

# Differential Roles of SWI/SNF Complexes in Metal Regulation

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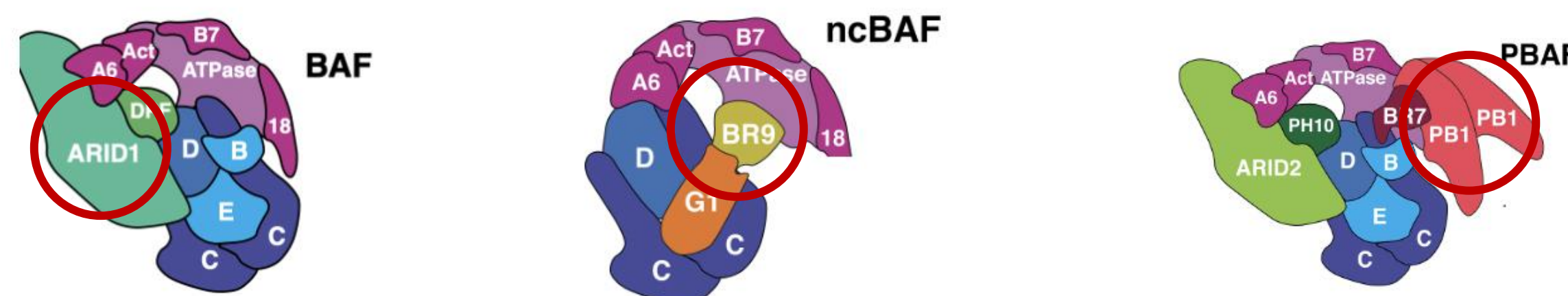
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## Abstract

SWI/SNF complexes play a major role in chromatin remodeling in mammalian cells. Mammalian DNA is compacted into chromatin by nucleosomes and other factors. The level of compaction is regulated largely by SWI/SNF complexes. These complexes remodel nucleosomes by displacing or evicting them, or by exchanging histones. By controlling which areas of the chromatin are available for transcription, SWI/SNF complexes have a large impact on gene expression. There are three main classes of SWI/SNF complexes: Brg/Brm-associated factor (BAF), polybromo-associated BAF (PBAF), and non-canonical BAF (ncBAF)<sup>1</sup>. We hypothesized that these distinct classes of SWI/SNF complexes may have different roles and respond to environmental and cellular signals in a specific manner. Preliminary data from our lab showed that the PBAF, and potentially the BAF complexes, may play a role in stress, homeostasis, and metal regulation in proliferating and differentiating myoblasts<sup>2</sup>. We used cultured C2C12 myoblasts partially depleted of a representative subunit of each SWI/SNF complex, as these cells have an intrinsic high demand for transition metals for proper growth, differentiation, and function<sup>3,4</sup>. Knock-down myoblasts were treated with sublethal concentrations of different metals, and we analyzed the correlation of each SWI/SNF complex with the expression of the metal protective transcription factor (*Mtf1*) and metal responsive metallothionein-1 (*Mt1*) genes. Our work provides the first mechanistic evidence of the contribution of specific chromatin remodelers to the expression of the main metal protective transcription factor and effector genes necessary for surviving metal toxicity.

## Introduction

- The BAF, ncBAF, and PBAF classes of SWI/SNF complexes are involved in chromatin remodeling (Fig. 1)<sup>1</sup>
- Knocking down specific subunits of each one of these complexes renders differential phenotypes in the skeletal muscle lineage<sup>2</sup>.



- Previous data from our lab shows a possible relationship between *Baf180* and expression of *Mtf1* (Fig. 2)
- We propose a mechanism whereby metal protective transcription factors may interact and associate differentially with SWI/SNF complexes (Fig. 3)

Fig. 2: Fold change in gene expression of the metal protective genes *Mtf1* and *Mt1* determined from RNA-seq of proliferating and differentiating myoblasts<sup>2</sup>

Gene name	Scr Prol-1	Scr Prol-2	Scr Dif-1	Scr Dif-2	Baf180 Prol-1	Baf180 Prol-2	Baf180 Dif-1	Baf180 Dif-2	Baf250 Prol-1	Baf250 Prol-2	Baf250 Dif-1	Baf250 Dif-2	Brd9 Prol-1	Brd9 Prol-2	Brd9 Dif-1	Brd9 Dif-2
<i>Mtf1</i>	3.76	3.83	2.06	2.64	2.09	1.84	1.47	1.33	2.75	3.19	2.46	2.57	3.81	3.55	3.54	2.9
<i>Mt1</i>	163.18	154.61	530.66	424.18	126.30	122.62	179.35	148.59	187.11	219.95	79.87	87.22	138.13	126.98	3	68.14

Fig. 3: Proposed mechanism for interaction between metal protective factors and SWI/SNF complexes

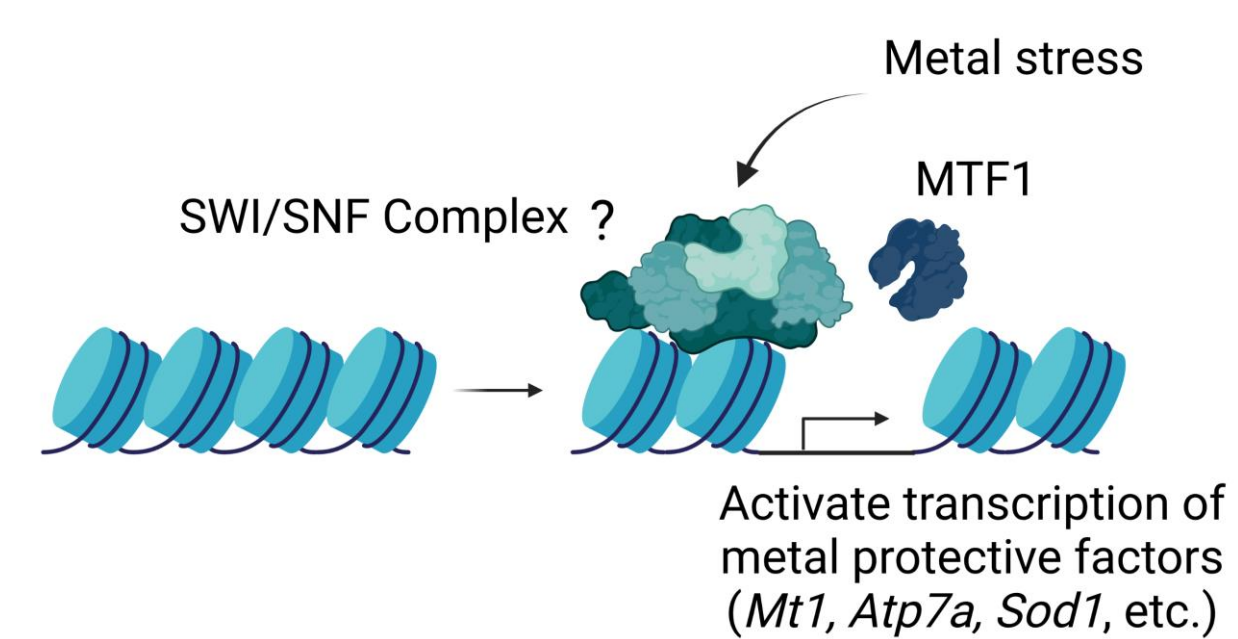
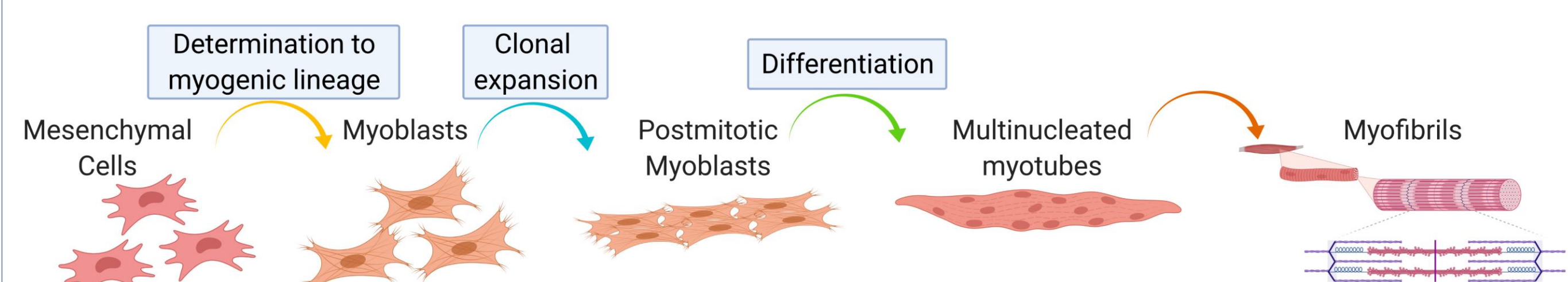


Fig. 4: C2C12 cells as a model for skeletal muscle differentiation



## Results

Fig. 4: Validation of knockdown cells via qPCR

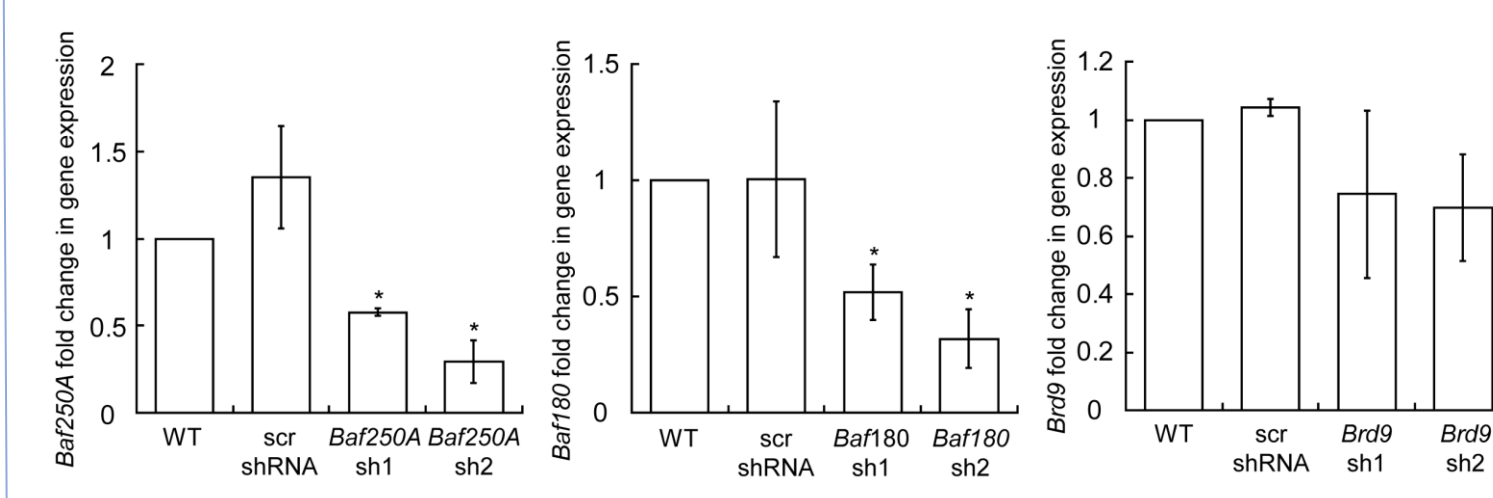


Fig. 5: Validation of knockdown cell lines via Western Blot

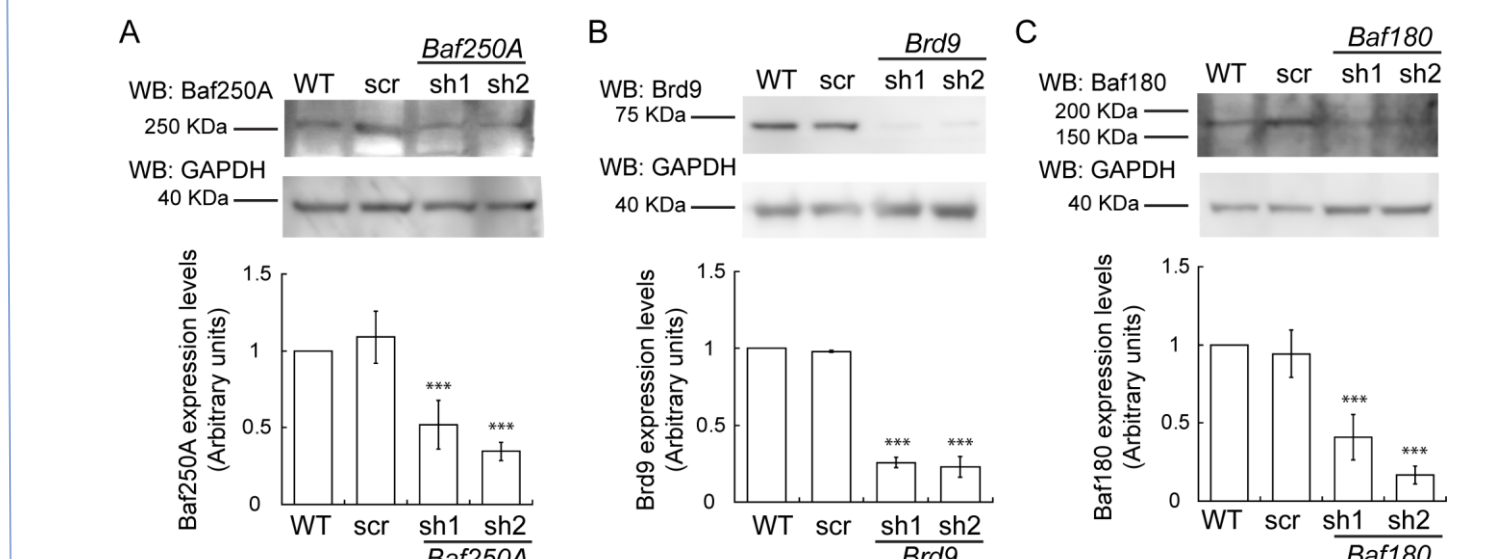


Fig. 6: Fold change in cell number over time in proliferating cells

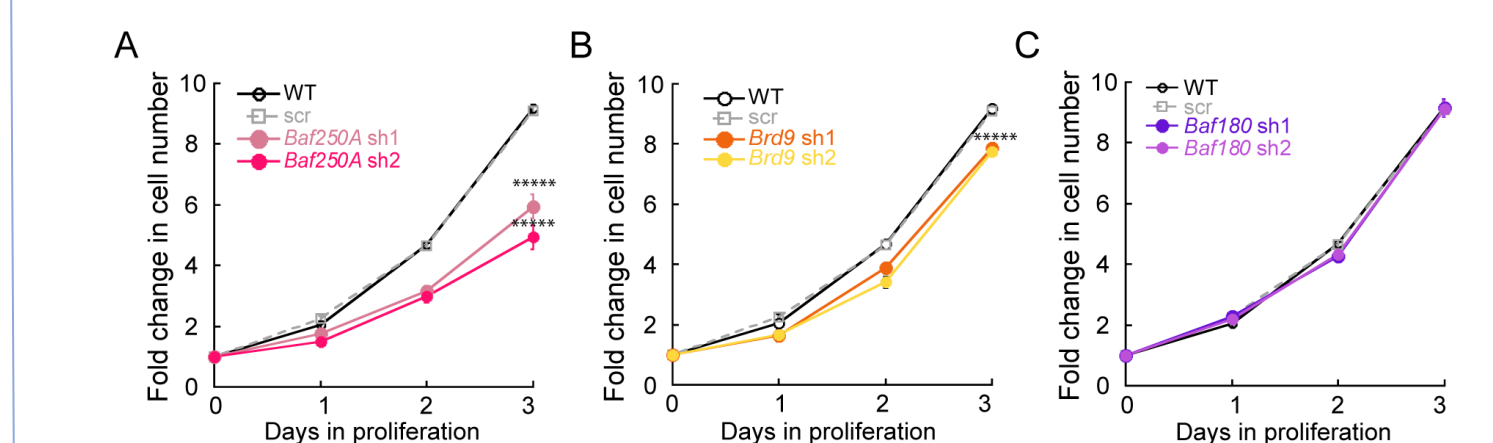


Fig. 7: ZnSO<sub>4</sub> supplementation impairs *Baf180* KD myoblasts growth, but enhanced *Baf250A* and *Brd9* KDs growth. Pax7 staining.

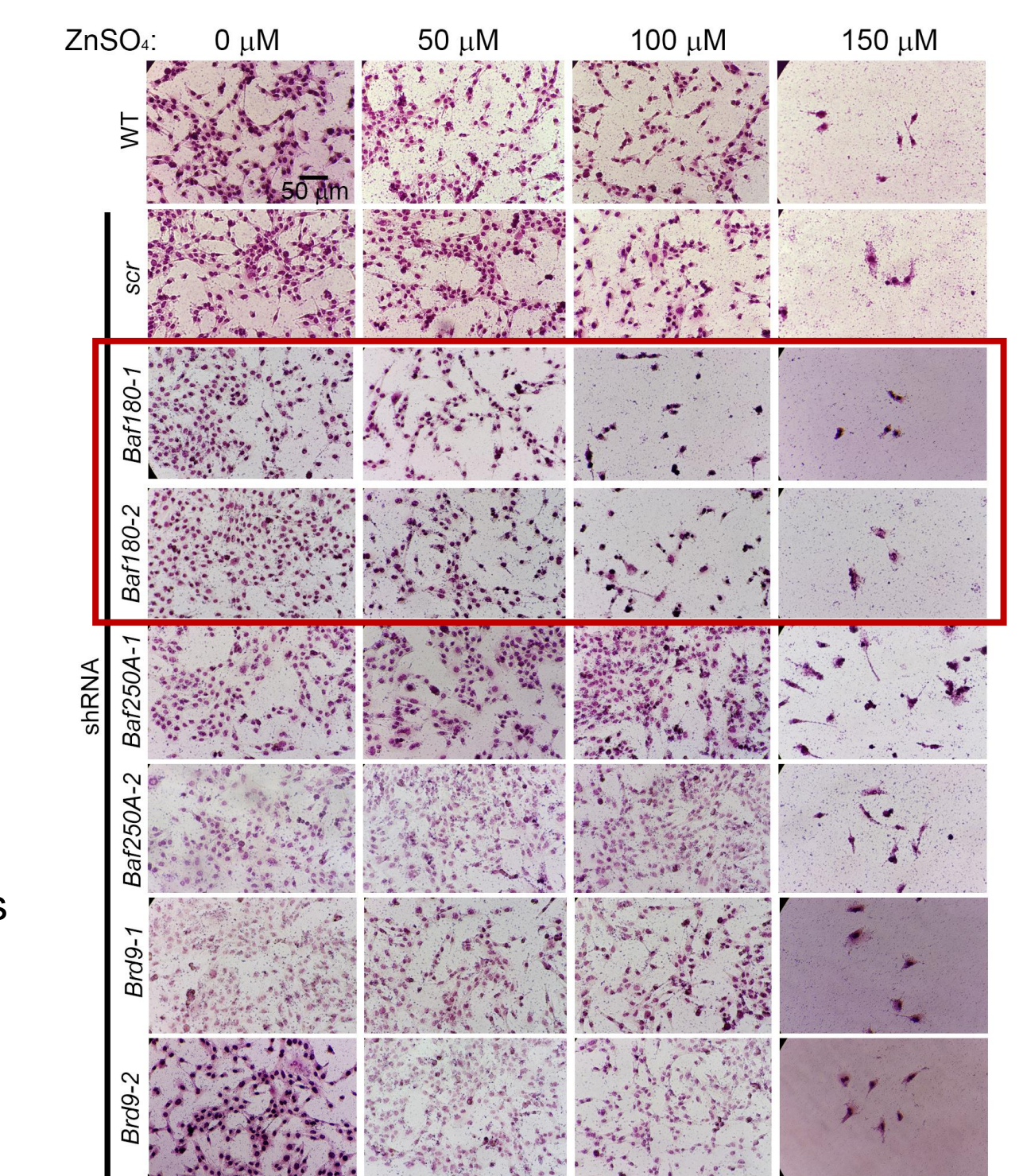


Fig. 8: CuSO<sub>4</sub> supplementation impairs *Baf180* KD myoblasts growth, but enhanced *Baf250A* and *Brd9* KDs growth.

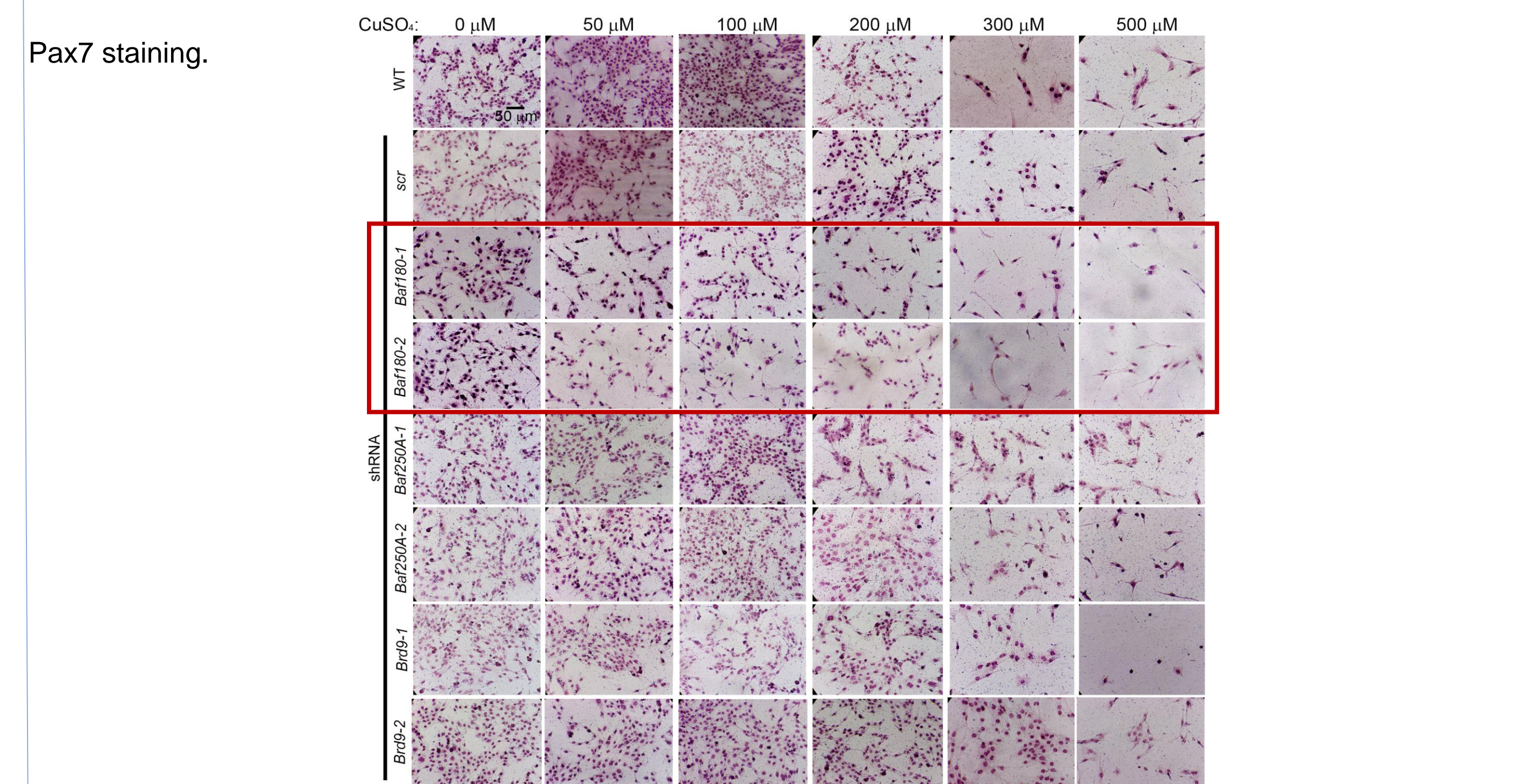


Fig. 9: CoCl<sub>2</sub> supplementation impairs *Baf180* KD myoblasts growth, but enhanced *Baf250A* and *Brd9* KDs growth; however, cell morphology is affected

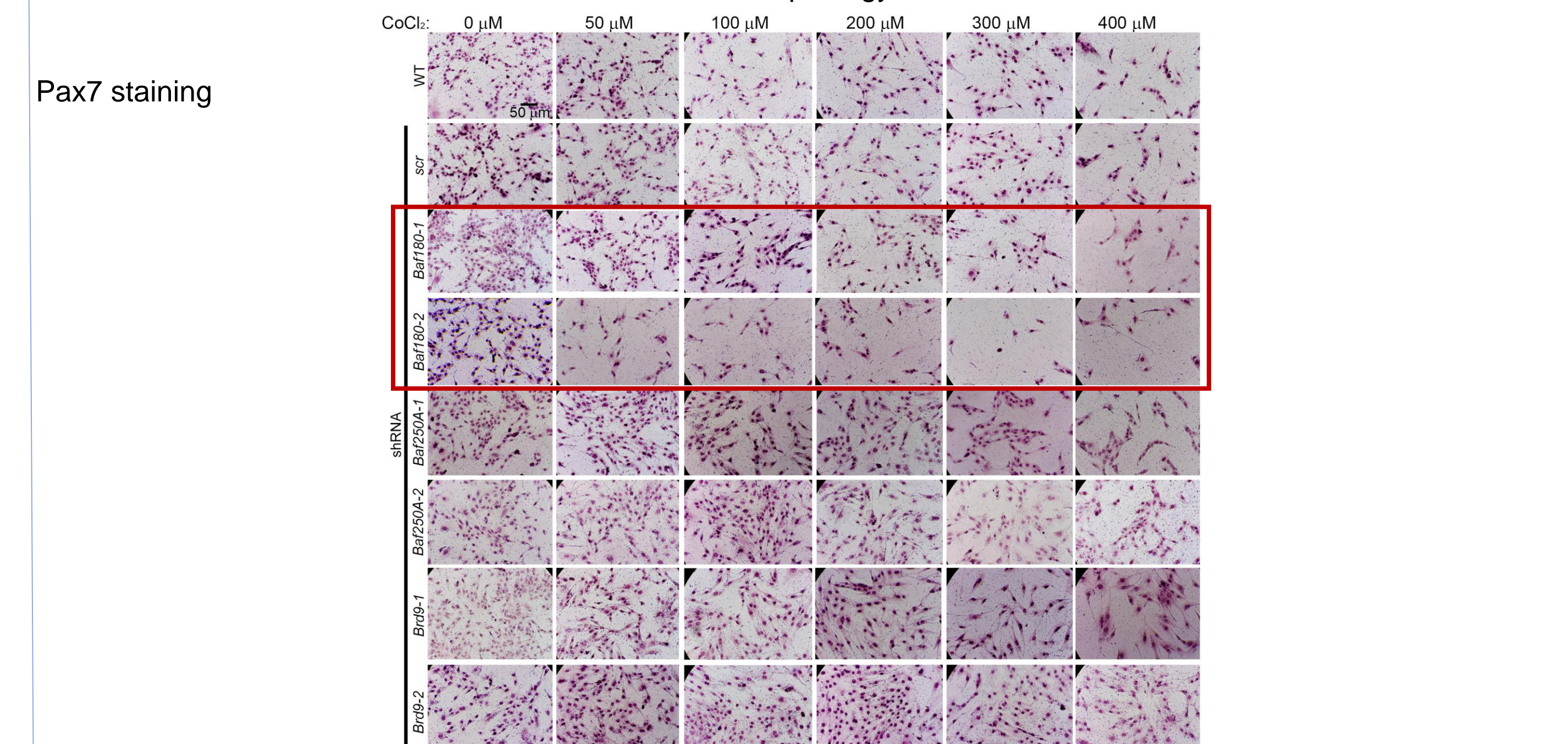


Fig. 10: *Mtf1* and *Mt1* gene expression profile in proliferating myoblasts supplemented with sublethal concentrations of metals

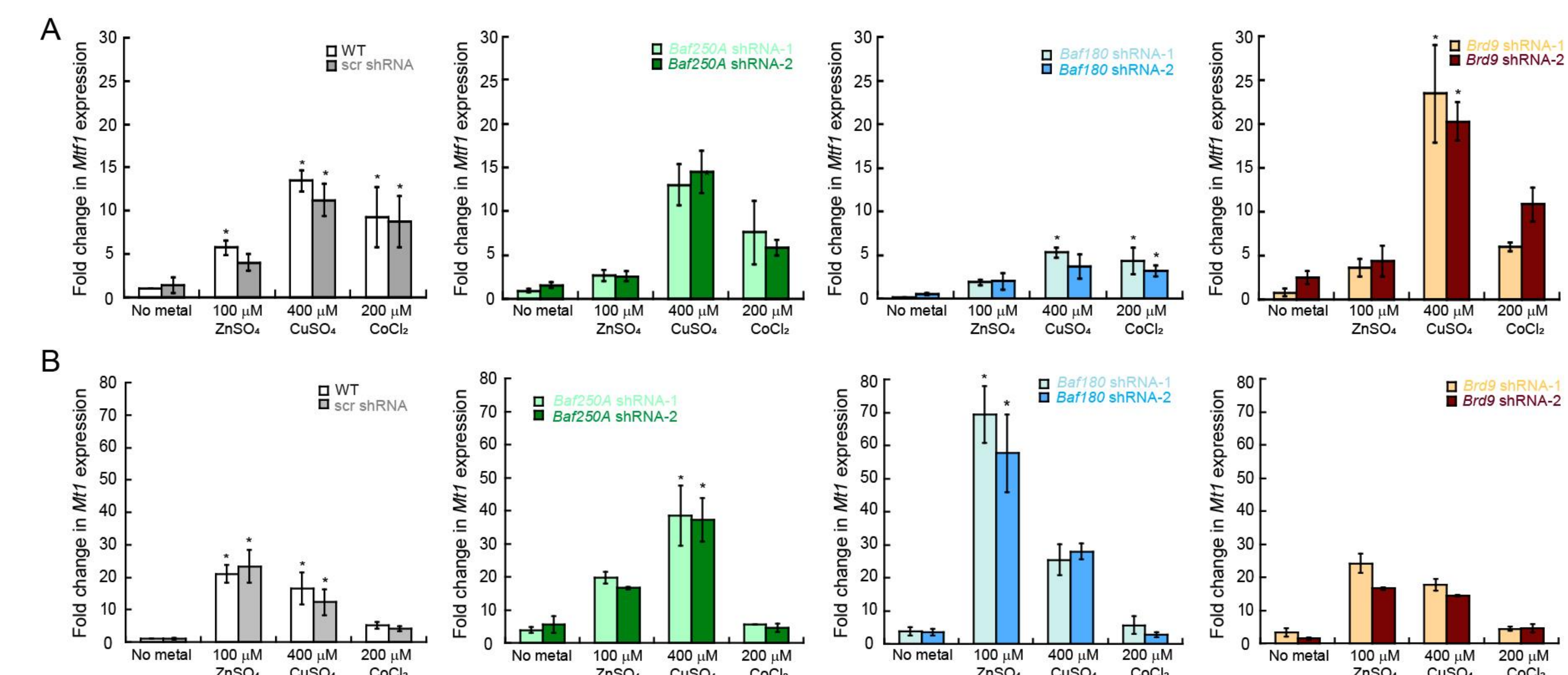
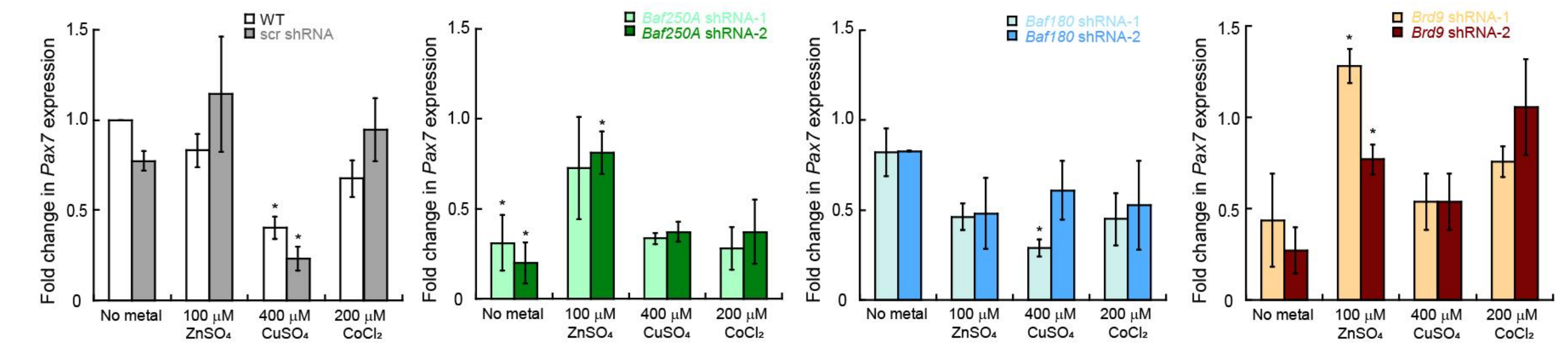


Fig. 11: *Pax7* gene expression is enhanced by ZnSO<sub>4</sub> in proliferating *Baf250A* KD myoblasts



## Conclusions

- Mtf1* expression decreases in *Baf180* KDs, while *Mt1* expression is increased
- Baf250A* KD appears to not significantly effect *Mtf1* and *Mt1* expression compared to wild type cells
- Copper stimulus results in larger induction of *Mtf1* in *Brd9* KD cells
- Zinc treatment appears to rescue proliferation defect phenotype and *Pax7* expression in cells partially depleted of *Baf250A*

## Future Directions

- Validate *Mtf1* data with new set of primers
- Investigate *Mtf2* as a potential activator of *Mt1* in the *Baf180* KD
- Repeat cell culturing and treat cells with non-lethal levels of copper to observe *Pax7* expression and cell growth for proliferating cells

## Acknowledgements

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- Wesleyan University MB&B Department

## References

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- Padilla-Benavides *et al.*, in prep.
- Vest *et al.*, 2018. Metallomics
- Tavera *et al.*, 2019. The FASEB Journal.

## Materials & Methods

**Cell culture:** Various C2C12 cell lines were cultured in DMEM 10% FBS in a humid atmosphere supplemented with 5% CO<sub>2</sub> and with or without the indicated concentrations of metals.  
**qPCR:** After purification of RNA from the metal-treated cultured cell lines, 1 µg of RNA was reverse transcribed into cDNA and analyzed by qPCR with *Mtf1*, *Mt1* and *Pax7* specific primers