Rapamycin supplementation of *Drosophila melanogaster* larvae results in less viable adults with smaller cells

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SUPPLEMENTARY MATERIALS

An extended description of GAMM statistical methods

GAMMs are an extension to the Generalized Linear Mixed Models (GLMM) and use penalized smoothing functions of some predictor variables (smooths) to model nonlinear relationships. Using GAMMs enabled us to analyse age differences via log mortality rate rather than differences in the cumulative survival curves shown in Kaplan-Meier plots (see also Fig. 3 in the paper). The mortality rate in generalized models is modelled using count data models with number of deaths as dependent variable and offset set to log of individuals days lived (exposures, classic life table approach). We use a negative binomial distribution (log as the link function) to account for potential over-dispersion and Restricted Maximum Likelihood (REML) as a fitting method. Mixed effect models have two kinds of terms: fixed and random. We considered four fixed terms across all tested models: sex, treatment, an interaction between these two variables, and a smooth function of age modelled separately for each combination of sex and treatment levels (`s(Age,by=Tr:Sex, bs="ps")` in R notation, where "*ps*" denotes P-spline basis). We tested several combinations of random smooth terms using AIC selection (Tab. S2) (e.g. [1]). We considered two categories of models: (i) based on simple, independent, smooth random effects ("*re*" basis), which included random intercepts and various random slopes, and (ii) models based on factor smooth interactions ("*fs*" basis). We selected the most parsimonious model (Tab. S3) according to the minimum AIC (Tab. S2). The model includes such random effect terms as (i) factor smooth interaction between isoline and age for sex (`s(Age,Iso,by=Sex,bs="fs",m=1)` in R notation), and (ii) the analogical interaction for treatment (`s(Age,Iso,by=Tr,bs="fs",m=1)` in R notation). The effects of all smooth terms on the linear predictor scale (log mortality rate scale) are shown in Fig. S2. For different application of very similar models please see [2] and [3].

The fitted GAMM model was used to calculate marginal mortality rates. The marginal mortality rates is based on the concept of population heterogeneity [4], which states that the population changes its composition with time, because individuals / population groups of the highest frailty (i.e., mortality rates) are expected to die out at faster rates. The marginal

mortality rate was used to determine the pattern of mortality rate that includes the effect of all isolines (Figs. 4 & 5 in the paper) and to test the goodness of fit (Fig. S1). The marginal mortality $\mu_M(x)$ was calculated according to the formula:

$$\mu_M(x) = \Sigma_z n_z s_z(x) \mu_z(x) / \Sigma_z n_z s_z(x), \quad (1)$$

where x is age, n_z is an initial abundance of the isoline z and $s_z(x)$ and $\mu_z(x)$ are survivorship and mortality rate of the isoline z at age x. $\mu_z(x)$ is derived from the model predictions (*mgcv* package, `predict.gam()` function, "*response*" type), while $s_z(x)$ is computed as $\exp(-h_z(x))$, where $h_z(x)$ is the age cumulative isoline z mortality rate.

To analyse the „base line" mortality rates with the effects of isolines excluded from model predictions, we utilized the concept of conditional mortality. The differences in conditional log mortality rates between studied groups (see Fig. 6 in the paper) were calculated using model predicted values of log mortality rates for different combinations of sex and treatment with the exclusion of random effects. The piece-wise confidence intervals of the differences were calculated using a bootstrap percentile method (described below). The differences between the estimated marginal mortality rates (Fig. 5 in the paper) were calculated analogically, however the procedure included also the averaging across the effects of all isolines using eq. 1.

To calculate the confidence intervals for the differences in log marginal mortality rates (Fig. 5) as well as for the differences in conditional log mortality rates (Fig. 6), we used predicted log mortality rates (with included or excluded random effects, respectively) and their piece-wise standard errors for each isoline, sex, treatment and age *(mgcv* package, `predict.gam()` function, "*link*" type). These predictions or their modifications (e.g., eq. 1) as well as standard errors were used in the bootstrap parametric percentile algorithm (100,000 replicates for each combination of sex, treatment, and isolines) (e.g. [5]). The Bootstrap method allowed us to calculate the median as well as the 0.025 and 0.975 percentiles of each type of age-specific log mortality rates, which were used as 95% confidence intervals.

The goodness of fit of the presented model was analysed graphically, comparing the predicted marginal survivorship ($\exp(-h_M(x))$ calculated analogously to $\exp(-h_z(x))$) with the Kaplan-Meier curves (Fig. S1a). We found almost the perfect coverage of the fitted model and the Kaplan-Meier estimator. As expected, there also were no problems with the autocorrelation of the time series (Fig. S1b) [6].

The dataset used (*rda* R format) and the R code of the entire analysis are available in the separate supplementary material files. In addition, the reproductive materials for all analyses, including our dataset, R code, and the results of fitted models and bootstrap are available online at https://github.com/MaciejDanko/Rapamycin.

References

1. Burnham KP, Anderson DR. 2004 Multimodel inference: Understanding AIC and BIC in model selection. *Sociol. Methods Res.* **33**, 261–304. (doi:10.1177/0049124104268644)
2. Sóskuthy M. 2021 Evaluating generalised additive mixed modelling strategies for dynamic speech analysis. *J. Phon.* **84**, 101017. (doi:10.1016/j.wocn.2020.101017)
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4. Vaupel JW, Missov TI. 2014 Unobserved population heterogeneity: A review of formal relationships. *Demogr. Res.* **31**, 659–686. (doi:10.4054/DemRes.2014.31.22)
5. Efron B, Tibshirani RJ. 1993 *An Introduction to the Bootstrap*. Boca Raton, Florida, USA: Chapman \& Hall/CRC.
6. van Rij J, Wieling M, Baayen RH, van Rijn H. 2020 itsadug: Interpreting Time Series and Autocorrelated Data Using GAMMs.

Supplementary figures and tables

Figure S1

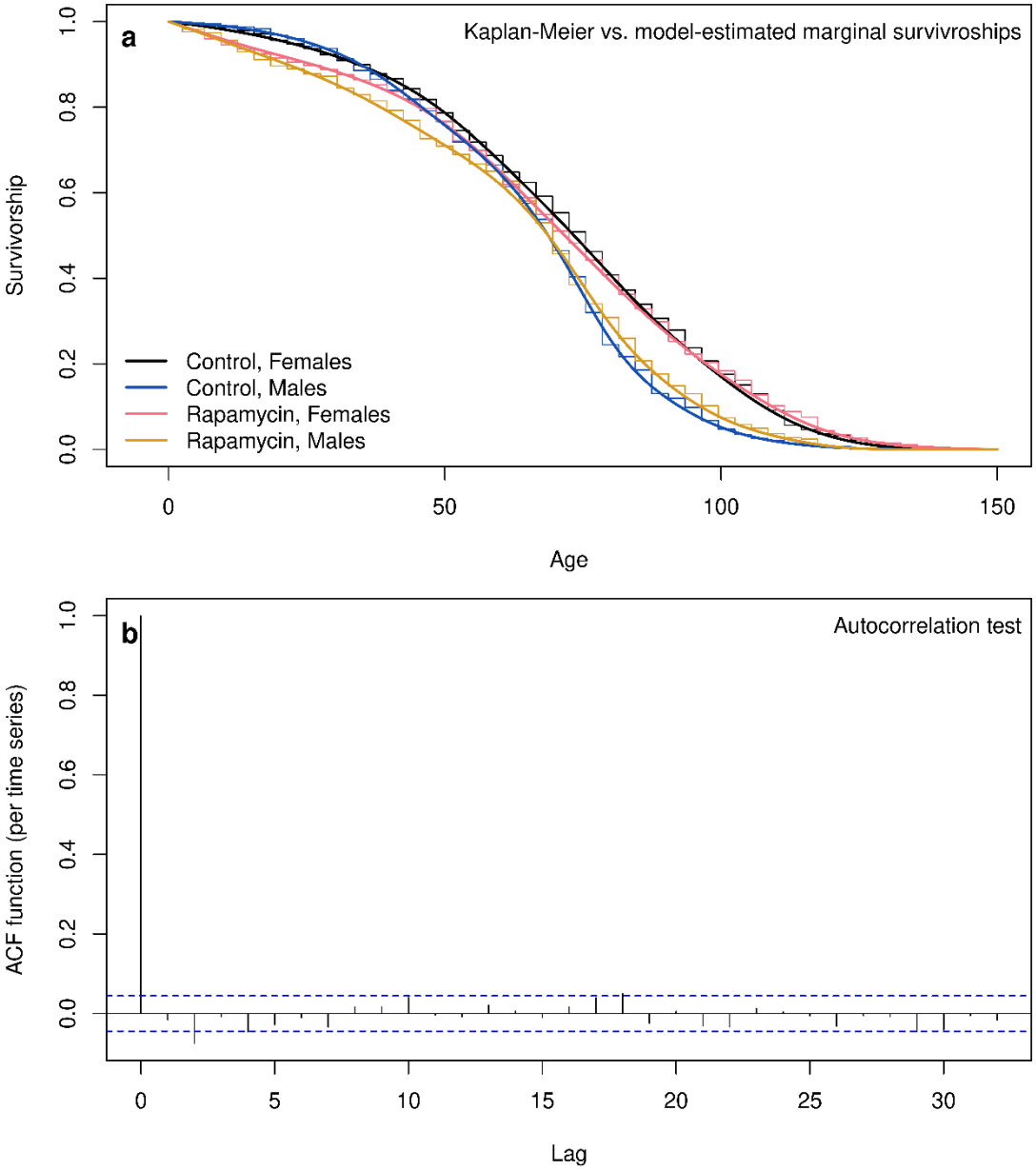
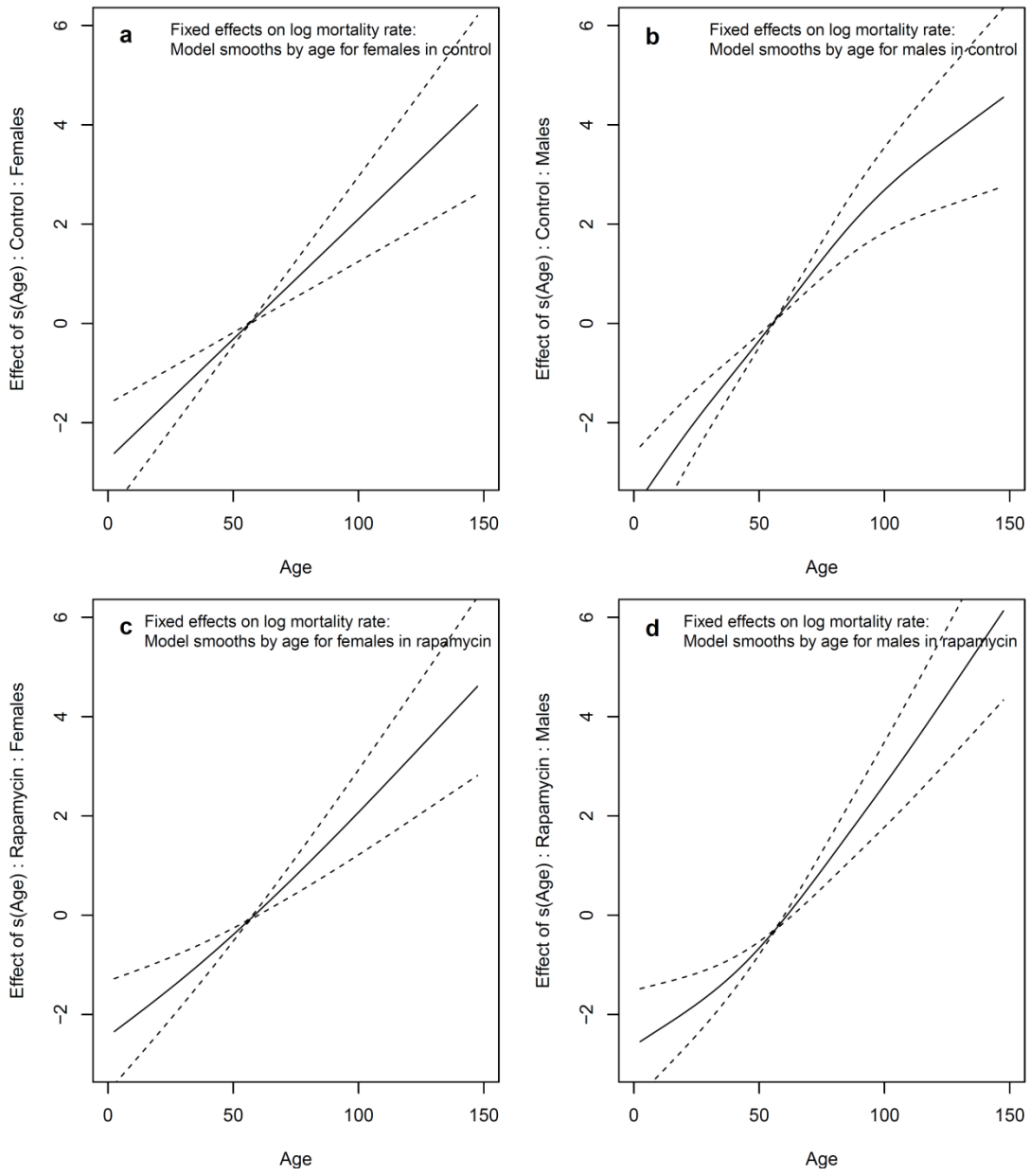


Figure S1. Diagnostic plots assessing the fit of statistical models to the survivorship data of adult *Drosophila melanogaster* in the experiment. Flies from each of the 14 isolines were raised on diets with or without rapamycin (rapamycin vs. control flies). Females and males emerging from the two developmental treatments were then maintained in same-sex groups until death on standard food without rapamycin to compare mortality between developmental treatments. Initially, each of the 14 isolines was represented by approximately 90 adult males and 90 adult females in each treatment. **a.** The graph shows a comparison of Kaplan-Meier survivorship for the combined isolines and the marginal survivorship obtained from the model. The fitted model and the Kaplan-Meier estimator have almost perfect coverage, indicating that the model fitted the empirical data well. **b.** The graph shows the results of the time series autocorrelation test. It can be seen that there is no evidence of autocorrelation in the time series, indicating that the model has captured the dynamics of the empirical data well.

Figure S2


a
Fixed effects on log mortality rate:
Model smooths by age for females in control
Effect of s(Age) : Control : Females
b
Fixed effects on log mortality rate:
Model smooths by age for males in control
Effect of s(Age) : Control : Males
c
Fixed effects on log mortality rate:
Model smooths by age for females in rapamycin
Effect of s(Age) : Rapamycin : Females
d
Fixed effects on log mortality rate:
Model smooths by age for males in rapamycin
Effect of s(Age) : Rapamycin : Males
Age
−2
0
2
4
6
0
50
100
150

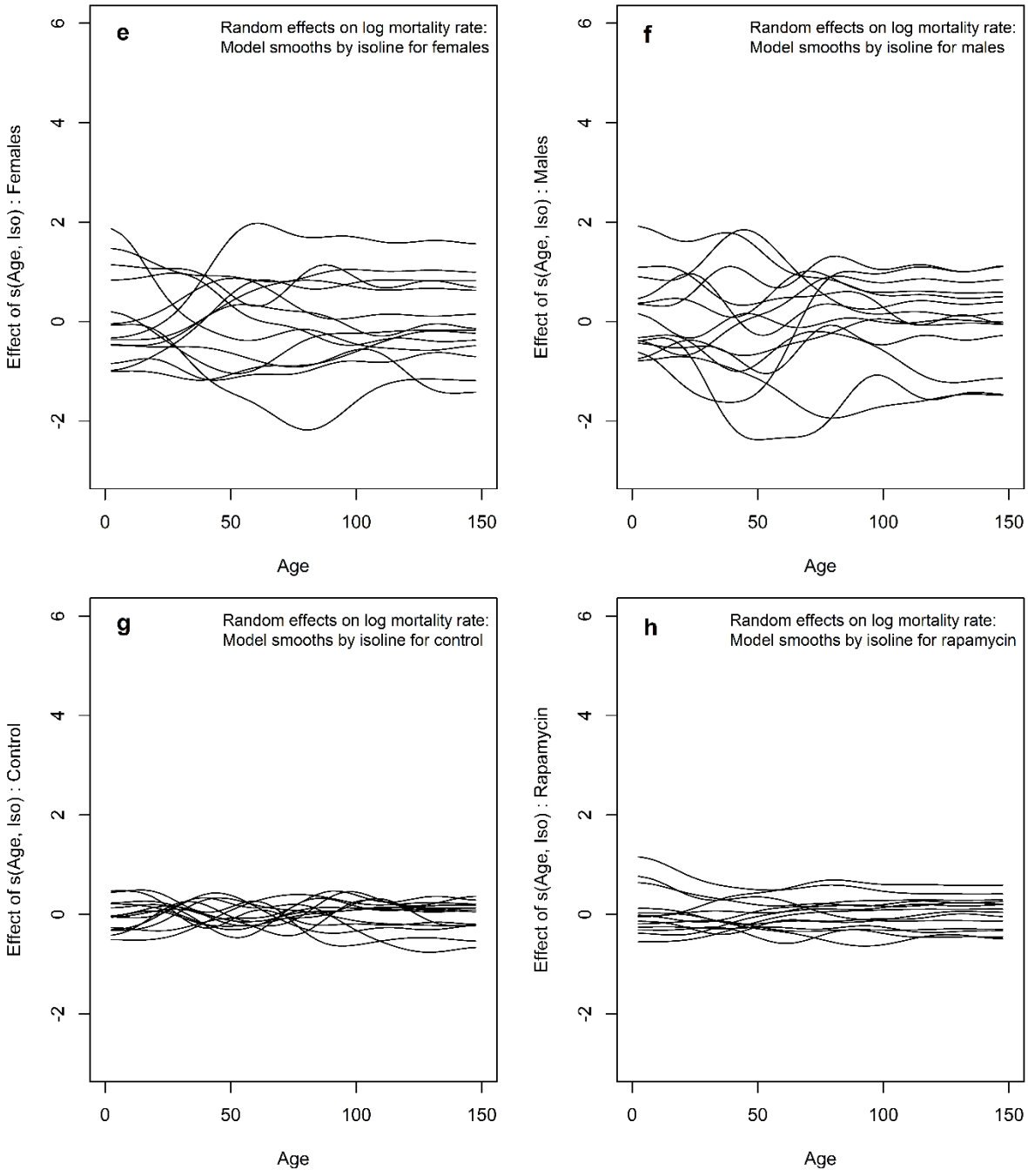

e
Random effects on log mortality rate:
Model smooths by isoline for females
Effect of s(Age, Iso) : Females
Age
f
Random effects on log mortality rate:
Model smooths by isoline for males
Effect of s(Age, Iso) : Males
Age
g
Random effects on log mortality rate:
Model smooths by isoline for control
Effect of s(Age, Iso) : Control
Age
h
Random effects on log mortality rate:
Model smooths by isoline for rapamycin
Effect of s(Age, Iso) : Rapamycin
Age

Figure S2. The figure shows graphically the fixed and random age-specific smooth functions in the GAMM shown in Table S3. The degrees of freedom reported in Table S3 for the smooth functions offer limited information about their shape, making it difficult to fully understand how the predictor variables affect the response variable without looking at the graphical representation of the modelled smooth functions shown here. GAMM analysed differences in (log) mortality of adult *Drosophila melanogaster* in the experiment. Flies from each of the 14 isolines were raised on diets with or without rapamycin (rapamycin vs. control flies). Females and males emerging from the two developmental treatments were then maintained in same-sex groups until death on standard food without rapamycin to compare mortality between developmental treatments. Initially, each of the 14 isolines was represented by approximately 90 adult males and 90 adult females in each treatment. Panels **a-d** show the effects of smooths of age for different combinations of sex and treatment levels. Panels **e-h** show the random effect of isolines modelled as factor smooth interactions for different sex and treatment levels.

Table S1. Results of stratified two-sample log-rank type tests.

Name	Test	Ties method	Z	P
Sex stratified by treatment	Gehan-Breslow	Hothorn-Lausen	7.43	**<0.0001**
Treatment stratified by sex	Gehan-Breslow	Hothorn-Lausen	2.00	**0.0458**

Table S2. Results of the AIC model selection.

Models	**df**	**AIC**
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Age,Iso,by=Sex,bs="fs",m=1) + s(Age,Iso,by=Tr,bs="fs",m=1)	**200.42**	**6735.63**
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Age,Iso,by=Sex,bs="fs",m=1)	152.71	6833.08
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Iso,bs="re") + s(Iso,Age,bs="re") + s(Iso,TrSexInteraction,bs="re")	81.12	7034.34
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Iso,bs="re") + s(Iso,Age,bs="re") + s(Iso,Sex,bs="re") + s(Iso,Tr,bs="re")	69.39	7046.49
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Iso,bs="re") + s(Iso,Age,bs="re") + s(Iso,Sex,bs="re")	58.63	7090.20
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Age,Iso,by=Tr,bs="fs",m=1)	142.77	7102.87
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Age,Iso,bs="fs",m=1)	90.84	7158.67
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Iso,bs="re") + s(Iso,Age,bs="re") + s(Iso,Tr,bs="re")	54.44	7274.01
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Iso,bs="re") + s(Iso,Age,bs="re")	43.72	7299.24
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps") + s(Iso,bs="re")	33.08	7537.66
~Tr * Sex + s(Age,by=Tr:Sex,bs="ps")	22.31	8145.80

Tr - treatment, Iso - isoline. Tr:Sex – interaction between treatment and sex, bs – spline basis: “ps” – p-splines, “re” - simple random effects, “fs” - factor smooth interactions. For detailed description of parameters in used smooths please see description of s() function in mgcv R-package (or type ?mgcv::s in R console).

Table S3. Estimated parameters of the fitted GAMM. To better understand how the predictor variables, affect the response variable, refer to the graphical representation of the smooth functions in Figure S2.

Non-smooth terms	**Estimate**	**Std. Error**	**z value**	**P**
(Intercept)	-4.37	0.21	-20.75	**<0.0001**
Treatment (Rapamycin)	0.10	0.11	0.91	0.3654
Sex (Males)	0.32	0.32	1.01	0.3128
Tr(Rapamycin):Sex(Males)	0.19	0.09	2.09	**0.0363**

Control as the reference for treatment. Females as the reference for Sex.

Smooth terms	**edf**	**Ref.df**	**Chi.sq**	**P**
s(Age):Control:Females	1.01	1.01	92.82	**<0.0001**
s(Age):Control:Males	2.72	3.12	105.85	**<0.0001**
s(Age):Rampamycin:Females	1.96	2.23	113.96	**<0.0001**
s(Age):Rampamycin:Males	2.71	3.08	104.98	**<0.0001**
s(Age,Isoline):Females	57.39	125.00	399.31	**<0.0001**
s(Age,Isoline):Males	57.01	125.00	452.49	**<0.0001**
s(Age,Isoline):Control	32.45	125.00	58.57	**<0.0001**
s(Age,Isoline):Rampamycin	28.42	125.00	76.40	**<0.0001**

":" - denotes an interaction