

Supplementary Text

Altered CNT-1 activity affects apoptosis induced by *smIs82*

We scored cell corpses in *smIs82* embryos to examine if loss of *cnt-1* is capable of suppressing cell death after the CED-3 caspase is globally activated. In the absence of the heat shock treatment, *smIs82* embryos showed a cell corpse profile similar to that of N2 embryos (**Supplementary Fig. 4c**). After the heat shock treatment, the numbers of cell corpses in *smIs82* embryos were dramatically increased (**Supplementary Fig. 4d**), indicating that many cells are induced to undergo apoptosis. We found that loss of *cnt-1* partially suppressed and expression of tCNT-1a enhanced ectopic cell death induced by *smIs82* (**Supplementary Fig. 4d**), providing further supporting evidence that *cnt-1* acts downstream of CED-3 to promote apoptosis. A similar partial suppression of cell death was accomplished by a gain-of-function *akt-1(mg144gf)* mutation (**Supplementary Fig. 4d**), confirming that *akt-1* can also inhibit cell death downstream of EGL-1 and CED-3.

Supplementary Tables

Supplementary Table 1 RNAi treatment of *cnt-1* caused a delay-of-cell-death phenotype similar to that of the *cps-2(sm8)* mutant.

RNAi clone	Cell death defect
vector RNAi	–
<i>tpi-1</i>	–
Y17G7B.10	–
<i>arrd-7</i>	–
<i>arrd-8</i>	–
<i>cnt-1</i>	+
Y17G7B.17	–
Y17G7B.18	–
Y17G7B.19	–
Y43F11A.1	–

N2 animals were treated with RNAi and cell corpses in embryos were scored as described in METHODS. For each RNAi experiment, 15 embryos each at the comma, 1.5 fold, 2 fold, 2.5 fold, 3 fold, and 4 fold embryonic stages were scored. The candidate genes tested and corresponding RNAi results are summarized above. “+” indicates that the RNAi clone caused a delay-of-cell-death defect. “–” indicates that there was no obvious cell death defect.

Supplementary Table 2 Inactivation of *cnt-1* enhances suppression of cell death.

Genotype	No. of extra cells	Range of extra cells
N2	0.1 ± 0.1	0–1
<i>cnt-1(tm2313)</i>	0.1 ± 0.1	0–1
<i>ced-3(n2438)</i>	1.3 ± 0.3	0–3
<i>cnt-1(tm2313); ced-3(n2438)</i>	3.5 ± 0.3*	2–6
<i>ced-4(n2273)</i>	2.2 ± 0.4	0–5
<i>cnt-1(tm2313); ced-4(n2273)</i>	3.4 ± 0.4**	1–6

Extra cells were scored in the anterior pharynx of L4 hermaphrodites using Nomarski optics as described in METHODS. Data shown are mean ± s.e.m. Statistical significance values were determined by one-way ANOVA, followed by Tukey's test ($n = 20$ animals).

* $P < 0.001$, compared with *ced-3(n2438)*. ** $P < 0.05$, compared with *ced-4(n2273)*.

Supplementary Table 3 Expression of tCNT-1 causes ectopic cell death.

Genotype	No. of missing cells	Range of missing cells
N2	0 ± 0	0
<i>cnt-1(tm2313)</i>	0 ± 0	0
<i>cnt-1</i> ; ex[P _{dpy-30} tCNT-1a] #1	$0.3 \pm 0.1^*$	0–1
<i>cnt-1</i> ; ex[P _{dpy-30} tCNT-1a] #2	$0.35 \pm 0.1^*$	0–1
<i>cnt-1</i> ; ex[P _{dpy-30} tCNT-1a] #3	$0.35 \pm 0.1^*$	0–1
<i>cnt-1</i> ; ex[P _{dpy-30} tCNT-1b] #1	$0.3 \pm 0.1^*$	0–1
<i>cnt-1</i> ; ex[P _{dpy-30} tCNT-1b] #2	$0.3 \pm 0.1^*$	0–1
<i>cnt-1</i> ; ex[P _{dpy-30} tCNT-1b] #3	$0.35 \pm 0.1^*$	0–1

Missing cells were scored in the anterior pharynx of L4 transgenic hermaphrodites using Nomarski optics. Data shown are mean \pm s.e.m. Statistical significance values were determined by one-way ANOVA, followed by Tukey's test ($n = 20$ animals). $*P < 0.001$, compared with *cnt-1(tm2313)* animals.

Supplementary Table 4 Increased activity of *akt-1* and loss of *daf-18* both inhibit apoptosis.

Genotype	No. of extra cells	Range of extra cells
N2	0.1 ± 0.1	0–1
<i>akt-1(mg144gf)</i>	0.1 ± 0.1	0–1
<i>ced-3(n2438)</i>	1.3 ± 0.3	0–3
<i>ced-3(n2438); akt-1(mg144gf)</i>	3.4 ± 0.4*	1–6
<i>daf-16(mu86)</i>	0.1 ± 0.1	0–1
<i>daf-16(mu86); ced-3(n2438)</i>	3.5 ± 0.4*	1–6
<i>daf-18(e1375)</i>	0.1 ± 0.1	0–1
<i>ced-3(n2438) dpy-4(e1166)</i>	1.4 ± 0.3	0–3
<i>daf-18(e1375) ced-3(n2438) dpy-4(e1166)</i>	3.5 ± 0.3**	1–5

Extra cells were scored in the anterior pharynx of L4 hermaphrodites using Nomarski optics. Data shown are mean ± s.e.m. Statistical significance values were determined by one-way ANOVA, followed by Tukey's test ($n = 20$ animals). * $P < 0.001$; compared with *ced-3(n2438)*. ** $P < 0.001$; compared with *ced-3(n2438) dpy-4(e1166)*.

Supplementary Table 5 *cnt-1* acts downstream of *age-1* and upstream of *akt-1*, *akt-2* and *sgk-1* to promote apoptosis.

Genotype	No. of missing cells	Range of missing cells
N2	0 ± 0	0
<i>cnt-1(tm2313)</i>	0 ± 0	0
<i>age-1(mg44)</i>	0.35 ± 0.11*	0–1
<i>age-1(mg44) cnt-1(tm2313)</i>	0 ± 0	0
<i>akt-1(tm399); akt-2(tm1975) sgk-1(ok538)</i>	0.35 ± 0.11*	0–1
<i>cnt-1(tm2313); akt-1(tm399); akt-2(tm1975) sgk-1(ok538)</i>	0.40 ± 0.11**	0–1

Missing cells were scored in the anterior pharynx of L4 hermaphrodites using Nomarski optics. Data shown are mean ± s.e.m. Statistical significance values were determined by one-way ANOVA, followed by Tukey's test ($n = 20$ animals). * $P < 0.001$, compared with N2. ** $P < 0.001$, compared with *cnt-1(tm2313)*.