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

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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 µM 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).

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) = 659 X 455
Image Size (Scaled) = 72.18 µm X 49.83 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

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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 @ 20.00%
Exposure Time = 200 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.96 µm
Binning Mode = 2,2

488 -
Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
Exposure Time = 200 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.80 µm
Binning Mode = 2,2

DAPI -
Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - *Panel A*

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The top-left panel shows Rabbit anti-Human BAP1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Rabbit, IgG (H+L). The top-right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Donkey anti-Mouse, IgG (H+L). The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Colocalization View - *Panel B*

Horizontal Axis - AF594, Threshold 225, Range Auto - BAP1
Vertical Axis - AF488, Threshold 104, Range Auto - H2AX
Image Measurement = Entire Field

Colocalization analysis of BAP1 (X-axis) and H2AX (Y-axis) was performed across the entire image acquisition.

Summary

Panel A shows Rabbit anti-Human BAP1 Polyclonal, IgG detected with the Identifyn® goat anti-rabbit 594 secondary in the upper left panel, Mouse anti-Human H2AX Monoclonal, IgG1 – (1H1D9/B7) counterstained with the Identifyn® donkey anti-mouse 488 secondary in the upper right panel, DAPI in the lower left, and the three-channel merge in the lower right. Following etoposide-induced DNA damage, BAP1 demonstrates marked nuclear recruitment and focal enrichment within H2AX-positive damage-associated regions.

Panel B shows the widefield colocalization analysis comparing BAP1/594 signal intensity on the X-axis against H2AX/488 intensity on the Y-axis, demonstrating strong nuclear colocalization between H2AX-marked DNA damage regions and recruited BAP1. The vertical skew observed within the distribution reflects the relative abundance and broader nuclear occupancy of H2AX compared with the more spatially restricted recruitment profile of BAP1 at damage-associated repair domains.

BAP1 is a tumor suppressor deubiquitinase involved in chromatin remodeling and DNA double-strand break repair, where it is recruited to sites of DNA damage and promotes homologous recombination-associated repair processes through regulation of H2A ubiquitination and repair-complex assembly. The observed focal overlap between H2AX and BAP1 is therefore consistent with BAP1 recruitment into DNA damage response microenvironments following etoposide-induced genomic stress.

Image Correction

NONE

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

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