permanent increase in viral production capacity.
- Modified Vero and MDCK cells have been thoroughly characterized for viral production phenotype, stability of STAT1 knockout genotype, lack of Cas9 plasmid integration, and absence of off-target mutations.
- Enhanced viral production cell lines have broad applicability for basic research, production of high-titer viral stocks, and manufacturing of viruses and viral vaccines.



For more information visit www.atcc.org/vaccinedev

Ebola virus infecting Vero cell, image courtesy of NIAID

www.atcc.org/vaccinedev



www.atcc.org/coronavirus



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