bio-protocol

Published: Sep 20, 2022

In situ Dephosphorylation Assay with Recombinant Nil Phosphatase

Nilay Nandi[1, #], Charles Tracy[1, #] and Helmut Krämer[1, 2, *, #]

[1]Department of Neuroscience, UT Southwestern Medical Center, Dallas, USA
[2]Department of Cell Biology, UT Southwestern Medical Center, Dallas, USA
*For correspondence: helmut.kramer@utsouthwestern.edu
[#]Contributed equally to this work

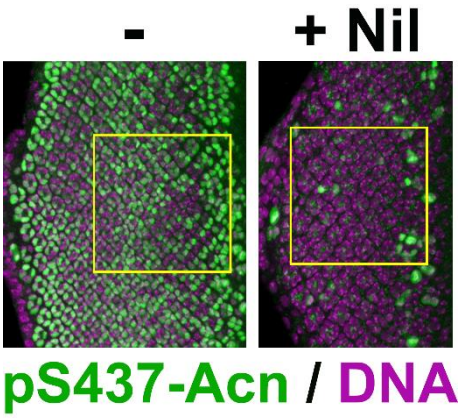
Abstract

The activity of numerous autophagy-related proteins depends on their phosphorylation status, which places importance on understanding the responsible kinases and phosphatases. Great progress has been made in identifying kinases regulating autophagy, but much less is known about the phosphatases counteracting their function. Genetic screens and modern proteomic approaches provide powerful tools to identify candidate phosphatases, but further experiments are required to assign direct roles for candidates. We have devised a novel protocol to test the role of purified phosphatases in dephosphorylating specific targets *in situ*. This approach has the potential to visualize context-specific differences in target dephosphorylation that are not easily detected by lysate-based approaches such as Western blots.

Keywords: Autophagy, Acinus, Nilkantha, Phosphorylation, Metal-dependent phosphatases, Cdk5, *Drosophila*

This protocol was validated in: eLife (2022), DOI: 10.7554/eLife.72169

Graphical abstract:



Background

Tight regulation of autophagy is important as both insufficient and excessive autophagic flux can doom individual cells and shorten organismal life span (Doherty and Baehrecke, 2018; Levine and Kroemer, 2019). This is well illustrated by the function of acinus, an upstream regulator of the Atg1 master regulator of autophagy (Tyra et al., 2020). In *Drosophila*, excessive acinus levels cause autophagy-dependent death (Haberman et al., 2010), whereas Cdk5-dependent phosphorylation and the resulting activation of acinus function contributes to the extension of life span by promoting basal autophagy (Nandi et al., 2017). A genetic screen identifying the phosphatase balancing Cdk5-mediated acinus activation pointed to the Nilkantha (Nil) phosphatase (Nandi et al., 2022), a functional homolog to mammalian metal-dependent PPM1A/B phosphatases (Kamada et al., 2020). In vitro phosphorylated and purified proteins or peptides have classically been used for testing the activity of phosphatases on specific substrates (e.g., Berndsen et al., 2019). This class of phosphatases poses challenges for the in vitro assessment of their activity towards specific targets. Their required N-terminal myristoylation (Chida et al., 2013) precludes simple bacterial expression. Furthermore, metal-dependent PPM-type phosphatases interact with substrates at sites distinct from the phosphorylated residue (Kamada et al., 2020). This constitutes a challenge for testing their activity on multiprotein complexes, such as the ASAP complex that contains the RNPS1 and SAP18 subunits in addition to acinus, and that has proven difficult to purify or reconstitute (Murachelli et al., 2012). Therefore, it may be important to present subunits of multiprotein complexes, such as acinus, in the appropriate functional context to assess their dephosphorylation. To address these issues, we describe here our protocol to purify *Drosophila* Nil and human PPM1B expressed in *Drosophila* S2 cells and use these phosphatases to assess dephosphorylation of endogenous acinus protein in its endogenous context in fixed and permeabilized tissue samples (Nandi et al., 2022). We expect that this protocol can be easily adapted to other phosphatase/substrate combinations, if phospho-specific antibodies are available for the site to be dephosphorylated.

Materials and Reagents

1. 500 mL vacuum filter/storage bottle (Corning, catalog number: 430769)
2. 25 cm^2 flask (Corning, catalog number: 430639)
3. Six-well plate (Falcon, catalog number: 353046)
4. 96-well assay plate (Costar, catalog number: 2595)
5. 1.5 mL microfuge tube (Eppendorf, catalog number: 0030119487)
6. 15 mL conical centrifuge tube (Thermo Scientific, catalog number: 339651)
7. Micro slides, single frosted, precleaned 75 × 25 mm (Corning, catalog number: 2948-75X25)

8. Cover glass, thickness 1.5, 22 × 40 mm (Corning, catalog number: 2980-224)
9. S2 cells [*Drosophila* Genomics Resource Center (DGRC): Stock Number 6]
10. Schneider's *Drosophila* medium (Gibco, catalog number: 21720)
11. Fetal bovine serum (FBS) (Sigma, catalog number: F4135)
12. TransIT-2020 (Mirus, catalog number: MIR 5400)
13. Nil expression plasmid: pPuro-MT-Nil-Twin-streptag (Nandi et al., 2022)
14. Nil^{D231N} mutant expression plasmid: pPuro-MT-Nil^{D231N}-Twin-streptag (Nandi et al., 2022)
15. Cupric sulfate pentahydrate (MP Biomedicals, catalog number: 0219511783)
16. NaCl (Fisher Scientific, catalog number: BP358-1)
17. KCl (Fisher Scientific, catalog number: BP366-500)
18. Na_2HPO_4 (Sigma-Aldrich, catalog number: S5136100G)
19. KH_2PO_4 (Sigma-Aldrich, catalog number: P5655100G)
20. HCl (Fisher Scientific, catalog number: A481-212)
21. Tris base (Fisher Scientific, catalog number: BP152-5)
22. NP40 (Accurate Chemical, catalog number: A56009)
23. Phenylmethylsulfonyl fluoride (PMSF) (Thermo Scientific, catalog number: 36978)
24. Protease inhibitor (Roche, catalog number: 05892970001)
25. Phosphatase inhibitor (Roche, catalog number: 04906837001)
26. MagStrep "type3" XT beads (IBA, catalog number: 2-4090-002)
27. Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid (EGTA) (RPI, catalog number: E57060-250.0)
28. Sodium acetate, anhydrous (Thermo Scientific, catalog number: A131840B)
29. Dithiothreitol (DTT) (RPI, catalog number: D11000-25.0)
30. 1 M manganese chloride ($MnCl_2$) (RPI, catalog number: M20100-50.0)
31. 1 M magnesium chloride ($MgCl_2$) (Thermo Scientific, catalog number: J61014EQE)
32. Puromycin dihydrochloride (MP Biomedicals, catalog number: 0219453980)
33. Paraformaldehyde (Electron Microscopy Sciences, catalog number: 19208)
34. NaOH, 50% w/w (Thermo Scientific, catalog number: 33382A1)
35. Sodium phosphate monobasic monohydrate (Sigma-Aldrich, catalog number: 71507-250g)
36. Sodium phosphate (Sigma-Aldrich, catalog number: 342483-500g)
37. L-Lysine (Sigma-Aldrich, catalog number: L-5626)
38. di-sodium hydrogen phosphate anhydrous (Fluka, catalog number: 71636)
39. Sodium m-periodate (Sigma, catalog number: S-1147)
40. Halt™ protease inhibitor single-use cocktail (100×) (Thermo Scientific, catalog number: 1860932)
41. Vectashield mounting media with fluorescence with DAPI (Vector Laboratories, catalog number: H-1200)
42. Nail polish, advanced hard (Sally Hansen, catalog number: 45121)
43. Normal goat serum (MP Biomedicals, catalog number: 191356).
44. Rabbit anti-pS437-Acn (1:1000) (Nandi et al., 2017). Long-term storage of aliquots at -80 °C; aliquots in use stored at 4 °C
45. Goat anti-Rabbit IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 488 (Invitrogen, catalog number: A11088). Store at 4 °C
46. SYLGARD 184 (Sigma, catalog number: 761036)
47. Magnesium chloride hexahydrate (Fisher Chemical, catalog number: M33-500)
48. Cadmium chloride (Fisher Chemical, catalog number: C10-500)
49. Saponin from quillaja bark (Sigma, catalog number: S7900-100G)
50. Parafilm (Thermo Scientific, catalog number: S37440)
51. 2× Laemmli sample buffer (Bio-Rad, catalog number: 1610737)
52. Bovine serum albumin (BSA) (RPI, catalog number: 9048-46-8)
53. 10% protein gel (Bio-Rad, catalog number: 4568035)
54. Nitrocellulose blotting membrane (Cytiva, catalog number: 10600003)
55. Blotting system (Bio-Rad, catalog number: 1703930)
56. Total protein stain (LiCor, catalog number: 926-11015)

57. S2 cell media (see Recipes)
58. 1× PBS (see Recipes)
59. Puromycin dihydrochloride (10 mg/mL) (see Recipes)
60. 0.7 M $CuSO_4$ (see Recipes)
61. Lysis buffer (see Recipes)
62. 2× stock phosphatase assay buffer (see Recipes)
63. Biotin elution buffer (see Recipes)
64. 8% paraformaldehyde (see Recipes)
65. 0.4 M Sorenson's phosphate buffer stock (see Recipes)
66. 0.1 M di-sodium hydrogen phosphate buffer (see Recipes)
67. Lysine stock (see Recipes)
68. Periodate-lysine-paraformaldehyde fixative (see Recipes)
69. 10% saponin (see Recipes)
70. 1 M DTT (see Recipes)
71. Phosphatase assay buffer (see Recipes)

Equipment

1. Electrophoresis chamber (Bio-Rad, catalog number: 1658004)
2. Incubator set at 37 °C (Thermo Scientific, Heratherm IGS60, catalog number: 51028063)
3. Biosafety cabinet (The Baker Company, model: SG403)
4. Incubator set at 25 °C (Shel Lab, model: SRI3)
5. Countess™ automated cell counter (Invitrogen, model: AMQAX2000)
6. Inverted microscope for cell culture (Nikon, TMS: 213340)
7. Microfuge tube rotator (Thermo Scientific, catalog number: 88881001)
8. Magnetic microfuge tube rack (Thermo Scientific, catalog number: MR02)
9. Western blot scanner (Li-Cor, Odyssey: 9120)
10. Leica L2 microscope (Leica Microsystems)
11. Orbital shaker complete (Bellco Biotechnology; SKU: 7744-01010)
12. Fine forceps #5 (Fine Science Tools, catalog number: 11254-20)
13. Fine forceps #55 (Fine Science Tools, catalog number: 11255-20)
14. LSM 710 confocal microscope (Zeiss), with a 63× NA 1.4 objective

Software

1. Image Studio (Li-Cor, https://www.licor.com/bio/image-studio/)
2. ImageJ (NIH, https://imagej.nih.gov/ij/)
3. Adobe Photoshop (Adobe, https://www.adobe.com/products/photoshop.htmL)
4. Zen (black edition) (Zeiss, https://www.micro-shop.zeiss.com/en/us/softwarefinder/software-categories/zen-black/)

Procedure

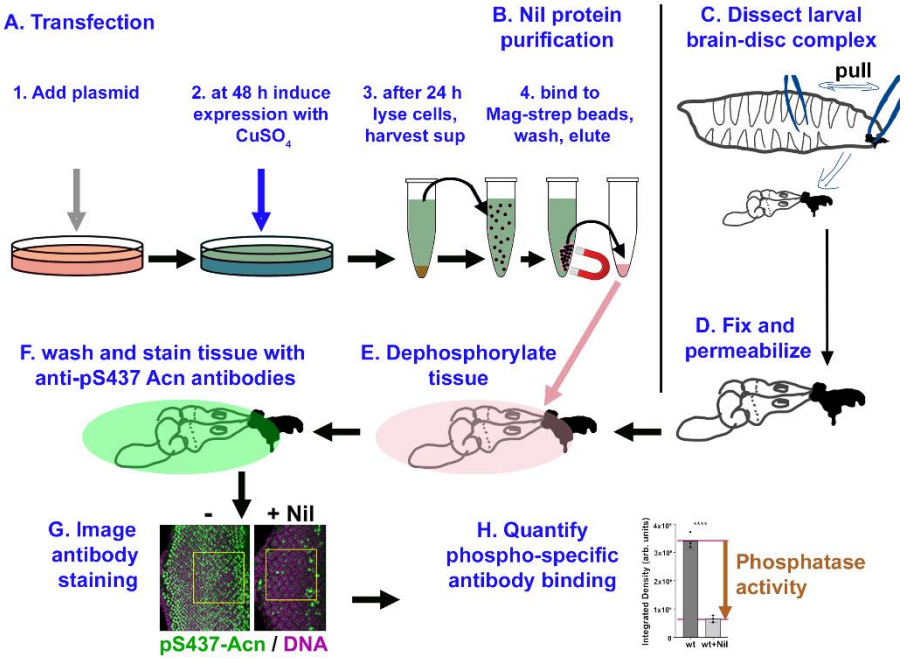


Figure 1. Flow chart of key steps in protocol.
Notice that purified phosphatase after step B and mounted tissues after step F can be stored frozen, constituting possible breakpoints in the procedure.

A. S2 cell transfection

Note: All steps should be done in a biosafety cabinet.

1. Prepare S2 cell media + 10% FBS (see Recipe 1).
2. Revive S2 cells following DGRC instructions, available at https://dgrc.bio.indiana.edu/include/file/general_maintenance.pdf.
3. Maintain cells in a 25 °C incubator at a density between 2×10^6 and 1×10^7 (6 mL final volume) in a 25 cm^2 flask.
4. Transfect and harvest S2 cells using the following 5-day protocol.
   Day 1: Passage cells into a new 25 cm^2 flask.
   Day 2: Count the cells that were passaged on day 1.
   a. Determine cell density using Countess™ automated cell counter.
   b. Plate 2×10^6 cells per well of a six-well plate in 2.5 mL final volume.
   Day 3: Transfect S2 cells.
   a. Warm transIT-2020 reagent to room temperature and vortex gently.
   b. Pipette 250 µL of serum-free Schneider's *Drosophila* medium into a microfuge tube.
   c. Pipette 2.5 µg of DNA of phosphatase expression plasmid (pPuro-MT-Nil-Twin-streptag) to media in step b and mix gently.
      i. Pipette 7.5 µL of TransIT-2020 into the DNA/media mixture and mix gently.
   d. Incubate at room temperature for 30 min.
   e. Pipette transfection mixture dropwise to cells plated on day 2 and cover entire well.
   f. Swirl plate gently to mix.
   g. Place six-well plate in 25 °C incubator.
   h. Select for transfected cells using puromycin (optional).
      i. Allow transfected cells to recover for 48 h.

ii. Prepare a 10 mg/mL puromycin solution in water.
iii. Transfer cells from six-well plate to a 25 cm^2 flask; add S2 cell media up to a final volume of 6 mL.
iv. Allow cells to grow until confluent.
v. Remove S2 cell media and floating cells and transfer to new 25 cm^2 flask.
    1) Allow cells to grow to reach confluency.
    2) Keep as a backup if the first selection fails.
vi. Begin selection of confluent cells by adding 6 mL of fresh S2 cell media + puromycin [10 µg/mL final concentration (see Recipe 3)].
    1) Remove S2 media every two days and add fresh S2 cell media + 10 µg/mL puromycin.
    2) Be sure to remove all floating debris.
vii. Monitor cell death daily using inverted microscope.
viii. Continue selection with 10 µg/mL puromycin until 90%–95% of cells are dead.
ix. Add fresh S2 cell media + 2 µg/mL puromycin to the few remaining cells and allow the transfected cells to grow until confluent. This may take 2–3 weeks. Maintain selected cells at normal density with 2 µg/mL puromycin after selection is complete.
x. Plate 2.5 × 10^6 cells (2.5 mL) per well in a six-well plate to be induced.

Day 4: Induce phosphatase expression from pMT plasmid for 24 h. Pipette 2.5 µL of a 0.7 M $CuSO_4$ solution (see Recipe 4) to each well with transfected cells.
Day 5: Harvest cells after 24 h of induction.
a. Dislodge cells by pipetting the medium up and down.
b. Transfer cell suspension to a 15 mL conical centrifuge tube.
c. Centrifuge 5 min at 1,000 × *g* at room temperature.
d. Remove supernatant.
e. Wash cells by suspending in 1 mL of 1× PBS (see Recipe 2).
f. Transfer cell suspension to a 1.5 mL microfuge tube.
g. Centrifuge 5 min at 1,000 × *g* at room temperature.
h. Remove supernatant.
i. Freeze cells at -80 °C or proceed to protein purification.

B. Nil protein purification

1. Prepare lysis buffer (see Recipe 5).
2. Add 1 mL of lysis buffer to cell pellet and suspend cells by pipetting up and down.
3. Incubate cells/lysis buffer at 4 °C for 1 h with rotation.
4. Centrifuge homogenate at 20,000 × *g* and 4 °C for 20 min.
5. Wash 20 µL MagStrep “type3” XT magnetic beads three times with lysis buffer.
6. Transfer lysate to washed beads.
7. Incubate beads/lysate for 1 h at 4 °C with rotation.
8. Prepare biotin elution buffer (see Recipe 7). Warm to 37 °C.
9. Wash beads three times with 500 µL lysis buffer.
10. Add 25 µL of biotin elution buffer to beads.
11. Incubate 10 min with occasional mixing/vortexing.
12. Remove beads from eluate using magnetic microfuge tube rack.
13. Aliquot eluate containing phosphatase (5 µL each) and store at -20 °C.
14. Quantify phosphatase concentration.
    a. Mix 10 µL of eluates with 10 µL of 2× Laemmli sample buffer and DTT to 0.1 M.
    b. Prepare serial dilutions of BSA in 20 µL of 1× Laemmli sample buffer ranging from 1 µg–0.1 ng. Include DTT to 0.1 M.
    c. Boil samples for 3 min at 100 °C.
    d. Load eluate samples and BSA dilution series samples on a 10% protein gel.

e. Run gel for 1 h at 150 V in electrophoresis chamber.
f. Transfer protein contents from gel onto a nitrocellulose membrane using blotting system.
g. Stain membrane with a total protein stain.
h. Image membrane with a Western blot scanner.
i. Use software associated with Western blot scanner to quantify BSA and eluate bands.
j. Determine eluate protein concentration by comparing quantification of band of interest to those of BSA serial dilutions.

C. *In situ* dephosphorylation assay

1. Dissect third instar larvae in 300 µL of ice-cold 1× PBS (see Recipe 2) under a dissecting microscope on a silicone dish. Gently hold the larva at the middle with forceps and firmly hold the larval mouth hooks with other forceps. Then, pull the mouth parts away from the rest of the body to get a single mass of brain with attached eye-antennal imaginal discs and salivary glands, as well as other tissues (larval carcass). For a comprehensive description of such dissection see the video in Hsiao et al. (2012).
2. Fix 10–15 larval carcasses in a 200 µL drop of periodate-lysine-paraformaldehyde for 20 min on the same silicone dish (at room temperature; no shaking required) (see Recipe 12 for fixative preparation).
3. Transfer fixed larval carcasses in a well of a 96-well plate with 200 µL of PBS and wash for 10 min with gentle rotation on an orbital shaker.
4. For each of the following washes and incubations, gently use forceps to move carcasses into the next well on the 96-well plate with the indicated solution, and then incubate on an orbital shaker with gentle rotation unless otherwise specified.
5. Wash two times in 200 µL of 1× PBS for 10 min (see Recipe 2).
6. Permeabilize larval carcasses in 200 µL of PBSS (PBS + 0.3% saponin) for 10 min.
7. Repeat the previous step one more time.
8. Wash fixed and permeabilized tissues twice in 200 µL of 1× PBS for 10 min.
9. Treat larval carcasses with 50 ng of wild-type Nil phosphatase without or with 100 µM $CdCl_2$ or with 50 ng of the inactive Nil^{D231N} phosphatase in 100 µL of phosphatase assay buffer (see Recipe 15), for 3 h in 37 °C incubator. Nil, like other metal-dependent phosphatases, including its human homologs PPM1A/B, is potently inhibited by cadmium (Kamada et al., 2020; Nandi et al., 2022).
10. Wash phosphatase-treated larval carcasses three times in 200 µL of 1× PBS for 10 min.
11. To block unspecific antibody binding, incubate larval carcasses in PBSS + 5% normal goat serum for 30 min.
12. Stain blocked larval carcasses with primary antibody (phospho-specific antibody for pS437-acinus raised in rabbit, 1:1,000) in 200 µL of PBSS + 5% normal goat serum overnight at room temperature on an orbital shaker. Cover wells with parafilm for overnight incubation.
13. Wash antibody-stained tissues four times in PBSS for 10 min to remove unbound primary antibody.
14. Stain blocked larval carcasses with goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody (1:500) in PBSS + 10% normal goat serum for 3 h in the dark at room temperature.
    Note: Protect specimen from unnecessary light exposure during and after secondary antibody staining with aluminum foil.
15. Wash secondary antibody–stained tissues four times in PBSS for 10 min to remove unbound secondary antibody.
16. Add a small drop of Vectashield with DAPI to a microscope slide. Mount eye discs from the stained larval carcasses in that Vectashield with DAPI. DAPI stains the nuclei.
17. Slowly lower the coverslip onto the drop of Vectashield in which eye discs are mounted. Take care to minimize air bubbles.
18. Seal the edges of the coverslip to the slide with clear nail polish.
19. Store slides in the dark at -20 °C until imaging.
20. Image eye discs on a confocal microscope with a 63× objective for pS437-Acn staining. Acquire Confocal Z-stacks at 1 µm step size using Zen software.

Data analysis

To examine pS437-Acn staining in larval eye discs with different phosphatase treatments, open confocal images with ImageJ and smooth with a Gaussian blur of one. Generate Z-projection of three optical sections of eye discs encompassing mainly photoreceptor cells and examine pS437-Acn level in eye discs. Repeat experiments at least three times with three samples each. In complex tissues, such as eye imaginal discs, take care to select equivalent regions of interest for quantification (see Figure 2 for an example).

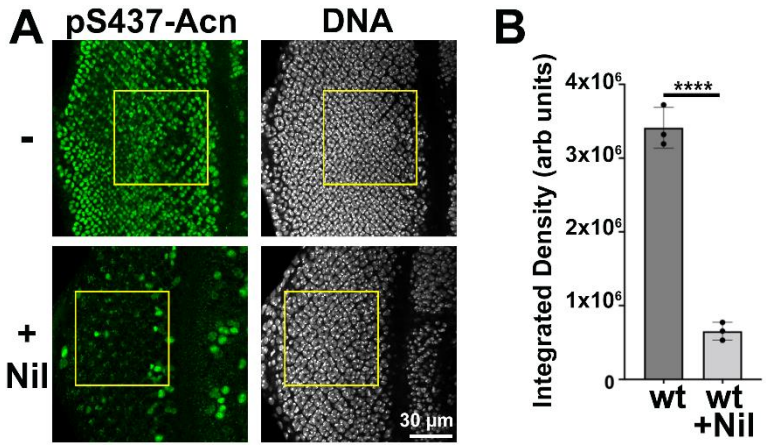


Figure 2. *In situ* dephosphorylation of acinus.
(A) Micrographs of representative eye imaginal discs, treated with (+) or without (-) Nil phosphatase and stained for pS437-Acn and DNA, as indicated. Yellow squares point to equivalent regions of interest, just posterior to the morphogenetic furrow. (B) Quantification of pS437-acinus staining from three different eye discs.

Recipes

1. **S2 Cell Media**
   500 mL of S2 cell media and 50 mL of FBS. Assemble 500 mL vacuum filter/storage bottle and connect to vacuum. Filter S2 cell media and FBS into storage container. Store at 4 °C.

2. **1× PBS**
   137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4. Adjust pH to 7.4 with HCl before finalizing volume with water. Autoclave and store at room temperature.

3. **Puromycin dihydrochloride (10 mg/mL)**
   Dissolve 100 mg of puromycin dihydrochloride in 10 mL of ddH_2O. Aliquot and store at -20 °C.

4. **0.7 M $CuSO_4$**
   Combine 174.78 g of cupric sulfate pentahydrate and water up to 1 L. Autoclave and store at room temperature.

5. **Lysis buffer**
   50 mM Tris pH 8.0, 150 mM NaCl, 1.0% NP40, 0.1 mM PMSF, 1× protease inhibitor, and 1× phosphatase inhibitor. Add water to desired volume. Store at 4 °C.

6. **2× stock phosphatase assay buffer** (used in biotin elution buffer and phosphatase assay buffer below)
   100 mM Tris-HCl (pH 7.5), 80 mM NaCl, and 0.2 mM EGTA. Store at 4 °C.

7. **Biotin elution buffer**
   Add 0.12 g of biotin to 5 mL of 2× stock phosphatase assay buffer. Adjust pH to 8.0 with 1 M NaOH to dissolve biotin. Add ddH_2O to final volume of 10 mL. Store at 4 °C.

8. **8% paraformaldehyde**
   Heat 200 mL of ddH_2O to 55 °C. Add two drops of 50% w/w NaOH. Add 20 g of paraformaldehyde and stir continuously until solution is clear. Do not exceed 60 °C. Filter through vacuum filter/storage bottle. Top off to 250 mL. Aliquot and freeze at -80 °C.

9. **0.4 M Sorenson's phosphate buffer stock**
   Dissolve 7.176 g of sodium phosphate monobasic monohydrate in 100 mL of ddH_2O.
   Dissolve 49.4 g of sodium phosphate in 750 mL of ddH_2O.
   Combine the two solutions above and top off to 1 L with ddH_2O. The resulting solution should have a pH of 7.6.
   Autoclave and store at room temperature.
   Dilute to 0.1 M by adding 3 volumes of ddH_2O before use.

10. **0.1 M di-sodium hydrogen phosphate buffer**
    Dissolve 14.196 g of Na_2HPO_4 in 1 L of ddH_2O final volume. Autoclave and store at room temperature.

11. **Lysine stock**
    Dissolve 16.4 g of L-Lysine in 300 mL of ddH_2O. Adjust pH to 7.4 by adding 0.1 M Na_2HPO_4.
    Add ddH_2O to 450 mL. Add 0.1 M Sorenson's phosphate buffer to 900 mL. Aliquot and store at -80 °C.

12. **Periodate-lysine-paraformaldehyde fixative**
    Note: Always make fresh.
    a. Mix 3 mL of 0.1 M lysine solution with 1 mL of 8% paraformaldehyde/H_2O.
    b. Add 10 mg of sodium meta-periodate.
    This makes the following final concentrations:
    2% paraformaldehyde, 0.01 M Na meta periodate, 0.075 M lysine, and 0.035 M phosphate buffer.

13. **10% saponin**
    10 g of saponin dissolved in 100 mL of deionized water. Aliquot and store at -20 °C.

14. **1 M DTT**
    Combine 1.55 g of DTT with 10 mM sodium acetate, pH 5.2, to a final volume of 10 mL. Completely dissolve DTT, aliquot, and store at -20 °C.

15. **Phosphatase assay buffer**
    Note: Always make fresh.
    500 µL of 2× stock phosphatase assay buffer
    1 µL of 1 M DTT
    40 µL of 1 M $MnCl_2$
    40 µL of 1 M $MgCl_2$
    1 µL of 0.1 M PMSF
    10 µL of 100× Halt™ protease inhibitor single-use cocktail (EDTA-free)

408 µL of dH_2O
This makes the following final concentrations:
40 mM $MgCl_2$, 40 mM $MnCl_2$, 50 mM Tris pH 8.5, 40 mM NaCl, 0.1 mM EGTA, 1× EDTA-free protease inhibitor, and 0.1 mM PMSF.

Acknowledgments

We thank Zuhair Zaidi for help with original analysis of the *nil* phenotype. This work was funded by NIH grants R01EY010199 and R01AI155426.
This protocol was derived from the original research paper "A phosphoswitch at acinus-serine(437) controls autophagic responses to cadmium exposure and neurodegenerative stress" (Nandi et al., 2022).

Competing interests

We declare no competing interest.

References

Berndsen, K., Lis, P., Yeshaw, W. M., Wawro, P. S., Nirujogi, R. S., Wightman, M., Macartney, T., Dorward, M., Knebel, A., Tonelli, F., et al. (2019). PPM1H phosphatase counteracts LRRK2 signaling by selectively dephosphorylating Rab proteins. *Elife* 8: e50416.

Chida, T., Ando, M., Matsuki, T., Masu, Y., Nagaura, Y., Takano-Yamamoto, T., Tamura, S. and Kobayashi, T. (2013). N-Myristoylation is essential for protein phosphatases PPM1A and PPM1B to dephosphorylate their physiological substrates in cells. *Biochem J* 449(3): 741-749.

Doherty, J. and Baehrecke, E. H. (2018). Life, death and autophagy. *Nat Cell Biol* 20(10): 1110-1117.

Haberman, A. S., Akbar, M. A., Ray, S. and Krämer, H. (2010). *Drosophila* acinus encodes a novel regulator of endocytic and autophagic trafficking. *Development* 137(13): 2157-2166.

Hsiao, H.Y., Johnston, R.J., Jr., Jukam, D., Vasiliauskas, D., Desplan, C., and Rister, J. (2012). Dissection and immunohistochemistry of larval, pupal and adult *Drosophila* retinas. *J Vis Exp* 69: 4347.

Kamada, R., Kudoh, F., Ito, S., Tani, I., Janairo, J. I. B., Omichinski, J. G. and Sakaguchi, K. (2020). Metal-dependent Ser/Thr protein phosphatase PPM family: Evolution, structures, diseases and inhibitors. *Pharmacol Ther*: 107622.

Levine, B. and Kroemer, G. (2019). Biological Functions of Autophagy Genes: A Disease Perspective. *Cell* 176(1-2): 11-42.

Murachelli, A.G., Ebert, J., Basquin, C., Le Hir, H., and Conti, E. (2012). The structure of the ASAP core complex reveals the existence of a Pinin-containing PSAP complex. *Nat Struct Mol Biol* 19(4): 378-386.

Nandi, N., Tyra, L. K., Stenesen, D. and Krämer, H. (2017). Stress-induced Cdk5 activity enhances cytoprotective basal autophagy in *Drosophila melanogaster* by phosphorylating acinus at serine(437). *Elife* 6: pii: e30760.

Nandi, N., Zaidi, Z., Tracy, C. and Krämer, H. (2022). A phosphoswitch at acinus-serine(437) controls autophagic responses to cadmium exposure and neurodegenerative stress. *Elife* 11: e72169.

Tyra, L. K., Nandi, N., Tracy, C. and Krämer, H. (2020). Yorkie Growth-Promoting Activity Is Limited by Atg1-Mediated Phosphorylation. *Dev Cell* 52(5): 605-616.e607.