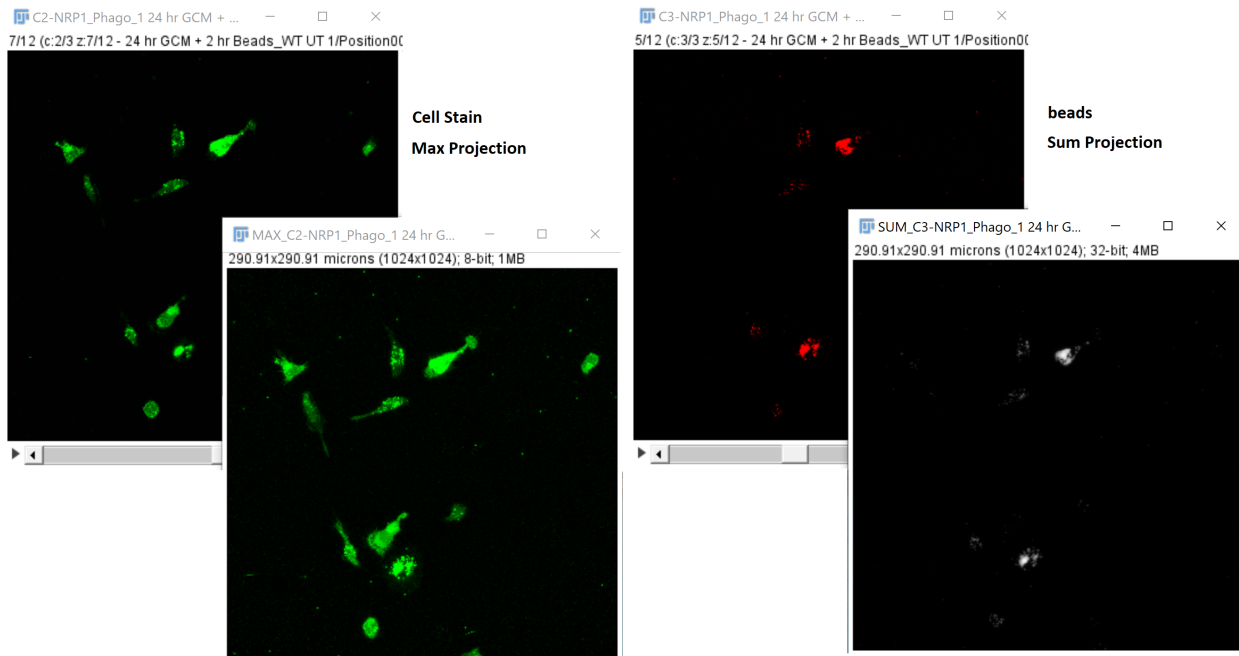
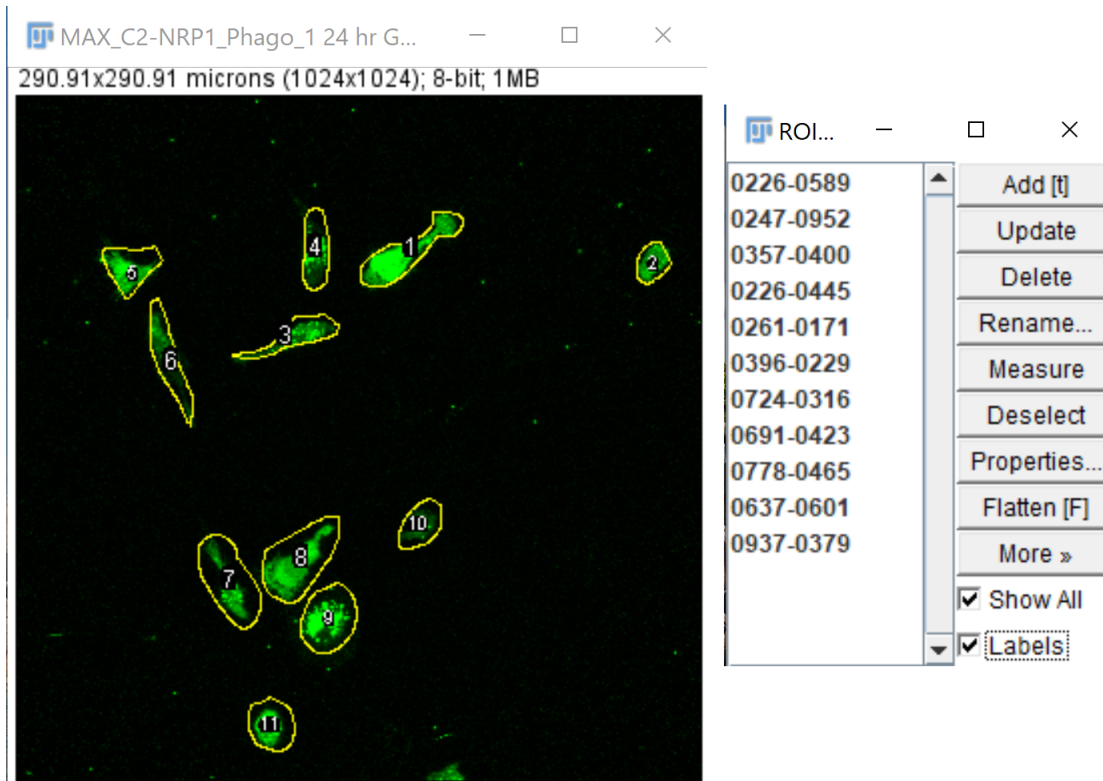


Supplementary Document 1: Image Analysis Protocol

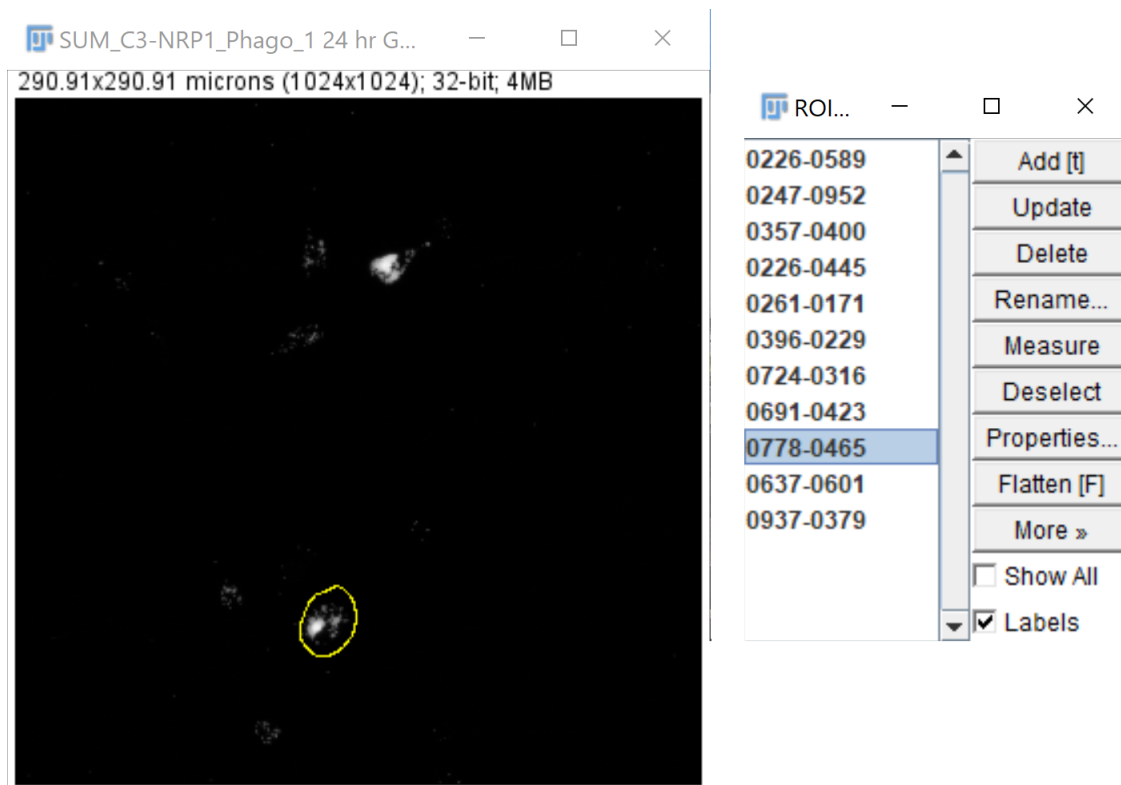
1. Import image into ImageJ (FIJI)
2. Split the channels of the image
3. On **cell stained channel** image (see green stain): *Image > Stacks > Z-Project > Projection type: Max Intensity*
4. On **bead channel** image (grayscale of beads): *Image > Stacks > Z-Project > Projection type: Sum Slices*



5. Open ROI manager: *Analyze -> tools -> ROI manager*
6. Trace cells of interest (cells that are completely in frame and do not overlap with other cells) using free hand selection tool. Add each cell to ROI manager by clicking 'add' or by pressing "T"



7. In **sum projected beads image**, click on an ROI. This should select the same cell in the grayscale sum projection.



8. To collect fluorescent signal value: *Analyze -> set measurements -> check area, mean gray value, and integrated density.*
9. To collect data: *Analyze -> measure or press Ctrl+M*

Set Measurements

Area Mean gray value

Standard deviation Modal gray value

Min & max gray value Centroid

Center of mass Perimeter

Bounding rectangle Fit ellipse

Shape descriptors Feret's diameter

Integrated density Median

Skewness Kurtosis

Area fraction Stack position

Limit to threshold Display label

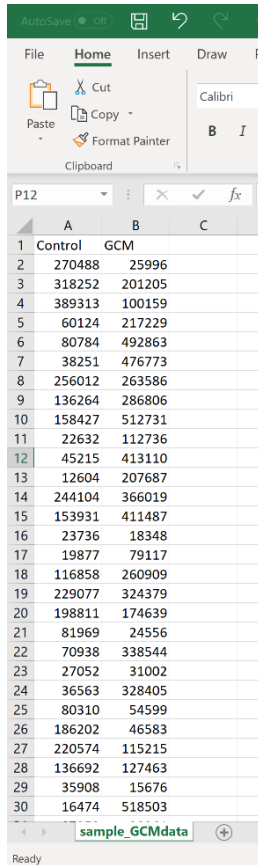
Invert Y coordinates Scientific notation

Add to overlay NaN empty cells

Redirect to:

Decimal places (0-9):

OK Cancel Help



10. Copy and paste results into Excel spreadsheet. We use the RawIntDen value, as it does not normalize to mean fluorescence of the selected cell. Thus, it captures the *sum* of the pixels generated across the z-stack.

11. To upload data to our KS UI, make a single column for each treatment/condition containing the RawIntDen values. Save this file as tab delimited .txt

Supplementary Table 1

A.

	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se
dist1	1	100	50	50.2518907629606	50	50	74.13	0	100	100	0	-2.0199	5.02518907629606
dist2	1	100	50.6	24.7337335587973	50	49.5	0	10	100	90	0.288785796296112	-0.176186756032821	2.47337335587973

B.

	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se
Control	1	184	108860.467391304	95322.0949728333	73540	94902.0135135135	74820.1503	6397	430396	423999	1.18851149405518	0.718687855257543	7027.23807378872
GCM	1	143	184355.629370629	150297.774662416	140567	165178.295652174	145948.6266	9683	598962	589279	0.872976096310908	-0.177242276540173	12568.5313183291

Supp table 1. Descriptive statistics from A. hypothetical data sets *dist1* and *dist2*, and B. Data collected from BMDM in experiment 1.