Research using rodents is an essential tool for advancing biomedical research on Earth and in space. Rodent Research (RR)-1 was conducted to validate flight hardware, operations, and science capabilities that were developed at the NASA Ames Research Center. Twenty C57BL/6J adult female mice were launched on Sept 21, 2014 in a Dragon Capsule (SpaceX-4), then transferred to the ISS for a total time of 21-22 days (10 commercial mice) or 37 (10 validation mice). Tissues collected on-orbit were either rapidly frozen or preserved in RNAlater at ≤-80˚C (n=2/group) until their return to Earth. Remaining carcasses were rapidly frozen for dissection post-flight. The three controls groups at Kennedy Space Center consisted of: Basal mice euthanized at the time of launch, Vivarium controls, housed in standard cages, and Ground Controls (GC), housed in flight hardware within an environmental chamber. FLT mice appeared more physically active on-orbit than GC, and behavior analysis are in progress. Upon return to Earth, there were no differences in body weights between FLT and GC at the end of the 37 days in space. RNA was of high quality (RIN>8.5). Liver enzyme activity levels of FLT mice and all control mice were similar in magnitude to those of the samples that were optimally processed in the laboratory. Liver samples collected from the intact frozen FLT carcasses had RNA RIN of 7.27 +/- 0.52, which was lower than that of the samples processed on-orbit, but similar to those obtained from the control group intact carcasses. Nonetheless, the RNA samples from the intact carcasses were acceptable for the most demanding transcriptomic analyses. Adrenal glands, thymus and spleen (organs associated with stress response) showed no significant difference in weights between FLT and GC. Enzymatic activity was also not significantly different. Over 3,000 tissues collected from the four groups of mice have become available for the Biospecimen Sharing Program. Together, these validation flight findings demonstrate the capability to support long-duration RR on the ISS to achieve both basic science and biomedical objectives.