Role of MuscleBlind-Like proteins in the regulation of expression of CaVβ1 isoforms in adult skeletal muscle

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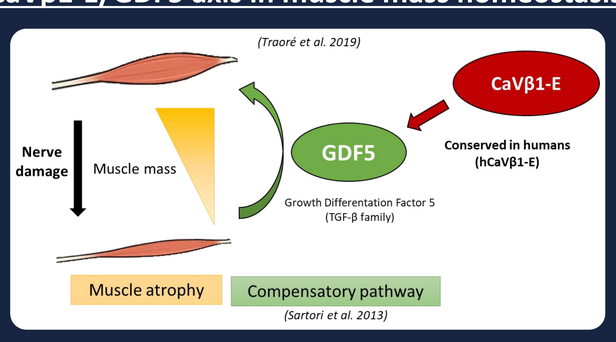
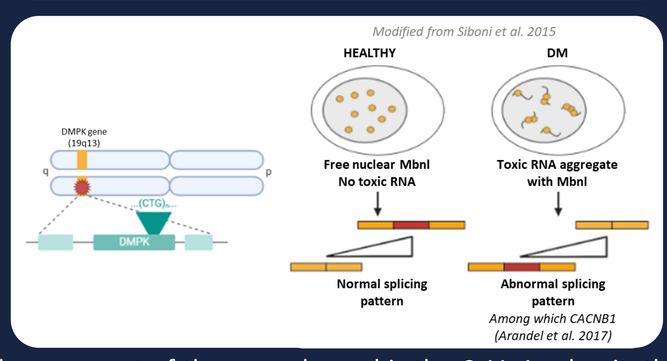
INTRODUCTION

CaVβ1-E/GDF5 axis in muscle mass homeostasis



Cacnb1 isoforms in skeletal muscle

Implication of MBNLs in DM1 pathophysiology



Voltage-gated calcium channels (CaVs or VGCCs) are major regulators of calcium-related cellular functions. In skeletal muscle, though the essential component of the pore channel is the CaVα1 subunit, the CaVβ1 subunit is an essential subunit guaranteeing CaV fine-tuning activity.

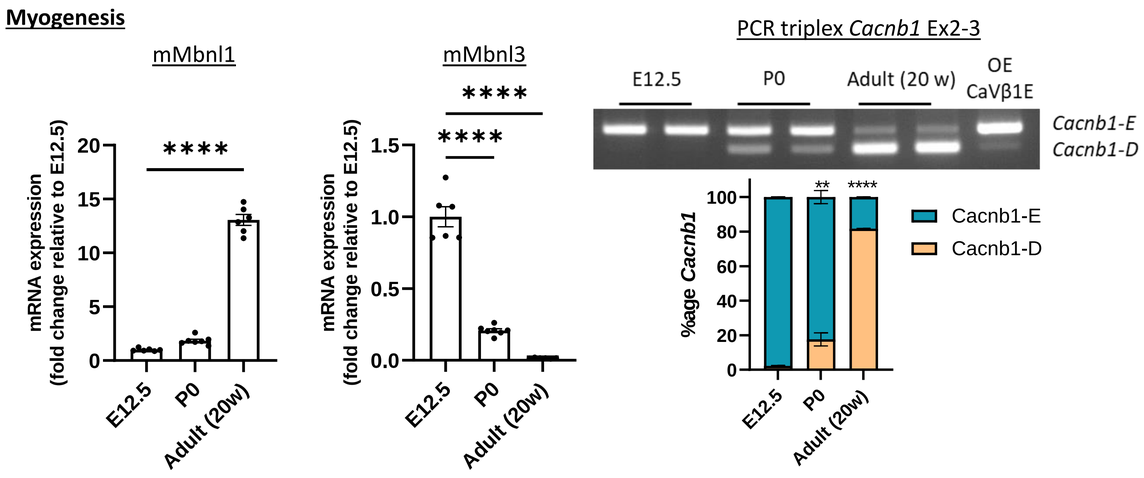
CaVβ1-E and CaVβ1-D are two different isoforms of CaVβ1 protein in skeletal muscle, expressed during embryogenesis and in healthy innervated adult muscle, respectively. Importantly, our recent study demonstrated that the embryonic CaVβ1-E expression increases after a nerve damage in adult skeletal muscle and enables the expression of GDF5 (Growth Differentiation Factor 5) to counteract excessive muscle wasting (*Traoré et al. 2019*). However, the mechanisms leading to the increase in CaVβ1-E expression are unknown to date.

Our RNAseq data analysis in innervated *versus* denervated muscles revealed MuscleBlind-Like (MBNL) proteins as potential candidates regulating CaVβ1 expression in skeletal muscle. Interestingly, in a human model of Dystrophy Myotonic 1 (DM1), the sequestration of MBNLs in toxic nuclear aggregates is related to an impaired splicing of CaVβ1 transcript (*CACNB1*) (*Arandel et al. 2017*).

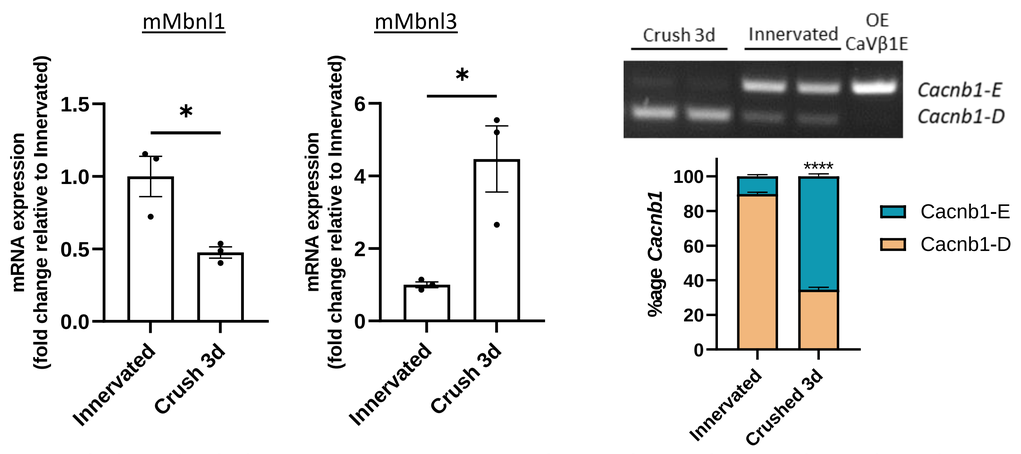
Here, we evaluate the effect of a modulation of MBNLs protein levels on the expression of CaVβ1 isoforms in both *in vitro* and *in vivo* systems as well as in pathological mouse models of DM1.

Correlation between MBNLs and *Cacnb1* expressions

Myogenesis

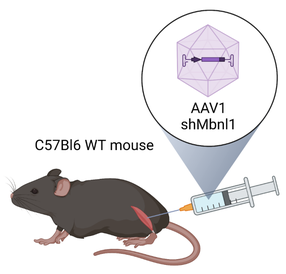


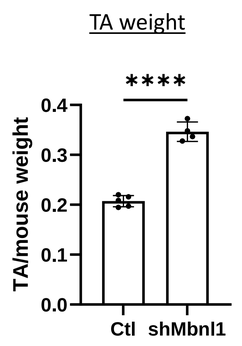
Nerve crush model

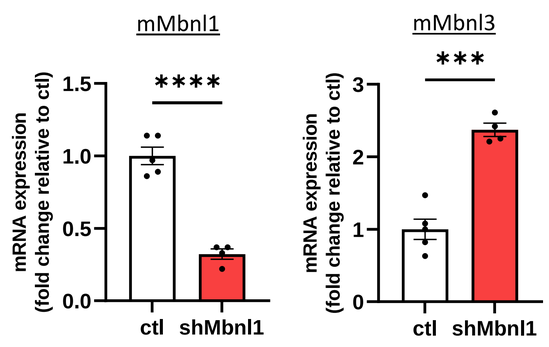


Mbnl1 and *Mbnl3* mRNA expressions correlate with *Cacnb1-D* and *Cacnb1-E* during myogenesis and after sciatic nerve crush.

Mouse model of Mbnl1 downregulation

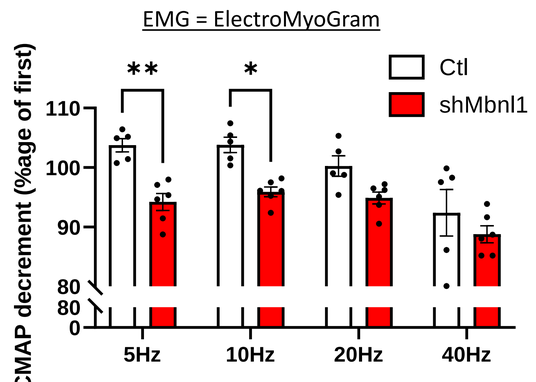


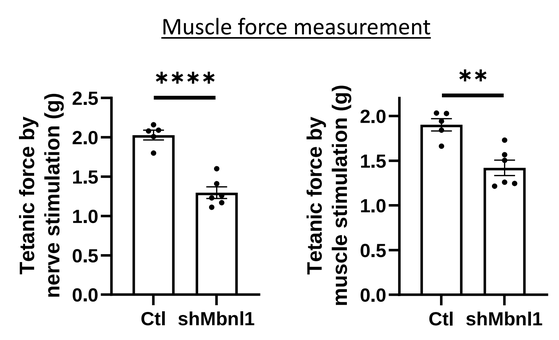


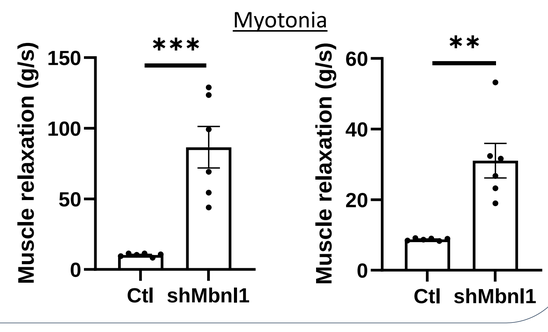


Downregulation of Mbnl1 induces an increase of Mbnl3 expression and an increased muscle mass.

The functional analysis shows an impaired neuromuscular transmission, a decreased muscle force and a myotonic profile.

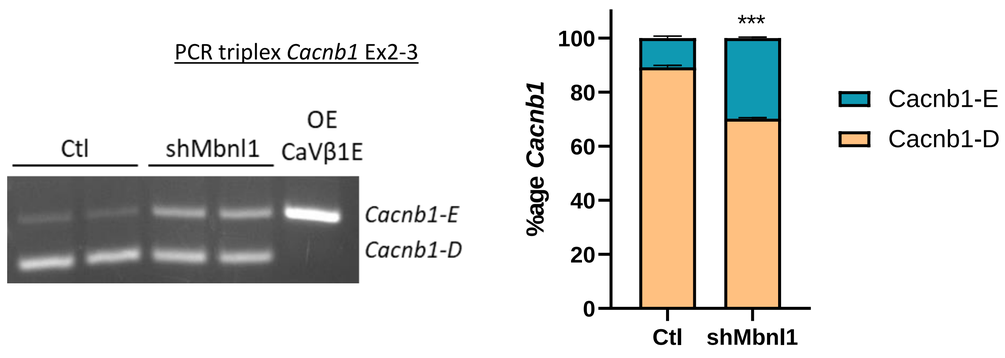




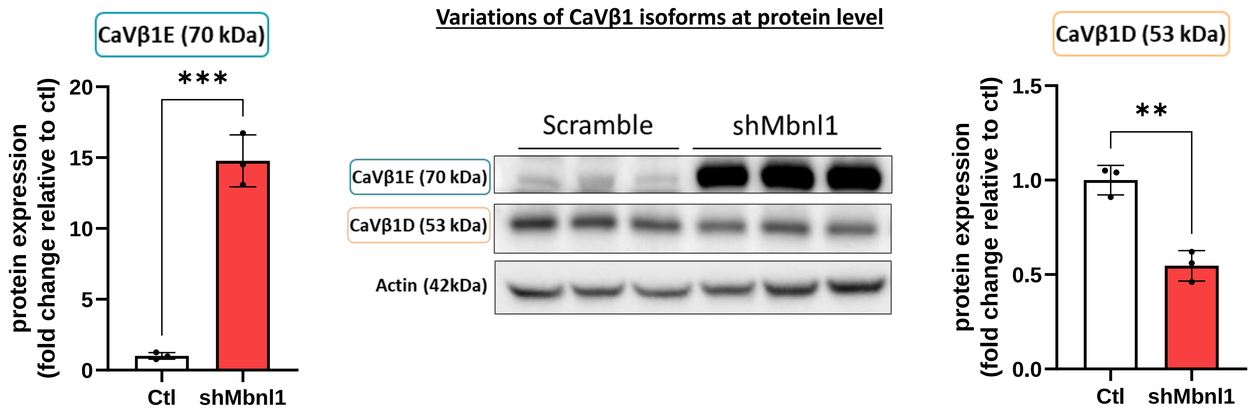


MBNLs modulates the expression of CaVβ1 isoforms *in vivo*

Variations of *Cacnb1* isoforms at transcriptional level

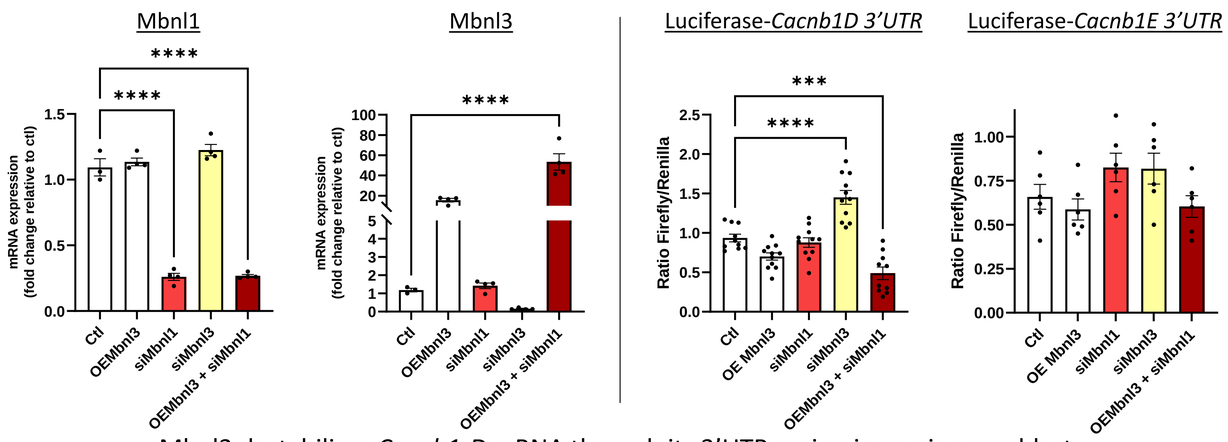


Variations of CaVβ1 isoforms at protein level



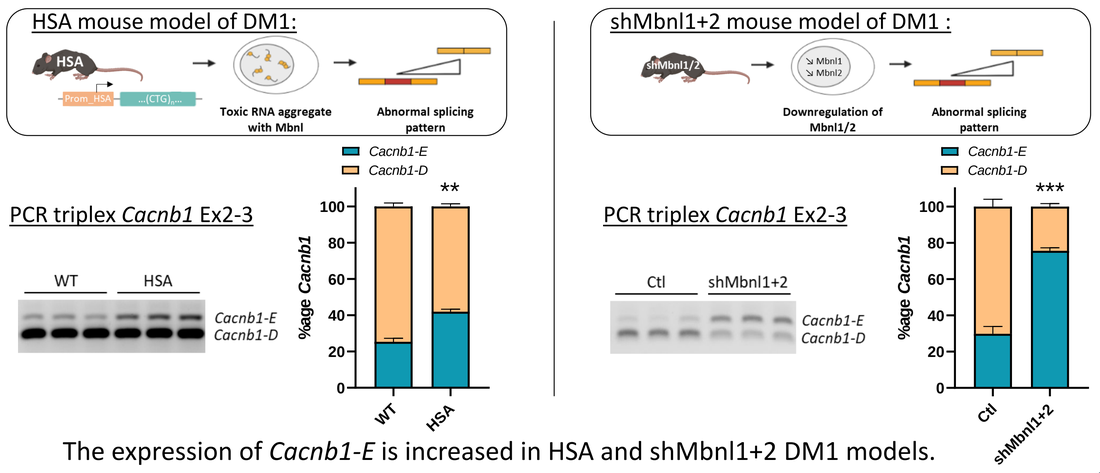
A downregulation of Mbnl1 induces an increase of the expression of CaVβ1-E in mouse skeletal muscle through a direct or indirect mechanism.

MBNLs impacts *Cacnb1* mRNA stability



Mbnl3 destabilizes *Cacnb1-D* mRNA through its 3'UTR region in murine myoblasts.

Cacnb1-E increases in DM1 pathological models



The expression of *Cacnb1-E* is increased in HSA and shMbnl1+2 DM1 models.

CONCLUSIONS & PERSPECTIVES

- Mbnl1 negatively regulates Mbnl3
- Downregulation of Mbnl1 *in vivo* is associated with impaired muscle and neuromuscular functions
- A downregulation of Mbnl1, associated with an increase of Mbnl3, leads to increased CaVβ1-E and decreased CaVβ1D expression levels *in vivo*
- *Cacnb1* transcripts stability is modulated by MBNLs *in vitro* through their 3'UTR
- Mouse models of DM1 are associated with an increased *Cacnb1-E* expression

- Characterization of the splicing events occurring at *Cacnb1* Ex2-3 and Ex13-14
- Studying a potential cross-regulation of CaVβ1-D on CaVβ1-E expression
- Deciphering the role of CaVβ1-E in DM1 pathophysiology

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