(In some pictures appears “x” instead of 10 µM of extracellular zinc -> 2x means 20 µM of extracellular zinc)

1. Figure1
2. Original images of Live/Dead assay of C2C12 cells cultured during 1,3 and 5 days in presence of different concentrations of zinc.
3. Excel data sheet with the fluorescence emission (only calcein-AM, associated to living cells) values correspondent to Live/Dead assay.
4. Original images of cells/field (cell nuclei were stained with Hoechst) at 10 magnifications used to calculate cell density at different time lapses.
5. Figure2
   1. –f) Original images of immunofluorescence experiment of the figure 2 and set of images used to calculate C2C12 differentiation and the parameters associated to differentiation.
6. Bright field images at 10 magnifications of C2C12 cells after 3 and 6 days of differentiation experiment.
7. Data associated to real time q-PCR experiments of C2C12 cells after 6 days of differentiation assay.
8. Figure3
   1. Bright field and fluorescence images of intracellular zinc of C2C12 cells in presence of different concentrations of extracellular zinc after 24 h.

Excel data sheet of intracellular zinc values obtained through zinc marker fluizin3-AM by means a plate reader after to be cultured during 24 h in presence of different concentrations of extracellular zinc.

* 1. Bright field images and fluorescence images of intracellular zinc of C2C12 cells differentiated during 6 days in presence of different concentrations of extracellular zinc.

Excel data sheet of intracellular zinc values obtained through zinc marker fluizin3-AM by means a plate reader after myoblasts differentiation during 6 days in presence of different concentrations of extracellular zinc.

* 1. Live/Dead assay images of C2C12 differentiated during 6 days and exposed to different concentrations of zinc in order to evaluate cytotoxicity.

1. Figure4
   1. Immunofluorescence original images of zinc transporter Zip7 after 24 h culture and after 6 days of differentiation assay.
   2. –e) Western blot original bands correspondent to figure 4.
2. Figure5
   1. Immunofluorescence original images of zinc transporter Zip7 subsequent to be silenced with RNAi during 3 days.
   2. Western blot original bands correspondent to figure 5.
3. Figure6
   1. Data sheet of intracellular zinc measured with FluoZin3-AM after expose C2C12 cells and C2C12 cells treated with RNAi to different concentrations of extracellular zinc. Measurements were done each 40 seconds approximately.
   2. Original images of BrDU incorporation assay used to quantify cell proliferation.
4. Figure7
   1. –f) Immunofluorescence original images represented in figure 7a and images used to quantify C2C12 cells differentiation.
5. Figure8

Hypothetical chain of events occurred when myoblasts were exposed to high concentration of extracellular zinc.

Supplementary information

1. Supplementary figure1
   1. –f) Set of images used to calculate C2C12 cells differentiation with an initial culture density of 10.000 cells/cm2
2. Supplementary figure2

Data sheet obtained by the RT-qPCR system for the analysis of the expression of MyoD and Myogenin after differentiation of C2C12 exposed to different concentration of extracellular zinc.

1. Supplementary figure3

Original images used for the panel of images correspondent to BrDU incorporation assay.

1. Supplementary figure4 and figure5

Original western blot developed images of the bands correspondent to figures 4 and 5 with the explanation of the molecular weight and which band corresponds to each sample.

1. Supplementary figure6

Set of images used to quantify C2C12 proliferation in media supplemented with 1% ITS and different concentrations of zinc after 1 and 3 days.