Analysis of Longevity from Muscle TSC1/Raptor Flies

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Experimental Design

These data are stored in the **Data** subfolder. This script was most recently run on Mon Feb 24 09:33:55 2014. There has been a total of **3487** deaths, with **3435** of natural causes and **3385** 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 and looking at the controls in Figure 2. To examine the controls, and whether there are effects of the GAL4 or the shRNA we examined the median age of the controls from each cross. These data are shown in Figure 3. Still need to do some statistics to test if this is relevant.

Driver	Gene	shRNA/GAL4	shRNA/Tm3	GAL4/Tm6B	Tm3/Tm6B	NA
24B-Gal4	Raptor	0	2	4	2	0
24B-Gal4	Tsc1	354	314	246	143	12
C179-Gal4	Raptor	230	0	260	0	3
Hand-Gal4	Raptor	310	313	241	133	39
Hand-Gal4	Tsc1	310	237	209	58	5

Table 1: Total Natural Deaths 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].
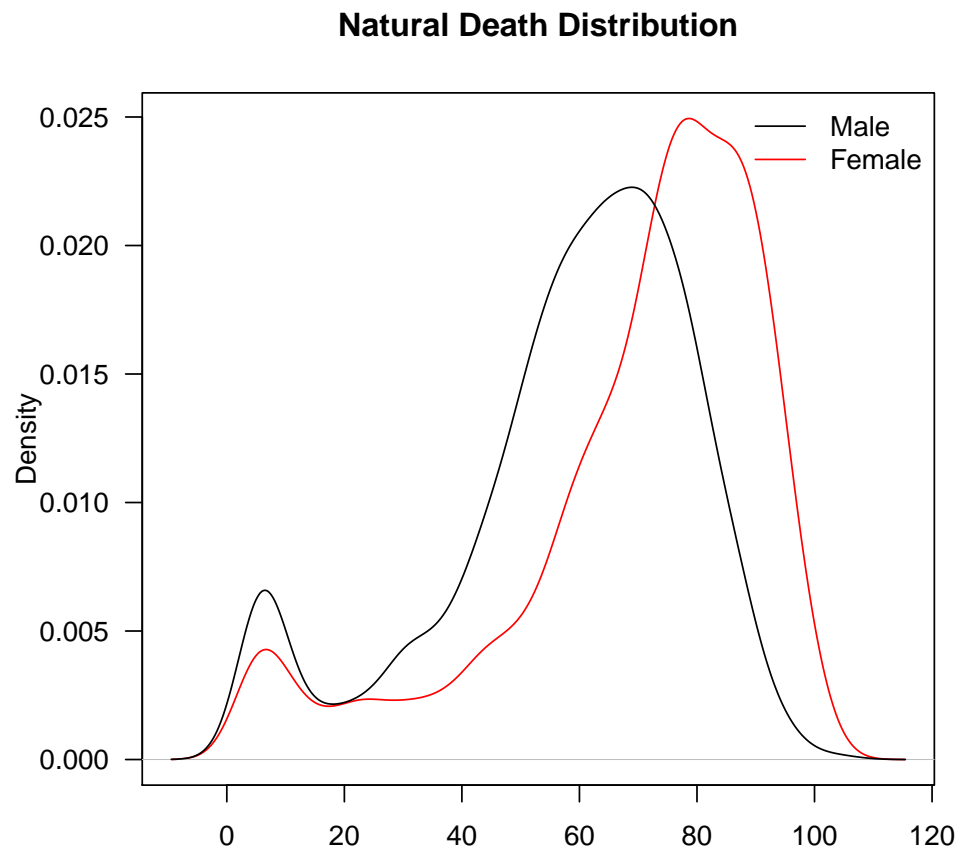
Natural Death Distribution

Figure 1: Histogram of Age Ranges for All Genotypes

Natural Death Distribution – Female Controls

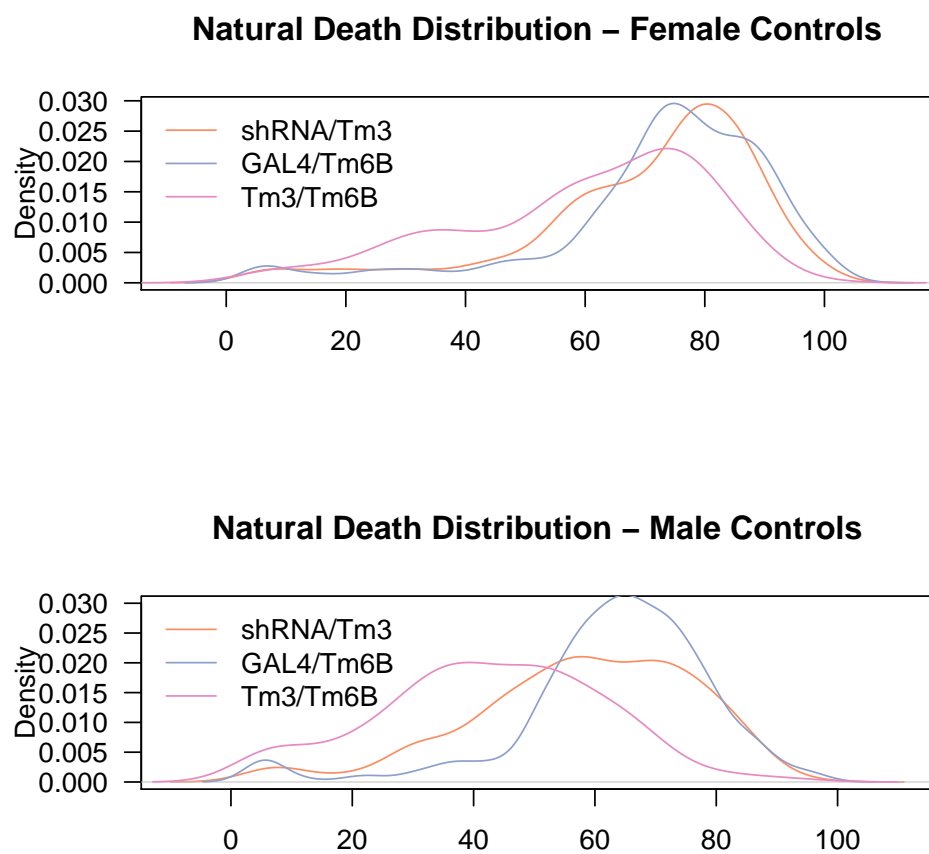**Natural Death Distribution – Male Controls**

Figure 2: Histogram of Age Ranges for Control Genotypes
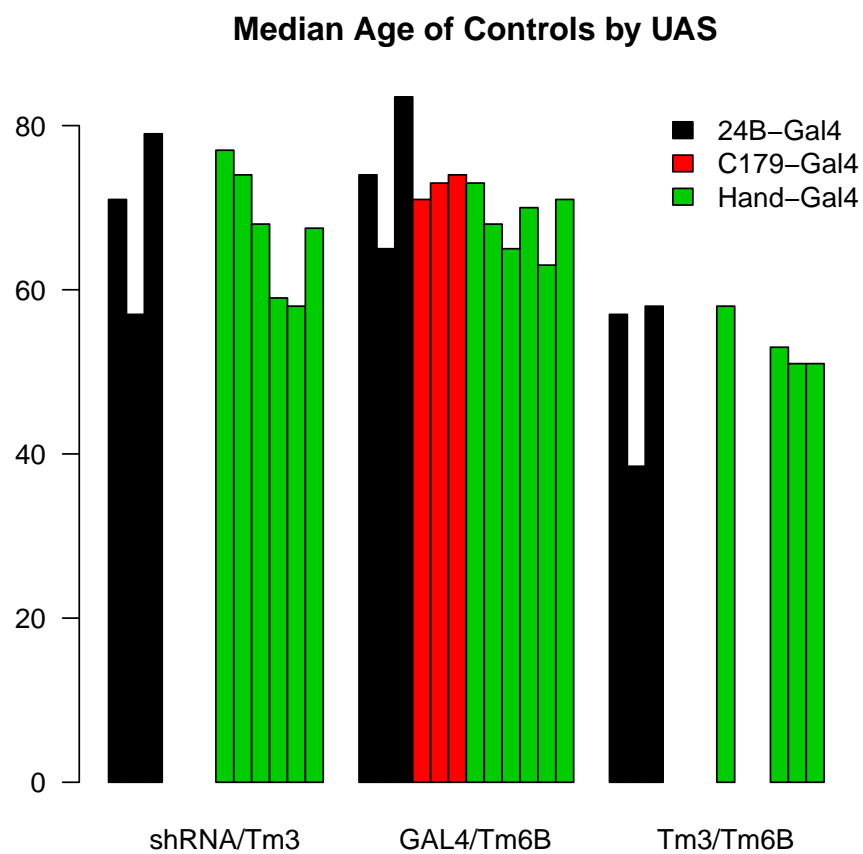
Figure 3: Median Ages for Control Genotypes

Driver	Gene	Gender	shRNA/GAL4	shRNA/Tm3	GAL4/Tm6B	Tm3/Tm6B	NA
24B-Gal4	Raptor	female	0	1	3	1	0
24B-Gal4	Raptor	male	0	1	1	1	0
24B-Gal4	Tsc1	female	179	172	133	87	7
24B-Gal4	Tsc1	male	175	142	113	56	5
C179-Gal4	Raptor	female	150	0	140	0	1
C179-Gal4	Raptor	male	80	0	120	0	2
Hand-Gal4	Raptor	female	166	184	135	80	21
Hand-Gal4	Raptor	male	144	129	106	53	18
Hand-Gal4	Tsc1	female	169	129	128	30	5
Hand-Gal4	Tsc1	male	141	108	81	28	0

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

24B-Gal4 Driver Analysis

This analysis is only for the three *Tsc1* shRNA alleles, since there were so few births in the *Raptor* knockdown alleles. The summary statistics from this analysis are shown in Table 3.

	n	logtest.p	waldtest.p	sctest.p
Raptor	6	0.52898	0.55256	0.54576
Tsc1	913	0.00000	0.00000	0.00000

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

	n	logtest.p	waldtest.p	sctest.p
Tsc1 male	429	0.000641609544611	0.00064	0.00058
Tsc1 female	484	0.000000000000000	0.00000	0.00000
Raptor male	2	0.239031891659252	0.99958	0.31731
Raptor female	4	0.095890967234548	0.99951	0.08326

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

We performed a variety of survival tests for the was a 24B-Gal4 driven *Tsc1* knockdowns (see Tables 3 and 4). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was highly significant (p-=0) for the combined comparasons and both males (p=0.0006416) and females (p=2.2e-16).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 5, 6 and 7. All comparasons were highly significant all less than 0.01695. The instantaneous hazard ratios ranged from 1.31 to 2.66, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 4 and 5.
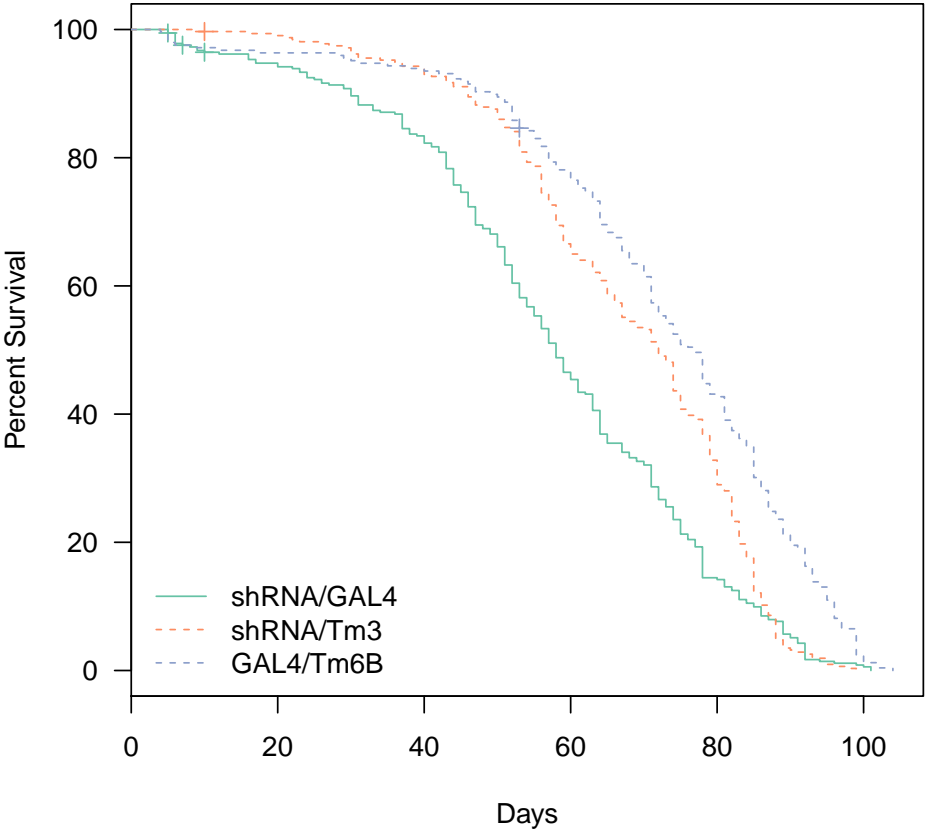
Figure 4: Survival Curve for 24B Driven Tsc1 Knockdown
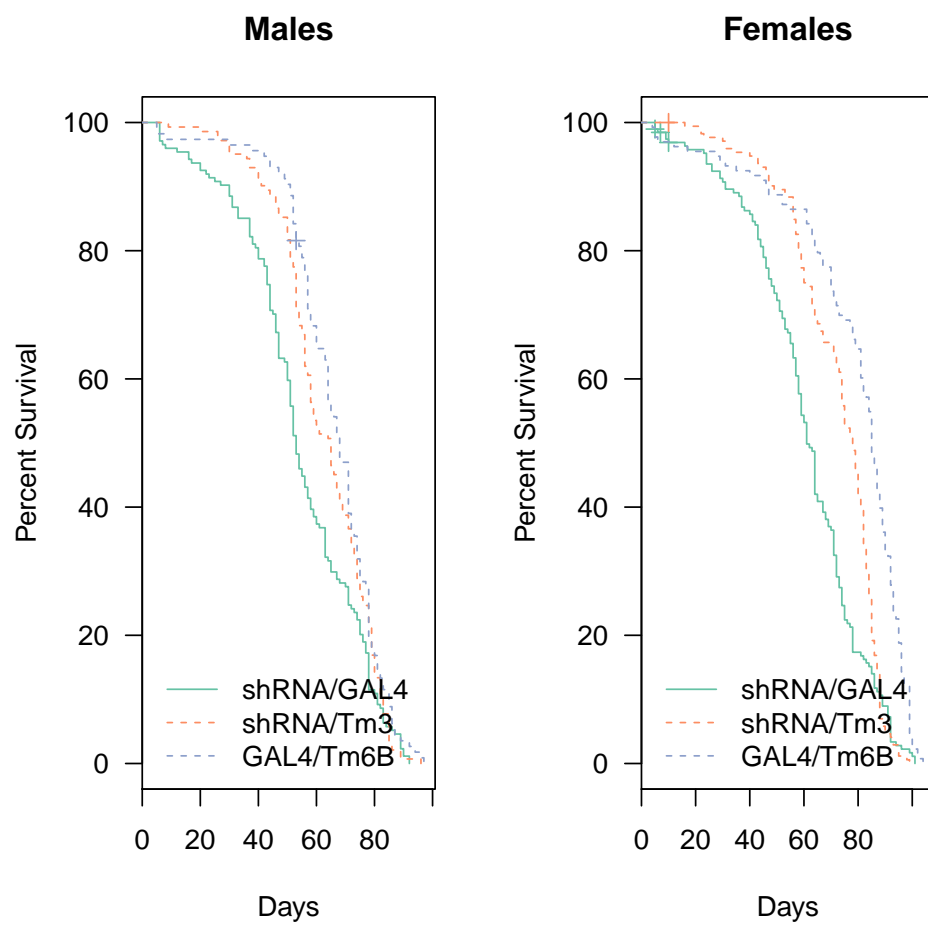
Figure 5: Survival Curves for 24B Driven Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	-0.336	0.078	1.399	0.000017529131172
GenotypeGAL4/Tm6B	-0.785	0.085	2.193	0.000000000000000

Table 5: Gene Level Cox Proportional Hazard Tests for 24B-Gal4 Drivers and Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	-0.272	0.114	1.312	0.016951567991777
GenotypeGAL4/Tm6B	-0.455	0.122	1.576	0.000191753055309

Table 6: Gene Level Cox Proportional Hazard Tests for Males with 24B-Gal4 Drivers and Tsc1 Knockdown

C179 Driver Analysis

This analysis is only for the three *Raptor* shRNA alleles, since we did not examine the effects of Tsc1 knockdown with this driver. The summary statistics from this analysis are shown in Table 8.

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We performed a variety of survival tests for the was a C179-Gal4 driven *Raptor* knockdowns (see Tables 8 and 9). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was not significant (p=0.123) for the combined comparason and females (p=0.052). There was a significant difference with the males (p=0.0881342).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 10, 11 and 12. The instantaneous hazard ratios ranged from 0.79 to 1.3, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 6 and 7.
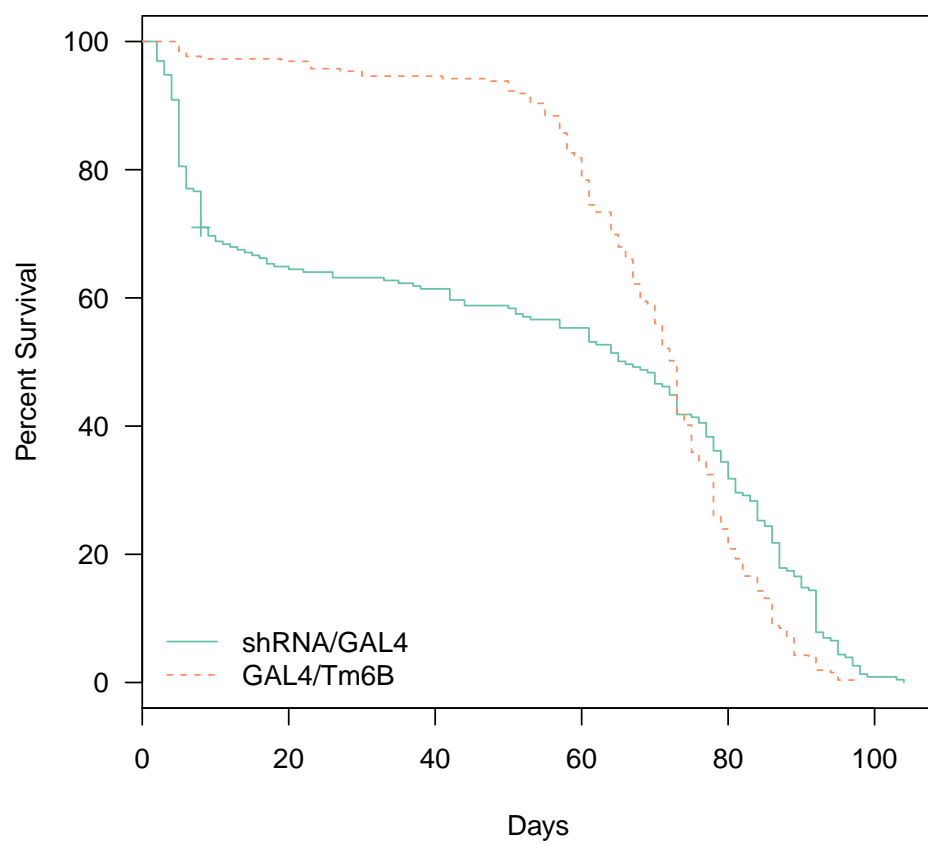
Figure 6: Survival Curve for C179 Driven Raptor Knockdown

Males

Females
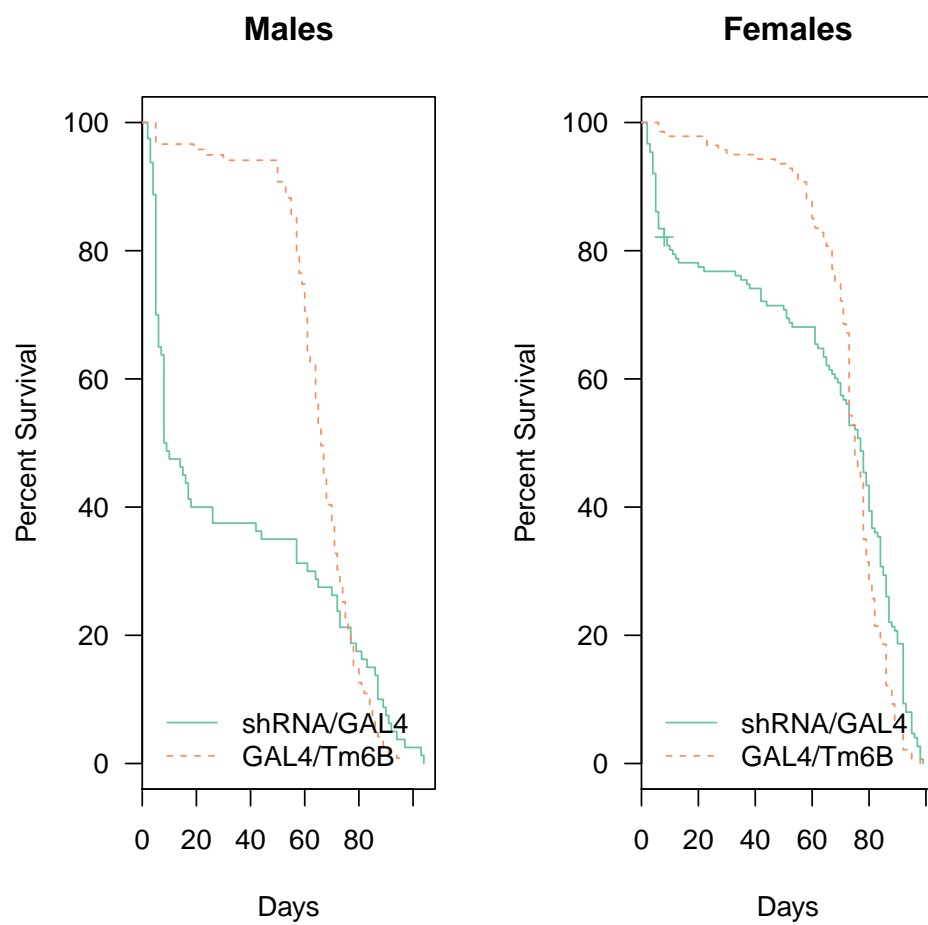
Figure 7: Survival Curves for C179 Driven Raptor Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	-0.316	0.109	1.372	0.00357969788482937
GenotypeGAL4/Tm6B	-0.977	0.119	2.657	0.00000000000000022

Table 7: Gene Level Cox Proportional Hazard Tests for Females with 24B-Gal4 Drivers and Tsc1 Knockdown

	n	logtest.p	waldtest.p	sctest.p
Raptor	489	0.12310	0.12371	0.12344
Tsc1				

Table 8: Gene Level Tests for C179-Gal4 Drivers

Hand-Gal4 Analysis

This analysis is for both the three *Tsc1* and the three *Raptor* shRNA alleles. The summary statistics from this analysis are shown in Table 13.

Tsc1 Knockdown with Hand-Gal4

We performed a variety of survival tests for the was a Hand-Gal4 driven *Tsc1* knockdowns (see Tables 13 and 14). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was highly significant (p=5.7e-14) for the combined comparasons and both males (p=0) and females (p=2.7e-11).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 15, 16 and 17. All comparasons were highly significant all less than 0.0133. The instantaneous hazard ratios ranged from 0.37 to 0.75, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 8 and 9.

Raptor Knockdown with Hand-Gal4

We performed further survival tests for the was a Hand-Gal4 driven *Raptor* knockdowns (see Tables 13 and 14). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was highly significant (p=0.000471) for the combined comparasons and both males (p=8.96e-06) and females (p=2.7e-05).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 18, 19 and 20. All comparasons were highly significant all less than 0.0417. The instantaneous hazard ratios ranged from 0.598 to 1.46, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 10 and 11.
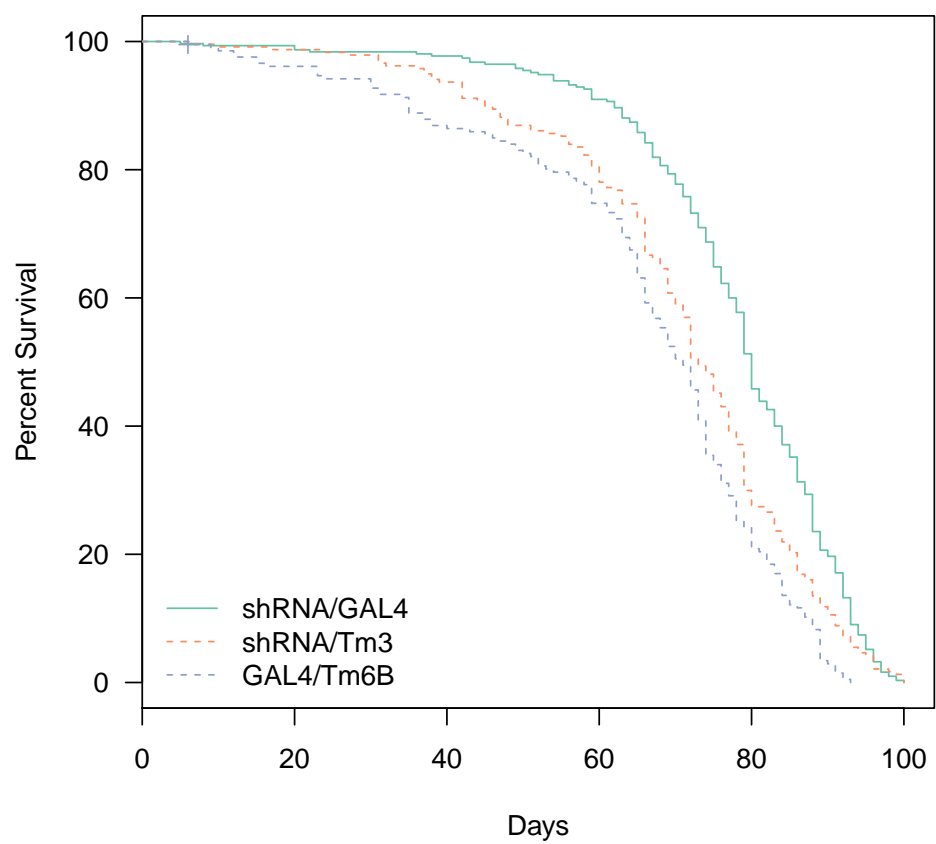
Figure 8: Survival Curve for Hand Driven Tsc1 Knockdown
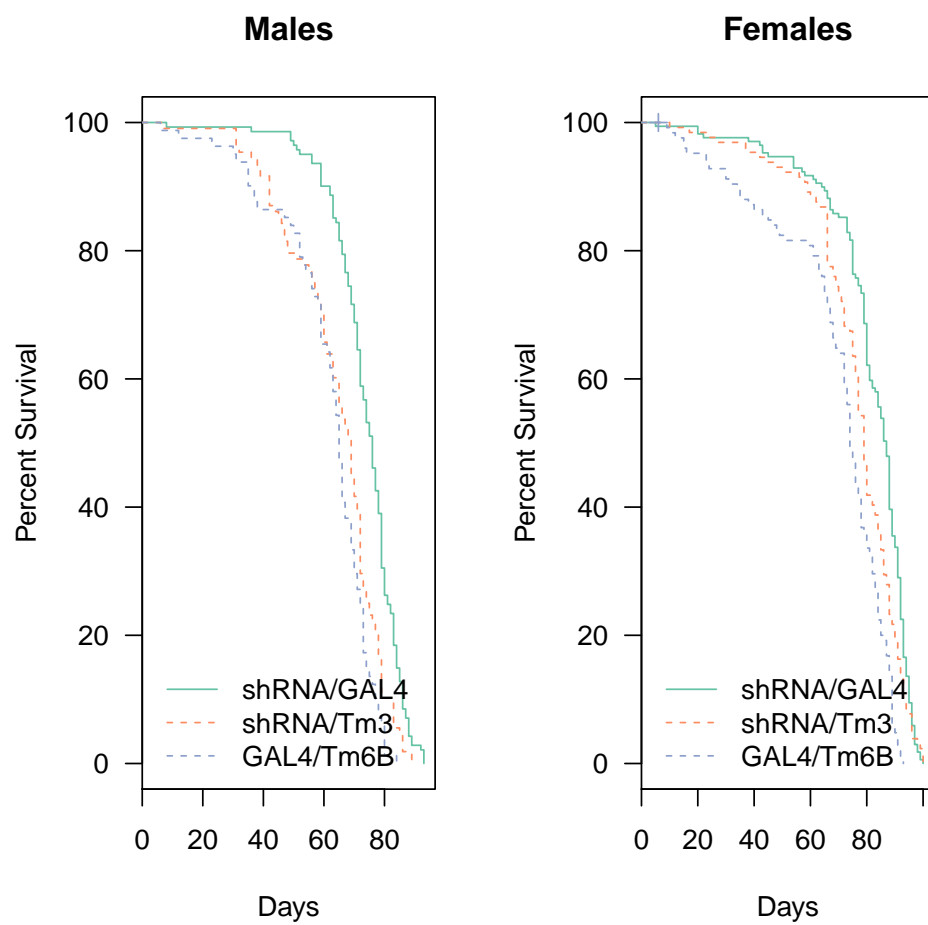
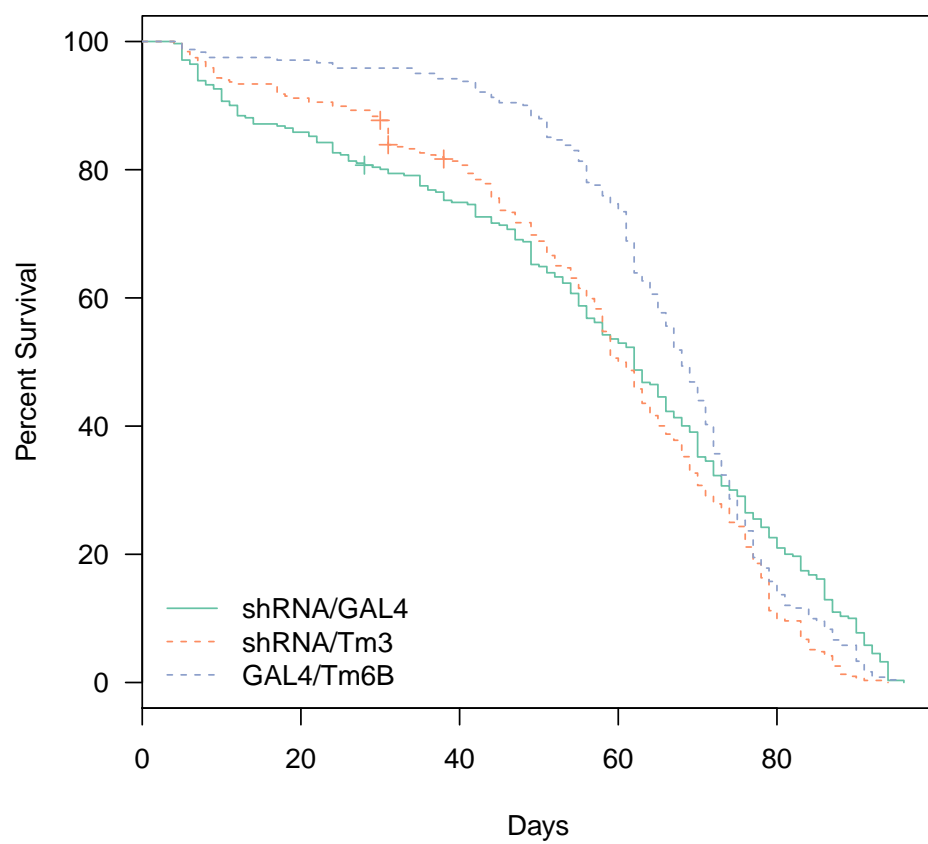Figure 9: Survival Curves for Hand Driven Tsc1 Knockdown

Figure 10: Survival Curve for Hand Driven Raptor Knockdown
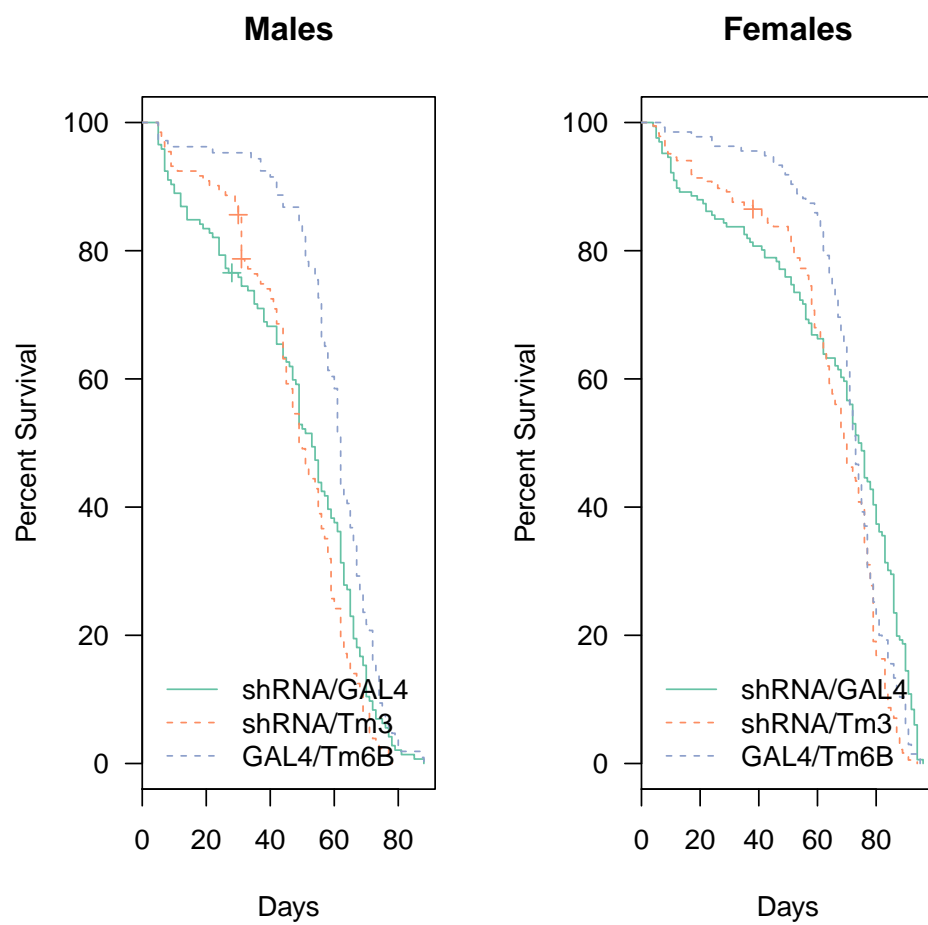
Figure 11: Survival Curves for Hand Driven Raptor Knockdown

	n	logtest.p	waldtest.p	sctest.p
Raptor male	199	0.08813	0.08499	0.08427
Raptor female	290	0.05241	0.05209	0.05162

Table 9: Gene and Gender Level Tests for C179-Gal4 Drivers

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	0.146	0.095	0.865	0.124

Table 10: Gene Level Cox Proportional Hazard Tests for C179-Gal4 Drivers and Raptor Knockdown

Strain Level Analysis

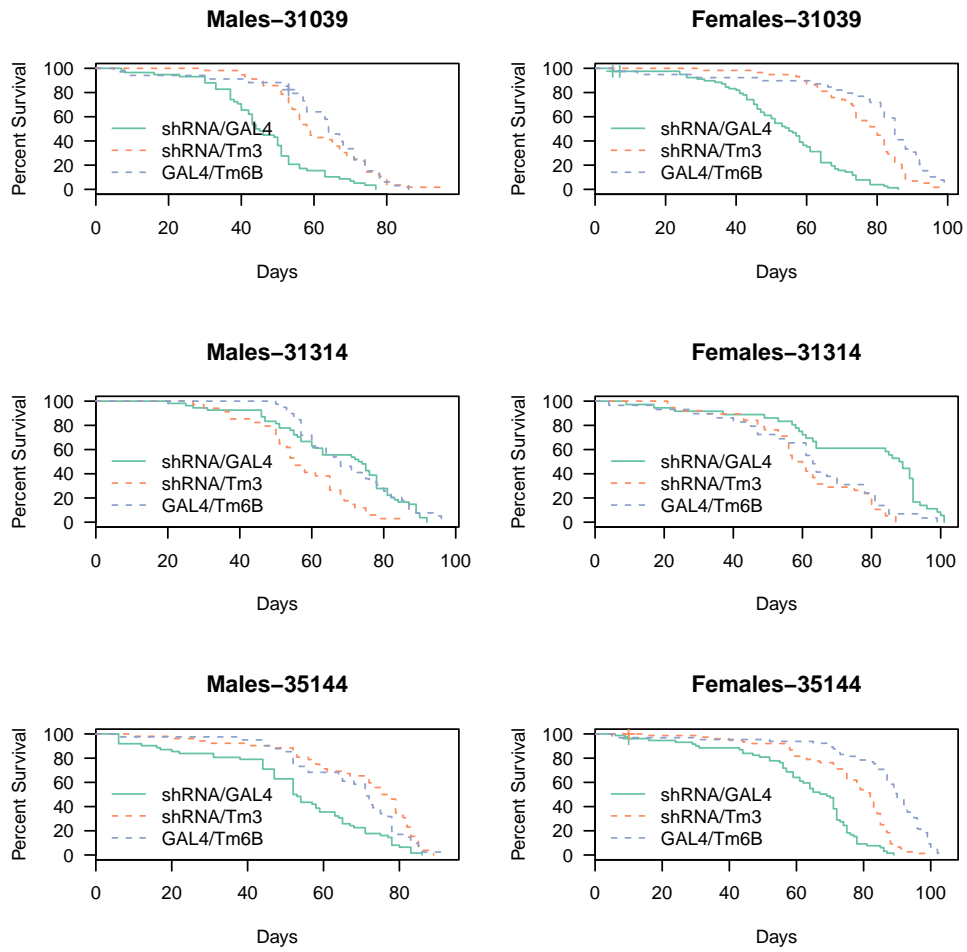## Strain Level Graphs for C179-Gal4 Driven Knockdowns

16

Figure 12: Survival Curves for 24B Driven Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	-0.261	0.152	1.299	0.0849881

Table 11: Gene Level Cox Proportional Hazard Tests for Males with C179-Gal4 Drivers and Raptor Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	0.236	0.122	0.790	0.052

Table 12: Gene Level Cox Proportional Hazard Tests for Females with C179-Gal4 Drivers and Raptor Knockdown

## Strain Level Graphs for C179-Gal4 Driven Knockdowns
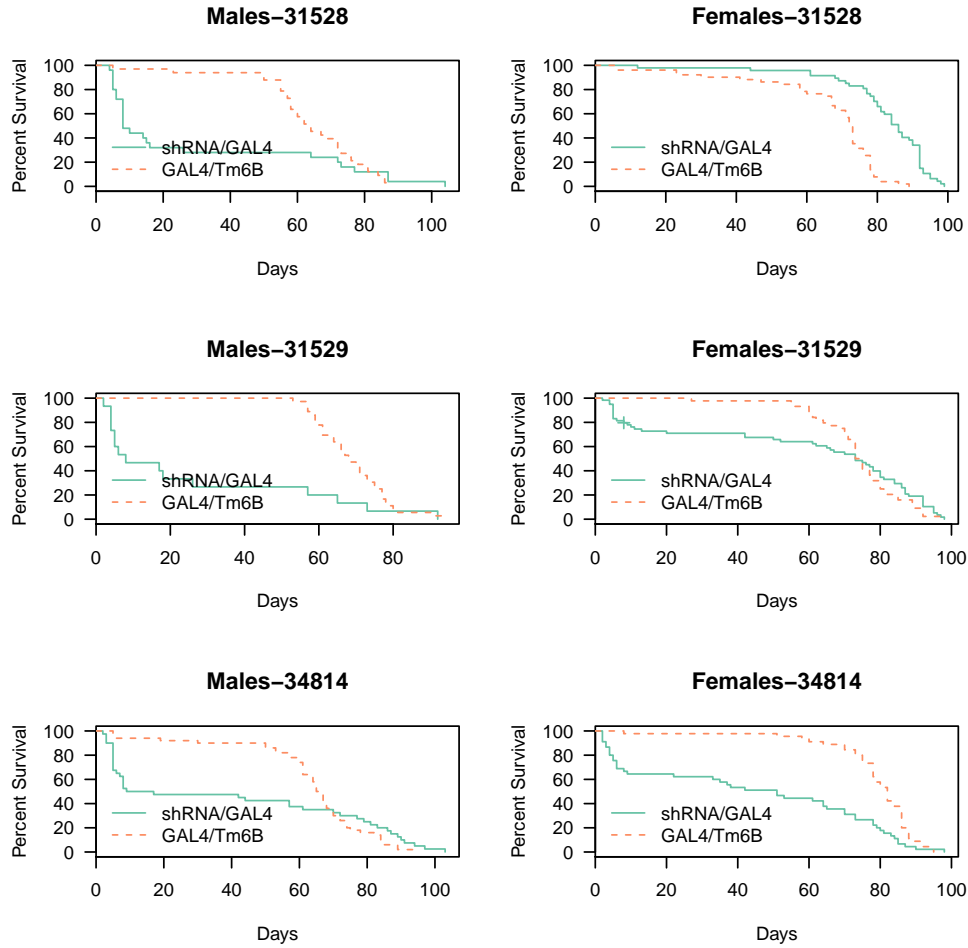
	n	logtest.p	waldtest.p	sctest.p
Raptor	864	0.00047077489618619	0.00039	0.00037
Tsc1	753	0.00000000000005718	0.00000	0.00000

Table 13: Gene Level Tests for Hand-Gal4 Drivers

	n	logtest.p	waldtest.p	sctest.p
Tsc1 male	330	0.00000000001070799	0.00000	0.00000
Tsc1 female	423	0.00000000002700662	0.00000	0.00000
Raptor male	379	0.00000896462415934	0.00001	0.00001
Raptor female	485	0.00002698327196304	0.00003	0.00002

Table 14: Gene and Gender Level Tests for Hand-Gal4 Drivers
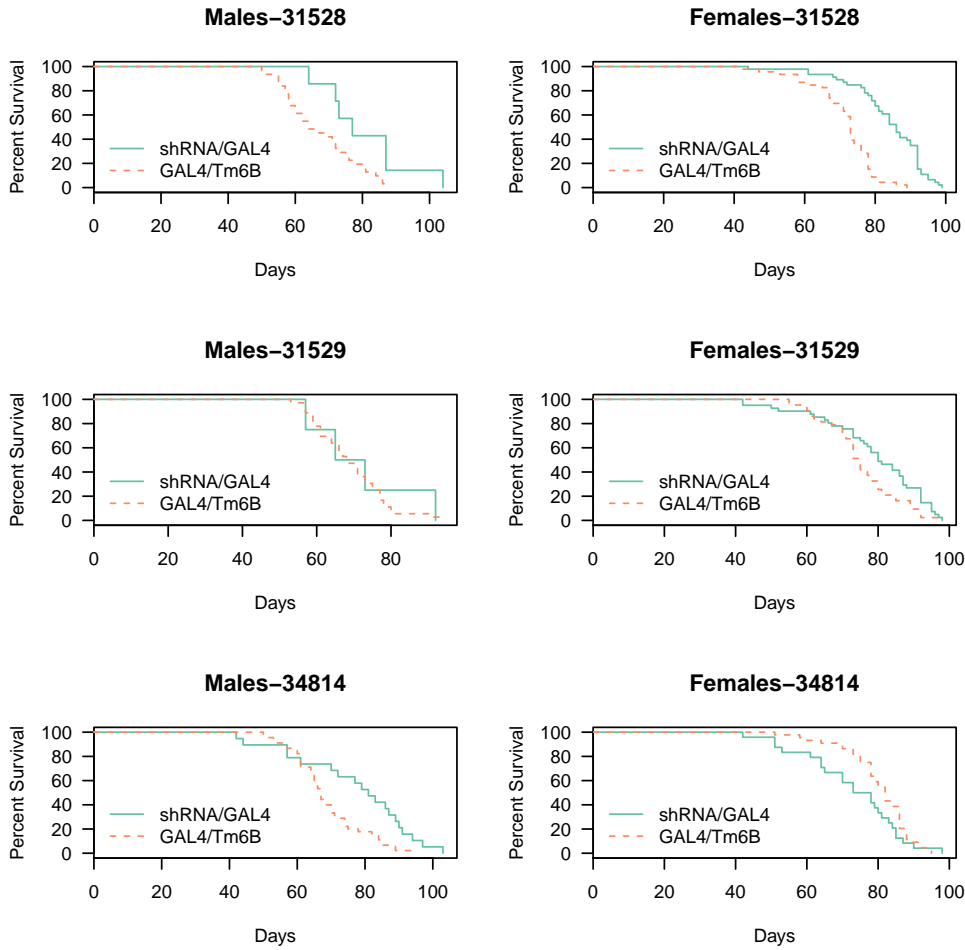
	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	0.343	0.087	0.710	0.000
GenotypeGAL4/Tm6B	0.731	0.092	0.481	0.000

Table 15: Gene Level Cox Proportional Hazard Tests for Hand-Gal4 Drivers and Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	0.633	0.130	0.531	0.00000121085825067
GenotypeGAL4/Tm6B	1.002	0.147	0.367	0.00000000000946199

Table 16: Gene Level Cox Proportional Hazard Tests for Males with Hand-Gal4 Drivers and Tsc1 Knockdown

## Strain Level Graphs for Hand-Gal4 Driven Knockdowns
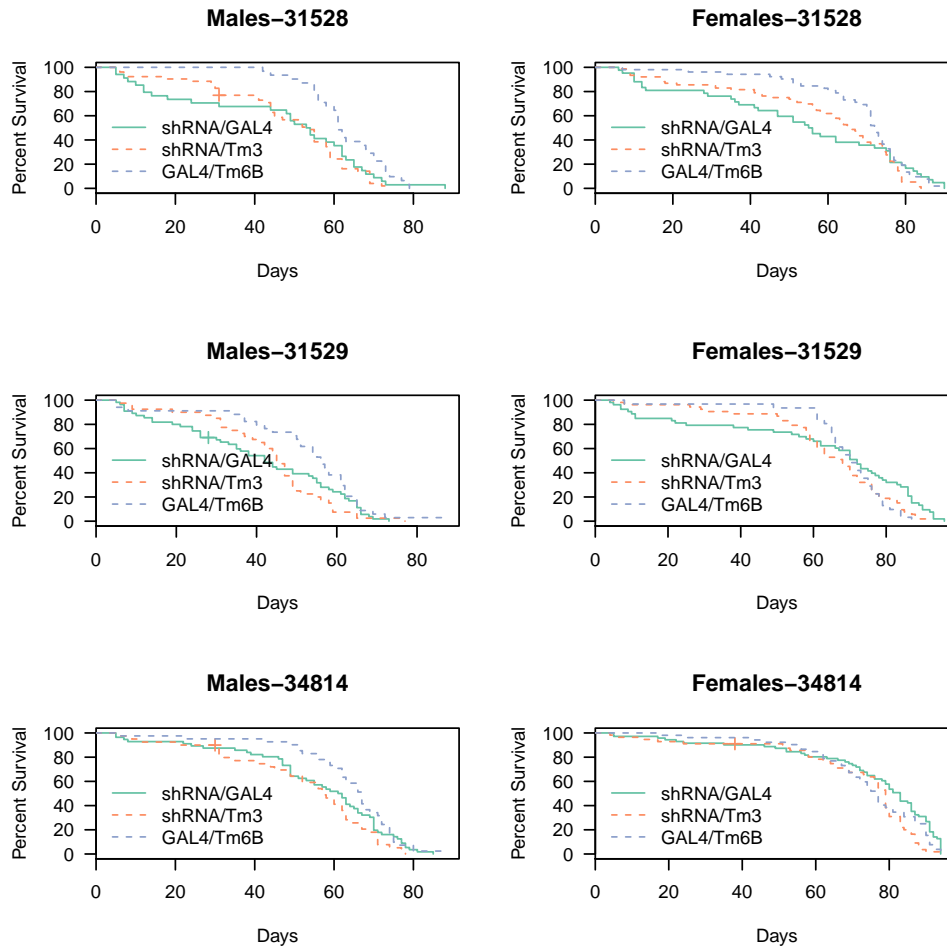
	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	0.292	0.118	0.747	0.01329324928242348
GenotypeGAL4/Tm6B	0.889	0.124	0.411	0.00000000000081579

Table 17: Gene Level Cox Proportional Hazard Tests for Females with Hand-Gal4 Drivers and Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	0.287	0.082	0.751	0.0005080187
GenotypeGAL4/Tm6B	0.002	0.087	0.998	0.9818157265

Table 18: Gene Level Cox Proportional Hazard Tests for Hand-Gal4 Drivers and Raptor Knockdown
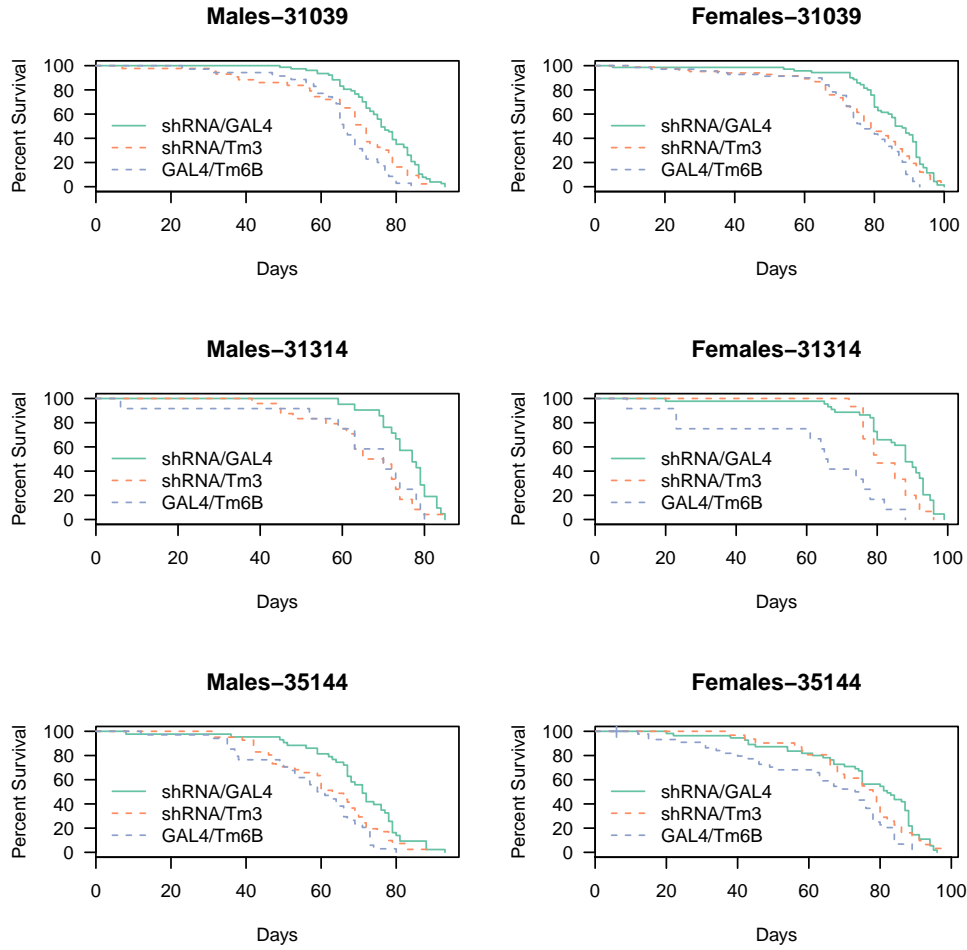
	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	0.250	0.123	0.779	0.0416921408
GenotypeGAL4/Tm6B	-0.382	0.128	1.465	0.0029149791

Table 19: Gene Level Cox Proportional Hazard Tests for Males with Hand-Gal4 Drivers and Raptor Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeshRNA/Tm3	0.514	0.112	0.598	0.0000047329
GenotypeGAL4/Tm6B	0.244	0.118	0.784	0.0388410327

Table 20: Gene Level Cox Proportional Hazard Tests for Females with Hand-Gal4 Drivers and Raptor Knockdown

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.

[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