Animal Return

0.0 PC1 (25.2%)

0.1

-0.1

-0.2

Dataset





Supplementary Figure 2. BatchQC results for uncorrected and corrected data. BatchQC skew kurtosis results for sample data A) before correction B) after correcting for the library preparation batch variable using ComBat; C) after correcting for the library preparation batch variable using ComBat-seq; D) after correcting for the mission batch variable using ComBat; E) after correcting for the mission batch variable using ComBat; E) after correcting for the mission batch variable using ComBat-seq; F-H) after correcting for the library preparation batch variable using MBatch Empirical Bayes (EB), ANOVA, and Median Polish (MP), respectively; I-K) after correcting for the mission batch variable using MBatch Empirical Bayes (EB), ANOVA, and Median Polish (MP), respectively.



Supplementary Figure 3. **PCA plots of corrected data.** PCA plots of sample data after using MBatch Empirical Bayes (EB) to correct for the A) library preparation batch variable and B) the mission batch variable; after using MBatch ANOVA to correct for the C) library preparation batch variable and D) the mission batch variable; after using MBatch Median Polish (MP) to correct for the E) library preparation batch variable and F) the mission batch variable. For plots A, C, and E samples are colored by library preparation method. For plots B, D, and F samples are colored by Mission.



Supplementary Figure 4. DSC scores of corrected data compared to uncorrected data. Barplots of sample data grouped by biological (blue) or technical (orange) variables for uncorrected DSC values subtracted from corrected DSC values after using MBatch Empirical Bayes (EB) to correct for the A) library preparation batch variable and B) the mission batch variable; after using MBatch ANOVA to correct for the C) library preparation batch variable and D) the mission batch variable; after using MBatch Median Polish (MP) to correct for the E) library preparation batch variable and F) the mission batch variable. A blue star indicates the biological condition of interest (FLT or GC) and the orange star indicates the technical batch variable that was corrected for.



Supplementary Figure 5. **FLT vs. GC LFC correlation between liver datasets after batch correction.** Correlation plots comparing space flight (FLT) versus ground control (GC) log₂ fold change (LFC) in gene expression between original datasets after using MBatch Empirical Bayes (EB) to correct for the A) library preparation batch variable and B) the mission batch variable; after using MBatch ANOVA to correct for the C) library preparation batch variable and D) the mission batch variable; after using MBatch Median Polish (MP) to correct for the E) library preparation batch variable and F) the mission batch variable. FLT and GC samples from datasets GLDS-48 and GLDS-245 were each split into two sub-datasets as described in Methods.



Supplementary Figure 6. Comparison of preserved DEGs in FLT vs. GC groups across datasets after correction. Plots showing the pairwise comparisons of the percent overlapping DEGs preserved in FLT versus GC groups across datasets after using MBatch Empirical Bayes (EB) to correct for the A) library preparation batch variable and B) the mission batch variable; after using MBatch ANOVA to correct for the C) library preparation batch variable and D) the mission batch variable; after using MBatch Median Polish (MP) to correct for the E) library preparation batch variable and F) the mission batch variable. FLT and GC samples from datasets GLDS-48 and GLDS-245 were each split into two sub-datasets as described in Methods.