BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

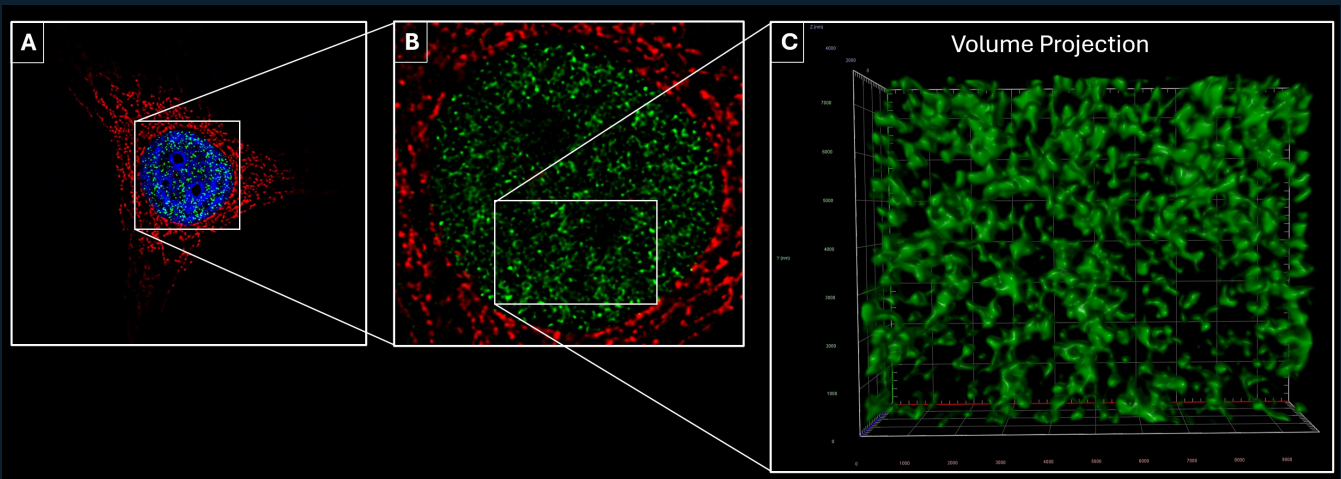
BAP1.P

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human BAP1.p (Serine 592) Polyclonal - Identifyn® SRM Alexa Fluor™ 488

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN SIM -> SIM²

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A - This panel displays a 2-dimensional view at z-plane 68 of 135, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green). Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red) and DAPI nuclear staining (blue) employing super resolution SIM Squared. A white box indicates a region of interest and will further reduce the area investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 68 of 135 (same z-plane as previous panel), from the Region of Interest shown in Panel A. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green) and the Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the control for the resolution, as mitochondrial structure is well-documented and reproducible. A white box indicates a region of interest and will be further reduced and investigated in Panel C.

Panel C - This panel showcases the region of interest indicated in Panel B, displaying a three-dimensional volume projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate.

Note - It is evident that super-resolution SIM, in this case, SIM Squared, provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. And using the same sample as was imaged for SIM, we show here a gain in resolving the three-dimensional structural localization details of BAP1.p localization following etoposide-induced DNA damage. Again, we make no claims as to what this image represents, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is DNA damage-induced localization at or near the DNA break sites.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000049 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

UNPUBLISHED / PENDING SCIENTIST REVIEW / NO PUBLICATION AUTHORIZED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for BAP1.p, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM²

BENNETT ASSESSMENT

The preserved DC-000049 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000049 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 7 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

The record supports retrospective, source-bounded microscopy review. Target identity, specificity, treatment effect, binding, interaction and mechanism are not inferred from presentation images alone.

CONTROL ASSESSMENT

No separate control record is preserved; none is inferred from method or template language.

CLAIM CEILING

This draft supports historical capability documentation, source registration, modality ordering, settings review and bounded interpretation. It does not independently validate antibody specificity, quantify a population response, establish binding, interaction, causation or mechanism, or authorize publication.

PROCESSING DISCLOSURE

Retrospective BENNETT architectural evaluation of preserved Identifyn source evidence. BENNETT did not perform native-file reprocessing, new deterministic measurement or the historical image post-processing represented in this record.

REAGENTS AND ACQUIRED CHANNELS

Preparation reagents are reported separately from channels actually documented in the acquisition settings. Fluorophores mentioned in staining are not automatically treated as acquired channels.

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	3; 38 eGFP; 50 Cy 5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 - 550
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 38 HE eGFP; 50 Cy 5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 - 550
Confocal	405/DAPI; 488; 647	3; None; 488 nm @0.3%; 639 nm @0.08%; 405 nm @0.2%; 493; 653; 353
Airyscan	405/DAPI; 488; 647	3; None; 488 nm @0.8%; 639 nm @0.2%; 405 nm @0.2%; 493; 653; 353
SIM	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 488
SIM²	405/DAPI; 488; 647; 680	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 488

MODALITY ASSESSMENT / PART 1 OF 7

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s). Structured source metadata values: 11.

VISIBLE OBSERVATION

A preserved presentation image is available for Western blot. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 488.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Band position and displayed intensity do not independently establish analytical specificity, target identity, linearity, or treatment effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Western blot evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 2 OF 7

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 994 X 743; Image Size (Pixels): 994 X 743; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 200 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4; 1.25. Structured source metadata values: 40.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

A presentation image supports gross localization and source-bounded co-occurrence, not direct binding, molecular colocalization, or native quantitative measurement.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 3 OF 7

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 82 Slices (8.1 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 906 X 688; Image Size (Pixels): 906 X 688; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 250 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20.00%; X-Cite Xylis Lamp Intensity @8.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 43.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield SIM — Apotome. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Improved optical sectioning does not by itself establish reconstructed super-resolution or molecular interaction.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield SIM — Apotome evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 4 OF 7

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 151 Slices (4.5 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1066 X 1066; 574 X 889; Image Size (Pixels): 1066 X 1066; 574 X 889; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.32 µs; Frame Time: 3.52 s. Illumination: Laser Wavelength: 488 nm @0.3%; 639 nm @0.08%. Optical/scan: Pinhole: 1.00 AU / 50 µm; 1.00 AU / 65 µm; Scan Zoom: 2.1; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.1 (3D, Auto); 7.7 (3D, Auto). Structured source metadata values: 65.

VISIBLE OBSERVATION

A preserved presentation image is available for Confocal. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Confocal evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 5 OF 7

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 104 Slices (3.09 µm); 57 Slices (1.68 µm); Channels: 3; Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm; Image size (Pixels): 1711 X 1711; 851 X 604; Image Size (Pixels): 1711 X 1711; 851 X 604; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.21 µs; Frame Time: 17.06 s. Illumination: Laser Wavelength: 488 nm @0.8%; 639 nm @0.2%. Optical/scan: Pinhole: 5.00 AU / 250 µm; 5.00 AU / 328 µm; Scan Zoom: 2.2; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 9.0 (3D, Auto); 9.9 (3D, Auto). Structured source metadata values: 69.

VISIBLE OBSERVATION

A preserved presentation image is available for Airyscan. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Displayed feature separation remains reconstruction dependent; no unsupported numerical resolution or molecular architecture claim is permitted.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Airyscan evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 6 OF 7

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 135 Slices (4.02 µm); Channels: 3; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1665 X 1830; Image Size (Pixels): 5056 X 5056; 1665 X 1830; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 488 nm @2.50%; 640 nm @2.00%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 65.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Reconstruction-dependent structural separation requires native reconstruction QC before numerical resolution or molecular dimensions can be claimed.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 7 OF 7

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 135 Slices (4.02 µm); Channels: 3; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1342 X 1342; Image Size (Pixels): 5056 X 5056; 1342 X 1342; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 488 nm @2.50%; 640 nm @1.00%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 28°, Scale Z 82%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 64.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM². BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 647; 680.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Further reconstruction-dependent segmentation does not establish molecular dimensions, structure identity, or mechanism without independent support.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM² evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

CROSS-MODALITY SYNTHESIS

The preserved DC-000049 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

Different acquisitions are not treated as a quantitative intensity ladder. Any continuity statement remains qualitative and source bounded.

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

BENNETT uses independently verified primary scientific sources to test whether the interpretation is scientifically consistent and properly bounded. Literature correlation does not retroactively validate the Identifyn dataset or replace scientist review.

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.

- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

1. Uhlen M, et al. A proposal for validation of antibodies. Nat Methods. 2016;13:823-827. doi:10.1038/nmeth.3995.
2. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198:82-87. doi:10.1046/j.1365-2818.2000.00710.x.
3. Sage D, et al. Quantitative evaluation of software packages for single-molecule localization microscopy. Nat Methods. 2015;12:717-724. doi:10.1038/nmeth.3442.

RELEASE BOUNDARY

This assessment remains a draft pending scientist review. No publication is authorized. Any future native-file analysis must enter BENNETT as a new evidence snapshot with routed engine authority, native-data QA, methods, limitations, and an independently reviewed claim ceiling.

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

Historical Identifyn source material was subjected to BENNETT retrospective QA. Original source state and correction history remain preserved in provenance.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm -> 0.03529 µm X 0.03529 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1711 X 1711; Image Size (Scaled)=60.37 µm X 60.37 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm -> 0.01689 µm X 0.01689 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=85.40 µm X 85.40 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm -> 0.01689 µm X 0.01689 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=85.40 µm X 85.40 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM; 2µM; 60 nm -> Etoposide = 2 µM. The governing scientist identified every 200 µM occurrence as a technician transcription error. The canonical historical concentration is 2 µM. Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

HIGH CONFIDENCE - SOURCE-DERIVED METADATA CANONICALIZATION

Product identity / phosphosite: Blank structured canonical field -> Serine 592. The phosphorylation site is explicitly stated in the preserved source product identity. Supporting evidence: Rabbit anti-Human BAP1.p (Serine 592) Polyclonal - Identifyn® SRM Alexa Fluor™ 488

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

BENNETT has completed the bounded retrospective assessment.
The following pages switch ownership to the preserved Identifyn record.

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

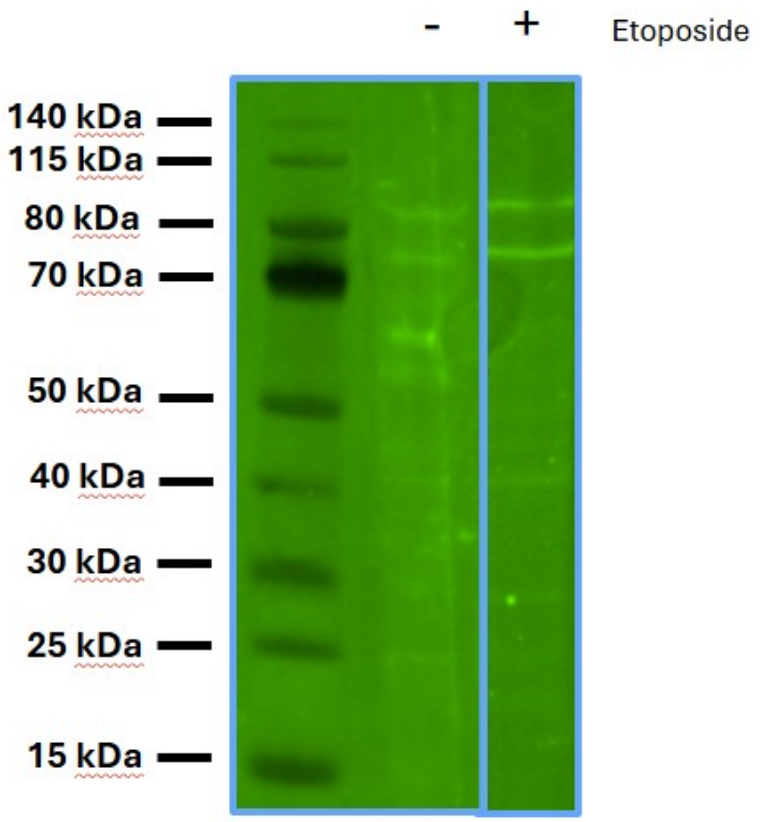
This dossier preserves the complete available evidence progression for DC-000049. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	488
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	488nm
Emission Filter	513±17nm
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000049

Expected Molecular Weight: 100 kDa

HeLa cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet down the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80°C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12%, Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank The Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and washed 5X with PBS. The blocked membrane was incubated with Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG for 2 hours, followed by 5X PBS washes. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)

Scan Settings

Detection mode = Fluorescence
Laser = 488nm
Emission Filter = 513±17nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: K. Small
Data Presentation by P.D. Amistoso

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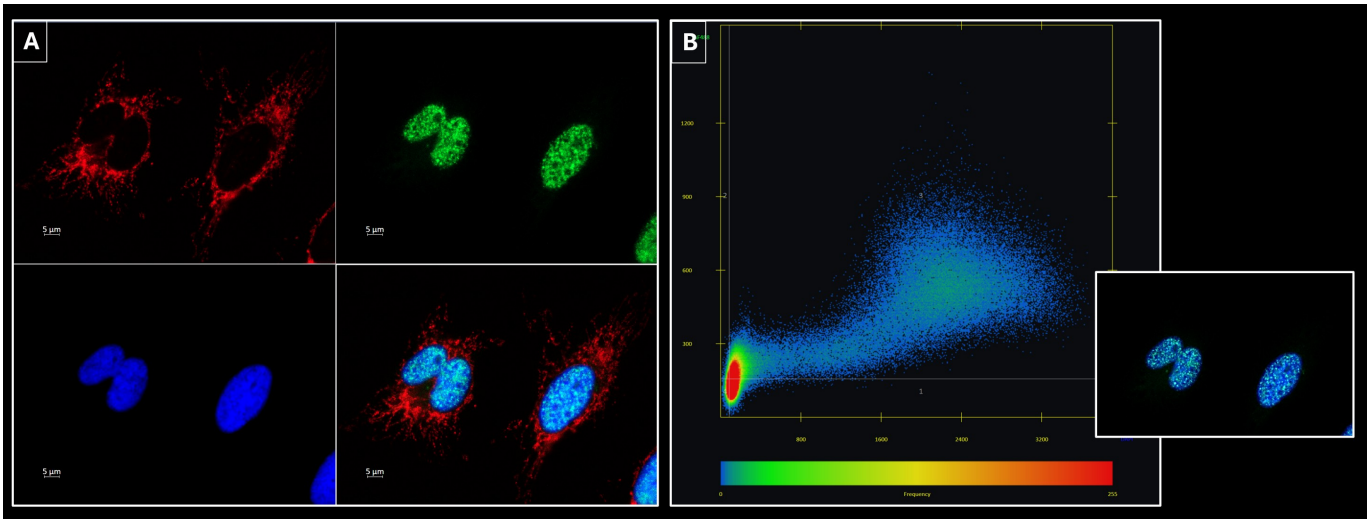
SOURCE PROVENANCE

Catalog	DC-000049
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D6F617C0F3FD71FB56D0FC8EC26202FB5BD9785BC0B62023F8F6CB33E68A0F81
Method PDF SHA-256	930414356B6504E1A93FEAFD0B4D62FDBA6199B2CD80C0241886D6BA88BDC34D

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A - Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate. The upper left section features Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). The upper right displays Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 (green), the lower left section shows DAPI nuclear staining (blue), while the lower right presents a merged image of the three channels.

Panel B – Displays a colocalization view of the DAPI (X-axis) and Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 (Y-axis), showing that BAP1.p is strongly colocalized with DAPI and is thereby, via this 2-dimensional image, within the nuclear compartment as expected for this protein following Etoposide induced DNA damage.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	40

STRUCTURED ACQUISITION METADATA / WIDEFIELD

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	994 X 743
Image Size (Scaled)	108.87 µm X 81.38 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	38 eGFP | 50 Cy 5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450 - 490 | 625 - 655 | 335 - 383
Filter Emission Wavelength	500 - 550 | 665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00% | X-Cite Xylis Lamp Intensity @8.00%
Exposure Time	200 ms | 100 ms | 20 ms
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4 | 1.25
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.80 µm | 1.03 µm | 0.72 µm
Binning Mode	2,2

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000049

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR, although its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody followed this, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 994 X 743
Image Size (Scaled) = 108.87 µm X 81.38 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 200 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel features Mitochondria visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the top-right panel shows Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels.

Colocalization View - Panel B

Horizontal Axis - DAPI, Threshold 92, Range Auto
Vertical Axis - AF488, Threshold 158, Range Auto

Summary

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal -Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate. The upper left section features Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right displays Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 (green), the lower left section shows DAPI nuclear staining (blue), while the lower right

presents a merged image of the three channels.

Panel B - Displays a colocalization view of the DAPI (X-axis) and Rabbit anti-Human BAP1.p Polyclonal -Identifyn® SRM Alexa Fluor™ 488 (Y-axis), showing that BAP1.p is strongly colocalized with DAPI and is thereby, via this 2-dimensional image, within the nuclear compartment as expected for this protein following Etoposide-induced DNA damage.

Image Correction

None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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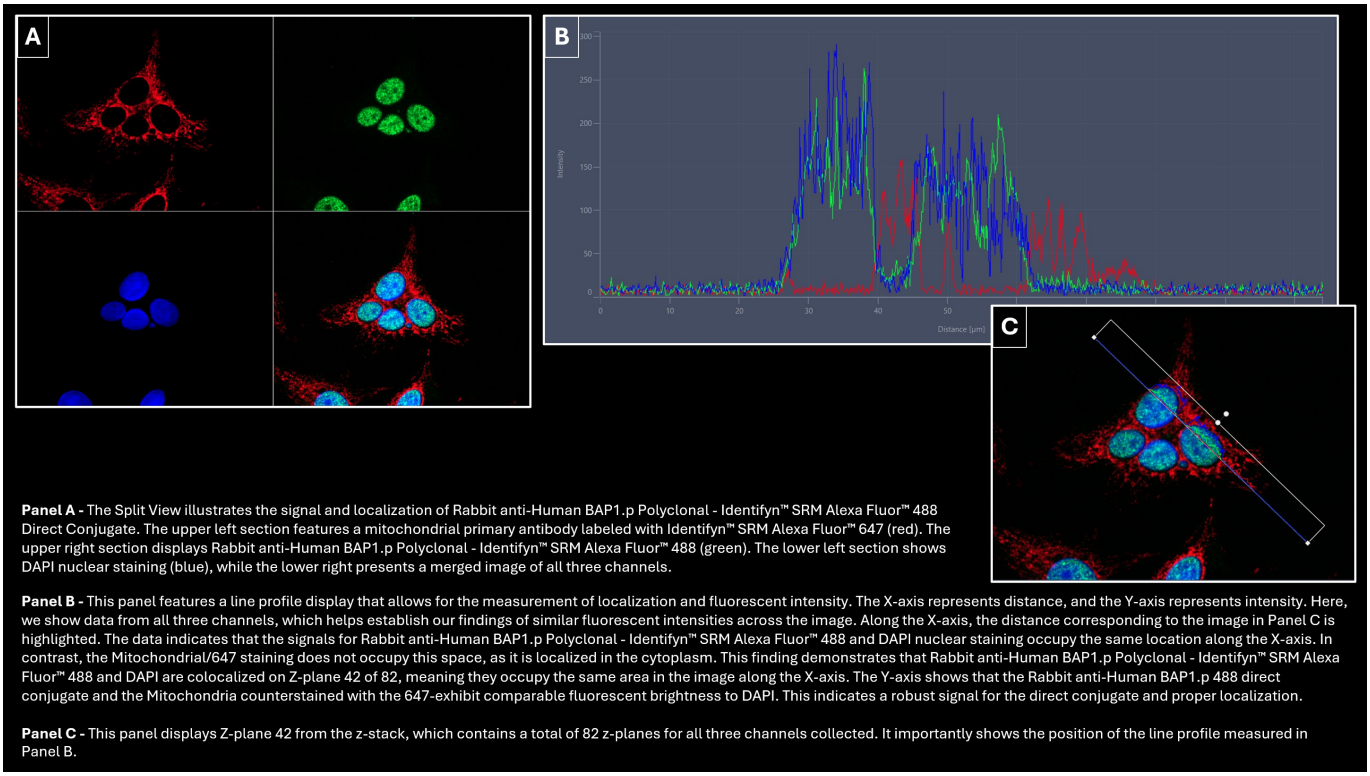
SOURCE PROVENANCE

Catalog	DC-000049
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	46243F8DFE42276E4EBA4F9709905ED898F654B96E73B0174053574EDE4FA87C
Method PDF SHA-256	2B191CE0A97CA3978711937C67337BAEA8C57EE959F423B962E494D1C32FFAC1

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A - The Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate. The upper left section features a mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). The upper right section displays Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 (green). The lower left section shows DAPI nuclear staining (blue), while the lower right presents a merged image of all three channels.

Panel B - This panel features a line profile display that allows for the measurement of localization and fluorescent intensity. The X-axis represents distance, and the Y-axis represents intensity. Here, we show data from all three channels, which helps establish our findings of similar fluorescent intensities across the image. Along the X-axis, the distance corresponding to the image in Panel C is highlighted. The data indicates that the signals for Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 and DAPI nuclear staining occupy the same location along the X-axis. In contrast, the Mitochondrial/647 staining does not occupy this space, as it is localized in the cytoplasm. This finding demonstrates that Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 and DAPI are colocalized on Z-plane 42 of 82, meaning they occupy the same area in the image along the X-axis. The Y-axis shows that the Rabbit anti-Human BAP1.p 488 direct conjugate and the Mitochondria counterstained with the 647-exhibit comparable fluorescent brightness to DAPI. This indicates a robust signal for the direct conjugate and proper localization.

Panel C - This panel displays Z-plane 42 from the z-stack, which contains a total of 82 z-planes for all three channels collected. It importantly shows the position of the line profile measured in Panel B.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	43

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	82 Slices (8.1 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	906 X 688
Image Size (Scaled)	129.43 µm X 98.29 µm
Bit Depth	14 Bit
Image Center Position	X: 40.74 mm, Y: 52.73 mm
Stage Position	X: 40.74 mm, Y: 52.73 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	38 HE eGFP | 50 Cy 5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450 - 490 | 625 - 655 | 335 - 383
Filter Emission Wavelength	500 - 550 | 665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20.00% | X-Cite Xylis Lamp Intensity @8.00% | X-Cite Xylis Lamp Intensity @6.00%
Exposure Time	250 ms | 100 ms | 15 ms
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.00 µm | 1.30 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mod" "Optical Sectioning" - enabled correction selected.
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 104.1), Y (Min 0 and Max 306)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000049

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR. However, its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. hosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 42 of 82 total - Panel A

Z Stack - (3D)

Z-Stack = 82 Slices (8.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 906 X 688
Image Size (Scaled) = 129.43 µm X 98.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 40.74 mm, Y: 52.73 mm
Stage Position = X: 40.74 mm, Y: 52.73 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 250 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 100 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.00%
Exposure Time = 15 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mod" "Optical Sectioning" - enabled correction selected. Normalization, Phase Corrector, Fourier Filter, and Deconvolution were NOT used.

Split View - Panel A

The top-left panel features Mitochondria visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the top-right panel shows Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels.

Profile View Display - Panel B & C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 104.1), Y (Min 0 and Max 306)

Summary

Panel A - The Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate. The upper left section features a mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right section displays Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 (green). The lower left section shows DAPI nuclear staining (blue), while the lower right presents a merged image of all three channels.

Panel B - This panel features a line profile display that allows for the measurement of localization and fluorescent intensity. The X-axis represents distance, and the Y-axis represents intensity. Here, we show data from all three channels, which helps establish our findings of similar fluorescent intensities across the image. Along the X-axis, the distance corresponding to the image in Panel C is highlighted. The data indicate that the signals for Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 and DAPI nuclear staining occupy the same location along the X-axis.

In contrast, the Mitochondrial/647 staining does not occupy this space, as it is localized in the cytoplasm. This finding demonstrates that Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™488 and DAPI are colocalized on Z-plane 42 of 82, meaning they occupy the same area in the image along the X-axis. The Y-axis shows that the Rabbit anti-Human BAP1.p 488 direct conjugate and the Mitochondria counterstained with the 647 exhibit comparable fluorescent brightness to DAPI. This indicates a robust signal for the direct conjugate and proper localization.

Panel C - This panel displays Z-plane 42 from the z-stack, which contains a total of 82 z-planes for all three channels collected. It importantly shows the position of the line profile measured in Panel B.

Image Correction

None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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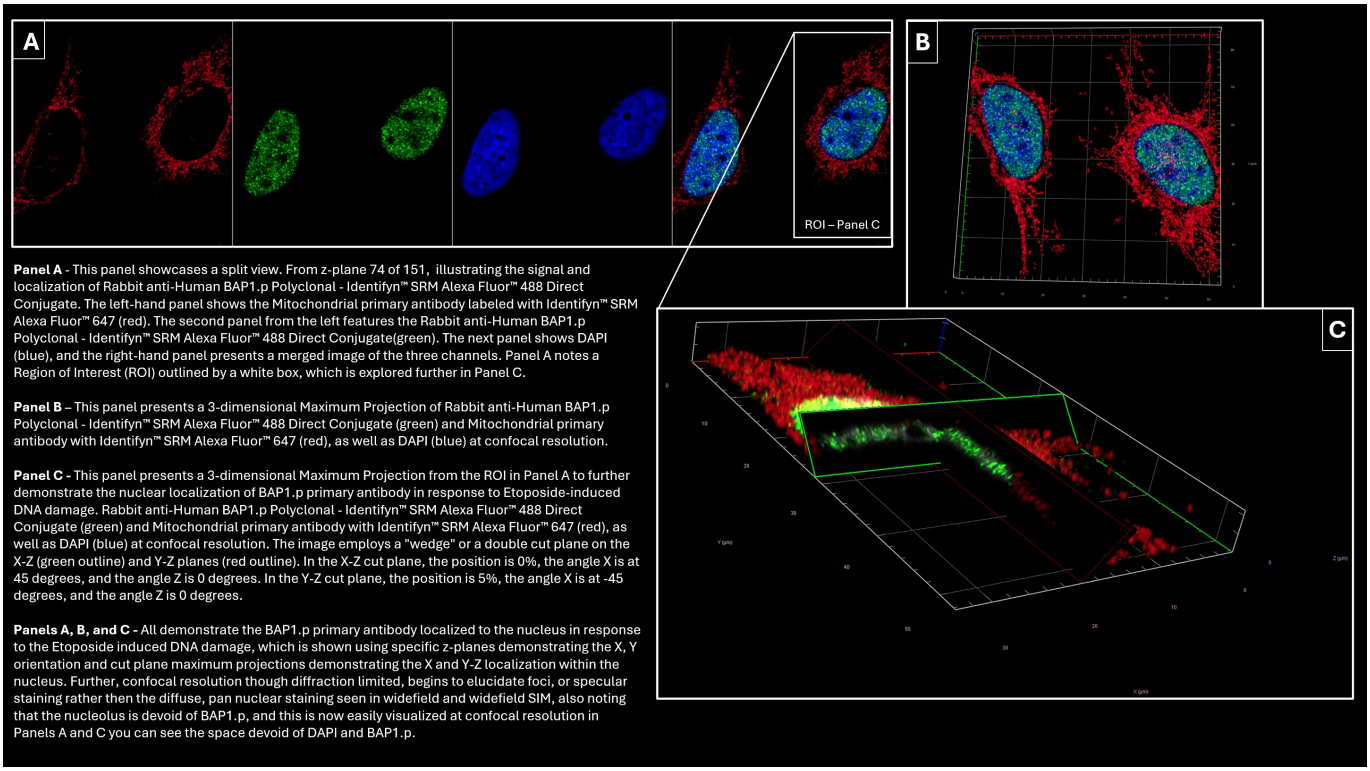
SOURCE PROVENANCE

Catalog	DC-000049
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	43
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C1995A4C961F2FA59A151734BEC2CCCC32E8BA50F7D3E52C05262570AAD00586
Method PDF SHA-256	2DA2ACDCBB6CD06AE7042B7F93054605F7FA47909E3FAF758F5D3FB5A2F77DB0

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A - This panel showcases a split view. From z-plane 74 of 151, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate. The left-hand panel shows the Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). The second panel from the left features the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate(green). The next panel shows DAPI (blue), and the right-hand panel presents a merged image of the three channels. Panel A notes a Region of Interest (ROI) outlined by a white box, which is explored further in Panel C.

Panel B – This panel presents a 3-dimensional Maximum Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green) and Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 647 (red), as well as DAPI (blue) at confocal resolution.

Panel C - This panel presents a 3-dimensional Maximum Projection from the ROI in Panel A to further demonstrate the nuclear localization of BAP1.p primary antibody in response to Etoposide-induced DNA damage. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green) and Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 647 (red), as well as DAPI (blue) at confocal resolution. The image employs a "wedge" or a double cut plane on the X-Z (green outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is 0%, the angle X is at 45 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is 5%, the angle X is at -45 degrees, and the angle Z is 0 degrees.

Panels A, B, and C - All demonstrate the BAP1.p primary antibody localized to the nucleus in response to the Etoposide induced DNA damage, which is shown using specific z-planes demonstrating the X, Y orientation and cut plane maximum projections demonstrating the X and Y-Z localization within the nucleus. Further, confocal resolution though diffraction limited, begins to elucidate foci, or specular staining rather then the diffuse, pan nuclear staining seen in widefield and widefield SIM, also noting that the nucleolus is devoid of BAP1.p, and this is now easily visualized at confocal resolution in Panels A and C you can see the space devoid of DAPI and BAP1.p.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	65

STRUCTURED ACQUISITION METADATA / CONFOCAL

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	151 Slices (4.5 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1066 X 1066 | 574 X 889
Image Size (Scaled)	62.71 µm X 62.72 µm | 33.77 µm X 52.31 µm
Bit Depth	16 Bit
Image Center Position	X: 77.54 mm, Y: 49.37 mm
Stage Position	X: 77.54 mm, Y: 49.37 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 50 µm | 1.00 AU / 65 µm | 1.00 AU / 43 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	Mirror | SBS SP 615
Laser Wavelength	488 nm @0.3% | 639 nm @0.08% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.1
Rotation	0°
Sampling	1.20
Pixel Time	0.32 µs
Frame Time	3.52 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4
Detection Wavelength	489-550 | 636-732 | 440-470
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.1 (3D, Auto) | 7.7 (3D, Auto) | 6.3 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced in Panel C to pronounce regions of special interest.
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	0% | 5%
Clip X	45°
Clip Z	0°
Y-Z	Activate was selected, textured opaque was chosen, and a dark red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	-45°

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000049

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR. However, its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. hosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 74 of 151 total - Panel A

Z Stack - (3D) - Panel A & B

Z-Stack = 151 Slices (4.5 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1066 X 1066
Image Size (Scaled) = 62.71 µm X 62.72 µm
Bit Depth = 16 Bit
Image Center Position = X: 77.54 mm, Y: 49.37 mm
Stage Position = X: 77.54 mm, Y: 49.37 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 151 Slices (4.5 µm)
Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 574 X 889
Image Size (Scaled) = 33.77 µm X 52.31 µm
Bit Depth = 16 Bit
Image Center Position = X: 77.54 mm, Y: 49.37 mm
Stage Position = X: 77.54 mm, Y: 49.37 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 50 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 489-550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.1 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639 nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 636-732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.7 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 440-470
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.3 (3D, Auto)

Split View - Panel A

The first panel on the left features Mitochondria visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the second panel shows Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, the third panel features DAPI nuclear staining, and the last panel on the right features the merged image of all channels. This image was taken at Z-Position 74 of 151.

3D Image Processing - Panels B & C

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = Clipping planes are introduced in Panel C to pronounce regions of special interest.

X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%
Clip X = 45°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a dark red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 5%
Clip Y = -45°
Clip Z = 0°

Summary

Panel A - This panel showcases a split view. From z-plane 74 of 151, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate. The left-hand panel shows the Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The second panel from the left features the Rabbit anti-Human BAP1.p Polyclonal- Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green . The next panel shows DAPI (blue), and the right-hand panel presents a merged image of the three channels. Panel A notes a Region of Interest (ROI) outlined by a white box, which is explored further in Panel C.

Panel B - This panel presents a 3-dimensional Maximum Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green) and Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 647 (red), as well as DAPI (blue) at confocal resolution.

Panel C - This panel presents a 3-dimensional Maximum Projection from the ROI in Panel A to further demonstrate the nuclear localization of BAP1.p primary antibody in response to Etoposide-induced DNA damage, Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green) and Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 647 (red), as well as DAPI (blue) at confocal resolution. The image employs a "wedge" or a double cut plane on the X-Z (green outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is 0%, the angle X is at 45 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is 5%, the angle X is at -45 degrees, and the angle Z is 0 degrees.

Panels A, B, and C - All demonstrate the BAP1.p primary antibody localized to the nucleus in response to the Etoposide-induced DNA damage, which is shown using specific z-planes demonstrating the X, Y orientation, and cut plane maximum projections demonstrating the X and Y-Z localization within the nucleus. Further, confocal resolution though diffraction limited, begins to elucidate foci, or specular staining rather then the diffuse, pan nuclear staining seen in widefield and widefield SIM, also noting that the nucleolus is devoid of BAP1.p, and this is now easily visualized at confocal resolution in Panels A and C you can see the space devoid of DAPI and BAP1.p.

Image Corrections

None

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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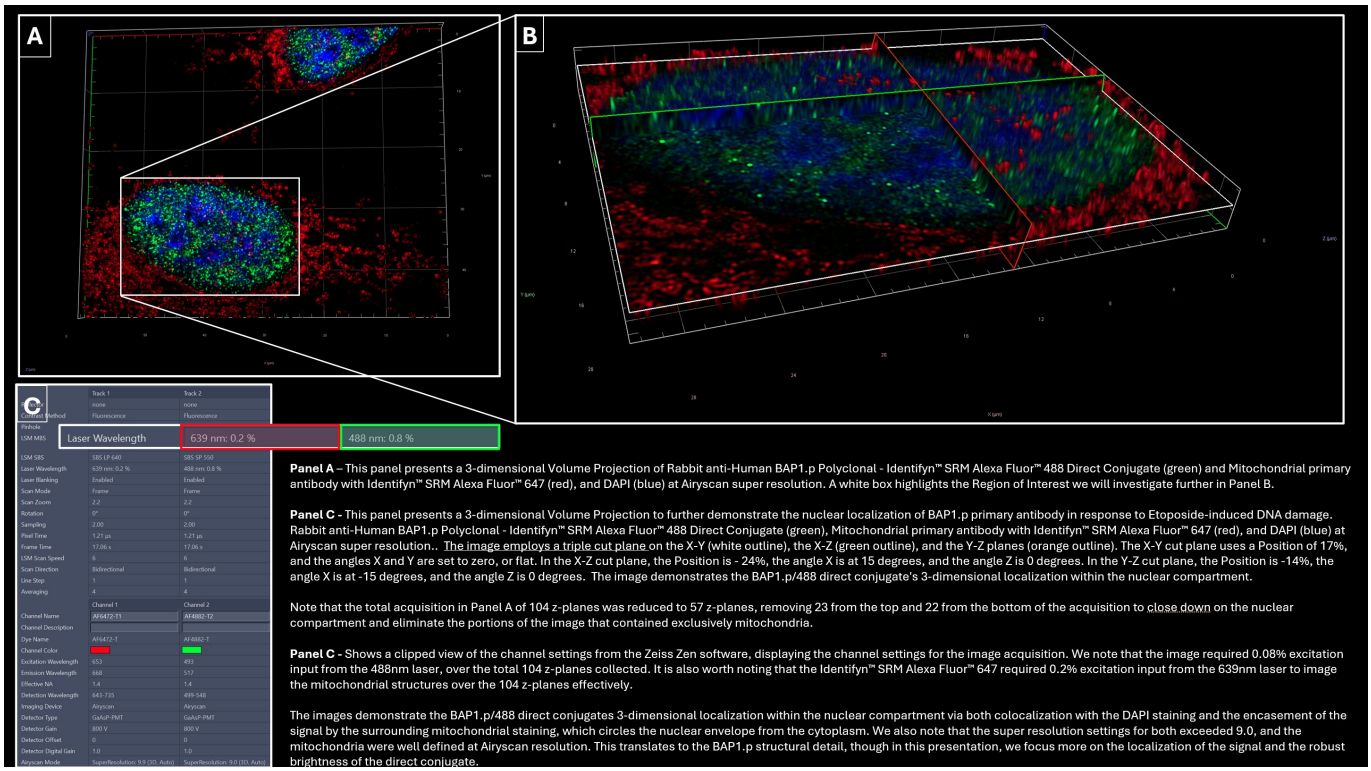
SOURCE PROVENANCE

Catalog	DC-000049
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4F1B5B8B79FF1B3500F5581BF6864FF8D6F420148223CE728EE64E46DFF50BBA
Method PDF SHA-256	0383D5DB3E1E5DCA14ED1E03C31662EAB3CBEE6A35B3A1D194BD316FC7C33DB1

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A – This panel presents a 3-dimensional Volume Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green) and Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 647 (red), and DAPI (blue) at Airyscan super resolution. A white box highlights the Region of Interest we will investigate further in Panel B.

Panel C - This panel presents a 3-dimensional Volume Projection to further demonstrate the nuclear localization of BAP1.p primary antibody in response to Etoposide-induced DNA damage. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green), Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 647 (red), and DAPI (blue) at Airyscan super resolution.. The image employs a triple cut plane on the X-Y (white outline), the X-Z (green outline), and the Y-Z planes (orange outline). The X-Y cut plane uses a Position of 17%, and the angles X and Y are set to zero, or flat. In the X-Z cut plane, the Position is - 24%, the angle X is at 15 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is -14%, the angle X is at -15 degrees, and the angle Z is 0 degrees. The image demonstrates the BAP1.p/488 direct conjugate's 3-dimensional localization within the nuclear compartment.

Note that the total acquisition in Panel A of 104 z-planes was reduced to 57 z-planes, removing 23 from the top and 22 from the bottom of the acquisition to close down on the nuclear compartment and eliminate the portions of the image that contained exclusively mitochondria.

Panel C - Shows a clipped view of the channel settings from the Zeiss Zen software, displaying the channel settings for the image acquisition. We note that the image required 0.08% excitation input from the 488nm laser, over the total 104 z-planes collected. It is also worth noting that the Identifyn™ SRM Alexa Fluor™ 647 required 0.2% excitation input from the 639nm laser to image the mitochondrial structures over the 104 z-planes effectively.

The images demonstrate the BAP1.p/488 direct conjugates 3-dimensional localization within the nuclear compartment via both colocalization with the DAPI staining and the encasement of the signal by the surrounding mitochondrial staining, which circles the nuclear envelope from the cytoplasm. We also note that the super resolution settings for both exceeded 9.0, and the mitochondria were well defined at Airyscan resolution. This translates to the BAP1.p structural detail, though in this presentation, we focus more on the localization of the signal and the robust brightness of the direct conjugate.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	69

STRUCTURED ACQUISITION METADATA / AIRYSCAN

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	104 Slices (3.09 µm) | 57 Slices (1.68 µm)
Channels	3
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.03000 µm
Image Size (Pixels)	1711 X 1711 | 851 X 604
Image Size (Scaled)	60.37 µm X 60.37 µm | 30.03 µm X 21.31 µm
Bit Depth	16 Bit
Image Center Position	X: 77.15 mm, Y: 48.83 mm
Stage Position	X: 77.15 mm, Y: 48.83 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 250 µm | 5.00 AU / 328 µm | 5.00 AU / 214 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS SP 550 | SBS LP 640 | Mirror
Laser Wavelength	488 nm @0.8% | 639 nm @0.2% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	2.00
Pixel Time	1.21 µs
Frame Time	17.06 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4
Detection Wavelength	499-548 | 643-735 | 422-477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	9.0 (3D, Auto) | 9.9 (3D, Auto) | 8.2 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Y	Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	17% | -24% | -14%
Clip X	0° | 15° | -15°
Clip Y	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Y-Z	Activate was selected, textured opaque was chosen, and an orange outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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Cat ID# - DC 000049

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Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

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Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR. However, its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. hosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 104 Slices (3.09 µm)

Channels = 3
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 1711 X 1711
Image Size (Scaled) = 60.37 µm X 60.37 µm
Bit Depth = 16 Bit
Image Center Position = X: 77.15 mm, Y: 48.83 mm
Stage Position = X: 77.15 mm, Y: 48.83 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 57 Slices (1.68 µm)

Channels = 3
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 851 X 604
Image Size (Scaled) = 30.03 µm X 21.31 µm
Bit Depth = 16 Bit
Image Center Position = X: 77.15 mm, Y: 48.83 mm
Stage Position = X: 77.15 mm, Y: 48.83 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 550
Laser Wavelength = 488 nm @0.8%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.21 µs
Frame Time = 17.06 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499-548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 9.0 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 640
Laser Wavelength = 639 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.21 µs
Frame Time = 17.06 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 9.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.21 µs
Frame Time = 17.06 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422-477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.2 (3D, Auto)

3D Image Processing - Panel A

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Y = Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 17%
Clip X = 0°
Clip Y = 0°

X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -24%
Clip X = 15°
Clip Y = 0°

Y-Z = Activate was selected, textured opaque was chosen, and an orange outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -14%
Clip X = -15°
Clip Y = 0°

Summary

Panel A - This panel presents a 3-dimensional Volume Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green) and Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 647 (red), and DAPI (blue) at Airyscan super resolution. A white box highlights the Region of Interest we will investigate further in Panel B.

Panel B - This panel presents a 3-dimensional Volume Projection to further demonstrate the nuclear localization of BAP1.p primary antibody in response to Etoposide-induced DNA damage, Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green), Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 647 (red), and DAPI (blue) at Airyscan super resolution. The image employs a triple cut plane on the X-Y (white outline), the X-Z (green outline), and the Y-Z planes (orange outline). The X-Y cut plane uses a Position of 17%, and the angles X and Y are set to zero, or flat. In the X-Z cut plane, the Position is -24%, the angle X is at 15 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is -14%, the angle X is at -15 degrees, and the angle Z is 0 degrees. The image demonstrates the BAP1.p/488 direct conjugate's 3-dimensional localization within the nuclear compartment.

Note that the total acquisition in Panel A of 104 z-planes was reduced to 57 z-planes, removing 23 from the top and 22 from the bottom of the acquisition to close down on the nuclear compartment and eliminate the portions of the image that contained exclusively mitochondria.

Panel C - Shows a clipped view of the channel settings from the Zeiss Zen software, displaying the channel settings for the image acquisition. We note that the image required 0.08% excitation input from the 488nm laser, over the total 104 z-planes collected. It is also worth noting that the Identifyn® SRM Alexa Fluor™ 647 required 0.2% excitation input from the 639nm laser to image the mitochondrial structures over the 104 z-planes effectively.

The images demonstrate the BAP1.p/488 direct conjugates 3-dimensional localization within the nuclear compartment via both colocalization with the DAPI staining and the encasement of the signal by the surrounding mitochondrial staining, which circles the nuclear envelope from the cytoplasm. We also note that the super resolution settings for both exceeded 9.0, and the mitochondria were well defined at Airyscan resolution. This translates to the BAP1.p structural detail, though in this presentation, we focus more on the localization of the signal and the robust brightness of the direct conjugate.

Image Corrections

None

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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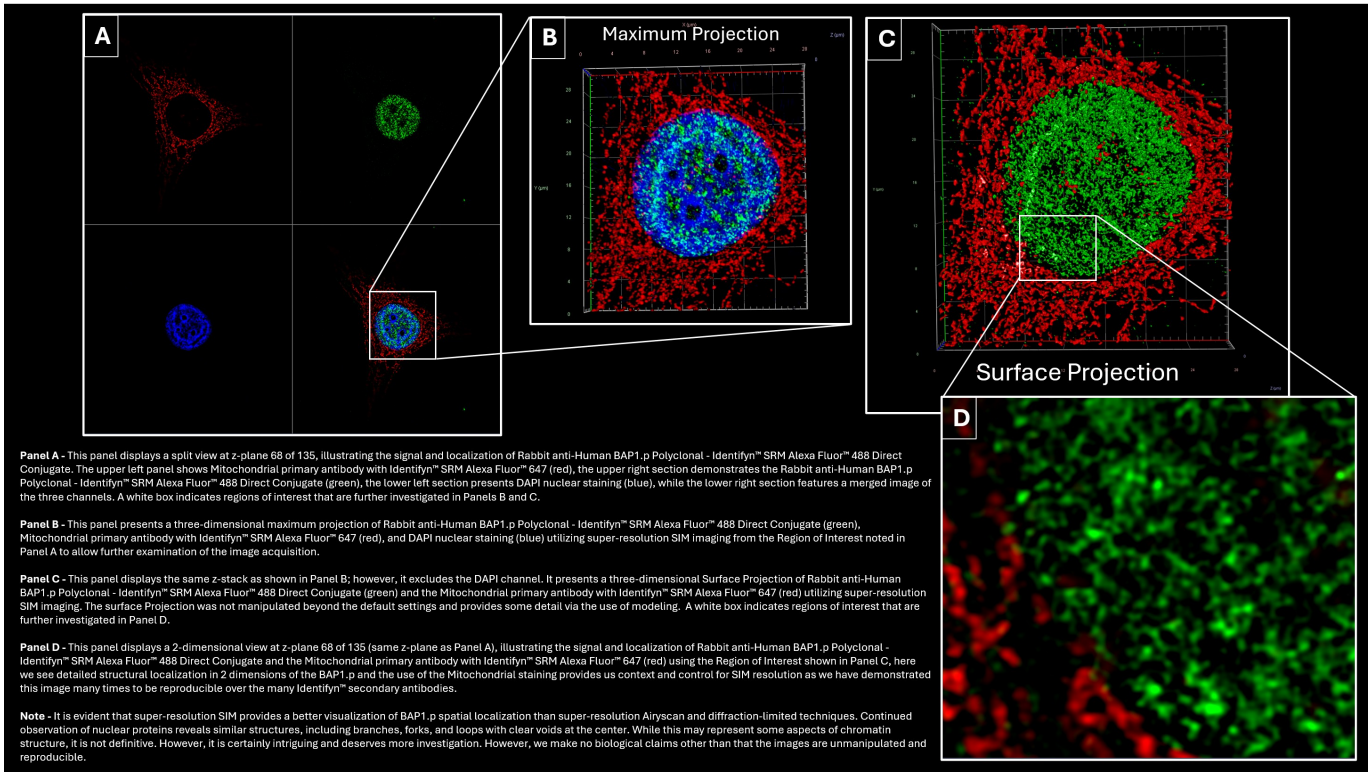
SOURCE PROVENANCE

Catalog	DC-000049
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F9AB248F02713716E2A330A0C4FD0C32124304723DFBAE0378849AC38F93CA2B
Method PDF SHA-256	5C0532826ACC215AB4D084C9345BFCDCF1AFAADBE5F9DB1D834E11A629C6BAF8

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	65

STRUCTURED ACQUISITION METADATA / SIM

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	135 Slices (4.02 µm)
Channels	3
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1665 X 1830 | 549 X 478
Image Size (Scaled)	85.40 µm X 85.40 µm | 28.12 µm X 30.91 µm | 9.27 µm X 8.07 µm
Bit Depth	16 Bit
Image Center Position	X: 64.90 mm, Y: 37.41 mm
Stage Position	X: 64.90 mm, Y: 37.41 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 640 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 50 ms
Depth of Focus	0.81 µm | 1.13 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @2.50% | 640 nm @2.00% | 405 nm @2.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.7393 (647), 9.4176 (488), 8.4787 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%
Background	Black
Light	Brightness 100%, Tone Mapping Enabled | Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000049

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR. However, its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. hosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 68 of 135 total - Panel A

Z Stack - (3D)

Z-Stack = 135 Slices (4.02 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 85.40 µm X 85.40 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.90 mm, Y: 37.41 mm
Stage Position = X: 64.90 mm, Y: 37.41 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panels B & C

Z-Stack = 135 Slices (4.02 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 1665 X 1830
Image Size (Scaled) = 28.12 µm X 30.91 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.90 mm, Y: 37.41 mm
Stage Position = X: 64.90 mm, Y: 37.41 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 68 of 135 total - Panel D

Z Stack - (3D)

Z-Stack = 135 Slices (4.02 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 549 X 478
Image Size (Scaled) = 9.27 µm X 8.07 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.90 mm, Y: 37.41 mm
Stage Position = X: 64.90 mm, Y: 37.41 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @2.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @2.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @2.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.7393 (647), 9.4176 (488), 8.4787 (DAPI)
Fitting = Fast Fit
Normalization = Auto

Split View - Panel A

The top-left panel features Mitochondria visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the top-right panel shows Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels. This image was taken at Z-Position 68 of 135.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise
Appearance = Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%
Background = Black
Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel displays a split view at z-plane 68 of 135, illustrating the signal and localization of Rabbit anti-Human BAP1.pPolyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate. The upper left panel shows the Mitochondrial primary antibody with Identifyn SRM Alexa Fluor 647 (red), and the upper right section demonstrates the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™SRM Alexa Fluor™ 488 Direct Conjugate (green), the lower left section presents DAPI nuclear staining (blue), while the lower right section features a merged image of the three channels. A white box indicates regions of interest that are further investigated in Panels B and C.

Panel B - This panel presents a three-dimensional maximum projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green), Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 647 (red), and DAPI nuclear staining (blue) utilizing super-resolution SIM imaging from the Region of Interest noted in Panel A to allow further examination of the image acquisition.

Panel C - This panel displays the same z-stack as shown in Panel B; however, it excludes the DAPI channel. It presents a three-dimensional Surface Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green) and the Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 647 (red) utilizing super-resolution SIM imaging. The surface projection was not manipulated beyond the default settings and provides some detail via the use of modeling. A white box indicates regions of interest that are further investigated in Panel D.

Panel D - This panel displays a 2-dimensional view at z-plane 68 of 135 (same z-plane as Panel A), illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate and the Mitochondrialprimary antibody with Identifyn® SRM Alexa Fluor™ 647 (red) using the Region of Interest shown in Panel C, here we see detailed structural localization in 2 dimensions of the BAP1.p and the use of the Mitochondrial staining provides us context and control for SIM resolution as we have demonstrated this image many times to be reproducible over the many Identifyn® secondary antibodies.

Note - It is evident that super-resolution SIM provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. Continued observation of nuclear proteins reveals similar structures, including branches, forks, and loops with clear voids at the center. While this may represent some aspects of chromatin structure, it is not definitive. However, it is certainly intriguing and deserves more investigation. However, we make no biological claims other than that the images are unmanipulated and reproducible.

Image Corrections

None

Image by P. Raut
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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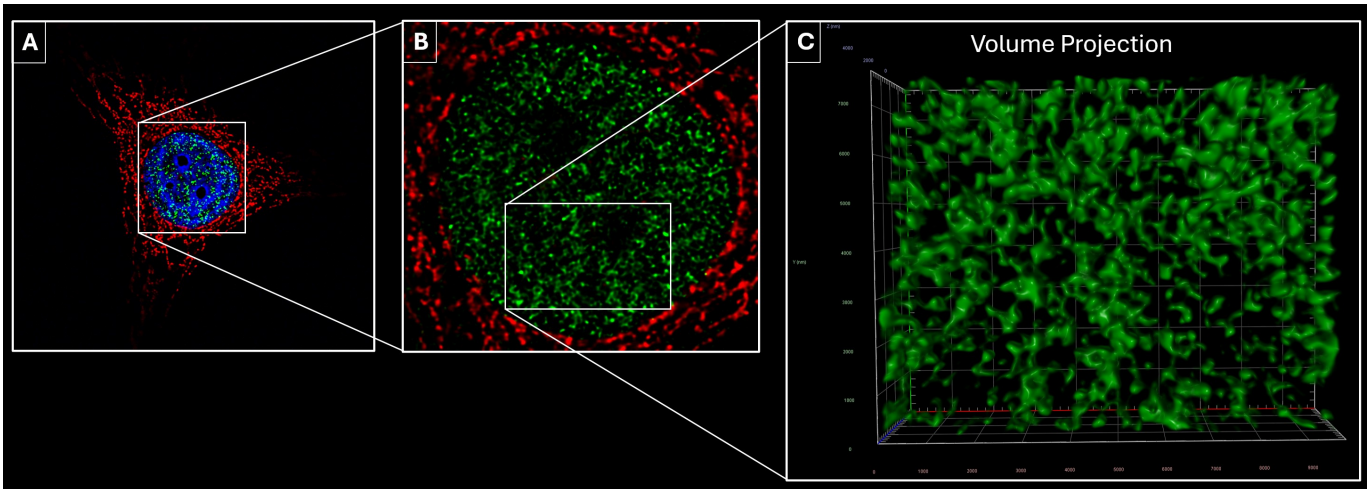
SOURCE PROVENANCE

Catalog	DC-000049
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	46F740977B53332F9E9994792683850A345D267112576E76F45EF372E29405CB
Method PDF SHA-256	86C61E8BC6C93D57B36A666F564BAF2FDA44074B9C0A55882E76F7DB58342B55

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A - This panel displays a 2-dimensional view at z-plane 68 of 135, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green). Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red) and DAPI nuclear staining (blue) employing super resolution SIM Squared. A white box indicates a region of interest and will further reduce the area investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 68 of 135 (same z-plane as previous panel), from the Region of Interest shown in Panel A. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate (green) and the Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the control for the resolution, as mitochondrial structure is well-documented and reproducible. A white box indicates a region of interest and will be further reduced and investigated in Panel C.

Panel C - This panel showcases the region of interest indicated in Panel B, displaying a three-dimensional volume projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 488 Direct Conjugate.

Note - It is evident that super-resolution SIM, in this case, SIM Squared, provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. And using the same sample as was imaged for SIM, we show here a gain in resolving the three-dimensional structural localization details of BAP1.p localization following etoposide-induced DNA damage. Again, we make no claims as to what this image represents, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is DNA damage-induced localization at or near the DNA break sites.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 680
METADATA VALUES	64

STRUCTURED ACQUISITION METADATA / SIM²

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	135 Slices (4.02 µm)
Channels	3
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1342 X 1342 | 579 X 456
Image Size (Scaled)	85.40 µm X 85.40 µm | 22.67 µm X 20.84 µm | 9.78 µm X 7.70 µm
Bit Depth	16 Bit
Image Center Position	X: 64.90 mm, Y: 37.41 mm
Stage Position	X: 64.90 mm, Y: 37.41 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 640 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 50 ms
Depth of Focus	0.81 µm | 1.13 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @2.50% | 640 nm @1.00% | 405 nm @3.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM²
SIM² Settings	Standard
Input SNR	Low
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 28°, Scale Z 82%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000049

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR. However, its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. hosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 68 of 135 total - Panel A

Z Stack - (3D)

Z-Stack = 135 Slices (4.02 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 85.40 µm X 85.40 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.90 mm, Y: 37.41 mm
Stage Position = X: 64.90 mm, Y: 37.41 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 68 of 135 total - Panel A

Z Stack - (3D)

Z-Stack = 135 Slices (4.02 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 1342 X 1342
Image Size (Scaled) = 22.67 µm X 20.84 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.90 mm, Y: 37.41 mm
Stage Position = X: 64.90 mm, Y: 37.41 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 135 Slices (4.02 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 579 X 456
Image Size (Scaled) = 9.78 µm X 7.70 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.90 mm, Y: 37.41 mm
Stage Position = X: 64.90 mm, Y: 37.41 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @2.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

680 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @3.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM² Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM²
SIM² Settings = Standard
Input SNR = Low
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 28°, Scale Z 82%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel displays a 2-dimensional view at z-plane 68 of 135, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green). Mitochondrial primary antibody labeled with Identifyn™SRM Alexa Fluor™ 647 (red) and DAPI nuclear staining (blue) employing super resolution SIM Squared. A white box indicates a region of interest and will further reduce the area investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 68 of 135 (same z-plane as the previous panel) from the Region of Interest shown in Panel A. Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate (green) and the Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red) DAPI is omitted to allow for better visualization of the BAP1.p. We also note the control for the resolution, as mitochondrial structure is well-documented and reproducible. A white box indicates a region of interest and will be further reduced and investigated in Panel C.

Panel C - This panel showcases the region of interest indicated in Panel B, displaying a three-dimensional volume projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 488 Direct Conjugate.

Note - It is evident that super-resolution SIM, in this case, SIM Squared, provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. Using the same sample as was imaged for SIM, we show here a gain in resolving the three-dimensional structural localization details of BAP1.p localization following etoposide-induced DNA damage Again, we make no claims as to what this image represents, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is DNA damage-induced localization at or near the DNA break sites.

Image Corrections

None

Image by P. Raut
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000049
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5CDA2D0F87E5E075FB806F561103F48F7890472F8FC6EC3863259B1964BCBF8E
Method PDF SHA-256	812990057AAE4A069A328636FC4312450F4720F9B9CEFA452667E404E4A2FE26