Supplementary Text
Colocalization Macro

```
r thresholdUbi = 40;
r thresholdMito =20;
r thresholdDAPI = 6;

    roiManager("Reset");
    //get image attributes
    name = getInfo("image.filename    ");
    original = getImageID();
    originalTitle = getTitle();
    shortTitle= originalTitle
   CellROI="Cells.zip";

    //Set the directory where files will be pulled from and saved to
    DataDir=getDirectory("Choose the folder where data will be saved");
    run("8-bit");
    run("Duplicate...", "title=UbiImage duplicate channels=2");
    UbiImage = getImageID();
    UbiImageTitle = getTitle();
    changeValues(0, thresholdUbi, 0);

    //Mask on the Mitochondria
    selectImage(original);
    run("Duplicate...", "title=MitoImage duplicate channels=3");
    MitoImage = getImageID();
    MitoImageTitle = getTitle();
    run("Median...", "radius=1");
    changeValues(0, thresholdMito, 0); //KEY difference with non-norm macro
   //-->run("Convert to Mask", " black");
    rename("MitoMaskImage");
    MitoMaskImage = getImageID();
   MitoMaskTitle = getTitle();

    // divide image1 by image2
    run("Misc...", "divide=NaN"); //dividing by zero gives NaN
    imageCalculator("Divide create 32-bit", UbiImage, MitoMaskImage);
    rename("RatioImage");
    RatioImage = getImageID();
    RatioImageTitle = getTitle();

    if (File.exists(DataDir + CellROI) == 1) open(DataDir + CellROI);
    else {
    selectImage(original);
    run("Duplicate...", "title=DAPI duplicate channels=1");
     setThreshold(thresholdDAPI, 255);
    //setAutoThreshold("Mean dark");
   run("Convert to Mask", " black");
    DAPIImage = getImageID();
    rename("DapiMaskImage");
   run("Watershed");
   run("Analyze Particles...", "size=40-100 clear include add");
   waitForUser("Delete squished cells");
   //Number cells
   ROICount=roiManager("Count");
   for (i=0; i<ROICount; i++) {
      roiManager("select", i);
      CellNumber=i+1;
      roiManager("Rename", "cell" + CellNumber);
          }
```

```
roiManager("Save", DataDir+CellROI);
    }
waitForUser("Note which are squished cells");
//Measure the cell ratios
run("Set Measurements...", "area mean standard integrated display redirect=None decimal=3");
selectImage(RatioImage);
roiManager("Deselect");
roiManager("Measure");
selectImage(UbiImage);
roiManager("Deselect");
roiManager("Measure");
i = nResults;    // variable for counting, initialising with 0
setResult("Image name", i, originalTitle);
setResult("thresholdUbi", i, thresholdUbi); // add a "Label" column to the results table and name the entry "point1"
setResult("thresholdMito", i, thresholdMito);
setResult("thresholdDAPI", i, thresholdDAPI);
  selectWindow("Results");
  saveAs("Results", DataDir + shortTitle + ".xls");
  }
```