Statistical analyses and data rigor/reproducibility. The experiments in this proposal include HTS assays, IC_{50} determinations of selected compounds in recombinant protein and cell-based assays, various biochemical and toxicology studies in cell lines and animals, *in vitro* inhibition studies using live parasites and *in vivo* efficacy studies using animal models. Preliminary and published data (33, 77, 84) from our lab using all of these methods demonstrate that the approaches are robust and can be used to identify compounds that are statistically significantly more potent, selective or biologically active than vehicle controls in each assay. Identification of potential hit compounds will be assessed in the HTS platform using plate Z-factor (Z = 1 - 3SD of sample + 3SD of control/mean of sample - mean of control) statistics to measure the quality and power of the HTS assay (85). Hits from the HTS are defined as >30% inhibition of rSmNACE and/or compounds that fall 3SD from the data mean. All IC_{50} values of HTS hits will be calculated from a 10-point dose response concentration curve by a 4-parameter logistic fit of the data. We will use 3-5 replicates in individual recombinant protein/cell-based assays and biochemical assays (i.e. NAD measurements) and 8-10 individual adult parasites in viability assays as our preliminary results and published studies (86-91) indicate that our sample size will be large enough to detect statistically significant differences between groups in these assays ($p<0.05$). Group sizes in the *in vivo* PK studies will enable, at a minimum, calculation of mean and standard deviation values for data parameters, while the number of dose groups will be consistent with the recommendations of the FDA, the OECD and the International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH). *In vivo* efficacy studies will include 5-10 mice/timepoint as suggested by our sample size calculations (see Vertebrate Animal section) based on published data from other groups. This number of animals will be sufficient to detect differences between groups with at least an 80% power and 0.05 significance. Statistical analyses will include unpaired *t*-tests or non-parametric tests when comparing two independent groups (i.e. placebo and drug treated) and ANOVA tests when comparing multiple independent groups (i.e. multiple drug comparisons to placebo).

To ensure *rigor in our analyses*, our data sets will be reviewed by our biostatistics team (see LOS from Dr. Redden, Chair Biostatistics UAB) and we will consult with them in both the design and analysis phases of our project. To *ensure reproducibility* in our experiments, all compound hits will be retested following resynthesis of new compound and IC_{50} values will be validated in secondary orthogonal screens as well as in cell-based assays. Key *in vitro* biochemical and biological assays using live parasites will be repeated with adult worms that are isolated from independent batches of infected source mice. All *in vivo* efficacy studies will be confirmed in up to three independent experiments. *In vitro* experiments will be performed using both male and female parasites and all *in vivo* efficacy experiments will be conducted with infected male and female mice.