Abstract 500:

# Gene Therapy Rescues Disease Phenotype in the Oculopharyngeal Muscular Dystrophy Mouse Model

George Dickson, Alberto Malerba, Pierre Klein, Susan Jarmin, Houria Bachtarzi, Arnaud Ferry, Gillian Butler-Browne, **David Suhy**, Michael Graham, Capucine Trollet

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# Clinical features of OPMD

Rare autosomal dominant inheritance

- 1:100,000 (Europe)
- As high as 1:600 in specific populations
- Founder effect in Quebec, Canada

Typically onset occurs in the fifth to early sixth decade of life

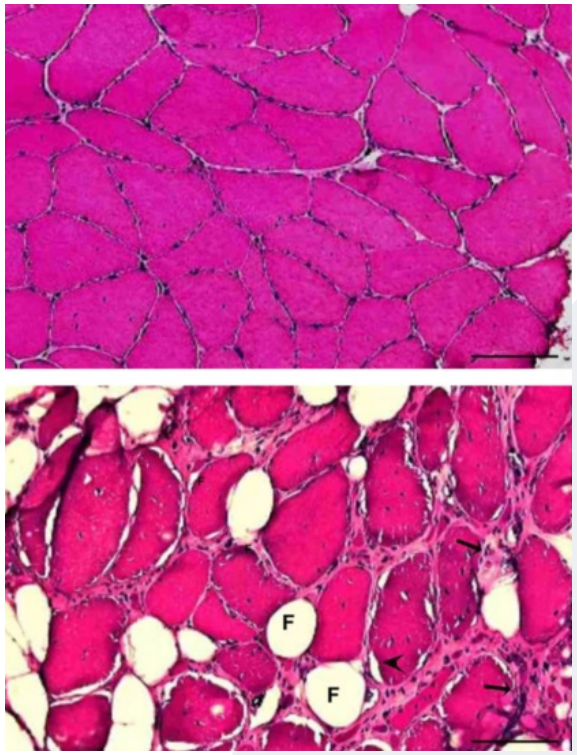
Characterised by:

- eyelid drooping (ptosis)
- swallowing difficulty (dysphagia)
- proximal limb weakness
- death due to aspiration pneumonia & malnutrition

Histopathology

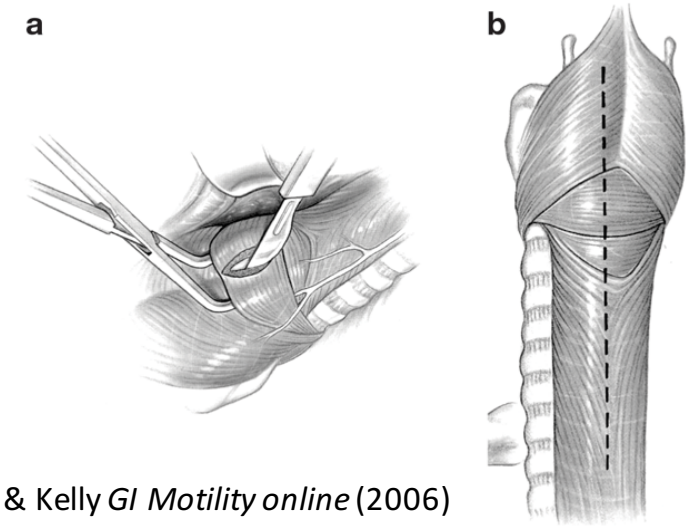
- Decrease of muscle fiber number
- Variation in the size of muscle fibers
- Fibrosis (connective tissue)

Raz et al., *BMC Neurology* 2013, **13**:70



# Current treatment

- **Cricopharyngeal myotomy :** a surgical intervention to improve swallowing, but does not correct the progression of the disease since it has a genetic basis.




Chu & Kelly *GI Motility online* (2006)

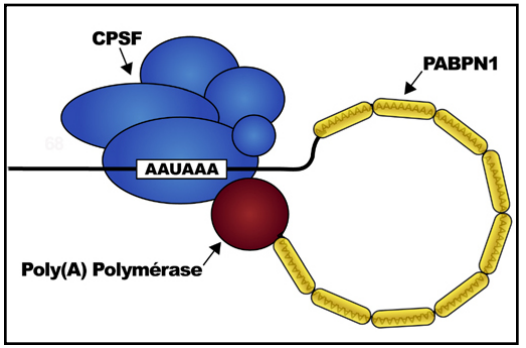
- **Stem cell transplants**: grafting of autologous myoblasts isolated from unaffected quadriceps or sternocleidomastoid muscles into the esophagus of the patient. Some short term efficacy but transplanted cells still carry the genetic defect.

*Molecular Therapy* (2014); **22** 1, 219–225

# Genetic basis of OPMD: expansion of the poly-alanine tract within PABPN1

**PABPN1:**

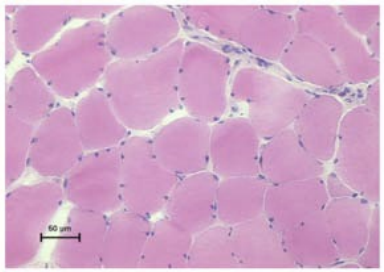
- a ubiquitous factor that promotes interaction between the poly(A) polymerase and CPSF (cleavage and polyadenylation specificity factor) and thus controls the length of mRNA poly(A) tails, mRNA export from the nucleus, and alternative poly(A) site usage.



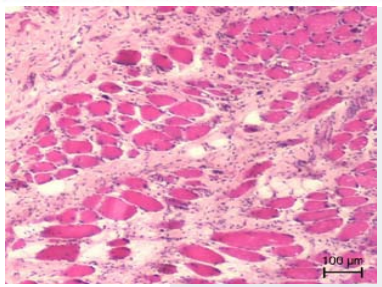

**In OPMD:**

- a genetic mutation results in trinucleotide repeat expansion within exon 1 of PABPN1 and results in an expanded poly-alanine tract at the N-terminal end of PABPN1.

WT ATG $(GCG)_6$----------$(GCA)_3$GCG GGG GCT GCG..
MUT ATG $(GCG)_6$ $(GCG)_{1-7}$ $(GCA)_3$GCG GGG GCT GCG…--



Non-affected



Affected

# INIs, the hallmark of OPMD

Expansion of the short (GCG) trinucleotide repeat in the coding sequence of PABPN1

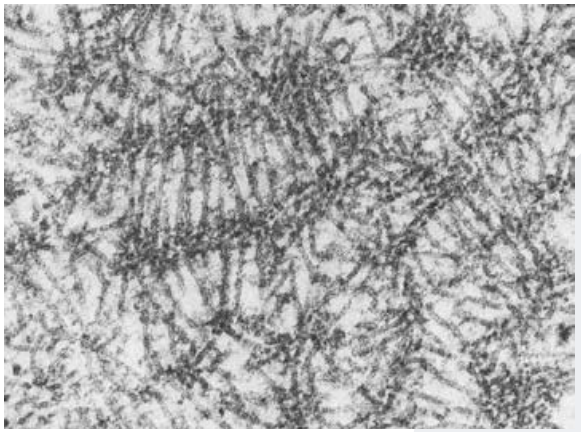
↓

The mutated protein has 11-17 alanines in the N-Terminal domain instead of 10

↓

Protein aggregation forms intranuclear inclusions (INIs)

- Tubular filaments
- Resistant to degradation
- INIs found in the nuclei of skeletal muscle fibres (both affected and non-affected)



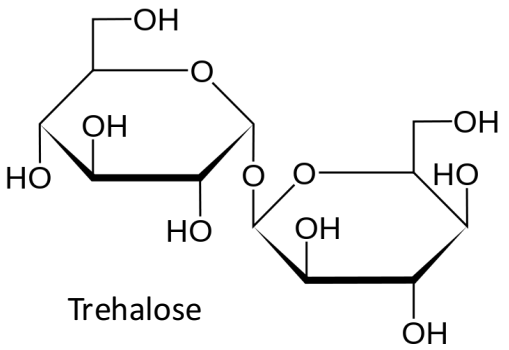
*Tomé & Fardeau, 1980*

# Other therapies under development

## Trehalose

- BioBlast is in Phase II clinical testing of Cabaletta, a chemical chaperone that prevents pathological aggregation of proteins within cells. The active ingredient Trehalose, a disaccharide of glucose, is thought to induce autophagy and stimulate intracellular clearance of the protein aggregates.

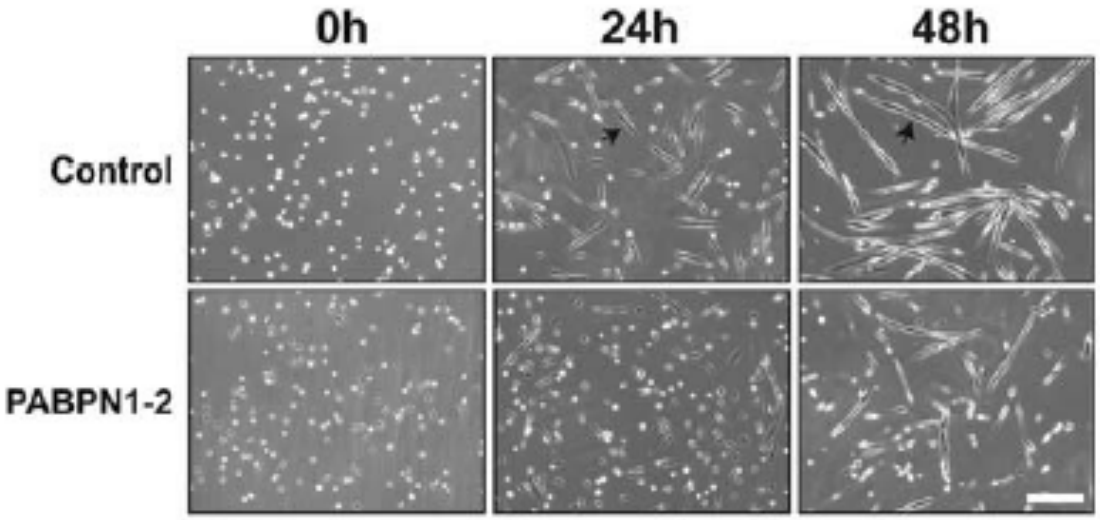
- The drug is administered weekly by intravenous infusion.



Trehalose

# Development of a ddRNAi-based therapeutic for OPMD

# A disease that is more than nuclear aggregation: PABPN1 is required to maintain muscle function




*Human Molecular Genetics, 2010, Vol. 19, No. 6* **1058–1065**
*doi:10.1093/hmg/ddp569*
*Advance Access published on December 24, 2009*

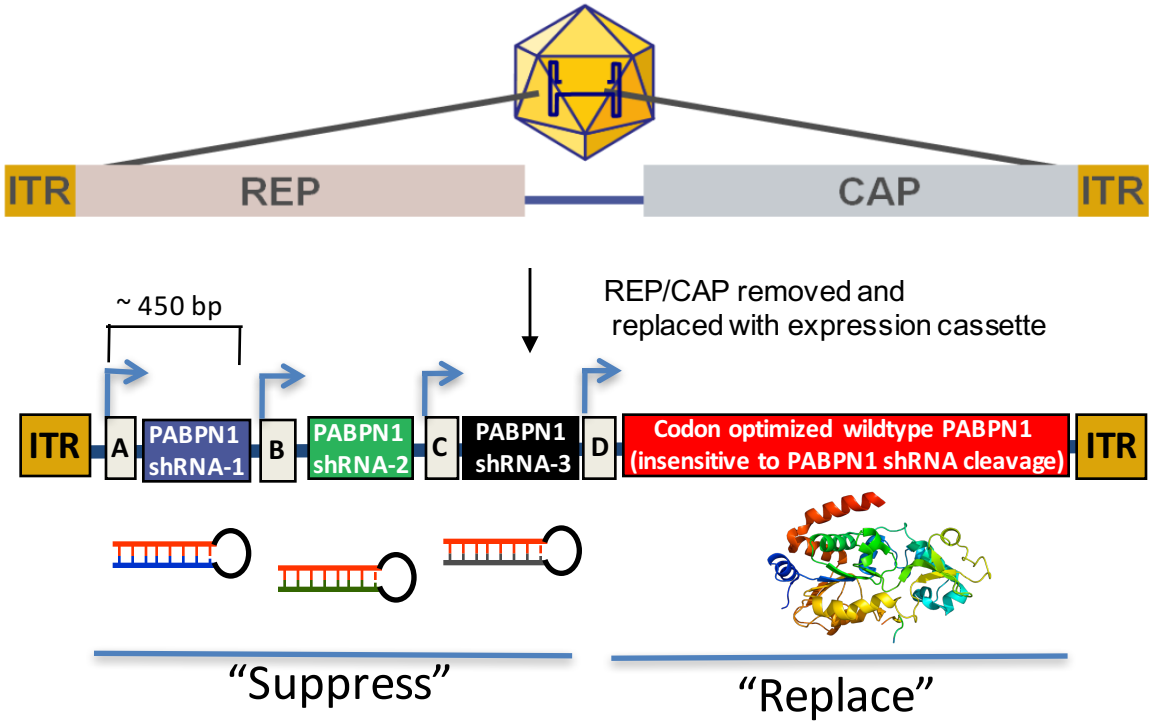
**Loss of nuclear poly(A)-binding protein 1 causes defects in myogenesis and mRNA biogenesis**

Luciano H. Apponi[1], Sara W. Leung[2], Kathryn R. Williams[1], Sandro R. Valentini[3], Anita H. Corbett[2,*] and Grace K. Pavlath[1,*]

- PABPN1 is required for normal myoblast proliferation and differentiation
- PABPN1 is required for proper polyadenylation in muscle cells
- PABPN1 is required for proper poly(A) RNA export from the nucleus

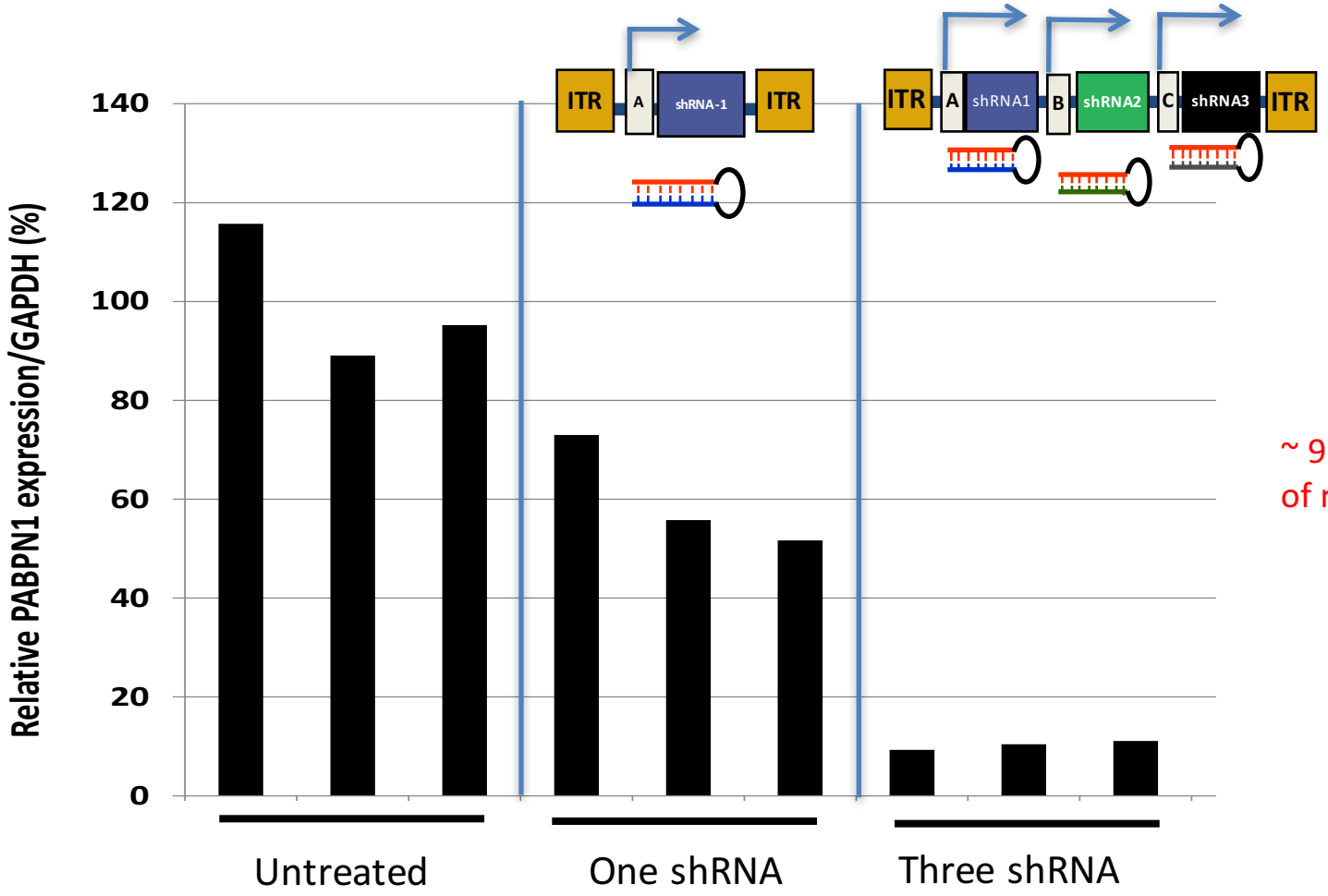
****Thus, an effective treatment likely requires maintaining endogenous function in addition to eliminating mutant protein aggregates**

# A ‘Suppress and Replace’ approach



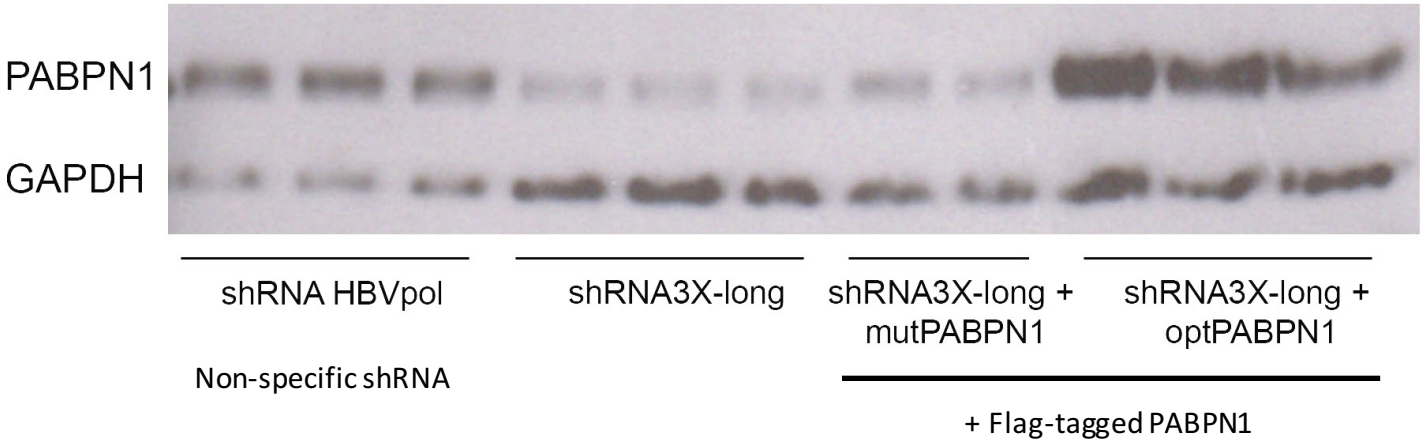

- Non-integrating, non-pathogenic viral delivery system
- To date, AAV has been used in over 137 clinical trials with excellent safety record
- Sustained expression (years) following single injection

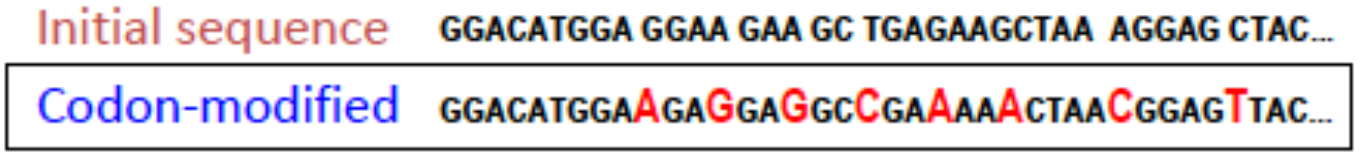
# Use of multi-targeting properties of ddRNAi knockdown of PABPN1 in a 293T cell line




~ 90% knockdown of mutant PABPN1

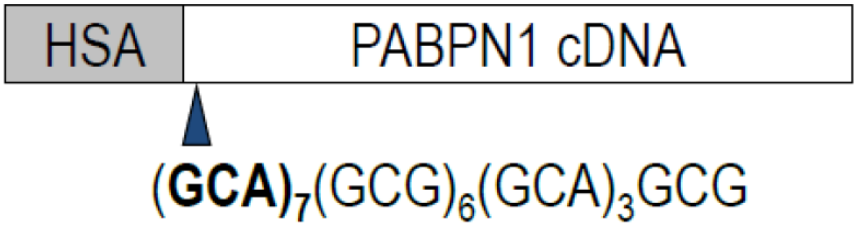
# Expression of “codon optimized” wildtype PABPN1 is not knocked down by shRNA




**Codon modified wildtype PABPN1 is resistant to knockdown with shRNA

# An animal model of OPMD: The 'A17' mouse




- Transgenic mouse: express a mutated bovine PABPN1 driven by the human skeletal actin promoter in addition to the endogenous PABPN1
- Recapitulates severe muscle atrophy
- Mimics many of the disease pathologies:
  - Progressive muscle weakness/ Atrophy
  - Fibrosis
  - Mitochondrial / Ubiquitin-Proteasome defects
  - Muscles contain intranuclear inclusions

*Nature Medicine* **11**, 672 - 677 (2005)
Published online: 1 May 2005 | doi:10.1038/nm1242

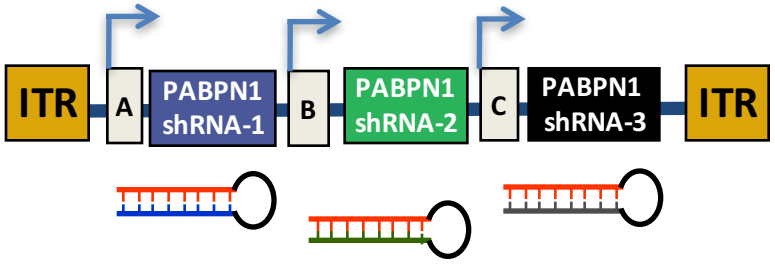
Doxycycline attenuates and delays toxicity of the oculopharyngeal muscular dystrophy mutation in transgenic mice

Janet E Davies[1], Lin Wang[1], Lourdes Garcia-Oroz[1], Lynnette J Cook[1], Coralie Vacher[1], Dominic G O'Donovan[2] & David C Rubinsztein[1]

# Assessment of efficacy in the A17 mouse model

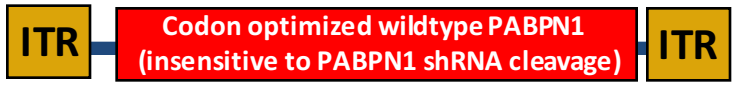
Mice TA Muscles injected with:

2.5e10 vg scAAV8-shRNA3X
1.3e11 vg ssAAV9-optPABN1
Analyses at week 18 post injection

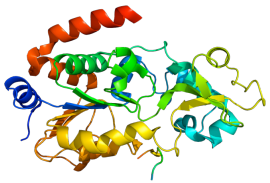



shRNA3x

AND / OR

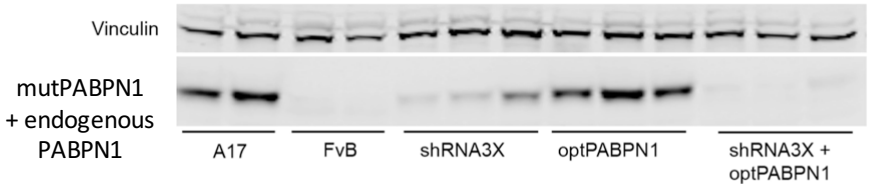



optPABPN1

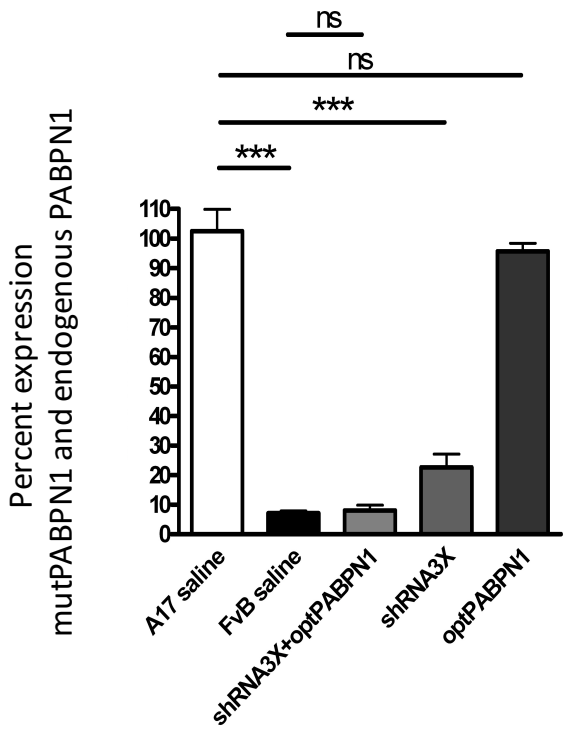


# Analyses of mutant PABPN1 protein levels and codon optimized PABPN1 RNA in A17 treated mice

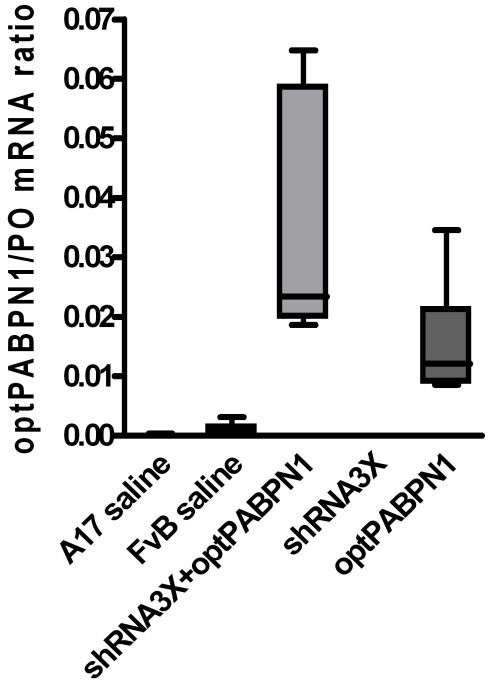
## Western Blot confirms knockdown of mutPABPN1



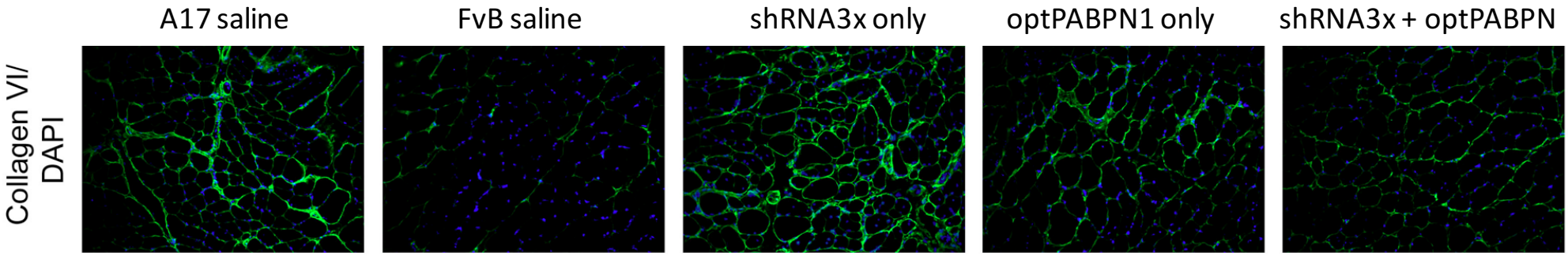

* Antibody does not recognize optPABPN1

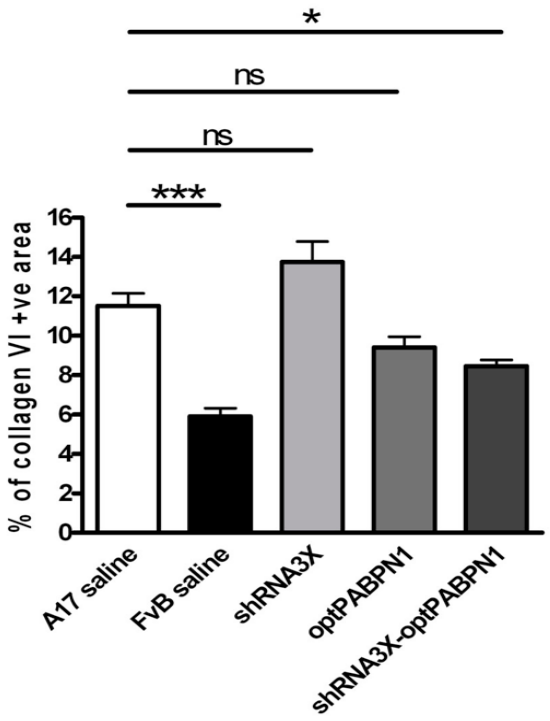



## QPCR confirms expression of optPABPN1



# Assessment of Collagen VI, a marker of fibrosis, in transverse muscle sections of treated A17 mice

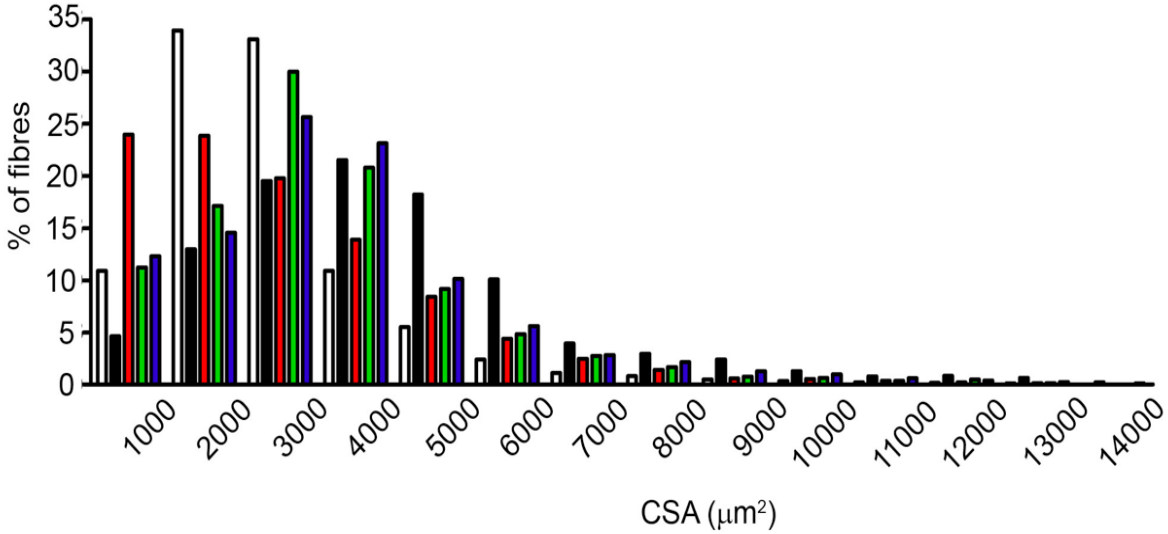


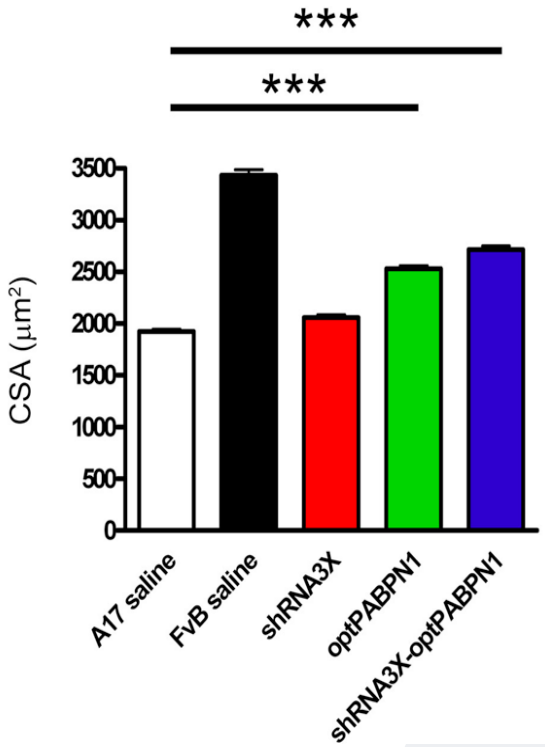




**Expression of codon optimized PABPN1 with concomitant knockdown of mutant PABPN1 reduces muscle fibrosis.**

# Partial reversal of atrophy: a cross section analysis of muscle fibers in treated A17 mice

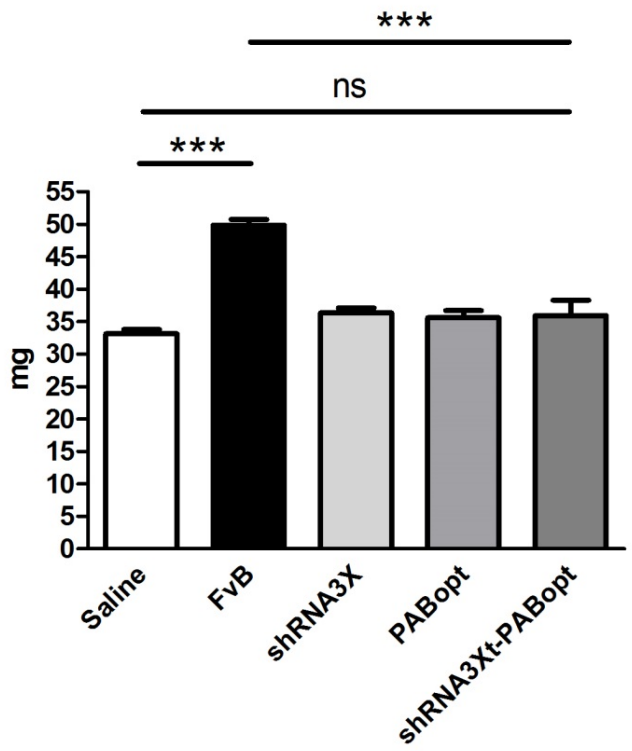
muscle fiber size assessed by quantifying fiber cross-sectional area (CSA)



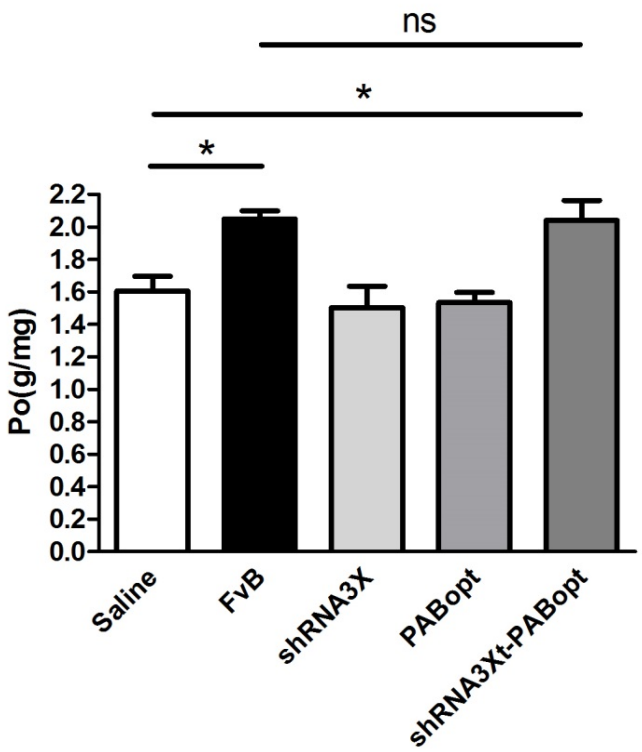



# Assessment of muscle atrophy and restoration of specific force treated A17 mice

Muscle weight




Specific Force

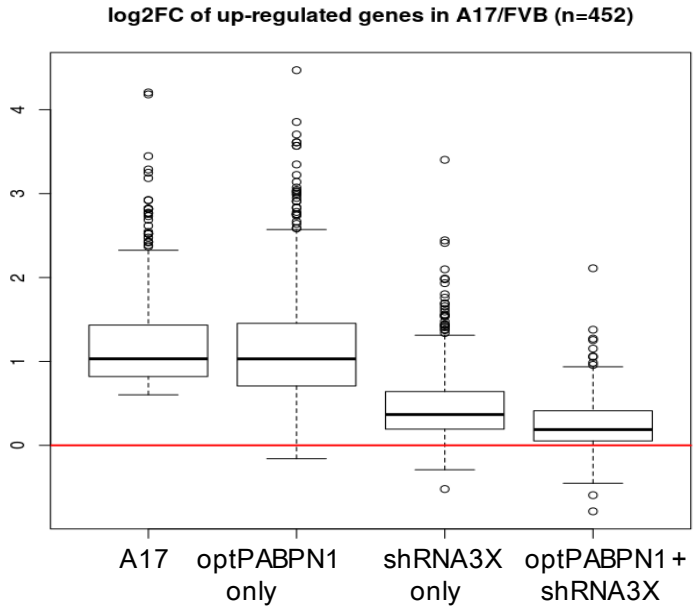


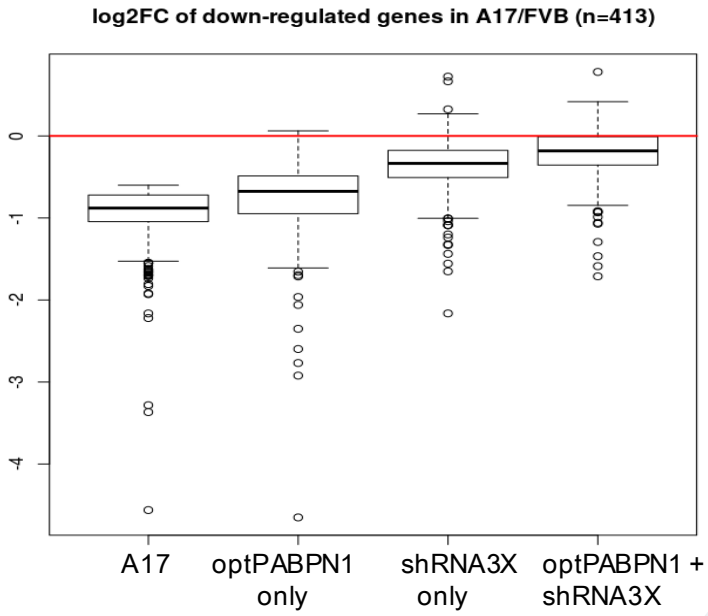

Reduced Fibrosis +
Partial Reversal of Atrophy

Specific force calculated by normalizing maximal force for muscle weight

# Impact of treatment of A17 mice: a microarray analyses

- expPABPN1 expression in A17 mice causes extensive remodelling of muscle transcriptome
  (Trollet et al. *HMG* 2010; Anvar et al. *Sk Muscle* 2011; Chartier et al. *Plos Genet* 2015)
- Transcriptome analyzed from current experiment




- In A17 mice vs wildtype, 865 transcripts were deregulated FC>2; p<0.05
- Dual treatment with shRNA3X + optPABPN1 results in only 12 genes deregulated, a 98% "correction"

# Acknowledgments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