Abstract
In protein quality control, heat shock proteins, such as heat shock protein 70 (HSP70), and their interactions with co-chaperones, such as carboxyl-terminus of HSP interacting protein (CHIP) and HSP organizing protein (HOP), play an important role in determining protein fate. These interactions are influenced by the phosphorylation status of HSP70. In this study, the interaction of both non-phosphorylated HSP70 (70A) and phosphomimetic HSP70 (70D) with different versions of the CHIP protein were investigated through a co-immunoprecipitation (co-IP) and NanoBit assay. A mutant form of CHIP, G132N, does not rescue the decrease in interaction that is caused by phosphorylation (HSP70D), as seen in the decreased protein level in the co-IP pull-down. In the co-current NanoBit assay, the luminescence between HSP70D and G132N CHIP decreased, indicating less interaction. The matching results of the co-IP and NanoBit imply NanoBit is an effective way of measuring protein interaction. Additionally, this study involved an attempt to engineer a hemagglutinin (HA) tagged form of the HOP protein. This version of HOP would be useful for future studies on HOP interactions with HSP70 protein and the preference for HSP70 for HOP versus CHIP. Although currently unsuccessful, changes to the protocol, such as melting temperature or new primers will allow for future success of this HA addition. These experiments provide knowledge on how phosphorylation status of HSP70 affects protein quality control in cancer cells. Understanding these phosphorylation changes will provide opportunities to further engineer chemotherapeutics to better treat cancer.