

A black and white photograph of a person sitting on a rock at night, holding a flashlight that illuminates the dark landscape. The person is in profile, looking towards the left. The background shows rolling hills under a starry night sky. The overall mood is contemplative and focused.

**Functional  
characterization  
and therapeutic  
targeting of gene  
regulatory  
elements**

**Nadav Ahituv  
UCSF**

# Disclosures

Equity holder and scientific advisor for Encoded Therapeutics

Equity holder and member of scientific advisory board for Regel Therapeutics

Equity holder and member of scientific advisory board for Neomer Diagnostics

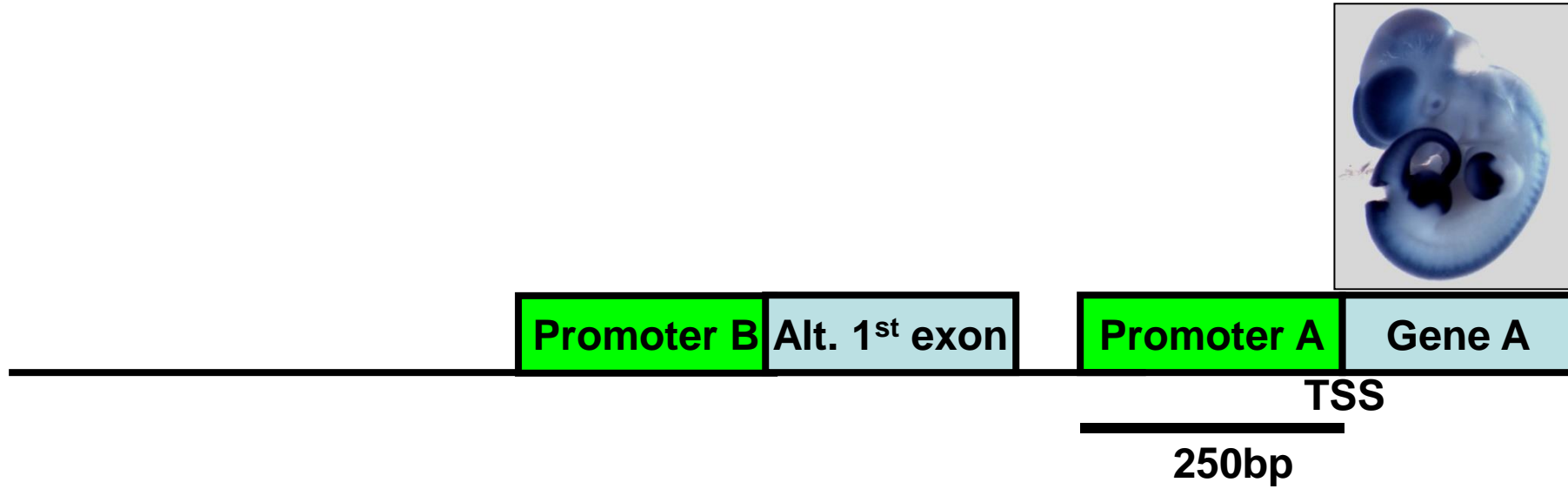




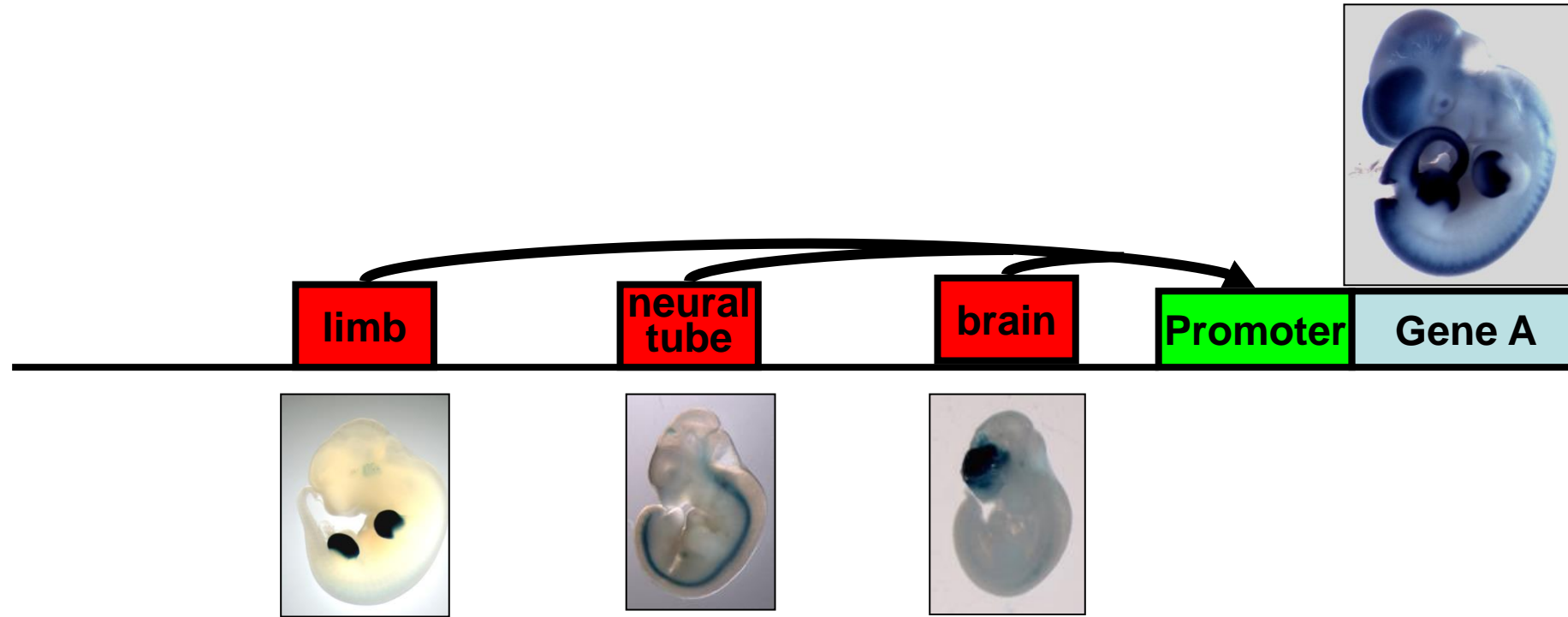




# Promoters

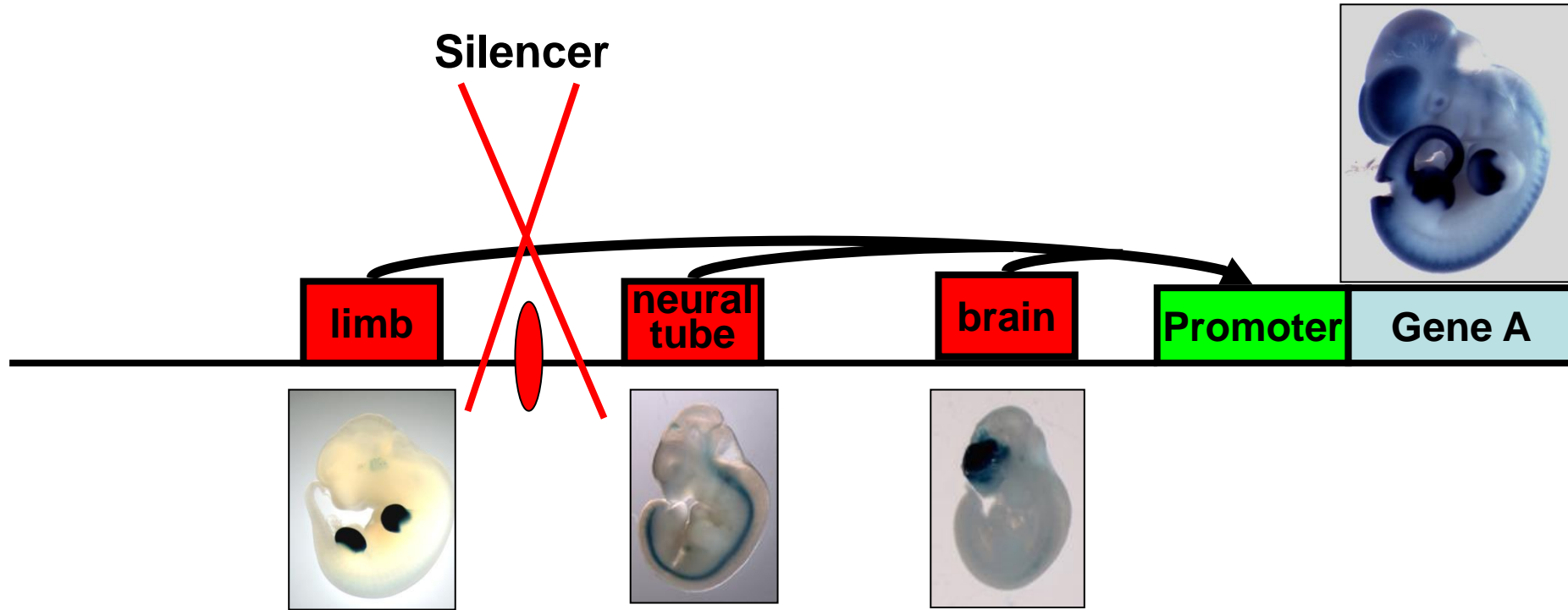


# Enhancers



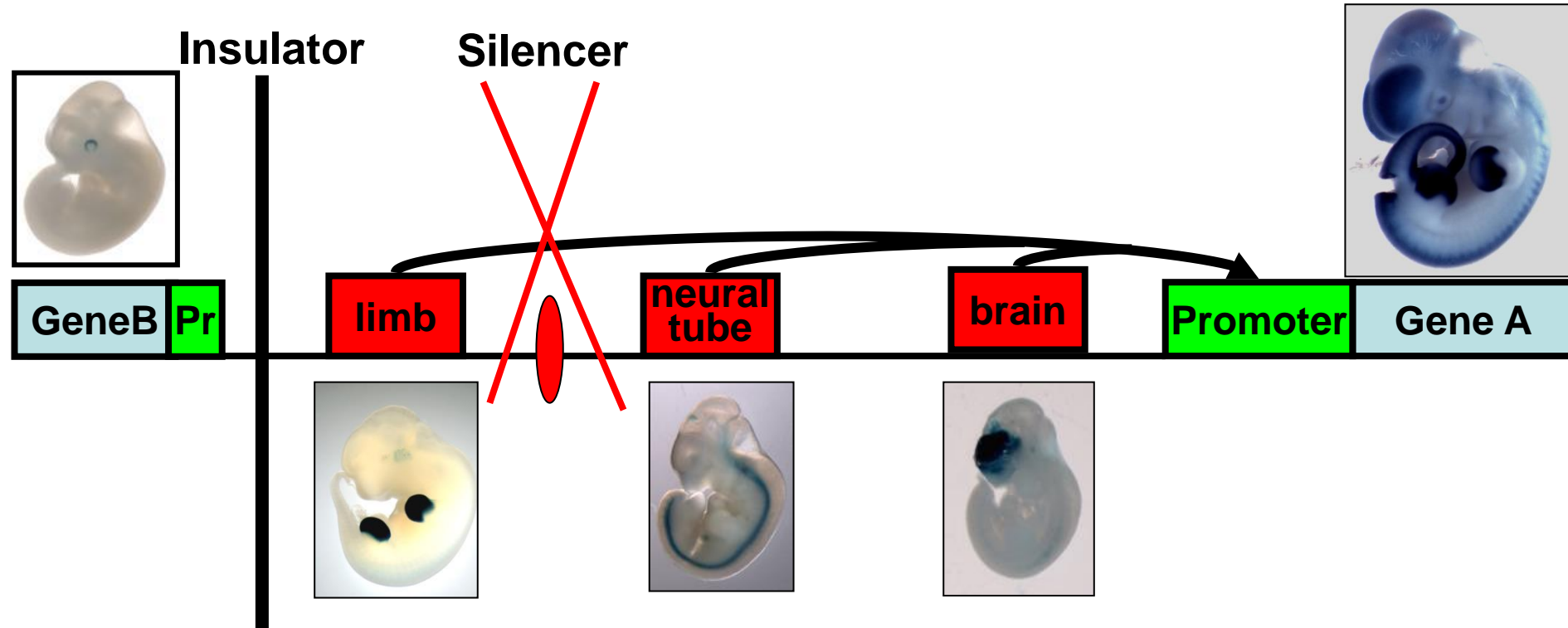
# Enhancers

# Silencers



# Enhancers

# Insulators



# Enhancers



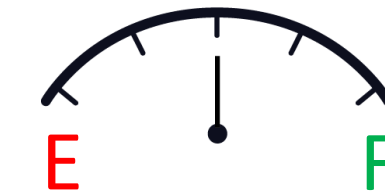
# Haploinsufficiency:

## When one functional copy of a gene is not enough

2 normal  
copies



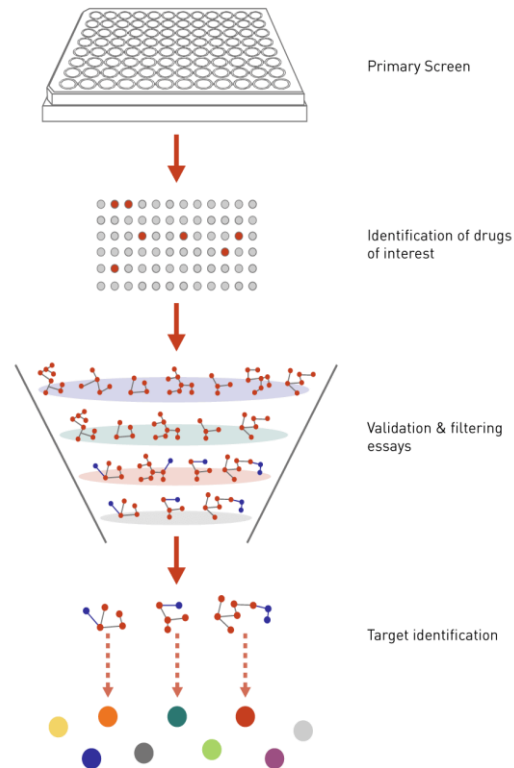
1 normal  
& 1 mutant copy



- Over 660 genes lead to human disease due to haploinsufficiency

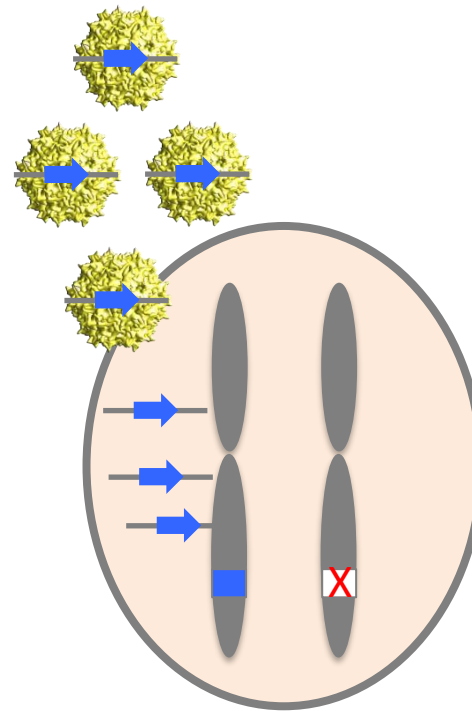
# Current treatments for haploinsufficient diseases

## 'Standard' Drugs

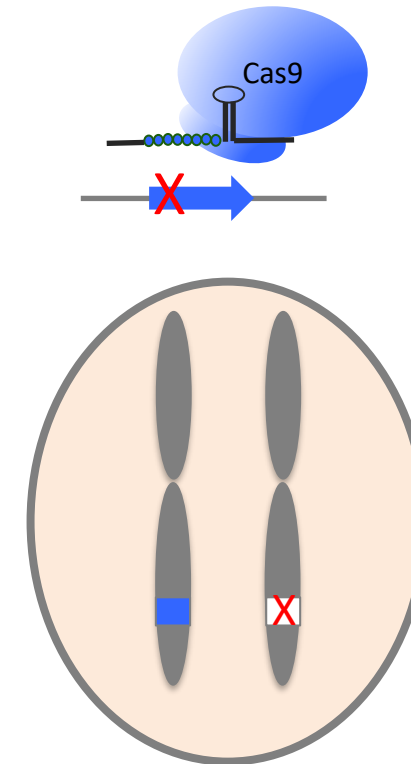


Source: Chemical Genetics and Drug Target Discovery.  
Jay Yang, Singer Instruments, Roadwater, TA23 ORE.

## Gene Therapy



## CRISPR gene editing

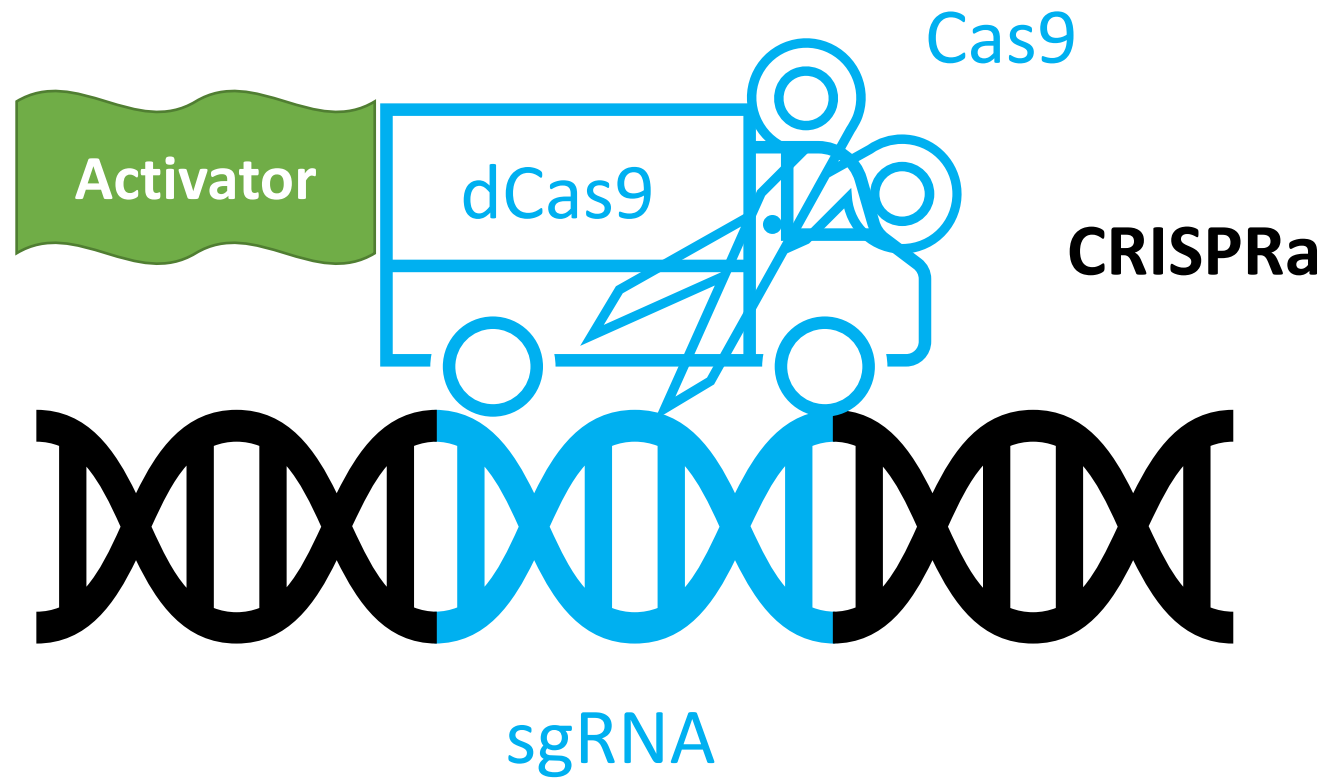


## Limitations

- Time
- Cost
- Primarily to treat symptoms

- Dosage
- Tissue-specificity
- Gene length

- Low HDR frequency
- Custom tailoring





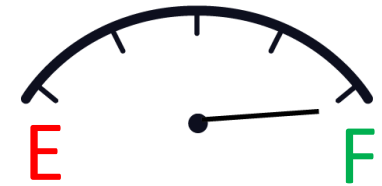
# CRISPRa as a therapeutic for haploinsufficiency?

CRISPRa

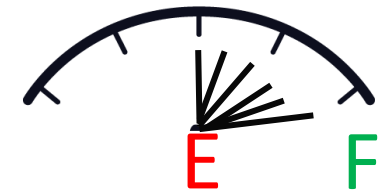
2 normal  
copies



Gene levels

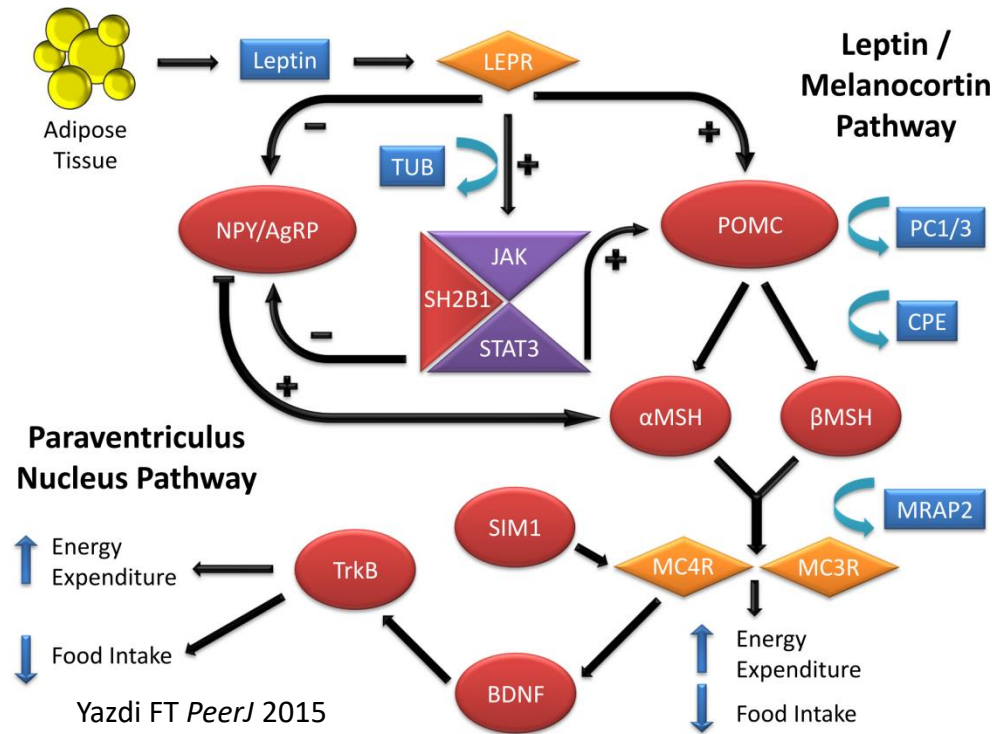


1 normal  
& 1 mutant copy



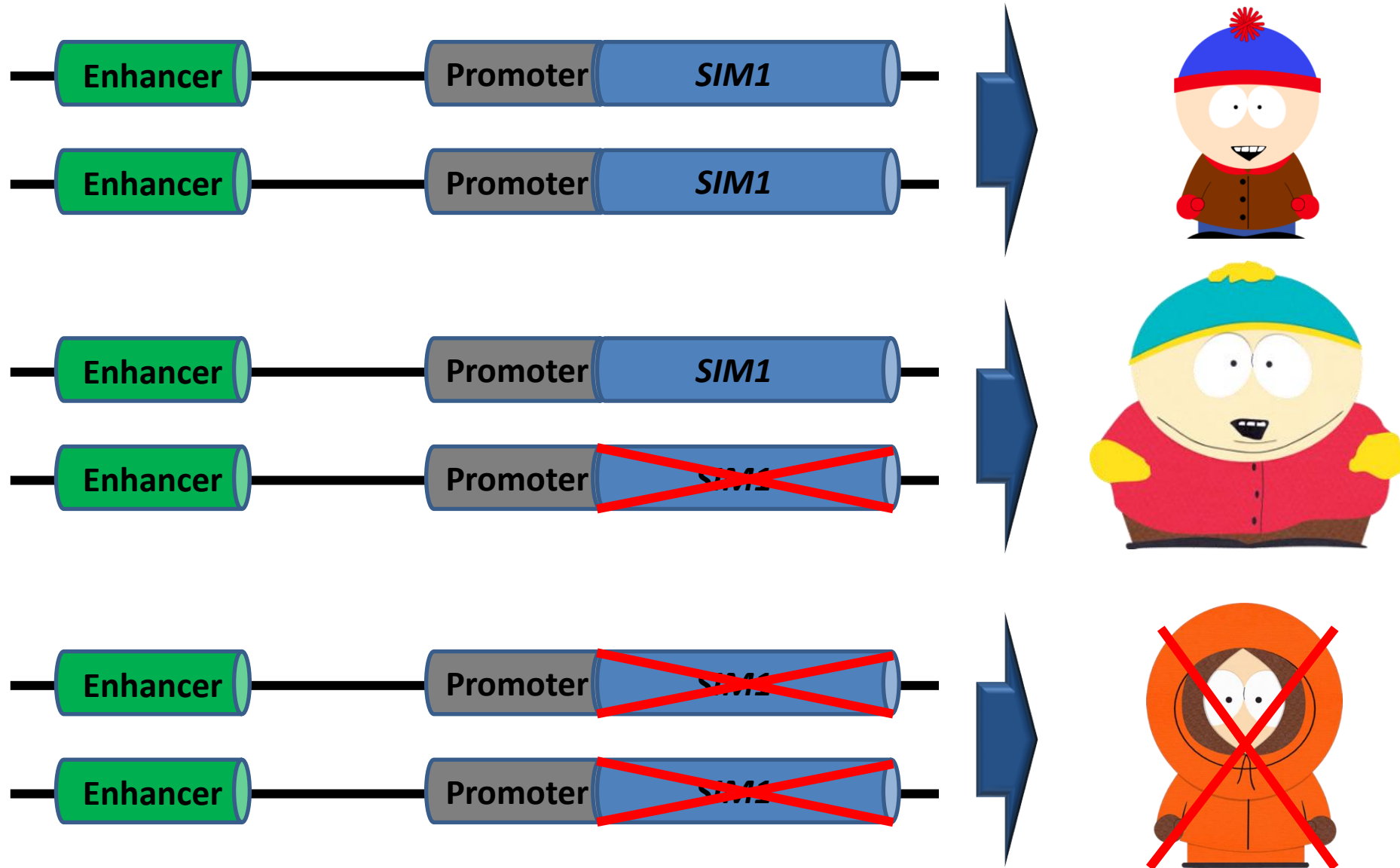
# *SIM1*, an important obesity gene

- Transcription factor that is:
  - Required for hypothalamic development
  - Part of the Leptin pathway and controls food intake



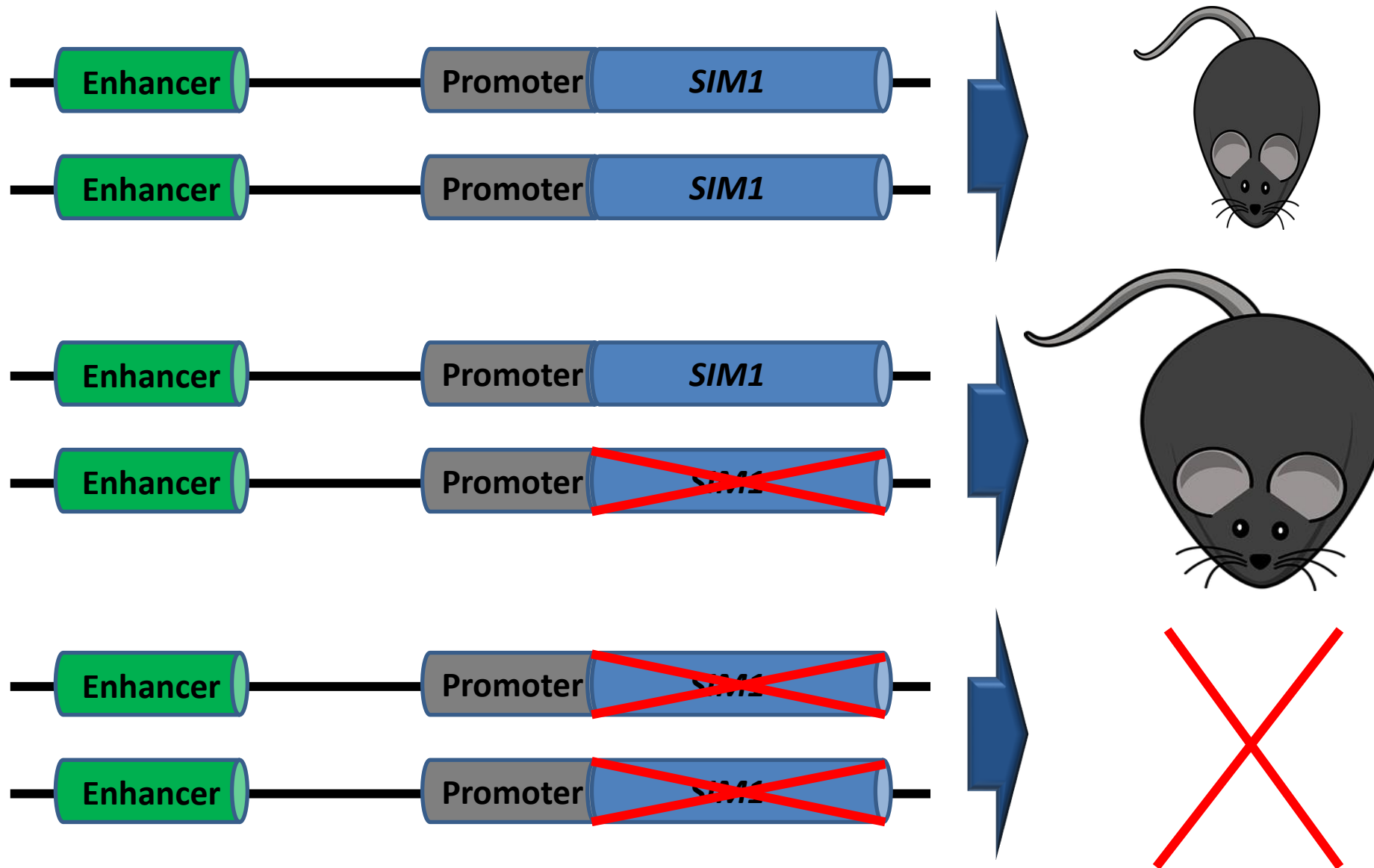
- *SIM1* mutations are the second most prevalent coding mutations found in severely obese individuals.

# *SIM1* haploinsufficiency leads to severe obesity



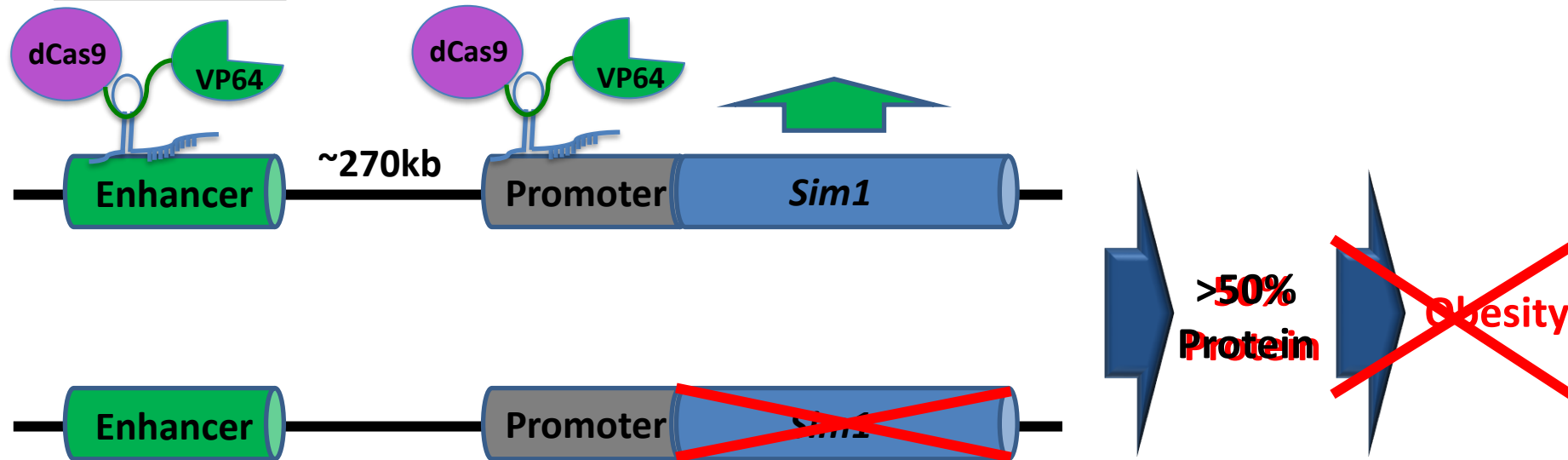


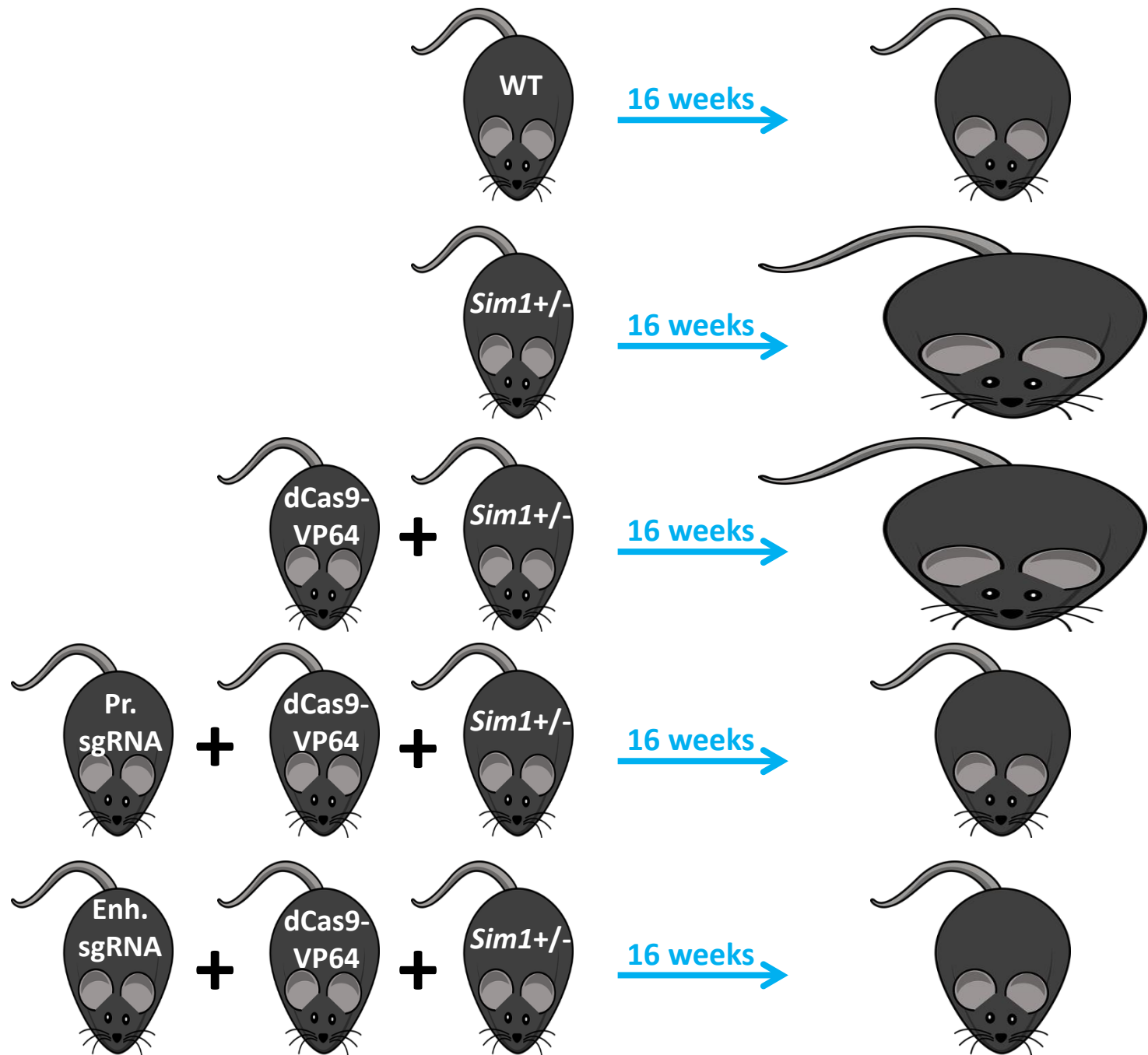
# *Sim1* haploinsufficiency leads to severe obesity



# Can we rescue *Sim1* haploinsufficiency with CRISPRa?

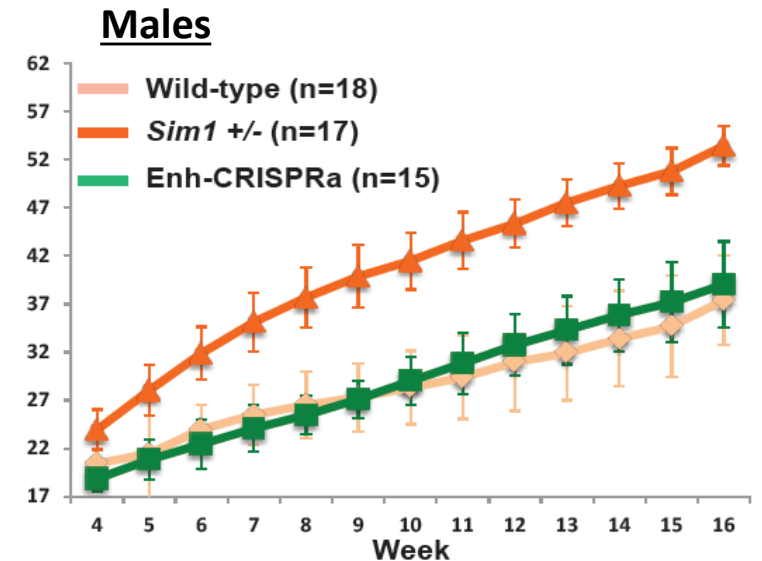
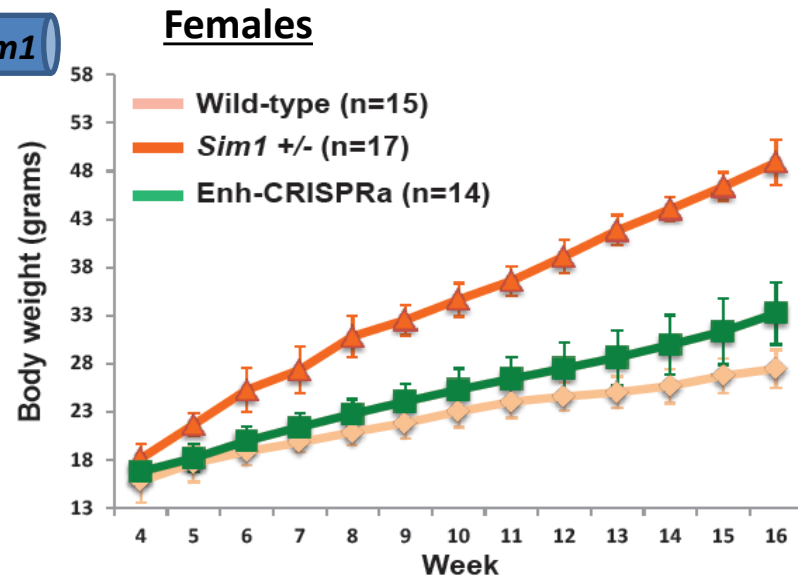
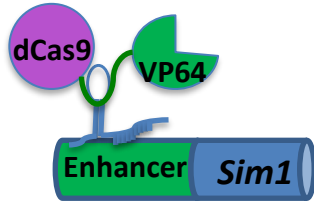
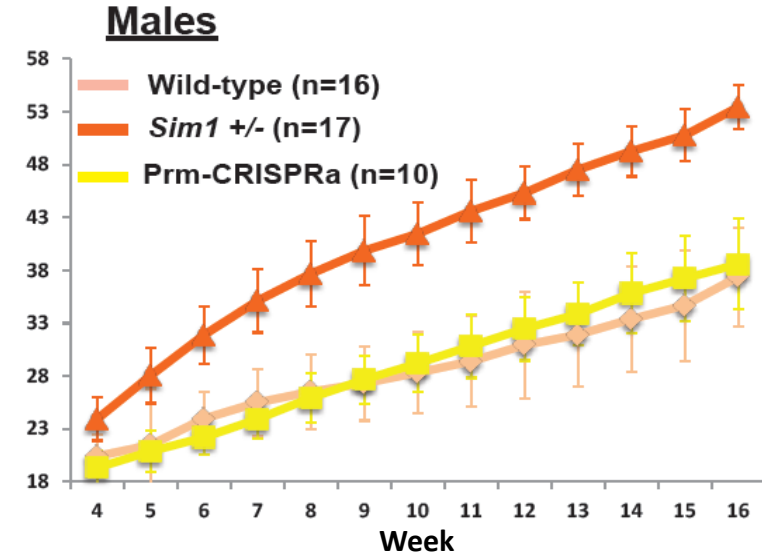
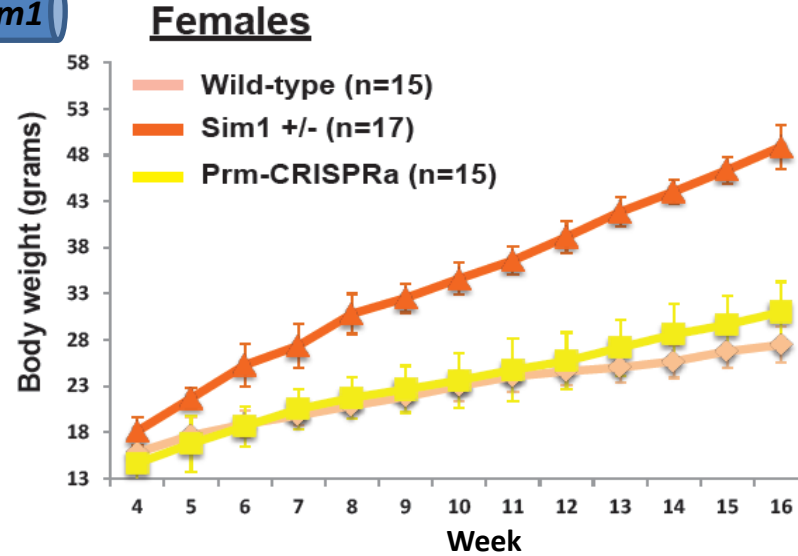
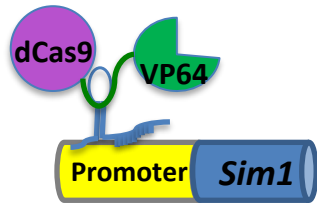
Hypothalamus enhancer  
(Kim M. *HMG* 2013)







# CRISPRa for either *Sim1* promoter or enhancer rescues the obesity phenotype





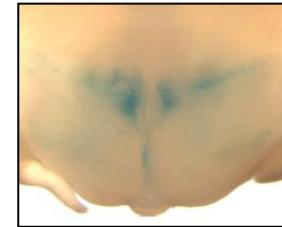
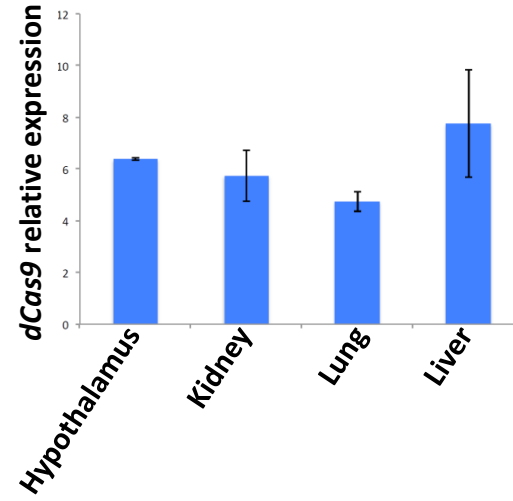
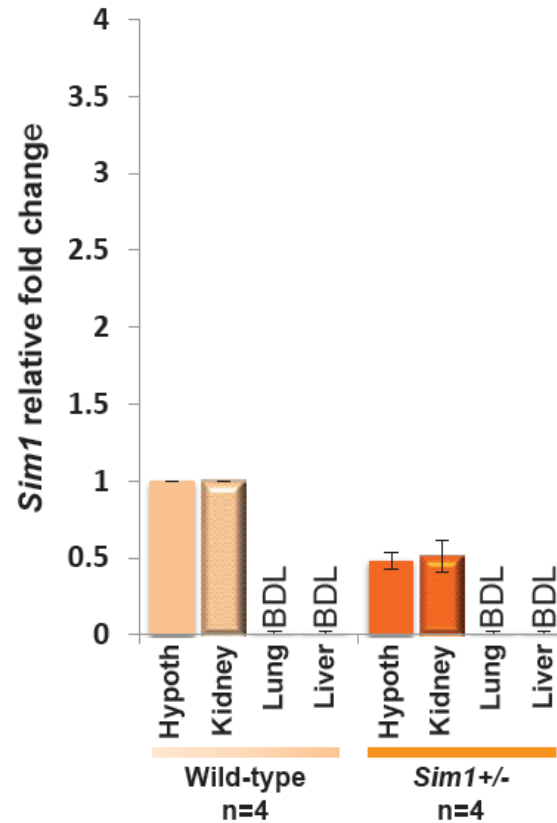
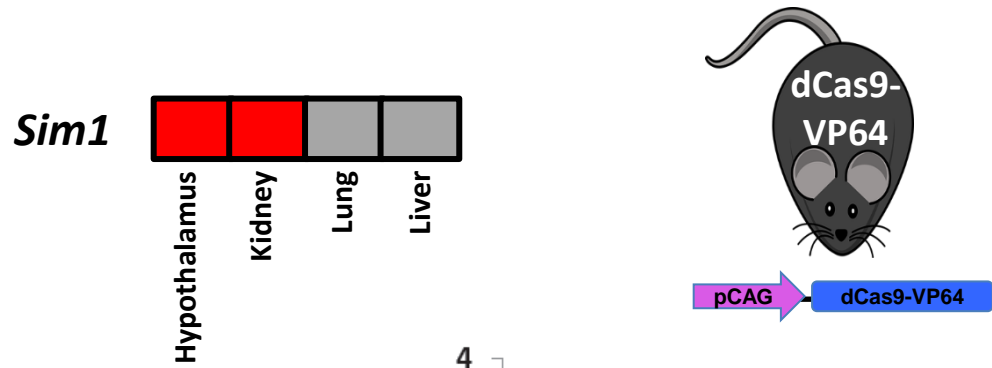
***Sim1 +/-***

***Sim1 +/-*  
**CRISPRa****

**Wild-type**

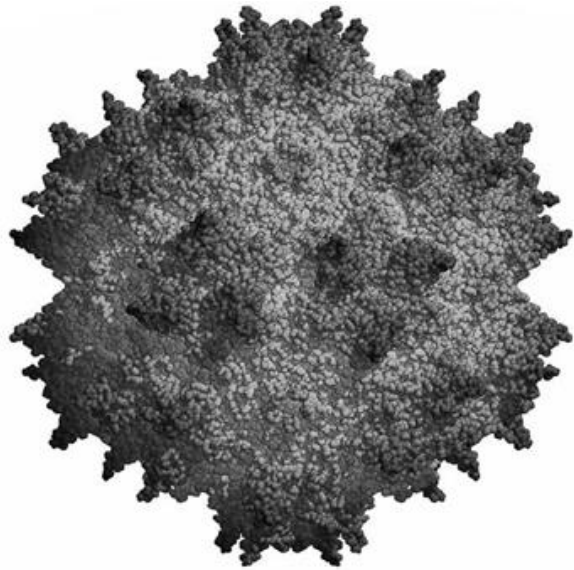
INCHES  
1  
2  
3  
4  
5  
CENTIMETERS  
1  
2  
3  
4  
5  
UNITED STATES  
800.521.2109 • 650.349.1636  
FAX 650.349.3729  
CANADA  
800.665.5355 • 604.980.2481  
FAX 604.987.3299  
EUROPE  
(+49) 62 21 / 90 56  
FAX (+49) 62 21 / 90

# Cis-regulatory elements determine dCas9-VP64 tissue activation

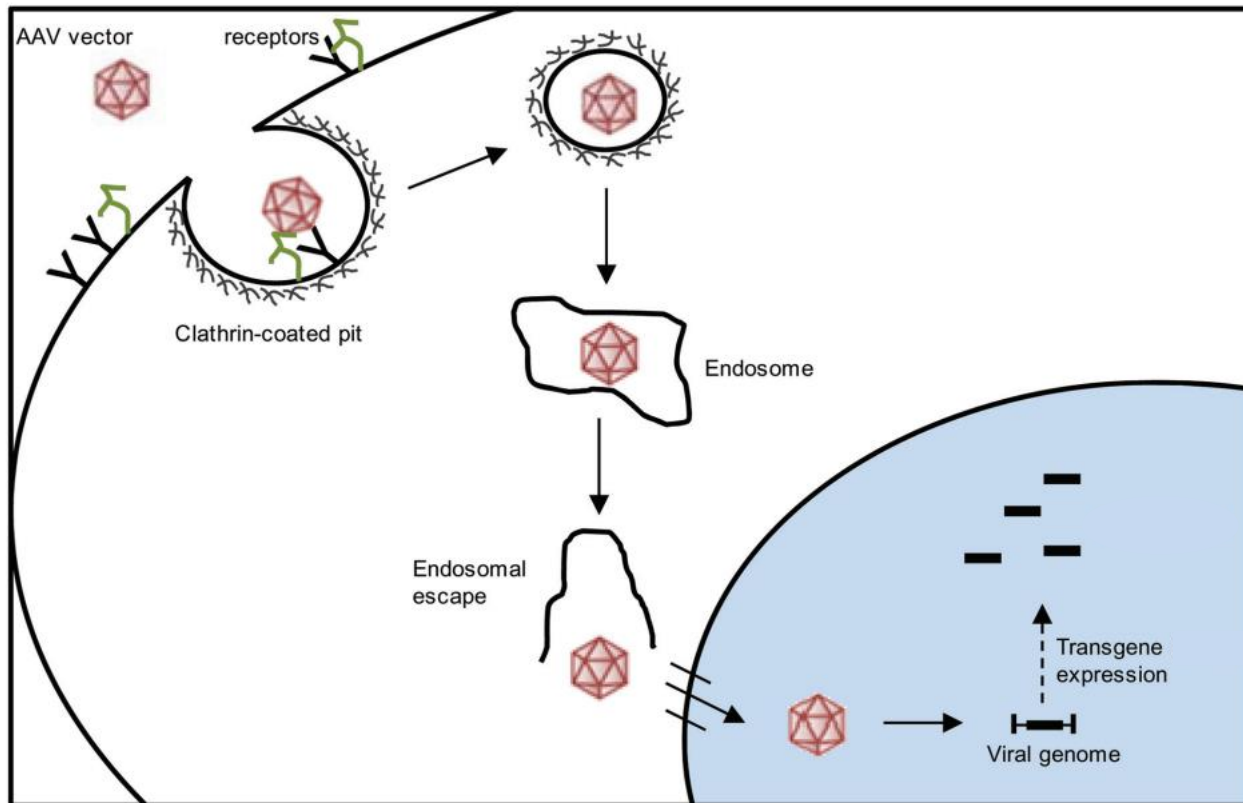


# Can we develop it as a therapeutic?

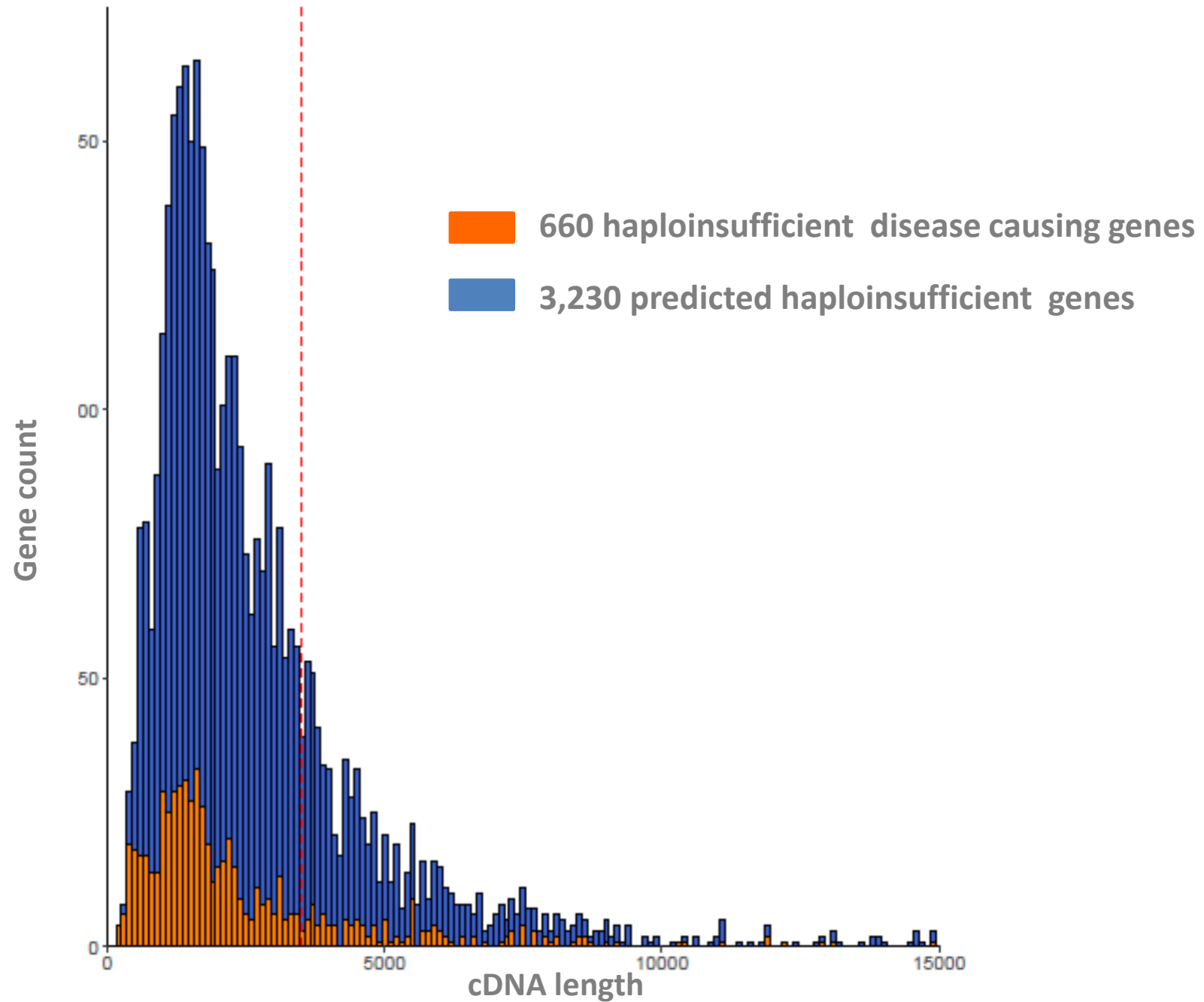
AAV



4.7kb packaging capacity

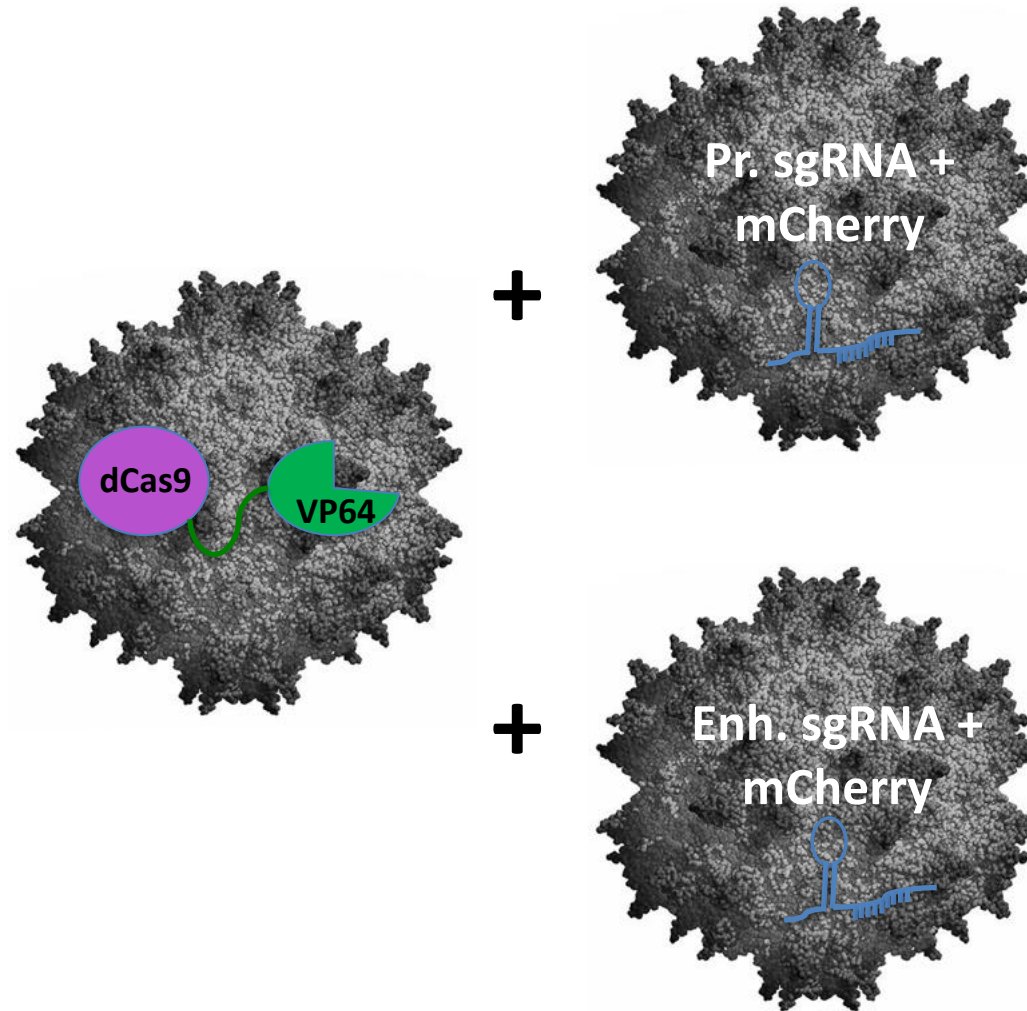


# Many genes cannot get packaged in AAV



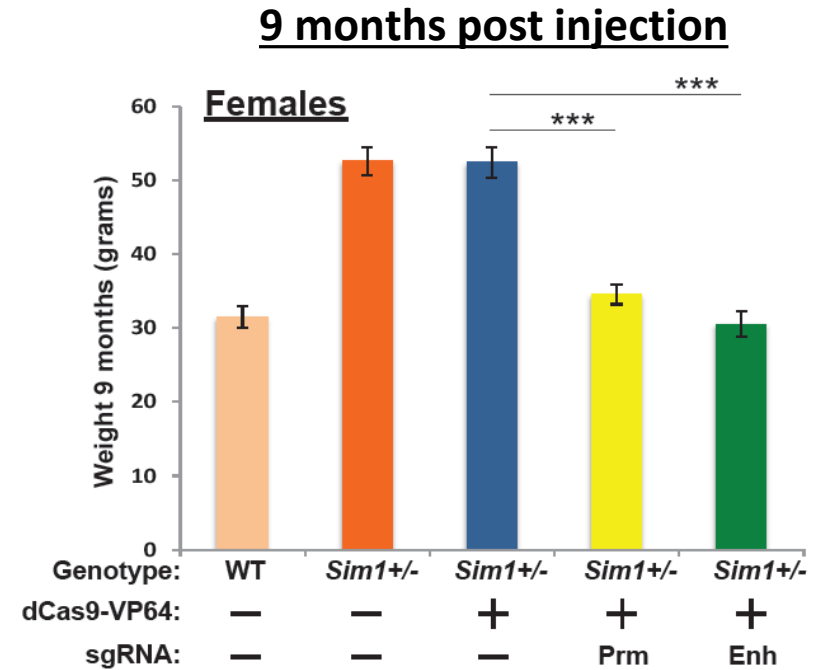
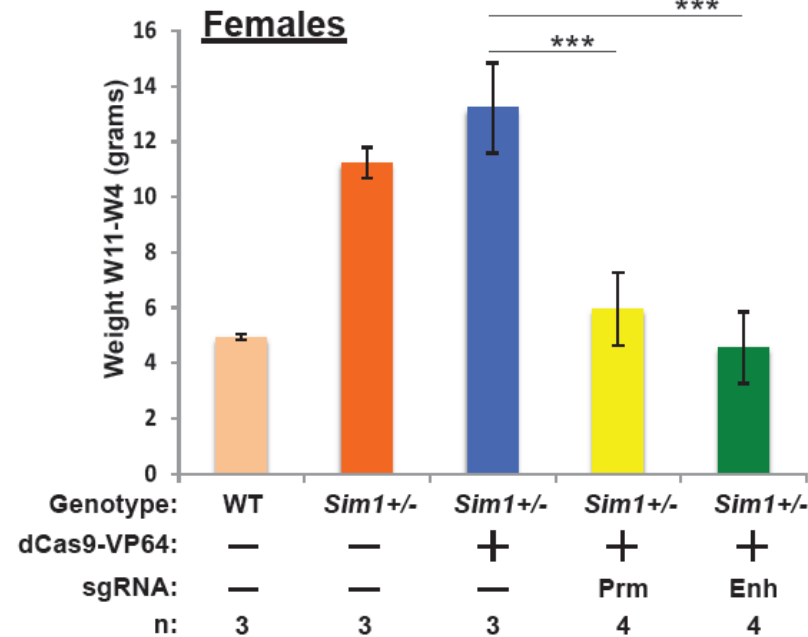
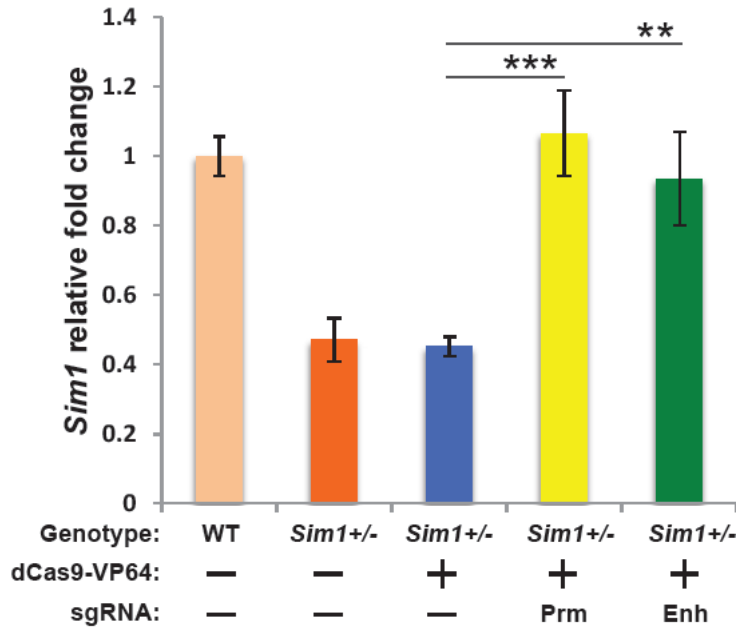
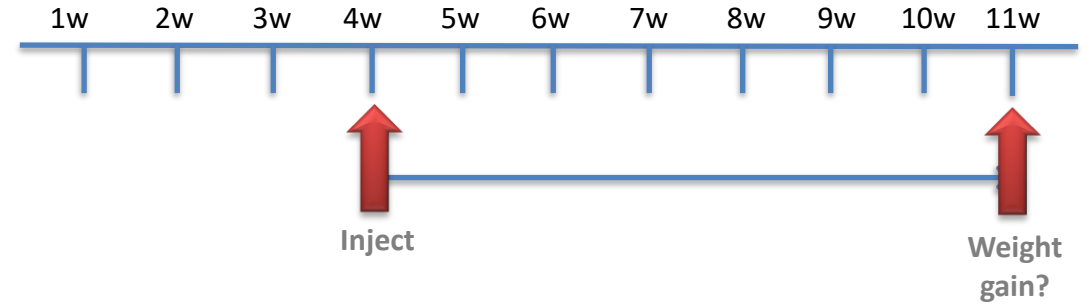
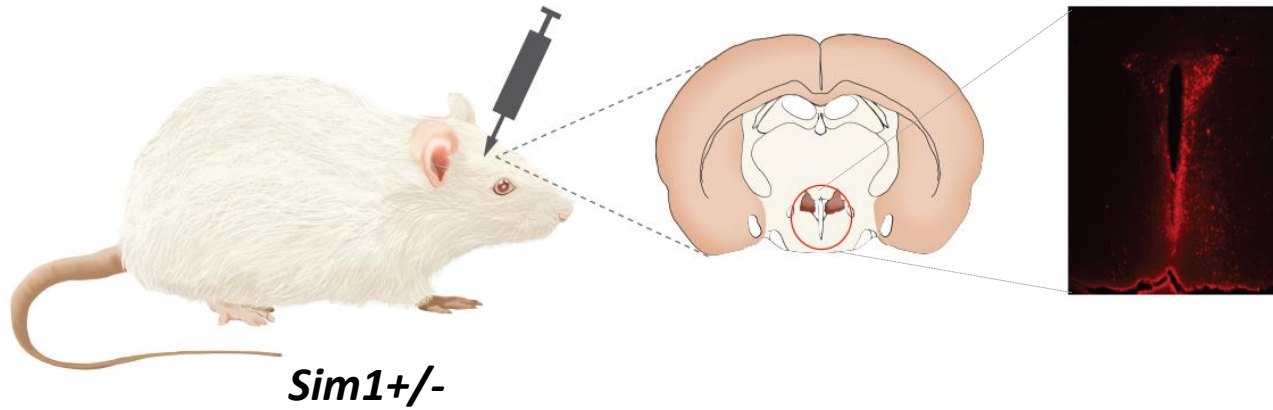


# Generated AAV for dCas9-VP64 and also for either sgRNA (targetting *Sim1* promoter or enhancer)

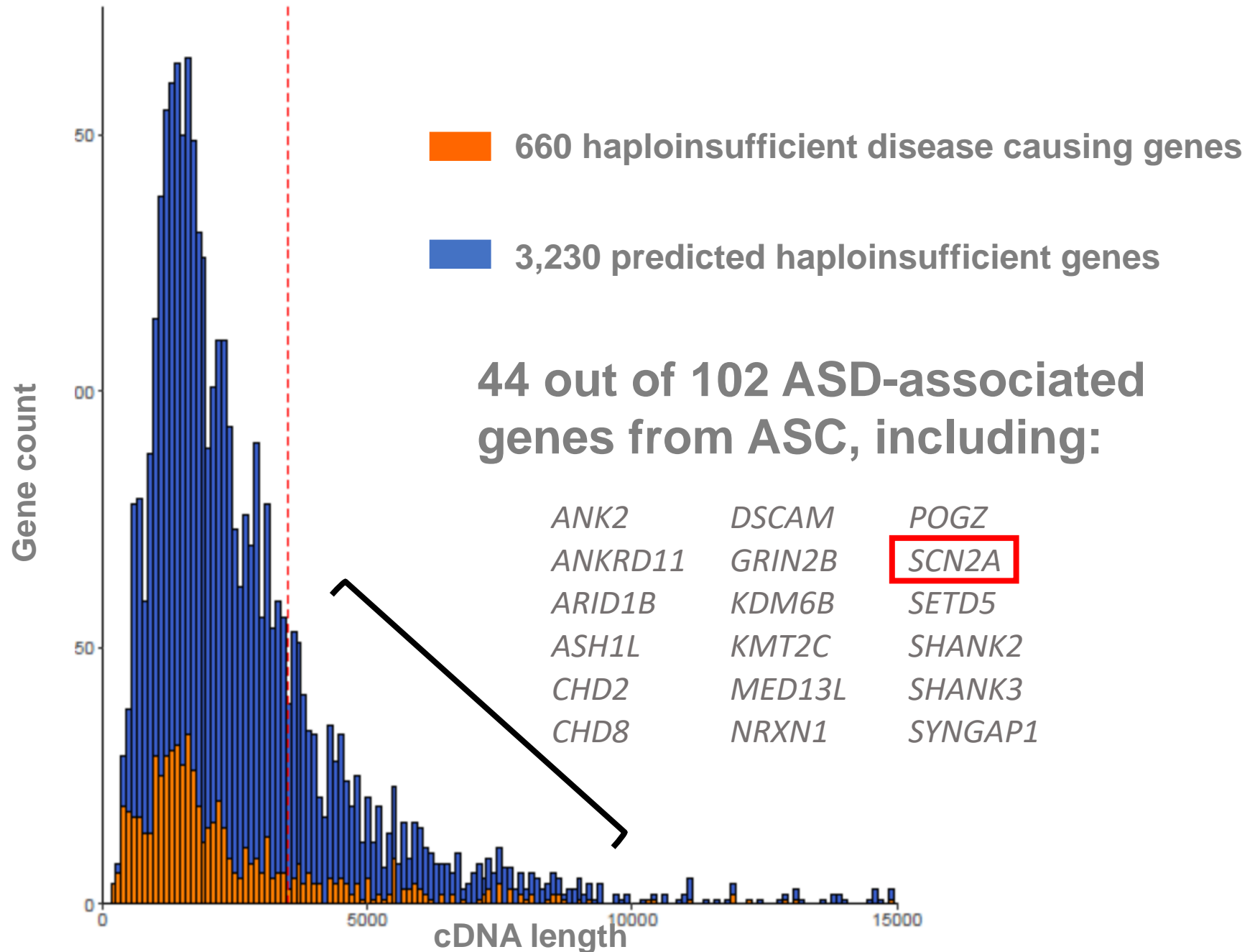


AAV-DJ serotype (chimera of type 2, 8 and 9)

# AAV CRISPRa hypothalamus stereotactic injections reduce food intake



# Many neurodevelopmental genes are too long to fit into an AAV



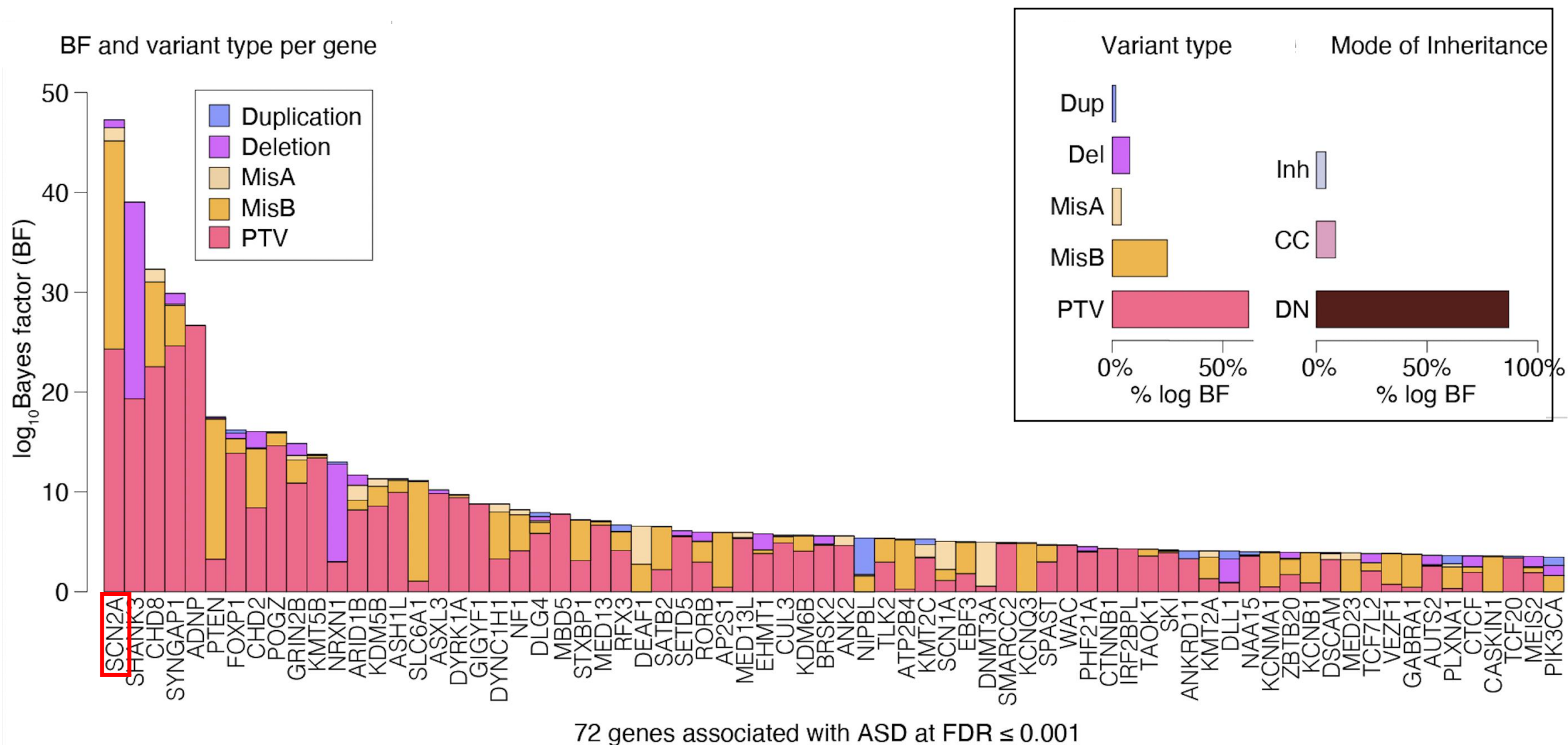


Perry  
Spratt



Serena  
Tamura

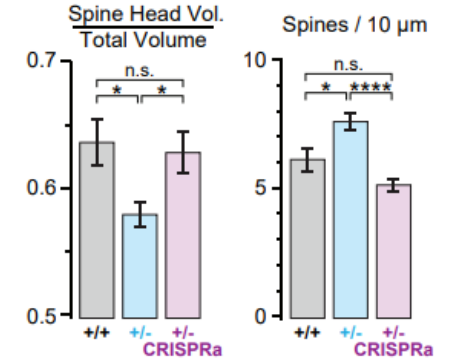
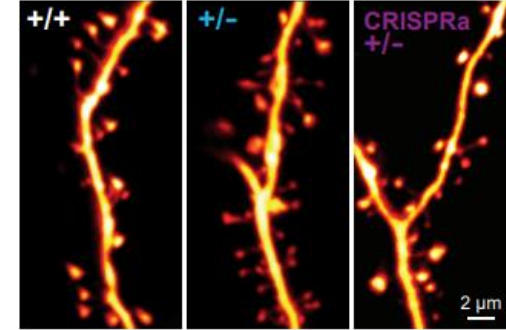
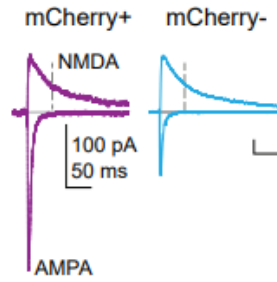
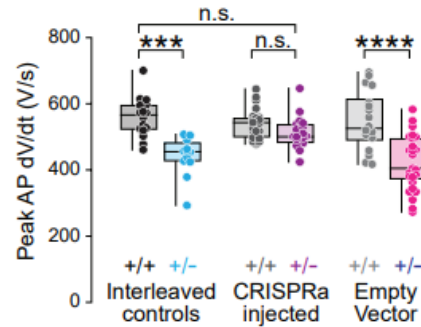
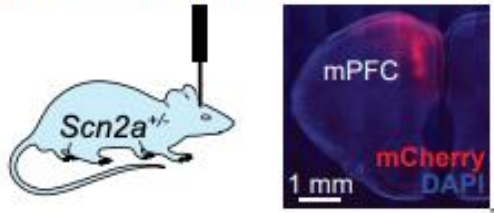
# SCN2A is the top associated gene with ASD



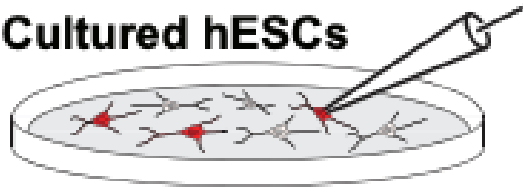


# Scn2a CRISPRa rescues synaptic deficit in mature mice

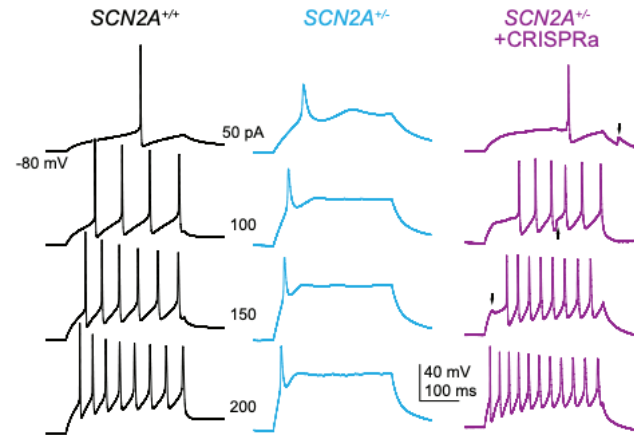
Intracranial CRISPRa delivery

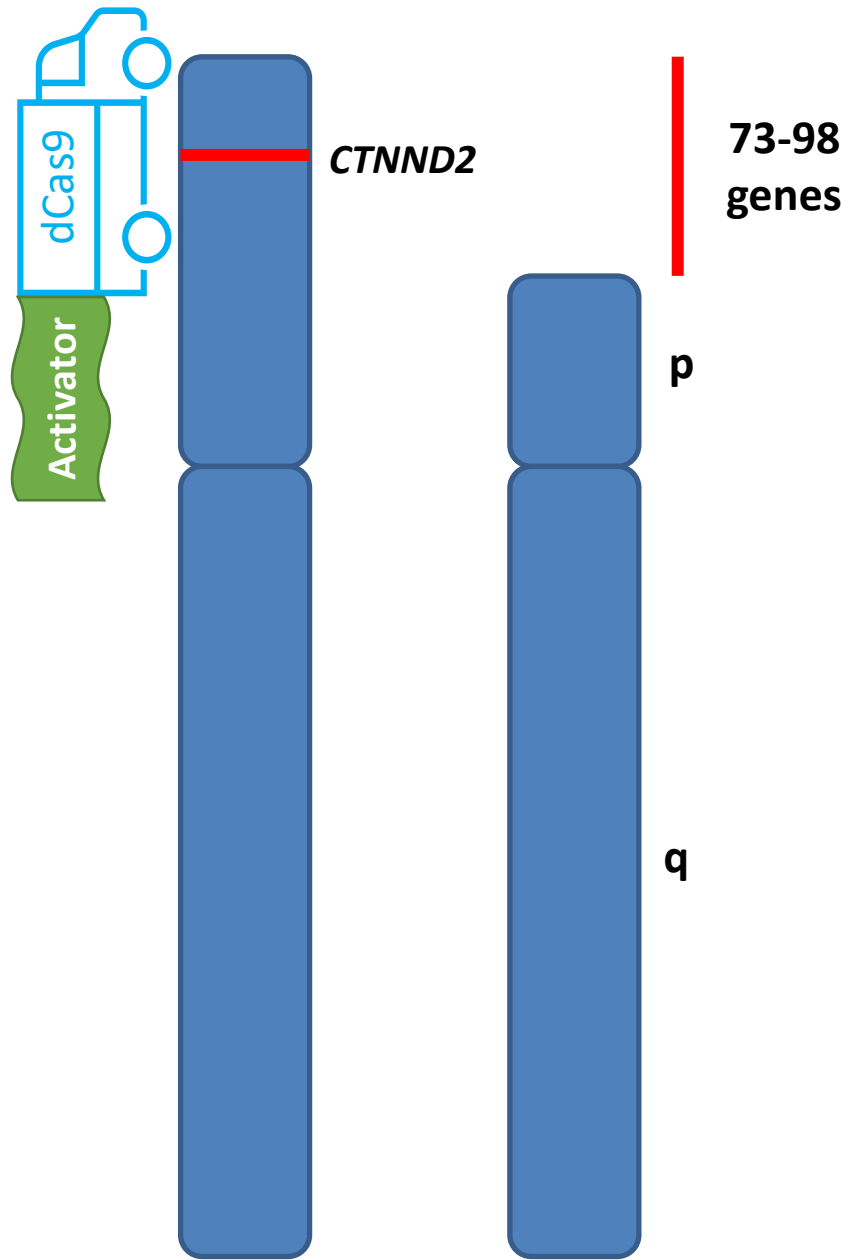


Cultured hESCs



CRISPRa





Chromosome 5

Coline Arnould  
Andrew Blair  
Rachael Bradley  
Candace Chan  
Chengyu Deng  
Ryder Easterlin  
Sarah Fong  
Ilias Georgakopoulos-Soares  
Yarden Golan-Maor  
Wei Gordon  
Yelena Guttman  
Lana Harshman  
Dianne Laboy Cintron  
Weiyu Li  
Navneet Matharu  
Elizabeth Murray  
Hai Nguyen  
Mai Nobuhara  
Nick Page  
Rory Sheng  
Jasmine Sims  
Serena Tamura  
Aki Ushiki  
Ofer Yizhar-Barnea  
Jingjing Zhao  
Xujia Zhou

**UCSF**

Kevin Bender  
Tom Nowakowski  
Katie Pollard  
Alex Pollen  
Jennifer Rosenbluth  
Stephan Sanders  
Christian Vaisse

**Harvard**

Martin Hemberg

**UW**

Vikram Agarwal  
Florence Chardon  
Jason Klein  
Jean-Benoit Lalanne  
Beth Martin  
Troy McDiarmid  
Samuel Regalado  
Jay Shendure

**Berlin Institute of Health**

Martin Kircher  
Max Schubach

**Postdoc positions available**

Funding: NHGRI, NCI, NIMH, NICHD, NHLBI, NIDDK, SFARI, UCSF, BioMarin, Weill Neurohub, Cri Du Chat foundation