

Elucidation of sAnk1 as a transcriptional and/or epigenetic regulator of sarcoplasmic reticulum formation during myogenic development

Introduction

- Small ankryn 1 (sAnk1) is an alternatively spliced product of ANK1, abundantly expressed in striated muscle. sAnk1 predominantly localizes to the sarcoplasmic reticulum (SR) where it directly interacts with obscurin.

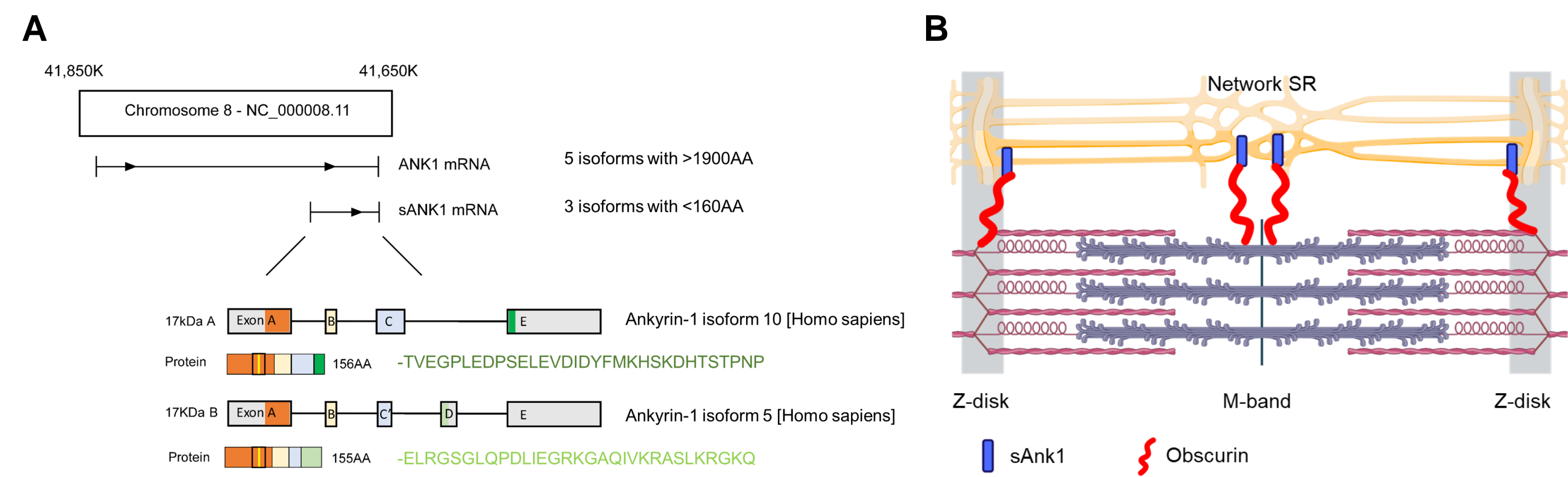


Figure 1. Schematic representation of A) sAnk1 mRNA and protein, and B) its subcellular localization in the sarcomere.

- Partial knockdown of sAnk1 in skeletal muscle results in altered longitudinal SR structure [1], akin to what is observed in obscurin knockout muscles [2].

Table 1. phosphorylation level alteration (site-specific)

Gene	P Site	Domain	Ratio	P-value
Ank1	Ser55	Non-modular	1.39429	0.022526
Myom2	Ser76	Non-modular	0.688883	0.0476216
Cds2	Ser32	Non-modular	1.45837	0.006472
Klhdc7a	Ser361	Non-modular	2.18531	0.01033
Prkra	Ser18	Non-modular	1.24809	0.035211
Emi1	Ser128	Non-modular	0.913426	0.039107

- Phosphoproteomic analysis of heart tissue from obscurin Δ Ig58/59 mice revealed altered phosphorylation of sAnk1 at serine 55 (Table 1).

- The Ser55 sAnk1 phosphosite was confirmed in 5 other references (Table 2).

Table 2. Site information from PhosphoSitePlus Database

Gene	ACC_ID	Organism	P-site	Reference
ANK1	P16157-17	Human	S55-p	[3],[4]
ANK1	P16157-17	mouse	S55-p	[5],[6],[7]

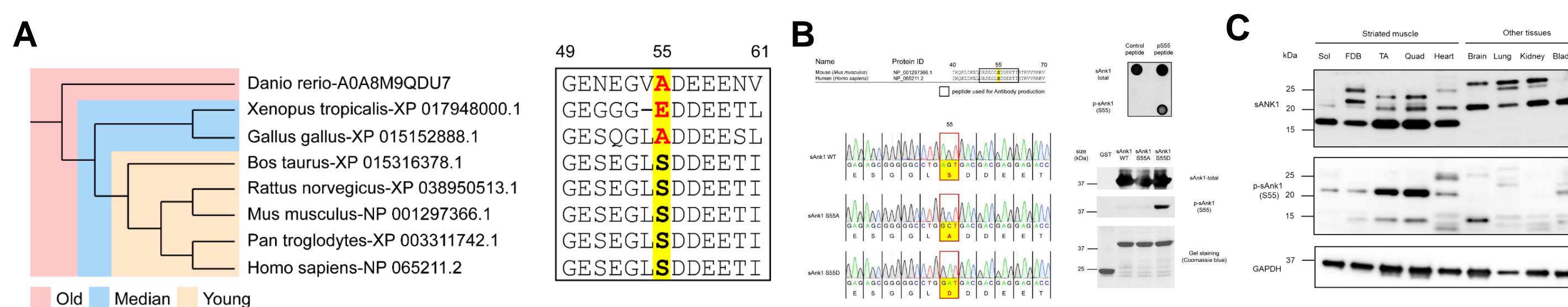


Figure 2. Phosphorylation of Ser55 on sAnk1 is conserved in mammals and is striated muscle specific. **A)** Phylogenetic tree and multiple sequence alignment of sAnk1 from different species revealed that sequences near Ser55 are well conserved in mammals. **B)** We generated a custom antibody against p-sAnk1 Ser55 and tested its specificity against control and phosphorylated sAnk1 peptide, along with recombinant wild-type, phospho-ablated, and phospho-mimetic sAnk1 proteins. **C)** p-sAnk1 is abundantly expressed in striated muscle tissues but not in brain, lung, or kidney.

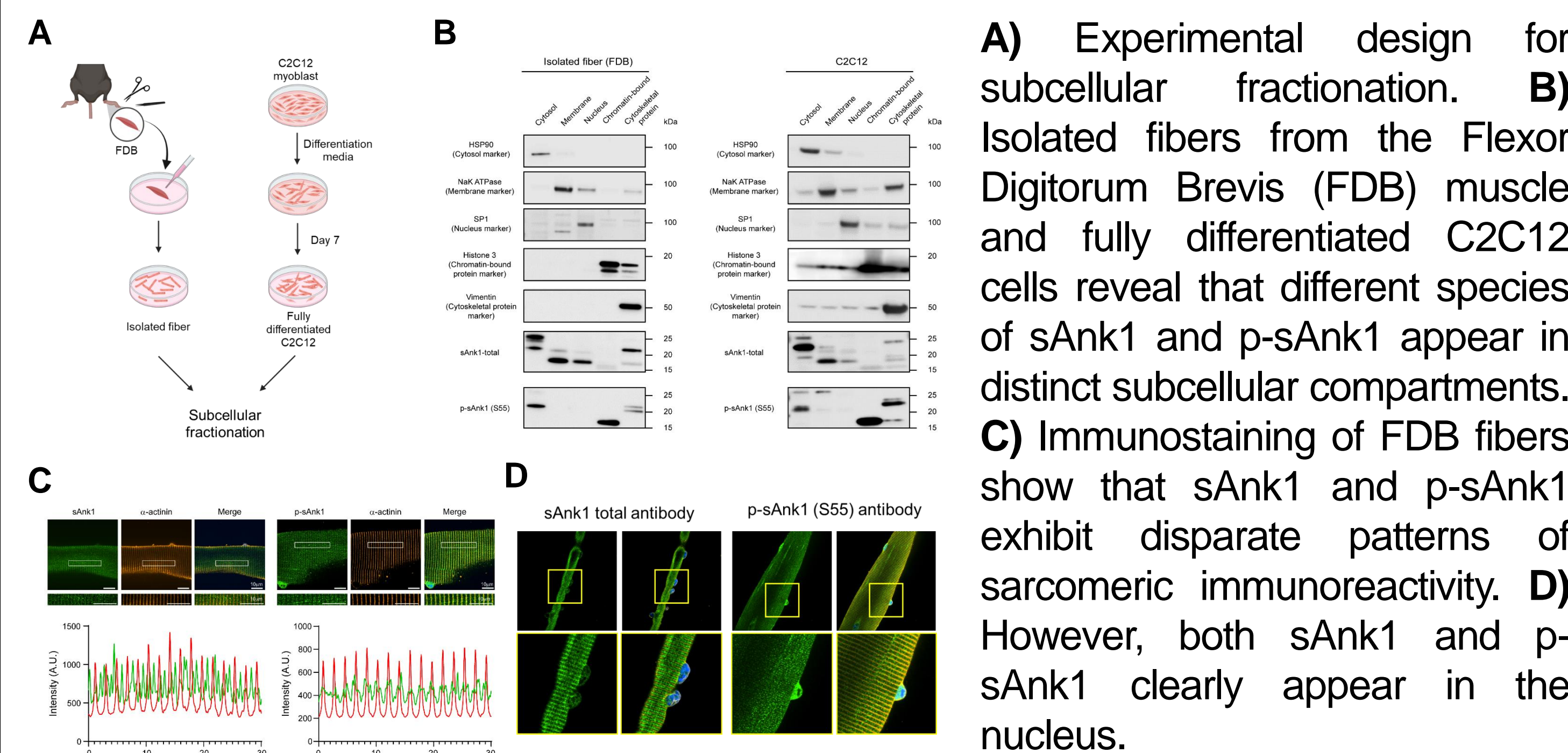
Hypothesis

1) sAnk1 and its phosphorylation are upregulated during the process of myogenic differentiation.

2) The role of sAnk1 in SR network formation emerges during striated muscle myogenesis.

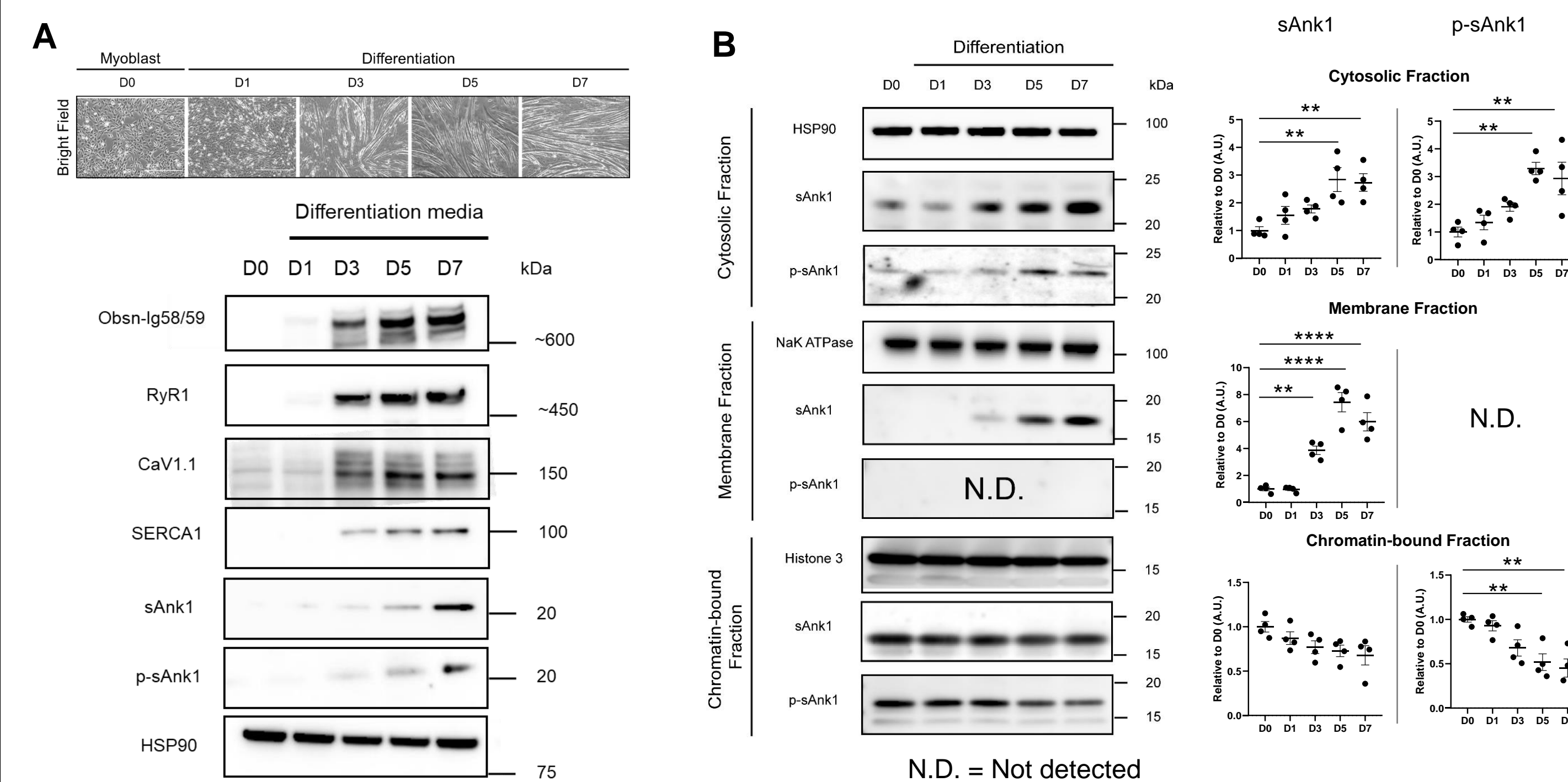
Results

Figure 3. Subcellular fractionation reveals the presence of sAnk1 in nuclear and chromatin-bound fractions.



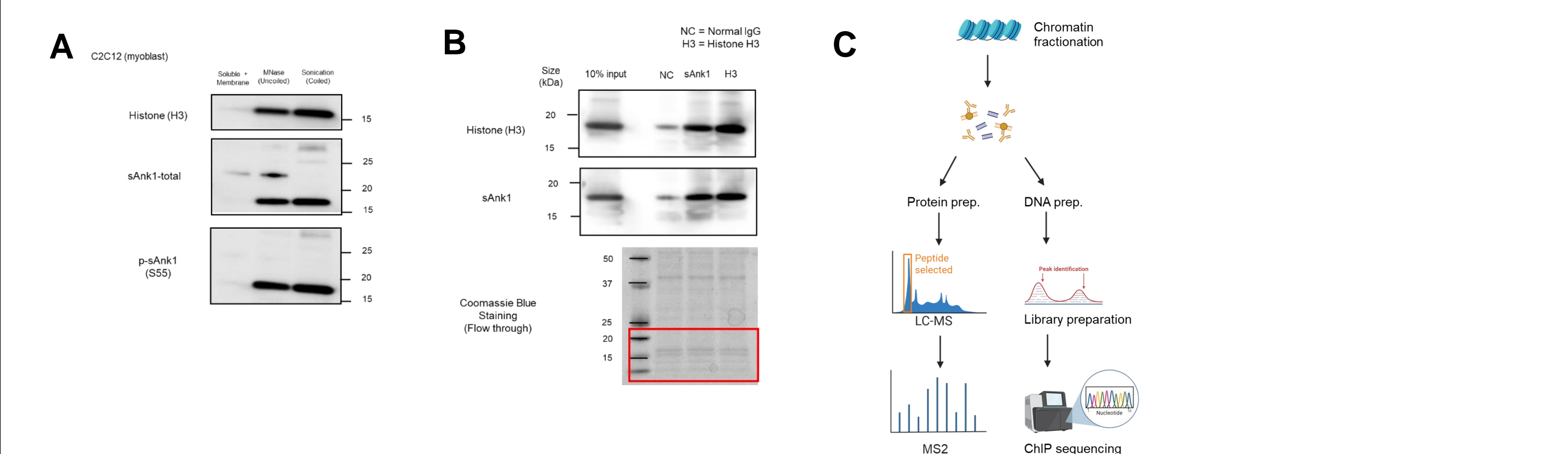
A) Experimental design for subcellular fractionation. **B)** Isolated fibers from the Flexor Digitorum Brevis (FDB) muscle and fully differentiated C2C12 cells reveal that different species of sAnk1 and p-sAnk1 appear in distinct subcellular compartments. **C)** Immunostaining of FDB fibers show that sAnk1 and p-sAnk1 exhibit disparate patterns of sarcomeric immunoreactivity. **D)** However, both sAnk1 and p-sAnk1 clearly appear in the nucleus.

Figure 4. sAnk1 and p-sAnk1 expression changes during myogenic differentiation.



A) During myogenic differentiation, the expression of obscurin, and SR proteins RyR1, SERCA1, and sAnk1 are gradually upregulated. **B)** Temporal analysis of sAnk1 and p-sAnk1 pools in cytosolic, membrane, and chromatin-bound compartments reveals that while sAnk1 expression increases in cytosolic and membrane fractions during differentiation, chromatin-bound p-sAnk1 decreases.

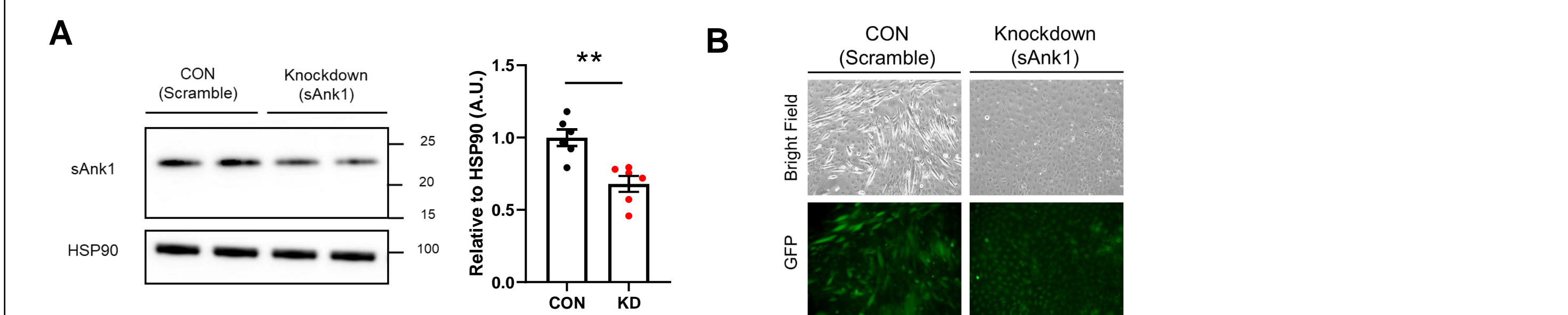
Figure 5. Immunoprecipitation of sAnk1 with chromatin fraction revealed its potential roles in transcriptional and/or epigenetic role through histone binding



A) sAnk1 and p-sAnk1 are abundantly expressed in the heterochromatin fraction. **B)** Immunoprecipitation experiments reveal that sAnk1 and histone 3 bind to one another. **C)** Schematic drawing for our ongoing experiments.

Results

Figure 6. Knockdown of sAnk1 impairs myogenic differentiation



A) Lentiviral transduction of sAnk1 shRNA induced a ~40% knockdown of sAnk1 in undifferentiated C2C12 myoblast. **B)** CON (scrambled) lentivirus did not affect the differentiation process of C2C12 myoblasts when growth media was switched to the differentiation media. However, sAnk1 shRNA lentivirus significantly impaired the myogenic fusion of myoblasts during differentiation.

Summary and Current Directions

- sAnk1 and p-sAnk1 expression are upregulated during myogenic differentiation in cytosol and membrane fractions of striated muscle.
- Differentiation is accompanied by a decrease in p-sAnk1 expression within the chromatin-bound fraction, implicating that p-sAnk1 may play a potential transcriptional and/or epigenetic role through histone 3 binding.
- sAnk1 knockdown significantly impairs the myogenic fusion of myoblasts.
- To understand why sAnk1 is critical during differentiation, we will evaluate the temporal expression of myogenic markers in control and sAnk1 shRNA-treated myoblasts.
- To investigate the role of sAnk1 and p-sAnk1 in the nucleus, proteomics and ChIP sequencing will be used to validate its binding partners and binding sites.

References

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