

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P)***Cat ID# PA-RR 000014***Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)***Cat ID# - PA-MM 000002*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, DNA damage was induced by treatment with 2 μM Etoposide-spiked complete growth media for 20 hours, followed by -20°C methanol fixation and blocking with [Thermo Fisher Fish Serum Blocker cat#37527X3](#).

The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in 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 3X in 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 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI cat#P36962](#).

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 82 Slices (8.1 μm)

Channels = 3

Scaling (Per Pixel) = 0.143 μm X 0.143 μm X 0.100 μm

Image Size (Pixels) = 981 X 879

Image Size (Scaled) = 140.14 μm X 125.57 μm

Bit Depth = 14 Bit

Image Center Position = X: 57.29 mm, Y: 45.89 mm

Stage Position = X: 57.29 mm, Y: 45.89 mm

ROI Center Offset = X: 1.14 μm , Y: 0.00 μm

Z Stack - (3D) - Panel B, Wedge Cut, Region of Interest Panel A

Z-Stack = 82 Slices (8.1 μm)

Channels = 3

Scaling (Per Pixel) = 0.143 μm X 0.143 μm X 0.100 μm

Image Size (Pixels) = 274 X 300

Image Size (Scaled) = 39.14 μm X 42.86 μm

Bit Depth = 14 Bit

Image Center Position = X: 57.29 mm, Y: 45.89 mm

Stage Position = X: 57.29 mm, Y: 45.89 mm

ROI Center Offset = X: 1.14 μm , Y: 0.00 μm

647 -

Reflector = 50 Cy 5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = AxioCam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.30 μm
Binning Mode = 2,2

488 -

Reflector = He 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 @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = AxioCam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 μm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = AxioCam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.72 μm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

3D Image Processing - Panel A

3D Maximum Projection = Precise

Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

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

Clipping Image Process - Panel B

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P) within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

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

Position of Clip = -2%

Clip X = 0°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = 30°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = -30°

Clip Z = 0°

Panel B shows a wedge-cut view displayed from the top with a slight backward rotation, highlighting the region of wedge removal and revealing the full depth of signal across the z-stack. This orientation provides an alternative view of wedge geometry and confirms intranuclear localization of Phospho-BAP1 (Serine 592), as indicated by colocalization with H2Ax.

Summary

Widefield structured illumination microscopy (SIM) demonstrates coordinated intranuclear organization between phosphorylated BAP1 and H2Ax following etoposide-induced DNA damage. Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P) was visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified

Goat Anti-Rabbit, IgG (H + L). In contrast, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) was visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L). Maximum-intensity and wedge-cut volumetric projections reveal overlapping yet structurally complex intranuclear signal organization, with discrete punctate regions of apparent colocalization distributed throughout DNA damage-associated chromatin territories consistent with activation of the DNA damage response following etoposide exposure.

Although widefield SIM improves lateral resolution and volumetric interpretation relative to conventional widefield microscopy, substantial signal convolution remains present within dense nuclear repair domains. Nevertheless, orthogonal X-Y, X-Z, and Y-Z reconstructions demonstrate coordinated spatial distribution between phosphorylated BAP1 and H2Ax, supporting their functional association within DNA damage-associated chromatin remodeling and repair microenvironments.

Biologically, H2Ax is a core histone variant involved in chromatin organization and DNA damage response signaling within the nucleus. Following genotoxic stress, H2Ax distribution may become reorganized within damage-associated chromatin environments that support recruitment and coordination of DNA repair machinery. BAP1 is a tumor suppressor deubiquitinase involved in chromatin remodeling, transcriptional regulation, and the homologous recombination-associated DNA repair pathway. Phosphorylation of BAP1 following genotoxic stress has been associated with modulation of chromatin accessibility and recruitment into DNA damage-responsive nuclear microenvironments. The overlapping intranuclear architecture observed here is therefore consistent with coordinated spatial association between phosphorylated BAP1 and H2Ax within chromatin regions undergoing DNA damage response signaling following etoposide-induced genotoxic stress.

Image Correction

NONE

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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