MAU2 is required for zebrafish neurodevelopment

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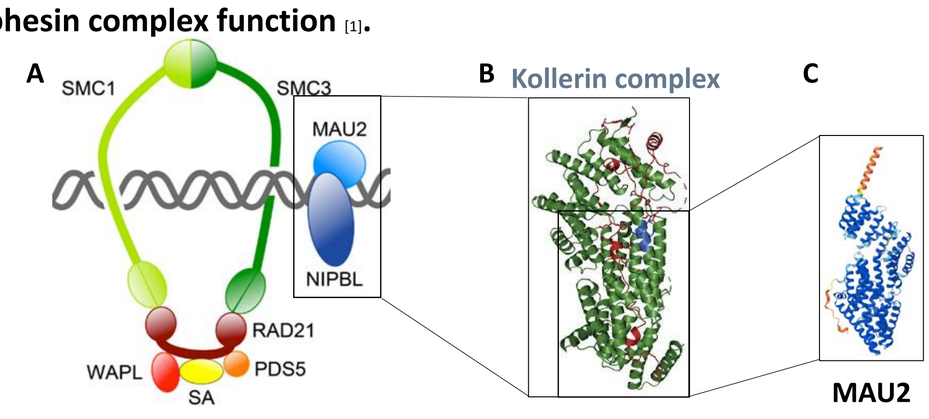
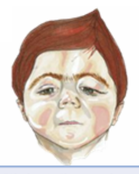
Introduction

Cohesin is a multisubunit protein complex involved in chromatin dynamics.
The cohesin complex loads on DNA via the kollerin complex.
NIPBL-MAU2 forms the kollerin complex and their heterodimerization is required for the cohesin complex function [1].



A) Schematic structure of the cohesin complex and the kollerin loading complex modified from Horsfield et al. 2012, **B)** protein complex structure from Parenti et al. 2020 and **C)** MAU2 prediction from Alphafold. [1] Ciosk R et al. Mol Cell. 2000 [2]Parenti I et al. Cell Rep. 2020

Variants in NIPBL are known to be causative of Cornelia de Lange syndrome (CdLS).
***mau2* variants have been found in patients presenting CdLS phenotype, that include microcephaly, distinctive facial feature, limb malformation and psychomotor delay[2].**
MAU2 protein sequence is evolutionarily conserved across vertebrates, including at the sites of variants found in patients.



MAU2 patients

The Cornelia de Lange Syndrome (CdLS)
MAU2 (NM_015329.4)
Chr19:19455722 19p13.11 - c.1142T>C; p.(Leu381Pro)

	Patient 1	Patient 2
Clinical diagnosis	CdLS	CdLS
Mutation	p.(Gln310_Ala316del)	p.(Leu381Pro)
ID	Severe	Mild
Motor delay	+	-
Speech delay	+	-
Facial dysmorphism	+	+
Microcephaly	+	+
Hirsutism	+	+

MAU2 protein sequence

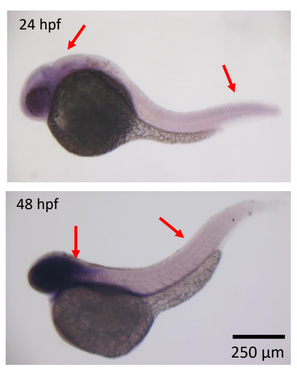
SNHAAQLHTL	Homo sapiens
SNHAAQPHTL	Homo sapiens mut.
SNHAAQLHTL	Mus musculus
SNHAAQLHTL	Danio Rerio

Aim

Elucidating MAU2 role in neurodevelopment by establishing a loss of function zebrafish model and compare phenotypic and molecular features to CdLS patients.

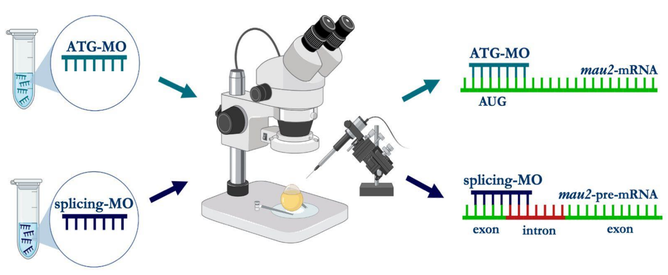
Results

1. *mau2* spatial localization in zebrafish

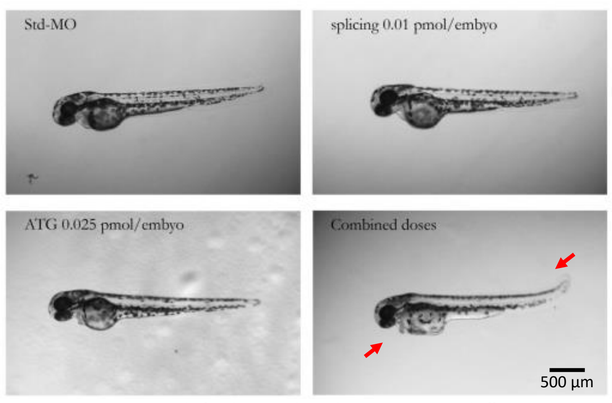


Whole-embryo *in situ* hybridization, red arrows highlight MAU2 probe deposits in the central nervous system (CNS) and in the spinal cord. Signal shown in purple.

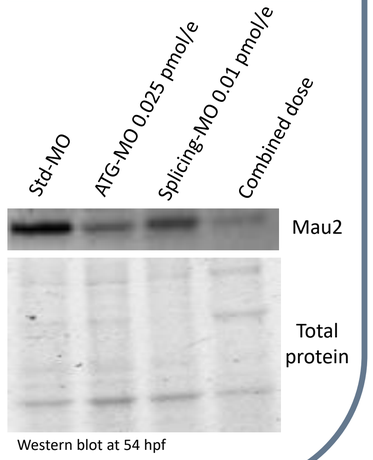
2. Establishing a MAU2 loss of fuction (LoF) zebrafish model by combining two antisense oligonucleotide morpholinos (MO)



Injection of subcritical doses of *mau2* **ATG** and **splicing MO,** alone and in combination, into zebrafish Tg(*-3.1neurog1*:GFP) strain induce an efficient MAU2 protein knockdown in MAU2 LoF embryos.

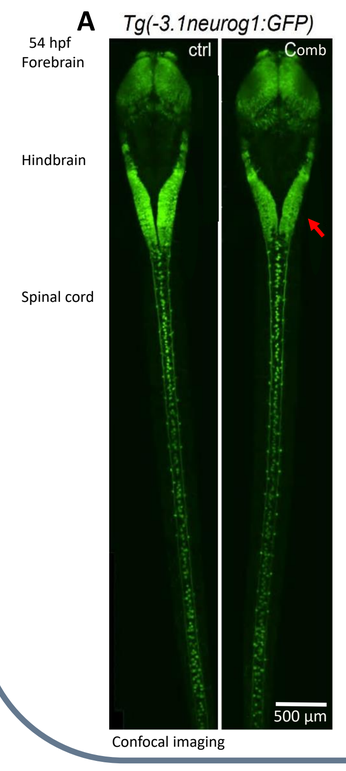


Stereoscopic imaging at 54 hpf, red arrows show phenotype-associated effect

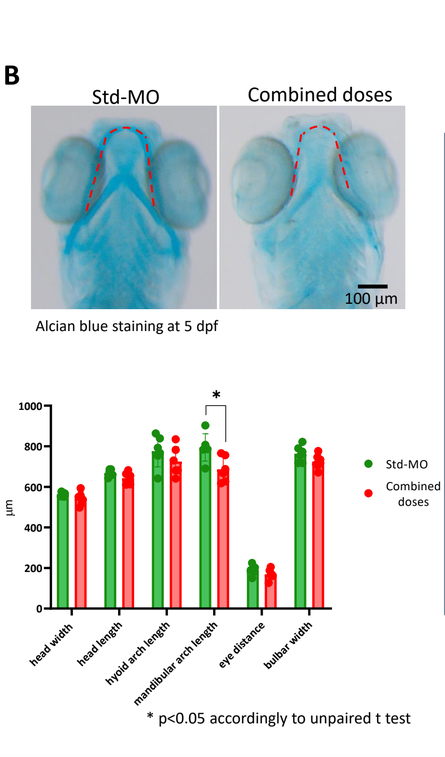


Western blot at 54 hpf

3. Morphologic alterations in hindbrain structure and cartilages resemble CdLS patient phenotype



Confocal imaging

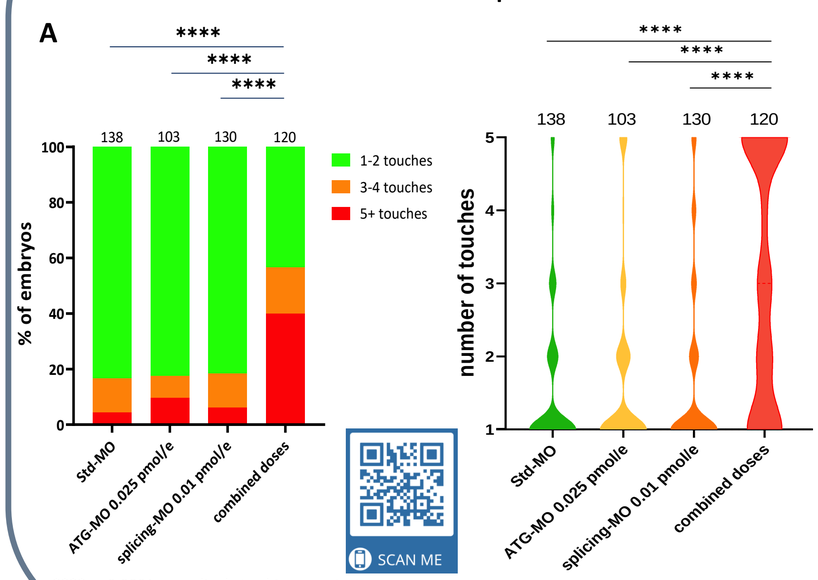


A. Whole embryo confocal imaging reveals shorter hindbrain and reduce expression of neurodifferentiation marker neurog1 in LoF embryos.

B. Alcian blue staining for cartilages shows reduced mandibular arch length in MAU2 LoF.

4. MAU2 loss of fuction results in defective escaping instinct following mechanical and phototropic stimuli

Touch-evoked response

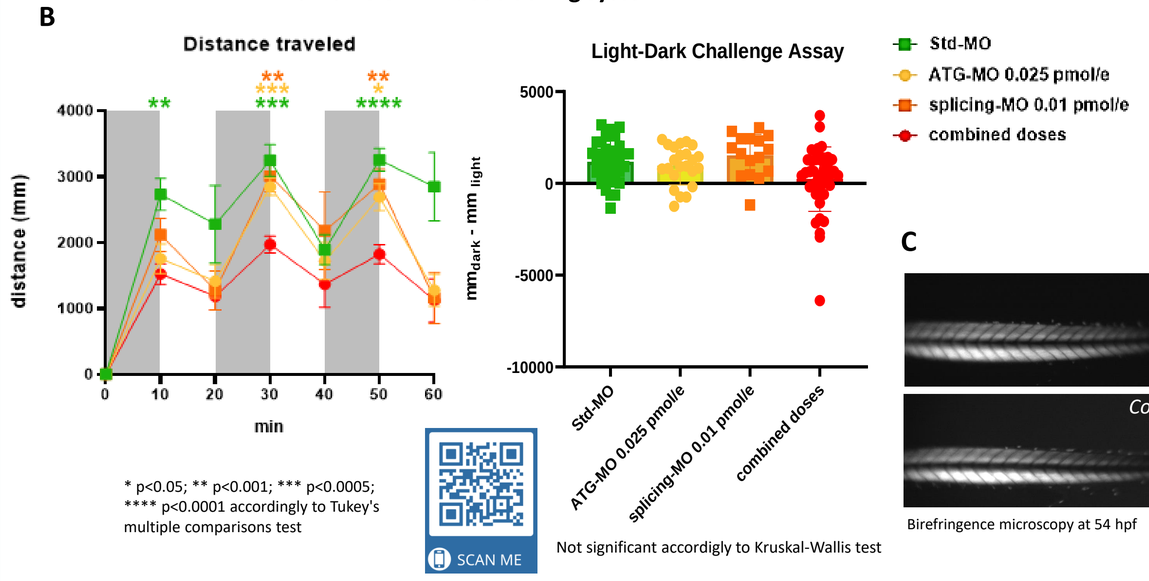


A. Touch-evoked response behavioural test shows a significant higher percentage of embryos with delayed escaping response and a higher number of touches required to escape upon mechanical stress in MAU2 LoF embryos.

B. DanioVision videotracking system displays reduced travelled distance in MAU2 LoF embryos, but no difference in the response to light-dark cycles.

C. Birefringence microscopy shows that motility defects are not due to alterations in trunk muscle structure.

DanioVision – videotracking system



Conclusions

We obtained MAU2 LoF zebrafish model that recapitulates CdLS patients' phenotypic and molecular features:
- Alteration of hindbrain structures and cartilages
- Delayed neuromotor behaviour
- Reduced motility

Take-home message

- Our zebrafish LoF model could be useful to broaden our knowledge about the molecular basis of CdLS and the impact of MAU2 pathogenic variants.
- Our MAU2 LoF model could be exploited to test new therapeutic strategies for CdLS.

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