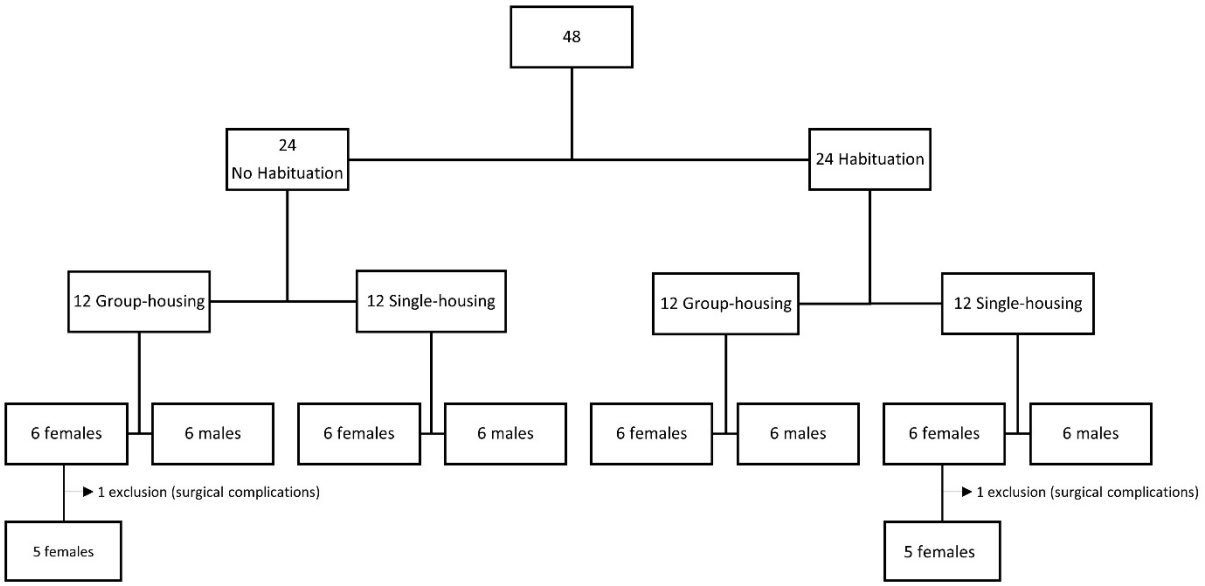
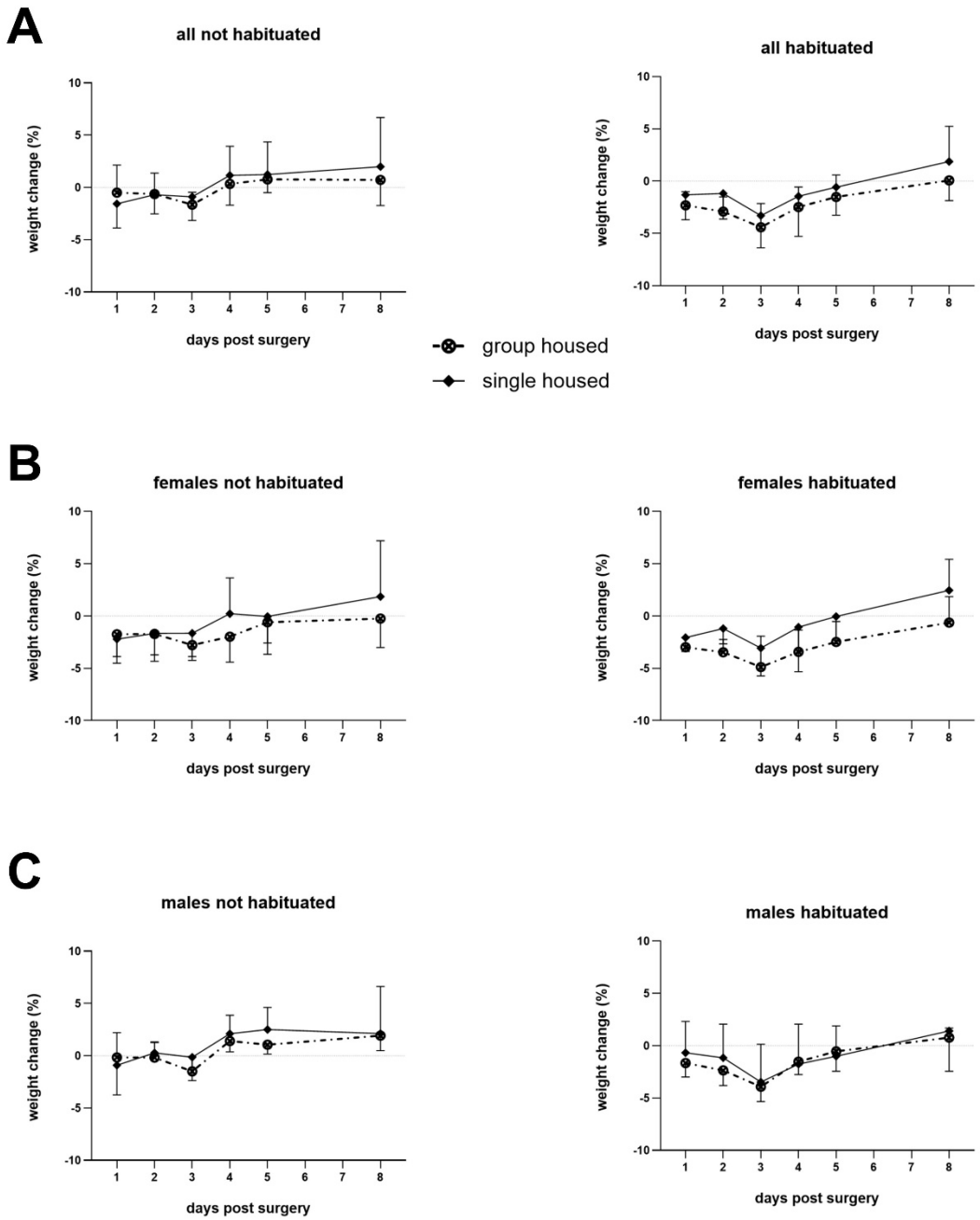
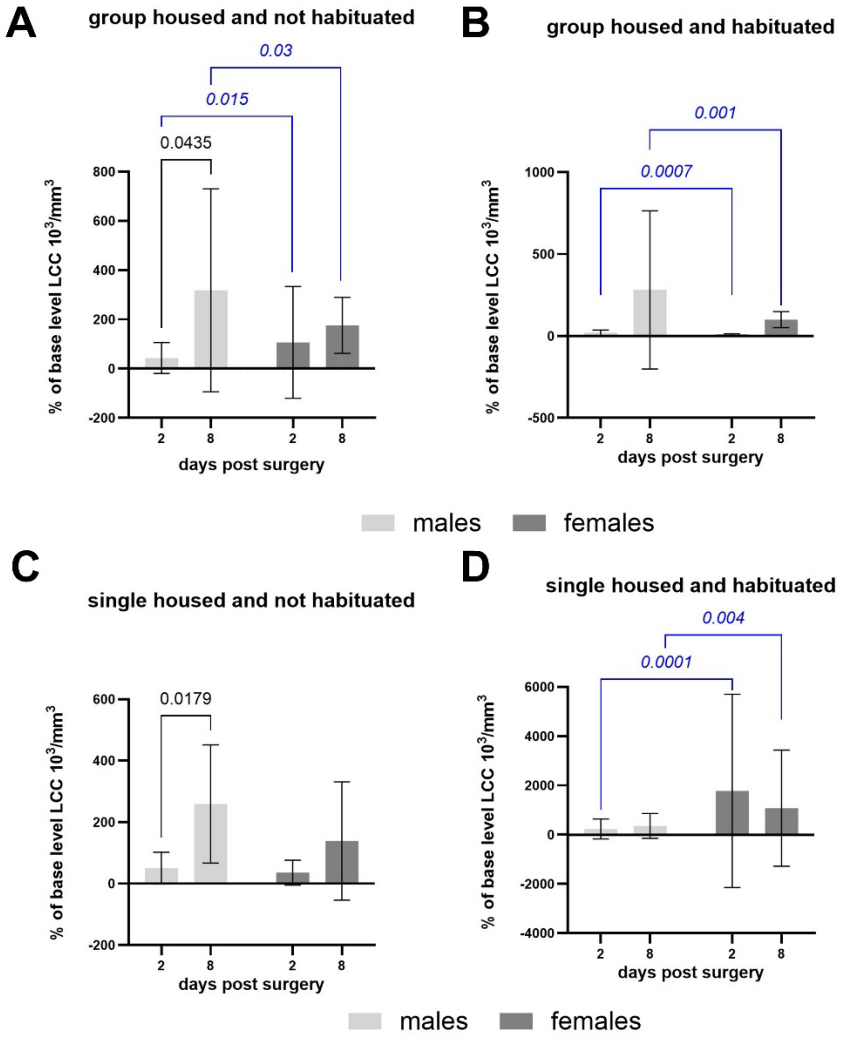


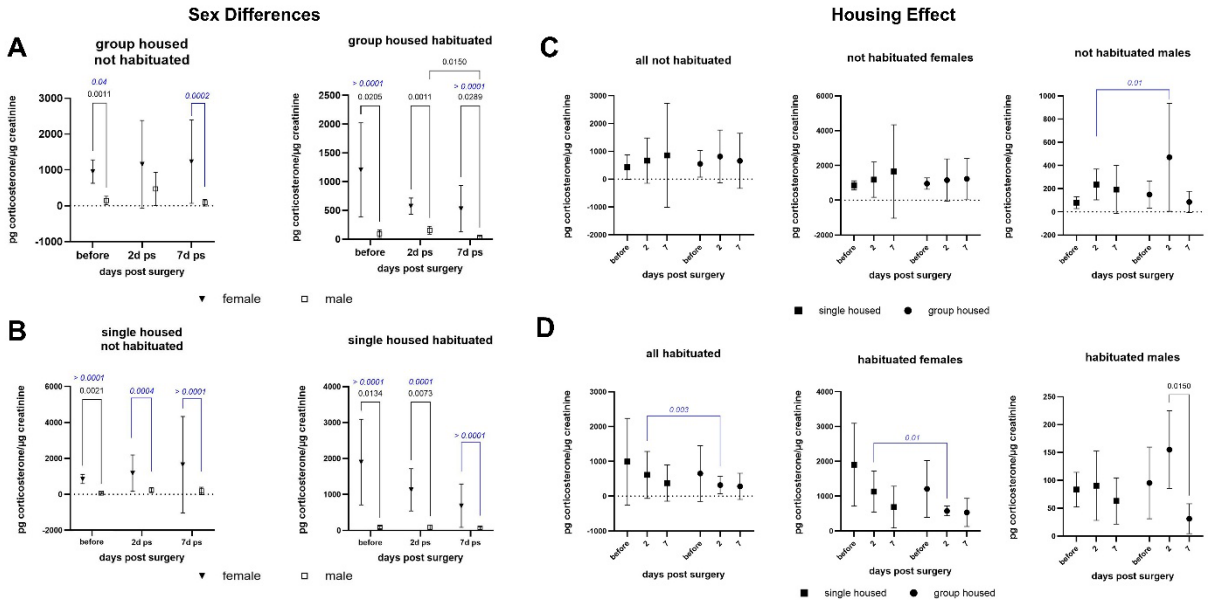
Supplementary Figure 1. Flow chart of animal enrollment and group allocation.
Forty-eight mice were allocated to habituation (habituated/non-habituated) and housing (group-housed/single-housed) conditions, with six males and six females per subgroup. One female was excluded from the non-habituated group-housed subgroup and one female from the habituated single-housed subgroup due to surgical complications, resulting in n = 5 females in these two subgroups.



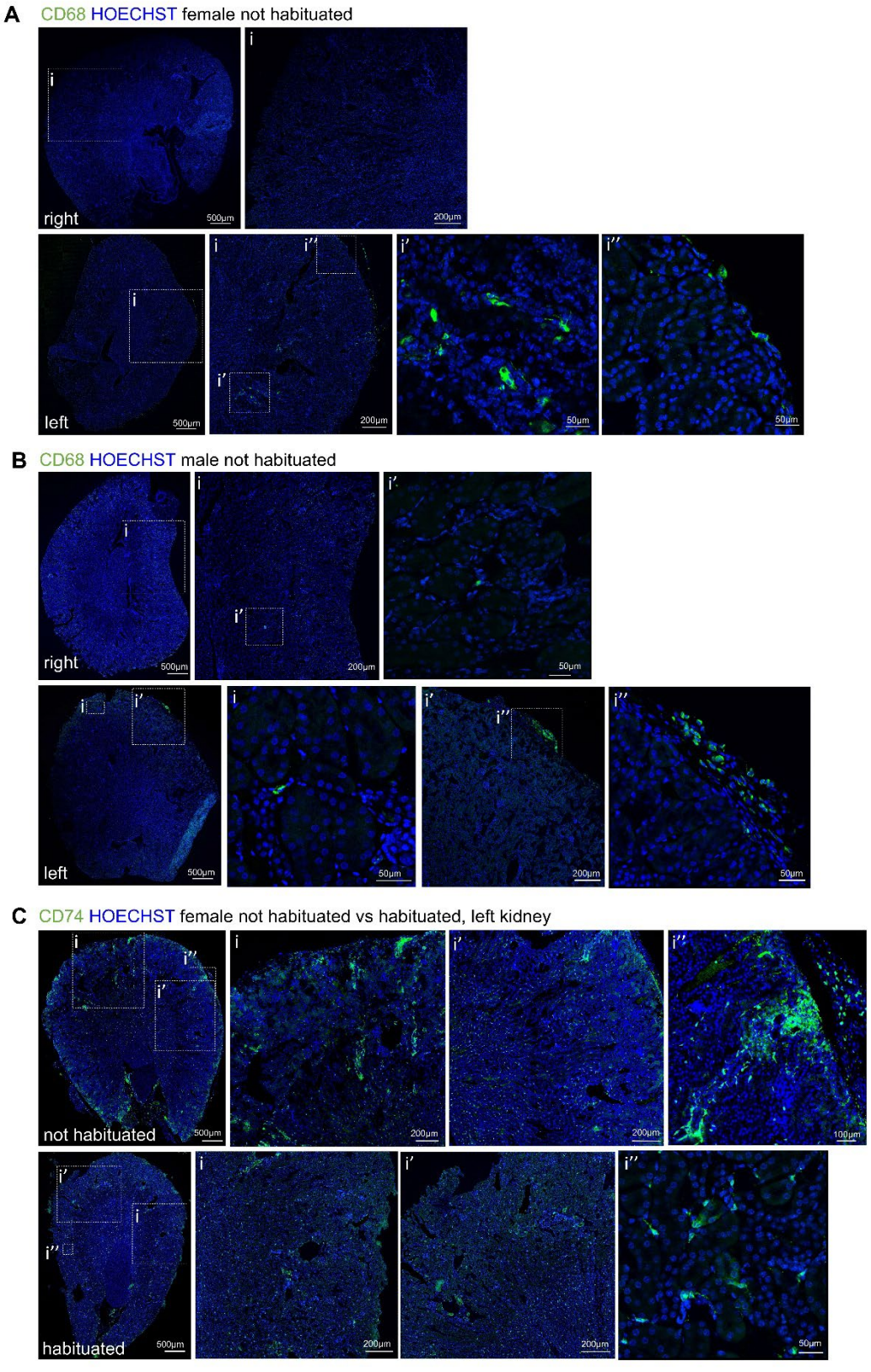
Supplementary Figure 2. Effect of housing condition on post-operative body weight change. Body weight change (%) relative to the day of surgery over the eight-day post-operative period. Group-housed animals are shown as open circles with dashed lines; single-housed animals as filled diamonds with solid lines. **(A)** All animals, non-habituated (left) and habituated (right). **(B)** Females, non-habituated (left) and habituated (right). **(C)** Males, non-habituated (left) and habituated (right). (n = 6 males and 6 females per subgroup, except n = 5 females in the non-habituated group-housed and habituated single-housed subgroups due to exclusion; mean ± SD).



Supplementary Figure 3. Sex differences in peripheral blood leukocyte counts. Peripheral blood leukocyte counts (LCC) expressed as percentage of individual pre-surgical baseline values ($\times 10^3/mm^3$) on post-operative days 2 and 8, compared between males (light grey) and females (dark grey) within each experimental subgroup. **(A)** Group-housed not habituated. **(B)** group-housed habituated. **(C)** Single-housed not habituated. **(D)** Single-housed habituated. Black p-values indicate significant differences in means (mixed-effects analysis with Fisher's LSD post-hoc test); blue italic p-values indicate significant differences in variance (F-test of equality of variances). (n = 6 males, 5 females per subgroup; mean ± SD).



Supplementary Figure 4. Sex differences and housing effects on urinary corticosterone levels. Urinary corticosterone concentration normalized to urinary creatinine (pg/µg creatinine) in spot urine collected 1 day before surgery and on post-operative days 2 and 7. Black p-values indicate significant differences in means (two-way ANOVA with Tukey's multiple comparison test); blue italic p-values indicate significant differences in variance (F-test of equality of variances). Mean ± SD. **(A)** Sex differences in group-housed animals. Left: group-housed non-habituated mice. Right: group-housed habituated mice. Filled triangles, females; open squares, males. (n = 5 females, 6 males per subgroup). **(B)** Sex differences in single-housed animals. Left: single-housed non-habituated mice. Right: single-housed habituated mice. Filled triangles, females; open squares, males. (n = 5 females, 6 males per subgroup). **(C)** Effect of housing condition in non-habituated animals. Left: all non-habituated mice. Middle: non-habituated females. Right: non-habituated males. Filled squares, single-housed; filled circles, group-housed. In non-habituated males, group housing was associated with significantly increased variance at day 7 post-surgery. (n = 12 males, 6 per housing group; 10 females, 5 per housing group). **(D)** Effect of housing condition in habituated animals. Left: all habituated mice. Middle: habituated females. Right: habituated males. Filled squares, single-housed; filled circles, group-housed. (n = 12 males, 6 per housing group; 10 females, 5 per housing group).



Supplementary Figure 5. Representative fluorescence immunostaining of transverse kidney cryosections. Kidney cryosections stained with anti-CD68 or anti-CD74 antibodies (green) and Hoechst nuclear counterstain (blue). CD68 and CD74 were stained on sequential sections from the same tissue block. **(A)** CD68 immunostaining in a non-habituated female mouse. Right (contralateral) kidney (upper panel) shows sparse or absent labeling. Left (operated) kidney (lower panel) shows

CD68-positive cells in the cortex and capsule. **(B)** CD68 immunostaining in a non-habituated male mouse. Right (contralateral) kidney (upper panel) shows sparse or absent labeling. Left (operated) kidney (lower panel) shows CD68-positive cells in the cortex and capsule. **(C)** CD74 immunostaining in left (operated) kidneys from a non-habituated (upper panel) and a habituated (lower panel) female mouse. CD74-positive cells are distributed throughout the cortex and concentrated in superficial and peritubular locations — most likely capillaries — and in the capsule. Fewer foci of accumulated CD74-positive cells are observed in the kidney from the habituated mouse. Boxed areas are shown in the adjacent magnified insets. Scale bars are indicated directly on individual panels.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Groups compared: Habituated vs non-habituated; Group-housed vs individually housed; Four groups in a 2 × 2 factorial design; Sex-stratified analyses; Controls: non-habituated (for habituation), group-housed (for housing); Location in manuscript- Results: Experimental design, habituation efficacy, and post-operative Experimental unit: Individual mouse; except approach test assessing habituation efficacy where the experimental unit was the cage.
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	a) Methods: Mice and husbandry; Results: Experimental design; Figure1 and Supplementary Figure 1 b) Methods: Sample size and power
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Humane endpoint criteria were established a priori according to the institutional welfare scoring system. Animals reaching the predefined intervention threshold (a cumulative health score of 0.4 per observation) would have been removed from b) Methods: Mice & husbandry, Results: Experimental design, Supplementary Fig.1 Results, Supplementary Fig.1, Figures 1-6
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	allocation to habituation was randomized cage-wise within each cohort by drawing lots, whereas post-operative housing was determined by cohort. Male and female mice were equally represented across experimental groups. All animals were maintained under identical husbandry conditions before surgery, and all surgical, sampling, and monitoring procedures were standardized. Outcome assessment and data analysis were performed blinded to group allocation
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	During allocation and conduct of the animal experiments, two of the three investigators were aware of group allocation, as knowledge of treatment and housing conditions was required to perform the experimental procedures. Biological samples were subsequently coded by a one of them. Outcome assessments were performed by
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	a) body weight, peripheral blood leukocyte count, urinary corticosterone (pg per µg Creatinine), number of CD68- and CD74-positive objects per mm2, numer of approaches per 10 min (per cage) and lag body weight, peripheral blood leukocyte count, urinary corticosterone
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	a) Methods: Statistical Analysis b) Methods: Statistical Analysis
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	a) Methods: Mice & husbandry b) not relevant
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	a) Methods: Mice & husbandry, Habituation, Urine Alanysis, Peripheral blood analysis, Kidney-window implantation b) Methods: Mice & husbandry, Habituation, Urine Alanysis, Peripheral blood analysis, Kidney-window implantation c) Methods: Mice & husbandry, d) Introduction, Results and Methods section
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	a) Results and Figure s 1 to 6, Supplementary Figures 2-4 b) not applicable

Stockholm University

ECF-R-041

Scoring template and acclimatization time at ECF

1. Summary

This routine provides information regarding parameters included in the KI-template, which is used to assess animal health for rats and mice at ECF, as well as information on acclimatization time.

2. Content

2.1 Acclimatization time

Animals who will be part of survival experiments should be acclimatized for one week after arrival to the facility. If this is not possible for your experimental design, please consult the University Veterinarian.
Animals who will be part of terminal experiments should be acclimatized for one week after arrival to the facility, if this is possible for your experimental design.

2.2. Scoring template background

The assessment template often called “KI-mallen” - was developed by the KI veterinary department in 2003 and was modified in 2016 by the Veterinary group within the Comparative Medicine department.

The assessment template is generic and may, if required, be supplemented with more specific assessment criteria which are tailored to a specific experimental model e.g. research involving arthritis, tumor growth, MS, ALS and diabetes.
This assessment template is recommended to be used in ethics applications in order to establish the humane endpoints or for the assessment of animal welfare in general.

A humane endpoint of 0.3 points is considered standard for breeding animals and animals within an animal facility’s permit. When animals participate in a research experiment, one must always follow the humane endpoint that is specified in the approved ethics application.

When using the assessment template to set a humane endpoint, it is important to set a well-grounded and realistic humane endpoint. The endpoint should take into account the experimental design and research purpose in order to avoid unnecessary euthanasia of individual animals. It is possible for humane endpoints to be set higher during shorter periods of a research experiment e.g. the few days following a surgical procedure.

As a complement to this assessment template, there is one additional, detailed template for body condition scoring, see appendix 1.
Appendix 1: Body condition scoring (BCS) for the assessment of body condition. This is recommended to be used when performing body condition scoring (BCS) during cancer studies. The growth of a tumor will often obscure the weight loss of an individual animal.

3. Scoring template

Veterinär plan för bedömning av djurens fysiska och psykiska välbefinnande (för smågnagare och kanin) ***Veterinary plan for the assessment of animals' physiological and psychological well-being (for rodents and rabbits)***		
Bedömnings-parameter ***Assessment parameters***	**Poäng** ***Points***	**Exempel på observationer** ***Examples of observations***
Allmäntillstånd ***General condition***	**0.0**	Pigg, alert, aktiv, reagerar på sin omgivning. *Alert, lively, active, reacts to their surroundings.*
	0.1	Slö, hängig, mindre aktiv. *Slow, weak, less active.*
	0.4	Stillasittande, rör sig ogärna, känns kall, piper, visar stark smärtreaktion vid beröring, orörlig. *Immobile, limited or no voluntary movement, cold upon touch, vocalises, the animal displays extreme pain reaction when touched, lying motionless.*
Porfyrifärgning[1] och ögon ***Porphyrin staining[1] and the eyes***	**0.0**	Ingen missfärgning, klara rena ögon. *No discolouration, clear and clean eyes.*
	0.1	Lindrig mängd kladd runt ögon och nos. Hos vita djur syns rödaktig missfärgning (porfyri). *Slight discharge around eyes and nose. Red staining (porphyrin) observed in white animals.*
	0.4	Kladdig i "ansiktet" och/eller på ben och tassar, kniper med ögonen, svullna ögon. Kraftig rödfärgning nära ögonen på vita möss. *Discharge on the face and/or legs and paws. Squint with eyes, swollen eyes. Large amounts of red staining near the eyes of white mice.*
Rörelser och kroppshållning ***Movement and posture***	**0.0**	Normal. *Normal.*
	0.1	Kan inte helt samordna rörelser (inkoordination[2]). *Cannot fully coordinate movements (incoordination[2]).*
	0.4	En eller flera av följande: Tydlig inkoordination[2], permanent snett buret huvud, går i cirklar, sitter ihopkrupen, ligger orörlig, skjuter rygg, kraftig hälta, stödjer ej på alla ben, visar förlamningssymtom, uppsvullen buk ("bukighet"), upprepade eller längre (>4 s) kramper och/eller faller ihop. *One or more of the following: Marked incoordination[2], permanent head tilt, circling, hunched posture or back, lying motionless, severe lameness, not standing on all legs, signs of paralysis, marked swollen abdomen, seizure of long duration (> 4 seconds) or repeated seizures and / or collapse.*
Piloerektion[3] ***Piloerection[3]***	**0.0**	Pälsen slät och välskött. *Fur smooth and well-groomed.*
	0.1	Lindrigt ruggig päls (piloerektion). *Mild piloerection.*
	0.4	Kraftigt ruggig päls (piloerektion). *Severe piloerection.*

Bedömnings-parameter *Assessment parameters*	Poäng *Points*	Exempel på observationer *Examples of observations*
Hud *Skin*	**0.0**	Hud pälsbeklädd utan sår eller andra tecken på skada. *Fur-covered skin without sores or any signs of injury.*
	0.1	Enstaka små och ytliga sår, skorpor eller liknande utan tecken på infektion eller klåda. *A single or a few, small and superficial sores, scabs or similar without signs of infection or scratching.*
	0.4	Flera, större eller djupare sår, samt sår med infektionstecken såsom röd, irriterad eller död hud. Variga eller vätskande sår, kladdig och ovårdad päls eller klåda. Operationssår som ej läker eller suturer som gått upp. *Multiple, large or deep sores. Sores with signs of infection such as red, irritated or dead skin. Purulent or oozing sores. Sticky and ungroomed fur or scratching. Operation wounds that do not heal or the sutures have loosened.*
Hull[4] och vikt[5] *Body condition[4] and weight[5]*	**0.0**	Hull normalt eller något över normalt (BC3-4). Viktförlust < 5% jämfört med vikt "före ingrepp". *Well-conditioned or over-conditioned (BC3-4). Weight loss < 5% compared with weight "prior to procedure".*
	0.1	Hull under eller kraftigt över normalt (BC2 eller BC5). Viktförlust ≥ 5% jämfört med vikten "före ingrepp". *Under-conditioned or obese (BC2 or BC5). Weight loss ≥ 5% compared with weight "prior to procedure".*
	0.4	Hull kraftigt under normalt (BC1). Viktförlust ≥ 15% jämfört med vikten "före ingrepp". *Severely under normal body condition (BC1). Weight loss ≥ 15% compared with weight "prior to procedure".*
Mått på uttorkning (hudturgor)[6] *Measurement of dehydration (skin turgor)[6]*	**0.0**	Spänstig, elastisk hud. *Supple, elastic skin.*
	0.1	Vid kontroll av hudturgor har huden nedsatt elasticitet. *When checking the skin turgor, there is reduced skin elasticity.*
	0.4	Vid kontroll av hudturgor "tältar" huden[7], insjunkna ögon, torra slemhinnor. *When checking the skin turgor, the skin forms a tent[7], sunken eyes, dry mucous membranes.*

Bedömnings-parameter *Assessment parameters*	Poäng *Points*	Exempel på observationer *Examples of observations*
Tarm- och urinfunktion ***Intestinal- and urinary function***	**0.0**	Tarm och urinering fungerar normalt. *Normal intestinal- and urinary functions.*
	0.1	Avföring lösare än normalt. *Faeces are looser than normal.*
	0.4	Avföring saknas, blod i avföringen eller tecken på diarré. Kvarstående utbuktning av ändtarmen (rektalprolaps). Tecken på att djuret inte kan urinera/kraftigt fylld urinblåsa. Illaluktande, blod i urinen eller på annat sätt onormal urin. Kvarstående penisframfall (prolaps). *Constipation, blood in the faeces or signs of diarrhoea. Persistent rectal prolapse. Signs that the animal cannot urinate/ greatly distended urinary bladder. Foul-smelling, bloody or abnormal urine. Persistent penis prolapse.*
Andning ***Respiration***	**0.0**	Normal andning. *Normal respiration/breathing.*
	0.4	Andas med öppen mun, ökade andningsljud, bukandning eller flämtande och/eller rosslande andhämtning. *Breathing with an open mouth, increased breathing sounds, abdominal breathing or panting and/or wheezing/rales.*
Tänder ***Teeth***	**0.0**	Normal tandstatus. *Normal teeth.*
	0.1	Lindrig felställning av framtänderna (bettfel) som inte påverkar foderintaget. *Slight malocclusion of front teeth (incisors) which does not hinder food intake.*
	0.4	Kraftigt förvuxna och felställda framtänder som påverkar foderintaget. Avsaknad av framtänder. *Severely overgrown and malocclusion of front teeth (incisors) which hinders food intake. Missing front teeth.*

4. Key to assessment template

1. Porphyrin = red colored secretion from the eyes (and nose). This is not seen in rabbits or guinea pigs. Observed relatively easier in white animals (albinos) but more difficult to observe in black (pigmented) animals.
2. Incoordination = wobbly, has difficulty in controlling movements.
3. Piloerection = erection of the hairs of the skin, it looks like the animal is cold.
4. See Appendix 1 for Body condition scoring.
5. Weighing is normally not performed at the daily welfare assessment, and is done only if required in the ethics permit and/or the study plan. For non-weaned and immature, growing animals, the weight loss must be assessed in relation to the normal growth, either by referring to available weight curves or by comparison to healthy animals of the same age, sex and strain.
6. Skin turgor = control of dehydration, performed by pinching the skin and assessing how quickly the skin returns to normal.
7. When checking the skin turgor, the skin does not return to normal and remains erect like a tent.

5. Training programs

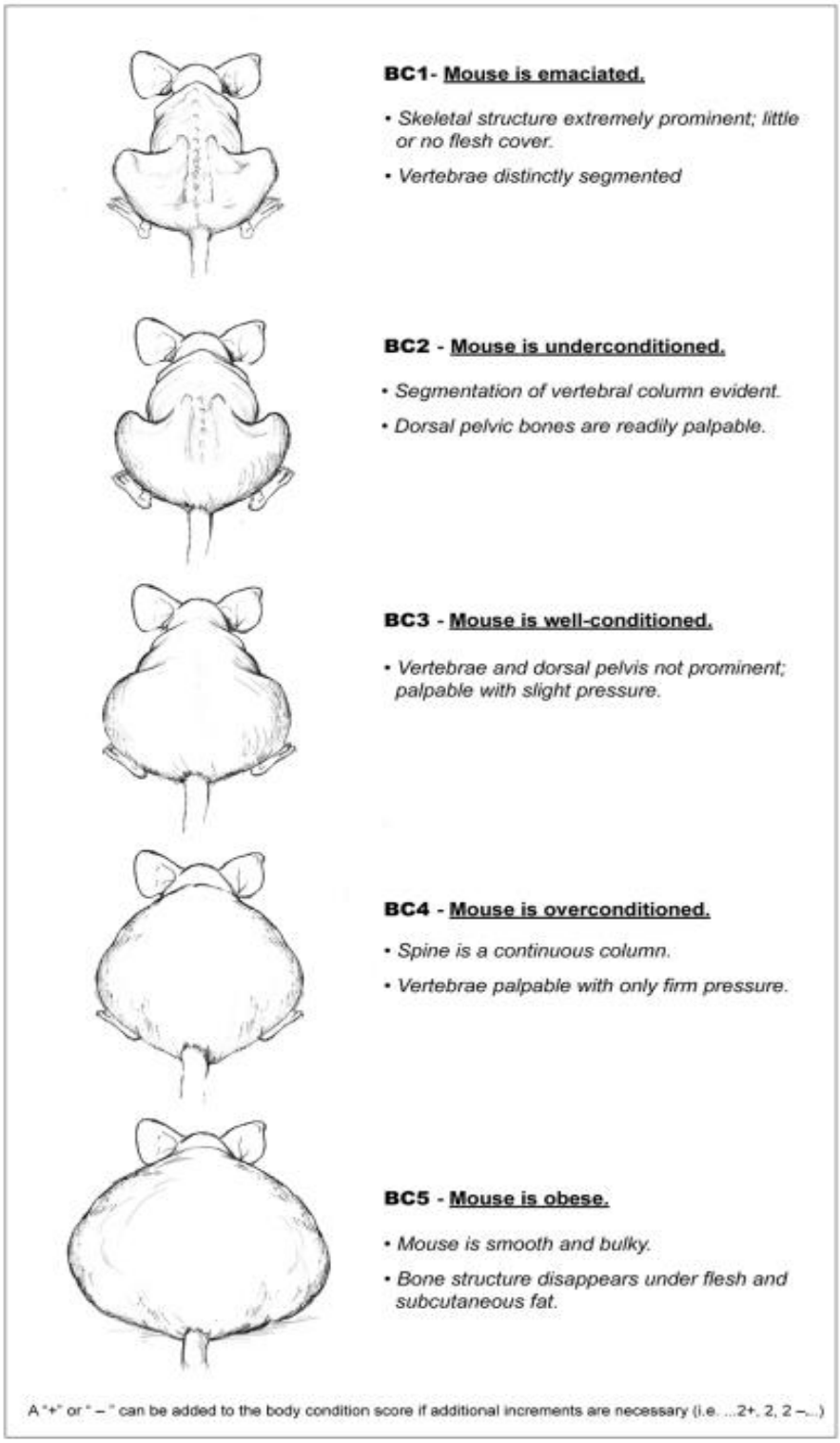
If specified in the ethics application, or if otherwise appropriate, the animals will be habituated to specific procedures via training programs. The training will be performed as described in the ethics permit, or as decided by the University veterinarian.

6. Contact

University Veterinarian	See contact list
ECF Director	See contact list

Document updated 210422, 210510

Appendix 1. Body Condition Scoring (BCS) for the assessment of body conditioning



Mollie H. Lillma-Cullere and Charmain J Foltz, Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice Lab. Animal Science; Vol 49 (3) 319-323, 1999