Dr. Navneet Matharu – University of California San Francisco, USA

1

Modulating Gene Expression to Treat Diseases

Dr. Navneet Matharu, PhD
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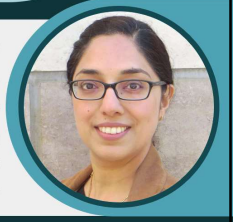
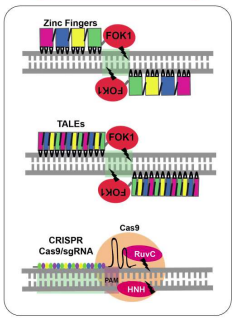


2

Programmable DNA targeting modules

Editors



Zinc finger nucleases
- C2H2 zinc finger domains
- Fusing FOK1 restriction enzyme

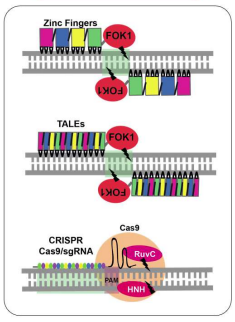
TALENs
- TAL effector DNA binding domains
- Fusing FOK1 restriction enzyme

CRISPR system
- A ribonucleo-complex where target recognition is guided by sgRNA
- Contains 2 nuclease domains that can create a double stranded break

Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

Programmable DNA targeting modules

Editors



- These editors can be programmed into nuclease deficient versions (DNA targeting modules)

Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

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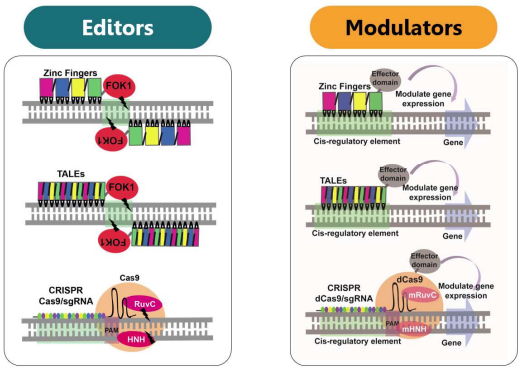
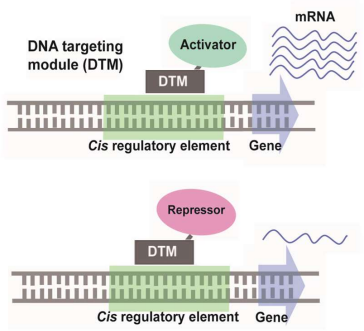
Programmable DNA targeting modules



Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

3

Effector domains: transcription modulators

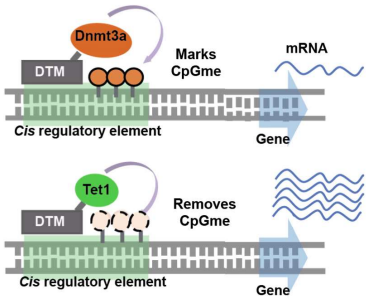


Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

4

Fusions with effector domains

DNA methylation modulators



Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

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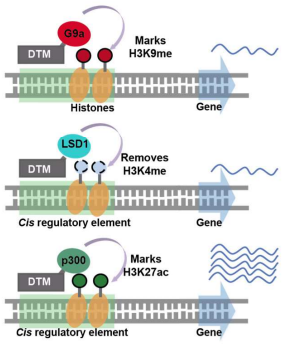
Fusions with effector domains

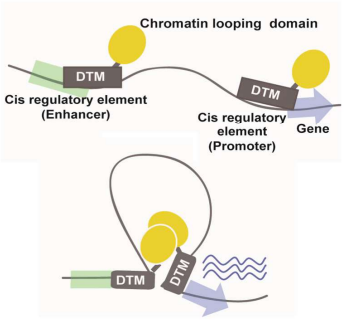
Histone PTM modulators



Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

Fusions with effector domains

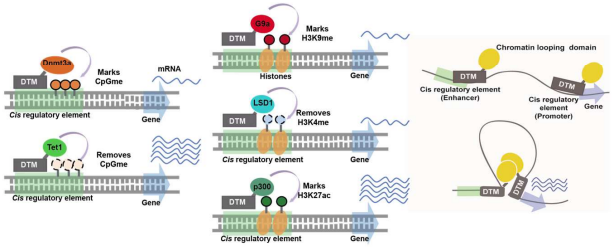
Chromatin looping modulators



Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

Fusions with effector domains

DNA methylation modulators | **Histone PTM modulators** | **Chromatin looping modulators**



Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

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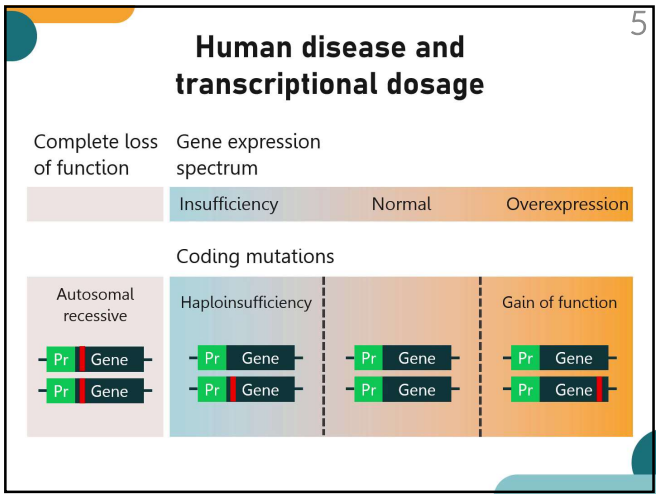

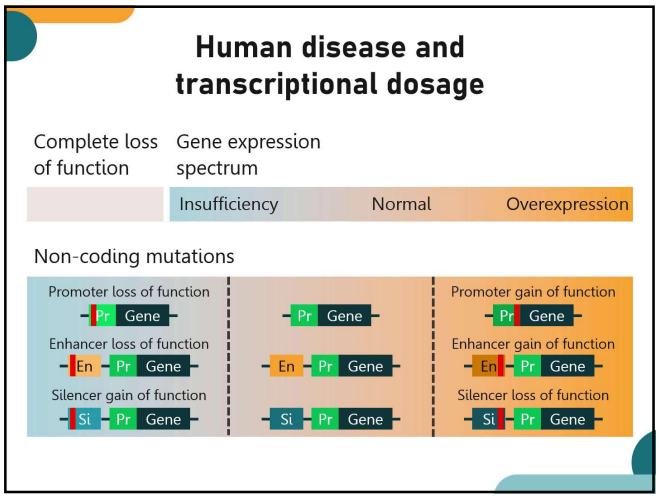

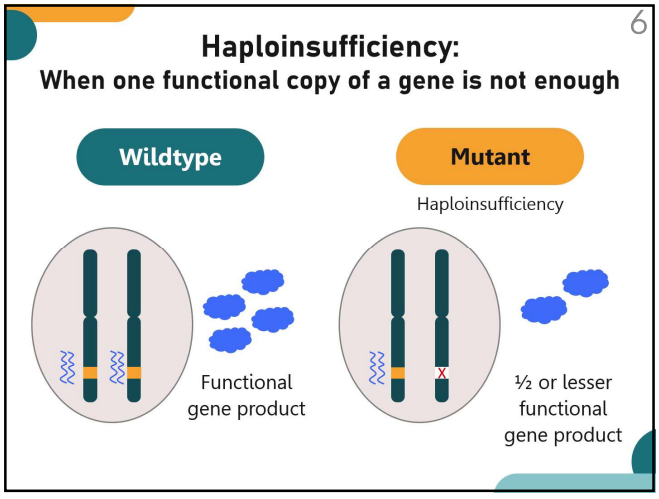

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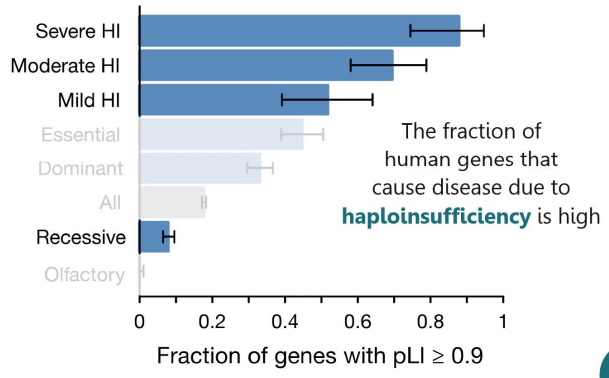
7

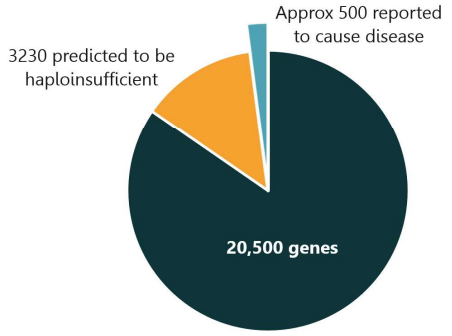
Zygosity of pathogenic genes



Lek M. *et al., Nature.* 2016; **536**(7616):285-91

8

Around 15% of our genes could be haploinsufficient

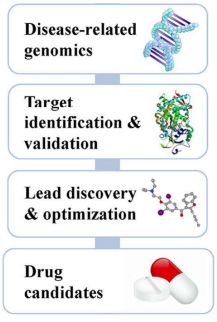


Based on data from Lek M. *et al., Nature.* 2016; **536**(7616):285-91

9

Treatments for haploinsufficient disease

Chemical drug screens



- Drug target screening is a lengthy process
- Lower efficacy

Wu F. *et al., Front. Chem.* 2020; **8**:726

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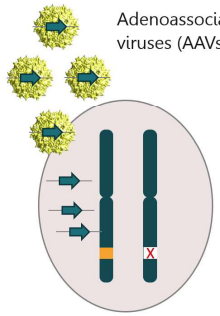
Treatments for haploinsufficient disease

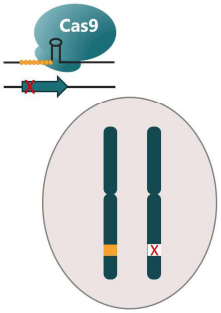
Gene therapy



- Ectopic expression
- Dosage
- Tissue specificity

Treatments for haploinsufficient disease

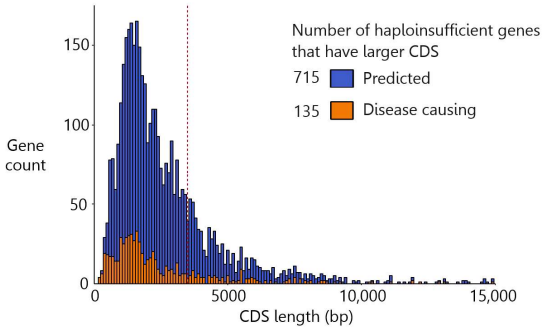
CRISPR editing



- Low HDR frequency especially in CNS tissue

10

AAV vectors have a 4.7Kb limit on cargo



Many haploinsufficient genes are **too big to be packaged into AAV**

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

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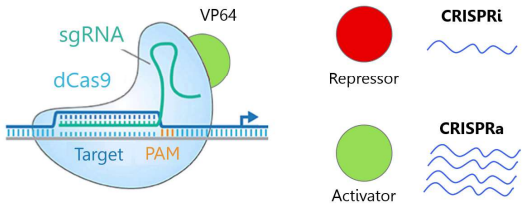
11

Transcription modulation-dCas9-fusions

Non-editing CRISPR that can target a specific genome location but cannot cut the DNA

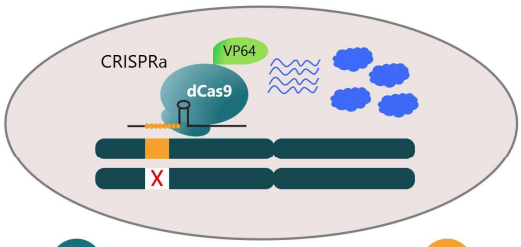


CRISPR interference or CRISPR activation system

Duke CG. *et al, Front Genome Ed.* 2020; **2**:9
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

12

Can CRISPRa correct haploinsufficiency?



01 Gene dosage rescue?

Neuroendocrine obesity

02 Disease rescue?

13

Haploinsufficiency of *SIM1* leads to obesity

SIM1 single-minded family bHLH transcription factor 1

Bonnefond A. *et al., J Clin Invest.* 2013; **123**(7):3037-3041

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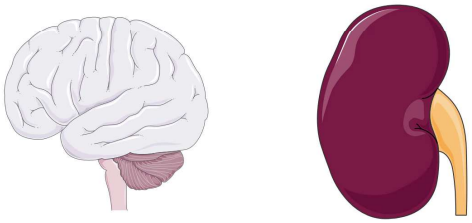
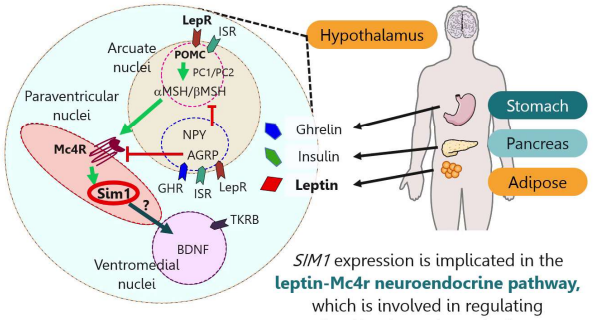
14

SIM1 is downstream of the Leptin-Mc4r pathway

SIM1 is a transcription factor expressed mainly in **brain** and **kidney**



SIM1 is downstream of the Leptin-Mc4r pathway

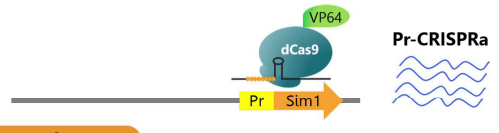


SIM1 expression is implicated in the **leptin-Mc4r neuroendocrine pathway,** which is involved in regulating food intake and metabolism

15

CRISPRa targeting to rescue *Sim1* obesity

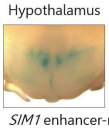
Target promoter



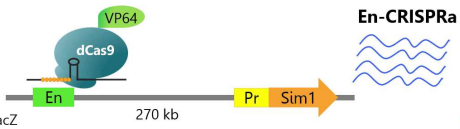
Pr-CRISPRa

Target enhancer

PVN Hypothalamus



SIM1 enhancer-mP-LacZ



En-CRISPRa

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

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CRISPRa targeting to rescue *Sim1* obesity

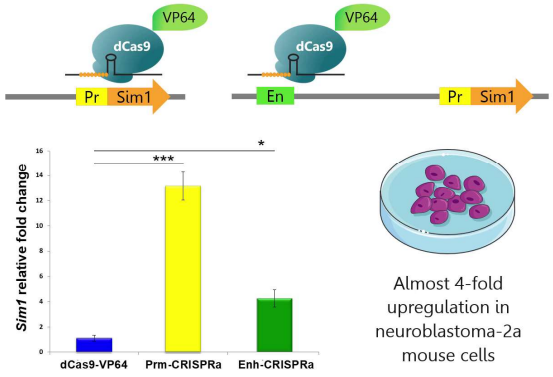
01 *In vitro*

02 Transgenic studies

03 Postnatal studies

16

CRISPRa upregulates *Sim1 in vitro*

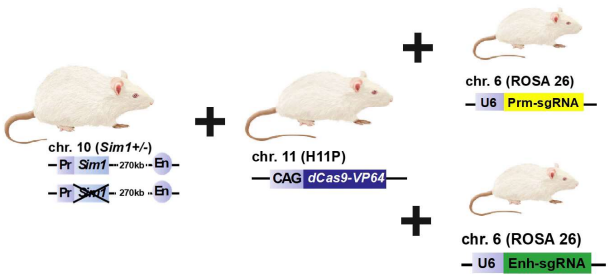


Almost 4-fold upregulation in neuroblastoma-2a mouse cells

Matharu N. *et al*, *Science*. 2019; **363**(6424):eaau0629

17

Sim1 CRISPRa upregulation strategy: *in vivo*



Matharu N. *et al*, *Science*. 2019; **363**(6424):eaau0629

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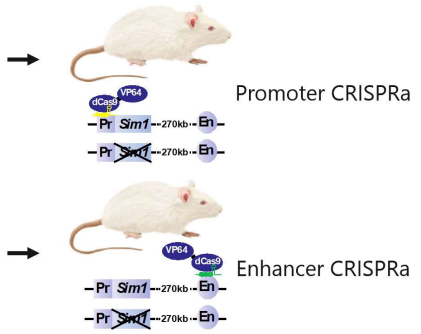
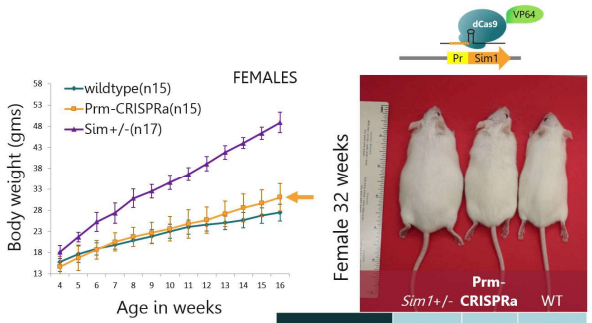
Sim1 CRISPRa upregulation strategy: *in vivo*



Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

18

Sim1 promoter CRISPRa rescues obesity

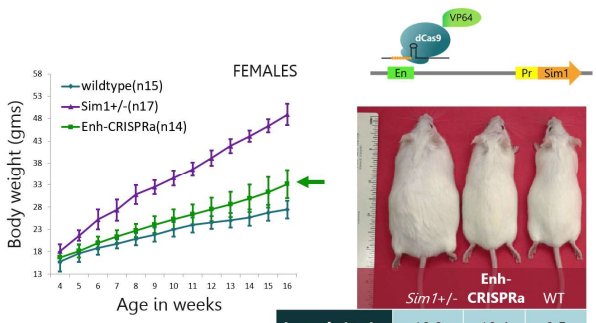


Length (cm)	10.5	9.8	9.5
Weight (g)	61.3	38.6	36.2

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

19

Sim1 enhancer CRISPRa rescues obesity



Length (cm)	10.8	10.4	9.5
Weight (g)	63.5	37.4	37.0

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

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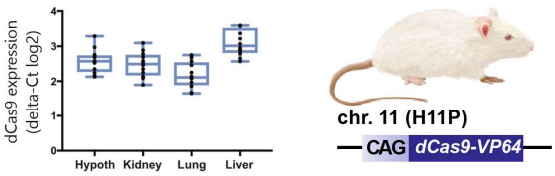
20

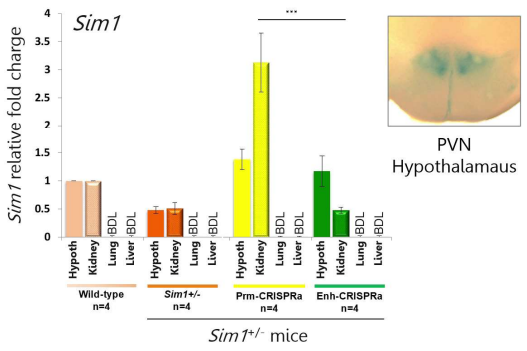
Sim1 CRISPRa targeting can define tissue specificity



We made dCas9-VD64 cross gene under a ubiquitous protomer

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

Sim1 CRISPRa targeting can define tissue specificity

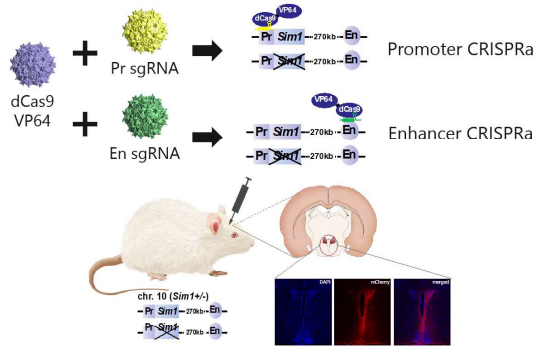


PVN
Hypothalamaus

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

21

CRISPRa-AAV upregulates *Sim1* in the hypothalamus



Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

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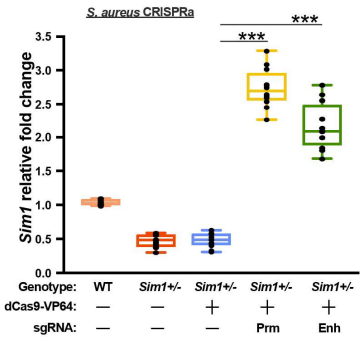
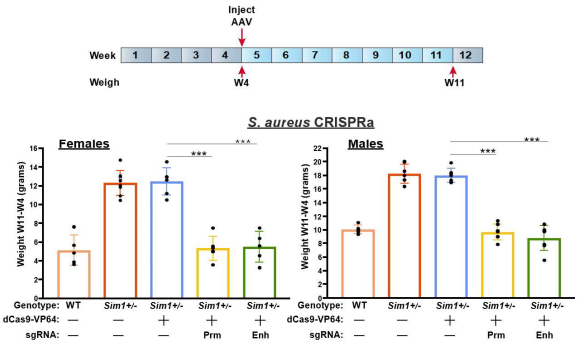
CRISPRa-AAV upregulates *Sim1* in the hypothalamus



Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

22

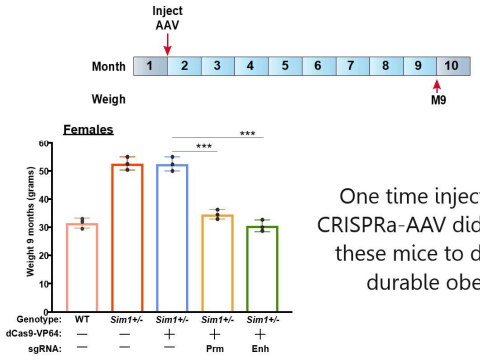
Postnatal rescue of neuroendocrine obesity



Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

23

Sim1-CRISPRa-AAVs have long lasting effect



One time injection of CRISPRa-AAV didn't allow these mice to develop durable obesity

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

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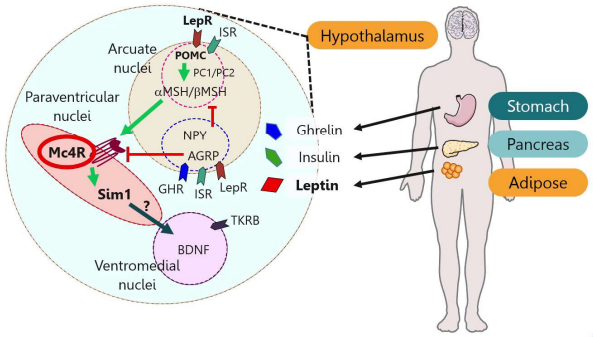
24

Can CRISPRa strategy work for other haploinsufficient genes?

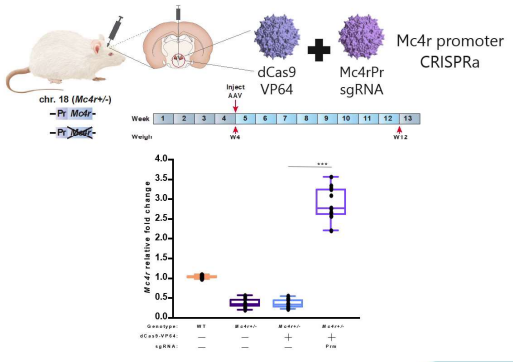
25

Leptin-Mc4r pathway



26

CRISPRa-AAV rescues Mc4r haploinsufficiency



Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

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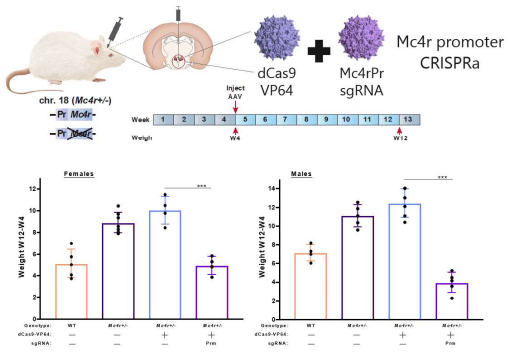
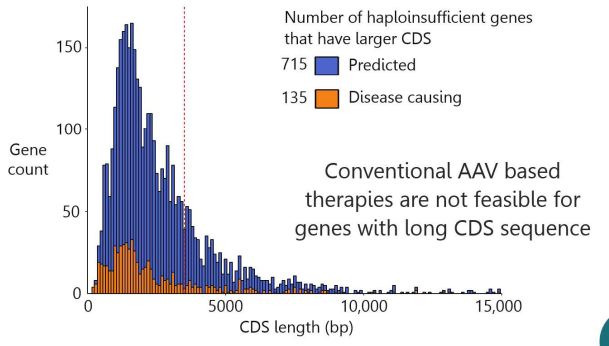
CRISPRa-AAV rescues Mc4r haploinsufficiency



Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

27

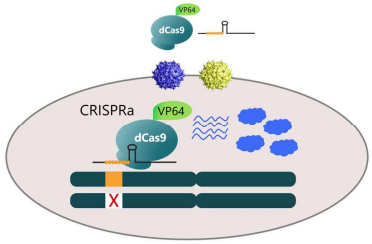
Overcoming size limitation of AAV gene therapy



Conventional AAV based therapies are not feasible for genes with long CDS sequence

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

Overcoming size limitation of AAV gene therapy



CRISPRa overcomes this limitation as it can target any gene, regardless of its size

Matharu N. *et al., Science.* 2019; **363**(6424):eaau0629

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28

Summary

01 *Cis*-regulatory elements as therapeutic targets to correct haploinsufficieny

02 Targeting these elements can determine tissue specificity in tissues only where they are active

03 Non-editing CRISPR can be developed for therapeutic applications

29

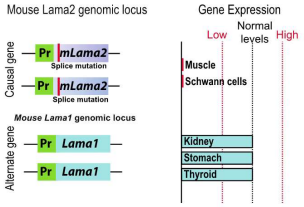
Loss of function of *Lama2*-MDC1A

Complete loss of function

Gene expression spectrum

Insufficiency | Normal | Overexpression

Gene involved in congenital muscular dystrophy type 1



- The cDNA sequence of this gene exceeds the packaging capacity of an AAV
- A traditional gene therapy approach wouldn't be viable in this case

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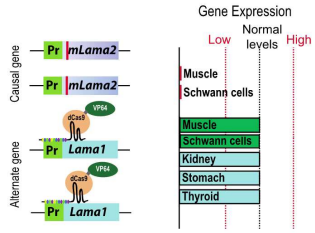
30

CRISPRa targeting alternative/redundant gene



Degenerating muscle fibre

Degenerating peripheral nerves

Muscle fibrosis

- Delivery vehicle, AAV
- Route of administration, temporal vein @ P2
- *Lama1* expression in muscle and schwann cells has been shown to compensate for the loss of the *Lama2* gene

Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

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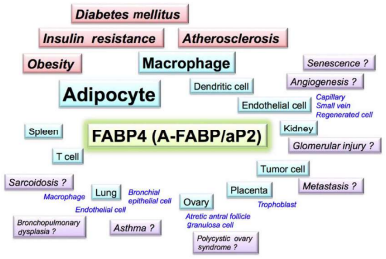
31

FABP4 expression is linked to many diseases

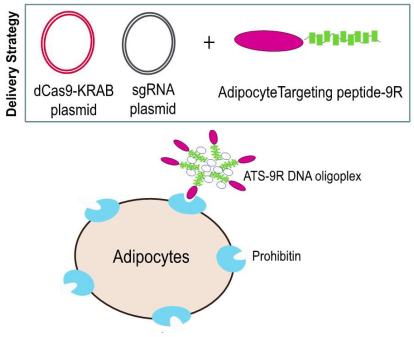


Potential association of FABP4 in several pathological conditions - FABP4 (A-FABP/aP2) is expressed not only in adipocytes and macrophages but also in several types of tissues and cells under physiological and pathophysiological conditions and may contribute to several aspects of metabolic and cardiovascular diseases as well as renal, respiratory, gynecological, and oncological diseases

Furuhashi M. *et al., Clin Med Insights Cardiol.* 2015; **8**(suppl 3):23-33

32

CRISPRi to downregulate the biomarker genes

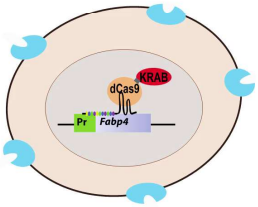


Chung JY. *et al., Genome Res.* 2019; **29**(9):1442-1452
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

CRISPRi to downregulate the biomarker genes

- Delivery vehicle, non-viral
- Route of administration, IP @ adult

Internalization of oligoplex



Fabp4 expression ↓
Lipogenesis ↓
Inflammation ↓
Steatosis ↓
Amelioration of obesity on high fat diet

Chung JY. *et al., Genome Res.* 2019; **29**(9):1442-1452
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

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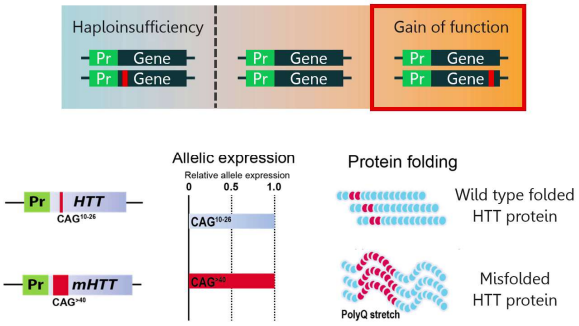
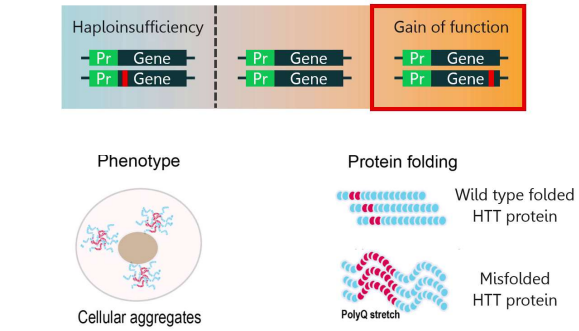
33

Autosomal dominant: Huntington's Disease



Garriga-Canut M. *et al., Proc Natl Acad Sci USA.* 2012; **109**(45):e3136-45
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

Autosomal dominant: Huntington's Disease

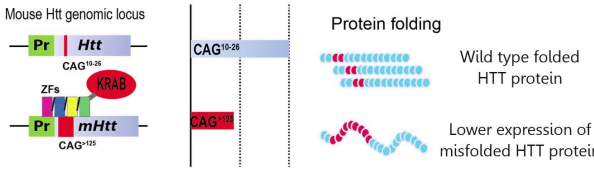


Garriga-Canut M. *et al., Proc Natl Acad Sci USA.* 2012; **109**(45):e3136-45
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

34

ZF-KRAB to downregulate the pathogenic gene

- Delivery vehicle, AAV
- Route of administration, I.C (striatum) @ P28



Garriga-Canut M. *et al., Proc Natl Acad Sci USA.* 2012; **109**(45):e3136-45
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

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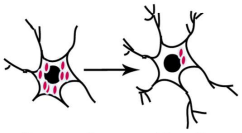
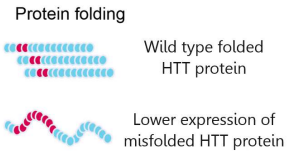
ZF-KRAB to downregulate the pathogenic gene

- Delivery vehicle, AAV
- Route of administration, I.C (striatum) @ P28



Rescue of neuronal function in HD mouse models



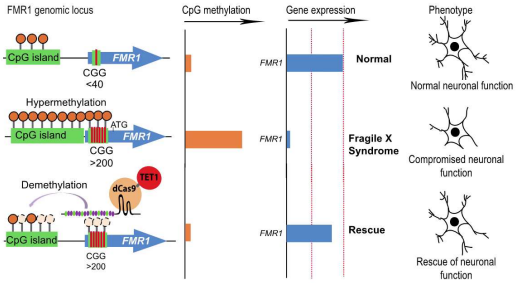
Garriga-Canut M. *et al., Proc Natl Acad Sci USA.* 2012; **109**(45):e3136-45
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

35

DNA methylation modulation

Insufficiency | Normal | Overexpression

Fragile X syndrome



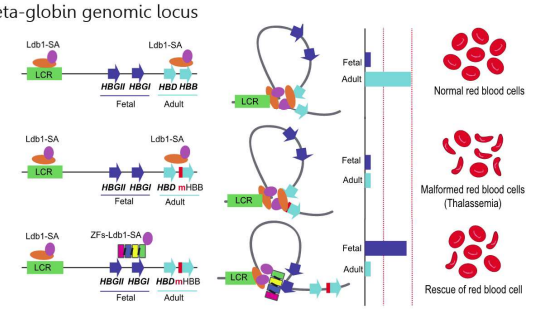
Lui XS. *et al., Cell.* 2016; **167**(1):233-247
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

36

DNA looping modulation

Insufficiency | Normal | Overexpression

Beta-globin genomic locus



Deng W. *et al., Cell.* 2014; **158**(4):849-860
Matharu N. and Ahituv N. *Nat Rev Drug Discov.* 2020; **19**(11):757-775

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37

Advantages and disadvantages

01 Does not edit/cut DNA
No off-target 'DNA scars'

02 Upregulate/downregulate endogenous gene
De-risking ectopic expression

03 *Cis*-regulatory elements as targets
Built-in tissue specificity

Advantages and disadvantages

01 Immunogenicity

02 Dosing

38

Acknowledgements

Thank you

Nadav Ahituv lab
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Stanley Qi lab

Kevin Bender Lab
Perry Spratt
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Stephan Sanders lab

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