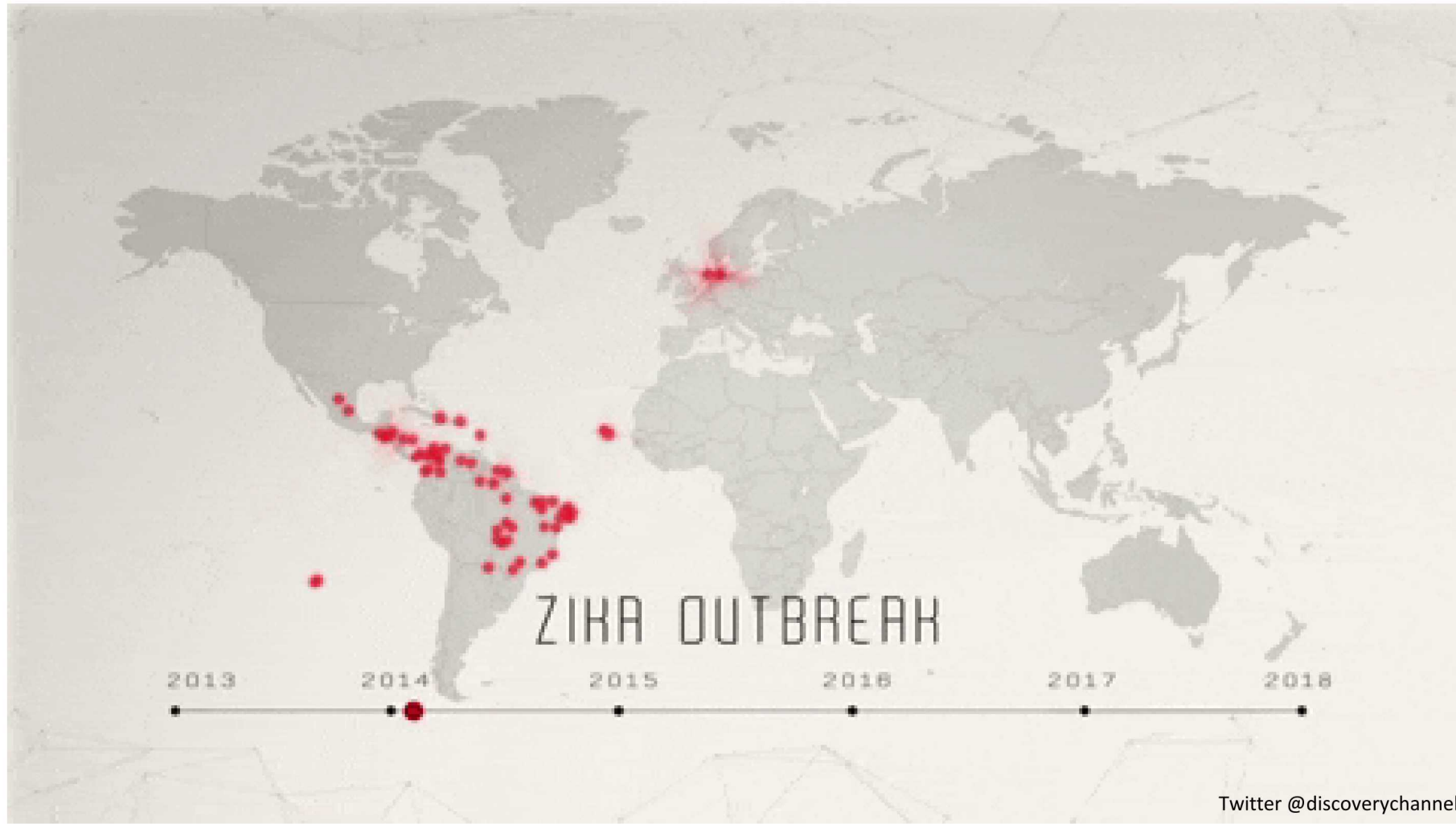


Development and delivery of RNA-targeting CRISPR systems for anti-viral countermeasures

Edwin A. Saada, PhD

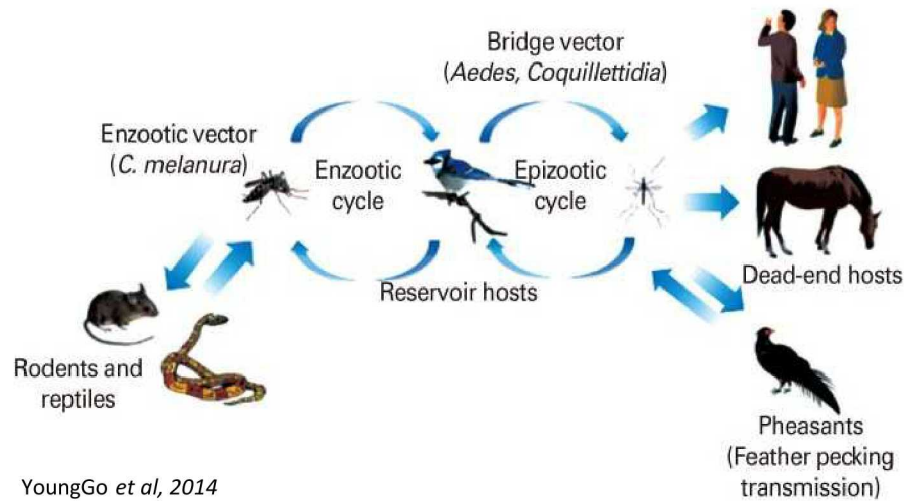
Systems Biology Department

Pathogen outbreaks are difficult to predict....

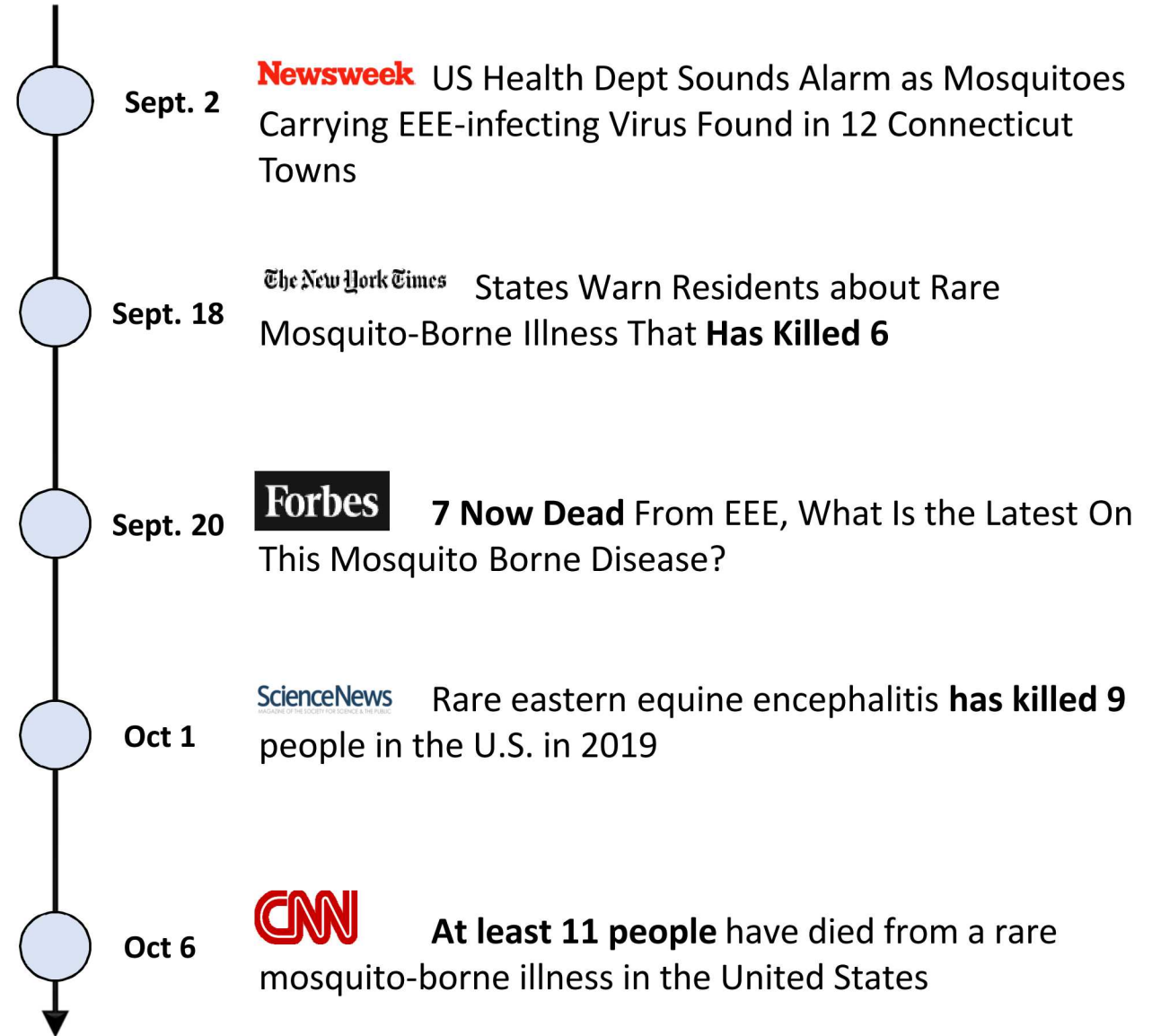
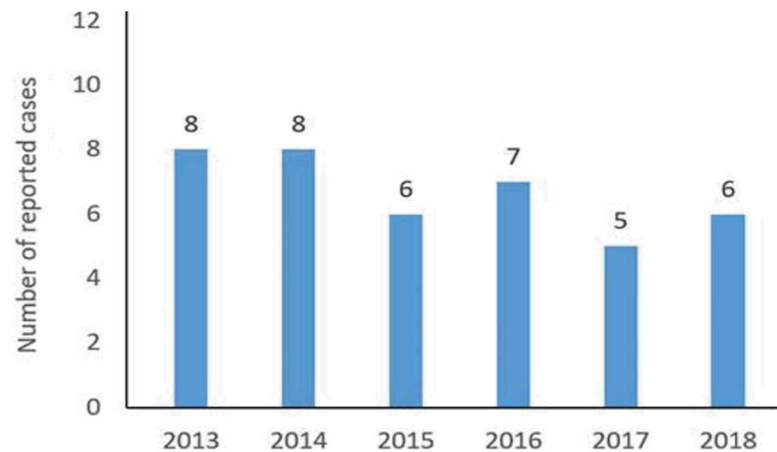


Pathogen outbreaks are difficult to predict and require a rapid response

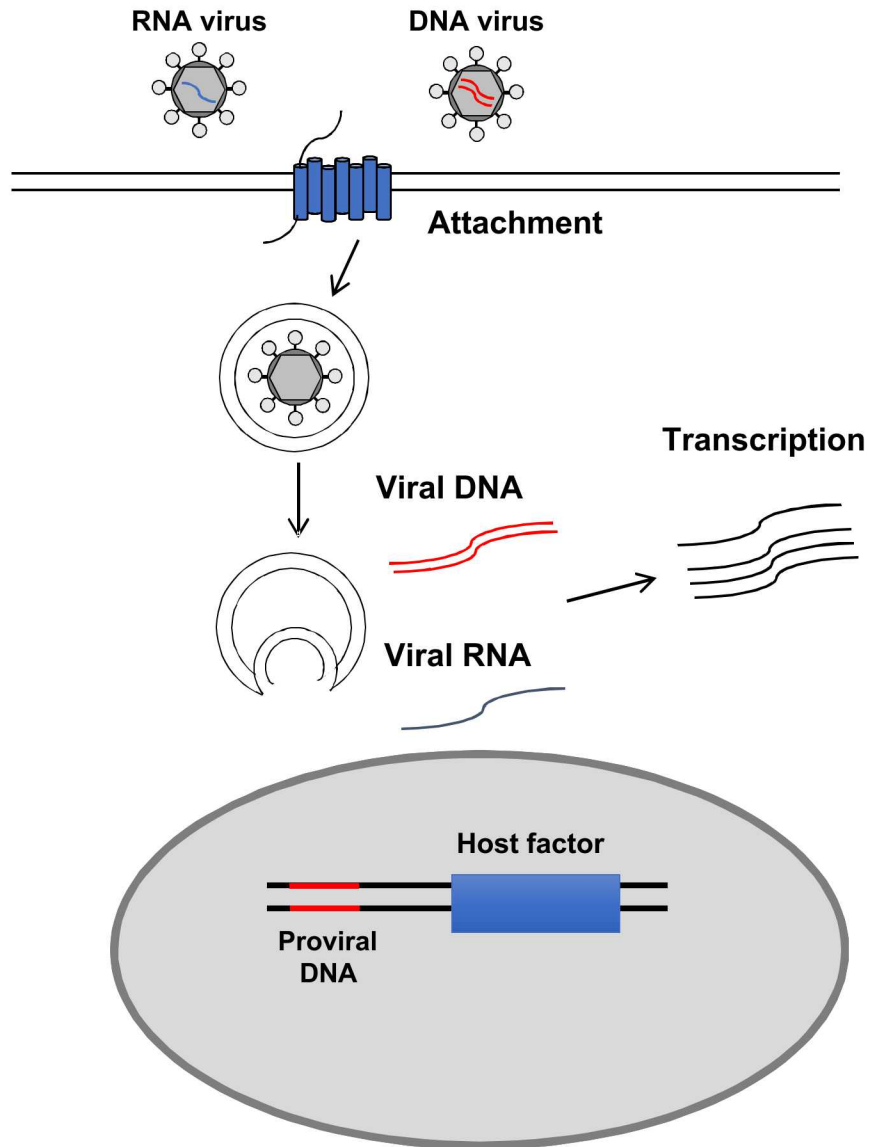
Eastern Equine Encephalitis Virus



CDC Confirmed Case Reports by Year



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

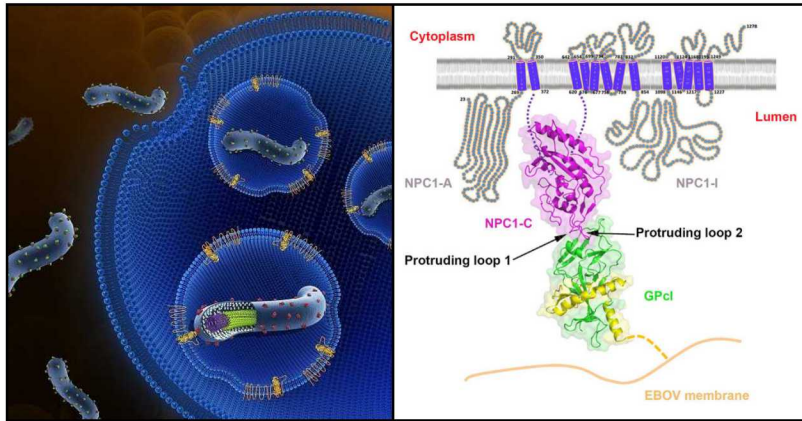
mRNA

Sa/CjCas9

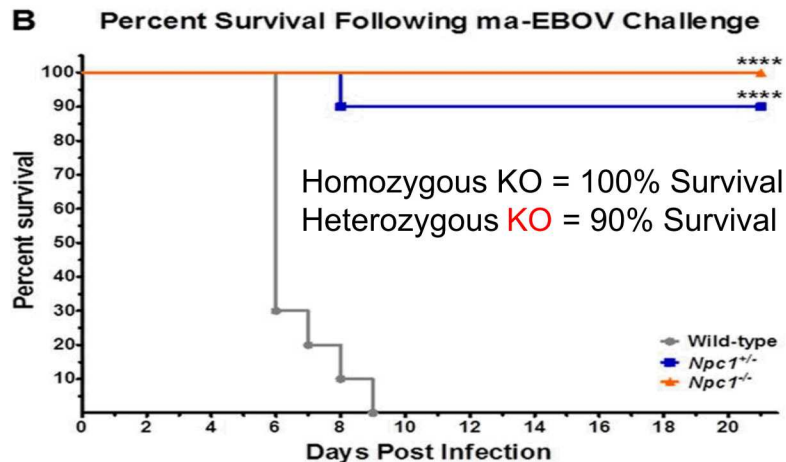
Cas13a/d

Host-directed countermeasures: targeting the Ebola receptor

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

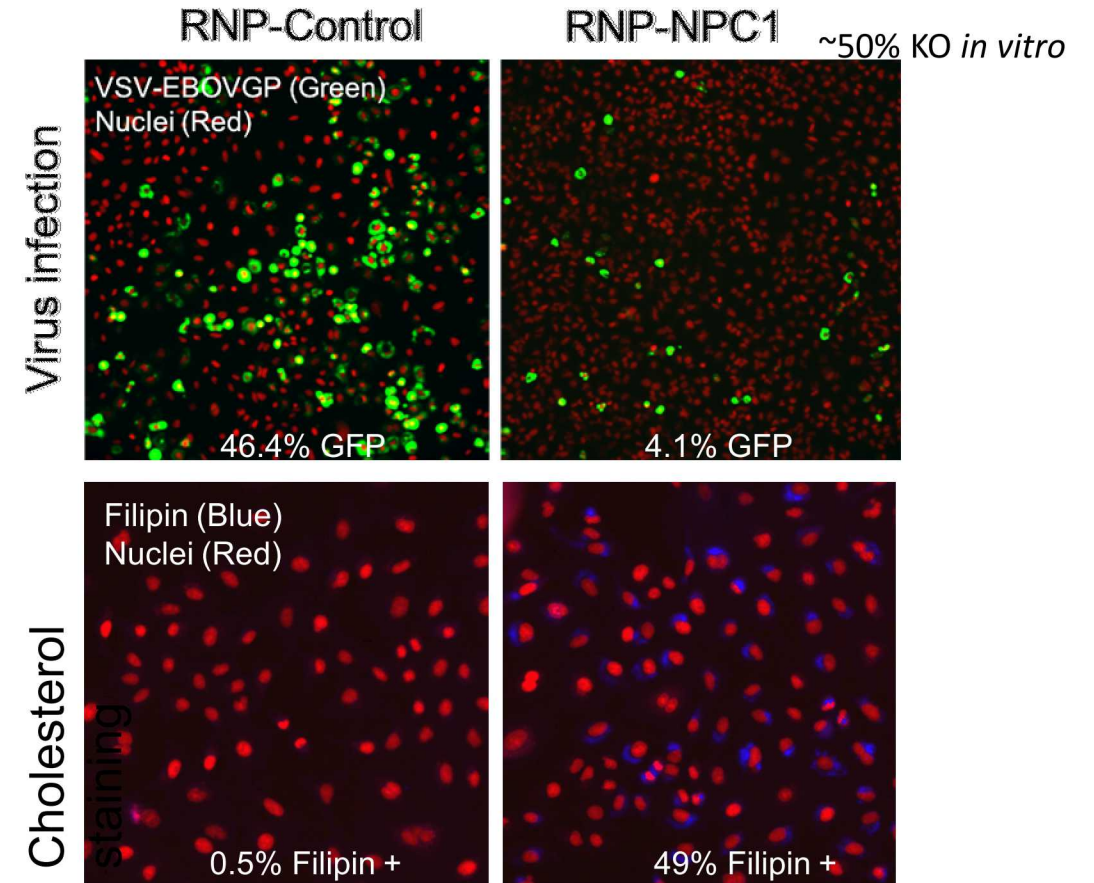


Wang *et al*, *Cell* 2016



Herbert *et al*, *mBio* 2015

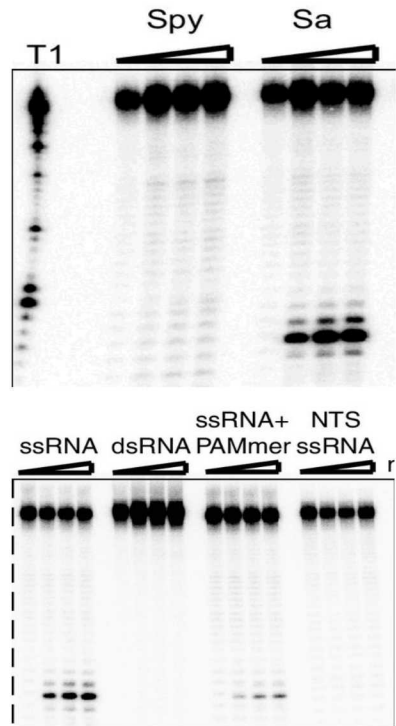
NPC1 as a target for genome editing



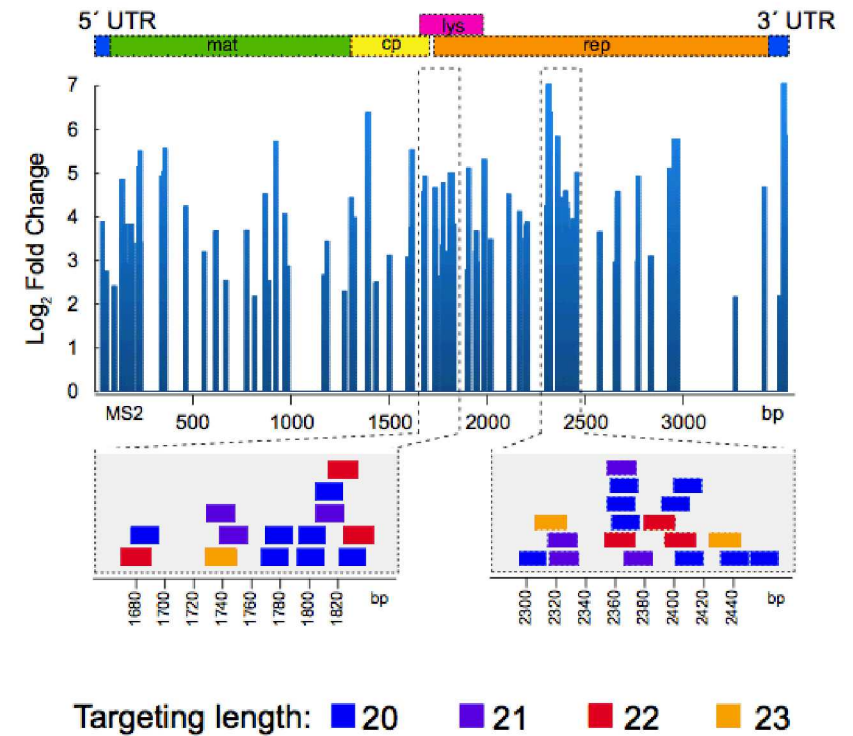
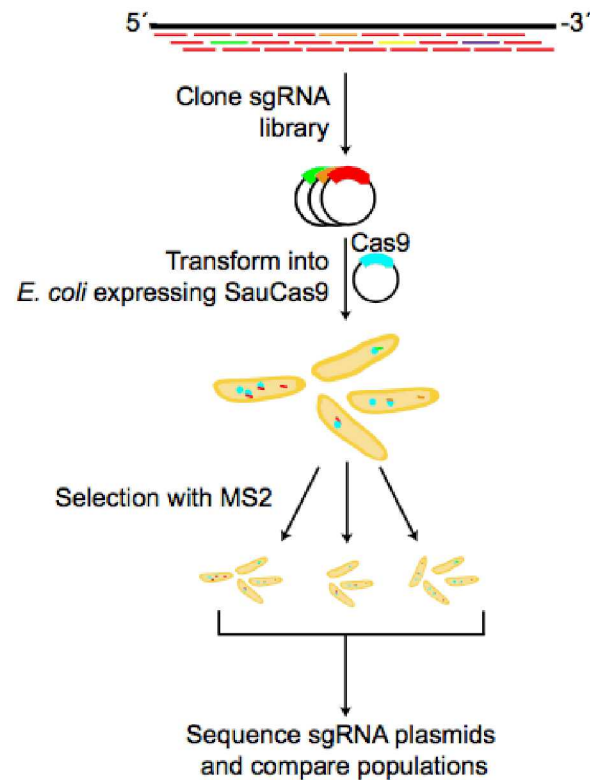
in vivo delivery experiments underway

Viral-directed countermeasures: RNA targeting Cas?

RNA Cleavage Assays



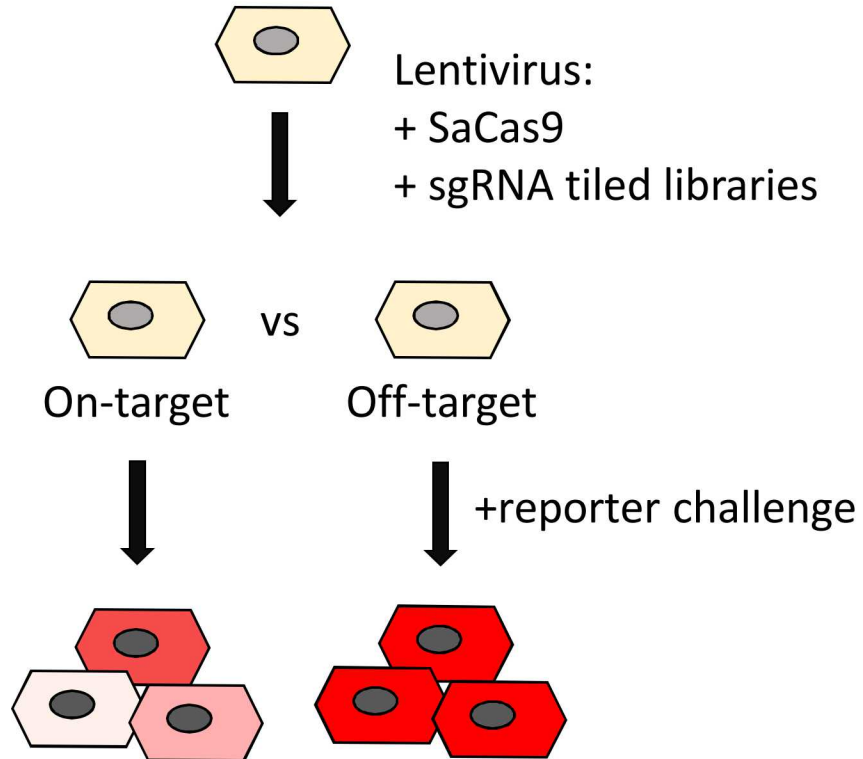
SaCas9 protects *E. coli* from MS2 Phage



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

Understanding RNA-targeting dynamics in mammalian cells

Cell-based screening of sgRNA libraries



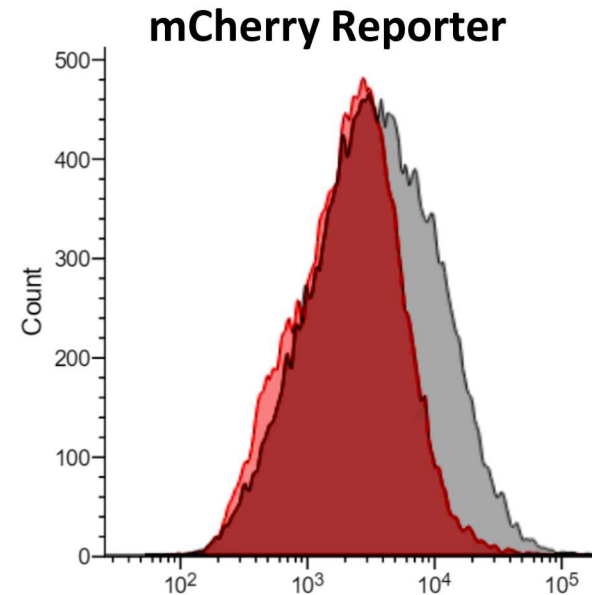
Challenge conditions being optimized

Transient
Constitutive

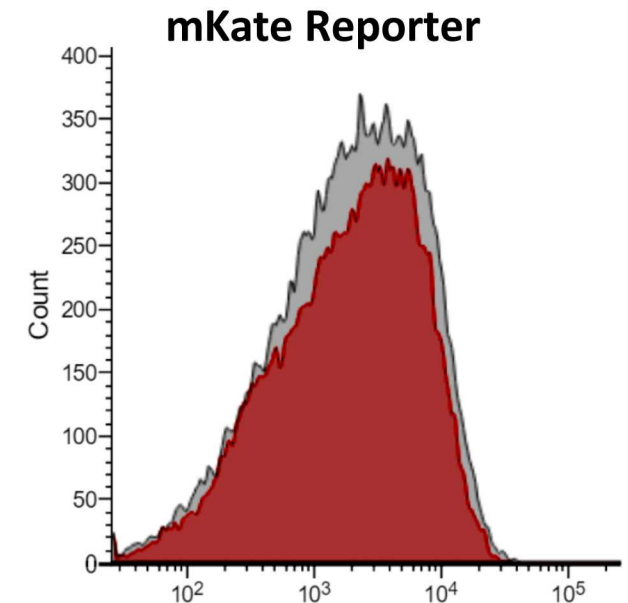
Plasmid or mRNA transfection
Stable Cell Line

Population Level analysis via FACS

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



51.6% mean fluorescence

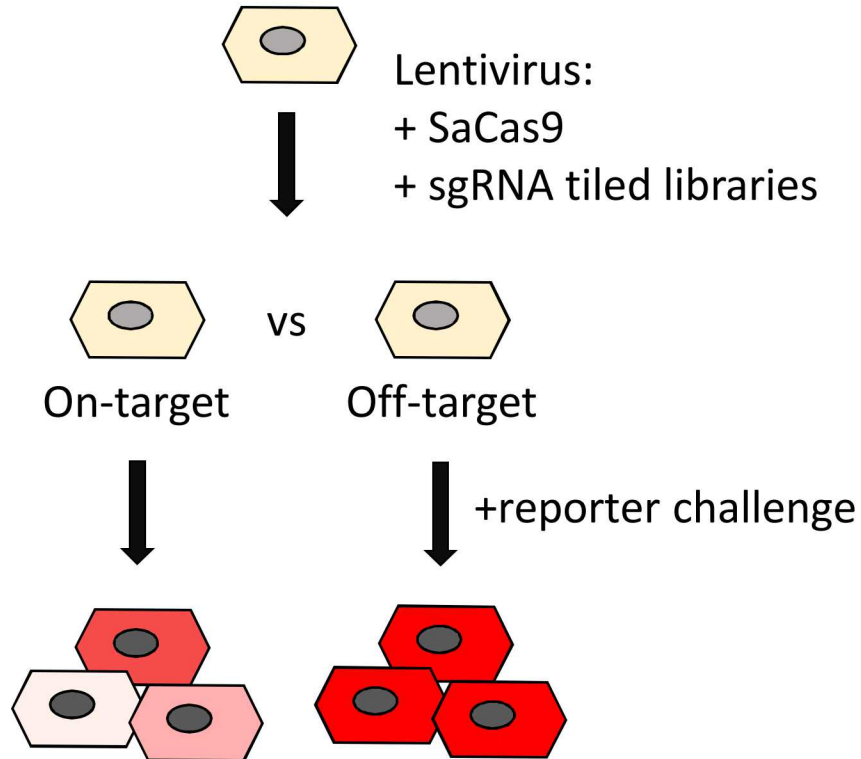


95.4% mean fluorescence

Optimization and enrichment screening underway

Understanding RNA-targeting dynamics in mammalian cells

Cell-based screening of sgRNA libraries



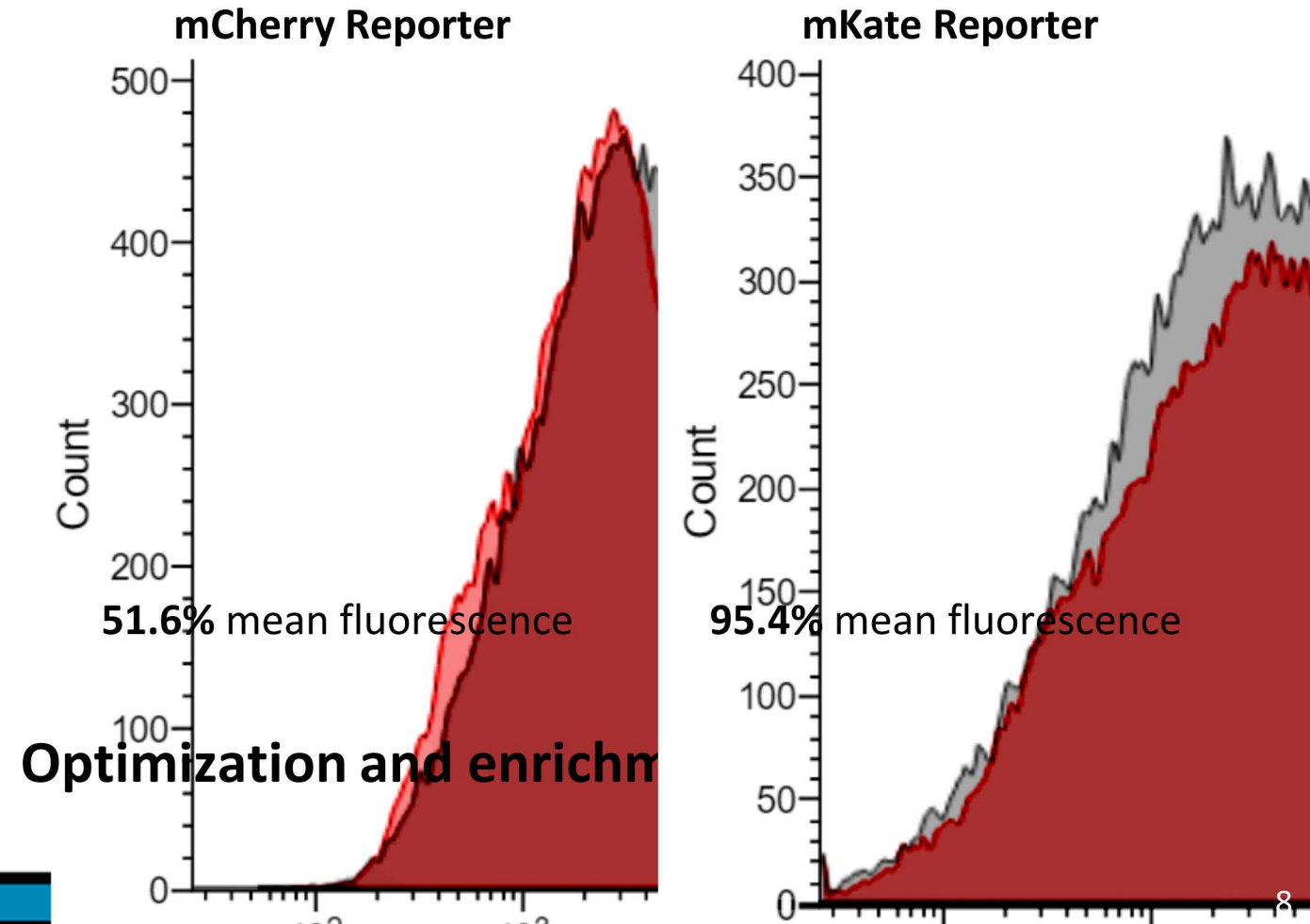
Challenge conditions being optimized

Transient
Constitutive

Plasmid or mRNA transfection
Stable Cell Line

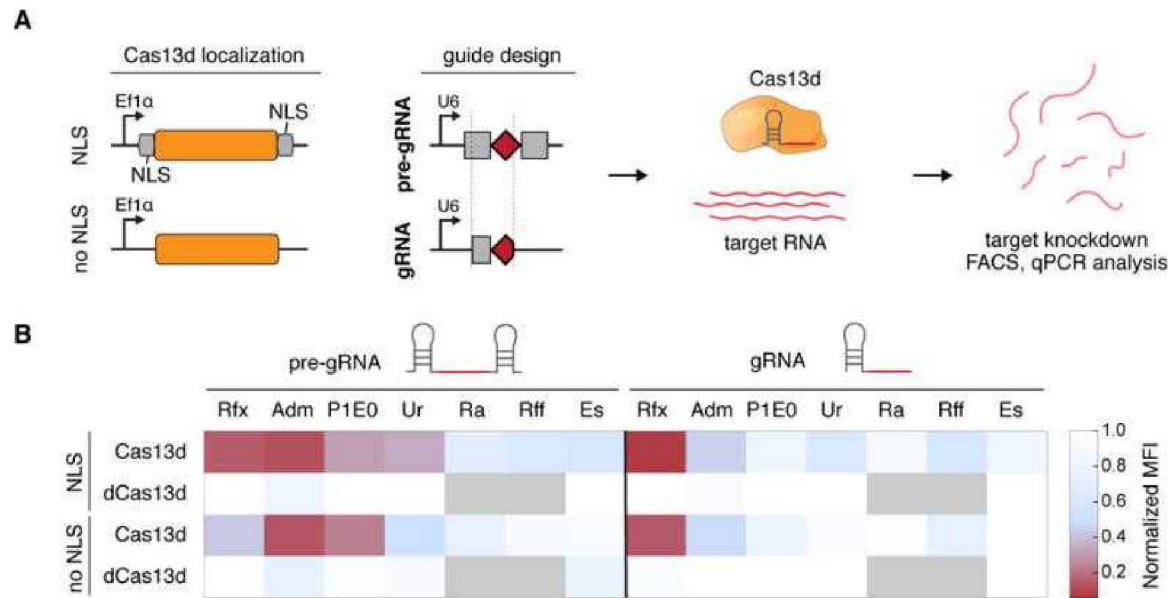
Population Level analysis via FACS

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



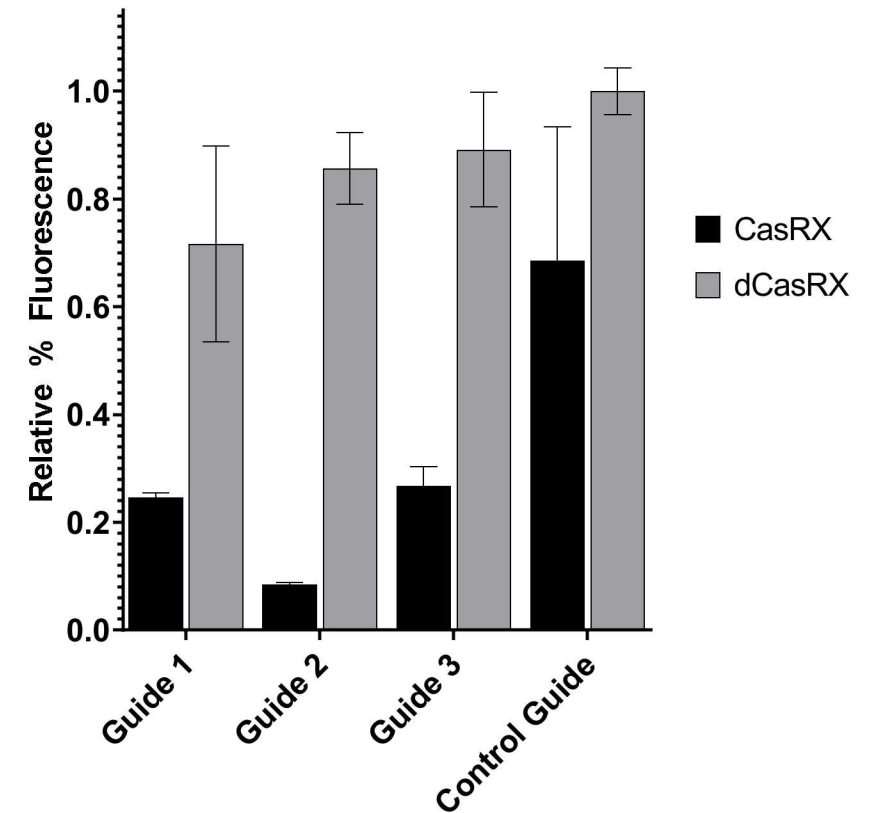
Cas13s are RNA-guided RNAases and promising anti-viral candidates

Cas13d variants targeting mCherry



Konermann *et al*

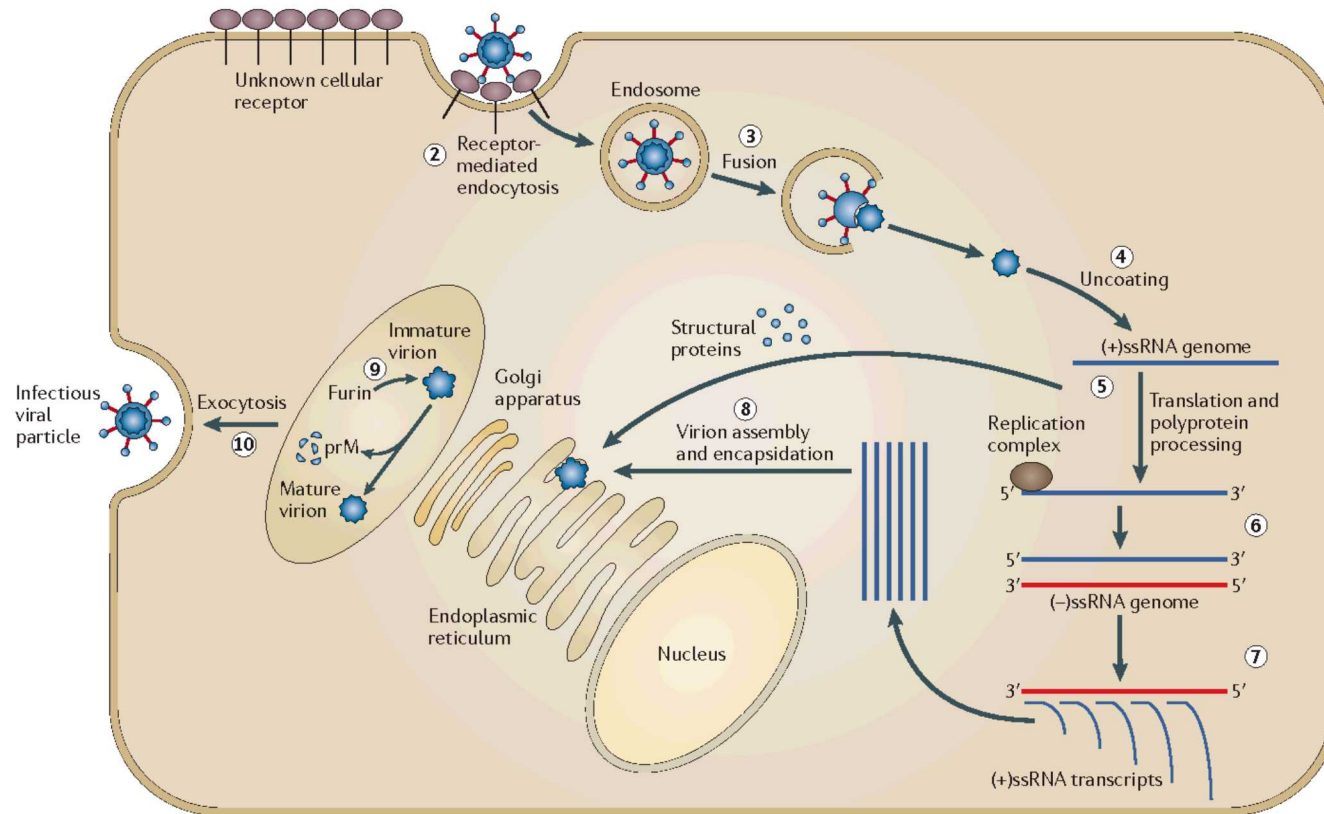
CasRX inhibition of mCherry



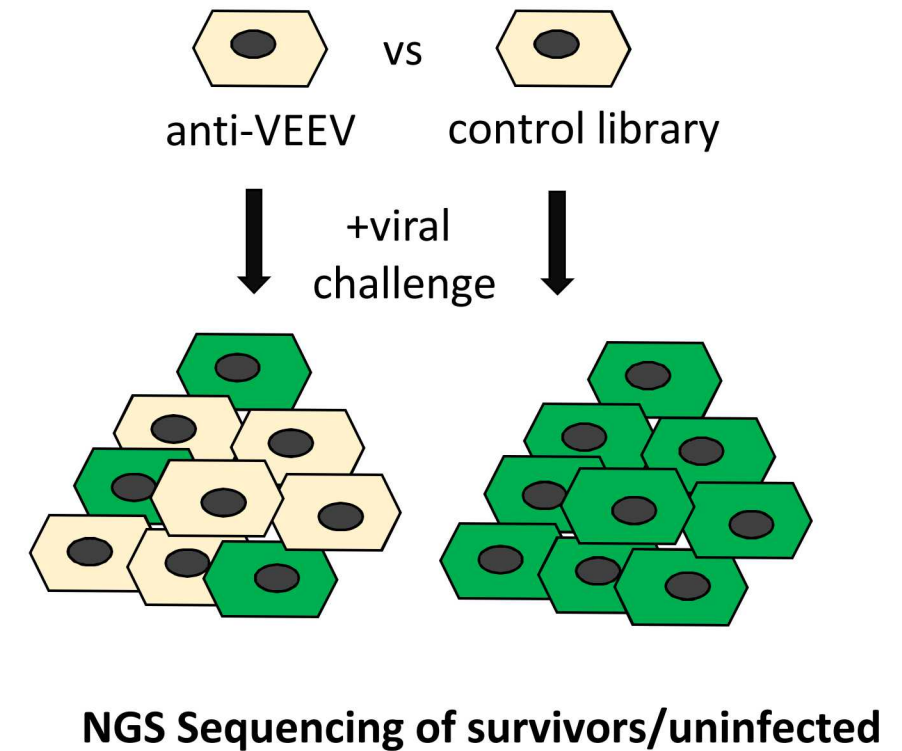
Now screening CasRX guides targeting NPC1

Viral RNAs are a challenging, moving target

Simplified ssRNA viral life cycle

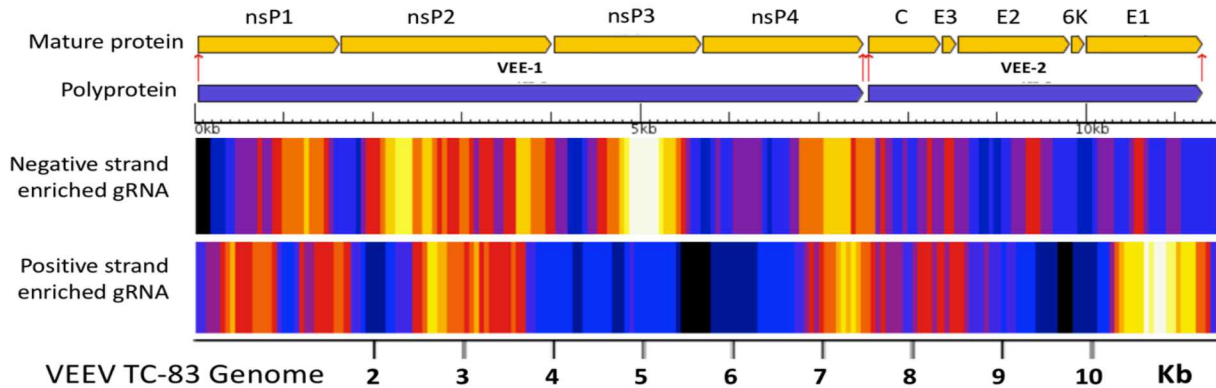


Cell-based viral survival screening



RNA-targeting SaCas9 mitigates VEEV infection *in vitro*

Heatmap of anti-VEEV library enrichment

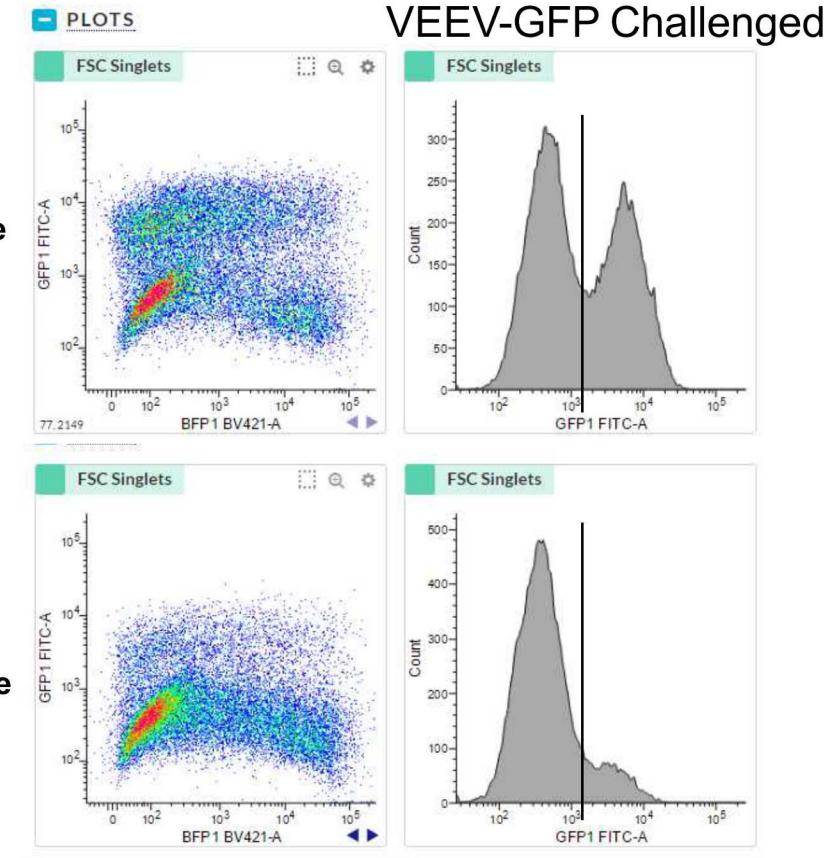


- Identified over 100 significant hits from the primary screens
- Divided nearly equally into genome and anti-genome targeting sequences

Single anti-VEEV guides reduce viral infection

SaCas9-BFP
+ scramble-guide

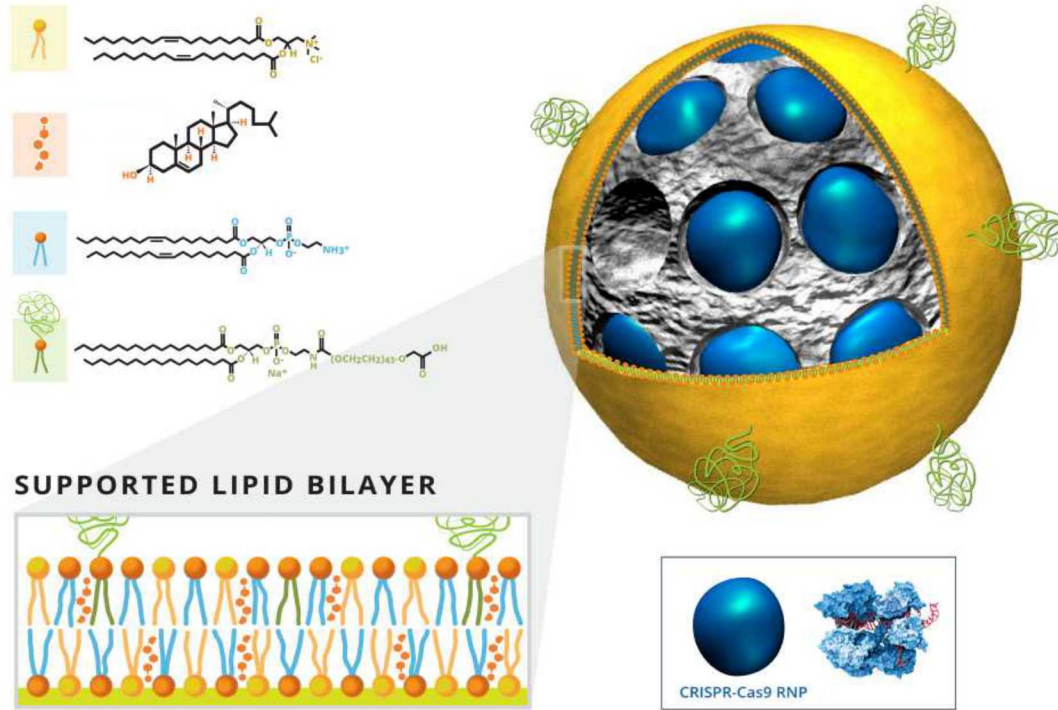
SaCas9-BFP
+ anti-VEEV guide



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)

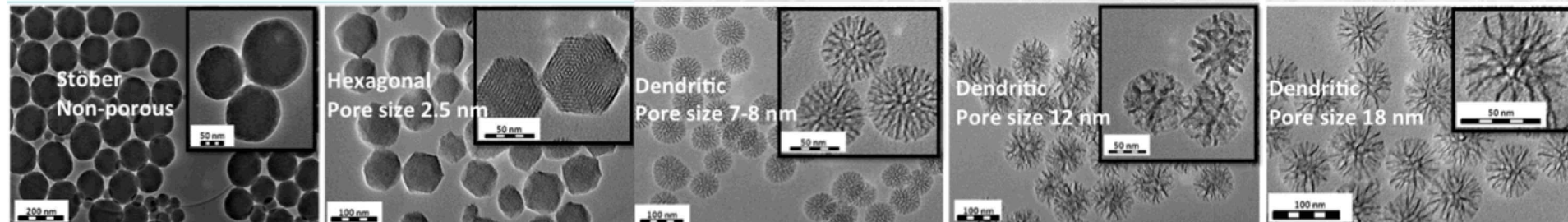


Lipid-Coating

Protects cargo
Cationic or Zwitterionic
Functionalizable with targeting ligands

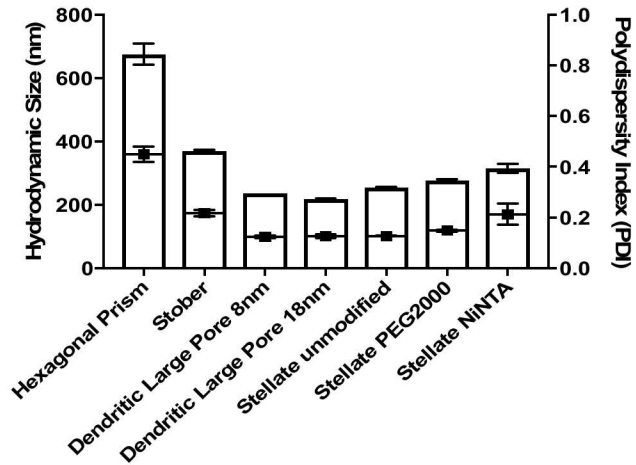
Silica

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

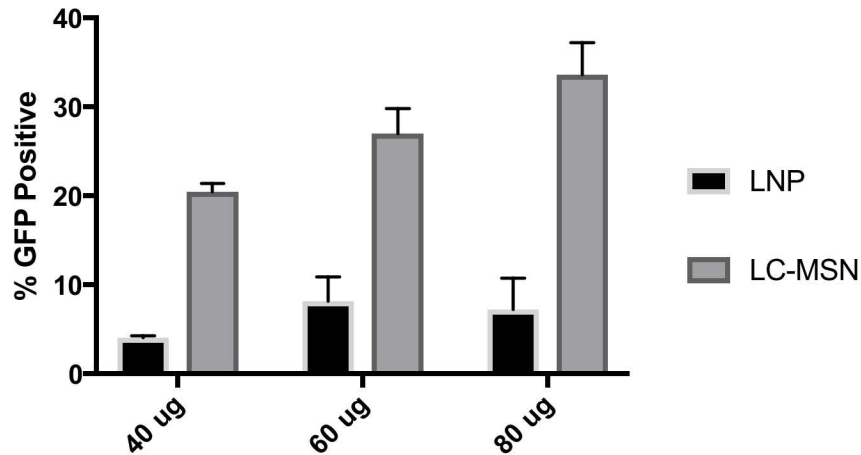


Pilot LC-MSN *in vivo* efficacy studies are underway

LC-MSNs are 200-400nm in size



LC-MSN *in vitro* reporter assays



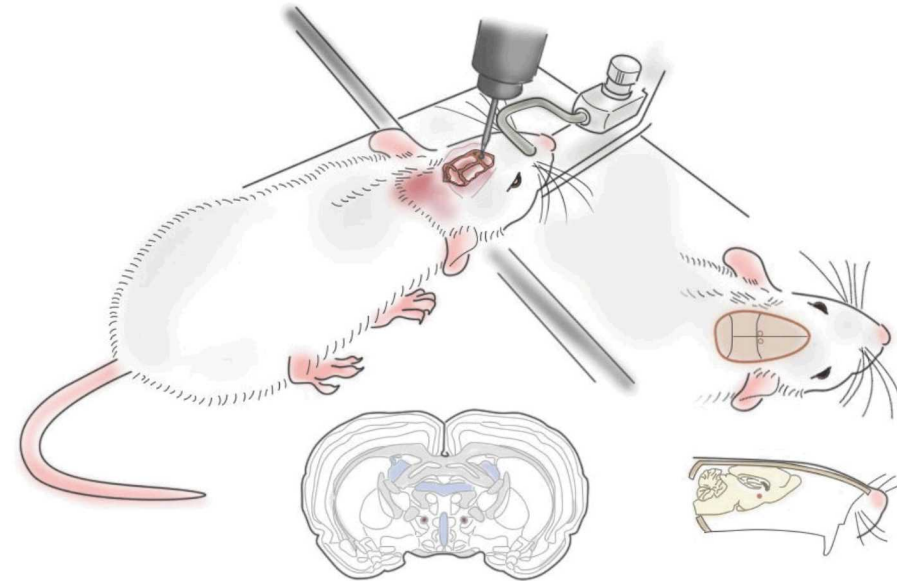
Systemic delivery – tail vein injections

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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UCSF

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Luke Gilbert
Jonathan Weissman



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GC LDRD Award (190245- NanoCRISPR)



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Exemption #. Category Name.

Department of Energy review required
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Guidance (if applicable) _____



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