**Mechanical loading of tissue engineered skeletal muscle prevents dexamethasone induced myotube atrophy**

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**Supplementary material**

The following annotated text describes the application of the in-house macro described within the methods (2.5 Histology). Text in red is descriptive annotation and not part of the macro.

**MyHC % Calculation Macro**

run("Clear Results");

roiManager("Reset");

title=getTitle();

// Clears existing data from prior runs

delimiters =".";

stringResult = split(title, delimiters);

title2=stringResult[0];

selectWindow(title);

s=nSlices();

run("Set Scale...", "distance=1392 known=1283 pixel=1 unit=um");

// Sets scale to 10x magnification.

run("Duplicate...", "title="+title2+"\_mask duplicate range=1-"+s+"");

// Duplicates the image file.

run("8-bit");

// Converts image file to 8 bits.

run("Despeckle");

run("Smooth");

// De-speckles and smooths image to remove noise.

run("adaptiveThr ");

// Runs adaptive threshold macro (see below).

run("Set Measurements...", "area ellipse feret's stack redirect="+title2+"\_mask decimal=3");

// Defines measurements.

run("Invert", "stack");

// Inverts image for analysis.

Answer=getBoolean("Apply Watershed?");

if (Answer==true) run("Watershed", "stack");

// Applies the watershed feature to separate features.

run("Analyze Particles...", "size=25-50000000 show=Outlines display exclude add stack");

// Analyses particles for defined size range.

showStatus ("Done");

// Completes analysis and displays data.

The threshold macro utilised above is available for download free of charge from imagej.net.