# **Supplementary material**

**To manuscript: Dermal penetration analysis of curcumin in an ex-vivo porcine ear model using epifluorescence microscopy and digital image processing**

**S1:** Threshold algorithm to quench the autofluorescence of the skin to a minimum. Programmed macro for auto-thresholding the image of curcumin skin biopsies obtained from epifluorescence microscopy and analyzed using the DAPI HC filter block system (excitation filter: 340 – 390 nm (LP), dichroic mirror: 410 nm, emission filter: 420 nm (LP)). **M1:** Macro for setting of the RGB-threshold, **M2:** Macro for white to black inversion of images.

**S2:** Representative images for blank, untreated skin and skin biopsies treated with ethanolic solutions containing different concentrations of curcumin after auto threshold.

# **S1:**

**M1:**

/ Color Thresholder 1.52a

// Autogenerated macro, single images only!

min=newArray(3);

max=newArray(3);

filter=newArray(3);

a=getTitle();

run("RGB Stack");

run("Convert Stack to Images");

selectWindow("Red");

rename("0");

selectWindow("Green");

rename("1");

selectWindow("Blue");

rename("2");

min[0]=33;

max[0]=255;

filter[0]="pass";

min[1]=0;

max[1]=0;

filter[1]="stop";

min[2]=0;

max[2]=0;

filter[2]="stop";

for (i=0;i<3;i++){

selectWindow(""+i);

setThreshold(min[i], max[i]);

run("Convert to Mask");

if (filter[i]=="stop") run("Invert");

}

imageCalculator("AND create", "0","1");

imageCalculator("AND create", "Result of 0","2");

for (i=0;i<3;i++){

selectWindow(""+i);

close();

}

selectWindow("Result of 0");

close();

selectWindow("Result of Result of 0");

rename(a);

// Colour Thresholding-------------

**M2:**

run("Invert");

# **S2:**













