

## **Indicaxanthin induces autophagy in intestinal epithelial cancer cells by epigenetic mechanisms involving DNA methylation**

### **Nutrients**

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**Supplemental material: Tables S1-S8 and Figures S1-S4**

**Table S1:** Complete list of selected genes and chromosomal ranges corresponding to their promoters and gene bodies

gene	strand	hg38.coordinates	chr	prom.start	prom.end	body.start	body.end
MTOR	-1	chr1:11,106,535-11,262,551	chr1	11261551	11263551	11106535	11261551
ULK1	1	chr12:131,894,622-131,923,150	chr12	131893622	131895622	131895622	131923150
ULK2	-1	chr17:19,770,830-19,867,936	chr17	19866936	19868936	19770830	19866936
ATG13	1	chr11:46,617,577-46,674,520	chr11	46616577	46618577	46618577	46674520
ATG101	1	chr12:52,069,971-52,077,494	chr12	52068971	52070971	52070971	52077494
RB1CC1	-1	chr8:52,622,458-52,714,435	chr8	52713435	52715435	52622458	52713435
ATG9A	-1	chr2:219,219,380-219,229,717	chr2	219228717	219230717	219219380	219228717
BECN1	-1	chr17:42,810,134-42,824,282	chr17	42823282	42825282	42810134	42823282
PIK3C3	1	chr18:41,955,234-42,087,830	chr18	41954234	41956234	41956234	42087830
PIK3R4	-1	chr3:130,678,934-130,746,829	chr3	130745829	130747829	130678934	130745829
ATG14	-1	chr14:55,366,392-55,411,858	chr14	55410858	55412858	55366392	55410858
AMBRA1	-1	chr11:46,396,414-46,591,364	chr11	46592948	46594948	46396414	46592948
UVRAG	1	chr11:75,815,210-76,144,232	chr11	75814210	75816210	75816210	76144232
NRFB2	1	chr10:63,133,328-63,155,024	chr10	63132328	63134328	63134328	63155024
TFEB.1	-1	chr6:41,683,978-41,735,608	chr6	41734608	41736608	41683978	41734608
TFEB.2	-1	chr6:41,683,978-41,735,608	chr6	41733570	41736782	41683978	41733570
BCL2.1	-1	chr18:63,123,346-63,320,128	chr18	63319128	63321128	63123346	63319128
BCL2.2	-1	chr18:63,313,802-63,318,812	chr18	63317812	63319812	63313802	63317812
ATG3	-1	chr3:112,532,510-112,561,962	chr3	112560962	112562962	112532510	112560962
ATG5	-1	chr6:106,184,476-106,325,760	chr6	106324760	106326760	106184476	106324760
ATG7	1	chr3:11,272,416-11,557,665	chr3	11271416	11273416	11273416	11557665
ATG12	-1	chr5:115,828,200-115,841,565	chr5	115840565	115842565	115828200	115840565
ATG16L1	1	chr2:233,251,673-233,295,669	chr2	233250673	233252673	233252673	233295669
ATG10	1	chr5:81,972,023-82,256,133	chr5	81971023	81973023	81973023	82256133
MAP1LC3B.1	1	chr16:87,383,953-87,404,779	chr16	87382953	87384953	87384953	87404779
MAP1LC3B.2	1	chr16:87,383,953-87,404,779	chr16	87391745	87392506	87392506	87404779
WIPI1	-1	chr17:68,421,281-68,457,496	chr17	68456496	68458496	68421281	68456496
WIPI2	1	chr7:5,190,196-5,231,524	chr7	5189196	5191196	5191196	5231524
WDR45B	-1	chr17:82,614,562-82,648,444	chr17	82647444	82649444	82614562	82647444
WDR45.1	-1	chrX:49,074,640-49,101,121	chrX	49100121	49102121	49074640	49100121
WDR45.2	-1	chrX:49,074,640-49,101,121	chrX	49079073	49081073	49074640	49079073
RUBCN.1	-1	chr3:197,668,867-197,736,904	chr3	197735904	197737904	197668867	197735904
RUBCN.2	-1	chr3:197,668,867-197,736,904	chr3	197748699	197750699	197668867	197748699
RAB7A	1	chr3:128,726,122-128,814,796	chr3	128725122	128727122	128727122	128814796

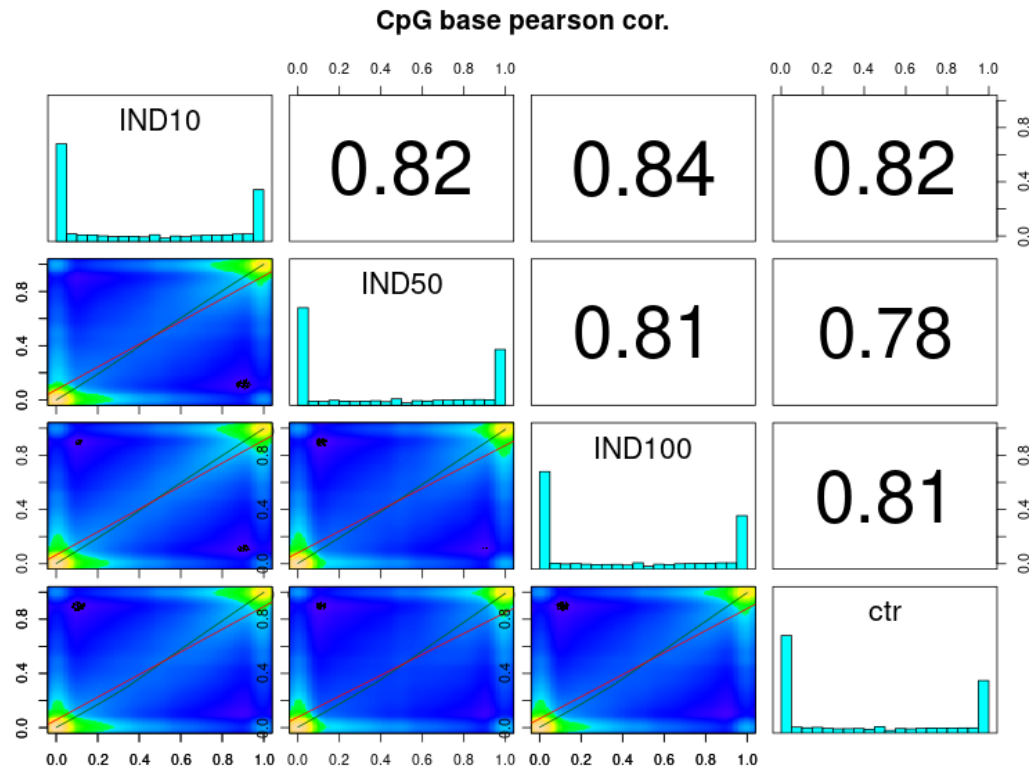
PLEKHM1	-1	chr17:45,435,900-45,490,721	chr17	45489721	45491721	45435900	45489721
TECPR1	-1	chr7:98,214,624-98,252,232	chr7	98251232	98253232	98214624	98251232
EPG5	-1	chr18:45,847,609-45,967,329	chr18	45966329	45968329	45847609	45966329
INPP5E	-1	chr9:136,428,619-136,439,845	chr9	136438845	136440845	136428619	136438845
STX17	1	chr9:99,906,654-99,974,534	chr9	99905645	99907645	99907645	99974534
SNAP29	1	chr22:20,859,007-20,891,214	chr22	20858007	20860007	20860007	20891214
VAMP8.1	1	chr2:85,577,586-85,582,031	chr2	85576491	85578491	85578491	85582031
VAMP8.2	1	chr2:85,577,586-85,582,031	chr2	85560720	85562493	85562493	85582031
VPS11	1	chr11:119,067,692-119,081,978	chr11	119066692	119068692	119068692	119081978
VPS16	1	chr20:2,840,745-2,866,732	chr20	2839745	2841745	2841745	2866732
VPS18	1	chr15:40,894,450-40,903,975	chr15	40893450	40895450	40895450	40903975
VPS33A	-1	chr12:122,229,564-122,266,494	chr12	122265494	122267494	122229564	122265494
VPS39	-1	chr15:42,158,701-42,208,316	chr15	42207316	42209316	42158701	42207316
VPS41	-1	chr7:38,722,974-38,909,191	chr	38908191	38910191	38722974	38908191
SH3GLB1	1	chr1:86,704,576-86,748,184	chr1	86703576	86705576	86705576	86748184
MAPK8.1	1	chr10:48,401,648-48,439,360	chr10	48400612	48402612	48402612	48439360
MAPK8.2	1	chr10:48,401,648-48,439,360	chr10	48305743	48307743	48307743	48439360
DAPK3.1	-1	chr19:3,958,453-3,971,099	chr19	3970099	3972099	3958453	3970099
DAPK3.2	-1	chr19:3,958,453-3,971,099	chr19	3968828	3970828	3958453	3968828
GABARAPL2	1	chr16:75,566,379-75,577,881	chr16	75565379	75567379	75567379	75577881
MAP1LC3A	1	chr20:34,558,718-34,560,345	chr20	34557718	34559718	34559718	34560345
TSNARE1.1	-1	chr8:142,212,080-142,403,182	chr8	142402182	142404182	142212080	142402182
TSNARE1.2	-1	chr8:142,212,080-142,403,182	chr8	142353805	142355805	142212080	142353805
YKT6	1	chr7:44,200,978-44,214,294	chr7	44199978	44201978	44201978	44214294
USE1	1	chr19:17,215,357-17,219,829	chr19	17214357	17216357	17216357	17219829
SNAP25	1	chr20:10,218,830-10,307,418	chr20	10217830	10219830	10219830	10307418

**Table S2.** Oligonucleotides used in this study, related to MSRE-PCR method.

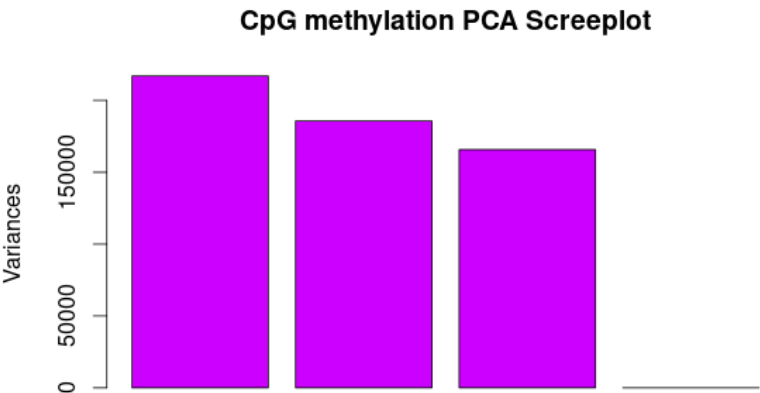
<i>BECN1</i> CpG sites	Primer sequences	Expected fragment size (bp)
<i>HpaII</i> / <i>MspI</i> -126	Forward: 5'-GACTTGGGTGACGGGCTCCG-3'	145
	Reverse: 5'-ACAGCCTGAGCAAAGGCAGTTTAGAGC-3'	
<i>HpaII</i> / <i>MspI</i> -255	Forward: 5'-TCCCAGGTGAAGAGCATCG-3'	189
	Reverse: 5'-GAGGGAAGTCCCAACCTGCG-3'	
<i>HhaI</i> -299	Forward: 5'-CCGAGGCCACAGTGCCTCCG-3'	302
	Reverse: 5'-AAGCGACGCCCTTGACCTCCG-3'	
<i>HhaI</i> -472	Forward: 5'-GGGACTTCCCTCCCTTTACCATC-3'	282
	Reverse: 5'-ACCTTCCACATTCTTGACCACCC-3'	

Primers used for amplification of *BECN1* promoter CpG sites. Genome coordinates of CpG analysed: 472=42,823,819/20; 299=42,823,991/2; 255=42,824,036/7; 126=42,824,165/6

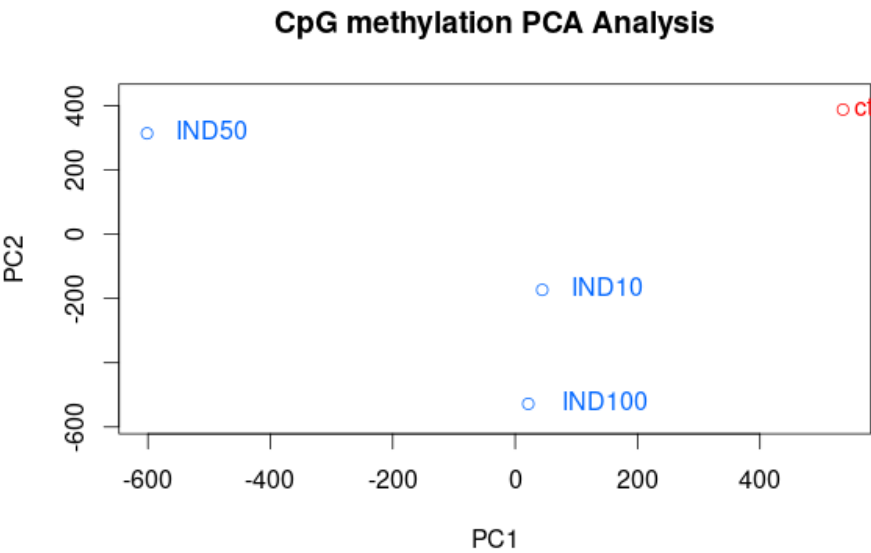
**Figures S1-S3.** RRBS datasets statistical analysis:



**Figure S1.** Pearson Correlation Coefficients calculated using the *methyKit* R package: IND10, IND50, and IND100 RRBS datasets.



**Figure S2.** RRBS datasets - Principal Component Analysis (PCA) calculated using the *methyKit* R package: scree plot.



**Figure S3.** RRBS datasets – PCA calculated using the *methyKit* R package: scatter plot.

**Table S3.** Averaged differential methylation data (log2 weighted average values) of each CpG (min.cov=10) located in the promoter region of genes involved in the autophagy induction.

INDUCTION group	IND10	IND50	IND100	5-azaC
<i>MTOR</i>	0.95	-0.61	-1.52	1.69
<i>ULK1</i>	-0.16	-	0.36	-0.45
<i>ULK2</i>	-0.08	-0.01	-0.16	-0.02
<i>ATG13</i>	0.68	0.88	0.25	0.26
<i>ATG101</i>	0.65	-	-	1.23
<i>RB1CC1</i>	0.4	-	1.6	-

**Table S4.** Averaged differential methylation data (log2 weighted average values) of each CpG (min.cov=10) located in the promoter region of genes involved in the phagophore nucleation process.

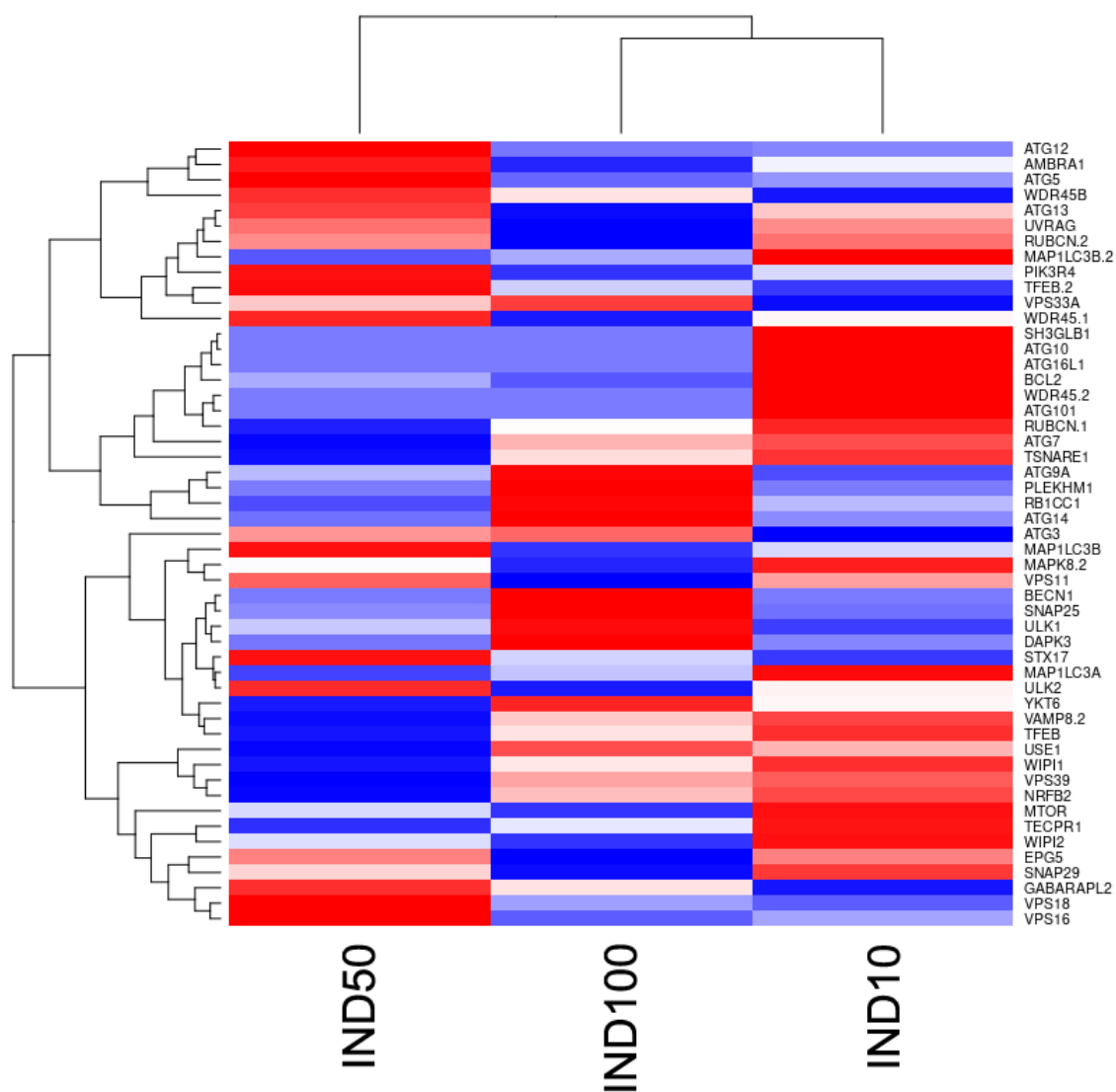
NUCLEATION group	IND10	IND50	IND100	5-azaC
<i>BECN1</i>	-	-	0.30	0.29
<i>PIK3R4</i>	0.85	1.43	0.49	0.75
<i>ATG14</i>	0.61	0.51	2.46	2.83
<i>AMBRA1</i>	1.01	2.07	0.12	0.95
<i>UVRAG</i>	0.73	0.76	0.29	0.18
<i>NRFB2</i>	-0.10	-1.14	-0.39	0.63
<i>TFEB.1</i>	0.01	-0.67	-0.27	-0.10
<i>TFEB.2</i>	-0.08	1.00	0.29	-0.03
<i>BCL2.1</i>	1.37	-	-0.33	1.55
<i>RUBCN.1</i>	0.81	-0.31	0.26	0.30
<i>RUBCN.2</i>	0.53	0.50	0.16	-0.01
<i>SH3GLB1</i>	1.11	-	-	1.60
<i>MAPK8.2</i>	0.29	-0.15	-0.57	1.31
<i>DAPK3.1</i>	-0.20	-0.22	0.38	-0.32

**Table S5.** Averaged differential methylation data (log2 weighted average values) of each CpG (min.cov=10) located in the promoter region of genes involved in the phagophore elongation process.

ELONGATION group	IND10	IND50	IND100	5-azaC
<i>ATG9A</i>	-0.18	0.16	1.18	0.27
<i>ATG3</i>	-1.76	0.02	0.24	0.39
<i>ATG5</i>	0.24	2.18	-	1.68
<i>ATG7</i>	1.14	-1.35	0.56	0.86
<i>ATG12</i>	0.58	2.20	0.53	0.17
<i>ATG16L1</i>	1.26	-	-	0.93
<i>ATG10</i>	1.03	-	-	0.01
<i>MAP1LC3B.1</i>	-0.10	0.53	-0.47	0.23
<i>MAP1LC3B.2</i>	0.87	0.39	0.49	0.69
<i>WIPI1</i>	-0.16	-1.62	-0.78	0.83
<i>WIPI2</i>	-0.66	-1.30	-1.72	-1.01
<i>WDR45B</i>	-	2.53	1.50	0.26
<i>WDR45.1</i>	0.13	1.21	-1.09	0.36
<i>WDR45.2</i>	0.83	-	-	0.99
<i>GABARAPL2</i>	-0.96	-0.54	-0.71	-0.90
<i>MAP1LC3A</i>	-0.04	-0.04	-0.04	-0.05

**Table S6.** Averaged differential methylation data (log2 weighted average values) of each CpG (min.cov=10) located in the promoter region of genes involved in the fusion process.

FUSION group	IND10	IND50	IND100	5-azaC
<i>PLEKHM1</i>	-	-	0.95	0.41
<i>TECPR1</i>	-0.64	-1.56	-1.18	-0.32
<i>EPG5</i>	-	-	-1.34	-1.39
<i>STX17</i>	-0.18	0.05	-0.09	-0.12
<i>SNAP29</i>	-0.38	-0.66	-1.18	-0.57
<i>VAMP8.2</i>	0.30	-0.67	0.00	0.59
<i>VPS11</i>	-0.08	0.00	-0.53	-0.27
<i>VPS16</i>	-0.80	0.00	-0.97	-0.31
<i>VPS18</i>	-0.87	-	-0.73	-0.63
<i>VPS33A</i>	0.04	0.38	0.54	0.32
<i>VPS39</i>	0.04	-1.40	-0.20	-0.54
<i>TSNARE1.1</i>	2.24	-	1.37	1.02
<i>YKT6</i>	-0.17	-0.48	0.10	0.10
<i>USE1</i>	-0.30	-1.90	0.22	0.10
<i>SNAP25</i>	-0.01	-	0.17	-0.03



**Figure S4:** Heatmap obtained using the average values of methylation difference of the studied promoters. *PIK3C3*, *RAB7A*, *VPS41*, *INPP5E* genes were excluded due to the absence of significant data.



**Table S7.** Promoter DMRs (tiles) and their features, related to Table 1.

Gene symbol	strand	tile chr	tile start	TSS	IND 10	IND 50	IND 100	EH38 cCRE	classification CGI	hg38. oreganno.id	TFBS
<i>MTOR</i>	-1	chr1	11263201	11262551	30			E1318739	enhP		
<i>ULK2</i>	-1	chr17	19867801	19867936	24		28	E1851797 ***	prom CGI	OREG1175743 OREG1609744 OREG1853806 OREG1482646 OREG0399094	TFAP2C, FOXP1, RB1, E2F4, E2F4
<i>BECN1</i>	-1	chr17	42823401	42824282	26	49			intron		
<i>PIK3R4</i>	-1	chr3	130747201	130746829	31	36			up-stream	OREG1230891	SMARCA4
<i>BCL2</i>	-1	chr18	63320201	63320128	13		3	E1923297 **	prom CGI	OREG1250101 OREG1219989 OREG1488348 OREG0395794 OREG1340917	SMARCA4, SMARCA4, EGR1, EGR1, CTCF
<i>BCL2.2</i>	-1	chr18	63318401	63318812	-35	-51		E1923293	prom CGI	OREG1937747 OREG1250101 OREG0396743	VDR, SMARCA4, EGR1
<i>ATG3</i>	-1	chr3	112562801	112561962	-40		-20	E2227361 E2227362	enhP		
<i>ATG5</i>	-1	chr6	106325201	106325760				E2490641 E2490642	enhP/ prom CGI	OREG1173894	TFAP2C, SMARCA4, STAT1
<i>ATG7</i>	1	chr3	11271401	11272416	-13	-15			up-stream		
<i>WDR45.2</i>	-1	chrX	49079601	49080073			-15	E2754334	enhP CGI	OREG1600364 OREG1793948 OREG1823967	FOXA1, RBL2, RBL2
<i>EPG5</i>	-1	chr18	45966201	45967329	34				intron		
<i>VAMP8.2</i>	1	chr2	85562201	85561720	16	-9	12		intron	NEAR TO OREG1944064	ZNF263
<i>VPS11</i>	1	chr11	119068401	119067692	28	31			intron	OREG1260042 OREG1081042	SMARCA4, TCF7L2
<i>VPS33A</i>	-1	chr12	122266401	122266494			10	E1651497	prom CGI	OREG1250692 OREG1812689 OREG1791744 OREG1778450 OREG1220514 OREG1797023 OREG1945509 OREG1762962 OREG1612756 OREG1543101 OREG1139438 OREG1112385 OREG0231119 OREG1112386	SMARCA4, RBL2, RBL2, RBL2, SMARCA4, RBL2, ZNF263, MITF, FOS, ETS1, ZNF263, USF2, USF1, USF2

<i>MAPK8.2</i>	1	chr10	48306201	48306743				<b>E1466099</b> **	prom CGI	OREG1493767 OREG1173286 OREG1766204 OREG0273979 OREG0273980 OREG0367292	EGR1, TFAP2C, MITF, ZEB1, ZEB1, EBF1
<i>MAP1LC3A</i>	1	chr20	34558801	34558718	14	12	15	E2108528	enhP CGI		
<i>SNAP25</i> (a)	1	chr20	10217801	10218830			12	E2097209	enhP CGI		
<i>SNAP25</i> (b)	1	chr20	10219601	10218830		-13	11	E2097215	prom	OREG1893190 OREG1181996 OREG1844288 OREG1848497 OREG1856839 OREG1232693 OREG1485028 OREG0004318 OREG0004317 OREG1100322	STAT1, TFAP2C, RB1, RB1, RB1, SMARCA4, E2F4, REST, REST, TFAP2C

UCSC hg38 functional annotations:

EH38 CCRE: ENCODE Candidate Cis-Regulatory Elements (cCREs) combined from all cell types [1]:

enhP: proximal enhancer-like signature

prom: promoter-like signature

\*\* DNase Z-score, H3K4me3 Z-score >1.64 in CaCo-2 cells

\*\*\* DNase Z-score, H3K4me3 Z-score, CTCF Z-score >1.64 in CaCo-2 cells.

ORegAnno: Regulatory elements from ORegAnno [2].

Abbreviations:

TSS: Transcription Start Site

TFBS: Transcription Factor Binding Site

CGI: CpG Island

**Table S8.** Gene body DMRs (tiles) and their features, related to Table 2.

Gene symbol	strand	tile chr	tile start	IND 10	IND 50	IND 100	EH38 cCRE	classification CGI	hg38. oreganno.id	TFBS
MTOR	-1	chr1	11119401	13		-22		intron		
		chr1	11122601		-47			intron		
		chr1	11193601	-13	-10	-17	E1318692	enhD	ANGPTL7 exon	
		chr1	11250001			23		intron		
		chr1	11261201		-23			intron		
ULK1	1	chr12	131896401	14			E1657097 **	prom	OREG1193847 OREG1601983 OREG1660293 OREG1824583 OREG1789197 OREG1854905 OREG0833612 OREG0986170 OREG0880598 OREG1809256 OREG1846437 OREG1843192	ULK1, TFAP2C, FOXA1, RBL2, RB1, MAFF, NFE2::MAF, MAFK, RB1
		chr12	131908201			17		intron CGI		
		chr12	131909401		-17			intron CGI		
		chr12	131919801			-26		intron		
		chr12	131919801			-26		intron		
ULK2	-1	chr17	19772601					intron		
		chr17	19772801		-20	-21		intron	near to E1851762	
		chr17	19836201	16	20	14		intron		
		chr17	19838801					intron		
		chr17	19842401			24		intron		
ATG9A	-1	chr2	219219401	15		51	E2075977	enhP	near to ABCB6 promoter	
PIK3R4	-1	chr3	130727401		74		E2238076 E2238077	enhD		
AMBRA1	-1	chr11	46429001			13		intron	H3K27Ac	
		chr11	46520601		21			intron		
		chr11	46548201		17	19		exon2		
		chr11	46553001					intron		
		chr11	46566201		-16			intron		
UVRAG	1	chr11	75877401	20	20	22		intron		
		chr11	75887401	-8		-18		intron		
		chr11	75974401	24				intron		
ATG7	1	chr3	11290801	-22			E2178358	enhP	OREG1616328 OREG0665690 OREG0741992	ATG7,FOS, FOSL1, JUNB

		chr3	11379801	-37	-29	E2178411	enhD	OREG1256740 OREG0563863 OREG0730235 OREG1675345	ATG7, SMARCA4, DUX4, JUN, ATG7, GATA2
ATG10	1	chr5	82037401	-18			intron		
MAP1LC3B	1	chr16	87390401	-17	-15		intron		
WIPI2	1	chr7	5222801	-11		<b>E2531309</b> **	enhD/intron	OREG1602525 OREG1660808 OREG1194239	WIPI2, FOXA1, TFAP2C
WDR45B	-1	chr17	82646801	17		E1893683	intron		
		chr17	82647001		21	E1893683	intron		
WDR45.1/.2	-1	chrX	49099601	30	24		intron	H3K27Ac in K562	
RUBCN.1/.2	-1	chr3	197707201		-12		intron		
		chr3	197729201		-9	-24	intron		
RUBCN.2	-1	chr3	197739401		-20	E2273633 E2273634	intron		
RAB7A	1	chr3	128743201	14			intron	OREG1926731	TRIM28
PLEKHM1	-1	chr17	45437001	10			last exon	OREG0844941 OREG0890141	MAFF, MAFK
		chr17	45453401		-20	E1866153	exon		
		chr17	45485801		-17		intron		
EPG5	-1	chr18	45966201	34			intron		
INPP5E	-1	chr9	136430201		-12	E2736673	enhD		
		chr9	136432601				intron CGI		
		chr9	136432801	-18	-20		intron	OREG1510897	EGR1
SNAP29	1	chr22	20860401	-24	-14	-22	intron	OREG1489467 OREG1946017	PI4KA, EGR1, TOP3B, ZNF263
VAMP8.2	1	chr2	85562801		21	20	intron		
		chr2	85563001			11	intron		
		chr2	85563201	-12		-16	intron		
		chr2	85563601		-10		intron	OREG1303251	CEBPA
VPS11	1	chr11	119072401	14			intron		
VPS18	1	chr15	40899601		16	15	exon		
		chr15	40899801	-19			exon		
VPS33A	-1	chr12	122241201		11		intron		
MAPK8.2	1	chr10	48346401			E1466129	CTCF		
DAPK3	-1	chr19	3964801				exon CGI	OREG1505997	EGR1
GABARAPL2	1	chr16	75571801	-18	-38	E1828346	intron		
TSNARE1.1/.2	-1	chr8	142237601		18		intron	OREG1516995	EGR1
		chr8	142243201	-20			intron		
		chr8	142244001				intron		

		chr8	142249201		-27		intron		
		chr8	142252001			-17	intron CGI		
		chr8	142253001		21		intron		
		chr8	142253201		17		intron		
		chr8	142257201				intron		
		chr8	142261601	37			intron	OREG1946662	ZNF263
		chr8	142271201	61	63	28	exon or intron	OREG1517008	EGR1
		chr8	142325801		-16		E2674338 E2674339	enhD CGI	
		chr8	142326001			11	E2674339	enhD CGI	
		chr8	142326201			13	13	E2674339	enhD CGI
		chr8	142326401	-15			E2674339	enhD CGI	
		chr8	142346401		-16	-19	E2674353	K4m3	
TSNARE.1	-1	chr8	142361001	-11				intron	
		chr8	142375201			36		intron	
SNAP25	1	chr20	10278801			15		intron	

UCSC hg38 functional annotations:

EH38 CCRE: ENCODE Candidate Cis-Regulatory Elements (cCREs) combined from all cell types [1]:

enhP: proximal enhancer-like signature

enhD: distal enhancer-like signature

K4m3: DNase-H3K4me3

CTCF: CTCF-only

\*\* DNase Z-score, H3K4me3 Z-score >1.64 in CaCo-2 cells.

ORegAnno: Regulatory elements from ORegAnno [2].

Abbreviations:

TFBS: Transcription Factor Binding Site

CGI: CpG Island.

## References:

1. ENCODE Project Consortium. Expanded Encyclopedias of DNA Elements in the Human and Mouse Genomes. *Nature*. 2020 July 30;583(7818):699-710. DOI: 10.1038/s41586-020-2493-4
2. Lesurf R, Cotto KC, Wang G, Griffith M, Kasaian K, Jones SJ, Montgomery SB, Griffith OL, Open Regulatory Annotation Consortium. ORegAnno 3.0: a community-driven resource for curated regulatory annotation. *Nucleic Acids Res*. 2016 Jan 4;44(D1):D126-32. DOI: 10.1093/nar/gkv1203