

# A Rational Approach of Early Humane Endpoint Determination in a Murine Model for Cholestasis

## Supplementary Data

Tab. S1: Consequences according to the score sheet

Single score	Total score	Distress level	Consequences
A		mild	Anesthetize animal and shorten teeth. Document it.
B		mild	Inform the person in charge of the experiment. If necessary, anesthetize the animal and close the wound. Document it.
1		mild	Inform the person in charge of the experiment. A sufficient frequency of observation is necessary, consider treatment options and document it.
2-4		moderate	Inform the person in charge of the experiment. Daily observation of the animal is necessary, consider treatment options and document it.
5		severe	In agreement with the person in charge euthanasia (preferably painless after anesthesia) has to be performed. Document it.
	3-4	mild	Inform the person in charge of the experiment. Daily observation of the animal is necessary, consider treatment options and document it.
	5-15	moderate	Inform the person in charge of the experiment. Euthanasia or treating the animal plus daily observation of the animal is necessary. Document it.
	> 15	severe	In agreement with the person in charge euthanasia (preferably painless after anesthesia) has to be performed. Document it.

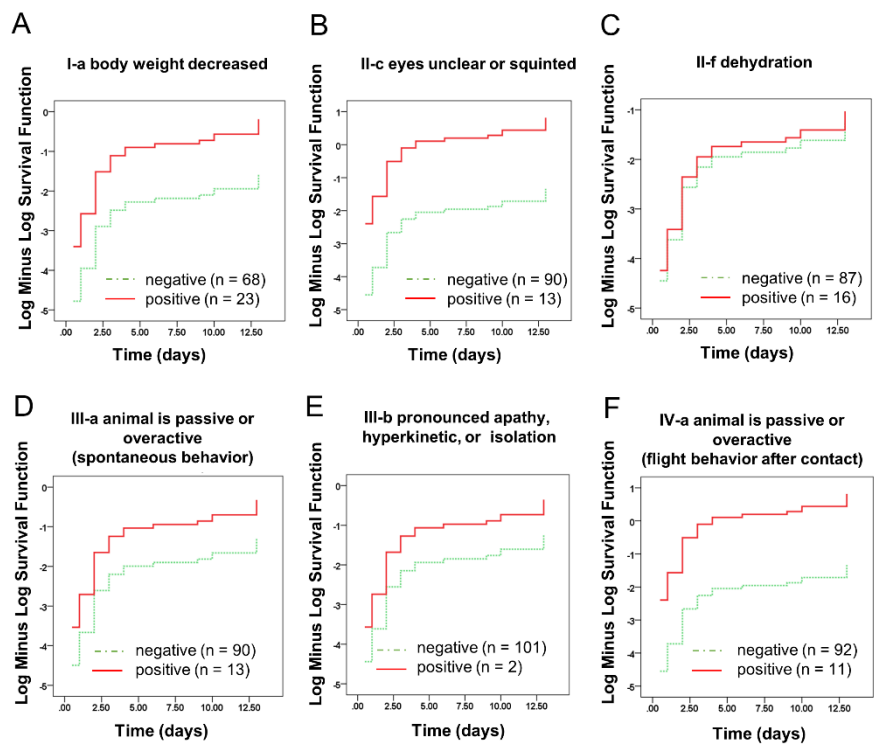
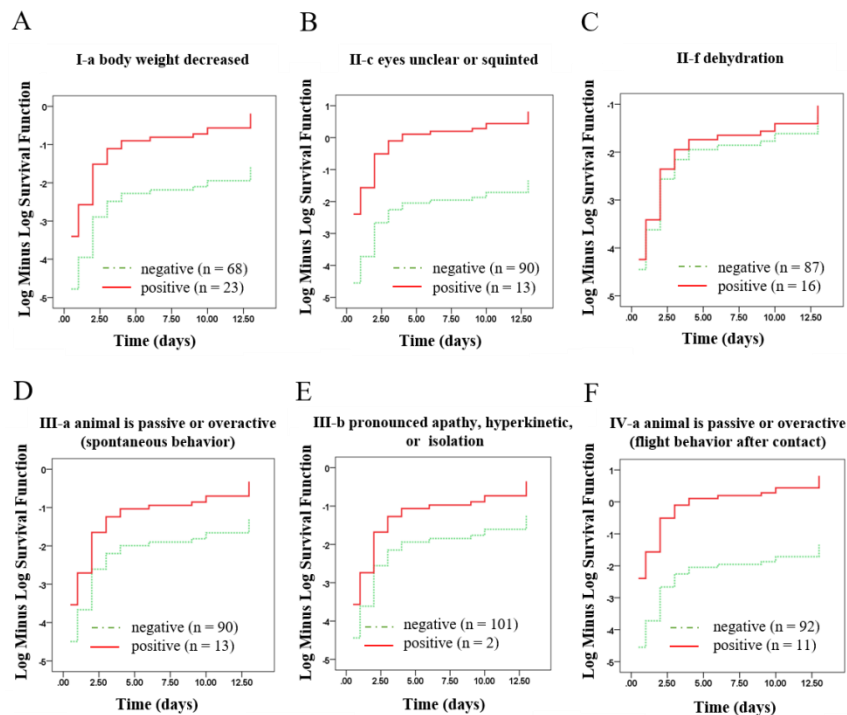


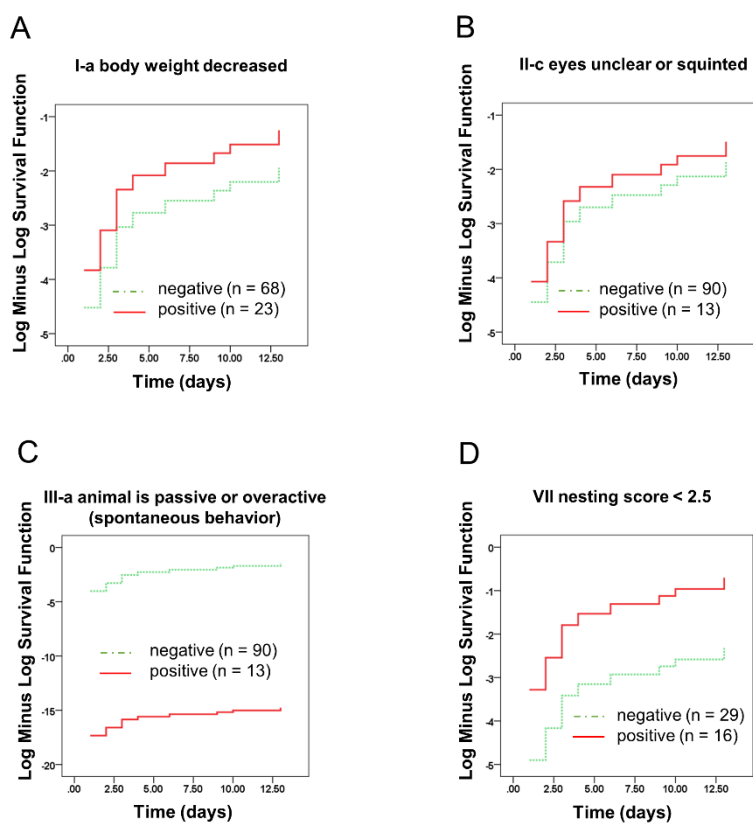
Fig. S1: Log minus log plots of strategy 1

For the criteria I-a (body weight decreased 10 to 20%) (A), II-c (eyes unclear and squinted) (B), II-f (dehydration) (C), III-a (spontaneous behavior: animal is passive and over active) (D), III-b (pronounced apathy, hyperkinetic or isolation) (E), and IV-a (flight behavior after contact: animal is passive or overactive) (F), log minus log survival function was performed for strategy 1 in Tab. 2 to assess the assumption of proportional hazard functions.



**Fig. S2: Log minus log plots of strategy 2**

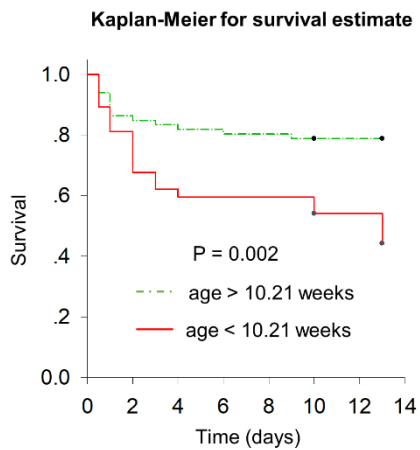
For the criteria I-a (body weight decreased 10 to 20%) (A), II-c (eyes unclear and squinted) (B), II-f (dehydration) (C), III-a (spontaneous behavior: animal is passive and over active) (D), III-b (pronounced apathy, hyperkinetic or isolation) (E), and VI (burrowing decreased > 79.4%) (F), log minus log survival function was performed for strategy 2 in Tab. 2 to assess the assumption of proportional hazard functions.



**Fig. S3: Log minus log plots of strategy 3**

For the criteria I-a (body weight decreased 10 to 20%) (A), II-c (eyes unclear and squinted) (B), II-f (dehydration) (C), and III-a (spontaneous behavior: animal is passive and over active), VII (nesting score < 2.5) (D), log minus log survival function was performed for strategy 3 in Tab. 2 to assess the assumption of proportional hazard functions.

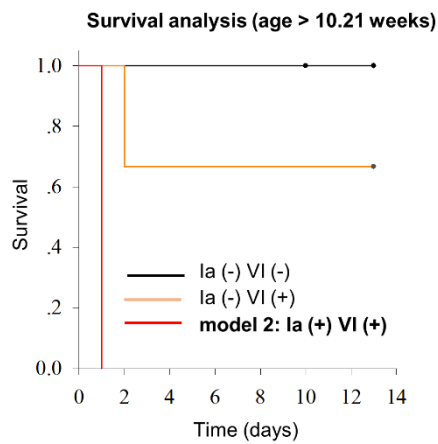
A



**Fig. S4: Kaplan-Meier curve, comparing the survival of old and young mice as well as the survival prediction of each age group by the multivariate Model 2**

Young mice (age < 10.21 weeks) displayed a significant shorter survival time in the BDL model than old mice (age > 10.21 weeks) (A). Old mice (age > 10.21 weeks) with a body weight loss of more than 10 to 20% (variable I-a) and decreased burrowing activity of more than 79.4% (variable VI) died within 1 day (B). Young mice (age < 10.21) with positive status for both variables (I-a, VI) died within 2 days (C). The *P*-values were determined by log-rank test (*P* < 0.05).

B



C

