BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

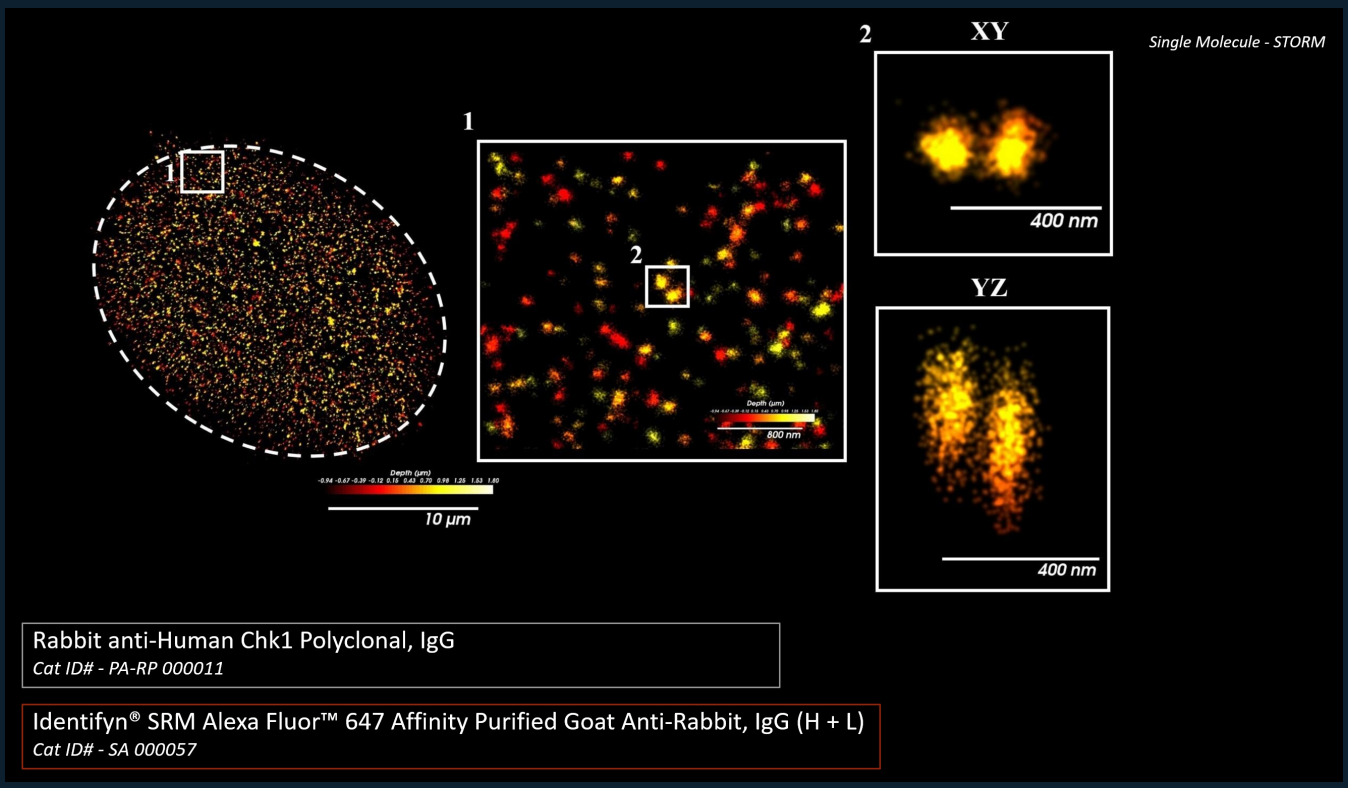
CHK1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human Chk1 Polyclonal

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

8	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Rabbit anti-Human Chk1 Polyclonal, IgG
Cat ID# - PA-RP 000011

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

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RP-000011 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

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

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

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for CHK1, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM² -> Single-molecule STORM -> Control

BENNETT ASSESSMENT

The preserved PA-RP-000011 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved PA-RP-000011 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594	3; 45 Texas Red; 38 eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 45 Texas Red; 38 HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Confocal	405/DAPI; 488; 594	3; None; 561 nm @ 0.6%; 488 nm @ 1.0%; 405 nm @ 0.4%; 590; 493; 353
Airyscan	405/DAPI; 488; 594	2; None; 561 nm @ 3.50%; 488 nm @ 2.1%; 590; 493; 618; 517
SIM	405/DAPI; 488; 594	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820-SR; 561; 488
SIM²	405/DAPI; 488; 594	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820-SR; 561; 488
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 594	1; None; 561 nm @ 0.6%; 590; 618

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.. 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 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

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 1029 X 889; Image Size (Pixels): 1029 X 889; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150 ms; 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 15.00%; X-Cite Xylis Lamp Intensity @ 20.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 49.

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; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63x/1.25 Oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 86 Slices (8.5 µm); 40 Slices (3.9 µm) - Gallery Subset was Used; Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 1058 X 979; 322 X 301; Image Size (Pixels): 1058 X 979; 322 X 301; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 150 ms; 250 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 15.00%; X-Cite Xylis Lamp Intensity @ 25.00%. Optical/scan: Effective NA: 1.25. 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: 60.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 156 Slices (4.65 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1612 X 1612; Image Size (Pixels): 1612 X 1612; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsp-Pmt3; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.24 µs; Frame Time: 6.04 s. Illumination: Laser Wavelength: 561 nm @ 0.6%; 488 nm @ 1.0%. Optical/scan: Pinhole: 1.00 AU / 59 µm; 1.00 AU / 51 µm; Scan Zoom: 1.4; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.9 (3D, Auto); Filter: 7.7 (3D, Auto). Structured source metadata values: 56.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 9

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 161 Slices (4.8 µm); Channels: 2; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 766 X 683; 164 X 147; Image Size (Pixels): 766 X 683; 164 X 147; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.15 µs; Frame Time: 17.86 s. Illumination: Laser Wavelength: 561 nm @ 3.50%; 488 nm @ 2.1%. Optical/scan: Pinhole: 5.00 AU / 286 µm; 5.00 AU / 250 µm; Scan Zoom: 2.1; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution: 9.1 (3D, Auto). Structured source metadata values: 50.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 134 Slices (3.99 µm); Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1694 X 1267; 239 X 219; Image Size (Pixels): 1694 X 1267; 239 X 219; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 200 ms; 120 ms; Frame Time: 1.59 s. Illumination: Laser Wavelength: 561 nm @ 15.00%; 488 nm @ 5.00%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 50%, 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 SIM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 9

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 148 Slices (4.41 µm); 134 Slices (3.99 µm) - Gallery was Reduced to Highlight Signal; Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1374 X 1522; 285 X 288; Image Size (Pixels): 1374 X 1522; 285 X 288; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 200 ms; 120 ms; Frame Time: 1.59 s. Illumination: Laser Wavelength: 561 nm @ 15.00%; 488 nm @ 5.00%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 45°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 56.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60x/1.30 Silicon Oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20 ms Selected; Super Resolution Camera Exposure Time: 20 ms; Widefield Camera Exposure Time: 100 ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 80.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 156 Slices (4.65 µm); Channels: 1; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 512 X 572; 492 X 519; Image Size (Pixels): 512 X 572; 492 X 519; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.24 µs; Frame Time: 6.04 s. Illumination: Laser Wavelength: 561 nm @ 0.6%. Optical/scan: Pinhole: 1.00 AU / 59 µm; Scan Zoom: 1.4; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.9 (3D, Auto); Filter: 8.3 (3D, Auto). Structured source metadata values: 50.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved PA-RP-000011 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=766 X 683; Image Size (Scaled)=27.04 µm X 24.11 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

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)=1694 X 1267; Image Size (Scaled)=35.76 µm X 26.75 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

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)=1374 X 1522; Image Size (Scaled)=29.01 µm X 32.13 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["24 hours"], "Control": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

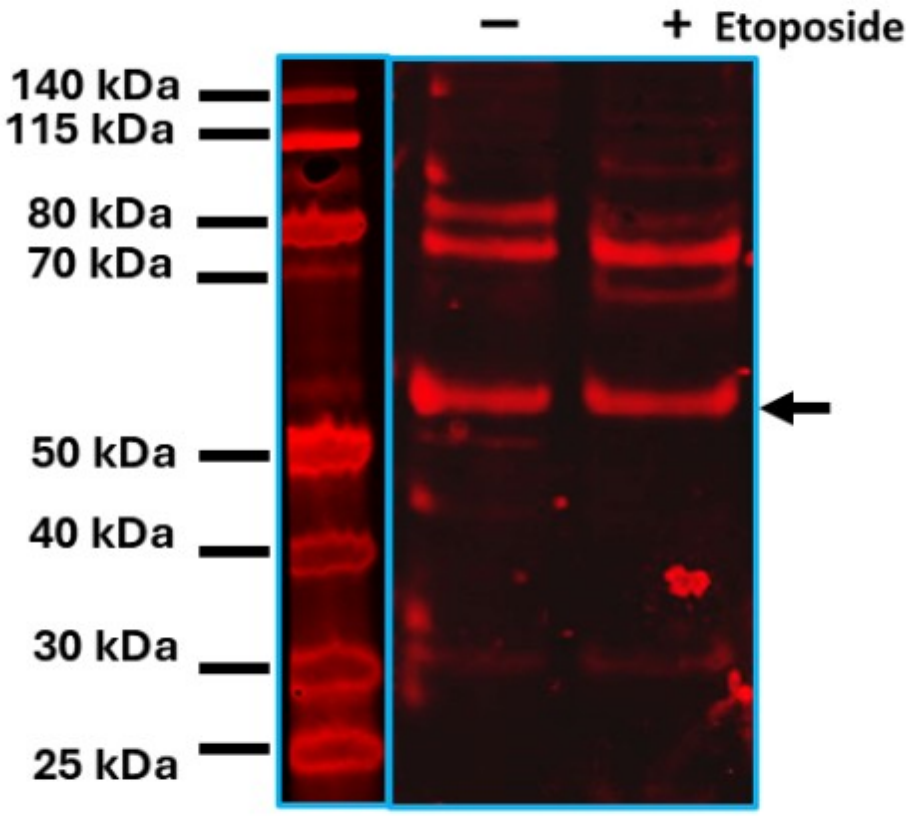
This dossier preserves the complete available evidence progression for PA-RP-000011. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	647
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 Imaging.
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Detection mode	Fluorescence
Laser	638 nm
Emission Filter	676 ± 37 nm
Detector	PMT
Intensity	L2
Pixel size	100 µm
Scan Speed	Highest
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	S. Khanal

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Chk1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Expected MW: 55 kDa

HeLa cells were treated with 2 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a 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 on NuPAGE™ 4-12% Bis-Tris, 1.0-1.5 mm Mini Protein Gels in a Mini Gel Tank. Thermo Fisher™ PageRuler™ Prestained Protein Ladder, 10-180 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 Rabbit anti-Human Chk1 Polyclonal, IgG for 20 hours, followed by 5 washes with 1X PBS. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 638 nm
Emission Filter = 676 ± 37 nm
Detector = PMT
Intensity = L2
Pixel size = 100 µm
Scan Speed = Highest
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

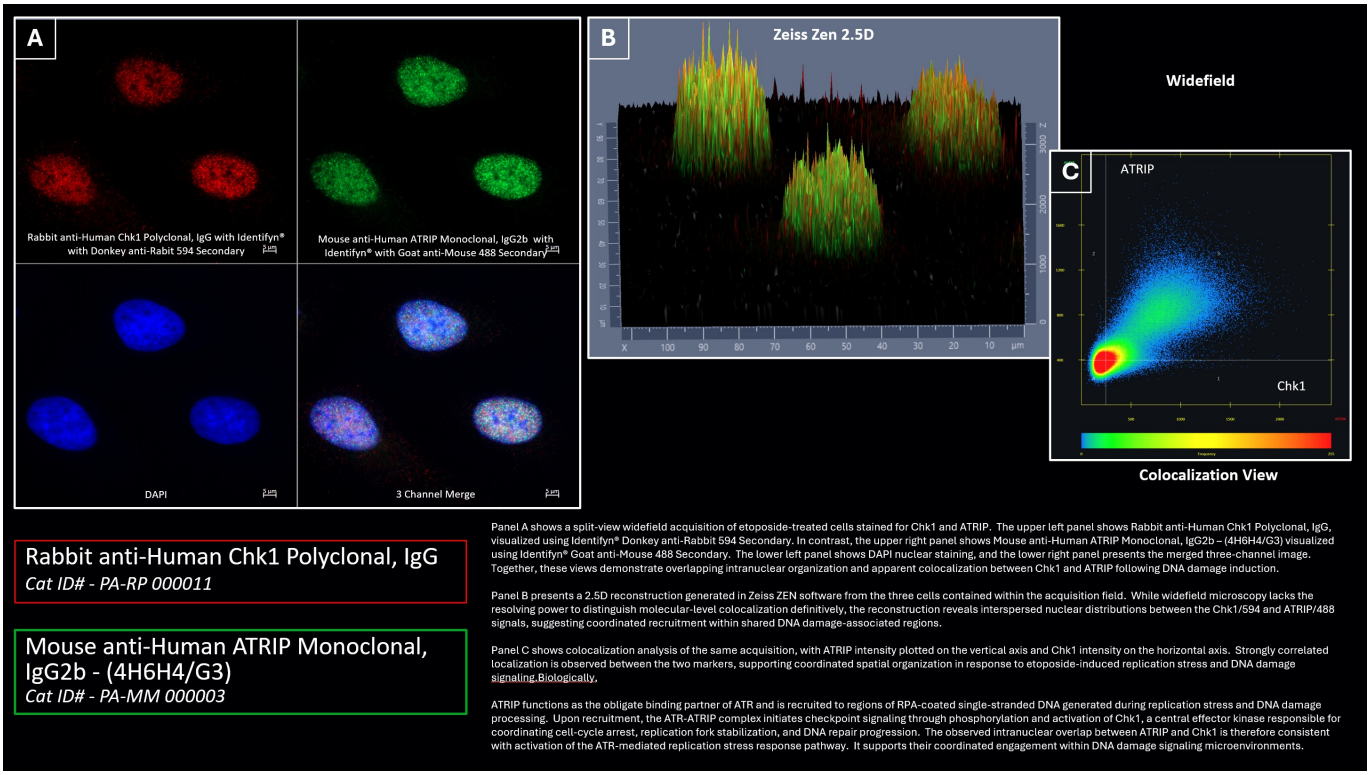
NONE

Image: S. Khanal

SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	3E58A7B6139B96AEAE2B0C9F8C6902E4038393A57F5822F342AAAF567B009E5C
Method PDF SHA-256	11A25EE3118887511D0BD4A328E612FB527AB2E2BB212E338DFCE1148AFC2611

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	49

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)	1029 X 889
Image Size (Scaled)	112.70 µm X 97.37 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	45 Texas Red | 38 eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 15.00% | X-Cite Xylis Lamp Intensity @ 20.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	150 ms | 200 ms | 20 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1x
Depth of Focus	0.96 µm | 0.80 µm | 0.72 µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	54
Angle Y	-180
Z Scaling	1.2
Ambient	50
Specular	20
Shininess	50
Light Height	2.0

FIELD	SOURCE-DOCUMENTED VALUE
Image Measurement	Entire Field

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Chk1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

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

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 1029 X 889
Image Size (Scaled) = 112.70 µm X 97.37 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

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.96 µm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 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 = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 affinity-purified Donkey anti-Rabbit IgG (H+L). The top-right panel shows Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Goat anti-Mouse IgG (H+L). The lower-left panel shows DAPI nuclear staining, and the bottom-right panel shows the three-channel merged image.

2.5D Image Processing - Panel B

2.5D Display

Render Mode = Surface
Grid distance = 5
Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 54
Angle Y = -180
Z Scaling = 1.2
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.0

Colocalization View - Panel C

Horizontal Axis - AF594, Threshold 250, Range Auto - Chk1
Vertical Axis - AF488, Threshold 396, Range Auto - ATRIP
Image Measurement = Entire Field

Colocalization analysis of Chk1 (X-axis) and ATRIP (Y-axis) was performed across the entire image acquisition.

Summary

Human Chk1 Polyclonal, IgG, visualized using Identifyn® Donkey anti-Rabbit 594 Secondary. In contrast, the upper right panel shows Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3) visualized using Identifyn® Goat anti-Mouse 488 Secondary. The lower-left panel shows DAPI nuclear staining, and the lower-right panel shows the merged three-channel image. Together, these views demonstrate overlapping intranuclear organization and apparent colocalization between Chk1 and ATRIP following DNA damage induction.

Panel B presents a 2.5D reconstruction generated in Zeiss ZEN software from the three cells contained within the acquisition field. While widefield microscopy lacks the resolving power to definitively distinguish molecular-level colocalization, the reconstruction reveals interspersed nuclear distributions between the Chk1/594 and ATRIP/488 signals, suggesting coordinated recruitment within shared DNA damage-associated regions.

Panel C shows colocalization analysis of the same acquisition, with ATRIP intensity plotted on the vertical axis and Chk1 intensity on the horizontal axis. Strongly correlated localization is observed between the two markers, supporting coordinated spatial organization in response to etoposide-induced replication stress and DNA damage signaling.

Biologically, ATRIP functions as the obligate binding partner of ATR and is recruited to regions of RPA-coated single-stranded DNA generated during replication stress and DNA damage processing. Upon recruitment, the ATR-ATRIP complex initiates checkpoint signaling by phosphorylating and activating Chk1, a central effector kinase that coordinates cell-cycle arrest, replication fork stabilization, and DNA repair progression. The observed intranuclear

overlap between ATRIP and Chk1 is therefore consistent with activation of the ATR-mediated replication stress response pathway. It supports their coordinated engagement within DNA damage signaling microenvironments.

Image Correction

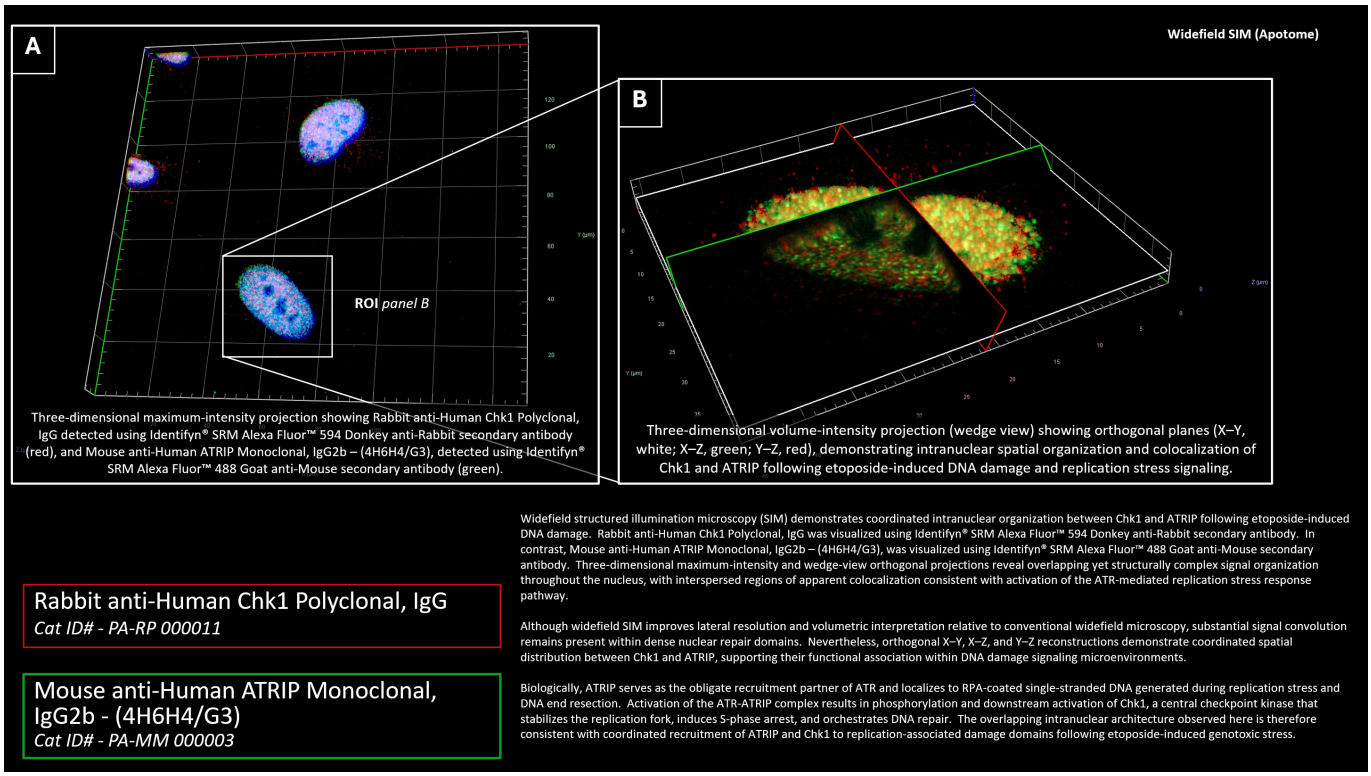
NONE

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

SOURCE PROVENANCE

Catalog	PA-RP-000011
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	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	31C8DD2385F961926D6BB019B8C4D69E9582C6D0A322743E18B435DF27F50E23
Method PDF SHA-256	52339BDA8CF36E6CFEA844D446E9DE2AB50F53648B55292B46B4EAC743496F4D

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Three-dimensional maximum-intensity projection showing Rabbit anti-Human Chk1 Polyclonal, IgG detected using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody (red), and Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), detected using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody (green).

Three-dimensional volume-intensity projection (wedge view) showing orthogonal planes (X–Y, white; X–Z, green; Y–Z, red), demonstrating intranuclear spatial organization and colocalization of Chk1 and ATRIP following etoposide-induced DNA damage and replication stress signaling.

Rabbit anti-Human Chk1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Widefield structured illumination microscopy (SIM) demonstrates coordinated intranuclear organization between Chk1 and ATRIP following etoposide-induced DNA damage. Rabbit anti-Human Chk1 Polyclonal, IgG was visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody. In contrast, Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), was visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody. Three-dimensional maximum-intensity and wedge-view orthogonal projections reveal overlapping yet structurally complex signal organization throughout the nucleus, with interspersed regions of apparent colocalization consistent with activation of the ATR-mediated replication stress response pathway.

Although widefield SIM improves lateral resolution and volumetric interpretation relative to conventional widefield microscopy, substantial signal convolution remains present within dense nuclear repair domains. Nevertheless, orthogonal X–Y, X–Z, and Y–Z reconstructions demonstrate coordinated spatial distribution between Chk1 and ATRIP, supporting their functional association within DNA damage signaling microenvironments.

Biologically, ATRIP serves as the obligate recruitment partner of ATR and localizes to RPA-coated single-stranded DNA generated during replication stress and DNA end resection. Activation of the ATR-ATRIP complex results in phosphorylation and downstream activation of Chk1, a central checkpoint kinase that stabilizes the replication fork, induces S-phase arrest, and orchestrates DNA repair. The overlapping intranuclear architecture observed here is therefore consistent with coordinated recruitment of ATRIP and Chk1 to replication-associated damage domains following etoposide-induced genotoxic stress.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	60

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63x/1.25 Oil M27
Z-Stack	86 Slices (8.5 µm) | 40 Slices (3.9 µm) - Gallery Subset was Used
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	1058 X 979 | 322 X 301
Image Size (Scaled)	151.14 µm X 139.86 µm | 46.00 µm X 43.00 µm
Bit Depth	14 Bit
Image Center Position	X: 44.75 mm, Y: 44.46 mm
Stage Position	X: 44.75 mm, Y: 44.46 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	45 Texas Red | 38 HE eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 15.00% | X-Cite Xylis Lamp Intensity @ 25.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	150 ms | 250 ms | 25 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 Mono
Camera Adapter	1x
Depth of Focus	1.20 µm | 1.00 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)

FIELD	SOURCE-DOCUMENTED VALUE
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	-20% | -8% | 11%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	45° | -30°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

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

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack =86 Slices (8.5 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 1058 X 979
Image Size (Scaled) = 151.14 µm X 139.86 µm
Bit Depth = 14 Bit
Image Center Position = X: 44.75 mm, Y: 44.46 mm
Stage Position = X: 44.75 mm, Y: 44.46 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 40 Slices (3.9 µm) - Gallery Subset was Used

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 322 X 301
Image Size (Scaled) = 46.00 µm X 43.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 44.75 mm, Y: 44.46 mm
Stage Position = X: 44.75 mm, Y: 44.46 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1x
Depth of Focus = 1.20 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 25.00%
Exposure Time = 250 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

DAPI - Acquired but not shown in Data Analysis

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 = 1.25
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.

3D Image Processing - Panel A

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 spatial organization of Rabbit anti-Human Chk1 Polyclonal, IgG and Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3) within the nuclear compartment by applying a triple-wedge cut through the X-Y, X-Z, and Y-Z planes, selectively removing portions of the volumetric reconstruction to improve visualization of intranuclear signal distribution and colocalization.

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 = -20%
Clip X = 0°
Clip Z = 0°

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

Position of Clip = -8%
Clip Y = 45°
Clip Z = 0°

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

Position of Clip = 11%
Clip Y = -30°
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 Chk1, as indicated by colocalization with ATRIP.

Summary

Widefield structured illumination microscopy (SIM) demonstrates coordinated intranuclear organization between Chk1 and ATRIP following etoposide-induced DNA damage. Rabbit anti-Human Chk1 Polyclonal, IgG was visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody. In contrast, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody. Three-dimensional maximum-intensity and wedge-view orthogonal projections reveal overlapping yet structurally complex signal organization throughout the nucleus, with interspersed regions of apparent colocalization consistent with activation of the ATR-mediated replication stress response pathway.

Although widefield SIM improves lateral resolution and volumetric interpretation relative to conventional widefield microscopy, substantial signal convolution remains present within dense nuclear repair domains. Nevertheless, orthogonal X-Y, X-Z, and Y-Z reconstructions demonstrate coordinated spatial distribution between Chk1 and ATRIP, supporting their functional association within DNA damage signaling microenvironments.

Biologically, ATRIP serves as the obligate recruitment partner of ATR and localizes to RPA-coated single-stranded DNA generated during replication stress and DNA end resection. Activation of the ATR-ATRIP complex results in phosphorylation and downstream activation of Chk1, a central checkpoint kinase that stabilizes the replication fork, induces S-phase arrest, and orchestrates DNA repair. The overlapping intranuclear architecture observed here is therefore consistent with coordinated recruitment of ATRIP and Chk1 to replication-associated damage domains following etoposide-induced genotoxic stress.

Image Correction

NONE

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

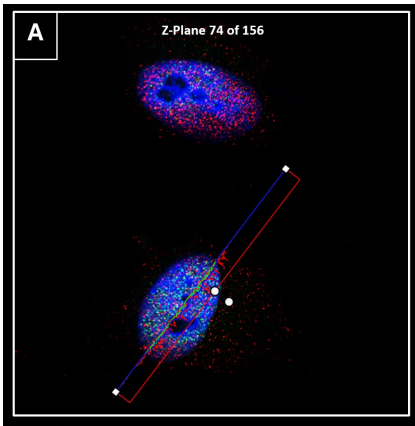
SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	60
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

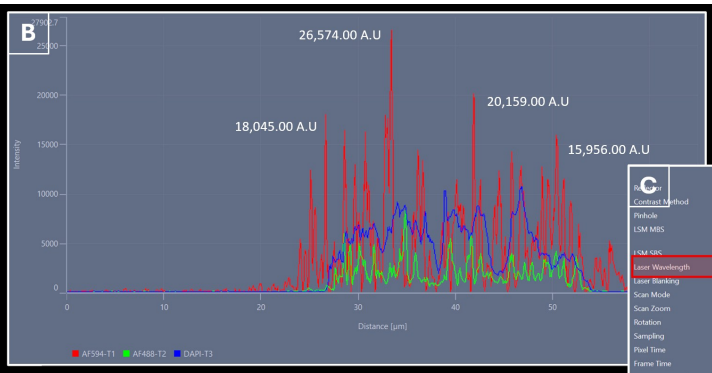
SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	3DEA8172960E0DDEEF256EFAB2881AC9E27ED0318BC531B233C97580BEE3E0E0
Method PDF SHA-256	1A5B3E9283673BA20AD6A5C955A817A477EEE7D4E733FAE55F6BB98882CC1559

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Rabbit anti-Human Chk1 Polyclonal, IgG detected using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody (red), Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), with Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody (green), and DAPI nuclear staining (blue).



Line Profile of Chk1, ATRIP, and DAPI Fluorescent Intensities Across the Nucleus

Panel A shows an LSM980 confocal optical section acquired at Z-plane 74 of 156, demonstrating Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody, and DAPI nuclear staining. A line profile traversing the lower nucleus measures fluorescent intensity across all three channels, demonstrating that Chk1, ATRIP, and DAPI signals remain confined to the nuclear compartment. The line profile additionally reveals punctate regions of elevated Chk1 intensity associated with intranuclear aggregate formation following etoposide-induced DNA damage.

Panel B shows histogram-based quantitative analysis of the measured fluorescent intensities from the line profile shown in Panel A. Four prominent Chk1-associated aggregate regions were identified with measured fluorescent intensities of 26,674.00, 20,159.00, 18,045.00, and 15,956.00 arbitrary units (A.U.), respectively. These findings support localized redistribution and concentration of Chk1 into discrete intranuclear signaling domains rather than a uniform increase in total nuclear protein abundance. While Chk1 is constitutively distributed throughout the nucleus under basal conditions, DNA damage and replication stress promote its spatial reorganization into punctate repair-associated microenvironments, coordinated by ATR-ATRIP signaling.

Panel C provides acquisition transparency by including the Zeiss ZEN imaging parameters used to collect the dataset. Recorded excitation inputs included 0.6% laser power for the 561 nm channel corresponding to Alexa Fluor™ 594, 1.00% laser power for the 488 nm channel corresponding to Alexa Fluor™ 488, and 0.4% laser power for the 405 nm DAPI channel. **See Full Summary Methods Section**

Rabbit anti-Human Chk1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	156 Slices (4.65 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	1612 X 1612
Image Size (Scaled)	94.79 µm X 94.79 µm
Bit Depth	16 Bit
Image Center Position	X: 81.40 mm, Y: 51.78 mm
Stage Position	X: 81.40 mm, Y: 51.78 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 59 µm | 1.00 AU / 51 µm | 1.00 AU / 44 µm
LMS MBS	MBS 488/561 & 405/730
LMS SBS	SBS SP 550 | SBS SP 505 | SBS LP 525
Laser Wavelength	561 nm @ 0.6% | 488 nm @ 1.0% | 405 nm @ 0.4%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.4
Rotation	0°
Sampling	1.20
Pixel Time	0.24 µs
Frame Time	6.04 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Detection Wavelength	590 - 645 | 511 - 549 | 435 - 490
Imaging Device	GaAsP-Pmt3 | GaAsp-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	600 V | 550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.9 (3D, Auto) | Filter: 7.7 (3D, Auto) | Filter: 6.9 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 65.0), Y (Min 0 and Max 27903)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

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

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D) - Panels A, B, and C, and Showing Z-Plane 74 of 156

Z-Stack = 156 Slices (4.65 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 1612 X 1612
Image Size (Scaled) = 94.79 µm X 94.79 µm
Bit Depth = 16 Bit
Image Center Position = X: 81.40 mm, Y: 51.78 mm
Stage Position = X: 81.40 mm, Y: 51.78 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 561 nm @ 0.6%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.24 µs
Frame Time = 6.04 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590 - 645
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.9 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 51 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488 nm @ 1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.24 µs
Frame Time = 6.04 s
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 - 549
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.7 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS LP 525
Laser Wavelength = 405 nm @ 0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.24 µs
Frame Time = 6.04 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 435 - 490
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.9 (3D, Auto)

2 Dimensional View - Panel A

Shows an LSM980 confocal optical section acquired at Z-plane 74 of 156 demonstrating Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody, and DAPI nuclear staining. A line profile traversing the lower nucleus measures fluorescent intensity across all three channels.

Profile View Display - Panels B and C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 65.0), Y (Min 0 and Max 27903)

Measured at Z-plane 74 of 156

Zen Acquisition Info Tab - Panel C

Panel C shows the Zeiss ZEN acquisition parameters used for image collection, including 0.6% laser power for the 561 nm channel (Alexa Fluor™ 594), 1.00% laser power for the 488 nm channel (Alexa Fluor™ 488), and 0.4% laser power for the 405 nm DAPI channel.

Summary

Panel A shows an LSM980 confocal optical section acquired at Z-plane 74 of 156, demonstrating Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody, and DAPI nuclear staining. A line profile traversing the lower nucleus measures fluorescent intensity across all three channels, demonstrating that Chk1, ATRIP, and DAPI signals remain confined to the nuclear compartment. The line profile additionally reveals punctate regions of elevated Chk1 intensity associated with intranuclear aggregate formation following etoposide-induced DNA damage.

Panel B shows histogram-based quantitative analysis of the measured fluorescent intensities from the line profile shown in Panel A. Four prominent Chk1-associated aggregate regions were identified with measured fluorescent intensities of 26,674.00, 20,159.00, 18,045.00, and 15,956.00 arbitrary units (A.U.), respectively. These findings support localized redistribution and concentration of Chk1 into discrete intranuclear signaling domains rather than a uniform increase in total nuclear protein abundance. While Chk1 is constitutively distributed throughout the nucleus under basal conditions, DNA damage and replication stress promote its spatial reorganization into punctate repair-associated microenvironments, coordinated by ATR-ATRIP signaling.

Panel C provides acquisition transparency by including the Zeiss ZEN imaging parameters used to collect the dataset. Recorded excitation inputs included 0.6% laser power for the 561 nm channel corresponding to Alexa Fluor™ 594, 1.00% laser power for the 488 nm channel corresponding to Alexa Fluor™ 488, and 0.4% laser power for the 405 nm DAPI channel.

Biologically, ATRIP functions as the obligate recruitment partner of ATR and localizes to regions of RPA-coated single-stranded DNA generated during replication stress and DNA end resection. Upon recruitment, ATR-mediated phosphorylation activates Chk1, initiating checkpoint signaling pathways that stabilize the replication fork, trigger intra-S phase arrest, and coordinate DNA repair. The observed intranuclear aggregation and colocalization patterns are therefore consistent with activation of the ATR-ATRIP-Chk1 replication stress response pathway following etoposide exposure. Importantly, the data suggest that the observed Chk1 response reflects redistribution and focal enrichment within damage-associated signaling domains rather than wholesale increases in total nuclear Chk1 protein volume, a finding consistent with the established biology of checkpoint kinase activation.

Image Correction

NONE

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

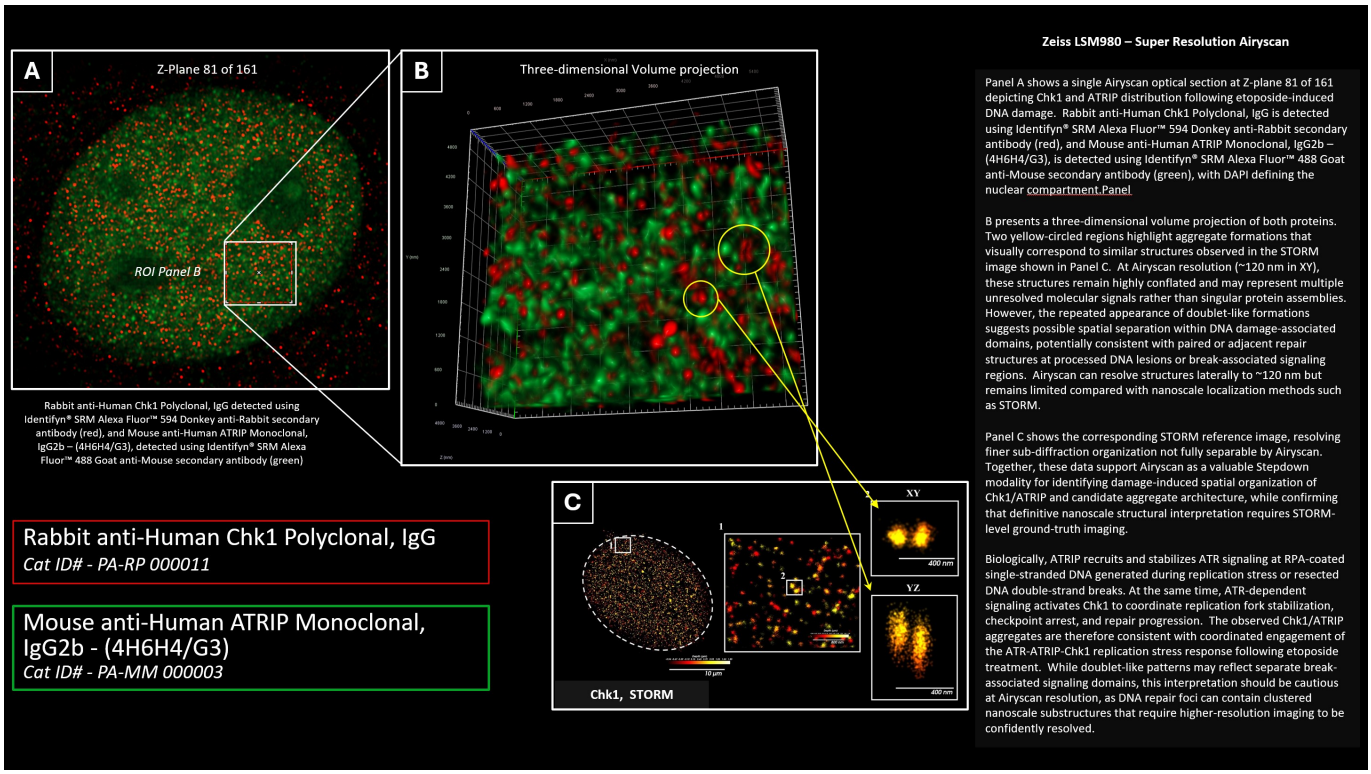
SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	56

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8607B4A4F379542AA6085B10C7746C52D375FCC0BE02CE0FDDED8559971F096E
Method PDF SHA-256	C15E54F71B280AF7C6B51CAF349F52BAA1384539C82D67B406ACA3443F2060AD

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	50

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	161 Slices (4.8 µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	766 X 683 | 164 X 147
Image Size (Scaled)	27.04 µm X 24.11 µm | 5.79 µm X 5.19 µm
Bit Depth	16 Bit
Image Center Position	X: 75.22 mm, Y: 49.76 mm
Stage Position	X: 75.22 mm, Y: 49.76 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 286 µm | 5.00 AU / 250 µm
LMS MBS	MBS 488/561 & 405/730
LMS SBS	SBS LP 525 | SBS SP 550
Laser Wavelength	561 nm @ 3.50% | 488 nm @ 2.1%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.1
Rotation	0°
Sampling	2.00
Pixel Time	1.15 µs
Frame Time	17.86 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	590 | 493
Emission Wavelength	618 | 517
Effective NA	1.4
Detection Wavelength	573 - 627 | 499 - 548
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	Filter: 10.0 (3D, Auto)
Airyscan Mode	Super Resolution: 9.1 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

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

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

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

Z Stack - (3D) - Panel A, Z-Plane 81 of 161

Z-Stack = 161 Slices (4.8 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 766 X 683
Image Size (Scaled) = 27.04 µm X 24.11 µm
Bit Depth = 16 Bit
Image Center Position = X: 75.22 mm, Y: 49.76 mm
Stage Position = X: 75.22 mm, Y: 49.76 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, Volume Projection

Z-Stack = 161 Slices (4.8 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 164 X 147
Image Size (Scaled) = 5.79 µm X 5.19 µm
Bit Depth = 16 Bit
Image Center Position = X: 75.22 mm, Y: 49.76 mm
Stage Position = X: 75.22 mm, Y: 49.76 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 286 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS LP 525
Laser Wavelength = 561 nm @ 3.50%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15 µs
Frame Time = 17.86 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 573 - 627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = Filter: 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488 nm @ 2.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15 µs
Frame Time = 17.86 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 9.1 (3D, Auto)

2 Dimensional View - Panel A

Shows an LSM980 confocal optical section acquired at Z-plane 81 of 161, demonstrating Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody. A white box shows the region of interest to be examined in panel B.

3D Image Processing

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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows a single Airyscan optical section at Z-plane 81 of 161 depicting Chk1 and ATRIP distribution following etoposide-induced DNA damage. Rabbit anti-Human Chk1 Polyclonal, IgG is detected using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody (red), and Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), is detected using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody (green), with DAPI defining the nuclear compartment.

Panel B presents a three-dimensional volume projection of both proteins. Two yellow-circled regions highlight aggregate formations that visually correspond to similar structures observed in the STORM image shown in Panel C. At Airyscan resolution (~120 nm in XY), these structures remain highly conflated and may represent multiple unresolved molecular signals rather than singular protein assemblies. However, the repeated appearance of doublet-like formations suggests possible spatial separation within DNA damage-associated domains, potentially consistent with paired or adjacent repair structures at processed DNA lesions or break-associated signaling regions. Airyscan can resolve structures laterally to ~120 nm, but remains limited compared with nanoscale localization methods such as STORM.

Panel C shows the corresponding STORM reference image, which resolves finer sub-diffractional organization not fully separable by Airyscan. Together, these data support Airyscan as a valuable Stepdown modality for identifying damage-induced spatial organization of Chk1/ATRIP and candidate aggregate architecture, while confirming that definitive nanoscale structural interpretation requires STORM-level ground-truth imaging.

Biologically, ATRIP recruits and stabilizes ATR signaling at RPA-coated single-stranded DNA generated during replication stress or resected DNA double-strand breaks. At the same time, ATR-dependent signaling activates Chk1 to coordinate replication fork stabilization, checkpoint arrest, and repair progression. The observed Chk1/ATRIP aggregates are therefore consistent with coordinated engagement of the ATR-ATRIP-Chk1 replication stress response following etoposide treatment. While doublet-like patterns may reflect separate break-associated signaling domains, this interpretation should be treated with caution at Airyscan resolution, as DNA repair foci can contain clustered nanoscale substructures that require higher-resolution imaging to be confidently resolved.

Image Correction

NONE

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

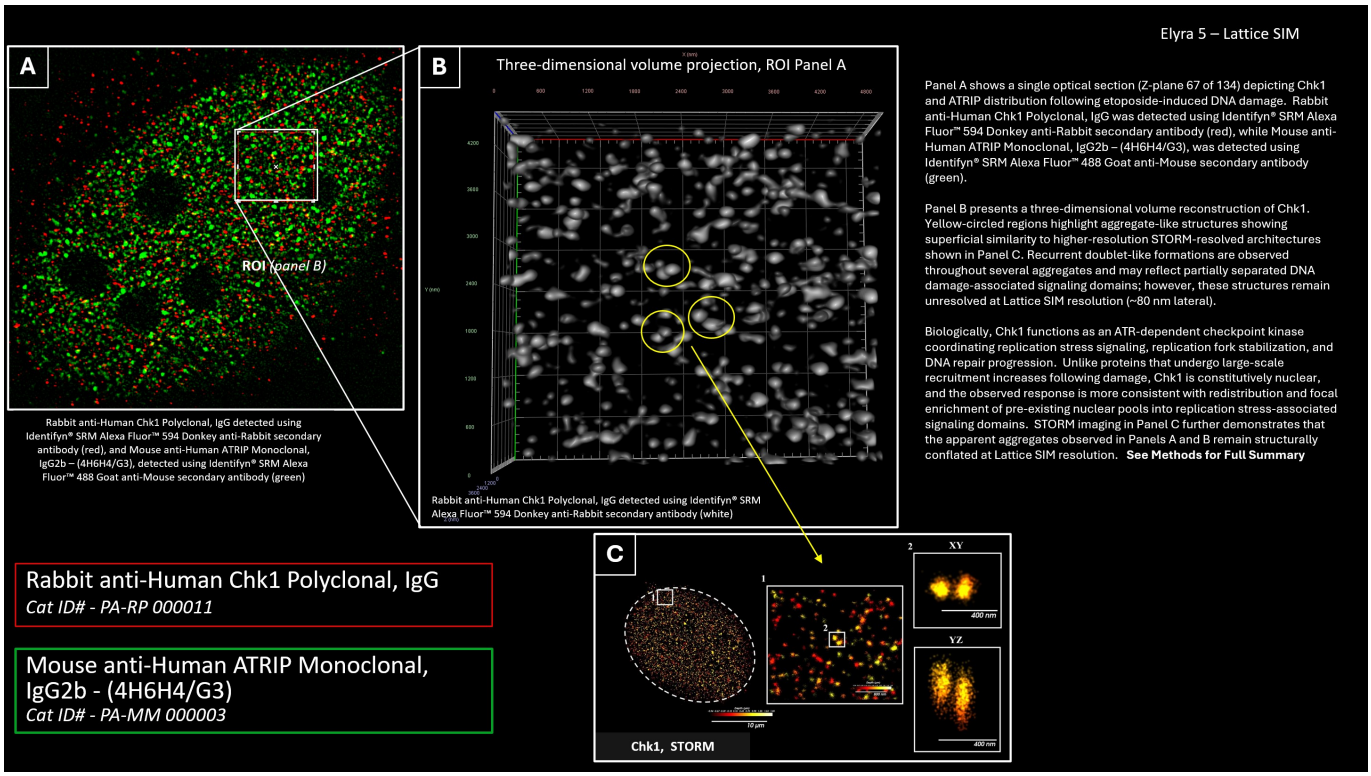
SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	746E008D6C50199963A32207CB1060CF91576BAD633984870325B190254B9079
Method PDF SHA-256	CBC74E520E30E64A345BC9984D128023E04B0072EDB46617E9B33488287C1384

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	55

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	134 Slices (3.99 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1694 X 1267 | 239 X 219
Image Size (Scaled)	35.76 µm X 26.75 µm | 5.05 µm X 4.62 µm
Bit Depth	16 Bit
Image Center Position	X: 64.98 mm, Y: 35.05 mm
Stage Position	X: 64.98 mm, Y: 35.05 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 | 488
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR
Excitation Wavelength	561 | 488
Emission Wavelength	597 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	200 ms | 120 ms
Depth of Focus	0.92 µm | 0.81 µm
Binning Mode	2,2
Laser Wavelength	561 nm @ 15.00% | 488 nm @ 5.00%
Frame Time	1.59 s
Scan Direction	Unidirectional
Grating	32.5 µm grating | 27.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.1066 (594), 11.0384 (488)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 4%, Ramp 96%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

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

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

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

Z Stack - (3D) - Panel A, Z-Plane 67 of 134

Z-Stack = 134 Slices (3.99 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1694 X 1267
Image Size (Scaled) = 35.76 µm X 26.75 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.98 mm, Y: 35.05 mm
Stage Position = X: 64.98 mm, Y: 35.05 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, Volume Projection

Z-Stack = 134 Slices (3.99 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 239 X 219
Image Size (Scaled) = 5.05 µm X 4.62 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.98 mm, Y: 35.05 mm
Stage Position = X: 64.98 mm, Y: 35.05 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

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 = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 200 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 15.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 32.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 503 - 546, 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 @ 5.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 11.1066 (594), 11.0384 (488)
Fitting = Fast Fit
Normalization = Auto

2 Dimensional View - Panel A

Shows an Elyra 5, Lattice SIM, optical section acquired at Z-plane 67 of 134, demonstrating Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody. A white box shows the region of interest to be examined in panel B.

3D Image Processing - Panel B, Showing Chk1/594 only

3D Volume Projection = Precise
Appearance = Threshold 4%, Ramp 96%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows a single optical section (Z-plane 67 of 134) depicting Chk1 and ATRIP distribution following etoposide-induced DNA damage. Rabbit anti-Human Chk1 Polyclonal, IgG is detected using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody (red), and Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), is detected using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody (green).

Panel B presents a three-dimensional volume reconstruction of Chk1 from the same region. Regions of interest (ROIs), indicated by yellow circles, highlight concentrated aggregate-like signal patterns that demonstrate superficial similarity to higher-resolution STORM-resolved structures. The observed Chk1 aggregates reflect localized enrichment within DNA damage-associated nuclear compartments; however, the structures remain diffraction-limited and do not resolve discrete molecular subunits. Recurrent doublet-like signal configurations are observed throughout several aggregate regions. They may reflect partially separated DNA damage-associated signaling domains or adjacent repair-associated structures, although these features remain unresolved at this resolution scale.

Despite improved resolution relative to conventional confocal microscopy, Lattice SIM still limits lateral resolution to ~80 nm and can still conflate closely spaced molecular assemblies into a single apparent structure. As a result, Chk1 signals appear as compact foci or aggregates without fully resolving the underlying nanoscale organization. In contrast, Panel C (STORM) resolves these apparent aggregates into more refined structural architectures, demonstrating that the structures observed in Panels A and B represent broader spatial organization rather than definitive molecular detail.

Biologically, Chk1 functions as a central ATR-dependent checkpoint kinase coordinating replication fork stabilization, intra-S phase arrest, and DNA repair progression following replication stress and DNA damage. Unlike proteins that undergo dramatic recruitment increases following damage induction, Chk1 is constitutively present throughout the nucleus under basal conditions. The observed response is therefore more consistent with the redistribution and focal enrichment of pre-existing nuclear Chk1 pools into replication-stress-associated signaling microenvironments rather than with substantial increases in total protein abundance. ATRIP, shown in Panel A, functions as the obligate recruitment partner of ATR and localizes to RPA-coated single-stranded DNA, where it facilitates ATR-mediated activation of Chk1 during the DNA damage response.

Image Correction

NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

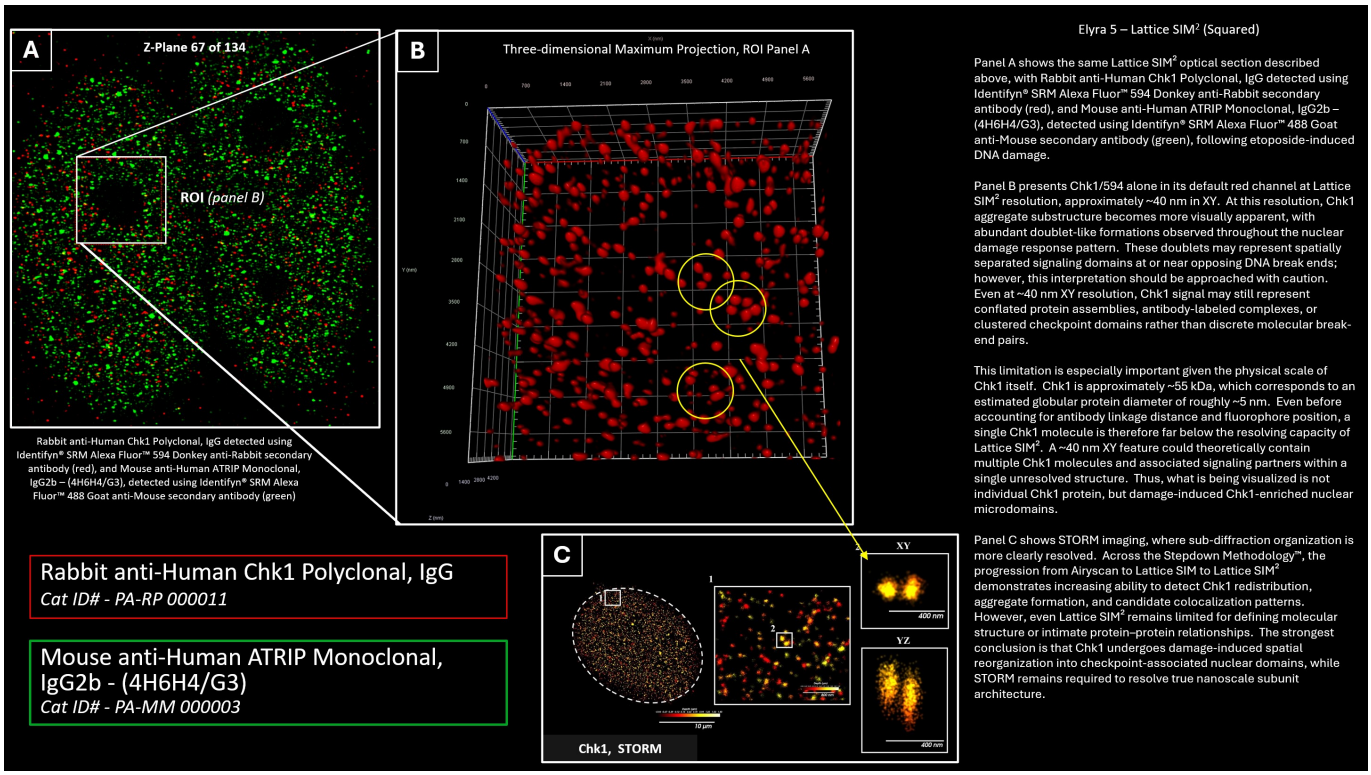
SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	55

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	78616AE973BFA803060640F57B22D904432EBE416F66C31FABC89D020C5C5FE5
Method PDF SHA-256	DDCCA1022B657A6C9114309B1306D189FEC33E221EA9A6A9A4340B048B235777

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	148 Slices (4.41 µm) | 134 Slices (3.99 µm) - Gallery was Reduced to Highlight Signal
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1374 X 1522 | 285 X 288
Image Size (Scaled)	29.01 µm X 32.13 µm | 6.02 µm X 6.08 µm
Bit Depth	16 Bit
Image Center Position	X: 64.52 mm, Y: 37.48 mm
Stage Position	X: 64.52 mm, Y: 37.48 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 | 488
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR
Excitation Wavelength	561 | 488
Emission Wavelength	597 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	200 ms | 120 ms
Depth of Focus	0.92 µm | 0.81 µm
Binning Mode	2,2
Laser Wavelength	561 nm @ 15.00% | 488 nm @ 5.00%
Frame Time	1.59 s
Scan Direction	Unidirectional
Grating	32.5 µm grating | 27.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2%, Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 65%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 45°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

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

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

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

Z Stack - (3D) - Panel A, Z-Plane 67 of 134

Z-Stack = 148 Slices (4.41 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1374 X 1522
Image Size (Scaled) = 29.01 µm X 32.13 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.52 mm, Y: 37.48 mm
Stage Position = X: 64.52 mm, Y: 37.48 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, Volume Projection

Z-Stack = 134 Slices (3.99 µm) - Gallery was Reduced to Highlight Signal

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 285 X 288
Image Size (Scaled) = 6.02 µm X 6.08 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.52 mm, Y: 37.48 mm
Stage Position = X: 64.52 mm, Y: 37.48 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

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 = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 200 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 15.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 32.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 503 - 546, 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 @ 5.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

2 Dimensional View - Panel A

Shows an Elyra 5, Lattice SIM, optical section acquired at Z-plane 67 of 134, demonstrating Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody. A white box shows the region of interest to be examined in panel B.

3D Image Processing - Panel B, Showing Chk1/594 only

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 65%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows the same Lattice SIM² optical section described above, with Rabbit anti-Human Chk1 Polyclonal, IgG detected using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody (red), and Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), detected using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody (green), following etoposide-induced DNA damage.

Panel B presents Chk1/594 alone in its default red channel at Lattice SIM² resolution, approximately ~40 nm in XY. At this resolution, Chk1 aggregate substructure becomes more visually apparent, with abundant doublet-like formations observed throughout the nuclear damage response pattern. These doublets may represent spatially separated signaling domains at or near opposing DNA break ends; however, this interpretation should be approached with caution. Even at ~40 nm XY resolution, the Chk1 signal may still represent conflated protein assemblies, antibody-labeled complexes, or clustered checkpoint domains rather than discrete molecular break-end pairs.

This limitation is especially important given the physical scale of Chk1 itself. Chk1 is approximately ~55 kDa, which corresponds to an estimated globular protein diameter of roughly ~5 nm. Even before accounting for antibody linkage distance and fluorophore position, a single Chk1 molecule is therefore far below the resolving capacity of Lattice SIM². A ~40 nm XY feature could theoretically contain multiple Chk1 molecules and associated signaling partners within a single unresolved structure. Thus, what is being visualized is not individual Chk1 protein, but damage-induced Chk1-enriched nuclear microdomains.

Panel C shows STORM imaging, in which sub-diffractional organization is more clearly resolved. Across the Stepdown Methodology™, the progression from Airyscan to Lattice SIM to Lattice SIM² demonstrates increasing ability to detect Chk1 redistribution, aggregate formation, and candidate colocalization patterns. However, even Lattice SIM² remains limited for defining molecular structure or intimate protein-protein relationships. The strongest conclusion is that Chk1 undergoes damage-induced spatial reorganization into checkpoint-associated nuclear domains, while STORM remains required to resolve true nanoscale subunit architecture.

Even STORM, at an approximate ~20 nm resolution, may still struggle to resolve individual Chk1 molecules or define true molecular boundaries, given that Chk1 is ~55 kDa and estimated to occupy only ~5 nm as a globular protein. Therefore, even STORM-resolved substructures likely represent nanoscale Chk1-enriched signaling domains, clustered assemblies, or antibody-accessible repair microenvironments rather than single-protein events.

Accordingly, the Stepdown Methodology™ is strongest for measuring response, redistribution, aggregate formation, and relative spatial organization across imaging modalities. It can support colocalization and structural inference, but it should not be overinterpreted as direct visualization of individual Chk1 molecules, opposing break ends, or definitive protein-protein interaction without orthogonal validation.

Image Correction

NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

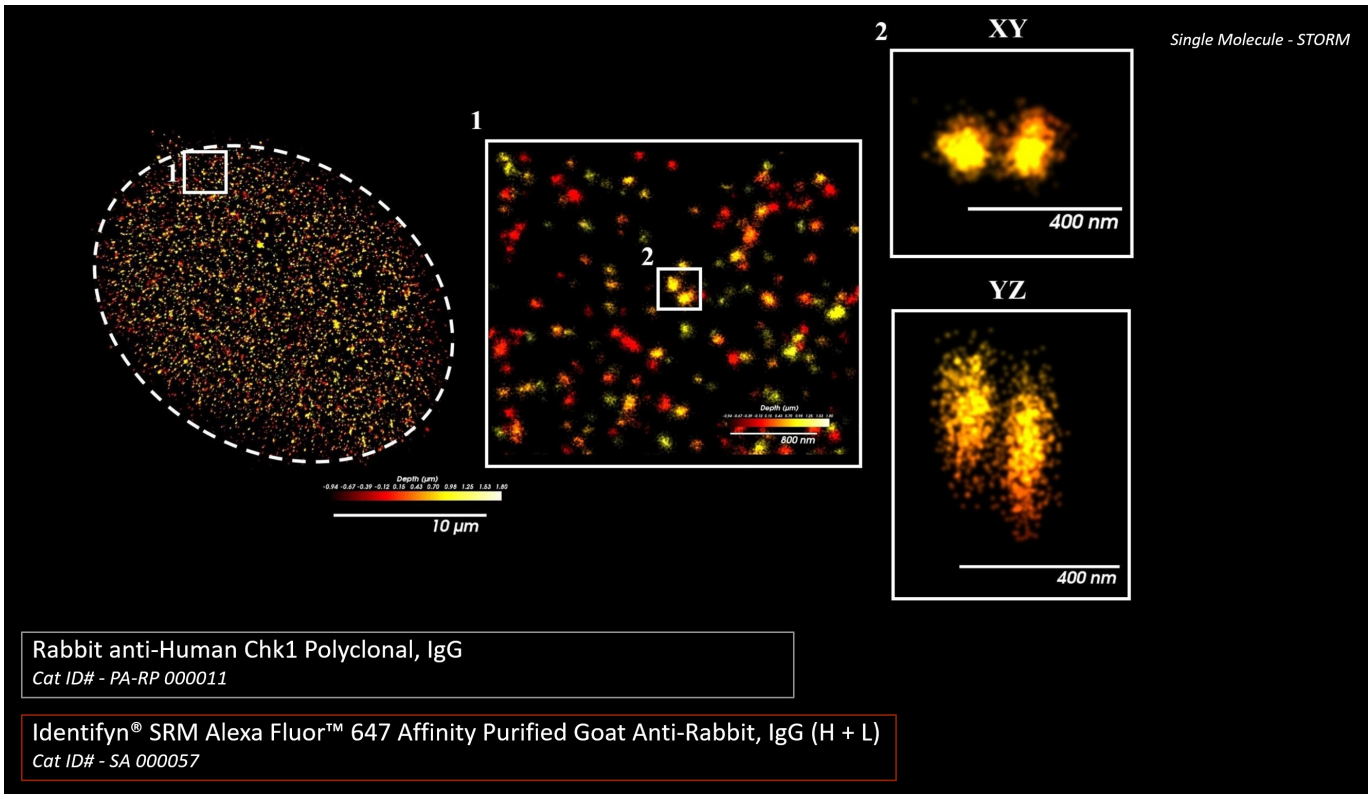
SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	SIM²

SOURCE PROVENANCE

Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	25978BE615A77AF07C7E9E4D12FD230FAC267B65B7254A87F0E584405AFF2CEA
Method PDF SHA-256	0F171EE5C6C6A7B4AD052BFF807F7AF3917509A833CCDBBD7EE7C0ADC75F41B1

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	80

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:
Objective	Olympus 60x/1.30 Silicon Oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635 nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100 ms | 20 ms
Super Resolution Camera Exposure Time	20 ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20 ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200 µm
Capture Mode	Live
Piezo Current	183.2 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640 nm	0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100 ms
Widefield Laser	"Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50 µm is selected
Cell(s) or Cellular Structure Localization	Positioning of the target Cell or Subsection of the Target Cell is optimized and focused.
Piezo Record	Begin: 182.2; End: 183.2
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	5
Cycles	30
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	40
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1 px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 25
Sizing Mode	Constant
Particle size	20 nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.50
Front-to-back Attenuation	Not Selected

FIELD	SOURCE-DOCUMENTED VALUE
Method	Particle (direct)
Time Intervals	8 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	30
Axial precision	65
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1 µm
Maximum Particle Count to Form Cluster	10
Compute Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	Deselected

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Chk1 Polyclonal
Cat ID# - PA-RP 000011

Identifyn® Secondary Antibody

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

CHK1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of CHK1's role in single-strand break repair and its broader role in maintaining genomic stability.

Overview of CHK1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown in a µ-Slide 8 Well High Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM Etoposide-spiked complete growth media for 24 hours,
followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Chk1 Polyclonal was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn®'s proprietary Wash Buffer, and Identifyn®'s 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:200. Cells were washed 5 times in Identifyn®'s proprietary Wash Buffer followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope Configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of the Sample

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

Piezo Current: 183.2 µm

Probe 1 Laser control 640 nm (635 nm Dichroic)

Widefield 640 nm: 0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100 ms
Default values are maintained unless otherwise noted.

Widefield Laser: "Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

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

View Mode

SuperRes: 50 µm is selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 182.2; End: 183.2

Probe 1 Laser control 640 nm (635 nm Dichroic)

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

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

Not Selected

Advanced Tools

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

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 5
Cycles: 30
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 40

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

All parameters were carried over from previous tabs and are kept except:

Probe1: BG threshold: 25

Region of Interest -

Not selected

Display -

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Constant
Particle size: 20 nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.50
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 8 Intervals
Pixel Size: 50 nm
Smoothing: 8.00

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

Radial precision: 30
Axial precision: 65

The other parameters were not applied.

Statistical Analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1 µm
Maximum Particle Count to Form Cluster: 10
Compute Hulls: Not Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

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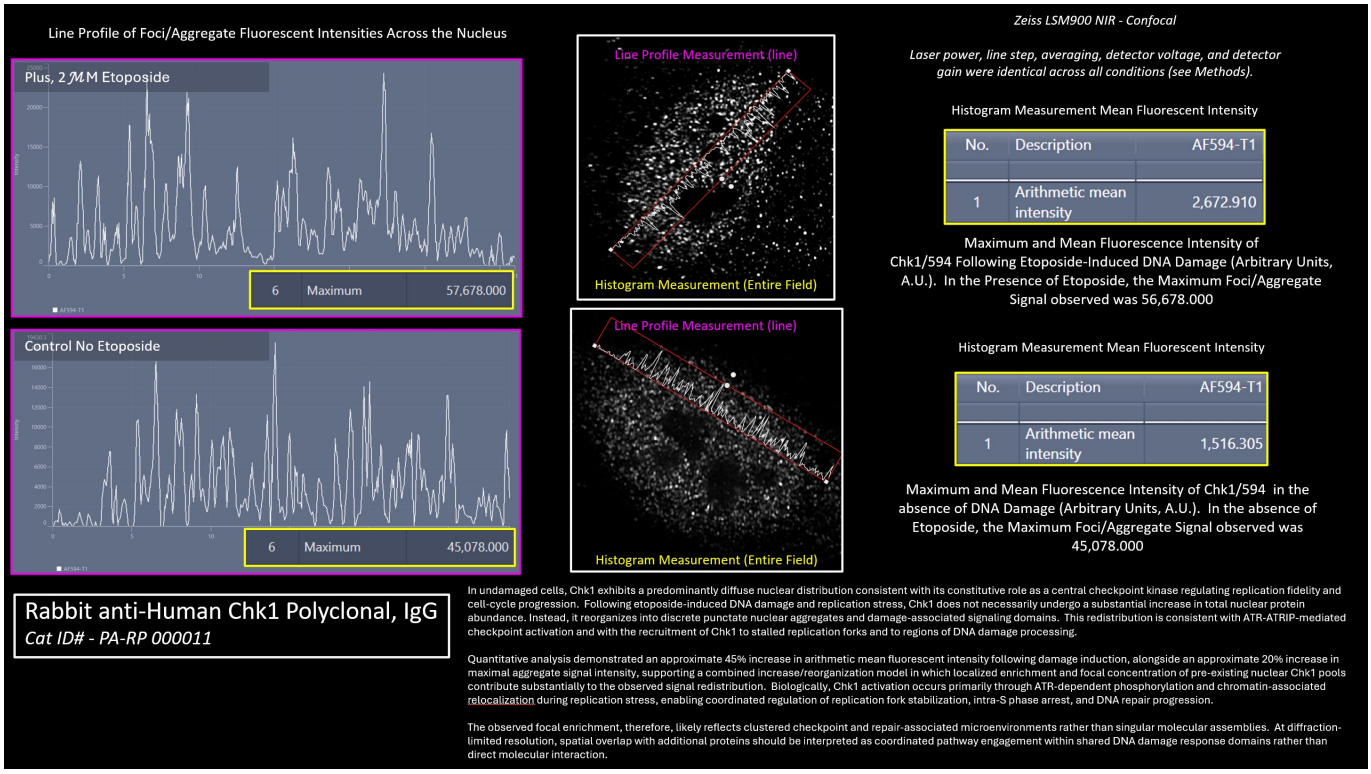
Image by P. Raut

SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:
Structured source metadata values	80
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	54EE318FB4B9A3098716C4588A07AE654B554CD8DDBF778E25E26A48B0E6C389
Method PDF SHA-256	4D5CC88523BB57BBE10F3F81D0794EF463C3C8EE1B9D13BAECF0DF643A6183EB

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	50

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	156 Slices (4.65 µm)
Channels	1
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	512 X 572 | 492 X 519
Image Size (Scaled)	30.11 µm X 33.64 µm | 28.93 µm X 30.52 µm
Bit Depth	16 Bit
Image Center Position	X: 81.40 mm, Y: 51.78 mm | X: 91.81 mm, Y: 52.99 mm
Stage Position	X: 81.40 mm, Y: 51.78 mm | X: 91.81 mm, Y: 52.99 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 59 µm
LMS MBS	MBS 488/561 & 405/730
LMS SBS	SBS SP 550
Laser Wavelength	561 nm @ 0.6%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.4
Rotation	0°
Sampling	1.20
Pixel Time	0.24 µs
Frame Time	6.04 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	590
Emission Wavelength	618
Effective NA	1.4
Detection Wavelength	590 - 645
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.9 (3D, Auto) | Filter: 8.3 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 | 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 31.0), Y (Min 0.0 and Max 25515) | X and Y axes were set to Auto - X (Min 0.0 and Max 28.9), Y (Min 0.0 and Max 19430)
Histogram Definition	Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535
Histogram View	X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 1000000) | X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000000)
Arithmetic Mean intensity	2,679.910 | 1,516.305

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

Plus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 µM Etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Chk1 Polyclonal, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Minus Damage -

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, Rabbit anti-Human Chk1 Polyclonal, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Microscope

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

Z Stack - (3D) - Showing Z-Plane 79 of 156, Plus Etoposide

Z-Stack = 156 Slices (4.65 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 512 X 572
Image Size (Scaled) = 30.11 µm X 33.64 µm
Bit Depth = 16 Bit
Image Center Position = X: 81.40 mm, Y: 51.78 mm
Stage Position = X: 81.40 mm, Y: 51.78 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Showing Z-Plane 75 of 161, Plus Etoposide

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 492 X 519
Image Size (Scaled) = 28.93 µm X 30.52 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.81 mm, Y: 52.99 mm
Stage Position = X: 91.81 mm, Y: 52.99 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594- Plus Etoposide-Induced DNA Damage, from Confocal Data Set

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 561 nm @ 0.6%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.24 µs
Frame Time = 6.04 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590 - 645
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.9 (3D, Auto)

594- Minus Damage - noting the same laser input power and conditions

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 561 nm @ 0.6%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.24 µs
Frame Time = 6.04 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590 - 645
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.3 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 31.0), Y (Min 0.0 and Max 25515)

This panel measures Chk1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~57K A.U. at its maximum.

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Profile View Display - Minus DNA Damage, Bottom

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 28.9), Y (Min 0.0 and Max 19430)

This panel measures Chk1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~45K A.U. at its maximum.

Histogram Display - Plus DNA Damage, Top

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 1000000)

Arithmetic Mean intensity = 2,679.910

The histogram was generated from the yellow-boxed region shown to quantify the overall Chk1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - Minus DNA Damage, Bottom

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000000)

Arithmetic Mean intensity = 1,516.305

The histogram was generated from the yellow-boxed region shown to quantify the overall Chk1 protein within a defined nuclear area in the absence of DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In the absence of etoposide-induced DNA damage, Chk1 exhibits a predominantly diffuse nuclear distribution, consistent with its constitutive role as a surveillance kinase that regulates replication fidelity and normal cell-cycle progression. Under control conditions, Chk1 signal remains broadly distributed throughout the nucleoplasm with comparatively reduced punctate organization and lower aggregate fluorescent intensity relative to the damage-induced state.

Quantitative line-profile analysis demonstrated that maximal aggregate fluorescent intensity under control conditions reached approximately ~45K A.U., compared with ~57K A.U. following etoposide exposure, representing an approximate ~20% increase in maximal aggregate signal associated with DNA damage induction. Histogram analysis further demonstrated an arithmetic mean intensity of 1,516.305 A.U. in the absence of damage compared with 2,679.910 A.U. following etoposide treatment, supporting an approximate ~45% increase in overall nuclear signal intensity associated with checkpoint activation and spatial reorganization.

Importantly, the observed increase is biologically consistent with redistribution and focal enrichment of pre-existing nuclear Chk1 pools rather than wholesale upregulation of total protein abundance. CHK1 is constitutively expressed and maintained within the nucleus under basal conditions, where it monitors replication integrity and coordinates normal S-phase progression. Following replication stress and activation of the ATR-ATRIP pathway, Chk1 undergoes phosphorylation-dependent relocalization to chromatin-associated signaling domains and stalled replication forks, resulting in punctate aggregate formation and enhanced local signal intensity.

Thus, the control condition establishes the expected baseline diffuse nuclear architecture of Chk1 before checkpoint activation. In contrast, the etoposide-induced condition demonstrates biologically consistent redistribution into damage-associated repair and signaling microenvironments characteristic of the ATR-Chk1 replication stress response pathway.

Image Correction

The Chk1 channel (594) was pseudo-colored white to enhance visual contrast in displaying measurements.

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

SOURCE PROVENANCE

Catalog	PA-RP-000011
Stage	Control
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	EE1582B5F021D03E2EE328AD9DB73CB37C6C5C69F3498C389EF80A85D1C2D35D
Method PDF SHA-256	116203B4BAFB6FEF6EB6C43C3D3619EB71795F7CAECFC868E6283D295AAC2069