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Endogenous Expression of the E3 Ubiquitin Ligase EEL-1 in *Caenorhabditis elegans*

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Abstract

The conserved HECT E3 ubiquitin ligase EEL-1 (human HUWE1) coordinates DNA damage-induced apoptosis, neuronal architecture, and embryogenesis in *Caenorhabditis elegans*. However, its endogenous spatiotemporal distribution remains uncharacterized. Using CRISPR/Cas9 genome editing, we generated an endogenous EEL-1::GFP knock-in strain. Confocal microscopy reveals nuclear-enriched fluorescence across the germline syncytium, oocytes, and early embryonic blastomeres, alongside prominent expression in head neurons and the pharynx. These localization patterns visually substantiate genetic requirements for EEL-1 in checkpoint apoptosis, transcriptional regulation, and neurodevelopment, establishing an *in vivo* anatomical framework to identify conserved HUWE1/EEL-1 degradation substrates

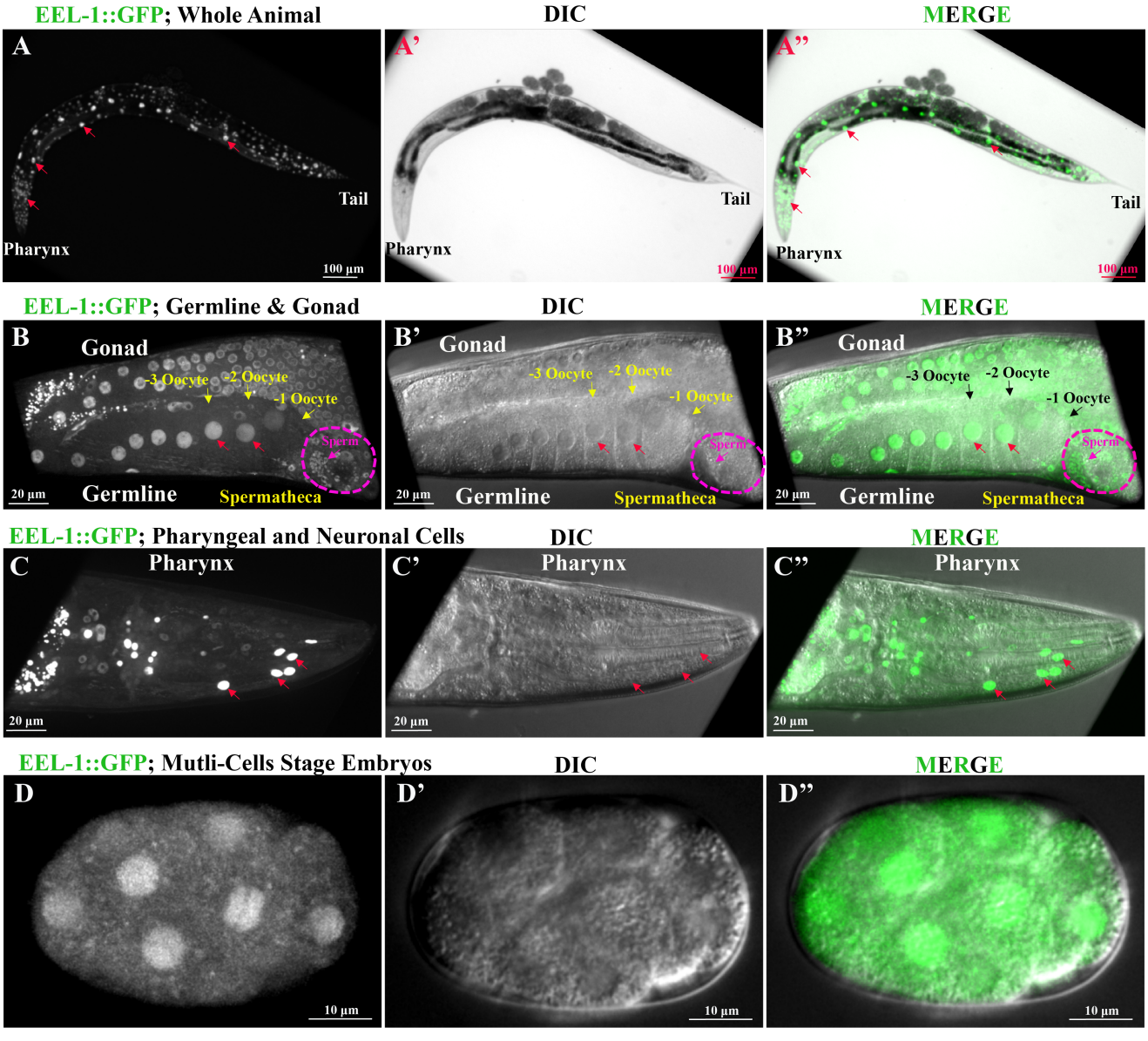


Figure 1. Endogenous expression and subcellular localization of EEL-1::GFP across *C. elegans* tissues:

(A-A") Representative confocal images of a live adult hermaphrodite expressing EEL-1::GFP (green in merge, A") across the whole animal. The monochromatic channel shows EEL-1::GFP fluorescence (A) alongside the corresponding Differential Interference Contrast (DIC) image (A'). Red arrows indicate prominent tissue-specific expression foci along the anterior-posterior axis. (B-B") High-magnification confocal images of the germline and proximal gonad. Monochromatic EEL-1::GFP (B), DIC (B'), and merged channels (B") reveal robust nuclear and cytoplasmic enrichment in pachytene germline nuclei (red arrows) and developing proximal oocytes (-1, -2, and -3 oocytes marked by yellow arrowheads). The spermatheca filled with sperm is outlined by a dashed magenta circle. (C-C") Confocal sections of the anterior region demonstrating EEL-1::GFP localization in the pharynx and head neuronal soma (red arrows). Monochromatic EEL-1::GFP (C), DIC (C'), and merged images (C"). (D-D") Representative imaging of a multi-cell stage embryo displaying ubiquitous blastomere distribution with prominent nuclear localization. Monochromatic EEL-1::GFP (D), DIC (D'), and merged channels (D") are shown. Scale bars are indicated in each panel.

Description

Ubiquitin (Ub) is a highly conserved 76-amino-acid protein, and ubiquitination is an essential post-translational modification that attaches a single ubiquitin moiety or polyubiquitin chains to target substrates (Kipreos 2005; Ross, 2011; Cecile, 2001). Ubiquitination is catalyzed through a sequential three-enzyme cascade comprising E1 activating, E2 conjugating, and E3 ligating enzymes (Cecile, 2001). E3 ubiquitin ligases confer substrate specificity and are classified into four major families: HECT-domain, monomeric RING, multi-subunit RING finger complexes, and U-box proteins (Ross, 2011; Cecile, 2001). As the terminal step in the cascade, E3 ligases target specific proteins for selective degradation by the 26S proteasome, thereby coordinating cell cycle progression, immune and inflammatory signaling, and antigen presentation (Ross, 2011). Consequently, aberrant ubiquitin-dependent proteolysis drives tumorigenesis and neurodegeneration by disrupting DNA repair, cellular differentiation, and programmed cell death (PCD) (Ross, 2011; Cecile, 2001). In humans, pathogenic variants in E3 ligases, including the HECT-domain HUWE1, underlie neurodevelopmental conditions, such as Turner-type X-linked syndromic intellectual disability (MRXST), which are associated with craniofacial dysmorphisms, microcephaly or macrocephaly, and variable delays in speech and gross motor milestones (Dai, 2026; De Falco, 2025).

Among the cellular pathways governed by ubiquitin-dependent proteolysis, the surveillance of genomic integrity and DNA damage-induced apoptosis represent two of the most critical regulatory mechanisms. In higher eukaryotes, this checkpoint is largely executed by the p53 tumor suppressor (encoded by the *TP53* gene), whose stability and function are tightly regulated by ubiquitin ligases. Post-translational modifications, including ubiquitination, phosphorylation, and acetylation, govern p53 stability, subcellular localization, and transcriptional activation (Aubrey, 2016). p53 is activated in response to oncogenic and genotoxic stress, initiating PCD in response to DNA damage induced by ionizing radiation (Ross, 2011; Aubrey, 2016). Under basal conditions, p53 levels are kept low by the RING E3 ligase MDM2, which ubiquitinates p53 to direct its proteasomal degradation via an autoregulatory negative feedback loop; upon genotoxic stress, this interaction is rapidly uncoupled, allowing p53 stabilization and checkpoint activation (Ross, 2011; Aubrey, 2016).

This critical link between E3 ubiquitin ligases and p53-dependent apoptosis is evolutionarily conserved. In *Caenorhabditis elegans*, *cep-1* is the homolog of *TP53* and is required for DNA-damage-induced germline apoptosis by activating pro-apoptotic genes to induce apoptosis (Ross 2011; Baruah, 2014). Previous studies established that *eel-1* acts in a parallel pathway to *cep-1* to coordinate this checkpoint response (Ross, 2011; Baruah, 2016). The *C. elegans eel-1* gene encodes a HECT-type E3 ubiquitin ligase orthologous to mammalian HUWE1 (HECT, UBA, and WWE domain-containing protein 1) (Ross, 2011). EEL-1 positively regulates apoptotic kinetics and sensitivity to genotoxic stress (Ross, 2011). Loss of *eel-1* function leads to hypersensitivity to genotoxic stress and neuronal abnormalities, including excessive dendrite branching and aberrant synaptic architecture (Ross, 2011; Opperman, 2017; Xie, 2023).

C. elegans provides a powerful genetic model to dissect conserved ubiquitination mechanisms due to its invariant cell lineages, well-defined apoptotic cascade, and a conserved repertoire of the majority of annotated E3 ligases (Conradt 2005; Kipreos, 2005). Although *eel-1* is genetically implicated in germline checkpoint signaling, neuronal development, and embryogenesis, the spatial distribution and endogenous subcellular localization of the native protein remain unresolved.

To determine the endogenous expression pattern of EEL-1, we generated an endogenous GFP knock-in, *eel-1::gfp*, at the endogenous *eel-1* locus using CRISPR/Cas9 genome editing. Confocal microscopy of adult hermaphrodites revealed widespread, punctate, and nuclear-enriched EEL-1::GFP signal distributed across distinct reproductive and somatic tissues (Figure A–A"). Within the reproductive system, robust EEL-1::GFP fluorescence was detected throughout the germline syncytium, pachytene-stage germ cell nuclei, and developing proximal oocytes (spanning the –3 to –1 oocytes), along with distinct localization in the spermatheca (Figure B–B").

This pronounced germline accumulation is consistent with the established genetic requirement for *eel-1* in driving DNA damage-induced germ cell apoptosis and preserving oocyte quality (Ross, 2011). In somatic tissues, a prominent EEL-1::GFP signal was observed in anterior neuronal cell bodies within the head ganglion and in the pharynx (Figure C–C"). The pronounced neuronal localization corroborates reports identifying EEL-1 as a key regulator of axon termination, neurite pruning, and synaptic remodeling (Opperman, 2017; Xie, 2023). In early multi-cell stage embryos, EEL-1::GFP was ubiquitously expressed and distinctly enriched in blastomere nuclei (Figure D–D"). This embryonic accumulation underpins the essential developmental role of EEL-1 in governing the spatial asymmetry and targeted proteolysis of the maternal transcription factor SKN-1, which is indispensable for proper mesendodermal and pharyngeal cell fate specification during early embryonic cleavage (Page, 2007).

In summary, our endogenous CRISPR-tagged reporter provides an *in vivo* spatiotemporal map of EEL-1 in *C. elegans*. The enriched expression of EEL-1::GFP in the germline, nervous system, and developing embryonic blastomeres visually confirms prior functional genetic models and provides a direct anatomical foundation for identifying novel, tissue-specific HUWE1/EEL-1 degradation substrates.

Methods

Microscopy. All fluorescence imaging was performed using a spinning-disk confocal system with a Nikon 10x objective, a 60× 1.2 NA water-immersion objective, a Hamamatsu C15440 ORCA-Fusion BT Digital camera, and a Yokogawa CSU-X1 confocal scanner unit. Imaging acquisition was controlled by Nikon's NIS-Elements software (Version 6.10). Image processing and channel merging were conducted using the ImageJ/FIJI Bio-Formats plugin (Fiji/ImageJ: ImageJ version 1.54f; Bio-Formats plugin: version 7.0.0) (National Institutes of Health) (Linkert et al., 2010; Schindelin et al., 2012).

Mounting *C. elegans* for imaging. Animals were mounted on 7% agarose pads in M9 buffer containing 7.5 mM levamisole and covered with a coverslip. The synchronized day-one adult animals were used for imaging in panels A–D.

Generation of CRISPR knock-in strains. CRISPR/Cas9 editing was performed using the Bristol N2 strain as the wild type. Repair templates and single-stranded DNA oligos were synthesized by Integrated DNA Technologies (IDT). Synthetic crRNAs and tracrRNA were purchased from Horizon Discovery. Approximately 25 young adult hermaphrodites were injected with the CRISPR/Cas9 injection mix. The C-terminal tag was amplified from the plasmid pDD282, which contains *C. elegans* codon-optimized GFP. We co-injected the *dpy-10(cn64)* roller marker with the EEL-1::GFP injection reagent (Arribere et al., 2014). F1 roller progeny of injected animals were initially selected based on visible GFP expression and subsequently screened by single-worm PCR across the insertion boundaries using the primers:

Forward 5' tccctgttcgttttcagCTTC 3'

Reverse 5' taacggggaccacgtatcag 3'.

The amplification sequence is shown below, with green letters indicating the GFP sequence.

tccctgttcgttttcagCTTCAATCAACTGGATTTACCGCAATACGAGTCATACGAAAAGCTACGTCAATCACTTCTC
TTGGCAATTCGTGAATGCACAGAAGGCTTTGGATTCGCCggagcatcgggagcctcaggagcatcgatgagtaaaggaga
agaattgttcactggagttgtcccaatcctcgtcgagctcgacggagacgtcaacggacacaagttctccgtctccggagagggagagggagacgccacc
tacggaaagctcaccctcaagttcatctgcaccaccggaaagctcccagtcccatggccaaccctcgtcaccaccttctgctacggagtccaatgcttct
cccgttacccagaccacatgaagcgtcacgacttcttcaagtccgccatgccagagggatacgtccaagagcgtaccatcttcttcaaggtaagtttaaa
catatatatactaactactgattatttaaattttcaggacgacggaaactacaagacccgtgccgaggtcaagttcgagggagacaccctcgtcaaccgt
atcgagctcaaggtaagtttaaacagttcggtactaactaaccatacatatttaaattttcagggaatcgacttcaaggaggacggaaacatcctcggac
acaagctcgagtacaactacaactcccacaacgtctacatcatggccgacaagcaaaagaacggaatcaaggtcaacttcaaggtaagtttaaacatgat
tttactaactaactaatctgatttaaattttcagatccgtcacaacatcgaggacggatccgtccaactcgccgaccactaccaacaaaacaccccaatc
ggagacggaccagtcctcctcccagacaaccactacctctccacccaatccgccctctccaaggacccaaacgagaagcgtgaccacatggtcctcctcg
agttcgtcaccgccgccggaatcacccacggaatggacgagctctacaagTAAatctccctctgtatacaccaattctggaataattattcccgatatttttttattttttttttttcgtt
tcttaaagcttctcttattttccctgatacgtggtccccgtta

Candidate insertions were validated by Sanger sequencing of both the 5′ and 3′ genomic junctions. All EEL-1::GFP lines used for imaging were established as homozygous knock-in stocks before imaging.

EEL-1::GFP CRISPR information: Guide RNA: 5' GTGTATACAGAGGGAGATTT 3'. Repair template primer F1: 5' CAATTCGTGAATGCACAGAAGGCTTTGGATTCGCC ggagcatcgggagcctcagg 3'. Repair template primer R1: 5' taattattccagaattggtgtatacagagggagatTTA CTTGTAGAGCTCGTCCATTC 3'. Genotype Primer F1: 5' tccctgttcgttttcagCTT C 3'. Genotype Primer R1: 5' taacggggaccacgtatcag 3'. wt amplicon size: 227 bp EEL-1::GFP amplicon size: 1121 bp. *dpy-10(cn64)* CRISPR information: Guide RNA: 5' GCTACCATAGGCACCACGAG". Repair template: 5' CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCT
ATGGTAGCGGAGCTTCACATGGCTTCAGACCAACAGCCTAT 3'

Reagents

N2, Bristol wild-type; XFB150 *eel-1(xmb15[eel-1::gfp])* IV. XFB150 was generated by CRISPR/Cas9 genome editing in this study.

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References

Aubrey BJ, Strasser A, Kelly GL. 2016. Tumor-Suppressor Functions of the TP53 Pathway. Cold Spring Harb Perspect Med 6(5): 10.1101/cshperspect.a026062. PubMed ID: 27141080

Baruah A, Chang H, Hall M, Yuan J, Gordon S, Johnson E, et al., Lee SS. 2014. CEP-1, the Caenorhabditis elegans p53 homolog, mediates opposing longevity outcomes in mitochondrial electron transport chain mutants. PLoS Genet 10(2): e1004097. PubMed ID: 24586177

Conradt B, Xue D. 2005. Programmed cell death. WormBook: 1-13. PubMed ID: 18061982

Dai Y, Zhang J, Wu D, Chen R, Li R. 2026. Novel HUWE1-Related Neurodevelopmental Disorder: Genotypic and Phenotypic Expansion. Int J Dev Neurosci 86(1): e70106. PubMed ID: 41664155

De Falco A, Minale EMP, Meossi C, Pagano S, Trovato R, Agolini E, et al., Piscopo C. 2025. Exploring the Clinical Spectrum of HUWE1 -Related Neurodevelopmental Disorder: Five New Patients and Literature Review. Am J Med Genet A 197(4): e63959. PubMed ID: 39629746

Kipreos ET. 2005. Ubiquitin-mediated pathways in C. elegans. WormBook: 1-24. PubMed ID: 18050424

Linkert M, Rueden CT, Allan C, Burel JM, Moore W, Patterson A, et al., Swedlow JR. 2010. Metadata matters: access to image data in the real world. J Cell Biol 189(5): 777-82. PubMed ID: 20513764

Opperman KJ, Mulcahy B, Giles AC, Risley MG, Birnbaum RL, Tulgren ED, et al., Grill B. 2017. The HECT Family Ubiquitin Ligase EEL-1 Regulates Neuronal Function and Development. Cell Rep 19(4): 822-835. PubMed ID: 28445732

Page BD, Diede SJ, Tenlen JR, Ferguson EL. 2007. EEL-1, a Hect E3 ubiquitin ligase, controls asymmetry and persistence of the SKN-1 transcription factor in the early C. elegans embryo. Development 134(12): 2303-14. PubMed ID: 17537795

Pickart CM. 2001. Mechanisms underlying ubiquitination. Annu Rev Biochem 70: 503-33. PubMed ID: 11395416

Ross AJ, Li M, Yu B, Gao MX, Derry WB. 2011. The EEL-1 ubiquitin ligase promotes DNA damage-induced germ cell apoptosis in C. elegans. Cell Death Differ 18(7): 1140-9. PubMed ID: 21233842

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(7): 676-82. PubMed ID: 22743772

Xie J, Zou W, Tugizova M, Shen K, Wang X. 2023. MBL-1 and EEL-1 affect the splicing and protein levels of MEC-3 to control dendrite complexity. PLOS Genetics 19: e1010941. PubMed ID: 37729192

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