Supplemental information

Maternal gut microbiota mediate

intergenerational effects of high-fat

diet on descendant social behavior

Claudia M. Di Gesù, Lisa M. Matz, Ian J. Bolding, Robert Fultz, Kristi L. Hoffman, Antonella Marino Gammazza, Joseph F. Petrosino, and Shelly A. Buffington

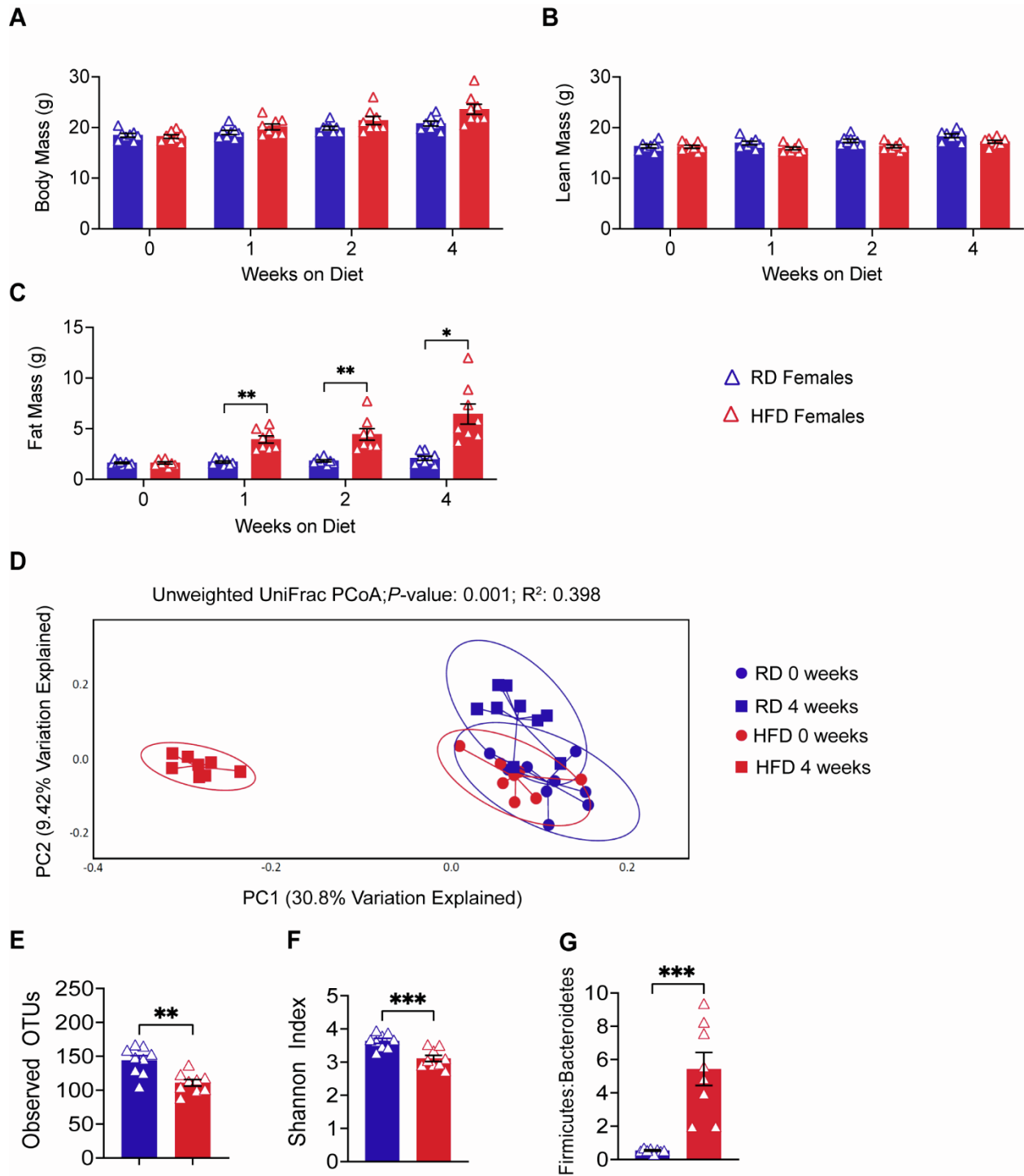**Supplemental Figure 1** (Di Gesù, Matz et al.)

Figure S1. Four weeks HFD-feeding increases fat mass and shifts gut microbiome composition in female mice.

Related to Figure 1. (**A**) RD- and HFD-fed female total body mass is not significantly different at four weeks on

diet (week 0: $t(14) = 0.4557$, $p = 0.9859$; week 1: $t(12.45) = 1.574$, $p = 0.4545$; week 2: $t(9.588) = 1.717$, $p = 0.3951$;

week 4: $t(9.979) = 2.504\ p = 0.1194$). (**B**) No significant differences in lean mass were observed between RD- and HFD-fed females at four weeks on diet (week 0: $t(13.94) = 0.2018$, $p = 0.9994$; week 1: $t(13.45) = 2.272$, $p = 0.151$; week 2: $t(13.76) = 2.451$, $p = 0.1082$; week 4: $t(13.26) = 2.451$, $p = 0.1104$). (**C**) HFD-fed mice showed a significant increase in fat mass within one week on diet that was sustained at four weeks on diet (week 0: $t(12.42) = 0.04358$, $p > 0.9999$; week 1: $t(8.111) = 6.061$, $p = 0.0011$; week 2: $t(7.51) = 4.518$, $p = 0.0091$; week 4: $t(7.634) = 4.287$, $p = 0.0118$) as determined by a mixed-effects model (see Materials and Methods). (**D**) Principal coordinate analysis (PCoA) of Unweighted UniFrac distances from the averaged rarefied 16S rRNA gene amplicon sequencing dataset (3,341 reads/sample, n=1,000 rarefactions) reveal a significant shift in maternal gut microbial ecology within one week on high-fat diet that is maintained at four weeks on diet. ($p=0.001$, $R^2 = 0.398$). (**E–F**) Alpha diversity metrics reveal a statistically significant loss of microbial diversity in HFD compared to RD dams, as measured by (**E**) observed OTUs ($t(16) = 3.942$, $p = 0.0012$) and (**F**) Shannon diversity index ($t(16) = 4.412$, $p = 0.0004$). (**G**) Phylum-level changes, characterized by a significant increase in the *Firmicutes:Bacteroidetes* ratio are evident after four weeks on diet (Mann-Whitney U = 0, $p = 0.0002$). Bar graphs show mean ± SEM with individual data points representing biological replicates. N = 8–9 subjects per group.
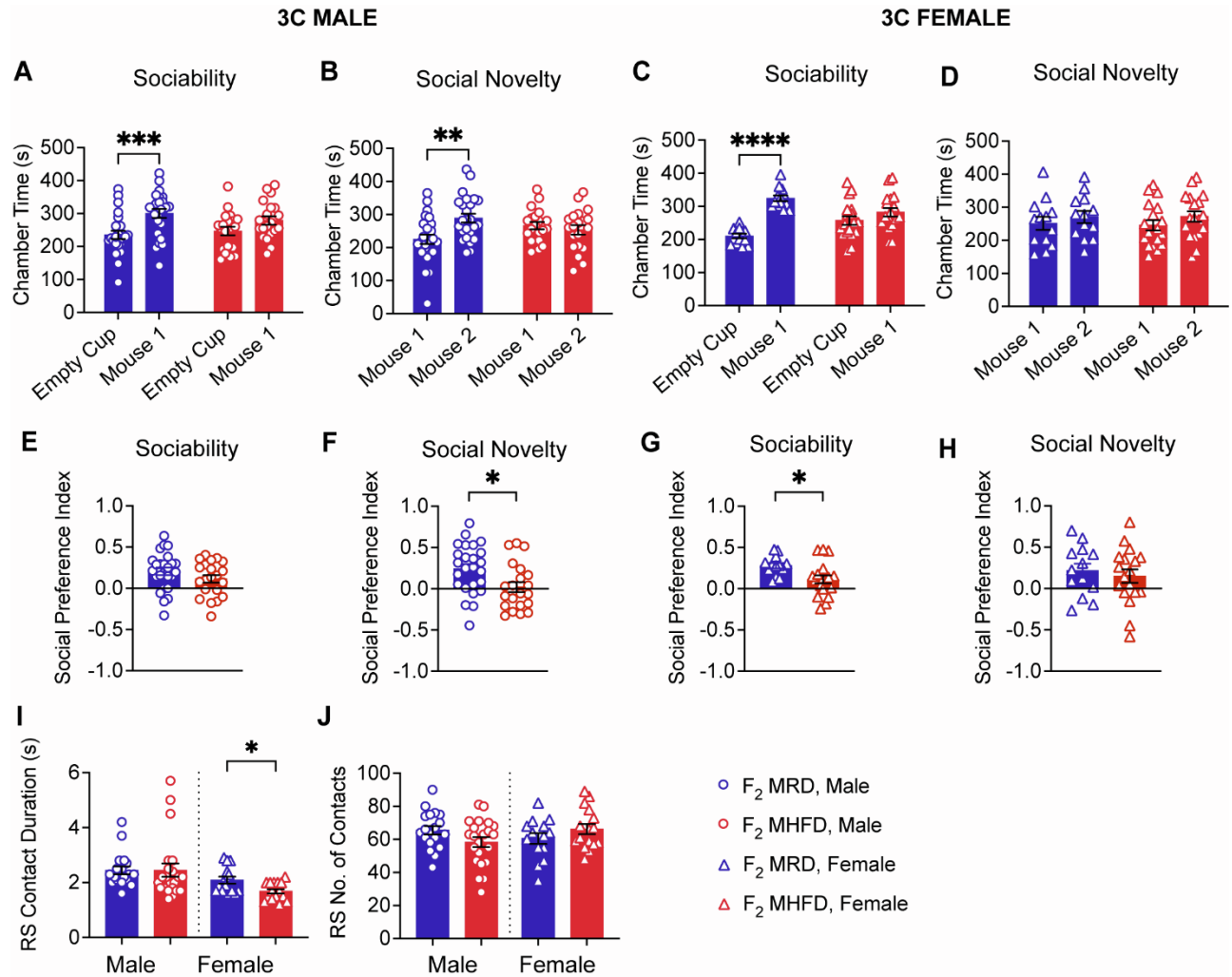
Figure S2. Automated 3C time in chamber data corroborate MHFD-descendant F₂ male social deficits measured by trained observers. Related to Figure 1. 3C ANY-maze chamber times for (**A, B**) male and (**C, D**) female sociability (Male MRD F₂: $t(86) = 3.740$, $p = 0.0007$; Male MHFD F₂: $t(86) = 1.654$, $p = 0.1932$; Female MRD F₂: $t(56) = 6.277$, $p < 0.0001$; Female MHFD F₂: $t(56) = 1.473$, $p = 0.2713$) and preference for social novelty (Male MRD F₂: $t(86) = 3.534$, $p = 0.0013$; Male MHFD F₂: $t(86) = 0.6284$, $p = 0.7804$; Female MRD F₂: $t(56) = 0.7172$, $p = 0.7257$; Female MHFD F₂: $t(56) = 1.178$, $p = 0.4283$), respectively. Social Preference Index for (**E, F**) male and (**G, H**) female sociability (Male MHFD *vs* MRD F₂: $t(43) = 1.4$, $p = 0.1686$; Female MHFD *vs* MRD F₂: $t(28) = 3.079$, $p = 0.0046$) and preference for social novelty (Male MHFD *vs* MRD F₂: $t(43) = 2.901$, $p = 0.0058$; Female MHFD *vs* MRD F₂: $t(8) = 0.8534$, $p = 0.4182$), respectively. F₂ male and female F₂ reciprocal social (**I**) contact duration (Male MHFD *vs* MRD F₂: Mann-Whitney U = 157, $p = 0.1763$; Female MHFD *vs* MRD F₂: Mann-

Whitney U = 57.50, p = 0.0214) and (**J**) number of contacts (Male MHFD *vs* MRD F$_2$: t(39) = 1.796, p = 0.0803; Female MHFD *vs* MRD F$_2$: t(28) = 1.258, p = 0.2188). Bar graphs show mean ± SEM with individual data points representing biological replicates. A–H, N = 13–25 subjects per group, I and J, N = 14–22 subject pairs per group.
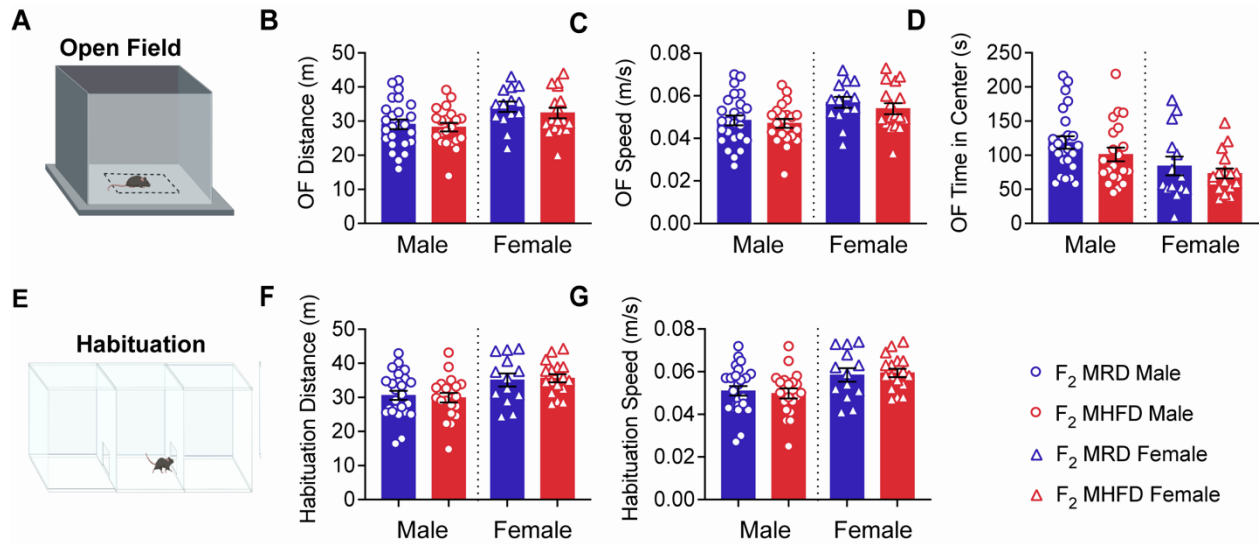
Supplemental Figure 3 (Di Gesù, Matz et al.)

Figure S3. Anxiety-like behavior and locomotor activity are unaltered in MHFD-descendant F₂ offspring.

Related to Figure 1. (**A**) Open field (OF) schematic. No statistically significant differences in OF (**B**) distance traveled (Males: $t(11) = 0.3246$, $p = 0.7516$; Females: $t(8) = 0.7396$, $p = 0.4807$), (**C**) speed (Males: $t(11) = 0.3431$, $p = 0.7380$; Females: $t(8) = 0.7397$, $p = 0.4806$, females), or (**D**) time spent in center (Males: $t(11) = 1.018$, $p = 0.3307$; Females: $t(8) = 0.6608$, $p = 0.5273$, females) were observed between cohorts. (**E**) Schematic of the 3C habituation stage. Neither (**F**) distance traveled (Male MRD *vs* MHFD: $t(43) = 0.3626$, $p = 0.7186$; Female MRD *vs* MHFD: $t(8) = 0.004881$, $p = 0.9962$) or (**G**) speed (Male MRD *vs* MHFD: $t(43) = 0.3706$, $p = 0.7128$; Female MRD *vs* MHFD: $t(8) = 0.01752$, $p = 0.9865$) differed between maternal diet lineages in the habituation phase of the 3C task. Bar graphs show mean ± SEM with individual data points representing biological replicates. A–H, N = 13 – 25 subjects per group; I and J, N = 14–22 subject pairs per group.

A Weighted UniFrac PCoA;*P*-value: 0.009; R²: 0.129

B Weighted UniFrac PCoA;*P*-value: 0.002; R²: 0.446

C Weighted UniFrac PCoA;*P*-value: 0.002; R²: 0.15

Figure S4. Weighted UniFrac analysis of beta diversity in F_1 and F_2 cohorts ± *L. reuteri*, MRD or MHFD F_2-FMT colonized males. Related to Figures 2, 4, and 5. (A) Principal coordinate analysis (PCoA) of Weighted UniFrac distances from the averaged rarefied 16S rRNA gene amplicon sequencing dataset (8,310 reads/sample; n=1,000 rarefactions) revealed statistically significant clusters based on diet and generation (p = 0.006, R^2 = 0.129).

(B) Principal coordinate analysis (PCoA) of Weighted UniFrac distances from the averaged rarefied 16S rRNA gene amplicon sequencing dataset (3,370 reads/sample; n=1,000 rarefactions) revealed statistically significant clusters based on diet ($p = 0.002$, $R^2 = 0.446$). **(C)** Principal coordinate analysis (PCoA) of Weighted UniFrac distances from the averaged rarefied 16S rRNA gene amplicon sequencing dataset (5,508 reads/sample; n=1,000 rarefactions) revealed statistically significant clusters based on diet ($p = 0.002$, $R^2 = 0.15$). A, N = 5–15 subjects per group; B, N = 7–8 subjects per group; C, N = 24–28 subjects per group. Data points represent biological replicates
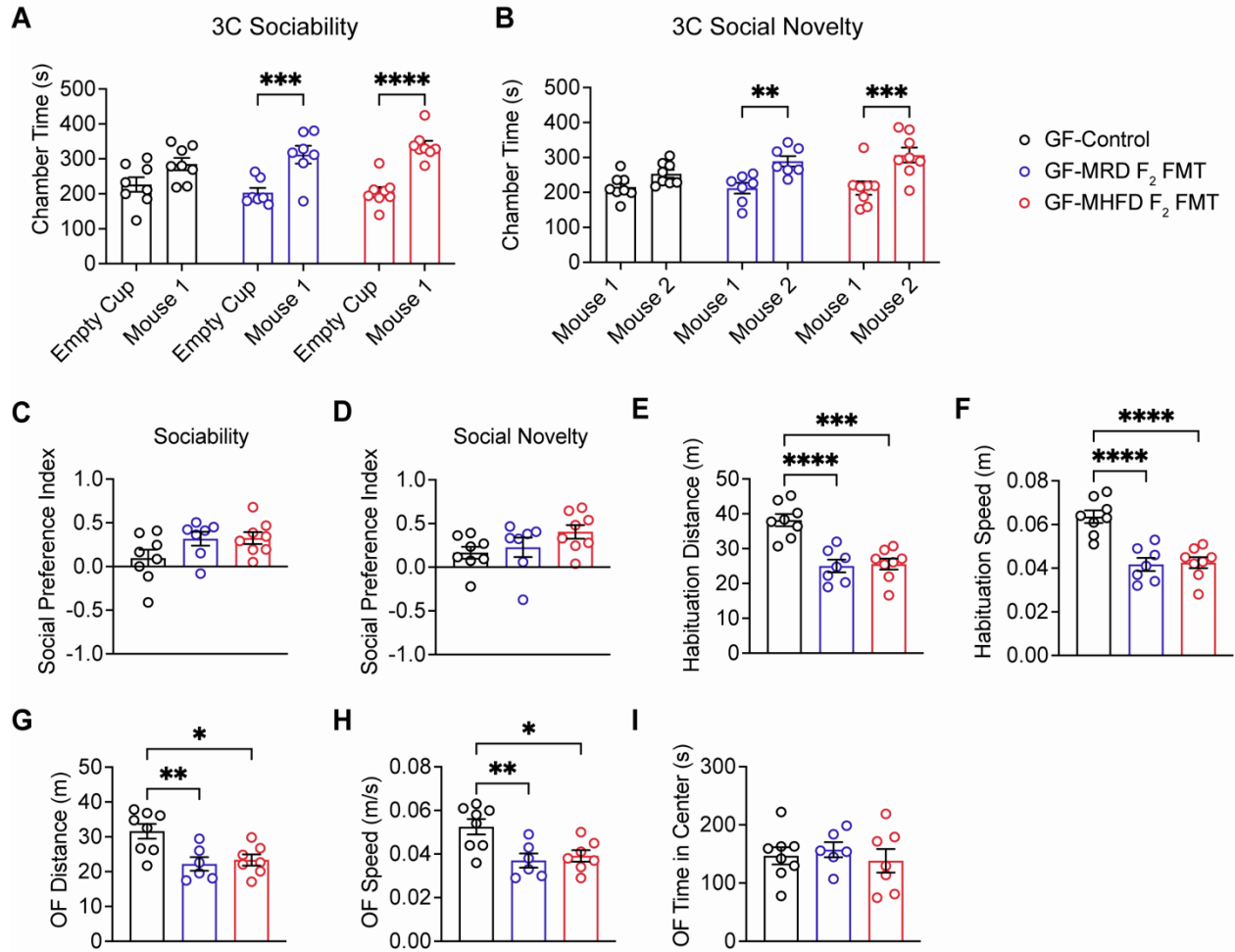
Figure S5. Automated 3C chamber times and locomotor data in germ-free (GF)-control, MRD F_2-FMT colonized, or MHFD F_2-FMT colonized males. Related to Figure 4. Automated ANY-maze 3C chamber times for GF-Control and MRD F_2- or MHFD F_2-FMT recipient (**A**) sociability (GF-control: $t(40) = 2.336$, $p = 0.0719$; MRD F_2-FMT: $t(40) = 4.053$, $p = 0.0007$; MHFD F_2-FMT: $t(40) = 5.294$, $p < 0.0001$) and (**B**) preference for social novelty (GF-control: $t(40) = 1.698$, $p = 0.2646$; MRD F_2-FMT: $t(40) = 3.3221$, $p = 0.0076$; MHFD F_2-FMT: $t(40) = 4.211$, $p = 0.0004$). Social preference index for F_2 male (**C**) sociability (GF-control vs MRD-FMT: $q(20) = 2.66$, $p = 0.1701$; GF-control vs MHFD-FMT: $q(20) = 2.85$, $p = 0.1343$; MRD F_2-FMT vs MHFD F_2-FMT: $q(20) = 0.09341$, $p = 0.9976$) and (**D**) social novelty (GF-control vs MRD F_2-FMT: $q(20) = 0.7191$, $p = 0.8681$; GF-control vs MHFD F_2-FMT: $q(20) = 2.855$, $p = 0.1335$; MRD F_2-FMT vs MHFD F_2-FMT: $q(20) = 2.039$, $p = 0.3393$). During 3C habituation, GF-control mice travel significantly farther than their colonized counterparts, as seen in (**E**) distance

(GF-control *vs* MRD F_2-FMT: $q(20) = 7.551$, $p < 0.0001$; GF-control *vs* MHFD F_2-FMT: $q(20) = 7.483$, $p = 0.0001$; MRD F_2-FMT *vs* MHFD F_2-FMT: $q(20) = 0.3218$, $p = 0.9719$) and (**F**) speed (GF-control *vs* MRD F_2-FMT: $q(20) = 7.687$, $p < 0.0001$; GF-control *vs* MHFD F_2-FMT: $q(20) = 7.671$, $p < 0.0001$; MRD F_2-FMT *vs* MHFD F_2-FMT: $q(20) = 0.2757$, $p = 0.9793$) compared to MRD F_2- or MHFD F_2-FMT males, as determined by one-way ANOVA with Tukey's correction for multiple comparisons. In the open field test, GF-control mice have significantly higher (**G**) distance traveled (GF-control *vs* MRD F_2-FMT: $q(18) = 4.821$, $p = 0.0084$; GF-control *vs* MHFD F_2-FMT: $q(18) = 4.425$, $p = 0.0152$; MRD F_2-FMT *vs* MHFD F_2-FMT: $q(18) = 0.5635$, $p = 0.9166$) and (**H**) speed (GF-C *vs* MRD F_2-FMT: $q(18) = 4.797$, $p = 0.0087$; GF-control *vs* MHFD F_2-FMT: $q(18) = 4.313$, $p = 0.018$; MRD F_2-FMT *vs* MHFD F_2-FMT: $q(18) = 0.6437$, $p = 0.8927$), but not (**I**) time spent in center (GF-control *vs* MRD F_2-FMT: $q(18) = 0.6256$, $p = 0.8984$; GF-control *vs* MHFD F_2-FMT: $q(18) = 0.5261$, $p = 0.9269$; MRD F_2-FMT *vs* MHFD F_2-FMT: $q(18) = 1.097$, $p = 0.7224$) compared to MRD F_2- or MHFD F_2-FMT males, as determined by one-way ANOVA with Tukey's correction for multiple comparisons. Bar graphs show mean ± SEM with individual data points representing biological replicates. A–F, N = 7–8 subjects per group; G–I, N = 6–8.
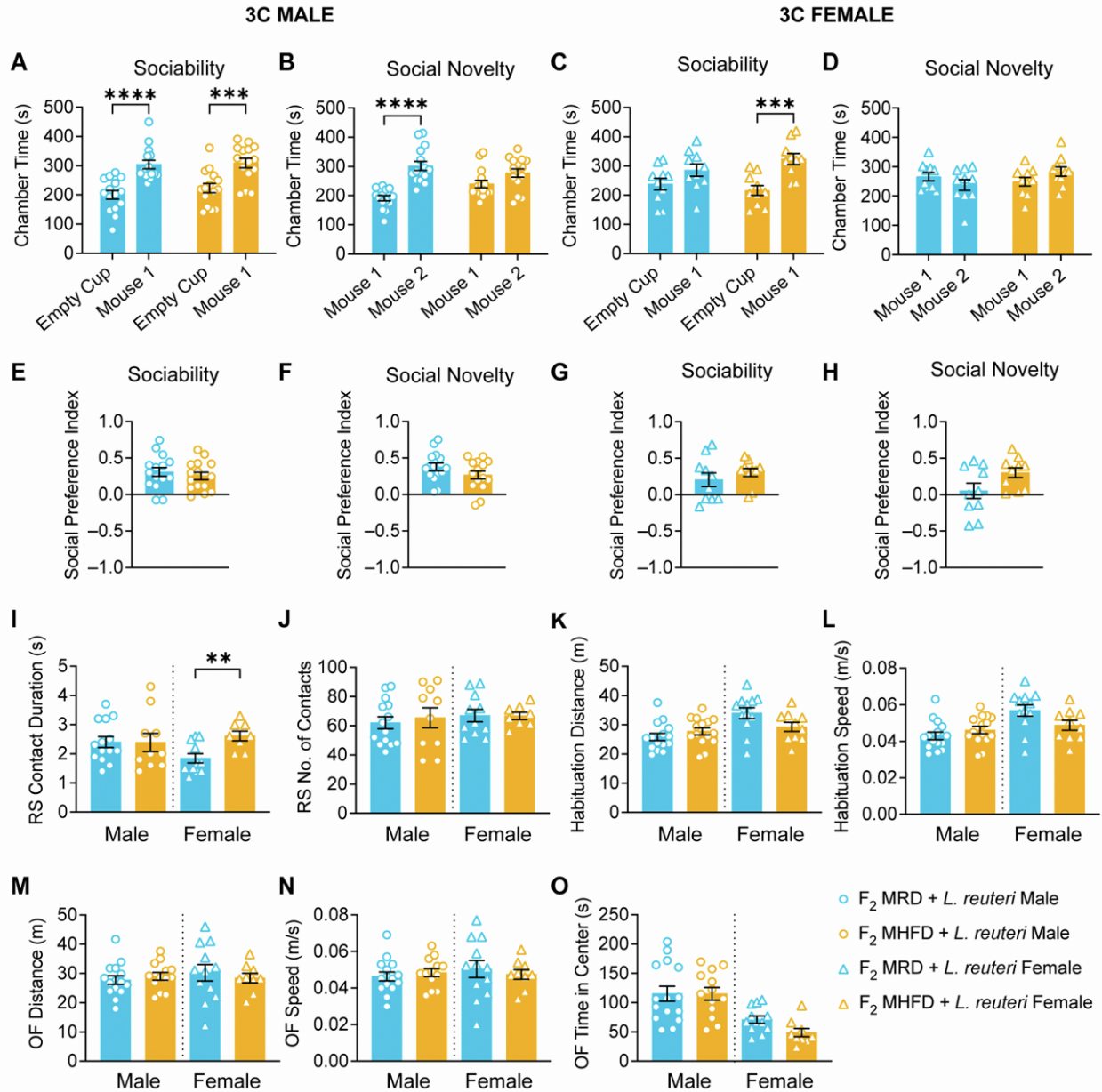
Figure S6. *L. reuteri*-**treated MRD- and MHFD-descendant F$_2$ mice do not display anxiety-like behavior or abnormal locomotor activity. Related to Figure 6.** 3C ANY-maze automated chamber times for F$_2$ + *L. reuteri* (**A, B**) male and (**C, D**) female sociability (Male MRD F$_2$ + *L. reuteri*: $t(56) = 4.783$, $p <0.0001$; Male MHFD F$_2$ + *L. reuteri*: $t(56) = 3.972$, $p = 0.0004$; Female MRD F$_2$ + *L. reuteri*: $t(36) = 1.747$, $p = 0.1703$; Female MHFD F$_2$ + *L. reuteri*: $t(36) = 3.952$, $p = 0.0007$) and preference for social novelty (Male MRD F$_2$ + *L. reuteri*: $t(56) = 5.969$, $p <0.0001$; Male MHFD F$_2$ + *L. reuteri*: $t(56) = 2.047$, $p = 0.0887$; Female MRD F$_2$ + *L. reuteri*: $t(36) = 1.276$, $p =$

0.3762; Female MHFD F_2 + *L. reuteri*: $t(36) = 1.549, p = 0.2435$), respectively, in the 3C task. Social preference

indices for (**E, F**) male and (**G, H**) female sociability (Male MHFD *vs* MRD F_2: $t(28) = 0.6876, p = 0.4973$; Female

MHFD *vs* MRD F_2: $t(4) = 0.7951, p = 0.4711$) and social novelty (Male MHFD *vs* MRD F_2: $t(5) = 1.313, p = 0.2463$; Female MHFD *vs* MRD F_2: $t(4) = 1.497, p = 0.2088$). F_2 + *L. reuteri* male and female reciprocal social (**I**)

contact duration (Male MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(22) = 0.02922, p = 0.9770$; Female MHFD

F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(17) = 3.261, p = 0.0046$) and (**J**) number of contacts (Male MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(22) = 0.4516, p = 0.6559$; Female MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*:

$t(17) = 0.04579, p = 0.9640$). Neither male nor female F_2 3C habituation (**K**) distance (Male MHFD F_2 + *L. reuteri*

vs MRD F_2 + *L. reuteri*: $t(5) = 0.9683, p = 0.3774$; Female MHFD F_2 + *L. reuteri vs* MRD F_2 + *Lr*: $t(18) = 2.064, p = 0.0537$) or (**L**) speed (Male MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(5) = 0.9711, p = 0.3761$; Female

MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(18) = 2.097, p = 0.0504$) differ between diets. Open field (**M**)

distance (Male MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(26) = 0.6324, p = 0.5326$; Female MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(4) = 0.2862, p = 0.7890$), (**N**) speed (Male MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(26) = 0.6384, p = 0.5288$; Female MHFD F_2 + *L. reuteri vs* MRD F_2 + *L. reuteri*: $t(4) = 0.2752, p = 0.7968$), and (**O**) time spent in the center (Male MHFD F_2 + *L. reuteri vs.* MRD F_2 + *L. reuteri*: $t(5) = 0.1939, p = 0.8539$; Female MHFD F_2 + *L. reuteri vs.* MRD F_2 + *L. reuteri*: $t(4) = 2.024, p = 0.1129$) did not significantly differ

between cohorts. Bar graphs show mean ± SEM with individual data points representing biological replicates. A–H,

K, and L, N = 10–15 subjects per group; I and J, N = 8–14 pairs per group; M–O, N = 9–15 subjects per group.

Supplemental Figure 7 (Di Gesù, Matz et al.)

A Unweighted UniFrac PCoA; *P*-value: 0.001; R²: 0.128

PC2 (14.6% Variation Explained)
PC1 (26.2% Variation Explained)

F₂ MRD + *L. reuteri* M   F₂ MHFD + *L. reuteri* M

B Weighted UniFrac PCoA; *P*-value: 0.294; R²: 0.0378

PC2 (11.4% Variation Explained)
PC1 (77.4% Variation Explained)

F₂ MRD + *L. reuteri* M   F₂ MHFD + *L. reuteri* M

C Observed OTUs *
D Chao1 Index *
E Shannon index

○ F₂ MRD + *L. reuteri* M   ○ F₂ MHFD + *L. reuteri* M

F LDA score (log10)

Roseburia
Family *Lachnospiraceae* (UCG 001)
Class *Clostridia* (vadinBB60 group)
Colidextribacter
Parasutterella
Order *Gastranaerophilales*
Family *Anaerovoracaceae* (XIII AD3011 group)
Family *Anaerovoracaceae* (*Eubacterium nodatum* group)
Family *Oscillospiraceae* (NK4A214 group)
Family *Defluviitaleaceae* (UCG 011)
Anaerofustis
Ruminococcus
Family *Lachnospiraceae* (*Eubacterium ventriosum* group)

F₂ MRD + *L. reuteri* M
F₂ MHFD + *L. reuteri* M

G Unweighted UniFrac PCoA;*P*-value: 0.001; R²: 0.301

PC2 (12.1% Variation Explained)
PC1 (35% Variation Explained)

F₂ MRD + *L. reuteri* F   F₂ MHFD + *L. reuteri* F

H Weighted UniFrac PCoA; *P*-value: 0.001; R²: 0.541

PC2 (12.3% Variation Explained)
PC1 (78% Variation Explained)

F₂ MRD + *L. reuteri* F   F₂ MHFD + *L. reuteri* F

I Observed OTUs ****
J Chao1 Index ***
K Shannon index ****

△ F₂ MRD + *L. reuteri* F   △ F₂ MHFD + *L. reuteri* F

L LDA score (log10)

Family *Muribaculaceae*
Family *Lachnospiraceae*
Family *Lachnospiraceae* (NK4A136 group)
Family *Lachnospiraceae* (UCG 008)
Family *Lachnospiraceae* (UCG 006)
Roseburia
Lachnoclostridium
Family *Lachnospiraceae* (*Eubacterium xylanophilum* group)
Order *Oscillospirales* (*Eubacterium coprostanoligenes* group)
Family *Lachnospiraceae* (FCS020 group)
Parvibacter
Parabacteroides
Family *Oscillospiraceae*
Family *Defluviitaleaceae* (UCG 011)
Class *Bacilli* (RF39)
Family *Anaerovoracaceae* (*Eubacterium nodatum* group)
Family *Ruminococcaceae*
Oscillibacter
Family *Ruminococcaceae* (*Incertae Sedis*)
Blautia
Order *Oscillospirales* (UCG 010)
Family *Lachnospiraceae* (A2)
Erysipelatoclostridium
Family *Lachnospiraceae* (GCA 900066575)
Parasutterella
Colidextribacter
Order *Gastranaerophilales*
Anaerotruncus
Acetitomaculum
Family *Erysipelotrichaceae*
Family *Anaerovoracaceae* (XIII UCG 001)
Faecalibaculum
Family *Lachnospiraceae* (UCG 001)
Family *Anaerovoracaceae* (XIII AD3011 group)
Anaeroplasma
Staphylococcus
Jeotgalicoccus
Ligilactobacillus
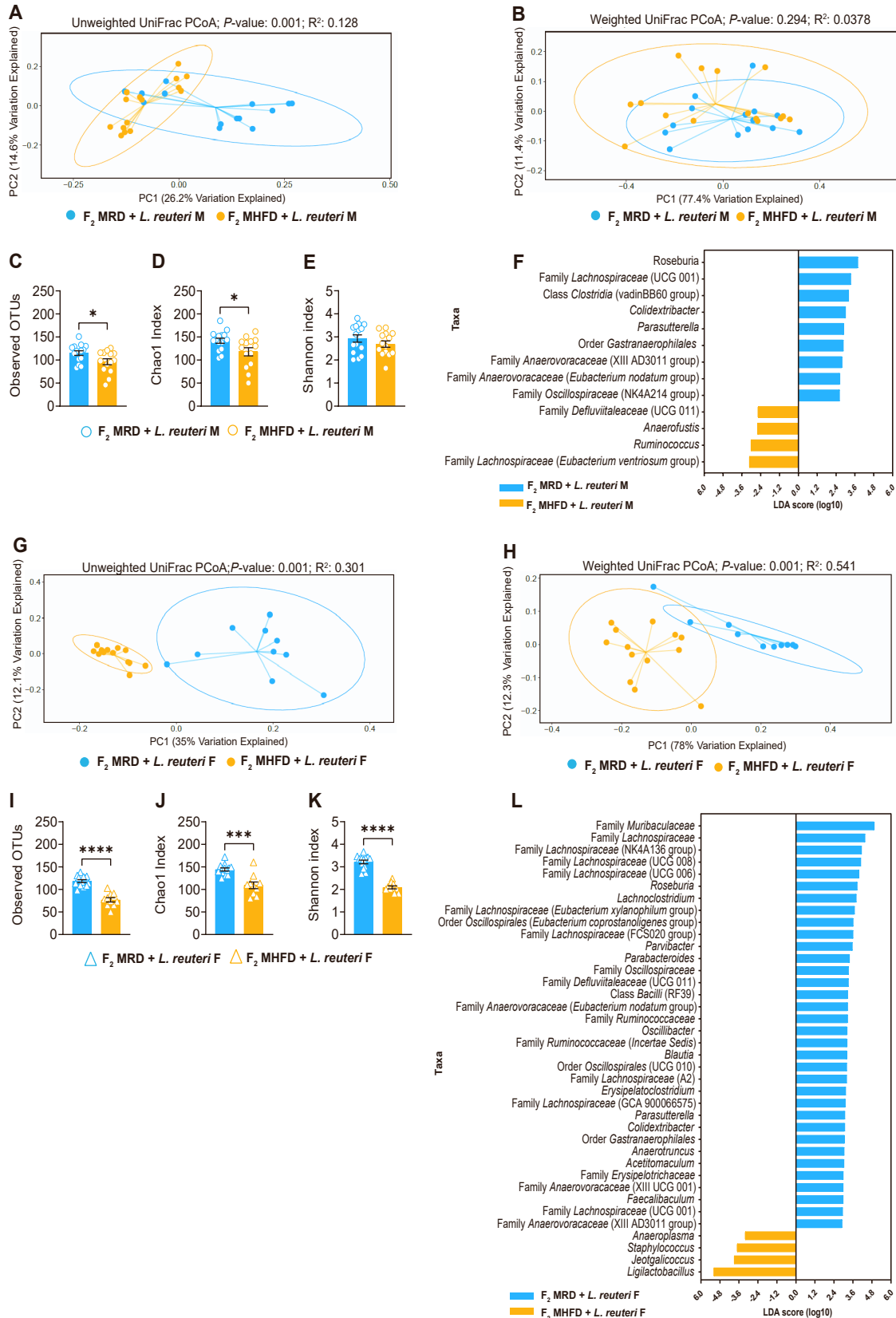
F₂ MRD + *L. reuteri* F
F₂ MHFD + *L. reuteri* F

Figure S7. F$_2$ microbiome remodeling in response to *L. reuteri* exhibits sex specificity, with females showing greater divergence between diet cohorts. Related to Figures 5 and 6. (A–B) Principal coordinate analysis (PCoA) of Unweighted (A), but not Weighted (B), UniFrac distances from the averaged rarefied 16S rRNA gene amplicon sequencing dataset (5,508 reads/sample; n=1,000 rarefactions) revealed statistically significant clusters based on diet (Unweighted Unifrac $p = 0.001$, $R^2 = 0.128$; Weighted Unifrac $p = 0.294$, $R^2 = 0.0378$) in F$_2$ male mice treated with *L. reuteri*. (C–E) Total number of OTUs (C) (MHFD F$_2$ + *L. reuteri* vs MRD F$_2$ + *L. reuteri*: $t(27)$ = 2.331, $p = 0.0275$) and Chao1 index (D) (MHFD F$_2$ + *L. reuteri* vs MRD F$_2$ + *L. reuteri*: $t(27)$ = 2.326, $p = 0.0278$), but not Shannon Index (E) (MHFD F$_2$ + *L. reuteri* vs MRD F$_2$ + *L. reuteri*: $t(27)$ = 1.147, $p = 0.2613$), are decreased in *L. reuteri*-treated MHFD F$_2$ male offspring compared to MRD group. (F) Histogram of the LDA scores (log10) computed for genus-level taxa with differential abundance in *L. reuteri*-treated MHFD and MRD F$_2$ male offspring. (G–H) Principal coordinate analysis (PCoA) of Unweighted (G) and Weighted (H) UniFrac distances from the averaged rarefied 16S rRNA gene amplicon sequencing dataset (5,508 reads/sample; n=1,000 rarefactions) revealed statistically significant clusters based on diet (Unweighted Unifrac $p = 0.001$, $R^2 = 0.301$; Weighted Unifrac $p = 0.294$, $R^2 = 0.541$) in F$_2$ female mice treated with *L. reuteri*. (I–K) Total number of OTUs (I) (MHFD F$_2$ + *L. reuteri* vs MRD F$_2$ + *L. reuteri*: $t(21)$ = 7.392, $p< 0.0001$), Chao1 index (J) (MHFD F$_2$ + *L. reuteri* vs MRD F$_2$ + *L. reuteri*: $t(21)$ = 4.674, $p = 0.0001$), and Shannon Index (K) (MHFD F$_2$ + *L. reuteri* vs MRD F$_2$ + *L. reuteri*: $t(21)$ = 10.25, $p< 0.0001$), are decreased in *L. reuteri*-treated MHFD F$_2$ female offspring compared to MRD group. (L) Histogram of the LDA scores (log10) computed for genus-level taxa with differential abundance in *L. reuteri*-treated MHFD and MRD F$_2$ female offspring. Bar graphs show mean ± SEM with individual data points representing biological replicates. N = 10–15 subjects per group.
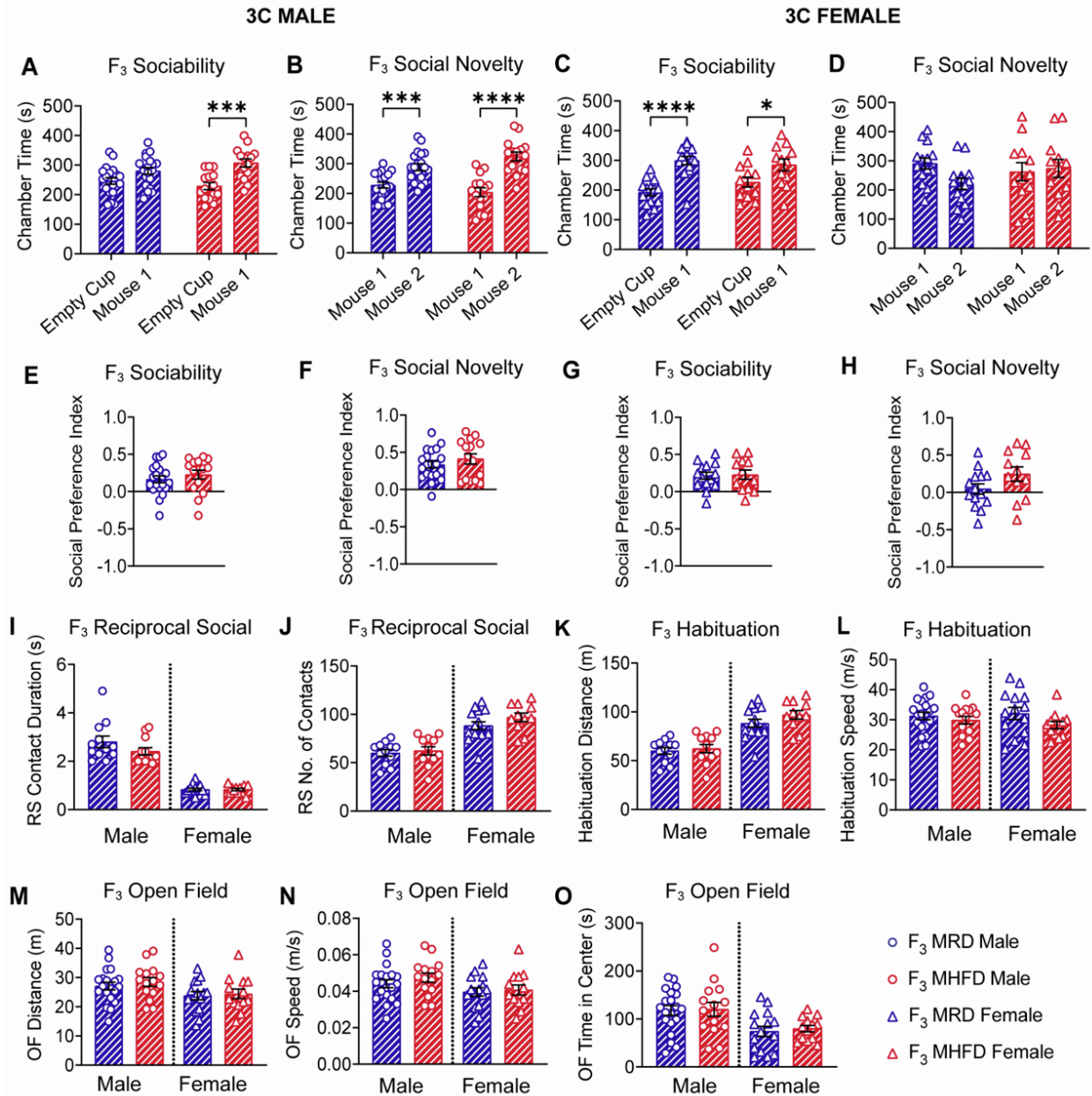
Figure S8. Consistent with F₂, MHFD-descendant F₃ mice do not display anxiety-like behavior or abnormal locomotor activity. Related to Figure 7. 3C ANY-maze automated chamber times for F_3 (A, B) male and (C, D) female sociability (Male MRD F_3: $t(66) = 2.166$, $p = 0.0667$; Male MHFD F_3: $t(66) = 4.299$, $p < 0.0001$; Female MRD F_3: $t(48) = 5.050$, $p < 0.0001$; Female MHFD F_3: $t(48) = 2.534$, $p = 0.0290$) and social novelty (Male MRD F_3: $t(66) = 3.691$, $p = 0.0009$; Male MHFD F_3: $t(66) = 6.330$, $p < 0.00001$; Female MRD F_3: $t(48) = 2.038$, $p = 0.0919$; Female MHFD F_3: $t(48) = 0.3118$, $p = 0.9407$), respectively. Social Preference Index for (E, F) male and (G,

H) female sociability (Male MHFD *vs* MRD F$_3$: $t(8) = 0.8067$, $p = 0.4432$; Female MHFD *vs* MRD F$_3$: $t(24) = 0.1777$, $p = 0.8605$) and social novelty (Male MHFD *vs* MRD F$_3$: $t(33) = 0.9657$, $p = 0.3412$; Female MHFD *vs* MRD F$_3$: $t(24) = 1,739$, $p = 0.0949$). F$_3$ male and female reciprocal social (I) contact duration (Male MHFD *vs* MRD F$_3$ Mann-Whitney U=45.50, $p = 0.2140$; Female MHFD *vs* MRD F$_3$ $t(24) = 0.03989$, $p = 0.9685$) and (J) number of contacts (Male MHFD *vs* MRD F$_3$: $t(22) = 0.4158$, $p = 0.6816$; Female MHFD *vs* MRD F$_3$: $t(24) = 1.426$, $p = 0.1668$). Neither male nor female 3C habituation (K) distance (Male MHFD F$_3$ *vs* MRD F$_3$: $t(8) = 0.4871$, $p = 0.6392$; Female MHFD *vs* MRD F$_3$: $t(4) = 1.223$, $p = 0.2885$) or (L) speed (Male MHFD *vs* MRD F$_3$: $t(8) = 0.5126$, $p = 0.6221$; Female MHFD *vs* MRD F$_3$: $t(4) = 1.205$, $p = 0.2947$) differ between diets. Open field (M) distance traveled (Male MHFD *vs* MRD F$_3$: $t(8) = 0.6214$, $p = 0.5516$; Female MHFD *vs* MRD F$_3$: $t(4) = 0.2135$, $p = 0.8414$), (N) speed (Male MHFD *vs* MRD F$_3$: $t(8) = 0.454$, $p = 0.7860$; Female MHFD *vs* MRD F$_3$: $t(4) = 0.2161$, $p = 0.8395$), and (O) time spent in center (Male MHFD *vs* MRD F$_3$: $t(8) = 0.04288$, $p = 0.9668$; Female MHFD *vs* MRD F$_3$: $t(4) = 0.3881$, $p = 0.7177$) are not statistically different between cohorts. Bar graphs show mean ± SEM with individual data points representing biological replicates. A–H, K, and L, N = 12–20 subjects per group; I and J, N = 12–15 pairs per group; M and O, N = 13–20 subjects per group.