ERN ITHACA

Webinar 2025

European Commission

EUROPEAN REFERENCE NETWORKS

Helping patients with rare or low-prevalence complex diseases

Webinar
NON-CODING GENOME AND HUMAN DISEASE

Tuesday Sept 23rd 2025 from 5pm to 6.30 pm (Central European Time)

Chaired by Prof. Florence PETIT

European Reference Networks ITHACA

Welcome – Technical points

- We are please to be numerous
  - 229 people registered and 146 participated online

- Webinar being recorded, we Thank you to :
  - *Turn off your microphone and disconnect your camera*
  - *Raise your hand at the time of the questions and discussions*
  - *We will answer the questions sent in the registration form*
  - *A satisfaction survey at the end of the meeting*

- Webinars # are available on the ITHACA website with authors' consent.
  - https://ern-ithaca.eu/documentation/educational-resources/

- Webinar Team Link to connect
  - https://teams.microsoft.com/l/meetup-join/19%3ameeting_YmM1YmNhZDQtMmIwMS00M2RjLWE1MjgtZTMzNmE1NjQzNzdj%40thread.v2/0?context=%7b%22Tid%22%3a%222461c129-d44f-406d-a778-d1a3c4c1527c%22%2c%22Oid%22%3a%223fe3bbab-4860-4b0b-bdc7-6ebc8a2f8f7b%22%7d

- Anne Hugon Project Manager ERN ITHACA - anne.hugon@aphp.fr

Welcome and Introduction

- **NON-CODING GENOME AND HUMAN DISEASE**
  - Recent developments in genomic technologies have enabled the genome-wide identification of regulatory elements and chromatin interactions, controlling the spatiotemporal gene expression. In this webinar, we will explore the significant involvement of non-coding genome in various human diseases, a rapidly evolving field of human genomics.
- Chaired by Prof. Florence PETIT (ERN ITHACA, HCP Lille, France)

Agenda

- **Welcome and Introduction**
  - Prof. Florence PETIT ; Lille University Hospital, Lille, France
- **1. Non-coding genome in limb malformations**
  - Prof. Florence PETIT ; Lille University Hospital, Lille, France
- **2. Enhancer hijacking: A key driver of congenital disorders**
  - Prof. Malte SPIELMANN ; Institut für Humangenetik, Lübeck, Germany
- **3. Interpreting the impact of noncoding structural variants in neurodevelopmental disorders -**
  - Prof. Sarah VERGULT, Ghent University, Ghent, Belgium
- **4. Finding causes of missing heritability in neurogenetic disorders: exploring the dark matter of the genome**
  - Dr Stefan BARAKAT; Erasmus MC, Rotterdam, Netherlands
- *Discussion time*
- **Conclusion with speakers and moderator**

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Helping patients with rare or low-prevalence complex diseases

1.Introduction to enhanceropathies

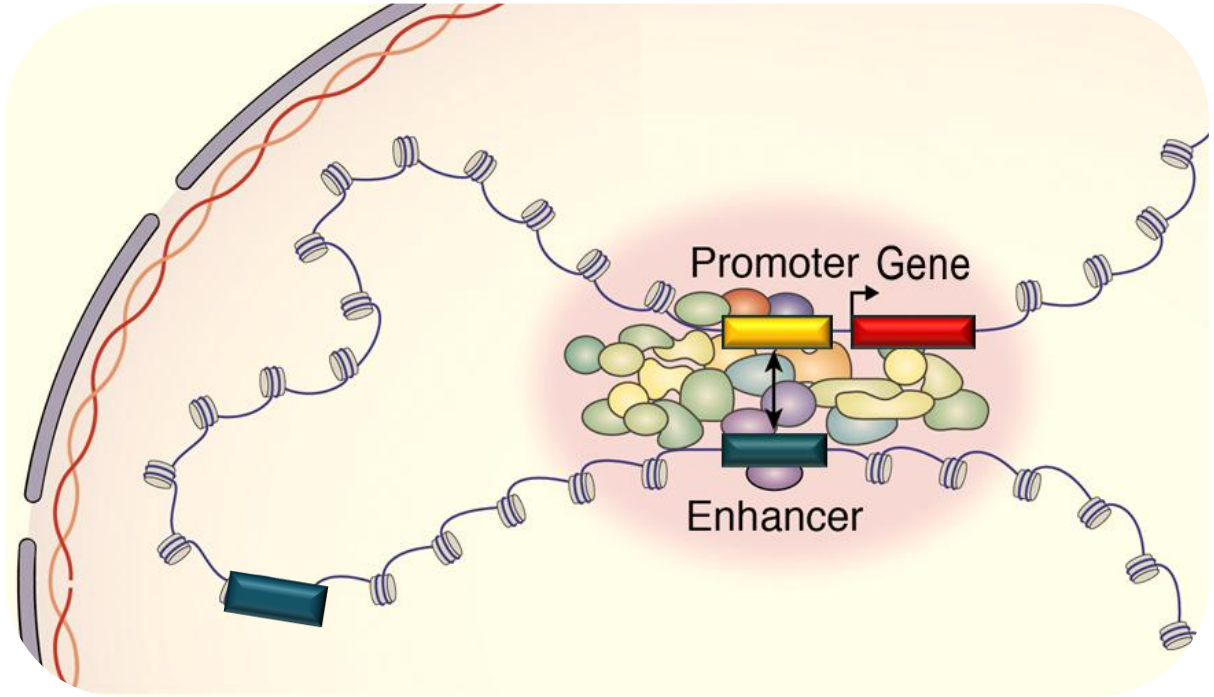
Non-coding genome in limb malformations

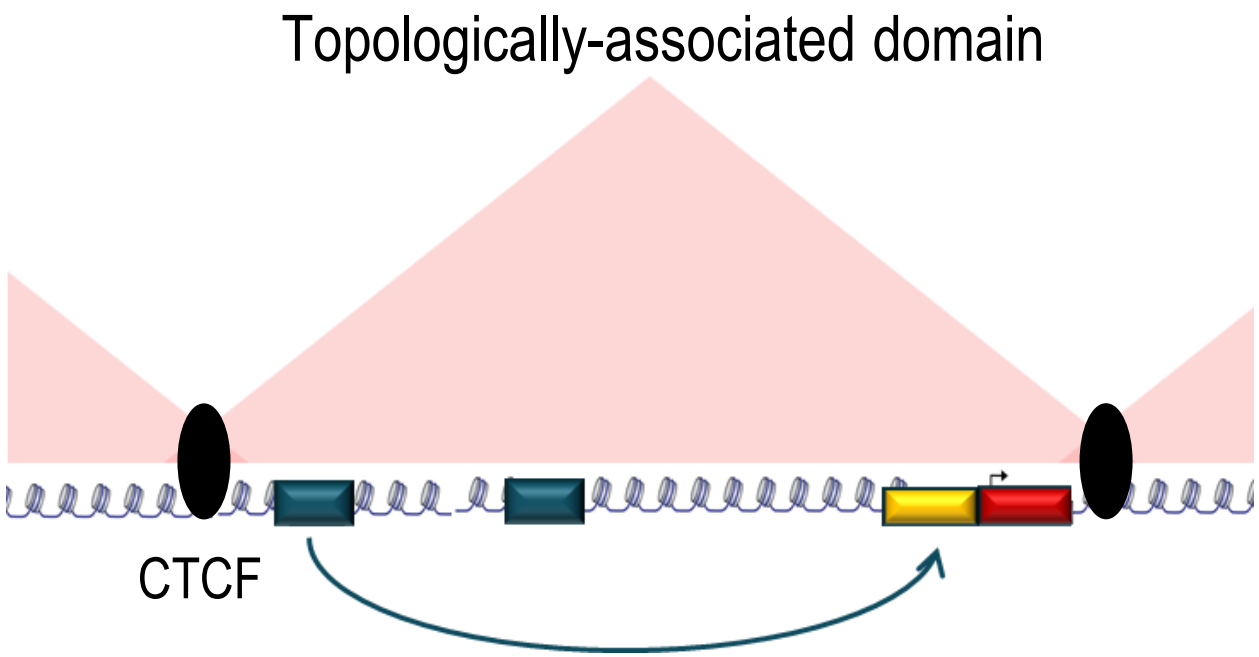
Florence PETIT

Lille University Hospital, Lille, France

Enhancers: tissue-specific promoters of the promoter

20,000 genes
400,000 promoters and enhancers ? (GeneHancer)





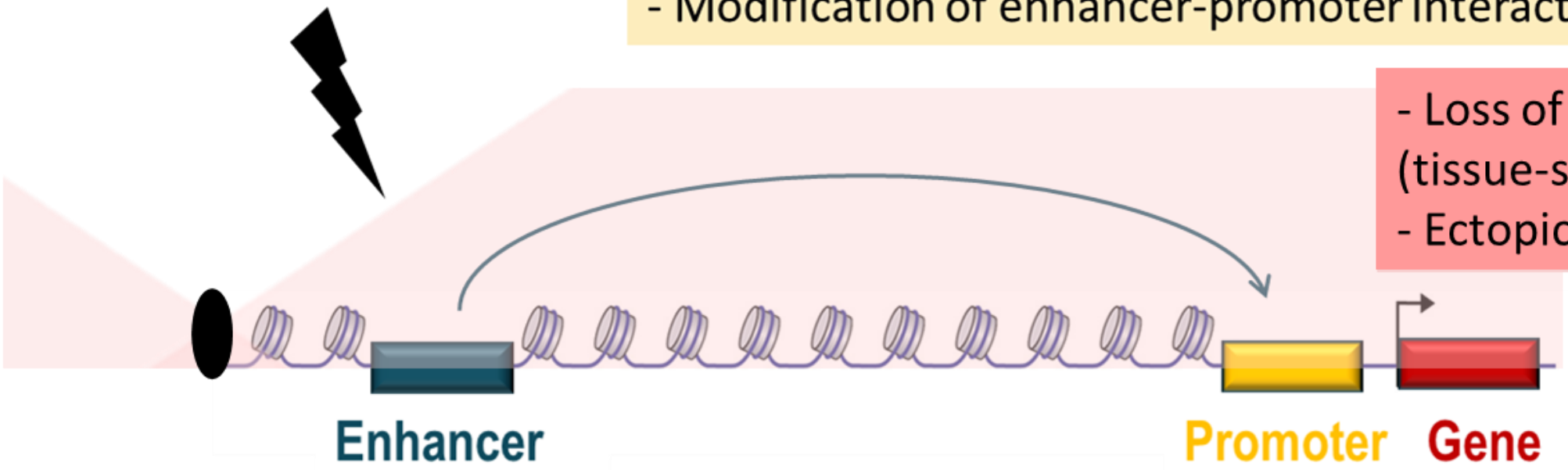
Enhanceropathies

- Sequence variations
- Copy Number Variations
- Structural Variations

- Loss of enhancer activity
- Gain of enhancer activity

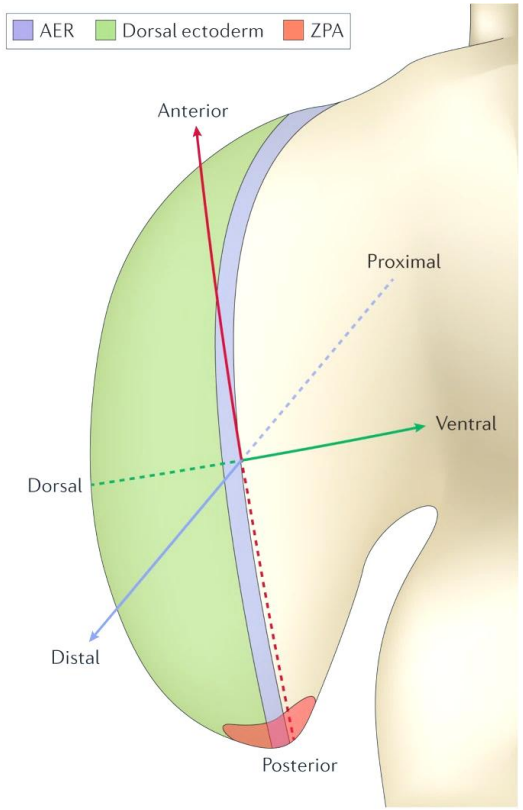
- Modification of enhancer-promoter interaction

- Loss of gene expression (tissue-specific)
- Ectopic gene expression



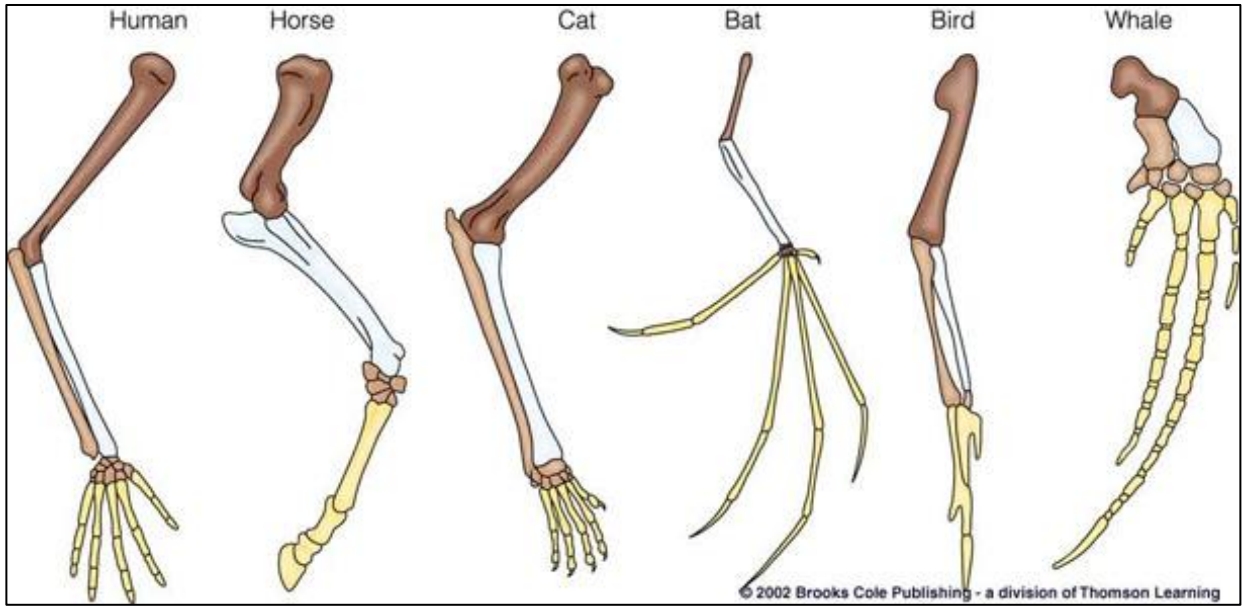
The limb : a model organ in developmental biology

Complex anatomy
Various signaling pathways
Easily observable
Non lethal malformations

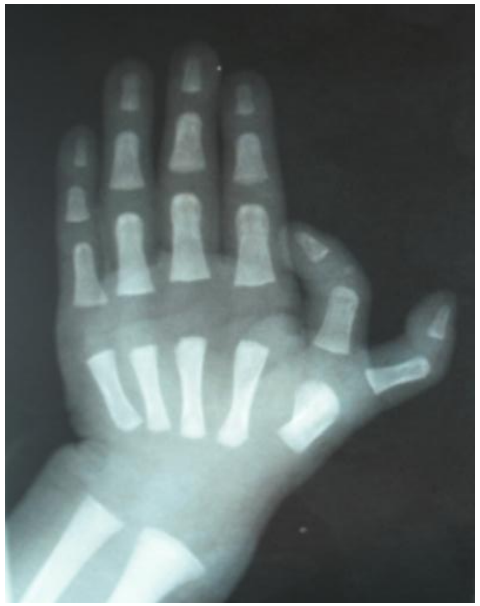


Variations in regulatory elements

Morphological evolution



Malformations



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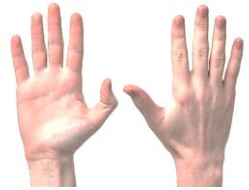
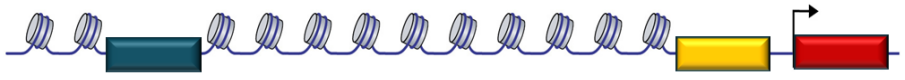
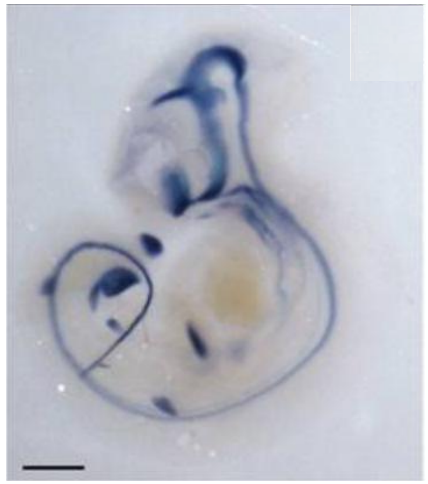
Cis-regulation of limb polarization



Antero-posterior

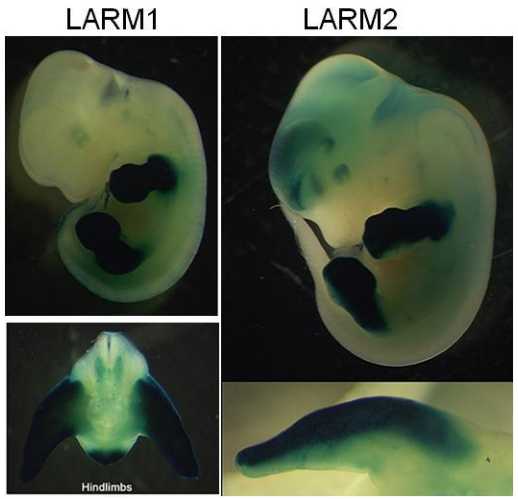


ZRS — ***Shh***

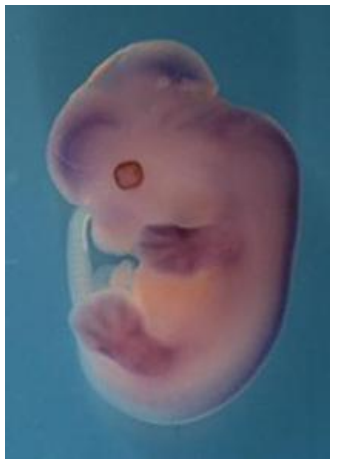


Dorso-ventral

LARM — ***Lmx1b***



E12.5



Haro et al., Development, 2017
Haro et al., Nat Commun, 2021

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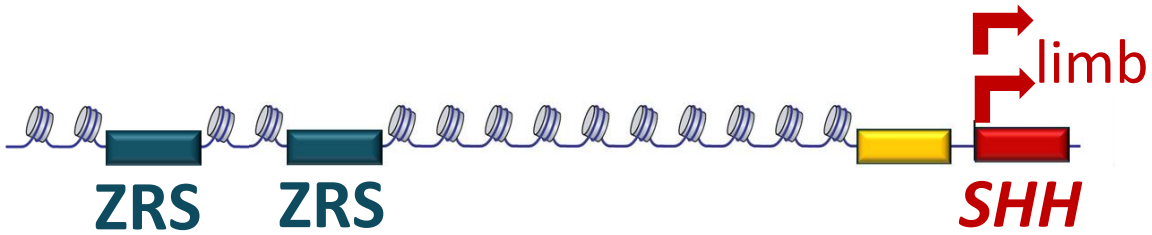
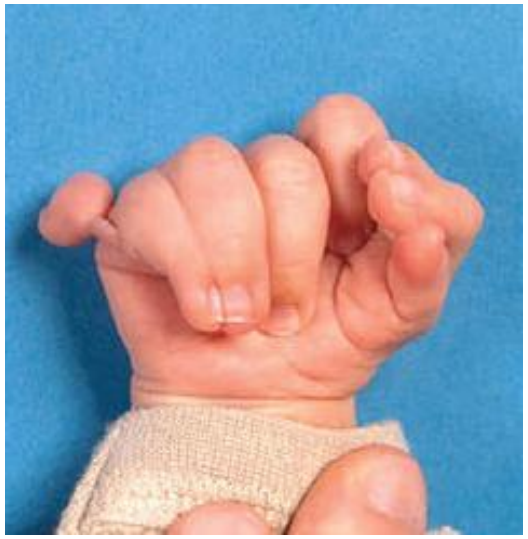
Copy Number Variations involving enhancers

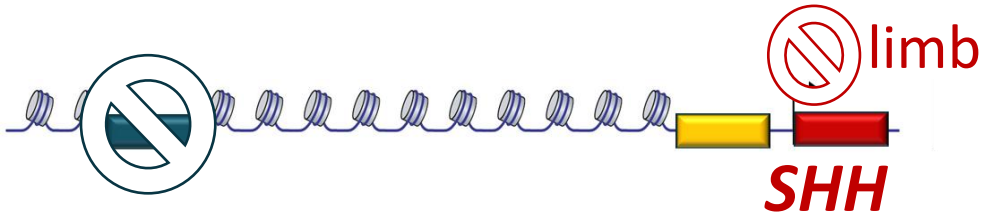
Enhancer duplication



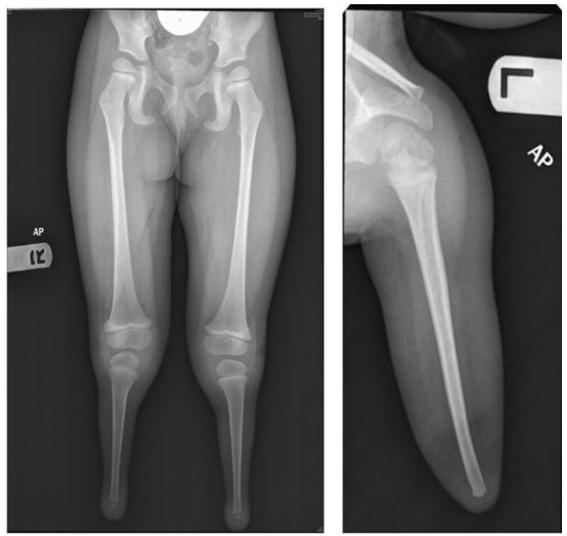
Ectopic *SHH* expression
in the anterior limb bud



Enhancer deletion



Loss of *SHH* expression in
the posterior limb bud



Shamseldin et al., AJMG, 2016

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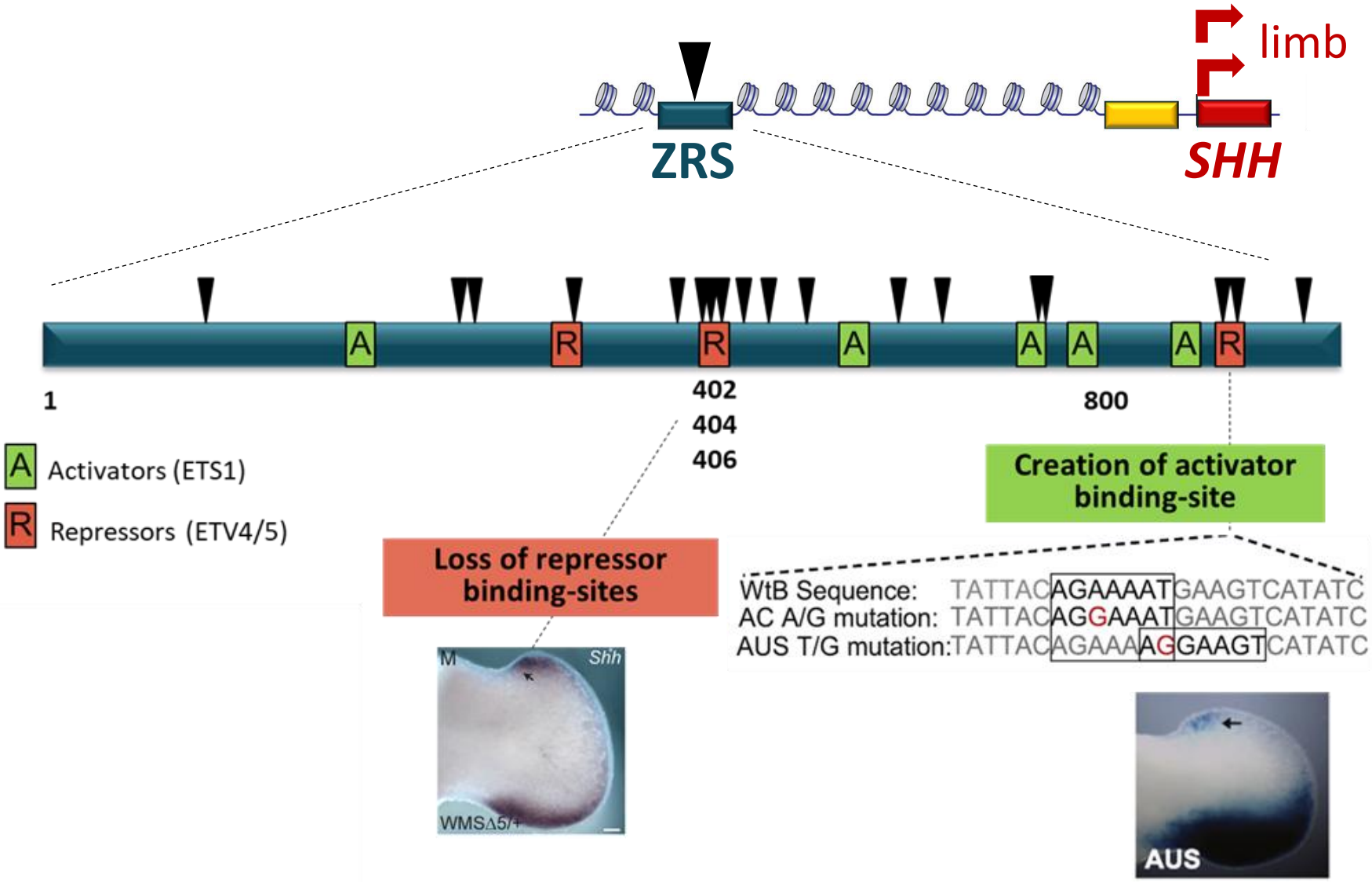
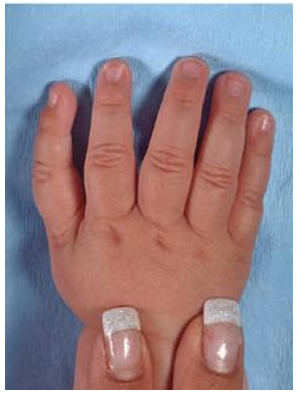
Sequence variations: enhancer gain-of-function





Modified from: Lettice et al., Dev Cell, 2012 ; Lettice et al., Cell Reports, 2017

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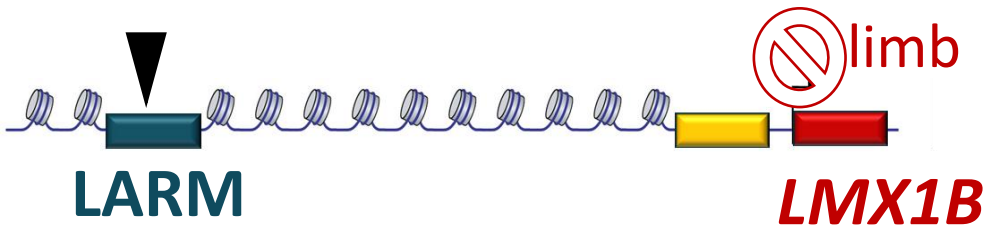
Sequence variations: enhancer loss-of-function

Consanguineous case
Homozygous for rare variant in LARM2
NC_000009.11:g.129291376C>T, MAF 0.00154%

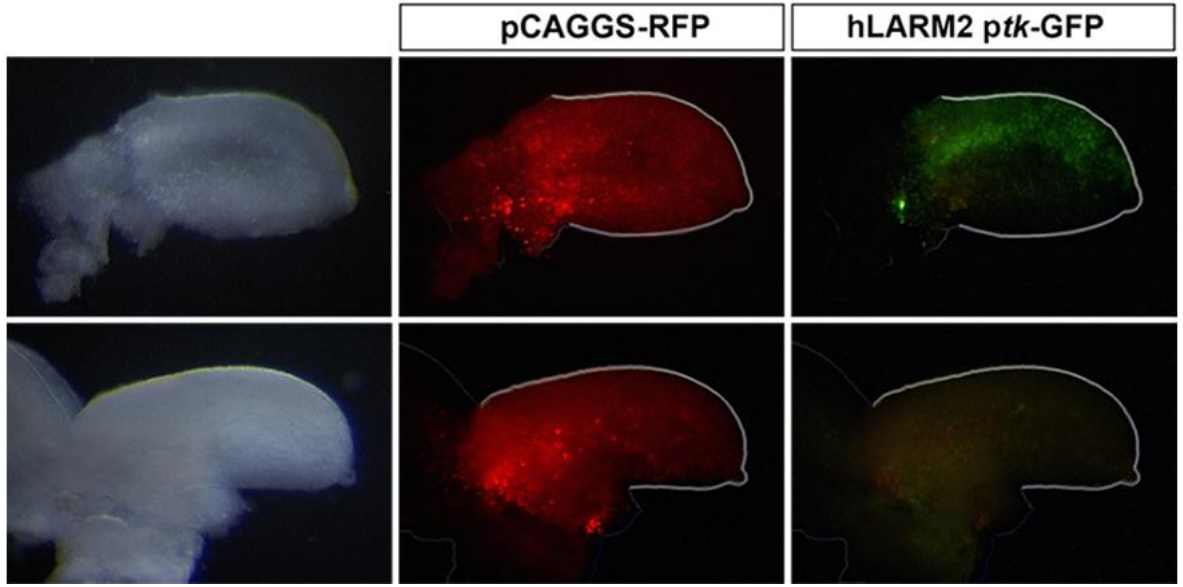


Clinics :
Dorsal limb defects
No kidney / eye defects

Nail-Patella syndrome,
limb-only phenotype

Recessive autosomal

Chick enhancer assay



Haro et al., Nat Commun, 2021

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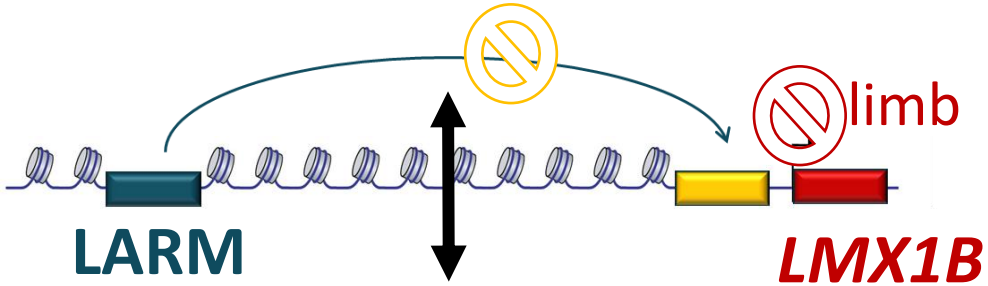
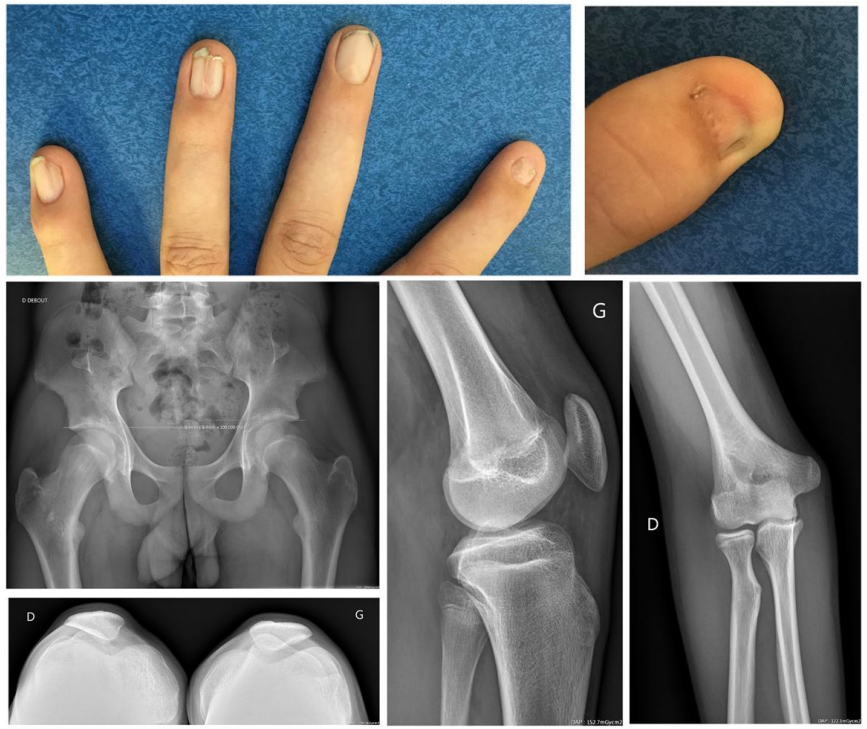
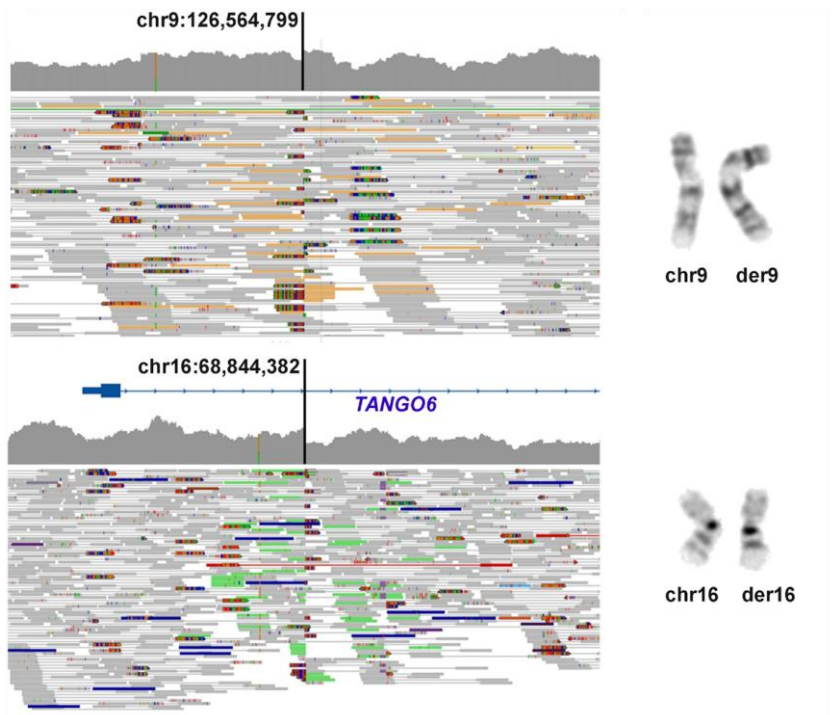
Structural Variations: loss of enhancer-promoter interaction

SV disrupting the LARM/*LMX1B* interaction



Dorsal limb defects
No kidney / eye defects





Nail-Patella syndrome, limb-only phenotype

Chromosomal

Brunelle et *al., in prep.*

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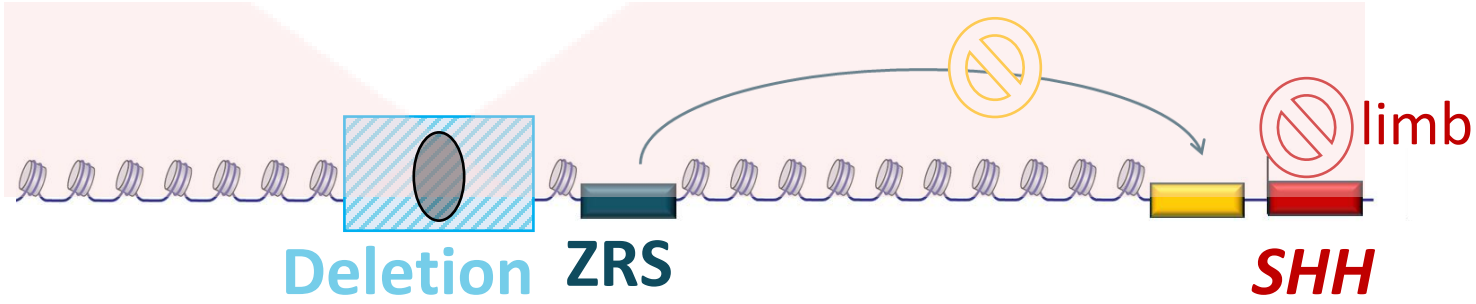
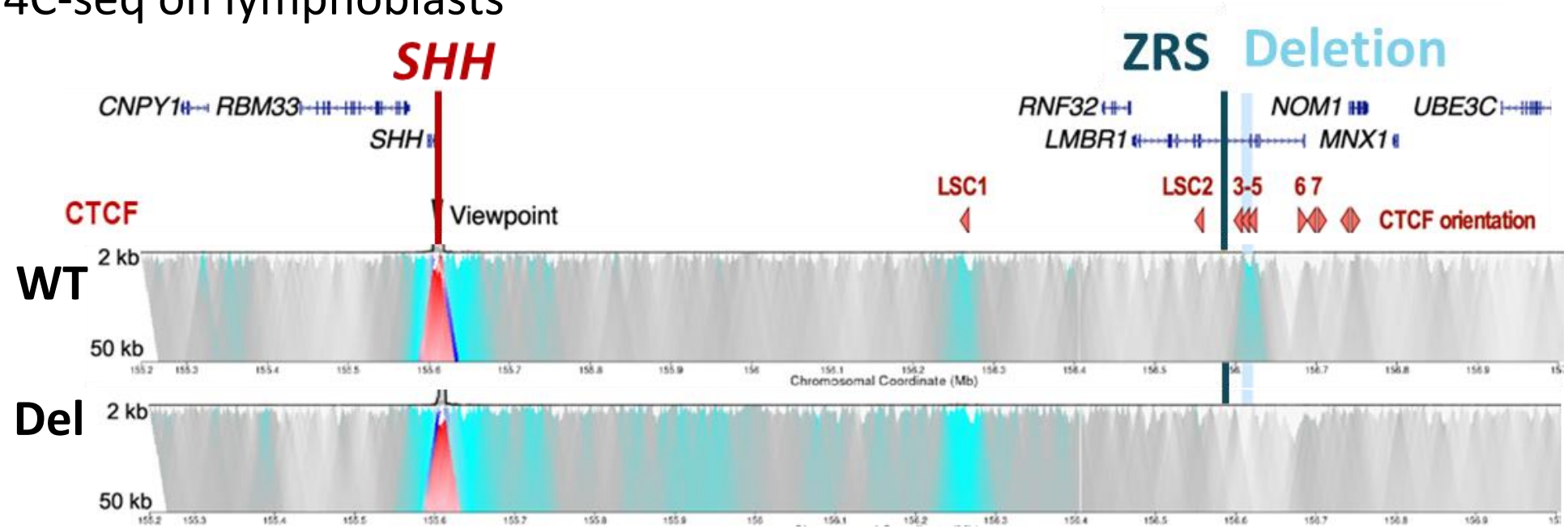
Structural Variations: loss of enhancer-promoter interaction



4C-seq on lymphoblasts



Ushiki et al., Nat Commun, 2021

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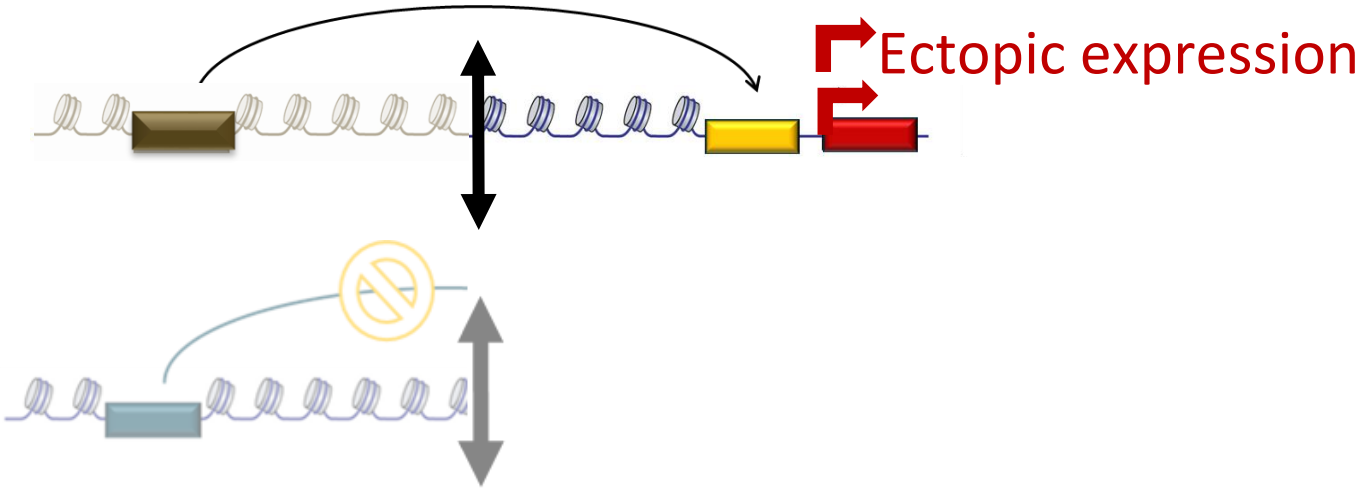
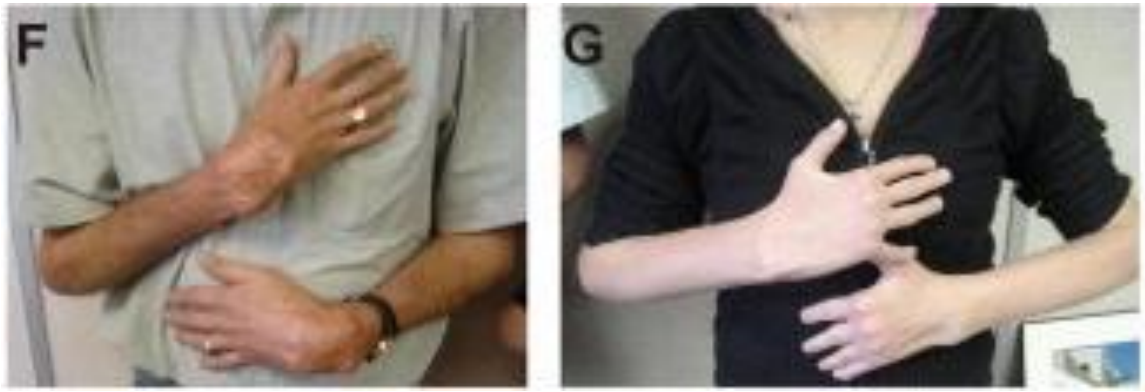
Structural Variations: ectopic enhancer-promoter interaction

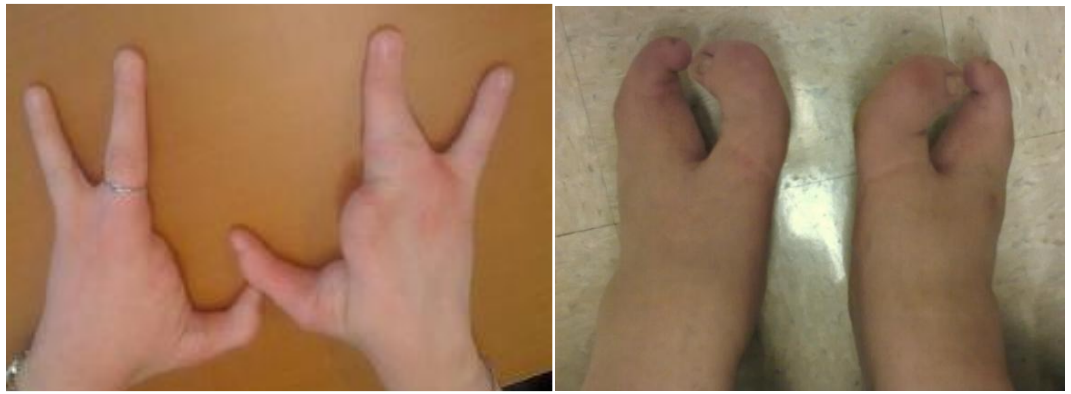
« Enhancer hijacking »



Liebenberg syndrome



SHFM3 (dup10q24)



Spielmann et al., AJHG, 2012

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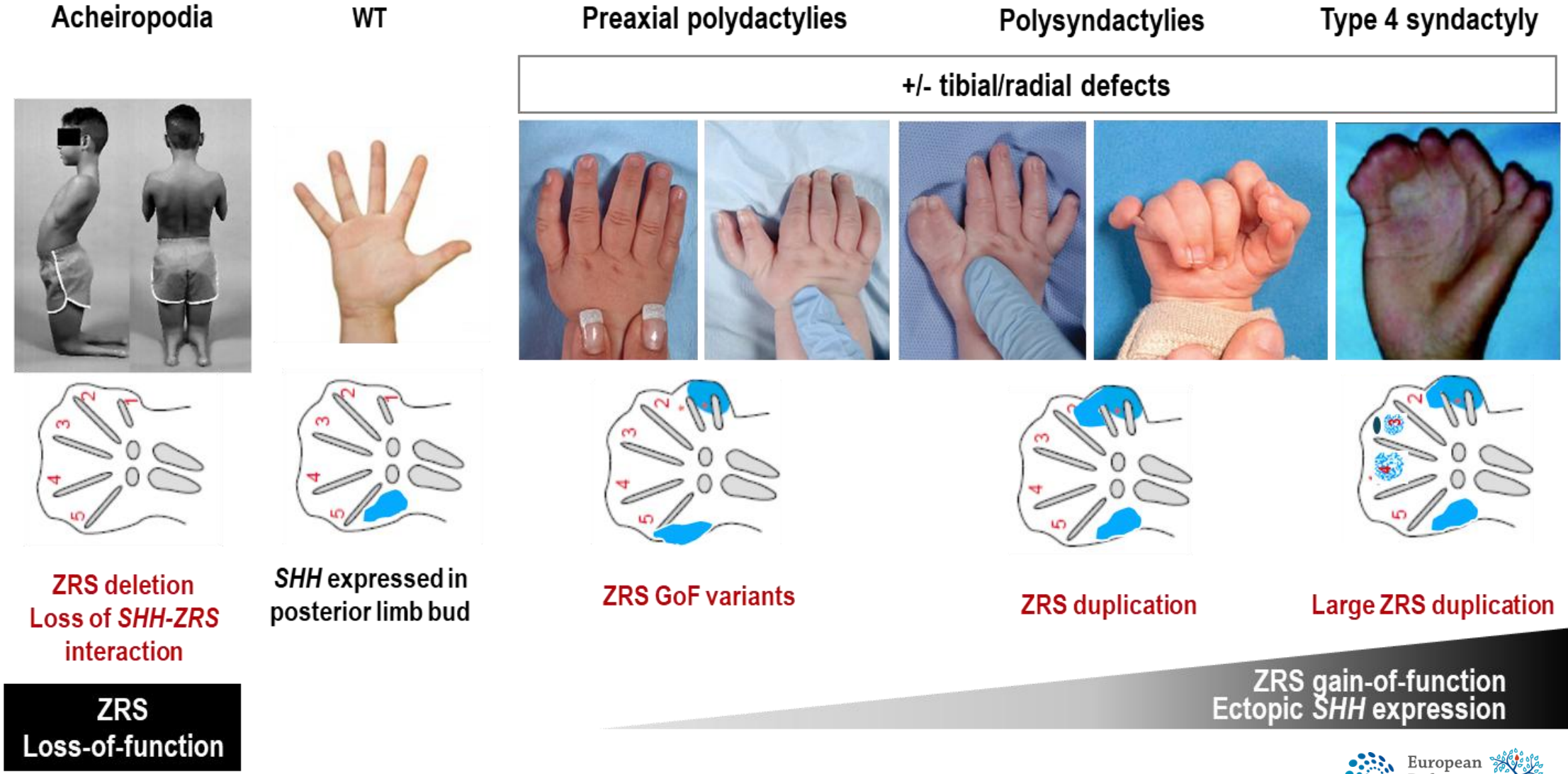
Summary: *SHH* regulation and limb malformations



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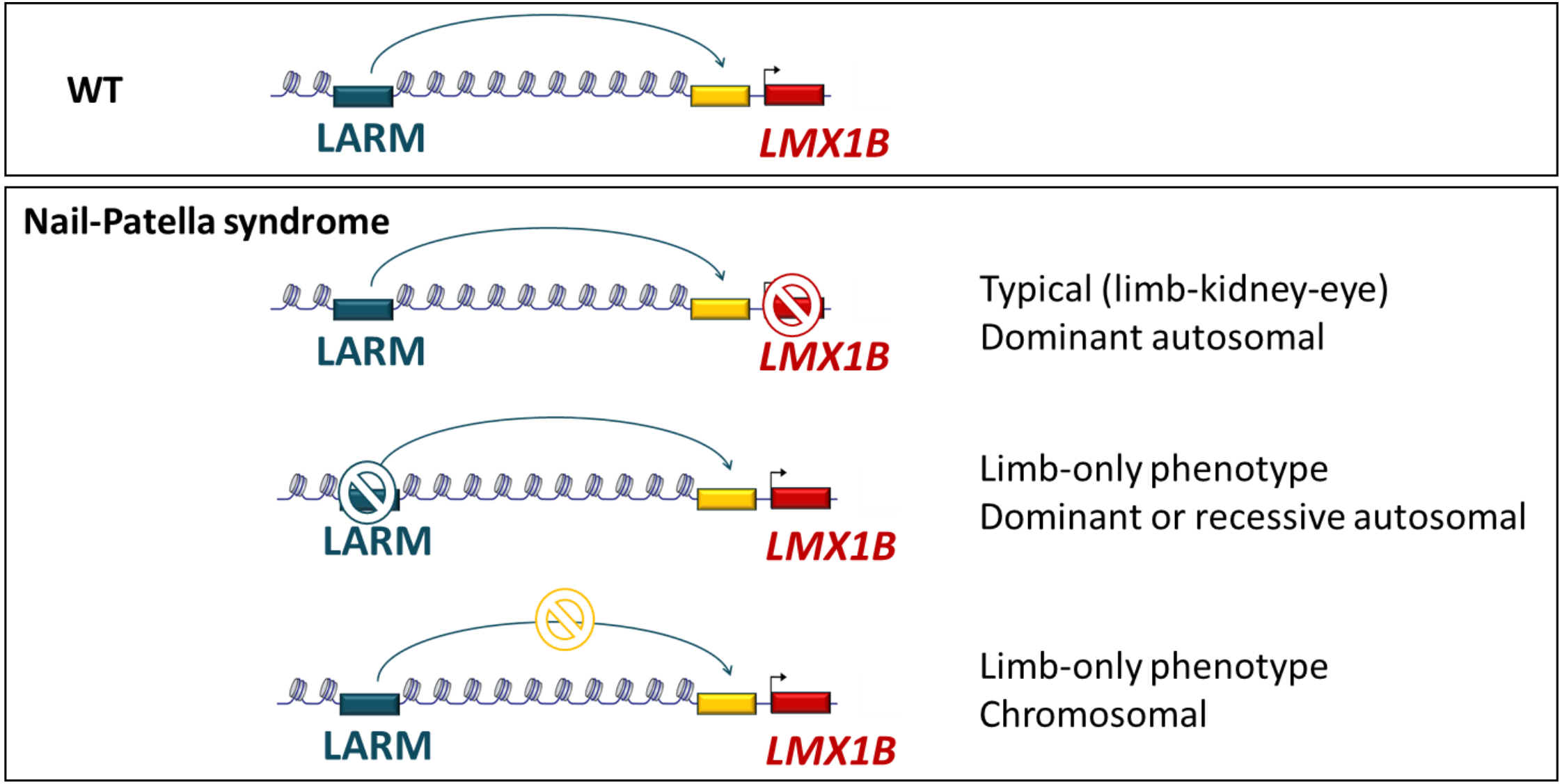
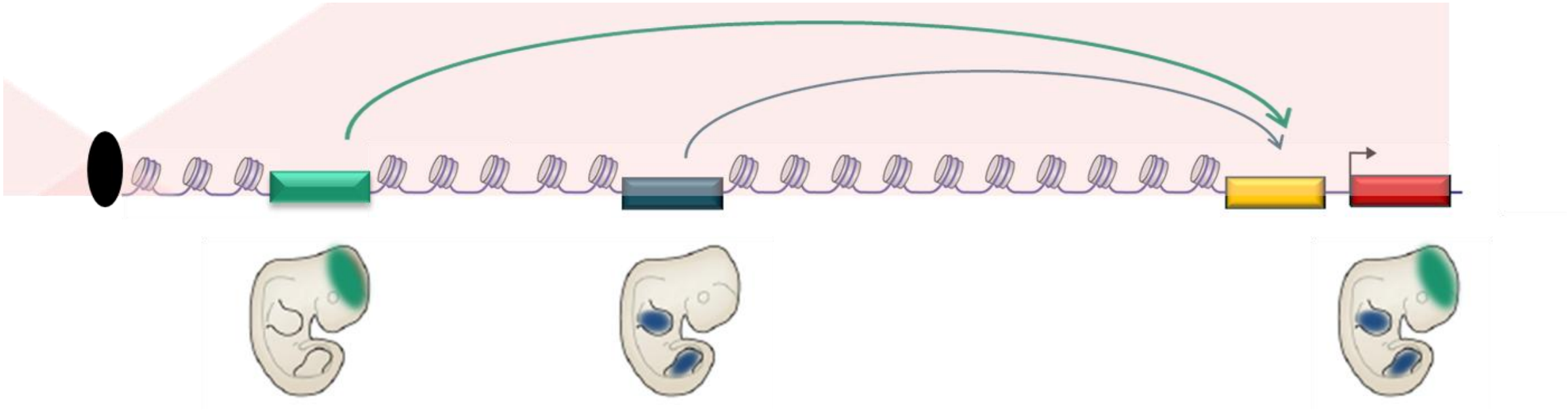
Summary: *LMX1B* regulation and limb malformations



Brunelle et *al., in prep*

Non-coding genome and human disease

Thousands of syndromes to dismember



Enhancers
Tissue-specific anomalies

5000 genes
8000 syndromes

Non-coding genome and human disease

Importance of the clinical-biological expertise and cooperation for data interpretation, particularly for non-coding genome alterations

Need for high-throughput functional testing of enhancers variations

Importance of genomic diagnosis for precision medicine and genetic counselling in rare diseases

Cis-regulation therapy perspectives

References and Acknowledgments

- Lettice LA, Williamson I, Wiltshire JH, Peluso S, Devenney PS, Hill AE, Essafi A, Hagman J, Mort R, Grimes G, DeAngelis CL, Hill RE. **Opposing functions of the ETS factor family define Shh spatial expression in limb buds and underlie polydactyly.** Dev Cell. 2012;22(2):459-67.
- Lettice LA, Devenney P, De Angelis C, Hill RE. **The Conserved Sonic Hedgehog Limb Enhancer Consists of Discrete Functional Elements that Regulate Precise Spatial Expression**. Cell Rep. 2017;20(6):1396-1408.
- Ushiki A, Zhang Y, Xiong C, Zhao J, Georgakopoulos-Soares I, Kane L, Jamieson K, Bamshad MJ, Nickerson DA; University of Washington Center for Mendelian Genomics; Shen Y, Lettice LA, Silveira-Lucas EL, Petit F, Ahituv N. **Deletion of CTCF sites in the SHH locus alters enhancer-promoter interactions and leads to acheiropodia.** Nat Commun. 2021;12(1):2282.
- Haro E, Petit F, Pira CU, Spady CD, Lucas-Toca S, Yorozuya LI, Gray AL, Escande F, Jourdain AS, Nguyen A, Fellmann F, Good JM, Francannet C, Manouvrier-Hanu S, Ros MA, Oberg KC. **Identification of limb-specific Lmx1b auto-regulatory modules with Nail-patella syndrome pathogenicity.** Nat Commun. 2021;12(1):5533.
- Haro E, Watson BA, Feenstra JM, Tegeler L, Pira CU, Mohan S, Oberg KC. **Lmx1b-targeted cis-regulatory modules involved in limb dorsalization.** Development. 2017;144(11):2009-2020.

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Helping patients with rare or low-prevalence complex diseases

2. Enhancer hijacking: A key driver of congenital disorders

Prof. Malte SPIELMANN ; Institut für Humangenetik, Lübeck, Germany

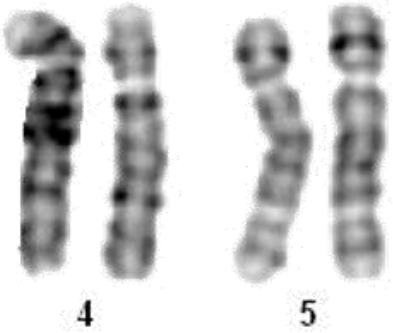
Enhancer hijacking in the 3D genome as driver of disease

Malte Spielmann, Institute for Human Genetics,
Universitätsklinikum Schleswig-Holstein, Kiel & Lübeck

One Test For All

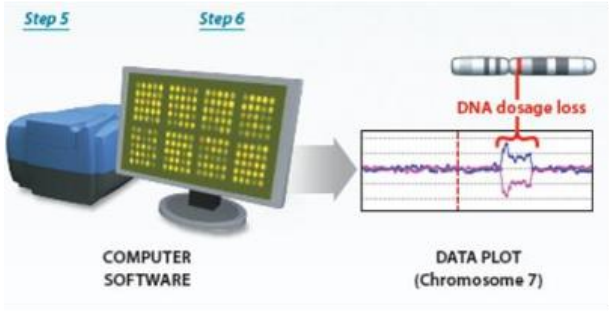
Whole Genome Sequencing

Repeats

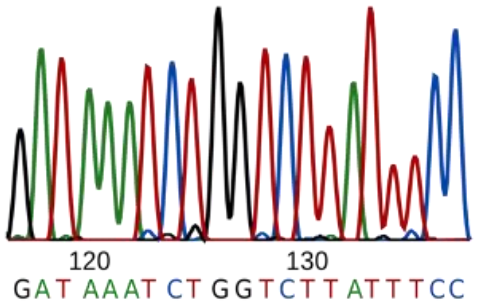


Karyotype

Array CGH



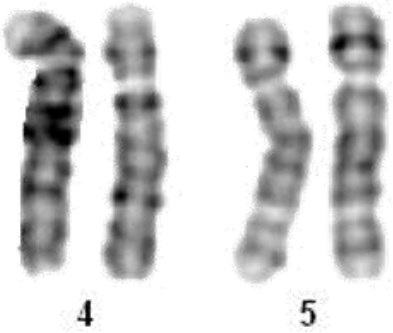
Sequencing



Kaschta et al. *Genome Medicine* 2025

Whole Genome Sequencing

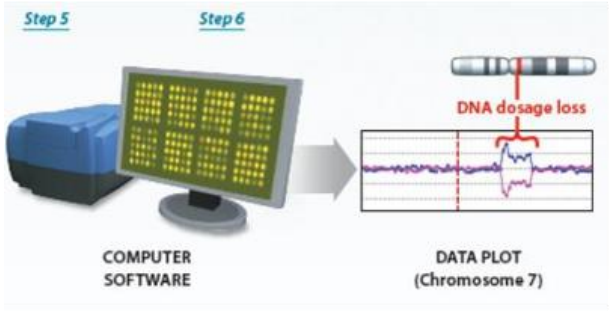
Repeats



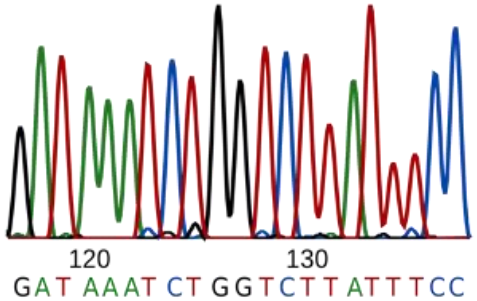
Karyotype

Diagnostic Yield 41%

Array CGH



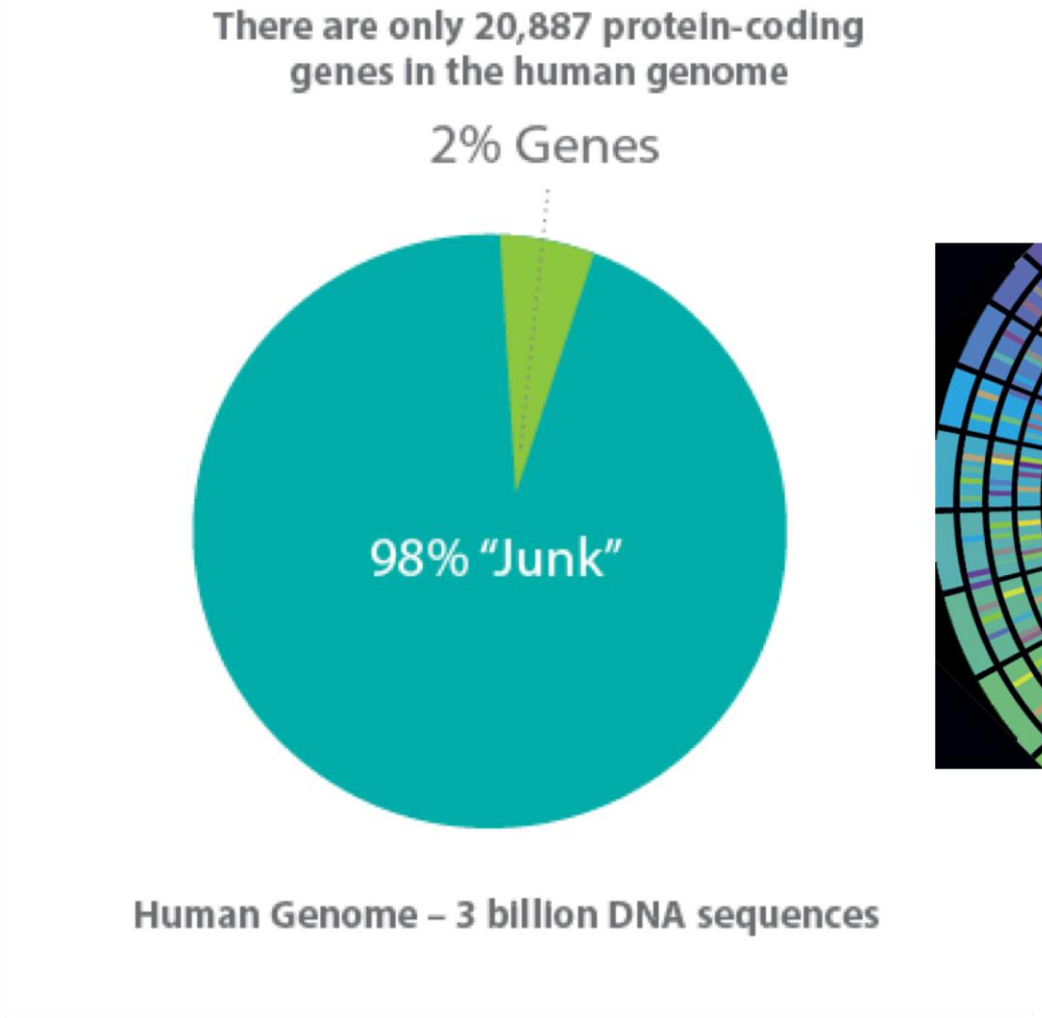
Sequencing

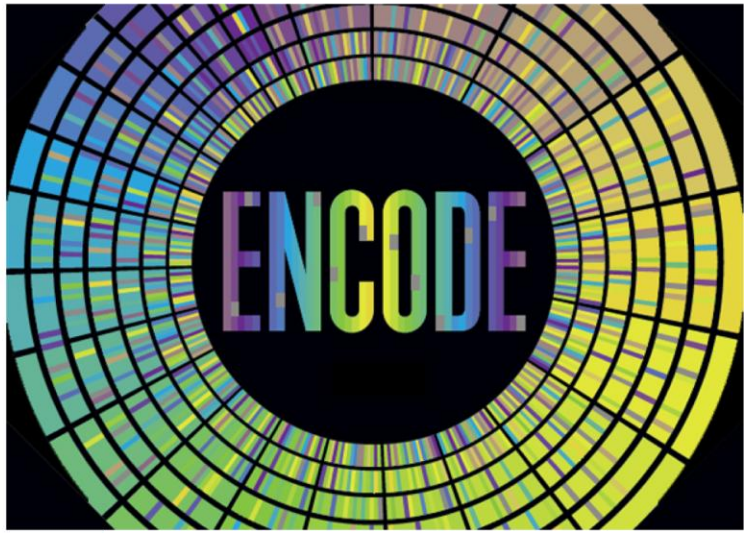


Kaschta et al. *Genome Medicine* 2025

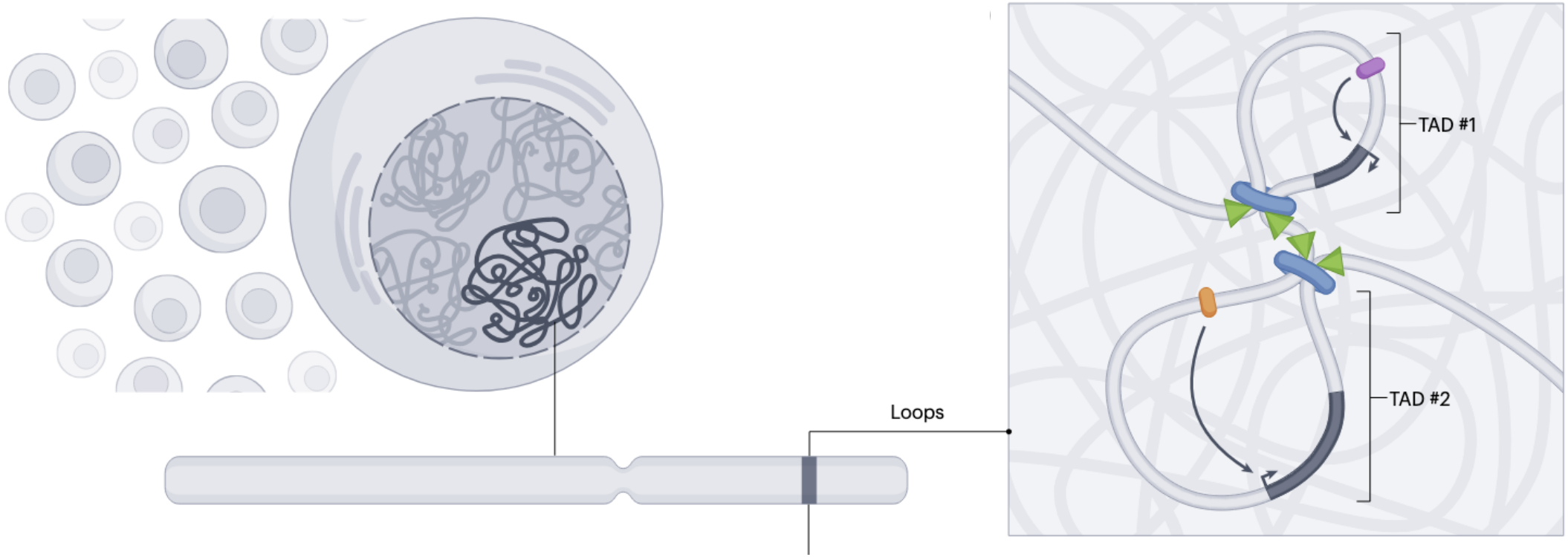
What are we missing ?

The non-coding genome: gene deserts



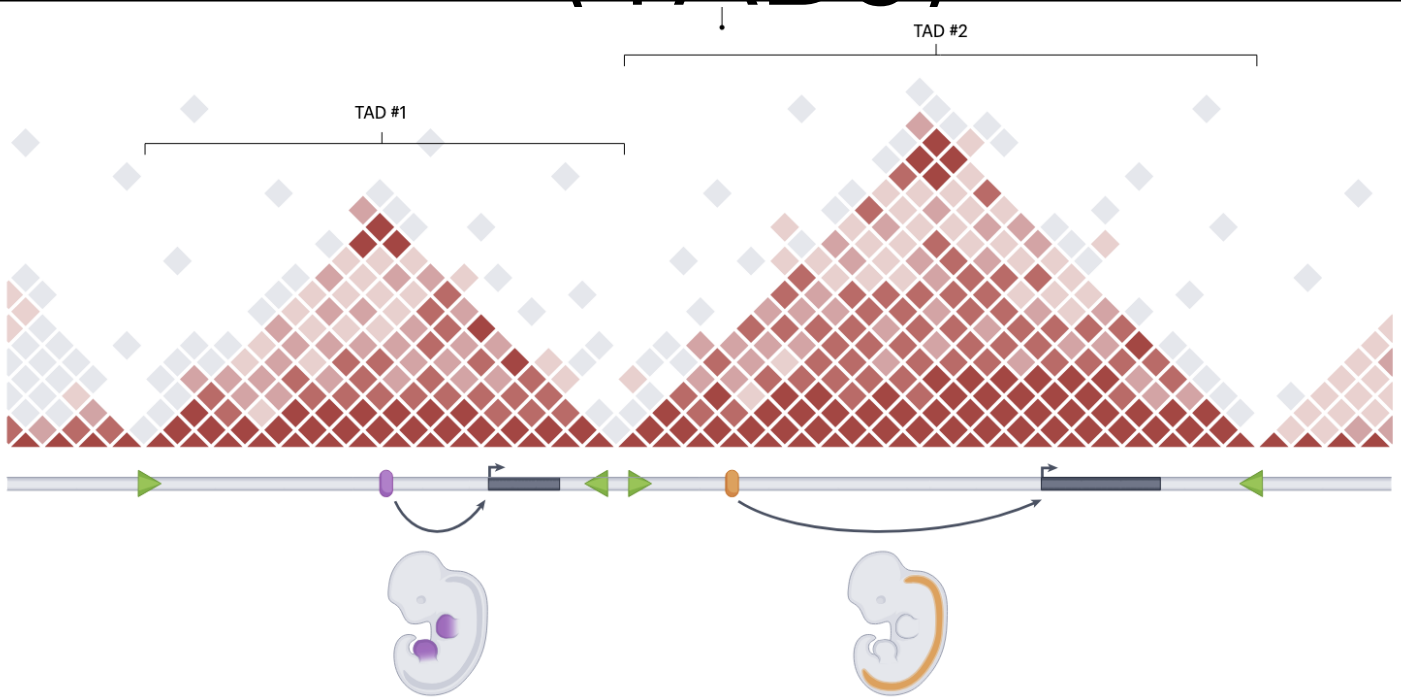


Structural Variants in the 3D genome



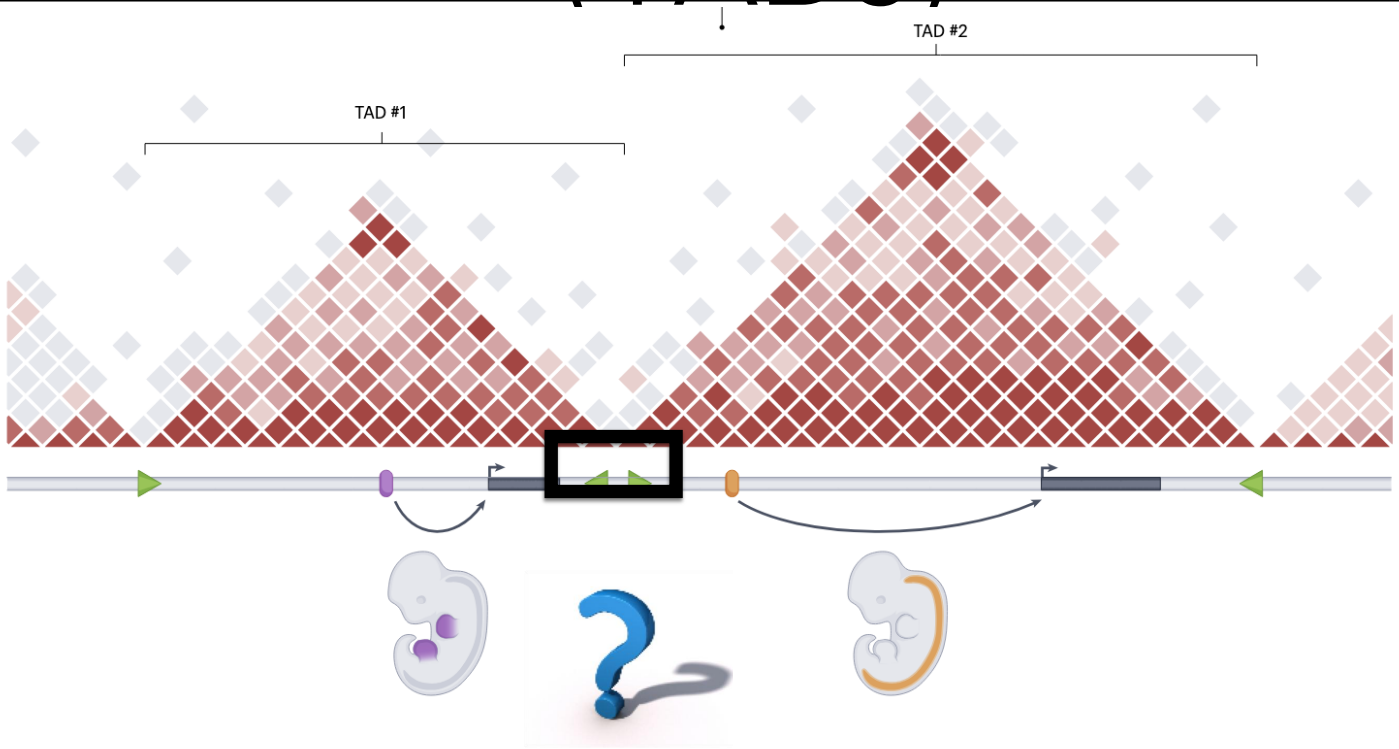
Sreenivasan et al, *Nat. Rev. Genet*. 2025

Topological Associating Domains (TADs)



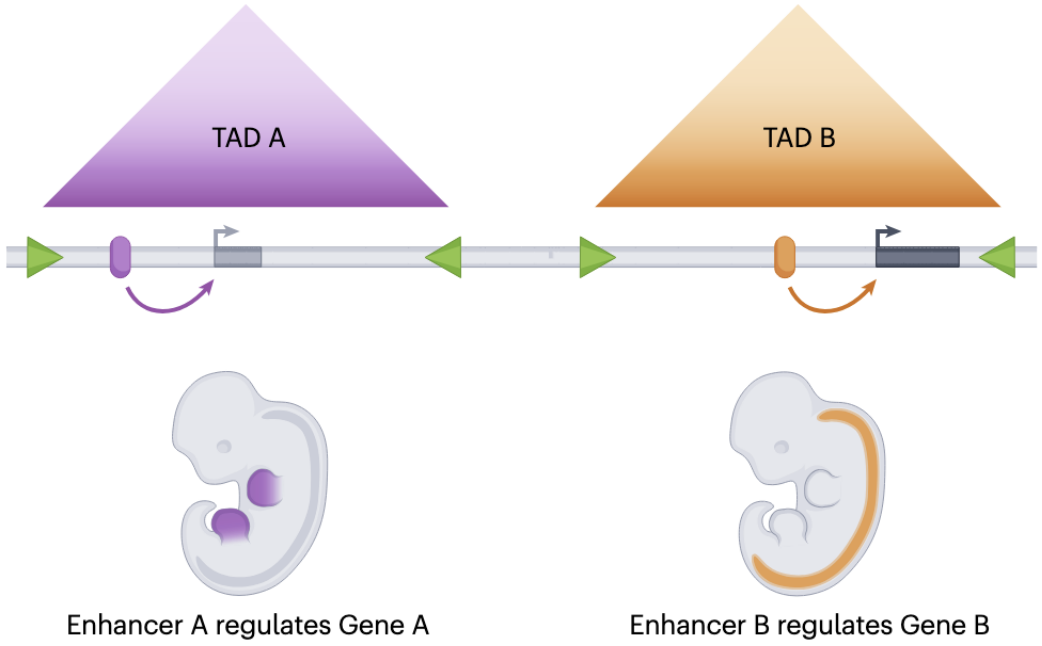
Sreenivasan et al, *Nat. Rev. Genet*. 2025

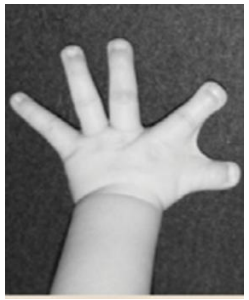
Topological Associating Domains (TADs)



Sreenivasan et al, *Nat. Rev. Genet*. 2025

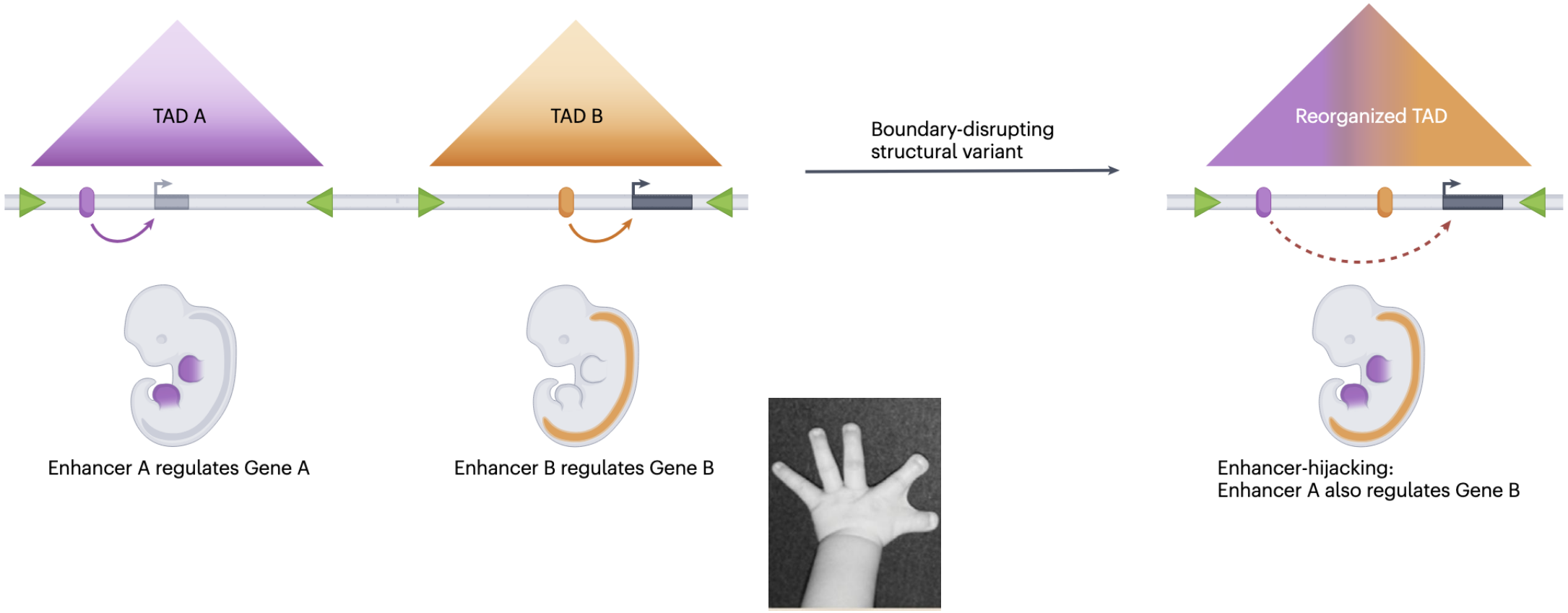
Enhancer hijacking





Sreenivasan et al, *Nat. Rev. Genet*. 2025

Enhancer hijacking



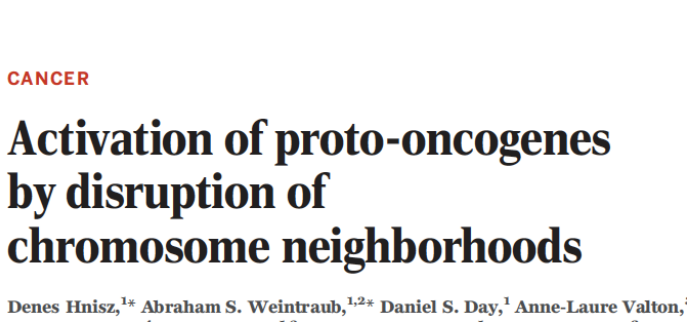
Sreenivasan et al, *Nat. Rev. Genet*. 2025

Structural Variants in the 3D genome

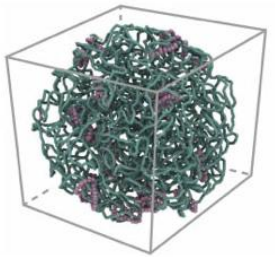
Spielmann et al, *Nat. Rev. Genet*. 2018

Structural Variants in the 3D genome

Spielmann et al, *Nat. Rev. Genet*. 2018

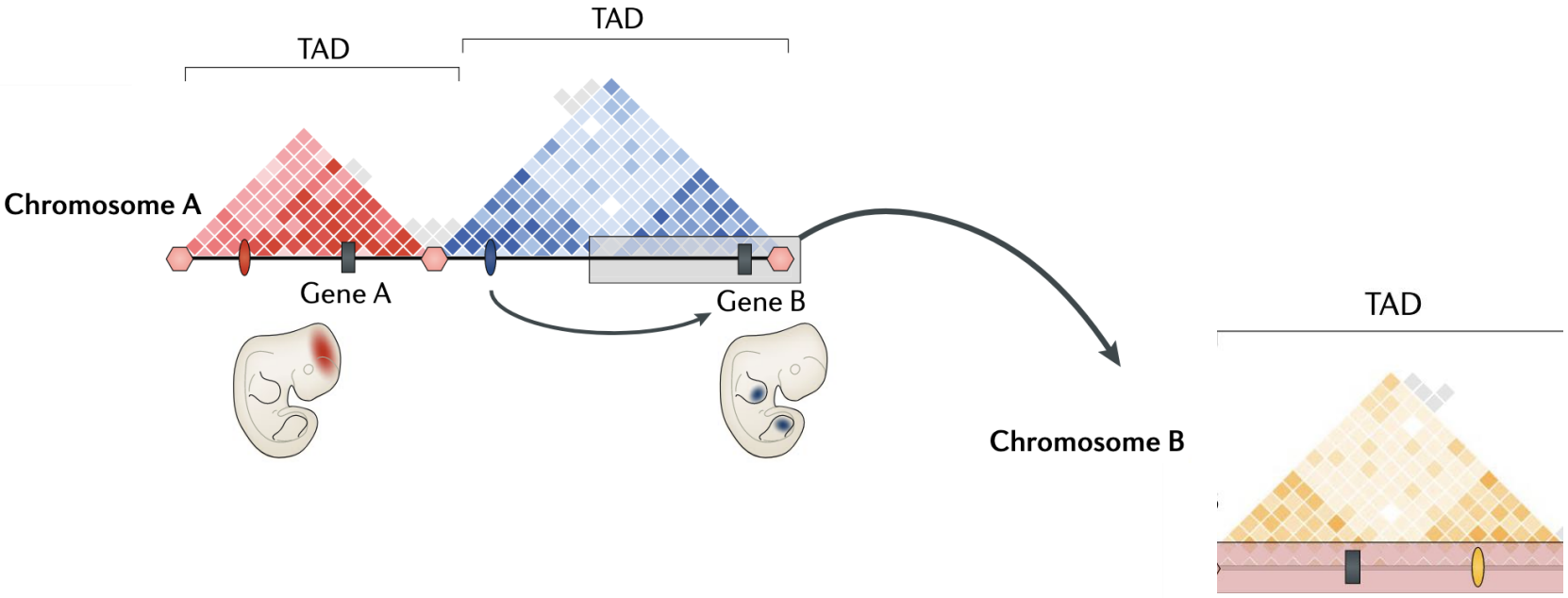


- **New Mutational mechanism in Cancer**



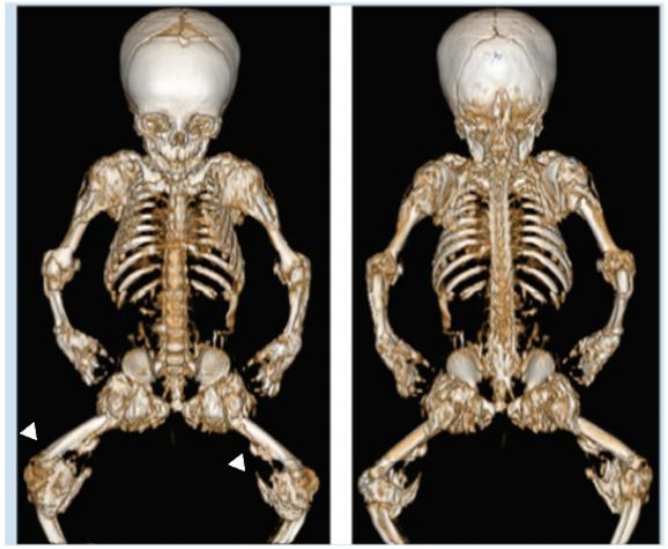
Genome^3
SPP2202

What about Insertions ?

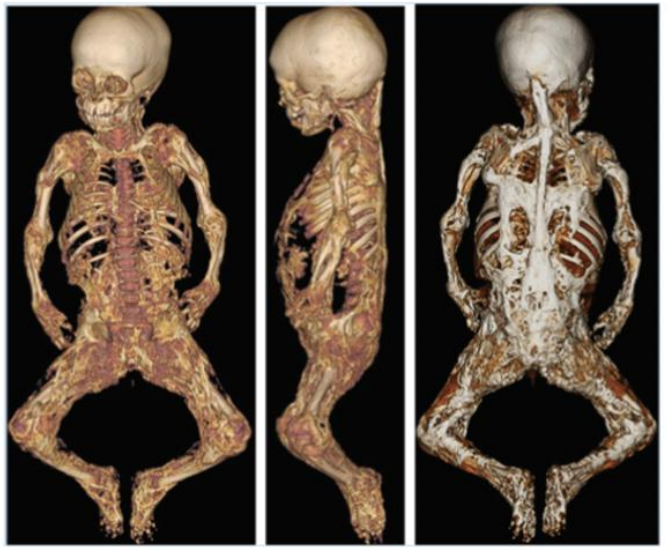


Ossificans Progressiva

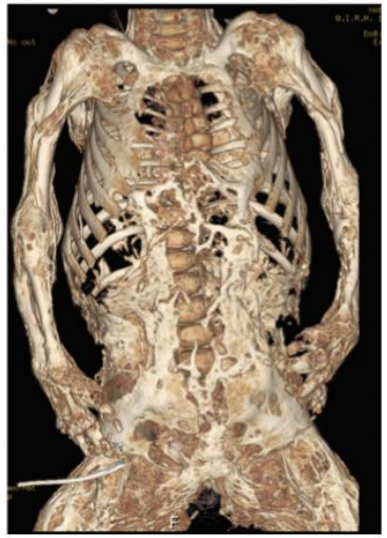
MRI - 10 months old



CT-scan - 3 years old

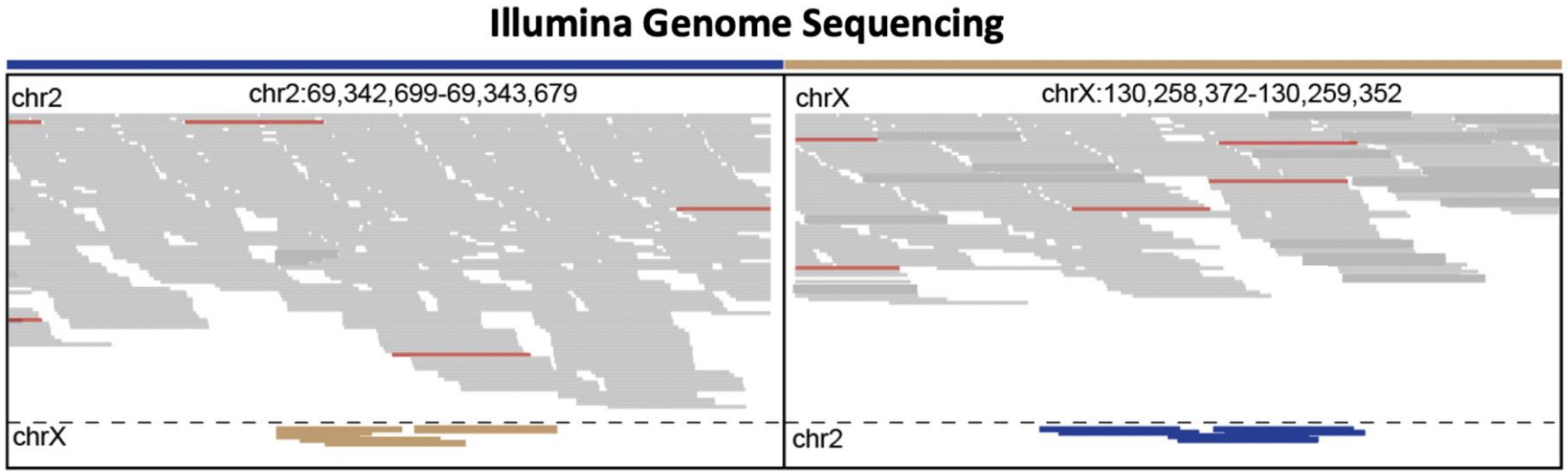


CT-scan - 5 years old



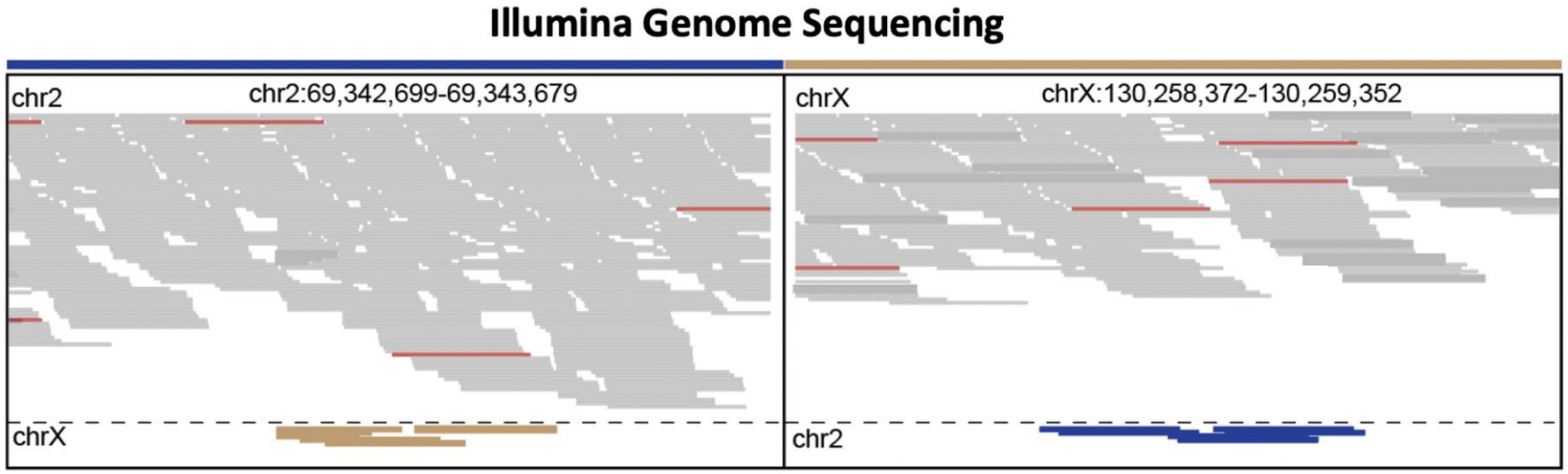
Uirá Melo Elisa Giorgio

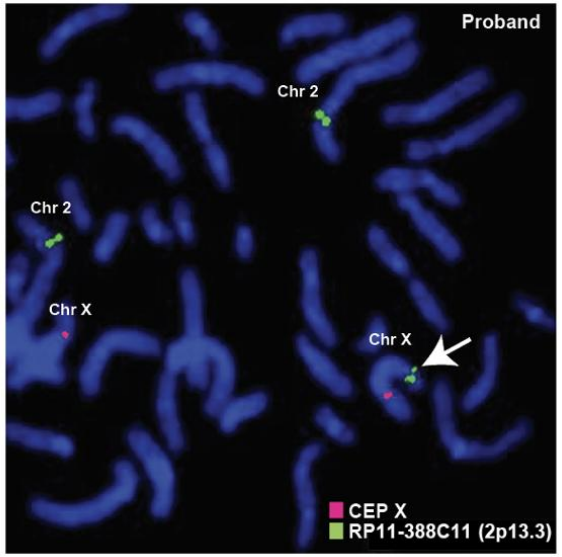
Insertion



Melo et al, *Nat. Commun. 2023*

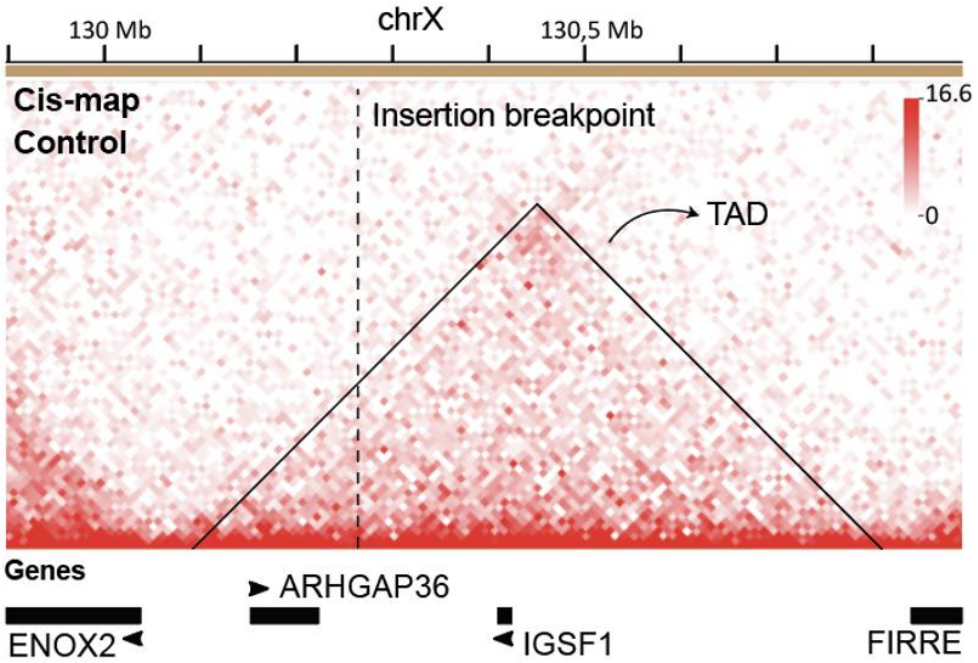
Insertion

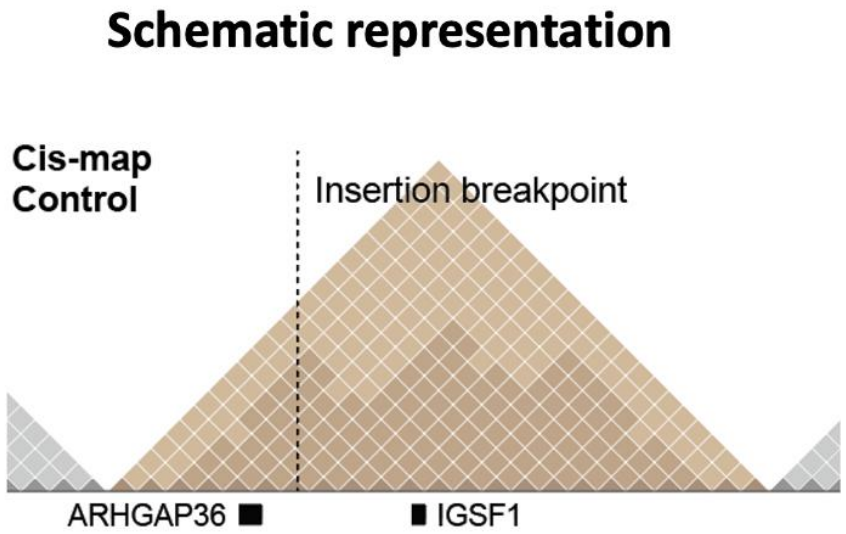




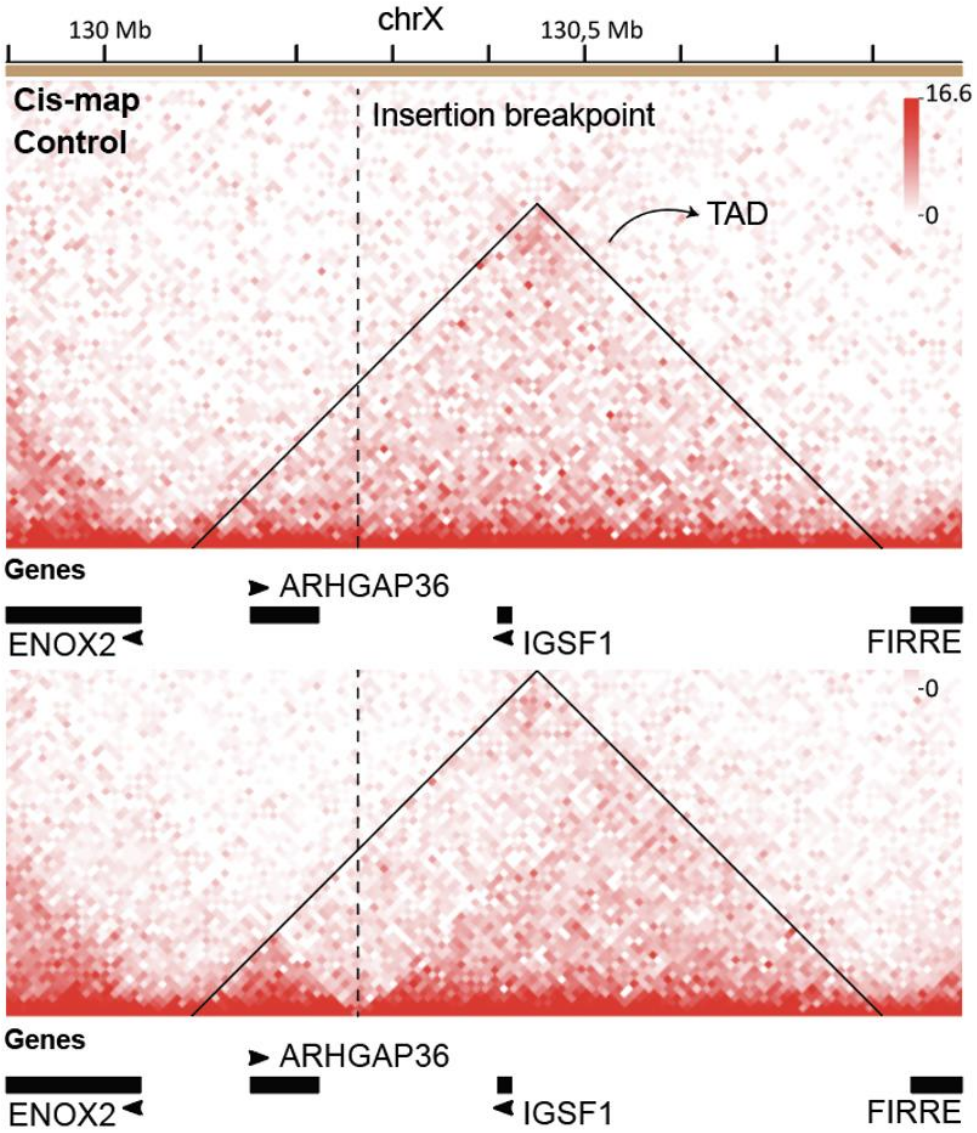
Melo et al, *Nat. Commun. 2023*

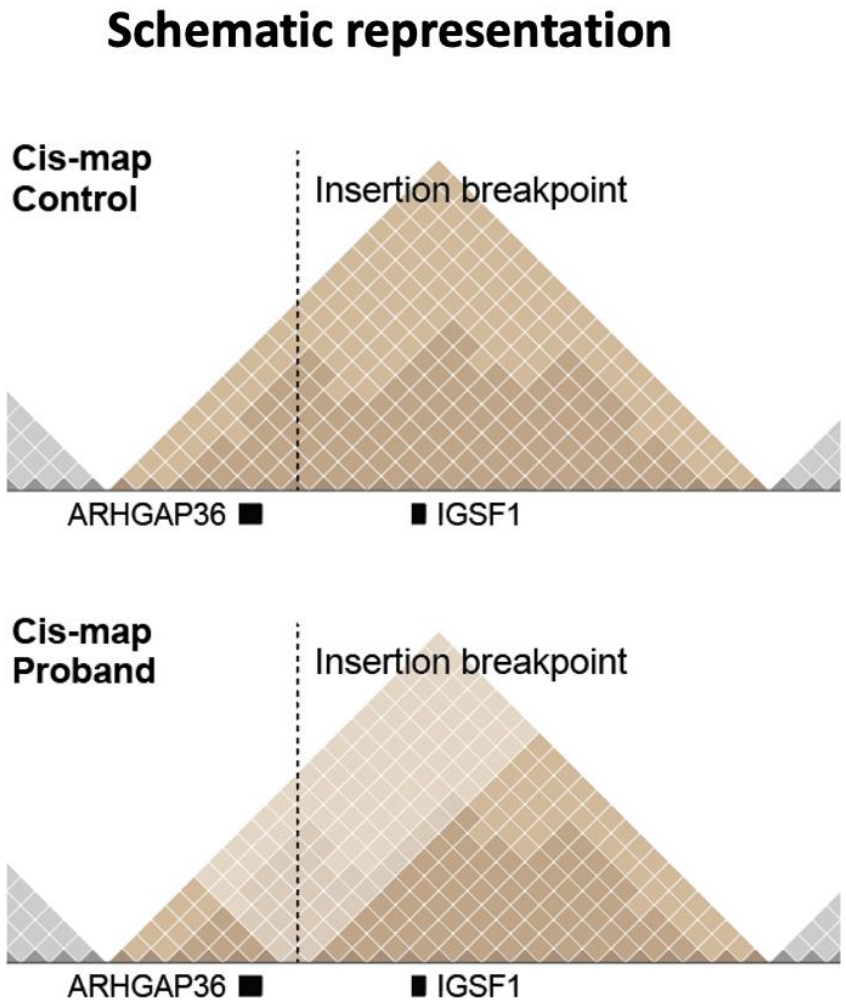
Changes in TAD structure



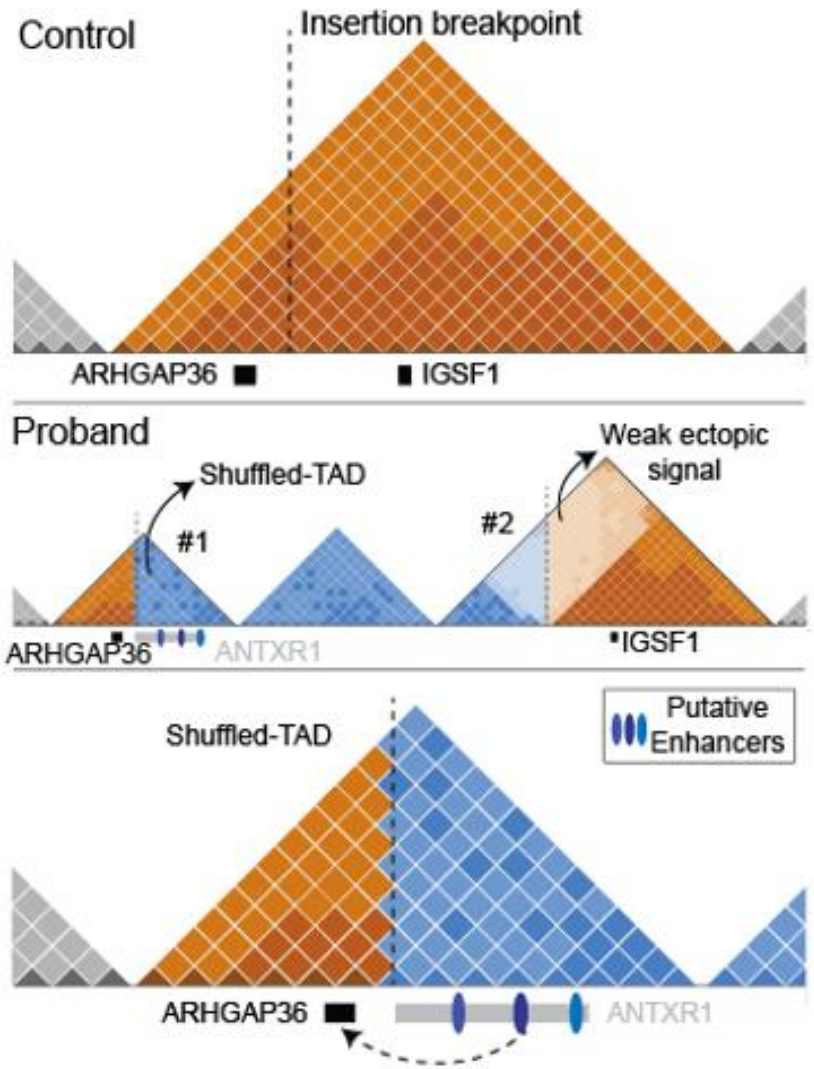


Changes in TAD structure

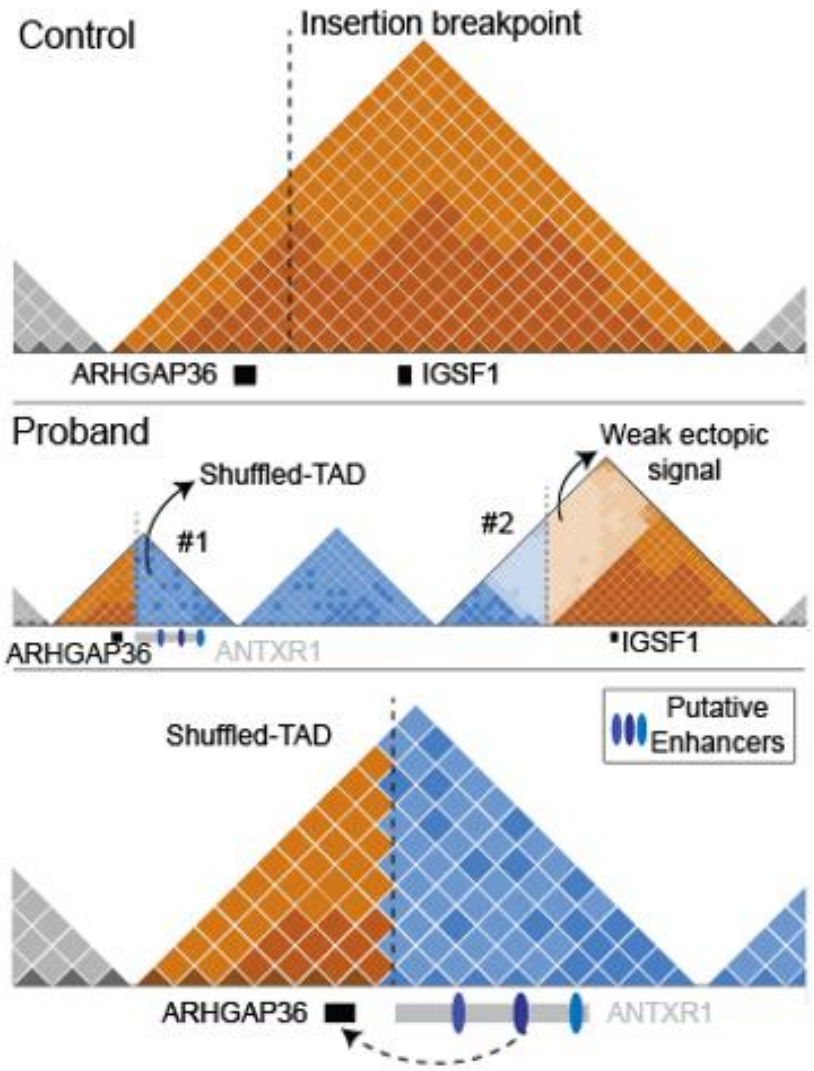


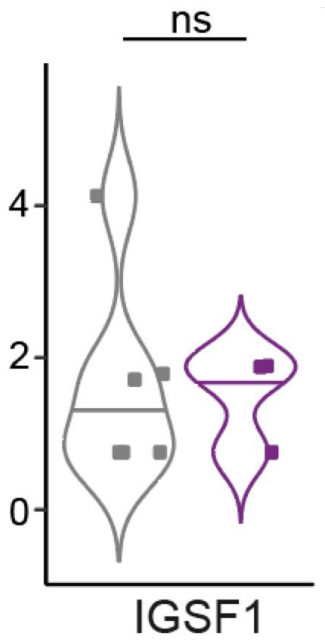


Neo-TAD formation



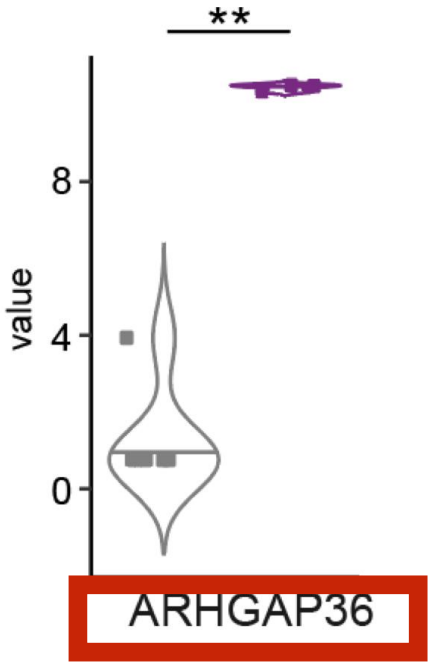
Enhancer Hijacking

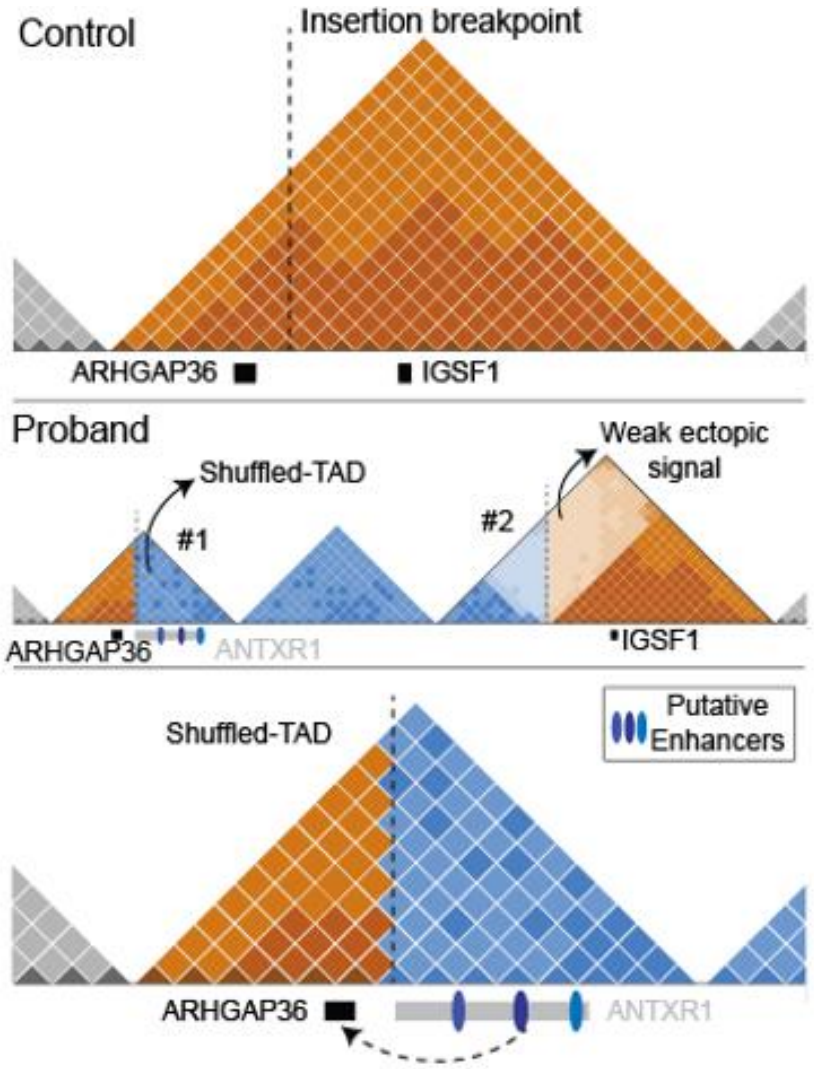


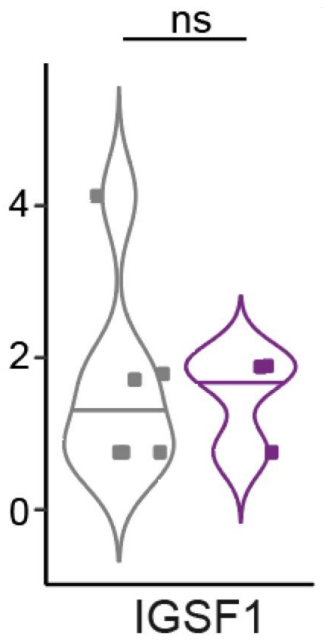


Melo et al, *Nat. Commun. 2023*

Enhancer Hijacking

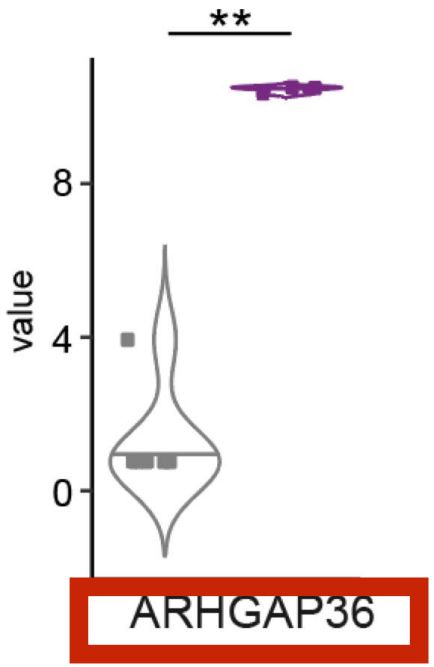


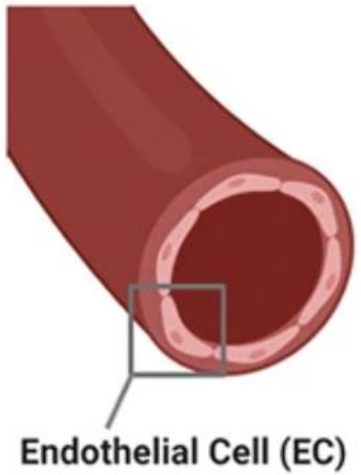


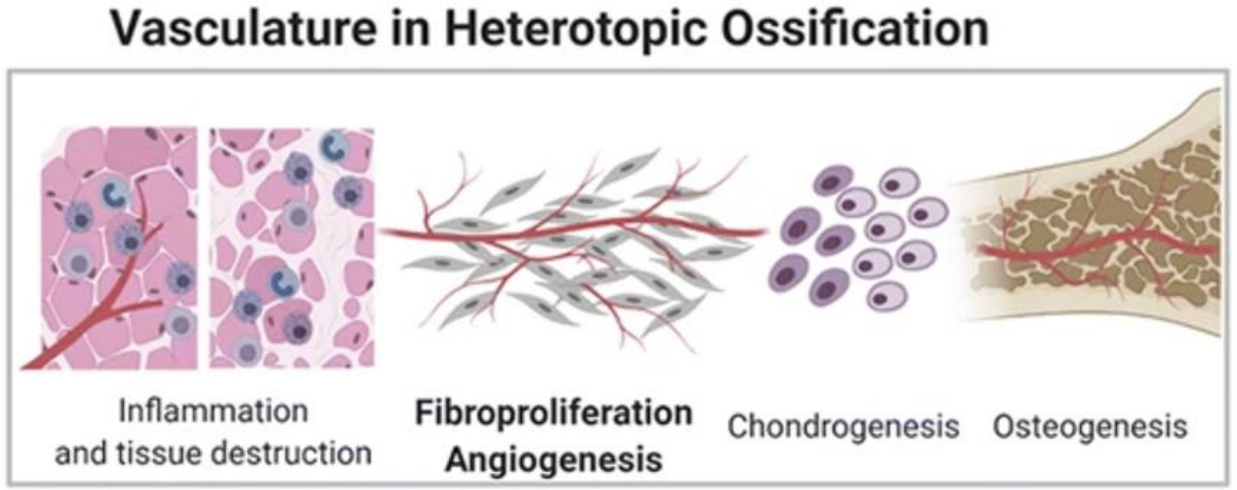


Melo et al, *Nat. Commun. 2023*

Enhancer Hijacking



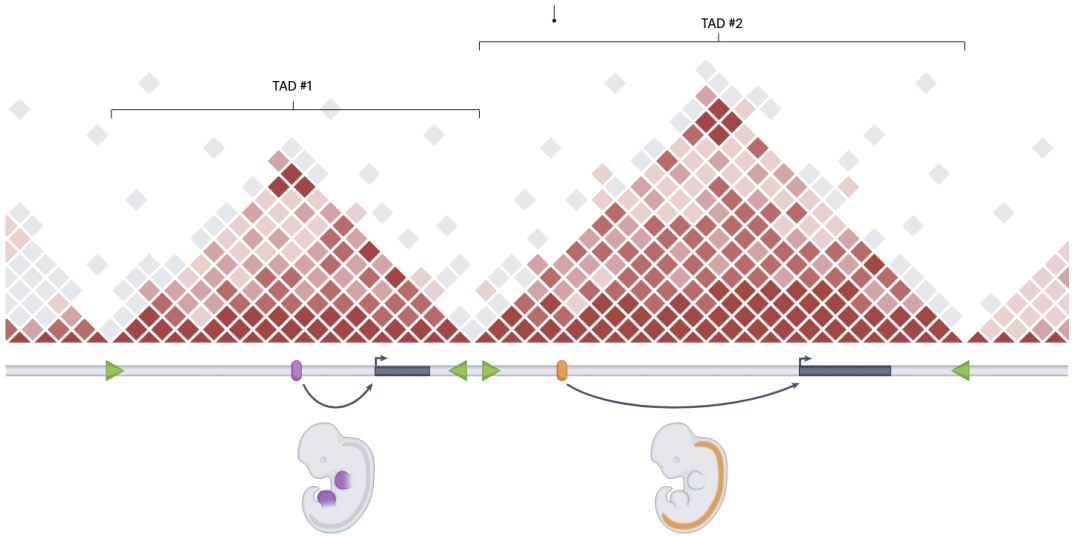




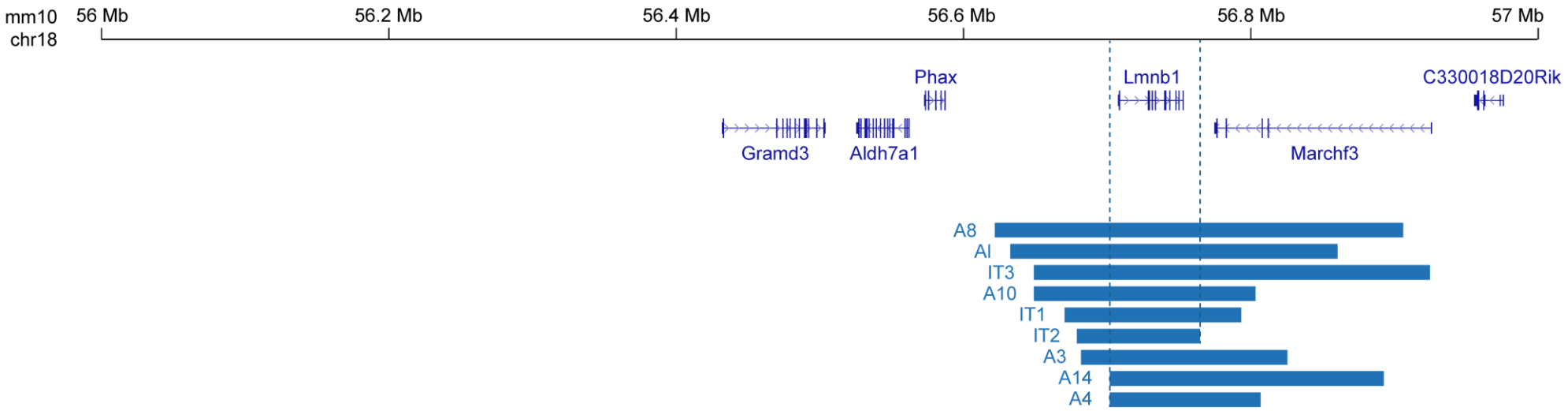
Melo et al, *Nat. Commun. 2023*

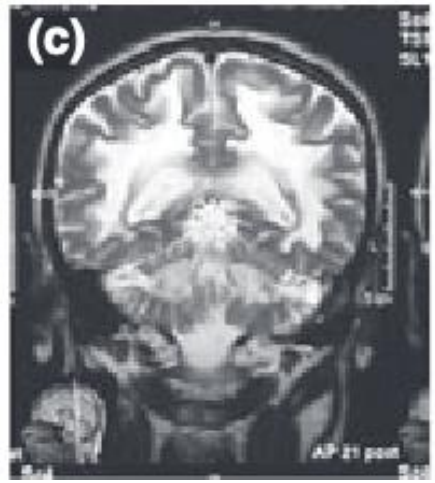
3D position effects in neurological disorders:

more than just bones ?



LMNB1 Duplications cause ADLD

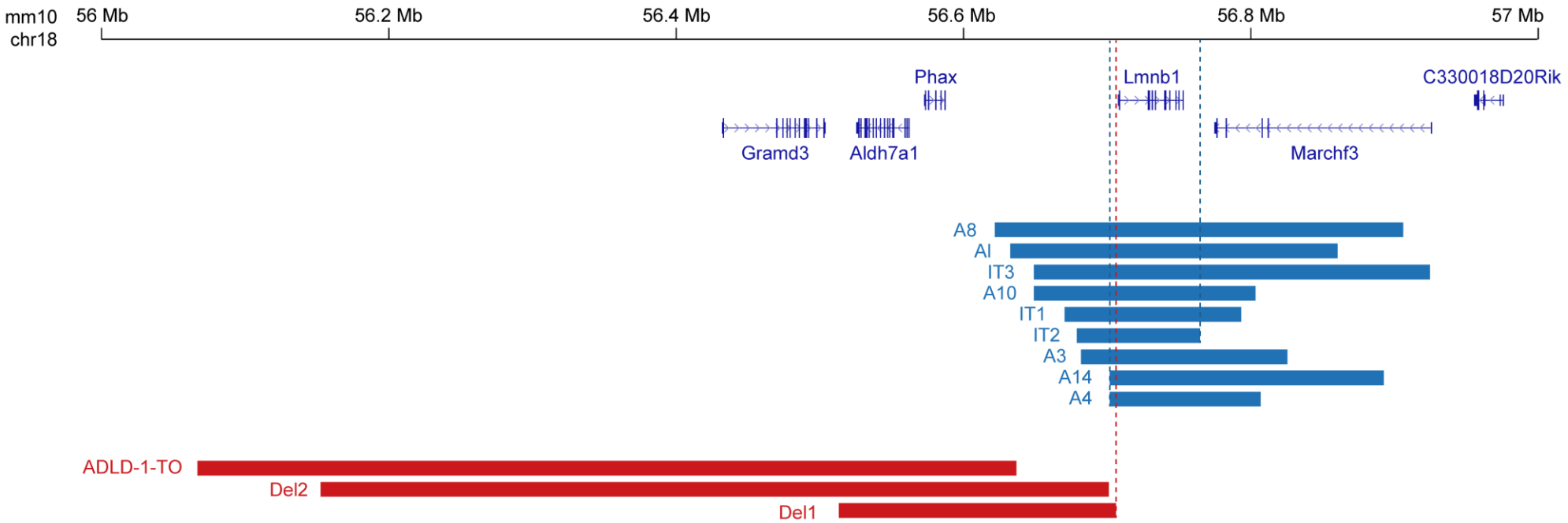


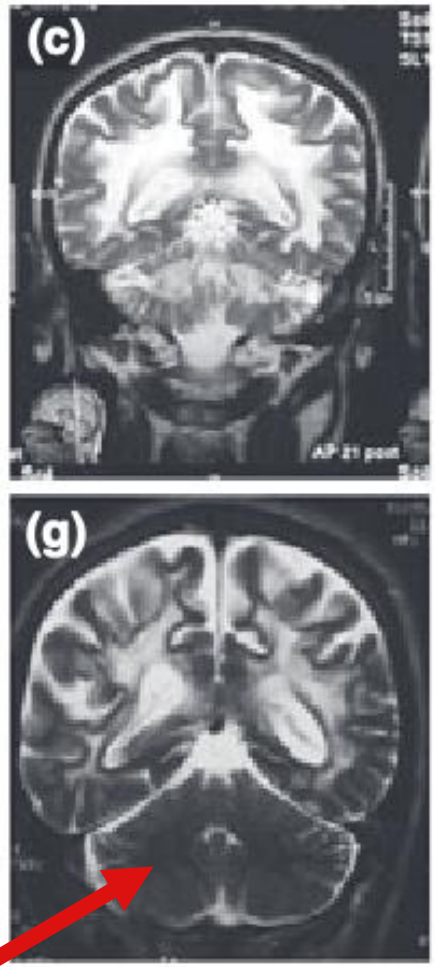


Elisa Giorgio

Brussino et al., Eu. J. Neurol., 2010

Upstream Deletions cause Atypical ADLD

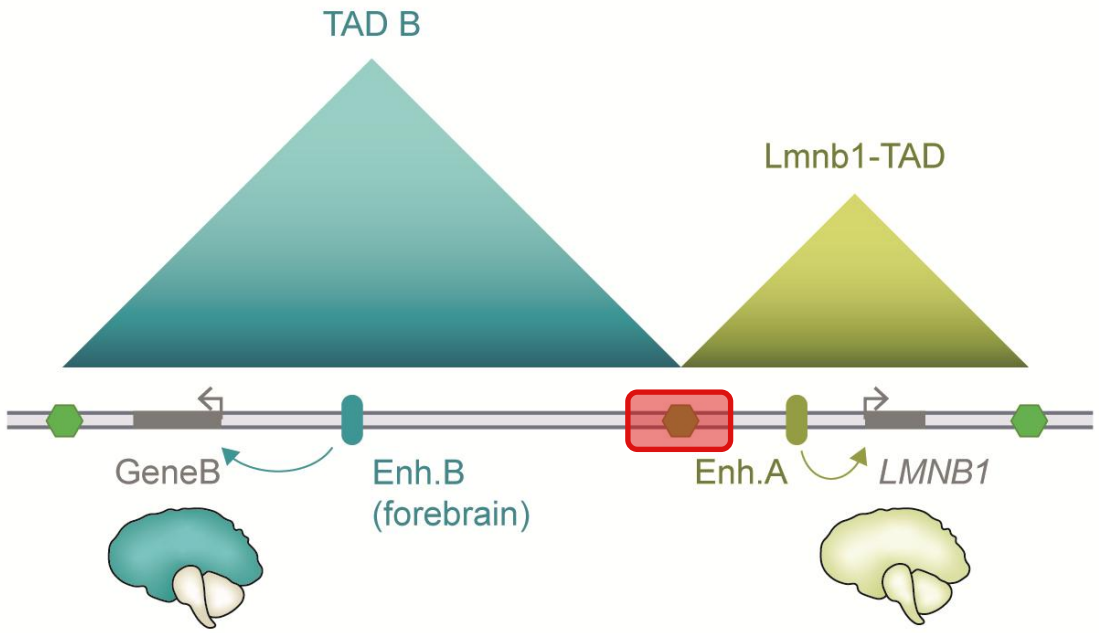




Cerebellum spared

Brussino et al., Eu. J. Neurol., 2010
Giorgio et al., 2013
Nmezi et al., 2019

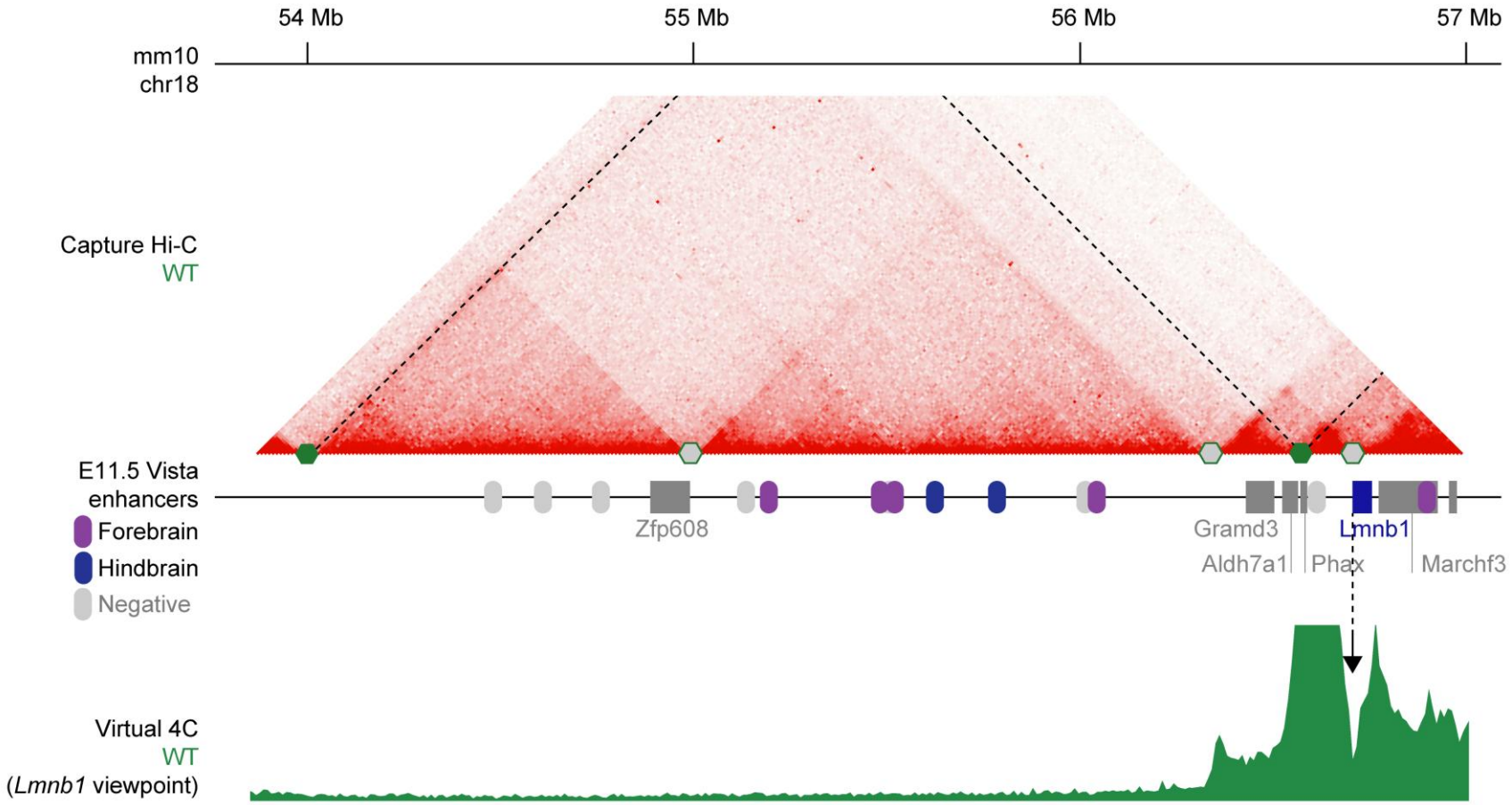
Enhancer hijacking:
A mechanism of *LMNB1* over-expression in Deletions



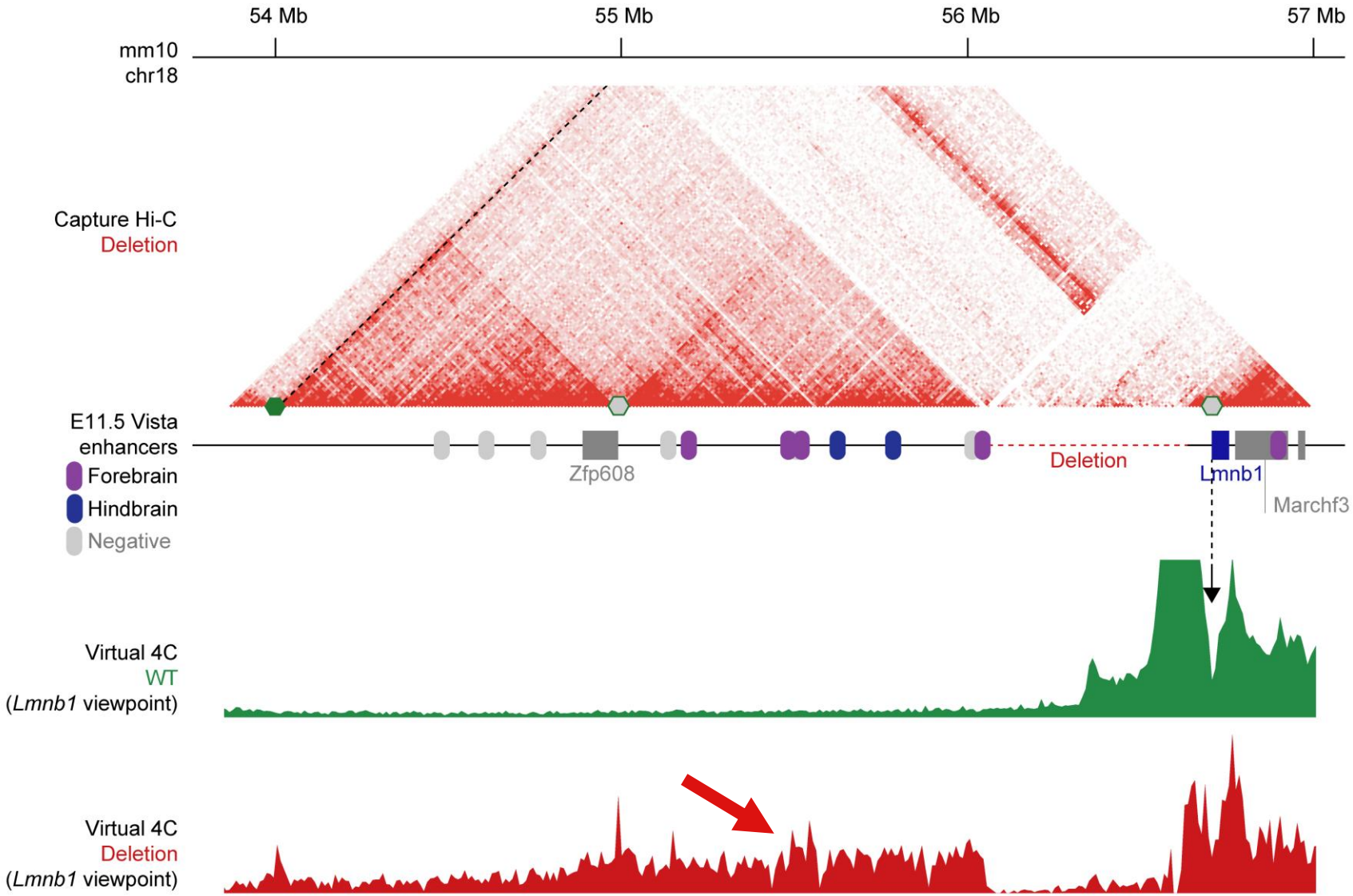
Varun Sreenivasan

Unbiased and multiplexed approach

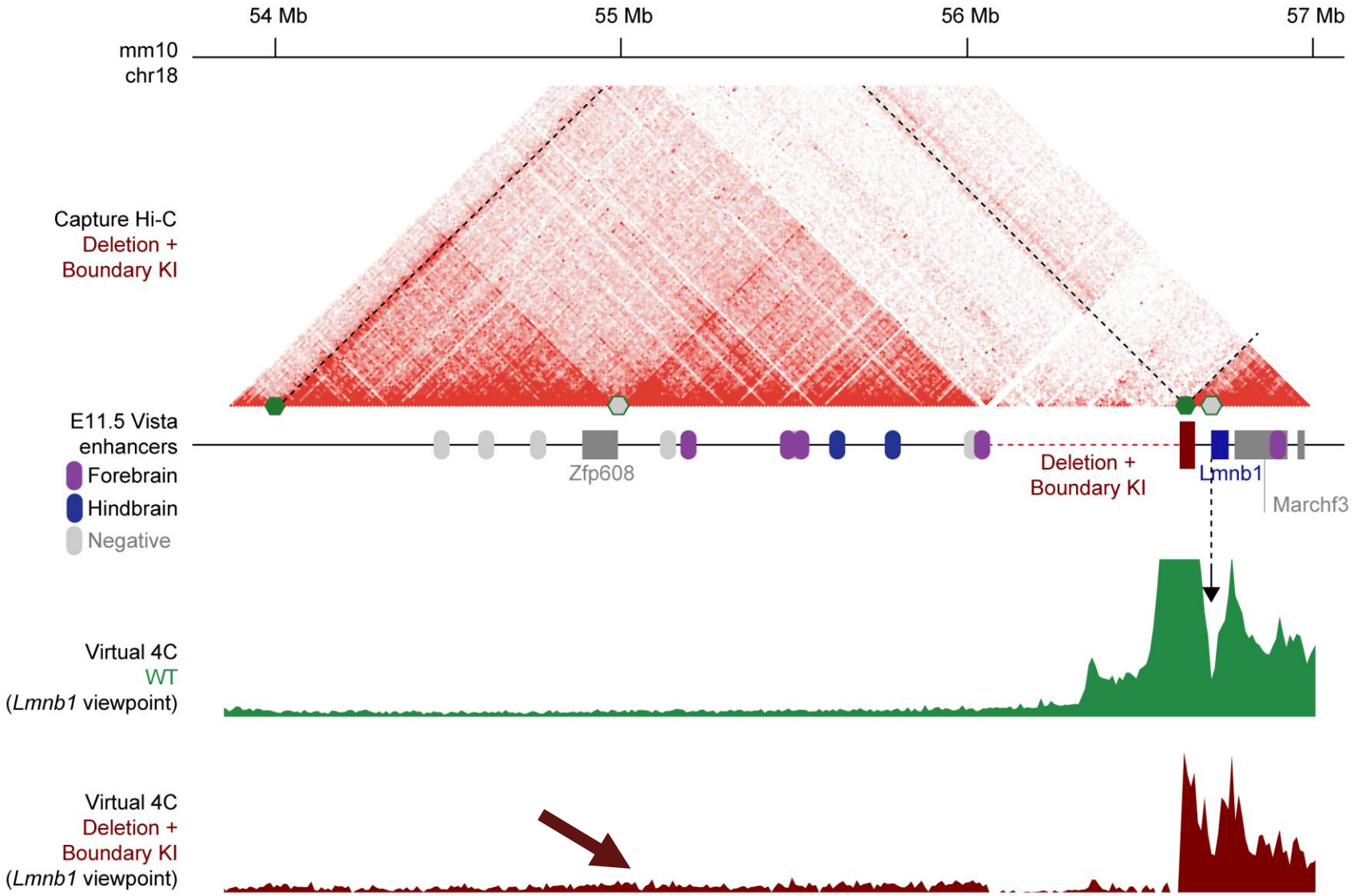
Capture Hi-C of WT



Capture Hi-C of Deletion



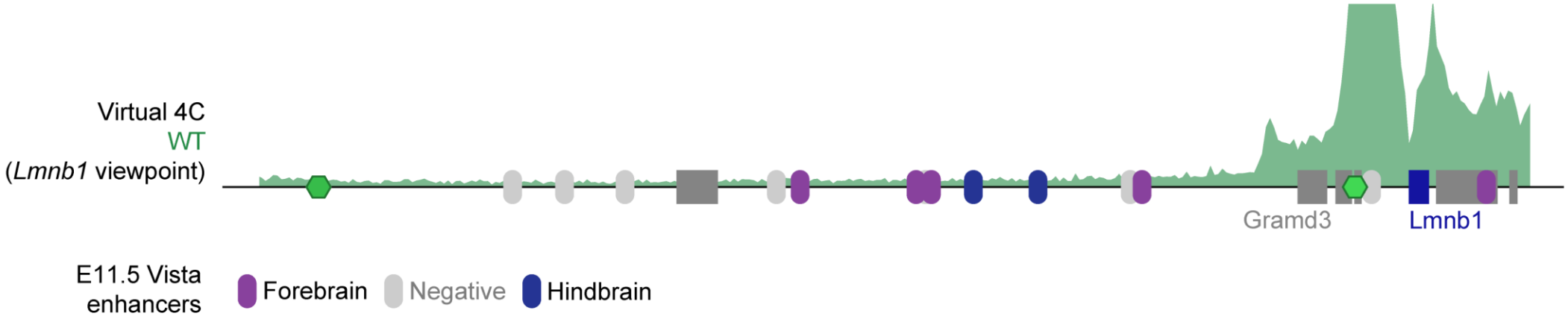
Deletion + Boundary knock-in

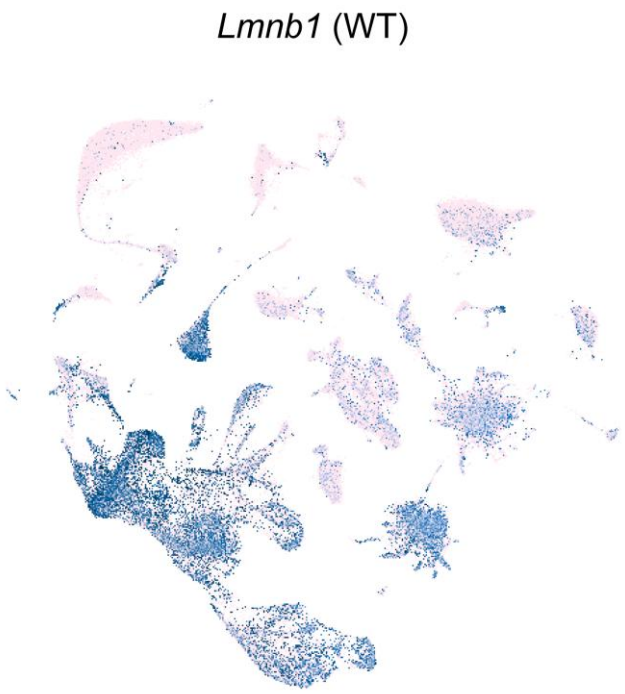


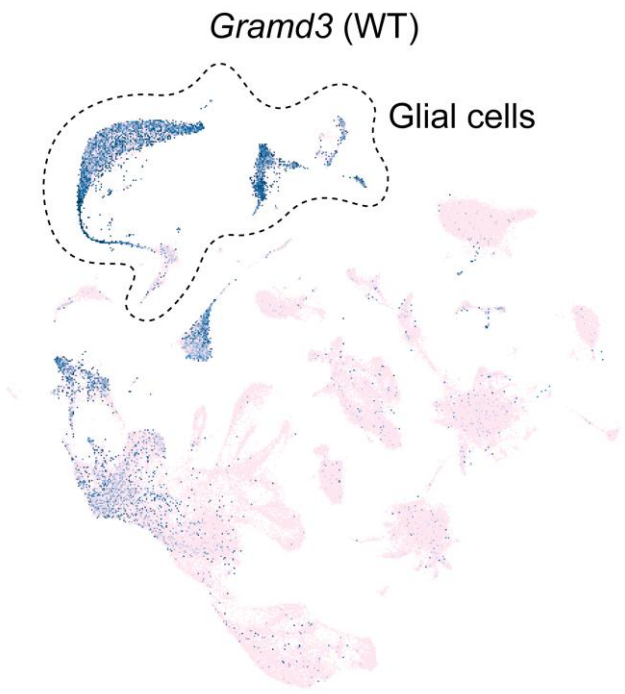
Cell types in the single-cell dataset

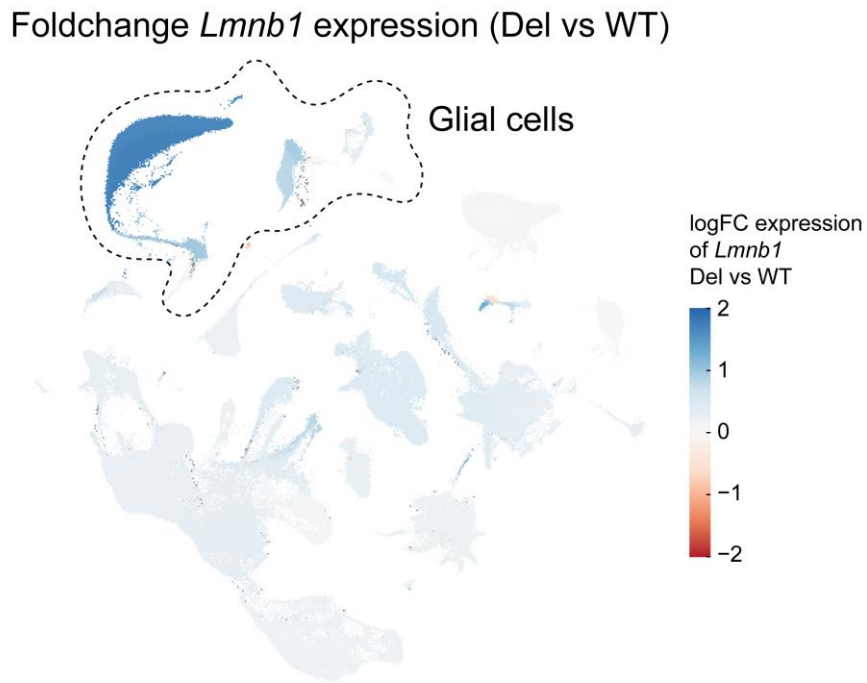
- CbGlutGranNeur
- RadGlia
- Mid-/Hind-GlutNeur
- ODC
- L2/3-GlutNeur
- NeurBlast
- Imm-Gaba/Glut-Neur
- Imm-DG-GlutNeur
- EarlyFibro
- Astro
- GlioblAstroFate
- CbGabaNeur1
- Cx-Gaba/Glut-Neur
- Tiam2+ImmGabaNeur1
- L5/6-GlutNeur
- ImmGabaNeur2
- COP
- Microglia
- Cntnap4+GabaNeur
- Sst/Npy+GabaNeur
- VLMC
- Lamp5/Crh/Vip+GabaNeur
- BergGlia
- Endoth
- CbGabaNeur2
- ImmDopNeur
- Pericyte
- OPC
- ChorPlex
- Cb(Dop)GlutNeur
- PrimErythro
- OlfEnshCell
- Astro2

Lmnb1 hijacks *Gramd3* enhancers

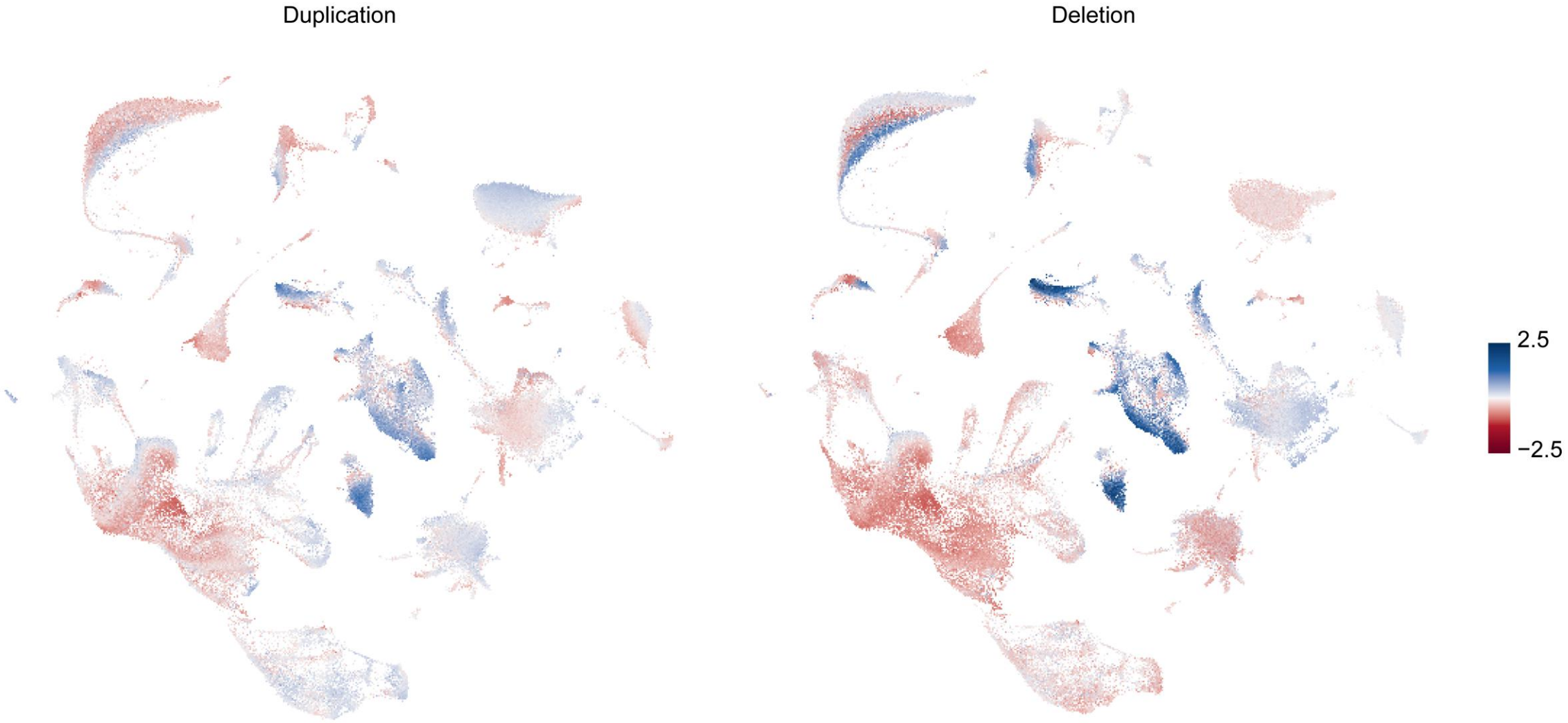








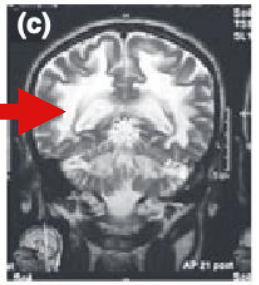
LochNESS analysis shows celltype specific changes

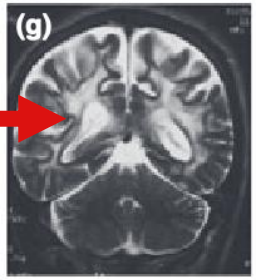


Shared phenotype of L2-6 Neurons of the cerebral cortex

Duplication

Deletion





2.5

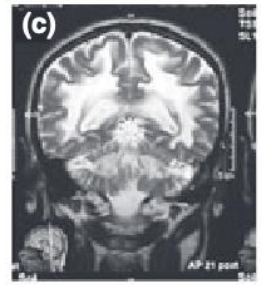
−2.5

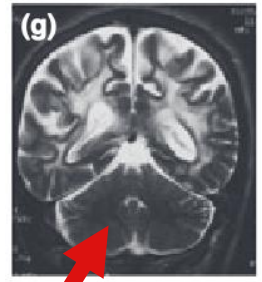
Cerebrum

Divergent phenotypes of excitatory neurons of the cerebellum

Duplication

Deletion



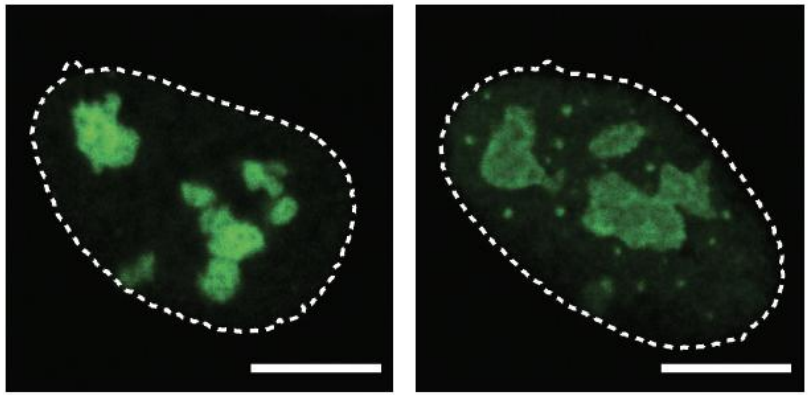


2.5

−2.5

Cerebellum

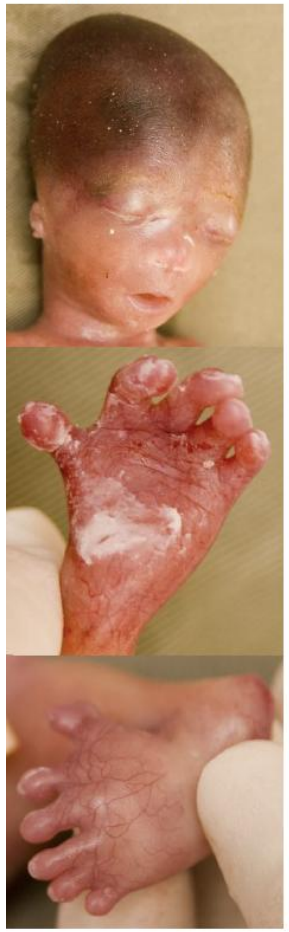
More than just TADs ?



Could coding mutations also changes 3D architecture?

Mutations in *HMGB1*

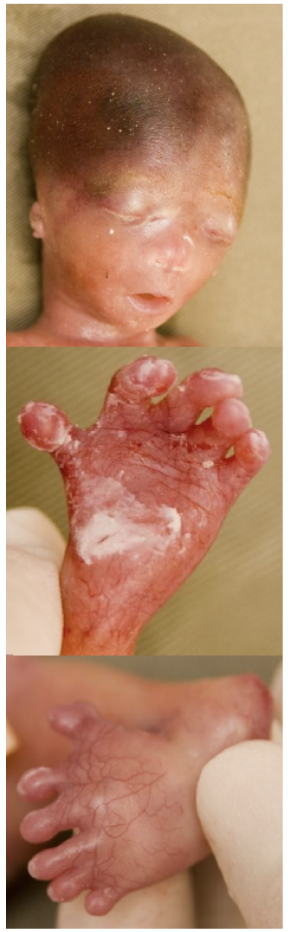
Individual 4

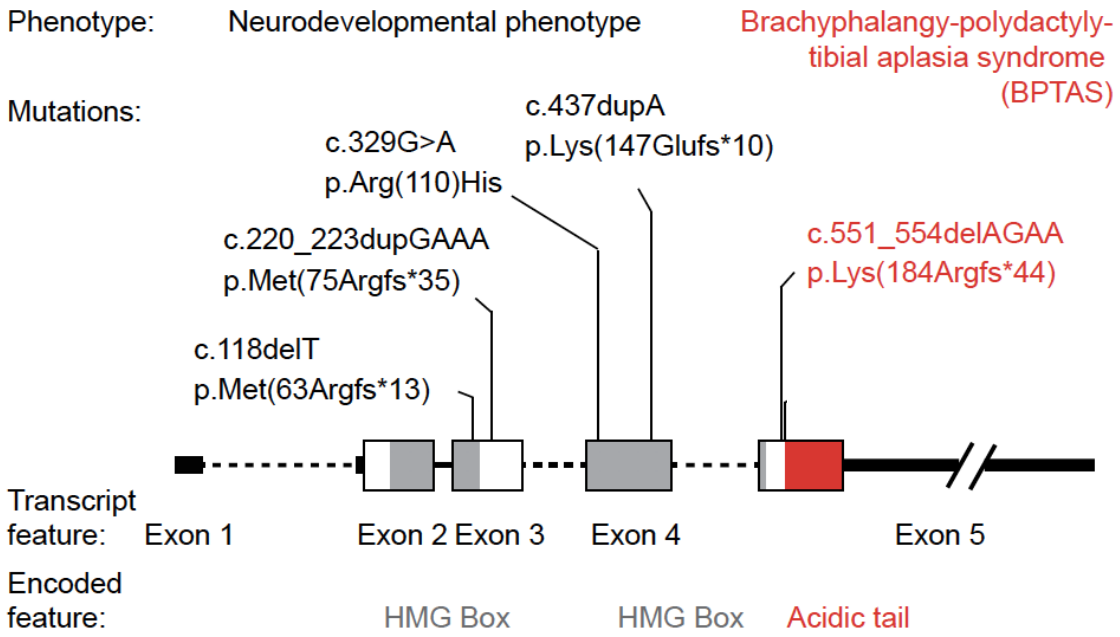


Martin Mensah

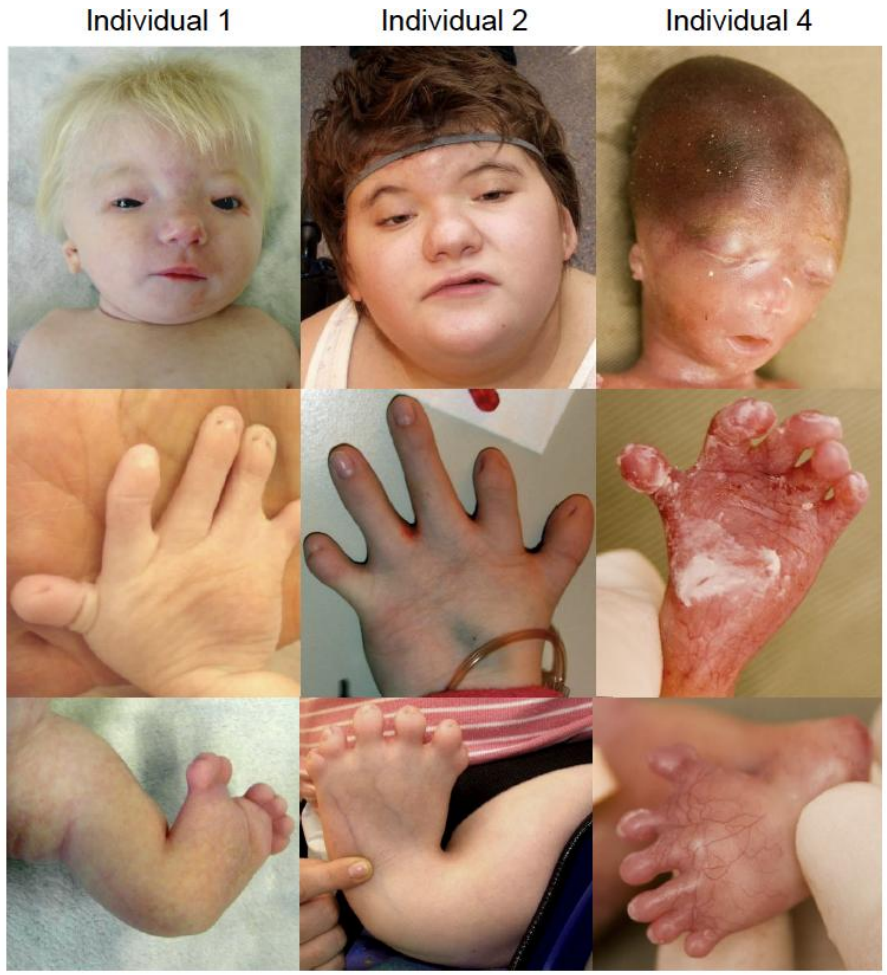
Mutations in *HMGB1*

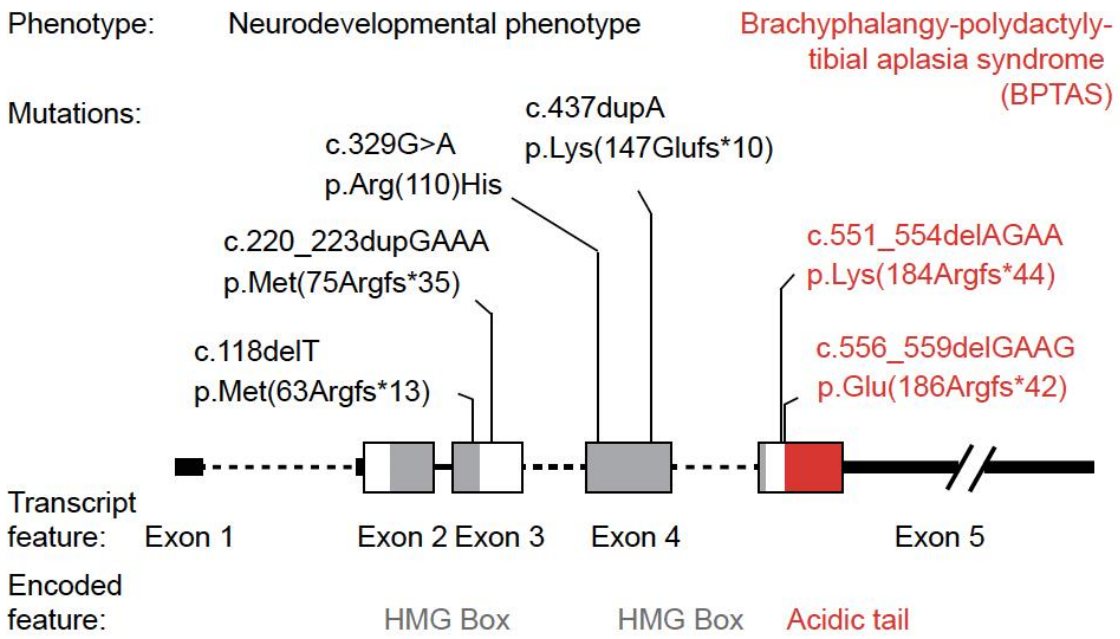
Martin Mensah





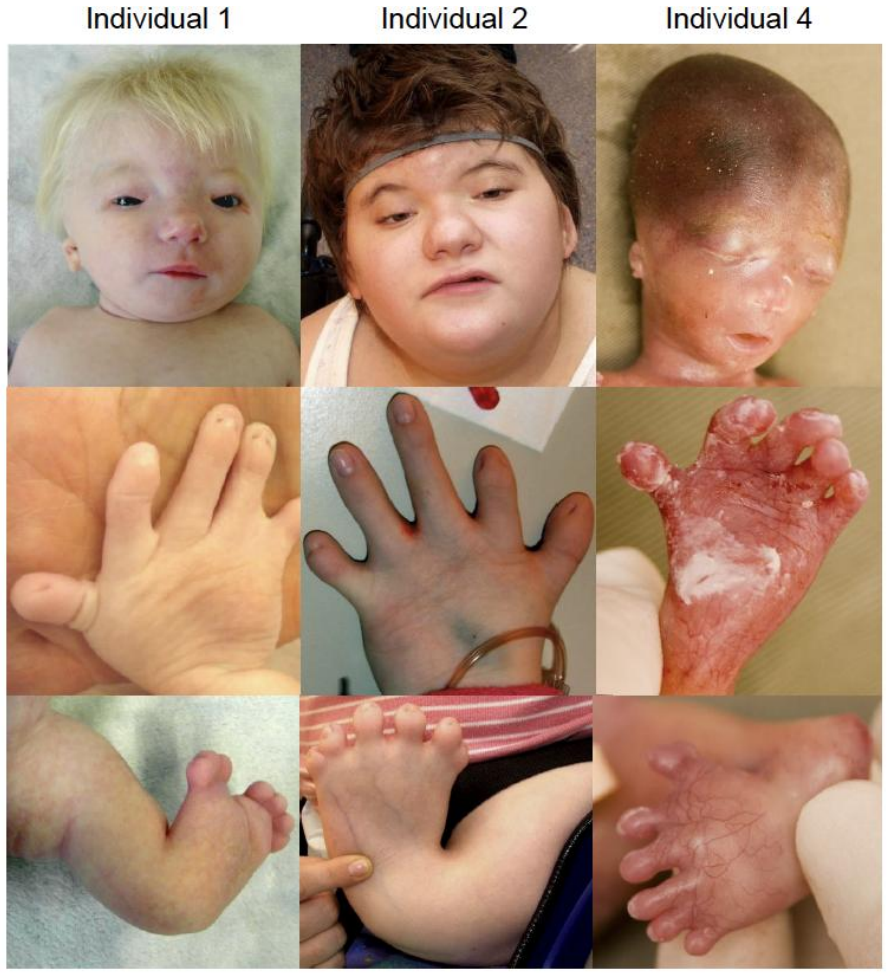
Mutations in *HMGB1*

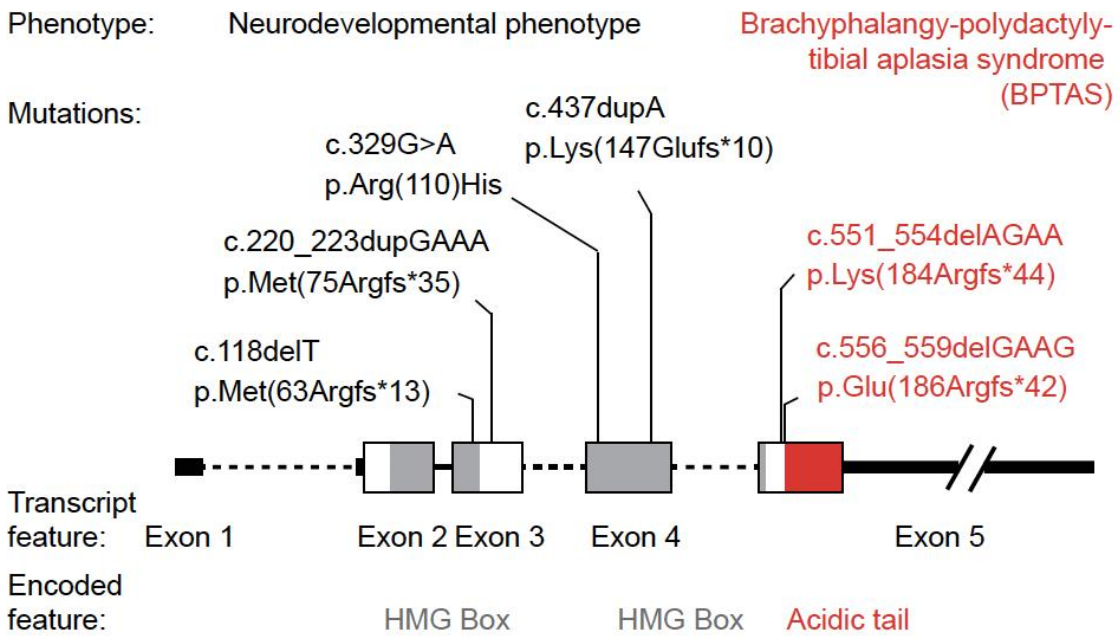


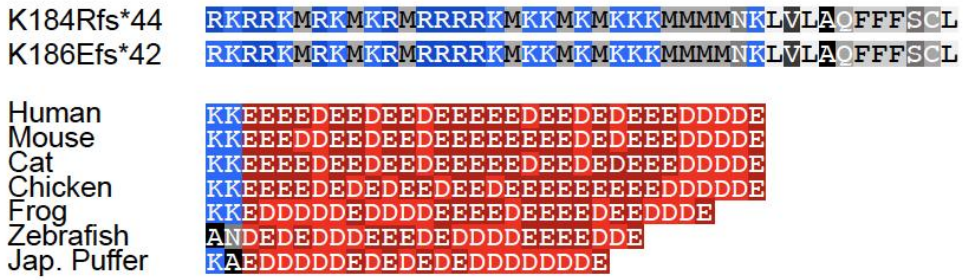


Mensah et al. *Nature* 2023

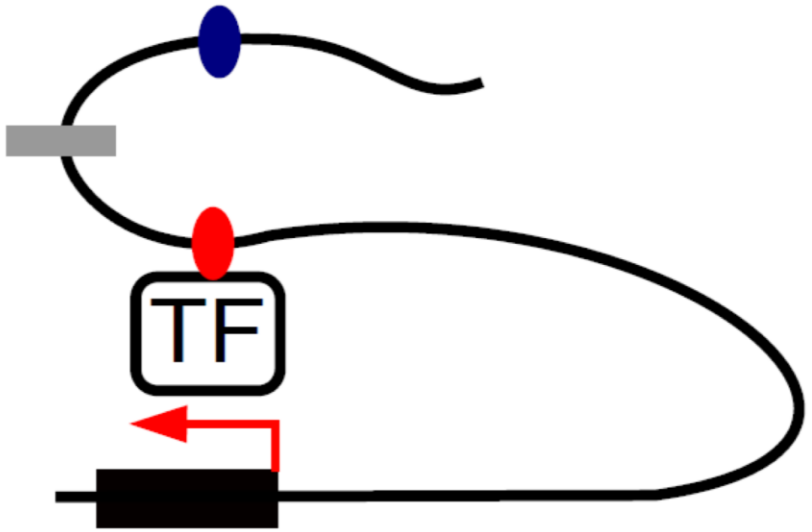
Mutations in *HMGB1*







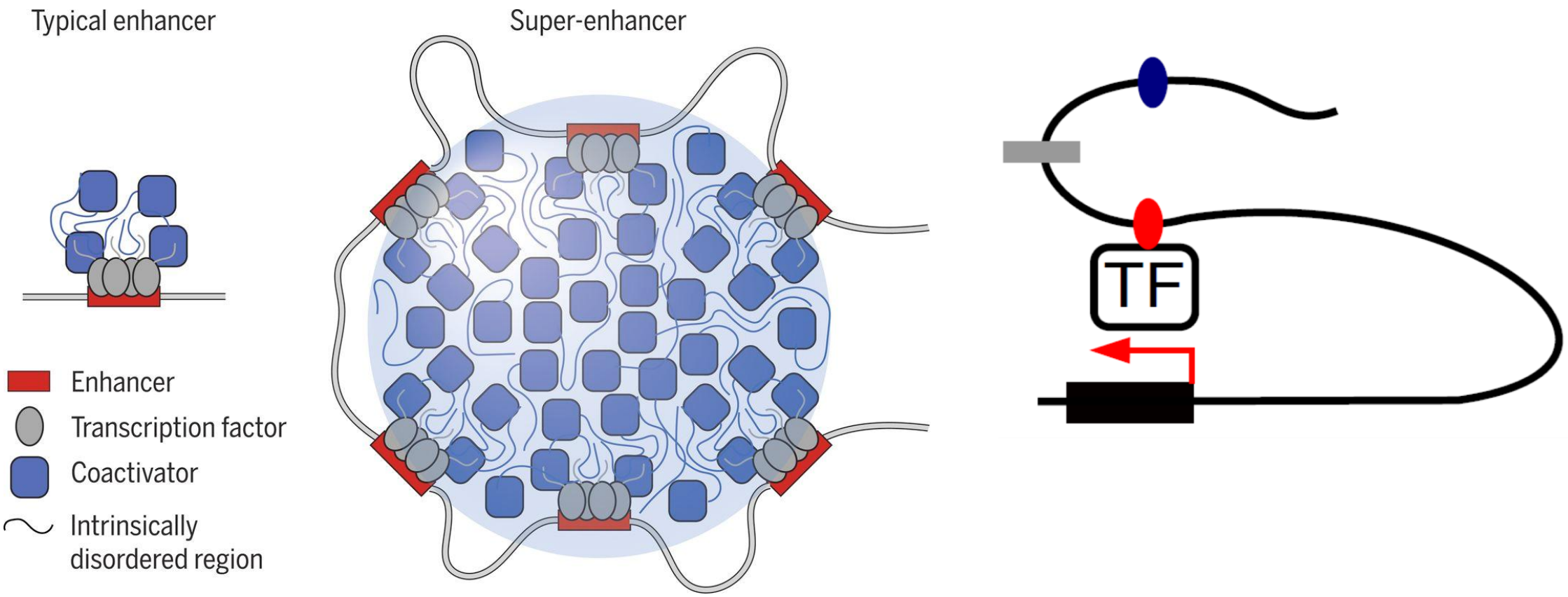
Mutations in *HMGB1*



Denes Hnisz

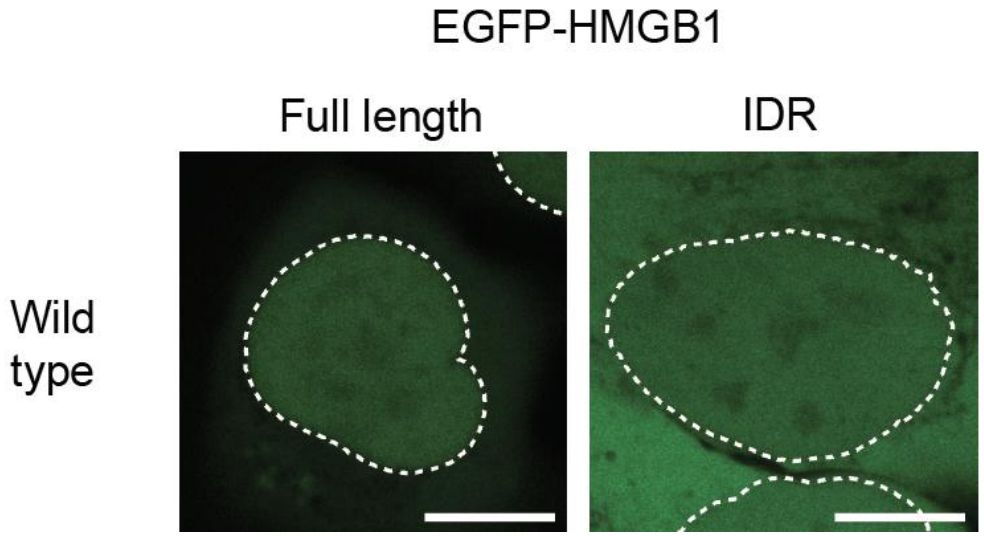
Spielmann et al, *Nat. Rev. Genet*. 2018

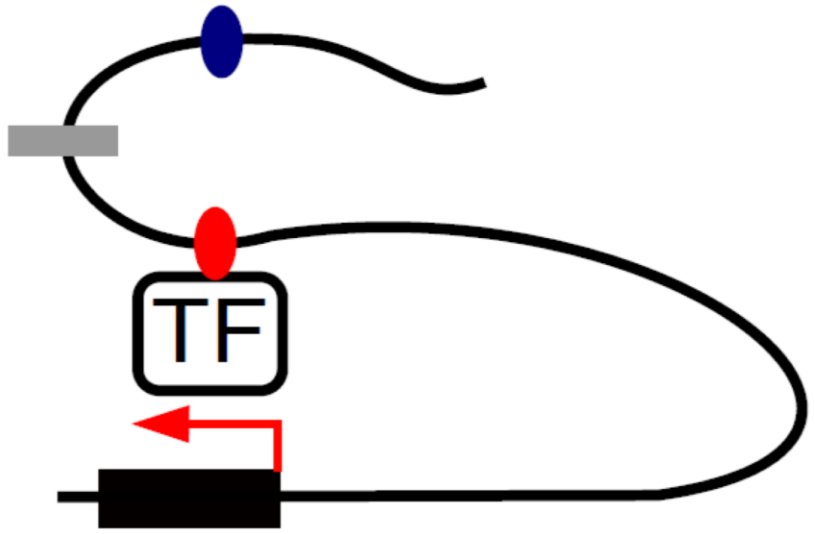
Phase separation



Sabari et al, *Science*. 2018

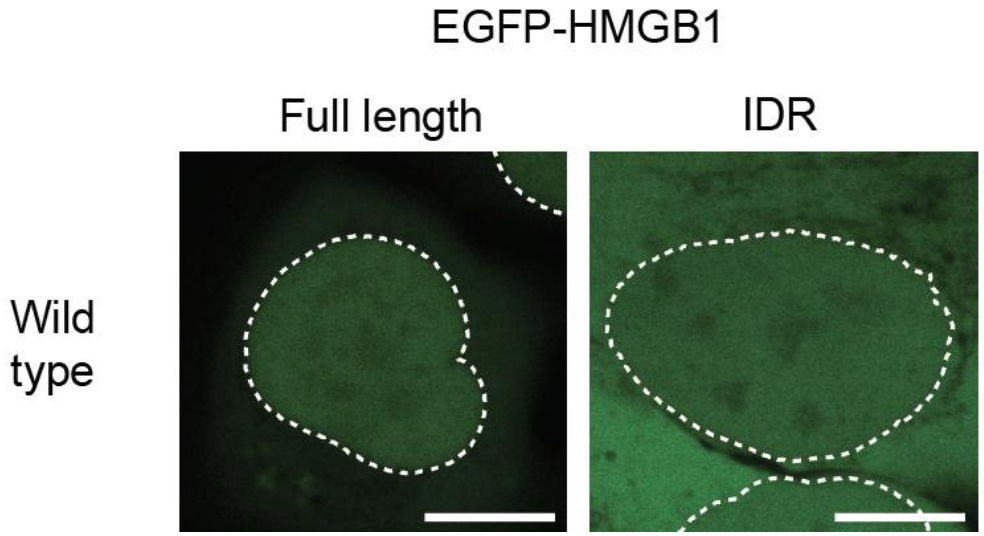
Mutations change phase separation capacity

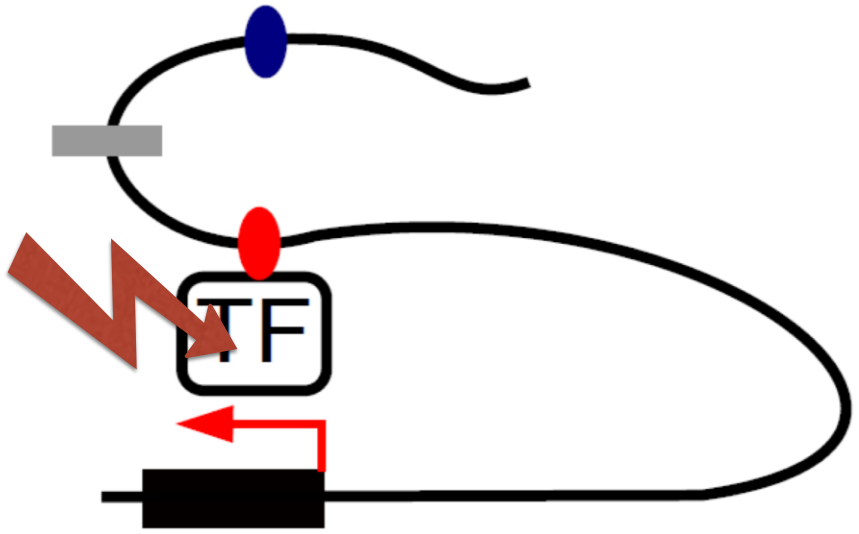




Mensah et al. *Nature* 2023

Mutations change phase separation capacity





Mensah et al. *Nature* 2023

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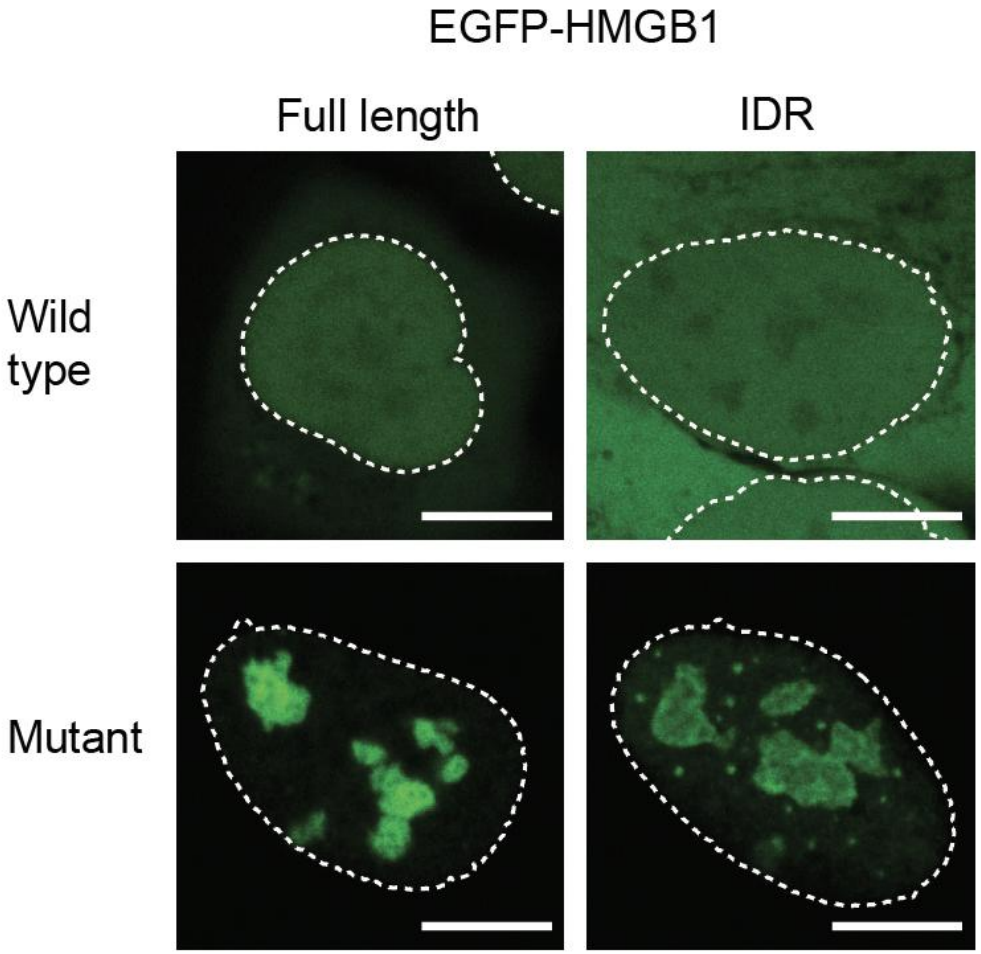
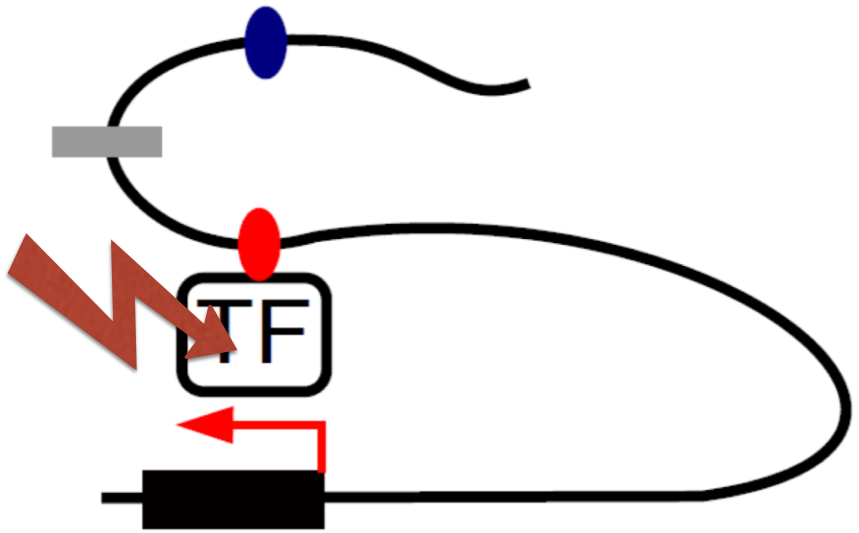
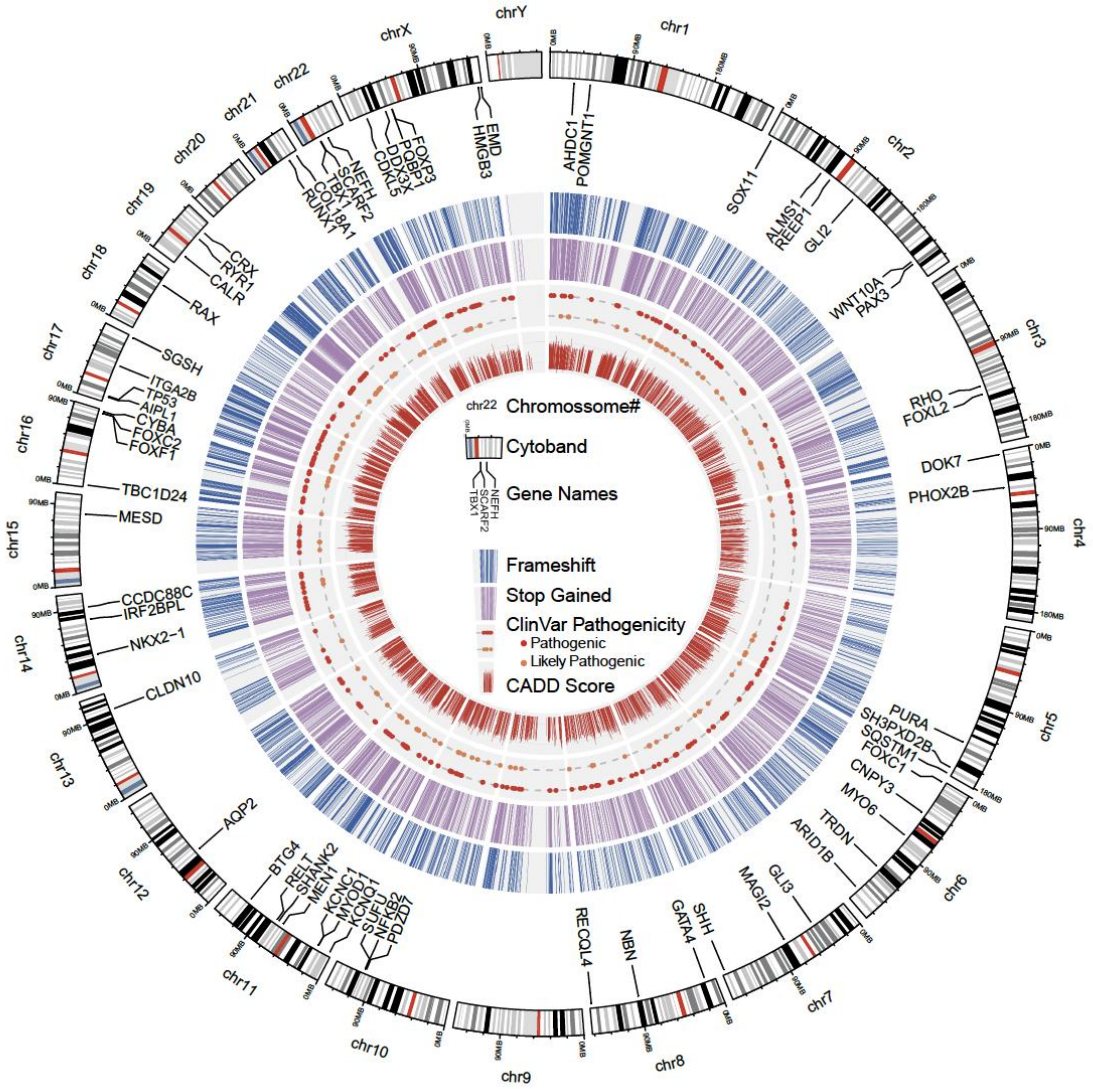
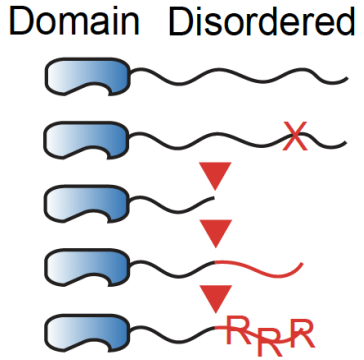




Mensah et al. *Nature* 2023

Over 600 mutations change phase separation capacity

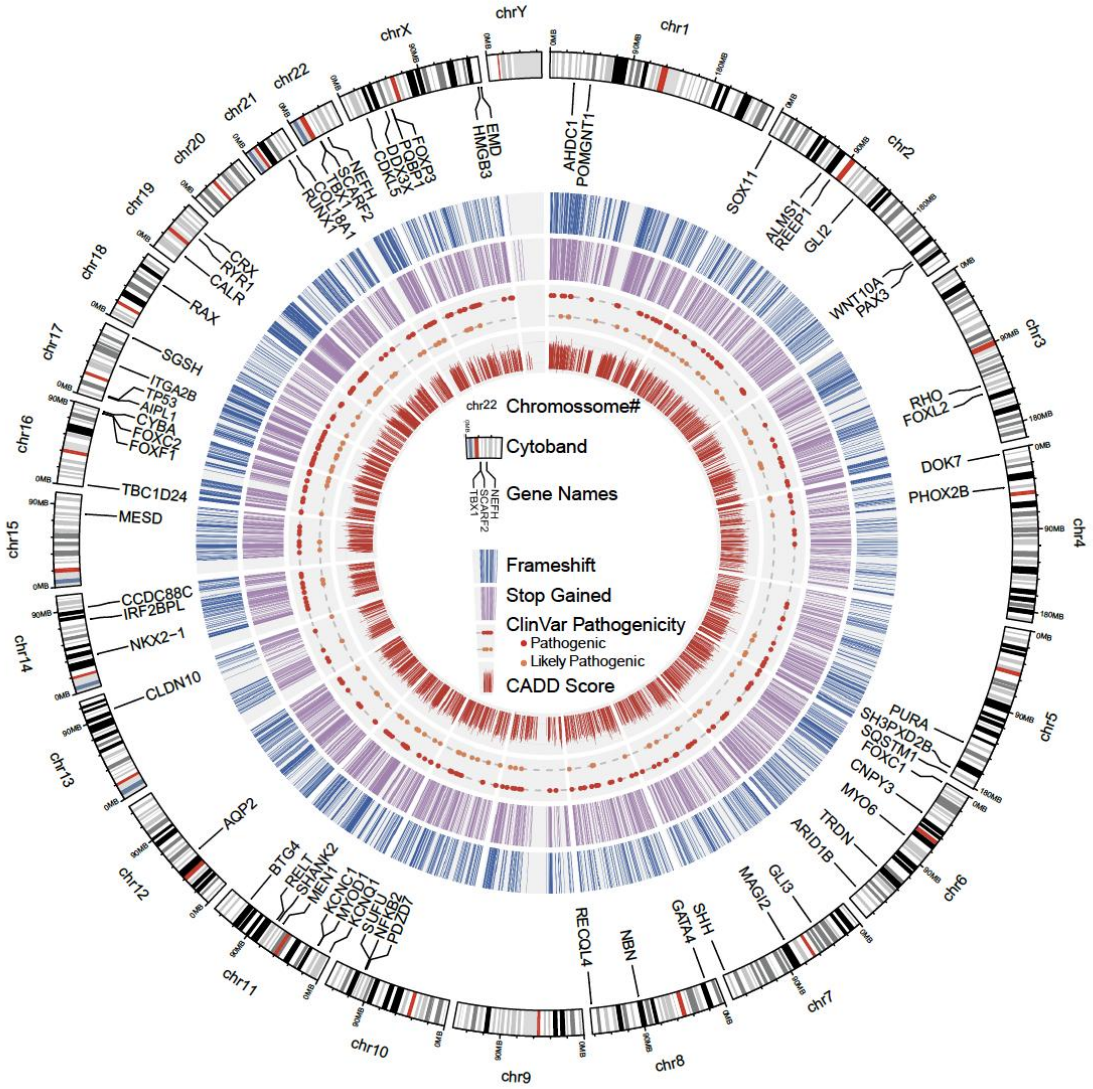




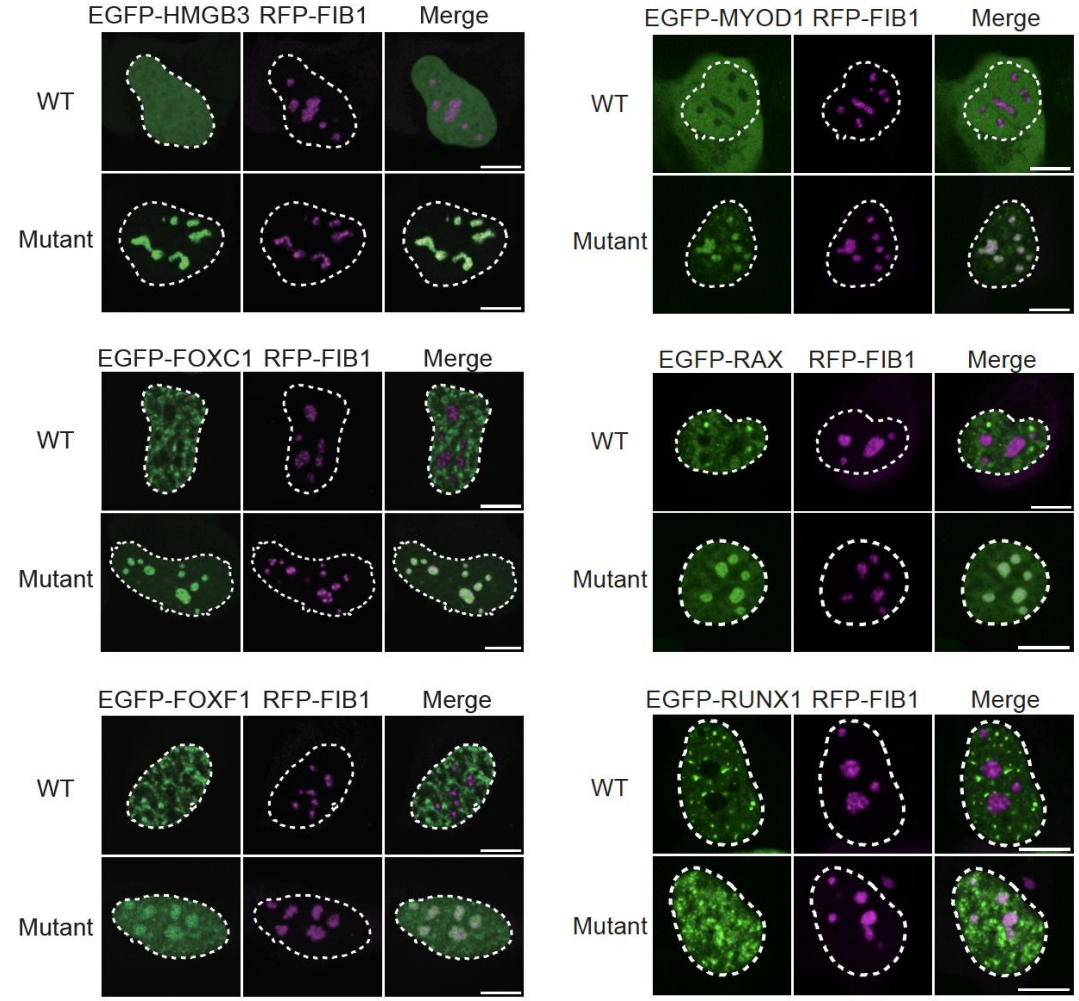
5,618 genes	
SNP	222,983
Stop gained	10,023
Frameshift (>20aa)	3,890
Frameshift (>15%R)	625

Mensah et al. *Nature* 2023

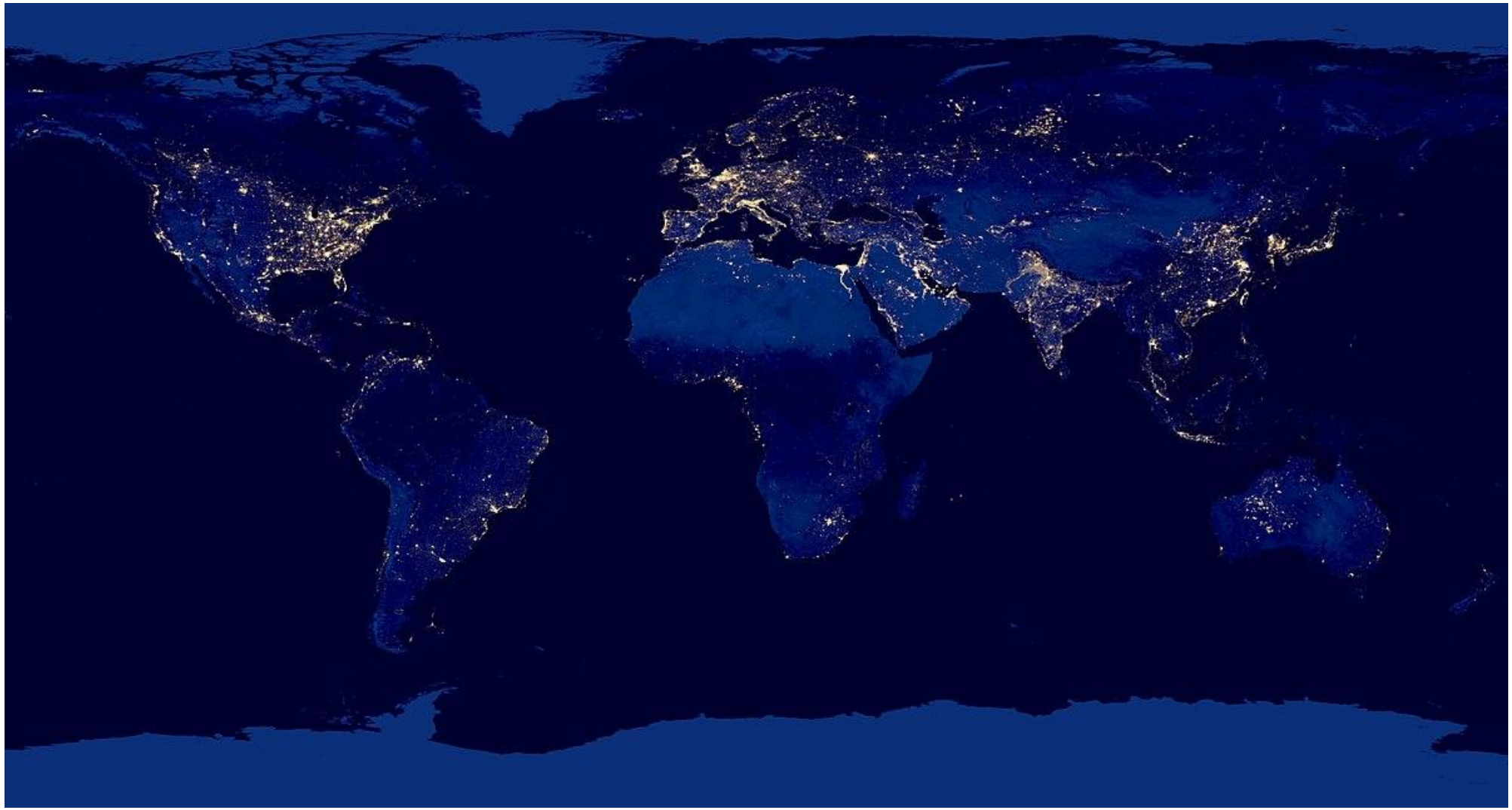
Over 600 mutations change phase separation capacity



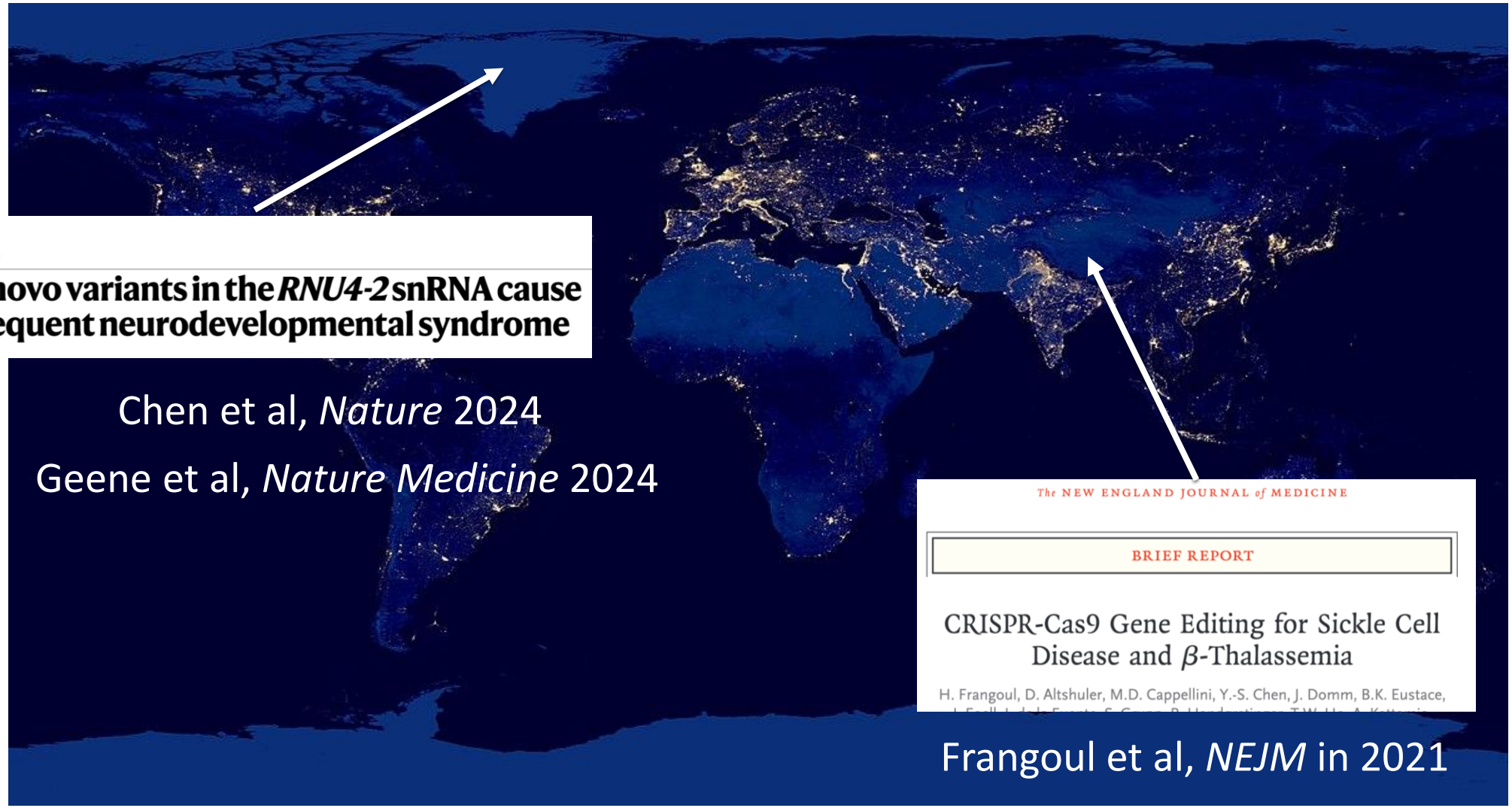
12 out of 13



The non-coding genome



The dark side of the genome



Article

De novo variants in the *RNU4-2* snRNA cause a frequent neurodevelopmental syndrome

Chen et al, *Nature* 2024

Geene et al, *Nature Medicine* 2024

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

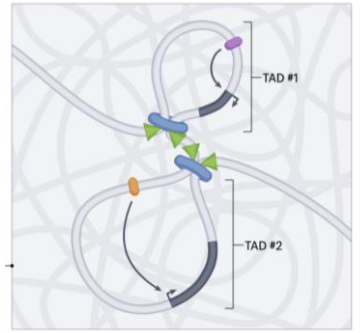
CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia

H. Frangoul, D. Altshuler, M.D. Cappellini, Y.-S. Chen, J. Domm, B.K. Eustace,

Frangoul et al, *NEJM* in 2021

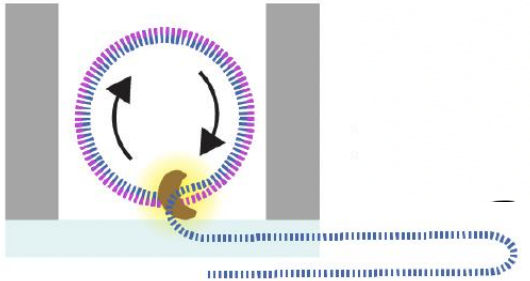
Summary

1: Structural Variants need to be studied in 3D



2: Think beyond the Exome

3: The non-coding genome is the next frontier in human genetics



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Kirstin Schulz
Jelena Pozojevic
Anna Liegmann
Nina Mellmann
Annelie Warnacke
Daniel Kaschta

Martin Mensah
Kristin Schultz
Verónica Corral

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DFG

Heisenberg-Programm

EUROPEAN JOINT PROGRAMME RARE DISEASES

IMPRS BAC

Genome^3 SPP2202

DZHK
DEUTSCHES ZENTRUM FÜR HERZ-KREISLAUF-FORSCHUNG E.V.

Acknowledgements

Universitätsklinikum Schleswig-Holstein, Kiel & Lübeck


Questions?

European Commission

EUROPEAN REFERENCE NETWORKS

Helping patients with rare or low-prevalence complex diseases

3. Interpreting the impact of noncoding structural variants in neurodevelopmental disorders -

Prof. Sarah VERGULT, Ghent University, Ghent, Belgium

European Reference Networks ITHACA

FUNGEN
functional genomics lab
at Ghent University

ITHACA Webinar| 23 September 2025

Interpreting the impact of noncoding structural variants in neurodevelopmental disorders

Sarah Vergult

Ghent University | Department of Biomolecular Medicine
Center for Medical Genetics

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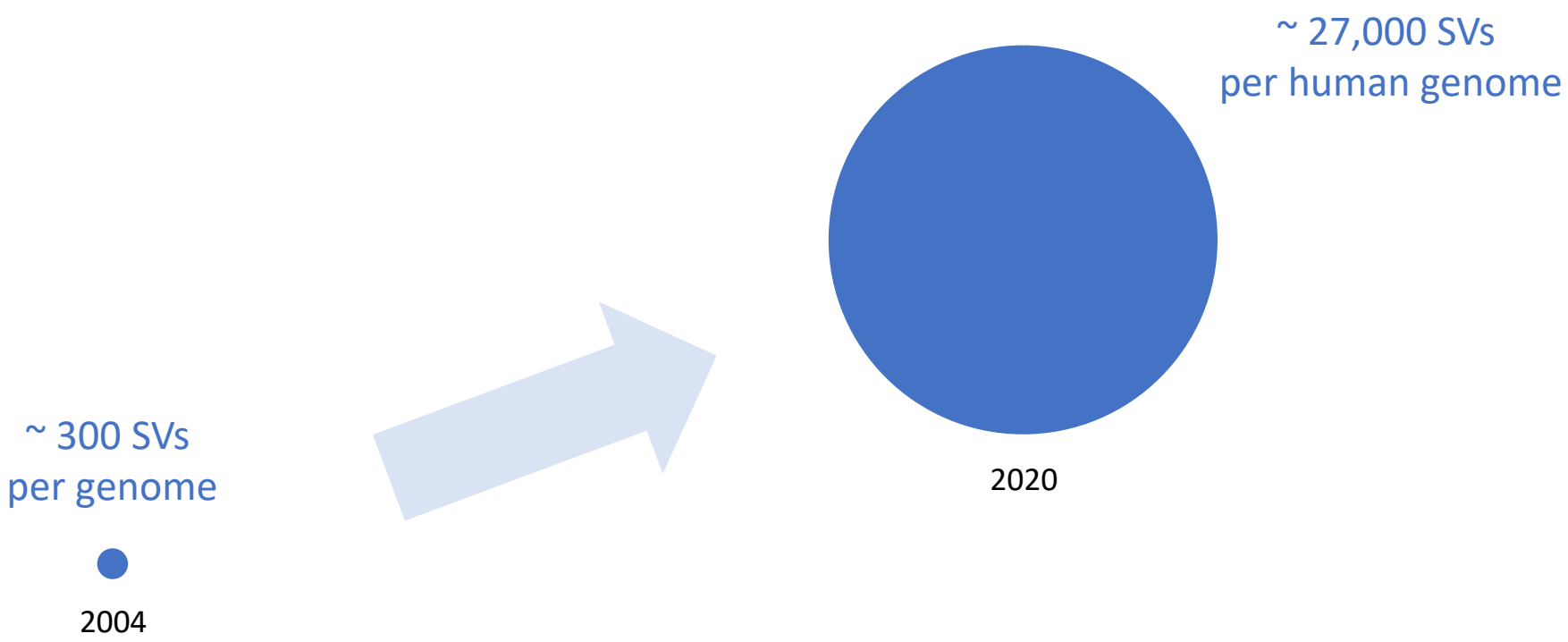
Advances in sequencing technologies have tremendously improved SV detection



Ho, Urban & Mills, Nat Rev Genet, 2019

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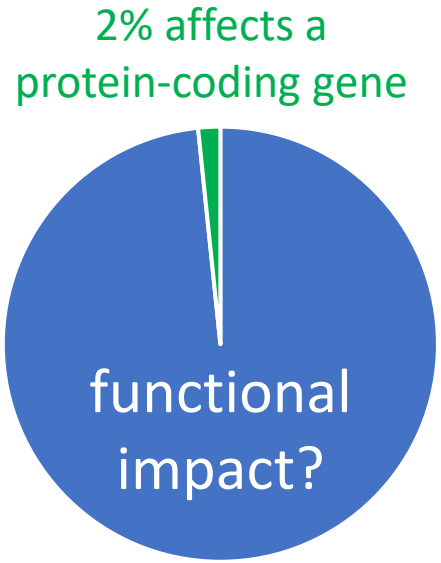
Advances in sequencing technologies have tremendously improved SV detection

~ 300 SVs
per genome

2004



~ 27,000 SVs
per human genome

2020

Chaisson et al., Nat Comm, 2019

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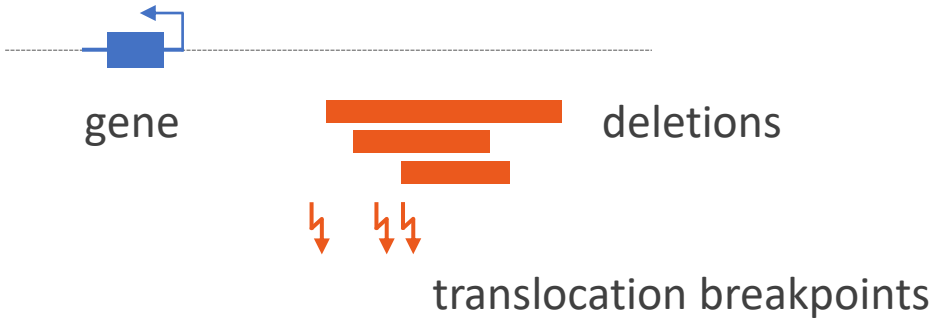
Non-coding SVs disrupt chromatin topology and gene regulation



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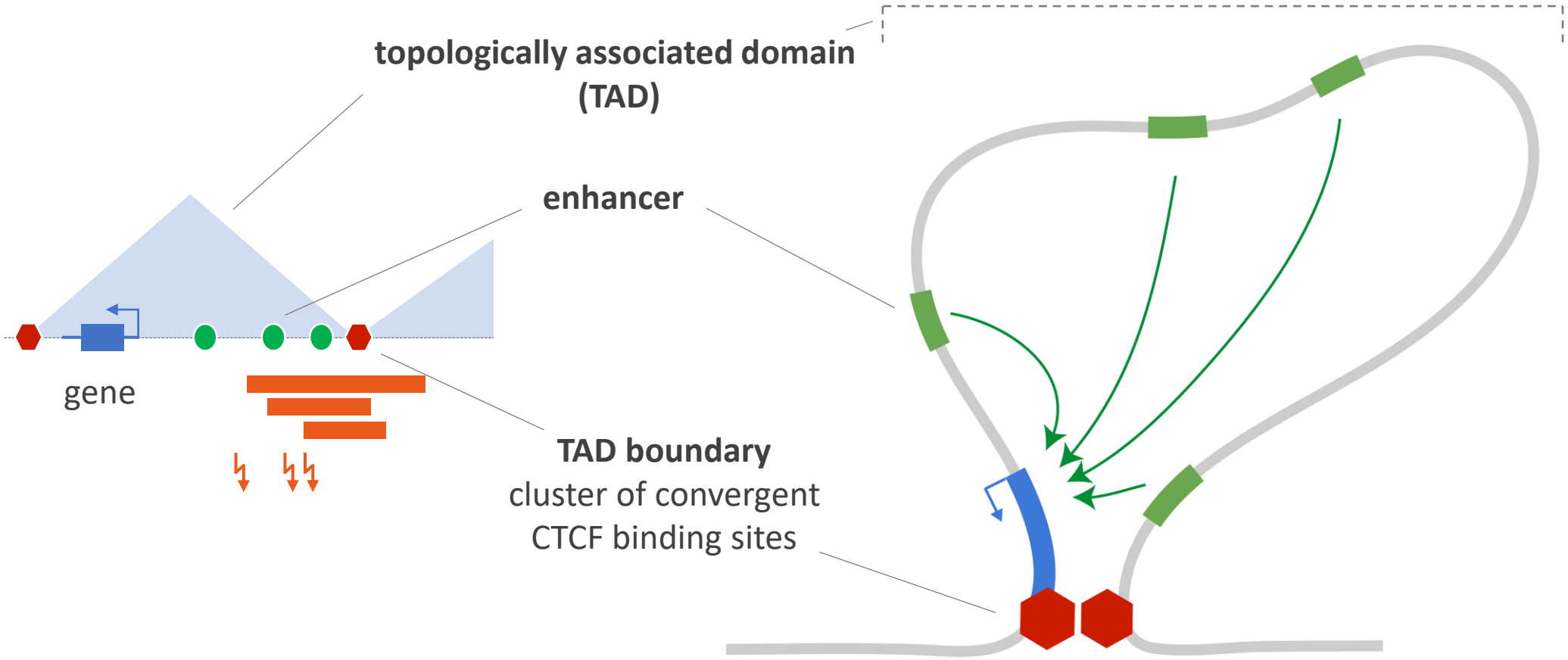

D'haene E & Vergult S, *Genet Med* (2021)

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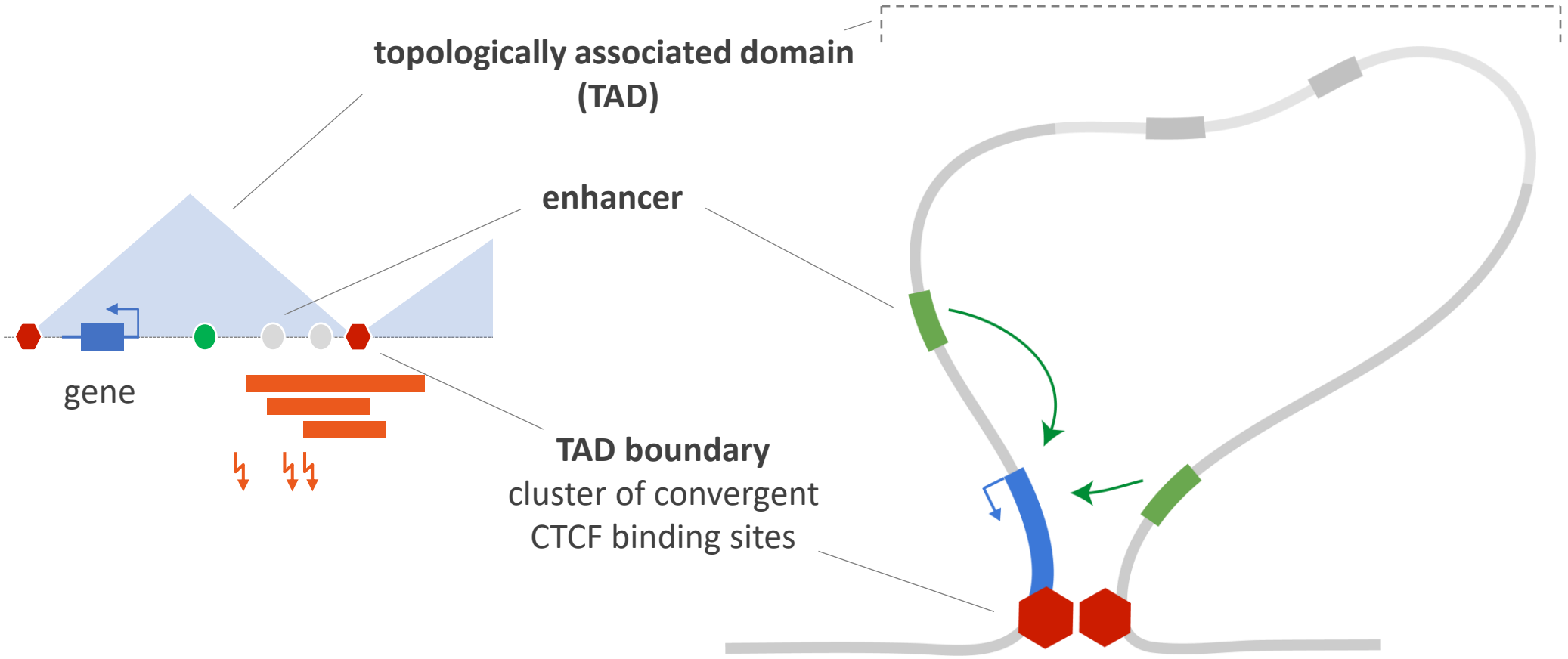

D'haene E & Vergult S, *Genet Med* (2021)

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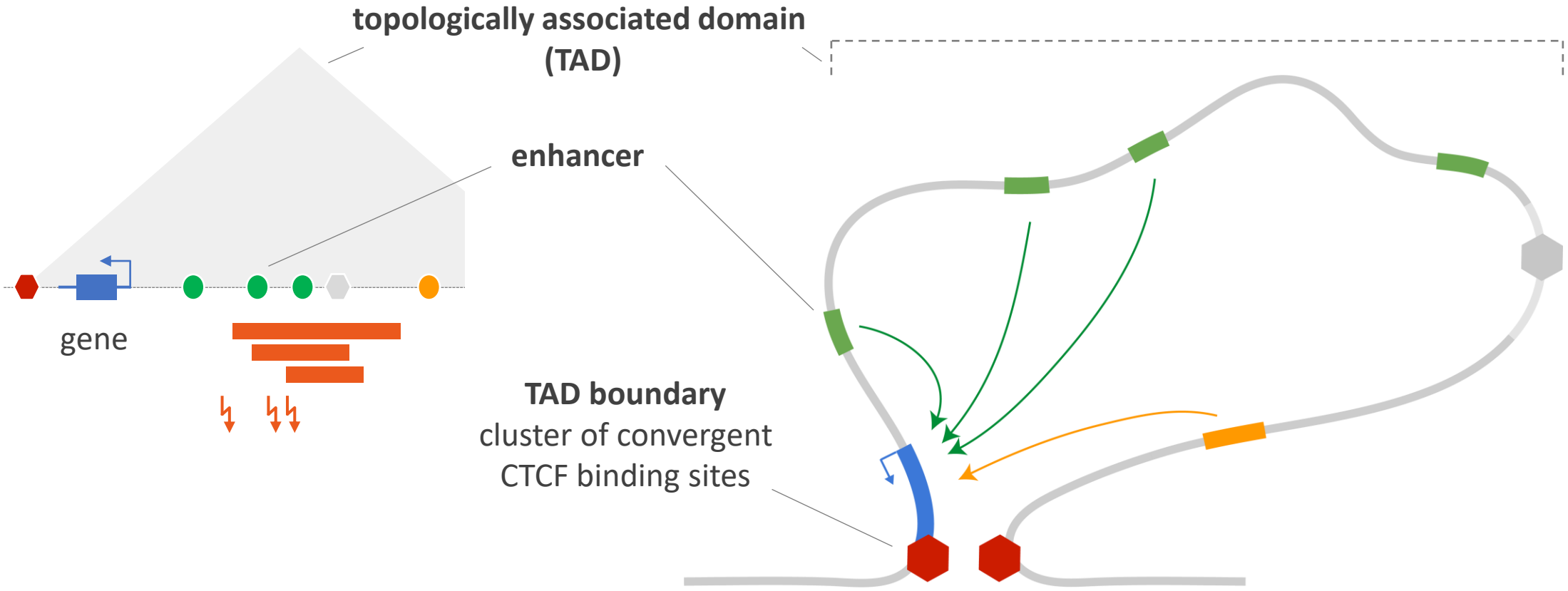

D'haene E & Vergult S, *Genet Med* (2021)

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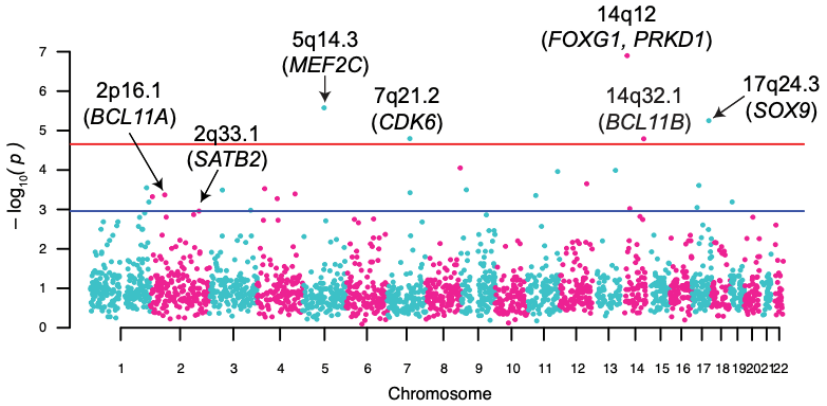
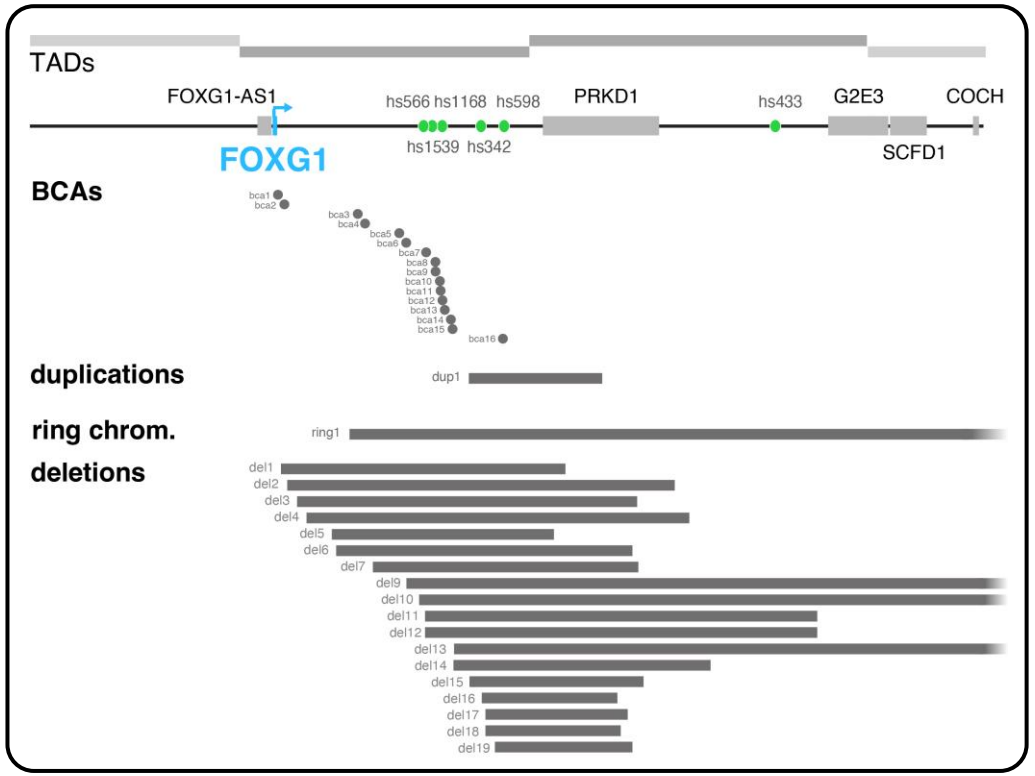
Enrichment of intergenic SV breakpoints in NDD cases at the 14q12 *FOXG1* locus



Redin et al., Nat Genet, 2017
Lowther et al., medRxiv, 2022



38 NDD cases with SVs downstream of FOXG1

Shoichet et al. (2005); Allou et al. (2012); Ellaway et al. (2013); Takagi et al. (2013); Goubau et al. (2013); Ibn-Salem et al. (2014); Alosi et al. (2015); Redin et al. (2016); Mehrjouy et al. (2018); Craig et al. (2020); Lu et al. (2022)

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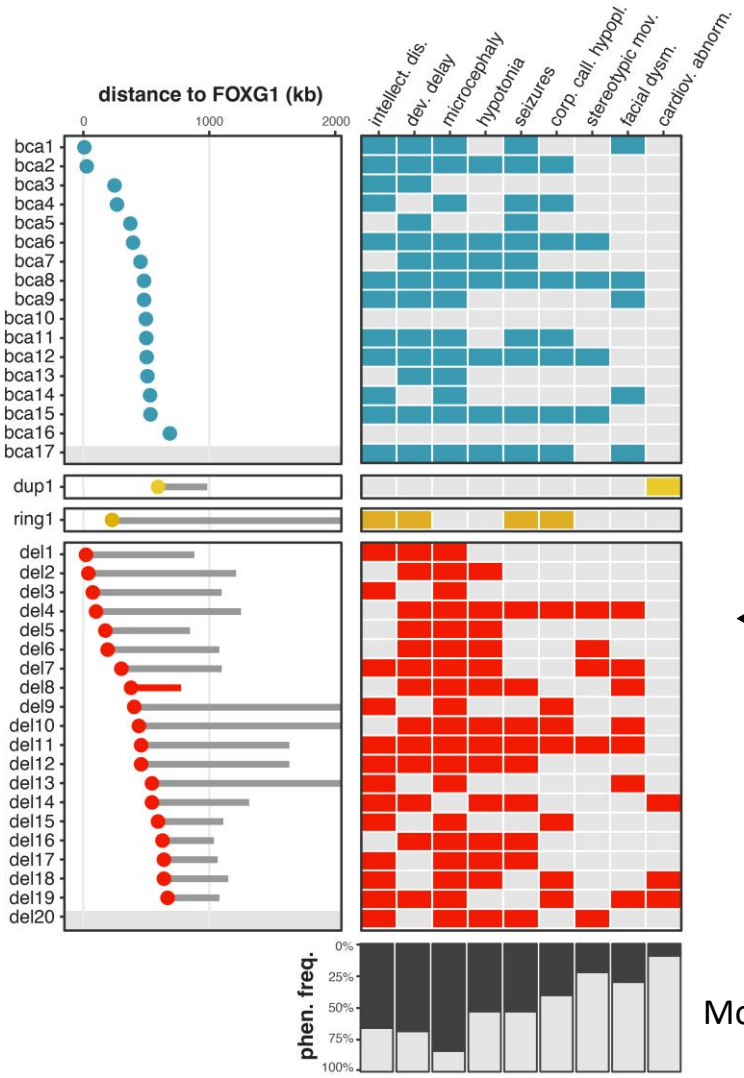

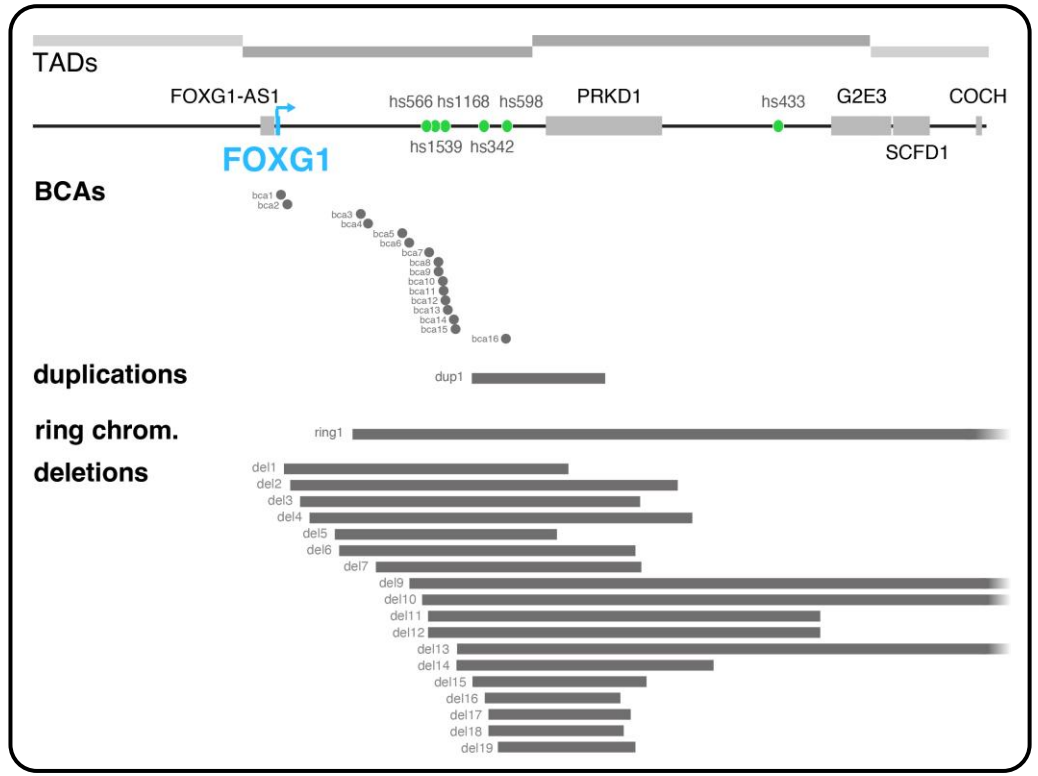

Most cases display hallmarks of *FOXG1* syndrome

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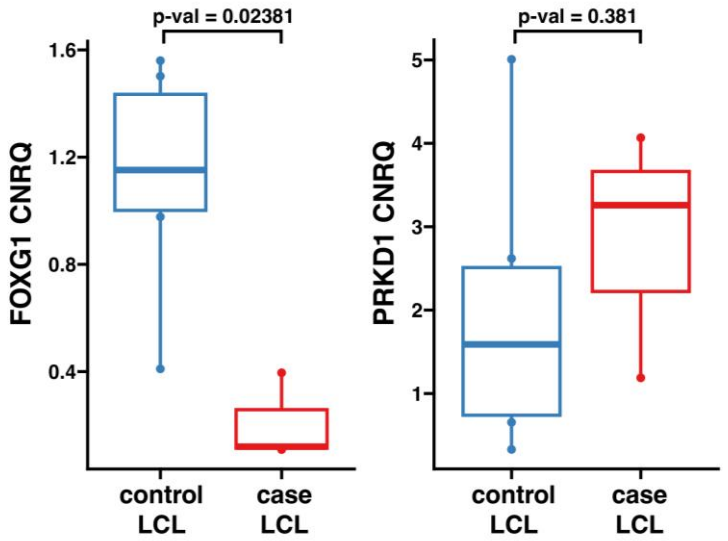
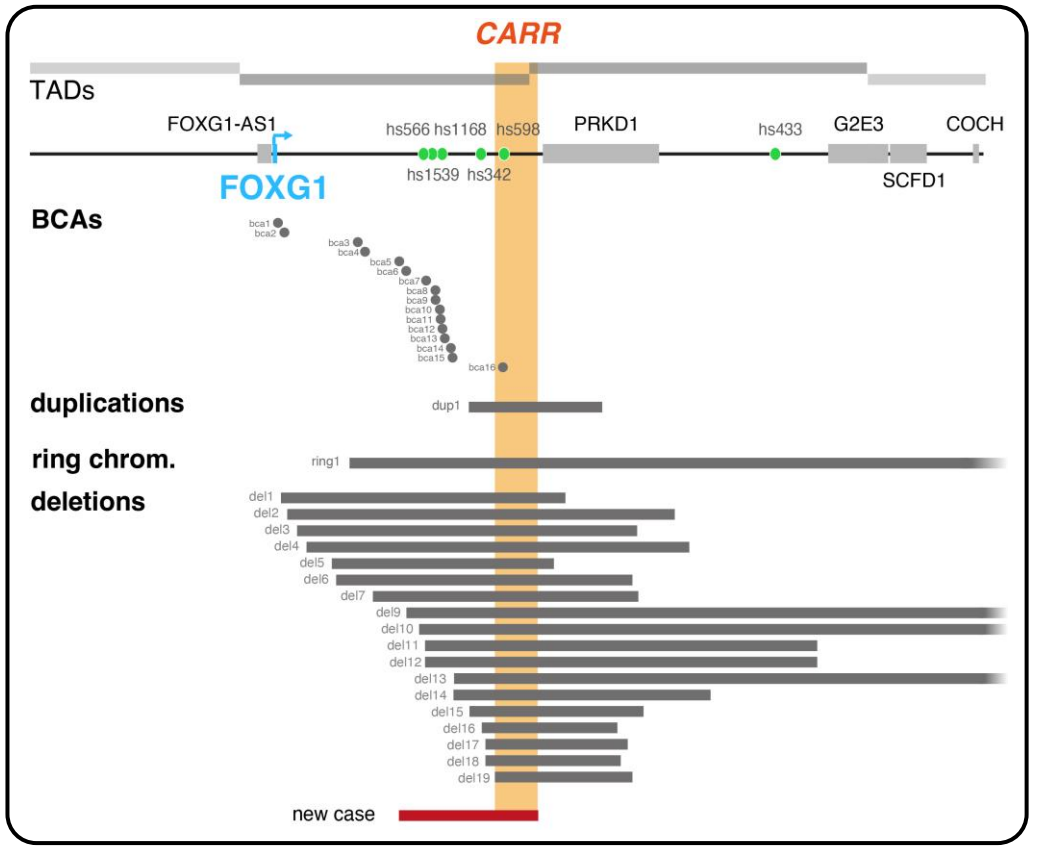
Non-coding deletion in individual with *FOXG1*-like syndrome narrows down commonly affected regulatory region (CARR) 3' of *FOXG1*

Reduced *FOXG1* expression in patient cells





Novel non-coding deletion delineates critical regulatory region downstream of FOXG1

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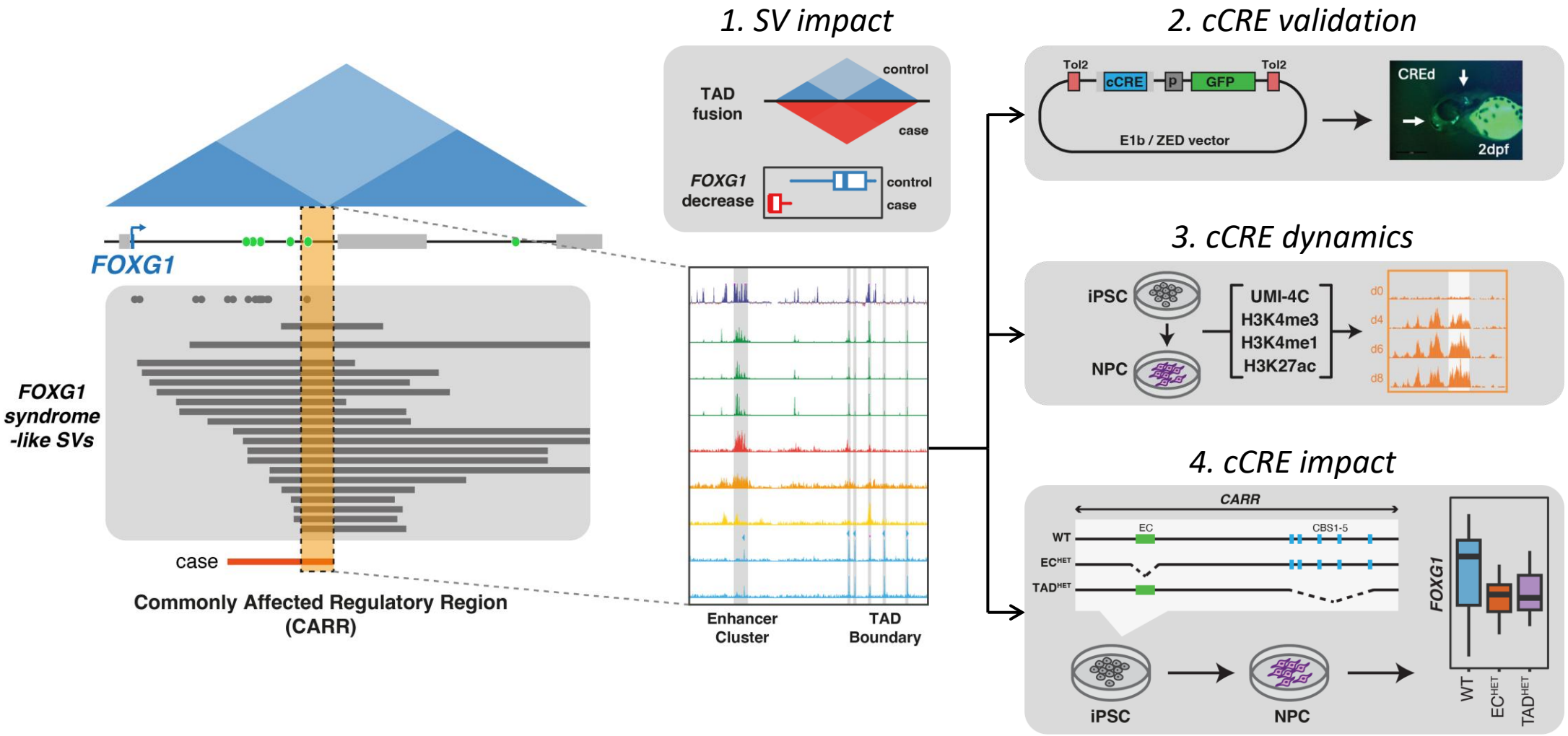
Non-coding structural variants affect *FOXG1* regulation in neurodevelopment



Hamerlinck, D'haene et al., medRxiv (2025)

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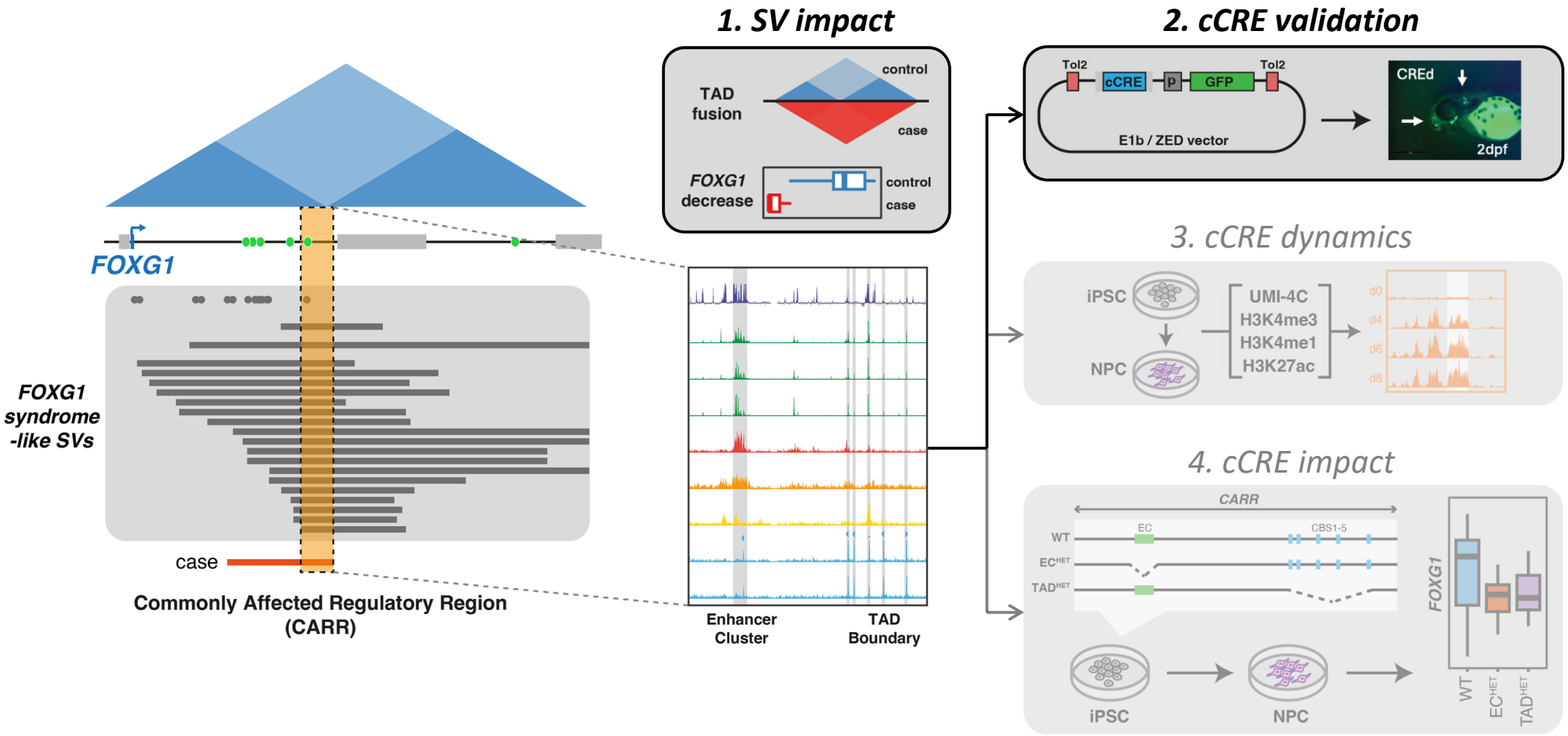
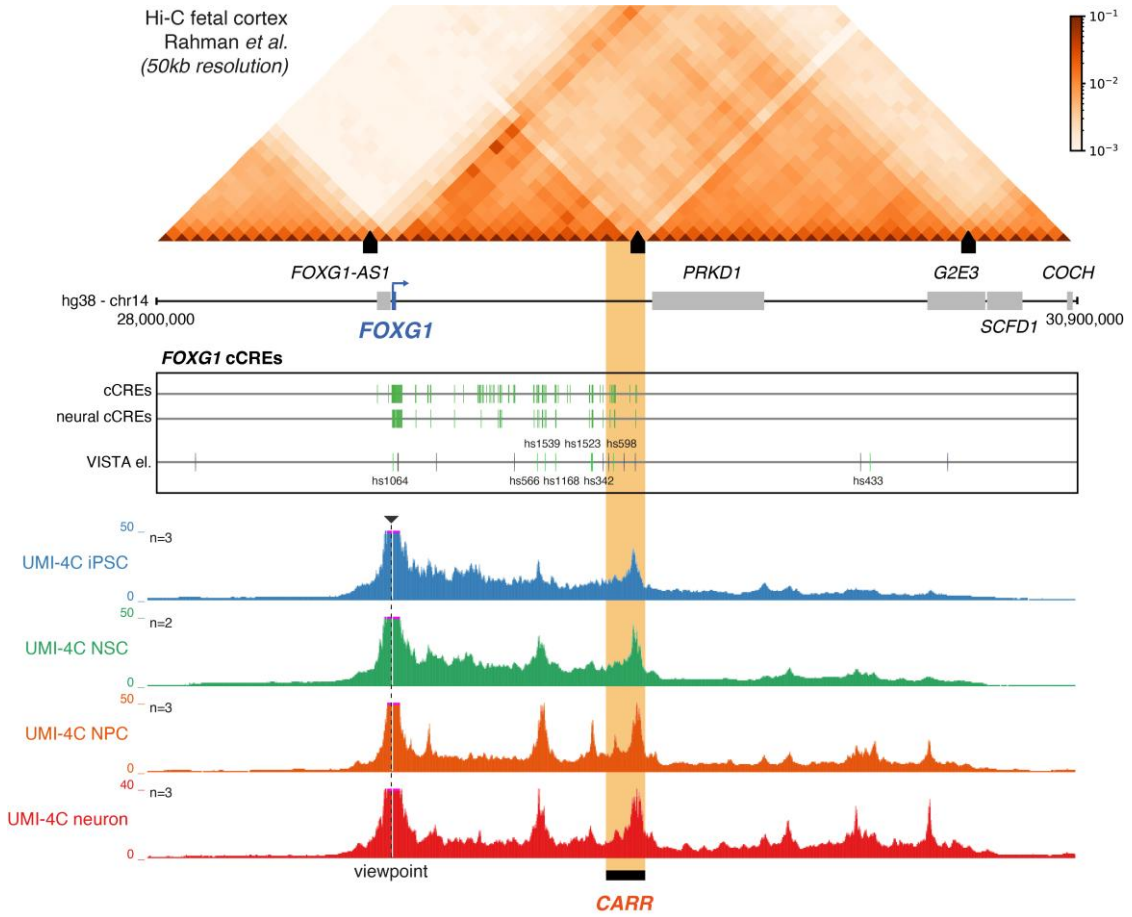


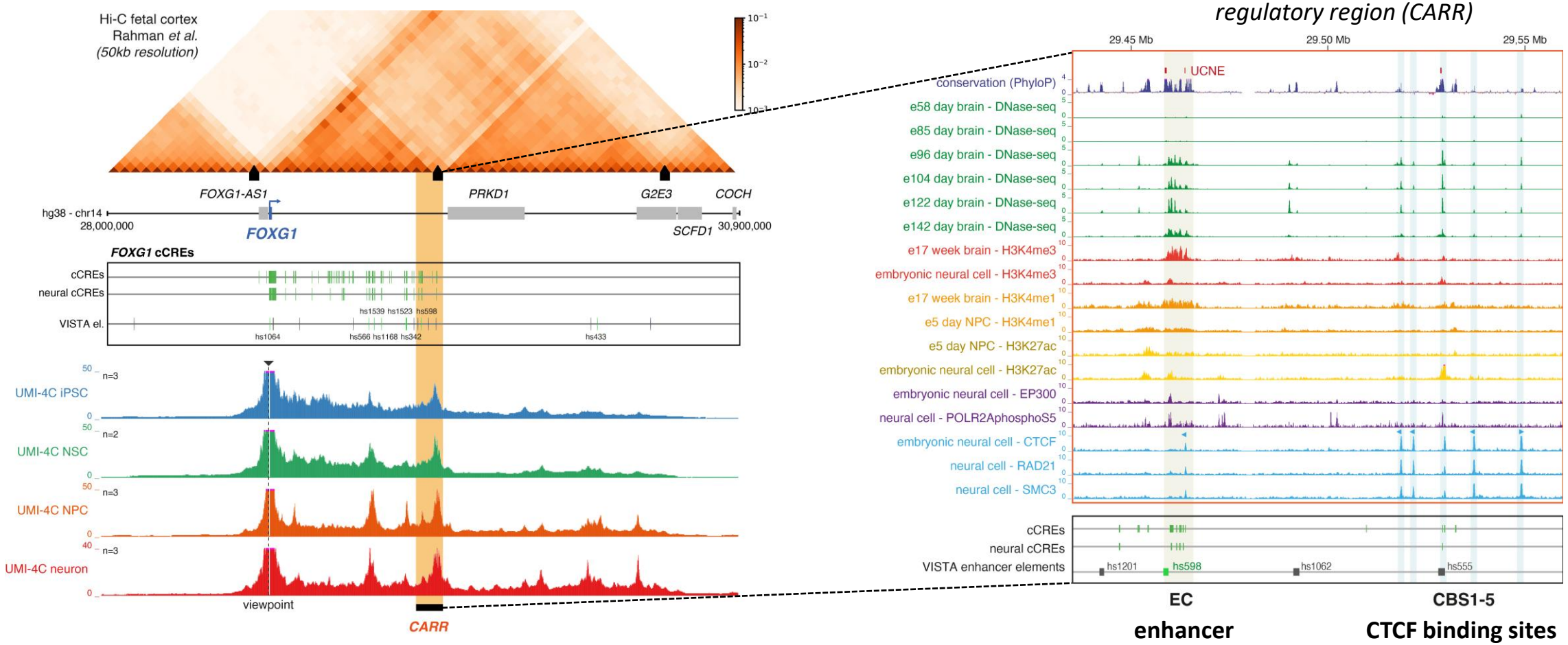
Hamerlinck, D'haene et al., medRxiv (2025)

CARR interacts with *FOXG1* during neuronal differentiation



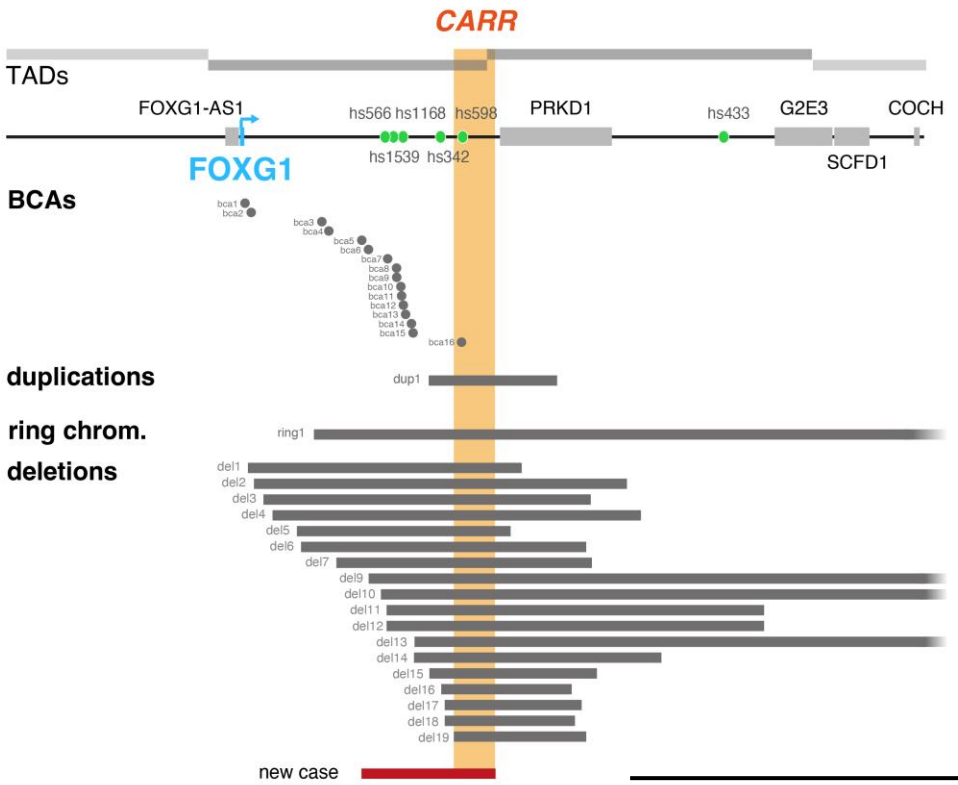
FOXG1 3D genomic interactions (UMI-4C)

CARR harbors enhancer cluster and TAD boundary

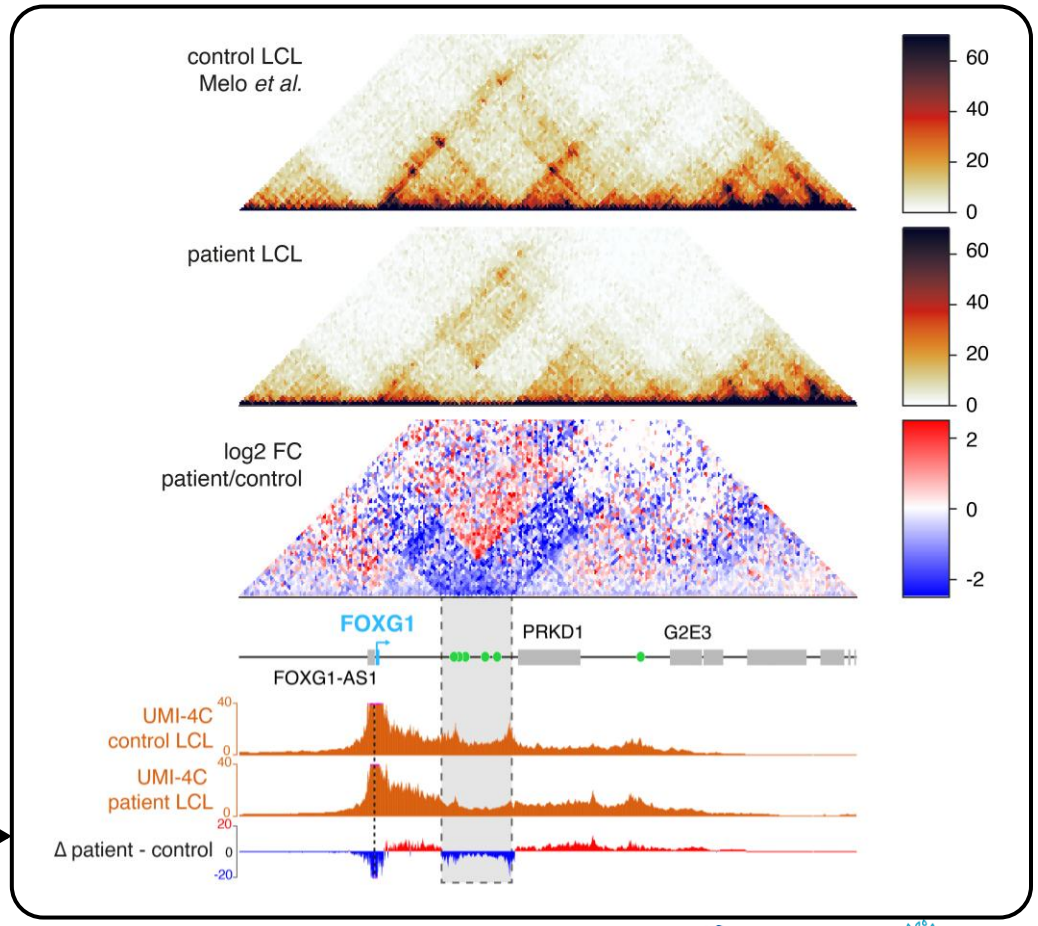


FOXG1 3D genomic interactions (UMI-4C)

TAD boundary deletion in patient cells results in TAD fusion



3D genome mapping (Hi-C & UMI-4C) in patient vs control cell lines



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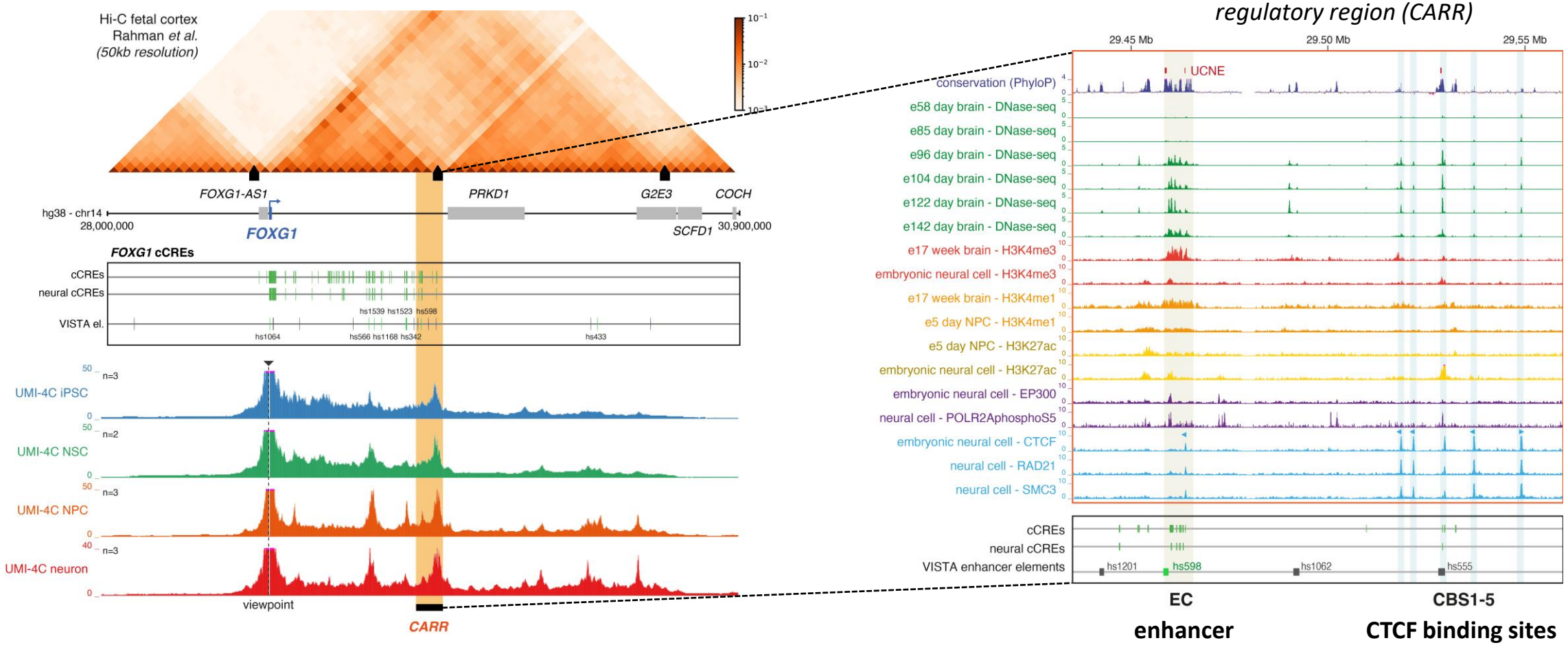
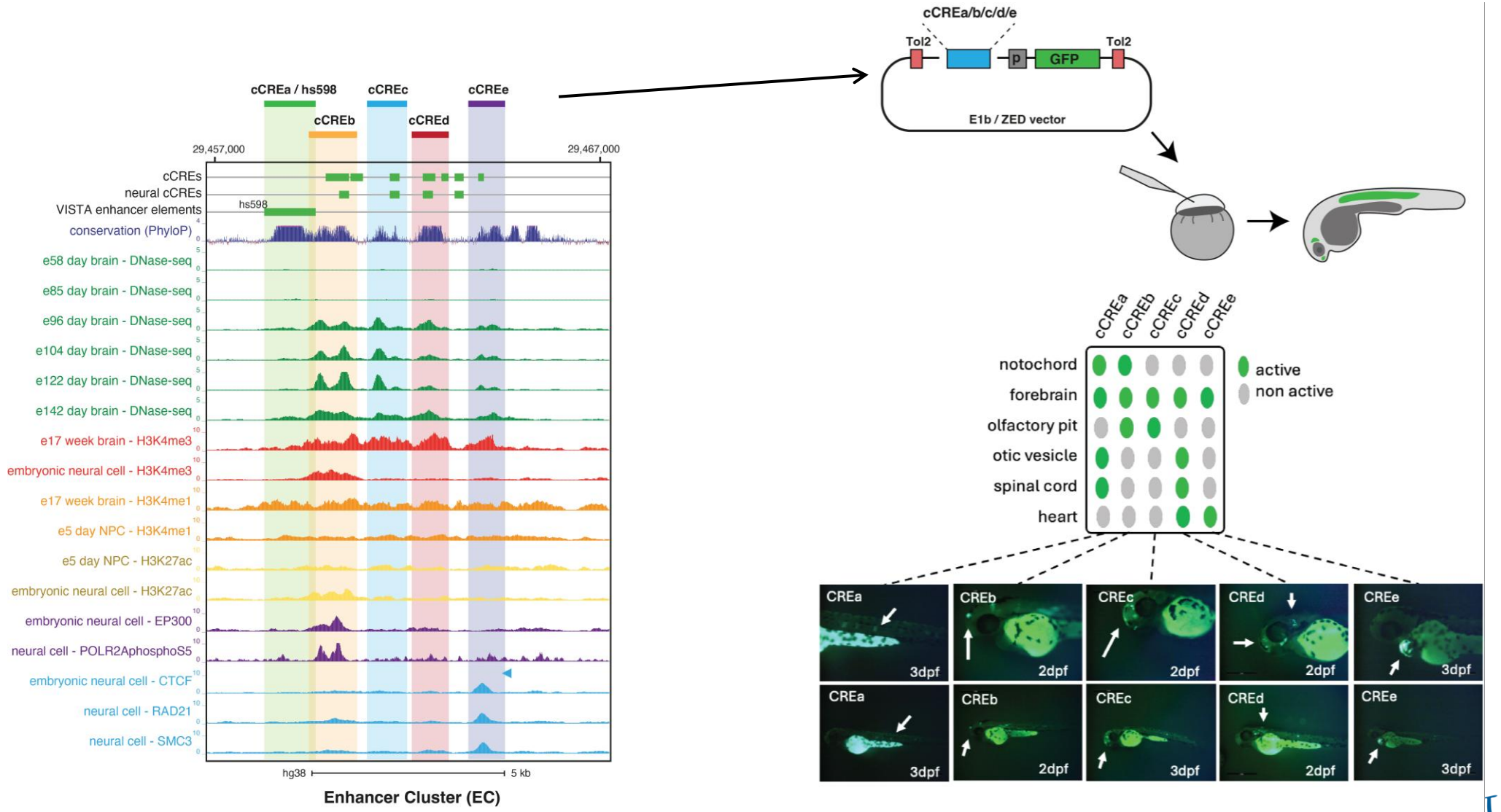
CARR harbors enhancer cluster and TAD boundary

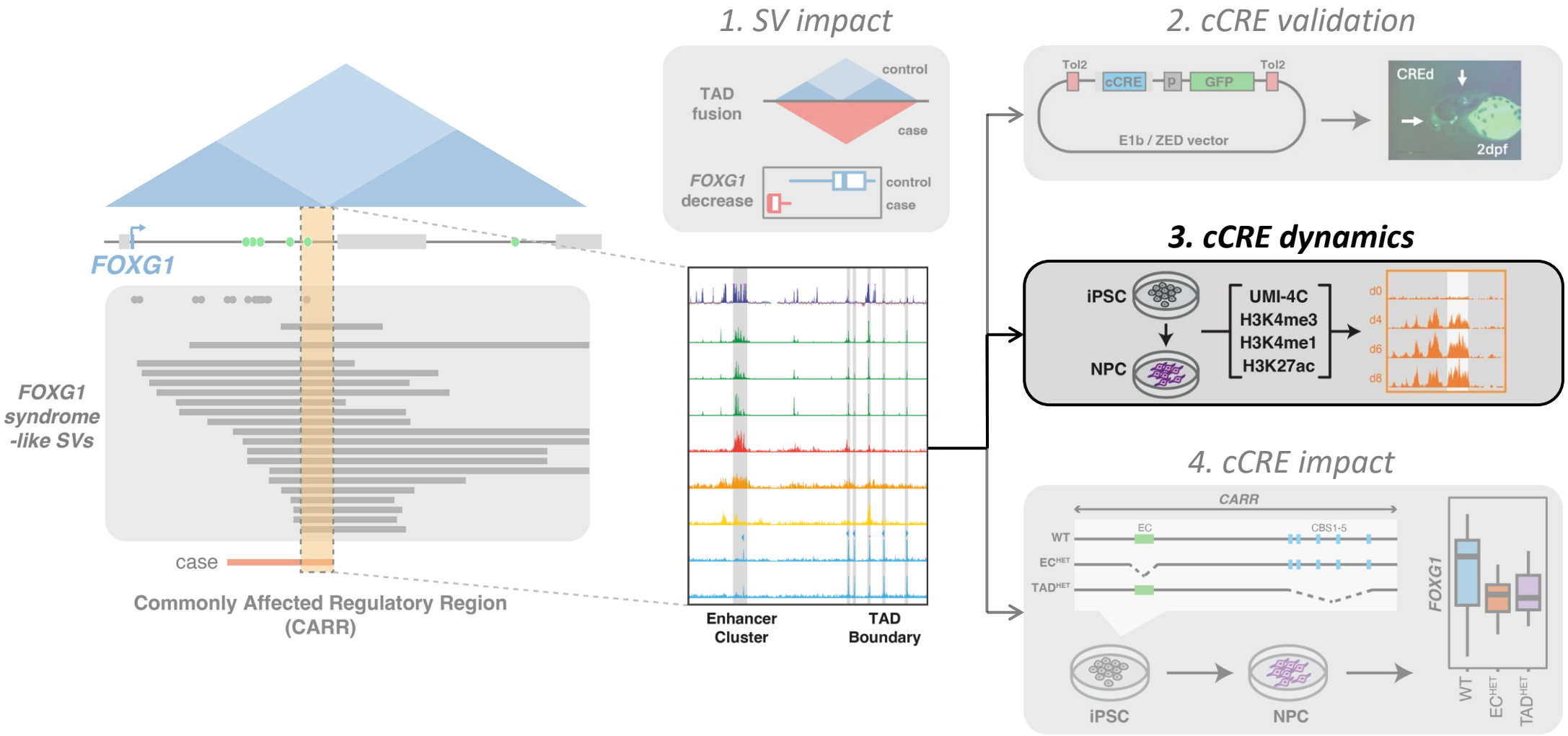


FOXG1 3D genomic interactions (UMI-4C)

Candidate enhancers display activity in neural tissues during zebrafish development

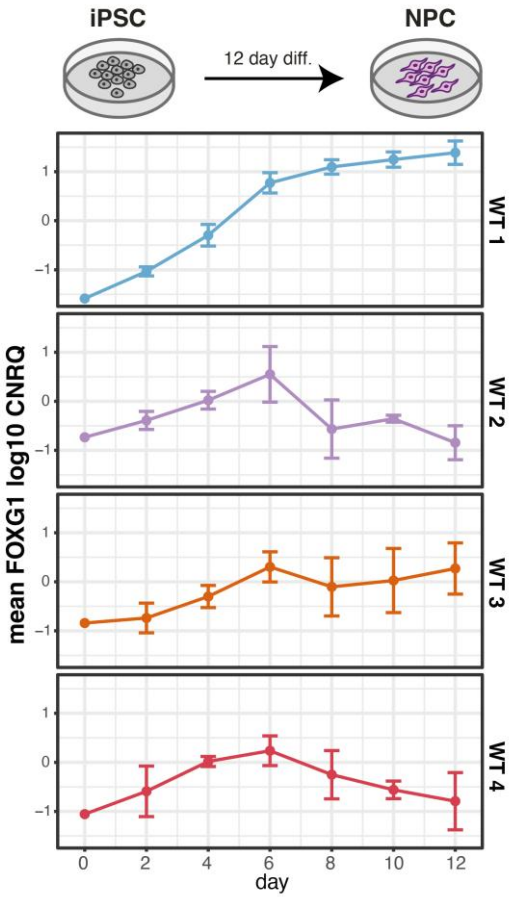


Non-coding structural variants affect *FOXG1* regulation in neurodevelopment

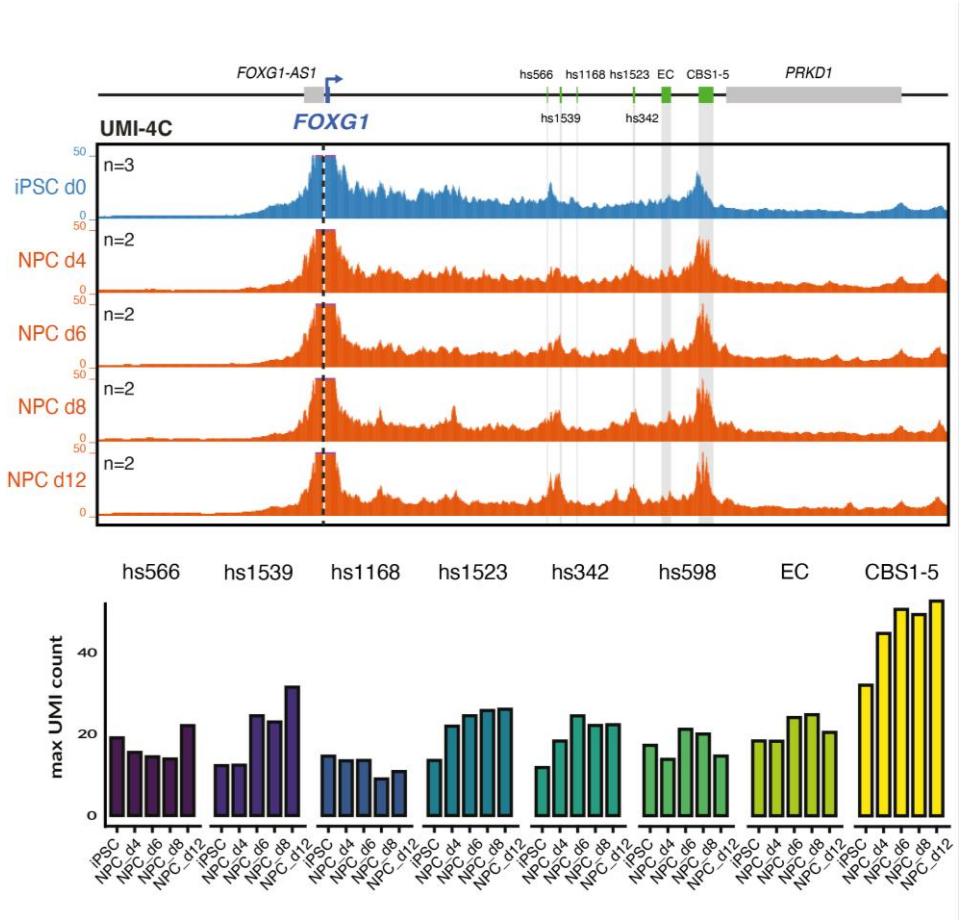


Hamerlinck, D'haene et al., medRxiv (2025)

FOXG1 is dynamically regulated during the early phases of NPC differentiation



FOXG1 transcription is activated during NPC differentiation



FOXG1 interaction with regulatory elements increases during NPC differentiation

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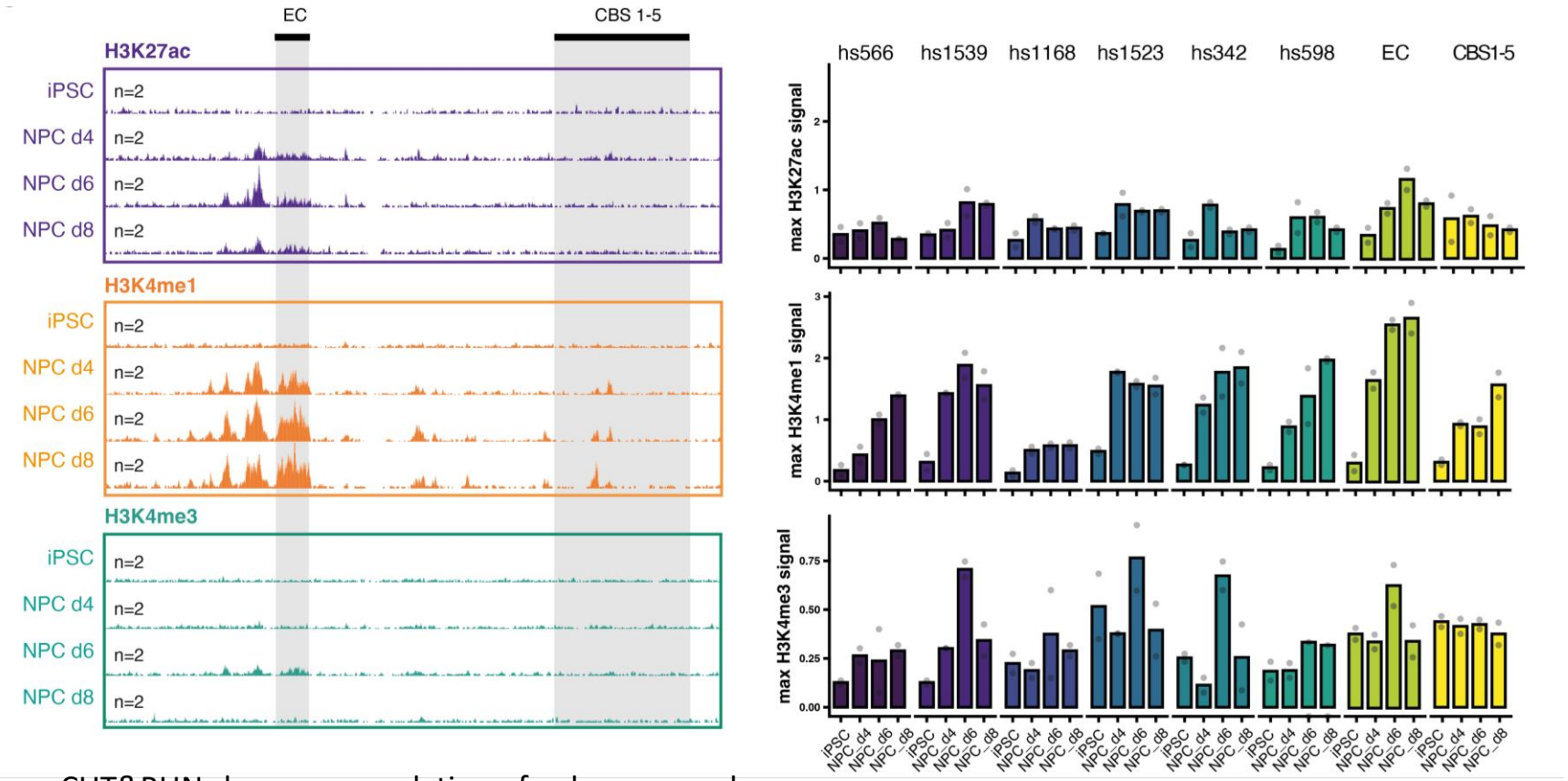
FOXG1 is dynamically regulated during the early phases of NPC differentiation



CUT&RUN shows accumulation of enhancer marks during NPC differentiation

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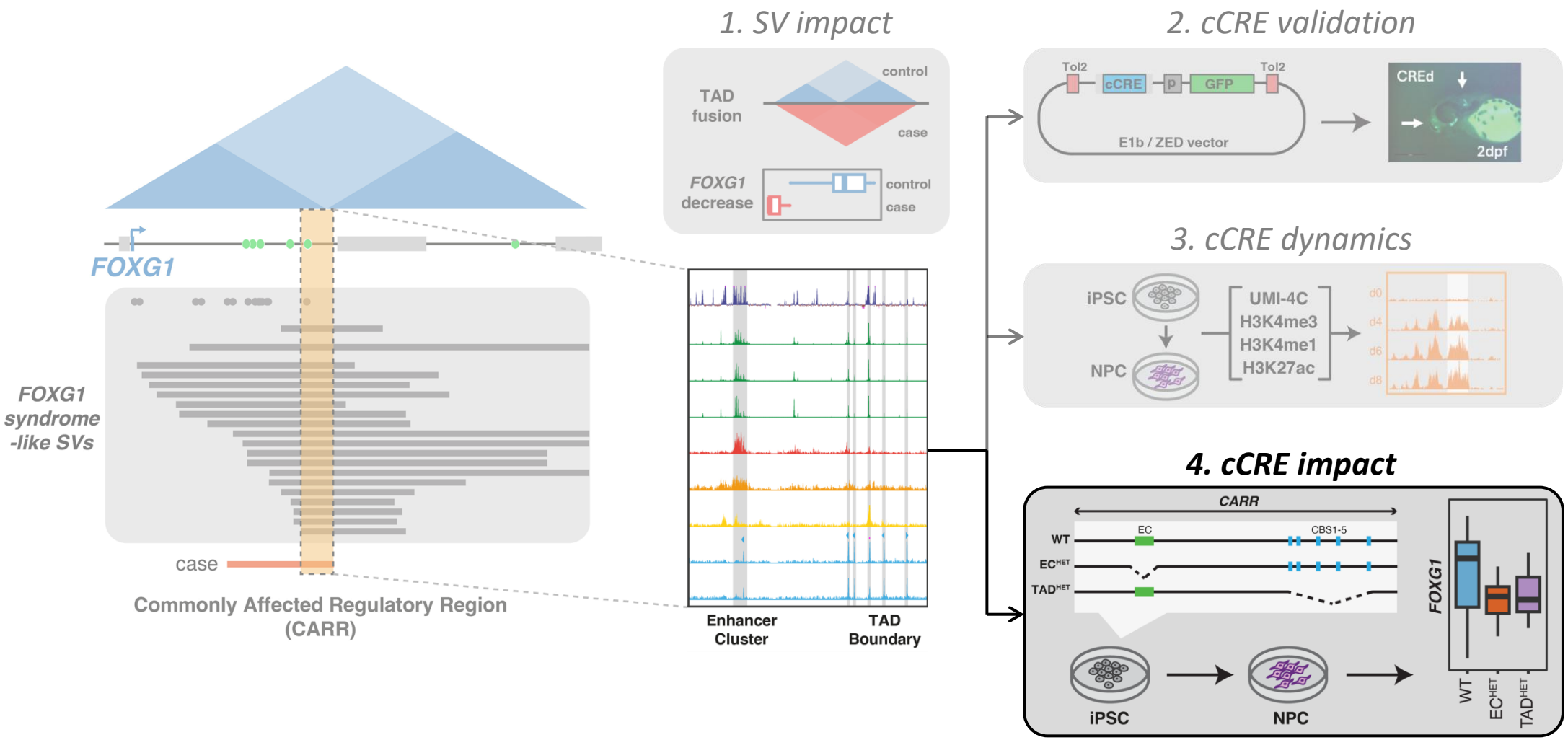
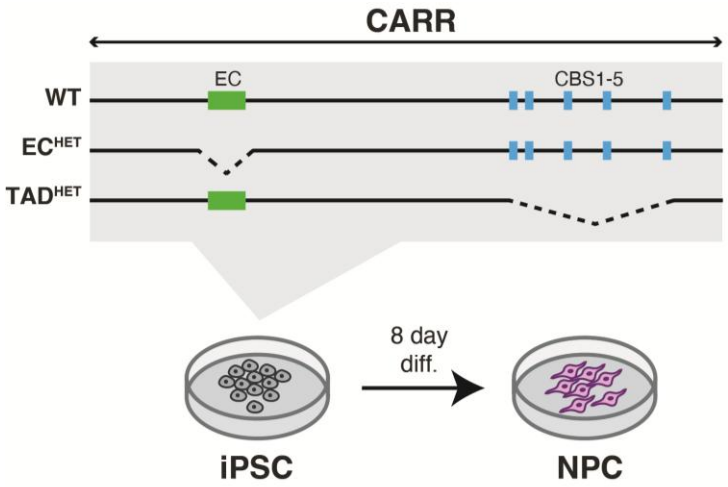
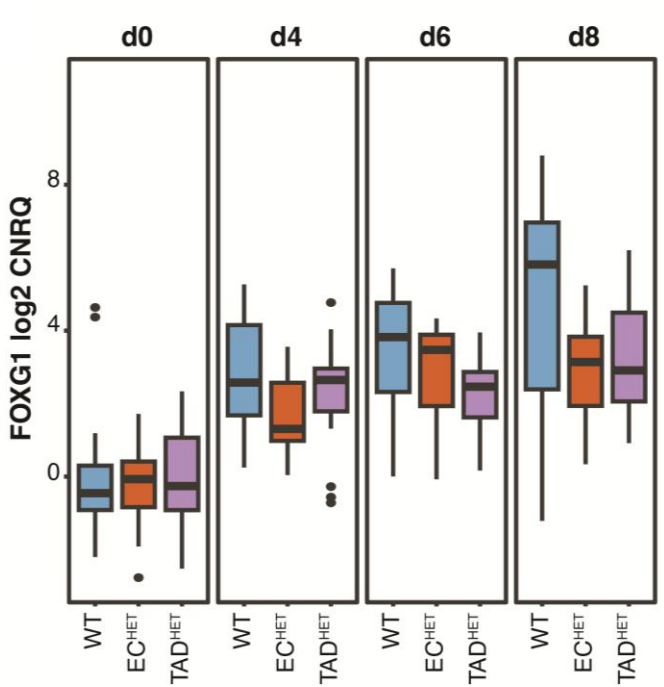
Non-coding structural variants affect *FOXG1* regulation in neurodevelopment

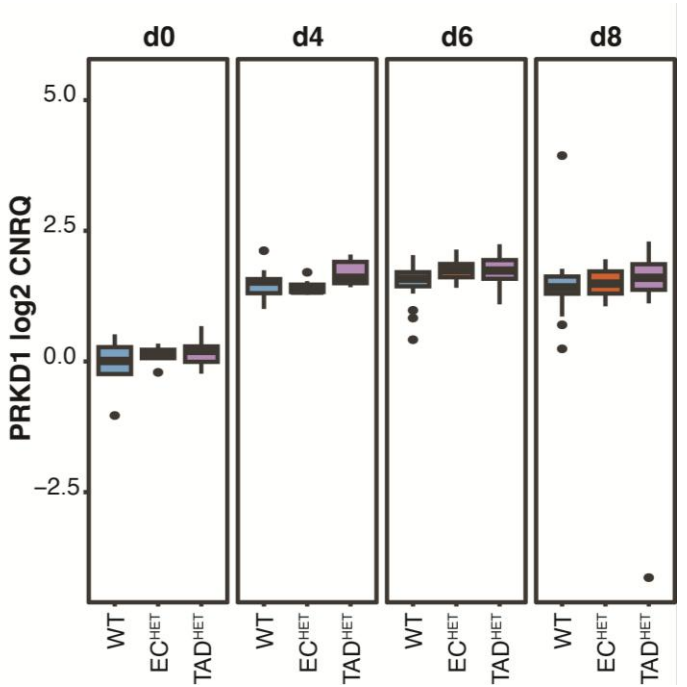


Hamerlinck, D'haene et al., medRxiv (2025)

Deletion of CARR functional elements results in decreased FOXG1 expression







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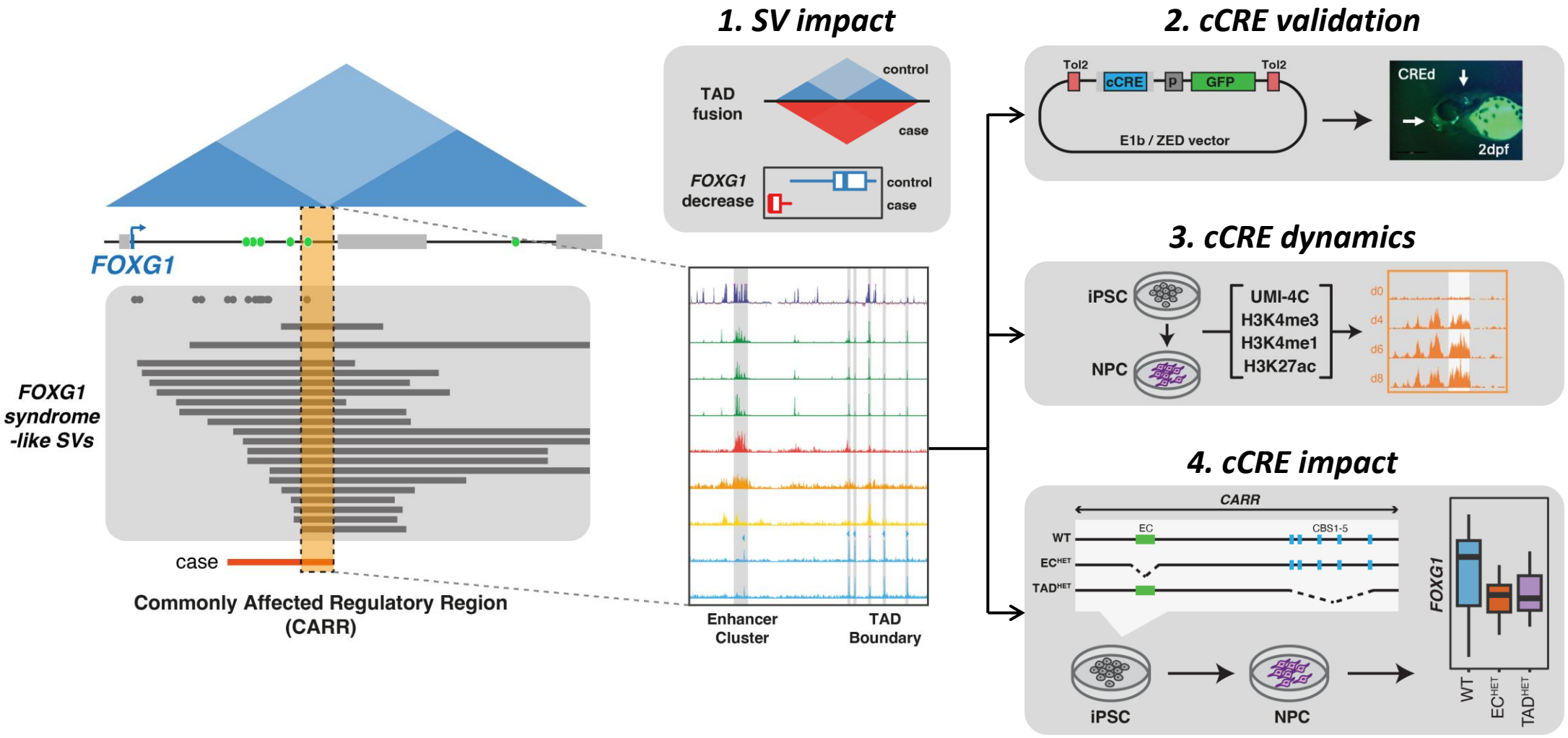

Hamerlinck, D'haene et al., medRxiv (2025)

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Non-coding structural variants identify a commonly affected regulatory region steering *FOXG1* transcription in early neurodevelopment

Lisa Hamerlinck, Eva D'haene, Nore Van Loon, Michael B Vaughan, Maria del Rocio Pérez Baca, Sebastian Leimbacher, Lara Colombo, Lies Vantomme, Esperanza Daal, Annelies Dheedene, Himanshu Goel, Björn Menten, Bert Callewaert, Sarah Vergult

doi: https://doi.org/10.1101/2025.03.10.25323301

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Interesting case and looking to collaborate?

> Reach out to:

Sarah.Vergult@ugent.be

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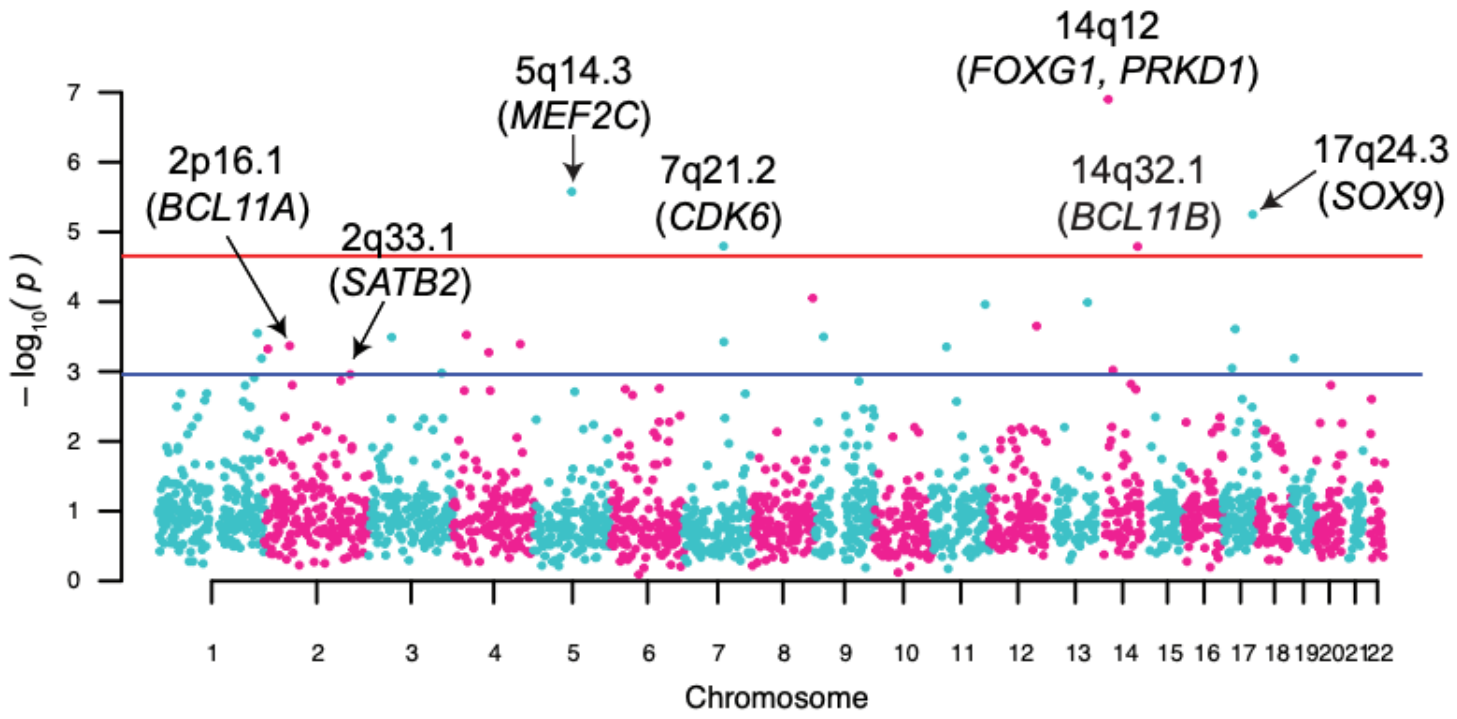
Enrichment of intergenic BCA breakpoints in NDD cases



Redin et al., Nat Genet, 2017
Lowther, Mehrjour, Collins, Bak, Dudchenko et al, medRxiv

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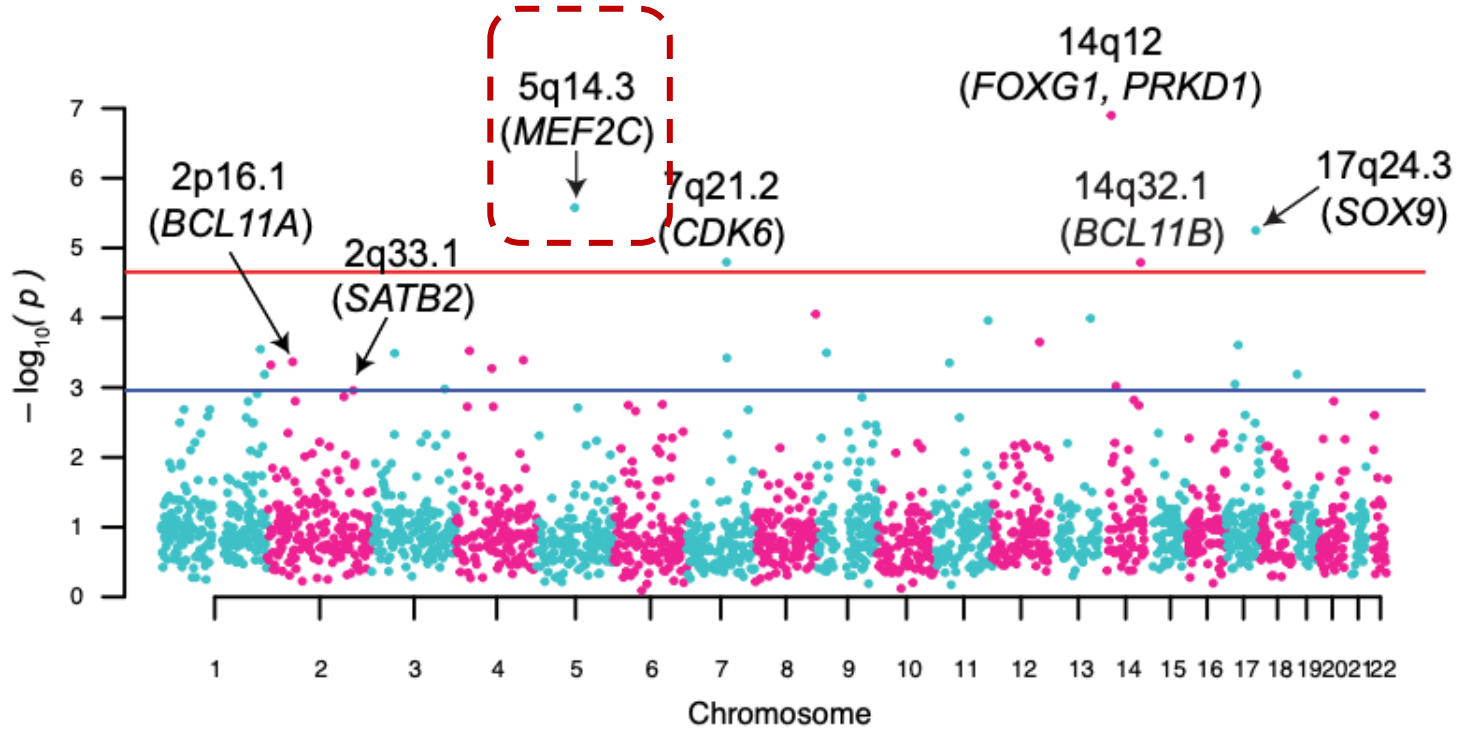
D'haene et al., 2019
Mohajeri et al., 2022



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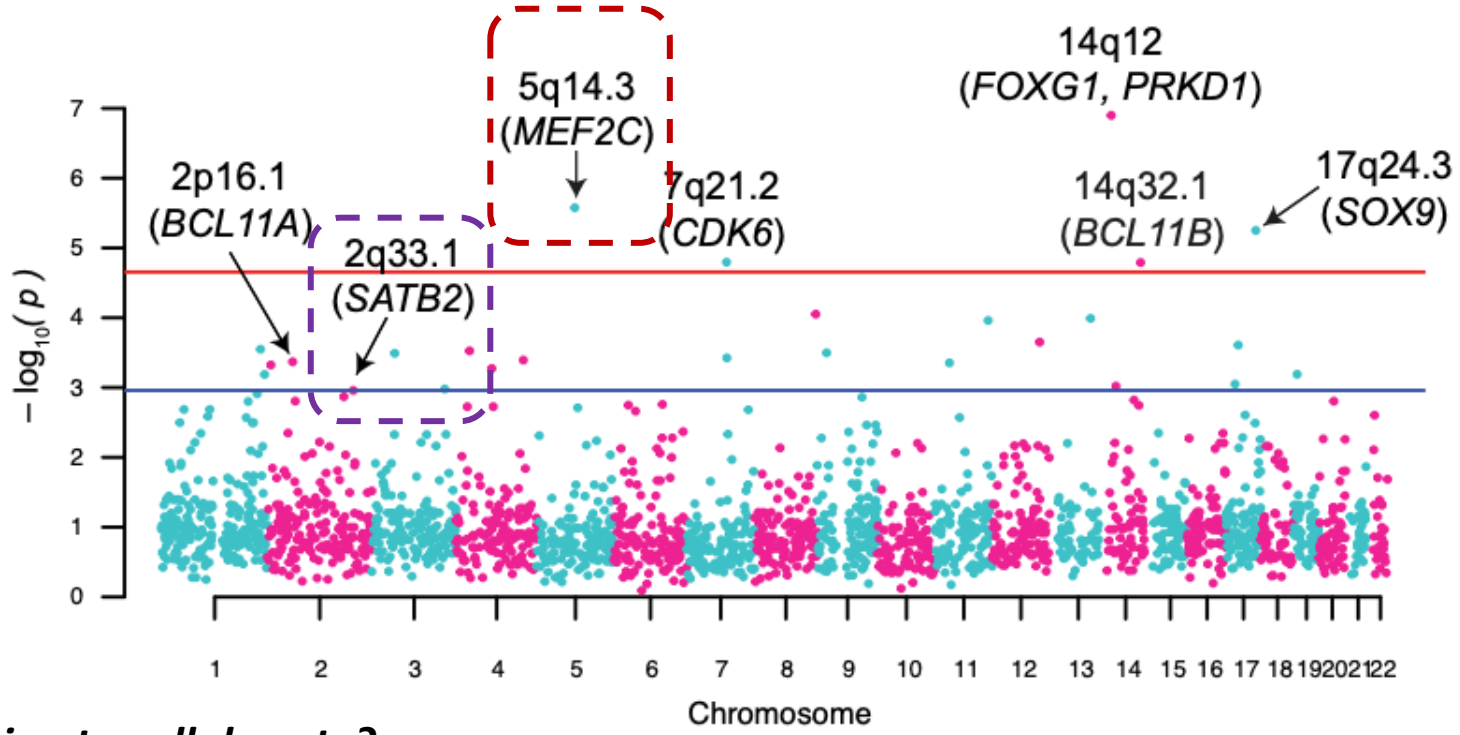
Mohajeri et al., 2022

Hamerlinck et al., in preparation



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Michael B Vaughan
Rocío Perez Baca
Sebastian Leimbacher
Lara Colombo
Lies Vantomme
Esperanza Daal

Center for Medical Genetics Ghent (CMGG)

Annelies Dheedene
Björn Menten
Bert Callewaert

University of Newcastle, Callaghan, Australia

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FACULTY OF MEDICINE AND HEALTH SCIENCES

UZ GENT
CENTRUM MEDISCHE GENETICA GENT

fwo
Opening new horizons

STICHTING Marguerite-Marie DELACROIX
uw zorg, onze zorg

RARE-MED

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4. Finding causes of missing heritability in neurogenetic disorders: exploring the dark matter of the genome

Dr Stefan BARAKAT; Erasmus MC, Rotterdam, Netherlands

European Commission

EUROPEAN REFERENCE NETWORKS

Helping patients with rare or low-prevalence complex diseases

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Finding Causes of Missing Heritability in Neurogenetic Disorders: Exploring the Dark Matter of The Genome

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2025

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We fail to genetically diagnose most of our patients

OPEN NATURE REVIEWS | NEUROLOGY

International consensus recommendations on the diagnostic work-up for malformations of cortical development

Renske Oegema[1]✉, Tahsin Stefan Barakat[2], Martina Wilke[2], Katrien Stouffs[3], Dina Amrom[4,5], Eleonora Aronica[6,7], Nadia Bahi-Buisson[8], Valerio Conti[9], Andrew E. Fry[10,11], Tobias Geis[12], David Gomez Andres[13], Elena Parrini[9], Ivana Pogledic[14], Edith Said[14,15], Doriette Soler[16,17], Luis M. Valor[18], Maha S. Zaki[19], Ghayda Mirzaa[20,21], William B. Dobyns[20,21], Orly Reiner[21], Renzo Guerrini[9], Daniela T. Pilz[22], Ute Hehr[23], Richard J. Leventer[24], Anna C. Jansen[25], Grazia M. S. Mancini[2,26] and Nataliya Di Donato[27]✉

Table 2 | **Diagnostic yield across Neuro-MIG**

MCD entity	Diagnostic yield (%)[a]
Microcephaly[b]	18–20
Lissencephaly	75–81
Cobblestone malformation	75
Polymicrogyria	20
Periventricular nodular heterotopia	30–37
Total cohort ($n = 737$)	15–37

-Current diagnostic yield: 30-50%
-most genetic testing is exome focused
-even when WGS, analysis often exome based

What is causing “missing heritability”?

-variants of unknown significance
-somatic mosaicism
-epigenetic alterations
-”missed genes”
-new disease genes (incl. non-coding genes)
-non-coding genetic alterations

Nature Reviews Neurology 2020

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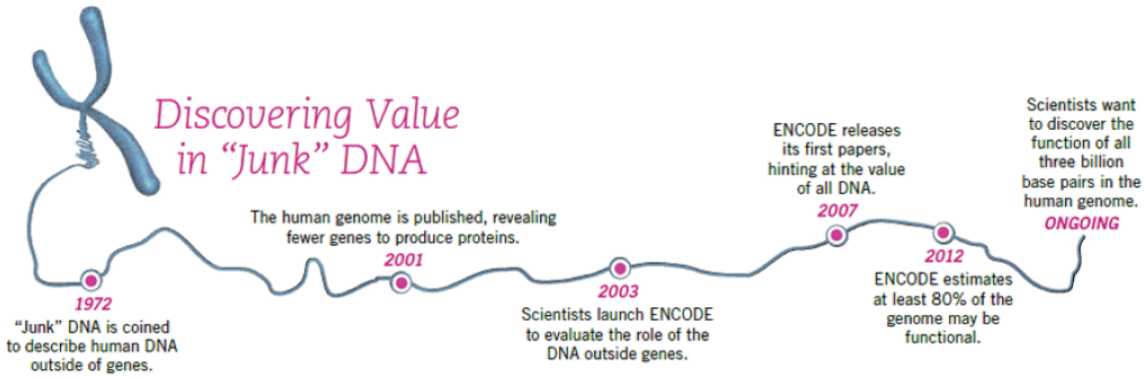
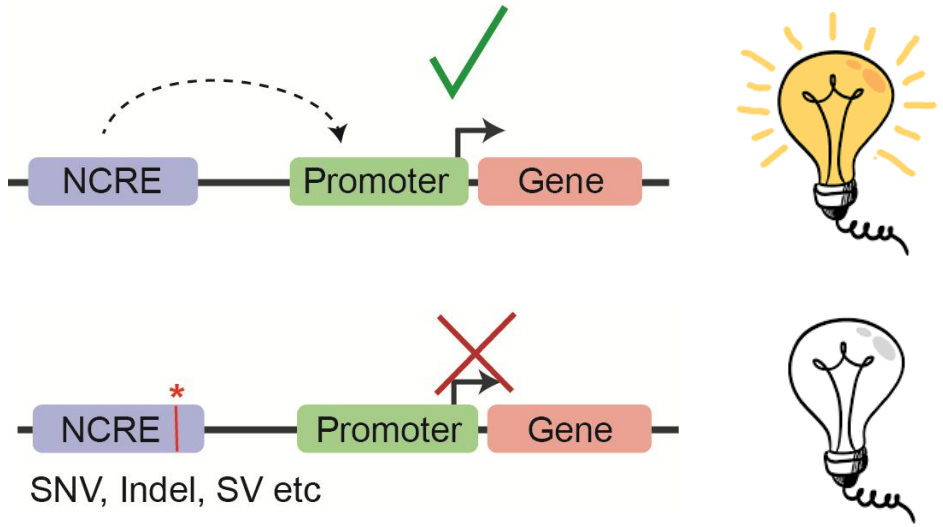
The unexplored mystery in genetics: the non-coding genome and non-coding regulatory

2% of human genome protein encoding – what does the rest do?





-Alterations of NCREs explain part of the missing heritability
-already plenty of examples
-different disease mechanisms

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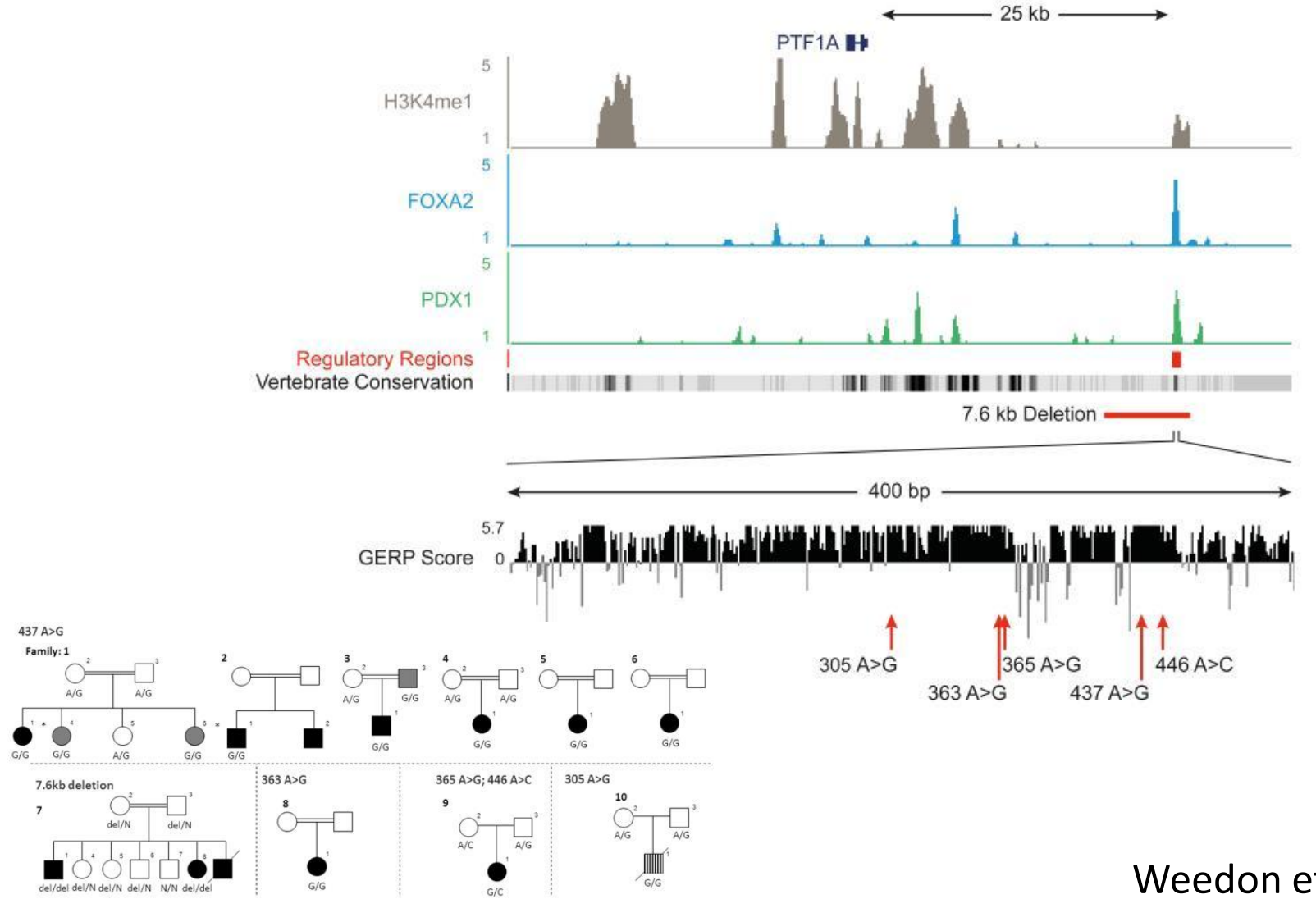
Even point mutations can cause enhanceropathies: Recessive mutations in a distal PTF1A enhancer cause isolated pancreatic agenesis



Weedon et al Nature Genetics 2014

How to find single nucleotide enhancer mutations?

Amongst thousands of non-coding variants...

Different ways to identify enhancers

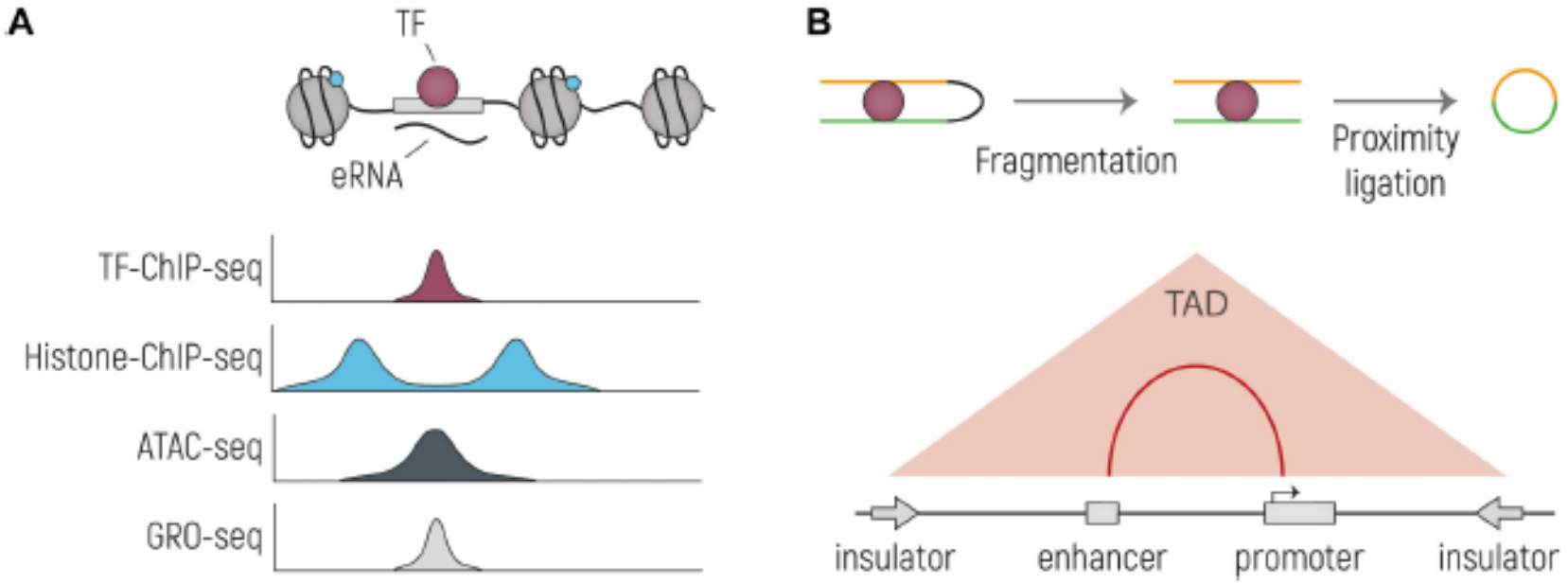


FIGURE 2 | Overview of the main techniques currently used to identify putative enhancer sequences and their interacting genes. **(A)** Schematic drawing of an TF-bound enhancer, located in nucleosome depleted DNA from which eRNA is transcribed. Below are representative genome browser tracks shown, illustrating expected profiles for the same genetic region. Histone-ChIP-seq is illustrative for marks such as H3K27ac and H3K4me1. **(B)** Cartoon representing the main steps of the workflow of Chromosome conformation capture technologies: nuclei are cross-linked, chromatin is then digested and re-ligated by proximity ligation. The two stretches of DNA that are normally located far away from each other (yellow and green), are now ligated together and can be tested by PCR or sequencing. In the bottom part is indicated the output of the experiment, with which TADs and enhancer-promoter interactions can be identified.

Perenthaler al 2019 Frontiers in Cellular Neuroscience

Finding active enhancers in the human genome

1) We need to find them
2) We need to interpret effects of mutations in them

How to reduce the search space?

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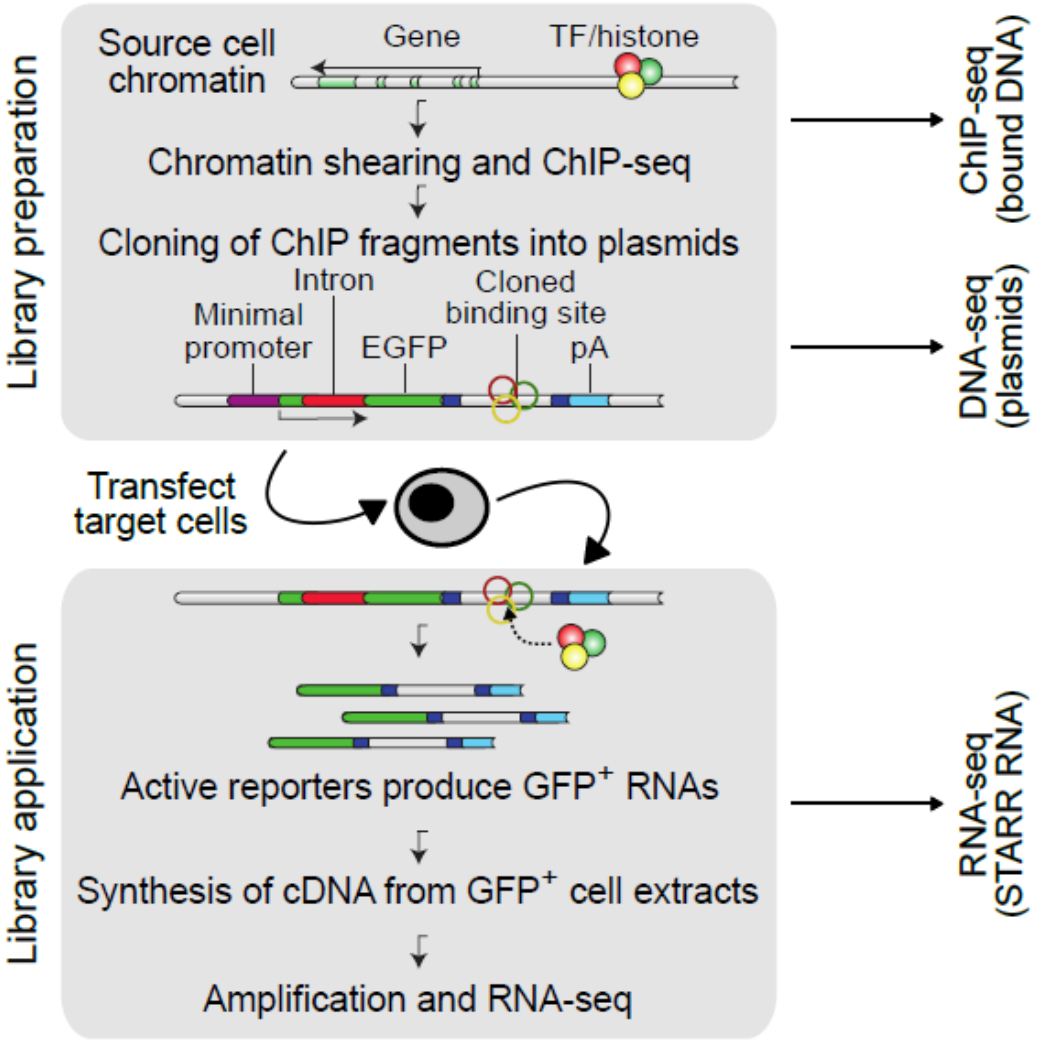
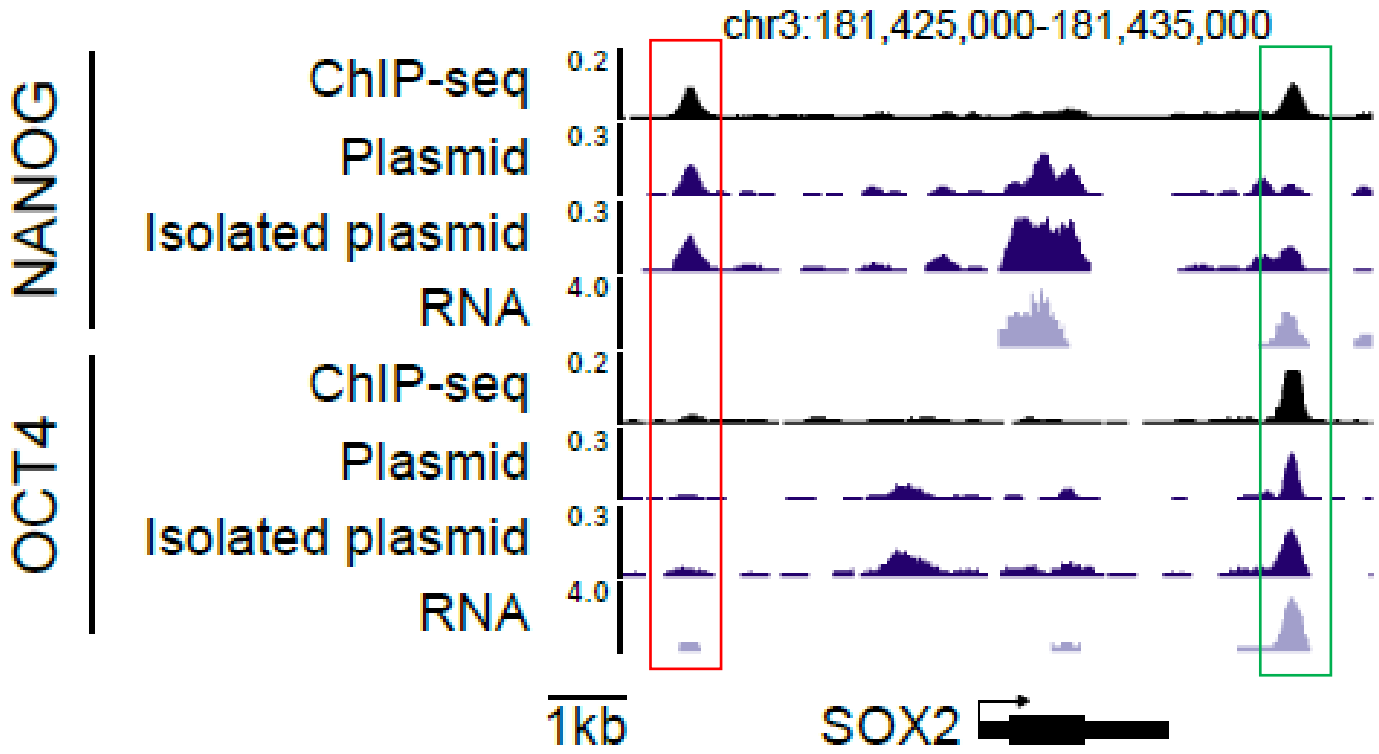
ChIP-STARR-seq approach to identify functional enhancers genome-wide & quantitative in ESCs

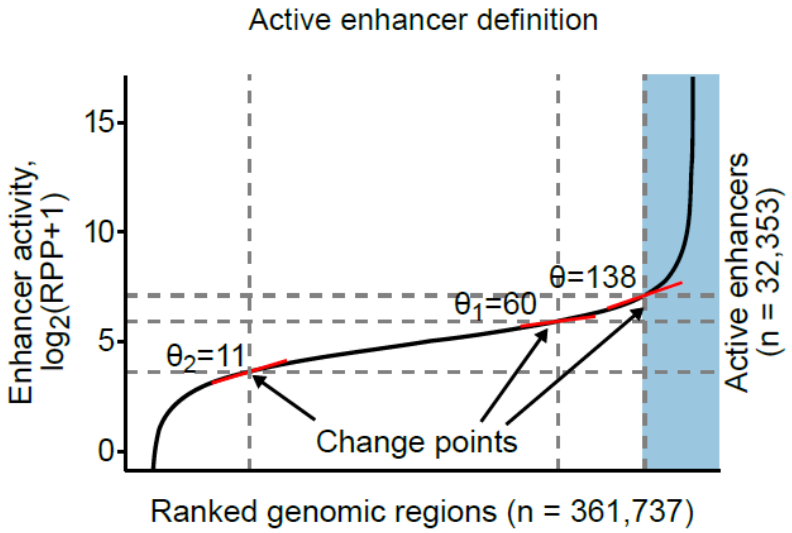


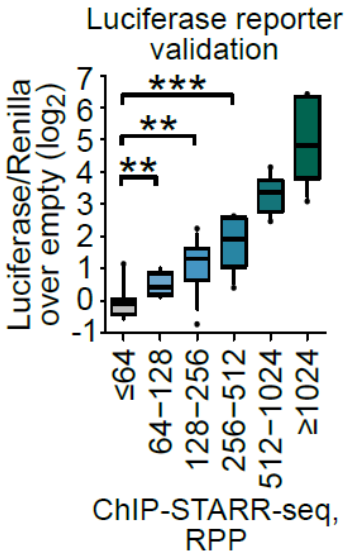


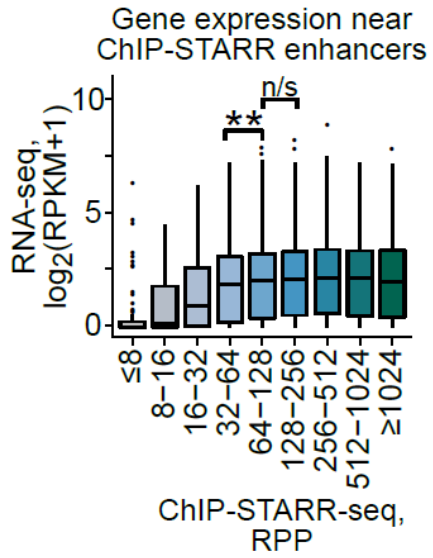
STARR-seq plasmid: Arnold et al 2013

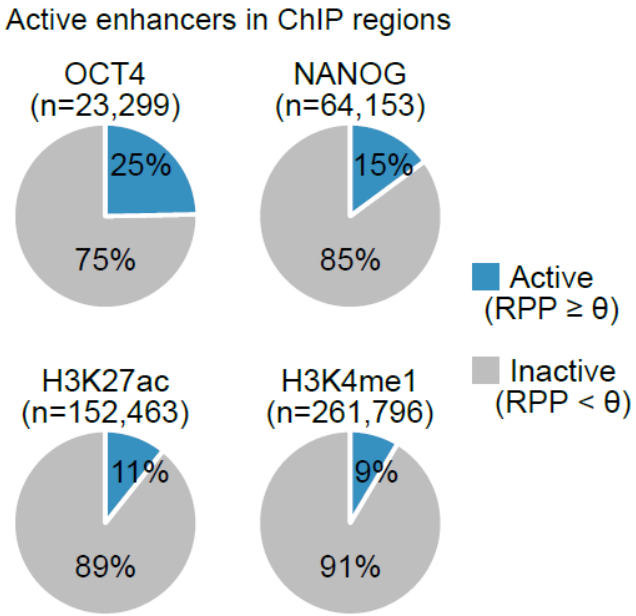
Barakat*, Halbritter* et al., Cell Stem Cell 2018

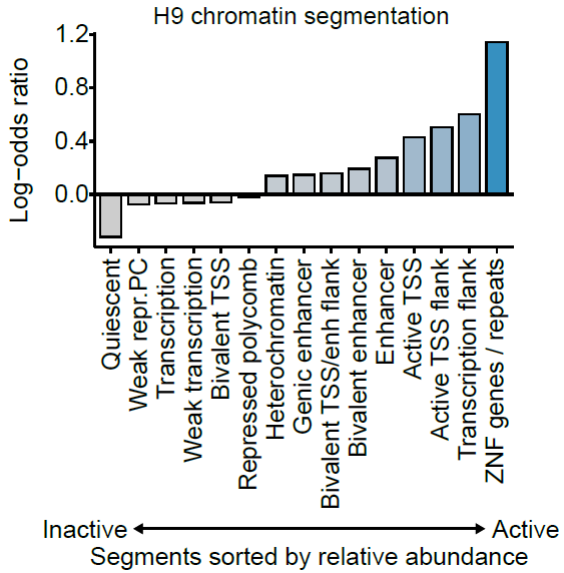
Genome-wide assessment of enhancer functionality by ChIP-STARR-seq in hESCs











Barakat*, Halbritter* et al., Cell Stem Cell 2018

Finding active enhancers in the human genome using ChIP-STARR-seq and related approaches

Active functional enhancers are likely candidate sequences that might contain mutations in those patients that are currently genetically unexplained

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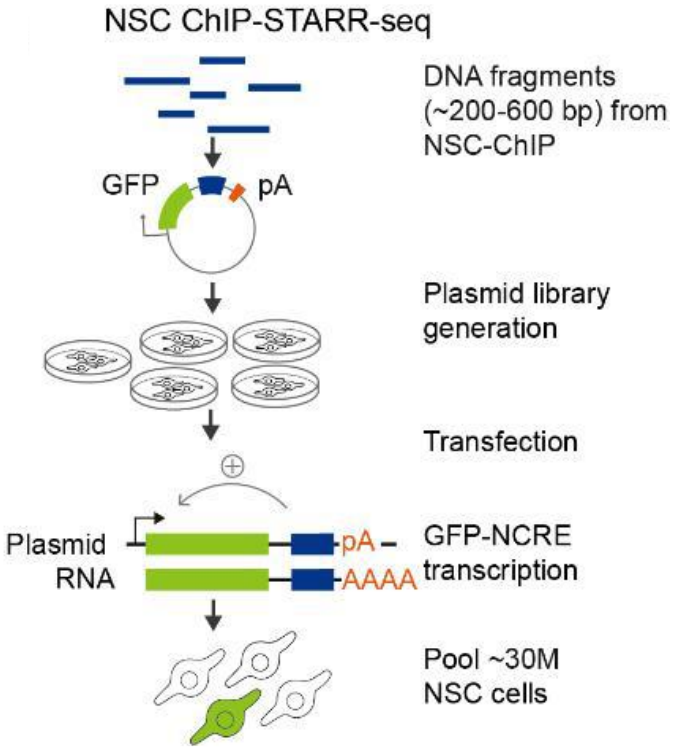
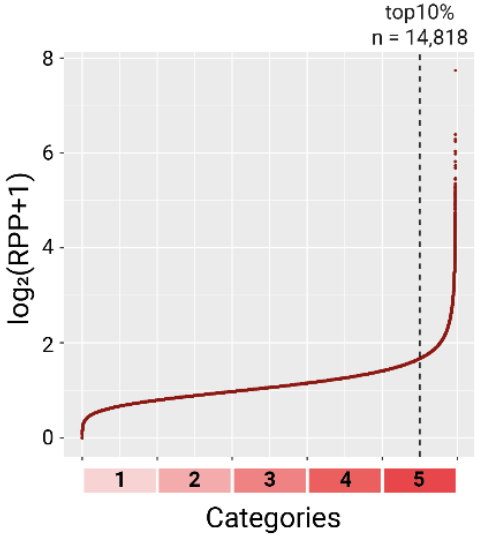
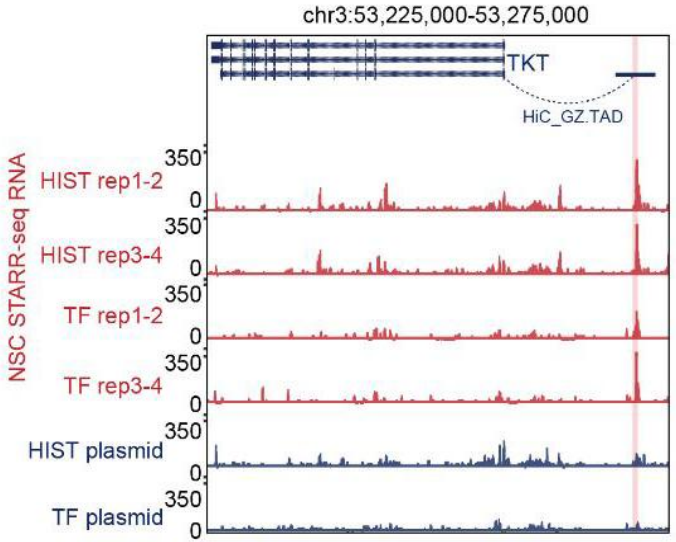
Finding active enhancers using ChIP-STARR-seq in neural stem cells

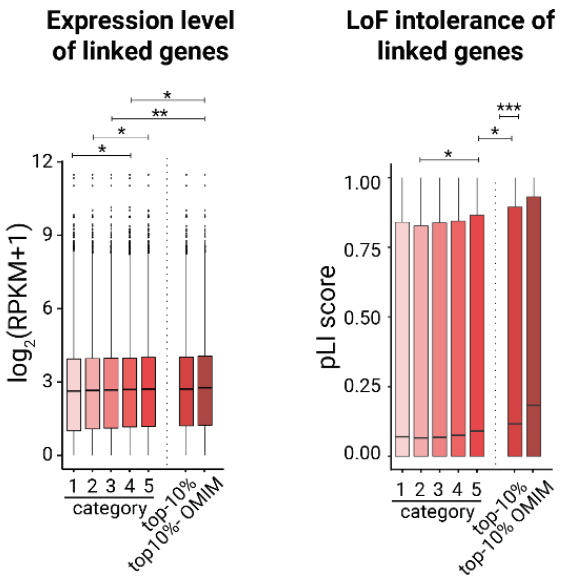


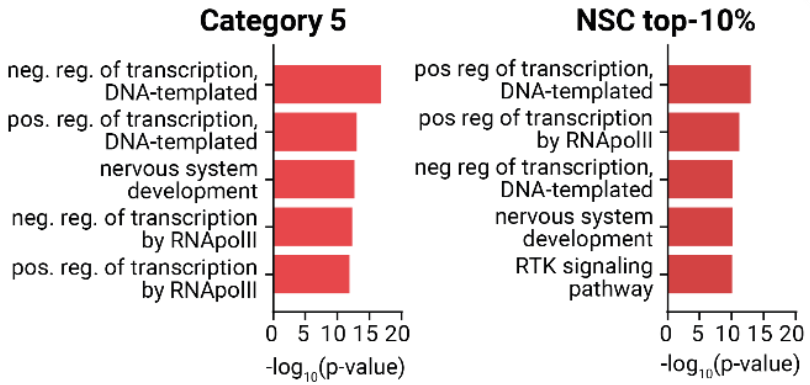
Histone library: H3K27ac and H3K4me
TF library: YY1 and SOX2





Elena Perenthaler





Ruizhi Deng

Deng*, Perenthaler* et al, preprint on medRxiv 2024

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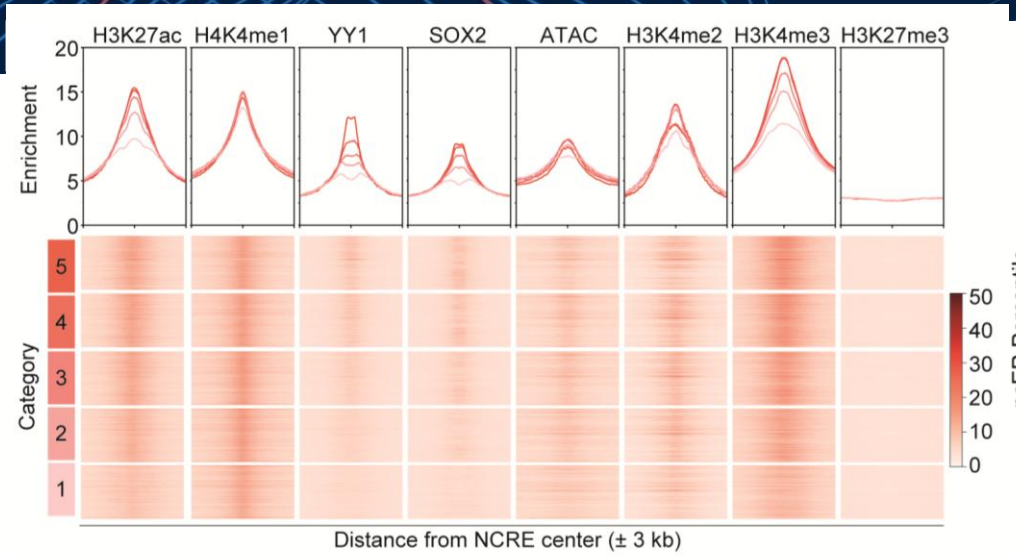
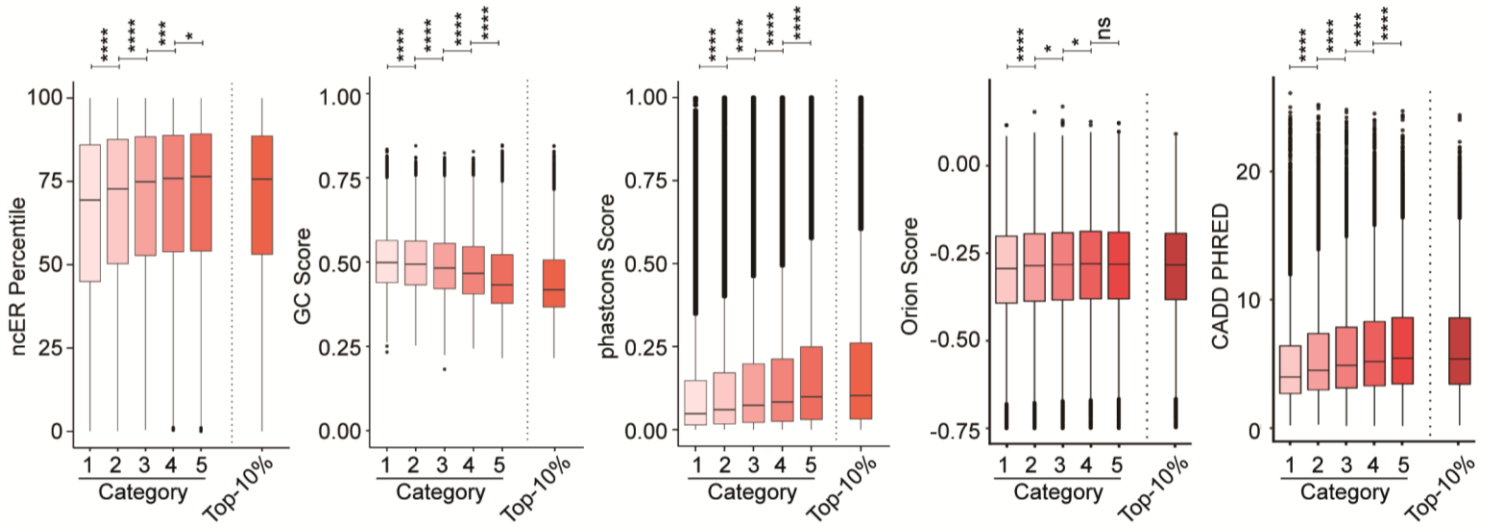
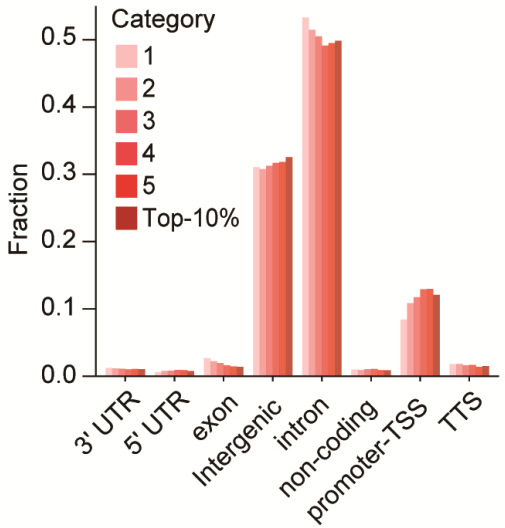
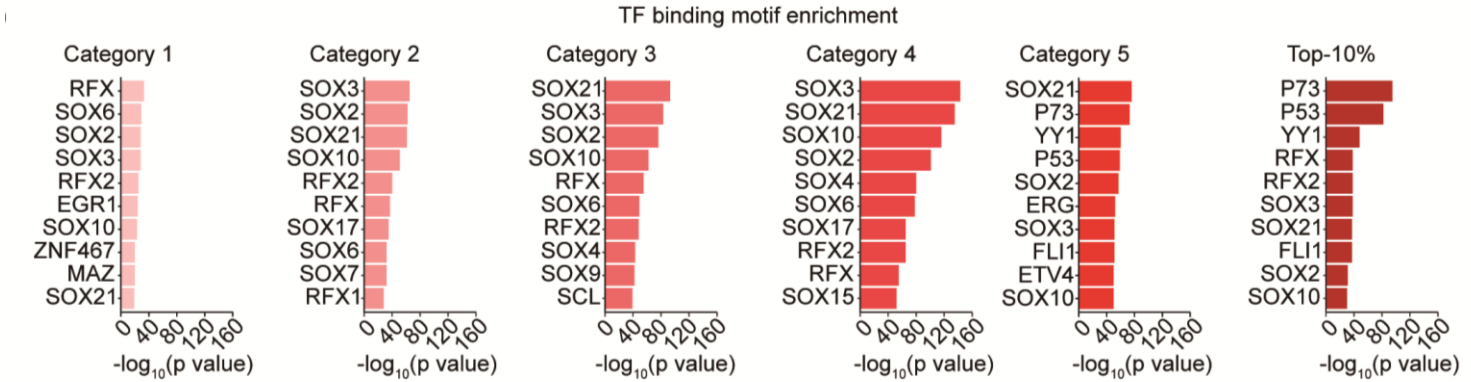
Differences in sequence composition between enhancer activity groups









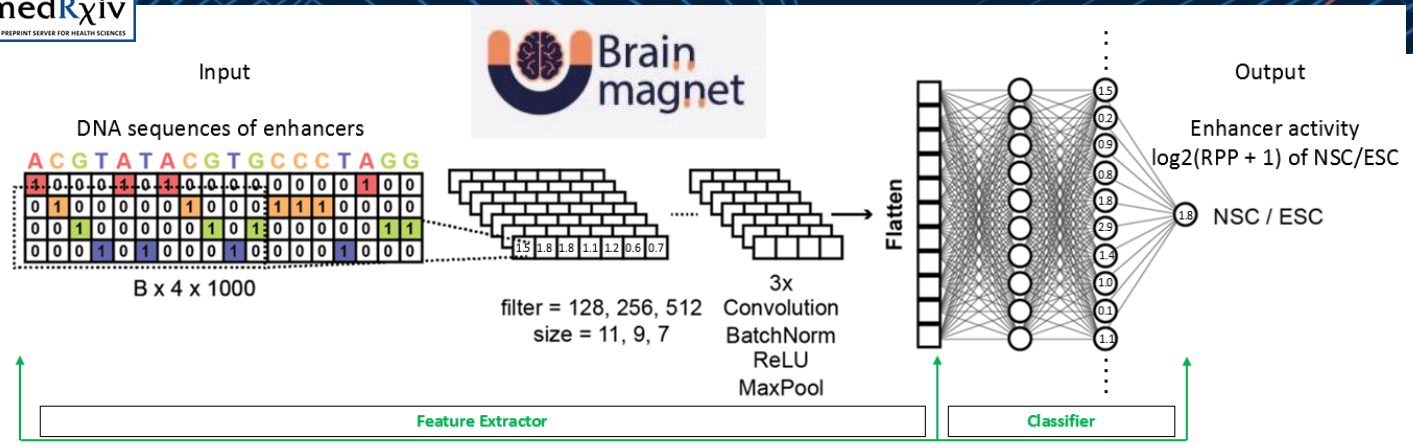
Finding active enhancers in the human genome

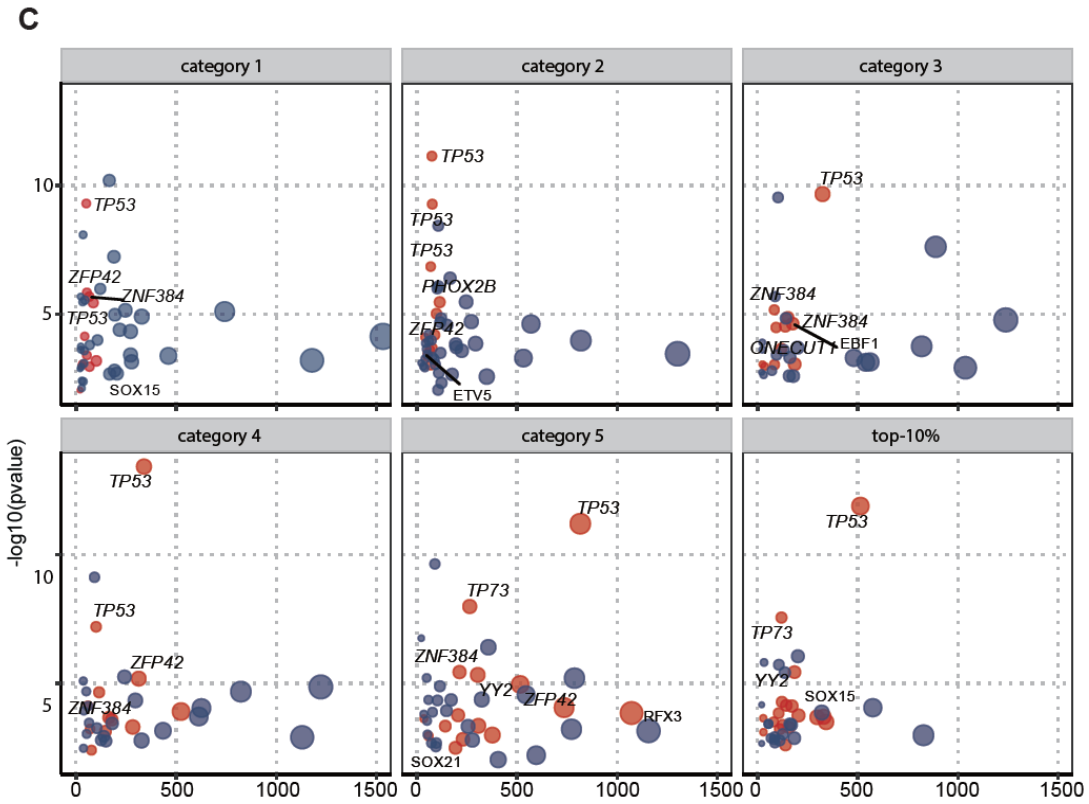
BRAINMAGNET:
BRain-focussed Artificial INtelligence Method to Analyse Genomes for Non-coding regulatory Element mutation Targets

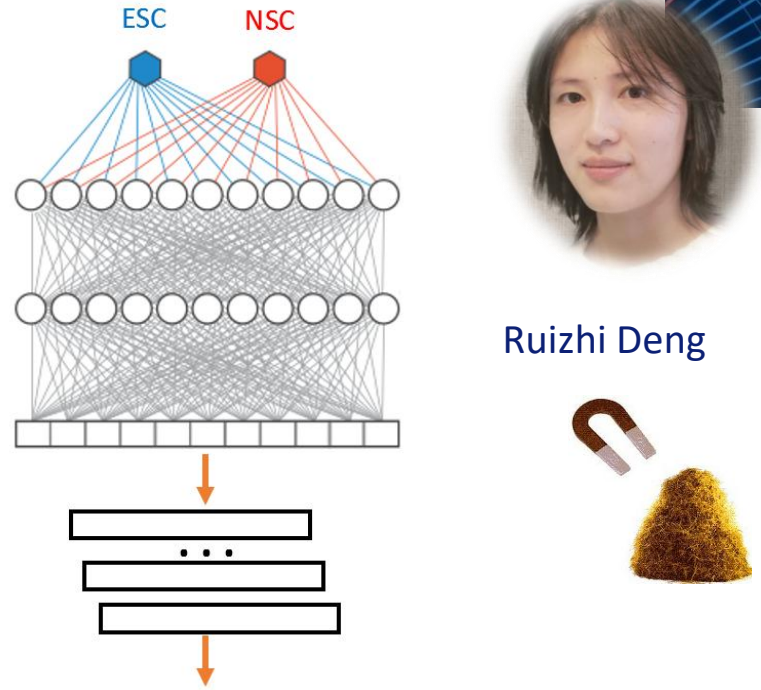
1) We need to find them
2) We need to interpret effects of mutations in them

Deng*, Perenthaler* et al., medRxiv 2024

Sequence-based Convolutional Neuron Network model to predict and interpret enhancer activity (BRAIN-MAGNET)







Ruizhi Deng

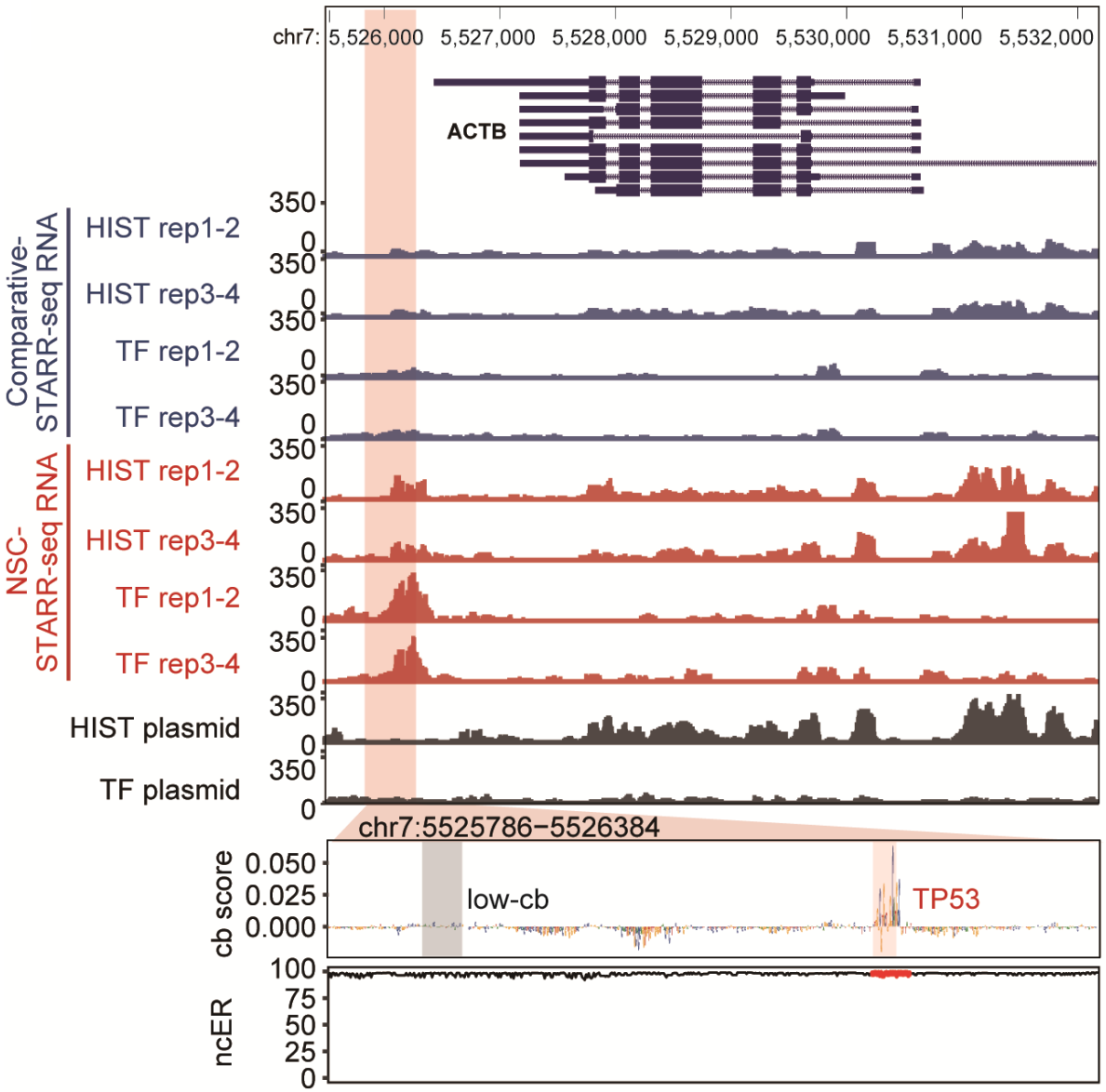
Explainable AI

DeepLIFT (Shrikumar et al., 2017)
DeepExplainer (Lundberg et al., 2020)

TF-Modisco (Shrikumar et al., 2017)
Position weight matrix of JASPAR

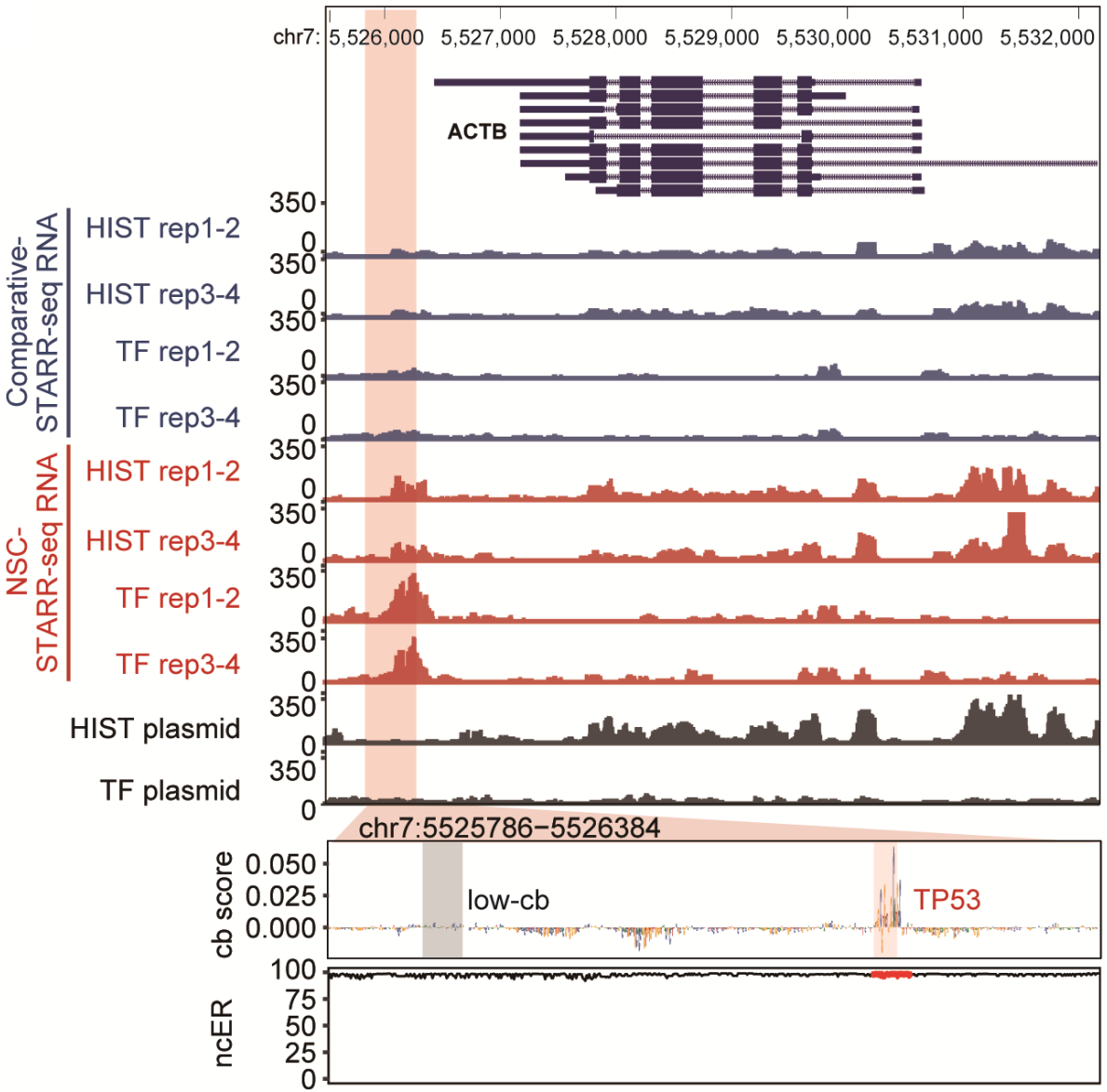
$$M = \begin{matrix} A \\ C \\ G \\ T \end{matrix} \begin{bmatrix} 0.26 & 1.26 & -1.32 & -\infty & -\infty & 1.26 & 1.49 & -0.32 & -1.32 \\ -0.32 & -0.32 & -1.32 & -\infty & -\infty & -0.32 & -1.32 & -1.32 & -0.32 \\ -1.32 & -1.32 & 1.49 & 2.0 & -\infty & -1.32 & -1.32 & 1.0 & -1.32 \\ 0.68 & -1.32 & -1.32 & -\infty & 2.0 & -1.32 & -1.32 & -0.32 & 1.26 \end{bmatrix}.$$

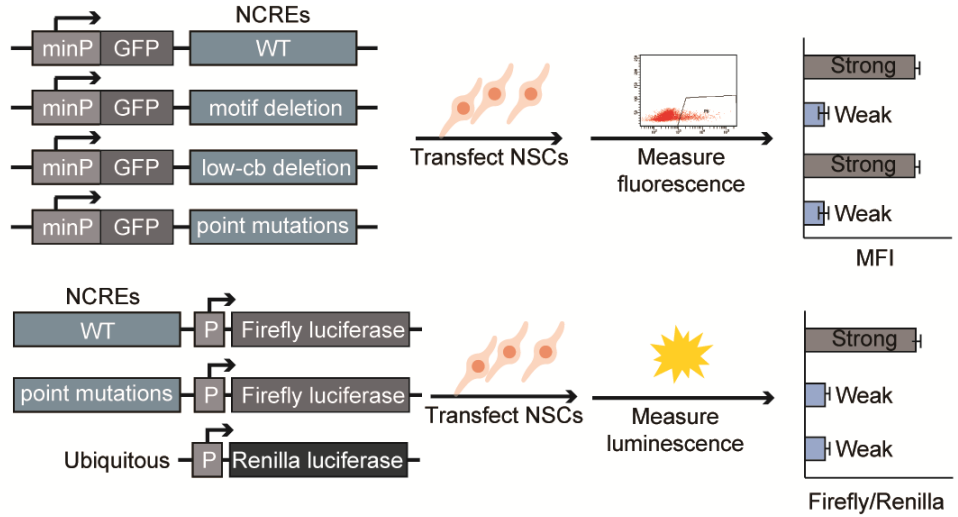
BRAIN-MAGNET allows zooming-in to important nucleotides motifs for enhancer activity

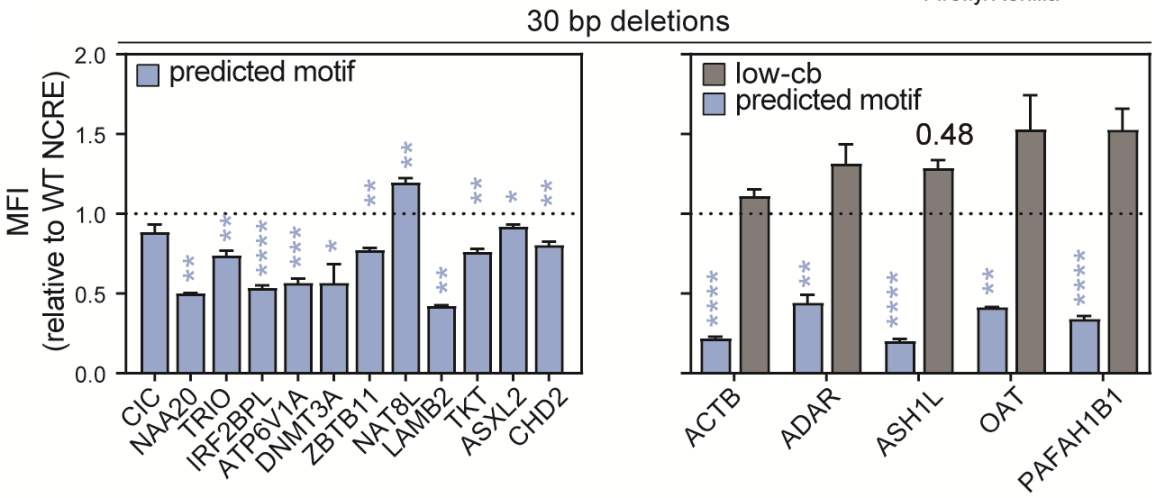


Deng*, Perenthaler* et al., medRxiv 2024

BRAIN-MAGNET allows zooming-in to important nucleotides motifs for enhancer activity

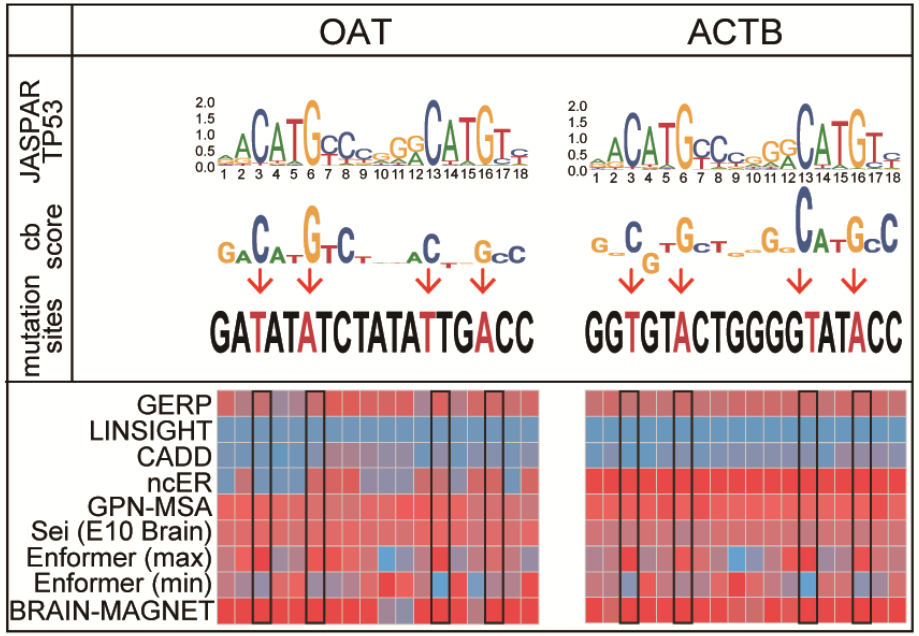


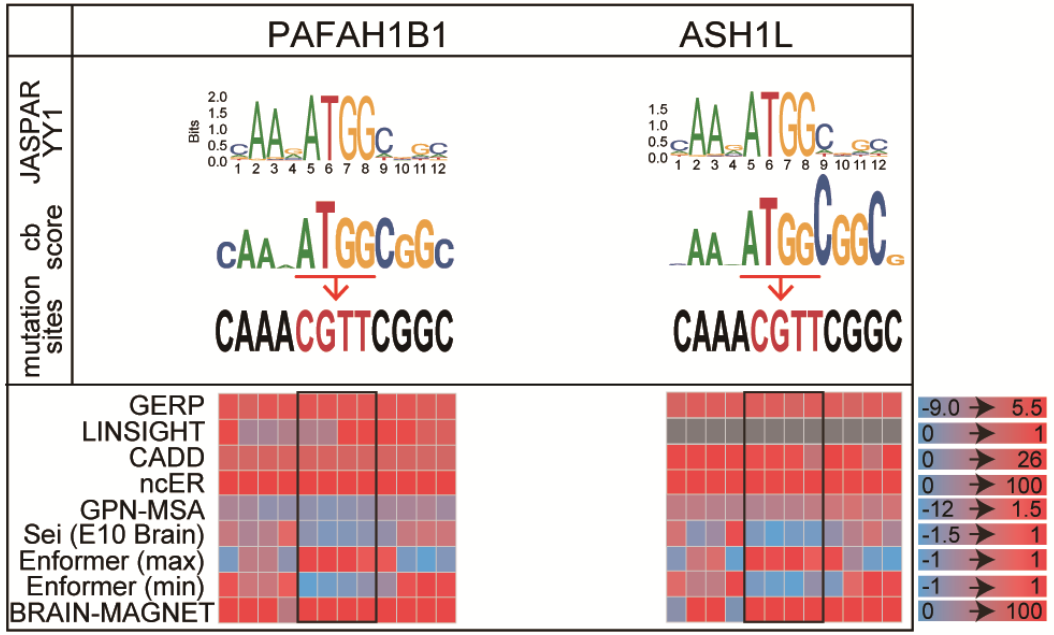


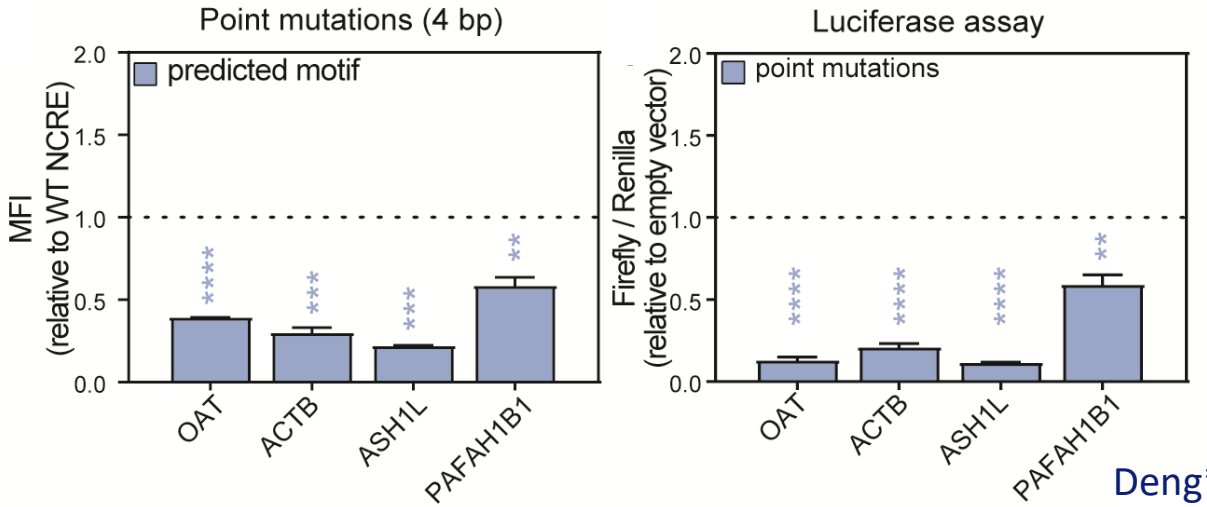


Deng*, Perenthaler* et al., medRxiv 2024

BRAIN-MAGNET allows zooming-in to important nucleotides motifs for enhancer activity



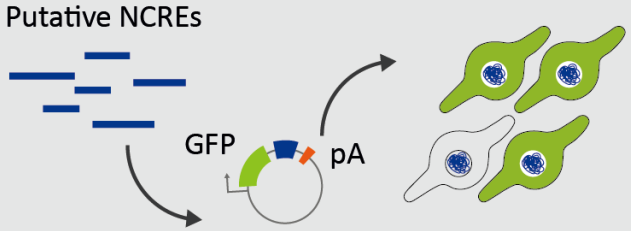




Deng*, Perenthaler* et al., medRxiv 2024

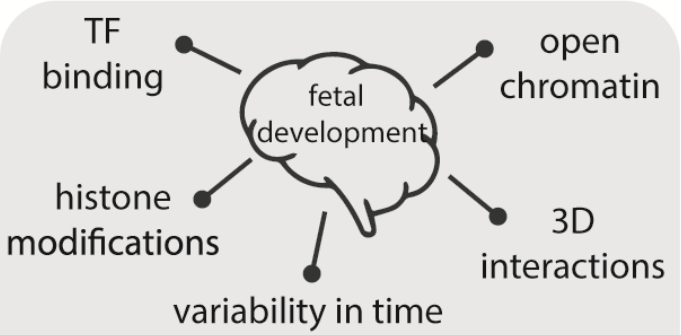
Moving to the future: exploring junk DNA using an atlas of non-coding regulatory elements (NCREs) to understand unexplained disease

Functional genomics



Deng et al., medRxiv
Barakat et al., Cell Stem Cell

Computational genomics



Yousefi et al Genome Med 2021

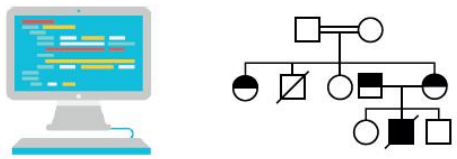
Genomics england

Erasmus MC
Universitair Medisch Centrum Rotterdam

Exclude cases with disease-causing exonic or splice mutations

Prioritize non-coding variants

Overlap variants with high resolution NCRE map



Machine learning and in silico predictors of pathogenecity

Segregation

Functional studies

Investigating the 98% of the human genome where current diagnostics stops

-improving diagnostics
-understand pathology
-new targets for therapy

Barakat et al Cell Stem Cell 2018, Perenthaler et al Front. Cell Neuroscience 2019, Yousefi et al Genome Medicine 2021

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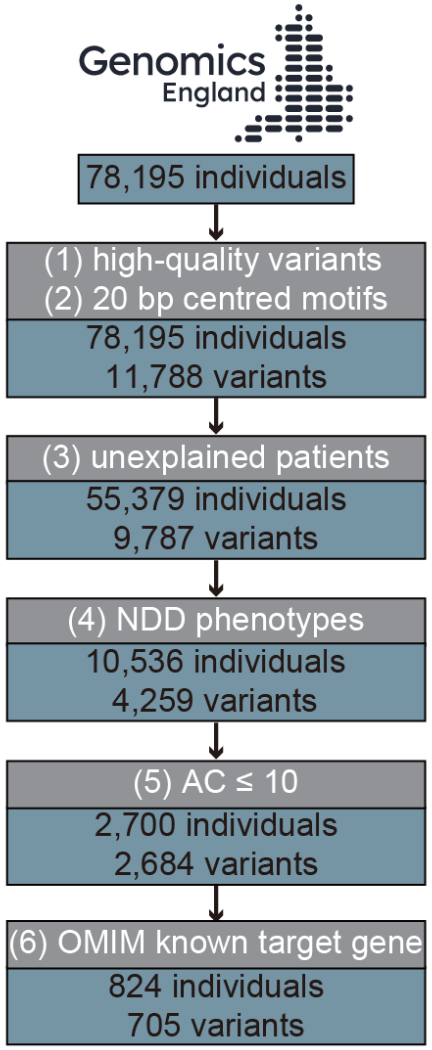
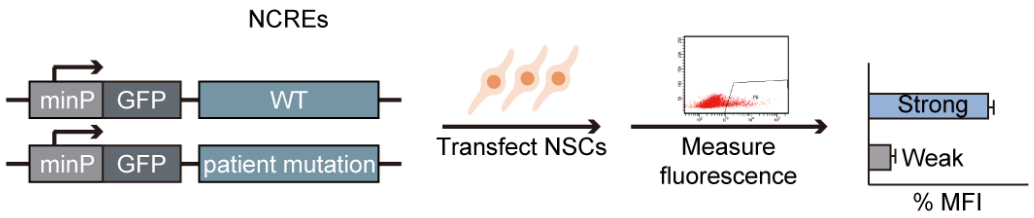
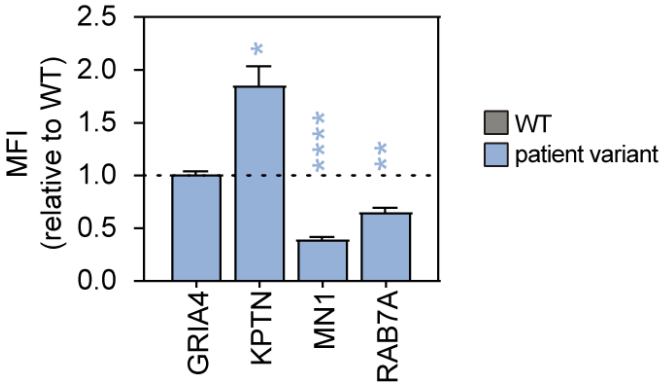
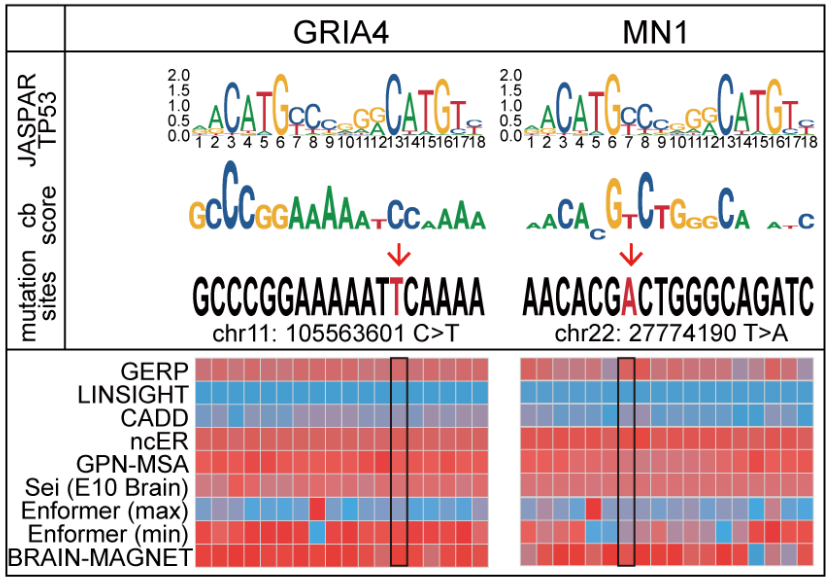
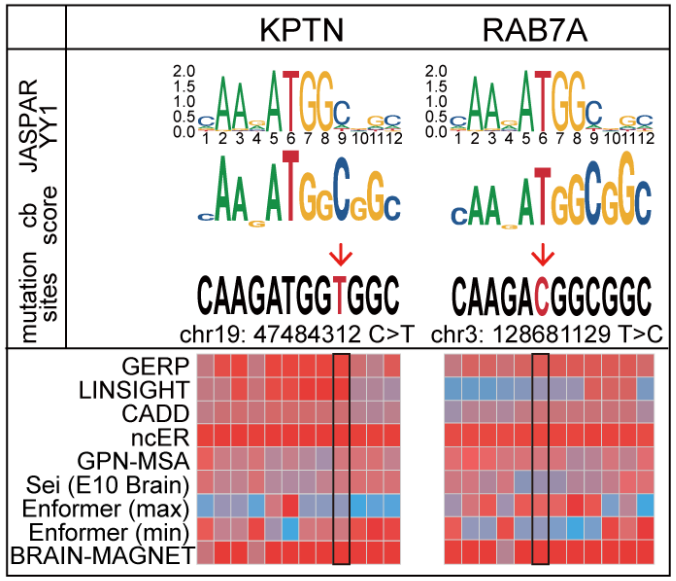
Applying BRAIN-MAGNET to the Genomics England 100,000 Genome Project identifies possible enhancer opathies

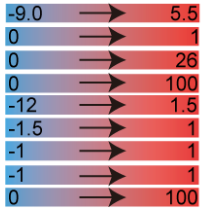






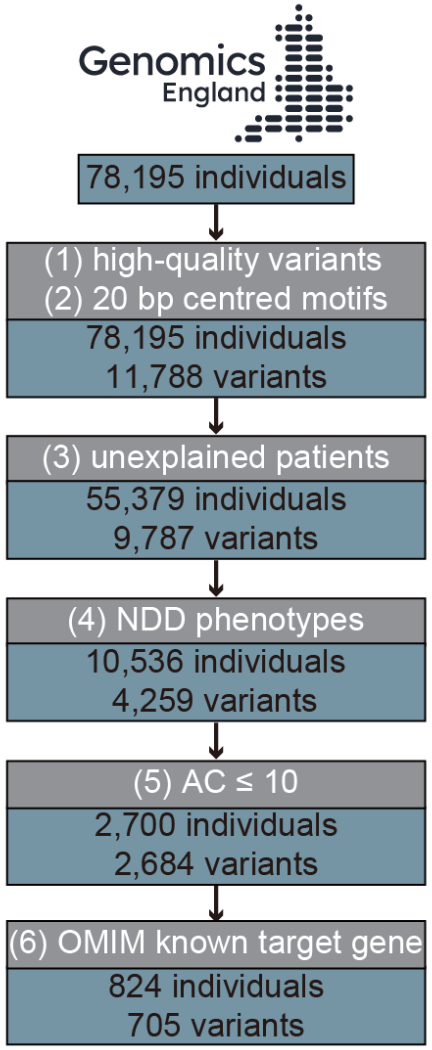


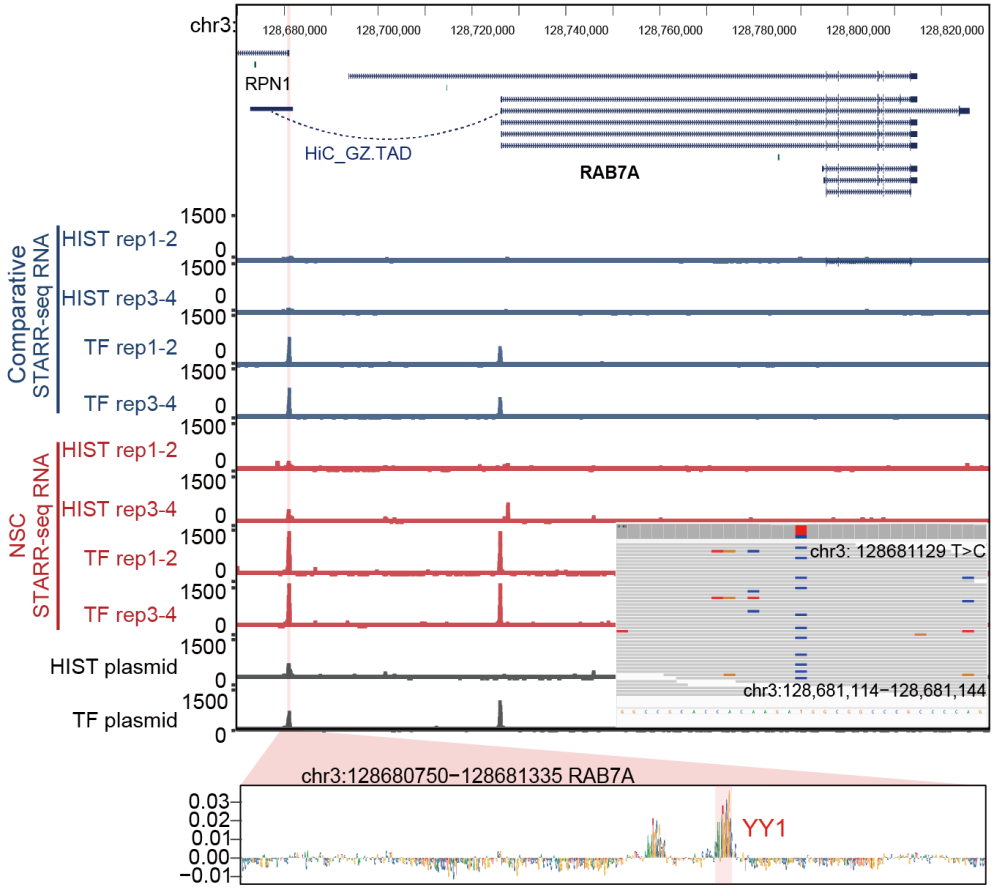


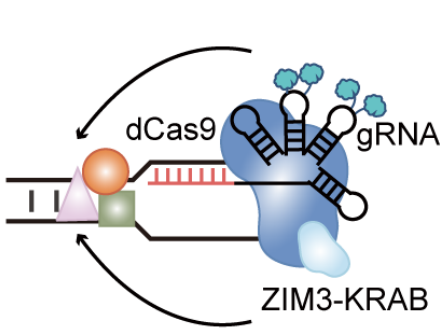


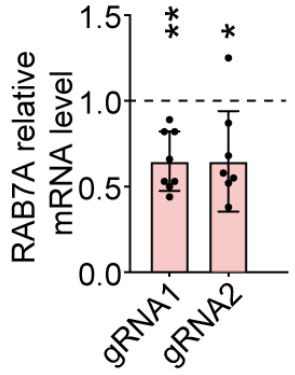
Deng*, Perenthaler* et al., medRxiv 2024

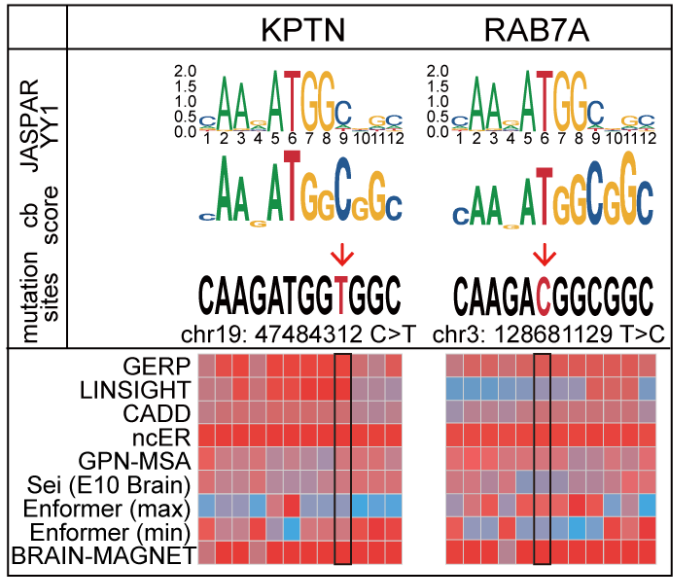
Applying BRAIN-MAGNET to the Genomics England 100,000 Genome Project identifies possible enhancer opathies









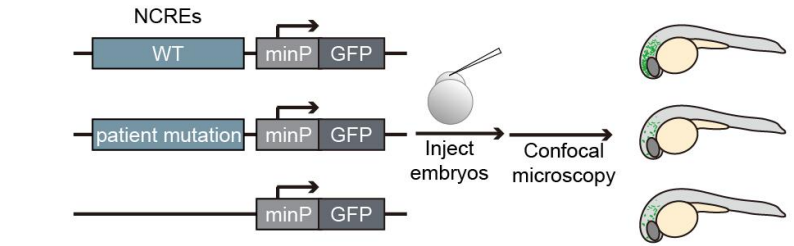


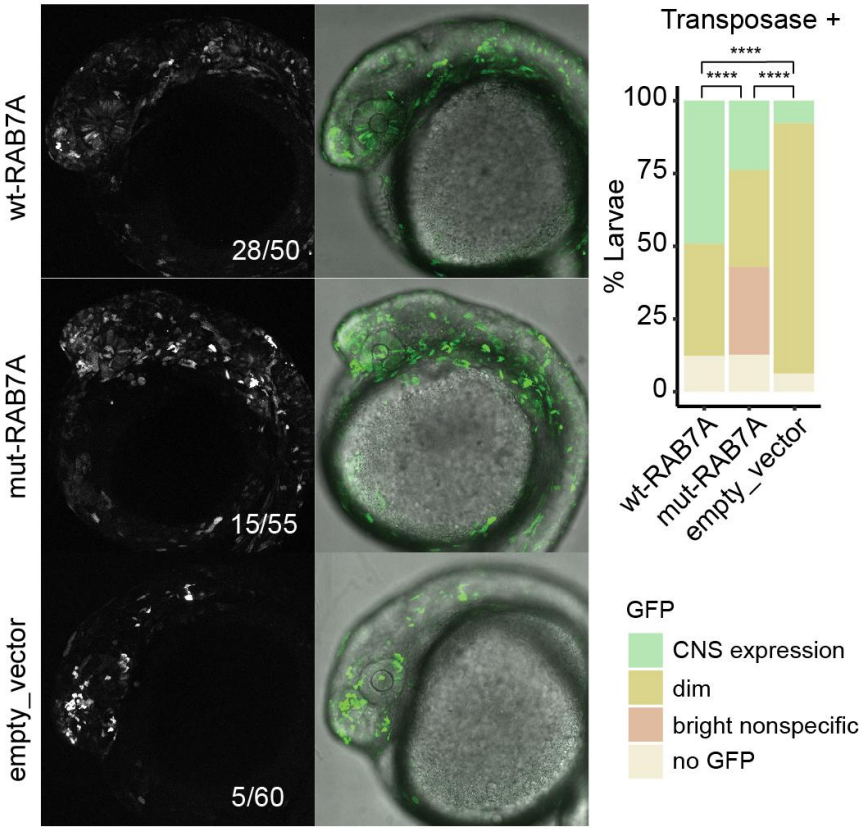
-gnomAD v4: absent.
-GEL: 1x
-RAB7A: OMIM #600882: Charcot-Marie-Tooth disease, type 2B
-HPO terms of individual fitting the phenotype

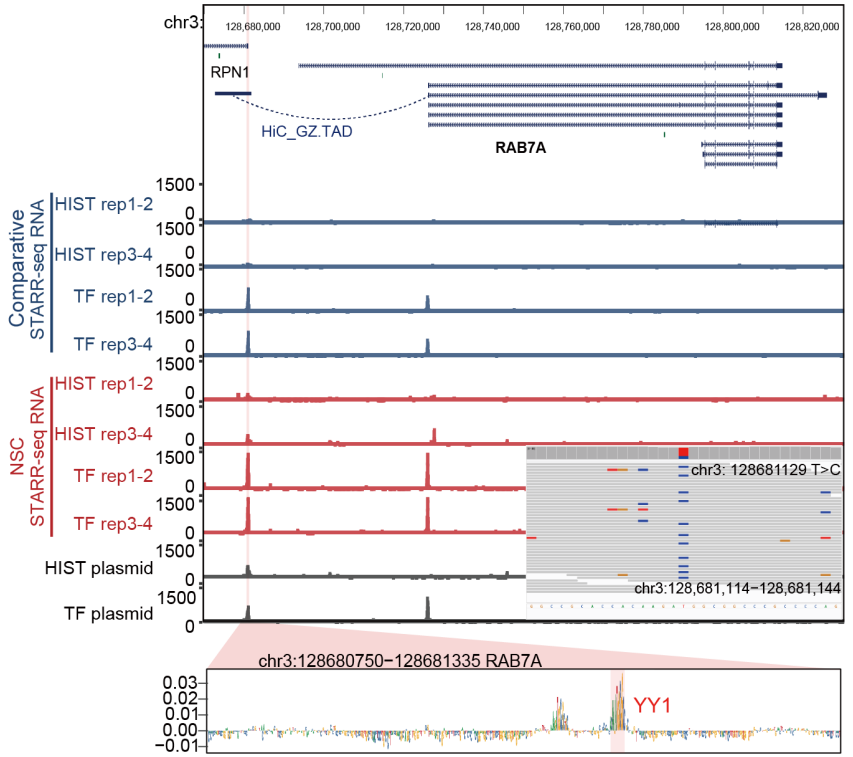
Patient of Michael Parker, Sheffield

Deng*, Perenthaler* et al., medRxiv 2024

Applying BRAIN-MAGNET to the Genomics England 100,000 Genome Project identifies possible enhanceropathies





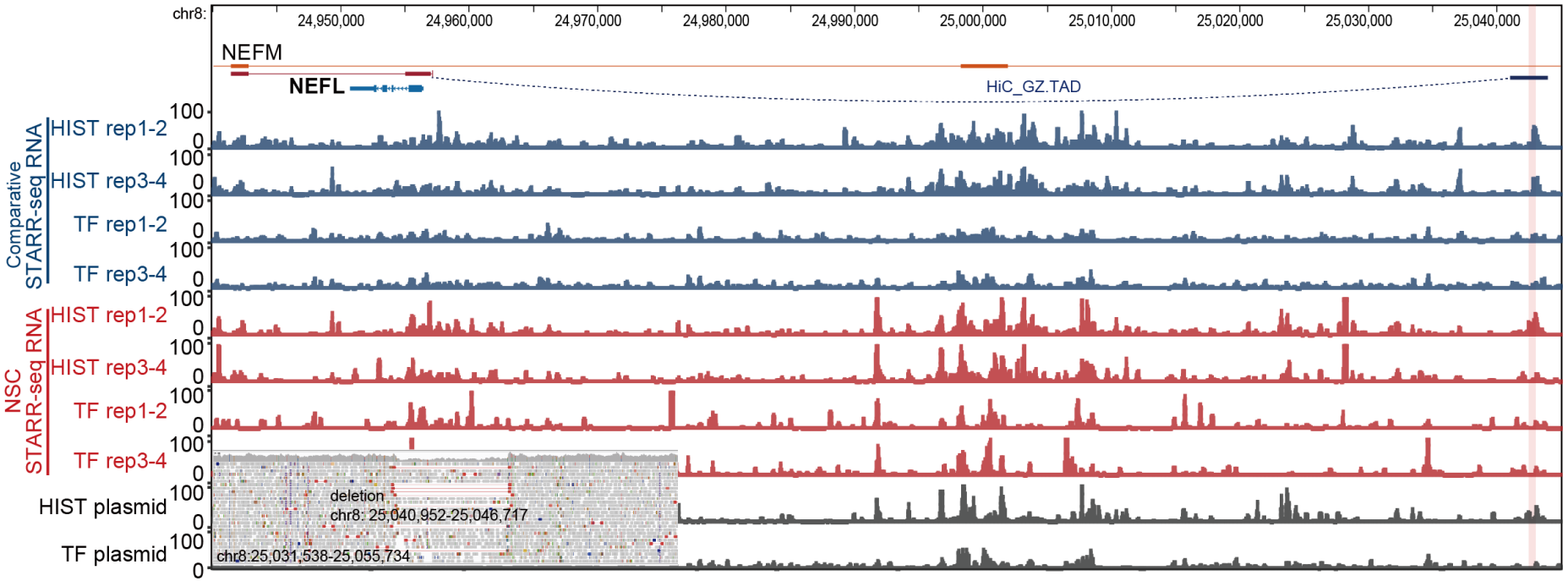


-gnomAD v4: absent.
-GEL: 1x
-RAB7A: OMIM #600882: Charcot-Marie-Tooth disease, type 2B
-HPO terms of individual fitting the phenotype

Deng*, Perenthaler* et al., medRxiv 2024

Applying BRAIN-MAGNET to other rare disease cohorts

Screening through 3,971 singleton WGS of unexplained rare disease patients, for deletions overlapping with high confidence NCREs identifies several candidates

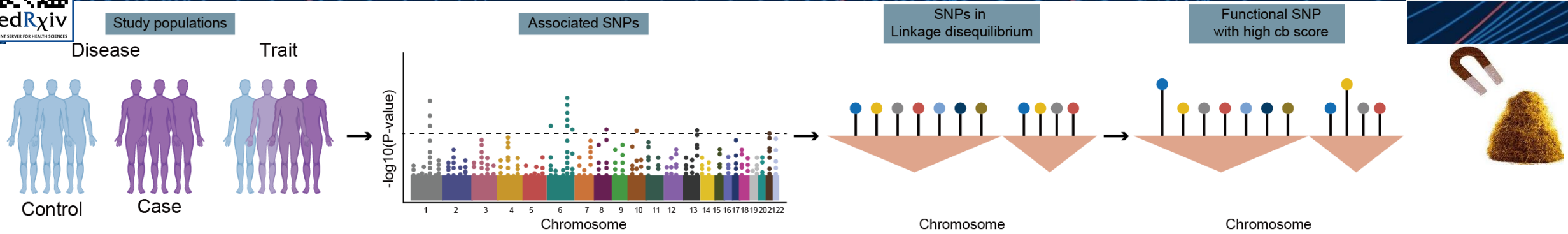


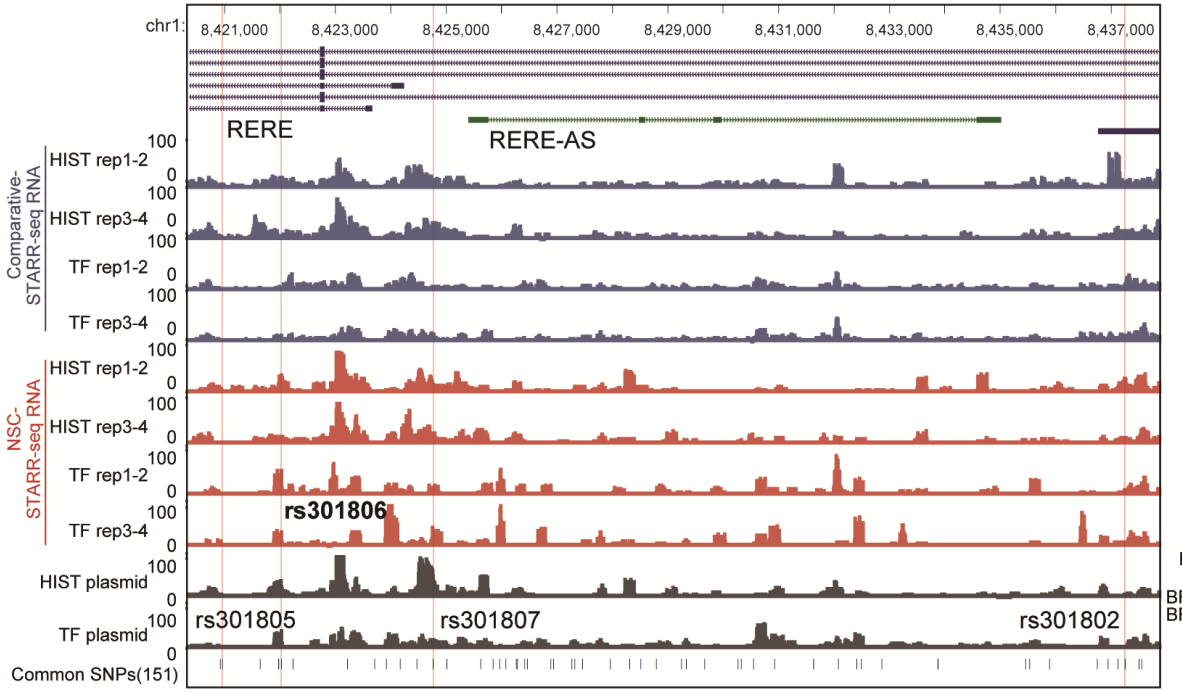
Example:
-5.7 kb deletion of an NCRE for NEFL
-NEFL involved in dominant forms of Charcot Marie Tooth
-found in genetically unsolved adult with motor and sensory neuropathy

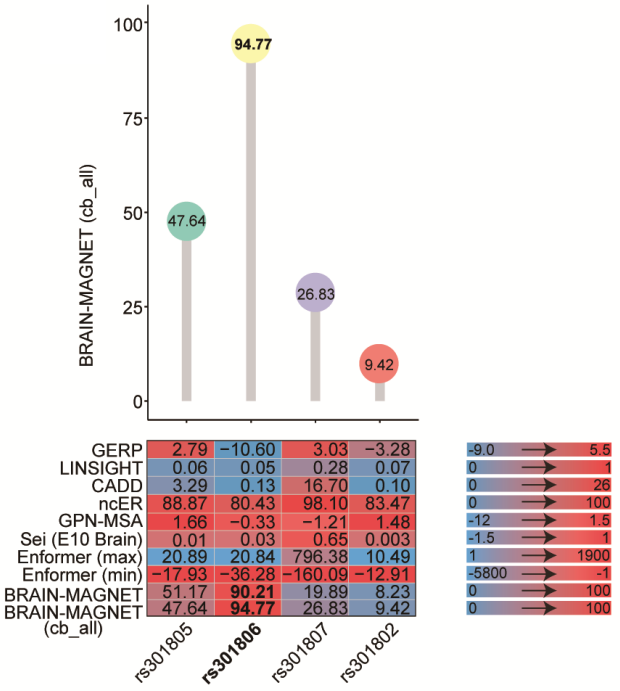
Collaboration with Joohyun Park, Marc Sturm, Tobias B. Haack, Tuebingen

Deng*, Perenthaler* et al., medRxiv 2024

Applying BRAIN-MAGNET to fine-map GWAS loci





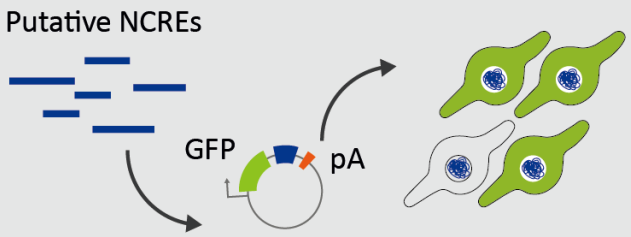


-GWAS locus on chr 1
-4 SNPs in LD, associated with depression
-recent study validates experimentally that rs301806 is the biological relevant SNP
(Guo et al Nat. Genet. 2023)

Deng*, Perenthaler* et al., medRxiv 2024

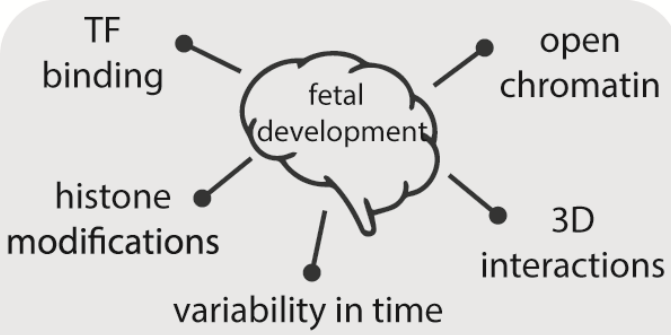
Call for collaboration: exploring junk DNA using an atlas of non-coding regulatory elements (NCREs) to understand unexplained disease

Functional genomics

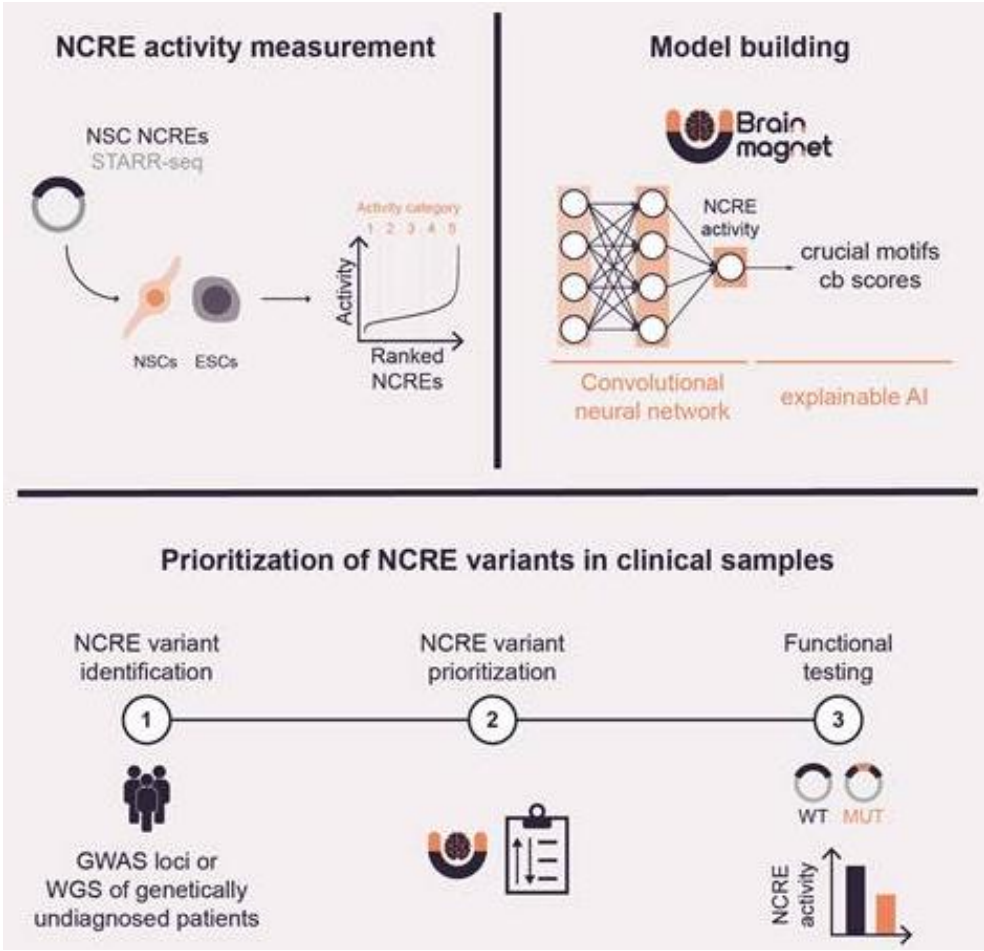


Deng et al., medRxiv
Barakat et al., Cell Stem Cell

Computational genomics



Yousefi et al Genome Med 2021



-looks like syndrome X but no coding mutation
(metabolic, episignatures, typical dysmorphic features)
-AR disease, but no second hit

Investigating the 98% of the human genome where current diagnostics stops

-improving diagnostics
-understand pathology
-new targets for therapy

BRain-focussed **A**rtificial **IN**telligence **M**ethod to **A**nalyse **G**enomes for **N**on-coding regulatory **E**lement mutation **T**argets

Barakat et al Cell Stem Cell 2018, Yousefi et al Genome Medicine 2021, Deng*, Perenthaler* et al., medRxiv 2024

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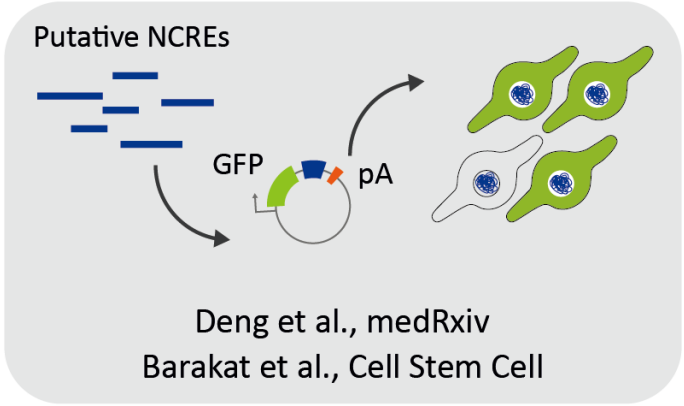
Call for collaboration: exploring junk DNA using an atlas of non-coding regulatory elements (NCREs) to understand unexplained disease

Functional genomics



Deng et al., medRxiv
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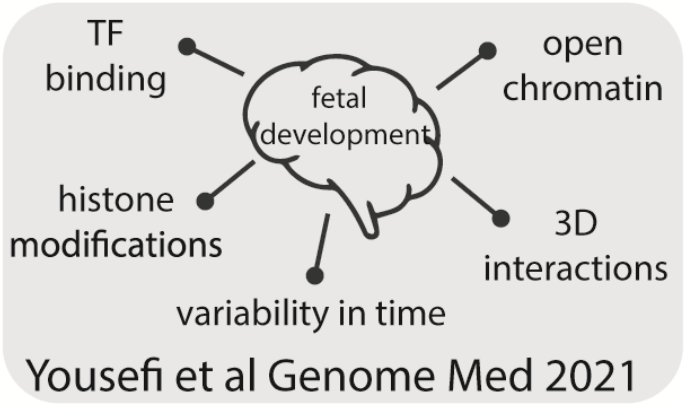
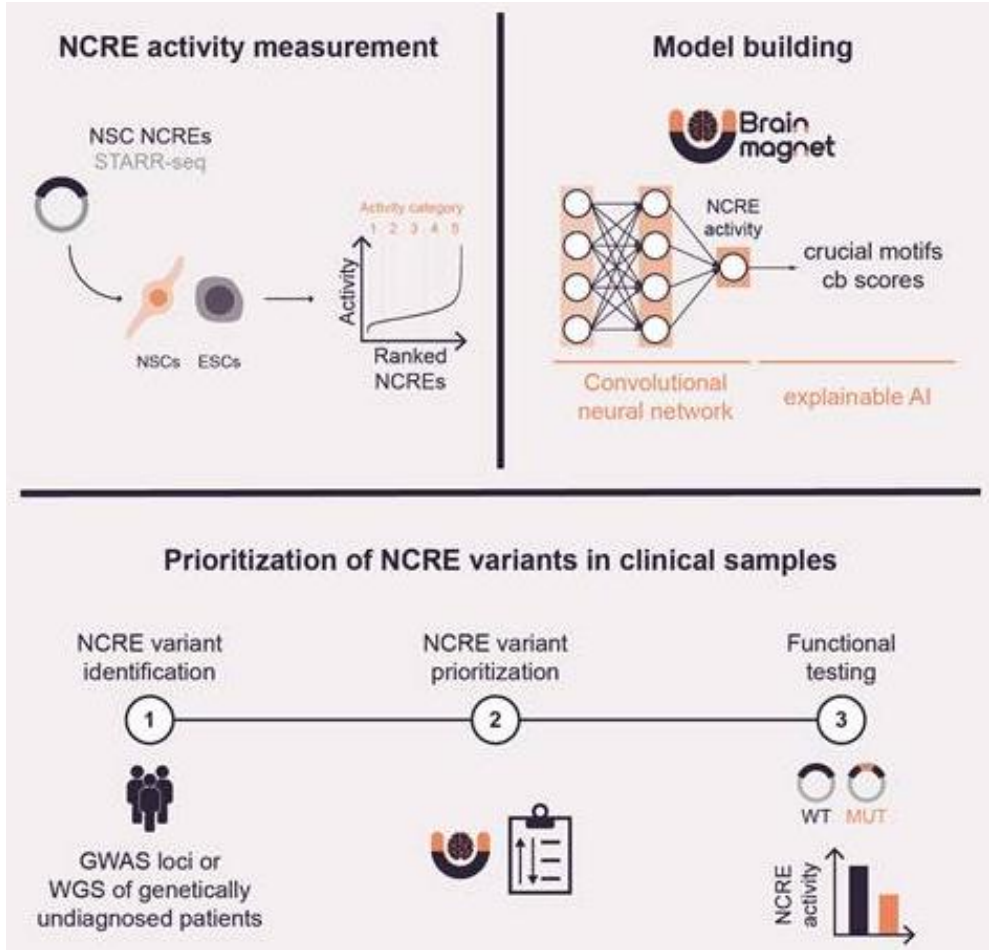
Computational genomics



Yousefi et al Genome Med 2021



Ongoing:

-integrating BRAIN-MAGNET cb scores and other noncoding variant prediction tools (SEI, ENFORMER, GPN-MSA etc.) into one integrated WGS research pipeline

BRain-focussed **A**rtificial **IN**telligence **M**ethod to **A**nalyse **G**enomes for **N**on-coding regulatory **E**lement mutation **T**argets

Barakat et al Cell Stem Cell 2018, Yousefi et al Genome Medicine 2021, Deng*, Perenthaler* et al., medRxiv 2024

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Barakat lab 2025

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-and many many others!!!

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Klinische Genetica

-Erasmus MC MRACE pilot 2019
-Human Disease Model Award 2018
-Erasmus MC Fellowship 2017

KNAW Early Career Award 2021

https://www.erasmusmc.nl/en/research/groups/barakat-lab-non-coding-genome-in-clinical-genetics

Links to BRAIN-MAGNET preprint:

A novel functional genomics atlas coupled with convolutional neural networks facilitates clinical interpretation of disease relevant variants in non-coding regulatory elements

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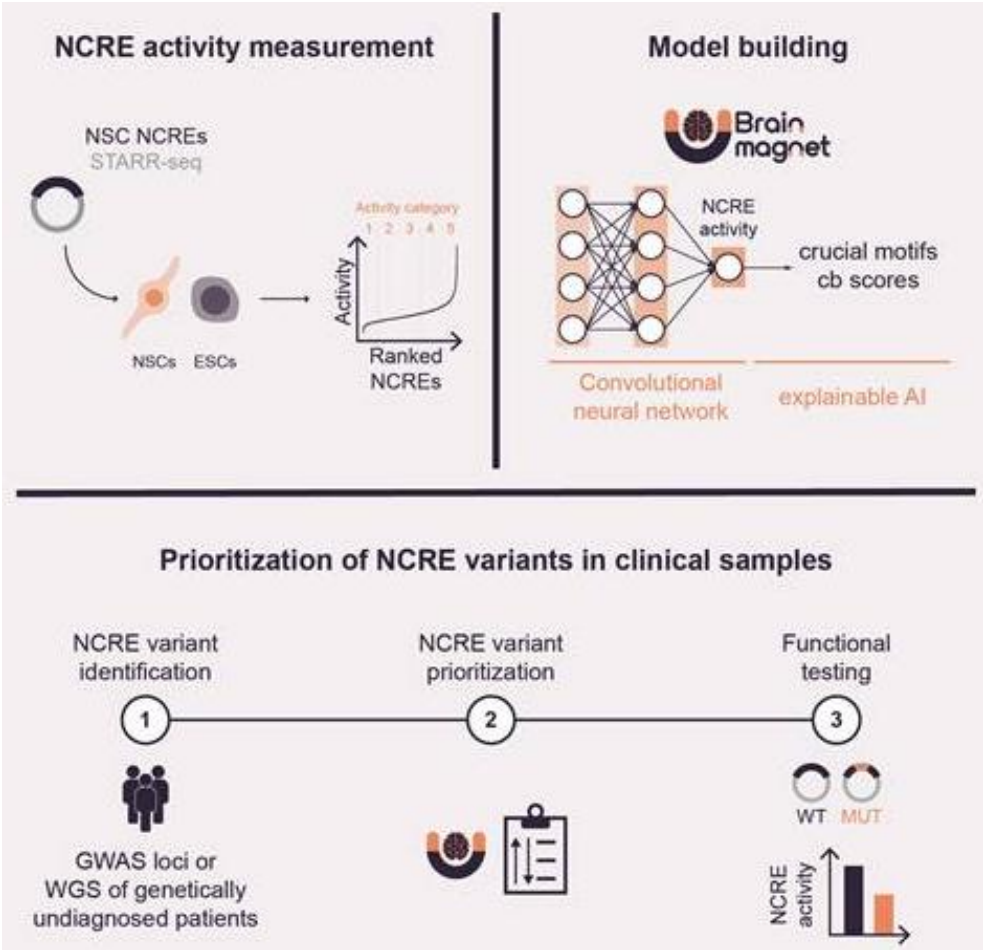

Deng*, Perenthaler et al, medRxiv,16th April 2024

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Discussion & Conclusion

- Time for questions

Thank you for your participation

- Satisfaction Survey :
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- Website :
  - https://ern-ithaca.eu
  - https://ern-ithaca.eu/webinars/