Connecting Calcium Signaling Alterations to Mitochondrial Dysfunction in Spinal Muscular Atrophy

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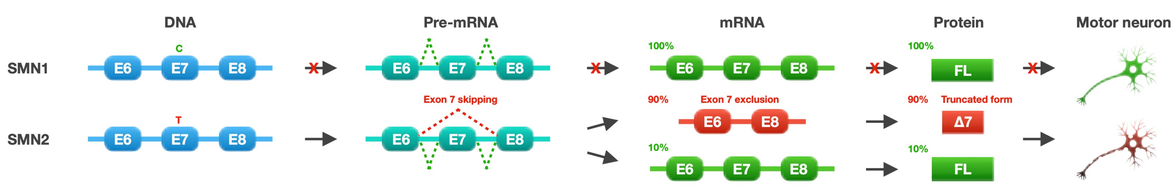
GUÉRIR LES MALADIES GÉNÉTIQUES

Introduction

Background 5q-linked spinal muscular atrophy (SMA), is a monogenic neuromuscular disorder caused by mutations of *SMN1*, which encodes the ubiquitously expressed and multifunctional SMN protein. Although SMN-restoring gene therapies have been available since 2016, treated patients continue to exhibit symptoms or even disabilities, and it is yet unclear which SMN-related molecular pathways and cellular processes are the major drive of neurodegeneration.

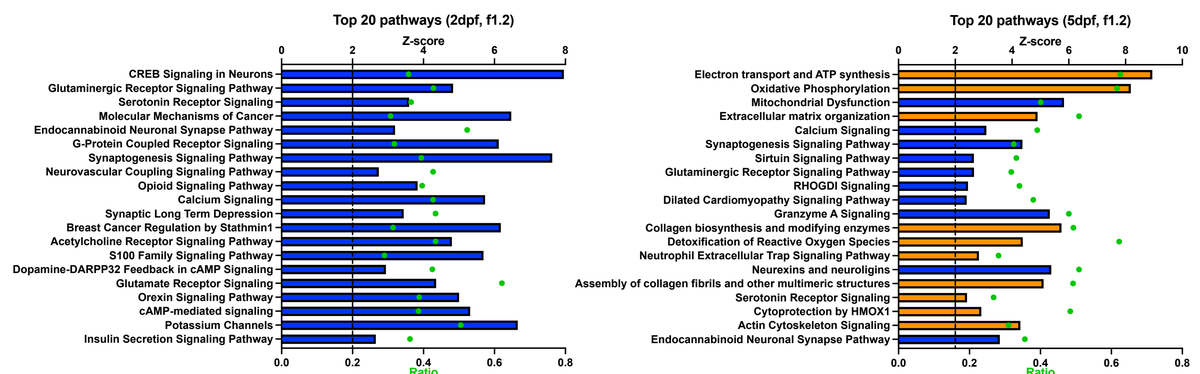
Objective Using CRISPR/Cas9 mediated gene knockout, we aimed to generate a novel zebrafish model for SMA and to identify potential therapeutic pathways through transcriptomics study.

Genetic basis of SMA

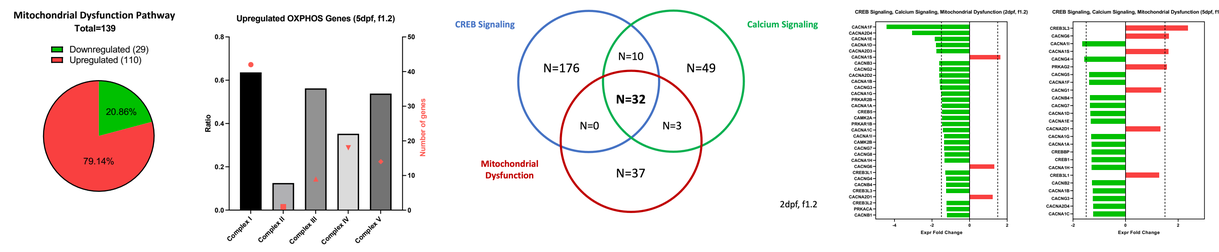


Transcriptomics revealed calcium signaling alterations

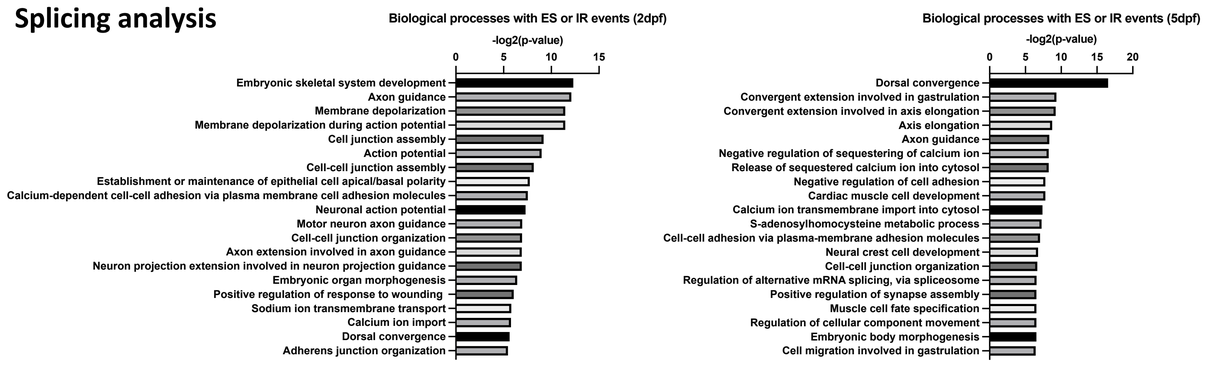
Top transcriptomic pathways (IPA)



Major pathways of interest

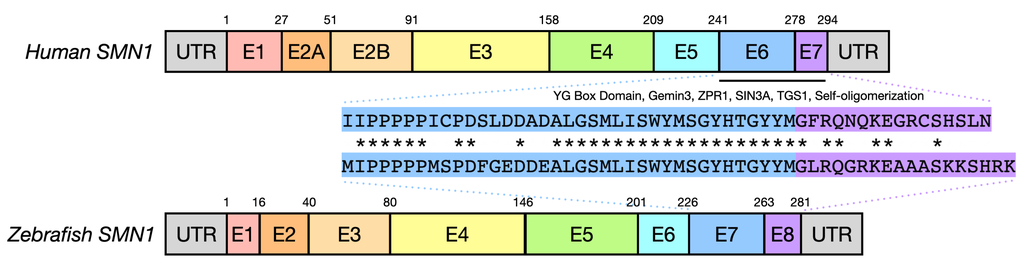


Splicing analysis

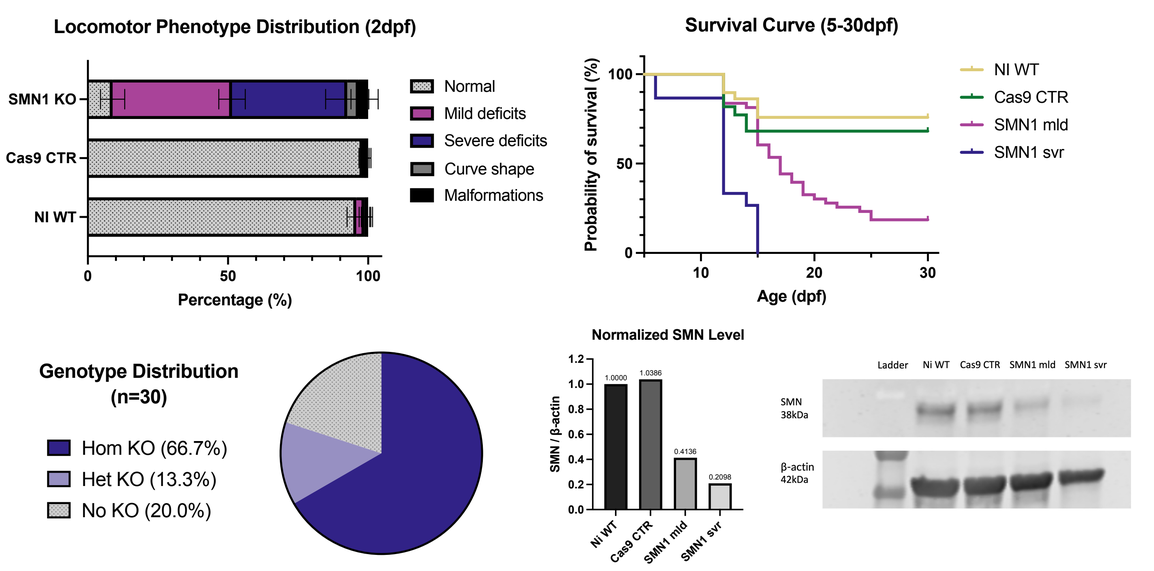


The generation of a novel zebrafish crispant model

Double-sgRNA knockout strategy

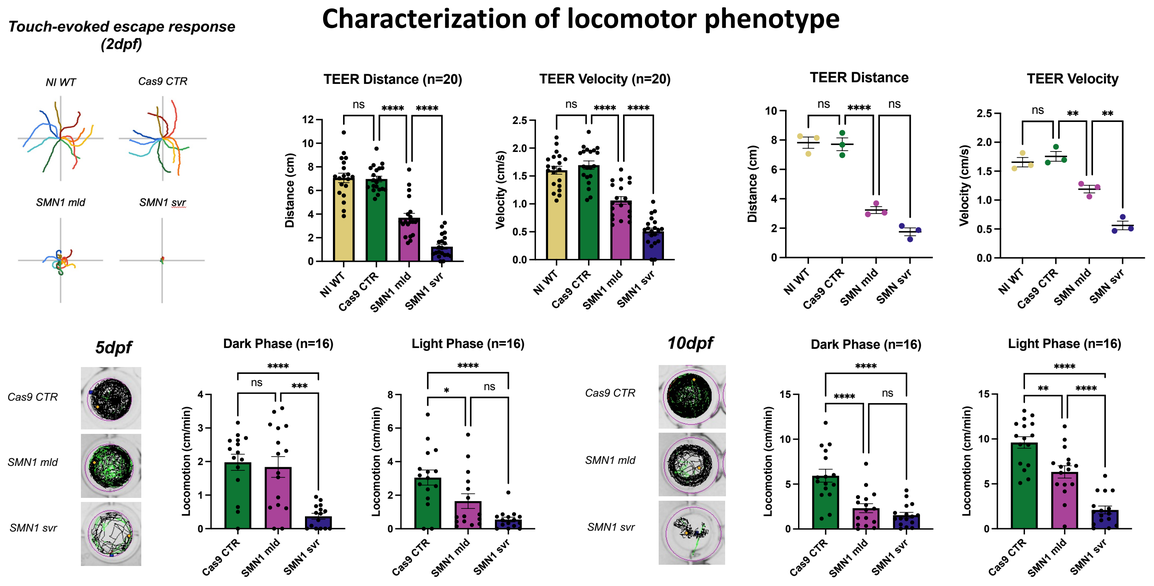


Genotype-phenotype correlation

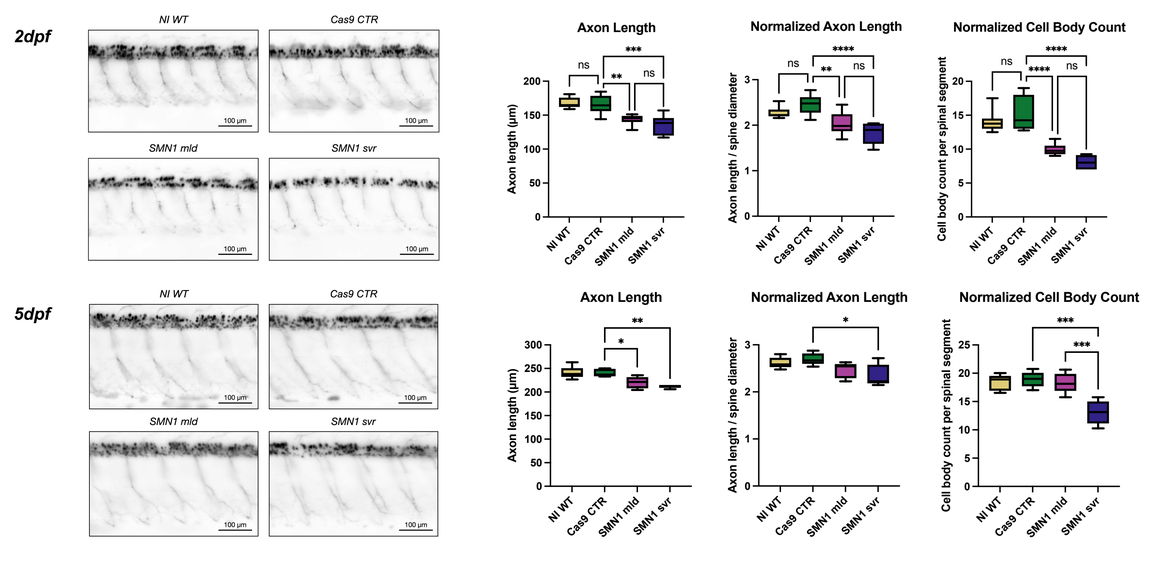


SMN1 KO resulted in disease-relevant phenotypes

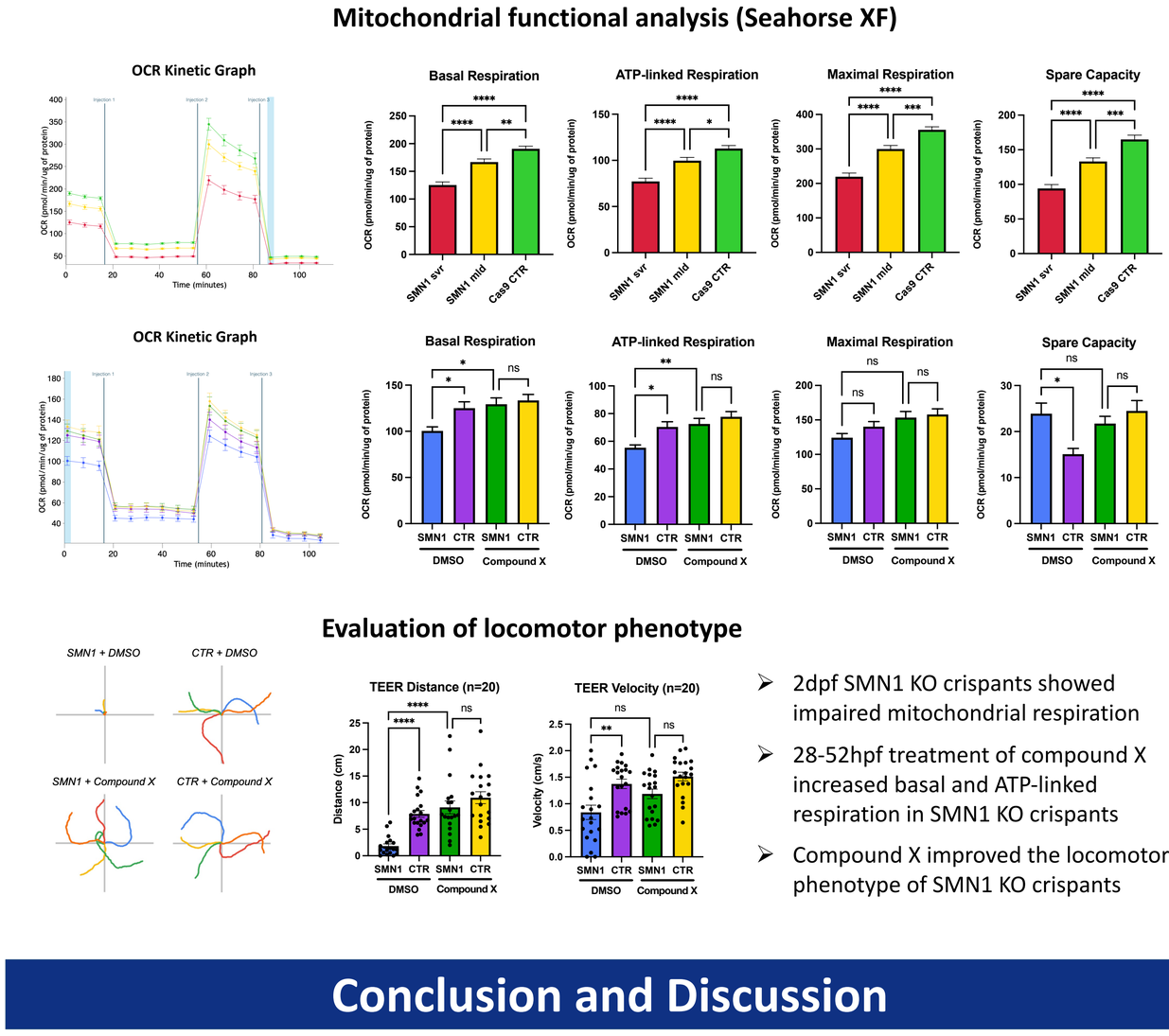
Characterization of locomotor phenotype



Characterization of motor neuron phenotype



Mitochondrial functional analysis (Seahorse XF)



Evaluation of locomotor phenotype

- 2dpf SMN1 KO crispants showed impaired mitochondrial respiration
- 28-52hpf treatment of compound X increased basal and ATP-linked respiration in SMN1 KO crispants
- Compound X improved the locomotor phenotype of SMN1 KO crispants

CaMK targeting compound X showed rescue effects

Conclusion and Discussion

- ✓ By targeting the zebrafish SMN1 exon 7 for CRISPR/Cas9 knockout, we generated a robust crispant model with well-characterized locomotor deficits and motor neuron defects.
- ✓ Bulk RNA-seq revealed that despite using whole embryo samples, the SMN1 KO model is highly representative of neurological disorder and neuronal alterations.
- ✓ Based on transcriptomics, novel pathways of interest are mitochondrial dysfunction, calcium signaling and CREB signaling, with CaMK at the crossroads of these 3 pathways.
- ❑ We currently focus on investigating the connection between calcium signaling alterations and mitochondrial dysfunction through exploring a CaMK-mediated therapeutic pathway.

Discussion Zebrafish is an efficient model organism to study genetic diseases, but in the context of SMA, there is only *SMN1* but not its paralogous gene *SMN2* in the zebrafish genome. To bring to light the effect of CaMK-mediated therapeutic pathway on *SMN2* splicing, we will incorporate drug testing on SMA patient iPSC-derived motor neurons using different compounds, evaluating (1) motor neuron survival; (2) neuritic network; (3) CaMK level; (4) SMN level; (5) *SMN2* splicing.

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