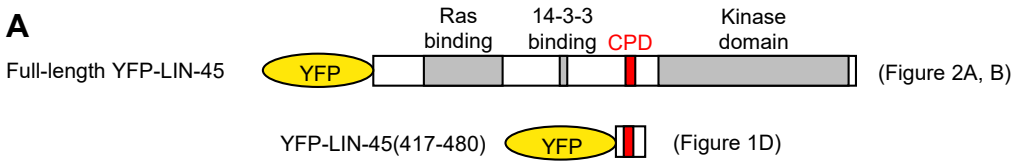
Table S1: *C. elegans* strains used in this study

Click here to Download Table S1

Table S2: *C. elegans* transgenes used in this study

Click here to Download Table S2



B **LIN-45(417-480) region**

CPD motif

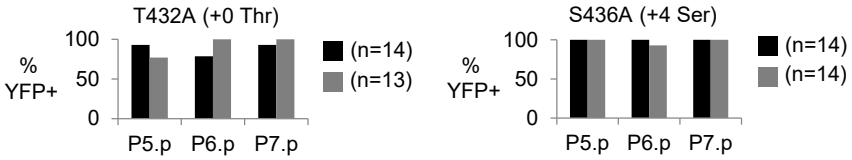
420 430 440 450 460 470 480

TTSSLVAAHLHTLPLTPPQSAPPQKISPGFFRNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE

Alanine substitutions made in YFP-LIN-45(417-480) reporter

		Summary of reporter stability:
TTSSLVAAHLHTLPLAPPQSAPPQKISPGFFRNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE	T432A	Stabilized
TTSSLVAAHLHTLPLTPPQAAPPQKISPGFFRNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE	S436A	Stabilized
TTSSLVAAHLHTLPLTPPQSAAAQKISPGFFRNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE	P438A+P439A	Degraded in P6.p
TTSSLVAAHLHTLPLTPPQSAPPAAISPGFFRNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE	Q440A+K441A	Degraded in P6.p
TTSSLVAAHLHTLPLTPPQSAPPQKAAPGFFRNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE	I442A+S443A	Degraded in P6.p
TTSSLVAAHLHTLPLTPPQSAPPQKISAAFFRNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE	P444A+G445A	Degraded in P6.p
TTSSLVAAHLHTLPLTPPQSAPPQKISPGAARNRSRSPGERLDAQRPRPPQKPHHEDWEILPNE	F446A+F447A	Stabilized

C **Stability of mutant forms of YFP-LIN-45(417-480) reporter: mutation of +0 Thr and +4 Ser**



D **Stability of mutant form of YFP-LIN-45(417-480) reporter: alanine scan**

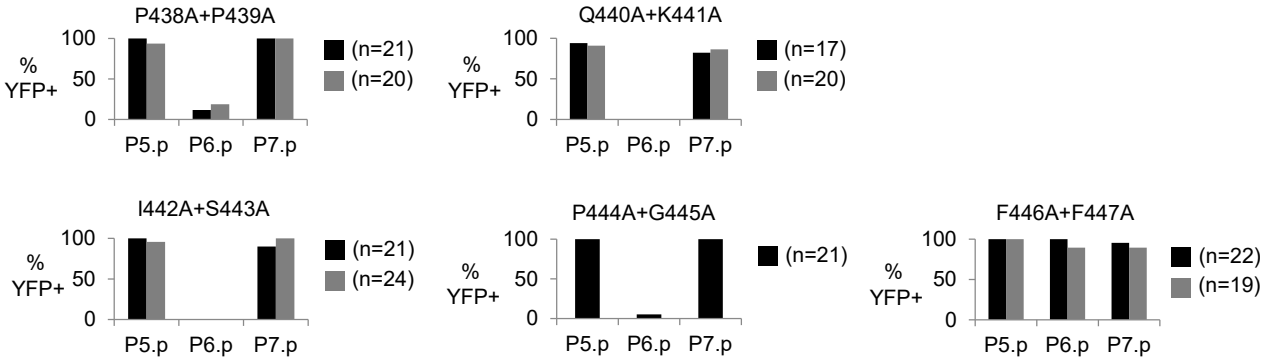


Figure S1. YFP-tagged LIN-45 protein reporters. A) Schematic of full-length YFP-LIN-45 and truncated YFP-LIN-45(417-480) proteins. **B)** Amino acid sequence of LIN-45(417-480) region. The +0 Thr and +4 Ser CPD residues are shown in red. In mutant forms tested, alanine substitutions are shown in blue. **C-D)** Stability of mutant forms of the truncated YFP-LIN-45(417-480) reporter. With exception of the P444A+G445A form, two independent strains were scored for each mutant form tested. Percentage of VPCs with visible YFP fluorescence, and number larvae scored (n) is shown for each strain. (C) Mutations at the +0 Thr or +4 Ser abolish degradation in P6.p. (D) Mutations at F446 and F447 abolish degradation in P6.p.

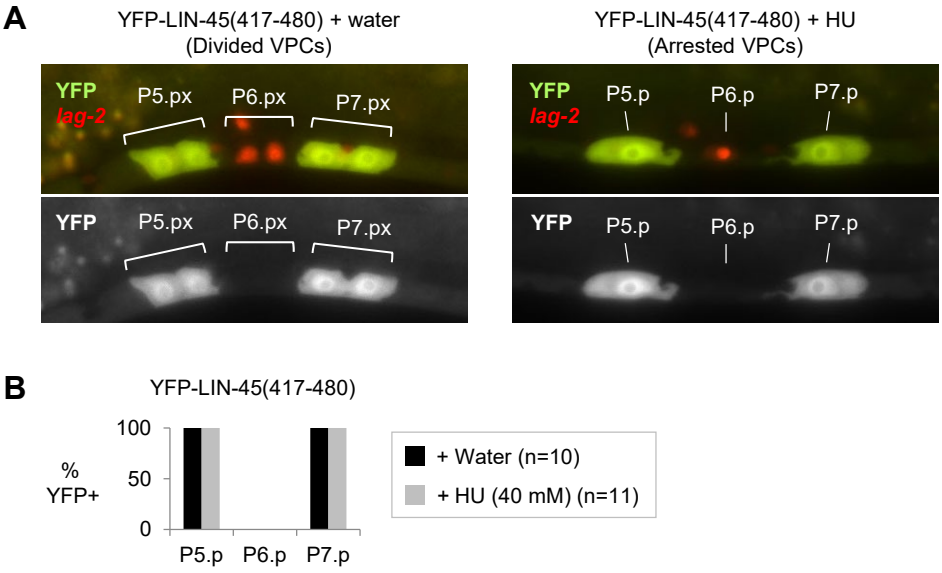


Figure S2. Blockade of S phase does not prevent degradation of the truncated LIN-45(417-480) reporter. Larvae were picked during L2 lethargus based on their lack of locomotion and pharyngeal pumping, transferred to plates with either water (control) or 40 mM hydroxyurea (HU), and grown for 6 hours. At that time, VPCs of all larvae on control plates had divided. For 11/12 larvae grown on HU plates, the VPCs did not divide. We excluded the larva in which VPCs had divided from our analysis. **A)** YFP-tagged LIN-45(417-480) (green) and *arIs222[lag-2p::2xNLS-tagRFP]* (red) proteins in representative images of L3 stage larvae. Top, merged image; bottom, YFP-LIN-45(417-480) alone in grayscale. For the water control, VPCs had divided, and the locations of daughters P5.px, P6.px, and P7.px are indicated with brackets. For the HU-treated larva, undivided VPCs P5.p, P6.p, and P7.p are indicated. **B)** Percentage of VPCs positive for YFP-LIN-45(417-480) in either the water control or HU-treated larvae, and the number of larvae scored (n) is shown.

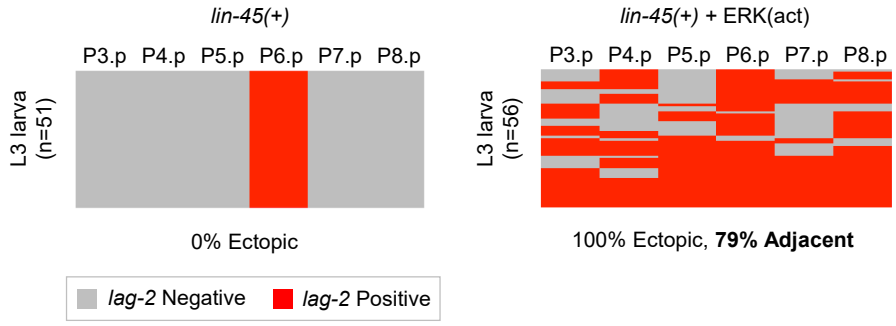


Figure S3. *lag-2* transcription is induced by ERK(act). The *gaIs37* transgene, referred to here as ERK(act), leads to highly active MPK-1/ERK and causes ectopic expression of a transcriptional reporter for *lag-2*, *arIs222[lag-2p::2xNLS-tagRFP],* a direct target of the EGFR-Ras-Raf-ERK signaling pathway and marker of 1° fate. Expression was scored in L3 stage larvae. Each panel represents the *lag-2* positive (red) or negative (gray) status in individual VPCs. Each larva is represented in a row, and VPC is represented in a column. The number of L3 larvae scored (n) is shown at left of each panel. Based on ectopic *lag-2* expression, larvae were categorized as displaying either alternating or adjacent 1° fate. The percentage of larvae that displayed any ectopic 1° fate (% Ectopic), and adjacent 1º fate (% Adjacent) is indicated at each panel.

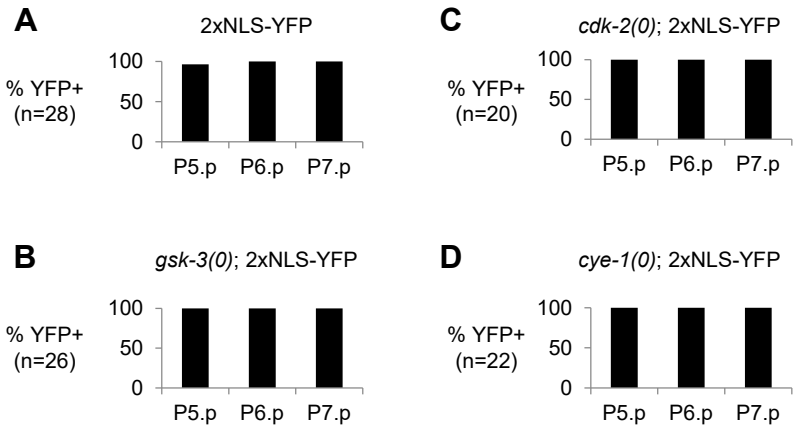


Figure S4. Expression of *lin-31p* is unaffected in *gsk-3*, *cdk-2*, and *cye-1* mutants. In wild-type L3 stage larvae, the *lin-31* promoter (*lin-31p*) is expressed approximately uniformly in P5.p, P6.p, and P7.p. Activity of *lin-31p* was assessed using *arTi88[lin-31p::2xNLS-YFP],* which drives a nuclear-localized YFP. **A)** Percentage of VPCs positive for 2xNLS-YFP in otherwise wild-type larvae, and the number of larvae score (n) is shown. **B-D)** To confirm that the *lin-31p* expression system used to drive YFP-LIN-45 reporters throughout this work is active in the genotypes examined, *arTi88[lin-31p::2xNLS-YFP],* was scored in *gsk-3*, *cdk-2*, and *cye-1* null mutants. Percentage of VPCs positive for 2xNLS-YFP, and the number of larvae scored (n) is shown for (B) *gsk-3(0)* larvae, (C) *cdk-2(0)* larvae, and (D) *cye-1(0)* larvae.