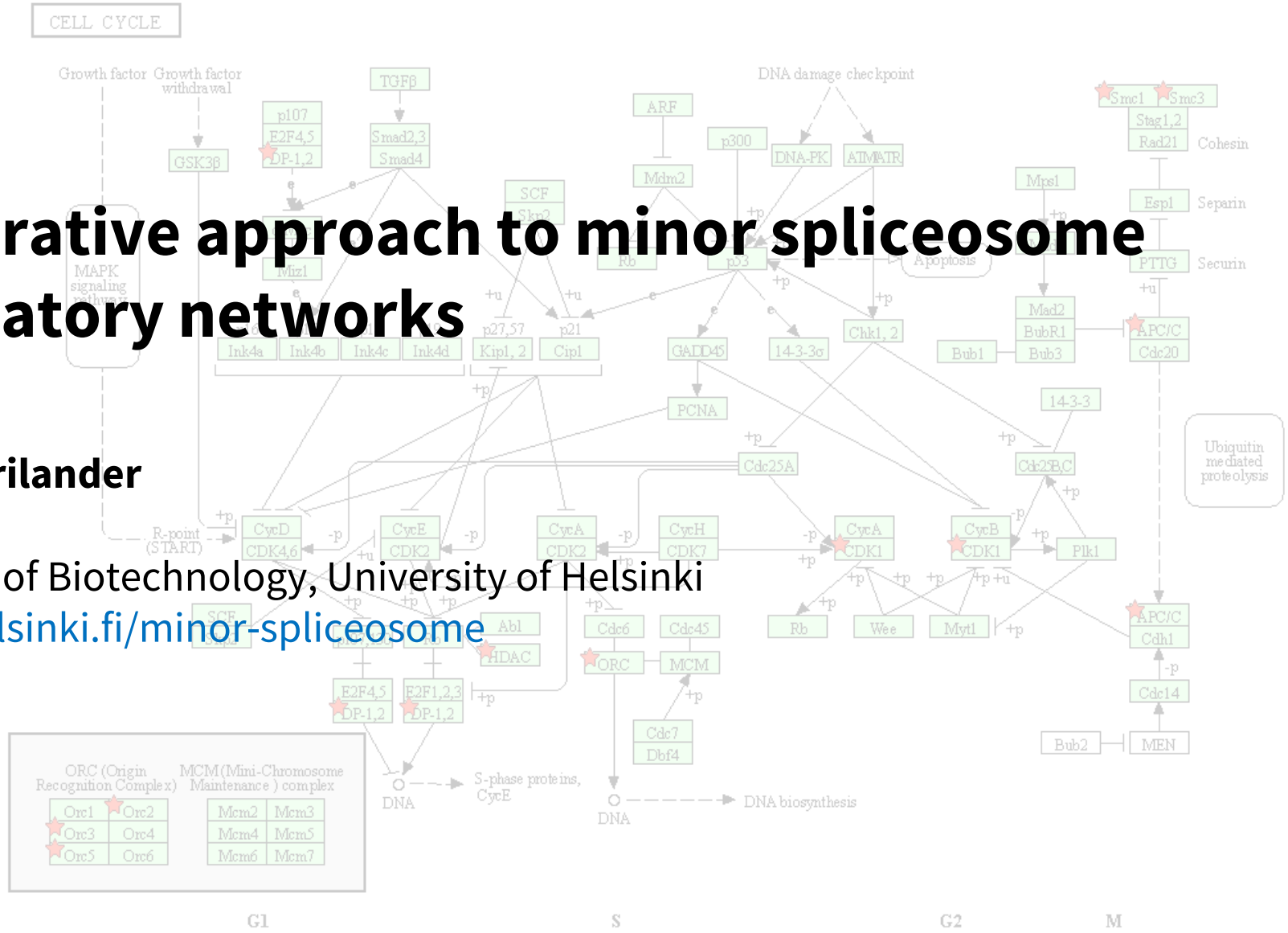
R’Life Kick-off Seminar, Nov 26, 2020



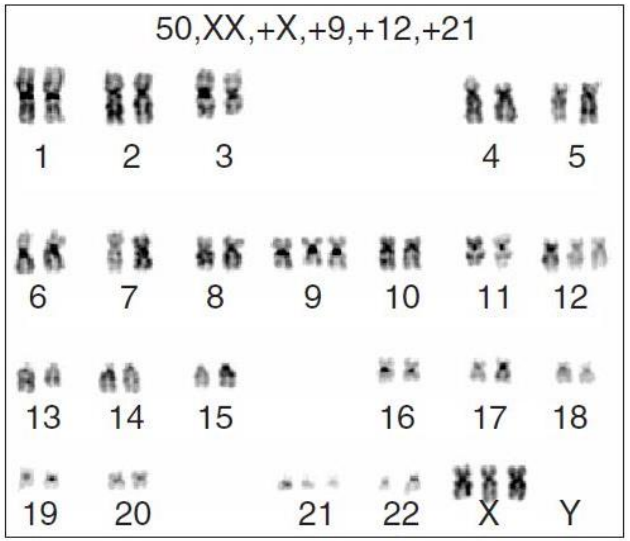
Integrative approach to minor spliceosome regulatory networks

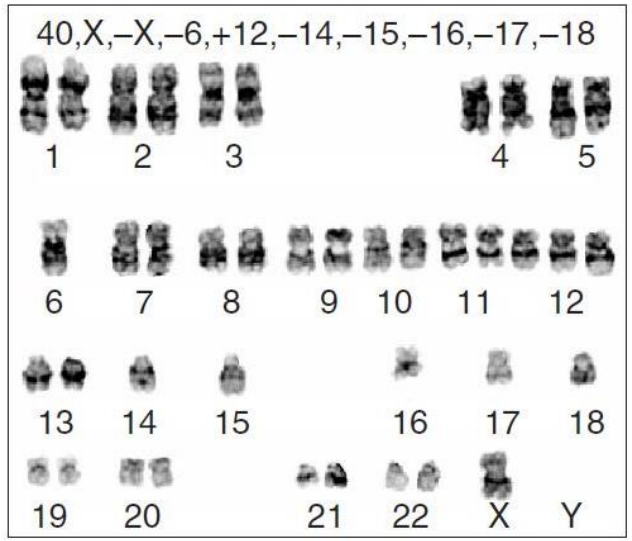
Mikko Frilander

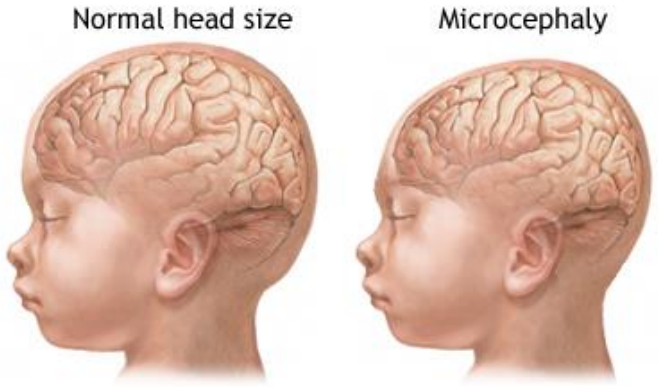
Institute of Biotechnology, University of Helsinki
www.Helsinki.fi/minor-spliceosome

Mosaic Variegated Aneuploidy (MVA)

- Rare Autosomal recessive disease
- Random loss individual chromosomes during mitosis.
- Microcephaly, pre/post natal growth restriction, global developmental delay and dysmorphic facial features
- Cancer: Wilm's Tumor, Rhabdomyosarcorma and in some case Myelodysplastic syndrome







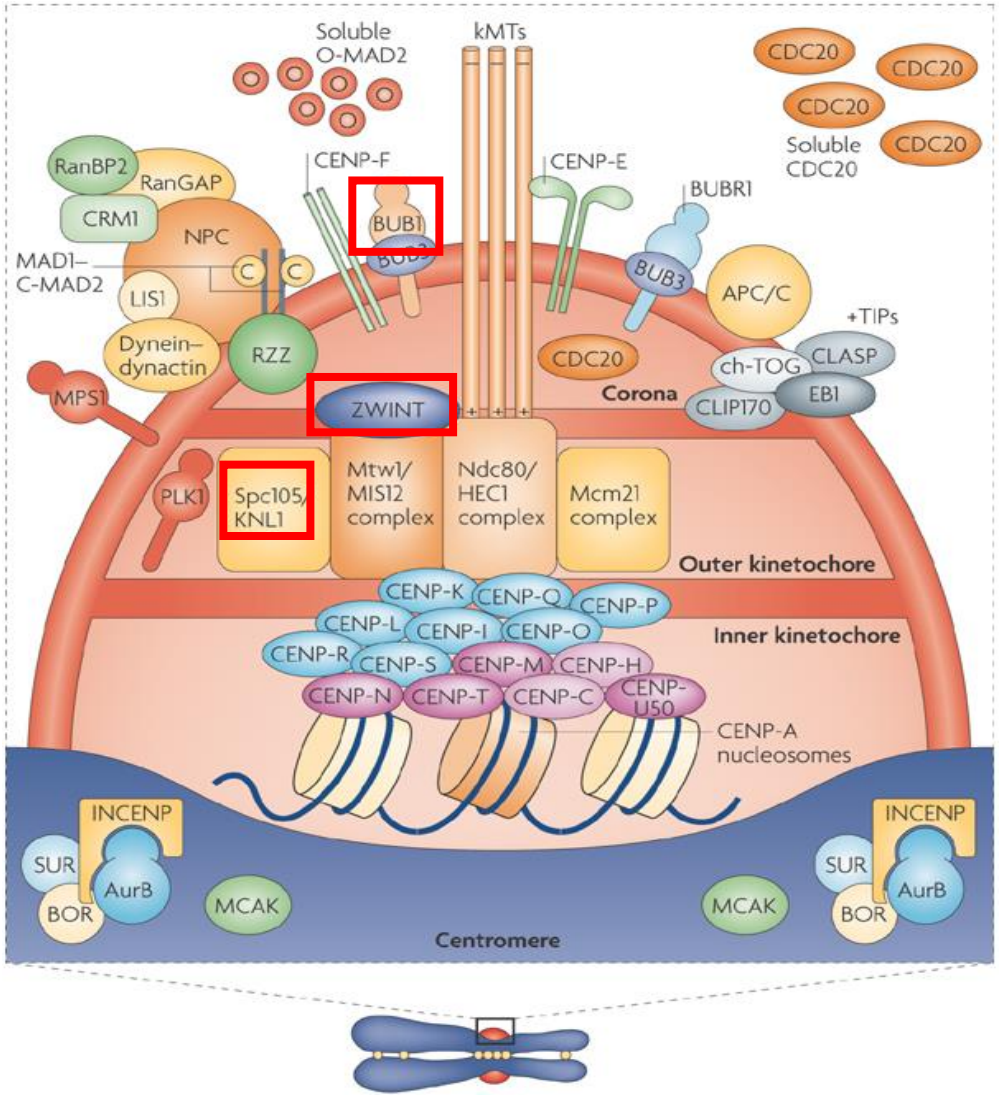
Bas de Wolf & Geert Kops (Hubrecht &Oncode Institute); Atlas Genetic Oncology.org

CCDC84/CENATAC: A novel disease gene causing MVA

Most MVA genes are part of Kinetochore or involved in accurate chromosomal segregation.

Mutated gene	# patients	Aneuploidy
BUB1B	21	9-83%
CEP57	5	15-32%
TRIP13	7	10-46%
KNL1	4	9-13%
ZWINT	2	92%
CCDC84	2	8%

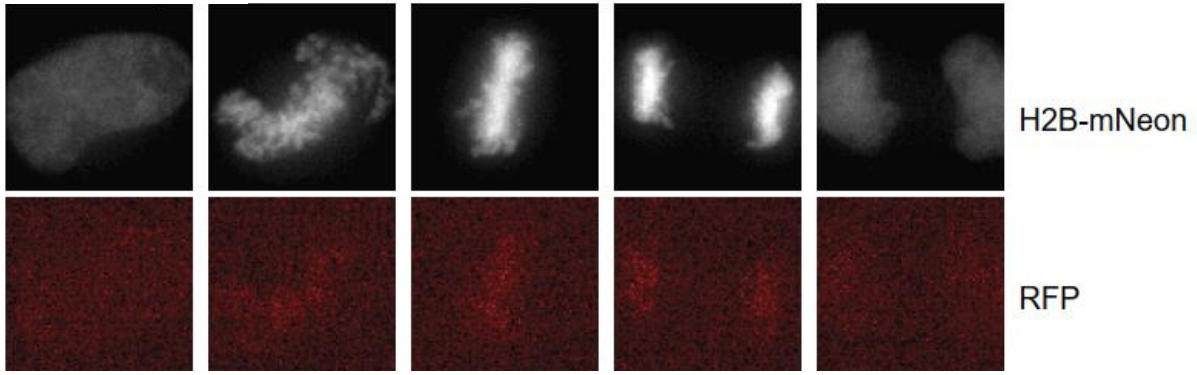
Hanks et al. *Nature Genetics*, 2004
Snape et al. *Nature Genetics*, 2011
De Wolf, Yost & Hanks et al. *Nature Genetics*, 2017



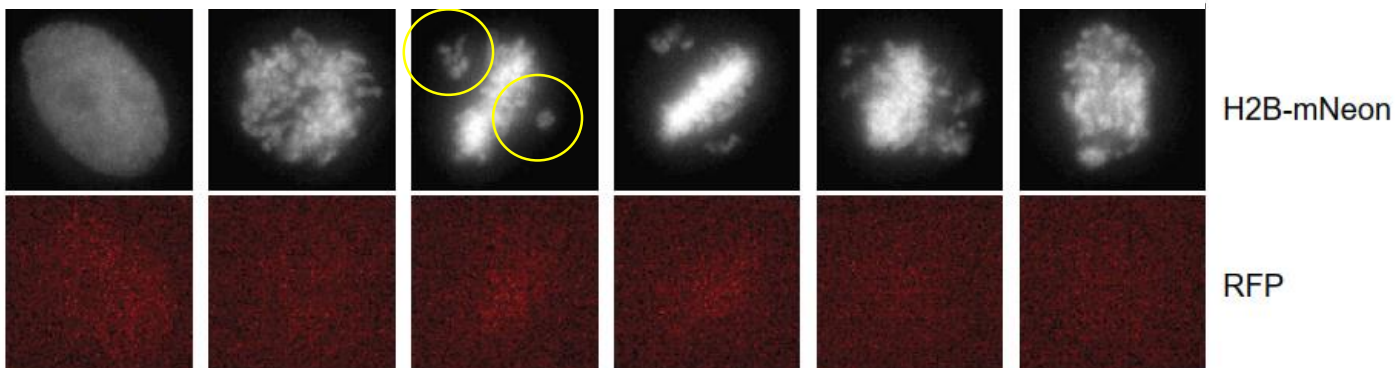
Musacchio, A. *et al. Nature Reviews Molecular Cell Biology* **8,** 385 (2007).

CCDC84/CENATAC loss leads to chromosome segregation defects

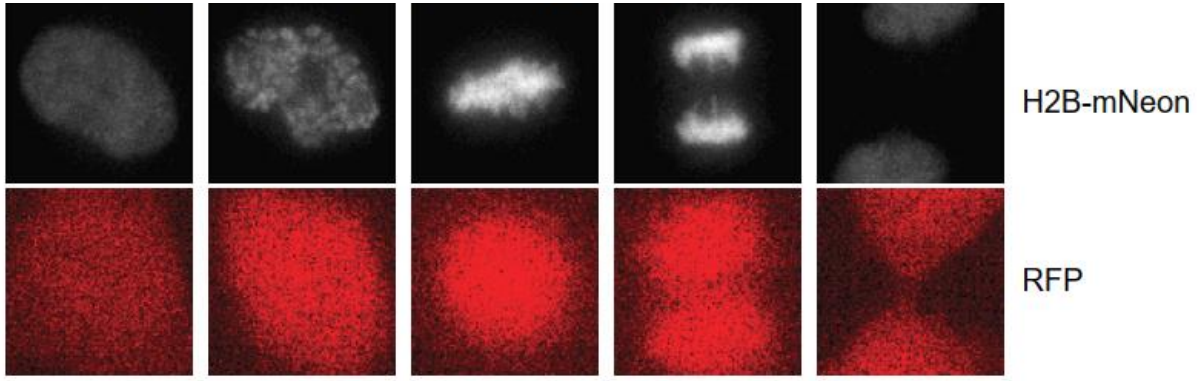
Control



CENATAC depletion



Rescue w/ wt CENTAC expression



0:00 0:15 0:30 0:45 1:00 3:00 h:mm

- Chromosome congression defects
- Mitotic delay

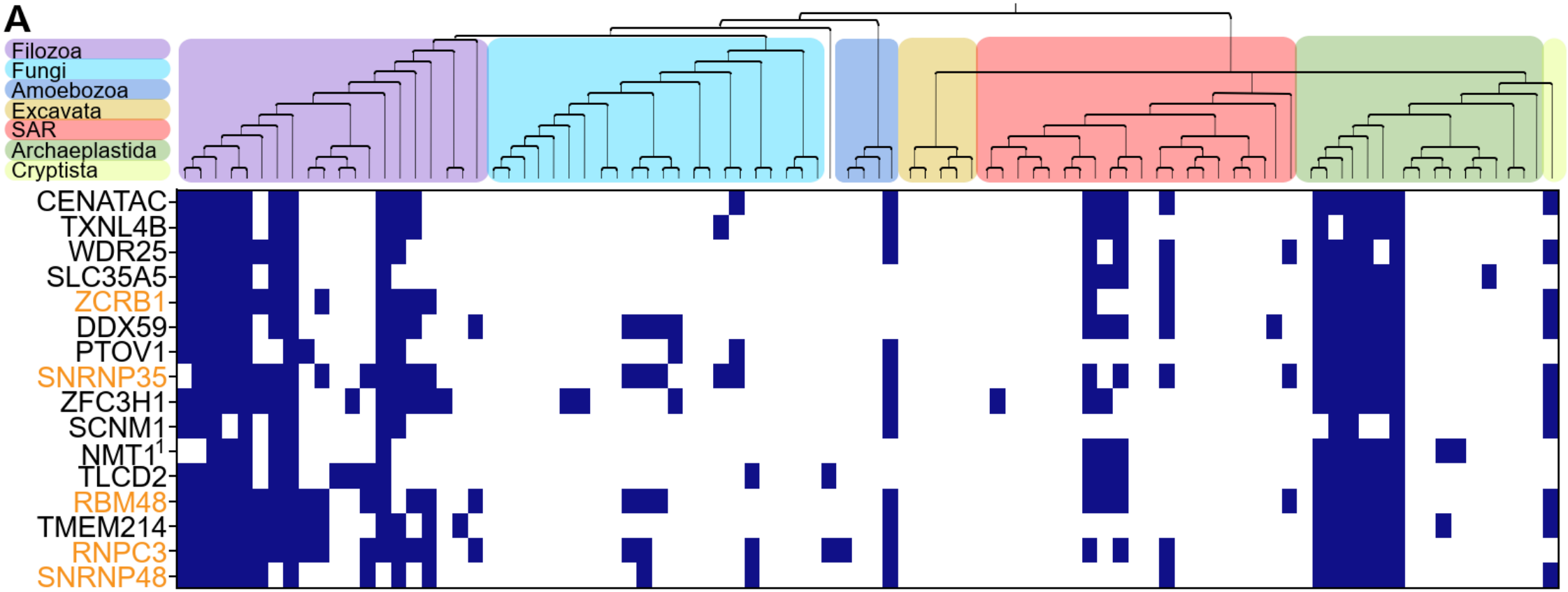
CCDC84 = coiled-coil containing 84
- Function unknown
- Nuclear but also centrosome localisation

de Wolf, B., et al. (2020). "Chromosomal instability by mutations in a novel specificity factor of the minor spliceosome." BioRxiv. DOI:2020.2008.2006.239418.

CCDC84/CENATAC is linked to U12-type spliceosome (‘minor spliceosome’)

Evolutionary co-occurrence analysis:

A



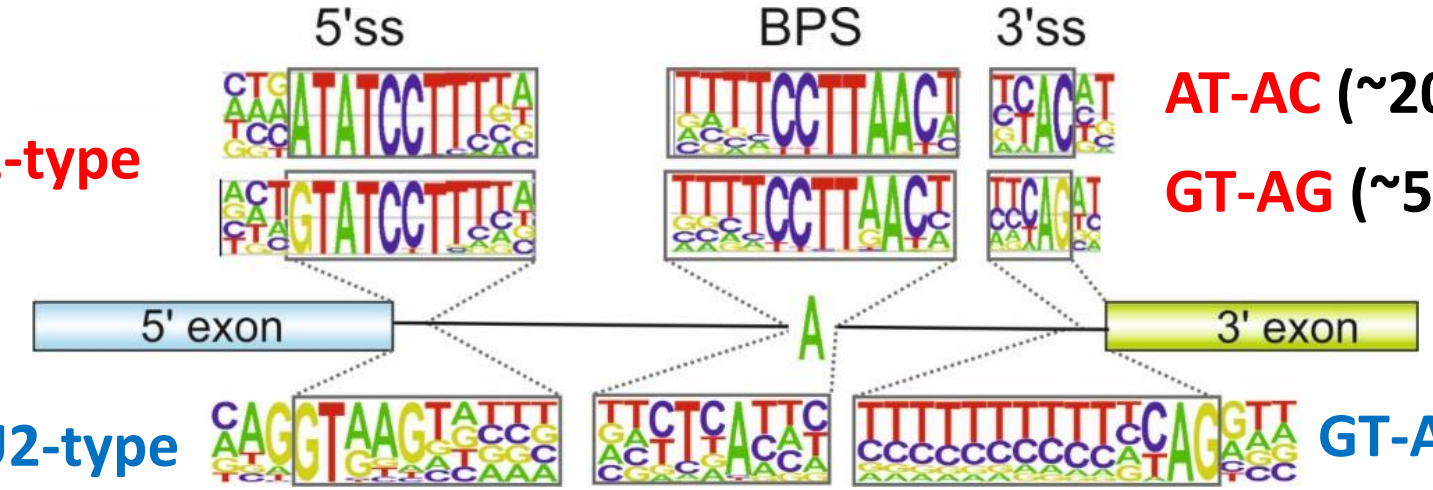
B

GO cellular component complete	Hits	Fold Enrichment	FDR
U12-type spliceosomal complex	5/26	89.73	1.16E-05
Nucleoplasm	20/3994	2.34	2.51E-02
U2-type spliceosomal complex	0/94	-	1.00E00

de Wolf, B., et al. (2020). "Chromosomal instability by mutations in a novel specificity factor of the minor spliceosome." BioRxiv. DOI:2020.2008.2006.239418.

Major and minor introns

Minor: U12-type



AT-AC (~200 genes in humans)

GT-AG (~550 genes in humans)

Major: U2-type

GT-AG

Major introns - U2-type

- ~250 000 in human genome
- ”GT-AG rule” for intron termini
- Otherwise low splice site conservation
- On average ~10 introns/gene [min 0, max 363]

Minor introns - U12-type

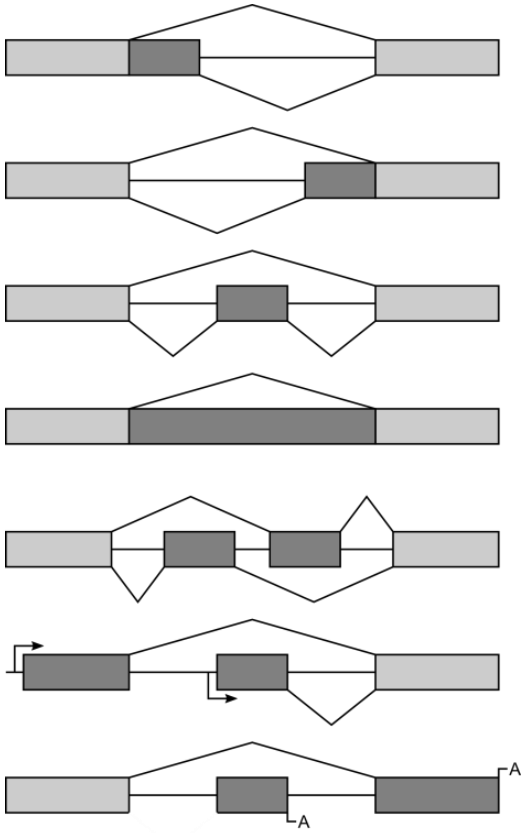
- Rare, 700–800 in the human genome
- Splice sites (5’ss and BPS) are highly conserved
- GT-AG or AT-AC termini
- **Typically one intron/gene**
- **Positions evolutionary conserved**

A major functional difference between the spliceosomes

Major spliceosome

Alternative splicing

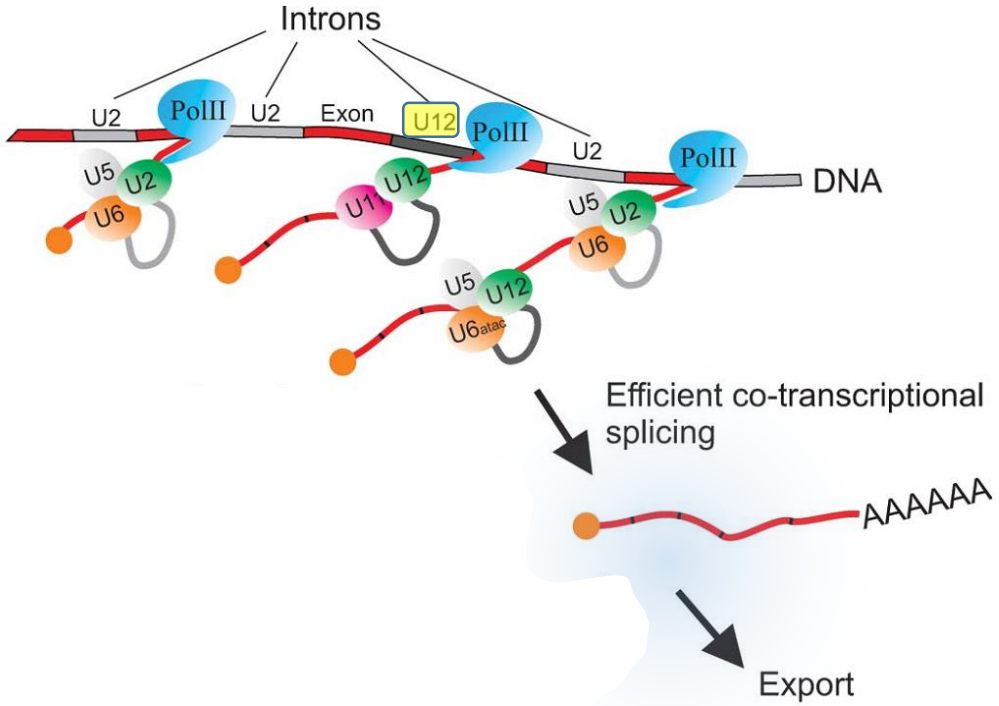
- Proteome diversification



Minor spliceosome

Inefficient splicing

- mRNA level regulation

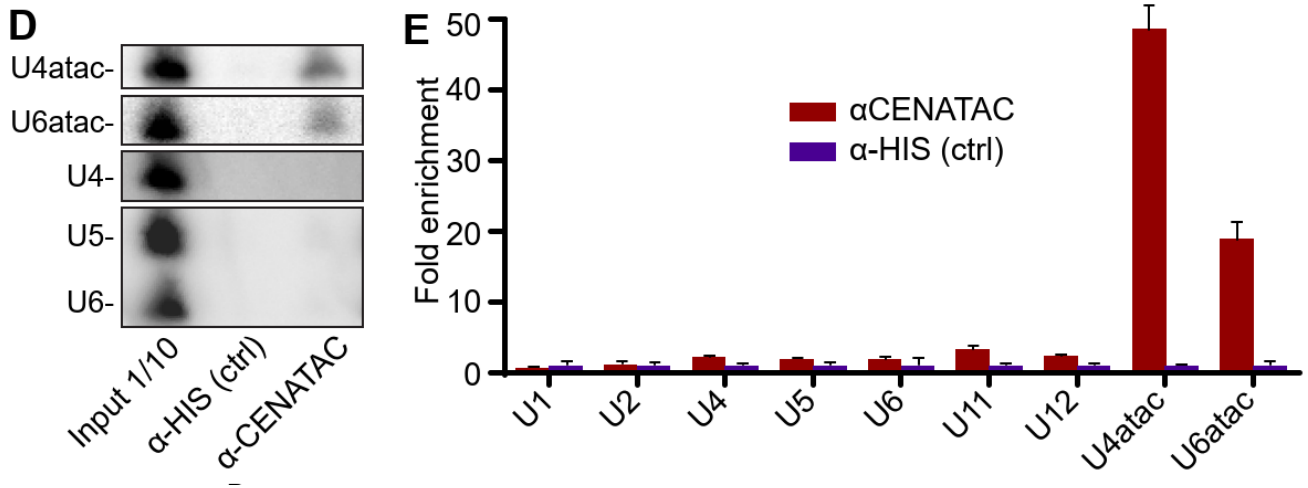


Niemelä , E., Frilander, M. (2014). Regulation of gene expression through inefficient splicing of U12-type introns. RNA Biol 11(11):1325-1329

Is CCDC84/CENATAC a (novel) component of minor spliceosome?

IP → Northern blot:

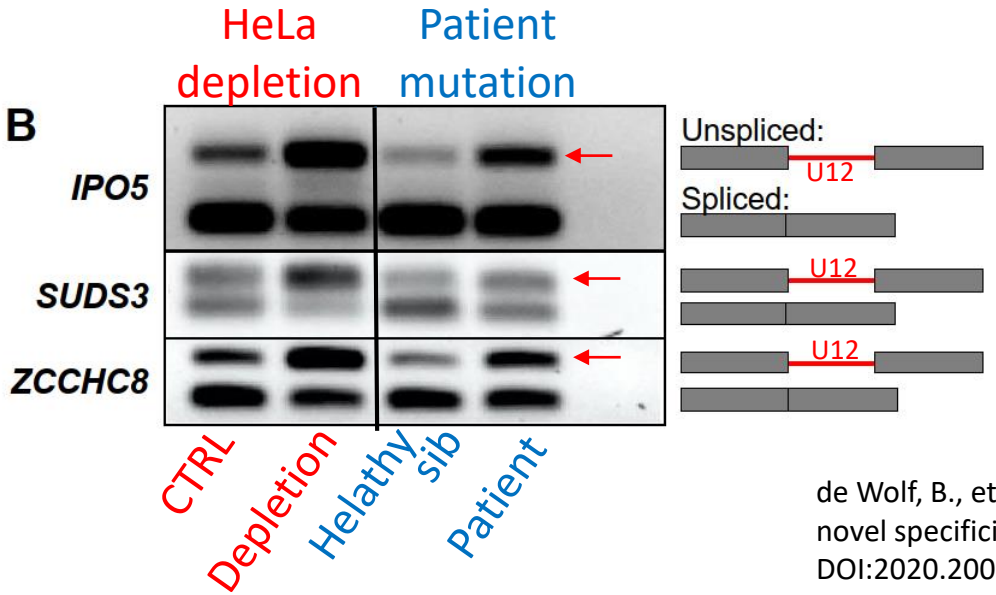
YES



Does loss of CCDC84/CENATAC cause a splicing defect?

RT-PCR:

YES

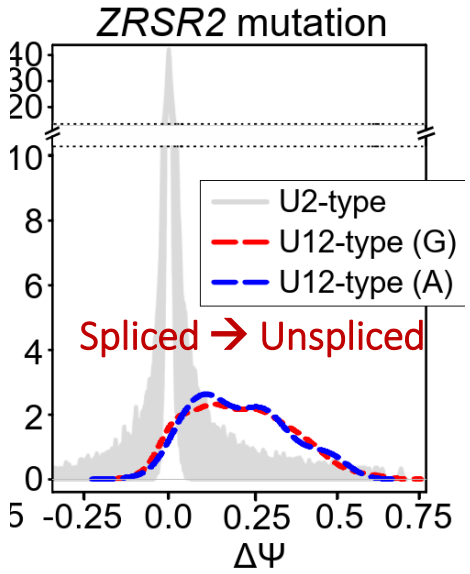


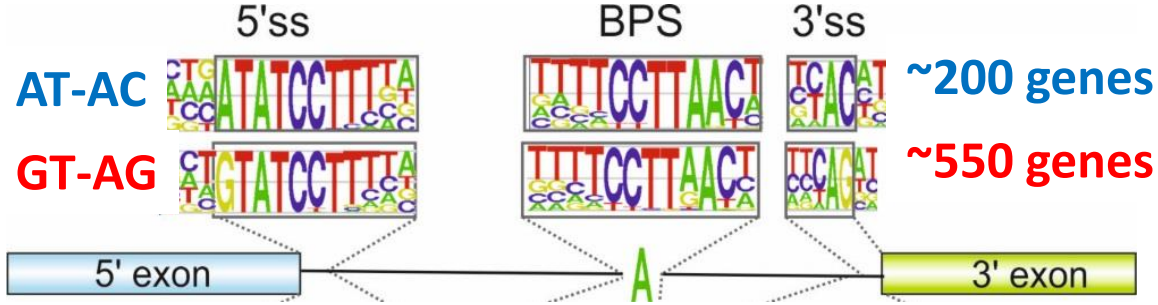
de Wolf, B., et al. (2020). "Chromosomal instability by mutations in a novel specificity factor of the minor spliceosome." BioRxiv. DOI:2020.2008.2006.239418.

RNAseq analysis: CCDC84/CENATAC is a novel specificity factor

“Normal” minor spliceosome disease:
Myelodysplastic syndrome

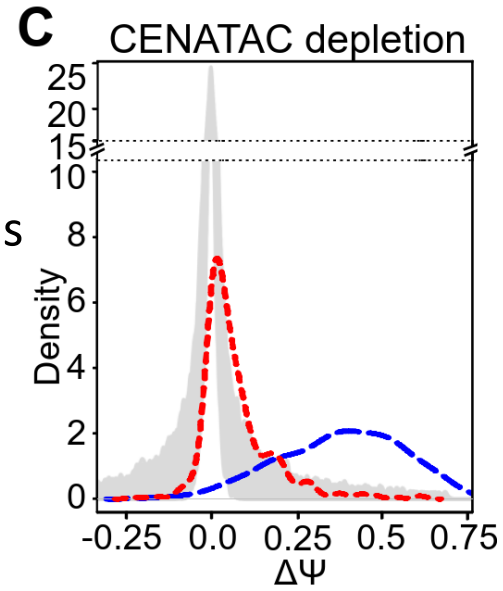
Affects equally
AT-AC and GT-AG introns

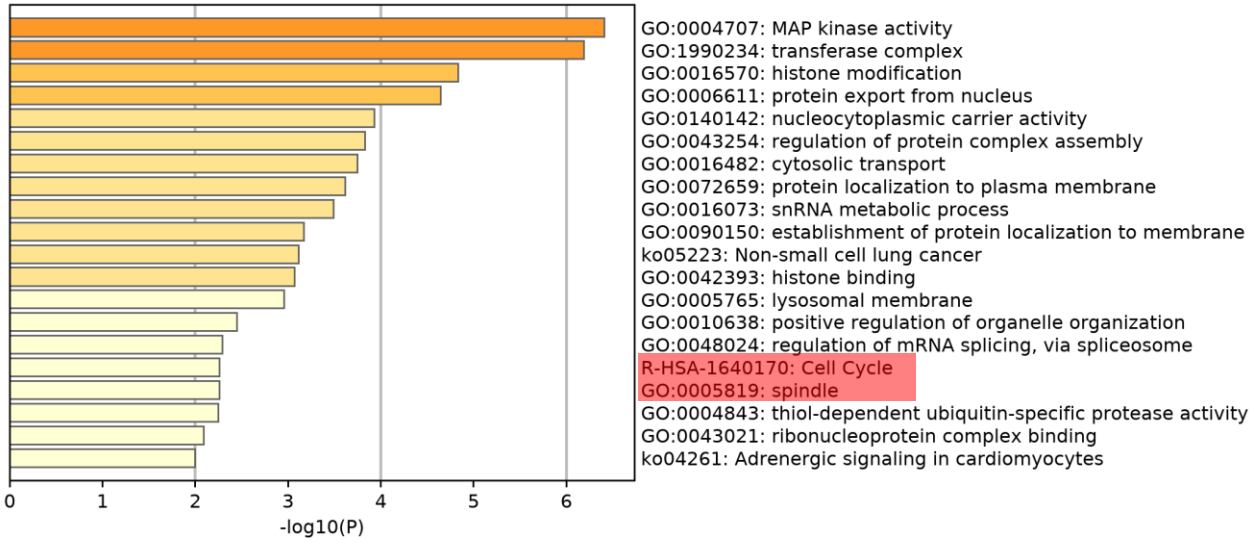




CENATAC depletion

Specificity for AT-AC introns
→separate regulation
via CENATAC?





de Wolf, B., et al. (2020). "Chromosomal instability by mutations in a novel specificity factor of the minor spliceosome." BioRxiv. DOI:2020.2008.2006.239418.

Summary of CCDC84/CENATAC present data

CENATAC = CENtrosomal AT-AC splicing factor

First splicing factor that differentiates between AT-AC and GT-AG introns

- → Separate regulation?
- → Subnetworks: genes related to cell cycle regulation?

Post-translational modifications

- → Regulation?

Aims of the R'Life application

Existing resources

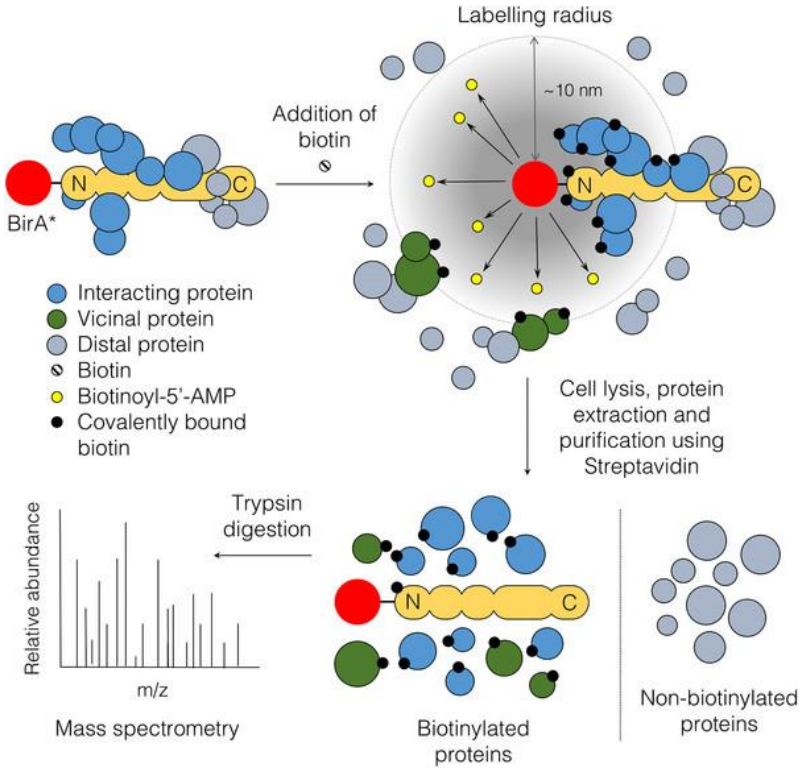
Deep RNAseq dataset: - CENATAC depletion time course
- MVA patient lymphocytes
- Additional minor spliceosome diseases

Edited cell line for Auxin-mediated depletion

Integrate exisiting CENATAC data with

Proteome analysis (quantitative mass spectrometry)

Interaction partners: protein pulldowns and **BioID analysis:**



To address

Molecular basis of CENATAC-associated MVA

Mechanism of AT-AC intron selectivity

- Upstream regulation
- Separate regulation for AT-AC and GT-AG introns

The big question

Connection between minor spliceosome and cell cycle regulation

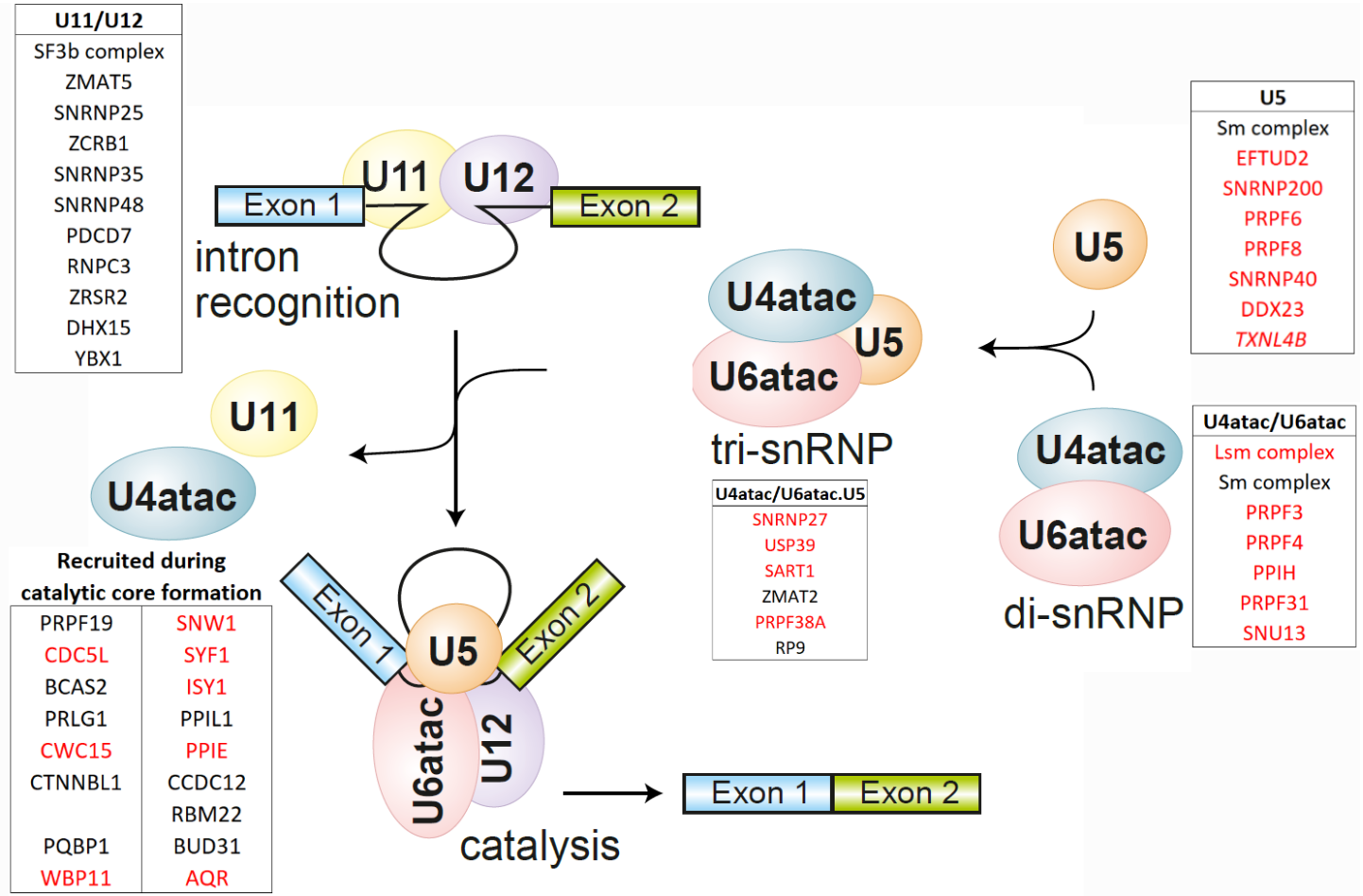
Preliminary CENATAC BioID interactome analysis

No hits in the intron recognition complex!

Catalytic complexes light up heavily

→ Unexpected role of for splice site selection/specificity

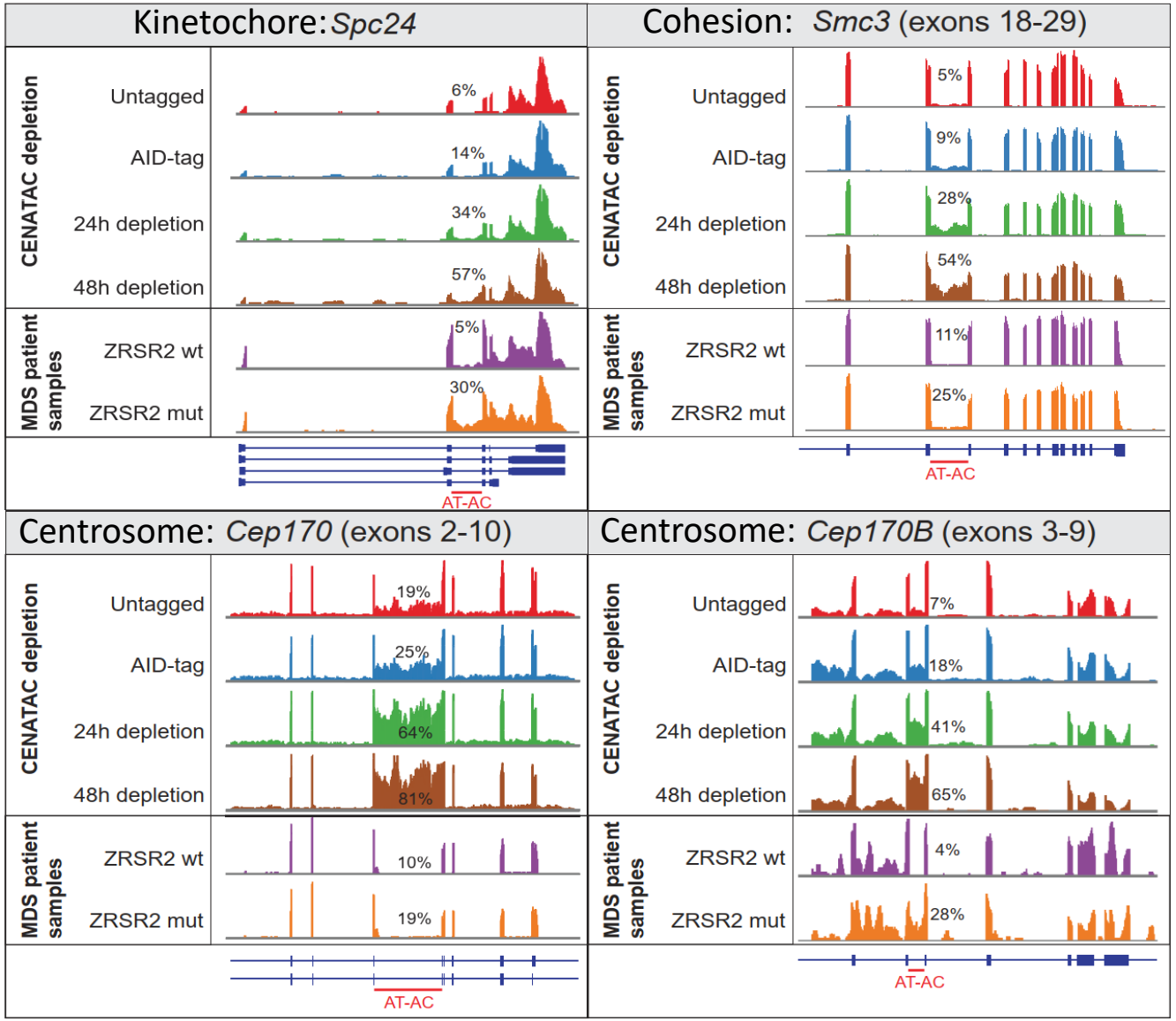
→ Proofreading?



Additional proteins: - Post-translational modifiers → Upstream regulators?

- Centrosomal proteins → Is CENTAC a dual-function protein?

Splicing defect – MVA phenotype data integration



Candidate genes for MVA phenotype

- Kinetochore components
- Cohesion complex
- Centrosomal proteins

Global analysis

RNAseq:

→ high level of AT-AC intron retention

→ nonfunctional mRNAs

Proteome:

→ impact on protein levels?

→secondary/downstream effects

FRILANDER LAB

Mikko Frilander, Institute of Biotechnology, University of Helsinki, Finland

Ali Oghabian

Maureen Akinyi

Michael Algie

KOPS LAB

Geert Kops, Oncode & Hubrecht Institute and University of Utrecht, Netherlands

Bas de Wolf

SNEL LAB

Berend Snel, University of Utrecht, Netherlands

Laura van Rooijen

[Biocenter Finland nodes at BI]

- DNA sequencing and genomics unit
- Proteomics unit
- Light microscopy unit

ACADEMY OF FINLAND

SIGRID JUSELIUS STIFTELSE

J&AE JANE AND AATOS ERKKO FOUNDATION