Abstract

The human centrosome is a vital component in cellular function. It is important for microtubule cytoskeleton morphology, as a base for primary cilium formation, and it is involved in signaling processes important for the cell cycle regulation, development, and in physiology. Dysregulation of the centrosome and centrosomal processes are implicated in not only cancer but several diseases and cellular dysfunctions. This has sparked great interest in a better understanding of centrosome functions and how they are regulated. One important aspect of the centrosome is centriole duplication, which is restricted to once per cell cycle. The master regulator of centriole duplication is Plk4, and identification of novel Plk4 substrates and phosphorylation sites can lead to a better understanding of this process. In this study, centrosome phosphorylation and especially centriole duplication was investigated by using an unbiased approach based on quantitative phosphoproteomics to identify novel Plk4 phosphorylation sites and substrates. Using this strategy, we identified 933 phosphorylation sites on centrosomal proteins, among which close to 40% were novel, and we show that several of these are regulated in a Plk4 dependent manner. We investigated the application of different CRISPR techniques to clarify the best setup for functional investigation of candidate phosphorylation sites, where knockin with homology-directed repair was found to be the most successful in this study. Cep152 Ser was identified as being regulated in a Plk4 dependent manner, and was mutated into a phospho-mimic and a phospho-dead mutant, where the phospho-dead mutant showed increased protein level of Plk4 at the centrosome.