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**Title:** LEVERAGING TARGETED PROTEOMICS TO ELUCIDATE CONSERVED MECHANISMS OF SIRT6 REGULATION AND DEVELOP NOVEL THERAPEUTICS FOR AGE-RELATED DISEASE

**Abstract**

Sirtuin 6 (SIRT6) is a protein deacetylase and ribosyltransferase that is a vital hub for maintaining the epigenome, regulating the transcriptome, and repairing double stranded breaks (DSBs). Enhanced SIRT6 activity has been correlated with longer organismal lifespan in a number of different aging model systems. For example, it has been shown that longer-lived rodent species have intrinsically higher SIRT6 activities and that these differences confer more efficacious DSB repair. However, it remains unclear whether enhanced SIRT6 activities in longer-lived organisms are primarily due to cross-species differences in intrinsic enzymatic efficiencies or differences in SIRT6 abundance. Accurate quantitation of SIRT6 protein levels across species has been hampered by its low abundance, low sequence homology across organisms, and challenging physical properties. We have therefore developed a mass-spectrometry based workflow that is capable of accurately quantifying SIRT6 protein levels across a large number of diverse biological samples. Thus far, we have calculated the steady-state abundance of SIRT6 to be ~20,000 molecules per nucleus of human skin fibroblasts. We plan to use this mass spectrometric approach to comprehensively quantify SIRT6 protein levels across 20+ mammalian species' skin fibroblasts. Furthermore, we will determine the relative contributions of transcriptional, translational, and post-translational regulation on SIRT6 protein levels across the analyzed mammalian species. We will accomplish this by comparing the gene expression (qPCR), translational efficiency (polysome profiling), and turnover kinetics (dynamicSILAC) of SIRT6 for each species. Future studies will expand these analyses to knockdowns of endogenous proteostasis machinery, specific post-translational modifications that may influence the stability of SIRT6, and human cancer cell lines (e.g. glioblastoma) that have been shown to have altered SIRT6 protein levels. Taken together, we have developed a powerful targeted proteomics workflow capable of comprehensively analyzing the expression landscape of SIRT6 across a large number of species and cell types. We believe that the regulatory mechanisms identified through this study will yield valuable and actionable therapeutic strategies for treating age-related

diseases by rationally altering the cellular expression levels of SIRT6.