Redox signaling, the translation of an oxidative intracellular environment into a cellular response, is mediated by the reversible oxidation of specific cysteine thiols. The latter can result in disulfide formation between protein (hetero)dimers that alter protein function until the cellular redox state has returned to basal. We have previously shown that this mechanism plays an important role in the regulation of localization and activity of the FOXO4 transcription factor [1,2]. Here, we present evidence that the human FOXO transcription factor family members FOXO3 and FOXO4 have acquired paralog-specific functional cysteines through vertebrate evolution. Using a quantitative mass spectrometry based proteome-wide screen for disulfide dependent protein complexes that we recently developed we identified previously unknown redox-dependent FOXO interaction partners, some of which are selective for FOXO3. Of these, the nuclear import receptor IPO7 forms a disulfide-dependent heterodimer with a specific cysteine in FOXO3 (but not with FOXO4), which is required for ROS-induced nuclear translocation of FOXO3. Indeed, we can make FOXO4 also bind to IPO7 by introduction of the cysteine at the homologous position. These findings suggest that evolutionary acquisition of cysteines could contribute to functional divergence of FOXO paralogs. In a broader perspective, our data indicate that phylogenetic analysis of cysteine acquisition of cysteines in unstructured protein regions can predict a potential role in redox signaling.