Supplementary tables

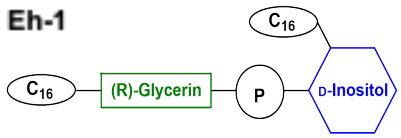
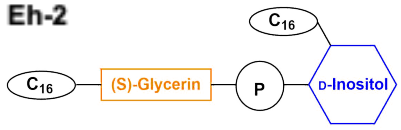
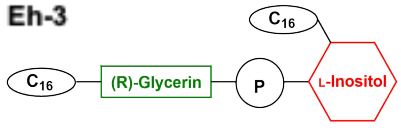
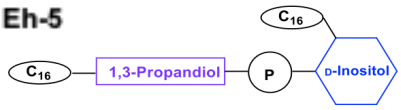
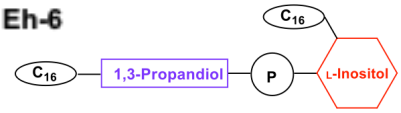
Table S1: Image analysis sequence established in Harmony software.

Analysis building block	Objective/ Specification	Parameters		
FIND NUCLEI	**Image segmentation:** Determines host cell nuclei	Channel: ROI: Method: Common Threshold: Area: Split Factor: Individual Threshold: Contrast: Output Population:	DAPI None B 0.02 > 40 µm² 16.9 0.14 > -0.71 Macrophages	
FIND CYTOPLASM	**Image segmentation:** Identifies host cell cytoplasm and defines single cells	Channel: Nuclei: Method: Individual Threshold:	Alexa 647 Macrophages A 0.06	
CALCULATE INTENSITY PROPERTIES	**Define regions of interest:** Quantifies properties in regions and removes border objects	Channel: Population: Region: Method: Mean Property Prefix:	DAPI Macrophages Nucleus Standard Nucleus DAPI	Alexa 647 Macrophages Cell Standard Cell Alexa 647
CALCULATE MORPHOLOGY PROPERTIES		Population: Region: Method: Property Prefix:	Macrophages Nucleus Standard (area/ roundness) Nucleus	
FIND SPOTS	**Image Segmentation:** Identifies intracellular spots within the region of interest	Channel: ROI: ROI Region: Method: Detection Sensitivity: Splitting Coefficient: Calculate Spot Properties Output Population:	Alexa 647 Macrophages Cell B 0.11 0.844 Spots	
CALCULATE MORPHOLOGY PROPERTIES	**Define properties of spots:** Quantification and calculation	Input Population: Region: Method: Output Property Prefix:	Spots Spot Standard (area / roundness/ width/ length/ ratio width to length) Spot	
		Input Channel: Population:	Alexa 647 Spots	DAPI Spots

CALCULATE INTENSITY PROPERTIES	of intracellular parasites	Region: Method: Output: Property Prefix:	Spot Standard (mean/ standard deviation/ coefficient of variance/ median) Intensity Spot Alexa 647	Spot Standard (mean) Intensity Spot DAPI
CALCULATE PROPERTIES		Population: Method: Formula: Variable A: Variable B: Output Property:	Spots By Formula A/B Spot Alexa 647 mean Spot DAPI mean ALEXA/ DAPI intensity ratio	
SELECT POPULATION I	**Identify intracellular *Leishmania* parasites:** Selects parasites from false-positive spots	Input Population: Method: Number of Classes:	Spots Linear Classifier 2	
		Relative Spot Intensity Corrected Spot Intensity Uncorrected Spot Peak Intensity Spot Contrast Spot Background Intensity Spot Area [px²] Region Intensity Spot to Region Intensity Spot Area [µm²]	Spot Roundness Spot Width [µm] Spot Length [µm] Spot Ratio Width to Length Spot Alexa 568 Mean Spot DAPI Mean ALEXA/DAPI intensity ratio Intensity Surrounding Alexa 568 Mean Intensity Surrounding Alexa 568 Median	
		Output Population A: Output Population B:	Likely *Leishmania* False-positive	
		Population:	Spots	
		Method: Likely *Leishmania:* Output Population:	Filter by property >0 Spots selected	
		Population: Method: Spot Area [µm²]: ALEXA/DAPI intensity ratio: Spot Area [µm²]: Corrected Spot Intensity: Boolean Operations:	Spots selected Filter by Property > 4 > 0.3 < 35 > 100 (Staining-dependent) F1 and F2 and F3 and F4	
CALCULATE PROPERTIES	**Macrophages and parasites are brought into relation**	Population: Method: Related Population: Number of *Leishmania* Output: Property Suffix:	Macrophages By related population *Leishmania* Per Cell	
		Population: Method:	Macrophages Filter by Property	

SELECT POPULATION II	**Identify subpopulation:** Defines infected cells within the total population	Number of Leishmania – per cell: Output Population: Number of Leishmania – per cell: Output Population: Number of Leishmania – per cell: Output Population:	>0 Infected macrophages >=2 Double infected macrophages >0 Seriously infected macrophages
DEFINE RESULTS	**Readout values:** Calculates readout values and measures the biological effects visible in the input image	Method: Population: Population: Population: Population:	List of outputs Macrophages - number of objects Leishmania - number of objects Infected macrophages - number of objects Seriously infected macrophages - number of objects
		Method: Formula: Population Type: Variable A: number of objects Variable B: number of objects Output name:	Formula Output 1. a/b 2. to 4. a/b*100 Objects 1. Leishmania 2. Infected macrophages 3. Seriously infected macrophages 4. Double infected macrophages Macrophages 1. Leishmania per infected macrophage 2. % infected macrophages 3. % seriously infected macrophages 4. Double infected macrophages

Table S2: Structures of *Eh*PIb compounds and IC_{50} determination.

Compound	*Leishmania ssp.*	IC50 [95% CI]	r^2
Eh-1 	*L. major* *L. braziliensis* *L. donovani* *L. infantum*	NA NA NA NA	< 0.5 < 0.5 < 0.5 < 0.5
Eh-2 	*L. major* *L. braziliensis* *L. donovani* *L. infantum*	NA NA NA NA	< 0.5 < 0.5 < 0.5 < 0.5
Eh-3 	*L. major* *L. braziliensis* *L. donovani* *L. infantum*	NA NA NA NA	< 0.5 < 0.5 < 0.5 < 0.5
Eh-4 	*L. major* *L. braziliensis* *L. donovani* *L. infantum*	NA NA NA NA	< 0.5 < 0.5 < 0.5 < 0.5
Eh-5 	*L. major* *L. braziliensis* *L. donovani* *L. infantum*	NA NA 0.93 [0.48 to 1.48] NA	< 0.5 < 0.5 0.9260 < 0.5
Eh-6 	*L. major* *L. braziliensis* *L. donovani* *L. infantum*	NA 4.685 [2.91 to 7.16] 2.294 [1.20 to 3.73] NA	< 0.5 0.5929 0.7778 < 0.5

Abbreviations: NA = not available, CI = confidence interval

Supplementary figures

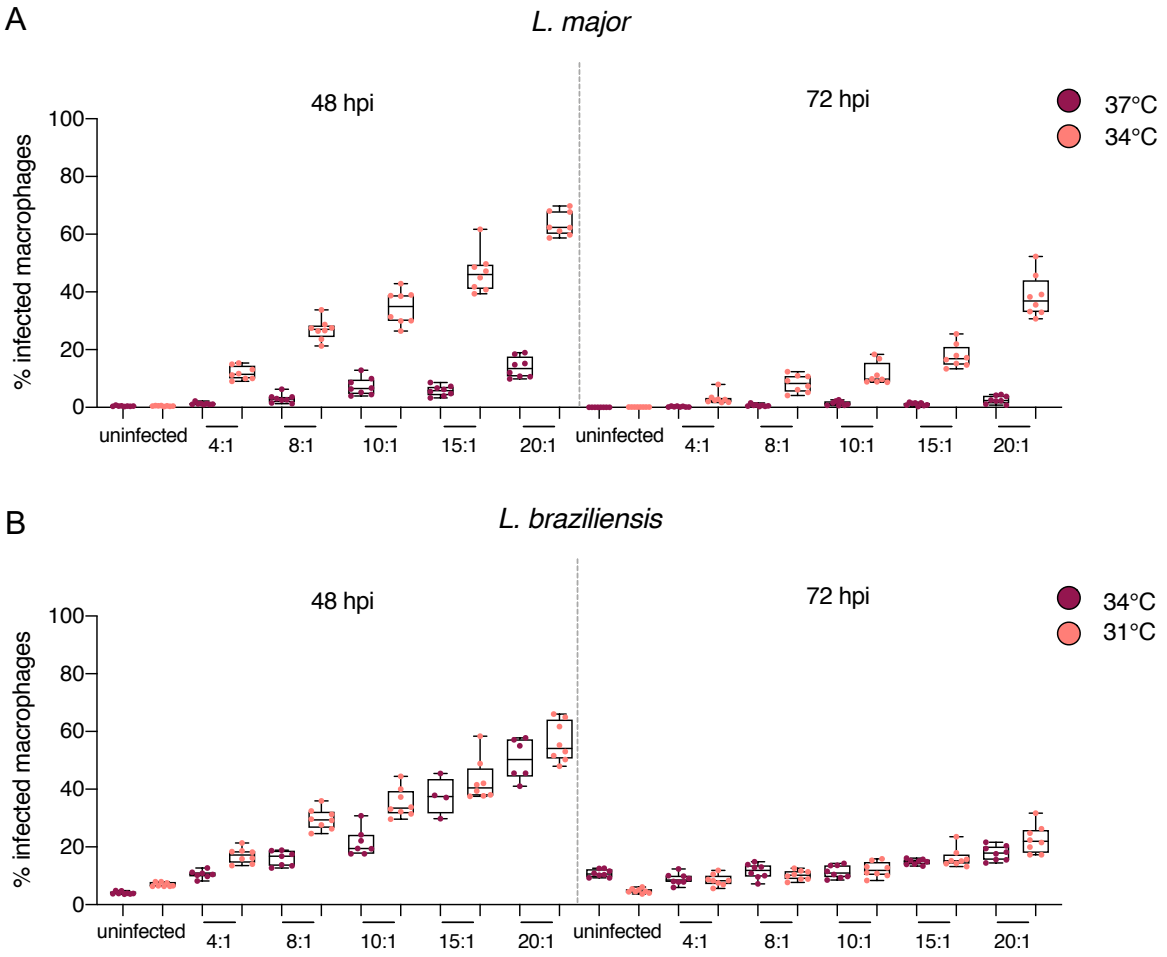


Figure S1: Influence on assay temperature for infection with cutaneous *Leishmania* species.

Percentage of infected bone-marrow derived macrophages (BMDMs) infected with (**A)** *L. major* (37°C, 34°C) or (**B)** *L. braziliensis* (34°C, 31°C) at different incubation temperatures and different multiplicities of infection (MOIs; 4:1/8:1/10:1/15:1/20:1) after 48 h and 72 h post infection. Data is expressed as boxplots with whiskers from minimum to maximum of percent of infected macrophages (n=8).