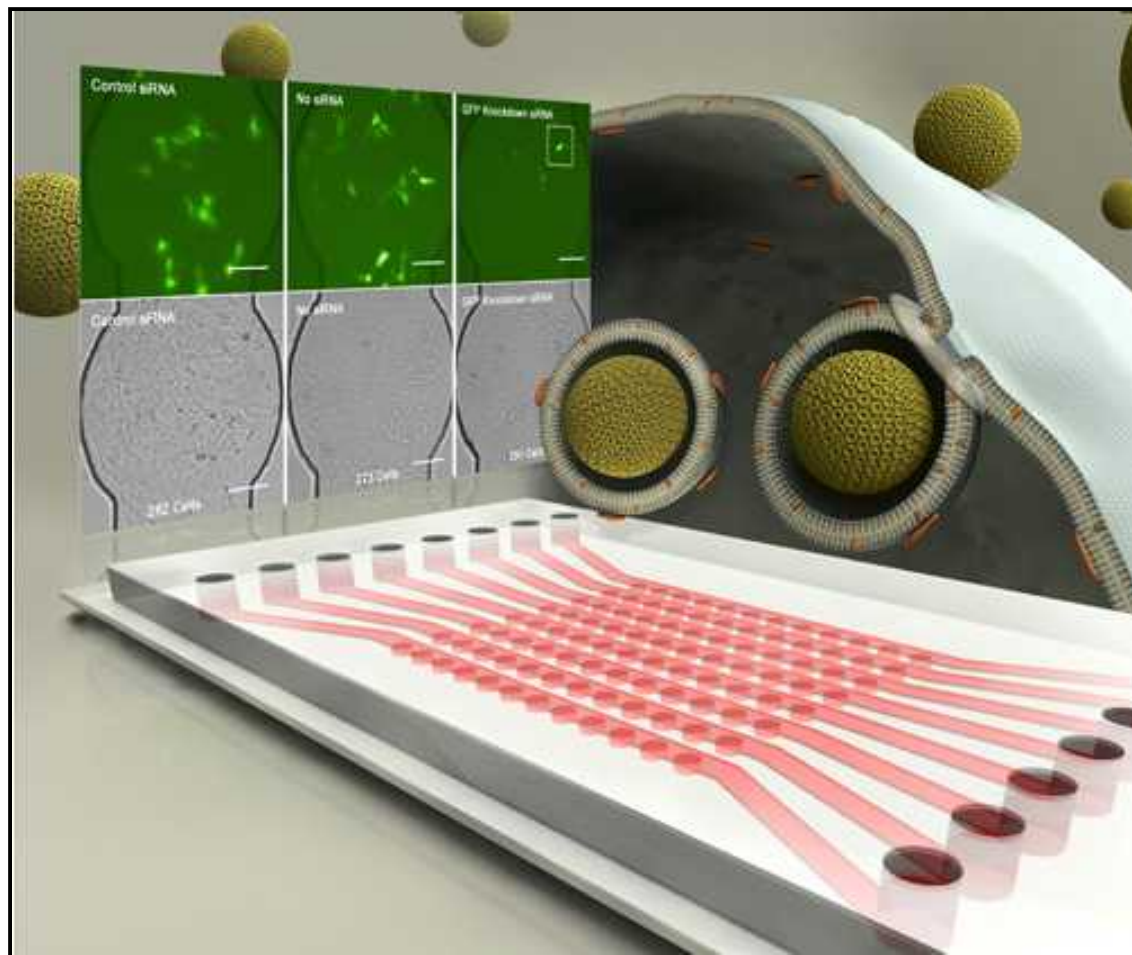


# Microfluidic devices for genetic screening of positionally-controlled cell cultures

SAND2013-3133C

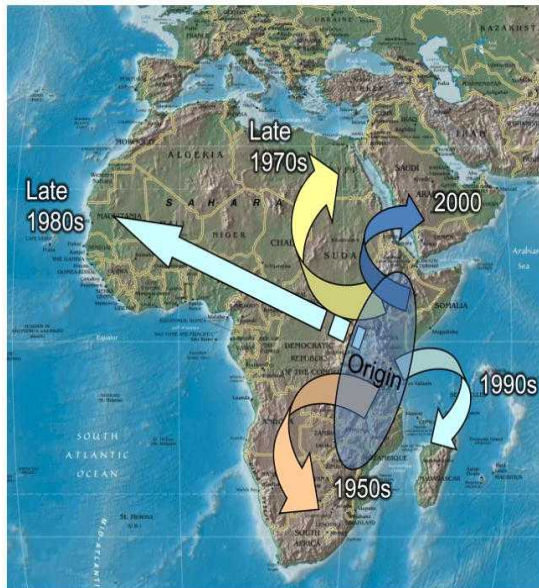
Benjamin R. Schudel  
Brooke Harmon  
Vinay V. Abhyankar  
Benjamin Pruitt  
Oscar A. Negrete  
Anup K. Singh



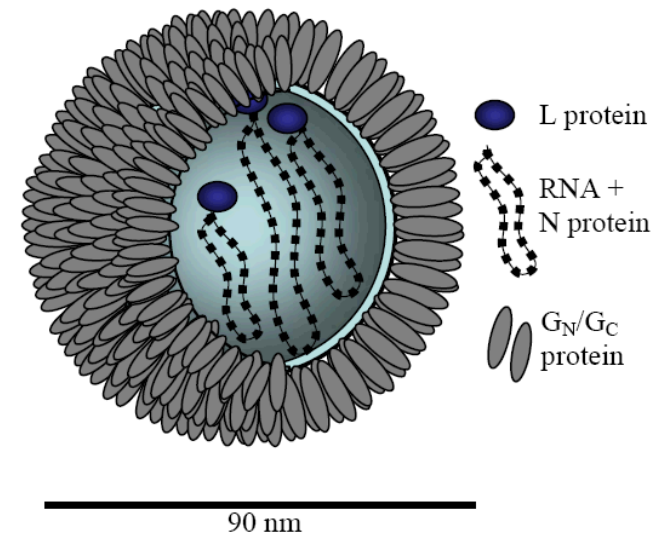
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

# Motivation: Rift Valley Fever virus (RVFV)

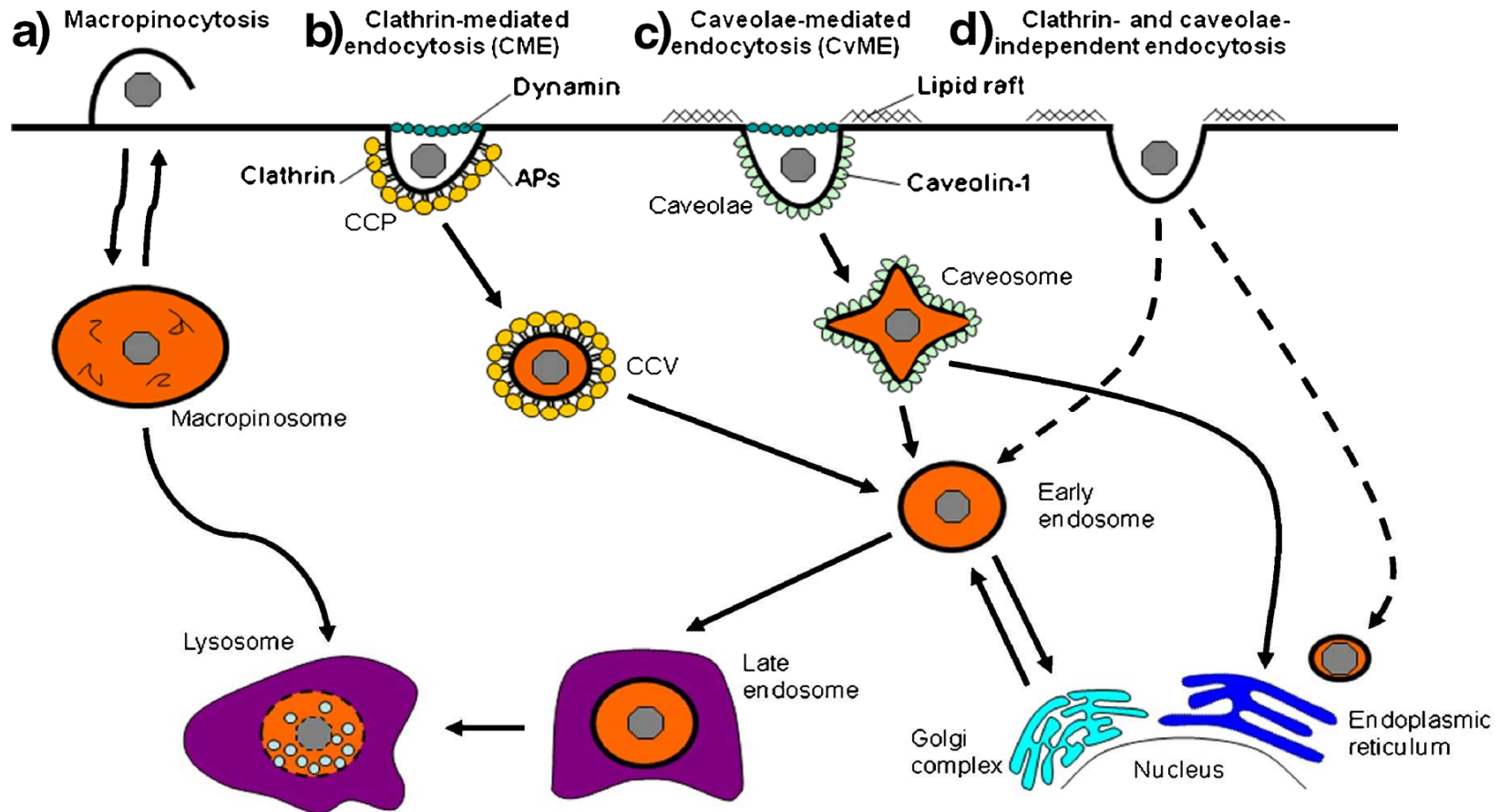
Sub-Saharan Africa, zoonosis, enveloped virus, potentially stable



Weaver S, Antiviral Res. 2010

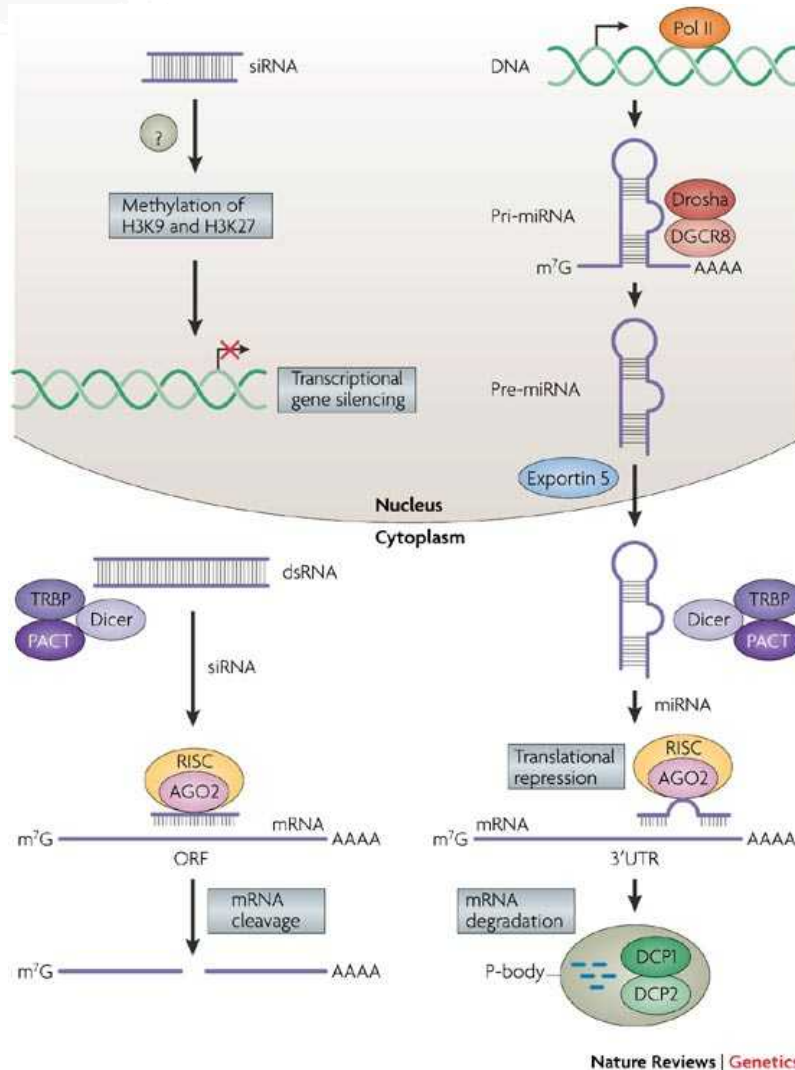


# Rift Valley Fever Virus – What we don't know





# RNA interference (RNAi) is the technique used to knock down a specific gene using small interfering RNA (siRNA)



## siRNA:

- RNA strands are small enough not to trigger an immune response from the cell
- Specifically interfere with genetic expression
- Ideal for examining which genes participate within an infection process

Figure Credit: Kim, Rossi, *Nature Reviews Genetics*, **2007**, 8, 173.

Due to the vast parameter space, smaller and faster screening techniques are desired for cheaper discoveries

ABLM1	ATF3	CAMTA1	CRYBA2	DPH2	FBLN2	HAAO	IGLL1	KRTAP5-2	MCM7	NUDT1	PUM2	SLC16A8	TTC33
ABLM3	ATF4	CAMTA2	CRYM	DSC1	FBXO47	HABP4	IGSF1	LAIR1	MDN1	NUDT2	QPRT	SLC35A3	TTL12
ABR	ATF6	CAPRIN1	CS	DSC2	FCAR	HADHB	IGSF3	LAIR2	MEIS2	NUP160	R3HDM1	SLC35D1	TTL15
ACAN	ATG2A	CAPS	CSH1	DSC3	FCGR2B	HARS2	INCA	LARP5	MEOX1	NUP205	RAB3GAP1	SLC39A14	UBR4
ACIN1	ATMIN	CARHSP1	CSH1	DSCAM	FCH01	HAS3	INPP4A	LDHC	MEST	NUP210	RAB6IP1	SLC4A1AP	UBXD2
ACOT9	ATP13A2	CAST	CSHL1	DST	FGF12	HBM	IQCE	LDOC1	MFAP2	OR10C1	RABGAP1	SMC5	UBXD8
ACSBG1	ATP1B1	CBFA2T3	CSTF2T	DTNB	FGF13	HCG3	IQSEC2	LEMD3	MGAT2	OR10J3	RAD54L2	SMG5	UNC84A
ACSL3	ATP1B4	CBX5	CTAGE5	DULLARD	FGF14	HDLBP	IRF5	LGALS8	MGC70857	OR13G1	RBM34	SMG6	UNC84B
ACYF1	ATP5C1	CRX6	CTBP1	DUSP4	FJX1	HEBP2	IRF7	LGALS9	MGRN1	OR2B3P	RBPJ	SMPX	WAPAL
ADD3	ATP5F5	CCBL3	CTH1	FCM1	FLNA	HEP2	ISLR	LHX8	MLXIP	OR2W5	RCAN1	SNAPIN	WBP2
ADP1	ATP5F5	CCBL3	CTH1	FCM1	FLNA	HEP2	KCTD2	LILRA4	MMP19	OR4Q3	RCOR1	SPANXN1	WDR37
AGT	ATP5G3	CD42EP4	CRYM	EEF1D	FBNP1	HIST1H2BD	KIAA0082	LIMCH1	MMRN1	OR5B3	RFTN1	SPANXN4	WDR47
ALAS1	ATP5G3	CD42EP4	CRYM	EEF1D	FBNP1	HIST1H2BD	KIAA0090	LIMX1A	MN1	OR5U1	RGL1	SPECC1L	WDTCL
ALG11	ATP5J	CDH7	EEF1D	FBNP1	HIST1H2BD	KIAA0157	LOC441150	MOBP	OR9K2	RHOBTB2	SPG20	WSCD1	
AMPH	ATP6V0A2	CENTR2	YETB1	FENM4	FOXA2	HMLA-A	KIAA0182	LOC441476	MOC51	OTP	RIMBP2	SRRM2	WMC1
AMY1A	ATP6V	CENTR2	YETB1	FENM4	FOXA2	HMLA-A	KIAA0240	LOC492311	MOC5	PADI4	RIMS2	SSBP2	XPO6
ANGEL1	BACH1	CENTR2	YETB1	FENM4	FOXA2	HMLA-A	KIAA0241	LOC493869	MON1B	PALLD	RNASEK	STGC3	ZBTB1
ANK1	BAHD1	CENTR2	YETB1	FENM4	FOXA2	HMLA-A	KIAA0265	LOH11CR2A	MON2	PANX1	RP4-	STMN1	ZBTB43
ANKRD12	BAT2D1	CHAC2	CIC	EIF4G1	FSTL4	HNRNP3	KIAA0323	LPIN1	MORC2	PAXIP1	691N24.1	STOM	ZC3H13
ANKRD6	BCKDHB	CIC	EIF4G1	FSTL4	HNRNP3	HNRNP3	KIAA0409	LRCH1	MORC3	PCNX	RPGRIP1L	SUGT1P	ZC3H3
ANKS1A	BHMT2	CIC	EIF4G1	FSTL4	HNRNP3	HNRNP3	KIAA0423	LRRC18	MRPS27	PDS5A	RPH3A	SULT1C3	ZC3H7B
ANXA11	BICD1	CLC1	EIF5	FUT8	HNRPK	KIAA0460	KIAA0460	LRRC52	MS4A13	PDSS1	RPIA	SUZ12	ZCCHC14
ANXA13	BIN1	CLC1	EIF5	FUT8	HNRPK	KIAA0460	KIAA0467	LRRC6	MSMB	PDXDC1	RPL13A	SWAP70	ZFPM2
ANXA6	BLID	CLCC1	ELF5	GABARAPL1	HOXA10	KIAA0556	KIAA0556	LRRC8B	MSRB2	PEF1	RRP12	SYNGR4	ZFYVE26
ANXA7	BOP1	CLEC1E	ELF5	GABARAPL1	HOXA3	KIAA0746	KIAA0746	LSM5	MT1F	PES1	RRP18	SYT11	ZHX2
AOAH	BRDT	CLTA	ELF5	GABARAPL1	HOXA3	KIAA0776	KIAA0776	LSL1	MTCH1	PHC2	RPH3A	TARD8P	ZHX3
AOC2	BRF1	CLTB	ELF5	GABARAPL1	HOXA3	KIAA0802	KIAA0802	LY96	MTCH2	PHF3	RTF1	TBC1D28	ZIM2
AP1B1	BTF3	CNKSK1	ENP4	GGT1	HPS1	KIAA0892	KIAA0892	LYPLA3	MTCP1	PHLDA3	RUFY3	TBC1D9	ZNF281
AP2A1	C10orf55	CNN2	DDX58	ENTPD2	GGT1	KIAA0895	KIAA0895	MAGEA11	MTHFD2L	PHLD81	RUNX1T1	TCF25	ZNF318
AP2S1	C10orf62	CNTN1	DENND3	EPB41L3	GGT1	KIAA0907	KIAA0907	MAGEA2	MTIF2	PHLPP	RUSC1	TDRD7	ZNF423
AP4E1	C15orf2	COBL	DFFA	ETHE1	GJA5	KIAA0913	KIAA0913	MAGEA3	MTMR15	PHLPPL	SAMD4A	TGDS	ZNF510
APBB1	C1orf186	COL11A1	DHP5	ETV3L	GLE1	KIAA0922	KIAA0922	MAGEB1	MTX1	PHOSPHO2	SAPS1	TLX2	ZNF705A
APOBEC1	C20orf103	COL12A1	DHX9	EVC	GLT25D2	KIAA1012	KIAA1012	MAL	MYBPC1	PISD	SASH1	TMED3	ZZEF1
ARC	C22orf31	COL17A1	DIP2C	EXOSC2	GPATCH8	KIAA1024	KIAA1024	MAP1B	MYH11	PITPNB	SATB2	TMEFF2	
ARHGAP6	C2orf64	COL2A1	DIS3	EXOSC7	GPD1L	KIAA1033	KIAA1033	MAP1LC3C	MYL1	PIWIL3	SBN02	TMEM194	
ARHGEF12	C3orf27	COL4A5	DLX4	EXPH5	GPM6A	KIN	KIN	MAP4	MYL4	PLD3	SCMH1	TMEM2	
ARHGEF15	C3orf63	COL4A6	DMD	EYA2	GPM6B	KIR2DL3	KIR2DL3	MAP6	MYT1L	PLEKHA6	SDF2L1	TMEM50A	
ARHGEF18	C4BPB	COL6A2	DMXL2	EYA3	GRP	KLHDC2	KLHDC2	MAPRE3	NAP1L1	PLEKHG7	SEC14L2	TMEM89	
ARHGEF9	C8orf22	COMMD3	DNAJC9	FAIM2	GSPT2	KLHL18	KLHL18	MAST1	NAT6	POLA2	SEC61G	TRAK1	
ARL2BP	C9orf103	CMO1C	DNM2	FAM116B	GSTM4	KLRCL1	KLRCL1	MAP3	PD3	PPWD1	SEPHS2	TRIM2	
ARL6IP1	C9orf5	COTL1	DNMBP	FAM120A	GTF2A1	KLRCL1	KLRCL1	MAP3	PD3	PPWD1	SEPHS2	TRIM2	
ASCC3L1	CALB2	COX15	DNTT	FANCB	GTF2I	KLRD1	KLRD1	MBNL1	NCDN	PRMT1	SF3B3	TSC22D3	
ASTL	CALD1	CRISP1	DOCK9	FASTKD2	GYPE	KLRK1	KLRK1	MBNL1	NCDN	PRMT1	SF3B3	TSC22D3	
ASTN1	CAMSAP11	CRMP1	DPAGT1	FBLN1	H3F3B	KRT13	KRT13	MCM4	NUCD3	PSME4	SKIV2L2	TSPYL4	

Host Factors that inhibit RVFV virus entry and replication

~22000 siRNAs- Qiagen genome-wide library

Primary Screen – UC Berkeley

2556 hits

Secondary Screen - Sandia

413 hits

Hits in Human and Mosquito cells

105 hits

357 Hits

(Dr. Brooke Harmon)

Large scale screening is often performed using robotic handling in screening facilities

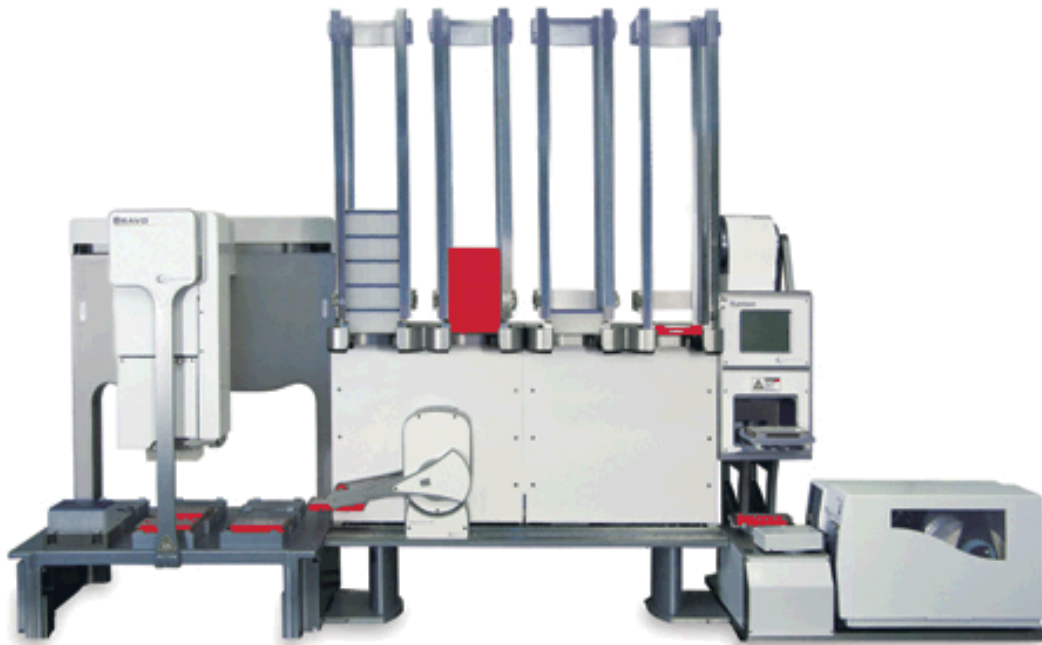


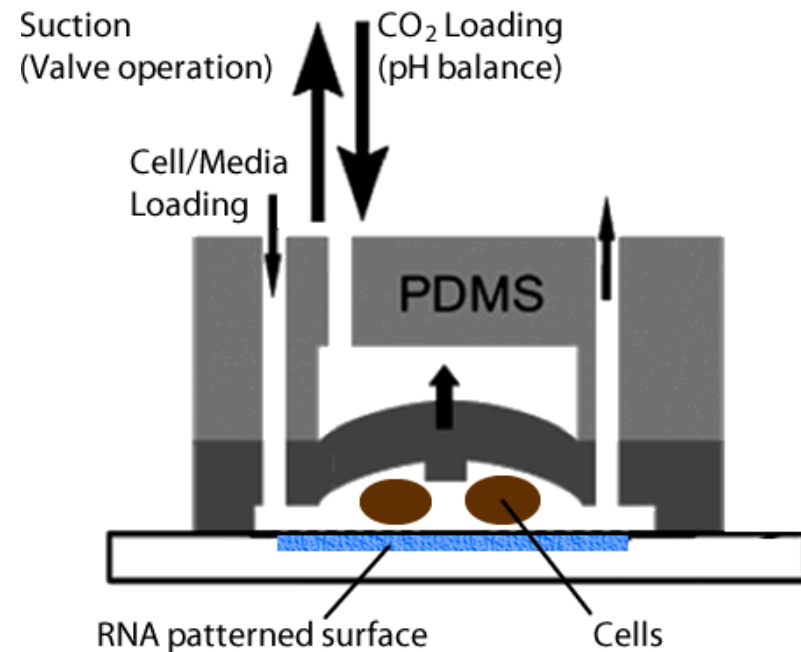
Image Credit: [www.velocity11.com](http://www.velocity11.com), Agilent



Schudel *et al.*, *Lab Chip* **2009**.

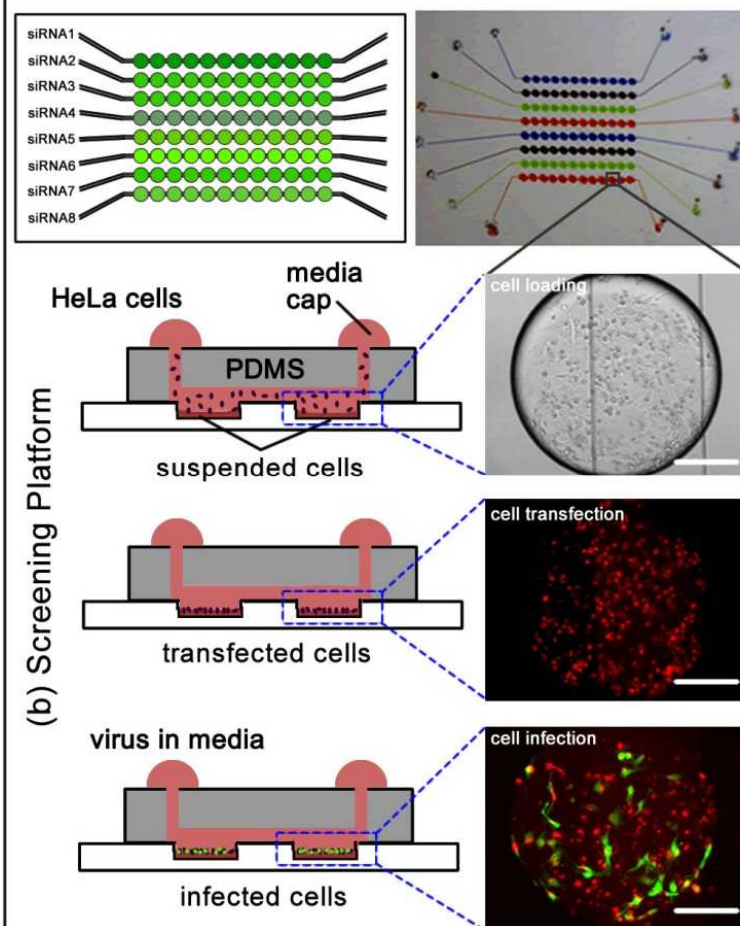
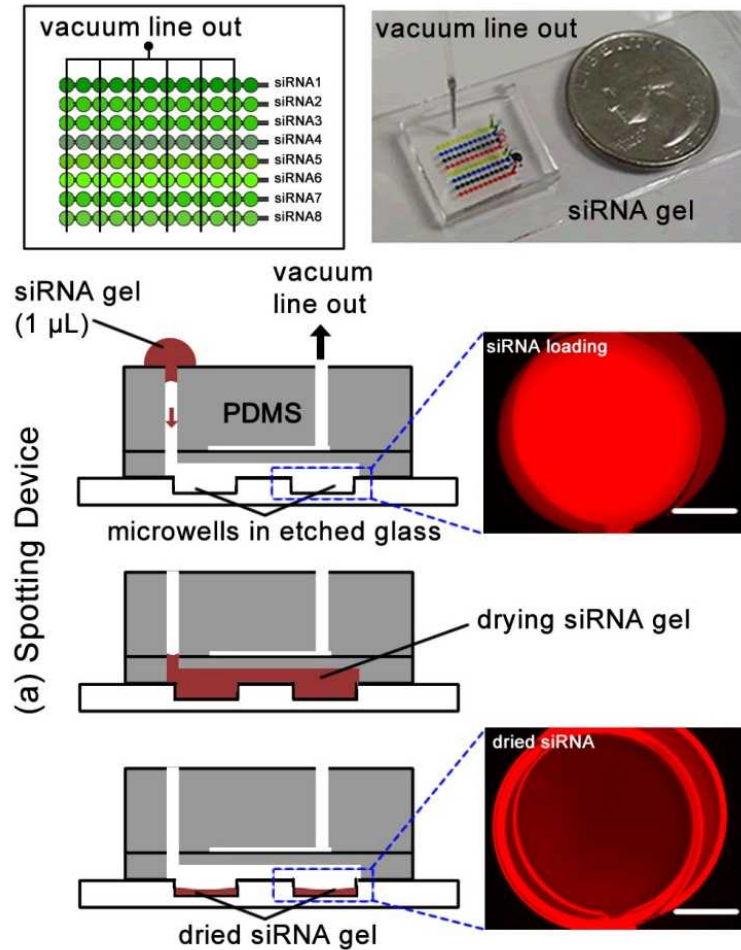
The knockdown process and infection evaluation can take up to four days, which requires a stable cell culture platform

1. Cell seeding/culture
2. siRNA transfection
3. Lower gene expression
4. Infection with GFP-based virus



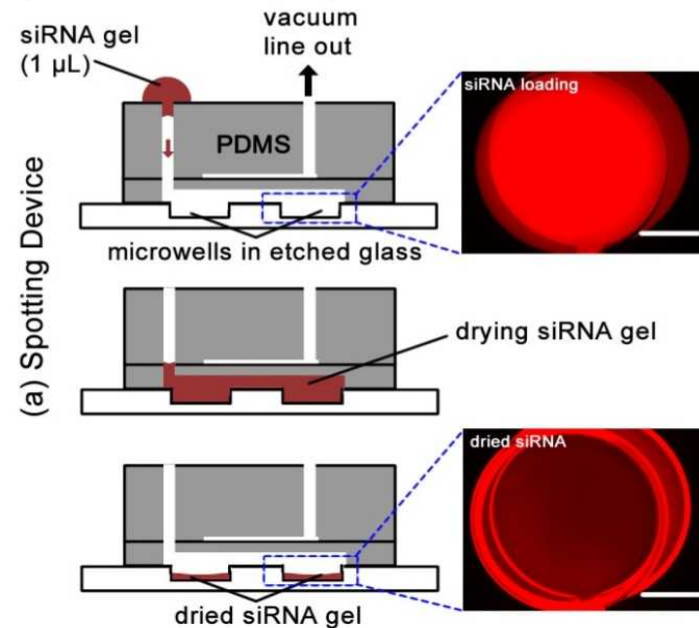
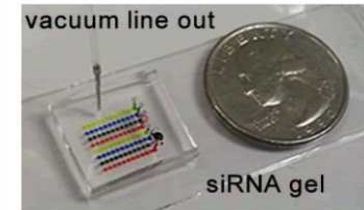
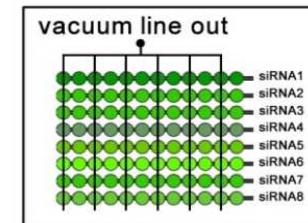
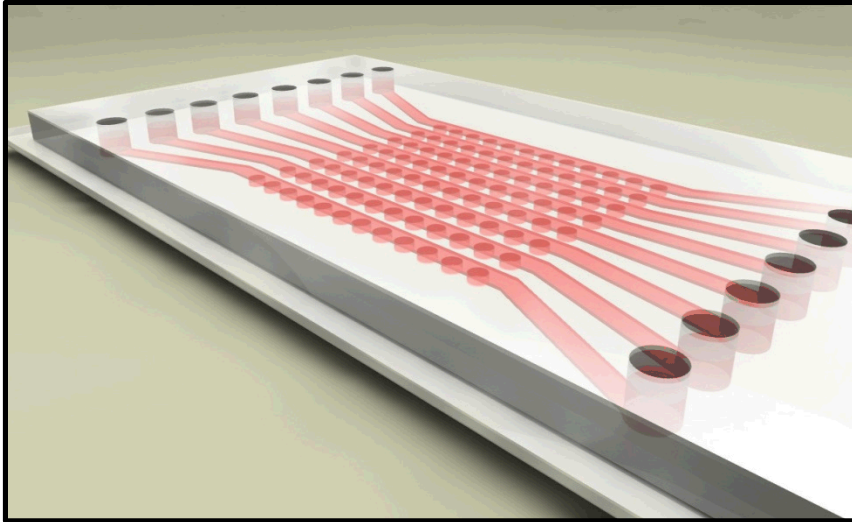


# Microfluidic siRNA Screening System: Spotting Device and Screening Platform

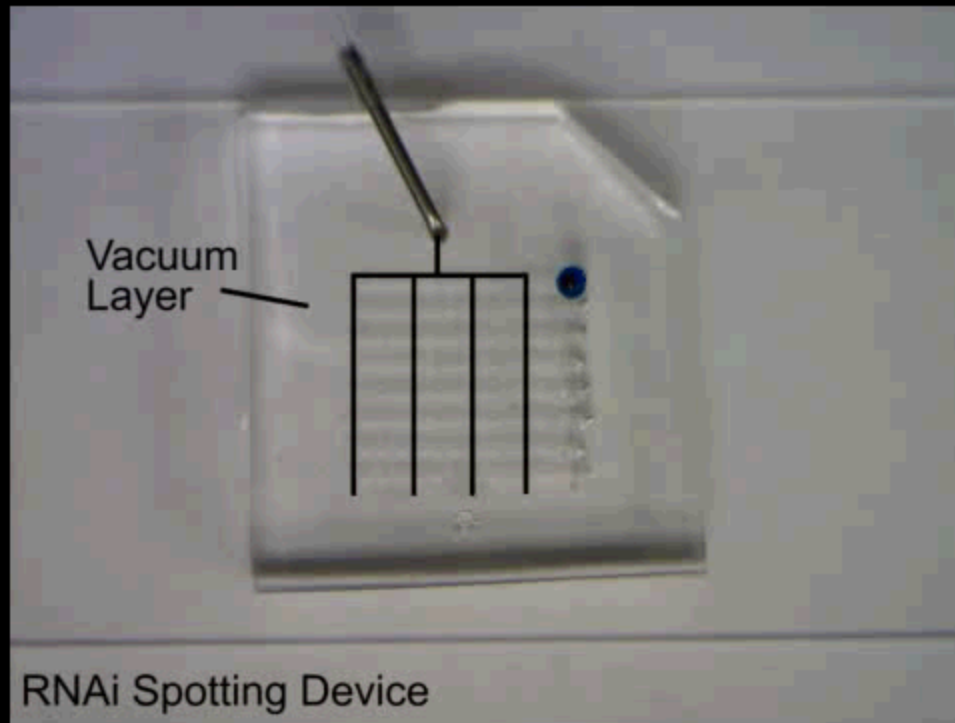




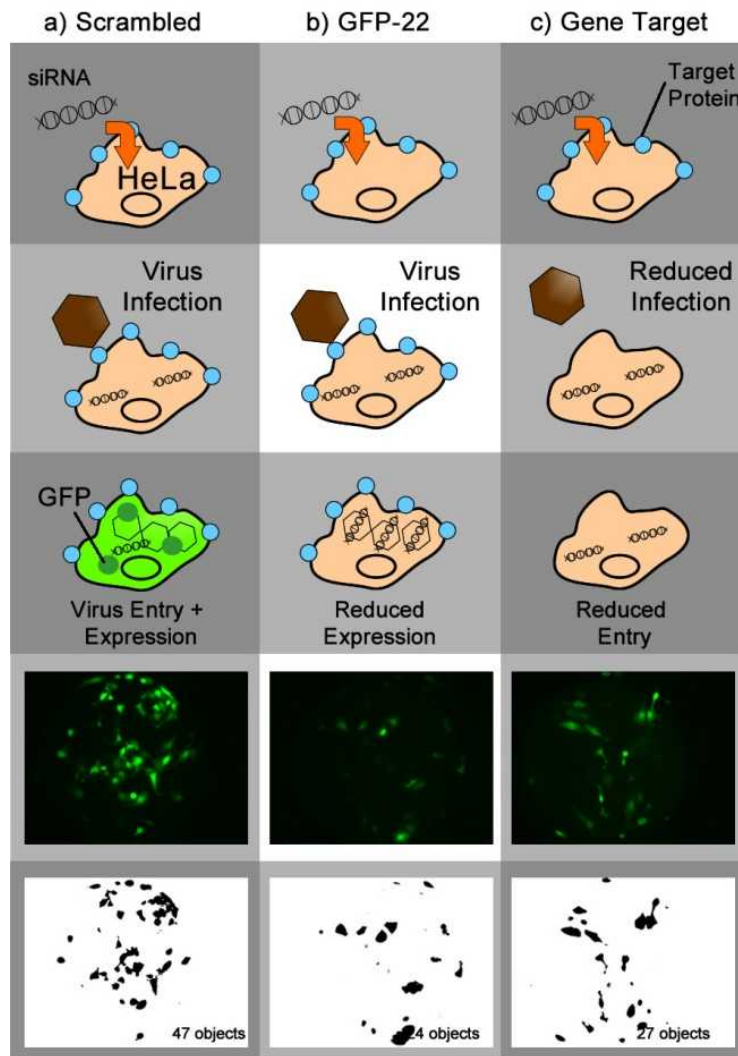
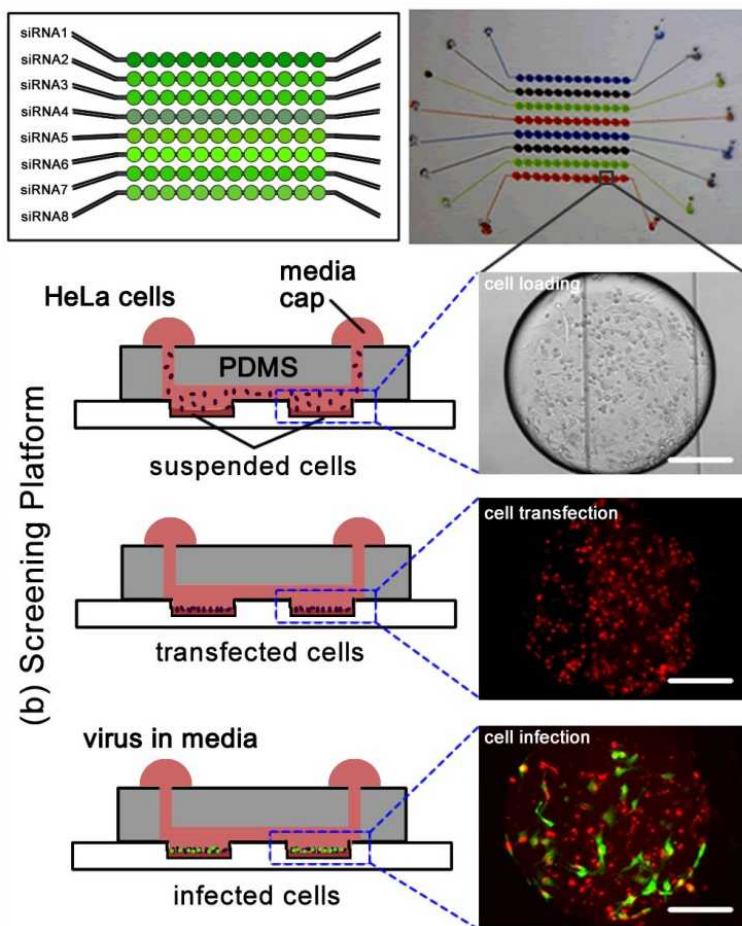
# siRNA libraries are patterned using a multilayer microfluidic spotting device



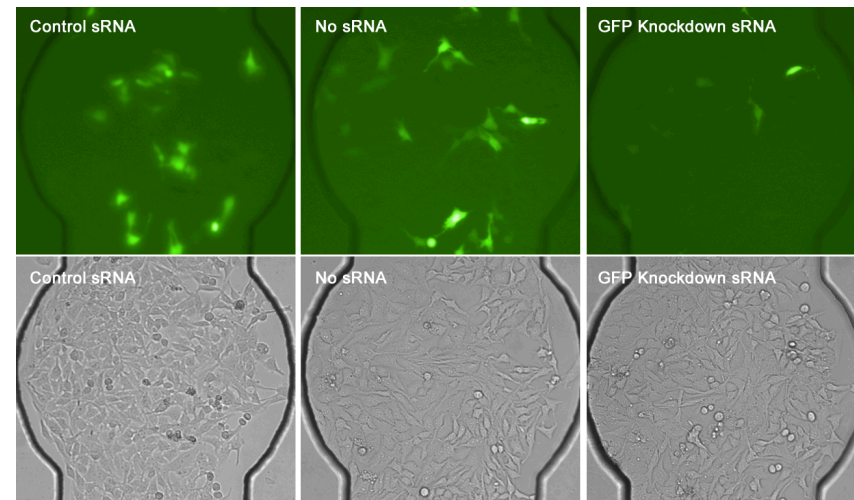
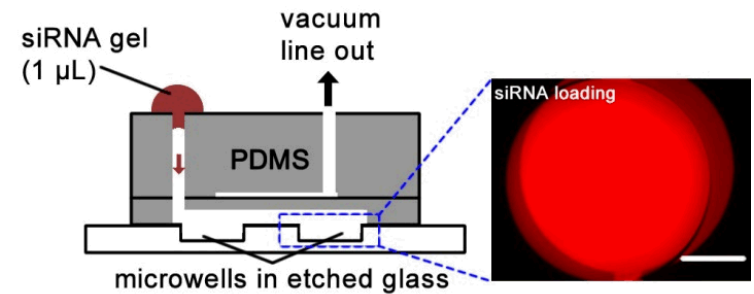
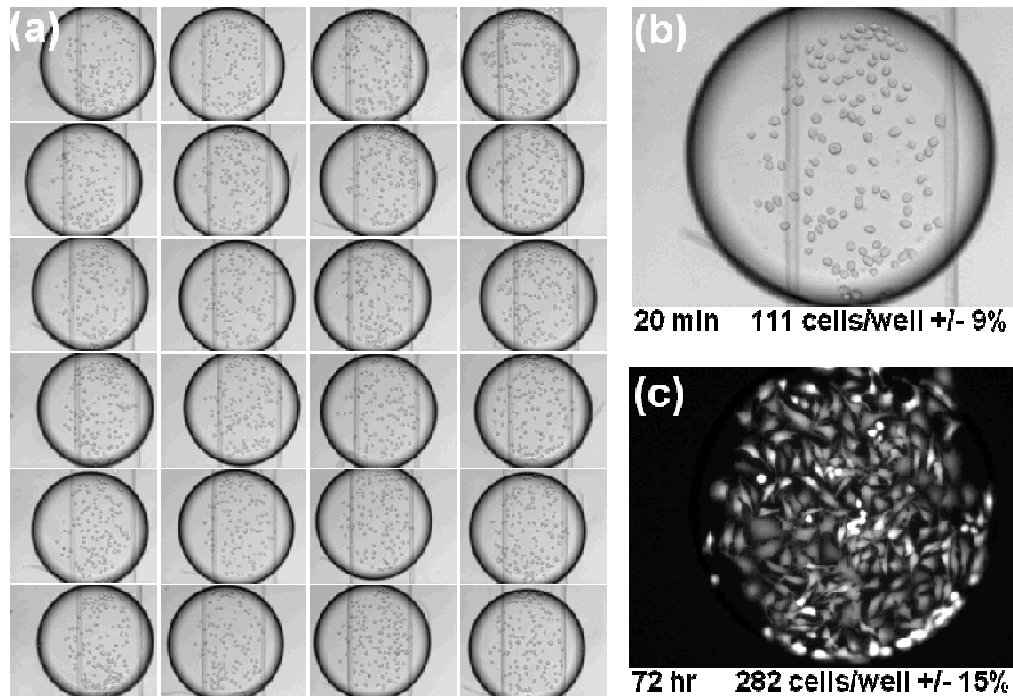
# Demonstration of RNAi Spotting Device



# Microfluidic screening using controlled wells



# HeLa cells transfected with siRNA demonstrate knockdown properties of GFP associated siRNA analyzed on-chip



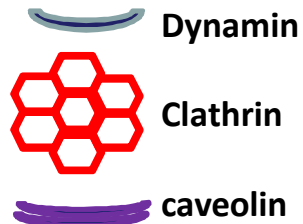


# Routes of Virus Entry

Driven by formation  
of clathrin coat,  
clathrin, AP2, EPS15,  
epsin 1 and  
cholesterol

Dynamin  
Dependent

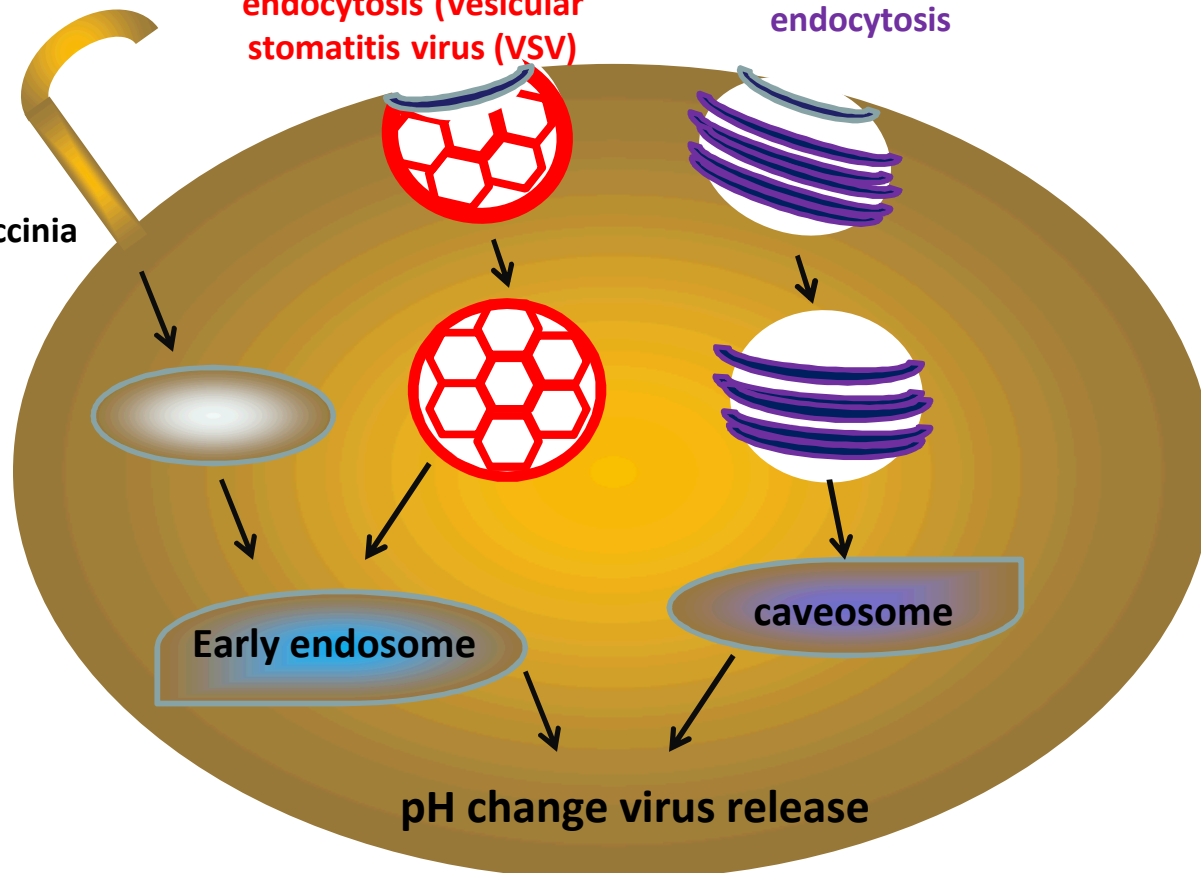
Coat protein:  
caveolin1, reg by tyr  
kinases,  
phosphatases, PKC,  
RhoA and cholesterol



Macropinocytosis (Vaccinia  
virus (VacV))

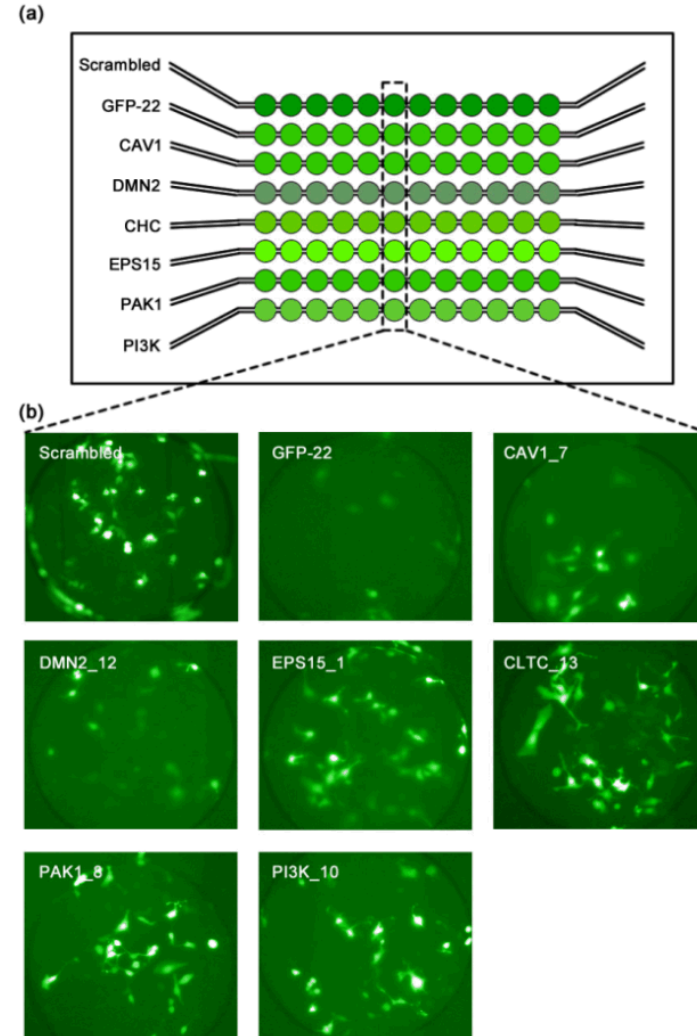
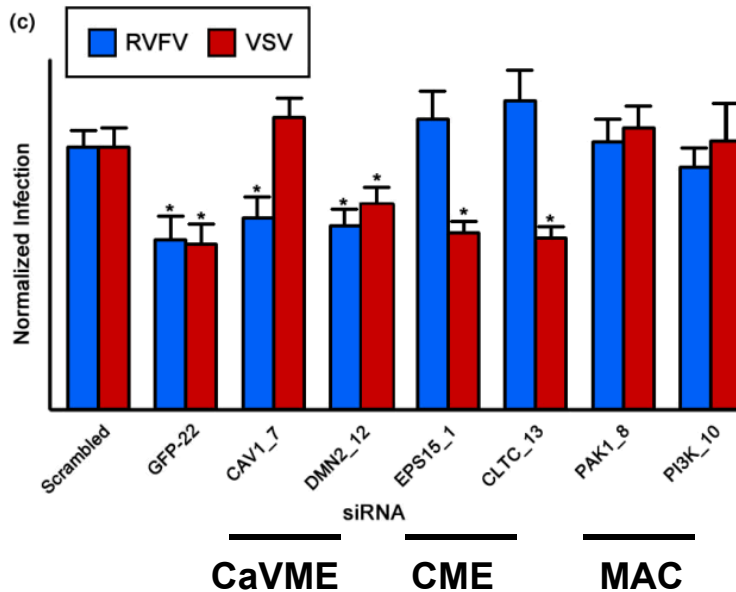
Clathrin-mediated  
endocytosis (Vesicular  
stomatitis virus (VSV))

Caveolin-mediated  
endocytosis

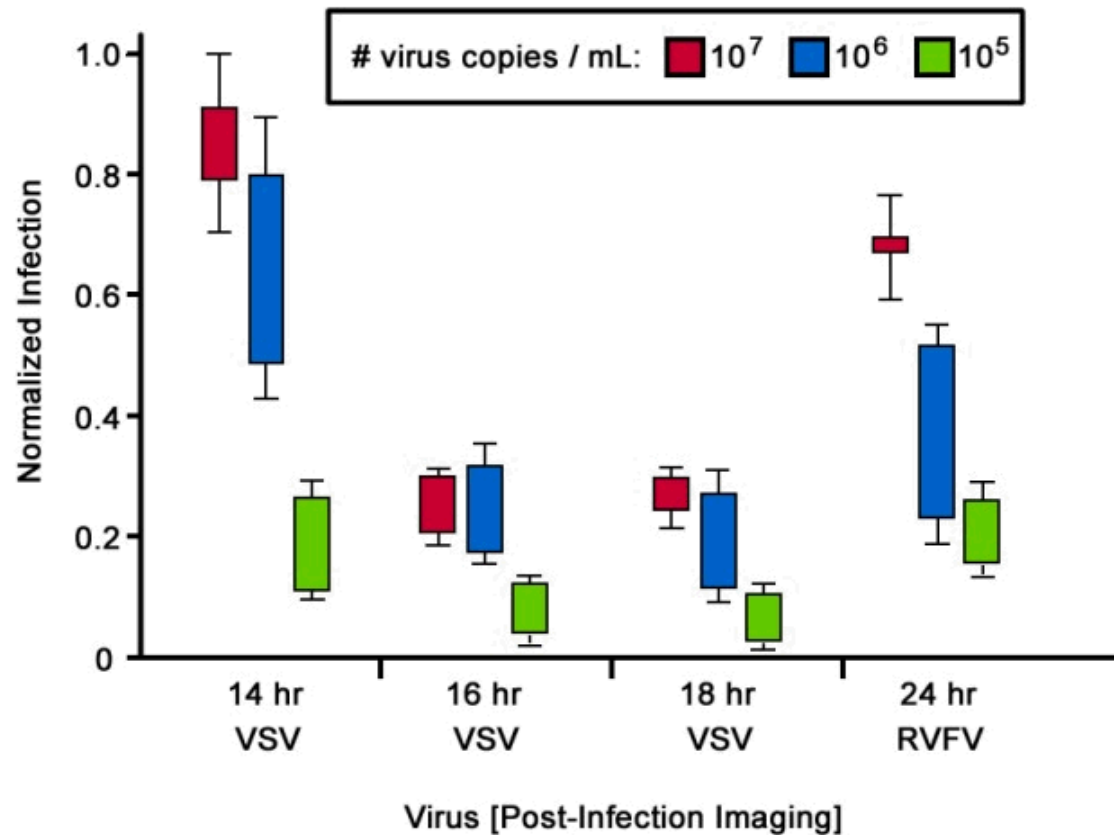


Actin mediated  
process with pm  
ruffles, regulated by  
Na<sup>+</sup>/H<sup>+</sup> exchange—  
PI3K—Rac1, PAK1,  
and cholesterol

# Virus Entry Screen: Multiple experiments per siRNA



## Sensitivity experiments – bandwidth of GFP response



## Current Results / Forward Path

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- Assay using RVFV-MP12-GFP can demonstrate knockdown using siRNA in cell culture well plates
- Demonstrated cells on platform being infected by RVFV
- Future work: Demonstrate knockdown of RVFV on chip
- Integrate wells into a microfluidic device, demonstrating cell capture, transfection and knockdown



# Acknowledgements

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Oscar Negrete  
Brooke Harmon  
Dianna Maar  
Anup Singh  
Carrie Kozina  
Vinay Abhyankar