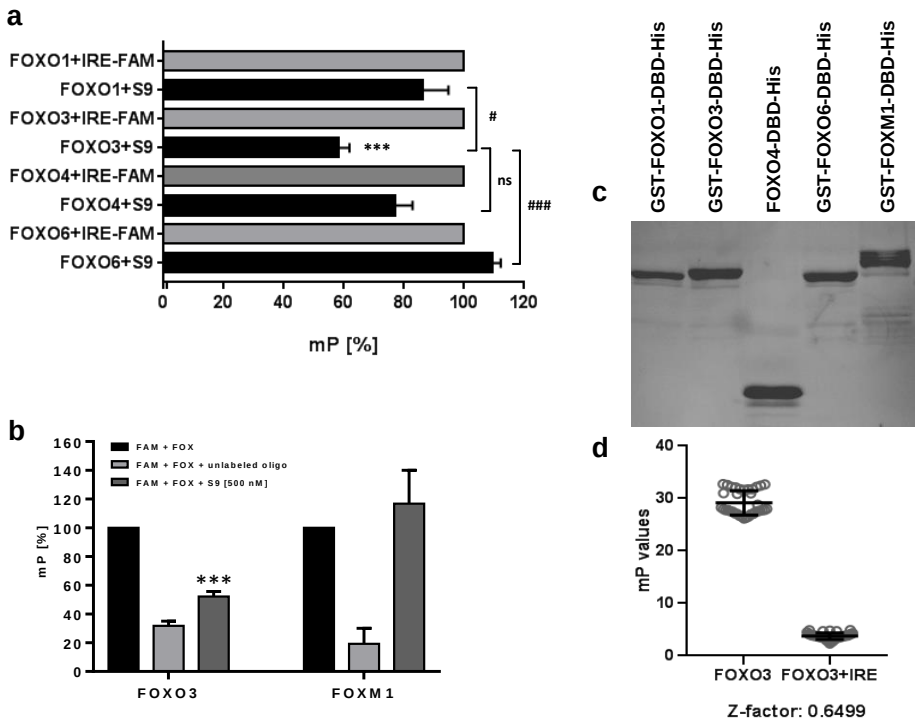

Figure 1 – figure supplement 2: Effect of S9 on protein-DNA interaction of different FOX family members. Recombinant FOXO1-DBD (159 - 272) FOXO4-DBD (82-207) and FOXO6-DBD (87 - 200) were prepared as described for FOXO3-DBD in materials and methods. FPAs to measure binding of FAM-labeled IRE oligonucleotide (25 nM) to recombinant FOXO1-DBD, FOXO3-DBD, FOXO4-DBD and FOXO6-DBD (each 125 nM) were performed as described. A final concentration of 250 nM of S9 was used for all FOXO proteins. mP value of freely rotating IRE-FAM oligonucleotide was subtracted and the mP-value of FOXO-protein + IRE-FAM oligonucleotide complex was calculated as 100%. Shown is the mean +/- SEM of three independent experiments, statistical differences between FOXO3 +/- S9 or between FOXO1+S9 *versus* FOXO3+S9 or FOXO3+S9 *versus* FOXO6+S9 were assessed with unpaired student's t-test. FOXO3 +/- S9: ***p<0.001, FOXO1+S9 *versus* FOXO3+S9: $^{\#}$p<0.05, two-tailed, FOXO6+S9 *versus* FOXO3+S9: $^{\#\#\#}$p<0.001, two-tailed, (a). An FPA using recombinant FOXM1-DBD (222-360) with the FAM-labeled oligonucleotide TTT GTT TAT TTG TTT GTT TAT TTG was established to test efficacy of S9 on the DBD of this non-FOXO family protein. FPAs of FOXO3 (with IRE-FAM oligonucleotide) and FOXM1 were performed in parallel with a concentration of 500 nM S9 (b) controls were 100fold excess of unlabeled oligonucleotide. Whereas S9 inhibitory effect on FOXO3-DBD FPA was clearly visible, no effect was observed on FOXM1-DBD FPA suggesting that S9 does not interfere with FOXM1-DBD –DNA interaction. Shown is the mean of three independent experiments (student`s t test, ***p<0.001). Recombinant FOXO1-DBD, FOXO3-DBD, FOXO4-DBD, FOXO6-DBD and FOXM1-DBD were separated on PAGE and Coomassie-stained (c). 36 FOXO3-DBD FPAs were performed with FOXO3-DBD (125 nM) and FAM-IRE-oligonucleotide (25 nM) in presence or absence of a 100fold excess of unlabeled IRE-oligonucleotide and Z´factor was calculated using the formula $Z' = 1-(3\sigma_+ + 3\sigma_-)/(\mu_+ - \mu_-)$. μ_+, σ_+ and μ_-, σ_- are mean values and standard deviations for the high and low controls (+ unlabeled IRE oligonucleotide), respectively. The analysis provided a Z´factor of 0.6499 which proves that the FPA is reliable for high-throughput screening (Z´> 0.5).