

Supplementary material

Supplementary table 1 – Normalized expression values per sample, mean and standard deviation of Forkhead box N1 (*FOXN1*) gene.

FOXN1			
Group	Sample Age	Normalized gene expression value	Mean SD per group
Posnatal	5 days-old	0,266258729	0,2940 0,06941
	1 year-old	0,242802884	
	1 year-old	0,373019084	
Child-Adolescent	10 years-old	0,240222927	0,2534 0,02782
	17 years-old	0,285327986	
	14 years-old	0,234575866	
Adult	49 years-old	0,067409705	0,1056 0,04663
	57 years-old	0,113462441	
	59 years-old	0,122242775	
	66 years-old	0,054146431	
	75 years-old	0,170809434	

Supplementary table 2 – Non-parametric One-Way ANOVA (Kruskal-Wallis test) followed by Dunn’s multiple comparison test of normalized expression values of *FOXN1* gene.

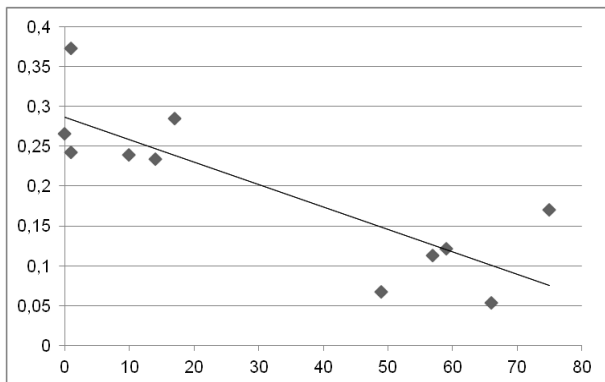
Non-parametric One-Way ANOVA - <i>FOXN1</i> expression			
Kruskal-Wallis test			
P value	0,0195		
Exact or approximate P value?	Gaussian Approximation		
P value summary	*		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	3		
Kruskal-Wallis statistic	7,879		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Posnatal vs Child-Adolescent	1,667	No	Ns
Posnatal vs Adult	6,333	Yes	0,0195*
Child-Adolescent vs Adult	4,667	No	Ns

Supplementary table 3 – Non-parametric *t* test of normalized expression values of *FOXN1* gene.

Non-parametric 't" test - <i>FOXN1</i> expression	
Mann Whitney test	<i>p</i> value (two-tailed)
Posnatal vs Child-Adolescent	0,4000
Posnatal vs Adult	0,0357*
Child-Adolescent vs Adult	0,0357*

Supplementary figure 1 - Spearman linear regression analysis of *FOXN1* expression data set. A: regression plot, B: statistical summary, C: input data. The results show that the data is statistically significant in fact without age grouping.

A



B

Age	FOXN1 normalized expression values
0,01	0,266258729
1	0,242802884
1	0,373019084
10	0,240222927
17	0,285327986
14	0,234575866
49	0,067409705
57	0,113462441
59	0,122242775
66	0,054146431
75	0,170809434

C

Number of XY Pairs	11
Spearman r	-0,7517
95% confidence interval	-0.9342 to -0.2573
P value (two-tailed)	0,0098
P value summary	**
Exact or approximate P value?	Exact
Is the correlation significant? (alpha=0.05)	Yes

Supplementary table 4 – Normalized expression values per sample, mean and standard deviation (SD) of Delta-like 1 (*DLL1*) gene.

<i>DLL1</i>			
Group	Sample age	Normalized expression value	Mean SD per group
Posnatal	5 days-old	0,070445716	0,05495 0,03622
	1 year-old	0,098867962	
	1 year-old	0,02614221	
	1 year-old	0,024350335	
Child-Adolescent	7 years-old	0,08201404	0,1605 0,09197
	10 years-old	0,084885469	
	17 years-old	0,266056597	
	14 years-old	0,209041312	
Adult	49 years-old	0,17860024	0,5932 0,4084
	57 years-old	1,199760048	
	66 years-old	0,269715036	
	75 years-old	0,742344941	
	78 years-old	0,575636735	

Supplementary table 5 – Non-parametric One-Way ANOVA (Kruskal-Wallis test) followed by Dunn's multiple comparison test of normalized expression values of *DLL1* gene.

Non-parametric One-Way ANOVA - <i>DLL1</i> expression			
Kruskal-Wallis test			
P value	0,0139		
Exact or approximate P value?	Gaussian Approximation		
P value summary	*		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	3		
Kruskal-Wallis statistic	8,558		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Posnatal vs Child-Adolescent	-3,500	No	ns
Posnatal vs Adult	-7,600	Yes	0,0139*
Child-Adolescent vs Adult	-4,100	No	ns

Supplementary table 6 – Non-parametric *t* test of normalized expression values of *DLL1* gene.

Non-parametric 't' test - <i>DLL1</i> expression	
Mann Whitney test	<i>p</i> value (two-tailed)
Posnatal vs Child-Adolescent	0,1143
Posnatal vs Adult	0,0159*
Child-Adolescent vs Adult	0,0635

Supplementary table 7 – Normalized expression values per sample, mean and standard deviation (SD) of Delta-like 4 (*DLL4*) gene.

<i>DLL4</i>				
Group	Sample age	Normalized expression value	Mean	SD per group
Posnatal	1 year-old	0,060775	0,06134	0,006366
	1 year-old	0,055274		
	1 year-old	0,067968		
Child-Adolescent	7 years-old	0,384012	0,4568	0,07953
	10 years-old	0,43852		
	17 years-old	0,570134		
	14 years-old	0,434496		
Adult	49 years-old	0,480831	0,7606	0,3624
	57 years-old	0,293505		
	57 years-old	0,922305		
	66 years-old	0,622254		
	75 years-old	0,959233		
	78 years-old	1,285413		

Supplementary table 8 – Non-parametric One-Way ANOVA (Kruskal-Wallis test) followed by Dunn's multiple comparison test of normalized expression values of *DLL4* gene.

Non-parametric One-Way ANOVA - <i>DLL4</i> expression			
Kruskal-Wallis test			
P value	0,0205		
Exact or approximate P value?	Gaussian Approximation		
P value summary	*		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	3		
Kruskal-Wallis statistic	7,775		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Posnatal vs Child-Adolescent	-4,750	No	ns
Posnatal vs Adult	-7,667	Yes	0,0205
Child-Adolescent vs Adult	-2,917	No	ns

Supplementary table 9 – Non-parametric *t* test of normalized expression values of *DLL4* gene.

Non-parametric 't' test - <i>DLL4</i> expression	
Mann Whitney test	<i>p</i> value (two-tailed)
Posnatal vs Child-Adolescent	0,0571
Posnatal vs Adult	0,0238*
Child-Adolescent vs Adult	0,1714

Supplementary table 10 – Normalized expresssion values per sample, mean and standard deviation (SD) of Wingless-type MMTV integration site family, member 4 (*WNT4*) gene.

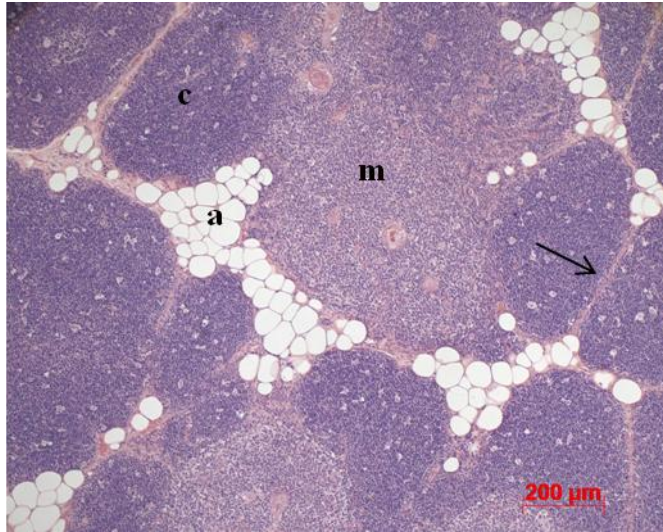
WNT-4			
Group	Sample Age	Normalized expression value	Mean SD per group
Posnatal	5 days-old	0,446514	0,3564 0,1295
	1 year-old	0,207967	
	1 year-old	0,414811	
Child-Adolescent	7 years-old	0,431773	0,5333 0,1531
	10 years-old	0,409908	
	17 years-old	0,744537	
	14 years-old	0,546798	
Adult	49 years-old	0,145398	0,3843 0,2591
	57 years-old	0,293505	
	57 years-old	0,173372	
	75 years-old	0,73409	
	78 years-old	0,575031	

Supplementary table 11 – Non-parametric One-Way ANOVA (Kruskal-Wallis test) followed by Dunn's multiple comparison test of normalized expression values of *WNT4* gene.

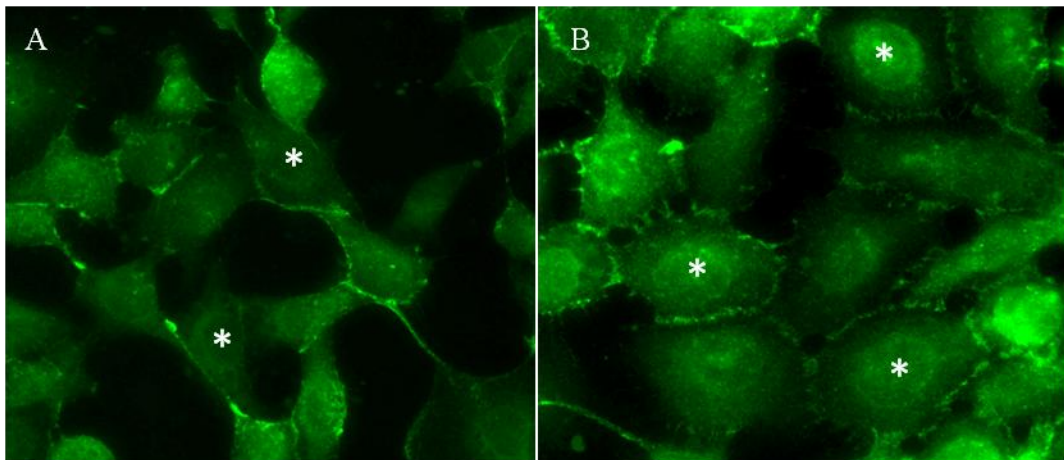
Non-parametric One-Way ANOVA - WNT-4 expression			
Kruskal-Wallis test			
P value	0,4931		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	3		
Kruskal-Wallis statistic	1,414		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Posnatal vs Child-Adolescent	-2,583	No	ns
Posnatal vs Adult	0,06667	No	ns
Child-Adolescent vs Adult	2,650	No	ns

Supplementary table 12 – Non-parametric *t* test of normalized expression values of *WNT4* gene.

Non-parametric 't' test - WNT-4 expression	
Mann Whitney test	p value (two-tailed)
Posnatal vs Child-Adolescent	0,4000
Posnatal vs Adult	1,0000
Child-Adolescent vs Adult	0,4127



Supplementary figure 2 – Human thymus histology. Photomicrograph show morphological aspects of a thymus sample from 14 years-old donor (“Child-Adolescent” group). Paraffin sections were stained with haematoxylin-eosin stain and analyzed by light microscopic examination. (4x, objective magnification). c= cortical region; m= medullary region; a= Adipose tissue; arrow= connective tissue septum.



Supplementary figure 3 – Beta-catenin nuclear translocation in hTEC after treatment with LiCl for 6 hrs. Non-treated TEC monolayer (**A**) and LiCl-treated cells (**B**) were incubated with a monoclonal anti- β -catenin antibody followed by secondary reaction with Alexa 488 conjugated anti-mouse Ig antibody. (*) β -catenin staining in hTEC nucleus. 40x, objective magnification.

A

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C20_Gen_Ed      --AGGCGTATGTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCGA
[2] C20_A03      -GAGGCGTATGTTTTTCGGTTTTTCGTTTAGTTTCGTTTGGTTGTGTTGTGCGA
[3] C20_A07      --AGGTCATGTTTTTTTGGTTTTTCGTTTA-TTTCGTTTGGTTCCGCTTGTTTGTGCGA
[4] C20_A09      -GAGGTCATGTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCGA
[5] C20_B01      AGAGGCGTATGTTTTTTGGTTTTTCGTTTAGTTTCGTTTGGTTGTGTTGTGCGA
[6] C20_B03      AGAGGCGTATGTTTTTCGGTTTTTCGTTTAGTTTCGTTTATTCCGCTTGTTTGTGCGA
                  ****
C20_Gen_Ed      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTTT-
[2] C20_A03      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTATGTTGATTTTTAGGTTGGGTTTTTA
[3] C20_A07      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTATGTTGATTTTTAGGTTCCGCGTTTTTA
[4] C20_A09      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTATGTTGATTTTTAGGTTCCGCGTTTTTA
[5] C20_B01      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTTTTA
[6] C20_B03      GTTTAGTTTTTCGTTTATAGGTTTGTGAGGACGTTGATTTTTAGGTTGGGTTTTTA
                  ****
C20_Gen_Ed      -----
[2] C20_A03      TTTATTTTTAGAGTTAGAAATTTGTTTTTA
[3] C20_A07      TTTATTTTTAGAGTTAGAAATTTGTTTTTC
[4] C20_A09      TTTATTTTTAGAGTTAGAAATTTGTTTTTA
[5] C20_B01      TTTATTTTTAGAGTTAGAAATTTGTTTTTA
[6] C20_B03      TTTATTTTTAGAGTTAGAAATTTGTTTTTA

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B

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C20_Gen_Ed      ---AGGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCG
[2] C20_B05      ----GGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCG
[3] C20_B07      ----AGGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCG
[4] C20_B09      ----AGGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCG
[5] C20_C05      GGAGAGGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCG
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[7] C20_C09      ----CGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCG
                  ****
C20_Gen_Ed      GAGTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTT
[2] C20_B05      GAGTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTT
[3] C20_B07      GAGTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTT
[4] C20_B09      GAGTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTT
[5] C20_C05      GAGTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTT
[6] C20_C07      GAGTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTT
[7] C20_C09      GAGTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTT
                  ****
C20_Gen_Ed      T-----
[2] C20_B05      TATTTATTTTTAGAGTTAGAAATTTGTTTTTA
[3] C20_B07      TATTTATTTTTAGAGTTAGAAATTTGTTTTTA
[4] C20_B09      TATTTATTTTTAGAGTTAGAAATTTGTTTTTA
[5] C20_C05      TATTTATTTTTAGAGTTAGAAATTTGTTTTTA
[6] C20_C07      TATTTATTTTTAGAGTTAGAAATTTGTTTTTG
[7] C20_C09      TATTTATTTTTAGAGTTAGAAATTTGTTTTT-
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C

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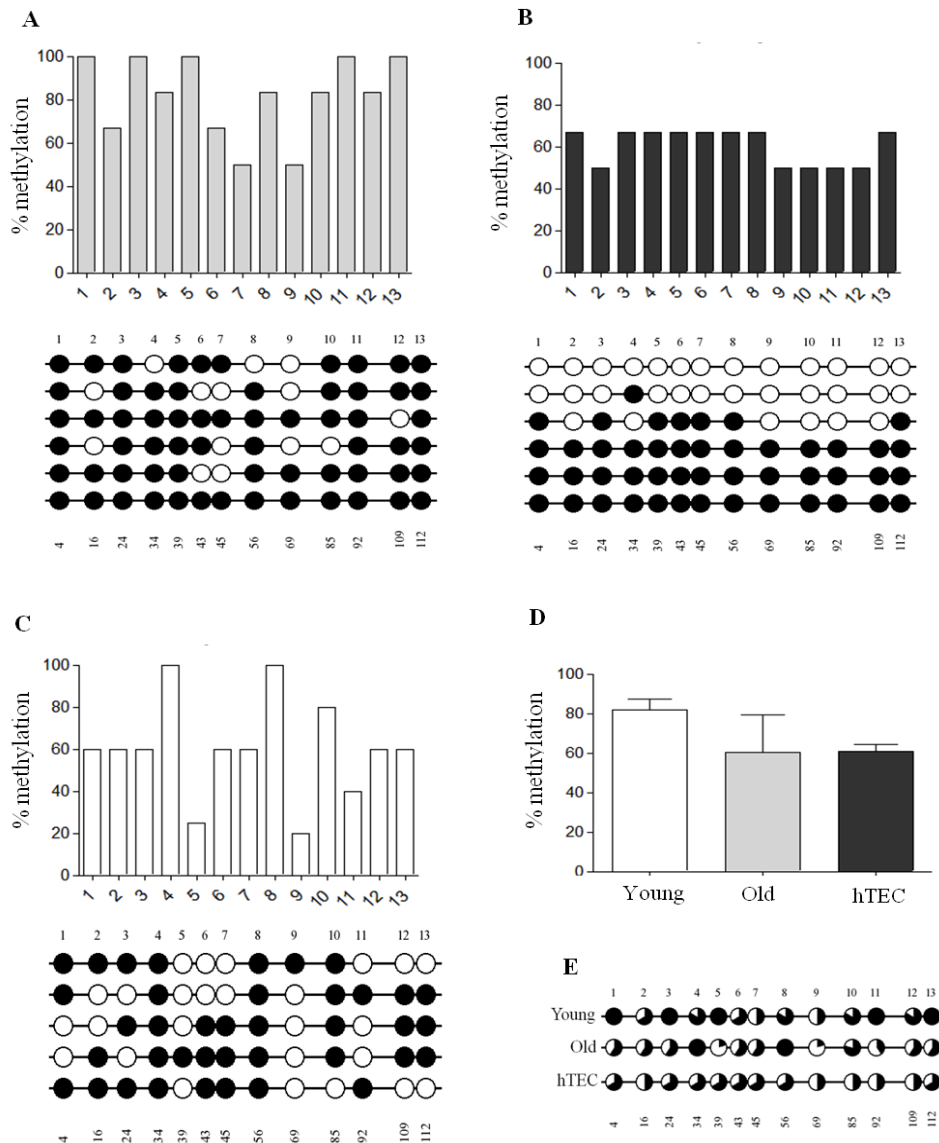
C20_Gen_Ed      --AGGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCGA
[2] C20_D01      --AGGTCATGTTTTTTTCGGTTTTTTTCGTTTAGTTTCGTTTGGTTGTGTTGTGCGA
[3] C20_D03      ----GCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCGA
[4] C20_D05      ----CGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCGA
[5] C20_D07      AGAGGTCATGTTTTTTTCGGTTTTTTTCGTTTAGTTTCGTTTGGTTGTGTTGTGCGA
[6] C20_D09      -GAGGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCGA
[7] C20_E01      -GAGGCGTATGTTTTTTTCGGTTTTTCGTTTAGTTTCGTTTCGTTCCGCTTGTTTGTGCGA
                  ****
C20_Gen_Ed      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTTT-
[2] C20_D01      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTATGTTGATTTTTAGGTTGGGTTTTTA
[3] C20_D03      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTATGTTGATTTTTAGGTTGGGTTTTTA
[4] C20_D05      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTTTTA
[5] C20_D07      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTATGTTGATTTTTAGGTTGGGTTTTTA
[6] C20_D09      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTTTTA
[7] C20_E01      GTTTAGTTTTTCGTTTATAGGTTTGTCCAGGTACGTTGATTTTTAGGTTCCGCGTTTTTA
                  ****
C20_Gen_Ed      -----
[2] C20_D01      TTTATTTTTAGAGTTAGAAATTTGTTTTTA
[3] C20_D03      TTTATTTTTAGAGTTAGAAATTTGTTTTT-
[4] C20_D05      TTTATTTTTAGAGTTAGAAATTTGTTTTTC
[5] C20_D07      TTTATTTTTAGAGTTAGAAATTTGTTTTTA
[6] C20_D09      TTTATTTTTAGAGTTAGAAATTTGTTTTT-
[7] C20_E01      TTTATTTTTAGAGTTAGAAATTTGTTTTT-

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Supplementary figure 4– Sequence alignment from FOYN1/C20 gene region. Panels show the multiple alignment of the sequence clones on FOYN1/C20 region from bisulfite-converted DNA fragments. DNA samples were obtained from hTEC (A), and thymus samples from 5 days-old (B) and 59 years-old donors (C).

(legend continues at the next page).

Methylated CpGs are represented in orange (CG), while in purple are presented the non-methylated CpG (TG), characterized by cytosine conversion in thymine during bisulfite treatment followed by PCR. Non-converted cytosines are showed in green. The multiple alignment was performed with ClustalW 1.83, included in the BiQ analyzer package.



Supplementary figure 5 – FOXN1/C20 region CpG methylation status on human thymic samples. Graphs represents the percentage of CpG methylation, for each residue, in young thymus (5 days-old; “Postnatal” group) (A-), old thymus (59 years-old ; “Adult” group) (B), and in hTEC cell line (C) clone sequences. Lower panels (A-C) show the 13 CpG-s presented as lollipop diagrams, where the methylated residue is shown in black circles, while the non-methylated residue is in open circles.

(legend continues at the next page).

Panel **D** represents the percent mean $\pm$ SEM of CpG methylation per clone sequence for each sample and **E** shows the percent mean per each residue per sample expressed as small pie-charts in the lollipop diagrams. The black label represents the percent mean of methylated CpG and white label is marked for percent mean of unmethylated CpG. Percent values were obtained after multiple sequence alignment using ClustalW algorithm followed for methylated CpG quantification using *BiQ analyzer* software. Difference between the samples was analyzed through Kruskal-Wallis non-parametric test followed by Dunn's multiple test.