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Α



**Appendix Figure S1 | Pten-deficient B cells are capable of acquiring an anergic phenotype** (related to Fig. 2) **A** | Mice of the indicated genotypes were sacrificed and mature splenic B cells were enriched by magnet-activated cell sorting (MACS). The purification efficiency was determined by flow cytometry (CD19/B220). IgM/IgD surface expression was analyzed in the Thy1.2<sup>-</sup> population, which was used for measurement of Ca<sup>2+</sup> mobilization in Fig. 2B. Mature splenic B cells that did not show Ca<sup>2+</sup> mobilization in Fig. 2B were stimulated with ionomycin to verify their capacity to induce Ca<sup>2+</sup> influx (right).

**B** | Splenic B cells from Pten<sup>ff</sup> x mb1-cre x 3-83<sup>ki</sup> mice on the respective background (H2-K<sup>d</sup>: w/o Ag, n = 4; H2-K<sup>b</sup>: + Ag, n = 5) were purified and cultured for 7 days in presence of either IL-4 (10 ng/ml), LPS (2.5  $\mu$ g/ml), the combination of both substances, or were left untreated (n.t. = non-treated). Percentages of viable B cells, determined at days 2, 3, 5 and 7 by flow cytometry, were normalized to those measured at day 1, (RU = relative units, mean ±SEM). Statistical significance was calculated by applying the Mann-Whitney U-test, n.s. = not significant, \* p ≤ 0.05.



Appendix Figure S2 | Pten is important for development of Fo.B cells (related to Fig. 3)

Immunohistochemistry of sections from spleens of *Pten<sup>tif</sup>*, *Pten<sup>tif</sup>*x mb1-cre, *Pten<sup>tif</sup>*x mb1-cre x *3-83<sup>ki</sup>* and *Pten<sup>tif</sup>* x mb1-cre x *MD4<sup>tg</sup>* mice for CD169 (green), CD5 (red) and IgM (cyan) at 10x magnification. Shown pictures are representative of 2-3 mice per genotype and display an overview of the whole section. Pictures shown in Fig. 3E represent enlarged views of the areas indicated by the white squares, respectively.



#### Appendix Figure S3 | Investigating direct FoxO1-binding to Fo.B cell-specific genes (related to Fig. 4)

A | Sequence of FoxO1-binding motif obtained from the JASPAR database.

**B** | Sequence of two FoxO1-binding motifs in the *Pax5* gene.

**C** | Schematic overview of the *Zfp318*, *Ell2* and *Fcer2* gene loci showing exons (gray boxes), untranslated regions (UTR, white boxes) and potential FoxO1-binding sites (arrows): 8/8 nucleotides matching FoxO1 consensus-sequence shown in Fig. EV3A (red), 7/8 nucleotides matching FoxO1 consensus-sequence (orange), sequences similar to P1 (blue) and P2 (green) shown in Fig. EV3B.

**D** | FoxO1 ChIP from mature B cells purified from spleens of WT mice (n = 4, except for Ell2-10 n = 3, mean  $\pm$ SD). Sequences comprising the potential FoxO1-binding sites (BS) were amplified by quantitative PCR (for primer sequences see Appendix Tables S12 - 14). Amplification of a sequence containing no predicted FoxO1-binding site 3.5 kb downstream of the 3'-UTR in each gene was used as a negative control (-3.5 kb ctrl). Statistical significance was calculated by using the Kruskal-Wallis test between the data obtained for the individual binding sites and the -3.5 kb ctrl, n.d. = product not detectable on fragmented ChIP-enriched DNA, n.s. = not significant.

## Supplemental information

#### Appendix Table S1: related to Fig. 2C

Kruskal-Wallis test: Dunn's multiple comparisons test		Adjusted p	value
Pten <sup>f/f</sup>	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> -Ag	> 0.9999	n. s.
Pten <sup>f/f</sup>	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> +Ag	< 0.0001	****
<i>Pten<sup>t/f</sup>x</i> mb1-cre	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> -Ag	> 0.9999	n. s.
<i>Pten<sup>f/f</sup></i> x mb1-cre	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> +Ag	< 0.0001	****

## Appendix Table S2: related to Fig. 2D

Kruskal-Wallis test: Dunn's multiple comparisons test		Adjusted p	value
Pten <sup>f/f</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> -Ag	0.0129	*
Pten <sup>t/f</sup>	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> +Ag	0.0288	*
<i>Pten<sup>f/f</sup></i> x mb1-cre	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> -Ag	0.0723	n. s.
<i>Pten<sup>f/f</sup></i> x mb1-cre	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> +Ag	0.0003	***

#### Appendix Table S3: related to Fig. 2E

Kruskal-Wallis test: Dunn's multiple comparisons test		Adjusted p	value
Pten <sup>t/f</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> -Ag	0.0720	n. s.
Pten <sup>f/f</sup>	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> +Ag	> 0.9999	n. s.
Pten <sup>t#</sup> x mb1-cre	<i>Pten<sup>t/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> -Ag	0.1240	n. s.
<i>Pten<sup>f/f</sup></i> x mb1-cre	<i>Pten<sup>f/f</sup></i> x mb1-cre x 3-83 <sup>ki</sup> +Ag	< 0.0001	****

#### Appendix Table S4: related to Fig. 3D

Kruskal-Wallis test: Dunn's multiple comparisons test		Adjusted p	value
5 mg/ml (row 1)			
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0003	***
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.
Dilution 1:3 (row 2)			
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	Pten <sup>f/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0003	***
<i>Pten<sup>f/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>f/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.
Dilution 1:9 (row 3)			
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0011	**
<i>Pten<sup>f/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>f/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.
Dilution 1:27 (row 4)			
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0017	**
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.
Dilution 1:81 (row 5)			
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	Pten <sup>f/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0024	**
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.
Dilution 1:243 (row 6)			
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	Pten <sup>f/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0014	**
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.

Dilution 1:729 (row 7)			
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	Pten <sup>f/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0006	***
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.
Dilution 1:2187 (row 8)			
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0005	***
<i>Pten<sup>f/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>f/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.

## Appendix Table S5: related to Fig. 6A

Kruskal-Wallis test: Dunn's multiple comparisons test		Adjusted p value	
IgM MFI			
Pten <sup>f/f</sup>	Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	0.0002	***
Pten <sup>t/f</sup> x mb1-cre	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	< 0.0001	****
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	< 0.0001	****
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	0.0627	n. s.
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.
IgD MFI			
Pten <sup>f/f</sup>	Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	0.1001	n. s.
Pten <sup>t/f</sup> x mb1-cre	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	0.2980	n. s.
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	> 0.9999	n. s.
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	<i>Pten<sup>f/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	0.0032	**
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	> 0.9999	n. s.

## Appendix Table S6: related to Fig. 6C

Mann-Whitney U-test:		P value	
cHEL			
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	Pten <sup>f/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.2468	n. s.
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	0.5368	n. s.
Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	0.9307	n. s.
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	0.1255	n. s.
sHEL			
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.0043	**
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	0.0087	**
Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	0.0087	**
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	0.2468	n. s.
Anti-Kappa			
Pten <sup>t/f</sup> x MD4 <sup>tg</sup>	Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	0.2468	n. s.
Pten <sup>f/f</sup> x MD4 <sup>tg</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	0.5368	n. s.
Pten <sup>t/f</sup> x MD4 <sup>tg</sup> x ML5 <sup>tg</sup>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	0.6623	n. s.
<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i>	<i>Pten<sup>t/f</sup></i> x mb1-cre x <i>MD4<sup>tg</sup></i> x <i>ML5<sup>tg</sup></i>	0.5368	n. s.

Mann-Whitney U-test:	P value	
WT: PBS vs. TNP-Ova		
10 µg/ml (row 1)	0.0095	**
Dilution 1:3 (row 2)	0.0095	**
Dilution 1:9 (row 3)	0.0095	**
Dilution 1:27 (row 4)	0.0095	**
Dilution 1:81 (row 5)	0.0095	**
Dilution 1:243 (row 6)	0.0095	**
Dilution 1:729 (row 7)	0.0143	*
Dilution 1:2187 (row 8)	0.1333	n. s.
IgD <sup>≁</sup> : PBS vs. TNP-Ova		
10 µg/ml (row 1)	0.1017	n. s.
Dilution 1:3 (row 2)	0.1212	n. s.
Dilution 1:9 (row 3)	0.0931	n. s.
Dilution 1:27 (row 4)	0.1320	n. s.
Dilution 1:81 (row 5)	0.0281	*
Dilution 1:243 (row 6)	0.1429	n. s.
Dilution 1:729 (row 7)	0.5000	n. s.
Dilution 1:2187 (row 8)	0.1786	n. s.
Overlay: WT vs. lgD <sup>-/-</sup>		
10 µg/ml (row 1)	0.0022	**
Dilution 1:3 (row 2)	0.0022	**
Dilution 1:9 (row 3)	0.0022	**
Dilution 1:27 (row 4)	0.0411	*
Dilution 1:81 (row 5)	0.1320	n. s.
Dilution 1:243 (row 6)	0.1320	n. s.
Dilution 1:729 (row 7)	0.1234	n. s.
Dilution 1:2187 (row 8)	0.1905	n. s.

## Appendix Table S7: related to Fig. 7G

## Supplemental Experimental Procedures

#### Appendix Table S8 | Antibodies used in flow cytometric analyses

Antigen	Species	Conjugate	Clone	Company
CD19	mouse	PerCP-Cy5.5	1D3	BD
CD21/CD35	mouse	APC or PE-Cy7	7E9	BioLegend
CD23	mouse	PE or Biotin	B3B4	BD
CD38	mouse	APC-Cy7	90	BioLegend
CD43	mouse	FITC	S7	BD
CD45R (B220)	mouse	PE-Cy7	RA3-642	eBioscience
CD45R (B220)	mouse	BV510	RA3-6B2	BioLegend
CD90.2	mouse	PE	53-2,1	BD
CD95	mouse	PE-Cy7	Jo2	BioLegend
GL-7	mouse	PerCP-Cy5.5	GL7	BioLegend
lgD	human	APC	IA6-2	BioLegend
lgD	mouse	FITC or Biotin	11-26	SouthernBiotech
lgD	mouse	APC	11-26	eBioscience
lgM	human	Alexa Fluor 647	polyclonal	Jackson
IgM	mouse	FITC	polyclonal	SouthernBiotech
IgM	mouse	eFluor 450	eB121-15-F9	eBioscience
IgM	mouse	PE-Cy7	2/41	eBioscience
кLC	mouse	APC-Cy7	187.1	BD
кLC	mouse	PE	polyclonal	SouthernBiotech
кLC	human	Biotin	polyclonal	SouthernBiotech
λLC	human	Biotin	polyclonal	SouthernBiotech

#### Appendix Table S9 | Antibodies used for immunohistochemistry

#### (related to Experimental Procedures)

Antigen	Conjugate	Clone	Company
CD169	FITC	MOMA-1	AbD serotec
CD5	PE	53-7.3	eBioscience
lgM	Cy5	polyclonal	Jackson Immunoresearch
PNA	СуЗ	-	vector laboratories

#### Appendix Table S10 | Primers used for analysis of *LC* gene recombination

Primers	Sequence $5' \rightarrow 3'$	
5' Vĸ	ggc tgc ags ttc agt ggc agt ggr tcw ggr ac	
3' of J <i>ĸ</i> 5	atg cga cgt caa ctg ata atg agc cct ct cc	
Srp20-F	gat tac cgc agg agg agt	
Srp20-R	ag aac gga tga ttg gga a	

Appendix Table S11 | TaqMan-probe mixes used for qPCR analyses

(related to Experimental Procedures)

TaqMan-probe mixes (Applied Biosystems)

lghm	Mm01718957_g1
lghd	Mm03979980_s1
Cstf64	Mm00500041_m1
Ell2	Mm00507237_m1
Zfp318	Mm01273666_m1
Fcer2	Mm00442792_m1
CD79b	Mm00434143_m1
Gapdh	Mm99999915_g1

#### Chromatin Immunoprecipitation (ChIP)

Mature B cells were purified from spleens of control mice. Proteins and DNA were crosslinked in 0.75% formaldehyde (Roth). Lysates were prepared and subjected to sonication by using a Bioruptor (Diagenode). DNA fragmentation (100 - 700 bp) was verified by gel electrophoresis. Lysates were pre-cleared with protein G dynabeads (invitrogen) and half of the volume was immunoprecipitated with  $\alpha$ -FoxO1-antibody (C29H4; Cell Signaling). Precipitated protein-DNA complexes were isolated with protein G dynabeads, decrosslinked and enriched DNA was further purified by using the QIAquick PCR purification kit (Qiagen).

Quantitative PCR was performed by using Maxima SYBR qPCR Master Mix (Thermo Fisher Scientific) and combinations of the primers listed in Appendix Tables S12-14. Specificity of the primer pairs was previously verified by PCR on unfragmented genomic DNA.

# **Appendix Table S12** | Primers used for qPCR amplification of sequences with potential FoxO1-binding sites in the *Zfp318* gene (related to Experimental Procedures)

Binding site	Sequence $5' \rightarrow 3'$ for	Sequence 5' $\rightarrow$ 3' rev
Zfp318-1/2	cac ttc ctg tga gct gat gga tt	cca cct cac tgt tga ccc aaa
Zfp318-3	cag ggg cta cct gta agg tct ta	cat gct cag aga ggc tca tgt
Zfp318-4	gct tat gac att tat aaa taa gca aat cgg ctt	ggc att ggt ggc aca aac ttt
Zfp318-5	ccc tta ccg tga tag aca aca at	ccg ctc tgg tgt gct aac aca
Zfp318-6	ggc aaa cac tat ggc att gag cta t	cgc atg tta gat tgt att ttc cca ttg
Zfp318-7	ggg ctg cta gtc ctg aca aa	cac agc acc cat atg gtg gtt
Zfp318-8	ggg ctg ata ata cat ggc ttt tct	gag ttt tct agg cca gct agg aat
Zfp318-9	gcc cac agc gtt ctg gga ta	cct ccc gag cta cac agt aa
Zfp318-10	ccg aaa ctt ctc cca agc taa taa t	cac ctc cat cag aag tgg atc ttt
Zfp318-11	tgc cta ggt ggt ccc agt t	ggg tct ggt gga tgt tgt tg
Zfp318 -3.5 kb ctrl	cca gta tcc aga tac ctt gac cca tat	gag ggc aat tgt gca cta tga at

## Appendix Table S13 | Primers used for qPCR amplification of sequences with

potential FoxO1-binding sites in the *Ell2* gene

Binding site	Sequence 5' $\rightarrow$ 3' for	Sequence 5' $\rightarrow$ 3' rev
Ell2-1	atg gtg gga cag gac tgt aat	ggg gag ggg ata gtg gat ttt
Ell2-2	gga gat ttc taa gtg cct gtg tga aa	agc aag cac gtg gca gaa a
Ell2-3	cag agt gcc tat ccc tgc ttt	cgc ctg ggc ttc aaa cca a
Ell2-4	cat gac cac cac tca aaa cac att	agg ctg tct caa gag agc tttt
Ell2-5	ggt tca ccg aga gtt agg aat ata taa gc	gca tcc cat gcc cat tta caa t
Ell2-6/7	agg agc cac agt tac agc taa	gac aag gga aaa tac cac ttt ttc aaa cat
Ell2-8	cat gga tgg ggt cct act aga aat	tgc tca cca ggg ttc tag gat t
Ell2-9	gtg tgg aaa atg ctt agg gtt t	agg act acc ata atc gtg cag act ta
Ell2-10	gca agc aca cac cca caa	cca cca tac cca gtt gag aac taa ac
Ell2-11	cca cct cgg agt tta gaa cca aa	ccc agc agg aaa gga tca agt
Ell2-12	agt act gtt ttg ccc atg caa at	cca aag ggt cag act aag gag att
Ell2-13	acc aag gta gca gga gag aag t	cac gtg cgc ata gta gga tt
Ell2-14	tcc ctg gaa gct gtg tgt taa g	gta gtg gta ctg agt agg acg ttt
Ell2-15	gtg ctg cca gtt gaa aag g	ctg cca caa ggc ctc agt act aa
Ell2-16	cac agt ggc tgc ctg tag aa	cct ata tgc aag ttg cga cca atg
Ell2-17	gga ggt gag agc cta gtg cat aa	tgg gct gat tac tga cgt cag a
Ell2 -3.5 kb ctrl	caa tga cct tgt ctg gcc tct at	gac tct cta gtg agg ggc tgt aa

## Appendix Table S14 | Primers used for qPCR amplification of sequences with

potential FoxO1-binding sites in the *Fcer2* gene

Binding site	Sequence $5' \rightarrow 3'$ for	Sequence 5'→ 3' rev
Fcer2-1	gtg tga cga tgt gtc tgg ctt a	agg gct cac aca gaa gca tat
Fcer2-2	gac agg tga cca gag gca aa	gca gga ggt cct gac ttg tat aag
Fcer2-3	act ggg caa agc tga gca ta	acc cat ggg aaa tgg cac tat t
Fcer2-4	ggc agg ccc att aga ata aca t	ggg tgc agt taa ctt ctt cca ac
Fcer2-5	gcc aca acc cca tag cat t	cac tgg gcc tgg gtt tt
Fcer2-6	gga agg cgg ctc agt agg taa a	gtg ctt acc cac tga acc ttg t
Fcer2-7	gga tcc aaa gcc tgg gga gat a	acc aga ccc gtt gtt aca gat
Fcer2 -3.5 kb ctrl	ggt aca gtg cac tcc gag cat a	cca tgt ggg tgc tgg aat