BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

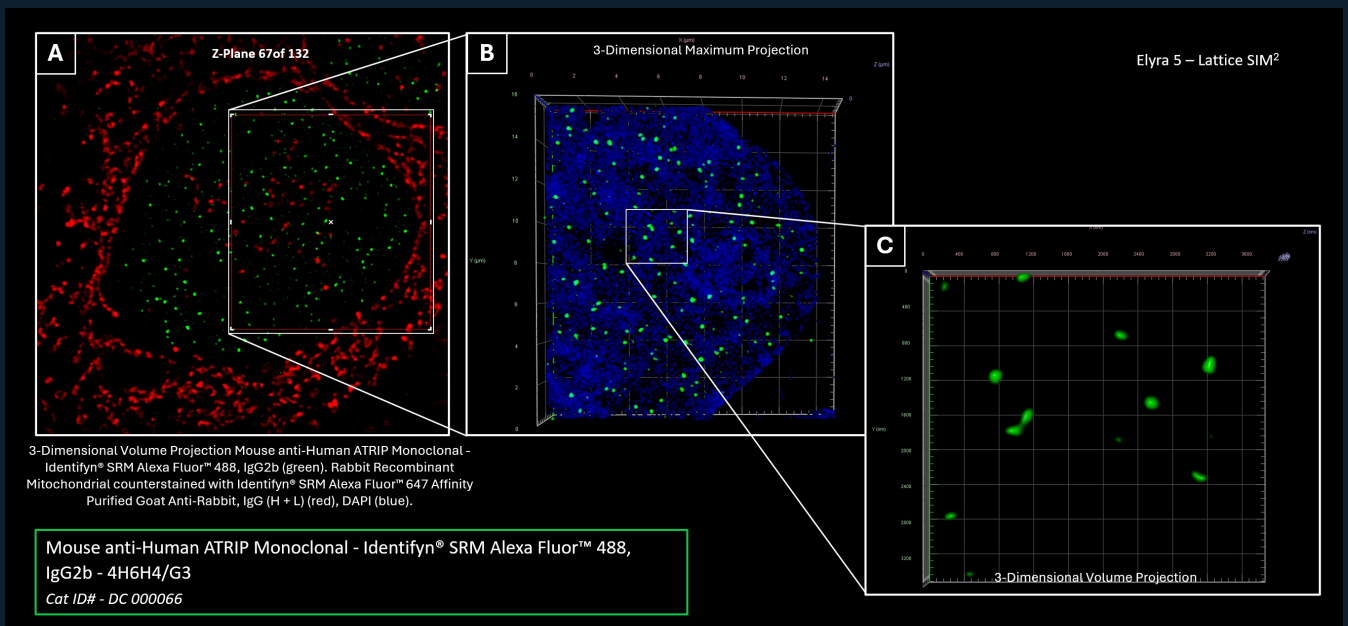
ATRIP

MICROSCOPY EVIDENCE ASSESSMENT

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488

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

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



3-Dimensional Volume Projection Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b (green). Rabbit Recombinant Mitochondrial counterstained with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) (red), DAPI (blue).

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - 4H6H4/G3

Cat ID# - DC 000066

Super-resolution lattice structured illumination microscopy squared (SIM^2) of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment is shown. Panel A presents a three-dimensional maximum-intensity projection of ATRIP 488 together with a rabbit recombinant monoclonal mitochondrial marker visualized using Identifyn® goat anti-rabbit Alexa Fluor™ 647 and DAPI, confirming extranuclear exclusion and nuclear confinement consistent with the unconjugated ATRIP control (Rabbit anti-Human ATRIP, Cat. ID# PA-MM-000003). Panel B shows a maximum projection with a white box denoting the region of interest examined in Panel C, displayed as a three-dimensional volume projection of ATRIP alone. At SIM^2 lateral resolution (~40–60 nm in X–Y), ATRIP foci exhibit increased spatial separation relative to SIM, confirming damage-dependent enrichment and recruitment; however, fine subunit architecture remains unresolved. These data establish ATRIP nuclear recruitment and enrichment beyond diffraction-limited modalities while explicitly marking the transition point at which single-molecule localization microscopy is required for definitive structural resolution within the CDx step-down framework.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM^2

DC-000066 | 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
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

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

EVIDENCE AVAILABLE

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

BENNETT ASSESSMENT

The preserved DC-000066 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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 / NIR-BOUNDED PARTIAL STEPDOWN

The preserved DC-000066 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488	3; 38 eGFP; 49 DAPI; 450 - 490; 335 - 383; 500 - 550; 420 - 470; 493
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 38 eGFP; 50 Cy 5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 - 550
Confocal	405/DAPI; 488; 647	3; None; 488nm @0.5%; 639nm @0.09%; 405nm @0.2%; 493; 653; 353
Airyscan	405/DAPI; 488; 647; 750	3; None; 488nm @1.10%; 639nm @0.07%; 405nm @0.2%; 493; 653; 353
SIM	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820 -SR; T3-Axiocam 820 #2-SR; 488
SIM²	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820 -SR; T3-Axiocam 820 #2-SR; 488

MODALITY ASSESSMENT / PART 1 OF 7

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 7

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 678 X 694; Image Size (Pixels): 678 X 694; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 200 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 30.

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.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 7

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Not stated in extracted source metadata. Objective: not explicitly stated. Acquisition: Z-Stack: 84 Slices (8.3 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 800 X 742; Image Size (Pixels): 800 X 742; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 300 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @30.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25; 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. Structured source metadata values: 55.

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 not stated, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 7

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Not stated in extracted source metadata. Objective: not explicitly stated. Acquisition: Z-Stack: 71 Slices (2.1 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 550 X 667; Image Size (Pixels): 550 X 667; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsp-Pmt3; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.29µs; Frame Time: 4.97s. Illumination: Laser Wavelength: 488nm @0.5%; 639nm @0.09%. Optical/scan: Pinhole: 1.00AU / 51µm; 1.00AU / 65µm; Scan Zoom: 1.7; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 8.2 (3D, Auto); Filter: 7.2 (3D, Auto). Structured source metadata values: 55.

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 not stated, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 7

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Not stated in extracted source metadata. Objective: not explicitly stated. Acquisition: Z-Stack: 113 Slices (3.36µm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1566 X 1566; 145 X 128; Image Size (Pixels): 1566 X 1566; 145 X 128; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.66µs; Frame Time: 7.82s. Illumination: Laser Wavelength: 488nm @1.10%; 639nm @0.07%. Optical/scan: Pinhole: 5.00AU / 250µm; 5.00AU / 328µm; 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); View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 63.

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 not stated, 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 7

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Not stated in extracted source metadata. Objective: not explicitly stated. Acquisition: Z-Stack: 132 Slices (3.93 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 2667 X 2573; 674 X 668; Image Size (Pixels): 2667 X 2573; 674 X 668; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 100 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 488 nm @1.00%; 640 nm @0.5%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 35°, Scale Z 10%, 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 not stated, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 7

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Not stated in extracted source metadata. Objective: not explicitly stated. Acquisition: Z-Stack: 132 Slices (3.93 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1471 X 1428; 708 X 763; Image Size (Pixels): 1471 X 1428; 708 X 763; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 100 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 488 nm @1.00%; 640 nm @0.5%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 25°, Scale Z 20%, 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 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 not stated, 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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000066 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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)=2667 X 2573; Image Size (Scaled)=56.31 µm X 54.32 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)=1471 X 1428; Image Size (Scaled)=31.06 µm X 30.15 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 -> 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.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

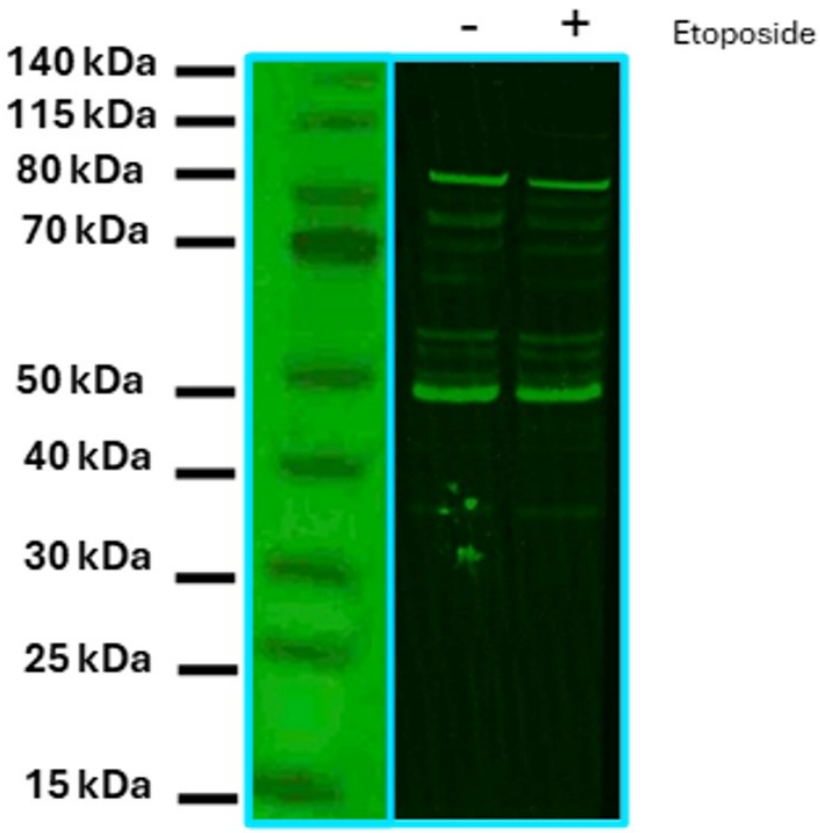
This dossier preserves the complete available evidence progression for DC-000066. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	Not stated	PRESERVED
4	Confocal	Not stated	PRESERVED
5	Airyscan	Not stated	PRESERVED
6	SIM	Not stated	PRESERVED
7	SIM²	Not stated	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



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

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

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

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3)
Cat ID# - DC 000070

Expected MW: 82 kDa

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and nuclear lysis buffer, 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, then washed 5 times with PBS. The blocked membrane was incubated with Mouse anti-Human ATRIP Monoclonal - Identifyn™ SRM Alexa Fluor™ 488 - Direct Conjugate for 2 hours, followed by five washes in 1X 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

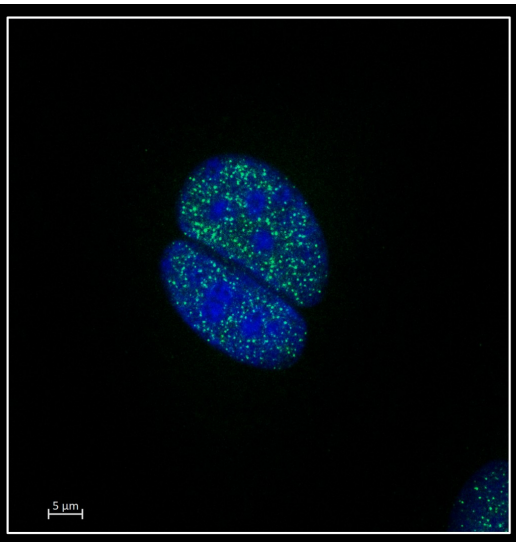
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000066
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CE95E96304E100BAE22257A6747A95F310AF7EFEFF51E7575000DBA4AC2DE0AE
Method PDF SHA-256	F0FEB8255D6082895EDE106B356139B1B82B332705CA360BECFA27EFA9B8C391

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - 4H6H4/G3
Cat ID# - DC 000066

Reflector	38 eGFP	49 DAPI
Beam Splitter	495	395
Filter Ex. Wavelength	450 - 490	335 - 383
Filter Em. Wavelength	500 - 550	420 - 470
Contrast Method	Fluorescence	Fluorescence
Light Source	X-Cite Xylis Lamp	X-Cite Xylis Lamp
Light Source Intensity	20.00 %	10.00 %
Channel Name	AF488	DAPI
Channel Description		
Dye Name	AF488	DAPI
Channel Color		
Excitation Wavelength	493	353
Emission Wavelength	517	465
Effective NA	1.4	1.4
Imaging Device	Axiocam 705	Axiocam 705
Camera Adapter	1x Camera Adapter	1x Camera Adapter
Exposure Time	200 ms	25 ms
Depth of Focus	0.80 µm	0.72 µm
Binning Mode	2.2	2.2

Widefield

Widefield imaging establishes baseline ATRIP detectability and nuclear compartmentalization following etoposide-induced DNA damage, satisfying the CCR Gate (go/no-go) criterion for biological responsiveness. ATRIP, detected using the Identifyn® ATRIP Alexa Fluor™ 488 Direct Conjugate (referencing the unconjugated ATRIP monoclonal, Cat. ID# PA-MM-000003), exhibits clear nuclear localization relative to DAPI without detectable cytoplasmic signal or imaging artifacts. Acquisition metadata acquired in Zeiss ZEN demonstrate low-stress imaging conditions (20% LED excitation, 200 ms exposure, Axiocam 705), confirming that the observed signal is not acquisition-forced and reflects genuine biological behavior. Under etoposide treatment, ATRIP transitions from a diffuse nuclear distribution to punctate intranuclear foci, consistent with its established role in ATR pathway activation at sites of replication stress and DNA damage. This redistribution satisfies the CCR Enrichment gate, indicating functional recruitment rather than static expression. Collectively, these data support ATRIP as a damage-responsive, enrichment-qualified DDR biomarker, appropriate for advancement within the CCR step-down workflow toward higher-resolution modalities where spatial organization and pathway engagement can be further interrogated.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	30

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)	678 X 694
Image Size (Scaled)	74.26 µm X 76.01 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	38 eGFP | 49 DAPI
Beam Splitter	495 | 395
Filter Excitation Wavelength	450 - 490 | 335 - 383
Filter Emission Wavelength	500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	200 ms | 25 ms
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	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

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3)
Cat ID# - DC 000070

ATRIP (ATR Interacting Protein) is a key partner and co-activator of ATR (Ataxia Telangiectasia and Rad3-related), a crucial kinase in the DNA damage response, particularly in response to replication stress and single-strand breaks (SSBs). ATRIP plays a pivotal role in recognizing and binding to single-stranded DNA (ssDNA), facilitating ATR activation at DNA damage sites.

Overview of ATRIP

ATRIP is the regulatory subunit that associates with ATR and directs it to sites of DNA damage. It contains a domain that allows it to bind to Replication Protein A (RPA), a complex that binds and stabilizes single-stranded DNA. This interaction with RPA-coated ssDNA is crucial for ATRIP's ability to guide ATR to regions where the DNA damage response is needed.

Role of ATRIP in Single-Strand Break Repair

ATRIP plays a central role in the activation of ATR, which is critical to the cellular response to single-strand breaks and replication stress:

Recognition of Single-Stranded DNA: ATRIP, through its interaction with RPA, identifies regions of ssDNA. These regions are commonly found at stalled replication forks, resected DNA ends, or sites of single-strand breaks.

Recruitment of ATR: Once ATRIP binds to RPA-coated ssDNA, ATR is recruited to the site. This recruitment is crucial for ATR activation, leading to a cascade of events that coordinate the DNA damage response and facilitate DNA repair.

Activation of Checkpoints: By facilitating ATR activation, ATRIP indirectly contributes to checkpoint signaling. This leads to CHK1 phosphorylation, which plays a role in cell cycle arrest, providing time for the repair of single-strand breaks before the cell cycle progresses.

ATRIP's Broader Role in the DNA Damage Response

Beyond its specific role in single-strand break repair, ATRIP's interaction with ATR has broader implications for the DNA damage response:

Coordination of DNA Repair: The ATR/ATRIP complex orchestrates the recruitment of DNA repair proteins. This coordination ensures that repair processes like base excision repair (BER) and homologous recombination can proceed efficiently at sites of single-strand breaks.

Response to Replication Stress: ATRIP is crucial in responding to replication stress, which can lead to single-strand breaks or stalled replication forks. By facilitating ATR activation, ATRIP helps stabilize replication forks and prevent more severe DNA damage, such as double-strand breaks.

ATRIP is a critical regulatory protein that guides ATR to sites of single-strand breaks and other forms of replication stress. By recognizing RPA-coated ssDNA and recruiting ATR, ATRIP plays a pivotal role in activating the DNA

damage response, leading to checkpoint activation and coordination of DNA repair processes. Its role in stabilizing replication forks and promoting genomic stability underscores its importance in maintaining genome integrity.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 678 X 694
Image Size (Scaled) = 74.26 µm X 76.01 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 200 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 µm
Binning Mode = 2,2

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 @10.00%
Exposure Time = 25 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

Zen Acquisition Info Tab - Panel B

Shown is the acquisition of the 488 nm channel (ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488) using an X-Cite Xylis illumination source operated at 20% output and detected with an Axiocam 705 camera using a 200 ms exposure time.

Summary

Widefield imaging establishes baseline ATRIP detectability and nuclear compartmentalization following etoposide-induced DNA damage, satisfying the CCR Gate (go/no-go) criterion for biological responsiveness. ATRIP, detected using the Identifyn® ATRIP Alexa Fluor™ 488 Direct Conjugate (referencing the unconjugated ATRIP monoclonal, Cat. ID# PA-MM-000003), exhibits clear nuclear localization relative to DAPI without detectable cytoplasmic signal or imaging artifacts. Acquisition metadata acquired in Zeiss ZEN demonstrate low-stress imaging conditions (20% LED excitation, 200 ms exposure, Axiocam 705), confirming that the observed signal is not acquisition-forced and reflects genuine biological behavior.

Under etoposide treatment, ATRIP transitions from a diffuse nuclear distribution to punctate intranuclear foci, consistent with its established role in ATR pathway activation at sites of replication stress and DNA damage. This redistribution satisfies the CCR Enrichment gate, indicating functional recruitment rather than static expression. Collectively, these data support ATRIP as a damage-responsive, enrichment-qualified DDR biomarker, appropriate for advancement within the CCR step-down workflow toward higher-resolution modalities where spatial organization and pathway engagement can be further interrogated.

Image Correction

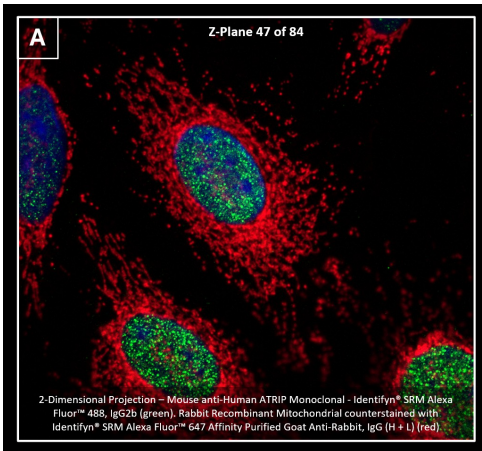
NONE

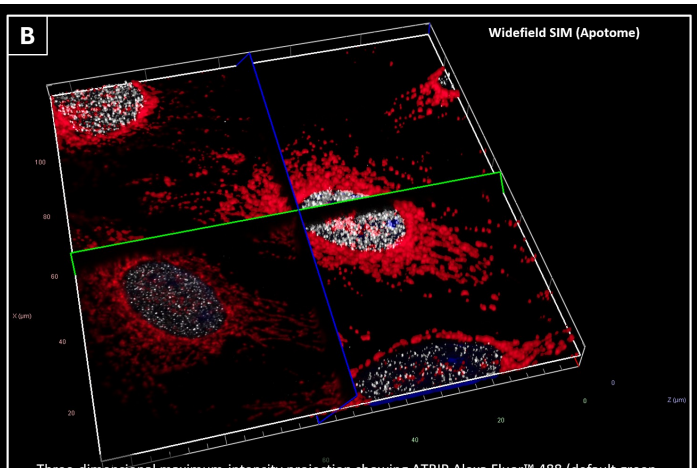
Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000066
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	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AA2AD6C3CD8A358D8A90424DDB2BEB6357DA3480435163197 39B6235A5CF444D
Method PDF SHA-256	DE067DA1CB3F759AA42AA45E8F912AC99B95A602B79A52537D6AC921AD9C8E9C

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME





Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - 4H6H4/G3
Cat ID# - DC 000066

Widefield SIM (Apotome) imaging of the ATRIP Alexa Fluor™ 488 Direct Conjugate is shown in Panel A as a 2D optical section at z-plane 47 of 85, demonstrating ATRIP nuclear localization consistent with the baseline widefield ATRIP dataset. A recombinant rabbit monoclonal antibody directed against mitochondria, visualized with an Identifyn® goat anti-rabbit Alexa Fluor™ 647 secondary, is included exclusively as a cytoplasmic exclusionary reference, while DAPI marks nuclear DNA. ATRIP signal remains confined to the nuclear compartment and is spatially segregated from mitochondrial labeling, supporting specificity and nuclear inclusion.

Panel B presents a 3D maximum-intensity projection with a wedge cut, illustrating orthogonal views of ATRIP localization across X–Y (white), X–Z (blue), and Y–Z (green) planes. Across all axes, the ATRIP signal is restricted to the nuclear volume, with no detectable cytoplasmic contribution, confirming its compartmental confinement. Compared with standard widefield imaging, widefield SIM improves optical sectioning and depth resolution, enabling more precise visualization of ATRIP distribution throughout the nuclear volume while remaining below the resolution threshold required for subnuclear structural interpretation. Collectively, these data reinforce the nuclear localization observed in widefield ATRIP imaging and establish a consistent spatial baseline prior to progression into higher-resolution modalities.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	Not stated
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	55

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	Not stated
Z-Stack	84 Slices (8.3 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	800 X 742
Image Size (Scaled)	114.29 µm X 106.00 µm
Bit Depth	14 Bit
Image Center Position	X: 62.72 µm, Y: 48.71 µm
Stage Position	X: 62.72 µm, Y: 48.71 µm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	38 eGFP | 50 Cy 5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450 - 490 | 625 - 655 | 335 - 383
Filter Emission Wavelength	500 - 550 | 665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @30.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	300 ms | 100 ms | 25 ms
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.25 | 1.4
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.00 µm | 1.30 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, 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)
X-Y	Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	5% | -11%

FIELD	SOURCE-DOCUMENTED VALUE
Clip X	0°
Clip Z	0°
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.
Clip Y	45°
Y-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

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

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3)
Cat ID# - DC 000070

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

ATRIP (ATR Interacting Protein) is a key partner and co-activator of ATR (Ataxia Telangiectasia and Rad3-related), a crucial kinase in the DNA damage response, particularly in response to replication stress and single-strand breaks (SSBs). ATRIP plays a pivotal role in recognizing and binding to single-stranded DNA (ssDNA), facilitating ATR activation at DNA damage sites.

Overview of ATRIP

ATRIP is the regulatory subunit that associates with ATR and directs it to sites of DNA damage. It contains a domain that allows it to bind to Replication Protein A (RPA), a complex that binds and stabilizes single-stranded DNA. This interaction with RPA-coated ssDNA is crucial for ATRIP's ability to guide ATR to regions where the DNA damage response is needed.

Role of ATRIP in Single-Strand Break Repair

ATRIP plays a central role in the activation of ATR, which is critical to the cellular response to single-strand breaks and replication stress:

Recognition of Single-Stranded DNA: ATRIP, through its interaction with RPA, identifies regions of ssDNA. These regions are commonly found at stalled replication forks, resected DNA ends, or sites of single-strand breaks.

Recruitment of ATR: Once ATRIP binds to RPA-coated ssDNA, ATR is recruited to the site. This recruitment is crucial for ATR activation, leading to a cascade of events that coordinate the DNA damage response and facilitate DNA repair.

Activation of Checkpoints: By facilitating ATR activation, ATRIP indirectly contributes to checkpoint signaling. This leads to CHK1 phosphorylation, which plays a role in cell cycle arrest, providing time for the repair of single-strand breaks before the cell cycle progresses.

ATRIP's Broader Role in the DNA Damage Response

Beyond its specific role in single-strand break repair, ATRIP's interaction with ATR has broader implications for the DNA damage response:

Coordination of DNA Repair: The ATR/ATRIP complex orchestrates the recruitment of DNA repair proteins. This coordination ensures that repair processes like base excision repair (BER) and homologous recombination can proceed efficiently at sites of single-strand breaks.

Response to Replication Stress: ATRIP is crucial in responding to replication stress, which can lead to single-strand breaks or stalled replication forks. By facilitating ATR activation, ATRIP helps stabilize replication forks and prevent more severe DNA damage, such as double-strand breaks.

ATRIP is a critical regulatory protein that guides ATR to sites of single-strand breaks and other forms of replication stress. By recognizing RPA-coated ssDNA and recruiting ATR, ATRIP plays a pivotal role in activating the DNA damage response, leading to checkpoint activation and coordination of DNA repair processes. Its role in stabilizing replication forks and promoting genomic stability underscores its importance in maintaining genome integrity.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

Control Data - Primary Unconjugated Antibody

Z Stack - (3D) - Panels A, Z-plane 47 of 84, and Panel B, 3-Dimensional Maximum Projection

Z-Stack = 84 Slices (8.3 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 800 X 742
Image Size (Scaled) = 114.29 µm X 106.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 62.72 µm, Y: 48.71 µm
Stage Position = X: 62.72 µm, Y: 48.71 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @30.00%
Exposure Time = 300 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 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 @10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

2- Dimensional View - Panel A

Panel shows Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3) and Rabbit Recombinant Monoclonal against Mitochondira with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) and DAPI staining.

Image from Z-plane 47 of 84.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, 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 - Panel B

Clipping planes are introduced in Panel B to highlight the Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3) within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

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

Position of Clip = 5%
Clip X = 0°
Clip Z = 0°

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 = 5%
Clip Y = 45°
Clip Z = 0°

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

Position of Clip = -11%
Clip Y = 45°
Clip Z = 0°

Panel B shows a wedge-cut view displayed from the top with a slight backward rotation, highlighting the region of wedge removal and revealing the full depth of signal across the Z-stack. This orientation provides an alternative view of wedge geometry and confirms intranuclear localization of ATRIP.

Summary

Widefield structured illumination microscopy (SIM) was used to evaluate ATRIP recruitment following etoposide-induced DNA damage as an initial CDx gating and enrichment assessment. Panel A shows a two-dimensional optical section acquired at Z-plane 47 of 85, where the ATRIP Alexa Fluor™ 488 Direct Conjugate exhibits clear nuclear localization relative to DAPI. A recombinant rabbit monoclonal antibody targeting mitochondria, visualized with an Identifyn® goat anti-rabbit Alexa Fluor™ 647 secondary, is included solely as a nuclear exclusion reference, confirming the absence of ATRIP signal in extranuclear compartments.

At this resolution, ATRIP displays a predominantly nuclear distribution with localized signal intensification consistent with damage-dependent recruitment, rather than diffuse expression. While individual ATRIP assemblies are not structurally resolved at widefield SIM resolution, the observed nuclear confinement and enrichment satisfy CDx gate criteria, confirming biological responsiveness under genotoxic stress.

Panel B presents a three-dimensional maximum-intensity projection with a wedge cut, displaying orthogonal planes (X-Y, white; X-Z, blue; Y-Z, green). Across all axes, ATRIP signal remains restricted to the nuclear compartment, further validating compartment-specific recruitment and excluding cytoplasmic or mitochondrial contribution. This orthogonal analysis supports CDx enrichment criteria, establishing ATRIP as functionally engaged in the DNA damage response following etoposide treatment.

Collectively, these widefield SIM data provide a positive go/no-go readout for ATRIP recruitment and nuclear enrichment. While higher-resolution modalities (confocal, Airyscan, SIM, SIM²) are required to interrogate sub-nuclear organization or potential ATRIP foci architecture, the current dataset establishes the necessary biological foundation for advancement within a stepwise CDx evaluation workflow.

Image Correction

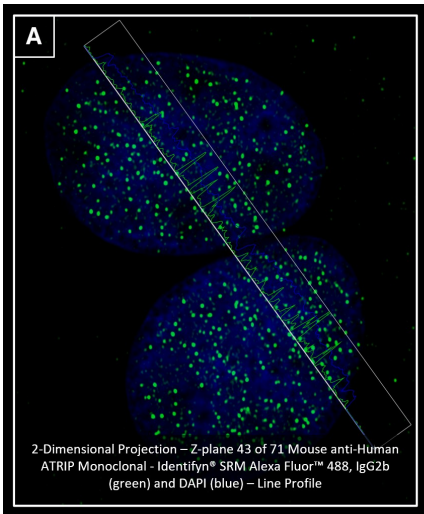
NONE

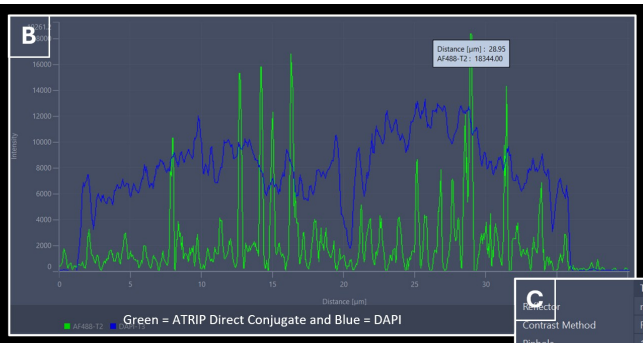
Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

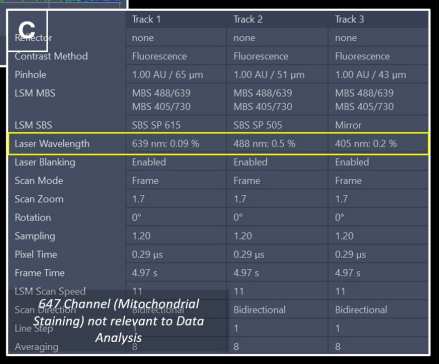
Catalog	DC-000066
Stage	Widefield SIM - Apotome
Instrument	Not stated in extracted metadata
Microscope configuration	Not stated in extracted metadata
Structured source metadata values	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	60109D202BD275AD6CFDF43E1CE266DCDB6332CA5F9417A400E4A9F9CFBDBCD5
Method PDF SHA-256	08222D5CA94B990988CF29F55473836C3CBEF5E92AD4395AE77B798D872C3BA9

ORIGINAL IMAGE EVIDENCE / CONFOCAL





Zeiss LSM980 NIR - Confocal



647 Channel (Mitochondrial Staining) not relevant to Data Analysis

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - 4H6H4/G3
Cat ID# - DC 000066

Confocal imaging of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment demonstrates damage-dependent nuclear enrichment consistent with the baseline response observed in the unconjugated ATRIP antibody condition (Cat. ID# PA-MM-000003). A representative 2D optical section (z-plane 43 of 71) shows robust intranuclear ATRIP signal with punctate foci/aggregate formation, confirming activation and recruitment during the DNA damage response. Quantitative analysis indicates a high fluorescence intensity (maximum 18,344 a.u.) acquired under low excitation conditions (488 nm at 0.5% laser power; Zeiss ZEN metadata shown for transparency), supporting a genuine biological response rather than acquisition-driven amplification. At confocal resolution, ATRIP recruitment is clearly detectable and meets CDx enrichment criteria; however, the observed foci remain structurally unresolved at the diffraction limit, necessitating progression to super-resolution modalities for definitive organizational interpretation, consistent with the step-down imaging framework.

EVIDENCE TIER	Confocal
INSTRUMENT	Not stated
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	55

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	Not stated
Z-Stack	71 Slices (2.1 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	550 X 667
Image Size (Scaled)	32.35 µm X 39.23 µm
Bit Depth	16 Bit
Image Center Position	X: 84.11 µm, Y: 50.16 µm
Stage Position	X: 84.11 µm, Y: 50.16 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 51µm | 1.00AU / 65µm | 1.00AU / 43µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 505 | SBS SP 615 | Mirror
Laser Wavelength	488nm @0.5% | 639nm @0.09% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	1.20
Pixel Time	0.29µs
Frame Time	4.97s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4
Detection Wavelength	511 - 550 | 640 - 732 | 430 - 470
Imaging Device	GaAsP-Pmt3 | GaAsp-Pmt3
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	550 V | 500 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.2 (3D, Auto) | Filter: 7.2 (3D, Auto) | Filter: 6.7 (3D, Auto)
Profile Definition	Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 40.1), Y (Min 0 and Max 19261)

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

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3)
Cat ID# - DC 000070

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

ATRIP (ATR Interacting Protein) is a key partner and co-activator of ATR (Ataxia Telangiectasia and Rad3-related), a crucial kinase in the DNA damage response, particularly in response to replication stress and single-strand breaks (SSBs). ATRIP plays a pivotal role in recognizing and binding to single-stranded DNA (ssDNA), facilitating ATR activation at DNA damage sites.

Overview of ATRIP

ATRIP is the regulatory subunit that associates with ATR and directs it to sites of DNA damage. It contains a domain that allows it to bind to Replication Protein A (RPA), a complex that binds and stabilizes single-stranded DNA. This interaction with RPA-coated ssDNA is crucial for ATRIP's ability to guide ATR to regions where the DNA damage response is needed.

Role of ATRIP in Single-Strand Break Repair

ATRIP plays a central role in the activation of ATR, which is critical to the cellular response to single-strand breaks and replication stress:

Recognition of Single-Stranded DNA: ATRIP, through its interaction with RPA, identifies regions of ssDNA. These regions are commonly found at stalled replication forks, resected DNA ends, or sites of single-strand breaks.

Recruitment of ATR: Once ATRIP binds to RPA-coated ssDNA, ATR is recruited to the site. This recruitment is crucial for ATR activation, leading to a cascade of events that coordinate the DNA damage response and facilitate DNA repair.

Activation of Checkpoints: By facilitating ATR activation, ATRIP indirectly contributes to checkpoint signaling. This leads to CHK1 phosphorylation, which plays a role in cell cycle arrest, providing time for the repair of single-strand breaks before the cell cycle progresses.

ATRIP's Broader Role in the DNA Damage Response

Beyond its specific role in single-strand break repair, ATRIP's interaction with ATR has broader implications for the DNA damage response:

Coordination of DNA Repair: The ATR/ATRIP complex orchestrates the recruitment of DNA repair proteins. This coordination ensures that repair processes like base excision repair (BER) and homologous recombination can proceed efficiently at sites of single-strand breaks.

Response to Replication Stress: ATRIP is crucial in responding to replication stress, which can lead to single-strand breaks or stalled replication forks. By facilitating ATR activation, ATRIP helps stabilize replication forks and prevent more severe DNA damage, such as double-strand breaks.

ATRIP is a critical regulatory protein that guides ATR to sites of single-strand breaks and other forms of replication stress. By recognizing RPA-coated ssDNA and recruiting ATR, ATRIP plays a pivotal role in activating the DNA damage response, leading to checkpoint activation and coordination of DNA repair processes. Its role in stabilizing replication forks and promoting genomic stability underscores its importance in maintaining genome integrity.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

Mitochondrial Primary, Identifyn® SRM Alexa Fluor™ 647 Staining not represented in data analysis

Control Data - Primary Unconjugated Antibody

Z Stack - (3D) - Panels A, Z-plane 43 of 71

Z-Stack = 71 Slices (2.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 550 X 667
Image Size (Scaled) = 32.35 µm X 39.23 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.11 µm, Y: 50.16 µm
Stage Position = X: 84.11 µm, Y: 50.16 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29µs
Frame Time = 4.97s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.2 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.09%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29µs
Frame Time = 4.97s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.2 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29µs
Frame Time = 4.97s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 470
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.7 (3D, Auto)

2- Dimensional View - Panel A

Panel shows Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3) and DAPI staining.

Image from Z-plane 43 of 71 and the Line Profile graphic showing the location of where the measurement was taken.

Profile View Display - Panel B

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 40.1), Y (Min 0 and Max 19261)

This panel also measures DAPI relative fluorescence and defines the nuclear compartment in 2 dimensions, as well as the known nucleolar PARP1 aggregate signals.

Zen Acquisition Info Tab -

Shown is the acquisition of the 488 channel (ATRIP 488 direct conjugate) using the 488nm laser at 0.5%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Confocal imaging of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment demonstrates damage-dependent nuclear enrichment consistent with the baseline response observed in the unconjugated ATRIP antibody condition (Cat. ID# PA-MM-000003). A representative 2D optical section (Z-plane 43 of 71) shows a robust intranuclear ATRIP signal with punctate foci/aggregate formation, confirming activation and recruitment during the DNA damage response. Quantitative analysis indicates a high fluorescence intensity (maximum 18,344 A.U.) acquired under low excitation conditions (488 nm at 0.5% laser power; Zeiss ZEN metadata shown for transparency), supporting a genuine biological response rather than acquisition-driven amplification. At confocal resolution, ATRIP recruitment is clearly detectable and meets CDx enrichment criteria; however, the observed foci remain structurally unresolved at the diffraction limit, necessitating progression to super-resolution modalities for definitive organizational interpretation, consistent with the step-down imaging framework.

Image Correction

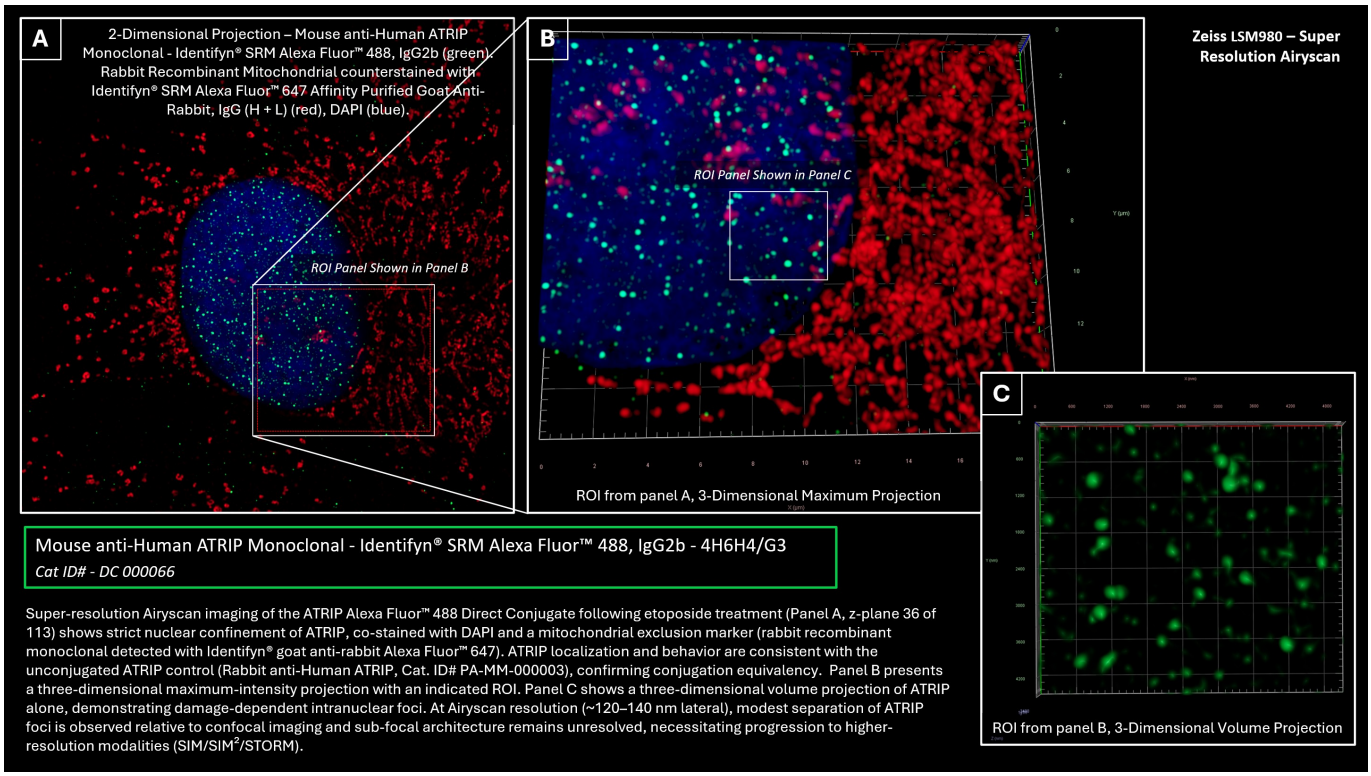
NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000066
Stage	Confocal
Instrument	Not stated in extracted metadata
Microscope configuration	Not stated in extracted metadata
Structured source metadata values	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7BEF02AB039D8385771D3CB34826BEABA0E54BD9CC721B61C7CABAD3D10EDC03
Method PDF SHA-256	617FA1C8AA5FB06A84DF63735BDE0B5704187F017F7C76754BBF499703C197C9

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	Not stated
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 750
METADATA VALUES	63

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	Not stated
Z-Stack	113 Slices (3.36µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image size (Pixels)	1566 X 1566 | 145 X 128
Image Size (Scaled)	55.27 µm X 55.27 µm | 5.12 µm X 4.52 µm
Bit Depth	16 Bit
Image Center Position	X: 86.11 µm, Y: 51.38 µm
Stage Position	X: 86.11 µm, Y: 51.38 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 250µm | 5.00AU / 328µm | 5.00AU / 214µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 550 | SBS LP 640 | SBS SP 505
Laser Wavelength	488nm @1.10% | 639nm @0.07% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66µs
Frame Time	7.82s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4
Detection Wavelength	499 - 548 | 643 - 735 | 422 - 477
Imaging Device	Airyscan
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	800 V | 750 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	9.7 (3D, Auto) | 9.0 (3D, Auto) | 8.0 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, 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) | View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

SOURCE METHOD

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Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3)
Cat ID# - DC 000070

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

ATRIP (ATR Interacting Protein) is a key partner and co-activator of ATR (Ataxia Telangiectasia and Rad3-related), a crucial kinase in the DNA damage response, particularly in response to replication stress and single-strand breaks (SSBs). ATRIP plays a pivotal role in recognizing and binding to single-stranded DNA (ssDNA), facilitating ATR activation at DNA damage sites.

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ATRIP is a critical regulatory protein that guides ATR to sites of single-strand breaks and other forms of replication stress. By recognizing RPA-coated ssDNA and recruiting ATR, ATRIP plays a pivotal role in activating the DNA damage response, leading to checkpoint activation and coordination of DNA repair processes. Its role in stabilizing replication forks and promoting genomic stability underscores its importance in maintaining genome integrity.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

Control Data - Primary Unconjugated Antibody

Z Stack - (3D) - Panels A, Z-plane 36 of 113, and Panel B, Maximum Projection

Z-Stack = 113 Slices (3.36µm)

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: 86.11 µm, Y: 51.38 µm
Stage Position = X: 86.11 µm, Y: 51.38 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 113 Slices (3.36µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image size (Pixels) = 145 X 128
Image Size (Scaled) = 5.12 µm X 4.52 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.11 µm, Y: 51.38 µm
Stage Position = X: 86.11 µm, Y: 51.38 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @1.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
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 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 9.7 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639nm @0.07%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
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
Super Resolution = 9.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 214µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
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
Super Resolution = 8.0 (3D, Auto)

2- Dimensional View - Panel A

Panel shows Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3) and Rabbit Recombinant Monoclonal against Mitochondria with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) and DAPI staining.

Image from Z-plane 36 of 113. A white box denotes a region of interest to be displayed in panel B.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, 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 C

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Super-resolution Airyscan imaging of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment is shown in Panel A as a two-dimensional optical section at Z-plane 36 of 113, co-stained with DAPI and a rabbit recombinant monoclonal mitochondrial marker visualized using the Identifyn® goat anti-rabbit Alexa Fluor™ 647 secondary, serving as an extranuclear exclusion control. ATRIP signal remains strictly confined to the nuclear compartment, consistent with the localization observed in the unconjugated ATRIP control (Rabbit anti-Human ATRIP, Cat. ID# PA-MM-000003) under widefield and confocal conditions, thereby confirming conjugation equivalency and preservation of biological behavior.

Panel B presents a three-dimensional maximum-intensity projection of all three channels, with a region of interest indicated for higher-order examination. Panel C shows a corresponding three-dimensional volume projection of the ATRIP 488 Direct Conjugate alone, isolating the ATRIP signal to evaluate spatial organization independent of chromatin or mitochondrial context. At Airyscan lateral resolution (~120-140 nm in the X-Y plane), ATRIP appears as discrete intranuclear foci with modest signal separation relative to confocal imaging, confirming damage-dependent recruitment and enrichment while remaining resolution-limited with respect to sub-focal architecture.

Consistent with the CCR step-down CDx framework, these data satisfy enrichment and transitional-resolution criteria by demonstrating reproducible, damage-dependent nuclear recruitment across conjugation state and imaging modality. However, definitive resolution of ATRIP substructures and higher-order organization is not achievable at the Airyscan level. It requires progression into Lattice SIM, SIM², and ultimately single-molecule localization microscopy (STORM) to resolve underlying assemblies. As such, Airyscan functions as a structural credibility checkpoint rather than a terminal structural readout for ATRIP.

Image Correction

NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

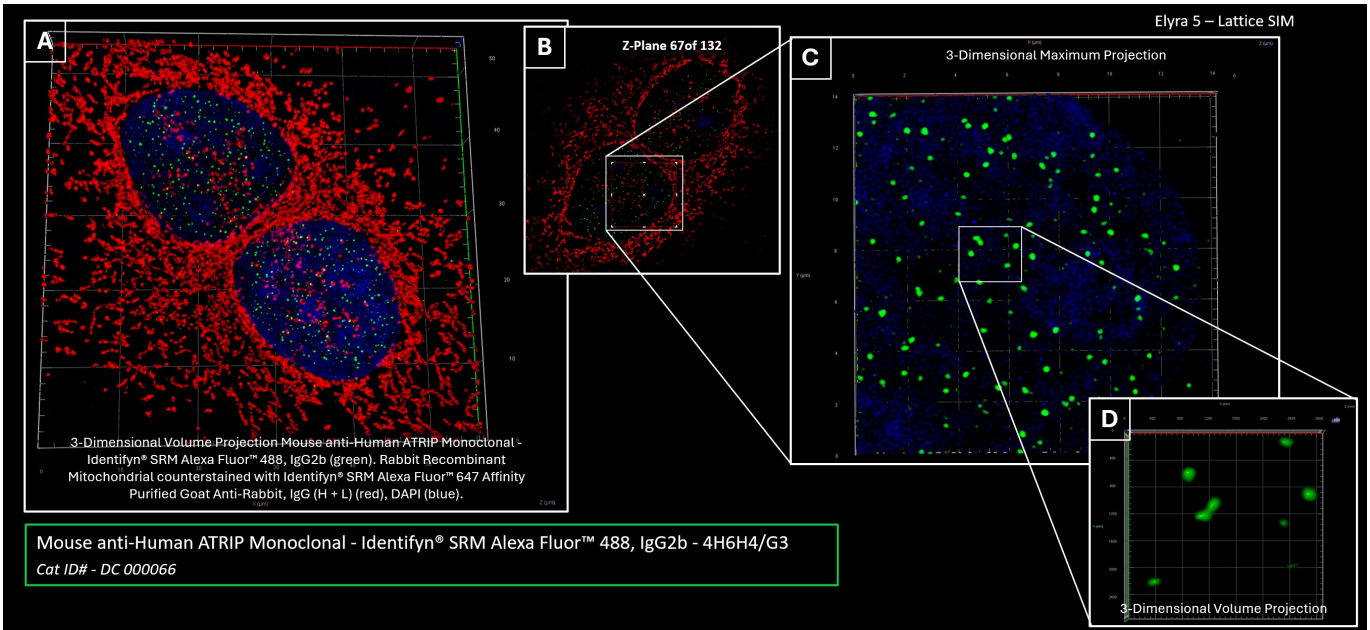
SOURCE PROVENANCE

Catalog	DC-000066
Stage	Airyscan
Instrument	Not stated in extracted metadata
Microscope configuration	Not stated in extracted metadata

SOURCE PROVENANCE

Structured source metadata values	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8DD889548236E06971F414865A72227626B37E3DDA7C7FEF0D5FFB95CFD4DB68
Method PDF SHA-256	E741F66D35E2BE7C26775A6B1EC0F8B38706E2FE390183972F7D078A73D9A6DD

ORIGINAL IMAGE EVIDENCE / SIM



Lattice structured illumination microscopy (SIM) of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment is shown across Panels A–D. Panel A presents a three-dimensional maximum-intensity projection of ATRIP 488 (green), a rabbit recombinant monoclonal mitochondrial marker visualized with Identifyn® goat anti-rabbit Alexa Fluor™ 647 (red) as an extranuclear exclusion control, and DAPI (blue). Panel B shows a two-dimensional optical section at z-plane 67 of 132, with a white box denoting the region of interest examined in subsequent panels. Panel C displays a maximum-intensity projection of ATRIP 488 and DAPI from the same acquisition, highlighting damage-dependent intranuclear enrichment consistent with the unconjugated ATRIP control (Rabbit anti-Human ATRIP, Cat. ID# PA-MM-000003) observed under widefield and confocal conditions, confirming conjugation equivalency. Panel D presents a three-dimensional volume view of the ROI, where ATRIP foci begin to separate into spatially distinct assemblies at SIM resolution (~60–80 nm lateral), indicating enrichment and recruitment following DNA damage. While increased resolution relative to Airyscan enables partial focal separation, sub-unit architecture remains unresolved at this stage and requires progression to higher-resolution modalities (SIM^2 or STORM) for definitive structural interpretation.

EVIDENCE TIER	SIM
INSTRUMENT	Not stated
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 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	Not stated
Z-Stack	132 Slices (3.93 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image size (Pixels)	2667 X 2573 | 674 X 668 | 137 X 131
Image Size (Scaled)	56.31 µm X 54.32 µm | 14.23 µm X 14.10 µm | 2.89 µm X 2.77 µm
Bit Depth	16 Bit
Image Center Position	X: 57.64 µm, Y: 33.91 µm
Stage Position	X: 57.64 µm, Y: 33.91 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 640 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820 -SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 100 ms | 50 ms
Depth of Focus	0.81 µm | 1.13 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @1.00% | 640 nm @0.5% | 405 nm @1.0%
Frame Time	1.59 s | 1.33 s
Scan Direction	Unidirectional
Grating	36.5 µm grating | 27.5 µm grating
Processing Dimension	3D
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.1667 (488), 10.4360 (647), 10.1213 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, 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) | View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) | View Angle 10°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
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

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3)
Cat ID# - DC 000070

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

ATRIP (ATR Interacting Protein) is a key partner and co-activator of ATR (Ataxia Telangiectasia and Rad3-related), a crucial kinase in the DNA damage response, particularly in response to replication stress and single-strand breaks (SSBs). ATRIP plays a pivotal role in recognizing and binding to single-stranded DNA (ssDNA), facilitating ATR activation at DNA damage sites.

Overview of ATRIP

ATRIP is the regulatory subunit that associates with ATR and directs it to sites of DNA damage. It contains a domain that allows it to bind to Replication Protein A (RPA), a complex that binds and stabilizes single-stranded DNA. This interaction with RPA-coated ssDNA is crucial for ATRIP's ability to guide ATR to regions where the DNA damage response is needed.

Role of ATRIP in Single-Strand Break Repair

ATRIP plays a central role in the activation of ATR, which is critical to the cellular response to single-strand breaks and replication stress:

Recognition of Single-Stranded DNA: ATRIP, through its interaction with RPA, identifies regions of ssDNA. These regions are commonly found at stalled replication forks, resected DNA ends, or sites of single-strand breaks.

Recruitment of ATR: Once ATRIP binds to RPA-coated ssDNA, ATR is recruited to the site. This recruitment is crucial for ATR activation, leading to a cascade of events that coordinate the DNA damage response and facilitate DNA repair.

Activation of Checkpoints: By facilitating ATR activation, ATRIP indirectly contributes to checkpoint signaling. This leads to CHK1 phosphorylation, which plays a role in cell cycle arrest, providing time for the repair of single-strand breaks before the cell cycle progresses.

ATRIP's Broader Role in the DNA Damage Response

Beyond its specific role in single-strand break repair, ATRIP's interaction with ATR has broader implications for the DNA damage response:

Coordination of DNA Repair: The ATR/ATRIP complex orchestrates the recruitment of DNA repair proteins. This coordination ensures that repair processes like base excision repair (BER) and homologous recombination can proceed efficiently at sites of single-strand breaks.

Response to Replication Stress: ATRIP is crucial in responding to replication stress, which can lead to single-strand breaks or stalled replication forks. By facilitating ATR activation, ATRIP helps stabilize replication forks and prevent more severe DNA damage, such as double-strand breaks.

ATRIP is a critical regulatory protein that guides ATR to sites of single-strand breaks and other forms of replication stress. By recognizing RPA-coated ssDNA and recruiting ATR, ATRIP plays a pivotal role in activating the DNA damage response, leading to checkpoint activation and coordination of DNA repair processes. Its role in stabilizing replication forks and promoting genomic stability underscores its importance in maintaining genome integrity.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

Mitochondrial Primary, Identifyn® SRM Alexa Fluor™ 647 Staining not represented in data analysis

Control Data - Primary Unconjugated Antibody

Z Stack - (3D) - Panels A, 3-dimensional Maximum Projection, and B, Z-plane 67 of 132

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image size (Pixels) = 2667 X 2573
Image Size (Scaled) = 56.31 µm X 54.32 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.64 µm, Y: 33.91 µm
Stage Position = X: 57.64 µm, Y: 33.91 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image size (Pixels) = 674 X 668
Image Size (Scaled) = 14.23 µm X 14.10 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.64 µm, Y: 33.91 µm
Stage Position = X: 57.64 µm, Y: 33.91 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image size (Pixels) = 137 X 131
Image Size (Scaled) = 2.89 µm X 2.77 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.64 µm, Y: 33.91 µm
Stage Position = X: 57.64 µm, Y: 33.91 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @0.5%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 27.5 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 @1.0%
Frame Time = 1.33 s
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 = 11.1667 (488), 10.4360 (647), 10.1213 (DAPI)
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, 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 C

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 10%, 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 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 10°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Lattice structured illumination microscopy (SIM) of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment is shown across Panels A-D. Panel A presents a three-dimensional maximum-intensity projection of ATRIP 488 (green), a rabbit recombinant monoclonal mitochondrial marker visualized with Identifyn® goat anti-rabbit Alexa Fluor™ 647 (red) as an extranuclear exclusion control, and DAPI (blue). Panel B shows a two-dimensional optical section at Z-plane 67 of 132, with a white box denoting the region of interest examined in subsequent panels. Panel C displays a maximum-intensity projection of ATRIP 488 and DAPI from the same acquisition, highlighting damage-dependent intranuclear enrichment consistent with the unconjugated ATRIP control (Rabbit anti-Human ATRIP, Cat. ID# PA-MM-000003) observed under widefield and confocal conditions, confirming conjugation equivalency. Panel D presents a three-dimensional volume view of the ROI, where ATRIP foci begin to separate into spatially distinct assemblies at SIM resolution (~60-80 nm lateral), indicating enrichment and recruitment following DNA damage. While increased resolution relative to Airyscan enables partial focal separation, sub-unit architecture remains unresolved at this stage and requires progression to higher-resolution modalities (SIM² or STORM) for definitive structural interpretation.

Image Correction

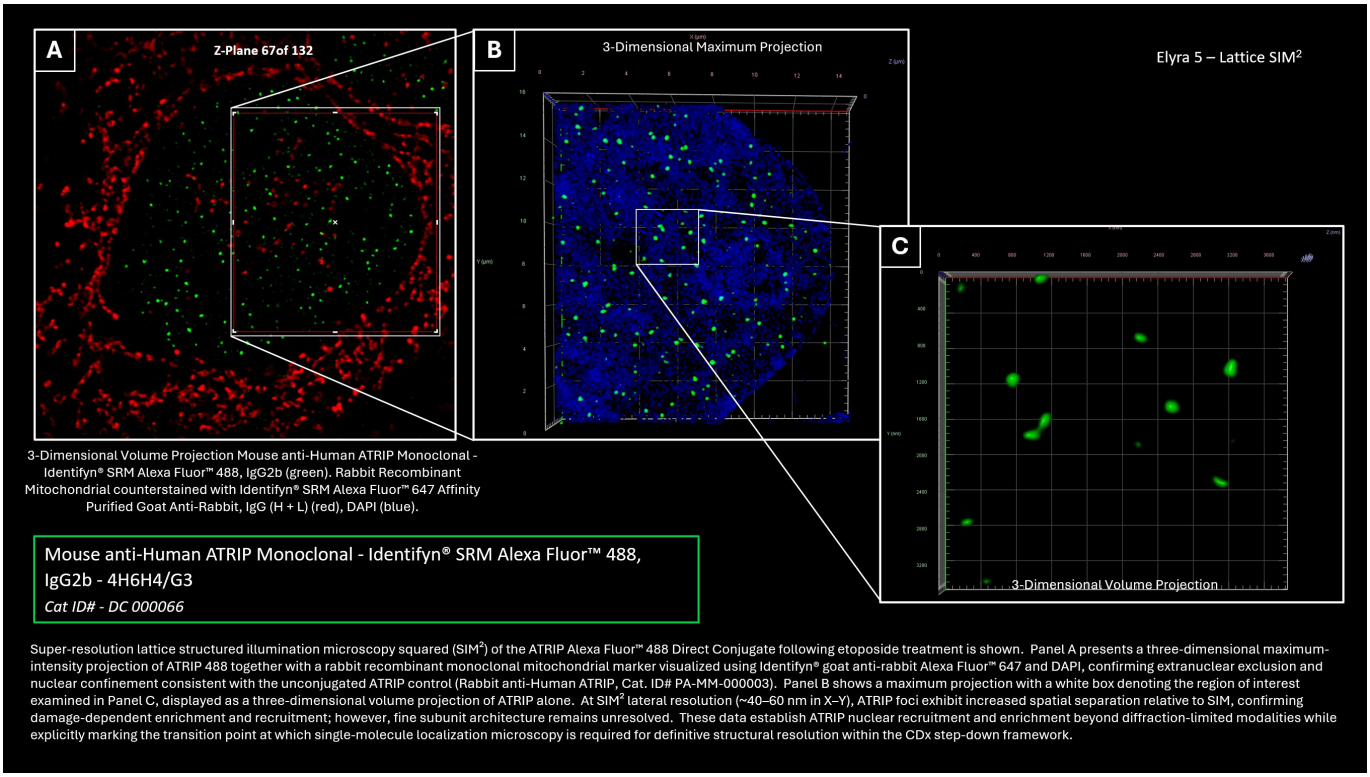
NONE

Image by P. Raut
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000066
Stage	SIM
Instrument	Not stated in extracted metadata
Microscope configuration	Not stated in extracted metadata
Structured source metadata values	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	827D12BFCB56CBD6834E1CED4B8A2997E9B8C13DECD01CE728CD60C54316F924
Method PDF SHA-256	36D4DE497EA9D3D7197A176CCF33FF87F7D4E8B6E24A6C47F80CFCFC936754E3

ORIGINAL IMAGE EVIDENCE / SIM²



Super-resolution lattice structured illumination microscopy squared (SIM²) of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment is shown. Panel A presents a three-dimensional maximum-intensity projection of ATRIP 488 together with a rabbit recombinant monoclonal mitochondrial marker visualized using Identifyn® goat anti-rabbit Alexa Fluor™ 647 and DAPI, confirming extranuclear exclusion and nuclear confinement consistent with the unconjugated ATRIP control (Rabbit anti-Human ATRIP, Cat. ID# PA-MM-000003). Panel B shows a maximum projection with a white box denoting the region of interest examined in Panel C, displayed as a three-dimensional volume projection of ATRIP alone. At SIM² lateral resolution (~40–60 nm in X–Y), ATRIP foci exhibit increased spatial separation relative to SIM, confirming damage-dependent enrichment and recruitment; however, fine subunit architecture remains unresolved. These data establish ATRIP nuclear recruitment and enrichment beyond diffraction-limited modalities while explicitly marking the transition point at which single-molecule localization microscopy is required for definitive structural resolution within the CDx step-down framework.

EVIDENCE TIER	SIM²
INSTRUMENT	Not stated
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	69

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	Not stated
Z-Stack	132 Slices (3.93 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image size (Pixels)	1471 X 1428 | 708 X 763 | 183 X 167
Image Size (Scaled)	31.06 µm X 30.15 µm | 14.95 µm X 16.11 µm | 3.86 µm X 3.53 µm
Bit Depth	16 Bit
Image Center Position	X: 57.64 µm, Y: 33.91 µm
Stage Position	X: 57.64 µm, Y: 33.91 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 640 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820 -SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 100 ms | 50 ms
Depth of Focus	0.81 µm | 1.13 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @1.00% | 640 nm @0.5% | 405 nm @1.0%
Frame Time	1.59 s | 1.33 s
Scan Direction	Unidirectional
Grating	36.5 µm grating | 27.5 µm grating
Result Sampling	4
Process Sampling	4
Algorithm	SIM2
Input SNR	Low

FIELD	SOURCE-DOCUMENTED VALUE
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 1% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, 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) | View Angle 25°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
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

Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3)
Cat ID# - DC 000070

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

ATRIP (ATR Interacting Protein) is a key partner and co-activator of ATR (Ataxia Telangiectasia and Rad3-related), a crucial kinase in the DNA damage response, particularly in response to replication stress and single-strand breaks (SSBs). ATRIP plays a pivotal role in recognizing and binding to single-stranded DNA (ssDNA), facilitating ATR activation at DNA damage sites.

Overview of ATRIP

ATRIP is the regulatory subunit that associates with ATR and directs it to sites of DNA damage. It contains a domain that allows it to bind to Replication Protein A (RPA), a complex that binds and stabilizes single-stranded DNA. This interaction with RPA-coated ssDNA is crucial for ATRIP's ability to guide ATR to regions where the DNA damage response is needed.

Role of ATRIP in Single-Strand Break Repair

ATRIP plays a central role in the activation of ATR, which is critical to the cellular response to single-strand breaks and replication stress:

Recognition of Single-Stranded DNA: ATRIP, through its interaction with RPA, identifies regions of ssDNA. These regions are commonly found at stalled replication forks, resected DNA ends, or sites of single-strand breaks.

Recruitment of ATR: Once ATRIP binds to RPA-coated ssDNA, ATR is recruited to the site. This recruitment is crucial for ATR activation, leading to a cascade of events that coordinate the DNA damage response and facilitate DNA repair.

Activation of Checkpoints: By facilitating ATR activation, ATRIP indirectly contributes to checkpoint signaling. This leads to CHK1 phosphorylation, which plays a role in cell cycle arrest, providing time for the repair of single-strand breaks before the cell cycle progresses.

ATRIP's Broader Role in the DNA Damage Response

Beyond its specific role in single-strand break repair, ATRIP's interaction with ATR has broader implications for the DNA damage response:

Coordination of DNA Repair: The ATR/ATRIP complex orchestrates the recruitment of DNA repair proteins. This coordination ensures that repair processes like base excision repair (BER) and homologous recombination can proceed efficiently at sites of single-strand breaks.

Response to Replication Stress: ATRIP is crucial in responding to replication stress, which can lead to single-strand breaks or stalled replication forks. By facilitating ATR activation, ATRIP helps stabilize replication forks and prevent more severe DNA damage, such as double-strand breaks.

ATRIP is a critical regulatory protein that guides ATR to sites of single-strand breaks and other forms of replication stress. By recognizing RPA-coated ssDNA and recruiting ATR, ATRIP plays a pivotal role in activating the DNA damage response, leading to checkpoint activation and coordination of DNA repair processes. Its role in stabilizing replication forks and promoting genomic stability underscores its importance in maintaining genome integrity.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Mouse anti-Human ATRIP Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG2b - (4H6H4/G3), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

Mitochondrial Primary, Identifyn® SRM Alexa Fluor™ 647 Staining not represented in data analysis

Control Data - Primary Unconjugated Antibody

Z Stack - (3D) - Panels A, 2-dimensional Projection, Z-plane 67 of 132

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image size (Pixels) = 1471 X 1428
Image Size (Scaled) = 31.06 µm X 30.15 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.64 µm, Y: 33.91 µm
Stage Position = X: 57.64 µm, Y: 33.91 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image size (Pixels) = 708 X 763
Image Size (Scaled) = 14.95 µm X 16.11 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.64 µm, Y: 33.91 µm
Stage Position = X: 57.64 µm, Y: 33.91 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image size (Pixels) = 183 X 167
Image Size (Scaled) = 3.86 µm X 3.53 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.64 µm, Y: 33.91 µm
Stage Position = X: 57.64 µm, Y: 33.91 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @0.5%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 27.5 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 @1.0%
Frame Time = 1.33 s
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
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel B

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

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, 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 C

3D Volume Projection = Precise
Appearance = Threshold 1% 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 25°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Lattice structured illumination microscopy squared (SIM²) imaging of the ATRIP Alexa Fluor™ 488 Direct Conjugate following etoposide treatment is shown using the same field of view and region of interest examined by lattice SIM, enabling direct step-down comparison across modalities. Panel A presents a three-dimensional maximum-intensity projection including ATRIP 488, DAPI, and a rabbit recombinant monoclonal mitochondrial marker visualized with Identifyn® goat anti-rabbit Alexa Fluor™ 647, serving as an extranuclear exclusion control. ATRIP signal remains strictly confined to the nuclear compartment, consistent with localization observed for the unconjugated ATRIP antibody (Rabbit anti-Human ATRIP, Cat. ID# PA-MM-000003) under widefield, confocal, and Airyscan conditions, confirming conjugation equivalency and preservation of biological behavior.

Panel B shows a three-dimensional maximum projection with a white box denoting the region of interest examined in Panel C. Panel C presents a three-dimensional volume projection of the ATRIP 488 Direct Conjugate alone, isolating the ATRIP signal for structural evaluation. At SIM² lateral resolution (~40-60 nm in the X-Y plane), ATRIP foci exhibit modestly increased spatial separation relative to lattice SIM, confirming damage-dependent enrichment and improved macro-scale resolution of intranuclear assemblies.

However, despite enhanced spatial separation of the ATRIP signal at SIM² resolution, the underlying sub-focal architecture remains unresolved. The observed assemblies likely represent resolution-limited composites of smaller subunits that cannot be definitively separated at this stage of the step-down workflow. In the CDx context, SIM² therefore represents the upper bound of actionable structural enrichment for ATRIP, while definitive resolution of ATRIP subunits and mechanistic assembly states requires progression to single-molecule localization microscopy (STORM). Collectively, these data satisfy CDx enrichment and structural-credibility criteria while explicitly defining the resolution boundary between SIM² and single-molecule confirmation.

Image Correction

NONE

Image by P. Raut
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000066
Stage	SIM²
Instrument	Not stated in extracted metadata
Microscope configuration	Not stated in extracted metadata
Structured source metadata values	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	BFA68EB4A38AD0A343B7D1F604799B448151E778FEDAA61145C5E0AF69E99E55
Method PDF SHA-256	94DD83CB9590FDD61EDB471F2FCA8A15B2A47F7CAA7E2C77863BC9A36C37DA11