BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

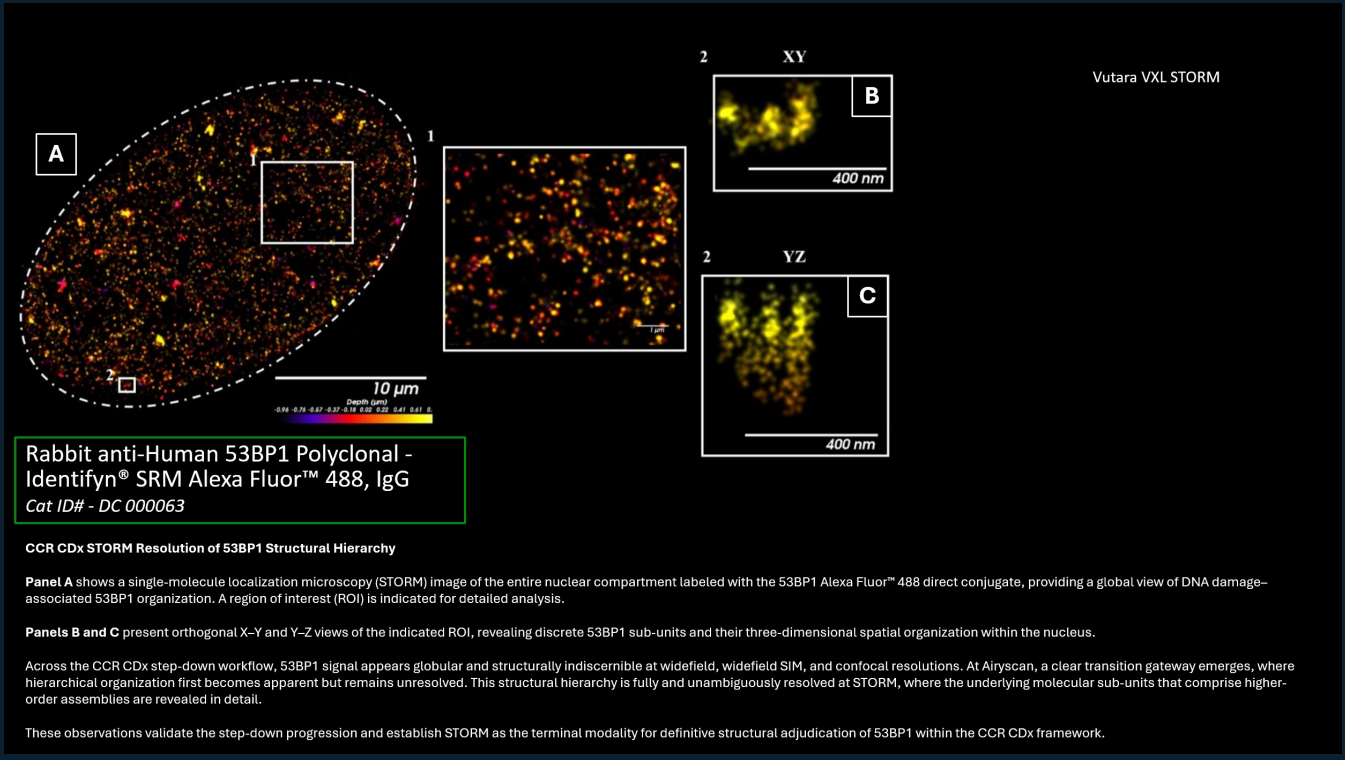
53BP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488

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

8	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



CCR CDx STORM Resolution of 53BP1 Structural Hierarchy

Panel A shows a single-molecule localization microscopy (STORM) image of the entire nuclear compartment labeled with the 53BP1 Alexa Fluor™ 488 direct conjugate, providing a global view of DNA damage–associated 53BP1 organization. A region of interest (ROI) is indicated for detailed analysis.

Panels B and C present orthogonal X–Y and Y–Z views of the indicated ROI, revealing discrete 53BP1 sub-units and their three-dimensional spatial organization within the nucleus.

Across the CCR CDx step-down workflow, 53BP1 signal appears globular and structurally indiscernible at widefield, widefield SIM, and confocal resolutions. At Airyscan, a clear transition gateway emerges, where hierarchical organization first becomes apparent but remains unresolved. This structural hierarchy is fully and unambiguously resolved at STORM, where the underlying molecular sub-units that comprise higher-order assemblies are revealed in detail.

These observations validate the step-down progression and establish STORM as the terminal modality for definitive structural adjudication of 53BP1 within the CCR CDx framework.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000063 | 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
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for 53BP1, 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

BENNETT ASSESSMENT

The preserved DC-000063 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 DC-000063 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

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594	3; 38 eGFP; 45 Texas Red; 49 DAPI; 450 - 490; 540 - 580; 335 - 383; 500 - 550
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 38 eGFP; 45 Texas Red; 49 DAPI; 450 - 490; 540 - 580; 335 - 383; 500 - 550
Confocal	405/DAPI; 488; 594	3; None; 488nm @0.2%; 561nm @0.4%; 405nm @0.2%; 493; 590; 353
Airyscan	405/DAPI; 488; 594; 750	3; None; 488nm @0.4%; 561nm @1.0%; 405nm @0.2%; 493; 590; 353
SIM	405/DAPI; 488; 594	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820 #2-SR; T3-Axiocam 820 #2-SR; 488
SIM²	405/DAPI; 488; 594	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820 #2-SR; T3-Axiocam 820 #2-SR; 488
Single-molecule STORM	488	Both Channels

MODALITY ASSESSMENT / PART 1 OF 8

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 8

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): 830 X 858; Image Size (Pixels): 830 X 858; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 37.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 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 8

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: 46 Slices (4.5 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 867 X 744; 212 X 269; Image Size (Pixels): 867 X 744; 212 X 269; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 150 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25; 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 48.

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 8

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: 88 Slices (2.61 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 534 X 28.17; Image Size (Pixels): 534 X 28.17; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsp-Pmt3; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.35µs; Frame Time: 4.15s. Illumination: Laser Wavelength: 488nm @0.2%; 561nm @0.4%. Optical/scan: Pinhole: 1.00AU / 51µm; 1.00AU / 59µm; Scan Zoom: 1.7; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.1 (3D, Auto); Filter: 7.5 (3D, Auto). Structured source metadata values: 55.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as 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 8

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: 111 Slices (3.36 µm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 908 X 602; 173 X 144; Image Size (Pixels): 908 X 602; 173 X 144; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66µs; Frame Time: 7.82s. Illumination: Laser Wavelength: 488nm @0.4%; 561nm @1.0%. Optical/scan: Pinhole: 5.00AU / 250µm; 5.00AU / 286µm; Scan Zoom: 2.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 57.

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; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 8

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: 110 Slices (3.27 µm); Channels: 3; Scaling (Per Pixel): 21.10 nm X 21.10 nm X 30.00 nm; Image size (Pixels): 1809 X 1852; 583 X 620; Image Size (Pixels): 1809 X 1852; 583 X 620; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 150 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 488 nm @1.00%; 561 nm @4.00%; Illumination Wavelength: 488; 561. 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 25%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 64.

VISIBLE OBSERVATION

A preserved presentation image is available for 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 8

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: 54 Slices (1.59 µm); Channels: 3; Scaling (Per Pixel): 21.10 nm X 21.10 nm X 30.00 nm; Image size (Pixels): 2455 X 2562; 649 X 676; Image Size (Pixels): 2455 X 2562; 649 X 676; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 150 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 488 nm @1.00%; 561 nm @4.00%; Illumination Wavelength: 488; 561. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 25%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 64.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 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 8

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 81.

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 488.

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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000063 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)=908 X 602; Image Size (Scaled)=32.05 µm X 21.60 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 1.62%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.10 nm X 21.10 nm X 30.00 nm -> 0.02110 µm X 0.02110 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)=1809 X 1852; Image Size (Scaled)=38.19 µm X 39.10 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.06%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.10 nm X 21.10 nm X 30.00 nm -> 0.02110 µm X 0.02110 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)=2455 X 2562; Image Size (Scaled)=51.83 µm X 54.09 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.06%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Single-molecule STORM": ["6 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. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

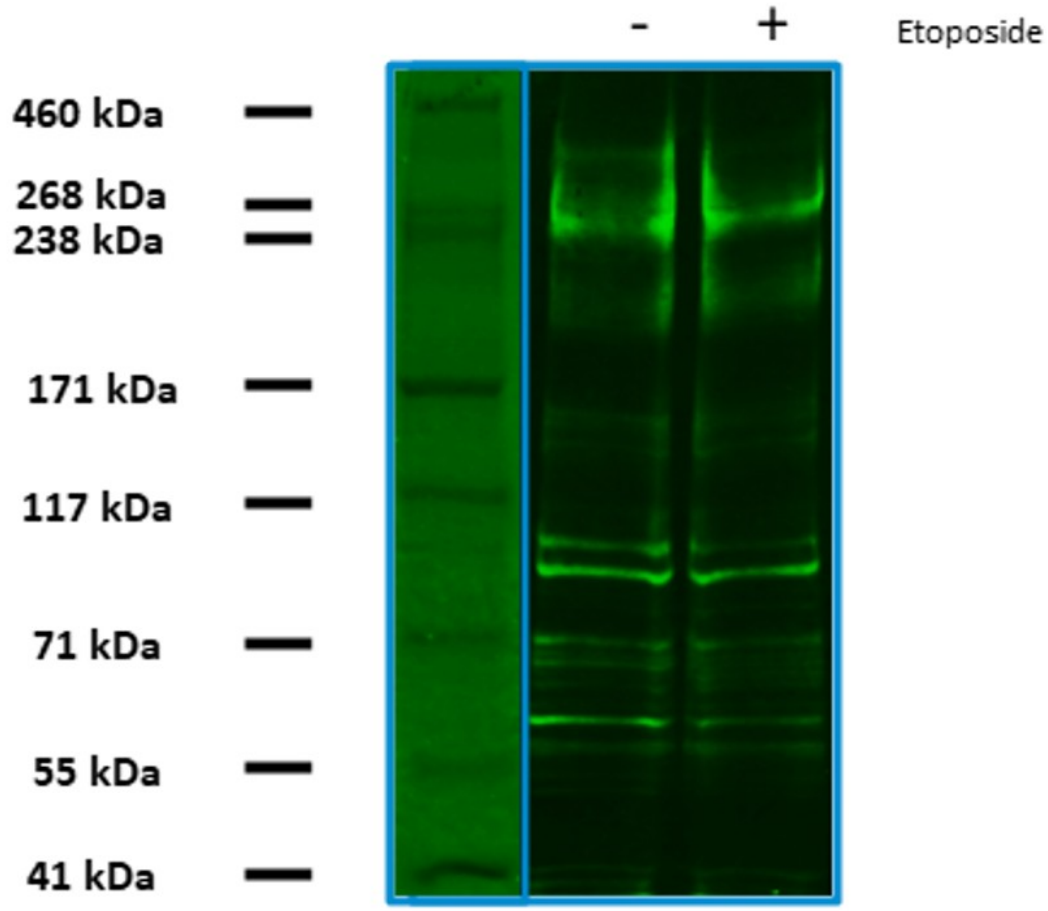
This dossier preserves the complete available evidence progression for DC-000063. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



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

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	488nm
Emission Filter	513±17nm
Detector	PMT
Intensity	3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000063

Expected MW: 214 kDa

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were then homogenized using an ultrasonication probe and nuclear lysis buffer, and then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm in a Mini Gel Tank. The Thermo Fisher's HiMark™ Pre-stained Protein Standard 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 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488 for 20 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence
Laser = 488nm
Emission Filter = 513±17nm
Detector = PMT
Intensity = 3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

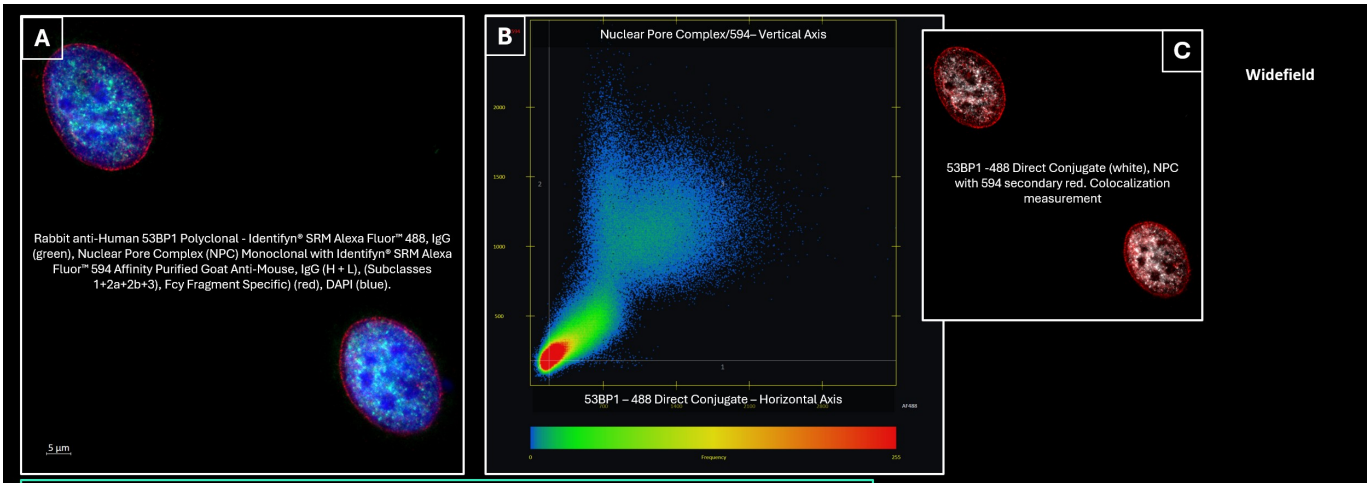
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000063
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A70265FEE3911870D4E3B760C791B7E57A594DBF2319D37E0C47AAA9A74B33AB
Method PDF SHA-256	20593AF56FA161CE2E46B17D1E7BA66DDFDDD5F4568F32A09DE8C0729993C291

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG

Cat ID# - DC 000063

Panel A shows a three-channel merged widefield image acquired as part of the CCR CDx step-down assay framework, demonstrating DNA damage response (DDR) nuclear architecture. Rabbit anti-Human 53BP1 Polyclonal – Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Mouse IgG (H+L; subclasses 1, 2a, 2b, 3; Fcγ fragment–specific) is shown in red, and DAPI nuclear counterstain is shown in blue. NPC staining serves as a nuclear inclusion and boundary reference, enabling spatial context for CCR-relevant DDR signal interpretation.

Panel B presents a CCR CDx colocalization analysis, with NPC (Alexa Fluor™ 594) plotted on the vertical axis and the 53BP1 Alexa Fluor™ 488 direct conjugate plotted on the horizontal axis. Thresholds were set automatically in Zeiss ZEN software (AF488 = 2212; AF594 = 2389). This analysis demonstrates that 53BP1 signal distribution is spatially organized relative to nuclear architecture, rather than randomly distributed fluorescence, supporting biologically meaningful DDR engagement.

Panel C displays the complete field used for CCR CDx analysis, with the 53BP1 Alexa Fluor™ 488 signal rendered in white to enhance visual contrast and structural interpretation at the widefield level. NPC staining continues to function as a nuclear reference marker. The observed 53BP1 localization and nuclear organization are consistent with prior step-down datasets and with results obtained using the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008), confirming reagent concordance and suitability for CCR CDx assay development.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	37

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	830 X 858
Image Size (Scaled)	90.90 µm X 93.97 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	38 eGFP | 45 Texas Red | 49 DAPI
Beam Splitter	495 | 585 | 395
Filter Excitation Wavelength	450 - 490 | 540 - 580 | 335 - 383
Filter Emission Wavelength	500 - 550 | 593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	150 ms | 20 ms
Excitation Wavelength	493 | 590 | 353
Emission Wavelength	517 | 618 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.80 µm | 0.96 µm | 0.72 µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000063

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting the end resection process (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in the S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 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 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 830 X 858
Image Size (Scaled) = 90.90 µm X 93.97 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 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

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

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

2- Dimensional View - Panel A

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Mouse IgG (H+L; subclasses 1, 2a, 2b, 3; Fcγ fragment-specific) is shown in red, and DAPI nuclear counterstain is shown in blue.

Colocalization View - Panel B, and Image Panel C, measurement was the entire image acquisition.

Horizontal Axis - AF488, Threshold 2212, Range Auto
Vertical Axis - AF594, Threshold 2389, Range Auto

Summary

CCR CDx Step-Down Validation of 53BP1 Nuclear Organization

Panel A shows a three-channel merged widefield image acquired as part of the CCR CDx step-down assay framework, demonstrating DNA damage response (DDR) nuclear architecture. Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Mouse IgG (H+L; subclasses 1, 2a, 2b, 3; Fcγ fragment-specific) is shown in red, and DAPI nuclear counterstain is shown in blue. NPC staining serves as a nuclear inclusion and boundary reference, enabling spatial context for CCR-relevant DDR signal interpretation.

Panel B presents a CCR CDx colocalization analysis, with NPC (Alexa Fluor™ 594) plotted on the vertical axis and the 53BP1 Alexa Fluor™ 488 direct conjugate plotted on the horizontal axis. Thresholds were set automatically in Zeiss ZEN software (AF488 = 2212; AF594 = 2389). This analysis demonstrates that 53BP1 signal distribution is spatially organized relative to nuclear architecture, rather than randomly distributed fluorescence, supporting biologically meaningful DDR engagement.

Panel C displays the complete field used for CCR CDx analysis, with the 53BP1 Alexa Fluor™ 488 signal rendered in white to enhance visual contrast and structural interpretation at the widefield level. NPC staining continues to function as a nuclear reference marker. The observed 53BP1 localization and nuclear organization are consistent with prior step-down datasets and with results obtained using the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008), confirming reagent concordance and suitability for CCR CDx assay development.
Biological and CDx Relevance:

At the widefield stage of the step-down workflow, this figure establishes signal specificity, nuclear context, and DDR engagement, forming the baseline required for subsequent higher-resolution CCR CDx validation. Preservation of spatial organization across resolution scales supports the use of Identifyn 53BP1 reagents in resolution-aware DDR diagnostics, where nuclear architecture and pathway organization are critical to interpretation.

Image Correction

None

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

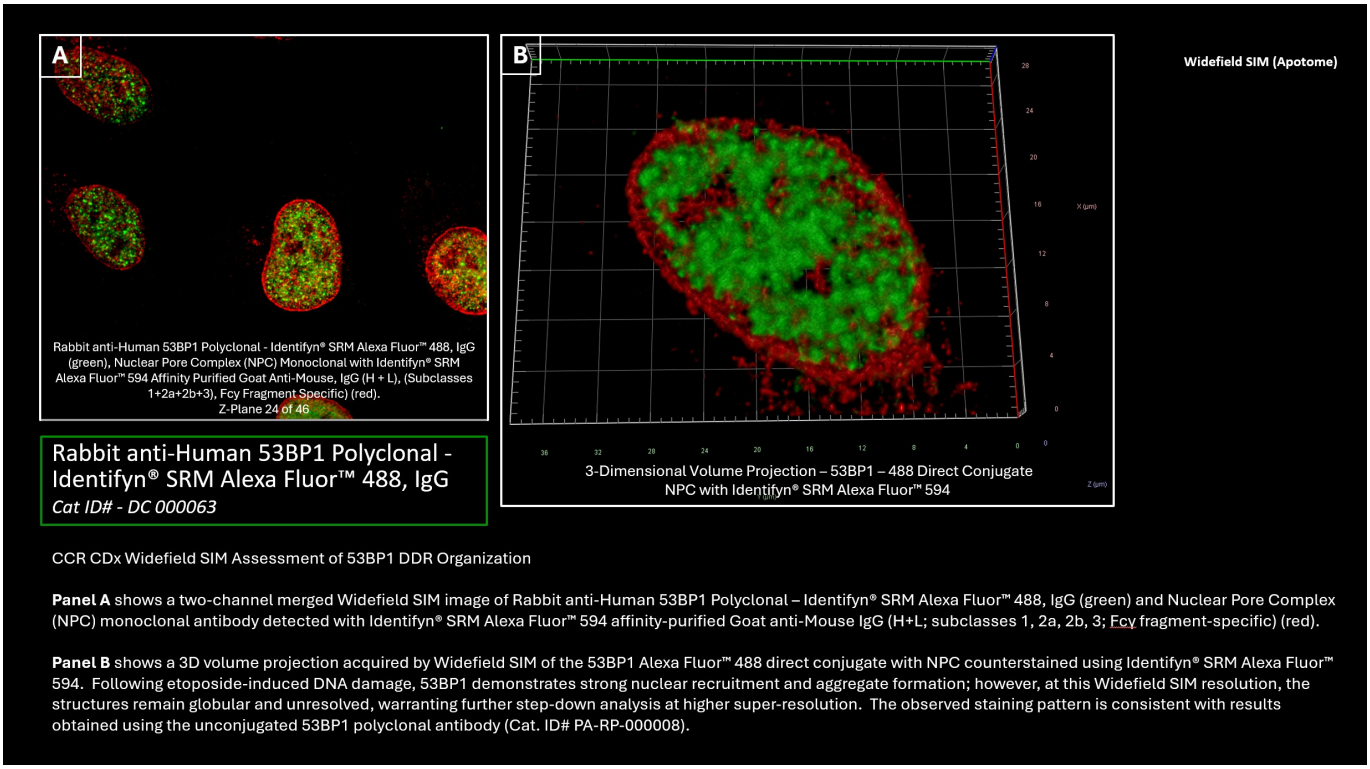
SOURCE PROVENANCE

Catalog	DC-000063
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	20D8EE9C7E243926091B758783E8A6D8832E5A8297D31923BF7B3FCEE9CE84B8
Method PDF SHA-256	7AD9588E3BF097BCBA96FEFC8375266B30A4041892ADA4AAF40D9CA46DAF37BD

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



CCR CDx Widefield SIM Assessment of 53BP1 DDR Organization

Panel A shows a two-channel merged Widefield SIM image of Rabbit anti-Human 53BP1 Polyclonal – Identifyn® SRM Alexa Fluor™ 488, IgG (green) and Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Mouse IgG (H+L; subclasses 1, 2a, 2b, 3; Fcγ fragment-specific) (red).

Panel B shows a 3D volume projection acquired by Widefield SIM of the 53BP1 Alexa Fluor™ 488 direct conjugate with NPC counterstained using Identifyn® SRM Alexa Fluor™ 594. Following etoposide-induced DNA damage, 53BP1 demonstrates strong nuclear recruitment and aggregate formation; however, at this Widefield SIM resolution, the structures remain globular and unresolved, warranting further step-down analysis at higher super-resolution. The observed staining pattern is consistent with results obtained using the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008).

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	48

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	46 Slices (4.5 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	867 X 744 | 212 X 269
Image Size (Scaled)	123.86 µm X 106.29 µm | 30.29 µm X 38.43 µm
Bit Depth	14 Bit
Image Center Position	X: 68.35 µm, Y: 49.01 µm
Stage Position	X: 68.34 µm, Y: 49.01 µm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	38 eGFP | 45 Texas Red | 49 DAPI
Beam Splitter	495 | 585 | 395
Filter Excitation Wavelength	450 - 490 | 540 - 580 | 335 - 383
Filter Emission Wavelength	500 - 550 | 593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	150 ms | 25 ms
Excitation Wavelength	493 | 590 | 353
Emission Wavelength	517 | 618 | 465
Effective NA	1.25 | 1.4
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.00 µm | 1.20 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000063

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting the end resection process (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in the S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 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 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 24 of 46

Z-Stack = 46 Slices (4.5 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 867 X 744
Image Size (Scaled) = 123.86 µm X 106.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 68.35 µm, Y: 49.01 µm
Stage Position = X: 68.34 µm, Y: 49.01 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

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

Z-Stack = 46 Slices (4.5 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 212 X 269
Image Size (Scaled) = 30.29 µm X 38.43 µm
Bit Depth = 14 Bit
Image Center Position = X: 68.35 µm, Y: 49.01 µm
Stage Position = X: 68.34 µm, Y: 49.01 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 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

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

DAPI - (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 = 1.4
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

2- Dimensional View - Panel A

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Mouse IgG (H+L; subclasses 1, 2a, 2b, 3; Fcγ fragment-specific) is shown in red.

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

CCR CDx Widefield SIM Assessment of 53BP1 DDR Organization

Panel A shows a two-channel merged Widefield SIM image of Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG (green) and Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Mouse IgG (H+L; subclasses 1, 2a, 2b, 3; Fcγ fragment-specific) (red).

Panel B shows a 3D volume projection acquired by Widefield SIM of the 53BP1 Alexa Fluor™ 488 direct conjugate with NPC counterstained using Identifyn® SRM Alexa Fluor™ 594. Following etoposide-induced DNA damage, 53BP1 demonstrates strong nuclear recruitment and aggregate formation; however, at this Widefield SIM resolution, the structures remain globular and unresolved, warranting further step-down analysis at higher super-resolution. The observed staining pattern is consistent with results obtained using the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008).

CCR CDx Step-Down Gate Summary:

Widefield SIM confirms signal specificity, nuclear localization, and DDR engagement, while indicating that higher-resolution modalities are required to resolve sub-structural organization. This dataset passes the Widefield SIM gate and advances to subsequent step-down stages for CCR CDx assay development.

Image Correction

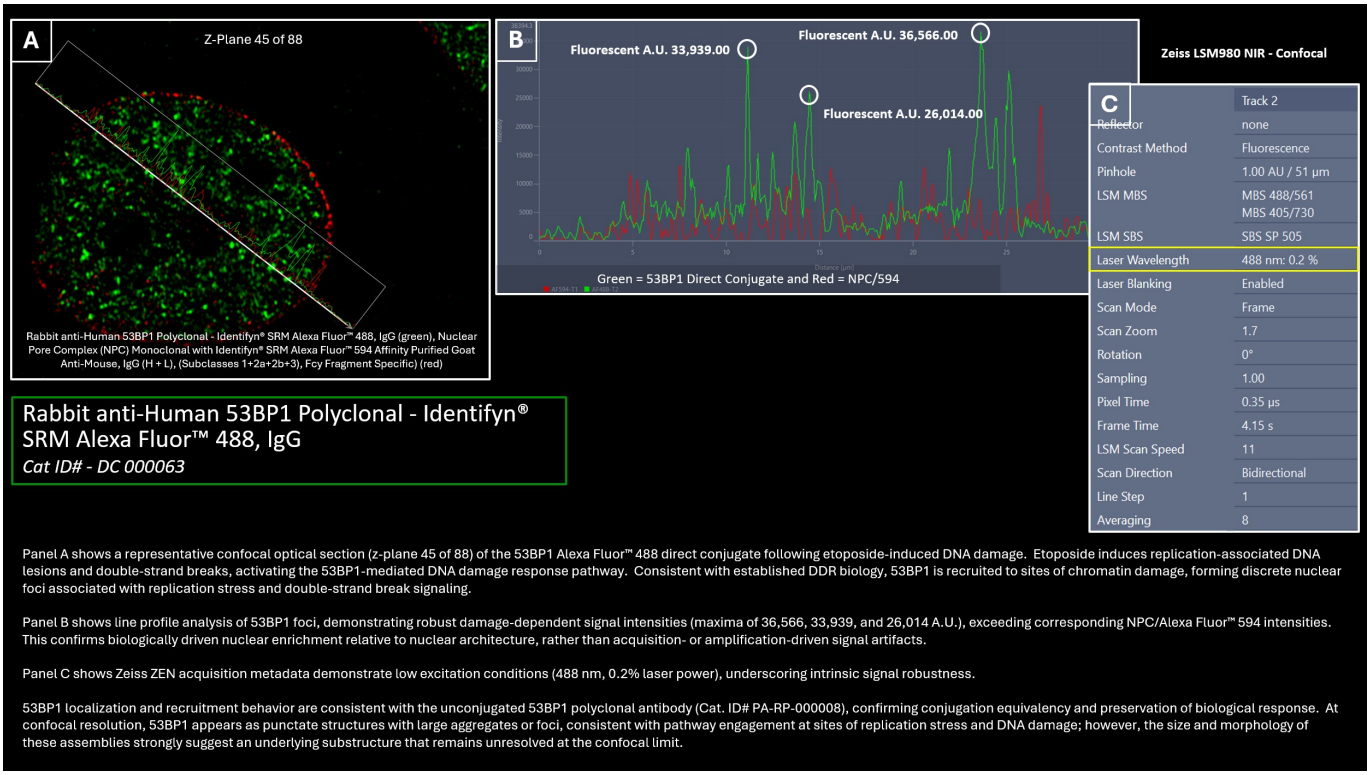
NONE

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

SOURCE PROVENANCE

Catalog	DC-000063
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	48
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7D46875AAF4FB54A026BEC86DC20D74D3D431786D73C2A27F6D01B9A3A89A8DF
Method PDF SHA-256	607C05F14291D106C5F232D292A0C1CC8BD2F2E6C775E2D67B316BED39520474

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A shows a representative confocal optical section (z-plane 45 of 88) of the 53BP1 Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage. Etoposide induces replication-associated DNA lesions and double-strand breaks, activating the 53BP1-mediated DNA damage response pathway. Consistent with established DDR biology, 53BP1 is recruited to sites of chromatin damage, forming discrete nuclear foci associated with replication stress and double-strand break signaling.

Panel B shows line profile analysis of 53BP1 foci, demonstrating robust damage-dependent signal intensities (maxima of 36,566, 33,939, and 26,014 A.U.), exceeding corresponding NPC/Alexa Fluor™ 594 intensities. This confirms biologically driven nuclear enrichment relative to nuclear architecture, rather than acquisition- or amplification-driven signal artifacts.

Panel C shows Zeiss ZEN acquisition metadata demonstrate low excitation conditions (488 nm, 0.2% laser power), underscoring intrinsic signal robustness.

53BP1 localization and recruitment behavior are consistent with the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008), confirming conjugation equivalency and preservation of biological response. At confocal resolution, 53BP1 appears as punctate structures with large aggregates or foci, consistent with pathway engagement at sites of replication stress and DNA damage; however, the size and morphology of these assemblies strongly suggest an underlying substructure that remains unresolved at the confocal limit.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	55

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	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	88 Slices (2.61 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	534 X 28.17
Image Size (Scaled)	37.61 µm X 28.17 µm
Bit Depth	16 Bit
Image Center Position	X: 84.35 µm, Y: 48.24 µm
Stage Position	X: 84.35 µm, Y: 48.24 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 51µm | 1.00AU / 59µm | 1.00AU / 43µm
LMS MBS	MBS 488/561 & 405/730
LMS SBS	SBS SP 505 | SBS SP 550 | Mirror
Laser Wavelength	488nm @0.2% | 561nm @0.4% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	1.00
Pixel Time	0.35µs
Frame Time	4.15s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 | 590 | 353
Emission Wavelength	517 | 618 | 465
Effective NA	1.4
Detection Wavelength	511 - 549 | 590 - 645 | 435 - 470
Imaging Device	GaAsP-Pmt3 | GaAsp-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.1 (3D, Auto) | Filter: 7.5 (3D, Auto) | Filter: 6.2 (3D, Auto)
Profile Definition	Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 31.6), Y (Min 0 and Max 38394)

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 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000063

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting end resection (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 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 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 45 of 88, Line Profile Panel B

Z-Stack = 88 Slices (2.61 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 534 X 28.17
Image Size (Scaled) = 37.61 µm X 28.17 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.35 µm, Y: 48.24 µm
Stage Position = X: 84.35 µm, Y: 48.24 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.35µs
Frame Time = 4.15s
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: 7.1 (3D, Auto)

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 59µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 561nm @0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.35µs
Frame Time = 4.15s
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 = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.35µs
Frame Time = 4.15s
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 - 470
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.2 (3D, Auto)

2- Dimensional View - Panel A

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Mouse IgG (H+L; subclasses 1, 2a, 2b, 3; Fcγ fragment-specific) is shown in red.

Image from Z-plane 45 of 88 and the Line Profile graphic showing the location where the measurement was taken.

Profile View Display - Panels B & C

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 31.6), Y (Min 0 and Max 38394)

This panel also measures NPC counterstained with the 594 relative fluorescence and defines the nuclear compartment in 2 dimensions.

Zen Acquisition Info Tab -

Shown is the acquisition of the 488 channel (53BP1 488 direct conjugate) using the 488 nm laser at 0.20%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

CCR CDx Confocal Assessment of 53BP1 DDR Recruitment

Panel A shows a representative confocal optical section (Z-plane 45 of 88) of the 53BP1 Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage. Etoposide induces replication-associated DNA lesions and double-strand breaks, activating the 53BP1-mediated DNA damage response pathway. Consistent with established DDR biology, 53BP1 is recruited to sites of chromatin damage, forming discrete nuclear foci associated with replication stress and double-strand break signaling.

Panel B shows line profile analysis of 53BP1 foci, demonstrating robust damage-dependent signal intensities (maxima of 36,566, 33,939, and 26,014 A.U.), exceeding corresponding NPC/Alexa Fluor™ 594 intensities. This confirms biologically driven nuclear enrichment relative to nuclear architecture, rather than acquisition- or amplification-driven signal artifacts.

Panel C shows Zeiss ZEN acquisition metadata demonstrate low excitation conditions (488 nm, 0.2% laser power), underscoring intrinsic signal robustness.

53BP1 localization and recruitment behavior are consistent with the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008), confirming conjugation equivalency and preservation of biological response. At confocal resolution, 53BP1 appears as punctate structures with large aggregates or foci, consistent with pathway engagement at sites of replication stress and DNA damage; however, the size and morphology of these assemblies strongly suggest an underlying substructure that remains unresolved at the confocal limit.

CCR CDx Step-Down Gate Summary:

This dataset confirms 53BP1 activation, nuclear recruitment, and damage-dependent enrichment, meeting CDx go/no-go criteria at the confocal stage. Definitive structural interpretation requires progression to higher-resolution modalities within the CCR step-down workflow.

Image Correction

NONE

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

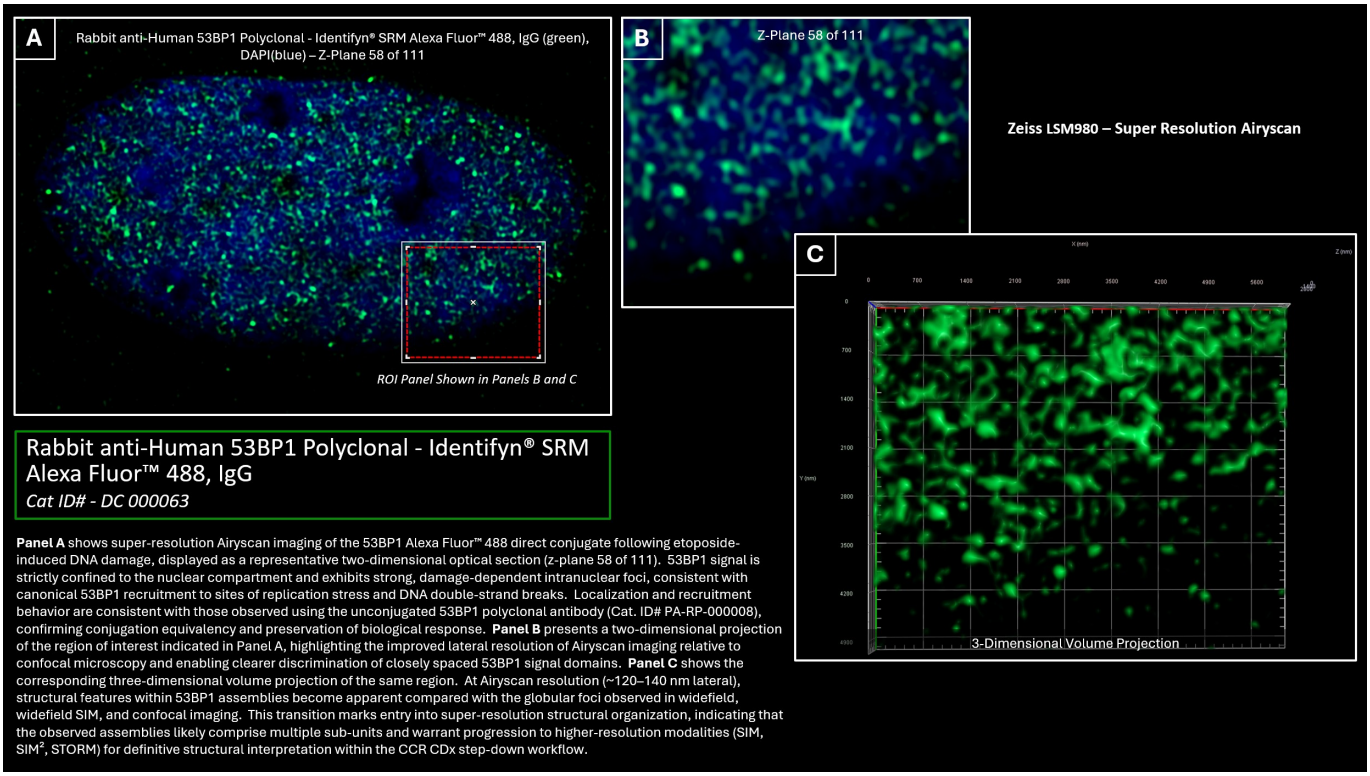
SOURCE PROVENANCE

Catalog	DC-000063
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	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	0E26CB04A0182C5A06105B86F42E744ACE32D1A0C755E7A7487AF0B1AE5EC53F
Method PDF SHA-256	C2767C121A948F334B345F12FC1412F501A2A42E5A76EFDD40918CF134918F08

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 750
METADATA VALUES	57

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	111 Slices (3.36 µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image size (Pixels)	908 X 602 | 173 X 144
Image Size (Scaled)	32.05 µm X 21.60 µm | 6.11 µm X 5.08 µm
Bit Depth	16 Bit
Image Center Position	X: 83.10 µm, Y: 46.95 µm
Stage Position	X: 83.10 µm, Y: 46.95 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 250µm | 5.00AU / 286µm | 5.00AU / 235µm
LMS MBS	MBS 488/561 & 405/730
LMS SBS	SBS SP 550 | SBS LP525 | SBS SP 505
Laser Wavelength	488nm @0.4% | 561nm @1.0% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66µs
Frame Time	7.82s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	493 | 590 | 353
Emission Wavelength	517 | 618 | 465
Effective NA	1.4
Detection Wavelength	499 - 548 | 573 - 627 | 420 - 480, 495 - 499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	Filter: 9.2 (3D, Auto) | Filter: 7.9 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000063

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting end resection (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 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 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Nuclear Pore Complexes (NPCs) counterstained with Identifyn® SRM Alexa Fluor™ 594, not shown in Data Analysis.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 58 of 111

Z-Stack = 111 Slices (3.36 µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image size (Pixels) = 908 X 602
Image Size (Scaled) = 32.05 µm X 21.60 µm
Bit Depth = 16 Bit
Image Center Position = X: 83.10 µm, Y: 46.95 µm
Stage Position = X: 83.10 µm, Y: 46.95 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, 2-Dimensional view at Z-plane 58 of 111, and Panel C, 3-Dimensional Volume Projection

Z-Stack = 111 Slices (3.36 µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image size (Pixels) = 173 X 144
Image Size (Scaled) = 6.11 µm X 5.08 µm
Bit Depth = 16 Bit
Image Center Position = X: 83.10 µm, Y: 46.95 µm
Stage Position = X: 83.10 µm, Y: 46.95 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = Filter: 9.2 (3D, Auto)

594 - (Not Shown in Data Analysis)

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 286µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS LP525
Laser Wavelength = 561nm @1.0%
Laser Blanking = Enabled
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 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: 9.2 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 235µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 420 - 480, 495 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = Filter: 7.9 (3D, Auto)

2- Dimensional View - Panels A and the Region of Interest Panel B

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, DAPI is shown in blue.

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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

CCR CDx Airyscan Super-Resolution Assessment of 53BP1 DDR Organization

Panel A shows super-resolution Airyscan imaging of the 53BP1 Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage, displayed as a representative two-dimensional optical section (Z-plane 58 of 111). 53BP1 signal is strictly confined to the nuclear compartment and exhibits strong, damage-dependent intranuclear foci, consistent with canonical 53BP1 recruitment to sites of replication stress and DNA double-strand breaks. Localization and recruitment behavior are consistent with those observed using the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008), confirming conjugation equivalency and preservation of biological response.

Panel B presents a two-dimensional projection of the region of interest indicated in Panel A, highlighting the improved lateral resolution of Airyscan imaging relative to confocal microscopy and enabling clearer discrimination of closely spaced 53BP1 signal domains.

Panel C shows the corresponding three-dimensional volume projection of the same region. At Airyscan resolution (~120-140 nm lateral), structural features within 53BP1 assemblies become apparent compared with the globular foci observed in widefield, widefield SIM, and confocal imaging. This transition marks entry into super-resolution structural organization, indicating that the observed assemblies likely comprise multiple sub-units and warrant progression to higher-resolution modalities (SIM, SIM², STORM) for definitive structural interpretation within the CCR CDx step-down workflow.

Image Correction

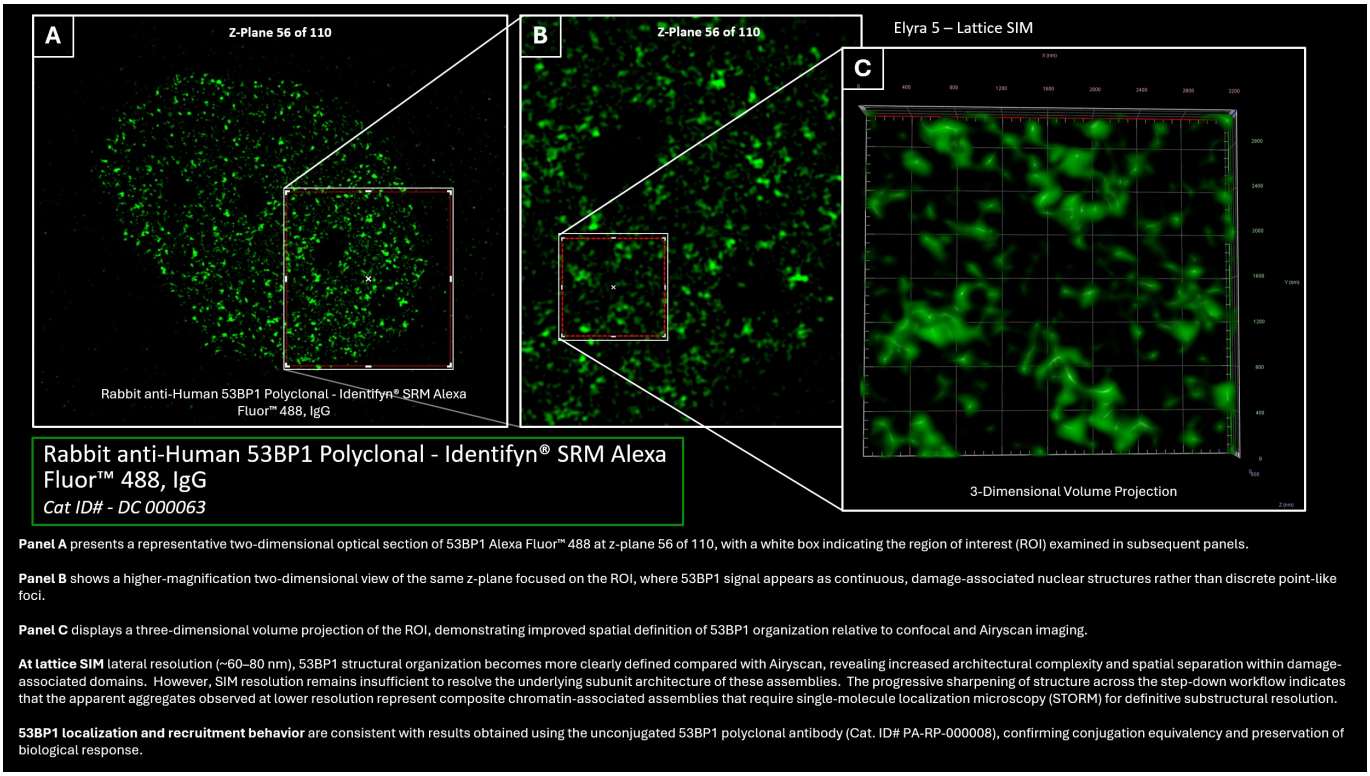
NONE

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

SOURCE PROVENANCE

Catalog	DC-000063
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	57
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	77A36D3CB0A6BE8FD50A464B4B5E713238AF72E2BB7F72999FE49F8CD374A79E
Method PDF SHA-256	567146F0236F3DE1B36B921F744B1A1D0DE1D5E1B70D6F95C635045C3DF16A9A

ORIGINAL IMAGE EVIDENCE / SIM



Panel A presents a representative two-dimensional optical section of 53BP1 Alexa Fluor™ 488 at z-plane 56 of 110, with a white box indicating the region of interest (ROI) examined in subsequent panels.

Panel B shows a higher-magnification two-dimensional view of the same z-plane focused on the ROI, where 53BP1 signal appears as continuous, damage-associated nuclear structures rather than discrete point-like foci.

Panel C displays a three-dimensional volume projection of the ROI, demonstrating improved spatial definition of 53BP1 organization relative to confocal and Airyscan imaging.

At lattice SIM lateral resolution (~60–80 nm), 53BP1 structural organization becomes more clearly defined compared with Airyscan, revealing increased architectural complexity and spatial separation within damage-associated domains. However, SIM resolution remains insufficient to resolve the underlying subunit architecture of these assemblies. The progressive sharpening of structure across the step-down workflow indicates that the apparent aggregates observed at lower resolution represent composite chromatin-associated assemblies that require single-molecule localization microscopy (STORM) for definitive substructural resolution.

53BP1 localization and recruitment behavior are consistent with results obtained using the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008), confirming conjugation equivalency and preservation of biological response.

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	64

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	110 Slices (3.27 µm)
Channels	3
Scaling (Per Pixel)	0.02110 µm X 0.02110 µm X 0.03000 µm
Image size (Pixels)	1809 X 1852 | 583 X 620 | 135 X 119
Image Size (Scaled)	38.19 µm X 39.10 µm | 12.31 µm X 13.09 µm | 2.85 µm X 2.51 µm
Bit Depth	16 Bit
Image Center Position	X: 63.85 µm, Y: 36.02 µm
Stage Position	X: 63.85 µm, Y: 36.02 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 561 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820 #2-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 561 | 405
Emission Wavelength	525 | 597 | 445
Effective NA	1.4
Detection Wavelength	503 - 546, 657 - 800 | 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 150 ms | 50 ms
Depth of Focus	0.81 µm | 0.92 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @1.00% | 561 nm @4.00% | 405 nm @2.0%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.2937 (488), 11.3045 (594), 10.0540 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2%, Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 25%, 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 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000063

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting end resection (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 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 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Nuclear Pore Complexes (NPCs) counterstained with Identifyn® SRM Alexa Fluor™ 594, not shown in Data Analysis.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 56 of 110

Z-Stack = 110 Slices (3.27 µm)

Channels = 3
Scaling (Per Pixel) = 21.10 nm X 21.10 nm X 30.00 nm
Image size (Pixels) = 1809 X 1852
Image Size (Scaled) = 38.19 µm X 39.10 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.85 µm, Y: 36.02 µm
Stage Position = X: 63.85 µm, Y: 36.02 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, 2-Dimensional view at Z-plane 56 of 110

Z-Stack = 110 Slices (3.27 µm)

Channels = 3
Scaling (Per Pixel) = 21.10 nm X 21.10 nm X 30.00 nm
Image size (Pixels) = 583 X 620
Image Size (Scaled) = 12.31 µm X 13.09 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.85 µm, Y: 36.02 µm
Stage Position = X: 63.85 µm, Y: 36.02 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C, 3-Dimensional Volume Projection

Z-Stack = 110 Slices (3.27 µm)

Channels = 3
Scaling (Per Pixel) = 21.10 nm X 21.10 nm X 30.00 nm
Image size (Pixels) = 135 X 119
Image Size (Scaled) = 2.85 µm X 2.51 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.85 µm, Y: 36.02 µm
Stage Position = X: 63.85 µm, Y: 36.02 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 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 @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

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 = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @4.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @2.0%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 11.2937 (488), 11.3045 (594), 10.0540 (DAPI)
Fitting = Fast Fit
Normalization = Auto

2- Dimensional View - Panels A and the Region of Interest Panel B

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green.

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 25%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

CDx Lattice Structured Illumination Microscopy (SIM) Assessment of 53BP1 DDR Structural Organization

Lattice structured illumination microscopy (SIM) of the 53BP1 Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage is shown across Panels A-C.

Panel A presents a representative two-dimensional optical section of 53BP1 Alexa Fluor™ 488 at Z-plane 56 of 110, with a white box indicating the region of interest (ROI) examined in subsequent panels. Panel B shows a higher-magnification two-dimensional view of the same Z-plane focused on the ROI, where 53BP1 signal appears as continuous, damage-associated nuclear structures rather than discrete point-like foci. Panel C displays a three-dimensional volume projection of the ROI, demonstrating improved spatial definition of 53BP1 organization relative to confocal and Airyscan imaging.

At lattice SIM lateral resolution (~60-80 nm), 53BP1 structural organization becomes more clearly defined compared with Airyscan, revealing increased architectural complexity and spatial separation within damage-associated domains. However, SIM resolution remains insufficient to resolve the underlying subunit architecture of these assemblies. The progressive sharpening of structure across the step-down workflow indicates that the apparent aggregates observed at lower resolution represent composite chromatin-associated assemblies that require single-molecule localization microscopy (STORM) for definitive substructural resolution.

53BP1 localization and recruitment behavior are consistent with results obtained using the unconjugated 53BP1 polyclonal antibody (Cat. ID# PA-RP-000008), confirming conjugation equivalency and preservation of biological response.

CCR CDx Step-Down Interpretation:

This dataset demonstrates resolution-dependent refinement of 53BP1 structural organization, satisfying CCR CDx enrichment and localization criteria at the SIM stage while justifying progression to STORM for final structural adjudication within the step-down workflow.

Image Correction

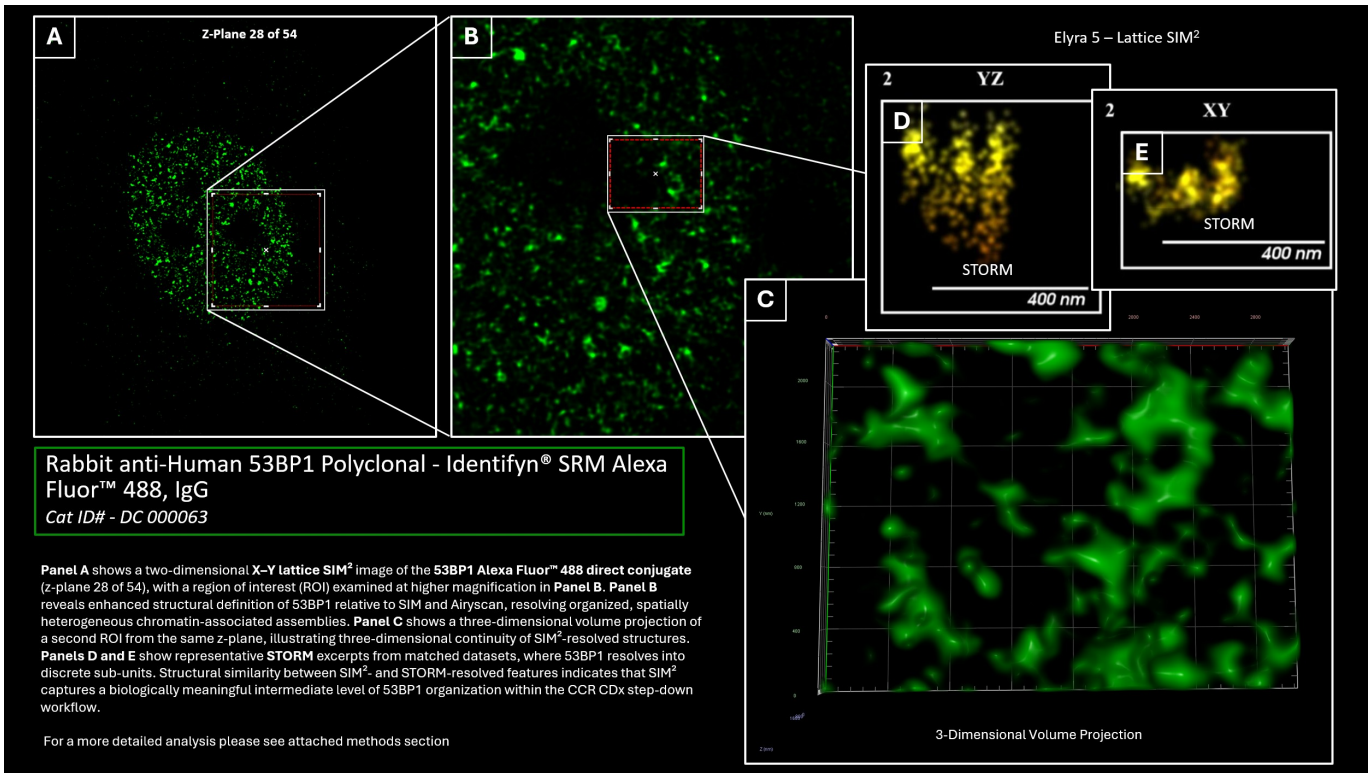
NONE

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

SOURCE PROVENANCE

Catalog	DC-000063
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	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	187402F5AFE4DD76F494A9F350D56C87FC34F79BF547B2D61A6A7813B39176CB
Method PDF SHA-256	ED320E4C10C5D43643E814B0F09990B0C3F4A8335FCE61CD2027BABB9B4A9376

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	64

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	54 Slices (1.59 µm)
Channels	3
Scaling (Per Pixel)	0.02110 µm X 0.02110 µm X 0.03000 µm
Image size (Pixels)	2455 X 2562 | 649 X 676 | 145 X 108
Image Size (Scaled)	51.83 µm X 54.09 µm | 13.70 µm X 14.27 µm | 3.06 µm X 2.28 µm
Bit Depth	16 Bit
Image Center Position	X: 62.07 µm, Y: 34.28 µm
Stage Position	X: 62.07 µm, Y: 34.28 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 561 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820 #2-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 561 | 405
Emission Wavelength	525 | 597 | 445
Effective NA	1.4
Detection Wavelength	503 - 546, 657 - 800 | 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 150 ms | 50 ms
Depth of Focus	0.81 µm | 0.92 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @1.00% | 561 nm @4.00% | 405 nm @2.0%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
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 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 25%, 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 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000063

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting end resection (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 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 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Nuclear Pore Complexes (NPCs) counterstained with Identifyn® SRM Alexa Fluor™ 594, not shown in Data Analysis.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 28 of 54

Z-Stack = 54 Slices (1.59 µm)

Channels = 3
Scaling (Per Pixel) = 21.10 nm X 21.10 nm X 30.00 nm
Image size (Pixels) = 2455 X 2562
Image Size (Scaled) = 51.83 µm X 54.09 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.07 µm, Y: 34.28 µm
Stage Position = X: 62.07 µm, Y: 34.28 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, 2-Dimensional view at Z-plane 28 of 54

Z-Stack = 54 Slices (1.59 µm)

Channels = 3
Scaling (Per Pixel) = 21.10 nm X 21.10 nm X 30.00 nm
Image size (Pixels) = 649 X 676
Image Size (Scaled) = 13.70 µm X 14.27 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.07 µm, Y: 34.28 µm
Stage Position = X: 62.07 µm, Y: 34.28 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C, 3-Dimensional Volume Projection

Z-Stack = 54 Slices (1.59 µm)

Channels = 3
Scaling (Per Pixel) = 21.10 nm X 21.10 nm X 30.00 nm
Image size (Pixels) = 145 X 108
Image Size (Scaled) = 3.06 µm X 2.28 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.07 µm, Y: 34.28 µm
Stage Position = X: 62.07 µm, Y: 34.28 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 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 @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

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 = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @4.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @2.0%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM2 Processing

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

2- Dimensional View - Panels A and the Region of Interest Panel B

Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green.

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 25%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panels D and E - STORM Images of the Rabbit anti-Human 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG

Summary

CCR CDx Lattice SIM² and STORM Assessment of 53BP1 Structural Organization

Panel A shows a two-dimensional X-Y lattice SIM² image of the 53BP1 Alexa Fluor™ 488 direct conjugate, acquired from Z-plane 28 of 54. A region of interest (ROI) is indicated and examined at higher magnification in Panel B, representing the same Z-plane.

Panel B displays a magnified SIM² view of the ROI from Panel A, revealing enhanced structural definition of 53BP1 assemblies relative to SIM and Airyscan. At SIM² resolution, 53BP1 no longer appears as a diffuse or globular signal, but instead exhibits organized, spatially heterogeneous structures, consistent with higher-order chromatin-associated assemblies formed during DNA damage response (DDR) signaling.

Panel C shows a three-dimensional volume projection of a second ROI from the same Z-plane, further illustrating the three-dimensional continuity and internal organization of 53BP1 structures resolved by SIM².

Panels D and E present representative single-molecule localization microscopy (STORM) excerpts of 53BP1 from matched datasets. At STORM resolution, 53BP1 assemblies resolve into discrete sub-units, revealing molecular-scale organization that underlies the larger structures observed at SIM². Comparison across modalities demonstrates clear structural similarity between SIM²-resolved features and STORM-resolved sub-units, indicating that SIM² captures an intermediate but biologically meaningful level of organization.

Resolution Context and Biological Interpretation

53BP1 is a large chromatin-associated protein (~213 kDa) that functions as part of multi-protein assemblies spanning tens to hundreds of nanometers at sites of DNA damage.

Airyscan (~120-140 nm lateral) begins to reveal non-random organization but cannot resolve internal sub-structure.

SIM (~60-80 nm) improves boundary definition and begins to separate composite domains.

SIM² (~40-60 nm) resolves reproducible internal structural features that correspond to underlying molecular organization.

STORM (~10-20 nm) resolves individual sub-units and protein clusters, providing definitive molecular architecture.

Across extensive step-down datasets, recurring structural motifs observed at STORM resolution are recognizable at SIM² and begin to emerge even at Airyscan, demonstrating continuity of biological organization across resolution scales rather than modality-specific artifacts.

CCR CDx Step-Down Interpretation

These data demonstrate progressive resolution-dependent refinement of 53BP1 structural organization, with SIM² providing a critical bridge between ensemble-level organization and single-molecule architecture. The observed continuity between SIM² and STORM supports the biological validity of the structures and satisfies CCR CDx step-down criteria, justifying STORM as the terminal modality for definitive structural adjudication of 53BP1 assemblies at sites of replication stress and DNA damage.

Image Correction

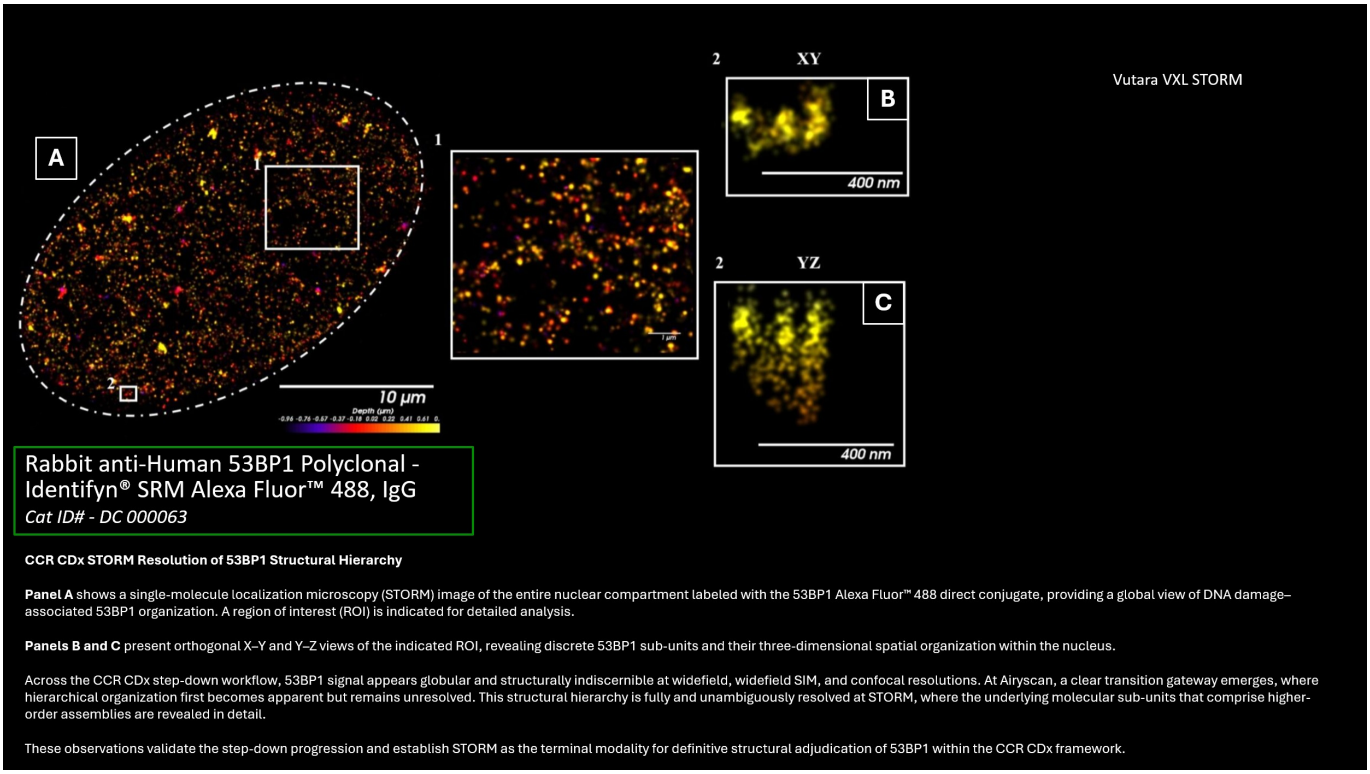
NONE

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

SOURCE PROVENANCE

Catalog	DC-000063
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	58A2028BCF0423784B5E4D6441FB027B1771110D4AFAF486031B07A292AD52DD
Method PDF SHA-256	BF88C9D887B658FEEE9D062357278B38A05CFA4F510AC022E9CA50FB72F98E94

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	488
METADATA VALUES	81

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 488nm (635nm Dichroic); First reference probe: 488nm (635nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	180.5µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 488nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 488nm (635nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 488nm" 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	50µm is selected
Piezo Record	Begin: 180.5; End: 180.5
SuperRes 488nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 20000
Z Positions	1 using Steps: 0.2µm (200nm)
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	10%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 7
Sizing Mode	Radial Precision
Particle size	4nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.02
Front-to-back Attenuation	Not Selected
Method	Particle (direct)

FIELD	SOURCE-DOCUMENTED VALUE
Time Intervals	10 Intervals
Pixel Size	30nm
Smoothing	8.00
PSF Z offset	-999 to +999
Radial precision	60
Axial precision	120
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1µm (1000nm)
Maximum Particle Count to Form Cluster	10
Computer Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Hull Alpha shape Radius	Not Selected
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 53BP1 Polyclonal - Identifyn® SRM Alexa Fluor™ 488
Cat ID# - DC 000063

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to sites of DNA damage. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.
Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends to prepare them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting the end resection process (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in the S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 6 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human 53BP1 Polyclonal-Identifyn® SRM Alexa Fluor™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5X in Identifyn's® proprietary Wash Buffer followed by the addition of Identifyn's® proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Microscope

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

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope configuration

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

Camera Configuration

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

Probe Configuration

Predefined Probe: First probe: 488nm (635nm Dichroic); First reference probe: 488nm (635nm Dichroic)
Photoactivation/Imaging Laser: 0% Selected
Exposure Time: 20ms Selected

Experiment Configuration

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

Stage and Piezo Selection

Examination of the Sample

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

Piezo Current: 180.5µm

Probe 1 Laser control 488 nm (635 nm Dichroic)

Widefield 488nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 488nm (635nm Dichroic): Not Selected

Advanced Tools Option

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

Widefield Laser: "Widefield 488nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

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

View Mode

SuperRes: 50µm is selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 180.5; End: 180.5

Probe 1 Laser control 488 nm (635 nm Dichroic)

SuperRes 488nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

Reference Probe 1 Laser control 488 nm (635 nm Dichroic)

Not Selected

Advanced Tools

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

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 20000
Z Positions: 1 using Steps: 0.2µm (200nm)
Cycles: 1
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 488 nm (640 nm Dichroic)

Laser Power: 10%

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 7

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: Radial Precision
Particle size: 4nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.02
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 10 Intervals
Pixel Size: 30nm
Smoothing: 8.00

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

PSF Z offset: -999 to +999
Radial precision:60
Axial precision:120

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: 0.1µm (1000nm)
Maximum Particle Count to Form Cluster: 10
Computer Hulls: Not Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Hull Alpha shape Radius: Not Selected
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

Summary

CCR CDx STORM Resolution of 53BP1 Structural Hierarchy

Panel A shows a single-molecule localization microscopy (STORM) image of the entire nuclear compartment labeled with the 53BP1 Alexa Fluor™ 488 direct conjugate, providing a global view of DNA damage-associated 53BP1 organization. A region of interest (ROI) is indicated for detailed analysis.

Panels B and C present orthogonal X-Y and Y-Z views of the indicated ROI, revealing discrete 53BP1 sub-units and their three-dimensional spatial organization within the nucleus.

Across the CCR CDx step-down workflow, 53BP1 signal appears globular and structurally indiscernible at widefield, widefield SIM, and confocal resolutions. At Airyscan, a clear transition gateway emerges, where hierarchical organization first becomes apparent but remains unresolved. This structural hierarchy is fully and unambiguously resolved at STORM, where the underlying molecular sub-units that comprise higher-order assemblies are revealed in detail.

These observations validate the step-down progression and establish STORM as the terminal modality for definitive structural adjudication of 53BP1 within the CCR CDx framework.

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

SOURCE PROVENANCE

Catalog	DC-000063
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	81
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	BD75974AF142F23F34952A75B44A0BC3F418AB5DE1AB2783F5D0C5674F5CA0F5
Method PDF SHA-256	63EE5E79BF75D330A872BB7825D2BECD25C7163E2A1A842074012634C5D94CF1