

Table 5: Effect of RNAi-knockdown of CUL-1-complex genes on transcriptional regulation of DAF-16-target gene *sod-3*

<i>daf-2(e1370); Psod-3::gfp</i>					
RNAi treatment [*]	Total Number of Worms Assayed [†]	Percentages [‡]			Summary [§]
		High	Moderate	Low	
Trial # 1[¶]					
Control	33	52	18	30	
<i>daf-16</i>	30	0	0	100	strong reduction
<i>cul-1</i>	40	17	30	53	strong reduction
<i>skr-1/2</i>	34	0	26	74	strong reduction
<i>lin-23</i>	42	12	36	52	strong reduction
<i>phi-3</i>	24	25	29	46	moderate reduction
<i>F59B2.8</i>	15	33	34	33	mild reduction
<i>fbxa-121</i>	23	61	30	9	no effect or increase
Trial # 2					
Control	25	80	16	4	
<i>daf-16</i>	25	0	0	100	strong reduction
<i>cul-1</i>	23	52	22	26	mild reduction
<i>skr-1/2</i>	28	64	0	36	mild reduction
<i>lin-23</i>	27	67	22	11	mild reduction
<i>phi-3</i>	29	100	0	0	no effect or increase
<i>F59B2.8</i>	27	74	19	7	no effect
<i>fbxa-121</i>	28	50	36	14	mild reduction
Trial # 3					
Control	23	44	43	13	
<i>daf-16</i>	36	0	0	100	strong reduction
<i>cul-1</i>	54	4	37	59	strong reduction
<i>skr-1/2</i>	88	1	32	67	strong reduction
<i>lin-23</i>	24	0	46	54	strong reduction
<i>phi-3</i>	37	43	49	8	no effect
<i>F59B2.8</i>	32	53	47	0	no effect or increase
<i>fbxa-121</i>	31	45	55	0	no effect
Trial # 4					
Control	34	88	12	0	
<i>daf-16</i>	20	0	0	100	strong reduction
<i>cul-1</i>	25	0	4	96	strong reduction
<i>skr-1/2</i>	69	13	42	45	moderate reduction
<i>lin-23</i>	29	79	17	4	no effect
<i>phi-3</i>	68	51	34	15	mild reduction
<i>F59B2.8</i>	84	74	11	15	no effect
<i>fbxa-121</i>	34	50	50	0	mild reduction

RNAi treatment [*]	Total Number of Worms Assayed [¶]	Percentages [‡]			Summary [§]
		High	Moderate	Low	
Trial # 5					
Control	49	81	19	0	
<i>daf-16</i>	33	9	6	85	strong reduction
<i>cul-1</i>	37	10	35	55	strong reduction
<i>skr-1/2</i>	32	28	15	57	strong reduction
<i>lin-23</i>	43	37	11	52	strong reduction
<i>phi-3</i>	38	81	13	6	no effect
<i>F59B2.8</i>	30	83	17	0	no effect
<i>fbxa-121</i>	46	42	48	10	mild reduction

^{*}Worms were grown on normal food till L4 stage and then transferred to bacteria expressing dsRNA for target genes. GFP levels were screened after 48hrs on Day2 of adulthood. Worms were kept at 20°C throughout the experiment.

[¶]GFP levels were scored as High (high levels visible in intestine and hypodermal cells; Fig. 5B), Moderate (GFP signal reduced in intestinal cells; Fig. 5G) and Low (intestinal GFP significantly reduced or abolished; hypodermal GFP also diminished; Fig. 5C- F). All assays were scored blind after initial familiarization with the control (empty vector) plate.

[‡]Based on the number of worms in each class, and the total number of worms assayed for a given RNAi treatment, the percentage of worms exhibiting high, moderate or low GFP levels was calculated.

[§]The inference about the effect of a given RNAi treatment on the level of *Psod-3::gfp*. It was based on the percentage of worms scored to exhibit high, moderate or low GFP levels and the overall impression of the experimenter during the blind assays.

[¶]Experiment depicted in Fig. 5 and SI7.

Background information on the constitutively nuclear DAF-16^{AM}-GFP fusion protein

The DAF-2/PI 3-kinase cascade regulates DAF-16 nuclear localization via the phosphorylation of consensus AKT sites on DAF-16. Changing these sites to alanine residues produces a functional DAF-16-GFP fusion protein (DAF-16^{AM}-GFP) that accumulates in the nucleus constitutively. In a *daf-16; daf-2* double mutant background, this construct rescues the extended lifespan of *daf-2* mutants (1).

1. Lin, K, Hsin, H, Libina, N & Kenyon, C (2001) *Nat Genet* **28**, 139-145.