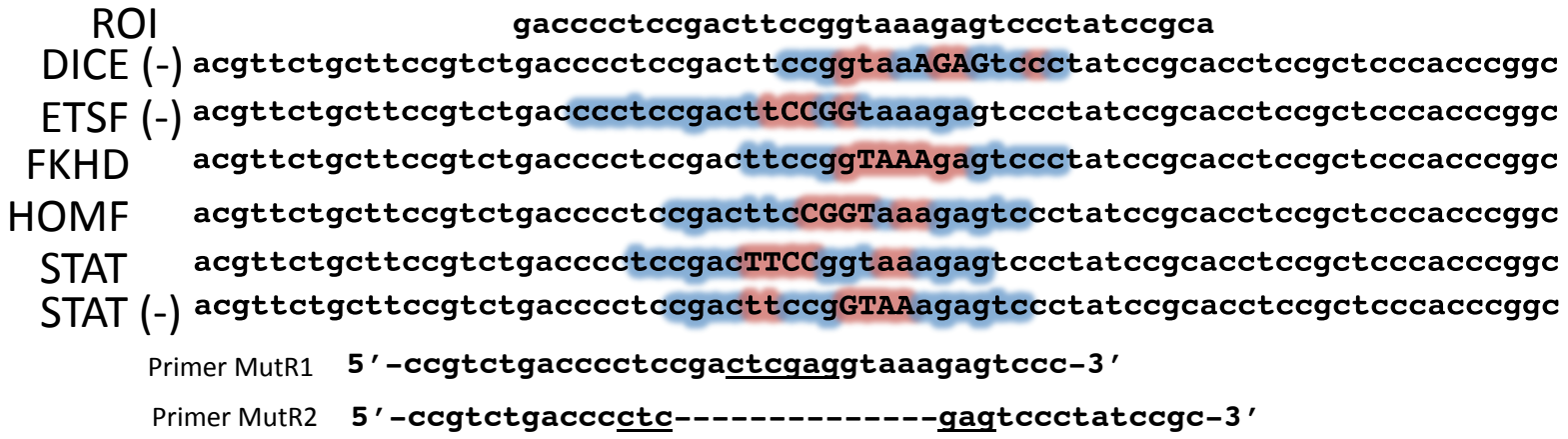
Lab Meeting
Dan
Wednesday May 4, 2011

Review of TFs acting at this ROI



- Ben inhibits ATXN2-luc (data today)
- ETS inhibit ATXN2-luc and bind this region by EMSA
- FKHD activate ATXN2-luc (most of them) (data revealed today)
- HOMF – not clear to me why this region is considered a HOMF binding site
- STAT– Do not bind this region by EMSA nor do they modulate ATXN2-luc

Homeodomain Transcription Factors

TABLE 2. *Described high-affinity binding sites of various Homeobox transcription factors*

Homeobox factor name	Homeobox factor species	High-affinity binding site described								Original description (ref.)
vnd/NK-2	*Drosophila melanogaster*	T	T/C	A	A	G	T	G	G/C	59
Engrailed	*Drosophila melanogaster*	G	T	A	A	T	G	A	C	60
OTX5	*Drosophila rerio*	N	T	A	A	G	A	C	T	61
Dlx3	*Xenopus tropicalis*	A	T	A	A	T	T	G/A	C/G	62
Xom	*Xenopus tropicalis*	C	T	A	A	T	T	A/G	G/C	63
CdxA (CTO)	*Gallus gallus*	A	T	A	A	A	T/G	N	N	64
CdxA (CTS)	*Gallus gallus*	A	T	T/A	A	T/A	T	N	N	64
CDX3	*Gallus gallus*	C	T	A	A	T	T	N	N	64
CAD	*Gallus gallus*	A	T	A	A	A	N	N	N	64
HOXA13	*Homo sapiens*	A	T	A	A	A	C/G	C/G	N	65
TGIF	*Homo sapiens*	T	C	A	A/T	T/A	A/T	C	N	66
SHOX	*Homo sapiens*	N	T	A	A	T	G	N	N	67
Homeobox	General consensus	A	T	A	A	T	T	N	N	

Georges et al., 2010.

TAAAG matches ATXN2 but not much else

5’-**cgacttcCGGTaaagagtc**

5’-CGACTTCCGGTAAAGAGTC

5’-GACTCTTTACCGGAAGTCG

Forkhead Transcription Factors

Forkheads are characterized by GTAAACA

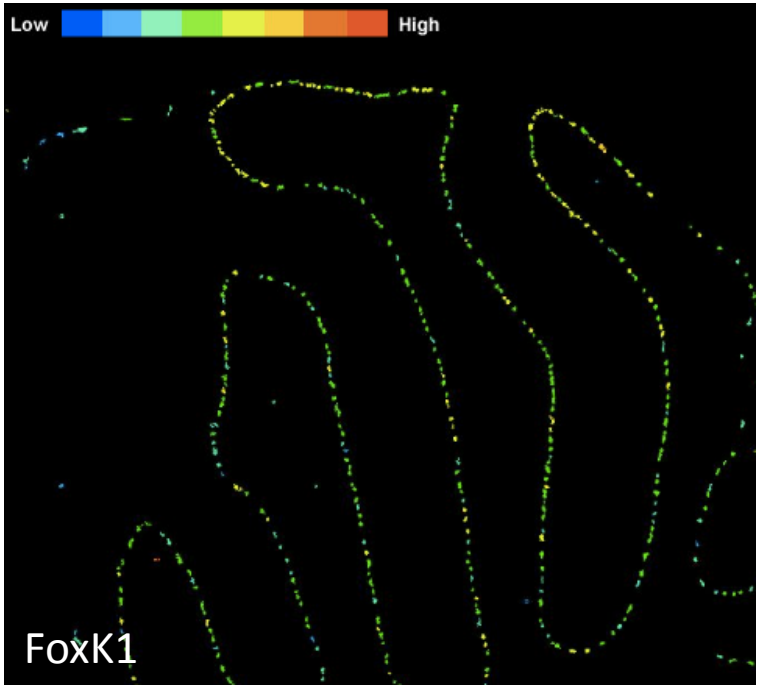
TABLE 1. *Described high-affinity binding sites of various Forkhead transcription factors*

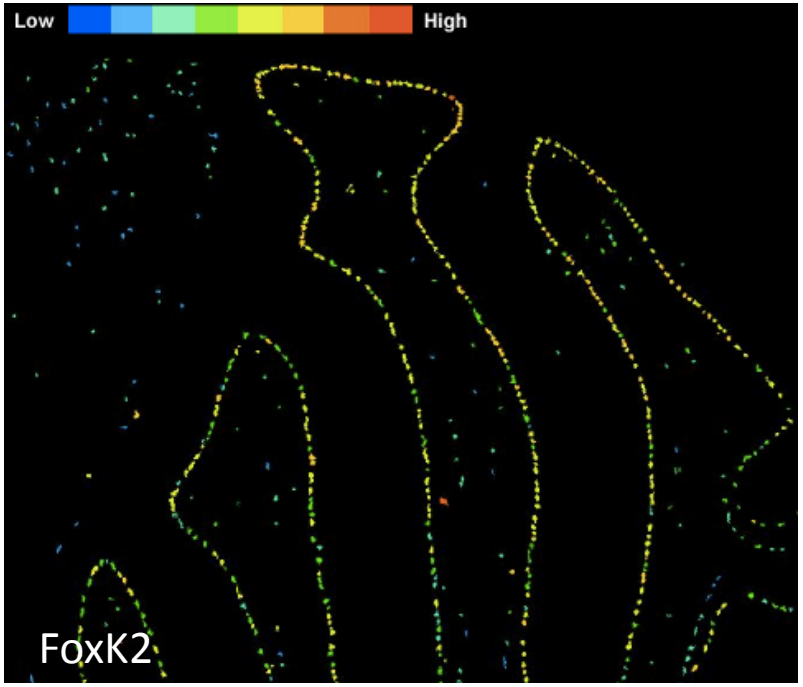
FOX factor name	Name in original description	High-affinity binding site described								Original description (ref.)
FOXF2	FREAC-2	G/A	T	A	A	A	C/T	A	A	54
FOXC1	FREAC-3	G	T	A	A	A	C/T	A	A	54
FOXD1	FREAC-4	G/A	T	A/C	A	A	C	A	N	54
FOXL1	FREAC-7	G/A	T/C	A/C	A	A	C/T	A	N	54
FOXQ1	HFH-1	A/C	T	A	A	A	C	A	A/T	55
FOXD3	HFH-2	A/T	T	A	A	A	C	A	A/T	55
FOXA3	HNF-3	G/A	T/C	A/C	A	A	C/T	A	A/T	55
FOXI1	N/A	G/A	C	C	A	A	T	C/G	A	56
FOXK2	ILF-1	G	T	A	A	A	C	A	A	57
FOXO1	FKHR	G	T	A	A	A	C	A	A	58
FOXO3A	FKHRL1	G	T	A	A	A	C	A	A	58
FOXO4	AFX	G	T	A	A	A	C	A	A	58
FOXP1	N/A	A	T/C	A	A	A	C	A	A	35
FOXL2	N/A	G	T	C/G	A	A	G	G	T	5
Forkhead	General consensus	G/A	T/C	A/C	A	A	C/T	A	N	

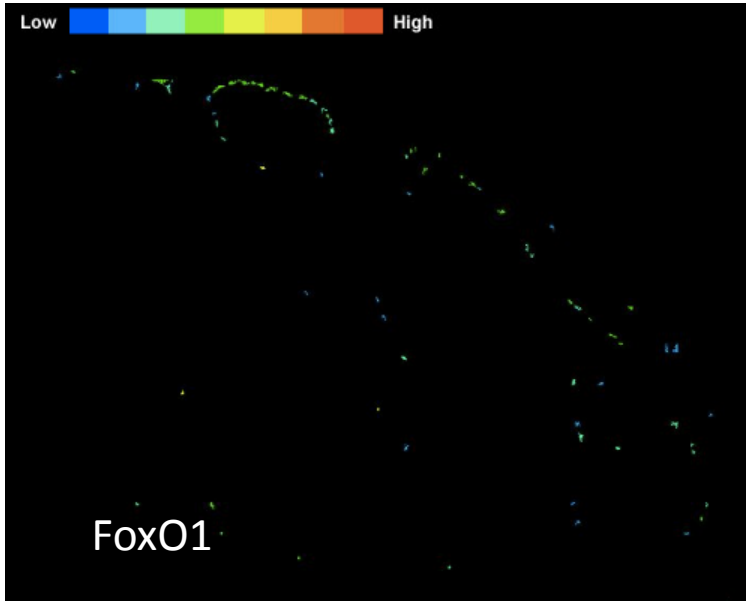
N/A, not available.

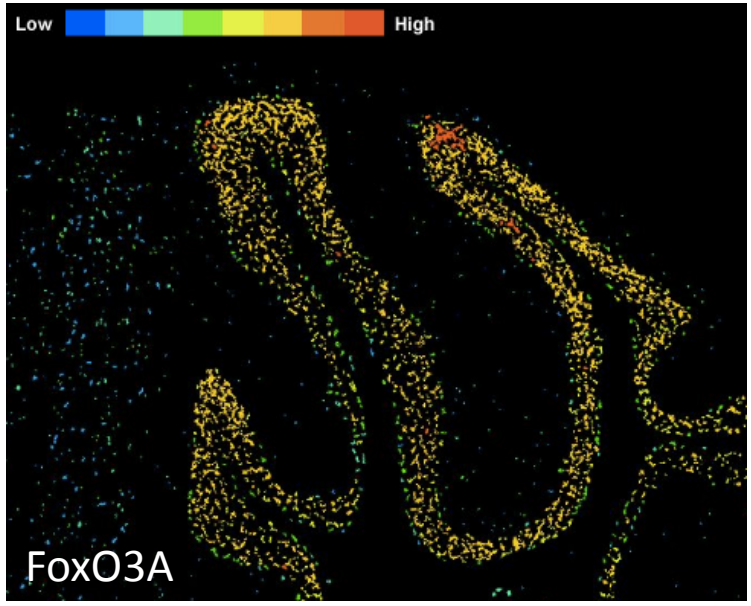
Georges et al., Generic binding sites, generic DNA-binding domains: where does specific promoter recognition come from? FASEB Journal: 24,246-56; 2010

GACCCCTCCGACTTCCGGTAAAGAGTCCCTATCCGCA	ATXN2 5’-UTR FRAG
GACCCCTCCGACTCGAGGTAAAGAGTCCCTATCCGCA	our “CGA” mutation in ETS core
GACCCCTC--------------GAGTCCCTATCCGCA	our “14bp del” mutation
GACCCCTCCGACTTCCGGTAAAGAGTCCCTATCCGCA	Forkhead prediction
GTAAACAN	Forkhead Consensus
GTAAACA	FoxK1
GTAAACA	FoxK2
GTAAACA	FoxO1
GTAAACA	FoxO3A
GTCAAGG	FoxL2







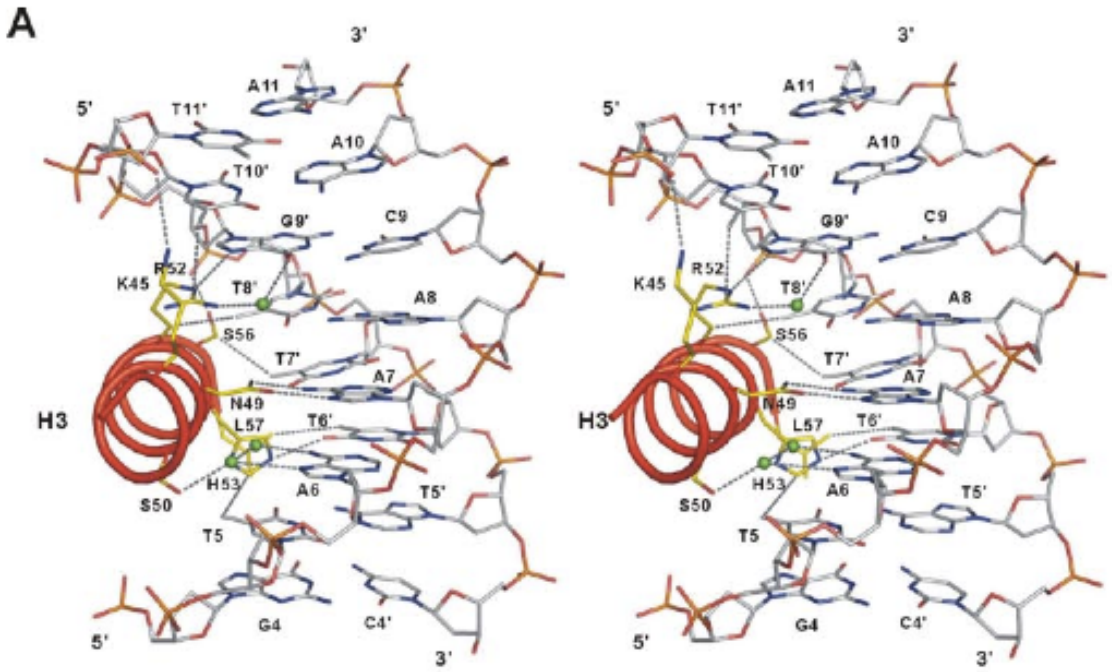


No data for FoxL2

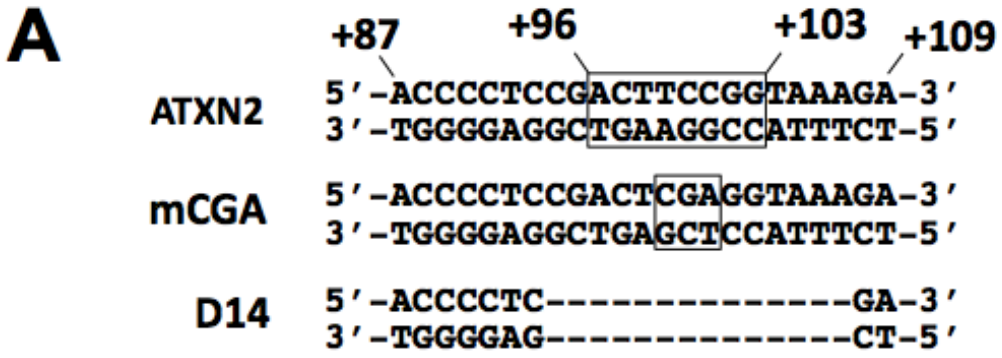
Forkhead box, 80-100 aa binding DNA
aka winged helix or helix turn helix (HTH)

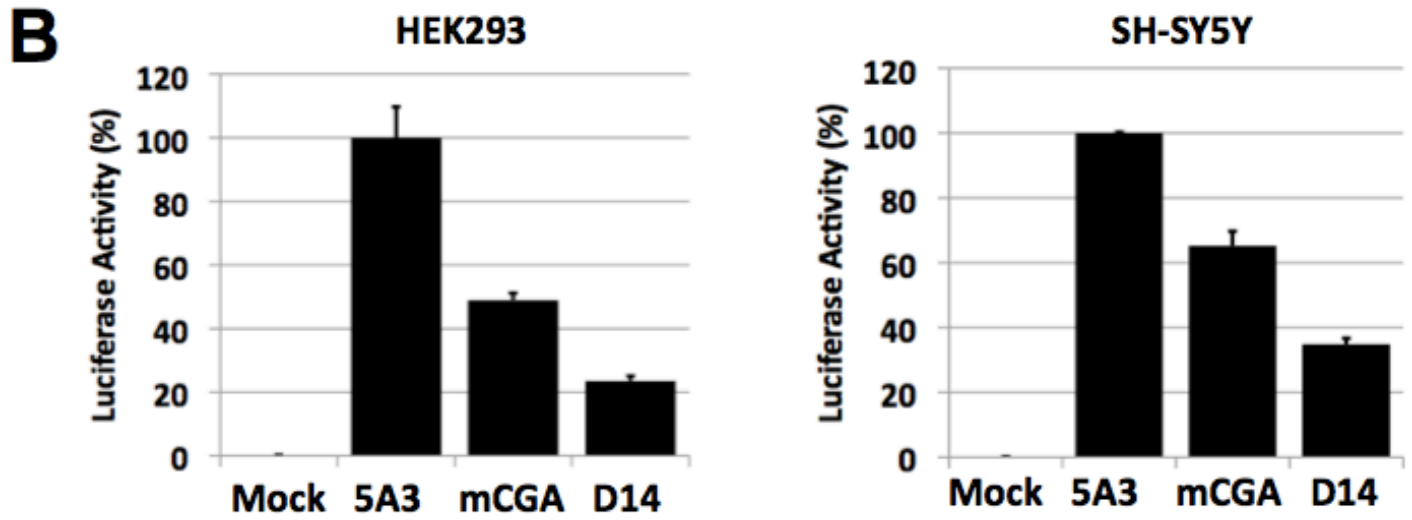
C9's paired G'9
interacts w/ FoxK1

Tsai et al., 2006 JBC

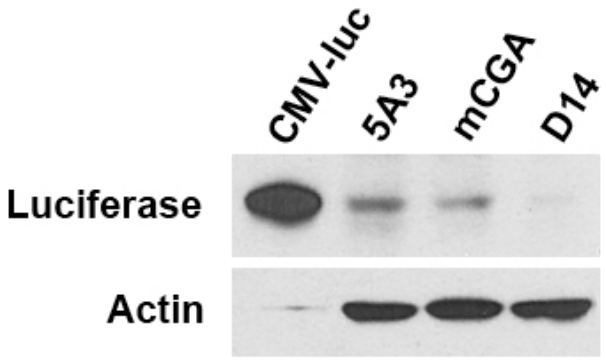


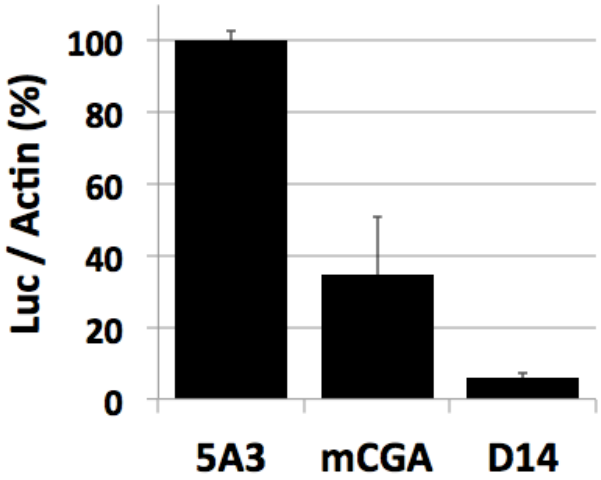
Effect of CGA mutation or D14 mutation on ATXN2-luc
LUCIFERASE ASSAY





Effect of CGA mutation or D14 mutation on ATXN2-luc
WESTERN BLOT

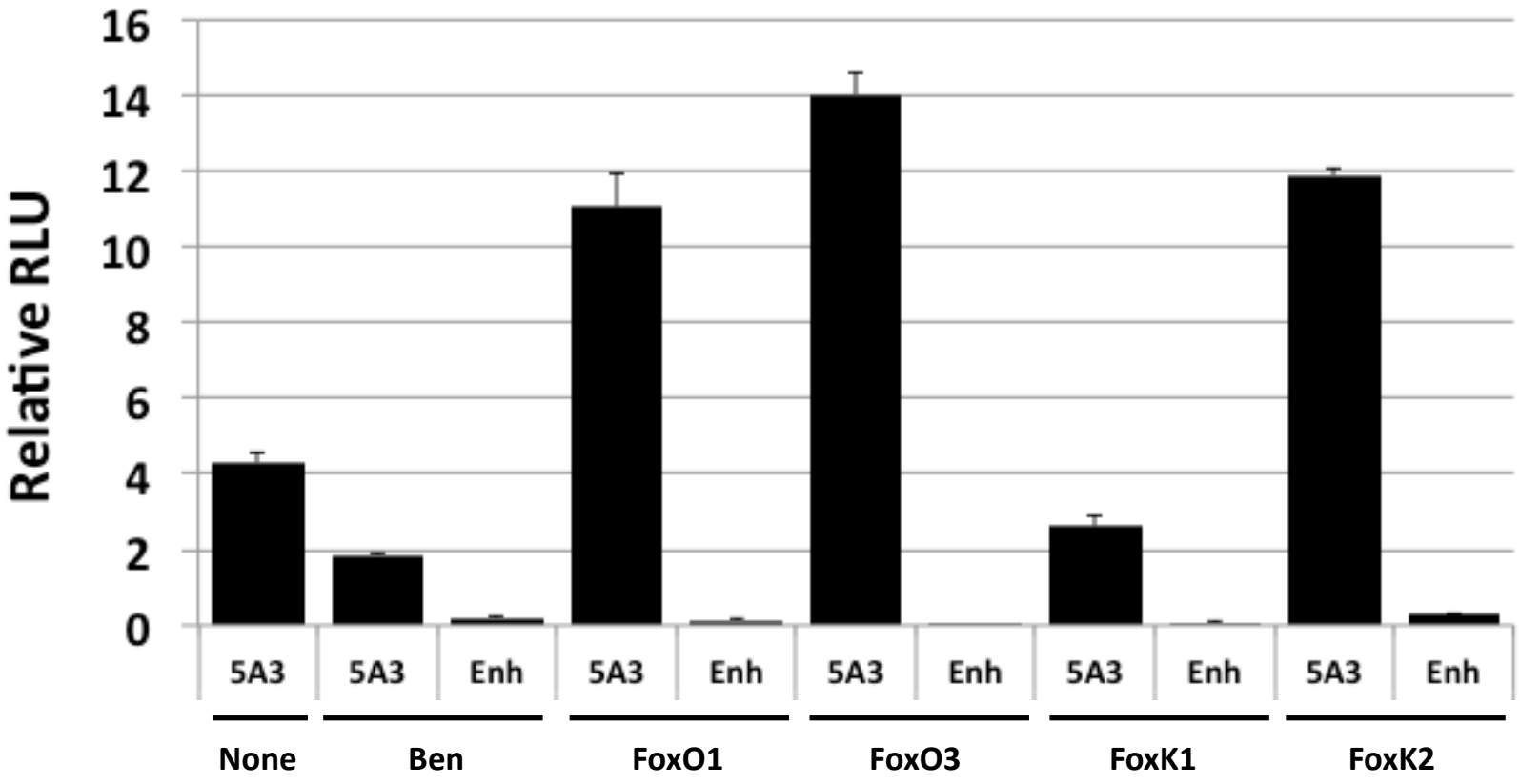




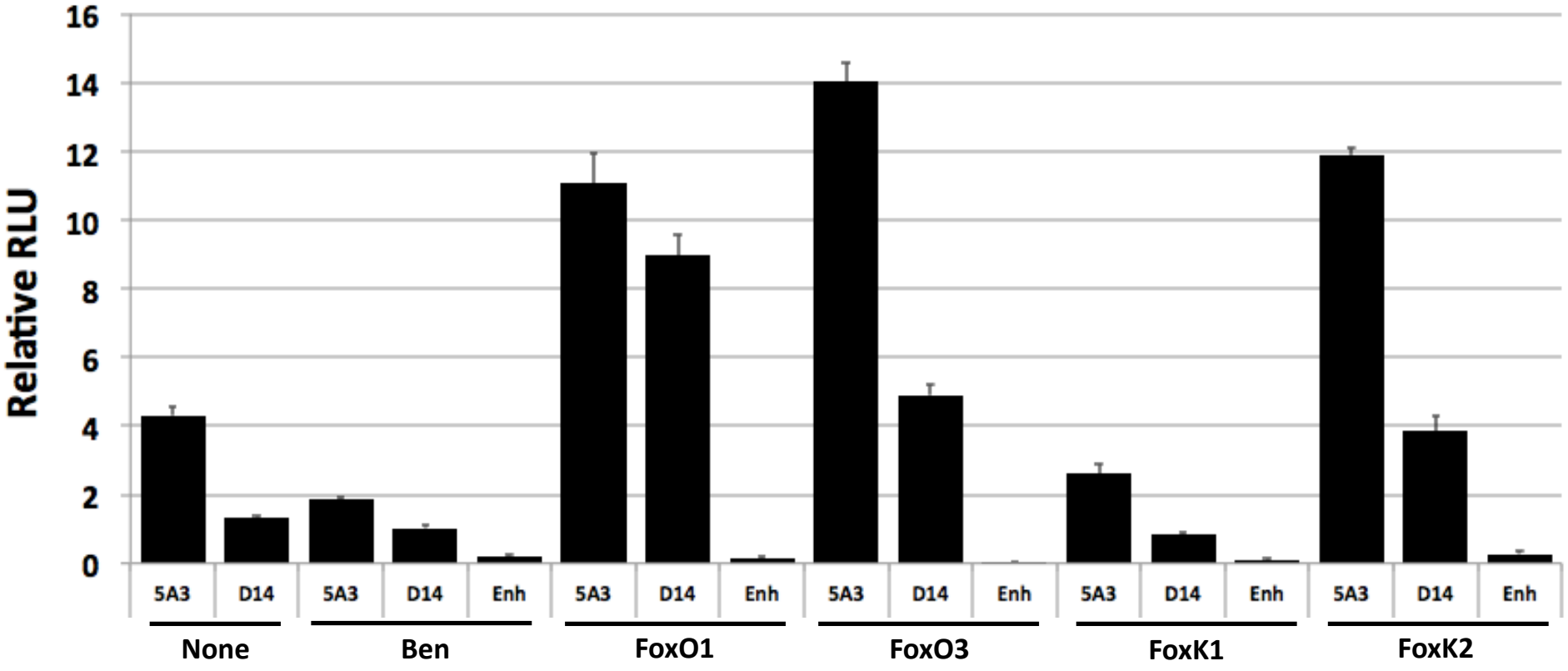
FoxO Expression Plasmids

FoxO1	pcDNA-FLAG-FKHR	Addgene
FoxO3a	pHA-FoxO3a-WT	Addgene
FoxK1	Andy Sharrocks	
FoxK2	Andy Sharrocks	

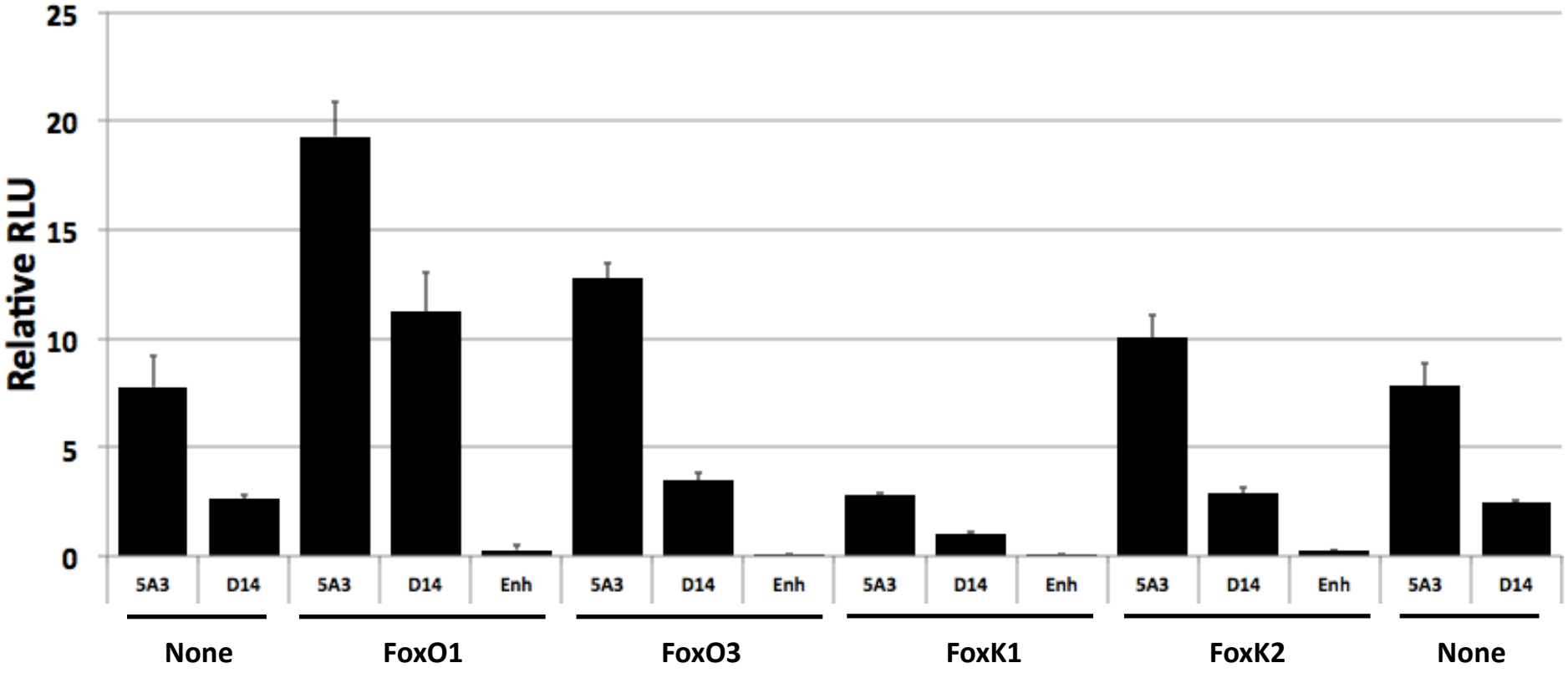
Transcription Factors Activating pGL2-5A3: FoxO1, FoxO3, FoxK2
Transcription Factors Inhibiting pGL2-5A3: Ben, FoxK1



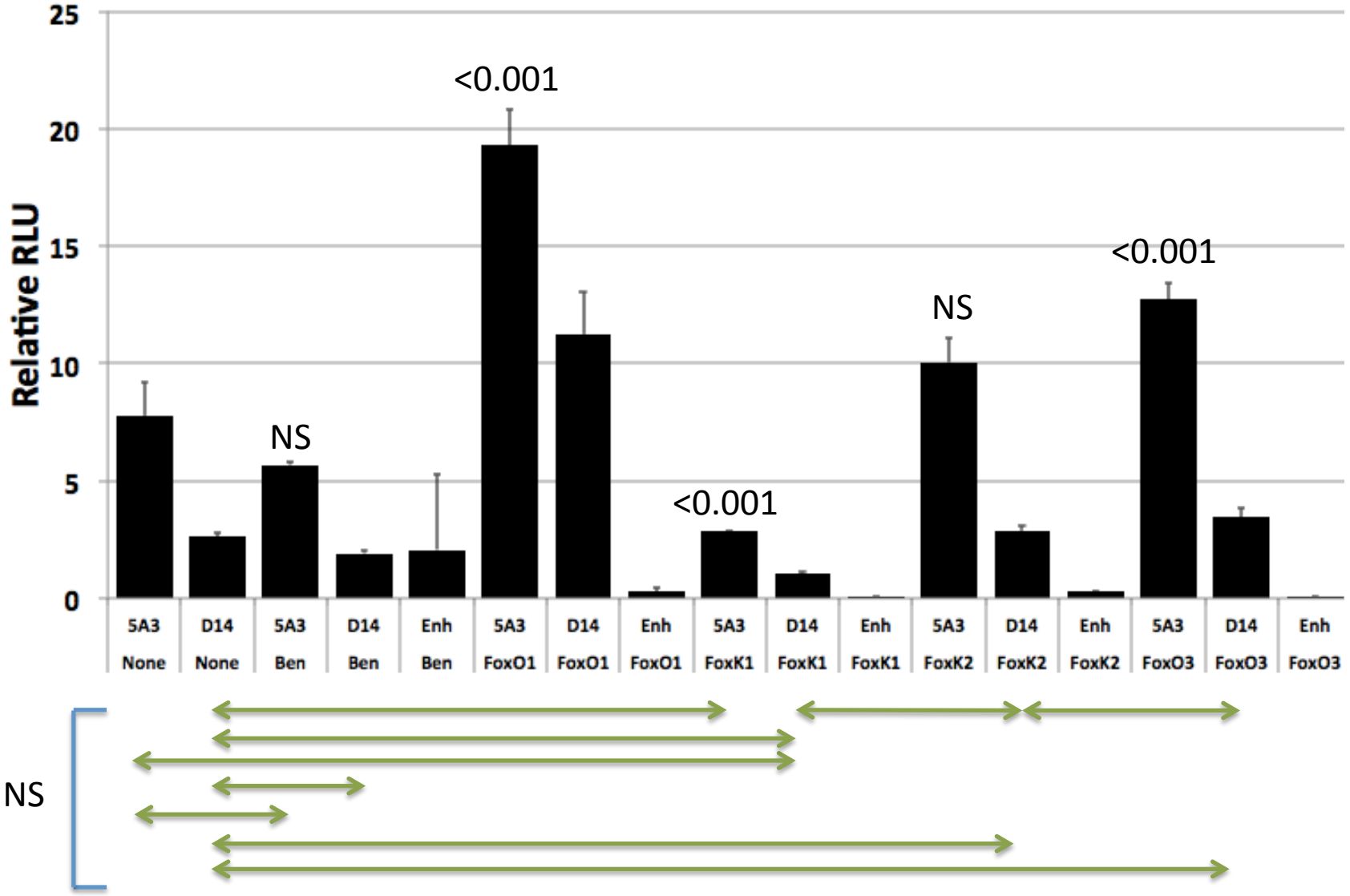
Effect of D14 deletion mutation in 5A3



Retrial

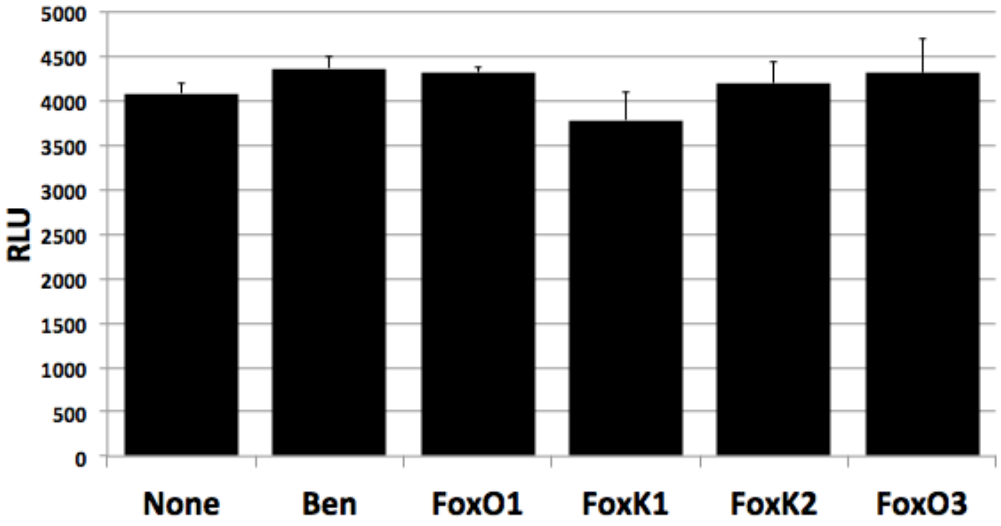

Relative RLU
25
20
15
10
5
0
5A3
D14
5A3
D14
Enh
5A3
D14
Enh
5A3
D14
Enh
5A3
D14
Enh
5A3
D14
None
FoxO1
FoxO3
FoxK1
FoxK2
None

One-Way ANOVA with Bonferroni post tests
Probablities shown are for comparisons with the 5A3 control



Other data

S2 cells



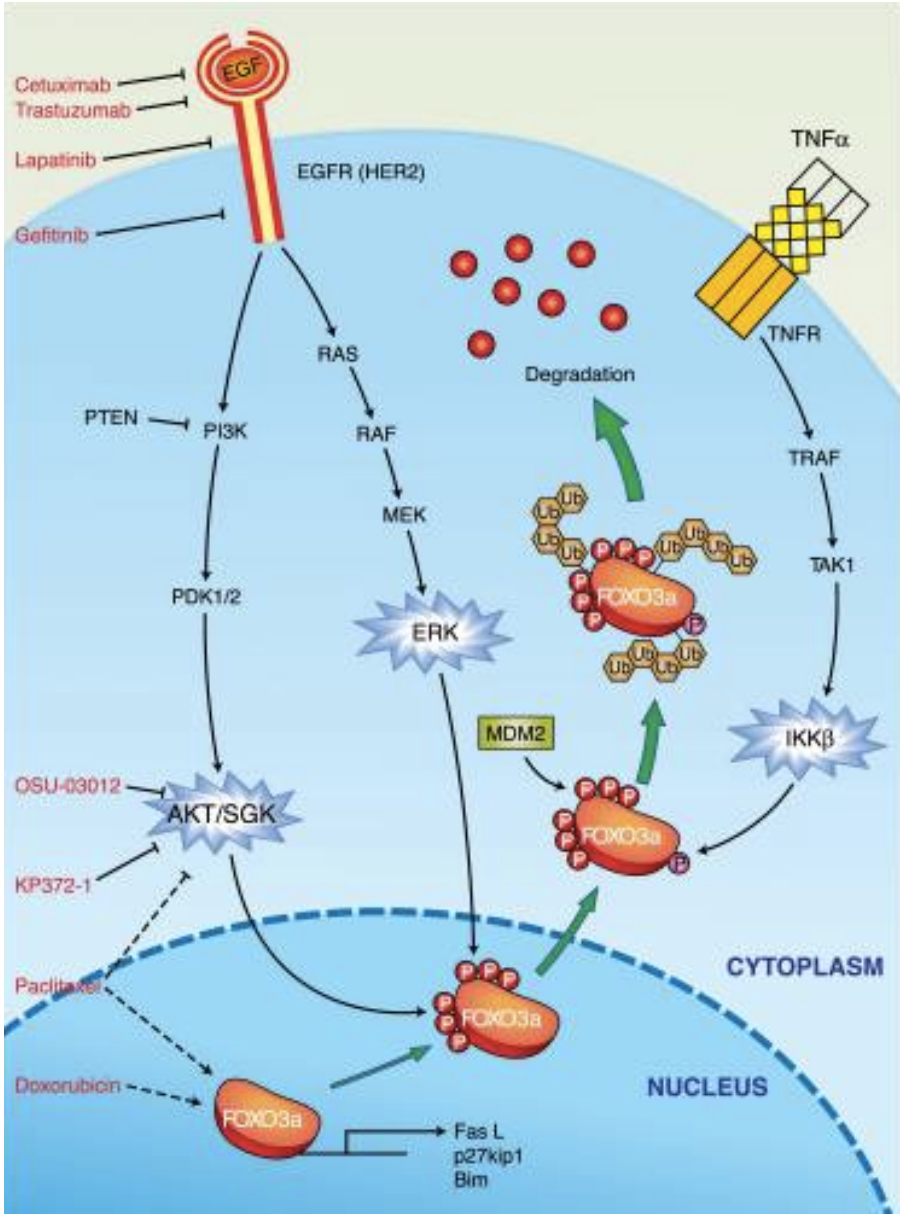
- Foxi1 regulates H^+-ATPase in inner ear and kidney (Vidarsson et al., PlosOne 2009)
- FoxB1 regulates TLR10 expression in B-cells (Bell et al., J. Immunology, 2007)

-

The Bad on FoxO

FoxO3 considered a tumor suppressor by some. Others limit the description to “mediator of apoptosis”.

Drugs targeting EGFR signaling that inhibit proliferation are predicted to dephosphorylate FoxO3 and activate growth inhibitors like p27(Kip)



Jer-Yen Yang and Mien-Chie Hung. A New Fork for Clinical Application:Targeting Forkhead Transcription Factors in Cancer. Clin Cancer Res. 2009 February 1; 15(3): 752–757.

- FoxO3 mutations reduce fertility but these women do not have tumors: Analysis of FOXO3 mutation in 114 Chinese women with premature ovarian failure. Wang et al., Reprod Biomed Online. 2010 Apr;20(4):499-503. Epub 2010 Feb 1.

- Number of tumors with mutations (Sanger Catalogue Of Somatic Mutations In Cancer)
  FoxO3: 4 grade IV astrocytomas, 2 lung adenocarcinomas (808 surveyed) (0.7%)
  FoxO1: 1 grade IV glioblastoma multiforme out of 808 surveyed (0.12%)
  NF2: 667 out of 4028 surveyed (16.5%)
  KRAS: 18076 out of 82910 surveyed (21.8%)

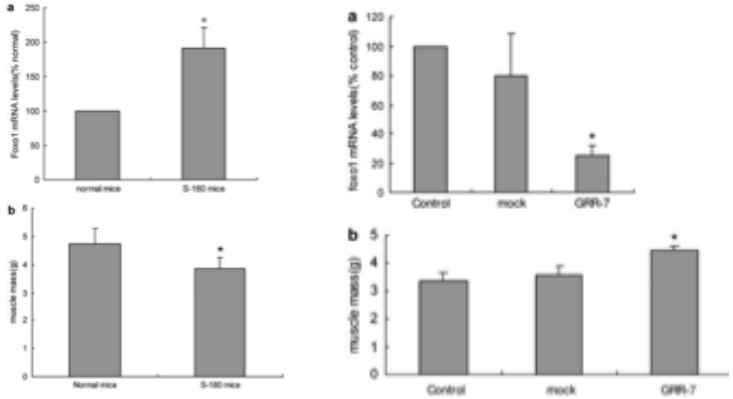
- FoxO3 regulates PINK: FOXO3a-dependent regulation of Pink1 (Park6) mediates survival signaling in response to cytokine deprivation. Mei et al., PNAS March 31, 2009 vol. 106 no. 13 5153-5158

- FoxO3a upregulation supports longevity. Anselmi et al., Association of the FOXO3A Locus with Extreme Longevity in a Southern Italian Centenarian Study Rejuvenation Research12, 2009. SIRT3 → FoxO3a, discovered in C. Elegans in 2001. FoxO3a is protective of ROS. SNPs in FoxO3a associate with longevity.

The Good on FOXO

- Foxo1 ASO therapy improved both hepatic insulin and peripheral insulin action in mice. Foxo1 is a potential therapeutic target for improving insulin resistance. (Samuel et al. Targeting Foxo1 in Mice Using Antisense Oligonucleotide Improves Hepatic and Peripheral Insulin Action. Diabetes 55, 2006.)

- FoxO1 is upregulated in skeletal muscle in cancer cachexia. FoxO1 ASO prevented cachexia in a S180 allograft model of cachexia (S180 cells are a mouse soft tissue sarcoma line lacking MHC surface expression that are highly aggressive) (Liu et al., Cancer Gene Therapy (2007) 14, 945–952).



Cells delivered IP into 6-8 wk old BALB/c
ASO delivered IV 3wks post cells 2/wk
Experimentation 4 wks post cells
N=6

- FoxO1 upregulates glycogen-6-phosphatase in liver (gluconeogenesis)
- FoxO1 upregulates Glucagon-like peptide-1 (GLP-1) antihyperglycemic hormone, inducing glucose-dependent stimulation of insulin secretion in pancreas

- Cook, Joshua R. FoxO1: The most important metabolic player you're not hearing about? College of Physicians and Surgeons, Columbia Medical Review, Fall 2010. http://juno.cumc.columbia.edu/psreview/node/94.

Domenico Accili, M.D. - Department of Medicine, Columbia Univ

FoxO1 and diabetes & obesity

Selected Publications:

1. L. Plum, H.V. Lin, R. Dutia, J. Tanaka, K.S. Aizawa, M. Matsumoto, A.J. Kim, N.X. Cawley, J-H. Paik, Y.P. Loh, R.A. DePinho, S.L. Wardlaw and D. Accili. (2009) The obesity susceptibility gene Carboxypeptidase E links FoxO1 signaling in hypothalamic pro-opiomelanocortin neurons with regulation of food intake. Nature Medicine 15:1195-1201

2. A.S. Banks, N. Kon, C. Knight, M. Matsumoto, R. Gutiérrez-Juárez, L. Rossetti, W. Gu and D. Accili. (2008) SirT1 gain-of-function increases energy efficiency and prevents diabetes in mice. Cell Metabolism 8:333-241

3. M. Matsumoto, A.Pocai, L. Rossetti, R.A. DePinho and D. Accili. (2007) Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor Foxo1 in liver. Cell Metabolism 6:208-216

4. T. Kitamura, Y. Ido-Kitamura, Y. Funahashi, C.L. Shawber, D.H. Castrillon, R. Kollipara, R.A. DePinho, J. Kitajewski and D. Accili. (2007) A Foxo/Notch pathway controls myogenic differentiation and fiber type specification. Journal of Clinical Investigation 117:2477-2485

5. T. Kitamura, Y. Feng, Y. Ido-Kitamura, S.C. Chua, A.W. Xu, G.S. Barsh, L. Rossetti and D. Accili. (2006) Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake. Nature Medicine 12:534-540

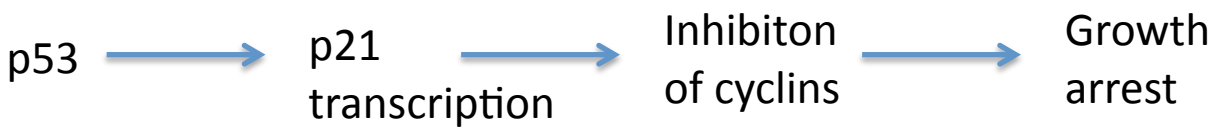
6. M. Matsumoto, S. Han, T. Kitamura and D. Accili. (2006) Dual role of the transcription factor Foxo1 to regulate insulin sensitivity and lipid metabolism. Journal of Clinical Investigation 116:2464-2472

- Very little is known on FoxK1/K2. So far only characterized as transcriptional inhibitors.

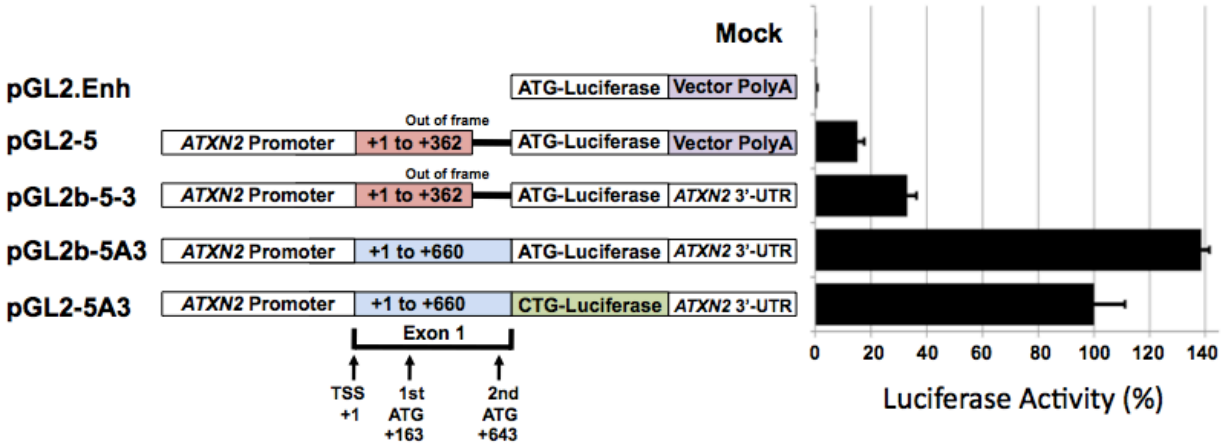
- FoxK1 and FoxK2 have a forkhead associated domain (FHA), not in FoxOs, that recognizes phosphothreonine epitopes. (Durocher D, Jackson SP. The FHA domain. FEBS Lett. 513(1):58-66; 2002.)

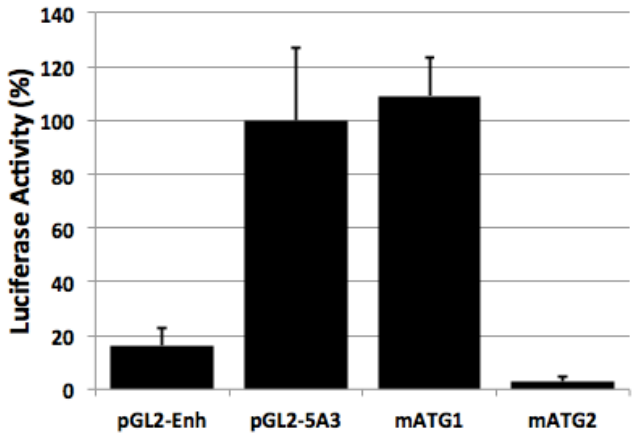
- FoxK1 KO mice have impaired skeletal muscle regeneration and are small. Myogenic progenitor cell isolates have reduced $p21^{CIP}$ suggesting FoxK1 is important for the cell cycle and that $p21^{CIP}$ may be a downstream target. (Hawke TJ et al. J Biol Chem. 278(6):4015-20. 2003.)

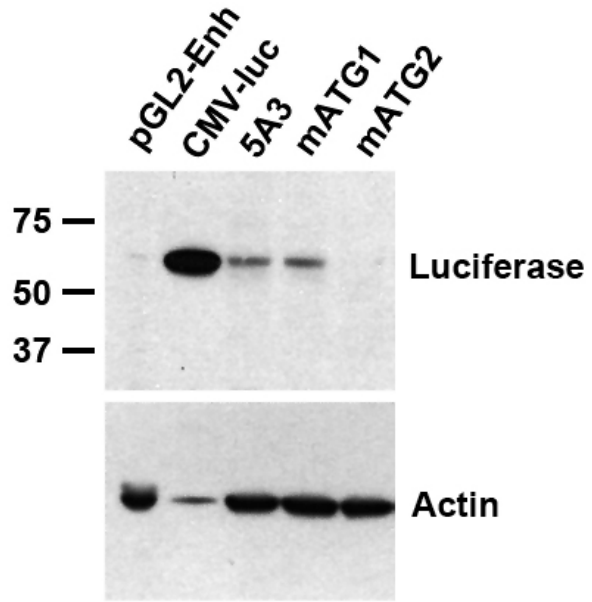
- A later paper on FoxK2 found that FoxK2 phosphorylation followed Cyclin E expression in osteosarcoma cells, then made the supposition that FoxK2 could also be an inhibitor of $p21^{CIP}$ but didn't test it. (Marais A, Ji Z, Child ES, Krause E, Mann DJ, Sharrocks AD. Cell cycle-dependent regulation of the forkhead transcription factor FOXK2 by CDK·cyclin complexes. J Biol Chem. 285(46):35728-39. 2010).



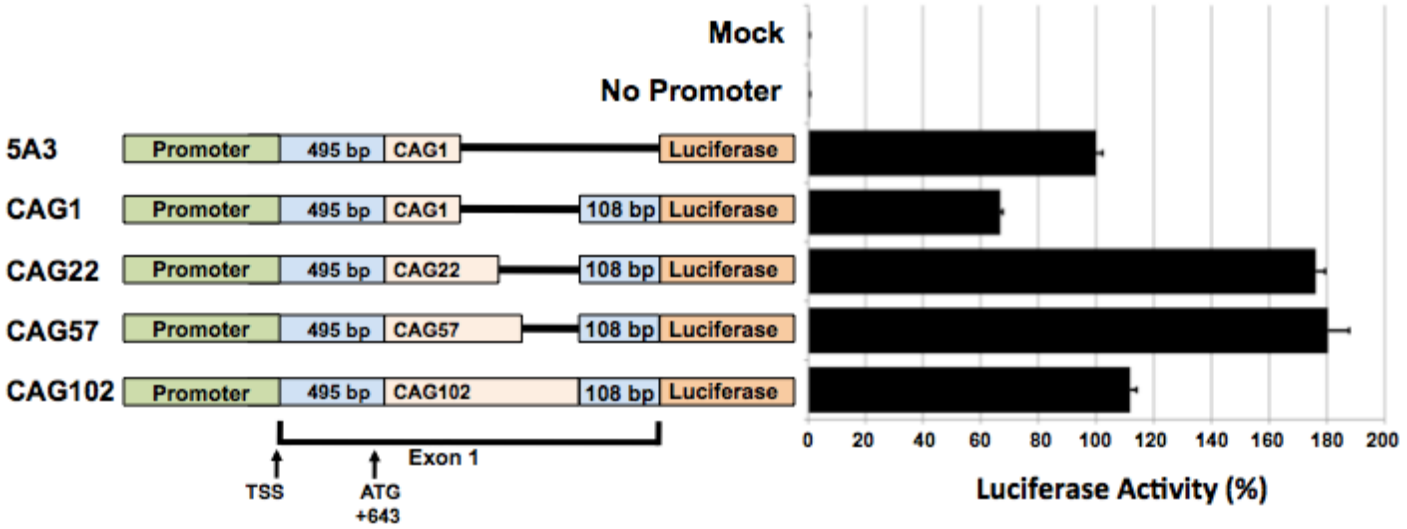
Start Codon 2 is used

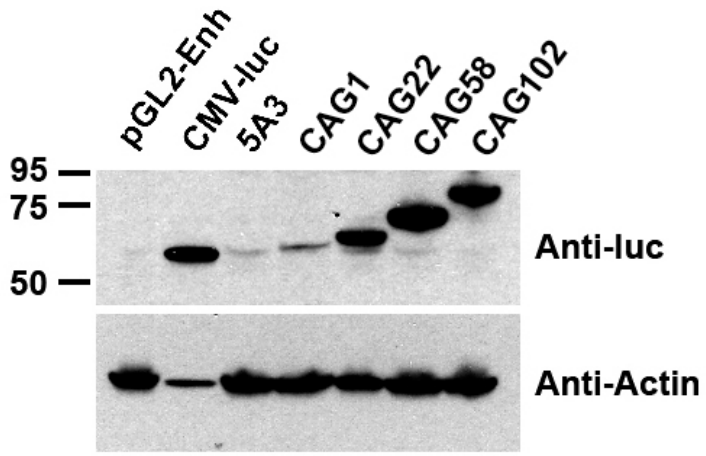


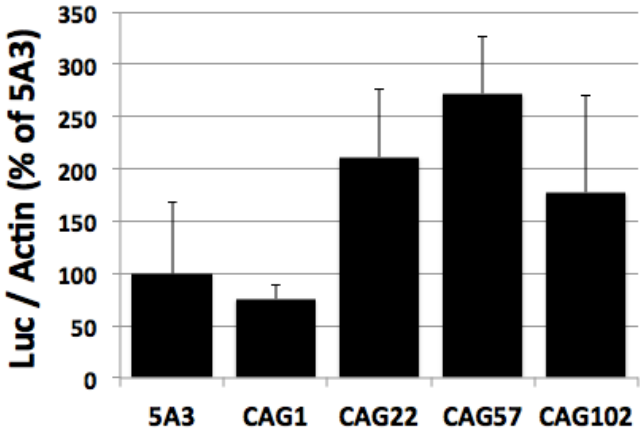




ATG-tcg-ctg-aag-ccc-CAG-CAG-CAG-CAG-CAG-CAG---------


Mock
No Promoter
5A3
CAG1
CAG22
CAG57
CAG102
Promoter
495 bp
CAG1
CAG22
CAG57
CAG102
108 bp
Luciferase
Exon 1
TSS
ATG
+643
0
20
40
60
80
100
120
140
160
180
200
Luciferase Activity (%)


pGL2-Enh
CMV-luc
5A3
CAG1
CAG22
CAG58
CAG102
95
75
50
Anti-luc
Anti-Actin


Luc / Actin (% of 5A3)
350
300
250
200
150
100
50
0
5A3
CAG1
CAG22
CAG57
CAG102

SBIR Grant

Phase I
$ 150,000 for 6 months including IDC
At least 67 % done by SBC, No more than 33% done by UTAH

Breakdown:
ISIS: $ 100,500
UTAH: $ 49,500 ($ 33,100 Direct, $16,390 Indirect)

Aims:
- HTS for ASO done by ISIS.
- Confirmation tests done by ISIS.
- Validations in relevant cells done by UTAH.

Submission: Aug 5, 2011
Start Date: April 1, 2012

Phase II
- $ 1,000,000 including IDC (50% ISIS / 50% Utah)
- Aims: Evaluation in mice

SBIR Grant

ISIS has to provide an update on what they have done with SBIR funds in the past with regard to commercialization

Raymond Ranken
Executive Director
R43 in 2004
HTS to detect donor blood pathogens

David Ecker
IBIS CEO, ISIS VP, ISIS Founder
RO1 in 2005
HTS for Bioterrorism

Lawerence Blyn
UC1 Challenge Grant in 2005
Biodefense (works with Ecker)

Susan Freier
Executive Director
R43 in 2007-8
miRNA Inhibitor

Eric Swayze
Vice President of Medicinal Chemistry
Chemical modifications to improve RNAi
R43 2006
R44 2007-2009