Legends to Supplementary Figures

Figure S1. Geographically Distinct Trapping locations of *Mus musculus domesticus*, Related to Figure 1 and 2

Mice were trapped in 8 different geographically distinct locations throughout Maryland and the District of Columbia (USA) in the fall of two consecutive years. Green dots indicate the three primary trapping locations (B, A, C from left to right), purple dots indicate additional trapping sites.

See also Table S1.

Figure S2. The Laboratory Mouse Bacterial Gut Microbiome from Commercial Vendors is Significantly Different from that of their Wild Living Kin, Related to Figure 2 and Figure S4

16S rRNA gene profiling data comparing the gut microbiome of *Mus musculus domesticus* from locations A, B and C in Maryland, USA (Wild) to that of C57BL/6 mice from Taconic Biosciences, Charles River and the Jackson Laboratory (Lab). (A and B) The *Mus musculus domesticus* gut microbiome in Maryland was stable in the fall of two consecutive years as illustrated by comparison of wild mice trapped in the fall of 2014 and 2015 with C57BL/6 mice from commercial vendors (Lab)**.** (A) Unweighted UniFrac PCoA. (B) Weighted UniFrac PCoA. (C) The unweighted pairwise UniFrac distances among gut communities from geographically distinct wild mouse locations were significantly decreased compared to distances between all wild and laboratory groups. Median and IQR are presented. $P<0.0001$ for each comparison green versus orange, Kruskal-Wallis with FDR adjustment. (D) Relative abundance at the rank of phylum. Median and IQR are presented. $^{**}P<0.05$, $^{****}P<0.0001$. Statistics within Table S3. (E) Relative abundance of the 10 most abundant orders. Relative abundance of the orders included in “Other” are provided along with the statistical analysis as Table S4.

Figure S3. Wild Mice from Maryland (USA) Locations A, B and C Represent Genetically Differentiated Populations and are not close Relatives within the Locations, but Display a Similar Pathogen Exposure History, Related to Figure 2 and Figure 3A

(A) PCoA on SNP genotypes showing that *Mus musculus domesticus* from the trapping sites A, B and C in Maryland, USA are genetically differentiated populations. (B) Distribution of pairwise kinship coefficients within and between Maryland primary trapping locations A, B, and C. Vertical lines indicate approximate cutoffs for various degrees of relatedness (first-degree, second-degree, third-degree). (C) Heatmap displaying the fraction of wild mice at location A (n=8), B (n=8), C (n=30) that tested positive by either PCR or ImmunoAssay® (MFIA®). *N.t.*, not tested.
See also Figure S1 and Table S1.

Figure S4. SubOTU Analysis Confirms that the Laboratory Mouse Bacterial Gut Microbiome from Commercial Vendors is Significantly Different from that of their Wild Living Kin, Related to Figure 2 and Figure S2

16S rRNA gene profiling data (subOTU analysis) comparing the gut microbiome of *Mus musculus domesticus* from locations A, B and C in Maryland, USA (Wild) to that of C57BL/6 mice from Taconic Biosciences, Charles River and the Jackson Laboratory (Lab). (A) Unweighted UniFrac PCoA. (B) Weighted UniFrac PCoA. (C) Relative abundance of the 12 most abundant phyla. (D) Relative abundance of the 9 most abundant orders. (E) Relative abundance at the rank of phylum. (F) Relative abundance at the rank of order. Median and IQR are presented. ***P<0.001, ****P<0.0001. Significance was determined using DESeq2 analysis.

Figure S5. The *Mus musculus domesticus* Bacterial Gut Microbiome can be Transferred to and Stably Maintained in the Multigenerational Offspring of C57BL/6 Laboratory Mice, Related to Figure 3 and Figure S6

16S rRNA gene profiling data comparing the gut microbiome of *Mus musculus domesticus* (Wild) or C57BL/6NTac (Lab), the recipient mice (WildR and LabR recipients [R]), and their subsequent generations (WildR F1, F2, F3, F4 and LabR F1, F2, F3, F4). (A) Unweighted pairwise UniFrac distances between corresponding WildR and LabR generations remained stable over time and were significantly greater than the stable UniFrac distances between subsequent WildR and LabR generations and their respective recipient mice. Median and IQR are presented. P<0.0001, orange versus green and blue, Kruskal-Wallis with FDR adjustment. (B) Relative abundance of the 10 most abundant orders. Relative abundance of the orders included in “Other” are provided along with the statistical analysis as Table S7.

Figure S6. SubOTU Analysis Confirms that the *Mus musculus domesticus* Gut Microbiome can be Transferred to Pregnant GF C57BL/6 Mice and Maintained in their Multigenerational Offspring, Related to Figure 3 and Figure S5

16S rRNA gene profiling data (subOTU analysis) comparing the gut microbiome of *Mus musculus domesticus* (Wild) or C57BL/6NTac (Lab), the recipient mice (WildR and LabR recipients [R]), and their subsequent generations (WildR F1, F2, F3, F4 and LabR F1, F2, F3, F4). (A) Unweighted UniFrac PCoA. (B) Weighted UniFrac PCoA. (C) Relative abundance of the 12 most abundant phyla. (D) Relative abundance of the 9 most abundant orders. (E) Relative abundance at the rank of phylum. (F) Relative abundance at the rank of order. Median and IQR are presented. ****P<0.0001. Significance was determined using DESeq2 analysis.

Figure S7. The *Mus musculus domesticus* Gut Microbiome Confers a Survival Advantage after Lethal IAV Infection, Related to Figure 5

(A) 400 $TCID_{50}$ IAV PR8 for Female and 600 $TCID_{50}$ IAV PR8 for Male C57BL/6 is a Lethal Infection Regardless of Vendor. Female and male mice from Taconic Biosciences, Charles River and the Jackson Laboratory were intranasally inoculated with 400 $TCID_{50}$ (female) and 600 $TCID_{50}$ (male) mice. Mice were monitored daily for 18 days with mice losing 30% or more of their body weight euthanized (Taconic Biosciences, n=36; Charles River, n=36; the Jackson Laboratory, n=36). Kaplan Meier survival curves for-female and male mice. No significant mortality differences between mice of different sex and/or sourced from different commercial vendors were observed (by log rank [Mantel-Cox] analysis). (B - D) WildR, LabR and Lab female mice were inoculated with 400 $TCID_{50}$ and male mice with 600 $TCID_{50}$ of PR8 (female Lab, n=27; LabR, n=23; WildR, n=22; male Lab, n=31; LabR, n=23; WildR, n=26). (B) Kaplan Meier survival curves, ****P<0.0001 comparing WildR with either LabR or Lab. (C) Weight loss curves, ****P<0.0001 comparing the slope of the weight loss (day 0 to day 7) of WildR to that of LabR or Lab in a linear regression analysis. Weight loss of LabR did not significantly differ from that of Lab. Median and IQR are presented. (D) Lung viral titer at day 3 post IAV infection assessed via MDCK monolayers in 96-well plates using an antibody-based assay. Lab, n=51; LabR, n=49; WildR, n=48. Median and IQR are presented. $^{**}P<0.01$, $^{***}P<0.001$. Significance was determined using Kruskal-Wallis with Dunn’s multiple comparison test, only significant (P-values <0.05) are indicated. All data shown are from three independent experiments using both female (X) and male (O) mice.

Legends to Supplemental Tables

Table S1. Age Class Scoring System and Age and Sex Classification of sampled Mus musculus domesticus, Related to Figures 1, 2 and 3A.

Table S2. The Laboratory Mouse Bacterial Gut Microbiome from Commercial Vendors is Significantly Different from that of their Wild Living Kin, Related to Figure 2.

Table S3. The Laboratory Mouse Bacterial Gut Microbiome from Commercial Vendors is Significantly Different from that of their Wild Living Kin, Related to Figure 2C and Figure S2D.

Table S4. The Laboratory Mouse Bacterial Gut Microbiome from Commercial Vendors is Significantly Different from that of their Wild Living Kin, Related to Figures 2 and S2E.

Table S5. Specific-Pathogen Free (SPF) Definition of Animal Facility and Pathogen Screening Results of WildR and LabR Populations, Related to Figure 3A.

Table S6. The Mus musculus domesticus Bacterial Gut Microbiome can be Transferred to Pregnant GF C57BL/6 Mice and Maintained in their Multigenerational Offspring, Related to Figure 3B, C and D.

Table S7. The Mus musculus domesticus Gut Microbiome can be Transferred to and Stably Maintained in the Multigenerational Offspring of C57BL/6 Laboratory Mice, Related to Figures 3 and S5B.