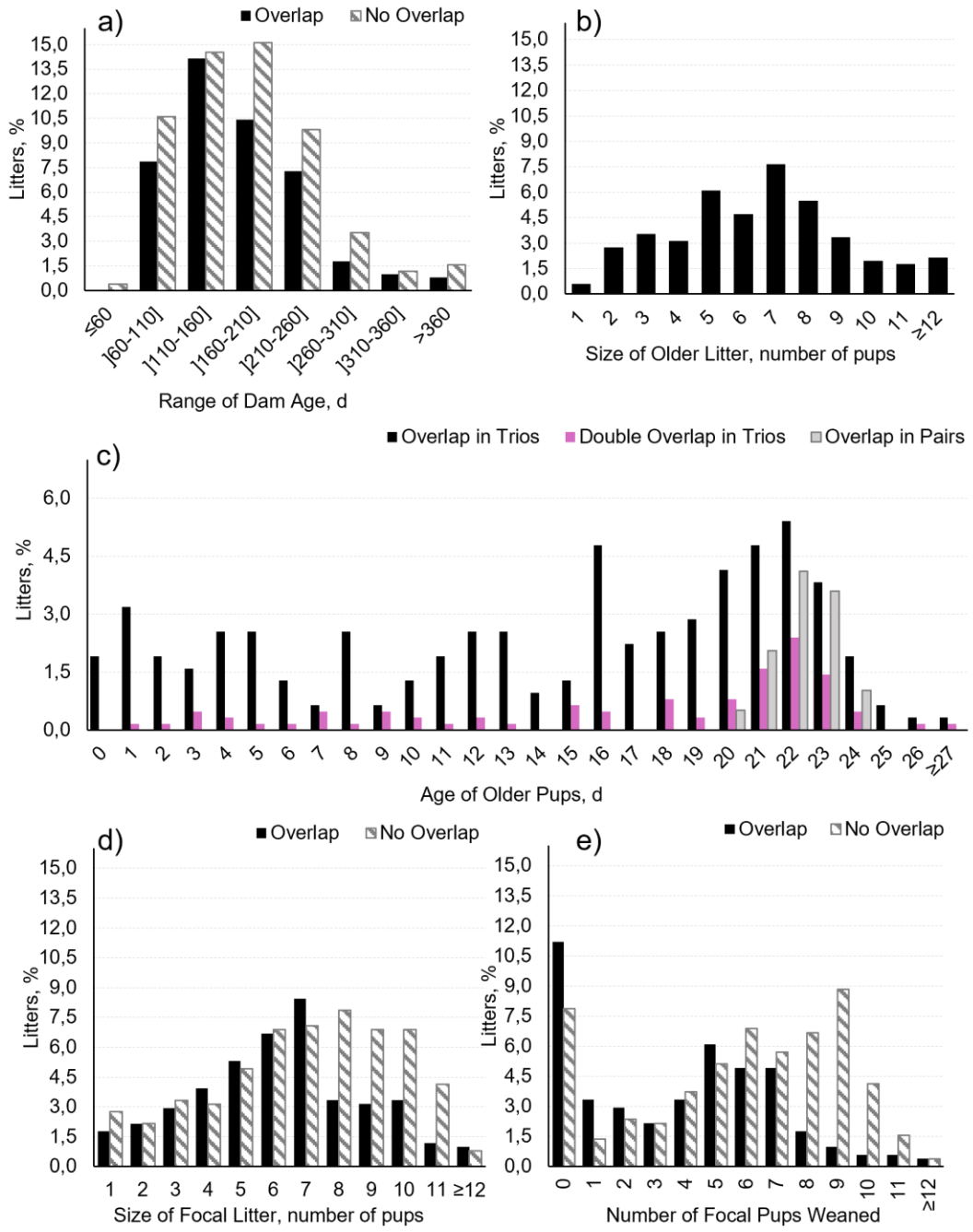
SUPPLEMENTARY MATERIAL
LITTER CHARACTERISTICS

Note 1. Litter Social Characteristics

Note 1.1 Dams

Litters were born to dams which were 174 ± 73 d old on average. The youngest and oldest dams in this study were 58 d and 493 d old, respectively. Young dams (≤ 80 d of age) gave birth to 7.3% of the studied litters, while older dams (≥ 250 d of age) gave birth to 12.6% of the litters. Most (83.1%) of the litters were born to dams of 80 to 260 days of age, with 54.6% of the litters being born to dams between 110 and 210 days of age. Suppl. Fig. 1a depicts the percentage of litters born per distinct category of Dam Age considering overlapped and non-overlapped litters.



Suppl. Fig. 1. Overall litter characteristics. Percentage of litters by category of: a) Dam Age; b) Size of Older Litter on the day of the Focal Litter's birth; c) Age of the Older Litter and overlap condition. Double Overlap means that the Focal Litter was born in the presence of two Older Litters. In Trio Double Overlapped litters, the Age of the Older litter represents the weighted age of both older litters; d) Size of Focal Litter, and; e) Number of Pups Weaned.

Note 1.2 Size and Age of overlapped Older Litters

A total of 43.2% of all the studied litters (pairs and trios included) were overlapped (n=220 litters), which means that 40.7% (n=1377) of all pups (n=3380) were born in the presence of an older litter. Litter Overlap occurred in 63.1% and 11.3% of all trio- (n=314) and pair-housed (n=195) litters, respectively.

Mean size of the older litters (number of pups) in situations of Litter Overlap was 6.7 ± 3.1 pups on the day that the focal litter was born. Most (55.5%) of the older litters had five to eight pups. Approximately 23.2% and 21.4% of the overlapped litters were born in the presence of older litters with four or less pups and nine or more pups, respectively. Approximately 4.1% of the overlapped focal litters were born in cages with large older litters, i.e. of 15 or more pups with a maximum of 18 older pups. The 18-pup litter happened in a trio-cage where two older litters were present (double overlap). Double overlapped litters happened either when the cage-mate female had pups (Older Litter 2) just before her previous litter was weaned (Older Litter 1), or when the dam gave birth to the focal litter just before weaning her previous litter (Older Litter 1) and in the presence of the cage-mate female´s litter (Older Litter 2). Suppl. Fig. 1b depicts the percentage of litters per category of size of the older litter present in the cage at the time of the birth of the focal litter in overlapped conditions.

Older pups in situations of Litter Overlap were on average 15.0 ± 7.7 d old on the day that the focal litter was born (Day 0). The mode age for older litters on Day 0 was 22.0 d, which happened in 24 litters, corresponding to 10.9% of the total overlapped litters (n=220). Most of the new-born overlapped litters (72.3%) were born in the presence of older litters of 10 d to 25 d of age. The breeding facility's protocol was to wean pups when these were approximately 21.0 days old (see Table 1) but would sometimes wean pups at an older age for reasons including low weight/vigour at 21.0 d of age or welfare and logistic issues in the breeding rooms that prevented litters being weaned at the target age. Minimum Age of the Older Litters was 0 d (older litters that were born on the same day as the focal litter), while the observed maximum Age of the Older Litters was 32

d in only one cage. Approximately 25.9% of the older litters in situations of Litter Overlap were 22.0 d-old or older, but only 1.8% of the older litters had 25.0 d-old pups or older. A total of 19.5% of the older litters had pups which were five or less days old.

Overlap in cages with pairs had a smaller distribution of age gaps between litters (within 20 d to 24 d), as pair-overlapped litters were necessarily from the same dam which had given birth just before weaning the older litter. In trios, however, litters could be from both the two dams present in the cage, which means that there was a much wider range for the distribution of age gap between litters (from 0 d to 32 d), as depicted in Suppl. Fig. 1c. Still, approximately 19.8% of the trio-litters were overlapped with litters aged within the same range as in pairs (20 d to 24 d), and 21.3% of trio-litters had Litter Overlap with age gaps of 20 d or more. Also, 20.2% (n=40) of all trio-overlapped litters (n=198) had double overlap, corresponding to 12.7% of all studied trios (Fig. 1c).

Note 1.3 Size of Focal Litter at birth and at weaning

A total of 3380 pups were born during the present study. The mean number of pups born in focal litters (i.e. number of pups found in the first cage check after birth) was 6.6 ± 2.8 (range 1-14 pups). The majority of litters (70.7%) were born having five to 10 pups, of which 56.9% were six to eight pups. The smallest litters had only one pup (4.5% of the litters) while the largest litters had a total of 14 pups (two cases, 0.4% of litters). A total of 15.1% of the litters were born with three pups or less, and 17.1% of litters had at least 10 pups. Suppl. Fig. 1d depicts the distribution of litters by Size of Focal Litter, considering litters with and without overlap.

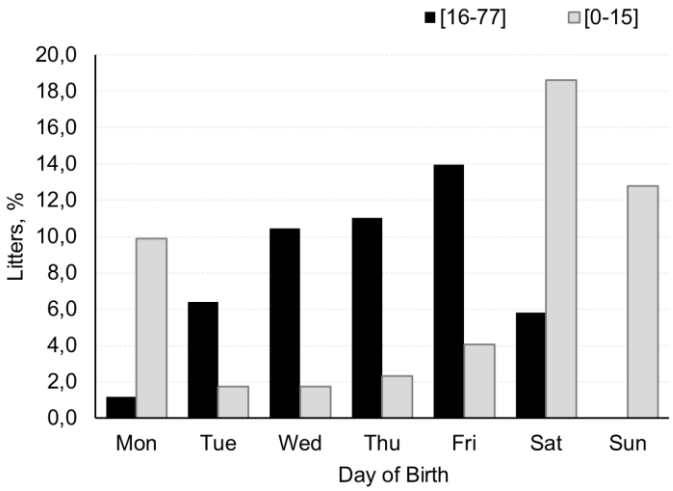
A total of 2515 pups were weaned during this study. The mean number of pups weaned in focal litters was 4.9 ± 3.0 (range 0-13 pups). A total of 19.1% of the litters were totally lost, meaning that these litters weaned zero pups, while 33.6% of the litters weaned five to seven pups, and 25.1% of litters weaned eight to 11 pups. Suppl. Fig. 1e depicts the distribution of focal litters per

69 category of number of pups weaned per focal litter, considering overlapped and non-overlapped
70 litters.
71 Approximately 30.0% and 22.7% of all the studied litters were weaned on Fridays and
72 Mondays, respectively. The same pattern was found for overlapped (Fridays: 33.2%, Mondays:
73 24.1%) and double overlapped litters (Monday and Friday: 29.8%).
74
75

Note 2. Litter Micro-Environment

Note 2.1 Motion Events near the Cage

A total of 3308 Motion Events were detected by the proximity logger, between the racks that contained the study cages, during the litters' respective Day 0. Mean incidence of Motion Events on the litters' Day 0 was 19.2 ± 15.7 events per litter with a mean duration of 103.1 ± 51.1 s per event. Nearly half (51.2%, n= 88) of the litters experienced 15 human Motion Events or less (low incidence), while the remaining litters experienced 16 to 77 human Motion Events (high incidence) on Day 0. Most of the litters which experienced low incidence of Motion Events were born either on weekends (61.4%, n=54) or on Mondays (19.3%, n=17). On the other hand, most of the litters that experienced high incidence of Motion Events were born between Tuesdays and Fridays (n=72, 81.8%), as illustrated on Suppl. Fig. 2.



Suppl. Fig. 2. Human movements in the room. Percentage of litters by category of Motion Events detected throughout Day 0, being 0 – 15 events and 16-77 events considered low and high categories, respectively.

The lower incidence of movements during the weekend can be explained by the fact that the breeding facility only had one animal caretaker on weekends. The reduced incidence of movements on Mondays may be reflecting the reduced cage changes during this weekday, in

comparison with the remaining weekdays. Also, the weekly staff meetings shortened the workday inside the breeding rooms by at least one hour on Mondays.

Note 2.2 Cage Vibration

Acceleration loggers registered - 0.2 to 0.0 g-force (-2.0 to 0.0 $m \cdot s^{-2}$) for 93.4 ± 24.5%, 90.9 ± 26.1% and 71.2 ± 38.8% of the litters respective Day 0 on the vertical, and horizontal directions of the cage's width and length, respectively. Mean Cage Vibration on Day 0 was 0.1 ± 0.3 g-force (1.1 ± 2.5 $m \cdot s^{-2}$) and 0.3 ± 0.3 g-force (2.9 ± 3.1 $m \cdot s^{-2}$) considering the vertical axis alone and the three directions together, respectively. The maximum absolute level of acceleration recorded during Day 0 was 2.0 g-force (19.6 $m \cdot s^{-2}$), 0.9 g-force (9.1 $m \cdot s^{-2}$) 1.28 g-force (12.5 $m \cdot s^{-2}$), on the vertical, horizontal cage width and length directions, respectively.

Note 2.3 Cage Light Intensity

All litters experienced complete or near complete darkness (0.00-2.00 Lx) for at least 60% of Day 0. On average, litters experienced 2.00 Lx or less for 97.0 ± 7.5% of Day 0, which means that the inside of cages remained dark for most of the diurnal and nocturnal periods. Cage Light Intensity was generally low, 0.90 ± 1.90 Lx, but a total of 20.4%, 11.6%, 7.0%, 4.1%, and 2.9% of all studied litters experienced light levels of or over 25.00 Lx, 50.00 Lx, 100.00 Lx, 200.00 Lx, and 400.00 Lx, respectively, at least once during their respective Day 0. The minimum and maximum light intensities experienced by a litter during this period was 0.00 Lx and 979.50 Lx, respectively.

Note 2.4 Cage Temperature

Mean Cage Temperature for the entire Day 0 ranged from 20.3°C to 23.9°C. Litters experienced momentary cage temperatures from 18.8°C to 24.7°C within Day 0. The most recurrent temperature range inside the cages was 22.0°C to 22.9°C, within which 52.3% of the litters spent at least half of their respective Day 0. However, only 35.5% and 12.2% of the litters spent at least 70.0% and 90.0% of their Day 0 within this temperature interval, respectively. Cage

Temperature was highly variable within this period. On average, new-born pups experienced less than 21.0°C for 8.3 ± 20.6%, 21.0-21.9°C for 37.3 ± 35.1%, 22.0-22.9°C for 47.0 ± 36.0%, and over 23.0°C for 7.2 ± 15.9% of their Day 0. The minimum and maximum variation in temperature experienced by the same litter within Day 0 was of 0.4°C and 4.0°C, respectively. Cage temperature fluctuations of at least 2.0°C, ≥1.5°C, ≥1.0°C, and ≥0.5°C were experienced by 8.1%, 29.1%, 66.3%, and 98.3% litters, respectively, within Day 0.

Note 2.5 Nest Score

Mean nest score across all 172 litters was 2.45 ± 1.52. Nests scores of 0.00-0.75, 1.00-1.75, 2.00-2.75, 3.00-3.75, 4.00-5.00 were observed on Day 0 for 16.9%, 18.6%, 26.2%, 16.3%, and 22.1 of litters, respectively.

SUPPLEMENTARY MATERIAL

TABLES AND STATISTICAL RESULTS

Suppl. Table 1. Final model solutions for fixed effects for predicting the odds of pup death fitted in the whole dataset (Model 1). n.a.: not applicable. DF: Degrees of Freedom.

Effect	Estimate	Standard Error	DF	t-value	Adj. P < |t|
Intercept	1.5341	0.9682	504	1.58	0.1137
No Overlap	-0.2911	0.7584	2893	-0.38	0.7012
Overlap	0.0000	n.a.	n.a.	n.a.	n.a.
Size of Focal Litter	-1.0738	0.2352	2893	-4.56	<0.0001
Size of Focal Litter × Size of Focal Litter (second order term)	0.0675	0.0160	2893	4.21	<0.0001
Dam Age, d	0.0095	0.0020	2893	4.64	<0.0001
Size of Focal Litter × No Overlap (interaction)	-0.2416	0.1033	2893	-2.34	0.0194
Size of Focal Litter × Overlap (interaction)	0.0000	n.a.	n.a.	n.a.	n.a.

Suppl. Table 2. Final model solutions for fixed effects for predicting the odds of pup death fitted in the dataset containing only overlapped litters (Model 2). n.a.: not applicable. DF: Degrees of Freedom.

Effect	Estimate	Standard Error	DF	t-value	Adj. P < |t|
Intercept	-5.5246	1.6075	211	-3.44	0.0007
Size of Focal Litter	-0.7624	0.3183	1144	-2.40	0.0168
Size of Focal Litter × Size of Focal Litter (quadratic term)	0.0478	0.0222	1144	2.16	0.0313
Dam Age, d	0.0096	0.0033	1144	2.87	0.0041
Age of Older Pups, d	0.5209	0.1165	1144	4.47	<0.0001
Age of Older Pups × Age of Older Pups (quadratic term)	-0.0140	0.0042	1144	-3.33	0.0009
Number of Older Pups	0.3071	0.0677	1144	4.54	<0.0001

Suppl. Table 3. Animal husbandry practices of the studied animal facility.

	i3S Animal Breeding Facility's Characteristics
Mouse Strain	C57BL/6J
Housing configuration	Trios (two adult females and one male) /pairs (one adult female and one male)
Type of cages	Standard Type II (pair-housing, 268 x 215 x 141 mm) and III (trio-housing, 425 x 276 x 153 mm) Tecniplast Filter-Top Static Cages
Bedding	Corncob bedding (grade 12, ULTRAGENE, Portugal)
Nest material	Two to three sheets of white paper towel
Enrichment	Shelter (GLP Semi-detached Dome Home, LBS Biotechnology, United Kingdom)
Water	*Ad libitum* from a drinking bottle with nipple, sterilized by reverse osmosis, filtered with a Polyethersulfone filter (SPECTRUM Filtration, England) and ultraviolet light disinfection
Food	*Ad libitum* as 12 mm dry pellets in a hopper (2018S – Mucedola Tecklad for Inotiv, Italy)
Cage change routine	Once a week on a fixed weekday for each room
Room temperature (target)	21°C (20°C-22°C)
Light schedule	Cycles of 12 hours of light (7:00h to 19:00h/ 8:00h to 20:00h between end of March and end of October) and 12 hours of dark
Weaning age (target)	21 days (delayed if pups were too small or if completion of 21 d was on a weekend or holiday)
Breeding start age (target)	Dam between eight to nine weeks of age
Retirement age (target)	Dams are retired at one year of age or sooner if presenting welfare/health problems
Facility pup-counting routine	Pups are counted once between their birth and day seven post-partum by a trained technician, with minimal handling, during cage-change.
Study pup-counting routine	Pups were counted daily by a trained scientist at 09.00h, and by the animal care-taker at 16,00h if litters were born either past 09.00h, or on weekends.

Suppl. Table 4. Confusion Matrix, calculated sensibility and precision for the obtained Decision Tree describing mouse pre-weaning death.

	Number of pups classified as:		Sensibility	Precision
True:	0	1		
0	756	91	756×100÷(756+91)=89.3%	756×100÷(756+191)=79.8%
1	191	441	441×100÷(191+441)=69.8%	441×100÷(91+441)=82.9%