A new toolkit to visualize and perturb endogenous LIN-12/Notch signaling in *C. elegans*

Ariel M Pani¹,²§, Theresa V Gibney¹, Taylor N Medwig-Kinney³, David Q Matus³,⁴, Bob Goldstein⁵,⁶

¹Department of Biology, University of Virginia, Charlottesville, VA, USA
²Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA, USA
³Department of Biochemistry & Cell Biology, Stony Brook University, Stony Brook, NY, USA
⁴D.Q.M. is a paid consultant of Arcadia Science
⁵Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
⁶Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

§To whom correspondence should be addressed: amp2na@virginia.edu

Abstract

Notch signaling mediates cell-cell interactions during development and homeostasis. Methods for visualizing and manipulating Notch activity *in vivo* are essential to elucidate how the Notch pathway functions. Here, we provide new tools for use in *C. elegans* to visualize and perturb Notch signaling *in vivo* using endogenously tagged alleles of the Notch receptor *lin-12*. Tagging the endogenous LIN-12 intracellular domain with the fluorescent protein mNeonGreen (mNG) allowed for visualization of both its membrane-localized state and translocation of the Notch intracellular domain into the nucleus upon ligand activation. LIN-12::mNG localized to the nucleus in cells where and when Notch signaling is known to be active and provided a real-time readout of Notch activity *in vivo* that complements existing biosensors and transcriptional reporters. We also report an allele of endogenous *lin-12* that we tagged with both mNG and an auxin-inducible degron, to facilitate conditional LIN-12 protein degradation. This toolkit provides novel reagents for the *C. elegans* research community to investigate mechanisms of Notch signaling and its functions *in vivo.*
A, schematic of the endogenous lin-12 locus in lin-12::mNG::3xFlag and lin-12::mNG::3xFlag::AID strains; The intracellular domain of LIN-12 is represented in dark gray with the transmembrane (TM) and PEST domains annotated. mNG is fused to the C-terminal end of the intracellular domain using a 9 amino acid flexible linker; B-D, nuclear localization of LIN-12::mNG::3xFlag in specific cells correlates with known Notch signaling events in C. elegans larval development. Arrowheads indicate cells with nuclear signal; B, LIN-12::mNG::3xFlag shows nuclear localization in ventral, but not dorsal, M lineage progenitors; C, LIN-12::mNG::3xFlag localization in VPCs is consistent with genetically determined functions in VPC specification. LIN-12::mNG::3xFlag exhibits clear nuclear localization in P5.p and P7.p cells where Notch signaling is known to be active, but localization is restricted to cell membrane and cytoplasmic domains in the neighboring P4.p, P6.p, and P8.p cells where Notch signaling is not active at this time; D, LIN-12::mNG::3xFlag nuclear localization in presumptive spermatheca (left) and central somatic gonad precursors (center); E, time-lapse imaging of LIN-12 intracellular domain localization in a dividing P7.p cell and its daughters reveals dynamic Notch activity in real time. Asterisk at 30 minutes indicates P7.p division. Nuclear LIN-12::mNG::3xFlag is apparent in P7.p prior to division and in both daughter cells immediately afterwards. Nuclear signal diminished over time in the posterior daughter P7.pp and was maintained in the anterior daughter P7.pa. Nuclei are outlined at key time points (0, 35, 85 minutes), and time elapsed is displayed as minutes:seconds; F-I, K-NAA treatment can be used to deplete endogenously tagged LIN-12::mNG::3xFlag::AID in vivo; F, nuclear LIN-12::mNG::3xFlag::AID fluorescence is visible in P5.p, P7.p, and several somatic gonad cells in a control animal imaged with moderate laser power; G, mCherry::His-11 fluorescence marks nuclei of cells that express TIR1; H, merged image showing LIN-12::mNG::3xFlag::AID, mCherry::His-11, and DIC. Arrowheads indicate cells with visible nuclear NICD signal; I, even with maximal laser power, LIN-12::mgNG::3xFlag::AID fluorescence was barely visible in a representative animal treated with the synthetic auxin analog K-NAA. The bright circular punctae are due to autofluorescence in intestinal cells that is more apparent with higher excitation intensity; J, mCherry::His-11 marks nuclei of cells that express TIR1; K, merged image showing LIN-12::mNG::3xFlag::AID, mCherry::His-11, and DIC. Nuclear NICD signal was not observed in any cells. All images are oriented with anterior to left and dorsal to top. Scale bars = 10 um.

Description

Cell-cell communication through evolutionarily conserved signaling pathways orchestrates many aspects of animal development and homeostasis. To understand signaling mechanisms at the cellular level, it is important to develop tools to visualize endogenous signaling pathway activity and to manipulate signaling in time and space. Notch signaling is one widely conserved cell-cell signaling pathway that mediates cellular interactions in numerous contexts. Receptors in the Notch family play important roles in key developmental events including cell fate specification, differentiation, proliferation, apoptosis, migration, and morphogenesis (reviewed in Artavanis-Tsakonas et al., 1999, and Hori et al., 2013). Understanding mechanisms of Notch signaling is also clinically relevant as Notch signaling has been implicated in multiple human diseases (reviewed in Radtke & Raj, 2003, and Siebel & Lendahl, 2017).

Among metazoan cell-cell signaling pathways, the mechanism of Notch signal transduction is startlingly direct. The Notch protein is a transmembrane receptor that, upon activation by its extracellular ligand, undergoes proteolytic cleavages that release the Notch intracellular domain (NICD) from the cell membrane. The NICD then translocates into the nucleus where it acts as part of a transcriptional complex to regulate expression of target genes (Struhl et al., 1993; Struhl & Adachi, 1998). C. elegans has been used for decades as a tractable and powerful model to elucidate mechanisms of Notch signaling in vivo (reviewed by Greenwald, 2005; Greenwald & Kovall, 2013). The C. elegans genome encodes two Notch proteins, LIN-12 and GLP-1, that have largely non-overlapping functions. During development, GLP-1 functions primarily in the germline and early embryo, and LIN-12 regulates multiple cell interactions and fate decisions in embryonic and larval somatic tissues (reviewed by Greenwald, 2005; Kimble & Crittenden, 2005; Priess, 2005).

Researchers have developed several methods to visualize dynamic Notch activity in C. elegans using live imaging of Notch target gene expression (Kershner et al., 2014; Lee et al., 2019) or changes in biosensor localization (Shaffer & Greenwald, 2022). Because nuclear translocation of the NICD is directly related to its activity, it is logical that live imaging of an endogenously tagged NICD could also provide a readout for Notch signaling in real-time. Such an approach has been demonstrated with live imaging of GLP-1:: sfGFp and GLP-1:: HaloTag transgenes (Sorensen et al., 2020), but has been thought to be impractical for endogenous LIN-12 based on low levels of endogenous expression in key cell types combined with high protein turnover rates (Attner et al., 2019; Shaffer & Greenwald, 2022). Here, we report a novel allele of lin-12 endogenously tagged with the rapidly maturing, bright green/yellow fluorescent protein mNeonGreen (mNG) (Shaner et al., 2013), which we found made it possible to visualize dynamic nuclear localization of the endogenous LIN-12 intracellular domain in vivo using spinning disk confocal microscopy. We also report an endogenously tagged lin-12::mNG::3xFlag::AID
allele that made it possible to conditionally degrade endogenous LIN-12 protein using well-characterized auxin-inducible degradation methods (Zhang et al., 2015; Martinez et al., 2020). This toolkit complements existing methods and provides a direct approach to investigate LIN-12/Notch signaling mechanisms in *C. elegans*.

To visualize LIN-12 intracellular domain localization in *vivo*, we used CRISPR/Cas9-triggered homologous recombination (Dickinson et al., 2013; Dickinson et al., 2015) to insert mNG::3xFlag at the carboxy-terminus of endogenous *lin-12* (Fig. 1A). Homozygous *lin-12::mNG::3xFlag* animals were phenotypically indistinguishable from wild-type, lacking *lin-12* loss of function phenotypes. We conclude this knock-in allele encodes a biologically functional Notch protein. Spinning disk confocal microscopy revealed that tagged LIN-12 was expressed in patterns generally consistent with its known functions. As expected, LIN-12::mNG::3xFlag localized primarily to the cell membrane, along with localized cytoplasmic domains that may represent endosomes. Strikingly, we also observed clear nuclear localization of the endogenously tagged NICD in cells where and when LIN-12 has well-documented functions (Fig. 1B-E).

Focusing on larval development, we found that LIN-12::mNG::3xFlag was expressed in all postembryonic mesoderm progenitor cells at the 4M and 8M stages, but was only localized to the nucleus in ventral M-lineage cells (Fig. 1B), consistent with functions in dorsoventral patterning of the M lineage (Foehr & Liu, 2008). LIN-12::mNG::3xFlag was subsequently visible in somatic gonad cells and in several PN.p cells (Fig. 1C) where *lin-12* regulates cell fates (reviewed by Sternberg, 2005). LIN-12::mNG::3xFlag was visible at the cell membrane and in internalized punctae in the P3.p – P8.p cells but localized to the nucleus only in P5.p and P7.p (Fig. 1C). NICD nuclear localization specifically in these cells is consistent with functions for *lin-12* in specifying 2º vulval precursor cells (reviewed by Sternberg, 2005). An accompanying Micropublication by Medwig-Kinney et al. describes LIN-12 dynamics during AC/VU specification and presents additional tools for visualizing Notch/Delta feedback in these cells (Medwig-Kinney et al., in press).

Following vulval precursor cell patterning, LIN-12::mNG::3xFlag was highly expressed in numerous somatic gonad cells, with nuclear localization in several spermatheca and uterine precursor cells (Fig. 1D). To assess the utility of endogenously tagged LIN-12 for visualizing changes in Notch activity over time, we examined time-lapse images of LIN-12::mNG::3xFlag localization in the P7.p cell and its descendants over 90 minutes during the process of VPC specification. Nuclear signal was prominent in P7.p, diminished during the process of cell division, and initially reappeared in both daughter cells. Nuclear signal subsequently disappeared in the posterior daughter P7.pp but was maintained in the anterior daughter P7.pa (Fig. 1E), which remains in contact with ligand-expressing P6.p descendants. Although we observed transient nuclear NICD localization in both daughter cells immediately after P7.p cell division, it is unclear whether LIN-12 has developmental functions in P7.pp after its birth. One possibility is that cleaved NICD may be present in the cytoplasm during P7.p division, is inherited equally, and translocates into the nuclei of both daughter cells after division is complete. Subsequent loss of nuclear signal in P7.pp could represent normal protein turnover. NICD localization exhibited similar dynamics in P5.p (not shown). These observations suggest that endogenous NICD localization can be used as a direct readout of LIN-12 signaling in *vivo*.

Thoroughly understanding how signaling pathways function requires the ability to manipulate signaling in space and time. To make progress towards this goal, we generated an additional strain where *lin-12* is endogenously tagged with mNG::3xFlag and an auxin-inducible degron (AID). The AID system allows for conditional degradation of degron-tagged proteins in cells that express TIR1, which mediates proteasomal degradation of AID-tagged targets upon treatment with auxin (Zhang et al., 2015; Martinez et al., 2020). As a proof of principle experiment, we combined *lin-12::mNG::3xFlag::AID* with an existing strain that ubiquitously expresses *TIR1::T2A::mCherry::his-11* and tested for the ability to degrade LIN-12 protein (Hills-Muckey et al., 2022). In control animals treated with water, LIN-12::mNG::3xFlag::AID expression and subcellular localization patterns appeared indistinguishable from LIN-12::mNG::3xFlag (Fig. 1F-H). Following treatment with the synthetic auxin K-NAA (1 mM in solid media), we observed a strong reduction of LIN-12::mNG::3xFlag::AID fluorescence (Fig. 1I-K), which indicates this approach is a promising tool to conditionally target LIN-12 signaling. We did not observe uterine or vulval cell phenotypes in the absence of auxin, although we recognize that in other contexts Notch signaling may be more sensitive to auxin-independent degradation that has been observed with wild-type *AtTIR1* (Martinez et al., 2020). Therefore, we also generated strains with a mutant version of the TIR1 transgene (*AtTIR1(F79G)*) that improves specificity (Hills-Muckey et al., 2022). Upon treatment with a modified auxin (5-Ph-IAA), these strains exhibited phenotypes at comparable penetration (not shown). Additional TIR1 and TIR1(F79G) lines available through the *Caenorhabditis* Genetics Center, or generated by users, could be used to leave the red channel open for visualization of other proteins and/or for tissue-specific degradation (Ashley et al., 2021; Negishi et al., 2022).

In summary, we expand the toolkit for investigating Notch signaling in *C. elegans* by providing new strains to visualize and conditionally perturb LIN-12/Notch signaling *in vivo*. Given the evolutionarily conserved nature of Notch signal transduction, we expect that similar approaches may be feasible in other animals amenable to live imaging. In comparison to previous methods for visualizing Notch activity in living *C. elegans*, live imaging of NICD localization has advantages and...
disadvantages that should be considered in the context of specific experimental designs and goals. A unique advantage is that visualizing the NICD itself allows researchers to explore additional questions related to how Notch proteins are localized within cells, how endocytosis is regulated (Shaye & Greenwald, 2002), and the potential for non-canonical Notch signaling processes that do not rely on nuclear translocation of the NICD (Sieiro et al., 2016; Polacheck et al., 2017). Compared to a complementary red/green biosensor-based approach for visualizing LIN-12 activity (Schaffer & Greenwald, 2022), our method requires imaging only a single tagged protein and allows researchers to utilize red fluorescent proteins for other purposes. However, SALSA may be the preferred method for visualizing LIN-12 activity in cases where automated nuclear segmentation is desired or where membrane and/or endosome-localized LIN-12::mNG interferes with imaging nuclear localization. It is our hope that these new reagents will be useful to the C. elegans community to further study mechanisms of Notch signaling and its functions during development and homeostasis.

Methods

Strain maintenance

C. elegans were reared on NGM plates under standard conditions and cultured at 20°C. Strains generated in this study can be found in the Strain Table and will be deposited at the Caenorhabditis Genetics Center for use by the C. elegans community.

Genome engineering

mNG::3xFlag and mNG::3xFlag::AID were inserted at the carboxy-terminus of endogenous lin-12 locus using CRISPR/Cas9-triggered homologous recombinant with the self-excising selection cassette (SEC) method (Dickinson et al., 2015). The guide RNA targeting sequence 5’- GGTTCGGAGTATCGCGTCAT-3’ was inserted into pDD162 (Peft-3>Cas9 + empty sgRNA, Addgene plasmid #47549) using Q5 site-directed mutagenesis (New England Biolabs). Homologous repair templates were generated by cloning PCR-amplified genomic homology arms into the vector backbones pDD268 (mNG^SEC^3xFlag, Addgene plasmid #132523) or pUA77 (mNG^SEC^3xFlag::AID) as described in detail previously (Dickinson et al., 2015).

To generate knock-in strains, young adult worms were microinjected with an injection mix consisting of 50 ng/ml sgRNA + Cas9 plasmid, 10 ng/ml homologous repair template, and a co-injection mix of fluorescent array markers (Dickinson et al., 2015). Knock-in animals were selected using hygromycin-B resistance, a roller phenotype, and lack of extrachromosomal arrays derived from the co-injection markers as described in detail previously (Dickinson et al., 2015). After isolating homozygous roller strains, L1 animals were heat shocked at 34°C for four hours to excise the SEC. Genome edits were confirmed by visualization of fluorescence and genotyping.

Auxin-inducible degradation:

Nematode growth media plates were treated with naphthaleneacetic acid (K-NAA) (PhytoTechnology Laboratories) diluted in water to provide a final concentration of ~1 mM K-NAA in solid media (Martinez & Matus, 2020). To degrade LIN-12::mNG::3xFlag::AID, L1 stage animals were moved to treated plates seeded with OP50 E. coli. Control plates were treated with an equivalent volume of water. The orthogonal auxin analog, 5-Ph-IAA, should be paired with the AtTIR1(F79G) transgenes (Hills-Muckey et al., 2022).

Live imaging

Imaging was performed using a Yokogawa CSU-W1 SoRa spinning disk confocal and Hamamatsu ORCA Fusion camera mounted on a Nikon Ti2 inverted microscope. We utilized 514 nm laser excitation and a 545/40 emission filter to image mNG in order to minimize autofluorescence that is prominent with 488 nm excitation (Heppert et al., 2016). For imaging, we utilized the SoRa disk with either a 60x 1.27 NA water immersion objective and 2.8x SoRa magnifier or a 100x 1.49 NA oil immersion objective and 1.0x SoRa magnifier. Images were acquired using Nikon NIS Elements 5.3 software. For imaging, animals were anesthetized with 0.1 mmol/L levamisole in M9 buffer or immobilized using 0.1 mm polystyrene beads (Polysciences) (Kim et al., 2013) and mounted on 4% agarose pads.

Image processing

Image brightness and contrast were adjusted using Fiji/ImageJ (v2.3.0) (Schindelin et al., 2012). Figure was prepared using Adobe Illustrator (v24.1).

Reagents

Strain table
### Strains

| Strain   | Genotype                                                                 | Source  |
|----------|---------------------------------------------------------------------------|---------|
| APL31    | lin-12(jf31[lin-12::mNeonGreen[C1]^loxP^3xFLAG]) III.                     | This study |
| DQM1070  | cshIs128[Prpl-28>TIR1::T2A::mCherry::his-11] II; lin-12(jf33[lin-12::mNeonGreen[C1]^loxP^3xFLAG::AID]) III; lag-2(bmd202[lag-2::P2A::H2B::mTurquoise2^lox511^2xHA]) V.; | This study |
| DQM1072  | cshIs140[Prpl-28>TIR1(F79G)::T2A::mCherry::his-11] II; lin-12(jf33[lin-12::mNeonGreen[C1]^loxP^3xFLAG::AID]) III; lag-2(bmd202[lag-2::P2A::H2B::mTurquoise2^lox511^2xHA]) V. | This study |

### Plasmids

| Plasmid | Description                                   | Source  |
|---------|-----------------------------------------------|---------|
| pAP163  | Peft-3>Cas9 + PU6>lin-12 sgRNA plasmid         | This study |
| pAP162  | lin-12 mNeonGreen[C1]^SEC^3xFLAG repair plasmid | This study |
| pTG024  | lin-12 mNeonGreen[C1]^SEC^3xFLAG::AID repair plasmid | This study |

### Acknowledgements:
Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

### References

Artavanis-Tsakonas S, Rand MD, Lake RJ. 1999. Notch signaling: cell fate control and signal integration in development. Science 284: 770-6. PubMed ID: [10221902](https://pubmed.ncbi.nlm.nih.gov/10221902/)

Ashley GE; Duong T; Levenson MT; Martinez MAQ; Johnson LC; Hibshman JD; et al.; Ward JD. 2021. An expanded auxin-inducible degron toolkit for Caenorhabditis elegans. Genetics 217: . PubMed ID: [33677541](https://pubmed.ncbi.nlm.nih.gov/33677541/)

Attner MA, Keil W, Benavidez JM, Greenwald I. 2019. HLH-2/E2A Expression Links Stochastic and Deterministic Elements of a Cell Fate Decision during C. elegans Gonadogenesis. Curr Biol 29: 3094-3100.e4. PubMed ID: [31402303](https://pubmed.ncbi.nlm.nih.gov/31402303/)

Dickinson DJ, Ward JD, Reiner DJ, Goldstein B. 2013. Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination. Nat Methods 10: 1028-34. PubMed ID: [23995389](https://pubmed.ncbi.nlm.nih.gov/23995389/)

Dickinson DJ, Pani AM, Heppert JK, Higgins CD, Goldstein B. 2015. Streamlined Genome Engineering with a Self-Excising Drug Selection Cassette. Genetics 200: 1035-49. PubMed ID: [26044593](https://pubmed.ncbi.nlm.nih.gov/26044593/)

Foehr ML, Liu J. 2008. Dorsoventral patterning of the C. elegans postembryonic mesoderm requires both LIN-12/Notch and TGFbeta signaling. Dev Biol 313: 256-66. PubMed ID: [18036582](https://pubmed.ncbi.nlm.nih.gov/18036582/)

Greenwald I. 2005. LIN-12/Notch signaling in C. elegans. WormBook : 1-16. PubMed ID: [18050403](https://pubmed.ncbi.nlm.nih.gov/18050403/)

Greenwald I, Kovall R. 2013. Notch signaling: genetics and structure. WormBook : 1-28. PubMed ID: [23355521](https://pubmed.ncbi.nlm.nih.gov/23355521/)

Heppert JK, Dickinson DJ, Pani AM, Higgins CD, Steward A, Ahringer J, Kuhn JR, Goldstein B. 2016. Comparative assessment of fluorescent proteins for in vivo imaging in an animal model system. Mol Biol Cell 27: 3385-3394. PubMed ID: [27385332](https://pubmed.ncbi.nlm.nih.gov/27385332/)

Hills-Muckey K; Martinez MAQ; Stec N; Hebbar S; Saldanha J; Medwig-Kinney TN; et al.; Hammell CM. 2022. An engineered, orthogonal auxin analog/AfTIR1(F79G) pairing improves both specificity and efficacy of the auxin degradation pathway. Mol Biol Cell 33: 3769-3786.
system in Caenorhabditis elegans. Genetics 220: . PubMed ID: 34739048
Hori K, Sen A, Artavanis-Tsakonas S. 2013. Notch signaling at a glance. J Cell Sci 126: 2135-40. PubMed ID: 23729744
Kershner AM, Shin H, Hansen TJ, Kimble J. 2014. Discovery of two GLP-1/Notch target genes that account for the role of GLP-1/Notch signaling in stem cell maintenance. Proc Natl Acad Sci U S A 111: 3739-44. PubMed ID: 24567412
Kim E, Sun L, Gabel CV, Fang-Yen C. 2013. Long-term imaging of Caenorhabditis elegans using nanoparticle-mediated immobilization. PLoS One 8: e53419. PubMed ID: 23301060
Kimble J, Crittenden SL. 2005. Germline proliferation and its control. WormBook : 1-14. PubMed ID: 18050413
Lee C, Shin H, Kimble J. 2019. Dynamics of Notch-Dependent Transcriptional Bursting in Its Native Context. Dev Cell 50: 426-435.e4. PubMed ID: 31378588
Martinez MAQ; Kinney BA; Medwig-Kinney TN; Ashley G; Ragle JM; Johnson L; et al.; Matus DQ. 2020. Rapid Degradation of Caenorhabditis elegans Proteins at Single-Cell Resolution with a Synthetic Auxin. G3 (Bethesda) 10: 267-280. PubMed ID: 31727633
Medwig-Kinney, TN; Sirotta, SS; Gibney, TV; Pani, AM; Matus, DQ (2022). An in vivo toolkit to visualize endogenous LAG-2/Delta and LIN-12/Notch signaling in C. elegans. microPublication Biology. 10.17912/micropub.biology.000602
Negishi T; Kitagawa S; Horii N; Tanaka Y; Haruta N; Sugimoto A; et al.; Kanemaki MT. 2022. The auxin-inducible degron 2 (AID2) system enables controlled protein knockdown during embryogenesis and development in Caenorhabditis elegans. Genetics 220: . PubMed ID: 34865044
Polacheck WJ, Kutys ML, Yang J, Eyckmans J, Wu Y, Vasavada H, Hirschi KK, Chen CS. 2017. A non-canonical Notch complex regulates adherens junctions and vascular barrier function. Nature 552: 258-262. PubMed ID: 29160307
Priess JR. 2005. Notch signaling in the C. elegans embryo. WormBook : 1-16. PubMed ID: 18050407
Radtke F, Raj K. 2003. The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nat Rev Cancer 3: 756-67. PubMed ID: 14570040
Schindelin J; Arganda-Carreras I; Frise E; Kaynig V; Longair M; Pietzsch T; et al.; Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9: 676-82. PubMed ID: 22743772
Shaffer JM, Greenwald I. 2022. SALSA, a genetically encoded biosensor for spatiotemporal quantification of Notch signal transduction in vivo. Dev Cell 57: 930-944.e6. PubMed ID: 35413239
Shaner NC; Lambert GG; Chammas A; Ni Y; Cranfill PJ; Baird MA; et al.; Wang J. 2013. A bright monomeric green fluorescent protein derived from Branchiostoma lanceolatum. Nat Methods 10: 407-9. PubMed ID: 23524392
Shaye DD, Greenwald I. 2002. Endocytosis-mediated downregulation of LIN-12/Notch upon Ras activation in Caenorhabditis elegans. Nature 420: 686-90. PubMed ID: 12478297
Siebel C, Lendahl U. 2017. Notch Signaling in Development, Tissue Homeostasis, and Disease. Physiol Rev 97: 1235-1294. PubMed ID: 28794168
Siegéro D, Rios AC, Hirst CE, Marcelle C. 2016. Cytoplasmic NOTCH and membrane-derived β-catenin link cell fate choice to epithelial-mesenchymal transition during myogenesis. Elife 5: . PubMed ID: 27218451
Sorensen EB, Seidel HS, Crittenden SL, Ballard JH, Kimble J. 2020. A toolkit of tagged glp-1 alleles reveals strong glp-1 expression in the germline, embryo, and spermatheca. MicroPubl Biol 2020: . PubMed ID: 32626848
Sternberg PW. 2005. Vulval development. WormBook : 1-28. PubMed ID: 18050418
Struhl G, Adachi A. 1998. Nuclear access and action of notch in vivo. Cell 93: 649-60. PubMed ID: 9604939
Struhl G, Fitzgerald K, Greenwald I. 1993. Intrinsic activity of the Lin-12 and Notch intracellular domains in vivo. Cell 74: 331-45. PubMed ID: 8343960
Zhang L, Ward JD, Cheng Z, Dernburg AF. 2015. The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in C. elegans. Development 142: 4374-84. PubMed ID: 26552885
Funding: NIH R35GM142880 (A.M.P.); NIH R35GM134838 (B.G); NIH R01GM121597 (D.Q.M.); Damon Runyon-Rachleff Innovator award DRR-47-17 supported in part by the Damon Runyon Cancer Research Foundation (D.Q.M.); NIH predoctoral fellowship F31HD100091 (T.N.M.-K.); Stony Brook University Presidential Critical Research Funds (T.N.M.-K.)
Author Contributions: Ariel M Pani: conceptualization, formal analysis, funding acquisition, investigation, methodology, project, resources, supervision, visualization, writing - original draft, writing - review editing. Theresa V Gibney: investigation, visualization, writing - review editing. Taylor N Medwig-Kinney: investigation, resources, writing - review editing, funding acquisition. David Q Matus: funding acquisition, project, resources, supervision, writing - review editing. Bob Goldstein: funding acquisition, resources, writing - review editing.

Reviewed By: Anonymous

History: Received June 28, 2022 Revision Received July 12, 2022 Accepted July 24, 2022 Published Online July 28, 2022 Indexed August 11, 2022

Copyright: © 2022 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Pani, AM; Gibney, TV; Medwig-Kinney, TN; Matus, DQ; Goldstein, B (2022). A new toolkit to visualize and perturb endogenous LIN-12/Notch signaling in C. elegans. microPublication Biology. 10.17912/micropub.biology.000603