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Identifying Protein-Protein Associations at the Nuclear Envelope with BioID

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Summary

The nuclear envelope (NE) is a critical cellular structure whose constituents and roles in a myriad of cellular processes seem ever expanding. To determine the underlying mechanisms by which these NE constituents participate in various cellular events, it is necessary to understand the nature of their protein-protein associations. BioID (proximity-dependent biotin identification) is a recently established method to generate a history of protein-protein association as they occur over time in living cells. BioID is based on fusion of a bait protein to a promiscuous biotin ligase. Expression of the BioID-fusion protein in a relevant cellular environment enables biotinylation of vicinal and interacting proteins of the bait protein, permitting isolation and identification by conventional biotin-affinity capture and mass-spec analysis. In this way, BioID provides unique capabilities to identify protein-protein associations at the NE. In this chapter we provide a detailed protocol for the application of BioID to the study of NE proteins.

Keywords

BioID; biotinylation; protein-protein interactions; nuclear envelope; nuclear lamina; nuclear pore complex

1. Introduction

The nuclear envelope (NE) is a highly organized organelle consisting of the inner nuclear membrane (INM) and outer nuclear membrane (ONM) separated by a lumen called the perinuclear space (PNS). The ONM and INM are connected to form annular junctions of high membrane curvature where large protein complexes, the nuclear pore complexes (NPCs), regulate nucleo-cytoplasmic trafficking. In Metazoa a protein meshwork called the nuclear lamina underlies the INM. Consisting of type-V intermediate filament proteins called lamins, the nuclear lamina serves as a structural framework for many NE-associated proteins as well as elements of chromatin. Enriched in the INM is a class of transmembrane proteins called NETs (nuclear envelope transmembrane proteins), retained at the NE in part

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²¹Magnetic beads may be resuspended in different solutions depending of the analysis method. At this point, the magnetic bead containing samples can be stored at -80°C

through associations with nuclear proteins. Spanning the NE is the LINC complex (linker of nucleoskeleton and cytoskeleton) that physically couples the cytoskeleton to the nucleus. This complex is fundamentally formed by interactions in the PNS lumen between INM SUN-domain proteins bound to nucleoskeletal proteins and ONM resident KASH-domain proteins that interact with elements of the cytoskeleton. An overview of key NE structures is depicted in Figure 1.

The NE plays important roles in various cellular mechanisms including nuclear positioning and migration (reviewed in [1]), chromatin organization (reviewed in [2]), transcriptional regulation (reviewed in [3]), cell signaling (reviewed in [4]), cytoskeleton organization (reviewed in [5]), and mechanotransduction (reviewed in [6]). Thus, perhaps not surprisingly, mutations in many constituents of the NE lead to a myriad of human diseases that impact a variety of cells and tissue (reviewed in [7]). Clearly, an important goal is to define the interaction of the various protein networks at the NE, which will help determine the mechanisms of human diseases-associated with the NE and shed light on the various functions of the NE in normal cellular functions.

There are currently two primary approaches widely used to identify protein-protein interactions (PPIs): yeast two-hybrid (Y2H, or protein complementation) and affinity-protein complex purification. Fundamentally, Y2H utilizes bait (a protein of interest) and prey (binding partners) each conjugated with a fragment of a functional protein. Upon direct binding of the bait and prey the fragments are joined together to create a functional protein that generates a selectable biological effect. Y2H is a powerful method for monitoring PPIs since it can be employed on a genome-wide screening and has potential to identify weak PPIs. However, Y2H frequently suffers from high false positive and false negative rates, perhaps in part because it often requires using fragments of proteins as bait and/or prey and screens them in an unnatural setting [8]. Protein complex purification relies on the solubilization of stable intact protein complexes to allow for their selective enrichment by affinity purification. However, this method needs to be optimized to balance the solubility and stability of the complexes. Transient and weak interactors can easily be lost by harsh lysis/wash conditions while false positive are increased with milder lysis/wash conditions. Given that NE proteins are frequently insoluble under more physiological conditions, often due to direct/indirect association with the lamina and/or membrane integration, it can be difficult to accomplish solubilization of meaningful protein complexes. To overcome the solubility issue of the NE proteins, chemical crosslinking has been employed to the protein complex purification approach [9]. However, the crosslinking approach intrinsically has a potential drawback to complicate interpretation of the identified candidates since they might just exist in a larger meshwork of protein associations. In addition, protein complex purification permits only a snapshot of PPIs at the time of lysis which can mask meaningful associations that are transient or of low frequency but nevertheless biologically relevant. Collectively, the limitations with existing approaches to study PPIs stimulated development of a new approach.

BioID (for proximity-dependent biotin identification) was developed to circumvent some of the limitations of current methods for PPI detection and provide a complementary approach [10]. Based on cellular expression of a protein of interest fused with a promiscuous bacterial

biotin ligase, BioID enables biotinylation of proximal proteins over time in living cells. Proteins biotinylated in this way can be selectively analyzed using conventional biotin affinity-capture methods followed by mass-spectrometry (Figure 1). In *E. coli*, biotin ligase (BirA) biotinylates acetyl-CoA carboxylase and regulates transcriptional activity of the biotin operon [11]. To this ends, BirA generates and delivers reactive biotin (biotinoyl-5'-AMP) to a specific substrate. BioID overcomes this specificity to generate a promiscuous biotinylation by utilizing a mutant BirA (R118G) that lacks DNA binding ability and has substantially decreased affinity to reactive biotin, leading to its release and ability to react with primary amines on proximate proteins [12,13]. Using a stable subcomplex of the NPC as a molecular ruler the practical biotinylation range of BioID appears to be ~10nm [14].

The BioID method provides practical advantages over traditional methods in PPI detection. First, BioID can overcome the solubility issue since it allows for biotin labeling to occur in living cells prior to cell lysis. Secondly, since the BioID fusion proteins can theoretically be expressed in any cells, PPIs can be screened in more relevant contexts, thus reducing false positives/negatives. In addition, since the biotin labeling occurs over time to generate a history of protein associations, BioID has the potential to detect weak PPIs and transient PPIs. However, BioID is not without limitations. Like other methods, the candidates identified by BioID need to be validated in order to determine the nature of their association. Since the BioID system is known to biotinylate primary amines of lysines, these post-translational modifications may impact the functions of both bait and candidates. Perhaps the most appreciated limitation of BioID is that this system relies on overexpression of BioID fusion proteins. Abnormally expressed fusion proteins may induce aberrant localization, something especially relevant for INM proteins that have nucleoplasmic-domain size constraints to permit proper targeting [15]. To improve the targeting ability of BioID, the Roux lab has developed a smaller variant of a promiscuous biotin ligase (manuscript in preparation).

Due to its unique capabilities, BioID is becoming a widely accepted method to identify PPIs. At the NE, BioID has been used for the nuclear lamina [10], NPC [14], isoforms of lamins (A, B1, B2, and C), and other INM proteins including LBR, emerin, TMPO, MAN1, SUN1, SUN2, and LAP1 (unpublished data, Kim and Roux) (Fig 1). BioID has also been applied to the study of PPIs at chromatin [16], cell junctions [17–22], centrosomes [23,24], Hippo signaling [25], HIV-host cell interactions [26], *Chlamydia psittaci*-host cell interactions [27], and in cell culture and mouse xenografted tumors to study c-Myc [28]. In addition to these mammalian applications, BioID has been used to study protein associations in *Trypanosoma brucei* [29] and *Toxoplasma gondii* [30]. Here we provide a detailed protocol for the application of BioID to the study of the NE.

2. Materials

All reagents should be prepared using autoclaved DNase/RNase free tubes while wearing gloves to minimize excess keratin contamination. It is also suggested that ultrapure water be used when preparing the various solutions. It is recommended to store reagents at room temperature (RT) unless otherwise specified.

2.1. Reagents

1. 20X biotin: 1mM biotin, bring final volume up with serum-free media, sterilize with 0.22 μ m filter, store at 4 °C for up to 8 weeks (see Note 1)
2. Lysis buffer: 50 mM Tris (pH 7.4), 500 mM NaCl, 0.2% SDS (w/v), store up to two weeks. Freshly add 1mM DTT and 1X combined protease inhibitor solution at the time of cell lysis (see Note 2).
3. Wash buffer 1: 2% SDS (w/v), store up to two weeks (see Note 2)
4. Wash buffer 2: 0.1% (w/v) deoxycholic acid, 1% (w/v) Triton X-100 (TX-100), 1 mM EDTA, 500 mM NaCl, 50 mM Hepes (pH 7.5), store up to two weeks (See Note 3)
5. Wash buffer 3: 250 mM LiCl, 10 mM Tris (pH 7.4), 1 mM EDTA, 0.5% (w/v) NP-40, 0.5% (w/v) deoxycholic acid, store up to two weeks (see Note 3)
6. Tris wash buffer: 50mM Tris (pH 7.4)
7. Ammonium bicarbonate (NH_4HCO_3): 50 mM ammonium biocarbonate, store at 4 °C for up to two weeks.
8. SDS-PAGE sample buffer: 50mM Tris-Cl (pH 6.8), 12% (w/v) sucrose, 2% (w/v) SDS, 20mM dithiothreitol (DTT), 0.004% bromophenol blue, make fresh
9. BSA blocking buffer: 1% (w/v) bovine serum albumin, fraction V, 0.2% (w/v) TX-100, bring final volume up with 1 X PBS, store at 4 °C for up to two weeks
10. ABS blocking buffer: 10% (w/v) adult bovine serum, 1% (v/v) TX-100, bring final volume with 1X PBS, store at 4 °C for up to two weeks
11. Quenching solution: 3% (v/v) hydrogen peroxide bring the final volume up with 1 X PBS.
12. PFA fixing buffer: 3% (w/v) paraformaldehyde (PFA), bring the final volume up with 1X PBS. Dissolve PFA while heating at 80°C in a fume hood. Once the buffer is cooled, add MgCl_2 and CaCl_2 (final 0.1mM each). Store at 4°C.

2.2. Equipment

1. Sonicator (Sonifier-250, Branson)
2. Centrifuge (Legend Micro 21R, Thermo Scientific)
3. Dynabeads® MyOne™ Streptavidin C1 (Life Technologies)
4. MagneSphere® Technology Magnetic Separation Stand (Promega)
5. SDS-PAGE Electrophoresis Unit, Mini-PROTEAN® II Electrophoresis Cell (Bio-Rad)

¹Use cell culture grade biotin. Dissolve biotin with pipetting or agitation.

²SDS often precipitates below 25°C. Warm the solutions at 37°C to dissolve SDS again.

³Deoxycholic acid precipitates under pH 7.1. Using a stock solution of Deoxycholic acid (10%) is recommended to make wash buffer 2 and 3. Deoxycholic acid must be protected from light.

6. Trans-Blot® Turbo™ Blotting System (Bio-Rad)

3. Methods

Unless otherwise noted, all steps should be conducted at RT unless otherwise specified. To minimize keratin contamination, wear gloves and use DNase/RNase free materials.

3.1. Generation of BioID fusion protein

1. It is critical not to disrupt any known targeting motifs and post-translational modifications by the fusion of BioID. If GFP has been fused to the bait (BioID is about the size of GFP) and shown not to perturb targeting and/or function then this inform BioID placement (see Note 4).
2. Cloning a BioID-fusion protein typically requires PCR. Using recombinational cloning techniques, PCR products can be inserted to the BioID vectors without restriction enzyme digestions. This technique provides more freedom to choose restriction enzymes in the vector.
3. The biotinylation range can be modulated by flexible linkers (e.g. BioID-flexible linker-bait). The length of the flexible linker may vary depending on the goals of the experiment. Short flexible linkers may also relieve any steric hindrance between the bait and BioID ligase.
4. If ectopic expression of the fusion protein needs to be inducibly regulated due to unwanted biological effects, consider using an inducible expression system. The BioID system itself is functional without inducible expression since biotinylation is regulated by the addition of excess biotin.

3.2. Validation of BioID fusion protein

- 1 Assess the expression of the BioID fusion protein and biotinylation of endogenous proteins using transient transfection. Since abnormal expression may lead to aberrant targeting or aggregation of the fusion protein, care must be taken to monitor the localization and biotinylation capability of the fusion protein. Often proper targeting may not become clear until a lower level of expression is obtained by stable expression.
- 2 Plate cells including non-transfected (or mock transfected) controls. Deliver the BioID constructs using Lipofectamine 2000 (Life Technologies) to the cells. Apply biotin to a final concentration of 50 μ M (1X) at the time of transfection. If transfection protocol requires media change, add 1X biotin at the time of media change.
- 4 After overnight incubation, analyze the cells with immunofluorescence (IF) and Western blot (WB) (Figure 2) (see Note 5)

⁴The mammalian expression systems for N-terminal and C-terminal BioID are available at Addgene https://www.addgene.org/Kyle_Roux/.

- 5 For IF, fix the cells for 10 min using 3% PFA fixing buffer followed by permeabilization with 0.4% TX-100 for 15 min. Label the fusion protein with appropriate primary and secondary antibodies. Detect biotinylated proteins using fluorescently labeled streptavidin (Streptavidin–Alexa Fluor). To label the nucleus, add fluorescent DNA binding reagents (see Note 6 and 7).
- 6 For WB, wash the cells briefly with PBS to remove BSA in the culture media. Lyse the cells using SDS sample buffer (200 μ L/ 1×10^6 cells). Boil the samples at 95 °C for 5 min and subsequently sonicate the samples to shear the genomic DNA. Perform SDS-PAGE and transfer to nitrocellulose membranes. After transfer, incubate the membrane with BSA blocking buffer for 20 min while agitating the membrane. Incubate the membrane with streptavidin-HRP for 40 min. Wash membrane with PBS twice for 5 min each and with ABS blocking buffer once for 5 min. Visualize the biotinylated proteins using enhanced chemiluminescence (ECL) reagents (see Note 8).
- 7 Quench the HRP activity to permit further probing since the streptavidin-biotin interaction is not easily perturbed by stripping. Apply the quench buffer to the membrane and agitate for 20 min. Wash the membrane three times with 1X PBS for 5 min each. Consider reapplication of ECL to the membrane and visualization to confirm success of quenching. It is recommended to incubate the membrane with the ABS blocking buffer for 20 min (see Note 9).
- 8 Apply appropriate primary and secondary antibodies to detect the fusion protein. Visualize the fusion proteins using ECL reagents (see Note 7).

3.3. Stable cell line generation

- 1 Stable cell line generation can begin with viral transduction or transient transfection, dependent on cell type and expression vector.
- 3 Carefully monitor the expression level of the fusion proteins. It is highly recommended to utilize cells expressing minimal levels of the fusion protein to foster physiologically relevant associations (see Note 10).
- 4 Generate a cell line expressing BioID (enzyme only) for control.

⁵Biotinylation generally reaches its maximum level approximately 16 hr post incubation with 1X biotin solution. However, biotinylation can be observed after 2 hr of incubation at a considerably reduced level. Biotin incubation time may vary depending on the purpose of experiment, abundance of the fusion protein and nature of protein interactions.

⁶Methanol fixation increases mitochondria background with fluorescently labeled streptavidin, likely by exposing the naturally biotinylated mitochondrial carboxylases.

⁷The N-terminal BioID vector was designed with a myc-tag (myc-BioID) while the C-terminal one has a HA-tag (BioID-HA). Tagging peptides can be re-engineered if necessary. Fusion proteins can also be detected using a commercially available BirA antibody (Abcam, 1:2000 for IF and WB).

⁸A brief wash with high salt (300 mM in 1X PBS) after ABS wash can reduce background.

⁹Since the quenching solution inactivates the HRP activity, it is important to remove the quenching solutions completely. The residual quenching solution might impair the sensitivity of HRP-conjugated antibodies in further probings.

¹⁰It is recommended to check the functionality of the fusion protein if necessary (e.g. knockdown, targeting, biotinylation, etc.).

3.4. BioID pull-down

- 1 This protocol is optimized for 4×10^6 plates of adherent cells (approximately 4×10^7 cells). Modification might be needed depending on cell types.
- 2 Begin with 4×10^6 plates of stable cells expressing BioID fusion protein. In parallel, set up control cells expressing BioID (enzyme only) with the same condition (see Note 11).
- 3 Once these cells reach 80% confluence, exchange the media with fresh media containing 1X biotin.
- 4 Incubate the cells for 16–18 hr in 1X biotin (see Note 5).
- 5 Before starting lysis make sure all the reagents and equipment are set up.
- 6 Remove media from cells by aspiration and rinse the cells in each dish twice with 3 ml of fresh autoclaved 1X PBS. Rock cells back and forth with 1X PBS prior to aspiration. This step ensures that any excess biotin is removed from cells. Residual free biotin binds streptavidin magnetic beads, impairing the efficiency of the BioID pull-down. Remove 1X PBS completely prior to lysis (see Note 12).
- 7 Apply 540 μ L of lysis buffer to each plate of the cells.
- 8 Collect the cells by scraping the bottom of the dish and transfer the cell lysate into a 15 ml conical tube.
- 9 Add 240 μ L of 20% TX-100 and mix briefly by vortex (see Note 13).
- 10 Immediately put the 15 ml conical tube on ice and keep there until step 15.
- 11 Sonicate the sample for 1 min at 30% duty cycle and an output level of 4. Make sure that the sonicator's probe tip is just above the bottom of the 15 ml conical tube and centered.
- 12 Rinse the sonicator probe with deionized water and repeat step 11. Check the sample after sonication. If the sample is still cloudy, sonicate the sample until the sample is mostly clear.
- 13 Once the sample is clear, add 2.4 mL of 4 °C 50 mM Tris-HCl (pH 7.4) (see Note 14).
- 14 Sonicate once with the same conditions as step 11 and evenly aliquot the lysate into 3 prechilled 2 mL microcentrifuge tubes (approximately 1.8 mL per tube).
- 15 Spin the samples at $16,500 \times g$ for 10 min at 4 °C.
- 16 During the centrifugation, place 15 mL conical tubes on ice to prechill.

¹¹This protocol is optimized for 4×10^7 cells per pull-down. Cell numbers can be scaled depending on the purpose and the resolution of pull-down; however, the buffer volumes should also be scaled accordingly.

¹²Residual PBS will increase the volume of pull-down. Lean the 10 cm plate (or the container of cells) on its edge so the PBS congregates at the bottom. Then it can be aspirated out, efficiently removing all excess biotin containing PBS.

¹³Adding a five-fold excess of Triton X-100 dilutes out the SDS and prevents its precipitation at 4°C.

¹⁴Dilution with 50 mM Tris (pH 7.4) will provide more favorable conditions for biotin-capture.

- 17 Once the centrifugation is finished, transfer and pool together the supernatant to a 15 mL conical tube prepared in step 16. Do not disturb any insoluble pellet (if present) at the tube wall when transferring the supernatant (Note 15).
- 18 In parallel, prepare the magnetic beads for capture of biotinylated proteins. Place 1.5 mL microcentrifuge tubes to a magnetic stand and add 500 μ L of lysis buffer and 500 μ L of 50 mM Tris-HCl (pH 7.4).
- 19 Agitate Dynabeads stock. Once evenly distributed in the bottle, take 300 μ L of the magnetic beads and transfer to a 1.5 mL microcentrifuge tube set up at step 18. Wait for 3 min (see Note 16).
- 20 Remove supernatant from the 1.5mL microcentrifuge tubes prepared at step 19. Avoid disturbing the magnetic beads on the tube wall. Take 1 mL of lysate from a 15 mL conical tube from step 17 and resuspend the magnetic beads. Transfer the resuspended the magnetic beads to its original 15ml conical tube (see Note 17 and 18).
- 21 Recap the 15 mL conical tube, and wrap the top with parafilm.
- 22 Incubate the magnetic beads on rotator overnight (~18 hr) at 4 °C (see Note 19).
- 23 After incubation with the magnetic beads, place the 15 mL conical tubes onto magnetic stand and allow the magnetic beads to be collected on side of tube for 3 min.
- 24 Remove supernatant, paying close attention to not disturb the magnetic beads on the side of the 15 mL conical tube.
- 25 Resuspend the magnetic beads in 1 mL of wash buffer 1 and transfer from 15 mL conical to 1.5 mL microcentrifuge tube.
- 26 Wash the samples on a rotator at RT for 8 min.
- 27 Place tubes on magnetic stand for 3 min and remove supernatant. Try not to disturb the magnetic beads at the tube wall (see Note 20).
- 28 Add wash buffer 1 and resuspend the magnetic beads with gentle pipetting.
- 28 Repeat step 26–27.
- 29 Remove wash buffer 1. Resuspend the magnetic beads with 1 mL of wash buffer 2.
- 30 Repeat step 26–27.

¹⁵This step is to remove azide from the Dynabeads storage solution and to equilibrate the magnetic beads with the condition of affinity capture.

¹⁶After centrifugation, pool the supernatants together. The combined lysate can be quickly frozen with liquid nitrogen and stored at –80°C if one wishes to capture biotinylated peptides at a later date.

¹⁷It is important to collect the magnetic beads on the magnetic stand yet watch to ensure that the magnetic beads are completely submerged to keep the magnetic beads and associated proteins hydrated.

¹⁸It is important to do this step quickly to avoid drying the magnetic beads and potentially hindering their ability to bind biotinylated peptides.

¹⁹Incubation time may vary depending on the purpose of experiment. Shorter incubation times yield fewer candidates.

²⁰It is recommended to use a microcentrifuge tube opener to minimize keratin contamination.

- 31 Remove wash buffer 2. Resuspend the magnetic beads with 1 mL of wash buffer 3.
- 32 Repeat step 26–27.
- 33 Remove wash buffer 3. Resuspend with 1 mL of 50 mM Tris-HCl (pH 7.4).
- 35 During this step, take 100 μ L (10%) of the resuspended mixture and transfer it to a newly labeled 1.5 mL microcentrifuge tube. This will be reserved for WB analysis to monitor success of pull-down.
- 36 Spin tubes including 10% samples at $2,000 \times g$ for 5 min.
- 37 Remove supernatant from the samples for mass-spec analysis. Resuspend the magnetic beads with 150 μ L of ammonium bicarbonate buffer. Quickly flash-freeze the samples in liquid nitrogen and store at -80°C (see Note 22).
- 38 Remove supernatant from the 10% samples. Resuspend the magnetic beads with 100 μ L of fresh sample buffer and boil at 95°C for 5 min.
- 39 Perform WB (see 3.2) on the 10% samples to ensure that proper biotinylation and fusion proteins are present before sending samples off for mass-spectrometry analysis (see Note 23).

3.5. Mass spec analysis of BioID candidates

1. This protocol is optimized for on-bead digestion followed by 1D-LC MS/MS [10,14] (see Note 24).
2. There are five recognized abundant naturally biotinylated proteins in eukaryotes: pyruvate carboxylase (PC), methylcrotonoyl-CoA carboxylase subunit alpha (MCC1), acetyl-CoA carboxylase 1 (ACACA), acetyl-CoA carboxylase 1 (ACACB), propionyl-CoA carboxylase alpha chain (PCCA).
3. There are some commonly identified background proteins from BioID. The use of a BioID-only control will permit their identification (see Note 24).

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²²Spinning the sample separates the magnetic beads and SDS sample buffer. Do not disturb the magnetic beads when removing supernatant.

²³Candidates can be identified by MS using proteins separated by SDS-PAGE. However, it is recommended to utilize on-bead digestion to bypass the difficulty of releasing biotinylated proteins from the biotin-capturing molecule coated materials. The on-bead digestion approach can also minimize keratin contamination introduced during SDS-PAGE.

²⁴It is noted that number and abundance of candidates identified by BioID do not imply the biological relevance. The candidates need to be validated.

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Figure 1.

Overall scheme of the BioID method at the NE. The status of BioID fusion proteins and biotinylated proteins is depicted on the left. Experimental procedures are depicted on the right. The NE proteins that have been analyzed by BioID are indicated with BioID fusion (red). Nup107-160 complex at the NPC is colored in red. (A) Generate expression BioID vector. (B) Stably express BioID fusion in cells. (C) Induce biotinylation (pink circle) by adding 1X biotin. (D) Lyse stable cells. At this point, cell lysate contains mixture of biotinylated proteins and non-biotinylated proteins. (E) Isolate biotinylated proteins using biotin-affinity capture. (F) Identify isolated proteins using mass-spectrometry.

Figure 2.

Validation of BioID fusion proteins. HEK293 cells stably expressing myc-BioID fused with the NPC constituents Nup107, Nup133, and Nup160 were analyzed using WB and IF. (A) Following SDS-PAGE, biotinylated proteins were probed with Streptavidin-HRP (top). The same blot was quenched and subsequently probed with anti-BioID antibody (bottom). Compared to the few naturally biotinylated proteins in the cells that do not express BioID proteins (parental), extensive biotinylation was observed from the lysate of cells expressing BioID-fusion protein. (B) Biotinylation and localization of the BioID fusion proteins were monitored by confocal microscopy. Biotinylated proteins were labeled with streptavidin-Alexa Fluor 488 (green, top). In parallel, the BioID fusion proteins were detected with anti-myc antibody (green, bottom). Biotinylated proteins and the fusion protein colocalizes with the NPC, labeled with anti-Nup153 antibody (red). DNA was detected with Hoechst dye (blue). Scale bar is 10 μ m.