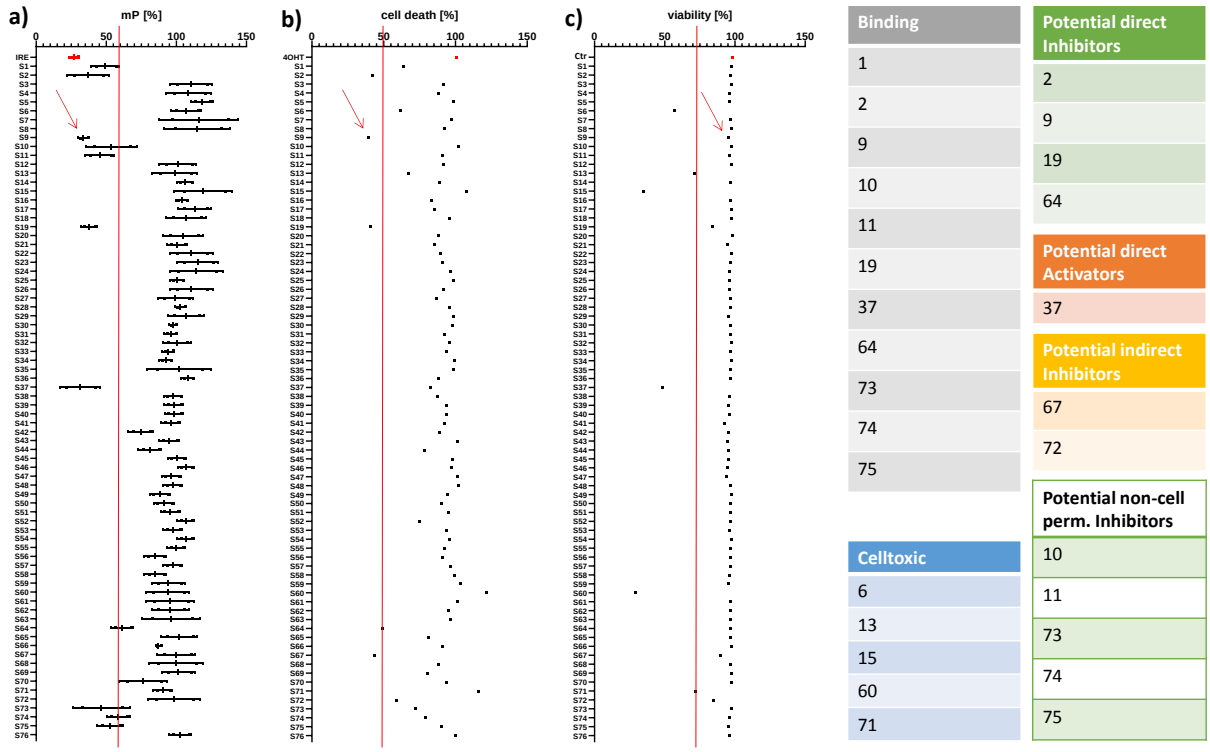

Figure 1 – figure supplement 1: Overview of combined fluorescence polarization assay (FPA)- and live/dead flow cytometry-based validation of candidate compounds. FPAs were performed with 125 nM FOXO3-DBD and 25 nM FAM-labeled IRE-oligonucleotide as described in materials and methods. Compound concentration was 1 µM. mP value of freely rotating IRE-FAM oligonucleotide was subtracted. Threshold for FPA was set to 60% mP value reduction (a). For flow cytometry, SH-EP/FOXO3 cells were treated with 20 nM 4OHT and 20 µM of each compound for 48 hours. Apoptotic cells were detected using propidium-iodide staining of fragmented nuclei. Threshold for cell death inhibition was set to 50% of FOXO3-induced cell death (b), threshold for viability/toxicity was set to 75% of DMSO-treated controls (c). From those compounds that reduced mP in the FPA, S2, S9, S19 and S64 demonstrated a clear death-inhibitory effect in the conditional FOXO3 system suggesting that these are "Potential direct inhibitors" of FOXO3, S37 induced cell death and triggered FOXO subcellular shuttling (data not shown) suggesting that this compound triggers FOXO3 activation ("Direct activator"). Compounds not affecting cellular FOXO3 effects (S10, S11, S73, S74, S75) despite mP reduction in the FPA apparently do not transit the cytoplasm membrane. Compounds S67 and S72 did not demonstrate significant effects in the FPA, but inhibited cell death induction by ectopic FOXO3, suggesting that these compounds rather inhibit FOXO3-induced cell death indirectly in this system ("Indirect Inhibitors").