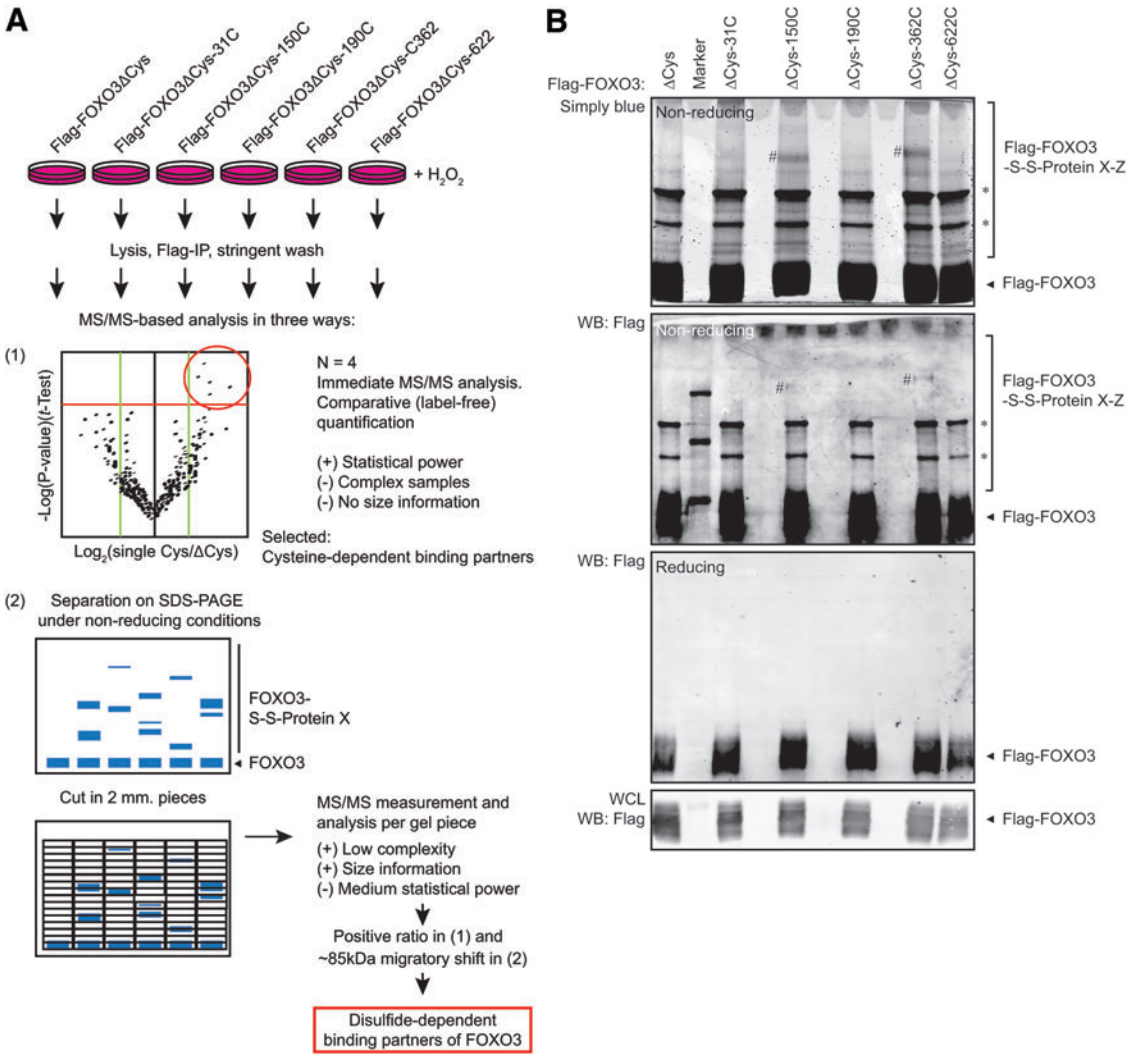

SUPPLEMENTARY FIG. S4. Identification of FOXO3 cysteine-disulfide-dependent binding partners. (A) Schematic representation of the setup of the screen. HEK293T cells overexpressing different Flag-tagged FOXO3 cysteine mutants were incubated with H_2O_2 (200 μM) before lysis to induce disulfide formation. Immunoprecipitation (IP) was performed using anti-Flag beads in a buffer containing an excess of the Cys-directed alkylator iodoacetamide (to prevent postlysis oxidation and to inactivate Thioredoxin) and stringently washed. Cysteine-disulfide-dependent binding partners were identified by two different MS/MS-based approaches as depicted by (1) and (2) in the figure. **(B)** Actual result of the experiment depicted in **(A)**. The upper panel shows the nonreduced IPs after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) separation and simply blue staining (90% of the sample), the middle panel shows a Western blot of the other 10% of the nonreduced sample stained for Flag-FOXO3. Both show that FOXO3 displays several cysteine-dependent (and specific) apparent mass shifts, suggesting the existence of FOXO3-containing cysteine-disulfide-mediated complexes. The clearest examples are marked by a #. Bands marked with asterisk represent (nonreduced) antibody chains.