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The atypical Alpha Kinase 3 regulates sarcomeric protein metabolism in cardiomyopathy

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Muscle contraction is driven by tightly regulated interactions between sarcomeric proteins. While the constituent components of the sarcomere are well characterized, it is less clear how the activity and turnover of sarcomeric proteins is regulated. Muscle contraction is regulated strongly by phosphorylation cascades which converge upon sarcomeric to modulate their activity. Thus, identification of regulatory kinases is key to understanding sarcomere function. *Alpha kinase 3* (*ALPK3*) is an atypical kinase that is associated with cardiomyopathy and musculoskeletal disease, but little is known about its underlying biology or role in muscle pathology.

Here, using human pluripotent stem cells (hPSCs), we demonstrate that ALPK3 is a novel component of the M-Band of the sarcomere and define the ALPK3-dependent phosphoproteome. ALPK3 deficiency disrupted sarcomeric organization and calcium handling in hPSC-derived cardiomyocytes and reduced contractile performance in cardiac organoids. Phosphoproteomic profiling identified ALPK3-dependant phospho-peptides that were enriched for sarcomeric components of the M-band and the ubiquitin-binding protein SQSTM1. Analysis of the ALPK3 interactome confirmed binding to M-band proteins as well as protein quality control regulators, including SQSTM1. Importantly, in hPSC-derived cardiomyocytes modeling *ALPK3* deficiency and cardiomyopathic *ALPK3* mutations, sarcomeric organization and M-band localization of SQSTM1 were abnormal. These data suggest ALPK3 has an integral role in maintaining sarcomere integrity and protein turnover in striated muscle. We propose this mechanism may underly disease pathogenesis in patients with *ALPK3* variants.