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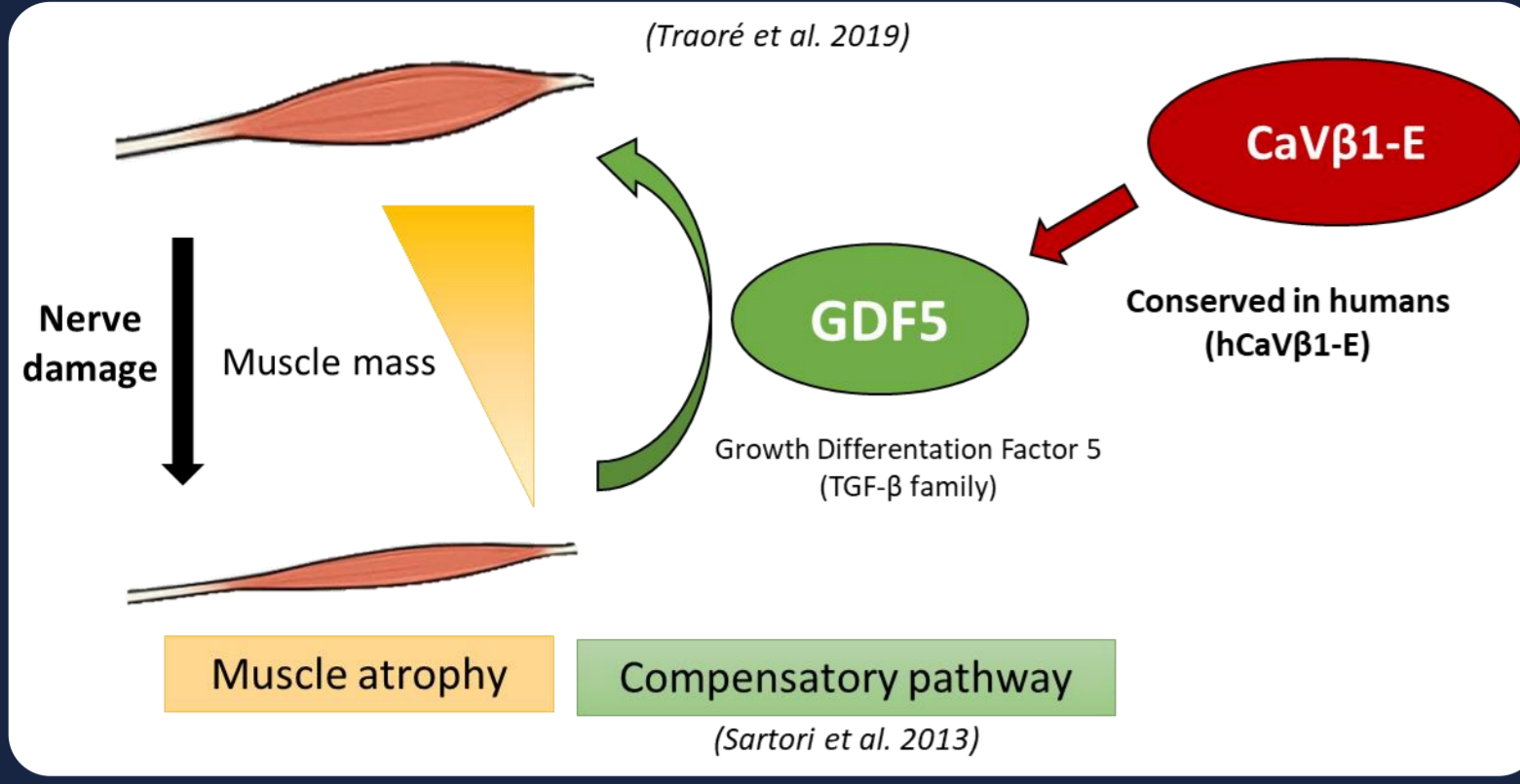
Role of MuscleBlind-Like proteins in the regulation of expression of CaVβ1 isoforms in adult skeletal muscle

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INTRODUCTION

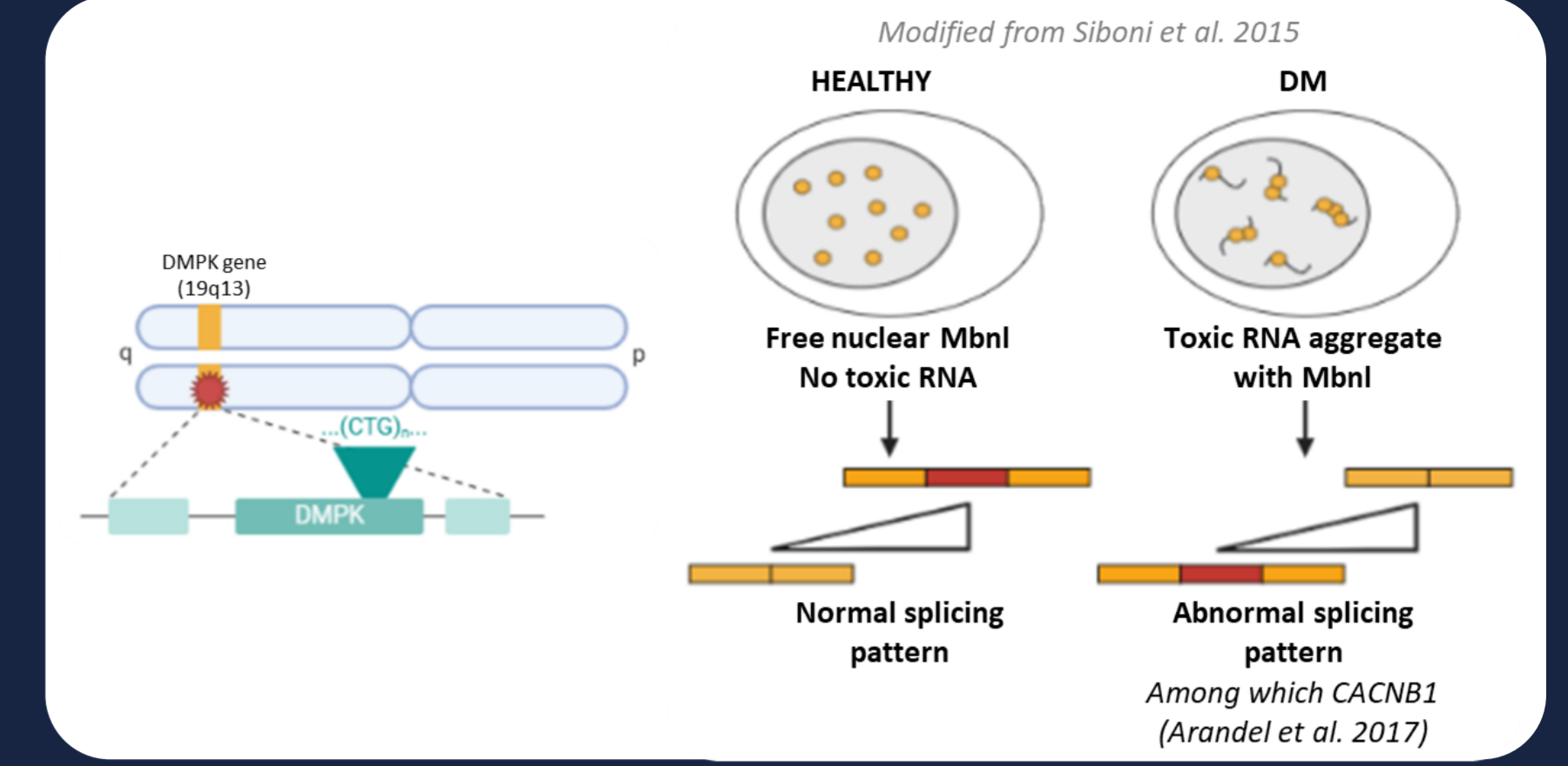
CaVβ1-E/GDF5 axis in muscle mass homeostasis



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.

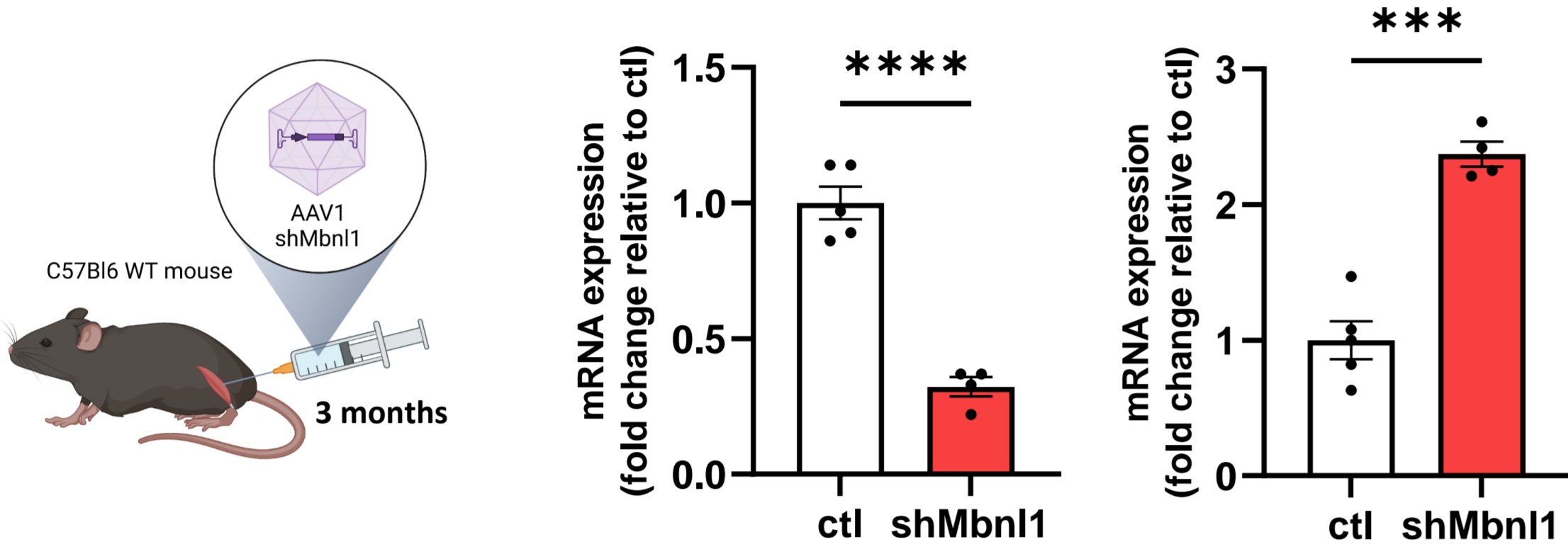
CaVβ1-E and CaVβ1-D are two different isoforms of CaVβ1 protein in skeletal muscle, expressed during embryogenesis and in healthy innervated adult muscle, respectively. Importantly, our recent study demonstrated that the embryonic CaVβ1-E isoform expression increases after a nerve damage in adult skeletal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

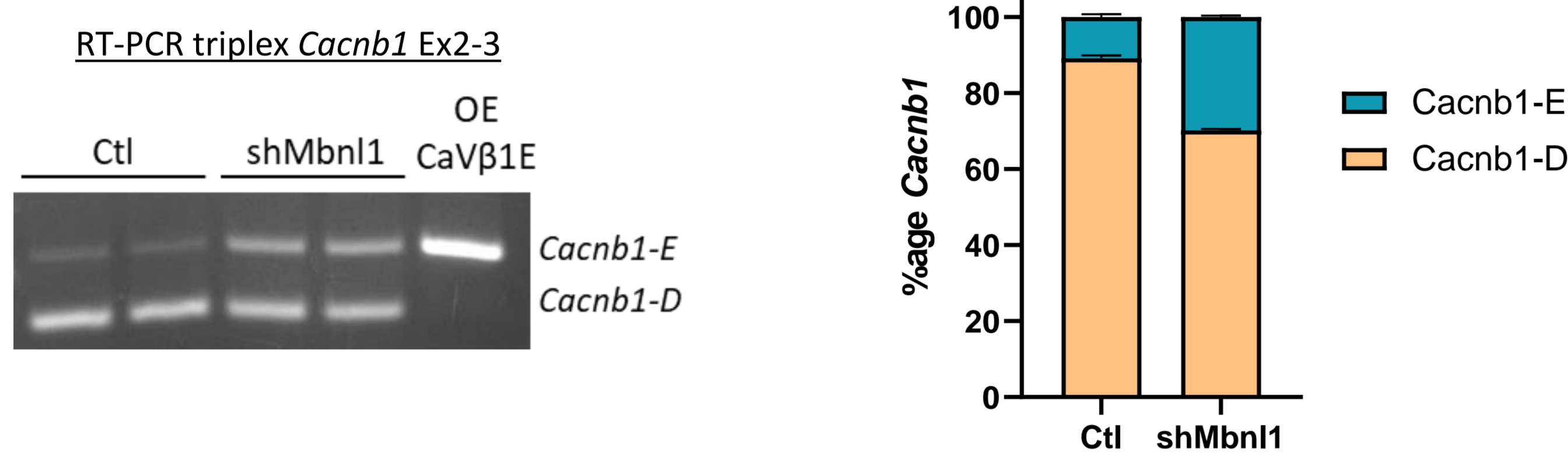
Mbnl1 modulates the expression of CaVβ1 isoforms *in vivo*

Mouse model of Mbnl1 downregulation

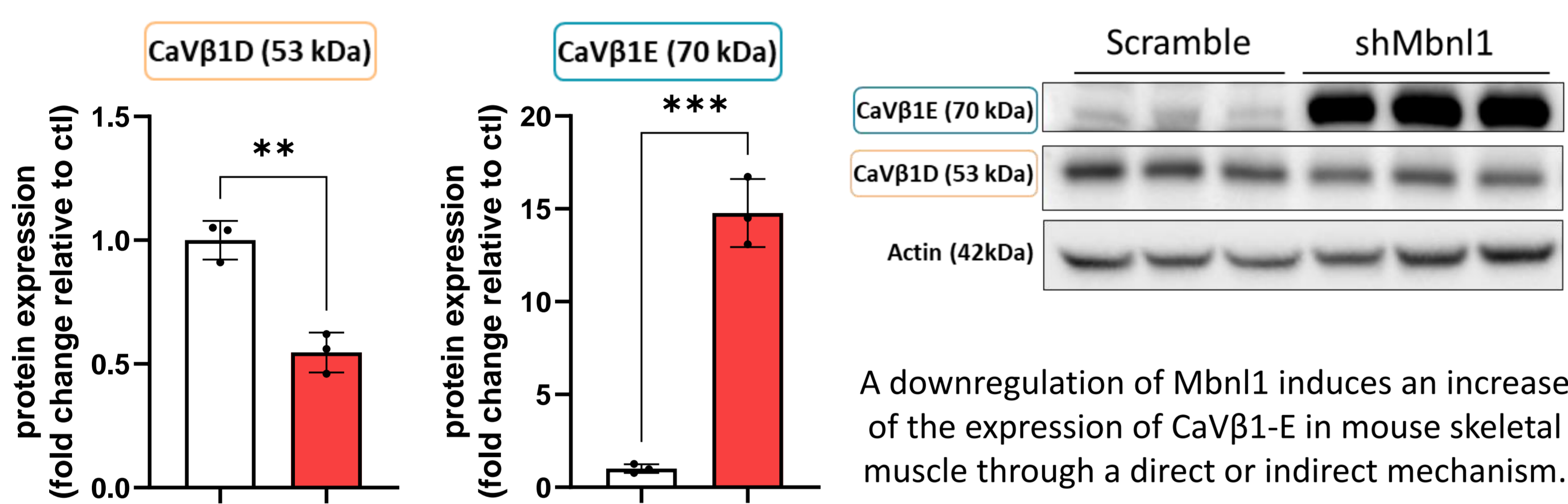


Downregulation of Mbnl1 induces an increase of Mbnl3 expression.

Variations of Cacnb1 isoforms at transcriptional level

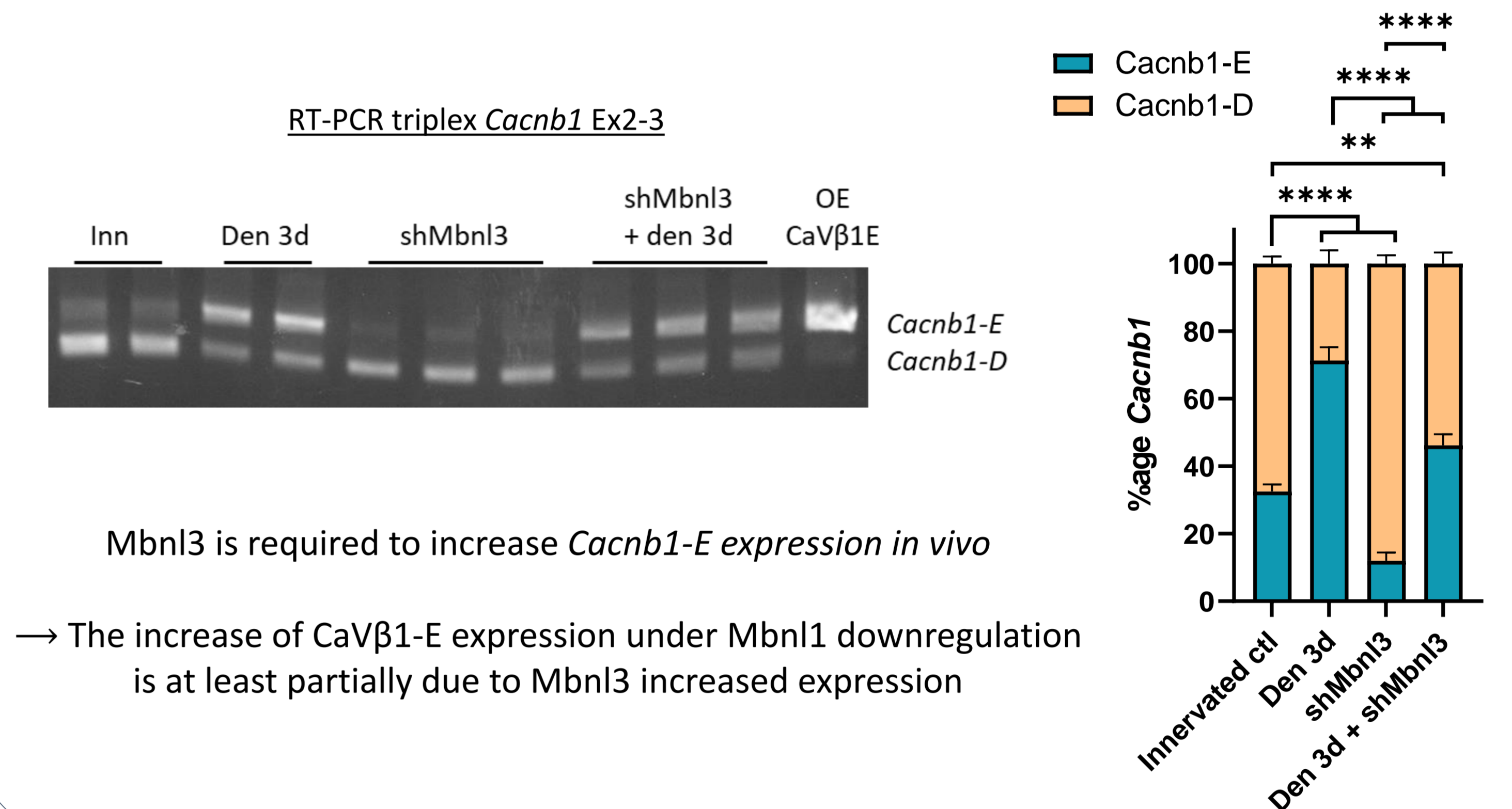
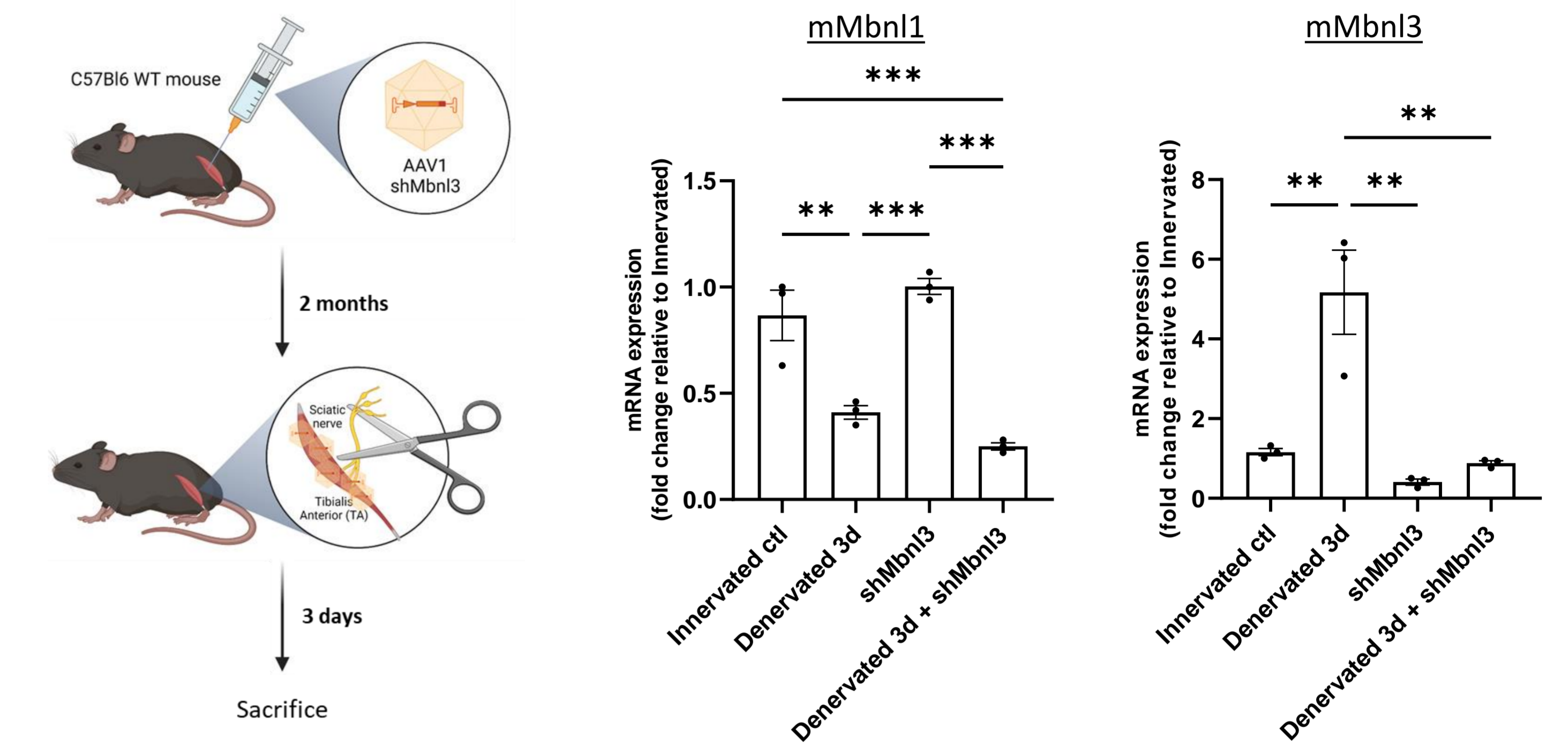


Variations of CaVβ1 isoforms at protein level



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

Mbnl3 modulates the expression of CaVβ1 isoforms *in vivo*

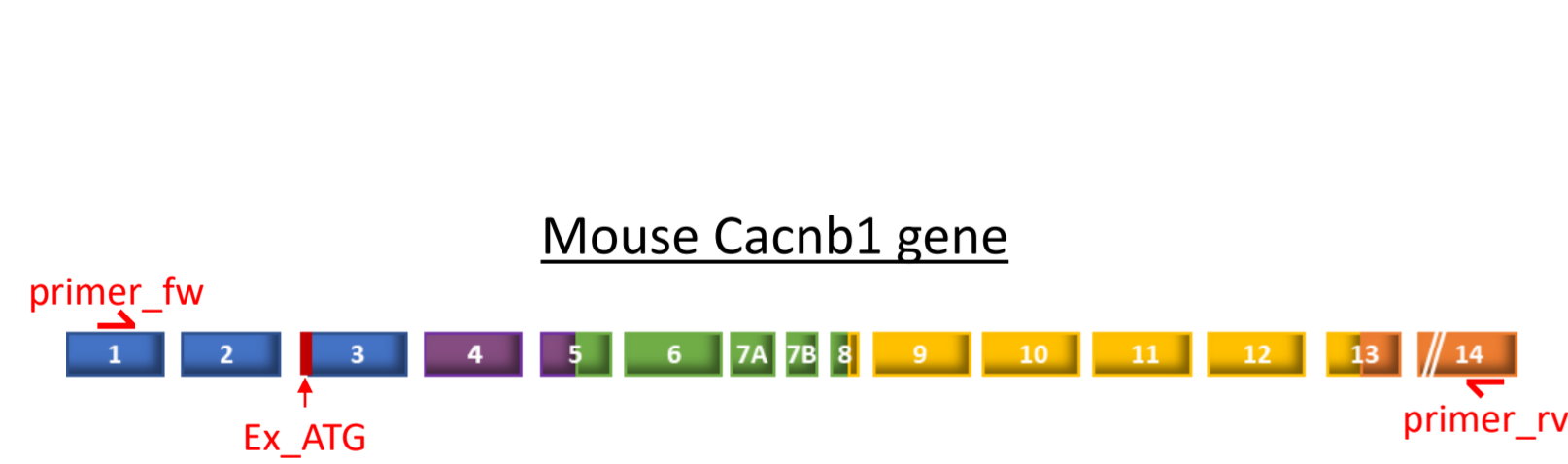


Mbnl3 is required to increase Cacnb1-E expression *in vivo*

→ 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



Ex_ATG	Mouse				100,00
	TA Inn	TA den	GAS Inn	GAS den	
11000111111111110000	94,58	92,01	89,96	82,83	89,84
1100011100000011001	0,00	0,00	0,00	11,19	2,80
11000111101111111001	0,44	2,84	5,37	1,37	2,50
11000111111101111001	2,12	1,90	1,68	1,58	1,82
11000111011111111001	0,76	0,81	0,70	0,69	0,74
11000111110111111001	0,49	0,73	0,66	0,73	0,65
1100011111111101001	0,53	0,59	0,55	0,45	0,53
110001111111111101001	0,42	0,34	0,32	0,39	0,37
11000111111111111001	0,18	0,19	0,17	0,18	0,18
11000111111111011001	0,15	0,11	0,15	0,19	0,15
11000101111111111001	0,18	0,13	0,13	0,09	0,13
11000111111100111001	0,13	0,14	0,14	0,10	0,13
11000111010111111001	0,02	0,04	0,08	0,08	0,05
110001111111111110001	0,02	0,05	0,09	0,03	0,05
110001111111111111111	0,00	0,12	0,01	0,00	0,03
110011100000001001	0,00	0,00	0,00	0,10	0,03

0 = Exon absent / 1 = Exon present

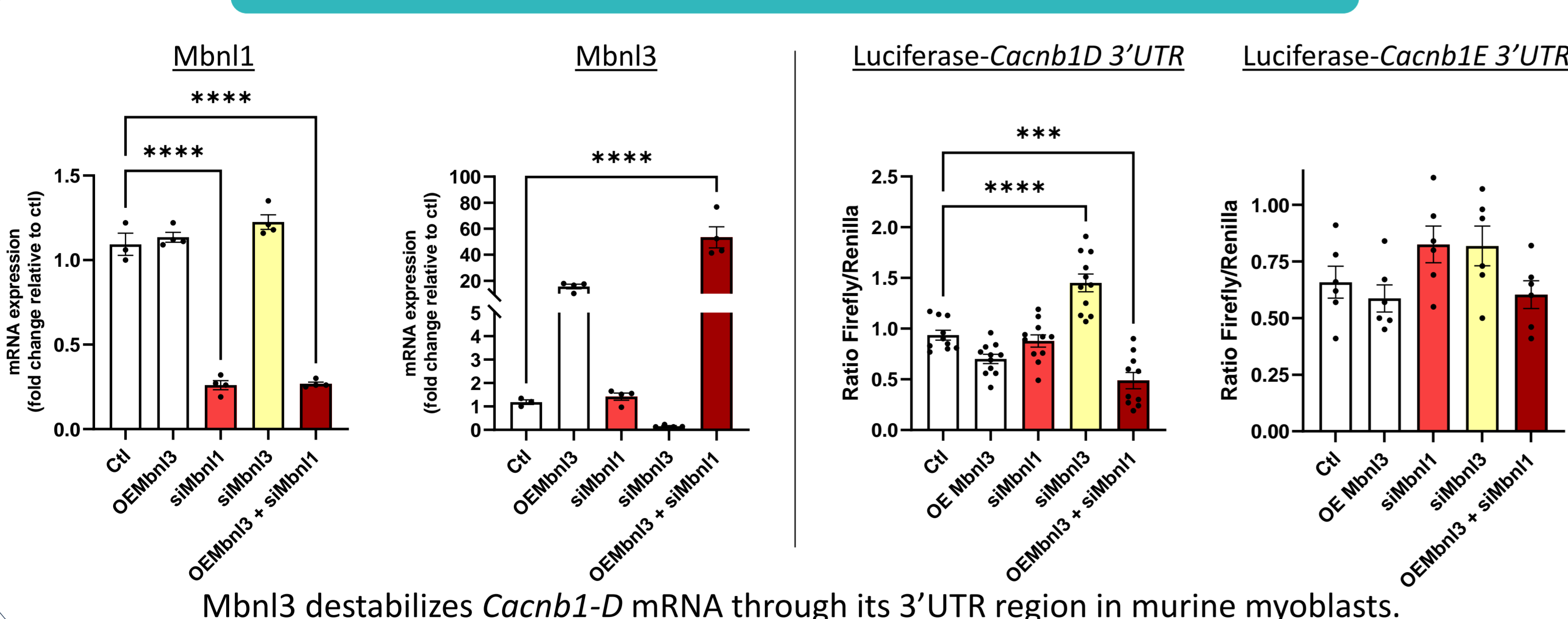
Human

Ex_ATG	BC1	BC2	BC3	BC4	100,00
	100,00	100,00	100,00	100,00	
1100010101111111010101	90,85	93,86	88,45	88,20	84,60
1100010101010101010101	0,26	0,35	0,36	0,32	6,61
1100010101111100110101	2,08	2,20	1,94	2,04	1,85
1000000000000000000001	1,58	0,23	0,71	2,36	0,74
1100010101001101010101	0,81	0,85	0,67	0,61	0,71
11000101011010101010101	0,68	0,73	0,72	0,62	0,63
1100000000000000000001	0,01	0,01	2,61	1,70	0,51
1100010100000000000001	0,01	0,02	1,45	0,00	0,50
1100010101111101010001	0,30	0,35	0,40	0,31	0,38
1000010101010101010001	0,31	0,34	0,48	0,37	0,33
11000101000110101010101	0,03	0,08	0,03	0,06	0,31
1100010101010101010001	0,01	0,00	0,01	0,01	0,31
1100000000000000000101	0,66	0,01	0,00	1,50	0,25
1100010101000000000001	0,01	0,00	0,14	0,00	0,19
110001010110100101010101	0,01	0,02	0,02	0,02	0,16

The « Ex_ATG » intronic region is never associated with an initiation of transcription in Ex1.

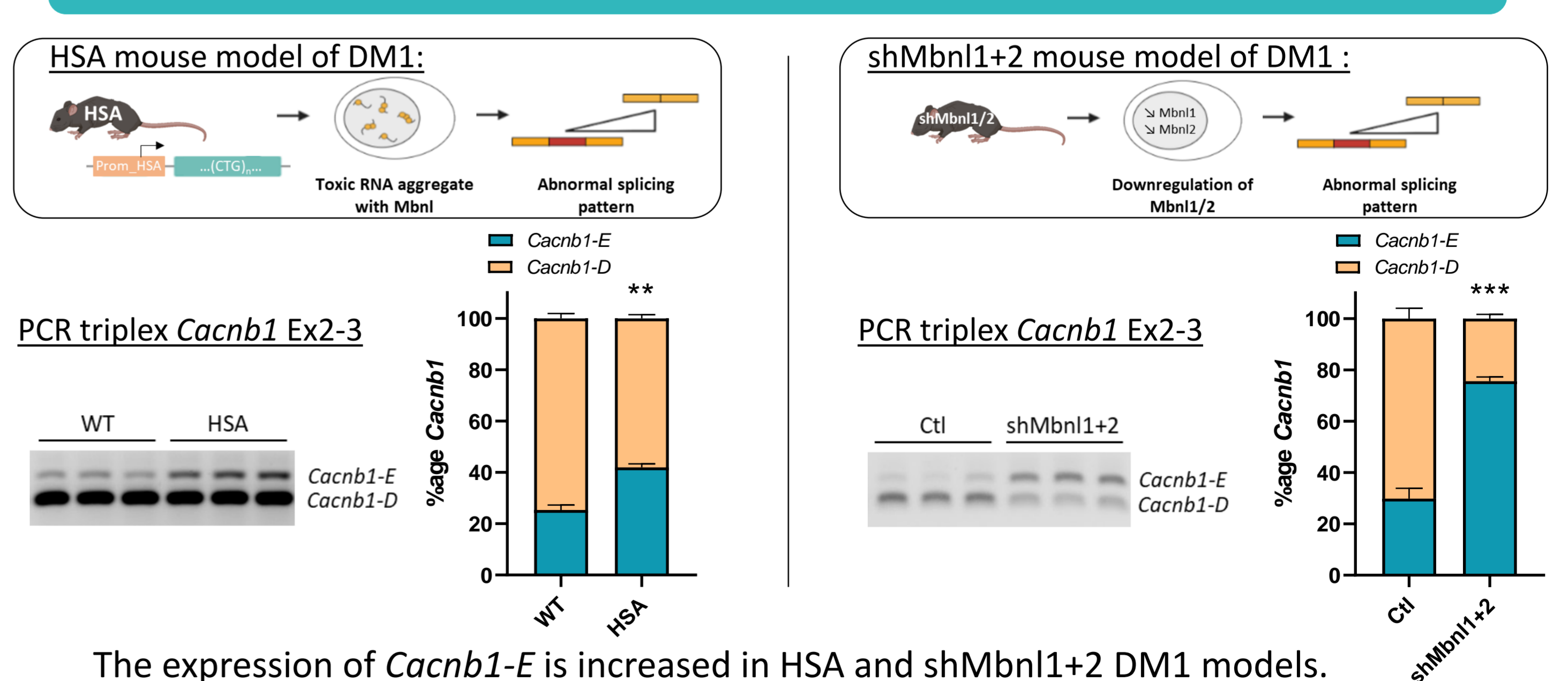
→ The expression of Cacnb1 isoforms could be transcriptionally regulated, with the existence of an alternative promoter.

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