

**Identifyn Standards for Optical Microscopy Methods™**Identifyn® Primary Antibody**Rabbit anti-Human BAP1 Polyclonal, IgG***Cat ID# - PA-RP 000012***Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)***Cat ID# - PA-MM 000002*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000046***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

BAP1 (BRCA1-associated protein 1) is a deubiquitinase with critical roles in DNA repair, chromatin dynamics, and cell cycle regulation. Its ability to interact with other proteins central to the DNA damage response is significant in maintaining genomic stability. BAP1 has several identified protein-protein interactions within the HR pathway, which are critical to DNA Damage Response (DDR) signaling.

**BAP1 and BRCA1, BARD1, Rad51 and Others**

BRCA1 is a tumor suppressor gene critical for homologous recombination (HR), a high-fidelity DNA repair pathway for double-strand breaks. BAP1's interaction with BRCA1 and other DNA repair proteins, such as BARD1 and Rad51, is linked to its deubiquitinating activity, suggesting that it regulates critical protein function during DNA repair. This relationship may influence BRCA1's stability, recruitment to DNA damage sites, or ability to mediate HR. This interaction underlines the importance of BAP1 in promoting accurate DNA repair and maintaining genomic stability.

**BAP1 and ATM**

ATM (ataxia-telangiectasia mutated) is a central kinase in the DNA damage response, primarily activated by DNA double-strand breaks. It phosphorylates various downstream targets to initiate DNA repair pathways, including homologous recombination and non-homologous end joining (NHEJ). While the direct interaction between BAP1 and ATM isn't as well-documented as other interactions, BAP1 may indirectly influence ATM's activity by affecting the stability or function of proteins involved in DDR. Deubiquitination (BAP1) and phosphorylation (ATM) may well affect how cells respond to DNA damage.

**BAP1 and HERC2**

HERC2 is an E3 ubiquitin ligase involved in regulating protein ubiquitination. It has a known role in genomic stability and is implicated in the DNA damage response. BAP1's interaction with HERC2 likely involves deubiquitination, suggesting that BAP1 could regulate HERC2's activity or modulate its effects on other proteins in the DNA repair pathways. This interaction could be critical in balancing ubiquitination and deubiquitination, impacting DNA repair processes and cell cycle control.

**ASXL1/ASXL2**

ASXL1 and ASXL2 (additional sex combs-like 1 and 2) are other key interactors of BAP1. This complex can influence chromatin remodeling, potentially affecting the accessibility of DNA repair proteins to sites of DNA damage.

### Implications in DNA Repair

These interactions underscore BAP1's involvement in DNA repair pathways and the broader DNA damage response. By interacting with BRCA1, BAP1 may facilitate homologous recombination, while interactions with ATM and HERC2 highlight its role in broader DDR signaling. Critical interaction with ASXL1/ASXL2 highlights its importance in chromatin remodeling, potentially affecting the accessibility of DNA repair proteins to sites of DNA damage. BAP1's deubiquitinating activity can stabilize essential proteins, promote the proper assembly of repair complexes, and maintain chromatin structure, all of which are necessary for efficient DNA repair and genomic integrity. Mutations in BAP1 that disrupt these interactions can increase genomic instability and are associated with various cancers, underscoring the clinical relevance of these protein interactions for understanding disease mechanisms and developing therapeutic strategies.

### Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, DNA damage was induced by treatment with 2  $\mu$ M etoposide-spiked complete growth media for 24 hours, then fixed in -20 °C methanol and blocked with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human BAP1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI \(cat#P36962\)](#).

*H2Ax Acquired, however, not shown in Data Analysis - Focus on BAP1 Polyclonal Structural Details.*

### 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) -

**Z-Stack = 119 Slices (3.49  $\mu$ m)- 2D at Z-Plane 54 of 119, Panel A**

Channels = 2

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 1187 X 2157

Image Size (Scaled) = 38.63  $\mu\text{m}$  X 45.54  $\mu\text{m}$   
Bit Depth = 16 Bit  
Image Center Position = X: 61.24 mm, Y: 40.07 mm  
Stage Position = X: 61.24 mm, Y: 40.07 mm  
ROI Center Offset = X: 0.00  $\mu\text{m}$ , Y: 0.00  $\mu\text{m}$

**Z Stack - (3D) - Region of Interest Panels B and C**  
**Z-Stack = 119 Slices (3.49  $\mu\text{m}$ )**

Channels = 2  
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm  
Image Size (Pixels) = 387 X 352  
Image Size (Scaled) = 8.17  $\mu\text{m}$  X 7.43  $\mu\text{m}$   
Bit Depth = 16 Bit  
Image Center Position = X: 61.24 mm, Y: 40.07 mm  
Stage Position = X: 61.24 mm, Y: 40.07 mm  
ROI Center Offset = X: 0.00  $\mu\text{m}$ , Y: 0.00  $\mu\text{m}$

**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 = 120 ms  
Depth of Focus = 0.92  $\mu\text{m}$   
Binning Mode = 2,2  
Laser Wavelength = 561 nm @ 1.50%  
Frame Time = 1.33 s  
Scan Direction = Unidirectional  
Grating = 32.5  $\mu\text{m}$  grating

**488 - Acquired but not shown in Data Analysis.**

Reflectors = SR HE LBF 405/488/561/642  
Beam Splitter = 405, 488, 561, 642  
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642  
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
Contrast Method = Fluorescence  
Illumination Wavelength = 488  
Channel Name = T2-Axiocam 820-SR  
Excitation Wavelength = 488  
Emission Wavelength = 525

Effective NA = 1.4  
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
Imaging Device = AxioCam 820  
Camera Adapter = 1X  
Exposure Time = 120 ms  
Depth of Focus = 0.81  $\mu\text{m}$   
Binning Mode = 2,2  
Laser Wavelength = 488 nm @ 1.00%  
Frame Time = 1.59 s  
Scan Direction = Unidirectional  
Grating = 27.5  $\mu\text{m}$  grating

### **Post Processing - SIM Processing**

Processing Dimension = 3D  
Result Sampling = 4  
Process Sampling = N/A  
Channels = 2  
Algorithm = SIM  
SIM Settings = Standard  
Auto Sharpness = On  
Sharpness = 11.1529 (594), 11.2053 (488)  
Fitting = Fast Fit  
Normalization = Auto

### **2-Dimensional View- Panel A**

Shows Rabbit anti-Human BAP1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Rabbit, IgG (H + L), with a region of interest denoted by a white box. Image is at z-plane 54 of 119

### **3D Image Processing - Panel B**

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)

### **2-Dimensional View - Panel C**

Rabbit anti-Human BAP1 Polyclonal, IgG was visualized using Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Rabbit IgG (H + L). The native 594 fluorescence signal was pseudocolored white to improve structural contrast and facilitate visualization of sub-resolution architectural features within the selected region of interest shown in Panel B. The image corresponds to z-plane 54 of 119 from the original Lattice SIM acquisition and highlights focal BAP1-enriched nuclear repair-associated domains following etoposide-induced DNA damage.

### **STORM - Panel D**

STORM image shown from the same product data (BAP1), set to demonstrate the resolution improvements of Lattice SIM vs. Airyscan; however, it still lacks the ability to resolve the subunit structure seen in STORM.

## Summary

Panel A shows a single optical section (z-plane 54 of 119) from a Lattice SIM acquisition of Rabbit anti-Human BAP1 Polyclonal, IgG detected with the Identifyn® Goat anti-Rabbit 594 secondary antibody. The white box denotes the region of interest (ROI) examined further in Panels B and C. BAP1 demonstrates focal nuclear enrichment consistent with recruitment into DNA damage-associated repair domains following etoposide-induced damage.

Panel B shows a three-dimensional volume projection of the ROI. Yellow-circled regions begin to approximate broader structural morphologies observed in the corresponding STORM reconstruction shown in Panel D. Compared with confocal and Airyscan imaging, Lattice SIM provides improved volumetric resolution and enhanced separation of repair-associated signal domains; however, the signal remains partially conflated and below true single-molecule resolving capability.

Panel C shows the same optical section as Panel A, with the native 594 signal displayed in white for improved structural visualization. The yellow-circled region again demonstrates superficial similarity to structures observed in the STORM image shown in Panel D. These similarities suggest underlying repair-domain organization. Still, the structures should be interpreted cautiously, as unresolved subunit assemblies may contribute to the observed morphology.

Panel D shows STORM imaging of BAP1, revealing sub-diffraction structural organization not fully resolved by Lattice SIM. Together, these observations demonstrate that while Lattice SIM substantially improves the spatial interpretation of BAP1 recruitment domains, accurate interpretation of nanoscale repair architecture requires true single-molecule localization microscopy.

## Image Correction

NONE

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

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