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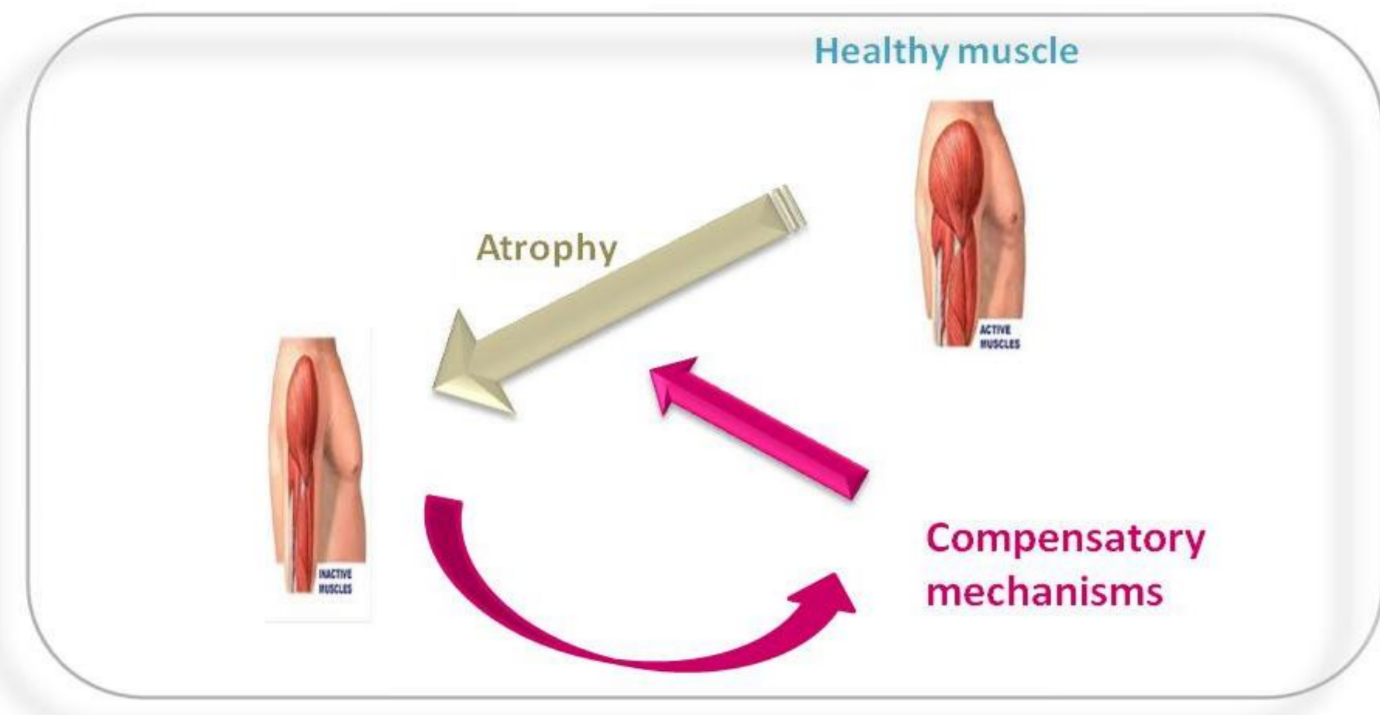
From voltage sensing to gene expression in the control of muscle mass homeostasis

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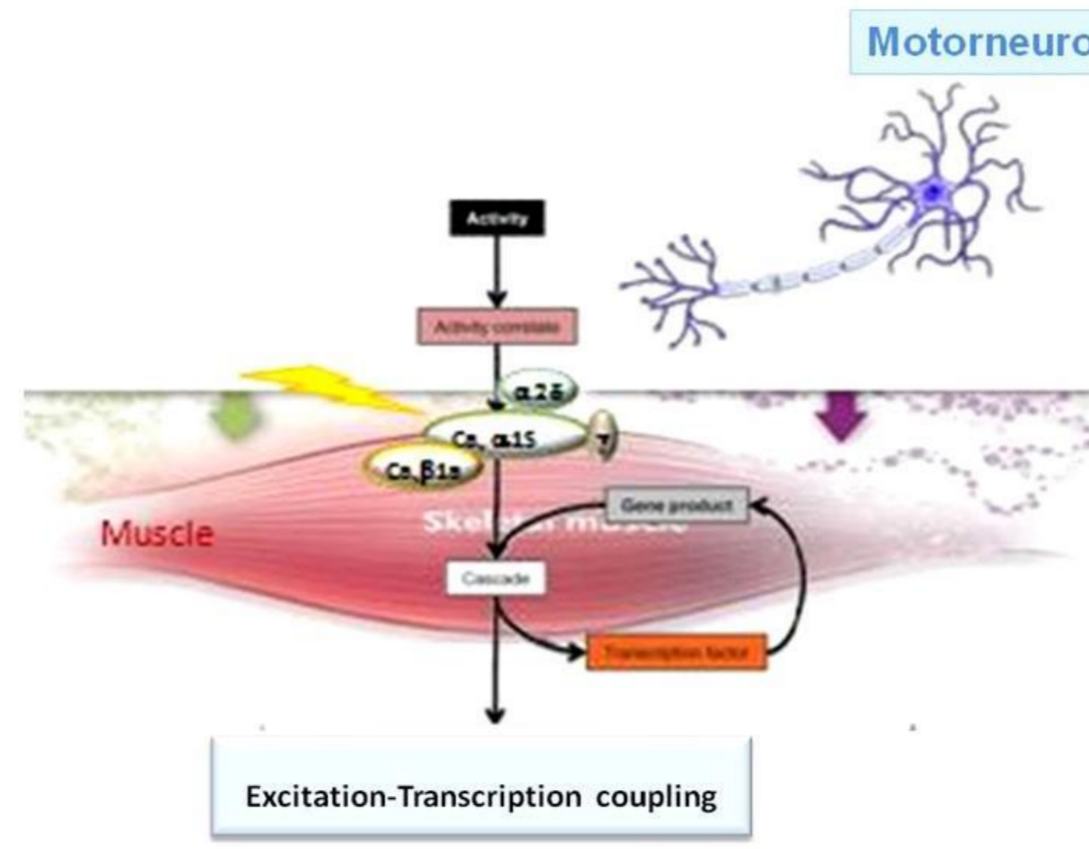
INTRODUCTION

Muscle plasticity and compensatory response

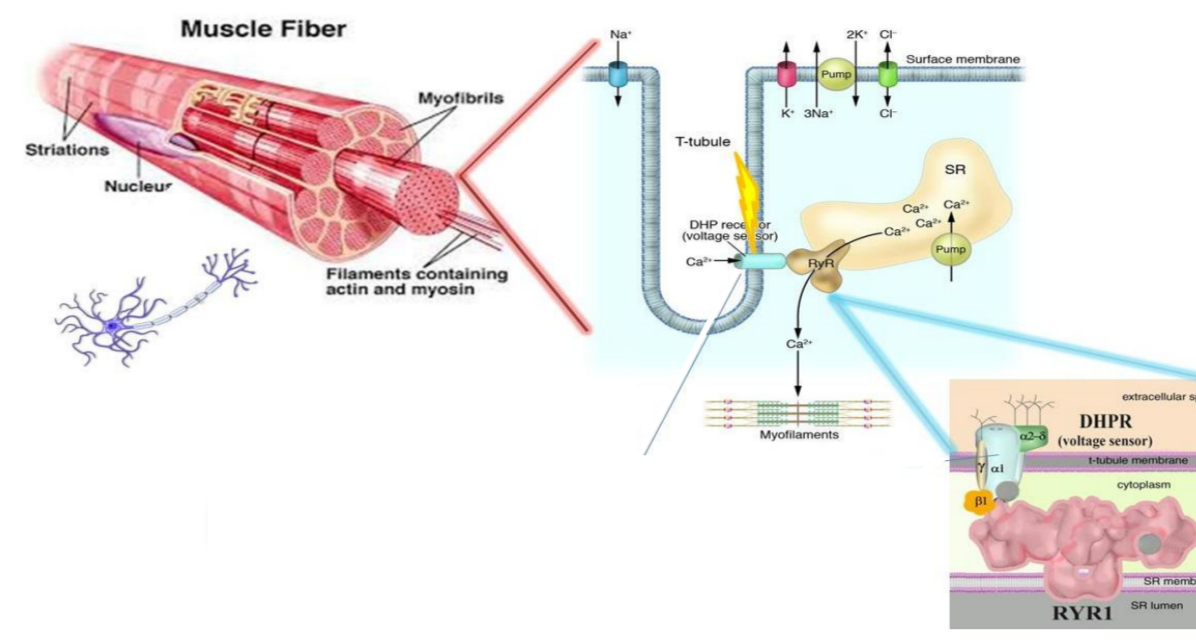


- Causes:**
- ✓ Myopathies-neuromuscular diseases
 - ✓ Nerve damage
 - ✓ Disuse-immobilization

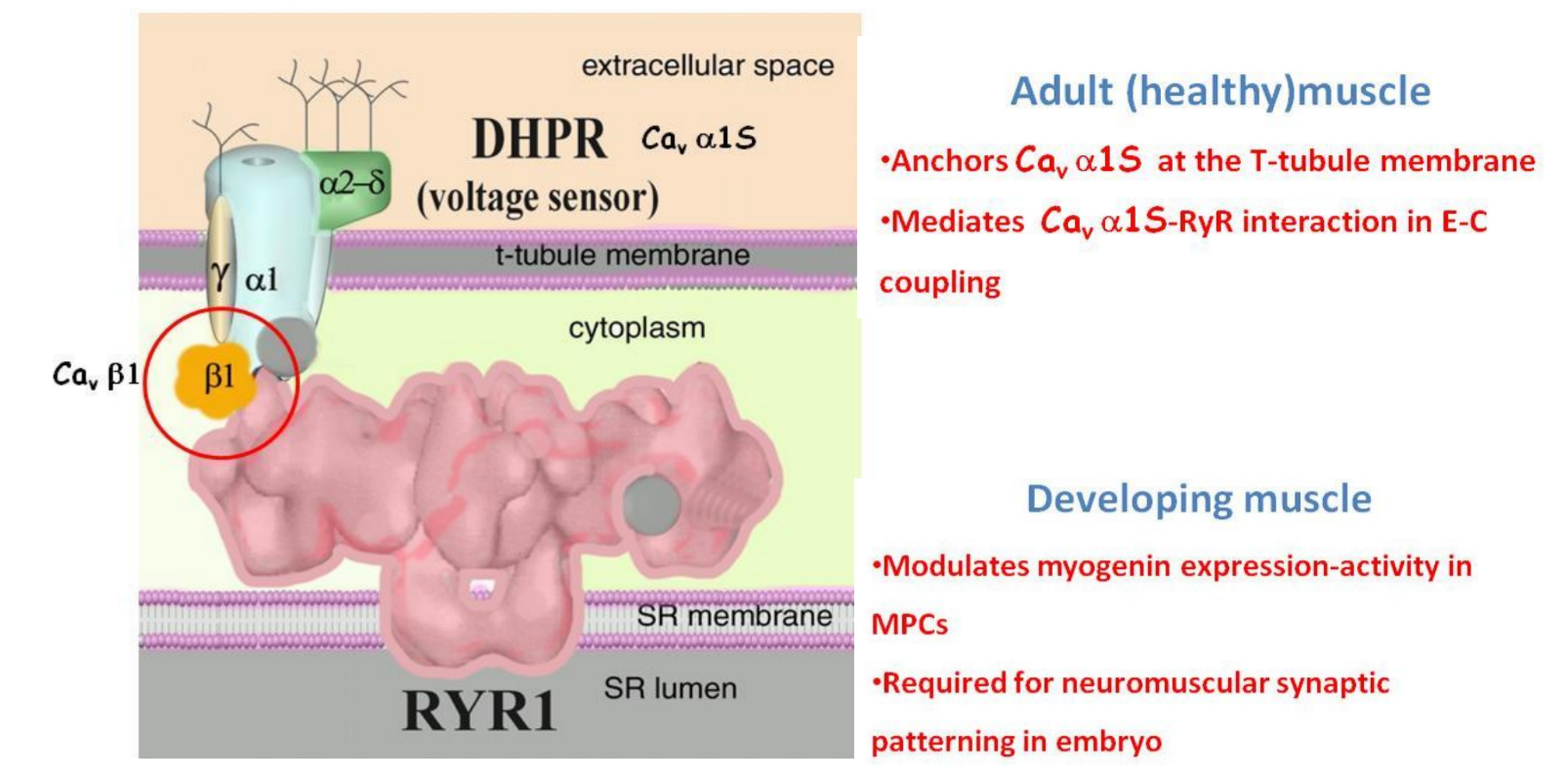
Electrical activity and Excitation-Transcription coupling



Excitation-Contraction coupling



BEST CANDIDATE: essential for voltage sensor function
Dayal et al., PNAS 2013

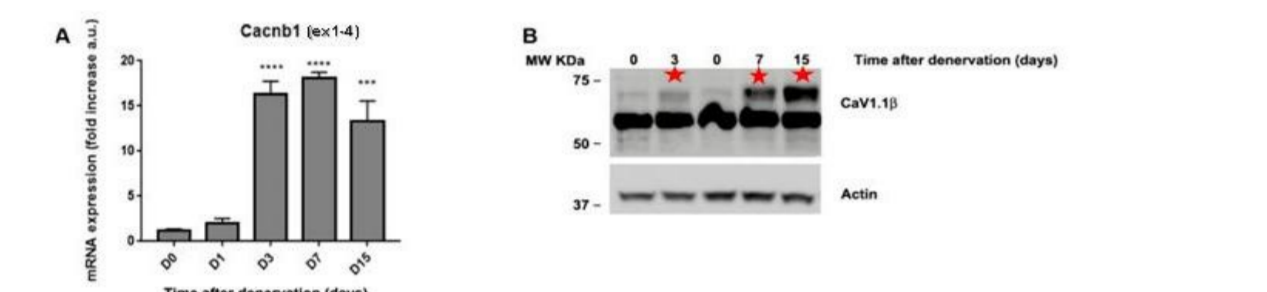


- Adult (healthy) muscle**
- Anchors Ca_v1.5 at the T-tubule membrane
 - Mediates Ca_v1.5-RyR interaction in E-C coupling
- Developing muscle**
- Modulates myogenin expression-activity in MPCs
 - Required for neuromuscular synaptic patterning in embryo

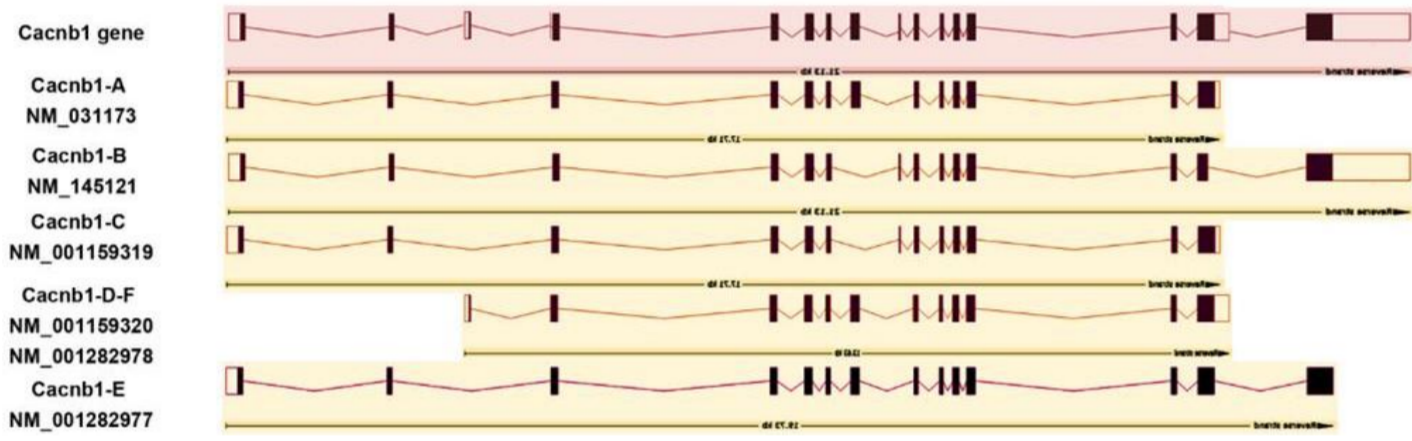
Muscle mass and fiber size undergo to rapid and significant changes according to environmental and pathological conditions. Among the various trophic signals, intrinsic muscle contractile activity, neurotransmission and neurotrophic factors are crucial components regulating the integrity of muscle mass. Alterations in the pattern of nerve-evoked electrical activity convey in a modulation of the signal, due to modification in the gene expression by switching on and off specific transcription factors. This excitation-transcription (E-T) coupling is crucial for isotypic determination of muscle fibers but also for plastic adaptation and compensation after the loss of muscle mass. When electrical activity is impaired, such as during neuromuscular diseases, disuse or aging, massive muscle atrophy is observed. Few components of this compensatory response have been identified; in particular, a crucial role is played by the GDF5/Smad4 pathway. Nevertheless, it is still unknown which protein acts as first trigger of the atrophic-compensatory response after an electrical activity alteration. Good candidates could be molecules implicated in the muscle voltage sensing, and we focused our study on the beta subunit of the L-type of calcium channel complex Ca_v1.1. Ca_vβ1,

Ca_vβ1 in adult skeletal muscle after electrical activity alteration

Ca_vβ1 expression in muscle after denervation



Denervation induces a strong increase of mRNA levels of Ca_vβ1 related to the appearance of an upper extra band: splicing isoform expression?



RNAseq : illumina sequencing, NextSeq500, Paired-end 2x75bp

Exon level analysis: Summary

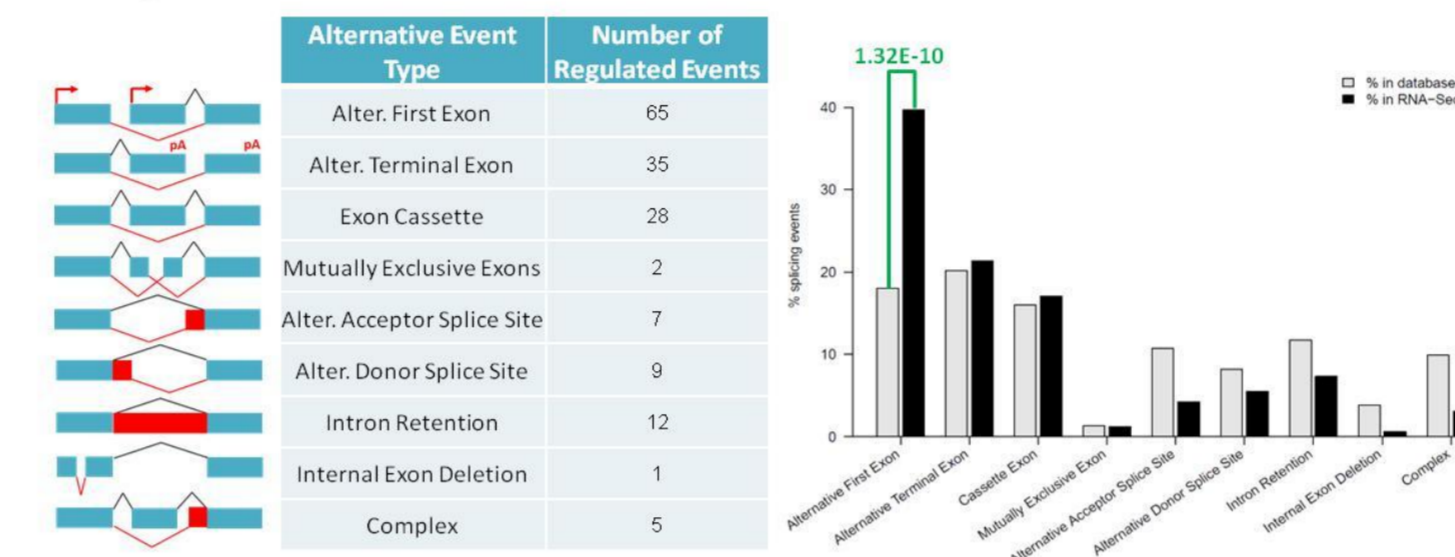
- ▶ 1,022 events/exons were found significantly regulated:
 - 164 alternative events from "Pattern" analysis;
 - 858 from "Exon" analysis only (i.e., without a priori on alternative event information).
- ▶ 706 distinct genes are involved.

Many regulated alternative events



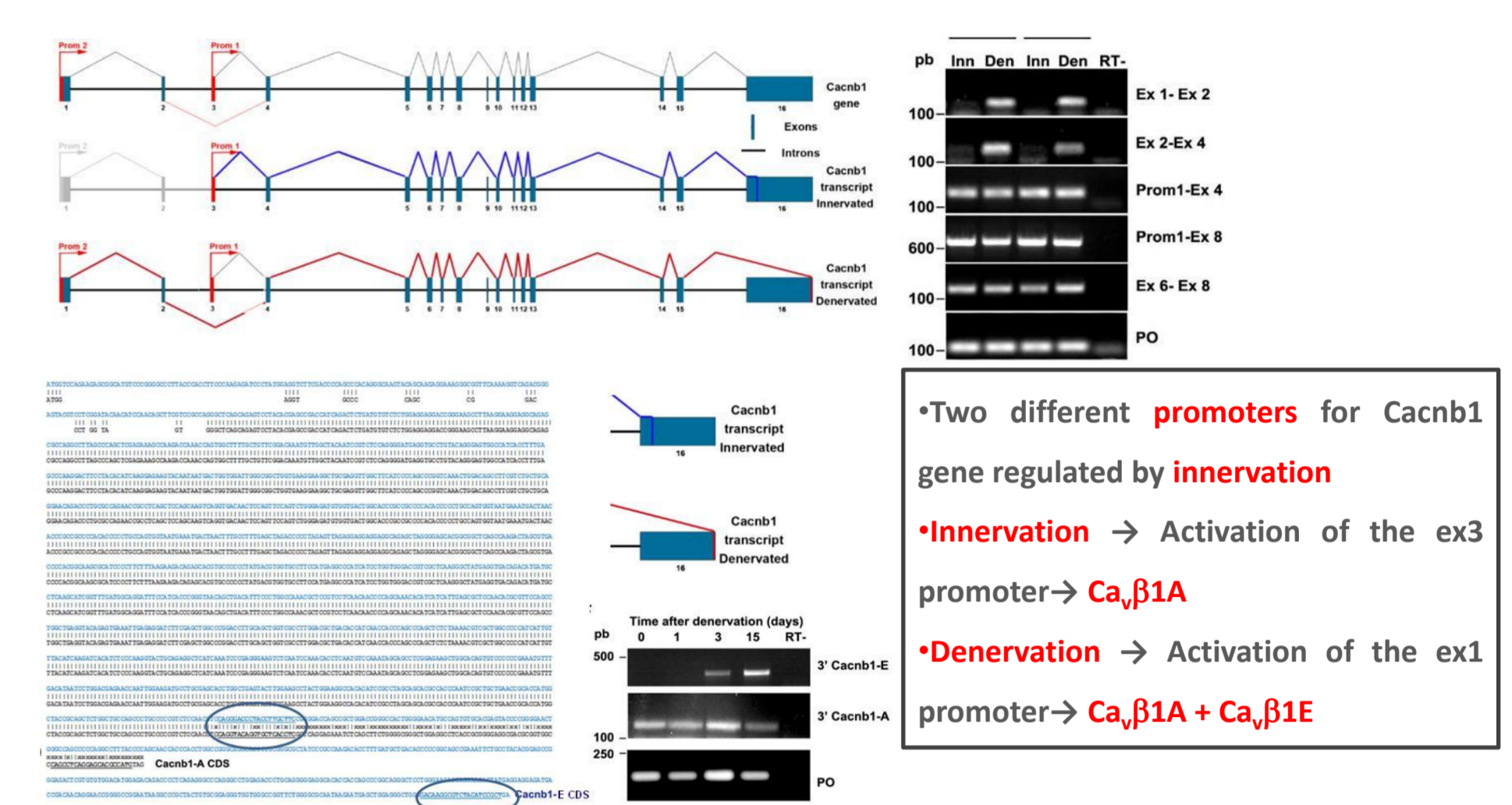
Exon level analysis: Distribution of alternative events

Based on pattern analysis, most types of alternative event are retrieved but with a high enrichment in alternative first:



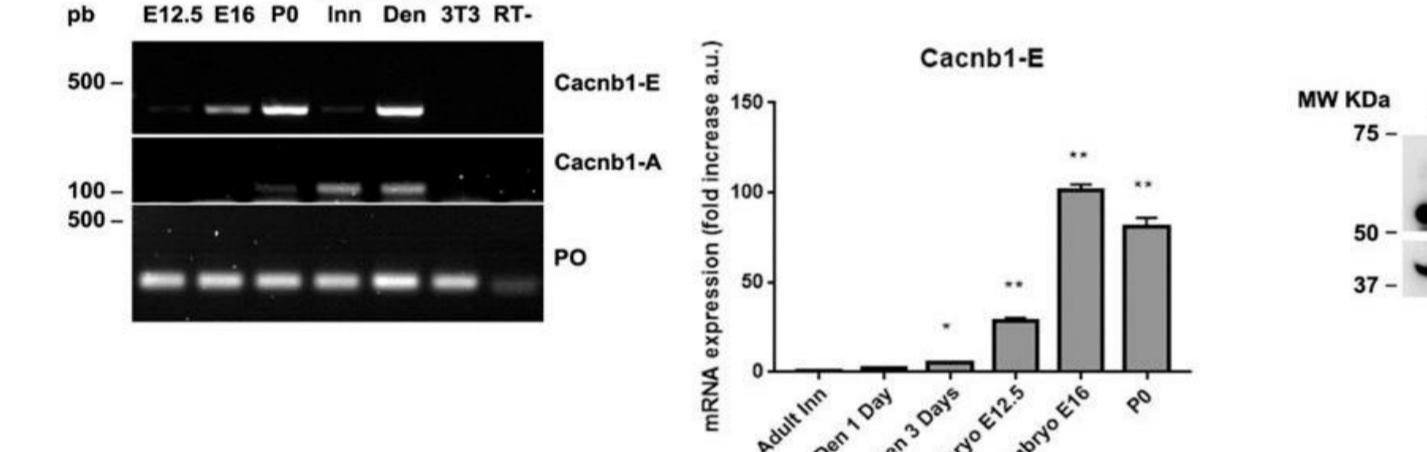
High enrichment in alternative first exons

Alternative first exon of Cacnb1 transcript in muscle after denervation

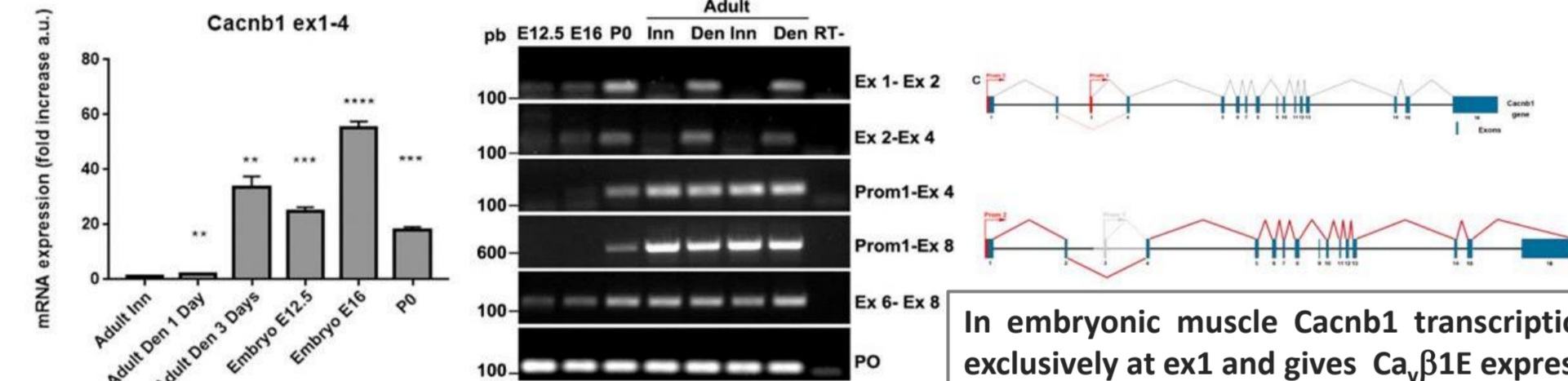


- Two different promoters for Cacnb1 gene regulated by innervation
- Innervation → Activation of the ex3 promoter → Ca_vβ1A
- Denervation → Activation of the ex1 promoter → Ca_vβ1A + Ca_vβ1E

A new Ca_vβ1 isoform: embryonic?



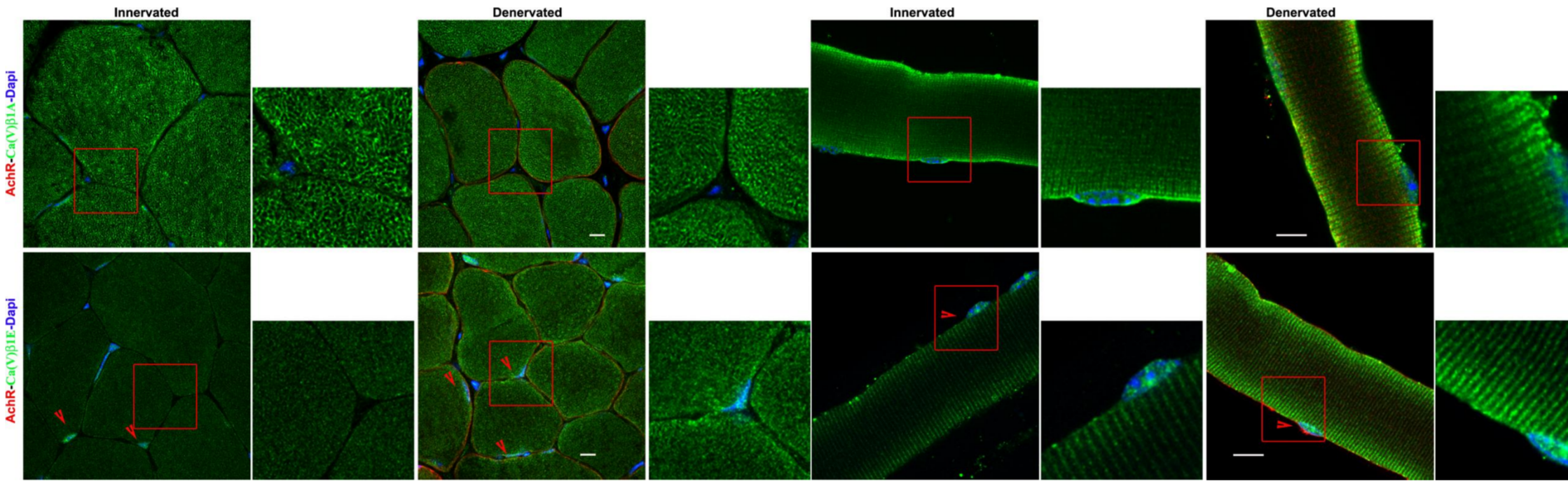
An embryonic isoform of Ca_vβ1 is specifically expressed in the adult muscle after denervation



In embryonic muscle Cacnb1 transcription starts exclusively at ex1 and gives Ca_vβ1E expression

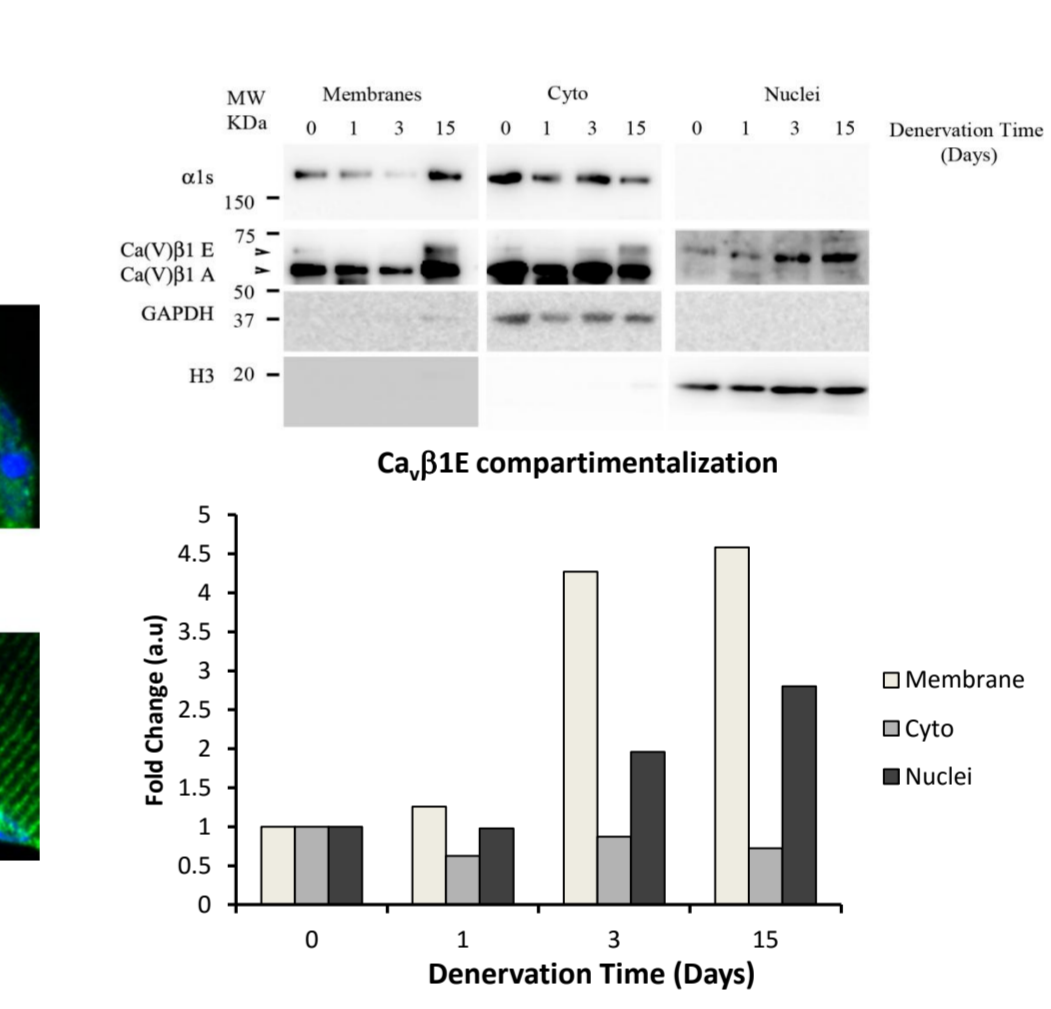
Characterization of Ca_vβ1E in adult skeletal muscle

Ca_vβ1 isoforms localization in adult skeletal muscle



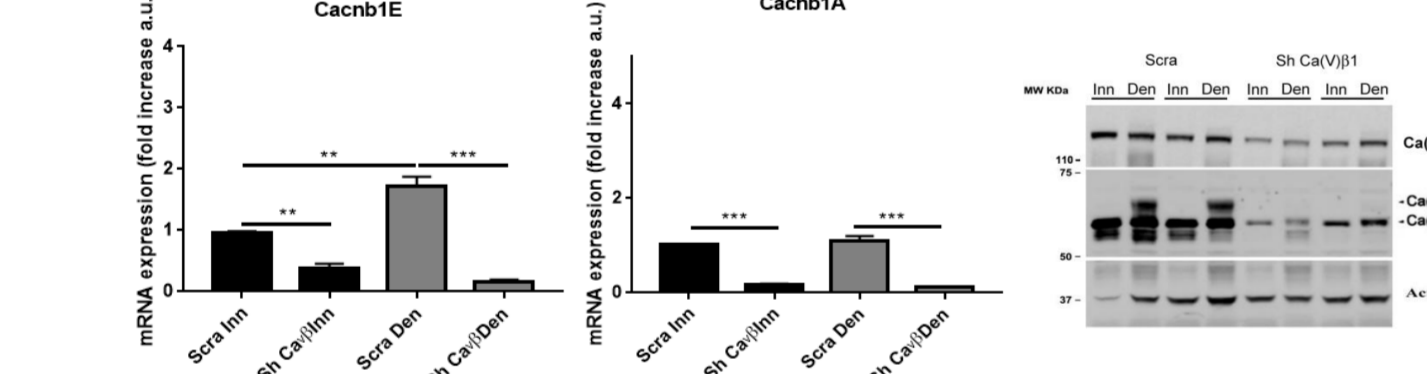
Ca_vβ1E shows different localization and nuclear staining compared to Ca_vβ1A

Ca_vβ1E in subcellular fractions in innervated and denervated muscles

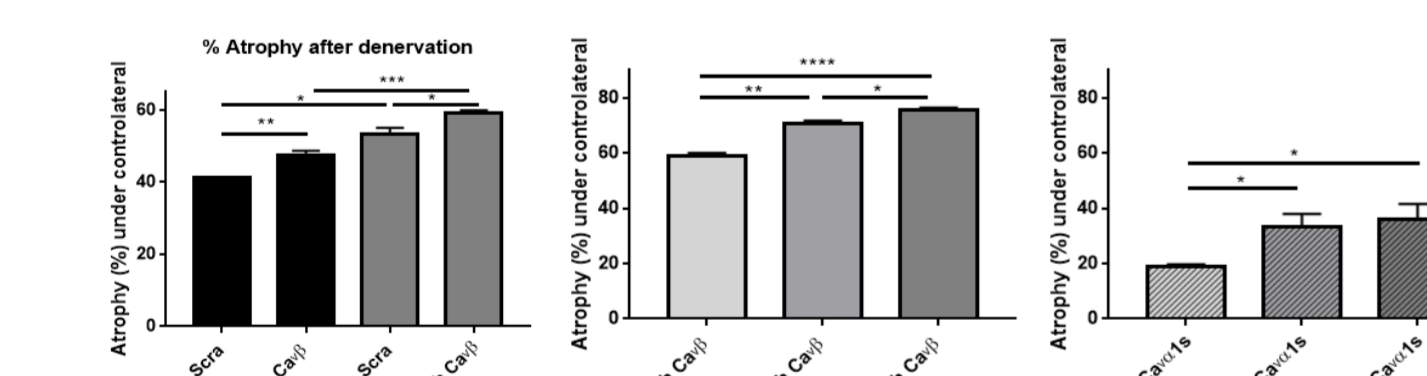


- Ca_vβ1E → embryonic Ca_vβ1 variant → activation of ex1 promoter exclusively
- Intracellular localization of Ca_vβ1E looks different from Ca_vβ1A in adult
- Denervation induces re-distribution of Ca_vβ1E → Nuclear and membrane enrichment

Down regulation of Ca_vβ1 in adult skeletal muscle



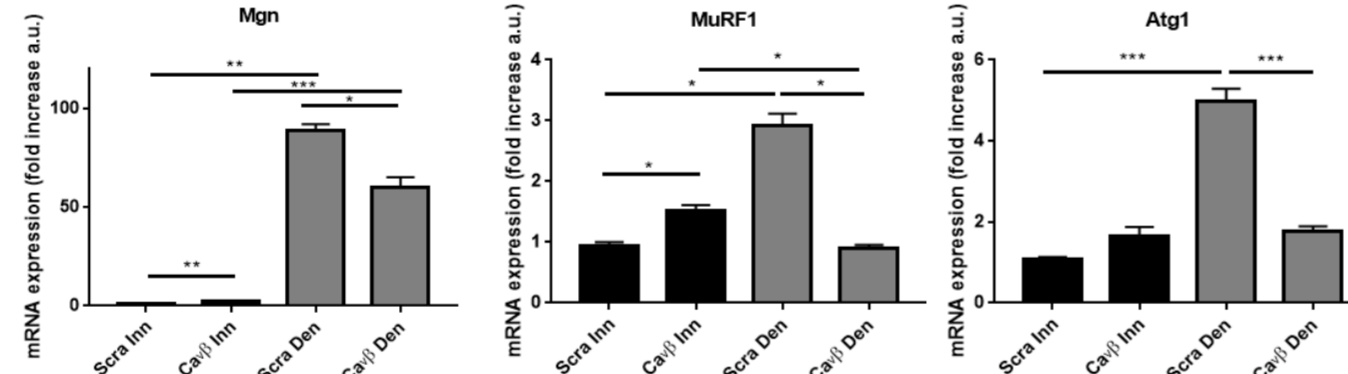
Targeting Cacnb1A transcript down regulates both isoforms in adult skeletal muscles



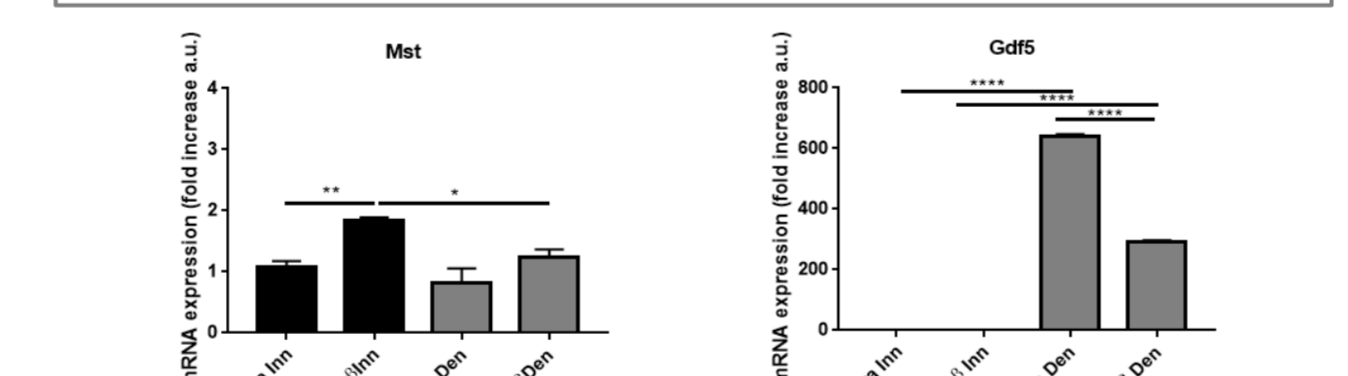
Ca_vβ1 ablation dramatically exacerbates denervation atrophy and induces massive muscle mass loss independently of Ca_vα1s reduction

Fonction of Ca_vβ1E in adult skeletal muscle

Atrogens and compensatory response to denervation in Ca_vβ1 KO muscles

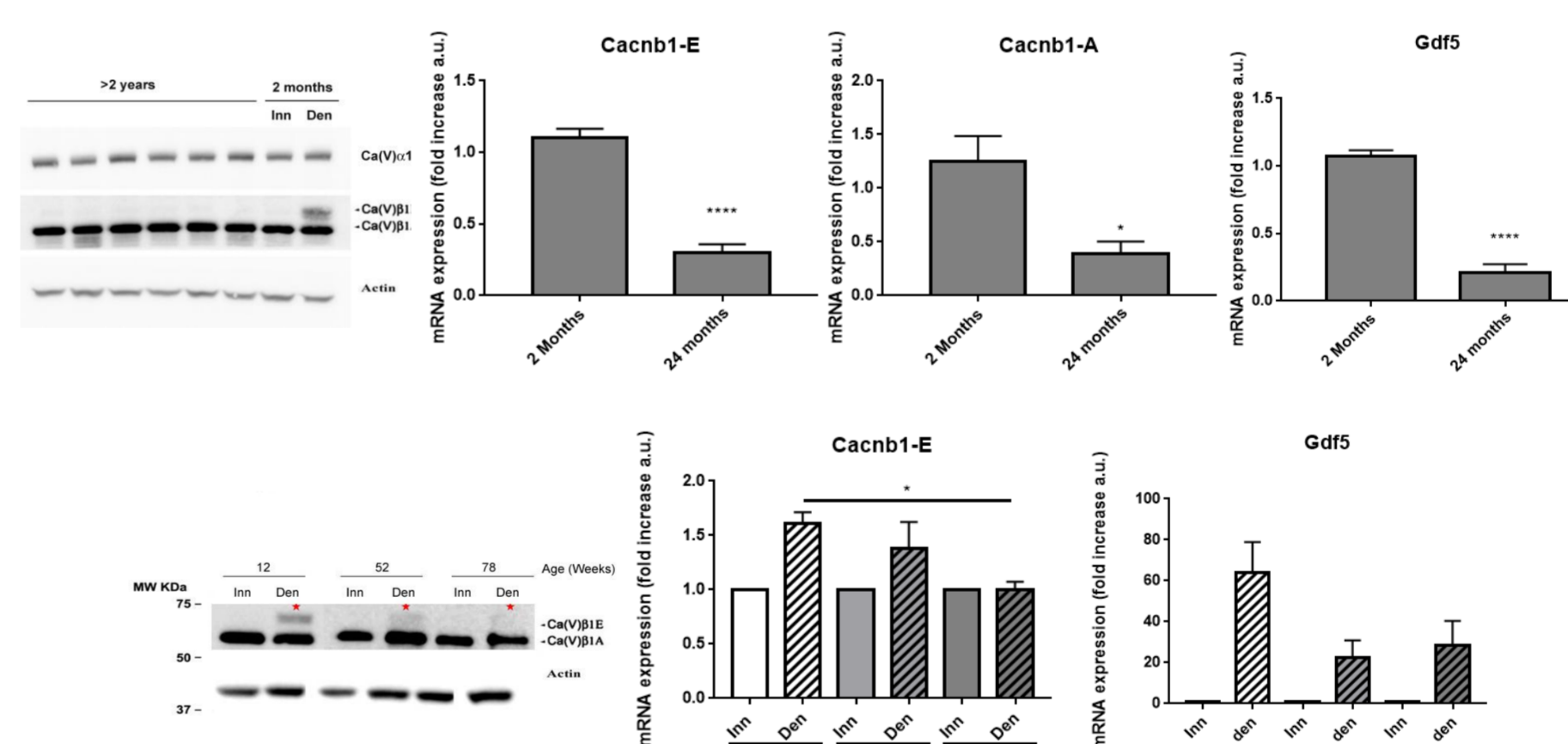


Myogenin-dependent signaling is altered by Ca_vβ1 down-regulation



The loss of compensatory response to muscle waste in the absence of Ca_vβ1 is probably dependent on increased myostatin and decreased Gdf5/BMP14 signaling

Ca_vβ1E in ageing muscle



In ageing muscle Ca_vβ1 is strongly downregulated in parallel to decreased Gdf5/BMP14 expression: the origin of sarcopenia?

- Ca_vβ1A shRNA → down-regulation of Ca_vβ1E and Ca_vβ1A
- Ca_vβ1 KO → muscle mass loss and exacerbation of atrophy
- Ca_vβ1 KO → myogenin signaling altered; Gdf5/Gdf8 signaling altered
- Ageing muscle → physiological Ca_vβ1 KD

Conclusion and perspectives

What we know:

- Electrical activity alteration → embryonic Ca_vβ1E expression by alternative first exon event
- Ca_vβ1A shRNA → down-regulation also Ca_vβ1E and splicing → Ca_vβ1E expression depends on Ca_vβ1A
- Ca_vβ1A/E KO → muscle mass loss and exacerbation of atrophy
- Ca_vβ1A/E KO → myogenin signaling altered; Gdf5/Mst signaling altered
- Ageing muscle → physiological Ca_vβ1 KD and splicing; Gdf5 signaling altered
- Overexpression of Ca_vβ1E → no impact on Ca_vβ1A expression

What we must know:

- Ca_vβ1E effector of compensation? → overexpression of Ca_vβ1E + sh Ca_vβ1A (ongoing in ageing model)
- Ca_vβ1A effector of compensation? → overexpression of Ca_vβ1A in sh Ca_vβ1A
- Role of alternative promoter in Ca_vβ1 fonction and expression → sh Ca_vβ1 ex2 versus sh Ca_vβ1E
- Is Gdf5 the key mediator of Ca_vβ1 effect on muscle mass? → overexpression of Gdf5/BMP14 in sh Ca_vβ1
- Can Ca_vβ1A act on its own promoter and/or Gdf5 promoter? → Luciferase-based assay

Perspectives:

- Role of Ca_vβ1 in sarcopenia → overexpression of Ca_vβ1 in ageing model (ongoing for Ca_vβ1E)
- Ca_vβ1 in reinnervation and disease models (ALS, SMA)
- Ca_vβ1 in human neuromuscular disease/sarcopenia
- Ca_vβ1 in myogenesis /embryogenesis

Ca_vβ1E over-expression does not modify Ca_vβ1E endogenous expression in adult muscle and can be rescued by a specific shRNA targeting the 3' end