

Development and delivery of RNA-targeting CRISPR systems for anti-viral countermeasures

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Systems Biology Department



Exceptional service in the national interest
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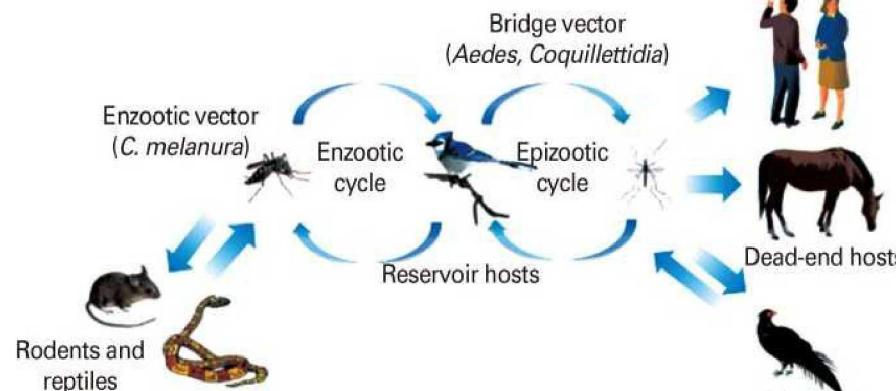


Pathogen outbreaks are difficult to predict....



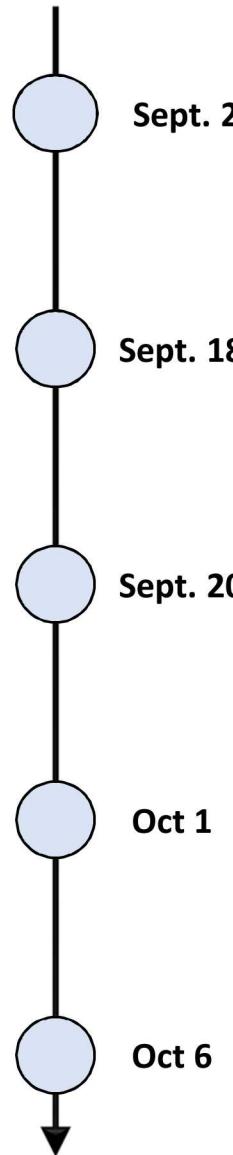
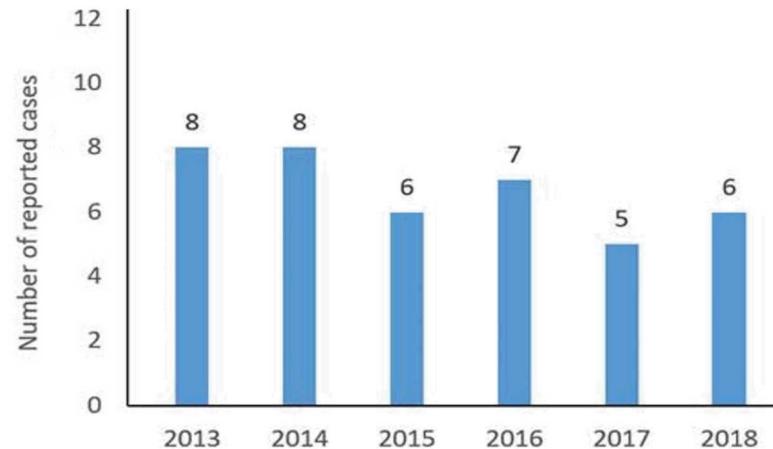
Pathogen outbreaks are difficult to predict and require a rapid response

Eastern Equine Encephalitis Virus



YoungGo *et al*, 2014

CDC Confirmed Case Reports by Year



Newsweek US Health Dept Sounds Alarm as Mosquitoes Carrying EEE-infecting Virus Found in 12 Connecticut Towns

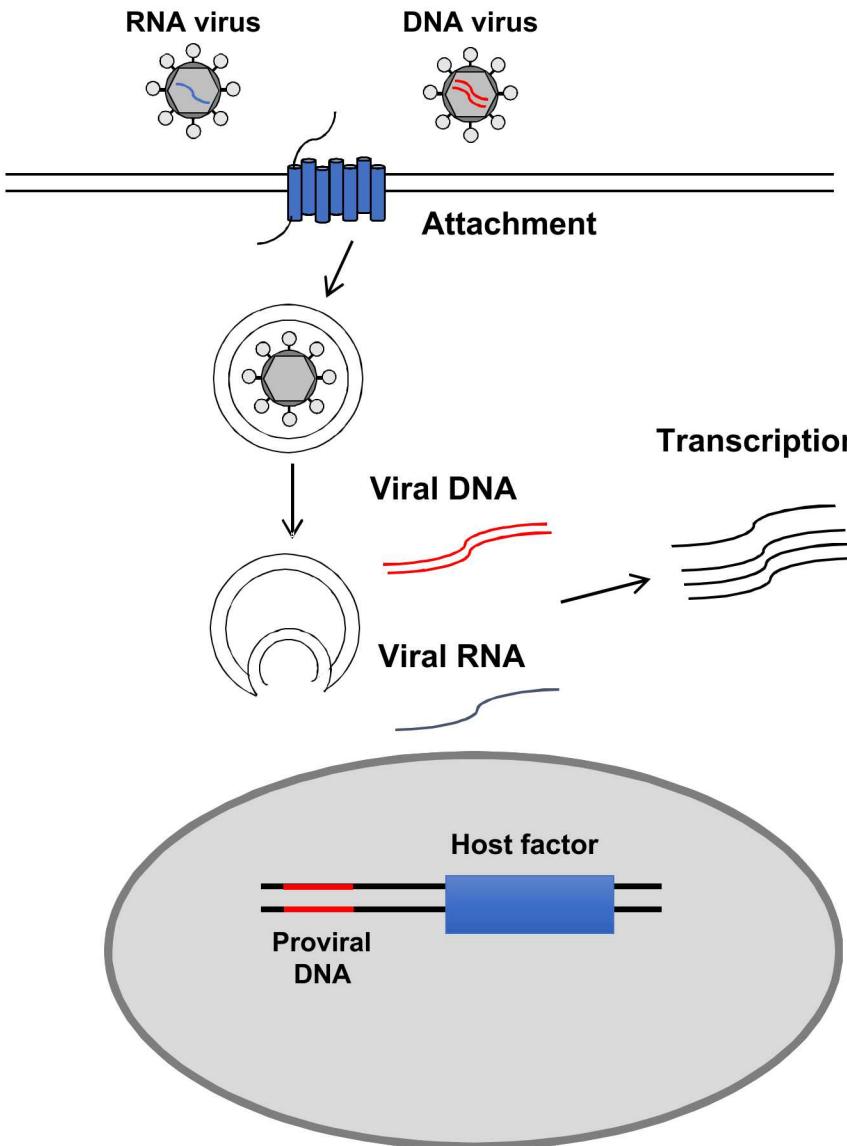
The New York Times States Warn Residents about Rare Mosquito-Borne Illness That **Has Killed 6**

Forbes **7 Now Dead** From EEE, What Is the Latest On This Mosquito Borne Disease?

ScienceNews Rare eastern equine encephalitis **has killed 9** people in the U.S. in 2019

CNN **At least 11 people** have died from a rare mosquito-borne illness in the United States

CRISPR as a modular, rapidly adaptable anti-viral countermeasure



GOALS:

Target host and viral genomes for anti-viral development

Optimize CRISPR/Cas tools and for safely targeting DNA/RNA

DNA targeting

Host factor



Controlled gene disruption:

Ligand inducible Cas9

Long term gene silencing:

Epigenetic dCas fusion

RNA targeting

Viral RNA

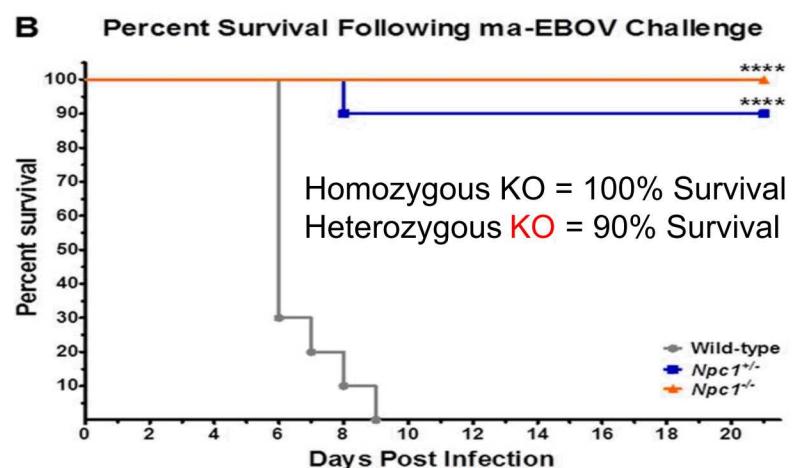
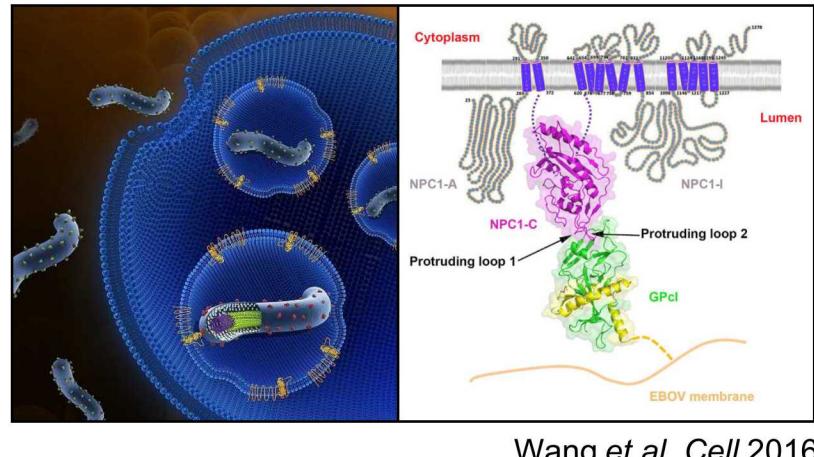
mRNA

Sa/CjCas9

Cas13a/d

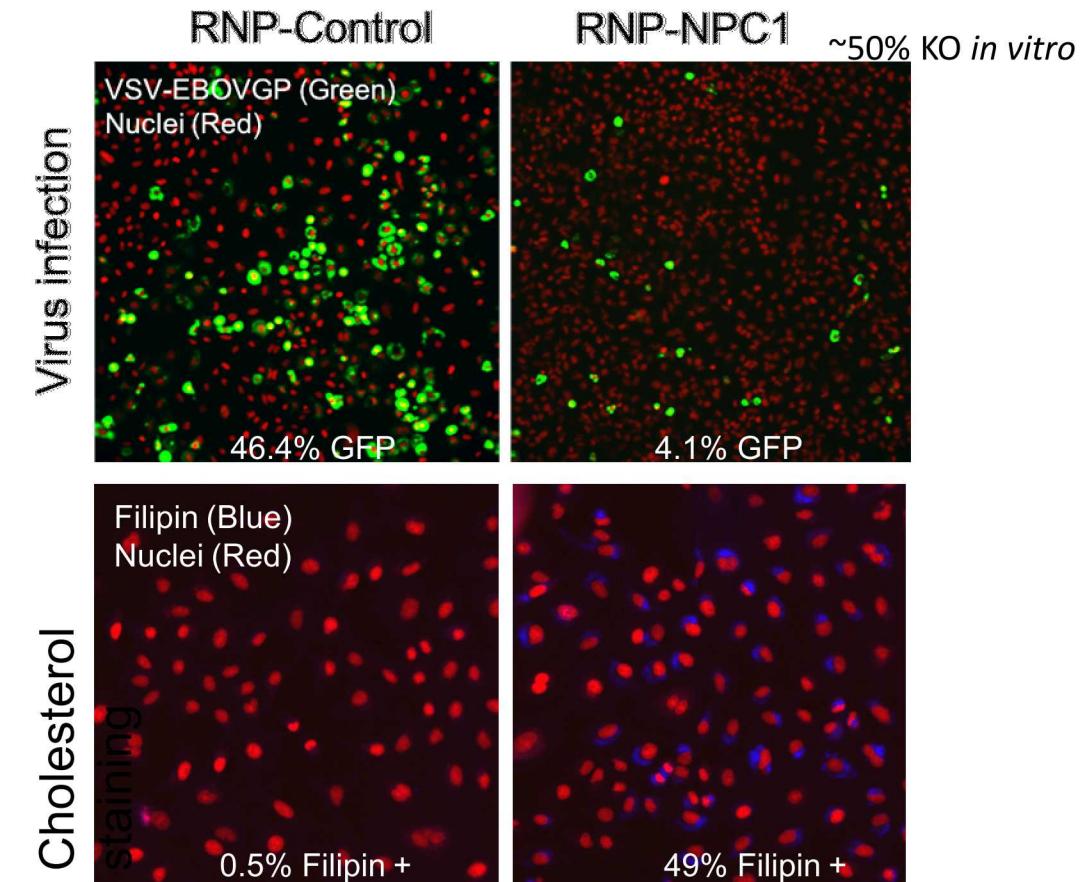
Host-directed countermeasures: targeting the Ebola receptor

Niemann-Pick C1 (NPC1) is required for filoviral infection



Herbert et al, *mBio* 2015

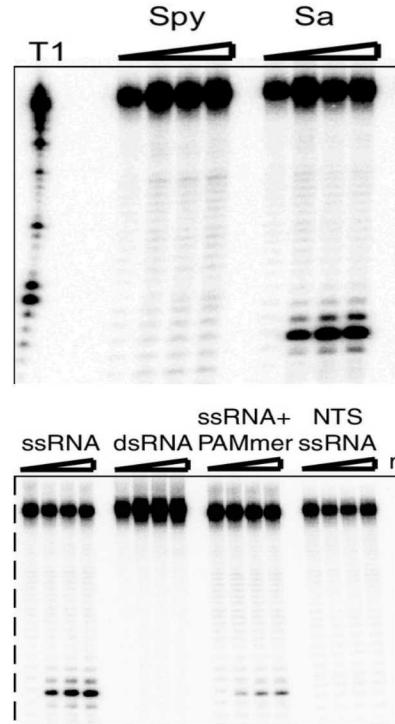
NPC1 as a target for genome editing



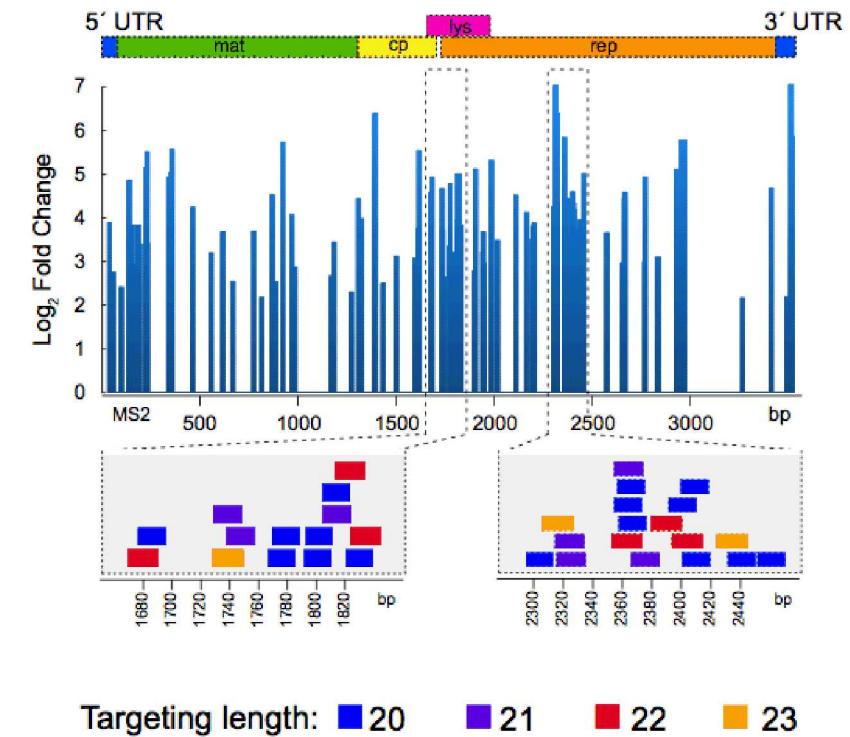
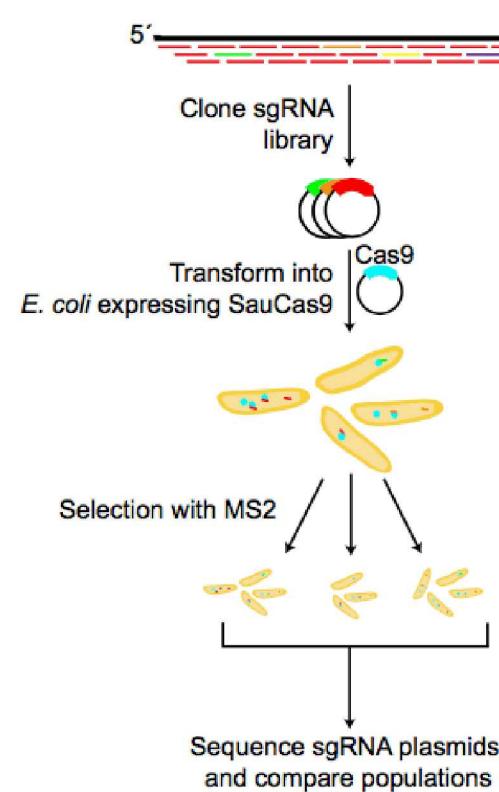
in vivo delivery experiments underway

Viral-directed countermeasures: RNA targeting Cas?

RNA Cleavage Assays



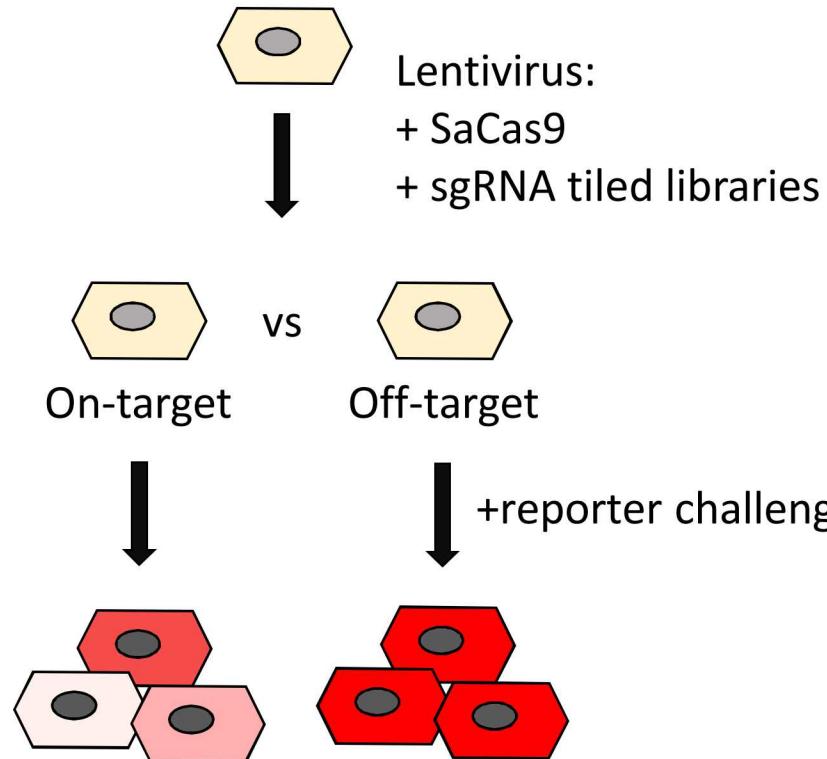
SaCas9 protects *E. coli* from MS2 Phage



Can this be exploited to protect mammalian cells from viral infection?

Understanding RNA-targeting dynamics in mammalian cells

Cell-based screening of sgRNA libraries

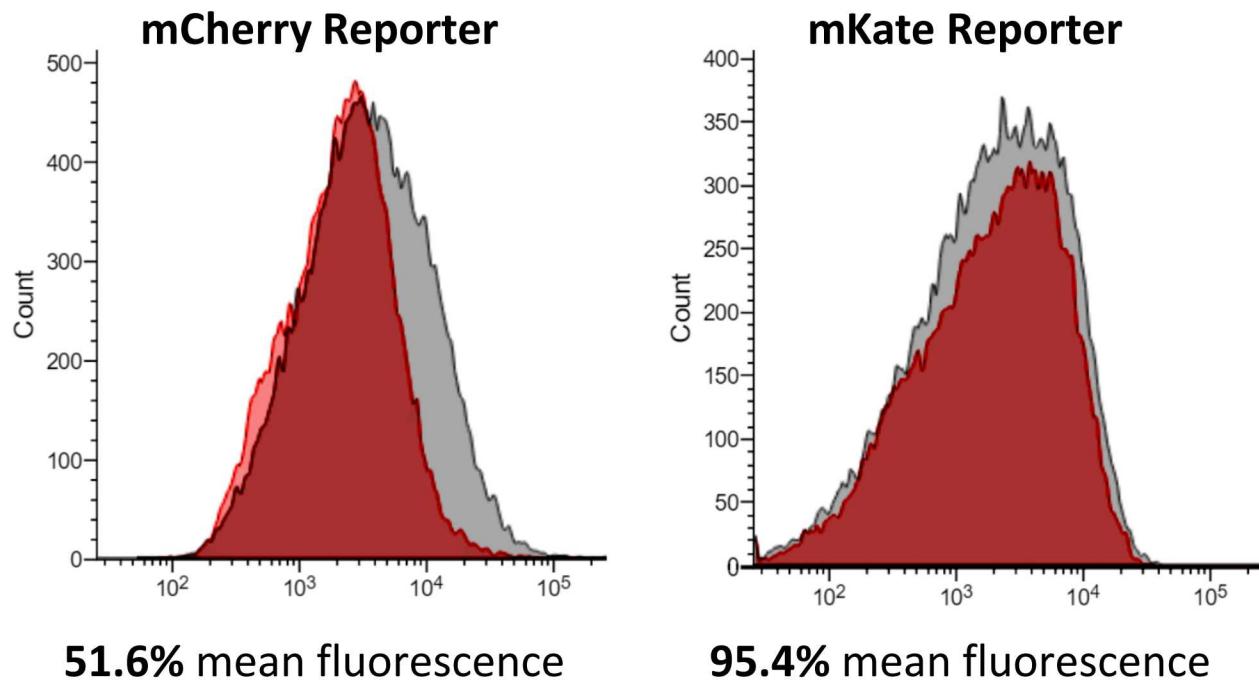


Challenge conditions being optimized

Transient	Plasmid or mRNA transfection
Constitutive	Stable Cell Line

Population Level analysis via FACS

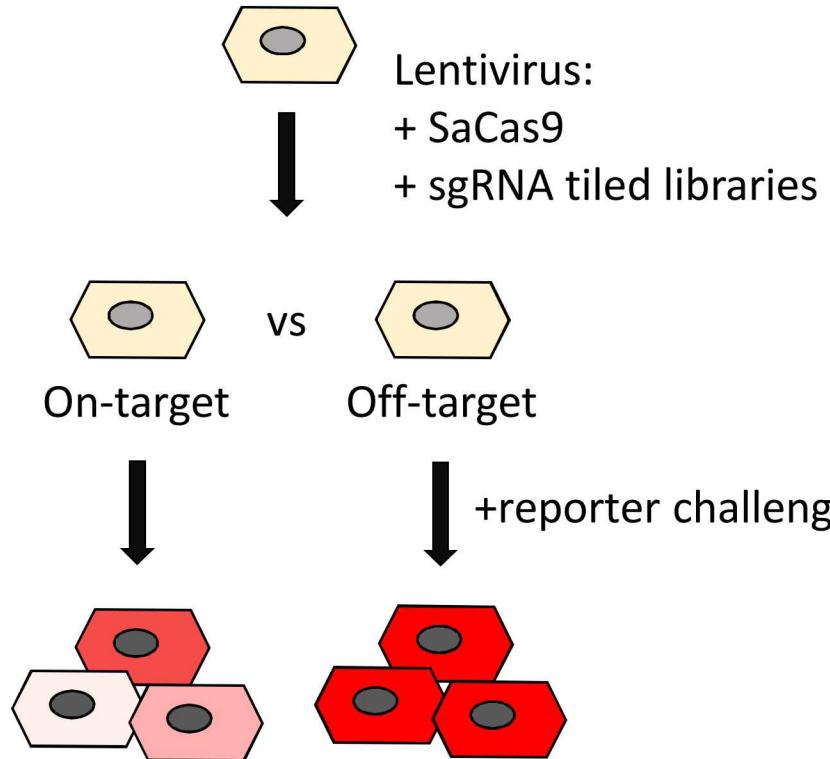
SaCas9 with **anti-mCherry** vs control-sgRNA libraries



Optimization and enrichment screening underway

Understanding RNA-targeting dynamics in mammalian cells

Cell-based screening of sgRNA libraries

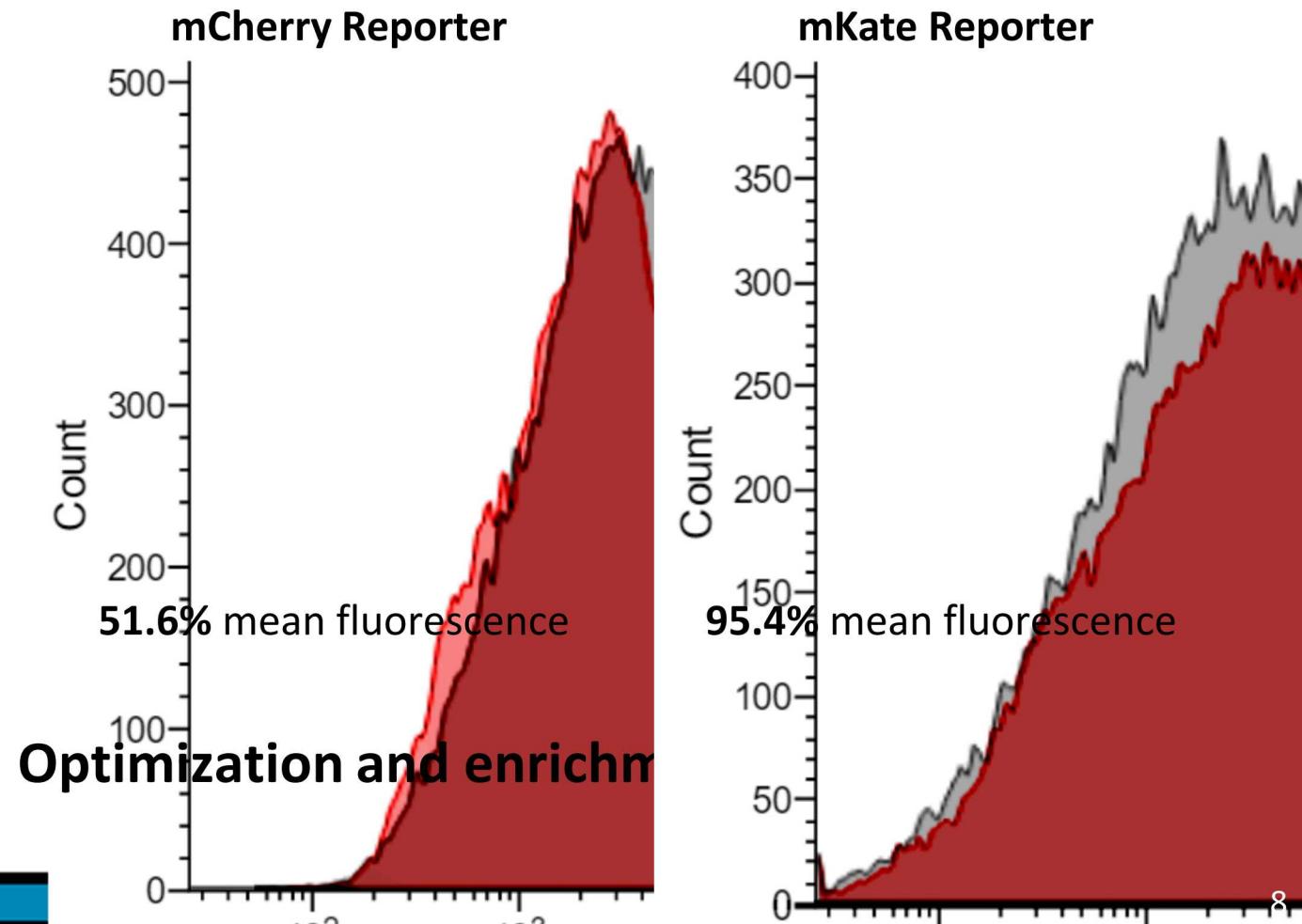


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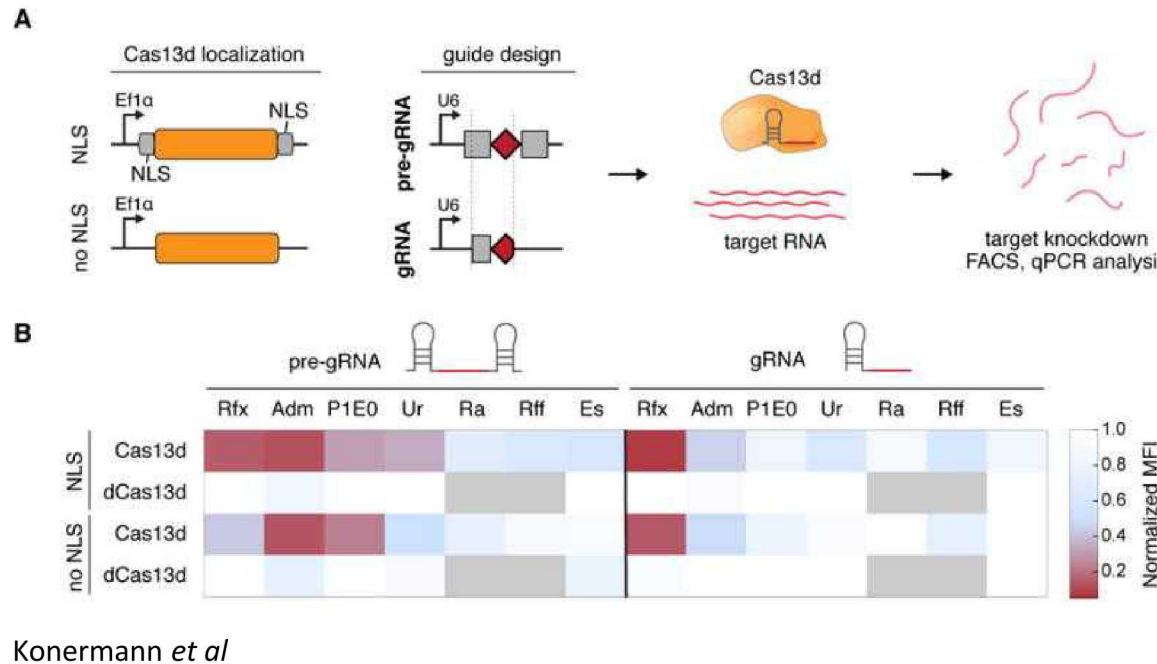
Population Level analysis via FACS

SaCas9 with **anti-mCherry** vs control-sgRNA libraries

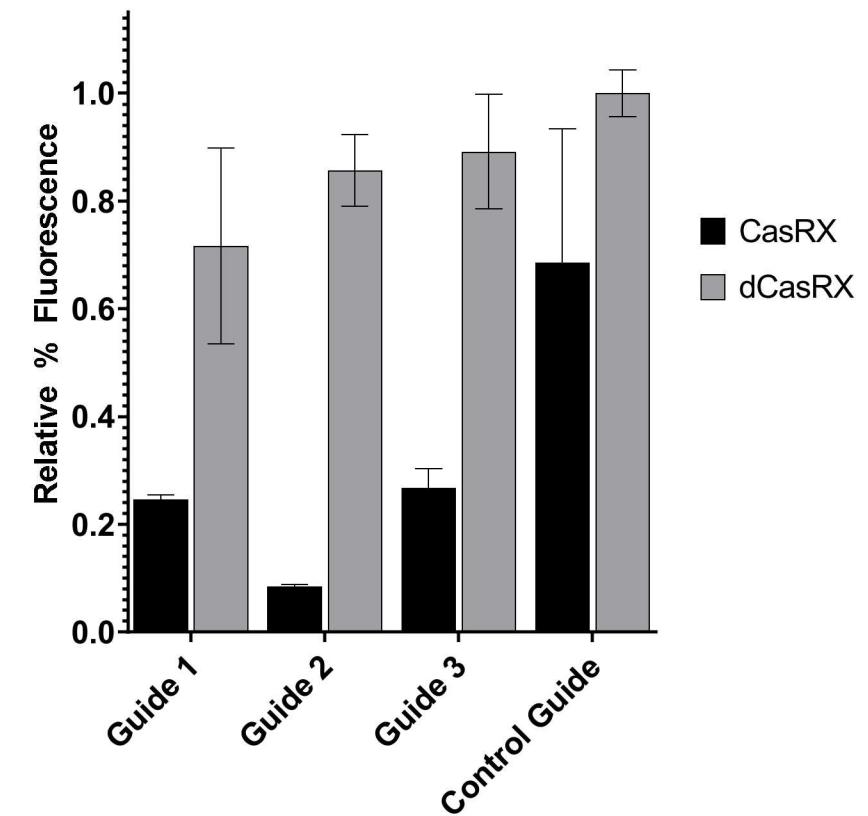


Cas13s are RNA-guided RNAases and promising anti-viral candidates

Cas13d variants targeting mCherry



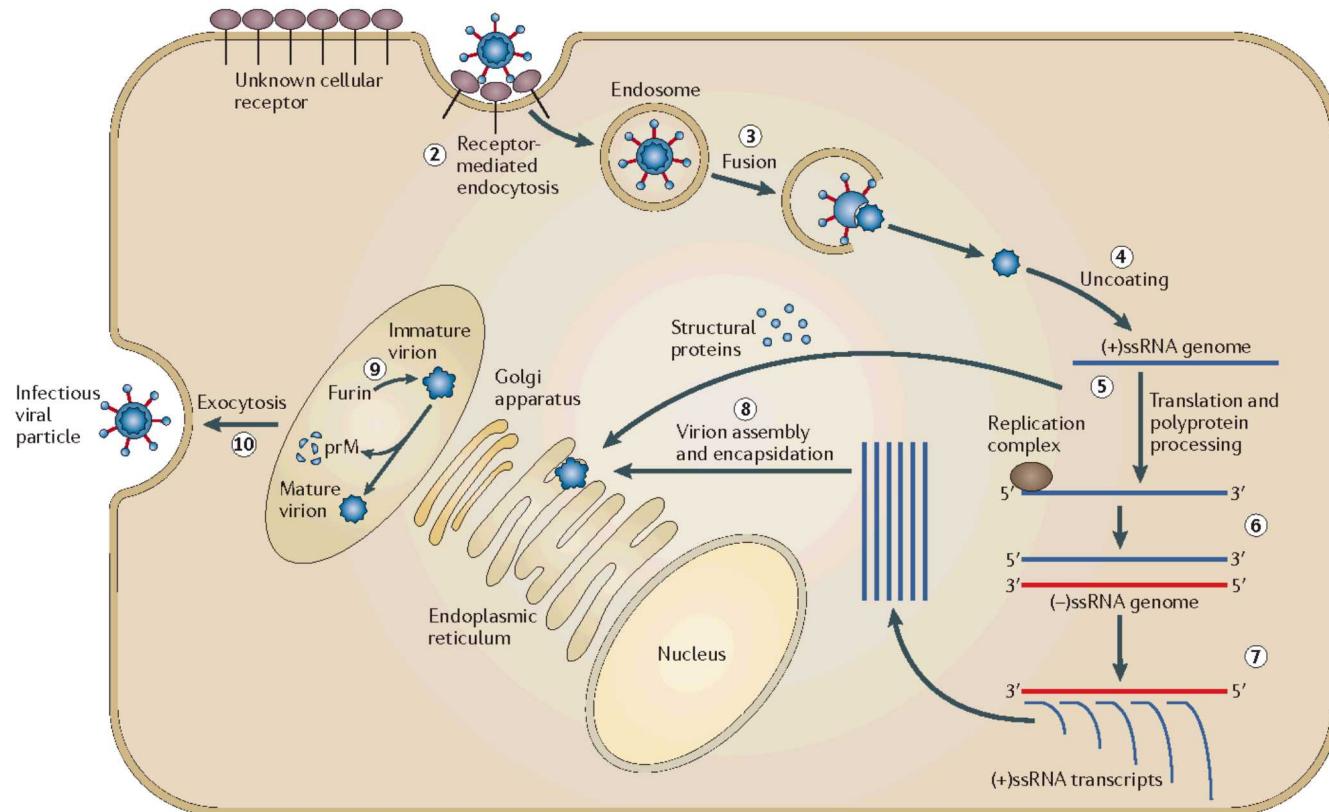
CasRX inhibition of mCherry



Now screening CasRX guides targeting NPC1

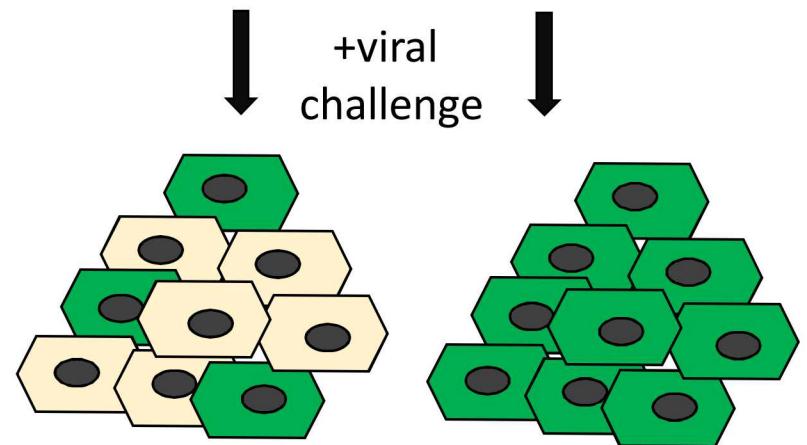
Viral RNAs are a challenging, moving target

Simplified ssRNA viral life cycle



Cell-based viral survival screening

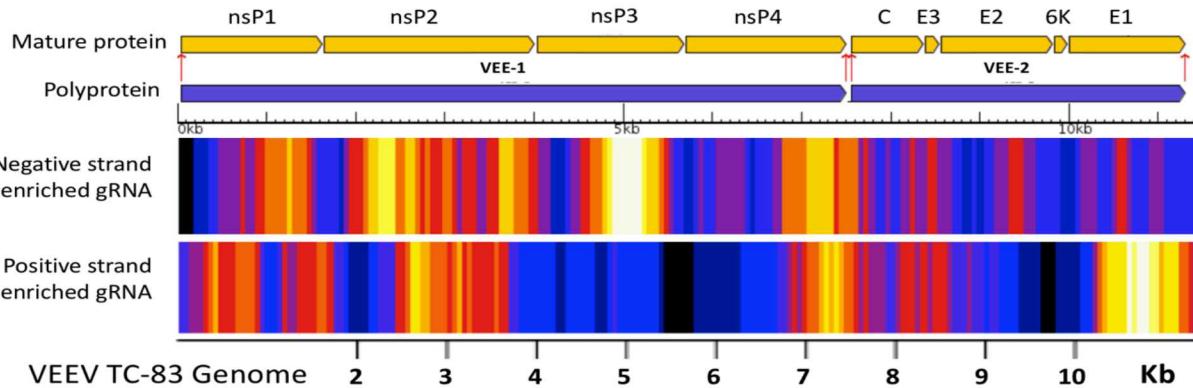
anti-VEEV vs control library



NGS Sequencing of survivors/uninfected

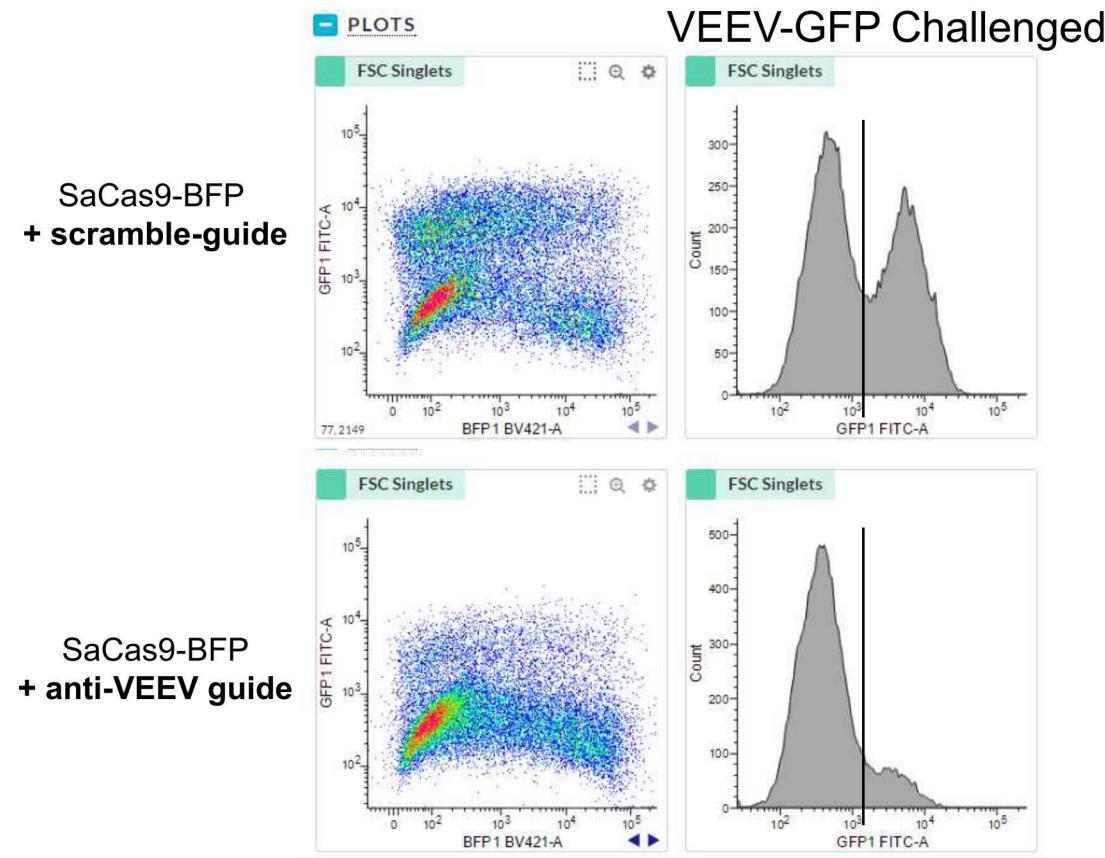
RNA-targeting SaCas9 mitigates VEEV infection *in vitro*

Heatmap of anti-VEEV library enrichment



- Identified over 100 significant hits from the primary screens
- Divided nearly equally into genome and anti-genome targeting sequences

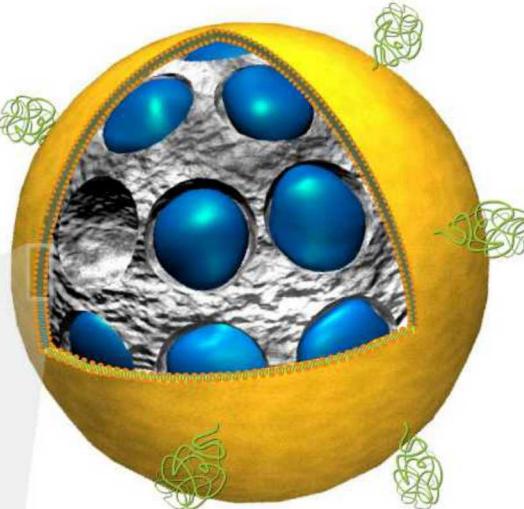
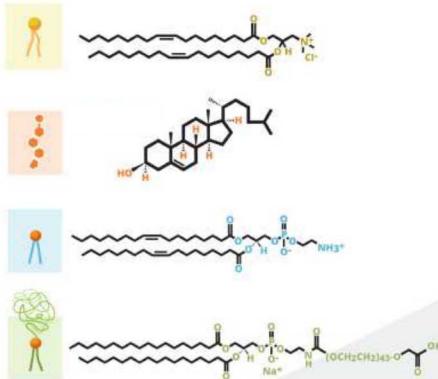
Single anti-VEEV guides reduce viral infection



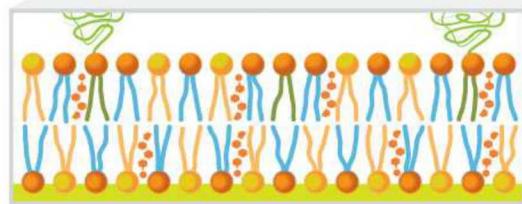
Validating guides against different viruses in different *in vitro* systems

Development of a scalable *in vivo* delivery platform

Lipid-Coated Mesoporous Silica Nanoparticles (LC-MSN)



SUPPORTED LIPID BILAYER

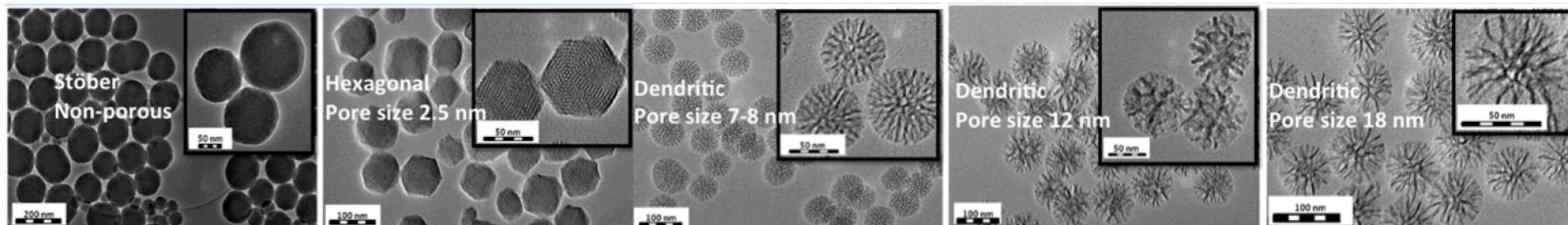


Lipid-Coating

Protects cargo
Cationic or Zwitterionic
Functionalizable with targeting ligands

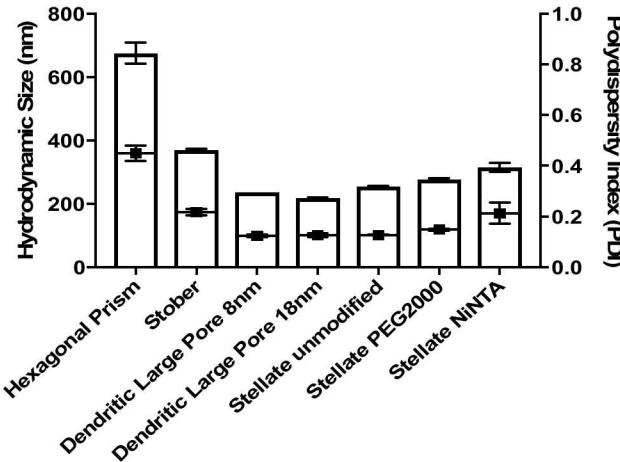
Silica

Hydrolyzes intracellularly / “GRAS”
Scaffold for various attachment chemistries
Porous – can fit large cargo

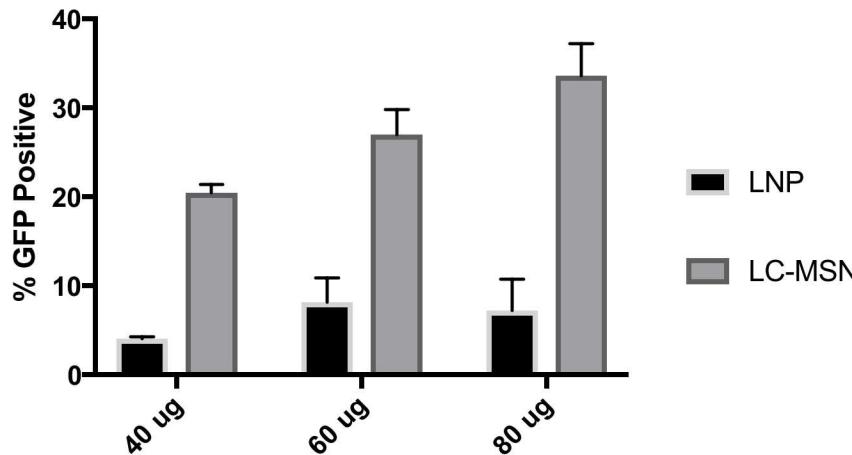


Pilot LC-MSN *in vivo* efficacy studies are underway

LC-MSNs are 200-400nm in size



LC-MSN *in vitro* reporter assays



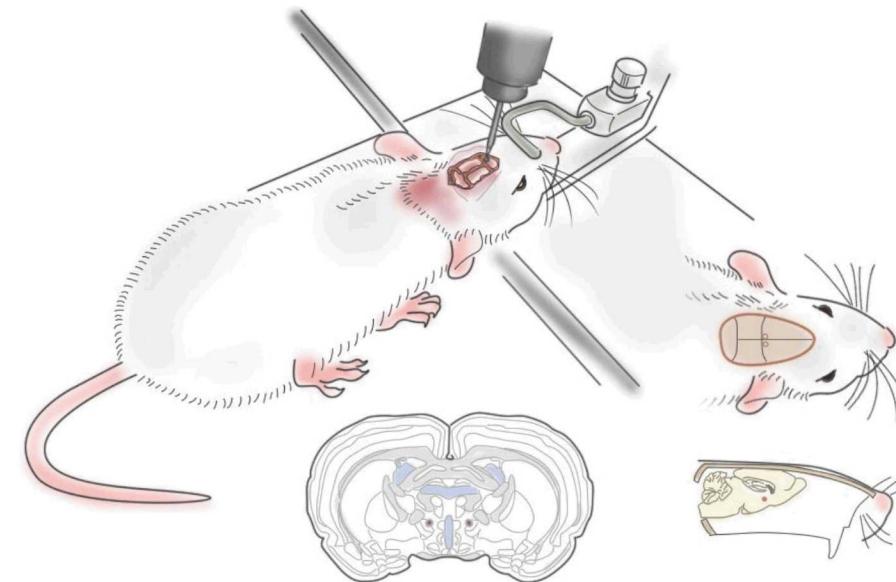
Systemic delivery – tail vein injections

Localized delivery – oropharyngeal aspiration, muscle injection

Safety and biodistribution studies –

Veterinary pathology of multiple organs

Direct application to the brain/CNS via IC and ITC



Summary

CRISPR is a modular, rapidly-adaptable tool to use against emerging pathogen outbreaks

RNA and DNA-editing

Capable of targeting both host and viral-targets directly

Developing the LC-MSN delivery technology for *in vivo* efficacy

Packaging RNP and/or nucleic acids

Scalable and easy to chemically modify both core and coating

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Department of Energy review required
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