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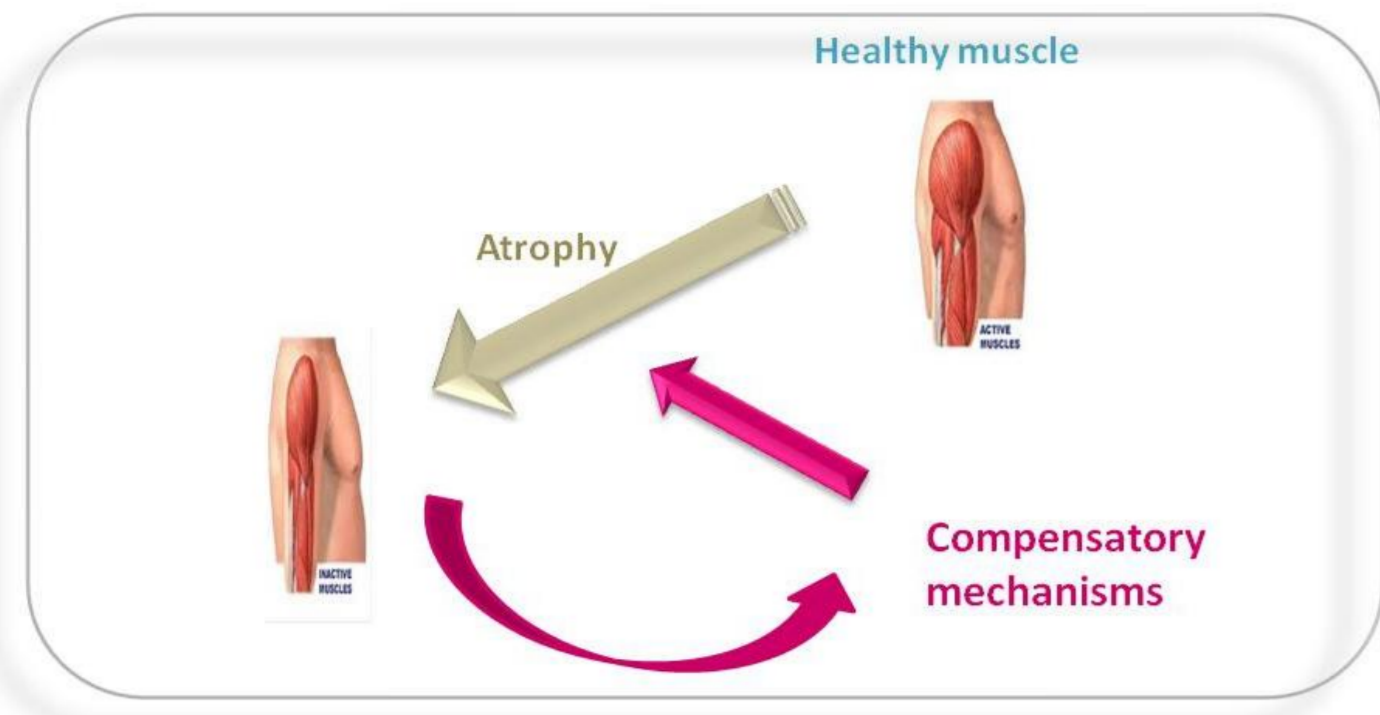
From voltage sensing to gene expression in the control of muscle mass homeostasis

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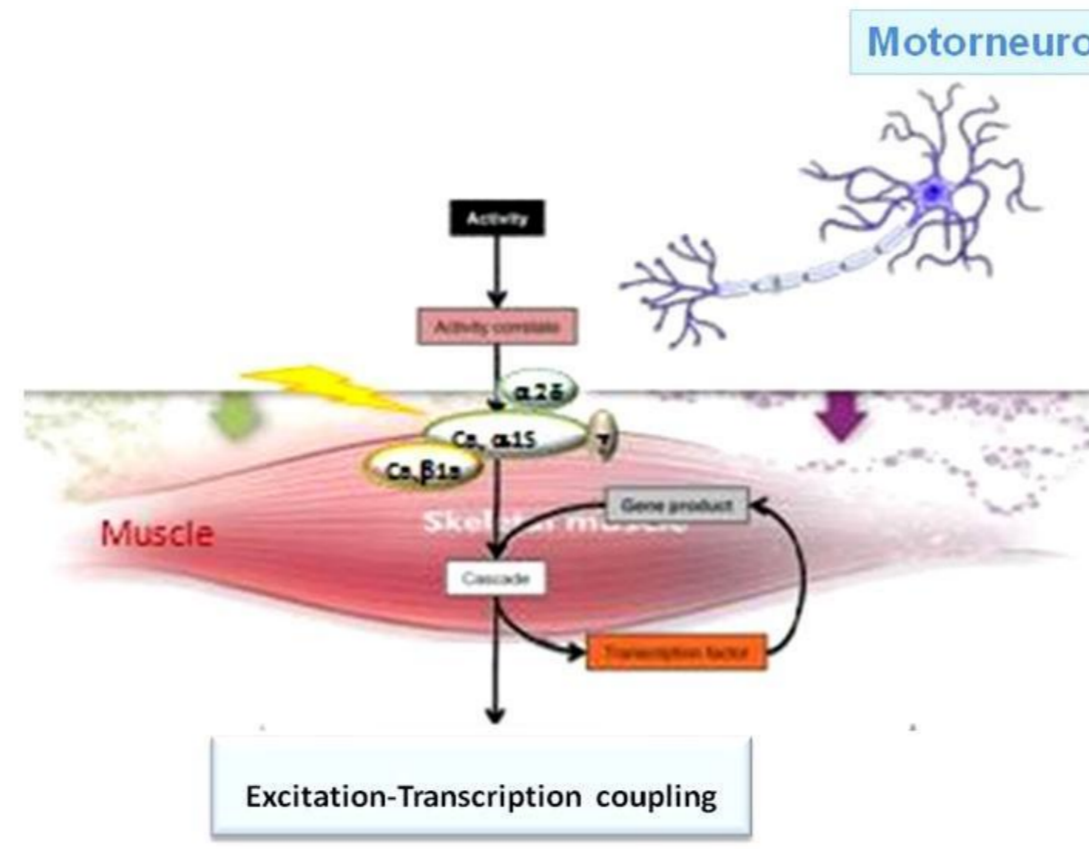
INTRODUCTION

Muscle plasticity and compensatory response

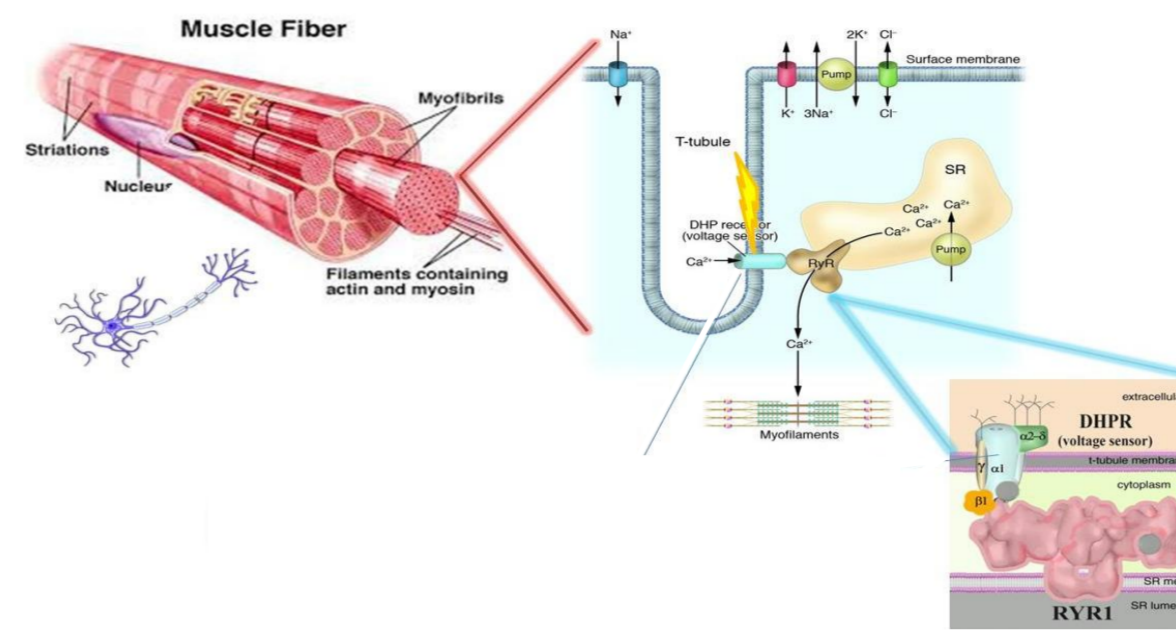


- Causes:**
- ✓ Myopathies-neuromuscular diseases
 - ✓ Nerve damage
 - ✓ Disuse-immobilization

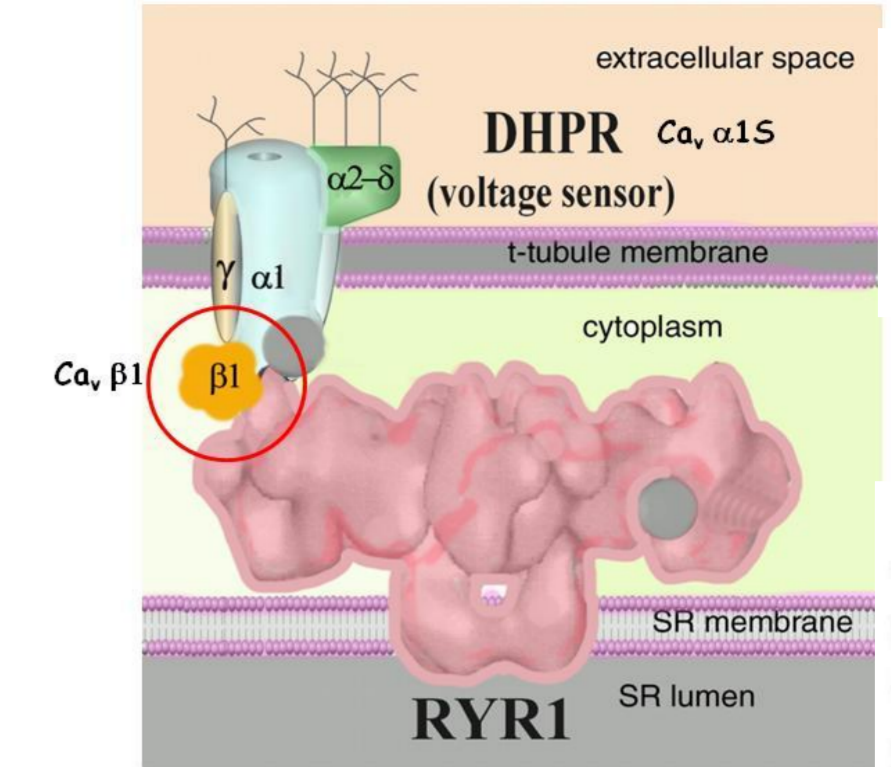
Electrical activity and Excitation-Transcription coupling



Excitation-Contraction coupling



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

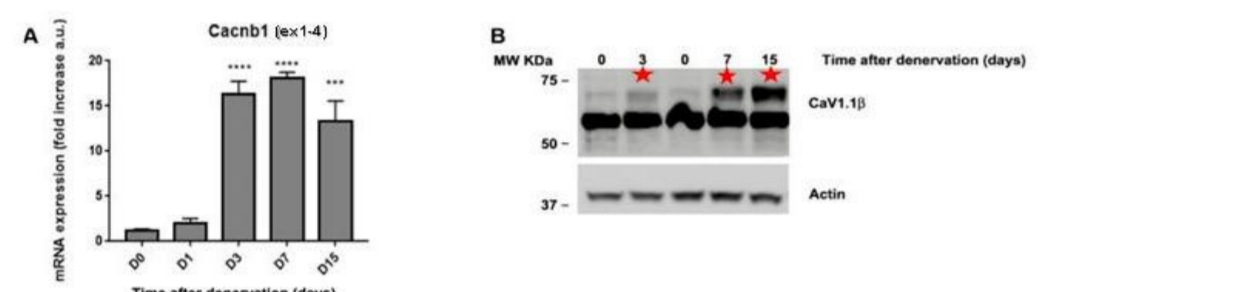


- Adult (healthy) muscle**
- Anchors $Ca_v1.1$ at the T-tubule membrane
 - Mediates $Ca_v1.1$ -RyR interaction in E-C coupling
- Developing muscle**
- Modulates myogenin expression-activity in MPCs
 - Required for neuromuscular synaptic patterning in embryo

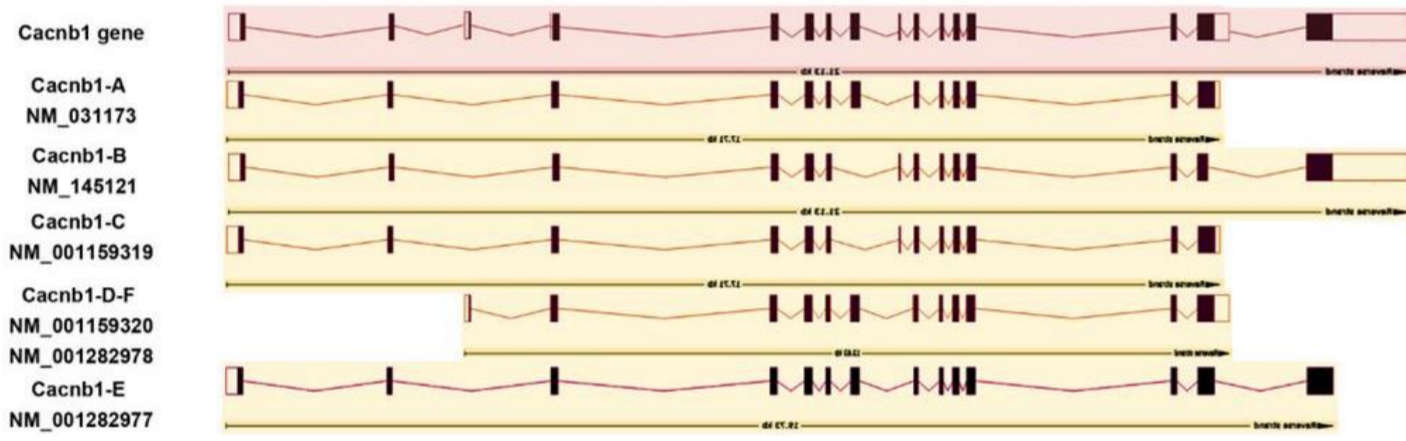
Muscle mass and fiber size undergo to rapid and significant changes according to environmental and pathological conditions. Among the various trophic signals, intrinsic muscle contractile activity, neurotransmission and neurotrophic factors are crucial components regulating the integrity of muscle mass. 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 $Ca_v1.1$. $Ca_v1.1$.

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 $Ca_v\beta1$ related to the appearance of an upper extra band: splicing isoform expression?



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

Exon level analysis: Summary

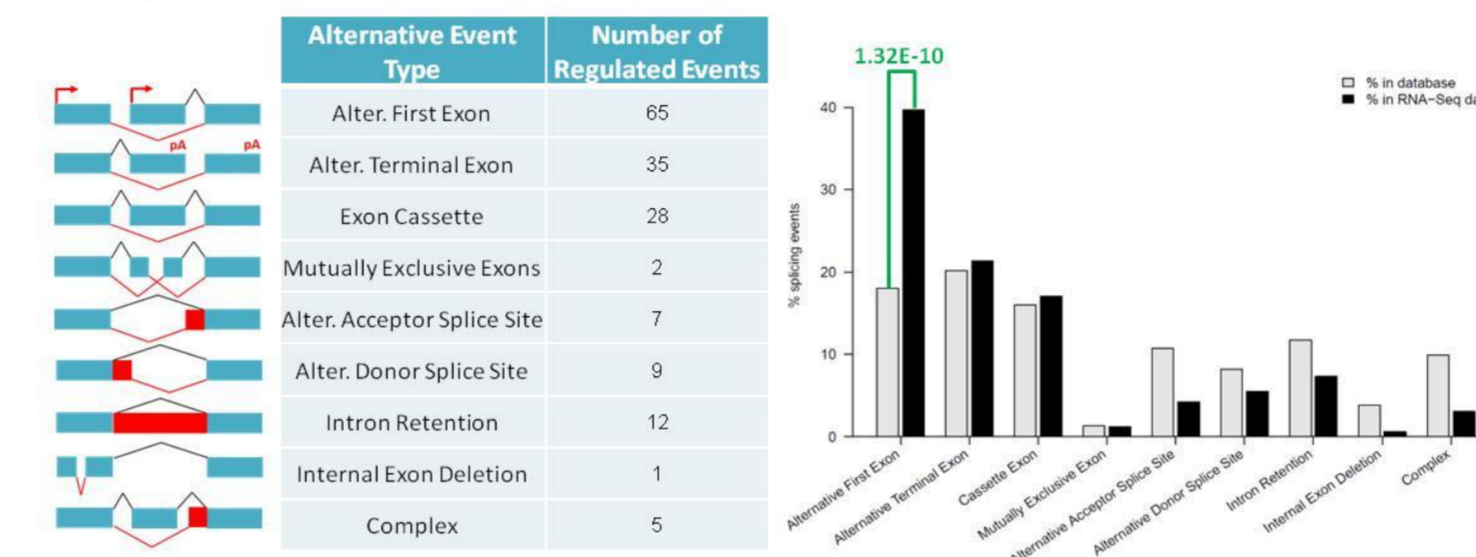
- ▶ 1,022 events/exons were found significantly regulated:
 - 164 alternative events from "Pattern" analysis;
 - 858 from "Exon" analysis only (i.e., without a priori on alternative event information).
- ▶ 706 distinct genes are involved.

Many regulated alternative events



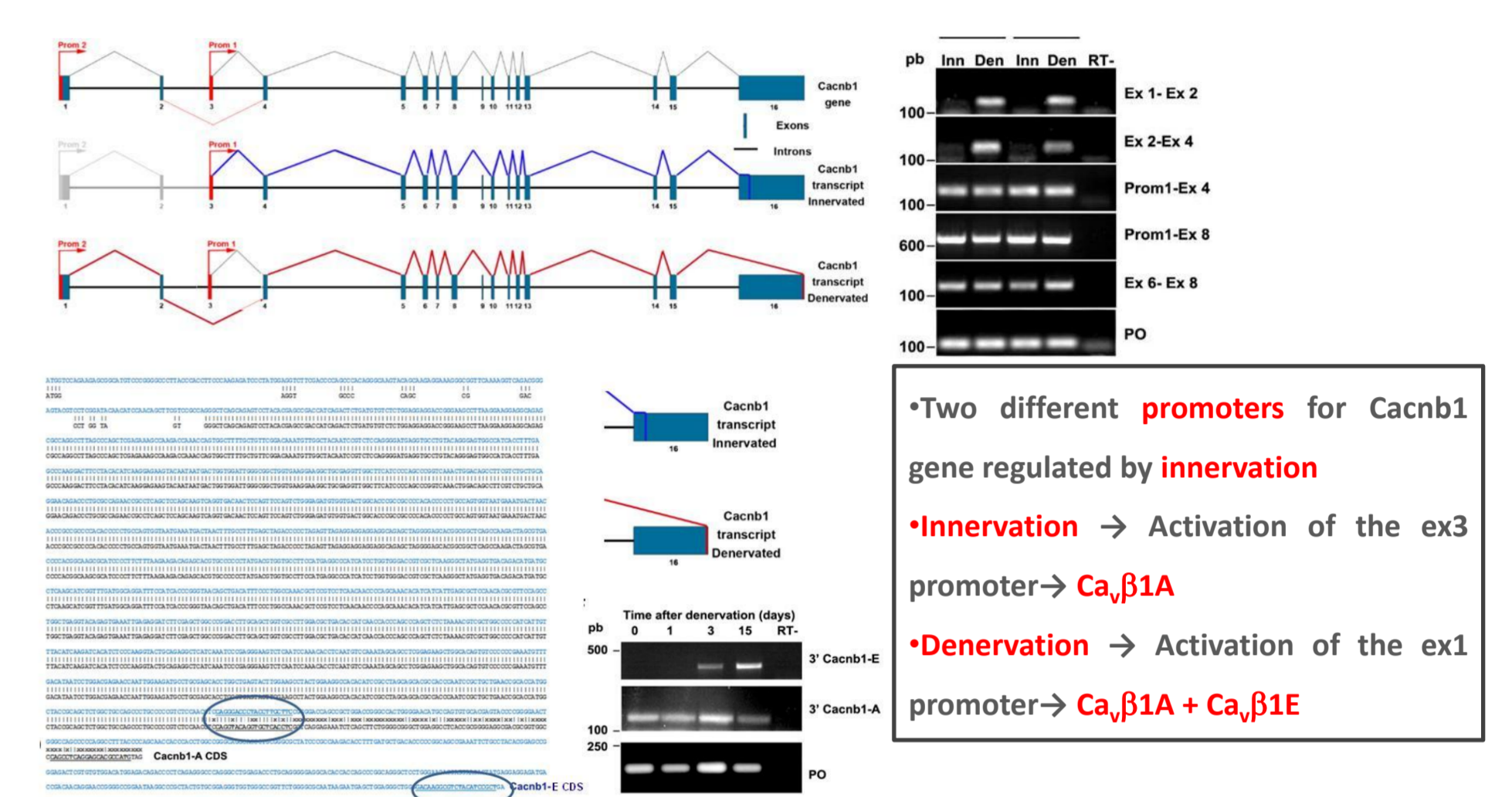
Exon level analysis: Distribution of alternative events

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



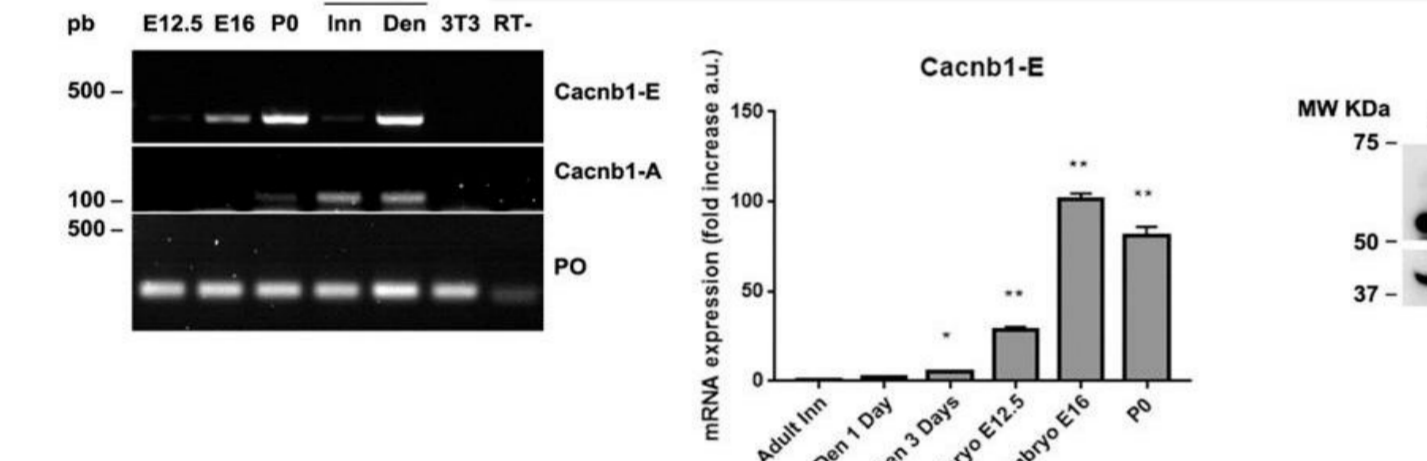
High enrichment in alternative first exons

Alternative first exon of Cacnb1 transcript in muscle after denervation

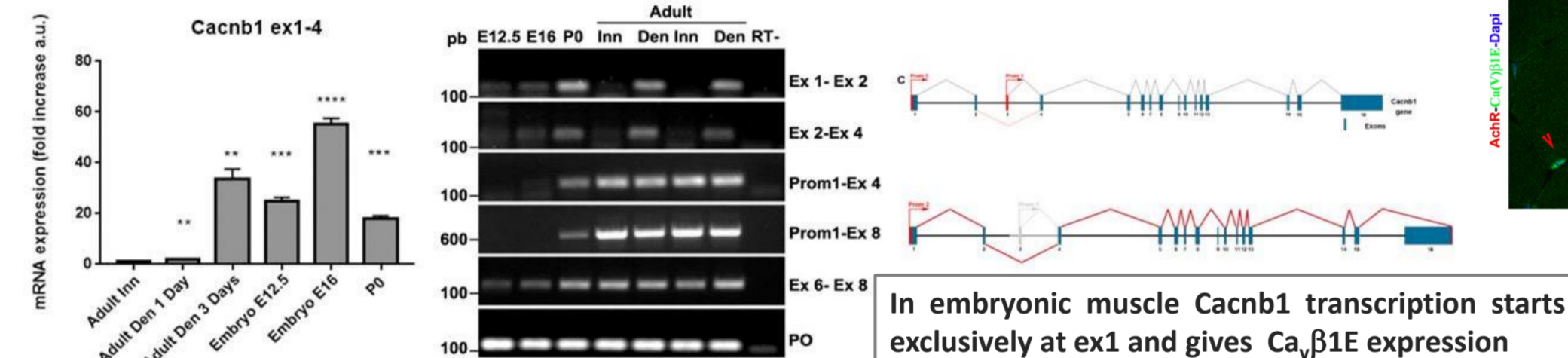


- Two different promoters for $Ca_v\beta1$ gene regulated by innervation
- Innervation → Activation of the ex3 promoter → $Ca_v\beta1A$
- Denervation → Activation of the ex1 promoter → $Ca_v\beta1A + Ca_v\beta1E$

A new Ca_vβ1 isoform: embryonic?



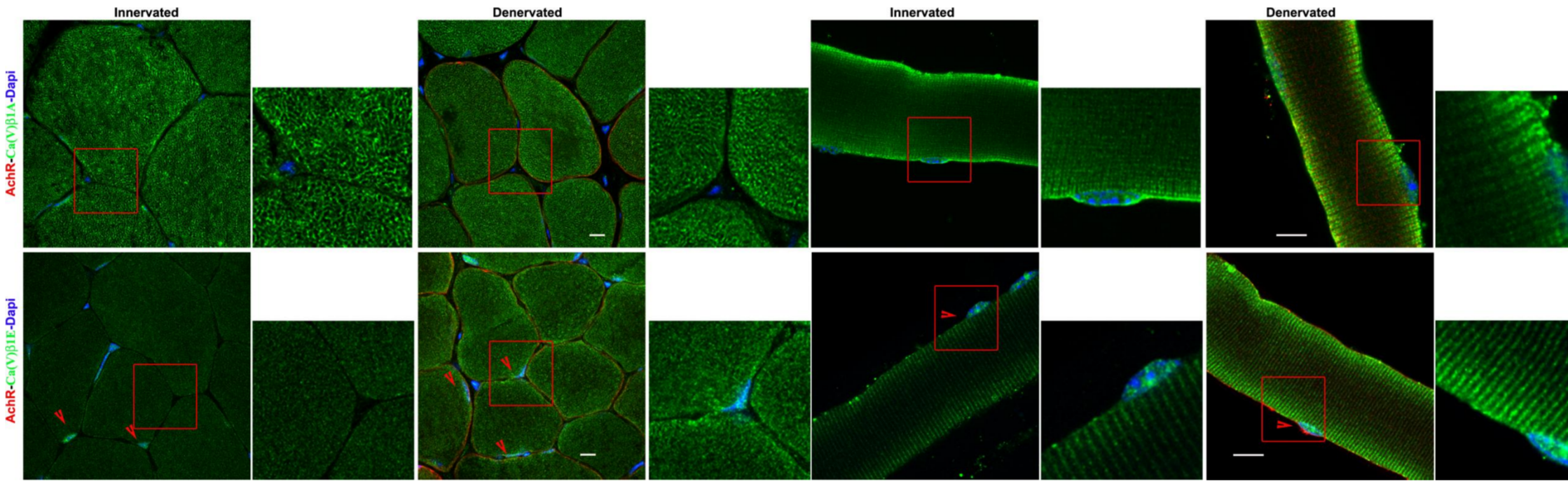
An embryonic isoform of $Ca_v\beta1$ is specifically expressed in the adult muscle after denervation



In embryonic muscle $Cacnb1$ transcription starts exclusively at ex1 and gives $Ca_v\beta1E$ expression

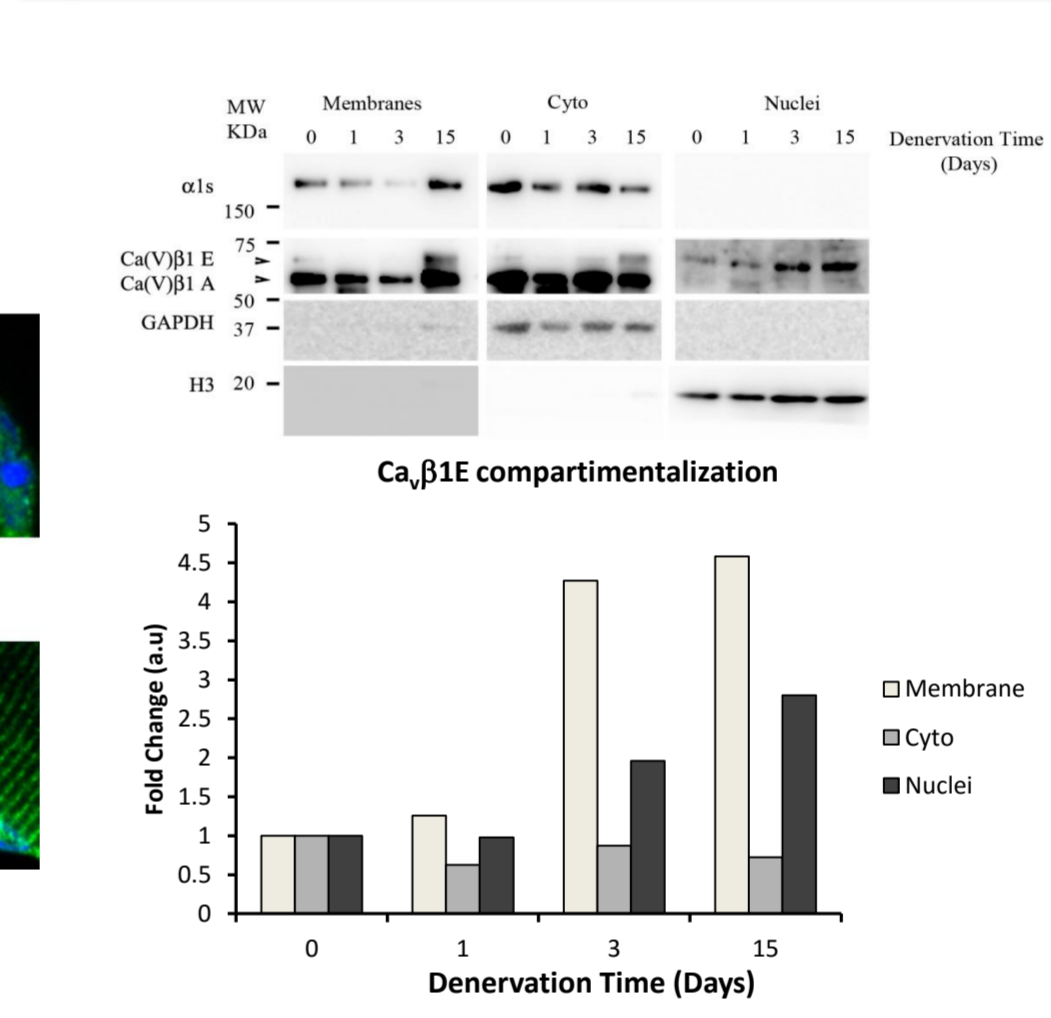
Characterization of Ca_vβ1E in adult skeletal muscle

Ca_vβ1 isoforms localization in adult skeletal muscle



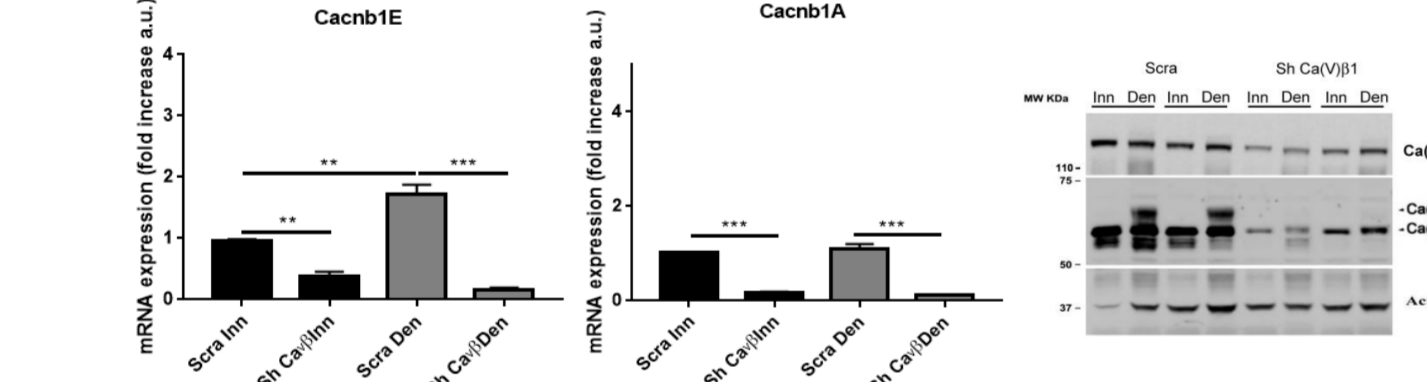
$Ca_v\beta1E$ shows different localization and nuclear staining compared to $Ca_v\beta1A$

Ca_vβ1E in subcellular fractions in innervated and denervated muscles

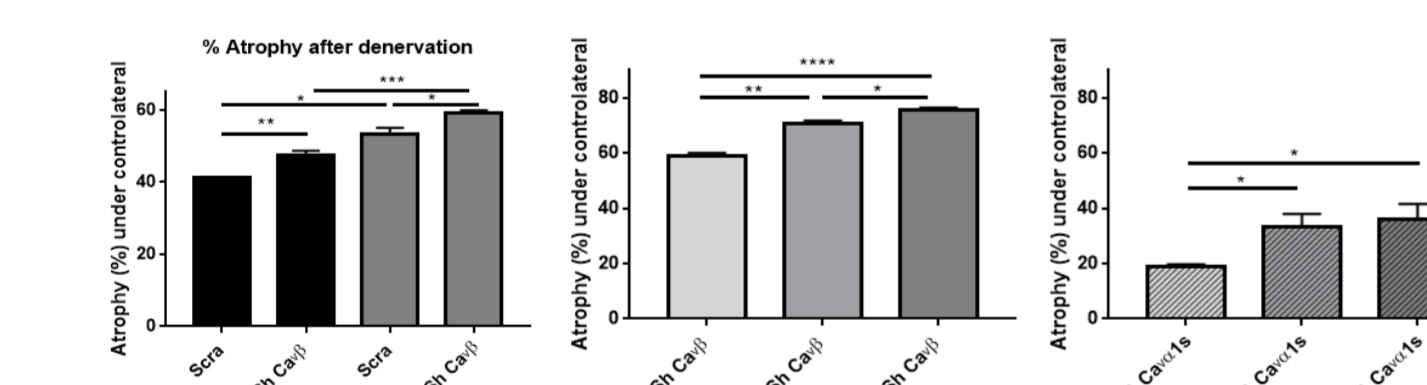


- $Ca_v\beta1E$ → embryonic $Ca_v\beta1$ variant → activation of ex1 promoter exclusively
- Intracellular localization of $Ca_v\beta1E$ looks different from $Ca_v\beta1A$ in adult
- Denervation induces re-distribution of $Ca_v\beta1E$ → Nuclear and membrane enrichment

Down regulation of Ca_vβ1 in adult skeletal muscle



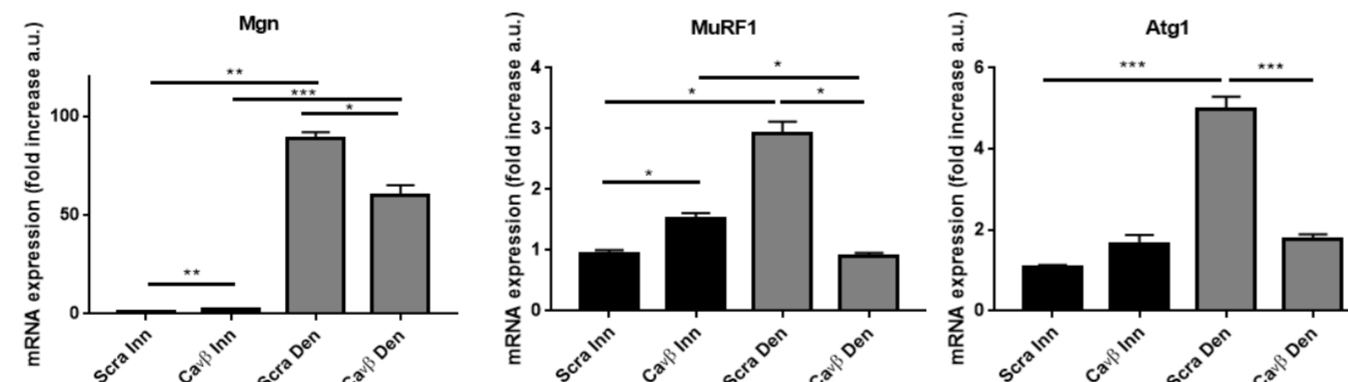
Targeting $Cacnb1A$ transcript down regulates both isoforms in adult skeletal muscles



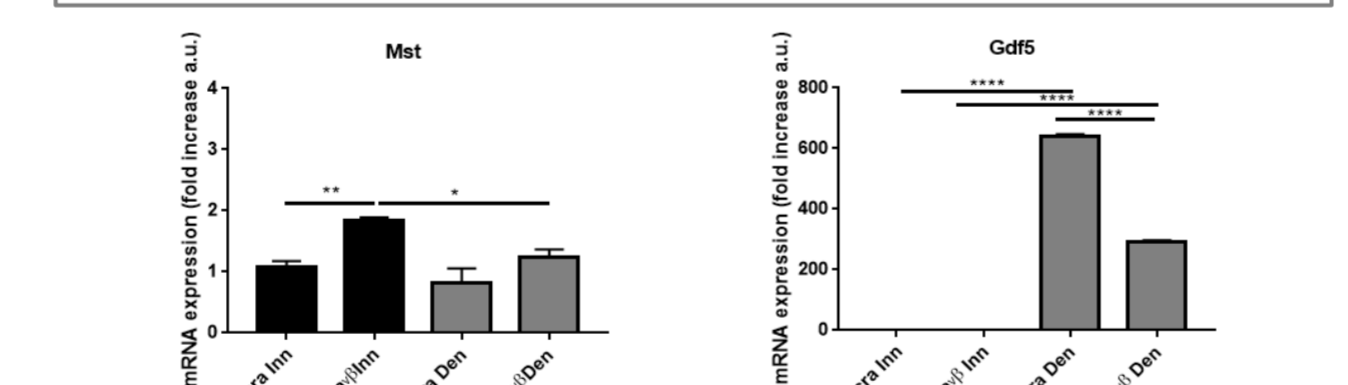
$Ca_v\beta1$ ablation dramatically exacerbates denervation atrophy and induces massive muscle mass loss independently of $Ca_v\alpha1s$ reduction

Fonction of Ca_vβ1E in adult skeletal muscle

Atrogenes and compensatory response to denervation in Ca_vβ1 KO muscles

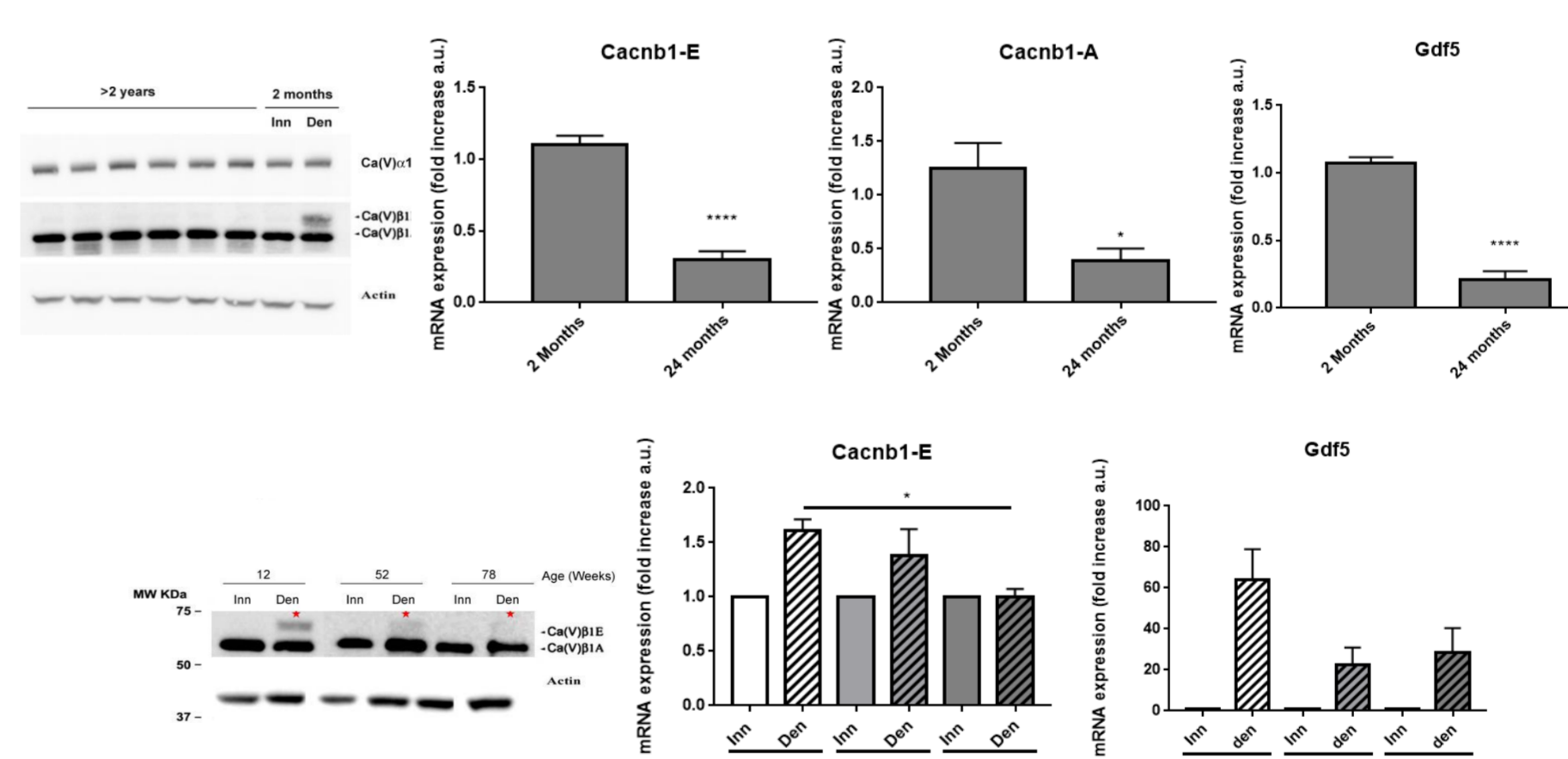


Myogenin-dependent signaling is altered by $Ca_v\beta1$ down-regulation



The loss of compensatory response to muscle waste in the absence of $Ca_v\beta1$ is probably dependent on increased myostatin and decreased Gdf5/BMP14 signaling

Ca_vβ1E in ageing muscle



In ageing muscle $Ca_v\beta1$ is strongly downregulated in parallel to decreased Gdf5/BMP14 expression: the origin of sarcopenia?

- $Ca_v\beta1A$ shRNA → down-regulation of $Ca_v\beta1E$ and $Ca_v\beta1A$
- $Ca_v\beta1$ KO → muscle mass loss and exacerbation of atrophy
- $Ca_v\beta1$ KO → myogenin signaling altered; Gdf5/Gdf8 signaling altered
- Ageing muscle → physiological $Ca_v\beta1$ KD

Conclusion and perspectives

What we know:

- Electrical activity alteration → embryonic $Ca_v\beta1E$ expression by alternative first exon event
- $Ca_v\beta1A$ shRNA → down-regulation also $Ca_v\beta1E$ and splicing → $Ca_v\beta1E$ expression depends on $Ca_v\beta1A$
- $Ca_v\beta1A/E$ KO → muscle mass loss and exacerbation of atrophy
- $Ca_v\beta1A/E$ KO → myogenin signaling altered; Gdf5/Mst signaling altered
- Ageing muscle → physiological $Ca_v\beta1$ KD and splicing; Gdf5 signaling altered
- Overexpression of $Ca_v\beta1E$ → no impact on $Ca_v\beta1A$ expression

What we must know:

- $Ca_v\beta1E$ effector of compensation? → overexpression of $Ca_v\beta1E + sh Ca_v\beta1A$ (ongoing in ageing model)
- $Ca_v\beta1A$ effector of compensation? → overexpression of $Ca_v\beta1A$ in sh $Ca_v\beta1A$
- Role of alternative promoter in $Ca_v\beta1$ fonction and expression → sh $Ca_v\beta1$ ex2 versus sh $Ca_v\beta1E$
- Is Gdf5 the key mediator of $Ca_v\beta1$ effect on muscle mass? → overexpression of Gdf5/BMP14 in sh $Ca_v\beta1$
- Can $Ca_v\beta1A$ act on its own promoter and/or Gdf5 promoter? → Luciferase-based assay

Perspectives:

- Role of $Ca_v\beta1$ in sarcopenia → overexpression of $Ca_v\beta1$ in ageing model (ongoing for $Ca_v\beta1E$)
- $Ca_v\beta1$ in reinnervation and disease models (ALS, SMA)
- $Ca_v\beta1$ in human neuromuscular disease/sarcopenia
- $Ca_v\beta1$ in myogenesis /embryogenesis

$Ca_v\beta1E$ over-expression does not modify $Ca_v\beta1E$ endogenous expression in adult muscle and can be rescued by a specific shRNA targeting the 3' end