

Table S1. Dauer entry phenotypes of wild-type and insulin-deficient worms

Strain/treatment	Mean fraction of dauers $\pm$ s.e.m.	Total no. of animals observed (no. of trials)	P-value against control*	P-value against specified groups
<b>25°C</b>				
Wild type*	0.00 $\pm$ 0.00	1005 (10)		
<i>ins-1(nr2091)</i>	0.00 $\pm$ 0.00	368 (4)	n.s.	
<i>ins-6(tm2416)</i>	0.00 $\pm$ 0.00	566 (6)	n.s.	
<i>daf-28(tm2308)</i>	0.02 $\pm$ 0.01	705 (7)	0.004	
<i>ins-6; ins-1</i>	0.00 $\pm$ 0.00	277 (3)	n.s.	
<i>ins-1; daf-28</i>	0.04 $\pm$ 0.01	494 (5)	0.004	n.s. <sup>†</sup>
<i>ins-6; daf-28</i>	0.36 $\pm$ 0.04	1061 (10)	<0.0001	0.0006 <sup>†</sup>
<i>ins-6; ins-1; daf-28</i>	0.30 $\pm$ 0.06	392 (3)	0.002	n.s. <sup>‡</sup>
<b>20°C</b>				
Wild type	0.00 $\pm$ 0.00	608 (6)	n.s.	
<i>daf-2(e1368)*</i>	0.00 $\pm$ 0.00	595 (6)		
<i>daf-2; daf-28</i>	0.76 $\pm$ 0.08	444 (4)	0.002	
<i>daf-2; ins-1; daf-28</i>	0.44 $\pm$ 0.09	621 (6)	0.004	0.06 <sup>§</sup>
<i>ins-6; daf-2; daf-28</i>	0.92 $\pm$ 0.05	437 (4)	0.004	n.s. <sup>§</sup>
<i>ins-6; daf-2; ins-1; daf-28</i>	0.93 $\pm$ 0.04	419 (4)	0.004	n.s. <sup>¶</sup>
<b>27°C</b>				
Wild type*	0.01 $\pm$ 0.01	1262 (13)		
<i>ins-1</i>	0.02 $\pm$ 0.01	730 (8)	n.s.	
<i>ins-6</i>	0.06 $\pm$ 0.02	849 (9)	0.007	
<i>daf-28</i>	0.74 $\pm$ 0.04	873 (9)	<0.0001	
<i>ins-6; ins-1</i>	0.11 $\pm$ 0.05	353 (4)	0.04	
<i>ins-1; daf-28</i>	0.79 $\pm$ 0.07	605 (6)	0.0003	n.s. <sup>†</sup>
<i>ins-6; daf-28</i>	1.00 $\pm$ 0.00	903 (9)	<0.0001	0.0001 <sup>†</sup>
<i>ins-6; ins-1; daf-28</i>	1.00 $\pm$ 0.00	328 (3)	0.004	n.s. <sup>‡</sup>
<b>22.5°C</b>				
Wild type	0.00 $\pm$ 0.00	488 (5)	0.009	
<i>daf-2*</i>	0.29 $\pm$ 0.07	487 (5)		
<i>daf-2; ins-1</i>	0.10 $\pm$ 0.03	476 (5)	0.03	
<i>ins-6; daf-2</i>	0.36 $\pm$ 0.08	487 (5)	n.s.	
<i>ins-6; daf-2; ins-1</i>	0.14 $\pm$ 0.03	487 (5)	0.08	0.03**
<i>daf-2; daf-28</i>	0.99 $\pm$ 0.00	509 (5)	0.009	
<i>daf-2; ins-1; daf-28</i>	0.97 $\pm$ 0.01	510 (5)	0.009	0.05 <sup>§</sup>
<i>ins-6; daf-2; daf-28</i>	1.00 $\pm$ 0.00	503 (5)	0.005	
<i>ins-6; daf-2; ins-1; daf-28</i>	1.00 $\pm$ 0.00	299 (3)	0.02	n.s. <sup>¶</sup>
<b>25°C</b>				
Wild type	0.00 $\pm$ 0.00	486 (5)		
<i>daf-16*</i>	0.00 $\pm$ 0.00	501 (5)	n.s.	
<i>ins-6; daf-28</i>	0.21 $\pm$ 0.05	504 (5)	0.005	0.005 <sup>††</sup>
<i>daf-16; ins-6; daf-28</i>	0.01 $\pm$ 0.01	491 (5)	n.s.	0.008 <sup>‡</sup>
<i>ins-6; ins-1; daf-28</i>	0.25 $\pm$ 0.06	426 (5)	0.005	n.s. <sup>‡</sup>
<i>daf-16; ins-6; ins-1; daf-28</i>	0.00 $\pm$ 0.00	458 (5)	n.s.	0.005 <sup>‡‡</sup>
<b><i>ins-6</i> rescues</b>				
<b>25°C</b>				
<i>ofm-1p::gfp</i> (25 ng)				
<i>wild type; jxEx18*</i>	0.00 $\pm$ 0.00	594 (5)		
<i>wild type; jxEx21*</i>	0.00 $\pm$ 0.00	477 (5)		
<i>wild type; jxEx22*</i>	0.00 $\pm$ 0.00	477 (5)		
<i>daf-28; jxEx18</i>	0.03 $\pm$ 0.02	337 (5)	n.s. <sup>§§</sup>	0.008***
<i>daf-28; jxEx21</i>	0.02 $\pm$ 0.01	516 (5)	0.05 <sup>§§</sup>	0.009***
<i>daf-28; jxEx22</i>	0.01 $\pm$ 0.01	414 (4)	n.s. <sup>§§</sup>	0.01***
<i>ins-6; daf-28; jxEx18</i>	0.47 $\pm$ 0.05	554 (5)	0.005 <sup>§§</sup>	
<i>ins-6; daf-28; jxEx21</i>	0.31 $\pm$ 0.06	559 (5)	0.005 <sup>§§</sup>	
<i>ins-6; daf-28; jxEx22</i>	0.34 $\pm$ 0.06	532 (5)	0.007 <sup>§§</sup>	
Full rescue (low)				
<i>ins-6p::ins-6</i> (2 ng); <i>ofm-1p::gfp</i> (25 ng)				

<i>ins-6; daf-28; jxEx27</i>	0.01±0.00	638 (5)	<0.01 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx28</i>	0.01±0.01	682 (5)	<0.01 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx29</i>	0.01±0.00	571 (5)	<0.01 <sup>¶¶</sup>	
Full rescue (high)				
<i>ins-6p::ins-6 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; yxEx163</i>	0.00±0.00	175 (2)		
<i>ins-6; daf-28; yxEx174</i>	0.02±0.02	264 (2)		
<i>ins-6; daf-28; yxEx175</i>	0.01±0.01	215 (2)		
ASI-specific rescue (low)				
<i>str-3p::ins-6 (2 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx53</i>	0.23	47 (1)		
<i>ins-6; daf-28; jxEx54</i>	0.01	96 (1)		
<i>ins-6; daf-28; jxEx64</i>	0.10	114 (1)		
<i>ins-6; daf-28; jxEx65</i>	0.23	105 (1)		
<i>ins-6; daf-28; jxEx66</i>	0.26	100 (1)		
ASI-specific rescue (high)				
<i>str-3p::ins-6 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx50</i>	0.00±0.00	479 (2)		
<i>ins-6; daf-28; jxEx51</i>	0.00±0.00	300 (2)		
<i>ins-6; daf-28; jxEx52</i>	0.01±0.01	264 (2)		
ASJ-specific rescue (low)				
<i>trx-1p::ins-6 (2 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx61</i>	0.01±0.01	378 (2)		
<i>ins-6; daf-28; jxEx62</i>	0.01±0.01	284 (2)		
<i>ins-6; daf-28; jxEx63</i>	0.00±0.00	219 (2)		
ASJ-specific rescue (high)				
<i>trx-1p::ins-6 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx58</i>	0.00	134 (1)		
<i>ins-6; daf-28; jxEx59</i>	0.00±0.00	412 (2)		
<i>ins-6; daf-28; jxEx60</i>	0.00±0.00	241 (2)		
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27°C				
<i>ofm-1p::gfp (25 ng)</i>				
<i>wild type; jxEx18*</i>	0.01±0.01	320 (3)		
<i>wild type; jxEx21*</i>	0.01±0.01	312 (3)		
<i>wild type; jxEx22*</i>	0.01±0.01	345 (3)		
<i>daf-28; jxEx18</i>	0.21±0.00	232 (2)	0.08 <sup>§§</sup>	0.08***
<i>daf-28; jxEx21</i>	0.44±0.04	328 (3)	0.05 <sup>§§</sup>	0.05***
<i>daf-28; jxEx22</i>	0.40±0.05	333 (3)	0.05 <sup>§§</sup>	0.05***
<i>ins-6; daf-28; jxEx18</i>	0.99±0.00	286 (3)	0.05 <sup>§§</sup>	
<i>ins-6; daf-28; jxEx21</i>	0.98±0.00	292 (3)	0.05 <sup>§§</sup>	
<i>ins-6; daf-28; jxEx22</i>	0.99±0.01	326 (3)	0.05 <sup>§§</sup>	
Full rescue (low)				
<i>ins-6p::ins-6 (2 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx27</i>	0.29±0.03	360 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx28</i>	0.13±0.08	308 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx29</i>	0.28±0.05	312 (3)	<0.05 <sup>¶¶</sup>	
Full rescue (high)				
<i>ins-6p::ins-6 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; yxEx163</i>	0.13±0.02	373 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; yxEx174</i>	0.10±0.02	357 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; yxEx175</i>	0.13±0.02	344 (3)	<0.05 <sup>¶¶</sup>	
ASI-specific rescue (low)				
<i>str-3p::ins-6 (2 ng); ofm-1p::gfp (25 ng)</i>				

<i>ins-6; daf-28; jxEx53</i>	0.82±0.18	70 (2)	n.s. <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx54</i>	0.12±0.09	303 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx64</i>	0.73±0.09	310 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx65</i>	0.93±0.02	152 (2)	0.08 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx66</i>	0.86±0.09	179 (2)	0.08 <sup>¶¶</sup>	
ASI-specific rescue (high)				
<i>str-3p::ins-6 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx50</i>	0.14±0.10	339 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx51</i>	0.16±0.14	350 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx52</i>	0.15±0.08	372 (3)	<0.05 <sup>¶¶</sup>	
ASJ-specific rescue (low)				
<i>trx-1p::ins-6 (2 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx61</i>	0.12±0.10	325 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx62</i>	0.15±0.11	301 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx63</i>	0.22±0.14	342 (3)	<0.05 <sup>¶¶</sup>	
ASJ-specific rescue (high)				
<i>trx-1p::ins-6 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx58</i>	0.11±0.07	301 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx59</i>	0.14±0.08	310 (3)	<0.05 <sup>¶¶</sup>	
<i>ins-6; daf-28; jxEx60</i>	0.15±0.09	351 (3)	<0.05 <sup>¶¶</sup>	
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<b>daf-28 rescues</b>				
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<b>25°C</b>				
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<i>ofm-1p::gfp (25 ng)</i>				
<i>wild type; jxEx18</i>	0.00±0.00	315 (3)		
<i>wild type; jxEx21</i>	0.00±0.00	320 (3)		
<i>ins-6; daf-28; jxEx18</i>	0.55±0.04	416 (3)	0.04 <sup>§§</sup>	
<i>ins-6; daf-28; jxEx21</i>	0.30±0.02	347 (3)	0.04 <sup>§§</sup>	
Full rescue (low)				
<i>daf-28p::daf-28 (2 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx107</i>	0.27±0.05	391 (3)	0.04 <sup>+++</sup>	0.05 <sup>+++</sup> n.s. <sup>§§§</sup>
<i>ins-6; daf-28; jxEx108</i>	0.40±0.02	344 (3)	0.04 <sup>+++</sup>	0.05 <sup>+++</sup> ,§§§
Full rescue (high)				
<i>daf-28p::daf-28 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>ins-6; daf-28; jxEx103</i>	0.05±0.02	379 (3)	0.04 <sup>+++</sup>	0.05 <sup>+++</sup> ,§§§
<i>ins-6; daf-28; jxEx105</i>	0.15±0.07	352 (2)	0.05 <sup>+++</sup>	0.08 <sup>+++</sup> ,§§§
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<b>27°C</b>				
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<i>ofm-1p::gfp (25 ng)</i>				
<i>wild type; jxEx18</i>	0.04±0.03	255 (3)		
<i>wild type; jxEx21</i>	0.04±0.03	282 (3)		
<i>daf-28; jxEx18</i>	0.74±0.08	257 (3)	0.02 <sup>§§</sup>	
<i>daf-28; jxEx21</i>	0.82±0.08	308 (3)	0.02 <sup>§§</sup>	
Full rescue (low)				
<i>daf-28p::daf-28 (2 ng); ofm-1p::gfp (25 ng)</i>				
<i>daf-28; jxEx112</i>	0.67±0.14	353 (3)	0.03 <sup>+++</sup>	n.s. <sup>¶¶¶</sup>
<i>daf-28; jxEx108</i>	0.68±0.06	352 (3)	0.03 <sup>+++</sup>	n.s. <sup>¶¶¶</sup>
Full rescue (high)				
<i>daf-28p::daf-28 (25 ng); ofm-1p::gfp (25 ng)</i>				
<i>daf-28; jxEx110</i>	0.86±0.05	174 (2)	0.06 <sup>+++</sup>	n.s. <sup>¶¶¶</sup>
<i>daf-28; jxEx111</i>	0.77±0.07	186 (2)	0.06 <sup>+++</sup>	n.s. <sup>¶¶¶</sup>
<i>daf-28; jxEx103</i>	0.59±0.21	299 (2)	0.06 <sup>+++</sup>	n.s. <sup>¶¶¶</sup>
<i>daf-28; jxEx105</i>	0.73±0.15	221 (2)	0.06 <sup>+++</sup>	n.s. <sup>¶¶¶</sup>

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We assayed wild-type, mutant and rescued worms in parallel in independent trials at different temperatures and show statistics from the cumulative experiments. We used the Wilcoxon Mann-Whitney rank sum test to determine the statistical significance of the differences among the groups.

\*The control to which the different worms were compared in each trial.

<sup>†</sup>Compared with *daf-28(tm2308)* mutants.

<sup>‡</sup>Compared with *ins-6(tm2416)*; *daf-28(tm2308)* mutants.

<sup>§</sup>Compared with *daf-2(e1368)*; *daf-28(tm2308)* mutants.

<sup>¶</sup>Compared with *ins-6(tm2416)*; *daf-2(e1368)*; *daf-28(tm2308)* mutants.

\*\*Compared with *ins-6(tm2416)*; *daf-2(e1368)* mutants.

<sup>††</sup>Compared with wild type.

<sup>‡‡</sup>Compared with *ins-6(tm2416)*; *daf-28(tm2308)*; *ins-1(nr2091)* mutants.

<sup>§§</sup>Compared with wild type carrying the corresponding transgene.

<sup>¶¶</sup>Compared with *ins-6*; *daf-28*; *jxEx18*, *ins-6*; *daf-28*; *jxEx21* or *ins-6*; *daf-28*; *jxEx22*.

\*\*\*Compared with *ins-6*; *daf-28* mutants carrying the corresponding transgene.

<sup>†††</sup>Compared with wild type carrying *jxEx18* or *jxEx21*.

<sup>‡‡‡</sup>Compared with *ins-6*; *daf-28*; *jxEx18*.

<sup>§§§</sup>Compared with *ins-6*; *daf-28*; *jxEx21*.

<sup>¶¶¶</sup>Compared with *daf-28*; *jxEx18* or *daf-28*; *jxEx21*.

n.s., not significant ( $P > 0.1$ ). See the Fig. S1 legend concerning the lack of rescue in some animals carrying low levels of the ASI-specific *ins-6* expression construct.

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