Supporting Information

Precision electrophile tagging in C. elegans

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General materials and methods

All primers were from IDT. *C. elegans* wild-type strains and OP50 *E. coli* strains were procured from the University of Minnesota’s Caenorhabditis Genetics Center. Phusion HotStart II polymerase was from Thermo Scientific. All restriction enzymes were from NEB. Complete EDTA free protease inhibitor was from Roche. 1X Bradford dye was from BioRad. LDEs (4–6) The HaloTag-targetable photocaged precursors to LDEs (1–3) were synthesized as described previously1-3. Cyanine5 (Cy5)-azide, FAM-Azide, and Cu(TBTA) were from Lumiprobe. Dithiothreitol (DTT), streptomycin sulfate, isopropyl β-D-1-thio-galactopyranoside (IPTG), TCEP-HCl were from GoldBiochem. 1X Bradford dye was from BioRad.

The HaloTag® targetable photocaged precursors to LDEs (1–3) were synthesized as described previously1-3. Cyanine5 (Cy5)-azide, FAM-Azide, and Cu(TBTA) were from Lumiprobe. Dithiothreitol (DTT), streptomycin sulfate, isopropyl β-D-1-thio-galactopyranoside (IPTG), TCEP-HCl were from GoldBiochem. 1X Bradford dye was from BioRad.

Dyes 7 (HaloTag® TMR Ligand) and 8 (HaloTag® diAcFAM Ligand) were from Promega. Biotin-dPEG®11-azide was from Quanta Biodesign. Streptavidin agarose beads (high-capacity) were from Pierce. Bovine Serum Albumin (BSA) powder was from Thermo Scientific. EDTA, K$_2$HPO$_4$, and KH$_2$PO$_4$ was from Fisher Scientific. MnCl$_2$, ZnSO$_4$, CuSO$_4$, MgSO$_4$, and sodium citrate were from JT Baker. Penicillin/streptomycin solution was from Life Technologies. NaCl was from EMD. Tris for Tris-buffered saline (TBS) and HEPES was from Chem-Impex. All other chemicals were from Sigma. The plasmid for recombinant expression of TEV protease (pRK793, Addgene #8827) was from Addgene. His$_6$-TEV S219V protease was recombinantly expressed and purified from BL21(DE3)–RIL cells (Invitrogen) using TALON resin (Clontech). Information on antibodies and cloning primers and used are listed in Supplementary Table S1 and S2, respectively. Petri dishes for preparation of agar plates were from VWR, and plasticware for preparing OP50 was from CellTreat. 365 nm UV lights were from Spectroline (for handheld size, ENF240C and if larger surface area is needed, XX15N). For T-REX™ experiments, the lamps were positioned above suspended *C. elegans* populations in 6-well plates such that the power of UV irradiation was ~ 5 mW/cm$^2$ (as measured by a hand-held power sensor [Spectroline, XDS-1000]). In vivo imaging of *C. elegans* was performed using a Leica M205 FA stereomicroscope with fluorescent imaging capability. Quantitation of fluorescence intensity was performed using Image-J software (NIH, version 1.50g). In-gel fluorescence analysis and imaging of western blots and coomassie-stained gels were performed using BioRad Chemi-Doc MP Imaging system. Densitometric quantitation was performed using ImageJ software (version 1.50i, NIH). Cy5 excitation source was epi illumination and 695/55 emission filter was used. S-complete media, used for worm incubations and liquid cultures, was prepared using the following final concentrations: 1x S basal media (100 mM NaCl [EMD], 5.7 mM K$_2$HPO$_4$ [Fisher], 44.1 mM KH$_2$PO$_4$ [Fisher], 5 mg/L cholesterol), 1x trace metal solution (50 µM EDTA [Fisher], 25 µM FeSO$_4$•7 H$_2$O, 10 µM MnCl$_2$•4 H$_2$O [JT Baker], 10 µM ZnSO$_4$•7 H$_2$O [JT Baker], 1 µM CuSO$_4$•5 H$_2$O [JT Baker]), 3 mM CaCl$_2$, 3 mM MgSO$_4$ [JT Baker], 10 mM sodium citrate [JT Baker], 1x penicillin/streptomycin [Life Technologies], and 1x nystatin.
Validation of antibodies
For many experiments requiring antibody-based detection, such as western blot analysis (detailed below), an additional condition consisting of transgenic worms that have not undergone heat shock was performed. This condition was collected, lysed, and analyzed according to protocols detailed below, alongside all lysates of heat shock-induced conditions. Signals from anti-Keap/anti-Halo or Cy5-Click, etc. were compared in control (no heat shock) versus induced (heat shock) to verify antibody (Table S1) specificity.

Construction of plasmids
Ligase-free cloning method was used to clone various plasmids (Table S2) for expression in *C. elegans*. In order to clone any desired fusion genes in any vector of choice, the gene of interest (GOI) was PCR-amplified from the original plasmid using the indicated forward (fwd-1) and reverse primers (rev-1) in Table S2. The resultant PCR product was extended using the indicated fwd-2, and rev-2 primers. The resultant “megaprimer” was inserted into the destination vector of interest that had been linearized with an appropriate restriction enzyme (NEB) using PCR. The plasmid was verified by sequencing the entire gene at the genomics facility of Cornell Institute of Biotechnology. Plasmids were purified using EZ-10 spin column plasmid DNA miniprep kits (Bio Basic, BS614).

Strains
*C. elegans* strains were cultured using standard methods and were grown at 17°C unless otherwise indicated. Wild-type animals were Bristol N2. *C. elegans* culture methods.

*C. elegans microinjection*
5–7 days prior to injection, 3–4 healthy L4 worms were picked and placed on a worm plate with OP50. Worms were grown to the second generation to the point where there were a high number of young adults on the plate. 1–3 days prior to injection, agarose pads were made by placing 2–3 drops of agarose onto coverslip slides and allowing the slides to thoroughly dry out (at least 24 h) at room temperature. The day of injection, young adults were picked and moved to a plate with no OP50 and allowed to crawl around to dry. 2 drops of mineral oil was placed on the desiccated agarose pads, and the worm pick was placed in this mixture, then 3–4 worms were picked and placed on the pad and aligned such that their gonads were clearly visible. 150 ng/µL of DNA mix (1:1 mixture of expression plasmid and dominant marker plasmid, centrifuged 18000 x g for 20 min prior to injection) were injected into 20–60 worms typically via single gonad injection. Worms were recovered from the pad in recovery buffer (5mM HEPES pH 7.2, 3 mM CaCl₂, 3 mM MgCl₂, 66 mM NaCl, 2.4 mM KCl, 4% glucose (w/v), then placed 3–4 per plate onto a fresh plate with food. Worms were incubated at 16 ºC for 3 days, then worms were
screened for expression of dominant phenotypic markers (for Mec7 expression, it is clearly visible even at the L1 stage) every day for next 5 days at 20°C. Transgenic worms were individually moved from the source plate to separate screening plates and progeny were scored for transmission. Most of our extrachromosomal arrays gave 30–50% transmission.

**Generation of non-mosaic transgenic Halo C. elegans**

Transgenic worms with appropriate transmission efficiency (less than 50%) were grown until populations consisted of several hundreds of transgenic L4 worms within age-asynchronous populations. The worms were gamma-irradiated, and then allowed to recover for several hours. From these populations, transgenic young adult worms were picked onto new plates and grown (20 per plate) until OP50 was near depletion. Transgenic young adults from those populations were then passaged and grown similarly. After repeating this growth step at least once, individual transgenic young adult worms were picked and their immediate progeny screened for Mendelian inheritance of the transgene. From the progeny that contained the suspected integrated transgene, worms were selected and screened for homozygous transgenic progeny. Progeny with the homozygous integrated transgene were then backcrossed with the parent strain. This was done by plating 4–5 young adult homozygous transgenic hermaphrodites with 14–16 young adult male worms from the parent strain, then screening for hermaphroditic progeny that are heterozygous for the integrated transgene. From these progeny, homozygous transgenic worms were recovered through screening. The backcross is then repeated several (at least 3) times.

**C. elegans culture method**

20 L4-to-young adult *C. elegans* (strain: Bristol N2) with integrated extrachromosomal arrays expressing *tom70::mcherry::halo* or *halo::tev::keap1* were picked using platinum picks and grown on 100 mm nematode growth medium (NGM/agarose) plates on a lawn of freshly-plated *E. coli* OP50. Worm age was not synchronized. Worms were maintained for 4–5 days at either 20 °C or 17 °C to coordinate growth until harvesting, which was performed prior to starvation.

**T-REX in C. elegans**

For Click assays or western blot, 1 x 10 cm plate with dense coverage of worms, but OP50 lawn intact, is sufficient. For pulldown (biotin Click) conditions, 5 x 10 cm plates are suggested. Typically for pulldown experiments, we also prepare non-heat-shocked control (2 plates; designated as “– induction” in figures). These worms were retained at either 20 °C or 17 °C while other plates undergoing heat shock induction (typically 10–20 plates; “+ induction”) are incubated at 37 °C for 60 minutes. To produce enough OP50 for the collected worms, 1 mL of OP50 was spun down into a pellet per worm plate harvested. The pellet was resuspended in S-complete media (see general Materials and Methods) equal to half the starting volume to make
a 2x OP50 stock. After heat shock, all plates (i.e., + and – induction) were washed with 2 mL of S-Complete each, and the mixtures were pipetted into 2 mL clear microfuge tubes. The tubes were centrifuged from 2000-6000 x g for 30 seconds. The supernatant was removed and the worms were washed once in S-complete if necessary. Heat-shocked worms were pooled, split evenly among all treatment conditions into 15-mL conical tubes, then diluted with S-complete to 2.0 mL per condition. Following, 2.5 mL of the 2x OP50 stock was added to each condition, for a volume of 4.5 mL. Then, HaloTag-targetable photocaged precursors were diluted in S-Complete to make a 10x stock (300 µM), and 0.5 mL of this solution was added to the appropriate 4.5 mL samples resulting in final precursor concentration of either 25 or 30 µM¹ (equal across all samples in a biological replicate) in 5 mL. “–Induction” and no precursor-treated samples received 0.5 mL of S-Complete buffer instead. At this point, HNE(alkyne) 4 samples (bulk-exposure conditions) received 0.5 mL of S-Complete buffer, but were then spiked with stock HNE(alkyne) 4 to a final concentration of 30 µM one hour before the end of incubation, to better mimic the minutes-long availability of the released HNE in T-REX setting. An additional 4.5 mL negative control sample received a 10x stock of DMSO to match the concentration of vehicle derived from the 10x probe stocks (for a final concentration of 0.03% DMSO in 5 mL across all treatments). The samples were rotated end-to-end in the dark for 6 hours at room temperature. Subsequently, the samples were washed with S-complete 2x for 30 minutes, then once with 50 mM HEPES for 30 minutes. Between each wash, the worms were spun down into a pellet via short centrifuge pulses between 2000–4000 x g. For this process, it is best to use a 15 mL conical tube with swinging bucket rotor, as it removes OP50 most efficiently. Each pellet was then resuspended in 1.5–2 mL HEPES and transferred to a multi-well plate (6-well plates were used in our experiments). The uncovered plate was then placed underneath a UV light source (365 nm, ~ 5 mW/cm²) (see Materials and Methods) that had been powered on for at least 10 minutes beforehand. The plate was exposed for 5 minutes. Following, the worms were collected into 2mL tubes and pelleted. The pellets were then flash-frozen in liquid nitrogen, transferred to −80°C storage without thawing, and used within 1 week.

**Worm lysis**

Worm pellets were removed from −80°C and thawed on ice. To each pellet, 2–8 volumes of Lysis Buffer [containing in final concentrations: 1.5% NP-40, 50 mM HEPES (pH 7.6), 2x Roche protease inhibitors, 0.50 mM TCEP] was added along with approximately ¼ volume of zirconia beads (0.7 mm dia., from BioSpec Products) is used. For pulldown, worm volume is typically 60–100 µL; for Cy5 click/western blot, it is typically 10 µL. The samples were vortexed for 15

¹25 µM of probe was initially used in pilot studies; 30 µM was used in subsequent biological repeats as this more consistently saturated the Halo binding site.
seconds at room temperature and subsequently freeze–thawed with liquid nitrogen. The cycle was repeated three times in total. Afterwards, the samples were centrifuged for 10 min at 20000 $\times$ g at 4 °C. The protein concentration was estimated using the Bradford assay. Briefly, 1 µL of lysate was mixed with 1 mL Bradford reagent. Absorbance at 595 nm was measured using a plate reader and protein concentration was assessed relative to BSA as a standard. The lysates were immediately used after Bradford quantitation.

**Click coupling assay with fluorescent-azide**

10x Click mixture was prepared by mixing 50 µL 20% SDS, 10 µL 100 mM CuSO$_4$, 10 µL of 10 mM TBTA (LumiProbe), 20 µL of 100 mM TCEP (GoldBio) and 20 µL of 0.5 mM FAM- or Cy5-Azide (Figure S1B). 2.5 µL of 10x Click mixture and 1.25 µL of t-BuOH were added to 21.3 µL of 1 mg/mL lysate mixture in PCR tubes. The final concentrations in the 25 µL samples were 5% t-BuOH, 1% SDS, 1 mM CuSO$_4$, 0.1 mM TBTA, 2 mM TCEP, 10 µM FAM- or Cy5-Azide, 5% t-BuOH, and 0.85 mg/mL lysate. The samples were incubated at 37°C for 30 min. To quench the reaction, 8 µL of Laemmli buffer with 6% BME was added to each tube. The tubes were either stored at −80°C or proceeded to be analyzed by gel.

**Blocking assay**

22 µL samples were prepared with final concentrations of 0.91 mg/mL lysate and 2 µM of either HaloTag® TMR Ligand 7 or HaloTag® diAcFAM Ligand 8. The samples were then incubated at 37 °C for 30 min. 6 µL of 4X Laemmli buffer with 6% BME was added to quench the reaction.

**In-gel fluorescence**

After incubation at 37 °C for 5 min, 20 µL of Click and Blocking Assays were analyzed through 10–well 10% SDS-PAGE gels. The gel was run under dark conditions at 70 V for 30 min and 120 V until the dye front ran out of the gel. After electrophoresis, the gels were washed with ddH$_2$O 3 times on a platform shaker for 5 min each.

**Western blot**

10% SDS-PAGE gels were transferred to a PVDF membrane either at 90 V on ice for 60 min or 33 V overnight at 4 °C. The membrane was subsequently blocked with 5 or 10% milk overnight at 4 °C. 1:1000 monoclonal Halo (Promega G9211) in 1% milk in TBS buffer [100 mM Tris (from Chem-Impex), 150 mM NaCl (from EMD), pH 7.6] with 0.02% Tween-20 was added and incubated overnight at 4 °C. Afterwards, the membrane was washed 3x with TBS and 0.02% tween for 10 min per rinse. 1:10000 Gt to Ms secondary HRP antibody (abcam 6789) in 1% milk in TBS and 0.02% Tween-20 was added, and the membrane was incubated for 40 minutes at room temperature. The membrane was subsequently washed 2x with TBS and 0.02% Tween-20
for 10 min each. After a final wash with TBS (no Tween-20) for 10 min, dye reagents Peroxidase and Luminol (ThermoFisher#32132) were added to the membrane. The membrane signals were measured using a Bio-Rad Chemi-doc-MP Imager. To measure actin loading, monoclonal anti β-actin-HRP (Sigma A3854) 1:3000 was loaded overnight at 4°C. The membrane was subsequently washed 2x with TBS and .02% tween for 10 min. After a final wash with TBS and no tween for 10 min, the membrane was imaged as before.

**Biotin-Click pulldown**

After T-REX and worm lysis (described above), the concentration of each lysate was standardized to the lowest-concentration lysate (typically 1.0 mg/mL) using lysis buffer (composition described above), to a final volume of 300–500 μL. Afterwards, TEV protease was added to each lysate to a final concentration of 0.3 mg/mL of protease. The lysates were then incubated at 37°C for 30 minutes. Following this, streptavidin beads that had been pre-rinsed in ddH₂O (1:1 beads:ddH₂O, 30 min, room temperature, 2x) and 50 mM HEPES buffer (1:1 beads:buffer, 30 minutes, room temperature, 1x) were added to the lysates to pre-clear endogenous biotinylated proteins. The lysates were incubated with the beads on a rotator for 2 hours at room temperature. Afterwards, the lysates were spun down (2000 x g, 1 min) and removed from the beads.

To perform the biotin Click reaction, the lysates were prepared such that each sample mixture contained in 350–600 L typical final volume, in final concentrations/% volume, 1% SDS, 5% tert–butanol, 1 mM CuSO₄, 0.1 mM CuTBTA, 2 mM TCEP, and 0.2 mM biotin azide. Biotin azide was added last, with TCEP immediately preceding it. The mixture was incubated at 37°C for 30 min. Following this, ethanol precipitation was performed for each lysate; pure ethanol (pre-chilled at −20°C) was added to each lysate at a 1:4 lysate:ethanol ratio, followed by overnight storage at −80°C.

Following precipitation, the lysates were spun down at 20000 x g for 30 min at 4 °C. The resultant pellet was washed twice with 70% ethanol, then once with acetone; each wash entailed resuspension of the pellet followed by spin-down (20000 x g for 30 minutes at 4 °C). After the final wash, acetone was removed, and the pellets were allowed to stand until excess acetone had evaporated. Next, for each pellet, 50 mL of resuspension buffer (8% LDS and 1 mM EDTA in 50 mM HEPES) was added. To resolubilize the pellets, they were sonicated for 5–15 minutes at 55 °C. This process was repeated until the pellet was fully solubilized, with a spin-down performed between each sonication (15000 x g, 5 min.). Each sample was then diluted in 50 mM HEPES to a final concentration of 0.5% LDS. After centrifugation at 15000 x g for 5 mins, the samples were moved to a fresh tube and pre-rinsed streptavidin beads (see above) were added at a 1:10 sample:bead ratio by volume. After incubation on a rotator for 2 hours (room
temperature), the beads were washed with 0.5% LDS in 50 mM HEPES 3 times for 30 min per wash. Finally, the beads were boiled in 2x Lamelli dye with 3% β-mercaptoethanol for 5 min to elute the protein. The eluates are then run on a gel and transferred for western blot analysis as shown above.

**Statistical methods**
Within each TMR- or FAM-Halo-ligand blocking data set, a ratio of TMR/FAM fluorescence to Halo blot intensity was obtained for each condition. These ratios were then normalized to non-treated, heat shock-induced controls, providing a readout for the proportion of HaloTag labeled by LDE or photocaged-LDE-precursor. These readouts were then averaged across all datasets that featured that condition. For pulldown experiments, the amount of eluted Keap1 for each condition was calculated by densitometry, and normalized to Keap1 pulled down upon treatment with 1 (which was always the highest intensity band).

**Data analysis**
Data were analyzed using Graphpad Prism v.6.01. Western blot and fluorescence gel quantification were performed using ImageJ (1.50i, NIH). Data are presented as mean ± s.d. or mean ± s.e.m. as indicated. A two-tailed unpaired t-test was used to determine differences between control and experimental samples. A P value of <0.05 was considered significant. No statistical tool was used to pre-determine sample size.
### Supporting Tables

#### Table S1. Summary of antibodies

| Antibody                          | Catalog number; Supplier          | WB Dilution (C. elegans) |
|-----------------------------------|-----------------------------------|--------------------------|
| Mouse monoclonal anti-β-Actin    | A4700; Sigma                      | 1:5000 – 1:10000         |
| Rabbit polyclonal anti-Halo      | G9281; Promega                    | 1:1000                   |
| Mouse monoclonal anti-Halo       | G921A; Promega                    | 1:1000                   |
| Mouse monoclonal anti-Keap1<sup>2</sup> | Ab119403, Abcam NBP2-13309, Novus | 1:1000                   |
| Mouse monoclonal anti-GFP        | Sc-9996; Santa Cruz Biotechnology | 1:1000                   |
| Secondary antibody to rabbit IgG, HRP linked | 7074; Cell Signaling Technology | 1:5000-1:10000          |
| Secondary antibody to mouse IgG, HRP linked | Ab6789; Abcam                     | 1:5000                   |

<sup>2</sup>The 1BF anti-Keap clone from both suppliers performed comparably well in our analyses
### Table S2. List of cloning primers

| Primer name                  | Sequence                                                                 | Type of primer |
|------------------------------|--------------------------------------------------------------------------|----------------|
| hKeap1-Lig778-rev            | ATGACAGCGGCGATGCGGAGCTCTTTATAACACAGGTACAGTTCTGTCTGGTCAAT                 | rev-1          |
| Lig778-His6-Fwd-MetFix       | TTGTGAATAATGAAATCTCTTAAAATTTAATCATCTCATACTATGGGCAACACCATCCCGTGCAAAGAC  | fwd-1          |
| Halo r utr                   | ATGACAGCGGCGATGCGGAGCTCTTTAGCCGAAATCTCGAAGCCGAGCG                      | rev-1          |
| Gfp f lig                    | AAATTAGAAATCTCCAATCTAATCATCACTATGGTGAGCAAGGGCG                         | fwd-1          |
| Gfp f HLH                    | AACTGACCGATTTCAAAATATGATAATTCACAGATGGTGAGCAAGGGCG                      | fwd-1          |
| Lig778-mito                  | GAAATTAGAAAAATCTCCTTCAATACATCACTATCATGAAAGACTTCAATCACAGGAACCAAAGCA    | fwd-1          |
| Lig778 -fex                  | TACCGAACACATTTGGCTTAAATTGAAATTGAAATCTTTCAAGCTTCCAATCACAGGAACCAAAGCA  | fwd-2          |
| Lig u trex                   | TGGGACTTGAAGTCAGAGGGCACGGCAGGATGGGCAGATGCCGAGATGCCGAGATTGGAGGCG        | rev-2          |
| HALO r hlh utr               | TAGAAGTCAGAGGGCACGGCAGGATGGGCAGATGCCGAGATTGGAGGCG                      | rev-2          |
| HLH f ex                     | TGTGGTTACTTTTTATTTTATTACTTTGAACTGACCGATTTTACAGGTCAGAGGGCGAAGTTGGACT  | fwd-2          |
| R ex HLH utr                 | GAAAGAGCATGTAGGGATGTGAGTAGAAGAATTTGGAGAAGAAGGCGAAGATGGAGGCG            | rev-2          |
Figure S1. (A) The generalizable T-REX method previously established in cells¹, ³, ⁵, ⁶ and fish⁷ is transferrable to C. elegans and can be used with several different lipid-derived electrophiles (LDEs)³ ⁴–⁶ through their photocaged precursors ¹–³ (see Figure 1), respectively. Each LDE precursor (inset, top left) contains an anthraquinone photocage (purple) and a 15-atom linker (blue and grey) containing a hexyl chloride terminus (grey), allowing for specific binding to the HaloTag active-site and solvent exposure of the photocage motif (see inset, bottom right: docking model of Ht-PreHNE covalently bound to Halo⁶). Light exposure releases the LDE within the "solvent cage" of Halo-fusion protein of interest (POI). Proximity enhancement⁸ enables capture of the reactive LDE by the POI provided the POI is kinetically privileged to react with LDE prior to diffusion. (B) Chemical structures of fluorescent ligands used in this work. Halo–targetable fluorescent dyes (top row) and azido-functionalized dyes (lower row).
Figure S2. Ht-PreHNE 1 (with alkyne functionalization) and 1-na (with no alkyne functionalization), Ht-PredHNE 2 and Ht-PreHDE 3 saturate functional Halo binding site in live worms. Induction designates heat-shock treatment. See SI methods for experimental details. Worm lysates were treated with TMR-Halo (A and C) or diFAM-Halo (B) ligands (see Figure S1B for chemical structures). Any free Halo binding sites that had not been occupied by the photocaged probes bind with the dye. Worms pretreated with photocaged compounds display a loss or reduction in TMR or diFAM fluorescence (see Inset, bottom right, and also Readout A in Figure 1). Western blots show Halo expression exclusively in heat-shocked worms. Actin blot accounts for lysate loading. The band at ~105 kDa corresponds to Halo::tev::keap1 (A and C), whereas ~66 kDa corresponds to Tom70::mCherry:Halo (B) (indicated by arrows). Gel is representative of 8 experiments (see also Figure 2).
Figure S3. Photocaged probes covalently bind to functional HaloTag in live worms [analyzed by Click coupling assay (Readout B, Figure 1)]. Inset shows workflow of the experiment. See SI methods for details. Induction designates heat shock. (Top) Following T-REX procedure in vivo, worm lysates were clicked with FAM-azide (Figure S2B). Only samples that originate from worms treated with Ht-PreHNE 1 and Ht-PredHNE 3 showed FAM signal. Consistent with blocking results (Figure 2A and S2), FAM-signal resulting from Ht-PredHNE was lower, despite largely similar level of Halo-POI transgene induced upon heat-shock [based on western blot using anti-Halo antibody (Bottom)]. Anti-actin blot serves as loading control. ~105 kDa, halo::tev::keap1 fusion protein; ~66 kDa, tom70::mcherry::halo (marked by arrows). Note: The band labelled (⌘) seen in tom70::mcherry::halo worms is presently unclear but likely represents a modified state of the tom70::mcherry::halo gene product. The strength of this band varies from worm batch to batch (e.g. it is less apparent in Figure S2B, for instance); however, it is selectively upregulated only in this construct. Since the tom70::mcherry::halo construct is not used for delivery, but mainly as a tool to help optimize the click labeling and validate expression, we do not feel this level of complexity requires further investigation.
**Figure S4.** Whole-worm bathing with HNE-alkyne (red dots) modifies many proteins and prevents spatiotemporal control in LDE exposure to cells/animals, whereas T-REX photouncaging enables precision release of a specific LDE at a specific time and for a specific duration (stoichiometric to Halo at maximum). Photouncaging [in gfp::halo transgenic worms (Figure 1) after transgene-expression induction and probe introduction] triggers time-dependent liberation of HNE in basal amounts (stoichiometric to transgene) specifically to the subcellular locale where HaloTag protein is expressed. Click coupling of alkyne-modified protein(s) with Cy5-azide (see workflow in Figure S3) enables a direct readout with gel-based analysis. Western blot (bottom) confirms GFP–Halo expression.
Figure S5. The T-REX concept of proximity-targeted on-demand release of HNE in situ under electrophile-limited conditions selectively HNEylate Keap1 protein expressed in live C. elegans. See Fig. 3A for workflow of the experiment. Additional representative data sets that accompany Fig. 3 (Main Manuscript). Actin serves as loading control. Induction designates heat shock for transgene expression. Bulk HNE exposure and T-REX give different extent of HNEylation on Keap1, suggesting that uptake/metabolism is a more significant variable in living model organisms than in living cells where HNEylation efficiencies are largely found to be comparable between the two conditions.3
Supporting References

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