

1 **Supplemental Method 2**

2 The annotated ImageJ macro for measuring GR+ and total cell counts in MDA-
3 MB-231 xenografted mammary gland tumors is shown below. Images were
4 captured using CRi Panoramic Scan Whole Slide Scanner (PerkinElmer Life
5 Sciences) with a 40x objective. Twenty images per tumor slide were exported
6 using the 3DHistech viewer software at a final magnification of 25x. Objects were
7 corrected for background illumination and a threshold based on DAPI illumination
8 was determined (Cy5 threshold values: 0.15, 0.45 and 0.96). Automated cell
9 counts were validated by manual counts of at least 100 objects per image.

```
10
11 //Total cell count (blud) = DAPI
12 //GR= cell count (red)
13 //Start with an RGB merge image blue, NO GREEN and red
14 //Designed to split RGB, count number of cells only in blue and red only
15 dir = getDirectory("Choose a Directory ");
16 list = getFileList(dir);
17 setBatchMode(true); // runs up to 6 times faster
18 for (f=0; f<list.length; f++) { //main files loop
19     path = dir+list[f];
20     if (!endsWith(path,"/") && endsWith(path,"p")) open(path); //do only bitmap files
21     if (nImages>=1) {
22         t=getTitle();
23         s=lastIndexOf(t, '.');
24         t=substring(t, 0, s);
25         t1=replace(t, " ", "_");
26         t2= t1 + ' DAPI outlines.tif';
27         t3= t1 + ' DAPI masks.tif';
28         t4= t1 + ' Cy5 outlines.tif';
29         t5= t1 + ' Cy5 masks.tif';
30         run("Split Channels");
31         run("Subtract Background...", "rolling=50");
32         setAutoThreshold("Default dark");
33         setThreshold(34, 255); // change DAPI threshold here
34         run("Convert to Mask");
35         run("Watershed");
36         run("Set Measurements...", " redirect=None decimal=0");
37         run("Analyze Particles...", "size=100-Infinity pixel circularity=0.00-1.00
38         show=Outlines summarize");
39         P1= path + t2;
40         save(P1);
41         close();
42         P2= path + t3;
```

```
43 save(P2);
44 close();
45 close();
46 run("Subtract Background...", "rolling=10");
47 run("Gaussian Blur...", "sigma=1");
48 setAutoThreshold("Default dark");
49 setThreshold(20, 255); // change Cy5 threshold here
50 run("Convert to Mask");
51 run("Watershed");
52 run("Set Measurements...", "area display redirect=None decimal=0");
53 run("Analyze Particles...", "size=100-Infinity pixel circularity=0.00-1.00
54 show=Outlines summarize");
55 P3= path + t4;
56 save(P3);
57 close();
58 P4= path + t5;
59 save(P4);
60 close();}}
```