

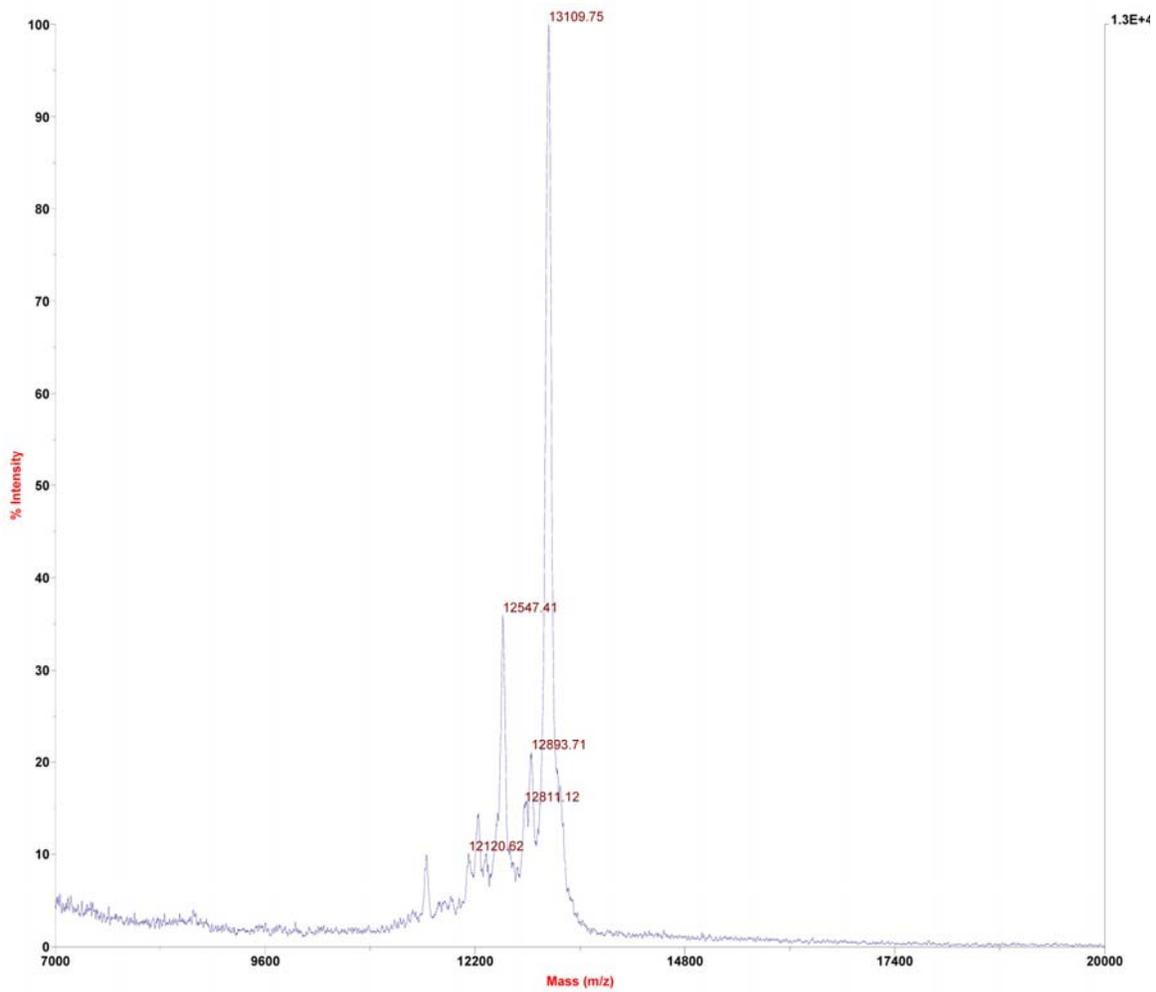
Structure 16

Supplemental Data

Structural Basis for DNA Recognition by FoxO1 and Its Regulation by Posttranslational Modification

Michael M. Brent, Ruchi Anand, and Ronen Marmorstein

A



B

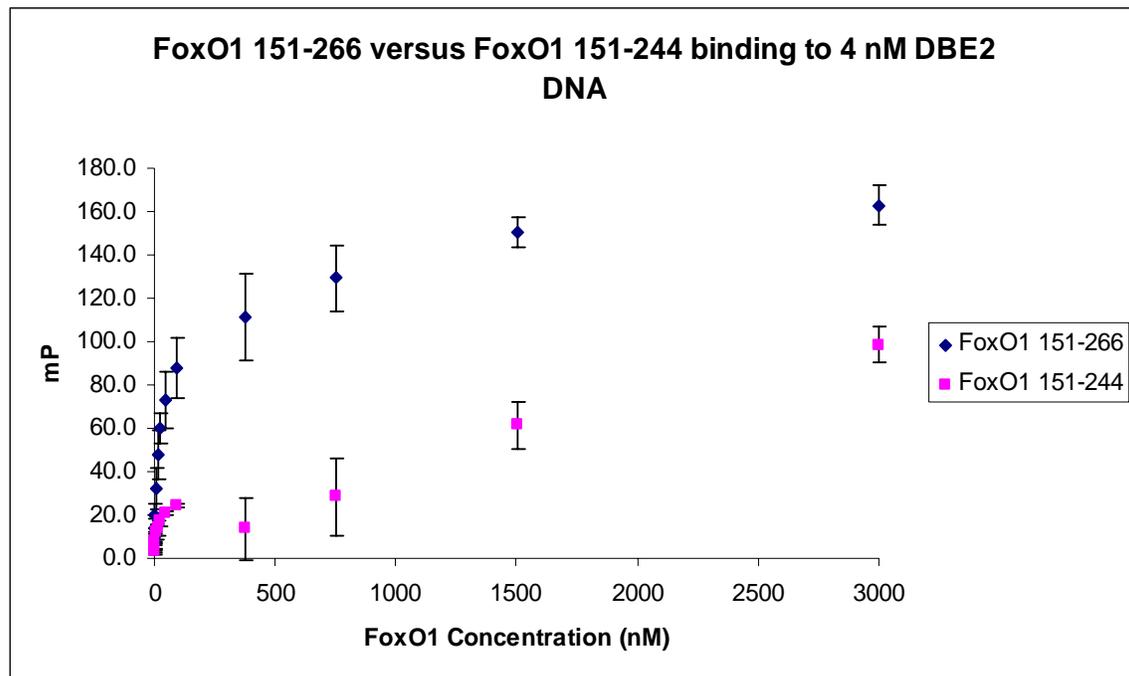
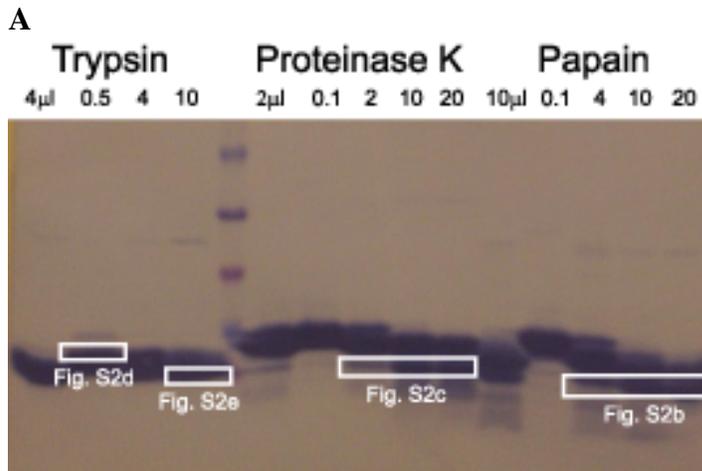


Figure S1. TOF Mass Spectrometry Results for FoxO1 151-266/IRE DNA Crystals Dissolved in Water

The vector encodes an additional serine on the N-terminus for a total predicted mass of 13130.7 Da (A). Fluorescence polarization results confirming the EMSA finding that FoxO1 151-244 has severely reduced DNA affinity compared with FoxO1 151-266 (B). Measurements were taken for a 3000 nM to 0.4 nM concentration range of FoxO1 binding to 4 nM 5' fluorescently labeled DBE2 DNA using a BEACON 2000 variable temperature fluorescence polarization system. Samples were allowed to equilibrate for 30 minutes in buffer containing 20 mM HEPES pH 7.5, 50 mM NaCl, 2 mM β -mercaptoethanol, and 0.1 $\mu\text{g}/\mu\text{L}$ BSA before measurement. Measurements were done in triplicate.



B S07046 Papain

Cycle #	1 ^o	2 ^o	3 ^o	4 ^o
1	S 35	N 9	S 7	S 0.5
2	K 60	E 7.5	S	V 0.4
3	S 25	G 5.8	R 5	P 0.75
4	S	T 4.8	R 4.5	Y 1
5	S	G 4	N 14	F 0.8
6	S	K 5	A 15	K 0.5
7	R 10.5	S	W 5.7	D 0.7
8	R 6.5	S	G 8	K 0.6

1^o=SKSSSR
 2^o=NEGTGKSS
 3^o=SSRRNAWG
 4^o=SVPYFKDK

C S07047 Proteinase k

Cycle #	1 ^o	2 ^o	3 ^o
1	S 14	S 0.7	
2	K 25	S	
3	S 9	R 1.4	
4	S	R 1	
5	S	N 1.9	
6	S	A 2	
7	R 6.8	W 0.2	
8	R 6.5	G 0.9	

1^o=SKSSSR
 2^o=SSRRNAWG
 3^o=none

D**S07049 Trypsin Upper**

Cycle #	1 ^o	2 ^o	3 ^o
1	S 30		
2	K 61		
3	S 25		
4	S		
5	S		
6	S		
7	R 15		
8	R 12		

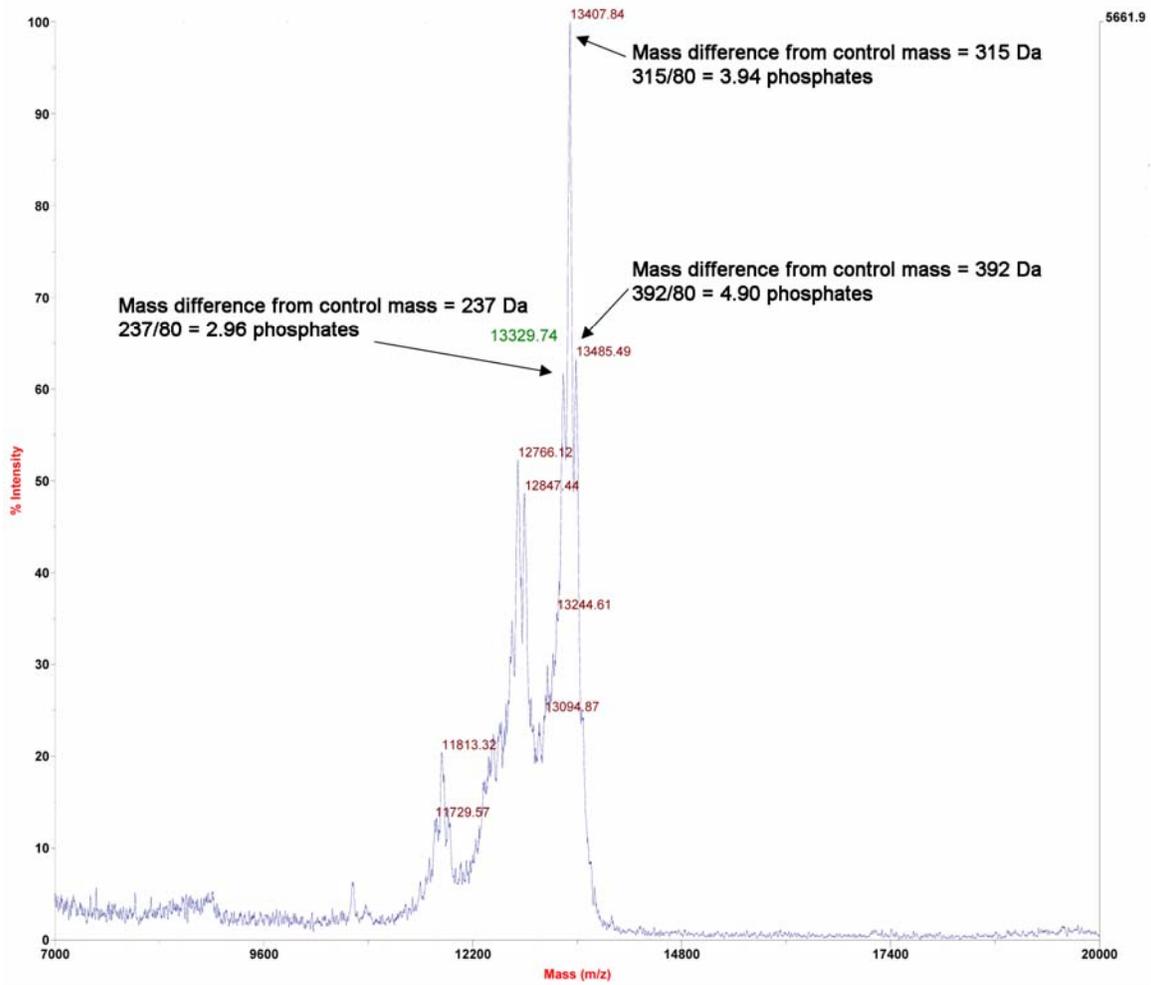
1^o=SKSSSSRR2^o=none3^o=none**E****S07048 Trypsin lower**

Cycle #	1 ^o	2 ^o	3 ^o	4 ^o
1	N 90	R 4	S 1	A 0.5
2	A 95	N 7	V 1.3	W 0.45
3	W 60	A 8	P 1.6	G 0.5
4	G 61	W 5	Y 1	N 0.45
5	N 70	G 6.5	F 0.7	L 1.1
6	L 75	N 7	K 1.4	X
7	S 33	L 8	X	Y 0.6
8	Y 60	S 4	K 0.6	A 0.4

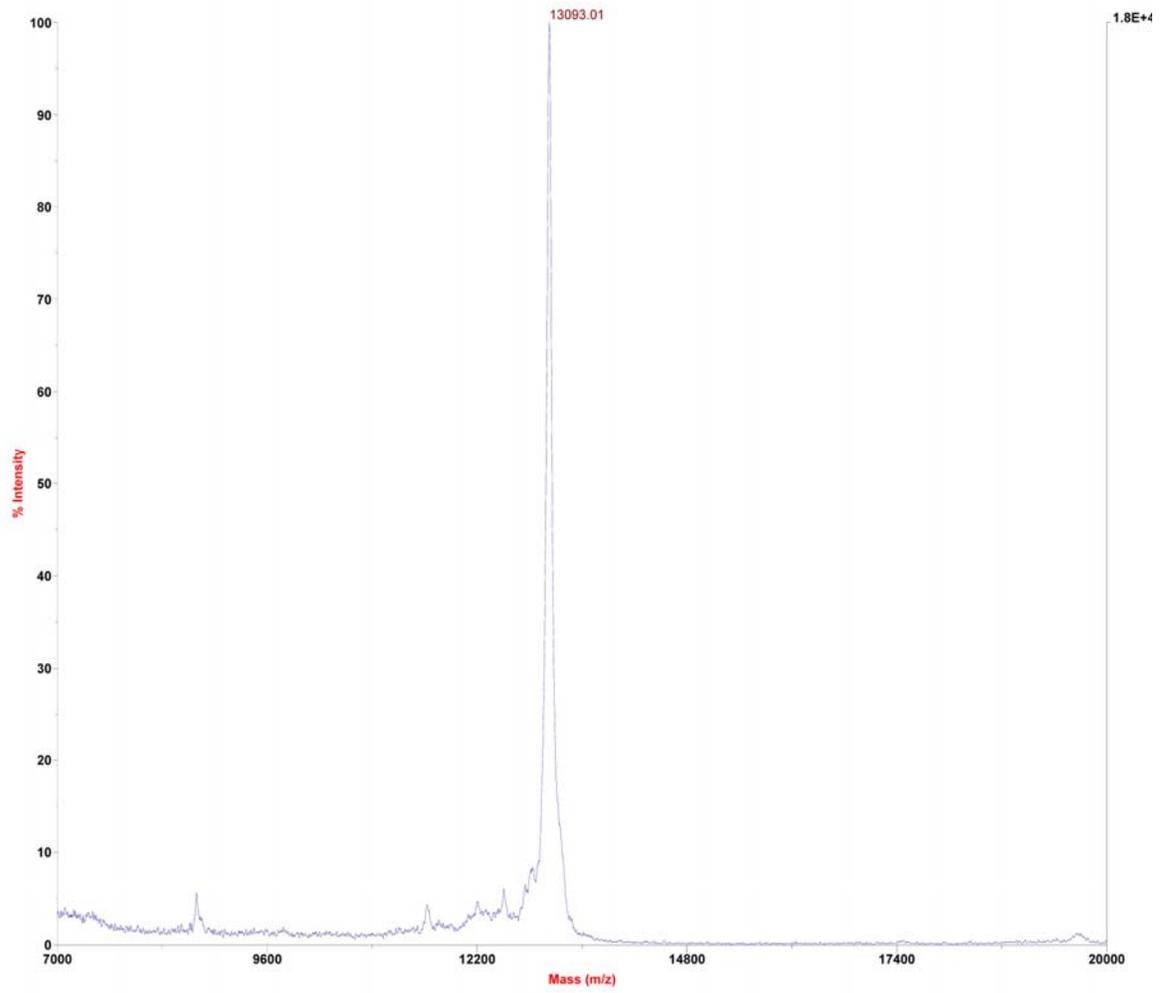
1^o=NAWGNLSY2^o=RNAWGNLS3^o=SVPYFKKK4^o=AWGNLXYA**Figure S2. Results for FoxO1 151-266 Digested in the Presence of a 3-Fold Molar Excess of DBE2 DNA**

Digestion products separated by SDS PAGE, electroblotted onto a PVDF membrane and stained with Amido Black (A). N-terminal sequencing report for papain product (B), proteinase K product (C), trypsin larger product (D), trypsin smaller product (E). The SKSSSSRR sequence represents the first eight residues of the FoxO1 DBD construct. The number immediately following the amino acid designation is the approximate background-corrected yield for that residue in the cycle noted. There is a very strong carryover of S, so accurate yields could not be assigned for SSSS sequences.

A



B



C

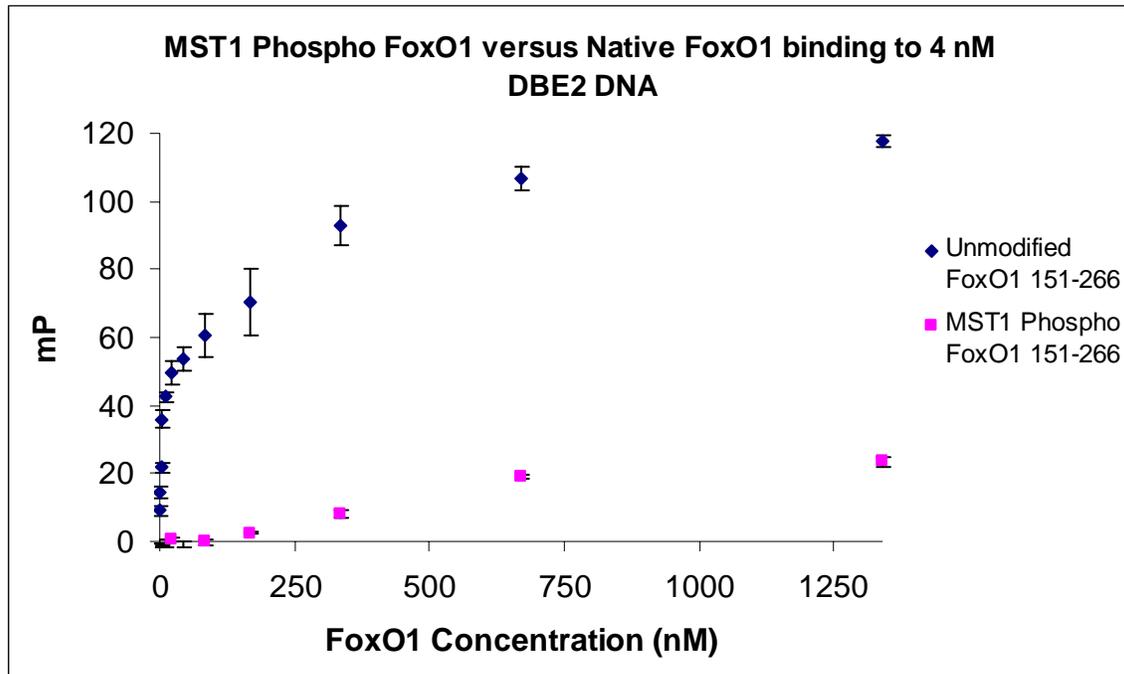
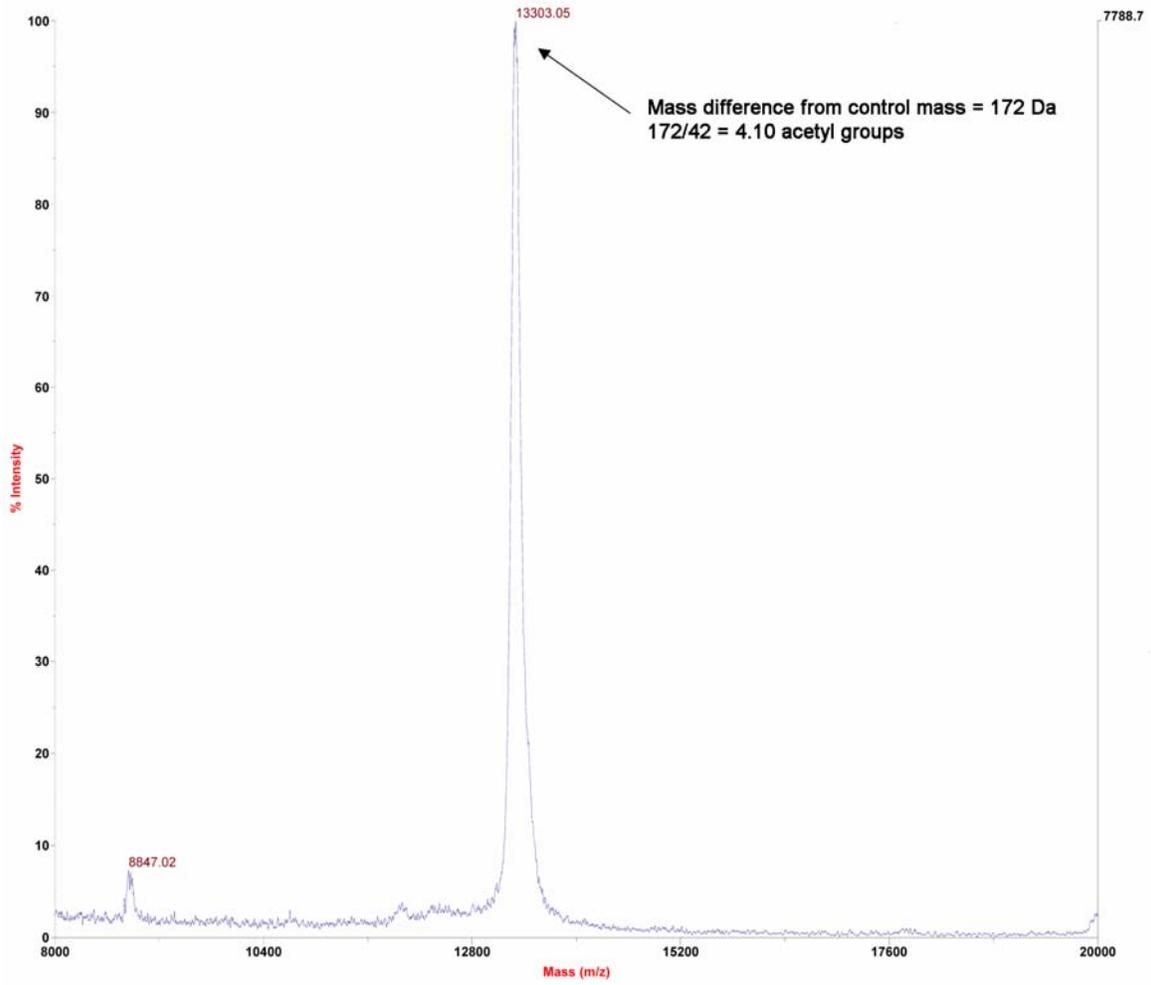
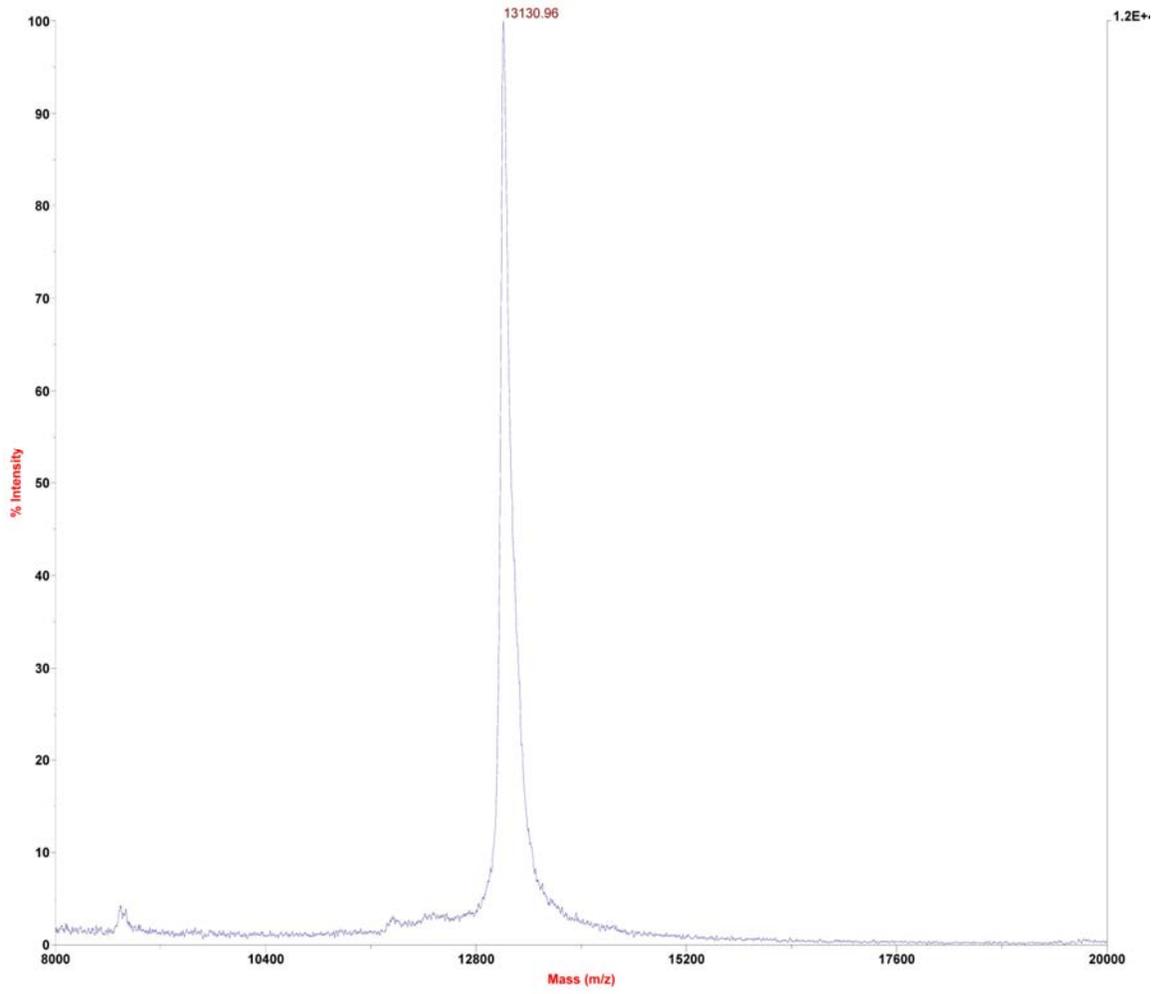


Figure S3. TOF mass spectrometry results for MST phosphorylated FoxO1 DBD (151-266) (A), a control FoxO1 DBD (151-266) that was incubated at 30 °C for 4 hours in the absence of MST or ATP (B), and fluorescence polarization results confirming the EMSA finding that MST1 phosphorylation of FoxO1 nearly eliminates DNA binding (C). Measurements were taken for a 1340 nM to 0.7 nM concentration range of FoxO1 binding to 4 nM 5' fluorescently labeled DBE2 DNA using a BEACON 2000 variable temperature fluorescence polarization system. Samples were allowed to equilibrate for 30 minutes in buffer containing 20 mM HEPES pH 7.5, 50 mM NaCl, 2 mM β -mercaptoethanol, and 0.1 μ g/ μ L BSA before measurement. Measurements were done in triplicate.

A



B



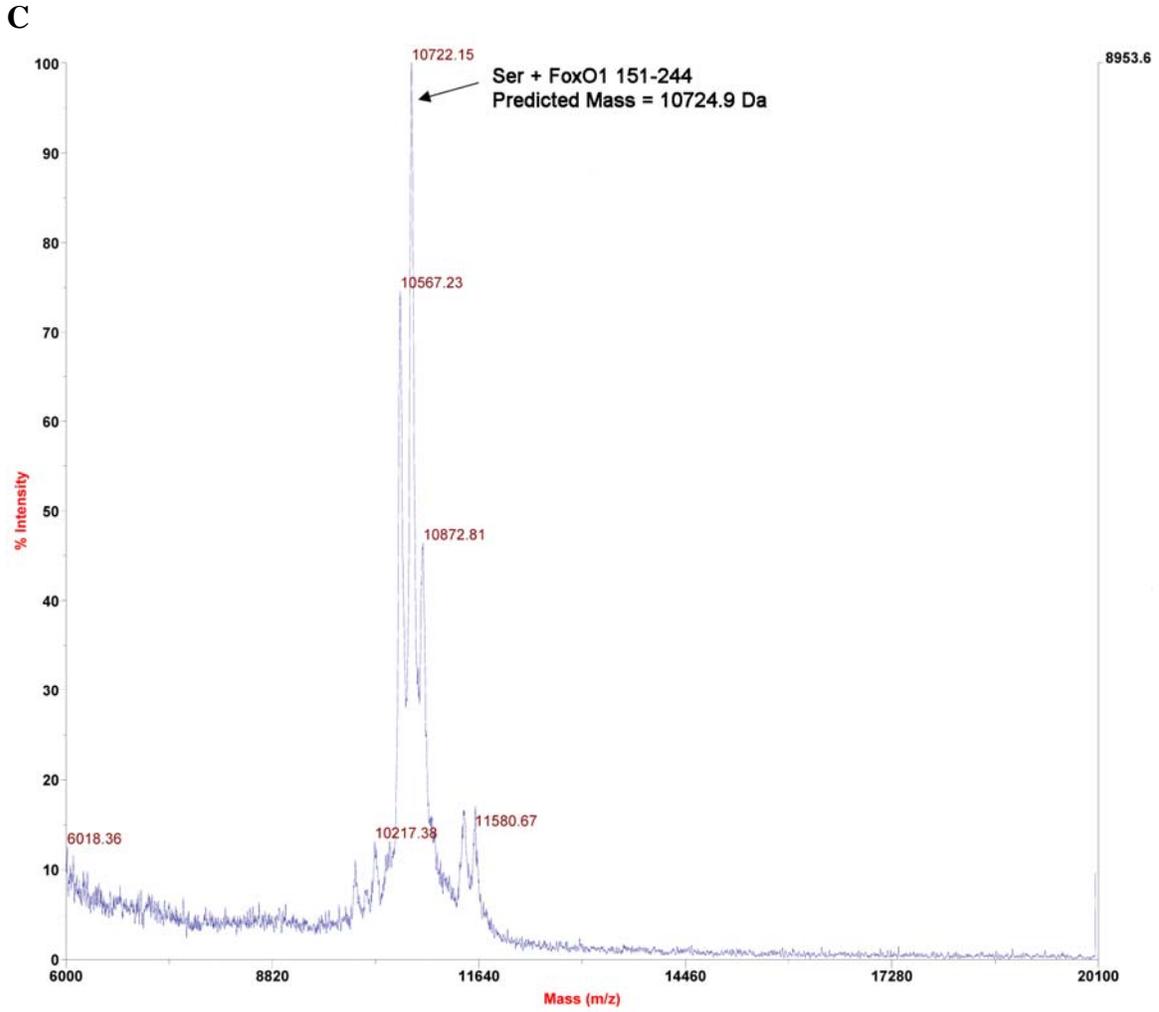
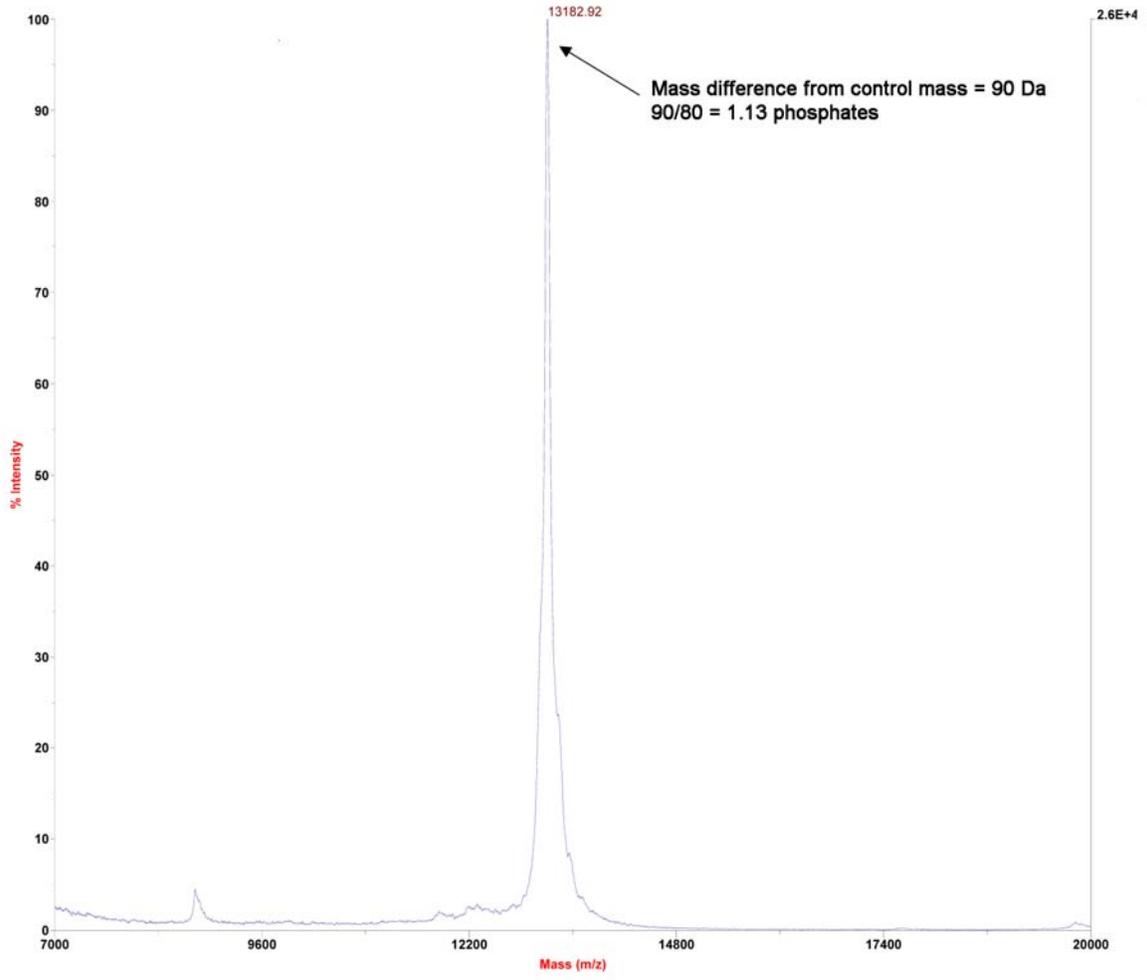


Figure S4. TOF mass spectrometry results for p300 Acetylated FoxO1 DBD (151-266) (A), a control FoxO1 DBD (151-266) that was incubated at 30 °C for 3 hours in the absence of p300 (B), and an acetylated FoxO1 DBD (151-266) that has been digested with papain that indicates a C-terminally truncated product of unacetylated FoxO1 (151-244) (C).

A



B

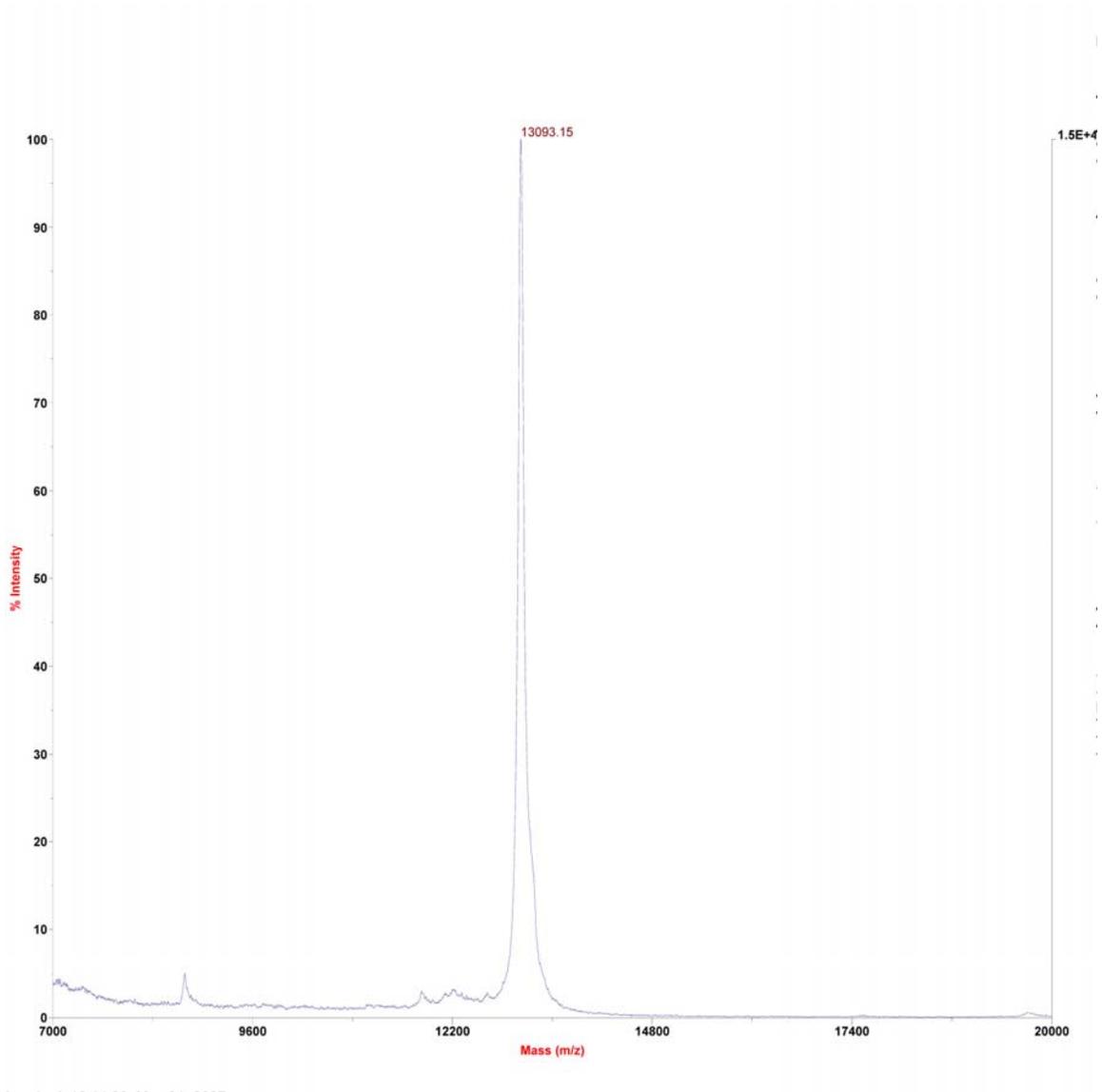


Figure S5. TOF mass spectrometry results for Akt phosphorylated FoxO1 DBD (151-266) (A) and a control FoxO1 DBD (151-266) that was incubated at 30 °C for 3 hours in the absence of Akt (B).