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# Exploration of muscle from GRMD dogs transplanted with MuStem cells using “omics” approaches

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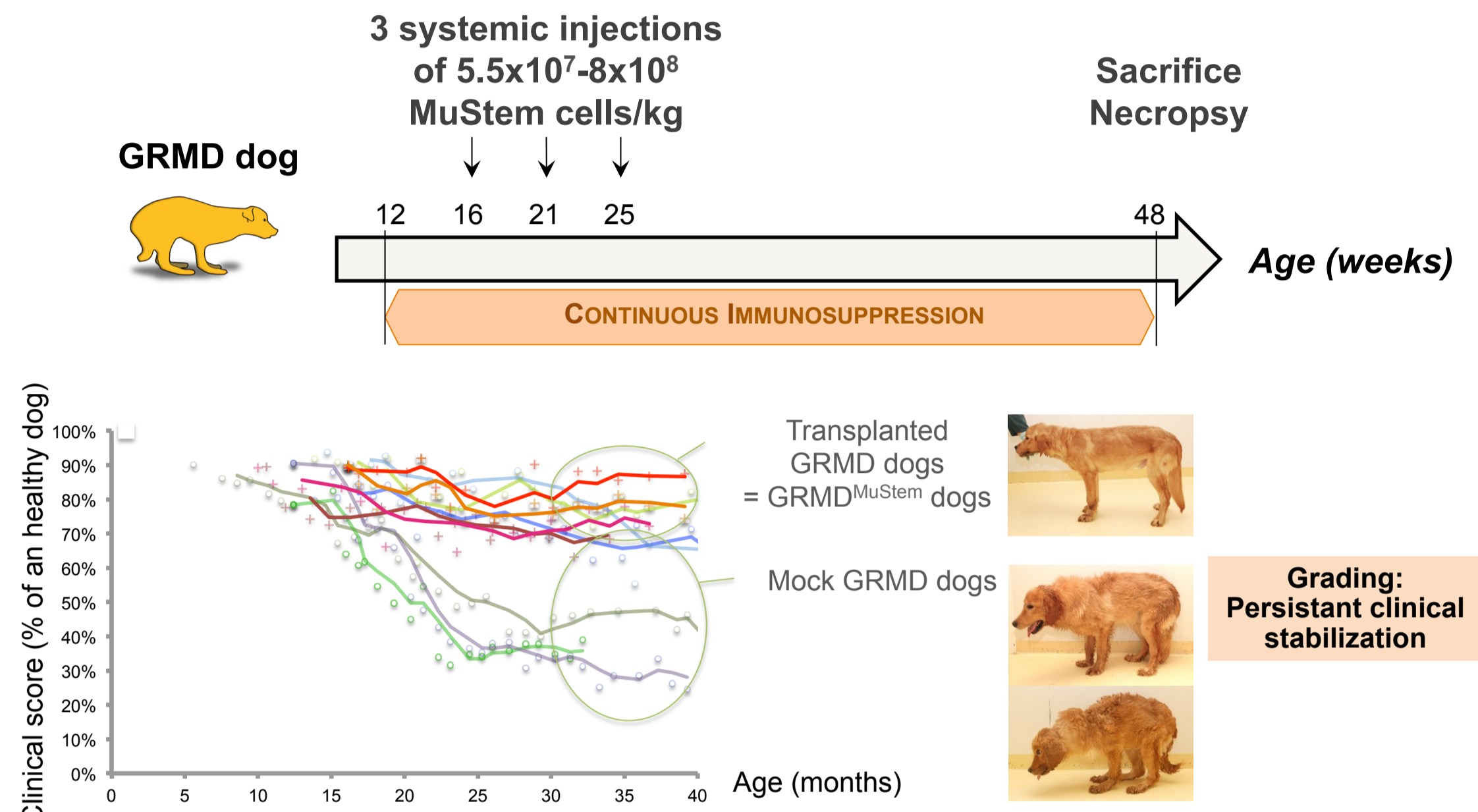
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**Introduction - Duchenne Muscular Dystrophy (DMD)**, the most common form of inherited neuromuscular disorder, is caused by mutations in the dystrophin gene leading to the protein lack. Membrane disorganization and subsequent alterations in signaling pathways and energy metabolism play important roles in muscle fibre necrosis. Systemic delivery of **MuStem cells**, skeletal muscle-resident stem cells isolated from healthy dog, generates a remodeling of muscle tissue and gives rise to striking clinical benefits in Golden Retriever Muscular Dystrophy (**GRMD**) dog (Rouger *et al.*, 2011). To pursue investigation of the consequences on the skeletal muscle tissue 6 months after cell transplantation with undedicated approach, we used here a combined analysis of **transcriptomics** (gene expression microarrays) and **quantitative proteomics** (ICPL/LC-MS/MS) (Robriquet *et al.*, 2015; Lardenois *et al.*, in preparation). In addition, we establish that the analysis of a limited set of miRNAs in skeletal muscle discriminates MuStem cell therapy effects on GRMD dogs.

**Objective -** To pave the way to the understanding of the **mechanisms underlying the therapeutic effect of the MuStem cells** and identify **potential biomarkers of the treatment efficiency** we employed combined “omics” approaches that allowed us to identify changes in transcript and protein profiles in Healthy, mock GRMD and Treated GRMD muscles (GRMD<sup>MuStem</sup>).

## Experimental context: Systemic delivery of MuStem cells



## Material and methods

We performed comparative **transcriptomic** and **proteomic** studies between healthy, mock GRMD (received continuous immunosuppressive regimen) and GRMD<sup>MuStem</sup> dog muscles (MuStem cell transplantation under the same immunosuppression) in order to identify changes in transcript and protein profiles. **Biceps femoris** muscle samples were collected surgically from the middle portion of the muscle in **9-month-old** healthy, mock GRMD dogs and GRMD<sup>MuStem</sup> dogs (n=2: transcriptomic; n=3: proteomic).

### • Transcriptomic study

-The transcriptome experiment was performed by the integrative Genomic core facility (Nantes, France). mRNA expression profiling was obtained using the **Agilent-021193 Canine (V2) Gene Expression Microarray**.

- **Statistical filtration** and **classification** were performed. The detectable probes yielding signals  $\geq 7.32$  were identified and probes displaying a high expression variation (fold change  $\geq 2$ ) were then selected. A LIMMA statistical test was done.

### • Proteomic study

•The proteomic experimentation was performed by the Biogenouest Proteomic core facility (Rennes, France). We used isotope-coded protein labeling for quantitative proteomic analysis. LC-MS/MS analyses performed on an ESI-LTQ-Orbitrap mass spectrometer led to the relative quantification of 492 proteins.

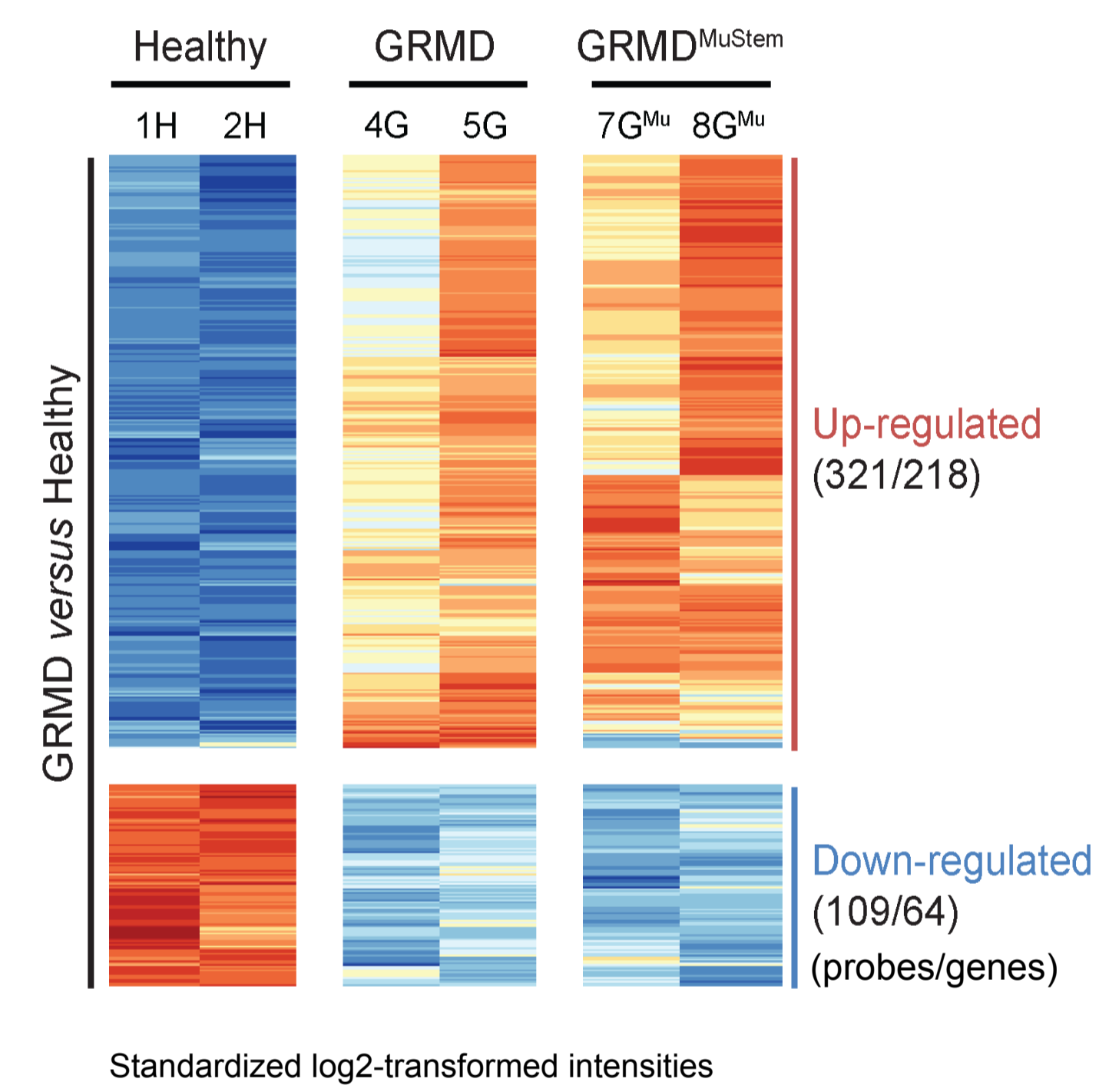
•**Statistical filtration** and **classification** were performed. The quantified proteins displaying a high ratio (absolute value of the median of the ratio data in a triplex  $\geq 1.5$ ) in at least two triplex were identified. A LIMMA statistical test was then used to identify the proteins that were significantly differentially expressed (F-value adjusted with the False Discovery Rate,  $p \leq 0.05$ ).

### • miRNA study

- The **expression levels** of 5 miRNAs (**miR-1, miR-133, miR-206, miR-222 and miR-486**) were determined by RT-qPCR in **Biceps femoris** of **9-month-old** healthy, mock GRMD dogs and GRMD<sup>MuStem</sup> dogs (n=6).

## Transcriptomic study

### A- Profiling of differentially expressed transcripts

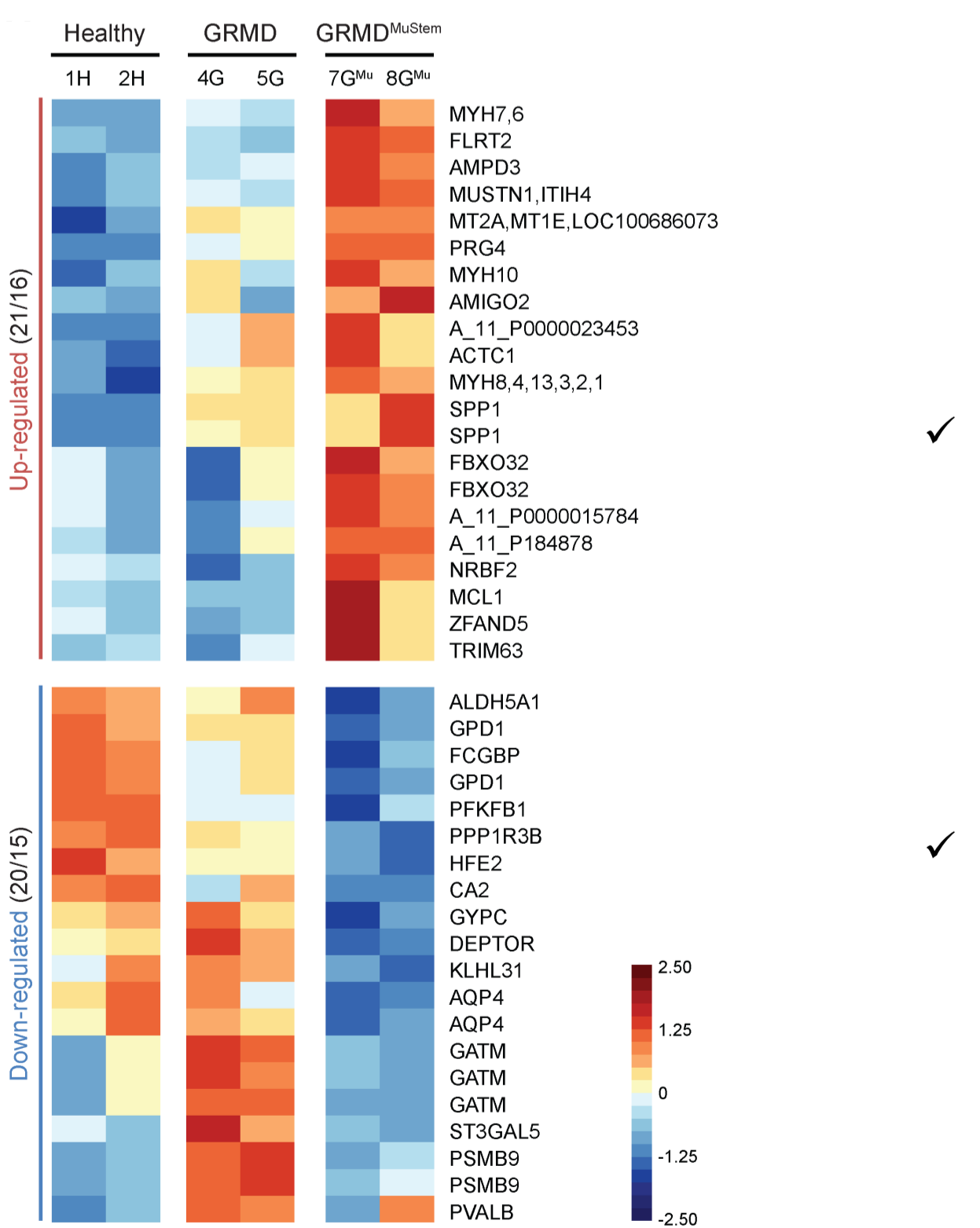


### B- Functional characterization of GRMD dog model

Gene Ontology enrichment analysis

Biological process	Up-regulated	Down-regulated
immune system process	1531	6/7
inflammatory response	288	0/1
response to stimulus	6161	29/26
response to stress	3118	18/13
response to cytokine stimulus	424	1/2
response to lipid	756	6/3
regulation of cell migration	398	2/2
cell adhesion	651	0/3
extracellular matrix organization	258	1/1
regeneration	172	2/1
cell differentiation	3242	11/14
regulation of protein secretion	127	0/1
small molecule metabolic process	2350	30/10
oxidation-reduction process	863	23/4
carboxylic acid catabolic process	226	10/1
fatty acid beta-oxidation	93	8/9
regulation of transmembrane transporter activity	92	5/6
Molecular function	Up-regulated	Down-regulated
calcium ion binding	531	2/2
structural molecule activity	539	1/2
integrin binding	75	1/0
serine hydrolase activity	138	1/1
oxidoreductase activity	621	18/13
heat shock protein binding	70	5/6
Cellular component	Up-regulated	Down-regulated
extracellular region	1584	7/7
proteinaceous extracellular matrix	312	1/1
collagen	75	0/0
plasma membrane	3598	13/15
membrane-bounded vesicle	987	2/4
perinuclear region of cytoplasm	527	1/2
cytoplasmic part	6512	42/27
mitochondrion	1583	29/17

### C- Impact of MuStem cell delivery



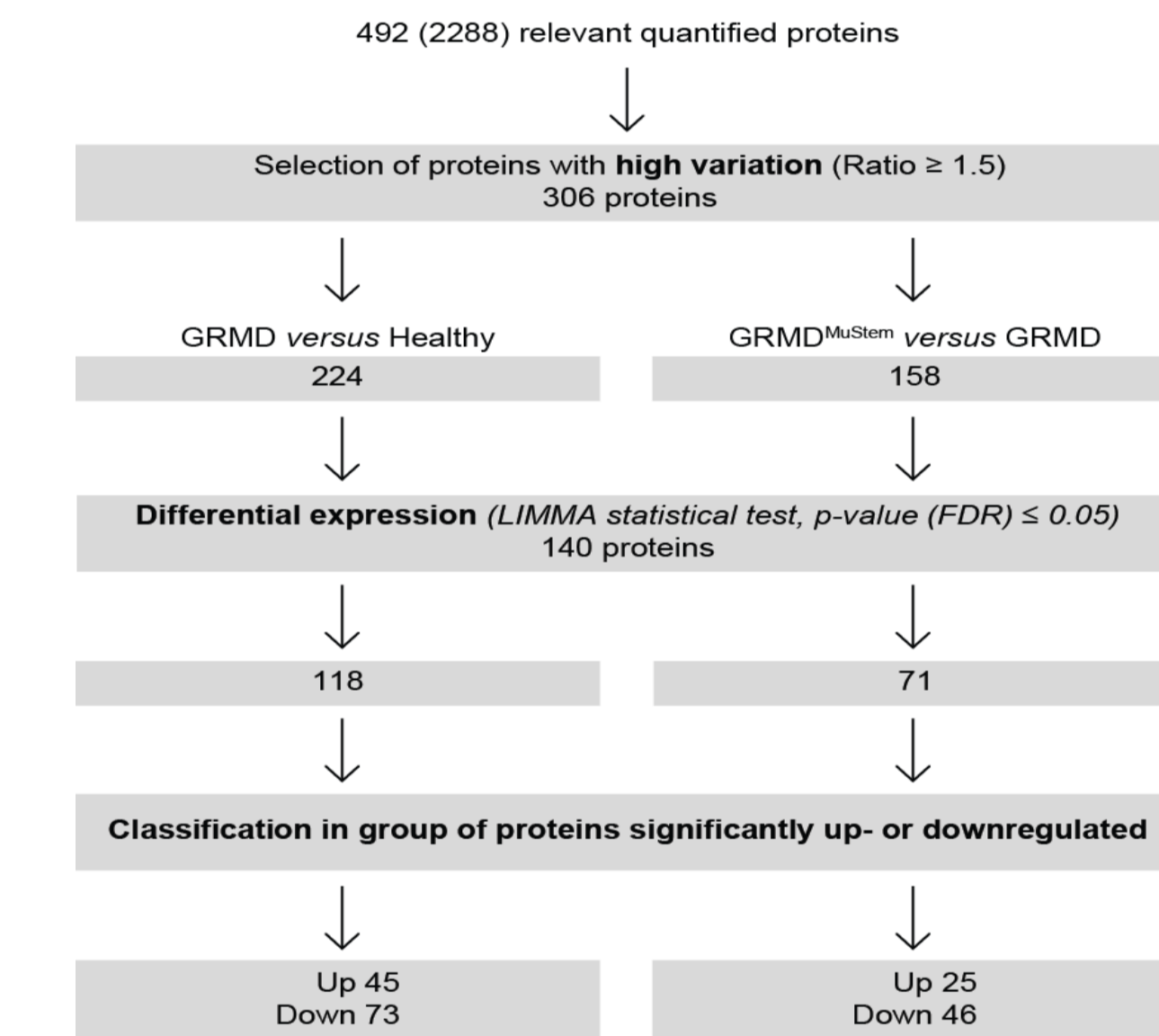
✓ The high-throughput characterization of the **GRMD dog model** reveals an altered expression profile of **282 genes**. Up-regulated genes are significantly associated with genes implicated in **regeneration, extracellular matrix organization, inflammatory response, cell adhesion** and **cell migration**.

✓ MuStem cell administration is associated with **gene expression modification of 31 factors** implicated in different biological functions, among them: **regeneration, protein degradation and folding, myogenesis, lipid and glucose metabolism, homeostasis**.

Robriquet *et al.*, PLoS One (2015)

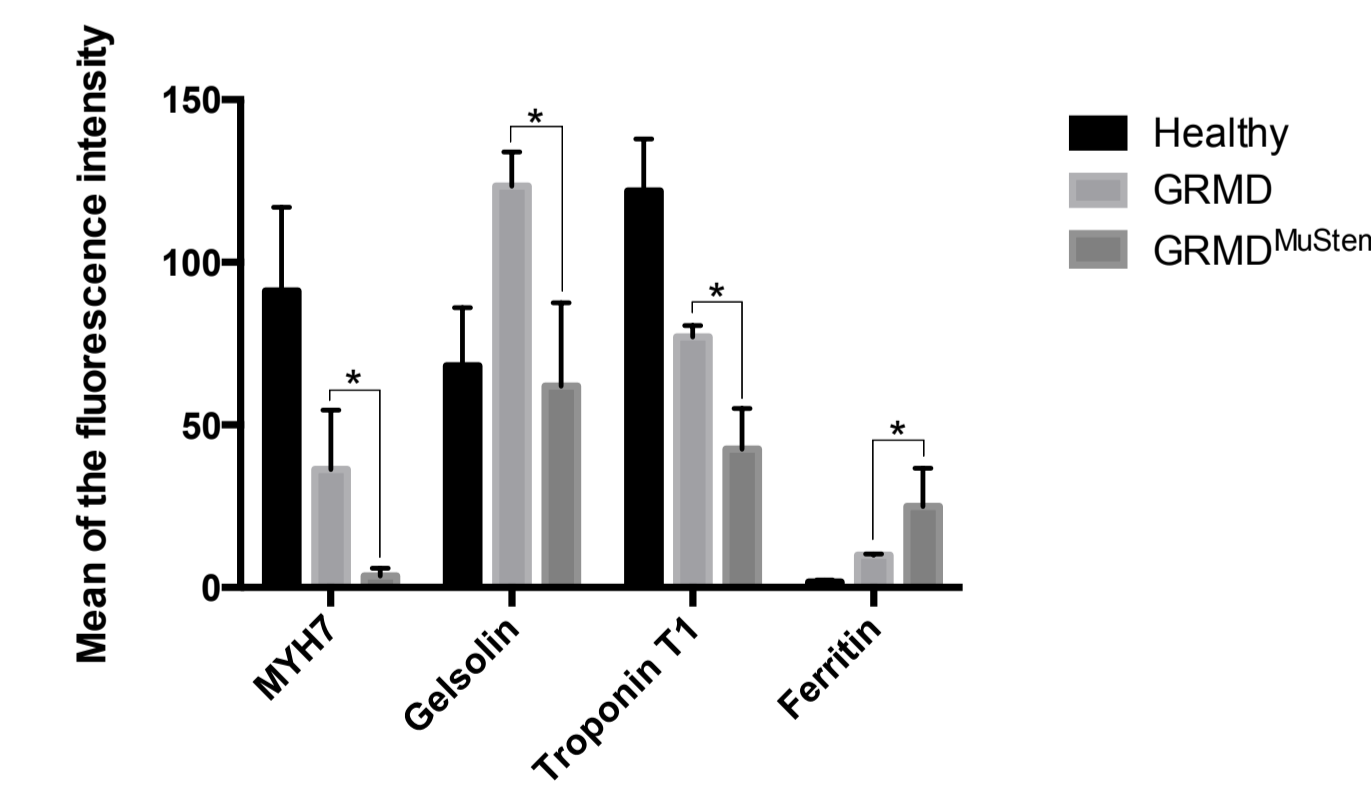
## Proteomic study

### A- Flow chart of the statistical filtration of the ICPL data



✓ The **492 proteins** considered as correctly quantified were filtered up to groups of **up- and downregulated** proteins in the GRMD *versus* Healthy comparison and in the GRMD<sup>MuStem</sup> *versus* GRMD comparison.

### C- Immunoblotting quantification



### B- ICPL data validation. Ratios ICPL versus Western Blot

Protein name	Triplex	ICPL ratio	Average ICPL ratio	WB ratio	Average WB ratio
MYH7	T1	0.55	0.27±0.25	0.13	0.23±0.09
	T2	0.18		0.29	
	T3	0.07		0.27	
Gelsolin	T1	0.42	0.60±0.2	0.77	0.7±0.15
	T2	0.81		0.8	
	T3	0.56		0.52	
Vimentin	T1	0.36	0.56±0.24	0.31	0.57±0.23
	T2	0.48		0.64	
	T3	0.83		0.76	
Haptoglobin	T1	3.58	0.29±0.33	3.35	1.83±1.33
	T2	2.36		1.20	
	T3	1.82		0.93	
Troponin T1	T1	0.18	0.29±0.33	0.67	0.65±0.09
	T2	0.03		0.73	
	T3	0.66		0.55	
Ferritin	T1	20.97	11.65±8.07	4.08	3.01±1
	T2	6.96		2.86	
	T3	7.01		2.09	

✓ **Quantitative MS and WB data comparison.** To validate the protein changes detected by proteomic analyses, immunoblotting experiments were performed on a representative range of protein types

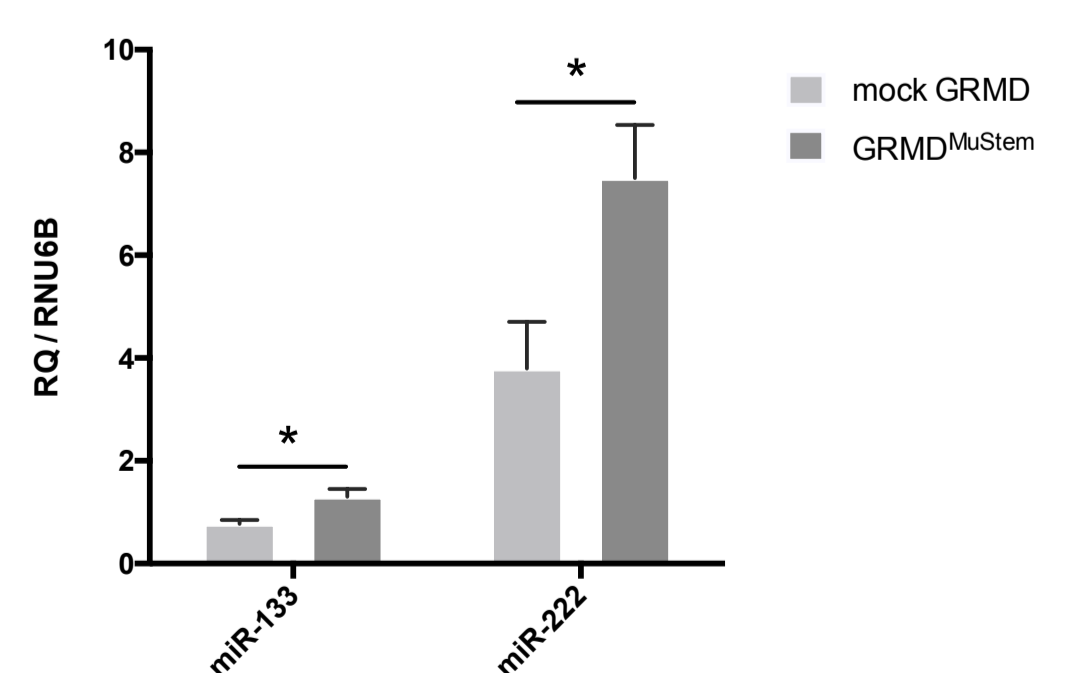
✓ **Myofibrillar reorganization.** Inhibition of myogenic and sarcomeric proteins after MuStem cell transplantation (MYH7, MHC, Troponin)

✓ **Protection of oxidative stress.** MuStem cell administration enhances skeletal muscle cells protection against oxidative stress (Haptoglobin, Ferritin)

## miRNA study

✓ The **expression levels** of 5 miRNAs (**miR-1, miR-133, miR-206, miR-222, and miR-486**) and some targets of relevant miRNA were determined by RT-qPCR and WB in **Biceps femoris** of all dogs.

✓ The analysis of a miR-133 and miR-222 in skeletal muscle discriminates MuStem cell therapy effects on GRMD dogs



## Conclusions

Overall, the combination of transcriptomics, proteomics and miRNA approaches allowed to pave the way to the understanding of MuStem cell action modalities.

Interestingly, we show that MuStem cell administration is associated with expression pattern modification of many factors belonging to different biological functions such as muscle mass regeneration, myofibrillar reorganization, metabolism, lipid and energy homeostasis. In addition, we demonstrate an effect of MuStem cell transplantation on the muscle expression of miR-133 and miR-222.

This strategy has a great potential to considerably contribute to the identification of therapeutic biomarkers of MuStem cell transplantation and thus represents an interesting tool to monitor therapeutic effects during DMD-dedicated preclinical studies.

## Fundings

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