BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

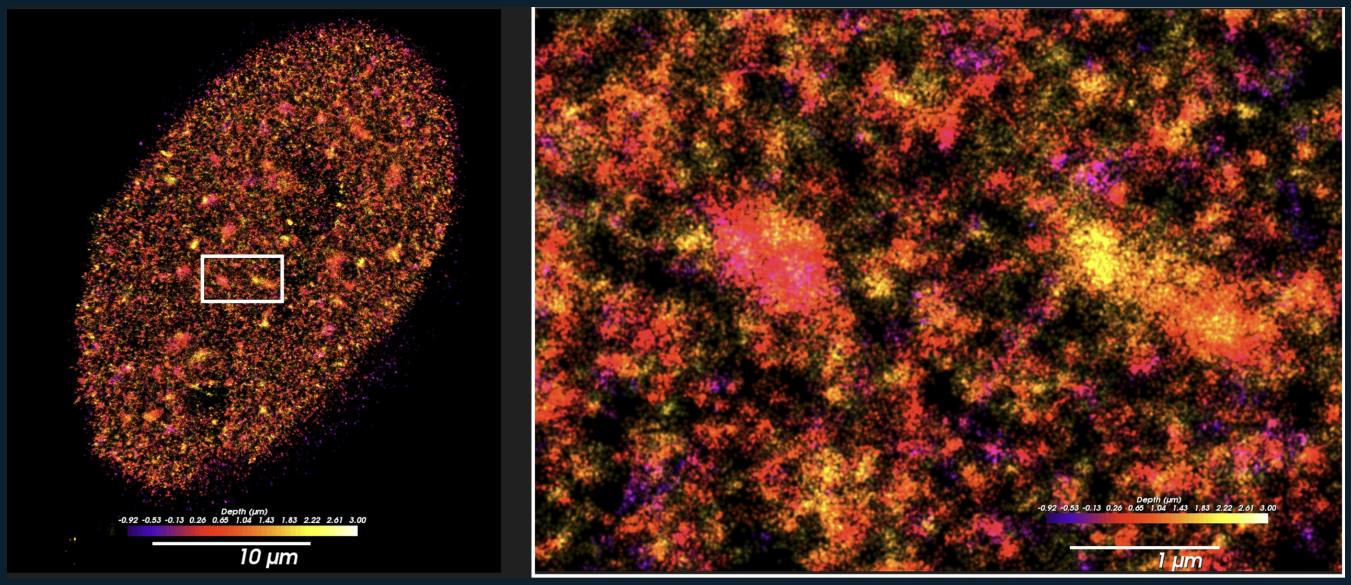
BAP1.P

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human BAP1.p (Serine 592) Polyclonal

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN
SIM -> SIM² -> SINGLE-MOLECULE STORM

8	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RP-000005 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

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
- Single-molecule STORM presentation workflow
- Preserved control experiment
- 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 -> Single-molecule STORM -> Control

BENNETT ASSESSMENT

The preserved PA-RP-000005 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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 / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved PA-RP-000005 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 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

A true preserved control record is present and appears last.

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; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 555; 647	3; 50 Cy 5; AF532-49014; 49 DAPI; 625 - 655; 515 - 545; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 647	3; None; 639 nm @0.08%; 488 nm @0.05%; 405 nm @0.2%; 653; 493; 353
Airyscan	405/DAPI; 488; 647; 750	3; None; 639 nm @0.08%; 488 nm @0.08%; 405 nm @0.2%; 653; 493; 353
SIM	405/DAPI; 488; 555; 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; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
SIM²	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; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 647	2; 50 Cy 5; 49 DAPI; 625 - 655; 335-383; 665 - 715; 420-470; 653

MODALITY ASSESSMENT / PART 1 OF 9

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 9

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): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 100 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 37.

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 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: EC Plan-Neofluar 63x/1.25 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, 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: 91 Slices (9 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 572 X 573; Image Size (Pixels): 572 X 573; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 40 ms; 120 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @6.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 44.

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 Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 555; 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 9

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: 171 Slices (5.1 µm); Channels: 3; Scaling (Per Pixel): 0.043 µm X 0.043 µm X 0.030 µm; Image size (Pixels): 1276 X 1276; 641 X 490; Image Size (Pixels): 1276 X 1276; 641 X 490; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.26 µs; Frame Time: 4.20 s. Illumination: Laser Wavelength: 639 nm @0.08%; 488 nm @0.05%. Optical/scan: Pinhole: 1.00 AU / 65 µm; 1.00 AU / 50 µm; Scan Zoom: 2.4; 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: 7.3 (3D, Auto); 8.6 (3D, Auto). Structured source metadata values: 71.

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 9

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: 161 Slices (4.8 µm); 161 Slices (4.8µm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1566 X 1566; 744 X 589; Image Size (Pixels): 1566 X 1566; 744 X 589; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66 µs; Frame Time: 7.82 s. Illumination: Laser Wavelength: 639 nm @0.08%; 488 nm @0.08%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 268 µm; Scan Zoom: 2.4; LSM Scan Speed: 7; 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: 8.8 (3D, Auto); 8.6 (3D, Auto). Structured source metadata values: 64.

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; 750.

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 9

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: 162 Slices (4.83 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 4950 X 4321; 1246 X 1553; Image Size (Pixels): 4950 X 4321; 1246 X 1553; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.33 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.50%; Illumination Wavelength: 640; 488. 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: 69.

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; 555; 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 9

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: 162 Slices (4.83 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1406 X 1521; Image Size (Pixels): 5056 X 5056; 1406 X 1521; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100 ms; 120 ms; Frame Time: 1.33 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 45°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 66.

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.

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.

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 71.

VISIBLE OBSERVATION

A preserved presentation image is available for Single-molecule STORM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Without localization tables, uncertainty, blinking, drift, and independent cluster evidence, the presentation cannot establish molecule count, cluster truth, exact precision, or molecular architecture.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / 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: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 761 X 707; 1080 X 741; Image Size (Pixels): 761 X 707; 1080 X 741; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 40 ms; 18 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @6.00%; X-Cite Xylis Lamp Intensity 6.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 36.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

Consistent with peer-reviewed antibody-control guidance that a no-primary or related control bounds background but is not a complete specificity system.

INTERPRETIVE LIMIT

A control bounds the recorded comparison only; it does not establish universal specificity, zero background, zero cross-reactivity, or absence of free dye.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Control 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 PA-RP-000005 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 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)=1566 X 1566; Image Size (Scaled)=55.27 µm X 55.27 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): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 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)=4950 X 4321; Image Size (Scaled)=104.51 µm X 91.23 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 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)=106.75 µm X 106.75 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM; 2µM; 2 µM -> 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.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["24 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["6 hours"], "Control": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

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

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.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

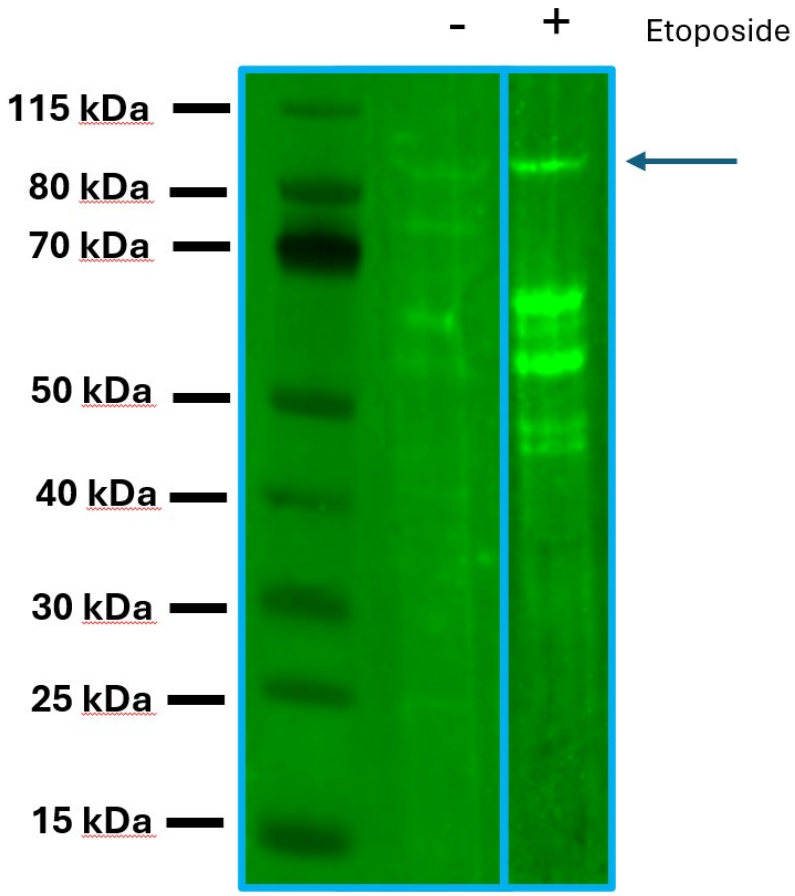
This dossier preserves the complete available evidence progression for PA-RP-000005. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 Imager.M1	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	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™

Rabbit anti-Human BAP1.p Polyclonal
Cat ID# - PA-RP 000005

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 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 then washed five times with PBS The blocked membrane was incubated with Rabbit anti-Human BAP1p Polyclonal, followed by five washes in 5X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5X with PBS. 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

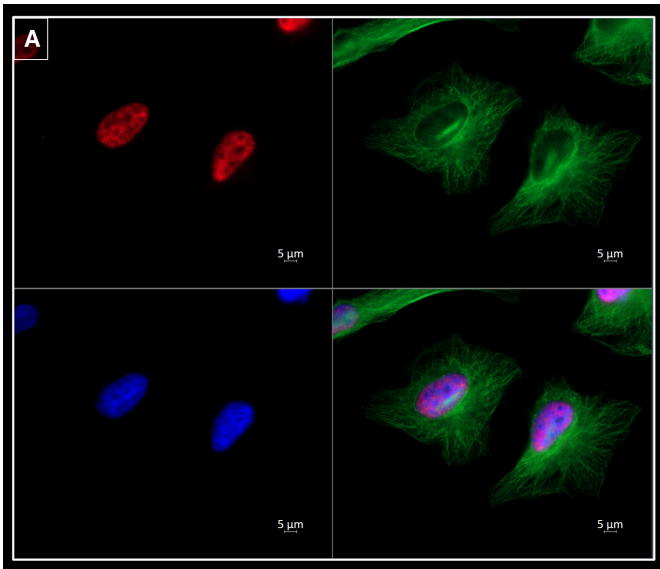
SOURCE PROVENANCE

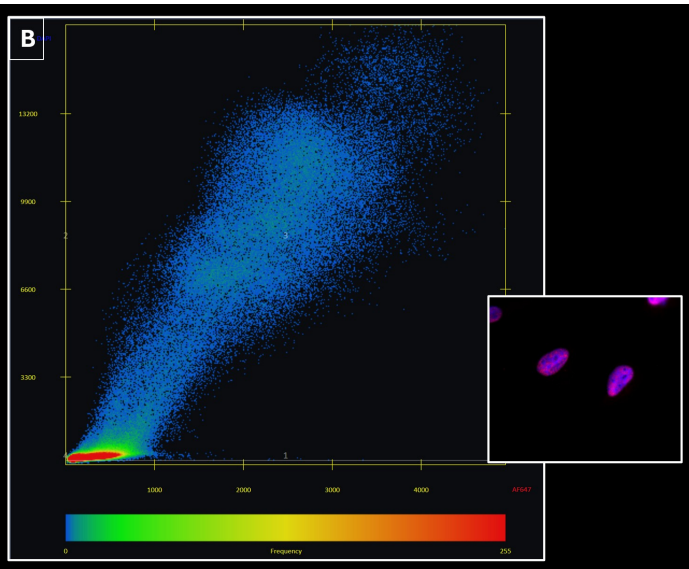
Catalog	PA-RP-000005
Stage	Western blot

SOURCE PROVENANCE

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	1B5E2A92420BA05708453399FAD17AFBC4EDEB93264ED3E2C67CCEC07156D534
Method PDF SHA-256	5945ABFBC63731F85B51AA0148243FF416FC92AFD3F18EB6FADACF3C381C1291

ORIGINAL IMAGE EVIDENCE / WIDEFIELD





Panel A - Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section features the Rabbit anti-Human BAP1.p Polyclonal IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647(red). The upper right displays an Alpha Tubulin primary antibody labeled with 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 (Y-axis) and Rabbit anti-Human BAP1.p Polyclonal IgG with an Identifyn™ SRM Alexa Fluor™ 647channel (-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	37

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)	1216 X 1028
Image Size (Scaled)	133.18 µm X 112.59 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	50 Cy 5 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10.00% | X-Cite Xylis Lamp Intensity @15.00%
Exposure Time	100 ms | 20 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 µm | 0.80 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, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

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, IgG, 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-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), 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™ 488 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 1:100 . Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

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) = 1216 X 1028
Image Size (Scaled) = 133.18 μm X 112.59 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

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

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 @10.00%
Exposure Time = 100 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

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 @15.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
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 Rabbit anti-Human BAP1.p Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L), the top-right panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, 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 - AF647, Threshold 8, Range Auto
Vertical Axis - DAPI, Threshold 191, Range Auto

Summary

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section features the Rabbit anti-Human BAP1.p Polyclonal IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right displays an Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa

Fluor™ 488 (green). The lower left section shows DAPI nuclear staining (blue). In contrast, the lower right presents a merged image of the three channels.

Panel B - Displays a colocalization view of the DAPI (Y-axis) and Rabbit anti-Human BAP1.p Polyclonal IgG with an Identifyn® SRM Alexa Fluor™ 647 channel (X-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

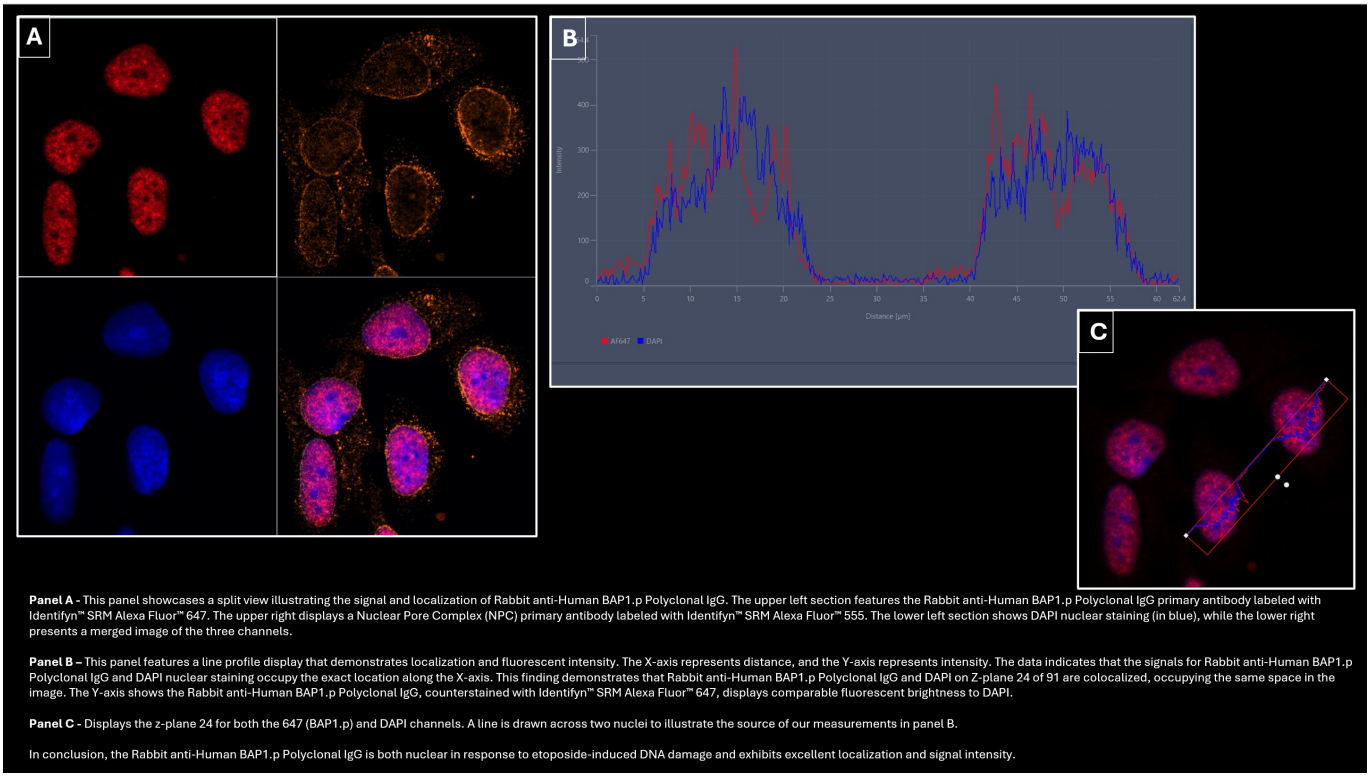
None

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

SOURCE PROVENANCE

Catalog	PA-RP-000005
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	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	EB04F6F69E1F9ABDD355952B22C47B644CF8C268ADCF54D08C829AC5697E1C7A
Method PDF SHA-256	AFDE2923E85F9A5ABF0872732C414F5C14EBF2DAF72C754F59CDF42969FA3DCC

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A - This panel showcases a split view illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section features the Rabbit anti-Human BAP1.p Polyclonal IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647. The upper right displays a Nuclear Pore Complex (NPC) primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 555. The lower left section shows DAPI nuclear staining (in blue), while the lower right presents a merged image of the three channels.

Panel B – This panel features a line profile display that demonstrates localization and fluorescent intensity. The X-axis represents distance, and the Y-axis represents intensity. The data indicates that the signals for Rabbit anti-Human BAP1.p Polyclonal IgG and DAPI nuclear staining occupy the exact location along the X-axis. This finding demonstrates that Rabbit anti-Human BAP1.p Polyclonal IgG and DAPI on Z-plane 24 of 91 are colocalized, occupying the same space in the image. The Y-axis shows the Rabbit anti-Human BAP1.p Polyclonal IgG, counterstained with Identifyn™ SRM Alexa Fluor™ 647, displays comparable fluorescent brightness to DAPI.

Panel C - Displays the z-plane 24 for both the 647 (BAP1.p) and DAPI channels. A line is drawn across two nuclei to illustrate the source of our measurements in panel B.

In conclusion, the Rabbit anti-Human BAP1.p Polyclonal IgG is both nuclear in response to etoposide-induced DNA damage and exhibits excellent localization and signal intensity.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 647
METADATA VALUES	44

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 Imager.M1 Widefield, 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	91 Slices (9 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	572 X 573
Image Size (Scaled)	81.71 µm X 81.86 µm
Bit Depth	14 Bit
Image Center Position	X: 49.39 mm, Y: 51.35 mm
Stage Position	X: 49.39 mm, Y: 51.35 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | AF532-49014 | 49 DAPI
Beam Splitter	660 | 550 | 395
Filter Excitation Wavelength	625 - 655 | 515 - 545 | 335 - 383
Filter Emission Wavelength	665 - 715 | 555 - 595 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @6.00% | X-Cite Xylis Lamp Intensity @15.00%
Exposure Time	40 ms | 120 ms | 20 ms
Excitation Wavelength	653 | 553 | 353
Emission Wavelength	668 | 568 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 1.10 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mod" "Optical Sectioning" - enabled correction selected.
Z-Position	24 of 91
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 62.4), Y (Min 0 and Max 554)

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, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000042

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, IgG, 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-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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™ 555 Affinity Purified Donkey Anti-Mouse, F(a'')□ (H + L) 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.

Microscope

Zeiss Axio Imager.M1 Widefield, 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 24 of 91 total - Panel A

Z-Stack = 91 Slices (9 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 572 X 573
Image Size (Scaled) = 81.71 µm X 81.86 µm
Bit Depth = 14 Bit
Image Center Position = X: 49.39 mm, Y: 51.35 mm
Stage Position = X: 49.39 mm, Y: 51.35 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

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 @6.00%
Exposure Time = 40 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

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 120 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.10 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 = 20 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 Rabbit anti-Human BAP1.p Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(a'')□ (H + L), the top-right panel shows Nuclear Pore Complexes visualized with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
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 24 of 91.

Profile View Display - Panel B & C

Z-Position = 24 of 91
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 62.4), Y (Min 0 and Max 554)

Summary

Panel A - This panel showcases a split view illustrating the signal and localization of Rabbit anti-HumanBAP1.p Polyclonal IgG. The upper left section features the Rabbit anti-Human BAP1.p Polyclonal IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647. The upper right displays a Nuclear Pore Complex (NPC) primary antibody tagged with Identifyn® SRM Alexa Fluor™ 555. The lower left section shows DAPI nuclear staining (in blue), while the lower right presents a merged image of the three channels.

Panel B - This panel features a line profile display that demonstrates localization and fluorescent intensity. The X-axis represents distance, and the Y-axis represents intensity. The data indicate that the signals for Rabbit anti-Human BAP1.p Polyclonal IgG and DAPI nuclear staining occupy the exact location along the X-axis. This finding demonstrates that Rabbit anti-Human BAP1.p Polyclonal IgG and DAPI on Z-plane 24 of 91 are colocalized, occupying the same space in the image. The Y-axis shows the Rabbit anti-Human BAP1.p Polyclonal IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647, displays comparable fluorescent brightness to DAPI.

Panel C - Displays the z-plane 24 for both the 647 (BAP1.p) and DAPI channels. A line is drawn across two nuclei to illustrate the source of our measurements in panel B.

In conclusion, the Rabbit anti-Human BAP1.p Polyclonal IgG is both nuclear in response to etoposide-induced DNA damage and exhibits excellent localization and signal intensity.

Image Correction

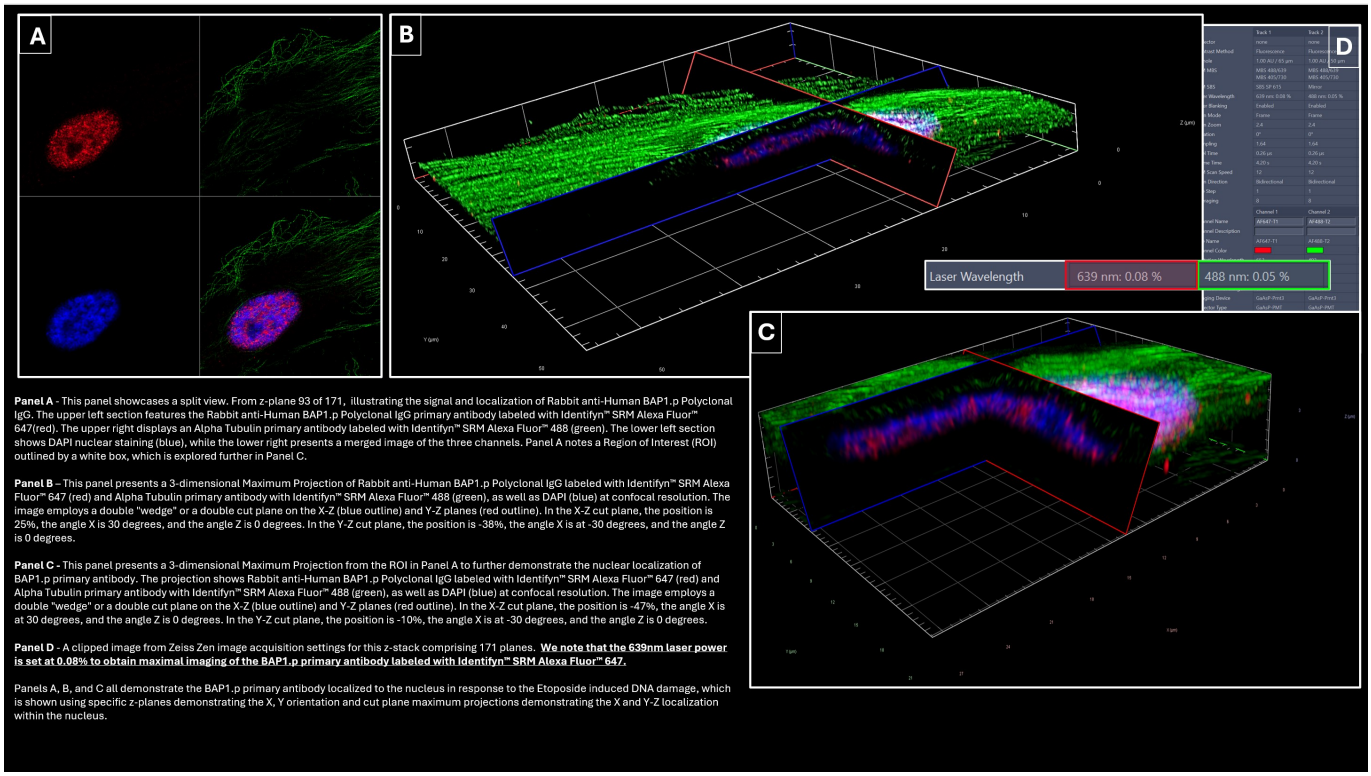
None

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

SOURCE PROVENANCE

Catalog	PA-RP-000005
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, 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	44
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D78602997B67C51C76CB4CA5BCCF9126DD8580E7A5F5DB8719C3DD6156D6D63E
Method PDF SHA-256	E164E243BD8C9BBC995EC855990451EDBF63457D31000731AF028911F40C90B2

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A - This panel showcases a split view. From z-plane 93 of 171, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section features the Rabbit anti-Human BAP1.p Polyclonal IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647(red). The upper right displays an Alpha Tubulin primary antibody labeled with 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 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 IgG labeled with Identifyn™ SRM Alexa Fluor™ 647 (red) and Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution. The image employs a double "wedge" or a double cut plane on the X-Z (blue outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is 25%, the angle X is 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is -38%, the angle X is at -30 degrees, and the angle Z is 0 degrees.

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. The projection shows Rabbit anti-Human BAP1.p Polyclonal IgG labeled with Identifyn™ SRM Alexa Fluor™ 647 (red) and Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution. The image employs a double "wedge" or a double cut plane on the X-Z (blue outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is -47%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is -10%, the angle X is at -30 degrees, and the angle Z is 0 degrees.

Panel D - A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 171 planes. **We note that the 639nm laser power is set at 0.08% to obtain maximal imaging of the BAP1.p primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647.**

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.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	71

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	171 Slices (5.1 µm)
Channels	3
Scaling (Per Pixel)	0.043 µm X 0.043 µm X 0.030 µm
Image Size (Pixels)	1276 X 1276 | 641 X 490
Image Size (Scaled)	55.08 µm X 55.08 µm | 27.67 µm X 21.15 µm
Bit Depth	16 Bit
Image Center Position	X: 82.48 mm, Y: 48.63 mm
Stage Position	X: 82.48 mm, Y: 48.63 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 65 µm | 1.00 AU / 50 µm | 1.00 AU / 44 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS SP 615 | Mirror
Laser Wavelength	639 nm @0.08% | 488 nm @0.05% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	1.64
Pixel Time	0.26 µs
Frame Time	4.20 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	636-732 | 489-556 | 440-475
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.3 (3D, Auto) | 8.6 (3D, Auto) | 6.8 (3D, Auto)
LMS MBS	MBS 488/639, 405/730
3D Maximum Projection	Precise
Appearance	Threshold 4% 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)
Wedge	X-Z and Y-Z
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Z	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	25% | -38% | -47% | -10%
Clip X	30° | -30°
Clip Z	15° | 0°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	-30° | 0°

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, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

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, IgG, 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-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), 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™ 488 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.

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 93 of 171 total - Panel A

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

Z-Stack = 171 Slices (5.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.043 µm X 0.043 µm X 0.030 µm
Image Size (Pixels) = 1276 X 1276
Image Size (Scaled) = 55.08 µm X 55.08 µm
Bit Depth = 16 Bit
Image Center Position = X: 82.48 mm, Y: 48.63 mm
Stage Position = X: 82.48 mm, Y: 48.63 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 171 Slices (5.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.043 µm X 0.043 µm X 0.030 µm
Image Size (Pixels) = 641 X 490
Image Size (Scaled) = 27.67 µm X 21.15 µm
Bit Depth = 16 Bit
Image Center Position = X: 82.48 mm, Y: 48.63 mm
Stage Position = X: 82.48 mm, Y: 48.63 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 2.4
Rotation = 0°
Sampling = 1.64
Pixel Time = 0.26 µs
Frame Time = 4.20 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 = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.3 (3D, Auto)

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 @0.05%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.64
Pixel Time = 0.26 µs
Frame Time = 4.20 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 = 489-556
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.6 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LMS MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.64
Pixel Time = 0.26 µs
Frame Time = 4.20 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 = 440-475
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.8 (3D, Auto)

Split View - Panel A

The top-left panel features Rabbit anti-Human BAP1.p Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L), the top-right panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, 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 93 of 171.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 4% 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)
Wedge = X-Z and Y-Z

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

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

Position of Clip = 25%
Clip X = 30°
Clip Z = 15°

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

Position of Clip = -38%
Clip Y = -30°
Clip Z = 0°

3D Image Processing - Panel C

3D Maximum Projection = Precise
Appearance = Threshold 4% 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)
Wedge = X-Z and Y-Z

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

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

Position of Clip = -47%
Clip X = 30°
Clip Z = 0°

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

Position of Clip = -10%
Clip X = -30°
Clip Y = 0°

Summary

Panel A - This panel showcases a split view. From z-plane 93 of 171, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG The upper left section features the Rabbit anti-Human BAP1.p Polyclonal IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right displays an Alpha Tubulin primary antibody labeled with 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 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 IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (red) and Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution. The image employs a double "wedge" or a double cut plane on the X-Z (blue outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is 25%, the angle X is 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is -38%, the angle X is -30 degrees, and the angle Z is 0 degrees.

Panel C - This panel presents a 3-dimensional Maximum Projection from the ROI in Panel A, further to demonstrate the nuclear localization of BAP1.p primary antibody. The projection shows Rabbit anti-Human BAP1.p Polyclonal IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (red) and Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution. The image employs a double "wedge" or a double cut plane on the X-Z (blue outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is -47%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is -10%, the angle X is at -30 degrees, and the angle Z is 0 degrees.

Panel D - A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 171 planes. We note that the 639 nm laser power is set at 0.08% to obtain maximal imaging of the BAP1.p primary antibody labeled with Identifyn® SRM Alexa Fluor™647.

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.

Image Corrections

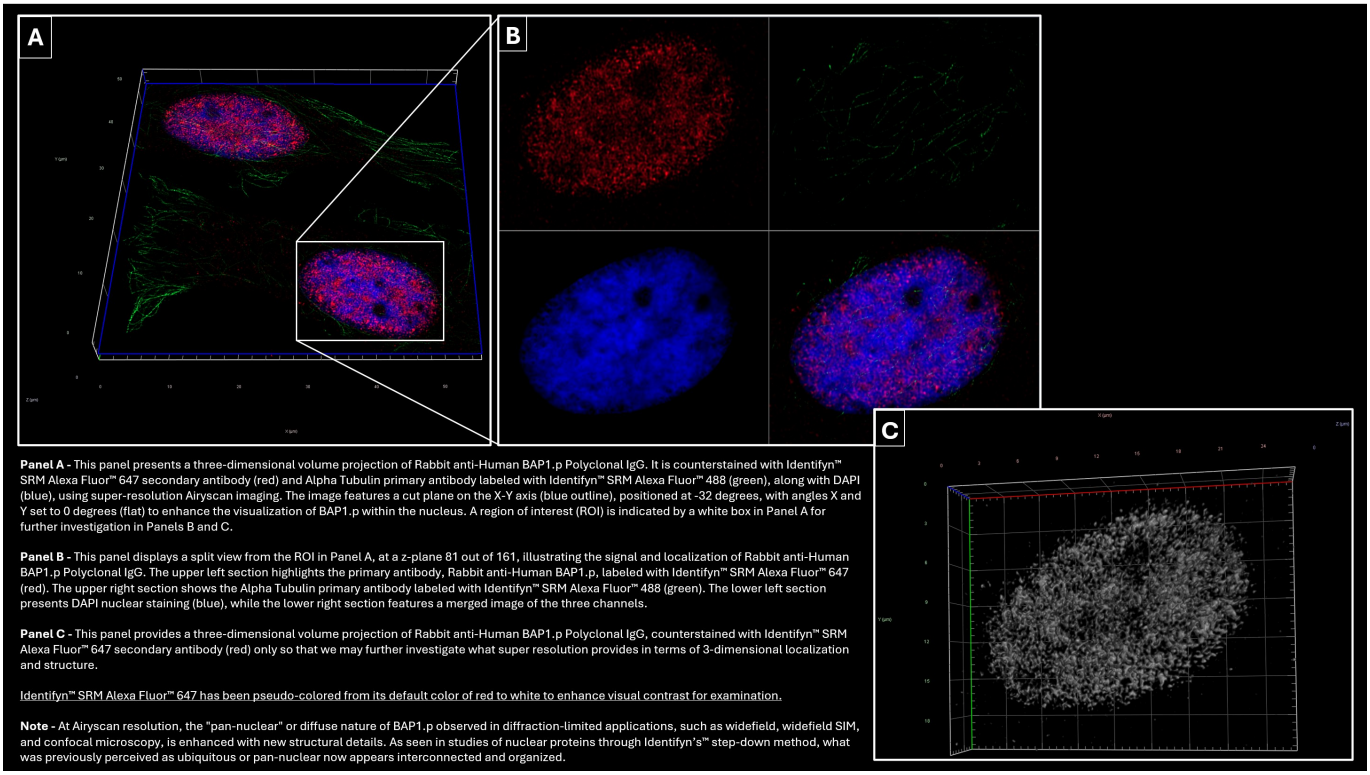
None

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

SOURCE PROVENANCE

Catalog	PA-RP-000005
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	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0157B9D3900A4579C45DC136F9658CD56E8B2747640DDDDE4B8597AFE1C26B9B
Method PDF SHA-256	13167E30F65E8D39FC0A2F06258FC011D5C186758D6A7E69D90C76306AF8EB87

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A - This panel presents a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG. It is counterstained with Identifyn™ SRM Alexa Fluor™ 647 secondary antibody (red) and Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green), along with DAPI (blue), using super-resolution Airyscan imaging. The image features a cut plane on the X-Y axis (blue outline), positioned at -32 degrees, with angles X and Y set to 0 degrees (flat) to enhance the visualization of BAP1.p within the nucleus. A region of interest (ROI) is indicated by a white box in Panel A for further investigation in Panels B and C.

Panel B - This panel displays a split view from the ROI in Panel A, at a z-plane 81 out of 161, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section highlights the primary antibody, Rabbit anti-Human BAP1.p, labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). The upper right section shows the Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). The lower left section presents DAPI nuclear staining (blue), while the lower right section features a merged image of the three channels.

Panel C - This panel provides a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG, counterstained with Identifyn™ SRM Alexa Fluor™ 647 secondary antibody (red) only so that we may further investigate what super resolution provides in terms of 3-dimensional localization and structure.

Identifyn™ SRM Alexa Fluor™ 647 has been pseudo-colored from its default color of red to white to enhance visual contrast for examination.

Note - At Airyscan resolution, the "pan-nuclear" or diffuse nature of BAP1.p observed in diffraction-limited applications, such as widefield, widefield SIM, and confocal microscopy, is enhanced with new structural details. As seen in studies of nuclear proteins through Identifyn's™ step-down method, what was previously perceived as ubiquitous or pan-nuclear now appears interconnected and organized.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 750
METADATA VALUES	64

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	161 Slices (4.8 µm) | 161 Slices (4.8µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1566 X 1566 | 744 X 589
Image Size (Scaled)	55.27 µm X 55.27 µm | 26.26 µm X 20.79 µm
Bit Depth	16 Bit
Image Center Position	X: 84.20 mm, Y: 47.85 mm
Stage Position	X: 84.20 mm, Y: 47.85 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm | 5.00 AU / 268 µm | 5.00 AU / 214 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS LP 640 | SBS Plate 10° | SBS SP 505
Laser Wavelength	639 nm @0.08% | 488 nm @0.08% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66 µs
Frame Time	7.82 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	643-735 | 499-549, 573-627 | 422-477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	8.8 (3D, Auto) | 8.6 (3D, Auto) | 7.5 (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 define regions of special interest.
X-Y	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-32%
Clip X	0°
Clip Y	0°

SOURCE METHOD

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Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

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).

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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, IgG, 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-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), 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™ 488 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.

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 = 161 Slices (4.8 µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 1566 X 1566
Image Size (Scaled) = 55.27 µm X 55.27 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.20 mm, Y: 47.85 mm
Stage Position = X: 84.20 mm, Y: 47.85 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 81 of 161 total - Panel B

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

Z-Stack = 161 Slices (4.8µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 744 X 589
Image Size (Scaled) = 26.26 µm X 20.79 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.20 mm, Y: 47.85 mm
Stage Position = X: 84.20 mm, Y: 47.85 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
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 = 8.8 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 268 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS Plate 10°
Laser Wavelength = 488 nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499-549, 573-627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.6 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
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 = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.5 (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)

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

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

Position of Clip = -32%
Clip X = 0°
Clip Y = 0°

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)

Summary

Panel A - This panel presents a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG. It is counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red) and Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), along with DAPI (blue), using super-resolution Airyscan imaging. The image features a cut plane on the X-Y axis (blue outline), positioned at -32 degrees, with angles X and Y set to 0 degrees (flat) to enhance the visualization of BAP1.p within the nucleus A region of interest (ROI) is indicated by a white box in Panel A for further investigation in Panels B and C.

Panel B - This panel displays a split view from the ROI in Panel A, at a z-plane 81 out of 161, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section highlights the primary antibody, Rabbit anti-Human BAP1.p, labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right section shows the Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The lower left section presents DAPI nuclear staining (blue), while the lower right section features a merged image of the three channels.

Panel C - This panel provides a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red) only so that we may further investigate what super resolution provides in terms of 3-dimensional localization and structure.

Identifyn® SRM Alexa Fluor™ 647 has been pseudo-colored from its default color of red to white to enhance visual contrast for examination.

Note - At Airyscan resolution, the "pan-nuclear" or diffuse nature of BAP1.p observed in diffraction-limited applications, such as widefield, widefield SIM, and confocal microscopy, is enhanced with new structural details. s seen in studies of nuclear proteins through Identifyn's™ step-down method, what was previously perceived as ubiquitous or pan-nuclear now appears interconnected and organized.

Image Corrections

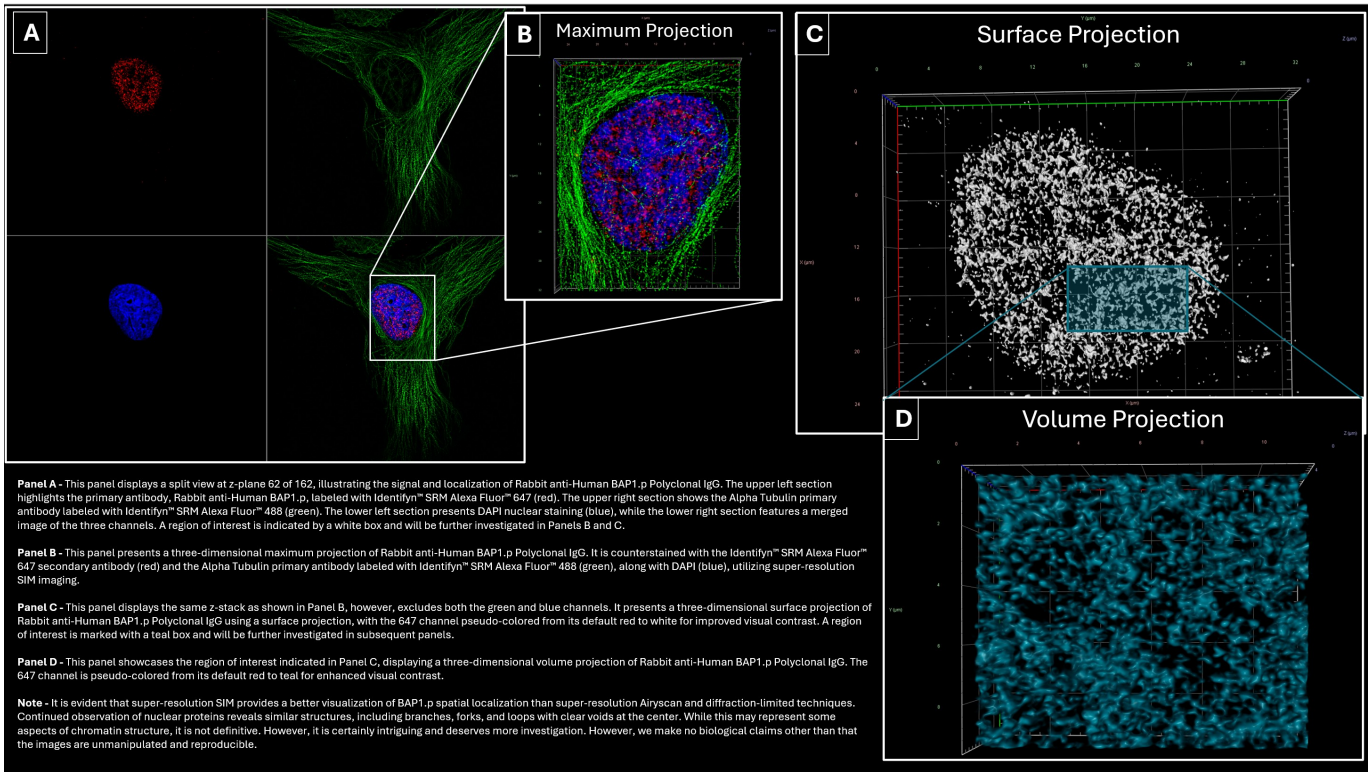
None

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

SOURCE PROVENANCE

Catalog	PA-RP-000005
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	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7488CF2784B9783384D7140EAE16DC4E1795CAE6A5083581445EFE1B4BC25D58
Method PDF SHA-256	5AC989595D7D81511B4E5F599E8CBA417D8309406FCCF536C7C9BB89A25FD0A0

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	69

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	162 Slices (4.83 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	4950 X 4321 | 1246 X 1553 | 555 X 548
Image Size (Scaled)	104.51 µm X 91.23 µm | 26.31 µm X 32.79 µm | 11.72 µm X 9.67 µm
Bit Depth	16 Bit
Image Center Position	X: 56.44 mm, Y: 35.21 mm
Stage Position	X: 56.44 mm, Y: 35.21 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	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 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	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% | 488 nm @1.50% | 405 nm @3.00%
Frame Time	1.33 s | 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	10.5081 (647), 11.1781 (488), 9.7530 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 4% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30% | Threshold 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled | Azimuth 80°, Elongation -130°, Enabled Tone Mapping | 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 Surface Projection	Precise
3D Volume 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, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

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, IgG, 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-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), 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™ 488 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.

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 62 of 162 total - Panel A

Z Stack - (3D)

Z-Stack = 162 Slices (4.83 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 4950 X 4321
Image Size (Scaled) = 104.51 µm X 91.23 µm
Bit Depth = 16 Bit
Image Center Position = X: 56.44 mm, Y: 35.21 mm
Stage Position = X: 56.44 mm, Y: 35.21 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 162 Slices (4.83 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1246 X 1553
Image Size (Scaled) = 26.31 µm X 32.79 µm
Bit Depth = 16 Bit
Image Center Position = X: 56.44 mm, Y: 35.21 mm
Stage Position = X: 56.44 mm, Y: 35.21 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D

Z-Stack = 162 Slices (4.83 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 555 X 548
Image Size (Scaled) = 11.72 µm X 9.67 µm
Bit Depth = 16 Bit
Image Center Position = X: 56.44 mm, Y: 35.21 mm
Stage Position = X: 56.44 mm, Y: 35.21 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

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 @1.50%
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 - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 10.5081 (647), 11.1781 (488), 9.7530 (DAPI)
Fitting = Fast Fit
Normalization = Auto

Split View - Panel A

The top-left panel features Rabbit anti-Human BAP1.p Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(a'')□ (H + L), the top-right panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, 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 62 of 162.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 4% 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)

3D Image Processing - Panel D

3D Volume Projection = Precise
Appearance = Threshold 4% all channels, Ramp 96% 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)

Summary

Panel A - This panel displays a split view at z-plane 62 of 162, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section highlights the primary antibody, Rabbit anti-Human BAP1.p, labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right section shows the Alpha Tubulin primary

antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The lower left section presents DAPI nuclear staining (blue). In comparison, the lower right section features a merged image of the three channels. A white box indicates a region of interest and will be further investigated in Panels B and C.

Panel B - This panel presents a three-dimensional maximum projection of Rabbit anti-Human BAP1.p Polyclonal IgG. It is counterstained with the Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red) and the Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), along with DAPI (blue), utilizing super-resolution SIM imaging.

Panel C - This panel displays the same z-stack as shown in Panel B, but excludes both the green and blue channels. It presents a three-dimensional surface projection of Rabbit anti-Human BAP1.p Polyclonal IgG using a surface projection, with the 647 channel pseudo-colored from its default red to white for improved visual contrast. A region of interest is marked with a teal box and will be further investigated in subsequent panels.

Panel D - This panel showcases the region of interest indicated in Panel C, displaying a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG. The 647 channel is pseudo-colored from its default red to teal for enhanced visual contrast.

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

Panel C - Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast.

Panel D - Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L) was pseudo-colored in Zen Software from red, the default color, to teal to provide visual contrast.

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

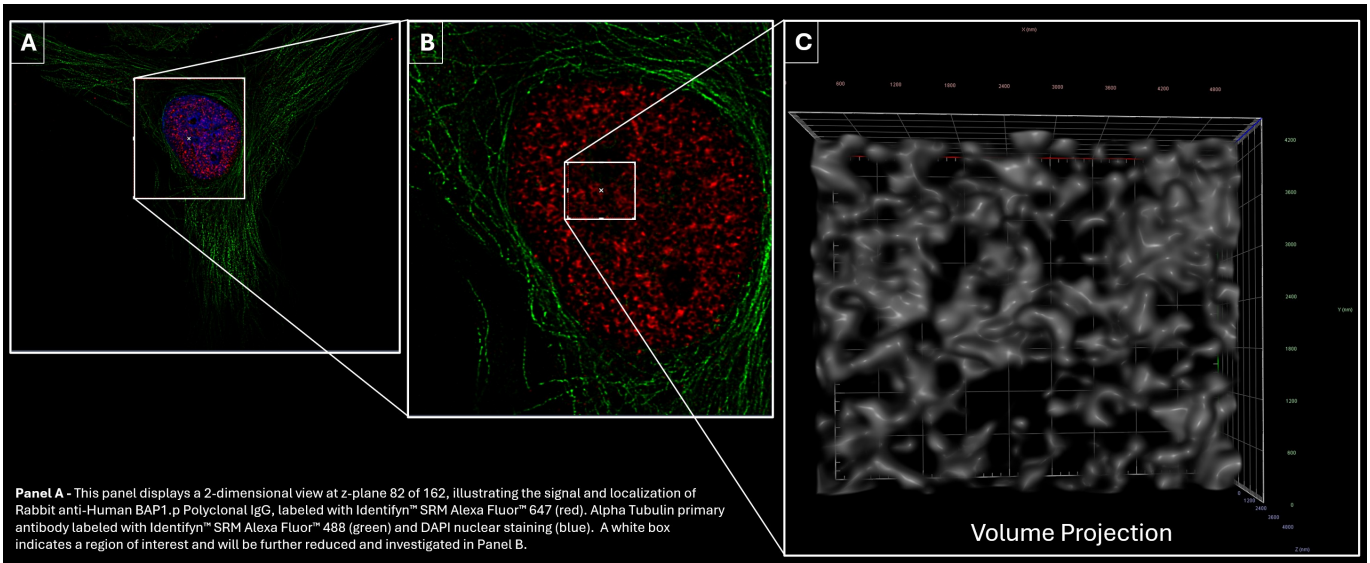
SOURCE PROVENANCE

Catalog	PA-RP-000005
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	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	6389D2EABC27C102B6F50B13ABEC9A7233589992C4B16AF6D672A4CC9433F3E0
Method PDF SHA-256	22695FC17BE7B2C959B5F58F7C10D0BC60DF35803C92DBC8E2F7F5B3157B91D7

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A - This panel displays a 2-dimensional view at z-plane 82 of 162, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG, labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green) and DAPI nuclear staining (blue). A white box indicates a region of interest and will be further reduced and investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 82 of 162, illustrating Rabbit anti-Human BAP1.p Polyclonal IgG. It is counterstained with the Identifyn™ SRM Alexa Fluor™ 647 secondary antibody (red) and the Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the resolution, as alpha tubulin segmentation is well-documented and reproducible, and serves as an internal control for resolution. 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 Rabbit anti-Human BAP1.p Polyclonal IgG. The 647 channel is pseudo-colored from its default red to light grey for enhanced visual contrast.

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 being recruited following etoposide-induced DNA damage to the breaks, or areas of DNA breaks. Again, we make no claims as to what this image represents, however, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is representative of the chromatin localization.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	66

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	162 Slices (4.83 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1406 X 1521 | 255 X 212
Image Size (Scaled)	106.75 µm X 106.75 µm | 29.68 µm X 32.11 µm | 5.38 µm X 4.48 µm
Bit Depth	16 Bit
Image Center Position	X: 56.44 mm, Y: 35.21 mm
Stage Position	X: 56.44 mm, Y: 35.21 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	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 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	100 ms | 120 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% | 488 nm @1.50% | 405 nm @3.00%
Frame Time	1.33 s | 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 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 90%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 45°, Scale Z 50%, 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.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

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, IgG, 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-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), 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™ 488 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.

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 82 of 162 total - Panel A

Z Stack - (3D)

Z-Stack = 162 Slices (4.83 μm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 106.75 μm X 106.75 μm
Bit Depth = 16 Bit
Image Center Position = X: 56.44 mm, Y: 35.21 mm
Stage Position = X: 56.44 mm, Y: 35.21 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Single Plane (2D) - Z-plane 82 of 162 total - Panel B

Z Stack - (3D)

Z-Stack = 162 Slices (4.83 μm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1406 X 1521
Image Size (Scaled) = 29.68 μm X 32.11 μm
Bit Depth = 16 Bit
Image Center Position = X: 56.44 mm, Y: 35.21 mm
Stage Position = X: 56.44 mm, Y: 35.21 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Z Stack - (3D) - Panel C

Z-Stack = 162 Slices (4.83 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 255 X 212
Image Size (Scaled) = 5.38 µm X 4.48 µm
Bit Depth = 16 Bit
Image Center Position = X: 56.44 mm, Y: 35.21 mm
Stage Position = X: 56.44 mm, Y: 35.21 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

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 @1.50%
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 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 90%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 50%, 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 82 of 162, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (red), Alpha Tubulin primary antibody labeled with Identifyn™SRM Alexa Fluor™ 488 (green), and DAPI nuclear staining (blue). A white box indicates a region of interest and will be further reduced and investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 82 of 162, illustrating Rabbit anti-Human BAP1.p Polyclonal IgG It is counterstained with the Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red) and the Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the resolution, as alpha tubulin segmentation is well-documented and reproducible, and serves as an internal control for resolution. 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 Rabbit anti-Human BAP1.p Polyclonal IgG. The 647 channel is pseudo-colored from its default red to light grey for enhanced visual contrast.

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 demonstrate a gain in resolving the three-dimensional structural localization details of BAP1.p recruited following etoposide-induced DNA damage to the breaks or areas of DNA breaks. 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 coating chromatin.

Image Corrections

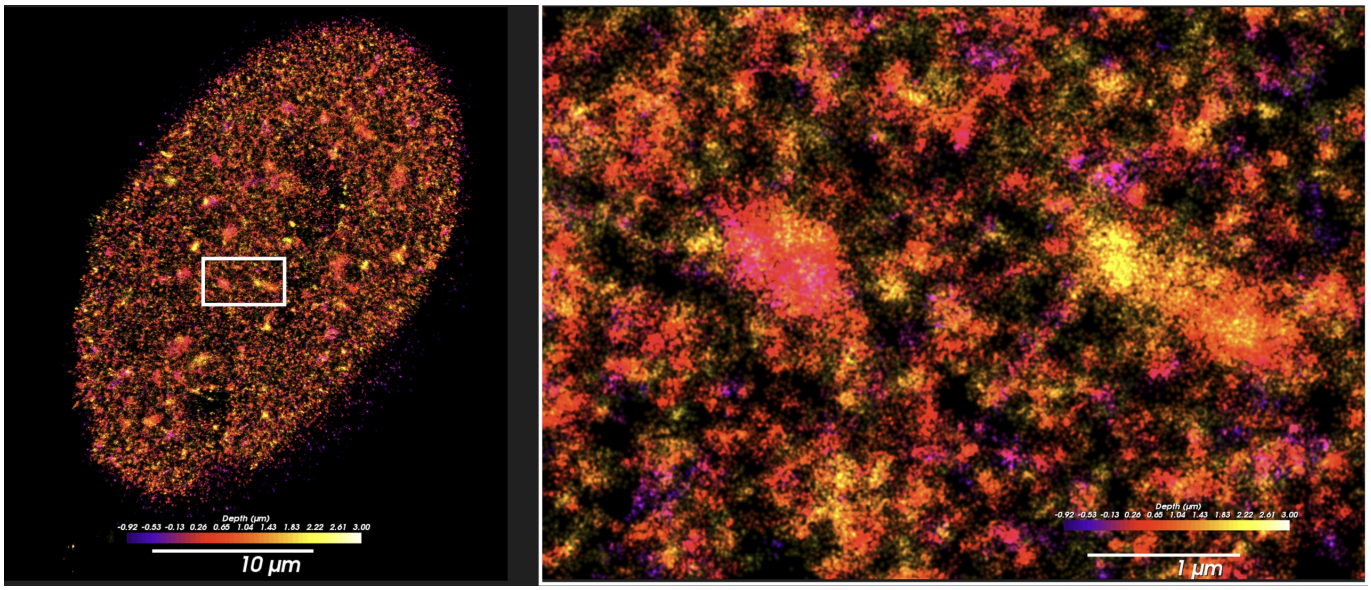
Panel C - Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L) was pseudo-colored in Zen Software from red, the default color, to light grey to provide visual contrast.

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

SOURCE PROVENANCE

Catalog	PA-RP-000005
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	66
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F846B84B66F03AD7DD92620A068DC96765074DAEC40FEF06380E5EAA39ADB762
Method PDF SHA-256	546F019C198B88525AA4A2C7AE1FF5FC3E912A6D24AB14B282C31FC3142008AC

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	71

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	179 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is selected
Piezo Record	Begin: 175; End: 177.2
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	11 using Steps: 0.2µm (200nm)
Cycles	15
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	70%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 15
Sizing Mode	Constant
Particle size	20nm
Color by	Constant
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.4
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	10 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-600 to +600
Radial precision	25
Axial precision	65

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, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

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 µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 6 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, was incubated for 1 hour at room temperature at a dilution of 1:200. Cells were washed 3X in Identifyn's™ proprietary Wash Buffer, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:200 Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:

Calibration -

Calibration follows Identifyn's Calibration Standard Operating Procedure, which can be found here.

https://identifyn.com/storm-calibration

Image Acquisition -

SRX Software - Under the 'Single Molecule Localization' tab, 'Record' is selected and contains the following workflow tabs and parameters.

Configuration Tab - Capture Configuration

Number of Probes: 1
Number of Simultaneous Probes: 1
Number of Reference Probes: 1
Camera: Both Cameras
Color Channel: Both Channels
Probe Capture Mode: Interleaved
Z -Stack Capture Mode: Interleaved
Multiple Location Capture: Not selected
Post Record Reference: Not Selected
Fluidics: Not Selected
Autofocus Drift Correction: On

Microscope configuration

Biplane Module: 635nm Dichroic
Objective: Silicon Selected
Stage Insert: Rectangular Selected
Course Focus Position: 8-Well Chamber Selected
Dichroic: SuperRes
PSF: (Red, Orange)
Heater: Current: 26

Camera Configuration

Recording Vertical Field of View: 100%
Widefield Camera Exposure Time: 100ms
Super Resolution Camera Exposure Time: 20ms
Widefield Camera Binning: 1
Per-Probe Exposure Time: Selected

Probe Configuration

Predefined Probe: First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser: 0% Selected
Exposure Time: 20ms Selected

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200µm
Capture Mode: Live

Stage and Piezo Selection

Examination of the Sample

Cell(s) or Cellular Structure Localization - The red-bordered box identifies the area of the SuperRes view and defines a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.
Piezo Current: 179 µm

Probe 1 Laser control (640 nm (635 nm Dichroic)

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100ms
Default values are maintained unless otherwise noted.
Widefield Laser: "Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

Current Position" -0.979
Current Intensity: 4.5
Calibration Value: -5070 nm/unit
Autofocus Mode: Pre-Capture
View Mode

SuperRes: 50µm is selected

Capture Mode

Live

Stage and Piezo Selection

Cell(s) or Cellular Structure Localization - Positioning of the target Cell or Subsection of the Target Cell is optimized and optimally focused.

Piezo Record: Begin: 175; End: 177.2

Probe 1 Laser control (640 nm (635 nm Dichroic)

SuperRes 640nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

Reference Probe 1 Laser control (640 nm (635 nm Dichroic)

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 11 using Steps: 0.2µm (200nm)
Cycles: 15
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control (640 nm (640 nm Dichroic)

Laser Power: 70%

Expert Configuration Options - The Localization Tab

Beyond the values shown, all other parameters were set to their default values.

Localization

Cutout Width: 16px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1px
Fiducial at Max Accumulation: Not selected
Max Particles Per Frame: No Max
Background SLPE Removal: Not Selected
Cutout/Background Removal: Not Selected
Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

The folder is created automatically with the localization file for this image acquisition.

Recording Summary

All parameters were carried over from previous tabs and are kept except:
Probe1: BG threshold: 15

Region of Interest -

Not selected

Display

Was deployed to inspect if the BG threshold applied chooses localizations in an expected manner

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Constant
Particle size: 20nm
Color by: Constant
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.4
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 10 Intervals
Pixel Size: 50nm
Smoothing: 8.00

Advanced Particle Filters - Applied to the drift-corrected data with the following parameters

PSF Z offset: -600 to +600
Radial precision: 25
Axial precision: 65
The other parameters were not applied.

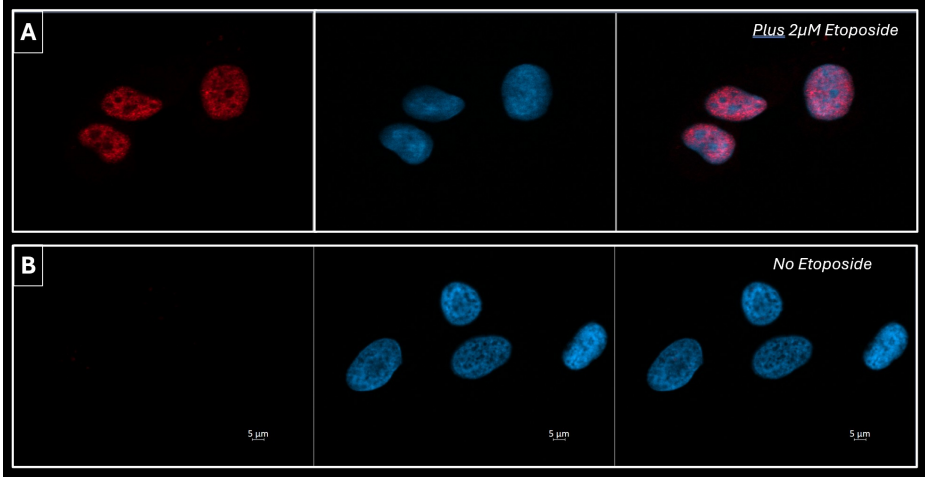
Statistical analysis - NA

Image by P. Raut

SOURCE PROVENANCE

Catalog	PA-RP-000005
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E0E7EABFB5823689B0F602ED4A31BBC75B653286B093796A4467FD871AA874A8
Method PDF SHA-256	3E435F2ADA7C10D846EF18AFBCB3611B7271CCC742E6002F851986A7C2B060CA

ORIGINAL IMAGE EVIDENCE / CONTROL



C

	Track 1	Track 2
Reflector	50 Cy 5	49 DAPI
Beam Splitter	660	395
Filter Ex. Wavelength	625 - 655	335 - 383
Filter Em. Wavelength	665 - 715	420 - 470
Contrast Method	Fluorescence	Fluorescence
Light Source	X-Cite Xylis Lamp	X-Cite Xylis Lamp
Light Source Intensity	6.00 %	6.00 %
Line Step		
	Channel 1	Channel 2
Channel Name	AF647	DAPI
Channel Description		
Dye Name	AF647	DAPI
Channel Color		
Excitation Wavelength	653	353
Emission Wavelength	668	465
Effective NA	1.25	1.25
Imaging Device	807 mono	807 mono
Camera Adapter	1x Camera Adapter	1x Camera Adapter
Exposure Time	40 ms	18 ms
Depth of Focus	1.30 μm	0.90 μm
Binning Mode	2,2	2,2

Panel A – DNA damage was induced by treating cells with 2 μM etoposide-spiked complete growth media for 24 hours. This panel presents a split-channel view of z-plane 21 out of 44 (same was used for both Panel A and B). The left-hand panel shows the Rabbit anti-Human BAP1.p antibody, IgG, counterstained with Identifyn™ SRM Alexa Fluor™ 647 secondary (red), while the middle panel displays the DAPI nuclear staining (blue). The right panel merges both channels. The introduction of DNA damage demonstrates a discernible response, evidenced by the formation of pan-nuclear staining for BAP1.p. Notably, the protein's absence in the nucleolar space is expected. Furthermore, while widefield SIM is diffraction-limited, we observe the formation of aggregates or foci, which we intend to investigate further using our step-down methodology, particularly through super-resolution Airyscan, SIM, SIM2, and STORM techniques.

Panel B – DNA damage was not induced in this panel. It presents a split-channel view of the two-dimensional image collected. The left-hand panel again shows the Rabbit anti-Human BAP1.p antibody, IgG, counterstained with Identifyn™ SRM Alexa Fluor™ 647 secondary (red), while the middle panel displays the DAPI nuclear staining (blue). The right panel merges both channels. The lack of DNA damage results in little to no BAP1.p signal being detected. Although there is some negligible signal under the same imaging conditions, it does not match the robust protein aggregation observed following DNA damage. This leads us to conclude that BAP1.p was activated and recruited following DNA damage and is strongly localized within the nuclear compartment.

Panel C – This panel displays the acquisition settings used for both images, confirming that they are identical, with the same excitation input and exposure times. Additionally, we note that the image acquisition was performed in a z-stack, using the same z-plane for both cases and all other settings remained the same.

EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	36

STRUCTURED ACQUISITION METADATA / CONTROL

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
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image size (Pixels)	761 X 707 | 1080 X 741
Image Size (Scaled)	108.71µm X 101.00µm | 152.29µm X 105.86µm
Bit Depth	14 Bit
Image Center Position	X: 48.10µm, Y: 49.96µm | X: 70.95µm, Y: 44.96µm
Stage Position	X: 48.10µm, Y: 49.96µm | X: 70.95µm, Y: 44.96µm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	50 Cy 5 | 49 DAPI
Beam Splitter	660 | 395
Filter Excitation Wavelength	625 - 655 | 335-383
Filter Emission Wavelength	665 - 715 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @6.00% | X-Cite Xylis Lamp Intensity 6.00%
Exposure Time	40 ms | 18 ms
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mod" "Optical Sectioning" - enabled correction 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.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal, IgG
Cat ID# - PA-RP 000005

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

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

Plus Damage - Panel A

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, Rabbit anti-Human BAP1.p antibody, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody 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.

No Damage - Panel B

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human BAP1.p antibody, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody 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.

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) - Panel A (Z-plane 21 of 44)

Channels = 2
Scaling (Per Pixel) = 0.143μm X 0.143μm
Image size (Pixels) = 761 X 707
Image Size (Scaled) = 108.71μm X 101.00μm
Bit Depth = 14 Bit
Image Center Position = X: 48.10μm, Y: 49.96μm
Stage Position = X: 48.10μm, Y: 49.96μm
ROI Center Offset = X: 1.14μm, Y: 0.00μm

Single Plane (2D) - Panel B (Z-plane 21 of 44)

Channels = 2
Scaling (Per Pixel) = 0.143μm X 0.143μm
Image size (Pixels) = 1080 X 741
Image Size (Scaled) = 152.29μm X 105.86μm
Bit Depth = 14 Bit
Image Center Position = X: 70.95μm, Y: 44.96μm
Stage Position = X: 70.95μm, Y: 44.96μm
ROI Center Offset = X: 1.14μm, Y: 0.00μm

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 @6.00%
Exposure Time = 40 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 = 18 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.

Summary

Panel A - DNA damage was induced by treating cells with 2 µM etoposide-spiked complete growth media for 24 hours. This panel presents a split-channel view of z-plane 21 out of 44, which was used for both Panel A and Panel B. The left-hand panel shows the Rabbitanti-Human BAP1.p antibody, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary (red), while the middle panel displays the DAPI nuclear staining (blue). The right panel merges both channels. The introduction of DNA damage demonstrates a discernible response, evidenced by the formation of pan-nuclear staining for BAP1.p. Notably, the protein's absence in the nucleolar space is expected. Furthermore, while widefield SIM is diffraction-limited, we observe the formation of aggregates or foci, which we intend to investigate further using our step-down methodology, particularly through super-resolution Airyscan, SIM, SIM2, and STORM techniques.

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Panel C - This panel displays the acquisition settings used for both images, confirming that they are identical, with the same excitation input and exposure times. Additionally, we note that the image acquisition was performed in a z-stack, using the same z-plane for both cases.

Image Corrections

None

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

SOURCE PROVENANCE

Catalog	PA-RP-000005
Stage	Control
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	36
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1CE8A4D4C5B4FFCDB2B0775E798C73B8523C21E46687356675E96AFD9C143ED2
Method PDF SHA-256	DB46C161F067C542A028DAA0362D3BE984B080B6BB6977D717048AF8196B50C7