RYR1-related myopathies, the most common non-dystrophic muscle disease subtype, are a heterogeneous group of muscle disorders united by the presence of disease-causing mutations in the skeletal muscle ryanodine receptor (*RYR1*). Affected individuals can exhibit severe disabilities that include the need for a wheelchair for ambulation, severe spine curvature requiring surgery, breathing difficulties necessitating the use of a ventilator, and in some cases, can result in premature death in childhood. Despite great advances in our understanding of RYR1 function and the pathologic mechanisms associated with disease causing mutations in RYR1, there are currently no effective treatments for these devastating myopathies and there is an alarming paucity of candidate therapies in the development pipeline. We recently showed that homology-independent targeted integration (HITI) mediated CRISPR/Cas9 gene editing allows for targeted knock-in within the context of both dividing and non-dividing cells *in vitro* and *in vivo*. This approach employs a single double-strand break at a specific genomic locus induced by the CRISPR/Cas9 system, while the desired knock-in fragment flanked with inverted target sequence is cut from donor vector at the same time. Oriented integration at a specific genomic locus occurs based on non-homologous end joining (NHEJ) repair machinery. As homology-directed repair (HDR) only occurs in dividing cells, post-mitotic tissues like skeletal muscle are difficult to adapt to using HDR to repair a genomic mutation. Our central hypothesis is that HITI-mediated gene editing can be used to correct RYR1 mutations, and thus, restore normal RYR1 channel function and localization. Therefore, successful optimization of HITI-mediated CRISPR/Cas9 gene repair is an essential preclinical step needed in order to justify future studies designed to use this approach to treat genetically-inherited skeletal muscular diseases. **The overall goal of this proposal is to develop and validate the *in vitro* efficacy of homology-independent targeted integration (HITI) CRISPR/Cas9 gene editing to correct mutations that result in RYR1 myopathy.**

IV. Specific Aims and Rationale

RYR1 Research Priority Area:

- Utilization of CRISPR/Cas9 as a Therapeutic Strategy for RYR-1 Myopathy and Related Diseases