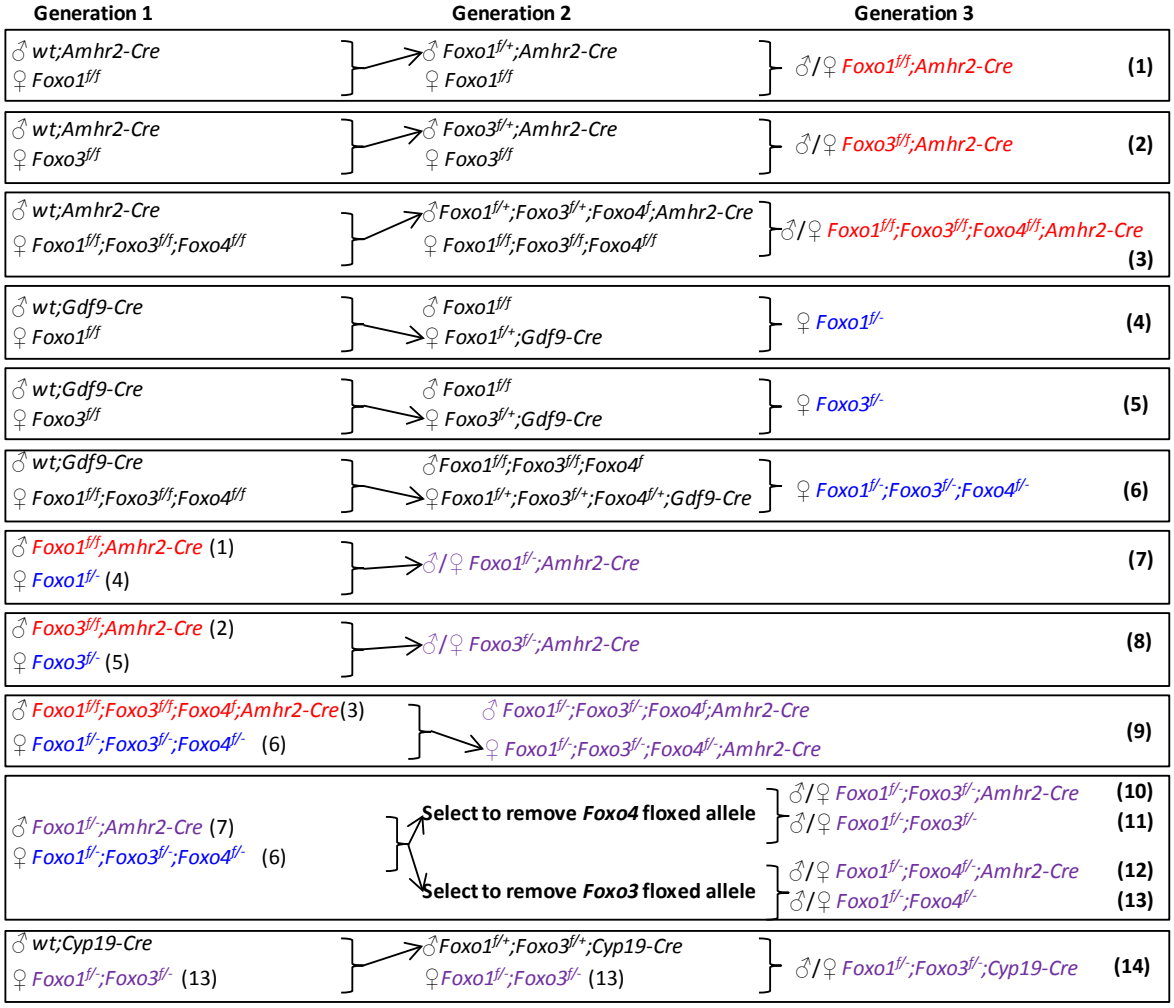
Supplemental Data



Maintenance breeding scheme I for *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$; *Amhr2-Cre* : (same for *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$;*Cyp19-Cre*)

♂ *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$;*Amhr2-Cre* (12) × ♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$ (13) (Only showing potential female genotypes)

♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$	(control)	♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$; *Amhr2-Cre*	(*Foxo1/3* dKO)	(10)
♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/f}$	(control)	♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/f}$; *Amhr2-Cre*		(15)
♀ *Foxo1*$^{f/-}$;*Foxo3*$^{-/-}$	(discard)	♀ *Foxo1*$^{f/-}$;*Foxo3*$^{-/-}$;*Amhr2-Cre*		(16)
♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/-}$	(control)	♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/-}$;*Amhr2-Cre*	(discard)	
♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/f}$	(control)	♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/f}$;*Amhr2-Cre*	(discard)	
♀ *Foxo1*$^{f/f}$;*Foxo3*$^{-/-}$	(discard)	♀ *Foxo1*$^{f/f}$;*Foxo3*$^{-/-}$;*Amhr2-Cre*	(discard)	

Maintenance breeding scheme II for *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$; *Amhr2-Cre* : (same for *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$;*Cyp19-Cre*)

♂ *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$;*Amhr2-Cre* (12) × ♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/f}$ (13) (Only showing potential female genotypes)

♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$	(control)	♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/-}$; *Amhr2-Cre*	(*Foxo1/3* dKO)	(10)
♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/f}$	(control)	♀ *Foxo1*$^{f/-}$;*Foxo3*$^{f/f}$; *Amhr2-Cre*		(15)
♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/-}$	(control)	♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/-}$;*Amhr2-Cre*	(discard)	
♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/f}$	(control)	♀ *Foxo1*$^{f/f}$;*Foxo3*$^{f/f}$;*Amhr2-Cre*	(discard)	

Supplemental Figure 1: Outlined breeding scheme of *Foxe* mutant mice.

Briefly, we obtained the *Foxo1*$^{fl/fl}$, *Foxo3*$^{fl/fl}$, and *Foxo1*$^{fl/fl}$; *Foxo3*$^{fl/fl}$;*Foxo4*$^{fl/fl}$ mice (1) from Dr. Diego H. Castrillon , *Amhr2-Cre* mice (2) from Dr. Richard R. Behringer, and *Gdf9-Cre* mice (3) from Dr. Austin J. Cooney. The *Cyp19-Cre* mice, originally generated at Organon (4), have been characterized and developed in our own laboratory.

We initially mated the *Foxo1*$^{fl/fl}$, *Foxo3*$^{fl/fl}$, and *Foxo1*$^{fl/fl}$; *Foxo3*$^{fl/fl}$;*Foxo4*$^{fl/fl}$ female mice (1) to *Amhr2-*Cre+ males (2) to generate *Foxo1*$^{f/f}$*;Amhr2-Cre* (**Line 1**), *Foxo3*$^{f/f}$*;Amhr2-Cre* (**Line 2**) and *Foxo1*$^{f/f}$*;Foxo3*$^{f/f}$*; Foxo4*$^{f/f}$*;Amhr2-Cre* (**Line 3**) mice. There mice are viable and fertile, likely due to the low efficiency of *Amhr2-Cre*.

To overcome this, we employed a strategy used by many (5-7) to generate germ-line depletion of *Foxo1* and *Foxo3* alleles. Specifically *Gdf9-Cre+* males (3) were crossed with *Foxo1*$^{fl/fl}$ and *Foxo3*$^{fl/fl}$ mice to generate germ-line depletion of the *Foxo1* and *Foxo3* alleles (**Line 4, 5 and 6**). These mice were then crossed with the mice carrying *Amhr2-Cre* to ultimately obtain the mice with one null allele as well as the *Amhr2-Cre gene*, as illustrated (**Line 7, 8, and 9**). (Notice: *Foxo4* is on chromosome X. So in the male, it has just one allele, as demonstrated in Line 9. In the following lines, this was not pointed out again as the female and male were mentioned together in Line 12 and 13).

As *Foxo4* is not critical for the ovarian function, we removed the *Foxo4* floxed allele from the triple mutant strains and maintained it as wild type allele to get the *Foxo1* and *Foxo3* double mutant mice (**Line 10 and 11**). **Line 10 is our experimental line, designated as *Foxo1/3* dKO**.

The same strategy was used to get *Foxo1* and *Foxo4* double mutant mice (**Line 12 and 13**). These mice were similar to the *Foxo1* single mutant mice (data not shown).

To support the observed phenotype in *Amhr2-Cre* strains and to exclude the possibility that the phenotype was due to leakage of *Amhr2-Cre* in other tissues, we also used *Cyp19-Cre* mice (4) to generate the *Foxo1*$^{f/-}$*;Foxo3*$^{f/-}$*;Cyp19-Cre* (**Line 14**). The *Cyp19* promoter is more ovarian-granulosa-cell-specific but expressed at a later stage of follicle development than *Amhr2-Cre*. These mice exhibit same phenotype as *Foxo1*$^{f/-}$*;Foxo3*$^{f/-}$*;Amhr2-Cre* mice.

Two breeding strategies were used to generate and maintain the appropriate mutant mice. The male *Foxo1*$^{f/-}$*;Foxo3*$^{f/-}$*;Amhr2-Cre* mice were fertile and used for breeding. In the first scheme, female *Foxo1*$^{f/-}$*;Foxo3*$^{f/-}$ mice were used to obtain the *Foxo3* germ line depletion mice: *Foxo1*$^{f/-}$*;Foxo3*$^{-/-}$*;Amhr2-Cre* (**Line 16**). In the second scheme, female *Foxo1*$^{f/-}$*;Foxo3*$^{f/f}$ mice were used to generate more *Foxo1*$^{f/-}$*;Foxo3*$^{f/-}$*;Amhr2-Cre* (**Line 10**) and *Foxo1*$^{f/-}$*;Foxo3*$^{f/f}$*;Amhr2-Cre* (**Line 15**) mice.

References:

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4. Fan HY, Liu Z, Cahill N, Richards JS 2008 Targeted disruption of Pten in ovarian granulosa cells enhances ovulation and extends the life span of luteal cells. Mol Endocrinol 22:2128-2140
5. Pangas SA, Jorgez CJ, Tran M, Agno J, Li X, Brown CW, Kumar TR, Matzuk MM 2007 Intraovarian activins are required for female fertility. Mol Endocrinol 21:2458-2471
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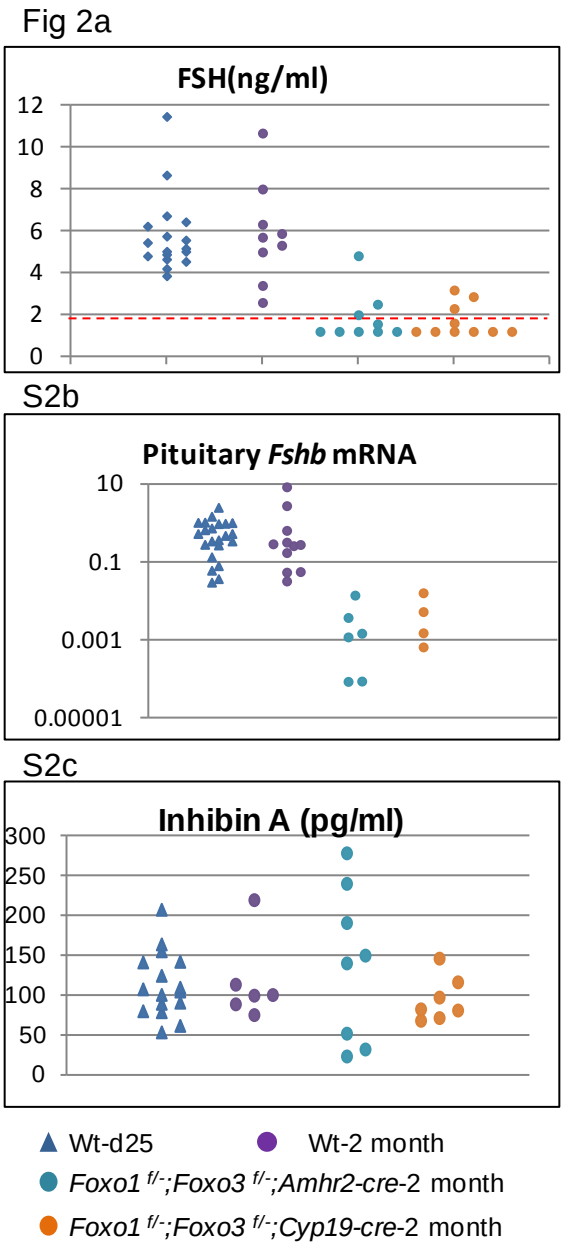
7. Li Q, Pangas SA, Jorgez CJ, Graff JM, Weinstein M, Matzuk MM 2008 Redundant roles of SMAD2 and SMAD3 in ovarian granulosa cells in vivo. Mol Cell Biol 28:7001-7011

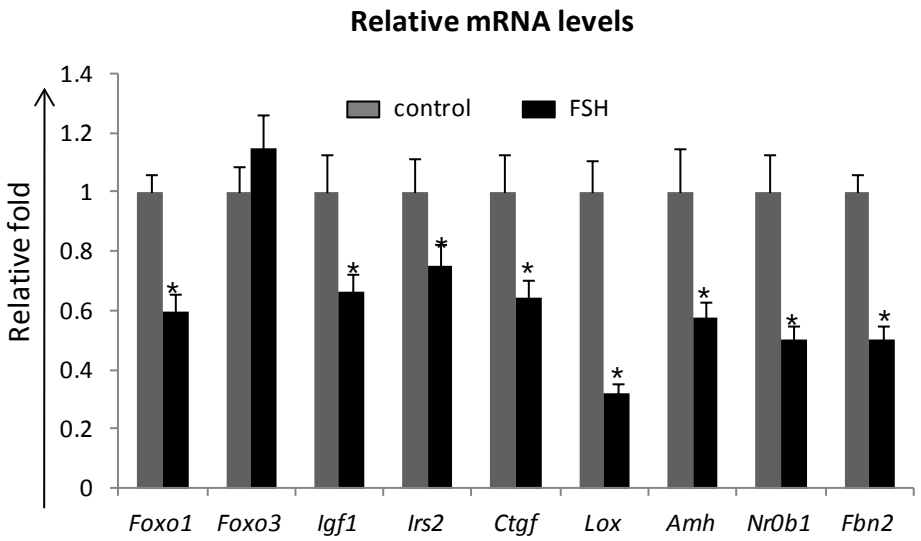


Supplemental Figure 2: *Foxo1/3 dKO* mice have reduced serum FSH levels, reduced pituitary *Fshb* mRNA expression, yet comparable serum inhibin A levels.

A: Serum FSH levels were significantly reduced in both *Foxo1/3 dKO* (*Amhr2-Cre*) and *Foxo1/3 dKO* (*Cyp19-Cre*) mice.

B: Pituitary *Fshb* mRNA levels were significantly reduced in both *Foxo1/3 dKO* (*Amhr2-Cre*) and *Foxo1/3 dKO* (*Cyp19-Cre*) mice.

C: Serum inhibin A levels were comparable in control mice, *Foxo1/3 dKO* (*Amhr2-Cre*) and *Foxo1/3 dKO* (*Cyp19-Cre*) mice (P>0.4 for both when compared to control samples).



Supplemental Figure 3: **FSH down regulates FOXO1 and *Foxo1/3* target genes.**

Granulosa cells were isolated from wild type mice and cultured with or without FSH (100ng/ml) for 24h. RNA was isolated and mRNA transcripts were measured by qPCR. The values shown represent the mean fold induction from 3 experiments. Asterisk * denotes genes significantly down-regulated by FSH.

Supplemental Table 1: Primer Sets for Real-time RT-PCR.

Gene	Primer-Forward	Primer-Reverse
Amh	ACCCTTCAACCAAGCAGAGA	AAGCGAGTGAGGGTCTCTAGG
Bcl2l11	TTCCACTTGGATTCACACCA	GCTGCAATTGTCCACCTTCT
BMP2	GCTCCACAAACGAGAAAAGC	AGCAAGGGGAAAAGGACACT
Cga	TTGCTTCTCCAGGGCATATC	GCGCTCAGAAGCTACGACTT
Ctgf	AAGACACATTTGGCCCAGAC	GACAGGCTTGGCGATTTTAG
Egr1	GAGCGAACAACCCTATGAGC	AGGCCACTGACTAGGCTGAA
Fbn2	GAATGGGCTTCAACAAAGGA	TACGCTCTCCAGGCTGATTT
Fos	GGGGCAAAGTAGAGCAGCTA	GGCTGCCAAAATAAACTCCA
FosB	AAAACAAACAAACCCGCAAG	AGAAAACCAGAGACGGAGCA
Foxl2	ACATGTTCGAGAAGGGCAAC	GCCAGGAGTTGTTGAGGAAC
Foxo1	GTGAACACCATGCCTCACAC	TGGACTGCTCCTCAGTTCCT
Foxo1	TATTGAGCGCTTGGACTGTG	TGGACTGCTCCTCAGTTCCT
Foxo4	CAGTGACCTCATGGATGGTG	GAGGGCTCAAGGAGGAAAAG
Fst	AGAGGTCGCTGCTCTCTCTG	TCCTCCTCCTCCTCTTCCTC
Gata2	GCACCTGTTGTGCAAATTGT	GCCCCTTTCTTGCTCTTCTT
Gh	CTGGCTGCTGACACCTACAA	AAGCGAAGCAATTCCATGTC
Igf1	TGGATGCTCTTCAGTTCGTG	GTCTTGGGCATGTCAGTGTG
INHA	ATGCACAGGACCTCTGAACC	GGATGGCCGGAATACATAAG
INHBA	ATCATCACCTTTGCCGAGTC	CCCTTTAAGCCCATTTCCTC
INHBB	CGAGATCATCAGCTTTGCAG	TCCACCTTCTTCTCCACCAC
Irs2	GTAGTTCAGGTCGCCTCTGC	CAGCTATTGGGACCACCACT
Jun	AAAACCTTGAAAGCGCAAAA	CGCAACCAGTCAAGTTCTCA
Junb	GCAGCTACTTTTCGGGTCAG	TTCATCTTGTGCAGGTCGTC
Klf5	GCCAGTTAATTCGCCAACTC	GGTGCACTTGTAGGGCTTCT
Lhb	AGTTCTGCCCAGTCTGCATC	GACCCCCACAGTCAGAGCTA
Lhcgr	CTGAAAACTCTGCCCTCCAG	AATCGTAATCCCAGCCACTG
Lox	CAGGGATTGAGTCCTGGATG	ACTGGGAACTGGGCTTCTTT
Mapk10	GATGAAAGGGAGCACACCAT	AGTGTCAGATGCGAGGGTCT
Pdk4	GCTTGCCAATTTCTCGTCTC	CCTGCTTGGGATACACCAGT
Pomc	GAAGATGCCGAGATTCTGCT	TTTTCAGTCAGGGGCTGTTC
Prl	CAAGGAACAAGCCCTGAAAG	ATCCCATTTCCTTTGGCTTC
Star	AGAGGTTCCACCTGTGTGCT	GGTTGGCGAACTCTATCTGG
Tshb	TCAACACCACCATCTGTGCT	TCTGACAGCCTCGTGTATGC

Supplemental Table 2:
Verification of microarray data for selected genes by qPCR.

Gene Symbol	Foxo-d25/wt (array/qPCR)	Foxo-2m/wt (array/qPCR)
Foxo family members		
Foxo1	-6.7|-8.2	-40.6|-66.8
Foxo3	1.9|-12.9	1.4|-8.4
Foxo4	-1.1|-3.1	-1.1|-48.6
TGF beta family		
Inha	1|1	1|1.4
Inhba	1.4|2.1	1.2|2.1
Inhbb	1.2|1.4	1.1|1.7
Fst	-1.1|1.1	-1.9|-1.9
Gdf9	1.9|3.5	-1.3|1.1
Steroids		
Cyp11a1	1.6|2.4	5.2|11.5
Cyp17a1	1.9|3.6	9.9|33
Cyp19a1	-2.2|-2.3	-1.1|2.2
Akr1c14	-2.3|-2.1	-3.5|-3.6
Star	-1.6|-1.1	5.2|16.2
Transcription Factor		
Nr0b1	-3.5|-2.9	-5.5|-3.4
Nr5a1	-1.5|-1.1	1.6|3.1
Nr5a2	-2.1|1	-2.3|1
Apoptosis		
Arnt2	-1.9|-1.6	-5.1|-4.4
Bcat1	-5.0|-3.0	-10.6|-6.9
Bcl2l11	-1.5|-3.8	-1.5|-4.9
Mapk10	-2.1|-2	-2.5|-1.9
Cancer		
Myc	-2.1|-1.6	-3.5|-2.9
Trp53	-1.5|-1.1	-1.8|-1.2
DNA damage		
Gadd45a	-2.1|-8.1	-2.6|-4.5
Other genes of Interest		
Stc1	24.7|66.1	19.1|51.1
Mylk	4.2|9.6	5.3|16.9
Fbn2	-6.0|-5.2	-9.2|-9.8
Rgs13	-5.8|-3.9	-5.2|-4
Fshr	1.3|1.9	1.2|2.1
Lhcgr	1.7|2.4	5.5|14.8
Ccnd2	-1.4|-1.3	-1.9|-1.6
Ctgf	-2.4|-2.2	-2.8|-2.3
Hoxc8	2.7|3.4	3.3|6.1
Hoxd8	2.6|4.6	4.7|9.7
Igf1r	-2|-2.4	-2.6|-3.6

Note: Data are the average fold change for the indicated samples.

Supplemental Table 3: Top bio functions of genes changed in d25 dKO samples.

Category	Gene Names	# Genes
Cardiovascular Disease	ANGPT2,BCAN,BCAT1,CASQ2,CDH13,CNTF,CRYAB,CTNNA2,DDAH1,FAM19A5,FAM40B,FHL2,FOXO1,FOXP1,HBA1/HBA2,IRS2,ITIH5,KCNQ5,LIMCH1,LRP8,MTUS2,MYBPC3,MYLK,MYOM1,NT5E,PDE5A,PIK3R1,PLA2G5,PLN,PNPT1,RGS13,SERPINA5,SLC25A48,THBS2,TNNC1,UNC13D	36
Gastrointestinal Disease	CNTF,CXCL2,FOXO1,IRS2,MYLK,NEU3,NT5E,PDE5A,PIK3R1,SERPINA1,SMPX,THBS4,TNNC1	13
Developmental Disorder	ANGPT2,CASQ2,CNTF,CRYAB,FHL2,H19,ITGA7,MYBPC3,MYOM1,NR0B1,NT5E,PDE5A,PIK3R1,PLN	14
Endocrine System Disorders	BCAT1,CD7,CDH13,CNTF,CTNNA2,DDAH1,FAM19A5,FOXO1,FOXO4,FOXP1,HBA1/HBA2,IRS2,ITIH5,KCNQ5,LIMCH1,LRP8,MTUS2,MYLK,MYOM1,NEU3,NR0B1,PDE5A,PIK3R1,SERPINA1,SERPINA5,SPECC1,TDGF1,TRIM15	28
Metabolic Disease	ASS1,CNTF,FOXO1,FOXO4,IRS2,NEU3,PIK3R1	7

Supplemental Table 4: Top bio functions of genes changed in 2 month old *Foxo1/3dKO* mice.

Category	Gene Names	# Genes
Cancer	1600029D21Rik, ADH1C, ADM, AGTR1, ALDH1A1, ANG, AOC3, BCAN, BCL11A, CAMK2N1, CAPN6, CDH5, CENPA, Clca1/Clca2, CTSC, CXCL2, CXCR7, CYP11A1, CYP17A1, CYTIP, DPYSL4, EDN1, EFEMP1, EFS, ENPEP, EPHX1, ETS1, FBN2, FHL2, FOLR1, FOXO1, FOXO4, FST, FUBP1, H19, HMGA2, HSD17B11, HSD17B3, IGF1R, IL13RA1, IRS2, ITIH5, JAK1, KCNJ5, KCNK2, KRT20, LGALS3, LHCGR, LRP8, MAF, MGLL, MLF1, MSI1, MYC, MYF5, NR0B1, NR3C1, NRP1, PDE5A, PDK4, PIK3R1, PLP1, RGNEF, RNASEL, SECTM1, SERPINA3, SERPINA5, SLC9A3R1, SMC4, ST5, STAR, STAT1, STC1, SULT1A1, TDGF1, TEK, TGM2, TLR2, TM4SF1, TNC, TNS4, TUBA8, ZBTB16	83
Cardiovascular Disease	ABLIM1,ADAMTS19,ADM,AFF3,AGTR1,ALDH1A1,ANK2,AOC3,AQP2,BCAN,BCAT1,BCL11A,CASQ2,CNTF,CNTN4,COL9A2,CTSC,CXCR7,DDAH1,DNAH7,EDN1,ENPEP,EPHX1,ETS1,FBN2,FGD5,FHL2,FOLR1,FOXO1,HJURP,HMGA2,IGF1R,IRS2,ITIH5,JPH1,KCNJ5,KCNK2,KCNQ5,LHCGR,LIMCH1,LRP8,MGLL,MLF1,MSI1,MYH6,MYLK,MYOM1,NCF2,NR3C1,PDE5A,PID1,PIK3R1,PLN,PPP1R12B,RGS13,RNF182,SERPINA5,SLC26A7,SYBU,TAC1,TEK,TLR2,TNFAIP6,TNNT2,TUBA8,UNC13D	66
Developmental Disorder	ADM,AGTR1,ALDH1A1,ANK2,CASQ2,CNTF,COL9A2,CTSC,CYP11A1,EDN1,FBN2,FHL2,FST,H19,HMGA2,IGF1R,KCNJ5,LGALS3,LHCGR,MYC,MYH6,MYOM1,NR0B1,PDE5A,PIK3R1,PLN,STC1,TLR2,TNNT2,WNT4	30
Endocrine System Disorders	ABLIM1,AFF3,AGTR1,ALDH1A1,ANK2,AOC3,BCAT1,CENPE,CNTF,CNTN4,COL27A1,CXCR7,CYP11A1,CYP17A1,DDAH1,DNAH7,EFEMP1,ETS1,FADS2,FAM129A,FBN2,FOXO1,FOXO4,FST,HSD17B3,IGF1R,IRS2,ITIH5,KCNJ5,KCNK1,KCNQ5,LGALS3,LHCGR,LIMCH1,LRP8,MLF1,MYC,MYLK,MYOM1,NCF2,NR0B1,NR3C1,NRP1,NTNG1,PDE5A,PIK3R1,PLAGL1,PLCH1,PPP1R12B,RGNEF,SERPINA3,SERPINA5,SPECC1,STAR,TDGF1,TGM2,TMEFF1,TNNT2,TUBA8,ZBTB16,ZNF385B	61
Genetic Disorder	ABCD2,ABLIM1,ADAMTS19,ADH1C,ADM,AFF3,AGTR1,ALAS2,ALDH1A1,ANG,ANK2,AOC3,AQP2,ARNT2,BCAN,BCAT1,BCL11A,CAMK2N1,CAPN6,CASQ2,CDC42EP3,CENPA,CENPE,CNTF,CNTN4,COL14A1,COL27A1,COL9A2,CTSC,CXCL2,CXCR7,CYP11A1,CYP17A1,CYTIP,DDAH1,DNAH7,EDN1,EFEMP1,ENPEP,EPHX1,FADS2,FAM129A,FAM81A,FBN2,FGD5,FHL2,FOLR1,FOXO1,FST,FUBP1,FXYD1,H19,HJURP,HLA-DMB,HMGA2,HSD17B11,HSD17B3,IGF1R,IL13RA1,IRS2,ITIH5,IVNS1ABP,JAK1,JPH1,KCNJ5,KCNK1,KCNK2,KCNQ5,KRT20,LDB3,LGALS3,LHCGR,LIMCH1,LRP8,LRRC3B,MAF,MGLL,MLF1,MYC,MYH6,MYLK,MYOM1,NCF2,NR0B1,NR3C1,NRP1,NTNG1,PDE5A,PDK4,PIK3R1,PLA2G1B,PLAGL1,PLN,PLP1,PPARGC1B,PPP1R12B,PTPRB,RGNEF,RGS13,RNASEL,SERPINA3,SERPINA5,SLC26A7,SLC9A3R1,SLCO2A1,SLITRK1,SMC4,SNCAIP,SPECC1,STAR,STAT1,SULT1A1,SYBU,TAC1,TDGF1,TEK,TGM2,TLR2,TMEFF1,TMPRSS13,TNC,TNFAIP6,TNNT2,TNS4,TUBA8,TUFT1,UBA7,UNC13D,WNT4,ZBTB16,ZNF385B	131

Supplemental Table 5:
Number of putative transcription factor DNA binding response elements in the proximal promoters of genes of interest.

Gene promoter	SMAD	FOXO	SP1	AP1	SF1
Growth-related Genes					
*Ctgf(ccn2)**	1	6	1	1	0
Amh	1	2	6	0	4
Lox	5	2	6	0	1
Foxo1	1	2	8	0	0
Foxo3	1	1	13	4	0
Igf1	4	9	4	20	2
*Nr0b1**	0	3	1	0	0
Inhba	1	3	1	2	0
Irs2	2	9	20	4	2
Metabolism & Apoptosis-related Genes					
Bmp2	2	2	7	0	0
*Klf5**	1	6	9	2	1
*Bcl2l11(Bim)**	1	1	13	1	0
Mapk10	1	3	7	6	1
*Pdk4**	2	2	4	3	1

Note: Putative transcription factor DNA binding response elements in the proximal promoters (-800bp) of growth-related, metabolism and apoptosis-related genes. ***** Denotes genes known to be up-regulated by FOXO1. The results are summarized according to the promoter information obtained from Genomatix (http://www.genomatix.de/en/index.html).