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

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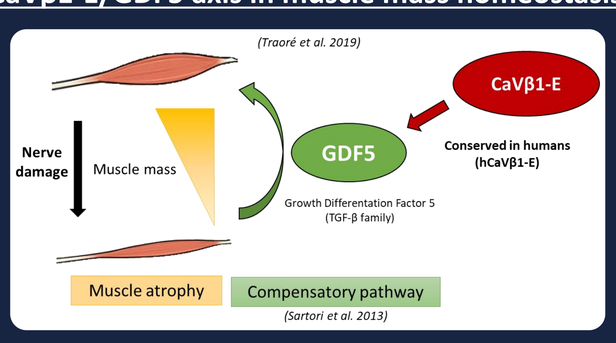
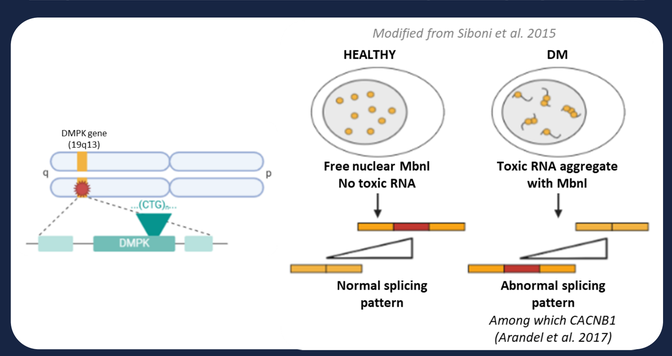
INTRODUCTION

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.

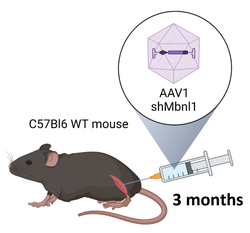
CaVβ1-E and CaVβ1-D are two different isoforms of CaVβ1 protein in skeletal muscle, expressed during embryogenesis and in healthy innervated adult muscle, respectively. Importantly, our recent study demonstrated that the embryonic CaVβ1-E isoform expression increases after a nerve damage in adult skeletal 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*).

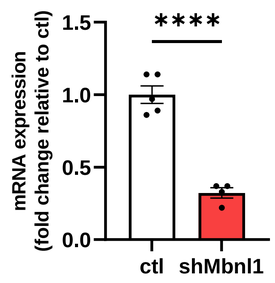
Here, we evaluate the effect of a modulation of MBNLs protein levels on the expression of CaVβ1 isoforms in both *in vitro* and *in vivo* systems as well as in pathological mouse models of DM1.

Mbnl1 modulates the expression of CaVβ1 isoforms *in vivo*

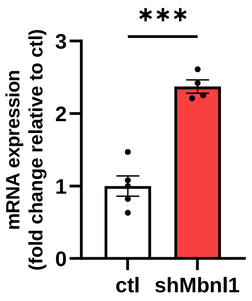
- **Mouse model of Mbnl1 downregulation**



mMbnl1



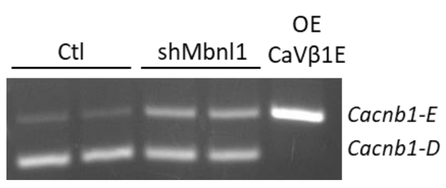
mMbnl3

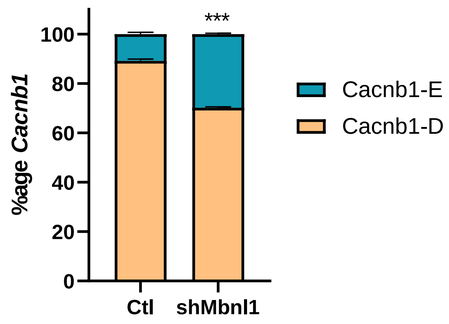


Downregulation of Mbnl1 induces an increase of Mbnl3 expression.

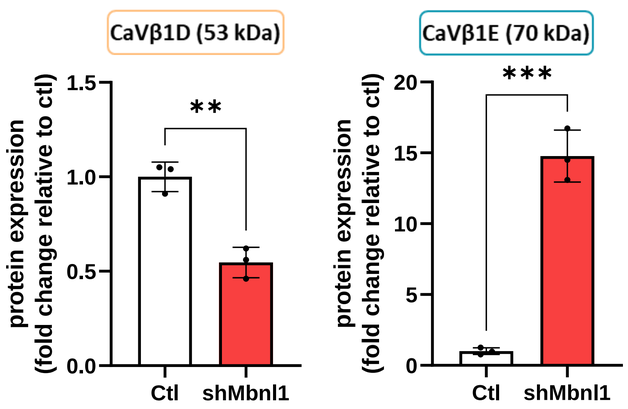
- **Variations of *Cacnb1* isoforms at transcriptional level**

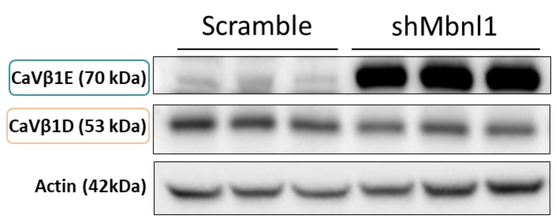
RT-PCR triplex *Cacnb1* Ex2-3





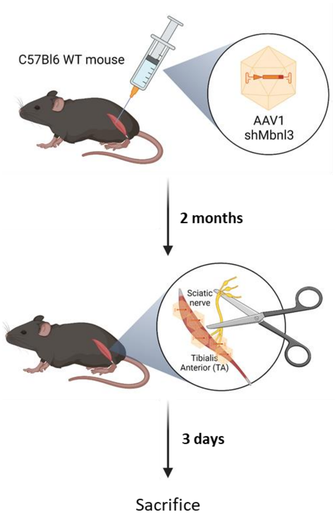
- **Variations of CaVβ1 isoforms at protein level**



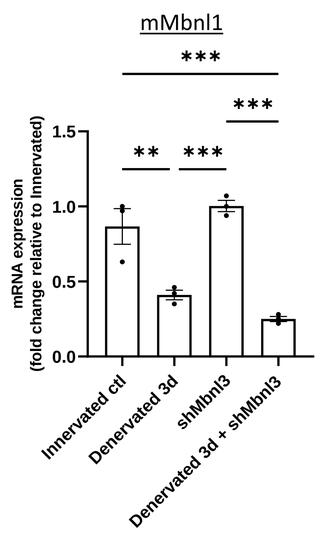


A downregulation of Mbnl1 induces an increase of the expression of CaVβ1-E in mouse skeletal muscle through a direct or indirect mechanism.

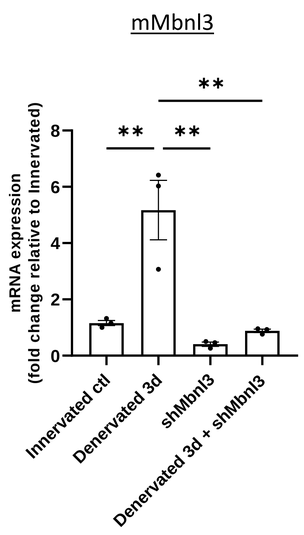
Mbnl3 modulates the expression of CaVβ1 isoforms *in vivo*



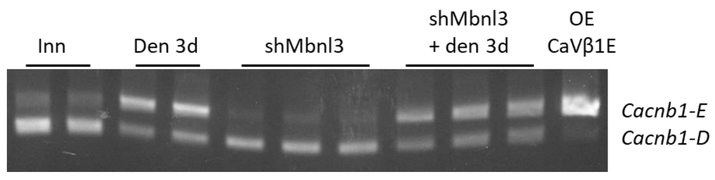
mMbnl1

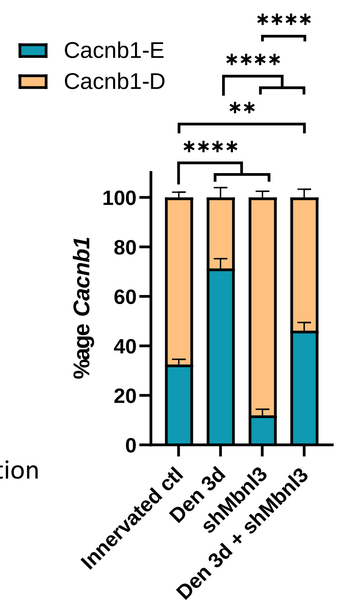


mMbnl3



RT-PCR triplex *Cacnb1* Ex2-3



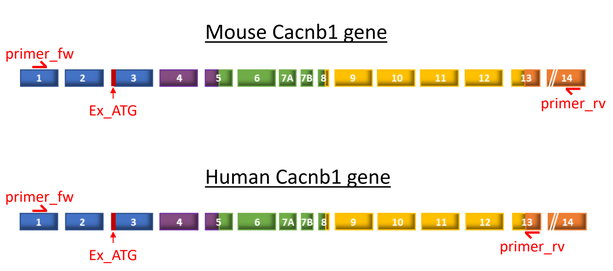


Mbnl3 is required to increase *Cacnb1-E expression in vivo*

⟶ The increase of CaVβ1-E expression under Mbnl1 downregulation is at least partially due to Mbnl3 increased expression

Cacnb1-D seems to be expressed after the activation of an alternative promoter

- **Nanopore sequencing on mouse and human muscles**



Mouse

Ex_ATG	TA inn	TA den	GAS inn	GAS den	
	100,00	100,00	100,00	100,00	100,00
1100011111111110000	94,58	92,01	89,96	82,83	89,84
1100011100000011001	0,00	0,00	0,00	11,19	2,80
1100011110111111001	0,44	2,84	5,37	1,37	2,50
1100011111011111001	2,12	1,90	1,68	1,58	1,82
1100011101111111001	0,76	0,81	0,70	0,69	0,74
1100011111011111001	0,49	0,73	0,66	0,73	0,65
1000011111111111001	0,53	0,59	0,55	0,45	0,53
1100011111111101001	0,42	0,34	0,32	0,39	0,37
1100001111111111001	0,18	0,19	0,17	0,18	0,18
1100011111110111001	0,15	0,11	0,15	0,19	0,15
1100010111111111001	0,18	0,13	0,13	0,09	0,13
1100011111001111001	0,13	0,14	0,14	0,10	0,13
1100011110101111001	0,02	0,04	0,08	0,08	0,05
1100011111111110001	0,02	0,05	0,09	0,03	0,05
1100011111111111111	0,00	0,12	0,01	0,00	0,03
1100011100000001001	0,00	0,00	0,00	0,10	0,03

Human

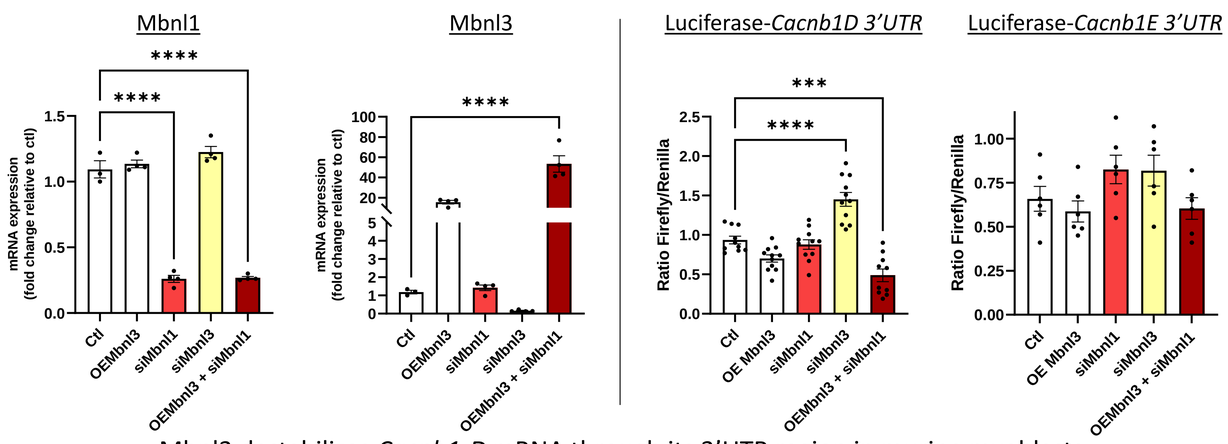
Ex_ATG	BC1	BC2	BC3	BC4	
	100,00	100,00	100,00	100,00	100,00
1100001011011110110101	90,85	93,86	88,45	88,20	84,60
1100001011010110110101	0,26	0,35	0,36	0,32	6,61
1100001011011100110101	2,08	2,20	1,94	2,04	1,85
1000000000000000000001	1,58	0,23	0,71	2,36	0,74
1100001011001110110101	0,81	0,85	0,67	0,61	0,71
1100001011011010110101	0,68	0,73	0,72	0,62	0,63
1100000000000000000001	0,01	0,01	2,61	1,70	0,51
1100001010000000000001	0,01	0,02	1,45	0,00	0,50
1100001011011110110001	0,30	0,35	0,40	0,31	0,38
1000001011011110110101	0,31	0,34	0,48	0,37	0,33
1100001010000111110101	0,03	0,08	0,03	0,06	0,31
1100001011010110110001	0,01	0,00	0,01	0,01	0,31
1100000000000000000101	0,66	0,01	0,00	1,50	0,25
1100001011010000000001	0,01	0,00	0,14	0,00	0,19
1100001011010100110101	0,01	0,02	0,02	0,02	0,16

0 = Exon absent / 1 = Exon present

The « Ex_ATG1 » intronic region is never asociated with an initiation of transcription in Ex1.

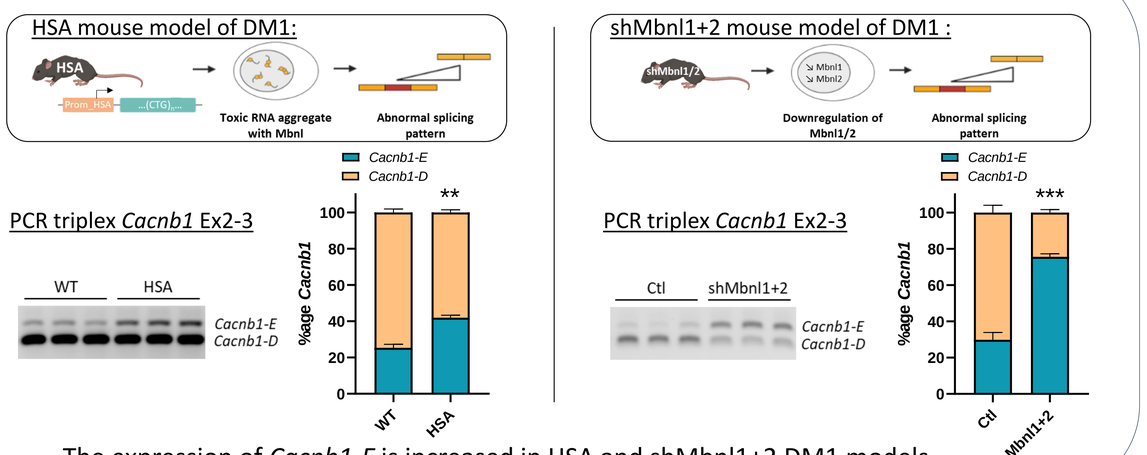
⟶ The expression of *Cacnb1* isoforms could be transcriptionally regulated, with the existence of an alternative promoter.

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
- A downregulation of Mbnl1, and the subsequent increase of Mbnl3, leads to increased CaVβ1-E and decreased CaVβ1D expression levels *in vivo*
- The expression of *Cacnb1-D* seems to be under the control of an alternative promoter
- *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

- Studying the potential alternative promoter controlling *Cacnb1-D* 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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