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Post-weaning maternal presence exerts a protective effect against social isolation-induced behavioural alterations in mice

Hiroshi Ueno[1]✉, Eriko Kitano[2], Yu Takahashi[2], Sachiko Mori[2], Shinji Murakami[2], Kenta Wani[2], Yosuke Matsumoto[3], Motoi Okamoto[4] & Takeshi Ishihara[2]

Parental "watchful presence" is considered an important factor influencing behavioural and emotional development in offspring across mammalian species, including humans. However, the effects of such parental presence remain insufficiently understood, even in human studies. In laboratory mice, offspring are typically weaned at approximately three weeks of age, leaving the impact of post-weaning maternal presence on behavioural development largely unexplored. This study aimed to investigate whether maternal presence in an adjacent cage after weaning can attenuate behavioural effects of social isolation stress in mice. Furthermore, we sought to assess whether this experimental paradigm could serve as a novel animal model for studying the effects of parental watchful presence, with potential relevance to human parent–child relationship research. Mouse pups were weaned at postnatal day 21 and housed either adjacent to their mother (maternal presence group) or without maternal presence (control group). The pups were subsequently housed either in groups with littermates or individually until eight weeks of age. After maturation, behavioural tests were conducted to assess locomotor activity, anxiety-like behaviour, social behaviour, and depression-like behaviour. In group-housed mice, maternal presence did not influence behavioural outcomes. However, in individually housed mice, maternal presence partially attenuated social isolation-induced behavioural alterations, suggesting a subtle protective effect, including hyperlocomotion, reduced anxiety-like behaviour, and abnormal social interactions. Our findings demonstrate that maternal presence during the post-weaning period can offer a protective effect against certain behavioural abnormalities induced by social isolation stress in mice. This simple adjacent-cage paradigm provides a novel and practical model for elucidating the impact of parental watchful presence on behavioural and emotional development, offering insights relevant to the understanding of parent–child relationships in humans.

Keywords Maternal presence, Social isolation, Post-weaning period, Resilience, Mice

Abbreviations

ANOVA	Analyses of variance
P	Postnatal day
SI	Social isolation
HPA axis	Hypothalamic–pituitary–adrenal axis
EPM	Elevated plus maze
LD box	Light/dark transition box
OF	Open field
YM	Y-maze
FST	Forced swim test
TS test	Tail suspension test

[1]Department of Medical Technology, Kawasaki University of Medical Welfare, 288, Matsushima, Kurashiki, Okayama 701-0193, Japan. [2]Department of Psychiatry, Kawasaki Medical School, Kurashiki 701-0192, Japan. [3]Department of Neuropsychiatry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama 700-8558, Japan. [4]Department of Medical Technology, Graduate School of Health Sciences, Okayama University, Okayama 700-8558, Japan. ✉email: dhe422007@s.okayama-u.ac.jp

In humans, parental presence plays a critical role in regulating emotional and behavioural development long after weaning, providing a secure base from which children explore their environment[1,2]. The very presence of caregivers enhances children's sense of control over their environment and emotional state[3]. In contrast to human children, many non-human mammals are thought to transition rapidly to independence after weaning, acquiring survival skills and separating from their parents relatively quickly. However, in laboratory studies using mice, pups are typically transferred to separate cages from their mothers after the nursing period, which may have precluded investigations of the direct effects of maternal presence on post-weaning development.

Rodent studies have greatly contributed to our understanding of mother-infant interactions and social behaviour[4]. However, most of this research has focused on maternal separation as a model of neglect or abuse in humans[5,6]. The presence of the mother until weaning is considered necessary for normal emotional development in pups, including the suppression of excessive anxiety. Extended or repeated separation from the mother during the early postnatal period (postnatal days [P] 2–3 weeks) induces learning and memory deficits[7,8], as well as emotional alterations reminiscent of depression and anxiety in humans[9,10], which persist into adulthood. Consequently, research on mother-infant interactions in mice has primarily focused on the pre-weaning period. However, it has also been reported that post-weaning pups maintain a strong attachment to their mothers and show a clear preference for being with them[11]. Evidence regarding the persistence of the parent–offspring bond in mice after weaning remains inconclusive.

Adolescence in rodents is a transitional developmental stage extending from approximately postnatal day 28 to 60, during which substantial neuroendocrine and behavioural changes occur. Corticosterone secretion increases progressively from the juvenile stage through early and late adolescence, contributing to heightened stress sensitivity and plasticity of the hypothalamic–pituitary–adrenal (HPA) axis[12,13]. These hormonal changes are associated with increased novelty-seeking, risk-taking, and social exploration[14,15]. Such characteristics render adolescence a particularly vulnerable period in which adverse experiences, such as social isolation, can alter typical developmental trajectories and lead to long-lasting behavioural consequences. Situating our study within this framework highlights the potential importance of maternal presence as a buffering factor during adolescence.

During the early nursing period (P0–9), pups exhibit low stress sensitivity. As they progress through the mid-nursing period (P10–15), stress sensitivity increases, and maternal presence becomes important for mitigating stress responses[16,17]. By the late nursing period (P16 and beyond), this maternal buffering effect diminishes[18]. The timing of psychological independence in mice remains unclear[19]. Importantly, under standard laboratory housing conditions, it is common practice to separate pups from their mothers at P21–P28, even though their psychological independence at this stage is not well defined. In laboratory research, mice are generally weaned and separated from their dams at postnatal days 21–23, whereas in the wild they typically remain within the maternal territory until adolescence, maintaining ongoing contact with their mother[20]. This discrepancy highlights the need to examine maternal influence beyond standard laboratory weaning practices. Although the maternal role during this transitional period remains largely unexplored, studies in rats have shown that nursing can persist up to P35[21,22]. The post-weaning stage is also characterized by progressive behavioural and neurodevelopmental changes—including increased exploration, social play, affiliative behaviours, and altered responses to isolation[23]. Here, we focused on maternal presence rather than active caregiving, proposing that such passive presence may provide a modest buffering influence on offspring during this extended developmental phase.

In this study, we use the term 'maternal watchful supervision' to describe the perceptual presence of the mother, in which she is perceptually available through visual, olfactory, and auditory cues, but physically separated by a transparent barrier from the offspring, and does not engage in direct caregiving behaviours[24]. Traditional maternal behaviours encompass both this type of supervision and more active forms of care, including nursing, licking, and grooming[21–23]. This framework allows us to distinguish the potential influence of sensory-based maternal presence itself from the effects of active maternal care.

Maternal behaviour comprises two major components: passive "watchful supervision" (presence and monitoring) and active caregiving (nursing, grooming, pup retrieval, etc.). Nursing provides direct nutritional support, while grooming stimulates physiological development. Maternal licking and grooming regulate pup autonomic function (e.g., heart rate and respiration) and modulate serotonergic activity, altering cortical activity patterns and influencing sensory and emotional development[25]. While the mother's presence until weaning is known to be essential for normal emotional development (including the suppression of excessive anxiety)[5], it remains unclear whether maternal "watchful supervision" is still necessary for emotional development after weaning.

Laboratory mice are widely used in modelling human behavioural disorders[26]. To successfully translate findings from mouse studies to humans, it is essential to fully understand mouse developmental trajectories, behavioural characteristics, and appropriate handling practices. Although mice are not primarily visual animals, many cognitive behavioural tests for mice rely on visual cues (e.g., open field, elevated plus maze, light/dark box, Morris water maze, radial arm maze, Barnes maze). The visual system of mice supports a range of adaptive behaviours, from detecting predators to locating shelter, food, and conspecifics, across diverse environments[27].

Our previous studies demonstrated that mice housed in transparent cages can recognise conspecifics in neighbouring cages and modulate their social behaviour accordingly[28]. Furthermore, when enrichment objects were placed in adjacent cages, observer mice exhibited increased anxiety-like behaviour[29]. These findings enabled us to establish a novel experimental model using adjacent transparent cages.

In the present study, we applied this model to investigate the effects of post-weaning maternal "watchful supervision" on emotional development in pups. Pups were weaned at P21 and housed in a cage adjacent to their mother's cage until adulthood (P56). This design allowed offspring to receive sensory cues from their mother without physical contact, approximating a form of social buffering through perceptual familiarity rather than

direct caregiving. After maturation, a battery of behavioural tests was conducted to assess how maternal presence influenced emotional behaviour. This study investigated whether post-weaning maternal presence exerts a perceptual buffering effect on socioemotional outcomes in offspring, particularly under social isolation stress. We targeted the post-weaning to adolescent period as a critical window of vulnerability. We hypothesized that the passive presence of the mother in an adjacent cage could partially modulate behavioural alterations induced by social isolation. To provide comparison conditions, we included group housing with siblings—representing social buffering—and exposure to an unfamiliar adult male, which could act as a potential social stressor. Because male pheromones are known to influence pubertal development and behaviour in young mice[30,31], this factor was considered in interpreting the findings. We anticipated that social isolation would produce behavioural abnormalities, that maternal presence would partially buffer these effects, that sibling housing would offer additional protection, and that exposure to an unfamiliar adult male would exacerbate stress-related outcomes.

Experimental procedures

Ethics statements

All animal experiments were conducted in accordance with relevant guidelines and regulations and adhered to ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines) and the U.S. National Institutes of Health's (NIH) *Guide for the Care and Use of Laboratory Animals* (8th edition, 2011). The study was approved by the Committee for Animal Experiments of Kawasaki Medical School (Approval No. 24–026, approved in 2024). Every effort was made to minimise the number of animals used and their suffering. The experimental design aimed to reduce animal usage while ensuring scientific rigour and statistical validity, employing the smallest possible number of animals per group and the minimum number of experimental groups.

Animals

Male C57BL/6N mice at postnatal day 14 (P14), during the lactation period, were purchased from The Jackson Laboratory Japan (Tokyo, Japan). A total of 36 C57BL/6N mouse pups were used in each experimental series. Each group (n = 12) consisted of offspring derived from all three litters, thereby minimizing potential litter-related confounds. In Experiment 1, each group cage housed four pups from the same litter, and each cage was positioned adjacent to either their biological dam (MS), an unfamiliar adult male (NUM), or no adjacent cage (control). In Experiment 2, one male pup from each litter was assigned to each condition (control, SI, and MS–SI), and pups in the MS–SI group were placed adjacent to their biological dam. The same dams were not reused between experiments. Transparent plastic mesh cages (220 × 340 × 150 mm) equipped with a non-woven fabric filter cap on the upper mesh were used. Nesting material, food (MF-R; Oriental Yeast Co., Tokyo, Japan), and water were provided ad libitum. Animals were maintained under a 12-h light/dark cycle (lights on at 08:00 and off at 20:00) at a temperature of 23–26 °C. Because behavioural variability is partly sex-dependent, and the present study did not aim to compare male and female behaviours, only male mice were used. Cages were cleaned once per week. All behavioural experiments were conducted in a room separate from the housing room to avoid potential environmental influences such as odor, sound, or position effects. Within the housing room, cages for all experimental groups were placed on racks at the same height to minimize positional variation. For each experiment, all groups were run simultaneously rather than in separate cohorts.

Housing with a maternal or unfamiliar mouse in an adjacent cage

Male C57BL/6N mice at postnatal week 3 were randomly assigned to one of three groups (control group, maternal supervision group, or neighboring unfamiliar mouse group) using an online randomisation tool (https://www.randomizer.org) (Fig. 1A). Subject mice were housed four per cage. In the control group (n = 12), mice were housed in opaque cages with no visual access to the surrounding environment. Group housing with siblings was included to provide a social buffering condition. Thus, maternal effects were evaluated not only in comparison with isolated animals but also in relation to sibling-housed animals. In the maternal supervision group (n = 12), mice were housed in a cage adjacent to a single cage containing their mother. After weaning, pups assigned to the maternal supervision group were housed in a transparent cage positioned directly adjacent to their dam's home cage. This arrangement allowed the pups to maintain continuous visual contact with the dam. Although direct physical interaction and nursing were prevented by the cage walls, it is possible that the pups were also exposed to limited olfactory and auditory cues from the mother due to the proximity of the cages. This configuration enabled multi-modal sensory exposure, which we refer to as "maternal watchful supervision" in the context of this study. In the neighboring unfamiliar mouse group (n = 12), mice were housed in a cage adjacent to a single cage containing an unfamiliar adult male mouse (10 weeks old, C57BL/6N). The unfamiliar male condition was chosen to introduce a potential social stressor, given that male pheromones and cues can elevate arousal and stress responses in younger mice. Mice were housed under these conditions from postnatal week 3 to postnatal week 8. At 8 weeks of age, the mice underwent behavioural testing (Fig. 1B). In addition to maternal presence, two comparison conditions were used: group housing of male siblings, to provide a form of social buffering, and housing adjacent to an unfamiliar adult male, which was expected to act as a potential social stressor through visual, olfactory, and pheromonal cues.

Housing with a maternal mouse in an adjacent cage under social isolation stress conditions

Male C57BL/6N mice at postnatal week 3 were randomly assigned to one of three groups (control group, social isolation under maternal supervision group, or social isolation group) using an online randomisation tool (https://www.randomizer.org) (Fig. 6A). In the control group (n = 12), mice were group-housed (four per cage) in opaque cages with no visual access to the surrounding environment. In the social isolation under maternal supervision group (n = 12), mice were individually housed (one per cage) in a cage adjacent to a cage containing their mother. In the social isolation group (n = 12), mice were individually housed (one per cage) in opaque

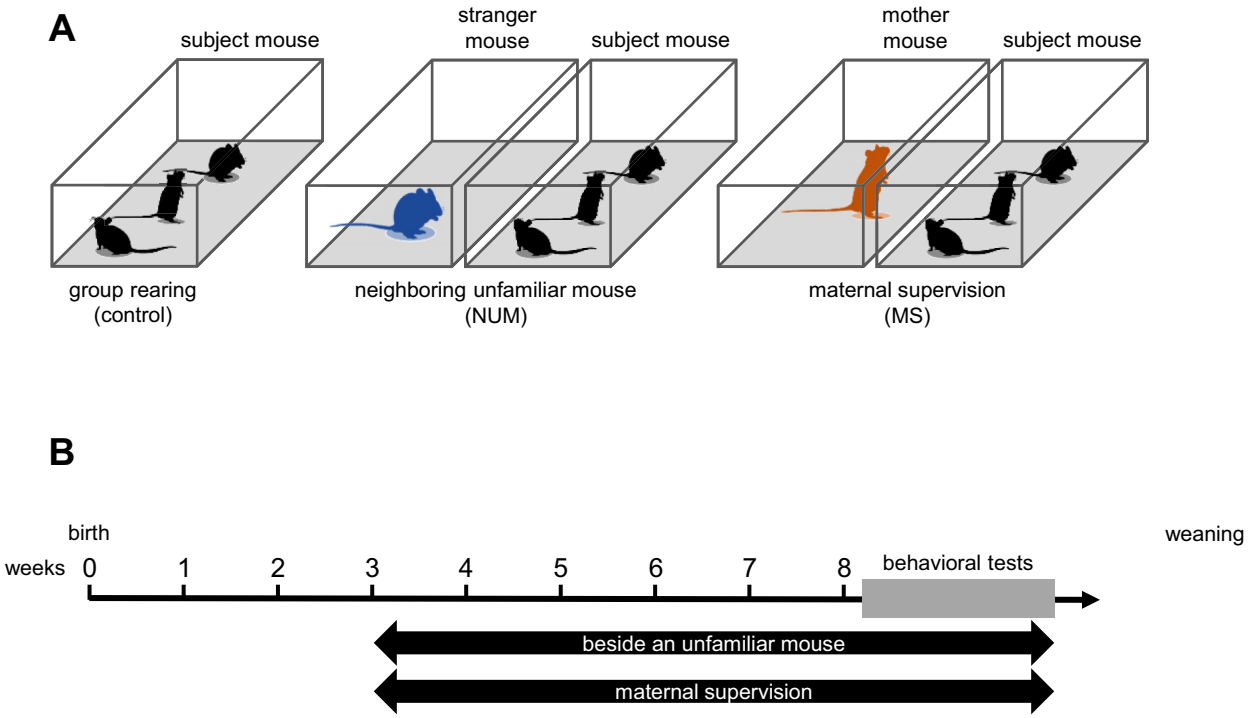

Fig. 1. Experimental design. (**A**) Schematic representation of the housing conditions used in the study. After weaning at postnatal week 3, male C57BL/6N mice were assigned to one of three groups: group rearing (control), maternal supervision (MS), or neighboring unfamiliar mouse (NUM). (**B**) Experimental timeline. From postnatal week 3 to week 8, mice were maintained under the assigned housing conditions. Behavioural testing was conducted at 8 weeks of age. The time axis is expressed in weeks.

cages with no visual access to the surrounding environment. Mice were maintained under these conditions from postnatal week 3 to postnatal week 8. At 8 weeks of age, the mice underwent behavioural testing (Fig. 6B). In both experiments, exposure to maternal presence, an unfamiliar adult male, or isolation was conducted continuously from postnatal week 3 to week 8.

Primary outcomes were defined as follows: maternal presence was hypothesized to reduce anxiety-like behaviour and normalize social interaction, sibling housing to attenuate but not eliminate isolation-induced changes, unfamiliar adult male exposure to increase stress-related responses, and social isolation to cause robust abnormalities across multiple behavioural domains.

Behavioural tests

All behavioural tests were conducted in dedicated behavioural testing rooms between 09:00 and 16:00 during the light phase of the light/dark cycle. To minimise potential confounding effects from olfactory cues, all testing apparatus and objects were thoroughly cleaned with 70% ethanol and superhypochlorous water after each use. Behavioural assessments were performed on experimentally naïve mice (i.e., mice with no prior exposure to any of the behavioural tests). Behavioural tests were conducted in the following order to minimize carry-over effects: grip-strength test (postnatal day 57, P57), elevated plus maze (P58), light/dark transition test (P59), open field test (P60), Y-maze, social interaction test (P61), tail-suspension test (P62), hotplate test (P63), cotton bud biting test (P64), and Porsolt forced swim test (P65). The order was chosen so that less stressful tasks preceded more stressful ones. All procedures were performed according to established protocols[26,32]. All behavioural assessments were performed by trained observers who were blinded to the experimental conditions of the subjects. The behavioural battery was chosen to assess multiple domains relevant to our hypotheses. Anxiety-like behaviours were evaluated using the elevated plus maze, light/dark transition, and open field tests, as these measures are sensitive to social isolation and maternal buffering. Social interaction was assessed in a novel environment, since isolation is known to strongly affect affiliative behaviours. Cognitive function was evaluated using the Y-maze to test spatial working memory, which may be altered by adolescent stress. Grip strength was measured as an indicator of physical function, which can be reduced under chronic stress conditions. Depression-like behaviours were assessed with the tail suspension and forced swim tests, widely used paradigms for evaluating stress-induced immobility.

Grip-strength test

Neuromuscular strength was assessed using a grip-strength test[33]. Forelimb grip strength was measured with a grip dynamometer. Each mouse was gently lifted by the tail and allowed to grasp the wire grid of the dynamometer with its forepaws. The mouse was then slowly pulled backward until it released its grip. The peak force exerted

by the forelimbs during the task was recorded in centinewtons (cN). To control for potential differences in body size, grip strength values were normalized to the body weight of each animal prior to statistical analysis.

Hotplate test

The hotplate test was used to assess nociceptive responses[34]. Mice were placed on a hotplate maintained at 55.0 ± 0.3 °C, and the latency to the first paw response (such as paw licking or shaking) was recorded. A cutoff latency of 30 s was applied to prevent tissue damage and was considered indicative of complete analgesia.

Cotton bud biting test

Aggressive behaviour was assessed using the cotton swab bite test. Each mouse was gently held in the experimenter's hand, and a sterile cotton swab was presented near its face. Biting of the swab was considered an aggressive response. Each mouse was tested ten times, and the total number of biting responses was recorded for analysis.

Elevated plus maze test

Anxiety-like behaviour was assessed using the elevated plus maze[35]. The apparatus consisted of two open arms (8 × 25 cm) and two closed arms of the same size, each enclosed by transparent walls 30 cm in height. The arms were constructed from white plastic plates and elevated 50 cm above the floor, with opposing arms of the same type. The illumination level at the centre of the maze was set to 100 lx. Each mouse was initially placed in the central square of the maze, facing a closed arm, and allowed to explore freely among the four arms for 6 min. Behaviour was recorded on video and analysed using ANY-maze software (ANY-maze, Stoelting Co., Wood Dale, IL, USA): number of arm entries, distance travelled (m), and time spent in the open arms (s).

Light/dark transition test

The light/dark transition test assessed anxiety-related behaviour and exploratory activity[36]. The apparatus for the light/dark transition test consisted of an acrylic cage (22 × 44 × 40 cm) divided into two equal sections by a partition with a door. One chamber had white acrylic walls and was brightly illuminated (200 lx), while the other chamber had black acrylic walls and remained dim (50 lx). Both chambers had white plastic floors. Mice were initially placed in the dark chamber and allowed to move freely between the two chambers for 6 min with the door open. The following parameters were analysed using ANY-maze software to assess the distance travelled (m), total number of transitions, and time spent in the light chamber (s).

Open field test

Exploratory activity and anxiety-like behaviour were assessed using an open field apparatus[37]. The open field test was used to assess exploratory behaviour, anxiety-like behaviour, and general locomotor activity. Each mouse was placed in the centre of a square arena (45 × 45 × 40 cm). The following parameters were recorded: distance travelled (m), number of entries into the central area, and time spent in the central area (s). The central area was defined as the central 20 × 20 cm portion of the field. The test chamber was illuminated at 100 lx, and data were collected over a 20-min period. Data analysis was performed using ANY-maze software.

Y-maze test

Spatial working memory was assessed using a Y-maze apparatus (arm length, 40 cm; bottom arm width, 3 cm; upper arm width, 10 cm; wall height, 12 cm)[38]. Each mouse was placed in the centre of the Y-maze and allowed to explore freely for 6 min. Visual cues were placed around the testing chamber and remained visible throughout the test period. Mice were tested without any prior exposure or habituation to the maze. The following parameters were recorded and analysed using ANY-maze software: total distance travelled (m), number of arm entries, and number of alternations.

Social interaction test in a novel environment

Social behaviour was assessed by allowing two unfamiliar mice to interact freely in a novel environment for 10 min[39]. For the social interaction test, two mice from the same experimental group but housed in separate cages were placed together in a novel box (45 × 45 × 40 cm) and allowed to interact freely for 10 min. Their behaviours were recorded, and the following parameters were analysed: total distance travelled (m), total number of contacts, and mean duration per contact (s). Data were analysed using SMART software, version 3.0 (PanLab, Harvard Apparatus, Spain).

Tail-suspension test

Depressive-like behaviour was assessed using the tail-suspension test[40]. Each mouse was suspended by the tail in a white plastic chamber positioned 60 cm above the floor, secured with adhesive tape placed less than 1 cm from the tail tip. Behaviour was recorded for 6 min using a video camera, and immobility time was measured. Immobility was defined as the period during which the mouse ceased struggling for ≥ 1 s. Data acquisition and analysis were performed using ANY-maze software.

Porsolt forced-swim test

The Porsolt forced-swim test was used to assess depressive-like behaviour. The apparatus consisted of four Plexiglas cylinders (20 cm in height × 10 cm in diameter) filled with water (23 °C) to a depth of 7.5 cm, based on previous studies[41,42]. Mice were positioned in the cylinders for 6 min, and their behaviour was recorded. Consistent with the tail-suspension test, immobility time was assessed using ANY-MAZE software.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). The normality of all data sets was assessed using the Shapiro–Wilk test, and all groups met the assumptions of normality required for parametric testing. Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Data are presented as box plots showing the median and interquartile range. This format was chosen to robustly visualize the data distribution and minimize the influence of potential outliers. Statistical significance was defined as $p < 0.05$.

Results

Effects of maternal supervision and exposure to an unfamiliar mouse on body weight, grip strength, pain sensitivity, and aggressive behaviour

We examined the effects of post-weaning maternal supervision and exposure to an unfamiliar mouse on body weight, grip strength, pain sensitivity, and aggressive behaviour (Fig. 2**,** Table 1). At the start of the experiment (postnatal week 3), there were no significant differences in body weight among the groups (mean ± SEM; control: 8.37 ± 0.16 g, SI: 8.67 ± 0.0.37 g, MS-SI: 9.24 ± 0.36 g; one-way ANOVA, $F(_{2, 33}) = 2.493$, $p = 0.104$). No significant differences in body weight at 8 weeks of age were observed among the groups (Fig. 2A). In contrast, body weight gain from postnatal week 3 to week 8 was significantly higher in the MS group compared with both the control group and the NUM group ($p < 0.0001$; Fig. 2B). Normalized forelimb grip strength was significantly higher in the NUM group compared with both the control group ($p < 0.001$) and the MS group ($p < 0.05$; Fig. 2C). There were no significant differences among the groups in pain sensitivity as assessed by latency to paw response in the hot plate test (Fig. 2D), or in aggressive behaviour as measured by the number of biting responses in the cotton swab bite test (Fig. 2E).

Effects of maternal supervision and exposure to an unfamiliar mouse on anxiety-like behaviour and exploratory activity

Anxiety-like behaviour and exploratory activity were assessed using the elevated plus maze test, light/dark transition test, and open field test (Fig. 3**,** Table 1). In the elevated plus maze test, no significant differences were observed among the groups in total distance travelled, number of arm entries, time spent in the open arms, or

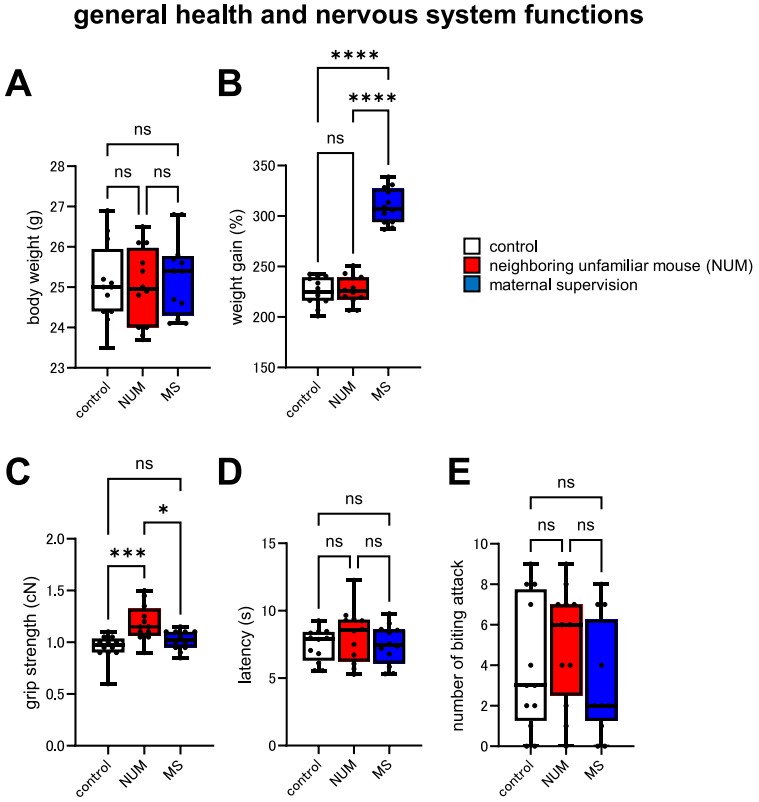

Fig. 2. Effects of maternal supervision and exposure to an unfamiliar mouse on body weight, grip strength, pain sensitivity, and aggressive behaviour. (**A**) Body weight at 8 weeks of age. (**B**) Percentage of body weight gain from postnatal week 3 to week 8. (**C**) Grip strength as measured by the grip-strength test. (**D**) Pain sensitivity as assessed by latency to paw response in the hot plate test. (**E**) Aggressive behaviour assessed by the number of biting responses in the cotton swab bite test. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). $p^* < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$, $p^{****} < 0.0001$; ns, not significant.

			F (DFn, DFd)	ANOVA p-value				p Value
Fig. 2	body weight	A	F (2, 33) = 0.2481	0.7817	control	vs.	MS	0.8979
					control	vs.	NUM	0.9654
					MS	vs.	NUM	0.7676
	weight gain	B	F (2, 33) = 119.5	<0.0001	control	vs.	MS	<0.0001
					control	vs.	NUM	0.9617
					MS	vs.	NUM	<0.0001
	grip strength test	C	F (2, 33) = 9.605	0.0005	control	vs.	MS	0.42
					control	vs.	NUM	0.0004
					MS	vs.	NUM	0.0139
	hot plate test	D	F (2, 33) = 0.7999	0.4579	control	vs.	MS	0.9991
					control	vs.	NUM	0.5116
					MS	vs.	NUM	0.5364
	cotton but	E	F (2, 33) = 1.365	0.2694	control	vs.	MS	0.7736
					control	vs.	NUM	0.6072
					MS	vs.	NUM	0.2416
Fig. 3	elevated plus maze test	A	F (2, 33) = 1.907	0.1646	control	vs.	MS	0.1483
					control	vs.	NUM	0.4287
					MS	vs.	NUM	0.7853
		B	F (2, 33) = 0.8040	0.4561	control	vs.	MS	0.9653
					control	vs.	NUM	0.6134
					MS	vs.	NUM	0.46
		C	F (2, 33) = 1.261	0.297	control	vs.	MS	0.9322
					control	vs.	NUM	0.4764
					MS	vs.	NUM	0.2931
		D	F (2, 33) = 1.548	0.2286	control	vs.	MS	0.2029
					control	vs.	NUM	0.7779
					MS	vs.	NUM	0.5187
	light/dark transition test	E	F (2, 33) = 4.254	0.023	control	vs.	MS	0.7396
					control	vs.	NUM	0.1065
					MS	vs.	NUM	0.0231
		F	F (2, 33) = 1.393	0.2626	control	vs.	MS	0.9061
					control	vs.	NUM	0.4696
					MS	vs.	NUM	0.2557
		G	F (2, 33) = 3.384	0.0464	control	vs.	MS	0.2214
					control	vs.	NUM	0.6514
					MS	vs.	NUM	0.0391
		H	F (2, 33) = 0.1449	0.8665	control	vs.	MS	0.9651
					control	vs.	NUM	0.8544
					MS	vs.	NUM	0.9932
	open field test	I	F (2, 33) = 0.1559	0.8563	control	vs.	MS	0.8431
					control	vs.	NUM	0.9634
					MS	vs.	NUM	0.9524
		J	F (2, 33) = 0.9589	0.3937	control	vs.	MS	0.366
					control	vs.	NUM	0.8544
					MS	vs.	NUM	0.6823
		K	F (2, 33) = 0.1209	0.8865	control	vs.	MS	0.8782
					control	vs.	NUM	0.9823
					MS	vs.	NUM	0.9497

Table 1. Significance levels for the effects reported in Figs. 2–5.

latency to first entry into an open arm (Fig. 3A–D). In the light/dark transition test, the NUM group exhibited significantly greater distance travelled ($p < 0.05$) and time spent in the light compartment ($p < 0.05$) compared with the MS group (Fig. 3E,G). Other parameters did not differ significantly among groups (Fig. 3F,H). In the open field test, no significant differences were found among groups in total distance travelled, number of entries into the central area, or time spent in the central area (Fig. 3I–K).

			F (DFn, DFd)	ANOVA p-value				p Value
Fig. 4	Y-maze test	A	F (2, 33) = 0.9193	0.4088	control	vs.	MS	0.4082
					control	vs.	NUM	0.9524
					MS	vs.	NUM	0.5841
		B	F (2, 33) = 1.140	0.3321	control	vs.	MS	0.4939
					control	vs.	NUM	0.9578
					MS	vs.	NUM	0.34
		C	F (2, 33) = 0.9766	0.3872	control	vs.	MS	0.4076
					control	vs.	NUM	0.9794
					MS	vs.	NUM	0.52
		D	F (2, 33) = 0.2633	0.7701	control	vs.	MS	0.7946
					control	vs.	NUM	0.999
					MS	vs.	NUM	0.8175
	social interaction test in a novel environment	E	F (2, 33) = 4.068	0.0263	control	vs.	MS	0.2226
					control	vs.	NUM	0.4965
					MS	vs.	NUM	0.0207
		F	F (2, 15) = 0.5507	0.5878	control	vs.	MS	0.6315
					control	vs.	NUM	0.6556
					MS	vs.	NUM	0.9991
		G	F (2, 15) = 1.742	0.2088	control	vs.	MS	0.1934
					control	vs.	NUM	0.8211
					MS	vs.	NUM	0.4551
		H	F (2, 15) = 0.2010	0.82	control	vs.	MS	0.956
					control	vs.	NUM	0.8044
					MS	vs.	NUM	0.936
Fig. 5	tail suspension test	A	F (2, 33) = 0.2141	0.8084	control	vs.	MS	0.9642
					control	vs.	NUM	0.9189
					MS	vs.	NUM	0.7938
	Porsolt forced swim test	B	F (2, 33) = 1.758	0.1881	control	vs.	MS	0.388
					control	vs.	NUM	0.8824
					MS	vs.	NUM	0.1823

Fig. 1. (continued)

Effects of maternal supervision and exposure to an unfamiliar mouse on spatial working memory and social behaviour

Spatial working memory and social behaviour were assessed using the Y-maze test and the social interaction test in a novel environment (Fig. 4, Table 1). In the Y-maze test, no significant differences among groups were observed in total distance travelled, number of arm entries, total number of alternations, or percentage of spontaneous alternations (Fig. 4A–D). In the social interaction test, the total distance travelled was significantly lower in the NUM group compared with the MS group ($p < 0.05$), while no significant difference was observed between the NUM and control groups (Fig. 4E). In the social interaction test, there were no significant differences among groups in number of contacts, total contact time, or mean duration per contact (Fig. 4F–H).

Effects of maternal supervision and exposure to an unfamiliar mouse on depressive-like behaviour

Depressive-like behaviour was assessed using the tail suspension test and the Porsolt forced swim test (Fig. 5, Table 1). No significant differences among groups were found in total immobility percentage in either test (Fig. 5A,B).

While maternal supervision did not induce substantial behavioural changes in mice raised under standard group housing conditions (Fig. 2–5), it remained unclear whether maternal presence could exert protective effects under more stressful environments. To address this, we subjected offspring to social isolation stress after weaning, and examined whether maternal supervision in this context would alleviate stress-induced behavioural alterations (Fig. 6).

Effects of maternal supervision under social isolation on body weight, grip strength, pain sensitivity, and aggressive behaviour

We next examined the effects of maternal supervision under social isolation (MS-SI) on body weight, grip strength, pain sensitivity, and aggressive behaviour (Fig. 7, Table 2). At the start of the experiment (postnatal week 3), there were no significant differences in body weight among the groups (mean ± SEM; control: 10.93 ± 0.10 g, NUM: 10.95 ± 0.18 g, MS: 10.44 ± 0.17 g; one-way ANOVA, $F_{(2, 33)} = 0.613$, $p = 0.55$). Body weight at 8 weeks

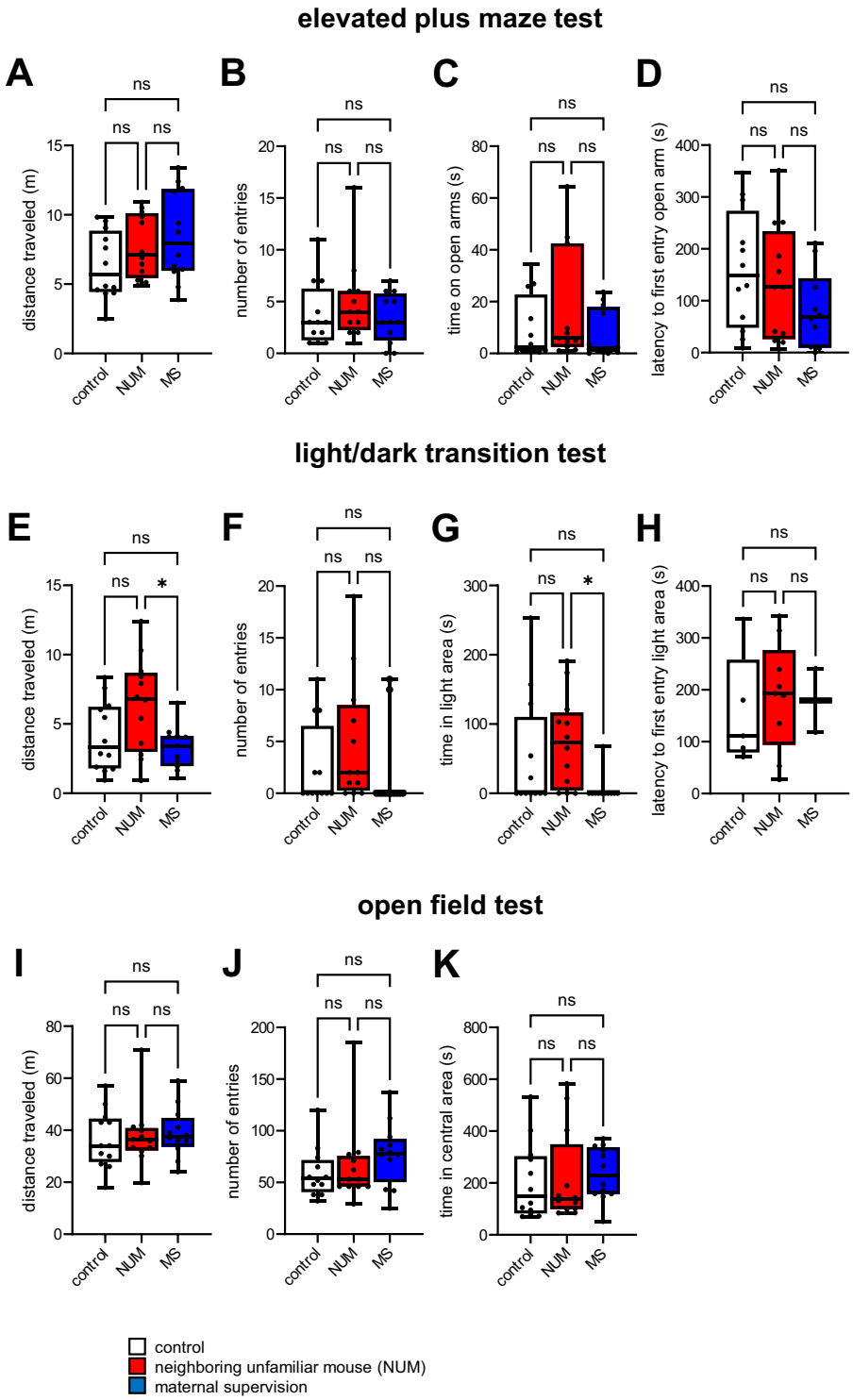

Fig. 3. Effects of maternal supervision and exposure to an unfamiliar mouse on anxiety-like behaviour and exploratory activity. Elevated plus maze test: (**A**) Total distance travelled. (**B**) Number of arm entries. (**C**) Time spent in the open arms. (**D**) Latency to first entry into an open arm. Light/dark transition test: (**E**) Total distance travelled. (**F**) Number of transitions between light and dark compartments. (**G**) Time spent in the light compartment. (H) Latency to first entry into the light compartment. Open field test: (**I**) Total distance travelled. (**J**) Number of entries into the central area. (**K**) Time spent in the central area. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). $p^* < 0.05$; ns, not significant.

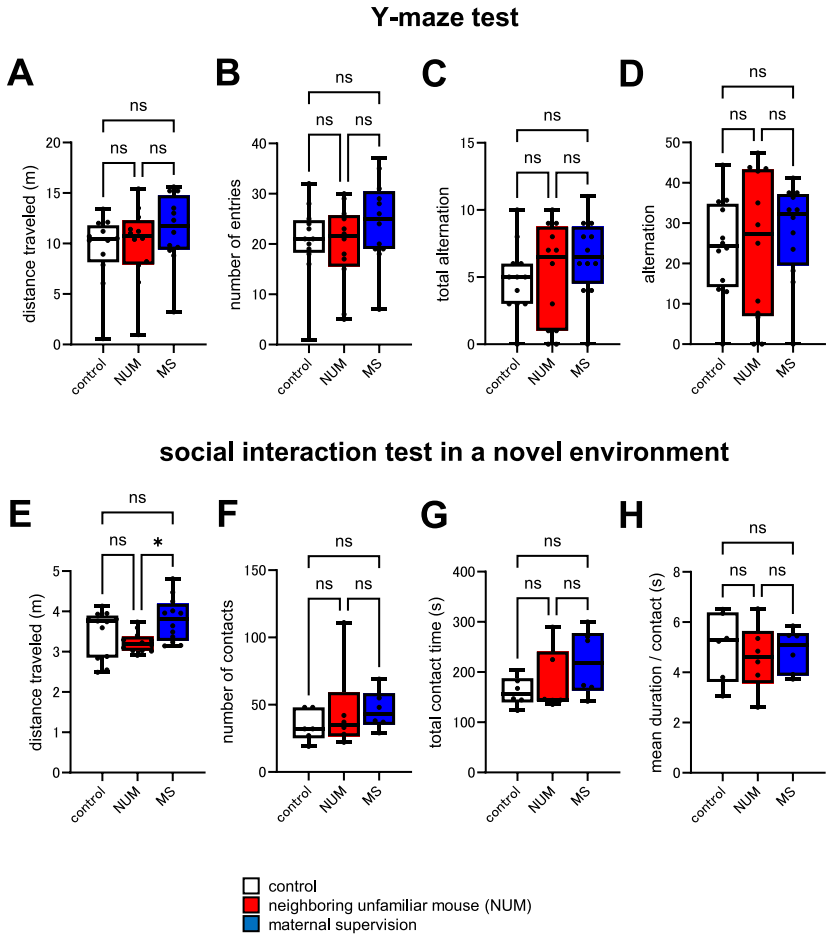

Fig. 4. Effects of maternal supervision and exposure to an unfamiliar mouse on spatial working memory and social behaviour. Y-maze test: (**A**) Total distance travelled. (**B**) Number of arm entries. (**C**) Total number of alternations. (**D**) Percentage of spontaneous alternations. Social interaction test in a novel environment: (**E**) Total distance travelled. (**F**) Number of contacts. (**G**) Total contact time. (**H**) Mean duration per contact. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). $p^* < 0.05$; ns, not significant.

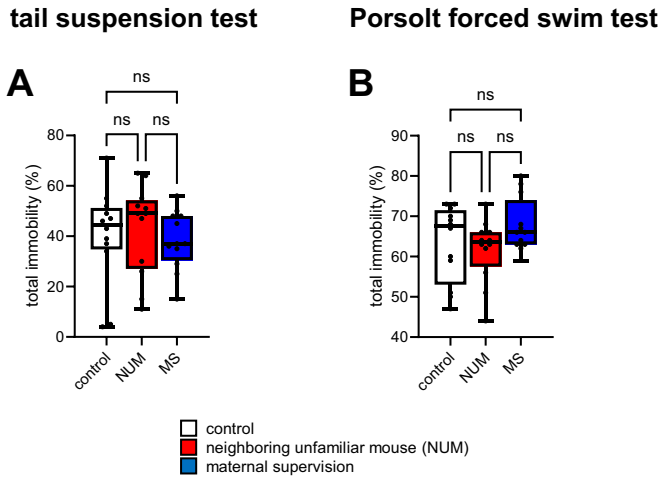

Fig. 5. Effects of maternal supervision and exposure to an unfamiliar mouse on depressive-like behaviour. (**A**) Total immobility percentage in the tail suspension test. (**B**) Total immobility percentage in the Porsolt forced swim test. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). ns, not significant.

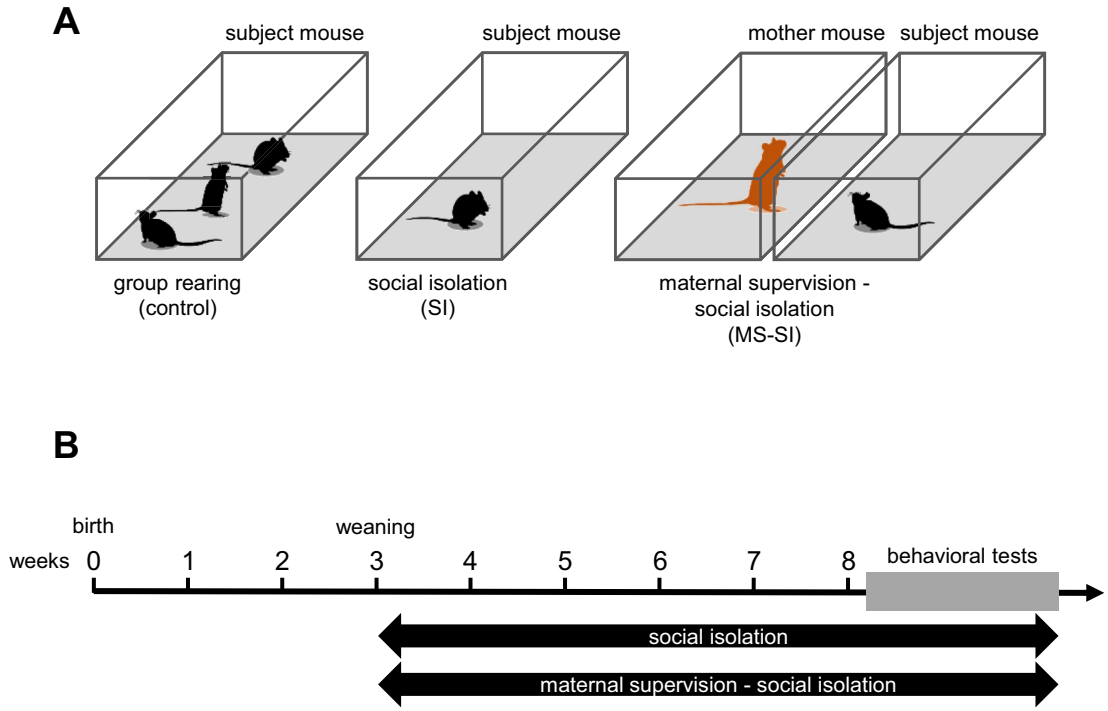

Fig. 6. Experimental design under social isolation conditions. (**A**) Schematic representation of the housing conditions used in the social isolation experiment. After weaning at postnatal week 3, male C57BL/6N mice were assigned to one of three groups: group rearing (control), maternal supervision under social isolation (MS-SI), or social isolation (SI). (**B**) Experimental timeline. From postnatal week 3 to week 8, mice were maintained under the assigned housing conditions. Behavioural testing was conducted at 8 weeks of age.

of age did not differ significantly among groups (Fig. 7A). Body weight gain from postnatal week 3 to week 8 was significantly greater in the SI group compared with both the control group ($p < 0.01$) and the MS-SI group ($p < 0.05$), whereas no significant difference was observed between the MS-SI and control groups (Fig. 7B). Grip strength was significantly lower in the MS-SI group compared with the control group ($p < 0.05$; Fig. 7C). No significant differences were observed in the hot plate test (Fig. 7D). Aggressive behaviour, as measured by the number of biting responses in the cotton swab bite test, was significantly lower in the SI group compared with the control group ($p < 0.01$; Fig. 7E). Maternal supervision under social isolation (MS-SI) normalized aggressive behaviour, with no significant difference from the control group.

Effects of maternal supervision under social isolation on anxiety-like behaviour and exploratory activity

Anxiety-like behaviour and exploratory activity were assessed under social isolation conditions (Fig. 8, Table 2). In the elevated plus maze, the total distance travelled was significantly greater in the SI group compared with both the control and MS-SI groups ($p < 0.05$), whereas no significant difference was observed between the MS-SI and control groups (Fig. 8A). Similarly, time spent in the open arms was significantly increased only in the SI group compared with controls ($p < 0.05$), while the MS-SI group did not differ from the control group (Fig. 8C). The number of arm entries was significantly higher in the SI group compared with the control group ($p < 0.05$; Fig. 8B). Latency to first entry into an open arm did not differ significantly (Fig. 8D). In the light/dark transition test, the SI group travelled a significantly greater distance compared with both the MS-SI and control groups ($p < 0.01$), whereas no significant difference was observed between the MS-SI and control groups (Fig. 8E). In the light/dark transition test, the latency to first entry into the light compartment was significantly shorter in the MS-SI group compared with the control group ($p < 0.05$; Fig. 8H). Other parameters showed no significant differences (Fig. 8F,G). No significant differences were found in the open field test (Fig. 8I–K).

Effects of maternal supervision under social isolation on spatial working memory and social behaviour

In the Y-maze test, the SI group exhibited significantly greater total distance travelled and number of arm entries compared with the control group ($p < 0.01$; Fig. 9A,B, Table 2), whereas no significant difference was observed between the MS-SI and control groups. No significant differences were observed in total number of alternations or percentage of spontaneous alternations (Fig. 9C,D). In the social interaction test, the SI group exhibited significantly greater total distance travelled compared with the control group ($p < 0.05$; Fig. 9E), whereas no significant difference was observed between the MS-SI and control groups. In the social interaction test, the SI group exhibited significantly more contacts ($p < 0.05$) and longer total contact time ($p < 0.05$) compared with the

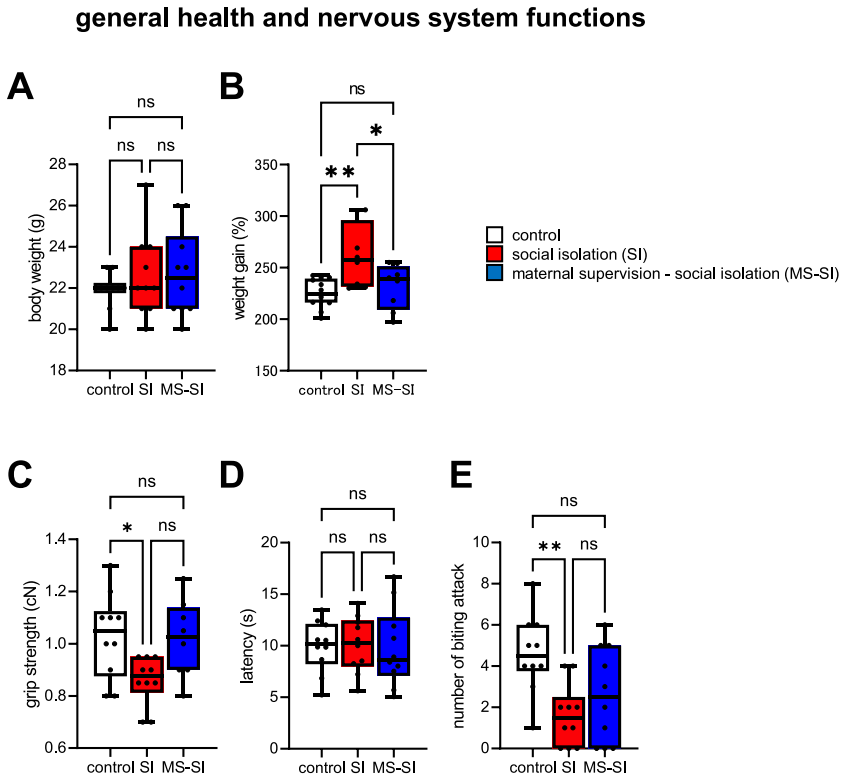

Fig. 7. Effects of maternal supervision under social isolation on body weight, grip strength, pain sensitivity, and aggressive behaviour. (**A**) Body weight at 8 weeks of age. (**B**) Percentage of body weight gain from postnatal week 3 to week 8. (**C**) Grip strength as measured by the grip-strength test. (**D**) Pain sensitivity as assessed by latency to paw response in the hot plate test. (**E**) Aggressive behaviour assessed by the number of biting responses in the cotton swab bite test. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). $p^* < 0.05$, $p^{**} < 0.01$; ns, not significant.

control group (Fig. 9F,G), whereas no significant differences were observed between the SI and MS-SI groups. No significant differences were found in mean duration per contact (Fig. 9H).

Effects of maternal supervision under social isolation on depressive-like behaviour

No significant differences among groups were observed in total immobility percentage in either the tail suspension test or the Porsolt forced swim test (Fig. 10A,B, Table 2).

Discussion

The present study examined the effects of post-weaning maternal supervision on behavioural development in mice, both under standard group housing conditions and under social isolation stress. Our findings suggest that maternal presence had limited impact on behaviour when offspring were reared in a standard environment. However, under social isolation stress, maternal supervision showed a context-dependent buffering effect against certain stress-induced behavioral alterations. These results suggest that the protective role of maternal presence may be particularly evident in adverse environmental contexts. It should be noted that sibling cohousing itself provides social buffering, which may attenuate stress-related behaviours. Therefore, the effects of maternal presence were interpreted relative to both sibling and isolation groups, to better distinguish maternal-specific influences from general social buffering.

Group-housed mice reared adjacent to their mother tended to exhibit greater weight gain, which reached statistical significance only at specific time points. However, there were no significant differences in body weight at 8 weeks among the three groups. These results indicate that maternal presence during post-weaning group housing influences the rate of weight gain. Significant changes in eating patterns and body weight are commonly observed among individuals experiencing depression, anxiety, or stress[43,44]. Stress is well known to alter body weight and food intake in animal models. Our results suggest that post-weaning maternal presence affects these processes. Further research is needed to clarify the mechanisms underlying this increased weight gain.

Mice reared with an unfamiliar mouse in an adjacent cage exhibited increased grip strength compared to other groups. Grip strength testing is a reliable measure of forelimb strength in mice[45]. Peak forelimb grip strength in mice is reported to occur around 4 months of age[46]. Grid climbing behaviour in the home cage is known to maintain muscle strength in mice[47]. The presence of an unfamiliar mouse likely promoted exploratory or arousal-related behaviours, including grid climbing, which may have secondarily enhanced forelimb strength. Notably, the use of an unfamiliar adult male, rather than a female, may have played a distinctive role in shaping

			F (DFn, DFd)	ANOVA p-value				p Value
Fig. 7	body weight	A	F (2, 33) = 0.6151	0.548	control	vs.	NUM	0.5722
					control	vs.	MS	0.6507
					NUM	vs.	MS	0.9911
	weight gain	B	F (2, 33) = 6.874	0.0042	control	vs.	NUM	0.8152
					control	vs.	MS	0.0038
					NUM	vs.	MS	0.0301
	grip strength test	C	F (2, 33) = 4.624	0.0196	control	vs.	NUM	0.9838
					control	vs.	MS	0.0279
					NUM	vs.	MS	0.057
	hot plate test	D	F (2, 33) = 0.02680	0.9736	control	vs.	NUM	0.9946
					control	vs.	MS	0.9905
					NUM	vs.	MS	0.9711
	cotton but	E	F (2, 33) = 6.225	0.006	control	vs.	NUM	0.0714
					control	vs.	MS	0.0049
					NUM	vs.	MS	0.4896
Fig. 8	elevated plus maze test	A	F (2, 33) = 4.960	0.0157	control	vs.	NUM	>0.9999
					control	vs.	MS	0.0303
					NUM	vs.	MS	0.0259
		B	F (2, 33) = 4.192	0.0274	control	vs.	NUM	0.6117
					control	vs.	MS	0.0234
					NUM	vs.	MS	0.1362
		C	F (2, 33) = 4.753	0.0182	control	vs.	NUM	0.9304
					control	vs.	MS	0.0241
					NUM	vs.	MS	0.0529
		D	F (2, 33) = 0.4314	0.6547	control	vs.	NUM	0.9509
					control	vs.	MS	0.793
					NUM	vs.	MS	0.6421
	light/dark transition test	E	F (2, 33) = 6.560	0.0058	control	vs.	NUM	0.8912
					control	vs.	MS	0.0238
					NUM	vs.	MS	0.0063
		F	F (2, 33) = 1.418	0.2603	control	vs.	NUM	0.3482
					control	vs.	MS	0.9934
					NUM	vs.	MS	0.2976
		G	F (2, 33) = 0.6609	0.5245	control	vs.	NUM	0.6319
					control	vs.	MS	0.5487
					NUM	vs.	MS	0.99
		H	F (2, 33) = 3.319	0.0459	control	vs.	NUM	0.0455
					control	vs.	MS	0.6058
					NUM	vs.	MS	0.2709
	open field test	I	F (2, 33) = 0.5702	0.5721	control	vs.	NUM	0.9906
					control	vs.	MS	0.5934
					NUM	vs.	MS	0.6743
		J	F (2, 33) = 0.5659	0.5745	control	vs.	NUM	0.7228
					control	vs.	MS	0.9669
					NUM	vs.	MS	0.5711
		K	F (2, 33) = 0.1549	0.8572	control	vs.	NUM	0.9984
					control	vs.	MS	0.8685
					NUM	vs.	MS	0.8938

Table 2. Significance levels for the effects reported in Figs. 7–10.
The ANOVA p-value column indicates the overall significance level from the one-way ANOVA.

these behavioural outcomes. Male-derived pheromones and social cues are known to accelerate pubertal onset and enhance stress sensitivity in young mice, potentially eliciting heightened arousal or exploratory activity[31]. Therefore, the unfamiliar male condition likely functioned not merely as a source of social stimulation, but also as a stress-inducing factor—a consideration that is important for interpreting the behavioural data in this context.

			F (DFn, DFd)	ANOVA p-value				p Value
Fig. 9	Y-maze test	A	F (2, 33) = 5.155	0.013	control	vs.	NUM	0.2549
					control	vs.	MS	0.0095
					NUM	vs.	MS	0.2503
		B	F (2, 33) = 5.688	0.0089	control	vs.	NUM	0.6926
					control	vs.	MS	0.0085
					NUM	vs.	MS	0.053
		C	F (2, 33) = 2.722	0.0845	control	vs.	NUM	0.8419
					control	vs.	MS	0.0812
					NUM	vs.	MS	0.2222
		D	F (2, 33) = 0.01321	0.9869	control	vs.	NUM	0.9987
					control	vs.	MS	0.9862
					NUM	vs.	MS	0.9937
	social interaction test in a novel environment	E	F (2, 33) = 4.289	0.0246	control	vs.	NUM	0.57
					control	vs.	MS	0.0212
					NUM	vs.	MS	0.1587
		F	F (2, 15) = 3.712	0.0486	control	vs.	NUM	0.2885
					control	vs.	MS	0.0482
					NUM	vs.	MS	0.4791
		G	F (2, 15) = 5.241	0.0252	control	vs.	NUM	0.143
					control	vs.	MS	0.0223
					NUM	vs.	MS	0.4631
		H	F (2, 15) = 1.055	0.3783	control	vs.	NUM	0.4253
					control	vs.	MS	0.4645
					NUM	vs.	MS	0.9971
Fig. 10	tail suspension test	A	F (2, 33) = 0.3989	0.6749	control	vs.	NUM	0.6989
					control	vs.	MS	0.9961
					NUM	vs.	MS	0.7491
	Porsolt forced swim test	B	F (2, 33) = 2.324	0.1171	control	vs.	NUM	0.5431
					control	vs.	MS	0.5281
					NUM	vs.	MS	0.0975

Fig. 2. (continued)

Behavioural differences among the three group-housed conditions were limited. After two weeks of age, mice show increased exploratory behaviour outside the nest, marking the onset of psychological independence[48–50]. Psychological independence promotes social development. It has been reported that pups separated from both the mother and littermates experience greater stress than those separated from the mother alone. The presence of littermates after weaning is known to buffer stress even in the absence of the mother[48]. Our results are consistent with previous findings indicating that littermate interactions play a dominant role in behavioural development. Under enriched social conditions, maternal presence alone may not confer additional behavioural advantages.

In contrast, maternal presence exerted marked protective effects under social isolation stress. Early-life social experiences influence social functioning, and social isolation (SI) in childhood induces social deficits in both animals and humans. To better understand these adverse effects, researchers have explored how SI affects behavioural, psychological, and physiological mechanisms[51]. Early-life social experiences significantly affect brain function and behaviour in rodents, dogs, and humans[52–54]. Adverse social experiences in early development can severely impair brain development and maturation, resulting in morphological and functional abnormalities in the central nervous system[55].

Socially isolated mice exhibited increased weight gain, while mice housed in isolation adjacent to their mother did not show this increase. Significant changes in body weight are common among individuals with depression, anxiety, or stress [56,57]. Stress is known to affect body weight and food intake in animal models. Weight gain has also been observed under chronic intermittent psychosocial stress[58,59]. Our results suggest that maternal presence mitigates the stress of social isolation. In Experiment 1, the greater body weight observed in the NUM group may reflect a beneficial outcome, potentially indicating the supportive influence of maternal presence during the adolescent period. Conversely, in Experiment 2, socially isolated mice also exhibited increased weight gain compared to controls—a finding that may represent a maladaptive physiological response to chronic stress, as reported in previous models of early-life adversity[60]. While these outcomes may initially seem inconsistent, they likely stem from distinct underlying mechanisms: the former possibly reflecting enhanced developmental support, and the latter indicating stress-related dysregulation of metabolic processes. These contrasting patterns underscore the importance of considering the surrounding environmental and social context when evaluating changes in body weight within developmental frameworks.

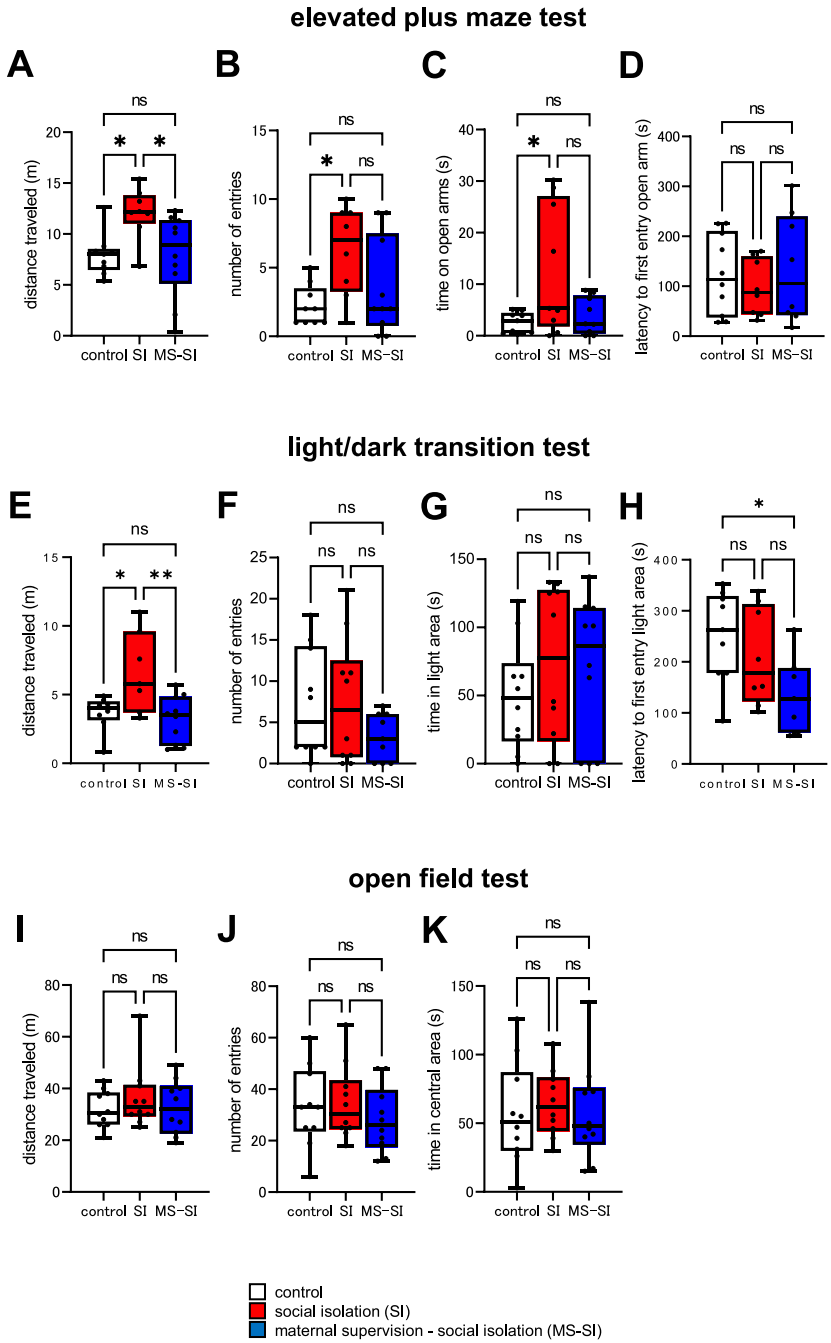

Fig. 8. Effects of maternal supervision under social isolation on anxiety-like behaviour and exploratory activity. Elevated plus maze test: (**A**) Total distance travelled. (**B**) Number of arm entries. (**C**) Time spent in the open arms. (**D**) Latency to first entry into an open arm. Light/dark transition test: (**E**) Total distance travelled. (**F**) Number of transitions between light and dark compartments. (**G**) Time spent in the light compartment. (**H**) Latency to first entry into the light compartment. Open field test: (**I**) Total distance travelled. (**J**) Number of entries into the central area. (**K**) Time spent in the central area. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). $p^* < 0.05$, $p^{**} < 0.01$; ns, not significant.

In Experiment 2, socially isolated mice showed both reduced forelimb grip strength and fewer biting responses in the cotton swab test compared with controls, consistent with previous reports[61]. Grip strength assessment is an important tool for studying neuromuscular impairment in rodent models[62]. These findings may reflect reduced motivation or arousal rather than a specific decline in aggression. However, the difference between the SI and MS-SI groups did not reach statistical significance for either measure. This indicates that any potential buffering effect of maternal presence was not statistically detectable in our study, although it is possible that a larger sample size could reveal more subtle effects.

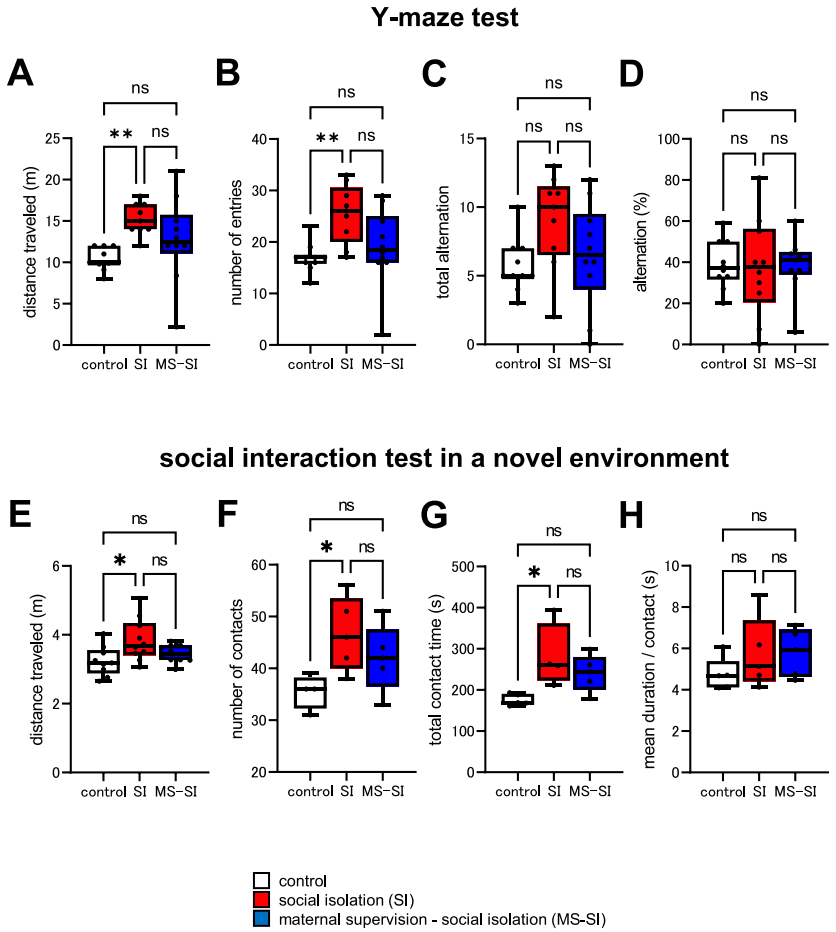

Fig. 9. Effects of maternal supervision under social isolation on spatial working memory and social behaviour. Y-maze test: (**A**) Total distance travelled. (**B**) Number of arm entries. (**C**) Total number of alternations. (**D**) Percentage of spontaneous alternations. Social interaction test in a novel environment: (**E**) Total distance travelled. (**F**) Number of contacts. (**G**) Total contact time. (**H**) Mean duration per contact. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). $p^{*} < 0.05$, $p^{**} < 0.01$; ns, not significant.

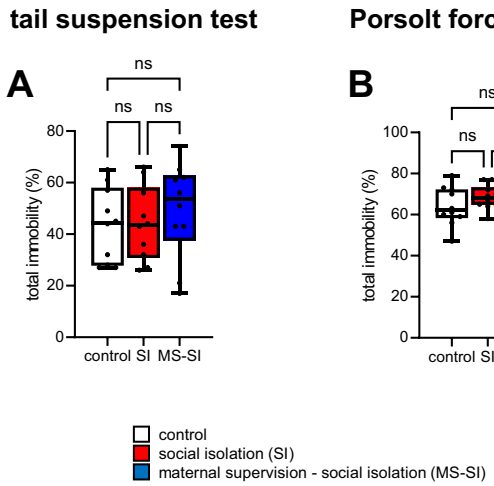

Fig. 10. Effects of maternal supervision under social isolation on depressive-like behaviour. (**A**) Total immobility percentage in the tail suspension test. (**B**) Total immobility percentage in the Porsolt forced swim test. Data are presented as box plots showing the median and interquartile range for each group (n = 12 mice per group). ns, not significant.

A key finding in Experiment 2 was the significant increase in locomotor activity in socially isolated (SI) mice across multiple tests, including the elevated plus maze (EPM) and light/dark transition test[32]. Crucially, this hyperactivity likely confounds the interpretation of anxiety-related measures[63,64]. For example, the increased time SI mice spent in the open arms of the EPM may not reflect reduced anxiety, but rather a general behavioral disinhibition. Our results support this interpretation, as maternal supervision appeared to normalize this hyperactivity rather than directly altering anxiety-like behavior. Interestingly, this hyperactivity was not observed in the 20-min open field test, which may suggest that the effect is most prominent in novel, moderately stressful environments like the EPM and LDT, or that a more granular, time-resolved analysis of habituation is needed to uncover subtle patterns.

Notably, increased activity was not observed in isolated mice reared adjacent to their mother. Post-weaning SI is known to alter anxiety-like behaviour in rodents[65]. Parental buffering of children's stress responses involves prefrontal cortex and amygdala circuitry[66,67]. Parental presence continues to influence emotional regulation into adolescence, providing social buffering[68]. Our results indicate that maternal presence exerts a subtle protective effect against stress-induced behavioural alterations in offspring. Although the SI group consistently exhibited elevated activity levels across the elevated plus maze (EPM), light–dark transition test, and Y-maze compared to the control group, the MS-SI group did not show significant differences from the SI group in several of these measures. In the anxiety-related tests, socially isolated mice exhibited increased locomotor activity, which may reflect general hyperactivity or disinhibition rather than a simple reduction in anxiety. Therefore, the apparent "anxiolytic-like" phenotype in SI mice should be interpreted with caution. Maternal presence appeared to modestly modulate these alterations in a context-dependent manner, rather than directly reducing anxiety. Notably, the lack of significant differences in total locomotor activity during the 20-min open field test implies that a more granular, time-resolved analysis—such as minute-by-minute habituation curves—may be necessary to uncover subtle temporal patterns not evident from overall distance traveled. Consistent with the elevated activity observed in the anxiety-related tests, socially isolated mice also exhibited increased locomotion in the Y-maze and social interaction tests. These findings further support the interpretation that social isolation induced general hyperactivity or behavioural disinhibition, rather than a simple reduction in anxiety. Thus, maternal presence may have mitigated excessive arousal or impulsive exploration, rather than directly reducing anxiety-like behaviour. Furthermore, these behavioural outcomes should be contextualized within adolescence, a developmental stage characterized by heightened novelty-seeking and exploratory behaviours, which may modulate the effects of both maternal presence and social isolation during this critical period[69]. In Experiment 1, the presence of an unfamiliar adult male (NUM) increased grip strength compared with controls but did not alter anxiety-related behaviour in the elevated plus maze, light/dark transition, or open field tests. Maternal supervision (MS) slightly reduced exploration in the light/dark test and increased locomotion during social interaction, yet these effects were not consistent across other measures. Therefore, MS does not appear to reverse a specific behavioural effect of the NUM condition but may instead modulate exploration and social responsiveness in a context-dependent manner. These findings suggest that maternal presence influences certain behavioural domains selectively, without producing a general alteration in anxiety or locomotor activity.

Overall, the effects of maternal supervision on behavioural outcomes were modest, and no statistically significant differences were observed between the MS-SI and SI groups in several tests. Thus, the findings should be interpreted as indicating a subtle protective or buffering effect of maternal presence, rather than a direct influence on anxiety or locomotor behaviour. This partial attenuation nonetheless suggests that the presence of a familiar maternal figure may help stabilize behavioural responses under social isolation stress, even if the effect is small in magnitude.

Acquisition of social competence is a key developmental milestone in adolescence[70,71]. In rodents, social behaviour increases after postnatal day 21 (Terranova & Laviola, 2005)[50]. Multiple brain regions—including the prefrontal cortex, amygdala, and hippocampus—are involved in regulating social behaviour[72–74]. The presence of caregivers is known to buffer stress responses in children[75,76], and the quality of attachment modulates the efficacy of this buffering effect[77]. In our study, socially isolated mice exhibited increased social contact compared to controls, a behavior not seen in the maternal supervision group. While this suggests maternal presence may influence social behavior, it is important to note that there was no statistically significant difference in social contact between the MS-SI and SI groups. Therefore, we cannot conclude that maternal presence significantly mitigated this social alteration induced by isolation stress.

Although the need for attachment to the mother is thought to disappear as pups approach weaning and gain independence[78,79], our study indicates that psychological development continues after weaning and may be influenced by maternal presence. Importantly, separation from the mother at weaning is not typically associated with adverse psychological outcomes. Our results suggest that revisiting post-weaning rearing environments may be important for promoting optimal development.

Watchful supervision and active caregiving are not mutually exclusive and typically operate in tandem. In humans, parental presence is known to buffer children's stress responses during social stressors such as school or exams[80]. Psychological independence usually develops after physical weaning in humans; however, this developmental stage has not been well studied in other species. Maturation rates differ between mice and humans[81]. One month of mouse development corresponds to approximately 150 times the human rate; from 1–6 months, it corresponds to 45 times the human rate. Mature adult mice are equivalent to humans aged 20–30 years. Although precise correspondence is not established, 1–2-month-old mice are considered developmentally comparable to 12–17-year-old humans[82]. Postnatal week 3 in mice corresponds roughly to 8–10 human years, and week 4 to early adolescence (10–12 years)[83,84]. Our results suggest that maternal influence remains important for psychological development after weaning in mice, providing insights into mechanisms underlying adolescent development in humans. Under standard group-housed conditions, we did not anticipate substantial behavioural modulation by maternal presence, and our findings aligned with this expectation. Conversely, in

the context of isolation-induced stress, maternal presence appeared to partially buffer select socioemotional outcomes. However, these effects should be interpreted with caution due to the broad developmental timeframe examined and the lack of additional social control groups. In the present study, 'maternal watchful supervision' was conceptualized as non-contact perceptual co-presence, allowing visual, olfactory, and auditory perception of the mother without physical interaction, and its potential role is best understood as a form of contextual social buffering rather than as a primary behavioural determinant[85,86]. It is also important to acknowledge that maternal–offspring signalling is dynamic and evolves throughout development. As such, the significance and potential impact of maternal presence may vary by developmental stage, with post-weaning cues likely having different meanings than those perceived later in adolescence.

It should be acknowledged that maternal cues may be interpreted differently depending on the developmental stage of the offspring, and that maternal presence itself may vary across time as mothers transition through their own reproductive cycles. These factors may alter the meaning and impact of maternal presence during the post-weaning to adolescent period.

Our experimental model offers a novel and tractable paradigm for investigating the effects of passive parental presence—specifically 'watchful supervision'—on offspring behaviour. While parental monitoring and availability are recognized as critical during human adolescence, few experimental models exist to explore these mechanisms. Our paradigm provides a controlled framework for studying how parental presence influences behavioural resilience and stress coping in post-weaning development.

Our results should be interpreted within the broader framework of adolescent neurobehavioral development. This developmental stage is marked by elevated corticosterone levels and heightened tendencies toward risk-taking and novelty-seeking, which are believed to reflect ongoing maturation of stress-regulatory and reward-related neural circuits[69]. Disruption of these processes through adolescent social isolation has been shown to induce long-lasting impairments in emotional and social functioning[13,65]. Within this context, the buffering role of maternal presence observed in our study may hold particular relevance for modulating stress responses during this critical period. The mechanisms underlying this effect may involve familiarity-driven sensory cues, including visual recognition, olfactory signals, and attenuation of hypothalamic–pituitary–adrenal (HPA) axis activity. Additionally, it remains possible that such buffering is not exclusive to maternal figures; similar effects might be conferred by other familiar conspecifics such as adult females or littermates. Further investigations are warranted to differentiate maternal-specific pathways from general social buffering processes.

Although our original hypothesis posited a protective function of maternal watchful supervision during the post-weaning to adolescent period, the observed effects were limited, with maternal presence failing to significantly modulate several behavioural outcomes. These findings suggest that maternal cues in adolescence may exert a diminished regulatory role compared to the robust impact of active maternal care during the pre-weaning stage. The developmental maturation of both the mother and offspring likely alters the functional relevance of maternal presence across time. From an applied standpoint, our results imply that visual access to the mother alone may be insufficient to counteract stress-induced behavioural disturbances post-weaning, underscoring the need for more comprehensive strategies to enhance post-weaning care and improve animal welfare in laboratory environments.

Several limitations warrant consideration. First, our study used only male mice, and the relatively small sample size per litter and use of only male subjects may have limited statistical power and generalizability. Future studies should include female mice and larger cohorts to examine sex differences and individual variation. Weaning at postnatal day 21 may yield different outcomes than later weaning. Although we focused on specific behavioural endpoints, continuous monitoring of home-cage activity would provide a more comprehensive understanding of maternal presence effects. Finally, the neural mechanisms underlying the protective effects of maternal presence remain to be elucidated. Future research should investigate stress hormone dynamics, neural circuit plasticity, and gene expression changes. It is important to emphasize that the current study does not equate post-weaning maternal absence with early-life maternal separation, as these occur during distinct developmental periods and likely engage different neurobiological mechanisms. Furthermore, because behavioural assessments were conducted only in adulthood, the precise timing and trajectory of developmental alterations remain undetermined. This temporal limitation warrants caution in interpreting the findings and underscores the risk of overgeneralization. Another notable limitation is the use of group housing with four male siblings, which may have facilitated the emergence of social hierarchies or dominance interactions. Such dynamics could have confounded the behavioural outcomes by either obscuring or amplifying the effects attributable to maternal or unfamiliar male presence. Future research should consider alternative housing paradigms to more accurately delineate the specific contributions of maternal presence during the post-weaning period.

In conclusion, housing weaned mice adjacent to their mother until adulthood did not markedly alter behaviour under group housing but offered a protective effect against certain abnormalities induced by isolation, specifically normalizing weight gain and hyperlocomotion. These findings demonstrate that maternal presence after weaning can partially buffer specific socioemotional outcomes, though the effects were not global. Overall, our findings suggest that maternal presence may continue to provide partial buffering of stress responses after weaning. This study provides a scientific foundation for understanding the effects of parental supervision on offspring behaviour and introduces a new experimental model for exploring these effects. While our findings suggest that maternal presence may mitigate some of the behavioural alterations induced by social isolation, we acknowledge that the present study did not include conditions involving other potential social partners such as littermates. Therefore, it cannot be concluded that maternal presence was the unique mitigating factor. Future studies should compare maternal presence with other forms of social buffering to clarify the relative contributions of mothers versus other conspecifics to post-weaning socioemotional outcomes.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Received: 13 June 2025; Accepted: 30 October 2025
Published online: 19 December 2025

References

1. Walle, E. A., Reschke, P. J. & Knothe, J. M. Social referencing: Defining and delineating a basic process of emotion. *Emotion Rev.* **9**(3), 245–252 (2017).
2. Gunnar, M. R. & Stone, C. The effects of positive maternal affect on infant responses to pleasant, ambiguous, and fear-provoking toys. *Child Dev.* **55**(4), 1231 (1984).
3. Stansbury, K. & Gunnar, M. R. Adrenocortical activity and emotion regulation. *Monogr. Soc. Res. Child Dev.* **59**(2–3), 108–134 (1994).
4. Stoesz, B. M., Hare, J. F. & Snow, W. M. Neurophysiological mechanisms underlying affiliative social behavior: insights from comparative research. *Neurosci. Biobehav. Rev.* **37**(2), 123–132 (2013).
5. Kikusui, T. & Mori, Y. Behavioural and neurochemical consequences of early weaning in rodents. *J. Neuroendocrinol.* **21**(4), 427–431 (2009).
6. Tractenberg, S. G. et al. An overview of maternal separation effects on behavioural outcomes in mice: Evidence from a four-stage methodological systematic review. *Neurosci. Biobehav. Rev.* **68**, 489–503 (2016).
7. Wang, L., Jiao, J. & Dulawa, S. C. Infant maternal separation impairs adult cognitive performance in BALB/cJ mice. *Psychopharmacology* **216**(2), 207–218 (2011).
8. Nishi, M., Horii-Hayashi, N. & Sasagawa, T. Effects of early life adverse experiences on the brain: implications from maternal separation models in rodents. *Front Neurosci.* **8**, 166 (2014).
9. Lee, J. H. et al. Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation. *Neurosci. Res.* **58**(1), 32–39 (2007).
10. Shin, S. Y. et al. Adolescent mice show anxiety- and aggressive-like behavior and the reduction of long-term potentiation in mossy fiber-CA3 synapses after neonatal maternal separation. *Neuroscience* **316**, 221–231 (2016).
11. Slamin MM, Bishnoi IR, Bankowski IM, Norris HA, Bordt EA. Where's my mom? Resilient maternal preference in post-weaning male and female mice within a multi-chamber social behavior task. Biorxiv, (2025).
12. Schmidt, M. V. et al. The postnatal development of the hypothalamic–pituitary–adrenal axis in the mouse. *Int. J. Dev. Neurosci.* **21**(2), 125–132 (2003).
13. McCormick, C. M., Mathews, I. Z., Thomas, C. & Waters, P. Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. *Brain Cogn.* **72**(1), 73–85 (2010).
14. Spear, L. P. The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* **24**(4), 417–463 (2000).
15. Doremus-Fitzwater, T. L., Varlinskaya, E. I. & Spear, L. P. Motivational systems in adolescence: possible implications for age differences in substance abuse and other risk-taking behaviors. *Brain Cogn.* **72**(1), 114–123 (2010).
16. Rincon-Cortes, M. & Sullivan, R. M. Early life trauma and attachment: immediate and enduring effects on neurobehavioral and stress axis development. *Front Endocrinol. (Lausanne).* **5**, 33 (2024).
17. Schmidt, M. V. et al. The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. *Int. J. Dev. Neurosci.* **21**(3), 125–132 (2003).
18. Moriceau, S. & Sullivan, R. M. Maternal presence serves as a switch between learning fear and attraction in infancy. *Nat. Neurosci.* **9**(8), 1004–1006 (2006).
19. Ost'adalova I, Babicky A. Periodization of the early postnatal development in the rat with particular attention to the weaning period. Physiol. Res. 61(6)673–681. (2012).
20. Bechard, A. & Mason, G. Leaving home: A study of laboratory mouse pup independence. *Appl. Anim. Behav. Sci.* **125**, 181–188 (2010).
21. Cramer, C. P., Thiels, E. & Alberts, J. R. Weaning in rats: I Maternal behavior. *Dev. Psychobiol.* **23**(6), 479–493 (1990).
22. Thiels, E., Alberts, J. R. & Cramer, C. P. Weaning in rats: II Pup behavior patterns. *Dev. Psychobiol.* **23**(6), 495–510 (1990).
23. Terranova, M. L., Laviola, G. & Alleva, E. Ontogeny of amicable social behavior in the mouse: gender differences and ongoing isolation outcomes. *Dev. Psychobiol.* **26**(8), 467–481 (1993).
24. Navarro-Moreno, C. et al. Becoming a mother shifts the activity of the social and motivation brain networks in mice. *Iscience* **25**, 104525 (2022).
25. Courtiol E, Wilson DA, Shah R, Sullivan RM, Teixeira CM. Maternal Regulation of Pups' Cortical Activity: Role of Serotonergic Signaling. eNeuro. 2018;5(4):ENEURO.0093–18. (2018).
26. Crawley, J. N. *What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice* 2nd edn. (Wiley, 2007).
27. Boursot, P., Auffray, J. C., Britton-Davidian, J. & Bonhomme, F. The evolution of house mice. *Annu. Rev. Ecol. Syst.* **24**, 119–152 (1993).
28. Ueno, H. et al. Mice Recognise Mice in Neighbouring Rearing Cages and Change Their Social Behaviour. *Behav. Neurol.* **2024**, 9215607 (2024).
29. Ueno, H. et al. Rearing in an envy-like environment increases anxiety-like behaviour in mice. *Transl. Neurosci.* **16**(1), 20220364 (2025).
30. Zilkha, N. et al. Sex-dependent control of pheromones on social organization within groups of wild house mice. *Curr. Biol.* **33**, 1407-1420.e4 (2023).
31. Mucignat-Caretta, C., Caretta, A. & Cavaggioni, A. Acceleration of puberty onset in female mice by male urinary proteins. *J. Physiol.* **486**, 517–522 (1995).
32. Komada, M., Takao, K. & Miyakawa, T. Elevated plus maze for mice. *J. Vis. Exp.* **22**, 1088 (2008).
33. Takeshita, H. et al. Modified forelimb grip strength test detects aging-associated physiological decline in skeletal muscle function in male mice. *Sci. Rep.* **8**(7), 42323 (2017).
34. O'Callaghan, J. P. & Holtzman, S. G. Quantification of the analgesic activity of narcotic antagonists by a modified hot-plate procedure. *J. Pharmacol. Exp. Ther.* **192**, 497–505 (1975).
35. Pellow, S., Chopin, P., File, S. E. & Briley, M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods.* **14**(3), 149–167 (1985).
36. Takao, K. & Miyakawa, T. Light/dark transition test for mice. *J Vis Exp.* **1**, 104 (2006).
37. Prut, L. & Belzung, C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol.* **463**(1–3), 3–33 (2003).
38. Conrad, C. D., Grote, K. A., Hobbs, R. J. & Ferayorni, A. Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobiol. Learn Mem.* **79**(1), 32–40 (2003).
39. Tanda, K. et al. Abnormal social behavior, hyperactivity, impaired remote spatial memory, and increased D1-mediated dopaminergic signaling in neuronal nitric oxide synthase knockout mice. *Mol. Brain* **2**, 19 (2009).

40. Can, A. et al. The tail suspension test. *J. Vis. Exp.* **59**, e3769 (2011).
41. Matsuda, I., Shoji, H., Yamasaki, N., Miyakawa, T. & Aiba, A. Comprehensive behavioral phenotyping of a new semaphorin 3 F mutant mouse. *Mol. Brain* **9**, 15 (2016).
42. Abbas, M. G. et al. Comprehensive behavioral analysis of male Ox1r (-/-) mice showed implication of orexin Receptor-1 in mood, anxiety, and social behavior. *Front. Behav. Neurosci.* **9**, 324 (2015).
43. Konttinen, H. et al. Emotional eating, depressive symptoms and self-reported food consumption. *Appetite* **54**, 473–479 (2010).
44. Luppino, F. S. et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch. Gen. Psychiatry.* **67**, 220–229 (2010).
45. Rogers, D. C. et al. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome.* **8**, 711–713 (1997).
46. Tavoian, D., Lozier, N. R. & de Lacalle, S. Age of Peak Performance Differs by Functional Task in Mice Tracked Over 2 Years. *J. Gerontol. A Biol. Sci. Med. Sci.* **76**(7), 1179–1183 (2021).
47. Ueno, H. et al. The prevention of home-cage grid climbing affects muscle strength in mice. *Sci. Rep.* **12**(1), 15263 (2022).
48. Katori, S., Nishizumi, H., Noguchi-Katori, Y. & Sakano, H. Separation of pups from their mother mice enhances odor associative learning at the late lactation stage. *Sci. Rep.* **15**(1), 6700 (2025).
49. Goodwin GA, Yacko H. Emergence of the exploratory motive in rats. Dev Psychobiol. 45, 34–48.
50. Terranova, M. L. & Laviola, G. Individual-Differences in Mouse Behavioral-Development - Effects of Precocious Weaning and Ongoing Sexual Segregation. *Anim. Behav.* **50**, 1261–1271 (1995).
51. Valtorta, N. K. et al. Loneliness and social isolation as risk factors for coronary heart disease and stroke: systematic review and meta-analysis of longitudinal observational studies. *Heart* **102**, 1009–1016 (2016).
52. Han, X., Wang, W., Xue, X., Shao, F. & Li, N. Brief social isolation in early adolescence affects reversal learning and forebrain BDNF expression in adult rats. *Brain Res. Bull.* **86**, 173–178 (2011).
53. Bick, J., Fox, N., Zeanah, C. & Nelson, C. A. Early deprivation, atypical brain development, and internalizing symptoms in late childhood. *Neuroscience* **342**, 140–153 (2015).
54. Chugani, H. T. et al. Local brain functional activity following early deprivation: a study of postinstitutionalized Romanian orphans. *Neuroimage* **14**, 1290–1301 (2001).
55. Murai, T., Okuda, S., Tanaka, T. & Ohta, H. Characteristics of object location memory in mice: behavioral and pharmacological studies. *Physiol. Behav.* **90**, 116–124 (2007).
56. Konttinen, H., Männistö, S., Sarlio-Lähteenkorva, S., Silventoinen, K. & Haukkala, A. Emotional eating, depressive symptoms and self-reported food consumption A population-based study. *Appetite* **54**(3), 473–479 (2010).
57. Luppino, F. S. et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch. Gen. Psychiatry.* **67**(3), 220–229 (2010).
58. Finger, B. C., Dinan, T. G. & Cryan, J. F. The temporal impact of chronic intermittent psychosocial stress on high-fat diet-induced alterations in body weight. *Psychoneuroendocrinology* **37**, 729–741 (2012).
59. Tramullas, M., Dinan, T. G. & Cryan, J. F. Chronic psychosocial stress induces visceral hyperalgesia in mice. *Stress* **15**, 281–292 (2012).
60. Queen, N. J. et al. Social isolation exacerbates diet-induced obesity and peripheral inflammation in young male mice under thermoneutrality. *Iscience* **26**(3), 106259 (2023).
61. Farbstein, D. et al. Social isolation in mice: behavior, immunity, and tumor growth. *Stress* **24**(2), 229–238 (2021).
62. Smith, J. P., Hicks, P. S., Ortiz, L. R., Martinez, M. J. & Mandler, R. N. Quantitative measurement of muscle strength in the mouse. *J. Neurosci. Methods.* **62**, 15–19 (1995).
63. Hu, Y. Y. et al. Analysis of the influences of social isolation on cognition and the therapeutic potential of deep brain stimulation in a mouse model. *Front Psychiatry.* **14**, 1186073 (2023).
64. Shishelova, A. Y. & Raevsky, V. V. Effects of Early Social Isolation on Locomotor Activity and Learning Ability in Adult WAG/Rij Rats. *Neurosci. Behav. Physiol.* **52**, 89–96 (2022).
65. Lukkes, J. L., Watt, M. J., Lowry, C. A. & Forster, G. L. Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. *Front Behav. Neurosci.* **3**, 18 (2009).
66. Gunnar, M. R. et al. Parental buffering of fear and stress neurobiology: Reviewing parallels across rodent, monkey, and human models. *Soc. Neurosci.* **10**, 474–478 (2015).
67. Callaghan, B. L. & Tottenham, N. The neuro-environmental loop of plasticity: a cross-species analysis of parental effects on emotion circuitry development following typical and adverse caregiving. *Neuropsychopharmacology* **41**, 163–176 (2016).
68. Gee, D. G. et al. Maternal buffering of human amygdala-prefrontal circuitry during childhood but not during adolescence. *Psychol. Sci.* **25**, 2067–2078 (2014).
69. Fahlström, A., Zeberg, H. & Ulfhake, B. Changes in behaviors of male C57BL/6J mice across adult life span and effects of dietary restriction. *Age (Dordr)* **34**, 1435–1452 (2012).
70. Crone, E. A. & Dahl, R. E. Understanding adolescence as a period of social-affective engagement and goal flexibility. *Nat. Rev. Neurosci.* **13**, 636–650 (2012).
71. Nelson, E. E., Jarcho, J. M. & Guyer, A. E. Social re-orientation and brain development: an expanded and updated view. *Dev. Cogn. Neurosci.* **17**, 118–127 (2016).
72. Ferri, S. L. et al. Activation of basolateral amygdala in juvenile C57Bl/6J mice during social approach. *Neuroscience* **335**, 184–194 (2015).
73. Ko, J. Neuroanatomical substrates of rodent social behavior: the medial prefrontal cortex and its projection patterns. *Front Neural Circuits.* **11**, 41 (2017).
74. Felix-Ortiz, A. C. & Tye, K. M. Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. *J. Neurosci.* **34**(2), 586–595 (2014).
75. Nachmias, M., Gunnar, M., Mangelsdorf, S., Parritz, R. H. & Buss, K. Behavioral inhibition and stress reactivity: the moderating role of attachment security. *Child Dev.* **67**, 508–522 (1996).
76. Hostinar, C. E., Johnson, A. E. & Gunnar, M. R. Early social deprivation and the social buffering of cortisol stress responses in late childhood: an experimental study. *Dev. Psychol.* **51**, 1597–1608 (2015).
77. Gunnar, M. R. Social buffering of stress in development: a career perspective. *Perspect. Psychol. Sci.* **12**, 355–373 (2017).
78. Yoshida, S. et al. Transport Response is a filial-specific behavioral response to maternal carrying in C57BL/6 mice. *Front Zool.* **10**(1), 50 (2013).
79. Gubernick, D. J. Parent and infant attachment in mammals. In *Parental Care in Mammals* (eds Gubernick, D. J. & Knopfler, P. H.) 243–305 (Springer, 1981).
80. Parenteau, A. M. et al. Parenting matters: Parents can reduce or amplify children's anxiety and cortisol responses to acute stress. *Dev. Psychopathol.* **32**(5), 1799–1809 (2020).
81. Flurkey, K., Currer, J. M. & Harrison, D. E. The mouse in biomedical research. In *American College of Laboratory Animal Medicine Series* (ed. Fox, J. G.) (Elsevier Academic Press, 2007).
82. McWain, M. A., Pace, R. L., Nalan, P. A. & Lester, D. B. Age-dependent effects of social isolation on mesolimbic dopamine release. *Exp. Brain Res.* **240**(10), 2803–2815 (2022).
83. Choy, K. H. C. et al. Cognitive behavioral markers of neurodevelopmental trajectories in rodents. *Transl. Psychiatry.* **11**(1), 556 (2021).

84. Romijn, H. J., Hofman, M. A. & Gramsbergen, A. At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby?. *Early Hum. Dev.* **26**, 61–67 (1991).
85. Fomby, P. & Musick, K. Mothers' time, the parenting package, and links to healthy child development. *J. Marriage Fam.* **80**, 166–181 (2018).
86. Kiyokawa, Y. & Hennessy, M. B. Comparative studies of social buffering: A consideration of approaches, terminology, and pitfalls. *Neurosci. Biobehav. Rev.* **86**, 131–141 (2018).

Acknowledgements
We thank M. Okamoto and Y. Honda for technical assistance. We thank the Kawasaki Medical School Central Research Institute for providing the instruments used in this study.

Author contributions
All authors had full access to all study data and take full responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: H.U., E.K., M.O., and T.I. Acquisition of data: H.U., E.K., Y.T., S.M., A.O., and T.H. Analysis and interpretation of data: H.U., E.K., and Y.T. Drafting of the manuscript: H.U. and E.K. Critical revision of the manuscript for important intellectual content: S.M., K.W., Y.T., and Y.M. Statistical analyses: H.U. and E.K. Study supervision: M.O. and T.I. Acquisition of data: H.U., E.K., Y.T., S.M., A.O., and T.H. Analysis and interpretation of data: H.U., E.K., and Y.T. Drafting of the manuscript: H.U. and E.K. Critical revision of the manuscript for important intellectual content: S.M., K.W., Y.T., and Y.M. Statistical analyses: H.U. and E.K. Study supervision: M.O. and T.I.

Funding
This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors also declare that no funding source had any role in the study design, data collection, analysis, interpretation, or writing of the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
All animal experiments were conducted in accordance with the ARRIVE guidelines and the U.S. National Institutes of Health's (NIH) *Guide for the Care and Use of Laboratory Animals* (8th edition, 2011). The study was approved by the Committee for Animal Experiments of Kawasaki Medical School (Approval No. 24–026, approved in 2024). The need for consent to participate does not apply to this study.

Additional information
Correspondence and requests for materials should be addressed to H.U.