
Supplementary information

Long-lasting geroprotection from brief rapamycin treatment in early adulthood by persistently increased intestinal autophagy

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Supplementary Information

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Supplementary Table 1. Statistical analysis of survival data presented in Fig. 1a.

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30-day switch – data before day 30 were censored
n=400/treatment

p-value (log rank)	control	rapamycin	rapamycin 30-end	rapamycin 1-30
median (d)	72.5	82	78	82
mean (d)	73.2	82.3	77.6	81.3
control		6.84E-27	2.13E-06	2.39E-13
rapamycin chronic			1.04E-13	0.087215
rapamycin 30-end				2.88E-06

Cox Proportional Hazard (CPH) analysis Risk Ratios

Level 1	/Level 2	Risk Ratio	p	Lower 95%	Upper 95%
rapamycin	control	0.4309059	<0.0001	0.3734110	0.4977745
rapamycin 1-30	rapamycin chronic	1.1543417	0.1266	0.9601738	1.3877745
rapamycin 30-end	rapamycin chronic	1.4612031	<0.0001	1.2173019	1.7539727
rapamycin 1-30	rapamycin 30-end	0.6843676	<0.0001	0.5701343	0.8214889

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Supplementary Table 2. Statistical analysis of survival data presented in Fig. 1b,c.

n=400/treatment

Rapamycin day 45-end – data before day 45 were censored
p-value (log rank)

	control	rapamycin	rapamyc in 45- end
median (d)	75.5	82	78
mean (d)	73.8	83.2	76.3
control rapamycin chronic		1.22E-28	0.00030 3.7E-12

Rapamycin day 60-end – data before day 60 were censored
p-value (log rank)

	control	rapamycin	rapamyc in 60- end
median (d)	75.5	84	75.5
mean (d)	76.9	84	77.7
control rapamycin chronic		1.09E-21	0.25631 5 5.38E-16

**Cox Proportional Hazard (CPH) analysis
Parameter Estimates**

	Term	Estimate	SE	p
d30 v d45 start	rapamycin	0.13680	0.025308	<0.0001
	timing	0.00355	0.024808	0.8861
	rapa*timing	0.00975	0.024808	0.6943
d45 v d60 start	rapamycin	0.07538	0.027004	0.0052
	timing	0.05899	0.026677	0.0267
	rapa*timing	0.08447	0.026677	0.0016

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Supplementary Table 3. Statistical analysis of survival data presented in Fig. 1d,e and 2a.

15-day switch
n=400/treatment

p-value (log rank)	control	rapamycin	rapamycin 1-30	rapamycin 15-30	rapamycin 1-15
median (d)	72.5	77.5	80	77.5	77.5
mean (d)	72.1	77.7	77.5	76.0	77.5
maximum (d)	89	91.5	91.5	91.5	91.5
control		1.2944E-10	2.0562E-13	7.585E-07	1.2016E-11
rapamycin chronic			0.14736386	0.1908386	0.50923849
rapamycin 1-30				0.0153711	0.51293879
rapamycin 15-30					0.07282181

Cox Proportional Hazard (CPH) analysis

Risk Ratios

Level 1	/Level 2	Risk Ratio	p	Lower 95%	Upper 95%
rapamycin	control	0.6522501	<0.0001	0.565943	0.751711
rapamycin 1-15	rapamycin chronic	0.9632981	0.6074	0.834742	1.111281
rapamycin 15-30	rapamycin chronic	1.0933126	0.2117	0.950452	1.257805
rapamycin 1-15	rapamycin 15-30	0.8809997	0.0792	0.764565	1.014886

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Supplementary Table 4. Statistical analysis of survival data presented in Extended Data Fig. 2e,f.

TiGS/UAS-S6K const.act.

n=200/treatment

p-value (log rank)	EtOH	RU	Rapa	Rapa + RU	RU 1-30	Rapa 1-30	Rapa + RU 1-30
median (d)		67	67	69	71	65	69
mean (d)		66.5	65.8	70.4	71.4	63.8	67.3
maximum (d)		75.5	75.5	82.5	82.5	75.5	80
EtOH		0.37293	9.9E-05	9.27E-06	0.424	0.029619	0.001376
RU			0.00438	0.002346	0.8139	0.396167	0.071006
Rapamycin				0.863832	0.0017	0.016441	0.16678
Rapamycin + RU					0.0013	0.011603	0.229435
RU 1-30						0.225444	0.020668
Rapamycin 1-30							0.23175

Cox Proportional Hazard (CPH) analysis

Risk Ratios

Level 1	/Level 2	Risk Ratio	p	Lower 95%	Upper 95%
rapa chronic	control	0.69202	<0.0001	0.587187	0.8156
induced chronic	non-induced chronic	0.94363	0.4864	0.801264	1.1112
rapa 1-30	control	0.0799	0.0064	0.680384	0.9388
induced 1-30	non-induced 1-30	0.90581	0.2265	0.771479	1.0633

Parameter Estimates

	Term	Estimate	SE	p
chronic	rapamycin	0.18407	0.0418	<0.0001
	induction	-0.0290	0.0416	0.4864
	rapamycin*induction	-0.0271	0.0418	0.5164
day 1-30	rapamycin	0.11219	0.0410	0.0064
	induction	-0.0494	0.0400	0.2265
	rapamycin*induction	-0.0045	0.0408	0.911

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Supplementary Table 5. Statistical analysis of survival data presented in Fig. 4a,b.

5966GS/UAS-Atg5^{RNAi}
n=400/treatment

p-value (log rank)	EtOH	RU	Rapa	Rapa + RU	RU 1-15	Rapa1-15	Rapa+ RU 1-15	
median (d)		57	59	61.5	61.5	61.5	64	61.5
mean (d)		58.2	59.1	62.7	59.9	59.1	63.7	60.9
maximum (d)		68	70.5	70.5	68	70.5	70.5	70.5
EtOH		0.467679	1.53E-05	0.156857	0.43617	8.8E-10	0.012342	
RU			7.48E-05	0.253446	0.84502	2.4E-08	0.056321	
rapamycin				0.000263	9.8E-05	0.03165	0.030291	
rapamycin + RU					0.51559	5.4E-09	0.327552	
RU 1-15						7.4E-09	0.097373	
rapamycin 1-15							8.44E-05	
rapamycin + RU 1-15								

Cox Proportional Hazard (CPH) analysis

Risk Ratios

Level 1	/Level 2	Risk Ratio	p	Lower 95%	Upper 95%
rapa chronic	control	0.740356	0.0006	0.624593	0.87815
induced chronic	non-induced chronic	1.123287	0.1774	0.948662	1.33023
rapa 1-15	control	0.553101	<0.0001	0.461037	0.66278
induced 1-15	non-induced 1-15	0.960025	0.6559	0.802404	1.149

Parameter Estimates

	Term	Estimate	SE	p
chronic	rapamycin	0.150312	0.043435	0.0006
	induction	0.05813	0.043093	0.11774
	rapamycin*induction	-0.083593	0.043034	0.0516
day 1-15	rapamycin	0.203859	0.044964	<0.0001
	induction	0.0543	0.044685	0.2246
	rapamycin*induction	-0.074401	0.044552	0.0948

1 **Supplementary Table 6.** Statistical analysis of survival data presented in Fig. 4c,d.

5961GS/UAS-Atg5^{RNAi}
n=200/treatment

p-value (log rank)	EtOH	RU	Rapa	Rapa + RU	RU 1-15	Rapa 1-15	Rapa + RU 1-15
median (d)		54.5	54.5	61.5	61.5	54.5	61.5
mean (d)		54.1	55.4	59.7	60.3	55.3	62.4
maximum (d)		68	70.5	69	68	61.5	70.5
EtOH		0.233667	3.4E-06	3.49E-06	0.5718	8.2E-13	1.93E-12
RU			0.001097	0.001491	0.4706	2.8E-09	1.1E-08
rapamycin				0.73857	1E-05	0.00529	0.011967
rapamycin + RU					2.4E-05	0.00053	0.001288
RU 1-15						8.2E-12	5.4E-12
rapamycin 1-15							0.386465
rapamycin + RU 1-15							

Cox Proportional Hazard (CPH) analysis

Risk Ratios

Level 1	/Level 2	Risk Ratio	p	Lower 95%	Upper 95%
rapa chronic	control	0.630867	<0.0001	0.526928	0.75437
induced chronic	non-induced chronic	0.929143	0.4213	0.776204	1.11124
rapa 1-15	control	0.553101	<0.0001	0.461037	0.66278
induced 1-15	non-induced 1-15	0.960025	0.6559	0.802404	1.149

Parameter Estimates

	Term	Estimate	SE	p
chronic	rapamycin	0.23033	0.045737	<0.0001
	induction	-0.036746	0.045736	0.4213
	rapamycin*induction	-0.022517	0.045743	0.6225
day 1-15	rapamycin	0.138892	0.03734	0.0002
	induction	0.15442	0.037407	<0.0001
	rapamycin*induction	-0.024520	0.03722	0.5101

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Supplementary Table 7. Statistical analysis of survival data presented in Fig. 5b,c.

5966GS/UAS-Atg1^{OE}

n=200/treatment (Fig. 5b) and 160/treatment (Fig. 5c)

p-value (log rank)					
	EtOH	RU	Rapa	Rapa+ RU	
median (d)		60	67	64.5	64.5
mean (d)		59.2	66.4	64.4	63.5
maximum (d)		71.5	71.5	71.5	71.5
EtOH			3.6E-11	5.68E-06	8.49E-05
RU				0.046318	0.004648
rapamycin					0.506055
rapamycin + RU					

	EtOH	RU 1-15	Rapa 1-15	Rapa + RU 1-15	
median (d)		60.5	67.5	67.5	67.5
mean (d)		60.3	66.8	67.3	67.8
maximum (d)		74.5	74.5	74.5	74.5
EtOH			6.72E-5	1.00E-06	1.84E-07
RU 1-15				0.371120	0.212741
rapamycin 1-15					0.697781
rapamycin + RU 1-15					

Cox Proportional Hazard (CPH) analysis

Risk Ratios

Level 1	/Level 2	Risk Ratio	p	Lower 95%	Upper 95%
rapa chronic	control	0.567652	<0.0001	0.461557	0.70218
induced chronic	non-induced chronic	0.380319	<0.0001	0.307387	0.47305
rapa 1-15	control	0.725947	0.0002	0.613941	0.85838
induced 1-15	non-induced 1-15	0.790212	0.0059	0.668278	0.93439

Parameter Estimates

	Term	Estimate	SE	p
chronic	rapamycin	-0.283123	0.053447	<0.0001
	induction	-0.483371	0.054918	<0.0001
	rapamycin*induction	0.156973	0.052663	0.0036
day 1-15	rapamycin	0.160138	0.042750	0.0002
	induction	-0.117726	0.042755	0.0059
	rapamycin*induction	-0.107443	0.042741	0.0119

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Supplementary Table 8. Statistical analysis of survival data presented in Extended Data Fig. 4c.

5966GS/UAS-H3/H4^{OE}
n=200/treatment

p-value (log rank)	EtOH	RU	RU 1-15
median (d)	66.6	71.0	66.6
mean (d)	66.9	71.0	67.5
maximum (d)	82.0	89.0	86.0
control		7.38E-06	0.59709
RU			0.00011

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Supplementary Table 9. Statistical analysis of survival data presented in Extended Data Fig. 7c.

5966GS/UAS-BCAT^{RNAi}
n=180/treatment

p-value (log rank)	EtOH	RU 1-15	Rapa 1-15	Rapa + RU 1-15
median (d)	79.0	79.0	81.5	79.0
mean (d)	80.6	80.5	82.2	80.9
maximum (d)	86.0	88.5	91.0	88.5
EtOH		0.699135	7.83E-04	0.050575
RU 1-15			0.002971	0.138767
rapamycin 1-15				0.150979
rapamycin + RU 1-15				