Native PAGE immunoblot (uncropped) anti-proteasome

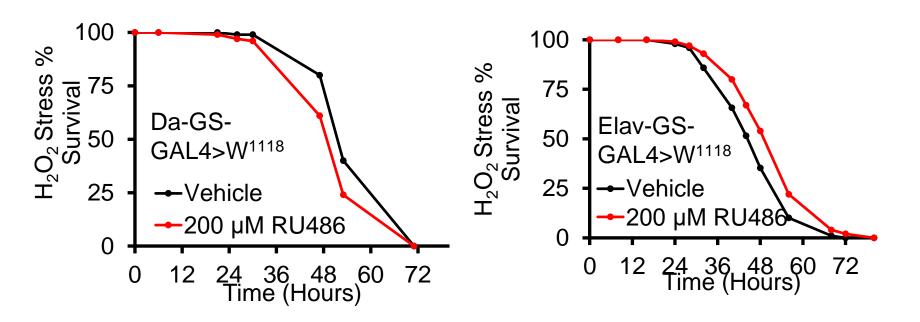
Control

Prosβ5 O.E.

Prosβ5 O.E.

Protein loading (india ink stain)

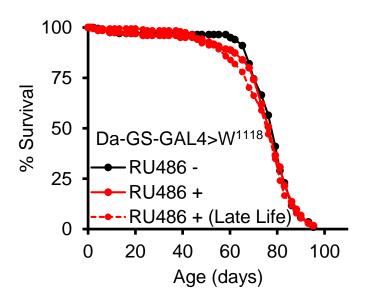


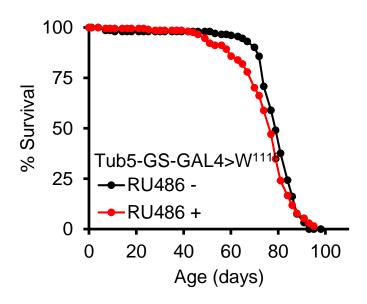


## Supplemental Figure 2:

 $H_2O_2$  stress survival assay RU486 controls for Figures 1E and 2F. RU486 treatment produces minimal changes to oxidative stress under control of the drivers Da-GS-GAL4 and Elav>GS-GAL4. Flies were fed 4.4M  $H_2O_2$  mixed with 5% sucrose, survival was monitored every 8 hr, N = 75. Flies were removed from RU486 during stress assay to prevent potential differences in consumption.

	- RU486	+ RU486	%	Chi-Square	Logrank
DA-GS-GAL4>UAS-Prosβ5					
N ≈ 200					
Median (50%)	75.4	75.3	-0.2	2.5	0.11
Maximum (10%)	87.1	84.9	-2.6		
Tub5-GS-GAL4>UAS-Prosβ5					
N ≈ 250					
Median (50%)	66.0	66.5	8.0	0.2	0.64
Maximum (10%)	74.7	73.7	-1.3		

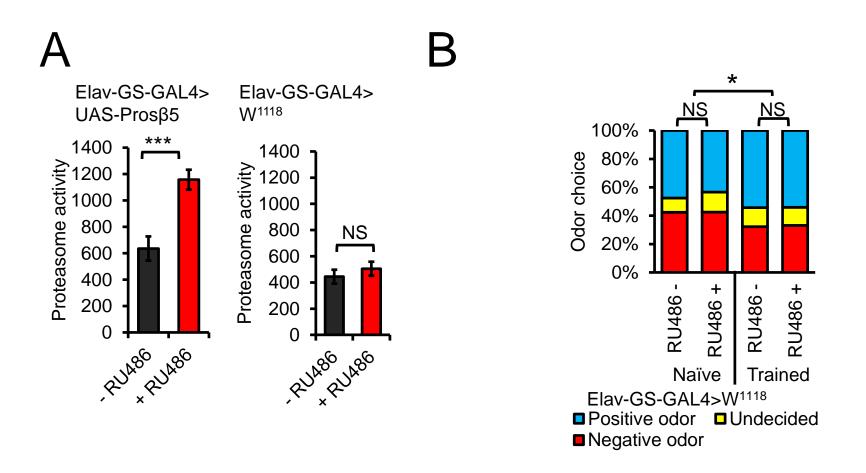




Supplemental Figure 4: Lifespan RU486 controls for Figure 1F-G.

	Elav-GS-GAL4>UAS-Prosβ5						
	- RU486	+ RU486	%	Chi-Square	Logrank		
Lifespan 1 N ≈ 250							
Median (50%)	77.4	86.5	11.9	66	4.44x10 <sup>-16</sup>		
Maximum (10%)	89.1	98.4	10.4				
Lifespan 2 N ≈ 175							
Median (50%)	63.3	68.7	8.5	77.8	<1x10 <sup>-18</sup>		
Maximum (10%)	68.7	77.6	12.9				
Lifespan 3 N ≈ 225							
Median (50%)	67.0	77.8	16.1	176	<1x10 <sup>-18</sup>		
Maximum (10%)	70.0	85.3	21.7				
Lifespan 4 N ≈ 250							
Median (50%)	83.9	88.9	5.9	22.6	2.03x10 <sup>-6</sup>		
Maximum (10%)	93.1	99.2	6.5				

	t				<u> </u>		
	Elav-GS-GAL4>W <sup>1118</sup>						
	- RU486	+ RU486	%	Chi-Square	Logrank		
Lifespan 1 N ≈ 175							
Median (50%)	98.0	97.3	-0.7	2.4	0.12		
Maximum (10%)	104.5	104.4	-0.2				
Lifespan 2 N ≈ 175							
Median (50%)	85.5	86.6	1.2	77.8	0.04		
Maximum (10%)	92.2	95.6	3.6				



## Supplemental Figure 6:

A. RU486-induced Prosβ5 overexpression increases under control of the Elav-GS-GAL4 (Elav-GS-GAL4>Prosβ5) driver increases 20S proteasome based on an Suc-LLVY-AMC activity assay. No increase in activity seen in flies lacking the transgene (Elav-GS-GAL4>W<sup>1118</sup>), N = 12. **B**. RU486 treatment does not alter behavior in olfaction aversion training. Assay performed in 10 day old Elav-GS-GAL4>W<sup>1118</sup> flies, N = 150.