

Figure S1. Multiple sequence alignment of human forkhead domain. The protein sequences were aligned using Clustal Omega webserver (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The alignment was presented by Jalview. Residue color coding: blue, hydrophobic residues; red, positively charged residues; magenta, negatively charged residues; green, polar residues; orange, glycines; yellow, prolines; cyan, aromatic residues; and white, unconserved residues.

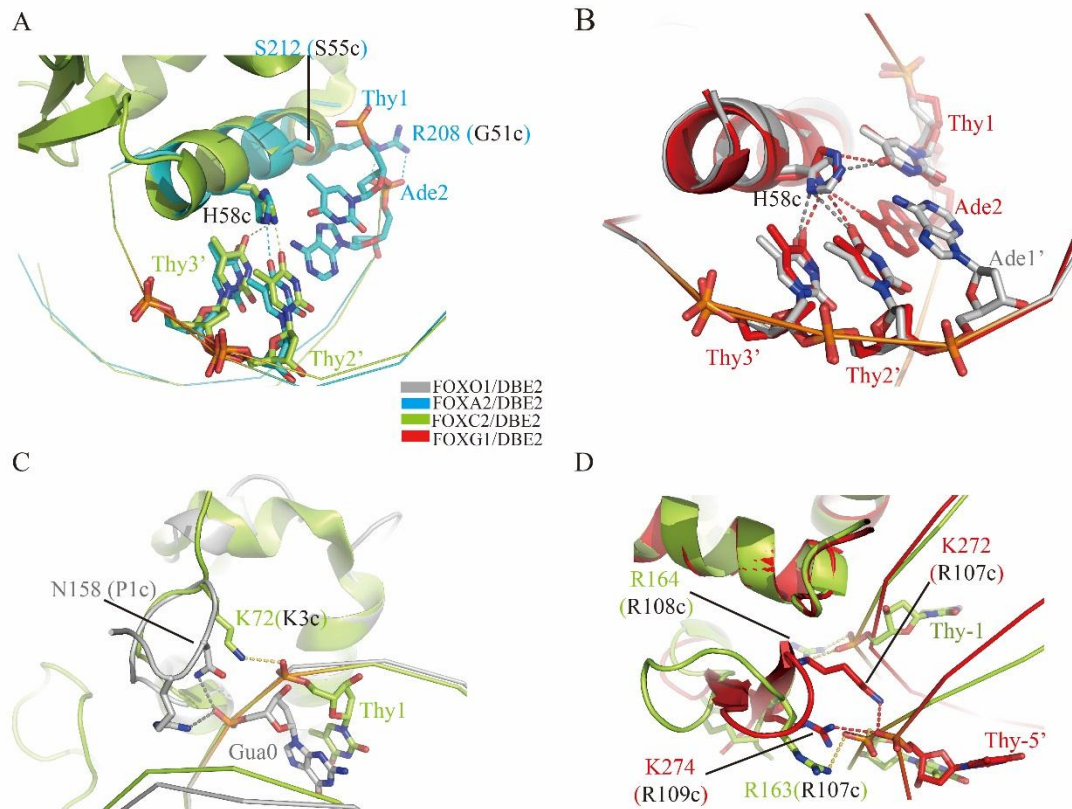


Figure S2. Comparison of different FOX DBDs binding to the same DNA site. (A) H3 helix-DNA contacts in the FOXA2 (cyan) and FOXC2 (green) structures. (B) H3 helix-DNA contacts in the FOXO1 (gray) and FOXG1 (red) structures. (C) protein-DNA interaction in the N termini of FOXO1 and FOXC2 DBDs. (D) DNA recognition of the FOXC2 and FOXG1 wing2 regions. Corresponding residues in the FKH consensus are shown in Parentheses.

Table S1. List of identified DBD mutations of selected FOX proteins.

Protein	Mutation	PubMed ID	COSMIC accession number
FOXA1	P171S	28481359, 28825054	COSV51647442
	S174L	N/A	COSV99163462
	Y175C	30205045	COSV51647165
	I176V/M	29807833, 30537512	COSV51644739, COSV51646232
	P205L	26214590	COSV51649162
	Y207H	N/A	COSV99163432
	R213H/C	27103403	COSV51649232, COSV99163750
	W214C	30181556	COSV51646120
	S217C	31243370	COSV99163604
	R219S/C	24823478, 26542946	COSV51647749, COSV51646097
	H220Q	N/A	COSV99163516
	F224L	27148695	COSV51645367
	D226G/N/V/E	22610119, 27135926	COSV51643222
	V229I	N/A	COSV51646752
	V231A	N/A	COSV99163547
	A232V/S	22610119	COSV99163540
	K237R	N/A	COSV99163230
	P238S	N/A	COSV104565547
	G239S	29807833	COSV99163640
	G241S	27890785	COSV51644269, COSV51645075
	Y243S	N/A	COSV51644631
	H247Q/R/Y	26000489	COSV51645490, COSV51644535
	D249G/V	30181556	COSV51643974
	S250F/Y	30531871, 30205045	COSV51644190, COSV51646200
	M253K	22610119, 26542946	COSV51647482
	F254V/S/L	26000489, 30181556	COSV51644341, COSV51647202
	Y259C	26000489	COSV51644341
	R261G/C	26928463, 30181556	N/A
F266C/S/L	27103403	N/A	
FOXC1	P79L/T/R	14506133	N/A
	S82T	11179011	N/A
	A85T/P	32631953	COSV66515893
	L86F/P	14578375	N/A
	I87M	11179011	N/A
	I91T/S	14506133	N/A
	M109V/I	29487724	COSV66516385
	F112S/I	11179011	COSV101083228
	Y115S	16936096	N/A
	I126M/S	11179011	N/A
	R127H/L	14506133	N/A
	L130F/P	17210863	COSV66516720

	S131L/W/	11179011	COSV66516570
	C135Y/R	15277473	COSV66516241
	K138E/N	29487724	COSV101083299
	G149D	32631953	COSV101083387
	W152G	19279310	N/A
	M161K/V	15277473	N/A
	G165R	15277473	N/A
	R169P/W	15277473	COSV66515642
	R170W	32631953	N/A
FOXG1	K181N	26938784, 25741868	N/A
	P183L	26804919	COSV57397623
	F184L	N/A	COSV57399493
	S185T/I	25741868	N/A
	N187K	25356970	N/A
	A188V/T	23525077, 22895190	COSV57395156, COSV57398945
	A193T	27267998	COSV100486832
	R195W	N/A	COSV100487048
	G206S	27149842	COSV57397070
	E209G	N/A	COSV100486593
	F210L	N/A	COSV57399812
	F215S	19578037, 18414213	N/A
	G224C	21270142	N/A
	I229L	25741868	N/A
	R230H/L	22810696, 22129046	COSV57398603, COSV5739696
	N232D/Y	25741868	N/A
	S234P	21441262	COSV57395902
	L235F	26168399	COSV57397834
	C238Y	N/A	COSV57397792
	V242M	25344691	COSV57399961
	R244S	25741868	COSV57398832
	G252V	18414213	N/A
	N253D	21441262, 18414213	N/A
	F265L	26873401	COSV57400212
	G267S	18414213	COSV100487141
	G271D	N/A	COSV57397176
R274Q/W	N/A	COSV57395324	
R275H/P	26071483, 27149842	COSV57394796	
FOXP3	R337Q	18931102	COSV66052168
	P339A	18931102	N/A
	A343T	11137992	N/A
	R347H	18951619	COSV66051279
	I363V	11768393	N/A
	H365N/Q	27149842	COSV100873129

	F367L/C	18820676	N/A
	R369C	30709738	COSV100873061
	M370L	21595732	N/A
	F371C	11137992	COSV100873291
	F373A/V	28778586	N/A
	F374Y/V	28296713	COSV66051770
	T380I	18316354	N/A
	A384T	11137992	N/A
	R386H	20650610	COSV105319177
	S390N	16371377	N/A
	R397W/Q	11295725	COSV66050988
	V405G	N/A	COSV100873195
	V408M	18931102	N/A
	D409G	16990602	N/A
	L411P	N/A	COSV100873211
	R417W	27149842	COSV66050838

N/A stands for not available.