Analysis of Longevity from Muscle mTORC1 Effector Flies

Isabelle Hatfield, Erika Yates, Matt Peloquin and Dave Bridges

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Experimental Design

These data are stored in the **Data** subfolder. This script was most recently run on Mon Feb 24 09:34:53 2014. There has been a total of **1117** deaths, with **1113** of natural causes and **1109** of identifiable genotypes excluding accidental deaths. The oldest fly recorded so far was **104** days old at time of death.

Gene Level Analysis

Number of Flies Examined

The total number of deaths for each cross and genotype, removing deaths that were accidental or not due to natural causes, the data is shown in Table 1 and 2.

The distribution of deaths in the samples are shown at a gender level in Figure 1.

Driver	Gene	shRNA/GAL4	shRNA/Tm3	NA
24B-Gal4	Atg5	140	39	0
24B-Gal4	Atg8a	57	72	3
24B-Gal4	Atg8b	100	81	2
24B-Gal4	HLH106	187	147	1

Table 1: Total Natural Deaths for Each Gene and Genotype

Driver	Gene	Gender	shRNA/GAL4	shRNA/Tm3	NA
24B-Gal4	Atg5	female	59	15	0
24B-Gal4	Atg5	male	81	24	0
24B-Gal4	Atg8a	female	10	29	0
24B-Gal4	Atg8a	male	47	43	3
24B-Gal4	Atg8b	female	54	46	2
24B-Gal4	Atg8b	male	46	35	0
24B-Gal4	HLH106	female	101	56	0
24B-Gal4	HLH106	male	86	91	1

Table 2: Total Natural Deaths by Gender for Each Gene and Genotype

Survival Analysis

All of these are relative to the reference Genotype which is the knockdown (GAL4/shRNA). The key packages used in this analysis were R [1], lubridate [2], plyr [3] and survival [4, 5]. The summary statistics from this analysis are shown in Tables 3 and 4.

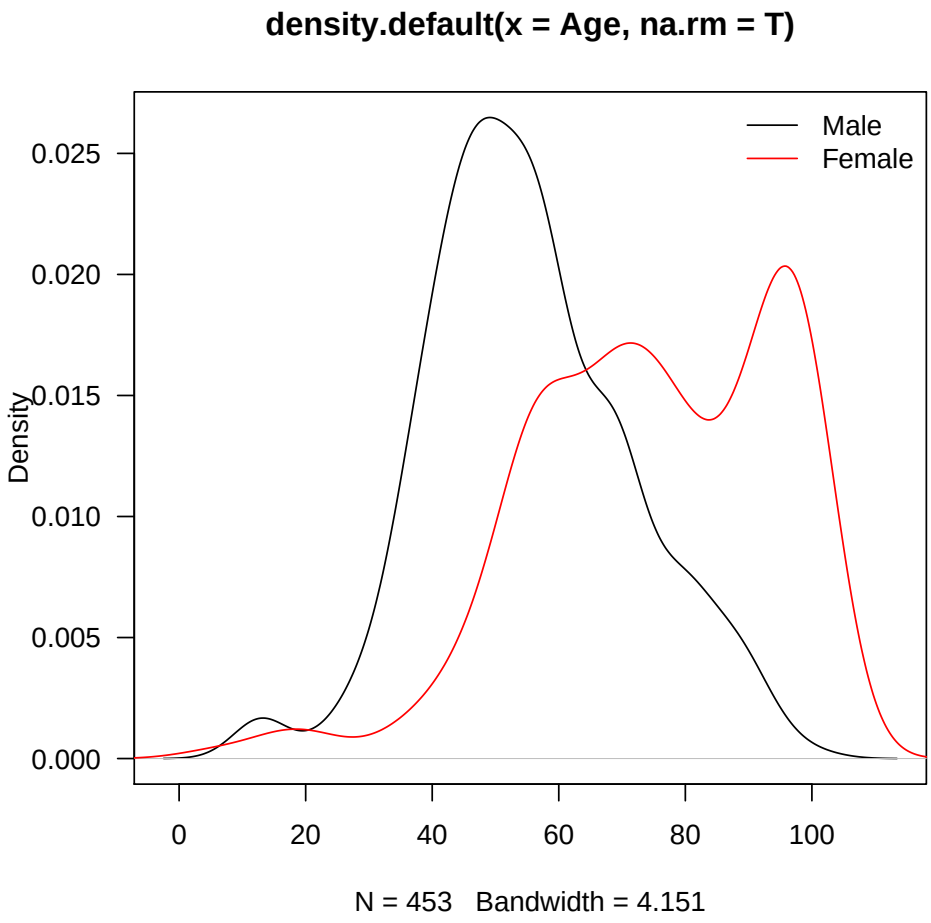

Figure 1: Histogram of Age Ranges for All Genotypes

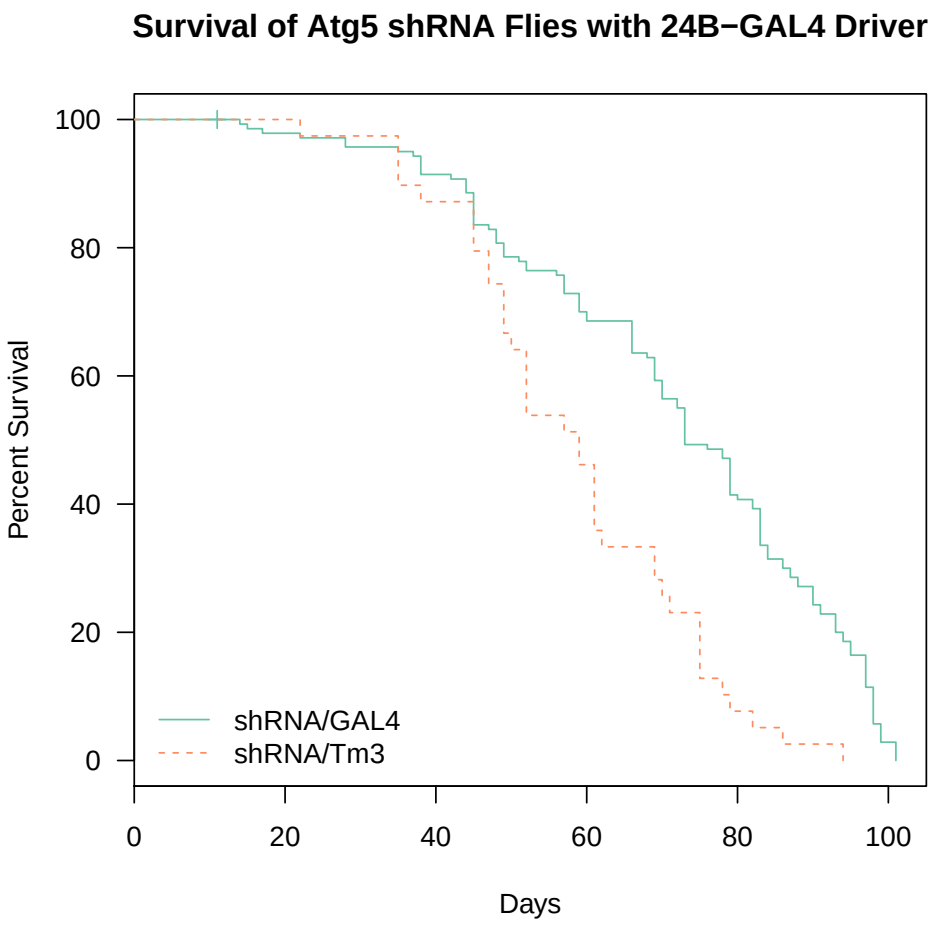

Figure 2: Survival Curve for 24B Driven Atg5 Knockdown

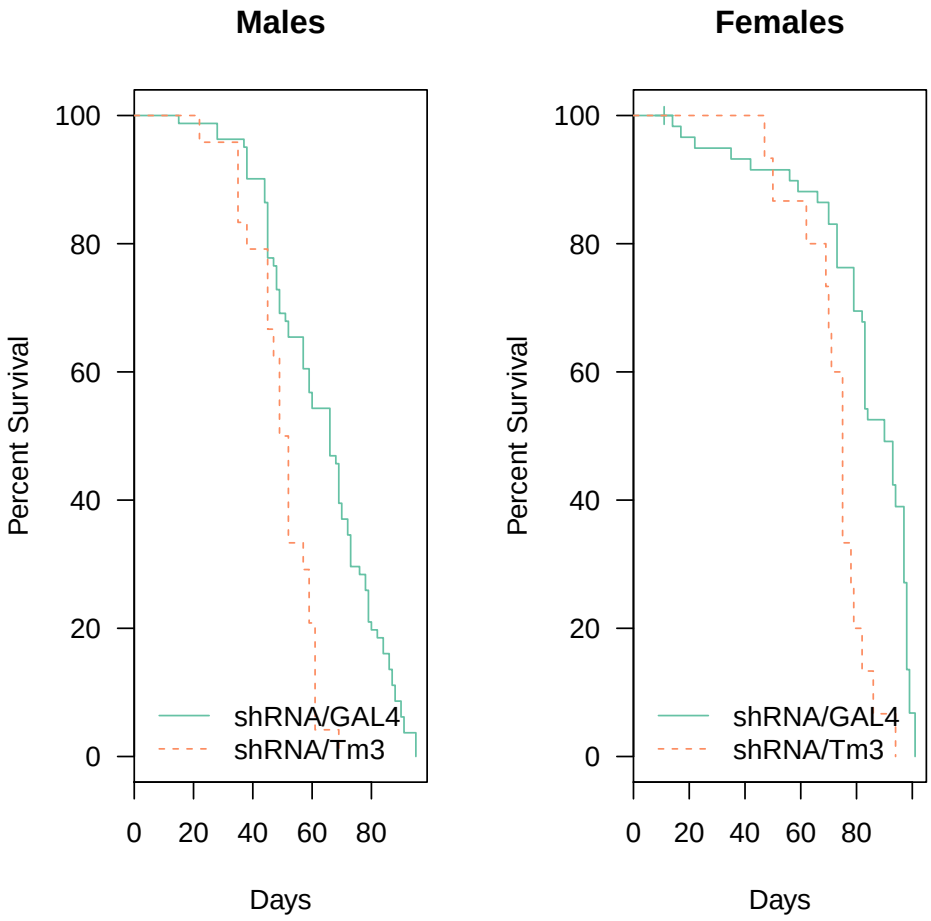

Figure 3: Survival Curves for 24B Driven Atg5 Knockdown

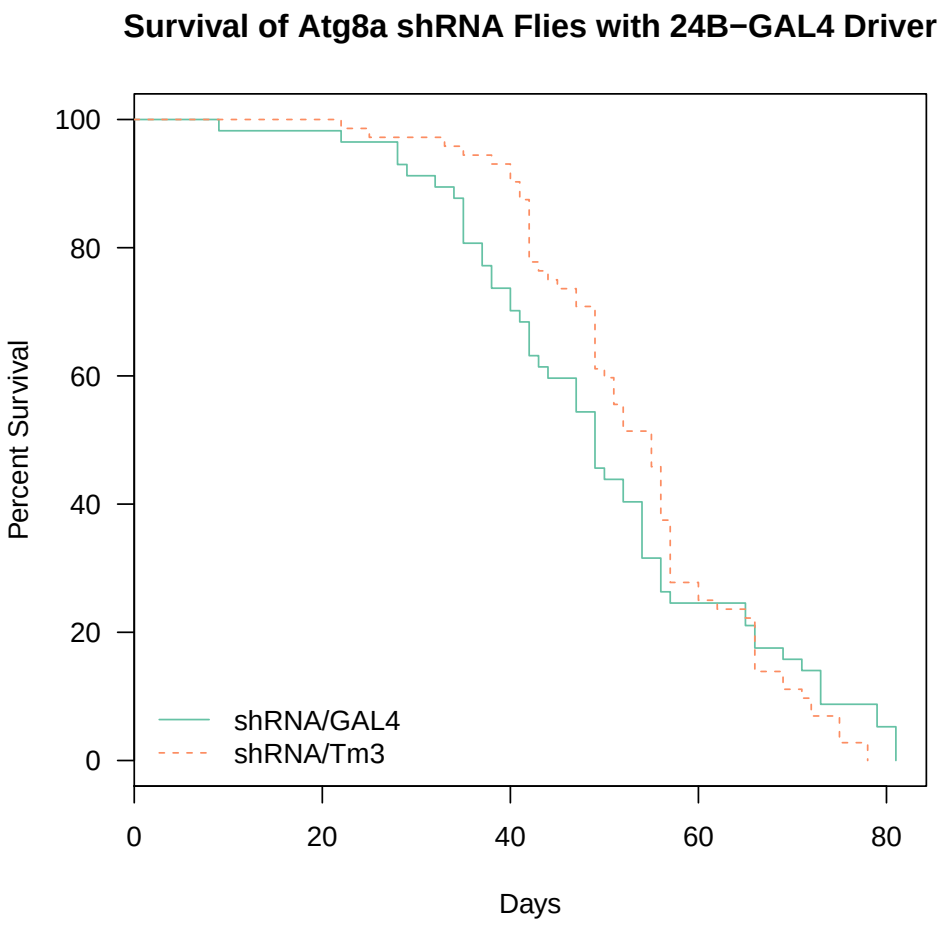

Figure 4: Survival Curve for 24B Driven Atg8a Knockdown

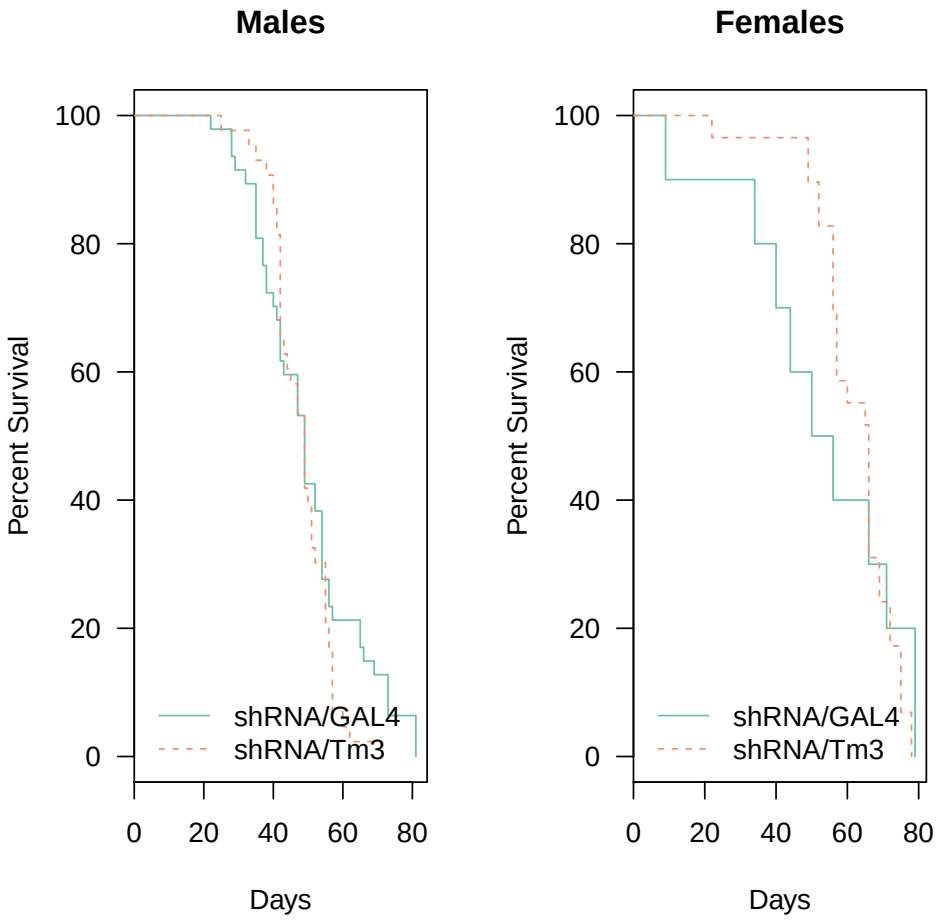

Figure 5: Survival Curves for 24B Driven Atg8a Knockdown

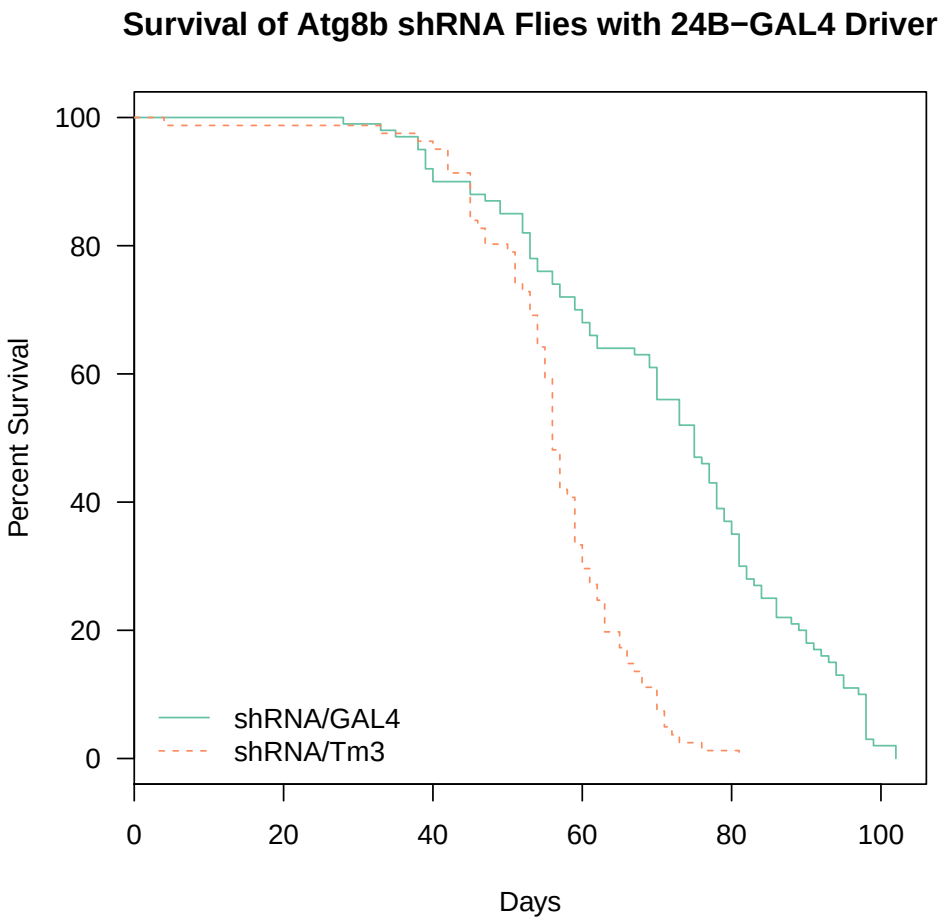

Figure 6: Survival Curve for 24B Driven Atg8a Knockdown

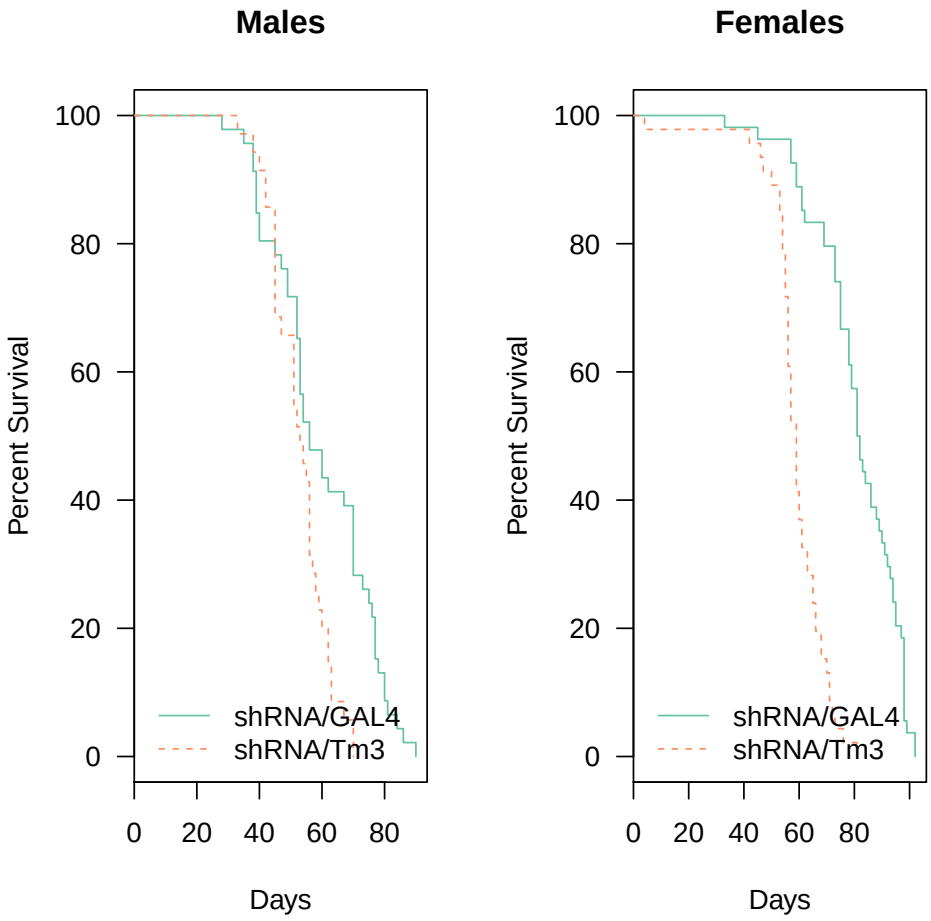

Figure 7: Survival Curves for 24B Driven Atg8b Knockdown

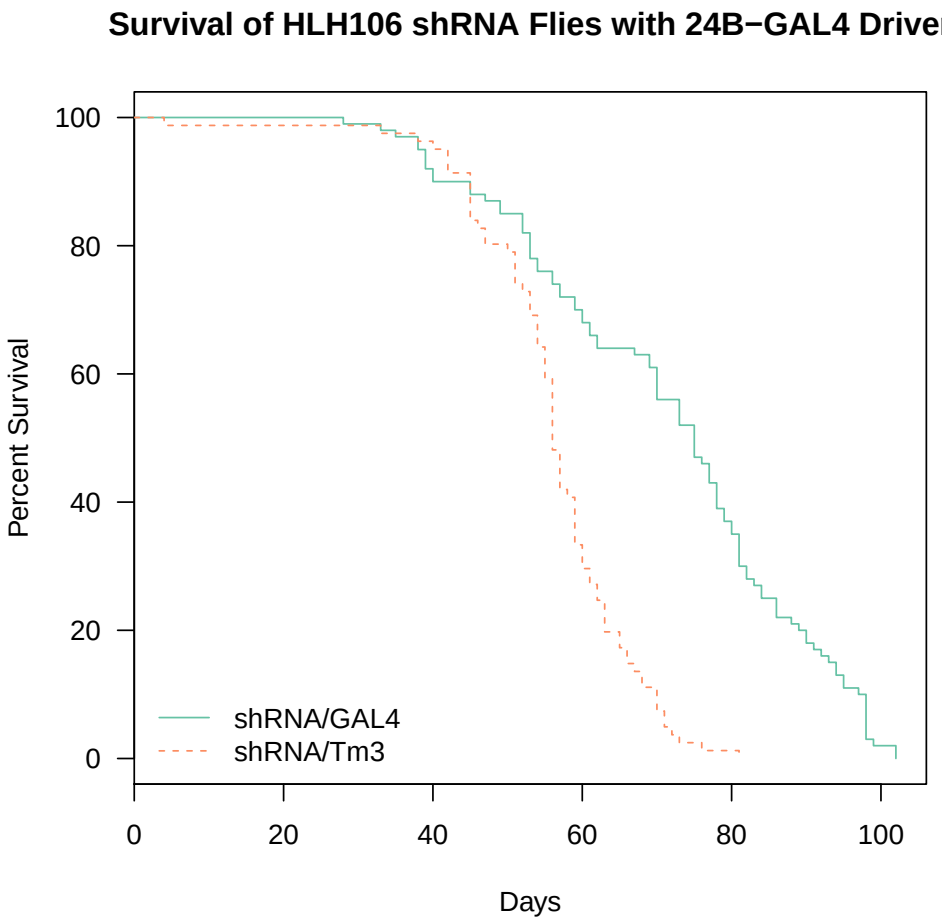

Figure 8: Survival Curve for 24B Driven HLH106 Knockdown

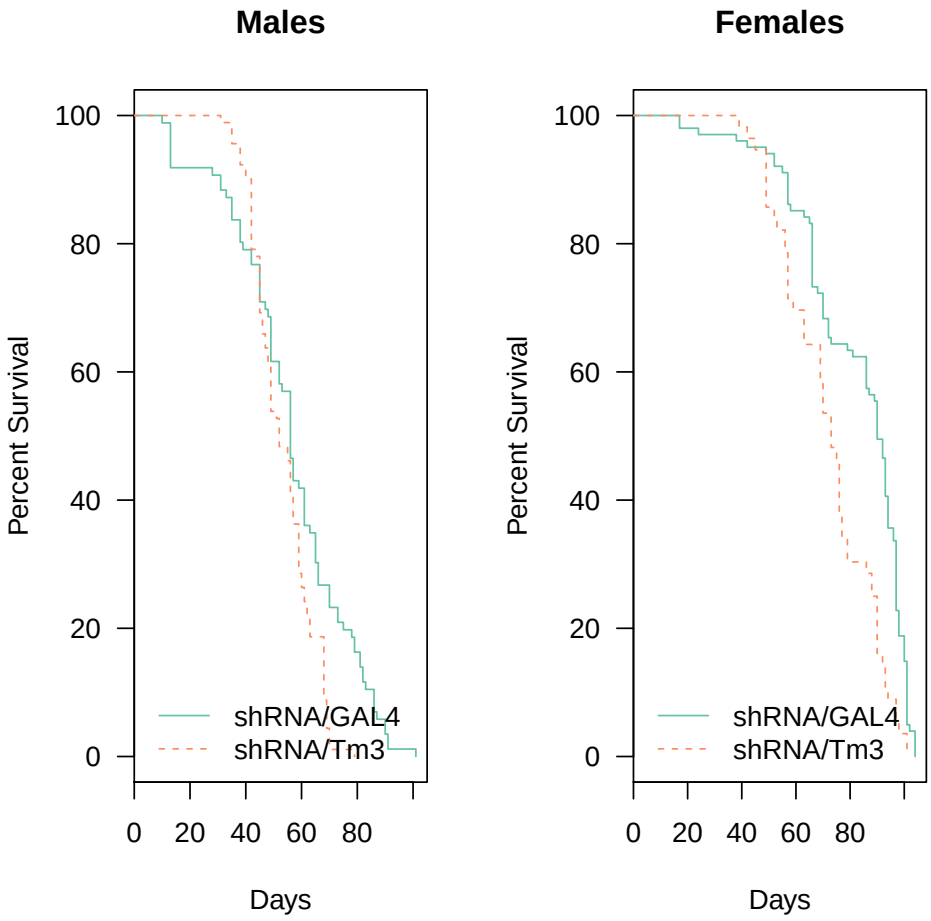

Figure 9: Survival Curves for 24B Driven HLH106 Knockdown

	n	logtest.p	waldtest.p	sctest.p
Atg5	179	0.000010893	0.000002431	0.000001138
Atg8a	129	0.907539650	0.907485407	0.907483753
Atg8b	181	0.000000000	0.000000000	0.000000000
HLH106	334	0.000000019	0.000000012	0.000000007

Table 3: Gene Level Tests for 24B-Gal4 Drivers

	n	logtest.p	waldtest.p	sctest.p
Atg5 male	105	0.000064349	0.000021212	0.000008170
Atg5 female	74	0.000296796	0.000081610	0.000028967
Atg8a male	90	0.201970740	0.202348648	0.200953953
Atg8a female	39	0.733739760	0.736828039	0.736655774
Atg8b male	81	0.002128659	0.002124445	0.001683301
Atg8b female	100	0.000000000	0.000000000	0.000000000
HLH106 male	177	0.001456808	0.001603990	0.001438531
HLH106 female	157	0.000048352	0.000026097	0.000018073

Table 4: Gene and Gender Level Tests for 24B-Gal4 Drivers

Atg5 Knockdown

The summary statistics from this analysis are shown in Table 5. In the case of muscle *Atg5* knockdown flies, we observed the knockdown flies dying later than the wildtypes with a hazard ratio of 0.402 (p=2.43e-06). This was true for both Males (HR=0.279, p=8.16e-05) and Females (HR=0.328, p=2.12e-05).

Atg8a Knockdown

The summary statistics from this analysis are shown in Table 6. In the case of muscle *Atg8b* knockdown flies, we observed no significant differences with a hazard ratio of 1.022 (p=0.907). This was true for both Males (HR=0.871, p=0.737) and Females (HR=0.751, p=0.202).

Atg8b Knockdown

The summary statistics from this analysis are shown in Table 7. In the case of muscle *Atg8b* knockdown flies, we observed the knockdown flies dying later than the wildtypes with a hazard ratio of 0.255 (p=3.62e-13). This was true for both Males (HR=0.119, p=6.5e-14) and Females (HR=0.455, p=0.00212).

HLH106 Knockdown

The summary statistics from this analysis are shown in Table 8. In the case of muscle *HLH106* knockdown flies, we observed the knockdown flies dying later than the wildtypes with a hazard ratio of 0.515 (p=1.15e-08). This was true for both Males (HR=0.48, p=2.61e-05) and Females (HR=0.594, p=0.0016).

References

[1] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2013.

[2] Garrett Grolemund and Hadley Wickham. Dates and times made easy with lubridate. *Journal of Statistical Software*, 40(3):1–25, 2011.

	Coef	SE	Hazard.Ratio	p
Total	0.912	0.194	0.402	0.00000243
Male	1.278	0.324	0.279	0.00008161
Female	1.115	0.262	0.328	0.00002121

Table 5: Gene Level Cox Proportional Hazard Tests for 24B-Gal4 Drivers and Atg5 Knockdown

	Coef	SE	Hazard.Ratio	p
Total	-0.021	0.184	1.022	0.907
Male	0.138	0.410	0.871	0.737
Female	0.286	0.224	0.751	0.202

Table 6: Gene Level Cox Proportional Hazard Tests for 24B-Gal4 Drivers and Atg8a Knockdown

[3] Hadley Wickham. The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40(1):1–29, 2011.

[4] Terry M Therneau. *A Package for Survival Analysis in S*, 2014. R package version 2.37-7.

[5] Terry M. Therneau and Patricia M. Grambsch. *Modeling Survival Data: Extending the Cox Model*. Springer, New York, 2000.

Session Information

- R version 3.0.2 (2013-09-25), `x86_64-apple-darwin10.8.0`
- Locale: `en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8`
- Base packages: base, datasets, graphics, grDevices, methods, splines, stats, utils
- Other packages: bibtex 0.3-6, bitops 1.0-6, lubridate 1.3.3, plyr 1.8, RColorBrewer 1.0-5, RCurl 1.95-4.1, reshape2 1.2.2, survival 2.37-7, xtable 1.7-1
- Loaded via a namespace (and not attached): digest 0.6.4, memoise 0.1, stringr 0.6.2, tools 3.0.2

	Coef	SE	Hazard.Ratio	p
Total	1.365	0.188	0.255	0.000000000000362
Male	2.130	0.284	0.119	0.000000000000065
Female	0.787	0.256	0.455	0.002124445047858

Table 7: Gene Level Cox Proportional Hazard Tests for 24B-Gal4 Drivers and Atg8b Knockdown

	Coef	SE	Hazard.Ratio	p
Total	0.663	0.116	0.515	0.000000011537224
Male	0.734	0.175	0.480	0.000026096889752
Female	0.521	0.165	0.594	0.001603989549559

Table 8: Gene Level Cox Proportional Hazard Tests for 24B-Gal4 Drivers and HLH106 Knockdown