Editorial comments:

More specifically, we focused on the following editorial requirements:

- Language was improved to reach publication grade.
- Summary was shortened to no more than 50 words.
- The advantages of the presented method are further developed.
- All abbreviations are defined before use, spacing is edited, and an ethics statement is included accordingly.
- More details are provided to the protocol steps, including mouse age, muscle collection, freezing steps, and the nature of surgical tools.
- Discussion was significantly rearranged so that the critical steps within the protocol are highlighted: the significance of the technique, its advantages over the alternative methods, and the extent of application.
- Journal titles are not abbreviated in the References.
- The Table of Materials is updated and items are sorted in alphabetical order.

Reviewers' comments:

We thank the reviewers and the editor for their careful evaluation of the manuscript.
The language in the manuscript is not publication grade. Please employ professional copy-editing services.
The language is improved in the edited version.

Reviewer 1
Major Concerns:
The authors should establish the utility of this technique for labeling of different stages of fiber degeneration/regeneration by inducing acute muscle injury by cardiotoxin injection and then sampling the muscle for staining at different time points after the injury. (Currently, the authors do include some discussion on this topic but provide no data on which this discussion is based.)

We thank the reviewer for their suggestion. However, the relevance of this technique has already been demonstrated by Kevin Campbell's group (Straub et al.1997). Its utility has been confirmed by several independent groups, including ours[1-6].

We indeed made the choice to include in the discussion some relevant precisions on the typical histological features of regenerative muscles. Not all readers are necessarily familiar with the histology of the skeletal muscle tissue in health and disease. We believe that these "reminders" are useful to fully appreciate the phenotype of the muscle cross sections which have been labelled with our technique. As so, we think this part is most appropriate in the discussion.

We agree that the description of the kinetics of IgG uptake in myofibres following an acute injury would be of interest. However, the purpose of this manuscript is to describe a reliable method to assess myofibre demise, not the downstream events. The regenerative events following an experimental injury are already extensively investigated and described in the literature [7-9], while cell death processes are largely overlooked and therefore remain poorly understood. This manuscript aims to present a simple and specific tool for assessing myofibre cell death, and regenerative events are only taken as indirect indications of past myofibre death event.
Therefore, we do not believe that such a characterization is crucial in this manuscript.

Minor Concerns:

The manuscript emphasis on muscular dystrophies is too narrow, as necrotic fibers are encountered in many myopathies and this technique should be applicable to all of them.

We fully agree that this technique applies to a broad range of muscular disorders.

Please note that this article is entitled "Immunolabelling myofibre degeneration in muscle biopsies » and the technique is not restricted to muscular dystrophies. Furthermore, the abstract begins with the following statement (Line 50-51): "… myonecrosis has a central role in the pathogenesis of muscle conditions, including in muscular dystrophies".

A decision was made to apply this method in this manuscript to a broadly used model for muscle degeneration. Duchenne muscular dystrophy is the most investigated and the best known muscle pathology involving chronic myonecrosis. We recently provided the first demonstration of the involvement of a regulated form of cell death in the muscle tissue, using this model.

To fully address the reviewer's concern, we clarified this point in the introduction with the following statements:

> *Line 69: "Myofibre demise can occur in multiple muscle conditions, including mechanical trauma, ischemia-reperfusion injuries or muscular dystrophies, and is associated with the necrotic morphology of dead cells".*
>
> *Line 262: "Myofibre necrosis is a common consequence of traumatic exercise in normal muscles. It is well compensated by a powerful regenerative capacity of the local muscle progenitors. However, in several muscle conditions such as in MDs…"*
>
> *Line 321: "Considering its specificity and its general lack of counter indications, we recommend its use as a gold standard for myonecrosis assessment, regardless of the nature of the necrotic injury."*

How does this technique compare with immunostaining for the membrane attack complex C5b-9, which is routinely used to label degenerating/regenerating fibers in tissue sections?

We wish to emphasize the fact that degeneration and regeneration are two distinct processes. A better understanding of the degeneration mechanism(s) in neuromuscular disorders requires a strict separation of these concepts and the use of distinct and specific tools. Membrane attack complex immunoreactivity can indeed be found on both regenerating and degenerating myofibres[10]. Strong C5b-9 deposition is observed on living muscle fibres in pathological conditions[11,12].

We do not necessarily consider that C5b-9 labelling is a reliable method to accurately identify muscle degeneration. It is clearly more associated with some disorders than others[13], and therefore represents a poor marker for muscle necrosis in some muscle conditions.

Together, this indicates that complement deposition lacks specificity for cell death. In contrast, blood protein uptake by myofibres specifically indicates necrotic myofibres.

Proofreading of manuscript by a native or near-native English speaker is recommended.

The revised version of the article was edited accordingly.

Reviewer #2:

This is a very nice study

We thank the reviewer for his/her very positive evaluation of the manuscript.

I wonder why you chose the tibialis anterior which is not much affected in the MDX mouse and why not also use in the diaphragm to compare.

We fully agree with the comment of the reviewer 2: the diaphragm muscle is much more affected than the hindlimb muscles in dystrophin-deficient mice.
We chose tibialis anterior (TA) muscles over diaphragm for two main reasons:

1. Experimentally, the harvesting and the analysis of TAs are much easier than the diaphragm: TA muscle is an external muscle, with easy access for collection. Its shape is longitudinal and is easier to mount and freeze, whereas the diaphragm is a very thin muscle with an internal location. Diaphragm collection and mounting requires very specific skills. For newcomers to the field of muscle biology, we wanted this part as simple as possible for the manuscript.
2. We aimed to use a muscle for which we would be absolutely sure that ongoing myonecrosis can be observed in young mice. To our knowledge, the kinetics and the extent of myonecrosis is better described in TAs than in the diaphragm. Furthermore, the onset of myonecrosis is very early in the TA compared to the diaphragm. In our hands, one month-old mdx mice offered the advantage of significant cell death, inflammation and recent regenerative events, so that all features associated with myonecrosis and its consequences can be observed within the same cryosection.

There are several things that are not clear, for example, statements that need to be corrected. When it says in the introduction, last paragraph, "however the injection of vital dyes is counter indicated in some experimental procedures such as creatinine kinase", it is not clear and needs to be explained more clearly.
We thank the reviewer 2 for giving us the opportunity to clarify this point.
In one of our previous studies, we found that the reading of blood samples for CK activity was perturbed by the presence of Evan's blue dye in the mouse bloodstream. This observation was made on dysferlin-deficient mice. For this reason, blood CK measurement (reflecting general myofibre membrane permeability) and Evan's blue labelling were each performed at different timepoints in distinct animals. This limitation doesn't exist using the IgG uptake assay and this advantage is now more clearly specified in the edited version of the manuscript.

Line 98: *"The injection of vital dyes, such as Evan's blue dye (EBD), represents a useful alternative for assessing myofibres that have lost the integrity of sarcolemma, but is not necessarily convenient in some experimental protocols. For instance, the presence of EBD in blood samples can affect the results of CK measurement, a colorimetric assay."*

We also included in the discussion a larger part dealing with the advantages of the IgG uptake technique over alternative methods such as the Evan's Blue dye injections.
Line 309: "*The whole musculature of EBD injected animals contains the dye that can possibly affect further analysis such as fluorescent immunolabelling of muscles or the blood CK colorimetric assay."*

I wonder if the description of the freezing is necessary, as this is a very standard method.
We understand this comment. However, editorial requests were made to develop this part.

The English needs to be improved.
The manuscript has been edited accordingly.

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4 Morgan, J.E. *et al.* Necroptosis mediates myofibre death in dystrophin-deficient mice. *Nat Commun.* **9** (1), 3655, doi:10.1038/s41467-018-06057-9 (2018).
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