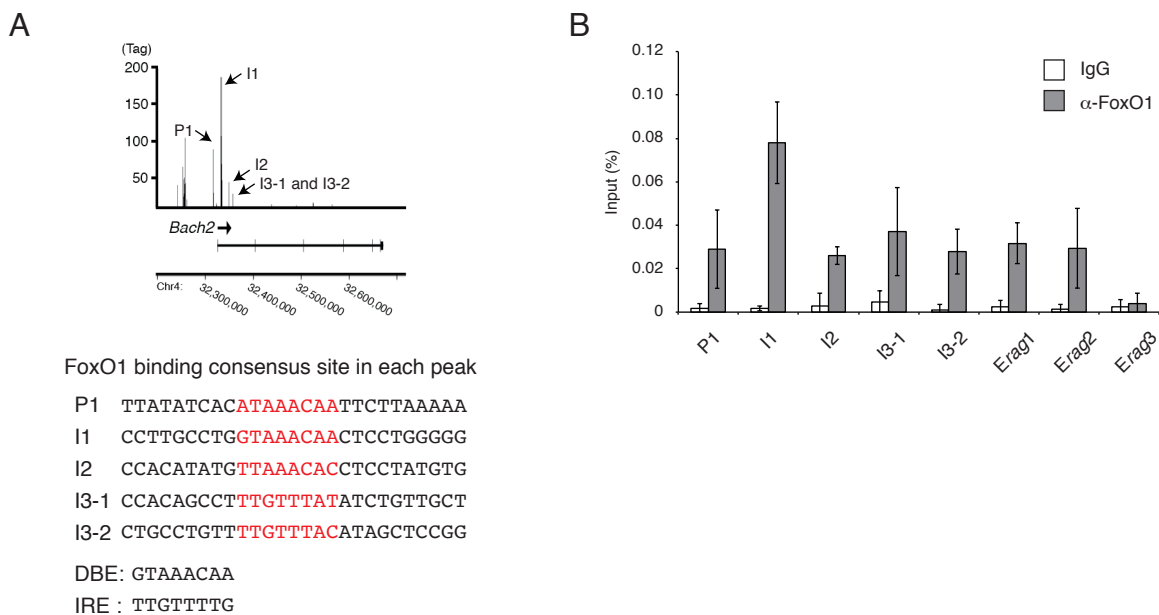


Supplemental Figure 1. Nuclear accumulation of Bach2 protein by attenuation of IL-7 signaling.

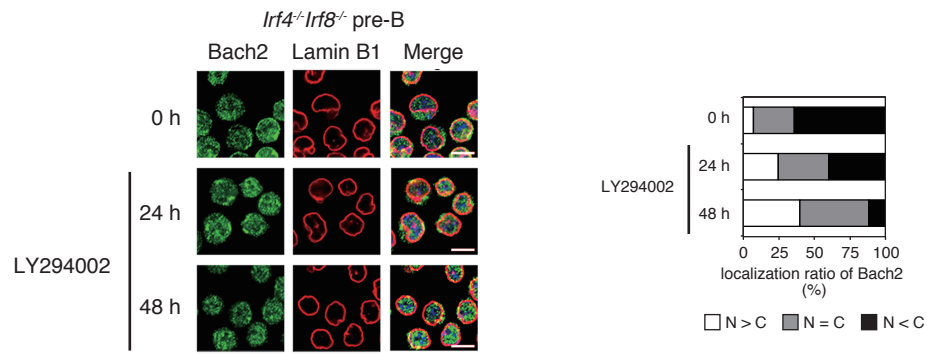
IL-7 signaling was attenuated in *Irf4^{-/-}Irf8^{-/-}* pre-B cells by changing the concentration of IL-7 in the culture medium from 5.0 ng/mL (IL-7 Hi) to 0.1 ng/mL (IL-7 Lo) for indicated hours, and subcellular localization of Bach2 protein were examined by using immunohistochemistry. Left; Bach2 (green) and Lamin B1 (red) distribution in cells. Right; the subcellular localization of Bach2 was evaluated by classification with counted 100 cells for each condition: nucleus-dominant (N > C), nucleus and cytoplasm (N = C), cytoplasm-dominant (N < C). Scale bar = 10 μ m.



Supplemental Figure 2. FoxO1 directly binds to *Bach2* gene locus.

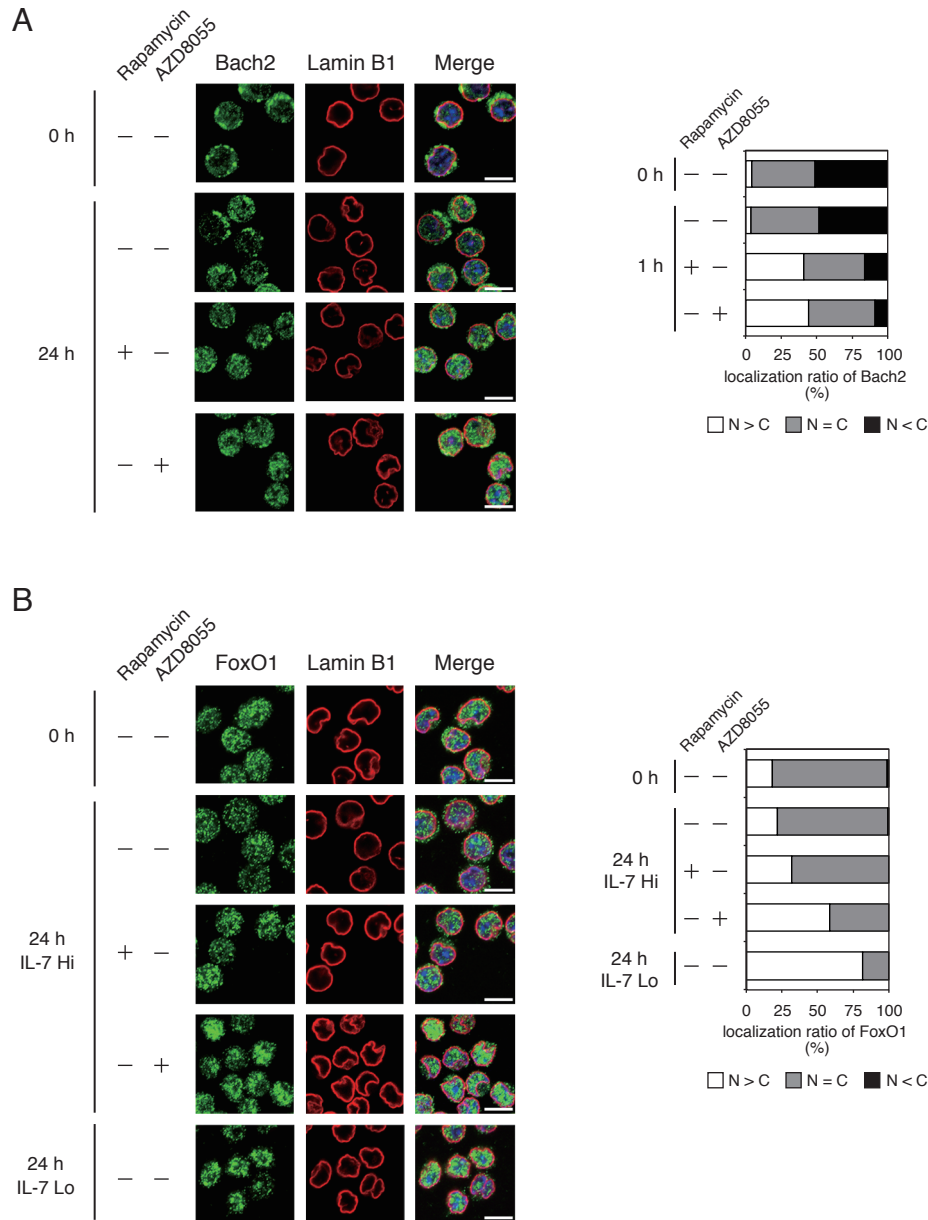
(A) Upper; FoxO1-binding peaks at *Bach2* locus identified by using previously reported ChIPseq (Ochiai K. 2012) and presented with Integrated Genome Browser (IGB) in terms of normalized tag counts. P, binding peak at promoter region; C, binding peaks at coding regions. Lower; FoxO1 consensus in identified regions. DBE, the DAF-16 binding element; IRE, the insulin response element.

(B) FoxO1 ChIP with control immunoglobulin G (IgG) and anti-FoxO1 (α -FoxO1) in *Irf4^{-/-}Irf8^{-/-}* pre-B cells cultured 48 h with IL-7 Lo as in Fig. S1, followed by quantitative PCR analysis of the binding of FoxO1 at *Bach2* locus. *Erag1* and *Erag2* as positive control regions, and *Erag3* as negative control region. The mean and SEM is from three independent experiments.



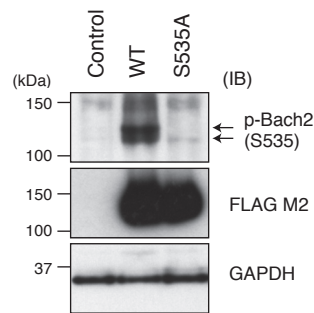
Supplemental Figure 3. PI3K inhibitor promotes nuclear accumulation of Bach2 protein under the presence of IL-7 signaling.

Irf4^{-/-}Irf8^{-/-} pre-B cells were cultured in the presence of LY294002 under IL-7 Hi condition for indicated time, and subcellular localization of Bach2 protein were examined by using immunohistochemistry. Left; Bach2 (green) and Lamin B1 (red) distribution in cells. Right; the subcellular localization of Bach2 was evaluated by classification with counted 100 cells for each condition: nucleus-dominant ($N > C$), nucleus and cytoplasm ($N = C$), cytoplasm-dominant ($N < C$). Scale bar = 10 μ m.



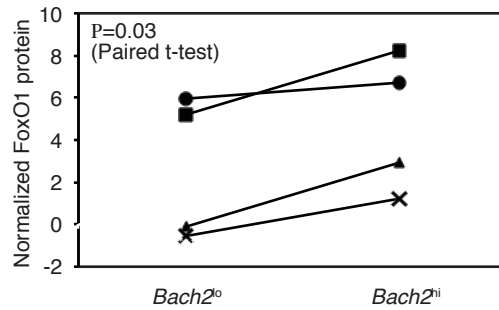
Supplemental Figure 4. Distinct protein regulation of Bach2 and FoxO1 under mTOR signaling.

Irf4^{-/-}*Irf8*^{-/-} pre-B cells were cultured in the presence of 20 nM rapamycin or 500 nM AZD8055 under IL-7 Hi condition for 24 h, and subcellular localization of Bach2 protein (A) or FoxO1 protein (B) were examined by using immunohistochemistry. Left; Bach2 or FoxO1 (green) and Lamin B1 (red) distribution in cells. Right; the subcellular localization of Bach2 or FoxO1 was evaluated by classification with counted 100 cells for each condition: nucleus-dominant (N > C), nucleus and cytoplasm (N = C), cytoplasm-dominant (N < C). Scale bar = 10 μm.



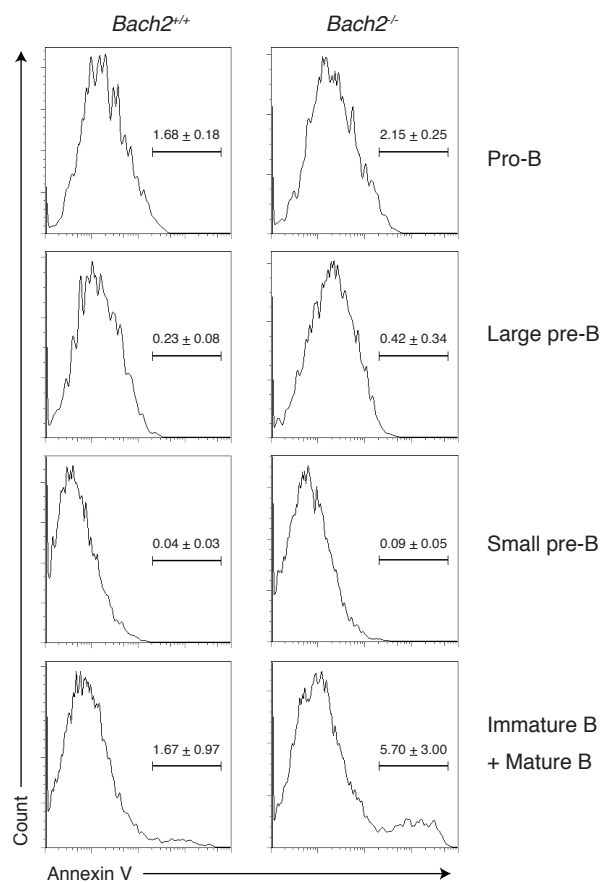
Supplemental Figure 5. Detection of Bach2 phosphorylation of S535 by a generated monoclonal antibody.

Immunoblot analysis using monoclonal antibody against Bach2 phosphorylated at S535. 293-derived Plat-E cells were transfected with pcDNA FLAG-Bach2WT or pcDNA FLAG-Bach2S535A, and whole cell extracts were prepared at 24 h post transfection. Expression of each plasmid was examined with anti-FLAG M2 antibody, and GAPDH was served as internal control. IB, immunoblot; kDa, kilodalton.



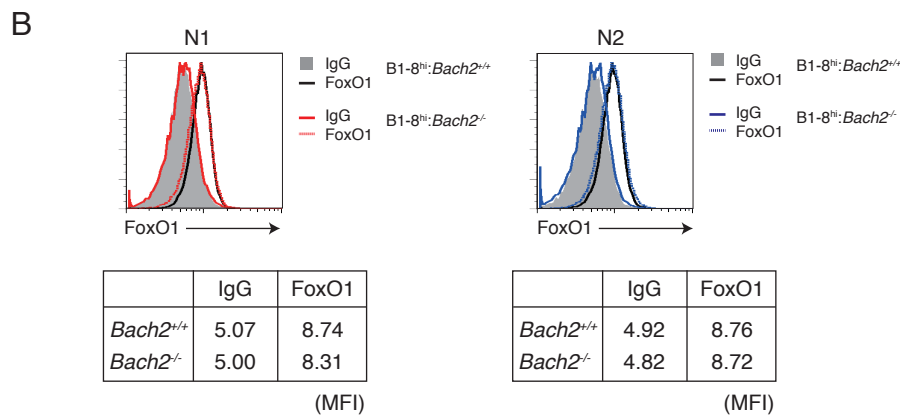
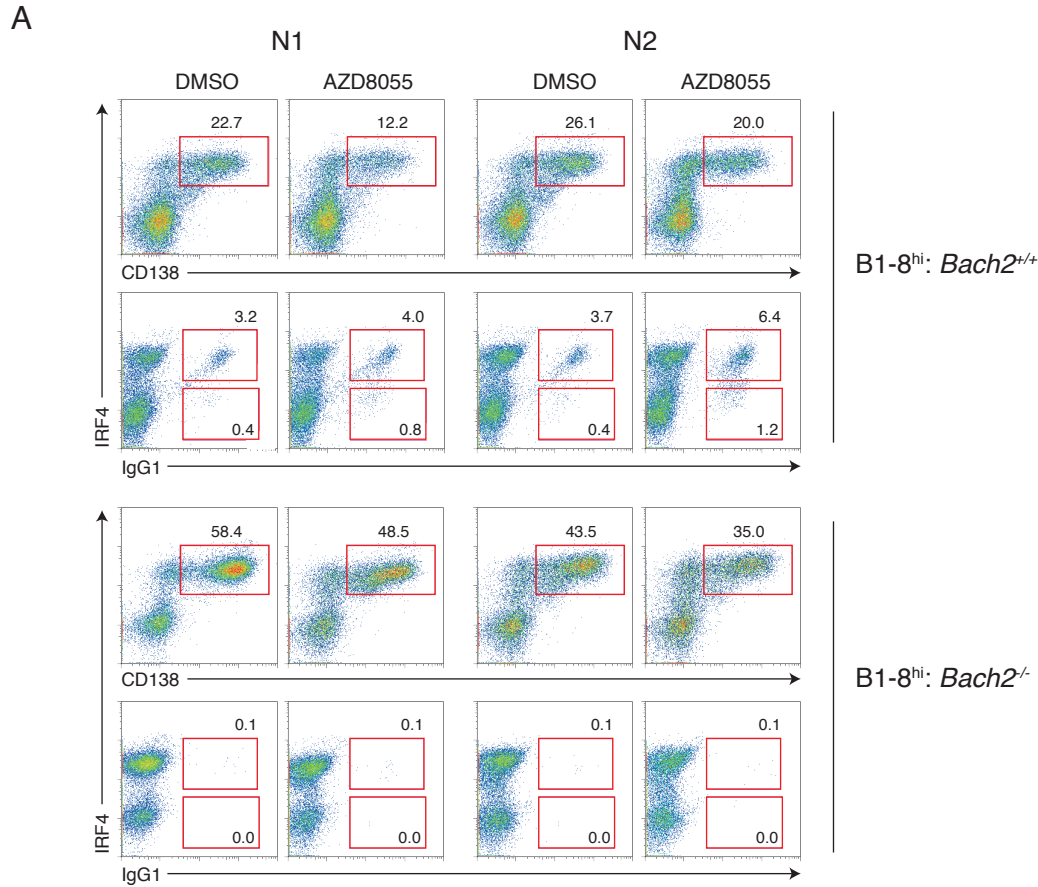
Supplemental Figure 6. The higher FoxO1 protein in *Bach2^{hi}* pre-B cells.

Intracellular staining of FoxO1 in pre-B cells obtained from *Bach2*-RFP reporter mice. *Bach2^{hi}* and *Bach2^{lo}*-RFP indicate population with higher and lower expression of RFP, respectively. FoxO1 protein amounts were analyzed, and the geometric mean fluorescence intensity were subtracted with control IgG staining. Normalized data were shown as a graph with marks which indicate each four mice used for this analysis.



Supplemental Figure 7. The frequency of apoptosis cells were increased in *Bach2^{-/-}* cells.

Bone marrow B cells from wild-type (*Bach2^{+/+}*) and *Bach2*-deficient (*Bach2^{-/-}*) mice were stained with Annexin V, and analyzed using flow cytometry. Data is representative of three mice analysis for each genotype in one experiment, and shown with the means and SEM respectively.



Supplemental Figure 8. *Bach2*-dependent increase of CSR frequency and inhibition of PC with AZD8055 treatment.

(A) Flow cytometry analysis of intracellular IRF4, CD138, and IgG1. Splenic B cells were isolated from *Bach2*^{+/+} and *Bach2*^{-/-} under B1-8^{hi} background, and cultured with IL-2, IL-4, IL-5, CD40L and NP-ficol in the presence of DMSO or AZD8055 for 72 h.

(B) Intracellular staining of FoxO1 protein in splenic B cells isolated from *Bach2*^{+/+} and *Bach2*^{-/-} under B1-8^{hi} background. FoxO1 protein amounts were shown with log scale (x-axis). The geometric mean fluorescence intensity of each data was shown at the bottom of histogram.

For A and B, data are from one experiment using two mice for each genotype.

Supplemental Table 1

GeneSymbol	FC ([4Bach2] vs [MigR])	FC ([Lo] vs [Hi])
Pqbp1	-5.09	-2.93
Fbp1	-2.87	-29.39
Cst7	-1.88	-48.81
Wfdc13	-1.75	-2.09
Iqgap3	-1.75	-35.79
Dhcr7	-1.71	-2.62
Nfix	-1.66	-2.90
Wee1	-1.65	-6.00
Prrc2a	-1.58	-2.30
Myom3	-1.56	-2.78
Xrcc2	-1.55	-6.42
Senp1	-1.50	-4.18
Sh3bgrl3	-1.49	-2.01
Cbx2	-1.49	-2.00
Kif18b	-1.47	-44.21
Cpne2	-1.46	-4.59
Cox6b1	-1.46	-2.62
Sit1	-1.46	-2.39
Ass1	-1.46	-5.81
2310075C17Rik	-1.45	-5.59
Nek6	-1.45	-8.13
Mbni3	-1.44	-2.03
Mt2	-1.44	-6.17
Nek6	-1.43	-6.07
Sh2d3c	-1.43	-2.04
Ppp4c	-1.43	-3.93
Rras2	-1.43	-2.35
Apex1	-1.42	-6.97
Dcakd	-1.41	-3.97
Aacs	-1.40	-4.45
Ubap2l	-1.39	-2.21
Sgsm2	-1.39	-3.46
Cbx5	-1.38	-21.99
Pmf1	-1.38	-14.74
Pgf	-1.38	-2.18
Dip2b	-1.38	-2.10
Ucp3	-1.37	-5.68
Hist1h1b	-1.37	-19.76
Slc43a3	-1.37	-2.50
Fntb	-1.36	-3.41
Crtap	-1.36	-2.42
Eif5a	-1.35	-5.39
McpH1	-1.34	-4.54
Gclm	-1.34	-2.81
Itgb4	-1.34	-3.58
Gapdh	-1.34	-4.01
Gtpbp1	-1.34	-3.12
Atic	-1.34	-4.89
Siah1b	-1.33	-3.06
Fzr1	-1.32	-2.83
Cand2	-1.32	-2.50
Cdk2ap1	-1.32	-5.72
Ube2c	-1.32	-44.32
Zbtb45	-1.32	-3.37
Ccne1	-1.32	-31.85
Kif11	-1.31	-42.62
Calu	-1.31	-2.28
Hcst	-1.31	-3.63
Mgat2	-1.31	-2.45
Ccnd3	-1.31	-2.46
Tmem180	-1.31	-4.06
Ndufa11	-1.31	-2.61
Hspa2	-1.31	-2.24
Stmn1	-1.30	-20.16
Jdp2	-1.30	-5.30

Supplemental Table 2

GO ACCESSION	GO Term	p-value	corrected p-value	Count in Selection	% Count in Selection	Count in Total	% Count in Total
GO:0000278	mitotic cell cycle	7.22E-08	4.27E-04	11	17.460318	446	2.1023853
GO:1903047	mitotic cell cycle process	5.35E-08	4.27E-04	11	17.460318	433	2.041105
GO:0005622	intracellular	1.65E-07	6.50E-04	54	85.71429	11610	54.72801
GO:0044424	intracellular part	1.33E-06	0.004	52	82.53968	11361	53.554256
GO:0007049	cell cycle	2.87E-06	0.007	13	20.63492	933	4.398039
GO:0022402	cell cycle process	4.14E-06	0.007	11	17.460318	673	3.1724334
GO:0051301	cell division	3.93E-06	0.007	10	15.873015	539	2.540775
GO:1902494	catalytic complex	5.26E-06	0.008	11	17.460318	690	3.252569
GO:0005634	nucleus	1.33E-05	0.018	32	50.79365	5391	25.412464
GO:0000280	nuclear division	2.09E-05	0.023	8	12.698413	394	1.857264
GO:0007067	mitotic nuclear division	2.04E-05	0.023	7	11.111111	283	1.3340247
GO:0048285	organelle fission	3.24E-05	0.032	8	12.698413	419	1.9751108
GO:0043229	intracellular organelle	6.77E-05	0.056	45	71.42857	9947	46.888847
GO:0010035	response to inorganic substance	6.79E-05	0.056	6	9.523809	234	1.1030451
GO:0051726 GO:0000000	regulation of cell cycle	7.05E-05	0.056	9	14.285714	605	2.8518903
GO:1990234	transferase complex	7.95E-05	0.059	8	12.698413	476	2.2438014
GO:0008152	metabolic process	8.95E-05	0.062	40	63.49206	8346	39.341946
GO:0043226	organelle	1.01E-04	0.066	45	71.42857	10081	47.520504
GO:0000152	nuclear ubiquitin ligase complex	1.53E-04	0.095	3	4.7619047	35	0.16498539

Supplemental Table 3

primer	Sequence 5' → 3'
[RT-PCR]	
<i>Bach2</i> Forward	AGTTCATCCACGACATCC
<i>Bach2</i> Reverse	AGGTGATTCTCTCCGAC
<i>β2-microglobulin</i> Forward	AGACTGATACATACGCCTGCA
<i>β2-microglobulin</i> Reverse	GCAGGTTCAAATGAATCTTCAG
<i>Pax5</i> Forward	TCTACAGGCTCCGTGACGCA
<i>Pax5</i> Reverse	GAACAGGTCTCCCGCATCT
<i>Rag1</i> Forward	CTGCAGACATTCTAGCACTC
<i>Rag1</i> Reverse	AACTGAAGCTCAGGGTAGAC
<i>Rag2</i> Forward	CCTTCAGTGCCAAAATAAGA
<i>Rag2</i> Reverse	TCATAAGTGAGAAGCCTGGT
<i>p27</i> Forward	CTGACTCGTCAGACAATCC
<i>p27</i> Reverse	TTCTTAATTCGGAGCTGTTT
<i>Aiolos</i> Forward	GGACACCTTAGGACACATTC
<i>Aiolos</i> Reverse	CTCTGCTTGTAGCTTCTTCC
<i>Ccnd3</i> Forward	GAAGCTTGCATCTATACGG
<i>Ccnd3</i> Reverse	ATCGCAAAGGTGTAATCTGT
<i>Ccne1</i> Forward	CCCCAGGACTGCATTTACAGC
<i>Ccne1</i> Reverse	TGACGCTGCAGAAAGTGCTCA
[ChIP-PCR]	
<i>Foxo1</i> P1 Forward	TTAGCACTGTTCGGGTTG
<i>Foxo1</i> P1 Reverse	CCCAAAGAGTCAGTGAGACA
<i>Foxo1</i> I1 Forward	GAAGAGAGGCTGACTGGTTA
<i>Foxo1</i> I1 Reverse	GGTAGCAAGCAACTCTGACT
<i>Foxo1</i> I2 Forward	TCTCCCTCATTCAACACTTC
<i>Foxo1</i> I2 Reverse	TAACCGAAGCACAGTTCCCT
<i>Foxo1</i> I3-1 Forward	GGGTGTGACTAGCAAGTGAAG
<i>Foxo1</i> I3-1 Reverse	AGGCTAGGCGCTTTGTTT
<i>Foxo1</i> I3-2 Forward	AAGATGCTGTACCTGACTT
<i>Foxo1</i> I3-2 Reverse	CAGCATTTCACTTTCTGA
<i>Erag1</i> Forward	ACACCCATAATGGGCCGTGAAC
<i>Erag1</i> Reverse	CAGAACCCGAGGGCTTAGCATT
<i>Erag2</i> Forward	AACTTCCTCCAGCAGGCGATCT
<i>Erag2</i> Reverse	ACCCATTTCCAAGCAGGAGAGG
<i>Erag3</i> Forward	AAGCCTCTCTTTGTACCCAACCTCAC
<i>Erag3</i> Reverse	TTGACTGTGCTAGTTCAGCCAAAGGAAT
<i>Ccne1</i> Forward	TGCATTTGGTCTTTCATCAT
<i>Ccne1</i> Reverse	TGGACCCTATAACCGCACT
<i>Ccnd3</i> -1Forward	TTACAGGCATGGAAAGTACC
<i>Ccnd3</i> -1Reverse	TAGGACTCACGTCATTAGGC
<i>Ccnd3</i> -2 Forward	CCCTTTCCGCCTAGAAAT
<i>Ccnd3</i> -2 Reverse	TGGACCAAGTGTGAATACCA
<i>Hmox1</i> Forward	GGGCTAGCATGCGAAGTGAG
<i>Hmox1</i> Reverse	AGACTCCGCCCTAAGGGTTC