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VIEWPOINT

Reproducibility

Better reporting is better science: Community-defined minimal reporting requirements for light microscopy

Paula Montero Llopis[1*], Chloë van Oostende-Triplet[2*], Nathalie Gaudreault[3], Caterina Strambio De Castillia[4], Julia Fernandez-Rodriguez[5], Gabriel Martins[6], Alison North[7], Luis Acevedo[8], Sergiy Avilov[9], Cristina Bertocchi[10,11], Ulrike Boehm[12], Lisa Cameron[13], Michael Cammer[14], Aurélie Cleret-Buhot[15], Steffen Dietzel[16], Orestis Faklaris[17], David Gaboriau[18], Thomas Guilbert[19], David Grunwald[20], Tingting Gu[21], Nadia Halidi[22,23], Mathias Hammer[20], Hella Hartmann[24], Janosch Heller[25,26], Helena Jambor[27], Ayse Aslihan Koksoy[28], Judith Lacoste[29], DeLaine Larsen[30], Sylvia Emmanuelle Le Dévédec[31], Penghuan Liu[32], Josh Moore[33], Glyn Nelson[34], Michael Nelson[35], Nils Norlin[36,37,38], Adam Parslow[39], Alexander L. Payne-Dwyer[40], John Peterson[41], Santosh Podder[42], Andrea Ravasio[43], Eduardo Rosa-Molinar[44], Britta Schroth-Diez[45], Olaf Selchow[46], Sathya Srinivasan[47], Douglas Taatjes[48], Kirstin Vonderstein[49], Christa Walther[50,51], and Roland Nitschke[52]

Incomplete reporting of microscopy methods undermines transparency, reproducibility, and data reuse. Despite recent initiatives, comprehensive, broadly endorsed, and accessible reporting guidelines are still lacking. Here, we present a bare minimal microscopy reporting requirements checklist that integrates human- and machine-readable input to provide clear, actionable guidance for researchers, reviewers, and publishers and to advance community standards in microscopy.

Introduction

Microscopy is central to discovery in the biomedical and life sciences. Continuously evolving to meet researchers' needs, it enables scientists to visualize and quantify the invisible and explore questions once thought beyond reach. Advances such as

[1]MicRoN Core Facility, Harvard Medical School, Boston, MA, USA; [2]CBIA Core Facility, Faculty of Medicine, University of Ottawa, Ottawa, Canada; [3]Allen Institute for Cell Science, Seattle, WA, USA; [4]Program in Molecular Medicine, UMass Chan Medical School, Worcester, MA, USA; [5]Centre for Cellular Imaging, University of Gothenburg, Gothenburg, Sweden; [6]Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal; [7]Bio-Imaging Resource Center, The Rockefeller University, New York, NY, USA; [8]Quantum Medical Diagnostics & UMKC, Overland Park, KS, USA; [9]Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany; [10]Pontificia Universidad Católica de Chile, Santiago, Chile; [11]University of Osaka, Osaka, Japan; [12]Carl Zeiss AG, Oberkochen, Germany; [13]Light Microscopy Core Facility, Duke University, Durham, NC, USA; [14]Microscopy Core, NYU Langone Grossman School of Medicine, New York, NY, USA; [15]Research Center of the Hospital, Center of Université de Montréal (CRCHUM), Montréal, Canada; [16]Core Facility Bioimaging and Institut für Kardiovaskuläre Physiologie und Pathophysiologie, Biomedical Center, Ludwig-Maximilians-Universität München, Munich Germany; [17]Institut Jacques Monod, Université Paris Cité, CNRS, Paris, France; [18]Facility for Imaging by Light Microscopy (FILM), Imperial College London, London, UK; [19]INSCOPER, Cesson-Sévigné, France; [20]RNA Therapeutics Institute, University of Massachusetts Chan Medical School, Worcester, MA, USA; [21]Samuel Roberts Noble Microscopy Laboratory, University of Oklahoma, Norman, OK, USA; [22]Advanced Light Microscopy Unit, Centre for Genomic Regulation (CRG), Barcelona, Spain; [23]The Barcelona Institute of Science and Technology, Barcelona, Spain; [24]CMCB Technology Platform, Light Microscopy Facility, TU Dresden, Dresden, Germany; [25]School of Biotechnology & DCU Life Sciences Institute, Dublin City University, Dublin, Ireland; [26]UCL Queen Square Insitute of Neurology, University College London, London, UK; [27]DAViS, University of Applied Sciences of the Grisons, Chur, Switzerland; [28]UT MD Anderson Cancer Center, Houston, TX, USA; [29]MIA Cellavie Inc., Montreal, Canada; [30]Eikon Therapeutics, Hayward, CA, USA; [31]Leiden Academic Centre for Drug Research (LACDR), Leiden University, Leiden, the Netherlands; [32]College of Optical and Electronic Technology, China Jiliang University, Hangzhou, China; [33]German BioImaging-Gesellschaft für Mikroskopie und Bildanalyse e.V., Constance, Germany; [34]Bioimaging Unit, Newcastle University, Newcastle Upon Tyne, UK; [35]BioMedical Engineering Department, University of Wisconsin-Madison, Madison, WI, USA; [36]Lund University bioimaging Centre, Lund University, Lund, Sweden; [37]Department of Experimental Medical Science, Lund University, Lund, Sweden; [38]NanoLund, Lund University, Lund, Sweden; [39]Baker Heart and Diabetes Institute, Melbourne, Australia; [40]Bioscience Technology Facility, Department of Biology, University of York, York, UK; [41]Imaging Core, Shared Laboratory Resources, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA; [42]Microscopy Facility, IISER, Pune, India; [43]Institute for Biological and Medical Engineering (IIBM) Schools of Engineering, Medicine and Biological Sciences Pontificia Universidad Católica de Chile, Santiago, Chile; [44]Department of Cell Biology & Physiology and Neuroscience, Washington University in Saint Louis School of Medicine, Washington University Center for Cellular Imaging, St. Louis, MO, USA; [45]Light Microscopy Facility, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; [46]Microscopy & BioImaging Consulting, Thuringia, Germany; [47]Imaging and Morphology Support Core - Integrated Pathology Core, Oregon National Primate Research Center, Beaverton, OR, USA; [48]Microscopy Imaging Center. Larner College of Medicine, University of Vermont, Burlington, VT, USA; [49]Biomedicum Imaging Unit (BIU), University of Helsinki, Helsinki, Finland; [50]GermanBioImaging, Konstanz, Germany; [51]GloBIAS, Klosterneuburg, Austria; [52]Life Imaging Center and Signalling Research Centres CIBSS and BIOSS, University of Freiburg, Freiburg, Germany.

*P. Montero Llopis and C. van Oostende-Triplet contributed equally to this paper. Correspondence to Paula Montero Llopis: paula_monterollopis@hms.harvard.edu; Chloë van Oostende-Triplet: cvanoost@uottawa.ca.

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super-resolution imaging, spatial-omics, tissue clearing, and volumetric imaging, to cite just a few, continue to expand our ability to uncover the complexity of biological systems in unprecedented detail. To ensure these powerful techniques effectively drive discovery, detailed and transparent reporting of microscopy methods, including hardware specifications and other key metadata, is essential. Yet, despite imaging's central role in most biomedical research, methodological details are still too often underreported (Marqués et al., 2020). Improving rigor and reproducibility in microscopy reporting is not merely a matter of compliance; it is an opportunity to strengthen the reliability and impact of our science: improving reporting accelerates discovery, enables others to build upon existing work, and reinforces confidence in published results.

Why, then, does rigorous microscopy methods reporting remain so challenging?

The rapid innovation in advanced microscopy techniques makes it difficult for researchers to stay current or develop deep expertise in every technology. Extensive training requirements often conflict with the pressure for rapid publication, leading to an incomplete understanding of how microscope configurations affect experimental reproducibility, data reuse, and comparability. Unfortunately, microscopes are often treated as "black boxes," offering little incentive or guidance for learning essential controls that ensure accurate, artifact-free results. Most critically, the absence of standardized publication guidelines prevents consistent, comprehensive reporting.

Encouragingly, efforts to strengthen both methods reporting and the broader understanding of what impacts reproducibility and interpretability in microscopy are gaining momentum. Recent publications and, notably, a pilot initiative by the *Nature Portfolio*, introducing updated microscopy reporting guidelines, reflect growing recognition of this need (Light microscopy reporting for reproducibility, 2025). However, while many valuable tools and frameworks have been created and made available (Larsen et al., 2023; Hammer et al., 2021; Ryan et al., 2021; Kunis et al., 2021; Montero Llopis et al., 2021; Czymmek et al., 2025; Konshin et al., 2025), their limited adoption or lack of universal endorsement has delayed the standardization of microscopy method reporting.

Building consensus and the development of the proposed standards

For reporting standards and guidelines to be effective and widely adopted, they must be accessible, understandable, and relevant across scientific disciplines. Most importantly, such standards must be developed through broad expert consensus and remain applicable across diverse microscopy methods and modalities.

Advancing accurate methods reporting, rigor, and reproducibility in light microscopy has been the central mission of Working Group 11 (WG11) of the Quality Assessment and Reproducibility for Instruments and Images in Light Microscopy (QUAREP-LiMi) Consortium. From its inception, QUAREP-LiMi has united a broad international community of imaging scientists, core facility staff experts, principal investigators, and industry partners committed to improving quality control and reproducibility in microscopy (Nelson et al., 2021; Boehm et al., 2021).

QUAREP-LiMi follows a rigorous, transparent consensus process described in detail in the QUAREP-LiMi bylaws (Grunwald et al., 2025): guidelines and protocols developed within each working group undergo continuous discussion, review, and agreement by all active members of the consortium before being approved by its Editorial Board and Steering Committee. Each QUAREP-LiMi publication thus represents a true, community-wide consensus among experts (https://quarep.org/resources/publications/).

Through this collaborative approach, WG11 developed the bare minimal microscopy reporting requirements checklist (Montero Llopis et al., 2025) (Table 1), a universal framework designed to enhance reproducibility and scientific rigor in light microscopy. The checklist is a learning resource for nonspecialists and experts alike, aimed at deepening the understanding of the essential information that should be included in any publication featuring microscopy data. Its design allows it to be applied broadly, independent of specific modalities or techniques.

The bare minimal microscopy reporting requirement checklist

The checklist organizes essential metadata into two main categories that capture key aspects of fluorescence light microscopy: how the specimen is prepared (specimen setup) and how images are collected (image acquisition, including hardware and acquisition setup) (Fig. 1). Its structure intentionally aligns with existing community efforts, such as the recommended metadata for biological images framework and the QUAREP-LiMi-endorsed light microscopy model (LiMi-model, formerly NBO-Q metadata model) (Hammer et al., 2021; Sarkans et al., 2021), ensuring consistency across ongoing initiatives. Additionally, it expands and complements the recently published QUAREP-LiMi Working Group 12 recommendations for image publication, processing, and analysis of image data (Schmied et al., 2023) (Fig. 1 image data, image processing, and analyzed data categories).

The checklist is guided by three key principles:

(1) Clarity: making microscopy metadata terminology understandable for all users, with representative examples of how to describe each parameter.
(2) Practicality: ensuring that requirements are achievable in everyday research environments by experts and nonexperts alike.
(3) Ease of adoption: enabling implementation across disciplines, journals, and institutions.

The checklist intentionally focuses on the minimum information essential for transparency and reproducibility in all light microscopy experiments. More advanced techniques will require additional metadata beyond what is captured in this checklist. QUAREP-LiMi WG11 is currently developing modality-specific checklists to meet those needs. Nonetheless, this framework represents an important first step toward a culture of open, rigorous, and reproducible microscopy reporting.

To enhance interoperability, the checklist includes a machine-readable column that aligns each metadata field with the LiMi-model, standardizing terminology. This facilitates integration with existing community-driven open microscopy environment (OME) tools and resources

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Table 1. **Minimal requirements for reporting microscopy methods, including machine-readable metadata, shaded (LiMi-model metadata specifications), and representative examples to facilitate microscopy methods reporting.**

Categories		Examples	Machine-readable LiMi-model alignment
Specimen setup			
Sample mounting	Cover glass (cover glass number or thickness; coating)	Samples were grown on #1.5H cover glass (Marienfeld), coated with 1 mg/ml collagen type I (C8919; Sigma-Aldrich)	CoverGlass/CoverGlassNo CoverGlass/Thickness CoverGlass/Coating
	Mounting medium or imaging medium (name and manufacturer)	Prior to imaging, samples were mounted in SlowFade Glass mounting medium (Thermo Fisher Scientific)	MountingMedium/Model MountingMedium/Manufacturer
Sample labeling[a]	Fluorescent protein (specific variant or probe)	mGFPmut3, GCaMP6f	
	Dye (name, manufacturer, and concentration)	MitoTracker Green at 1 µg/ml final Secondary antibody conjugated to Alexa Fluor 647	
Hardware setup			
Microscope stand	Description (manufacturer; model; inverted or upright)	Microscopy imaging experiments were performed on a Nikon Ti2 inverted microscope stand	MicroscopeStand/Manufacturer MicroscopeStand/Model MicroscopeStand subtype (Inverted, Upright)
Modalities and modules/add- on	Specify the modalities and modules used[b]	Microscope stand was equipped with a Yokogawa spinning disk CSU-W1 and a SORA module	Pixels/Channel/IlluminationType (Wide-field_Fluorescence, Confocal_ Fluorescence_array-raster- scan, Confocal_ Fluorescence_spinning disk)
		For phase contrast imaging, we used a phase contrast objective and a respective phase plate in the condenser	
		Fluorescent images were captured on a Zeiss Observer.Z1 widefield microscope equipped with an Apotome module	
Objective and additional magnification	Full designation (description, specification) found on the barrel of the objective (magnification, numerical aperture, correction type, and immersion type)	Images were acquired using a 100×/ 1.45 DIC Plan Apochromat, oil immersion objective	Objective/Magnification Objective/LensNA Objective/Correction Objective/ImmersionType
	Additional Magnification (magnification changer)	In addition, a 1.5× Optovar was inserted in the Lightpath	MagnificationChanger
Light source	Manufacturer and model (for non-laser light sources) Type (for non-laser light sources) (e.g., LED, mixed metal halide, and mercury)	Samples were illuminated using an LED light engine (Spectra X, Lumencor)	LightSource subtype (Arc, Filament, GenericLightSource, Laser, LightEmittingDiode, MultiLaserEngine) LightSource/Manufacturer
	Specify the excitation wavelength used (for laser-based)	DAPI excitation was performed using a 405 nm diode laser	LightSource/Model LightSource/Laser LightSource/PeakWavelength
Wavelength selection	Specific filters or filter cubes (excitation filter center wavelength/FWHM, emission filter center wavelength/ FWHM, and optionally company, filter name)	Alexa Fluor 488 was imaged using filter cube 38 HE (Zeiss, BP 470/40 Ex, DC495 dichroic, BP525/50Em)	FilterCube Filter/TransmittanceRange/Wavelength Filter/TransmittanceRange/FWHMBandwidth Filter/Manufacturer Filter/Model
	Adjustable wavelength selector (e.g., spectral detection in a point scanner), cut on/cutoff wavelengths	eGFP emission was detected with a spectral detector between 500 and 544 nm, with a spectral width of 8.9 nm for the single detection elements	Dichroic/TransmittanceRange/Wavelength
Detection system (as applicable)	Camera (manufacturer and model)	An Orca Flash 4.0 (Hamamatsu) monochrome camera	Camera/Manufacturer Camera/Model
	Point detector (type)	A 32-channel GaAsP detector was used to detect the emitted signal	PointDetector subtype (Multialkali/GaAsP)PhotomultiplierTube, PhotoDiode, HybridPhotoDetector)

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Table 1. **Minimal requirements for reporting microscopy methods, including machine-readable metadata, shaded (LiMi-model metadata specifications), and representative examples to facilitate microscopy methods reporting. (*Continued*)**

Categories		Examples	Machine-readable LiMi-model alignment
Acquisition setup	All acquisition settings optimized to acquire the image must be reported, and should include but not be limited to:		
Acquisition settings	Camera-based: exposure time	The DAPI channel was captured with 30 ms exposure time (camera based)	Pixels/Plane/ExposureTime
	Point detector–based: pinhole size (in AU), pixel dwell or scan speed, simultaneous or sequential acquisition	The images were captured sequentially, with a pixel dwell time of 2 µs and a 1 AU pinhole and no averaging	PinholeSettings/Aperture Pixels/Plane/PixelDwellTime ConfocalScannerSettings/ScanningFrequency ConfocalScannerSettings/ MultiChannelMode(Parallel or Sequential)
	Final effective image pixel size (in the image)	Final image pixel size was 0.065 um/pixel	Pixels/PhysicalSizeX Pixels/PhysicalSizeY
	Z-stack settings (z-step increment, number of steps, total range)	Images were acquired as 15 µm range z-stacks with a 100 nm z-step interval	Pixels/PhysicalSizeZ Pixels/SizeZ
	Time series settings (time increment and total acquisition time)	Time-lapse imaging was performed for 2.5 h with a 10 min interval	Pixels/Plane/TimeIncrement
	Tiling settings	Tiling was performed with a 10% overlap	
Acquisition software	Name, manufacturer, and version	NIS-Elements AR V5.21 (Nikon) Micro-manager 2.0.0	AcquisitionSoftware/Name AcquisitionSoftware/Developer AcquisitionSoftware/Version

[a]Sample preparation is essential for rigorous and reproducible experiments but is covered in other sections of the Materials and methods. This includes detailed protocols for growth, transfection, and imaging conditions (for live-cell experiments), as well as labeling methods such as fixation, permeabilization, and antibody concentrations. We include the fluorescent protein variant or dye used, as this information, often omitted, is crucial for evaluating the hardware setup and acquisition settings.
[b]Modality refers to a microscopy technique such as wide-field, confocal, light-sheet, two photon, STED, etc. Modules are hardware components that enable a modality, such as a confocal scan unit or TIRF arm.

(Zulueta-Coarasa et al., 2025; Goldberg et al., 2005; Allan et al., 2012; Moore et al., 2021, 2023), which already support the automatic capture, storage, and exchange of microscopy metadata in standardized formats. Aligning human-readable method descriptions with machine-readable metadata clarifies how the two complement each other and helps researchers meet FAIR principles (Konshin et al., 2025; Wilkinson et al., 2016), making data findable, accessible, interoperable, and reusable.

Together, these efforts promote and support consistency across imaging platforms and enhance the usability and adoption of the checklist within the broader microscopy community. They represent not only a step toward better reporting but a collective move toward a more open, transparent, and collaborative future for image-based science.

Impact, implementation, and next steps

For researchers, the bare minimal microscopy reporting requirements checklist provides a simple yet powerful tool to guide both the design and reporting of microscopy experiments. The checklist helps identify essential metadata and informs deliberate decisions about hardware and acquisition settings that may influence the interpretation and conclusion of results. After completion of the experiments, it ensures that all critical parameters are accurately recorded in the methods. Researchers can follow the clear sequence outlined in the checklist when reporting microscopy experiments: describing the sampling and mounting procedure, including the labeling technique; detailing the equipment hardware selection, including modality modules, optics, illumination, wavelength selection, and detection; specifying how images were captured; and listing all parameters needed to address the specific scientific question. By using the checklist throughout the experimental workflow, researchers not only improve the transparency and completeness of microscopy reporting but also foster thoughtful, conscious experimental design, ultimately improving education in microscopy and strengthening the reliability and reproducibility of their findings.

For reviewers, editors, and funding agencies, the checklist offers a clear structure to evaluate methodological rigor and transparency. Because it aligns with the LiMi-model and OME tools, it integrates seamlessly with existing data standards and review workflows. Its concise design encourages adoption by journals and reduces barriers to establishing common publication standards.

The introduction of this checklist is particularly timely. Across the scientific ecosystem, momentum is building to strengthen reproducibility and transparency in microscopy. Funding agencies such as the NIH and NSF in the United States and the European Commission through Horizon Europe's Open Science policy are prioritizing reproducibility, FAIR data reuse, open data, and metadata standards in research infrastructure programs. In Europe, initiatives such as the EU-funded project Open Science to Increase Reproducibility in Science further highlight this commitment. As mentioned, publishers

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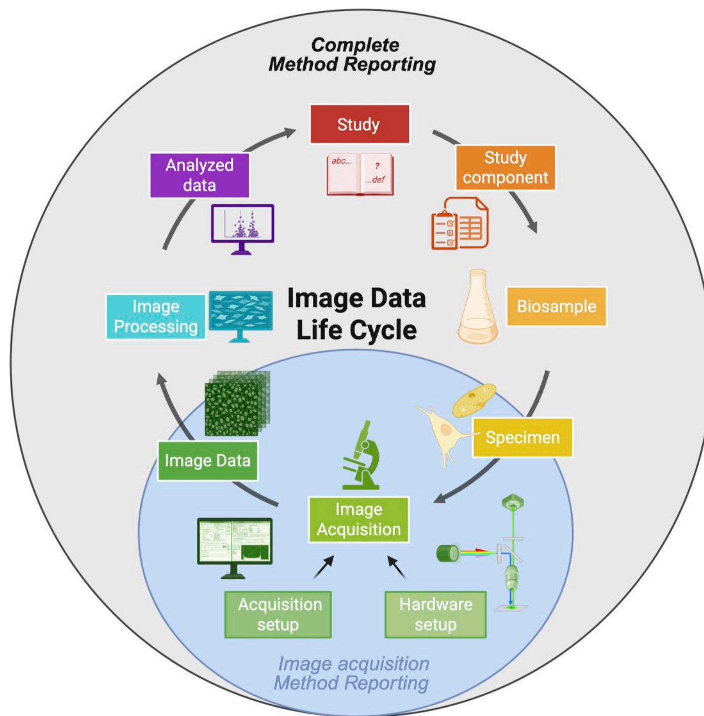

Figure 1. **Categories critical to producing complete and accurate microscopy methods reporting, structured according to the recommended metadata for biological images (REMBI) framework and the QUAREP-LiMi–endorsed LiMi-model.** The bare minimal microscopy reporting requirements checklist focuses on image acquisition metadata and aspects of the specimen that affect image acquisition (shaded blue). Created in https://BioRender.com.

are also responding to this movement: several *Nature Portfolio* journals have recently piloted microscopy reporting guidelines developed in collaboration with QUAREP-LiMi WG11. Meanwhile, community, consortia such as BioImaging North America, Global BioImaging, Euro-Bioimaging, and QUAREP-LiMi, continue to advance and promote shared standards, open data practices, and education in quantitative imaging while fostering international collaboration.

At a time when public trust in science faces mounting pressure, initiatives like this provide a constructive and unifying path forward. By making rigorous reporting more accessible, we empower researchers to communicate their work in traceable, reproducible ways. Each step toward openness strengthens the credibility of scientific discovery and fosters a culture of accountability and excellence. Ensuring that image data are truly FAIR expands the impact of research and enables AI-driven discovery (Zulueta-Coarasa et al., 2025). Through collaboration among scientists, imaging specialists, institutions, publishers, and founders, we can build a research ecosystem where every image, dataset, and result contributes to a more trustworthy and inspiring future for science.

Data availability

The authors apologize that, due to space limitations, it was not possible to cite all relevant literature. A more comprehensive list of references supporting this work is available at the Zenodo repository from QUAREP-LiMi https://doi.org/10.5281/zenodo.18289058.

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Author contributions: Paula Montero Llopis: conceptualization, data curation, methodology, project administration, supervision, visualization, and writing—original draft, review, and editing. Chloë van Oostende-Triplet: conceptualization, data curation, methodology, project administration, visualization, and writing—review and editing. Nathalie Gaudreault: conceptualization, data curation, methodology, and writing—review and editing. Caterina Strambio De Castillia: conceptualization, data curation, methodology, and writing—review and editing. Julia Fernandez-Rodriguez: conceptualization, data curation, methodology, and writing—review and editing. Gabriel Martins: conceptualization, data curation, methodology, and writing—review and editing. Alison North: conceptualization, data curation, methodology, visualization, and writing—review and editing. Luis Acevedo: conceptualization, methodology, resources, visualization, and writing—review and editing. Sergiy Avilov: conceptualization, methodology, and writing—review and editing. Cristina Bertocchi: conceptualization, data curation, methodology, and writing—review and editing. Ulrike Boehm: conceptualization, methodology, and validation. Lisa Cameron: conceptualization, data curation, methodology, and writing—review and editing. Michael Cammer: conceptualization and writing—review and editing. Aurélie Cleret-Buhot: conceptualization and writing—review and editing. Steffen Dietzel: conceptualization, data curation, methodology, and writing—review and editing. Orestis Faklaris: writing—review and editing. David Gaboriau: conceptualization, data curation, methodology, and writing—review and editing. Thomas Guilbert: validation and writing—review and editing.

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