Module	Attribute	Comments	Data entry method	Relevant existing standards and ontologies
Study				
(contains 1 or more Study components)	Study type	Type of the overall study, which may include other imaging and/ or non-imaging data	text, ontology	EDAM-BIOIMAGING, FBbi, EFO, IDR
	Study description	Study description, e.g., title of published paper	text	IDR
	General dataset info	Authors, publications, licenses etc	misc.	Dublin Core, DataCite Metadata, schema.org, IDR
Study component				
(contains Image data and Analysed data)	Imaging method	Technique used to acquire image data	ontology	EDAM-BIOIMAGING, FBbi, OME
	Study component description	Description specific to this image dataset component	text	IDR
Biosample				
	Identity	Internal unique ID		
	Biological entity	What is being imaged	text and/or ontology entry (multiple possible)	EFO
	Organism	Species (multiple possible)	taxonomy	NCBI Taxonomy
	Intrinsic variable	Intrinsic (e.g. genetic) alteration if applicable	text and/or ontology entry (multiple possible)	EFO
	Extrinsic variable	External biosample treatment (e.g. reagent) if applicable	text and/or ontology entry (multiple possible) or associated file	EFO, IDR
	Experimental variables	What is intentionally varied (e.g. time) between multiple entries in this study component	text and/or ontology entry (multiple possible)	EFO
Specimen				
(linked to Biosample)	Experimental status	Test/ control		
	Location within Biosample	Plate/dish coordinate or tissue location	text or associated file	OME
	Preparation method	Sample preparation protocol	text, file, ontology, or widget for specific method types	EDAM-BIOIMAGING, FBbi
	Signal/contrast mechanism	How is the signal generated by this sample	text, ontology	EDAM-BIOIMAGING, FBbi
	Channel - content	Specific specimen staining (e.g. IEM, DAB)	text	
	Channel - biological entity	What molecule is stained	text, ontology entries	EFO
Image acquisition				
(linked to Specimen)	Instrument attributes	Details about instruments used	text, file, ontology, or widget for specific instrument types	EDAM-BIOIMAGING, FBbi, OME, 4DN-BINA-OME
	Image acquisition parameters	Image acquisition details	text, file, ontology, or widget for specific acquisition method types	EDAM-BIOIMAGING, OME, 4DN-BINA-OME
Image data				
(result of Image acquisition, or processing of Image data)	Type	Primary image/processed image/segmentation	pull-down	EDAM-BIOIMAGING
	Format & compression	File type	extract from data if possible	EDAM-BIOIMAGING, OME
	Dimension extents	Volume in pixels: x, y, z, tilts	extract from data if possible	OME
	Size description	Physical size of image volume in x,y,z & units (pull-down), OR magnification	extract from data if possible	OME
	Pixel/voxel size description	Physical size of pixels in x, y, z & units (pull-down)	extract from data if possible	OME
	Channel information	How are individual channels represented in the image	extract from data if possible	OME
	Image processing method	Image registration, other processing applied to this dataset	text, file, ontology, or widget for specific method types	EDAM-BIOIMAGING, FBbi
	Contrast inversion to TEM	Y/N; N if stained features result in brighter (whiter) signal; Y if it looks like a TEM image	pull-down	
	QC info	QC score for uploaded image quality if applicable	text or controlled vocabulary	
Image Correlation				
(linked to 1 or more Image data)	Spatial and temporal alignment	Method used to correlate images from different modalities (e.g. manual overlay, alignment algorithm etc)	text, ontology	EDAM-BIOIMAGING
	Fiducials used	Features from correlated datasets used for colocalization	text	
	Transformation matrix/ other info	Correlation transformations	text, or related project files (e.g. .hx Amira files)	
	Related images and relationship	Correlated dataset or images	link	
Analysed data				
	Analysis result type	Numerical analyses, segmention (non-image), categorical features/phenotypes	text, ontology	EDAM-BIOIMAGING, OME
	Data used for analysis	Specific feature set used for analysis (e.g. volume measurements, locations of features)	text or file(s)	
	Analysis method and details	Analysis method	text, file, ontology, or pointer to Methods section	EDAM-BIOIMAGING

Referenced existing standards and ontologies

EDAM-BIOIMAGING - an extension to the EDAM ontology (edamontology.org) for bioimage analysis, bioimage informatics, and bioimaging - https://bioportal.bioontology.org/ontologies/EDAM-BIOIMAGING

FBbi - Biological Imaging Methods Ontology - https://www.ebi.ac.uk/ols/ontologies/FBbi

OME - Open Microscopy Environment model, a specification for storing data on biological imaging - https://docs.openmicroscopy.org/ome-model/5.6.3/index.html

IDR - Image Data Resource, a public repository of image datasets, and the associated data submission guidelines - https://idr.openmicroscopy.org/about/submission.html

EFO - Experimental Factor Ontology, provides a systematic description of experimental variables, combines parts of several biological ontologies - https://www.ebi.ac.uk/efo/

NCBI Taxonomy - curated nomenclature for all of the organisms in the public sequence databases - https://www.ncbi.nlm.nih.gov/taxonomy

4DN-BINA-OME - minimum Information guidelines for fluorescence microscopy - https://github.com/WU-BIMAC/MicroscopyMetadata4DNGuidelines

Dublin Core - a metadata element set for describing resources - https://dublincore.org/

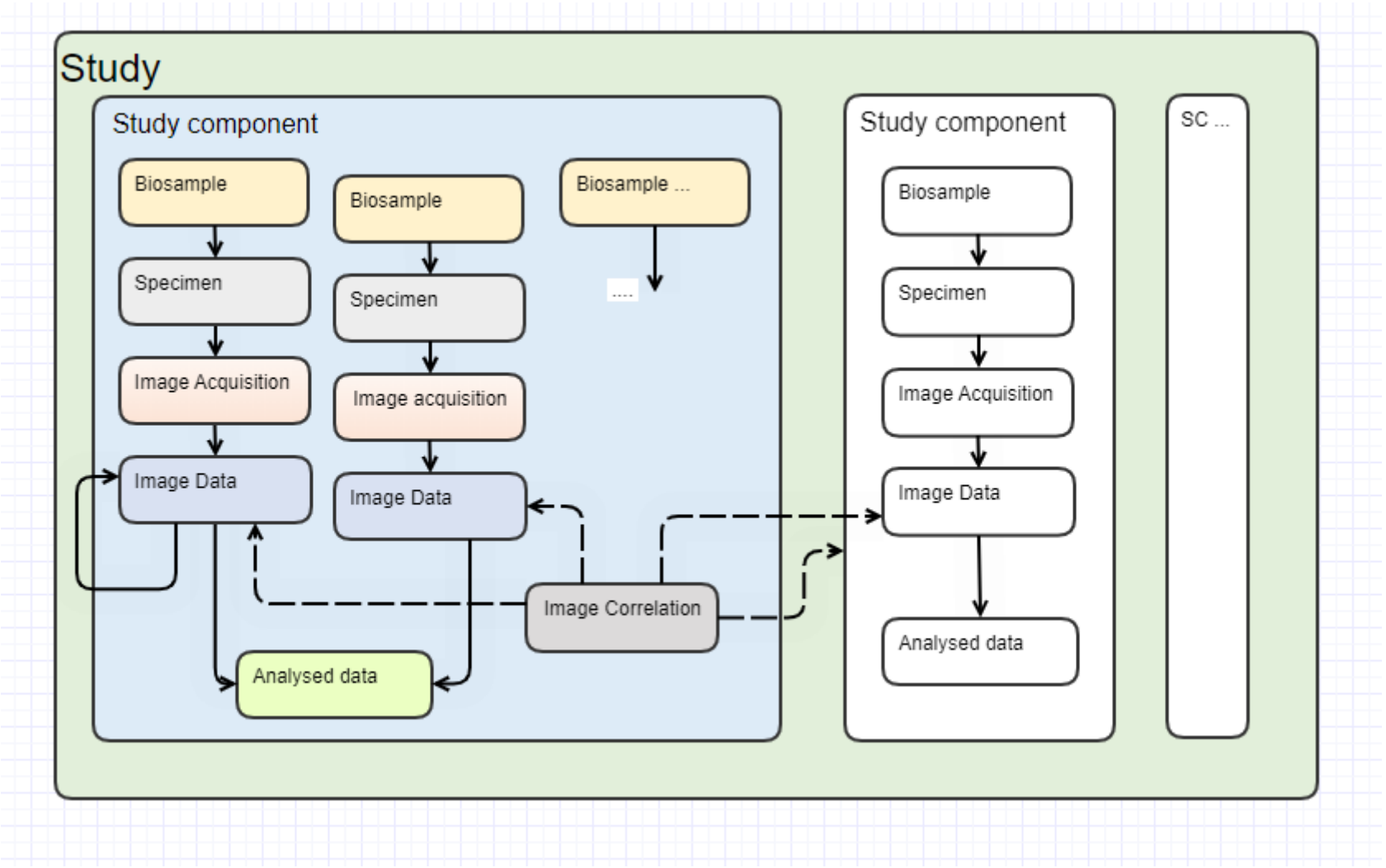
DataCite Metadata - core metadata properties for resource identification - https://schema.datacite.org/

schema.org - schemas for structured data on the Internet - https://schema.org/

Supplementary Figure 1. REMBI modules and attributes

Module	Attribute	Comments	WG1 - example 1	WG1 - example 2	WG2 - example 1	WG2 - example 2	WG3 - example 1	WG3 - example 2	WG3 - example 3	WG3 - example 4
Study										
(contains 1 or more Study components)	Study type	Type of the overall study, which may include other imaging and/ or non-imaging data	Single-particle cryo-EM	cryo-ET	CLEM/FIB-SEM of activated CD4+ T cells	FRAP/FIB SEM of parasitophorous vacuoles containing Toxoplasma gondii ΔCAP parasites	N/A	N/A	High-throughput/high-content screening	Atlas
	Study description	Study description, e.g., title of published paper	Single-particle cryo-EM at atomic resolution	Multi-particle cryo-EM refinement with M visualizes ribosome-antibiotic complex at 3.7 Å inside cells	Plasma Membrane LAT Activation Precedes Vesicular Recruitment Defining Two Phases of Early T-cell Activation	Differential requirements for cyclase-associated protein (CAP) in actin-dependent processes of Toxoplasma gondii	N/A	Ex vivo live cell tracking in kidney organoids using light sheet fluorescence microscopy		Data to build a mitotic cell atlas
	General dataset info	Authors, publications, licenses etc	https://doi.org/10.1101/2020.05.22.110189	https://doi.org/10.1101/2020.06.05.136341	PMID: 29789604	Hunt A , Russell MRG , Wagener J , Kent R, Carmeille R, Peddie CJ, Collinson L, Heaslip A, Ward GE, Treeck M Elife 8 (2019) PMID: 31577230 DOI: 10.7554/elife.50598	Images acquired by Jeffrey Skerker and annotated by Tom Morgan	PMID: 31527839	Hériché JK et al.. Mol Biol Cell. 2014 Aug 15;25(16):2522-2536. doi: 10.1091/mbc.E13-04-0221. PubMed PMID: 24943848	Cai Y, et al. An experimental and computational framework to build a dynamic protein atlas of human cell division. Nature. 2018 Sep 10. doi: 10.1038/s41586-018-0518-z. PubMed PMID: 30202089.
Study component										
(contains Image data and Analysed data)	Imaging method	Technique used to acquire image data	Single-particle cryo-EM	cryo-ET	FIB-SEM	FIB SEM	Differential interference contrast	Light sheet fluorescence microscopy	Epifluorescence microscopy	Confocal fluorescence microscopy + Fluorescence correlation spectroscopy
	Study component description	Description specific to this image dataset component	N/A	N/A	StudyComponentDescription.rtf	Processed FIB SEM images of a parasitophorous vacuole containing Toxoplasma gondii ΔCAP parasites	N/A	N/A	Image-based RNAi screen of 100 candidate genes predicted to be involved in mitotic chromosome condensation.	Image data used to build the mitotic cell atlas. This consists of three subsets: primary images, segmentation masks for the landmarks and protein concentration maps.
Biosample										
	Identity	Internal unique ID			pLAT_8O-2_FIBSEM	EM04226_2_U19	BBBC009			
	Biological entity	What is being imaged	apo-ferritin	ribosome-antibiotic complex	Jurkat E6.1 T cells; http://purl.obolibrary.org/obo/CL_0000084	Parasitophorous vacuole, in a human foreskin fibroblast, containing Toxoplasma gondii ΔCAP parasites	Red blood cells	Kidney tissue	HeLa cells	HeLa cells
	Organism	Species	Mouse	Homo sapiens	Homo Sapien; http://purl.obolibrary.org/obo/NCBITaxon_9606	Homo Sapiens, Toxoplasma gondii	Homo sapiens	CD1 Mus musculus embryonic, day 13.5		
	Intrinsic variable	Intrinsic (e.g. genetic) alteration if applicable	N/A	N/A	Jurkat E6.1 transfected with emerald-VAMP7	N/A	N/A	Wild type	stable overexpression of HIST1H2BJ-mCherry and LMNA-(	Homozygous GFP integration into mitotic genes + fluorescent dextran in medium + either stable overexpression of HIST1H2BJ-mCherry or SiR-Hoechst staining
	Extrinsic variable	External biosample treatment (e. g. reagent) if applicable	N/A	N/A	Plate-bound anti-CD3 activation	N/A	N/A	N/A	Library of 200 siRNAs, the sequences of which should be listed here as they are required for target gene inference.	
	Experimental variables	What is intentionally varied (e.g. time) between multiple entries in this study component	N/A	N/A	Time	Complementation of ΔCAP mutant with wild-type CAP (not done for this sample, hence mutant phenotype)	N/A	Genotype	time	time
Specimen										
(linked to Biosample)	Experimental status	Test/ control	N/A	N/A	N/A	Test (ΔCAP)	N/A	N/A		
	Location within Biosample	Plate/dish coordinate or tissue location	N/A	N/A	Plate2_8O	Experiment EM04226, dish 2, grid square U19	N/A	N/A	list of plate/wells coordinates	
	Preparation method	Sample preparation protocol	A frozen aliquot of 7mg/ml mouse apo-ferritin in 20mM HEPES pH 7.5, 150mM NaCl, 1mM dithiothreitol (DTT) and 5% trehalose, which we received from the Kikawa Lab at Tokyo University, was thawed at room temperature and cleared by centrifugation at 10,000g for 10 min. The supernatant was diluted to 5mg/ml with 20mM HEPES pH 7.5 150mM NaCl, and 3 µl of the diluted sample was applied onto glow-discharged R1.2/1.3 300 mesh UltrAuFoil gold grids (Quantifoil) for 30 s and then blotted for 5 s before plunge-freezing the grids into liquid ethane cooled by liquid nitrogen. Plunge-freezing was performed using a Vitrobot Mark IV (Thermo Fisher Scientific) at 100% humidity and 4 °C.	Mycoplasma pneu- moniae strain M129(ATCC 29342)cells were grown on 200 mesh gold grids coated with a holey carbon support (R 2/1, Quantifoil). Cells were cultivated at 37 °C in modified Hayflick medium: 14.7 g/L Difco PPLO (Becton Dickinson, USA), 20% (v/v) Gibco horse serum (New Zealand origin, Life Technologies, USA), 100 mM HEPES-Na (pH 7.4), 1% (w/w) glucose, 0.002% (w/w) phenol red and 1,000 U/mL freshly dissolved penicillin G. Chloramphenicol (Cm; Sigma-Aldrich, USA) was added 15 minutes prior to vitrification, at a final concentration of 0.5 mg/ml. Grids were quickly washed with PBS buffer containing 10 nm protein A-conjugated gold beads (Aurion, Netherlands), blotted from the back side for 2 seconds, and plunged into mixed liquid ethane/propane at liquid N2 temperature with a manual plunger (Max Planck Institute of Biochemistry, Germany). The cryo-EM grids were stored in a sealed box in liquid N2 before usage.	EM_SampProcProt_Revised_01.xls	RT fixation and megametal sample preparation for FIB SEM	N/A	CLARITY cleared		
	Signal/contrast mechanism	How is the signal generated by this sample	3D classification	Multiplying the FT of a particle image by the corresponding real-valued CTF	Heavy metal staining; m/z contrast detected by back-scatter electron detector	Osmium, uranium, lead, as contrast agents, m/z contrast detected by back-scatter electron detector	N/A	Antibodies	fluorescent proteins	fluorescent proteins and fluorescent dyes
	Channel - content	Specific specimen staining (e.g. IEM, DAB)	N/A	N/A	N/A	N/A	N/A	Megalin 1:200 overnight, Laminin 1:1000 overnight	red: HIST1H2BJ-mCherry, green: LMNA-eGFP	490-552:protein of interest-GFP; 587-621:HIST1H2BJ-mCherry | 622-695:SiR-DNA; 622-695:Dy-481XL-labelled 500 kDa dextran
	Channel - biological entity	What molecule is stained	N/A	N/A	N/A	N/A	N/A	Megalin: tubular lumen, green; Laminin: basement membrane, red	red: HIST1H2BJ-mCherry, green: LMNA-eGFP	490-552:protein of interest-GFP; 587-621:HIST1H2BJ-mCherry | 622-695:SiR-DNA; 622-695:Dy-481XL-labelled 500 kDa dextran
Image acquisition										
(linked to Specimen)	Instrument attributes	Details about instruments used							Automated Olympus IX-81 epifluorescence microscope with 20X objective	
	Image acquisition parameters	Image acquisition details	All cryo-EM data were collected on Falcon cameras in electron counting mode using Titan Krios microscopes (Thermo Fisher Scientific) operating at 300 kV. Before data acquisition, two-fold astigmatism was corrected and beam tilt was adjusted to the coma-free axis using the autoCTF program. All datasets were acquired automatically using EPU software (Thermo Fisher Scientific). Detailed data acquisition parameters for all data sets are given in Extended Data Table 1.	Tilt series data were collected on a Titan Krios TEM operated at 300 kV (Thermo Fisher Scientific) equipped with a field-emis- sion gun, a Gatan K2 Summit direct detector and a Quantum post-column energy filter (Gatan). Images were recorded in exposure-fractionation, counting mode using SerialEM 3.7.2. Tilt-series were acquired with a dose-symmetric scheme us- ing dedicated scripts51 with the following settings: TEM in na- no-probe mode, magnification 81,000 with a calibrated pixel size of 1.7 Å, energy filter in zero loss mode, defocus range 1.5 to 3.5 µm, tilt range -60° to 60° with 3° tilt increment and constant ex- posure per tilt, total exposure of 120 e-/Å2. In total, 65 tilt series were collected from Cm-treated cells.	FIB-SEM_Acquisition_Parameters_Samleson_pLAT_8O-2_correct.xls	5 nm isotropic, 10 µs dwell time. SEM at 1.5 kV with 1 nA current. EsB detector with grid voltage of 1.2 kV. Ion beam milling at accelerating voltage of 30 kV and current of 700 pA	N/A	N/A	Images acquired every 8.5 min for 44h.	
Image data										
(result of Image acquisition, or processing of Image data)	Type	Primary image/processed image/segmentation	micrographs	tilt series	Processed Image	Processed image	Primary image and segmentation outlines	Primary image	Primary image	Primary images, segmentation masks for the landmarks and protein concentration maps
	Format & compression	File type	EER	MRC	.mrc	.mrc	.tif	.czi	TIFF	
	Dimension extents	Volume in pixels: x, y, z, tilts	4096, 4096, 434	4096, 4096, 434	1000, 800, 546		800 x 600	x: 1920, y: 1920, z: 600, c: 2, v: 5, t: 1	x: 1344, y: 1024, t: variable, ~330	x: 256, y: 256, z: 31
	Size description	Physical size of image volume in x,y,z & units (pull down), OR magnification	N/A (can be calculated from voxel size)	N/A (can be calculated from voxel size)	15 x 12 x 8.719 um	15.62 x 16.70 x 3.61 µm	N/A	N/A (can be calculated from voxel size)		
	Pixel/voxel size description	Physical size of pixels in x, y, z & units (pull down)	14 14 14 nm	10 x 10 x 10 nm	15 x 15 x 15.97 nm	10 x 10 x 10 nm	N/A	0.57 um x 0.57 um x 1.30 um	0.32 um x 0.32 um	0.25 um x 0.25 um x 0.75 um
	Channel information	How are individual channels represented in the image								
	Image processing method	Image registration, other processing applied to this dataset	A total of 3370 movies in EER format were motion corrected with RELION's implementation of the MotionCor2 algorithm53. For this purpose, original hardware movie frames were dose fractionated into groups of 14 frames, corresponding to an accumulated dose of 1.3 e-/Å2 per fraction. CTF estimation was performed with CTFFIND-4.1.1354 using the sums of power spectra from combined fractions corresponding to an accumulated dose of 4 e-/Å2. Micrographs whose estimated resolution from CTFFIND was worse than 5 Å were removed, leaving 3080 micrographs for further processing.	Raw tilt movies were processed in Warp. De novo tilt series alignment was performed in IMOD using gold fiducials picked automatically with Warp's BoxNet, and the results were imported in Warp, where the tilt series CTFs were estimated. Using full tomograms reconstructed at 10 Å/px, two tomograms were de- noised using Warp's Noise2Map tool to pick the ribosome par- ticles manually. Using these coordinates, sub-tomograms were exported from Warp to RELION to obtain an initial reference.	cross-correlation based stack alignment + binning + inversion	Zeiss Atlas 5.2.1.25; Gradient align Export of cropped region of interest exported as 8 bit TIF files Fiji; Invert Gaussian blur: Sigma 0.75 Unsharp mask: Radius (Sigma) 1 pixel, Mask weight 0.6 Rotate to straighten XY (D19: no rotation) Reslice: YZ assigned to XY plane Scale to 10 nm isotropic voxels IMOD; tif2mrc	N/A	N/A		Segmentation, FCS-based intensity calibration
	Contrast inversion to TEM	Y/N; N if stained features result in brighter (whiter) signal; Y if it looks like a TEM image	N/A	N/A	Y	Y	N/A	N/A		
	QC info	QC score for uploaded image quality if applicable	N/A	N/A	N/A	N/A	N/A	N/A		
Image Correlation										
(linked to 1 or more Image data)	Spatial and temporal alignment	Method used to correlate images from different modalities (e.g. manual overlay, alignment algorithm etc)	N/A	Warp's BoxNet	Manual	Manual	N/A	N/A		
	Fiducials used	Features from correlated datasets used for colocalization	N/A	gold fiducials	Plasma membrane and nuclear membrane	Paraseite fluorescent reporter used for FRAP	N/A	N/A		
	Transformation matrix/ other info	Correlation transformations	N/A	N/A	N/A	N/A	N/A	N/A		
	Related images and relationship	Correlated dataset or images	N/A	N/A	pLAT_8O-2_Fluo.tif	FRAP, fluorescence after fixation, same area imaged (not deposited). EMPIAR-10326; raw data from which this entry was processed	N/A	N/A		
Analysed data										
	Analysis result type	Numerical analyses, segmention (non-image), categorical features/phenotypes	N/A	N/A	N/A	Decentralised residual body central axis skeletonisation (green), basal pole (orange), coarse segmenation of part of parasite surface membrane (yellow), putative ER in a region of the decentralised residual body (blue).	Segmentation outlines	N/A	Phenotypes	protein concentrations in time and space
	Data used for analysis	Specific feature set used for analysis (e.g. volume measurements, locations of features)	N/A	N/A	N/A	N/A	Primary image	N/A	associated HDF5 file	associated files: cell features, mitotic cell model, sequences used to edit the genomic loci (to identify the genes), protein concentrations (in nM) at each voxel of the mitotic cell model
	Analysis method and details	Analysis method	N/A	N/A	N/A	3dmod manual segmentation	Manual annotation	N/A	see Hériché JK et al.. Mol Biol Cell. 2014 Aug 15;25(16):2522-2536. doi: 10.1091/mbc.E13-04-0221. PubMed PMID: 24943848	Cai Y, et al. An experimental and computational framework to build a dynamic protein atlas of human cell division. Nature. 2018 Sep 10. doi: 10.1038/s41586-018-0518-z. PubMed PMID: 30202089.

Supplementary Figure 2. REMBI modules and attributes with examples.



Supplementary Figure 3. Digrammatic representation of REMBI modules.