Table 1: Effect of RNAi-depletion of core proteasomal subunits on the lifespans of long-lived mutants and wild-type worms‡

Subunit	Gene	Cosmid	*daf-2(mu150)*			*glp-1(e2141ts)*			N2		
			Mean LS ± SEM (days)	Events/ Obs*	P value vs Control†	Mean LS ± SEM (days)	Events/ Obs*	P value vs Control†	Mean LS ± SEM (days)	Events/ Obs*	P value vs Control†
1.A: RNAi-depletion of proteasome structural and enzymatic subunits											
	Control†		34.2 ± 0.1	77/89		22.2 ± 0.9	82/88		20.7 ± 0.7	42/88	
	daf-16		16.6 ± 0.4	80/83	<0.0001	17.8 ± 0.3	77/87	<0.0001	19.2 ± 0.2	51/90	0.01
19S Non-ATPase subunits											
Rpn1	*rpn-1*	T22D1.9	**15.0 ± 0.2**	78/88	<0.0001	**15.8 ± 0.8**	87/93	<0.0001	**13.8 ± 0.2**	52/90	<0.0001
Rpn2	*rpn-2*	C23G10.4	**15.3 ± 0.2**	83/88	<0.0001	**15.1 ± 0.2**	77/88	<0.0001	nt		
Rpn3	*rpn-3*	C30C11.2	**16.3 ± 0.2**	83/89	<0.0001	**15.8 ± 0.3**	88/91	<0.0001	**13.2 ± 0.2**	69/92	<0.0001
Rpn5	*rpn-5*	F10G7.8	35.3 ± 0.7	64/80	0.55	23.8 ± 0.7	66/89	0.78	nt		
Rpn6	*rpn-6*	F57B9.10	**13.4 ± 0.1**	87/90	<0.0001	**13.9 ± 0.2**	89/89	<0.0001	**14.1 ± 0.2**	72/90	<0.0001
Rpn7	*rpn-7*	F49C12.8	**14.2 ± 0.2**	82/87	<0.0001	**15.0 ± 0.2**	89/89	<0.0001	**13.1 ± 0.2**	75/90	<0.0001
Rpn8	*rpn-8*	R12E2.3	**15.8 ± 0.2**	84/90	<0.0001	**14.8 ± 0.2**	88/90	<0.0001	**13.4 ± 0.2**	58/90	<0.0001
Rpn9	*rpn-9*	T06D8.8	32.2 ± 0.8	79/84	0.01	**16.7 ± 0.3**	83/90	<0.0001	**13.7 ± 0.5**	55/75	0.001
Rpn10	*rpn-10*	B0205.3	32.6 ± 0.8	89/91	0.007	20.2 ± 0.4	81/82	0.0002	18.1 ± 0.6	70/90	0.03
Rpn11	*rpn-11*	K07D4.3	**15.6 ± 0.2**	69/90	<0.0001	**13.7 ± 0.3**	88/90	<0.0001	**13.6 ± 0.2**	46/90	<0.0001
Rpn12	*rpn-12*	ZK20.5	36.7 ± 0.7	85/90	0.17	21.0 ± 0.6	85/88	0.007	19.0 ± 0.8	56/90	0.4
19S ATPase subunits											
Rpt1	*rpt-1*	C52E4.4	**15.0 ± 0.2**	86/88	<0.0001	**15.0 ± 0.2**	80/90	<0.0001	**13.2 ± 0.2**	49/90	<0.0001
Rpt2	*rpt-2*	F29G9.5	33.2 ± 0.8	85/87	0.07	21.0 ± 0.6	84/87	0.01	nt		
Rpt3	*rpt-3*	F23F12.6	**16.0 ± 0.2**	83/90	<0.0001	**14.2 ± 0.3**	88/88	<0.0001	nt		
Rpt4	*rpt-4*	F23F1.8	**15.6 ± 0.2**	83/90	<0.0001	**12.8 ± 0.3**	88/88	<0.0001	**13.1 ± 0.1**	49/89	<0.0001
Rpt5	*rpt-5*	F56H1.4	**15.1 ± 0.2**	78/88	<0.0001	**15.0 ± 0.2**	73/88	<0.0001	**12.7 ± 0.2**	48/90	<0.0001
Rpt6	*rpt-6*	Y49E10.1	**15.8 ± 0.3**	54/59	<0.0001	**14.2 ± 0.6**	86/89	<0.0001	**12.8 ± 0.1**	77/91	<0.0001

Subunit	Gene	Cosmid	*daf-2(mu150)*			*glp-1(e2141ts)*			N2		
			Mean LS ± SEM (days)	Events/ Obs*	P value vs Control†	Mean LS ± SEM (days)	Events/ Obs*	P value vs Control†	Mean LS ± SEM (days)	Events/ Obs*	P value vs Control†
20S α-type subunits											
α1	*pas-1*	C15H11.7	33.8 ± 0.7	85/88	0.008	22.5 ± 0.8	66/85	0.78	20.0 ± 0.7	43/90	0.5
α5	*pas-5*	F25H2.9	**15.7 ± 0.2**	69/88	<0.0001	**14.8 ± 0.2**	65/90	<0.0001	**13.5 ± 0.1**	78/91	<0.0001
α6	*pas-6*	CD4.6	**13.5 ± 0.2**	84/89	<0.0001	**13.2 ± 0.2**	89/90	<0.0001	**14.1 ± 0.1**	75/91	<0.0001
α7	*pas-7*	ZK945.2	34.0 ± 0.8	84/89	0.34	21.1 ± 0.7	87/88	0.06	21.1 ± 0.7	75/90	0.3
20S β-type subunits											
β2	*pbs-2*	C47B2.4	**13.0 ± 0.1**	89/90	<0.0001	**14.0 ± 0.3**	83/86	<0.0001	**14.1 ± 0.1**	53/92	<0.0001
β3	*pbs-3*	Y38A8.2	**11.9 ± 0.1**	79/81	<0.0001	**12.6 ± 0.3**	84/89	<0.0001	**14.1 ± 0.1**	77/90	<0.0001
β4	*pbs-4*	T20F5.2	**12.2 ± 0.1**	65/89	<0.0001	**15.4 ± 0.2**	75/89	<0.0001	**14.3 ± 0.1**	72/88	<0.0001
β5	*pbs-5*	K05C4.1	**12.5 ± 0.1**	87/90	<0.0001	**14.1 ± 0.3**	83/90	<0.0001	**14.5 ± 0.5**	63/90	<0.0001
β6	*pbs-6*	C02F5.9	**16.9 ± 0.3**	81/86	<0.0001	**15.6 ± 0.2**	83/88	<0.0001	20.1 ± 0.7	53/89	0.88
β7	*pbs-7*	F39H11.5	**12.4 ± 0.1**	87/89	<0.0001	**14.9 ± 0.2**	67/78	<0.0001	**13.5 ± 0.1**	70/79	<0.0001
1.B: RNAi-depletion of E3 Ligase Cullins											
	Control†		28.8 ± 0.6	68/90		22.4 ±1.0	82/85		18.6 ± 0.8	70/88	
	daf-16		15.6 ± 0.4	87/89	<0.0001	16.1 ± 0.3	88/88	<0.0001	16.4 ± 0.3	51/90	0.005
	cul-1	D2045.6	**21.5 ± 0.4**	84/89	<0.0001	22.8 ±0.8	88/91	0.78	18.6 ± 0.8	67/85	0.8
	cul-2	ZK520.4a	27.6 ± 0.6	72/75	0.14	25.1 ±0.9	72/85	0.41	16.4 ± 0.6	82/91	0.4
	cul-3	Y108G3AL.1	**25.2 ± 0.3**	50/83	<0.0001	20.9 ±0.7	87/89	0.11	19.7 ± 0.9	72/93	0.1
	cul-4	F45E12.3	26.2 ± 0.5	84/90	0.002	24.6 ±1.0	83/89	0.42	19.7 ± 0.9	57/88	0.1
	cul-5	ZK856.1	27.7 ± 0.6	83/91	0.2	26.5 ±0.9	89/92	0.02	21.3 ± 0.9	68/87	0.01
	cul-6	K08E7.7	26.2 ± 0.6	88/90	0.007	25.9 ±1.0	91/91	0.03	19.7 ± 0.8	68/89	0.2

*Some animals were censored as described in Materials and Methods.

†Control refers to worms exposed to empty vector plasmid without an RNAi insert.

Bold red: Lifespan suppression with P <0.0001

Red: Significant lifespan suppression but P >0.0001

Green: Enhancement of lifespan

nt: not tested

‡The proteasome is a multi-subunit complex comprising a 20S core capped on either side by a 19S complex. The 20S core includes the structural α subunits and the enzymatic β subunits, whereas the 19S complex includes the ATPase Rpt subunits and the non-ATPase Rpn subunits. General proteasomal function was reduced by inactivating the structural and enzymatic subunits by RNAi. In most cases, RNAi-depletion caused a dramatic shortening of lifespan. The worms appeared unhealthy and quiescent soon after RNAi treatment, and did not resemble normal old animals in their appearance (data not shown).There were a few exceptions to this trend (e.g., *pas-7, rpn-9*). However, since inhibiting a significant majority of the general proteasomal subunits (~70% of the ones we tested) affected *daf-2(mu150)*, *glp-1(e2141ts)* and N2 worms similarly, this finding suggests that the proteasome is essential for the viability of adult animals.