Genetic sequencing enables the pinpointing of specific genetic mutations correlated with striated muscle diseases. However, such associations do not describe mechanistic relationships between specific mutations and clinical phenotypes. To address this, we use CRISPR-edited human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as a model system to probe how mutations in β-myosin heavy chain (MHC) lead to cardiac muscle disease. Preliminary data show that the specific force generation and activation rate of mutant G256E myofibrils is greater than isogenic control myofibrils. During relaxation, the initial, slow phase kinetics were decreased, indicating slower cross-bridge detachment rate. Additionally, stopped-flow mant-ATP assays on isolated myofibrils showed slower ATP binding for the G256E mutation, supporting delayed relaxation observed in myofibrils. Overall, these data suggest a hypercontractile phenotype with impaired early relaxation for the G256E mutation. To understand the molecular structure basis of these effects we have used molecular dynamics simulations of wild type and mutant β-MHC. In post-rigor (M.ATP) and rigor state simulations, we observed reduced stability in the transducer region of β-myosin, due to weakened hydrogen bonds between neighboring β-sheet strands as well as significant changes in local contacts. Pairing these in silico data with our in vitro data, our results suggest that the G256E mutation affects the transducer region of myosin such that it alters communication between the nucleotide binding pocket and the actin binding surface during the acto-myosin chemo-mechanical cycle, leading to hypercontractility and slowed relaxation.